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**Electrostatically Assembled Porphyrin Aggregates:
From Supramolecular Organization to Metal Ion
Sensing Applications**

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Introduction

Supramolecular Chemistry

Supramolecular chemistry represents a conceptual extension of traditional chemistry, in which the focus shifts from the formation and breaking of covalent bonds to the organization and collective behavior of multiple molecules connected by non-covalent interactions.¹ Sometimes referred to as “chemistry beyond the molecule,” this discipline aims to understand and control the processes of recognition, self-assembly, and emerging functionality in complex molecular systems.² One of the most important architectural concepts in nature is that an increase in structural complexity leads to the development of properties that are not predictable based on the individual constituent units. The construction of complex buildings occurs through a mechanism of molecular recognition, the ability of a molecular entity (host or receptor) to specifically select and bind another molecule (guest) through a set of directional, reversible, and cooperative interactions. (Figure 1) These interactions, which include hydrogen bonds, metal-ligand coordination, van der Waals forces, electrostatic interactions, and π - π stacking, are weak when considered individually but extremely powerful when acting in concert.^{3,4}

Fundamental in this context is the principle of molecular complementarity, which implies both geometric (three dimensional fit) and electronic (charge distribution, H-bond donors/acceptors, polarizability) compatibility between host and guest. The effectiveness of

the bond depends on the pre-organization of the receptor, its ability to present the binding sites in the most favorable conformation even before interaction.⁵

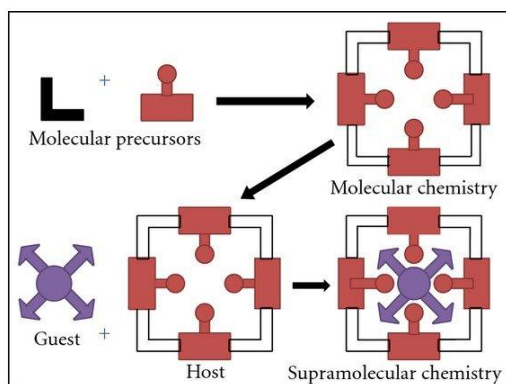


Figure 1. Formation of a host-guest complex through molecular and supramolecular chemical reactions.⁶

- Static vs. Dynamic Molecular Recognition

Molecular recognition processes are commonly divided into **static** and **dynamic** categories.

Static molecular recognition refers to a relatively simple 1:1 interaction, following the classical host–guest model (Lock and key), wherein a receptor binds a guest molecule at a specific, preorganized site. The efficiency of this process depends on the predefined structural features of the host, which are tailored to recognize a specific guest with high affinity and selectivity. This type of recognition is often described by equilibrium

binding constants (K_e), and its thermodynamics are well characterized by techniques such as isothermal titration calorimetry (ITC) or nuclear magnetic resonance (NMR) titrations.^{5,7,8}

Dynamic molecular recognition, on the other hand, introduces a level of complexity more akin to biological regulation. In these systems, the binding of the first guest molecule to the receptor alters the binding properties of the second site, often through conformational changes or allosteric effects. This interdependence of binding events leads to cooperativity, either positive (enhancing binding) or negative (hindering further binding), and represents a powerful strategy to modulate function in a controllable manner. Such behavior is central to many biological processes, including enzyme catalysis and signal transduction.⁹ Moreover, dynamic recognition provides an entry point into stimuli-responsive systems, where external triggers (pH, light, redox conditions, or the presence of a competitive guest) can modulate the strength or occurrence of binding. This adaptability is critical in the development of smart materials, molecular machines, and responsive drug delivery systems

- **Self-assembly and Pre-organization**

One of the key concepts in supramolecular chemistry is **self-assembly**, the spontaneous, thermodynamically driven process by which discrete molecular units organize themselves into ordered structures, such as lattices, fibers, or larger aggregates, without the direct intervention of an external agent. This mechanism allows the emergence of functional architectures, often highly symmetrical and dynamic, and is the basis of

numerous artificial and biological phenomena (such as the formation of cell membranes or secondary protein structures).¹⁰

The aggregation between molecules, which can be one of the outcomes of self-assembly, is regulated by a delicate balance between attractive and repulsive forces and can give rise to different structures depending on the chemical-physical environment, such as pH, ionic strength, temperature, or the presence of co-solvents. Although the topic of aggregation will be explored in more detail later, it is important to emphasize at this point that the ability of certain molecules (planar chromophores such as porphyrins) to organize themselves in an orderly manner is crucial for the design of new molecular materials with targeted optical, electronic, or catalytic properties.^{11,12}

Pre-organization, on the other hand, refers to the structural design of a molecule in such a way as to minimize the conformational changes necessary for binding, thereby maximizing affinity and selectivity. This strategy, introduced and developed by Jean-Marie Lehn, is now a guiding concept in the design of receptors and self-assembling systems.¹³

Chirality

Chirality is one of the most fundamental and pervasive properties in nature, playing a crucial role in numerous phenomena underlying life (Figure 2). It distinguishes between what is symmetrical and what is not, profoundly influencing biological, chemical, and even astrophysical systems. The concept of chirality has progressively gained importance in

multiple disciplines, including chemistry, agriculture, food and environmental sciences, materials science, as well as pharmaceutical and biomedical research.¹⁴

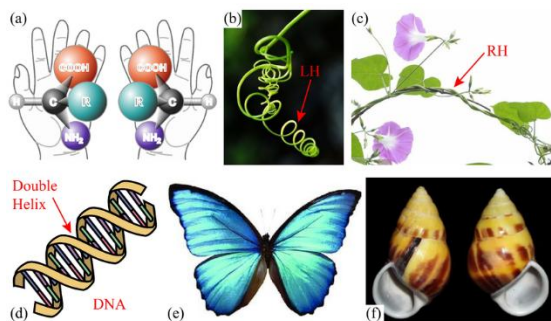


Figure 2. Representation of various examples of biological chirality found in nature.¹⁵

Chirality is a concept that describes an object that cannot be superimposed on its mirror image. In chemistry, a molecule is defined as chiral if it cannot be superimposed on its mirror image. In practical terms, this occurs when the molecule has a chiral center, an asymmetric carbon atom bonded to four different atoms or groups of atoms. However, chirality can also occur in the absence of a stereogenic center. For example, when chirality arises from the spatial arrangement of substituents around an axis and the molecule cannot freely rotate to interconvert mirror-related forms, this is referred to as axial chirality (as in atropisomeric systems). Conversely, when chirality originates from the three-dimensional arrangement of substituents with respect to a molecular plane that lacks symmetrical elements and cannot be rendered superimposable on its mirror image, this is referred to as planar chirality.

Since supramolecular chemistry is based on non-covalent bonds, supramolecular chirality derives from the asymmetric arrangement of molecules through these interactions. This can originate from systems formed by two chiral molecules, from a combination of chiral and achiral molecules, or even from achiral molecules alone. However, supramolecular chirality is strongly influenced by the self-assembly mode of the components, and the presence of chiral molecules plays a key role in determining geometry. In general, chiral molecules tend to generate supramolecular structures with defined chirality. When a chiral molecule interacts strongly with an achiral molecule, it can induce supramolecular chirality through a process known as chirality induction. In systems consisting solely of achiral components, supramolecular chirality can still emerge, but the macroscopic result will generally be racemic.

A crucial aspect of chiral self-assembly is the way in which chirality is generated or transferred. This process is known as chirality transfer and consists of the propagation of chiral information from an individual molecule to an entire supramolecular system, typically through specific interactions. Such transfer represents one of the main origins of supramolecular chirality. The transfer can occur in two ways: (i) the chirality of a center (often an asymmetric carbon or axial chirality) is extended to the entire aggregate or to assemblies containing chromophores, with chirality detectable by CD spectroscopy; (ii) the chiral information of a molecule is transmitted to achiral molecules, generating a complex system with supramolecular chirality. The main interactions

responsible for this transfer include hydrogen bonds, electrostatic interactions, metal-ion coordination, donor-acceptor interactions, guest-guest interactions, and van der Waals forces.

Induction of chirality in supramolecular system

During the self-assembly process, the organization of molecules can generate supramolecular chirality, detectable by CD spectroscopy. This occurs because the chromophores are packed together via non-covalent bonds, adopting a chiral spatial arrangement. In many cases, chiral nanostructures are clearly visible by AFM or SEM. It is important to note, however, that a CD signal does not necessarily imply the presence of chiral molecules: the transfer of chirality depends strictly on the molecular structure of the chiral components. This section therefore analyzes the correlation between supramolecular chirality and molecular structure in typical chiral systems.

Another relevant process is the transfer of chirality from a chiral molecule to an achiral one, and its extension to the entire supramolecular system. In these cases, the induction of chirality in achiral units is essential, and the interaction between chiral and achiral molecules is decisive. This transfer can occur simply by mixing the two components, which then form functional supramolecular systems.

Liu's group reported an example of chirality transfer mediated by hydrogen bonds between a pyridine group and a carboxylic acid.¹⁶ As shown in Figure 3, a simple supramolecular approach has been proposed

to achieve chiral transcription, with the formation of intertwined nanostructures in a two-component system composed of L-glutamic acid-based amphiphiles **64** and bipyridines **4Py**. Compound **64** can self-assemble into nanofibers in water. After co-assembly with bipyridine, the nanostructures show changes in chiral twists due to strong hydrogen bonds between the carboxylic group and the pyridine nitrogen atoms. The chirality of the gelator molecules is thus transferred to the bipyridine aggregates. The resulting supramolecular chirality manifests itself both in the CD signals of bipyridine and in the formation of chiral intertwined structures.¹⁶

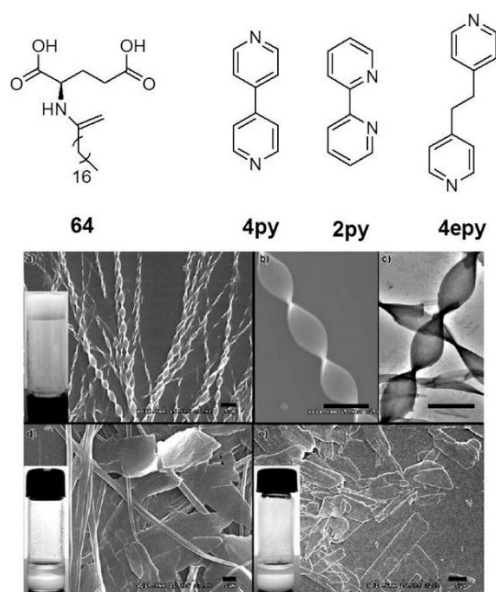


Figure 3. Example of supramolecular chirality induced in self-assembling systems. The molecular structures of the amphiphilic unit **64** and the pyridine co-assemblants **4py**, **2py**, and **4epy** are shown above. Below are electron microscope images and photographs of the samples, highlighting how small variations in the structure of the co-assemblants can drastically modulate the morphology of the supramolecular aggregates, leading to the formation of intertwined fibers, chiral helical nanostructures, sheets, or lamellar aggregates.

In another study, Liu et al. demonstrated that an enantiomer of diamminocyclohexane can induce a water soluble porphyrin (TPPS₄⁴⁻) to form helical nanorods in organic solvents.¹⁷ In this system, mirror-image structures were observed, confirming that chirality induced in achiral π -conjugated molecules can manifest itself not only in CD signals but also morphologically, in oriented helical nanostructures. This type of large-scale structural control from single molecules to nanostructures is particularly promising for the development of asymmetric nanocatalysts.¹⁷

1. Porphyrins

Porphyrins are [18 π] electron heteroaromatic compounds in which the aromatic character of the underlying tetrapyrrole portion and the reactivity of the functional groups present in their side chains govern their rich chemistry.¹⁸ Three different classes of 24 atom macrocyclic tetrapyrrole are found in nature, each differentiated by its respective degree of oxidation. The various classes of porphyrins are: porphyrins themselves, chlorins (chlorophylls and bacteriochlorophylls), and isobacteriochlorins. These structures have complex chemistry that develops around five main structural regions (Figure 4): i) the central nitrogen atoms, ii) any coordinated metal ions, iii) the α -positions (C α = 1, 4, 6, 9, 11, 14, 16, 19), iv) the β -positions (C β = 2, 3, 7, 8, 12, 13, 17, 18) and v) finally the meso positions (5, 10, 15, 20).

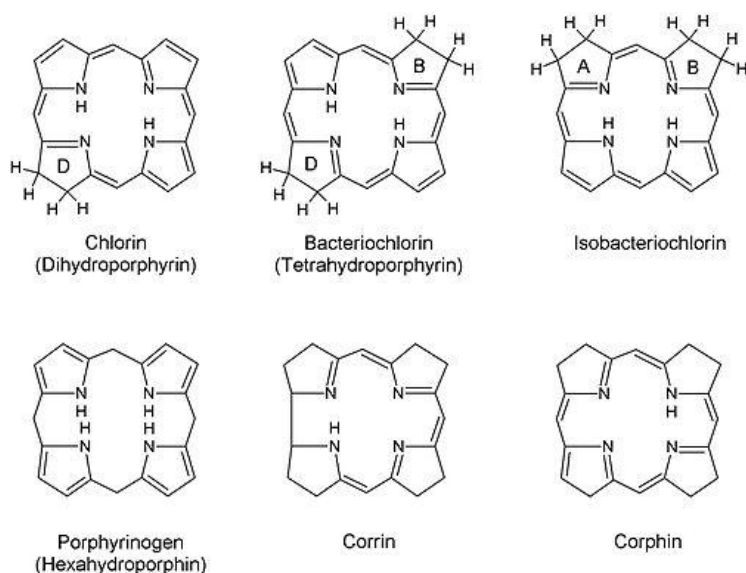


Figure 4. Structures representing the main tetrapyrrole macrocycles and their partially reduced derivatives, including porphyrins, chlorins, bacteriochlorins, porphyrinogens, and corrins.

The meso position are crucial for chemical functionalization and allow for a wide range of structural modifications.

Porphyrins have the ability to coordinate numerous metal ions within the macrocyclic nucleus, thanks to the presence of nitrogen atoms. This structural flexibility, combined with rich photochemical and electrochemical reactivity, explains their fundamental role in biology and their wide application in various scientific fields. Among related structures, there are even smaller porphyrinoids, such as the cofactor F430 and the corrin system (with 23 atoms), typical of vitamin B12. In addition to these, there are numerous synthetic porphyrinoids, which have modifications to the macrocycle (expansions, contractions, isomerism, etc.), further expanding the chemical potential of the class.^{19–21}

Synthesis of porphyrins

The synthesis of porphyrins has been a central theme in organic and bioinorganic chemistry for decades, given the importance of these compounds in fields ranging from catalysis to medicine. The synthesis of tetraphenyl derivatives was first developed from benzaldehyde and pyrrole²². The synthesis was carried out under pressure at 150 °C for 24 hours, but the results were poor. This fact prompted Adler and Longo, in 1967, to use benzaldehyde and pyrrole in propionic acid under reflux for 30 minutes.²³ This approach provided relatively easy access to a variety of symmetrical porphyrins, but suffers from rather harsh reaction conditions, which often compromise yield and compatibility with sensitive substrates.

Many of these limitations have been overcome thanks to the method developed by Lindsey et al. Using tetraphenylporphyrin (TPP) as a model molecule, Lindsey and colleagues demonstrated that the combination of pyrrole, benzaldehyde, and triethyl orthoacetate (as a dehydrating agent), in the presence of an acid catalyst such as boron trifluoride, at room temperature, provides optimal results. The reaction, conducted in an inert atmosphere in dichloromethane for one hour, is followed by oxidation with p-chloranil and reflux for a further hour. This protocol has enabled the synthesis of over 30 different porphyrins with average yields between 30% and 40%, using both $\text{BF}_3 \cdot \text{OEt}_2$ and trifluoroacetic acid (TFA) as catalysts (Figure 5).^{24,25}

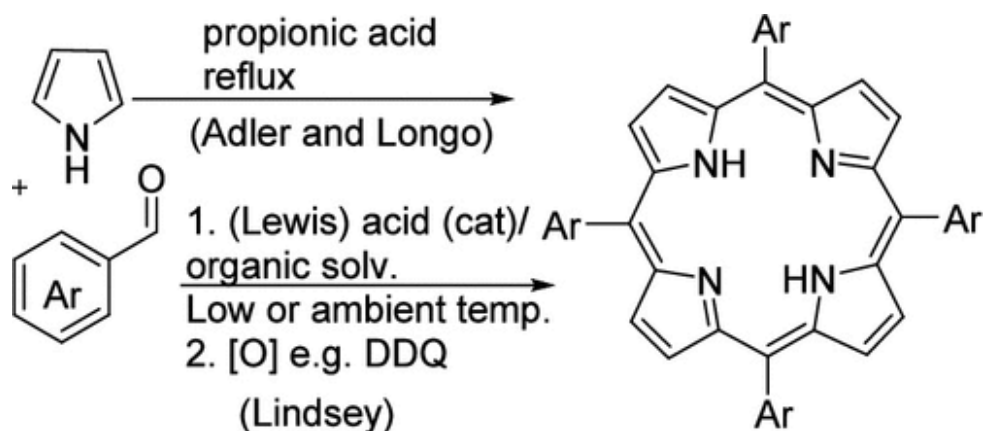


Figure 5. Synthesis of porphyrins by condensation of pyrrole and aromatic aldehydes in propionic acid, followed by oxidation with 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), according to the methods of Adler-Longo and Lindsey

One of the main advantages of the MacDonald method lies in the possibility of selectively introducing substituents both in the β positions of the pyrrole rings and on the bridge carbon of the dipyrromethanes. The MacDonald approach, also known as the 2+2 condensation strategy, consists of the acid-catalyzed coupling of two dipyrromethane units—typically bearing complementary α -functionalities such as formyl groups followed by cyclization and oxidation to afford the porphyrin macrocycle. For example, Honeybourne et al. used a modified version of this 2+2 synthesis to prepare structural analogues of natural porphyrins aimed at studying the enzyme ferrochelatase. The dipyrromethanes employed contained differentiated β -substituents and formyl groups at the α position; their coupling afforded porphyrins in yields of around 10%.²⁶

A further development is the use of monocarbon units such as trimethyl orthoformate, which allows the condensation of two dipyrromethanes without formyl groups. Baldwin et al. demonstrated that this approach

allows the formation of porphyrins linked in opposite meso positions, starting from a bisdipyrromethane and a source of “equivalent carbon.” Post-synthetic modifications of porphyrins are also of great interest.²⁷

The meso and β -positions of the macrocycle are both susceptible to electrophilic aromatic substitution reactions, but the selective introduction of substituents in the meso position often requires pre-substituted porphyrins in the β -position. One of the most common transformations is meso formylation, generally carried out via the Vilsmeier-Haack reaction on the metal complex of the porphyrin, usually based on copper or nickel.^{28–30} The aldehyde group thus introduced represents a strategic access point for further functionalization, such as Knoevenagel type condensations, Wittig reactions, or selective reductions, which will be discussed in detail in a later section of this introduction.

In recent years, more sustainable and green chemistry-oriented approaches have also been proposed, such as the use of solid acid catalysts, reactions in alternative solvents (water or ethanol), and microwave or ultrasound techniques, which allow for reduced reaction times and greater energy efficiency. The use of continuous flows (flow chemistry) is also gaining attention, especially to overcome the problem of dilution and improve the scalability of Lindsey based methods.^{31,32}

Over the last 40 years, interest has shifted to porphyrins with reduced symmetry, particularly 5,15-disubstituted ones (also called “trans porphyrins”). These are easily obtained by reacting aldehydes with simple or partially substituted dipyrromethanes.³³

A notable advance was the introduction of coordination chemistry and catalysis with transition metals, which allowed for a variety of transformations in the meso and β positions of the macrocycle. In particular, reactions such as Heck, Suzuki, and Sonogashira couplings, Buchwald–Hartwig reactions and classic functionalization such as Vilsmeier formylation and C-nitration have enabled a wide range of functional modifications, making it possible, in principle, to construct any desired meso-substituted porphyrin, even in a sequential and rational manner.³⁴ However, the introduction of halogens at well defined positions is still a challenge, making it easier to use partially substituted porphyrins as a starting point for further Pd-catalyzed functionalization.³⁵

Lindsey's approach offered a general route for the synthesis of ABCD meso-tetrasubstituted porphyrins, based on: i) condensation between a 1-acyldipyrromethane and a protected dipyrromethane carbinol, ii) final cyclization via a reaction promoted by $\text{Sc}(\text{OTf})_3$ or $\text{Yb}(\text{OTf})_3$, with the bromine group as the best leaving group and iii) the one-pot transformation allows, in the presence of a templating metal, the direct formation of metalloporphyrins (zinc or magnesium) with yields of 50% and 69%, respectively.^{36,37}

Finally, the combination of supramolecular chemistry with transition metal catalysis has paved the way for the construction of complex systems, such as the porphyrin synthesized by Anderson according to a Vernier model, starting from a pre-constructed tetramer.^{38,39}

Optical properties

Spectroscopically, porphyrins exhibit intense absorption in the visible region, dominated by a very intense band around 400 nm, known as the Soret band (or band B), and a series of lower intensity bands between 500 and 600 nm, called Q bands.

The Soret band is attributable to strongly allowed $\pi \rightarrow \pi^*$ electronic transitions and corresponds to the transition from the ground state S_0 to the second excited state S_2 ($S_0 \rightarrow S_2$), with a particularly high molar extinction coefficient, of the order of $10^5 \text{ M}^{-1}\cdot\text{cm}^{-1}$. The Q bands, on the other hand, are also attributable to $\pi \rightarrow \pi^*$ transitions, but involve the first singlet excited state ($S_0 \rightarrow S_1$) and are less intense due to unfavorable selection rules, with typical molar extinction coefficients of the order of $10^4 \text{ M}^{-1}\cdot\text{cm}^{-1}$.⁴⁰

It is important to note that Q bands are particularly sensitive to the surrounding chemical and physical environment: their position and intensity can vary depending on the solvent, protonation of the core, presence of central metal ions or substituent groups on the periphery of the macrocycle.

Structural modifications, such as the introduction of electron-donating or electron-withdrawing groups, can alter the distribution of electron density on the conjugated system, influencing the orbital energies involved in the transitions and, consequently, causing bathochromic or hypsochromic shifts of the Soret band and Q bands. Similarly, interaction

with the solvent environment, through solvation effects or aggregate formation, can induce significant spectral changes, making porphyrins highly versatile and sensitive tools for applications in spectroscopy, sensor technology, photochemistry and biomedicine.

Acid-base properties

Porphyrins exhibit particularly interesting acid-base behavior, closely related to their electronic structure and the nature of the macrocycle. From a basic point of view, they behave as significantly stronger nitrogenous bases than simple pyrroles, reaching a basicity comparable to that of pyridine. This behavior derives from the presence of four nitrogen atoms in the porphyrin nucleus, two of which can act as protonatable center, generally those not directly bonded to the internal hydrogen (NH). The protonation of porphyrins typically occurs in two successive stages, leading to the formation of mono- and dicationic species.

It is interesting to note that, contrary to what is observed in conventional acid-base systems, the addition of the second proton is more favored than the first. This apparently anomalous behavior has been attributed to the lower aromaticity and structural distortion induced by the first protonation, which reduces the electronic delocalization of the system and makes the monocationic species less energetically stable.⁴¹ The introduction of the second proton, on the other hand, partially restores the overall aromaticity of the system, improving electron delocalization and conferring greater stability to the dicationic species.

From a structural point of view, protonation has marked effects on the planarity of the macrocycle. The dicationic form deviates significantly from planarity, due both to the electrostatic repulsion between the added protons and the central nitrogen atoms (each of which assumes a partial positive charge, $+1/2$), and to the steric hindrance resulting from the proximity of the protons within the porphyrin cavity.^{42,43}

If these protons were coplanar with the ring, the repulsive interactions between them and with the internal hydrogens (NH) would be particularly unfavorable, thus inducing a conformational distortion of the macrocycle to minimize the energy of the system. The monoacidic form of porphyrins, the species with only one added proton, is difficult to isolate and characterize, as it is stable only in extremely narrow pH ranges. The first studies on this subject were conducted by Aronoff and Calvin, who hypothesized the instability of this species⁴⁴; this hypothesis was later confirmed by Walter et al. through spectroscopic investigations on the acidic form of protoporphyrin IX.^{45–47} Aronoff subsequently demonstrated that it is possible to detect the monoacidic species indirectly by UV-Vis spectrophotometric titrations, thanks to changes in the absorption bands associated with different protonation states.⁴⁷

Indeed, from a spectroscopic point of view, the protonation of porphyrins causes significant changes in their electronic spectra. In particular, the formation of the dicationic species leads to bathochromic shifts of the absorption bands, both of the Soret band and of the Q bands, a phenomenon that reflects an increase in electronic delocalization. This

effect is further accentuated in the presence of electron-donating or electron-withdrawing substituents in the meso positions of the macrocycle, which are able to modulate the electron density on the central nucleus and thus influence the strength of acid-base interactions. The presence of aromatic substituents, in particular, can further extend the conjugated system, stabilizing the protonated species through resonance and delocalization.⁴⁸

Overall, the acid–base behavior of porphyrins is a crucial aspect not only for understanding their fundamental chemistry, but also for the numerous applications in which proton reactivity plays a decisive role, such as chemical sensors, optoelectronic devices and catalysis. This is particularly relevant in biological systems, where porphyrins predominantly in their metal coordinated forms perform vital functions in pH sensitive contexts such as oxygen transport, photosynthesis, and enzymatic processes. In these systems, pH-dependent effects mainly arise from the protonation or deprotonation of axial ligands and peripheral functional groups, rather than from protonation of the porphyrin core itself.

Coordination chemistry of porphyrins

Porphyrins are a class of highly versatile macrocycles capable of coordinating a wide range of metal cations thanks to their planar structure. The central cavity, bounded by four inward facing nitrogen atoms, acts as a highly selective coordination center, capable of interacting efficiently with transition metals, alkaline earth metals, lanthanides, and actinides.⁴⁹

The metallation of porphyrins involves the replacement of the protons associated with the central pyrrole nitrogen atoms with a metal ion, which is inserted into the tetrapyrrole cavity (Figure 6). This process is, in most cases, thermodynamically favored, although its speed and reversibility depend on numerous factors, such as the nature of the metal, the solvent, the temperature, and the presence of coordinating agents or catalysts. From an electronic point of view, coordination mainly involves σ donation by the central nitrogen atoms, but may also include π -stabilizing contributions, particularly in the case of metals with d orbitals available for delocalized interaction.⁵⁰

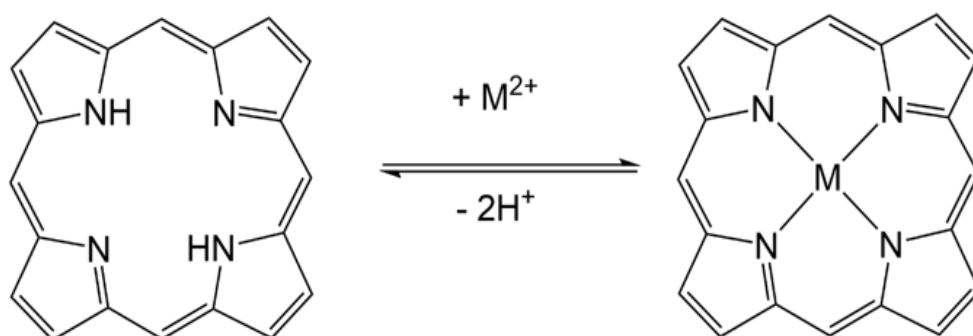


Figure 6. Formation of a metalloporphyrin when a metal ion M^{2+} inserts itself into the central cavity of porphyrin, releasing two protons.

The most commonly used methods for obtaining metalloporphyrins include direct metallation and trans-metallation. Direct metallation occurs through the reaction of a free porphyrin ("free base") with a metal salt in an apolar organic solvent (such as chloroform, toluene) or moderately polar solvent (such as dimethylformamide, DMF), at temperatures generally between 50 °C and 140 °C. The efficiency of the process depends

heavily on parameters such as the ionic size of the metal, its oxidation state, electronic affinity, and electronic configuration, which affect its suitability for insertion into the coordination center of the porphyrin.

Alternatively, trans-metallation involves the use of a preformed metalloporphyrin containing a labile coordinated metal, which is replaced by another metal cation with greater affinity for the tetrapyrrole center. This strategy is particularly useful in the synthesis of complexes containing metals that are poorly reactive or sensitive to high temperatures.^{51,52}

Once metallation has taken place, the structure of the porphyrin undergoes a significant change both in terms of plane and symmetry. The type of geometry assumed by the complex depends heavily on the axial coordination of the inserted metal: i) in the presence of cations such as Cu(II), a square planar geometry is typically observed, with the metal inserted perfectly into the plane of the porphyrin and coordinated exclusively by the four central nitrogen atoms; ii) for cations such as Zn(II), the coordination of an additional axial ligand (solvent or anion) leads to a square-based pyramidal pentacoordinate geometry; iii) metals such as Mn(III) and Fe(III), on the other hand, tend to form octahedral complexes, in which two axial ligands complete the six-coordinate environment.

From a symmetrical point of view, metallation can change and reduce the original symmetry of the system: if free porphyrin has D_{2h} symmetry, the introduction of a metal and axial ligands can lead to higher (D_{4h}) or lower

symmetries (C_{4v} , C_{2v} , or lower), also affecting the electronic, optical, and magnetic properties of the complex.

Metallation mechanisms: the “Sitting-Atop” model

One aspect of particular interest in the coordination chemistry of porphyrins concerns the mechanism of metal insertion into the macrocycle. Although in many cases the reaction occurs in a concerted manner, with simultaneous coordination and deprotonation, a two-step mechanism has been proposed to describe situations in which the process occurs more slowly or under suboptimal conditions (low solvent polarity, less labile metals, sterically hindered porphyrins).⁵³

This model, known as “sitting-atop” (SAT), predicts that the metal initially positions itself above the porphyrin plane, in a pre-coordinated position but not completely inserted into the cavity. In this intermediate state, the metal interacts weakly with one or more nitrogen atoms of the macrocycle, without yet causing complete deprotonation of the central pyrrole sites. Only in a second phase, favored by an increase in temperature or appropriate acid-base conditions, does the metal promote deprotonation, descend into the plane, and insert itself stably into the tetrapyrrole cavity, completing the formation of the metalloporphyrin complex.⁵⁴

The existence of these intermediates has been confirmed experimentally using spectroscopic techniques (UV-Vis, NMR, EPR) and X-ray crystallography. In some cases, the SAT complex can be isolated or observed in equilibrium with the fully metallated form, suggesting that

metallation can be described by a pre-associative equilibrium between free porphyrin and intermediate coordination.⁵³

Kinetic considerations and role of cations

The metallation of porphyrins is also influenced by ionic and solvent effects. Systematic studies have shown that the rate of metallation can be accelerated in the presence of alkali ions such as Li^+ , which presumably act by stabilizing the intermediate complex or facilitating deprotonation through electrostatic effects or conformational changes in the macrocycle. Furthermore, the use of water-soluble metal salts and aprotic polar solvents such as DMF improves the solubility of free porphyrin and facilitates metal access to the reaction center.⁵⁵

Recently, alternative and sustainable methods for metallation have been developed, including the use of microwaves in green solvents⁵⁶ and mechanochemistry (grinding-induced solid-state reactions), which allow metalloporphyrin complexes to be obtained quickly, with high yields and minimal production of by-products.⁵⁷

In addition to simple metal coordination, porphyrins are capable of self-assembling into complex supramolecular structures through non-covalent interactions or multiple coordinations. In the presence of monovalent metal ions such as Na^+ or K^+ , porphyrins modified with peripheral pyridyl groups can aggregate to form ordered lattices, guided by the formation of secondary coordination bridges. This type of architecture is influenced by

the shape of the porphyrin, its conformational flexibility, and the spatial arrangement of the donor sites.⁵⁸

Such assemblies can also interact with biological molecules, such as DNA, giving rise to chirally active structures, whose formation can be monitored using advanced spectroscopic techniques (circular dichroism, time-resolved fluorescence, absorption spectroscopy). In such contexts, metallation not only confers stability to the structure but can also modulate the affinity for biomolecules and overall photophysical activity.^{59–64}

Porphyrin based Self-assembled structures

Porphyrins, thanks to the previously described properties, significantly favor the establishment of non-covalent interactions. These include π -stacking interactions, hydrogen bonds, van der Waals forces, and electrostatic interactions, which form the basis for supramolecular self-assembly phenomena.⁶⁵

Through these types of interactions, porphyrins can aggregate to form structures of increasing complexity, such as dimers, trimers, or even extended aggregates. This behavior makes them ideal for applications in the fields of functional materials, optical sensors, photonics, and medicine, particularly in photodynamics and molecular nanotechnology. In the case of water-soluble porphyrins, aggregation can be finely modulated by varying the properties of the medium, such as ionic strength, pH, temperature, or the addition of specific co-solvents or additives. These

parameters influence the balance between attractive and repulsive interactions, and therefore the stability and morphology of the resulting aggregates. In the case of penta-coordinated or hexa-coordinated metalloporphyrins, in which an axial ligand is coordinated to the metal center outside the porphyrin plane, the steric hindrance introduced can inhibit supramolecular aggregation, preventing the efficient approximation of monomeric units. This steric effect is a useful tool for controlling or preventing self-assembly in systems where monomeric distribution is required.

The formation of porphyrin aggregates is accompanied by marked changes in their spectroscopic properties, particularly in the UV-Vis absorption bands, resulting from a perturbation of the electronic states compared to the isolated monomer. This variation can be rationalized through the exciton splitting model proposed by Kasha in 1965, which describes the interaction between transition dipoles of coupled chromophores.⁶⁶

According to this model, when two or more chromophores interact with each other in a defined geometry, their excited electronic levels (in particular the LUMO orbital) couple and split energetically, forming new absorption bands. The magnitude of the splitting (ΔE) depends on three main factors: i) the transition moment of each monomer (μ), ii) the angle α formed between the polarization axes of the two molecules, and iii) the angle θ , which represents the relative orientation between the polarization axes and the line connecting the dipole centers. Considering the two

limiting cases: i) when $\theta = 0^\circ$ (the chromophores are aligned face-to-face), the dipole transition moments are consistent (in phase) in the highest energy excitonic level. This leads to the formation of H-aggregates, characterized by a hypsochromic (blue) shift and hypochromic effect. ii) When $\theta = 90^\circ$ (edge-to-edge), the dipole transition moments are consistent (in phase) in the lowest energy excitonic level. This gives rise to J-aggregates, which show a bathochromic (red) shift.

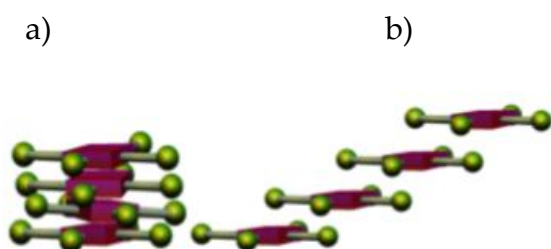


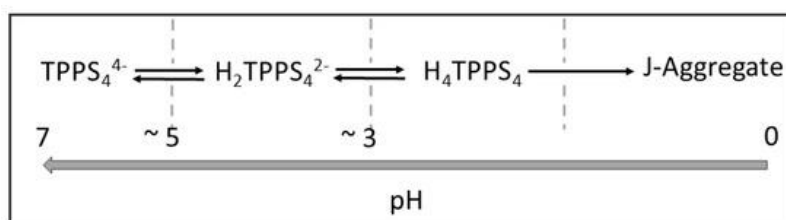
Figure 7. a) aggregate H formed by a face-to-face arrangement and b) aggregate J formed by an edge-to-edge arrangement.

J-aggregates of TPPS₄⁴⁻ porphyrin

A paradigmatic case study in the field of supramolecular self-assembly and induced chirality is that of 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (TPPS₄⁴⁻). This water-soluble porphyrin, characterized by sulfonate ($-\text{SO}_3^-$) groups in the para positions of the phenyl substituents, is an ideal model for studying aggregation processes in aqueous environments and for investigating induced supramolecular chirality phenomena.

Depending on the pH of the solution, TPPS₄⁴⁻ can undergo different stages of protonation, generating species with distinct overall charges.

At neutral pH, it exists as a stable tetra-anion (TPPS₄⁴⁻), since all four sulfonate groups are fully ionized. The pK_a value for protonation of the two central nitrogen atoms of the porphyrin macrocycle is 4.9; therefore, at pH values below 3.9, the corresponding dianion (H₂TPPS₄²⁻) is formed. Upon further acidification (pH < 3), partial protonation of two sulfonate groups leads to a neutral zwitterionic species (H₄TPPS₄). This latter species acts as the precursor of J aggregates, generated through supramolecular self-assembly of monomeric units, driven by non-covalent interactions such as π -stacking and electrostatic forces (Scheme 1).⁶⁷⁻⁶⁹

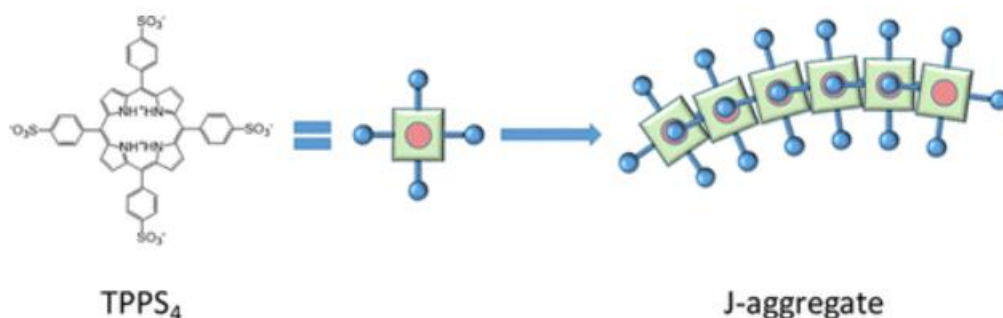


Scheme 1. Various forms of TPPS₄⁴⁻ porphyrin and formation of J-aggregate depending on the pH of the solutions.

The aggregation process develops in three stages: i) nucleation: initially, small supramolecular nuclei (dimers, trimers) are formed, stabilized by aromatic stacking and electrostatic interactions between the -SO_3^- groups and the protonated core of the neighboring unit. This phase is slow and determines the formation of the “seed” of the aggregate; ii) directional growth: once nucleation has occurred, the nuclei extend along a preferential direction, in an anisotropic manner, giving rise to molecular

ribbons, fibrils, or filamentous structures. π -interactions drive staggered stacking (typical of J-aggregates), while the negative charges of the sulfonate groups modulate the interplanar distance (Scheme 2); iii) hierarchical organization and complex morphologies: in the presence of chiral agents, the aggregate structures can further organize themselves into complex architectures, such as helical ribbons, tubules, or chiral fibers, which are often stable and persistently ordered.

At the spectroscopic level, the transition from monomer to aggregate is evidenced by a marked shift in the Soret band ($\sim 434 \text{ nm} \rightarrow \sim 490\text{--}494 \text{ nm}$), accompanied by fluorescence quenching and increased Rayleigh scattering (RLS).^{70–72}



Scheme 2. Molecular structure of diacid TPPS₄⁴⁻ and schematic representation of a J-aggregates.⁷³

Induction of chirality via chiral agents

Although TPPS₄⁴⁻ is an achiral molecule, its aggregation can be strongly influenced by the presence of external chiral agents, such as amino acids (L-arginine, L-serine, L-phenylalanine),⁷⁴ or chiral acid like tartaric

acid⁷⁵, even in sub-stoichiometric quantities. These trigger a process of chirality transfer and amplification at the supramolecular level, generating optically active aggregates, as evidenced by the appearance of Cotton signals in CD (Circular Dichroism) spectroscopy.

The mechanisms involved include: i) electrostatic interactions between the -SO_3^- groups and the amino/carboxyl groups of the inducers; ii) directional hydrogen bonds and iii) solvation and orientation effects in the aqueous bulk.

Effect of the mixing protocols in J-aggregates formation

It has been demonstrated in a study on the hierarchical self-assembly kinetics of 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin ($\text{H}_2\text{TPPS}_4^{4-}$) under acidic conditions and at high ionic strength, in the presence of a chiral templating agent such as tartrate, that the order of the mixing protocol plays a decisive role in both the aggregation kinetics and the expression of chirality in the final J-aggregates.⁷⁶ In particular, only when the chiral tartrate anion is premixed with the porphyrin do the resulting nanoarchitectures exhibit circular dichroism (CD) spectra clearly reflecting the chirality induced by the template.

The kinetics of high ionic strength aggregation in acidic conditions and, finally, in the presence of an added chiral templating agent (tartrate) were studied using UV/Vis spectroscopy, resonance light scattering, and circular dichroism (CD). The effect of changing the mixing order of the

various components in the solution on the kinetic parameters and on the expression of chirality on the final J aggregates was evaluated.⁷⁴

To study the kinetic growth of J aggregates, the Pasternack equation was used, which describes the sigmoidal trend typical of cooperative aggregation processes^{68,77}

$$\text{Ext} = \text{Ext}_\infty + (\text{Ext}_0 - \text{Ext}_\infty) (1 + (m - 1)\{k_0 t + (n + 1)^{-1} (k_c t)^{n+1}\})^{-1/(m-1)}$$

where k_0 and k_c represent the constants related to a non-catalysed and an autocatalytic pathway, respectively, m is the number of monomers present within the nucleus responsible for the aggregation process and, finally, n is the power of time that regulates the autocatalytic phase. Ext_0 and Ext_∞ are the extinction values at the beginning and end of the aggregation process.

Three distinct mixing protocols were analyzed, each of which differently influenced the self-assembly and chirality of the J aggregates. The first protocol involves premixing the tartrate anion with porphyrin before adding NaCl: this method proved to be the most effective, leading to the formation of well-defined chiral J aggregates, characterized by intense and structured circular dichroism (CD) spectra, indicative of strong chiral induction. In the second protocol, however, NaCl is added first to the porphyrin, followed only subsequently by the tartrate buffer: in this case, the ability of the chiral anion to induce chirality in the aggregates is significantly reduced. Finally, in the third protocol, a premixed solution of NaCl and tartrate is added directly to the porphyrin: again, the

induction of chirality is less efficient than in the initial premixing of porphyrin and tartrate, emphasizing how the order of mixing plays a crucial role in the formation of chiral supramolecular structures.⁷⁸

These results support a mechanism in which the chiral anion interacts with porphyrin already in the early stages of nucleation, forming preorganized ionic pairs or chiral dimers, which direct the growth of the aggregate along a stereoselective path. The mixing protocol, therefore, controls preferential access to chiral nanoarchitectures, with important implications for the design of functional supramolecular materials.

2. Self-assembling onto polymeric matrices

Numerous studies have shown that porphyrins can interact effectively with oppositely charged polyelectrolytes. These include biomolecules such as DNA and RNA, but also synthetic or semi-synthetic polyelectrolytes such as polyadenylate⁷⁹, polyglutamate^{80,81} and polystyrene⁸². These polymers act as molecular scaffolds, capable of organizing porphyrins into defined structures both in solution and in the solid state. The type of interaction, as well as the morphology of the resulting aggregates, strongly depends on the characteristics of the host polymer (charge, length, flexibility) and environmental conditions (pH, ionic strength, concentration).

Interaction between cationic porphyrins and polyglutamic acid

Cationic porphyrins, such as 5,10,15,20-tetrakis(N-methylpyridinium-4-yl)porphyrin (TMpyP), are among the most studied in terms of interaction with anionic polyelectrolytes, thanks to their high water solubility and symmetrical distribution of positive charges. In particular, poly(L-glutamic acid) (PGA) has been widely used as a biologically relevant polyelectrolyte model to study the interaction with TMpyP.^{80,83}

A study reported in literature showed that PGA can act as a catalytic matrix to accelerate the metallation of TMpyP with Zn^{2+} ions. Under weakly acidic conditions (pH ~5.4), where PGA assumes a highly charged random coil conformation, the rate of metal ion insertion into porphyrin increases by up to three orders of magnitude compared to free porphyrin in solution. This remarkable catalytic effect is attributed to the ability of PGA to spatially organize the reacting species, reducing the activation entropy and promoting the approach between TMpyP and Zn^{2+} .⁸¹

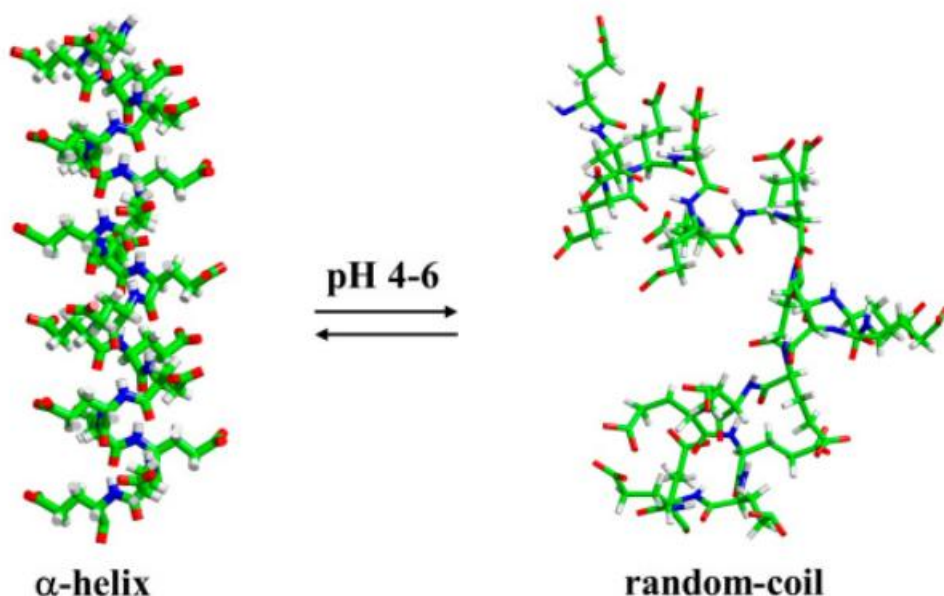


Figure 8. The structural transition of a PGA from an alpha-helix conformation to a random coil conformation, induced by an acidic pH environment.⁸¹

Experiments conducted at varying pH levels have shown that the efficiency of the metallation process is strongly correlated with the conformational transition of PGA from α -helix to random coil. The addition of salt to the solution, increasing the ionic strength, drastically reduces the observed effect, confirming the electrostatic nature of the interaction between porphyrin and the polymer matrix.

