Geometric and Optical Transformations of Supramolecular Host-Guest Amphiphiles

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ABSTRACT

Molecular self-assembly has been an area of recent interest due to its application in a variety of important contexts including drug delivery, regenerative medicine, energy applications, and others. Simultaneously, host-guest chemistry provides a robust and powerful mechanism for inducing switching on the molecular level. In this research, we demonstrate a new platform that combines molecular self-assembly of an amphiphilic chromophore guest molecule with its host molecule counterpart, CB[8] in water. We find that upon addition of CB[8] to a solution of the amphiphilic guest molecule, host-guest complexation occurs and a transition in the morphology of the observed self-assembled nanostructures occurs. Here we present the synthetic route to our amphiphilic guest molecule, in addition to the nanostructural characterization of the supramolecular nanostructures and the host-guest nanostructure by TEM, UV-Vis, and fluorescence spectra.
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1. Introduction

Molecular engineering of organic nanostructured materials has revolutionary potential for improving technology in a number of fields. The design and characterization of precisely synthesized molecules and polymers for their material properties has enabled entire new classes of materials to develop, from plastics to gels. Recently, the field has shifted towards design of soft materials on a nanoscale, inspired by precisely controlled materials and structures made by nature, such as membranes, proteins, and other biomaterials. Control over properties of nanoscale materials is still fairly rudimentary and addresses the challenge from many different angles, such as nanoparticles and artificial peptides in self assembled structures. This thesis explores the optical and geometric properties of push pull amphiphilic chromophores and its interactions in a host-guest complex with macrocycles.
1.1 Supramolecular Chemistry

Self assembly, a type of supramolecular assembly that involves the spontaneous formation of nanostructures from amphiphilic molecules, has several advantages over other techniques in developing nanoscale materials. It relies on thermodynamics and environmental conditions to assemble, and can be easily controlled by synthetic structure of the materials and molecules used to create the structure, a “bottom up” approach [1]. However, a “top down” approach, adjustments post –

*Figure 1 Geometrical Configurations of differently shaped amphiphiles can create different types of nanoscale architectures. By controlling the molecular structure of amphiphiles by designing different shaped head groups and tails, we can precisely tune the resulting structure [3]*
Research on everything from the basic physical properties and simulations of the science of self assembly to the development of its use in a number of applications ranging from energy to biomedical applications has become a large field.

This work in particular looks at small, amphiphilic molecules that self assemble, as opposed to self assembling polymers [2]. Amphiphilic molecules consist of two parts: a hydrophilic head group and a hydrophobic tail. The hydrophilic head group interacts with the solvent, typically water, and the hydrophobic tail tends to aggregate in the center of a structure, forming nanostructures. The type of nanostructure depends on the size of the tail and head groups, in addition to the chemistries itself, and are predicted by the packing parameters based on these size ratios. Figure 1 demonstrates how, geometrically, the different sizes of amphiphiles can create different structures [3]. We look to change two parameters that allow us to control the types of nanostructures observed. First, changing the tail length of the amphiphile by using different sizes of halogenated alkanes and differences in the head group based on the choice of peptide will change the packing parameter of the molecule, and therefore its final structure [4]. We can also quickly change the packing parameter by the addition of a macrocycle, a “host molecule” that can encapsulate two of the
amphiphilic molecules called cucurbituril[8] (CB[8]), which will increase the size of the headgroup and change the curvature angle of the structures.

1.2 Host Guest Chemistry

Host guest chemistry describes the phenomenon of complexes that form by two or more molecules by forces other than covalent bonds. CB[8], the “host” molecule used in this study, is a cyclodextrin and has the shape of a large ring. It interacts with other molecules because the interior contains high energy water molecules, and thus aromatic molecules commonly function as “guests” and displace the high energy water molecules, forming a stable complex [5]. This has been used in a variety of applications ranging from drug delivery, molecular “machinery”, and supramolecular hydrogels [6] [7] [8].

