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**PHOTOCONTROLLED
SUPRAMOLECULAR ASSEMBLY OF
STIFF-STILBENE BOLA AMPHIPHILES
IN AQUEOUS MEDIA**

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MPhil

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Photocontrolled Supramolecular Assembly of
Stiff-Stilbene Bola Amphiphiles in Aqueous Media

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A thesis submitted in partial fulfilment of the requirements for
the degree of Master of Philosophy

August, 2023

Certification of Originality

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Abstract

Molecular photoswitches are organic aromatic molecules which can be switched on/off by photoirradiation. Photoswitches are widely employed in different research areas such as chemical biology and smart materials due to their intrinsic photochemical properties. Amphiphiles, consisting of a hydrophobic tail and a hydrophilic head, are able to form various supramolecular structures e.g., vesicles, nanofibers, and nanotubes. Stimuli-responsive synthetic amphiphiles have been drawn attention of chemists for recent decades. Geometrical structural transformations of molecular switches would be a result of manipulation by external stimuli including pH, light, and heat. Light is regarded as a non-invasive stimulus that provides benefits in tunable wavelength and intensity. Photoresponsive molecular amphiphiles are able to assemble into supramolecular nanostructures and have a structural transformation upon photoirradiation at microscopic level.

When comparing to the design of the first generation of molecular motor, a five-membered ring fused stiff-stilbene is featured with a relatively planar structure. Therefore, the design of stiff-stilbene amphiphiles have a higher tendency to assemble nanostructures with high packing parameters. In this thesis, a series of stiff-stilbene amphiphiles (SAs) were synthesized and their photoswitchability in both organic and aqueous media were studied. On the other hand, the assemblies of SAs were examined by transmission electron microscopy and their critical aggregation concentrations were confirmed by dynamic light scattering.

The first chapter is the literature review of molecular photoswitches especially stiff-stilbene and photoswitchable amphiphiles.

The second chapter is about the co-assembly of oppositely charged SAs at different length scale. It is previously reported that motor amphiphiles functionalized with anionic phosphite and cationic quaternary groups were not able to form a macroscopic string. To study the potential of co-assembly of SAs, stiff-stilbene amphiphiles functionalized with anionic phosphite and cationic quaternary groups were synthesized. It is found out that both anionic and cationic SAs could not afford a macroscopic string. However, co-assembled of anionic and cationic SAs was feasible to form a stable macroscopic string in an inorganic salt solution even deionized water. On the other hand, the macroscopic string of co-assembled SAs retained stable upon photoirradiation. This approach demonstrates a new method to a photocontrolled stable soft material.

The third chapter is about multiple responsiveness of amino acid-based SAs. Currently, it remains challenging to control the supramolecular systems which respond to multiple stimuli. To achieve different functionalities, a series of SAs functionalized with different amino acid groups were synthesized. To study the morphological and helical transformation of SAs, different stimuli including pH, light, and solvent were applied. It is found out that the morphology of SAs is partially influenced by different stimuli when only a subtle change of helicity is observed. Indeed, the effect of the size of amino acids in co-assembly of SA achiral and chiral nanotubes is revealed. Moreover, those amino acid-based SAs are capable to form a stable macroscopic string in the presence of an inorganic salt solution. Indeed, they remain stable and show subtle changes upon photoirradiation. In short, it demonstrates self-assembly of amino acid-based SAs in water and their responsiveness to various external stimuli.

The forth chapter is an experimental section about the operation condition of instruments related to this thesis.

The fifth chapter is the conclusion of this thesis.

List of Publications

K. S.-Y. Kwan, W.-K. Wong, M. C. A. Stuart, T. Kajitani, F. K.-C. Leung*. Multifunctional Stiff-Stilbene Amphiphiles Functionalized with Amino Acids. In preparation.

K. S.-Y. Kwan, Y.-Y. Lui, T. Kajitani, F. K.-C. Leung*. Aqueous Supramolecular Co-Assembly of Anionic and Cationic Photoresponsive Stiff-Stilbene Amphiphiles. *Macromol. Rapid Commun.* 2022, 43, 2200438

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Abbreviations and Symbols

NMR	Nuclear Magnetic Resonance Spectroscopy
δ	Chemical Shift in NMR (ppm)
J	Coupling Constant in NMR
s	Singlet in NMR; second(s)
d	Doublet in NMR
t	Triplet in NMR
q	Quartet in NMR
m	Multiplet in NMR
ESI-MS	Electrospray Ionization-Mass Spectroscopy
CD	Circular Dichroism
mdeg	Ellipticity
OM	Optical Microscopy
POM	Polarized Optical Microscopy
TEM	Transmission Electron Microscopy
Cryo-TEM	Cryogenic Transmission Electron Microscopy
SEM	Scanning Electron Microscopy
AFM	Atomic Force Microscopy
XRD	X-ray Diffraction
WAXD	Wide-angle X-ray Diffraction
SAXS	Small-angle X-ray Scattering
rt	Room Temperature (293 K)
h	Hour(s)
min	Minute(s)
THF	Tetrahydrofuran
DMF	Dimethylformamide
DCM	Dichloromethane
EA	Ethyl Acetate
MeOH	Methanol

NaOH	Sodium Hydroxide
CaCl ₂	Calcium Chloride
HCl	Hydrochloric Acid
SA	Stiff-stilbene Amphiphile
Gly	Glycine
Ala	Alanine
Val	Valine
Phe	Phenylalanine
R _f	Retention Factor in Column Chromatography
<i>E</i> -	<i>trans</i> -
<i>Z</i> -	<i>cis</i> -

Chapter 1: Introduction

1.1 Introduction to Molecular Photoswitches

Molecular photoswitches are organic molecules containing aromatic systems that can interconvert between two or more states by light. The photoisomerization is usually accompanied with structural changes including *E-Z* isomerization about the double bond and photochemical cyclization reactions. The structural changes of photoswitch can be observed in the difference of UV-vis spectra and NMR spectral shift. Nowadays, there are plentiful photoswitch molecules including: azobenzene, spiropyran, diarylethene, stilbene, hydrazone, thioindigo, donor-acceptor Stenhouse adducts, stilbene, etc.

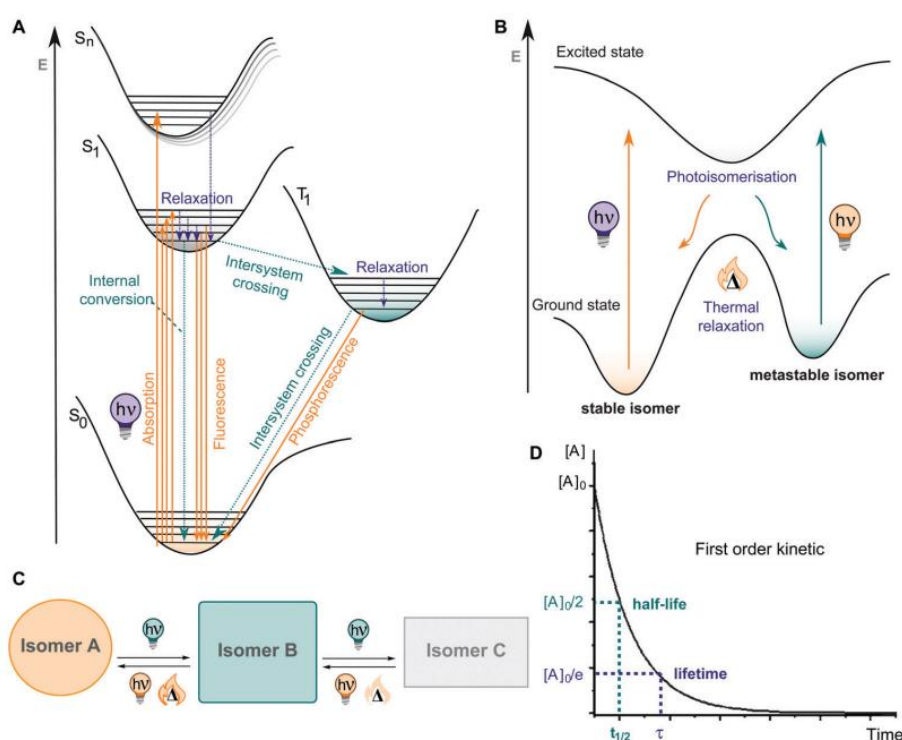


Figure 1.1. (a) The Jablonski diagram regarding the state of a photoswitch in photochemical processes as described in (c); (b) energy profile of a photoswitch during isomerization; (c) isomerization process of a photoswitch upon light irradiation. (d) a graph of concentration against time which depicting the first order kinetics of photoswitching with highlighted half-life and lifetime values. Adapted with permission from Ref.[1]. Copyright 2021 Royal Society of Chemistry.

The photoisomerization process is triggered by light which leads to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ electron transitions. When the chromophore is at excited state, different relaxation pathways

may occur as shown in Figure 1.1a. According to Figure 1.1b and 1.1c, a thermal stable photoswitch is excited to the first even second singlet excited state followed by relaxation. When a conical intersection resides, a photoswitch will reach the ground state of another isomer. Since the newly formed isomer has a greater energy level than the original one, the metastable isomer will isomerize back to the stable state following the first order kinetics with a rate constant k . After plotting the graph of concentration against time (Figure 1.1d), the half-life ($t_{1/2}$) of isomer is determined at $[A]_0/2$ while the lifetime is defined as $1/k$. When the energy barrier between the stable and metastable state is too low, thermal back reactions may occur at ambient condition. In order words, it is a T-type photoswitch. If the energy barrier between two photoisomers is too high, it is regarded as a P-type photoswitch. Since the photoisomers are bi-stable, the isomerization process is triggered by photochemical reactions.

1.1.1 Stiff-stilbene Molecular Photoswitch

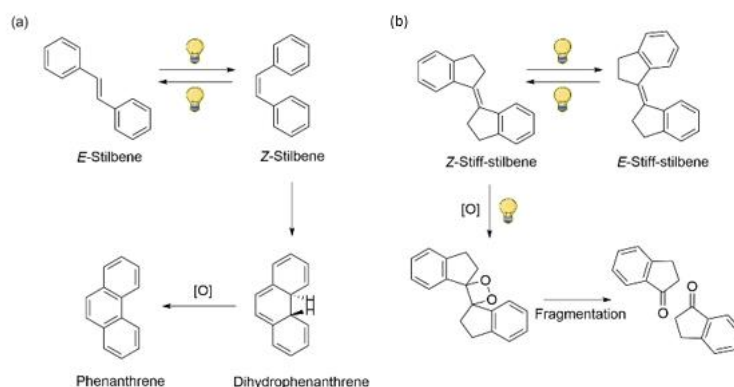


Figure 1.2. (a) Isomerization and oxidation process of stilbene and (b) Isomerization and oxidation process of stiff-stilbene.

Due to the gaining interest of molecular photoswitches, the modifications and applications of molecular photoswitches have become an attractive topic in chemical sciences. Stilbenes are molecular photoswitch with molecular formula $\text{Ar}-\text{CH}=\text{CH}-\text{Ar}$ (Figure 1.2a). The photoisomerization of stilbenes has been well explored for condensed-phase reaction dynamics. *E*-stilbene could photoisomerize to *Z*-stilbene upon UV light. Except for *Z*- to *E*-stilbene isomerization, 6π -electrocyclization may occur in *Z*-stilbene which results in the formation of dihydrophenanthrene. More importantly, dihydrophenanthrene is an easily oxidized compound that results in the formation of phenanthrene. Due to the side reactions of *Z*-stilbene, the applications of stilbene are limited.

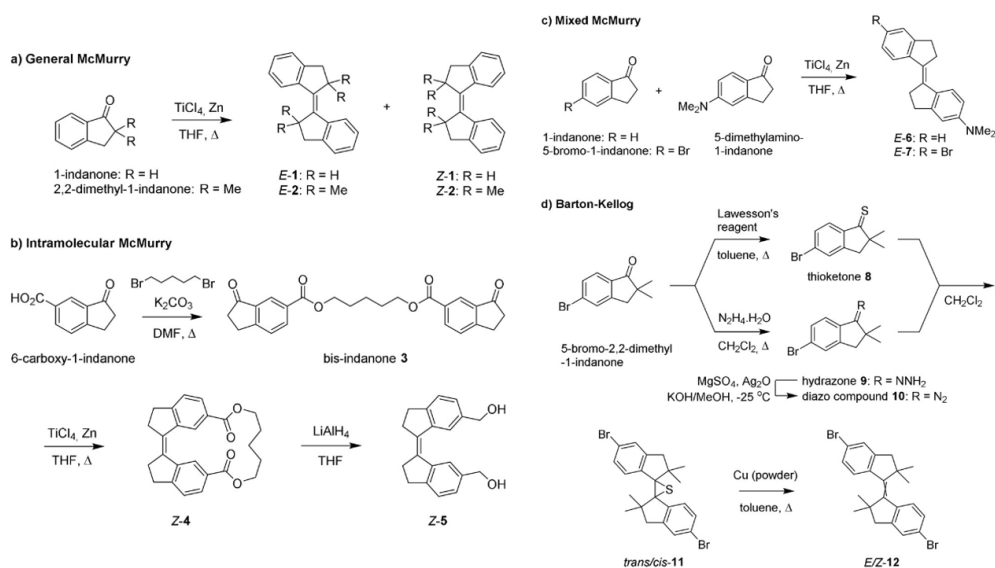


Figure 1.3. Synthetic pathways of stiff-stilbene. Adapted with permission from Ref.[2]. Copyright 2020 John Wiley & Sons.

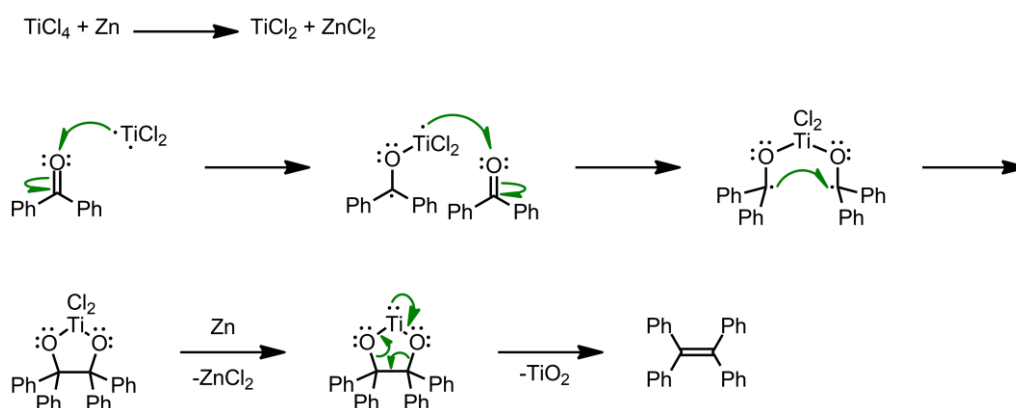


Figure 1.4. Mechanism of McMurry coupling reactions. Adapted with permission from Ref.[3]. Copyright 1974 American Chemical Society.

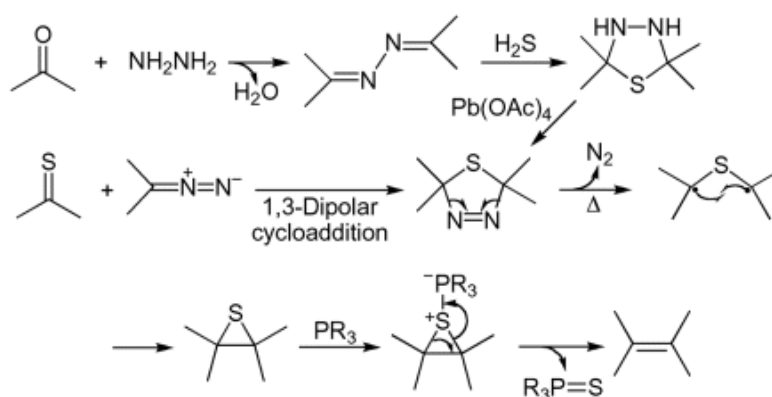


Figure 1.5. Mechanism of Barton-Kellogg coupling reactions. Adapted with permission from Ref.[4]. Copyright 2010 John Wiley & Sons.

Stiff-stilbene consisting of a 5-membered fused ring is more stable upon UV irradiation (Figure 1.2b). Oxidation of double bond may occur as a side reaction, so it is crucial to carry out the photoisomerization process in an air-free condition. The relative experimental design and other exceptional cases will be discussed. According to Figure 1.3, There are different approaches to synthesize stiff-stilbene and they are relatively straight forward and simple. McMurry coupling reactions including general McMurry, intramolecular McMurry, and mixed McMurry are commonly used to obtain stiff-stilbene. McMurry reactions are basically the coupling reactions of two ketone or aldehyde groups to form an alkene in the presence of TiCl_4 and Zn .^[5,6] Zn is acting as a reducing agent that reduces Ti^{4+} to Ti^{2+} while oxidizes itself to Zn^{2+} . Subsequently, low-valent Ti complexes react with either ketone or aldehyde groups to afford an alkene product (Figure 1.4).^[3] Barton-Kellogg reaction is another type of coupling reaction. Unlike McMurry coupling reactions, Barton-Kellogg is a coupling reaction between a diazo compound and a thioketone to give an alkene while an episulfide is formed as an intermediate (Figure 1.5).^[7,8] Initially, a diazo compound reacts with a thioketone to obtain a 5-membered thiadiazoline ring through dipolar cycloaddition. Due to the instability of thiadiazoline, a thiocarbonyl ylide is formed by the extrusion of a nitrogen-containing molecule. Afterwards, the cyclization of a thiocarbonyl ylide gives an episulfide intermediate.^[4] PR_3 , a nucleophile, opens the three-membered ring which results in the formation of a sulfaphosphatane. Finally, an alkene is obtained by removing triphenylphosphine sulphide. McMurry coupling reactions are a promising and straight-forward method to produce symmetric products while Barton-Kellogg reactions would result in asymmetric products.

Because of steric restrictions of the fused ring analogue, the photoisomerization of stiff-stilbene is simpler than stilbene.^[9] Although the structural motif of stiff-stilbene is similar to Feringa-type molecular motor, the photoisomerization of stiff-stilbene significantly differs to molecular motors.^[10–12] In contrast to molecular motor, stiff-stilbene shows high quantum yield in

photoisomerization.^[2] In spite of plentiful photoswitches based on double-bond isomerization, Z-stiff-stilbene has exceptionally high energy level that means Z-isomer is thermally stable. Being with the intrinsic photochemical properties, stiff-stilbene has been widely applied in different aspects including host-guest systems,^[13,14] catalysis,^[15,16] and self-assembly process^[17]. However, most of the photochemical studies were performed in organic solvents such as DMSO, DCM, and MeOH because of lacking of a water-solubilizing group. Although the photoisomerization was successfully reversed up to several cycles, photo-induced oxidation occurred in some cases. As mentioned previously, it is important to perform photoisomerization process in an inert atmosphere to prevent oxidation of stiff-stilbene in organic media. Therefore, degassing solvent and preparing the sample in a glove box are preferable.

There are abundant research showing the wide applications of stiff-stilbene photoswitch in organic media. Boulatov and his co-workers (2009) pioneered the method to study chemomechanical kinetics by using stiff-stilbene as a molecular force probe.^[18] The relationship between the restoring force in stiff-stilbene and the kinetics of localized reactions was studied by atomic force microscopy. The results revealed that the greater storing force would be accessible when isomerization kinetics of stiff-stilbene compound are less sensitive to restoring force. In 2014, the group of Stang demonstrated a new method to study photodynamic materials by using the coordination-driven self-assembly of stiff-stilbene molecules and platinum polymers.^[17] This metallosupramolecular polymer bearing with stiff-stilbene motif would undergo conformational transformation from linear polymer to cyclic oligomer during Z- to E- isomerization. Zimmerman (2015) illustrated the stiff-stilbene based proton gate to study the photoresponsive transmembrane for proton-transfer kinetics.^[19] During the E-/Z- photoisomerization, the transmembrane is more effective in proton delivery through a flipping mechanism. The results showed that the proton gate could be reversibly controlled by the photoisomerization of stiff-stilbene. The applications of stiff-

stilbene in organic media are well-explored, whereas the applications of stiff-stilbene in aqueous media are still in development due to the undesirable photochemical side reactions such as photooxidation and photocyclization.^[20,21] Recently, there are few cases about the stiff-stilbene with solubilizing groups which were characterized in the mixture of DMSO and water, buffer solutions, and even *in vivo*.^[21–23]

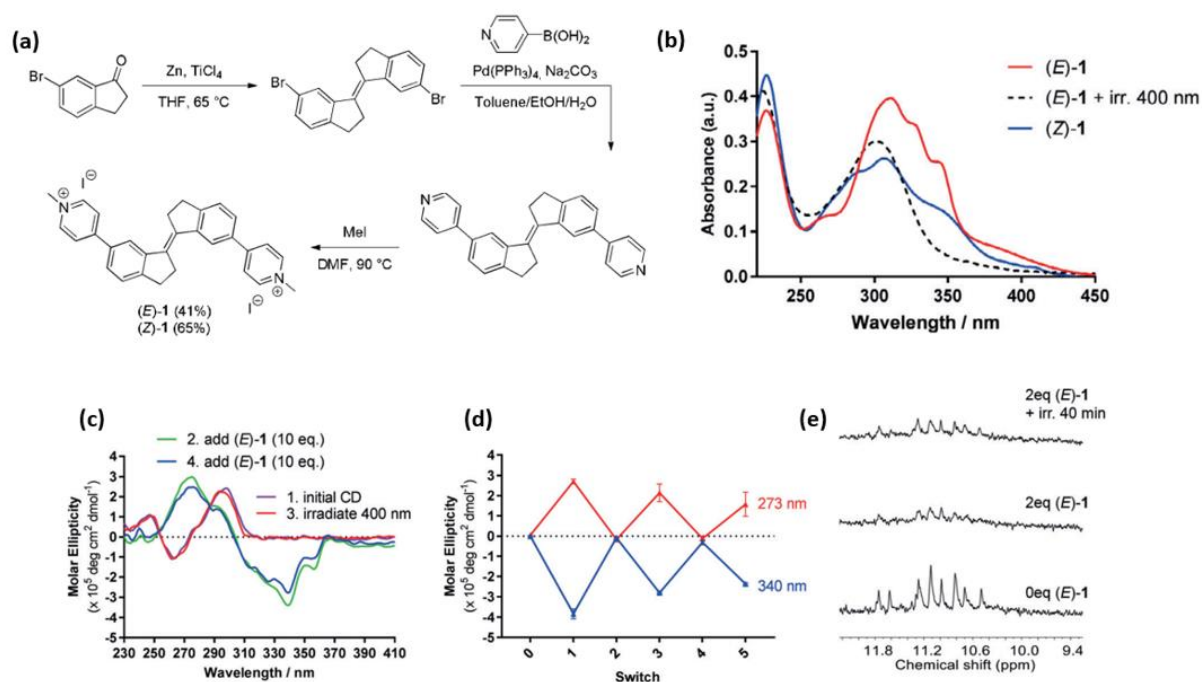


Figure 1.6. (a) Synthesis of **1**; (b) UV-vis spectra of *E*-**1** in buffer solution upon 400 nm light irradiation; (c) CD spectra of telo23 in the presence of *E*-**1** generated by 400 nm irradiation; (d) The change of ellipticity at 273 nm and 340 nm over irradiation cycles; (e) NMR spectra of *E*-**1** during photoirradiation. Adapted with permission from Ref.[21]. Copyright 2019 John Wiley & Sons.

Galan and her co-workers have applied stiff-stilbene motifs for biological studies. In 2019, they designed and synthesized **1** as a ligand for the regulation of G4 DNA-structures (Figure 1.6a).^[21] Interestingly, the change of UV-vis spectra of *E*-**1** upon 400 nm irradiation was significantly different to other stiff-stilbene compounds (Figure 1.6b). According to the results of mass spectrometry, it suggested that photooxidation of stiff-stilbene rather than *E/Z* photoisomerization. The fuelling/irradiation process of *E*-**1** was investigated by CD. There was no change in the CD spectra of a telomeric G4 sequence (telo23) upon 400 nm irradiation (Figure 1.6c, purple line). After introducing *E*-**1** to telo23, there was a spectral blue shift (Figure 1.6c, green line) and it could be restored as telo23 alone after photoirradiation (Figure 1.6c, red line). After the second addition of *E*-**1**, the CD spectrum blue-shifted again (Figure 1.6c, blue line). The fuelling/irradiation process could be reversed over several cycles (Figure 1.6d). Apart from CD, the fuelling/irradiation process

was confirmed by NMR analysis. The imino signals could be recovered and sharpened during photoirradiation that implies the regeneration of G4 via refolding of DNA (Figure 1.6e). This work reveals the applications of stiff-stilbene ligands for the regulation of G4 DNA-structures upon photoirradiation.

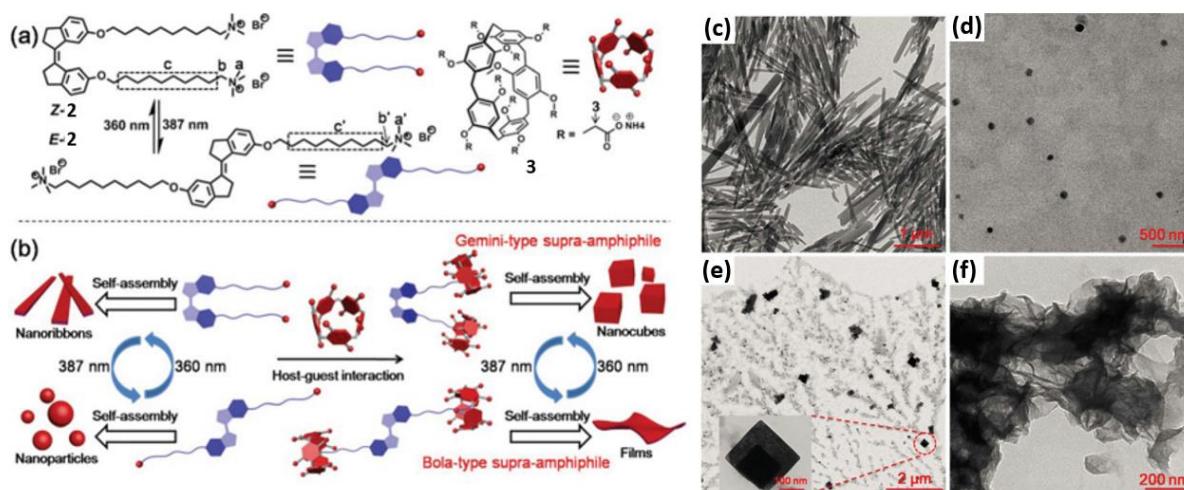


Figure 1.7. (a) Chemical structures of *E*-2, *Z*-2, and 3. (b) Schematic diagram of the host-guest complexations and morphological transformation upon photoirradiation. TEM images of (c) *Z*-2 in water; (d) *Z*-2 after 387 nm photoirradiation for 10 h; (e) 3:*Z*-2=2:1 in water; (f) 3 and *Z*-2 in water after 385 nm photoirradiation for 10 h. Adapted with permission from Ref.[23]. Copyright 2017 Royal Society of Chemistry.

Shi (2017) reported that the host-guest complexations of stiff-stilbene amphiphiles and pillar[5]arene which can be controlled through photochemical reactions in the mixture of DMSO and water even water alone (Figure 1.7a).^[23] Due to the good solubility of 2 and 3 in water, the host-guest reactions and photoisomerization studies between 2 and 3 were investigated by water. The morphology transformation of stiff-stilbene amphiphiles *E*-2 and host-guest amphiphiles of *E*-2 and 3 upon *E*-/*Z*- photoisomerization was briefly introduced in Figure 1.7b. The TEM image shows that *Z*-2 formed nanoribbons and transformed to nanoparticles after 387 nm photoirradiation (Figure 1.7c and 1.7d). It indicates that the morphology of bola-amphiphilic *E*-2 is nanoparticles with average diameter of ~ 90 nm. After adding 2 mole equivalents of 3 to *Z*-2, the formation of nanocubes were observed (Figure 1.7e). Upon 387 nm photoirradiation for 10 h, the mixture of 3 and *Z*-2 was transformed to the mixture of 3 and *E*-2 and those nanotubes turned to films because of the formation of bola *E*-2 and 3 (Figure 1.7f). This works opens the strategies to apply stiff-stilbene amphiphiles to host-guest system in aqueous media.

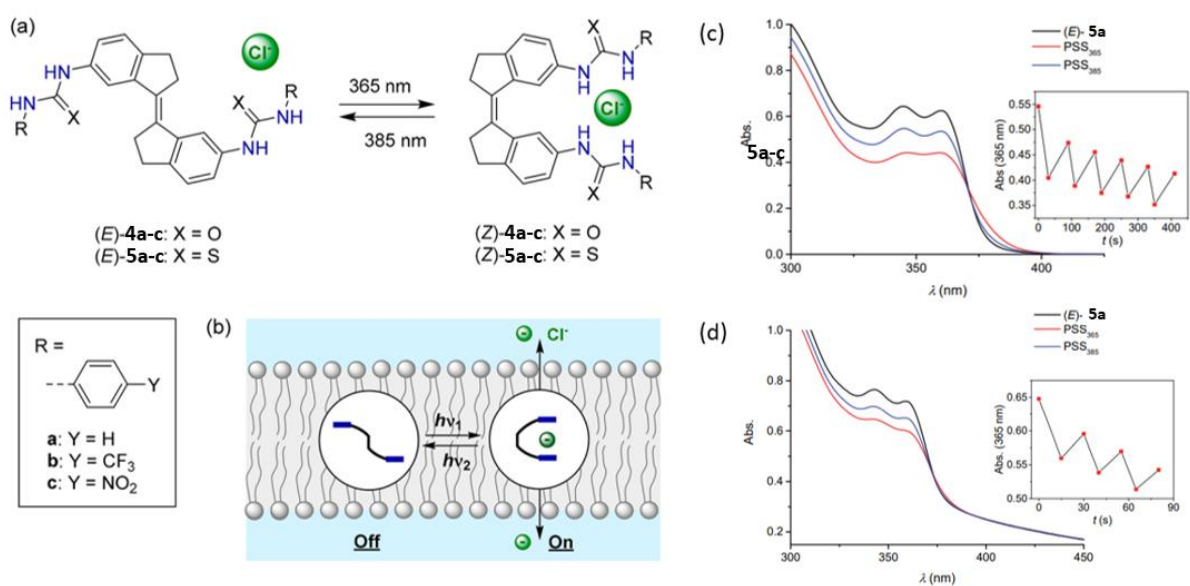


Figure 1.8.1. (a) Photoisomerization process of stiff-stilbene based bis(thio)ureas; (b) Schematic diagram of photocontrolled transmembrane transport; (c) The cyclability of **5a** upon 365/385 nm photoirradiation in degassed DMSO; (d) The cyclability of **5a** upon 365/385 nm photoirradiation in degassed KCl buffer. Adapted with permission from Ref.[24]. Copyright 2022 American Chemical Society.

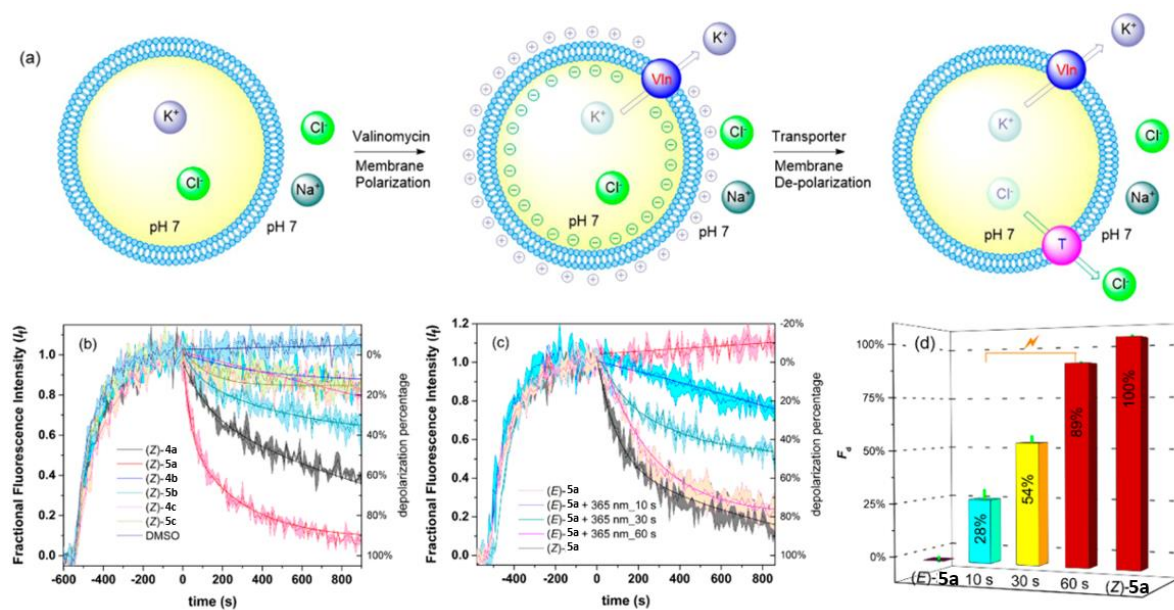


Figure 1.8.2. (a) Schematic diagram of the Safranin O fluorescence assay; The change in emission intensity of safranin O was monitored over time upon (b) addition of *Z*-4a-c and *Z*-5a-c and (c) 365 nm photoirradiation of *E*-5a; (d) Depolarization efficiencies (%) of photoirradiated *E*-5a related to *Z*-5a. Adapted with permission from Ref.[24]. Copyright 2022 American Chemical Society.

Last year, Wezenberg and his co-workers demonstrated the photocontrolled transmembrane transport by stiff-stilbene based bis(thio)ureas.^[24] Two types of stiff-stilbene based bis(thio)ureas **4** and **5** were synthesized and studied (Figure 1.8.1a). It is found out that the Z-isomer has higher reactivity in regulating transmembrane transport (Figure 1.8.1b). The photoisomerization of *E*-**5a** was examined in DMSO by UV-vis spectroscopy. According to Figure 1.8.1c, the photoisomerization of *E*-**5a** was successfully repeated for several cycles without significant fatigue. Before applying *E*-**5a** to biological studies, UV-vis photoisomerization of *E*-**5a** was performed in buffer solution and it was feasible to switch over several cycles (Figure 1.8.1d). Because of the scattering effect caused by liposomes, moderate fatigue effects may be observed. Safranin O fluorescence measurement is used to study the transportation within the biological membrane as shown in Figure 1.8.2a. Based on the fluorescence assay (Figure 1.8.2b), there was about 98% decrease of fluorescence intensity of Z-**5a** which means it exhibited the greatest depolarization. All Z-isomers illustrated depolarization activity in liposomes which resulted in Cl⁻ concentration gradient (KCl inside, Na₂SO₄ outside) to membrane. The selectivity of Cl⁻ ions of Z-isomers would dramatically decrease without a concentration gradient. Since Z-**5a** demonstrated the greatest depolarization degree, it was selected as a transporter. 365 nm photoirradiation of *E*-**5a** was monitored to study the depolarization activities. Initially, *E*-**5a** was inactive but the depolarization efficiency decreased to about 50% even 90% after 365 nm photoirradiation for 30 s and 60 s, respectively. The results indicated that the transportation of Cl⁻ ions through membrane is feasible to be controlled through *E*-/*Z*- photoisomerization. It provides a new way to control ion channel reversibly in the cellular membrane.

In order to exert the full potential of stiff-stilbene in aqueous media, the photochemical studies and certain applications of water-soluble stiff-stilbene amphiphiles will be envisioned in this thesis.

1.2 Introduction to Photoresponsive Amphiphiles

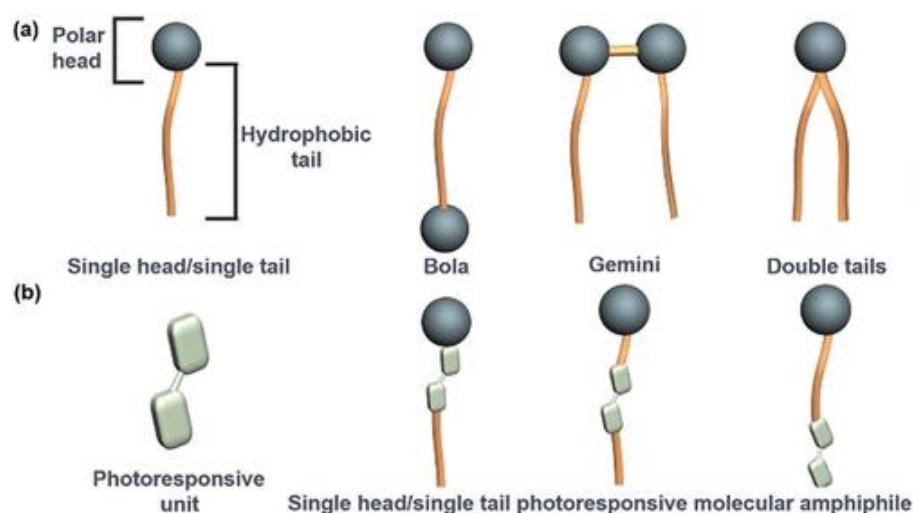


Figure 1.9. Schematic illustration of (a) different categories of molecular amphiphiles and (b) photoresponsive molecular amphiphiles. Adapted with permission from Ref.[25]. Copyright 2020 John Wiley & Sons.

Amphiphiles consisting of both hydrophobic and hydrophilic properties play an important role in nature^[25–27]; a class of amphiphiles called phospholipids is the main component of cell membrane. Besides, cholesterol, fatty acids and glycolipids are common biological amphiphiles. Amphiphiles are usually either natural or synthetic molecules, and they can form plentiful structures like micelles, nanotubes and nanofibers through self-assembly.^[28] In order to mimic living organisms, water is a crucial solvent for amphiphiles self-assembly and other biological processes. The hydrophilic head prefers interacting with the aqueous (polar) phase, and the hydrophobic tail would either expose to air or interact with the non-polar phase.^[25] Consequently, the aggregation of amphiphiles leads to a variety of molecular assemblies between the hydrophilic and hydrophobic

components through non-covalent interactions.^[25–27] Amphiphiles is not only a compulsory component for mimicking lipid layer, but also significant in making soap and detergent.^[27]

As mentioned above, amphiphiles normally consist of a hydrophilic head and a hydrophobic tail. To make the head groups dissolvable in polar solvent even aqueous media, ionic, polar or hydrogen bond groups should be contained.^[29] The hydrophobic tails, which dissolve in non-polar solvent, are always either saturated or unsaturated hydrocarbon chains.^[30] There is various connection between the hydrophilic moiety and hydrophobic moiety, which result in different structures of amphiphiles. Bolaamphiphiles, gemini amphiphiles, and double-tailed amphiphiles are the most common classes among all categories (Figure 1.9a).^[25] The morphology of self-assembled amphiphiles may change by external stimuli including light, pH and heat.^[25] Indeed, amphiphiles containing photoresponsive unit are commonly called photoresponsive amphiphile. The photoresponsive unit can be functionalized in either the head group or in the hydrophobic chain as shown in Figure 1.9b. There is a plenty of photoresponsive groups such as azobenzene, indigo, and stiff-stilbene and they would undergo photoisomerization upon light irradiation. UV, visible light, and near-infrared (NIR) light are the main light sources for photoinduced reactions.

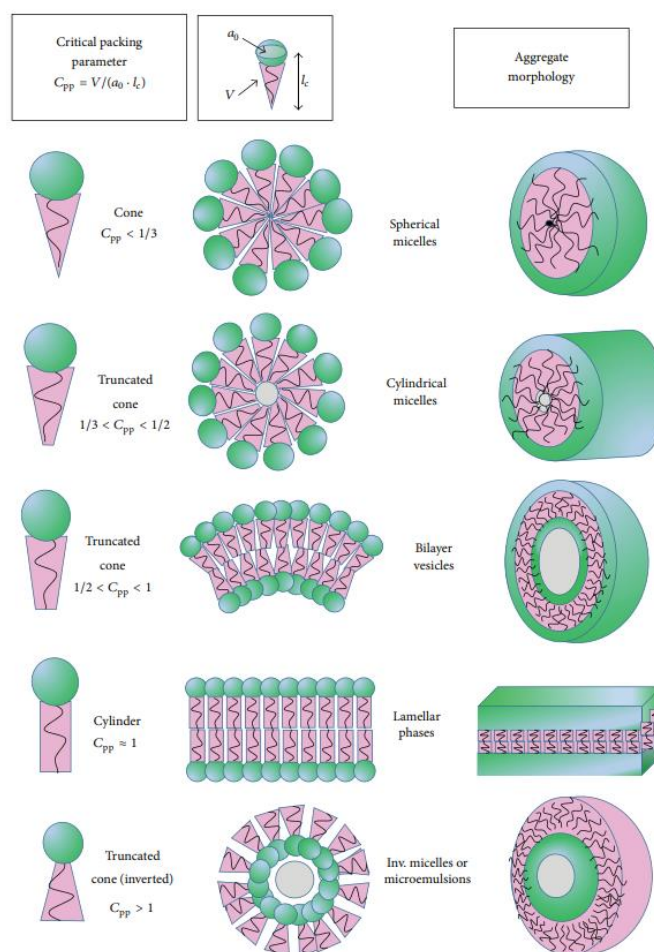


Figure 1.10. Schematic illustration of the aggregation of amphiphilic structure and packing parameter. Adapted with permission from Ref.[28]. Copyright 2015 Hindawi Publishing Corporation.

In order to achieve biomimetic system, self-assembly of amphiphiles gives a wide range of structure such as vesicles, nanotubes, nanofibers, lamellae and micelles.^[28] The morphology of assembled amphiphiles relies heavily on the molecular geometry of amphiphile. The assembled morphology could be predicted by critical packing parameters (C_{pp}) and the certain equation is $C_{pp} = V/a_0l_c$; where V_0 is the volume of hydrophobic area in the aggregation core, l_c is the critical chain length, and a_0 is the surface area of hydrophilic headgroup. According to Figure 1.10, it clearly shows that the cone-shaped amphiphiles with $C_{pp} < 1/3$ would aggregate to spherical micelles. The truncated cone amphiphiles with $1/3 < C_{pp} < 1/2$ could form cylindrical micelles while increasing C_{pp}

to $1/2 < C_{pp} < 1$ results in bilayer vesicles. Cylinder with C_{pp} about 1 would lead to the formation of lamellar phases. Inverted micelles are caused by the formation of inverted truncated cone amphiphiles with $C_{pp} > 1$.

Although stiff-stilbene has similar structural motif to molecular motor, the properties and applications of stiff-stilbene amphiphiles are not fully explored. On the contrary, the studies of photoresponsive amphiphiles particularly molecular motor amphiphiles have been increased in recent decades and they are mainly focusing on self-assembled soft materials towards biological applications.

1.2.1 Existing Examples of Photoresponsive Amphiphiles

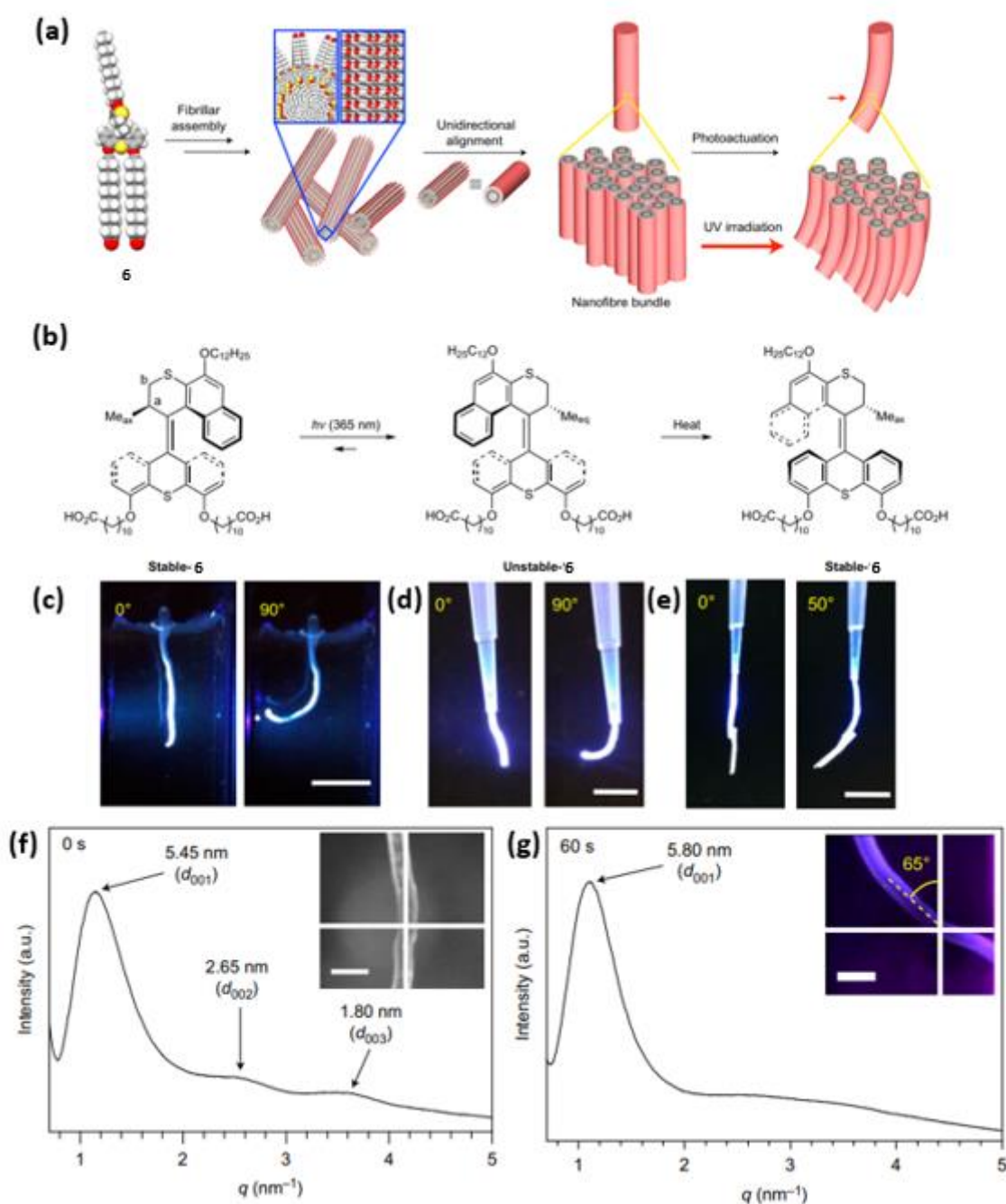


Figure 1.11. (a) Schematic diagram of the self-assembly process of molecular motor amphiphile **6** to nanofibers and its actuation process upon UV irradiation; (b) structural transformation of molecular motor amphiphile **6** during photoisomerization; a photo of macroscopic string of **6** bent towards the light source during photoirradiation (c) in aqueous media; (d) in air; (e) in air for weightlifting. *In-situ* SAXS of motor amphiphile **6** (f) before and (g) after UV irradiation for 60 s, respectively. Adapted with permission from Ref.[31]. Copyright 2018 Springer Nature.

In 2018, Feringa illustrated the use of molecular motor amphiphile **6** as a photoactuator that composed of nanofibers (Figure 1.11a).^[31] The structural transformation of motor amphiphile **6** during photoirradiation was shown in Figure 1.11b. Motor amphiphile **6** assembled into nanofibers with about 5 – 6 nm in diameter and several micrometers in length in aqueous media at microscopic level while a macroscopic string was formed by a shear-flow method in calcium chloride solution. The macroscopic string of motor amphiphile **6** showed unidirectional alignment which is confirmed by different microscopies and X-ray. As shown in Figure 1.11c, the string of motor amphiphile **6** bent towards to light source in aqueous media during photoirradiation. To study its actuation properties in air, the photoactuation experiment of motor amphiphile **6** was performed in air and similar observation was noted (Figure 1.11d). Indeed, the motor amphiphile **6** was able to lift a piece of paper and remained stable upon photoirradiation (Figure 1.11e). Motor amphiphile **6** showed unidirectional alignment as confirmed by *in-situ* SAXS experiment. After photoirradiation, the diffraction plane of d_{001} increased from 5.45 nm (Figure 1.11f) to 5.80 nm (Figure 1.11g). On the other hand, those high-ordered diffraction peaks were disappeared. It indicated that the packing arrangement of motor amphiphile **6** was disrupted because of the volume loss of motor amphiphile **6** during photoisomerization. It opens up a way of applying a macroscopic string of a motor amphiphile for mechanical motion and soft robotics.

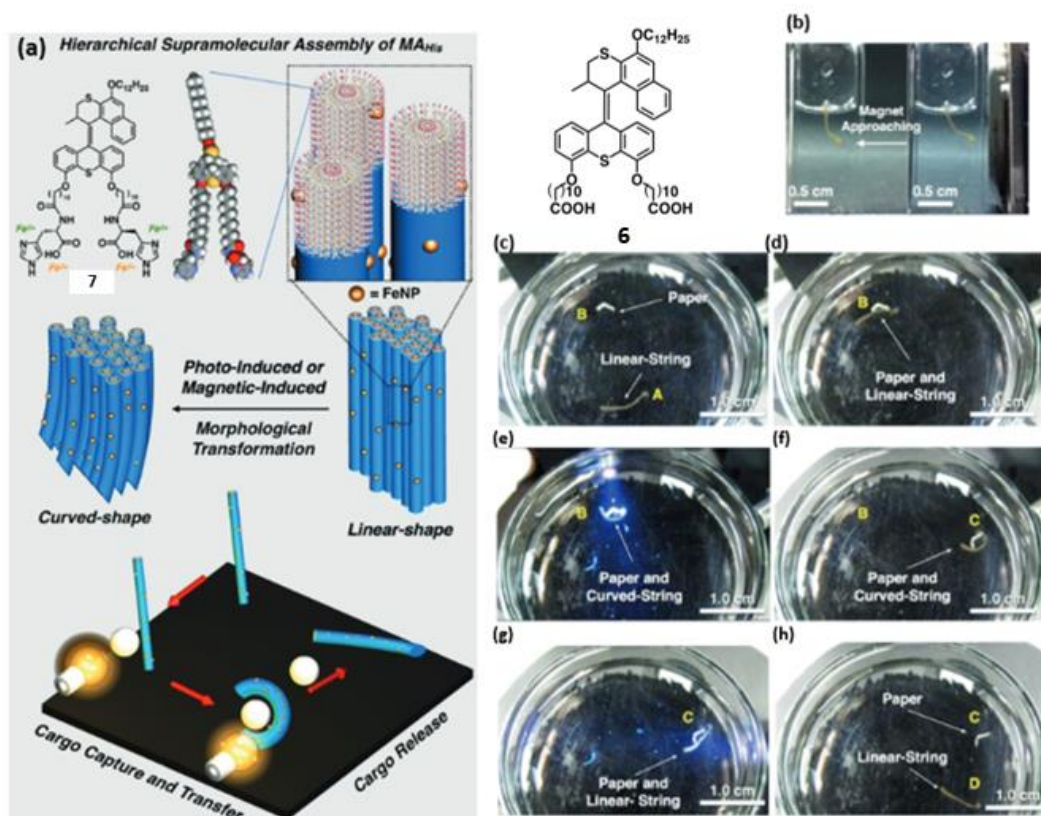


Figure 1.12. (a) Schematic illustration of the applications of **7** to cargo capture through magnetic interactions; (b) a photo of **7**/**FeNP**:**6** string bent towards a magnetic field in CaCl_2 solution; (c) a photo of a string of **7**/**FeNP** at position A and paper at position B and (d) a string moving towards paper; (e) a photo of a curved string after photoirradiation and (f) the captured paper was led by curved-string through magnetic forces at position C. A photo of (g) the string changed to linear after photoirradiation and (h) release the paper at position D. Adapted with permission from Ref.[32]. Copyright 2019 John Wiley & Sons.

Leung and Feringa (2019) demonstrated the a dual-controlled macroscopic actuation of a motor amphiphile for cargo release as shown in Figure 1.12a.^[32] **7** was acted as a template for the growth of icon nanoparticles (NPs). The nanofibers of **7** are formed with about 7 — 8 nm in diameter and several micrometers in length. After mixing **7** with $\text{Fe}^{2+}/\text{Fe}^{3+}$ in basic condition, FeNPs were directly attached on nanofibers of **7**. To improve the stability of a macroscopic string of **7**/**FeNP**, **6** was mixed with **7**/**FeNP** which were then studied by POM, SEM, and SAXS measurement. The string bent towards the light source (90°) after irradiation (Figure 1.12b). Initially, the linear **7**/**FeNP**:**6** string located at the A position, the paper located at position B (Figure 1.12c). Then, the **7**/**FeNP**:**6** string

moved to position B guided by a magnet (Figure 1.12d). Upon photoirradiation, the **7**/_{FeNP}:**6** string started to bend and capture the paper (Figure 1.12e) followed by moving the paper and curved string to position C (Figure 1.12f). Upon the photoirradiation to the backside of the string, the curved **7**/_{FeNP}:**6** string became linear (Figure 1.12g) to release paper. Finally, the linear **7**/_{FeNP}:**6** string moved to position D (Figure 1.12h). This work reveals that the macroscopic string of molecular motor amphiphile could function as a photoactuator for cargo transport process. Hopefully, it could be applied to the tissue engineering in the future.

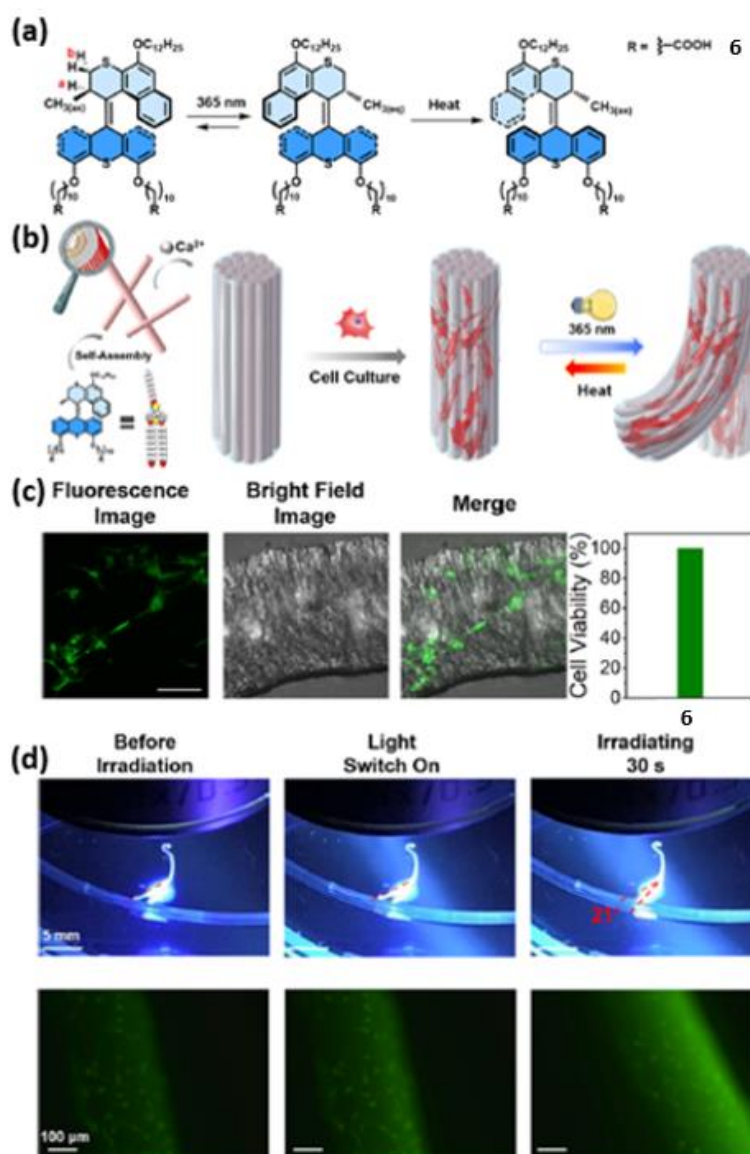


Figure 1.13. (a) Structural transformation of motor amphiphile **6** upon photoirradiation; (b) schematic diagram of the biological application of **6** photoactuator; (c) confocal microscopic images of a macroscopic string of **6** after cell culture and cell viability (right); (d) photos (top) and fluorescence microscopic images (bottom) of a macroscopic string of **6** with cells during photoactuation. Adapted with permission from Ref.^[33]. Copyright 2022 American Chemical Society.

After investigating the photoactuation properties by using different molecular motor amphiphile, the biological applications of molecular motor amphiphiles were investigated by Chen et al. in 2022.^[33] The structural changes of second-generation molecular motor amphiphile was stated in Figure 1.13a. A macroscopic string of **6** was found with good biocompatibility and the

string with cells could undergo actuation during photoirradiation (Figure 1.13b) while molecular motor amphiphiles with phosphite group and sulfate group could not be stabilized. After cell culture and staining, live cells were successfully attached on the surface of the macroscopic string of **6** as shown in Figure 1.13c. Afterwards, the photoactuation properties of **6** with cells were estimated. The photoactuation process of **6** with cells was monitored by fluorescence microscopy. According to Figure 1.13d, the cell-adhered **6** macroscopic string bent 21° towards the light source. It suggested that the **6** macroscopic string did not only have good biocompatibility but also underwent the photoactuation with cells.

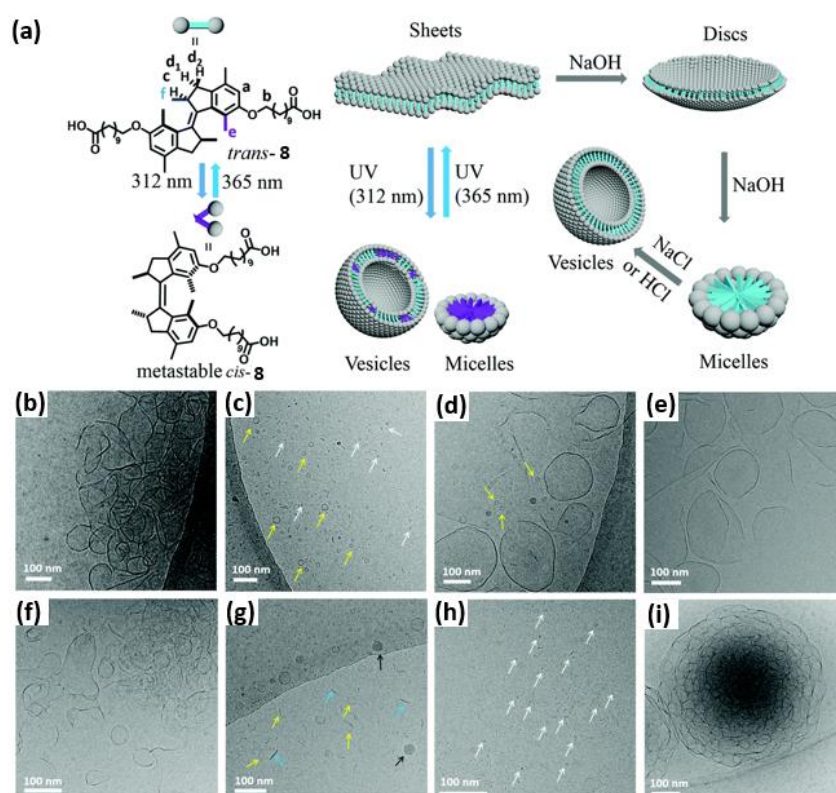


Figure 1.14. (a) Schematic diagram of the morphological transformation of motor amphiphile **8** upon photoirradiation and other external stimuli. Cryo-TEM images of (b) *trans*-**8**, (c) after 312 nm photoirradiation of *trans*-**8**, (d) after subsequent 365 nm photoirradiation, and (e) a heating-cooling cycle. Cryo-TEM images of *trans*-**8** at (f) pH ~ 9, (g) pH ~ 10, (h) pH = 11, and (i) after adjusting pH ~ 9 from pH ~ 11 by using HCl. Adapted with permission from Ref.^[34]. Copyright 2017 Royal Society of Chemistry.

Apart from using light as an external stimulus, Feringa (2020) demonstrated the molecular motor amphiphile **8** which would undergo morphological transformation during photoirradiation and pH variation (Figure 1.14a). As shown in Figure 1.14b, the structure of *trans*-**8** was nanosheet with $P \sim 1$. After 312 nm photoirradiation, *trans*-**8** was transformed to metastable *cis*-**8** which was revealed by the mixture of vesicles and micelles (Figure 1.14c). *trans*-**8** was recovered as the reformation of nanosheet after subsequent photoirradiation of 365 nm (Figure 1.14d). Moreover, the sheet-like structure of *trans*-**8** was able to recovered through a heating-cooling cycle. On the other hand, the carboxylic group of *trans*-**8** was pH-sensitive that led to the morphological transformation at different pH environment. According to the Cryo-TEM images (Figure 1.14f–g),

trans-**8** formed sheet-like structure at pH ~ 9 ($P \sim 1$), converted to disc-like structure at pH ~ 10 ($1/2 < P < 1$), finally micelles ($P < 1/3$) at pH = 11. Notably, the packing parameter of *trans*-**8** decreases as increasing pH values which could be explained by the deprotonation of *trans*-**8** at high pH environment.

Based on the existing examples, they demonstrated that motor amphiphiles functionalized with counter ions were barely able to form macroscopic strings. In an addition, molecular motor amphiphiles functionalized with carboxylate acid groups as a pH sensitive group could facilitate the multi-responsivity. When comparing with molecular motor, stiff-stilbene has a relatively planar structure. Therefore, stiff-stilbene amphiphiles have a greater potential to form a structure with greater packing parameter which may be beneficial for the macroscopic studies. In this thesis, the co-assembly of stiff-stilbene amphiphiles functionalized with positive charged and negative charged counter ions will be firstly envisioned. Additionally, the multiresponsivity of stiff-stilbene amphiphiles functionalized with amino acids across different length scales will be studied.

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Chapter 2: Co-Assembly of Oppositely Charged SAs

2.1 Introduction

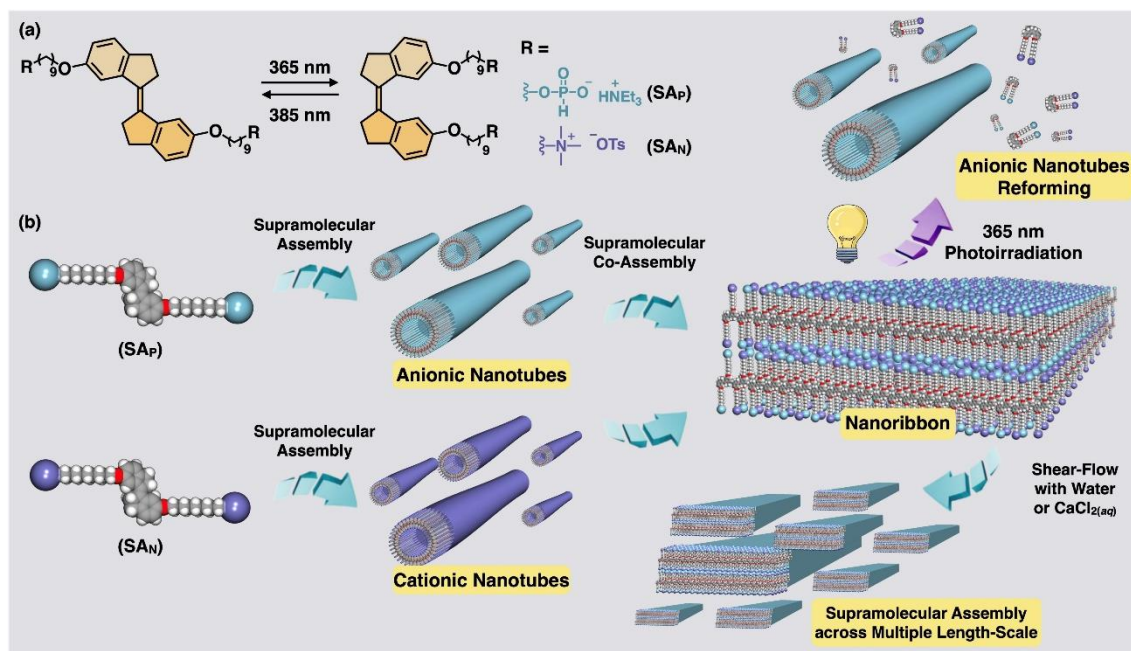
From the perspective of biological systems, supramolecular assembly and co-assembly play a crucial role in living systems, for example, lipids in cell membranes and cytoskeletons filaments in cell division.^[1,2] Biomimetic materials are naturally used to preprogram the supramolecular assembling processes in certain environment.^[3,4] In contrast, artificial supramolecular materials are able to capture non-biocompatible sources of stimulation, including ions, pH, and light.^[5–10] The functional applicability, tunability,^[11] and stimuli-responsiveness are more feasible to be enriched by synthetic supramolecular assemblies than natural organic molecules.^[5,12–16] Light is relatively a non-invasive stimulus which is featured with high-temporal and high-spatial precision.^[5,6,17] As a consequence, supramolecular transformation in both organic and aqueous conditions is easily controlled by light. Photoresponsive amphiphiles are usually functionalized with a photoresponsive unit including azobenzenes,^[18,19] spiropyrans,^[20,21] dithieylethenes,^[22] molecular motors,^[5,23,24] and stiff-stilbenes^[25] into molecular amphiphiles. A plenty of photoresponsive molecular amphiphiles in aqueous media has been constructed by photoresponsive molecular amphiphiles. It results in controlling assembly properties, reversible assembly, and artificial muscle.^[5] Notably, photoresponsive molecular amphiphiles are able to form nanostructure and undergo structural transformation during photoirradiation.^[5] Indeed, a part of photoresponsive molecular amphiphiles can assemble at different length-scale which results in the formation of photoresponsive soft materials or supramolecular actuators.^[26–28] To fabricate photoresponsive soft materials at macroscopic scale in inorganic salt solutions, a shear-flow method is commonly used.^[29] Supramolecular actuators, an example of macroscopic photoresponsive soft materials, are prepared from the solutions of motor amphiphiles in the presence of inorganic salt. Its structure is remained intact upon photoactuation.^[28] However, several charged end-groups of photoresponsive

molecular amphiphiles like phosphite, sulphate, and quaternary ammonium ions showed partially stable macroscopic structures.^[5,30,31]

To achieve supramolecular assembled structures with structural complexity and functional diversity, supramolecular co-assembly of two or more molecular amphiphiles is a common method.^[32–34] Stupp and his co-workers reported that co-assemblies of negatively-charged and positively-charged peptide amphiphiles resulting in supramolecular nanofibers.^[35–38] Tekinay and Guler stated similar co-assembly systems of peptide amphiphiles which can fabricate into macroscopic soft materials by varying the molar ratio of peptide amphiphiles and pH values.^[39,40] These examples have demonstrated that co-assembled peptide amphiphiles have great stability and excellent biocompatibility for biomedical studies.^[39,40] Although supramolecular co-assembly systems have been widely studied, the co-assembly of photoresponsive molecular amphiphiles in aqueous media remains questionable. Feringa stated that co-assemblies of non-charged amphiphiles with synthetic lipids led to the formation of nanotubes for attaining supramolecular transformation.^[41,42] In 2019, Leung and Feringa reported that the co-assembled of different anionic motor amphiphiles resulted in the formation of nanofibers. Consequently, those nanofibers would be utilized for iron nanoparticles growth.^[26] Based on the existing evidence, we are interested in the co-assembly of oppositely charged photoresponsive molecular amphiphiles and their stability towards macroscopic materials.