Other investigations, using circular dichroism (CD) spectroscopy, revealed that TMpyP, bound to PGA in a random coil conformation, does not induce significant CD signals. This suggests flexible and dynamic interaction, without rigid orientation, in contrast to what is observed in similar systems, for example between anionic porphyrins and cationic

polylysine, where more ordered supramolecular complexes are observed.⁸⁴

Interactions between porphyrins and poly(vinyl sulfonate) (PVS)

In addition to biological polyelectrolytes, synthetic models such as poly(sodium vinylsulfonate) (PVS) have also been widely used in the study of porphyrin self-assembly. PVS is a polyanion that is completely dissociated in aqueous solution, providing an intense and uniform electrostatic field, making it ideal for interaction with cationic species.

In the case of cationic porphyrins, recent studies have investigated the interaction between TMpyP and PVS, as well as its metalated derivatives (Cu^{2+} , Zn^{2+} , Mn^{3+} , Au^{3+}), under different pH and concentration conditions.⁸⁵ It has been observed that the high electrostatic field exerted by PVS is able to stabilize different aggregate forms of porphyrin depending on the pH: i) at $\text{pH} > 5$, an H-type aggregate is formed, characterized by planar stacking and a UV-Vis spectrum typical of exciton coupling between chromophores; ii) at $\text{pH} < 4$, the diacid form of porphyrin prevails, indicative of advanced protonation favored by proximity to the polymer's anionic groups. The interconversion between these two forms is completely reversible with pH variations, suggesting a dynamic equilibrium regulated by environmental conditions. At low PVS concentrations and in a strongly acidic environment, the development of compact J-aggregates consisting of porphyrin in hexa-cationic form has also been detected.

Interaction with nucleic acid (DNA or RNA)

Interactions between DNA and porphyrin are influenced by numerous factors, including the number of charges present on the porphyrin, the stoichiometric ratio between the two components, the ionic strength of the environment, and the nature of the solvent. The bond between DNA and porphyrin can occur in several ways, including intercalation between bases, interaction with the minor and major grooves of the double helix, the formation of external complexes, and association with structures such as G-quadruplexes. The first three modes are generally grouped together in the so called “three mode binding model” described by Fiel and colleague^{62,86}. Intercalation involves the insertion of a planar aromatic system, such as that of porphyrin, between DNA base pairs, a process that involves the partial opening of the double helix. This type of interaction can cause the length of the DNA to be extended, especially in shorter fragments. Bonding in the grooves, on the other hand, occurs through non-covalent interactions between cationic porphyrins and the negatively charged phosphate group of DNA. Generally, guanine-cytosine (GC) base pairs tend to favor intercalation, while adenine-thymine (AT) pairs show greater affinity for minor groove interaction. It has been observed that the competition between intercalation and external binding depends significantly on the ionic strength of the environment and the molar ratio between porphyrin and DNA bases. Other factors crucial in determining the mode and selectivity of interaction include the shape of the porphyrin cation, the local electric field generated by DNA, van der Waals forces,

potential hydrogen interactions with DNA functional groups, and hydrophobic effects.^{87,88}

The first systematic study on the interactions between nucleic acids and tetrakis(4-N-methylpyridyl)porphyrin, was published Fiel and Munson in 1980⁶². The analysis was based on data obtained from binding isotherms, thermal fusion curves, and circular dichroism measurements. Subsequently, Pasternack et al. provided a detailed description of the structural characteristics of metalloporphyrins in 1983⁶¹. It has been hypothesized that the mode of interaction with DNA may vary depending on the central metal and the size and position of the substituent groups on the periphery of the porphyrin⁸⁹. Porphyrins in their free base form or containing Cu and Ni tend to bind preferentially to regions rich in GC base pairs, while complexes with Zn, Mn, Fe, and Co show a greater affinity for AT sequences.

A particularly advantageous aspect of using porphyrins as structural probes for DNA in solution is the availability of numerous metal derivatives. This allows for a more detailed exploration of the nature of the bond with DNA, distinguishing, for example, between metalloporphyrins without axial ligands (such as copper(II) and nickel(II)), those with a single axial ligand (such as zinc(II) and iron(III)), and those with two axial ligands (such as manganese(III), iron(III), nickel(II), and cobalt(III)). Thanks to their structure, these derivatives can act as true “molecular gauges,” allowing the maximum thickness

compatible with the intercalation of a drug within the DNA double helix to be estimated.

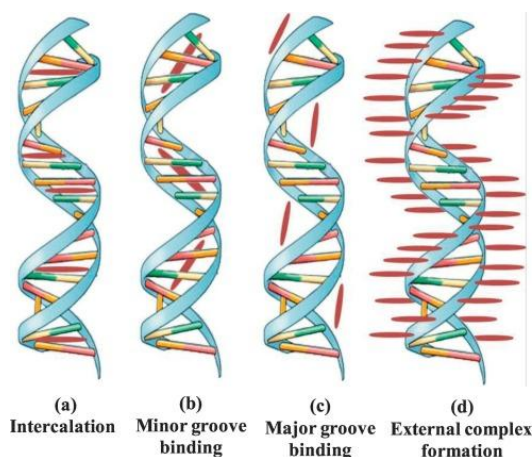


Figure 9. The image illustrates four ways in which molecules bind to DNA.

3. Quantum Dots

Quantum dots (QDs) are nanostructured materials consisting of particles measuring a few nanometers (typically between 2 and 10 nm). Their peculiarity lies in the fact that, of such sizes, electrons and holes are spatially confined within the particle. This phenomenon is called quantum confinement and occurs when the particle size approaches or becomes smaller than the Bohr radius of the exciton (the bound electron-hole pair).

Due to this confinement, QDs have an energy gap (band gap) that strongly depends on the size and shape of the particle, as well as its chemical composition. In particular, as the size of the QD increases, the band gap tends to decrease, and consequently the wavelength of the emitted light

increases (red shift). This makes quantum dots luminescent, with emissions that can be controlled dimensionally, a property exploited in various optical and electronic applications.^{90,91}

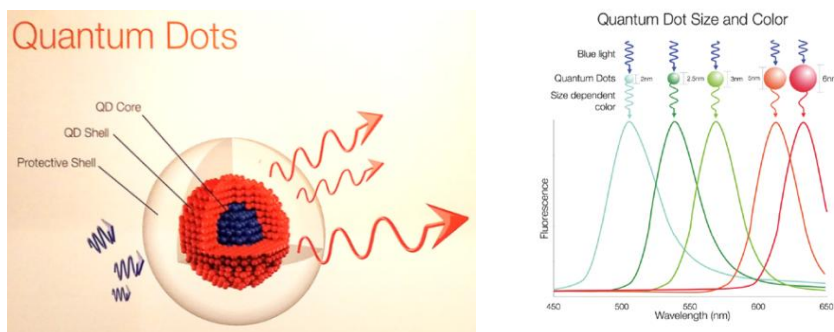


Figure 10. The size of quantum dots influences the color of light they emit when excited by blue light. Smaller dots emit light at shorter wavelengths (blue/green), while larger dots emit at longer wavelengths (red).

Traditionally, QDs are made of inorganic metal-based semiconductors (e.g. CdSe, CdTe, PbS). However, due to the toxicity and poor biocompatibility of many heavy metals, research is shifting towards alternative, metal-free materials, including carbon quantum dots (CQDs) and, in particular, graphene quantum dots (GQDs).⁹²

Structure and properties of graphene

Graphene is a material consisting of a single two-dimensional layer of carbon atoms arranged in a planar hexagonal structure. Each carbon atom is sp^2 hybridized and forms three σ bonds with adjacent carbon atoms, leaving one delocalized π electron per atom, which is responsible for the excellent electronic properties of graphene.

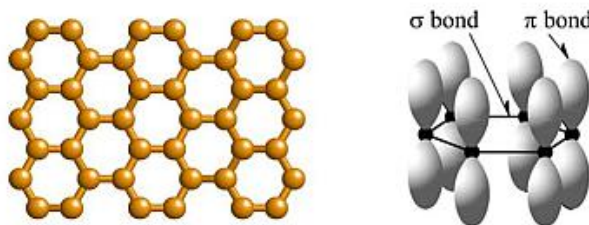


Figure 11. The graphene structure

Main characteristics of graphene are: i) very high electrical conductivity (thanks to delocalized π electrons); ii) high electron mobility; iii) no intrinsic band gap (semimetal), but the band gap can be opened by introducing defects, deformations, or quantum confinement; iv) excellent mechanical and thermal properties, and v) properties that depend heavily on the number of layers, size, and presence of defects or functional groups.^{93,94}

Graphene Quantum Dots (GQDs)

Graphene Quantum Dots (GQDs) are a class of graphene-based nanomaterials typically smaller than 10 nm, which exhibit quantum confinement and very pronounced edge effects. Unlike carbon quantum dots (CQDs), which are generally spherical, GQDs have an anisotropic structure, with planar shapes, often hexagonal or irregular.

Their structure is characterized by the presence of various functional groups, such as hydroxyl ($-\text{OH}$), carboxyl ($-\text{COOH}$), and amino ($-\text{NH}_2$) moieties, located at the edges or on the surface of the graphene domains, which originate from the synthesis processes and play a crucial role in determining their solubility, chemical reactivity, and interaction with

surrounding species. In addition, GQDs can be doped with heteroatomic elements including nitrogen, sulfur, phosphorus, or boron, a strategy that allows fine tuning of their electronic structure and optical properties, such as band gap modulation and enhanced photoluminescence. GQDs have graphite structures of one or more layers connected to chemical groups on the surface/edge. They consist of graphene lattices and are characteristic because, unlike typically spherical carbon quantum dots, they are anisotropic.

Optical properties of GQDs

Graphene quantum dots exhibit strong absorption in the ultraviolet region, typically between 200 and 400 nm. A characteristic absorption band around 230 nm is commonly attributed to the $\pi \rightarrow \pi^*$ electronic transitions of the C=C bonds within the sp^2 hybridized graphene lattice. In addition, a broader absorption feature appearing at approximately 300–330 nm is generally associated with $n \rightarrow \pi^*$ transitions, which arise from non-bonding electrons of surface functional groups, such as carbonyl (C=O) moieties, or from heteroatomic dopants, for instance C=N bonds in nitrogen doped GQDs. Photoluminescence represents one of the most intriguing properties of graphene quantum dots from both fundamental and applied perspectives. Unlike conventional semiconductor quantum dots, GQDs combine size-dependent optical emission with high chemical and photostability, excellent biocompatibility, and relatively low toxicity. These features make them particularly attractive for applications in bioimaging, sensing,

optoelectronics, and photocatalysis, while also stimulating ongoing theoretical investigations aimed at elucidating the origin of their emission mechanisms.⁹⁵

Synthesis of GQDs

Graphene quantum dots can generally be synthesized using two methods: “top-down” and “bottom-up” (Figure 11). The top-down approach refers to the breakdown of large carbon structures such as graphite, carbon nanotubes, and graphene oxide into nanometric dimensions of the material, with a size of less than 10 nm, using, for example, laser ablation, arc discharge, and electrochemical techniques. The bottom-up approach, on the other hand, involves the synthesis of CDs from small organic precursors such as molecules or polymers. This involves processes such as carbonization, dehydration, and polymerization through, for example, thermal decomposition, microwave pyrolysis, plasma treatment, and hydrothermal oxidation.^{96–98}

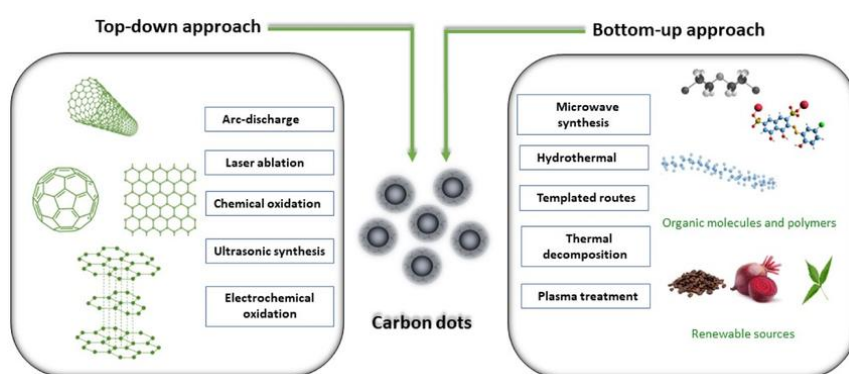


Figure 12. Diagram of the two main approaches for synthesizing carbon-based quantum dots, highlighting the different techniques and starting materials used.⁹⁹

To improve the fluorescent and stability characteristics of GQDs, and of CDs in general, various studies have been carried out on their functionalization. In particular, carbon-based quantum dots are doped, generally with N, S, B, and P. In this way, CDs/polymers are synthesized, where the polymer is considered as the continuous phase and the CDs are considered as nanofiller species.⁹⁸

Consequently, the central structure and properties of CD/polymer nanocomposites depend on the polymer matrix, which can be thermoplastic, linear or branched, or even thermosetting, and the CDs can be covalently attached to the polymers.¹⁰⁰

Depending on the polymer structure, the covalent interaction forces between the polymer and the quantum dots vary, which further influences the dispersion of the CDs and their preparation cost. Current approaches to constructing CD/polymer nanocomposites can be classified into mixing, chemical grafting, and in situ growth. The physical mixing approach is a simple method for preparing these systems and involves mixing tiny amounts of CDs with the polymer matrix by mixing solutions. The formation of the nanomaterial is mainly attributed to non-covalent interactions such as hydrogen bonding, electrostatic interactions, or π -interactions between the polymer and the CDs.¹⁰¹

Although this is currently the main approach for constructing functionalized CDs, it has some drawbacks in that the molecular interactions between CDs and polymer matrices are relatively weak and unstable. The grafting method provides strong bonds between CDs and

the polymer matrix, but it has disadvantages such as the involvement of multiple preparation steps, complicated reaction processes, and, in many cases, the use of non-eco-sustainable reagents. In situ growth of CDs within the polymer matrix, on the other hand, is an environmentally friendly and easy process, and the bond between the CDs and the polymer involves physical and chemical interactions, thus ensuring greater stability.¹⁰²

Supramolecular interactions between QDs and porphyrin

Porphyrins have been extensively studied as key elements in the design of functional nanomaterials, thanks to their highly conjugated electronic structure, photostability, and ability to selectively coordinate metals. These characteristics make them particularly suitable for integration into supramolecular architectures and nanostructured systems, where they can act both as active units and as molecular recognition modules.¹⁰³ Among the most promising conductive nanomaterials with which porphyrins interact are graphene-based ones, such as graphene itself, its oxides (GO, rGO), and graphene quantum dots (GQDs). The interaction between the extended π electron network of porphyrins and the planar surface of graphene or its derivatives is the basis for a series of synergistic effects of interest for optoelectronic, photovoltaic, and sensing applications.¹⁰⁴

Non-covalent supramolecular interactions can guide the orderly self-assembling process of porphyrins on the surface of carbon nanomaterials,

preserving the electronic integrity of both the porphyrin system and the conductive substrate.

Graphene quantum dots, in particular, represent an interesting platform for supramolecular interaction with porphyrins, thanks to their high surface area, controllable nanometric dimensions, and the presence of surface functional groups (carboxyl, hydroxyl, epoxy) that facilitate both physical interaction and chemical functionalization. Supramolecular coupling between porphyrins and GQDs has shown significant improvements in photoinduced charge separation, luminescence, and energy transfer efficiency, making such hybrids promising for use in photovoltaic devices, bioimaging, and photo redox catalysis.^{104,105}

In addition to non-covalent interactions, porphyrins can be covalently bound to graphene-based nanomaterials or carbon nanotubes through various chemical strategies.

Covalent functionalization allows for more stable hybrids, although it can locally alter the electronic conjugation of the components. Among the most commonly used methods for establishing covalent bonds between porphyrins and carbonaceous materials are: i) Esterification, which exploits the carboxyl groups present on the oxidized surface of nanotubes or graphene and the alcohol groups of porphyrins; ii) Suzuki coupling, based on the use of boronic or halogenated units present on porphyrins and on the modified surfaces of nanomaterials; iii) Huisgen 1,3-dipolar cyclization (azide-alkyne reaction), a bio-orthogonal strategy that allows “click” conjugation between porphyrins functionalized with alkynes and

azide surfaces; iv) Diazonio reactions, in which diazonio salts derived from porphyrins are reduced in situ on the surface of graphene or nanotubes, generating direct covalent bonds with the carbon lattice.^{106–108}

However, one of the main obstacles in the realization of these advanced hybrid systems is the difficulty of obtaining nanocrystals with a high degree of structural and compositional order while maintaining the monodispersity of the porphyrin units. The challenge is to balance the stability of covalent functionalization with the need to preserve the intrinsic photophysical properties of porphyrins and the conductivity of the host nanomaterials.

Electrostatic interactions between porphyrins and graphene quantum dots (GQDs)

In addition to π -stacking interactions and covalent functionalization, electrostatic interactions play also a fundamental role in the self-assembly and stabilization of porphyrin-GQD hybrids, especially in aqueous environments or under physiological conditions. These interactions arise from the presence of opposite charges or contrasting surface potentials between the two molecular components and can be exploited to obtain stable and functional complexes without the need for irreversible chemical modifications.^{104,109}

When porphyrins are mixed with oppositely charged GQDs, a strong Coulombic attraction occurs, which can drive the spontaneous formation of stable supramolecular complexes. These complexes rapidly self-

assemble in solution, often with favorable orientation to maximize further π -interactions, generating ordered multicomponent hybrids.

Factors influencing electrostatic interaction

Electrostatic interactions between graphene quantum dots and porphyrins are strongly affected by several environmental and molecular parameters. First, the pH of the solution plays a crucial role, as it governs the protonation or deprotonation state of the functional groups present on the surface of GQDs as well as on the porphyrin molecules, thereby modulating their net charge. The ionic strength of the medium is another key factor: increasing salt concentration leads to electrostatic screening effects, which weaken long-range Coulombic attractions and can destabilize the supramolecular assembly. In addition, the charge distribution on both interacting partners, including the density and spatial arrangement of ionic groups, influences not only the strength of the interaction but also the geometry and organization of the resulting complexes. Finally, the presence of competing ions or other charged molecules in solution may interfere with binding by occupying available interaction sites, further affecting the formation and stability of the electrostatically driven assemblies.

A notable example of the interaction between porphyrins and quantum dots (QDs), with advanced applications in molecular electronics, was reported by Duan et al. In this work, the authors designed and synthesized a hybrid nanomaterial structured according to a donor-acceptor (D-A) architecture, obtained by covalently modifying graphene

quantum dots (GQDs) with a metallated porphyrin, specifically ZnTPP (zinc tetraphenylporphyrin). The synthesis of the material, called ZnTPP-g-GQDs, was carried out by a 1,3-dipolar cycloaddition reaction, using ZnTPP-CHO, GQDs, and N-methylglycine.¹¹⁰

To ensure homogeneous dispersion of the nanomaterial within electronic devices, ZnTPP-g-GQDs was subsequently mixed with polyvinylpyrrolidone (PVP) in a proper ratio, thus obtaining a composite material (ZnTPP-g-GQDs:PVP) suitable for spin-coating deposition on ITO glass conductive substrates. The final architecture of the device was completed with the deposition of aluminum top electrodes, creating a memristor with an Al/ZnTPP-g-GQDs:PVP/ITO structure.¹¹⁰

Applications of Supramolecular Systems

Supramolecular materials, constructed through non-covalent interactions, offer numerous advantages over traditional covalent approaches. The weak and reversible interactions underlying supramolecular chemistry allow the spontaneous formation of complex structures through self-assembly, often under environmental conditions and using simple, rapid, and environmentally friendly synthetic methods, avoiding laborious synthesis and purification processes. Furthermore, these materials show remarkable structural adaptability in response to external stimuli, such as pH, light, temperature, or magnetic fields, reorganizing their architectures towards more energetically favorable states. This plasticity makes them ideal for the design of sensitive and functional systems, with applications

in areas such as sensors¹¹¹, optoelectronic devices (solar cells, LEDs)¹¹², and biomedical technologies such as bioimaging.¹¹²⁻¹¹⁵

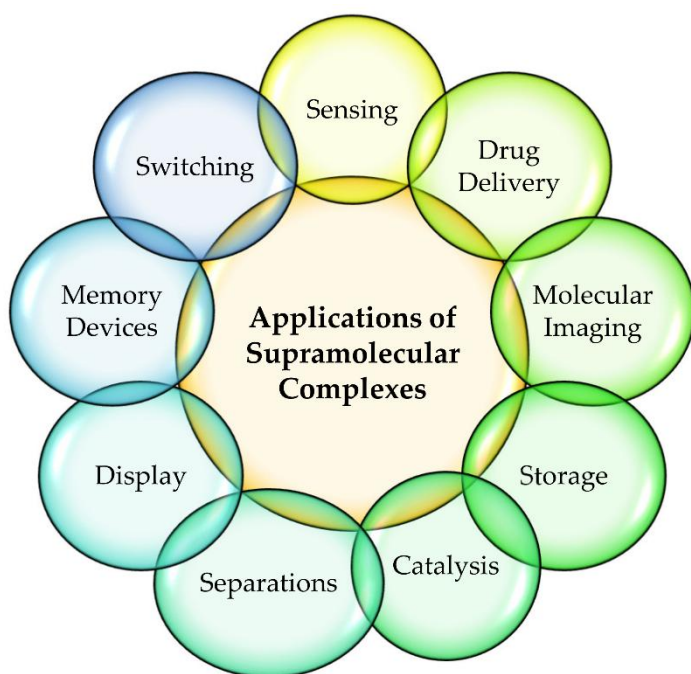


Figure 13. Diagram of the main applications of supramolecular systems¹¹⁶

In the field of molecular imaging, Smith et al have combined the potential of mechanically interconnected rotaxane complexes, in which a linear guest is trapped by a macrocycle, with highly stable and selective fluorophores. In particular, his group has encapsulated squaraine dyes, known for their optical properties in the red and near-infrared, within tetra-lactam macrocycles, forming highly stable squaraine rotaxanes.¹¹⁷ These systems, further functionalized with receptors such as zinc(II)-dicolylamine (ZnDPA), show specificity for biological targets and are currently in the preclinical phase, with over a dozen probes already available for studies in animal models.¹¹⁸

Among the most studied host-guest systems in supramolecular chemistry are cucurbiturils (CBs), which represent a class of barrel-shaped macrocycles derived from the condensation of formaldehyde and glycoluril. Over the past two decades, various CB homologues have been synthesized and characterized, exhibiting excellent inclusion properties and stability in aqueous environments. One of these in particular has found applications in areas such as drug delivery, catalysis, and materials chemistry.¹¹⁹ A recent study exploited this CB to construct a hybrid supramolecular hydrogel (HSH), combining catechol-functionalized chitosan polysaccharide (CAT-CS) and superparamagnetic $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles. In this system, the catechol side chains interact with CB, while the carbonyl groups of the cucurbituril bind to the magnetic nanoparticles via electrostatic interactions, generating a stable three-dimensional network. The resulting hydrogel exhibits alternating magnetic field-induced heating, representing a promising platform for stimulus-responsive chemotherapy, with experimental data obtained both in vitro and in vivo.^{119,120}

Another frontier of application is represented by supramolecular cross-linked polymers, which combine the mechanical strength of conventional polymers with the reversibility and dynamics of non-covalent interactions, such as hydrogen bonds, metal-ligand coordination, electrostatic interactions, and guest-guest interactions. Two main approaches have been developed for their synthesis: the use of traditional polymer scaffolds or supramolecular polymer blocks.¹²¹⁻¹²³

Supramolecular cross-linking induces a significant change in the structural and functional properties of materials, promoting the formation of organogels and hydrogels with self-healing capabilities, sensitivity to external stimuli, encapsulation capabilities, and controlled drug release. These materials are particularly promising for advanced applications in tissue engineering and regenerative medicine.¹²⁴

A prime example of the use of supramolecular chemistry in sensor technology is the pioneering work of A.P. de Silva, who, between the 1980s and 1990s, contributed to the development of optical detection based on host-host systems. His group combined crown ethers and photoinduced electron transfer (PET)-sensitive fluorophores, developing fluorescent probes capable of detecting metal ions such as sodium, potassium, and calcium. In the presence of sodium, for example, interaction with the corona ether inhibited the PET mechanism, allowing fluorescence to be triggered and the analyte to be quantified. These systems are now used in real-time monitoring of ionic concentrations in blood, both in humans and animals.^{125,126}

As described in the previous sections, supramolecular chemistry can be effectively exploited in a variety of sensing applications, owing to the high selectivity arising from non-covalent molecular recognition processes. Within this broad field, the development of supramolecular sensors for metal cations, including heavy metals, has attracted significant interest and constitutes the central objective of the present doctoral thesis.

4. Supramolecular systems for metal ions sensing

Supramolecular sensors for metal ions rely on macrocycles or coordinating ligands that selectively bind metal cations, enabling sensitive signal transduction through coordination-driven recognition processes.

This receptor is often integrated (immobilized or supported) on or in a low-dimensional material (quantum dots, photoluminescent nanotubes, graphene) that acts as a transducer: the change in optical or electrical properties (e.g. fluorescence or current) following the interaction is used as an analytical signal. A key advantage is that the high surface area/volume ratio of the nanostructured material allows for a high density of active receptors and high sensitivity. The constant challenge is to maintain the stability of the receptor-transducer bond without compromising the electronic/optical properties of the support material.¹²⁷

Among the various analytes detectable through supramolecular recognition, heavy metal ions represent one of the most critical targets due to their high toxicity, persistence, and widespread environmental presence.

Heavy metal contamination is a serious concern due to their toxic properties and lethal effects. Heavy metals contaminate water, air and soil and are a major contributor to modern environmental pollution. These elements enter the environment from natural or anthropogenic sources. Major anthropogenic sources include industrial effluents, construction

and demolition waste, agricultural chemicals (pesticides, fertilizers), mining, pharmaceutical and cosmetic industries.^{128,129} Natural sources include soil erosion, vulcanic activity, forest fires, rock and mineral alteration, biological processes and the decomposition of organic matter. From these sources, heavy metal particles are released into the air, water and soil. Heavy metals are not biodegradable and accumulate in the environment and in the tissues of flora and fauna. The accumulation of heavy metals in plants alters the normal process of chlorophyll synthesis.¹³⁰ Chronic or acute exposure to high doses of heavy metals causes mortality in flora and fauna.¹³¹ Heavy metals alter aquatic ecosystems and terrestrial plants. For example, heavy metal contaminants that accumulate in the gills of fish cause asphyxia, blood poisoning and, ultimately, death.¹³²

It is estimated that over 13% of the world's arable land and nearly 40% of surface water sources (lakes and rivers) are contaminated.¹³³

Exposure to heavy metals also poses a serious risk to human health. These toxic elements can enter the body through ingestion (contaminated water and food), inhalation (dust and suspended particles) or skin contact. Once absorbed, they tend to accumulate in various organs such as the liver, kidneys, lungs, brain and bones, where they can interfere with vital biochemical processes.

Several heavy metals are known for their specific and severe toxicological effects on human health. Lead (Pb^{2+}) is particularly harmful to the central nervous system, where it interferes with neuronal development and

function, causing cognitive and behavioral deficits, especially in children; in addition, chronic exposure is associated with kidney damage and cardiovascular disorders. Mercury (Hg^{2+}) is a highly neurotoxic element capable of inducing irreversible damage to the brain and nervous system, as well as impairing renal function. Cadmium (Cd^{2+}) tends to accumulate in the kidneys, leading to nephrotoxicity, bone demineralization and osteoporosis, and dysfunctions of the reproductive system. Arsenic, present mainly in its trivalent (As^{3+}) and pentavalent (As^{5+}) forms, is a well-established carcinogen and has been linked to cancers of the skin, lungs, and bladder, in addition to cardiovascular diseases and metabolic disorders such as diabetes. Finally, hexavalent chromium (Cr^{6+}) is particularly hazardous when inhaled, as it can cause severe damage to the respiratory system and exhibits strong mutagenic and carcinogenic properties.

Chronic exposure, even at low concentrations, can lead to subclinical or cumulative effects, making early diagnosis difficult. The most vulnerable population groups are children, pregnant women, the elderly and people with pre-existing medical conditions.

Furthermore, through bioaccumulation and biomagnification in the food chain, heavy metals can also reach humans through the consumption of contaminated agricultural or animal products, further aggravating the risks to public health. For this reason, it is necessary to develop specific sensors having a detection limit (LoD) in the nanomolar range, good selectivity, stability and a fast response time.^{134,135}

Studies reported in the literature show that some sensors based on supramolecular systems achieve sub-micromolar LoDs with good selectivity, but often in model solutions rather than complex matrices. Adaptation to real samples (natural, biological waters) is one of the challenges that still needs to be addressed.

Conventional methods for detecting heavy metal ions mainly include atomic absorption spectroscopy (AAS)¹³⁶, inductively coupled plasma atomic emission spectroscopy (ICP-AES)¹³⁷, and inductively coupled plasma mass spectrometry (ICP-MS).¹³⁸ These techniques offer very low detection limits and allow the simultaneous analysis of multiple ions. However, they have some disadvantages: they require highly specialized personnel, complex sample pretreatment procedures, and the use of bulky and expensive equipment, which is not suitable for in situ measurements. For this reason, portability, low cost, and high integration are now key directions for the development of new solutions in heavy metal ion detection.

Optical sensors are based on variations in the optical signal generated by the interaction between the recognition element and the target molecule, from which information on the concentration of the analyte can be obtained. The main optical techniques used include fluorescence spectroscopy, colorimetric spectroscopy, Raman spectroscopy, chemiluminescence, infrared spectroscopy, and refractive index-based spectroscopy. In recent years, optical detection has favored the

development of portable sensors for heavy metal ions, thanks to their low cost, rapid response, and ease of use.¹³⁹

Electrochemical sensors, on the other hand, are based on converting information about the concentration of a target molecule into an electrical signal. During the detection process, the solution to be analyzed acts as the active part of an electrochemical cell, and changes in the electrical parameters of the cell provide information about the concentration of the analyte.^{140,141}

With the evolution of photolithography and 3D printing technologies, microelectrodes such as screen-printed electrodes and fork electrodes have been developed for use in electrochemical detection. These can be integrated with analytical circuits and communication modules, enabling the creation of compact, highly integrated, and miniaturized devices.

In the context of chemical detection, selectivity is the ability of a sensor or analytical system to distinguish and respond preferentially to a specific analyte (e.g. a metal ion) even in the presence of other potentially interfering species, often present in much higher concentrations. A selective sensor is able to recognize the target analyte on the basis of specific chemical or physical interactions, minimizing unwanted signals from similar compounds or complex matrices¹⁴²

Once selectivity is ensured, the main parameters for evaluating a sensor's performance become the limit of detection (LoD) and sensitivity. A device is defined as highly sensitive when it is capable of detecting extremely low

concentrations of analyte, when it has a very low LoD. However, LoD and sensitivity are often confused, even though they are distinct concepts. The LoD indicates the minimum detectable concentration above which of the system's background noise, while sensitivity describes how effectively the sensor can discriminate between two similar concentrations of analyte.¹⁴³

Similarly, there is often ambiguity between the use of the theoretically calculated LoD and that obtained experimentally. In general, for a sensor with a linear response, the relationship between signal and concentration can be expressed by the equation:

$$S = mC + i$$

where S is the output signal (absorbance, Raman intensity, electric current), C is the concentration of the analyte, m represents the sensitivity, and i is the intercept, associated with noise or background error.

According to IUPAC, the detection limit can be defined by the equation:

$$\text{LoD} = 3.3SD/m$$

It should be noted that the LoD value obtained in this way correctly reflects the theoretical detection limit only if the slope m of the curve is well defined and sufficiently high compared to the noise. In practice, however, the value reported as LoD often does not correspond to that obtained experimentally, or the actual LoD is significantly higher.¹⁴⁴

Another crucial aspect for evaluating a sensor is responsiveness, understood as the time required to record the signal variation and its

return to the initial state. Ideally, an effective sensor should respond very quickly (<1 s) to the presence of the analyte. Systems based on non-covalent recognition tend to provide rapid responses and favour fast signal recovery, which are fundamental qualities for continuous monitoring applications. However, response and recovery times depend on the affinity between the receptor and the analyte: greater affinity leads to faster detection but can slow down the return of the signal to its initial state. For this reason, when developing reusable sensors, it is necessary to find a balance between detection speed and regeneration capacity.

5. Objective of the doctoral research thesis

Supramolecular systems represent an extremely promising class of systems in the field of functional materials chemistry, thanks to their ability to exhibit unique optical and photophysical properties resulting from non-covalent interactions and their molecular architecture. In light of these considerations, the main objective of this doctoral thesis is to study and characterize complex supramolecular systems obtained through the non-covalent self-assembly of porphyrins with other functional species, in order to develop new sensory platforms for the detection of metal cations, in particular heavy metals, in aqueous solutions and on electrochemical supports.

The research activity focused on the analysis of different classes of supramolecular systems:

1. Electrostatic interaction between cationic Graphene Quantum Dots (GQDs) and anionic meso-tetrakis(4-sulfonatophenyl)porphyrin (TPPS₄⁻) porphyrin.

This study required the development and optimization of synthetic protocols to obtain GQDs with controlled optical properties, in particular with opportune fluorescence characteristics. The GQDs were extensively characterized using various techniques: Dynamic Light Scattering (DLS) for size distribution, zeta potential for colloidal stability, X-ray Photoelectron Spectroscopy (XPS) for surface composition and oxidation state analysis, and Raman spectroscopy for the identification of carbon

structures and defects. The interaction with TPPS_4^{4-} was studied as a function of pH to evaluate its spectroscopic behavior and potential application in optical and electrochemical sensing of heavy metals. The GQDs@TPPS_4^{4-} system was analyzed using UV-Vis spectroscopy, stationary and time-resolved fluorescence, and fluorescence anisotropy. This system was investigated as an optical sensor exploiting the double fluorescence emission of the two chromophores and as an electrochemical sensor using techniques such as cyclic and linear voltammetry, as well as electrochemical impedance spectroscopy.

2. Non-covalent interaction between the disubstituted cationic porphyrin t-H₂Pagg and anionic carboxymethylcellulose (CMC).

This system allowed us to explore the relationship between the metal coordinated at the center of the porphyrin and the type of aggregate formed. Depending on the charge and coordination geometry of the metal, different modes of aggregation (dimers, oligomers or extended structures) mediated by π -interactions and electrostatic interactions with the polymer were observed. This approach highlighted the potential of the $\text{t-H}_2\text{Pagg@CMC}$ system as a spectroscopic probe sensitive to the nature of the central metal, making it suitable for preliminary and rapid screening for the presence of heavy metals in aqueous solution. The spectroscopic investigation was conducted using UV-Vis, fluorescence and circular dichroism (CD).

3. Investigation of the J-type aggregation of octabromo meso-tetrakis(4-sulphonatophenyl)porphyrin ($\text{Br}_8\text{TPPS}_4^{4-}$)

A significant part of the work involved analyzing the aggregation behavior of porphyrin containing eight bromine atoms in the β position under acidic conditions. The presence of bromine induces a structural distortion of the porphyrin plane, altering the electronic properties and favoring the formation of J-type aggregates, distinguished by a head to tail arrangement of the molecules and a marked bathochromic shift in the absorption bands.

The kinetic constants of the aggregation process were determined and compared with those of TPPS_4^{4-} , highlighting how the distortion introduced by bromine profoundly modifies the dynamics of the process. This distortion also makes porphyrins more reactive towards metalation with the Zn^{2+} ion, making it possible to use it as a valid candidate sensor for zinc in aqueous solutions.

4. Synthesis and characterization of new meso-substituted porphyrins with terminal triphenylphosphonium groups:

In this part of the work, cationic porphyrins (tetra- and octa-cationic) with alkyl chains of different lengths (C_3 , C_5 and C_{10}) terminated with triphenylphosphonium groups in the meso position were designed, synthesized and characterized. These molecules show a marked tendency to form electrostatic interactions with anionic nanostructures such as GQDs and have been proposed as sensory platforms for the optical and

electrochemical detection of metal cations. The synthesized porphyrins were characterized by ^1H NMR spectroscopy. Due to time restrictions during my research period in Portugal, the complete characterization through and ^{13}C NMR and mass spectrometry will be completed in the near future.

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Chapter 1

Electrostatic interaction between anionic meso-tetrakis(4-sulphonatophenyl) porphyrin and cationic Graphene Quantum Dots.¹

1.1. Synthesis and characterization of Graphene Quantum Dots (GQDs)

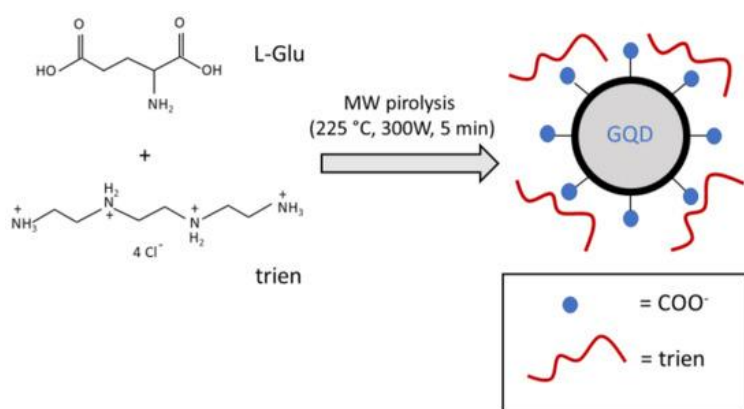
The interaction between positively charged GQDs and tetrakis(4-sulphonatophenyl)porphyrin (TPPS₄⁴⁺), a negatively charged water-soluble molecule has been studied.

This porphyrin, present in tetra-anionic form at neutral pH, has already demonstrated its ability to self-assemble with numerous complex systems, including polymers², proteins³ and nanomaterials such as carbon nanotubes⁴ and carbon quantum dots⁴.

GQDs were prepared following procedures reported in the literature and modifications thereof.⁵ In general, alternative routes based on the pyrolysis of an organic precursor or microwave (MW) irradiation of solid samples of precursors or their aqueous solutions were followed. Among the various syntheses studied, one proved to be better due to the colloidal stability over time of the products. This synthesis uses microwave-assisted pyrolysis (225°C, 300 W, 5 min) of L-glutamic acid as an organic precursor and triethylenetetramine tetrahydrochloride (trien) as a capping agent in equimolar quantities (Scheme 1). The dark brown powder was dissolved

in deionized water to obtain a brown solution, which was first purified by filtration and subsequently by dialysis.

The nanoparticles produced have a positively charged surface, attributable to the presence of residual ionizable cationic amino groups and traces of trien used as a capping reagent. As a result, they are able to interact quite efficiently with TPPS_4^{4-} , which has a negative charge.



Scheme 1. Schematic diagram of the MW-assisted synthesis of GQDs used in this study.

The synthesized GQDs were analyzed using various spectroscopic techniques, including UV/Vis absorption, fluorescence emission, Dynamic Light Scattering (DLS), X-rays Photoelectron Spectroscopy (XPS), RAMAN spectroscopy and FT-IR spectroscopy.

The UV/Vis absorption spectra of an aqueous dispersion of GQDs show a typical unstructured profile, characterized by a shoulder at approximately 330 nm (Figure 1.1, top). Fluorescence analyses, performed by varying the excitation wavelength, show that, when irradiated up to 310 nm, a single

emission band centered around 410 nm is observed, whose intensity increases with increasing excitation wavelength. Above 330 nm, the band shifts from 410 nm to approximately 430 nm, accompanied by a decrease in fluorescence intensity; pushing the excitation further towards the red, a second band appears around 470 nm. The intensity normalized emission spectra confirms this behavior (Figure 1.1, bottom)

The dependence of fluorescence on the excitation wavelength can be attributed to the presence of particles of different sizes in solution.^{6,7}

DLS measurements (Figure 1.2) show a heterogeneous size distribution, including very small particles (0.8 ± 0.2 nm), intermediate-sized particles (5.8 ± 0.9 nm) and significantly larger particles (260 ± 40 nm). The latter are likely the result of the aggregation of intermediate-sized particles.

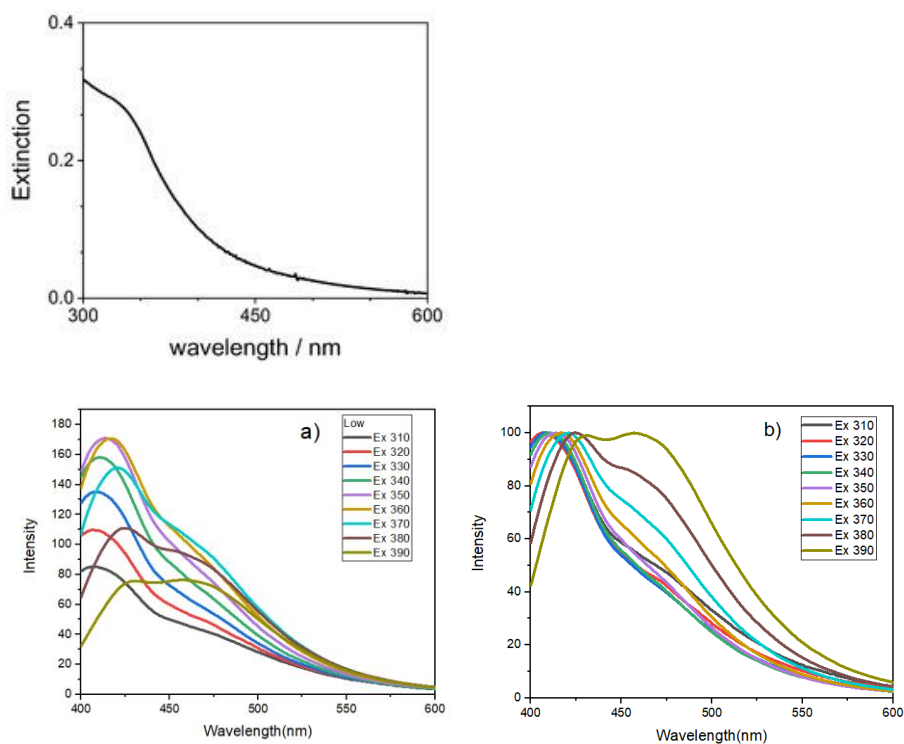


Figure 1.1 UV/Vis absorbance spectrum (top) and fluorescence spectra (a) as a function of excitation wavelength (excitation wavelength from 310 to 390 nm) and corresponding normalized spectra (b) for aqueous solutions of GQDs (0.14 mg/mL)

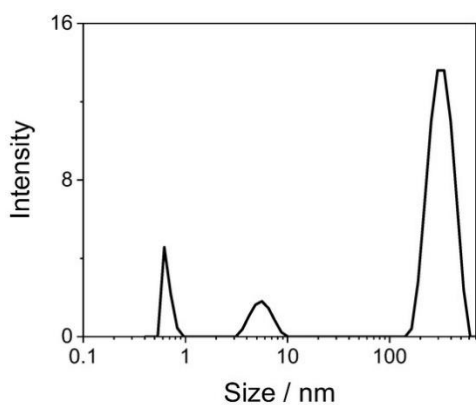
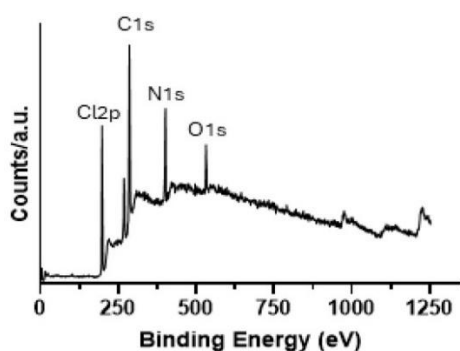


Figure 1.2. Distribution of particle sizes measured an aqueous solution of GQD (0.14 mg/mL) using the DLS technique.

The presence of trien as a capping agent is confirmed by the positive zeta potential (+24 mV) detected in samples at pH 7. Generally, GQDs synthesized from pure L-glutamic acid or other similar organic sources have a negative charge, attributable to residual ionized carboxyl groups on the surface of the nanoparticles. Under neutral pH conditions, the trien polyamine is partially protonated, allowing electrostatic interaction with the carboxylate groups (COO^-), thus contributing to the net positive charge of the GQDs.^{6,8}

Aqueous solutions containing GQDs show good stability in terms of both size and emissive properties, maintaining these characteristics for at least three months.

Additional characterization was performed using XPS (Figure 1.3) The analysis as reported reveals the predominant presence of the elements C (63.24%), N (12.19%), Cl (11.84%), and O (12.74%) in the samples.