In the amphiphile designed in this thesis, the headgroup consists of a charged biaryl (Fig 2). Biaryls of various types have been shown in previous work to engage in host guest chemical interactions with Cucurbituril[8] (CB[8]) [9] [2, 10]. X-ray crystallography (Fig 3) of methoxy functionalized biaryls have shown a offset pi-pi stacking configuration within the macrocycle (Fig 3) [9]. Self assembly and host guest interactions are highly prevalent in nature, enabling
everything from the formation of membranes to the catalysis of reactions within enzymes.

Nature often uses a combination of chemical and thermodynamic effects to “assemble” key components of the cellular system [11]. Borrowing nature’s strategies for synthesizing our own ordered structures allows us to design materials with increasing complexity and precision.

1.3 Push Pull Molecules

Molecules fluorescence when excited with radiation because the absorbed photons produce an excited state and in the transition back to the normal state, due to the arrangement of bonds in the molecule a photon may be emitted in turn. This occurs mostly in conjugated systems, such as interconnected aromatic rings or heterocyclic compounds. This compound is a particularly promising platform for fluorescent nanostructures because it also has a unique push pull property [12]. In push pull molecules, the aromatic ring is endcapped by an electron acceptor and electron donor, and their interaction causes formation of a low energy molecular orbital that can further enhance photochemical properties. These molecules, part of a broader class called chromophores, are tunable by changing the donor group, acceptor group, or conjugation pattern, allowing for finer control of the excitation and emission wavelength [12] [13]. The head group in our compound requires only synthetic step to produce, and can easily be changed through a number of different reagents known to react via Suzuki Miyauri coupling (Fig 4) [14]. This makes our platform
particularly ideal as a model for fluorescent probes because its properties are modifiable without major changes in the synthetic pathway.

1.4 Fluorescent Nanostructures

Examples of fluorescent nanoparticles today are polymer based vesicles with fluorescent molecules embedded, inorganic nanoparticles, and fluorescence functionalized polymeric self assemblies [15]. However, these approaches have some drawbacks. Synthesizing polymers often include difficulties to precisely control the molecular weight, and therefore their properties [16]. Fluorescent molecules or nanoparticles incased in nanostructures can leak out, making it difficult to determine the fluorescence signatures within the cell between individual particles and the larger vesicles. [17] [18] Some fluorescent small molecule-based assemblies have been researched for use, but are not in widespread use and vary wildly in use cases and structure [19] [20] [21]. Creating fluorescent molecules and nanostructures is an important field that has been critical for advances in many fields, such as advances in biochemistry, as it allows researchers to be able to image and thus determine the location of various important compounds within cells [17].

1.5 Synthetic pathway

For this project, two amphiphilic head groups were tested. The first head group H1, 4-(4-pyridinyl) benzoic acid (H1, Fig 6) was commercially available. The second head group, H2 was synthesized via a Suzuki-Miyauri coupling reaction (Fig 5).
After synthesis of H1 and H2, 1-bromo alkanes were attached to the head groups using a Suzuki-Miyaura coupling reaction with a palladium catalyst under an inert atmosphere. A pressure vessel is used to prevent loss of solvent and water is added to allow for sodium bicarbonate to solubilize into the reaction mixture.

Figure 6 Synthetic pathway of H2. The synthesis of this biaryl uses a Suzuki-Miyaura coupling reaction with a palladium catalyst under an inert atmosphere. A pressure vessel is used to prevent loss of solvent and water is added to allow for sodium bicarbonate to solubilize into the reaction mixture.

Figure 5 Synthetic pathway of carboxylic acid functionalized chromophore amphiphiles. a) Commercially purchased 4-(4-pyridinyl)benzoic acid undergoes nucleophilic substitution with bromo-alkanes of varying length to form amphiphiles, and are purified via precipitation. These compounds are primarily soluble in methanol. b) 4-(4-methoxyphenyl)pyridine, synthesized via Suzuki-Miyaura coupling, undergoes nucleophilic substitution with bromo-alkanes and purified via evaporation and precipitation. These compounds are primarily soluble in acetone.
After synthesis of H1 and H2, 1-bromo alkanes were attached to the head groups via a nucleophilic substitution reaction, serving as the hydrophobic tail group and generating a positive formal charge on the nitrogen, which increased the hydrophilicity of the head group.