Leung and Feringa indicated that the first-generation molecular motor amphiphiles usually self-assembled into worm-like micelles or vesicles.^[30,31] π - π interactions of non-planar molecular motor core could be reduced within the nanostructures. Therefore, the large aspect ratio nanostructures formation is disfavoured. In contrast, stiff-stilbene with five-membered fused ring analogue of stilbene shows distinctive photoisomerization.^[25] The photoisomerization of stiff-

stilbene shows higher quantum yield towards *Z-E* isomerization than molecular motor. Due to their excellent photochemical properties, stiff-stilbene derivatives have been studied in different aspects including supramolecular host-guest systems,^[43,44] dynamic assemblies,^[45,46] to biological applications.^[25,47–52] Leung and Feringa claimed that anionic phosphite and cationic quaternary ammonium groups in motor amphiphiles showed either no significant or weakened macroscopic structures formations.^[30,31] In order to investigate the potential of co-assembled photoresponsive molecular amphiphiles, we design and synthesize both anionic phosphite and cationic quaternary ammonium functionalized with the stiff-stilbene planar motif (Scheme 1). SAs with improved π - π interactions are guaranteed to assemble into nanotubes with large-aspect ratio. Varying the molar ratio of anionic and cationic SAs would result in stable co-assembled structures with greater packing parameter like nanoribbons with $P = 1$. Supramolecular co-assembly transformations occur during photoirradiation. Co-assembled SAs are quite stable without calcium chloride solutions when no nearly no stable macroscopic sole anionic SAs is formed in calcium chloride solution. By elucidating the structural design and co-assemblies of SAs, this paves a new way to develop photocontrolled soft functional materials and smart soft supramolecular robotic systems.

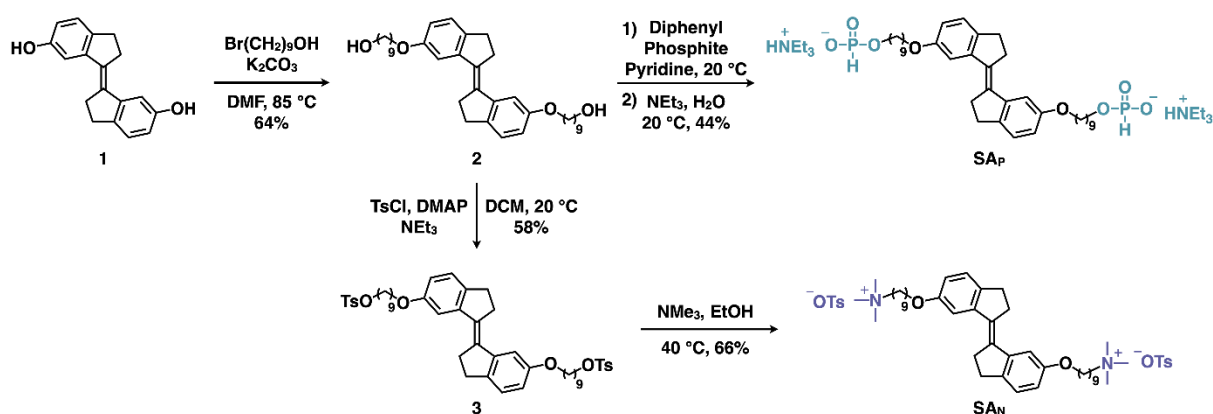


Scheme 1. Schematic illustration of the molecular structure and photoisomerization of SAs.

2.2 Results and Discussion

2.2.1 Design and Synthesis of SAs

The stiff-stilbene bola amphiphile (SA) was designed with a five-membered fused ring stiff-stilbene core and functionalized with different ionic groups on both sides of stiff-stilbene core. The hydrophobic stiff-stilbene core is surrounded with two hydrophilic parts to form a bola-amphiphilic structure. The synthesis is summarized in Scheme 2. Phenolic *trans*-stiff-stilbene **1** was prepared by McMurry coupling of 6-methoxy-1-indanone followed by deprotection. *trans*-Stiff-stilbene **2** was obtained by a Williamson ether synthesis. **SA_P** was obtained through the substitution reactions of *trans*-stiff stilbene **2** and diphenyl phosphite, when **SA_N** was yielded from a sulphate ester formation of *trans*-stiff stilbene **2** followed by hydrolysis. The structures of all compounds were characterized by ¹H, ¹³C nuclear magnetic resonance (NMR) spectroscopy, and high-resolution electrospray ionization-mass spectrometry (ESI-MS) (Figures A2.24–A2.32).



Scheme 2. Synthetic Scheme of **SA_P** and **SA_N**.

Compound **2**

A mixture of compound **1** (0.1 g, 0.4 mmol), Br(CH₂)₉OH (0.21 g, 0.94 mmol) and K₂CO₃ (0.15 g, 1.09 mmol) in dimethylformamide (5 mL) was heated at 85 °C for 18 h under nitrogen condition. The reaction mixture was cooled down to 20 °C, and then washed with water (40 mL x 3) and ethyl acetate (40 mL x 3). The combined organic layers were washed with brine, dried over MgSO₄, and the solvent was evaporated at reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/dichloromethane; v/v = 1/4, R_f = 0.34) to provide *trans*-**2** as an off-white solid (140 mg, 0.26 mmol, 64%).

¹H NMR (400 MHz, CDCl₃) δ 7.22 – 7.14 (m, 4H), 6.77 (dd, *J* = 8.2, 2.3 Hz, 2H), 3.99 (t, *J* = 6.5 Hz, 4H), 3.64 (t, *J* = 6.6 Hz, 4H), 3.22 – 3.14 (m, 4H), 3.08 – 3.00 (m, 4H), 1.86 – 1.74 (m, 4H), 1.64 – 1.53 (m, 7H), 1.52 – 1.42 (m, 6H), 1.35 (s, 18H).

¹³C NMR (101 MHz, CDCl₃) δ 158.2, 144.5, 139.4, 135.9, 125.3, 113.6, 111.1, 68.5, 63.2, 32.9, 32.6, 30.4, 29.7, 29.5, 29.5, 26.2, 25.9.

HR-MS (ESI+) calculated for C₃₆H₅₂O₄ [M+Na]⁺ 571.3758, found 571.3758.

Compound **3**

A mixture of compound **2** (50 mg, 0.09 mmol), TsCl (61 mg, 0.32 mmol), DMAP (5.5 mg, 0.05 mmol) and triethylamine (16 μ L, 0.12 mmol) was added in dichloromethane (5 mL) at 20 °C under nitrogen. The reaction mixture was stirred at 20 °C for 18 h under nitrogen, and then evaporated to give the crude product. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane; v/v = 1/1, R_f = 0.63) to provide *trans*-**3** as an off-white solid (45 mg, 0.05 mmol, 58%).

^1H NMR (400 MHz, CDCl_3) δ 7.79 (d, J = 8.2 Hz, 4H), 7.34 (d, J = 8.1 Hz, 4H), 7.23 – 7.12 (m, 4H), 6.77 (dd, J = 8.2, 2.1 Hz, 2H), 4.08 – 3.91 (m, 8H), 3.17 (d, J = 6.8 Hz, 4H), 3.12 – 2.97 (m, 4H), 2.44 (s, 6H), 1.85 – 1.71 (m, 4H), 1.71 – 1.55 (m, 7H), 1.52 – 1.39 (m, 5H), 1.39 – 1.17 (m, 18H).

^{13}C NMR (101 MHz, CDCl_3) δ 158.3, 144.8, 144.5, 139.4, 135.9, 133.4, 129.9, 128.0, 125.3, 113.6, 111.1, 70.8, 68.5, 32.6, 30.4, 29.5, 29.5, 29.4, 29.0, 29.0, 26.2, 25.5, 21.8.

HR-MS (ESI+) calculated for $\text{C}_{50}\text{H}_{64}\text{O}_8\text{S}_2$ $[\text{M}+\text{Na}]^+$ 879.3934, found: 879.3935.

SA_N

A mixture of compound **3** (20 mg, 0.02 mmol) and trimethylamine (3 mL, 0.03 mol) was in a 20 mL pressure tube. The reaction mixture was heated at 40 °C for 24 h, and then evaporated to give the crude product. The residue was washed by diethyl ether to provide *trans*-SA_N as an off-white solid by filtration (15 mg, 0.02 mmol, 66%).

¹H NMR (400 MHz, CDCl₃) δ 7.74 (d, *J* = 7.8 Hz, 3H), 7.34 (s, 2H), 7.24 – 7.12 (m, 7H), 6.78 (d, *J* = 7.9 Hz, 2H), 4.00 (t, *J* = 6.2 Hz, 4H), 3.35 – 3.26 (m, 4H), 3.16 (s, 20H), 3.05 (s, 4H), 2.36 (s, 5H), 1.88 – 1.63 (m, 8H), 1.36 (s, 20H).

¹³C NMR (101 MHz, CDCl₃) δ 158.0, 144.3, 141.9, 140.4, 139.4, 135.7, 128.9, 125.8, 125.2, 113.5, 110.9, 68.3, 67.0, 53.1, 32.5, 30.2, 29.3, 29.2, 29.1, 26.1, 26.0, 23.0, 21.2.

HR-MS (ESI+) calculated for C₅₆H₈₂N₂O₈S₂ [M–OTs]⁺ 803.5397, found: 803.5396.

SA_P

A mixture of compound **2** (100 mg, 0.18 mmol) and diphenyl phosphite (273 μ L, 1.43 mmol) in pyridine (5 mL) was stirred at 20 °C for 18 h under nitrogen condition. The reaction mixture was added triethylamine/water; v/v = 1/1 (2 mL for each), and then stirred at 20 °C for 18 hours under nitrogen condition. The residue was purified by column chromatography on silica gel (dichloromethane/methanol; v/v = 1/9, R_f = 0.26) to provide *trans*-SA_P as an off-white solid (69.4 mg, 0.08 mmol, 44%).

¹H NMR (400 MHz, MeOD-*d*₄) δ 7.51 (s, 1H), 7.18 (d, J = 8.2 Hz, 3H), 7.11 (s, 2H), 6.77 (dd, J = 8.2, 2.0 Hz, 2H), 5.93 (s, 1H), 4.03 – 3.95 (m, 4H), 3.94 – 3.80 (m, 4H), 3.24 – 3.16 (m, 8H), 3.16 – 3.10 (m, 4H), 3.03 (s, 4H), 1.84 – 1.71 (m, 4H), 1.71 – 1.59 (m, 4H), 1.57 – 1.46 (m, 4H), 1.45 – 1.27 (m, 30H).

¹³C NMR (101 MHz, CDCl₃) δ 158.2, 144.5, 139.4, 135.8, 125.3, 113.6, 111.1, 68.5, 64.4, 64.4, 45.6, 32.6, 30.9, 30.8, 30.4, 29.6, 29.5, 29.5, 29.4, 26.2, 25.9, 8.6.

³¹P NMR (162 MHz, CDCl₃) δ 4.62

HR-MS (ESI+) calculated for C₄₈H₈₄N₂O₈P₂ [M–2HNEt₃]^{2–} 337.1569, found: 337.1569.

2.2.2 Photoswitchability of Anionic and Cationic SAs.

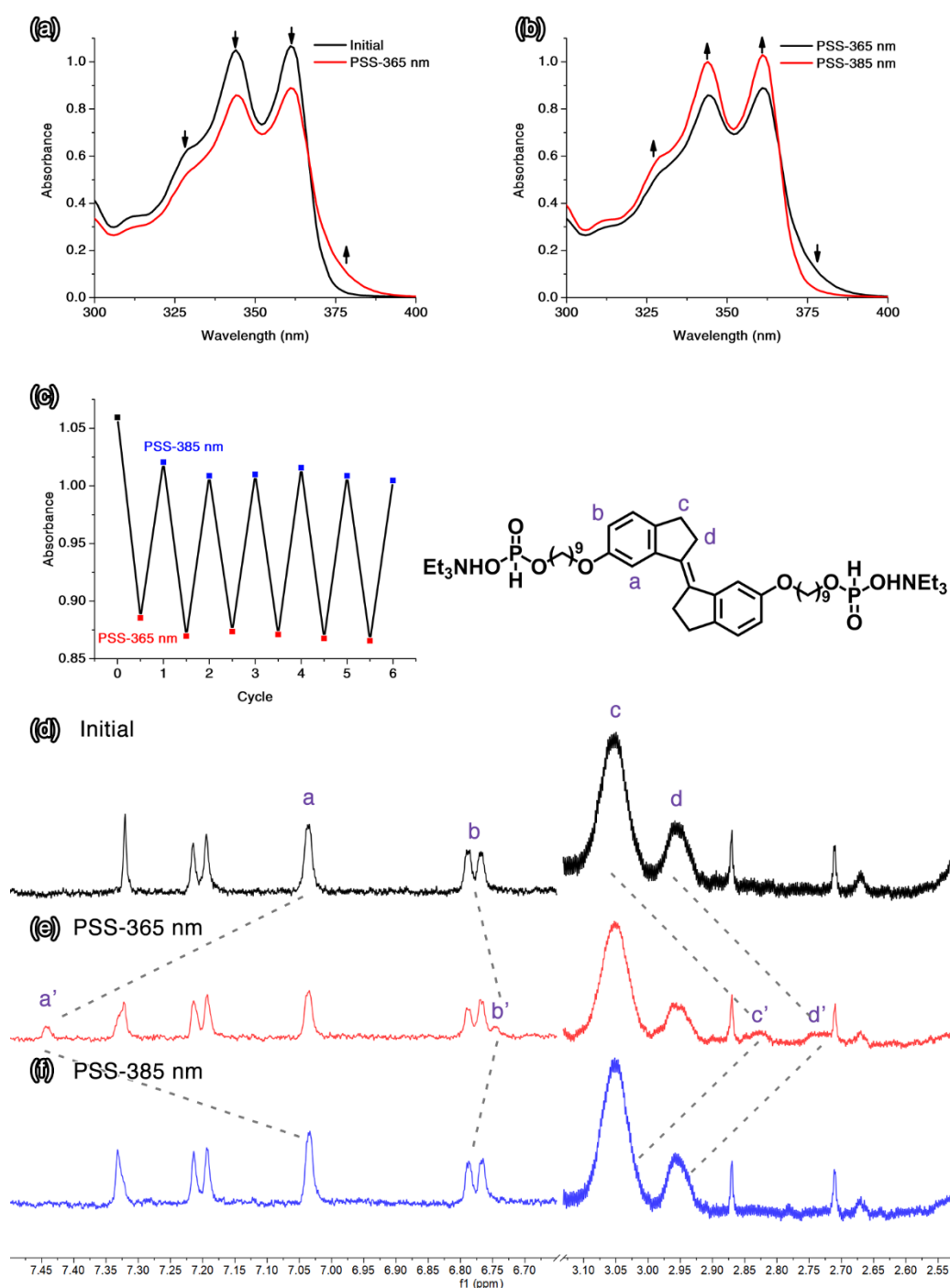


Figure 2.1. (a) UV-vis absorption spectra of SA_P (68 μM) in DMSO upon 365 nm photoirradiation (b) followed by 385 nm photoirradiation. (c) Absorbance change at $\lambda = 360$ nm upon several 365/385 nm switching cycles. Aromatic and aliphatic region in the ^1H NMR spectra of SA_P (3.2 mM in $\text{DMSO}-d_6$), (d) before photoirradiation, (e) irradiated with 365 nm for 3 h at 20 $^{\circ}\text{C}$, (f) followed by 385 nm irradiation for 2 h at 20 $^{\circ}\text{C}$.

The photoisomerization of **SA**s from *trans*-isomer to *cis*-isomer was mainly studied in DMSO. A solution of **SA_P** (68 μ M in DMSO) shows a strong absorption band at 320–375 nm in UV-vis absorption spectrum (Figures 2.1a and A2.1), the absorbance maxima at 365 nm and 345 nm were significantly decreased upon 365 nm photoirradiation (2 min, 20 °C) with a spectral shift to offer an absorption shoulder at 385 nm with isosbestic point at 370 nm. It indicated that a selective photoisomerization from *trans*-**SA_P** to *cis*-**SA_P** (Figure 2.1a). Subsequently, the resulting solution showed a reverse photoswitching process to *trans*-**SA_P** after 2 min 385 nm photoirradiation at 20 °C (Figure 2.1b). The photoisomerization processes between *trans*-**SA_P** and *cis*-**SA_P** was reversible and showed cyclability over five cycles (Figure 2.1c).

A solution of *trans*-**SA_P** (3.2 mM in DMSO-*d*₆) shows structural changes upon 365 nm photoirradiation in ¹H NMR spectrum (Figures 2.1d and A2.3). During 3 h photoirradiation with 365 nm, proton shifts were dramatically observed with the proton signals of H_b (δ = 6.78 ppm), H_c (δ = 3.05 ppm), and H_d (δ = 2.95 ppm) which were shifted upfield to δ = 6.75 ppm, δ = 2.83 ppm, and δ = 2.73 ppm, respectively (Figure 2.1d–1e) when H_a was shifted downfield in obtaining *trans*-**SA_P**/*cis*-**SA_P** = 8/2. A subsequent photoirradiation with 385 nm for 2 h, the small ratio of *cis*-**SA_P** isomer was feasible to switch back to *trans*-**SA_P** (Figure 2.1f). The photoisomerization of **SA_N** in DMSO-*d*₆ was also examined (Figure S4). The photoswitchability of **SA_N** was as good as observed for **SA_P** (Figure 2.1a–1c). Meanwhile, the photostationary state (PSS) of **SA_N** (2.9 mM, DMSO-*d*₆) upon 365 nm photoirradiation was determined with a *trans*-**SA_N**/*cis*-**SA_N** = 8/2 (Figure A2.5). The photoisomerization of **SA_N** can also be performed in MeOH (Figure A2.6). The results indicated that the photoswitchability of stiff-stilbene with anionic phosphite and cationic quaternary ammonium end-groups is still satisfactory in DMSO.

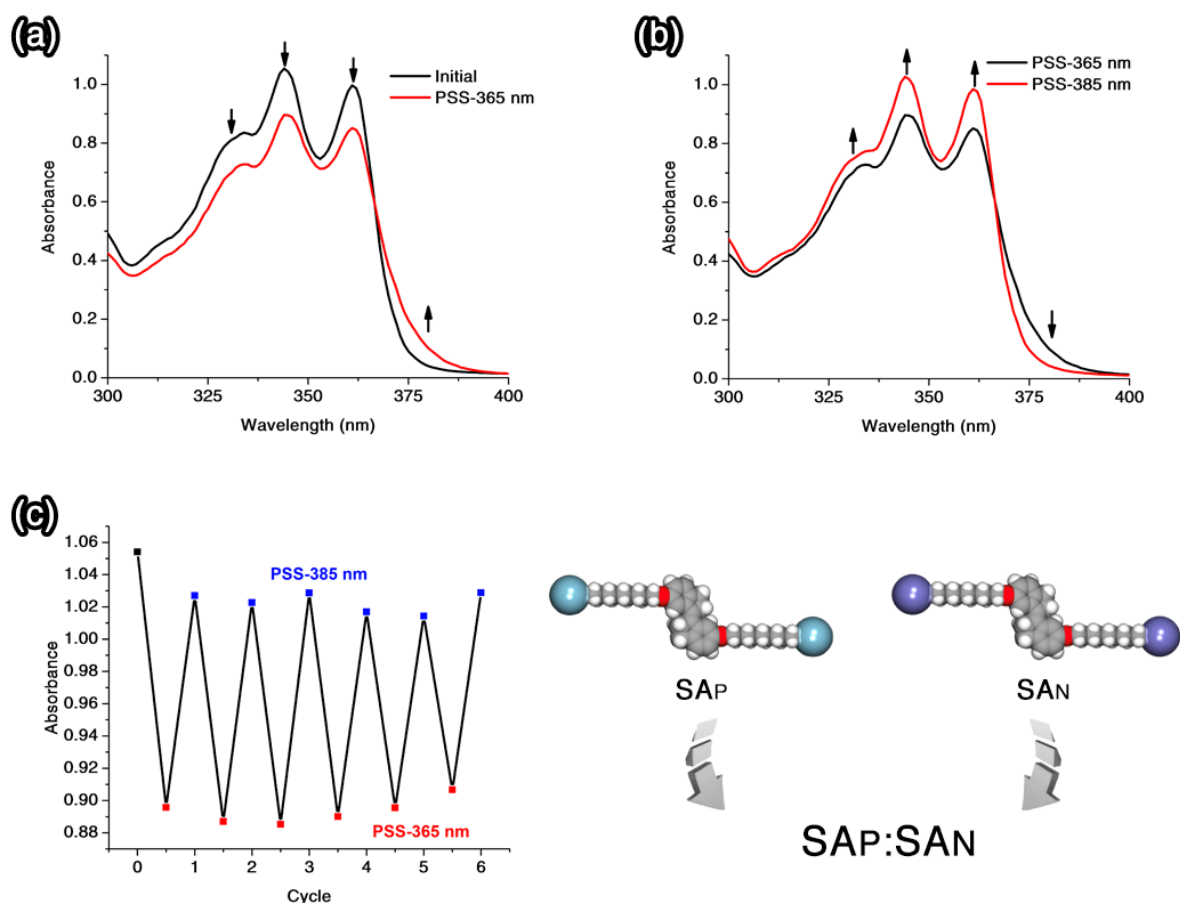


Figure 2.2. (a) UV-vis absorption spectra of **SAP:SAN** (1:1, 68 μM) in DMSO upon 365 nm photoirradiation (b) followed by 385 nm photoirradiation. (c) Absorbance change at $\lambda_{\text{max}} = 343 \text{ nm}$ upon several 365/385 nm switching cycles.

Due to excellent photoswitching properties of both **SAP** and **SAN**, a mixture solution of **SAP:SAN** (1:1) in DMSO was irradiated with 365 nm for 2.5 min, leading to a shoulder peak at 385 nm (Figures 2.2a and A2.7). A subsequent photoirradiation with 385 nm for 2.5 min was applied to show reversible process of *cis*-**SAP:SAN** to *trans*-**SAP:SAN** (Figure 2.2b). The photoisomerization processes between *trans*-**SAP:SAN** and *cis*-**SAP:SAN** was reversible and showed cyclability over five cycles (Figure 2.2c).

2.2.3 Supramolecular Assembly of SAs in Aqueous Media

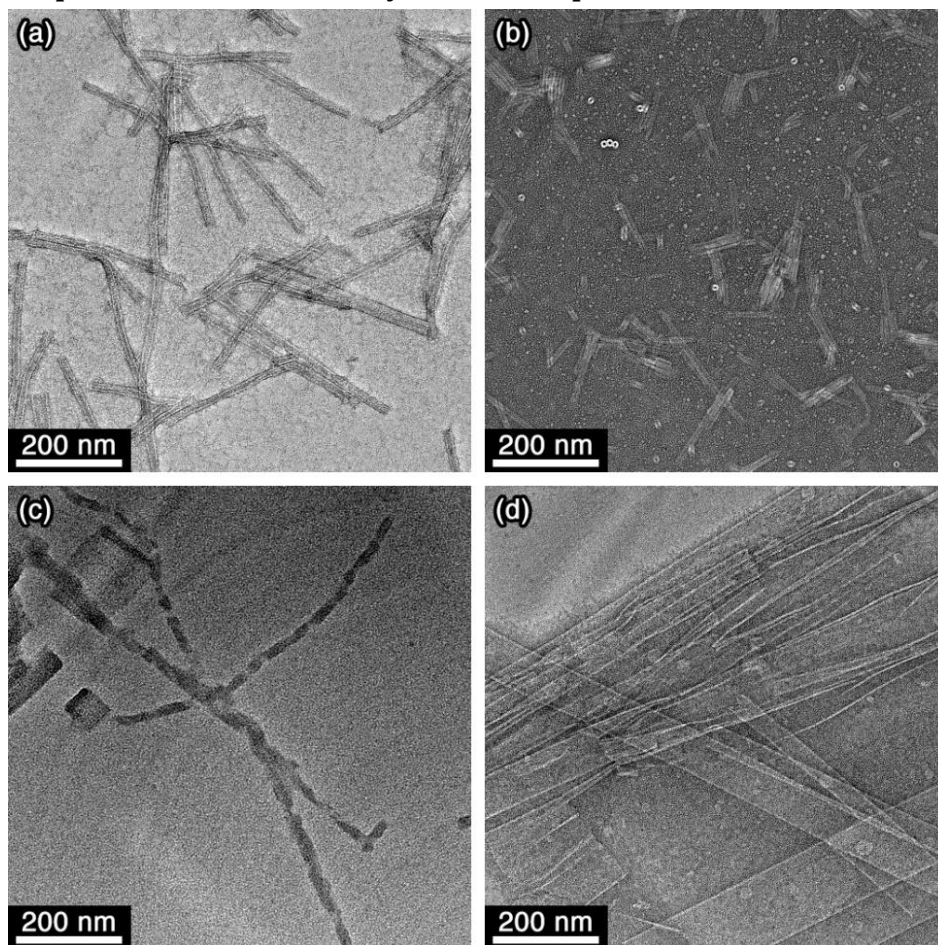


Figure 2.3. TEM images of (a) **SA_P** and (b) **SA_N** (2.0 mM, after annealing at 85 °C for 10 min), **SA_P:SA_N** (1:1, 2.0 mM) (c) before and (d) after annealing at 85 °C, 10 min.

To study the self-assembled morphology of **SA_P**, an aqueous solution of **SA_P** (5.0 wt.%, 56.9 mM) was annealed at 85 °C for 10 min and immediately diluted into 0.2 wt.% (2 mM) for transmission electron microscopy (TEM) measurement. The resulting images revealed nanotubes of **SA_P** (50–100 nm in length and ~15 nm in diameter) (Figure A2.3a). Meanwhile, **SA_N** showed nanotubes with 50–150 nm in length and ~13 nm in diameter (Figure 2.3b). On the other hand, non-annealed **SA_P** and **SA_N** were showed with aggregates and nanofibers (Figure A2.8) which means annealing process is necessary for nanotube formations of both SAs. To determine the critical aggregation concentration (CAC) of **SA_P**, the annealed solution was diluted ranging from 1.0×10^{-4} to 0.09 mM for dynamic light

scattering assay (DLSA). There was a significant rise of scattering intensity when the concentration of **SA_P** solution was higher than 0.004 mM (Figure A2.9a). The CAC of **SA_P** and **SA_N** in aqueous media were $\sim 9 \mu\text{M}$ and $\sim 7 \mu\text{M}$, respectively (Figures A2.9a–A2.9b), implying the concentration of solutions used in TEM were greater than CAC. It is observed that the formations of nanotubes in both SAs are likely caused by improved aromatic π - π interaction of SAs assemblies.

When mixing annealed solutions of **SA_P** and **SA_N** in equal molarity (5.0 mM), it resulted in a mixture of nanotubes even unwinding nano-helices (Figure 2.3c). The solution mixture of **SA_P** and **SA_N** was then annealed at 85 °C for 10 min, resulting in nanoribbons. It implied that the nanotubes of both SAs are unwinding and transforming into nanoribbons because of changing the packing parameter of nanostructures from $1/2 < P < 1$ to $P = 1$. Besides, chirality was not involved in this study (Figure A2.9c–A2.9e). However, when annealing stock solutions of **SA_P** and **SA_N** in different molarity ratio like 2:1 and 1:2, mixtures of nanotubes of SAs and nanoribbons were observed (Figure A2.10). It is likely caused by multiple packing parameters involved during co-assembly process.

2.2.4 Microscopic Supramolecular Assembled Soft Materials of SAs in an Inorganic Salt Solution

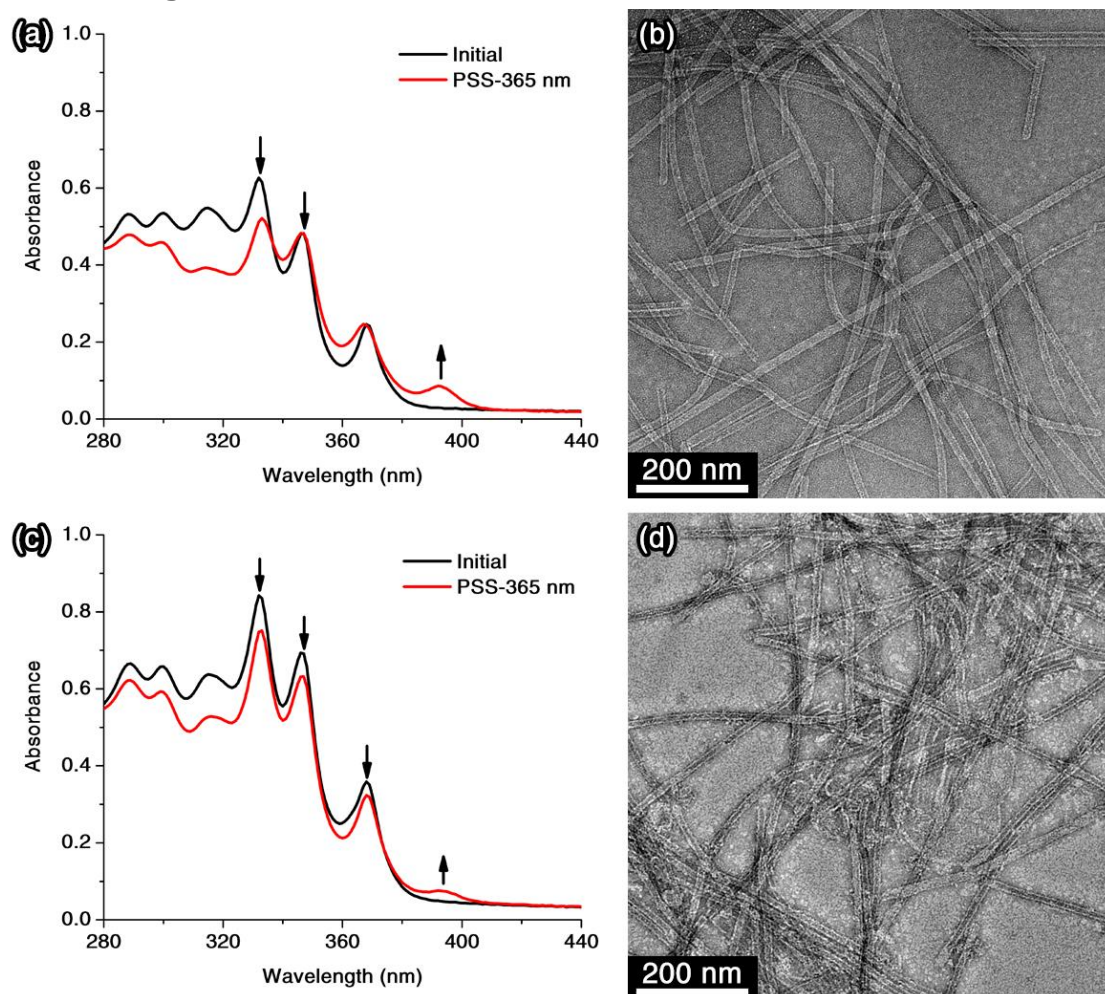


Figure 2.4. UV-vis absorption spectra of (a) SA_P (12 μM) and (c) $\text{SA}_\text{P}:\text{SA}_\text{N}$ (1:1, 9 μM) after 365 nm irradiation for 8 min at 20 $^\circ\text{C}$ in HEPES-buffer (25 mM, pH 7.1). TEM images of (b) SA_P (2.0 mM, annealed at 85 $^\circ\text{C}$, 10 min) and (d) $\text{SA}_\text{P}:\text{SA}_\text{N}$ (1:1, 2.0 mM, annealed at 85 $^\circ\text{C}$, 10 min) after 365 nm photoirradiation for 2 h and 1.5 h at 20 $^\circ\text{C}$, respectively.

SA_P (12 μM) in HEPES buffer solution (25 mM, pH $\sim$ 7.1) illustrates absorption bands at 320–355 nm and 360–375 nm in the UV-vis absorption spectrum (Figure 2.4a). The absorption spectra of SA_P at different concentration did not vary a lot (Figure A2.11). Additionally, there was an obvious spectral shift of SA_P when changing the solvent ratio of DMSO/water (Figure A2.12). It could be explained by solvent polarity effect. After 365 nm photoirradiation for 8 min, there were an absorption band occurring at 380–410 nm when absorptions bands at 320–355 nm and 360–375

decreased (Figure 2.4a, A2.13). Upon dual-light irradiation (385 nm & 405 nm) for 2.5 min, the absorption spectrum remained nearly unchanged which means the switching process of *cis*-**SA_P** was hindered due to the highly ordered structure of **SA_P** (Figure A2.13b). **SA_N** in HEPES buffer solution was extremely stable over 15 h (Figure A14a). However, there was a rapid drop of absorption band at 320–380 nm during 365 nm photoirradiation of **SA_N** in aqueous solution (Figure A2.14b). It implied that **SA_N** photodecomposed in aqueous media. After 365 nm photoirradiation of **SA_P** in aqueous solution for 2 h, there was no significant morphological transformation shown in TEM image (Figure 2.4b). In order to study the structural changes of **SA_P** with zeta potential measurement, the surface charge of **SA_P** was –38.4 mV (Figure A2.15a) and transformed to –23.4 mV (Figure A2.15b) after 365 nm photoirradiation. In spite of limited structural transformation of **SA_P** during photoirradiation, photoisomerization of **SA_P** involved the reduce of surface charge. On the other hand, **SA_N** had surface charge value of +21.3 mV (Figure A2.15c) that means nanotubes of **SA_N** were cationic. The surface charge value of **SA_N** was dramatically decreased to –1.5 mV (Figure A2.15d) upon 365 nm light irradiation which indicated the loss of cationic surface charge. Therefore, the photodecomposition of **SA_N** would lead to the structural transformation with reduced surface charge.

The absorption spectrum of **SA_P:SA_N** (1:1, 9 μ M) (Figure 2.4c) was nearly as same as **SA_P** (Figure 2.4a). **SA_P:SA_N** in HEPES buffer solution (25 mM, pH $\sim$ 7.1) showed exceptionally stability in aqueous media over 6 h (Figure A2.16a). Indeed, it showed spectral shift upon 365 nm photoirradiation (Figure 2.4c, A2.16b-A2.16d). However, the backward photoswitching process was promoted by 6 min dual-light irradiation (385 nm & 405 nm) (Figure A2.16c). **SA_P:SA_N** (1:1, 2.0 mM) in both HEPES buffer solution (25 mM, pH $\sim$ 7.1) and water showed nanoribbons formation (Figure A2.17). The surface charge value of **SA_P:SA_N** was +3.42 mV (Figure A2.15e) that is near to neutral when comparing with **SA_P** and **SA_N**. The nanoribbons of **SA_P:SA_N** (1:1, 2.0 mM) was transformed into

a mixture of nanotubes with diameter of about 15 nm and nanoribbons during 365 nm photoirradiation for 1.5 h (Figure 2.4d). Indeed, the surface charge value of **SA_P:SA_N** was changed to −22.4 mV (Figure A2.15f) which was much negative than before 365 nm photoirradiation (Figure A2.15f). The results implied that the photoisomerization of **SA_P**, reformation of nanotubes of **SA_P**, and photodecomposition of **SA_N** were possible causes of the supramolecular transformation of **SA_P:SA_N** (1:1).

2.2.5 Macroscopic Supramolecular Assembled Soft Materials of SAs in Calcium Chloride Solution

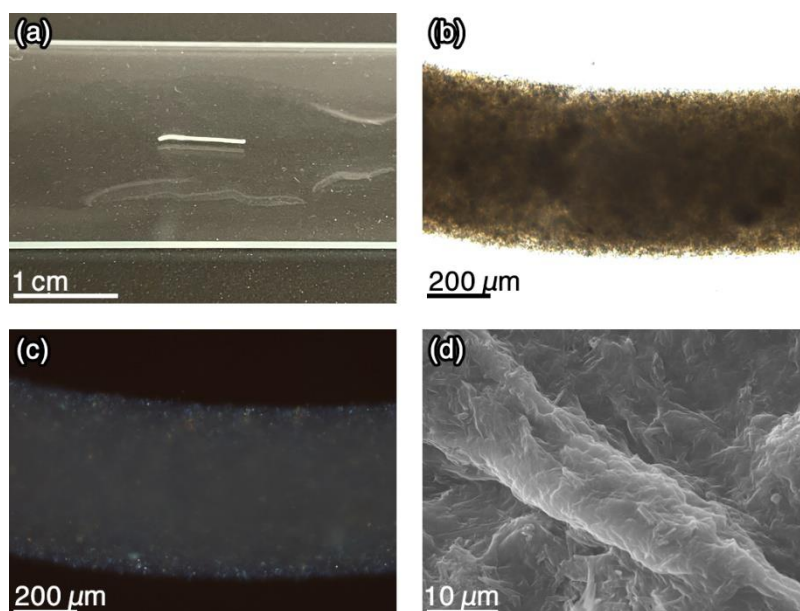


Figure 2.5. (a) Photograph of aqueous solutions of **SA_P:SA_N** (1:1, 5.0 wt.%, 50 mM, annealed at 85 °C, 10 min) ejected into a shallow pool of CaCl₂ solution (150 mM). (b) OM images of a macroscopic string composed of **SA_P:SA_N** prepared from a solution of CaCl₂ (150 mM) (b) without and (c) with crossed polarizers. (d) SEM image of **SA_P:SA_N** (1:1, 5.0 wt.%, 50 mM) prepared from a solution of CaCl₂ (150 mM).

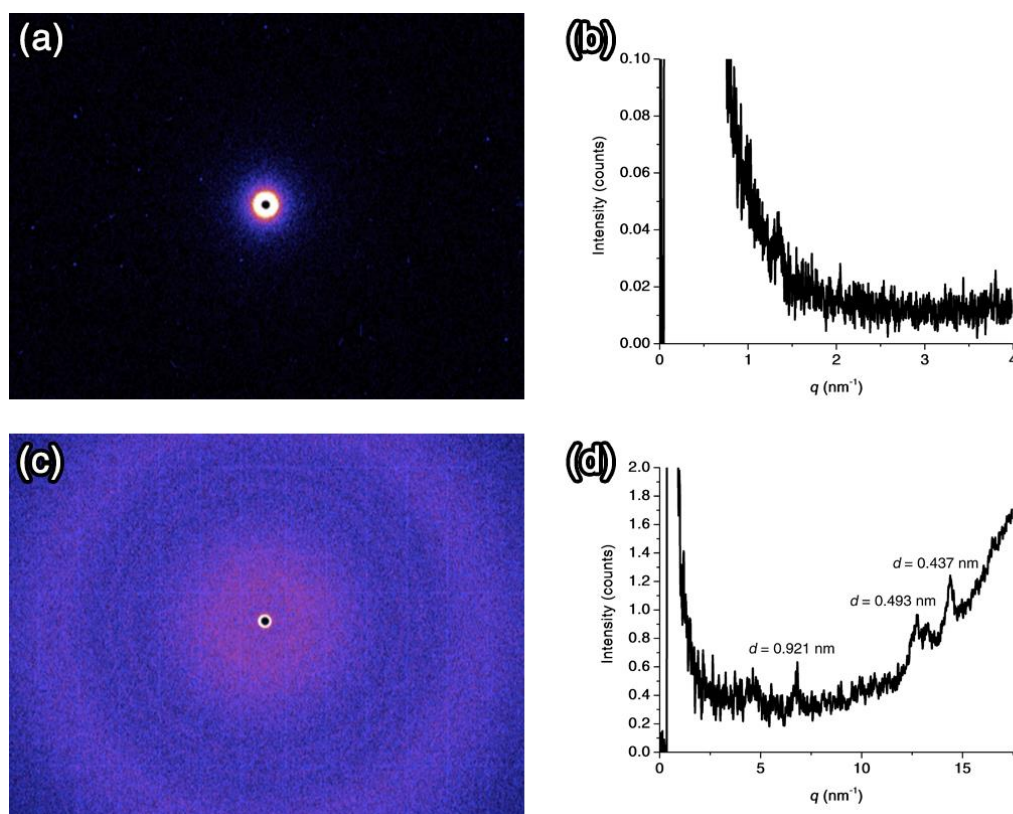


Figure 2.6. (a) 2D SAXS images of a macroscopic aligned string composed of **SA_P:SA_N** (1:1, 5.0 wt.%, 50 mM) prepared from a solution of CaCl₂ (150 mM) with a minute of exposure time. (b) 1D SAXS pattern of **SA_P:SA_N** (1:1, 5.0 wt.%, 50 mM) macroscopic string of the 2D SAXS image in (a). (c) 2D WAXD images of a macroscopic aligned string composed of **SA_P:SA_N** (1:1, 5.0 wt.%, 50 mM) prepared from a solution of CaCl₂ (150 mM) with a minute of exposure time. (d) 1D WAXD pattern of **SA_P:SA_N** macroscopic string (1:1, 5.0 wt.%, 50 mM) of the 2D WAXD image in (c).

Being with good stability of nanostructures of **SA_P:SA_N** in aqueous media, it was interesting to observe bundled nanoribbons with diameter of 4–6 μm and length of 200–600 μm under optical microscopy (Figure A2.18a). Meanwhile, there was no nanostructures of **SA_P** and **SA_N** observed at the same condition (Figure A2.18b and A2.18c). The results clearly indicated the excellent stability of co-assembled **SA_P:SA_N** which leads to assembly ability from nanoscale to microscale. In an addition, an aqueous solution of **SA_P:SA_N** (1:1) has exceptionally good stability to offer a milky

solution at high concentration (50 mM). A plenty of nanoribbons of **SA_P:SA_N** (1:1) were hierarchically formed a macroscopic string through a shear-flow method (Figure 2.5a). Shear-flow method means ejecting the solution of **SA_P:SA_N** (1:1, 5.0 wt.%, 50 mM) into calcium chloride solution (150 mM) followed by water wash. An off-white macroscopic string of **SA_P:SA_N** with diameter $\sim 400\ \mu\text{m}$ was observed under optical microscopy (OM) (Figure 2.5b). There was no birefringence observed with crossed polarizers (Figure 2.5c). Furthermore, nanoribbons with limited alignment of a macroscopic string of **SA_P:SA_N** (1:1) was observed by scanning electron microscopy (SEM) (Figure 2.5d).

Moreover, small-angle X-ray scattering (SAXS) was used to study the conformational diversity of a macroscopic string of **SA_P:SA_N** prepared from calcium chloride solution (1:1, 5.0 wt.%, 50 mM) since calcium ion could promote the formation of nanotubes at relatively low concentration” was added. A defused scattering was occurred in a region of $q = 0.5\text{--}2.0\ \text{nm}^{-1}$ in the 2D SAXS image of the **SA_P:SA_N** (1:1) macroscopic string exposing to X-ray for 1 min on a sapphire substrate (Figure 2.6a). A defuse scattering at $q = 0.2\text{--}1.5\ \text{nm}^{-1}$ of a macroscopic string of **SA_P:SA_N** was observed in the 1D SAXS pattern (Figure 2.6b). The results implied that nearly no unidirectional alignment of **SA_P:SA_N** nanoribbons with large diameters. There were three diffraction rings observed in a 2D through-view wide angle X-ray diffraction (WAXD) image (Figure 2.6c). There is a diffraction ring with d -spacing of 0.92 nm which is from the diffraction of high electron density stiff-stilbene core in 1D WAXD pattern (Figure 2.6d). The diffraction rings with d -spacings of 0.49 nm and 0.44 nm are likely from the intermolecular distances of **SA_P:SA_N** (Figure 2.6d). Electrostatic interactions of the anionic and cationic end-groups of **SA_P:SA_N** play a crucial role in lengthening the intermolecular distances of **SA_P:SA_N** (Figure 2.6d), that is a bit longer than the common aromatic π - π interaction of stiff-stilbene. Although the results implied nanoribbons of **SA_P:SA_N** can assemble across different length-scale in calcium chloride solution, the nanoribbons was not able to

unidirectionally aligned by shear-flow method because of the non-uniform size of nanoribbons. It is observable that a longer X-ray exposure time (20 min) to a macroscopic string of **SA_P:SA_N** prepared in calcium chloride solution (150 mM) in both SAXS and WAXD, respectively (Figure A2.19a-A2.19b and A2.19c-A2.19d). Some additional diffraction peaks in the 1D WAXD pattern were occurred because of drying effect (Figure A2.19d).

2.2.6 Macroscopic Supramolecular Assembled Soft Materials of SAs in Deionized Water

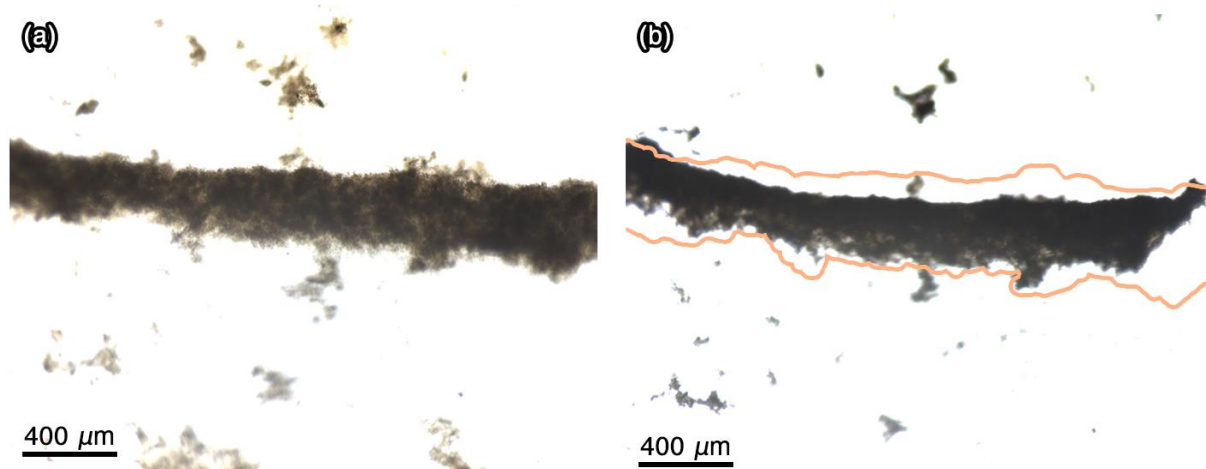


Figure 2.7. OM images of a macroscopic aligned string composed of **SA_P:SA_N** (1:1, 1.0 wt.%, 10 mM) prepared from H₂O (a) before, and (b) after 365 nm photoirradiation for 90 min. The shape of the macroscopic string at the initial state is indicated as the orange boundary. Irradiation of sample was carried out at 20 °C using a Thorlabs model collimated LEDs (M365LP-C1, 365 nm, 1.2 A).

Except for preparing a macroscopic string of **SA_P:SA_N** (1:1) in an inorganic salt solution, the morphology and structure of a macroscopic string of **SA_P:SA_N** (1:1) nanoribbons in water were also investigated. A 5.0 wt.% (50 mM) aqueous solution of **SA_P:SA_N** (1:1) was ejected into water resulting in a stable macroscopic string (Figure A2.20a). However, both 5.0 wt.% (50 mM) solutions of **SA_P** and **SA_N** was not able to form a macroscopic string even diffuse in water with the same preparation. OM image showed the morphology of a macroscopic string of **SA_P:SA_N** (1:1, 5.0 wt.%, 50 mM) in water (Figure A2.20b) was as same as prepared in calcium chloride solution (Figure 2.5b-2.5c). SEM image

of a macroscopic string of **SA_P:SA_N** (1:1, 5.0 wt.%, 50 mM) showed random ribbons (Figure A2.20c). The results indicated that the macroscopic string of **SA_P:SA_N** could be stabilized without an inorganic salt solution.

Lower concentrations of **SA_P:SA_N** (1:1) nanoribbons were also feasible to form a macroscopic string in water. The macroscopic string of **SA_P:SA_N** (1:1) at 10 mM (Figure A2.21a and A2.22b) had a similar morphology to **SA_P:SA_N** (1:1) at 50 mM (Figure A2.20b-A2.20c). Meanwhile, a less dense macroscopic string of **SA_P:SA_N** (1:1) (Figure A2.21b-A2.21c) nanoribbons was formed at 5 mM in water (Figure A2.22b-A2.22c). The morphological transformation of **SA_P:SA_N** (1:1, 2 mM) was triggered by photoisomerization of **SA_P** and photodecomposition of **SA_N** (Figure 2.4d). A macroscopic string of **SA_P:SA_N** (1:1, 10 mM) with diameter about $\sim 300\ \mu\text{m}$ (Figure 2.7a) and it remained 70% of original size after photoirradiation at 365 nm (Figure 2.7b). On the contrary, there was no significant changes of a macroscopic string of **SA_P:SA_N** in water without photoirradiation (Figure A2.23). The results illustrated that a macroscopic string of **SA_P:SA_N** consisting of both cationic and anionic SA mixture can be stabilized without an inorganic salt solution and it showed photoresponsive properties.

2.3 Summary

Stiff-stilbene amphiphiles connecting with anionic phosphite (**SA_P**) and cationic quaternary ammonium groups (**SA_N**) as ionic head groups were synthesized. Their photochemical and supramolecular assembly properties were also investigated. Photoswitchability of SAs were studied by UV-vis absorption and ^1H NMR spectroscopy. Self-assembled nanotube structure of both **SA_P** and **SA_N** were determined by TEM. Both SAs had low CAC were revealed by DLS assay. Co-assembly of **SA_P** and **SA_N** in equivalent molar ratio in aqueous media (pH = 7) resulted in nanoribbons. The co-assembled of **SA_P:SA_N** (1:1) could form a macroscopic string in CaCl_2 solution even in deionized water through a shear-flow method. Upon photoirradiation, the macroscopic string could be massively

retained. These results indicated that the possibility of fabricating a stable soft material by co-assembled anionic and cationic amphiphiles without the presence of inorganic salt solutions. It demonstrates a new method to offer photoresponsive macroscopic soft materials for the smart soft robotic systems.

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Chapter 3: Multifunctional Amino Acid-based SAs

3.1 Introduction

Amino acids are building blocks of peptide and important for formation of supramolecular self-assembled structures. Being with chemical simplicity, biocompatibility and thermodynamic stability, amino acids play a crucial role in self-assembled biomaterials. Since amino acids are simple building blocks that provide unique and tunable morphologies, self-assembly of single amino acid has been investigated to form functional biomaterials. Gazit and his co-workers have studied the assembly of different types of amino acid. Aromatic amino acids, *e.g.* phenylalanine, tyrosine, and tryptophan, self-assemble by noncovalent interactions such as van der Waals interactions, aromatic interactions, and hydrogen bonding to give a wide range of nanostructures including amyloids, nanoribbons, and nanotubes.^[1–3]

The chirality is regarded as a key factor to the assembled structure. From the perspective in nature, the handedness of α -helix in proteins and the double helix in DNA are determined by *D*-saccharides and *L*-amino acids.^[4] The unique sequence of amino acids significantly affects the hierarchical structures of proteins even living functions.^[5] The chirality of biomaterials can be observed at different length scales ranging from atomic level to macroscopic level even galactic level.^[6] Molecular chirality has already been well-investigated in drugs design and functional molecules. Because of the significant findings in supramolecular molecular self-assembly, supramolecular chirality has gained increasing attention in a wide range of research fields including biochemistry, supramolecular chemistry, and materials chemistry.^[7] Chirality amplification, chirality transfer, and asymmetric breaking of either chiral or achiral compounds are common examples of supramolecular chirality. Supramolecular chirality is the aggregation of either chiral or achiral building blocks which results in asymmetric spatial stacking.^[8,9] As a consequence, non-symmetric

structures, *e.g.* helical fibers, coiled-coil helix bundles, and dendritic twists will be formed for different perspectives such as recognition, ion transport, and catalysis.

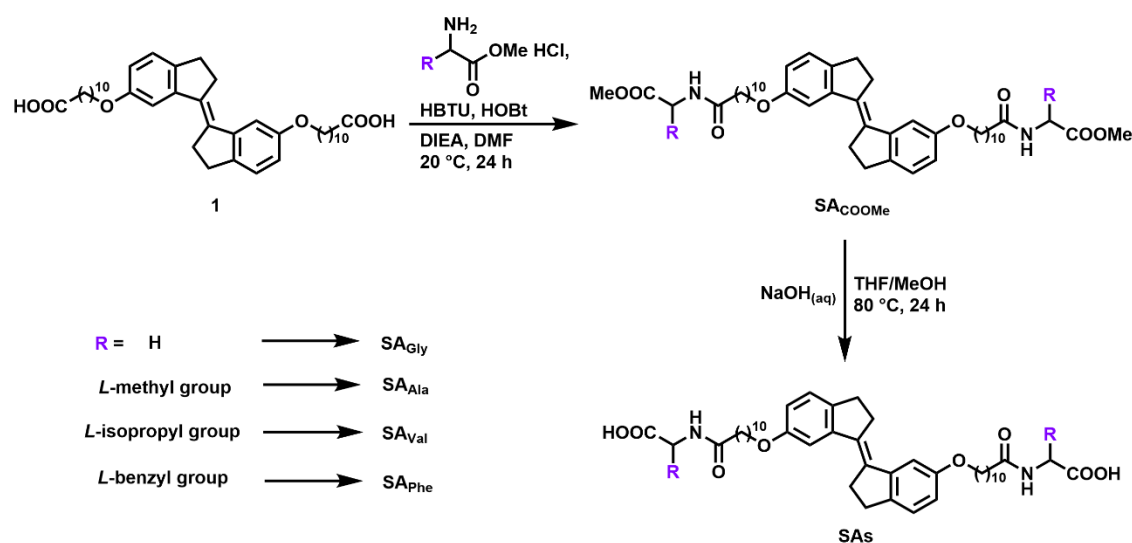
In order to mimic the features of living materials, stimuli-responsive artificial soft materials in aqueous media have been attracted to scientists for recent decades because of their unique and tunable properties.^[10] External stimulus *e.g.* light^[11–18], pH^[19–22], and ions^[14,15,17] plays an important role to control the properties of supramolecular soft materials which results in morphological transformation^[15,16,23–25], photogating system^[26], and cellular uptake^[18]. Nowadays, there are plentiful examples of stimuli-responsive soft materials.^[27–31] The group of Stupp has implemented the studies of hierarchical assembled peptides amphiphiles with different stimuli including light^[17,27–29] and pH^[35,36], respectively. Feringa's group has studied the assembly properties and functionalization of a series of photoswitchable amphiphiles.^[16,37–42] Due to their intrinsic properties in versatile applications^[43], multiple stimuli-responsive soft materials have been pioneered in recent decades. In 2018, Leung and Feringa reported the macroscopic strings of molecular motor amphiphiles prepared in calcium chloride solution among other metal ion solutions showed the best response rate upon photoirradiation.^[37] Leung and Feringa (2019) demonstrated the co-assembly of molecular motor histidine amphiphile and alkyl amphiphile could serve as a template for iron nanoparticles growth which can be controlled by dual-stimuli.^[15] The group of Besenius reported the assembly of a dendritic peptide amphiphile is responsive to pH and reactive oxygen species (ROS).^[43] Albertazzi and his co-workers reported that an azobenzene polymer conjugated with amino acids could respond to multiple stimuli including pH, light, ions, and temperature independently to control supramolecular system in aqueous media.^[44,45] The attention of multiple stimuli-responsive materials has been drawn to chemist; however, amino acid-based amphiphiles with multiresponsivity in aqueous media is not fully explored.

Herein, we demonstrate a series of biocompatible stiff-stilbene amphiphiles (SAs) with different amino acid groups, *i.e.*, glycine, alanine, valine, and phenylalanine which are responsive to different stimuli including solvent, light, and pH. Stiff-stilbene consisting of five-membered fused ring of stilbene is a planar aromatic motif which shows clean photoisomerization. When comparing with the non-planar molecular motor, stiff-stilbene has stronger aromatic π - π interactions and favors large aspect ratio nanostructure formation.^[46] Recently, our group reported SAs functionalized with anionic phosphite and cationic quaternary ammonium showed nanotubes structures which co-assemble into nanoribbons with greater packing parameters.^[25] To exert the potentials of highly ordered SAs, the effects of external stimuli and size of amino acids on SAs with amino acid groups are examined. Due to the intrinsic properties of stiff-stilbene, the photoresponsiveness of SA is promising across different length scales. Nanotubes formation of SA could be observed at neutral to slightly basic conditions. To examine the self-assembly structures of SAs, different assembly conditions were attempted. Although self-assembled nanotubes were observed at different approaches, the width and length of nanotubes can be controlled and regulated through ageing and annealing process. Noticeably, both short and long nanotubes show great biocompatibility. Apart from these, opposite cotton effects do not only observe in *L*- and *D*-amino acids but also SAs with different size of amino acids. Therefore, the interactions of SAs were investigated through co-assembly to induce and deduce chirality. By elucidation of the key structural design of multiple stimuli-responsive amphiphiles, this could open up new prospects towards the development of biocompatible multi-controlled supramolecular materials and smart soft robotic systems.

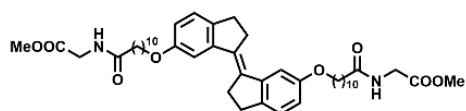
3.2 Results and Discussion

3.2.1 Design and Synthesis of SAs

The stiff stilbene amphiphile (SA) was designed with a five-membered fused ring stiff stilbene core and functionalized with amino acid groups connected via an alkyl-linker on both sides of stiff stilbene core. The hydrophobic stiff stilbene core is surrounded with two hydrophilic parts, affording a bola-amphiphilic structure. The synthesis is summarized in Scheme 1. *trans*-stiff-stilbene **1** was prepared according to previously reported procedure. *trans*-stiff-stilbene amino methyl ester (SA_{COOMe}) was obtained by amide coupling in the presence of HBTU, HOBt monohydrate, DIEA in DMF. SAs were obtained by hydrolysis of *trans*-SA_{COOMe}. The structures of all new compounds were characterized by ¹H, ¹³C nuclear magnetic resonance (NMR) and high-resolution electrospray ionization-mass spectrometry (ESI-MS) (Figures A3.55–A3.70).



Scheme 3. Synthetic Scheme of SAs.



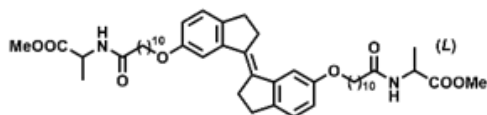
SA_{Gly}COOMe

A mixture of **1** (100 mg, 0.16 mmol), glycine methyl ester hydrochloride (39 mg, 0.31 mmol), hexafluorophosphate benzotriazole tetramethyl uronium (136 mg, 0.36 mmol), hydroxybenzotriazole monohydrate (48 mg, 0.36 mmol) and N,N-diisopropylethylamine (98 μ L, 0.56 mmol) in dimethylformamide (5 mL) was stirred at 20 °C for 24 h. The reaction mixture was then washed with water (40 mL x 3) and ethyl acetate (40 mL x 3). The combined organic layers were washed with brine, dried over Mg₂SO₄, and the solvent was evaporated at reduced pressure. The residue was recrystallized in ethyl acetate/hexane (1:9) to afford *trans*-**1** as an off-white solid (49 mg, 0.06 mmol, 40%).

¹H NMR (400 MHz, CDCl₃) δ 7.20 (d, *J* = 8.2 Hz, 2H), 7.16 (d, *J* = 2.3 Hz, 2H), 6.80 – 6.75 (m, 2H), 5.91 (s, 2H), 4.05 (d, *J* = 5.1 Hz, 3H), 3.99 (t, *J* = 6.5 Hz, 3H), 3.76 (s, 6H), 3.18 (m, 4H), 3.05 (m, 4H), 2.24 (m, 4H), 1.79 (m, 4H), 1.64 (m, 4H), 1.56 (s, 20H), 1.51 – 1.42 (m, 4H), 1.31 (s, 18H).

¹³C NMR (101 MHz, CDCl₃) δ 173.3, 170.6, 158.1, 144.4, 139.3, 135.7, 125.2, 113.5, 111.0, 68.4, 52.4, 41.2, 36.4, 32.5, 30.2, 29.5, 29.4, 29.3, 29.2, 26.1, 25.6.

HR-MS (ESI+) calculated for C₄₆H₆₆N₂O₈ [M+Na]⁺ 797.4711, found 797.4710.



SA_{AlaCOOMe}

Except for changing glycine methyl ester hydrochloride to *L*-alanine methyl ester hydrochloride,

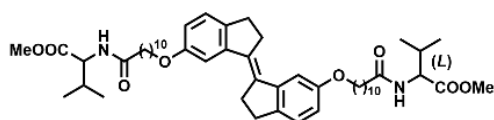
SA_{AlaCOOMe} was synthesized in the similar method as compound **SA_{GlyCOOMe}** to afford pure *trans*-

SA_{GlyCOOMe} compounds as off-white solid (53 mg, 0.07 mmol, 42%).

¹H NMR (600 MHz, CDCl₃) δ 7.20 (d, *J* = 8.2 Hz, 2H), 7.16 (d, *J* = 2.3 Hz, 2H), 6.78 (m, 2H), 5.98 (d, *J* = 7.5 Hz, 2H), 4.61 (m, 2H), 3.99 (t, *J* = 6.6 Hz, 4H), 3.75 (s, 6H), 3.18 (m, 4H), 3.09 – 2.99 (m, 4H), 2.24 – 2.14 (m, 4H), 1.79 (m, 4H), 1.63 (m, 5H), 1.58 (s, 17H), 1.47 (m, 4H), 1.40 (d, *J* = 7.2 Hz, 6H), 1.30 (m, 20H).

¹³C NMR (151 MHz, CDCl₃) δ 173.8, 172.6, 158.1, 144.4, 139.3, 135.7, 125.2, 113.5, 111.0, 68.4, 52.5, 47.9, 36.6, 32.5, 30.2, 29.5, 29.4, 29.3, 29.2, 26.1, 25.6, 18.6.

HR-MS (ESI⁺) calculated for C₄₈H₇₀N₂O₈ [M+H]⁺ 803.5205, found 803.5205.



SA_{ValCOOMe}

Except for changing glycine methyl ester hydrochloride to *L*-valine methyl ester hydrochloride,

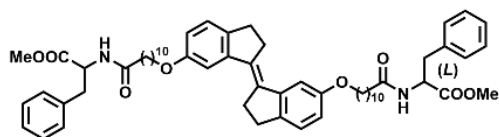
SA_{ValCOOMe} was synthesized in the similar method as **SA_{GlyCOOMe}** to afford pure *trans*-**SA_{ValCOOMe}**

compounds as off-white solid (98 mg, 0.1 mmol, 72%).