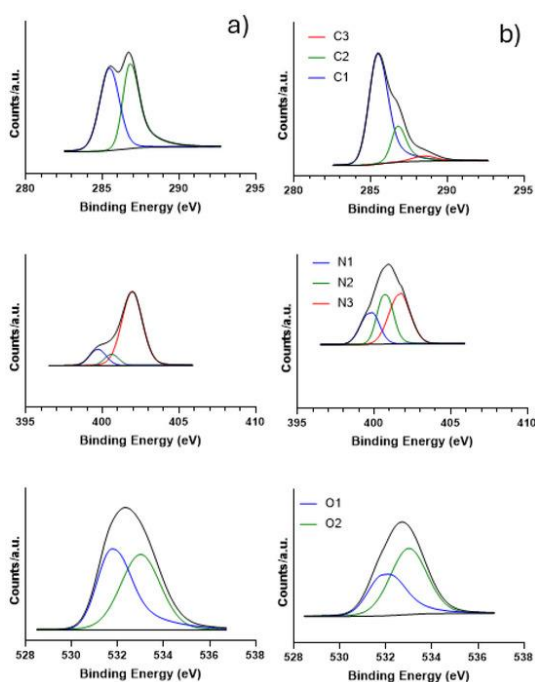


Figure 1.3. XPS spectrum for a blank mixture containing the precursors before the pyrolysis procedure(top) and XPS spectra (bottom) for the C1s, N1s, and O1s regions for (a) the sample before pyrolysis and (b) for GQDs

In the C1s core level, a decrease in the intensity of the C2 component is observed, from 48.64% in the untreated sample to 20.39% in the treated sample. In addition, a new component appears at 288.50 eV, attributed to the presence of conjugated N–C=N bonds, including species containing quaternary nitrogen. The most intense component is C1, whose percentage increases from 51.4% to 75.7% in the treated sample, suggesting an increase in the carbon phase with a highly defective sp^2 character.^{9,10}

As regards the N1s level, there is a reduction in the components with higher binding energy (BE) in favor of those with lower BE. In general, all components are shifted towards higher binding energies, indicating an

interaction between nitrogen species and the carbon lattice. The N1 and N2 components show an increase, while the N3 component is significantly reduced, from 78.4% to 43.0% compared to the reference sample, in accordance with decrease in chlorine content.

The Raman spectrum of GQDs in the solid state (Figure 1.4) shows two characteristic bands centered at 1567 cm^{-1} of G band and 1363 cm^{-1} of D band, consistent with what has been reported in the literature⁸. The G band is associated with the stretching vibrations of sp^2 C–C bonds within the plane of graphene nanostructures, while the D band indicates the presence of disorders in the sp^2 network, linked to the formation of C–C bonds with sp^3 hybridization.

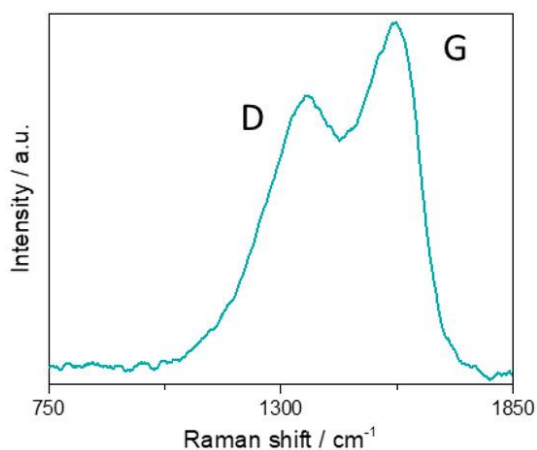


Figure 1.4. Raman spectrum of a solid state sample of GQDs

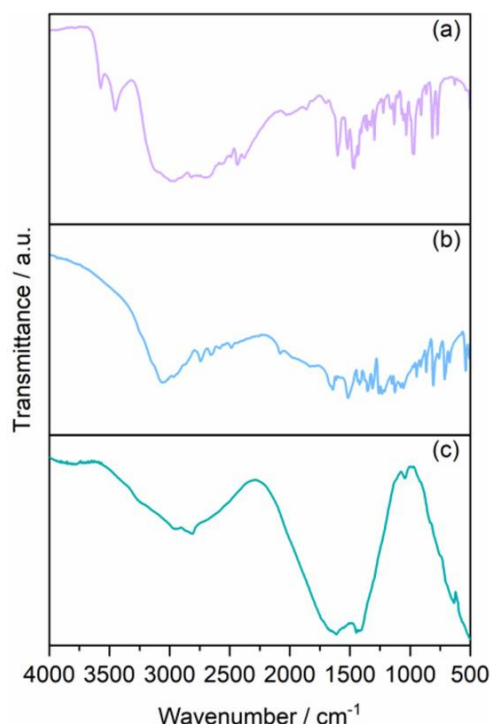


Figure 1.5. FT-IR spectra of (a) trien, (b) L-glutamic acid, and (c) GQDs as solids onto silicon windows.

FT-IR spectroscopy was also used to characterize the GQDs (Figure 1.5). The FT-IR spectra of the two solid precursors were compared with that of the solid sample obtained after the pyrolysis process. The signals were assigned based on a comparison with the data reported by Wu et al.⁸

The broad band around 3000 cm^{-1} is likely attributable to O–H, N–H, or C–H stretching vibrations of surface functional groups, whereas the band in the $1500\text{--}1600\text{ cm}^{-1}$ region can be assigned to the C=C stretching modes of the graphitic framework. The signal at 1456 cm^{-1} , also clearly visible in the trien spectra, can be associated with the bending vibration of the methylene groups. A broad band between 2600 and 2870 cm^{-1} is probably

attributable to the O–H stretching of the residual carboxyl groups present in GQDs, while the extended signal between 1560 and 1730 cm^{-1} is due to the overlap of the stretching modes of the C=O groups and amide functions. Finally, the broad band observed in the 4000–3600 cm^{-1} region and the peak at 3270 cm^{-1} are attributed to the N–H stretching of the amide and amine functions, respectively.

1.2. Supramolecular interactions with porphyrin

Given their particular colloidal stability and fluorescence emission characteristics, supramolecular interactions with cationic and anionic porphyrins were studied. The presence of the trien reagent as a capping system for GQDs not only contributes to their stabilization but also introduces positive charges due to the presence of residual amino groups that are already protonatable at neutral pH.

The various systems were investigated by means of UV/Vis spectrophotometric and fluorescence titrations. In addition, Zeta potential analysis, fluorescence lifetime, and time-resolved anisotropy were used during the titrations.

1.2.1. Interactions with meso-tetrakis(4-N-methylpyridyl)porphyrin (TMPyP⁴⁺) cationic porphyrin

- pH 7

As regards the study with the cationic porphyrin (TMPyP⁴⁺), various aliquots of a titrant solution containing GQDs and TMPyP⁴⁺ (3 μ M) at pH = 7 were added to a 3 μ M solution of TMPyP⁴⁺ in 1 mM phosphate buffer (PBS) at neutral pH. In this way, the porphyrin concentration was kept constant throughout the titration. UV/Vis and fluorescence spectra were recorded after each addition.

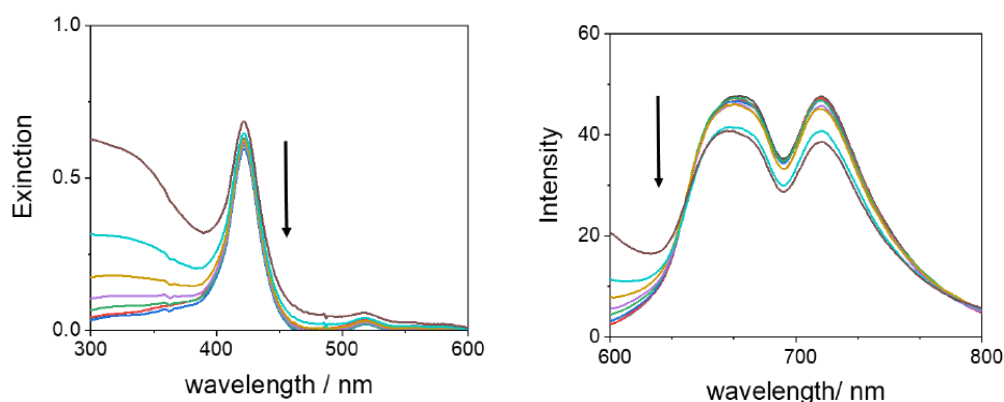


Figure 1.6. UV/Vis and fluorescence emission spectral changes during the titration of TMPyP⁴⁺ with GQDs at neutral pH (the arrow marks the increasing GQDs concentration). [TMPyP⁴⁺] = 3 μ M; [GQDs] = 0, 0.002, 0.01, 0.034, 0.056, 0.1, 0.18, 0.40 mg/mL; phosphate buffer 1 mM, pH = 7; T = 298 K; cell path length 1 cm;

From the spectrophotometric titrations performed, it can be seen that the Soret band (band B) originating from $\pi \rightarrow \pi^*$ transitions attributable to the second excited state $S_0 \rightarrow S_2$ of porphyrin TMPyP⁴⁺ in UV/Vis remains constant at 420 nm throughout the titration (Figure 1.6, left).

This can also be observed in the lower energy region relative to the Q bands given by $S_0 \rightarrow S_1$ transitions. Even the fluorescence spectra characterized by the two bands at 670 nm and 716 nm, characteristic of the tetracationic species, do not show significant variations after each addition of GQDs (Figure 1.6, right)

- pH 3

UV/Vis spectrophotometric and fluorescence titrations were also performed at pH = 3. In this case, different volumes of titrant were added to a 3 μ M TMPyP⁴⁺ solution in HCl at pH 3. In both samples, an acidic environment was obtained because the presence of quantum dots from L-glutamic acid generated a solution with a pH of about 3. The UV/Vis and fluorescence spectra did not show significant variations suggesting a weak or almost absent interaction between the two species (Figure 1.7)

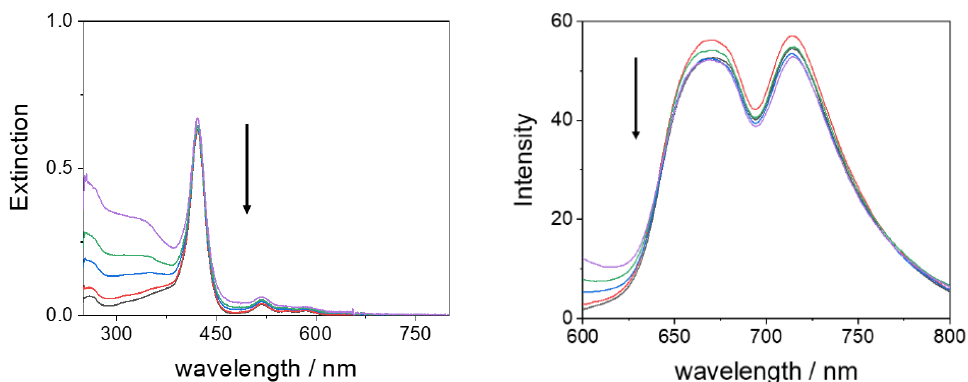


Figure 1.7. UV/Vis and fluorescence emission spectral changes during the titration of TMPyP⁴⁺ with GQDs at pH = 3 (the arrow marks the increasing GQDs concentration). The emission spectra are not corrected for the extinction of the samples. Experimental conditions: [TMPyP⁴⁺] = 3 μ M; [GQDs] = 0, 0.01, 0.056, 0.1, 0.18 mg/mL; [HCl] = 10⁻³ M; T = 298 K; cell path length 1 cm.

The scarce or total absence of interaction between GQDs and TMPyP⁴⁺ is attributed to the specific structure of the cationic species since, under neutral or moderately acidic conditions, the latter does not undergo further protonation phenomena and does not show a tendency to aggregate. The presence of four peripheral charges on the substituent groups does not allow for the formation of electrostatic interactions with GQDs, which are themselves positively charged by amino groups present on the surface due to the presence of trien.

1.2.2. Interaction with anionic meso-tetrakis(4-sulphonatophenyl)porphyrin (TPPS₄⁴⁻)

With regard to preliminary studies on the interaction between GQDs and anionic porphyrin, preliminary studies have shown that significant spectroscopic variations are observed. For this reason, these titrations were conducted in greater detail, through analysis of fluorescence emission lifetimes and time-resolved anisotropy. As already described in the introduction, TPPS₄⁴⁻ exhibits pH dependent behavior, forming J-aggregates under acidic conditions. Therefore, to study the interaction with GQDs, spectroscopic titrations were performed at pH = 7 (where porphyrin is mainly tetra-anionic) and at pH = 3 (favoring the formation of J-aggregates).¹¹ Further investigations were also conducted using inverse spectrophotometric titrations.

- pH 7

The UV/Vis spectra (Figure 1.8) show that there is a clear variation for each addition of GQDs. In particular, a progressive increase can be observed in the UV region around 330 nm, attributable to the increase in the concentration of graphene quantum dots. At the same time, the B-band of the porphyrin undergoes marked hypochromicity (~70%) and a clear bathochromic shift, moving from 413 to 424 nm ($\Delta\lambda = +11$ nm).

An isosbestic point at 420 nm is clearly visible, which remains stable up to a GQDs concentration of 0.04 mg/mL. Above this threshold, the isosbestic point is no longer observable, likely due to the distortion of the spectra caused by the increase in scattered light, combined with the increasing contribution of absorption by the GQDs.

The absorbance variations at 413 nm, recorded during the titration of TPPS₄⁴⁻ with GQDs, shows a breakpoint around 0.06 mg/mL of GQDs. The Q bands also show a slight bathochromic shift, although the spectral variation is less significant and the charge number remains four, indicating that the porphyrin does not undergo protonation of the nitrogen atoms of the macrocycle. However, the presence of a significant contribution from light scattering makes it difficult to accurately quantify the intensity variations, which are relatively modest¹²

The observed red shift of the B band is consistent with a J-type excitonic coupling between chromophores. This result differs from that reported for a similar system, where a hypsochromic shift was observed, probably due

to the formation of H type dimers. Furthermore, the amount of hyperchromicity in the system studied here is more than double that of carbon nanodots derived from ethylenediamine in the organic precursor.

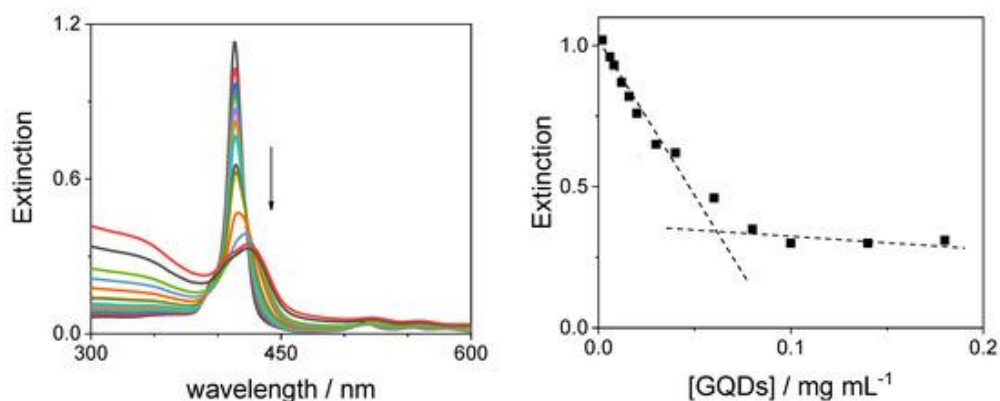


Figure 1.8. UV/Vis spectra (left panel) and absorbance at 413 nm (right panel) during titration of TPPS₄⁴⁻ with GQDs at neutral pH. Experimental conditions: [TPPS₄⁴⁻] = 3 μ M; [GQDs] = 0–0.18 mg/mL; 1 mM phosphate buffer, pH = 7; T = 298 K; cell path length 1 cm.

As can be seen with the addition of increasing amounts of GQDs, the fluorescence intensity relative to the two characteristic bands of porphyrin in the form of a free base at 645 and 707 nm decreases substantially until it is completely quenched (Figure 1.9). A breakpoint is clearly visible after the addition of 0.1 mg/mL of GQDs. The Stern-Volmer graph (Figure 1.10) of the ratio I_0/I , where I_0 and I represent the fluorescence intensities in the absence and in the presence of GQDs, shows an almost linear behavior for GQDs concentrations up to 0.06 mg/mL. From these data, it is possible to calculate a value for the Stern-Volmer constant $K_D = 13.7 \pm 0.2$ (mL/mg). Above this concentration, a large deviation can be easily observed, which could be attributed to porphyrin aggregation or static quenching. The

deviation from the linear trend with respect to the Stern-Volmer equation can be explained by a combination of static and dynamic quenching mechanism.

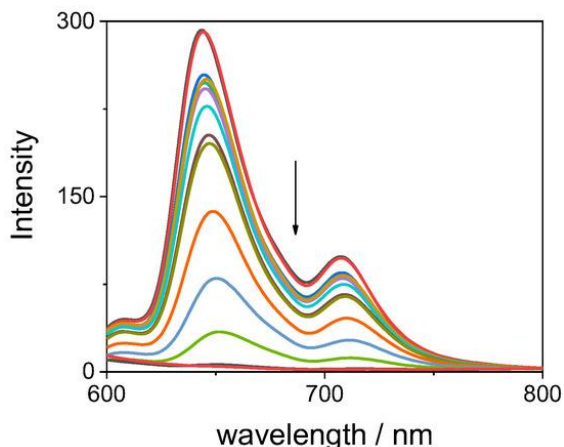


Figure 1.9. Spectral variations in fluorescence emission during titration of TPPS₄⁴⁻ with GQDs at neutral pH. Experimental conditions: [TPPS₄⁴⁻] = 3 μ M; [GQDs] = 0–0.18 mg/mL; 1 mM phosphate buffer, pH = 7; T = 298 K; cell path length 1 cm; excitation wavelength 420 nm (isosbestic point in UV/Vis titration).

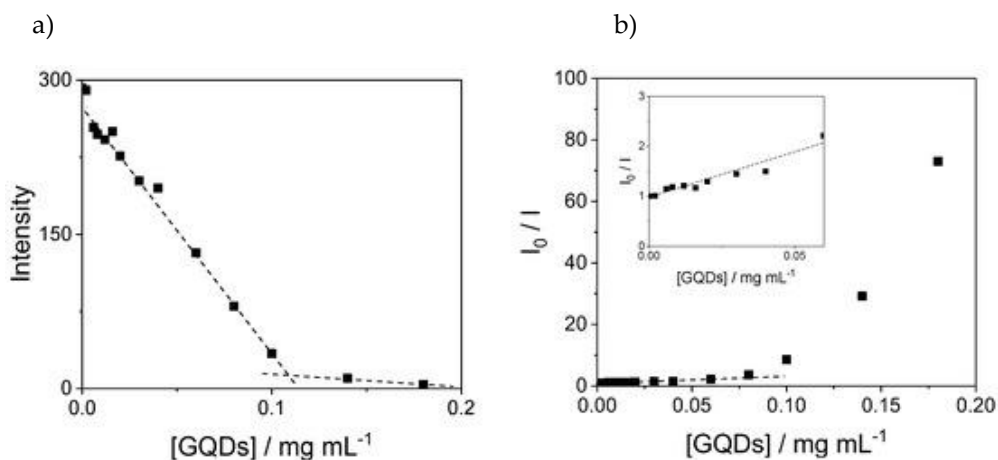


Figure 1.10 a) Variations in fluorescence emission intensity at 645 nm as a function of GQDs concentration at neutral pH and b) Stern-Volmer plot of I_0/I as a function of increasing GQDs concentration (the inset repots an expansion of the lower concentration range of added GQDs). Experimental conditions: [TPPS₄⁴⁻] = 3 μ M; [GQDs] = 0–0.18 mg/mL; 1 mM phosphate buffer, pH = 7; T = 298 K; cell path length 1 cm; excitation wavelength 420 nm (isosbestic point in UV/Vis titration).

In order to better characterize the nature of the species present in solution, an analysis was conducted in terms of time-resolved fluorescence and anisotropy (Table 1.1). The data related to these measurements were collected in the presence of volumes different from those reported for the previous titrations, as porphyrin was not present in the titrant. The decays of the fluorescence intensity signal were treated with a nonlinear best-fitting to a biexponential equation, yielding a major contributing lifetimes with $\tau_1 = 1.1 \pm 0.1$ ns ($A = 3\%$). A small rapid decaying contribution is also detectable in our solutions with a $\tau_2 = 10.0 \pm 0.1$ ns ($A = 97\%$), probably due to a small amount of porphyrin oligomers in solution. The relative amplitudes of the two contributions show a corresponding and parallel change: as the GQD concentration increases, the τ_1 values decrease slightly (from 97 to 86%), while τ_2 increases from 3 to 14%.¹³

The analysis of time-resolved fluorescence anisotropy allows us to obtain the value of the rotational correlation time τ_r , derived from Perrin's equation¹⁴, and, for porphyrin TPPS₄⁴⁻, the measured value ($\tau_r = 0.72$ ns) is in line with a monomeric species, free to rotate in solution. On the contrary, already from the first addition of GQDs, the τ_r values show a slight increase (~25%), suggesting that porphyrins interact with GQDs, even though they are essentially free to rotate in solution. This could imply that electrostatic interactions between the positively charged trien ammino groups on the surface of the GQDs keep the negatively charged TPPS₄⁴⁻ close to the graphene quantum dots, although without completely blocking them.

Table 1.1. Fluorescence lifetimes and rotational correlation time for the titration of TPPS₄⁴⁻ with GQDs at neutral pH. Experimental conditions: [TPPS₄⁴⁻] = 3 μ M at pH = 7; T = 298 K; cell path length 1 cm; λ_{ex} = 390 nm; λ_{em} = 644 nm.

[GQDs] mg/mL	Lifetime / ns		Anisotropy Decays / ns
0	$\tau_1 = 1.06$ (3%)	$\tau_2 = 10$ (97%)	$\tau_r = 0.72$
0.02	$\tau_1 = 1.30$ (7%)	$\tau_2 = 10.4$ (93%)	$\tau_r = 0.96$
0.08	$\tau_1 = 1.38$ (8%)	$\tau_2 = 9.99$ (92%)	$\tau_r = 1.00$
0.16	$\tau_1 = 1.83$ (14%)	$\tau_2 = 10.1$ (86%)	$\tau_r = 0.92$
0.78	$\tau_1 = 1.74$ (49%)	$\tau_2 = 7.67$ (51%)	$\tau_r = 0.93$

As regards the titration carried out on a sample of GQDs titrated with a TPPS₄⁴⁻ solution at neutral pH, the UV/Vis spectra show that as the concentration of TPPS₄⁴⁻ increases, a new band appears at 427 nm, together with a shoulder around 410 nm, which, however, becomes less evident as the additions proceed. The absorbance data measured at 427 nm shows a linear trend as the porphyrin concentration increases (Figure 1.11)

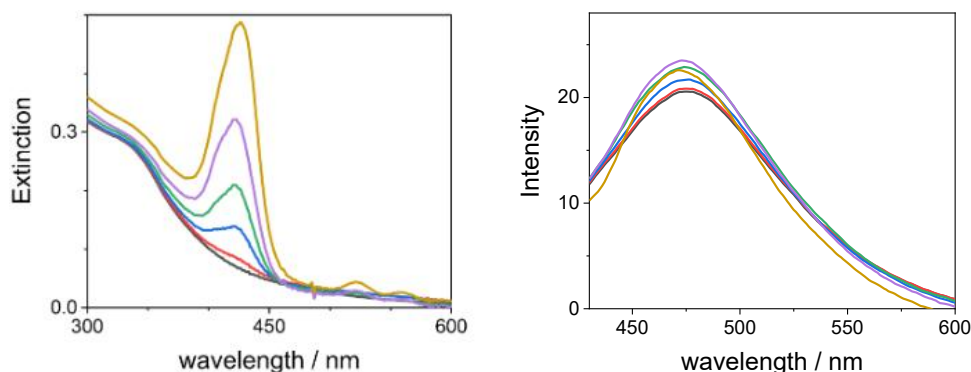


Figure 1.11. UV/Vis (left) and fluorescence emission (right) spectra during the titration of GQDs with TPPS₄⁴⁻ at neutral pH. Experimental conditions: [TPPS₄⁴⁻] = 0.07 μM, red line; 0.42 μM, blue line; 0.9 μM, green line; 1.8 μM, purple line; 3 μM, yellow line; [GQDs] = 0.14 mg/mL; phosphate buffer 1 mM, pH = 7; T = 298 K; cell path length 1 cm.

Table 1.2. Fluorescence lifetimes (τ_1 , τ_2 and τ_3) and relative percentage amplitudes for the titration of GQDs with TPPS₄⁴⁻ at neutral pH. Experimental conditions: [GQDs] = 0.14 mg/mL; phosphate buffer 10 mM, pH = 7; T = 298 K; cell path length 1 cm; λ_{exc} = 390 nm; λ_{em} = 474 nm.

[TPPS ₄] / μM	Lifetime / ns		
0	$\tau_1 = 15.4 \pm 0.1$ (14%)	$\tau_2 = 5.4 \pm 0.1$ (61%)	$\tau_3 = 1.8 \pm 0.1$ (25%)
0.07	$\tau_1 = 14.6 \pm 0.1$ (17%)	$\tau_2 = 5.0 \pm 0.1$ (64%)	$\tau_3 = 1.5 \pm 0.1$ (19%)
0.42	$\tau_1 = 14.9 \pm 0.1$ (16%)	$\tau_2 = 5.2 \pm 0.1$ (72%)	$\tau_3 = 1.6 \pm 0.1$ (22%)
0.90	$\tau_1 = 15.5 \pm 0.1$ (15%)	$\tau_2 = 5.3 \pm 0.1$ (61%)	$\tau_3 = 1.8 \pm 0.1$ (24%)
1.80	$\tau_1 = 16.3 \pm 0.1$ (14%)	$\tau_2 = 5.4 \pm 0.1$ (64%)	$\tau_3 = 1.7 \pm 0.1$ (22%)
3.00	$\tau_1 = 16.5 \pm 0.1$ (15%)	$\tau_2 = 5.6 \pm 0.1$ (63%)	$\tau_3 = 1.9 \pm 0.1$ (22%)

The fluorescence emission spectra were acquired by exciting the GQDs region at 330 nm, where the variation in absorbance is not significant following successive additions. The initial spectrum is characterized by an intense band at 404 nm, accompanied by a shoulder of moderate intensity at 470 nm. Following the addition of increasing amounts of TPPS₄⁴⁻, a

gradual hypochromic shift of the higher energy band to 394 nm is observed.

At the same time, the overall intensity of the emission decreases and the band at 470 nm becomes more evident. After correcting the emission spectra for the filter effect, the measured emission profiles are virtually unchanged by the increase in porphyrin concentration. Similar behavior is observed when the samples are excited at 330 nm. Time-resolved fluorescence measurements were performed on the samples under examination.

For the GQDs sample at the highest energy band, the analysis of the fluorescence intensity decay (Table 1.2) ($\lambda_{\text{exc}} = 390$ nm; $\lambda_{\text{em}} = 474$ nm) is tri-exponential with $\tau_1 = 15.4 \pm 0.1$ ns ($A_3 = 14\%$), $\tau_2 = 5.4 \pm 0.2$ ns ($A_2 = 61\%$), and a longer time $\tau_3 = 1.8 \pm 0.1$ ns ($A_1 = 25\%$). By increasing the amount of porphyrin in solution, these values remain essentially unchanged.

These experimental results suggest that no effective energy transfer occurs from GQDs to porphyrins bound to the surface of graphene quantum dots.

- pH 3

A titration was performed by adding GQDs to an aqueous solution of TPPS₄⁴⁻ porphyrin at pH = 3 (Figure 1.12). At this pH, the porphyrin is present as the diacid form H₂TPPS₄²⁻, characterized by a Soret band at 434 nm. As the concentration of GQDs increases, the B band remains almost centered at 434 nm, although it undergoes substantial hypochromicity.

Furthermore, at high GQDs loads, there is an increasingly consistent presence of a band at 410 nm, probably due to the interference of the growing GQDs absorption band, which partially overlaps the porphyrin B-band. At the same time, a new band appears at 491 nm, accompanied by a large shoulder at longer wavelengths, with a clear isosbestic point at 452 nm. This band is attributable to the formation of porphyrin J-aggregates^{15,16} and its concentration increases with increasing GQD concentration.

The typical J-band in such aggregates is usually quite sharp due to the restriction of motion, and the band width is related to the coherence length. The rather broad and less intense J-band has already been reported in the case of such aggregates in the presence of polyamines or other cationic species.¹⁷

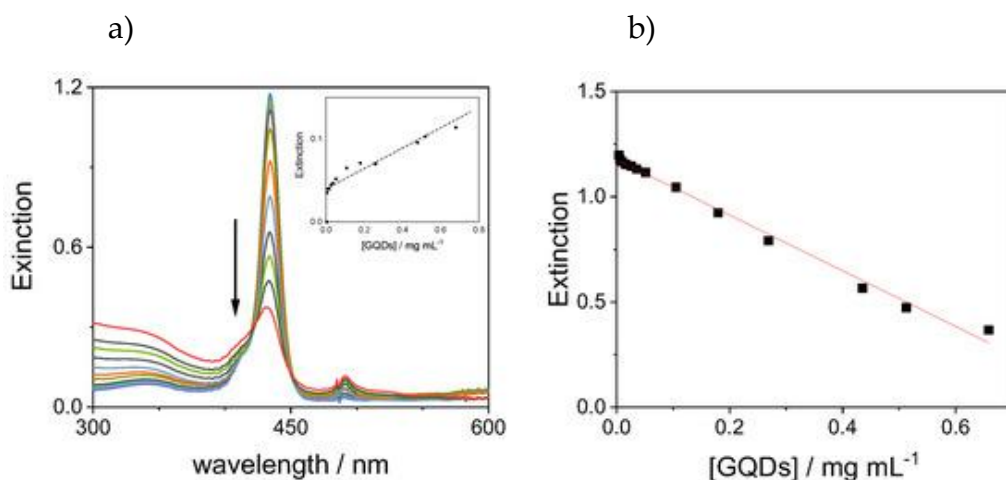


Figure 1.12. a) UV/Vis spectral variations and b) decrease in absorbance at 434 nm during titration of $\text{H}_2\text{TPPS}_4^{2-}$ with GQDs at pH = 3. The inset on the left shows the increase of the band at 491 related to the J-aggregate as a function of GQDs concentration. Experimental conditions: $[\text{H}_2\text{TPPS}_4^{2-}] = 3 \mu\text{M}$; $[\text{GQDs}] = 0\text{--}0.78 \text{ mg/mL}$; $[\text{HCl}] = 1 \text{ mM}$, pH = 3; $T = 298 \text{ K}$; cell path length 1 cm.

The emission spectra corresponding to UV/Vis titration are shown in Figure 1.13. The fluorescence emission spectrum characteristic of the diacid species of porphyrin has two bands at 670 and 709 nm, that decrease in intensity following the addition of GQDs.

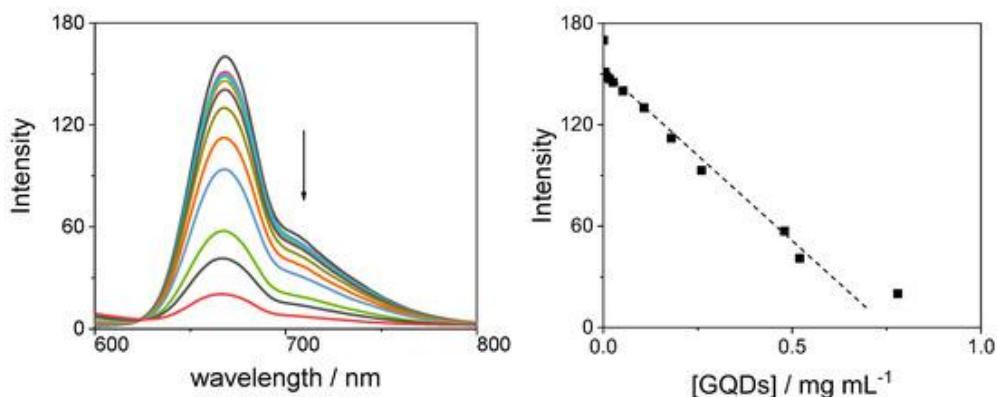


Figure 1.13. a) Spectral changes in fluorescence emission and b) fluorescence intensity at 668 nm during titration of $\text{H}_2\text{TPPS}_4^{2-}$ with GQD at pH = 3 Experimental conditions: $[\text{H}_2\text{TPPS}_4^{2-}] = 3 \mu\text{M}$; $[\text{GQDs}] = 0\text{--}0.78 \text{ mg/mL}$; $[\text{HCl}] = 1 \text{ mM}$, pH = 3; $T = 298 \text{ K}$; cell path length 1 cm; excitation wavelength 452 nm.

To confirm the hypothesis that the observed fluorescence quenching is simply due to porphyrin aggregation, fluorescence lifetime measurements were performed. The intensity profile for the diacid porphyrin $\text{H}_2\text{TPPS}_4^{2-}$ in aqueous solution follows a mono-exponential decay (Table 1.3) with a $\tau_1 = 2.0 \pm 0.1 \text{ ns}$ ($A = 8\%$).¹³ Again, a faster decay contribution is present in our samples with a $\tau_2 = 4.0 \pm 0.1 \text{ ns}$ ($A = 92\%$), indicating a small amount of porphyrin oligomers in solution. By increasing the GQD concentration, the values of both lifetimes remain almost unchanged. The measured value of the rotational correlation times for porphyrin $\text{H}_2\text{TPPS}_4^{2-}$ is $\tau_r = 0.72 \text{ ns}$, very similar to the data reported in the literature and consistent with the monomeric nature of this species in solution. In the presence of GQDs,

the τ_r values increase only slightly, indicating that diacid porphyrins interact weakly with GQDs.

Table 1.3. Fluorescence lifetimes (τ_1 and τ_2) and relative percentage amplitudes, together with the time constant of fluorescence anisotropy decays for the titration of TPPS₄⁴⁻ with GQDs at pH = 3. Experimental conditions: [TPPS₄⁴⁻] = 3 μ M; pH = 3 ([HCl] = 0.001 M); T = 298 K; cell path length 1 cm; λ_{exc} = 390 nm; λ_{em} = 670 nm.

[GQDs] / mg mL ⁻¹	Lifetime / ns		Anisotropy Decays / ns
0	τ_1 = 1.96 (8%)	τ_2 = 3.96 (92%)	τ_r = 0.88
0.02	τ_1 = 1.89 (10%)	τ_2 = 3.81 (90%)	τ_r = 0.99
0.08	τ_1 = 1.91 (10%)	τ_2 = 3.81 (90%)	τ_r = 1.02
0.28	τ_1 = 1.91 (8%)	τ_2 = 3.91 (92%)	τ_r = 1.05

The formation of J-aggregates has been described as a hierarchical process, strongly influenced by the mixing method used. In particular, when a defined amount of GQDs (0.052 mg/mL) is rapidly added to the porphyrin-containing solution, a more abundant formation of J-aggregates is observed, probably due to changes in the nucleation process. The growth of the J-band occurs progressively, following sigmoidal kinetics, characteristic of this type of aggregation. The kinetic data can be analyzed by fitting the experimental data using a model developed by Pasternack, already used to describe the self-assembly of dyes in supramolecular systems.^{18,19}

It should be noted that the mixing protocol used in these experiments corresponds to the so called “porphyrin first” (PF) approach, known to

promote aggregation even under strongly acidic conditions. Considering that at pH 3 the diacid form of porphyrin is stable and does not spontaneously tend to aggregate, it can be concluded that the self-assembly process is activated by the presence of GQDs. This behavior is consistent with what has already been documented for TPPS₄⁴⁻ aggregation in the presence of gold nanorods, cationic porphyrins, and polyamines such as trien.²⁰ In this case too, therefore, the polyamine used as a capping agent plays a fundamental role in promoting the self-assembly of monomeric porphyrins, facilitating the formation of the initial nuclei of the J-aggregates. Previous studies have shown that the distance between the protonated amino groups in these polyamines is around 3.8 Å, which is the optimal distance for promoting stacking interactions and electrostatic bonds between the porphyrin rings (Figure 1.14)

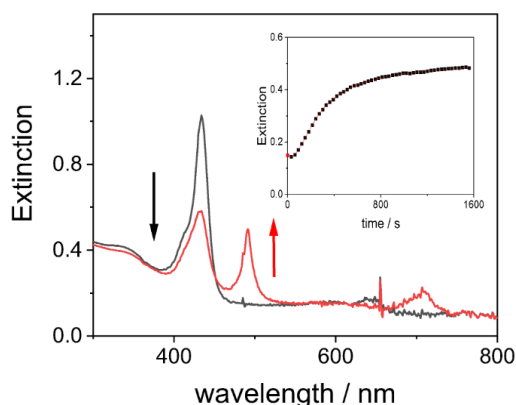


Figure 1.14. UV/Vis extinction spectra corresponding to the initial solution of H₂TPPS₄²⁻ and the formation of the J-type aggregate after the addition of GQDs at pH = 3. The inset shows the kinetic trace at 491 nm for the formation of J-aggregates (at eq. 1, $k_0 = 5.1 \pm 3.9 \times 10^{-4} \text{ s}^{-1}$; $k_c = 5.9 \pm 0.2 \times 10^{-3} \text{ s}^{-1}$; $m = 3.9 \pm 0.6$; $n = 1.9 \pm 0.4$). Experimental conditions: [H₂TPPS₄²⁻] = 3 μM; [GQDs] = 0.052 mg/mL; [HCl] = 1 mM, pH = 3; T = 298 K; cell path length 1 cm.

As regards the titration performed on a solution of GQDs with TPPS₄⁴⁻ porphyrin at pH 3, the UV/Vis spectra show the gradual growth of the Soret band of TPPS₄⁴⁻ porphyrin in its di-acid form, at 434 nm, and, in parallel, the onset of a band positioned at 491 nm, indicating the formation of the J-type aggregate, as previously discussed for the interaction between the two systems at acidic pH (Figure 1.15)

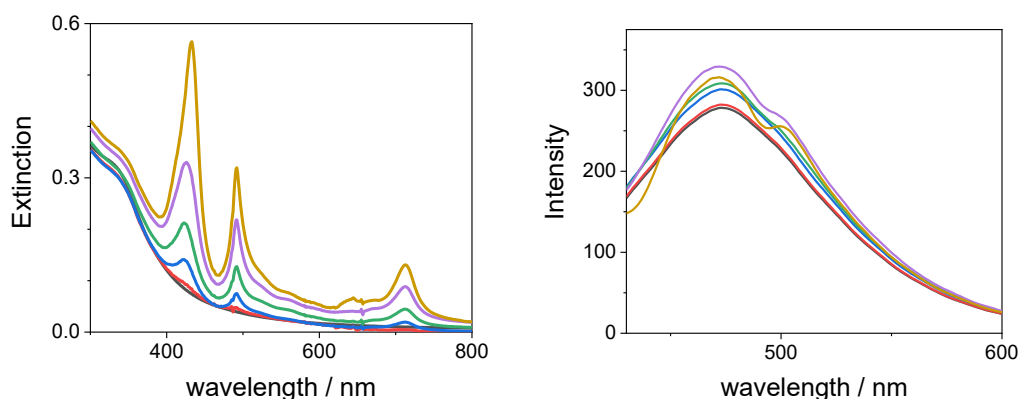


Figure 1.15 UV/Vis absorbance spectral changes (left panel) and fluorescence emission spectra exciting at $\lambda = 410$ nm (right panel) during the titration of GQDs (black line) with TPPS₄⁴⁻ at pH= 3. Experimental conditions: [TPPS₄⁴⁻] = 0.07 μ M, red line; 0.42 μ M, blue line; 0.9 μ M, green line; 1.8 μ M, purple line; 3 μ M, yellow line; [GQDs] = 0.14 mg/mL; [HCl] = 1 mM, pH = 3; T = 298 K; cell path length 1 cm.

The spectra relating to the fluorescence emissions of GQDs, after being corrected for the filter effect on incident radiation, do not show any significant variation as the concentration of TPPS₄⁴⁻ increases. From the time-resolved fluorescence analyses (Table 1.4), it can be observed that the lifetimes and relative percentages of GQDs are not altered by the pH of the solution. As the concentration of TPPS₄⁴⁻ increases, the lifetimes of the two species tend to decrease, although in this case, as at neutral pH, the

decrease observed for the short-living term is greater (80%) than that for the long-living term (23%).

Table 1.4. Fluorescence lifetimes (τ_1 and τ_2) and relative percentage amplitudes for the titration of GQDs with TPPS₄⁺ pH = 3. Experimental conditions: [GQDs] = 0.14 mg/mL; [HCl] = 1 mM, pH = 3; T = 298 K; cell path length 1 cm. λ_{exc} = 390 nm; λ_{em} = 474 nm.

[TPPS ₄ ⁺] / μ M	Lifetime / ns	
0	$\tau_1=2.31(47\%)$	$\tau_2=6.29(53\%)$
0.07	$\tau_1=2.14(42\%)$	$\tau_2=5.98(58\%)$
0.42	$\tau_1=2.00(42\%)$	$\tau_2=5.89(58\%)$
0.90	$\tau_1=1.42(35\%)$	$\tau_2=5.49(65\%)$
1.80	$\tau_1=0.82(35\%)$	$\tau_2=5.11(65\%)$
3.00	$\tau_1=0.45(42\%)$	$\tau_2=4.86(58\%)$

Experimental part

Materials

The porphyrins 5,10,15, 20-tetrakis(4-sulfonatophenyl)porphyrin (TPPS₄⁺), in the form of sodium salt, and 5,10,15,20-tetrakis(N-methylpyridinium-4-yl)porphyrin (TMPyP⁴⁺), in the form of tosylate salt, were purchased from Sigma-Aldrich (Milan, Italy). L-glutamic acid, triethylenetetramine tetrahydrochloride, sodium dihydrogen phosphate, sodium hydrogen phosphate, hydrochloric acid, and sodium hydroxide were of highest grade of purity and were used without further purification (Sigma-Aldrich, Milan, Italy). All aqueous solutions were prepared using high purity double-distilled water (HPLC grade, Fluka, Milan, Italy). The

porphyrin stock solutions (200 μM) were prepared at the time of use and stored in the dark to prevent photodegradation. The concentrations of porphyrins in the samples were determined by UV/Vis spectroscopy, using the molar extinction coefficients in the Soret band: TPPS₄⁴⁻: $\epsilon = 5.33 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ at 414 nm and TMPyP⁴⁺: $\epsilon = 2.26 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ at 422 nm.

Graphene quantum dots were synthesized by microwave-assisted pyrolysis using a CEM Discover synthesizer (Matthews, NC, USA). An equimolar amount of L-glutamic acid (147 mg) and triethylenetetramine tetrahydrochloride (292 mg) was placed in a glass test tube and subjected to microwave treatment for 5 minutes at 225 °C with a power of 300 W. The dark residue obtained was then suspended in 1 mL of deionized water and filtered through a Millipore filter (0.45 μm) to remove larger particles.

Conclusions

Graphene quantum dots (GQDs) are nanostructured systems of great interest for the construction of supramolecular architectures intended for a wide range of applications. The synthesized GQDs have a positively charged surface due to the presence of ionizable amino groups. At neutral pH, TPPS₄⁴⁻ porphyrin, in its tetra-anionic form, interacts electrostatically with GQDs, resulting in a progressive and complete quenching of its fluorescence emission. This phenomenon is probably attributable to a photoinduced electron transfer from the excited electron donor state of porphyrin to GQDs, which act as acceptors. As the pH decreases, porphyrin protonates and its overall charge is reduced, thus weakening the electrostatic interactions with the nanoparticles. At the same time, the

diacid form of TPPS₄⁴⁻ tends to self-aggregate, and the cationic terminal groups of the GQDs facilitate the formation of J-type aggregates. The supramolecular adduct studied is easily assembled in aqueous solution, and the degree of interaction between the components can be modulated simply by varying the pH. Furthermore, by using an appropriate ratio between quantum dots and porphyrin, it is possible to use this system as a potential ratiometric sensor using the double fluorescence emission of the two chromophores.

Chapter 2 will illustrate the use of this supramolecular system as fluorescence sensor, highlighting its performance and potential in various analytical contexts.

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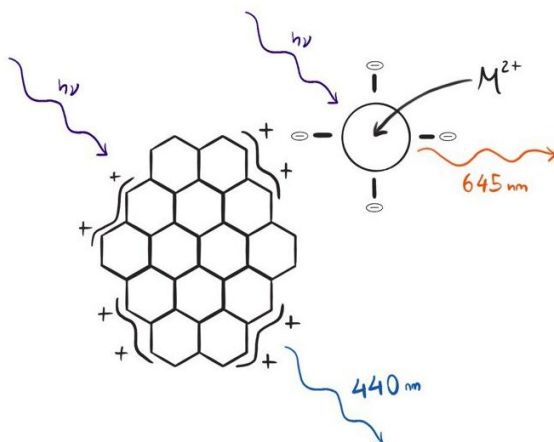
Chapter 2

Graphene Quantum Dots/Porphyrin (GQDs@TPPS₄⁴⁻) supramolecular assembly as a fluorescent probe for metal ion sensing.¹

As already described in the introduction, heavy metal ions pose a serious risk to human health and the environment. In order to effectively monitor their presence, the GQDs@TPPS₄⁴⁻ system has been studied as a possible ratiometric sensor for the detection of metal ions in solution.

As discussed in Chapter 1, positively charged Graphene Quantum Dots (GQDs), synthesized from L-glutamic acid and triethylenetetramine using a bottom-up approach, can easily interact with meso-tetrakis(4-sulfonatophenyl) porphyrin (TPPS₄⁴⁻) which is negatively charged due to the presence of sulfonate groups in the meso position. This interaction leads to the formation of stable supramolecular adducts, which as the acidity of the solution increases, promotes the self-aggregation of TPPS₄⁴⁻ diacid species, also through the catalytic action of GQDs.

It is hypothesized that TPPS₄⁴⁻ is condensed in the vicinity of the primary and secondary semiprotonated amino groups of trien² thus favoring the metalation process, in close analogy with similar systems reported in the literature.³



Scheme 2.1. General scheme of GQDs@TPPS₄⁴⁻ supramolecular system for metal ion sensing

2.1. Characterization of GQDs@TPPS₄⁴⁻ systems

In this context, with the aim of testing the GQDs@TPPS₄⁴⁻ (1:5 w/w) system as a potential fluorescent ratiometric sensor for metal ions, experimental conditions were appropriately chosen to ensure double fluorescence emission from both the two chromophores.

