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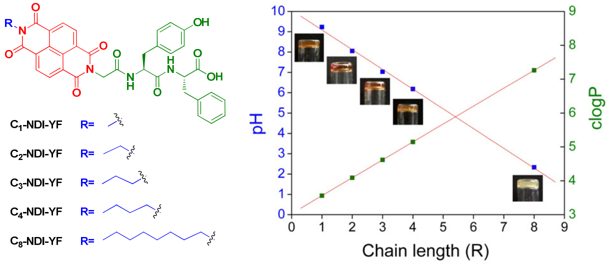
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Remarkable Influence of Alkyl Chain Lengths on Supramolecular Hydrogelation of Naphthalene Diimide-capped Dipeptides

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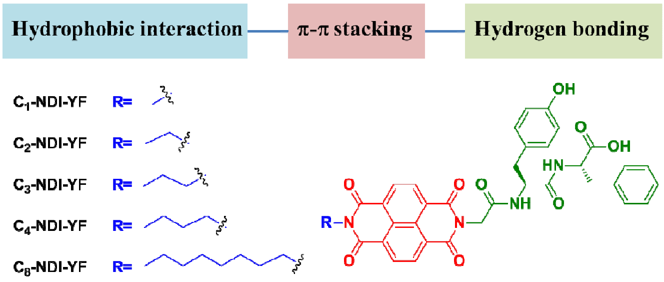
The influence of aliphatic chain lengths on supramolecular hydrogelation of naphthalene diimide-capped dipeptides is described. The dependence of the gelation pH as a function of chain lengths reveals an inverse linear relationship and the gelation pH values cover a broad range from pH 2 to pH 9. In addition, the presence of a *n*-octyl tail in the hydrogelator could enhance the ability of self-assembly in dilute solution.

During the past few years, supramolecular hydrogels self-assembled from peptide amphiphiles have become one of the active research fields in supramolecular chemistry due to their scientific insights and many potential biomedical applications such as tissue engineering and drug delivery.[1-6] Comparing to traditional polymeric hydrogel, supramolecular hydrogel is a new type of biomaterial with a stable three-dimensional network through weak intermolecular interactions such as hydrogen bonding, π–π stacking and van der Waals forces.[7-10] More interestingly, the gelation of peptide amphiphiles can be controlled by heat, light, redox, ultrasound or pH as external stimuli.[11-14] To design the chemical structure of a supramolecular hydrogelator, Xu *et al.* has proven that aromatic-capped peptide amphiphile is an efficient chemical strategy toward realizing a suparmolecular hydrogelator which is to covalently bind an aromatic group to the N-terminus of a short peptide.[15] Napthalene diimide (NDI) is an attractive aromatic building block owing to their n-type semiconducting property and air stability.[16,17] It is known that end-capped alkyl and heteroalkyl substitution of π-conjugated molecules is a general design strategy to achieve desired self-organization in the solid state.[18,19] For example, Ogawa *et al.* have systematically explored the superstructures of a series of NDI with various alkyl chain lengths.[18] Ichikawa *et al.* have studied the morphology and electric properties of long alkyl substituted NDI derivatives.[19] NDI is also a building block for supramolecular hydrogels, for instances, Parquette *et al.* have proven that the Fmoc-KK(C_4-NDI) is an efficient self-assembled hydrogelator.[20] Ulijn *et al.* have discovered that the amino-acid derivative of C_4-NDI-Y could form a self-supporting hydrogel.[21] More recently, we have demonstrated that the self-assembly of C_8-NDI-FF or C_8-NDI-FG is feasible to form a supramolecular hydrogel under physiological pH.[22] The previous studies were focused on the importance of aromatic moieties such as Fmoc group at N-terminus and aromatic rings in the side chain of amino acids. However, the role of aliphatic chain length in the formation of supramolecular hydrogels of NDI-capped peptides has not been systematically explored. In this study, we examine the influence of end-capped alkyl chains on intermolecular interactions of the hydrogelators in both gel and solution states. In Scheme 1, we have systematically synthesized various end-capped alkyl chains in NDI/peptide conjugates (C_n-NDI-YF, n= 1, 2, 3, 4, 8) and their hydrogelation properties were studied.



