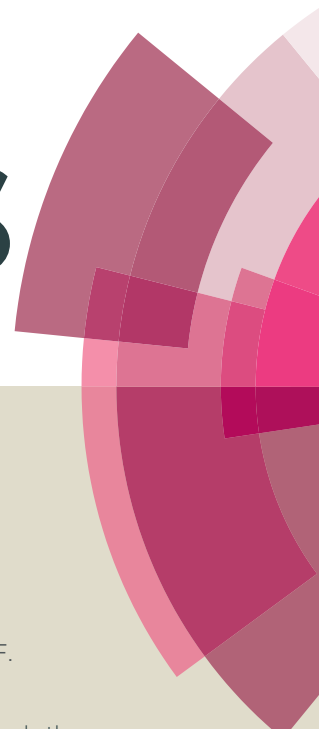


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COMMUNICATION

Nanofibrous Hydrogels Self-Assembled from Naphthalene Diimide (NDI)/Amino Acid Conjugates

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Ling-Huang Hsu, Shu-Min Hsu, Fang-Yi Wu, Yu-Hao Liu, Srinivasa Rao Nelli, Mei-Yu Yeh and Hsin-Chieh Lin*

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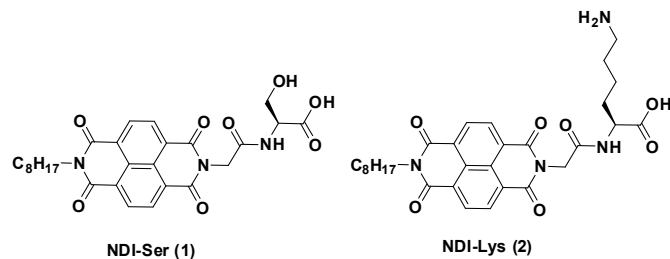
The combination of a naphthalene diimide (NDI) group and a single amino-acid of serine (Ser) or lysine (Lys) can be used to promote the formation of a supramolecular hydrogel, which indicates the aromatic amino acid is not necessary to be used in the structural design of NDI-capped small molecule hydrogelators. An equimolar ratio of NDI-Ser and NDI-Lys can form a stable hydrogel under neutral condition and its gel-to-sol transition temperatures is higher than 37°C. This work, as the first example of the NDI-containing hydrogelators without aromatic rings in the side chain of amino acids, illustrates a new approach to design NDI-capped supramolecular hydrogels.

Naphthalene diimide (NDI) is an attractive molecular unit due to their n-type semiconducting property and air stability.^[1-3] Recently, NDI molecules have been studied as key components in several systems, including light-emitting diodes,¹ solar cells,² field effect transistors,³ and ion sensors,⁴ as well as in cell imaging⁵ and photodynamic therapy.⁶ In addition, NDI motifs have been employed as components in various supramolecular nanostructures, including barrels, catenanes, rotaxanes, and vesicles,⁷ as well as one-dimensional nanostructures,⁸ which can be utilized to construct more promising three-dimensional materials such as organogels⁹ and hydrogels.^{8b,10} Because of their desired electronic and spectroscopic properties, NDI can act as one of the ideal components for the creation of supramolecular functional materials.

Xu *et al.* demonstrated that aromatic peptide amphiphile is an efficient strategy toward realizing a low-molecular-weight hydrogelator which is to covalently bind an aromatic hydrophobic group to a short peptide.¹¹ NDI/peptide conjugates have been proven an effective way to construct new functional hydrogels. For example, the symmetric NDI-peptide hydrogelators have been investigated previously by Tovar *et al.*^{10a} For asymmetric examples, Parquette *et al.* have found that the Fmoc-KK(NDI) can self-assemble to form hydrogel.^{8b} Ulijn *et al.* have demonstrated that the simple structure of NDI-Y can form a yellow-orange self-supporting hydrogel.¹²

More recently, we have proven that the NDI-FF and NDI-FG can promote the formation of one-dimensional nanostructures and three-dimensional hydrogels under physiological conditions.¹³ These successful examples indicate the necessity of aromatic amino acids or additional aromatic moiety in the structure of NDI-appended peptides.

