# CHAPTER 2

*meso*-Tetrakis(3,5-dihydroxyphenyl)N-Confused Porphyrin: Tautomeric Existence, Exchange and Influence of Tautomerism and Anions on Morphological Features

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2.1 Abstract

This chapter describes the synthesis, spectral and structural characterization, solvent dependent tautomeric existence and exchange of a novel octa-hydroxy N-confused porphyrin derivative; meso-Tetrakis(3,5-dihydroxyphenyl)N-Confused Porphyrin (NCPH). The tautomeric existence and exchange of NCPH was investigated through various spectroscopic techniques such as UV-Vis, fluorescence and FT-IR spectroscopy in different polar protic and aprotic solvent combinations. A combination of DMSO/ACN, DMSO/H$_2$O, MeOH/ACN and MeOH/H$_2$O were used for the investigations. Solvent driven aggregation in these combinations was also monitored through scanning electron microscopy (SEM) and atom force electron microscopy (AFM) analysis. The morphological features observed in the different solvent combinations was explained on the basis of difference in the hydrogen bonding formation possible due to the existence of different tautomeric forms in the particular medium. Further, the role of anions in determining the morphology during aggregation was studied.
2.2 Introduction

It was exactly five decades before Furuta and Latoń isolated NCP independently [62, 63], Melvin Calvin and Linus Pauling formulated the structure of N-confused porphyrin [115]. In their 1943 paper entitled “The porphyrin-like products of the reaction of pyrrole with benzaldehyde” Calvin and his graduate student Sam Aronoff described about a Rothemund-type condensation reaction between pyrrole and benzaldehyde and identified six condensed products, but unable to analyze the products [116]. Notably, they formulated several porphyrin isomers with one or two inverted pyrrole rings, which they named “carboporphyrins”, similar to the current nomenclature.

On the other hand, Linus Pauling, one amongst the most influential scientific personality in the modern era, contributed in various areas of science including structure and bonding of molecules, genetics, evolution, haematology, immunology, brain research, biomedicine and nutritional therapy [115]. Particularly to the porphyrin chemistry, porphyrin isomers and the prediction of stability of various isomers including “extroverted” pyrrole rings based on his analysis of the electronic structure was remarkable. In fact, he was trying to determine the number of possible resonance structures with eleven double bonds that can be drawn for each type of system to assess their stability, where he could predict the possible existence of porphyrin derivatives with one, two and three extroverted pyrrole rings [115].

2.2.1 Tautomerism, historical background

The phenomenon tautomerism was recognized in the 19\textsuperscript{th} century, where Gerhardt describes this phenomenon in his book on organic chemistry [117]. The name “tautomerism” was introduced by Laar [118], to describe the properties of organic compounds that can react as if they have two or more structures. The term “tautomerism” (in Greek, \textit{tauto}-same and \textit{meros}-part) refers to a compound existing in equilibrium
between two or more labile isomeric forms called the tautomers and usually the tautomers differ in the point of attachment of a hydrogen atom [119]. A common type of tautomer was found in ketones, where one proton transfer generates enolic form and is readily interconvertible constitutional isomers that exist in equilibrium with each other, usually referred as keto-enol tautomerism. The investigations on ‘keto-enol’ tautomerism initiated at the end of 19th century, when in 1863 Geuther proposed the enol structure for acetoacetate ester [118], but a few years later Frankland [120] and then Wislicenus [121] assigned the keto structure, revealed that acetoacetate can exist in these two forms. Further, the existence of keto-enol tautomerism was illustrated by Claisen in 1896 with acetyldibenzoylmethane and tribenzoylmethane [122], Wislicenus for methyl and ethyl formylphenylacetate [123] and by Knorr for ethyl dibenzoysuccinate and ethyl diacetylsuccinate [124]. The existence of the different tautomeric forms depends on the stability of that particular tautomer due to the $\pi$-electron delocalization and in fact, this extra stability helps to explain why a particular compound prefers a different tautomeric form than the other.

### 2.2.2 Tautomerism in N-confused porphyrin

The inversion of one pyrrolic ring in N-confused porphyrins (NCP) exhibit significant differences in their physical and chemical properties with respect to normal porphyrins. Interestingly, not so longer after the introduction of N-confused derivative, the possible tautomeric existence of NCP was initially demonstrated by Abhik Ghosh in 1995 [125]. While investigating the unexpected labile nature of the inside C-H bond in the confused porphyrin, he compared the inverted pyrrolic ring with an imidazolium carbene and calculated the energy difference between the tautomers. Later, Latos-Grażyński and co-workers exemplified the tautomeric existence of the molecule with nickel complexes of various oxidation state [126, 127], where, the paramagnetic nickel complexes of carba-
methylated and nitrogen-methylated NCP adopted different geometries to stabilizes the metal complexes [126].

![Figure 2.1. Possible tautomeric structures of NCP.](image)

In 1997, the same group predicted the existence of the ‘hypothetical’ tautomers of the carbaporphyrins [128] (used the term 2-aza-21-carbaporphyrin for NCP) and their structures. The electronic energies have been investigated by applying density functional theory (DFT) for idealized 21-carbaporphyrin derivatives such as, 21-CPH$_2$ (N-confused derivative with three hydrogens inside the core, one with inside carbon and two with nitrogen atoms), 21-H-21-CPH (three hydrogens inside the core, two with carbon and
one with nitrogen), **2-NH-CPH** (two hydrogens inside, one with carbon and another with nitrogen), **2-NH-CPH**₂ (carbenic form with two hydrogens with nitrogen atom), **2-NH-21-H-21-CP** (two hydrogen atoms with the inside carbon) with respect to normal porphine derivative, **PH**₂ as shown in Figure 2.1. The calculated total electronic energies, using the B3LYP/6-31-G***/B3LYP/6-31G approach, demonstrate that relative stability of the postulated tautomers decreases in the order **21-CPH**₂ > **21-H-21-CPH** > **2-NH-CPH** >> **2-NH-CPH**₂ > **2-NH-21-H-CP** [128].

