WATER SOLUBLE PORPHYRIN BASED MULTICROMOPHORE ARRAYS: ASSEMBLED ON G-QUADRUPLEX AND SILICON NANOPARTICLE SCAFFOLDS

AN ABSTRACT
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BY
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Abstract

A variety of multi-porphyrin containing arrays and nanostructures (see Chapter 1 for relevant examples) are being increasingly considered as attractive components for the development of functional materials. The construction of such defined multi-chromophore arrays in water, although a tall-order, has exciting potential applications in many fields such as molecular photonics, photodynamic therapy, solar energy harvesting, catalysis and sensing. In this dissertation two distinct approaches to construct water soluble porphyrin based multi-chromophore array systems are reported.

In the first part (Chapter 2), we detail the development of multi-chromophore (including porphyrins) containing photonic DNA nanowires. For the most part, photonic DNA nanostructures are prepared by the assembly of multiple sequences of long DNA strands that are linked covalently to various chromophores/fluorophores. In contrast, we introduce a non-covalent method for the construction of porphyrin-containing DNA nanowires and their networks that harnesses the programmed assembly of a single, very short, oligodeoxynucleotide sequence. This strategy uses a range of supramolecular binding modalities (including DNA base-pairing, metal-ion coordination, and β-cyclodextrin-adamantane derived host-guest interactions) for simultaneous nanowire assembly and porphyrin incorporation. Furthermore, we also describe how the resultant DNA-porphyrin assembly can be further functionalized with a complementary “off-the-shelf” DNA binding dye leading to structures that can facilitate energy transfer and display broadband absorption. Different spectroscopic and microscopic studies (such as
UV-Vis, circular dichroism, fluorescence, transmission electron microscopy (TEM), atomic force microscopy (AFM), scan tunneling microscopy (STM), confocal microscopy, and non-denaturing polyacrylamide electrophoresis (PAGE) were conducted to characterize and investigate the properties of the assemblies. The work described in Chapter 2 is based- and elaborates-on a recent full article published in *Langmuir* (2013).

The second focus of this dissertation (Chapter 3) describes the synthesis, characterization, and functional properties of a novel water-soluble silicon nanoparticle grafted with porphyrin chromophores. In particular, a 50% azide terminated silicon nanoparticle is “clicked” with a tetra-phenylporphyrin functionalized with poly-ethylene glycol (PEG) chains at *para*-position of three phenyl rings and an alkynyl handle on the last phenyl ring. Interestingly, the resultant nanoparticle can self-assemble into micelle-like structures with a diameter ~10-150 nm in water. Spectroscopic studies including UV-Vis and fluorescence demonstrated that the aggregation of porphyrins is largely hindered within the nanoparticle. Further, the nanoparticle is capable of producing singlet oxygen when the porphyrin units are excited by light. Preliminary, yet exciting, cell studies also demonstrated that the porphyrin-containing nanoparticle system is capable of translocating into HEK293T cells and can serve as an efficient photosensitizer to kill these cells (via photodynamic therapy).
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DEDICATION

TO MY FAMILY AND MY BEST FRIENDS
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Chapter 1: Background and Significance

1.1. Introduction

Inspired by the natural light harvesting complexes that absorb and funnel light-energy with exquisite precision and efficiency, much recent interest has been directed towards the design and construction of multiporphyrin arrays with well controlled inter-chromophoric distances, orientations, and defined donor-acceptor ratios. As a result of being composed of monomeric porphyrin units, that themselves have fascinating and tunable photochemical and photophysical properties, these synthetically derived multiporphyrin arrays are ideal candidates for developing functional materials that have potential applications in different fields including energy harvesting, molecular scale photonics and electronics, sensors, and therapeutic applications.

In the past decades, a variety of approaches have been developed, using either covalent or self-assembly strategies to construct multiporphyrin arrays. Various elegant multiporphyrin arrays, such as porphyrin dimers, trimers, one dimensional linear oligomers, two dimensional networks and macromolecular circles, with precisely defined structure and chromophore ratios have been explored using covalent chemistry. The main drawback of this approach is the tedious synthesis and purification steps required to achieve such large and complex macromolecules. On the other hand, the “bottom-up” self-assembly strategy is increasingly being considered as a promising technique for preparing nanoscale materials. Unlike the covalent method, supramolecular self-assembly does not require laborious synthesis, and highly ordered structures can be constructed from small “building blocks” via non-covalent interactions such as van der
Waals forces, hydrophobic interactions, dipole-dipole interactions, electrostatic interactions and hydrogen bonds.

However, as the target multiporphyrin systems become larger and increasingly complex, it is difficult to maintain the structural rigidity that is required to precisely position the monomeric porphyrin units by utilizing only the self-assembly of small components. The recent developments in the field of nanotechnology provides an additional level of control on how to precisely organize porphyrins by using nanostructures as scaffolds from which porphyrin units can be appropriately organized. This latter approach is a fast growing multidisciplinary field that blends synthetic chemistry, supramolecular chemistry and nanotechnology. In this regard, developing nano-scale scaffolds on which a large number of porphyrin units can be attached (either covalently or non-covalently) in a defined manner has received our interest. In addition, we are particularly focused in constructing water soluble multiporphyrin arrays not only because water is an environmentally friendly and cost-effective solvent, but also because water solubility can greatly extend the applications of the resultant porphyrin arrays, especially in biomedical fields.

Based on the abovementioned considerations, self-assembled DNA-based nanostructures first drew our attention because of (a) their attractive programmable assembly properties (i.e., high-fidelity architectures can be formed via canonical Watson-Crick base pairing rules), (b) their possibility of site specific modification (via simple synthetic chemistry), (c) their ability to bind and recognize target molecule (i.e., well-defined protein-DNA and small molecule-DNA interactions), as well as (d) their innate water solubility (due to the negatively charged phosphate backbone).
Our second focus is on using silicon nanoparticles as a nano-scale scaffold from which to append porphyrin moieties because silicon nanoparticles (a) can be readily prepared by a “top-down” method in large quantities (i.e., mechanochemical method\textsuperscript{35}, electrochemical or chemical etching\textsuperscript{36,37} and chemical vapor deposition\textsuperscript{38} etc.), (b) are easily functionalized with a variety of reactive functional group handles (c) are biodegradable and non-toxic.\textsuperscript{39–42} Further, ultra-small silicon nanoparticles possess distinct photophysical and photochemical properties\textsuperscript{43,44} that may enhance and complement the properties of the porphyrin macrocycles.

This chapter of the dissertation is dedicated to provide the reader with a brief introduction into porphyrins as well as an overview of the recent developments on multiporphyrin arrays prepared by the incorporation of porphyrin monomers onto various macromolecular scaffolds. Chapter two and three discuss the original contributions of the candidate and specifically focus on the construction of multiporphyrin arrays using DNA quadruplex or silicon nanoparticle scaffolds and details the synthesis, characterization, properties and applications of these multiporphyrin arrays. At the end of these two chapters, experimental details and characterization data are provided, as well as possible future directions.

1.2. Introduction to porphyrins

1.2.1. Background and structure

Porphyrins are a group of highly colored organic compounds comprising a particular conjugated core macrocycle, the porphine.\textsuperscript{45} The porphine macrocycle is made up of four pyrrole rings joined together via four methine bridges which also called α-
carbon (see Figure 1.1). The word Porphyrin comes from the Greek word for purple, *porphura*. The name implies an obvious property of most porphyrins, their intense purple-red color. This color is a result of its large conjugated system. Porphyrin has 22 π-electrons (or 18 π-electrons in the shortest conjugated path), making it aromatic according to Hückel’s rule (i.e., 4n+2 π-electrons). Considered as a tetra-dentate ligand, porphyrin can bind to various metals such as Zn, Mg, Fe, Ni, Co, and Cu to give various metalloporphyrins that show different color and other properties. Porphyrins and metalloporphyrins are widely found in Nature and play important roles in many biological systems. Perhaps the most well-known are (a) heme [Fe(II)porphyrin] found in red blood cells and (b) the reduced magnesium porphyrin, chlorophyll, present in the photosynthetic apparatus of plants. Porphyrins have also attracted considerable attention because their prospective applications in mimicking enzymes, catalytic reactions, photodynamic therapy, molecular electronic devices and conversion of solar energy.

![Figure 1.1 Structure of porphine and substituted porphyrins](image)

**Figure 1.1** Structure of porphine and substituted porphyrins

1.2.2. Nomenclature

The structure of porphyrins was first suggested by Küster in 1912, then widely accepted after the total synthesis of heme by Fischer in 1929\(^{46}\). Fischer was also the first
to introduce a nomenclature and numbering system for porphyrins. As shown in Figure 1.2, in the Fischer nomenclature system, carbons on the pyrrole ring that has a proton attached is named the β-carbon and numbered 1 to 8. The bridging carbons (the meso positions) were named α, β, γ and δ accordingly. Porphyrins can also be named via the IUPAC nomenclature system. In this system all of atoms are numbered (1-20).

![Figure 1.2 Fischer and IUPAC nomenclatures for porphyrins](image)

### 1.2.3. Spectral properties

#### 1.2.3.1. UV-Vis absorption spectra

Due to their highly conjugated π system, porphyrins and metalloporphyrins have very high extinction coefficients (~$10^5$) and characteristic absorption spectra. As shown in Figure 1.3, the UV-Vis absorption spectra of both free base porphyrin and metalloporphyrins consists of two distinct regions: an intense absorption band known as B-band (or the Soret band) in near UV regions (typically in 380-500 nm, depending on whether the porphyrin is β- or meso- substituted) and four (in the case of free base porphyrins) or two (for metalloporphyrins) weaker absorption band known as the Q-bands in the visible region (typically 500-750 nm).
Figure 1.3 UV-Vis absorption spectra of a free base porphyrin (left) and zinc porphyrin (right). Inset graphs shows the Q-band region for each porphyrin.

The absorption spectrum of porphyrins can be explained by the highly successful “four-orbital” (i.e., two highest occupied π orbitals and two lowest unoccupied π* orbitals) model that was proposed by Gouterman in 1963\(^47\). As shown in Figure 1.4, according to this model, the metalloporphyrin systems possess two HOMOs (\(a_{1u}\) and an \(a_{2u}\) orbital) and two LUMOs (\(e_{gx}\) and \(e_{gy}\) orbitals). Transitions between these orbitals give rise to two excited states. Orbital mixing splits these two states in energy, creating a higher energy state with greater oscillator strength, resulting in the Soret band (B bands), and a lower energy state with lower oscillator strength, resulting in the Q-bands.\(^{48}\). Free-base porphyrins have D\(_{2h}\) symmetry due to the presence of the pyrrole NH protons and metalloporphyrins such as zinc porphyrin have D\(_{4h}\) symmetry. This causes some of the energy levels to become degenerate increasing the number of Q bands for the freebase porphyrin case.
Figure 1.4 Porphyrin HOMOs and LUMOs. (A) represents the four molecular orbitals in porphyrins (B) shows the energy levels of four molecular orbitals for porphyrins and transitions corresponding to Q and B bands.

1.2.3.2. NMR spectra

Porphyrins are large aromatic macrocycles bearing extended conjugated systems. This conjugated macrocyclic system gives rise to characteristic 1H-NMR spectra for porphyrins. In specific, the β-protons on the pyrrole ring and meso-protons (that are located on the exterior of the macrocycle) are shifted to the far downfield due to the strong de-shielding effect of the paramagnetic ring current. On the other hand, the –NH protons of the pyrrole ring (located in the interior of the macrocycle) are strongly shifted up field to the negative region of the 1H-NMR spectrum as a result of the shielding effect of the ring current (see Figure 1.5).\textsuperscript{49,50}
Figure 1.5 $^1$H-NMR (CDCl$_3$,400MHz) spectrum of a substituted tetraphenyl porphyrin, Inset: a schematic illustration of the ring current of porphyrin and the shielding and deshielding effect

1.2.4. Synthesis of meso-substituted porphyrins

Porphyrrins can be considered as a diverse family of organic compounds because there are many ways of making substitutions. For instance, various substituents can be placed on all 20 different positions of the porphine core as well introducing metals to the center. There are two main classes of porphyrin core-substitution patterns i.e., β-substituted porphyrins and meso-substituted porphyrins. Since the second part of this thesis requires the development of a novel meso-substituted porphyrins, in this section we
will give a brief introduction to the fundamental synthetic methods for the construction of meso-substituted porphyrins. For further detailed reviews of porphyrin synthesis, the reader is referred to several excellent books and review articles on this topic.51,52

1.2.4.1. Monopyrrole tetrimerization pathway

*Meso*-substituted porphyrins are essentially synthesized from a mixture of pyrrole and aldehydes. One of the advantages to this strategy is that aldehydes are widely available, easy to manipulate and are ideal for making a huge range of porphyrins without complicated multistep syntheses of precursors. Substitution groups introduced by the aldehyde unit leads to convenient handles on the porphyrin for further modification.

Rothemund53 was one of the pioneers of synthesizing *meso*-substituted porphyrins. In 1935, Rothemund reported the synthesis of *meso*-tetramethylporphyrin by reacting pyrrole and acetaldehyde in pyridine. Later, he further expanded this approach by introducing substituted benzaldehydes, to achieve a large family of substituted tetraphenylporphyrins (TPP). The reaction condition for these reactions are 140-150 °C for 24 hr in a sealed glass tube under nitrogen atmosphere. This method was then modified by Adler and Lingo in 196754. These researchers discovered that if pyrrole and benzaldehydes were refluxed in acidic solvent such as propionic acid and in an open air atmosphere, the yield of TPP significantly increased to 25% (See Scheme 1.1).
In 1987, Lindsey and coworkers further optimized the Adler-Lingo method since they noticed that both pyrrole and benzaldehydes are quite reactive therefore the high reacting temperature is not necessary. The Lindsey method involves a two-step reaction between an arylaldehyde and pyrrole. First, a dilute solution of aldehyde and pyrrole was condensed using a Lewis acid such as BF$_3$OEt$_2$ as a catalyst to achieve the porphyrinogen intermediate, then the intermediate was further oxidized by a mild oxidant such as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to achieve the desired porphyrin with yields of 35-40% (see Scheme 1.2).
Due to its mild reaction conditions, the Lindsey method has significant advantages over the Alder-lingo method, such as less linear tar-like side products, broader choice of aldehydes that have acid sensitive functional groups or bulky functional groups, as well as being much easier to purify. Although the Lindsey method is quite successful in synthesizing porphyrins that have same meso-substituents, it is not very efficient in synthesizing porphyrins with different meso-substituents. For example, condensation of two types of aldehydes usually leads to a statistical mixture of six porphyrins (see Scheme 1.3). As a consequence, separation of desired porphyrin from this mixture is quite challenging, and the specific targeted porphyrin is always collected in unsatisfactory yield.

Scheme 1.3 Reaction of pyrrole with two type of aldehydes
1.2.4.2. The [2+2] route.

Based on the synthesis of dipyrollic intermediates such as dipyrromethanes and dipyrrromethenes, complicated meso- and β-substituted porphyrins can also be achieved through the so-called [2+2] route by reacting dipyrrolic intermediates and aldehydes. The [2+2] approach is also known as the “MacDonald method” because it was pioneered by MacDonald and coworkers while synthesizing analogues of heme\textsuperscript{56}. For example, the intermediate 1,9-di-unsubstituted dipyrromethane 17 are prepared via the condensation of pyrrole and aldehyde under conditions that stop unwanted polymerization. 1,9-diformyl dipyrromethane 18 is synthesized using the same method, followed by formylation reaction. With the dipyrromethane intermediates in hand, the target porphyrin 19 could be achieved through the condensation of 17 and 18 under acidic conditions in high yield (see Scheme 1.4). However, it should be noted that if unsymmetrical dipyrromethanes are used, mixtures of two different types of porphyrins will result since both ends of each dipyrromethane have equal reactivity which could lead to two condensation pathways.

![Scheme 1.4 synthesis of an analogue of heme via [2+2] route](image-url)
In addition, meso-substituted porphyrins could also be achieved by reacting 1,9-di-unsubstituted dipyrromethane and aldehydes through the [2+2] route. The number of distinct porphyrin products are also related to the number of both dipyrromethanes and aldehydes used. For example, reaction of one dipyrromethane with two different aldehydes results in a statistical mixture of three porphyrins (See Scheme 1.5).

1.3. Porphyrin based multichromophore arrays

The development of porphyrin based multichromophore arrays have been inspired and prompted by many interesting functional biological systems in which porphyrin derivatives play an important role. In these biological systems, porphyrin derivatives, including heme, chlorophylls, and bacteriochlorophylls, are precisely assembled with protein scaffolds to achieve specific functions. A particular example for this architecture-function relationship is the photosynthetic system of the purple bacteria, that is constituted of the light harvesting complex 2 (LH2) unit and a reaction center (RC).
surround by an LH1 complex. In LH1 and LH2 light harvesting complexes a large number of pigments, including bacteriochlorophylls and carotenoids, are precisely assembled within a peptide scaffold to form a circular structure. Largely as a consequence of the precise circular arrangement of these pigments, the LH2 unit displays an incredible efficiency to harvest the sunlight in a wide range of visible and near IR light spectrum (250nm-900nm). Because of the perfectly tuned energy gradient, the captured energy is efficiently transferred from LH2 to LH1, and finally transferred to the reaction center where the conversion of the excitation energy to chemical energy is conducted. As a consequence of the chromophores that are used in these systems and the precise position and orientation that are defined by the protein scaffold, this system exhibits extremely high efficiency for absorbing photons (95%) (Figure 1.6).
Figure 1.6 Top: Schematic representation of a natural light-harvesting antenna system. Absorption of light by LH2 (light harvesting II) complex is followed by sequential and directional energy transfer that leads to the excitation energy migrating to the reaction center (RC). Bottom: top view of the X-Ray structure of LH2.

Inspired by these biological systems, researchers have devoted significant effort to create artificial macromolecular multiporphyrin systems in which porphyrins and other chromophores are ordered with well controlled interchormophoric distances, orientations, and defined donor-acceptor ratios. These arrays are typically constructed by either (a) coupling of multiple porphyrin building blocks covalently or (b) by the self-assembly of small porphyrin building blocks through relatively weak non-covalent interactions.
including hydrophobic interactions, hydrogen bonds, van der Waals interactions, electrostatic interactions, metal ion co-ordination and host-guest interactions.\textsuperscript{16,27} However, to achieve large and complex systems (such as those found in LH1 or LH2) with large numbers of chromophores at precisely controlled positions and directions is challenging to construct by using only the purely synthetic or supramolecular techniques. Blending together both the synthetic and supramolecular strategies, however, is a promising alternative. In particular, a potentially highly efficient strategy is to use rigid scaffolds with nanoscale structure to precisely organize the individual chromophores. In this section, we will give an overview of recent reports on multiporphyrin systems either based on synthetic macromolecular scaffolds such as polymeric and dendritic systems, or based on supramolecular scaffolds such as DNA and virus like nanoparticle systems.