It has been demonstrated that short peptide sequences such as two or three amino acids with aromatic functionality can promote the formation of nanostructured hydrogels.¹⁴ In these hydrogelators, aromatic-aromatic interactions and hydrogen bonding play key roles behind the self-assembly.¹⁵ The choice of chemical composition of amino acid sequence have significant effects on the physical properties of the resulting gels.¹⁶ For example, the cell adhesion and proliferation of various cell types can be changed by judicious modification of hydrogel surface with simple chemical functionality, including hydroxyl (OH), amine (NH₂), and carboxyl (COOH).¹⁷ In this study, we have adopted a 'minimalist approach' to use a NDI group and a single amino-acid (Ser or Lys) instead of longer peptides or aromatic amino acids, therefore two NDI materials with serine (Ser, with side chain R = CH₂OH) or lysine (Lys, with side chain R = (CH₂)₄NH₂) were studied. Furthermore, a mixture of the two NDI-amino-acids were also investigated: NDI-Ser (**1**) and NDI-Lys (**2**) as the simplest co-assembling units reported so far in the formation of NDI-based supramolecular hydrogels at neutral pH. This hydrogel provides a highly hydrated, stiff and nanofibrous network that may mimic certain essential features of the extracellular matrix which would be potentially useful in the development of new nanostructured biomaterials.



Scheme 1. Chemical structures of NDI-Ser (1) and NDI-Lys (2).

The NDI/amino acid conjugates NDI-Ser (1) and NDI-Lys (2) have synthesized through solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin. The characterization data of 1 and 2 are collected in the Supporting Information. We have been prepared the hydrogels using the sequential change in pH values from 11 to 3 at the concentration of 2 wt%. Compound 1 and 2 formed self-supporting gels under pH 4.5 and 10.0, respectively. The structural and physical properties of amino acid derivatives were found to be dictated by the amino acid sequence. Furthermore, we found that the equal volume ratio of 1 and 2 were mixed and formed a hydrogel at pH 7.0 (2 wt%). The appearances of the hydrogels are shown in the inset of Fig. 1a–1c, the colors of the hydrogels are opaque brownish for 1, transparent yellow for 2 and opaque brownish for a 1 : 1 blend of 1 and 2. The microscopic nanostructures within the peptide hydrogels are demonstrated by transmission electron microscopy (TEM). In Fig. 1, the TEM analysis revealed that the hydrogels of 1, 2 as well as 3 consisted of a uniform fibrous network with fiber diameters of 9 ± 1 , 7 ± 1 and 11 ± 1 nm, respectively, and these nanofibers entangle to trap water molecules to form three dimensional environment under acidic, basic and neutral conditions.

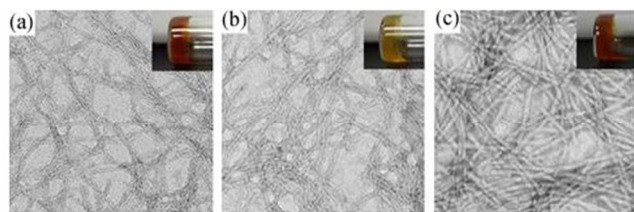


Fig. 1 Optical (in inset) and negative stained TEM images of (a) 1, (b) 2 and (c) a 1 : 1 blend of 1 and 2 at 2 wt%. Scale bar = 50 nm.

