Self-assembled Two-component Organic Tubes: Structures And Applications

2013

Wenlang Liang
University of Central Florida

Find similar works at: https://stars.library.ucf.edu/etd

University of Central Florida Libraries http://library.ucf.edu

Part of the Materials Science and Engineering Commons

STARS Citation

https://stars.library.ucf.edu/etd/2952

This Doctoral Dissertation (Open Access) is brought to you for free and open access by STARS. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of STARS. For more information, please contact lee.dotson@ucf.edu.
SELF-ASSEMBLED TWO-COMPONENT ORGANIC TUBES:
STRUCTURES AND APPLICATIONS

by

WENLANG LIANG
B.S. Huazhong University of Science and Technology, 2008
M.S. University of Central Florida, 2011

A dissertation submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy
in the Department of Materials Science and Engineering
in the College of Engineering and Computer Science
at the University of Central Florida
Orlando, Florida

Summer Term
2013

Major Professor: Jiyu Fang
ABSTRACT

Bile acids are physiologically important metabolites, which are synthesized in liver as the end products of cholesterol metabolism and then secreted into the intestines. They play a critical role in the digestion and absorption of fats and fat-soluble vitamins through emulsifications. The amphipathic and chiral nature of bile acids makes their unique building blocks for assembling supramolecular structures including vesicles, fibers, ribbons and hollow tubes.

Lithocholic acid (LCA) is a secondary bile acid. Our studies show LCA can self-assemble into helical tubes in aqueous solution by the linear aggregation and fusion of vesicles. The objective of this dissertation is to tune the structure of helical tubes and functionalize them by the co-assembly of ionic LCA and cationic cetyltrimethylammonium bromide (CTAB) and ionic LCA and cationic cyanine dye (CD), respectively. The first part of this dissertation focuses on the ionic-assembly of LCA and CTAB to synthesize the helical tubes with varied diameters and pitches. Our studies show that LCA and CTAB can self-assemble into helical tubes in NH₄OH aqueous solution. The diameter of the helical tubes can be changed by adjusting the molar ratio of LCA and CTAB. The pitch of the helical tubes can be tuned by varying NH₄OH concentrations. Differential scanning calorimetry studies indicate that there is a homogeneous composition distribution in the LCA/CTAB helical tubes. X-ray diffraction analysis studies show that the helical tubes have multibilayer walls with an average d-spacing of 4.11nm. We demonstrate that the helical tubes with varied diameters and pitches can be transformed into helical silica through the sol-gel transcription of tetraethoxysilane (TEOS). The second part of this dissertation is to use the ionic self-assembly of LCA and CD to design light-harvesting tubes.
by mimicking green sulfur bacteria that are known to be a highly efficient photosynthesizer. X-ray diffraction and optical spectra show that LCA and CD can co-assemble into J- or H-aggregate tubes, depending the condition under which the self-assembly occurs. We demonstrate the feasibility of using the J-aggregate nanotubes in the sensitive and selective detection of mercury (II) ions by the photoinduced electron transfer under sunlight. The presence of mercury (II) ions in aqueous solution could be detected for concentrations as low as 10 pM.
ACKNOWLEDGMENTS

First and foremost, I would like to take this opportunity to express my gratitude to Dr. Jiyu Fang for his help, support and valuable discussions throughout my research. It has been an honor to be his student. His constant guidance and persistent spirit helped me substantially in the research work. I would also like to thank Dr. Jihua Gou, Dr. Lei Zhai, Dr. Quangfang Chen, and Dr. Weiwei Deng for serving on my thesis committee and for their encouragement and insightful comments.

I thank my colleagues and friends, Sihui, Rebecca, Tanmay Bera, Xueping, Xuejun and Jianhua for their help and support.

I would like to give specially thank to my parents and my wife, without their love and support I would not be able to achieve what I have. Also, thank you for my brother, sister-in-law and my niece with their care and encouragement.
# TABLE OF CONTENTS

**LIST OF FIGURES** ......................................................................................................................... ix

**LIST OF TABLES** ........................................................................................................................... xvi

**CHAPTER 1 INTRODUCTION** ....................................................................................................... 1

1.1 Bile Acids......................................................................................................................................... 1

1.2 Self-Assembly .................................................................................................................................. 5

1.2.1 Self-Assembly of Surfactants ...................................................................................................... 7

1.2.2 Self-Assembly of Bile Acids ....................................................................................................... 10

1.3 Conclusion .................................................................................................................................... 13

**CHAPTER 2 SELF-ASSEMBLY OF LITHOCHOLIC ACID** ........................................................... 14

2.1 Introduction .................................................................................................................................... 14

2.2 Experimental Methods .................................................................................................................. 16

2.3 Results and Discussion .................................................................................................................. 17

2.3.1 Self-Assembly of LCA in Sodium Hydroxide Solutions .......................................................... 17

2.3.2 Self-Assembly of LCA in Ammonium Hydroxide Solutions .................................................... 21

2.4 Conclusion .................................................................................................................................... 30

**CHAPTER 3 CO-ASSEMBLY OF LITHOCHOLIC ACID AND A CATIONIC SURFACANT** .......... 31
3.1 Introduction ........................................................................................................................................31
3.2 Experimental Methods ....................................................................................................................31
3.3 Results and Discussion .....................................................................................................................33
  3.3.1 Self-Assembly of CTAB in Ammonium Hydroxide Solutions ..............................................33
  3.3.2 Study of Co-Assembled LCA with CTAB Mixtures in NH₄OH Solutions ..............................34
    3.3.2.1 Vesicle Fusion Study in the Initial Stage of the Co-assembly ........................................34
    3.3.2.2 Formation Process of the Co-Assembled Tubes .............................................................37
    3.3.2.3 Growing Process of the Co-Assembled Tubes ...............................................................41
    3.3.2.4 Effects of Molar Concentration Ratio on the Co-Assembled Tubes ..............................44
    3.3.2.5 Characterization of the Co-Assembled Tubes ...............................................................45
    3.3.2.6 Effects of Solvate Concentration on the Co-Assembled Tubes ......................................48
    3.3.2.7 Transformation of Co-Assembled Micro-structures into Silica ......................................50
  3.4 Conclusion ......................................................................................................................................51

CHAPTER 4 CO-ASSEMBLY OF LITHOCHOLIC ACID AND A CYANINE DYE ..................52
4.1 Introduction ..................................................................................................................................52
4.2 Experimental Methods ..................................................................................................................54
4.3 Results and Discussion ..................................................................................................................57
  4.3.1 Study of Co-Assembled LCA with CD Mixtures .................................................................57
  4.3.2 Photoinitiated Reduction of Silver Ions with Co-Assembled LCA/CD Mixtures ..............64
4.3.3 Hg (II) Detection by Photoinitiated Charge Transfer from Co-Assembled LCA/CD Mixtures .......................................................................................................................... 68

4.3.4 Dopamine Detection by Photoinitiated Charge Transfer from Co-Assembled LCA/CD Mixtures .......................................................................................................................... 73

4.3.5 Methylene Blue Decomposition .......................................................................................................................... 79

4.4 Conclusion ......................................................................................................................................................... 82

REFERENCES ......................................................................................................................................................... 84
LIST OF FIGURES

Figure 1 Schematic of enterohepatic circulation of bile acids\(^1\) .........................................................2
Figure 2 (a) 2D and (b) 3D chemical structure of a typical bile acid molecule. .........................3
Figure 3 (a) Dimer, (b) tetramer and (c) polymer formed by bile acids. .................................4
Figure 4 Mixed micelles formed by bile acids\(^8\) with phosphatidylcholine (PC), cholesterol, fatty
acid and monoglycerides. ..................................................................................................................5
Figure 5 Double-layer (a) tube and (b) vesicle structures formed by amphiphilic molecules
through self-assembly .....................................................................................................................7
Figure 6 Schematic image of a conventional surfactant structure .................................................8
Figure 7 (a) Single layer micelles, (b) single layer rod like micelles, (c) bilayer micelles and (d)
vesicles formed by conventional surfactant molecules through self-assembly .........................9
Figure 8 Molecular structures of common bile acids ......................................................................11
Figure 9 (a) Primary micelles and (b) aggregation of micelles formed by bile acid molecules....12
Figure 10 Chemical structure of LCA ..........................................................................................15
Figure 11 Optical microscopy images of LCA self-assembled structures in 0.01M NaOH
solutions after (a) 1 hour, (b) 10 hours and (c) 5 days. .................................................................18
Figure 12 Optical microscopy images of self-assembled LCA structures in NaOH solutions with
concentrations of (a) and (b) 0.1M, (c) and (d) at 0.01M and (e) 0.1M. (f) Polarizing
microscopy image of (e), direction of the polarizer and analyzer is indicated by white arrows.

Figure 13 Optical microscopy images of self-assembled structures of LCA in NH₄OH solutions with concentrations of (a) and (b) 1 wt%, (c) and (d) 5 wt% and (e) and (f) 10 wt%. ..........20

Figure 14 Optical microscopy images of self-assembled structures of LCA in NH₄OH solutions with concentrations of (a) and (b) 15 wt% and (e) and (f) 20 wt%. .........................23

Figure 15 Optical microscopy images of self-assembled single fan-like structures of LCA in NH₄OH solutions with concentration of 28wt% after (a) 2 days, (b) 5 days, (c) 8 days and (d) 15 days.................................................................25

Figure 16 Optical microscopy images of self-assembled double fan-like structures of LCA in NH₄OH solutions with concentration of 28wt% after (a) 1 day, (b) 3 days, (c) 7 days and (d) 10 days.................................................................26

Figure 17 Optical microscopy images of self-assembled spherulites structures of LCA in NH₄OH solutions with concentration of 28wt% after (a) 2 days, (b) 5 days, (c) 10 days and (d) 15 days.................................................................27

Figure 18 X-ray diffraction of self-assembled LCA tubes in NH₄OH solutions with concentrations of 1, 5, 10, 15 and 20wt% dried on silicon substrates. .........................28

Figure 19 Chemical structure of CTAB. .................................................................31
Figure 20 (a) An AFM image and (b) dynamic light scattering measurements of CTAB self-assembled structures in NH$_4$OH solutions. (d) Height profiles (green marks) of (c) two CTAB self-assembled aggregations (blue arrows).

Figure 21 Optical microscopy image of vesicles formed by LCA and CTAB mixed in NH$_4$OH solutions.

Figure 22 Optical microscopy images of vesicle fusion process of LCA/CTAB co-assembled mixtures in NH$_4$OH solutions. (a) Before fusion, (b) fused and (c) after fusion.

Figure 23 Schematic images of a typical vesicle fusion process. (a) Stalk, (b) hemifusion diaphragm, (c) fusion pore and (d) fused.

Figure 24 Optical microscopy images of the formation process of LCA/CTAB co-assembled tubes in NH$_4$OH solutions in a sealed micro channel. Images were taken after (a) 1 day, (b) 2 days, (c) 3 days and (d) 7 days.

Figure 25 Optical microscopy images of the formation process of an LCA/CTAB co-assembled spherulites nucleus in NH$_4$OH solutions in a sealed micro channel. Images were taken after (a) 1 day, (b) 3 days and (c) 7 days.

Figure 26 Optical microscopy images of the formation process of LCA/CTAB co-assembled tubes in NH$_4$OH solutions during vesicle fusion. Time intervals of (a), (b) and (c) was 10 seconds. (d) is the blown up image of the red box in (c).

