Electro-active Organic Dye Incorporating Dipeptides in the Formation of Self-assembled Nanofibrous Hydrogels

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Supporting Information

Contents **Page Number**

Synthesis of NDI-Phe-Gly (2). The peptide/dye conjugate derivative of **2** was prepared through SPPS using 2-chlorotrityl chloride resin, Fmoc-L-phenylalanine, Fmoc-L-glycine and NDIA (Figure 1).[57] The resin (1.2 g) was swelled in anhydrous CH_2Cl_2 for 30 min and then Fmoc-L-glycine (0.595 g, 2.000 mmol) was loaded onto the resin in anhydrous *N*,*N*-dimethylformamide and *N*,*N*-diisopropylethylamine (DIEA; 0.830 mL, 5.000 mmol) for 1 h. For deprotection of the Fmoc group, piperidine (20% in DMF) was added and the sample left for 20 min; this procedure was repeated twice (each time for 2 min). Fmoc-L-phenylalanine (0.775 g, 2.000 mmol) was coupled to the free amino group using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluraniumhexafluorophosphate (HBTU) (0.3793 g, 1.000 mmol) and *N*,*N*-diisopropylethylamine (DIEA) (0.4150 mL, 2.500 mmol) as coupling agents for 30 min. Again, the sample was treated with piperidine (20% in DMF) for 20 min; this procedure was repeated twice (each time for 2 min). Finally, NDIA (0.873 g, 2.000 mmol) was coupled to the free amino group using HBTU (0.7586 g, 2.000 mmol) and DIEA (0.830 mL, 5.000 mmol) as coupling agents. After the reaction mixture had been stirred overnight, the peptide derivative was cleaved through treatment with CF_3CO_2H (90% in DI water) for 3 h. The resulting solution was dried by air and then Et_2O was added to precipitate the target product. The solid was dried under vacuum to remove residual solvent (light-brown solid:

0.236 g). ^{1}H NMR (300 MHz, [D_6]DMSO, 25 °C): δ=0.80-0.95 (m, 3H; CH_3), 1.20-1.45 (m, 10H; CH_2), 1.60-1.75 (m, 2H; CH_2), 2.70-2.90 (m, 1H; CH_2), 3.00-3.15 (m, 1H; CH_2), 3.80-3.85 (m, 2H; CH_2), 4.09 (t, J=7.35 Hz, 2H; CH_2), 4.55-4.70 (m, 1H; CH), 4.72 (s, 2H; CH_2), 7.20-7.40 (m, 5H; CH), 8.40-8.50 (m, 1H; NH), 8.63 (d, J=8.4 Hz, 1H; NH),8.70-8.80 (m, 4H; CH); ^{13}C NMR (75 MHz, [D_6]DMSO, 25 °C): δ=14.0, 22.1, 26.5, 27.4, 28.6, 28.7, 31.2, 37.8, 40.7, 42.5, 53.9, 125.9, 126.3, 126.7, 128.1, 129.3, 130.4, 130.7, 137.7, 162.4, 162.6, 166.2, 171.1, 171.3; MS [FAB^-]: calcd. m/z 640.25, obsvd. 640.0 $[M - H]^-$.

Synthesis of NDI-Gly-Phe (3). The peptide/dye conjugate derivative of **3** was prepared through SPPS using 2-chlorotrityl chloride resin, Fmoc-L-phenylalanine, Fmoc-L-glycine and NDIA (Figure 1)**.**[57] The resin (1.2 g) was swelled in anhydrous CH_2Cl_2 for 30 min and then Fmoc-L-phenylalanine (0.775 g, 2.000 mmol) was loaded onto the resin in anhydrous *N*,*N*-dimethylformamide and *N*,*N*-diisopropylethylamine (DIEA; 0.830 mL, 5.000 mmol) for 1 h. For deprotection of the Fmoc group, piperidine (20% in DMF) was added and the sample left for 20 min; this procedure was repeated twice (each time for 2 min). Fmoc- L-glycine (0.595 g, 2.000 mmol) was coupled to the free amino group using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluraniumhexafluorophosphate (HBTU) (0.3793 g, 1.000 mmol) and *N*,*N*-diisopropylethylamine (DIEA) (0.4150 mL, 2.500