The UV-Vis spectra of the GQDs@TPPS₄⁴⁻ system, recorded in 10 mM PBS buffer at pH 7 (Figure 2.1, top) shows a characteristic band at 330 nm, attributable to carbon nanoparticles, and a B band at 414 nm, accompanied by four weaker Q bands centered at 516, 540, 580, and 612 nm, representing the typical extinction characteristics of TPPS₄⁴⁻.

The fluorescence emission spectra (Figure 2.1, bottom) obtained using an excitation wavelength of 380 nm, shows both the emission of GQDs (maximum at 440 nm) and that of TPPS₄⁴⁻, characterized by two bands at

645 and 707 nm. The stoichiometric ratio between the components is such that the addition of the anionic porphyrin causes a reversal of the overall charge of the system, which becomes globally negative, as confirmed by the zeta potential (-13 mV).

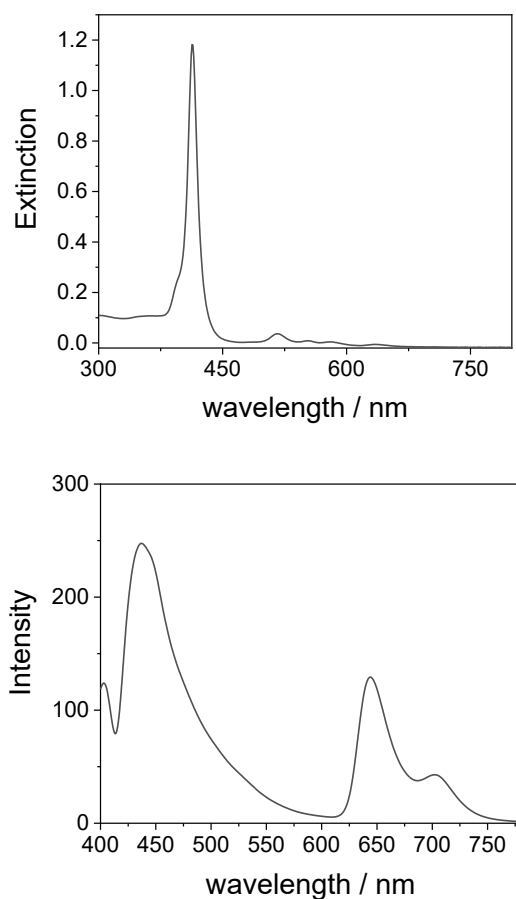


Figure 2.1. UV/Vis of GQDs@TPPS₄⁴⁺ system (top) and fluorescence emission spectra (bottom) using $\lambda_{\text{exc}} = 380$ nm; Experimental conditions: [TPPS₄⁴⁺] = 3 μ M; [GQDs] = 0.018 mg/mL; PBS 10 mM, pH = 7; T = 298 K; cell path length 1 cm.

2.2. Sensing of metal ions

- Sensing of Cu^{2+}

In this context, spectroscopic titration was performed by progressively adding micromolar aliquots of Cu^{2+} ions to a solution of GQDs@TPPS_4^{4-} .

From the analysis of the UV/Vis spectra, no significant changes were observed in the B band at 414 nm, while the Q-band region underwent marked modifications: the four bands gradually merged into two (at 540 and 580 nm) as the Cu^{2+} concentration increased. These spectral variations (reported in Figure 2.2, top) are indicative of the formation of the Cu(II)TPPS metal complex, in accordance with previous literature reports.⁴

Fluorescence emission spectra further confirmed this behavior, suggesting that the system can act as a non-ratiometric fluorescent probe for Cu^{2+} ions. As shown of the spectra (in Figure 2.2, bottom) the progressive addition of copper ions induces a slight decrease in the emission intensity of the GQDs (470 nm), while the fluorescence of TPPS_4^{4-} is almost completely quenched, an effect attributable to the formation of the Cu(II)-porphyrin derivative on the GQD surface. The evolution of the emission spectra recorded at 420 nm highlights a progressive fluorescence quenching with increasing Cu^{2+} concentration. Titration was carried out in the 0–11 μM concentration range. The limit of detection (LoD) was calculated from the fluorescence titration data according to the literature method.⁵ The linear response in the micromolar range demonstrates that

the GQDs@TPPS₄⁴⁻ probe can quantitatively detect low Cu²⁺ concentrations, with an LoD of 0.059 μM.

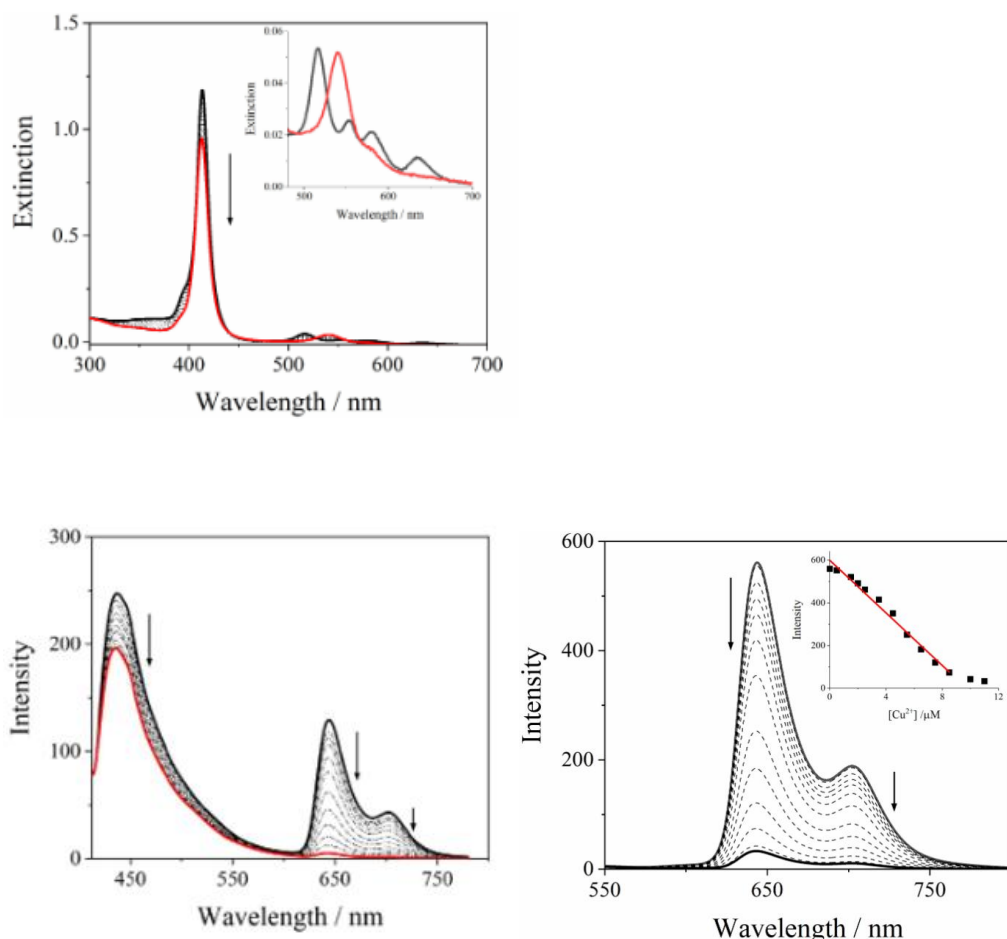


Figure 2.2. UV/Vis spectra(top) and fluorescence emission (bottom) spectral variation exciting at $\lambda = 380$ nm (left panel) and $\lambda = 420$ nm (right panel) during the titration of GQDs@TPPS₄⁴⁻ with Cu²⁺ ions at the beginning (black line) and at the end (red line) at neutral pH, and from which a LoD = 0.059 μM (the red line represents the linear fit of the data, while the arrow indicates the increasing concentration of metal ions). Experimental conditions: [TPPS₄⁴⁻] = 3 μM; [GQDs] = 0.018 mg/mL; [Cu²⁺] = 0–11 μM; PBS 10 mM, pH = 7; T = 298 K; cell path length 1 cm.

To confirm that the improved sensitivity is due to the presence of GQDs, a comparative titration of free TPPS₄⁴⁻ (without GQDs) was carried out under the same experimental conditions (Figure 2.3).

In this case, the Cu^{2+} concentration range was extended up to 185 μM , as higher concentrations were required to achieve a comparable fluorescence quenching.

From the corresponding calibration curve, an LoD value of 0.24 μM was obtained, which is about four times higher than that of the GQDs@TPPS₄⁴⁻ system. These results confirm that the presence of GQDs significantly enhances the sensitivity of the probe toward Cu^{2+} ions.

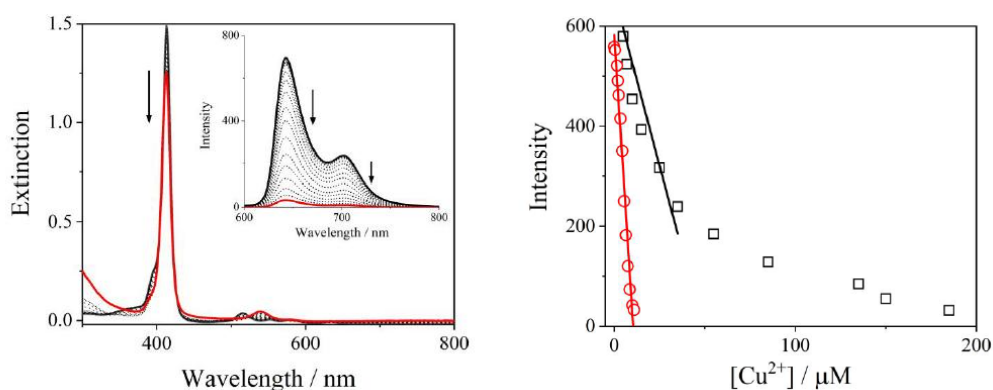


Figure 2.3. UV/Vis absorption spectra (left panel) and corresponding fluorescence emission spectra recorded upon excitation at $\lambda = 420$ nm (inset, left panel) during the titration of TPPS₄⁴⁻ with Cu^{2+} ions at neutral pH in bulk solution. The spectral variation of the emission band at 645 nm for free-base TPPS₄⁴⁻ (black line) as a function of Cu^{2+} concentration (LoD of 0.24 μM) is shown in comparison with the corresponding changes of the 645 nm emission band for the GQDs@TPPS₄⁴⁻ system (red line). The arrow indicates increasing metal ion concentration. Experimental conditions: $[\text{TPPS}_4^{4-}] = 3$ μM ; $[\text{Cu}^{2+}] = 0$ –200 μM ; PBS 10 mM, pH 7; $T = 298$ K; path length = 1 cm.

To verify whether the presence of GQDs in the system promotes the coordination reaction of Cu^{2+} ions in the porphyrin core of TPPS₄⁴⁻, kinetic metallation tests were conducted using a fixed concentration of copper ions (30 μM), in the presence and absence of GQDs (Figure 2.4).

The time trend of the absorbance at 540 nm shows first-order kinetics in both cases. The best fit of the experimental data according to a first-order law equation reported in experimental part (Chapter 7, Paragraph 7.2, method) allowed us to estimate a rate constant for the GQDs@TPPS₄⁴⁻ system that is approximately three times higher than that of bulk porphyrin.

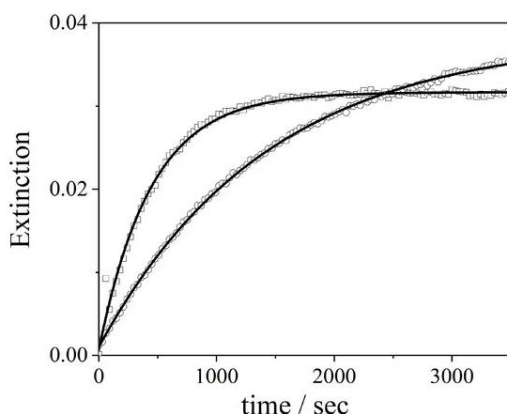


Figure 2.4. Kinetic traces collected at 540 nm for the metalation of TPPS₄ porphyrin catalyzed by the presence of GQDs (empty squares) and in bulk (empty circles). Experimental conditions: [TPPS₄] = 3 μ M; [GQDs] = 0.018 mg/mL; [Cu²⁺] = 30 μ M; PBS 10 mM, pH = 7; T = 298 K; cell path length 1 cm. The solid lines in the extinction profiles are the best fit of the experimental data according to Equation (1). (GQDs@TPPS₄): $k = (2.25 \pm 0.03) \times 10^{-3} \text{ s}^{-1}$; $R^2 = 0.99$. (TPPS₄): $k = (7.0 \pm 0.05) \times 10^{-4} \text{ s}^{-1}$; $R^2 = 0.99$

In order to evaluate the robustness and selectivity of the GQDs@TPPS₄⁴⁻ system towards the Cu²⁺ ion, a further study was conducted by varying the experimental conditions. In particular, the concentration of the PBS buffer was reduced from 10 mM to 1 mM, keeping the pH constant at 7.0, and 30 μ M of Zn²⁺ was added to the solutions used.

This metal was chosen because it is one of the main trace metal contaminants not only in glassware but also in laboratory instruments, including methacrylate cuvettes⁶ or commercial injectable waters. Based on these considerations, the response of the GQDs@TPPS₄⁴⁻ system in the presence of metal ions was tested by deliberately contaminating all the solutions used (including the solvent) with a known amount of Zn²⁺ equal to 30 μ M. Under these new experimental conditions, the reactivity of the system towards copper ions remained virtually unchanged. The variations observed in the UV/Vis spectra show that the copper derivative of porphyrin forms even at relatively low concentrations of Cu²⁺ (approximately 5 μ M). The corresponding fluorescence emission spectra show a marked attenuation of the intensity of the bands attributed to TPPS₄⁴⁻, accompanied by a slight increase in the band at 606 nm, attributable to the Zn(II) porphyrin derivative already present due to the controlled contamination of the solutions. These results confirm that the GQDs@TPPS₄⁴⁻ system maintains high sensing efficiency for Cu²⁺ ions even in the presence of potential metal interferents such as Zn²⁺.

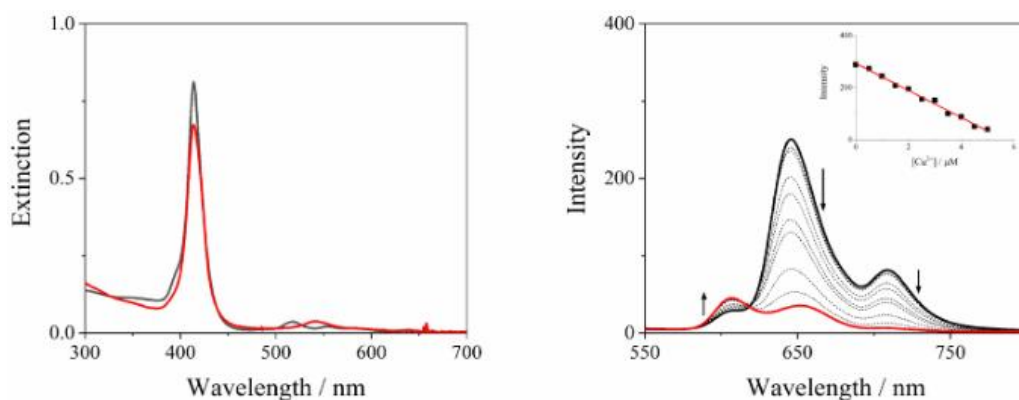


Figure 2.5. UV/Vis absorption spectral changes (left panel) and corresponding fluorescence emission spectra upon excitation at $\lambda = 418$ nm (right panel) for GQDs@TPPS₄⁴⁻, recorded at the beginning (black line) and at the end (red line) of a titration with Cu²⁺ ions at neutral pH (arrow indicates increasing metal ion concentration). The inset in the right panel shows the corresponding fluorescence intensity changes at 645 nm, corrected for the presence of the zinc(II) derivative, from which a LoD of 0.063 μ M is estimated (red line represents the linear fit of the data). Experimental conditions: [TPPS₄⁴⁻] = 3 μ M; [GQDs] = 0.018 mg/mL; [Zn²⁺] = 30 μ M; [Cu²⁺] = 0–5 μ M; PBS 1 mM, pH 7; T = 298 K; path length = 1 cm.

- Sensing of Hg²⁺

The GQDs@TPPS₄⁴⁻ system was further investigated in the presence of mercuric ions (Hg²⁺) in order to evaluate its sensitivity and selective response to this heavy metal. The UV/Vis spectra acquired for each aliquot of Hg²⁺ added show that the quantitative formation of the porphyrin metal derivative occurs at a concentration of approximately 20 μ M mercury ions.

The appearance of the new Soret band at 433 nm and the reduction in the number of Q bands from four to two, located at 590 and 628 nm respectively (Figure 2.6, top) are perfectly consistent with what is reported in the literature for the formation of the Hg(II)TPPS₄⁴⁻ complex.⁷

As regards the fluorescence emission spectra (in Figure 2.6, bottom), recorded by exciting at 380 nm, a marked quenching is observed in the porphyrin emission region, accompanied by a significant decrease in the fluorescence intensity of the GQDs as the concentration of mercury ions increases.

However, this latter variation does not reflect an actual quenching process of the GQDs but rather represents an artifact due to the internal filter effect caused by the absorption of the dominant porphyrin component, which reduces the amount of fluorescent light emitted and collected by the instrument. The fluorescence emission data were therefore corrected considering the absorption contribution of porphyrin, as described in the experimental section.

After this correction, the fluorescence intensity trend of GQDs as a function of Hg^{2+} ion concentration shows signal stability of the system, in line with the behavior previously observed in the presence of cupric ions (Cu^{2+}).

Furthermore, the variation in the fluorescence emission spectra was also recorded using excitation at 426 nm. Under these conditions, a progressive quenching of the emission intensity was observed, as shown in the insert, corresponding to the titration of porphyrin with Hg^{2+} ions in the concentration range between 0 and 40 μM .

From the processing of the fluorescence titration data, a limit of detection (LoD) of 0.078 μM was calculated, a value slightly improved compared to that obtained for TPPS_4^{4-} alone (LoD = 0.15 μM)

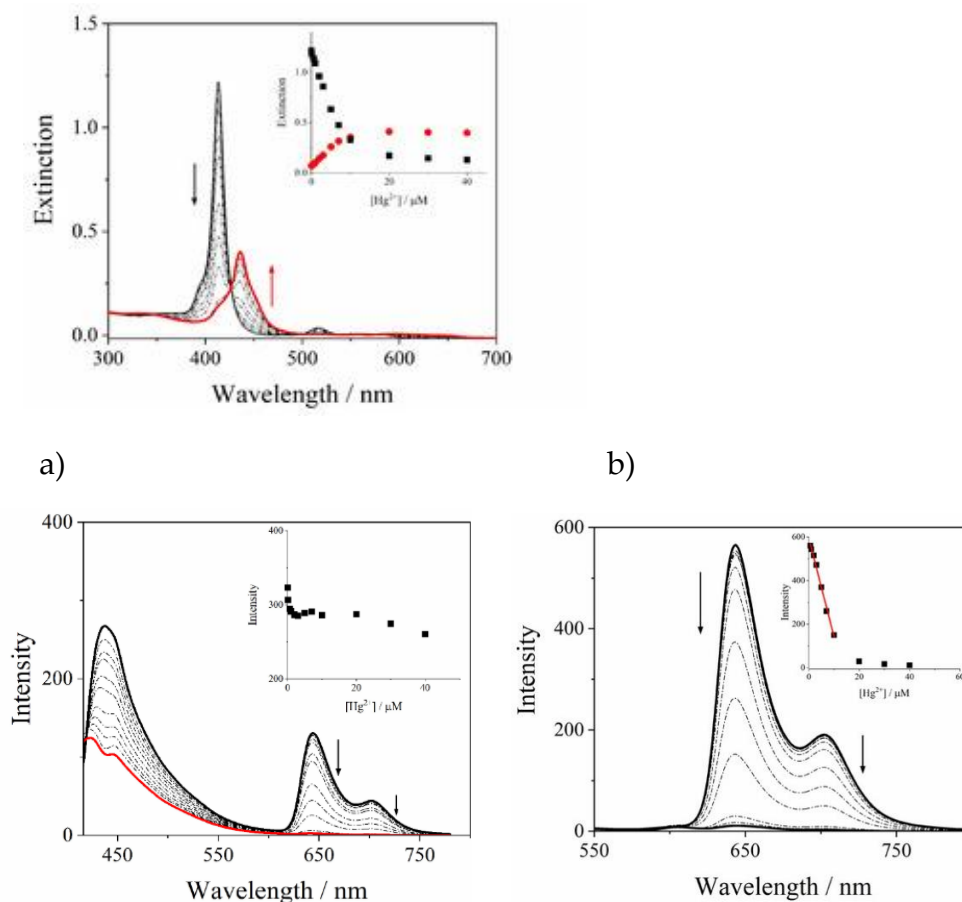


Figure 2.6. UV/Vis and fluorescence spectral changes of GQDs@TPPS_4^{4-} during titration with Hg^{2+} ions at neutral pH. The upper panel shows UV/Vis absorbance changes, with the inset displaying the extinction trends at 414 nm (black squares) and 436 nm (red circles). The lower panel (a) shows the corresponding fluorescence emission spectra recorded upon excitation at $\lambda = 380$ nm, with the inset corrected for the filter effect induced by the absorption of the porphyrin metal derivative. To better highlight the porphyrin fluorescence, emission spectra were also recorded upon excitation at $\lambda = 426$ nm (b), with the inset showing the fluorescence intensity changes at 645 nm, from which a LoD of 0.078 μM is estimated (red line represents the linear fit; arrow indicates increasing metal ion concentration). Experimental conditions: $[\text{TPPS}_4^{4-}] = 3 \mu\text{M}$; $[\text{GQDs}] = 0.018 \text{ mg/mL}$; $[\text{Hg}^{2+}] = 0\text{--}40 \mu\text{M}$; PBS 10 mM, pH 7; $T = 298 \text{ K}$; path length = 1 cm.

Again, in order to confirm the efficiency of the GQDs@TPPS₄⁴⁻ supramolecular system in detecting Hg²⁺ ions, UV/Vis (Figure 2.7, left panel) and fluorescence (Figure 2.7, right panel) spectroscopic titrations were performed on a solution containing only 3 μ M of TPPS₄⁴⁻, in the absence of GQDs, keeping the experimental conditions unchanged.

From the analysis of the UV/Vis spectra (in Figure 2.7, left panel) recorded for successive additions of Hg²⁺ ions, it was possible to observe the formation of the Hg(II)TPPS₄⁴⁻ complex, characterized by the onset of the Soret band at 433 nm and the variation in the pattern of the Q bands, in agreement with what was reported for the GQDs@TPPS₄⁴⁻ system. However, the quantitative formation of the metal derivative only occurs at significantly higher Hg²⁺ concentrations, approximately 150 μ M, compared to the 40 μ M sufficient for complete metalation in the GQDs@TPPS₄⁴⁻ system. This difference highlights the role of GQDs in promoting the interaction between porphyrin and mercury ions, improving the sensitivity and efficiency of the detection process.

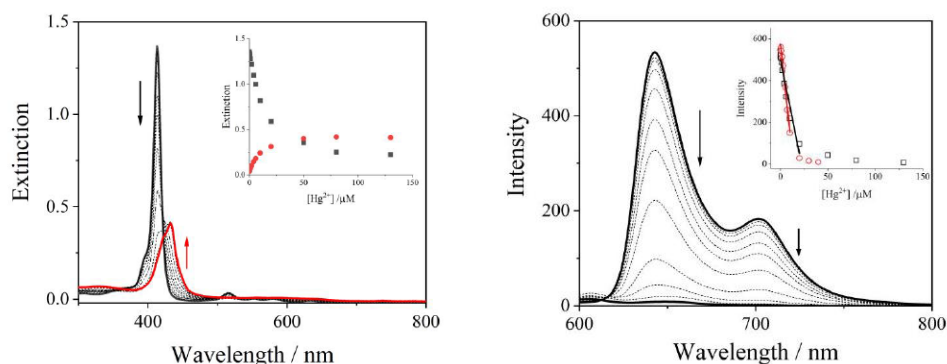


Figure 2.7. UV/Vis absorbance spectral changes (left panel) and corresponding fluorescence emission spectra upon excitation at $\lambda = 422$ nm (right panel) for the titration of TPPS₄⁴⁻ with Hg²⁺ ions in bulk solution at neutral pH. The arrow indicates increasing metal ion concentration. The inset in the left panel shows the concentration-dependent extinction changes at 414 nm (black squares) and 432 nm (red circles), while the inset in the right panel reports the corresponding fluorescence intensity changes at 645 nm (black squares), from which a LoD of 0.15 μ M is determined. Experimental conditions: [TPPS₄⁴⁻] = 3 μ M; [Hg²⁺] = 0–150 μ M; PBS 10 mM, pH 7; T = 298 K; path length = 1 cm.

To further assess the behaviour and selectivity of the GQDs@TPPS₄⁴⁻ system towards Hg²⁺ ions, additional experiments were performed under modified conditions. Specifically, the PBS buffer concentration was decreased from 10 mM to 1 mM while keeping the pH constant at 7.0, and all the solutions employed (including the solvent) were intentionally contaminated with a known amount of Zn²⁺ equal to 30 μ M. This metal was again selected to simulate the presence of trace contaminants commonly found in laboratory materials and analytical instruments.

Under these new experimental conditions, the behaviour of the GQDs@TPPS₄⁴⁻ system towards mercury ions differed significantly from that observed for copper. The UV/Vis spectral variations shown in Figure 2.8 (left panel) indicate that, in the case of Hg²⁺, the interaction does not lead to a classical metal insertion into the porphyrin core but rather

follows a *sitting-atop* mechanism. The coordination of Hg^{2+} on the outer surface of the porphyrin macrocycle induces a structural distortion that makes the ring non-planar. This distortion facilitates and accelerates subsequent metallation by smaller cations present in solution, such as Zn^{2+} . Infact it is possible to observe the increase of the Soret band at 422 nm and the formation of the new Q band 556 nm and 598 nm, respectively⁸, typically of the Zn(II) porphyrin⁸ derivative on the GQDs surface.

The corresponding fluorescence emission spectra in Figure 2.8 (right panel) support this interpretation: a progressive quenching of the TPPS_4^{4-} emission bands is observed, accompanied by a slight increase in the intensity of the band at 606 nm, attributable to the Zn(II)-porphyrin derivative formed under these conditions. These findings confirm that the GQDs@TPPS_4^{4-} system interacts efficiently with Hg^{2+} ions through a distinct *sitting-atop* mechanism, while maintaining high sensitivity and selectivity even in the presence of potentially interfering metal ions such as Zn^{2+} .

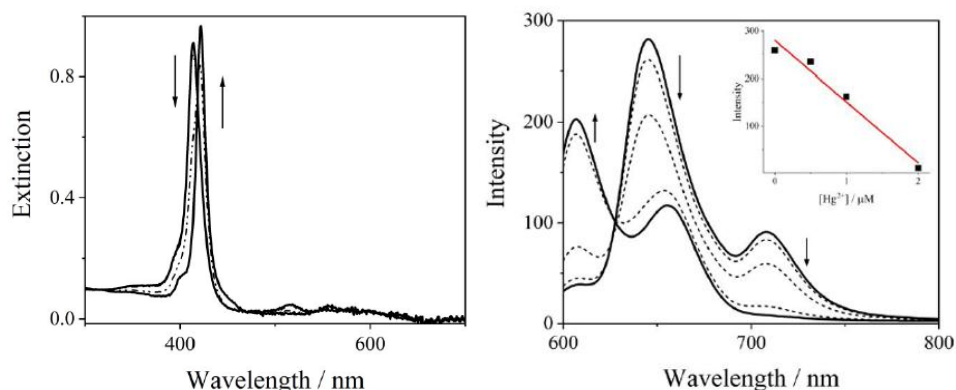


Figure 2.8. UV/Vis absorbance spectral changes (left panel) and corresponding fluorescence emission spectra recorded upon excitation at $\lambda = 418$ nm (right panel) for the titration of GQDs/TPPS₄⁴⁻ with Hg²⁺ ions in the presence of Zn²⁺ ions at neutral pH. The arrow indicates increasing metal ion concentration. The inset in the right panel shows the fluorescence intensity changes at 645 nm, corrected for the presence of the zinc(II) derivative, from which a LoD of 0.025 μ M is determined. Experimental conditions: [TPPS₄⁴⁻] = 3 μ M; [GQDs] = 0.018 mg/mL; [Zn²⁺] = 30 μ M; [Hg²⁺] = 0–12 μ M; PBS 1 mM, pH 7; T = 298 K; path length = 1 cm.

- Sensing of Cd²⁺

In the presence of PBS 10 mM, the supramolecular system GQDs@TPPS₄⁴⁻ does not show the ability to detect the presence of Cd(II) ions. This behavior is attributed to the strong interaction between the phosphate ions in the buffer and the metal ions, as the phosphates act as ligands, coordinating with the metals and reducing the concentration of free cadmium ions in solution.¹

The reduction in buffer concentration from 10 to 1 mM, on the other hand, causes a clear response of the GQDs@TPPS₄⁴⁻ system to Cd(II) ions, resulting from the increase in the fraction of ions available in free form. The UV/Vis spectra (Figure 2.9, left panel) show the formation of the metal

complex, marked by the emergence of a new absorption band centered at 430 nm. The corresponding fluorescence spectra (Figure 2.9, right panel), recorded with excitation at 423 nm, show a progressive quenching of the emission intensity of the characteristic bands of the system. From the analysis of the variation in fluorescence intensity as a function of Cd(II) concentration, a limit of detection (LoD) of 0.60 μM was estimated.

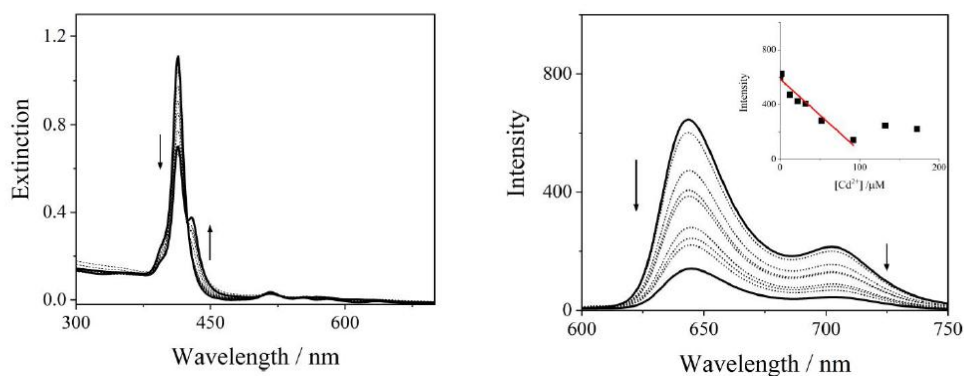


Figure 2.9. UV/Vis absorbance spectral changes (left panel) and corresponding fluorescence emission spectra recorded upon excitation at $\lambda = 423$ nm (right panel) for the titration of GQDs/TPPS₄⁴⁻ with Cd²⁺ ions at neutral pH. The arrow indicates increasing metal ion concentration. The inset in the lower panel shows the fluorescence intensity changes at 645 nm, from which a LoD of 0.60 μM is determined. Experimental conditions: [TPPS₄⁴⁻] = 3 μM ; [GQDs] = 0.018 mg/mL; [Cd²⁺] = 0–180 μM ; PBS 1 mM, pH 7; T = 298 K; path length = 1 cm.

The controlled addition of 30 μM Zn(II), necessary to simulate for contamination due to possible traces of metal present in the cuvettes, glassware, or water used (as discussed above), does not compromise the ability of the GQDs@TPPS₄⁴⁻ system to respond to the presence of Cd(II) ions. Similar to what was observed for Hg(II) ions, Cd(II) ions promote the insertion of Zn(II) into the porphyrin core, leading to the formation of the

Zn(II)-porphyrin derivative anchored to the surface of the GQDs (sitting a-top mechanism). As shown by the UV/Vis spectra, the addition of small amounts of Cd(II) ions induces the appearance of a new absorption band at 422 nm, accompanied by a change in the Q bands, which are reduced from four to two, located at 556 and 598 nm, respectively. (Figure 2.10, left panel). The corresponding fluorescence spectra show that, as the concentration of Cd(II) increases, there is an increase in the intensity of the emission band at 606 nm and the partial quenching of the emission band at 645 nm typical of free-base porphyrin. These spectroscopic changes are attributable to the formation of the Zn(II) derivative, in accordance with what has been previously described. A limit of detection (LoD) of 0.039 μM was determined, indicating a markedly improved sensitivity toward cadmium(II) ions. (Figure 2.10, right panel)

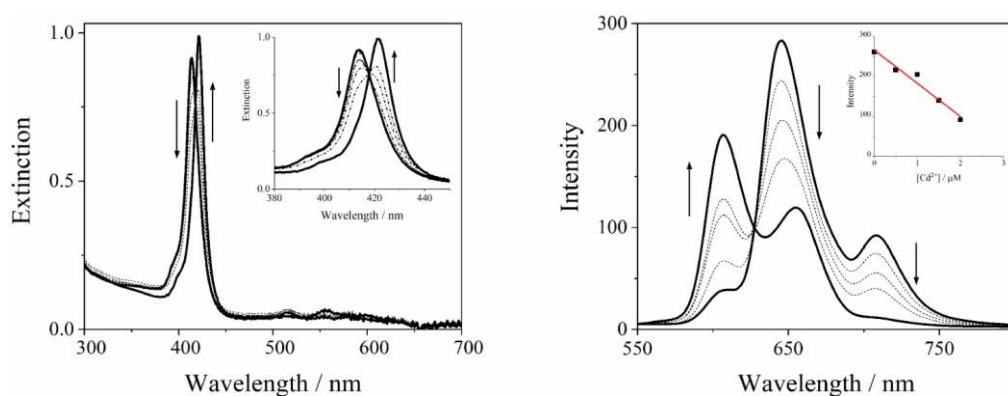


Figure 2.10. UV/Vis absorption changes (left panel) and corresponding fluorescence emission spectra upon excitation at $\lambda = 418$ nm (right panel) for the titration of GQDs@TPPS₄⁴⁻ with Cd²⁺ ions in the presence of Zn²⁺ ions at neutral pH. The arrow indicates increasing metal ion concentration. The inset in the right panel shows the fluorescence intensity changes at 645 nm, corrected for the presence of the zinc(II) derivative, from which a LoD of 0.039 μM is estimated (red line represents the linear fit). Experimental conditions: [TPPS₄⁴⁻] = 3 μM ; [GQDs] = 0.018 mg/mL; [Zn²⁺] = 30 μM ; [Cd²⁺] = 0–2 μM ; PBS 1 mM, pH 7; T = 298 K; path length = 1 cm.

- Sensing of Zn^{2+}

Even with regard to Zn(II) ions, the use of a 10 mM PBS concentration does not allow for effective zinc detection by the GQDs@TPPS_4^{4-} system, due to the strong interaction that occurs between the phosphate ions in the buffer and the Zn^{2+} ions present in solution, as previously described.¹

By reducing the buffer concentration from 10 mM to 1 mM, it was possible to perform titration and study the reactivity of the GQDs@TPPS_4^{4-} system towards Zn(II) ions, in the absence of larger metal cations that catalyze the formation of the zinc(II)-porphyrin derivative on the surface of the GQDs.

The UV/Vis spectra show in Figure 2.11 that, as the concentration of Zn^{2+} ions increases, the spectroscopic characteristics are in good agreement with those reported in the literature, highlighting a Soret band centered at 422 nm and two Q bands at 556 nm and 598 nm, respectively. However, a certain extension of Rayleigh scattering can be observed. The corresponding fluorescence emission spectra, recorded with excitation at 418 nm, show significant variations in the bands associated with free-based porphyrin. The panel insert shows (Figure 2.11, lower panel) the fluorescence intensity measured at 645 nm during the titration of porphyrin with Zn^{2+} ions in the concentration range 0–280 μM , from which a limit of detection (LoD) of 1.87 μM was calculated. In this case, as in the case of Cd(II) ions, any excitation at 380 nm will result in a moderate decrease in the fluorescence emission of GQDs attributable to a secondary filter effect.

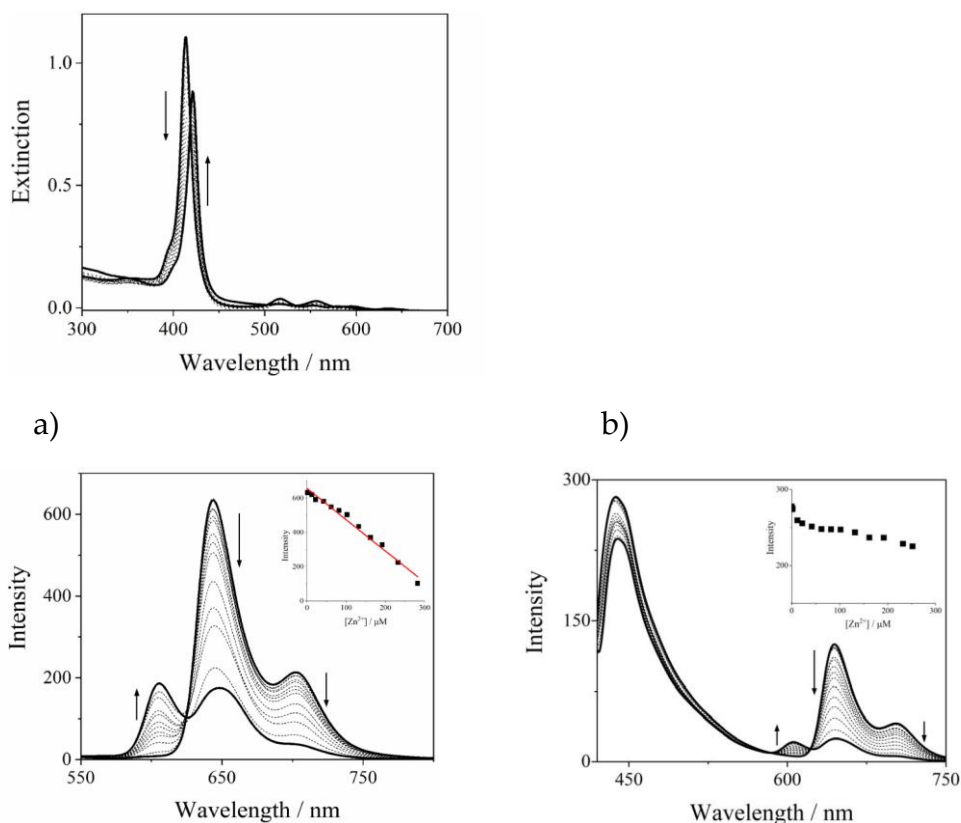
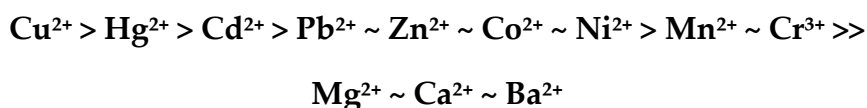


Figure 2.11. UV/Vis (upper panel) and fluorescence spectral changes (lower panels) of GQDs@TPPS₄⁴⁻ during titration with Zn²⁺ ions at neutral pH. The upper panel shows UV/Vis absorbance changes, while the lower panel a) shows fluorescence emission spectra recorded upon excitation upon excitation at $\lambda = 418$ nm. The inset in the right panel displays the corresponding fluorescence intensity changes at 645 nm, from which a LoD of 1.87 μ M is determined (red line represents the linear fit; arrow indicates increasing metal ion concentration). To better visualize the porphyrin emission, fluorescence spectra were also recorded at $\lambda = 380$ nm (b, lower panel), with the inset showing the emission trend at 435 nm, corrected for the filter effect induced by the absorption of the porphyrin metal derivative. Experimental conditions: [TPPS₄⁴⁻] = 3 μ M; [GQDs] = 0.018 mg/mL; [Zn²⁺] = 0–280 μ M; PBS 1 mM, pH 7; T = 298 K; path length = 1 cm

- Efficiency of Metal Ion Coordination on GQDs@TPPS₄⁴⁻ system

To test the selectivity of the developed method, experiments were performed using different metal cations, many of which are of environmental or biological interest. The data obtained, with a constant concentration of each ion equal to 4 μ M, showed that the efficiency of fluorescence quenching strongly depends on the type of cation (Figure 2.12), following the order:



In general, the ease of formation and stability of metalloporphyrins vary according to the size of the metal, with the following trend: medium-sized metals > large metals > alkali metals.^{7,9} In this context, the position of some cations appears unusual compared to what would be expected. Larger, less polarizing ions, such as alkaline earth metals, show a less pronounced quenching effect. This phenomenon can be explained by their lower affinity for the porphyrin coordination site, according to the Irving–Williams series¹⁰ which leads to less stable bonds and weaker complexes. Intermediate-sized ions, such as Cu^{2+} and Zn^{2+} , tend to coordinate in a 1:1 ratio with porphyrin, involving all four nitrogen atoms and fitting perfectly into the center of the ring.^{11,12} This interaction promotes metalation and causes significant changes in dye fluorescence, including the possibility of quenching. Conversely, larger cations such as Cd^{2+} and Hg^{2+} cannot fit completely into the porphyrin ring and are arranged on top of it, forming so-called “sitting-atop (SAT)” complexes.^{13–15}

Since porphyrin metalation is generally a slow process due to the rigidity of the ring, the effect observed with these ions, as well as their position in the quenching sequence, can be attributed to the additional deformation due to the formation of the SAT complex, which adds to the interaction already present between TPPS_4^{4-} and GQDs.

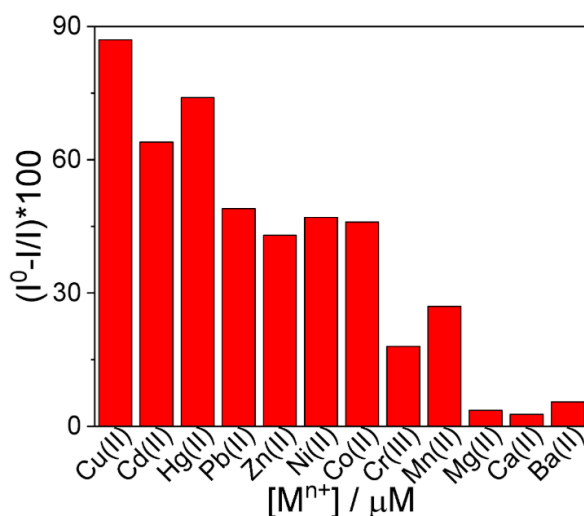


Figure 2.12. Fluorescence emission quenching efficiency of the GQDs@TPPS₄⁴⁻ system induced by the interaction with various divalent cations. All measurements were corrected for the presence of the zinc(II) derivative. Experimental conditions: [TPPS₄⁴⁻] = 3 μM; [GQDs] = 0.018 mg/mL; [Zn²⁺] = 30 μM; [M²⁺] = 4 μM; PBS 1 mM, pH 7; T = 298 K; path length = 1 cm; λ_{exc} = 418 nm; λ_{em} = 645 nm.

Experimental part

Materials

Graphene quantum dots (GQDs) were synthesized by microwave-assisted pyrolysis using a CEM Discover Synthesizer (Matthews, NC, USA), following a procedure previously reported in the chapter 1.

All reagents were of the highest commercially available purity ($\geq 99\%$) and were used as received. meso-Tetrakis(4-sulfonatophenyl)porphyrin (TPPS₄⁴⁻), in its sodium salt form, was obtained from Frontier Scientific (Halstenbek, Germany). L-Glutamic acid, triethylenetetramine tetrahydrochloride, sodium dihydrogen phosphate, and sodium hydrogen phosphate were purchased from Sigma–Aldrich (Milan, Italy). Zinc sulfate hexahydrate, mercury(II) chloride, cadmium chloride, manganese(II) sulfate monohydrate, cobalt(II) chloride hexahydrate, magnesium chloride hexahydrate, calcium chloride dihydrate, barium sulfate, lead(II) nitrate, and nickel(II) chloride hexahydrate were supplied by VWR International (Milan, Italy). Aqueous stock solutions of the porphyrin and the metal ions were prepared by dissolving the corresponding solids in high-purity doubly distilled water (HPLC grade, Fluka, Milan, Italy). TPPS₄⁴⁻ stock solutions ($\sim 200 \mu\text{M}$) were freshly prepared, protected from light to prevent photodegradation, and used within one week. The concentration of free TPPS₄ was determined by UV–Vis spectroscopy using an extinction coefficient of $5.33 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ at 414 nm

Conclusions

The supramolecular adduct GQDs@TPPS₄⁴⁻ can be suitably designed to exhibit fluorescence emission from both constituent fluorophores, namely graphene quantum dots (GQDs) and tetra(4-sulfonatophenyl)porphyrin (TPPS₄⁴⁻). In theory, this dual emission response could enable the creation of a ratiometric sensor for the detection of metal ions. However, although the currently available nanosystem shows the ability to interact selectively with different metal species of analytical relevance, the partial quenching of the emission associated with GQDs compromises direct ratiometric analysis, making it necessary to post process the spectroscopic data in order to compensate for the effects of the filter effect. Furthermore, the presence of GQDs has been shown to increase the rate of insertion of metal ions into the porphyrin macrocycle, with a particularly marked effect in the case of ions with a larger ionic radius, such as Hg²⁺ and Cd²⁺. These latter tend to form “sitting-atop” complexes, which promote and catalyze the incorporation of Zn²⁺ ions into the porphyrin. This behavior suggests the possibility of developing a highly sensitive sensor system based on the formation of the Zn(II)TPPS₄⁴⁻ complex, potentially capable of selectively detecting certain metal ions even at submicromolar concentrations.