Figure 27 Optical microscopy images of the growth of LCA/CTAB co-assembled tubes in NH$_4$OH solutions by multiple vesicle fusion. Time interval between these images was 20 seconds.
Figure 28 Optical microscopy images of the growth of red dye loaded LCA/CTAB co-assembled tubes NH₄OH solutions by single vesicle fusion. Time interval between (a) to (d) was 60 seconds; between (d) to (f) was 5 minutes. .................................................................43

Figure 29 (a) Formation time of LCA/CTAB co-assembled tubes. (b) Tubes diameter vs. LCA/CTAB molar concentration ratio ........................................................................................................45

Figure 30 DSC thermogram of LCA/CTAB co-assembled tubes in NH₄OH solutions. ..........46

Figure 31 ζ-potential studies of LCA self-assembled tubes and LCA/CTAB co-assembled tubes with molar concentration ratio of 1:1 in NH₄OH solutions .................................................................47

Figure 32 X-ray diffraction of co-assembled LCA/CTAB tubes with molar concentration ratio of 1:1 in NH₄OH solution dried on silicon substrates .................................................................47

Figure 33 Schematic images of LCA/CTAB co-assembled helical tubes in NH₄OH solutions. ..48

Figure 34 Optical microscopy images of LCA/CTAB with molar concentration ratio of 1:1 co-assembled helical tubes in NH₄OH solutions with concentration of (a) 6wt%, (b) 13wt%, (c) 20wt% and (d) 28wt% .................................................................49

Figure 35 Diagram of pitch lengths of LCA/CTAB with molar concentration ratio of 1:1 co-assembled tubes with diameter of 0.9μm± 0.1μm in NH₄OH solutions with concentration of 6wt%, 13wt%, 20wt% and 28wt% ..................................................................................50

Figure 36 SEM images of silica LCA/CTAB co-assembled microstructures ................................51

Figure 37 Chemical structure of DiSC₃(5) ........................................................................53
Figure 38 Absorption spectra of monomeric DiSC3(5) (black) and co-assembled LCA/DiSC3(5) with molar concentration ratio of 1:1 (red) in (a) NaOH (0.1M) solution; (b) NH4OH (13 wt%) solution. ................................................................. 58

Figure 39 X-ray diffraction analysis of LCA/DiSC3(5) co-assembled structures with molar concentration ratio of 1:1 in 0.1M NaOH solution. ......................................................... 59

Figure 40 Optical microscopy images of (a) J-aggregates tubes and (b) H-aggregates vesicles dried on glass substrates. .................................................................................. 60

Figure 41 (a) AFM images of J-aggregate nanotubes dried on a silicon substrate. (b) Corresponding height distributions. ............................................................................. 60

Figure 42 (a) Amphiphilic bile acid LCA and cyanine dye DiSC3(5). (b) DiSC3(5)+/LCA- ion pair. (c) Schematic illustrating of LCA/DiSC3(5) self-assembled nanotubular structures in NaOH solution. ...................................................................... 61

Figure 43 Absorption spectra of co-assembled LCA/DiSC3(5) with molar concentration ratios of 1:1, 2:1 and 1:2 in (a) 0.1M NaOH solutions; (b) NH4OH solutions with concentration of 13wt%. ....................................................................................................... 63

Figure 44 Absorption spectra of two-step (black) and one-step (red) assembly of LCA/DiSC3(5) with molar concentration ratio of 1:1 in 0.1M NaOH solutions. ........................................ 64

Figure 45 (a) Absorption spectra of LCA/DiSC3(5) in 0.1M NaOH solutions with 100μM AgNO3 with 0~68 minutes of exposure to white light. (b) Normalized intensity of the absorption peak at 710nm versus the expose time. Solid line is a linear fit. ........................................ 65
Figure 46  Absorption spectra of LCA/DiSC$_3$(5) in NH$_4$OH solutions with concentration of 13wt% with (a) 5000μM AgNO$_3$ with 0~45 minutes, (c) 100μM AgNO$_3$ with 0~52 minutes of exposure to visible light. Normalized intensity of the absorption peak at 450nm versus the expose time of (b) 5000μM and (d) 100μM AgNO$_3$. Solid line is a curve fit at (b) and linear fit at (d)..............................................................66

Figure 47 SEM images of (a) J-aggregates and (b) H-aggregates dried on silicon wafers after oxidation by Ag$^+$ ions with concentration of (a) 100μM and (b) 5000μM.................................67

Figure 48 (a) Absorption spectra of LCA/DiSC$_3$(5)/NaOH solution with 100μM HgCl$_2$ after 0~120 min of exposure to white light. (b) Normalized intensity of the absorption peak at 710nm versus the expose time. Solid line is a linear fit.................................................................69

Figure 49 (a) Effect of metal ions (100μM) on the change of absorbance at 711nm of LCA/DiSC$_3$(5)/NaOH solution after exposure to white light for 40 minutes.  (b) Reduction potentials of metal ions.................................................................71

Figure 50 (a) Absorption spectra of LCA/DiSC$_3$(5)/NaOH solution with 50μM AgNO$_3$ and HgCl$_2$ after 0~80 min of exposure to white light. (b) Normalized intensity of the absorption peak at 710nm versus the expose time. Solid line is a curve fit. ............................................................72

Figure 51 (a) Absorption spectra of LCA/DiSC$_3$(5)/NaOH solution with 0.01nM, 0.1nM, 0.5nM, 0.05μM and 0.5μM HgCl$_2$ after 40 min of exposure to white light. (b) Normalized intensity of the absorption peak at 710nm versus concentration. Solid line is a curve fit. .................72

Figure 52 Chemical structures of (a) dopamine, (b) L-ascorbic acid and (c) uric acid. ..............73
Figure 53 (a) Absorption spectra of J-aggregate solutions with 0.25μM to 50μM Dopamine. (b) Normalized intensity change of the absorption peak at 712nm versus dopamine concentration. (c) Normalized intensity change of the absorption peak at 712nm versus dopamine concentration in nanomolar range.

Figure 54 (a) Absorption spectra of J-aggregates solution with L-ascorbic acid (AA), uric acid (UA) and dopamine (DA). (b) Normalized change of absorption intensity at 712nm for DA, AA and UA in J-aggregates solution. (c) Normalized change of absorption intensity at 712nm for DA, AA, UA and their mixtures.

Figure 55 Chemical structures of oxidized and reduced forms of methylene blue.

Figure 56 Absorption spectra of methylene blue solution. Normalized intensity of the absorption peak at 660nm versus the expose time. Solid line is a curve fit at (b) and linear fit at (d).
LIST OF TABLES

Table 1 D-spacings and $2\theta$ angles of self-assembled LCA tubes in NH$_4$OH solutions with concentrations of 1, 5, 10, 15 and 20 wt%. ..........................................................29
CHAPTER 1 INTRODUCTION

1.1 Bile Acids

Bile acids are unique molecules that play several important physiological roles in the body. Researchers have been trying to understand the functions of bile acids in these physiological processes for over a century, and presently the roles they play in the liver and intestine have been partially understood. They are secreted into the bile after their synthesis in the liver, and later on be taken up from the intestine. Hepatocytes will absorb and resecrete bile acids in the portal blood, which establishes the enterohepatic circulation between liver and the intestine. Figure 1 shows the schematic description of the enterohepatic circulation. Because of their unique roles in various physiological processes, it is of great important to maintain the equilibrium of bile acids for the synthesis, secretion and the retake of the intestine. One of the physiological roles bile acids play is to maintain cholesterol homeostasis. The synthesis of bile acids is a complex process, which can be simplified as the formation of bile acids is by converting the neutral charged, water insoluble, hydrophobic cholesterol molecule to the ionized, water soluble, amphiphilic, soluble bile acid molecules. This catabolic process eliminates half of the total amount of cholesterol in the body, which signifies the most important physiological function of bile acids.
Bile acid molecules can be considered as planar molecules. Figure 2a shows the chemical structure of a typical primary or secondary bile acid molecule. Primary bile acids are synthesized in the hepatocyte from cholesterol. They are considered being the building block for secondary bile acids, which are made by bacterial biotransformations on the side chain and the nucleus. Primary bile acids are partially transformed into secondary bile acids in the colon. The “R” group on the steroid nucleus represents a hydroxyl group or a hydrogen atom depends on which specific type of bile acids. There are three hydroxyl groups for the case of the primary bile acid
cholic acid; while only one in the case of lithocholic acid, a secondary bile acid. As shown in Figure 2b, the upper surface of the molecule which contains the methyl groups is hydrophobic; the lower surface of the steroid ring which contains one or more hydroxyl groups is hydrophilic. Therefore, bile acid molecules are usually referred to as amphiphilic/amphipathic molecules. The carboxyl end group is polar and negatively charged when the solution reaches base condition due to the deprotonate process.

![Figure 2](attachment:image.png)

**Figure 2 (a) 2D and (b) 3D chemical structure of a typical bile acid molecule.**

Bile acids present in the monomer form when they exist in low concentrations. As the concentration increases, bile acid monomers will start to aggregate to form dimers (Figure 3a), tetramers (Figure 3b) and even larger structures such as polymers (Figure 3c) depending on the concentration. These small polymolecular aggregates are called micelles. Bile acid micelles, however, could not be composed exclusively by bile acid molecules in the human body. Conjugated bile acids, which are negatively charged anion molecules, will form mixed micelles with other lipids. Not all types of lipids will form mixed micelles with bile acids. There are
several types of lipids that will be favorable of forming mixed micelles with bile acids, as shown in Figure 4, such as phosphatidylcholine (PC), fatty acid-fatty acid mixtures and monoglycerides. Bile acids with phospholipids (consist mostly of phosphatidylcholine) can form mixed micelles in the bile, cholesterol can be dissolved by the micro-domains of these mixed micelles. Bile acids alone can convert cholesterol into bile acid, however, the mixed micelle formation greatly accelerates the cholesterol elimination.

Figure 3 (a) Dimer, (b) tetramer and (c) polymer formed by bile acids.
Figure 4 Mixed micelles formed by bile acids with phosphatidylcholine (PC), cholesterol, fatty acid and monoglycerides.

Pharmacological applications of bile acids have also been studied. Bile acids and their derivatives have been employed in treatments such as some liver diseases, bile acid deficiency and dissolving cholesterol gallstones. There are some promising results for their antifungal and antiviral properties.

1.2 Self-Assembly

Molecular self-assembly is a spontaneous process of molecules organizing themselves into order through cooperative weak interactions (non-covalent bonding) without any external intervention. Assume the molecules are building blocks, self-assembly is a processing that puts
these building blocks into order. In recent decades, the term self-assembly has been broadly discussed and in many cases it is confused with another term “formation”. Therefore, G. M. Whitesides made a more precise definition. He limits the term self-assembly only to reversible processes that use pre-existing components from separate or distinct parts of a disordered structure, which also can be controlled by the design of components\textsuperscript{13}. In molecular self-assembly, the structure of the assembly (usually in equilibrium states) is determined by the molecular structure\textsuperscript{14}.

Self-assembly can be divided into two types, static self-assembly and dynamic self-assembly\textsuperscript{14}. Static self-assembly happens in equilibrium systems. In static self-assembly no more external energy needs to be consumed after the ordered structure is formed, however energy maybe required during formation. In dynamic self-assembly, the order structure forms only when there is energy provided. Most of the study of self-assembly is focusing on the static self-assembly.

Most of the natural building blocks for self-assembly are amphiphiles. These amphiphilic molecules contain both distinct hydrophilic and hydrophobic segments, and will be able to form micelles, vesicles, monolayers and tubules in a solution environment or at the interface\textsuperscript{15}. The driving force for amphiphilic molecules to self-assemble is largely due to the hydrophobic part (non-polar) of the molecules driving them away from water and getting closer to one another. The self-assembled supramolecular structures formed by amphiphilic molecules depend on various factors, such as the coordination and repellence between the hydrophobic and hydrophilic segments to the ambient environment\textsuperscript{16}, the length and shape of the hydrophobic parts and the curvature and geometric factor of the hydrophilic parts\textsuperscript{15}. Figure 5 shows the
schematic draft of amphiphilic molecules self-assembled into double-layer tube (Figure 5a) and vesicle (Figure 5b) structures. The hydrophilic head groups in these two structures are pointing out to interact with the ambient solution while the hydrophobic parts of the molecules are getting away from the solution by interacting with the non-polar parts of one another.

Figure 5 Double-layer (a) tube and (b) vesicle structures formed by amphiphilic molecules through self-assembly.