1.3.1. Polymeric scaffolds.

Polymer backbones have long been considered as useful synthetic scaffolds towards the ordering of porphyrins units. In order to achieve multiporphyrin arrays, one straightforward strategy is functionalize the polymer backbones. Although soft and flexible linear polymers can also be used as the backbone, rigid polymers which often possess helical conformations\textsuperscript{60}, are considered as more ideal candidates for the arrangement of porphyrins into well-defined arrays because their structural rigidity provides a conduit for maintaining specific inter-chromophore distances and orientations.\textsuperscript{61} For example, porphyrin arrays based on the helical polyisocyanide backbone have been reported by Takahashi group (see Figure 1.7, \textbf{24-28}).\textsuperscript{62-65} Starting from various porphyrin monomers bearing isocyanides, helical polymers with precisely controlled molecular weight was achieved through living polymerization using a Pd-Pt $\mu$-
ethynediyl dinuclear catalyst. In this way, a polymer with ca. 20-200 attached porphyrins could be achieved by changing the monomer to catalyst ratio. In addition, di- and tri-block copolymers 26, 27, 28 containing both free base and zinc porphyrins could also be prepared using the same strategy, resulting in polymers with various block length and polydispersities of ~1.1. Photophysical studies revealed that porphyrins in these polymers are stacked in face-to-face manner and excitonically coupled. Due to the arrangement and energy gap between zinc and free base porphyrins, the resulting polymer displays good efficiency (35% in the case of 28) for the energy transfer from zinc to free base porphyrin. Takahashi and co-workers also incorporated chiral porphyrins as a central block in copolymer 29, wherein the flanking block domains contain enantiopure menthyl side chains attached. Due to the architecture of the polyisocyanide backbone, the pi-stacking of the porphyrins is now enforced in a chiral environment. As a consequence, an exciton coupled bisignate Cotton effect was observed in the Soret peak region of the CD spectrum.
Figure 1.7 (A) Chemical structures for polymers and (B) the top and side view of the energy minimized structure of 24 that shows the porphyrin stacks (50 units) parallel to the polymer backbone (Adapted with permission from ref\textsuperscript{65} copyright 2004 American Chemical Society)

The Nolte group also reported a similar multiporphyrin system based on polyisocyanide backbones.\textsuperscript{66,67} In this approach, a peptide based polyisocyanide polymer, that was also first reported by Nolte and co-workers\textsuperscript{68}, has been introduced as the macromolecular scaffold (see Figure 1.8). The peptide amide functionalities forms a hydrogen bonding network and allowed the peptide side chains to be aligned in one direction (see Figure 1.8B). This specific architecture resulted in a dramatic increase of rigidity of the overall structure as well as leading to extremely long polymer wires that are hundreds of nanometers to several micrometers in length, making it a better scaffold for anchoring porphyrins.
In another approach, Luh and coworkers\textsuperscript{69} have reported a multiporphyrin array system using isotactic double stranded polybisnorbornene as a scaffold. In particular, an isotactic polymeric ladderphane \textsuperscript{33}, which uses porphyrin moieties as linkers, was synthesized selectively from the corresponding bisnorbornene monomer \textsuperscript{31} by ring-opening metathesis polymerization (ROMP) using the first generation Grubbs catalyst \textsuperscript{32}. As shown in Figure 1.9B, because of the ladder-like structure, all porphyrin linkers are coherently aligned perpendicular to the longitudinal axis of the polymer. Strong exciton coupling between neighboring porphyrin linkers led to distinct photophysical characteristics. Later, another multiporphyrin array based on single strand polynorbornene \textsuperscript{34} was synthesized using the same strategy\textsuperscript{70}. Both \textsuperscript{33} and \textsuperscript{34} have been used for complexation with 1,4-diazabicyclo[2,2,2]octane (DABCO) to generate an array of 2:1 porphyrin–DABCO supramolecular sandwich complexes (Figure 1.9 D).
In addition to multiporphyrin arrays that are formed by covalent linkages to polymeric scaffolds, supramolecular porphyrin-polymer complex have also been reported. For example, Wang and coworkers\textsuperscript{71} reported the synthesis of a water soluble anionic polythiophene (PTP) \textit{35} and a cationic porphyrin (TPPN) \textit{36}. PTP \textit{35} and TPPN \textit{36} can form a supramolecular complex in water through electrostatic interactions. In this complex, PTP \textit{35} was designed as a light harvesting antennae and TPPN \textit{36} serving as a catalyst to convert oxygen to singlet oxygen. Upon excitation with visible light, energy transfer from PTP \textit{35} to TPPN \textit{36} occurs. Further, the quantum yield of singlet oxygen
generation in the supramolecular complex of 35 and 36 was significantly enhanced compared to TPPN 35 by itself. Furthermore, the positive charge of PTP/TPPN complex (mole ratio of PTP to TPPN is 9:1) facilitates its absorption to the negatively charged bacterial membranes of Gram-negative *Escherichia coli* and Gram-positive *Bacillus subtilis* bacteria through electrostatic interactions. Upon irradiation with white light (400-800 nm) at a fluence rate of 90 mW·cm$^{-2}$ for 5 mins, 70% reduction of bacterial viability was observed. This light harvesting antennae and photosensitizer constructed via supramolecular complexation provides a promising application in the photodynamic inactivation of bacteria.

**Figure 1.10** Schematic for the mechanism of enhanced singlet oxygen generating in PTP/TPPN complex and chemical structures of PTP and TPPN. (Note: ET stands for energy transfer)
1.3.2. Dendritic multiporphyrin arrays.

Dendrimers are characterized as a class of monodisperse, highly symmetric, branched three dimensional macromolecules with tunable size and shape. In addition, the unique step-by-step iterative synthesis of dendrimers allows functional groups, such as porphyrins, to be precisely placed within their nanoscopic three dimensional structures. Such functionality can be attached to (a) the core of the molecule, (b) at the branching point regions, (c) on the exterior surface, and (d) at a combination of the abovementioned regions (see Figure 1.11). Because of these unique properties dendrimers have been used to construct a variety of multiporphyrin arrays with the aim of mimicking the structure and function of natural photosynthesis systems.

Porphyrin-cored dendrimers were first reported by Inoue and coworkers (Jin, Aida, and Inoue) in 1993. In this approach, a porphyrin unit was covalently encapsulated into a poly(benzyl ether) dendrimer cage (Figure 1.12) through a convergent synthesis to form a sterically hindered dendrimer-porphyrin that mimics the structure and function of hemeproteins. Initial photophysical studies showed that highly efficient singlet energy transfer occurs in, from the poly(benzyl ether) dendritic wedges to the porphyrin
Later, Suslick et. al. reported the convergent synthesis of porphyrin-cored poly(phenyl ester) that possess a similar architecture. Instead of investigating energy transfer, these dendrimers have been examined as regioselective epoxidation catalysts, for both intramolecular and intermolecular selectivity. After these pioneering research directions, dendritic multiporphyrin arrays have begun to attract considerable attention (see below).

**Figure 1.12** Molecular structure of porphyrin-cored poly (benzyl ether) dendrimers.
Figure 1.13 Molecular structure of dendrimer 38 that contains eight naphthopyranone and 16 coumarin-3-carboxylate donor chromophores.

Although the porphyrin-cored dendrimers mentioned above have shown the ability of harvesting photons, their poly aryl dendritic wedges are not considered as ideal chromophores for the purpose of mimicking light harvesting and cascade energy transfer processes found in natural photosynthetic system. Based on this consideration, a variety of more complex porphyrin based multichromophoric dendrimers has been developed. For example, Fréchet and coworkers reported the divergent synthesis of a porphyrin-cored dendrimer 38 (Figure 1.13) that contains 8 naphthopyranone donor units in the
inner layer and 16 coumarin-3-carboxylate donor units in the outer layer. Arraying such a large number of chromophores gives rise to absorption of light in a wide spectral range. In addition, excitation of either the coumarin or the naphthopyranone donor units results in emission only from the porphyrin core, indicating a cascade energy transfer process from the coumarin donors in the outer layer to the naphthopyranones residing in the inner layer and finally to the porphyrin core.

**Figure 1.14** Molecular structure of dendrimer 39 that bears 24 porphyrin units on the exterior of the dendrimer. Inset: a depictive molecular model for a hexaarylbenzene-cored poly(aryl ester) dendrimer that bears 12 porphyrin units and the “intra-wheel” and “inter-wheel” energy transfer process.
With the aim of structurally mimicking the wheel-like arrays of pigments in LH1 and LH2 complexes, a series of compounds, such as 39, that repetitively bear 12, 18, 24 or 36 zinc porphyrin units on the periphery of a hexaarylbenzene-cored poly(aryl ester) dendrimer has been synthesized by Aida and coworkers. As a result of a tightly packed three dimensional dendrimer structure, porphyrins are separated into two multi-porphyrin wheels in the same molecule, that is above (red porphyrin moieties in 39) and below (black porphyrin moieties in 39) the planar of hexaarylbenzen core (see Figure 1.14, inset). As confirmed by time resolved fluorescence and absorption anisotropy measurements, excitation energy is efficiently transferred amongst the arrayed zinc porphyrin units. Interestingly, a two component decay of the anisotropy was observed for these compounds. This behavior was explained as there being two main energy transfer paths: inter-wheel (fast) and intra-wheel (slow) energy migration processes. However, due to the flexible dendrimer core and solvophobic effect, the structural and photophysical properties of dendrimer 39 are not well maintained in poor solvent. In order to amend this, the structural rigidity of dendrimer 39 can be further improved by adding bipyridyl guest 40, which is known to be bind in between two zinc porphyrins. Adding 40 to dendrimer 39 results in suppression of the direct porphyrin π - π interaction as well as an enhancement of the fluorescence quantum yield in poor solvents because of the well maintained inter-chromophore distance.
In a later development by the same group, supramolecular complexes involving the complexation of these abovementioned dendrimers with bipyridine guests possessing 1-3 fullerene units (e.g., 41 in Figure 1.15) were reported. For example, fullerene guest 41 strongly binds to dendrimer 39 to form an extremely stable supramolecular complex 42, which consists of a spatially segregated inner layer of photoactive electron donating porphyrin units and an outer layer of electron accepting fullerene arrays (Figure 1.15). These complexes was isolated with the aid of size-exclusion chromatography and were
also visualized by scanning tunneling microscopy (STM). Furthermore, when the zinc porphyrins are photo-excited, electron transfer from the zinc porphyrin units to the fullerene acceptors occurs, resulting in the formation of a charge-separated state. Upon increasing the number of the porphyrin donor units and fullerene acceptor units, the electron transfer process was remarkably facilitated, while the recombination of the resulting charge-separated state remained virtually intact. Among these complexes, the largest complex \(42\) has a charge separation rate constant of \(2.3 \times 10^{10} \text{ s}^{-1}\), which is almost ten times greater than that of the smallest complex consisting of 6 porphyrin units and 6 fullerene units \(\left(0.26 \times 10^{10} \text{ s}^{-1}\right)\).

**Figure 1.16** Schematic illustration of dendritic poly(L-lysine) functionalized with a mixture of zinc porphyrins and freebase porphyrins in a hemisphere fashion \((43)\) and random fashion \((44)\).
In addition, more complex system 43 was reported by Nishino and coworkers.\textsuperscript{80,81} In particular, a fifth generation poly(L-lysine) dendrimer bearing 16 zinc porphyrins on one hemisphere and 16 free base porphyrins on the other hemisphere was built through hemispherical synthesis that alternatively applied the Boc and Fmoc chemistries (Figure 1.16). Another dendrimer 44 that has a random mixture of zinc porphyrin units and free base porphyrin units on the surface was also synthesized\textsuperscript{82}. Upon selective photoexcitation of the zinc porphyrin units in dendrimer 43, excitation energy transfers from zinc porphyrin to the free base porphyrin units with an efficiency of 43\%. On the other hand, dendrimer 44 shows a much higher energy transfer efficiency (85\%). The lower energy transfer efficiency in 43 suggests that energy migration in the same hemisphere first occurs upon the photoexcitation of zinc porphyrin units, followed by energy transfer to the free base porphyrin units on the other hemisphere.
In another approach that uses porphyrins as the branching point, Aida and coworkers have reported a large dendritic multiporphyrin array 45 (Figure 1.17) consisting of four dendritic wedges each composed of zinc porphyrin heptamers connected to a core meso-substituted free base porphyrin. In dendrimer 45, the dendronized zinc porphyrin heptamer act as light harvesting antennae and energy donating unit, and the free base porphyrin in the core serves as an energy trap. The large number of energy donor porphyrins allows for efficient capture of photons. When the zinc porphyrin units are selectively photoexcited, the fluorescence emission from free base porphyrin acceptor is significantly enhanced compared to that of direct excitation of the free base porphyrin core. The dendrimer 45 exhibits a high efficiency (71%) of
energy transfer from the zinc porphyrin to free base porphyrin compared to a conical
dendron control (19%) that only contains 7 zinc porphyrins (see Figure 1.17). Thus the
number of donor porphyrins and the morphology of the chromophore array plays an
important role in determine the energy transfer efficiency. More recently, Harriman and
coworkers reported the synthesis of a similar porphyrin dendrimer via “click” chemistry\textsuperscript{83}. The resulting three-stage porphyrin dendrimer contained an Au\textsuperscript{+} porphyrin core, a second
layer of four free base porphyrins, and an outer layer of 12 zinc porphyrins.

![Molecular structure of a dendritic multiporphyrin array containing 20 zinc porphyrins and one free base porphyrin.](image)

**Figure 1.18** Molecular structure of a dendritic multiporphyrin array containing 20 zinc porphyrins and one free base porphyrin.

By far all the dendritic multiporphyrin arrays mentioned above are joined by
flexible linkages such as ethers, esters or amide bonds. In order to anchor multiple
porphyrin units onto rigid scaffolds, linear diarylethyn or diarylbutadiyne linkers have
been introduced through the very successful Sonogashira cross-coupling reaction. For example, Lindsey and coworkers reported the convergent synthesis of rigid dendrimeric multiporphyrin arrays containing \( n \) Zn-porphyrins (\( n=4, 8, \) or \( 20 \)) and one free base porphyrin in the center joined \( \text{via} \) diarylethyne linkers (Figure 1.18). The resulting arrays can be isolated by size exclusion chromatography and have sufficient solubility in toluene and other organic solvents for routine handling. With increase in size, the Soret band broadens, splits and red shifts due to inter-porphyrin exciton coupling. In contrast, the weaker Q bands remain essentially unchanged upon increasing the number of porphyrins that are incorporated. Similarly, the one-electron oxidation potential of the porphyrins are unchanged as well. These results suggest that the characteristic properties of the individual porphyrin units are largely retained as the porphyrin arrays increase in size. Moreover, highly efficient energy transfer occurs from the zinc porphyrin antennae units to the free base porphyrin core unit. The overall energy transfer efficiency was estimated to be 96%, 96% and 92% for multiporphyrin arrays with 4, 8, and 20 zinc porphyrins, respectively.

### 1.3.3. Virus Like nanoparticle as a scaffold.

Virus like particles (VLP) have recently received significant attention due to their enormous potential in the field of nanotechnology. VLPs are similar to dendrimers in that they are highly defined, and monodisperse. However, in contrast to dendrimers, VLPs are constructed by self-assembly of purified virus coat proteins (VCP) under suitable conditions.\(^4\) VLPs are highly attractive because of their simple preparation, uniform size and structure, and are highly programmable. Moreover, VLPs have modifiable surface handles on which one can precisely place a variety of functional molecules through
standard protein conjugation strategies or via non-covalent interactions. Although the use of VLPs for nanotechnology applications is still in an early stage, undoubtedly these are promising scaffolds to arrange chromophoric arrays in well defined spatial relationships. For example, the tobacco mosaic virus (TMV) coat protein is known to form a rod-like structure with a diameter of 18 nm and a length of 300 nm (Figure 1.19C). In 2005, Francis and co-workers were the first to demonstrate that a rhodamine derivative can be selectively attached to either the exterior or the interior of TMV nano-rod by applying two different protein conjugating strategies (reacting with lysine or cysteine residues, respectively). This approach opened the gate for constructing multiporphyrin arrays that are precisely defined in a VLP scaffold such as Bacteriophage M13, Bacteriophage MS2, and TMV mentioned above.

Figure 1.19 (A) Molecular model and (B) chemical structure of TMVCP-Porphyrin monomer. (C) Side view (top) and Top view (bottom) of molecular model of TMV rod assembly with N127 residues shown in red.
In 2007, Majima and coworkers demonstrated the construction of multiporphyrin arrays using the TMV supramolecular scaffold. As shown in Figure 1.19B, water soluble zinc or free base porphyrin was site-selectively incorporated on the cysteine residue (Cys127) of recombinant TMV coating proteins (TMVCP) through a maleimido-thiol coupling reaction. Similar to the native TMVCP, these porphyrin modified TMVCP building blocks assemble into double layer disk structures at pH 7 or rod structures at pH 5.5. In addition, a random mixture of zinc porphyrin donor and free base porphyrin accepter was packed in the TMV assembly. The photophysical properties of these multiporphyrin arrays were examined by time-resolved fluorescence spectroscopy, and the energy transfer rate were determined to be 3.1-6.4×10^9 s^-1.

**Figure 1.20** (A) Schematic illustration of cationic trans-(N-methyl-pyridinium-4-yl)diphenylporphyrin that binds to bacteriophage M13 and results in facilitating energy transfer from tryptophan to porphyrin. (B) Chemical structure of the porphyrin modification of bacteriophage M13 coat protein 48. (C) Schematic illustration of porphyrin grafted bacteriophage M13 nanorod and (D) porphyrin grafted bacteriophage M13 nanorod coated with IrO₂.
Another rod structured VLP is formed by the filamentous bacteriophage M13. This bacteriophage forms flexible rods, each containing ~2700 copies of an α-helical protein, arranged, around an axial circular single-stranded DNA molecule, in a helical array with 5-fold symmetry. The rods have a diameter of 6 nm and are 800-2000 nm in length depending on the exact length of the core axial DNA strand. In 2006, the first supramolecular approach to form multi-porphyrin arrays with this bacteriophage was shown. In particular, cationic trans-(N-methyl-pyridinium-4-yl)diphenylporphyrin that binds to both wild type and genetically modified bacteriophage scaffold via electrostatic interactions was reported by Felici and coworkers.88 (Figure 1.20A). Interestingly, here the protein scaffold itself serves as the light harvesting antennae, as energy transfer from the external tryptophan residue of the capsid to the non-covalently bound porphyrins on the outer surface of the VLP was clearly observed. In addition, covalent approaches were also reported by Belcher and coworkers.89 In particular, Zn(II) deuteroporphyrin IX 2,4-bis(ethylene glycol) (ZnDPEG) was conjugated to the exposed N-terminus and multiple lysine residues of bacteriophage M13 coat protein via the carbodiimide coupling reaction (Figure 1.20B). M13 VLPs with approximately 1564 and 2900 conjugated porphyrins was achieved. Compared to free ZnDPEG, the porphyrins assembled on the virus have distinctive spectroscopic changes, including broadening of Soret bands, fluorescence quenching, and the much shorter lifetime of the excited state. Later, this multi-porphyrin-M13 VLP complex was further assembled with iridium oxide (IrO$_2$) hydrosol clusters to create a photo catalytic nanostructure (Figure 1.20D). With the advantage of using porphyrins as a light harvesting antennae and the oxide (IrO$_2$) hydrosol clusters serving as the energy acceptor and photo catalyst. The assembled nanostructure was shown to
have significantly improved photocatalytic water-splitting activity with a turnover number (the total number of water molecules the catalyst splits) of $\sim 9.7 \times 10^4$. This value is about 12 times greater than that for free IrO$_2$ nanoparticles mixed with ZnDPEG($\sim 7.4 \times 10^3$).

In another approach, Francis and coworkers have demonstrated a multistep synthetic strategy to arrange fluorescent donor molecules on the inner surface and zinc porphyrin acceptors on the outer surface of the bacteriophage MS2 protein shell (see Figure 1.21). The protein shell of bacteriophage MS2 possess a hollow icosahedral structure that consists of 180 identical monomers with a diameter of 27 nm. In this approach, the author used a genetically modified bacteriophage MS2 coat protein (N87C/T19pAF MS2) with the native N87 residue and T19 residue changed to a cysteine residue and an artificial $p$-aminophenylalanine ($p$AF) residue, respectively. The cysteine
residue, which is located on the inner surface of the capsid, was functionalized with donor molecules such as Alexa Flour 350 and Oregon green 488 through maleimide-thiol conjugation. The $p$AF residue, which was located on the exterior surface of the capsid, was functionalized with a porphyrin acceptor through a two-step modification. In these systems, Alexa Fluor 350 can sensitize the porphyrin Soret band, and Oregon Green 488 was selected for sensitizing the first porphyrin Q band. Energy transfer from the donor molecules inside the capsid to the porphyrins on the outer surface was observed by monitoring the quenching of the donor emission by the porphyrin.