mmol) as coupling agents for 30 min. Again, the sample was treated with piperidine (20% in DMF) for 20 min; this procedure was repeated twice (each time for 2 min). Finally, NDIA (0.873 g, 2.000 mmol) was coupled to the free amino group using HBTU (0.7586 g, 2.000 mmol) and DIEA (0.830 mL, 5.000 mmol) as coupling agents. After the reaction mixture had been stirred overnight, the peptide derivative was cleaved through treatment with CF_3CO_2H (90% in DI water) for 3 h. The resulting solution was dried by air and then Et_2O was added to precipitate the target product. The solid was dried under vacuum to remove residual solvent (light-brown solid: 0.277 g). ^{1}H NMR (300 MHz, [D_6]DMSO, 25 °C): δ=0.85-1.00 (m, 3H; CH_3), 1.20-1.50 (m, 10H; CH_2), 1.65-1.80 (m, 2H; CH_2), 2.85-3.05 (m, 1H; CH_2), 3.05-3.20 (m, 1H; CH_2), 3.65-3.95 (m, 2H; CH_2), 4.09 (t, J=7.35 Hz, 2H; CH_2), 4.40-4.55 (m, 1H; CH), 4.77 (s, 2H; CH_2), 7.20-7.40 (m, 5H; CH), 8.22 (d, J=7.8 Hz, 1H; NH), 8.55-8.65 (m, 1H; NH),8.70-8.85 (m, 4H; CH); ^{13}C NMR (75 MHz, [D_6]DMSO, 25 °C): δ=13.9, 22.1, 26.5, 27.3, 28.7, 31.2, 36.8, 41.6, 42.5, 53.5, 125.9, 126.16, 126.19, 126.4, 126.6, 128.2, 129.1, 130.4, 130.6, 137.4, 162.45, 162.54, 166.6, 168.4, 172.7; MS [FAB$^-$]: calcd. m/z 640.25, obsvd. 639.8 $[M - H]^-$.

Synthesis of NDI-Gly-Gly (4). The peptide/dye conjugate derivative of **4** was prepared through SPPS using 2-chlorotrityl chloride resin, Fmoc-L-glycine and NDIA (Figure 1)**.**[57] The resin (1.2 g) was swelled in anhydrous CH_2Cl_2 for 30 min and then

Fmoc- L-glycine (0.595 g, 2.000 mmol) was loaded onto the resin in anhydrous *N*,*N*-dimethylformamide and *N*,*N*-diisopropylethylamine (DIEA; 0.830 mL, 5.000 mmol) for 1 h. For deprotection of the Fmoc group, piperidine (20% in DMF) was added and the sample left for 20 min; this procedure was repeated twice (each time for 2 min). Fmoc- L-glycine (0.595 g, 2.000 mmol) was coupled to the free amino group using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluraniumhexafluorophosphate (HBTU) (0.3793 g, 1.000 mmol) and *N*,*N*-diisopropylethylamine (DIEA) (0.4150 mL, 2.500 mmol) as coupling agents for 30 min. Again, the sample was treated with piperidine (20% in DMF) for 20 min; this procedure was repeated twice (each time for 2 min). Finally, NDIA (0.873 g, 2.000 mmol) was coupled to the free amino group using HBTU (0.7586 g, 2.000 mmol) and DIEA (0.830 mL, 5.000 mmol) as coupling agents. After the reaction mixture had been stirred overnight, the peptide derivative was cleaved through treatment with CF_3CO_2H (90% in DI water) for 3 h. The resulting solution was dried by air and then Et_2O was added to precipitate the target product. The solid was dried under vacuum to remove residual solvent (brown solid: 0.273 g). ^1H NMR (300 MHz, [D_6]DMSO, 25 °C): δ=0.85-0.95 (m, 3H; CH_3), 1.20-1.45 (m, 10H; CH_2), 1.65-1.80 (m, 2H; CH_2), 3.80-3.90 (m, 4H; CH_2), 4.09 (m, J=7.5 Hz, 2H; CH_2), 4.80 (s, 2H; CH_2), 8.20-8.30 (m, 1H; NH), 8.60-8.70 (m, 1H; NH), 8.70-8.80

(m, 4H; CH); ^{13}C NMR (75 MHz, [D_6]DMSO, 25 °C): δ=14.9, 23.0, 27.5, 28.3, 29.5, 29.6, 31.6, 32.2, 41.5, 42.7, 43.6, 126.8, 127.08, 127.14, 127.2, 127.5, 131.4, 131.6, 163.4, 163.5, 167.7, 169.9, 172.1; MS [FAB$^-$]: calcd. m/z 550.21, obsvd. 549.5 [M – H]$^-$.