1.2.1 Self-Assembly of Surfactants

Surfactants (surface active agents) are compounds that can significantly alter the interfacial energy of the system when they are absorbed into the interface at low concentration. Typically, they bear the amphiphilic structure as shown in Figure 6, with a non-polar tail that is
compatible with oil and a hydrophilic head group favors water. This distinct structure provides the surfactants with their unique interfacial characteristics.\textsuperscript{18}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{conventional_surfactant_structure.png}
\caption{Schematic image of a conventional surfactant structure.}
\end{figure}

Due to the hydrophobic effect,\textsuperscript{19} surfactant molecules tend to self-aggregate in water and form various supramolecular structures, such as spherical micelles, rodlike structures and spherical bilayer structures.\textsuperscript{18} Some typical micellar structures formed by self-aggregation of surfactants are shown in Figure 7. Among these aggregations, the hydrophilic polar groups of surfactant molecules are pointing outwards contacting with water, with the hydrocarbon chains interacting with one another in the middle avoiding contact with water. Due to the availability of hydrophobic domains provides by these micellar structures, surfactants have excellent capability of solubilizing hydrophobic solutes in solution,\textsuperscript{20} which signifies one of the distinguishable features of surfactants.
Tanford\textsuperscript{19} points out the cooperative growth of micelles is due to the hydrophobic effect; while due to the anti-cooperative interactions between the hydrophilic groups, the micelle size is controlled at a finite size. Both highly cooperative process and a critical micelle concentration (CMC) are needed for the micelle formation\textsuperscript{19}. CMC is defined as the point of a small concentration range, regarding to the solubility of the surfactants of the micelle phase\textsuperscript{21}. No
micelle structure can form below CMC while surfactant aggregates can take place when their concentration in solution goes beyond CMC.

1.2.2 Self-Assembly of Bile Acids

Bile acids are amphiphilic molecules which have a unique structure compared to conventional surfactants. The unique molecular structure of bile acids, as shown in Figure 8, has a rigid steroid ring system which has a hydrophobic convex surface and a less hydrophobic concave surface (hydrophilic compared to the convex surface) with one or more hydroxyl groups and a carboxyl group. The hydrophilic segments (hydroxyl and carboxyl group) are indicated as red in Figure 8, and the hydrophobic steroid ring is marked as blue. The number of hydroxyl groups has a large effect on the solubility of bile acids as well as their association behavior. The conventional surfactant molecules can be looked as a line structure with very long and flexible hydrophobic hydrocarbon chains and a small polar group; while the bile acids molecule is a planar structure with a polar (hydrophilic) side and a non-polar (hydrophobic) side, which is called bi-planar structure. The less hydrophobic of the concave surface is due the existence of hydrophilic hydroxyl group(s) which are oriented to the concave side. Also, the acidic group provides a strong hydrophilic moiety to the hydrophilic side when it is interacted with another interface.
Like conventional surfactants, bile salts will form micelles above the CMC. Below CMC, bile salts will present in the form of monomers. The CMC of bile salts is suggested to have a wider range region than the conventional surfactants\textsuperscript{26, 27}. For conventional surfactant molecules, their hydrophobic parts bury in the core of the micelle and the hydrophilic parts will be in contact with water. However, this is not what always the case when it comes to bile salt micelles. Some of the hydrophobic parts of the bile salts may remain in contact with the aqueous medium, while part of the hydrophilic sides will be remained inside the micelles. The main reason\textsuperscript{28} is that the domains of the hydrophobic and hydrophilic are not well separated due to the facial structure of bile salts.
Figure 9 (a) Primary micelles and (b) aggregation of micelles formed by bile acid molecules.

The most accepted model for bile salt micelles formation is a two steps model\textsuperscript{29}, which involves a primary micelle (Figure 9a) and secondary micelles (Figure 9b). Due to the driving force from the hydrophobic side of bile salts to reduce contact with the aqueous medium\textsuperscript{30}, bile salts molecules turn their back to each other to have back to back interaction to form aggregates with the hydrophilic surfaces with the hydroxyl and acidic groups pointing outward. The number of the bile salts molecules involve in the primary micelles is predicted to be up to ten\textsuperscript{31}. Primary micelles can form secondary micelles at a higher concentration. Through hydrogen bonding between hydroxyl groups of bile salts molecules, primary micelles aggregate and form secondary micelles, which can also be looked like elongated primary micelles in many cases.

Bile acids have been reported to form structures other than micelles, such as helical fibers\textsuperscript{32}, nanotubes\textsuperscript{33} and lamellar sheets\textsuperscript{34}. Due to the characteristic such as amphiphilicity, biocompatibility, structural rigidity and the capability of forming a variety of structures under different conditions\textsuperscript{35}, bile acids are being considered as ideal building blocks for synthesizing supramolecular structures. A considerable amount of research has been focusing on bile acids as a potential material of drug delivery\textsuperscript{36,37}. 
1.3 Conclusion

Bile acids and their derivatives, due to their distinct features such as unique amphiphilicity, high rigidity of the steroid structures, chirality, polarity, biocompatibility and availability, are ideal building blocks for constructing supramolecular structures. They can be self-assembled to form structures such as helical fibers, helical and straight nanotubes and ribbons.
CHAPTER 2 SELF-ASSEMBLY OF LITHOCHOLIC ACID

2.1 Introduction

Nature is in favor of helical structures. The most commonly known example may be the double helical nucleic acid deoxyribonucleic acid (DNA). Biological helical structures are formed by self-assembled of basic building blocks. Inspired by this marvelous synthetic art of nature, scientists have been attempting to achieve well-controlled morphology and geometry helical supramolecular structures by self-assembly of small molecules\textsuperscript{38}. Chiral amphiphilic molecules received exceptional attention among other basic building blocks due to the unique chirality of individual molecules is capable of forming helical supramolecular structures in aqueous medium.

Molecular self-assembly is based on cooperative weak, non-covalent interactions, such as hydrophobic interactions, water-mediated hydrogen bonds, electrostatic interactions and van der Waals interactions\textsuperscript{39}. These bonds are not isolated during the self-assembled process; in fact, they combine as a whole to establish the macromolecular structures. Among all these cooperative interactions, for biological systems the water mediated hydrogen interactions are of particular importance\textsuperscript{39} because water is indispensable.

Bile acids are facial amphiphilic, biologically active surfactants with distinct chirality and high structural rigidity that makes them ideal building blocks for supramolecular self-assembled structures. Differ from the hydrophilic head and hydrophobic tail structure of conventional surfactants; they embed a steroid ring structure that has a hydrophilic concave face with hydroxyl and carboxyl groups and a hydrophobic convex side. Upon a certain concentration, bile acids
self-aggregate to form micellar structures. The driving force for the aggregation is believed to be driven by the hydrophobic interactions of the hydrophobic parts of bile acid molecules.

Lithocholic acid (3α-hydroxyl-5β-cholan-24-oic acid, LCA) is a secondary bile acid, which was first isolated from a calf’s gallstone. As shown in Figure 10, LCA has a rigid hydrophobic steroid nucleus, with a hydroxyl group points towards the concave side and a carboxyl group connects to the other end through a short hydrocarbon chain staying on the convex surface.

![Chemical structure of LCA](image)

**Figure 10 Chemical structure of LCA.**

The solubility of LCA in pure water solution is low. However, with increasing pH value, the solubility of LCA increases. The concentration of LCA-COO⁻ and H⁺ is in the equilibrium state in the solution. As the pH value is raised, due to the deprotonation process of LCA dramatically increases, the concentration of the LCA-COO⁻ can increase exponentially when sufficient non-dissolved LCA is presented. Furthermore, when the LCA concentration reaches
the CMC, the limit of solubility will be practically removed as the formation of micelles takes place, and the solubility can be increased by further increasing the pH of the solution.

The self-assembly of LCA is very sensitive to the experimental conditions. It has been reported LCA can form vesicles of a uniform diameter about 1.5μm at pH 12 solutions. By dispersing LCA in sodium hydroxide (NaOH) solution, uniform distributed hollow nanotubes are synthesized with a diameter of 52nm and the tubule wall thickness is 3nm. Another study showed silica rods with mesoporous structure can be formed by mixing LCA and tetraethoxysilane (TEOS) in NaOH solution. One of our group’s previous works show that LCA can coil into spiral shape at pH 12 and turned back to the straight shape when the pH was decreased to 7.4; the transition can take place again while the pH was increased back to 12 by adding NaOH, which means the spiral to straight shape transition is reversible and controllable by changing the pH value.

2.2 Experimental Methods

**Chemicals and solutions.** 98% pure Lithocholic acid (LCA), sodium hydroxide (NaOH) and ammonium hydroxide (NH₃·H₂O, 28 wt%) were purchased from Sigma-Aldrich and used as received. Water used in our experiments was purified with an Easypure II system. Microscope cover glass slides were purchased from Fisher Scientific.

Stock solutions of NaOH for LCA self-assembly were prepared by dissolving NaOH pallets in water. Stock solutions of NH₄OH of 1 wt%, 5 wt%, 10 wt%, 15 wt% and 20 wt% for LCA self-assembly were made by diluting 28 wt% NH₄OH solution in water.
LCA (5mM) was dissolved in stock NaOH solutions and NH₄OH solutions with 30 minutes of sonication. The solutions were then stored in the dark at room temperature.

Unless otherwise stated, all chemicals are of analytical reagent grade and used without further purification.

**Optical microscopy.** Optical images were obtained by Olympus BX40 microscope equipped with a digital camera Olympus C2020 Zoom.

**X-ray diffraction analysis (XRD).** XRD patterns of LCA tubes and LCA/DiSC₃(5) complexes dried on silicon substrates were recorded with a Rigaku D/max diffractometer with CuKa radiation (λ = 1.542 Å) operated at 40 kV and 30 mA.

2.3 Results and Discussion

2.3.1 Self-Assembly of LCA in Sodium Hydroxide Solutions

The self-assembly of chiral amphiphilic molecules in aqueous solutions attracts special interest because of the chirality of individual molecules is often expressed in their supramolecular structures. For our study of the self-assembly of LCA in either NaOH or NH₄OH solutions, we fixed the LCA concentration at 5mM in order to investigate the influence of solvate concentration as well as how different types of solvate cations will effect on the self-assembled structures.

1 hour after LCA was dissolved in 0.01M NaOH solution, the LCA molecules form vesicles with an external diameter of about 1μm ± 0.5μm (Figure 11a). The LCA self-assembled
vesicle structure is different from the micelles formed by some of the other bile salts through the back-to-back hydrophobic interactions between the convex sides, with the hydrophilic concave side pointing outward in water\textsuperscript{47-48}. The vesicles linearly aggregate over time, and the fusion of these linearly aggregated vesicles will lead to the formation of hollow cylindrical tubes (Figure 11b). Eventually these trapped vesicles in the tubes disappear, possibly being integrated into the tube walls and becoming part of the tubes. The self-assembled mechanism of LCA tubes differs from that of other bile acid tubes in which the helical ribbons are a precursor\textsuperscript{49}. We find that the LCA tubes continuously grow until all vesicles are consumed (Figure 11c).

![Figure 11 Optical microscopy images of LCA self-assembled structures in 0.01M NaOH solutions after (a) 1 hour, (b) 10 hours and (c) 5 days.](image-url)
The self-assembled LCA structures in 0.01M, 0.1M and 1M NaOH solutions are shown in Figure 12. In 0.1M NaOH solution, both straight and helical tubes are formed (Figure 12a); also supramolecular spring structures are found (Figure 12b). The straight tubes are very flexible in this condition, which can be bent by the internal fluid motion. The helical structure formation is believed to be driven by the intrinsic chirality of the steroid ring of LCA molecule.

When the NaOH concentration increases to 0.1M, LCA self-assembled to form worm-like spherulite structures (Figure 12c) and all the tubes are found to be helical under this condition. An early stage worm-like spherulite structure is shown in Figure 12d. The spherulite structure contains a large amount of helical LCA tubes, with one of their ends ties to the center of the spherulites and the free ends pointing out and coil to form the worm-like shape.