¹H NMR (400 MHz, CDCl₃) δ 7.20 (d, *J* = 8.2 Hz, 2H), 7.16 (d, *J* = 2.3 Hz, 2H), 6.77 (dd, *J* = 8.2, 2.3 Hz, 2H), 5.90 (d, *J* = 8.9 Hz, 2H), 4.59 (dd, *J* = 8.8, 4.9 Hz, 2H), 3.99 (t, *J* = 6.6 Hz, 4H), 3.74 (s, 6H), 3.18 (m, 4H), 3.05 (m, 3H), 2.28 – 2.20 (m, 4H), 2.15 (td, *J* = 6.9, 5.0 Hz, 2H), 2.05 (s, 1H), 1.79 (m, 4H), 1.64 (m, 4H), 1.59 (s, 12H), 1.53 – 1.40 (m, 4H), 1.39 – 1.21 (m, 20H), 0.92 (dd, *J* = 14.2, 6.9 Hz, 12H).

¹³C NMR (101 MHz, CDCl₃) δ 173.4, 172.7, 158.1, 144.4, 135.7, 126.6, 126.5, 125.2, 116.8, 113.5, 111.5, 111.0, 68.4, 56.9, 52.2, 36.8, 32.5, 31.3, 30.2, 29.5, 29.4, 29.3, 29.2, 26.1, 25.7, 18.9, 17.8.

HR-MS (ESI⁺) calculated for C₅₂H₇₈N₂O₈ [M+H]⁺ 859.5831, found 859.5832.



SA_{PheCOOMe}

Except for changing glycine methyl ester hydrochloride to *L*-phenylalanine methyl ester hydrochloride, **SA_{PheCOOMe}** was synthesized in the similar method as **SA_{GlyCOOMe}** to afford pure *trans*-**SA_{PheCOOMe}** compounds as off-white solid (86 mg, 0.09 mmol, 57%).

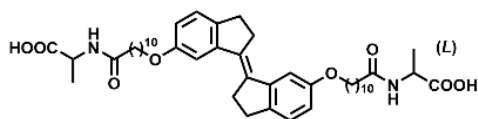
¹H NMR (400 MHz, CDCl₃) δ 7.22 – 7.14 (m, 4H), 6.77 (dd, *J* = 8.2, 2.3 Hz, 2H), 3.99 (t, *J* = 6.5 Hz, 4H), 3.64 (t, *J* = 6.6 Hz, 4H), 3.22 – 3.14 (m, 4H), 3.08 – 3.00 (m, 4H), 1.86 – 1.74 (m, 4H), 1.64 – 1.53 (m, 7H), 1.52 – 1.42 (m, 6H), 1.35 (s, 18H).

¹³C NMR (101 MHz, CDCl₃) δ 173.4, 172.7, 158.1, 144.4, 139.3, 135.7, 126.6, 126.5, 125.2, 116.8, 113.5, 111.5, 111.0, 68.4, 56.9, 52.2, 36.8, 32.5, 31.3, 30.2, 29.5, 29.4, 29.3, 29.2, 26.1, 25.7, 18.9, 17.8.

HR-MS (ESI+) calculated for C₆₀H₇₈N₂O₈ [M+Na]⁺ 977.5650, found 977.5655.



HR-MS (ESI+) calculated for $C_{44}H_{62}N_2O_8$ $[M+Na]^+$ 769.4398, found 769.4391.



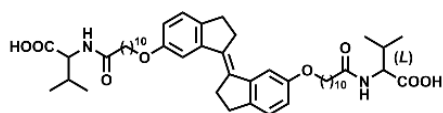
SA_{Ala}

Except for changing **SA_{Gly}COOMe** to **SA_{Ala}COOMe**, **SA_{Ala}** was synthesized in the similar method as **SA_{Gly}** (11 mg, 0.01 mmol, 38%).

¹H NMR (600 MHz, DMSO-*d*₆) δ 7.87 (s, 2H), 7.21 (d, *J* = 8.4 Hz, 2H), 7.07 (s, 2H), 6.80 (d, *J* = 8.4 Hz, 2H), 4.05 (s, 2H), 3.98 (s, 4H), 3.10 (s, 4H), 2.98 (s, 4H), 2.18 (t, *J* = 7.5 Hz, 2H), 2.06 (s, 4H), 1.71 (s, 5H), 1.52 – 1.38 (m, 11H), 1.37 – 1.08 (m, 40H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 174.6, 174.4, 171.8, 157.7, 143.8, 138.8, 135.3, 125.3, 113.9, 110.2, 69.8, 67.6, 47.7, 35.1, 33.7, 31.8, 29.7, 29.0, 28.9, 28.8, 28.8, 28.6, 28.6, 25.6, 25.3, 24.5, 22.1, 17.6.

HR-MS (ESI+) calculated for C₄₆H₆₆N₂O₈ [M+H]⁺ 775.4892, found 775.4909.



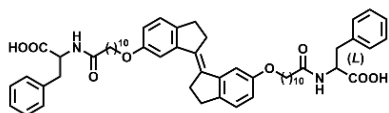
SA_{Val}

Except for changing **SA_{Gly}COOMe** to **SA_{Val}COOMe**, **SA_{Val}** was synthesized in the similar method as **SA_{Gly}** (20 mg, 0.02 mmol, 69%).

¹H NMR (600 MHz, DMSO-*d*₆) δ 7.79 (s, 2H), 7.22 (d, *J* = 8.4 Hz, 2H), 7.07 (s, 2H), 6.80 (s, 2H), 4.07 (s, 2H), 3.98 (s, 4H), 3.10 (s, 4H), 2.98 (s, 4H), 2.20 – 2.07 (m, 6H), 2.01 (d, *J* = 7.9 Hz, 3H), 1.71 (s, 6H), 1.54 – 1.37 (m, 10H), 1.25 (m, 27H), 0.83 (d, 16H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 173.27, 172.45, 137.83, 127.80, 123.65, 105.35, 67.83, 57.08, 54.92, 36.59, 34.98, 30.96, 29.79, 28.95, 28.92, 28.76, 28.59, 25.38, 24.65, 22.07, 19.20, 18.05, 13.98.

HR-MS (ESI+) calculated for C₅₀H₇₄N₂O₈ [M+H]⁺ 831.5518, found 831.5509.



SA_{Phe}

Except for changing **SA_{Gly}COOMe** to **SA_{Phe}COOMe**, **SA_{Phe}** was synthesized in the similar method as **SA_{Gly}**

(14 mg, 0.02 mmol, 48%).

¹H NMR (600 MHz, DMSO-*d*₆) δ 7.21 (d, *J* = 8.3 Hz, 2H), 7.15 (m, 6H), 7.11 – 7.05 (m, 8H), 6.80 (dd, *J* = 8.1, 2.2 Hz, 2H), 3.98 (m, 6H), 3.09 (m, 4H), 3.04 (dd, *J* = 13.3, 5.1 Hz, 3H), 2.97 (m, 4H), 2.87 (dd, *J* = 13.2, 6.2 Hz, 2H), 1.98 (q, 5H), 1.75 – 1.67 (m, 4H), 1.47 – 1.35 (m, 9H), 1.33 (m, 5H), 1.27 – 1.17 (m, 15H), 1.14 (m, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 170.7, 157.6, 143.7, 139.5, 138.7, 135.2, 129.5, 127.4, 125.4, 125.3, 113.8, 110.1, 67.6, 55.1, 40.0, 39.9, 39.8, 39.6, 39.5, 39.4, 39.2, 39.1, 37.4, 35.8, 31.8, 29.6, 29.0, 28.9, 28.9, 28.8, 28.8, 28.6, 25.6, 25.4.

HR-MS (ESI+) calculated for C₅₈H₇₄N₂O₈ [M+Na]⁺ 949.5337, found 949.5337.

3.3 Photoswitching of Stiff-Stilbene Amphiphiles in Organic Media

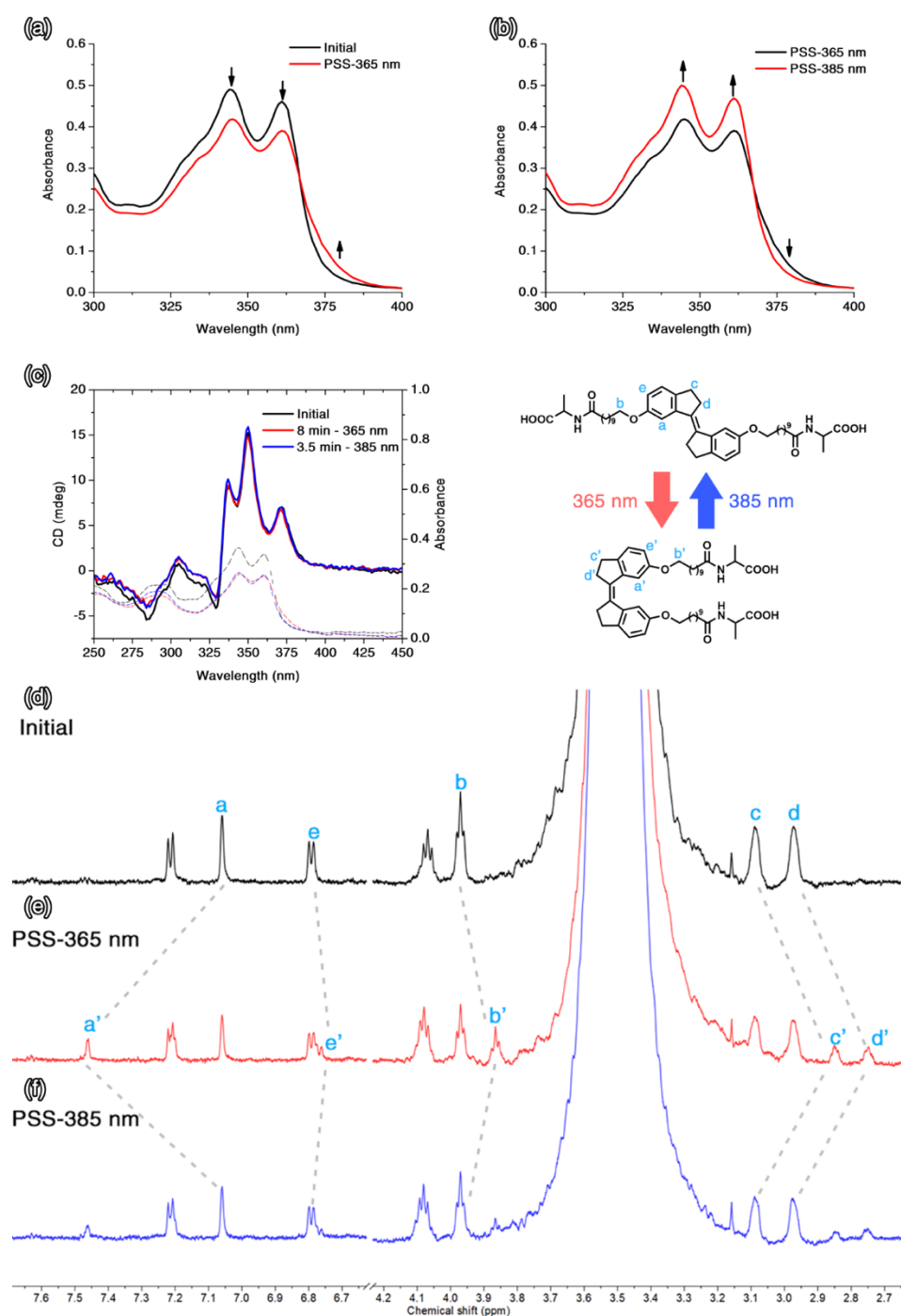


Figure 3.1. UV-vis absorption spectra of **SAAla** (65 μM), from *trans*-isomer to *cis*-isomer and *cis*-isomer to *trans*-isomer in DMSO. Absorption spectra of **SAAla** in PSS (red) (a) after 365 nm irradiation for 60 s (b) after 385 nm irradiation for 75 s. (c) CD (solid lines) and UV-vis spectra (dashed lines) of **SAAla** (0.69 mM) in DMSO after 365 nm irradiation (red) followed by subsequent 385 nm irradiation (line). Aromatic and aliphatic region in the ^1H NMR spectra during isomerization process of **SAAla** (5.5 mM, DMSO- d_6 , 600 MHz), (d) before photoirradiation, (e) irradiated with 365 nm for 1 h at 20 °C, (f) followed by 385 nm irradiation for 1 h at 20 °C.

The photoisomerization of SAs from *trans*-isomer to *cis*-isomer was studied in an organic medium. A DMSO solution of **SA_{Ala}** shows a significant absorption band at 320–370 nm in UV-vis absorption spectrum (Figure 3.1a and A3.1). Upon 365 nm photoirradiation (30 s, 20 °C), the intensity of absorption maxima at 344 nm was reduced with a spectral shift to provide a new absorption shoulder peak at 385 nm with a clear isosbestic point at 370 nm, indicating a selective photoisomerization from *trans*-**SA_{Ala}** to *cis*-**SA_{Ala}** (Figure 3.1a). The resulting solution showed a reverse switching process to *trans*-**SA_{Ala}** after irradiated with 385 nm light for over 1 min at 20 °C (Figure 3.1b). Due to the strong absorbance fluctuation of **SA_{Ala}** in DMSO over 4 h (Figure A3.1d–1e), cyclability of photoisomerization is not able to be performed.

A DMSO-*d*₆ solution of **SA_{Ala}** (5.5 mM) shows only *trans*-**SA_{Ala}** isomers in ¹H NMR spectrum (Figures 3.1d and A3.2). Distinctive proton shifts were observed with the proton signals of H_b (δ = 3.97 ppm), H_c (δ = 3.09 ppm), H_d (δ = 2.97 ppm), and H_e (δ = 6.80 ppm) shifted upfield to δ = 3.87 ppm, δ = 2.85 ppm, δ = 2.75 ppm and δ = 6.76 ppm, respectively (Figure 3.1d,e), along with a downfield shift of H_a in attaining *trans*-**SA_{Ala}**/*cis*-**SA_{Ala}** isomer ratio 75:25, upon 365 nm photoirradiation for 1 h at 20 °C. The obtained *cis*-**SA_{Ala}** isomer can be switched back to *trans*-**SA_{Ala}** upon 385 nm photoirradiation for 1 h at 20 °C (Figure 3.1f). The photoisomerization of other SAs in DMSO have been characterized (Figure A3.3–A3.5) to show good photoswitchability to that of observed for **SA_{Ala}** (Figure 3.1a–3.1c). Meanwhile, the photoisomerization of other SAs has similar peak shifts as **SA_{Ala}** in NMR spectroscopy (Figure A3.6–A3.8).

For the circular dichroism (CD) measurement, a DMSO solution of **SA_{Ala}** illustrated a significant Cotton effect at 300–375 nm, which is mainly caused by the stiff stilbene core (Figure 3.1c, A3.9). Upon 365 nm photoirradiation (8.5 min, 20 °C), there were no significant spectral

changes (Figure 3.1c, A3.9). Indeed, the UV-vis absorption spectra measured by CD spectroscopy showed the *trans*- to *cis*- photoisomerization (Figure A3.9b). These results indicate that the photoisomerization of **SA_{Ala}** could not lead to the change of helicity. The photoisomerization of **SA_{Val}** (Figure A3.10) in DMSO was also similar as observed for **SA_{Ala}**. **SA_{Phe}** did not significant CD signal (Figure A3.11) while **SA_{Gly}** lacking of a chiral center did not show any helicity in CD spectra (Figure A3.12).

3.3.1 Supramolecular Assembly of Stiff Stilbene Amphiphiles in DMSO

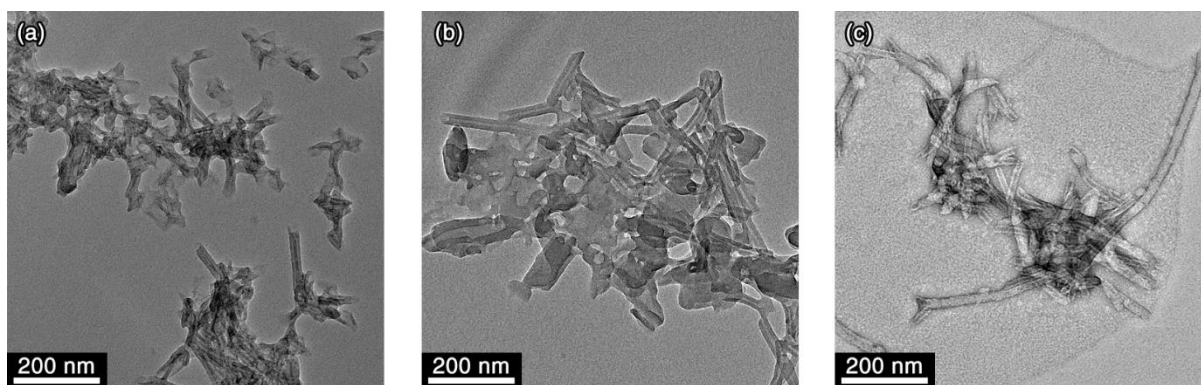


Figure 3.2. TEM image of **SA_{Ala}** (0.2 wt.%, 2.58 mM) in DMSO (a) without photoirradiation, (b) after 365 nm photoirradiation for 0.5 h, and (c) followed by 385 nm photoirradiation for 0.5 h. Irradiation of sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample.

After studying the photoswitchability of SAs in DMSO by UV-vis spectroscopy and CD spectroscopy, we would like to investigate the self-assembly of SAs in DMSO by negative-stained transmission electron microscopy (TEM). A DMSO solution of SAs showed assembly properties at 20 °C without annealing process. According to TEM images, **SA_{Ala}** (Figure 3.2a) formed nanotubes. On the other hand, **SA_{Gly}**, **SA_{Val}**, and **SA_{Phe}** formed nanoaggregates, helical ribbons and nanoribbons, respectively (Figure A3.13a, A3.14a, and A3.15a). It is interesting to observe that the morphology of SAs mainly depends on the type of amino acid. **SA_{Gly}** containing the smallest size of amino acid

could barely form nanoaggregates. In contrast, **SA_{Ala}** consisting of the second smallest size of amino acid is able to form nanotubes. When attaching amino acids with larger constituents, SAs would assemble to helical ribbons and ribbons. **SA_{Val}** containing the smallest three branched-chain amino acid forms helical ribbon. Indeed, **SA_{Phe}** with an aromatic amino acid forms nanoribbon. Previous research studies have revealed the size effect of amino acid on morphological formation. In 2013, Khushalani and her co-workers reported that achiral glycine did not show any significant morphology under electron microscopes.^[47] Imae (1992) stated that alanine with C₁₂ chain had tendency to contact side-by-side which resulted in neither twisted nor helical conformation.^[48] Gazit (2012) reported that a single phenylalanine was able to form amyloid-like fibrillar structure.^[49] These experimental results basically conclude that the morphology of self-assembled SAs is probably affected by the type of amino acids.

Because SAs show good switchability and self-assembled morphology in DMSO, we would like to draw an attention of the photochemical effects on morphological transformation. A degassed DMSO solution of *trans*-SAs was irradiated at 365 nm for 0.5 h, followed by 385 nm for 0.5 h. The photoirradiated sample was then examined by negatively stained TEM. As shown in Figure 3.2b and 3.2c, there was a significant morphological transformation of **SA_{Ala}** upon photoirradiation. After 365 nm photoirradiation of *trans*-**SA_{Ala}**, some nanotubes were transformed to ribbons as the formation of *cis*-**SA_{Ala}** (Figure 3.2b). Subsequent photoirradiation of 385 nm was conducted to study the morphological reversibility, most of the ribbons were converted back to nanotubes as *cis*- to *trans*-**SA_{Ala}** (Figure 3.2c). Since there was less than 30% of *trans*-to-*cis* photoisomerization efficiency of **SA_{Ala}** (Figure 3.1), the formation of *cis*-**SA_{Ala}** ribbons was not that significant (Figure 3.2b). Consequently, no apparent changes in helicity was observed (Figure 3.1c). The photoirradiation of other SAs could show similar photoswitchability and morphological transformation as **SA_{Ala}**. **SA_{Gly}** showed the formation of disordered aggregates after 365 nm

photoirradiation as *trans*-to-*cis* photoisomerization, and nanoaggregates could be partially reformed after 385 nm photoirradiation (Figure A3.13b and A3.13c). After photoirradiation at 365 nm of **SA_{Val}**, it showed nanotubes as the formation of *cis*-**SA_{Val}**, and it was reverted back to helical ribbons with subsequent photoirradiation of 385 nm (Figure A3.14b and A3.14c). Similarly, the nanoribbons of *trans*-**SA_{Phe}** would transform to disordered structure after 365 nm photoirradiation, and those nanoribbons were obviously reformed followed by 385 nm photoirradiation (Figure A3.15b and A3.15c). Based on the above results, SAs exhibited not only good self-assembly ability in DMSO but also morphological switchability.

3.4 Supramolecular Assembly of Stiff Stilbene Amphiphiles in Aqueous Media

Apart from organic media, the supramolecular self-assembly of SAs was performed in aqueous media. Critical aggregation concentration (CAC) of SAs were determined by dynamic light scattering assay (DLSA). The CAC of aqueous solutions of all SAs were below 10 μ M which means SAs could self-assemble at low concentration (Figure A3.16). SAs are bola-amphiphiles with two amino acids groups, so their self-assembled structures are strongly dependent on pH. Some SAs may decompose under strong basic conditions; however, **SA_{Ala}** nanotubes show exceptionally good stability in water.

3.4.1 pH-dependent assembly of SAs

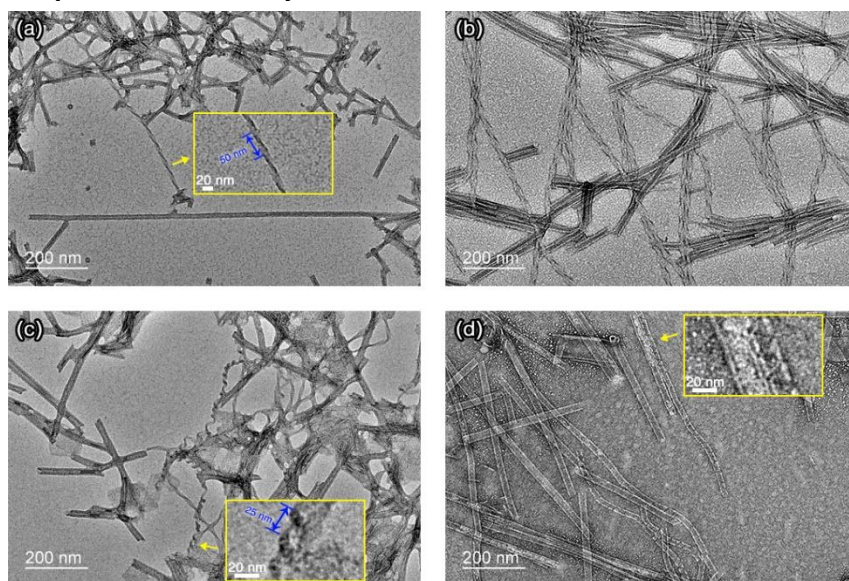


Figure 3.3. TEM images of **SA_{Ala}** (0.2 wt.%, 2.58 mM) in water at (a) pH = 7, (b) pH = 9, (c) pH = 11, (d) pH = 13.

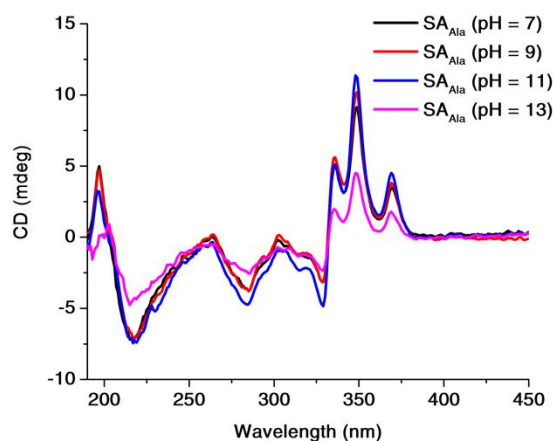


Figure 3.4. CD spectra of annealed **SA_{Ala}** (50 μ M) in water at pH = 7, 9, 11, and 13.

SA_{Ala} (0.2 wt.%, 2.58 mM) was dispersed in water at different pH values. Then, a clear solution was annealed at 85 $^{\circ}$ C for 70 min followed by 1 $^{\circ}$ C/min to acquire optimal self-assembled structure. The annealed solution was imaged using transmission electron microscopy (TEM), revealing nanotubes structure (Figure 3.3). Over 100 nanotubes were analyzed to study the changes of width and length at different pH. These results were summarized in histogram (Figure A3.17, A3.18). At neutral condition (pH = 7), the nanotubes are 16.7 ± 1.4 nm in width and 207.8 ± 21.6 nm

in length, and some left-handed helical ribbons as transformation to nanotubes with 50 nm of pitch could be observed (Figure 3.3a, A3.17a, and A3.18a). At moderate basic condition (pH = 9), similar left-handed helical ribbons and nanotubes could be observed and their width and length are 19.7 ± 4.1 nm and 345.6 ± 142.1 nm, respectively (Figure 3.3b, A3.17b, and A3.18b). To confirm the nanostructures in a near-native state, **SA_{Ala}** at pH = 9 was imaged by Cryogenic Transmission Electron Microscopy (Cryo-TEM) which showed nanotubes with similar width (15.8 ± 2.4 nm) and length (455.7 ± 350.2 nm) as in TEM (Figure A3.19). The morphological discrepancy between TEM image and cryo-TEM image could be caused by drying artifact and negative staining in TEM preparation.^[50,51] Similarly, the width and length of nanotubes were 21.0 ± 2.0 nm and 331.0 ± 112.8 nm at pH = 11 (Figure 3.3c, A3.17c, and A3.18c). However, helical coils with 25 nm of short helical pitch were observed. At extremely strong basic condition (pH = 13), a small portion of nanotubes were dramatically widened (width: 33.4 ± 19.8 nm) when the length (length: 400.2 ± 190.0 nm) was not significantly changed (Figure 3.3d, A3.17d, and A3.18d). More importantly, helical sheets were observed (Figure 3.3d). It is observable that the width of nanotubes increased and the pitch of helical ribbons decreased at basic conditions. The negative net charge effect of **SA_{Ala}** increases proportionally with the pH which results in charge repulsion, then tube widening. Furthermore, it is known that the formation of nanotubes is the result of decreasing helical pitch of helical ribbons.^[5,52] Growing-width model and closing-pitch model are two common processes of nanotubes formation.^[53,54] Closing-pitch model is observed in **SA_{Ala}** with varying pH values. Increasing pH value leads to better solubility of **SA_{Ala}**, so the helical ribbons had greater tendency to form nanotubes. The transformation from helices to nanotubes could be observed on **SA_{Ala}** with ageing time (Figure A3.20a and A3.20b).^[55–57]

Besides, the CD measurement of annealed **SA_{Ala}** varying with pH values was performed to study the helical changes during self-assembly (Figure 3.4). According to the TEM images as shown

in Figure 3.2, the nanotubes of **SA_{Ala}** is stable among all pH values. The CD spectra showed that there was a positive Cotton effect and had sharp peaks at 337 nm, 349 nm, and 370 nm at both neutral and basic condition at different pH conditions. The results implied that the nanotube of **SA_{Ala}** does not dramatically influence the helicity.

The morphological studies of other SAs were also studied in different pH environment. Amino acids, a pH-sensitive functional group, would undergo deprotonation at basic conditions. Therefore, the morphological transformation of peptide-based and amino-acid based soft materials are observed as a result of varying pH.^[35,44,45,58,59] According to TEM images (Figure A3.21a–c), **SA_{Gly}** is an achiral compound but it could also assemble to nanotubes as **SA_{Ala}** at pH = 7–11 (Figure A3.21a–c). The nanotube structure of **SA_{Gly}** at pH = 9 was confirmed by Cryo-TEM (Figure A3.21e). Only nanoaggregates could be observed at pH = 13 (Figure A3.21d). CD spectra showed that there is no helicity at different pH which means the nanotubes formation of **SA_{Gly}** does not involve chirality (Figure A3.22). Similarly, **SA_{Val}** could form nanotubes at neutral condition (pH = 7) and slightly basic condition (pH = 9) (Figure A3.23a, A3.23b, and A3.23e) but disordered structures were observed at strong basic condition (Figure A3.23c,23d). CD spectra showed positive cotton effect as similar to **SA_{Ala}** (Figure A3.24). **SA_{Phe}** could only form nanotubes at pH = 7 (Figure A3.25a,e) and turned to helical ribbons at pH = 9 (Figure A3.25b). However, the nanostructures decomposed at strong basic condition (Figure A3.25c and A3.25d). Unlike **SA_{Ala}** and **SA_{Val}**, **SA_{Phe}** showed negative Cotton effect which is symmetrically opposite to **SA_{Ala}** (Figure A3.26).

Only **SA_{Ala}** among all SAs could form nanotubes at different pH environment even DMSO. Indeed, the nanotubes formation of **SA_{Ala}** follows closing-pitch model as pH values vary. **SA_{Ala}** could exhibit the helical ribbons as the intermediate products during the transformation of nanotubes.^[54,60–62] It reveals the complexity of self-assembly pathway and hierarchical chiral

structures. Therefore, we are interested to investigate the tunable properties of **SA_{Ala}** nanotubes by different preparation methods.

3.4.2 Concentration-dependent assembly of SAs

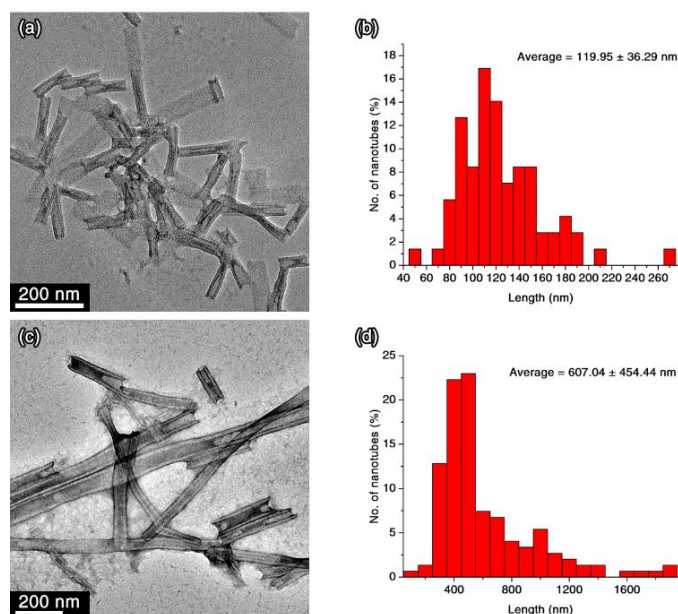


Figure 3.5. TEM images of **SA_{Ala}** nanotubes (0.2 wt.%, 2.58 mM) in water prepared by annealing aqueous solution of **SA_{Ala}** (5.0 wt.%, 65 mM) at 85 °C for (a) 10 min and (c) 30 min, respectively. (b) and (d) are the corresponding histograms of length distribution of **SA_{Ala}** nanotubes.

To study the thermodynamics driven assembly of **SA_{Ala}**, annealing a high concentrated solution of **SA_{Ala}** (5.0 wt.%, 65 mM) at 85 °C for 10 min and 30 min followed by fast cool was performed. TEM image illustrated that only nanotubes with 29.2 ± 4.0 nm width and 120.0 ± 36.3 nm length could be observed for 10 min incubation (Figure 3.5a and 3.5b). Ageing effects on the length of nanotubes were studied by TEM, and the results showed that there was an increase in the nanotubes length (Figure A3.27a,c). The length was increased by nearly 50% and 90% after ageing for 1 month and 2 months, respectively (Figure A3.27b and A3.27d). Although ageing process would result in longer nanotubes, it is not effective and efficient. Therefore, we also investigated the effect of incubation time because longer incubation time would result in longer nanotubes.^[63] As shown in Figure 3.5b and 3.5d, the nanotubes prepared by incubating high concentrated solution

(5.0 wt.%, 65 mM) at 85 °C for 30 min were much longer (607.0 ± 454.4 nm) but the width is relatively non-uniform (44.4 ± 16.1 nm). It reveals that the formation of **SA_{Ala}** nanotubes is concentration-dependent as varying the concentration of peptide may lead to different self-assembled nanostructures.^[64–68] Ghosh *et al.* reported that a biologically active peptide would form random coil at low concentration while twisted β -sheet was found at high concentration because of a slight difference in intermolecular interactions and self-assembly pathways.^[64] It is possible to claim that **SA_{Ala}** molecules are getting close and connected by strong intermolecular H-bonding at high concentration. As a consequence, long nanotubes with irregular width of **SA_{Ala}** were formed. Molecules in a crowded environment prefer to stack together which results in longer nanotubes with irregular width. Indeed, the variation of both width and length of nanotubes is quite large after one-month ageing. As shown in Figure A3.28, there were thick and long nanotube but some thin and short nanotubes observed at the same time. Therefore, these experiments suggested that uniform short and long nanotubes can be controlled by the concentration of **SA_{Ala}**.

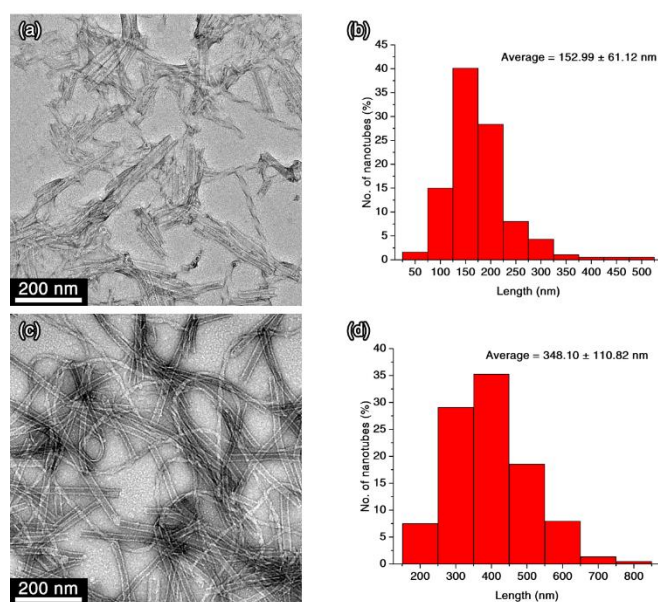


Figure 3.6. TEM images of **SA_{Ala}** nanotubes (0.2 wt.%, 2.58 mM) in water prepared by annealing a 0.2 wt.% solution at 85 °C for 10 min followed by (a) fast cooling and (c) cooling rate of 1 °C/min, respectively. (b) and (d) are the corresponding histograms of length distribution of **SA_{Ala}** nanotubes.

On the other hand, cooling rate is another factor to control self-assembly as alternating cooling rate may result in different morphology. Because molecules have sufficient time to form ordered structures in slow cooling, the resulting morphology usually has greater packing structure. On the contrary, fast cooling may decrease the liquid crystallinity of structure even lead to morphological defects.^[69] A clear solution of **SA_{Ala}** (0.2 wt.%, 2.58 mM) was annealed at 85 °C for 10 min followed by different cooling rates, which then was subject to TEM measurement. A mixture of helical ribbons and nanotubes could be observed by annealing a clear solution of **SA_{Ala}** (0.2 wt.%, 2.58 mM) (Figure 3.6a and 3.6c). The length of nanotubes obtained by slow cooling is 1.3 times longer than those nanotubes obtained by fast cooling (Figure 3.6b and 3.6d). It implies that slow cooling is beneficial to nanotubes lengthening. In short, low concentration, long incubation time, and slow cooling are probably the key factors to obtain nanotubes with regular width and long length.

3.5 Cell viability test of SAs

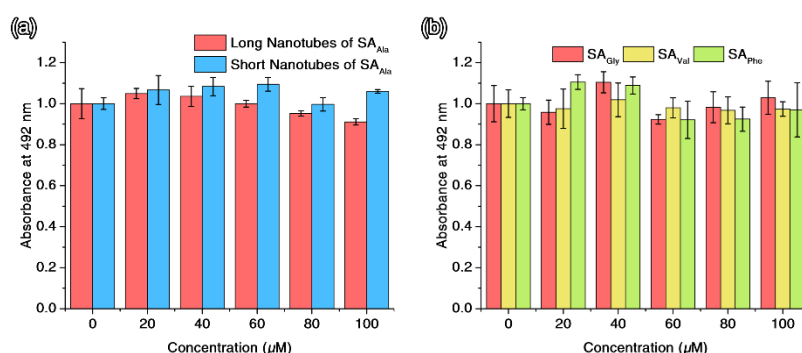


Figure 3.7. Bar chart of MTS assay of (a) short and long nanotubes of **SA_{Ala}** and (b) other SAs on the HeLa cell line.

As the length of nanotubes of **SA_{Ala}** differ by using different preparation methods, the cytotoxicity can be varied with length of nanotubes.^[70] To investigate the cytotoxicity between short and long nanotubes, MTS assay was performed to study the cell viability. In order to investigate the stability of both long and short nanotubes of **SA_{Ala}** at human temperature, stability

test was performed at 37 °C for 12 h prior to MTS assay. UV-vis spectra and CD spectra of both stability tests were not significantly changed, it implied that **SA_{Ala}** is stable at 37 °C (Figure A3.29a, A3.29b, A3.30a, and A3.30b). TEM images showed that both long and short nanotubes of **SA_{Ala}** slightly underwent tube lengthening (Figure A3.29c and A3.30c). As mentioned before, nanotubes of **SA_{Ala}** would grow longer during ageing process at 20 °C. Therefore, it is feasible to claim that storing nanotubes of **SA_{Ala}** at 37 °C could trigger them to grow faster and longer. As shown in Figure 3.7a, the cell viability of both short tubes and long tubes of **SA_{Ala}** is over 90% which implies that **SA_{Ala}** the cytotoxicity of **SA_{Ala}** does not vary too much by length variation. Besides, the cell viability of other SAs were similar as for **SA_{Ala}** (Figure 3.7b).

3.6 Photoisomerization of SAs in aqueous media

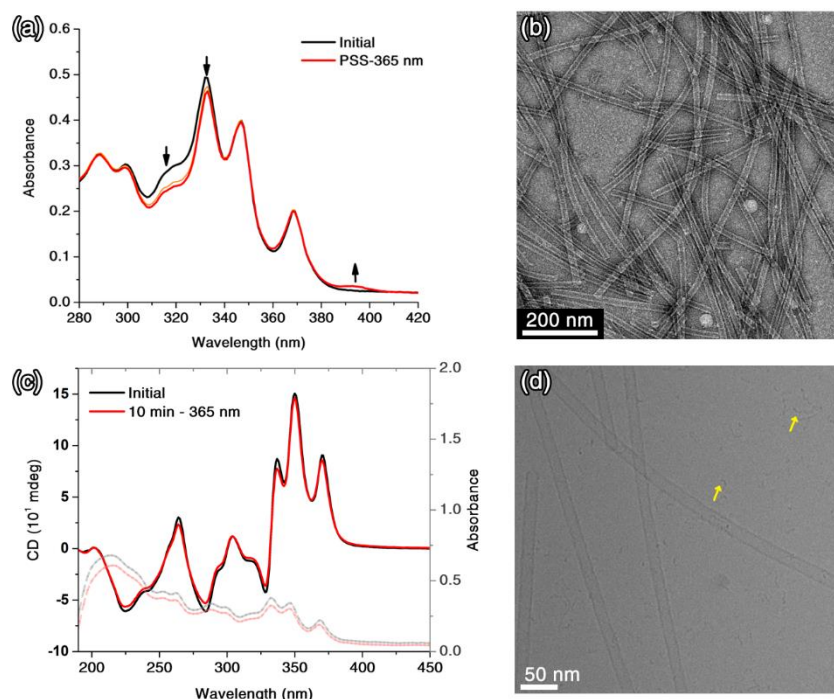


Figure 3.8. (a) UV-vis absorption spectra of **SA_{Ala}** (0.22 mM, pH = 9) after 365 nm irradiation for 6.5 min at 20 °C in water. (c) CD (left axis, solid lines) and UV-vis spectra (right axis, dashed lines) of **SA_{Ala}** (50 μM, pH = 9) after 365 nm irradiation for 10 min (red) at 20 °C in water. (b) TEM image of **SA_{Ala}** (0.2 wt.%, 2.58 mM, pH = 9) in water after 365 nm photoirradiation for 1 h, and (d) the corresponding cryo-TEM image (0.5 wt.%, 6.45 mM, pH = 9).

Since **SA_{Ala}** showed a great self-assembly ability in aqueous media, we would like to investigate the photoswitchability of **SA_{Ala}** in aqueous media. To ensure the solubility of **SA_{Ala}**, the study was performed at pH = 9. An aqueous solution of **SA_{Ala}** shows absorption bands at 320–355 nm and 360–375 nm in the UV-vis spectrum (Figure 3.8a and A3.31a). After 365 nm irradiation at 20 °C for 6.5 min, the absorption bands at 320–355 nm and 360–375 nm were decreased when a new absorption band at 380–410 nm was observed (Figure 3.8a and A3.31a). No further significant spectral shift was observed upon 385/405 nm irradiation which implies that the reverse switching process of **SA_{Ala}** was hindered (Figure A3.31b and A3.31c). In our previous work, we observed the similar phenomenon of a SA during photoirradiation in aqueous media which is mainly caused by the highly packed supramolecular structure of *cis*-SA.^[25] Consequently, the photoswitching back process of *cis*-SA was hindered. Similar photoswitching properties of **SA_{Gly}** could be observed (Figure A3.32). However, only the slow decrease absorption bands at 320–355 nm and 360–375 nm could be observed upon 365 nm irradiation of **SA_{Val}** and **SA_{Phe}** in water (Figure A3.33 and A3.34). Since both **SA_{Val}** and **SA_{Phe}** contain more bulky amino acid groups, the energy barrier of *trans*-to-*cis* isomerization is increased and *cis*-SA is destabilized especially in aqueous media.^[3] The decrease of absorption bands upon 365 nm irradiation of both **SA_{Val}** and **SA_{Phe}** are likely caused by either the decomposition of stiff-stilbene core or some photochemical effects.

Next, the photochemical effects on chirality of **SA_{Ala}** in aqueous media was measured by CD. A degassed aqueous solution of **SA_{Ala}** (50 μ M, pH = 9) illustrated a significant Cotton effect at 300–375 nm, which is mainly caused by the stiff-stilbene core (Figure 3.8c and A3.35). There were no significant changes in CD spectra upon 365 nm irradiation as similar as in DMSO. It reveals that the photoisomerization of **SA_{Ala}** does not affect the chirality because of a minor change upon photoirradiation. Similar CD spectra of other SAs upon 365 nm photoirradiation were obtained (Figure A3.36 and A3.37).

Besides, the morphological transformation of **SA_{Ala}** upon 365 nm light irradiation was investigated by both TEM and cryo-TEM. TEM image shows that the nanotubes of **SA_{Ala}** nearly retained the same after the photoirradiation for an hour (Figure 3.8b). Although TEM is a reliable tool to visualize the self-assembled nanostructures, negative staining and drying procedures can affect the morphology.^[12,51,71,72] To have a better understanding of the morphological transformation of photoirradiated **SA_{Ala}** in a solvated state, cryo-TEM was also performed. Cryo-TEM image shows that a mixture of nanotubes and worm-like micelles, which indicates the transformation of nanotubes to worm-like micelles (Figure 3.8d). Additionally, the photochemical effects on the short nanotubes of **SA_{Ala}** were investigated (Figure A3.38). TEM image shows a mixture of disordered short nanotubes and worm-like micelles and cryo-TEM image also reveals similar results (Figure A3.38). It indicates that the morphological transformation is probably irrelevant to the tubular length. Other SAs with nanotube structure also underwent morphological transformation under light irradiation. Nanotube bundles were observed after the light irradiation of **SA_{Gly}** (Figure A3.39). Upon 365 nm photoirradiation, the nanotubes of **SA_{Val}** were partially transformed to helical ribbons in cryo-TEM (Figure A3.40). The transformation of nanotubes to twisted ribbons was observed in **SA_{Phe}** in cryo-TEM (Figure A3.41). Although the changes of UV-vis and CD spectra of SAs upon photoirradiation were not significant, subtle morphological transformation of SAs nanotubes was observed to confirm the photochemical transformation.

3.7 Co-assembly of Achiral Nanotubes and Chiral Nanotubes

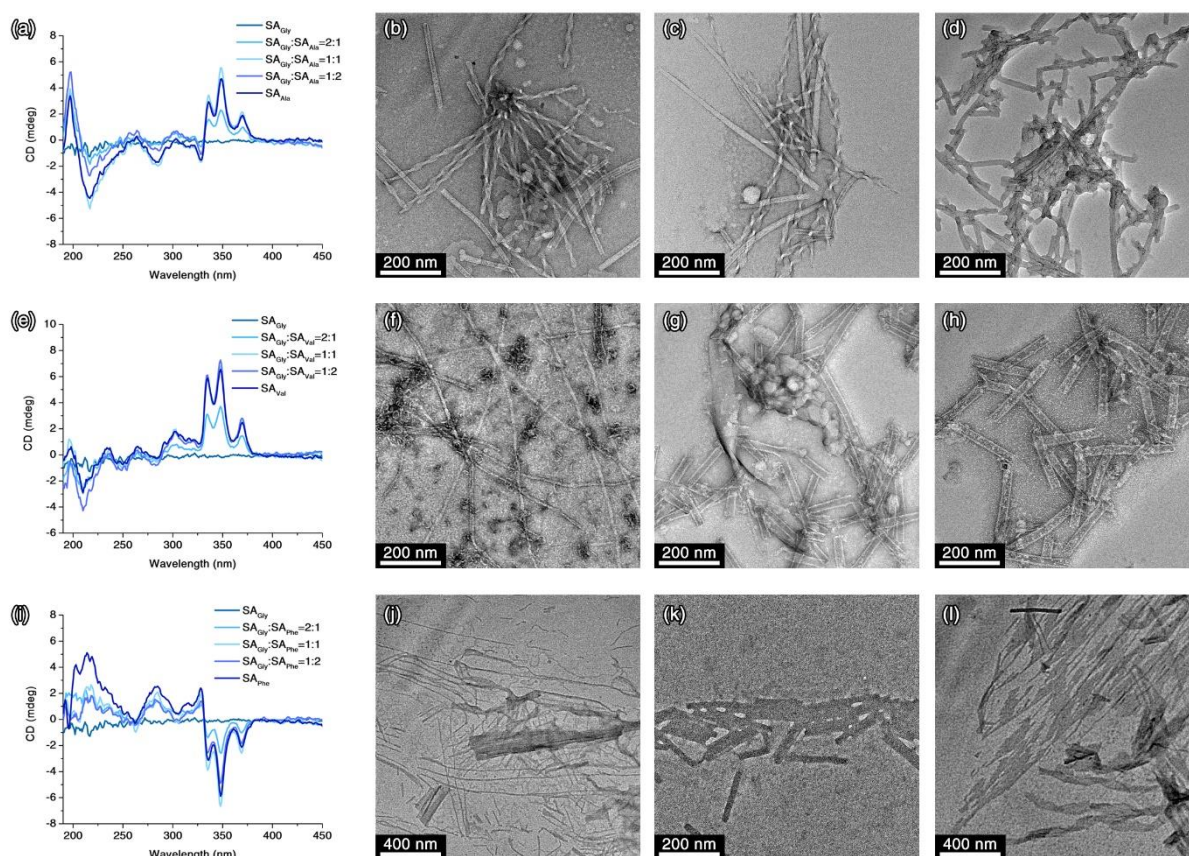


Figure 3.9. CD spectra of co-assembled (a) $\text{SA}_{\text{Gly}}:\text{SA}_{\text{Ala}}$, (e) $\text{SA}_{\text{Gly}}:\text{SA}_{\text{Val}}$, and (i) $\text{SA}_{\text{Gly}}:\text{SA}_{\text{Phe}}$ with different ratio in aqueous media. The corresponding TEM images of co-assembled $\text{SA}_{\text{Gly}}:\text{SA}_{\text{Ala}}$ at (b) 2:1, (c) 1:1, and (d) 1:2 (pH = 9); $\text{SA}_{\text{Gly}}:\text{SA}_{\text{Val}}$ at (f) 2:1, (g) 1:1, and (h) 1:2 (pH = 9), and (c) $\text{SA}_{\text{Gly}}:\text{SA}_{\text{Phe}}$ at (j) 2:1, (k) 1:1, and (l) 1:2 (pH = 7) in aqueous media.

Notably, SAs have tendency to form nanotubes at neutral to slightly basic conditions. SA_{Gly} is an achiral molecule which can assemble to nanotubes (Figure A3.21 and A3.22). On the contrary, SA_{Ala} shows strong CD signal and nanotubes in aqueous media at different pH values. Therefore, we are interested in the co-assembly of achiral nanotubes SA_{Gly} and chiral nanotubes SA_{Ala} . The co-assembly experiment was mainly performed in aqueous media at pH = 9. As shown in Figure 3.9a, the CD spectra of co-assembled $\text{SA}_{\text{Gly}}:\text{SA}_{\text{Ala}}$ exhibit similar Cotton effect as SA_{Ala} alone which means the chirality is from SA_{Ala} . TEM image shows that there are mixed nanotubes of SA_{Gly} and SA_{Ala} without annealing (Figure A3.42a). After annealing the premixed solution of $\text{SA}_{\text{Gly}}:\text{SA}_{\text{Ala}}$, there was

subtle morphological transformation observed (Figure 3.9b–3.9d). As increasing the ratio of **SA_{Ala}**, there is a favour to obtain nanotube structure. Starting with **SA_{Gly}:SA_{Ala}** = 2:1, there was a mixture of nanotubes and dendron-helical ribbons (Figure 3.9b). Increasing the amount of **SA_{Ala}**, a mixture of nanotubes and twisted ribbons was observed in **SA_{Gly}:SA_{Ala}** = 1:1 (Figure 3.9c). The mixture of **SA_{Gly}** and **SA_{Ala}** became nanotubes when **SA_{Gly}:SA_{Ala}** = 1:2 (Figure 3.9d). It is observable that the number of nanotubes increases as rising the amount of **SA_{Ala}**.

It is assumed that the larger size of amino acid could lead to more significant morphological transformation, so the chirality transfer from **SA_{Val}** and **SA_{Phe}** were performed accordingly. The CD spectra of the co-assembled **SA_{Gly}** and **SA_{Val}** are as similar to **SA_{Gly}:SA_{Ala}** (Figure 3.9a,e). However, the morphological transformation as increasing the amount of **SA_{Val}** was more obvious. Premixing equivalent ratio of **SA_{Gly}** and **SA_{Val}** without further treatment showed nanotubes (Figure A3.42b). After annealing the mixture of **SA_{Gly}** and **SA_{Val}** at different ratio, a tendency of nanotubes formation is preferred with rising the content of **SA_{Val}**. A majority of nanofibers was observed in **SA_{Gly}:SA_{Val}** = 2:1 (Figure 3.9f). A mixture of major nanotubes with minor left-handed helical stripe were revealed when **SA_{Gly}** and **SA_{Val}** are at equivalent ratio (Figure 3.9g). Only nanotubes were observed when increasing **SA_{Val}** to **SA_{Gly}:SA_{Val}** = 1:2 (Figure 3.9h). The co-assembly of **SA_{Val}** and **SA_{Gly}** exhibited significant morphological transformation from nanofibers to nanotubes as increasing **SA_{Val}**. Although valine is slightly larger than alanine, the CD spectra of **SA_{Val}** and **SA_{Ala}** are similar. Co-assembly of **SA_{Gly}:SA_{Val}**=1:1 at equivalent ratio would result in a majority of nanotubes when most of nanotubes were observed at **SA_{Gly}:SA_{Ala}**=1:2. It indicated that **SA_{Val}** has greater potential to form nanotubes when co-assembly with **SA_{Gly}**.

Apart from these, **SA_{Phe}** has the greatest size of amino acid among all SAs in this study. The co-assembly of **SA_{Phe}** and **SA_{Gly}** was performed in pH = 7 because the nanotubes of **SA_{Phe}** were only

formed at pH = 9. As the CD spectra of **SA_{Phe}** is totally inverse to **SA_{Ala}**, the CD spectra of co-assembled **SA_{Gly}:SA_{Phe}** showed negative Cotton effect (Figure 3.9i). Similarly, there was not significant changes in helicity after increasing the ratio of **SA_{Gly}:SA_{Phe}** to 1:1. According to TEM images, there was not obvious changes in morphology after premixing the **SA_{Gly}** and **SA_{Phe}** (Figure A3.42c). Unlike **SA_{Ala}** and **SA_{Val}**, co-assembled **SA_{Gly}:SA_{Phe}** = 2:1 and 1:1 would give nanotubes structures (Figure 3.9j and 3.9k). Whereas, increasing **SA_{Phe}** to **SA_{Gly}:SA_{Phe}** = 1:2 resulted in helical nanosheets (Figure 3.9l). In short, the results of co-assembly of **SA_{Gly}:SA_{Phe}** are opposite to **SA_{Ala}** and **SA_{Val}**. It is likely caused by the phenyl group of **SA_{Phe}** which leads to the negative Cotton effect even an inverse trend of morphological transformation in co-assembly with **SA_{Gly}**.

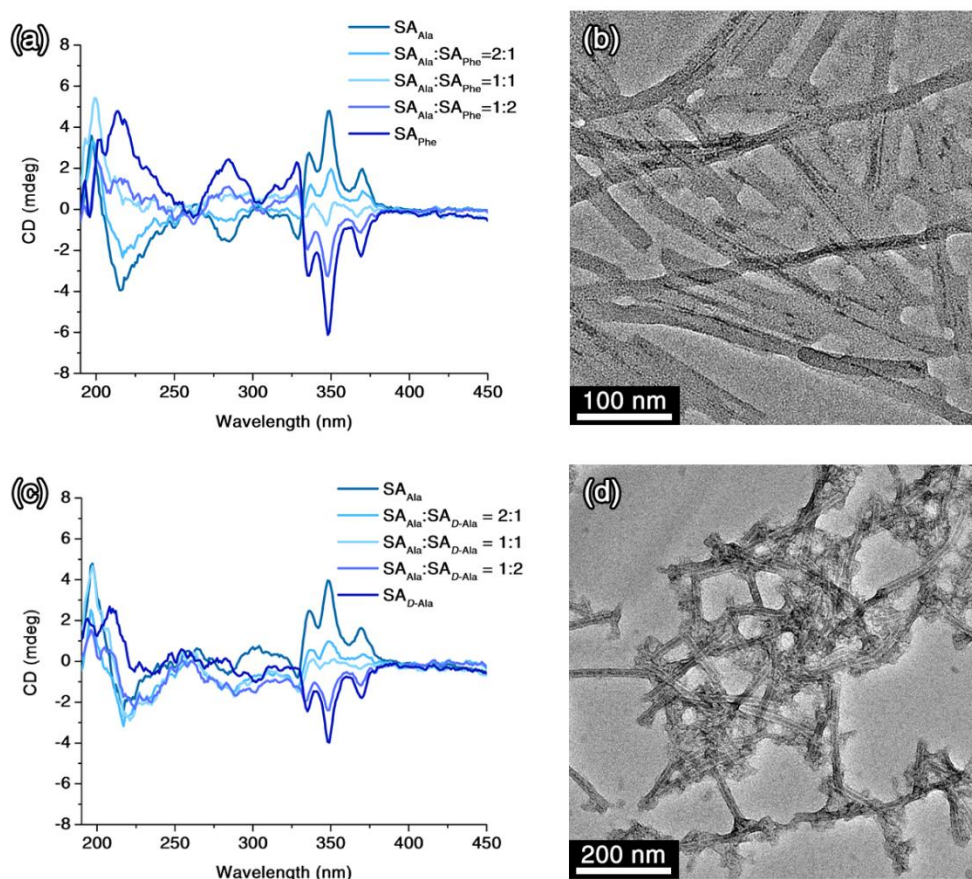


Figure 3.10. CD spectra of co-assembled (a) **SA_{Ala}:SA_{Phe}** (pH = 7) and (b) **SA_{Ala}:SA_{D-Ala}** (pH = 9) with equivalent ratio in aqueous media. The corresponding TEM images of co-assembled (c) **SA_{Ala}:SA_{Phe}** = 1:1 (pH = 7), (d) **SA_{Ala}:SA_{D-Ala}** = 1:1 (pH = 9).

As mentioned previously, there was a helicity inversion observed in **SA_{Ala}** and **SA_{Phe}** so we are interested in the chirality deduction of co-assembled **SA_{Ala}:SA_{Phe}**. It was clearly revealed that the helicity was reaching to zero when **SA_{Ala}** and **SA_{Phe}** are at equivalent ratio (Figure 3.10a). Then, we are interested in the chirality deduction of nanotubes of **SA_{Ala}** and **SA_{Phe}**. The nanotubes of **SA_{Ala}** and **SA_{Phe}** were observed after premixing **SA_{Ala}** and **SA_{Phe}** (Figure A3.42d). Surprisingly, nanotube structure was observed after annealing the premixed solution of **SA_{Ala}:SA_{Phe}** at equivalent ratio (Figure 3.10b). In order to have a better understanding of the effects of chirality deduction on self-assembly, the assembly of 1:1 racemic mixture of **SA_{Ala}** and **SA_{D-Ala}** was performed. The morphology of **SA_{D-Ala}** was nanotubes which were nearly as same as **SA_{Ala}** (Figure A3.43). The CD spectra of **SA_{D-Ala}** were opposite to **SA_{Ala}** as expected (Figure A3.44). As shown in the CD spectra (Figure 3.10c), the CD signals were approaching to zero if **SA_{Ala}** and **SA_{D-Ala}** were at equivalent ratio. Similar to previous studies, nanotubes were obtained after premixing **SA_{Ala}** and **SA_{D-Ala}** without further annealing (Figure A3.42e). More importantly, only nanotubes were observed from the co-assembled racemic **SA_{Ala}:SA_{D-Ala}** (Figure 3.10d). Although it looks rare to obtain the same structure from racemic mixture, it may indicate that the formed nanotubes of are nonhelical.^[73]

3.8 Macroscopic Supramolecular Assembled Soft Materials of Stiff Stilbene Amphiphiles in an Inorganic Salt Solution

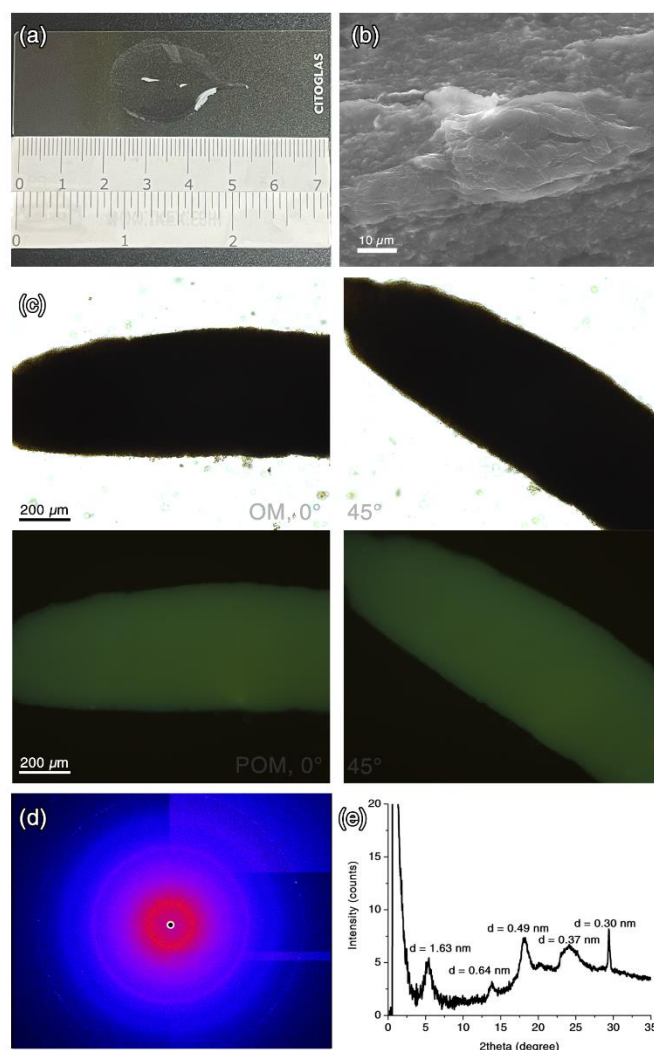


Figure 3.11. (a) Photograph of aqueous solutions of SA_{Ala} (5 wt.%, 65 mM, annealed at 85 °C for 10 min) was ejected into a shallow pool of CaCl_2 solution (150 mM). (b) SEM image of SA_{Ala} (5.0 wt.%, 65 mM) prepared from a solution of CaCl_2 (150 mM). (c) Optical microscopic images of a macroscopic string composed of SA_{Ala} prepared from a solution of CaCl_2 (150 mM) without (top) and with (bottom) crossed polarizers at 0° and 45° . (d) 2D WAXD images and (e) 1D WAXD pattern of a macroscopic aligned string of SA_{Ala} with 20 min of exposure time.

Being with high stability of SA_{Ala} in aqueous media, an off-white macroscopic string of SA_{Ala} (5.0 wt.%, 65 mM, annealed at 85 °C for 10 min) was prepared from a solution of CaCl_2 (150 mM) (Figure 3.11a). Nanoribbons of SA_{Ala} with rough surface and limited alignment were observed by scanning electron microscopy (SEM) (Figure 3.11b). The macroscopic string of SA_{Ala} with $\sim 400 \mu\text{m}$

diameter was revealed under optical microscopy (OM) but there was no significant birefringence with crossed polarizers (Figure 3.11c).

Although long incubation time was applied in microscopic studies, drying out may occur in concentrated solution. Besides, annealing a 5.0 wt.% solution at 10 min is enough for the macroscopic string formation. There was no significant difference in microscopic images of **SA_{Ala}** (5.0 wt.%, 65 mM) between annealed for 10 min and 70 min (Figure 3.11b, 3.11c, and A3.45). Therefore, a macroscopic string of **SA_{Ala}** is prepared by annealing a high-concentrated solution (5.0 wt.%, 65 mM) for 10 min. In the 2D WAXD image of **SA_{Ala}** with 20 min of exposure, several diffraction rings were observed (Figure 3.11d). The diffraction arcs with d -spacings of 1.63 nm is caused by half of the **SA_{Ala}** molecules. 0.49 nm and 0.37 nm correspond to π - π stacking between stiff-stilbene cores while 0.64 nm and 0.30 nm indicated the helical assembly of **SA_{Ala}**.

Other SAs could form a macroscopic string as **SA_{Ala}**. A macroscopic string of **SA_{Gly}** with non-uniform surface was formed in calcium chloride solution (Figure A3.46a and A3.46b). No birefringence was observed in microscopy (POM) (Figure A3.46c). Two diffraction rings were observed and the diffraction arcs with d -spacings 0.47 nm indicated π - π stacking between stiff-stilbene cores (Figure A3.46d,e). Similarly, an off-white macroscopic string of **SA_{Val}** (Figure A3.47a) showed smooth surface without birefringence (Figure A3.47b and A3.47c). Diffraction rings are observed in 2D WAXD and the diffraction arcs with d -spacings of 1.75 nm means half of **SA_{Val}** molecules, 0.48 nm reveals π - π stacking, and 0.30 nm indicates the helical assembly of **SA_{Val}** (Figure A3.47d and A3.47e). An off-white macroscopic string of **SA_{Phe}** (Figure A3.48a) with rough surface (Figure A3.48b) was formed and no birefringence was shown (Figure A3.48c). No diffraction rings are observed in 2D WAXD which indicates that no electron diffraction pattern is shown for **SA_{Phe}**.

(Figure A3.48d). Based on the WAXD results, **SA_{Ala}** has the strongest alignment among all SAs while other SAs show limited alignment.

The photoresponsiveness of **SA_{Ala}** macroscopic string was examined under OM. The macroscopic string of **SA_{Ala}** retained 95% of its original size upon 365 nm for 1.5 h (Figure A3.49) while no significant change in the size without photoirradiation (Figure A3.48). It implies that the macroscopic of **SA_{Ala}** is slightly responsive to light. Other SAs showed similar results as **SA_{Ala}** (Figure A3.51–A3.53).

3.9 Cell-gel interactions

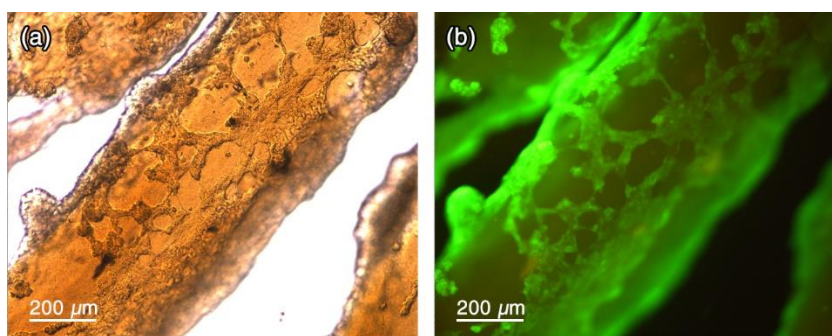


Figure 3.12. (a) OM and (b) fluorescent image of HeLa cell on a macroscopic string of **SA_{Ala}** (5 wt.%, 65 mM) at 10x magnification.

