Supporting Information

Discovery of Energy Transfer Nanostructures Using Gelation-Driven Dynamic Combinatorial Libraries †

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## Contents

Contents........................................................................................................................................... 2  
Materials and Methods......................................................................................................................... 3  
    Materials:........................................................................................................................................ 3  
    Methods:......................................................................................................................................... 3  
Synthesis: .......................................................................................................................................... 6  
    Synthesis of **Nap-Y**: .................................................................................................................... 7  
    Synthesis of **Nap-YF-NH₂**: ........................................................................................................... 9  
    Synthesis of **Nap-YL-NH₂**: ............................................................................................................ 10  
UV-vis and Fluorescence emission spectra:............................................................................................ 14  
Reversed-phase HPLC Analysis: .......................................................................................................... 18  
References ......................................................................................................................................... 20


**Materials and Methods**

**Materials:**

All commercial reagents were used as supplied. All solvents were used as supplied (analytical or HPLC grade) without further purification, unless otherwise mentioned. All reactions were carried out in oven-dried glassware and magnetically stirred. Thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} plates. All compounds were visualized either by UV light source (254 nm) or by dipping in basic permanganate solution. Column chromatography was carried out by using silica gel 60 (230-400 mesh). High resolution mass spectra (ESI-HRMS) were recorded on a Thermo Electron Exactive. \(^1\)H and \(^{13}\)C nuclear magnetic resonance (NMR) spectra were recorded on Bruker AV300 spectrometer in the deuterated solvents. All chemical shifts (\(\delta\)) are quoted in ppm and coupling constants (\(J\)) given in Hz. Residual signals from the solvents were used as an internal reference.

**Methods:**

**Enzyme-triggered hydrogellation:**

The precursors Nap-Y (20 mM) and each X-NH\(_2\) (80 mM, all amino acid amides were purchased from Bachem, Germany) were mixed (at 1:4 ratio) in a glass vial. The mixture was suspended in 1.0 mL of 100 mM phosphate buffer (pH 8) with the addition of 1 mg mL\(^{-1}\) lyophilised thermolysin powder (\textit{bacillus Thermoproteolyticus rokko} from Sigma-Aldrich, UK, mol wt 34.6 kDa by amino acid sequence). The mixture was vortex mixed for 20 s and sonicated for 1 min to ensure dissolution. Samples were incubated at room temperature for 24 h before analysis unless otherwise stated. Gelation was considered to have occurred when a homogeneous solid-like material was obtained that exhibited no gravitational flow.
In the case of dynamic combinatorial library (DCL), Nap-Y (20 mM) and various \( \text{X-NH}_2 \) (80 mM each) derivatives were used and the above procedure was followed to form a self-supporting hydrogel.

In the case of mixed donor-acceptor system in isolation as well as in DCL system, the dansyl-\( \beta \)-alanine (DA) acceptor was used at variable concentrations, 0.6 mM (33:1 donor-acceptor ratio), 2 mM (10:1 donor-acceptor ratio), 2.7 mM (7:1 donor-acceptor ratio) and 10 mM (2:1 donor-acceptor ratio), and mixed together with Nap-Y (20 mM) and \( \text{X-NH}_2 \) (80 mM each) by following the above procedure to form a self-supporting hydrogel. In this case (DCL + DA system), the corresponding amount of the acceptor was dissolved in the buffer (sonicated when necessary) and the amino acid derivatives were dissolved in the same solution followed by the addition of enzyme.

**High-performance liquid chromatography (HPLC):**

A Dionex P680 HPLC system was used to quantify the percentage conversion of the enzymatic reaction. A 50 \( \mu \)L sample was injected onto a Macherey–Nagel C18 column of 250 mm length with an internal diameter of 4.6 mm and 5 mm fused silica particles at a flow rate of 1 mL min\(^{-1} \) (eluting solvent system: linear gradient of 20\% (v/v) acetonitrile in water for 4 min, gradually rising to 80\% (v/v) acetonitrile in water at 35 min. This concentration was kept constant until 40 min when the gradient was decreased to 20\% (v/v) acetonitrile in water at 42 min.) Sample preparation involved mixing 20 \( \mu \)L of the sample with 1 mL acetonitrile-water (50:50 mixture) containing 0.1\% trifluoroacetic acid. The intensity of each identified peak was determined by UV detection at 280 nm. The experimental data was acquired in triplicate and the average data was shown. The samples were vortexed before collecting the aliquots for HPLC and the percentage yields are calculated from HPLC integrated peak areas.