In 1M NaOH solution, the self-assembled LCA tubes form noodle bundle-like structures (Figure 12e). When viewed between crossed polarizers, they show strong birefringence (Figure 12f), suggesting a high-order degree of the tubes. The birefringence of the noodle bundle-like structures is found to be the highest in the center, indicating large amounts of ordered LCA molecules pile up there.
Figure 12 Optical microscopy images of self-assembled LCA structures in NaOH solutions with concentrations of (a) and (b) 0.1M, (c) and (d) at 0.01M and (e) 0.1M. (f) Polarizing microscopy image of (e), direction of the polarizer and analyzer is indicated by white arrows.
2.3.2 Self-Assembly of LCA in Ammonium Hydroxide Solutions

Solvent may affect the growth rate as well as the structure formed by self-assembly\textsuperscript{50}. We investigated how the change of counterion from Na\textsuperscript{+} to NH\textsubscript{4}\textsuperscript{+} affects the LCA self-assembly. A series of NH\textsubscript{4}OH concentration dependent LCA self-assembled structures had been studied, in which LCA concentration was fixed at 5mM while the NH\textsubscript{4}OH concentration was varied between 1wt\% to 28wt\%.

LCA can be self-assembled to form worm-like shape structures with NH\textsubscript{4}OH concentration of 1wt\% (Figure 13a and Figure 13b). Flexible LCA tubules are knotted in the center of the spherulites, the free end of the tubules in the spherulites coils into a worm like shape. When the NH\textsubscript{4}OH concentration increases to 5wt\%, curled, spiral and flexible LCA tubules are formed and no worm-like shape structures are found (Figure 13c and Figure 13d). It is likely that the tied LCA tubules in the center of the spherulites are untied under this condition, therefore allows the LCA tubules to distribute individually and freely in the solution. When further increased the NH\textsubscript{4}OH concentration to 10wt\% (Figure 13e and Figure 13f), the LCA tubules are found to be more straight. Spiral shape LCA tubules are rare to be seen in this case, and the tubules seem to be less flexible and less coiled compared to the 5 wt\% samples.
Figure 13 Optical microscopy images of self-assembled structures of LCA in NH$_4$OH solutions with concentrations of (a) and (b) 1 wt%, (c) and (d) 5 wt% and (e) and (f) 10 wt%.
Figure 14 Optical microscopy images of self-assembled structures of LCA in NH$_4$OH solutions with concentrations of (a) and (b) 15 wt% and (c) and (d) 20 wt%.

The self-assembled LCA structures are mostly helical when the NH$_4$OH concentration is at 15 wt% (Figure 14a and Figure 14b). In this case, LCA tubes coil into the formation of supramolecular springs. The coiling of the tubes is a slow process; therefore, it is very difficult to track the coiling process of a single tube over days due to Brownian motion. The diameters of the tubes are mostly ranging from 0.3μm±0.1μm. It is uneasy to determine the handedness of the 3D helical tubes by using an optical microscope, because the apparent handedness of the helical tubes may change during a through-focus imaging. However, it is shown that with water
evaporization, 3D spiral tubes will deposit on the substrate and contract into 2D spirals\textsuperscript{46}. Therefore, the handedness of the helical tubes can be explicitly validated in this condition. All helical tubes found to be left-handed. The intrinsic chirality of the LCA steroid nucleus believed to be essential of the handedness of the helical tubes\textsuperscript{51}. From the optical contrast of the microscopy image in Figure 14b, it also shows that the helical structures are helical tubes rather than helical ribbons. Uniform optical contrast should be observed in 2D projection if they were ribbons, however, the optical contract between the tube walls and the inner aqueous core is found which prove they are tubes.

When the NH\textsubscript{4}OH concentration is increased to 20wt\%, the self-assembled LCA structures are a combination of straight, helical and spiral tubes (Figure 14c and Figure 14d). The pitch of the helical structures in the 20wt\% samples is much larger compared to the 15wt\% samples. It is very likely the helical spring tubes are stretched when the NH\textsubscript{4}OH concentration is increased to 20 wt\%. The straight tube can be considered as a helical structure with infinite pitch length. It is possible the increase of rigidity of the tubes leads to the stretch of the helical structures. The appearance of straight tubes is the extreme case of the stretched helical structures (infinite pitch length).

The LCA self-assembled structures are complicated in the 28wt\% NH\textsubscript{4}OH concentration solution. Single fan-like (Figure 15), double fan-like (Figure 16) and spherulite (Figure 17) structures are found to be coexisted. The splitting ends of the initially formed LCA rods will lead to the formation of fan-like structures\textsuperscript{43}. The formation process of a single fan-like bundle is shown in Figure 15. Two tubes are bound in one end initially (Figure 15a), which can also be considered as the split takes place in one end of the rod. Over time the splitting keeps going on,
and both the length and the number of the tubes in the bundle increases (Figure 15b and Figure 15c), finally the single fan-like structure is formed (Figure 15d).

![Figure 15 Optical microscopy images of self-assembled single fan-like structures of LCA in NH₄OH solutions with concentration of 28wt% after (a) 2 days, (b) 5 days, (c) 8 days and (d) 15 days.](image)

The double fan-like structures contain large number of tubes, the middle of which is tied with two tails fanning out. The formation process of a double fan-like bundle is shown in Figure 16. The split happens in both ends initially and gradually extends to the middle of the rods.
(Figure 16a) as time goes on. Similar to the formation of single fan-like bundles, the splitting continues with the length and the number of the tubes increase in process of time (Figure 16b and Figure 16c), in the end forms the double fan-like bundle (Figure 16d). The radius of the double fan-like bundles varies, which is depended on the length of the tubes.

Figure 16 Optical microscopy images of self-assembled double fan-like structures of LCA in NH₄OH solutions with concentration of 28wt% after (a) 1 day, (b) 3 days, (c) 7 days and (d) 10 days.
The formation process of a spherulite bundle is shown in Figure 17. Multiple ends splitting take place simultaneously in the original tube which leads to the formation of a pre-spherulites structure (Figure 17a). The splitting keeps taking place over time, and the growth of the spherulites continues as the length and the number of the tubes increase (Figure 17b and Figure 17c), finally leads to the formation the spherulites (Figure 17d). Like the double fan-like structure, the radius of the spherulites varies and is decided by the length of tubes.

Figure 17 Optical microscopy images of self-assembled spherulites structures of LCA in NH₄OH solutions with concentration of 28wt% after (a) 2 days, (b) 5 days, (c) 10 days and (d) 15 days.
Spherulite structures are commonly observed in polymer crystallization self-assembly\textsuperscript{52, 53}; bio-molecular self-assembled systems, such as proteins, DNA\textsuperscript{54, 55} and peptides\textsuperscript{56, 57} under crystallization\textsuperscript{58, 59}. Judging by the nucleation mechanisms of formation, spherulites can be separated into two types in general\textsuperscript{60}. Type I is an outcome of homogeneous nucleation, in which the needles form in solution first whereafter they split continuously at their ends to form spherulites. Type II is a consequence of heterogeneous nucleation, a spherical center forms first in solution and then subsequently the needles grow outward radially to form spherulites. The tubular spherulite structures in our study is belonged to the Type I category.

![X-ray diffraction graph]

*Figure 18 X-ray diffraction of self-assembled LCA tubes in NH$_4$OH solutions with concentrations of 1, 5, 10, 15 and 20wt% dried on silicon substrates.*
X-ray diffraction (XRD) of the LCA tubes self-assembled in 1, 5, 10, 15 and 20 wt% NH₄OH solutions show strong Bragg-peaks at $2\theta = 1.3^\circ \pm 0.15^\circ$ (Figure 18) with d-spacings of 6.58nm $\pm$ 0.49nm. The corresponding d-spacing and $2\theta$ angle for each sample is listed in Table 1. The XRD patterns indicate the tubes are crystalline. The small difference of the $2\theta$ angles among these samples implies similar molecular arrangement of their structures. Mesoscale simulation of chiral molecules self-assembly study shows that, the balance between chirality and elasticity determines the shape of one dimensional self-assembled supramolecular structures; furthermore, by varying the tilt direction with respect to the crystallographic axes, different pitch and diameter can be achieved even for the same material. For LCA self-assembly, the elastic modulus is provided by the hydrogen-bonding, hydrophobic and electrostatic interaction of LCA molecules. The intrinsic chirality of the LCA steroid nucleus is the main driving force for the coiling of the tubes. The interruption of the balance will lead to the shape change of the self-assembled structures.

**Table 1** D-spacings and $2\theta$ angles of self-assembled LCA tubes in NH₄OH solutions with concentrations of 1, 5, 10, 15 and 20 wt%.

<table>
<thead>
<tr>
<th>Sample</th>
<th>d</th>
<th>$2\theta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCA(5mM), 1 wt% NH₄OH</td>
<td>6.30nm</td>
<td>1.4°</td>
</tr>
<tr>
<td>LCA(5mM), 5 wt% NH₄OH</td>
<td>7.07nm</td>
<td>1.25°</td>
</tr>
<tr>
<td>LCA(5mM), 10 wt% NH₄OH</td>
<td>6.09nm</td>
<td>1.45°</td>
</tr>
<tr>
<td>LCA(5mM), 15 wt% NH₄OH</td>
<td>6.79nm</td>
<td>1.3°</td>
</tr>
<tr>
<td>LCA(5mM), 20 wt% NH₄OH</td>
<td>6.54nm</td>
<td>1.35°</td>
</tr>
</tbody>
</table>
In this chapter, self-assembled structures of LCA in NaOH and NH₄OH solutions were studied. The self-assembled process of LCA tubes in NaOH solutions was investigated. We found the self-assembled structures of LCA changed as the concentration of NaOH varied. The NH₄OH concentration dependent of the LCA self-assembled structures was also discussed. Worm-like spherulite, straight tube, helical tube including supramolecular spring, spherulite and single and double fan-like structures were found among the self-assembly of LCA in NH₄OH solutions study. The intrinsic chirality of the LCA steroid nucleus is believed to be essential of the handedness of the helical tubes. The helical structures are helical tubes rather than helical ribbons. The small difference of the $2\theta$ angles among these samples implies similar molecular arrangement within their structures. The balance between chirality and elasticity determines the shape of the one dimensional self-assembled supramolecular structures. The interruption of the balance will lead to the shape change of the self-assembled structures.
CHAPTER 3 CO-ASSEMBLY OF LITHOCHOLIC ACID AND A CATIONIC SURFACTANT

3.1 Introduction

The study of co-assembly in aqueous environments of bile salts with lipids and surfactants has great importance in understanding the physiology of bile and physical chemistry of mixed micelle systems. Secondary interactions, such as ionic interaction, play important roles in synthesizing supramolecular materials. It has been shown ionic self-assembly usually comes with a cooperative binding mechanism, in which bonds formed initially will promote the following bindings and finally leads to the formation of the self-assembled structures. Conventional surfactants are composed with a polar head and a non-polar tail, which makes them amphiphilic. Cetyltrimethylammonium bromide (CTAB) is a cationic surfactant (Figure 19).

![Chemical structure of CTAB](image)

Figure 19 Chemical structure of CTAB.

3.2 Experimental Methods

**Chemicals and solutions.** 98% pure Lithocholic acid (LCA), cetyltrimethylammonium bromide (CTAB), tetraethoxysilane (TEOS), Sudan III (red dye), sodium hydroxide (NaOH) and ammonium hydroxide (NH₃·H₂O, 28 wt%) were purchased from Sigma-Aldrich and used as
received. Water used in our experiments was purified with an Easypure II system. Microscope cover glass slides were from Fisher Scientific.

Stock solutions of NH$_4$OH of 6 wt%, 13 wt% and 20 wt% for LCA/CTAB co-assembly were made by diluting 28 wt% NH$_4$OH solution in water.

LCA and CTAB were dissolved in stock NH$_4$OH solutions (6 wt% ~ 28 wt%) with 5 to 30 minutes of sonication. The solutions were then stored in the dark at room temperature.