1.3.4. DNA nanostructures as a scaffold

Deoxyribonucleic acid (DNA) is the molecule that encodes the genetic information used by Nature to construct living organisms. Based on the canonical Watson-Crick base pairing and pi-stacking, DNA molecules can self-assemble to highly defined helical duplex structures. The DNA duplex has attractive characteristics for nanotechnology including that: (a) it has a defined diameter of about 2 nm, and the distance between pi-stacked base pairs are 3.4Å, (b) it is a rigid structure with a persistence distance (a measure of stiffness) of 50 nm, (c) the duplex structure can be reversibly assembled and disassembled, and (d) it has the ability to assemble into complex 2D- and 3D-structures. Furthermore, recent developments in automated solid phase synthetic protocols make it possible to readily modify DNA molecules at specific positions (see Figure 1.22A). Another important property of DNA is that (inter alia because of its negatively charged phosphate backbone and pi-stacked base pairs) it has the ability to bind to various molecules (e.g., proteins and small-molecules) through
non-covalent interactions. Due to these appealing properties, DNA molecules are considered as attractive building units for bottom-up nanotechnology.\textsuperscript{30).

A. Solid-phase oligonucleotide synthesis (3’ to 5’)

B. Covalent approach:

\begin{itemize}
\item Replacing the nucleoside or nucleobase with porphyrins
\item Modify the phosphate backbone
\item Modify the nucleobase
\end{itemize}

C. Non-covalent approach:

\begin{itemize}
\item Electrostatic interaction
\item Intercalation
\item Hydrogen bonding
\end{itemize}

**Figure 1.22** (A) Solid phase synthesis of oligonucleotide from 3’ to 5’. (B) and (C) Strategies for the construction of porphyrin arrays based on DNA scaffold.
In recent years, DNA nanostructures have been used as programmable scaffolds for the precise arrangement of chromophore arrays. In this section, we will provide a brief review on recent examples of multiporphyrin systems that have been assembled through the use of DNA scaffolds. These porphyrin arrays can be constructed by either covalent conjugation or non-covalent interactions. In the covalent approaches, the porphyrin unit can be introduced onto the DNA backbone by (a) totally replacing the nucleoside or nucleobase with porphyrins, (b) functionalizing on to the phosphate backbone, or (c) functionalizing on to the nucleobase (see Figure 1.22B). In the non-covalent approaches porphyrins (and other chromophores) are incorporated with the DNA scaffold through supramolecular interactions, including electron static interactions, hydrogen bonding, and intercalation etc. (see Figure 1.22C). Specifically, porphyrin arrays have been constructed by the porphyrin monomers binding via four major DNA binding modes: intercalation, minor groove binding, external stacking, and capping.

It is known that the DNA duplex structure and stability can be influenced by introducing aromatic capping molecules to the duplex termini. Capping is defined as pi-stacking interaction between aromatic molecules with the terminal base-pairs of the duplex. The capping molecules are typically covalently linked to the 5’ or 3’ DNA termini by introducing modified phosphoramidite building blocks to conventional solid phase oligonucleotide synthesis. For example, in 2005, Balaz and Berova et al. demonstrated the synthesis of 3’-tetraarylporphyrin-thymidine-5’-phosphoramidites and their application as building blocks in the synthesis of oligonucleotide-porphyrin conjugates using a simple ‘in flask’ modification of commercially available oligonucleotides on resin (Figure 1.23B). Based on this strategy, a self-complementary 8-
mer oligonucleotide 5’-ACGC CGCGT-3’ with porphyrin on 3’ (or 5’) terminus was synthesized. As a consequence of the chiral twist within a duplex, the two porphyrins on each terminus show exciton couplet interactions (see Figure 1.23C) in the circular dichroism (CD) spectrum. In particular, very strong induced cotton effects in the porphyrin Soret absorption region was observed.\textsuperscript{94,95} Furthermore, the exciton-coupled CD signal in the porphyrin Soret band region proved to be more sensitive than the conventional nuleobase absorption region (200-300 nm) for investigating the 3D structural changes of the ODNs. This signal change can serve as a sensitive probe for geometrical changes in the DNA backbone upon transitioning from double-strand to a single-strand and vice-versa.

\textbf{Figure 1.23} (A) Molecular structure of 3’-tetraarylporphyrin-thymidine-5’-phosphoramidites 53 (B) ‘In flask’ solid-phase synthesis by functionalization of oligonucleotide on the resin (C) Molecular model of Double-stranded (ds) oligonucleotides with the porphyrins attached on 3’ termini.

In another approach reported by Balaz and Berova et al.,\textsuperscript{96} they incorporated non-charged tetraarylporphyrin to the 5’ terminus of a non-self-complementary ODN duplex
((dGdA)_n, in this approach n=4) that consisted of only continuous non-canonical Watson-Crick guanine-adenosine (G-A) base pairs via phosphate links (54). Although guanine–adenine repeat strands ((dGdA)_n) are able to self-assemble into parallel- and antiparallel-homo-duplexes and hairpin structures under specific conditions (such as DMSO, ethanol, salt, and various pH values), these DNA secondary structures are extremely unstable in neutral aqueous phosphate buffer. However, due to end capping via pi-stacking interactions between the porphyrin and G-A base pairs (see Figure 1.24B), the anti-parallel homo-duplex is substantially stabilized. Compared to the self-assembly of (dGdA)_4 strand without end capping porphyrins, the T_m value of the porphyrin-modified duplex sequence formed by two strand of 54 increased by 10 °C. Moreover, the porphyrin units can also be used as a molecular “glue” due to the strong pi-stacking and hydrophobic interactions between porphyrins. Specifically, Balaz and co-workers demonstrated that a porphyrin-modified sequence similar to 54 (using an amide linker instead of the phosphate ester linker) can form highly ordered head to tail inter-molecular homoduplex assemblies under very high ionic strength (see Figure 1.24C). As a consequence of the strong excitonic interactions between pi-stacked porphyrins, the resultant supramolecular assemblies displayed characteristic multisignate CD profiles in the porphyrin Soret region.
Figure 1.24 (A) Structure of porphyrin-(dGdA)$_4$ conjugates (B) Diagram demonstrating the $pi$-stacking between porphyrin and non-canonical guanine-adenine base pair. (C) Schematic representation of the formation of porphyrin stabilized (using a sequence similar to 54) homoduplex followed by the formation of supramolecular inter-homoduplex nanoassemblies.

Porphyrrins monomers can also be introduced onto the DNA sequence by modifying the phosphate backbone. For example, Majima and coworkers reported the synthesis of four-way-branched DNA-porphyrin conjugates that form porphyrin dimers in the presence of a complementary DNA strand (see Figure 1.25)$^{98,99}$. In these DNA-porphyrin conjugates, a porphyrin is attached to the middle of four modified 10 mer ODN strands by coupling a porphyrin maleimide derivative with cysteine groups on the ODNs. Because the spacing between two porphyrins is 10 nucleosides long, and this corresponds to one helical turn of a B-helix, this arrangement ensures that the linkers are aligned on
the same side of the duplex. Upon adding four equivalents of a complementary 20 mer DNA strand, a supramolecular complex consisting of a co-facial zinc porphyrin/freebase porphyrin dimer is formed. The formation for this complex was confirmed by both absorption spectroscopy and non-denaturing PAGE studies. In addition, the singlet-singlet energy transfer from zinc porphyrin to freebase porphyrin was observed. According to fluorescence life time measurements, the energy transfer rate constant was calculated to be $1.3 \times 10^8 \text{ s}^{-1}$.

![Figure 1.25](image) Schematic illustration of the formation of a zinc and freebase porphyrin dimer 57 via the self-assembly of 55,56 and 4 equivalents of a complementary 20 mer DNA strand.

Based on a similar strategy, the same group developed a new preparation for structurally controlled DNA tubes by assembling DNA tiles 100 with a porphyrin connector functionalized with four DNA strands (see Figure 1.26)101. In particular, they designed and synthesized the porphyrin connector 58, in which the para- positions of the phenyl groups are attached to 10 mer ODN sequence that are complementary to a sequence in DNA tile system 60. DNA tiles (59 and 60) were first assembled into two-dimensional array structures. Upon adding the porphyrin connector, four DNA tile 60
units can be assembled and constrained together by forming complementary duplexes between porphyrin connector and 60. A DNA tube structure could be then induced during assembly with tile 59. The formation of DNA tube structure 61 was confirmed by AFM experiments.

![Diagram of molecular structures](image)

**Figure 1.26** (A) Structure of four way porphyrin connector 58 and the complementary sequence on DNA tile 60. (B) Two dimensional structures formed by DNA tile systems 59 and 60, followed by formation of a three dimensional DNA tube structure by adding porphyrin connector 58.

Porphyrrins can also be incorporated onto DNA by modifying the nucleobase region. For example, Stulz and coworkers have demonstrated the synthesis of a 2’-deoxyuridine phosphoramidite building block onto which tetraphenyl or diphenyl porphyrins (62,63) are attached (on the 5-position of the deoxyuridine via an acetylene linker; see Figure 1.27)\(^{102,103}\). These porphyrin substituted deoxyuridine building blocks were used to create discrete multiporphyrin arrays via site specific incorporation into
DNA sequences. Using this strategy, up to 11 porphyrin-deoxyuridine units were successfully incorporated onto a DNA single strand. Due to the pi-stacking interactions between porphyrins in the single strand, a stabilized single stranded helical structure was observed. Surprisingly, although CD studies and energy minimized molecular models showed that the attached porphyrins externally stacked in the major grove of double strand DNA with little perturbation to the overall duplex structure, the thermal stability of the duplex DNA (consisting of a porphyrin rich strand and an unmodified complementary strand) was significantly destabilized (by $\Delta T_m$ -5-7 °C per porphyrin modification) and this destabilization is accumulative along with the increase of porphyrin units. In order to regain the thermal stability, the researchers redesigned a “zipper” sequence by attaching porphyrins on complementary strands. Using this strategy, the thermal stability of the modified duplex DNA was remarkably enhanced. For example, modified complementary duplex 64 showed an increase in melting temperature of $\Delta T_m$ =+5.7 °C compared to the unmodified DNA duplex. Moreover, the porphyrins on the complementary strand can be selectively re-metallated to give an alternative zinc- and free base- porphyrin array (65). These researchers also observed energy transfer between the two types of porphyrins within this array.
In 2009, Albinsson and coworkers first reported the synthesis of another type of porphyrin conjugated deoxyuridine building block 66. In particular, the 5 position of 2'-deoxyuridine phosphoramidite building block was conjugated to the meso-position of a porphyrin via varying phenyl acetylene linkers (see Figure 1.28). These porphyrin substituted deoxyuridine building blocks were successfully incorporated into DNA strands that can then be used to form a duplex (67). Interestingly, due to the hydrophobic affinity of porphyrins to hydrophobic environments, linear DNA strand 67 and porphyrin incorporated hexagonal DNA structure can be firmly anchored to a large unilamellar lipid vesicle, resulting in the DNA being exposed to the aqueous phase and the porphyrin anchor being present in the membrane. The extremely strong non-covalent anchoring of the porphyrin units allows the DNA duplex to be reversibly disassembled and
reassembled on the surface of the lipid vesicle. Since the porphyrin anchor can be used as a redox center and energy accepter, two kinds of artificial light harvesting systems were constructed and their energy harvesting and transfer behavior were studied (via steady-state and time-resolved fluorescence emission measurements). For example, fluorescein was covalently conjugated to the termini of the DNA duplex as the light harvesting antennae unit (see structure of 68) and 4-t-butylpyridine (tBQ) was introduced to the lipid vesicle as zinc-porphyrin coordinating ligand as well as serving as an electron acceptor. Upon selective excitation of fluorescein, energy transfer from the antennae fluorescein units to the porphyrin “reaction center” and subsequent electron transfer from the porphyrin to the coordinated tBQ was observed. In a recent report by the same group, the DNA intercalating dye YO-PRO-1(69) was used to intercalate a 39-mer duplex DNA with one porphyrin attached in the middle of the sequence (70). Utilizing 69 as the donor chromophore to sensitize the porphyrin acceptor gives rise to an antenna effect of 12.

Figure 1.28 (A) Chemical structure of porphyrin 66, and YO-PRO-1 69. (B) Schematic representation of porphyrin-DNA system 67, fluorescein-DNA-porphyrin light harvesting system 68 and YO-DNA-porphyrin light harvesting system 70, all anchored to a lipid vesicle
In addition to the elegant covalent approaches mentioned above, porphyrins can also be incorporated onto DNA through non-covalent interactions. For example, it is well known that cationic tetrapyridyl porphyrin can bind to DNA structures by intercalation and by external electrostatic interactions and form supramolecular assemblies on DNA templates. Recently, Purrello and coworkers demonstrated that a tetraanionic nickel(II) meso-tetrakis(4-sulfonatophenyl)porphyrin 71 is able to selectively assemble with spermine induced Z-form DNA. In particular, poly(dG-dC)2 was selected as a tunable B-to-Z DNA scaffold and tetracationic spermine 72 as an inducer for the B-to-Z DNA conformational change. The conformational change process was monitored by CD spectroscopy. Left-handed Z-DNA was induced by spermine under low pH (pH=6.8), under this condition, porphyrin 71 assembles with Z-DNA-spermine complex and gives rise to a strong induced negative CD signal in the porphyrin Soret region, which indicates the formation of porphyrin 71-spermine-Z-DNA complex (see Figure 1.29B). When the Z-DNA is transformed to the right-handed B-DNA under pH 9.5, the induced porphyrin CD signal disappears. Using pH as an input signal and CD spectroscopy as an output, this system can be potentially used as a reversible AND logic gate.

![Chemical structure of 71 and spermine 72.](image)

**Figure 1.29** (A) Chemical structure of 71, and spermine 72. (B) Schematic illustration of modulating of 71-spermine-Z-DNA complex with pH.
In addition to electrostatic interactions, hydrogen bonding (in addition to pi-stacking) has also been applied for assembling chromophores to single strand DNA templates\textsuperscript{114}. For example, Balaz and coworkers reported the synthesis of a water soluble porphyrin-diaminopurine conjugate 73 and their use in assembling with a single stranded DNA template via hydrogen bonding (see Figure 1.30).\textsuperscript{115} In specific, 8-ethynyl-2,6-diaminopurine was conjugated to the meso-position of a diphenylporphyrin through palladium-catalyzed Sonogashira cross-coupling reaction. Non-self-complementary oligodeoxythymidine (dT40 74) was selected as the DNA template. Upon annealing the mixture of 73 and 74, porphyrin-DNA template nanoassemblies, with length of 8-16 nm and width of 4-6 nm, were formed. These nanostructures were characterized by UV-Vis absorption, fluorescence, RLS and TEM analysis. Impressively, the CD profiles indicated that left- and right-handed nanoassemblies can be selectively formed by controlling the ionic strength, porphyrin-template molar ratio, and the annealing rate.
Figure 1.30 (A). Chemical structure of porphyrin 73, and oligodeoxythymidine 74. (B) Schematic illustration of the formation of left-handed and right-handed porphyrin 73-dT40 nanoassemblies.

1.4. Summary.

As clearly shown in this chapter, a number of macromolecular scaffolds have been used to construct and organize large number of porphyrin units into functional nanoarchitectures. In addition, not discussed in this overview but also very important, are a
variety of alternative nanostructures (such as carbon nanotubes\textsuperscript{116}, gold nanoparticles\textsuperscript{117}, CdSe quantum dots\textsuperscript{118-120}, TiO\textsubscript{2} nanoparticles\textsuperscript{121,122}, silicon and silica nanoparticles\textsuperscript{123}) that can also be used to develop functional multiporphyrin nanostructures. Depending on the structural design and properties of the scaffold, the resultant multiporphyrin arrays possess varying structures, dimensions, number of porphyrin units, and photophysical/photochemical properties. These approaches give proof to the concept that the use of macromolecular scaffolding is a practical tool to develop functional nanomaterials decorated with porphyrins.

As mentioned in section 1.1, we are particularly interested in using (a) self-assembled DNA nanostructures and (b) ultra-small silicon nanoparticles as scaffolds to construct multiporphyrin arrays. Thus in next two chapters, we present two novel multiporphyrin systems designed and constructed by the candidate. Specifically, Chapter 2 discusses a photonic DNA-chromophore nanowire system that is assembled by multiple orthogonal supramolecular interactions and its potential applications. This chapter is based on an article which was published in \textit{Langmuir} (2013).\textsuperscript{124} Chapter 3 will present the synthesis, characterization, photochemical properties, and preliminary photodynamic therapeutic applications of a water soluble porphyrin grafted onto a silicon nanoparticle. At the end of each chapter, proposed future developments are discussed, and experimental details and characterization data are included. In all cases, the primary work was carried out by the candidate under the guidance of Prof. J. Jayawickramarajah. In the case of chapter 3, collaborations with other research groups was critical. In this regard, the author acknowledges the fruitful collaboration with Prof. Mark Fink’s group (and Zeijing Xu in particular) at the Department of Chemistry (Tulane University), especially
for providing the pre-cursor azido-silicon nanoparticle. The author also thanks Prof. Benjamin Hall’s group (Fading Chen in particular) at the Department of Cell and Molecular Biology, (Tulane University) for conducting the relevant cell studies.
Chapter 2: Photonic DNA-Chromophore Nanowire Networks:

Harnessing Multiple Supramolecular Assembly Modes

2.1. Introduction

As was highlighted in Chapter 1, Section 1.3, DNA derived self-assembly is considered an attractive modality for the construction of organized nanostructures since straightforward base-pairing rules and hierarchical self-organization can be utilized to construct high-fidelity architectures. Specifically, research effort has been dedicated to the development of functional DNA nanoarchitectures by imbuing functional entities (e.g., carbon nanotubes, polymers, viruses, and nanoparticles) onto DNA structures. In this regard, the Jayawickramarajah group has been interested in building DNA nanostructures containing precisely placed multiple chromophores with various possible applications (such as light-harvesting, photonics, information transfer, and biomolecular sensing).

A variety of attractive multichromophore arrays based on DNA nanostructures have recently been developed. However, the main method that is used to achieve such DNA-based multi-chromophore architectures is to assemble multiple unique sequences of oligodeoxyribonucleotide (ODN) strands that are covalently tethered with dye molecules (or incorporate fluorescent nucleobase analogs). An alternative and elegant strategy to install chromophores onto DNA nanostructures, without using elaborate
covalent synthesis, is to use supramolecular chemistry (i.e., non-covalent binding).

However, there has been no exploration of using orthogonal chromophore-initiated binding to a DNA scaffold as a trigger to facilitate the assembly of photonic DNA nanostructures. Such nanostructures that combine DNA assembly and orthogonal supramolecular functionalization are particularly important since such systems can result in diverse architectures and, more importantly engender novel properties (as a result of incorporating functional supramolecular species).

In addition, most of above mentioned functional DNA nanostructures are based on canonical DNA duplex structures, only a few approaches based non-canonical DNA structures (such as guanine rich quadruplex structures) have been reported.

Figure 2.1 (A) An example of monovalent cations stabilized G-Wires formed by single telomeric guanine rich strand d(GGGTTGGGG) (B) chemical structure and dimension of a G-quartet (C) chemical structure and dimension of a free base tetraphenyl porphyrin.
Guanine rich quadruplex structures (G-quadruplexes) are sets of well-defined structures that are formed by guanine rich DNA sequences in the presence of certain cations. Due to their inherent properties, such as defined structure, excellent water solubility and superior conductivity, G-quadruplexes are becoming promising materials for creating water-soluble linear nanostructures and bio-molecular electronics. For example, in 1994, Henderson and co-workers first reported G-Wires that formed by the self-assembly of a telomeric oligonucleotide d(GGGGTTGGGG) in an out of register (slipped) manner (see Figure 2.1A). After this pioneering work, several one dimensional G-wire systems were reported. However, traditional strategies of building G-quadruplex nanostructures primarily depend on the repetitive stacking of cation stabilized G-quartets (see Figure 2.1B). This assembly process makes it a challenge to construct defined nanostructures assembled together with various functional molecules in a controllable and addressable manner.