**UV-vis absorption spectroscopy:**

UV-vis absorption spectra were recorded on a Jasco V-660 spectrophotometer. Samples were prepared in PMMA cuvettes (from Fisher Scientific). The experimental data was acquired in triplicate and the average data was shown.
Fluorescence emission spectroscopy:

Fluorescence emission spectra were measured on a Jasco FP-6500 spectrofluorometer with light measured orthogonally to the excitation light, at a scanning speed of 200 nm min⁻¹. The excitation wavelength was 280 nm and emission data were recorded in the range between 300 and 700 nm. The spectra were measured with a bandwidth of 5 nm (or 3 nm) with a low (or medium) response and a 1 nm data pitch. Samples were prepared in PMMA cuvettes (from Fisher Scientific). The freshly prepared samples were directly taken in the cuvette and the time-dependent spectra were recorded immediately. The experiments were carried out directly under the described concentrations and the gels were not diluted for the experiments. The experimental data was acquired in triplicate and the average data was shown.

Circular dichroism (CD):

Circular dichroism (CD) spectra were measured on a Jasco J600 spectropolarimeter in a 0.1 mm pathlength cylindrical cell, with 1 s integration, step resolution of 1 mm, response of 0.5 s with a bandwidth of 1 nm and slit width of 1 mm. The freshly prepared samples were directly added to the cell using a pipette and the spectra were recorded after equilibration for 24 h. Due to the high opacity of the gels under these conditions, the High Tension (HT) voltage value reaches maximum below the wavelengths of 250 nm and the CD could not be measured.

Atomic force microscopy (AFM):

For AFM experiments, 20 µL of sample solution (as a gel) was diluted to 1000 µL of deionized water and then deposited onto a freshly cleaved mica surface (G250-2 Mica sheets 1” x 1” x 0.006”; Agar Scientific Ltd, Essex, UK). Each sample was air dried overnight in a dust-free environment prior to AFM imaging. The images were obtained by scanning the mica surface in air under ambient conditions using a Veeco diINNOVA Scanning Probe Microscope (VEECO/BRUKER, Santa Barbara, CA, USA) operated in tapping mode. The AFM measurements were obtained using sharp silicon probes (RTESPA; Veeco Instruments SAS, Dourdan, France). AFM scans were taken at 512 x 512 pixels resolution and produced topographic
images of the samples in which the brightness of features increases as a function of height. Typical scanning parameters were as follows: tapping frequency 326 kHz, integral and proportional gains 0.1 and 0.3, respectively, set point 0.5–0.7 V and scanning speed 1.0 Hz. AFM images (10 µm × 10 µm) were collected from two different samples and at random spot surface sampling (at least five areas). The images were analyzed using NanoScope Analysis software Version 1.40.

**Transmission electron microscopy (TEM):**

Transmission electron microscopy (TEM) images were captured using a LEO 912 energy filtering transmission electron microscope operating at 120kV fitted with 14 bit/2 K Proscan CCD camera. Carbon-coated copper grids (200 mesh) were glow discharged in air for 30 seconds. The support film was touched onto the gel surface for 3 seconds and blotted down using filter paper. Each sample was allowed to dry afterwards for few minutes in a dust-free environment prior to TEM imaging. Negative stain (20 µL, 1 % aqueous methylamine vanadate obtained from Nanovan, Nanoprobes) was applied and the mixture blotted again using filter paper to remove excess. The dried specimens were then imaged using the microscope.

**Synthesis:**

The molecules Nap-Y and Nap-YX-NH$_2$ were synthesized as shown in Scheme S1. The molecule dansyl-$\beta$-alanine (DA) was synthesized and characterized according to the literature procedure.$^{[1]}$
Scheme S1: (a) Synthetic route for the preparation of molecules Nap-Y and Nap-YX-NH₂ and (b) the molecular structure of dansyl-β-alanine (DA) acceptor.