100μL TEOS was mixed with 5mL LCA/CTAB/NH$_4$OH sample solution. The mixtures were incubated at room temperature for 24 hours. The products were washed with 20% ethanol and then calcinated at 500°C for 6 hours under nitrogen.

Unless otherwise stated, all chemicals are of analytical reagent grade and used without further purification.

**Optical microscopy.** Optical images were obtained by Olympus BX40 microscope equipped with a digital camera Olympus C2020 Zoom.

**Dynamic light scattering (DLS).** DLS (Precesion Detector PD 2000DLS) was used to measure the size distribution of CTAB micelles.

**X-ray diffraction analysis (XRD).** XRD patterns of LCA tubes and LCA/DiSC$_3$(5) complexes dried on silicon substrates were recorded with a Rigaku D/max diffractometer with CuKa radiation (\(\lambda=1.542\) Å) operated at 40 kV and 30 mA.

**Zeta potential (ζ – potential).** The ζ-potential of LCA/CTAB co-assembly structures was measured using a Zetasizer Nano ZS90 (Malvern Instruments Inc.) under a cell-driven voltage of 30V.
**Differential scanning calorimetry (DSC).** DSC studies were performed with a Seiko Instruments DSC100 differential scanning calorimeter. 10μL sample solution was sealed in a DSC pan and then transferred to the calorimeter. The sample was scanned in the range from 20 to 80 °C at a heating rate of 5 °C/min.

**Scanning electron microscopy (SEM).** Scanning electron microscopy measurements were carried out with a Zeiss Ultra-55 SEM operating at an acceleration voltage of 100 kV.

### 3.3 Results and Discussion

#### 3.3.1 Self-Assembly of CTAB in Ammonium Hydroxide Solutions

Our study shows the self-assembly of CTAB in ammonium hydroxide solutions forms spherical aggregates, with diameters range from 100nm to 500nm as shown in Figure 20a. Dynamic light scattering measurements show that the self-assembled CTAB structures are polydisperse (Figure 20b), with an average diameter of 136nm. The diameters of the two CTAB aggregates showed in Figure 20c are about 350nm. The height profiles in Figure 20d show the aggregates have heights about 350nm. Combining the diameters and the height profiles of the aggregates, the self-assembled CTAB aggregates are characterized with spherical shapes.
Figure 20 (a) An AFM image and (b) dynamic light scattering measurements of CTAB self-assembled structures in NH$_4$OH solutions. (d) Height profiles (green marks) of (c) two CTAB self-assembled aggregations (blue arrows).

3.3.2 Study of Co-Assembled LCA with CTAB Mixtures in NH$_4$OH Solutions

3.3.2.1 Vesicle Fusion Study in the Initial Stage of the Co-assembly

The self-assembly of LCA and CTAB in ammonia hydroxide had been studied. In both situations, there is only one component in the system accounts for the assembly, either LCA or
CTAB. With the deprotonated anionic LCA and cationic CTAB in the same system, the coupling between the charge groups could foreseeably take place.

LCA and CTAB formed vesicles in the initial stage of the co-assembly. As shown in Figure 21, after LCA and CTAB were dissolved in NH₄OH solution, wide distributed sizes of vesicles were found. The fusion of the vesicles was also observed. In Figure 22a, two vesicles were very close to each other right before the fusion took place. The fusing process was finished in the nanosecond scale that could not be observed by the optical microscope we used, the fused image was shown in Figure 22b. After fusion, the two vesicles became a single vesicle (Figure 22c).

![Optical microscopy image of vesicles formed by LCA and CTAB mixed in NH₄OH solutions.](image-url)
Vesicles are microscopic sacs that enclose a volume with a molecularly thin membrane\textsuperscript{63}. The basis of vesicle fusion is two vesicles comes in contact with each other and merge to form one vesicle. The is commonly agreed model of explaining the intermediate stages of vesicle fusion is the stalk-pore mechanism\textsuperscript{64}. According to the molecular dynamics simulations of the vesicle fusion\textsuperscript{65}, the initial contact between the two vesicles will quickly form a stalk (Figure 23a) which can be stable for about 10ns before transforming to a hemifusion diaphragm (Figure 23b). The inner monolayers merged during the formation of the hemifusion diaphragm. Fusion pore
(Figure 23c) appears in less than 15ns after the hemifusion diaphragm is formed. The completion of the fusion process is symbolized by the rupture of the bilayer (Figure 23d).

![Figure 23 Schematic images of a typical vesicle fusion process. (a) Stalk, (b) hemifusion diaphragm, (c) fusion pore and (d) fused.](image)

3.3.2.2 Formation Process of the Co-Assembled Tubes

The LCA/CTAB co-assembled tubes formation process observed by optical microscope in a sealed micro channel is shown in Figure 24. For the larger size vesicle in the upper right in Figure 24a, it seems like it has two layers, with the inner layer slowly grew to form tubes with one end attached to the outer layer and the free end pointing outward (Figure 24d). We believe this vesicle may finally lead to the formation of a worm-like spherulites or split into individual tubes / tubes bundles. However, due to the slow growing process in the sealed micro channel, it was difficult to track the specific tubes growing process for over one week.
Figure 25 shows the growing process of a spherulites nucleus. As shown in Figure 25a, large amount of short tubes were observed at the border of the vesicle, with one end attached to the edge of the vesicle and the free end sticking out. The free end grew in length of over time (Figure 25b and Figure 25d).

Figure 24 Optical microscopy images of the formation process of LCA/CTAB co-assembled tubes in NH$_4$OH solutions in a sealed micro channel. Images were taken after (a) 1 day, (b) 2 days, (c) 3 days and (d) 7 days.
Figure 25 Optical microscopy images of the formation process of an LCA/CTAB co-assembled spherulites nucleus in NH₄OH solutions in a sealed micro channel. Images were taken after (a) 1 day, (b) 3 days and (c) 7 days.
Figure 26 Optical microscopy images of the formation process of LCA/CTAB co-assembled tubes in NH₄OH solutions during vesicle fusion. Time intervals of (a), (b) and (c) was 10 seconds. (d) is the blown up image of the red box in (c).

The tubes formation process during vesicle fusion was observed and showed in Figure 26. Two types of vesicles were shown in Figure 26a, circular/elliptical shapes and irregular shapes. Vesicle fusion could take place in both of these two types of vesicles and even between them in some cases. With the irregular shaped vesicles moved away from the circular/elliptical shaped
vesicles (Figure 26c), from the blow up image of the red box in Figure 26c we noticed a tube was formed and it was chaining up the vesicles (Figure 26d).

3.3.2.3 Growing Process of the Co-Assembled Tubes

After the formation of the co-assembled tubes, the growth process of the tubes is discovered to have two types: one is by multiple vesicle fusion (Figure 27) and the other one is by single vesicle fusion (Figure 28). As shown in Figure 27a, a tube was found chaining up two vesicles (marked by orange arrows). Over time the two vesicles moved away from each other (Figure 27b to Figure 27d), with the tube grew in both length and width. During this process, vesicles surrounding the tube were absorbed into the tube. We believe vesicles that were absorbed would slowly fuse into the tube wall that leads to the growth of the tube.

The length and width of the co-assembled tube is very small in its early stage, which obstructs the observation by optical microscope. Therefore, we loaded the solution with red dye (Sudan III) which helped us to capture the whole growth process. Figure 28a shows a red dye loaded vesicle with two tubes protruding outward. Over time, not only the initial two tubes grew in length (Figure 28b), but the number of the tubes increased as well (Figure 28c and Figure 28d). The vesicle shrank as the tubes grew (Figure 28e) and disappeared in the end (Figure 28f).
Figure 27 Optical microscopy images of the growth of LCA/CTAB co-assembled tubes in NH₄OH solutions by multiple vesicle fusion. Time interval between these images was 20 seconds.
Figure 28 Optical microscopy images of the growth of red dye loaded LCA/CTAB co-assembled tubes NH$_4$OH solutions by single vesicle fusion. Time interval between (a) to (d) was 60 seconds; between (d) to (f) was 5 minutes.
3.3.2.4 Effects of Molar Concentration Ratio on the Co-Assembled Tubes

Change of concentration of the components for self-assembly has effects on both the formation time and the structures\textsuperscript{66}. Our studies of the LCA/CTAB vesicle fusion, the formation and growth of the tubes were achieved by keeping the LCA/CTAB molar concentration ratio constant at 1:1. In order to investigate how the concentration of the components effects on the co-assembly, we varied the LCA to CTAB molar concentration ratios to 0.5:1 and 2:1. Combining with the 1:1 ratio data, we plotted the formation time of the tubes versus LCA to CTAB molar concentration ratio in Figure 29a. The diagram of the diameters of the tubes versus LCA to CTAB molar concentration ratio is shown in Figure 29b. The formation time of the tubes for LCA:CTAB = 0.5:1, 1:1 and 2:1 is 9 days, 5 days and 2 days, respectively. It is interesting to notice that ratio of the formation time (9:5:2) is very close to the ratio of the LCA molar concentration (0.5:1:2) in reverse. With the molar concentration ratio of LCA doubles, the formation time reduces in half. The diameter of the tubes also has a trend, the increase of LCA molar ratio will lead to the increase in diameter of the co-assembled tubes.
3.3.2.5 Characterization of the Co-Assembled Tubes

Differential scanning calorimetry (DSC) thermogram of the LCA/CTAB co-assembled tubes in NH₄OH solution with concentration of 13wt% is shown in Figure 30. During heating, an endotherm appears with the phase transition temperature at 72.3°C, representing the melting temperature of the co-assembled tubes. In situ optical microscope images also show the tubes begin melting when heated to 73°C. Only one endotherm shows up from DSC thermogram also indicates a homogeneous composition distribution in the co-assembly system, which means all the LCA and CTAB molecules participate in the co-assembly instead of self-assembling into single-component aggregates.
In order to better understand how the LCA and CTAB molecules arrange in the co-assembled tubes, we carried out the $\zeta$-potential study. As shown in Figure 31, the LCA/CTAB co-assembled tubes show a positive $\zeta$-potential of 40mv, while the self-assembled LCA tubes show a negative $\zeta$-potential of -37.9mv. Again, the $\zeta$-potential studies indicate CTAB takes part in the co-assembly and the outer layer of the tubes is dominant by CTAB molecules. X-ray diffraction study shows the co-assembled tubes have well defined layer structures, with an average d-spacing of 4.11nm (Figure 32). A schematic image of the helical co-assembled tubes is shown in Figure 33.
Figure 31 $\zeta$-potential studies of LCA self-assembled tubes and LCA/CTAB co-assembled tubes with molar concentration ratio of 1:1 in NH$_4$OH solutions.

Figure 32 X-ray diffraction of co-assembled LCA/CTAB tubes with molar concentration ratio of 1:1 in NH$_4$OH solution dried on silicon substrates.
3.3.2.6 Effects of Solvate Concentration on the Co-Assembled Tubes

To further manipulate the helical structures of the co-assembled tubes, we study how the concentration of the solvate will effect on the morphology of the tubes. Figure 34 shows the optical microscopy images of LCA/CTAB with molar concentration ratio of 1:1 co-assembled tubes in NH$_4$OH solutions with concentrations of 6wt%, 13wt%, 20wt% and 28wt%. We notice the pitch of the helical tubes decreases as the concentration of NH$_4$OH increases. We collected
pitch lengths of 100 helical tubes with diameter at 0.9 μm ± 0.1μm in each of the four conditions with the result shown in Figure 35.

Figure 34 Optical microscopy images of LCA/CTAB with molar concentration ratio of 1:1 co-assembled helical tubes in NH₄OH solutions with concentration of (a) 6wt%, (b) 13wt%, (c) 20wt% and (d) 28wt%.
Figure 35 Diagram of pitch lengths of LCA/CTAB with molar concentration ratio of 1:1 co-assembled tubes with diameter of 0.9μm±0.1μm in NH₄OH solutions with concentration of 6wt%, 13wt%, 20wt% and 28wt%.