Scheme 1. Chemical structures of C_1-NDI-YF (**1**), C_2-NDI-YF (**2**), C_3-NDI-YF (**3**), C_4-NDI-YF (**4**) and C_8-NDI-YF (**5**).

The NDI/dipeptide conjugates C_1-NDI-YF (**1**), C_2-NDI-YF (**2**), C_3-NDI-YF (**3**), C_4-NDI-YF (**4**) and C_8-NDI-YF (**5**) have synthesized through solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin (see ESI for detailed). Hydrogels of **1**-**5** were prepared using the sequential change in pH values at the concentration of 1 wt%. As shown in inset of Fig. 1, the appearances of the self-assembled hydrogels are transparent brownish for **1**-**3**, semi-translucent brownish for **4** and semi-translucent yellow for **5**. Interestingly, we found that the dependence of the gelation pH as a function of alkyl chain length reveals an inverse linear relationship, indicating an increase in the end-capped alkyl chain lengths in the NDI-dipeptides resulted in

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hydrogelation at lower values of pH (Fig. 1 and Table 1). Short alkyl chains in **1** and **2** could gel water under basic conditions, the gelation of **3** occurred at neutral pH and relatively long alkyl chains in **4** and **5** formed self-assembled hydrogels under acidic pH. These results indicate the end-capped alkyl chains are essential to determine the gelation pH since it covers a broad range of pH from basic to acidic conditions. The evolution of hydrophobicity (clogP) as a function of end-capped alkyl chains showed a linear relationship with positive slope, showing the increase of hydrophobicity in the gelator would result in lower gelation pH. The length of the aliphatic chains in the NDI-capped dipeptides would alter the equilibrium between the carboxylic acid and carboxylate and enhance the alkyl intercalating effect between molecules in the assemblies, resulting to the different hydrogelation pH values as shown in Table 1. To further study the sol–gel transition of **1-5**, the hydrogelation characters were monitored over time. We observed that the aliphatic chain lengths can significantly affect the gelation time. For compounds with shorter chains such as **1** or **2** more than 15 h is needed for a solution turns into a stable hydrogel while with longer chain length such as **4** or **5** the stable hydrogel formed within 1 h (see Figure S1 for details) These results suggested that the presence of long alkyl chains in NDI-capped dipeptide would result in efficient hydrogelation. In addition, the lower minimum gel concentrations (mgc) of **1-5** are in the range of 0.4-1.0 wt%, depending on the hydrophobicity of the aliphatic chains as well as intercalating effect between molecules. In Table 1, we observed that the increase of chain length would result in lower mgc, pointing to the importance of end-capped alkyl chain on self-assembly in water.

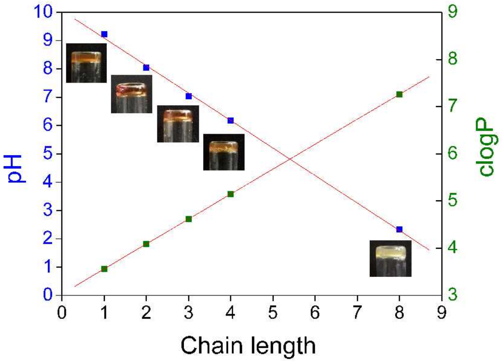

Fig. 1 The dependences of the gelation pH and clogP as a function of alkyl chain lengths of **1-5** at 1 wt % in water. Inset: gel images of **1-5**.

It has been proven that a supramolecular hydrogelator could construct self-organized nanostructures such as nanofibers, nanoribbons, or nanosheets in water through physical crosslinking.[23, 24] To verify the microstructures of gels **1-5**, the gels were studied by transmission electron microscopy (TEM). Fig. 2 showed the negatively stained TEM images of **1-5** under their hydrogelation conditions. TEM observations revealed that all the gels contained well-developed and uniform fibrous structures, and the apparent diameters were in the range of 5-10 nm. It is reasonable that these nanofibers entangle to trap water molecules in three-dimensional environment. Therefore, it may be concluded that all of the gels are constructed through physical crosslinking of nanofibers that formed from the self-assembly of small-molecule hydrogelators in water.