Later, Ghosh *et al.* have carried out a broad survey of the molecular structures of porphyrin derivatives including **NCP** and its core modified derivatives [129]. The relative energies of different tautomeric forms have been calculated and correlated with structural features of the respective macrocycles. The DFT calculations suggested that the internally protonated three hydrogen tautomer (**3H**) is more stable compared to the externally protonated analogue (**2H**) by ~5.7 kcal/mol, which was confirmed later by Modarelli and co-workers [129, 130]. Even though there is a macrocyclic conjugation break in the **2H** tautomer with respect to **3H** form, which is resonance and hydrogen bond stabilized, the less energy difference between the two forms was attributed to the steric crowding in the **3H** form. The carbenic tautomer (**2-NH-CPH**₂) was found to be destabilized by ~33 kcal/mol compared to **3H** tautomer, which in turn destabilized by a factor of 17.7 kcal/mol with respect to normal porphyrin as shown in Figure 2.2 [129].
Figure 2.2. Relative stabilization energy of different tautomers of NCP.

Figure 2.3. (a) Absorption spectra and the colour of two confused porphyrin tautomers [131] and (b) emission spectra of the tautomers [130].
In 2001, Furuta and co-workers demonstrated the tautomeric existence of NCP (3H and 2H) both in solution as well as in solids [131]. A DMF solution of the compound showed green colour in solution, where the CHCl₃ solution exhibited red colour and also differ in their absorption spectra as shown in Figure 2.3. The two N-confused tetraphenylporphyrin (NCTPP) tautomers observed in different solutions were stabilized by hydrogen bond formation. One tautomer has an external N-H group (2H) and is favoured in highly polar solvents such as DMF. The second tautomer has two internal N-H groups (3H) and is the preferred tautomer in aromatic and halogenated solvents.

Furuta and co-workers investigated the tautomeric existence of NCTPP in DMF-d7 and CDCl₃ using ¹H and ¹⁵N NMR spectroscopy. The ¹H NMR spectra recorded in CDCl₃ showed two peaks at -4.99 and -2.41 ppm, which was assigned to the inner CH and two NH protons respectively corresponding to the 3H tautomer. Where, ¹H NMR spectrum in DMF showed singlet peaks at 0.76, 2.27 and 13.54 ppm, assigned to the inner CH, inner and outer NH protons respectively corresponding to the 2H tautomer. The ¹⁵N NMR spectrum of 3H tautomer showed four signals at 135.64, 137.16, 238.09 and 303.58 ppm in CDCl₃ at ambient temperature. The first two sharp peaks (135.64 and 137.16 ppm) were assigned to the inner protonated nitrogens while the third peak (238.09 ppm) was for non-protonated inner pyrrolic nitrogen. The fourth broad peak (303.58 ppm) was corresponded to the exterior pyrrolic nitrogen that is magnetically coupled to the adjacent α-CH. The ¹⁵N signals for 2H tautomer was resonated at 129.82, 175.72, 267.54, and 271.38 ppm were observed in DMF-d7. The signal at 129.82 ppm was assigned to the inner NH, while the second peak (175.72 ppm) was corresponded to the outer NH. The third and fourth signals (267.54 and 271.38 ppm) was for the interior imine nitrogens, and mentioned that the weaker aromaticity of the 2H tautomer was supported by the smaller chemical shift difference between the interior and exterior.
nitrogens [131]. The structure of the tautomers in solid state were confirmed by single crystal X-ray structure, where the $2H$ form crystallized in DMF showed intermolecular hydrogen bonding with solvent and attained comparatively planar structure with respect to the $3H$ tautomer formed in DCM solution, which is significantly distorted from the planar structure as shown in Figure 2.4 [131].

![Figure 2.4. X-ray single crystal structure of NCTTP obtained from (a) DMF-MeOH ($2H$) and (b) DCM-MeOH ($3H$). For clarity meso-phenyl groups are omitted in the side views [131].](image)

As explained before, the geometry of the two tautomeric structures have distinct difference, which in turn influence the shape and energy of their occupied and unoccupied orbitals. However, both for $3H$ and $2H$ the HOMO and HOMO-1 orbitals are reversed in energy relative to tetraphenylporphyrin (TPP) and have $a_{2u}$ symmetry (HOMO) and $a_{1u}$ symmetry (HOMO-1). The energy difference between these orbital is 0.7156 for $2H$, where for $3H$ it is comparable with TPP. This energy difference is substantially larger than that found in either TPP (0.2220 eV) or tetraphenylchlorin (TPChl) (0.1412 eV) and according to Modarelli these energy difference arises from destabilization of the $a_{2u}$ HOMO which is 0.4528 and 0.3034 eV higher in energy than
the HOMO of TPP and TPChl, respectively. The HOMO-LUMO gap is 2.4580 eV (compared to 2.7609 eV for TPP) for the 3H form. On the other hand, the HOMO-LUMO gap in 2H tautomer was 2.122 eV [130].

Absorption spectra of both tautomers showed distinct difference in the Soret and Q-band pattern and were red-shifted significantly with larger extinction coefficients than those of TPP as shown in Figure 2.3. In polar solvents such as DMAc and DMF the externally protonated form (2H) is more stable, which is explained either by hydrogen-bonding or dipole-dipole interactions of the exocyclic N-H bond with solvent. Whereas, less polar solvent such as CHCl₃ favors the internally protonated 3H form. The Soret band of 2H tautomeric form was observed at 441 nm, where as that of 3H was at 438 nm. The Q-band pattern were also showed distinct difference for the two tautomeric structures [130, 132]. Both tautomers exhibit their characteristic emission bands (Figure 2.3.b) in different solvent systems and also differ significantly on their fluorescence quantum yield and singlet lifetime. The 3H tautomeric form give two bands in the emission spectrum with almost similar intensities at 744 and 815 nm, where as the 2H form shows a major band at 713 nm and a shoulder at 783 nm.