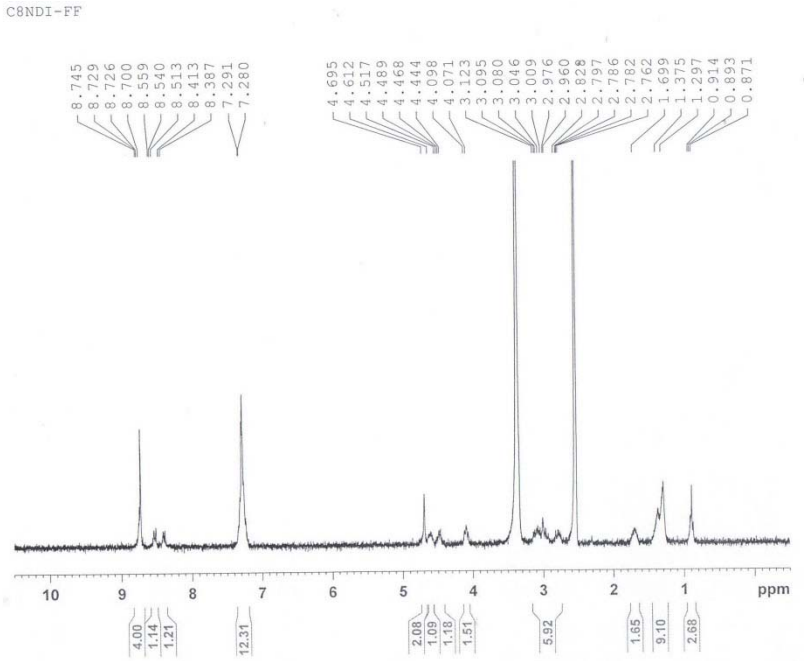

Fig. S1. ^{1}H NMR spectra of **1** in [D_6]DMSO.

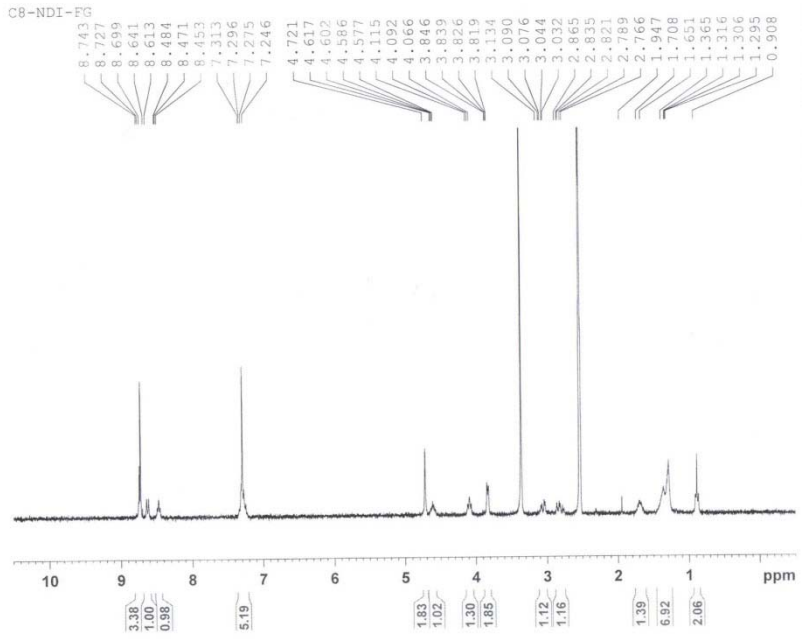

Fig. S2. ^{1}H NMR spectra of **2** in [D_6]DMSO.