**Synthesis of Nap-Y:**

2-Naphthoxy acetic acid (1.0 g, 5 mmol), L-Tyrosine tert-butylester (1.42 g, 6 mmol), HBTU (2.28 g, 6 mmol) and DIPEA (2.18 mL, 12.5 mmol) were dissolved in anhydrous dimethylformamide (20 mL) and left to stir at room temperature overnight. The resulting solution was taken up in ethyl acetate (70 mL) and sequentially washed with equal volumes (50 ml) of 1 M saturated sodium bicarbonate, brine, 1 M hydrochloric acid and brine. The organic layer was then collected, dried over magnesium sulphate and the solvent was removed by evaporation *in vacuo*. The residue was finally purified by silica gel column
chromatography (eluent, 40% ethyl acetate in n-hexanes) to afford 1.4 g (67% yield) of Nap-Y-OtBu as a white powder.

Nap-Y-OtBu (1.4 g, 3.32 mmol) was dissolved in trifluoroacetic acid (15 mL) and left to stir at room temperature overnight. The solvent was then removed by evaporation in vacuo and the residual trifluoroacetic acid was removed by azeotropic distillation with toluene. The crude product was then taken up in diethyl ether (100 ml) followed by sonication for few minutes resulted in a white emulsion. The pure product was then collected by filtration to afford Nap-Y as a white powder (900 mg, 74% yield).

$^1$H NMR (300MHz, DMSO): $\delta$ 12.83 (s, 1H, 17-H), 9.22 (s, 1H, 24-H), 8.26-8.24 (d, 1H, $J = 8.04$ Hz, 13-H), 7.85-7.83 (d, 2H, $J = 8.6$ Hz, 3,6-H), 7.76-7.74 (d, 1H, $J = 8.08$ Hz, 7-H), 7.49-7.45 (t, 1H, $J = 7.34$ Hz, 2-H), 7.39-7.37 (t, 1H, $J = 7.34$ Hz, 1-H), 7.24-7.23 (t, 2H, $J = 6.56$ Hz, 8,10-H), 7.03-7.01 (d, 2H, $J = 8.2$ Hz, 19,23-H), 6.65-6.63 (d, 2H, $J = 8.24$ Hz, 20,22-H), 4.62 (s, 2H, 11-H), 4.52-4.48 (q, 1H, $J = 5.4$ Hz, 14-H), 3.04-3.0 (m, 1H, 16-H), 2.93-2.88 (m, 1H, 16-H).

$^{13}$C NMR (75MHz, DMSO): $\delta$ 172.78 (C$_q$, 17-C), 167.51 (C$_q$, 12-C), 155.97 (C$_q$, 9-C), 155.54 (C$_q$, 21-C), 134.03 (C$_q$, 18-C), 130.05 (CH, 19-C), 129.32 (CH, 23-C), 128.74 (CH, 3-C), 127.49 (CH, 7-C), 127.40 (C$_q$, 4,5-C), 126.77 (CH, 6-C), 126.45 (CH, 1-C), 123.85 (CH, 2-C), 118.50 (CH, 8-C), 115.04 (CH, 20,22-C), 107.41 (CH, 10-C), 66.71 (CH$_2$, 11-C), 53.40 (CH, 14-C), 35.74 (CH$_2$, 16-C).

ESI-HRMS (m/z): Calculated for [C$_{21}$H$_{19}$NO$_5$H]$^+$: 366.1336; Found: 366.1339.

HPLC analysis: > 96% pure.
Synthesis of Nap-YF-NH₂:

Nap-Y (250 mg, 0.68 mmol), F-NH₂ (135 mg, 0.82 mmol), HBTU (311 mg, 0.82 mmol) and DIPEA (0.3 mL, 1.7 mmol) were dissolved in anhydrous dimethylformamide (10 mL) and left to stir at room temperature overnight. The resulting solution was taken up in ethyl acetate (50 mL) and sequentially washed with equal volumes (50 ml) of 1 M saturated sodium bicarbonate, brine, 1 M hydrochloric acid and brine. The organic layer was then collected, dried over magnesium sulphate and the solvent was removed by evaporation in vacuo. The crude product was finally purified by silica gel column chromatography (eluent, 20% methanol in chloroform) to afford Nap-YF-NH₂ as a white powder (123 mg, 35% yield).