2.2.3 Porphyrin aggregates

Compared to solution phase, surface-confined molecular assemblies display much greater coherence in their orientation, structure, and ultimately, function. Intermolecular and molecule substrate interactions govern these self-assembly processes and fine-tuning of the chemical and/or physical properties that influence these interactions can allow control over the structure and molecular distribution during self-assembly. On the other hand, there are systems that consist of molecules sensitive to aggregate with certain stimulus such as pH, temperature, light, solvents, guest molecule etc and the morphology can be modulated or controlled by these stimuli.
Solvent and other external input driven self-assembled nanostructures of organic molecules driven by various interactions are of great interest in the area of storage and conversion of solar energy, sensors, catalysis and biological applications [133-135]. For example, porphyrin assemblies play major role in biologically relevant systems such as chlorophyll and bacteriochlorophyll [136, 137]. In fact, molecules of bacteriochlorophylls, a porphyrin derivatives assemble to form the nanorods in the chlorosomes of green bacteria, and these chlorosomal rods are the largest and most efficient harvesters of light known [138, 139].

Usually, self-assembly of porphyrin nanostructures can be induced by various means such as reprecipitation, coordination polymerization, surfactant induced self-assembly and ionic self-assembly [136, 137, 140]. Here, in the reprecipitation method the compound is dissolved in a suitable solvent and reprecipitates with cooling, where in coordination and ionic and self-assembly methods the respective intermolecular interactions forms the assemblies. The solvent assisted and surfactant induced assemblies make use of the rapid exchange of solvents or mixing of solvents containing the chromophoric molecules with miscible solvents in which the compound is not soluble [137].

Shelnutt and co-workers showed robust porphyrin nanotubes, which was prepared by ionic self-assembly of two oppositely charged porphyrins in aqueous solution. The nanotubes are composed entirely of two ionic porphyrins as shown in the Figure 2.5. The electrostatic forces between these porphyrin units, in addition to the van der Waals, hydrogen bonding, axial coordination, and other weak intermolecular interactions that typically contribute to the formation of porphyrin aggregates, enhance the structural stability of these nanostructures [140].
Figure 2.5. (a) Structure of the charged porphyrin units (b) TEM images of porphyrin nanotubes formed through ionic interactions between the porphyrin units [140].

Later, the same group demonstrated photoinduced self-metallization process of porphyrin nanofibers and nanosheets. The photoactivity of porphyrin nanofibers were illustrated by their self-metallization reactions with various metals such as silver, gold and platinum [141, 142].

Figure 2.6. TEM images of cobalt-porphyrin nanorods at different temperatures: (a) 273 K, (b) 298 K, (c) 308 K, (d) 318 K, and (e) 328 K [143].
Guo and co-workers prepared the nanorods of a chiral cobalt porphyrin dimer by directly bridging with L-glutamic acid dimer using liquid-solid-solution technologies and found that the aggregate morphologies of the porphyrin dimer changes upon varying the temperatures (Figure 2.6). The porphyrin dimer transformed from H-aggregate to J-aggregate with an increase of the temperature under the same experimental conditions. The reason is that the J-aggregate is thermodynamically stable and hence promotes the formation of the J-aggregates with the increase in temperature [143].

In contrast to the above described physical stimuli, chemical stimuli, such as the incorporation of a specific species like anions and cations, could afford versatile supramolecular structures quite different from the previous conditions depending on the interactions between the additives and the molecules. There are several examples where the morphology transitions are controlled by cations [144, 145], but the design of anion receptors with high selectivity is challenging. To form anion-responsive dimensionally controlled organized structures, the molecules must act as anion receptors by possessing one or more of van der Waals interaction units (aliphatic chains), stacking \(\pi\)-planes, hydrogen-bonding sites, and metal-coordination units [146]. These interactions can be made possible by using complimentary binding sites with hydrogen-bonding groups, quaternary ammonium centres, Lewis acids and cationic metal ions. In particular, hydrogen bonding driven interaction, because of its directional nature [147]. Hence, neutral anion receptors having amide, urea, pyrrole units employed in a variety of both cyclic and acyclic receptors are an important subset of the anion binding agents [148-151].

In 2002, Král and co-workers reported the first self-assembly of porphyrin-bicyclic guanidine conjugate (PG) upon addition of small anions and forms chiral structures which was controlled by the type of anion used. Addition of anions such as \(\text{CH}_3\text{CO}_2^–\)
(Figure 2.7.c), H$_2$PO$_4$, terephthalate, or anthraquinone-1,5-disulfonate (Figure 2.7.d) to PG in water (Figure 2.7.b) causes an additional decrease and a split of the Soret band of the monomer accompanied by the appearance of two broad maxima at about 405 and 428 nm [152].

![Figure 2.7. I) Structure of PG, and II) absorption spectra of PG in (a) MeOH, (b) H$_2$O, (c) 10 mM acetate and (d) 0.5 mM anthraquinone-1,5-disulfonate [152].](image)

**Figure 2.8.** (a) Structure of [H$_4$T(4-STh)P$^{2-}$], (b) AFM images of the deposited J-aggregate prepared from the aqueous solution of [H$_4$T(4-STh)P$^{2-}$] with 2 M HCl and (c) scheme for the H- and J-aggregate formation [153].

Recently, Segawa and co-worker demonstrated the formation of both H- and J-type aggregate with porphyrin diacid, meso-tetrakis(4-sulfonatothienyl)porphyrin [H$_4$T(4-STh)P$^{2-}$, Figure 2.8.a] complexed with Cl$^-$ ion in aqueous solution. The H-aggregate is
formed preferably at dilute conditions, but further addition of Cl\(^-\) caused the transformation of H- into J-aggregate. AFM image showed that the J-aggregate as rod-shaped nanostructures composed of highly oriented molecules (Figure 2.8.b). The stacking structures of the H- and J-aggregates are proposed to be a slipped face-to-face dimer and an edge-to-edge polymer, respectively, where the porphyrins are mediated by two Cl\(^-\) anions (Figure 2.8.c) [153].