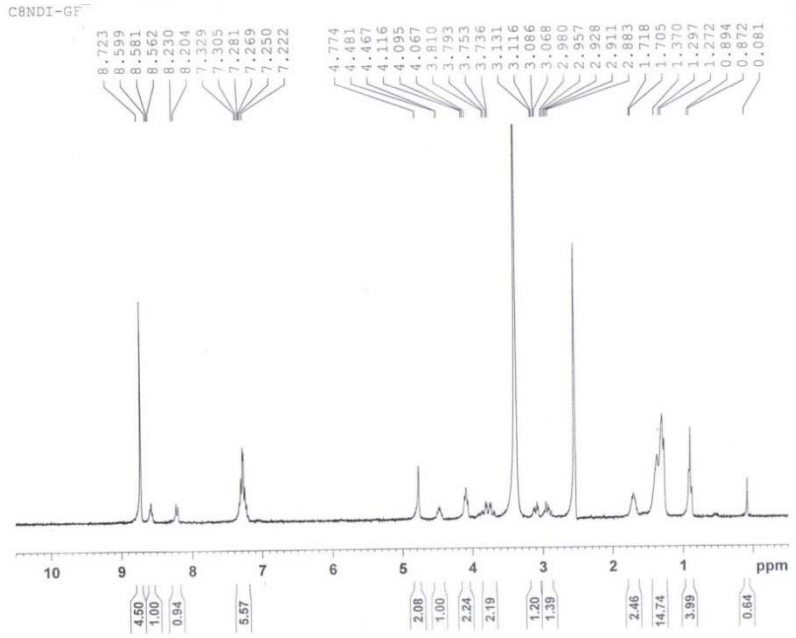

Fig. S3. ^{1}H NMR spectra of **3** in [D_6]DMSO.

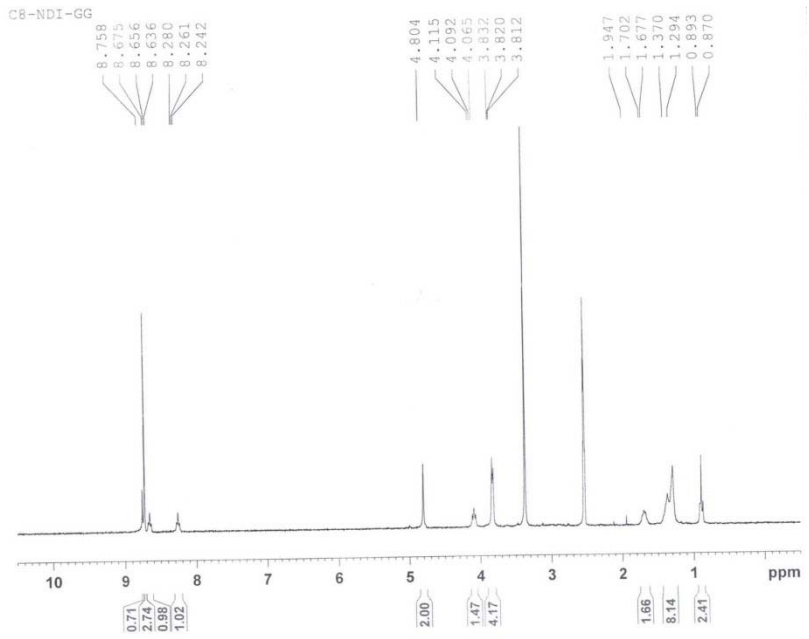

Fig. S4. ^{1}H NMR spectra of **4** in [D_6]DMSO.

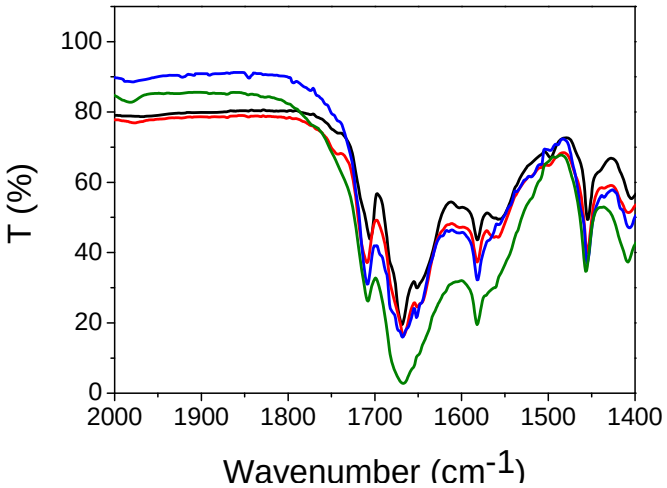

Fig. S5. FT-IR spectra of the hydrogels of **1** (black), **2** (red), **3** (blue) and **4** (green).