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Chapter 3

Electrochemical determination of heavy metals by a graphene quantum dots/porphyrin based supramolecular system.¹

As described in Chapter 2, the supramolecular system obtained from the electrostatic interaction between cationic quantum dots and the anionic meso-tetrakis(4-sulfonatophenyl) porphyrin (GQDs@TPPS₄⁴⁻) has proven to be an effective optical sensor for the detection of metal cations. Under these conditions, the fluorescence emission of GQDs remains essentially unchanged, even if a certain degree of experimental artifacts occur, while that of porphyrin is widely modulated according to the metal cation present.²

Numerous hybrid supramolecular systems based on porphyrins have been designed and analyzed, involving different materials such as carbon nanostructures^{3,4} and polymers.^{5,6} Among these, GQDs are excellent components for promoting the assembly of nanosystems with porphyrins for electrochemical sensing, particularly for applications in environmental monitoring.⁶⁻⁹

Based on these results, we then wondered whether the same nanostructured hybrid system could also function as an electrochemical sensor. To this end, some GQDs@TPPS₄⁴⁻ assemblies were prepared by varying the GQDs:TPPS₄⁴⁻ weight ratio between 1:3 and 1:9. Commercially available screen-printed carbon electrode (SPCE) platforms were then

modified by simple drop-casting of solutions containing the supramolecular adducts.

3.1 Characterization of GQDs@TPPS₄⁴⁻/SPCE electrode

Before evaluating the performance of GQDs@TPPS₄⁴⁻/SPCE sensors in heavy metal detection, their surface and electrochemical properties were analyzed. To this end, an ATR-FTIR analysis was performed to identify the functional groups present on the working electrode after modification of the SPCEs with the supramolecular adduct GQDs@TPPS₄⁴⁻. For direct comparison, unmodified electrodes and those coated exclusively with TPPS₄⁴⁻ porphyrin were also characterized.

The ATR-FTIR (Figure 3.1) spectrum of the TPPS₄⁴⁻ layer immobilized on the electrode surface shows typical vibrational signals, in accordance with what is reported in the literature.¹⁰ Two bands are observed at approximately 1600 and 1420 cm⁻¹, generally associated with C=C skeletal vibrations of the aromatic framework and with C–C stretching modes. These signals may also involve in-plane deformation (δ) modes of the aromatic C–H bonds and vibrational contributions from the meso-phenyl substituents. It should be noted that C–H stretching vibrations are typically observed in the 2850–3100 cm⁻¹ region. The presence of sulfonate groups is evidenced by peaks at 1151 and 980 cm⁻¹, attributable to the asymmetric and symmetric stretching vibrations of the OSO units, respectively. The spectrum obtained for GQDs@TPPS₄⁴⁻ deposited on SPCEs retains all the main spectroscopic characteristics of porphyrin observed previously. In addition, a broad and marked band appears

around 3400 cm^{-1} , typically attributed to the N–H stretching vibrations of the amino groups present on the GQDs capping.¹¹ In this same region, however, O–H vibrations due to possible traces of adsorbed water may also contribute, which can partially overlap with the band of the amino groups. There is also a broad signal extending between 1560 and 1730 cm^{-1} , assignable to the combination of C=O stretching vibrations and amide vibrational modes, in agreement with previous studies.¹¹

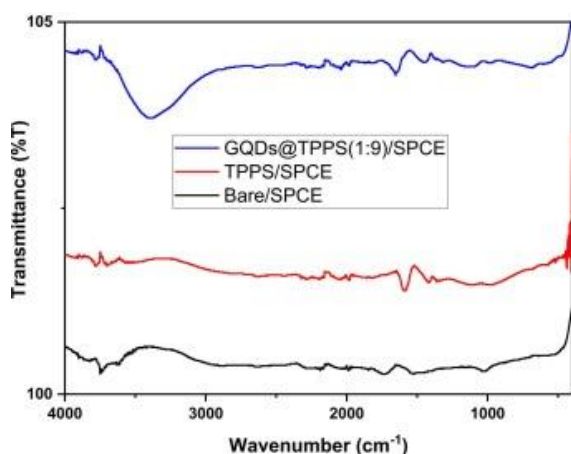


Figure 3.1. FT-IR spectra analysis of bare/SPCE (black line), TPPS₄⁴⁻/SPCE (red line) and GQDs@TPPS₄⁴⁻(1:9) /SPCE (blue line).

The electrochemical properties of the GQDs@TPPS₄⁴⁻ (1:y)/SPCE sensors were examined using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Figures 3.2 show the cyclic voltammograms obtained at a scan rate of 0.05 V/s , in the potential range between -0.2 V and 0.6 V , comparing the bare electrode with those modified with GQDs@TPPS₄⁴⁻, using a 5 mM ferrocyanide solution in PBS as the redox probe.

Analysis of the data shows a significant increase in the faradic current for the GQDs@TPPS₄⁴⁻(1:9)/SPCE electrode compared to the other conditions, highlighting an improvement in electron transfer kinetics.

Further information on the reversibility of the electrochemical process is obtained from the separation between the anodic and cathodic peak potentials ($\Delta E_p = E_{pa} - E_{pc}$). The lowest ΔE_p value, equal to 0.08 V, observed for the GQDs@TPPS₄⁴⁻ (1:9)/SPCE configuration, indicates the highest electron transfer rate at the electrode/solution interface, confirming the effectiveness of the hybrid material in facilitating the redox process.

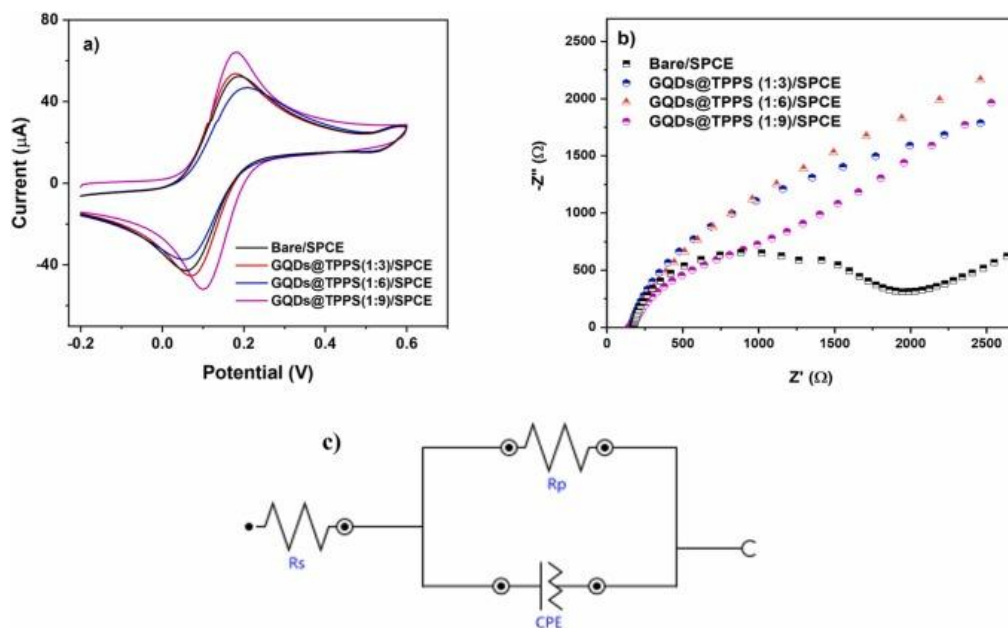


Figure 3.2. (a) CV curves, (b) EIS spectra of unmodified and modified GQDs@TPPS₄⁴⁻/SPCE electrodes in the presence of 5 mM [Fe(CN)₆]^{3/4-} and, (c) equivalent circuit of GQDs@TPPS₄⁴⁻(1:9)/SPCE.

The electrochemical response of the unmodified electrodes and those functionalized with GQDs@TPPS₄⁴⁻/SPCE were also analyzed using electrochemical impedance spectroscopy (EIS), applying a potential of

$E_{app} = 0.15$ V. The Nyquist diagrams obtained were processed using Nova software to identify the most appropriate equivalent circuit and derive the parameters of the Randles model.¹²

As is well known, the diameter of the semicircle in the high-frequency region, associated with charge transfer resistance (R_{CT}), is a direct indicator of the efficiency of the electron transfer process. Among all the configurations analyzed, the GQDs@TPPS₄⁴⁻ (1:9)/SPCE electrode shows the lowest Polarization Resistance (R_p value 2246 Ω), highlighting a significantly reduced charge transfer resistance. This result confirms that this surface modification more effectively promotes electron transfer at the electrode-electrolyte interface.

3.2 Influence of pH and ratio of GQDs:TPPS₄⁴⁻/SPCE for metal ions sensing:

- pH

The influence of both the type of metal ion and the pH of the solution (varying between 4 and 9) on the detection performance of the system was examined. Among the conditions tested, pH = 4 in 100 mM PBS buffer proved to be the most favorable, providing the highest electroanalytical response for ions such as Cu²⁺ and Cd²⁺.

To investigate this aspect further, the concentration of the cupric ion (4 μ M) was kept constant and the responses of the GQDs@TPPS₄⁴⁻/SPCE sensor were recorded as the pH varied. The results obtained in Figure 3.3 showed that the system has maximum detection efficiency at pH = 4,

confirming this value as the optimal condition for electrochemical measurements.^{13,14}

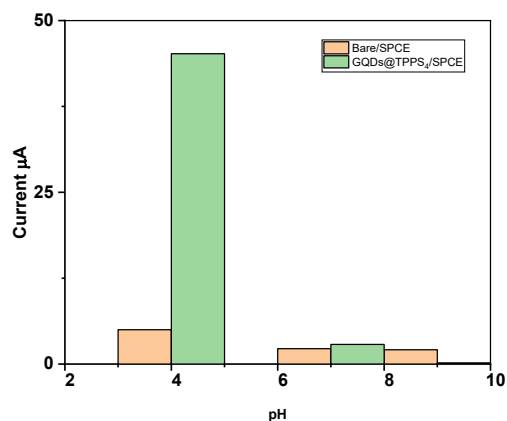


Figure 3.3. Bare/SPCE and GQDs@TPPS₄⁻/SPCE toward 4 μM of Cu²⁺ as function of pH (in the range 4 - 9). Experimental conditions: $t_{\text{dep}}=180$ s, $V_{\text{dep}}=-1$ V and scan rate (S_{rate})= 0.05 V.

The aim was therefore to verify whether this pH condition could also influence the supramolecular interaction underlying the system, conducting a spectroscopic titration of GQDs towards a solution of TPPS₄⁴⁻ 3 μM at pH 4.

In an aqueous environment at pH 4, porphyrin is partially protonated and shows a characteristic shoulder in the UV-Vis spectra attributable to the di-acid form. The UV-Vis spectrum of TPPS₄⁴⁻ (Figure 3.4 (a)) shows two main bands in the Soret region: an intense band at 434 nm, attributed to the di-acid species, and a shoulder at 414 nm, related to the residual free base in solution. The progressive addition of GQDs induces a marked hypochromic effect accompanied by a hypsochromic shift at 421 nm, similar to that reported for GQDs@TPPS₄⁴⁻ interaction at neutral pH,¹¹

highlighting the interaction between TPPS₄⁴⁻ and GQDs and suggesting a change in the electronic structure of the porphyrin. At the same time, an increase in absorption at 330 nm is observed, typical of GQDs and attributable to π - π^* transitions of the sp² domains.

Spectrofluorimetric titrations conducted by exciting at the isosbestic point further confirm this behavior. The increase in GQDs concentration causes a progressive and almost complete extinction of TPPS₄⁴⁻ emission at wavelengths of 645 and 707 nm, accompanied by an increase in emission at 480 nm, characteristic of GQDs. This trend, in agreement with what has been observed under neutral and acidic conditions,¹¹ is compatible with the simultaneous presence of static and dynamic quenching mechanisms, as suggested by the deviation from linearity in the Stern-Volmer analysis.(Figure 3.4 (b)). Overall, all the spectroscopic evidence collected supports the formation of supramolecular adducts between protonated TPPS₄⁴⁻ and GQDs even at pH 4, confirming the robustness of the GQD@TPPS₄⁴⁻ system as an effective hybrid platform, both optically and electrochemically, for the detection of metal ions.

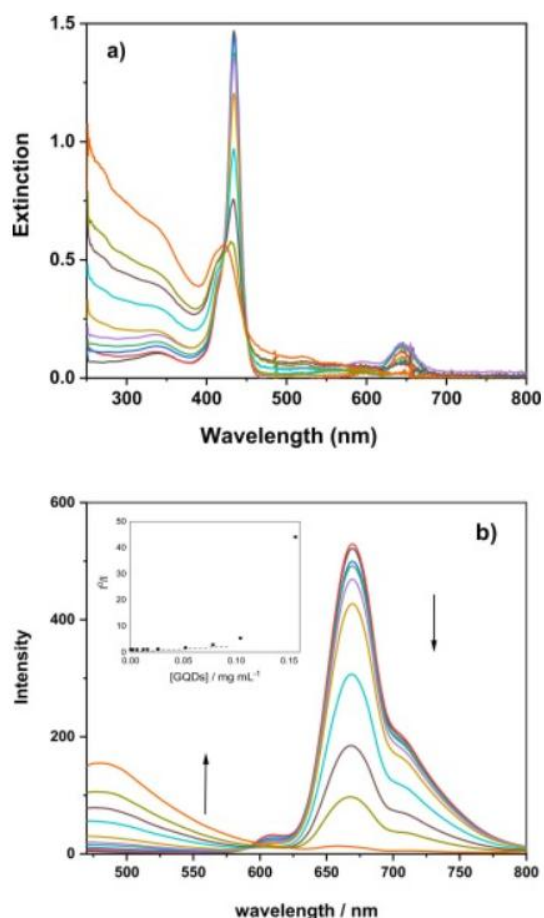


Figure 3.4. (a) UV/Vis extinction spectral changes and (b) fluorescence emission spectral changes during the titration of $\text{H}_2\text{TPPS}_4^{2-}$ with GQDs in phosphate buffer at pH = 4. Experimental conditions: $[\text{TPPS}_4^{4-}] = 3 \mu\text{M}$; $[\text{GQDs}] = 0 - 0.16 \text{ mg/mL}$; phosphate buffer 100 mM, pH = 4; $T = 298 \text{ K}$; cell path length 1 cm; excitation wavelength 425 nm (isosbestic point in the UV/Vis titration).

- Different Ratio (GQDs : TPPS_4^{4-})/SPCE

In order to develop electrochemical sensors for monitoring heavy metals, the performance of both modified and unmodified electrodes was investigated with respect to four different divalent metal ions (M^{2+}): cadmium (Cd^{2+}), copper (Cu^{2+}), lead (Pb^{2+}), and nickel (Ni^{2+}). The

measurements were performed by square wave voltammetry (SWV) using experimentally optimized parameters (frequency = 10 Hz, $E_{\text{step}} = 0.016$ V, $E_{\text{amp}} = 0.025$ V), and evaluating the influence of the different GQDs:TPPS₄⁴⁻ ratios used in the electrode modification.

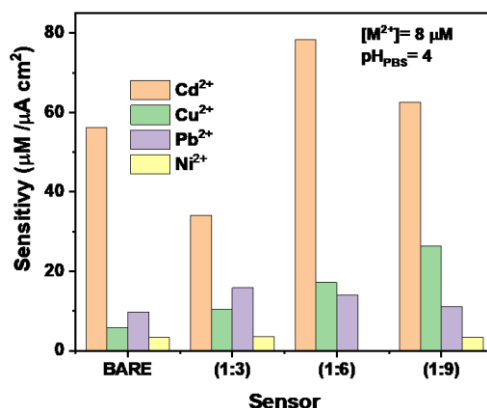


Figure 3.5. Electroanalytical performance toward four different heavy metal ions ($[M^{2+}] = 8 \mu\text{M}$) ions on bare and GQDs@TPPS₄⁴⁻ (1:y)/SPCE electrodes in PBS at pH = 4.

As shown in Figure 3.5, all electrodes exhibit different electroanalytical responses depending on the type of surface modification and the GQDs:TPPS₄⁴⁻ ratio used in the composite. In particular, it can be observed that an increase in the amount of TPPS₄⁴⁻ leads to a significant improvement in sensitivity towards Cd²⁺ and Cu²⁺ ions. The GQDs@TPPS₄⁴⁻ (1:6)/SPCE electrodes have a sensitivity to Cd²⁺ approximately 1.4 times higher than the unmodified SPCE, while GQDs@TPPS₄⁴⁻ (1:9)/SPCE show the best response to Cu²⁺, with a 4.5 fold increase in sensitivity compared to the reference SPCE. Regarding Ni²⁺ and Pb²⁺, the sensitivity of both modified and unmodified electrodes

remains substantially unchanged regardless of the GQDs:TPPS ratio. For this reason, Ni^{2+} and Pb^{2+} were not further investigated in subsequent analytical studies.

Based on these results, the modified GQDs@TPPS₄⁴⁻ (1:6)/SPCE and GQDs@TPPS₄⁴⁻ (1:9)/SPCE electrodes were selected for subsequent quantitative analyses of Cd^{2+} and Cu^{2+} , respectively.

Both selected electrodes were subjected to a scan rate study in a 5 mM ferrocyanide solution, in the potential range between -0.2 V and +0.6 V. In the scan rate range from 0.025 to 0.09 V/s, an increase in Faradaic current is observed, proportional to the square root of the scan rate. The linear relationship obtained (Figure 3.6) confirms that the electrode process is mainly governed by a diffusion-controlled mechanism.

The active surface area (A) of each electrode was then determined using the Randles–Ševčík equation, based on the experimental data obtained.¹⁵

$$I_{\text{pa}}(\text{A}) = 2.69 \times 10^5 \text{ A D}^{1/2} \text{ n}^{3/2} \text{ v}^{1/2} \text{ C}$$

where n denotes the number of electrons involved in the reaction, C is the concentration (mol/cm³), v is the scan rate, and D is the diffusion coefficient equal to $7.6 \times 10^6 \text{ cm}^2/\text{s}$.

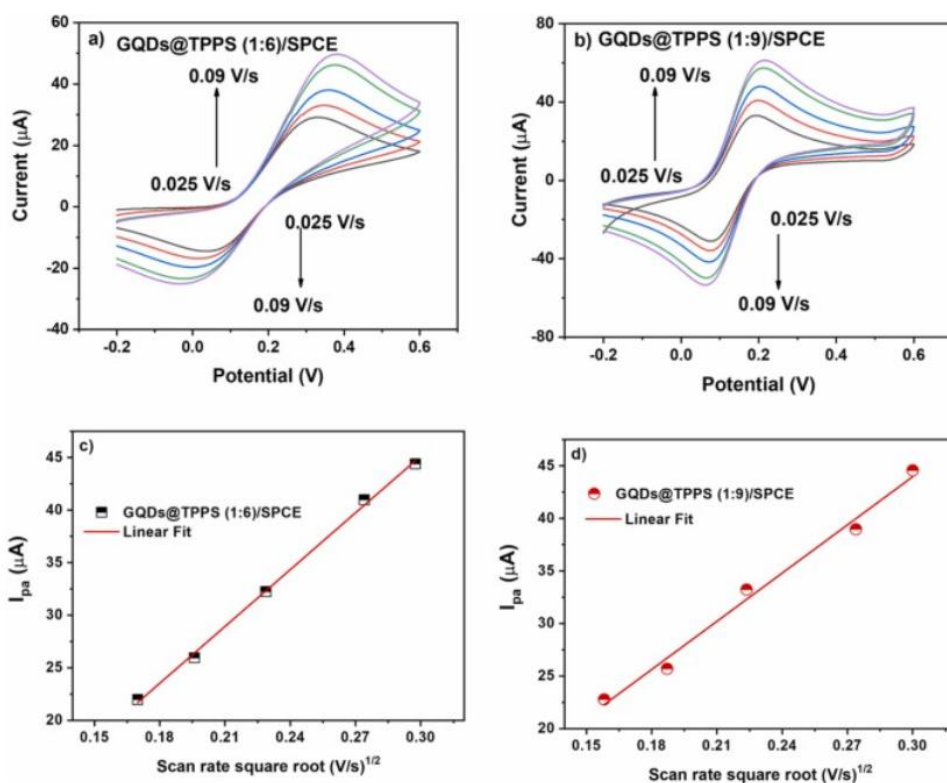


Figure 3.6. Scan rate study on (a) GQDs@TPPS $_4^{4-}$ (1:6)/SPCE and (b) GQDs@TPPS $_4^{4-}$ (1:9)/SPCE; (c,d) linear fit of the scan rate square root vs current peak.

3.3. Determination of Cd^{2+} and Cu^{2+}

Based on preliminary performance, SWV measurements were conducted in PBS buffer (pH = 4) containing increasing concentrations of Cd^{2+} and Cu^{2+} between 3 and 13 μM . The corresponding voltammograms are shown in Figure 3.7.

Both modified electrodes show a significant improvement in analytical response compared to the bare SPCE, with higher peak currents and clear separation of redox signals. Furthermore, a shift of the peak potential towards more positive values is observed, indicating that the presence of

the GQDs@TPPS₄⁴⁻ film facilitates the electrochemical oxidation of Cd²⁺ and Cu²⁺, requiring a lower potential than the unmodified electrode.

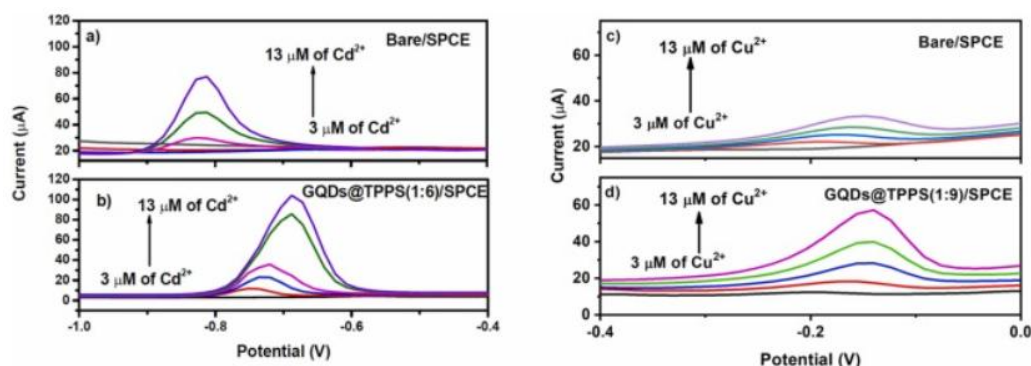


Figure 3.7. SWV curves of (a) bare/SPCE, (b) GQDs@TPPS₄⁴⁻ (1:6)/SPCE for the determination of Cd²⁺, and (c) bare/SPCE, (d) GQDs@TPPS₄⁴⁻ (1:9)/SPCE for the determination of Cu²⁺. Experimental conditions: PBS buffer at pH 4; $t_{\text{dep}} = 180$ s, $V_{\text{dep}} = -1$ V, and $S_{\text{rate}} = 0.05$ V/s.

The calibration curves obtained for the modified and unmodified electrodes show two distinct linearity ranges, one at low concentrations and one at high concentrations. Compared to untreated SPCE, GQDs@TPPS₄⁴⁻/SPCE electrodes exhibit a significant increase in sensitivity: approximately four times greater in the range between 0 and 8 μM and up to five times greater between 6 and 13 μM, for the measurement of Cd²⁺ and Cu²⁺, respectively.

The limit of detection (LoD) was estimated using the relationship $\text{LoD} = 3.3 \cdot \text{SD} / \alpha$, where SD represents the standard deviation and α the slope of the calibration line. Applying this equation, the LoD obtained for

GQDs@TPPS₄⁴⁻ (1:9)/SPCE is 0.172 μM , which reflects an improvement of about 1.5 times compared to the unmodified SPCE.

An even more marked increase, approximately fourfold, was observed when comparing the LoD of GQDs@TPPS₄⁴⁻ (1:6)/SPCE (0.436 μM) with that of the unmodified electrode.

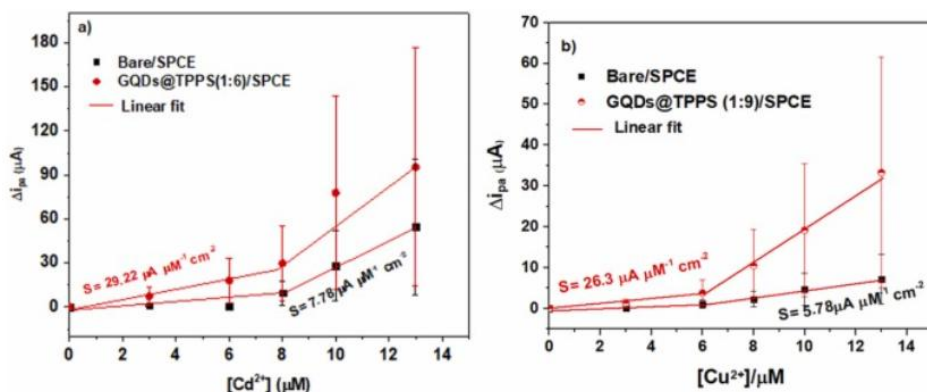


Figure 3.8. Calibration curve from SWV data of: (a) bare SPCE and GQDs@TPPS₄⁴⁻ (1:6)/SPCE for the determination of Cd²⁺; (b) bare SPCE and GQDs@TPPS₄⁴⁻ (1:9)/SPCE for the determination of Cu²⁺. Experimental conditions: PBS buffer 100 mM at pH 4; $t_{\text{dep}} = 180$ s, $V_{\text{dep}} = -1$ V, and $S_{\text{rate}} = 0.05$ V/s.

Table 3.1. Comparison between these systems and literature.

Metal ions	Sensing layer	Sensitivity	LoD	Ref
Cd²⁺	N-GQD	N.M	1.0×10^{-5} ppb	16
	MgFe ₂ O ₄ /reduced graphene oxide (rGO)	0.0172 $\mu\text{A nM}^{-1}$	0.2 nM	17
	RGO-CS/PLL/GCE	N.M	$0.01 \mu\text{g L}^{-1}$	18
	GQDs@TPPS ₄ ⁴⁻ (1:6)	μA $\mu\text{M}^{-1} \text{ cm}^{-2}$	$0.436 \mu\text{M}$	This system
Cu²⁺	RGO-CS/PLL/GCE	N.M	$0.02 \mu\text{g L}^{-1}$	18
	Layered graphene nanostructures	N.M	$0.35 \mu\text{M}$	19
	GQDs@TPPS ₄ ⁴⁻ (1:9)	μA $\mu\text{M}^{-1} \text{ cm}^{-2}$	$0.172 \mu\text{M}$	This system

N.M: not mentioned.

3.4 Stability and interference of GQDs@TPPS₄⁴⁻/SPCE electrode

Signal reproducibility, stability over time, and possible interference between ionic species are fundamental aspects for evaluating the practical reliability of an electrochemical sensor. The stability of the devices was monitored. As shown, both GQDs@TPPS₄⁴⁻/SPCE modified electrode configurations maintain reasonable stability even 100 days after the initial test. For a more detailed quantitative analysis, Table 3.2, that collects the numerical stability values measured on the same electrode at regular time intervals.

Table 3.2. Stability analysis of GQDs@TPPS₄⁴⁻ based sensors (1:6 and 1:9 ratios) in the presence of 13 μM of M^{2+} during a period of 100 days.

	time day	/ Current intensity/ μA	Relative stability %*	Analyte
GQDs@TPPS₄⁴⁻ (1:6)/SPCE	0	96	-	Cd^{2+}
	45	56	58	
	100	70	61	
GQDs@TPPS₄⁴⁻ (1:9)/SPCE	0	32	-	Cu^{2+}
	45	14	44	
	100	10	31	

*Calculated as percentage referred to the initial value ($t = 0$).

The interference of Cd^{2+} in the detection of Cu^{2+} was also evaluated using the GQDs@TPPS₄⁴⁻ (1:9)/SPCE sensor. The results, shown in Figure 3.9, show that for Cu^{2+} at 8 μM , an oxidation peak appears at -0.13 V . When Cd^{2+} is added at the same concentration, the peak potential for copper shifts to -0.21 V , but without significantly affecting the Cu^{2+} signal. The opposite situation was observed in the determination of Cd^{2+} with the GQDs@TPPS₄⁴⁻ (1:6)/SPCE sensor: in this case, the presence of Cu^{2+} causes a substantial attenuation of the cadmium peak.

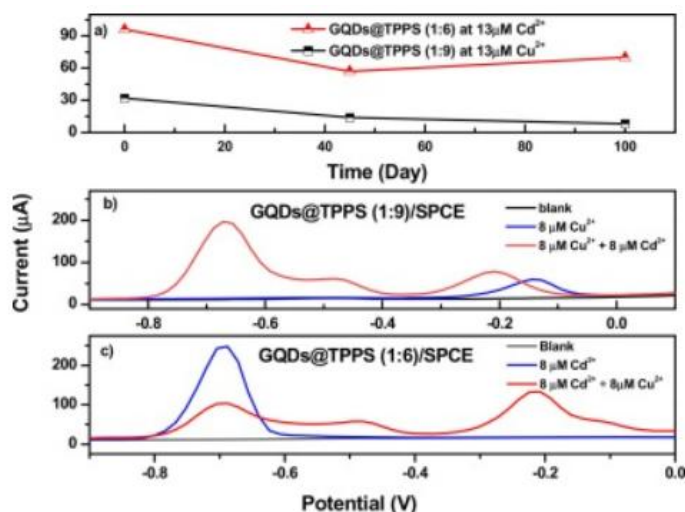


Figure 3.9. (a) Stability tests of both GQDs@TPPS₄⁴⁻ (1:6)/SPCE and GQDs@TPPS₄⁴⁻ (1:9)/SPCE at 13 μM of M²⁺. (b) for the determination of Cu²⁺, and (c) Cd²⁺. Experimental conditions: $t_{\text{dep}}=180$ s, $V_{\text{dep}}=-1$ V, and $S_{\text{rate}}=0.05$ V/s.

These results clearly indicate that the GQDs@TPPS₄⁴⁻/SPCE system has a high selectivity for Cu²⁺. This behavior is consistent with what is reported in the literature,²⁰ which describes a well-defined affinity sequence for the incorporation of metal ions into porphyrin at pH 4: Cu(II) > Zn(II) > Co(II) > Mn(II) > Ni(II). Based on the experimental data and references reported, it is plausible to assume that the porphyrin ligand complexed with Cu²⁺ is sufficiently stable to prevent the replacement of the copper ion with Cd²⁺.

The high preference for Cu²⁺ can be explained by considering the factors that regulate the chemical affinity between GQDs@TPPS₄⁴⁻ and this metal ion. In particular, the nitrogen sites of TPPS₄⁴⁻ can act as coordinative bond donors, forming particularly stable complexes with Cu²⁺. The typical coordination geometry of copper(II), combined with its smaller ionic

radius compared to Cd^{2+} , facilitates the entry of the ion into the porphyrin cavity and promotes greater stabilization of the complex. These considerations suggest that combining the porphyrin ligand with GQDs is a promising strategy for designing highly sensitive and selective Cu^{2+} sensors.

3.5 Determination of heavy metals in real sample

The good performance of the GQDs@TPPS₄⁴⁻/SPCE electrodes allowed us to verify their effectiveness in analyzing heavy metals in a real seawater sample taken in Messina (Italy) and used as is, without any preliminary treatment. Figure 3.10 show the results obtained for Cd^{2+} and Cu^{2+} in the same sample. As expected, under seawater operating conditions, the electrochemical signals are significantly lower than those recorded under optimal conditions at pH 4.5. However, the response for both ions is comparable to that observed in PBS buffer with a pH between 6 and 9, indicating that the matrix effect of seawater does not significantly compromise the performance of the sensor.

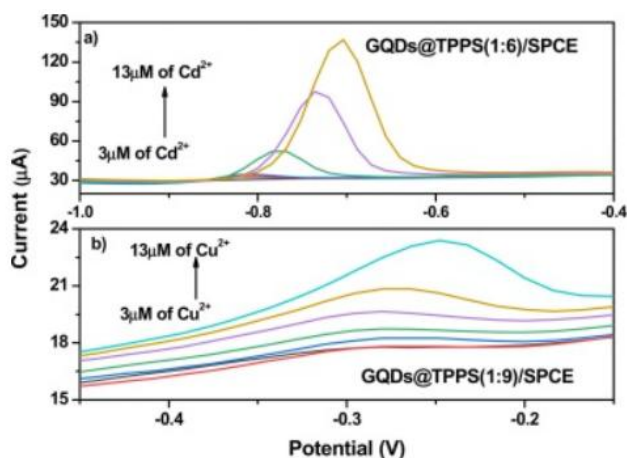


Figure 3.10. (a) Cd²⁺ determination on GQDs@TPPS₄⁴⁻ (1:6)/SPCE and (b) Cu²⁺ determination on GQDs@TPPS₄(1:9)/SPCE in seawater.

Experimental part

Materials

TPPS₄⁴⁻ solutions were prepared by dissolving 5,10,15,20-tetrakis(4-sulfophenyl)porphyrin (Na₄(H₂TPPS₄⁴⁻)·12H₂O, Sigma–Aldrich) in ultrapure water. Graphene quantum dots (GQDs) were synthesized using L-glutamic acid and triethylenetetramine, both of analytical grade and purchased from Sigma–Aldrich. Stock solutions of cadmium (Cd(NO₃)₂·4H₂O), copper (Cu(NO₃)₂), nickel (Ni(NO₃)₂·6H₂O), and lead (Pb(NO₃)₂), as well as sodium dihydrogen phosphate and sodium hydrogen phosphate, were prepared by dissolution in distilled water. All chemicals were obtained from Sigma–Aldrich.

GQDs were synthesized via a bottom-up approach, employing glutamic acid as the carbon precursor and triethylenetetramine as the surface passivating agent, in accordance with procedures described in chapter 1.

The GQDs@TPPS₄⁴⁻ supramolecular nano-hybrid system was subsequently obtained through electrostatic interactions between the anionic meso-tetrakis(4-sulfonatophenyl)porphyrin (TPPS₄⁴⁻) and positively charged GQDs.

Conclusions

An electrochemical sensor was developed using the GQDs@TPPS₄⁴⁻ nanohybrid, which showed excellent performance in the detection of heavy metals. SPCE electrodes functionalized with the GQDs@TPPS₄⁴⁻ supramolecular system, prepared in ratios of 1:6 and 1:9, proved particularly effective in the analysis of Cu²⁺ and Cd²⁺ ions. Compared to the unmodified SPCE, the GQDs@TPPS₄⁴⁻ (1:6)/SPCE electrode offers almost five times the sensitivity to Cd²⁺, while the GQDs@TPPS₄⁴⁻ (1:9)/SPCE version shows an approximately four-fold increase in response to Cu²⁺. Selectivity tests also reveal that the presence of Cd²⁺ does not interfere with the measurement of Cu²⁺, highlighting the greater stability of the complexes formed by TPPS₄⁴⁻ with the latter ion. Direct analysis of seawater samples, without the need for preliminary treatment, confirms the high potential of these sensors for environmental monitoring.

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Chapter 4

Non-covalent interaction between a cationic porphyrin and anionic carboxymethylcellulose.

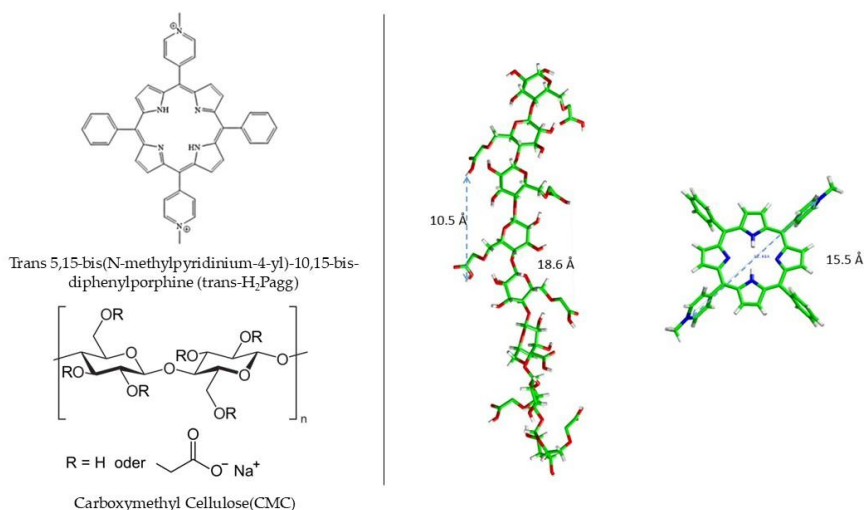
As mentioned in the Introduction, the interaction between porphyrins and polymers or biopolymers is an area of great interest due to the ability of these systems to modulate the formation of H- or J-type supramolecular aggregates of the chromophores.^{1,2}

In particular, the formation of aggregates in the presence of chiral polymers has been widely documented: the marked circular dichroism (CD) signals observed for J-aggregates of anionic tetrakis(4-sulfonatophenyl)porphyrin have been attributed to the presence of traces of optically active species, such as fragments of bacterial membranes even in ultrapure water or to the intentional addition of chiral templates.³

Within this context, water-soluble dicationic porphyrins, such as trans-bis(N-methylpyridinium-4-yl)diphenylporphyrin (t-H₂Pagg) and its copper(II) complex, also play an important role. These molecules have shown a high capacity to form extended supramolecular structures in the presence of nucleic acids⁴ or anionic polymers such as polyglutamate (PGA).^{5,6}

Based on these observations, it is natural to wonder how these porphyrins interact with other negative polymers, in particular carboxymethylcellulose (CMC), and whether this interaction can promote the formation of aggregates organized according to fractal morphologies.

The aim of this chapter is therefore to explore in detail the nature of these interactions and the resulting self-assembly mechanisms and evaluate their response, structural and spectroscopic, to the addition of metal ions, with particular attention to their possible use in sensing applications.



Scheme 4.1. Structure of *trans*-bis(N-methylpyridinium-4-yl)diphenylporphyrin (t-H₂Pagg) and carboxymethyl cellulose (CMC).

In aqueous solution, CMC assumes a helical conformation, stabilized by the regular distribution of carboxylate groups along the polymer chain. As shown in Scheme 4.1, the CH₂COO⁻ groups exhibit a distance of approximately 10.5 Å, when considering three adjacent repeating units, while a larger distance of 18.6 Å corresponds to the structural repetition associated with four repeating units. The dimensions of porphyrin (15.5 Å) are compatible with these periodicities, suggesting that each

macrocycle can interact electrostatically with multiple charged sites of CMC.

This geometric correspondence makes CMC an effective molecular template, capable of imposing a specific spatial organization on porphyrins and promoting their aggregation into ordered structures.

4.1 t-H₂Pagg@CMC supramolecular system

UV/Vis extinction spectra of the t-H₂Pagg porphyrin show a characteristic Soret band at 420 nm, accompanied by four Q bands at 520, 560, 590 and 644 nm.⁷ In order to investigate the interactions between porphyrin and polymers, spectrophotometric titrations were conducted by adding increasing amounts of carboxymethyl cellulose (CMC) to micromolar solutions of t-H₂Pagg in PBS at pH 7. Considering the pH of the solutions, CMC appears to be deprotonated and, consequently, able to interact electrostatically with the positive charges present on porphyrin.

Analysis of UV/Vis extinction spectra revealed significant changes induced by the addition of the polymer. In particular, a hypochromicity of the Soret band at 420 nm is observed, accompanied by the appearance of a new band at 443 nm. This phenomenon suggests the formation of J-type dimers on polymer in solution, a process favored as CMC concentration increases in the micromolar range. At polymer concentrations on the order of millimolar, the absorption spectrum reveals the presence of a new band at 423 nm, attributable to the formation of a new conformational rearrangement. This spectral transition suggests a

different degree of π -stacking interaction between the porphyrins, consistent with a more dispersed pattern on the polymer surface.

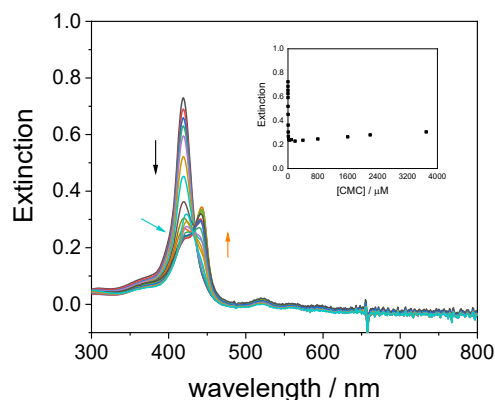


Figure 4.1. UV/Vis extinction spectral changes and CMC at neutral pH. In the inset, extinction (420 nm) as function of CMC concentration. Experimental conditions: [t-H₂Pagg] = 5 μ M; [CMC] = 0–3.7 mM ; phosphate buffer 1 mM, pH = 7; cell path length 1 cm.

Analysis of the RLS spectra shown in Figure 4.2a reveals that at low concentrations, the very small increase in signal at 480 nm indicates weak interactions between porphyrins in the form of small oligomers, probably dimers. As the concentration increases, the decrease in this signal and the concomitant increase in the intensity of the fluorescence bands at 660 and 720 nm (Figure 4.2b) suggest greater separation between porphyrins, favoring light emission.

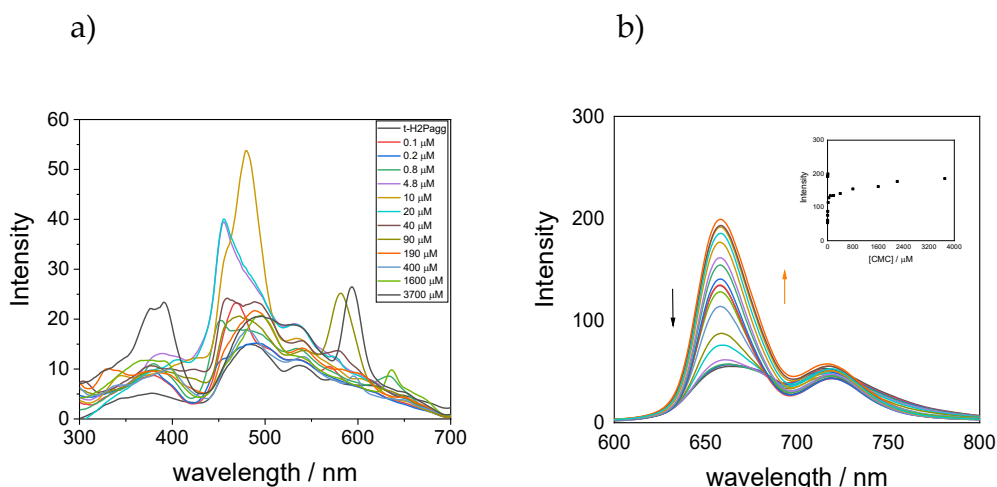


Figure 4.2. RLS (a) and emission spectra (b) of t-H₂Pagg during the titration with CMC at neutral pH. The inset represents the intensity of fluorescence emission at the maximum ($\lambda = 433$ nm), as function of CMC concentration. Experimental conditions: [t-H₂Pagg] = 5 μ M; [CMC] = 0 – 3 mM; [PBS] = 1 mM at pH 7; cell path length 1 cm.

The circular dichroism spectra (Figure 4.3) show a marked dependence on CMC concentration. At low concentrations, an intense negative CD signal couplet is observed, indicative of the formation of porphyrin dimers (oligomers) with defined chirality. As CMC concentration increases, the intensity of the negative CD signal couplet gradually decreases until it reverses, suggesting a progressive conformational rearrangement of these species, leading to a complete inversion of handedness. It is important to note, however, that at millimolar concentrations the positive CD signal couplet begins to decrease in intensity suggesting an initial loss of chirality due to a reduction in the structural order of the aggregates or a dispersion of porphyrins on the polymer surface; this is confirmed in UV/Vis spectra by the total formation of the species at 423 nm and the corresponding RLS and fluorescence signals.

These spectroscopic findings indicate that, at low CMC concentrations, weakly organized aggregates are formed. At intermediate concentrations, the occupation of carboxylate sites becomes optimal, favoring the formation of a well-defined helical architecture, accompanied by intense circular dichroism signals. At higher concentrations, however, variations in the charge⁺/charge⁻ ratio or in the packing of the chains cause a reorganization of the chromophores, which manifests itself in the inversion of the CD signal.

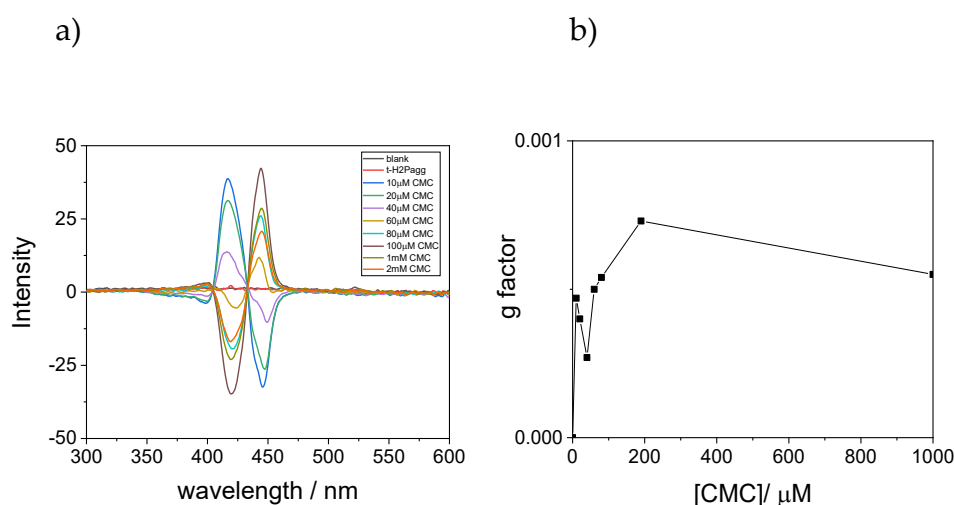


Figure 4.3. (a) CD spectra during titration of t-H₂Pagg with CMC (b) dissymmetry g-factor as a function of CMC concentration. Experimental conditions: [t-H₂Pagg] = 5 μM; [CMC] = 0 – 2 mM; [PBS] = 1 mM at pH 7; cell path length 1 cm.