As mentioned **SA_{Ala}** has good cell viability in MTS assay, the biocompatibility of macroscopic string of **SA_{Ala}** was investigated through attaching HeLa cells on the surface of string. A macroscopic string of **SA_{Ala}** (5 wt.%, 65 mM) was manually injected to CaCl_2 followed by adding HeLa cells. After incubation for 4 days, HeLa cells were attached and elongated on the surface of **SA_{Ala}** which was visualized by OM and fluorescent microscopy (Figure 3.12). It indicates that the macroscopic string of **SA_{Ala}** could serve as a cell culture platform for cell–material interface. The macroscopic string of **SA_{Gly}** also showed biocompatibility in HeLa cells (Figure A3.54) while **SA_{Val}** and **SA_{Phe}** destabilized in growth medium. The macroscopic string has potential to be applied for cell growth in the future.

3.10 Summary

A series of stiff-stilbene amphiphiles functionalized with different amino acids was synthesized and studied from atomic level to macroscopic level. The photochemical studies of SAs in both organic media and aqueous media were examined by UV-Vis spectroscopy, ^1H NMR, and TEM. Only self-assembled **SA_{Ala}** could form nanotubes structure in DMSO, and aqueous media with different pH among all SAs. Indeed, **SA_{Ala}** showed closing-pitch model as increasing the pH value. More importantly, the length of **SA_{Ala}** nanotubes could be controlled by annealing conditions such as concentration, time, and cooling rate; and both short and long nanotubes are biocompatible. Since all SAs could form nanotubes at neutral to slightly basic conditions, chirality induction to nanotubes of achiral **SA_{Gly}** was investigated. As increasing the ratio of **SA_{Ala}**, the co-assembled **SA_{Gly}:SA_{Ala}** has a greater tendency to form nanotubes. On the contrary, **SA_{Phe}**, being with an opposite helicity with **SA_{Ala}**, showed an inverse trend of structural change in chirality transfer. On the other hand, chirality deduction of racemic **SA_{Ala}** would resulted in nonhelical nanotubes. At macroscopic level, a macroscopic string of **SA_{Ala}** revealed diffractions in WAXD and showed limited photoresponsiveness. Moreover, the **SA_{Ala}** macroscopic string could act as a cell culture platform. This work illustrates multi-functional **SA_{Ala}** across different length scale and it has potential to be applied as biological functional materials.

3.11 References

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Chapter 4: Experimental Section

4.1 General section

All commercial reagents were purchased from Acros Organics, Aladdin, Alfa Aesar, Bidepharm, Dieckmann, Macklin, Sigma Aldrich and Tokyo Chemical Industry Co. Ltd, and were used as received. All solvents used in the reactions were purchased from Acros, Anaqua and Duksan. Analytical thin layer chromatography (TLC) was performed on ALUGRAM Xtra SIL G UV254 plates and visualized by UV light or staining with phosphomolybdic acid (PMA) solution followed by heating. Flash column chromatography was performed using Macherey-Nagel Silica gel 60 (230-400 mesh). Deuterated solvents were purchased from Cambridge Isotope Laboratories Inc. Photoirradiation experiment was performed in a dark box and repeated at least twice" was added.

NMR spectra were recorded at 25 °C on a Bruker Advance-III 400 MHz FT-NMR spectrometer (^1H : 400 MHz, ^{13}C : 101 MHz) and a Bruker Advance-III 600 MHz FT-NMR spectrometer (^1H : 600 MHz, ^{13}C : 151 MHz). The deuterated DMSO- d_6 was degassed by argon prior to use. Chemical shifts (δ) are expressed relative to the resonances of the residual non-deuterated solvent for ^1H NMR [CDCl_3 : $^1\text{H}(\delta) = 7.26$ ppm, DMSO- d_6 : $^1\text{H}(\delta) = 2.50$ ppm, MeOD- d_4 : $^1\text{H}(\delta) = 3.31$ ppm] and ^{13}C NMR [CDCl_3 : $^{13}\text{C}(\delta) = 77.2$ ppm, DMSO- d_6 : $^{13}\text{C}(\delta) = 39.5$ ppm, MeOD- d_4 : $^{13}\text{C}(\delta) = 49.0$ ppm]. Absolute values of the coupling constants are given in Hertz (Hz), regardless of their sign. Multiplicities are abbreviated as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), triplet of doublets (td), quartet (q), multiplet (m), and broad (br). High-resolution mass spectrometry (HRMS) was performed on an Agilent 6540 LC-ESI Quadrupole-Time-of-Flight Mass Spectrometer. All reactions were performed under anhydrous conditions under N_2 atmosphere.

UV-vis measurement was performed on an Agilent Cary 60 UV-Vis spectroscopy in a 1 cm path length quartz cuvette. Irradiation of samples in organic media was carried out at 293 K using Thorlabs M365F1 LED (4.1 mW) and M385F1 LED (10.7 mW) positioned at a distance of 1 cm from the samples. Irradiation process in aqueous phase was carried out by using Thorlabs M365LP-C1 (745 mW), M385LP-C1 (795 mW) and M405LP-C1 (750 mW) at 293 K. Dynamic light scattering assay was performed on a Wyatt Technology DynaPro NanoStar. Circular dichroism measurement was performed on a JASCO J-1500 CD Spectrophotometer in a 1 mm path length quartz cuvette (a 1 cm path length sealed quartz cuvette was used for photoisomerization experiment).

Transmission electron microscope (JEOL Model JEM-2010), field emission transmission electron microscope (JEOL Model JEM-2011F), scanning electron microscope (Tescan VEGA3), and optical microscope with polariser (Leica DM2700 P) were used to characterise the morphology.

4.2 Transmission Electron Microscopy

To study the morphology of self-assembled SA, the sample solutions (5.0 μL) were deposited onto a carbon grid (Micro to Nano, EMR Carbon support film on copper, 400 square mesh) for 20 s. After blotting to remove the solution, UranylLess EM stain solution (Electron Microscopy Science, 5.0 μL) was directly deposited onto the grid for 20 s and blotting to remove the stain. Grids were observed in a JEOL Model JEM-2010 Transmission Electron Microscope with hair pin type tungsten filament operating at 120 kV equipped with Gatan 794 CCD camera. The study of helicity of SA at different pH values was performed on a JEOL Model JEM-2100F operating at 200 kV FE (field emission) analytical electron microscope.

4.3 Cryogenic Transmission Electron Microscopy

To observe the self-assembly structures by cryo-transmission electron microscopy (cryo-TEM), 2.5 μL of SA solution was placed on a glow-discharged holey carbon coated grid (Quantifoil 3.5/1, QUANTIFOIL Micro Tools GmbH, Großlöbichau, Germany). After blotting, the corresponding grid was rapidly frozen in liquid ethane (Vitrobot, FEI, Eindhoven, The Netherlands) and kept in liquid nitrogen until measurement. The grids were observed with a Gatan model 626 cryo-stage in a Tecnai T20 (FEI, Eindhoven, The Netherlands) cryo-electron microscope operating at 200 keV. Cryo-TEM images were recorded under low-dose conditions on a slow-scan CCD camera.

4.4 Scanning Electron Microscopy and Polarized Optical Microscopy Analysis

Polarized optical microscopy (POM) was performed on a Leica DM2700-P optical polarizing microscope. Scanning electron microscopy (SEM) was performed on a Tescan VEGA3 Scanning Electron Microscope. Preparation of a string of SA on a glass substrate: SA (5 wt.%) with 7 equivalents of NaOH was annealed at 85 °C for 10 min, then cooled down to room temperature to form a milky solution. When an aqueous solution of SA (5.0 wt.%, 2 μL) was manually drawn into an aqueous solution of CaCl_2 (150 mM) from a pipette, a string with arbitrary length was formed. After removal of the solution of metal chloride, the string was washed with MilliQ water (three times), the resulting string was used directly for POM and SEM experiments. A string for SEM was directly prepared on conductive carbon adhesive tape and dried in air for 72 h before measurement. The air-dried samples were subject to gold sputtering (MCM-200 ion sputter coater) for 30 min prior to SEM measurement.

4.5 Dynamic Light Scattering

The scattering intensities of the samples were determined by dynamic light scattering measurement on a Wyatt Technology DynaPro NanoStar. The scattering intensities were recorded as a parameter for assembly size, since the objects in solutions are anisotropic and the models used by Wyatt software are fitting for spherical objects. To determine the critical aggregation concentration (CAC) of SA the scattering intensities of the solutions of SA (concentration: 1.0×10^{-4} to 1 mM) were recorded at 20 °C. This scattering rate was normalised by the concentration of the solution to yield the molar scattering intensity (on M Counts $s^{-1} M^{-1}$). Five replications were performed and the data was averaged to show the molar scattering intensity and its error standard deviation.

4.6 Small-Angle X-ray Scattering and Wide-Angle X-ray Diffraction

Small-angle X-ray scattering (SAXS) and wide-angle X-ray diffraction of a string of **SA_{Ala}** (5.0 wt.%, 65 mM) were measured on a sapphire substrate ($\varphi = 2.0$ cm) using the Rigaku NANOPIX equipped with a HyPix-6000 (Rigaku) detector. The scattering vector ($q = 4\pi\sin\vartheta/\lambda$), scattering angle ϑ and the position of the incident X-ray beam on the detectors were calibrated using several orders on layer reflections from silver behenate ($d = 58.380$ Å), where λ refers to the wavelength of the X-ray beam (CuK α , 1.54 Å). The sample-to-detector distances were 729 mm for SAXS and 101 mm for WAXD. The obtained diffraction patterns were integrated along the Debye-Scherrer ring to afford 1D intensity data using the Rigaku 2DP software.

4.7 Circular Dichroism

Circular dichroism (CD) measurement was performed on a JASCO J-1500 CD Spectrophotometer at 25 °C in a 1 mm path length quartz cuvette. CD spectra were recorded in the range of 190 – 450 nm. A scanning rate of 100 nm/min, a bandwidth of 1 nm, a data pitch of 1.0 nm, and single accumulation were applied. Photoisomerization experiment in aqueous media was performed at the same condition except for using a 1 cm sealed cuvette.

4.8 MTS Assay for Cell Viability

HeLa cell was cultured in growth medium: Dulbecco's Modified Eagle Medium (DMEM, Gibco™ thermofisher:11965092) supplemented with Fetal bovine serum (1:10 to DMEM, Gibco™ thermofisher:10270106) and Antibiotic-Antimycotic (1.1:100 to DMEM, Gibco™, thermofisher:15240062), under 37 °C, 5% CO₂. 5,000 HeLa cells were seeded on 96-well. For the treatment, cells were incubated with 140 µL of SA in growth medium for one day. After that, each well received 28 µL MTS solution (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega) and further incubated for one hour. Absorbance at 492 nm of the samples was measured via Labexim Products LEDETECT 96 Microplate Reader, referenced with 620 nm (n = 3).

4.9 Cytotoxicity Studies of Macroscopic Strings

The hydrogel was prepared by injecting 1 μ L of SA (5 wt.%) in 150 mM CaCl_2 on bioinert culture dish (μ -Dish. ibidi 81150). Hydrogels were prepared in each dish. For SA, 2.5 mL growth medium containing 500,000 HeLa cells was added to each bioinert culture dish. The sample was incubated for 3 days. After that, 1 mL medium was extracted and 1 mL growth medium with 1 μ M Calcein AM (Invitrogen™, thermofisher: C3100MP) was added to the samples. Live HeLa cells were labelled by incubation for 30 min. The Calcein AM containing medium was extracted and growth medium was added to the sample. Finally, most of the growth medium was removed and the samples were imaged under Leica DM 2700P.

Chapter 5: Conclusion

In this thesis, I aimed to study the photochemical and self-assembly of stiff-stilbene amphiphiles across different length scales. Because stiff-stilbene has a relatively planar structure among all photoswitches, it has preference to form morphology with higher packing parameters. The results showed that all stiff-stilbene bola amphiphiles in this study have potential to form nanotubes.

In chapter 1, stiff-stilbene amphiphiles functionalized with anionic phosphite (**SA_P**) and cationic quaternary ammonium (**SA_N**) were successfully synthesized. Both SAs showed good photoswitchability in organic media and only **SA_P** showed photoresponsive properties in aqueous media. Nanotubes formation of **SA_P** and **SA_N** was confirmed by TEM. The co-assembly of anionic nanotubes (**SA_P**) and cationic nanotubes (**SA_N**) would result in nanoribbons at microscopic level. Upon photoirradiation, the nanoribbons would transform to nanotubes which belong to **SA_P**. Although both **SA_P** and **SA_N** could not form macroscopic string in inorganic salt solution, the co-assembly of **SA_P** and **SA_N** gives a stable macroscopic string in the absence of inorganic salt. Indeed, the macroscopic string of **SA_P:SA_N** was photoresponsive which may be applied to smart soft robotics in the future.

In chapter 2, a series of stiff-stilbene amphiphiles functionalized with amino acids was synthesized. All SAs showed great photoswitchability in organic media while only **SA_{Ala}** and **SA_{Gly}** which bear smaller size of amino acids could undergo photoisomerization in aqueous media. Moreover, all SAs have preference to form nanotubes at neutral even slight basic conditions and they are biocompatible. Surprisingly, the nanotubes of **SA_{Ala}** are stable at all pH conditions and applicable to closing-pitch model. Indeed, the length of nanotubes was controlled by different assembly conditions to facilitate biological applications. On the other

hand, all SAs can form macroscopic string in inorganic salt solution and show limited photoreponsiveness. Moreover, the macroscopic string of **SA_{Gly}** and **SA_{Ala}** could be utilized as the cell culture platform. In short, SAs with smaller size of amino acids could show greater photoswitchability in aqueous media and form a stable macroscopic string for further biological studies.

In the future, it is expected to perform the detailed studies of photoresponsive SAs in different perspectives including tissue engineering, drug delivery, and phototherapies.

Appendices

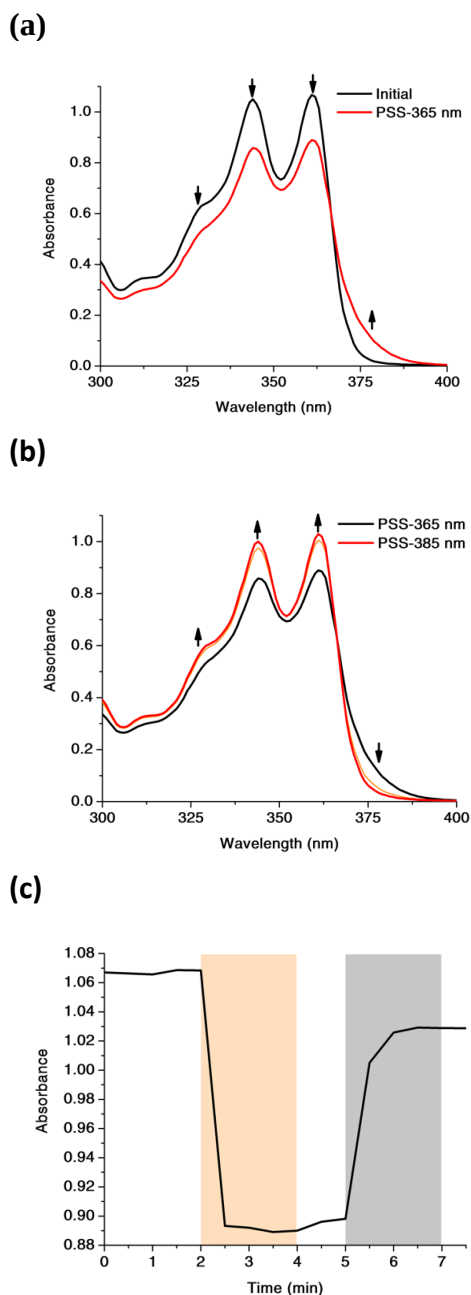
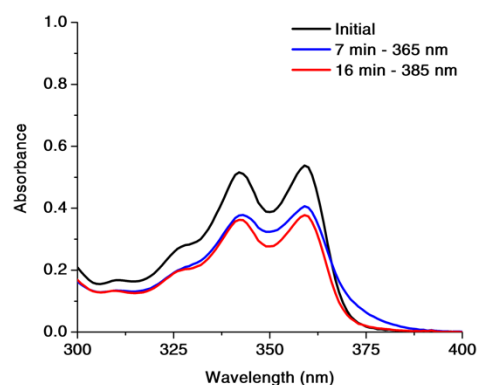
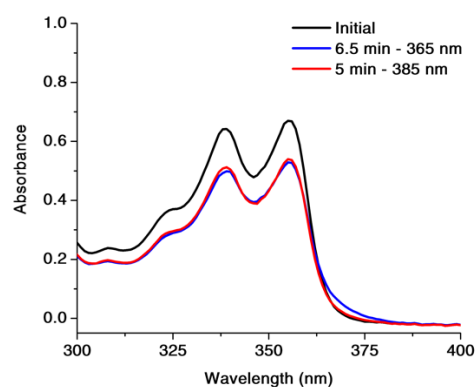


Figure A2.1: UV-vis absorption spectra of the **SA_P** in DMSO (68 μ M). (a) Absorption spectra of **SA_P** in PSS (red) after 365 nm irradiation for 120 s, (b) in PSS (red) after 385 nm irradiation over 120 s (orange). (c) Time-course of the photoisomerization process of **SA_P** in DMSO, monitored at λ_{max} 360 nm of *trans*-**SA_P** (orange area: photoirradiation at 365 nm period; grey area: photoirradiation at 385 nm period). Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample.

(a)



(b)



(c)

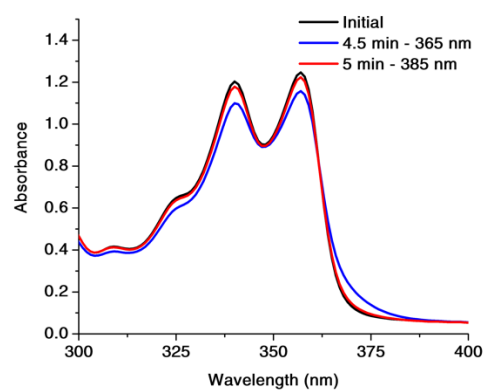


Figure A2.2: UV-vis spectra of SA_p upon photoirradiation at 365 nm, and followed by 385 nm in different organic solvent. (a) in DCM ($27 \mu\text{M}$), (b) in MeOH ($50 \mu\text{M}$), (c) in ACN ($170 \mu\text{M}$). Irradiation of UV-vis sample was carried out at 20°C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample.

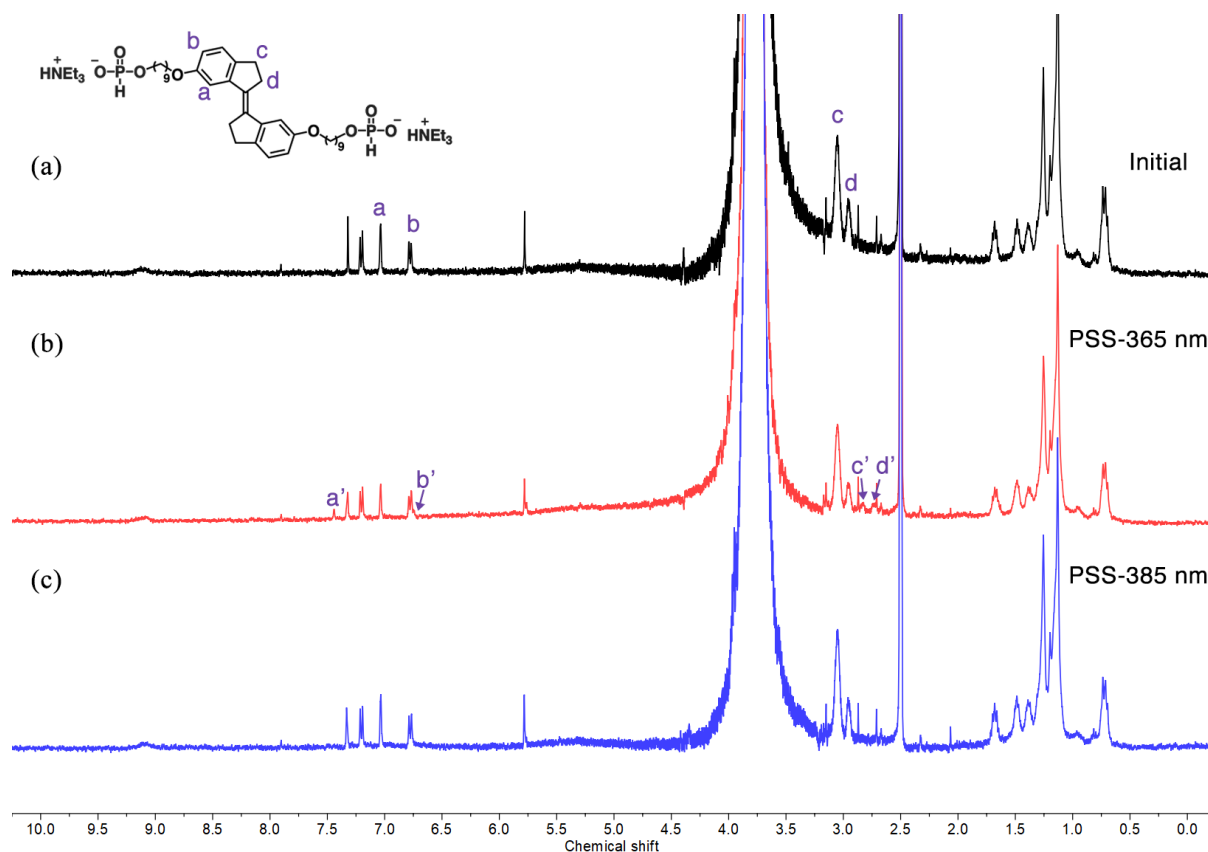


Figure A2.3: ^1H NMR spectra during isomerization process of SAp (3.2 mM, $\text{DMSO-}d_6$, 400 MHz), (a) before photoirradiation, (b) irradiated with 365 nm for 180 min at 20 °C, (c) followed by 385 nm irradiation for 120 min at 20 °C. Irradiation of sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample. The ^1H NMR signals at 0.68 – 0.8 ppm are from grease oil; 2.7 ppm and 2.9 ppm are from DMF residue.

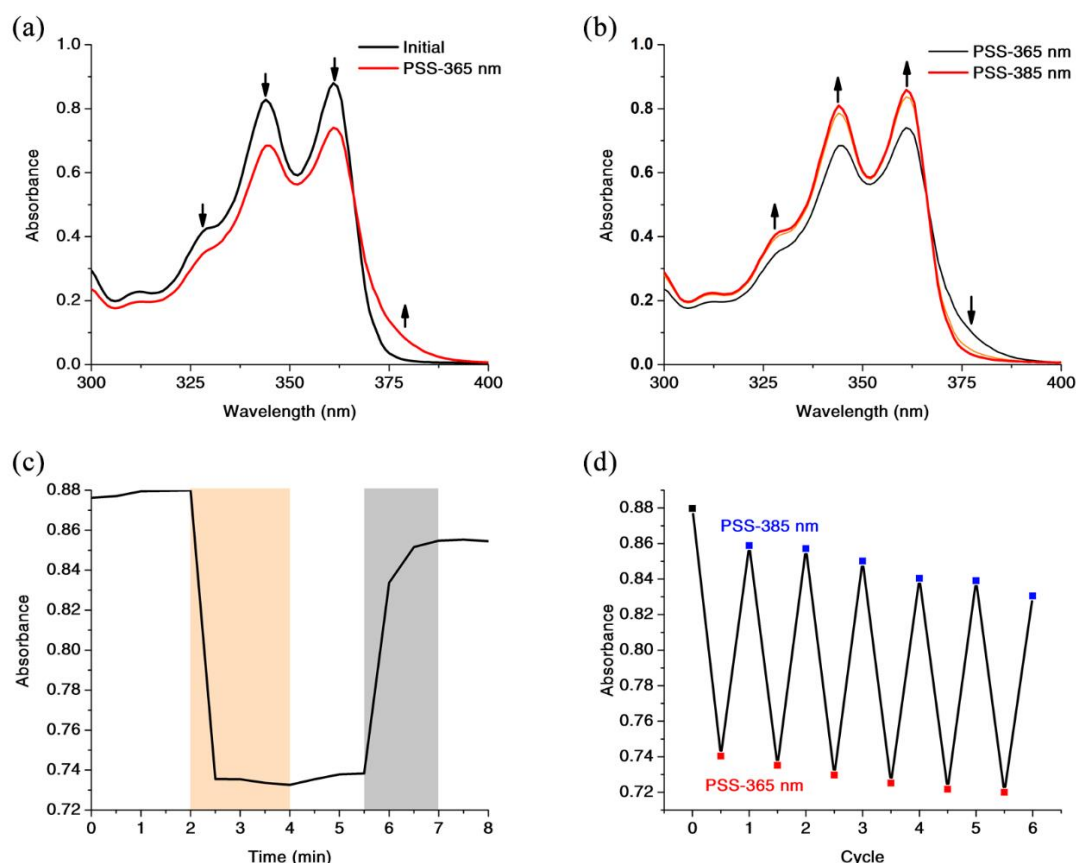


Figure A2.4: UV-vis absorption spectra of the SA_N in DMSO ($27 \mu\text{M}$). (a) Absorption spectra of SA_N in PSS (red) after 365 nm irradiation for 120 s, (b) in PSS (red) after 385 nm irradiation over 90 s (orange). (c) Time-course of the photoisomerization process of SA_N in DMSO, monitored at λ_{max} 360 nm of *trans*- SA_N (orange area: photoirradiation at 365 nm period; grey area: photoirradiation at 385 nm period). Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample.

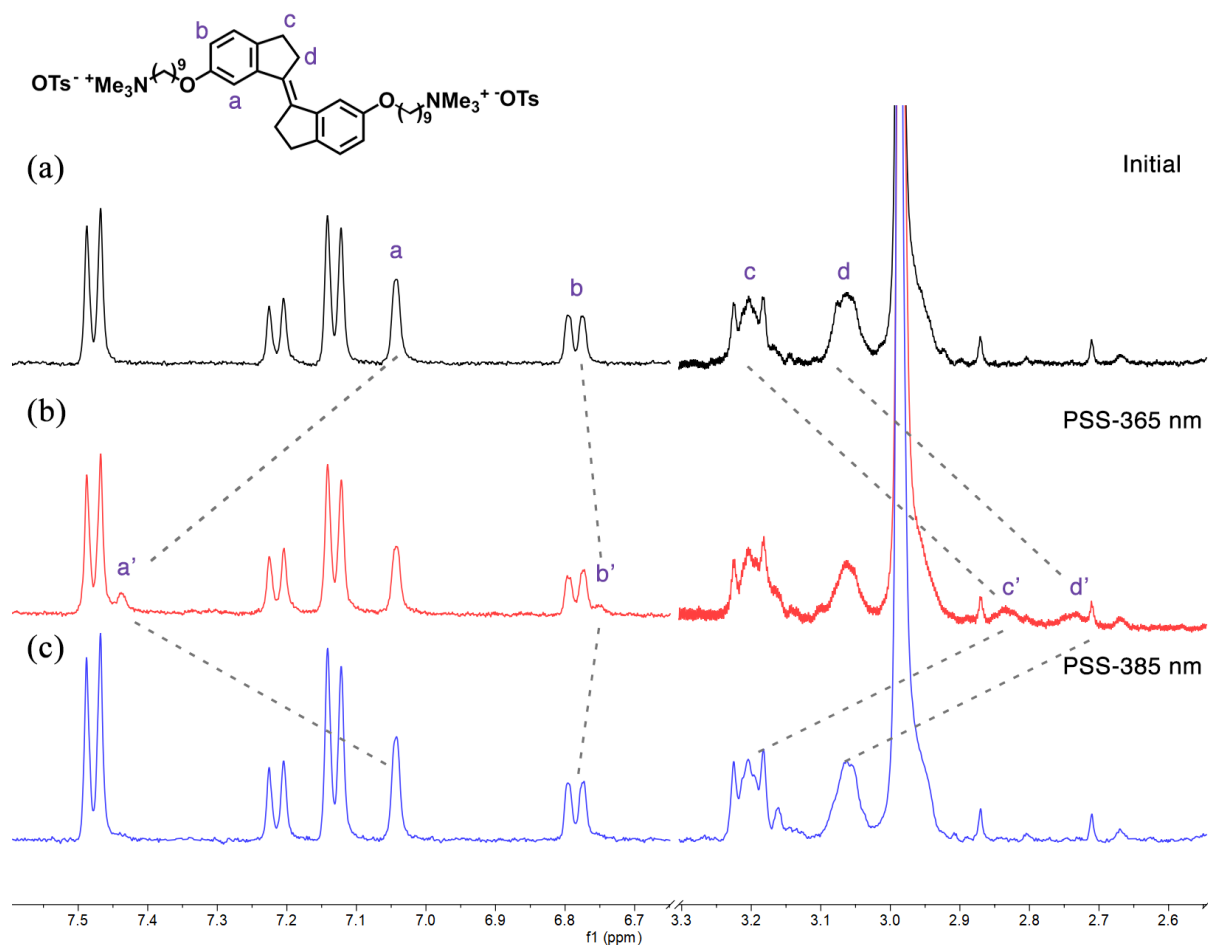
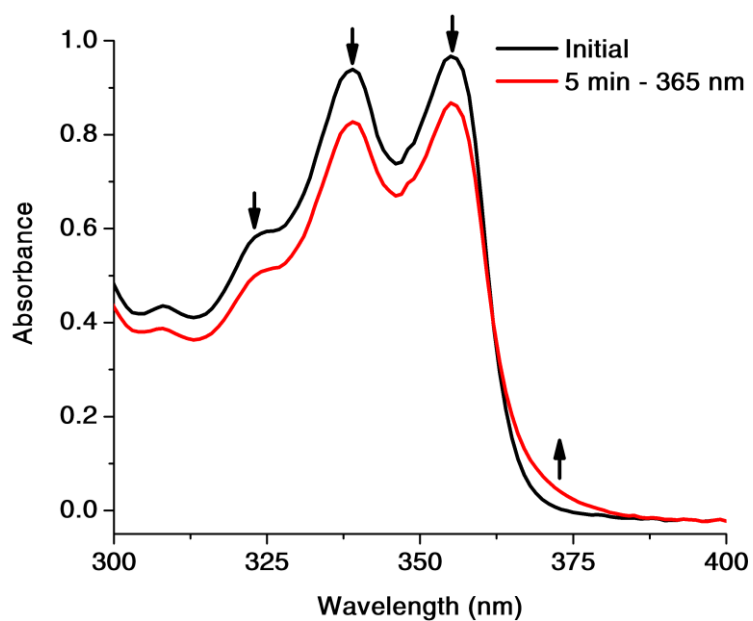


Figure A2.5: Aromatic and aliphatic region in the ^1H NMR spectra during isomerization process of SA_N (2.9 mM, $\text{DMSO}-d_6$, 400 MHz), (a) before photoirradiation, (b) irradiated with 365 nm for 180 min at 20 °C, (c) followed by 385 nm irradiation for 120 min at 20 °C. Irradiation of sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample. The ^1H NMR signals at 2.71 ppm and 2.87 ppm are from DMF residue.

(a)



(b)

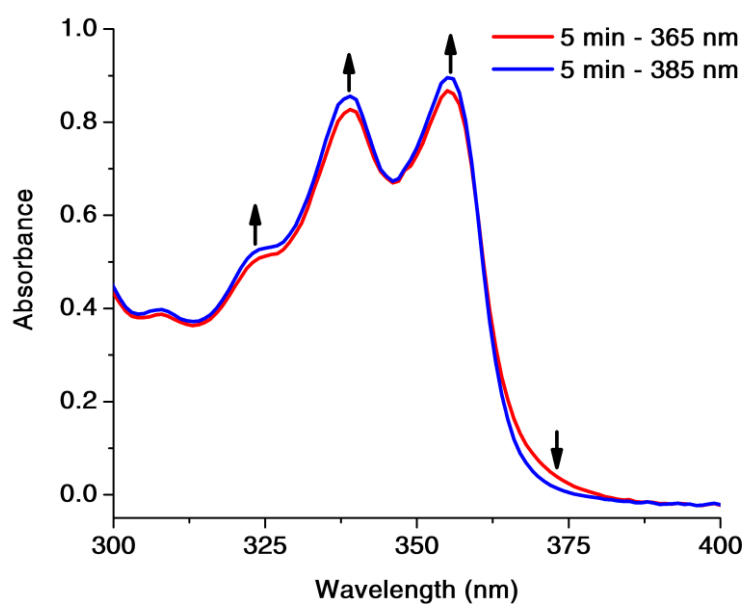


Figure A2.6: UV-vis spectra of SA_N upon photoirradiation at (a) 365 nm, and (b) followed by 385 nm in MeOH (50 μ M). Irradiation of UV-vis sample was carried out at 20 $^{\circ}$ C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample.

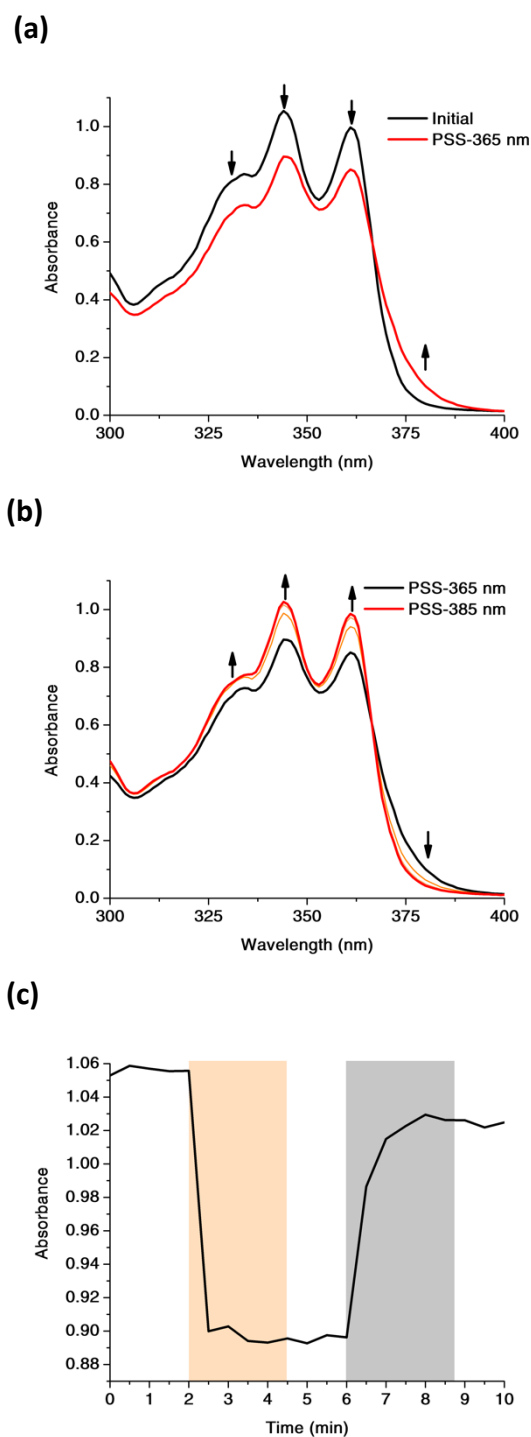


Figure A2.7: UV-vis absorption spectra of the **SA_P:SA_N** in DMSO (68 μ M). (a) Absorption spectra of **SA_P:SA_N** in PSS (red) after 365 nm irradiation for 150 s, (b) in PSS (red) after 385 nm irradiation over 150 s (orange). (c) Time-course of the photoisomerization process of **SA_P:SA_N** in DMSO, monitored at λ_{max} 344 nm of *trans*-**SA_P:SA_N** (orange area: photoirradiation at 365 nm period; grey area: photoirradiation at 385 nm period). Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample.

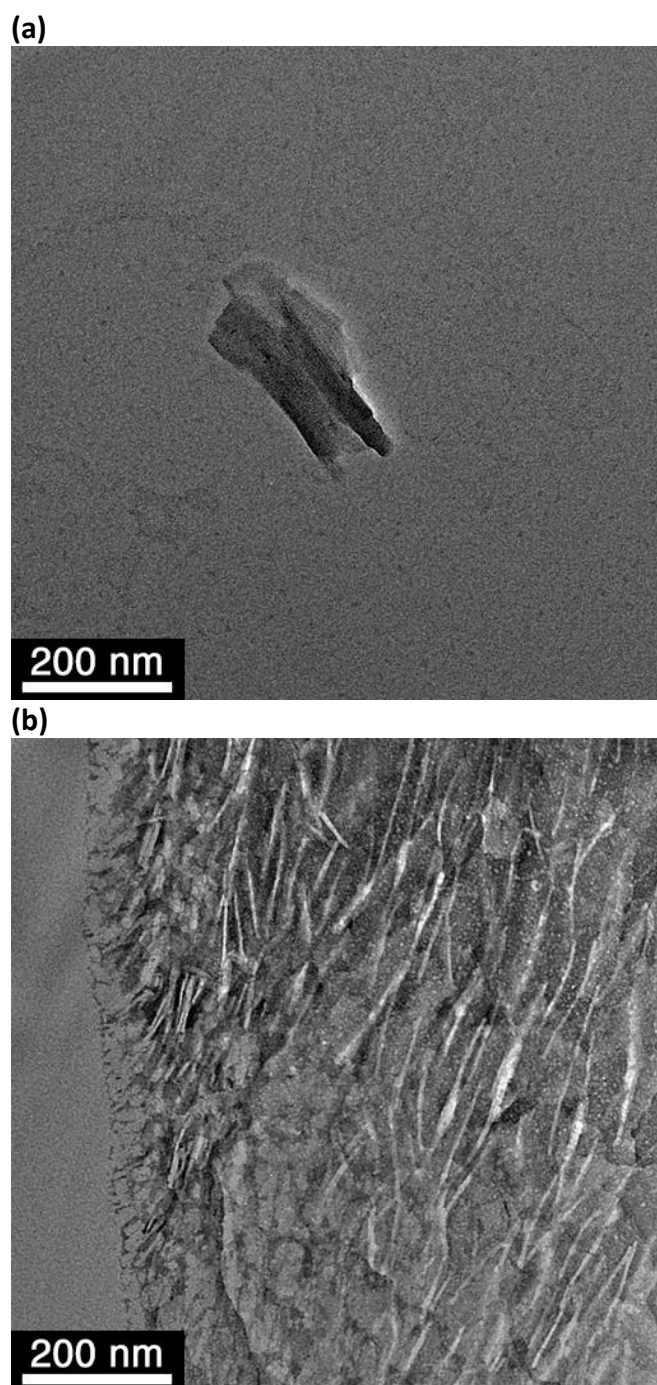


Figure A2.8: TEM images of unannealed (a) SA_P (2 mM), (b) SA_N (2 mM) in HEPES (25 mM, pH = 7.1).

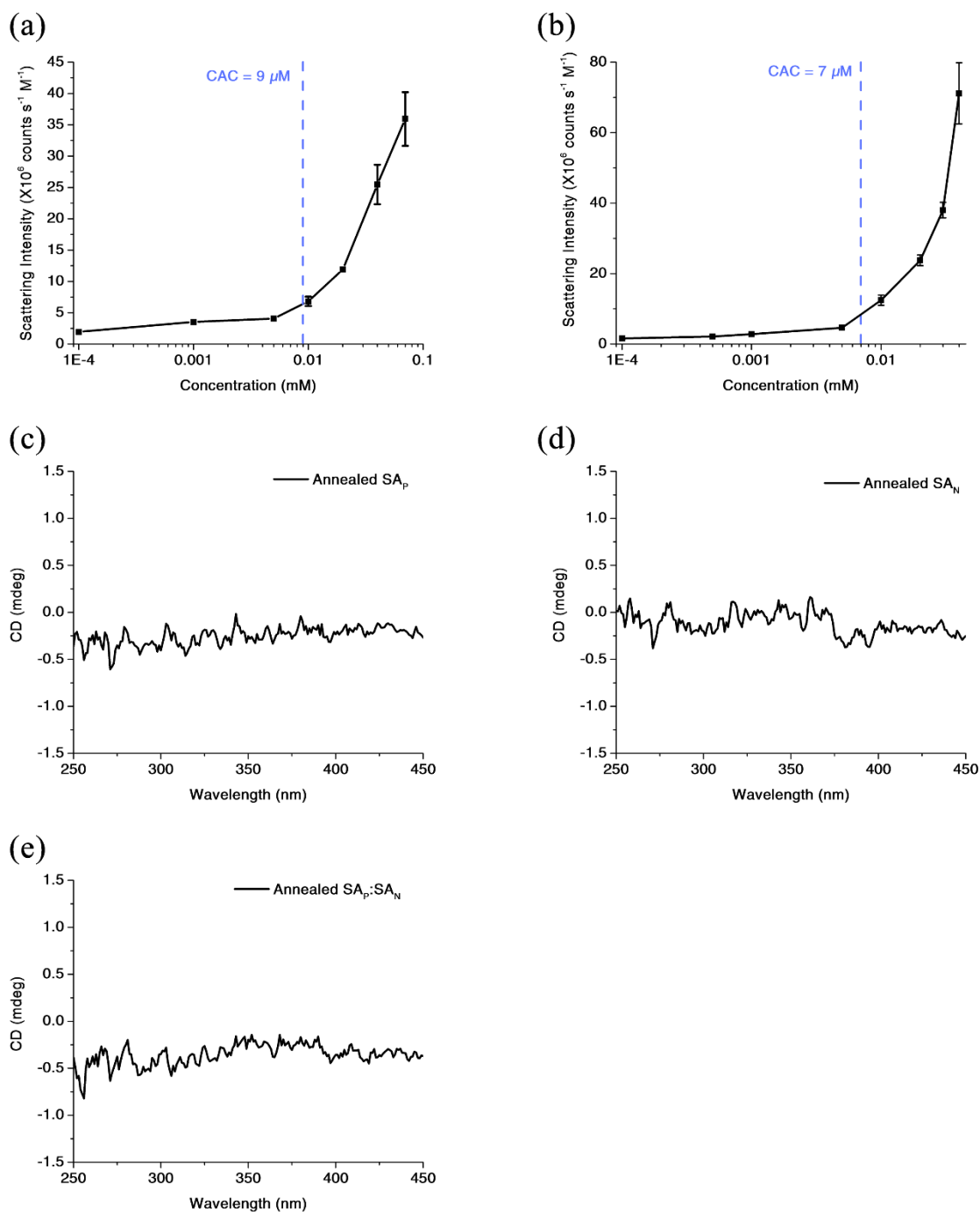


Figure A2.9: Dynamic light scattering assay for determination of critical aggregation concentration of (a) **SA_P** (concentration: 1.0×10^{-4} to 0.09 mM) and (b) **SA_N** (concentration: 1.0×10^{-4} to 0.04 mM). CD spectra of annealed (a) **SA_P** (50 μM), (b) **SA_N** (50 μM), (c) **SA_P:SA_N** (1:1, 50 μM) in HEPES (25 mM, pH = 7.1).

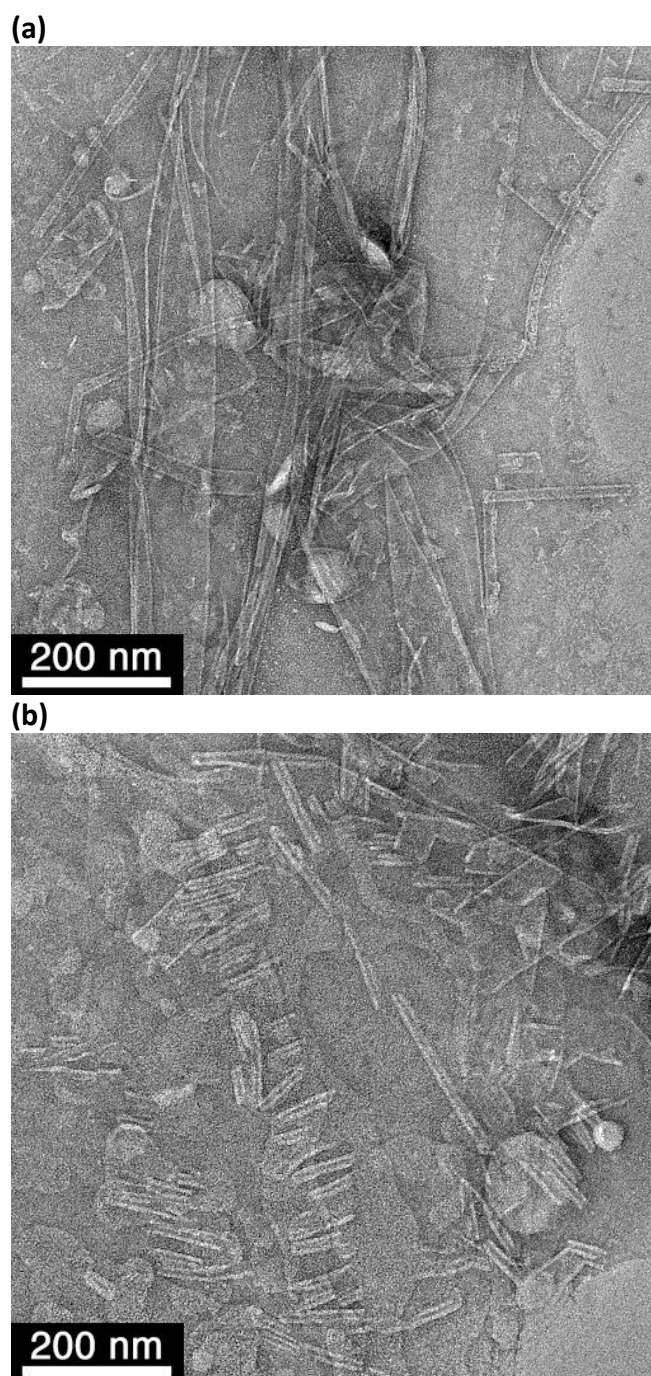


Figure A2.10: TEM images of different ratio $\text{SA}_\text{P}:\text{SA}_\text{N}$ in water. (a) $2:1 = \text{SA}_\text{P}:\text{SA}_\text{N}$ (SA_P : 2 mM, SA_N : 1 mM), (b) $1:2 = \text{SA}_\text{P}:\text{SA}_\text{N}$ (SA_P : 1 mM, SA_N : 2 mM).

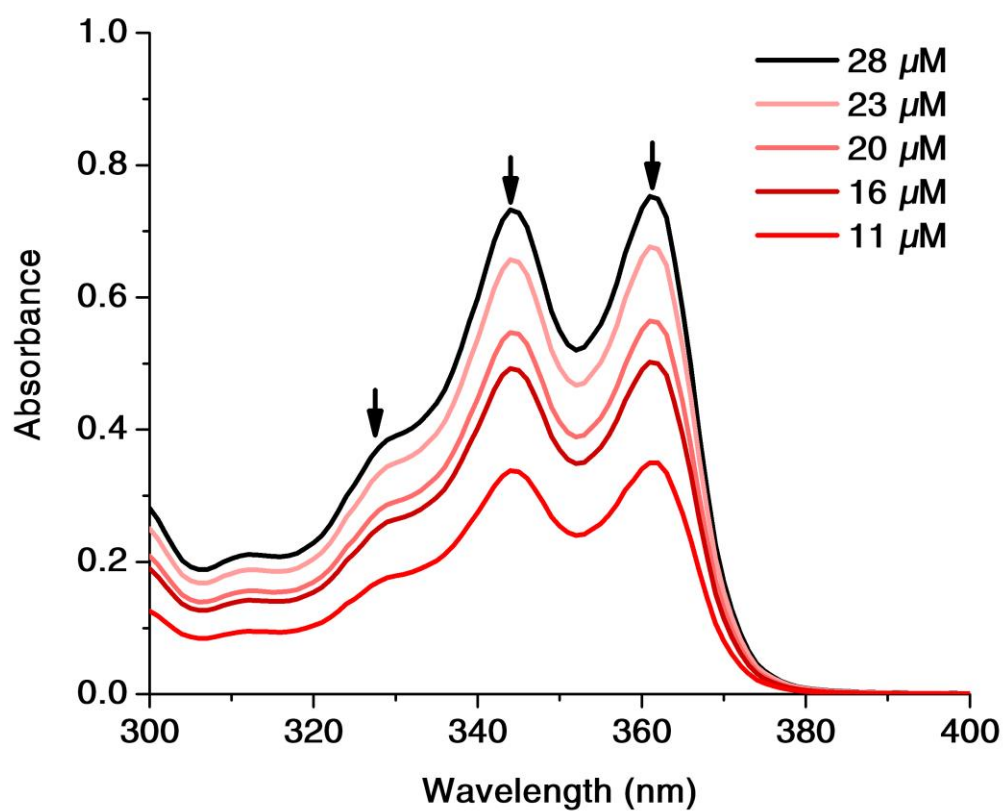


Figure A2.11: UV-vis spectra of SA_P in DMSO at various concentration.

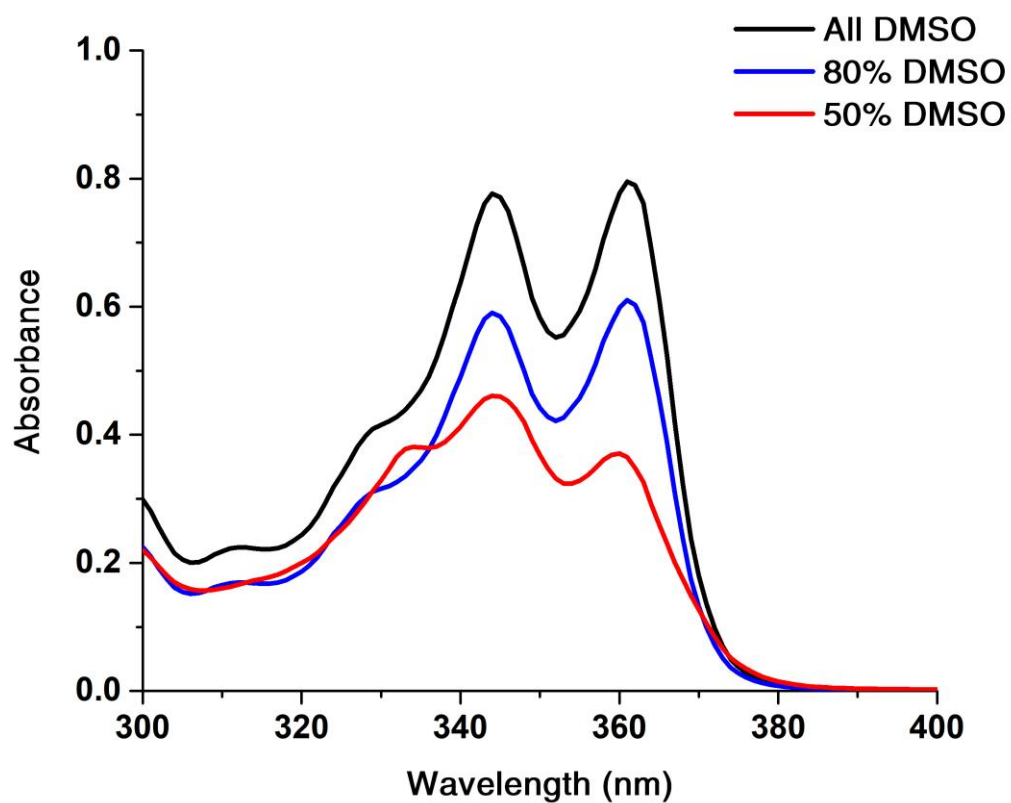


Figure A2.12: UV-vis spectra of SA_p (28 μM) by changing the ratio of DMSO and water.

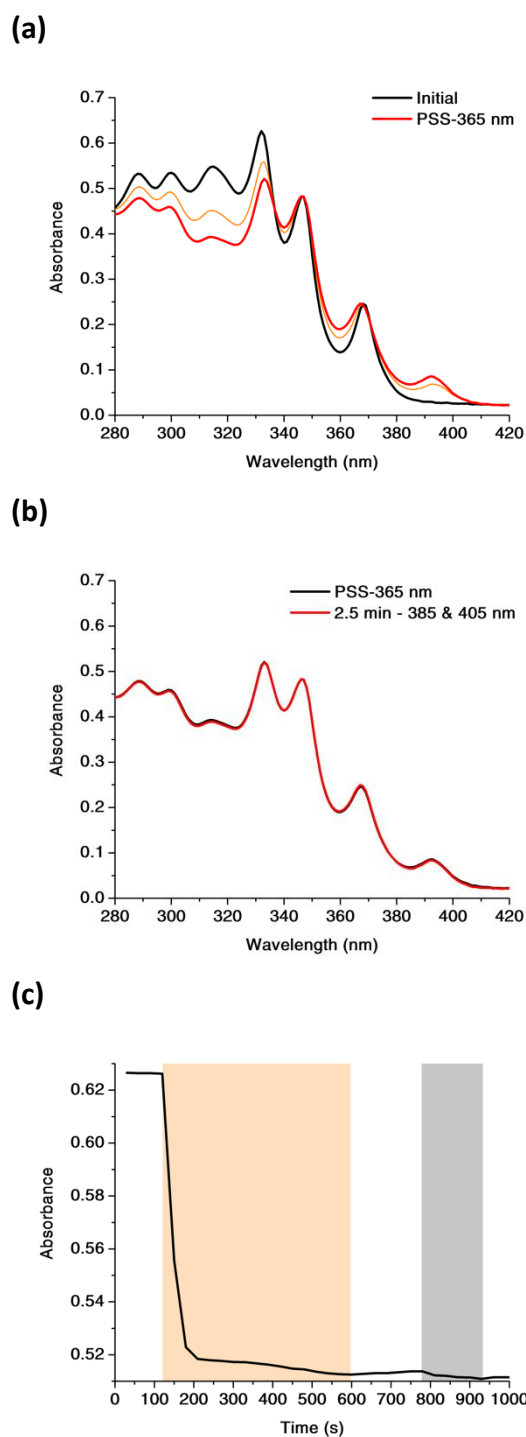
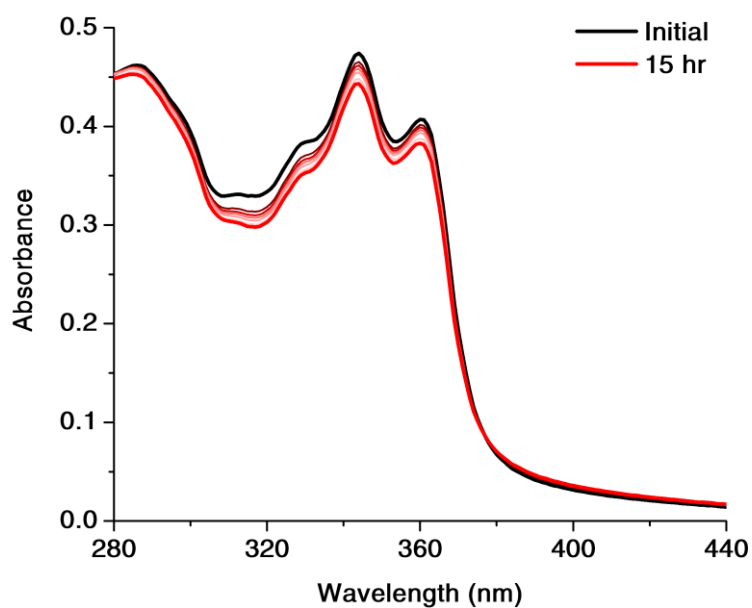


Figure A2.13: UV-vis absorption spectra of **SA_P** in HEPES (25 mM, pH = 7.1) (12 μ M). (a) Absorption spectra of **SA_P** in PSS (red) after 365 nm irradiation over 8 min (orange) (b) after a combination of 385 nm and 405 nm irradiation for 150 s (red). (b) Time-course of the photoisomerization process of **SA_P** in HEPES (25 mM, pH = 7.1), monitored at λ_{max} 332 nm of *trans*-**SA_P** (orange area: photoirradiation at 365 nm period; grey area: photoirradiation at 385 nm and 405 nm period). Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model collimated LEDs (M365LP-C1, 365 nm, 1.2 A; M385LP-C1, 385 nm, 1.2 A and 405 nm, M405LP-C1, 1.2 A) positioned at a distance of 1 cm from the sample.

(a)



(b)

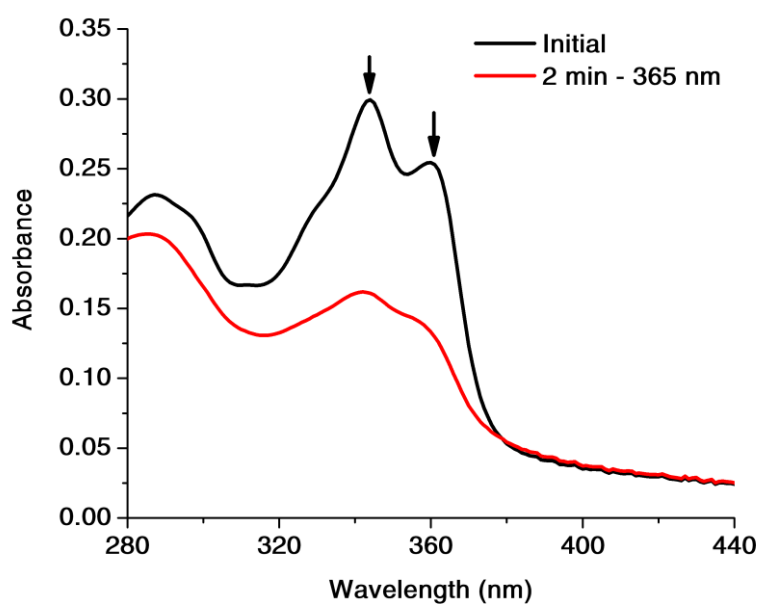


Figure A2.14: UV-vis absorption spectra of SA_N in HEPES (25 mM, pH = 7.1) (16 μM) over 15 h. UV-vis absorption spectra of SA_N in HEPES (25 mM, pH = 7.1) (12 μM) upon irradiation at 365 nm for 120 s. Irradiation of UV-vis sample was carried out at 20 $^\circ\text{C}$ using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A) positioned at a distance of 1 cm from the sample.

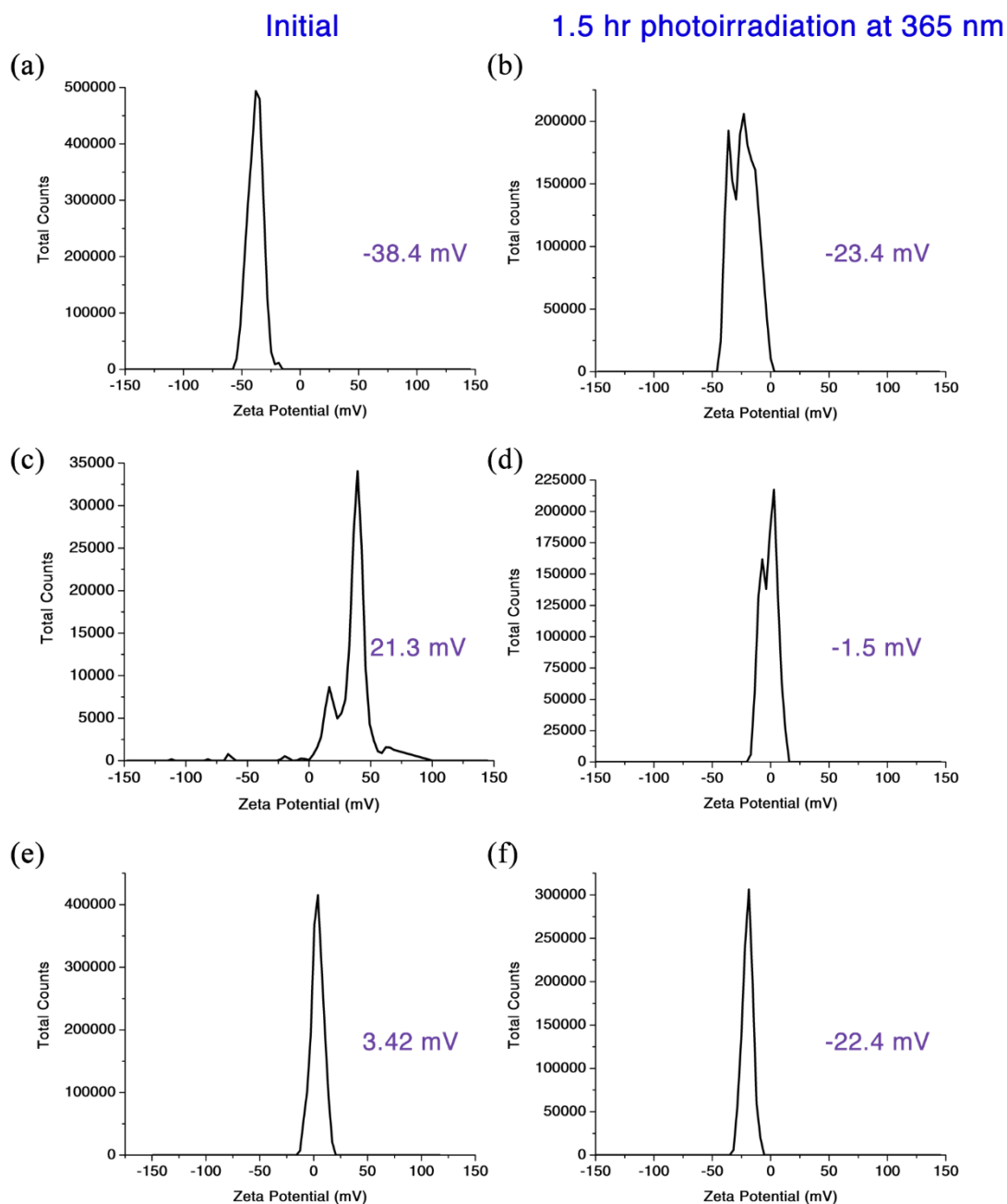


Figure A2.15: Zeta potential of (a) SA_P , (c) SA_N , (e) $SA_P:SA_N$ (1 μ M) in NaCl (1 mM) aqueous solution. Zeta potential of (b) SA_P , (d) SA_N , (f) $SA_P:SA_N$ (1 μ M) after 365 nm irradiation for 1.5 h in NaCl (1 mM) aqueous solution. Irradiation of sample was carried out at 20 $^{\circ}$ C using a Thorlabs model collimated LEDs (M365LP-C1, 365 nm, 1.2 A).

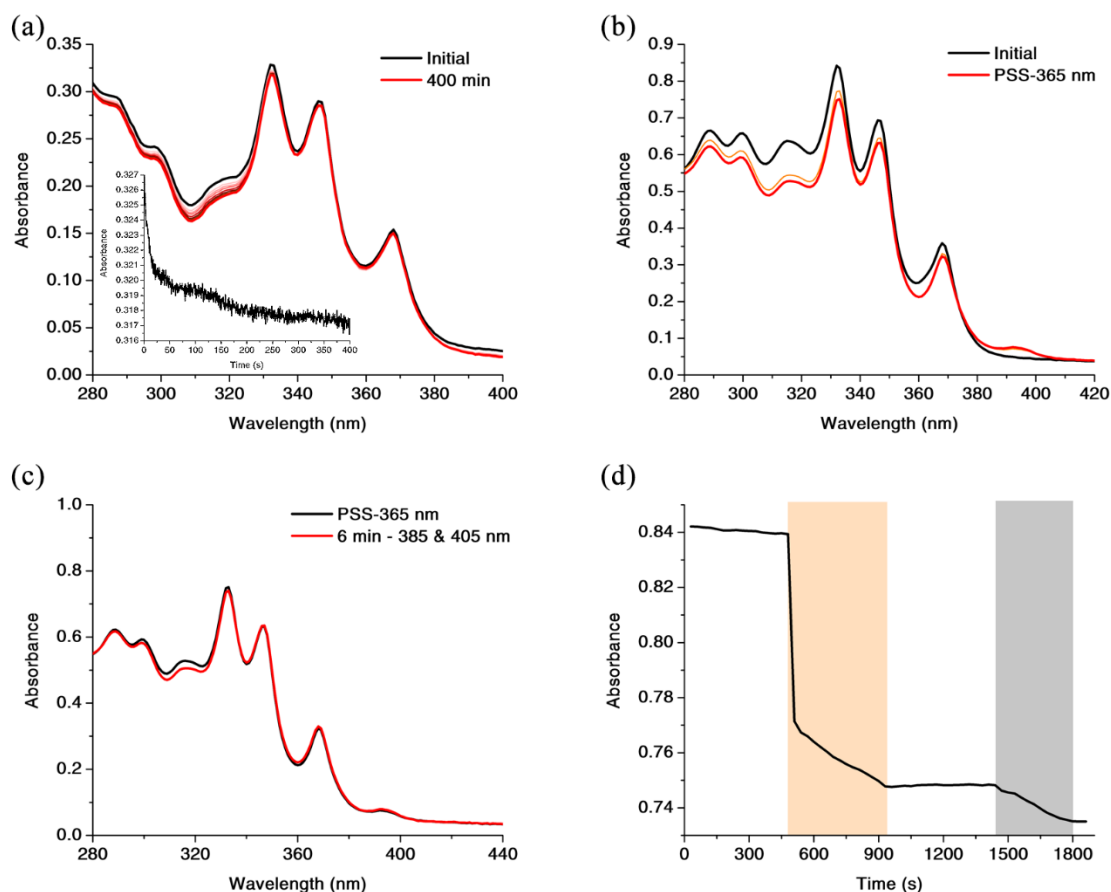


Figure A2.16: (a) UV-vis absorption spectra of $\text{SA}_P:\text{SA}_N$ in HEPES (25 mM, pH = 7.1) (9 μM) over 6 hours (inset: Time-course of the experiment, monitored at λ_{max} 332 nm of *trans*- $\text{SA}_P:\text{SA}_N$). (b) Absorption spectra of $\text{SA}_P:\text{SA}_N$ (17 μM) in PSS (red) after 365 nm irradiation over 7.5 min (orange) (c) after a combination of 385 nm and 405 nm irradiation for 6 min (red). (d) Time-course of the photoisomerization process of $\text{SA}_P:\text{SA}_N$ in HEPES (25 mM, pH = 7.1), monitored at λ_{max} 332 nm of *trans*- $\text{SA}_P:\text{SA}_N$ (orange area: photoirradiation at 365 nm period; grey area: photoirradiation at a combination of 385 nm and 405 nm period). Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model collimated LEDs (M365LP-C1, 365 nm, 1.2 A; M385LP-C1, 385 nm, 1.2 A and 405 nm, M405LP-C1, 1.2 A) positioned at a distance of 1 cm from the sample.