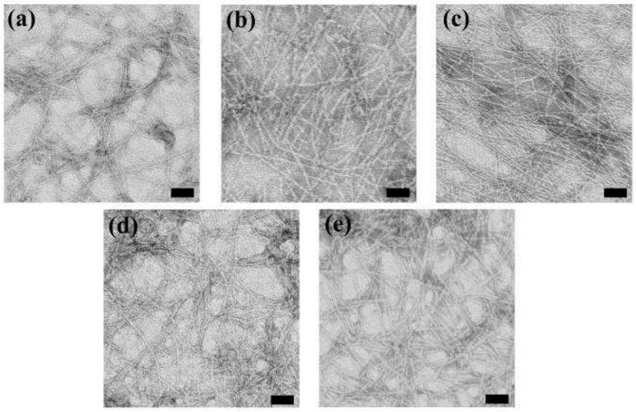

Fig. 2 Negative stained TEM images of (a) **1**, (b) **2**, (c) **3**, (d) **4** and (e) **5** at 1 wt%. (Scale bar= 50 nm)

Table 1. Physical properties of NDI compounds at 1 wt%

Entry	pH	clogP	Appr.[a]	mgc (wt%)	$T_{gel-sol}$ (°C)	G′, G″ (Pa)
1	9.23	3.56	TG	1.0	35	$9.1x10^{1}$, $2.3x10^{1}$
2	8.05	4.09	TG	0.9	43	$2.9x10^{3}$, $2.4x10^{2}$
3	7.03	4.62	TG	0.9	45	$5.3x10^{3}$, $5.9x10^{2}$
4	6.18	5.15	SG	0.7	52	$1.4x10^{4}$, $9.5x10^{2}$
5	2.33	7.26	SG	0.4	66	$1.7x10^{4}$, $1.5x10^{3}$

[a] TG: transparent gel. SG: semi-translucent gel.

The mechanical properties of gels **1-5** were measured by oscillatory rheology which provides the mechanical strength as well as the viscoelastic property of a gel. The viscoelastic properties of a hydrogel can be assessed by oscillatory rheology. In this experiment, two essential parameters of hydrogels are provided; they are shear storage modulus (G′) and loss modulus (G″). The G′ represents the stiffness of the material and G″ is the liquid-like response of the sample. The sample behaves like a viscous liquid when G″ > G′, and, however, the sample behaves like an elastic solid when G′ > G″.[25] Therefore, these two factors are critical for the applications of the hydrogel that will directly impact final uses of the materials. As shown in Fig. 3 and Table 1, it is important to note that the amplitudes of G′ and G″ increase with the end-capped chain lengths. The G′ moduli of **1-5** were in the range of 0.09-17.0 KPa, indicating that the elastic properties of these hydrogels can effectively be tuned by modulating the length of the end-capped alkyl chain in the NDI/dipeptide conjugates. Furthermore, all the G′ values exceed their G″, suggesting these five compounds are gels with a predominantly elastic component. The gel-to-sol transition temperature ($T_{gel-sol}$) was determined by the inverted tube method.[26] The $T_{gel-sol}$ of **1-5** at 1 wt% were all above 37 °C, as shown in Table 1, the trend of the $T_{gel-sol}$ for **1-5** is consistent with the tendency of their rheological data, and the relatively long alkyl chain length in **5** displayed a relatively high $T_{gel-sol}$. In addition, the gel of **3** formed at neutral pH was stable up to 45 °C, pointing to a potential scaffolding material for biomedical applications.[27]