The investigation of the effect of CMC on the chirality of t-H₂Pagg was extended to conditions of high ionic strength, promoting the formation of DLA aggregate.^{8,9}

Experiments were conducted either by adding 100 μM CMC to preformed DLA aggregates in the presence of 0.1 M NaCl or by reversing the order of reagent addition. In both cases, the presence of DLA aggregates, stabilized by ionic interactions under high ionic strength conditions (0.1 M NaCl), significantly inhibited the interaction between porphyrins and CMC. The CD data confirms the absence of induction of chirality by CMC in DLA aggregates, suggesting that the interactions responsible for aggregate formation are energetically more favorable than those between porphyrin and CMC.

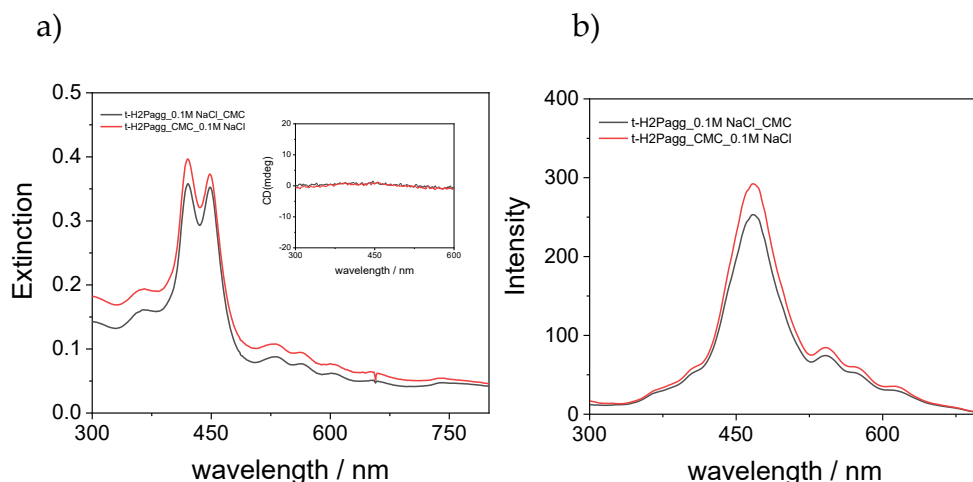


Figure 4.4. UV/Vis extinction (a) and RLS (b) spectra of t-H₂Pagg aggregated with NaCl, with subsequent addition of CMC (black line) and t-H₂Pagg in the presence of CMC, with subsequent addition of NaCl (red line). In the inset, the corresponding final CD spectra of the two samples. Experimental conditions: [t-H₂Pagg] = 5 μM ; [CMC] = 100 μM ; [NaCl] = 0.1 M ; cell path length 1 cm.

Considering the electrostatic interaction between t-H₂Pagg in free base form and CMC, as well as the resulting formation of oligomers, the goal was to evaluate how the introduction of a metal cation into the porphyrin core could influence these interactions and, consequently, the aggregation

processes. In particular, we wanted to understand how the nature of the metal, in terms of coordination number, geometry, and charge modifies the affinity towards CMC and promotes or inhibits the formation of supramolecular aggregates, including oligomers and J-type dimers. To this end, a complete series of metalloporphyrin complexes with different coordination characteristics was synthesized to systematically correlate the properties of the metal center with the observed aggregation behavior.

4.2 Square planar geometry: Cu(II)Pagg@CMC and Ni(II)Pagg@CMC

All metallic derivatives of t-H₂Pagg were prepared as previously reported in the literature.^{10,11}

UV/Vis extinction spectra of micromolar Cu(II)Pagg in pure water shows a B band at 418 nm, along with two Q bands at 545 and 590 nm (Figure 4.5, top) while the UV/Vis spectra of Ni(II)Pagg exhibit a Soret band at 416 nm and two Q bands at 534 and 580 nm (Figure 4.5, bottom).⁵ Increasing amounts of CMC in PBS at pH 7 were added to micromolar solutions of Ni(II)Pagg and Cu(II)Pagg to ensure electrostatic interactions between the negatively charged polymer and the positively charged porphyrins. In the case of the copper derivative, UV/Vis spectra revealed an initial hypochromicity followed by bathochromic shift of the B band to 434 nm ($\Delta\lambda = +16$ nm), likely attributable to aggregates formation, as previously reported in the literature.⁶ The nickel(II) derivative exhibited a considerably reduced hypochromic effect, accompanied by a red shift of the B band from 416 nm to 430 nm.

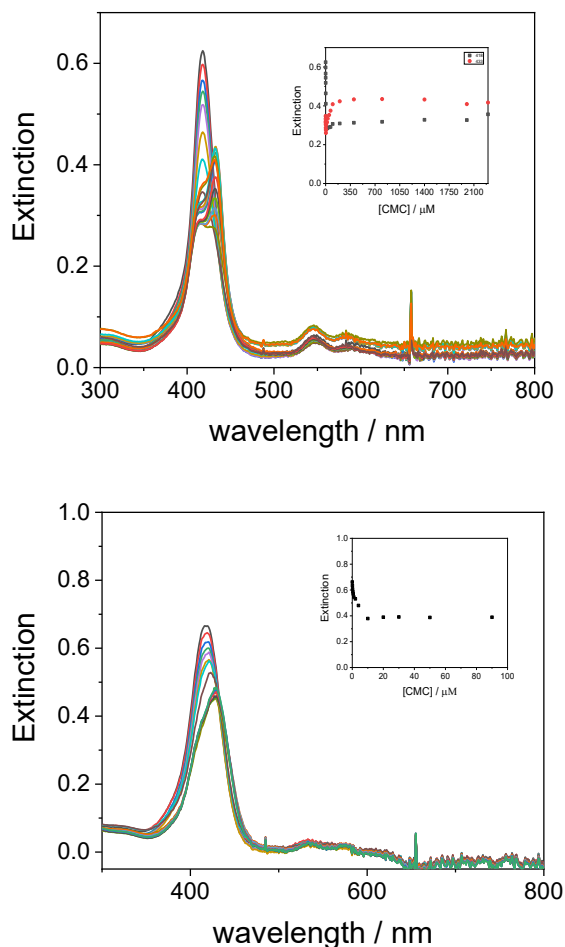


Figure 4.5. UV/Vis extinction spectra of Cu(II)Pagg (top) and of Ni(II)Pagg (bottom) during the titration with CMC at pH 7. The inset represents the extinction of the representative peaks as function of CMC concentration. Experimental conditions: $[M(II)Pagg] = 5 \mu M$; $[CMC] = 0 - 2 \text{ mM}$; $[PBS] = 1 \text{ mM}$ at pH 7; cell path length 1 cm.

RLS spectra revealed a consistent increase of intensity for the signal at 485 nm in the case of CuPagg (Figure 4.6a) and 490 nm for NiPagg (Figure 4.6b) as CMC concentration increased. The experimental findings strongly suggest large aggregates formation for both metal complexes, a process that is favored by the square planar geometry imposed by the metal, thus promoting effective π -stacking interactions between the porphyrin

aromatic rings. Similarly to what observed with t-H₂Pagg, on increasing further the concentration of CMC the RLS intensity drops indicating disaggregation and consequent dispersion of the monomeric porphyrin units onto the surface of the polymer. The maxima of the RLS intensity in the profiles occur close to a porphyrin/CMC ratio 1:2, suggesting that charge neutralization is playing an important role in the formation of the aggregated species.

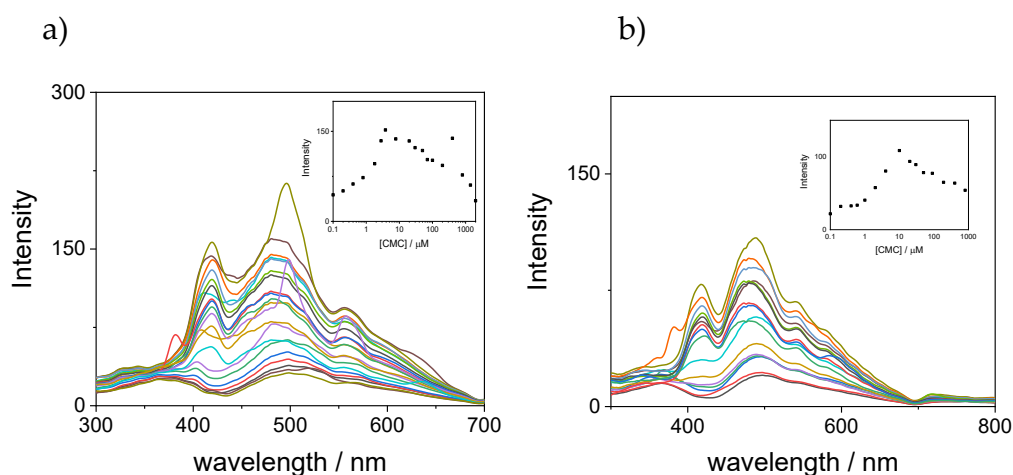


Figure 4.6. RLS spectra of a) Cu(II)Pagg and b) Ni(II)Pagg during the titration with CMC at neutral pH. The insets report the intensity of RLS signal as a function of CMC concentration. Experimental conditions: [M(II)Pagg] = 5 μ M; [CMC] = 0 – 2 mM; [PBS] = 1 mM at pH 7; cell path length 1 cm.

CD analysis revealed distinct spectral profiles for Cu(II)Pagg (Figure 4.7 a-b) and Ni(II)Pagg (Figure 4.7, c). The copper complex showed a complex profile, indicative of the formation of multiple species in equilibrium, with an initial increase in signal intensity as CMC concentration increases.

However, at higher concentrations, a decrease in intensity of the CD bands and the corresponding *g* dissymmetry factor, together with some spectral shifts are observed.

At difference with t-H₂Pagg, where a complete inversion of CD signs is observed, CuPagg aggregates seem to retain their initial handedness, probably due to slight structural rearrangements. Similarly to Cu(II)Pagg, the nickel complex showed a bisignate profile at the highest polymer concentration. The higher propensity of the copper derivative to form dimers, even at low concentration, could explain the complexity of its CD spectrum, suggesting the coexistence of different dimeric species with different spatial orientations.

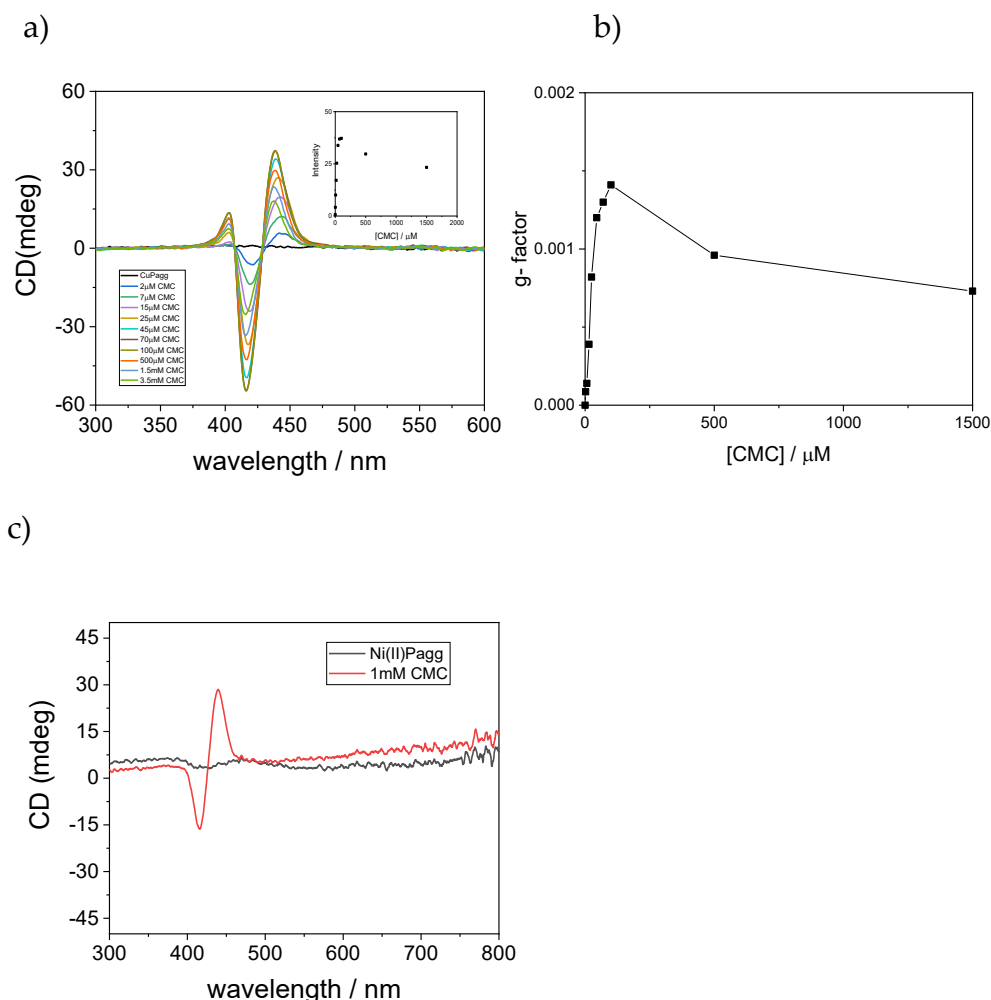
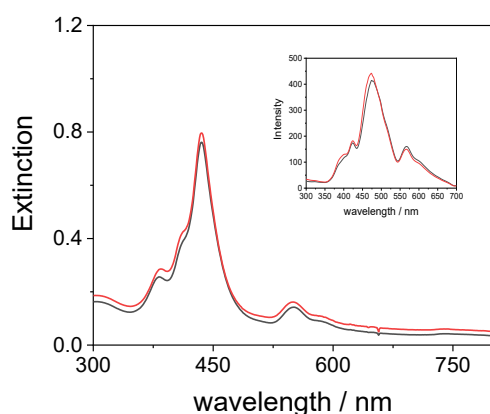


Figure 4.7. CD spectra during titration of Cu(II)Pagg (a), and Ni(II)Pagg (c) with CMC, in the inset the intensity of CD signal in function of CMC concentration b) g factor dissymmetry in function of CMC Experimental conditions: [Cu(II)Pagg] = 5 μ M; [CMC] = 0 – 2 mM; [PBS] = 1 mM at pH 7; cell path length 1 cm. c) CD spectra of Ni(II)Pagg with CMC. Experimental conditions: [Ni(II)Pagg] = 5 μ M; [CMC] = 1 mM; [PBS] = 1 mM at pH 7; cell path length 1 cm.

The presence of NaCl induced the formation of DLA type aggregates in the Cu(II) metal derivatives, consistent with their square planar geometry.

The addition of CMC, regardless of the order of addition, in the case of the Cu(II)Pagg, resulted in the formation of an aggregate species strictly interacting with the polymer, characterized by intense signals, noticeable from the CD spectra. These results suggest a synergism between NaCl and CMC in promoting the growth of fractal structures, with a particular propensity for aggregation, as already reported in the literature for the copper complex.

a)



b)

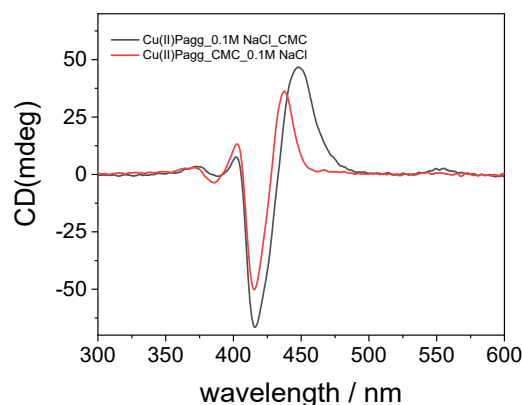


Figure 4.8. a) UV/vis absorption and RLS spectra (inset), and b) CD spectra of Cu(II)Pagg aggregated with NaCl, followed by the addition of CMC (black line), and Cu(II)Pagg with CMC, followed by the addition of NaCl (red line). Experimental conditions: [Cu(II)Pagg] = 5 μ M; [CMC] = 100 μ M; [NaCl] = 0.1 M ; cell path length 1 cm.

4.3 Square pyramidal geometry: Zn(II)Pagg@CMC

The UV/Vis absorption spectrum of the Zn(II)Pagg derivative shows a characteristic Soret band at 430 nm and two Q bands at 565 nm and 615 nm. Analysis of UV/Vis spectra obtained during titration with the polymer revealed hypochromicity of the Soret band at low polymer concentrations, followed by the appearance of a new peak at 434 nm at higher concentrations (Figure 4.9). This phenomenon can be attributed either to the different microenvironment offered by the polymer surface or to the formation of porphyrin dimers on the polymer chain, facilitated by the pentacoordinate geometry of the Zn(II) ion that makes a free face available for π -stacking interactions.

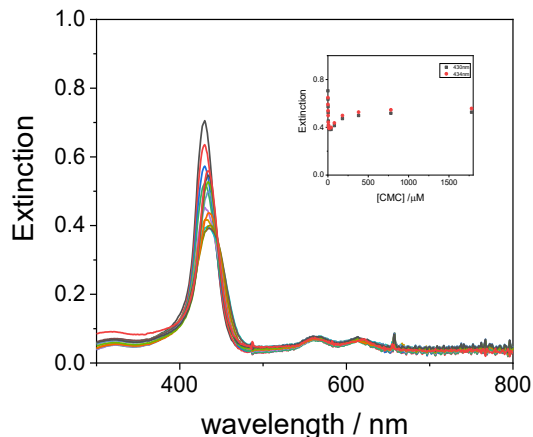


Figure 4.9. UV/Vis extinction spectra of Zn(II)Pagg during titration of CMC. In the inset, the extinction at 430 and 434 nm as function of CMC concentrations. Experimental conditions: [Zn(II)Pagg] = 5 μ M; [CMC] = 0 – 2 mM; [PBS] = 1 mM pH 7 ; cell path length 1 cm

The emission spectra of Zn(II)Pagg shows two bands at 648 nm and 710 nm (Figure 4.10) Gradual addition of CMC induces significant changes in the fluorescence profile. At low polymer concentrations, fluorescence quenching is observed, consistent with the results obtained from UV/Vis extinction measurements. This phenomenon can be attributed to the formation of more or less packed porphyrin dimers, facilitated by π -stacking interactions mediated by the pentacoordinate Zn(II) ion. As the concentration of CMC increases, a blue-shift of 10 nm is observed accompanied by a gradual increase in fluorescence intensity. These results suggest that, at high polymer concentrations, porphyrins arrange more dispersedly on the polymer chain, reducing excitonic interactions and promoting fluorescence emission. This behavior is in line with what has been observed for free base porphyrins, indicating that polymer concentration greatly influences porphyrin organization. The RLS spectra of Zn(II)Pagg show a different behavior than that observed for metal porphyrins with square planar metals (such as Cu(II) and Ni(II)), but similar to that found for t-H₂Pagg. In particular, as the concentration of CMC increases, there is only a slight increase in RLS intensity. This result indicates that dimers or small oligomers of Zn(II)Pagg are formed on the polymer surface, and these aggregates are less stable than the aggregates formed by metal complexes with square planar geometry. The low light scattered intensity of this metal derivative is in line with the RLS theory, that requires a certain amount of coupled chromophores in order to observe intense signals.¹²

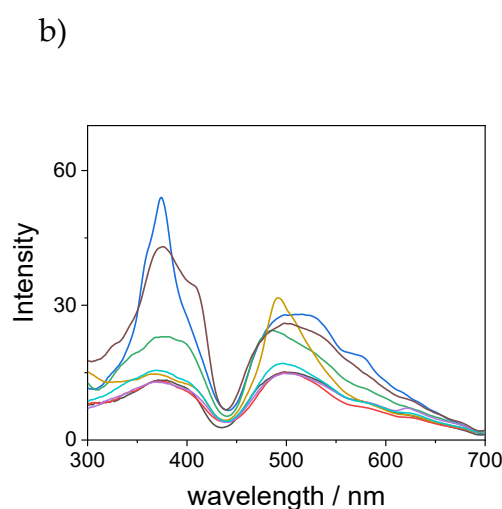
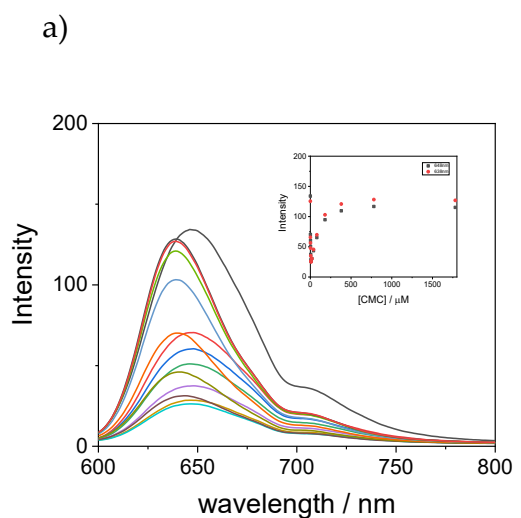


Figure 4.10. (a) Fluorescence spectral recorded using $\lambda = 445$ nm and (b) RLS spectra changes during titration of Zn(II)Pagg with CMC. In the inset, the intensity of the emission maxima as function of CMC concentration is shown. Experimental conditions: [Zn(II)Pagg] = 5 μ M; [CMC] = 0 – 2 mM; [PBS] = 1 mM pH 7; cell path length 1 cm

The CD spectra provide experimental evidence supporting the formation of porphyrin dimers (Figure 4.11, top), whose organization is strongly influenced by the presence of the pentacoordinate Zn(II) ion.

The characteristic bisignated CD signal, with an intensity and a calculated g dissymmetry factor (Figure 4.11, bottom) dependent on the concentration of CMC, suggests an initial formation of the dimers, followed by their loosening and dispersion at higher polymer concentrations. These results are in perfect agreement with the data obtained from light scattering and fluorescence measurements.

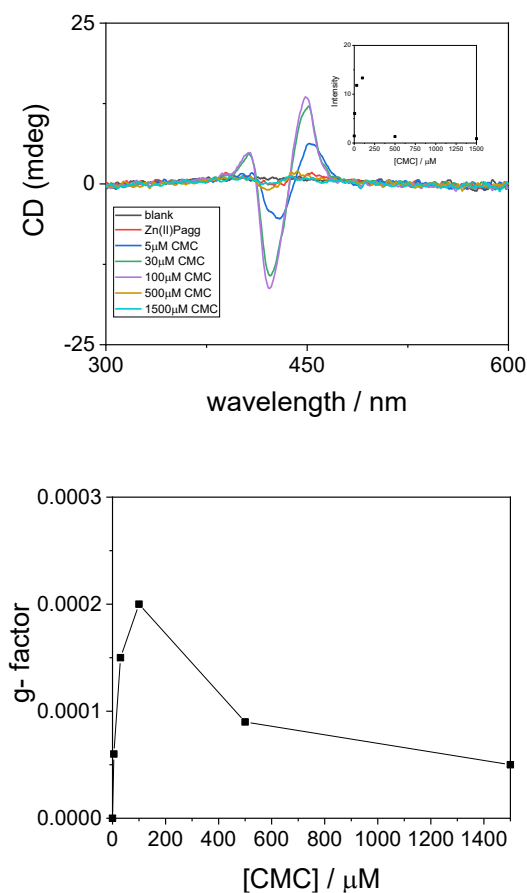


Figure 4.11. CD spectra (top) and dissymmetry g -factor (bottom) during titration of Zn(II)Pagg with CMC; in the inset the intensity of CD signal as a function of CMC. Experimental conditions: [Zn(II)Pagg] = 5 μ M; [CMC] = 0 – 1.5 mM; [PBS] = 1 mM pH 7 ; cell path length 1 cm.

Studies conducted under high ionic strength conditions in order to induce aggregation of Zn(II)Pagg did not show aggregate formation. These results suggest that the pentacoordinate geometry of the zinc ion in this porphyrin could stabilize a monomeric or dimeric structure in presence of CMC, inhibiting self-assembly processes, even in the presence of high salt concentrations.

4.4 Octahedral geometry: Au(III)Pagg@CMC, Mn(III)Pagg@CMC and Co(III)Pagg@CMC

Studies on the interactions between CMC and t-H₂Pagg derivatives were also conducted for the case of Au(III), Co(III) and Mn(III) as central metal ions. Increasing amounts of CMC were added to micromolar concentrations of solutions containing these metal species in PBS at pH 7. Specifically, Au(III)Pagg shows a B band centered at 406 nm, accompanied by two Q bands at 525 and 565 nm, Co(III)Pagg exhibits a B band at 430 nm, with Q bands at 550 and 605 nm and Mn(III)Pagg shows the B band at 465 nm and the Q bands at 560 and 608 nm.

UV/Vis extinction spectra obtained during the titrations in Figure 4.12 showed tiny or no significant changes in the position and intensity of the absorption bands, suggesting weak or absent interactions between the metal complexes and the CMC. In particular, a slight bathochromic shift of about 2 nm in the B band in the Co(III) and Au(III) complexes was observed as the CMC concentration increased. This behavior could be attributed to the electrostatic repulsion between the positive charges of the metals (Mn(III), Co(III), and Au(III)) and the polymer, as well as the

octahedral geometry of the Mn(III) and Co(III) complexes, which limits the formation of stronger intermolecular interactions between porphyrins; additionally, the high +3 charge enhances solvation and inter-porphyrin electrostatic repulsion, further hindering aggregation.

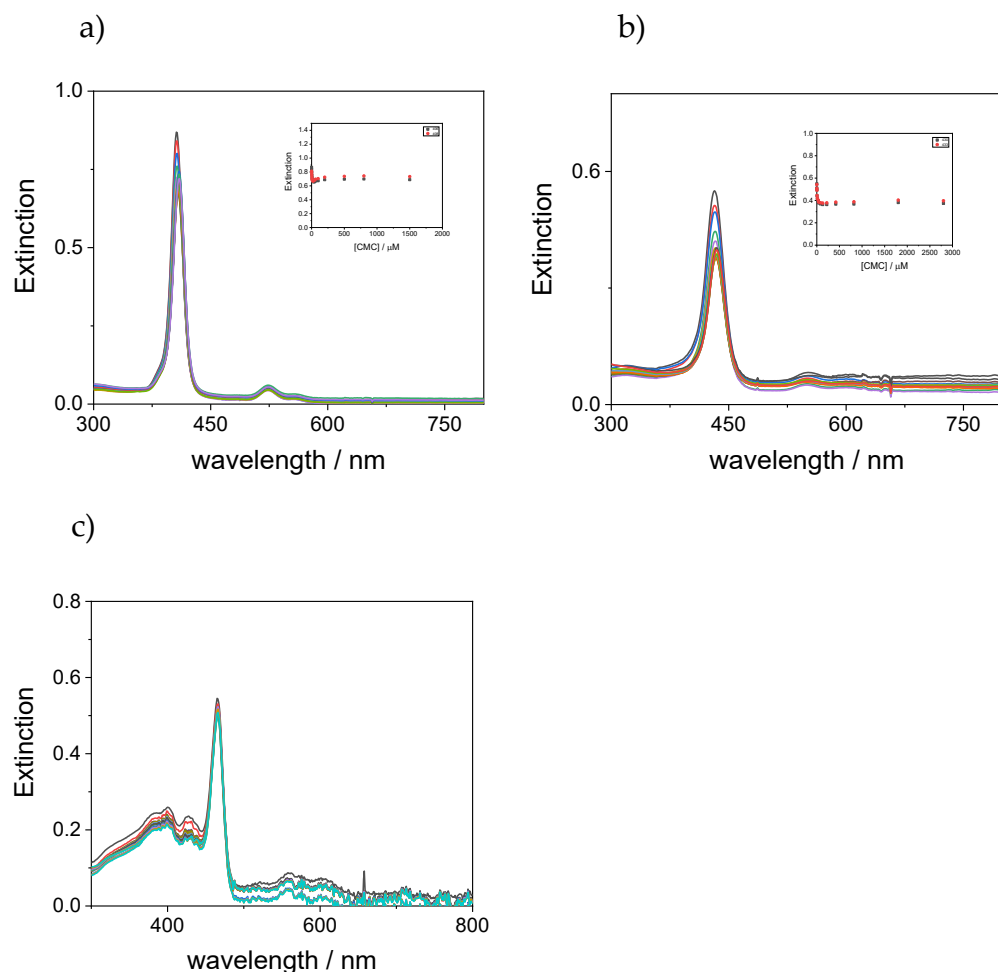


Figure 4.12. UV/Vis spectra of a) Au(III)Pagg b) Co(III)Pagg and c) Mn(III)Pagg during titration with CMC. In the inset, the extinction function of CMC concentrations. Experimental conditions: $[\text{M(III)Pagg}] = 5 \mu\text{M}$; $[\text{CMC}] = 0 - 1.5 \text{ mM}$; $[\text{PBS}] = 1 \text{ mM}$ pH 7; cell path length 1 cm

Similarly to extinction, RLS and CD spectra recorded during titration of Co(III) metal derivatives with CMC (Figures 4.13) show no significant changes. The other two metal complexes show strictly similar results. The RLS profiles of the complex in the presence of CMC display a very tiny increase in intensity, attributable to weak interactions among the chromophores, in agreement with the UV/Vis extinction data. In agreement with the other spectroscopic techniques, also CD spectra exhibit no significant change, suggesting the absence of significant interactions.

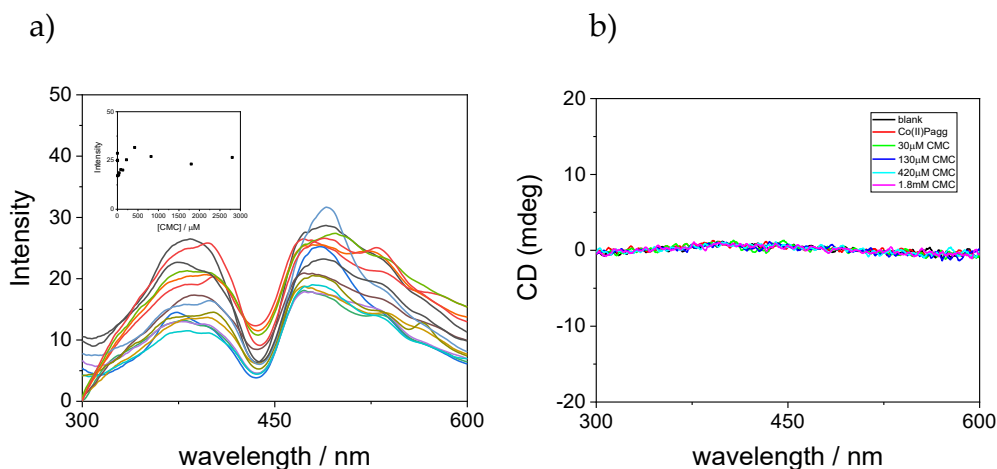


Figure 4.13. RLS spectra (left) and CD spectra (a) during titration of Co(III)Pagg with increasing amounts of CMC (b). In the inset, the RLS intensity at the maximum as function of CMC concentrations. Experimental conditions: $[\text{Co(III)Pagg}] = 5 \mu\text{M}$; $[\text{CMC}] = 0 - 1.5 \text{ mM}$; $[\text{PBS}] = 1 \text{ mM}$ pH 7 ; cell path length 1 cm.

All the experimental findings indicate that the studied trivalent cationic complexes maintained a monomeric structure even under conditions of high ionic strength and in the presence of CMC.

This behavior is probably attributable to their hexacoordinate geometry (Co(III)Pagg and Mn(III)Pagg) and 3+ charge, which make porphyrin-porphyrin interactions on the anionic polymer substantially unfavorable.

These investigations have made it possible to outline a preliminary screening platform for the recognition of metal cations, based on the analysis of the spectroscopic characteristics of the different porphyrins synthesized and immobilized on the surface of the CMC. The study of spectral variations induced by the presence of different metal centers has made it possible to evaluate their influence on the porphyrin-CMC interaction and, consequently, to identify their potential in the detection and discrimination of various cations.

4.5 Metal ions sensing

On the base of the results obtained in the study of t-H₂Pagg porphyrin metal complexes in the presence of CMC, we hypothesized a molecular recognition mechanism underlying the interaction between the supramolecular t-H₂Pagg@CMC system and metal ions, leading to the in situ formation of the characterized metal derivatives. Consequently, we decided to investigate the interaction of the metal cations Cu(II) and Ni(II) with the t-H₂Pagg@CMC supramolecular system.

UV/Vis spectrophotometric titrations of the t-H₂Pagg@CMC system in PBS at pH 7 with Cu²⁺ showed a significant hypsochromic shift of the Soret spectrum from 444 nm to 434 nm, accompanied by a concomitant reduction of the Q bands from four to two. (Figure 4.14, top) These results are indicative of the formation of the Cu(II)Pagg on the CMC polymer.

In parallel, fluorescence spectra showed a progressive quenching of the emission band at 660 nm as the Cu^{2+} concentration increased, confirming the formation of the metal complex (Figure 4.14,bottom)

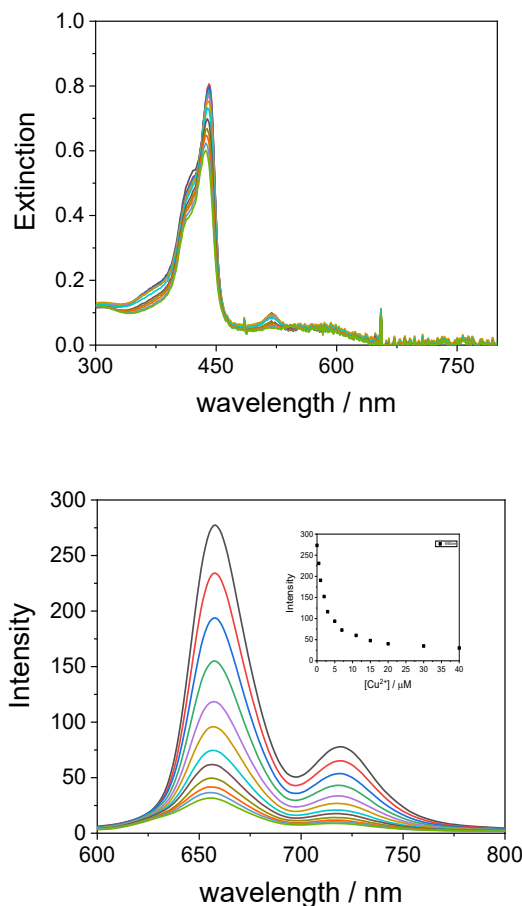


Figure 4.14. UV/Vis extinction spectra (top) and emission spectra (bottom) of t-H₂Pagg@CMC system during titration with increasing amounts of Cu(II) ion. In the inset, the intensity of the fluorescence emission maxima as function of Cu(II) concentrations. Experimental conditions: [t-H₂Pagg] = 5 μM; [CMC] = 100 μM; [Cu(II)] = 0 - 40 μM; [PBS] = 1 mM, pH 7 ; cell path length 1 cm

To confirm the role of the charged polymer on the complexation kinetics of Cu(II) towards the free base porphyrin, kinetic measurements were conducted. The experimental data, in Figure 4.15, show first-order kinetics

both in the presence and absence of CMC. However, the kinetic constant obtained in the presence of CMC ($k=1.8\times10^{-3}\text{s}^{-1}$) is slightly higher than that measured in the absence of polymer ($k=5.3\times10^{-4}\text{s}^{-1}$). This result highlights the moderate catalytic effect of CMC, which accelerates the formation of the Cu(II) complex by about 3 times. This acceleration can be attributed to the ability of the polymer's negative charges to concentrate the metal ion near the porphyrin center, facilitating the formation of the coordination bond. This effect is not unexpected since it has already been reported for the closely related tetrakis(N-methyl-pyridinium-4-yl)porphyrin metalation with zinc(II) onto poly(L-glutamate).¹³

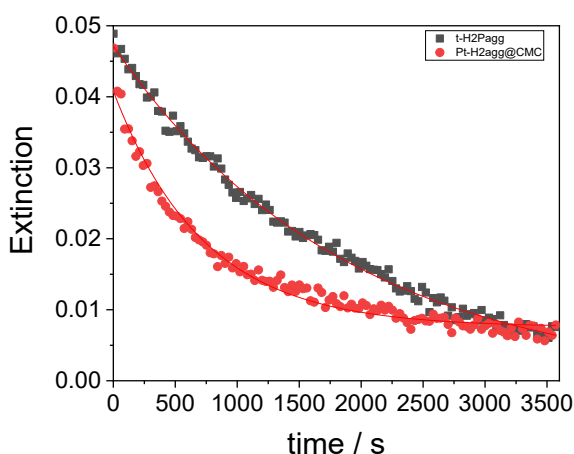


Figure 4.15. Extinctions kinetic traces of t-H₂Pagg (black line) and t-H₂Pagg@CMC (red line) in presence of Cu(II) ions (red lines represent the best fitting curves using a first order kinetic model). Experimental conditions: [t-H₂Pagg] = 5 μM; [CMC] = 100μM; [Cu(II)] = 500 μM; [PBS]= 1 mM pH 7 ; cell path length 1 cm.

To test the hypothesis that the coordination geometry of Zn(II) may hinder the formation of a stable complex with the porphyrin center, titrations with Hg(II) were conducted, in the presence of adventitious Zn(II) ions.

As reported in the literature,^{14,15} the mercury ion is known to induce a distortion of the porphyrin macrocycle, facilitating the access of other metal cations. The results obtained confirm this hypothesis: even at nanomolar concentrations, Hg²⁺ catalyzes the formation of the Zn(II)Pagg complex, as evidenced by the hypsochromic shift of the Soret band and the reduction of the Q bands in the UV/Vis spectrum (Figure 4.16 a). In parallel, quenching of the fluorescence of the 660 nm band and a concomitant increase in the characteristic emission of the Zn(II) complex at 640 nm are observed (Figure 4.16 b) These results suggest that Hg²⁺ induced distortion on the porphyrin macrocycle, even in the dimeric form present in the system and facilitates the access of Zn(II) into the porphyrin center, promoting the formation of the complex Zn(II)Pagg@CMC.

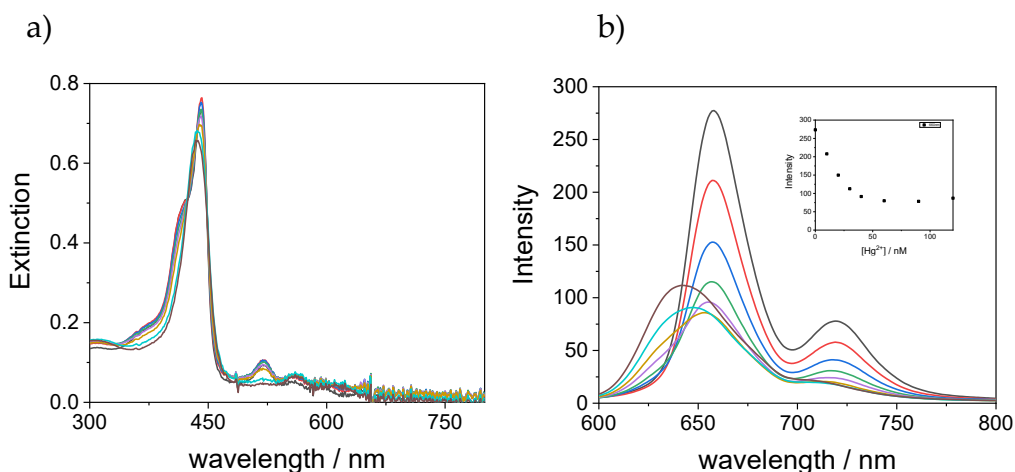


Figure 4.16. a) UV/Vis extinction spectra and b) emission spectra of t-H₂Pagg@CMC system titrated with Hg(II) ion, in the presence of adventitious Zn²⁺ ions. The inset represent the intensity of fluorescence emission at the maxima in function of Hg(II) concentrations. Experimental conditions: [t-H₂Pagg] = 5 μ M; [CMC] = 100 μ M; [Hg(II)] = 0 – 120 nM; [PBS] = 1 mM pH 7; cell path length 1 cm

The interaction of Ni(II) with the t-H₂Pagg@CMC system was investigated by spectrophotometric titrations (Figure 4.17). In contrast to what was observed for Cu(II), the spectral changes induced by the addition of Ni²⁺ were less pronounced, being limited to partial fluorescence quenching and B band hypochromicity in UV/Vis extinction spectra probably due to dilution phenomena. Considering that Cu(II) and Ni(II) both exhibit square planar geometry, similar behavior was expected.

However, the literature reports a higher affinity and reactivity of Cu(II) and Zn(II) toward the porphyrin center than Ni(II).¹⁶

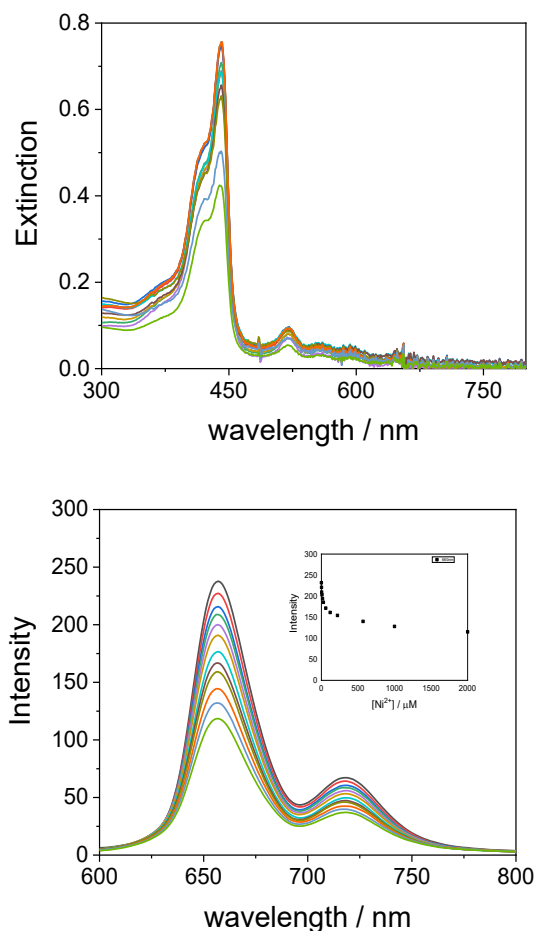


Figure 4.17. UV/Vis extinction spectra (top) and emission spectra (bottom) during titration of t-H₂Pagg@CMC system with increasing concentration of Ni(II) ions. In the inset, the intensity of emission as function of Ni(II) concentration is shown. Experimental conditions: [t-H₂Pagg] = 5 μM; [CMC] = 100 μM; [Ni(II)] = 0 – 3 mM; [PBS] = 1 mM pH 7; cell path length 1 cm.

Experimental part

Materials

5,15-bis(N-methylpyridinium-4-yl)-10,15-bis-diphenylporphine (t-H₂Pagg, chloride salt) was purchased from Frontier Scientific and used without further treatment. Metal derivatives Cu(II)Pagg, Ni(II)Pagg, Zn(II)Pagg, Co(III)Pagg, Mn(III)Pagg, and Au(III)Pagg were synthesized according to the procedure described in the literature.¹⁰ Stock solutions of porphyrins were prepared by dissolving the solids in ultrapure water (Galenica Senese). Concentrations were determined using the known molar extinction coefficient at the B-band maximum (t-H₂Pagg: $2.4 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$; Cu(II)Pagg: $2.34 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$; Ni(II)Pagg: $1.24 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$; Zn(II)Pagg: $1.4 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$; Co(III)Pagg: $1.23 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$; Mn(III)Pagg: $1.8 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$; Au(III)Pagg: $2.23 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$).

Carboxymethyl cellulose sodium salts (FW ~ 90,000), sodium dihydrogenophosphate and sodium hydrogenophosphate were purchased from Sigma-Aldrich (Milan, Italy). Aqueous stock solutions containing the metals of interest were prepared by dissolving the corresponding salts (CuSO₄, ZnSO₄, NiCl₂, HgCl₂) purchased from Sigma – Aldrich.

To prepare fractal aggregates of t-H₂Pagg, Cu(II)Pagg and Ni(II)Pagg, a concentrated aliquot of NaCl solution up to the final 0.1 M concentration has been added to a diluted aqueous solution of the porphyrin (5 μM). The kinetic growth of the aggregates have been monitored by UV/Vis extinction spectra, following the typical bands of the aggregated species.

Conclusions

In conclusion, the comparative analysis of the free base t-H₂Pagg and its metal derivatives showed that the coordination geometry and steric hindrance induced by the metal cation play a decisive role in modulating the interaction with CMC and, consequently, in the type of supramolecular aggregates that form on its surface. It emerged that cations with square planar geometry, such as Ni²⁺ and Cu²⁺, favor strong interaction with CMC, inducing the formation of well-defined J-type aggregates. On the contrary, Zn²⁺, in a predominantly pentacoordinated configuration, has only one accessible free face and leads to the formation of J-type dimers. Similarly, the free base system t-H₂Pagg@CMC also shows a tendency to form dimeric species, likely due to the absence of a central metal ion, which limits directional coordination and favors simpler aggregation motifs. Finally, hexacoordinated cations such as Mn³⁺ and Co³⁺, having a completely saturated porphyrin core have no sites available for interactions and are therefore unable to induce aggregation processes. Similar results are observed for the square planar Au(III) metal complex, where the 3+ charge plays a destabilizing role.

In-depth knowledge of these structure-interaction relationships, obtained through spectroscopic studies (UV/Vis extinction, fluorescence emission, RLS and CD), allows us to outline a preliminary screening platform for the recognition of metal cations based on the t-H₂Pagg@CMC system. Under the assumption that the cation to be detected inserts itself into the porphyrin core, reproducing the same interaction modes observed for the

synthesized metal derivatives, the aggregation behavior and the related spectroscopic features can in fact be used as discriminating signals.