However, aggregation properties and corresponding morphological changes of NCP derivatives were reported scarcely. Furuta and co-workers reported anion induced dimerization of a meso-unsubstituted NCP (3-oxo-NCP) which formed self-assembled dimer in DCM that is stabilized by complementary hydrogen-bonding interactions arising from the peripheral amide-like moieties [154, 155]. The protonated form of 3-oxo-NCP was found to bind halide anions such as F\(^-\) and Cl\(^-\) ions through the outer NH and the inner pyrrolic NH groups, thus affording an anion bridged dimer.

In 2008, Furuta and co-workers reported the aggregation behaviour of tetra glucamine appended N-confused derivative, TG-NCP. It exists as monocation in aqueous solution along with 6 mM sodium dodecyl sulfate (SDS) but forms aggregates in pure water. These properties were distinct from those of corresponding regular porphyrin, which exists as freebase in the micellar solution and practically insoluble in water. They elucidated the acid/base properties of TG-NCP under the monomeric and aggregated conditions, quantitatively by pH titrations in the presence/absence of 6 mM SDS and found that the dominant species of TG-NCP (in 6 mM SDS) solution at pH 11, 7.8-4.2, and 0.83 can be assigned as freebase, monocation and dication respectively. Whereas in acidic pH without SDS TG-NCP would represent a disassembly process of aggregated freebases to monomeric dications [156].

**2.3 Objective of the Work**
As per the above mentioned discussions, porphyrin based nanoarchitectures are well established. However, the supramolecular assemblies of N-confused porphyrin and its derivatives are not explored much in the literature. Hence, this chapter discusses the synthesis, spectral and structural characterization of an octahydroxy N-confused porphyrin derivative (NCPH), and demonstrates the tautomeric existence and exchange of NCPH in polar protic and aprotic solvents. Further, shows interesting morphological features during aggregation with respect to different stimuli such as tautomeric existence and presence of anions.

2.4 Results and discussion

2.4.1 Synthesis and structural characterization of NCPH

![Synthetic scheme for NCPH](image)

Scheme 2.1. Synthetic scheme for NCPH [157].
**NCPH** was prepared through a two step synthetic strategy as shown in Scheme 2.1, where in the first step corresponding methoxy derivative was prepared by using Lindsey’s method, followed by demethylation using borontribromide to form NCPH in 80% yield [64]. The compound was found to be highly soluble in polar solvents like MeOH, DMF and DMSO however, insoluble in H₂O and ACN. The structure of NCPH was confirmed through various spectroscopic techniques such as ¹H, ¹³C NMR, FT-IR, FAB-MS analysis. Purity of the compound was determined as > 95% using HPLC and elemental analysis.

**Figure 2.9.** ¹H-NMR spectrum of NCPH in DMSO-d₆ (a) structure of NCPH and (b) upfield region of ¹H NMR spectrum.

The ¹H NMR spectrum of NCPH in DMSO-d₆ is shown in Figure 2.9 [157]. The two broad peaks centered at 10.00 ppm corresponding to the phenolic OH protons. The confused pyrrolic NH proton is resonated as a broad singlet at δ = 14.3 ppm as expected for the 2H tautomer of the confused derivatives in polar solvents. The outer α and β-CH protons were resonated between 8.23-8.75 ppm and phenyllic CH’s between 6.77-7.27 ppm. The inner pyrrolic NH and β-CH were observed at δ = 2.14 and δ = -1.82 ppm respectively, which were downfield shifted compared to the other N-confused
derivatives. The presence of acidic protons such as pyrrolic NH and phenolic OH were further confirmed by deuterium exchange experiments with D₂O. The FAB-MS analysis showed the M + 1 peak at 743.83 and the phenolic OH stretching frequency observed at 3422 cm⁻¹ from the FT-IR spectral analysis confirms the structure of the molecule.

2.4.2 NH Tautomerism in NCPH

![Absorption spectra of NCPH in different solvent systems.](image)

**Figure 2.10.** Absorption spectra of NCPH in different solvent systems.

Absorption spectra of NCPH was recorded in three different solvents such as MeOH, DMF and DMSO (Figure 2.10) to investigate the effect of solvent polarity as well as to get an insight into the stable tautomer possible for the molecule in these solvents [157]. The spectra showed the Soret band at 443, 447 and 450 nm in MeOH, DMF and DMSO respectively with progressive red-shift as expected from the increase in solvent polarity. The molar extinction coefficient of NCPH was found to be 9.27 × 10⁴, 9.35 × 10⁴ and 9.53 × 10⁴ M⁻¹ cm⁻¹ for the Soret bands respectively. The Q-bands in MeOH solution exhibit peaks at 539 and 581 nm due to a Qₓ(1,0) and Qᵧ(0,0) transition whereas Qₓ(1,0) and Qᵧ(0,0) transitions were responsible for 653 and 727 nm bands.
which extend up to 900 nm. On the other hand, the Q-bands in DMSO solution show bands at 547, 595, 644 and 697 nm with Q-band oscillator strengths increases with decrease in energy in a regular fashion, where $Q_x(0,0)$ showing the maximum intensity and $Q_x(1,0)$ with the lowest. Interestingly, the Q-band pattern in MeOH and DMSO shows distinct discrimination and enlights the possible existence of different tautomeric form in these solvents irrespective of their high polar nature. The nature of NCPH Q-bands in MeOH was comparable with Q-band pattern of NCTPP in CHCl$_3$ to give indication about the existence of internally protonated 3H tautomer in MeOH, where, the Q-band pattern in DMSO was similar to that of NCTPP in DMF, which predicts the presence of NCPH in the externally protonated 2H tautomeric form in DMSO [157].