In earlier work by the Jayawickramarajah group, it was reported that the binding of the tetraphenyl porphyrin 77-Zn (see Scheme 2.1) containing eight cyclodextrin (host) arms with a complementary tetraphenyl porphyrin projecting eight adamantane (guest) arms leads to unidirectional nanowires as a result of good geometric matching (i.e., both supramolecular monomer cores are tetraphenyl porphyrins) and repetitive multivalent host-guest interactions. Furthermore, in the terms of dimensions and structures, the dimensions (~1.1 nm × 1.1 nm) and four fold symmetry of the porphyrin macrocycle perfectly match the geometry (~1.1 nm × 1.1 nm) and four fold symmetry of a guanine quartet (see Figure 2.1B and C). In this regard, the G-quadruplex structure could possibly
utilized as complimentary building block of porphyrin 77 to construct a linear nanostructure.

With these considerations in mind, here we describe an approach that uses a range of supramolecular assembly modalities to construct chromophore-containing DNA-based nanowires and networks of wires. Importantly, in terms of the DNA domain, we have used only one, very short (6 nt long) ODN sequence (75). Using only a short piece of DNA sequence to construct nanostructures is advantageous in terms of minimizing the cost associated with DNA synthesis. First, ODN 75, functionalized with two adamantane arms, self-assembles into a tetramolecular quadruplex 76 in the presence of templating potassium ions (Scheme 2.1). Second, as a result of multivalent β-CD-adamantane based host-guest interactions, incubation of quadruplex 76 with free-base porphyrin 77 containing eight permethylated β-cyclodextrin (PMβ-CD) arms leads to the formation of a porphyrin-containing DNA nanowire as well as higher-order networks of nanowires. In addition, the DNA-porphyrin nanostructures are further functionalized with a DNA-binding dye via non-covalent interactions. This hierarchical self-assembly strategy leads to photonic structures with broadband absorption properties in the visible spectrum (~300 – 665 nm) and energy transfer capability from the DNA-binding dye donor to the free-base porphyrin acceptor.
The design of multichromophore containing DNA assembly 79 starts with guanine rich ODN sequence 75 (d(AdTG₄TAd), Ad = adamantyl head group). The core sequence contains four repetitive guanine residues since, in the presence of K⁺ ions, such a track of guanines form stable parallel guanine quadruplexes. These quadruplexes are formed via repetitive stacks of guanine quartets (wherein the guanines are co-planar and are stabilized by hydrogen-bond driven base-pairing and metal ion-lone pair interactions). 153 In addition to the guanine residues, flanking thymine nucleobases and adamantane head-groups were also included to the design to destabilize potential
mismatched aggregation that can lead to conventional guanine wires.\textsuperscript{148,149} Importantly, the adamantane head-groups were specifically included since the self-assembled tetramolecular quadruplex 76 would then project four adamantane units from each of its two ends. Based on earlier work by our group and other researchers on multivalent host-guest interactions on non-DNA derived supramolecular nanostructures,\textsuperscript{151,154–156} it was envisaged that inclusion of a non-covalently “cross-linking” free-base porphyrin (77) that presents four PMβ-CD hosts from each face (and has dimensions that closely match the terminal face of a DNA quadruplex)\textsuperscript{152} should result in DNA-porphyrin array 78 as a result of repetitive multivalent host-guest interactions.

2.2. Results

2.2.1. Preparation and characterization of G-quadruplex 76

In order to realize a self-assembled nanowire structure, it is required that ODN 75 first forms a parallel tetramolecular quadruplex 76 (i.e., d(AdTG\textsubscript{4}TAd)\textsubscript{4} or 75\textsubscript{4}) in the presence of potassium ions (Scheme 2.1). In order to gather evidence for the formation of quadruplex 76, self-assembly experiments of 75 were conducted and then analyzed (a) \textit{via} non-denaturing polyacrylamide gel electrophoresis (PAGE), as well as (b) by circular dichroism spectroscopy. When ODN 75 was incubated under standard quadruplex forming conditions\textsuperscript{157} (see experimental section for details) in potassium containing buffer (80 mM KCl, 10 mM Tris-HCl, pH 7.5) and then loaded and run on a polyacrylamide gel, two distinct bands are observed (see Figure 2.2, lane 3). The weak band that migrates fast is ascribed to a small amount of single-stranded ODN 75, as this migrates similar to a single stranded control (lane 4: ODN 82; d(AdT\textsubscript{6}Ad)), that has same
length and terminal functionalization as ODN 75 but only contains thymine bases and thus cannot form a tetramolecular assembly. The presence of an intense slow-migrating band in lane 3 suggests the formation of a higher-order species i.e., tetramolecular quadruplex 76 (or 754). A well-established protocol to determine whether tetramolecular quadruplexes are formed is to co-incubate two putative tetramolecular quadruplex forming strands containing the same track of guanines but are of varying length and determine if a mixture of five unique quadruplexes are formed, each of distinct size and migration capacity.158 Thus, we incubated a long strand containing a track of four guanines ODN 81 (TG₄T₄: which is known to form a tetramolecular quadruplex in the presence of potassium cations)159 with ODN 75 under standard quadruplex forming conditions. As can be seen on lane 2, the resulting gel shows the presence of five slow migrating bands (corresponding to 81₄, 81₋₅₁, 8₁₋₅₂, 8₁₋₅₃, and 75₄), verifying that both ODN 81 and ODN 75 form tetramolecular quadruplexes. It is also interesting to note that the homomeric tetramolecular quadruplex 76 band stains a unique purple color (when exposed to the DNA binding dye Stains-all) while the other quadruplexes stain a blue color.
Further evidence for quadruplex formation came from circular dichroism spectroscopy. In particular, upon incubation in quadruplex forming conditions, both ODN 75 and control ODN 81 exhibited characteristic profile for a parallel quadruplex structure, with a strong positive circular dichroism peak at 263 nm and a relatively weak negative peak at 242 nm (Figure 2.3, inset). In an effort to discern the thermal stability of the quadruplexes, we performed thermal denaturation experiments. As can be gauged from Figure 2.3 both quadruplex 76 (754) and quadruplex 814 are thermally very stable and show no significant denaturation.
Figure 2.3 Thermal denaturation profile for quadruplex 76 (black squares) and quadruplex 814 (red triangles). Inset: Circular dichroism profile of quadruplex 76 (black line) and quadruplex 814 (red line) at 20 °C. All circular dichroism experiments were conducted in 80 mM KCl, 10 mM Tris-HCl, pH 7.5 buffer and concentration of the quadruplexes were 4 µM.

In order to confirm that the adamantane functional groups of ODN 75 do not affect the quadruplex formation another control experiment was conducted. Specifically, the CD spectra of quadruplex 76 was also compared with control ODN 83 (TGGGGT, structure shown in Scheme 2.4, in the experimental section) that does not have the adamantane head-groups. The quadruplex formed by ODN 83 (see Figure 2.3) also shows a similar CD profile as quadruplex 76 when exposed to standard quadruplex forming buffer conditions (80 mM KCl, 10 mM Tris-HCl, pH 7.5).
Figure 2.4 Circular dichroism spectra of quadruplexes formed by ODN 83 (black) and quadruplex 76 (red) in potassium containing buffer (80 mM KCl, 10 mM Tris-HCl, pH 7.5).

2.2.2. Preparation and Characterization of Nanowire 78

Since ODN 75 can form stable quadruplex 76, we subsequently incubated 76 for 48 hr (at room temperature) in the presence of water-soluble porphyrin 77 in a 1:1 ratio (assembly forming procedures are discussed in the experimental section). Circular dichroism spectroscopy of the resulting solution (shown in Figure 2.5) clearly indicates that the parallel quadruplex structure of 76 is not destabilized by the presence of porphyrin 77.
Figure 2.5 Circular dichroism spectra of quadruplex 76 (black), porphyrin 77 (blue), and a mixture of 76 and 77 (red) in potassium containing buffer (80 mM KCl, 10 mM Tris-HCl, pH 7.5).

Thermal denaturation experiments can best display whether the thermal stability of the quadruplex 76 is affected upon assembly formation. However, as described in Figure 2.3, potassium stabilized quadruplex 76 (by itself) is extremely stable. Hence, in order to destabilize quadruplex 76, we prepared a non cation stabilized quadruplex by diluting a high concentration solution of ODN 75 (1 mM) to 16µM (in ssDNA) in water. Both, this non-cation stabilized G-quadruplex and its mixture with porphyrin 77 (4 µM) showed the characteristic CD profile for parallel G-quadruplexes. More importantly, as shown in Figure 2.6 the melting profile of the mixture of non-cation stabilized G-quadruplex and porphyrin 77 displays a higher melting temperature ($T_m = 70.4 \, ^\circ C$) versus the non-cation stabilized G-quadruplex ($T_m = 67.3 \, ^\circ C$). In addition, the melting transition for the non-cation stabilized G-quadruplex and porphyrin 77 mixture displays higher cooperativity in its melting.
Figure 2.6 Thermal denaturation profile of non-cation stabilized G-quadruplex 76 only (black) and its mixture with porphyrin 77 (red) note: All circular dichroism experiments were conducted in deionized water and concentration of the quadruplexes and porphyrin 77 were 4 µM.

In order to evaluate whether the photophysical properties of porphyrin 77 were affected upon assembly formation, control UV-vis and fluorescence experiments were conducted. As can be seen by inspection of Figure 2.7, the porphyrin Soret- and Q-absorption bands and its fluorescence emission profile do not change appreciably upon incorporation into assembly 78. These control experiments support the notion that porphyrin 77 does not self-aggregate via pi-stacking interactions and its photophysical properties are not diminished upon assembly formation.
Figure 2.7 UV-Vis (Top) and Fluorescence (Bottom; excitation at 422 nm) spectra of quadruplex 76 (blue), porphyrin 77 (black), and assembly 78 (red). All of these samples are 4 µM (conc. of quadruplex and/or porphyrin units) in 10 mM Tris-HCl 80 mM KCl, pH 7.5.

Prior to various microscopic studies (i.e., AFM, TEM), a molecular model was constructed based on the X-ray crystal structure of the core quadruplex sequence (d[TGGGGT]₄) and an energy minimized molecular model of porphyrin 77. According to
this molecular model (see Figure 2.8), the diameter of single nanowire 78 was estimated to be 2.3-2.8 nm.

Figure 2.8 A representative model of a section of nanowire 78 (composed of two molecules of porphyrin 77 and one molecule of quadruplex 76). This model is based on the X-ray structure of the core quadruplex sequence (d[TGGGGT]₄); PDB code 1S45. Note: the space-filled atoms are the adamantane binding units. The structure on the Left is a side view and structure on the Right is a top-view. The model was energy minimized (AMBER94 forcefield) using the MOE software. Note: This model is only a depiction and not a rigorous calculation.

Atomic force microscopy (AFM) was first utilized to determine the structure of the DNA-porphyrin assembly. Since it is well established that Mg²⁺ ions can facilitate immobilization of ODN nanostructures onto mica, AFM measurements were conducted on Mg²⁺ treated, freshly cleaved, mica substrate under tapping mode in air. As can be seen on the AFM image shown in Figure 2.9a, when AFM studies were conducted on a 1:1 mixture of quadruplex 76 and porphyrin 77 (4 µM each), curvy single nanowires are observed. The apparent height of these single wires were found to be ca. 1.5 nm (see height profile on Figure 2.9d). Although this height is shorter than expected, it is not only consistent with previously reported STM results of porphyrin-only nanowires prepared from porphyrin 77-Zn, but is also in accord with previously reported AFM (~1.6
nm)\(^{149}\) and STM (~1.5 nm)\(^{162}\) data of conventional G-wires. In addition, it is known that soft nanomaterials assembled from DNA and/or cyclodextrin typically exhibit lower measured heights when imaged on a surface due to various artifacts including high humidity, indentation of the assembly by the tip, and substrate induced flattening.\(^{66,162,163}\) In this case, the decreased height of nanowire 78 is thought to be due to a combination of the abovementioned artifacts.

Figure 2.9 (a) A 0.75×1.4 \(\mu\text{m}^2\) zoom-in image from rectangle shown in panel b (b) 3×5 \(\mu\text{m}^2\) and (c) 3×3 \(\mu\text{m}^2\) AFM images of assembly 78 on mica substrate. Note: The concentrations of quadruplex 76 and porphyrin 77 were both 4 \(\mu\text{M}\). (d) Height profiles collected at the indicated white line (labeled 1,2,3, respectively) on panel a. (e) Height profile collected at the indicated white lines (labeled e) on panel b. (f) Histogram height distribution of the networks shown in panel c.
Furthermore, we also observed various aggregate states of the wires. For instance, networks of single wires are observed (Figure 2.9c and histogram on Figure 2.9f). Interestingly, these networks are composed of single wires with branching points every 50-200nm that show the same height of single wires. As shown on Figure 2.10a, five different height profiles of the branching points were collected. These branching points have apparent heights (Figure 2.10b) ranging from 1.25-1.87 nm, values that are close to the height of a single wire. Taken together, these experiments suggest that the nanowire networks are formed by one face of the quadruplex or porphyrin units binding to more than one partner molecule, in a mismatched fashion. In addition, larger networks composed of higher-order bundles of wires are also observed in these AFM images (see left hand portion of image shown in Figure 2.9b and height profile in Figure 2.9e).

![Figure 2.10](image)

**Figure 2.10** a) 3x3 µm² AFM image of assembly 78 on mica substrate. b) profiles 1-5 show the height profiles collected at lines 1-5 on panel a.

One potential foreseeable issue could be that the nanowires and nanowire networks observed are formed by the assembly of single-stranded ODN 75 and not its
quadruplex form (76). In order to rule out this possibility, we performed control AFM studies where non-quadruplex forming control ODN 82 was incubated with porphyrin 77. In general, these control experiments showed the presence of amorphous nanostructures (see “the “dot-like” morphologies shown in Figure 2.11a and height profile on Figure 2.11b. In one case, a wire-like structure was observed (Figure 2.11a), however, this structure displayed apparent heights between 0.3~0.6 nm (Figure 2.11b). Importantly, these values are much shorter than the heights observed for nanowire 78 (height of ~1.5 nm). These studies, taken in conjunction with the thermal stability of quadruplex 76, give evidence for the notion that the nanowires and networks observed for array 78 are due to the self-assembly of quadruplex 76 with porphyrin 77.

Figure 2.11 a) 2×2 µm² AFM image of mixture of ODN 82 (d(AdT₆Ad), 16µM ssDNA) and porphyrin 77 (4 µM) on mica substrate. b) Height profile collected at line 1 on panel a. c) Height profiles collected at the indicated arrows on panel a.

In order to probe whether an assembly was formed between quadruplex 76 and porphyrin 77 in conditions that better mimic the solution phase, cryo-transmission electron microscopy (Cryo-TEM) was utilized. As shown in Figure 2.12, the Cryo-TEM image of
a mixture of 76 and 77 (each diluted to 4 μM, after nanostructure forming conditions were employed) displayed wire-like structures from ~100 nm to 200 nm in length and have a width between 7-20 nm. The high aspect ratio of these nanostructures suggests a directional assembly composed of consecutive host-guest interactions between the quadruplex and porphyrin units.

![Image](image_url)

**Figure 2.12** Cryo-TEM image of assembly 78 in 160 mM KCl, 10 mM Tris-HCl, pH 7.5.

However, as we mentioned above, a molecular model for single nanowire 78 estimates the diameter to be 2.3-2.8 nm (see Figure 2.8), we postulated that the images seen in the Cryo-TEM are due to the higher-order association of single wires under high salt concentration (160 mM KCl). Interestingly, similar to our AFM observations, some of these bundles display branching also suggesting a flexible assembly where a single face of quadruplex 76 (or porphyrin 77) can bind to multiple partner molecules (i.e., via out-of-register host-guest interactions, see discussion section 2.3. for more detail). Unfortunately, we could not collect single wire images under low salt concentrations
since single wires with low salt also have low contrast on the Cryo-TEM since the wires lacks sufficient amounts of high atomic number atoms that can effectively absorb electrons for TEM studies.

In order to increase the single wire contrast for Cryo-TEM studies, nanowire 78-Zn was prepared. This nanowire is prepared by mixing the zinc porphyrin version (77-Zn) of free-base porphyrin 77 and quadruplex 76 under the standard nanowire forming conditions. The Cryo-TEM image of nanowire 78-Zn (Figure 2.13) in a buffer with significantly lower salt concentrations (8 mM KCl) displays multiple uniform nanowires that are 100-200nm long and ~ 4 nm wide (a width that is closer to that of the molecular model). Compare to the free base version nanowires (nanowire 78), these wires are more directional and less flexible. In addition, branching structures are also shown.

![Cryo-TEM images of 4µM nanowire 78-Zn solution in 8 mM KCl, 1 mM Tris HCl buffer](image)

**Figure 2.13** Cryo-TEM images of 4µM nanowire 78-Zn solution in 8 mM KCl, 1 mM Tris HCl buffer

In collaboration with the Group of Ulrike Diebold, Scanning Tunneling Microscopy (STM) studies were also utilized to collect more detailed structural information on these single wires. In order to avoid complications that can arise from the
salt present in the buffer, a solution of nanowire $78$-$Zn$ was first dialyzed. As shown in Figure 2.14, the STM image of $78$-$Zn$ clearly displays single nanowires on surface of HOPG (Highly Ordered Pyrolytic Graphite). This wire has an apparent height of ~1.5 nm, which is in accord with the above mentioned AFM results on assembly 78 and also agrees with previously reported STM (~1.5 nm)$^{51}$ data on other nanowires formed by porphyrin $77$-$Zn$. It also has a width of ~3.5 nm that is consistent with the above mentioned Cryo-TEM investigation. Although the height is lower and width is wider than values predicted by the molecular model in Figure 2.8, we postulated that this difference may be due to flattening of the nanowires on the surface. Further, from the STM image a periodicity of ~2.1 nm was discerned (Figure 2.14 d).

Figure 2.14 STM image of desalted nanowire $78$-$Zn$ on HOPG surface (a,b), image size are 100x100nm$^2$ and 25x50 nm$^2$. Sample bias voltage $V = 0.18$V, Tunneling current = 0.1nA. Lines on (b) indicates where profiles (c,d) were taken.


2.2.3. Sybr-Green and Quadruplex binding study.

The discussion so far has focused on a supramolecular strategy that uses the DNA domain as a self-assembling scaffold to project the adamantane arms in a multivalent fashion. However, the DNA domain can also be used for further molecular recognition. In this regard, we wanted to investigate whether the DNA domain can be used for molecular recognition to rapidly incorporate complementary dyes within the DNA-porphyrin array. In particular, the fluorescence properties of assembly 78 was probed upon addition of a commercial green fluorescent DNA binding dye, SYBR Green I (SG). This dye was specifically chosen because it exhibits significantly enhanced fluorescence upon binding (via stacking and groove-binding)\textsuperscript{54} to structured DNA assemblies but not to single stranded DNA. Furthermore, SG complements the porphyrin chromophore since it absorbs strongly between 450 and 540 nm (see Figure 2.15 for UV-Vis and fluorescence spectra of SG and porphyrin 77), a region of the visible spectrum where porphyrin 77 does not absorb significantly, thus the resultant supramolecular complex 79 possess broad band absorption from 300-665 nm (see Figure 2.15). Also, the emission spectrum of SG overlaps with all four Q-absorption bands of porphyrin 77 thereby providing the possibility of fluorescence resonance energy transfer. However, although the binding behavior of SG to duplex DNA has been described in the literature,\textsuperscript{164} the binding interaction of SG to tetramolecular quadruplexes has not been investigated.
Figure 2.15 Normalized UV-Vis (solid lines) and fluorescence spectra (dashed lines) of porphyrin 77 (red), SG + quadruplex 76 (green) and assembly 78 (black).