The physical properties of 1–3 are collected in Table 1. The gel-to-sol transition temperatures ($T_{\text{gel-sol}}$) of 1 and 2 at 2 wt % were both above 37 °C (Table 1), and the presence of lysine showed a relatively high $T_{\text{gel-sol}}$. In addition, the gel of a 1 : 1 blend of 1 and 2 at neutral condition was quite stable up to 55 °C, pointing to potential applications of the hydrogel for use in cell culturing. If a hydrogel is to provide a three-dimensional environment for cell growth, its mechanical properties should allow it to support the mass of a cell. Although the ideal storage modulus will vary depending on the type of cell, a storage modulus of approximately 100 Pa is the minimum required to support the mass of a cell.¹⁸ The mechanical properties of the hydrogels were tested using oscillatory rheology. The rheological data obtained for 1, 2 and a 1 : 1 blend of 1 and 2 gels were shown in Fig. 2a and Table 1, all of the storage moduli (G') were found to be higher than the loss modulus (G''), indicating the elastic properties rather than viscous ones. Note that the average elastic storage moduli were measured over the frequency range of 0.1–100 rad s^{-1} and they were all higher than 100 Pa, and the trend of rheological properties for 1,

2 and a 1 : 1 blend of 1 and 2 are consistent with the tendency of their $T_{\text{gel-sol}}$ data.

Table 1. Physical properties of NDI compounds at 2 wt%

entry	pH	Appr. ^b	Fiber width (nm)	$T_{\text{gel-sol}}$ (°C)	G' , G'' (Pa)
1	4.5	OG	9 ± 1	45	1.4×10^3 , 2.0×10^2
2	10.0	TG	7 ± 1	80	5.3×10^3 , 8.2×10^2
3 ^a	7.0	OG	11 ± 1	55	2.2×10^3 , 3.7×10^2

^a1:1 blend of 1 and 2. ^bOG: opaque gel. TG: transparent gel.

FT-IR was used for the analysis of the secondary structures of self-assembled nanostructures in hydrogels.^{15,19} We observed two peaks at around 1634 cm^{-1} and 1683 cm^{-1} in FT-IR spectra for 1, 2 and a 1 : 1 blend of 1 and 2, which is due to the absorptions of the β -sheet-like structures in the assemblies (See Fig. S4 and S5 for details). In addition, the presence of the amino acids might create the hydrogen-bonding interactions between carboxyl end groups of the hydrogelators as studied previously for NDI related systems.^{20,21} In addition, we observed two peaks at 1665 and 1709 cm^{-1} which correspond to symmetric and asymmetric stretching of the imide carbonyl of the NDI moiety in the hydrogelator, respectively (Fig. 2b).

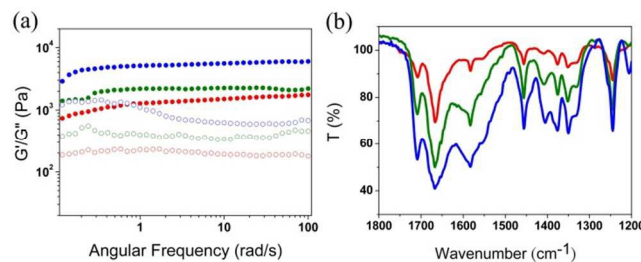


Fig. 2 (a) The frequency sweep of the hydrogels of 1 (pH=4.5, red), 2 (pH=10.0, blue) as well as a 1 : 1 blend of 1 and 2 (pH=7.0, green). All the concentration of hydrogels are prepared at 2 wt %; closed and open circles for G' and G'' . (b) FT-IR spectra of 1 (red), 2 (blue) as well as a 1 : 1 blend of 1 and 2 (green) in water.