The emission characteristics of NCPH were recorded in protic and aprotic polar solvents on excitation at the respective absorption maximum as shown in Figure 2.11. In DMSO and DMF, the compound was showing emission maxima at 720 and 717 nm respectively corresponding to a $Q_x(0,0)$ emission with a shoulder at around 780 nm for $Q_x(0,1)$ emission [157]. The spectral pattern were comparable with the emission characteristic of NCTPP in polar solvents like DMF and DMAc, where the 2H tautomer predominates over the 3H tautomer and confirms the existence of NCPH in the externally protonated form in DMSO and DMF. In contrast, MeOH solution of NCPH shows two bands of equal intensity at 725 and 785 nm similar to NCTPP in CHCl$_3$ substantiates the occurrence of NCPH in the internally protonated 3H tautomeric form. Further, the fluorescence quantum yield was measured with respect to TPP as reference and the yield obtained was 0.0013 in MeOH. Whereas, aprotic solvents showed a tenfold increase in the emission quantum yield as 0.013 and 0.019 in DMF and DMSO respectively. The fluorescence life time of NCPH in the different tautomeric form has been recorded using a picosecond time-correlated single-photon counting (TCSPC)
technique (Figure 2.11 inset). The life time of the internally protonated form showed a biexponential decay profile with life time of 0.7 and 1.27 ns having 31 and 69% contribution each respectively and an average life time of 1.1 ns. On the other hand, the life time values obtained in DMF and DMSO were 1.87 ns and 2.07 ns respectively.

Figure 2.11. Emission spectra of NCPH in different solvent systems. Inset shows the decay profile in different solvents.

The remarkable difference in quantum yield between MeOH and DMSO solution can be attributed mainly on two factors, planarity of the molecule as well as the difference in life time in the excited state, thereby difference in the extend of possible non radiative decay pathways which decrease the emission intensity. Even though the energy difference calculated between the two tautomers was less (∼3.4-5.7 kcal mol⁻¹), presence of three hydrogens inside the core contribute an extra destabilizing effect for the 3H tautomer and also disturb the planarity of the ring. Whereas, 2H tautomer is more planar due to the less steric crowding inside the core of the macrocycle, which contribute
towards the bathochromic shift in the absorption spectra as well as better emissive character. Also, a very short lived excited emissive state in the MeOH solution compared to the DMSO solution points at the various possible non-radiative decay channels as well as the accelerated rate of such decays in MeOH solution [157].

2.4.3 Tautomeric exchange and aggregation properties

The existence of a particular tautomeric form of NCPH in polar protic and aprotic solvents was confirmed by tautomeric exchange studies. As NCPH is insoluble in ACN (polar aprotic) as well as H₂O (polar protic) and the presence of eight hydroxyl group encouraged us to investigate the solvent assisted aggregation behavior. The investigation was conducted in solvent combinations such as DMSO/ACN, DMSO/H₂O, MeOH/ACN and MeOH/H₂O. The photophysical changes during aggregation were monitored through various spectroscopic techniques such as solution state FT-IR, UV-Vis absorption and emission spectroscopy.

The FT-IR spectral analysis of NCPH in MeOH/ACN showed OH stretching band at 3366 cm⁻¹, shifted to 3431 and then to 3541 cm⁻¹ as the ACN concentration increases from 0%, 50% and 90% as shown in Figure 2.12. a, b and c respectively [157]. The shift towards the higher energy side indicates existence of the strong solute-solvent hydrogen bonding interaction in the MeOH solution which weakens during aggregation. Analyses of IR spectra with higher ratio of ACN indicate the possibility of weak intermolecular hydrogen bonding that lead to self-assembled structures of NCPH. Higher ratio of ACN causes an exchange of tautomer from 3H form to 2H form. The 3H form of NCPH can easily make strong hydrogen bonding with MeOH through nitrogen lone pair and after the exchange, the 2H tautomer with an external NH proton prefers intermolecular interaction over the solvent-solute interaction. However, the DMSO/ACN mixture
showed a gradual drift in the OH stretching frequency as the aggregation progress. Where, the stretching frequency observed at 3449 cm\(^{-1}\) in DMSO decreased to 3444 and

![FT-IR spectra of NCPH](image)

**Figure 2.12.** FT-IR spectra of NCPH in (a) MeOH solution, (b) 1:1 mixture of MeOH/ACN (c) 1:9 mixture of MeOH/ACN, (d) DMSO solution, (e) 1:1 mixture of DMSO/ACN and (f) 1:9 mixture of DMSO/ACN.
3430 cm$^{-1}$ at 50% and 90% addition of ACN suggests that the intermolecular hydrogen bonding became stronger than the solute-solvent interaction as shown in Figure 2.12. d, e, and f respectively.

Figure 2.13. Change in the absorption profile of NCPH on aggregation in mixture of solvents (a) MeOH/ACN, (b) MeOH/H$_2$O, (c) DMSO/ACN and (d) DMSO/H$_2$O.

The UV-Vis absorption spectral changes in the mixture of solvents were recorded in 8 µM solution of NCPH (Figure 2.13). The absorption spectra displayed a hypsochromic shift in all situations with higher ratio of ACN and H$_2$O gives indication about formation of H-aggregates. The MeOH/ACN combination (Figure 2.13.a) was not showing considerable change for the Soret band owing to the combined effect of aggregation and tautomeric exchange. In other cases absorption spectra displayed slight hypsochromic
shift in initial stage, indicating the formation of H-aggregates. However, at higher ratio of both ACN and H$_2$O, spectral broadening occurs with red-shifted band.