2. Experimental Methods

2.1 Synthesis and Purification of Head Group

H1 was obtained commercially from Sigma Aldrich. H2 was synthesized from 4-pyridinyl boronic acid and 4-bromoanisole dissolved in a THF and milliQ water. Sodium bicarbonate was added and dissolved to form a basic environment, and tetrakis(triphenylphosphine)palladium(0) catalyst was added under inert atmosphere conditions. Nitrogen gas was bubbled into the reaction mixture for 2 minutes, and the pressure vessel was sealed and heated at 80°C for 2 hours. H2 was purified via column chromatography with an ethyl acetate/hexane eluting solution and characterized via NMR (Fig 7).

Figure 7 Flash Column Chromatography setup of synthesis of H2. A 3:1 mixture of Ethyl Acetate:Hexane was used as the mobile phase, and silica gel was used as the stationary phase. Eluent fractions were collected in test tubes and tested for presence of H2 via thin layer chromatography.
2.2 Synthesis and Purification of Derived Amphiphiles

A1 and B1 were synthesized in DMF. For A1, bromo-alkanes of lengths 4, 6, 8, 10, and 12 were dissolved in DMF with H1 and heated at 80°C with stirring overnight. For B1, bromo-alkanes of lengths 8, 10, 12, and 18 also followed the same protocol. DMF was evaporated and the resulting crude products were dissolved in methanol and filtered. The crude product was further purified via solvent extraction with ethyl acetate and deionized water, and then precipitated slowly in ethyl acetate or hexane overnight, while refrigerated.

Chemical structures were confirmed via NMR and FTIR. Samples were characterized using UV-Vis Spectroscopy, Fluorometry, and Transmission Electron Microscopy.

3. Results and Discussion

3.1 Screening of Biaryl Head Groups and Varying Alkane Tails

The construction of the supramolecular self assembling system in water was the first primary challenge in designing the platform. Following synthesis of B1 and confirmation of structure via NMR and an 18 carbon tail was attached via nucleophilic substitution. The resulting compound, a cream colored powder and characterized structurally by NMR (Fig 12), had low solubility in water which made it difficult to observe self assembly. Numerous forms of aggregation were observed, including a white, wispy solution and small, rod-like crystals observable under polarized microscopy [22]. This was likely due to the pi-pi stacking effect of the chromophores and heavy alkane tails limiting the accessibility of the amphiphile to solvent, causing crystallization. The pi-pi stacking effect likely had a larger effect, as this crystallization was not seen as readily when complexed with CB[8]. It was also observed at this time that when
complexed with CB[8], the solubility increased and crystallization was not present, as the CB[8] would limit pi-pi interactions. Due to this effect, it was postulated that the head group was too hydrophobic, and a new head group was proposed.

A1, a commercially available reagent, was chosen for the increased hydrophilicity of the carboxylic acid compared to the original methoxy functional group. Alkane tails of lengths 12, 10, 8, 6, and 4 were attached, however, due to the resulting differences in both the solubility of the compound, the A1 head group with carbon tail lengths of 4 and 6 were not purified. FTIR confirmed the presence of the carboxylic acid (Fig 8). The NMR spectra of the remaining three compounds (Fig 9, 10, 11, 12) suggested residual impurities but an otherwise successful synthesis. The compounds had a light to moderate orange color, with the heavier formula weight compounds having a deeper color, and the aqueous solubility was higher. However, the fluorescence observed from a 365 nm lamp was considerably lower than the methoxy compounds, so the methoxy chromophore amphiphiles were resynthesized, but with shorter tails to improve solubility (Fig 13, 14, 15, 16).