Figure 2.16 Normalized UV-Vis absorption profile of 4 µM SG in 80 mM KCl 10 mM TrisHCl pH 7.5 buffer (black), in EtOH (red) and a mixture of 4 µM SG, 4 µM quadruplex 76 in 80 mM KCl 10 mM Tris-HCl pH 7.5 buffer (blue).

Firstly, In order to determine whether SG forms dimers or higher order aggregates in aqueous buffer, preliminary UV-Vis studies were conducted in both Tris-HCl buffer
and in ethanol (Figure 2.16). These absorption profiles show no appreciable change between the high and lower polarity solvent systems suggesting that under these conditions, no significant aggregation was taking place in water. A slight bathochromic shift from 493 nm to 500 nm was observed when SG is mixed (in buffer) with quadruplex 76 indicating a binding event.

Further, we probed whether SG binds to quadruplex 76 via fluorescence spectroscopy. These studies showed that the fluorescence of SG is drastically enhanced in the presence of quadruplex 76 thus giving a convenient spectroscopic signature for quadruplex-dye interaction. The binding stoichiometry of SG to 76 was investigated using a Job plot performed in the same quadruplex forming buffer, and following the fluorescence enhancement (ΔF) of SG when complexed with quadruplex 76. This plot (Figure 2.17) clearly shows that under these concentrations a 1:1 binding stoichiometry is present for SG and quadruplex 76.

**Figure 2.17** Job plot for complexation of SG with quadruplex 76, based on the fluorescence enhancement (ΔF) of SG when it binds to quadruplex 76. For the Job plot, the total concentration of SG and quadruplex 76 were held constant at 4 µM.
In addition to the abovementioned fluorescence measurements, binding stoichiometry and binding constant \((K_a)\) were obtained by measuring the dependence of SG fluorescence upon titration of SG into quadruplex 76 (Figure 2.18, inset) and converting these results into Scatchard coordinates (see experimental section) followed by non-linear curve-fitting to equation 2.1 (Figure 2.18).\(^{164}\)

\[
\frac{v}{L} = K_a \frac{(1 - nv)^n}{(1 - nv + v)^{n-1}}
\]  
(2.1)

Equation 2.1 describes the conditional probability model for non-cooperative excluded site binding of ligand-lattice interactions, derived by McGhee and VonHippel.\(^{165}\) In equation 2.1, \(v\) is the number of bound SG per guanine quartet (a guanine quartet is the tetrad composed of four co-planar guanines), \(L\) is the concentration of free SG in solution at equilibrium, \(K_a\) is the observed association constant and \(n\) is the size of binding site in number of guanine quartets. Analyzing the titration data resulted in \(K_a\) of \(3.60 \pm 0.06 \times 10^5\ \text{M}^{-1}\). Further, the binding site size of SG to quadruplex 76 in terms of guanine quartets was found to be \(n = 3.66 \pm 0.08\) (i.e., close to 4 guanine-quartets), which further suggests that SG binds to DNA quadruplex 76 in a 1:1 stoichiometry since quadruplex 76 has, in total, only 4 guanine quartets.
Figure 2.18 Scatchard coordinates showing SG binding to quadruplex 76. The red line shows the non-linear curve-fit ($R^2 = 0.97$). Inset: Isotherms showing the binding of SG to quadruplex 76. The fluorescence intensity of SG (Ex $\lambda = 495$ nm, Em $\lambda = 525$ nm) was measured upon the titration of SG into 0.6 $\mu$M quadruplex 76 (i.e., 2.4 $\mu$M in terms of guanine-quartets, black line) and 15 $\mu$M quadruplex 76 (60 $\mu$M G-quartets, red line) in 10 mM TrisHCl buffer and 80 mM KCl, pH 7.5.

2.2.4. Energy Transfer Study

Upon determining that SG binds quadruplex 76 in a 1:1 fashion, we next set out to investigate the energy transfer capacity of SG to porphyrin 77 within the nanostructure. Prior to conducting the fluorescence studies, we performed UV-vis absorption experiments to ensure that excitation of (a) SG alone, (b) SG + quadruplex 76, (c) SG + assembly 78 (i.e., assembly 79), and (d) assembly 79 + excess $\beta$-CD (i.e., dis-assembly of assembly 79), were all conducted under relatively matched absorbance at wavelength of 480nm (<10% difference as shown in Figure 2.19).
Figure 2.19 UV-Vis spectra of SG only (black), SG + quadruplex 76 (green), assembly 79 (orange), assembly 79 + excess β-CD (blue), and assembly 78 (red) used in fluorescence studies shown in Figure 2.20. Note: All solutions were 4 μM in quadruplex DNA, porphyrin 77, and SG.

As shown in the fluorescence emission profiles (Figure 2.20a), SG displays no appreciable fluorescence when excited (at 480 nm) in the absence of quadruplex 76. In marked contrast, the fluorescence emission of SG is enhanced 200 fold upon addition to a solution of quadruplex 76 (SG/76 ratio = 1:1), again clearly suggesting binding of SG with the DNA quadruplex. Interestingly, when SG is added to assembly 78 (such that SG/76 ratio = 1:1) this enhancement in emission is diminished significantly and the presence of porphyrin-based emission bands at 650 and 715 nm is observed. The intensities of these porphyrin bands are ca. 10 fold higher than when only assembly 78 is excited at 480 nm (since porphyrin 77 has rather low absorption at 480 nm). Taken together, these experiments suggest that assembly 78 can be functionalized with SG.
resulting in a multi-chromophore array 79 and that an energy transfer process is operational from the SG donor to the porphyrin acceptor. Assuming that all of the quenching is due to FRET, an energy transfer efficiency of 75% was estimated based on the fluorescence emission profiles of SG:76 (donor) complex and assembly 79 (quenched state) according to the equation $K_{ET} = 1 - F_{79}/F_{SG+76}$, where $K_{ET}$ is energy transfer efficiency, $F_{79}$ and $F_{SG+76}$ are the areas of the emission profile from assembly 79 and the SG:76 complex, respectively.

In order to verify that the energy transfer was due to a host-guest derived assembly, we also mixed assembly 79 with a large excess of free β-CD (250 equiv. per each PMβ-CD unit on porphyrin 77). The fluorescence of the resulting solution shows that the high intensity emission (at 530 nm) corresponding to the complex of SG and quadruplex 76 is substantially restored and the emission from the porphyrin peaks is diminished.
Figure 2.20 (a) Solution based fluorescence (excitation $\lambda = 480$ nm) emission profile (inset shows the enlarged profiles around the porphyrin 77 emission region (from 600 nm - 750 nm)) and (b) excitation profile (observed at emission $\lambda = 715$ nm) of array 78 (red), SG only (black), SG + quadruplex 76 (green), assembly 79 (orange), and assembly 79 + excess $\beta$-CD (blue). Note: All solutions were 4 $\mu$M in quadruplex DNA, porphyrin 77, and SG. For excitation profiles a 500 nm cut off long pass filter was applied in front of the observation window.
The excitation spectra (followed at 715 nm, where porphyrin 77 predominantly emits) of assemblies 78, 79, and 79 after incubation with excess β-CD were also investigated. The results are displayed in Figure 2.20b and clearly show that array 79 (i.e., assembly 78 + SG) possesses broadband excitation from 300 nm to 665 nm. This enhanced absorption profile is due, *inter alia*, to the transfer of excitation energy from SG to porphyrin 77 within assembly 79. Another feature that is clear from the excitation spectra shown in Figure 2.20b is that the SG-associated excitation bands are significantly diminished in intensity when the wire is dis-assembled (i.e., assembly 79 + excess β-CD). Taken together, these results further support the notion that the energy transfer from SG to porphyrin 77 is a result of the structure of self-assembled array 79 that is held together through host-guest interactions between porphyrin 77 and quadruplex 76.

Based on the excitation spectra, antenna effects were also calculated. The antenna effect \( AE = I_{79,495 \text{ nm}}/I_{79, Q\text{-band excitation}} \), is defined as the ratio of the fluorescence emission intensity of the acceptor porphyrin 77 (at 715 nm) upon selective excitation of donor SG at its \( \lambda_{\text{max}} \) (495 nm) to that of direct excitation of the three longer wavelength Q-bands of porphyrin 77 (at 548, 588, and 650 nm, respectively) in nanowire 78. These three Q-bands were used to measure the antenna effect since the emission spectra of SG overlaps with these three Q bands of porphyrin 77. Based on the excitation spectra (see Figure 2.20) this antenna effect was found to be 593%, 547%, and 676%, respectively. We have also measured the antenna effect for the first Q-band at 525 nm (the antenna effect was calculated to be 188%), however, since SG chromophore also absorbs at 525 nm, we are not able to selectively excite porphyrin 77 via its first Q-band and thus the antenna effect is smaller than expected.
In addition, the decrease in emission of the SG:76 complex upon binding to porphyrin 77 provided a practical tool to probe the stoichiometry of quadruplex 76 and porphyrin 77 in the nanowire complex. A Job plot (Figure 2.21) gave evidence for a 1:1 stoichiometry of binding (with a maximum at 0.5).

![Job plot for binding of the SG:quadruplex 76 assembly with porphyrin 77, based on fluorescence quenching (ΔF) of SG:quadruplex 76 assembly, when it binds to porphyrin 77. Samples were held at 4 µM total concentration of SG:quadruplex 76 assembly and porphyrin 77 (the mixture was excited at 480 nm and emission was observed at 525 nm).](image)

**Figure 2.21** Job plot for binding of the SG:quadruplex 76 assembly with porphyrin 77, based on fluorescence quenching (ΔF) of SG:quadruplex 76 assembly, when it binds to porphyrin 77. Samples were held at 4 µM total concentration of SG:quadruplex 76 assembly and porphyrin 77 (the mixture was excited at 480 nm and emission was observed at 525 nm).

Further characterization of photonic supramolecular assembly 79 in terms of composition was obtained from non-denaturing gel-electrophoresis. Figure 2.22 (bottom) shows the result of a non-denaturing PAGE that visualizes the fluorescence emission (excitation using a broadband hand-held UV-lamp; excitation λ_max = 365 nm) of
quadruplex 76, porphyrin 77, and assembly 78, each pre-mixed with excess SG. It is evident that DNA quadruplex 76 (lane 1) migrates fast and displays green fluorescence, as a result of binding to SG. On the other hand, porphyrin 77 (lane 3) hardly migrates and displays only porphyrin-based red fluorescence since SG does not bind to the porphyrin. Importantly, assembly 78 + SG (lane 2) displays a broad band that migrates much slower than quadruplex 76 indicating a distribution of high molecular weight species. Furthermore, this new band, ascribed to assembly 79, exhibits fluorescence that is orange in color, suggesting that both the porphyrin and SG-bound quadruplex units are present. Interestingly, these distinct fluorescence emission colors are consistent with those observed in solution by the naked eye (Figure 2.22, top)

Figure 2.22 (top) Fluorescent solutions (from left to right) composed of quadruplex 76 + SG, assembly 78 + SG, and porphyrin 77 + SG, respectively. The solutions were excited with a broadband UV-lamp, $\lambda_{\text{max}} = 365$ nm. (bottom) 15% Non-denaturing PAGE. Lanes 1 through 3 correspond to SG pre-mixed with quadruplex 76, assembly 78, and porphyrin 77, respectively. The gel was imaged upon excitation with a broadband UV-lamp ($\lambda_{\text{max}} = 365$ nm).
PAGE studies were also used to show that supramolecular assembly 79 could be dis-assembled upon addition of excess free β-CD. As shown in Figure 2.23, the characteristic fast migrating quadruplex 76 band (shown in control lane 1) is clearly observed after assembly 79 (prepared by mixing assembly 78 and SG for 5 minutes) was exposed to 250 eq. of free β-CD (per each CD unit on Porphyrin 77) for 20 minutes (see lane 2). Also observed in lane 2 is a slow migrating reddish band that is moving slightly slower than expected for free porphyrin 77 (control shown in lane 3). The reason for this difference in migration may be due to the large excess of free β-CD present in lane 2.

![Figure 2.23](image.png)

**Figure 2.23** 15% PAGE. Lanes 1 through 3 correspond to quadruplex 76 + SG, assembly 78 + SG + free β-CD (250 equiv. per each PM-β-CD unit on porphyrin 77), and porphyrin 77 + SG, respectively.

In order to gather more detailed fluorescence data with respect to the structure of the assemblies, confocal microscopy was conducted. Assembly 78 shows the presence of red fluorescent micron-size structures (when excited at 488 nm) that are indicative of higher-order aggregation of 78 (Figure 2.24, top, inset). The confocal microscopy derived
emission spectra (Figure 2.24, top) clearly shows the fluorescence emission from porphyrin 77 (at 655 nm, and 715 nm). Assembly 79, also shows large aggregates but the emission is yellowish in color (Figure 2.24, bottom, inset). The confocal microscopy derived emission spectra of these micron size structures indicate that the yellowish color is due to a combination of SG(530 nm) and porphyrin 77 (655 nm, 715 nm) emission peaks.

Figure 2.24 Fluorescence emission spectra collected on a Nikon A1 confocal microscope (Excitation $\lambda = 488$ nm). Fluorescence emission spectra collected on the microscopic particle of assembly 78 (Top, particle shown in inset), and assembly 79 (Bottom, particle shown in inset).
2.3. Discussion and conclusion

In this chapter, we have demonstrated how self-assembled DNA quadruplex 76 projecting eight adamantane guest arms can assemble with porphyrin 77 and 77-Zn to form nanowires and their networks. As mentioned earlier, one rationale for using a DNA quadruplex scaffold was because the dimensions (~1.1 nm × 1.1 nm) and four fold symmetry of the porphyrin macrocycle nicely match the geometry (~1.1 nm × 1.1 nm)\(^{152}\) and four fold symmetry of a guanine quartet. As can be seen in Figure 2.8, we expected a linear nanowire to form when the self-assembling partners are properly aligned via multivalent host-guest interactions. However, these quadruplex-free base porphyrin assemblies (nanowire 78) exhibit regions that are unidirectional as well as regions containing junctions. Branching points were observed every 50-200 nanometers on the AFM image of the single wires (Figure 2.10). In contrast, the quadruplex-Zinc porphyrin assemblies (nanowire 78-Zn) show more directional and less flexible linear structures (Figure 2.13). Since it is reported that zinc coordination increases the structure rigidity of the porphyrin core and restricts the rotational motion of meso-phenyl groups.\(^{166,167}\) Thus the reason for this structural difference between nanowire 78 and 78-Zn is thought to be mainly due to the flexibility of freebase porphyrin 77. In addition, flexibility also comes from the flanking thymine residues and the alkyl spacers that connect the adamantane on both terminus of quadruplex 76 (see Scheme 2.4 for the full chemical structure of ODN 75). Such structural flexibility may lead to out-of-register binding. For example, two
adamantyl groups on one end of quadruplex 76 could bind to two cyclodextrin arms of one porphyrin 77 and the remaining two adamantyl arms could bind to two cyclodextrin arms of another porphyrin (see Scheme 2.2). This type of mismatched binding mode can explain the presence of branching regions as well as to the formation of a network of single wires.

Scheme 2.2 An illustration depicting the formation of linear wires, mismatched branched structures, and branched nanowire networks.

In addition to probing the formation of the quadruplex-porphyrin assembly, we have also investigated the possibility of non-covalently incorporating an additional complementary chromophore into the system, by using a DNA binding dye, Sybr Green I (SG). According to the fluorescence titration data, at the concentrations of SG used in our studies (< 4 μM) and the relatively large salt concentration (80 mM KCl), SG binds to quadruplex 76 in a 1:1 stoichiometry. In specific, it was shown that the binding site of SG to quadruplex 76 spans nearly all four guanine quartets (3.66 quartets). Furthermore, SG has been reported to bind to duplex DNA via a similarly large binding size (3.5 base-pairs)\textsuperscript{164}. Detailed studies with duplex DNA has led to the proposal that SG undergoes intercalation via the benzo-thiazole and phenyl-quinilinium aromatic moieties while the
propyl and dimethylaminopropyl arms span (and bind to) the minor groove. While the precise binding mode of SG to quadruplex DNA has not been elucidated, since SG binds with a similar binding size and shows substantial enhancement in fluorescence when bound to either duplex DNA or quadruplex DNA, we speculate that the aromatic rings on SG are likely to intercalate between two guanine quartets of quadruplex 76 with the positively charged dimethylaminopropyl arms interacting with the phosphate backbones.

Analysis of the fluorescence properties of the porphyrin/quadruplex/SG assembly 79 shows that an energy transfer mechanism is operational leading to funneling of energy into the porphyrin macrocycle. This behavior is of interest in terms of applications since such photoactive supramolecular complexes could lead to the development of self-assembled antenna structures for artificial light harvesting, or singlet oxygen generation (e.g., with potential in light-activated antibacterial applications)71. In addition, there is a need for the identification of quadruplex DNA specific binding agents168, thus such nanostructured assemblies incorporating quadruplex DNA could lead to high-throughput surface-based sensing platforms that can detect quadruplex binding agents via the disruption of an energy-transfer process.21 For instance, a small molecule or protein that binds to the quadruplex domain and displaces the dye will lead to loss of energy transfer to the porphyrin and thus can be detected.

In conclusion, we have developed a multifaceted supramolecular self-assembly strategy for the construction of photonic DNA-based nanowires and their networks. In contrast to traditional DNA-based photonic assemblies, this design starts from a single short ODN sequence without any chromophores covalently attached to it. It harnesses a number of important self-assembly modes in water including, DNA base-pairing, β-
CD/adamantane derived host-guest interactions, and DNA-dye binding, to both facilitate nanostructure assembly and to incorporate complementary porphyrin and SG chromophores. Together these chromophores show broad spectrum absorption, from 300 nm to 665 nm. Since there is a variety of commercially available DNA-binding dyes with different photophysical properties, this self-assembly strategy offers a simple route to investigate multi-chromophore containing photonic nanostructures by a simple mix and match protocol. Further, compare to conventional one dimensional G-wires,, this work is expected to serve as a stepping stone wherein better defined two- and three-dimensional G-quadruplex-based photonic nanostructures with directional FRET cascades can be constructed.

2.4. Future directions

Advantages of our photonic assembly are that a) the inter-chromophore distance can be potentially fine-tuned by modulating the length of G-quadruplex module, b) the energy donor-acceptor ratio and relative orientation can also be potentially adjusted by either changing the G-quadruplex module length or choosing a different DNA binding dye, and c) other functional DNA self-assembling modules such as the pH sensitive i-motif DNA structure could also be introduced. Hence, our photonic assembly lays the foundation for several future developing directions as shown below.

2.4.1. Modulating the inter-chromophores distance

One of our long term goals is to modulate the inter-chromophore distance by introducing G-quadruplexes with different lengths. In order to study the influence of interchromophore distance and chromophore ratio on the energy transfer efficiency
between different chromophores in the assembly, we will assemble different G-quadruplexes. For example, as shown in Scheme 2.3, we are currently investigating the effect of varying lengths of the G-quadruplexes, such as d(TGGT)$_4$ and d(TGGGGGGT)$_4$, which could modulate the distance between porphyrins, and also finely tune the ratio between porphyrin and SG.

Scheme 2.3 A schematic representation of potential nanowires that can be formed by assembling porphyrin 77 with d(TGGT)$_4$ or d(TGGGGGGT)$_4$

2.4.2. Fine-tuning the donor-acceptor ratio and relative orientation

SG is a member of the monomethine family of cyanine dyes and is routinely utilized to detect double stranded DNA, and as has been shown in this chapter quadruplex 76 can be bound by SG in a 1:1 stoichiometry. Other cyanine dyes can also interact with quadruplex DNA in a 1:1 fashion. For instance, the Armitage group has observed that the carbocyanine dye DiSC(3) binds to a folded intramolecular quadruplex with a 1:1 stoichiometry.