The effect of protonation on NCPH was analyzed with trifluoroacetic acid (TFA), both in MeOH and DMSO solution (Figure 2.14). The absorption spectra obtained in MeOH solution was different from that obtained from MeOH/H$_2$O mixture, indicating the reason behind the absorption changes in MeOH/H$_2$O mixture is aggregation of the molecule by hydrophobic interaction, rather than protonation. The absorption profile obtained for the acid titration in DMSO solution was similar to that obtained for DMSO/H$_2$O mixture, which can be attributed to the possible tautomeric change by the addition of acid.

![Figure 2.14. Change in the absorption spectra of NCPH during acid titration in (a) MeOH and (b) DMSO.](image-url)
Figure 2.15. Change in the emission characteristics of NCPH in (a) DMSO/ACN, (b) DMSO/H$_2$O, (c) MeOH/ACN and (d) MeOH/H$_2$O with increasing amount of ACN and H$_2$O.

The emission profile of NCPH (8 µM) upon addition of ACN and H$_2$O in DMSO and MeOH solution is shown in Figure 2.15. In DMSO/ACN mixture (Figure 2.15.a), when the ACN concentration increases from 20 to 90%, the emission intensity drops continuously due to solubility reasons. However, the emission profile ensures 2H tautomer of NCPH in the medium even in the aggregated state as both solvents are aprotic in nature. But, in DMSO/H$_2$O mixture (Figure 2.15.b) there was a clear display of exchange of 2H tautomer to 3H tautomer, which resulted in two equally intense emission peaks as observed in the MeOH solution. As the H$_2$O concentration increases from 20 to
90%, the emission intensity drops down due to aggregation. Interestingly, the gradual increase in the amount of ACN in MeOH/ACN mixture (Figure 2.15.c) shifted the emission maxima to the blue region with the maximum band at 720 nm as observed in DMSO solution of NCPH, indicate a tautomeric shift from 3H to 2H form. It was expected that, the insoluble nature of NCPH in ACN will cause aggregation and thus decreases the emission intensity as the ratio of ACN increases, but the transformation of the compound from 2H to 3H form, as explained earlier, increases the quantum yield four fold in the 1:9 mixture of MeOH/ACN as compared to MeOH solution. Further, the temperature dependent emission studies in the 1:9 MeOH/ACN mixture reveal the negligible influence of temperature on the emission intensity and concludes the tautomeric exchange as the reason for emission enhancement. In the MeOH/H2O mixture (Figure 2.15.d), the emission pattern remains same even at the 1:9 ratio with decrease in the emission intensity, attest the existence of 3H tautomer in the medium. The possible mechanism for the tautomeric existence of NCPH in different protic and aprotic solvents was shown in Figure 2.16, where the hydrogen bonding formation drives the respective forms in different solvents.

![Figure 2.16. Schematic representation of possible tautomeric structures in polar protic and aprotic solvents.](image)
2.4.4 The role of tautomeric structures in the morphology of NCPH aggregates

As discussed, reports associated with morphological changes in NCP derivatives during aggregation, with respect to any stimuli are not in the literature. Here, the tautomeric exchange of NCPH was studied with different solvent combinations, which was accompanied with aggregation of the molecules. Morphological features during aggregation process were investigated through SEM and AFM analysis as shown in Figure 2.17. An 80 µM solution of NCPH in the required solvent mixture were taken for the analysis. Interestingly, in a 1:9 MeOH/H2O (v/v) mixture of NCPH forms a cluster of

Figure 2.17. (a) SEM image of NCPH in MeOH/H2O (b) AFM image of NCPH in MeOH/H2O drop cast on mica sheet (c) SEM image of NCPH in MeOH/ACN (d) and (e) higher magnified images in MeOH/ACN mixture.
fibres with size less than one micrometer (Figure 2.17.a) as obtained from SEM imaging and the morphology was confirmed by AFM studies (Figure 2.17.b). Analysis of morphological features indicate that these extended fibre structures could be formed by the nonplanar 3H tautomer through inter molecular hydrogen bonding between OH groups as well as hydrophobic interaction. Whereas the same concentration of NCPH in a MeOH/ACN mixture, where NCPH in 2H form was self-assembled in shell morphology and the average size of the shell was found to be 200 nm ranges (Figure 2.17.c, d and e). This remarkable difference in the morphological features of the self-assembly of NCPH in MeOH/ACN mixture can be attributed to the planar nature of 2H tautomer, and thus favourable for π-π interaction between the units in addition to the other interactions possible as described. SEM analysis of NCPH in DMSO/ACN and DMSO/H$_2$O combinations was also exhibited similar trend as in the case of MeOH combinations (Figure 2.18). Overall, the investigations concluded that existence of the particular tautomeric form has major role in determining the morphology of NCPH in the aggregated state.

Figure 2.18. SEM images of NCPH in a mixture of (a) DMSO/ACN and (b) DMSO/H$_2$O.
2.4.5 Anion assisted aggregation of NCPH

It was an interesting observation that the tautomeric existence of the N-confused derivative has a key role in determining the morphology. On the other hand, reports with anion assisted self-assembled structure are attained much interest in recent years. The presence of eight hydroxyl group and outer NH offers suitable sites for hydrogen bonding interaction. Hence, this part of the chapter discuss about the role of anions on the aggregation behaviour of NCPH.

Figure 2.19. Changes observed in the absorption spectra of NCPH (8µM) in DMSO by the addition of (a) F ions, (b) CN ions, (c) CH₃CO₂⁻ ions, and (d) H₂PO₄⁻ ions.