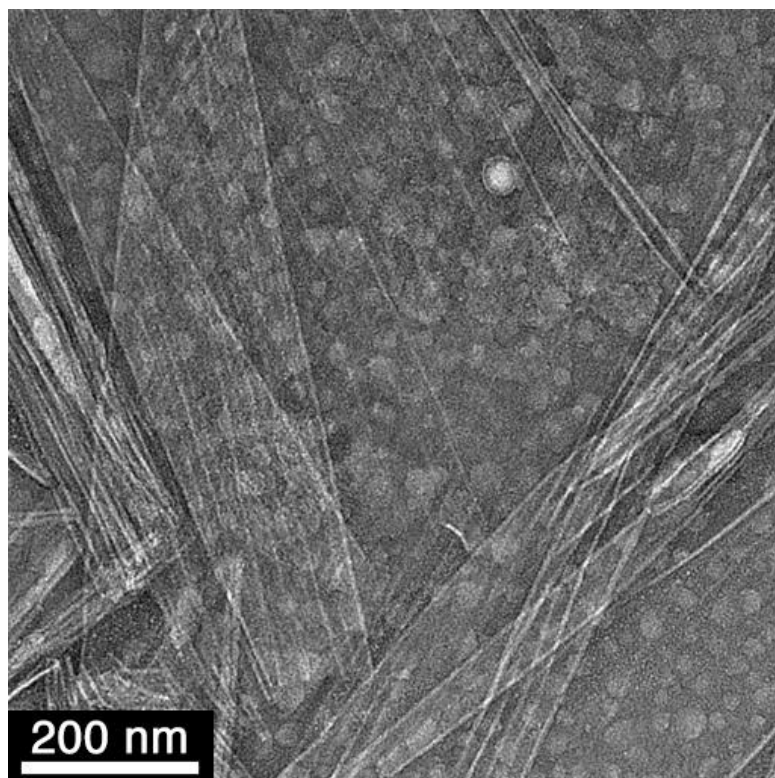


Figure A2.17: TEM image of $\text{SA}_\text{P}:\text{SA}_\text{N}$ (1:1, 2 mM) in HEPES (25 mM, pH = 7.1).

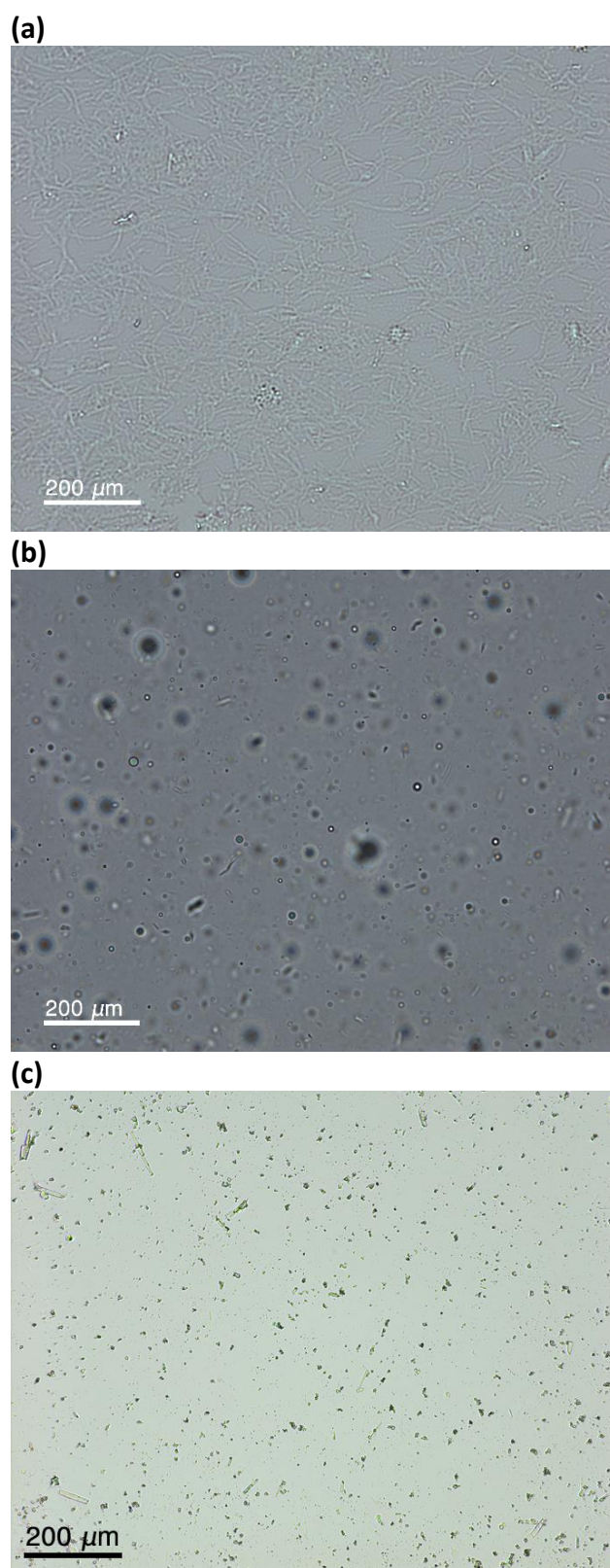


Figure A2.18: Optical microscopic images of solution of (a) $\text{SA}_\text{P}:\text{SA}_\text{N}$ (1:1, 0.5 wt.%, 5 mM), (b) SA_P (0.5 wt.%, 5 mM) and (c) SA_N (0.5 wt.%, 5 mM).

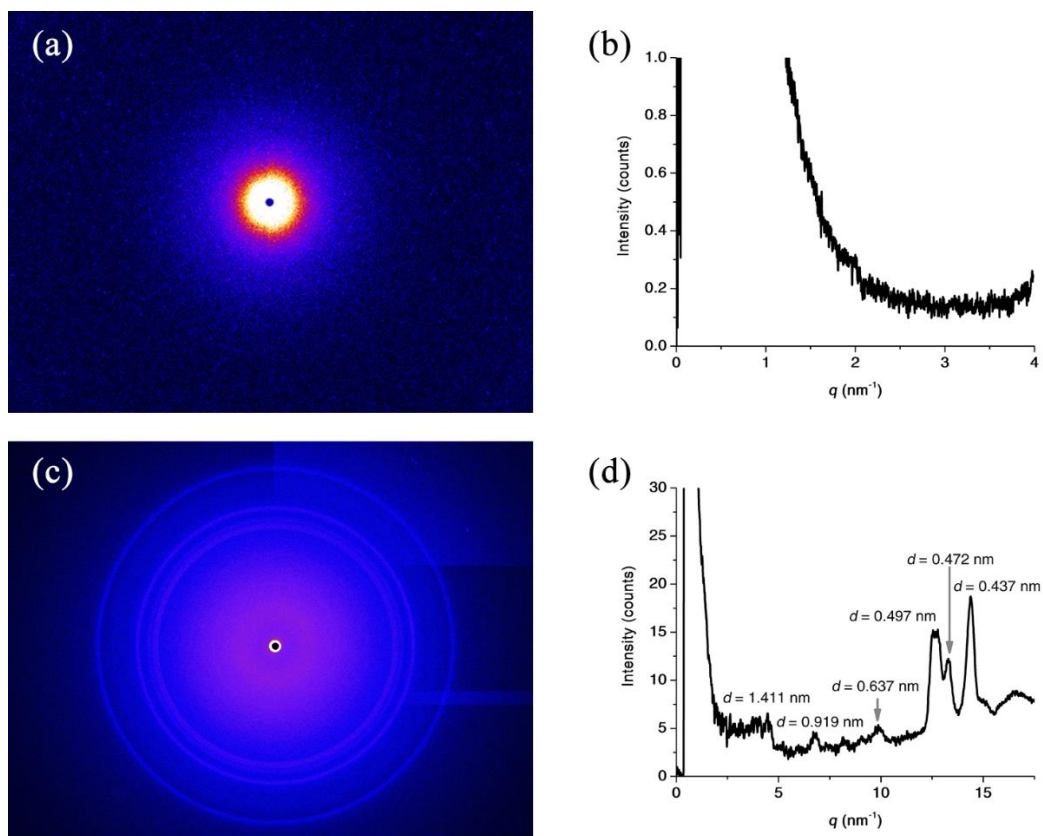


Figure A2.19: (a) 2D SAXS images of a macroscopic aligned string composed of **SA_P:SA_N** prepared from a solution of CaCl_2 (150 mM) with 20 min of exposure time. (b) 1D SAXS pattern of **SA_P:SA_N** macroscopic string of the 2D SAXS image in (a). (c) 2D WAXSD images of a macroscopic aligned string composed of **SA_P:SA_N** prepared from a solution of CaCl_2 (150 mM) with 20 min of exposure time. (d) 1D WAXD pattern of **SA_P:SA_N** macroscopic string of the 2D WAXD image in (c).

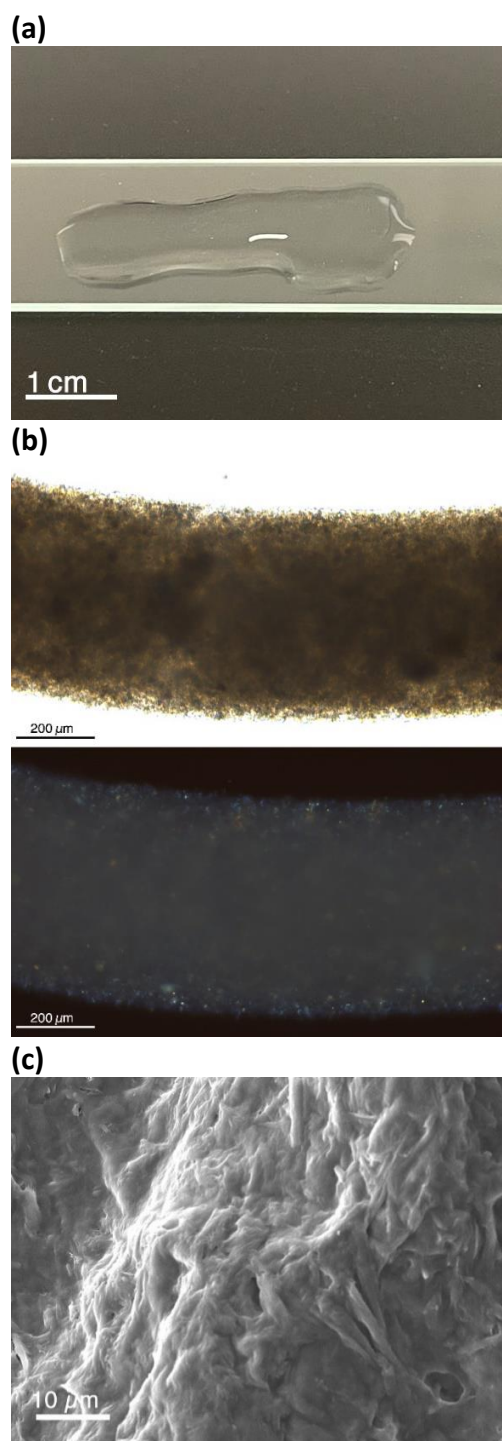


Figure A2.20: (a) Photograph of aqueous solutions of **SA_P:SA_N** (1:1, 5.0 wt.%, 50 mM, annealed at 85 °C, 10 min) was ejected into a shallow pool of H₂O. (b) Optical microscopic images of a macroscopic string composed of **SA_P:SA_N** prepared from H₂O without (top) and with (bottom) crossed polarizers. (c) SEM image of **SA_P:SA_N** (1:1, 5.0 wt.%, 50 mM, annealed at 85 °C, 10 min) in MilliQ water.

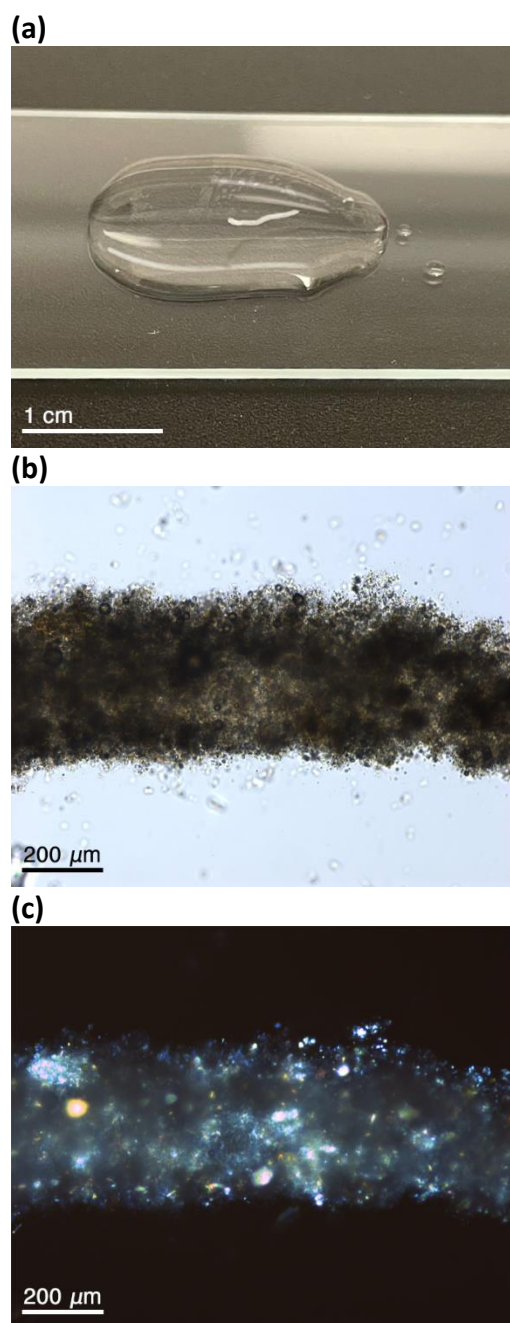


Figure A2.21: (a) Photograph of aqueous solutions of $\text{SA}_\text{P}:\text{SA}_\text{N}$ (1:1, 1.0 wt.%, 10 mM, annealed at 85 °C, 10 min) was ejected into a shallow pool of H_2O . Optical microscopic images of a macroscopic string composed of $\text{SA}_\text{P}:\text{SA}_\text{N}$ prepared from H_2O (b) without and (c) with crossed polarizers.

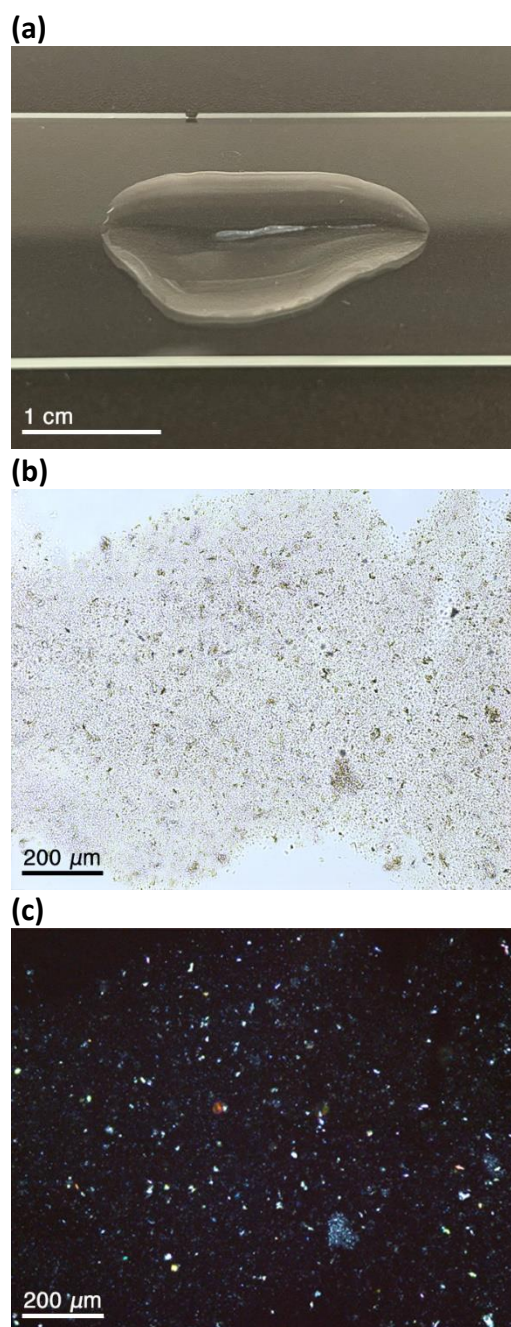


Figure A2.22: (a) Photograph of aqueous solutions of $\text{SA}_\text{P}:\text{SA}_\text{N}$ (1:1, 0.5 wt.%, 5 mM, annealed at 85 °C, 10 min) was ejected into a shallow pool of H_2O . Optical microscopic images of a macroscopic string composed of $\text{SA}_\text{P}:\text{SA}_\text{N}$ prepared from H_2O (b) without and (c) with crossed polarizers.

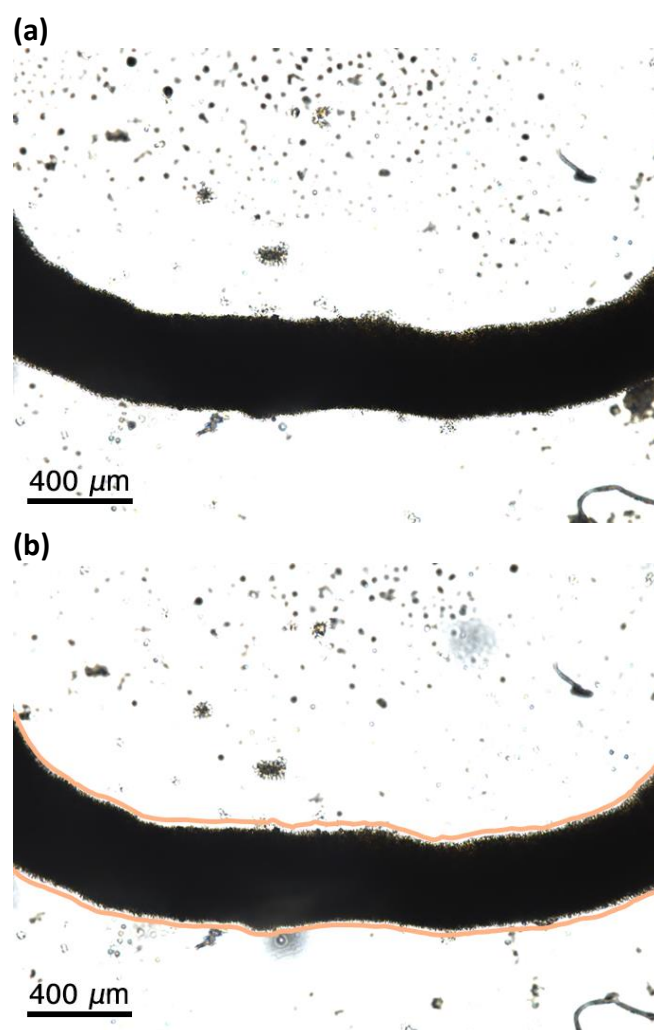


Figure A2.23: Optical microscopic images of a macroscopic string composed of $\text{SA}_\text{P}:\text{SA}_\text{N}$ (1:1, 1.0 wt.%, 10 mM) in H_2O (a) before, and (b) after 90 min. The shape of the macroscopic string at the initial state is indicated as the orange boundary.

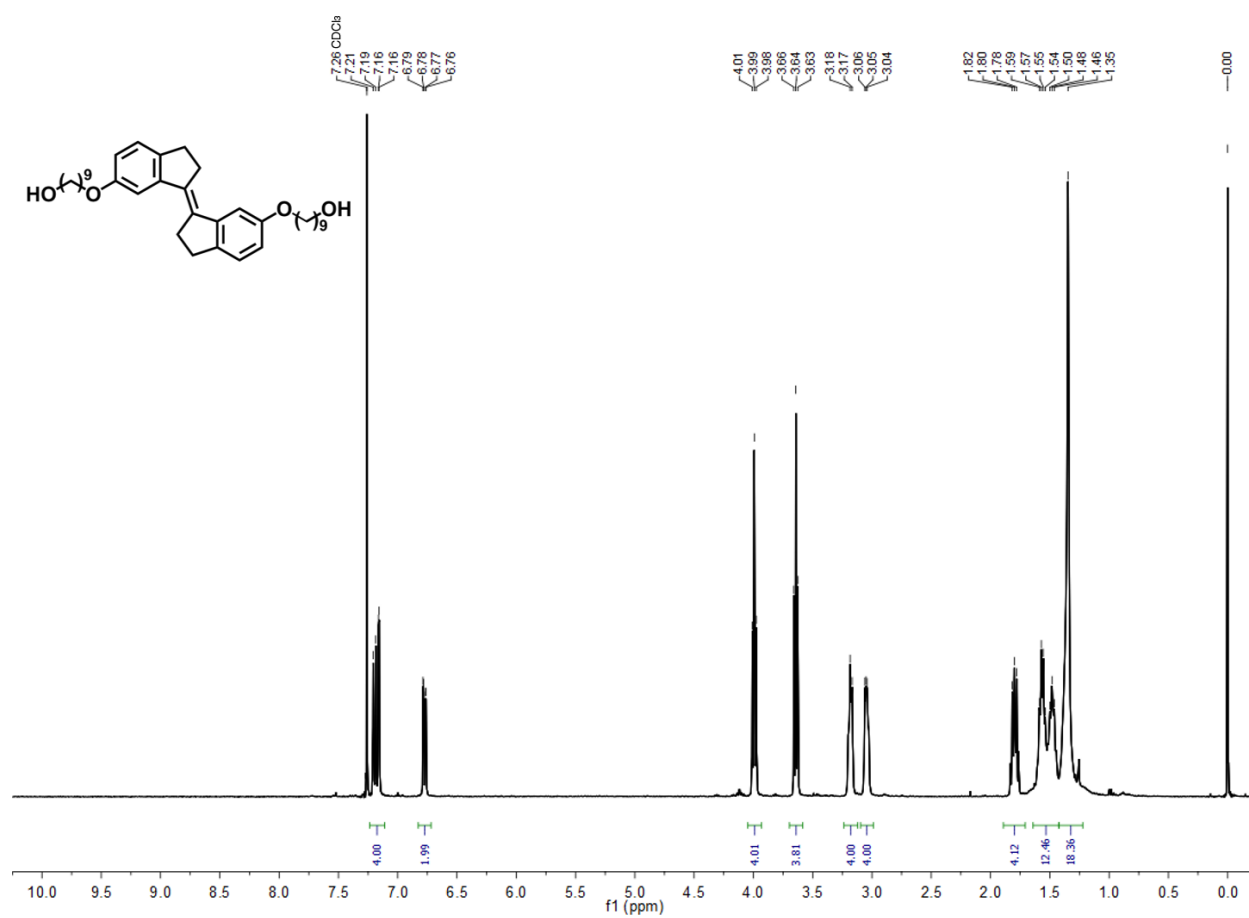


Figure A2.24: ¹H NMR spectrum (400 MHz) of compound **4** in CDCl₃ at 25 °C.

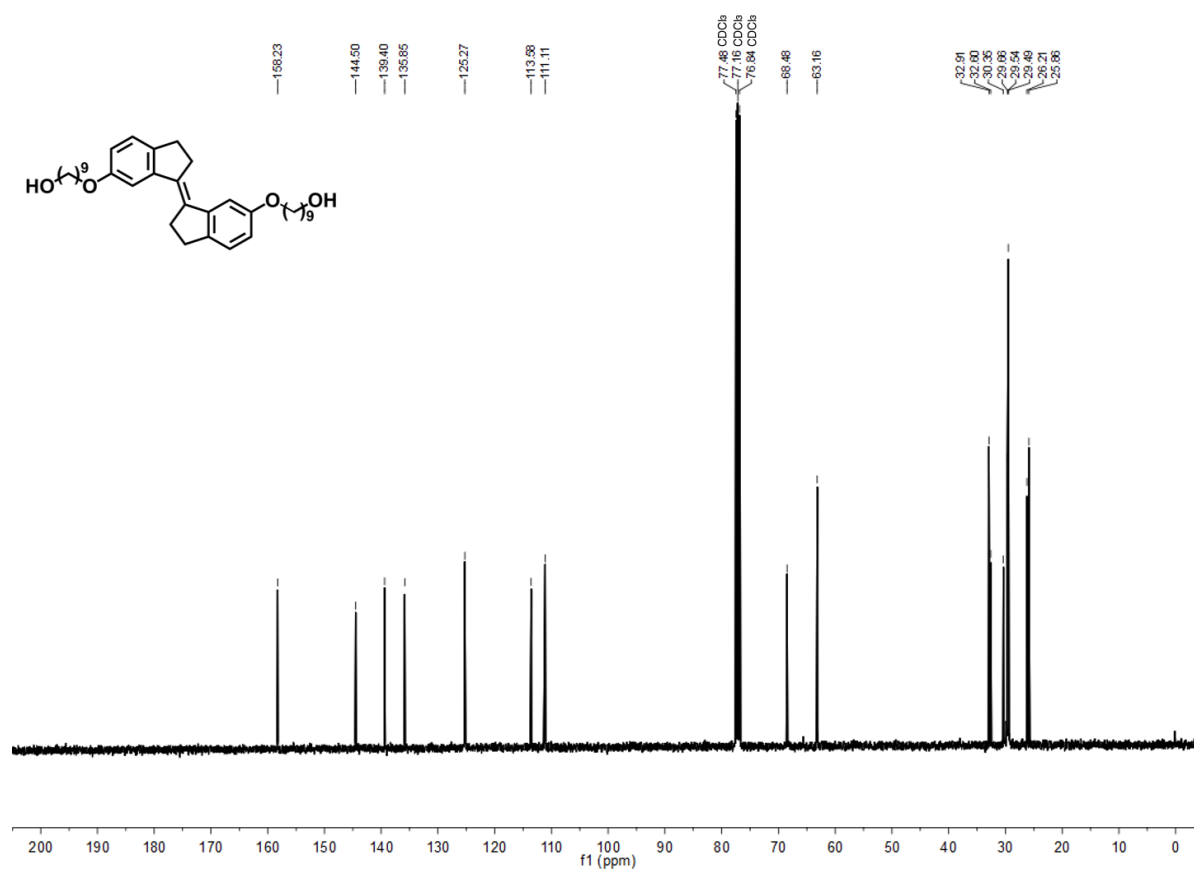
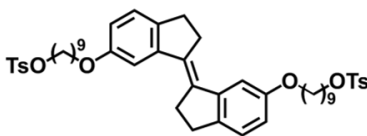
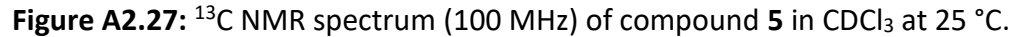


Figure A2.25: ¹³C NMR spectrum (100 MHz) of compound **4** in CDCl₃ at 25 °C.



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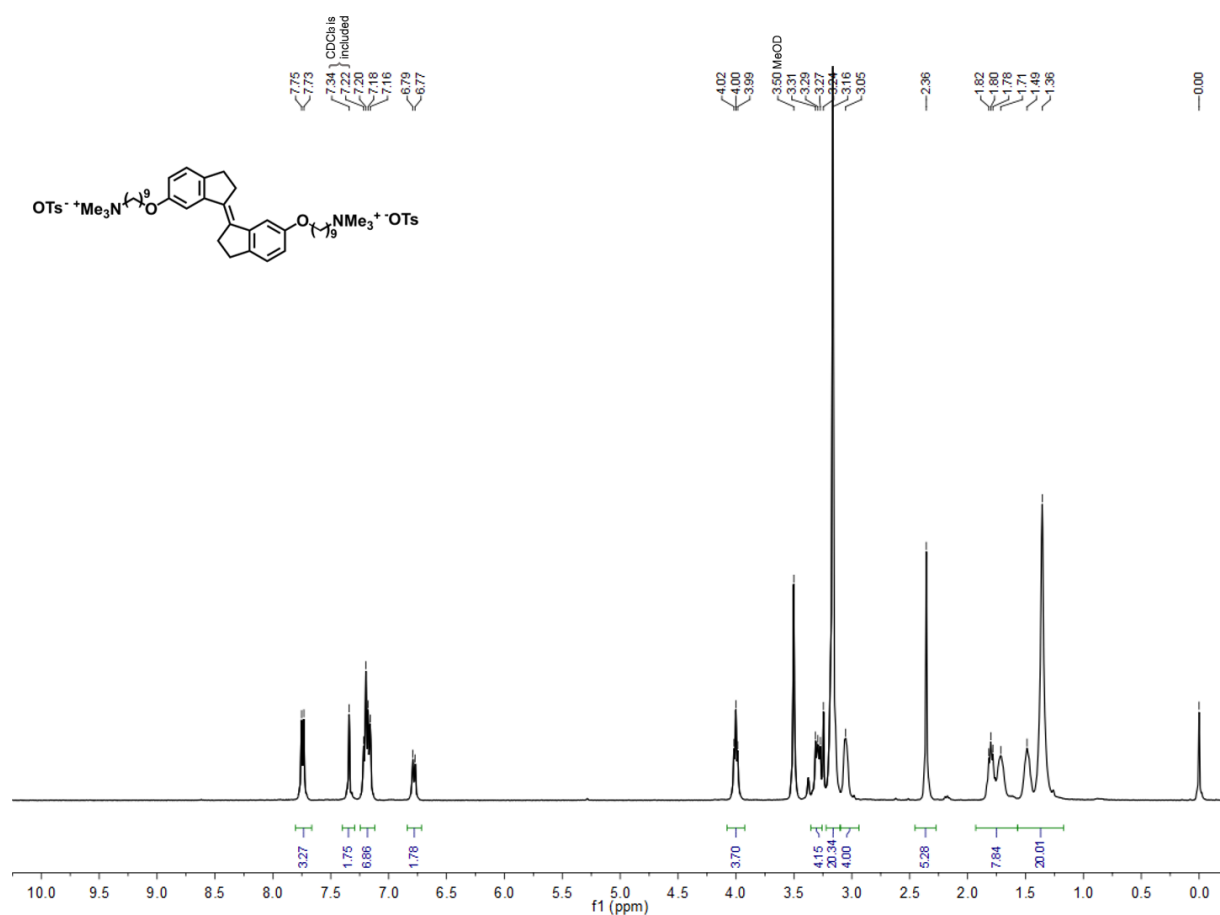


Figure A2.28: ¹H NMR spectrum (400 MHz) of **SA_N** in 90% CDCl₃ and 10% MeOD-*d*₄ at 25 °C.

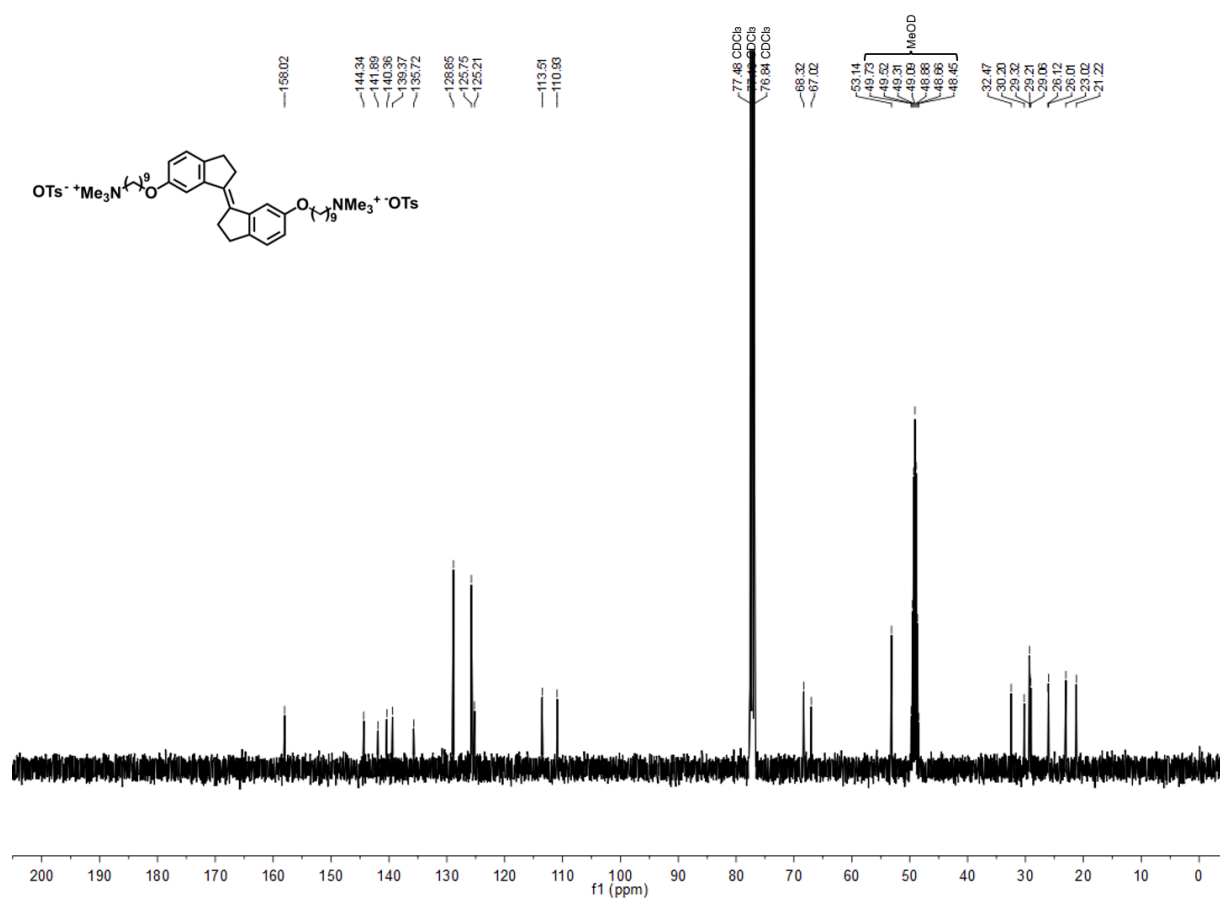
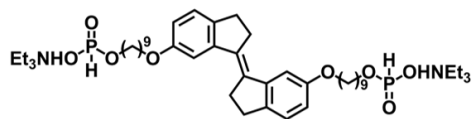
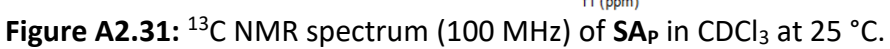


Figure A2.29: ¹³C NMR spectrum (100 MHz) of **SA_N** in 90% CDCl₃ and 10% MeOD-*d*₄ at 25 °C.



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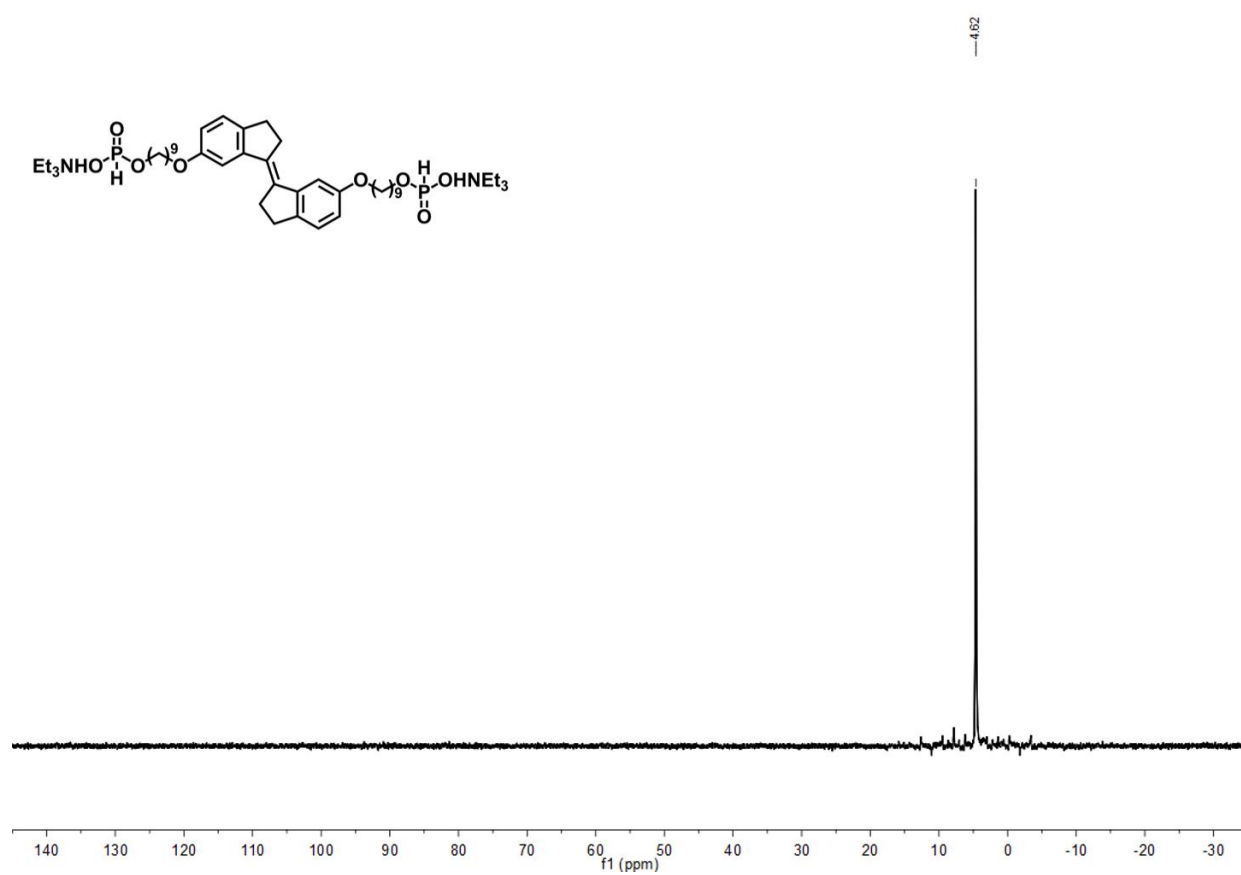
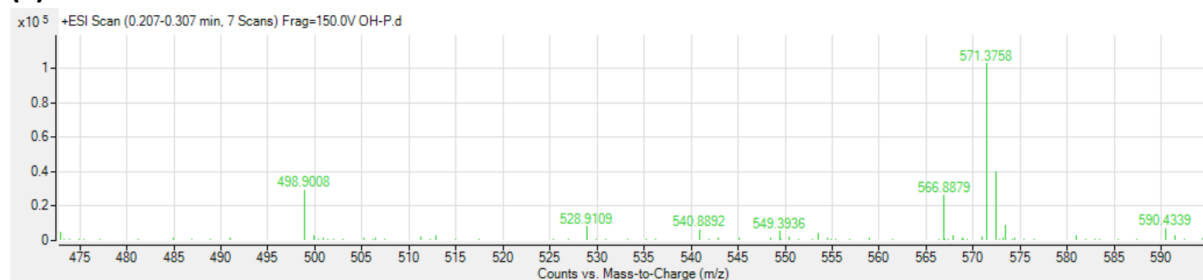


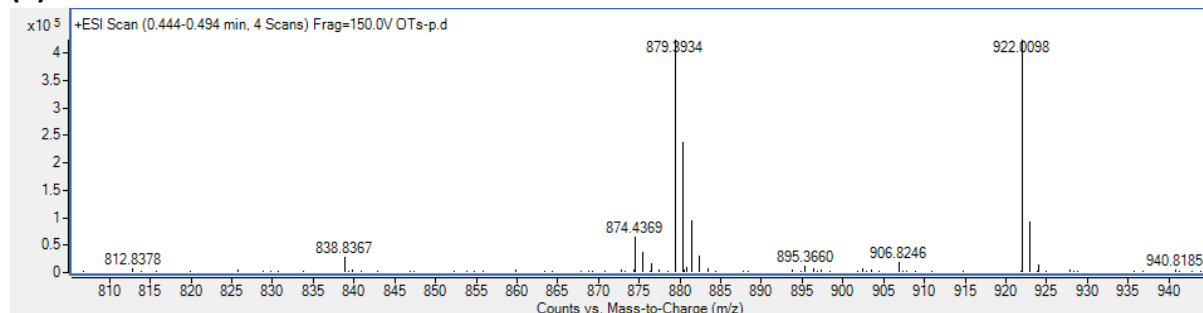
Figure A2.32: ³¹P NMR spectrum (162 MHz) of **SA_P** in CDCl₃ at 25 °C.

(a)



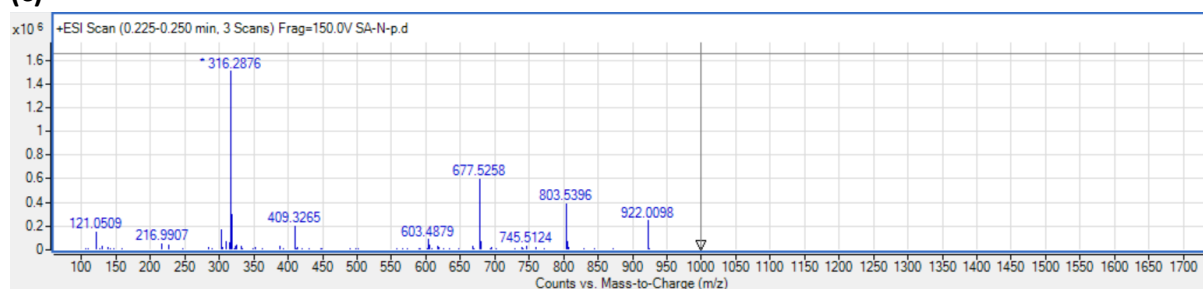
HR-MS (ESI+) calculated for $C_{36}H_{52}O_4$ $[M+Na]^+$ 571.3758, found 571.3758.

(b)



HR-MS (ESI+) calculated for $C_{50}H_{64}O_8S_2$ $[M+Na]^+$ 879.3934, found: 879.3934.

(c)



HR-MS (ESI+) calculated for $C_{56}H_{82}N_2O_8S_2$ $[M-OTs]^+$ 803.5397, found: 803.5396.

(d)



HR-MS (ESI+) calculated for $C_{48}H_{84}N_2O_8P_2$ $[M-2HNEt_3]^{2-}$ 337.1569, found: 337.1569.

Figure A2.33: HRMS spectrum of (a) compound **2**, (b) compound **3**, (c) **SA_N**, and (d) **SA_P**, respectively.

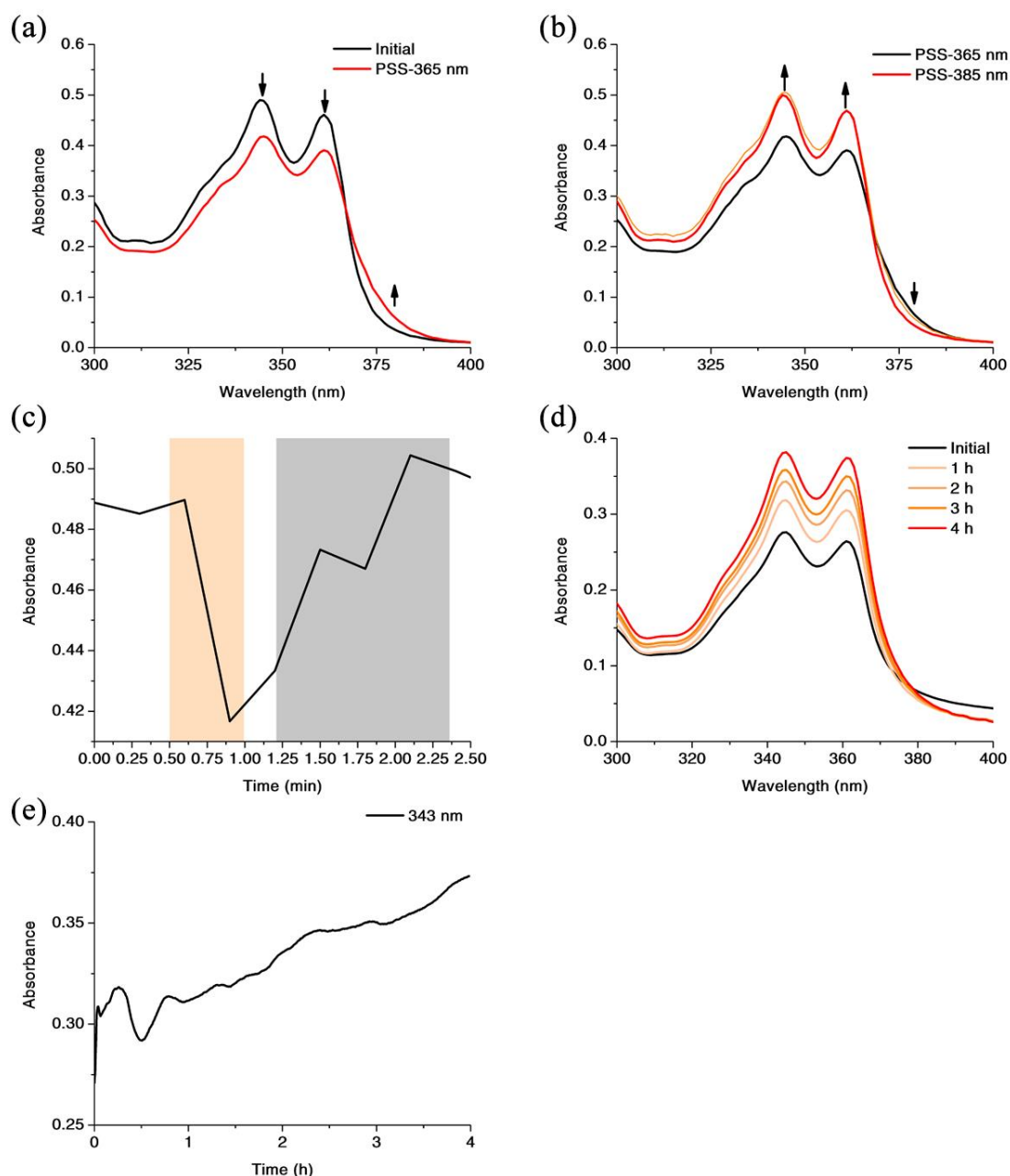


Figure A3.1: UV-vis absorption spectra of SA_{Ala} in DMSO (65 μM). (a) Absorption spectra of SA_{Ala} in PSS (red) after 365 nm irradiation for 0.5 min, (b) in PSS (red) after 385 nm irradiation over 1 min (orange). (c) Time-course of the photoisomerization process of SA_{Ala} in DMSO, monitored at λ_{max} 344 nm of *trans*- SA_{Ala} (orange area: photoirradiation at 365 nm period; grey area: photoirradiation at 385 nm period). (d) UV-vis absorption spectra of SA_{Ala} (32 μM) in DMSO at 20 °C over 4 h and (e) time-course of the stability experiment of SA_{Ala} monitored at λ_{max} 343 nm. Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample.

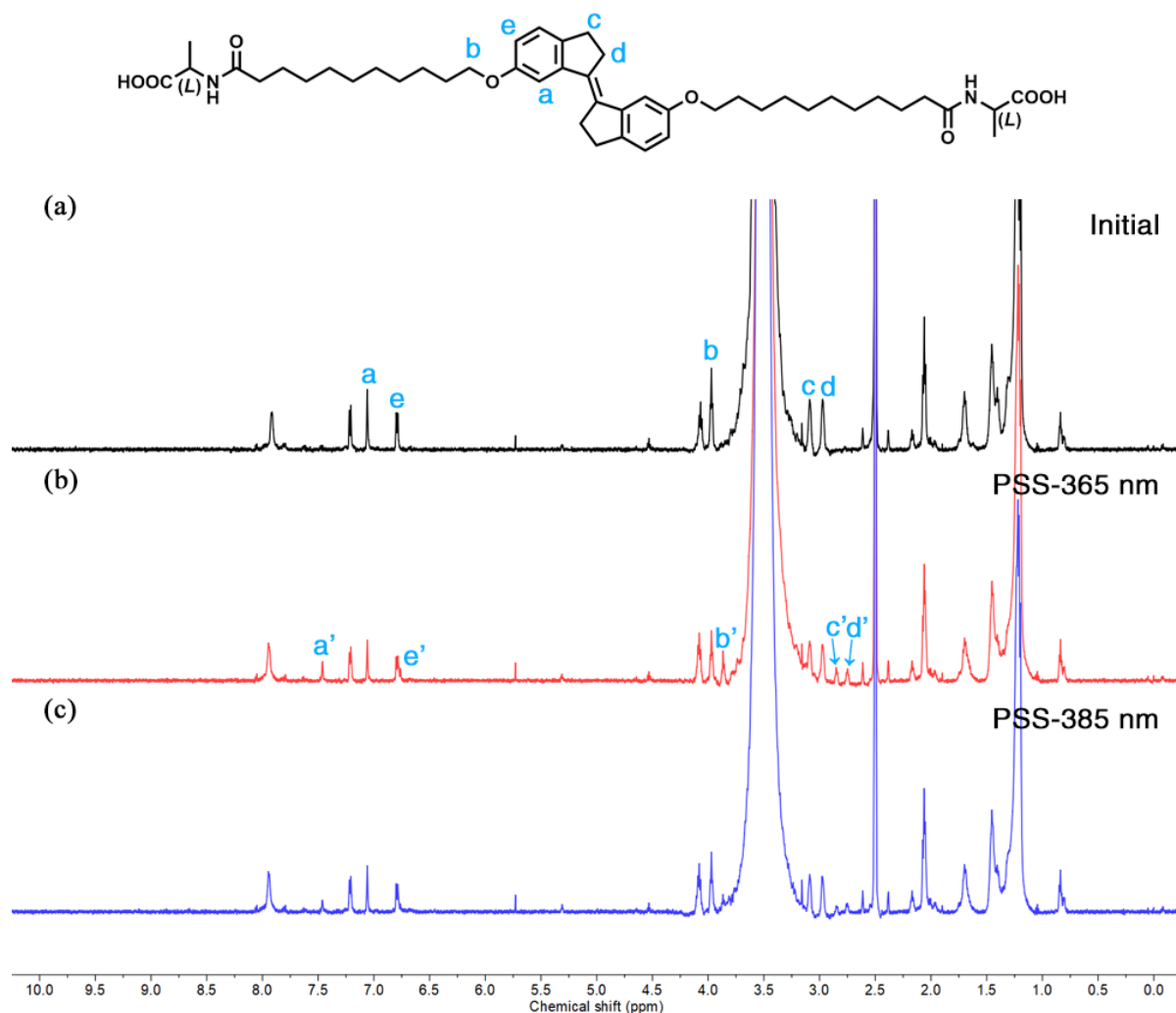


Figure A3.2: ¹H NMR spectra during isomerization process of **SA_{Ala}** (5.5 mM, DMSO-*d*₆, 600 MHz), (a) before photoirradiation, (b) irradiated with 365 nm for 1 h at 20 °C, (c) followed by 385 nm irradiation for 1 h at 20 °C. Irradiation of sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample. PSS₃₆₅ ratio of *trans*-**SA_{Ala}**/*cis*-**SA_{Ala}** was 75:25 and PSS₃₈₅ ratio of *trans*-**SA_{Ala}**/*cis*-**SA_{Ala}** was 85:15. The ¹H NMR signals at 0.77 – 0.86 ppm are from grease oil; 5.7 ppm is from DCM.

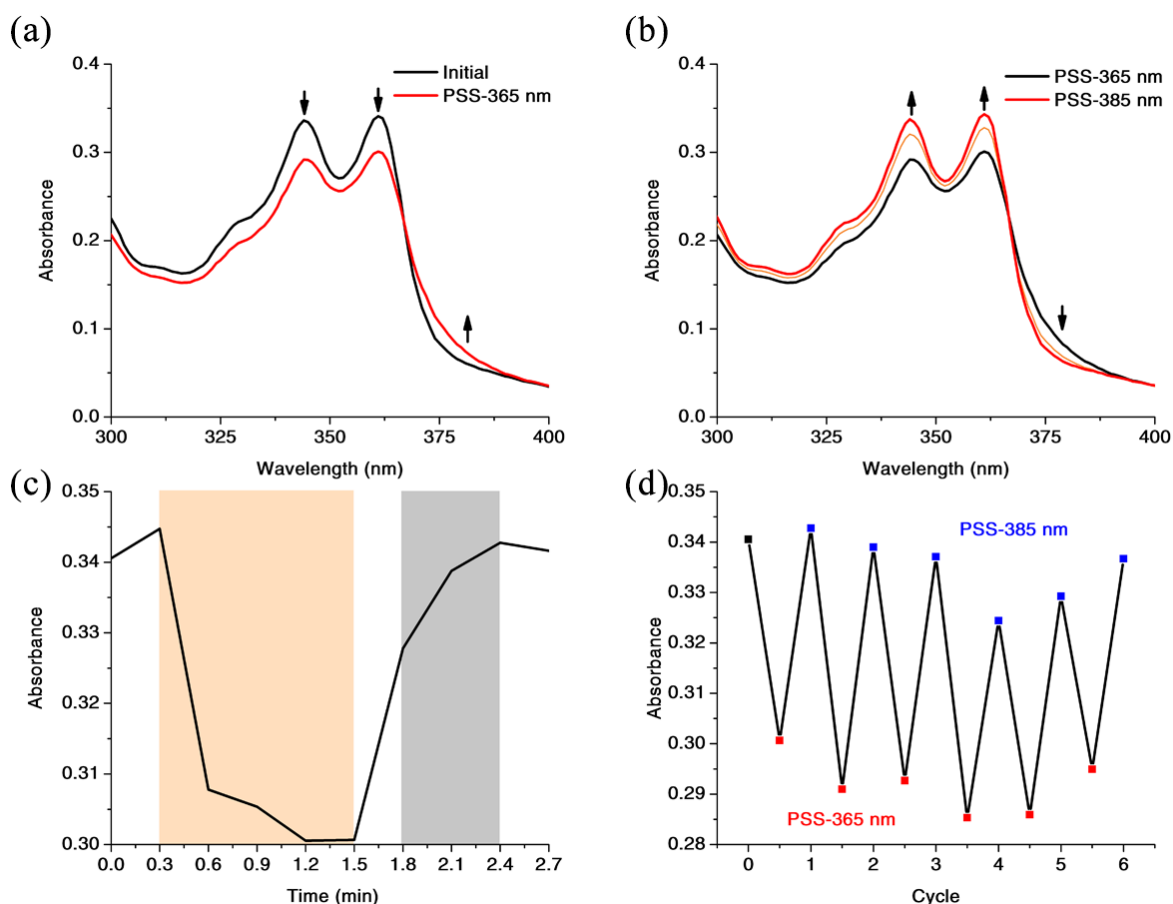


Figure A3.3: UV-vis absorption spectra of the SA_{Gly} in DMSO ($100 \mu\text{M}$). (a) Absorption spectra of SA_{Gly} in PSS (red) after 365 nm irradiation for 1.2 min, (b) in PSS (red) after 385 nm irradiation over 0.6 min (orange). (c) Time-course of the photoisomerization process of SA_{Gly} in DMSO, monitored at λ_{max} 361 nm of *trans*- SA_{Gly} (orange area: photoirradiation at 365 nm period; grey area: photoirradiation at 385 nm period). (d) Plot of the change in absorption of SA_{Gly} at λ_{max} 361 nm upon several 365/385 nm switching cycles. Irradiation of UV-vis sample was carried out at 20°C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample.

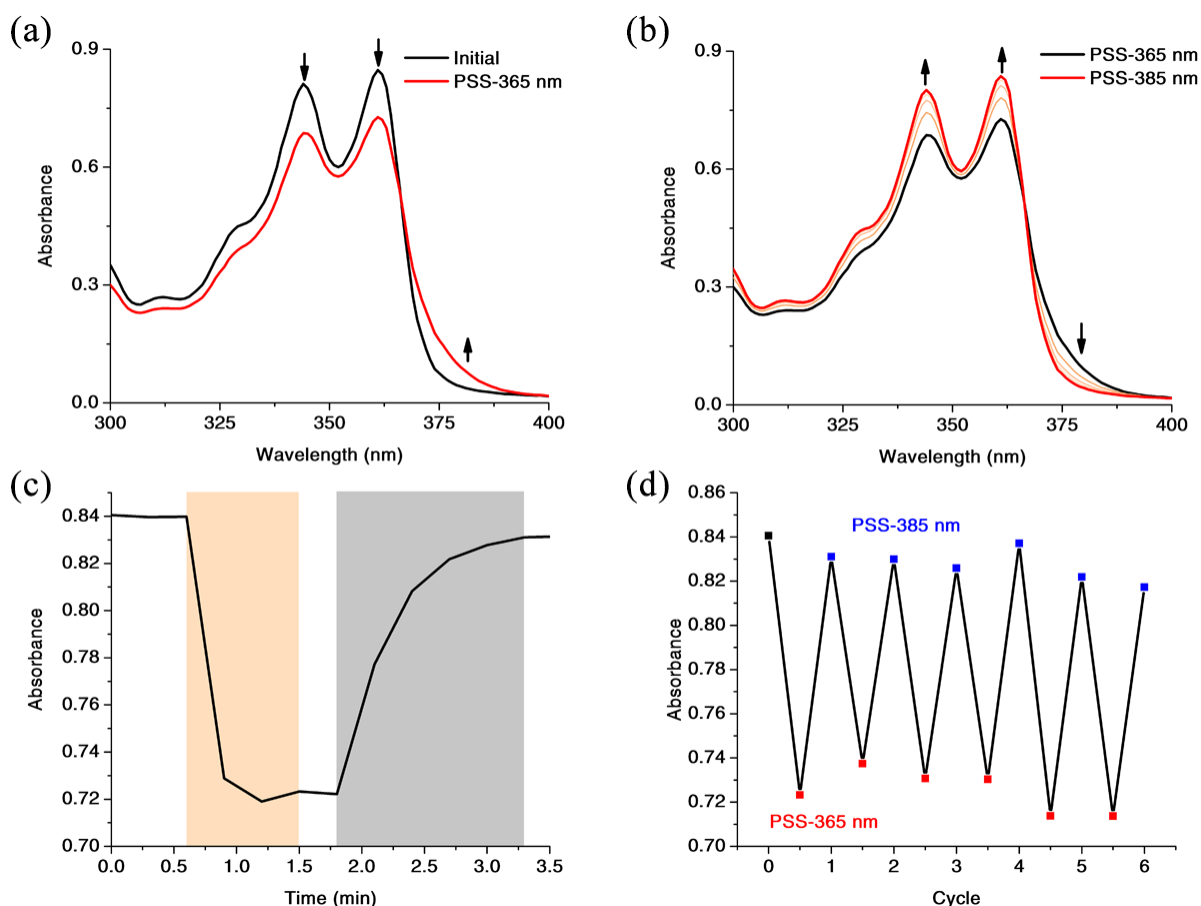


Figure A3.4: UV-vis absorption spectra of the SA_{val} in DMSO ($90 \mu\text{M}$). (a) Absorption spectra of SA_{val} in PSS (red) after 365 nm irradiation for 0.9 min, (b) in PSS (red) after 385 nm irradiation around 2 min (orange). (c) Time-course of the photoisomerization process of SA_{val} in DMSO, monitored at λ_{max} 362 nm of *trans*- SA_{val} (orange area: photoirradiation at 365 nm period; grey area: photoirradiation at 385 nm period). (d) Plot of the change in absorption of SA_{val} at λ_{max} 362 nm upon several 365/385 nm switching cycles. Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample.

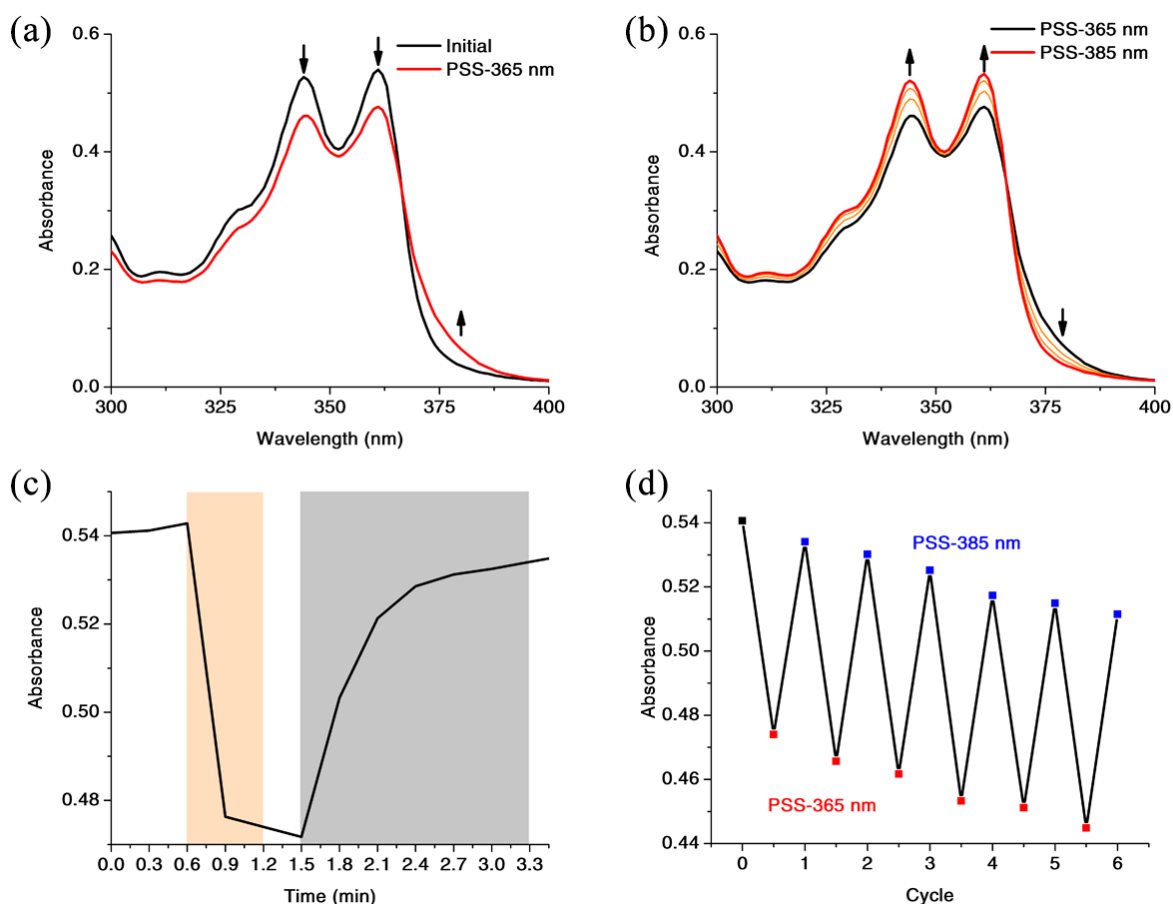


Figure A3.5: UV-vis absorption spectra of the SA_{Phe} in DMSO ($54 \mu\text{M}$). (a) Absorption spectra of SA_{Phe} in PSS (red) after 365 nm irradiation for 0.6 min, (b) in PSS (red) after 385 nm irradiation over 1.8 min (orange). (c) Time-course of the photoisomerization process of SA_{Phe} in DMSO, monitored at λ_{max} 361 nm of *trans*- SA_{Phe} (orange area: photoirradiation at 365 nm period; grey area: photoirradiation at 385 nm period). (d) Plot of the change in absorption of SA_{Phe} at λ_{max} 361 nm upon several 365/385 nm switching cycles. Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample.