However, the nature of the cyanine dye is salient since another cyanine-based chromophore, DMSB, is also capable of binding to tetramolecular quadruplex DNA in a 2:1 fashion (where the dye forms a stacked homo-dimer). The incorporation of such cyanine based homodimers, or even chromophore tethered quadruplex DNA binding ligands (such as Distamycin A) that bind to quadruplex DNA with higher
stoichiometry, may allow for further enhancement of the amount of light energy funneled into the porphyrin acceptor within the nanowire scaffold.

2.4.3. pH sensitive DNA nanowires based on i-motif units

Another attractive (antiparallel) quadruplex DNA structure is the i-motif DNA that consists of four d(Cn) strands. The structure of the quadruplex i-motif is described as a parallel-stranded duplex formed by non-canonical protonated cytosine pairs, and two such duplexes associate in a head-to-tail manner as a result of base-pair intercalation (Figure 2.25). Importantly, the i-motif structure is thermally stable, for example the d(TC5) tetramer has a $T_m = 48 \, ^\circ C$ at concentration of 11.5 μM in single strand DNA. Since this structure is typically formed under acidic conditions (< pH 6), the i-motif structure can dissociate upon an increase in pH. Thus, using the strategy we have described in Section 2.1, a pH sensitive DNA photonic nanowire can potentially be achieved via the self-assembly of porphyrin 77 with an i-motif moiety that functionalized with adamantane head-groups on both terminus (see structure of ODN 84).

Figure 2.25 (A). Chemical structure of non-canonical protonated C-C base pairs. (B) Side view of crystal structure consisting of one parallel duplex together with the intercalated basepairs from the other duplex. (C)
Chemical structure of ODN 84. (D) A descriptive molecular model of a nanowire composed of porphyrin 77 and adamantane functionalized i-motif (84).

2.5. Experimental Section

2.5.1. General Experimental.

Unless otherwise noted, all chemicals were purchased from Sigma-Aldrich or Acros Organics and solvents were purchased from Fischer Scientific. Sybr Green I (10,000x solution in DMSO, 1x = 0.68 μM) was purchased from Life Technologies. NMR spectra were recorded on a Varian 400 MHz spectrometer using CDCl₃ as solvent. MALDI-TOF spectra were recorded on a Bruker Autoflex 3 Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometer (MALDI-TOF MS). The matrices used were 2,5-dihydroxybenzoic acid (DHB) for oligodeoxyribonucleotide (ODNs) and α-cyano-4-hydroxycinnamic acid for the small molecules. UV-Vis studies were undertaken using a Hewlett Packard 8452A Diode Array Spectrophotometer. Fluorescence spectra were recorded on a Varian Cary Eclipse Fluorescence Spectrophotometer.

2.5.1.1. CD experiments.

Circular dichroism spectra were taken on an Olis RSM 1000 CD using a cylindrical cuvette with 1 mm path length. The quadruplex formation of ODN 75 was monitored by CD and the data was subtracted from the spectra of a solution containing only buffer (10 mM Tris-HCl, 80 mM KCl, pH 7.5).
2.5.1.2. PAGE Studies.

Non-denaturing polyacrylamide gel electrophoresis studies were conducted using a BioRad mini protean tetra cell equipped with BioRad PowerPac HC. For the gel shown in Figure 1a, in lanes 1, 3, and 4, \( \sim 8 \times 10^{-4} \) µmole of ssDNA was introduced, and in lane 2, \( \sim 16 \times 10^{-4} \) µmole of single strand ODN 75 and \( \sim 16 \times 10^{-4} \) µmole of single strand ODN 81 were used. All ODNs were applied to the gel after exposure to the standard quadruplex forming condition. The gel was stained using DNA binding dye Stains all, and imaged on a Nikon D200 camera.

For each lane of the gel shown in Figure 2.22 and Figure 2.23, \( \sim 2 \times 10^{-4} \) µmole of quadruplex DNA and/or porphyrin 77 were premixed with 2 µL of 300x SybrGreen I (~4 \( \times 10^{-4} \) µmole) for 15 min before applying to the gel. The running buffer was 1x TBE buffer containing 24 mM KCl at 110 V for 1.5 hrs. The gel was imaged on a Nikon D200 camera upon excitation with a broadband UV-lamp (\( \lambda_{\text{max}} = 365 \) nm). A 520 nm cut-off filter (blocks light < 520 nm) was applied in front of the camera lens to remove any background/scattered light.

2.5.1.3. HPLC conditions.

RP-HPLC purification was achieved using a Varian Prostar HPLC system, equipped with a Polymer Laboratories 100 Å 5 µm PLRP-S reverse phase column. The column was maintained at 65 °C for all runs. The flow rate was set at 0.75 mL/min. A gradient composed of two solvents (solvent A is 0.1 M TEAA in 5% acetonitrile and solvent B is 100% acetonitrile) was used.

Microscopy Protocols.
2.5.1.4. Cryo-TEM experiments

TEM experiments were performed on a FEI Tecnai G2 F30 Twin Transmission Electron Microscope Instrument (accelerating voltage = 120 kV). Sample was prepared on 200 mesh copper grids with lacey carbon film (purchased from Electron Microscopy Science). In order to facilitate the formation of higher order assemblies that show better contrast on TEM images, the sample was prepared in a 10 mM TrisHCl pH 7.5 buffer that contains 160 mM KCl.

2.5.1.5. AFM experiments

AFM experiments were carried out on a Veeco Bioscope AFM (Digital Instruments) under tapping mode in air. Bruker OTESPA AFM probes with nominal frequency, tip diameter, and spring constants of 300 KHz, 7 nm, and 42 N/m, respectively were used. Mica (highest grade V1 Mica disc, 10 mm diameter, was purchased from TED PELLA, Inc.) was used as the substrate and the mica plate was freshly cleaved via scotch tape to achieve a flat surface before use. Prior to sample introduction, a 2 mM MgCl₂ aqueous solution was applied for 5 minutes and then dried using a nitrogen gas flow. A solution of assembly 78 was subsequently applied on the mica substrate for 10 minutes, then washed 10 times with 50 μL aliquots of deionized water. After drying with nitrogen gas flow, the sample was imaged.

2.5.1.6. Confocal microscopy images

Confocal microscopy images were collected on a Nikon A1RSi equipped with a 32-channel multianode photomultiplier detector. 488 nanometer laser excitation was used. The spectral data was acquired in sequential bandwidths of 10 nm spanning the wavelength range from 492-742 nm using 26 PMT channels to generate a lambda stack.
2.5.1.7. Analysis of SG binding to quadruplex 76 via fluorescence spectroscopy.

Varying concentrations (0.02-1 µM) of SG was titrated into a solution containing quadruplex 76 (2.4 µM in terms of guanine-quartets) and the observed fluorescence intensity (F) of the SG:quadruplex 76 complex was used to generate an isotherm (black line, Figure 2.18, inset). In order to determine the fluorescence intensity of 100% bound SG (F_b), another titration was conducted where varying concentrations of SG was titrated into a solution containing a large excess of quadruplex 76 (60 µM in terms of guanine-quartets; red line, Figure 2.18, inset). Note: since the fluorescence of the free SG in solution is significantly lower than the fluorescence of the SG:quadruplex 76 complex (F_{complex}/F_{free} > 200 fold), the contribution of the fluorescence of the free SG dye to the total fluorescence intensity is negligible. Accordingly, the fraction of SG dye bound to quadruplex 76 can be expressed as:

\[ \theta \approx \frac{F}{F_b} \]  \hspace{1cm} (2.2)

Where F is the observed fluorescence intensity of the SG:quadruplex 76 complex and F_b is the observed fluorescence intensity of 100% SG bound to quadruplex 76.

At equilibrium, the concentration of free SG can be expressed as:

\[ L = (1-\theta)C_{SG} \]  \hspace{1cm} (2.3)

and the SG binding density (which is the number of bound SG per guanine-quartet) can be expressed as:

\[ v = \frac{\theta C_{SG}}{C_Q} \]  \hspace{1cm} (2.4)
where $C_{SG}$ and $C_Q$ correspond to the total concentrations of SG and guanine-quartets in solution, respectively.

After determining $v$ and $L$, a Scatchard plot was constructed, as shown in Figure 2.18, and non-linear regression analysis was used to fit the data to equation 2.1.

2.5.2. ODN Synthesis

2.5.2.1. General Protocol.

After synthesis (vide infra) the ODNs were first purified with Sephadex resin Microspin G-25 columns (GE Healthcare) and then were chromatographed on a Varian Prostar reverse-phase HPLC complete with a MetaTherm column heater. Once purified the ODNs were characterized by MALDI-TOF in linear negative mode. Concentrations of stock solutions of ODNs were quantified based on their 260 nm electronic absorption at 85°C and their molar extinction coefficients were obtained by standard nearest neighbor calculations.
Scheme 2.4 Chemical structures of ODNs 75, 80, 81, 82, and 83. Note: Calculated mass [M-H]⁻ is included.
2.5.2.2. Synthesis of core ODNs.

ODN 80 (structure shown in Scheme 2.4) was synthesized at the Keck Foundation Biotechnology Resource Laboratory at Yale University using standard automated solid phase synthesis. Modified phosphoramidites (5’-Amino-Modifier C3 and 3’-PT Amino-Mod C3) were purchased from Glen Research. After HPLC purification, ODN 80 was analyzed by MALDI-TOF under negative linear mode (See Figure 2.26; Found \( m/z = 2138.18 \text{ Da} \), calculated mass \([M-H]^+ = 2136.39 \text{ Da}\)).

2.5.2.3. General Synthesis of bis-adamantane functionalized ODN 75 and ODN 8.

The RP-HPLC purified core ODN 80 bearing primary amino groups on both the 3' and 5' ends, was reacted with excess 1-adamantyl isothiocyanate (10 mg in 1 mL of DMSO), DIPEA (50 µL) and 500 µL of 60 mM sodium carbonate buffer (pH = 8.5). The resulting solution was agitated overnight at 55°C and then unreacted 1-adamantyl isothiocyanate was precipitated by adding 2 mL of H₂O. The white precipitate was filtered off and the solvents were removed using a Savant SPD IIIV speed rotorvap. The resulting crude residue (ODN 75) was dissolved in 0.1 M TEAA buffer, desalted using a G-25 spin column, purified by HPLC, and analyzed by MALDI-TOF under negative linear mode (Figure 2.26; Found \( m/z = 2523.11 \text{ Da} \), calculated mass \([M-H]^+ = 2523.00 \text{ Da}\)).

ODN 82 was synthesized using the same procedure discussed above, starting from a modified core sequence that contains six thymidine (TTTTTT) bearing primary amino groups on both the 3’ and 5’ ends. The final product ODN 82 was analyzed by MALDI-TOF under negative linear mode (Figure 2.26; Found \( m/z = 2422.24 \text{ Da} \) calculated mass \([M-H]^+ = 2422.95 \text{ Da}\)).
Figure 2.26 MALDI-TOF mass spectra of ODNs 80, 75, 81, 82, and 83 respectively.
2.5.3. Standard Incubation Process for Formation of Quadruplex DNA

A stock solution of single strand ODN (75 or 81) was diluted to 160 μM in potassium containing Tris-HCl buffer (10 mM Tris-HCl, 80 mM KCl, pH 7.5) and sealed in an eppendorf tube. The tube was heated to 90°C for 10 minutes and then cooled at 4°C for 48 hr. The resultant solution containing 40 μM tetramolecular quadruplex (76 or 814) was diluted in potassium containing Tris-HCl buffer to an appropriate concentration prior to use in the various spectroscopic and microscopic studies.

2.5.4. Synthesis of Porphyrin 77.

The starting material, zinc porphyrin 77 (77-Zn) has been previously reported by our group. To a stirring solution of 77-Zn (50 mg, 0.004 mmol) in 100 mL chloroform was added 5 drops of concentrated HCl, and the stirring was continued for 10 min. The reaction mixture was then neutralized with triethylamine and washed with saturated sodium bicarbonate. Subsequently, the organic layer was dried over anhydrous Na₂SO₄. The solvent was evaporated in vacuo and the final product was purified by silica gel column (first eluted with hexanes to remove non-polar impurities and then the product was collected by eluting with CH₂Cl₂). The solvents were removed in vacuo to give 77 as a purple solid (41 mg, 81%). ³¹H NMR (400 MHz, CDCl₃): δ = 8.90 (br s, 8H), 7.81 (br s, 8H), 7.46 (br s, 8H), 7.06 (br s, 4H), 5.28 (m, 16H), 5.23-5.00 (m, 56H), 4.82 (m, 8H), 4.06-3.14 (m, the rest of PMβ-CDs protons ca. 808H), -2.91(br, 2H). MALDI-TOF MS (reflective positive mode): calculated for C₅₆₄H₉₁₈N₂₈O₂₈₀K m/z = 12602.8 Da, Found m/z = 12599.6 Da (M+K⁺). UV-Vis (H₂O): 422 nm (ε 203,489 dm³ mol⁻¹ cm⁻¹), 518 nm (ε
15,586 dm$^3$ mol$^{-1}$ cm$^{-1}$), 548 nm ($\varepsilon$ 8,787 dm$^3$ mol$^{-1}$ cm$^{-1}$), 588 nm ($\varepsilon$ 5,300 dm$^3$ mol$^{-1}$ cm$^{-1}$), 650 nm ($\varepsilon$ 5,326 dm$^3$ mol$^{-1}$ cm$^{-1}$).

**Scheme 2.5** Synthesis of porphyrin 77.

![Scheme 2.5 Synthesis of porphyrin 77.](image)

**Figure 2.27** MALDI-TOF mass spectra of porphyrin 77. The parent peak at 12599.6 Da (M+K$^+$) corresponds to porphyrin 77. The less intense peak at $m/z = 11167.4$ Da corresponds to MALDI-induced fragmentation of one PM-βCD arm of 77 (possibly via cleavage of the C-N bond between the triazole nitrogen and the 6'-carbon on the glucose unit of βCD); calculated m/z of the resulting fragment = 11166.2 Da).
Figure 2.28 $^1$H NMR spectra of porphyrin 77
2.5.5. Preparation of Assembly 78

100 μL of 40 μM quadruplex 76 in Tris-HCl buffer (10 mM Tris-HCl, 80 mM KCl, pH 7.5) was mixed with 40 μL of 100 μM porphyrin 77 in Tris-HCl buffer(10 mM Tris-HCl, 80 mM KCl, pH 7.5), to give an equal concentration of porphyrin 77 and quadruplex 76 (28.6 μM each). The mixture was covered with aluminum foil and kept at room temperature for 48 hrs. to form the assembly. This solution was diluted with Tris-HCl buffer to an appropriate concentration (for most spectroscopic and microscopic experiments 4 μM solution of the assembly, in terms of the concentration of porphyrin 77 or quadruplex 76, was used).

2.5.6. Preparation of Assembly 79

Assembly 79 was prepared by adding 1 μL of 300x SG in Tris-HCl buffer (10 mM Tris-HCl, 80 mM KCl, pH 7.5) to 50 μL of 4 μM solution of nanowire 78 (final SG: quadruplex 76 ratio ≈1:1) and mixed for 5 minutes. As can be seen from inspection of the solid black line in Figure 2.15, assembly 79 shows broadband absorption from 300nm to 665nm.
Chapter 3: Water Soluble PEGylated Porphyrin-grafted Silicon Nanoparticles for Photodynamic Therapy

3.1. Introduction

Photodynamic therapy (PDT) is a promising non-invasive method to treat small solid tumors (such as tumors of non-small cell lung cancer and esophageal cancer etc).\textsuperscript{174,175} As Shown in Figure 3.1, photodynamic therapy is a treatment that uses a combination of a photosensitizer and activating light at a specific wavelength.\textsuperscript{176} Upon capturing the light energy, the excited photosensitizer produces singlet oxygen (\(1^\text{O}_2\)) or superoxide (\(\text{O}_2^-\)) in the presence of molecular oxygen, and induces cell damage through direct and indirect cytotoxicity.\textsuperscript{176,177} PDT’s major advantages compared to traditional methods for cancer treatment are better selectivity, and low toxicity of the photosensitizers.

![Figure 3.1 A Schematic illustration of Photodynamic therapy.](image)

The photosensitizer, which is typically a small organic dye molecule, is considered a critical element in PDT. Ideal design of a photosensitizer should possess critical features such as follows: (a) the substance should have appreciable photophysical
and photochemical properties required for therapeutic and diagnostic applications. (b) the substance should have little or no dark cytotoxicity, (c) the substance should be a single, pure molecule and easy to prepare, (d) the photosensitizer should have absorption band(s) at the red end of visible spectrum (because light with longer wavelength can better penetrate body tissue), (e) it is better to possess an amphiphilic configuration to improve the selectivity to tumor tissues.\textsuperscript{13,178} In regard to the abovementioned points, porphyrins and their derivatives are considered as ideal photosensitizers for PDT because of their versatile photophysical and photochemical properties, low toxicity to human body, ease of synthesis and modification, as well as adjustable absorption properties.\textsuperscript{13,14} Indeed, most of the commercial second generation photosensitizers belong to the porphyrin family. However, traditional small molecule porphyrin photosensitizers have poor selectivity for tumor cells, and also show poor solubility and lower photophysical/chemical activity due to aggregation of porphyrins in aqueous media.\textsuperscript{179,180}

Therefore, developing porphyrin photosensitizers that can selectively accumulate in cancer cells and have minimum aggregation in water is a salient goal. Research has shown that the characteristic features of tumor tissue often can lead to the accumulation of nanoparticles in cancer cells, due to the enhanced permeability and retention (EPR) effects.\textsuperscript{181} Thus, incorporating photosensitizers onto nanocarriers such as Au nanoparticles\textsuperscript{182,183}, CdSe quantum dots\textsuperscript{118–120}, virus like nanoparticles\textsuperscript{86,87}, DNA nanostructures\textsuperscript{59}, and silica nanoparticles\textsuperscript{123} etc. or assembling photosensitizers into highly defined nanostructures, such as micelles\textsuperscript{181} has attracted great attention in recent years. The nanoparticle carrier not only can significantly enhance the selective accumulation in tumors due to the EPR effect (or through appropriate conjugation with a
targeting antibody or aptamer) but also can protect the photosensitizer during systemic circulation.

![Diagram of porphyrin grafted porous silicon nanoparticle (pSiNP)](image)

**Figure 3.2** Synthesis of porphyrin grafted porous silicon nanoparticle (pSiNP 86)

Because of their distinct photophysical and photochemical properties\textsuperscript{43,184} as well as their biodegradability and low toxicity,\textsuperscript{39–42} Silicon nanoparticles (SiNP) are attractive nano-systems for biological applications\textsuperscript{185} such as cellular assays\textsuperscript{39,186}, deep-tissue imaging,\textsuperscript{148–151} as well as nanocarriers for chemotherapeutic applications in cancer treatment\textsuperscript{191}. Sailor and coworkers have demonstrated that porous silicon nanoparticles are able to generate detectable amounts of singlet oxygen and also show phototoxicity when irradiated with blue light.\textsuperscript{192} They have also shown examples using large porous silicon nanoparticles (35nm-245nm) as nanocarriers to create water soluble porphyrin grafted nanoscale photosensitizers \textbf{86} (pSiNP, Figure 3.2).\textsuperscript{179} However, the porphyrin mass loading in the pSiNPs \textbf{86} is only 13.3μg per mg of nanoparticle. In this regard, ultra-small size (<10nm) silicon nanoparticles prepared \textit{via} mechanochemical synthesis using high energy ball milling (HEBM) has attracted our interest because of its appreciable properties such as large surface areas thereby allowing much higher porphyrin mass loading, easy preparation in large quantities, easy attachment of a variety
of functional groups, and size-dependent energy band gap resulting in versatile photophysical and photochemical properties.