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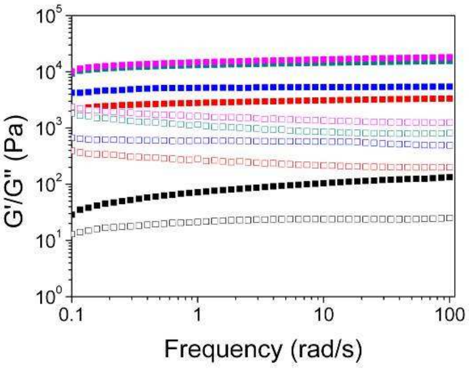

Fig. 3 The frequency sweep of the hydrogels of **1** (black), **2** (red), **3** (blue), **4** (cyan) and **5** (magenta) at 1 wt %; closed squares for G' and open squares for G".

The UV-Vis absorption, fluorescence emission and circular dichroic (CD) spectra were employed to explore the intermolecular interactions of NDI-capped dipeptides in the assemblies. In Fig. 4a, the UV-Vis spectra of **1**-**5** at 500 μM showed low-lying absorption maxima around 360 nm which are consistent with the π–π* transitions polarized along long axes of NDI moiety.[28] From the spectra, a bathochromic shift of the vibronic peak from 379 nm (C_1-C_4) to 384 nm (C_8) and relatively broad absorption band were observed for **5**. Fig. 4b showed the emission spectra of **1**-**5** at 500 μM (excitation at 360 nm), the emission maxima are centered at ca. 410 nm for **1**-**4**, and the emission maximum shifts to 420 nm for **5**. In Fig. 4c, the CD spectra of **5** at 500 μM showed a bisignated Cotton effect appeared in the range of π-π* absorption (300~400 nm), indicating the formation of ordered π–π stacking in the assemblies.[10] With increasing the concentration from 500 to 3000 μM (Fig. 4d), a enhancement of the Cotton effects for **3**-**5** were observed in the CD spectra. It is interesting to note that the intensity of the CD signal is proportional to the alkyl chain length in the NDI-dipeptide; that is, the gelator **5** has the strongest CD signal at 3000 μM compared with those of **1**-**4** (Fig. 4d). These results indicate that the presence of an end-capped long *n*-octyl tail in the NDI-dipeptide enhances the self-assembly ability in aqueous solution. Moreover, the temperature-dependent CD spectra of **5** were measured from 20°C to 80°C (Fig. S2). The CD signals decreased significantly with the increase of temperature, indicating that the molecular aggregates may disassemble at high temperature.

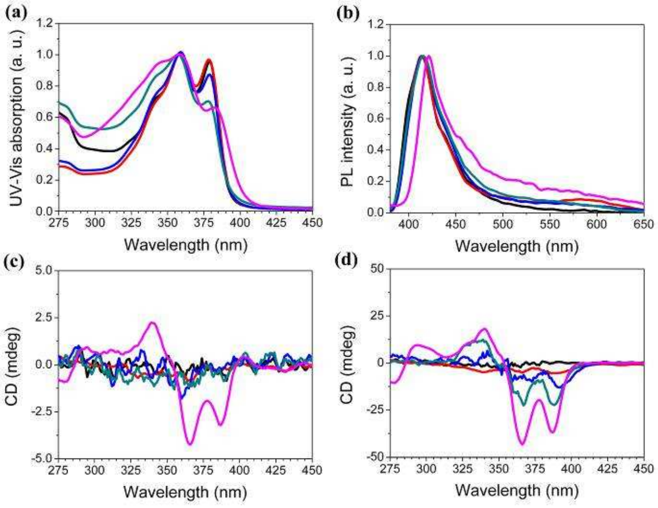

Fig. 4 Normalized (a) UV-Vis absorption and (b) emission spectra of **1** (black), **2** (red), **3** (blue), **4** (cyan) and **5** (magenta) at 500 μM in water. CD spectra of **1**-**5** at (c) 500 μM and (d) 3000 μM in water.