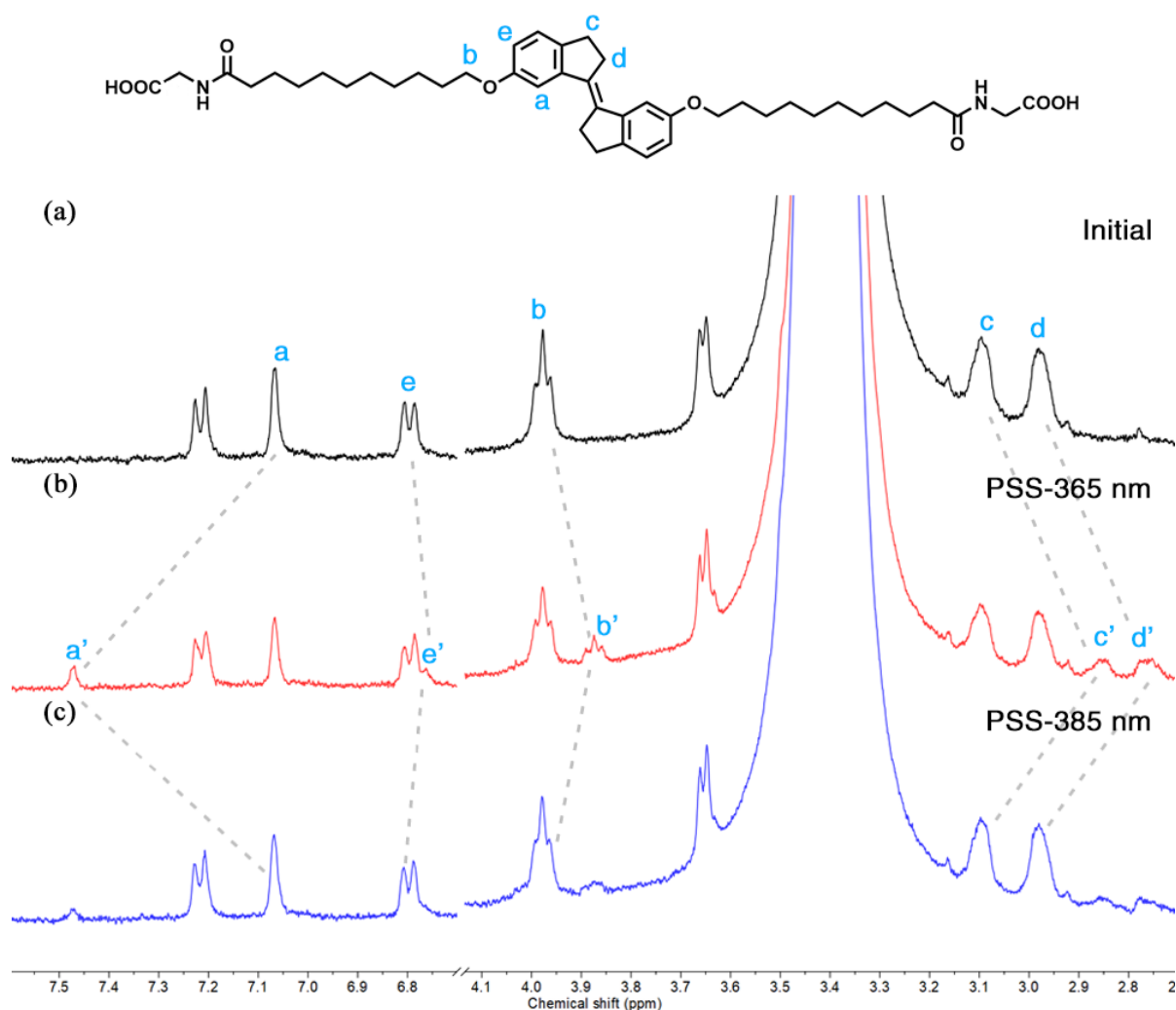


Figure A3.6: Aromatic and aliphatic region of ¹H NMR spectra during isomerization process of **SA_{Gly}** (5.5 mM, DMSO-*d*₆, 400 MHz), (a) before photoirradiation, (b) irradiated with 365 nm for 1 h at 20 °C, (c) followed by 385 nm irradiation for 1 h at 20 °C. Irradiation of sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample. PSS₃₆₅ ratio of *trans*-**SA_{Gly}**/*cis*-**SA_{Gly}** was 80:20 and PSS₃₈₅ ratio of *trans*-**SA_{Gly}**/*cis*-**SA_{Gly}** was 95:5.

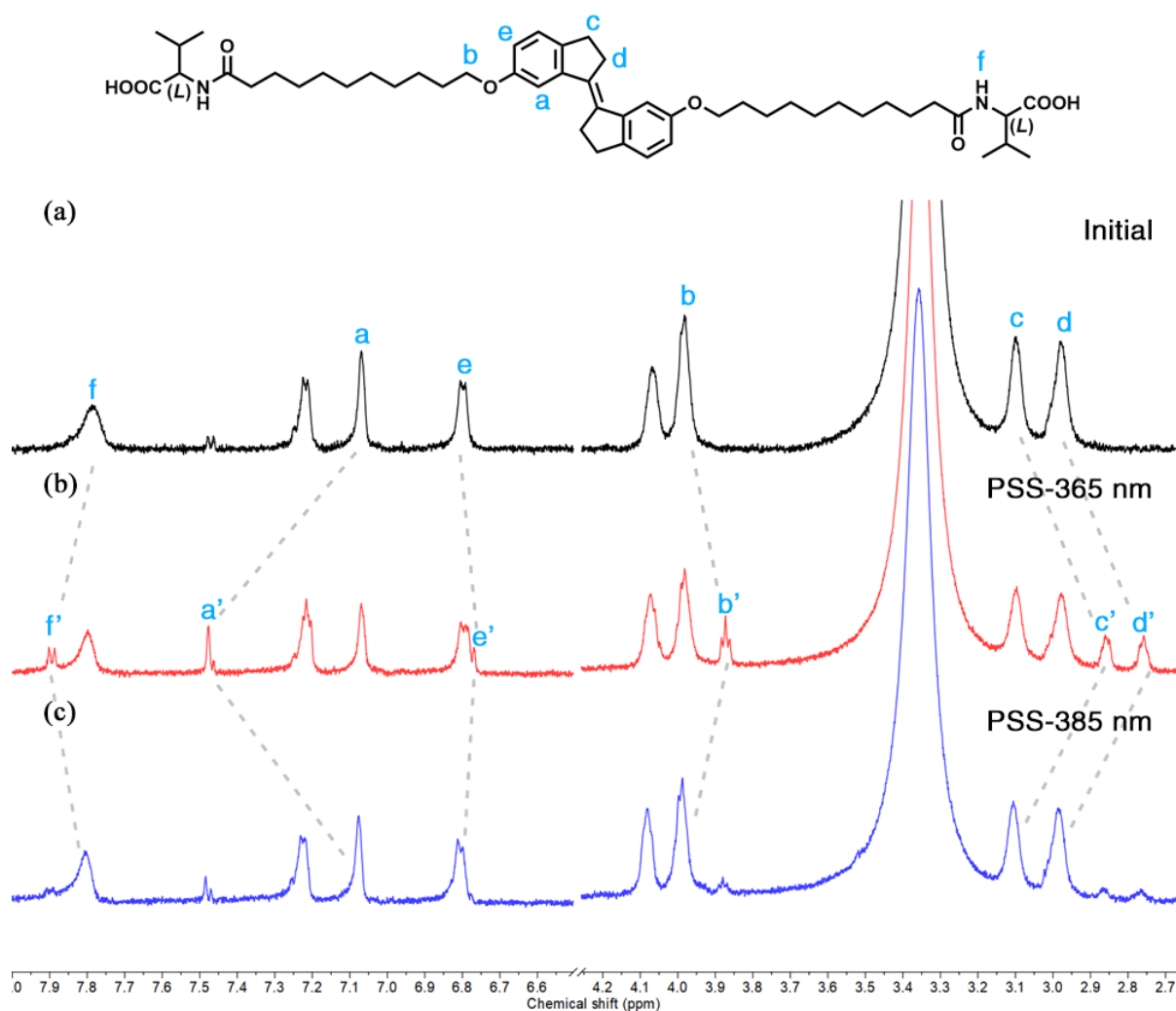


Figure A3.7: Aromatic and aliphatic region of ¹H NMR spectra during isomerization process of **SA_{Val}** (5.5 mM, DMSO-*d*₆, 600 MHz), (a) before photoirradiation, (b) irradiated with 365 nm for 1 h at 20 °C, (c) followed by 385 nm irradiation for 2 h at 20 °C. Irradiation of sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample. PSS₃₆₅ ratio of *trans*-**SA_{Val}**/*cis*-**SA_{Val}** was 74:26 and PSS₃₈₅ ratio of *trans*-**SA_{Val}**/*cis*-**SA_{Val}** was 90:10.

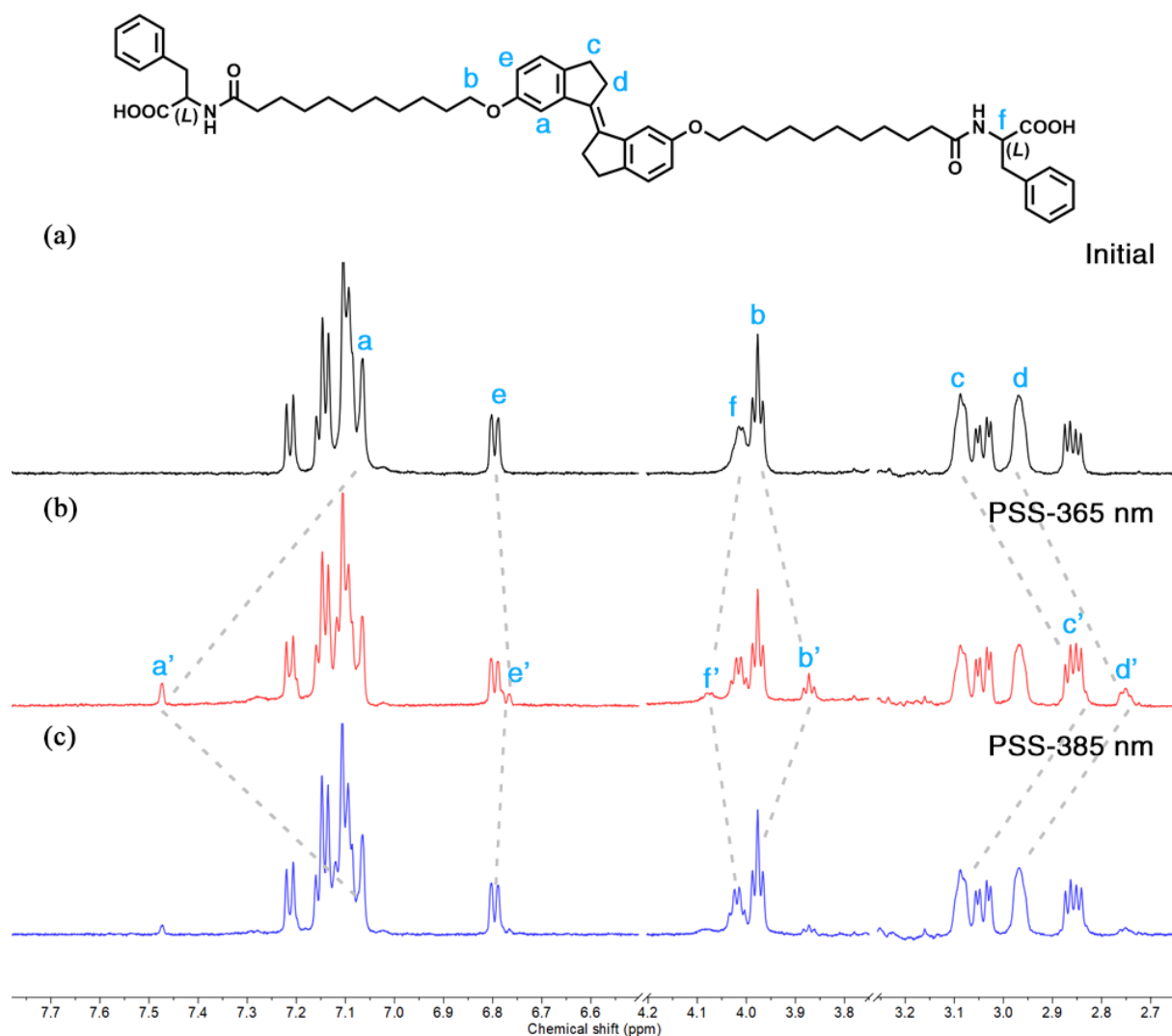


Figure A3.8: Aromatic and aliphatic region of ¹H NMR spectra during isomerization process of **SA_{Phe}** (5.5 mM, DMSO-*d*₆, 600 MHz), (a) before photoirradiation, (b) irradiated with 365 nm for 2 h at 20 °C, (c) followed by 385 nm irradiation for 2 h at 20 °C. Irradiation of sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample. PSS₃₆₅ ratio of *trans*-**SA_{Phe}**/*cis*-**SA_{Phe}** was 80:20 and PSS₃₈₅ ratio of *trans*-**SA_{Phe}**/*cis*-**SA_{Phe}** was 94:6.

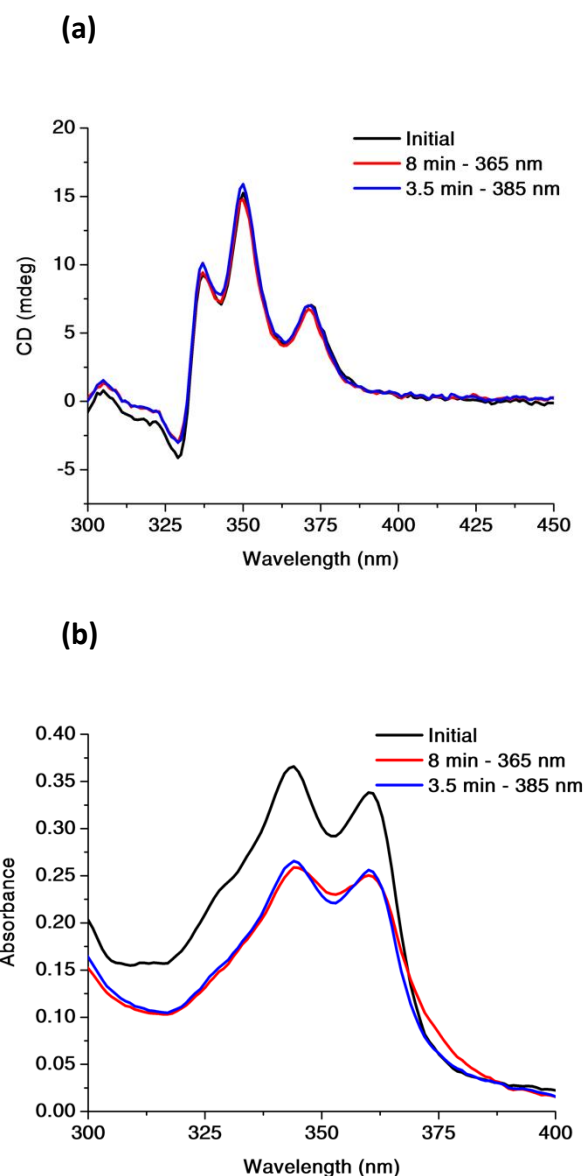


Figure A3.9: (a) CD spectra and (b) the corresponding UV-vis absorption spectra of the **SA_{Ala}** in DMSO (0.69 mM) without photoirradiation (black), after 365 nm photoirradiation for 8.5 min (red), and followed by 385 nm photoirradiation for 3.5 min (blue). Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample.

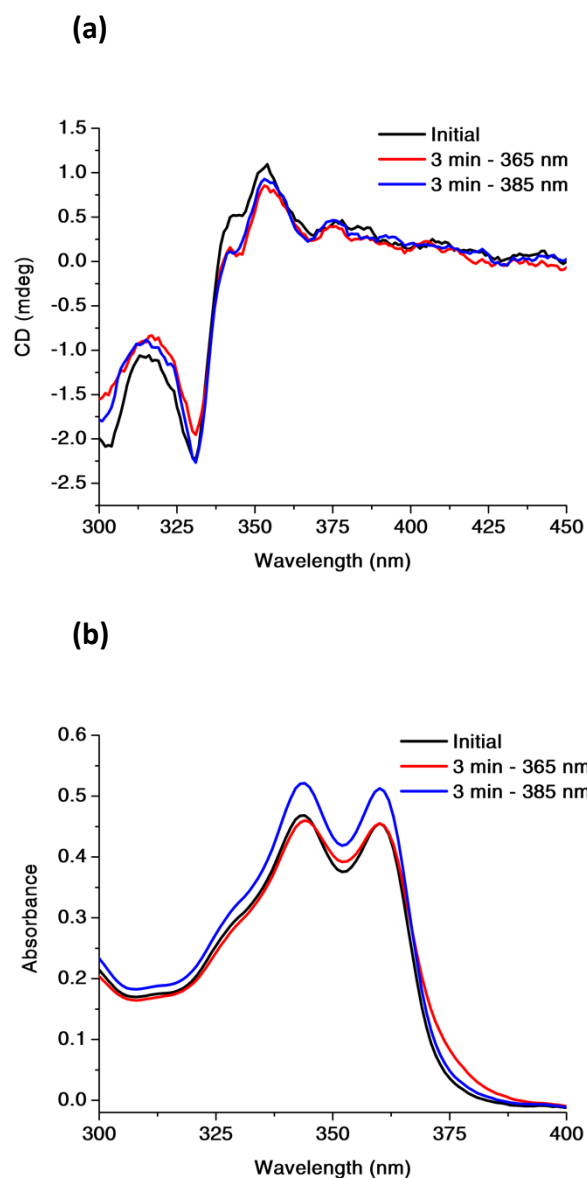


Figure A3.10: (a) CD spectra and (b) the corresponding UV-vis absorption spectra of the **SA_{val}** in DMSO (1.2 mM) without photoirradiation (black), after 365 nm photoirradiation for 3 min (red), and followed by 385 nm photoirradiation for 3 min (blue). Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample.

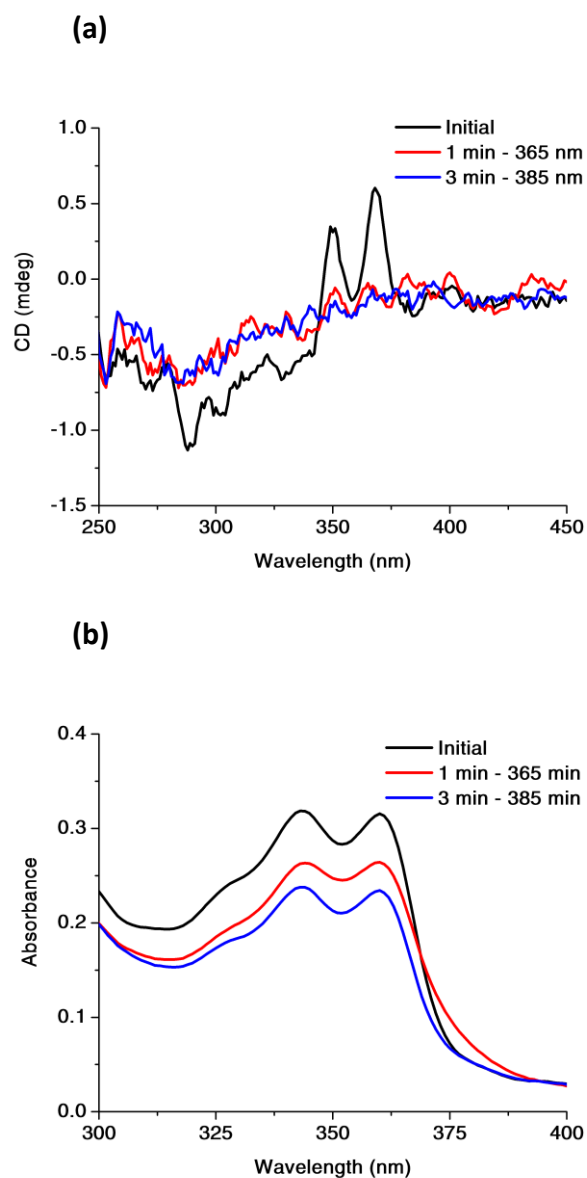


Figure A3.11: (a) CD spectra and (b) the corresponding UV-vis spectra of the **SA_{Phe}** in DMSO (0.80 mM) without photoirradiation (black), after 365 nm photoirradiation for 3 min (red), and followed by 385 nm photoirradiation for 3 min (blue). Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample.

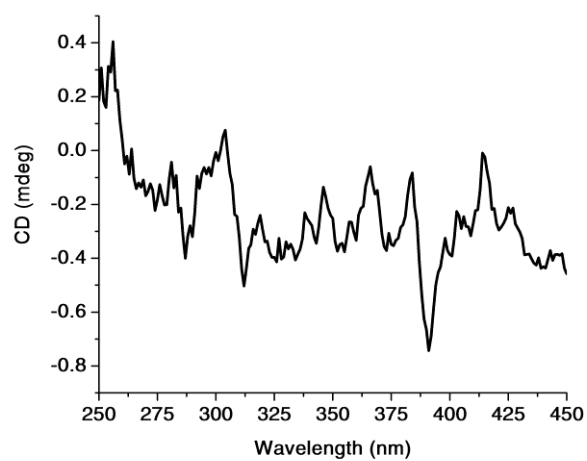


Figure A3.12: (a) CD spectra of the SA_{Gly} in DMSO (1.13 mM).

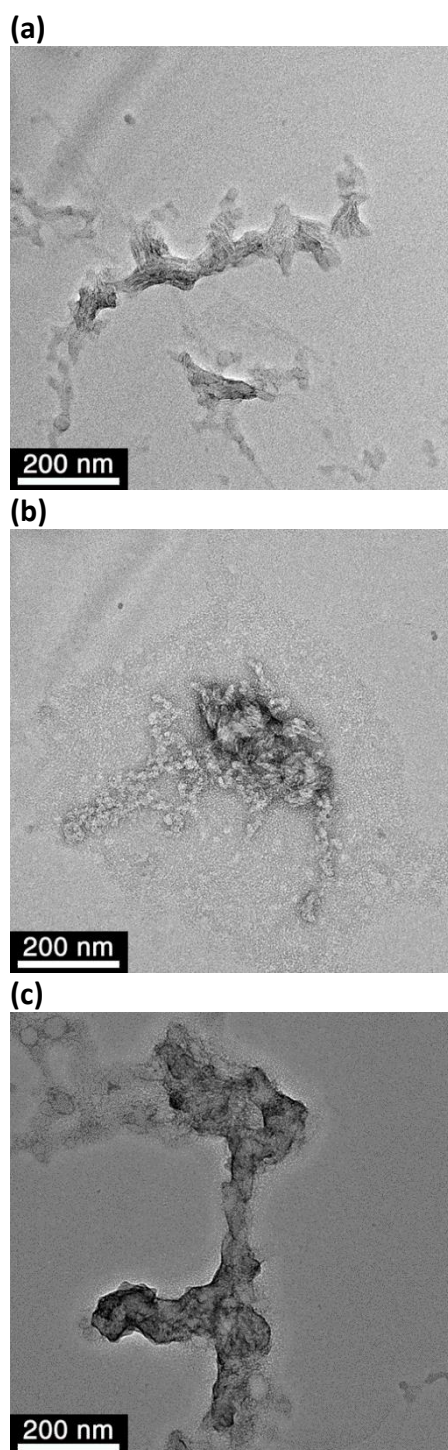


Figure A3.13. TEM images of SA_{Gly} (0.2 wt.%, 2.68 mM) in DMSO (a) without photoirradiation, (b) after 365 nm photoirradiation for 0.5 h, and (c) followed by 385 nm photoirradiation for 0.5 h. Irradiation of sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample.

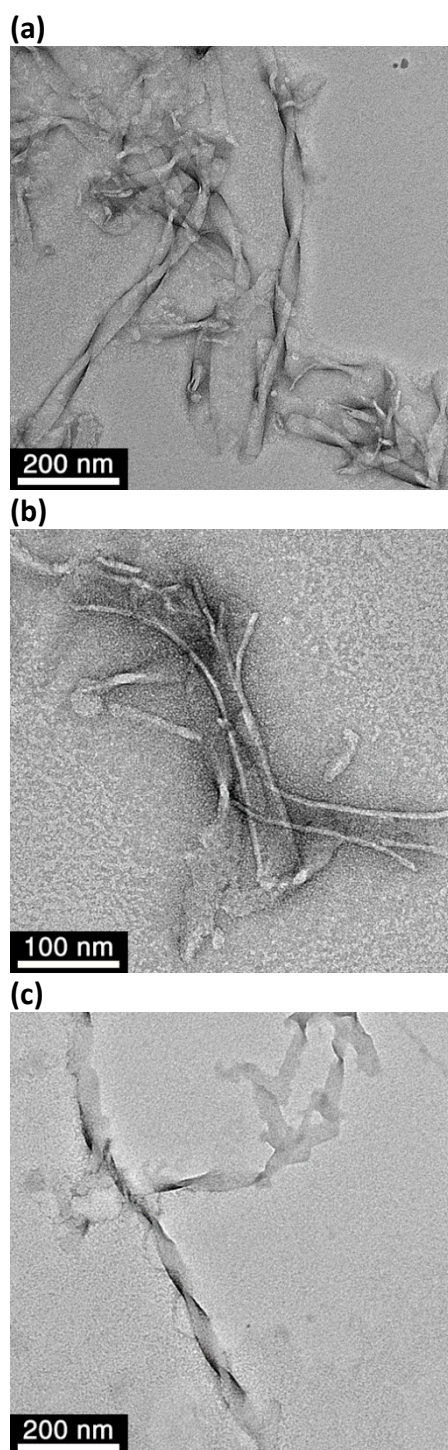


Figure A3.14. TEM images of **SA_{val}** (0.2 wt.%, 2.41 mM) in DMSO (a) without photoirradiation, (b) after 365 nm photoirradiation for 0.5 h, and (c) followed by 385 nm photoirradiation for 0.5 h. Irradiation of sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample.

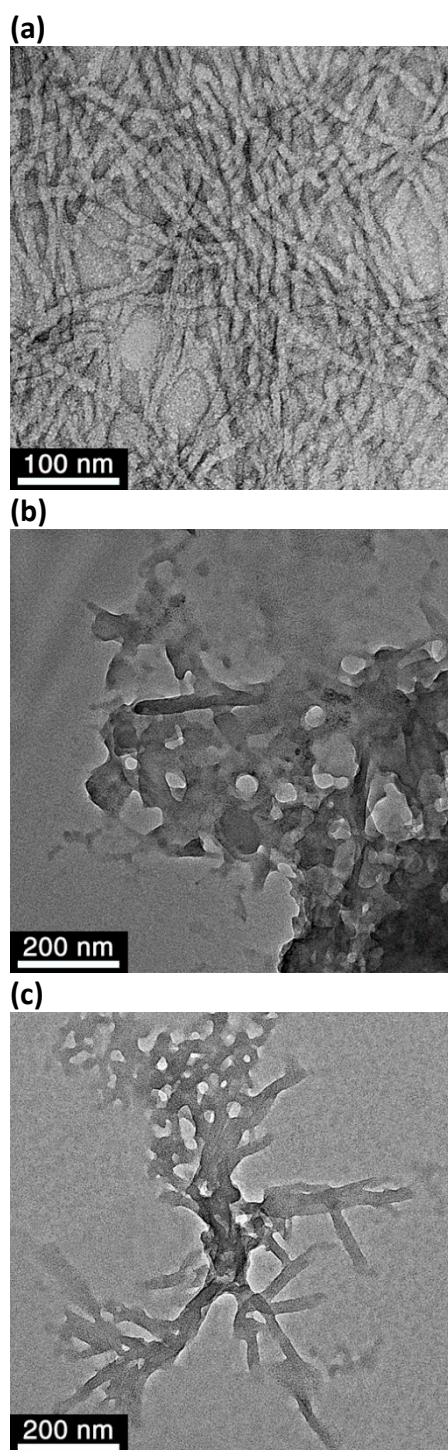


Figure A3.15. TEM images of SA_{Phe} (0.2 wt.%, 2.16 mM) in DMSO (a) without photoirradiation, (b) after 365 nm photoirradiation for 0.5 h, and (c) followed by 385 nm photoirradiation for 0.5 h. Irradiation of sample was carried out at 20 °C using a Thorlabs model fiber-coupled high-power LED (M365F1, 365 nm, 0.7 A; M385F1, 385 nm, 0.7 A) positioned at a distance of 1 cm from the sample.

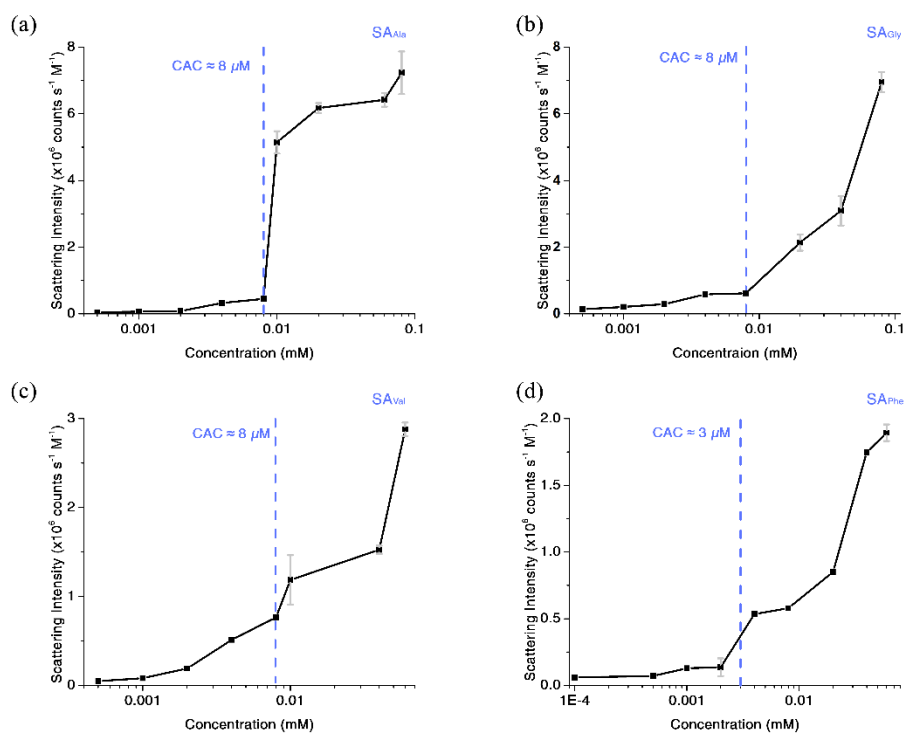


Figure A3.16. Dynamic light scattering assay for determination of critical aggregation concentration of (a) **SA_{Ala}** (concentration: 5.0×10^{-4} to 0.08 mM), (b) **SA_{Gly}** (concentration: 5.0×10^{-4} to 0.08 mM), (c) **SA_{Val}** (concentration: 5.0×10^{-4} to 0.06 mM), and **SA_{Phe}** (concentration: 1.0×10^{-4} to 0.1 mM).

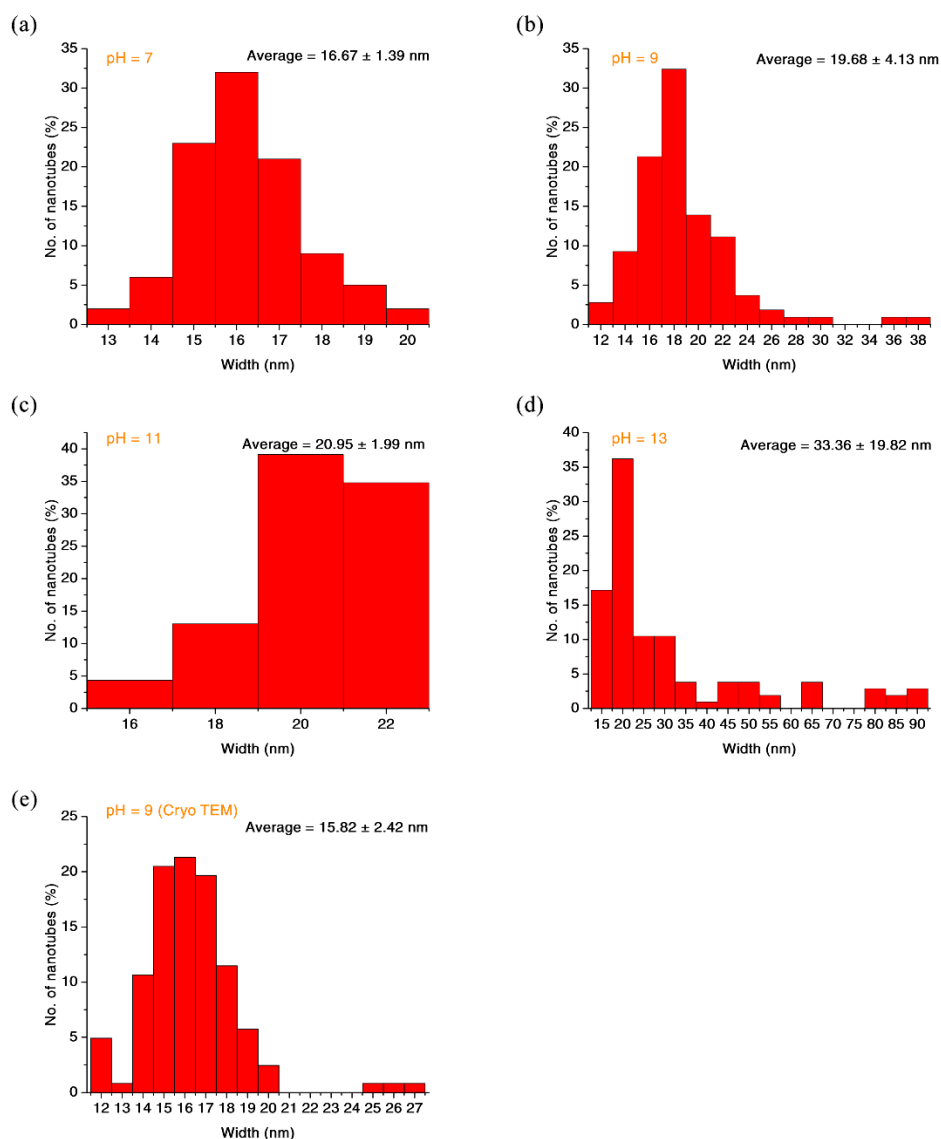


Figure A3.17. Histograms of width distribution of **SA_{Ala}** nanotubes (0.2 wt.%, 2.58 mM) at (a) pH = 7, (b) pH = 9, (c) pH = 11, and (d) pH = 13, respectively. (e) Histogram of width distribution of **SA_{Ala}** nanotubes (0.5 wt.%, 6.45 mM) at pH = 9 imaging by Cryo-TEM.

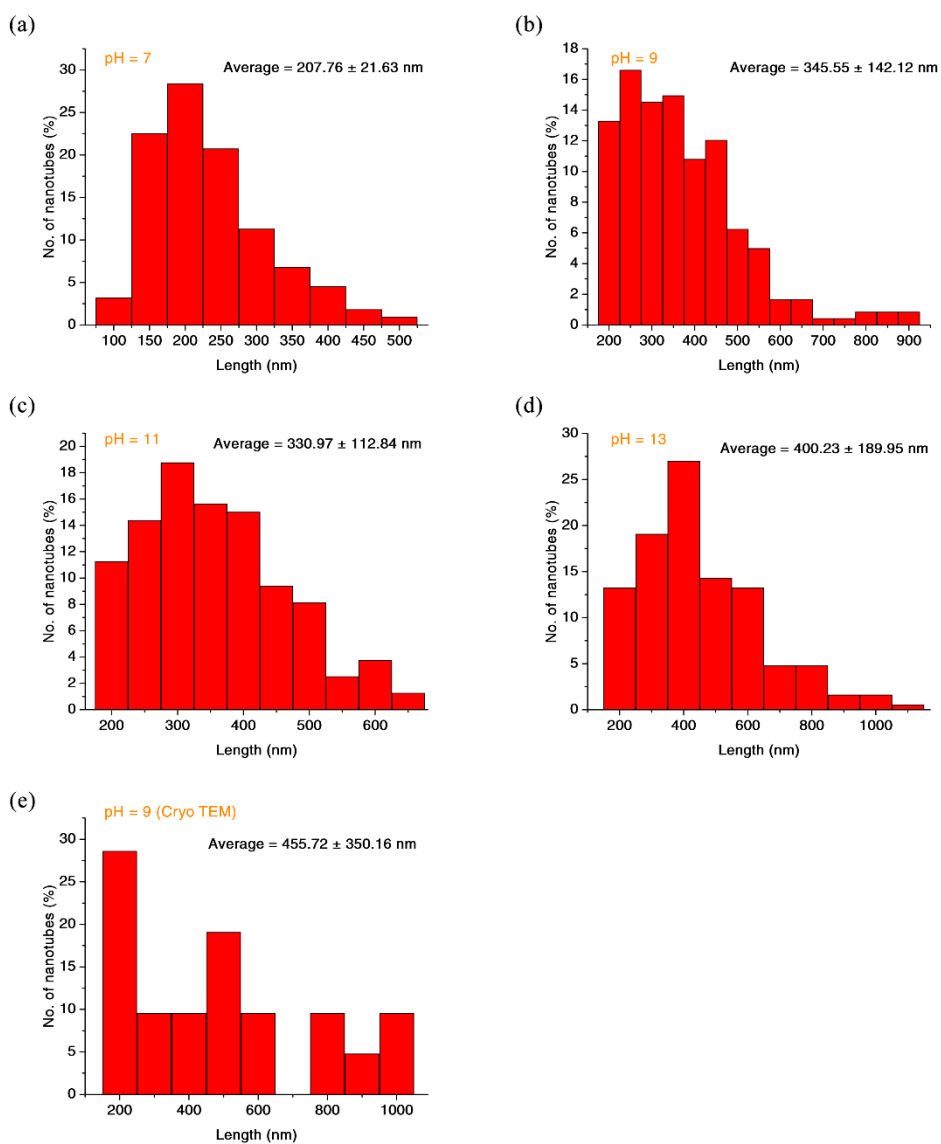


Figure A3.18. Histograms of length distribution of **SA_{Ala}** nanotubes (0.2 wt.%, 2.58 mM) at (a) pH = 7, (b) pH = 9, (c) pH = 11, and (d) pH = 13, respectively. (e) Histogram of width distribution of **SA_{Ala}** nanotubes (0.5 wt.%, 6.45 mM) at pH = 9 imaging by Cryo-TEM.

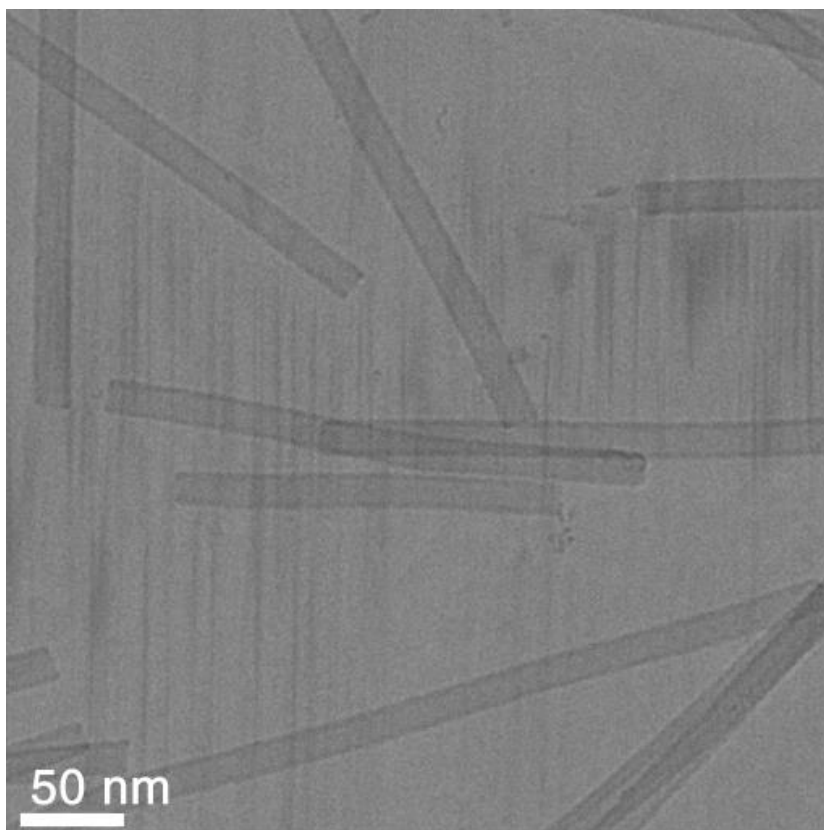
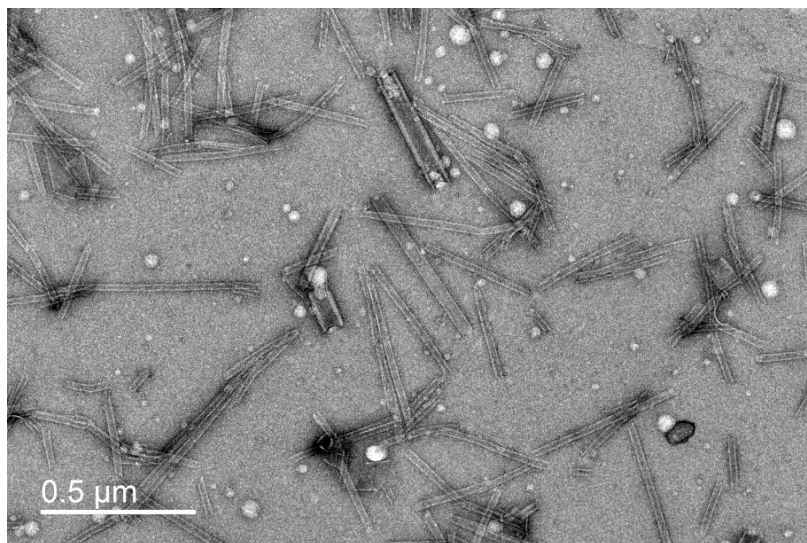


Figure A3.19. Cryo-TEM image of **SA_{Ala}** (0.5 wt.%, 6.45 mM) in water at pH = 9.

(a)



(b)

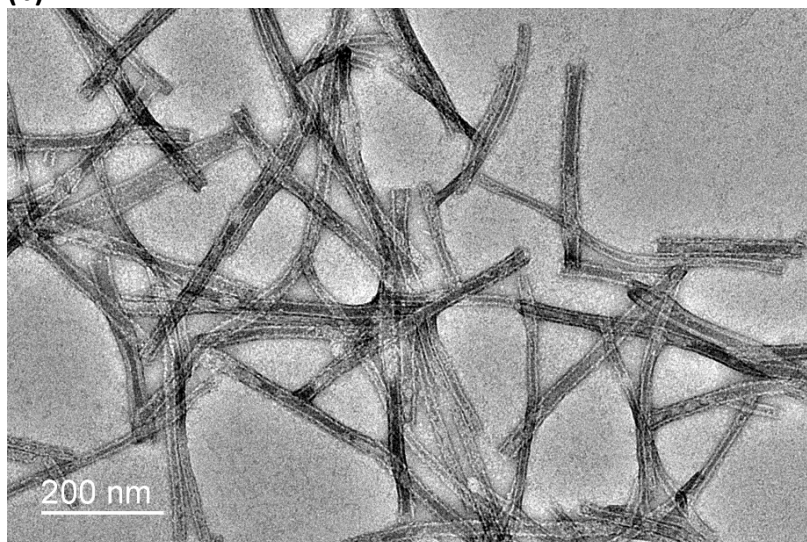


Figure A3.20. TEM image of **SA_{Ala}** (0.2 wt.%, 2.58 mM) in water at (a) pH = 11 after ageing for 2 month and (b) pH = 13 after ageing for 1 month.

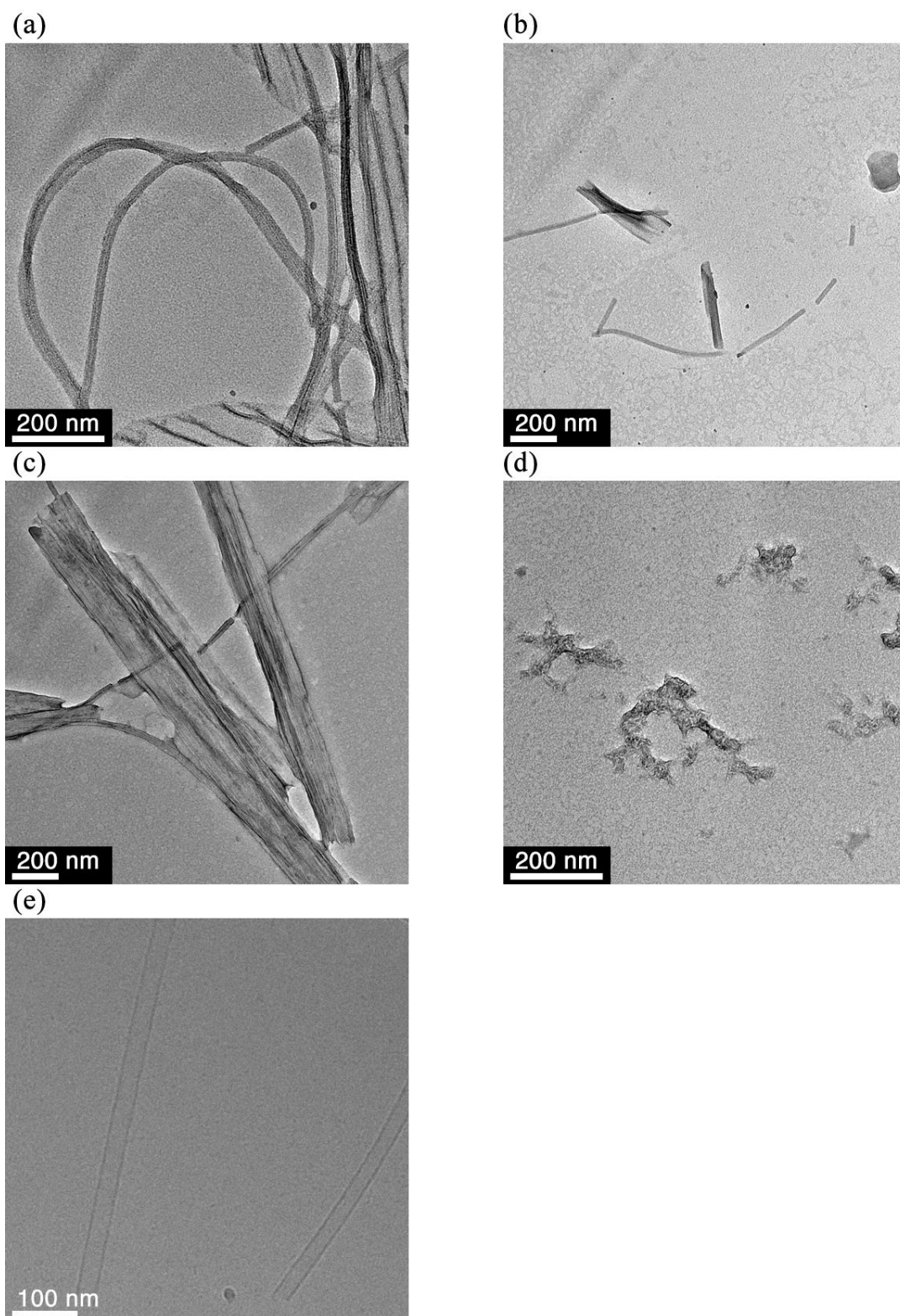


Figure A3.21. TEM images of SA_{Gly} (0.2 wt.%, 2.68 mM) in water at (a) pH = 7, (b) pH = 9, (c) pH = 11, and (d) pH = 13, respectively. (e) Cryo-TEM image of SA_{Gly} (0.5 wt.%, 6.69 mM) in water at pH = 9.

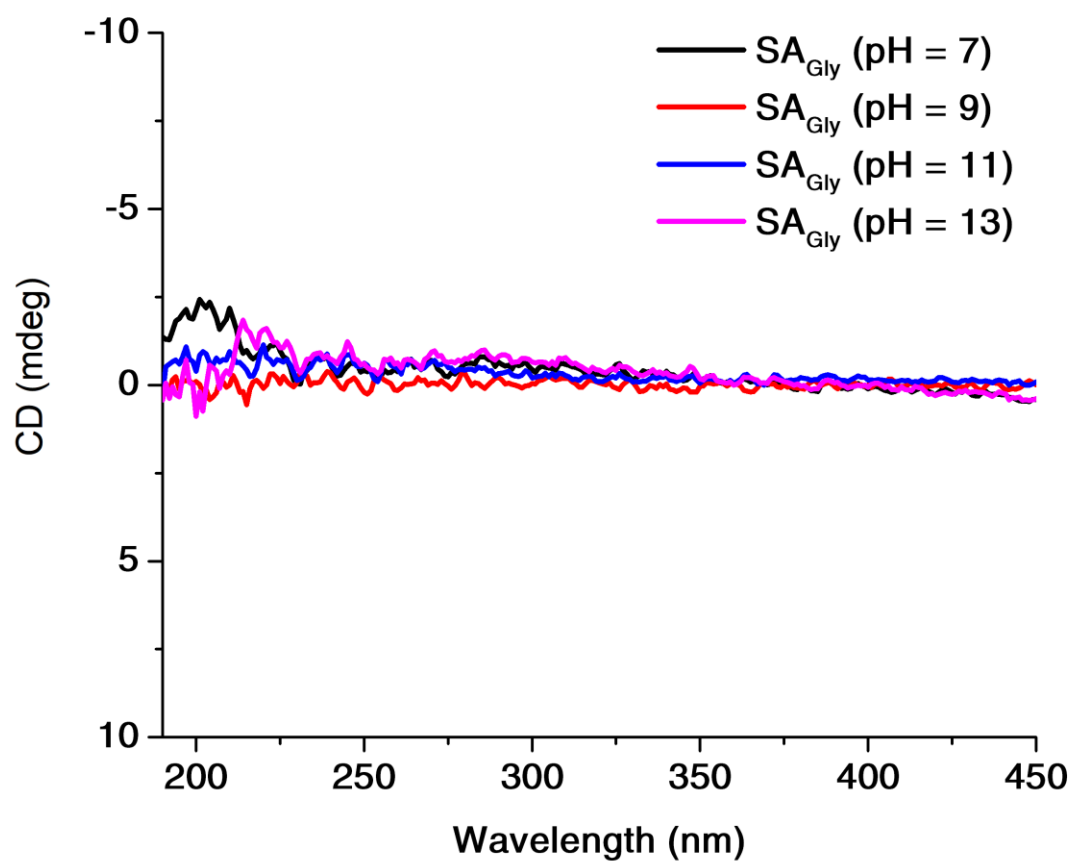


Figure A3.22. CD spectra of SA_{Gly} ($50 \mu\text{M}$) in water at different pH values.

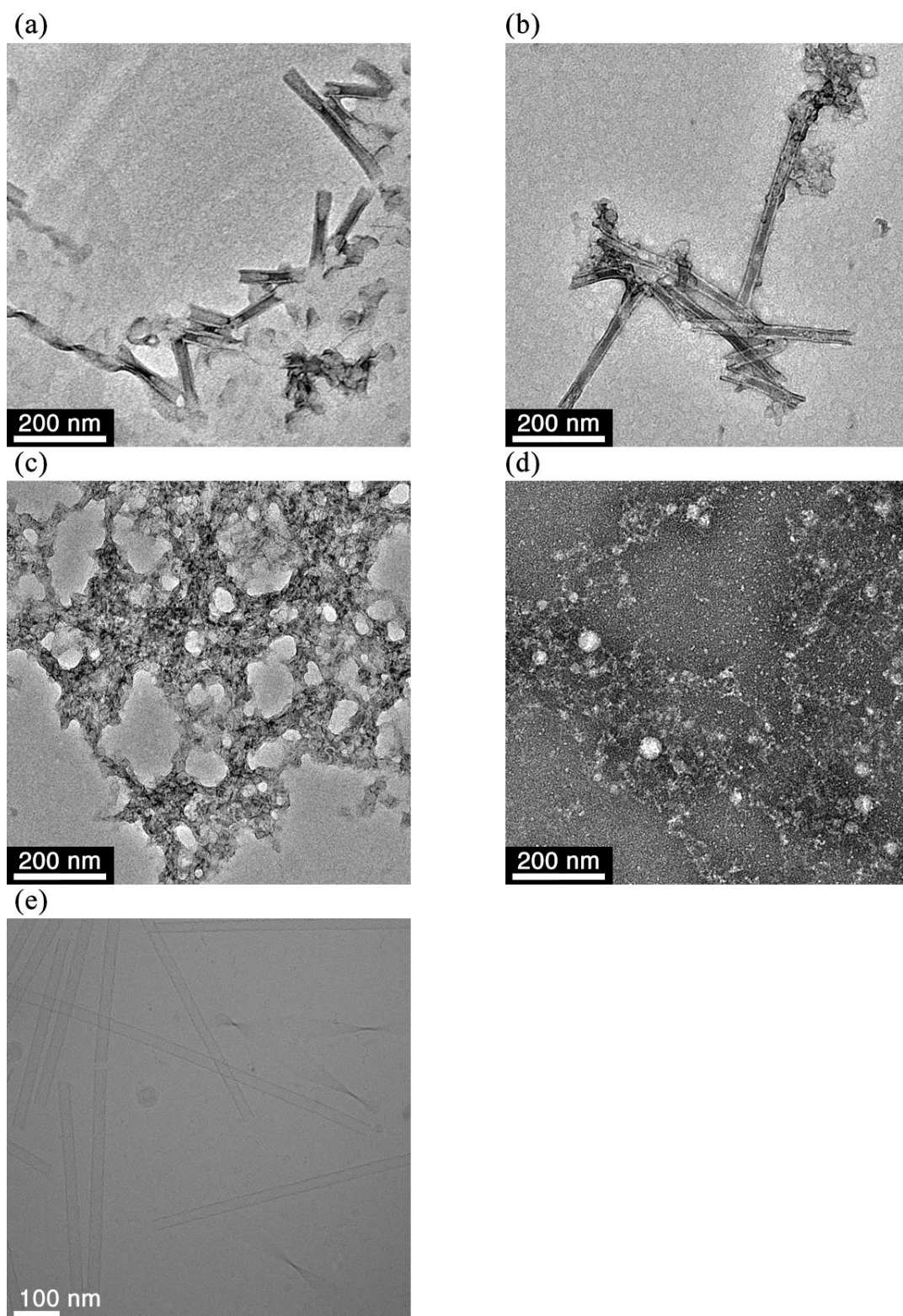


Figure A3.23. TEM images of SA_{val} (0.2 wt.%, 2.41 mM) in water at (a) pH = 7, (b) pH = 9, (c) pH = 11, and (d) pH = 13. (e) Cryo-TEM image of SA_{val} (0.5 wt.%, 6.02 mM) in water at pH = 9.

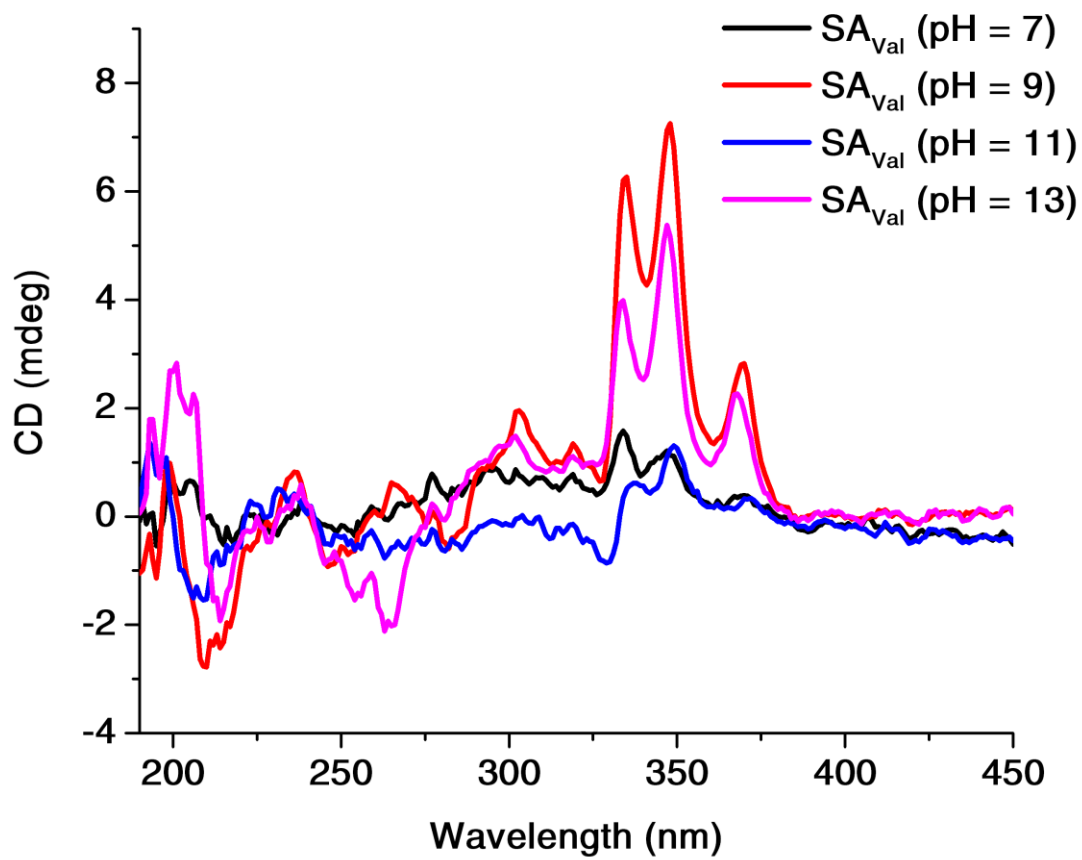


Figure A3.24. CD spectra of SA_{val} ($50 \mu\text{M}$) in water at different pH values.

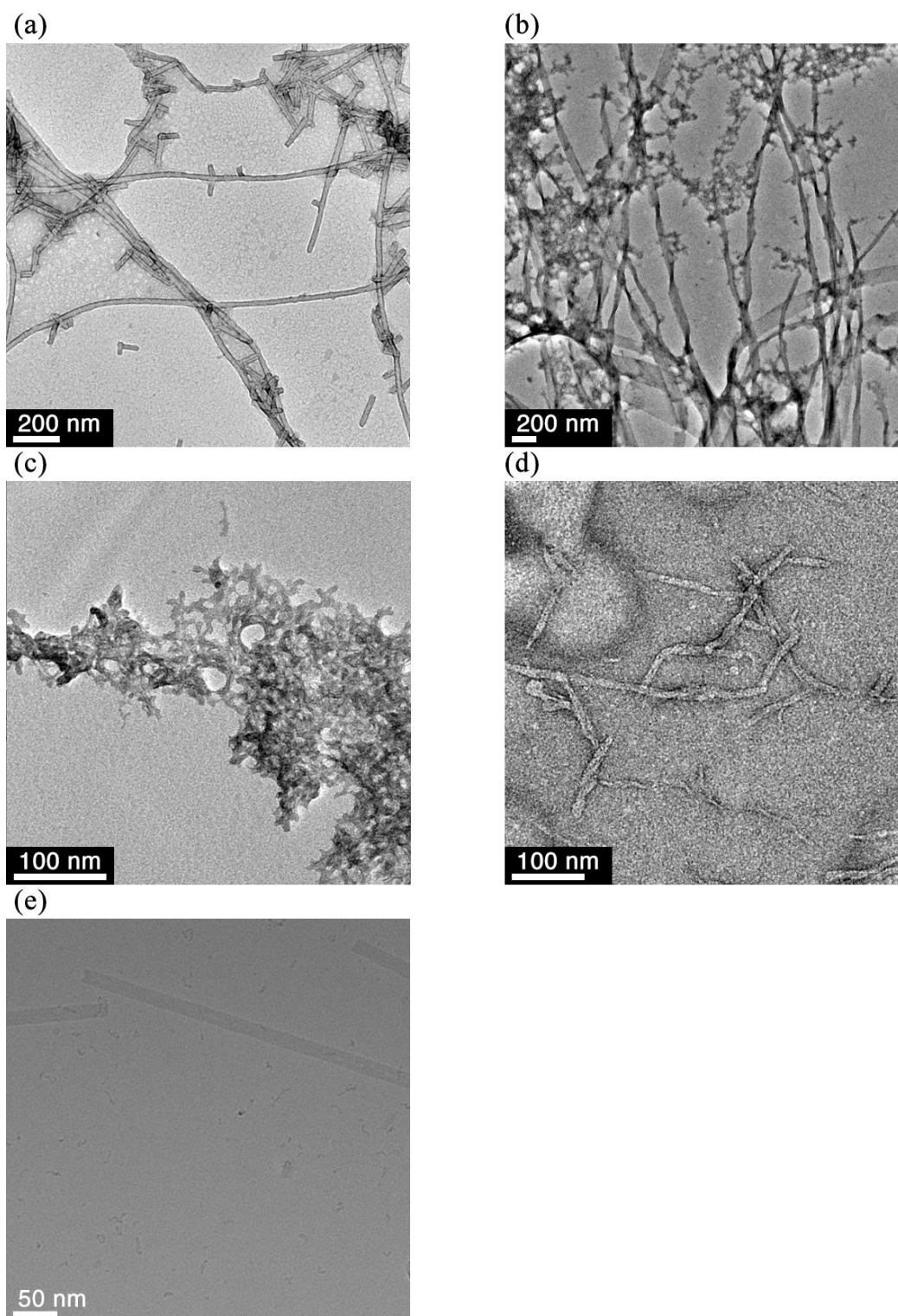


Figure A3.25. TEM images of SA_{Phe} (0.2 wt.%, 2.16 mM) in water at (a) pH = 7, (b) pH = 9, (c) pH = 11, (d) pH = 13. (e) Cryo-TEM image of SA_{Phe} (0.5 wt.%, 5.39 mM) in water at pH = 7.

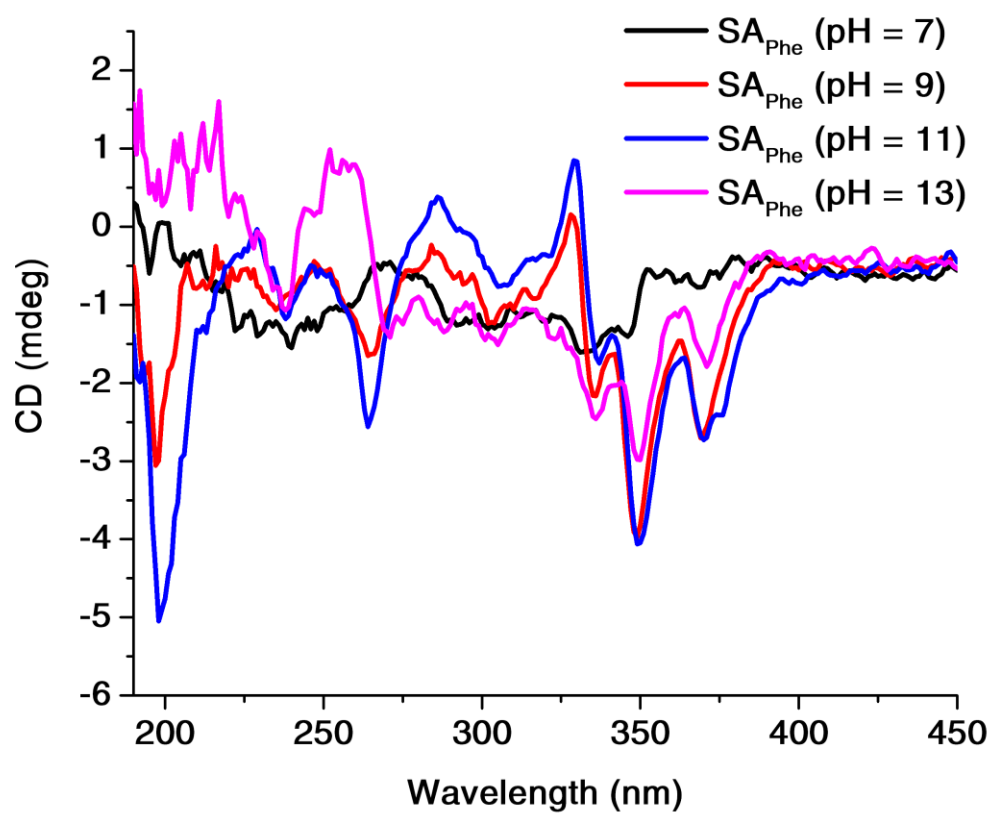


Figure A3.26. CD spectra of SA_{phe} (50 μM) in water at different pH values.