Based on these considerations, here we describe a new approach to water soluble PEGylated porphyrin grafted silicon nanoparticles (PPSiNP 95). In specific, as shown in Scheme 3.1, an azide-terminated SiNP (AzSiNP 87) was functionalized with a PEGylated zinc-tetraphenyl porphyrin 94 (via azide-alkyne “click” reaction) that has three PEG chains and one alkynyl group attached on the para position of the phenyl groups. In this system, polyethylene glycol (PEG) was chosen as the water solubilizing group not only because PEG is biocompatible and non-toxic, but also because PEG containing
amphiphilic structures are able to form large micelle-like nanostructures^{193–195}, and thus may improve the selectivity to tumor tissue. As importantly, large number of PEG substituents are also expected to create steric hindrance between the porphyrin units and hence minimize unwanted porphyrin aggregation.

3.2. Results and discussion

### 3.2.1. Synthesis of PEGylated porphyrin grafted silicon nanoparticles

Scheme 3.2 Synthesis of the ω-azido-pentenyl (50% coverage)-Si nanoparticles (AzSiNP 87) via high energy ball milling (HEBM)

**Figure 3.3** TEM image of AzSiNP 87 (left) and their corresponding particle size distribution histogram (right).
Azido-silicon nanoparticles 87 (AzSiNPs) were prepared via mechanochemical synthesis using high energy ball milling (HEBM). As shown in Scheme 3.2, chloride-terminated silicon nanoparticles were first synthesized by HEBM of Si wafers with a 1:1 mixture of 1-pentyne and 5-chloro-1-pentyne. Azide-terminated Si nanoparticles were subsequently obtained through the reaction of halide-terminated Si nanoparticles and NaN₃ in DMF. The azide terminated silicon nanoparticle (AzSiNP) 87 with the desired size range was isolated by gel permeation chromatography. Transmission electron microscopy (TEM) of the AzSiNP 87 shows nanoparticles with a size of 3-5nm (Figure 3.3). Atomic force microscopy (AFM) also showed that the nanoparticles have an apparent height of ca. 3nm (Figure 3.6A).

Scheme 3.3 Synthesis of PEGlyated porphyrin 94
The synthesis of PEGylated zincporphyrin (PZnP) 94 is outlined in Scheme 3.3. Briefly, aldehyde 88 and dipyrromethane 89 was prepared according to literature procedures. Both aldehyde 88 and dipyrromethane 89 were subsequently reacted with 4-formal methyl benzoate in the presence of BF3·OEt2 in deaerated chloroform, followed by oxidizing with DDQ. The resultant porphyrin mixture was purified via column chromatography (silica gel column using 4:1 hexane: methylene chloride as eluent) to afford porphyrin 90 in 19% yield. The TMS protecting groups and the methyl ester protecting group were deprotected by refluxing porphyrin 90 with KOH in 1:1 THF/water, followed by metallation of resulting porphyrin to afford Zn porphyrin 92. The PEGylated zinc porphyrin 94 was prepared by subsequently reacting Zn porphyrin 92 with O-(2-azidoethyl) nonadeca-ethylene glycol via the copper catalyzed Huisgen[3+2] cycloaddition. This was followed by coupling the acid unit with propargyl amine in the presence of DCC and NHS. The requisite PEGylated porphyrin grafted silicon nanoparticle (PPSiNP) 95 was prepared by the Huisgen [3+2] cycloaddition of porphyrin 94 and AzSiNP 87 and was isolated by gel permeation chromatography.

3.2.2. Characterization of PEGylated porphyrin grafted silicon nanoparticle 95

Successful attachment of porphyrin 94 to the silicon nanoparticle was first confirmed by NMR spectroscopy. For example, the NMR spectrum of nanoparticle 95 displays the characteristic peak at 8.67 ppm assigned to β-protons of the porphyrin unit, as well as peaks from 0.5 ppm-2 ppm assigned to the alkyl protons of the silicon nanoparticle. In addition, the ratio between the porphyrin units and the pentenyl group on the surface of silicon nanoparticle was estimated by comparing the integrated area of the aromatic proton peaks (~28.0) and the high field alkyl proton peaks (~153.2, from 0.5-2
ppm). The total number of aromatic protons of porphyrin, including β-protons, phenyl protons and triazole protons, are 28 protons in total. Since the azido silicon particle was covalently protected with a 1:1 mixture of ω-azido-pentenyl (4 high field alkyl protons) and pentenyl (7 high field alkyl protons) groups on the surface, the high field alkyl protons (2.5-0 ppm) was estimated to be 5.5 protons (average between the two types of pentenyl groups) per pentenyl chain. The ratio of porphyrin to pentenyl groups was then estimated to be 1:27.8, which indicates that only 7.2% of the azido groups on the silicon nanoparticle reacted. In addition, FT-IR profiles of nanoparticle 95 also exhibited a peak at 2098 cm⁻¹ that is assigned to unreacted azido groups and a small peak at 3111 cm⁻¹ that is assigned to triazole CH stretching peak. The reason that all the azido groups on the silicon nanoparticle do not fully react is likely because of steric hindrance caused by the bulky PEGylated porphyrin units.

Figure 3.4 FT-IR profile of AzSiNP 87(black) and nanoparticle 95(red)
In order to evaluate the photophysical properties of porphyrin 94 upon conjugating to the silicon nanoparticle, UV-vis and fluorescence experiments were conducted. As can be seen in Figure 3.5, the solution of porphyrin 94 and nanoparticle 95 in methylene chloride both show identical sharp Soret band and Q bands that are ascribed to monomeric porphyrin molecules. The Q absorption bands show increased absorption and also red shift from 557 nm to 562 nm and from 597 nm to 604 nm when porphyrin 94 and nanoparticle 95 are dissolved in water. In addition, the solution of porphyrin 94 and nanoparticle 95, in water, both show a broadened Soret absorption band that is constituted of the Soret band of monomeric porphyrin at 427 nm and a red shifted
peak at 438 nm (that belongs to the J-aggregates of porphyrin molecules). These peak can be reconstructed from the UV-Vis absorption profiles by applying Gaussian multi peak fitting function in Origin pro 8 (see Figure 3.5C and D). The ratio of integrated area between monomeric porphyrin absorption peak and porphyrin J-aggregate peak was calculated to be 1:0.36 for porphyrin 94 and 1: 0.16 for nanoparticle 95. These experiments indicate that although porphyrins in both porphyrin 94 and nanoparticle 95 undergo some self-aggregation via pi-stacking interactions in aqueous media, the porphyrin aggregation is minimized in nanoparticle 95. We believe it is the consequence of (a) the bulky design of porphyrin 94 as well as (b) the hydrophobic environment created by the alkyl chains on the surface of SiNP that lead to such an attenuation of porphyrin aggregation. Furthermore, the fluorescence emission spectra of nanoparticle 95 (Figure 3.5B) consists of a “blue” part (370 nm to 570 nm) and “red” part (590 nm to 750 nm) when excited at 300 nm, which representing the summary of both Si nanoparticles and Zn-porphyrin emissions.

In addition, the mass loading of porphyrin in the nanoparticle 95 system was also determined by UV-Visible spectroscopy. For example, the extinction coefficient of porphyrin 94 in methanol was determined to be 311,324 dm$^3$ mol$^{-1}$ cm$^{-1}$ at the wavelength of 430 nm. The absorption of a 4 mg/L solution of nanoparticle 95 in methanol in a 1 cm path length cuvette was 0.2732 at 430 nm (a region where the silicon portion of the nanoparticle has very weak absorption) and thus the concentration was determined to be 0.88 μmole/L or 3.16mg/L in porphyrin 94. So the mass loading of porphyrin 94 was calculated to be 0.79 mg per mg of nanoparticle 95 (mass loading = calculated mass of 94/ mass of 95).
Figure 3.6 (A) A 2.5×2.5 μm² AFM image of AzSiNP 87 (C) 5×5 μm² AFM image of PPSiNP 95 on mica substrate. (B) Histogram height distribution of the particles shown on panel A. (D) Height profiles collected at the indicated white lines (labeled 1,2,3 respectively) on panel C.

Tapping mode AFM was utilized to determine the structure of nanoparticle 95 on freshly cleaved mica substrate. As can be seen on the AFM image shown in Figure 3.6A, when AFM studies were conducted on AzSiNP 87, spherical particles are clearly observed. The apparent height of these particles was found to be ca. 3 nm, which is in accord with the TEM results mentioned above. More interestingly, the AFM image of nanoparticle 95 (Figure 3.6B) shows amorphous structures that have a bright core with an apparent height ~3 nm, which is in accord with the height of AzSiNP 87. These structures
are also have flexible surrounding area which likely corresponds to the PEGylated porphyrins conjugated to silicon nanoparticles.

![Image of nanoparticle structures](image)

**Figure 3.7** (A) Cryo-TEM of Image and (B) a zoom in image of nanoparticle 95 (20μM of Porphyrin) in water. (C) Histogram Size distribution of the particles shown on panel A. (D) Conventional TEM image of nanoparticle 95

In order to probe the structure of nanoparticle 95 in aqueous media, Cryo-TEM was employed. As shown in Figure 3.7, the Cryo-TEM image of nanoparticle 95 solution in water (20μM of porphyrin, determined by UV-Vis absorption) displayed solid sphere structures that have a diameter ca. 12 nm. Higher order aggregate states of these nanoparticles are also observed. As shown in Figure 3.7B, larger particles with diameters of ~36 nm display darker spots within the particle, which leads us to suggest that these particles are made up of multiple smaller particles. Since nanoparticle 95 has an
amphiphilic structure, they are able to form larger micelle-like nanostructures as a result of hydrophilic/hydrophilic interactions (i.e., PEG interactions) and hydrophobic/hydrophobic interactions (i.e., alkyl chain interactions). In order to verify that such interactions lead to aggregation of the nanoparticles into micelles, α-cyclodextrin, that can strongly thread into the PEG chain, was added. Indeed, Cryo-TEM results show that the micelle-like nanostructures are not present after adding α-cyclodextrin to the nanoparticle solution (data not shown). In addition, conventional TEM sample was prepared by drying aqueous solutions of nanoparticle (20 uM in porphyrin) on TEM grids. TEM image of this sample shows much larger spherical micelle-like structures with diameter of 50-150 nm which indicates formation of higher order aggregates during the drying process.

Prior to in vitro studies, millisecond phosphorescence emission spectra of singlet oxygen was collected to probe the photo reactivity of nanoparticle. As shown in Figure 3.8, a strong emission peak at ~1278 nm which corresponds to singlet oxygen emission was observed upon irradiating the first Q-band (557 nm) of air-saturated nanoparticle solution in deuterated acetone at room temperature.
**Figure 3.8** Emission spectra of singlet oxygen phosphorescence in air-saturated solution of PEG-Por-SiNP in deuterated acetone at room temperature. Absorbance of the PEG-Por-SiNP was ~0.5 at $\lambda = 430$ nm

### 3.2.3. Cellular uptake of nanoparticle 95

The Human Embryonic Kidney 293T (HEK293T) cell line was selected as the experimental model for *in vitro* studies. In order to determine whether nanoparticle 95 could be uptaken by the cells, a time dependent experiment was set up using HEK293T cells. To each of 5 confluent HEK293T cell samples were added a solution of nanoparticle 95 to achieve a concentration of 0.05 mg/mL (10$\mu$M in porphyrin) at time point of 0, 12, 18, 21, and 23 hours. A control was also set up in media without nanoparticle 95. In this case, these samples were incubated with same concentration of nanoparticle 95 for 24, 12, 6, 3, 1 and 0 hours respectively. Then the buffer was removed, and cells were washed with PBS buffer twice. UV-Vis spectroscopic analyses were performed on cell lysates. As shown in Figure 3.9, the time-dependent uptake curve of nanoparticle 95 is given by monitoring UV-Vis absorption at $\lambda=430$ nm. Importantly, these studies show that with time the cells are gradually uptaking the nanoparticle.
In addition, the internalization of nanoparticle 95 was also analyzed by confocal microscopy. As shown in Figure 3.10A, when nanoparticle 95 was selectively excited by a 633 nm excitation laser, very strong luminescent emission was observed in the fixed cells that were incubated with nanoparticle 95 for 24 hours. When these images are combined with the image of whole cells (Figure 3.10B) visualized by Cell Mask Green (that stains the cytoplasm and the nucleus) the composite image (Figure 3.10C) clearly shows that the bright red luminescent spots are located inside the cells. Taken together, these data indicate that nanoparticle 95 can efficiently be internalized and accumulates in HEK239T cells, and this process can be detected by the red fluorescence emission from the porphyrin moieties on the nanoparticles.
Figure 3.10 Confocal microscope image of fixed HEK293T cells that were incubated with nanoparticle 95 (10μM in porphyrin) for 24 hours and co-stained with a whole cell stain (Cell Mask green). Cell was visualized by 488 nm laser excitation, and a 515nm longpass (emission) filter was applied. Nanoparticle 95 was visualized by 633 nm laser excitation, and a 650-715 nm band pass (emission) filter was applied.

3.2.4. *In vitro* phototoxicity

Phototoxicity of nanoparticle 95 was probed by applying a filtered high intensity Xenon arch (200 W) light beam (with a 530 nm cut off long pass filter and a water IR filter (that filters light from 1000 to 3000 nm) was applied) to HEK293T cells. Two controls (cells only, in the absence of nanoparticle 95) and two samples (cells, in presence of nanoparticle 95) were incubated for 24 hours. They were separated into two groups (irradiate with light or keep in dark) with one control and one sample in each group. One group was kept in the dark, and the other group was irradiated with light for
30 minutes followed by a 30 minutes recovery period in an incubator to allow re-oxygenation of the media. This process cycle was repeated 6 times to achieve 3 hours of light dose. As can be seen in Figure 3.11, the nucleus of cells that were treated with both nanoparticle 95 and light show a dense, spherical morphology, a morphology that is indicative of cell death\(^{199}\). In marked contrast, the nucleus of both controls (with and without light irradiation in the absence of nanoparticle 95) and the sample (cells + nanoparticle 95) without light irradiation show a larger, amorphous morphology. Taken together this data shows that HEK293T cells can only be killed when both nanoparticle 95 and excitation light are applied. As importantly, no significant cell death was observed by applying only nanoparticle 95 to HEK239T, clearly indicating that nanoparticle 95 has good phototoxicity as well as no appreciable dark cytotoxicity.

**Figure 3.11** Fluorescence microscopy image of DAPI stained nucleus. (A) HEK293T cells without nanoparticle 95, kept in dark. (B) HEK293T cells incubated with 10μg/mL nanoparticle 95 for 24 hrs, kept
in dark. (C) HEK293T cell without nanoparticle 95, irradiated for 3 hrs. (D) HEK293T cells incubated with 10μg/mL nanoparticle 95 for 24 hours, irradiated for 3hrs.

### 3.3. Conclusion

In this chapter, we have disclosed the synthesis of a water soluble PEGylated porphyrin grafted silicon nanoparticles system 95 by conjugating a PEGylated zinc-porphyrin 94 to azido silicon nanoparticle 87 (prepared by HEBM) via the highly efficient azide-alkyne “click” reaction. The structure of nanoparticle 95 was confirmed by NMR and FT-IR spectroscopy as well as AFM and TEM characterization. Due to its amphiphilic structure, the resultant nanoparticle 95 shows excellent water solubility. Cryo-TEM and conventional TEM experiments proved that nanoparticle 95 can self-assemble into micelle-like structures with a diameter ~10-150 nm in water. Further, spectroscopic studies such as UV-VIS and fluorescence demonstrated that the aggregation of the porphyrins was actually hindered in the nanoparticle. The nanoparticle also exhibited the capability to covert oxygen to singlet oxygen when the porphyrin units are excited with light. Using the HEK293T cell line as an experimental model, preliminary in vitro studies was conducted. Cellular uptake results showed that nanoparticle 95 is significantly internalized into HEK293T cells. Additionally, in exciting results, in vitro photodynamic experiments demonstrated that this nanoparticle has excellent phototoxicity and low or no dark toxicity in HEK293T cell lines. Thus this porphyrin-silicon nanoparticle system has the potential to be used as an efficient photosensitizer for photodynamic therapy applications.
3.4. Future directions

In addition to well-known EPR effect, another practical strategy for enhancing selectivity of nanoparticles to cancer cells is conjugation of cellular targeting ligands to the surface of nanoparticles. For example, folic acid\textsuperscript{200, 201, 202}, natural\textsuperscript{203–205} bioactive peptides and large T antigen proteins\textsuperscript{206} have been conjugated onto gold nanoparticles to enhance localization and cellular uptake of gold nanoparticle to cancer cells. Recently, Nam and coworkers\textsuperscript{207} reported an iodine-labeled, cyclic RGD-PEGylated gold nanoparticle system that is designed for targeting cancer cells and imaging tumor sites. In this system cyclic RGD (arginine-glycine-aspartic acid) small peptides functioned as targeting ligand to $\alpha_v\beta_3$ integrin on cell membranes. \textit{In vivo} computed tomography (CT) imaging and biopsy of tumor tissue showed that this gold nanoparticle system can selectively accumulate in $\alpha_v\beta_3$ integrin expressing tumor cells. In addition, it is also known that $\alpha_v\beta_3$ integrin plays a crucial role in tumor angiogenesis, thus, inhibition of $\alpha_v\beta_3$ integrin by RGD containing peptide sequences could theoretically inhibit tumor angiogenesis.\textsuperscript{208} In this regard, herein we propose a new RGD-PEGylated porphyrin grafted silicon nanoparticle system as a multifunctional photosensitizer in photodynamic therapy. A potential synthetic route to such a new amino-PEGylated porphyrin 101 system is shown in Figure 3.12. Porphyrin 98 can be conjugated to a AzSiNP 87 via azide-alkyne “click” reaction. The resultant amino-PEGylated porphyrin grafted silicon nanoparticle could be subsequently treated with malic anhydride and conjugated with CRGD (cysteine-arginine-glycine-aspartic acid) peptide via the maleimide-thiol reaction.
Figure 3.12 Synthesis of amino-PEGylated porphyrin xx and RGD-PEGylated porphyrin grafted silicon nanoparticle.

3.5. Experimental

3.5.1. General experimental conditions

Unless otherwise noted, all chemicals were purchased from Sigma-Aldrich or Alfa Aesar, and employed without further purification unless specified. O-(2-Azidoethyl) nonadeca-ethylene glycol was purchased from Polypure Inc., Tetrahydrofuran (THF) was distilled over sodium-benzophenone prior to use. Dichloromethane was distilled over CaH₂ before use. Silicon wafers (undoped, mirror finish, orientation [111]) were obtained from Silarac. Gel permeation chromatography (GPC) was used for the purification of nanoparticles. Briefly, bio-beads S-X1 were swelled overnight in dry THF and packed into a 40cm x 1.3cm glass column. Dichloromethane was used as an elution solvent. A
concentrated nanoparticle solution (50mg in 1 ml CH₂Cl₂) was added to the column and eluted into 3 fractions collected in 10ml increments in separate vials. The first fraction was collected when the first colored band began to elute. This fraction contained nanoparticles and was used in the synthetic reactions. The second and third fractions, containing mostly impurities, were discarded.

UV-Vis absorption spectra were recorded in a quartz cuvette (1cm), using a Cary 50 spectrophotometer and were corrected for the solvent absorption. The scan range was 200-800 nm with a 300 nm min⁻¹ scan rate. Excitation-emission spectra were recorded in a quartz cuvette (1cm), using a Varian Cary Eclipse spectrofluorometer with a scan rate of 120 nm min⁻¹. FTIR spectra were recorded at 1 cm⁻¹ resolution with 1000 scans on a Thermo Nicolet NEXUS 670 FTIR instrument. NMR spectra were recorded on a Varian 400 MHz spectrometer using CDCl₃ as solvent. MALDI-TOF spectra were recorded on a Bruker Autoflex 3 Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometer (MALDI-TOF MS). The matrix used was α-cyano-4-hydroxycinnamic acid for the small molecules.