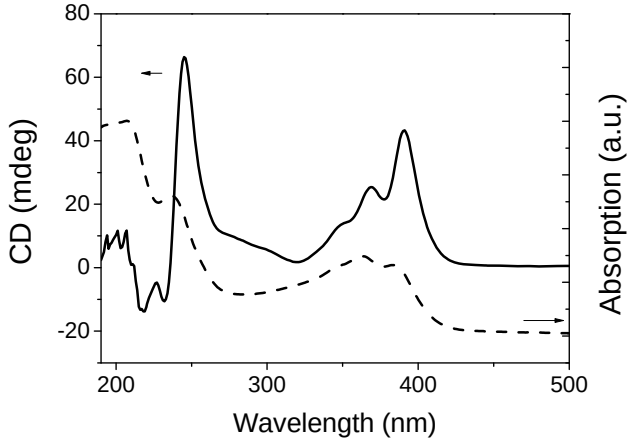

Fig. S6. CD (solid line) and UV-vis absorption (dashed line) spectra of **1** at 0.1 wt% solution (pH 4.8).

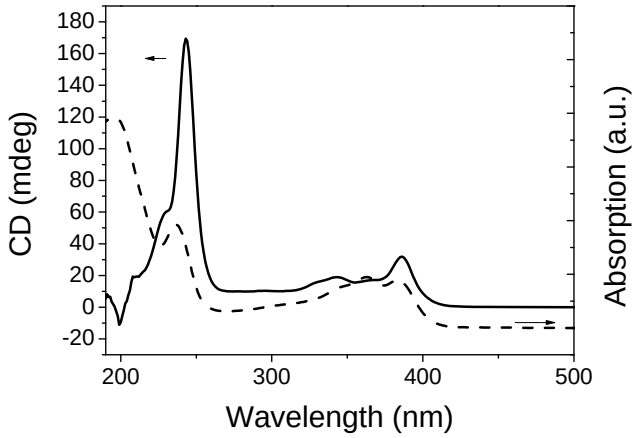

Fig. S7. CD (solid line) and UV-vis absorption (dashed line) spectra of **2** at 0.1 wt% solution (pH 4.7).

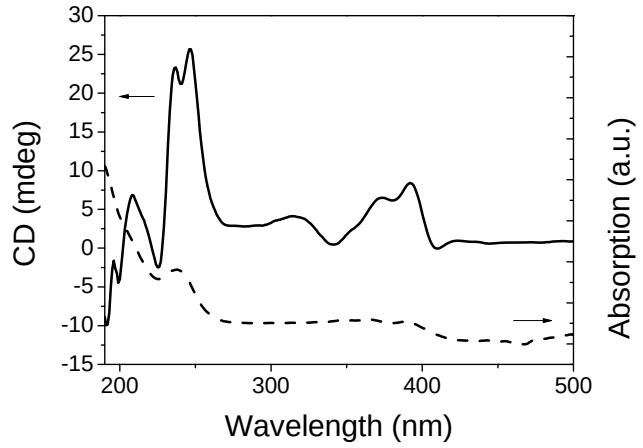

Fig. S8. CD (solid line) and UV-vis absorption (dashed line) spectra of **3** at 0.1 wt% solution (pH 4.3).

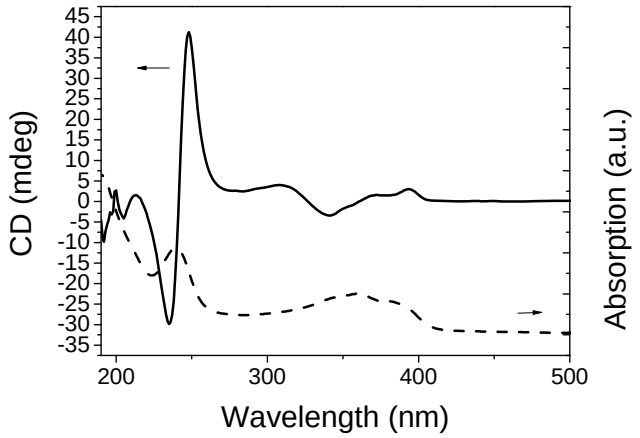

Fig. S9. CD (solid line) and UV-vis absorption (dashed line) spectra of **4** at 0.1 wt% solution (pH 3.8).