Our preliminary results suggest the potential of the t-H₂Pagg@CMC system as sensor for detecting specific metal cations, thanks to the direct correlation between the nature of the metal center, the mode of interaction with the polymer, and the spectroscopic response of the supramolecular system. Anyway, further experimental effort needs to be done to develop a precise protocol for effective use in metal ions sensing.

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Chapter 5

Investigation on J-Aggregates of 2,3,7,8,12,13,17,18-Octabromo -5,10,15,20-tetrakis(4-sulfonatophenyl) Porphyrin in Aqueous Solutions.¹

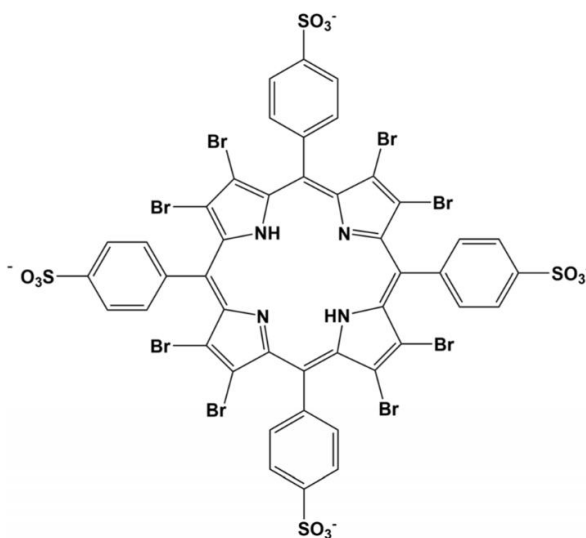
The aggregation of porphyrins, as mentioned in the introduction, is a phenomenon widely described in the literature and closely related to the considerable aromatic extension of the porphyrin macrocycle, which favors π -stacking between neighboring units, a process that is accentuated when the solvent conditions change from favorable to unfavorable. In this context, porphyrins functionalized with sulfonic groups on the meso *n* phenyl groups represent a privileged study system, as they are known to form both J- or H-type aggregates.²⁻⁴

As already extensively mentioned in the introduction, one of the most studied molecules in this context is tetrakis-(4-sulfonatophenyl)porphyrin (TPPS₄⁴⁻), which, thanks to its symmetry and sensitivity to experimental conditions, such as concentration, pH, ionic strength, and the presence of cationic species can self-assemble into a wide range of mesoscopic structures.⁴⁻⁸ Furthermore, chirality can be induced in aggregates through chemical or physical perturbations⁹⁻¹³ or even emerge spontaneously during out of equilibrium processes, in the absence of an external chiral source, by means of appropriate mixing protocols.¹⁴

Among the factors promoting the formation of J-aggregates, a crucial role is played by the preliminary protonation of the macrocycle, which involves a significant distortion of the porphyrin structure.

The introduction of two protons on the central nitrogen atoms reduces the overall negative charge, attenuating electrostatic repulsion and thus facilitating the formation of the first dimers. At the same time, the resulting adoption of a “saddle” conformation involves a rotation of the phenyls on the meso position, making the protonated molecule more planar than the neutral porphyrin.¹⁵

Based on these considerations, we thought it interesting to explore the self-assembling process into J-aggregates of a porphyrin that already exhibits marked structural distortion under neutral conditions. To this end, we focused our attention on 2,3,7,8,12,13,17,18-octabromo-5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin ($\text{Br}_8\text{TPPS}_4^{4-}$) (Scheme 1).



Scheme 5.1. Structure of 2,3,7,8,12,13,17,18-octabromo-5,10,15,20-tetrakis(4-sulfonatophenyl) porphyrin ($\text{Br}_8\text{TPPS}_4^{4-}$).

5.1 Species in solution as function of pH

In neutral aqueous solution, the free base $\text{Br}_8\text{TPPS}_4^{4-}$ exhibits a UV/Vis spectrum with an intense B band centered at 475 nm, accompanied by two Q bands at 660 and 760 nm (Figure 1, black trace), in agreement with what is reported in the literature.¹⁶ The addition of HCl to pH 1.3 leads to the formation of the diacid species $\text{H}_2\text{Br}_8\text{TPPS}_4^{2-}$, which exhibits a new, significantly red-shifted B band at 489 nm, as well as two Q bands located at 650 nm and 724 nm (Figure 1, red trace).¹⁶

A subsequent decrease in pH induces a further, albeit minimal, shift of band B (B at 490 nm, Figure 5.1, blue trace). This variation can be attributed to the formation of the neutral zwitterionic species $\text{H}_4\text{Br}_8\text{TPPS}_4$, similar to what was observed for TPPS_4^{4-} ¹⁷, or to solvatochromic effects.

In the presence of 6 M HCl, the spectrum shows another slight shift towards lower energies of both band B and bands Q (B at 492 nm, Figure 5.1, green trace), consistent with the formation of the species $\text{H}_6\text{Br}_8\text{TPPS}_4^{2+}$, in which four sulfonic groups are protonated. A similar species has been described for TPPS_4^{4-} under the same experimental conditions.¹⁸

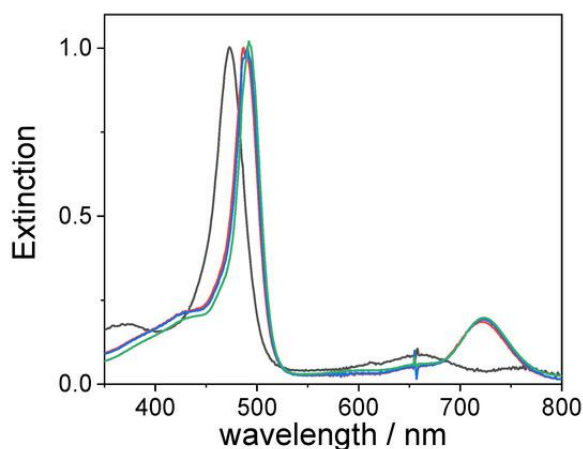
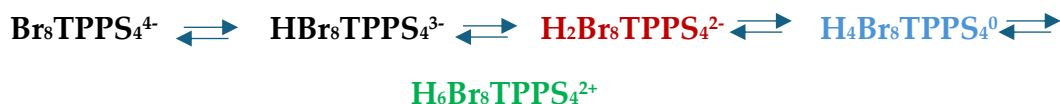


Figure 5.1. Normalized UV/Vis spectra of $\text{Br}_8\text{TPPS}_4^{4-}$ (pH = 7, black line), $\text{H}_2\text{Br}_8\text{TPPS}_4^{2-}$ ([HCl] = 0.05 M, red line), $\text{H}_4\text{Br}_8\text{TPPS}_4$ ([HCl] = 2 M, blue line) and $\text{H}_6\text{Br}_8\text{TPPS}_4^{2+}$ ([HCl] = 6 M, green line) in aqueous solution ($[\text{Br}_8\text{TPPS}_4^{4-}] = 3 \mu\text{M}$).



5.2 Kinetics of Self- Assembly

The growth of TPPS_4^{4-} J-aggregates is strongly influenced by both the overall ionic strength and the adopted mixing protocol.¹⁴ It is possible to observe that when porphyrin is added last to a solution already containing the acid (porphyrin last, PL condition), aggregation proceeds rapidly and the kinetics show an elongated exponential trend. The J-aggregates obtained in this way are optically achiral, as they show no response in circular dichroism.

Conversely, if the acid is added to a solution in which the porphyrin has already been pre-diluted (porphyrin first, PF), the temporal evolution of

absorption shows sigmoidal curves, and the aggregates formed are chiral, as evidenced by the appearance of CD bands in the solutions.

The addition of a salt, which further increases the ionic strength, introduces an additional level of complexity into the self-assembly process.¹⁴ In particular, under the experimental conditions described by Pasternack¹⁹, namely, sequential addition of 0.05 M NaCl and 0.5 M HCl to an aqueous solution of $\text{Br}_8\text{TPPS}_4^{4-}$, the initial species, which is diprotonated or zwitterionic in nature, evolves into a new species characterized by an absorption band centered at 521 nm (Figure 5.2), attributed to the formation of the J-aggregate

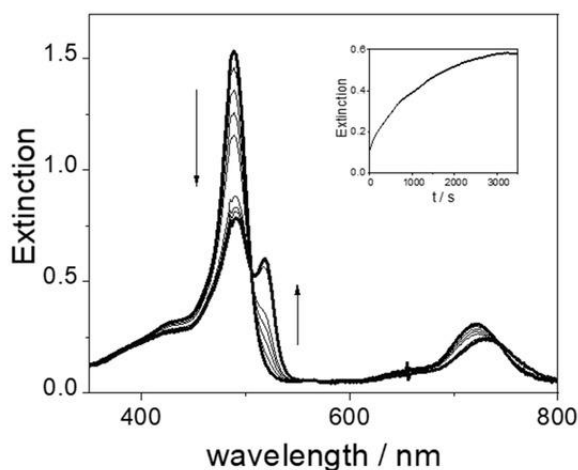


Figure 5.2. UV/Vis spectral changes for the conversion of the zwitterionic $\text{H}_4\text{Br}_8\text{TPPS}_4$ into its aggregate ($[\text{Br}_8\text{TPPS}_4^{4-}] = 10 \mu\text{M}$, $[\text{NaCl}] = 0.05 \text{ M}$, $[\text{HCl}] = 0.5 \text{ M}$, $T = 298 \text{ K}$). The inset shows the kinetic trace of the absorbance change at 521 nm.

The formation of the $\text{H}_4\text{Br}_8\text{TPPS}_4$ aggregate is supported by the recorded RLS spectra, which show a resonance peak at 530 nm, shifted towards the red compared to the corresponding absorption band and with an intensity

significantly higher than that of the pure solvent (Figure 5.3, green and black lines). By comparison, the free base porphyrin and its protonated forms (Figure 5.3, blue and red lines) show much weaker signals, with minimum at their respective absorption bands.

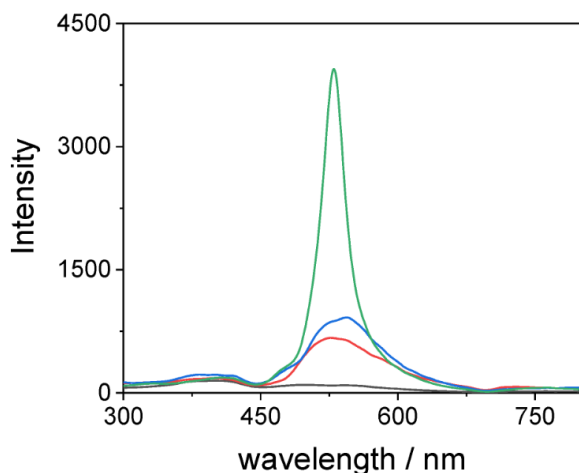


Figure 5.3. RLS profile for the neat solvent (black line), the neutral $\text{Br}_8\text{TPPS}_4^{4-}$ (blue line), its diacid form $\text{H}_2\text{Br}_8\text{TPPS}_4^{2-}$ (red line) and the J-aggregates (green line). Experimental conditions : $[\text{Br}_8\text{TPPS}_4^{4-}] = 10 \mu\text{M}$, $[\text{NaCl}] = 0.05 \text{ M}$, $[\text{HCl}] = 0.5 \text{ M}$, $T = 298 \text{ K}$.

The kinetic traces detected in the J-band region show an almost exponential behavior, accompanied by some instability in the final phase, a factor that makes experimental reproducibility more complex. To mitigate these difficulties, we modified the operating conditions by adopting a PF mixing protocol based exclusively on the use of HCl, to promote aggregate formation. Figure 5.4 shows a typical example of a sigmoidal trace, obtained by monitoring the extinction trend at 521 nm in this type of experiments.

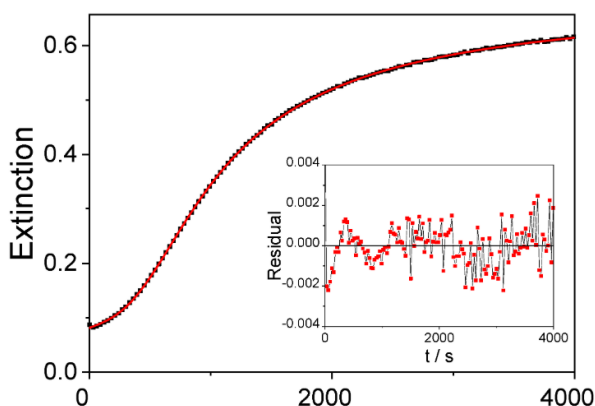


Figure 5.4. Sigmoidal profile for the time evolution of the extinction measured at 521 nm for the aggregation of $\text{H}_4\text{Br}_8\text{TPPS}_4$. The red line represents the best-fit curve of the experimental data with Equation (1) ($k_0 = (1.44 \pm 0.11) \times 10^{-4} \text{ s}^{-1}$, $k_c = (1.50 \pm 0.01) \times 10^{-3} \text{ s}^{-1}$, $m = 3.60 \pm 0.10$, $n = 2.10 \pm 0.07$, $R^2 = 0.9995$). In the inset are the residuals from the best-fitting procedure. Experimental conditions: $[\text{Br}_8\text{TPPS}_4^{4-}] = 10 \text{ } \mu\text{M}$; $[\text{HCl}] = 1.0 \text{ M}$; PF mixing protocol, $T = 298 \text{ K}$.

Pasternack proposed a kinetic model (Equation 7.1, in experimental part, Chapter 7, Paragraph 7.2, Method) to describe self-assembly processes characterized by an initial phase of slow nucleation, followed by rapid autocatalytic growth.²⁰ In this approach, the limiting step consists in the formation of a nucleus consisting of m monomers, followed by autocatalytic aggregation governed by the rate constant k_c and a time exponent n , related to the dimensionality of the process. The model also considers a non-catalytic pathway, defined by a constant k_0 .

Table 5.1 summarizes the main kinetic parameters obtained from the optimal fitting of the experimental extinction curves to Equation 7.1, both in the case where the aggregation of $\text{Br}_8\text{TPPS}_4^{4-}$ is induced by different concentrations of HCl ($[\text{Br}_8\text{TPPS}_4^{4-}] = 10 \text{ } \mu\text{M}$, PF protocol) and when the porphyrin concentration is varied while keeping $[\text{HCl}] = 1.0 \text{ M}$ constant.

Table 5.1. Values for the kinetic parameters k_0 , k_c , m and n calculated through a best-fitting procedure of extinction data to Equation (1), for the acid-induced J-aggregation of H₄Br₈TPPS₄ ([Br₈TPPS₄⁴⁻] = 8–15 μ M; [HCl] = 0.8–1.5 M; PF mixing protocol, T = 298 K).

$10^6[\text{Br}_8\text{TPPS}_4^{4-}]/\text{M}$	[HCl]/M	$10^4 k_0/\text{s}^{-1}$	$10^3 k_c/\text{s}^{-1}$	m	n
8	1	0.260 0.020	$\pm$ 0.594 $\pm$ 0.001	3.7 ± 0.1	3.5 ± 0.1
10	1	1.44 ± 0.11	1.50 ± 0.01	3.6 ± 0.10	2.1 ± 0.1
12	1	10.6 ± 0.7	4.55 ± 0.07	4.5 ± 0.2	1.8 ± 0.1
15	1	49.0 ± 0.5	12.0 ± 0.8	6.5 ± 0.7	1.8 ± 0.2
10	0.8	11.0 ± 2.3	3.70 ± 0.30	2.9 ± 0.3	0.9 ± 0.2
10	1.2	0.420 0.020	$\pm$ 0.657 $\pm$ 0.002	4.0 ± 0.1	2.9 ± 0.1
10	1.5	0.130 0.011	$\pm$ 0.320 $\pm$ 0.001	3.3 ± 0.1	3.0 ± 0.1

Analysis of the kinetic data shows that the average value of k_0 is lower, by about an order of magnitude, than k_c . The relative amplitudes of these two parameters influence the sigmoidal growth profile, which is particularly steep and different from that usually observed for TPPS₄⁴⁻ aggregation. For the latter porphyrin, in fact, the kinetic profiles typically have a much longer nucleation time.¹⁷ The number of monomers involved in the

nucleation phase that determines the speed is between 3 and 4, in agreement with what has been reported for non-brominated porphyrin.¹⁷ The time exponent n takes values between 0.8 and 3.5. For HCl concentrations below 0.8 M, the kinetics become extremely rapid, to the point of eluding the nucleation phase and making correct quantitative analysis impossible.

Like other related systems, a porphyrin concentration threshold has also been identified: below this value, the aggregation process does not occur or proceeds so slowly that accurate data collection is difficult. In our case, at $[HCl] = 1\text{ M}$, no aggregation is observed for concentrations of $[Br_8TPPS_4^{4-}] \leq 6\text{ }\mu\text{M}$.

The trend of k_c as a function of the concentration of $[Br_8TPPS_4^{4-}]$ (at $[HCl] = 1\text{ M}$) shows a monotonically increasing profile (Figure 5.5), which can be satisfactorily described by the expression:

$$k_c = a \times [Br_8TPPS_4^{4-}]^b$$

where $a = (3.81 \pm 2.47) \times 10^{-8}$, $b = 4.7 \pm 0.2$, and $R^2 = 0.997$. This concentration dependence is significantly different from that observed for $TPPS_4$, which instead follows quadratic behavior.¹⁷

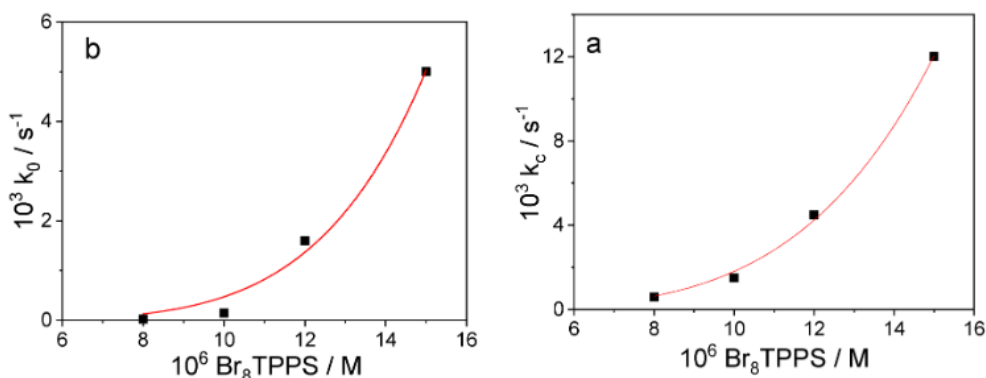


Figure 5.5. Plot of k_c (a) and k_0 (b) as functions of $[\text{Br}_8\text{TPPS}_4^{4-}]$ for aggregation of this porphyrin at $[\text{HCl}] = 1.0 \text{ M}$, $T = 298 \text{ K}$. The solid line represents the best-fitting curve to the equations $k_c = a \times [\text{Br}_8\text{TPPS}_4^{4-}]^b$ ($a = (3.8 \pm 2.5) \times 10^{-8}$; $b = 4.7 \pm 0.2$, $R^2 = 0.997$) and $k_0 = a \times [\text{Br}_8\text{TPPS}_4^{4-}]^b$ ($a = (6.9 \pm 15.3) \times 10^{-10}$; $b = 5.8 \pm 0.8$, $R^2 = 0.989$).

As the proton concentration increases, the k_c values decrease (Figure 5.6) and follow the trend described by the equation:

$$k_c = a \times [\text{HCl}]^b$$

with $a = (1.5 \pm 0.1) \times 10^{-3}$, $b = -4.0 \pm 0.10$, and $R^2 = 0.9993$. This behavior is similar to that already observed for TPPS_4^{4-} , where the pH range in which J aggregates can grow is narrow, being limited by a minimum acid concentration necessary to activate the aggregation process and by the formation of the species $\text{H}_6\text{Br}_8\text{TPPS}_4^{2+}$. In this latter species, the complete protonation of the sulfonate groups prevents the presence of those favorable electrostatic contacts that are essential for interaction with the protonated nitrogen atoms of the porphyrin nucleus.

The trends in the k_0 values are shown in Figure 5.6 and show a similar trend to that observed for the corresponding catalyzed constants.

In particular, they follow the relationships $k_0 = a \times [\text{Br}_8\text{TPPS}_4^4]^b$, with $a = (6.9 \pm 15.3) \times 10^{-10}$, $b = 5.8 \pm 0.8$, and $R^2 = 0.989$, and $k_0 = a \times [\text{HCl}]^b$ with $a = (1.5 \pm 0.1) \times 10^{-4}$, $b = -9.0 \pm 0.3$, and $R^2 = 0.999$.

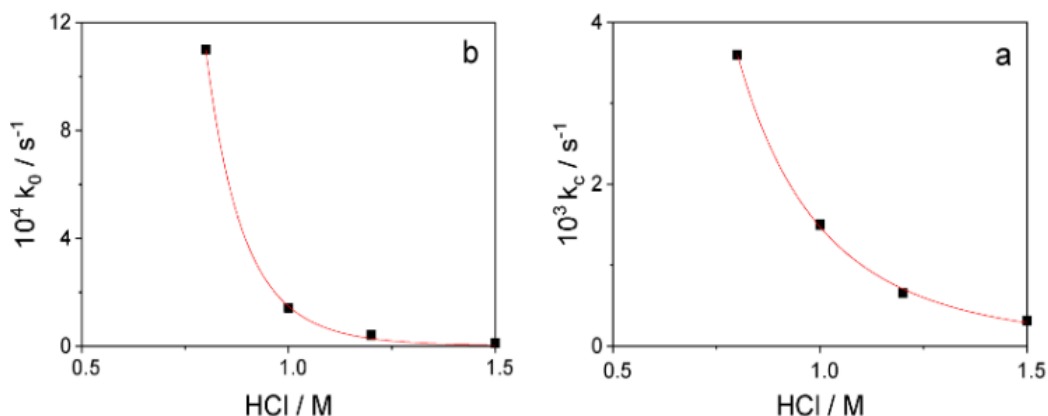


Figure 5.6. Plot of k_c (a) and k_0 (b) as functions of [HCl] for aggregation of this porphyrin at $[\text{Br}_8\text{TPPS}_4^4] = 10 \mu\text{M}$, $T = 298 \text{ K}$. The solid line represents the best-fitting curve to the equations $k_c = a \times [\text{HCl}]^b$ ($a = (1.5 \pm 0.1) \times 10^{-3}$; $b = -4.0 \pm 0.1$, $R^2 = 0.9993$) and $k_0 = a \times [\text{HCl}]^b$ ($a = (1.5 \pm 0.1) \times 10^{-4}$; $b = -9.0 \pm 0.3$, $R^2 = 0.999$).

The relative amount of J-aggregates obtained with respect to the residual monomer in solution was estimated from the ratio between the extinction values measured at 521 and 491 nm, respectively. The graphs of this ratio as a function of $[\text{Br}_8\text{TPPS}_4^4]$ and [HCl] are shown in Figure 5.7, indicating that aggregate formation is favored as the porphyrin concentration increases and the acid concentration decreases.

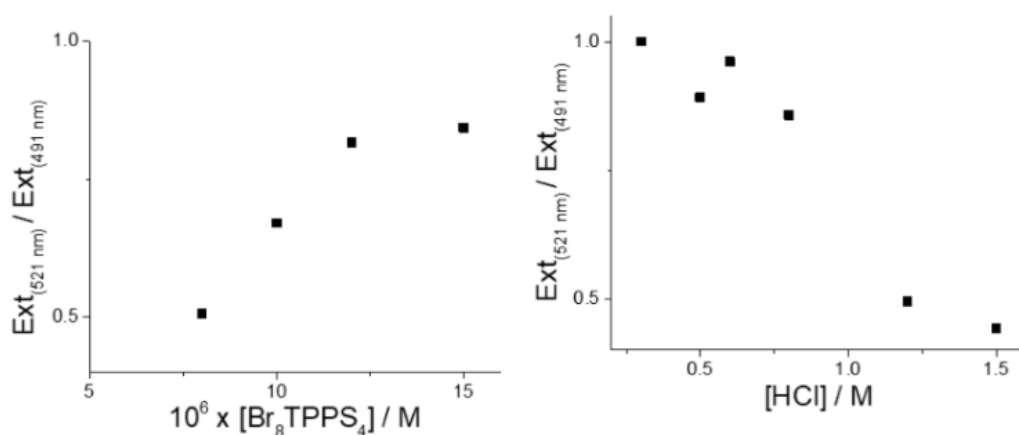


Figure 5.7. Plot of the ratio of the extinction values at 521 and 491 nm after completion of the aggregation process as a function of porphyrin (left) and hydrochloric acid concentration (right).

Unlike the TPPS_4^{4-} J-aggregates obtained using the same experimental protocol, the porphyrin aggregates studied here show no induced CD bands. This suggests the absence of chirality or the formation of racemic mixtures in conditions lacking chiral inducers. Even in the presence of 0.1 M L of tartaric acid, which in combination with 1 M HCl promotes the aggregation process, no chirality transfer was observed.

5.3 Depolarized Resonance Light Scattering Investigations

Resonance light scattering is an extremely effective technique for studying the aggregation of chromophores in solutions. A marked increase in the intensity of scattered light occurs when many monomeric units self-assemble ($N > 25$)²¹, and strong electronic coupling occurs. Parkash et al. described theoretically the dependence of the depolarization ratio in RLS measurements, thus allowing geometric information to be obtained on the arrangement of chromophores within the aggregates.²²

The depolarization ratio $\rho_v(90)$ is calculated using linearly polarized light as the excitation source and detecting the light scattered at 90° to the incident beam using an orthogonally oriented linear polarizer. It is defined as:

$$\rho_v(90^\circ) = \frac{I_{VH}}{I_{VV}},$$

where I_{VH} is the intensity of scattered light detected with horizontal polarization and I_{VV} is that detected with vertical polarization. The mechanical quantum model developed for different types of geometric arrangements can be applied both to chromophores characterized by an angular shift φ with respect to the axis connecting their centers in cylindrical aggregates.

Figure 5.8 shows the RLS spectrum of $H_4Br_8TPPS_4$ J aggregates (black line), flanked by the corresponding depolarization ratio profile (red line). From the average value obtained, $\rho_v(90) \approx 0.265$ and comparing it with the theoretical dependence of $\rho_v(90)$ on the angular slip²², a slip angle of approximately $\varphi \approx 20^\circ$ can be estimated for the J-aggregate of this porphyrin (Scheme 5.2).

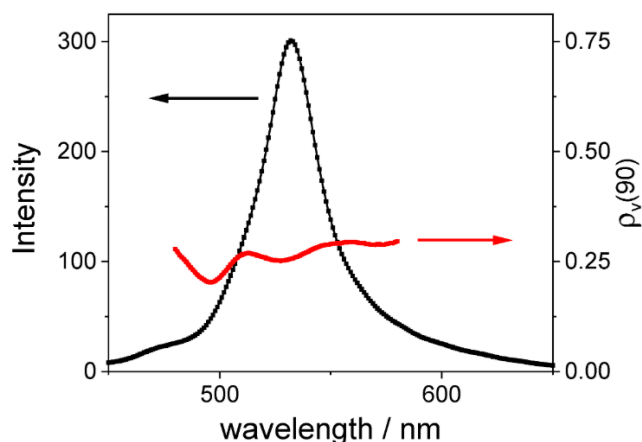
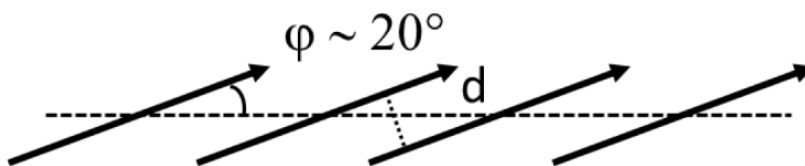


Figure 5.8. RLS spectrum (black line) and depolarization ratio $\rho_v(90)$ (red line) of J-aggregates of $\text{H}_4\text{Br}_8\text{TPPS}_4$ (Experimental conditions: $[\text{Br}_8\text{TPPS}_4^{4-}] = 10 \mu\text{M}$; $[\text{HCl}] = 1 \text{ M}$; $T = 298 \text{ K}$).



Scheme 5.2. Geometrical sketch of the electronic transition moments arrangement inside the J-aggregate from depolarized light-scattering measurements.

Further information on the electronic properties of J-aggregates can be obtained from the analysis of the line width of the J-band. In this class of aggregates, this band is typically very narrow due to the phenomenon of exchange narrowing. Knapp described the line shapes of exciton spectra in linear aggregates using a perturbative treatment.²³ According to this model, the line width of an aggregate composed of N strongly coupled monomers, $W(N)$, is related to the line width of the non-interacting monomer, $W(0)$, according to the relationship:

$$W(N) = [W(0) N^{-1/2}](2 \ln 2)^{1/2}.$$

The deconvolution of the UV/Vis spectra of the monomer and the J band yielded $W(0) = 875 \text{ cm}^{-1}$ e $W(N) = 463 \text{ cm}^{-1}$, respectively. From these values, it is possible to estimate a spectroscopic aggregation number of approximately $N \approx 5$. For comparison, Koti et al. reported values between 5 and 13 for TPPS₄⁴⁻ J-aggregates.²⁴

Assuming a linear arrangement of the chromophores, the coherence length L can be estimated as $(N+1)$ times the radius of a single monomer. Molecular simulations indicate a value of approximately 1 nm for the latter, resulting in $L \approx 6 \text{ nm}$.

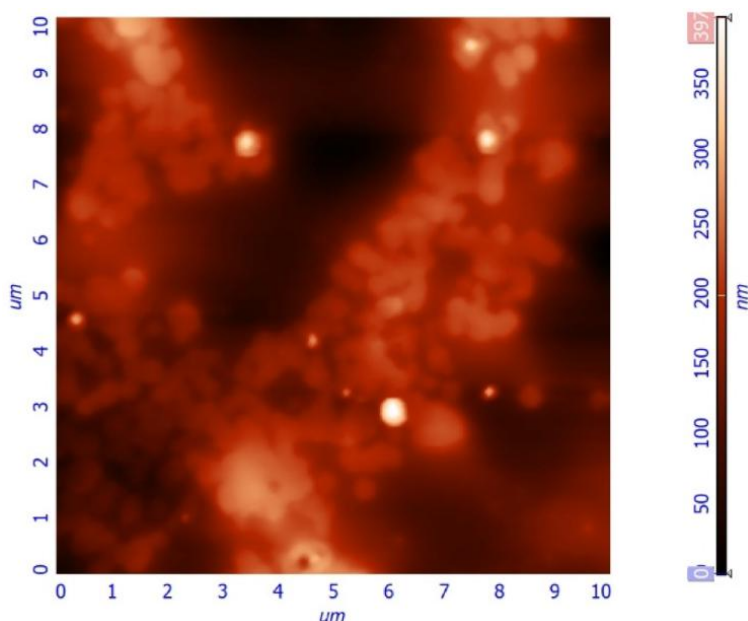


Figure 5.9. AFM image of J-aggregates of H₄Br₈TPPS₄ deposited on glass cover slides by solvent evaporation at room temperature.

The porphyrin J-aggregates were deposited on coverslips by slow evaporation of the solvent from equilibrated solutions. Atomic force microscopy (AFM) was used to analyze their morphology. As shown in Figure 5.9, a population of spherical aggregates with an average size of approximately 130 nm was observed. This value is in good agreement with the dynamic light scattering (DLS) measurements performed on identical samples in solutions, from which an average diameter of 150 nm (PDI = 0.51) was obtained.

It is particularly interesting to note that the Br₈TPPS₄⁴⁻ J-aggregates exhibit a bathochromic shift of approximately 30 nm, which is significantly lower than the 55 nm observed for TPPS₄⁴⁻ J-aggregates. This difference can be attributed to the different molecular architecture of the two systems, which results in a variation in the intensity of the electronic coupling responsible for their spectroscopic behavior.

5.4 Theoretical Calculations¹

For simplicity, molecular models of the porphyrin Br₈TPPS₄⁴⁻ in its neutral and diprotonated forms were used, maintaining its essential structural characteristics. Geometry optimizations performed using DFT at the B3LYP/6-31g(d,p) level of theory revealed a pronounced saddle-shaped conformation for both species, characteristic of β -substituted porphyrins. Protonation of the tetrapyrrolic core further induces significant structural rearrangements, particularly affecting the torsion angles of the phenyl groups and the lengths of the internal bonds.

Analysis of the molecular orbitals shows that protonation causes a lowering of the HOMO and LUMO energy levels and a reduction of the band gap, consistent with greater electronic stability of the diprotonated species with respect to the unprotonated one. This variation is also reflected in the optical properties: TD-DFT calculations predict a bathochromic shift of the absorption bands, generally in line with what was observed experimentally, although to a greater extent, probably due to the use of molecular models without sulfonate groups.

Finally, theoretical Raman analysis provides a useful reference for assigning the vibrational modes observed in these J-aggregates, showing good agreement in the main bands associated with out-of-plane torsions, porphyrin ring vibrations, and C–C and C–N bond stretches. Discrepancies in some spectral regions can be attributed to the absence of sulfonate groups in computational models.

Overall, the theoretical results support and reinforce the experimental interpretation, providing a coherent picture of the effect of protonation on the structural, electronic, and vibrational properties of Br₈TPPS₄⁴⁻ systems.

Experimental part

Materials

The porphyrin 2,3,7,8,12,13,17,18 octabromo 5,10,15,20 tetrakis (4sulfonatophenyl) porphyrin ($\text{Br}_8\text{TPPS}_4^{4-}$), in its acidic form, was obtained from PorphyChem (France). Sodium chloride and hydrochloric acid were purchased from Sigma-Aldrich (Milan, Italy) and were used as supplied, without any additional purification. All experiments were carried out using ultra-pure, double-distilled water provided by Galenica Senese (Siena, Italy).

Porphyrin stock solutions were freshly prepared by dissolving a small quantity of the solid compound in water and were kept in the dark until use. The concentration of the stock solutions was determined by UV–Vis spectroscopy, using a molar extinction coefficient $\varepsilon = 1.41 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ at 475 nm

Conclusions

The highly non-planar porphyrin $\text{Br}_8\text{TPPS}_4^{4-}$ forms J-aggregates in highly acidic aqueous solution, with self-assembling kinetics sensitive to the mixing protocol. In particular, the PF protocol induces early nucleation followed by rapid growth. Compared to TPPS_4^{4-} , the rate constants show a much more pronounced concentration dependence, and the aggregates exhibit neither spontaneous symmetry breaking nor chirality transfer from external inductors.

Spectroscopic analyses indicate an edge-to-edge arrangement of the chromophores, which are strongly coupled and regularly tilted, with delocalized excitation over approximately five units. AFM reveals an almost globular morphology, distinct from the nanotubular structure of the non-brominated analogue. DFT and TD-DFT calculations on simplified models confirm the effect of protonation on electronic properties and provide valuable support for the interpretation of Raman spectra. This J-aggregate thus represents a new example within the broader class of supramolecular self-assembled structures formed by porphyrins.

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Synthesis and characterization of new *meso*-substituted porphyrins with terminal triphenylphosphonium groups. *

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Compounds containing triphenylphosphonium groups are widely recognized for their high specificity in sensing and diagnostic applications.¹ However, low molecular weight systems functionalized with triphenylphosphonium units may have certain limitations, such as reduced thermal stability and poor persistence over time, which can result in a loss of effectiveness during use and raise of potential toxicity concerns.²

The functionalization of porphyrins with triphenylphosphonium groups has been the subject of increasing attention in literature.³ Thanks to their permanent positive charge, these groups have been used to improve solubility in aqueous environments, to modulate intermolecular interactions and, in some cases, to promote selective recognition processes of charged species⁴ In addition, porphyrin derivatives containing phosphonium units have been explored as probes for the detection of metal ions, exploiting the combination of the metal coordination site within the porphyrin core and the electrostatic interactions introduced by the cationic side chains.⁵

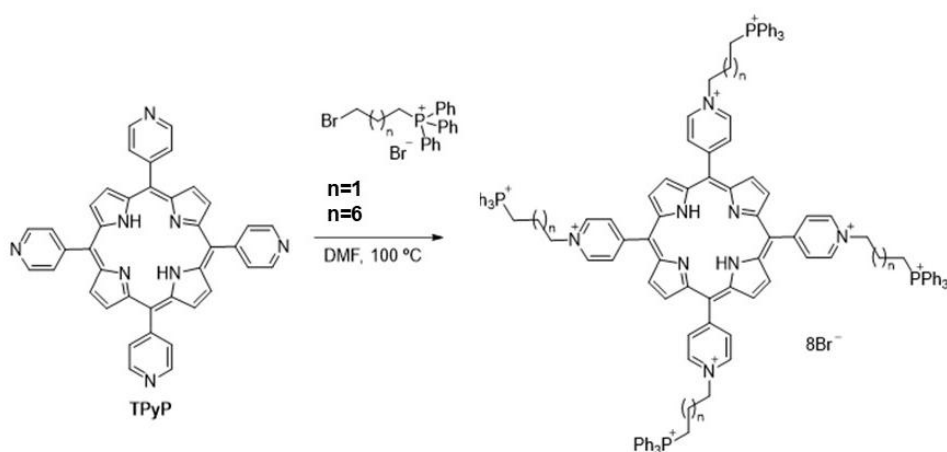
The presence of alkyl spacers of varying lengths allows modulation of the solubility, hydrophobicity, and supramolecular organization, making them potentially applicable in different contexts, from aqueous solutions to more complex matrices.^{6,7}

Based on these considerations, two different synthetic strategies for obtaining porphyrins functionalized with triphenylphosphonium groups were explored in this study. In the first approach, functionalization was achieved by direct *N*-alkylation of the *meso*-pyridyl units of 5,10,15,20-

tetra(4-pyridyl)porphyrin (TPyP), using commercial or synthesized triphenylphosphonium salts. In the second approach, the alkyl chain bearing the triphenylphosphonium group was introduced into the aromatic aldehyde, which was subsequently used as a building block for the synthesis of the porphyrin macrocycle via acid-catalysed condensation with pyrrole. These strategies made it possible to modulate both the number of charges (octa- *vs.* tetra-cationic porphyrins) and the length of the side alkyl chains, in order to evaluate the influence of these parameters on the structural and functional properties of the porphyrin systems obtained.

6.1 Synthesis of octacationic porphyrin–triphenylphosphonium (TMPyP–⁺PPh₃)

Octacationic porphyrins functionalized with triphenylphosphonium groups were synthesized using a post-functionalization approach based on *N*-alkylation of the *meso*-pyridyl units of a preformed porphyrin macrocycle. This strategy allows highly charged systems to be obtained while maintaining the porphyrin core unchanged and allowing direct control over the nature and length of the side chains introduced. In particular, two homologous derivatives were prepared, differentiated by the length of the alkyl linker, bearing 3 or 8 carbons, in order to evaluate the influence of the spacer length on the structural and functional properties of the octa-cationic porphyrins obtained.



Scheme 6.1. Synthesis of octacationic porphyrin TPyP-(CH₂)_n-⁺PPh₃ by *N*-alkylation of 5,10,15,20-tetra(4-pyridyl)porphyrin (TPyP) with the corresponding brominated bromoalkyltriphenylphosphonium salt.

6.1.1. Synthesis of the octacationic porphyrin derivative **C3** (TPyP-(CH₂)₃-⁺PPh₃)

The synthesis of the octa-cationic porphyrin–triphenylphosphonium conjugate was achieved by reacting TPyP with (3-bromopropyl)triphenylphosphonium bromide in *N,N*-dimethylformamide (DMF) used as the solvent. The reaction was carried out overnight at 100 °C, allowing the *N*-alkylation of the *meso*-pyridyl units by the bromopropyltriphenylphosphonium salt. At the end of the process, the porphyrin–triphenylphosphonium derivative **C3** was isolated with a quantitative yield, in accordance with the data reported in the literature.^{8,9}

The successful synthesis of this octa-cationic porphyrin–triphenylphosphonium conjugate was first confirmed by nuclear magnetic resonance (NMR) spectroscopy. The ¹H NMR spectrum shown in Figure 6.1 is consistent with the expected structure, showing the characteristic signals of the porphyrin macrocycle and the *meso*-pyridinium units functionalized with triphenylphosphonium groups.

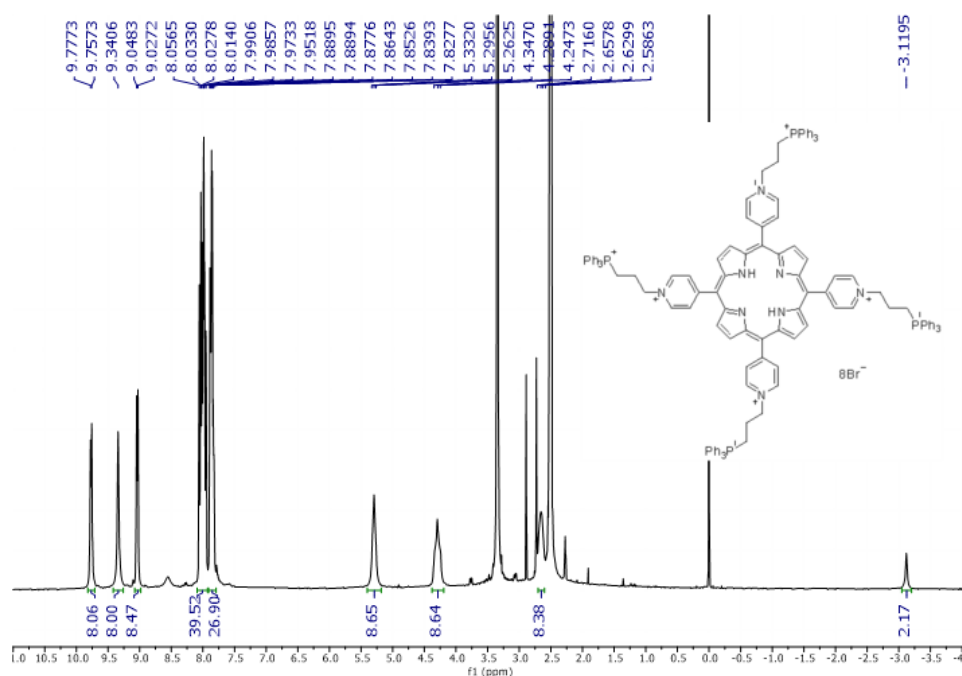


Figure 6.1. ^1H -NMR spectrum of the octa-cationic porphyrin TPyP⁺PPh₃ C3 obtained by *N*-alkylation with Br(CH₂)₃PPh₃ in DMSO-*d*₆.

The UV–Vis properties of the chromophore were analyzed and compared with those of reference porphyrins. The absorption spectra obtained for the TPyP⁺PPh₃ (C3) and TMPyP are typical of free-based *meso*-tetraarylporphyrins, characterized by D_{2h} symmetry (Figure 6.2). For the TPyP⁺PPh₃ compound, the Soret band, associated with π – π^* transitions from the ground state to the second excited state ($S_0 \rightarrow S_2$), shows a bathochromic shift of 6 nm compared to the neutral precursor TMPyP. As regards the Q band region, attributed to the less intense π – π^* transitions from the ground state to the first excited state ($S_0 \rightarrow S_1$), both compounds exhibit four characteristic bands, distributed in the range 516–645 nm. Overall, the introduction of triphenylphosphonium groups in the *meso*-pyridyl groups does not induce significant changes in the shape of the UV–Vis spectra and consequently on the UV–Vis properties.

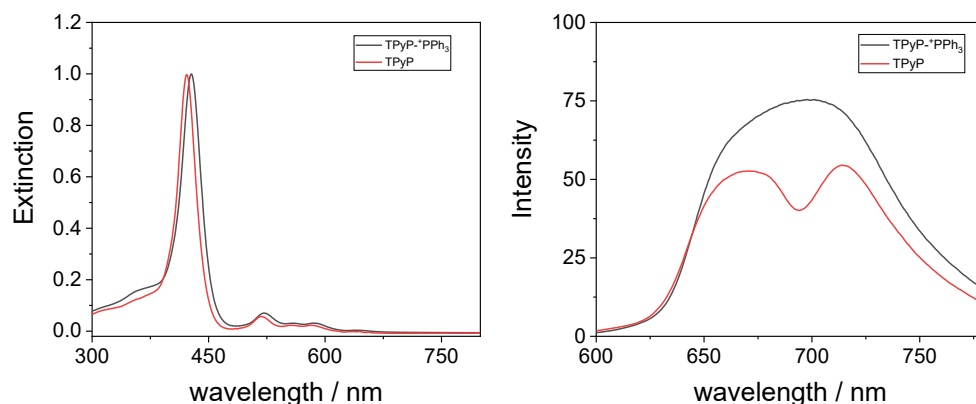


Figure 6.2. UV/Vis normalized spectra(left) and fluorescence (right) spectra of TMPyP and octa-cationic porphyrin **C3**-PPh₃, highlighting the effects of *N*-alkylation on the optical properties of the macrocycle. Experimental conditions: [TMPyP] = 3 μ M, [TPh⁺PPh₃] = 3 μ M, PBS 1 mM, pH 7; T = 298 K; path length = 1 cm

The fluorescence emission spectra of the neutral precursor TMPyP, used as reference, and the octacationic derivative TPyP⁺-PPh₃ **C3** are generally similar and show no substantial differences in terms of spectral position. However, some variations in the shape of the emission bands can be observed. In particular, the synthesized octa-cationic porphyrin exhibits a single broad fluorescence band, with a maximum emission centered around 700 nm, in contrast to the typical behavior of non-charged TMPyP, which shows two well-defined emission bands, located at approximately 690 and 714 nm, respectively.

This difference in the fine structure of the emission spectrum suggests a modification of the vibronic balance of the excited state, likely induced by the presence of triphenylphosphonium groups bound to the porphyrin pyridyl moieties, even in the absence of significant energy shifts of the main electronic transitions.