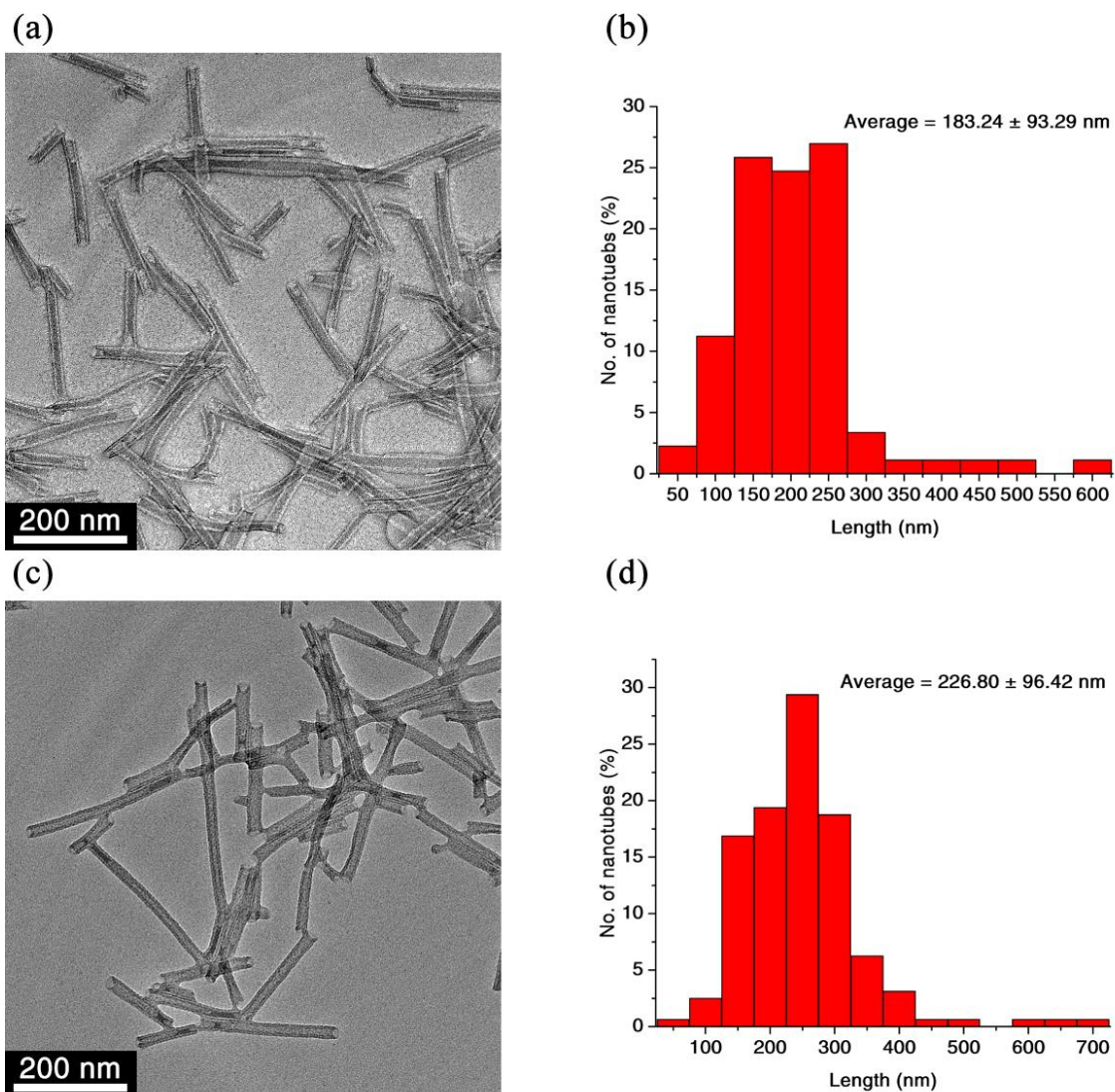


Figure A3.27. TEM images of SA_{Ala} nanotubes (0.2 wt.%, 2.58 mM) in water prepared by annealing SA_{Ala} (5.0 wt.%, 65 mM) at 85 °C for 10 min after ageing for (a) 1 month and (c) 2 months, respectively. (b) and (d) are the corresponding histograms of length distribution.

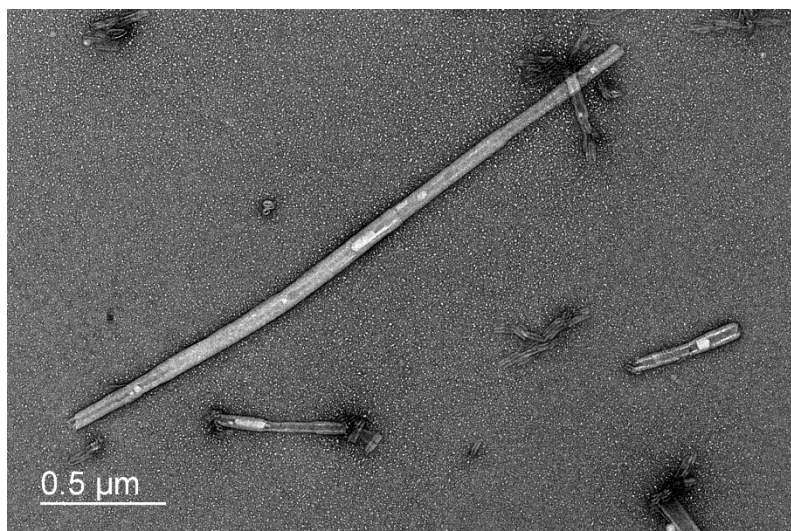


Figure A3.28. TEM image of **SA_{Ala}** (0.2 wt.%, 2.58 mM) in water prepared by annealing **SA_{Ala}** (5.0 wt.%, 65 mM) at 85 °C for 30 min after ageing for 1 month.

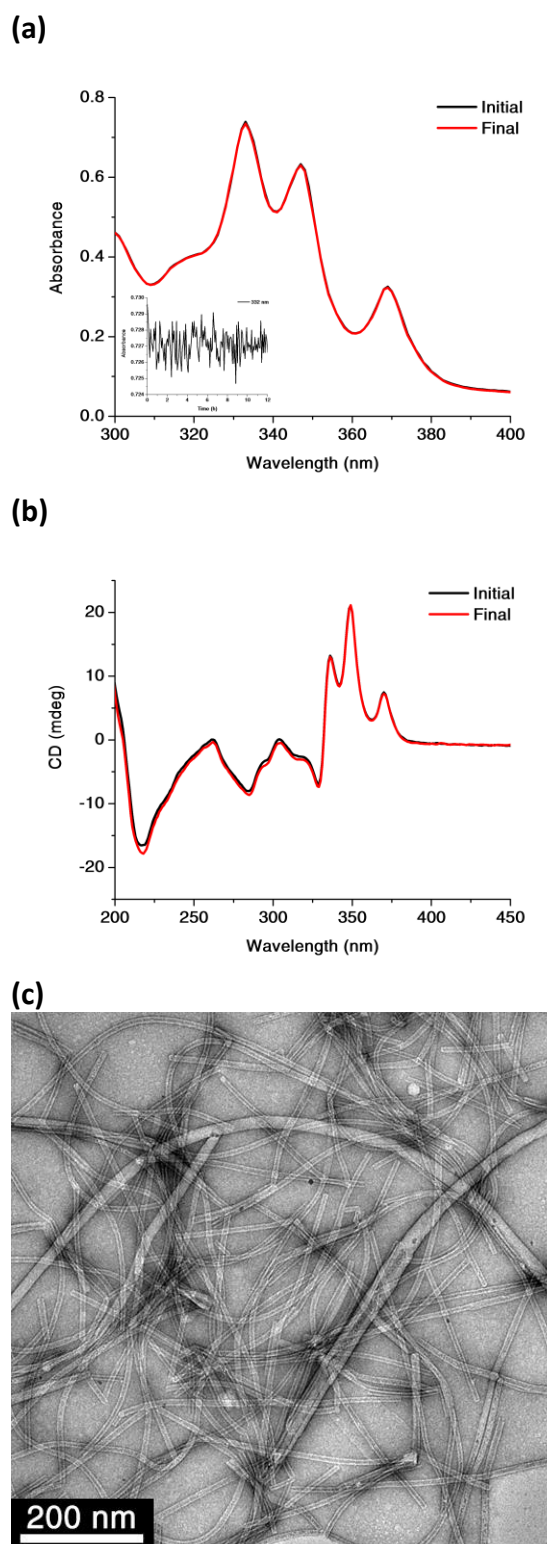


Figure A3.29. Stability test of **SA_{Ala}** prepared by annealing a 0.2 wt.% solution at 85 °C for 70 min followed by 1 °C/min to 20 °C. (a) UV-vis absorption spectra of **SA_{Ala}** (50 μ M, pH = 7) at 37 °C for 12 h (inset: time-course of the stability experiment of **SA_{Ala}** monitored at λ_{max} 332 nm) and (b) corresponding CD spectra. (c) TEM images of aqueous solutions of **SA_{Ala}** (50 μ M, pH = 7) after stability test at 37 °C for 12 h.

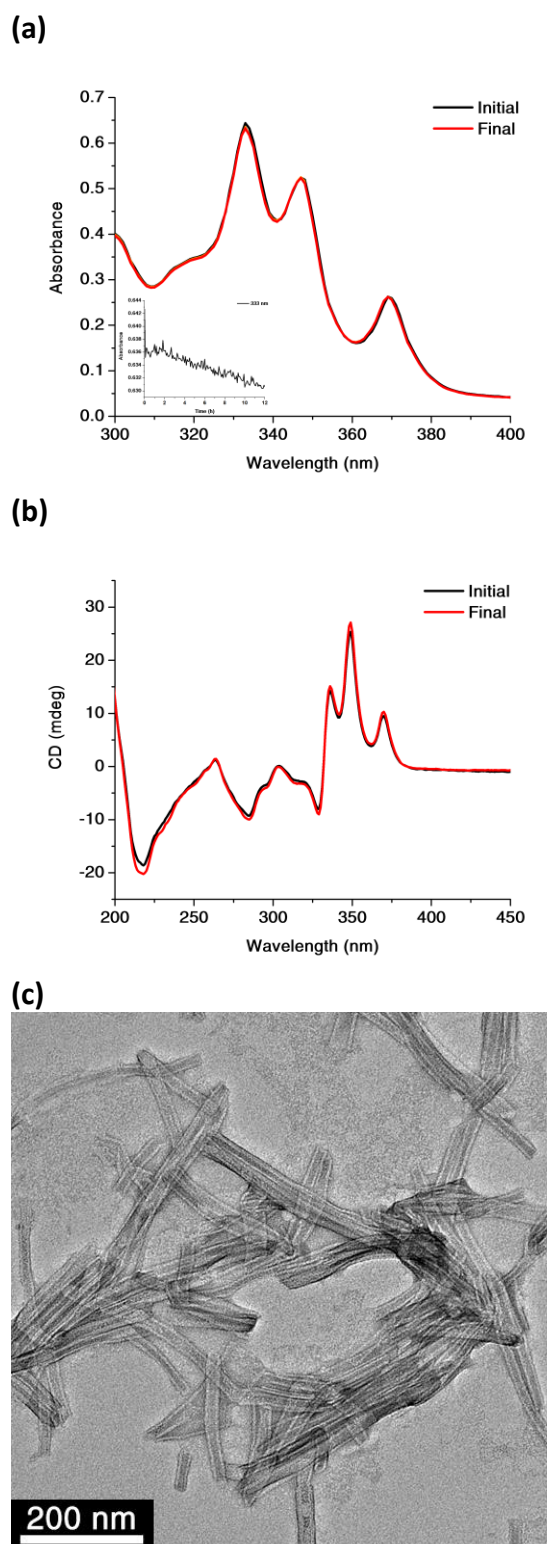


Figure A3.30. Stability test of **SA_{Ala}** prepared by annealing a 5.0 wt.% solution at 85 °C for 10 min followed by fast cooling. (a) UV-vis absorption spectra of **SA_{Ala}** (50 μ M, pH = 7) at 37 °C for 12 h (inset: time-course of the stability experiment of **SA_{Ala}** monitored at λ_{max} 333 nm) and (b) corresponding CD spectra. (c) TEM images of aqueous solutions of **SA_{Ala}** (50 μ M, pH = 7) after stability test at 37 °C for 12 h.

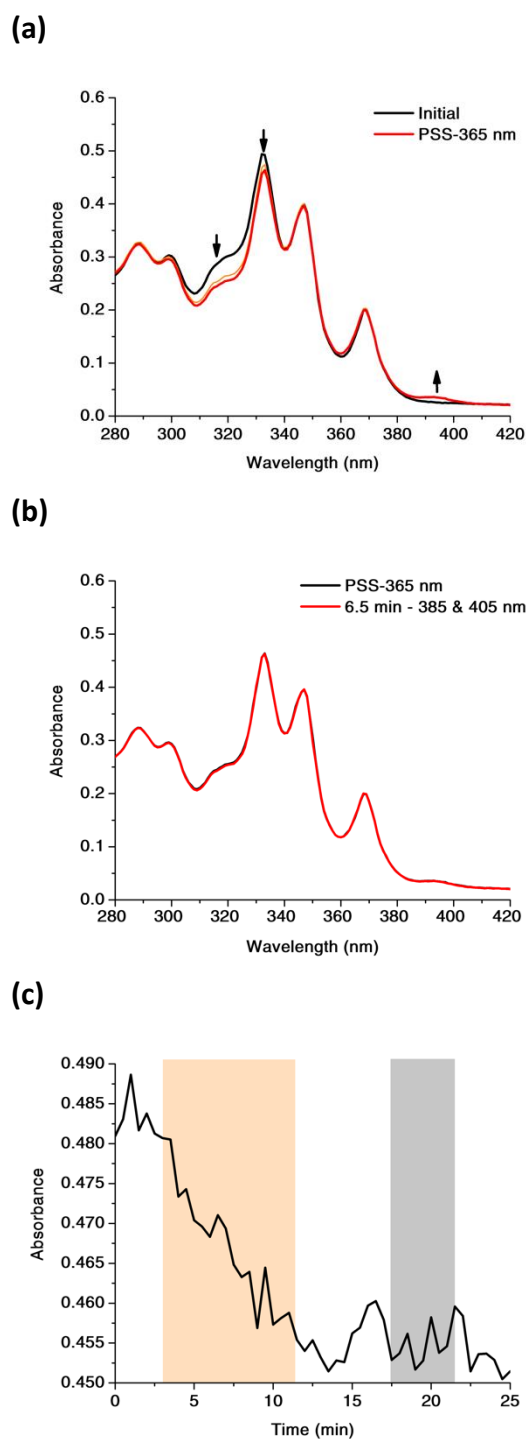


Figure A3.31. UV-vis absorption spectra of **SA_{Ala}** in water (0.22 mM, pH = 9). (a) Absorption spectra of **SA_{Ala}** in PSS (red) after 365 nm irradiation over 6.5 min (orange) (b) after a combination of 385 nm and 405 nm irradiation for 4 min (red). (c) Time-course of the photoisomerization process of **SA_{Ala}** in water, monitored at λ_{max} 333 nm of *trans*-**SA_{Ala}** (orange area: photoirradiation at 365 nm period; grey area: photoirradiation at 385 nm and 405 nm period). Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model collimated LEDs (M365LP-C1, 365 nm, 1.2 A; M385LP-C1, 385 nm, 1.2 A and 405 nm, M405LP-C1, 1.2 A) positioned at a distance of 1 cm from the sample.

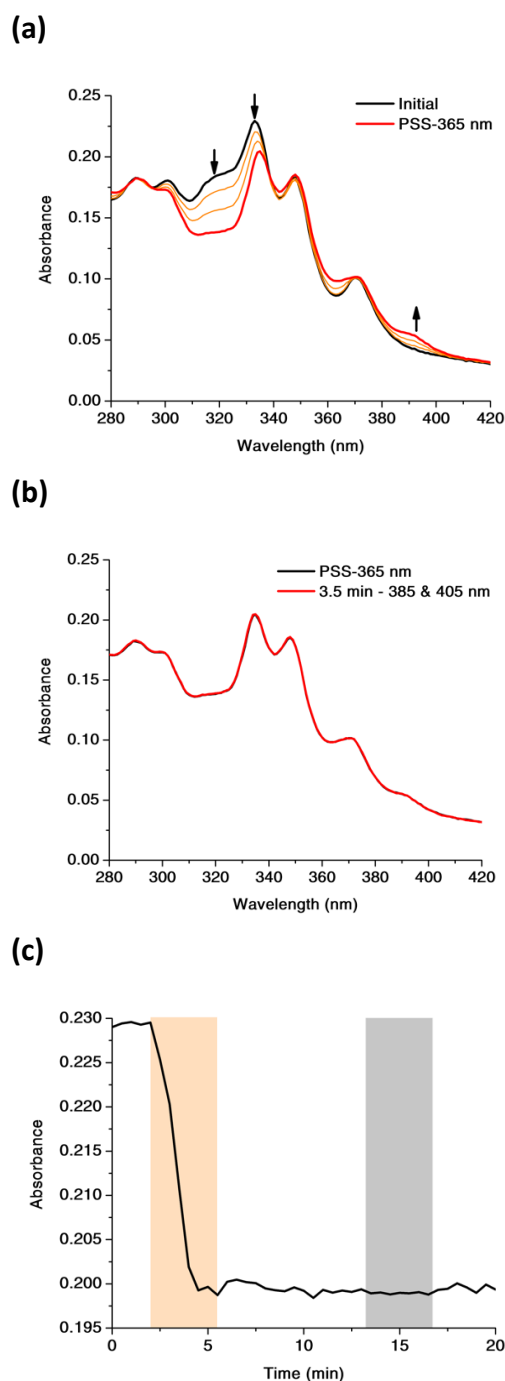


Figure A3.32. UV-vis absorption spectra of SA_{Gly} in water (0.12 mM, pH = 9). (a) Absorption spectra of SA_{Gly} in PSS (red) after 365 nm irradiation over 3.5 min (orange) (b) after a combination of 385 nm and 405 nm irradiation for 3.5 min (red). (c) Time-course of the photoisomerization process of SA_{Gly} in water, monitored at λ_{max} 333 nm of *trans*- SA_{Gly} (orange area: photoirradiation at 365 nm period; grey area: photoirradiation at 385 nm and 405 nm period). Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model collimated LEDs (M365LP-C1, 365 nm, 1.2 A; M385LP-C1, 385 nm, 1.2 A and 405 nm, M405LP-C1, 1.2 A) positioned at a distance of 1 cm from the sample.

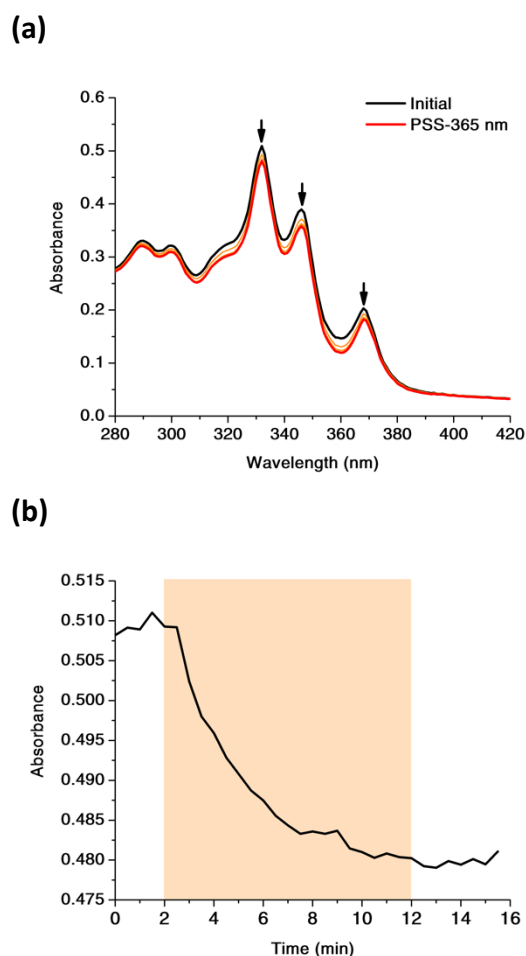


Figure A3.33. UV-vis absorption spectra of **SA_{Val}** in water (0.6 mM, pH = 9). (a) Absorption spectra of **SA_{Val}** in PSS (red) after 365 nm irradiation over 10 min (orange). (b) Time-course of the photoisomerization process of **SA_{Val}** in water, monitored at λ_{max} 332 nm of *trans*-**SA_{Val}** (orange area: photoirradiation at 365 nm period). Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model collimated LEDs (M365LP-C1, 365 nm, 1.2 A) positioned at a distance of 1 cm from the sample.

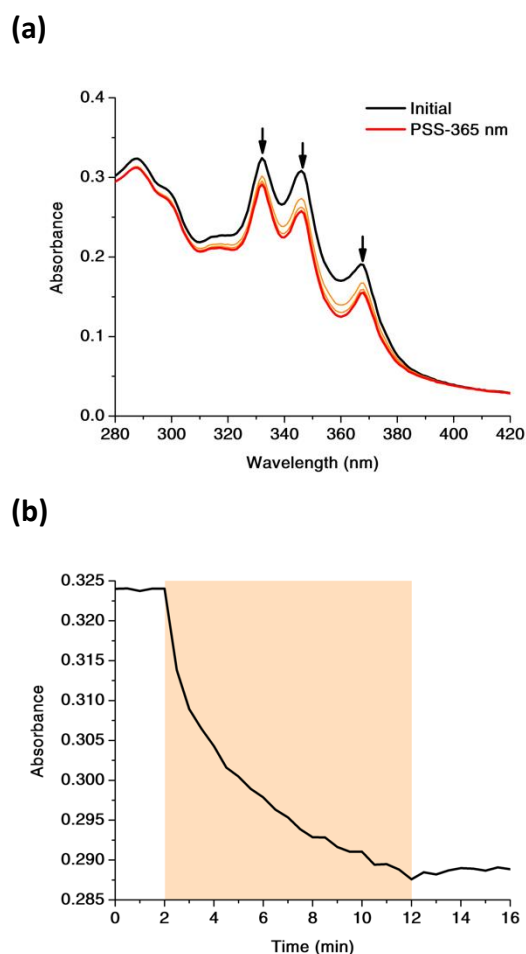
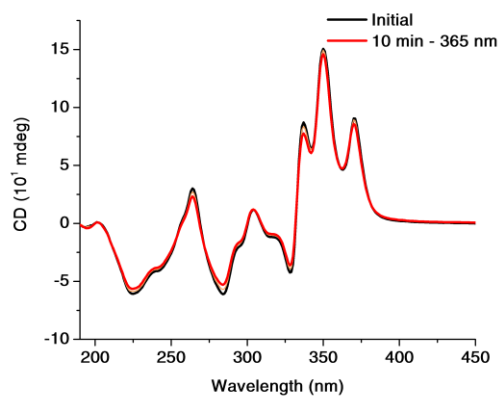


Figure A3.34. UV-vis absorption spectra of **SA_{Phe}** in water (0.43 mM, pH = 7). (a) Absorption spectra of **SA_{Phe}** in PSS (red) after 365 nm irradiation over 10 min (orange). (b) Time-course of the photoisomerization process of **SA_{Phe}** in water, monitored at λ_{max} 332 nm of *trans*-**SA_{Phe}** (orange area: photoirradiation at 365 nm period). Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model collimated LEDs (M365LP-C1, 365 nm, 1.2 A) positioned at a distance of 1 cm from the sample.

(a)



(b)

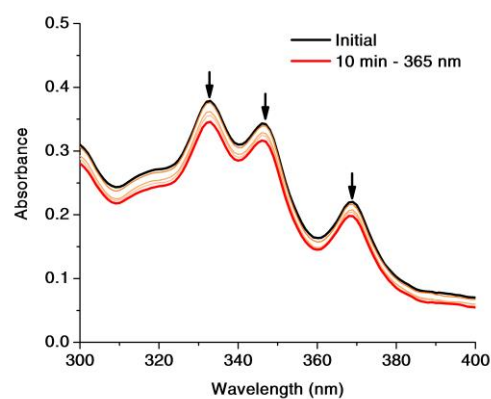


Figure A3.35. (a) CD spectra and (b) the corresponding UV-vis absorption spectra of the **SA_{Ala}** in water (50 μ M, pH = 9) without photoirradiation (black), after 365 nm photoirradiation for 10 min (red). Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model collimated LEDs (M365LP-C1, 365 nm) positioned at a distance of 1 cm from the sample.

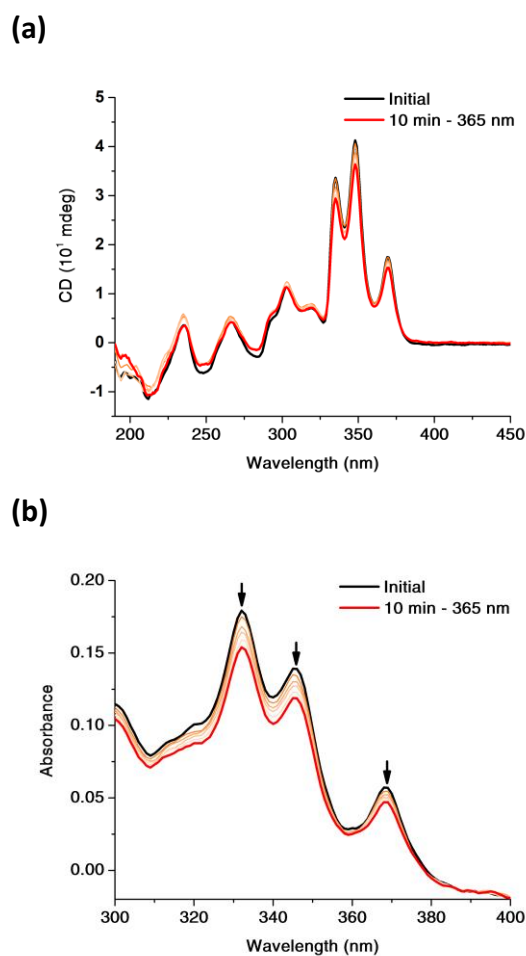


Figure A3.36. (a) CD spectra and (b) the corresponding UV-vis absorption spectra of the **SA_{val}** in water (50 μ M, pH = 9) without photoirradiation (black), after 365 nm photoirradiation for 10 min (red). Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model collimated LEDs (M365LP-C1, 365 nm) positioned at a distance of 1 cm from the sample.

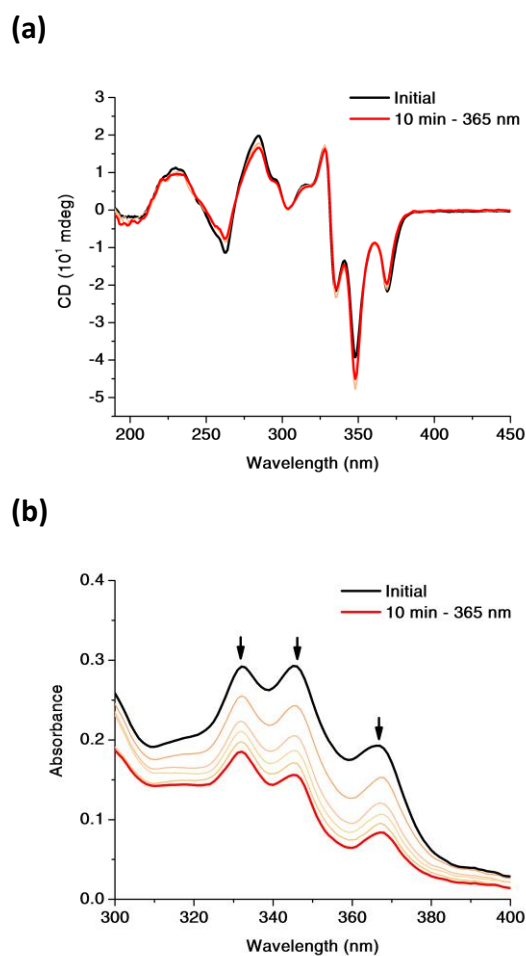
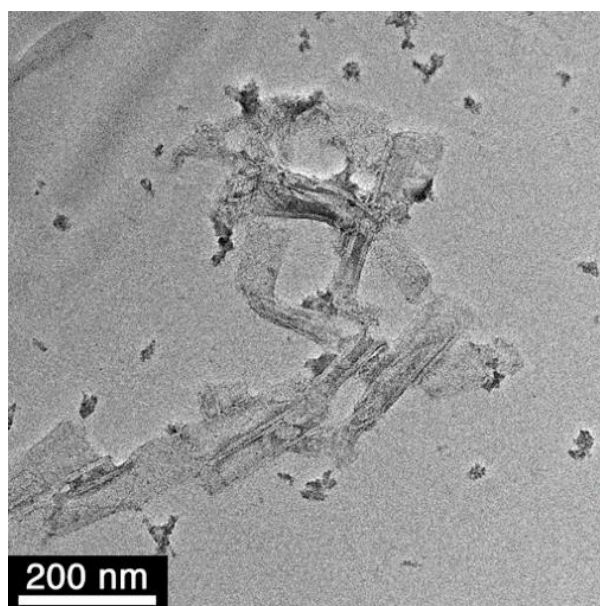


Figure A3.37. (a) CD spectra and (b) the corresponding UV-vis absorption spectra of the **SA_{Phe}** in water (50 μ M, pH = 7) without photoirradiation (black), after 365 nm photoirradiation for 20 min (red). Irradiation of UV-vis sample was carried out at 20 °C using a Thorlabs model collimated LEDs (M365LP-C1, 365 nm) positioned at a distance of 1 cm from the sample.

(a)



(b)

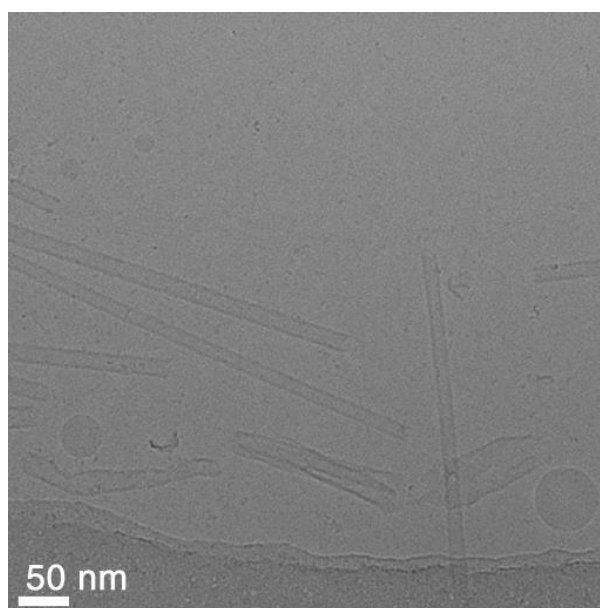
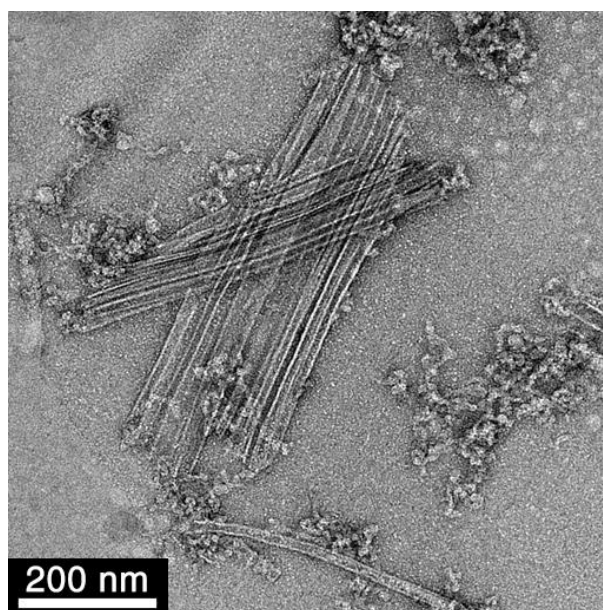


Figure A3.38. TEM image of **SA_{Ala}** (0.2 wt.%, 2.58 mM) annealed at 5 wt.% in water after 365 nm photoirradiation for 1 h, and (b) the corresponding Cryo-TEM image (0.5 wt.%, 5.39 mM, pH = 7). Irradiation of sample was carried out at 20 °C using a Thorlabs model collimated LEDs (M365LP-C1, 365 nm, 1.2 A) positioned at a distance of 1 cm from the sample.

(a)



(b)

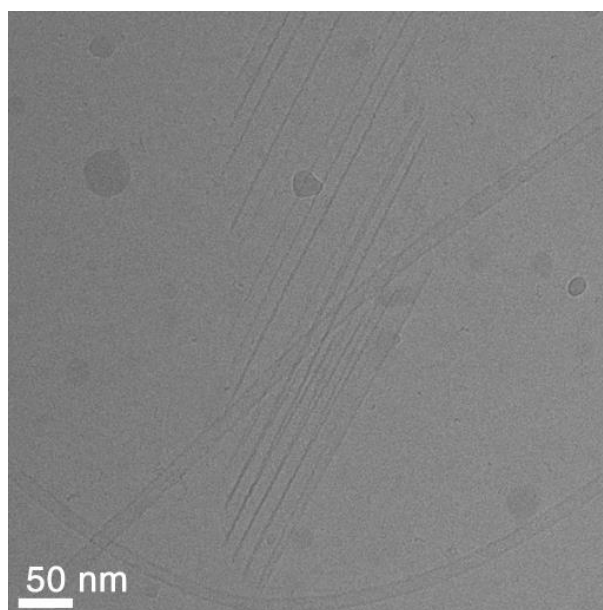
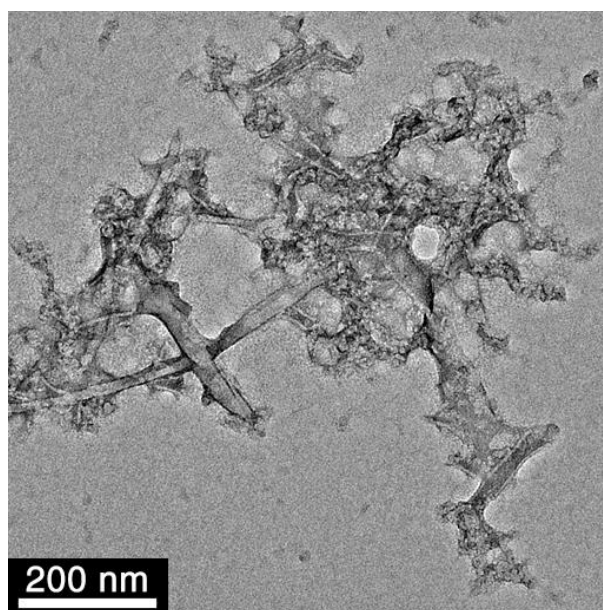


Figure A3.39. (a) TEM image of SA_{Gly} (0.2 wt.%, 2.68 mM, pH = 9) in water after 365 nm photoirradiation for 1 h, and (b) the corresponding Cryo-TEM image (0.5 wt.%, 6.69 mM, pH = 9). Irradiation of sample was carried out at 20 °C using a Thorlabs model collimated LEDs (M365LP-C1, 365 nm, 1.2 A) positioned at a distance of 1 cm from the sample.

(a)



(b)

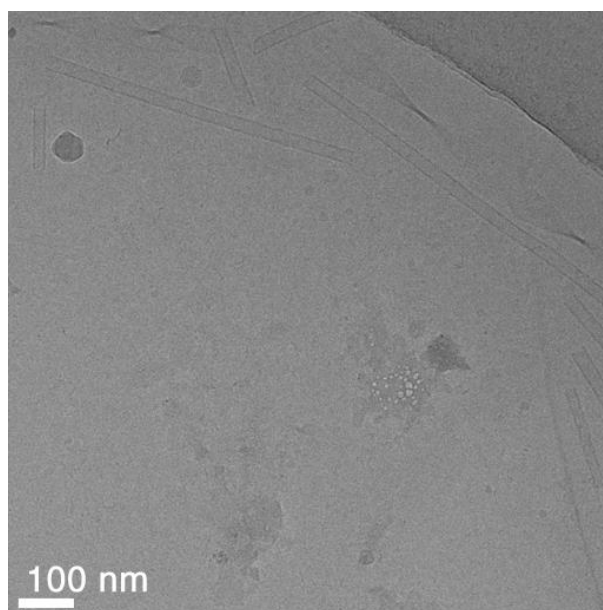
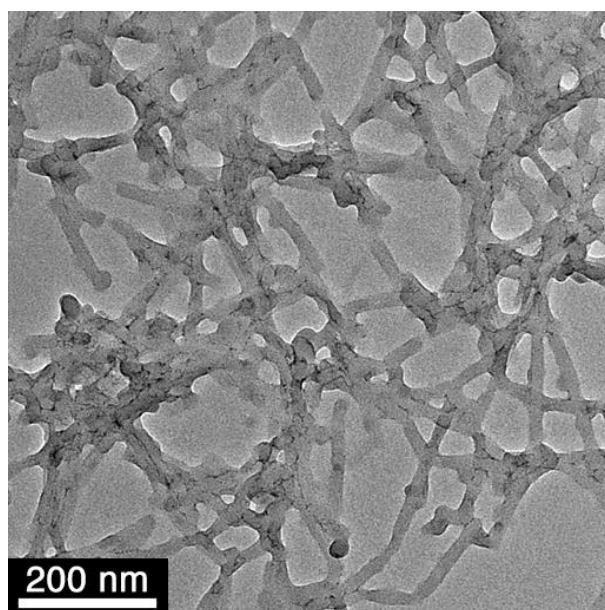


Figure A3.40. (a) TEM image of **SA_{val}** (0.2 wt.%, 2.41 mM, pH = 9) in water after 365 nm photoirradiation for 1 h, and (b) the corresponding Cryo-TEM image (0.5 wt.%, 6.02 mM, pH = 9). Irradiation of sample was carried out at 20 °C using a Thorlabs model collimated LEDs (M365LP-C1, 365 nm, 1.2 A) positioned at a distance of 1 cm from the sample.

(a)



(b)

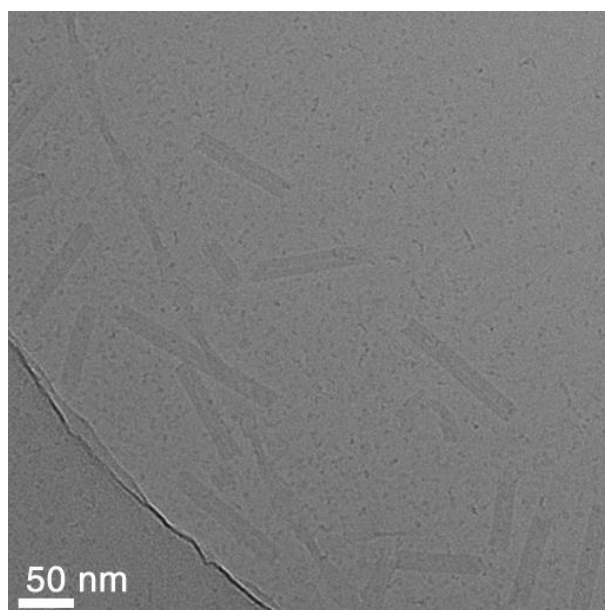


Figure A3.41. TEM image of **SA_{Phe}** (0.2 wt.%, 2.16 mM, pH = 7) in water after 365 nm photoirradiation for 1 h, and (b) the corresponding Cryo-TEM image (0.5 wt.%, 5.39 mM, pH = 7). Irradiation of sample was carried out at 20 °C using a Thorlabs model collimated LEDs (M365LP-C1, 365 nm, 1.2 A) positioned at a distance of 1 cm from the sample.

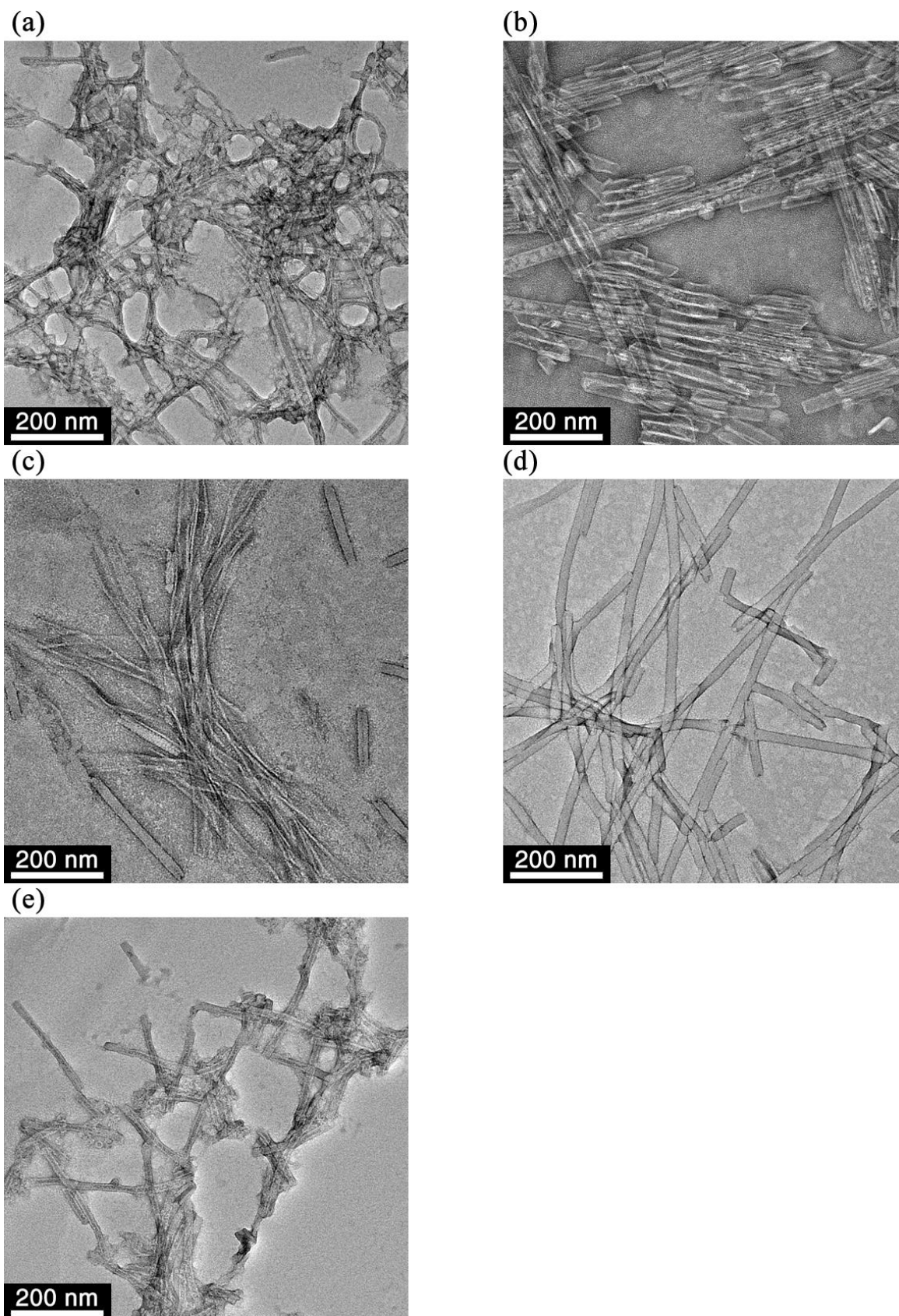


Figure A3.42. TEM image of premixed (a) $\text{SA}_{\text{Gly}}:\text{SA}_{\text{Ala}} = 1:1$ (pH = 9), (b) $\text{SA}_{\text{Gly}}:\text{SA}_{\text{Val}} = 1:1$ (pH = 9), and (c) $\text{SA}_{\text{Gly}}:\text{SA}_{\text{Phe}} = 1:1$ (pH = 7), (d) $\text{SA}_{\text{Ala}}:\text{SA}_{\text{Phe}} = 1:1$ (pH = 7), and (e) $\text{SA}_{\text{Ala}}:\text{SA}_{\text{D-Ala}} = 1:1$ (pH = 9) without further annealing.

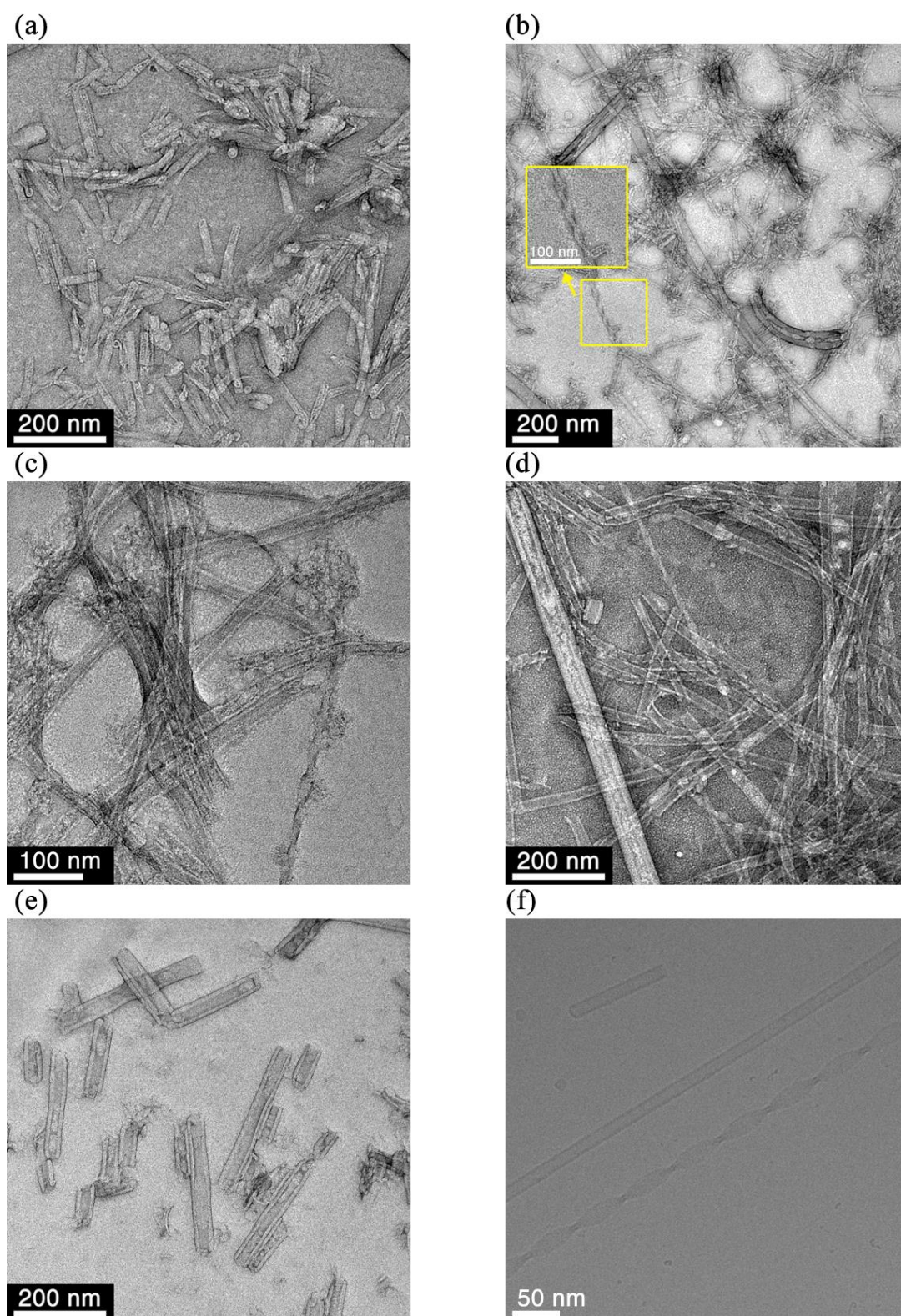


Figure A3.43. TEM images of **SA_{D-Ala}** (0.2 wt.%, 2.16 mM) in water at (a) pH = 7, (b) pH = 9 (inset: high magnification image to illustrate right-handed helicity), (c) pH = 11, (d) pH = 13, and (e) prepared by annealing **SA_{D-Ala}** (5.0 wt.%, 65 mM) at 85 °C for 10 min. (f) Cryo-TEM image of **SA_{D-Ala}** (0.5 wt.%, 6.45 mM) in water at pH = 9.

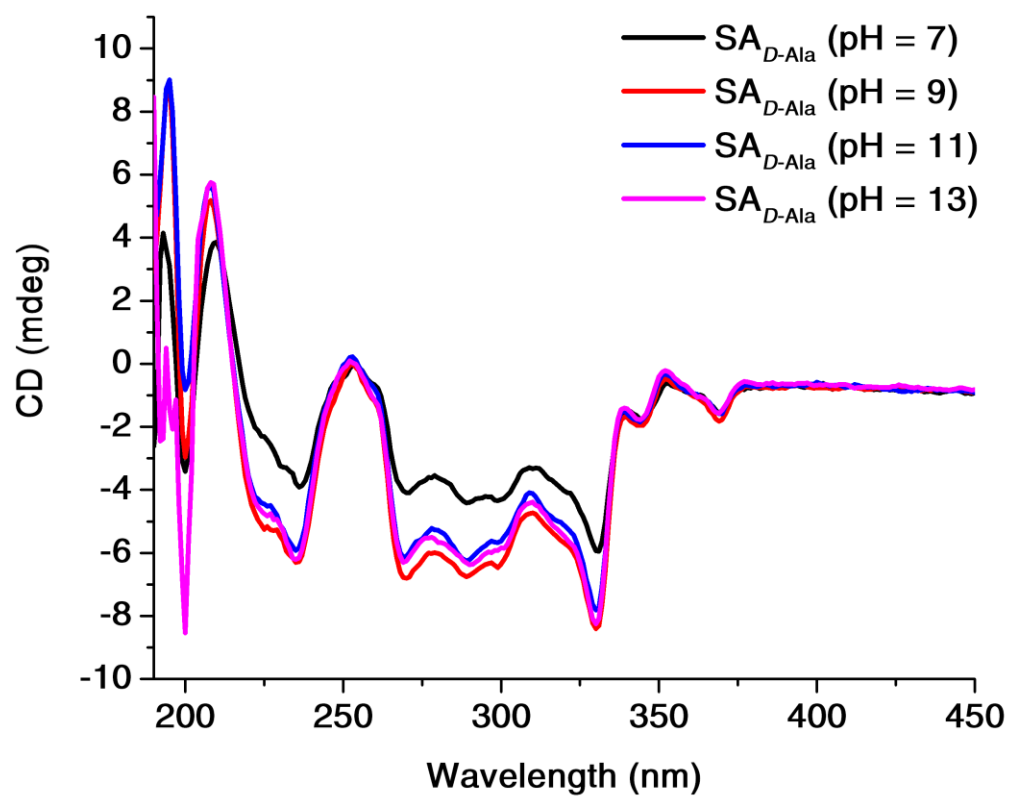
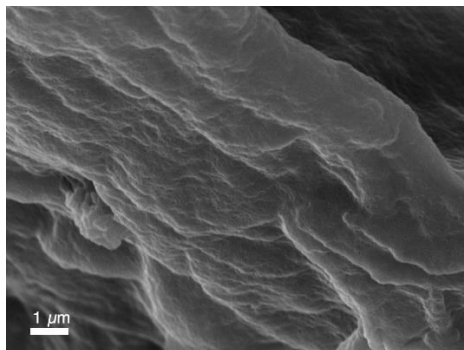


Figure A3.44: CD spectra of annealed $\text{SA}_{D\text{-Ala}}$ ($50\ \mu\text{M}$) in water at different pH values.

(a)



(b)

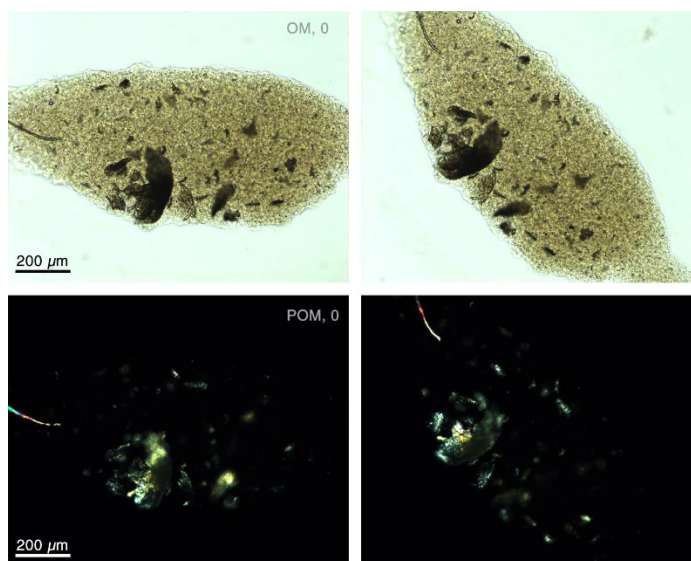


Figure A3.45. (a) SEM image of **SA_{Ala}** (5.0 wt.%, 65 mM, annealed at 85 °C for 70 min) prepared from a solution of CaCl_2 (150 mM). (b) Optical microscopic images of a macroscopic string composed of **SA_{Ala}** prepared from a solution of CaCl_2 (150 mM) without (top) and with (bottom) crossed polarizers. The OM and POM images of the macroscopic string were tilted at 0°, 45° relative to the transmission axis of the analyzer.

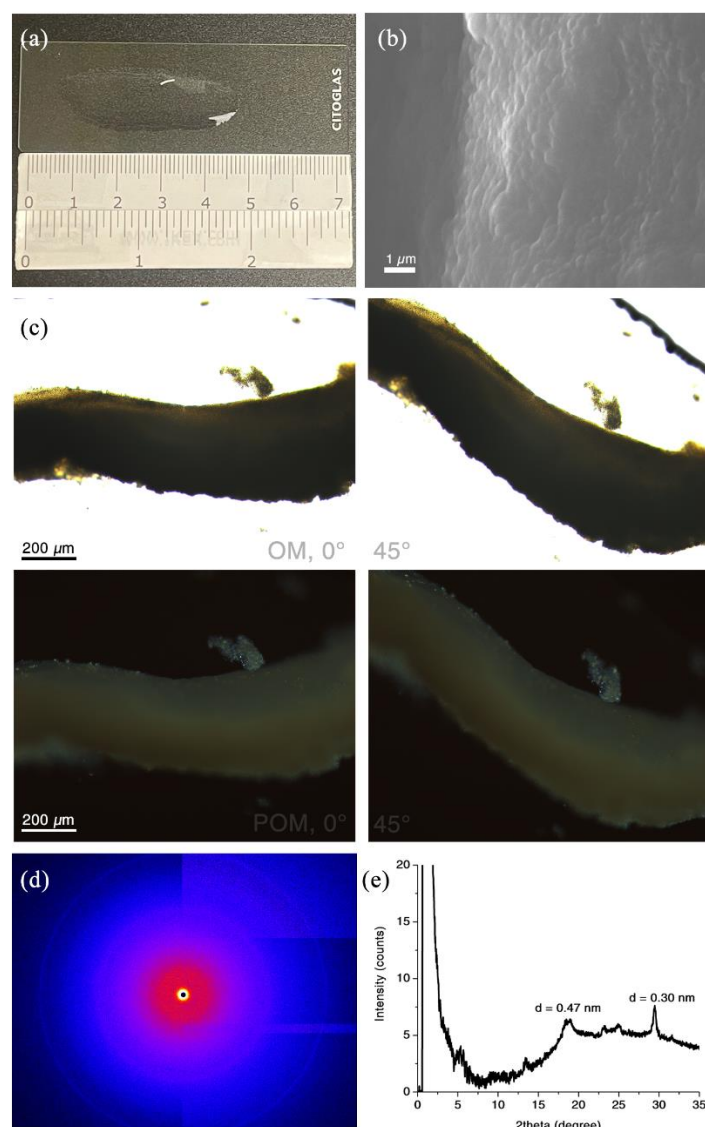


Figure A3.46. (a) Photograph of aqueous solutions of **SA_{Gly}** (5 wt.%, 67 mM, annealed at 85 °C for 10 min) was ejected into a shallow pool of CaCl_2 solution (150 mM). (b) SEM image of **SA_{Gly}** (5.0 wt.%, 67 mM) prepared from a solution of CaCl_2 (150 mM). (c) Optical microscopic images of a macroscopic string composed of **SA_{Gly}** prepared from a solution of CaCl_2 (150 mM) without (top) and with (bottom) crossed polarizers at 0°, 45°. (d) 2D WAXD images and (e) 1D WAXD pattern of a macroscopic aligned string of **SA_{Gly}** with 20 min of exposure time.

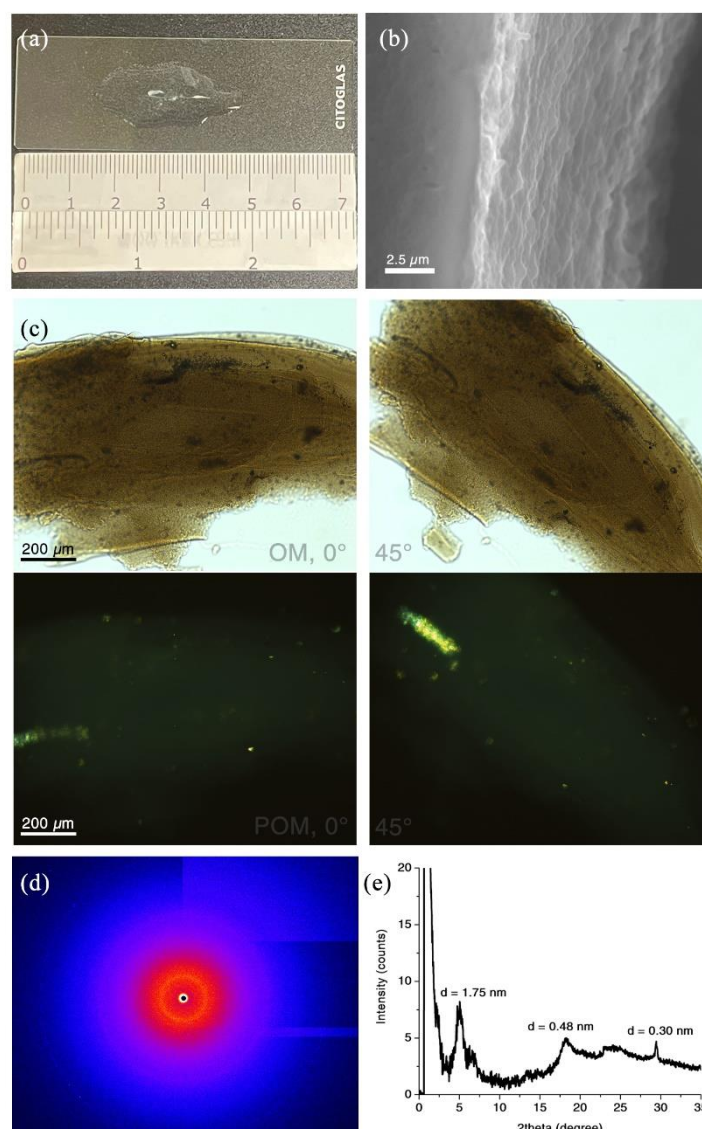


Figure A3.47. (a) Photograph of aqueous solutions of SA_{val} (5 wt.%, 60 mM, annealed at 85 °C for 10 min) was ejected into a shallow pool of CaCl_2 solution (150 mM). (b) SEM image of SA_{val} (5.0 wt.%, 60 mM) prepared from a solution of CaCl_2 (150 mM). (c) Optical microscopic images of a macroscopic string composed of SA_{val} prepared from a solution of CaCl_2 (150 mM) without (top) and with (bottom) crossed polarizers at 0°, 45°. (d) 2D WAXD images and (e) 1D WAXD pattern of a macroscopic aligned string of SA_{val} with 20 min of exposure time.

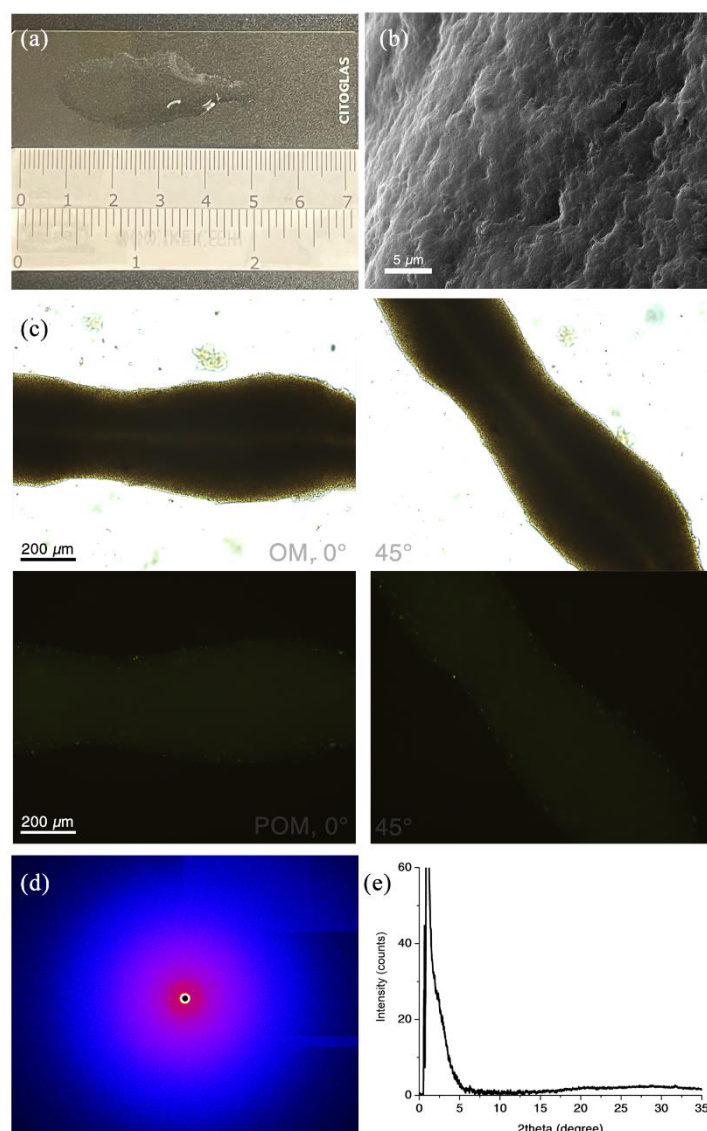


Figure A3.48. (a) Photograph of aqueous solutions of SA_{Phe} (5 wt.%, 54 mM, annealed at 85 °C for 10 min) was ejected into a shallow pool of CaCl_2 solution (150 mM). (b) SEM image of SA_{Phe} (5.0 wt.%, 54 mM) prepared from a solution of CaCl_2 (150 mM). (c) Optical microscopic images of a macroscopic string composed of SA_{Phe} prepared from a solution of CaCl_2 (150 mM) without (top) and with (bottom) crossed polarizers at 0°, 45°. (d) 2D WAXD images and (e) 1D WAXD pattern of a macroscopic aligned string of SA_{Phe} with 20 min of exposure time.

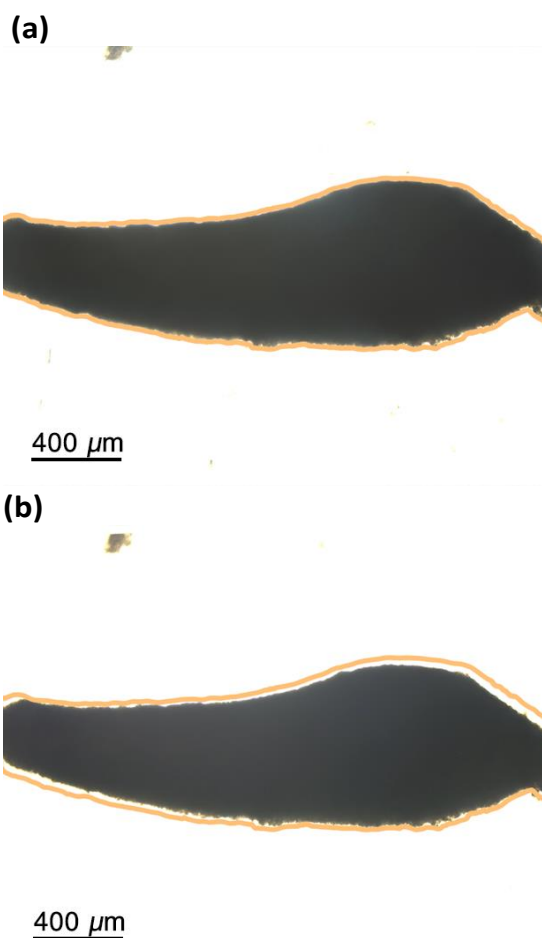
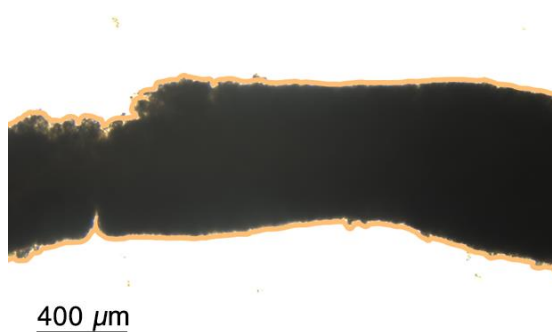


Figure A3.49. Optical microscopic images of a macroscopic string of **SA_{Ala}** (5.0 wt.%, 64.5 mM) in H₂O (a) before, and (b) after 365 nm photoirradiation for 90 min. The shape of the macroscopic string at the initial state is indicated as the orange boundary.