3.3.2.7 Transformation of Co-Assembled Micro-structures into Silica

The transcription of the co-assembled helical structures into silica by the sol-gel process of TEOS is shown in Figure 36. The silica transcription forms helical ribbon structures characterized by SEM study (Figure 36a). Transcription of the co-assembled microstructures into silica replicas implicates the existence of intermolecular interactions between the organic assemblies and the hydrolyzed TEOS.
3.4 Conclusion

In this chapter, we utilized the ionic-assembly of LCA and CTAB to synthesize helical tubes with varied diameters and pitches. Our studies show that LCA and CTAB can self-assemble into helical tubes in NH$_4$OH aqueous solution. The diameter of the helical tubes can be changed by adjusting the molar concentration ratio between LCA and CTAB. The pitch of the helical tubes can be tuned by varying NH$_4$OH concentrations. Differential scanning calorimetry studies indicate that there is a homogeneous composition distribution in the LCA/CTAB helical tubes. X-ray diffraction analysis studies show that the helical tubes have multibilayer walls with an average d-spacing of 4.11nm. We demonstrate that the helical tubes with varied diameters and pitches can be transformed into helical silica through the sol-gel transcription of tetraethoxysilane (TEOS).
CHAPTER 4 CO-ASSEMBLY OF LITHOCHOLIC ACID AND A CYANINE DYE

4.1 Introduction

Green sulfur bacteria are a highly efficient light harvesting system found in nature\textsuperscript{67,68}. Despite living in the deep ocean where the intensity of light is extremely low they are still able to harvest up to 95\% energy of the light that reaches them and perform photosynthesis with high efficiency. Chlorosomes, which are composed of large numbers of bacteriochlorophylls organized into multilayer nanotubes, serve a light-harvesting antenna of green sulfur bacteria. Once the bacteriochlorophylls organized in the nanotubes are excited by light, they are able to share their excited states and transfer light energy in a form of excitations to the reaction center.

Cyanine dyes have been synthesized and reported over a century\textsuperscript{40}. Due to their large extinction coefficients and moderate fluorescence quantum yields\textsuperscript{69}, they have been extensively used in spectral sensitizers for silver halide photography\textsuperscript{70}, solar cells\textsuperscript{71} and probes for biological systems\textsuperscript{72}.

Aggregation of cyanine dyes in polar solvent is known to change the absorption band compared to that of the monomer. With a narrow absorption band shifts to a longer wavelength relative to that of the monomer absorption band, these dye aggregates are termed J-aggregates or Scheibe aggregates\textsuperscript{73}. Dye aggregates with the absorption band hypsochromically shift (shift to shorter wavelength compared to the monomer) are characterized as H-aggregates\textsuperscript{70}.

J-aggregates are an organized supramolecular structure of dye molecules, in which the transition dipole moments of individual dye molecules are parallel aligned to the line joining their centers (end-to-end arrangement)\textsuperscript{74,75,76}. The excitation due to the interaction of the
transition dipole moments in J-aggregates leads to a sharp and intense absorption band (J-band), which is red-shifted in respect to the monomer absorption band. Recently, there has been great interest in the synthesis of tubular structures of J-aggregates by the self-assembly of amphiphilic dyes to mimic natural light-harvesting antennas for highly efficient energy transfer\textsuperscript{77-86}.

The unique spectroscopic properties of J-aggregates arise from the tightly packed dye molecules. With extended domains formed by their transition dipoles that are coherently coupled in the aggregates, which leads to the formation of a delocalized excitonic state\textsuperscript{87}. The incoherent transfer mechanism for the migration of the delocalized excitonic state within the J-aggregates is characterized as the hopping motion\textsuperscript{88} of the exciton. The extent of the exciton migration, which is determined by the exciton migration time and its life time, is believed to be hundreds of nanometers\textsuperscript{89} thus can be regulated by defects within the structure of the aggregates\textsuperscript{90}.

Cationic polymethine dye 3, 3'-Dipropylthiadicarbocyanine Iodide (DiSC\textsubscript{3}(5), Figure 37) is a membrane potential sensitive dye which is commonly used for measuring the cytoplasmic membrane permeability of biomolecules\textsuperscript{91, 92}.

![Chemical structure of DiSC\textsubscript{3}(5).](image)

Figure 37 Chemical structure of DiSC\textsubscript{3}(5).

The co-assembly of structurally different molecules has been proven to be a powerful method for the synthesis of organic tubes without requiring time-consuming chemical synthesis,
in which the interesting physical properties of self-assembled tubes can be designed by properly choosing molecules.\textsuperscript{16-25} In this paper, we report the synthesis of J-aggregate tubes by the co-assembly of lithocholic acid (LCA) and a commercially available cyanine dye (3, 3'-dipropylthiadicarbocyanine iodide (DiSC\textsubscript{3}(5)), in which the DiSC\textsubscript{3}(5) assembles into the multiple concentric cylinders of J-aggregates under the assistance of LCA.

4.2 Experimental Methods

**Chemicals and solutions.** The cyanine dye 3, 3'-Dipropylthiadicarbocyanine Iodide (DiSC\textsubscript{3}(5), MW=546.53 g/mol) was obtained from Invitrogen\textsuperscript{TM} and used as received. Metal salts AgNO\textsubscript{3}, HgCl\textsubscript{2}, CuCl\textsubscript{2}, NiSO\textsubscript{4}, KCl, MgCl\textsubscript{2}, CaCl\textsubscript{2}, SnCl\textsubscript{2}, FeCl\textsubscript{3}, ZnCl\textsubscript{2} and PdCl\textsubscript{2} were purchased from Sigma-Aldrich. Dopamine hydrochloride, methylene blue and sodium borohydride were purchased from Sigma-Aldrich.

**One-step assembly.** LCA (0.3mM) and DiSC\textsubscript{3}(5) (0.3mM) with molar ratio of 1:1 were dissolved together in NaOH (0.1M) solution and ammonium hydroxide solution (13 wt\%) with 30 minutes of sonication. The solutions were stored in the dark at room temperature for 48 hours.

**Two-step assembly.** LCA (0.3mM) was dissolved in NaOH (0.1M) solution with 30 minutes of sonication. The solution was then stored at room temperature for 72 hours before DiSC\textsubscript{3}(5) (0.3mM) was added. The mixed solution was stored in the dark at room temperature for 48 hours before taking absorption spectrum.

The stock solutions of interference ions (10mM) were prepared by dissolving CuCl\textsubscript{2}, NiSO\textsubscript{4}, KCl, MgCl\textsubscript{2}, CaCl\textsubscript{2}, SnCl\textsubscript{2}, FeCl\textsubscript{3}, ZnCl\textsubscript{2} and PdCl\textsubscript{2} in water, respectively.
The stock solutions of uric acid (UA) (1mM) and L-ascorbic acid (AA) (1mM) were prepared by dissolving UA and AA in water, respectively.

Unless otherwise stated, all chemicals are of analytical reagent grade and used without further purification.

**Reduction of Ag (I).** For 4mL J-aggregates and H-aggregates solution, 40μL 10mM AgNO₃ solution was added with a final AgNO₃ concentration of 100μM. To a 3.8mL H-aggregates solution, 200μL 100mM AgNO₃ solution was added with a final AgNO₃ concentration of 5000μM. Absorption spectra were taken within 60 min of adding AgNO₃. The solutions were illuminated with a white light fluorescent bulb.

**Detection of Hg (II).** Different volumes of HgCl₂ solution with concentration from 10nM to 10mM were added into J-aggregates solution to achieve a final Hg²⁺ concentration of 0.01nM to 100μM. Absorption spectra were taken within 60 min of adding HgCl₂. The solutions were illuminated with a white light fluorescent bulb.

**Detection of dopamine.** Dopamine solution was prepared freshly in DI water at room temperature. Different volumes of dopamine hydrochloride solution with concentration ranging from 10μM to 1mM were added into LCA (0.3mM)/DiSC₅(5) (0.3mM)/NaOH (0.1M) solution to achieve a final dopamine concentration of 0.25μM to 100μM.

**Degradation of methylene blue.** NaBH₄ solution (100mM) was prepared freshly in DI water at room temperature. 30uL LCA(0.3mM)/DiSC₅(5)(0.3mM)/NaOH (0.1M) and LCA (0.1mM)/DiSC₅(5) (0.1mM)/NH₄OH (13 wt%) with AgNO₃ (4.762mM) after reduction was added to 2mL 25μM methylene blue solution achieving a final silver concentration of 70μM, then 100μL 100mM of NaBH₄ was added.
Absorption spectroscopy. Absorption spectra from the solution were taken with a double-beam UV-VIS-NIR spectrophotometer (Cary 500, Varian Inc.) in a 10mm quartz cell from Hellmar.

Scanning electron microscopy (SEM). Scanning electron microscopy measurements were carried out with a Zeiss Ultra-55 SEM operating at an acceleration voltage of 100 kV.

Transmission electron microscopy (TEM). Transmission electron microscopy was performed with a JEOL JEM100SX-EM microscope operating at an acceleration voltage of 100 kV.

X-ray diffraction analysis (XRD). XRD patterns of LCA tubes and LCA/DiSC$_3$(5) complexes dried on silicon substrates were recorded with a Rigaku D/max diffractometer with CuKa radiation (\(\lambda =1.542\) Å) operated at 40 kV and 30 mA.

Atomic force microscopy (AFM). Atomic force microscope images were obtained by Dimension 3100 with built-in optical microscope (Veeco Instruments) on silicon substrates.

Optical microscopy. Optical images were obtained by Olympus BX40 microscope equipped with a digital camera Olympus C2020 Zoom.
4.3 Results and Discussion

4.3.1 Study of Co-Assembled LCA with CD Mixtures

The chemical structures of LCA and DiSC$_3$(5) dye together with a schematic for self-assembled J-aggregate tubes are shown in Figure 42. DiSC$_3$(5) is a cationic carbocyanine dye consisting of two nitrogen containing heterocyclic ring systems, which include a chain of conjugated double bonds and two short alkyl tails (Figure 42a). LCA is a secondary bile acid having a nearly planar hydrophobic steroid nucleus. The ionic head with a carboxyl group is linked to the steroid nucleus through a short alkyl chain (Figure 42a). It has been shown that LCA in 0.1 M NaOH aqueous solution is able to self-assemble into helical tubes with diameters in the range from 0.5 to 1.6 μm$^{93}$. Thus, we used LCA as a directing agent to assemble DiSC$_3$(5) into tubular structures. Briefly, LCA and DiSC$_3$(5) were mixed in 0.1 M NaOH aqueous solution in a 1:1 molar ratio with a total concentration of 0.6 mM at 60 °C and cooled to room temperature. In this condition, negatively charged LCA couples with the positively charged DiSC$_3$(5) to form a DiSC$_3$(5)$^+$/LCA$^-$ ion pair, which then self-assemble into tubular structures (Figure 42c).

Figure 38 shows the absorption spectra of two sets of LCA/DiSC$_3$(5) co-assembled samples, one set was prepared in 0.1M NaOH solution (Figure 38a) and the other set in NH$_4$OH solution with concentration of 13wt% (Figure 38b). The monomeric cyanine dye exhibits an absorption band at 648nm (FWHM: 51nm) in 0.1M NaOH solution, which is very close to the absorption band ($\lambda_{\text{max}} = 653\pm3$nm) for the dye in methanol solution. In addition to the absorption band at 648nm, a shoulder around 600nm is also found in the DiSC$_3$(5)/NaOH spectrum. The co-
assembled LCA/DiSC\textsubscript{3}(5) with molar concentration ratio of 1:1 in 0.1M NaOH solution shows a relatively narrow absorption band (FWHM: 30nm) with a maximum at 711nm, which indicates the formation of J-aggregates. In the ammonium hydroxide solution, the monomeric dye shows a wide absorption band around 650nm, while the LCA/DiSC\textsubscript{3}(5) with molar concentration ratio of 1:1 in NH\textsubscript{4}OH solution exhibits a blue shifted and narrower absorption peak at 452nm (FWHM: 42nm), indicating the formation of H-aggregates. Another absorption peak for the LCA/DiSC\textsubscript{3}(5)/NH\textsubscript{4}OH at 650nm is also found.