3.5.1.1. TEM experiments

High-resolution transmission electron microscopy (TEM) studies were performed with a JEOL 2011 TEM or a Tecnai G2 TEM using an accelerating voltage of 200 kV. TEM samples of azido silicon nanoparticle 87 were prepared by dropping a sonicated solution of 87 in ethanol onto a carbon-coated 400 mesh copper grid. Cryo-TEM sample of nanoparticle 95 was prepared on 200 mesh copper grids with lacey carbon film (purchased from Electron Microscopy Science). TEM samples of nanoparticle 95 were prepared by dropping an aqueous solution of 95 on carbon-coated 400 mesh copper grid.
3.5.1.2. AFM experiments

AFM studies were carried out on a Veeco Bioscope AFM (Digital Instruments) under tapping mode in air. Bruker OTESPA AFM probes with nominal frequency, tip diameter, and spring constants of 300 KHz, 7 nm, and 42 N/m, respectively were used. Mica (highest grade V1 Mica disc, 10 mm diameter, was purchased from TED PELLA, Inc.) was used as the substrate and the mica plate was freshly cleaved via scotch tape to achieve a flat surface before use. The sample of nanoparticles 87 and 95 was prepared by applying 20 μL solution of nanoparticles in dichloromethane (~1μg/mL) on mica surface. After drying with nitrogen gas flow, the sample was imaged.

3.5.2. *In vitro* experiments

3.5.2.1. Cell culture

Human Embryonic Kidney 293T cells (HEK-293T) were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum and 1000 U/mL Penicillin/Streptomycin. Cells were allowed to grow in a humidified atmosphere at 37°C under 5% CO₂.

3.5.2.2. Quantification of cell internalization

Confluent cells (100% confluency) were incubated with medium containing 10 μg/mL nanoparticle 95 for 1, 3, 6, 12 and 24 hours. Then cells were washed twice with PBS and lysed in buffer containing 25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS. After the cell extracts were centrifuged at 13,000 rpm for 10 min, the supernatant was taken for UV-Vis evaluation.
3.5.2.3. Cell imaging

HEK293T cells were incubated in medium containing nanoparticle 95 (10μM in porphyrin) for 24 hours and then fixed with 4% paraformaldehyde solution for 15 min at room temperature. For whole cell-staining, the fixed cells were incubated with PBS containing 2 μg/mL Cell Mask green (Invitrogen). Images were taken with a Zeiss LSM 510 confocal microscope.

3.5.2.4. Photodynamic therapy experiment.

Thermal Oriel 200W Xenon arch light system was used for this experiment. In order to filter off the short wave length UV and long wave length IR irradiation that can directly cause cell death, a 530 long pass filter and a water IR filter (filter off light from 1000nm to 3000nm) was applied in front of the light source (see Figure 3.13).

![Figure 3.13 Thermal Oriel 200W Xenon arch light system](image)

HEK-293T cells incubated with or without 10μg/mL nanoparticle 95 for 24 hours were subjected to light irradiation for 30 minutes followed by 30 minutes recovery period.
and repeated 6 times to achieve a total of 3 hours light dose. Dark controls were incubated with or without 10μg/mL nanoparticle 95, then were maintained in the dark for 3 hours. To visualize cell death, cells were stained with DAPI (a nucleus stain) for 5 min. Images of the nucleus were collected by an Olympus fluorescence microscope.

3.5.3. Synthesis of nanoparticle 95

**Synthesis of o-azido-pentenyl (50%)-Si nanoparticles 87.** Silicon wafer (1.0 g, 36 mmol) was ball milled with a mixture of 10 mL (94 mmol) 5-chloro-1-pentyne and 10 mL (101 mmol) 1-pentyne for 24 hours to synthesize the chloro-terminated silicon nanoparticles. Sodium azide (0.3 g, 4.6 mmol) was added to a solution of 50 mg chloro-terminated silicon nanoparticles in 20 mL of dry dimethylformamide (DMF). The solution was stirred at 65°C overnight under nitrogen atmosphere. DMF was removed by vacuum distillation and dry THF was added. The mixture was centrifuged to remove NaCl and unreacted NaN₃. The supernatant that contained azide-terminated silicon nanoparticles was collected and solvent was removed by rotary evaporation. The resultant nanoparticles were re-dispersed in CH₂Cl₂ and purified by GPC. **Safety note:**

*Large scale reactions of azides are prone to dangerous exothermic reactions. The use of chlorinated solvents in the presence of sodium azide must be avoided, as the byproducts may be highly explosive.*

**4-[(Trimethylsilyl)ethynyl]benzaldehyde 88:** This synthesis followed a standard procedure from Austin *et al* 209. A de-aerated solution of 4-bromobenzaldehyde (3 g, 16.5 mmol), Pd(PPh₃)Cl₂ (140 mg, 0.2 mmol), and CuI (130 mg, 0.7 mmol) in a mixture of 5 mL of anhydrous triethylamine and 25 mL anhydrous THF was treated with a
solution of ethynyl trimethylsilane (2.5 mL, 18 mmol) in 5mL anhydrous THF. The mixture was heated at reflux for 6 h. After cooling, the precipitated triethylamine hydrobromide was filtered off by passing through a pad of celite, and the solvent was evaporated to give a thick yellow oil. The crude product was purified on a silica gel column using Ethylacetate/Hexane (1:20) as eluent to give 2.1 g of 4-[(trimethylsilyl)ethynyl]benzaldehyde. Yield: 96%. Mp: 68-70 °C. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 9.97\) (s, 1H), 7.79 (d, 2H, \(J=8\)Hz), 7.58 (d, 2H, \(J=8\)Hz), 0.24 (s, 9H). EI-MS: Calculated for C\(_{12}\)H\(_{14}\)OSi (202.08, M), Found \(m/z\) = 201.1(M-H, 15% intensity), 187.1(M-O, 100% intensity).

5-[4-(Trimethylsilylethynyl)phenyl]dipyromethane 89. The synthesis followed a standard procedure from Muthukumaran et al.\(^{196,197}\). Briefly, a solution of 4-[(trimethylsilyl)ethynyl]benzaldehyde (2.2 g, 11 mmol) in pyrrole (125 mL) was degassed by bubbling with argon for 10 min. Then InCl\(_3\) (0.4 g, 1.4 mmol) was added. The mixture was stirred at room temperature under argon. After 1.5 h, NaOH (2.2 g, 55 mmol, 20–40 mesh beads) was added and the stirring was continued for an additional 45 min. The resultant mixture was filtered and the filtrate was concentrated under high vacuum. The resulting oil was triturated with hexanes and the volatile components were evaporated. This procedure was repeated four times, affording a white solid. Crystallization from ethanol and hexane afforded an off-white solid (7.66 g, 69%): mp 119–121 °C. \(^1\)H NMR spectral data of the product is consistent with reported literature values. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta \) 7.91 (br, 2H), 7.39 (d, \(J = 8.0\) Hz, 2H), 7.13 (d, \(J = 8.0\) Hz, 2H), 6.69 (m, 2H), 6.14 (dd, \(J = 5.9, 2.8\) Hz, 2H), 5.86 (br, 2H), 5.45 (s, 1H), 0.22 (s, 9H). EI-MS: Calculated for C\(_{20}\)H\(_{22}\)N\(_2\)Si (318.16, M), Found \(m/z\) = 318.1(M, 100% intensity).
intensity), 303.1(M-CH₃, 10% intensity), 252.1[M-C₄H₄N(pyrrole)], 145.0[M-C₁₁H₁₃Si(4-TMSPhenyl)].

**5-(Methyl-4-benzoate)-10,15,20-tris(4-((trimethylsilyl)ethynyl)phenyl)porphyrin 90.** A solution of 5-[4-((Trimethylsilyl)ethynyl)phenyl]dipyrromethane (956 mg, 3 mmol), methyl-4-formyl benzoate (246 mg, 1.5 mmol), and 4-[(trimethylsilyl)ethynyl]benzaldehyde (300 mg, 1.5 mmol) was degassed by bubbling with argon for 20 min, followed by addition of 400 uL of 2.5M BF₃(OEt₂). This solution was stirred at room temperature for 24 hrs. Subsequently, 900 mg of DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone, 4 mmol) was added and the reaction mixture was stirred for an additional 24 hrs. Then the final reaction mixture was treated with 1mL pyridine. The precipitated DDQ was filtered off and the filtrate was concentrated under vacuum and purified on a silica gel column using CH₂Cl₂/hexane (1:4) as the eluent. The product was further recrystallized from MeOH to afford a purple crystalline material (275 mg, 19%). ¹H NMR (400 MHz, CDCl₃) δ 8.84 – 8.76 (m, 8H), 8.43 (d, J = 8.1 Hz, 2H), 8.27 (d, J = 8.2 Hz, 2H), 8.13 (d, J = 8.1 Hz, 6H), 7.86 (d, J = 8.1 Hz, 6H), 4.10 (s, 3H), 0.36 (s, 27H), -2.85 (br, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 186.67, 146.71, 142.10, 134.29, 130.31, 127.89, 122.67, 119.63, 117.03, 111.15, 109.70, 104.81, 95.63, 52.4, 0.00. MALDI-TOF MS: Calculated for C₆₁H₅₆N₄O₂Si₃ (960.37, M), found m/z = 960.53 (M+H)⁺.

**5-(4-Carboxylicacid phenyl)-10,15,20-tris(4-ethynyl phenyl)porphyrin 91.** To a solution of 160 mg (0.17 mmol) of 5-(methyl-4-benzoate)-10,15,20-tris(4-((trimethylsilyl)ethynyl)phenyl) porphyrin 90 in 10 mL THF was added 2 mL of 2 mol/L KOH aqueous solution and refluxed overnight. The reaction mixture was subsequently
treated with HOAc to achieve a pH of ~ 5, and then was diluted with 100 mL of methylene chloride. This solution was subsequently washed three times with 20 mL water, 20 mL brine, and the organic layer was dried over Na$_2$SO$_4$ and filtered. Upon removal of the organic solvent a purple solid was isolated (115 mg, 93 %). $^1$H NMR (400 MHz, DMSO) $\delta$ 13.23 (br, 1H), 8.78 (br, 8H), 8.31 (d, $J = 8$ Hz, 4H), 8.24 (d, $J = 8$ Hz, 4H), 8.17 – 8.07 (m, 6H), 7.87 – 7.77 (m, 6H), 4.45 (s, 3H), -3.02 (br, 2H). MALDI-TOF MS: Calculated for C$_{51}$H$_{30}$N$_4$O$_2$ (730.24, M), found m/z =730.80 (M+H)$^+$.  

5-(4-Carboxylic acid phenyl)-10,15,20-tris(4-ethylphenyl)zincporphyrin 92: To a solution of 5-(4-carboxylic acid phenyl)-10,15,20-tris(4-ethylphenyl)porphyrin 91 (100 mg, 0.14 mmol) in 30 mL chloroform was added a solution of zinc acetate (0.25 g, 1.4 mmol) in 3 mL of methanol. The mixture was stirred at room temperature for 3 hrs and then was washed with water and dried over anhydrous Na$_2$SO$_4$. The solvent was then removed under vacuum to give the title product as a purple solid. $^1$H NMR (400 MHz, DMSO) $\delta$ 13.18 (br, 1H), 8.76 (br, 8H), 8.33 (d, $J = 8$ Hz, 4H), 8.26 (d, $J = 8$ Hz, 4H), 8.15 (d, $J = 8.0$ Hz, 6H), 7.86 (d, $J = 8.0$ Hz, 6H), 4.42 (s, 3H). $^{13}$C NMR (100 MHz, DMSO) $\delta$ 168.18, 149.72, 149.58, 143.87, 135.06, 132.45, 130.67, 128.22, 126.89, 121.66, 120.40, 120.36, 120.15, 84.26, 82.51. MALDI-TOF MS: Calculated for C$_{51}$H$_{28}$N$_4$O$_2$Zn (792.15, M), found m/z =792.17 (M+H)$^+$. 

PEGylated Porphyrin 93: To a solution of 5-(4-carboxylic acid phenyl)-10,15,20-tris(4-ethylphenyl)zinc porphyrin 92 (80 mg, 0.1 mmol) and O-(2-Azidoethyl) nonadeca-ethylene glycol (300 mg, 0.33 mmol) in 30 mL anhydrous DMF was added sodium ascorbate (200 mg, 1 mmol). The solution was degassed by bubbling with argon for 20 mins. A degassed solution of CuSO$_4$ (40 mg, 0.16 mmol) in 1 mL de-
ionized-water was then added to the reaction. The reaction mixture was protected under argon and stirred at 40 °C for 72 hrs. Subsequently, the solvent was removed under reduced pressure and the residue was separated by preparative TLC using 10% ethanol/CHCl₃ (with 2% Et₃N) as eluent to give the product as a purple oil. This oil was subsequently dissolved in a minimum amount chloroform and precipitated with a large excess of diethyl ether by centrifugation. The precipitate was then dried under high vacuum to give a thick purple oil (241 mg, 68%). ¹H NMR (400 MHz, CDCl₃) δ 11.83 (br, 1H), 8.95 (d, J = 6.8 Hz, 6H), 8.84 (d, J = 4.7 Hz, 2H), 8.43 (d, J = 8 Hz, 2H), 8.31 – 8.20 (m, 17H), 4.70 (br, 6H), 3.99 (br, 6H), 3.74 – 3.28 (m, the rest of PEG protons ca. 228H). MALDI-TOF MS: Calculated for C₁₇₁H₂₇₁N₁₃O₆₂Zn (3562.77, M), found m/z=3566.26 (M+H)+, 3589.40 (M +Na)+, 3605.38 (M +K)+.

PEGylated porphyrin 94. To a solution of porphyrin 93 (178 mg, 0.05 mmol), DCC (N,N'-dicyclohexylcarbodiimide, 42 mg, ~0.2 mmol) and NHS (24 mg, ~ 0.2 mmol) in 5 ml DMF was added 65 uL propargylamine (~1 mmol, large excess). The reaction mixture was stirred for 48 hrs at room temperature, then poured into 50 mL deionized water. The mixture was washed with ethyl acetate (20 mL×3). The aqueous layer was collected and water was removed by co-evaporating with toluene under reduced pressure. The resultant residue was separated by preparative TLC using 10% ethanol/CHCl₃ (with 2% Et₃N) as eluent to give the product as a purple oil. The residue was subsequently dissolved in a minimum amount chloroform, precipitated with a large excess of diethyl ether, and collected by centrifugation. The resulting precipitate was then dried under high vacuum to give a thick purple oil(148mg, 83%). ¹H NMR (400 MHz, CDCl₃) δ 8.92 (d, J = 7.5 Hz, 6H), 8.79 (d, J = 4.4 Hz, 2H), 8.27-8.15 (m, 17H), 7.90-7.81 (m, 2H), 4.67 (br,
6H), 4.33(br, 1H), 3.96 (br, 6H), 3.74 – 3.25 (m, the rest of PEG protons ca. 228H). MALDI-TOF MS: Calculated for C_{174}H_{274}N_{14}O_{61}Zn (3599.81, M), found \textit{m/z}=3602.43 (M+H)^+, 3625.4 (M +Na)^+, 3641.4 (M +K)^+.

**PEGylated porphyrin grafted silicon nanoparticle 95.** Azide-terminated passivated silicon nanoparticle 87 (20 mg) and porphyrin 94 (50 mg, 0.014 mmol) were dissolved in 15 mL of deaerated anhydrous THF. Cupric bromide (10 mg, 0.05 mmol) was added into the reaction mixture. The solution was stirred at room temperature overnight under nitrogen atmosphere. THF was removed by vacuum distillation and 20 mL dichloromethane and 1 mL ethylenediamine was added. The solution was subsequently washed three times with saturated NH₄Cl solution. Nanoparticle 95 was isolated by GPC using methylene chloride as eluent to remove unreacted silicon nanoparticles and Zn-porphyrin. Then it was dissolved in a minimum amount of chloroform, precipitated from 10 mL anhydrous diethyl ether, collected by centrifuge, and then dried under high vacuum to give a thick purple oil. ^1H NMR (400 MHz, CDCl₃) δ 8.8-8.1 (multiple broad peaks, 28 H, aromatic protons), 4.88(br, 2H), 4.75 (br, 6H), 4.62(br, 2H), 4.43 (br, 6H), 4.04(br, 6H), 3.91(br, 8H), 3.7-3.4 (m, all PEG methylene protons), 2.24-0.31(multiple broad peaks, 153H, alkyl protons of pentenyl groups on the surface of silicon nanoparticle).
3.5.4. NMR and Mass Spectroscopies

**Figure 3.14** $^1$H NMR (CDCl$_3$ 400MHz) spectrum of aldehyde 88. (Note: the aldehyde proton is the most deshielded proton and appears at 9.97ppm, TMS methyl protons are highly shielded and appears at 0.24ppm)
Figure 3.15 $^1$H NMR (CDCl$_3$ 400MHz) spectrum of dipyrromethane 89.
Figure 3.16 $^1$H NMR (CDCl$_3$, 400MHz) spectrum of porphyrin 90. (Note: $\beta$-protons of porphyrin are most deshielded protons and appear at 8.8 ppm. Pyrrole NH protons are highly shielded, and appears at -2.58ppm)
Figure 3.17 $^{13}$C NMR (CDCl$_3$ 100MHz) spectrum of porphyrin 90.
Figure 3.18 $^1$H NMR (DMSO 400MHz) spectrum of porphyrin 91. (Note: carboxylic protons are the most deshielded proton and appears at 13.23ppm, new alkyne protons appears at 4.45ppm)
Figure 3.19 $^1$H NMR (DMSO 400MHz) spectrum of porphyrin 92.
Figure 3.20 $^{13}$C NMR (DMSO 100MHz) spectrum of porphyrin 92
Figure 3.21 $^1$H NMR (CDCl$_3$, 400MHz) spectrum of porphyrin 93. (Note: due to the electron withdrawing effect of triazole groups, 6 $\beta$-protons are more deshielded and appears at 8.94ppm, and the other 2 $\beta$-protons appears at 8.84ppm. methylene protons of PEG appears at 3.25-3.75, but protons of methylene group connected with triazole group appears at 4.7ppm and 4.0ppm respectively.)
Figure 3.22 $^1$H NMR (CDCl$_3$, 400MHz) spectrum of PEGlyated porphyrin 94.
Figure 3.23 $^1$H NMR (CDCl$_3$ 400MHz) spectrum of PEGlyated porphyrin grafted silicon nanoparticle 95. (Note: due to the strong interaction between alkyl chains and porphyrins, all peaks are broadened. All aromatic protons from 8.25ppm-8.8ppm were integrated as 28, and 0.5ppm-2.5ppm that assigned as high field alkyl protons were integrated as 153)
Figure 3.24 EI mass spectrum of aldehyde 88
Figure 3.25 EI mass spectrum of dipyrromethane 89
Figure 3.26 MALDI-TOF-MS of porphyrin 90 (using α-cyano-4-hydroxycinnamic acid as matrix)
Figure 3.27 MALDI-TOF-MS of porphyrin 91 (using α-cyano-4-hydroxycinnamic acid as matrix)
Figure 3.28 MALDI-TOF-MS of porphyrin 92 (using α-cyano-4-hydroxycinnamic acid as matrix)
Figure 3.29 MALDI-TOF-MS of porphyrin 93 (using α-cyano-4-hydroxycinnamic acid as matrix)
Figure 3.30 MALDI-TOF-MS of porphyrin 94 (using α-cyano-4-hydroxycinnamic acid as matrix)
Chapter 4: Reference


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Biography

Nan Zhang was born in China on April 22, 1981. He received his BS in Chemistry in 2003 from Jilin University, China and his MS in 2006 from the same University. He started his graduate studies at Tulane University in September 2006 and started his research in September 2007 under the supervision of Prof. Jayawickramarajah. His graduate research involves the synthesis, properties studies, and applications of water soluble multiporphyrin arrays assembled on G-quadruplexes and silicon nanoparticle scaffolds.