Figure 8 FTIR spectra of A1 with C = 10 to confirm presence of carboxylic acid functional group
Figure 9 NMR spectra of A1 with carbon tail lengths of 12, 10, and 6 (from top to bottom)

Figure 10 NMR Spectra of A1 with carbon tail length of 6
Figure 12 NMR Spectra of A1 with carbon tail length of 10

Figure 11 NMR Spectra of A1 with carbon tail length of 12
Figure 13 NMR Spectra comparison of B1 with carbon tail lengths of 8 and 12

Figure 14 NMR Spectra of B1 with carbon tail length of 8
**Figure 15** NMR Spectra of B1 with carbon tail length of 10

**Figure 16** NMR Spectra of B1 with carbon tail length of 18
3.2 Chemical Properties of Chromophore Amphiphiles

These chromophores amphiphiles are unique due to their functionalization and push pull properties. A1 has a carboxylic acid attached to the para position of the biaryl, which would serve as a chemical handle for future functionalization, such as attaching peptides or other short functionally important groups. Furthermore, due to its aromaticity, it is still fluorescent, and the mechanism of adding tail groups is similar to the original synthesized head group. The critical micelle concentration (Fig 20, 22, 24) was measured via looking at the fluorescence spectra of various concentrations of the compound in water, and was observed to be between 0.1 mM and 0.01 mM. Its higher solubility in water made it an ideal candidate for geometric characterization.
3.2 Optical Characterization

The methoxy head group as a compound had a much higher fluorescence due to its push pull structure. Under a 365 nm fluorescent lamp, the methoxy compounds have a bright blue color. Upon addition of CB[8], the fluorescence shifts to a green color, as seen in Figure 18 and 19. The bathochromic shift and increase in fluorescence upon addition of CB[8] to the amphiphile may have occurred due several factors. CB[8] itself, prior to encapsulation, has several high energy water molecules within the cavity that guests such as this amphiphile to form

**Figure 18 Vials of B1 with C = 18 and the addition of CB[8]** Vials 1-3 and Vial 5 are B1 only in increasing concentrations, and vials 4, 5-8 are encapsulated with CB[8] and proceed in order of increasing concentration. Note the increase in brightness and shifts in color. The green color of vial 1 and 2 are due to an artifact of the 365 nm UV light and vial used to illuminate fluorescence, which is noticeable due to the more brightly lit bottom of the vial as opposed to a homogenous fluorescence.

**Figure 19 Emission spectrum of B1 with carbon = 18.** A bathochromic shift is observed with the addition of CB[8] Both of these spectra were taken at the same concentration (1mg/mL)
Figure 21 UV Vis Spectra for B1 with and without CB[8], showing a shift in the optical profile due to stabilization of the amphiphiles while encapsulated by CB[8].

Figure 20 Fluorescence spectrum of A1 with C = 10. The shift in peaks, known as a stokes shift, indicates the formation of a micelle. The excitation wavelength used was 355 nm.
a stable complex. The CB[8] increases the overall solubility of the complex because it encases the aromatic groups of the amphiphile.

Figure 22 Fluorescence spectra of B1 with C = 18. A very small shift occurs in the peaks, which suggests the formation of nanostructures. Excitation wavelength used was 330 nm.
Figure 24: Fluorescence Spectra of B1/CB[8] with C = 18. The change in profile suggests either a change in the orientation of encapsulated amphiphiles or formation of nanostructures.

Figure 23: Comparison of Figure 22 and Figure 23. Note that even without the formation of any nanostructures, encapsulated amphiphiles still have a different peak location than the encapsulated one, an offset of around 10 nm.
3.4 Geometric Characterization

The carboxylic acid amphiphile showed a very clear critical micelle concentration range and geometric data. Figure 25 was hypothesized to occur at a nanoscale level, where the difference in geometry of the molecule due to the addition of macrocycle should induce a change in curvature, and therefore a difference in the size of the micelle or other nanostructure. TEM images of A1 with the 10 carbon tail shows that the compound forms small micelles, with a radius of around 7 nm as measured from the TEM image using ImageJ (Figure 26, 27) [23]. As the tail of the amphiphiles get larger, the micelle size is expected to increase because head groups are planar, due to delocalization of the electrons. This was consistent with other amphiphiles tested with DLS (Fig 28, 29). So, as the tail group increases, we expect to see a corresponding decrease in the curvature of the micelle, potentially forming bilayers. The structure of A1 with a 10 carbon tail in complex with CB[8] is expected to be smaller in size, because the CB[8] has a much wider radius than the planar head group of the amphiphile.