![Figure 38 Absorption spectra of monomeric DiSC3(5) (black) and co-assembled LCA/DiSC\textsubscript{3}(5) with molar concentration ratio of 1:1 (red) in (a) NaOH (0.1M) solution; (b) NH\textsubscript{4}OH (13 wt\%) solution.](image)

Powder X-ray diffraction (XRD) of the J-aggregate nanotubes shows a strong Bragg peak at $2\theta = 3.7^\circ$, indicating that the tube walls have a regular layer stacking with a $d$-spacing of 2.3
nm (Figure 39). The \( d \)-spacing is close to the length of LCA measured from its hydroxyl to its carboxyl group (~ 1.5 nm) plus the width of DiSC\(_3\)(5) (~ 0.5 nm).

Figure 39 X-ray diffraction analysis of LCA/DiSC\(_3\)(5) co-assembled structures with molar concentration ratio of 1:1 in 0.1M NaOH solution.
Figure 40 Optical microscopy images of (a) J-aggregates tubes and (b) H-aggregates vesicles dried on glass substrates.

Figure 41 (a) AFM images of J-aggregate nanotubes dried on a silicon substrate. (b) Corresponding height distributions.

The J- and H-aggregates structures by optical microscopy study are shown in Figure 40. The J-aggregates have tubular structures (Figure 40a). Figure 40b shows vesicle structures
formed by H-aggregates. As can be seen from the atomic force microscopy (AFM) images in Figure 41a, the self-assembled J-aggregate nanotubes dried on a glass substrate exhibit a cylindrical shape with a smooth surface, suggesting that they are not grossly distorted after being dried on the silicon substrate. The external diameter of the J-aggregate nanotubes was measured from their heights to be in the range of 20-100 nm (Figure 41b). The length of the nanotubes varies 5 µm to 20 µm.

Combining the XRD characterization and the microscopy results, a possible scheme of molecular packing in the multilayer J-aggregate nanotubes is proposed (Figure 42).

![Figure 42](image)

Figure 42 (a) Amphiphilic bile acid LCA and cyanine dye DiSC₃(5). (b) DiSC₃(5)⁺/LCA⁻ ion pair. (c) Schematic illustrating of LCA/DiSC₃(5) self-assembled nanotubular structures in NaOH solution.
To better understand how the relation between LCA and DiSC$_3$(5) of the co-assembly affects the spectroscopic property of the aggregates, we modulate the DiSC$_3$(5) to LCA molar concentration ratio while keeping other conditions constant for the assembly. The absorption spectra is shown in Figure 43a for the co-assembled aggregates in 0.1M NaOH solutions. The J-band at 711nm is observed in both molar ratios of DiSC$_3$(5) to LCA of 1:2 and 2:1, which indicates the molecular packing in the aggregates is similar to the 1:1 case. The FWHM is 33nm and 37 nm for the molar ratios of DiSC$_3$(5) to LCA of 2:1 and 1:2, respectively, compared to 30nm for the case of 1:1. The change of the spectroscopic property is dramatic for the assembly in NH$_4$OH solutions with concentration of 13wt% (Figure 43b). For the molar concentration ratio of DiSC$_3$(5) to LCA of 2:1, the blue-shift of the absorption band is observed. However, the band is broadened compared to the 1:1 molar ratio and the peak shifts to 454nm. The absorption spectrum for the DiSC$_3$(5) to LCA molar ratio of 1:2 is with distinct feature that has never been observed, both J-band and H-band are possessed for the aggregates in this condition, with the H-band peak at 449nm and the J-band peak at 683nm.
Studies have shown absorption of cyanine molecules onto solid surfaces offers greater control of the aggregation processes.\textsuperscript{94, 95} The co-assembly of LCA and DiSC\textsubscript{3}(5) discussed earlier is what we called a one-step assembly, which is by mixing LCA and DiSC\textsubscript{3}(5) simultaneously in polar solvents. With the LCA nanostructures formed in the first place to provide solid templates for the aggregation of the dye, is what we called a two-step assembly. The absorption spectra of both one-step and two-step assembly of DiSC\textsubscript{3}(5)/LCA with molar ratio 1:1 are shown in Figure 44. The two-step assembled aggregates exhibit a red-shift J-aggregates spectral band with the maximum absorption at 712nm. The reduced sharpness of the J-band of the two-step assembly (FWHM: 62nm) indicates a less ordered packing of dye molecules.
Figure 44 Absorption spectra of two-step (black) and one-step (red) assembly of LCA/DiSC₃(5) with molar concentration ratio of 1:1 in 0.1M NaOH solutions.

4.3.2 Photoinitiated Reduction of Silver Ions with Co-Assembled LCA/CD Mixtures

Molecules in their exited states can act as electron donors. Electron transfer in solution can happen within a small distance between the donor and acceptor molecules. By diffusing acceptor ions into the J-/H-aggregates solutions, we studied the efficiency of photoinitiated electron transfers.

Ag⁺ was used as an external reducing agent for the J- and H-aggregates in solutions. J-aggregates of cyanine dye have been demonstrated to be photoinitiated to reduce silver ions (Ag (I)) to silver metal (Ag⁰), in which the cyanine dye molecules act as sacrificial electron
donors. Upon oxidation, the absorption spectrum will decrease in the exciton bands caused by the deprivation of electrons from the $\pi$-conjugated system of the dye chromophores.

Figure 45 (a) Absorption spectra of LCA/DiSC$_3$(5) in 0.1M NaOH solutions with 100$\mu$M AgNO$_3$ with 0–68 minutes of exposure to white light. (b) Normalized intensity of the absorption peak at 710nm versus the expose time. Solid line is a linear fit.
Figure 46 Absorption spectra of LCA/DiSC₃(5) in NH₄OH solutions with concentration of 13wt% with (a) 5000μM AgNO₃ with 0~45 minutes, (c) 100μM AgNO₃ with 0~52 minutes of exposure to visible light. Normalized intensity of the absorption peak at 450nm versus the expose time of (b) 5000μM and (d) 100μM AgNO₃. Solid line is a curve fit at (b) and linear fit at (d).

Upon the addition of 100μM AgNO₃ and exposure to white light, the changes of the spectra of J-aggregates are dramatic (Figure 45a), while there is only marginally changes are
observed for H-aggregates (Figure 46c). For J-aggregates, particularly the absorption peak at 710nm decreases significantly due to the oxidation process, while a broad silver plasmon absorption peak around ~420nm grows indicating the formation of silver nanostructures. With AgNO₃ concentration increased to 5000μM (Figure 46a) for the H-aggregates, the absorption peak at 450nm disappears almost completely and the plasmon resonance absorption peak rises at ~420nm. The slope of the intensity changes versus time indicates the speed of the oxidation process. The slope (-4.2×10⁻³) of J-aggregates (Figure 45b) is ten times of the slope (-4.0×10⁻⁴) of the H-aggregates (Figure 46d) with the same concentration of Ag⁺ ions. When the concentration of silver ions is raised 50 times for the H-aggregates, the slope is -1.392×10⁻² (Figure 46b), indicating the oxidation speed increases by 34 times.

Figure 47 SEM images of (a) J-aggregates and (b) H-aggregates dried on silicon wafers after oxidation by Ag⁺ ions with concentration of (a) 100μM and (b) 5000μM.
SEM images of the J- and H-aggregates structures after Ag\(^+\) oxidation are shown in Figure 46. It is critical that the morphology of the nanostructures of both J- and H-aggregates maintain undisturbed by the oxidation chemistry. Figure 46a shows the J-aggregates remained tubular structures after oxidation, with silver nanostructures in direct contact of the outer tube walls. However, the H-aggregates structures were destroyed after oxidation (Figure 46b). The difference between the J-aggregates and H-aggregates after oxidation can be explained by the concentration of silver ions. For the H-aggregates, the Ag\(^+\) concentration is 50 times to the J-aggregates. With higher Ag\(^+\) concentration, more electrons will be deprived upon oxidation. Therefore, the loss of electrons from the \(\pi\)-conjugated system exceeds the limit where the H-aggregates structures can be remained intact leads to the change of the morphology of the nanostructures.

4.3.3 Hg (II) Detection by Photoinitiated Charge Transfer from Co-Assembled LCA/CD Mixtures

Pollutions created by new technologies, especially heavy metal ions pollution, endanger human health and the environment. Mercury contamination can happen in many ways, such as gold mining which use large quantities of Hg to separate fine gold particles\(^98\), coal and wood combustion\(^99\), Zn and Pb production\(^100\) and solid waste incineration\(^101\).

The adding of Hg\(^{2+}\) into the J-aggregates solutions has a strong effect on the optical properties. Figure 48 shows the changes in the absorption spectrum with exposure time of a J-aggregates solution after adding HgCl\(_2\) (100\(\mu\)M). A loss in the total absorption of the aggregate is clearly demonstrated in the spectra as the redox reaction proceeds. We also notice the loss is
correlated with the increased absorption band at ~600nm, which we believe is related to the aggregate of Hg$^0$ atoms.

Figure 48 (a) Absorption spectra of LCA/DiSC$_3$(5)/NaOH solution with 100μM HgCl$_2$ after 0~120 min of exposure to white light. (b) Normalized intensity of the absorption peak at 710nm versus the expose time. Solid line is a linear fit.

We examined the effect of other metal ions on the optical properties of J-aggregates solutions under identical conditions. As shown in Figure 49a, only the addition of Hg$^{2+}$ resulted a significant absorption loss. No remarkable changes in the absorption intensity at 711nm of the J-aggregates solution were observed upon the addition of other metal ions, including Na$^+$, K$^+$, Mg$^+$, Ca$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, Sn$^{2+}$ and Fe$^{3+}$. We believe the redox potential is essential for the unique selectivity of J-aggregates to Hg$^{2+}$. The redox potential values$^{102}$ of the metal ions used in our study are shown in Figure 49b. The change of absorbance is negligible with the negative oxidation potential metal ions. The oxidation potential of Hg$^{2+}$ is very close to Ag$^+$, and the
absorbance change with Hg\textsuperscript{2+} is almost twice as much as with Ag\textsuperscript{+}. We believed this is due to each Hg\textsuperscript{2+} needs two electrons to be reduced to Hg\textsuperscript{0} while each Ag\textsuperscript{+} needs only one.

However, the addition of Ag\textsuperscript{+} also causes a certain amount of loss to the absorption intensity. To distinguish between Hg\textsuperscript{2+} and Ag\textsuperscript{+}, we carried out the measurement in the presence of both ions (Figure 50). As showed in our earlier study of the photoreduction of silver ions, the reduced silver atoms aggregated into the formation of nanostructures with a silver plasmon absorption band around 420nm. As shown in Figure 50a, upon the addition of both HgCl\textsubscript{2} (50μM) and AgNO\textsubscript{3} (50μM), the loss of total absorption is correlated with the appearance of two peaks at ~420nm and ~600nm, which is attributed to the plasmon absorption band of silver and mercury, respectively. The appearance of the plasmon absorption bands indicate light enhanced growth of silver and mercury nanostructures. Therefore, we can use the iconic silver and mercury plasmon absorption band to distinguish Hg\textsuperscript{2+} and Ag\textsuperscript{+}.

The Hg\textsuperscript{2+} concentration dependent absorbance change is shown in Figure 51. The lowest concentration of Hg\textsuperscript{2+} can be detected using this method is 10pM.
Figure 49 (a) Effect of metal ions (100µM) on the change of absorbance at 711nm of LCA/DiSC₃(5)/NaOH solution after exposure to white light for 40 minutes. (b) Reduction potentials of metal ions.
Figure 50 (a) Absorption spectra of LCA/DiSC$_3$(5)/NaOH solution with 50μM AgNO$_3$ and HgCl$_2$ after 0~80 min of exposure to white light. (b) Normalized intensity of the absorption peak at 710nm versus the expose time. Solid line is a curve fit.