6.1.2. Synthesis of the octacationic **C8** derivative of porphyrin (TPyP-(CH₂)₈⁺PPh₃)

The intermediate (8-bromooctyl)triphenylphosphonium bromide was prepared by reacting 1,8-dibromooctane and triphenylphosphine in DMF at 100 °C under an inert atmosphere. The formation of the salt was confirmed by ¹H NMR spectroscopy, as shown in Figure 6.3, through the appearance of characteristic signals due to the resonance of the alkyl chain protons and triphenylphosphonium groups. The ¹H NMR spectrum is consistent with those expected for a monobrominated triphenylphosphonium salt, confirming the complete formation of the product prior to its use in the *N*-alkylation of porphyrin.

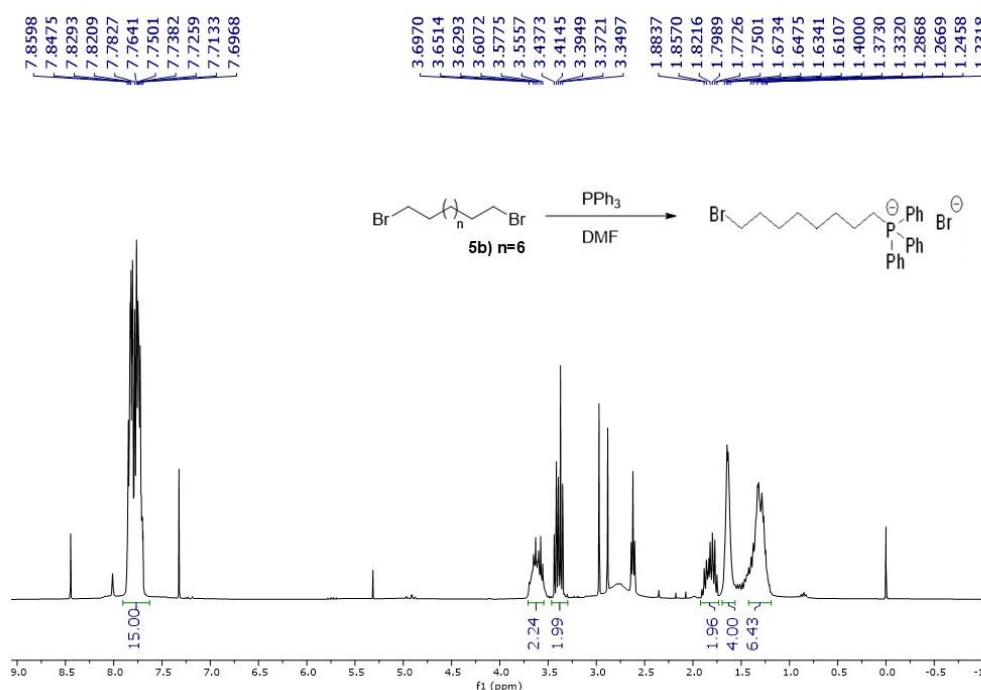


Figure 6.3. Synthesis of the phosphonium salt $\text{Br}(\text{CH}_2)_8\text{PPh}_3$ and its ¹H NMR spectrum in CDCl_3 .

Subsequently, TPyP was treated with (8-bromooctyl)triphenylphosphonium bromide in DMF at 100 °C overnight, allowing *N*-alkylation of the *meso*-pyridyl units. At the end of the reaction, the octacationic porphyrin-triphenylphosphonium derivative **C8** was obtained in high yield. The structure of the product was confirmed by ^1H NMR spectroscopy (Figure 6.4)

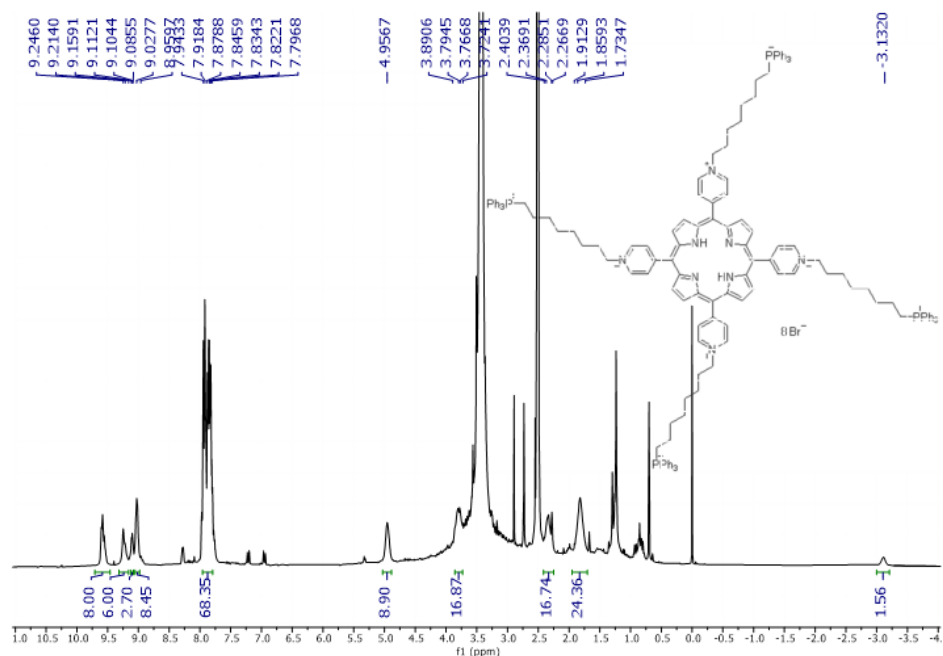
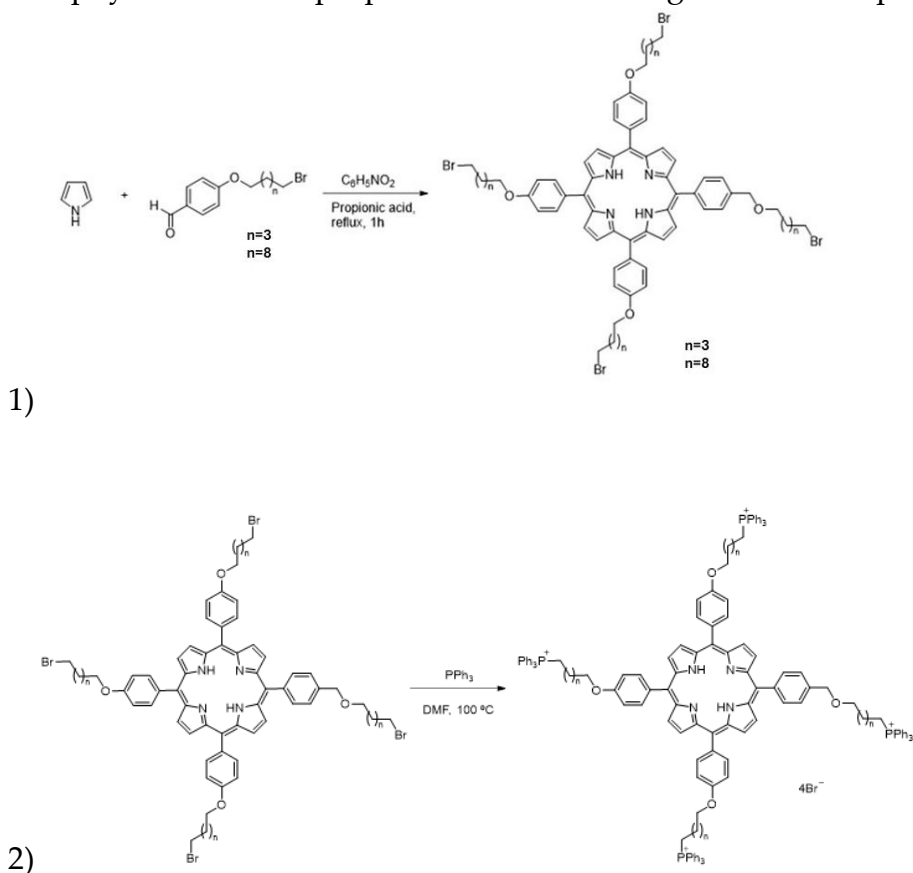


Figure 6.4. ^1H NMR spectrum of the octacationic porphyrin $\text{TPyP-}^+\text{PPh}_3$ (**C8**) obtained by *N*-alkylation with $\text{Br}(\text{CH}_2)_8\text{PPh}_3$ in $\text{DMSO-}d_6$

6.2 Synthesis of tetracationic porphyrin–triphenylphosphonium (TPPO- $^+\text{PPh}_3$)

In contrast to the octacationic systems, obtained through post-functionalization of preformed porphyrins, tetracationic porphyrin derivatives were synthesized following a different synthetic strategy, based on the preparation of alkylated aldehyde intermediates. This approach allows the introduction of the alkyl chains prior to macrocycle formation, providing a more controlled access to porphyrins bearing a

reduced number of charged substituents. In particular, two homologous derivatives bearing alkyl chains of different length (C5 and C10) were prepared in order to investigate the effect of chain length on the structural and physicochemical properties of the resulting tetracationic porphyrins.



Scheme 6.2. Synthesis scheme of bromoalkylporphyrins obtained by condensation of pyrrole with bromoalkyl aldehydes (**1**), which serve as precursors for subsequent functionalization with triphenylphosphonium groups via nucleophilic substitution (**2**).

6.2.1. Synthesis of the tetracationic porphyrin derivative C5 (TPPO-⁺PPh₃)

The synthesis of the bromopentyloxy-aldehyde represents the first key step in the modular strategy adopted to obtain tetracationic porphyrin derivatives. The introduction of a five-carbon alkyl chain allows for a moderate increase in the flexibility and hydrophobicity of the side substituent, while maintaining good reactivity of the aldehyde in the subsequent porphyrin condensation.

The successful alkylation of 4-hydroxybenzaldehyde was confirmed by ¹H NMR spectroscopy (Figure 6.5), which shows the characteristic resonance of the aromatic aldehyde proton, together with signals attributable to the resonances of the introduced alkyl chain protons. In particular, the appearance of multiplets in the aliphatic region and the disappearance of the phenolic proton resonance signal confirm the formation of the ether bond, in accordance with the expected molecular structure.

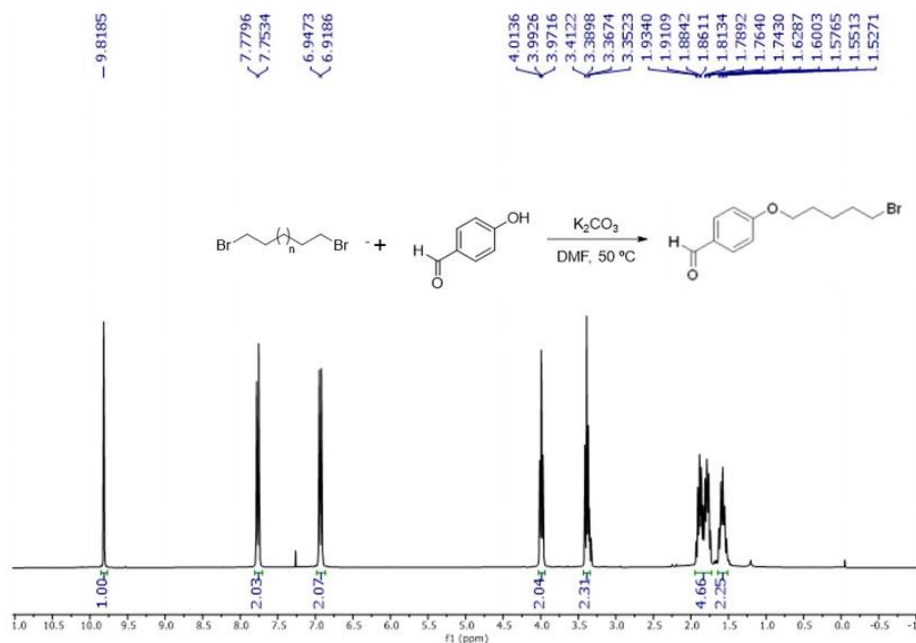


Figure 6.5. Synthesis of bromoalkyloxy-aldehyde by O-alkylation of 4-hydroxybenzaldehyde with Br(CH₂)₅Br, confirmed by ¹H NMR spectroscopy in CDCl₃.

The acid-catalysed condensation of bromoalkoxy-aldehyde with pyrrole led to the formation of the corresponding functionalized *meso*-tetrabromoalkoxyphenylporphyrin, obtained as a dark colored solid. The use of a solvent mixture of acetic acid and nitrobenzene proved effective in promoting the cyclisation and oxidation of the porphyrin macrocycle.

The structure of **C5** 5,10,15,20-tetrakis[4-(5-bromopentyloxy)phenyl]porphyrin was confirmed by ^1H NMR spectroscopy (Figure 6.6), which shows the characteristic resonances of the porphyrin nucleus and the alkyl side chains. The presence of signals attributable to the β -pyrrole protons (~ 9 ppm) and to the inner NH confirm (-2.7 ppm) the formation of the porphyrinic structure.

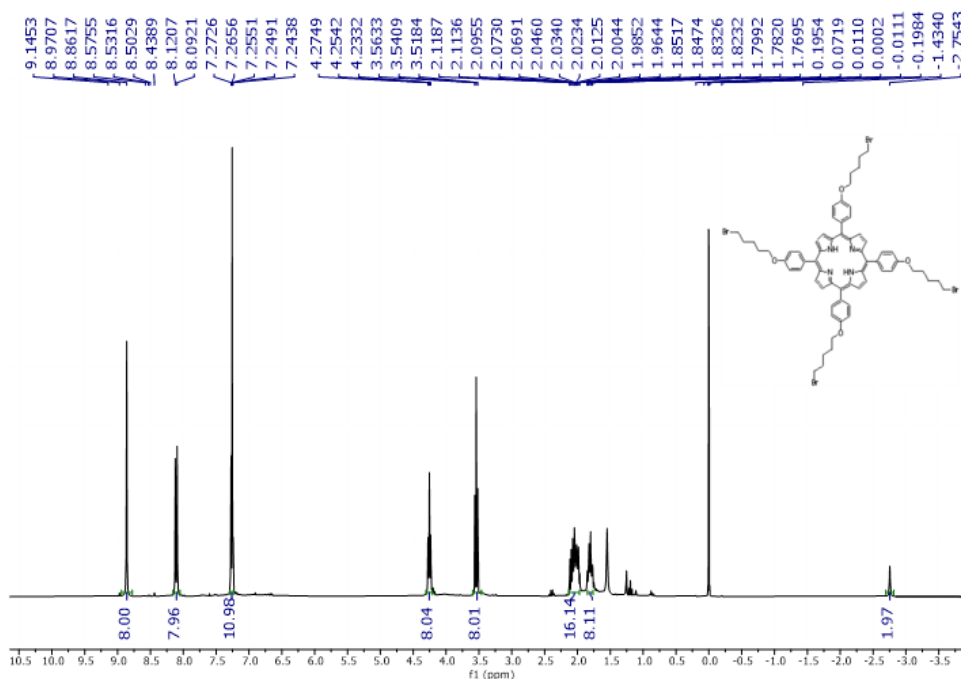


Figure 6.6. ^1H NMR spectrum of tetra-functionalized 5,10,15,20-tetrakis[4-(5-bromopentyloxy)phenyl]porphyrin in CDCl_3 .

The bromoalkoxy porphyrin with a five-carbon chain (C5) was subsequently functionalized by nucleophilic substitution reaction with triphenylphosphine, in order to obtain the terminal triphenylphosphonium groups. This step allows the transformation of the neutral porphyrin derivative into a tetra-cationic derivative, characterized by the presence of positive charges localized on the termini of the side chains.

The formation of the charged product was confirmed by ^1H NMR spectroscopy, which shows the disappearance of the signals associated with the $-\text{CH}_2\text{Br}$ groups and the appearance of the signals characteristic of the triphenylphosphonium units, in accordance with the expected structure (Figure 6.7).

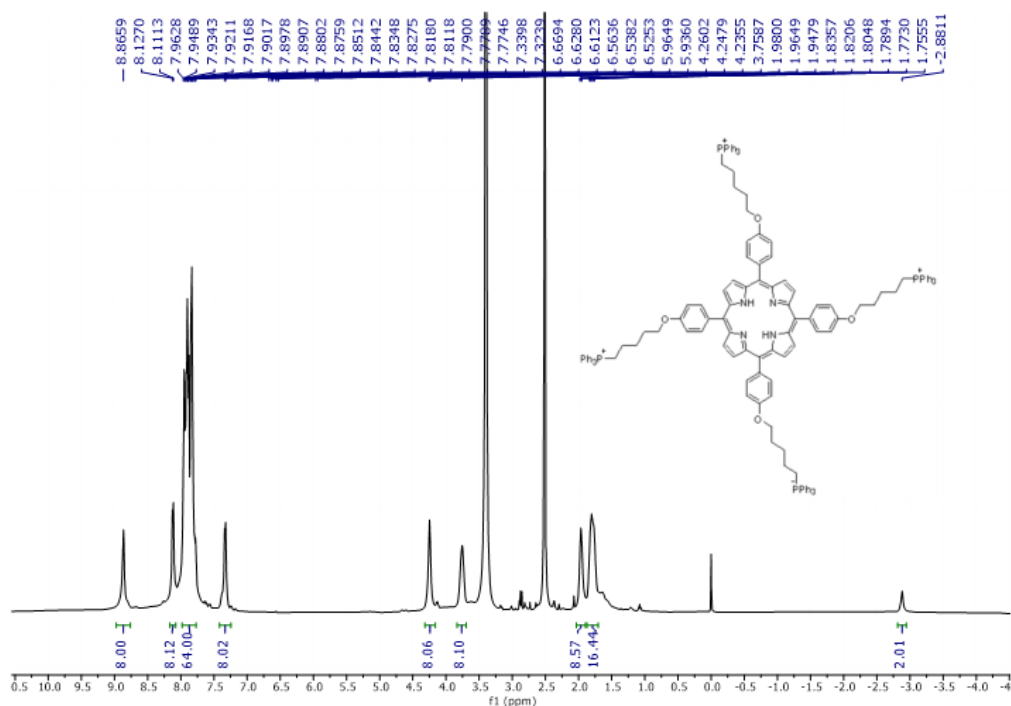


Figure 6.7. ^1H -NMR spectrum in DMSO-*d*₆ of the tetra cationic porphyrin **C5**-PPh₃ obtained by nucleophilic substitution with PPh₃.

6.2.2. Synthesis of the tetracationic porphyrin derivative **C10** (TPPO-⁺PPh₃)

Similarly, the 10-bromodecanoxy-aldehyde was synthesized by introducing a longer alkyl chain in order to evaluate the effect of the increased length of the substituent on the final properties of the tetracationic porphyrins. The extension of the chain to ten carbon atoms is expected to increase the hydrophobicity and conformational flexibility of the system, potentially influencing both the aggregative behavior and intermolecular interactions.

The ¹H NMR spectrum of the 10-bromodecanoxy-aldehyde (Figure 6.8) confirms the proposed structure, showing signals on the aromatic region due to resonance of the aromatic aldehyde protons and on the aliphatic region due to the resonance of the alkyl chain protons, consistent with the longer alkyl chain introduced. Again, the absence of the phenolic group signal and the presence of ether signals indicate the complete conversion of the starting reagent.

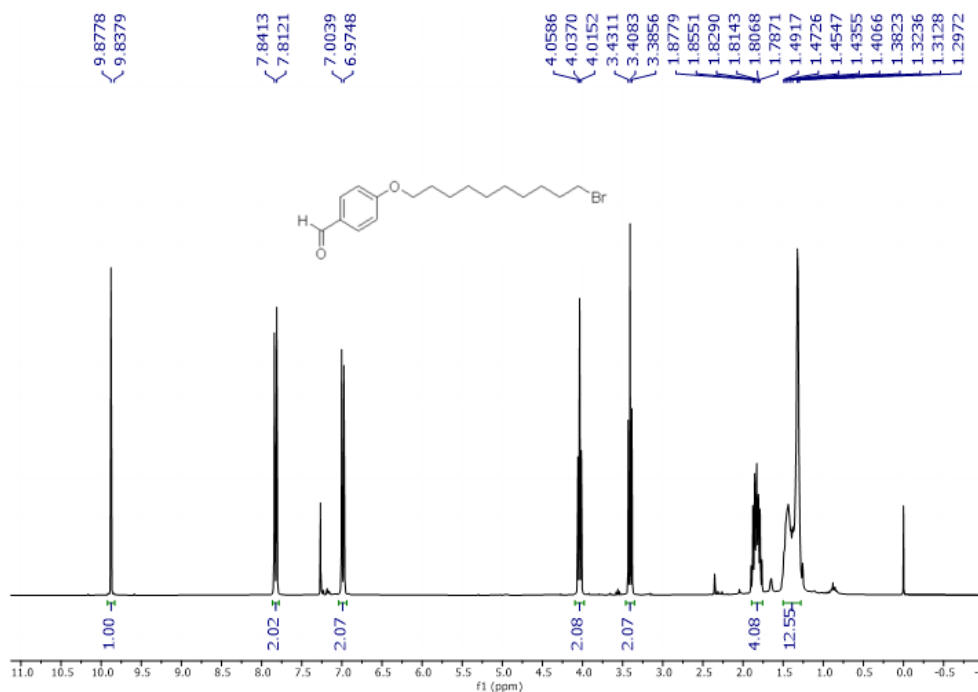


Figure 6.7. ¹H NMR spectrum of the alkylated aldehyde with an decyl chain (**C10**).

Similarly, the condensation of **C10** alkylated aldehyde with pyrrole allowed the preparation of tetrabromide porphyrin functionalized with longer side chains. The reaction conditions adopted allowed effective macrocycle formation, despite the increased flexibility and steric hindrance of the alkyl chains.

The ^1H NMR spectrum of **C10** tetrabromide porphyrin (Figure 6.8) is consistent with the proposed structure, showing an increase in the intensity of the aliphatic signals compared to the **C5** analogue, in accordance with the greater length of the chains. Again, the complete disappearance of the aldehyde signals confirms the success of the porphyrin condensation.

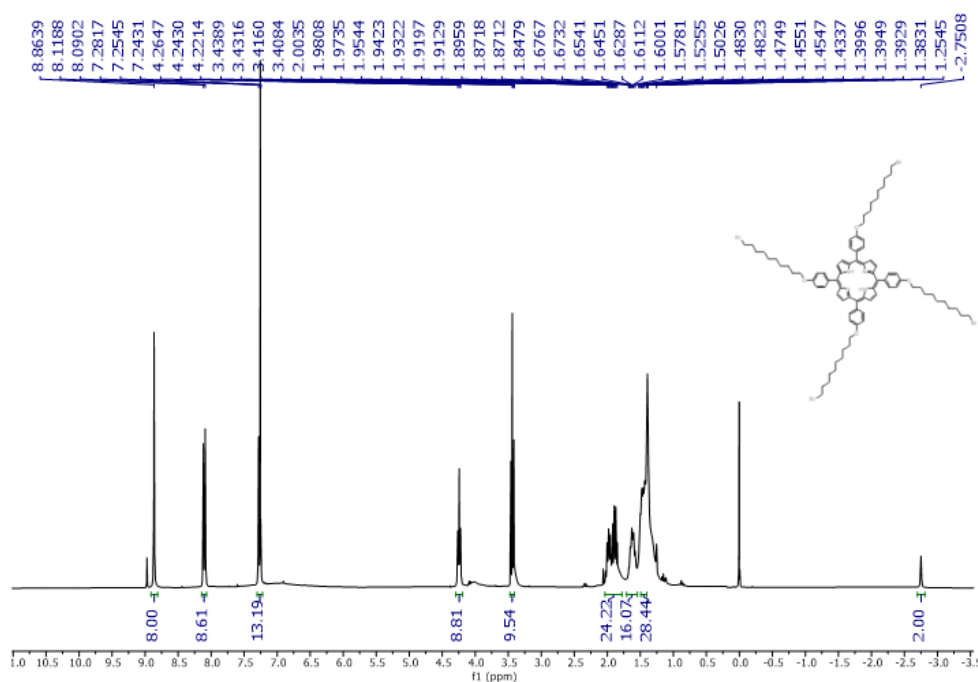


Figure 6.8. ^1H NMR spectrum of tetra-functionalized **C10-Br** porphyrin.

The brominated porphyrin derivative with ten-carbon alkyl chains (**C10**) was converted into the corresponding tetracationic compound through reaction with triphenylphosphine. This treatment allows the introduction of triphenylphosphonium groups at the ends of the side chains, giving the macrocycle a marked positive charge.

The successful transformation was confirmed by ^1H NMR analysis, which shows the complete disappearance of the signals attributable to bromine-bound methylene groups and the appearance of the characteristic resonances of the aromatic phosphonium units, in accordance with the expected structure.

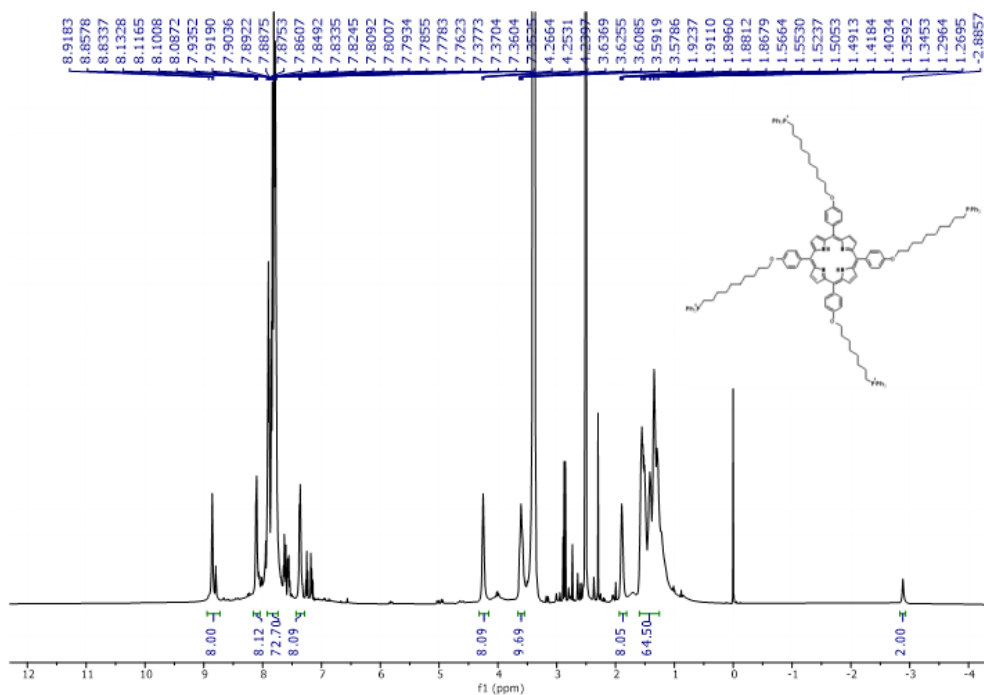


Figure 6.9. ^1H NMR spectrum in DMSO-d_6 of porphyrin **C10** after nucleophilic substitution of bromide with PPh_3 , confirming the formation of the corresponding phosphonium salt.

UV/Vis absorption spectroscopy was used to investigate the electronic properties of the tetracationic porphyrins **C5** and **C10** and to evaluate the influence of the alkyl chain length on their behavior in aqueous solution. Figure 6.10 shows the UV/Vis spectra of the two derivatives compared, recorded in water at comparable concentrations.

The tetracationic porphyrin **C5** shows a spectrum typical of a free porphyrin in monomeric form, characterized by an intense Soret band centered at 423 nm and four well-resolved Q bands at 520, 560, 597, and 653 nm. The presence of distinct and well-defined Q bands indicates limited intermolecular interaction in aqueous solution, suggesting that the moderate length of the alkyl chains allows good solubility of the porphyrin chromophore without promoting significant aggregation phenomena. On the contrary, the UV/Vis spectrum of the **C10 (x5)** derivative shows significantly different behavior. In this case, the longer alkyl chains cause a marked increase in the overall hydrophobicity of the molecule, promoting aggregation phenomena in an aqueous environment.

The comparison between the two spectra therefore highlights how the length of the alkyl chain plays a key role in determining the aggregation behavior of tetracationic porphyrins in water. While the **C5** derivative maintains predominantly monomeric behavior, the **C10** derivative tends to self-assemble, probably due to hydrophobic interactions between the side chains, resulting in a modification of the spectroscopic properties of the porphyrin chromophore.

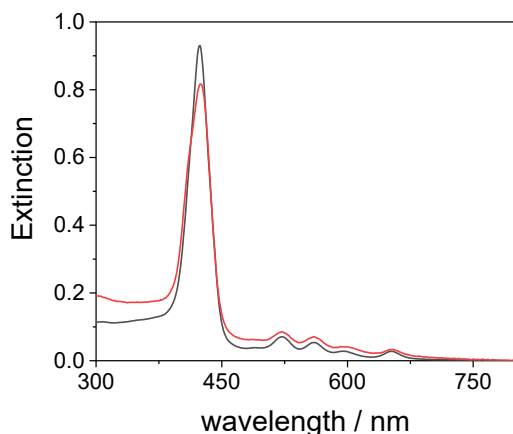


Figure 6.10. UV/Vis spectra of the tetracationic **C5** (black line) and **C10(x5)** (red line) derivative porphyrins in aqueous solution. Experimental condition [TPPO-⁺PPh3 **C5**]= 3 μ M ; [TPPO-⁺PPh3 **C10**]= 3 μ M T = 298 K; path length = 1 cm

6.3. Metal ion sensing

Preliminary studies on metal cation sensing were conducted using octa-cationic porphyrin functionalized with a three-carbon alkyl chain (**C3**). Attention was focused on copper(II) ions, chosen as a model metal cation due to their environmental and biological relevance.

The progressive addition of Cu^{2+} induced significant changes in the UV/Vis absorption spectra of **C3** porphyrin derivative (Figure 6.11, left). In particular, a progressive hypochromicity of the Soret band was observed, accompanied by a bathochromic shift of the absorption maximum, which changed from 428 nm to 431 nm as the concentration of the copper ion increased. At the same time, in the Q band region, there was a transition from four to two well-defined bands, a behavior typical of the formation of the metalloporphyrin complex, confirming the coordination of Cu^{2+} at the center of the macrocycle.

Fluorescence measurements show (in Figure 6.11, right) complementary behavior, revealing a marked quenching effect of porphyrin emission as the concentration of copper(II) increases. However, it is important to note that fluorescence quenching is observed at relatively high Cu^{2+} concentrations (approximately 50 μ M).

This result indicates that, although the octacationic porphyrin system shows a clear optical response to the presence of the cupric ion, the sensing performance can still be improved in terms of sensitivity. In this context, the integration of these porphyrins into more complex supramolecular systems could be an effective strategy for increasing recognition efficiency, amplifying the spectral response, and lowering the detection limit, paving the way for future applications in metal cation sensing.

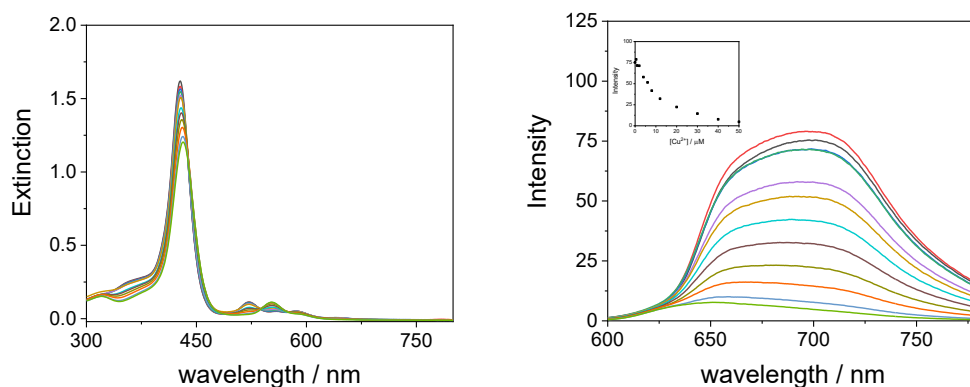


Figure 6.11. UV/Vis (left) and fluorescence (right) spectra of porphyrin **C3**–PPh₃ during titration with Cu^{2+} . Experimental conditions: [TPyP-⁺PPh₃ **C3**] = 5 μM ; $[\text{Cu}^{2+}]$ = 0–50 μM ; [PBS] = 1 mM; pH = 7; T = 298 K; cell path length 1 cm.

For reasons related to the time available, sensing studies were limited to octa-cationic porphyrin characterized by a shorter alkyl chain. However, the results obtained are sufficient to demonstrate the potential of the porphyrin platform functionalized with triphenylphosphonium groups in the recognition of metal cations.

Experimental part

Materials

For the synthesis of octacationic porphyrin TPyP (**C3** and **C8**) alkylated with propyl and octyl chains terminated by triphenylphosphonic groups, the procedure was performed following a protocol reported in the literature, with appropriate modifications in the experimental conditions. Specifically, 36 mg of TPyP was reacted with 600 mg of commercial salt (**C3**) or synthesized (**C8**) of (3-triphenylphosphonium)alkyl bromide $\text{Br}(\text{CH}_2)_n\text{PPh}_3$ ($n=3$ or 8), corresponding to 32 equivalents, in 1.5 mL of anhydrous DMF. The reaction mixture was kept at 100 °C under stirring overnight. After completion of the reaction, the derivative was purified by column chromatography using a $\text{CH}_2\text{Cl}_2/\text{MeOH}$ mixture as eluent.

(8-Bromooctyl)triphenylphosphonium bromide (**C8**) was synthesized by reacting triphenylphosphine (PPh_3) with 1,8-dibromoethane in a 1:2 molar ratio. The reaction was carried out at 100 °C for 24 h under a nitrogen atmosphere. Upon completion, the reaction mixture was cooled to room temperature, and the product was washed with diethyl ether (Et_2O). The solvent was added and the suspension was vigorously shaken until two phases formed; the supernatant phase was then removed. The washing procedure was repeated until complete purification of the triphenylphosphonium salt.

The alkyloxy-aldehyde was synthesized by alkylation reaction of 4-hydroxybenzaldehyde with 1,5-dibromopentane or 1,10-dibromodecane. In a flask, 4-hydroxybenzaldehyde was reacted with $\text{Br}(\text{CH}_2)_n\text{Br}$ ($n = 5$ or 10) in acetonitrile, in the presence of potassium carbonate (K_2CO_3 , 1 equivalent) as a base and tetrabutylammonium bromide (TBAB, 0.2 g) as a phase transfer catalyst. The reaction mixture was kept under reflux at 75 °C under magnetic stirring overnight. At the end of the reaction, the solvent was removed under reduced pressure, and the residue was subjected to liquid–liquid extraction by adding dichloromethane and water in order to remove inorganic salts. The organic phase was separated and concentrated, and the crude product was purified by silica gel column

chromatography using a dichloromethane/hexane (1:1, v/v) mixture as eluent, yielding the alkylated aldehyde as a pure compound in quantitative yield.

C5 and **C10** bromoalkyloxyporphyrin derivatives were obtained by acid-catalyzed condensation of bromoalkyloxy-aldehydes, with 5 and 10 carbon atoms, with pyrrole. The reactions were carried out in the presence of a mixture of propionic acid (2 equivalents)/acetic acid (1 equivalent) and nitrobenzene (1 equivalent) as a high boiling point solvent. The reaction mixtures were heated to 150 °C under stirring for 4 h, promoting the formation of the porphyrin macrocycle. At the end of the reaction, the two products were isolated by precipitation in excess methanol. The solids obtained were then recovered by filtration and washed with MeOH, yielding **C5** and **C10** bromoalkyloxy porphyrin derivatives as dark solid powders.

The tetrabrominated porphyrins **C5** and **C10** (were converted into the corresponding tetracationic porphyrins by nucleophilic substitution with triphenylphosphine, used in excess (1:9), at 110 °C overnight with stirring. At the end of the reaction, the cationic products were washed with methanol to remove excess reagent and then treated with toluene; the solvent was evaporated, yielding the pure dark products.

Conclusions

This chapter presented the synthesis, and characterization of a series of porphyrin derivatives functionalized with triphenylphosphonium groups, developed as molecular platforms for potential applications in metal cation sensing. The approach adopted allowed us to explore different synthetic strategies, based on both the post-functionalization of preformed porphyrin derivative and on the use of alkylated intermediates in the synthesis of the charged porphyrin macrocycle, allowing fine control of the number of positive charges and the length of the side chains.

In particular, octa- and tetra-cationic porphyrins were synthesized, differentiated by the length of the alkyl linker (**C3**, **C8**, **C5**, and **C10**), in order to evaluate the influence of these parameters on the structural and chemical-physical properties of the systems obtained. The success of the syntheses was confirmed by accurate ^1H NMR spectroscopic characterization, which showed full consistency between the proposed structures and the experimental data collected.

Preliminary sensing studies conducted on octacationic porphyrin **C3** demonstrated a clear optical response to copper(II) ions, evidenced by significant variations in the UV/Vis spectra and marked fluorescence quenching. Although these effects are observable at relatively high concentrations of Cu^{2+} , the results obtained confirm the ability of these porphyrins to interact effectively with metal cations and provide solid proof of concept for the use of triphenylphosphonium functionalized porphyrin systems in sensing.

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Experimental Part

7.1 General experimental techniques

Fourier transform infrared (FTIR) spectroscopy spectra in transmission mode were acquired in the mid-infrared range ($4000\text{--}380\text{ cm}^{-1}$) using a Bruker Vertex 80V spectrometer. Each spectrum was recorded with a resolution of 4 cm^{-1} , averaging 128 scans to improve the signal-to-noise ratio. Before each measurement, a background spectrum was collected in the absence of a sample, which was then subtracted from the experimental data to remove instrumental and atmospheric humidity contributions.

Micro-Raman measurements were performed using a Horiba LabRam HR800 spectrometer equipped with a confocal microscope and a liquid nitrogen-cooled CCD detector. The spectra were acquired at room temperature in the range $200\text{--}4000\text{ cm}^{-1}$, using a 600 gr/mm diffraction grating. Excitation was provided by a 532 nm laser focused through a $20\times$ microscope objective; the laser power, acquisition time, and number of accumulations were set at 5 mW , 5 s , and 20 , respectively.

X-ray photoelectron spectroscopy (XPS) analyses were performed using a PHI VersaProbe II instrument equipped with an Al $K\alpha$ source (1486.6 eV). Survey spectra were recorded with a pass energy of 117 eV , while the C1s, O1s, N1s, and Cl2p core levels were acquired at 23.5 eV .

To compensate for surface charge effects, a neutralization system using low-energy electron and ion beams was applied.

The binding energies were calibrated using a sheet of metallic Au (84.0 eV). Peak deconvolution was performed using Multipack software, adopting a Shirley-type background.

The UV/Vis extinction spectra were recorded with an Agilent 8453 diode array spectrophotometer and on a Shimadzu UV-2501 PC spectrophotometer. The temperature of the solutions was kept constant at 298 K using a water-circulating thermostatic bath.

Stationary fluorescence measurements were performed using a Jasco FP-750 spectrofluorometer, while time-resolved fluorescence measurements were performed using a Jobin Yvon-Spex Fluoromax 4 spectrofluorometer, employing the time-correlated single photon counting (TCSPC) technique. Excitation was provided by a 390 nm NanoLED. Time anisotropy measurements were performed on the same instrument using a motorized system of linear polarizers in excitation and detection.

The hydrodynamic dimensions of the samples were determined by Dynamic Light Scattering (DLS) using a Malvern Zetasizer Nano ZS instrument equipped with a 633 nm He-Ne laser and a detection angle of 173°. For each sample, 15 acquisitions of 10 s were performed, repeated three times.

The data were analyzed using Malvern's Dispersion Technology software, obtaining the intensity size distribution, the Z-average diameter, and the polydispersity index (PDI). The ζ potential was measured using a Malvern Zetasizer Nano ZS.

Electrochemical characterization was carried out by electrochemical impedance spectroscopy (EIS) using a galvanostatic potentiostat (Metrohm Autolab). Cyclic voltammetry (CV) and square wave voltammetry (SWV) measurements were performed with a DropSens μ Stat 400 potentiostat, controlled by DropView 8400 software for data acquisition and processing. The electrochemical response of the sensors was evaluated by CV and SWV in phosphate-buffered saline.

Circular dichroism (CD) spectra were recorded using a JASCO J-720 spectropolarimeter and corrected for solvent and cell contributions.

Atomic force microscopy (AFM) measurements were performed using an NTEGRA-Spectra NT-MDT microscope operating in tapping mode. The setup includes a confocal Raman spectrometer with a solid-state laser ($\lambda = 532$ nm). Scattered light from the sample was collected using a long-working-distance Mitutoyo objective (NA 0.75) and sent to a SOL MS3504I spectrometer equipped with an iDus Andor CCD camera.

Nuclear magnetic resonance (NMR) spectra were acquired using Bruker AVANCE 300 and Bruker AVANCE 500 spectrometers, operating at 300.13 MHz for ^1H -NMR. DMSO- d_6 was employed as the solvent, with tetramethylsilane (TMS) used as the internal standard.

7.2 Methods

- Chapter 1 – Electrostatic interactions and aggregation studies

Titration experiments were conducted by recording UV/Vis and fluorescence spectra on solutions contained in 1 cm quartz cuvettes, maintained at 298 K. In a typical procedure described, aliquots of a stock solution containing GQDs (4 mg/mL) and porphyrin (3 μ M) were progressively added to 2 mL of a pre-diluted solution of TPPS₄⁴⁻ (3 μ M). The pH of the solutions was controlled using a 1 mM phosphate buffer or HCl (pH 7 or 3) and checked by a Metrohm 827 pH meter calibrated with certified buffer solutions.

The kinetic data related to the aggregation of TPPS₄⁴⁻ under acidic conditions were analyzed using a cooperative kinetic model. The time trend of the extinction was described by the following equation:

$$\text{Ext}_t = \text{Ext}_\infty + (\text{Ext}_0 - \text{Ext}_\infty) \left[1 + (m - 1) \left(k_0 t + \frac{(k_c t)^{n+1}}{n + 1} \right) \right]^{-1/(m-1)} \quad (7.1)$$

where Ext_t , Ext_0 , and Ext_∞ represent the extinction at time t , at the initial time, and at the completion of the aggregation process, respectively; k_0 and k_c are the nucleation and auto-catalytic growth rate constants, while m and n are the cooperative parameters of the model.

- Chapter 2 – Metal ions titrations and metallation kinetics

The titrations with metal ions were performed by adding micromolar amounts of M^{2+} to solutions containing GQDs (0.018 mg/mL) and porphyrin (3 μ M), keeping the pH and ionic strength constant. The limit of detection (LoD) was calculated according to the following equation:

$$\text{LoD} = \frac{3.3 \text{ SD}}{\alpha} \quad (7.2)$$

where SD is the standard deviation of the white signal and α is the slope of the calibration curve.

The kinetic data relating to the metallation of TPPS_4^{4-} were analyzed assuming first-order kinetics. The temporal evolution of extinction was described by the equation:

$$\text{Ext}_t = \text{Ext}_\infty + (\text{Ext}_0 - \text{Ext}_\infty) e^{-k_{\text{obs}} t} \quad (7.3)$$

where k_{obs} represent the observed kinetic constant.

The fluorescence data were corrected for the secondary internal filter effect by applying the following relationship:

$$I_{\text{corr}} = I_{\text{obs}} \times 10^{\text{Ext}(\lambda_{\text{em}})} \quad (7.4)$$

When necessary, the contribution of Zn(II)TPPS₄ fluorescence was subtracted using the following expression:

$$I_{\text{corr}} = I_{645} - \frac{I_{606}}{1.71} \quad (7.5)$$

- Chapter 3 – Electrochemical measurements

Electrochemical measurements were performed using screen-printed carbon electrodes (SPCE), modified by depositing 10 µL of an aqueous solution of GQDs@TPPS₄⁴⁻ on the surface of the working electrode. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) measurements were performed in [Fe(CN)₆]^{3-/4-} solution. Electroanalytical determination of metal ions was performed by square wave voltammetry (SWV) in 0.1 M PBS at pH 4, using optimized deposition and scanning parameters.

- Chapter 4 – Metal ion titration and dissymmetry factor

Titration with metal ions were performed by incremental addition of micromolar amounts of M²⁺ to solutions containing 100 µM CMC and 5 µM porphyrin, in PBS 1 mM at pH 7.

The dissymmetry factor was calculated according to:

$$g = \frac{\Delta A}{A} \quad (7.6)$$

where ΔA represents the difference between the absorbance of left- and right-circularly polarized light ($\Delta A = A_L - A_R$), with A_L and A_R being the absorbances of left- and right-circularly polarized light, respectively. A is the total absorbance measured in the corresponding UV–Vis spectrum.

- Chapter 5 – Kinetics protocols and morphological studies

Kinetic measurements reported were obtained by repeatedly scanning UV/Vis spectra as a function of time at 298 K. The experiments were performed following a PF protocol, in which a defined volume of 6 M HCl was added to a pre-diluted aqueous porphyrin solution. The reagents were mixed by inverting the cuvette twice. Extinction data collected at 521 nm were fitted using Equation (7.1). Sterling Optics 105 UV polarizers were used to polarize the excitation beam and analyze the scattered light. The depolarization ratio, $q_v(90)$, was calculated as $q_v(90) = G \times I_{vH} / I_{vV}$, where $G = I_{HV} / I_{HH}$, I_{vV} and I_{vH} are the scattered light intensities for vertical and horizontal polarizations, and G corrects for instrumental sensitivity differences.

Thin films for morphological analysis were prepared by evaporating aqueous solutions containing J-aggregates onto microscope slides. AFM measurements were conducted to evaluate the film morphology. Raman measurements were performed in confocal mode using the integrated 532 nm solid-state laser, keeping the laser power in the μW range to prevent photodegradation of the molecules.

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