(a)



(b)

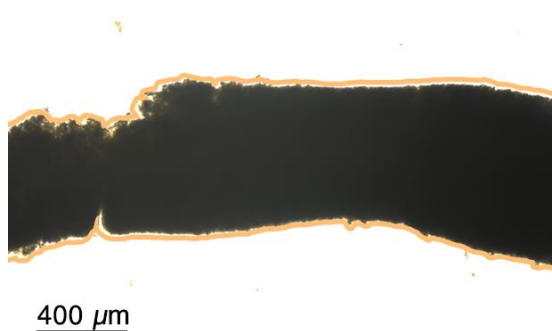


Figure A3.50. Optical microscopic images of a macroscopic string of **SA_{Ala}** (5.0 wt.%, 65 mM) in H₂O (a) before, and (b) after 90 min without photoirradiation. The shape of the macroscopic string at the initial state is indicated as the orange boundary.

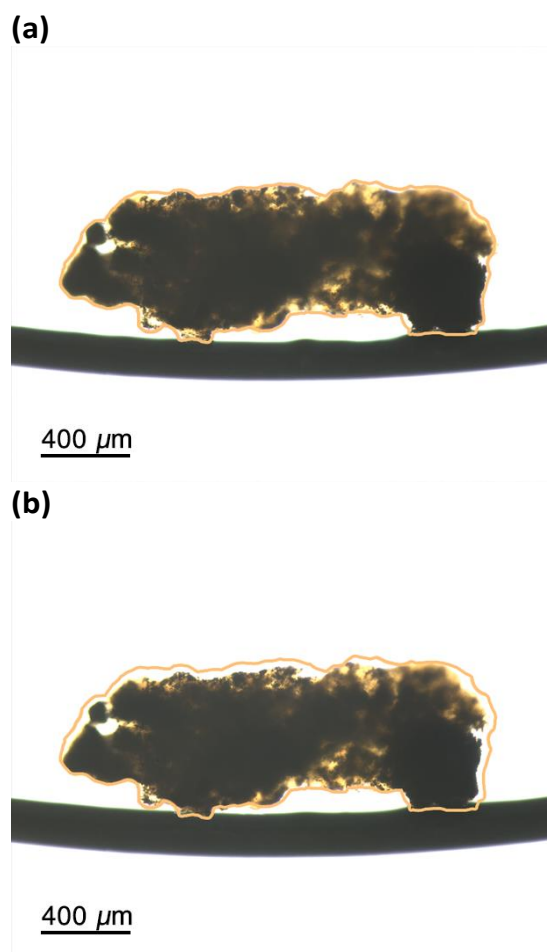


Figure A3.51. Optical microscopic images of a macroscopic string of **SA_{Gly}** (5.0 wt.%, 67 mM) in H₂O (a) before, and (b) after 365 nm photoirradiation for 90 min. The shape of the macroscopic string at the initial state is indicated as the orange boundary.

(a)



400 μ m

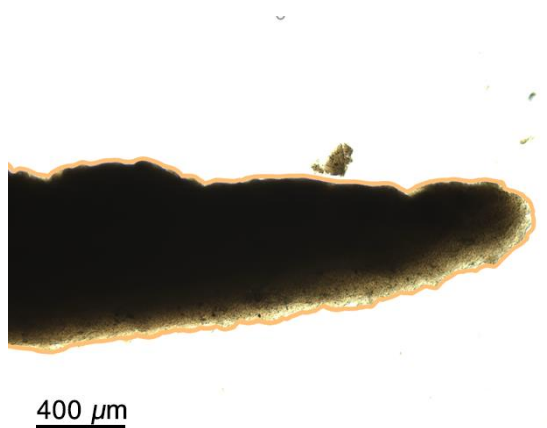
(b)



400 μ m

Figure A3.52. Optical microscopic images of a macroscopic string of **SA_{val}** (5.0 wt.%, 60 mM) in H₂O (a) before, and (b) after 365 nm photoirradiation for 90 min. The shape of the macroscopic string at the initial state is indicated as the orange boundary.

(a)



(b)

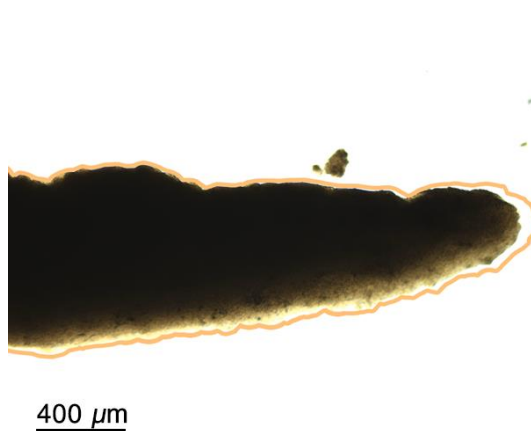


Figure A3.53. Optical microscopic images of a macroscopic string of **SA_{Phe}** (5.0 wt.%, 54 mM) in H₂O (a) before, and (b) after 365 nm photoirradiation for 90 min. The shape of the macroscopic string at the initial state is indicated as the orange boundary.

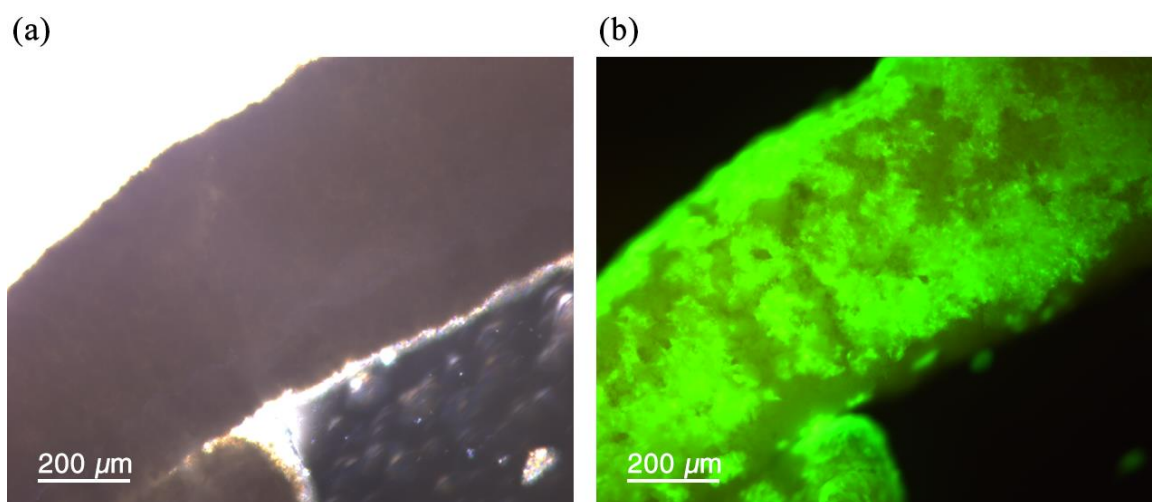


Figure A3.54. (a) OM and (b) fluorescent image of HeLa cell on a macroscopic string of SA_{Gly} (5 wt.%, 67 mM) at 10x magnification.

3. Analytical Data (A3.55 – A3.70)

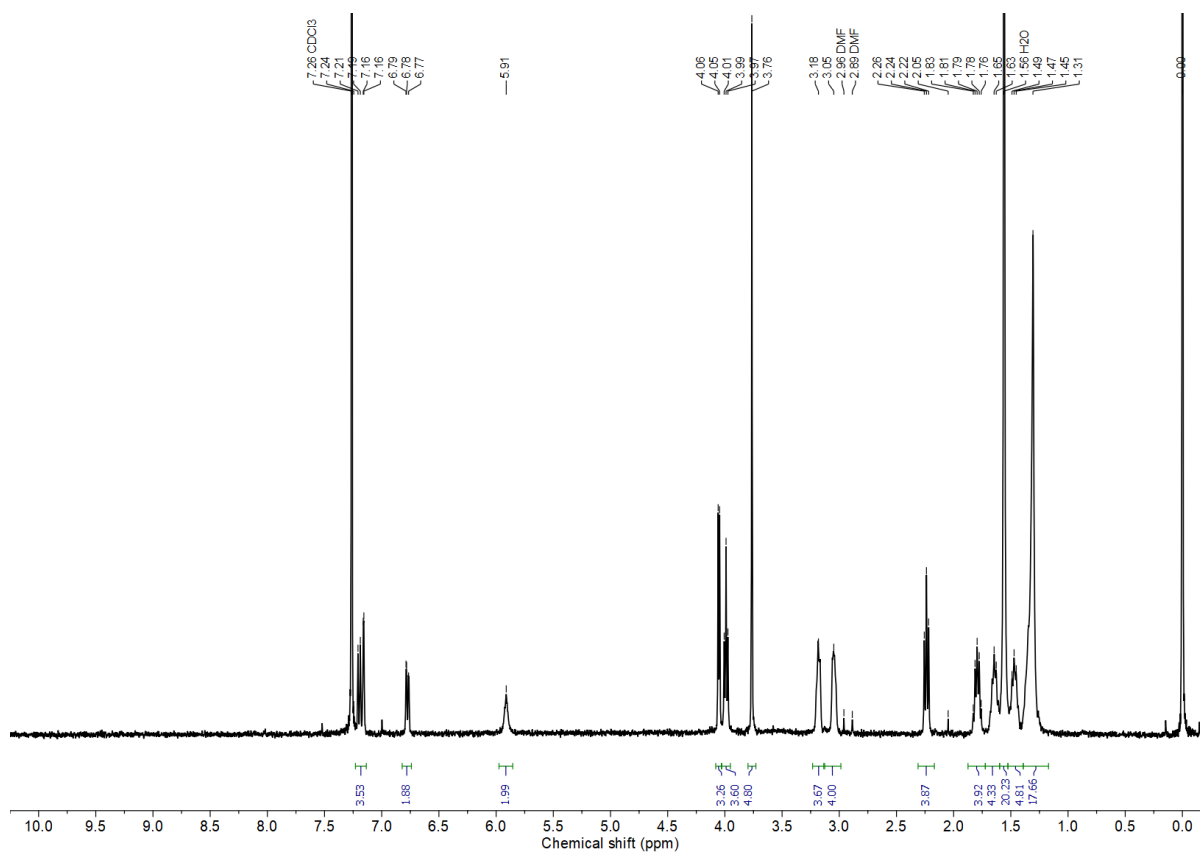


Figure A3.55: ¹H NMR spectrum (400 MHz) of **SA_{GlycooMe}** in CDCl₃ at 25 °C.

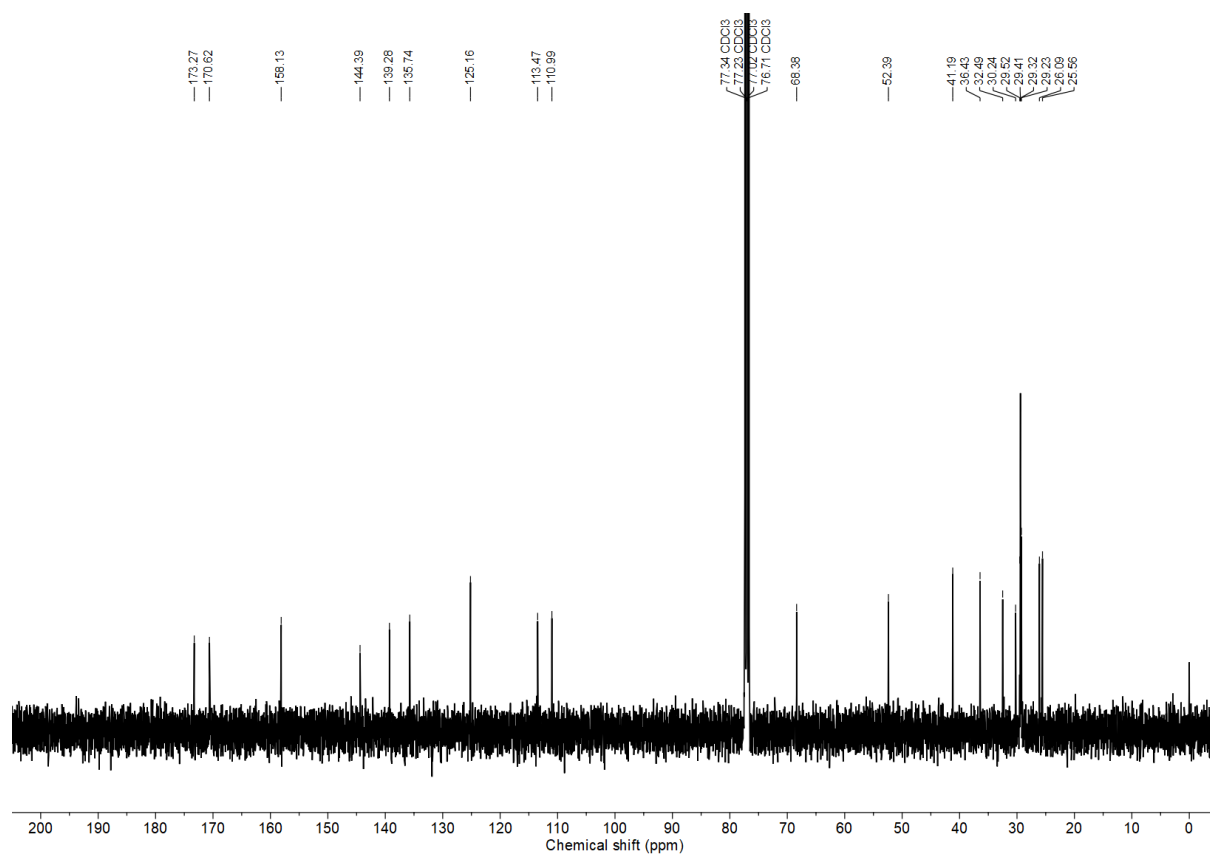


Figure A3.56: ^{13}C NMR spectrum (101 MHz) of **SA_{GlycoOMe}** in CDCl_3 at 25 °C.

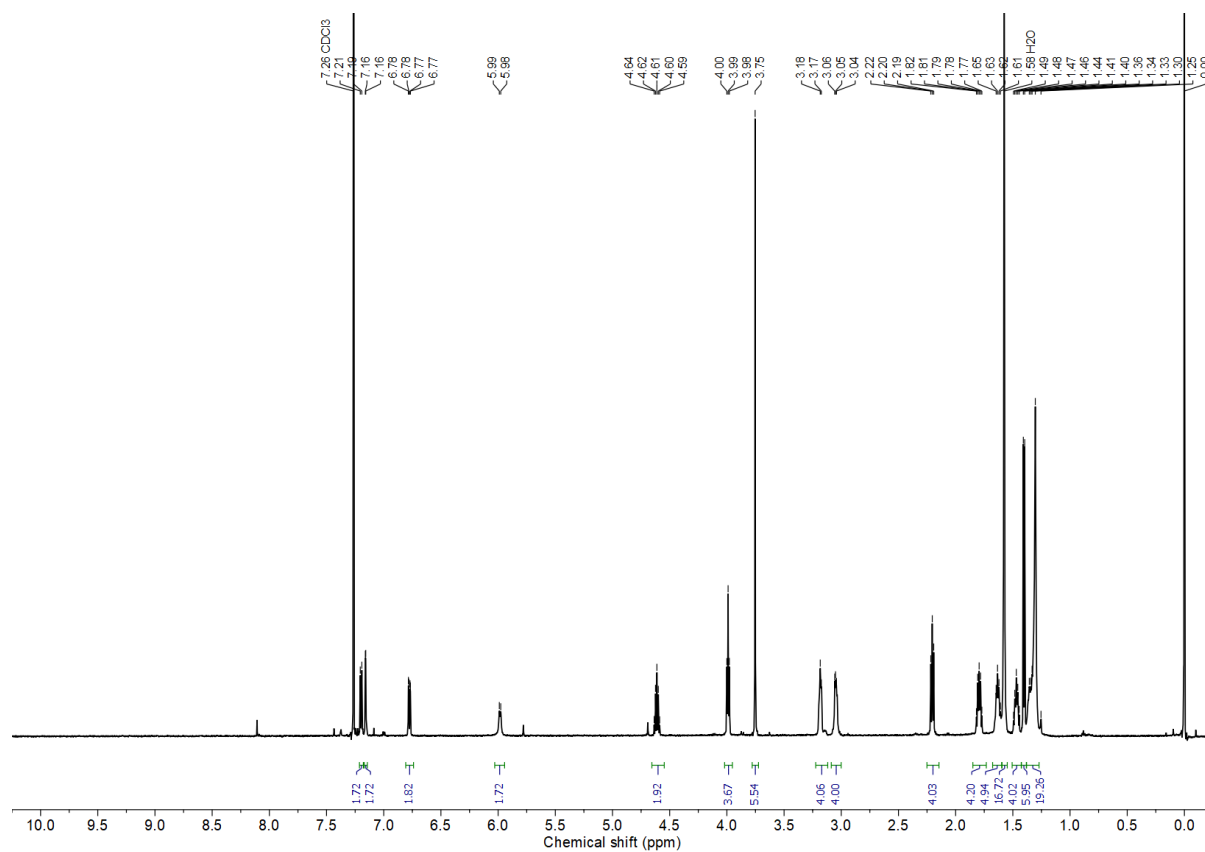


Figure A3.57: ^1H NMR spectrum (600 MHz) of $\text{SA}_{\text{AlaCOOMe}}$ in CDCl_3 at 25°C .

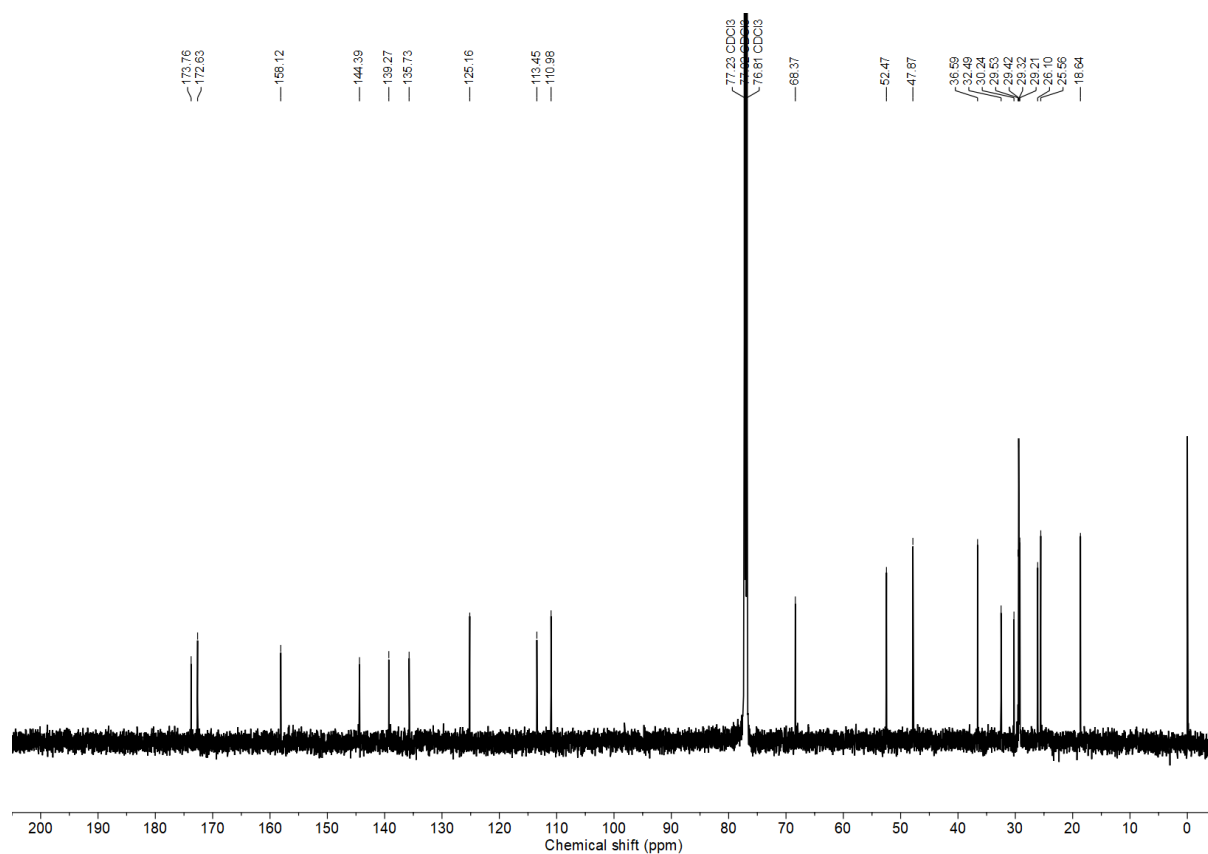


Figure A3.58: ^{13}C NMR spectrum (151 MHz) of **SA**_{Ala}COOMe in CDCl₃ at 25 °C.

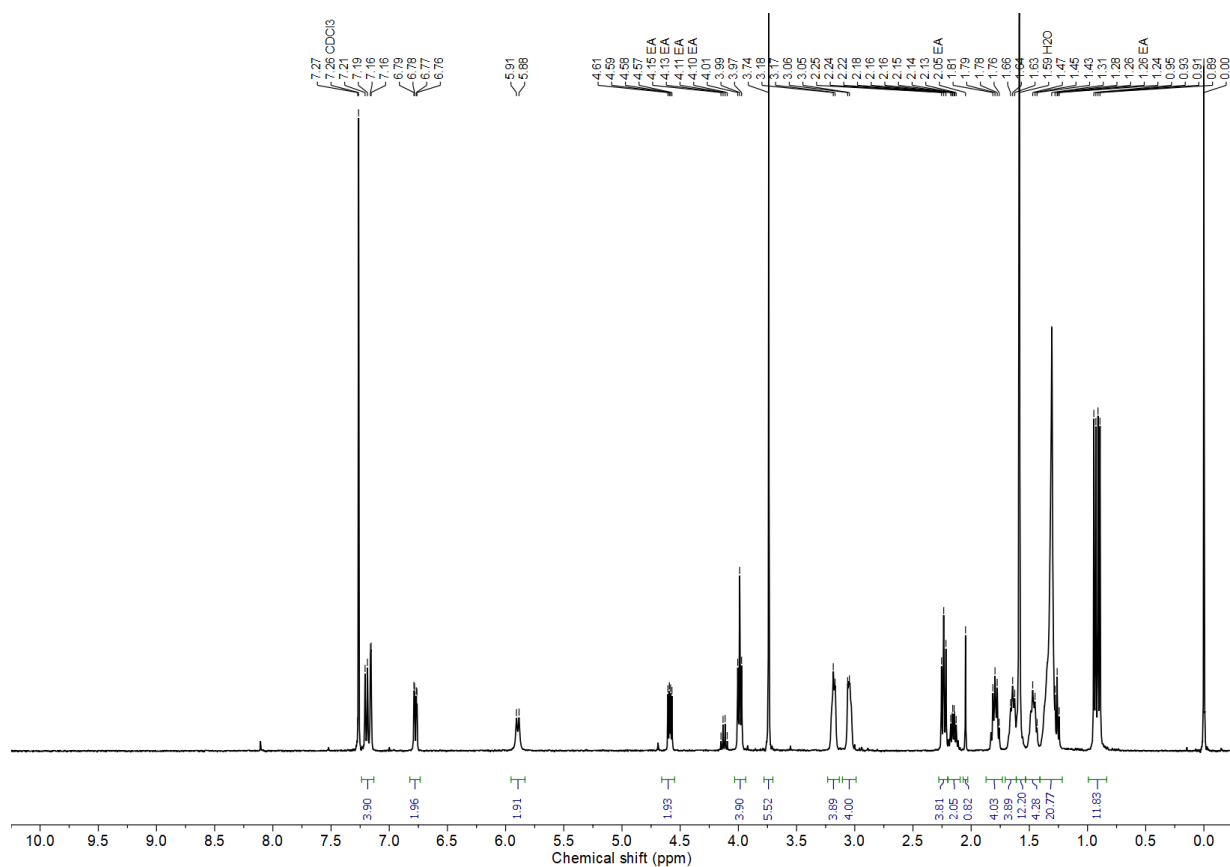


Figure A3.59: ^1H NMR spectrum (400 MHz) of $\text{SA}_{\text{valcooMe}}$ in CDCl_3 at 25°C .

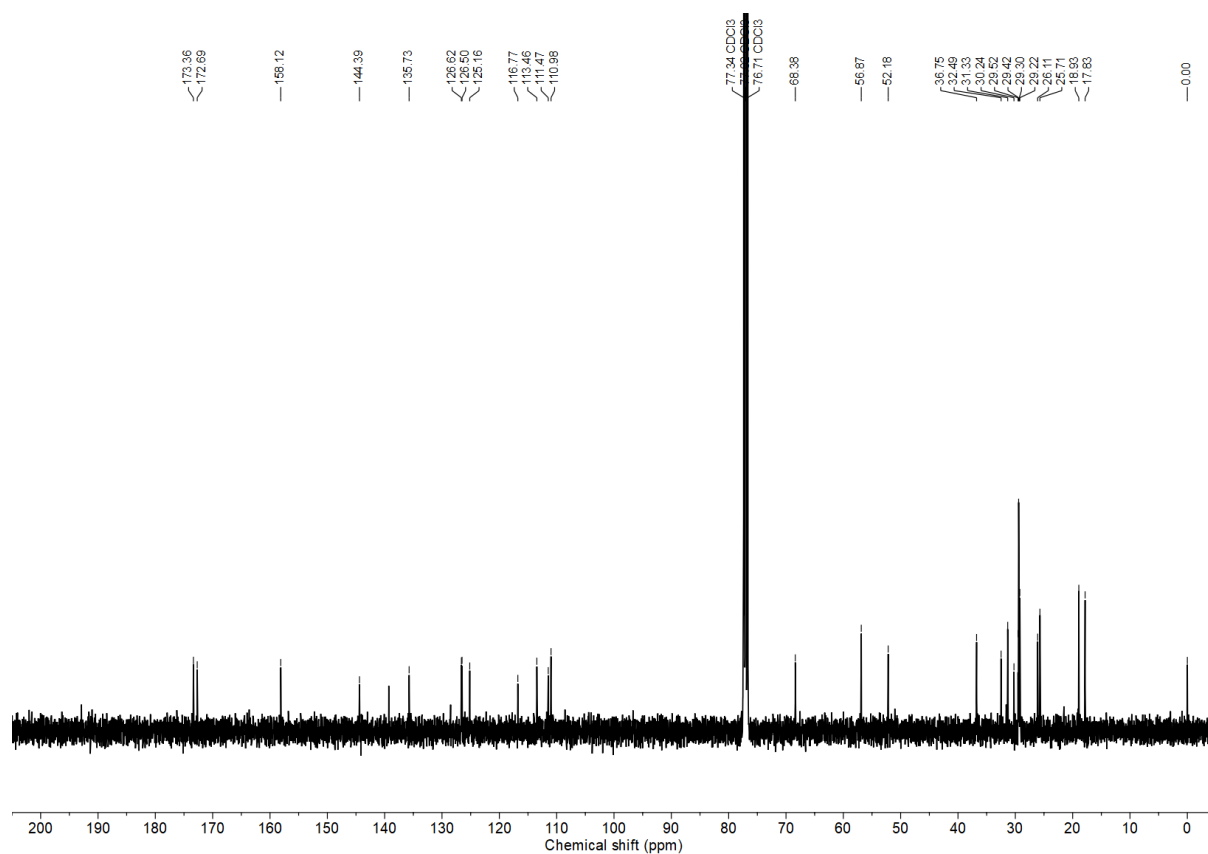


Figure A3.60: ^{13}C NMR spectrum (101 MHz) of **SA_{valcooMe}** in CDCl_3 at 25 °C.

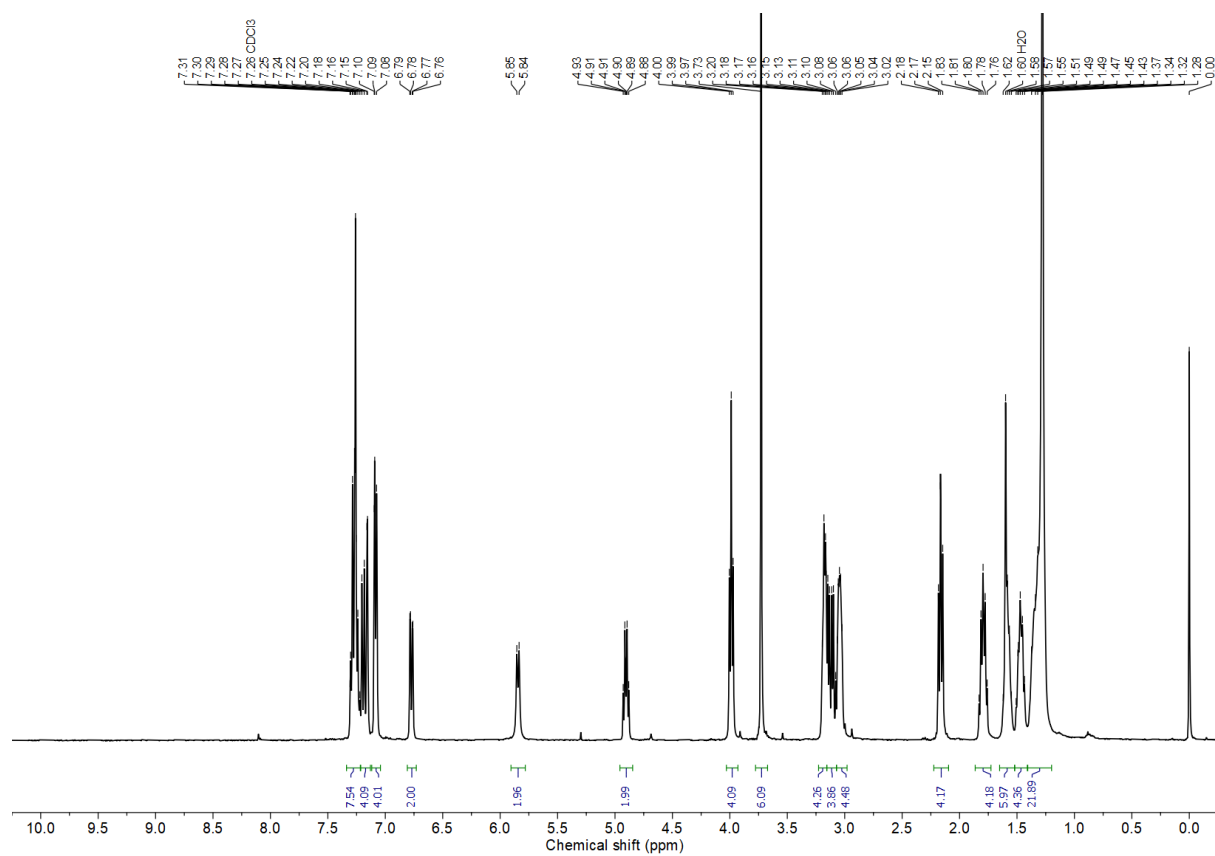


Figure A3.61: ^1H NMR spectrum (400 MHz) of $\text{SA}_{\text{PheCOOMe}}$ in CDCl_3 at 25 °C.

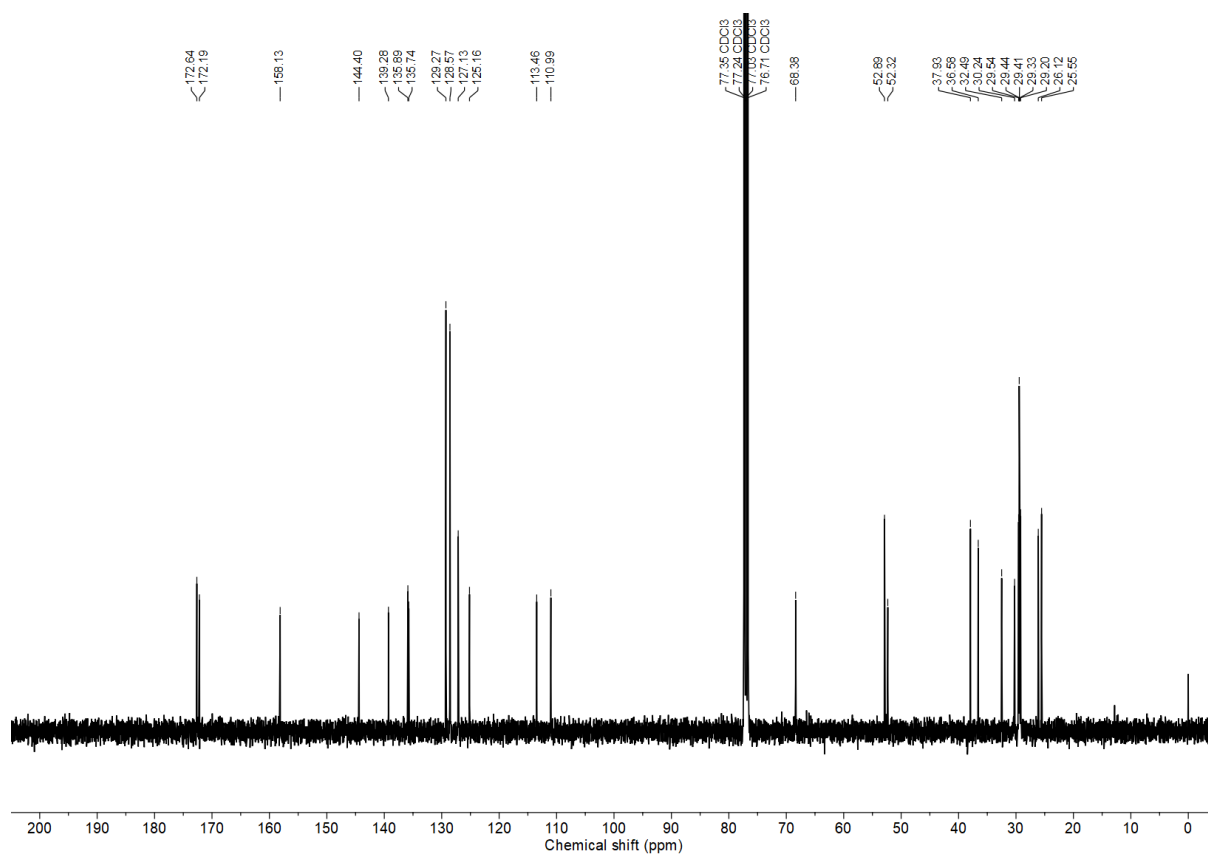


Figure A3.62: ^{13}C NMR spectrum (101 MHz) of **SA_{PheCOOMe}** in CDCl_3 at 25 °C.

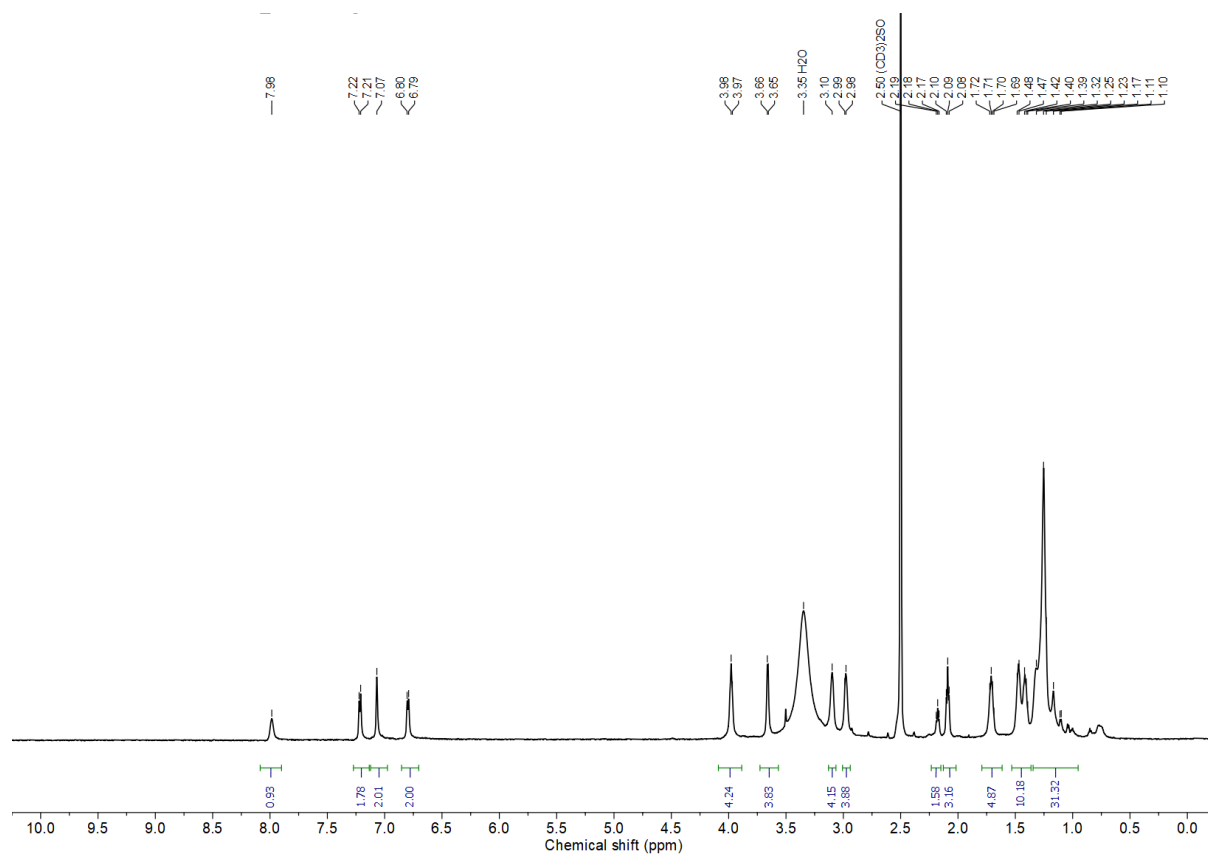


Figure A3.63: ^1H NMR spectrum (600 MHz) of compound **SA_{Gly}** in $(\text{CD}_3)_2\text{SO}$ at 25 °C.

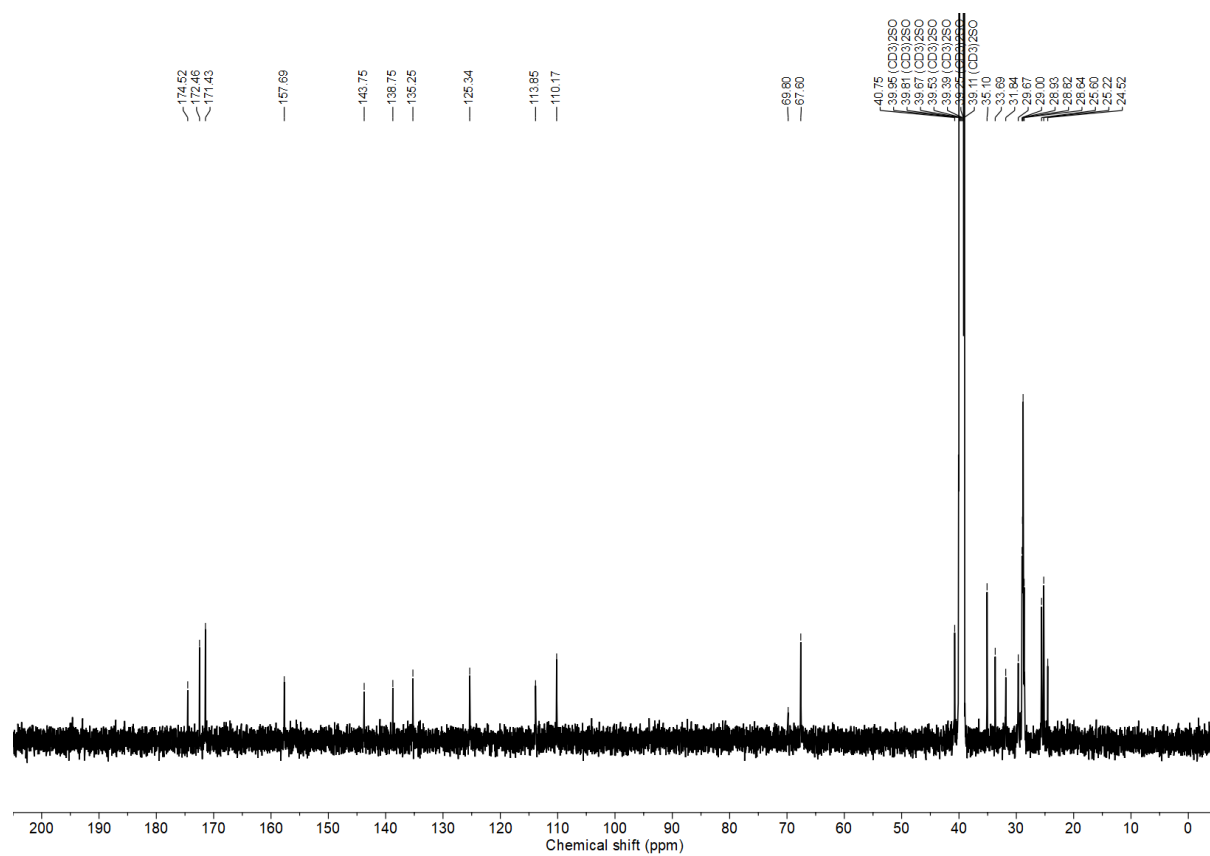


Figure A3.64: ^{13}C NMR spectrum (151 MHz) of **SA_{Gly}** in $(\text{CD}_3)_2\text{SO}$ at 25 °C.

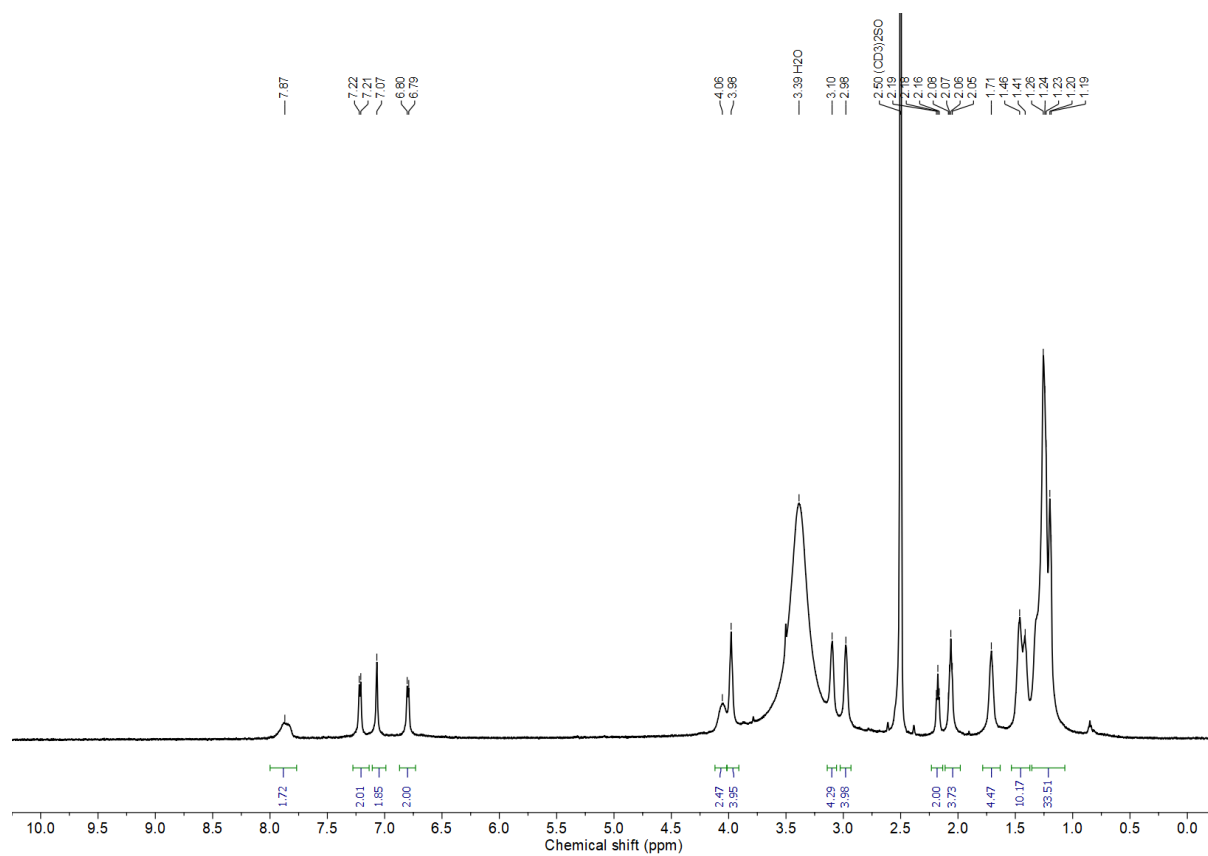


Figure A3.65: ^1H NMR spectrum (600 MHz) of compound **SAAla** in $(\text{CD}_3)_2\text{SO}$ at 25°C .

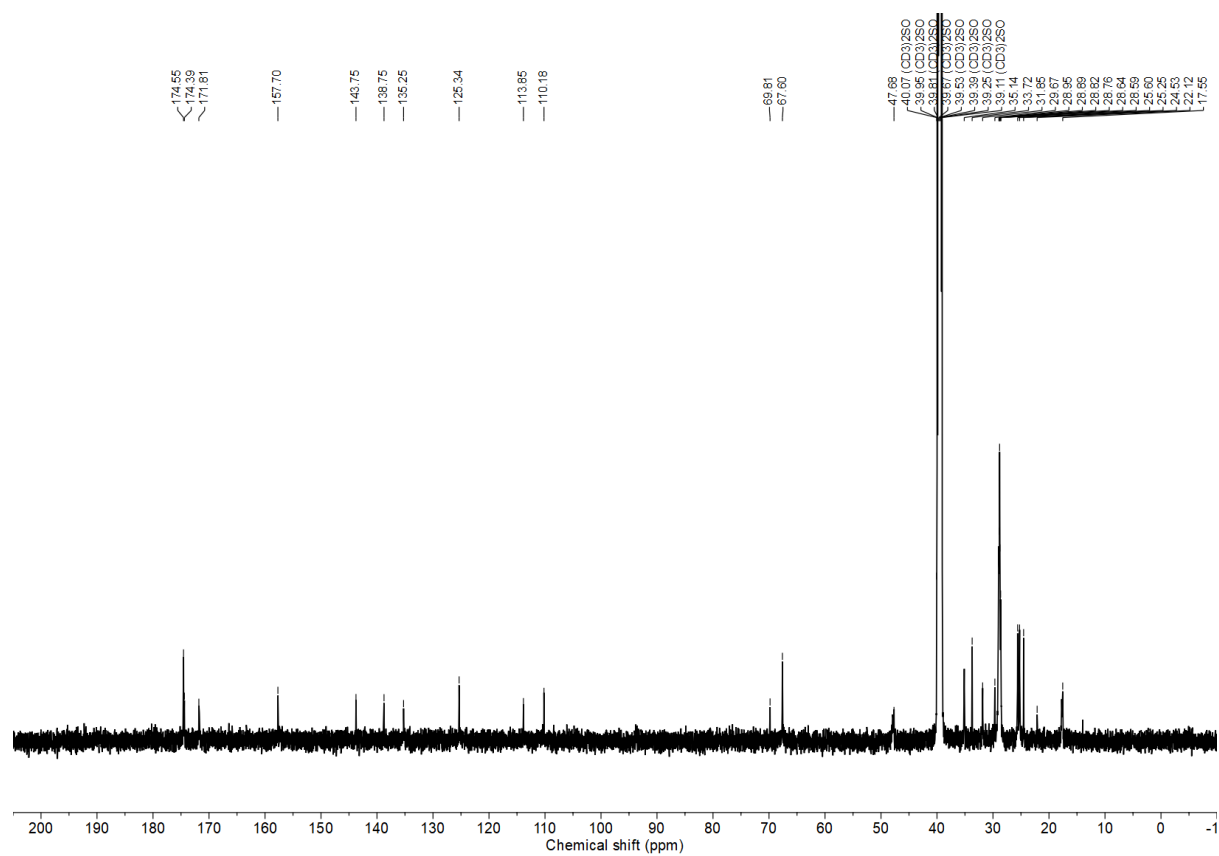


Figure A3.66: ^{13}C NMR spectrum (151 MHz) of **SA_{Ala}** in $(\text{CD}_3)_2\text{SO}$ at 25 °C.

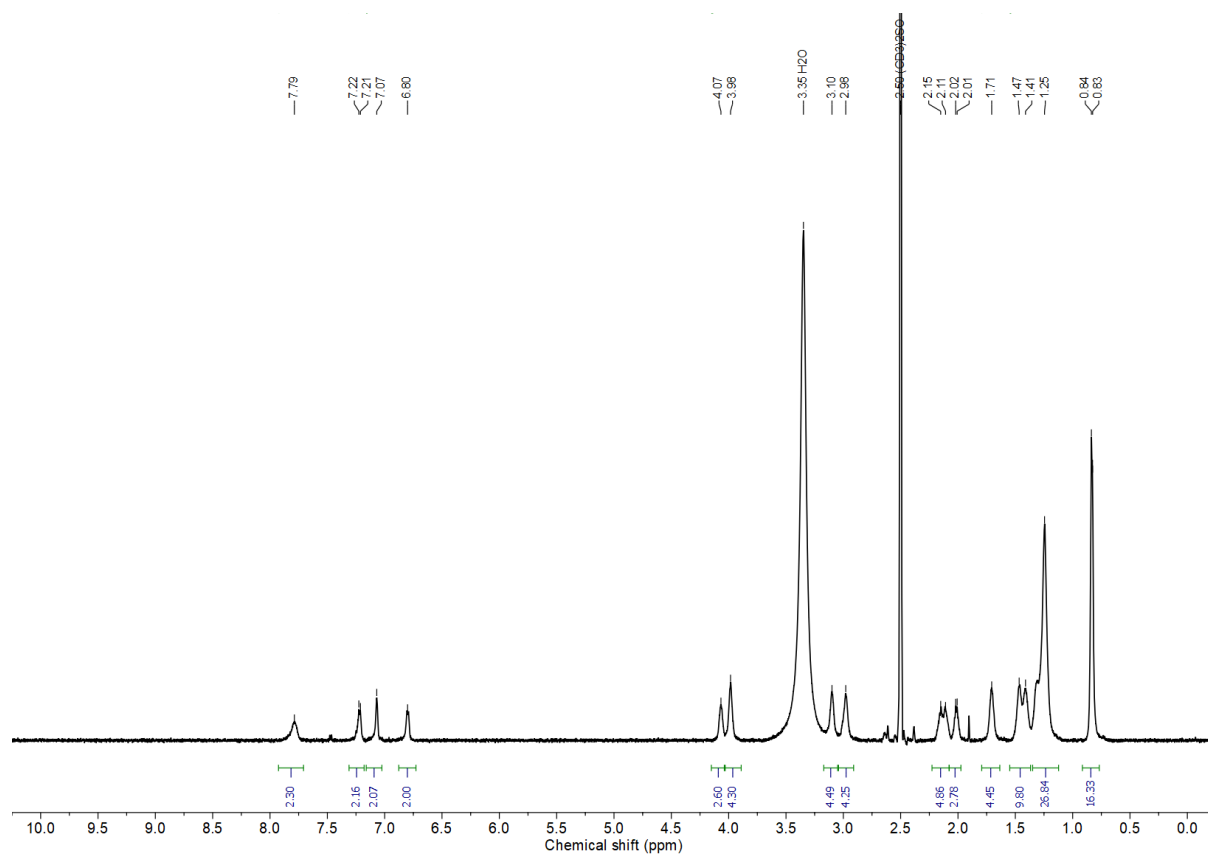
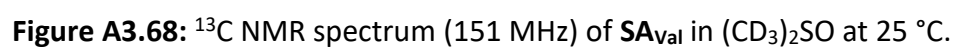


Figure A3.67: ^1H NMR spectrum (600 MHz) of compound **SA_{val}** in $(\text{CD}_3)_2\text{SO}$ at 25°C .



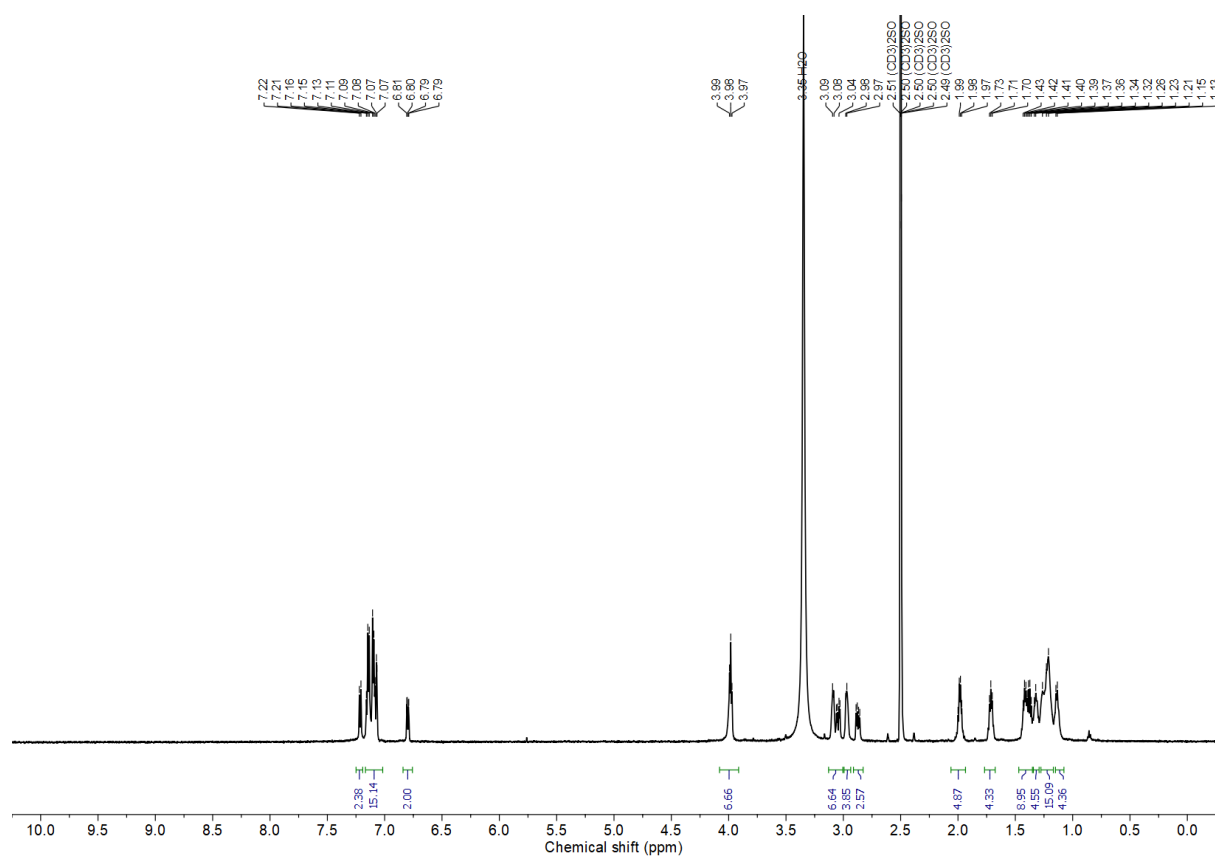
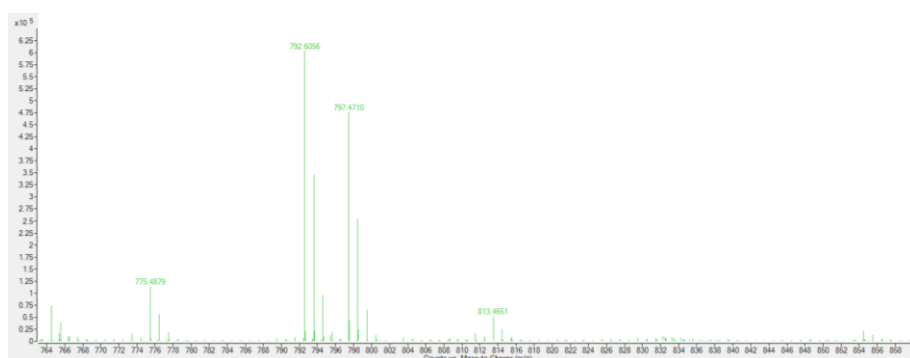


Figure A3.69: ^1H NMR spectrum (600 MHz) of **SA_{Phe}** in $(\text{CD}_3)_2\text{SO}$ at 25 °C.

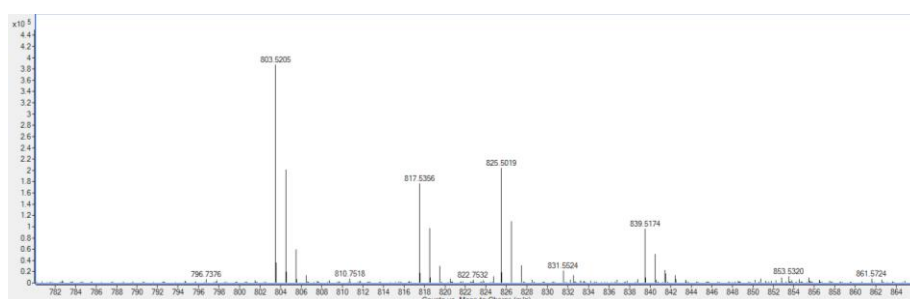


(a)



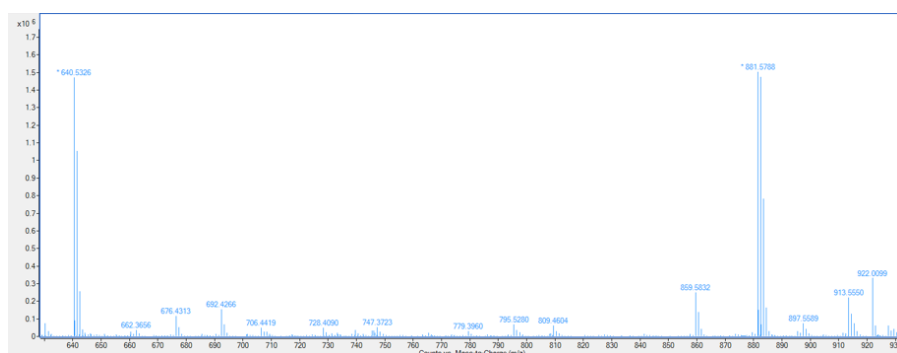
HR-MS (ESI+) calculated for C₄₆H₆₆N₂O₈ [M+Na]⁺ 797.4711, found 797.4710.

(b)



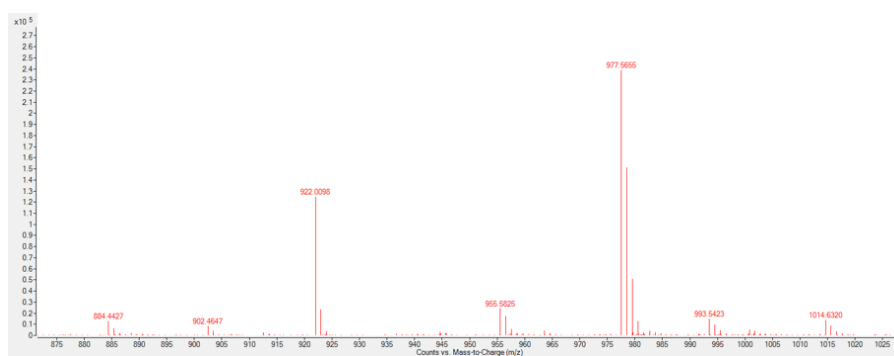
HR-MS (ESI+) calculated for C₄₈H₇₀N₂O₈ [M+H]⁺ 803.5205, found 803.5205.

(c)



HR-MS (ESI+) calculated for C₅₂H₇₈N₂O₈ [M+H]⁺ 859.5831, found 859.5832.

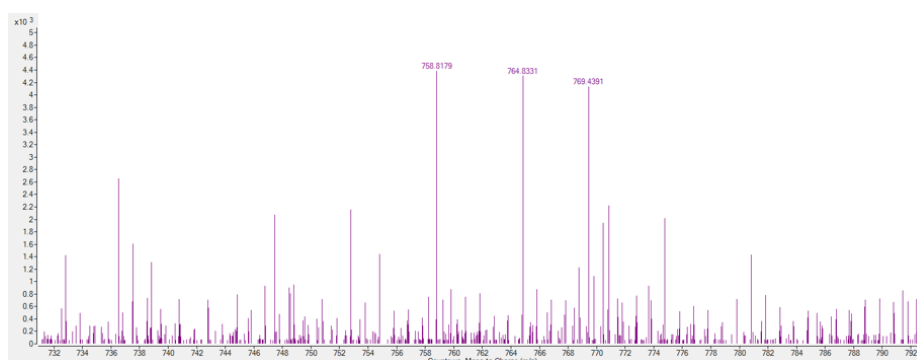
(d)



HR-MS (ESI+) calculated for C₆₀H₇₈N₂O₈ [M+Na]⁺ 977.5650, found 977.5655.

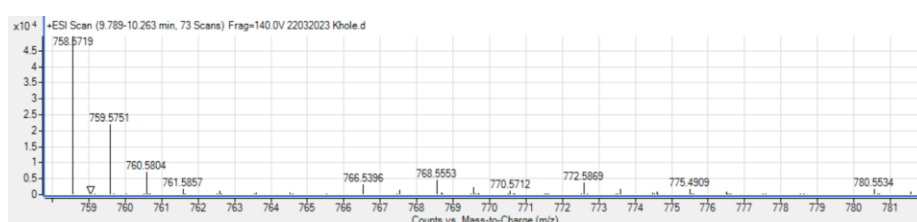
Figure A3.71: HRMS spectra of (a) **SA**_{GlyCOOMe}, (b) **SA**_{AlaCOOMe}, (c) **SA**_{ValCOOMe}, and (d) **SA**_{PheCOOMe}, respectively.

(a)



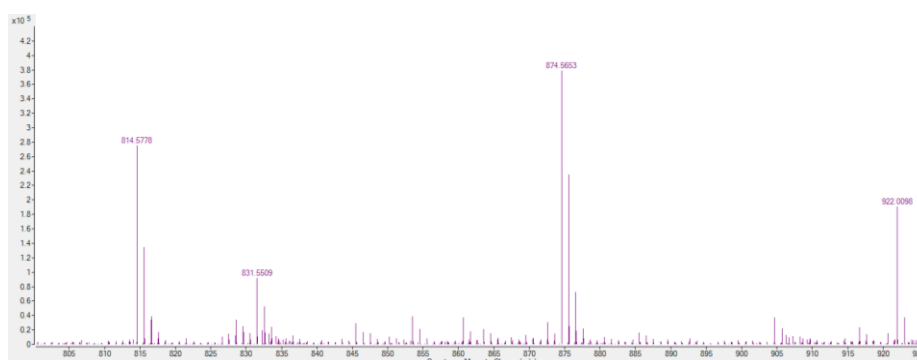
HR-MS (ESI+) calculated for C₄₄H₆₂N₂O₈ [M+Na]⁺ 769.4398, found 769.4391.

(b)



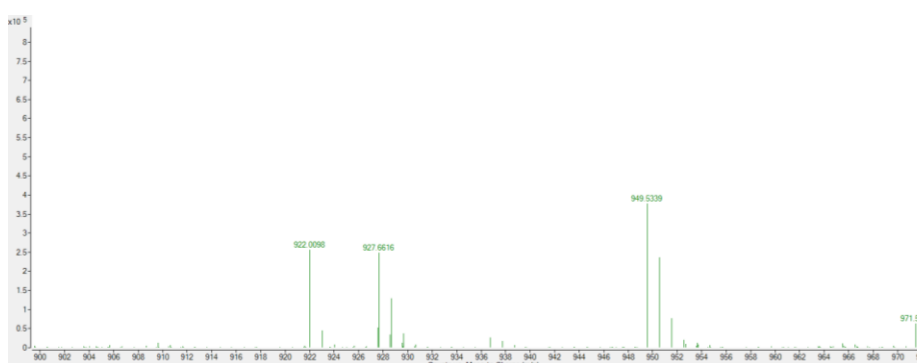
HR-MS (ESI+) calculated for C₄₆H₆₆N₂O₈ [M+H]⁺ 775.4892, found 775.4909.

(c)



HR-MS (ESI+) calculated for C₅₀H₇₄N₂O₈ [M+H]⁺ 831.5518, found 831.5509.

(d)



HR-MS (ESI+) calculated for C₅₈H₇₄N₂O₈ [M+Na]⁺ 949.5337, found 949.5339.

Figure A3.72: HRMS spectra of (a) **SA_{Gly}**, (b) **SA_{Ala}**, (c) **SA_{Val}**, and (d) **SA_{Phe}**, respectively.