Figure 51 (a) Absorption spectra of LCA/DiSC$_3$(5)/NaOH solution with 0.01nM, 0.1nM, 0.5nM, 0.05μM and 0.5μM HgCl$_2$ after 40 min of exposure to white light. (b) Normalized intensity of the absorption peak at 710nm versus concentration. Solid line is a curve fit.
4.3.4 Dopamine Detection by Photoinitiated Charge Transfer from Co-Assembled LCA/CD Mixtures

Dopamine (DA, Figure 52a) is discovered as a neurotransmitter in the late 1950s. Later, Parkinson’s disease is found to be associated with the dysfunctions of the dopamine system\textsuperscript{103}. The concentration of basal DA is very low (0.01\(\mu\)m to 1\(\mu\)m)\textsuperscript{104}. The main difficulty of detecting DA using electrochemical procedures is the lack of resolution between DA and uric acid (UA, Figure 52c) and ascorbic acid (AA, Figure 52b), as their oxidation potentials are very close\textsuperscript{105,106}. Here, we describe a DA detection process utilizing the oxidation property of the co-assembled LCA/CD complex. The majority of DA molecules have been shown to be electron acceptors in high pH aqueous solution\textsuperscript{107}, while UA and AA remain to be electron donors.

![Chemical structures of (a) dopamine, (b) L-ascorbic acid and (c) uric acid.](image)

The J-aggregate tubes show a sharp and intense J-band absorption at 714 nm, which is red-shifted with respect to the monomer absorption band. It has been shown that oxidized dopamine (DA) in alkaline solution can act as an electron acceptor\textsuperscript{107-109}. We find that light energy harvested by the J-aggregate tubes can be efficiently transferred into the DA adsorbed the surface of the tubes, leading to the decrease of the J-band absorption. The absorption quenching
of J-aggregate tubes due to the photo-induced electron transfer provides a novel sensing platform for the sensitive and selective detection of DA in aqueous solution with the detection limit of approximately 5 nM. DA is an important neurotransmitter in mammalian central nervous systems\textsuperscript{110}. A small fluctuations in DA concentrations causes several neurological disorders\textsuperscript{111}. Therefore, the precise determination of DA is of great clinical importance\textsuperscript{112}. In the past few decades, a number of sensing platforms including electrochemical methods\textsuperscript{112-113}, capillary electrophoresis\textsuperscript{33} and high-performance liquid chromatography–mass spectrometry\textsuperscript{114} have been developed for the sensitive and selective detection of DA. However, these methods share the common drawback of being time-consuming and often requiring specialized equipment. Furthermore, the detection limit of J-aggregate tubes for DA is at least one or two orders of magnitude lower than that of these methods and also superior to that of recently developed colorimetric sensors based on the plasmon absorption of Au and Ag nanoparticles\textsuperscript{115-118}. The low detection limit is a result of the efficient electron transfer from the J-aggregate tubes to the adsorbed DA. Our results indicate that light-harvesting J-aggregate tubes have the potential for constructing a new DA biosensor.

The J-aggregate nanotubes formed in 0.1M NaOH solution are stable after being suspended in PBS solution at pH 7.4. There is no change in their structures and optical properties observed. Dopamine (DA) is known to undergo autoxidation in basic solution to produce DA-quinone\textsuperscript{119-120}. It has been shown that amines interact with the carboxylate and hydroxyl groups of LCA via electrostatic interaction and hydrogen bonding, respectively\textsuperscript{121}. We find that the DA-quinone adsorbed on the surface of J-aggregate nanotubes in PBS solution can act as an electron acceptor.
The light energy harvested by the J-aggregate nanotubes can efficiently transfer to the DA acceptor, resulting in the significant decrease of J-band absorption (Figure 53a). In our experiments, DA was added into 2 mL of PBS solution containing 0.25 mg/mL J-aggregate nanotubes and then incubated for 40 mins in the dark. In the absence of white light exposure, the absorption band of the J-aggregate tubes at 714 nm remains unchanged. However, upon exposure to white light for 2 mins, the decrease of J-band absorption is clearly visible in Figure 53a. AFM images show that the morphology of the J-aggregate nanotubes is intact after the photo-induced electron transfer, confirming that the decrease of J-band absorption is not due to the disassembly of J-aggregate nanotubes. The intensity of the J-band absorption at 712 nm gradually decreases when the concentration of DA is raised from 0 to 50 μM. Figure 53b shows a plot of $I_0-I/I_0$ as a function of DA concentrations, where $I_0$ is the intensity of the J-band absorption in the absence of DA and $I$ is the intensity of the J-band absorption in the presence of DA at different concentrations. In the micromolar concentration range, the plot is nonlinear. When the DA concentration is reduced to the nanomolar range, the plot shows a good linear relationship (Figure 53c), giving a detection limit of approximately 5 nM, which falls within the basal level (nanomolar scale) of DA in cerebral systems. The detection limit of J-aggregate nanotubes is much lower than that of electrochemical methods (2.6 μM), capillary electrophoresis (120 nM), and high-performance liquid chromatography–mass spectrometry (54 nM).
Figure 53 (a) Absorption spectra of J-aggregate solutions with 0.25μM to 50μM Dopamine.

(b) Normalized intensity change of the absorption peak at 712nm versus dopamine concentration. (c) Normalized intensity change of the absorption peak at 712nm versus dopamine concentration in nanomolar range.
A major issue for the detection of DA is the effect of possible interfering compounds such as ascorbic acid (AA) and uric acid (UA), which coexist with DA in vivo at a concentration of 100 to 1000-fold higher than that of DA.\textsuperscript{124} AA and UA can also undergo oxidation in
aqueous solution. However, we find that the J-aggregate nanotubes in PBS solution are not sensitive to AA and UA. The addition of 100 µM AA or UA causes a negligible change in the J-band absorption, compared to the change induced by 0.1 µM DA (Figure 54a). Moreover, the presence of UA and DA does not affect the detection of J-aggregate nanotubes for DA in PBS solution. The detection limit of J-aggregate tubes for DA is found to be near the same with or without the presence of 10 µM AA or UA. It means that DA can still be detected even in the presence of 100 times higher of AA or UA than DA. The excellent selectivity of J-aggregate nanotubes for DA over AA and UA mainly attributed to the high affinity between the positively charged DA and the negatively charged J-aggregate nanotubes via electrostatic interaction. Compared to DA, AA and UA are negatively charged at the physiological pH 7.4. Thus, the electrostatic repulsion between the negatively charged AA/UA and the negatively charged J-aggregate nanotubes prevents their adsorption on the surface of the tubes. In addition to absorption spectrum analysis, the remarkable selectivity of J-aggregate nanotubes toward DA over interfering AA, UA and some metal ions can also be observed by the naked eye with the aid of a white light. Figure 54b shows photographs taken from the J-aggregate tube solutions in the presence of DA, UA, AA and metal ions, respectively. Under a white light excitation, the color difference of these tube solutions is apparent. The solution containing DA is transparent, while the tube solution containing other interfering species shows blue color. These results clearly indicates that the J-aggregate nanotubes can serve as a novel and facile sensing platform for the effectively identify DA with the aid of white light illumination.
In summary, we report the synthesis of J-aggregate nanotubes formed by the co-assembly of lithocholic acid (LCA) and cyanine dye, 3, 3'-dipropylthiadicarbocyanine iodide (DiSC\(_3\)(5)), in which the DiSC\(_3\)(5) dye is organized into the multiple concentric bilayers of J-aggregates. The ionic self-assembly offers a route to design J-aggregate tubes from a potentially wide range of charged dyes. Under white light irradiation, the light energy harvested by the J-aggregate tubes can efficiently transfer to the dopamine (DA), which absorbs on their surfaces as an electron acceptor. We demonstrate that the absorption quenching of J-aggregate nanotubes due to the efficient energy transfer can be used as a novel sensing platform for the highly sensitive and selective detection of DA in PBS solution in the presence of AA, UA and metal ions. The detection limit of the J-aggregate tube based sensing platform is at least one or two orders of magnitude lower than that of these instrumentation-based detection techniques such as electrochemical methods, capillary electrophoresis and high-performance liquid chromatography–mass spectrometry and also superior to that of recently developed colorimetric sensors based on the plasmon absorption of Au and Ag nanoparticles. This novel sensing strategy allows us to simply identify DA by the naked eye with high selectivity.

4.3.5 Methylene Blue Decomposition

About 10% to 15% world production of dye is lost due to the process of dyeing and is released as wastewater\(^\text{126}\). The removal techniques of these organic pollutant have been studied for over half a century, physical methods such as absorption by fiber carbon\(^\text{127}\) and
biodegradation methods\textsuperscript{128} are well studied and commonly used. Degradation of organic dyes via photocatalysis\textsuperscript{129, 130} is an emerging technology with the advantage of recyclability of the photo-catalyst particles. Here, the use of LCA/DiSC\textsubscript{3}(5)/NaOH and LCA/DiSC\textsubscript{3}(5)/NH\textsubscript{4}OH solutions containing reduced silver nanoparticles in the reduction of methylene blue (MB), a cationic nontoxic dye, has been demonstrated. The oxidized (blue color) and reduced (colorless) forms of methylene blue are shown in Figure 55.

![Chemical structures of oxidized and reduced forms of methylene blue.](image)

Even though NaBH\textsubscript{4} is a very strong reducing agent, it cannot reduce methylene blue in aqueous solution by itself\textsuperscript{131}. As showed in Figure 56a and Figure 56c, as time progress, the absorption intensity of MB decreases. The oxidation speed is higher in the case of H-aggregates/Ag solutions. As discussed earlier, the H-aggregates formed vesicles while J-aggregates formed tubes structures. Therefore, after reduction, it is very likely in the H-aggregates solutions small spherical silver nanoparticles were formed suspending in solution,
while silver particles would be sticking on the outside of the tubes in the case of the J-aggregates. The surface area of silver particles hence are larger in the H-aggregates solution, which explains why the oxidation speed of MB is faster.

Figure 56 Absorption spectra of methylene blue solution. Normalized intensity of the absorption peak at 660nm versus the expose time. Solid line is a curve fit at (b) and linear fit at (d).
4.4 Conclusion

In summary, we report the synthesis of J-aggregate nanotubes formed by the co-assembly of lithocholic acid (LCA) and cyanine dye, 3, 3'-dipropylthiadicarbocyanine iodide (DiSC$_3$(5)), in which the DiSC$_3$(5) dye is organized into the multiple concentric bilayers of J-aggregates. The ionic self-assembly offers a route to design J-aggregate tubes from a potentially wide range of charged dyes. X-ray diffraction and optical spectra show that LCA and CD can co-assemble into J- or H-aggregate tubes, depending the condition under which the self-assembly occurs. We demonstrate the feasibility of using the J-aggregate nanotubes in the sensitive and selective detection of mercury (II) ions by the photoinduced electron transfer under sunlight. The presence of mercury (II) ions in aqueous solution could be detected for concentrations as low as 10 pM. Under white light irradiation, the light energy harvested by the J-aggregate tubes can efficiently transfer to the dopamine (DA), which absorbs on their surfaces as an electron acceptor. We demonstrate that the absorption quenching of J-aggregate nanotubes due to the efficient energy transfer can be used as a novel sensing platform for the highly sensitive and selective detection of DA in PBS solution in the presence of AA, UA and metal ions. The detection limit of the J-aggregate tube based sensing platform is at least one or two orders of magnitude lower than that of these instrumentation-based detection techniques such as electrochemical methods, capillary electrophoresis and high-performance liquid chromatography–mass spectrometry and also superior to that of recently developed colorimetric sensors based on the plasmon absorption of Au and Ag nanoparticles. This novel sensing strategy allows us to simply identify DA by the naked eye with high selectivity. We also utilize the LCA/DiSC$_3$(5)/NaOH and
LCA/DiSC$_5$(5)/NH$_4$OH solutions containing reduced silver nanoparticles to reduce methylene blue.
REFERENCES