¹H NMR (300MHz, DMSO): δ 9.19 (s, 1H, 28-H), 8.2 (d, 1H, J = 8.28 Hz, 17-H), 8.06 (d, 1H, J = 8.28 Hz, 13-H), 7.84 (d, 2H, J = 8.56 Hz, 3,6-H), 7.75 (d, 1H, J = 8.12 Hz, 7-H), 7.49-7.45 (m, 1H, 2-H), 7.38-7.35 (m, 2H, 1,10-H), 7.22 (d, 4H, J = 4.36 Hz, 30,31,33,34-H), 7.19-7.12 (m, 4H, 8,20,32-H), 6.97 (d, 2H, J = 8.4 Hz, 23,27-H), 6.58 (d, 2H, J = 8.44 Hz, 24,26-H), 4.55-4.45 (m, 4H, 11,14,18-H), 2.9-2.7 (m, 4H, 16,21-H).

¹³C NMR (75MHz, DMSO): δ 172.7 (Cq, 19-C), 170.62 (Cq, 15-C), 167.24 (Cq, 12-C), 155.82 (Cq, 9-C), 155.50 (Cq, 25-C), 137.79 (Cq, 29-C), 134.04 (Cq, 22-C), 130.19 (CH, 23,27-C), 129.40 (CH, 7-C), 129.21 (Cq, 4,5-C), 128.74 (CH, 31,33-C), 127.52 (CH, 3-C), 126.83 (CH, 30,34-C), 126.5 (CH, 6,1-C), 126.26 (CH, 32-C), 123.89 (CH, 2-C), 118.44 (CH, 8-C), 114.88 (CH, 24,26-C), 107.37 (CH, 10-C), 66.72 (CH₂, 11-C), 53.86 (CH, 18-C), 53.78 (CH, 14-C), 37.65 (CH₂, 16-C), 36.61 (CH₂, 21-C).

HPLC analysis: > 98% pure.

**Synthesis of Nap-YL-NH$_2$:**

Nap-Y (250 mg, 0.68 mmol), L-NH$_2$ (106 mg, 0.82 mmol), HBTU (311 mg, 0.82 mmol) and DIPEA (0.3 mL, 1.7 mmol) were dissolved in anhydrous dimethylformamide (10 mL) and left to stir at room temperature overnight. The resulting solution was taken up in ethyl acetate (50 mL) and sequentially washed with equal volumes (50 mL) of 1 M saturated sodium bicarbonate, brine, 1 M hydrochloric acid and brine. The organic layer was then collected, dried over magnesium sulphate and the solvent was removed by evaporation *in vacuo*. The crude product was finally purified by silica gel column chromatography (eluent, 20% methanol in chloroform) to afford Nap-YL-NH$_2$ as an off-white solid (131 mg, 40% yield).

**$^1$H NMR (300MHz, DMSO):** $\delta$ 9.18 (s, 1H, 28-H), 8.11-8.08 (m, 2H, 13,17-H), 7.84 (d, 2H, $J = 8.6$ Hz, 3,6-H), 7.74 (d, 1H, $J = 8.08$ Hz, 7-H), 7.48-7.44 (m, 1H, 2-H), 7.38-7.34 (m, 1H, 1-H), 7.26 (s, 1H, 10-H), 7.19-7.12 (m, 2H, 20-H), 7.02 (d, 3H, $J = 8.4$ Hz, 8,23,27-H), 6.58 (d, 2H, $J = 8.44$ Hz, 24,26-H), 4.58-4.54 (m, 3H, 11,14-H), 4.26-4.24 (m, 1H, 18-H), 2.97-2.93 (m, 1H, 16-H), 2.88-2.78 (m, 1H, 16-H), 1.56-1.54 (m, 1H, 21-H), 1.47-1.35 (m, 2H, 21,29-H), 0.86-0.8 (m, 6H, 30,31-H).
$^{13}$C NMR (75MHz, DMSO): $\delta$ 173.88 (C$_q$, 19-C), 170.57 (C$_q$, 15-C), 167.29 (C$_q$, 12-C), 155.80 (C$_q$, 9-C), 155.48 (C$_q$, 25-C), 134.0 (C$_q$, 22-C), 130.17 (CH, 23,27-C), 129.33 (CH, 7-C), 128.68 (C$_q$, 4,5-C), 127.47 (CH, 3-C), 126.76 (CH, 6-C), 126.44 (CH, 1-C), 123.82 (CH, 2-C), 118.4 (CH, 8-C), 114.84 (CH, 24,26-C), 107.31 (CH, 10-C), 66.71 (CH$_2$, 11-C), 53.80 (CH, 14-C), 50.87 (CH, 18-C), 40.1 (CH$_2$, 21-C), 36.62 (CH$_2$, 16-C), 24.16 (CH, 29-C), 22.96 (CH$_3$, 30,31-C).