Since the presence of a relatively long *n*-octyl tail could enhance the ability of self-assembly in dilute solution. We then further study the concentration-dependent absorption, CD and fluorescence emission spectra of **5** as displayed in Fig. 5a-5c. In order to prevent the light scattering effect, the absorption and CD spectra for **5** were measured in the range of 500-5000 μM. The intensities of absorption were increased as a function of the concentrations of **5**, while the CD intensities were saturated ca. 3000 μM. The fluorescence emission was used to explore the π–π interactions between hydrogelators in the assemblies. The concentration-dependent study of **5** showed that the emission maxima gradually shift from 420 to 427 nm for the concentrations of 500 and 15000 μM, respectively. After photoexcitation of NDI chromophores, the decrease of the intensity in the emission peak at 427 nm for **5** might be assigned to the formation of H-type aggregation of the NDI chromophores in the assemblies (Fig. 5c).[29] Notably, a relative higher emission band at ca. 560 nm was observed for **5** at 15000 μM, which is due to typical π-stacking in the assemblies.[15] To explore the formation of secondary superstructures of the NDI-dipeptides in the gel state, FT-IR experiments were carried out in both the gel (1 wt% in water) and well-dispersed solution (1 wt% in MeOH). In Fig. 5d, the gel state of **5** displayed absorption bands of imide carbonyl of the NDI moiety centered at 1663 and 1649 cm^{-1}; these bands were shifted to a lower energy relative to those in homogeneous methanol solutions (1671 and 1652 cm^{-1}), suggesting hydrogen-boning, alkyl intercalating, and π–π interactions between hydrogelators in aqueous solution. There are two new peaks appeared at around 1633 and 1683 cm^{-1} upon transitioning from methanol to water, which might be assigned to the formation of anti-parallel β-sheet-like structures in the assemblies.[22] The spectroscopic data of **1**-**4** were similar to that of **5** (see ESI for detailed).

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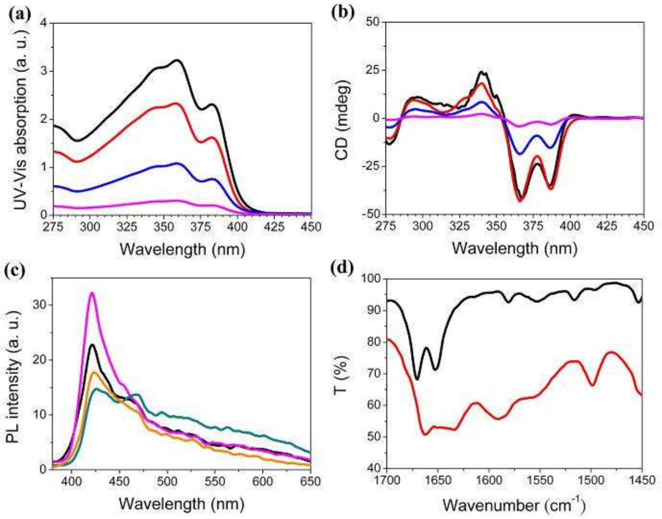

Fig. 5 Concentration-dependent (a) UV-Vis absorption, (b) CD and (c) emission spectra of **5** in water. (500 μM: magenta; 2000 μM: blue; 3000 μM: red; 5000 μM: black; 10000 μM: orange; 15000 μM: cyan). (d) FT-IR spectra of **5** at 1wt% in water (red) and MeOH (black).

In summary, we have systematically studied for the first time the influence of end-capped aliphatic chain length on supramolecular hydrogelation of NDI-capped dipeptides. The key finding in this study is that the dependence of the gelation pH as a function of chain lengths reveals an inverse linear relationship, and it covers a broad range of pH from basic to acidic conditions. The elongation of alkyl chain lengths in the NDI would result in hydrogelation at lower pH values compared with the corresponding shorter chains, and the NDI-dipeptide with end-capped propyl group can form a supramolecular hydrogel at neutral condition. In addition, the presence of a relatively long *n*-octyl tail could enhance the ability of self-assembly in dilute solution. This work highlights the importance of end-capped alkyl chains in the formation of supramolecular hydrogels and provides new insights into the design of functional self-assembled biomaterials.

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Notes and references

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