*Figure 25 Graphical representation of formation of nanostructures. The first image is amphiphile only, and the second is the addition of macrocycles, causing a change in the geometry and therefore a change in the structure*
The physical properties of the chemical compounds vary considerably by tail length. For instance, the solubility of each compound varied considerably with each two carbon increment in tail length, causing smaller compounds such as H1 paired with a four carbon tail to form an oil as opposed to a solid. Biological lipids tend to have larger tail lengths, on the order of sixteen to
twenty carbons. As the tail lengths shorten, solubility tends to increase, making the surfactant properties and the micellization of these lipids easier to study [24].

![Figure 28](image1.png)

*Figure 28* Radius distribution of B1 with C = 18 encapsulated by CB[8] measured with DLS, found to be around 1.850 nm

![Figure 29](image2.png)

*Figure 29* Radius distribution of B1 with C = 18 measured with DLS, found to be around 2.339 nm

The different geometries present of the amphiphile and the amphiphile with a host molecule demonstrate a novel method of quickly changing the geometry of precise, nanostructured self-assembly. Previous work had looked at the use of cucurbituril[8] in its ability to cross link polymers, change optical properties of materials, and create SOFs [17, 25, 26]. However, this is the first system that specifically uses the host-guest chemistry of macrocycles with self-assembling small amphiphiles. The ability of this system to quickly switch from one structure to
another without changing the chemical structure greatly expands the toolbox of supramolecular chemists in designing precisely sized, ordered nanostructures.

### 3.5 Impact and Future Work

Fluorescent amphiphiles, although a small field, are also critical to the field of chemical biology. Many major advances in chemical biology have occurred due to the development of new fluorescent probes or methods of attaching and tracking probes in the cell [17]. Fluorescent amphiphiles can be used in a variety of ways. Since they form micelles, they can easily enter and integrate with other vesicles within the cell [21, 27]. Bioactive molecules of interest, such as peptides and drugs, can be integrated into the micelle or attached to the end of the carboxylic...
acid group of A1, where chemical biologists can easily study its localization and targeting in the cell. For instance, labeling the end of the head group functionalized with peptides will help increase the hydrophilicity of the head group and also allow for micelles to be labeled for specific targeting in the cell (Fig 20)

The current molecular design can also be tailored in a number of different ways in order to target specific applications. Since B1 is a push-pull chromophore, its fluorescent properties can easily be tailored by changing the methoxy functional group to different electron donors, which can tune the fluorescent properties of the chromophore [12]. Tail lengths can be tuned to fit the application needed, and the head group can be extended by further coupling of aryls or design of coupled supramolecular polymers found in other studies [6]. Cucurbiturils can add an additional level of control, as they have been shown to be permeable to cell membranes and to be non toxic [7]. They can additionally contain other molecules in addition to the chromophores shown in this study, including various drug molecules, which can provide another level of drug loading and a layered release mechanism.

3. Conclusion

This thesis demonstrates the design, synthesis and characterization of a fluorescent host – guest supramolecular chemical platform. Eight different compounds of functionalized biaryl amphiphiles were synthesized and found to have different chemical properties based on functional group and tail length. UV-Vis and Fluorescence spectroscopy was used to characterize shifts in emission spectrum to determine encapsulation of amphiphiles via CB[8] and critical micelle concentration. TEM was used to determine size distribution of samples. This work can serve as the basis for a number of different applications and can work as a new tool for supramolecular chemists looking to achieve increasingly precise control over their structures.
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