The effect of various anions on the absorption spectrum of NCPH in DMSO was investigated. Anions such as F, CN⁻, CH₃COO⁻ and H₂PO₄⁻ have shown clear shift in the
absorption spectra of NCPH (Figure 2.19). However, other anions such as Cl\(^-\), Br\(^-\), SCN\(^-\), NO\(_3\)^-, NO\(_2\)^- and BH\(_2\)^- didn’t show much change. Up to 4 equiv. of F\(^-\) ions resulted in the gradual decrease in intensity of the Soret band of NCPH at 450 nm along with Q-bands at 644 and 697 nm. Further addition (up to 20 equiv.) of F\(^-\) ions accompanied with the appearance of red-shifted band at 472 nm. Interestingly, the Q-bands were blue-shifted to 638 and 683 nm. The red-shifted Soret band corresponds to the J-aggregates which were formed by the complexation of NCPH and anions using hydrogen bonds between anion and NH/OH groups of NCPH. Similar spectral changes were observed for other anions such as CN\(^-\), CH\(_3\)CO\(_2\)^-, and H\(_2\)PO\(_4\)^- ions. However, further investigations on aggregation were restricted to F\(^-\) ions because of its strong acidic nature.

![Figure 2.20](image)

**Figure 2.20.** (a) The emission spectral changes of NCPH (8µM) in DMSO by the addition of F\(^-\) ions. (b) The decay profile of NCPH and NCPH-F\(^-\) ions aggregate in DMSO.

Interestingly, the emission maximum of NCPH at 720 nm experienced a prominent blue-shift of about 30 nm by the addition of F\(^-\) ions to result in a new peak at 690 nm with significant increase in the fluorescence quantum yield (0.032) as shown in Figure 2.20. Increase in the quantum yield can be attributed to the enhanced rigidity, which can
be expected during aggregation by intermolecular hydrogen bonding. The blue-shifted emission spectrum provides the information about the possible stabilization of the higher excited state or ground state in the NCPH-F\textsuperscript{-} aggregates compared to the monomeric NCPH. The results were further confirmed by lifetime measurements. The fluorescence lifetime of NCPH in DMSO was found to be 2.07 ns, where upon addition of 10 equiv. of F\textsuperscript{-} increased the life time to 2.95 ns. The decay profile of NCPH and its aggregated form in DMSO solution were found to be single exponential.

![Figure 2.21. IR spectral change of NCPH with gradual addition of F\textsuperscript{-} ion.](image)

Figure 2.21. IR spectral change of NCPH with gradual addition of F\textsuperscript{-} ion.
The interaction of F ion with NCPH was monitored by using FT-IR spectroscopy (Figure 2.21) in solution state. The OH stretching frequency observed at 3449 cm$^{-1}$ in DMSO was shifted to 3413 cm$^{-1}$ by the addition of 4 equiv. of F$^-$, indicating the decrease in the OH bond strength, which can be attributed to the formation hydrogen bonding with the anion. Further addition of F$^-$ ion leads to increase in the OH stretching frequency to 3406 and 3387 cm$^{-1}$ as the hydrogen bonding interaction between the OH group and F$^-$ anion get stronger.

![NMR Spectra](image)

**Figure 2.22.** $^1$H NMR spectral change of NCPH with increased F$^-$ ion concentration.

$^1$H NMR titration analysis of NCPH has been performed with tetrabutyl ammonium salt of fluoride in varying concentration to get an insight into the structural change occurred in the presence of anion as well as to get information regarding the binding sites (Figure 2.22). Addition of 0.5 equiv. of F$^-$ to a 15 mM solution of NCPH in DMSO-$d_6$ resulted in significant change in the $^1$H NMR pattern. The outer NH proton ($\delta = 14.3$ ppm) of NCPH split into two with an unanticipated upfield shift, due to increased
electron density near NH proton. Further addition of F resulted in more shielding of NH, OH protons (two broad peaks centered at $\delta = 10$ ppm) and to the other phenylic and pyrrolic protons, attributed to the dramatic structural change experienced by the molecule during the process. But, the change in the splitting pattern of NH and OH protons give indication about the binding sites. At 3 equiv. of F both NH and OH peaks merge each other to give a broad peak, which disappears completely at 8 equiv. of $\text{F}^-$ ion. As there were many binding sites the whole binding process were complex to determine the stoichiometry by using NMR analysis.

Figure 2.23. ITC analysis for the complexation of NCPH and $\text{F}^-$ ions in DMSO.
The stoichiometry for the interaction of $F^-$ with NCPH during aggregation was quantitatively investigated by isothermal titration calorimetry (ITC) in DMSO (Figure 2.23). The addition of $F^-$ to a 0.4 mM solution of NCPH produced a heat response indicative of an exothermic process. The heat change measured during the binding process were $-5363 \pm 57$ cal/mol, where the equilibrium association constants ($K$) was calculated to be $8.77 \times 10^3$ M$^{-1}$. The binding ratio obtained was 1:9 which was matching with number of possible binding sites available in NCPH, including outer NH and phenylic OH.

![Figure 2.24. SEM images of NCPH in DMSO after the addition of F$^-$ ions.](image)

Morphological changes during anion assisted aggregation were investigated by SEM analysis (Figure 2.24). 30 µM solution of NCPH in DMSO with 10 equiv. of $F^-$ was used for SEM analysis. Formation of nano sized (~100 nm) spheres was observed during the analysis. The formation of spheres was consistent with aggregated structures formed for the 2H tautomeric structure during the solvent assisted aggregation as described in the previous section. The presence of nine hydrogen bonding making sites and the possible
\(\pi-\pi\) stacking interaction for the 2H tautomer of \textbf{NCPH} promotes the formation of nanospheres. The size of nanospheres formed was further confirmed by DLS measurement (Figure 2.25), where data show an average size of 130 nm.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{size_distribution.png}
\caption{DLS analysis of \textbf{NCPH} in DMSO with \text{F}^- ion.}
\end{figure}

\section*{2.5 Conclusions}

In conclusion, we have demonstrated the synthesis, spectral and structural characterization of a hitherto unknown octahydroxy derivative of N-confused porphyrin (\textbf{NCPH}). Also, apart from the previous reports, the switch over of the tautomeric forms from 3H to 2H and vice versa was controlled exclusively with respect to protic and aprotic solvents rather than polarity controlled transformation, which was probed through various spectroscopic techniques. Further, the role of tautomeric structures on generating self-assemblies of different size and shape, such as nanoshells and fibres, through solvent-assisted aggregation was described, which is unprecedented in N-confused prophyrin chemistry. Also, investigations revealed the anion-assisted aggregation of \textbf{NCPH} where \text{F}^- ion can complex with 2H tautomer of \textbf{NCPH} to generate nanospheres.
2.6 Experimental Section