ESI-HRMS (m/z): Calculated for [C$_{27}$H$_{31}$N$_3$O$_5$H]$^+$: 478.2336; Found: 478.2340.

HPLC analysis: > 97% pure.

Figure S1: Photographs of naphthoxy-substituted dipeptide derivatives (Nap-YX-NH$_2$) which formed self-supporting hydrogels when prepared by a thermolysin-catalyzed condensation reaction (top) but chemically synthesized molecules formed amorphous precipitates upon cooling a heated solution in buffer (bottom).
**Figure S2:** The percentage conversion of naphthoxy-substituted dipeptide derivatives (Nap-YX-NH$_2$) monitored over time by reversed-phase HPLC (yields are calculated from integrated HPLC peak areas).
Figure S3: Parts of the HPLC traces of Nap-Y DCL at variable concentrations of DA (2 mM, 2.7 mM and 10 mM) after 24 h.
**UV-vis and Fluorescence emission spectra:**

![UV-vis and Fluorescence emission spectra](image)

**Figure S4:** A) UV-vis absorption and B) fluorescence emission spectra of dansyl-β-alanine (DA) acceptor. The peak at 660 nm in B) is due to the emission monochromator transmitting at $2 \times \lambda_{ex}$. 

![AFM image](image)

**Figure S5:** AFM image showing the formation of spherical aggregates of naphthoxy-substituted tyrosine derivative (Nap-Y) alone in 100 mM phosphate buffer at 20 mM concentration. Inset: Photograph showing clear and transparent free-flowing solution.
**Figure S6:** A) Time-dependent fluorescence emission spectra and B) their normalized spectra of dynamic combinatorial library (DCL) of naphthoxy-substituted dipeptide derivatives (Nap-YX-NH$_2$) in the presence of 2 mM DA (i.e., 10:1 donor-acceptor ratio) upon the addition of thermolysin ($\lambda_{ex} = 280$ nm).

**Figure S7:** A) Time-dependent fluorescence emission spectra and B) their normalized spectra of dynamic combinatorial library (DCL) of naphthoxy-substituted dipeptide derivatives (Nap-YX-NH$_2$) in the presence of 10 mM DA (i.e., 2:1 donor-acceptor ratio) upon the addition of thermolysin ($\lambda_{ex} = 280$ nm).
Figure S8: A) Time-dependent fluorescence emission spectra and B) their normalized spectra of naphthoxy-substituted dipeptide derivative (Nap-YF-NH₂) in the presence of 0.6 mM DA (i.e., 33:1 donor-acceptor ratio) upon the addition of thermolysin (λ<sub>ex</sub> = 280 nm).

Figure S9: A) Time-dependent fluorescence emission spectra and B) their normalized spectra of naphthoxy-substituted dipeptide derivative (Nap-YF-NH₂) in the presence of 2 mM DA (i.e., 10:1 donor-acceptor ratio) upon the addition of thermolysin (λ<sub>ex</sub> = 280 nm).
**Figure S10:** A) Time-dependent fluorescence emission spectra and B) their normalized spectra of naphthoxy-substituted dipeptide derivative (Nap-YF-NH$_2$) in the presence of 10 mM DA (i.e., 2:1 donor-acceptor ratio) upon the addition of thermolysin ($\lambda_{ex} = 280$ nm).
**Reversed-phase HPLC Analysis:**

![HPLC Analysis Graph](image)

**Figure S11:** Reversed-phase HPLC analysis of chemically synthesized molecules, Nap-YF-NH$_2$ (top), Nap-YL-NH$_2$ (middle) and Nap-Y (bottom). Absorbance was monitored at 280 nm.
Figure S12: HPLC trace of Nap-Y DCL in the absence of DA at 24 h after the addition of thermolysin.

Figure S13: HPLC trace of Nap-Y DCL in the presence of DA at 1:1 donor-acceptor ratio at 24 h after the addition of thermolysin.
References