The UV-vis absorption and circular dichroic (CD) spectra were employed to study the intermolecular interactions and orientation of π -conjugated chromophores in the assemblies. As displayed in Fig. 3a, 3c and 3e, the UV-vis spectra of 1, 2 and a 1 : 1 blend of 1 and 2 at 0.05 wt% in water exhibited two bands at 235 nm (220–260 nm, band I) and at 377 nm (320–410 nm, band II) which are consistent with the π - π^* transitions polarized along short and long axes of the NDI group, respectively.²² For comparison, 2,2,2-trifluoroethanol (TFE) was used for UV-vis measurements since TFE is a good solvent for NDI/peptide conjugates.^{8b} We observed a profile change in vibronic progressions and lower absorption intensities when transition from TFE to water for gelators at 0.05 wt% (Fig. S3), thus indicating the hydrogelators aggregate at this concentration.²³ The CD spectra of the hydrogelators at 0.05 wt% revealed two excitonic Cotton effects appeared for band I and band II. In particular, the band II revealed a bisignated Cotton effect centered at 237 nm, corresponding to a right-handed, P-type helical orientation in the assemblies.¹³ Fluorescence spectroscopy was used to study the π - π interactions in both solution and gel states.^{11b} As shown in Fig. 3b, 3d, and 3f, the emission spectra of the NDI-capped materials were obtained with an excitation wavelength of 377 nm for NDI chromophores. In all three cases, the emission profiles of the solutions (0.025 wt%) are centered at ca. 408 nm, and the emission maximum shifts to ca. 420 nm at higher concentration (0.25 wt%) with lower intensity. The decrease of the intensity in the emission

peak at higher concentration can be attributed to the intermolecular interactions of the NDI-capped molecules in the assemblies.²³ Notably, the presence of the octyl group would increase the hydrophobic interactions between molecules which may facilitate the intermolecular packing in the assemblies.^{21,22} Furthermore, the ratios of the fluorescent emission intensities ($I_{0.25 \text{ wt}\%}$ to $I_{0.025 \text{ wt}\%}$) for NDI-Ser and NDI-Lys are 68.7% and 87.7%, respectively, suggesting that the bulky side chain and/or electrostatic repulsion (Lys) may prevent NDI chromophores from close proximity which results in the less decrease of fluorescence intensity. Since the π - π interactions, hydrophobic forces and hydrogen-bonding interactions are the major driving forces behind the self-assembly of nanostructures for the hydrogelators, we propose a plausible packing model for **1** in Fig. S6.

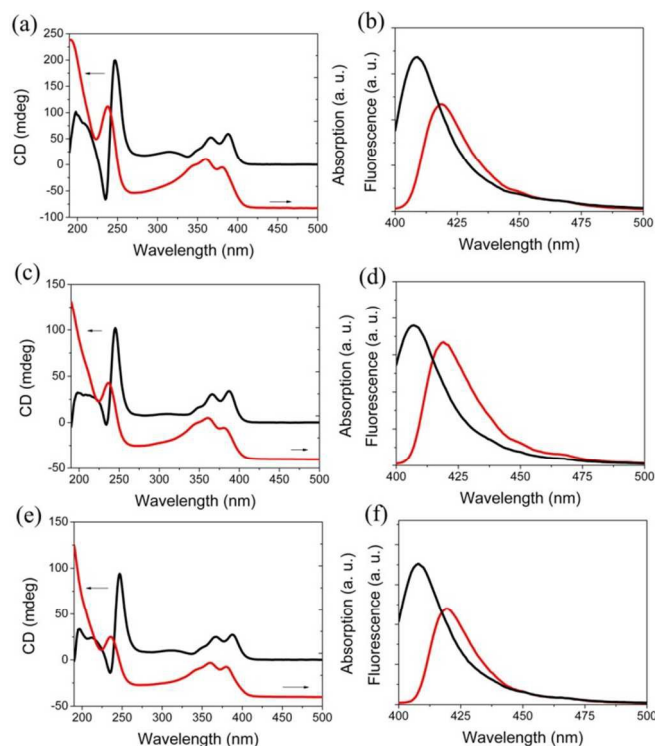


Fig. 3 UV-vis absorption (red) and CD (black) spectra of (a) **1**, (b) **2** and (c) a 1 : 1 blend of **1** and **2** at 0.05 wt%. Emission spectra of (d) **1**, (e) **2** and (f) a 1 : 1 blend of **1** and **2** at 0.025 wt% (black) and 0.25 wt% (red).