2.6.1 Materials and methods

The reagents for the synthesis as well as photophysical studies were obtained from Sigma-Aldrich and Merck, India and used as such. All solvents were distilled and dried before use. Deionized water was from Millipore. $^{1}\text{H}$ and $^{13}\text{C}$ NMR spectra were recorded on a Bruker Biospin 400 MHz spectrometer. NMR experiments were done in DMSO-$d_6$. Spectra were referenced internally by using the residual solvent ($^{1}\text{H}, \delta = 2.5$ and $^{13}\text{C}, \delta = 39.4$ for DMSO-$d_6$) resonances relative to Si(CH$_3$)$_4$ and the solvent peak were removed for clarity in the main text. Matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectra was recorded in Shimadzu Biotech Axima mass spectrometer. Infrared spectrum of the compound was recorded on a Perkin Elmer FT-IR spectrometer, spectrum RXI. Electronic absorption spectra and steady state fluorescence spectra were recorded on Perkin Elmer Lambda-750 UV-Vis absorption spectrometer and Perkin Elmer LS55 Fluorescence spectrometer respectively. Time-resolved fluorescence measurements were carried out by using a time-correlated single photon counting (TCSPC) spectrometer (Edinburgh, OB920). A diode laser ($\lambda_{\text{exc.}} = 445$ nm, FWHM = 98 ps) was used to excite the compound and MCP photomultiplier (Hamamatsu R3809U-50) was used as the detector (response time 40 ps). The lamp profile was recorded by using a scatterer (dilute ludox solution in water) in place of the sample. Decay curves were analyzed by a nonlinear least-squares iteration procedure using F900 decay analysis software. The quality of the fit was judged by the $\chi^2$ values and weighted deviation was obtained by fitting. ITC data were obtained from microcal iTC 200. The raw data obtained were fitted and analysed using origin 7.0 software provided along with the instrument. SEM imaging was performed on a Zeiss EVO 18 Cryo Special Edn. with variable pressure detector working at 20-30 kV. Atomic Force
Microscopy images were recorded under ambient conditions using a NTEGRA (NT-MDT) operating with a use tapping mode regime. Micro-fabricated TiN cantilever tips (NSG10) with a resonance frequency of 299 kHz and a spring constant of 20-80 Nm\(^{-1}\) were used. AFM section analysis was done offline. Samples for the imaging were prepared by drop casting the solution on freshly cleaved mica surface at the required concentrations at ambient conditions. DLS analyses were carried out with a Zetasizer Nano S from Malvern Instruments at 25 °C. The average hydrodynamic radii were calculated from Stork-Einstein equation (\(R_h= \frac{k_B T}{6\pi \eta D}\)), where \(k_B\) = Boltzmann’s constant, \(T\) = Absolute temperature, \(\eta\) = Viscosity, \(D\) = Diffusion constant.

2.6.2 Synthesis of NCPH

Synthesis of NCPH was achieved through a two step synthetic strategy. In the first step, meso-tetrakis(3,5-dimethoxyphenyl) N-Confused Porphyrin(NCP-OCH\(_3\)) was prepared by Lindsey’s method \([64]\) by using MSA (methane sulfonic acid) as catalyst. In the second step, dry DCM (15 mL) was taken in a 100 mL two neck RB flask at -78 °C under argon atmosphere and 0.5 mL boron tribromide (BBr\(_3\)) was added to this. Solution of NCP-OCH\(_3\) (100 mg) in 15 mL dichloromethane was added slowly to the BBr\(_3\) solution and allowed to stir for 2h at -78 °C and then gradually brought to RT. The mixture was refluxed at 65 °C for 8h and then room temperature for 12h. To the solution water was added and heated at 85 °C for 6h. The water layer was decanted and the compound recrystalised from dichloromethane and 2-propanol mixture. \(^1\)H NMR (400MHz, DMSO-\(d_6\)): \(\delta\) 14.30 (s, 1H, exch. D\(_2\)O, pyrrolic outer NH), 10.11 (s, 4H, exch. D\(_2\)O, phenolic OH), 9.9 (s, 4H, exch. D\(_2\)O, phenolic OH), 8.74-8.75 (d, J=4 Hz, 1H, pyrrolic \(\beta\) H), 8.62-8.63 (d, J=4 Hz, 1H, pyrrolic \(\beta\) H), 8.39 (s, 1H, pyrrolic \(\alpha\) H), 8.29-8.39 (m, 2H, pyrrolic \(\beta\) H), 8.23-8.28 (m, 2H, pyrrolic \(\beta\) H), 7.27 (s, 4H, phenyl), 7.09 (s, 4H, phenyl), 6.90-6.91 (d, J=4 Hz, 2H, phenyl), 6.77 (s, 2H, Phenyl), 2.14 (s, 1H, exch.
D$_2$O, pyrrolic inner NH), -1.82 (s, 1H, pyrrolic inner β H) $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 167.29, 158.45, 157.83, 138.84, 138.57, 137.62, 128.82, 124.86, 124.47, 124.00, 115.34. IR (KBr): 3422(br), 2929, 2856, 1606, 1479, 1368, 1308, 1167 cm$^{-1}$. FAB-MS: m/z 743.83 (C$_{44}$H$_{30}$N$_4$O$_8$ + H, M + 1). Anal. Calcd for C$_{44}$H$_{30}$N$_4$O$_8$: C, 71.15; H, 4.07; N, 7.54. Found: C, 71.01, H, 4.02, N, 7.31.