The emission spectra of aqueous solutions of **1** and **2** revealed that the emission bands are in the visible region. Accordingly, we were interested in exploring the cell imaging properties of water-soluble molecules **1** and **2** at relatively low concentration compared with that of the gel, using MCF-7 cells as the culture system. Before that, we have studied **1** and **2** for their biocompatibility with MCF-7 cells, using a colorimetric assay with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The experiments (Fig. 4a and b) revealed that after being incubated with the hydrogelators **1** and **2** in a concentration range of (10–500 μM) for 48 hours, cells that were grown in liquid medium showed the proliferation capacities with good viability for **1** and high viability for **2**. The survival ratio of **1** and **2** were all above 80% at 500 μM . These results greatly improved the cell viability ratios compared with NDI-

FF and NDI-FG as studied by our group previously.¹³ For example, the survival ratios for NDI-FF and NDI-FG at 50 μM are \sim 60% and \sim 50%, respectively. Therefore, removing the aromatic group in NDI-capped amino-acid-based hydrogelator may substantially reduce the cytotoxicity for MCF-7 cells. Since the concentrations of **1** and **2** required for IC_{50} after 48 h were greater than 500 μM , the biocompatibility tests of **1** and **2** are suitable for cell imaging tests of neutral condition (Table 1).²⁴

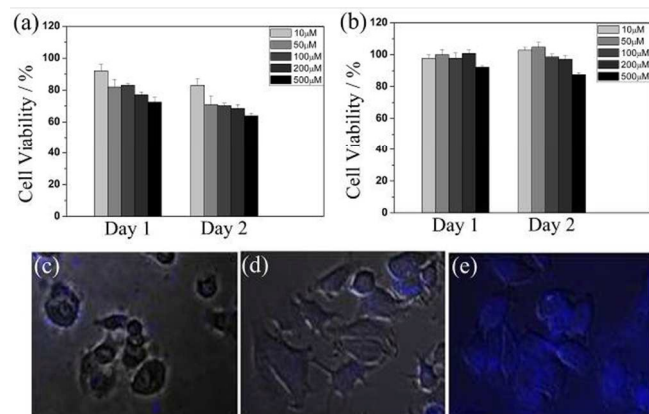


Fig. 4 MCF-7 cell viability in the presence of (a) **1** and (b) **2** (10–500 μM). Fluorescence images of MCF-7 cells incubated in the presence of (a) **1**, (b) **2** and (c) a 1:1 blend of **1** and **2** at 50 μM after 90 min.

Conclusions

In summary, we have synthesized amino acid-derived molecules of NDI-Ser and NDI-Lys. The combination of a naphthalene diimide (NDI) group and a single amino-acid of serine (Ser) or lysine (Lys) can be used to promote the formation of a supramolecular hydrogel, which indicates the aromatic amino acid is not necessary to be used in the structural design of NDI-capped small molecule hydrogelators. An equimolar ratio of NDI-Ser and NDI-Lys can form a stable hydrogel under neutral condition. All these gels are elastic materials with storage modulus of 1-5 KPa and the gel-sol transition temperatures are higher than 37 $^{\circ}\text{C}$. These results suggest that a 1:1 blend of **1** and **2** may be a potential nanostructured biomaterial. Cell viability ratios of newly discovered hydrogelators were greatly improved compared with those of NDI-dipeptides.¹³ This work, as the first example of the NDI-containing hydrogelators without aromatic rings in the side chain of amino acids, illustrates a new approach to design NDI-capped supramolecular hydrogels.

Acknowledgements

COMMUNICATION

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Notes and references

Department of Materials Science and Engineering, National Chiao Tung University, Hsinchu, 300, Taiwan, Republic of China.

E-mail: hclin45@nctu.edu.tw

† Electronic Supplementary Information (ESI) available: [Detail synthetic protocols and characterization data]. See DOI: 10.1039/c000000x/

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