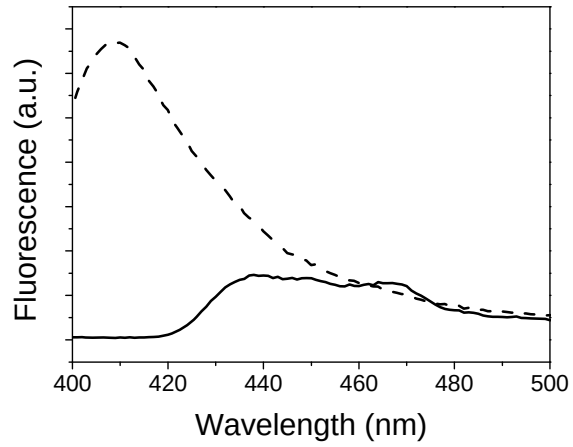

Fig. S10. Fluorescence emission spectra of **1** at 2 wt % gel (solid line) and 0.05 wt% solution (dashed line) (pH 4.8).

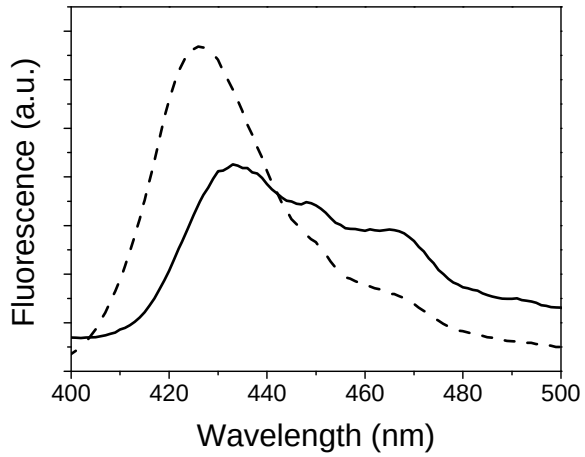

Fig. S11. Fluorescence emission spectra of **3** at 2 wt % gel (solid line) and 0.05 wt% solution (dashed line) (pH 4.3).

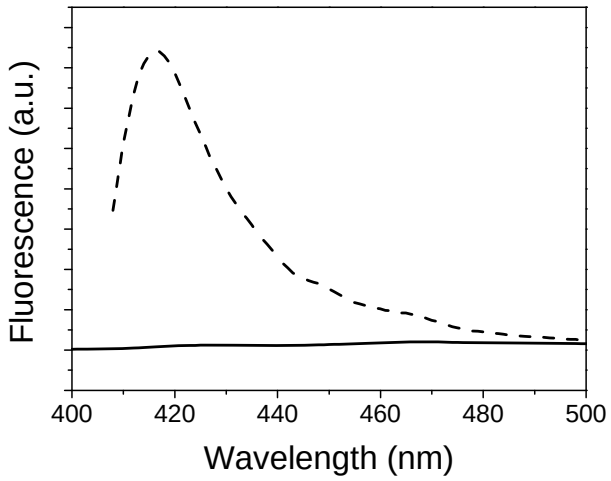

Fig. S12. Fluorescence emission spectra of **4** at 2 wt % gel (solid line) and 0.05 wt% solution (dashed line) (pH 3.8).

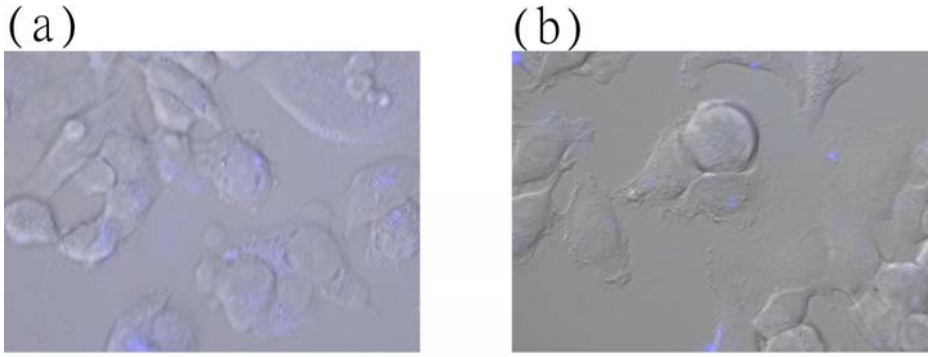

Fig. S13. Fluorescence images of MCF-7 cells incubated with 50 μM of (a) **3**, (b) **4** after 1.5 h.

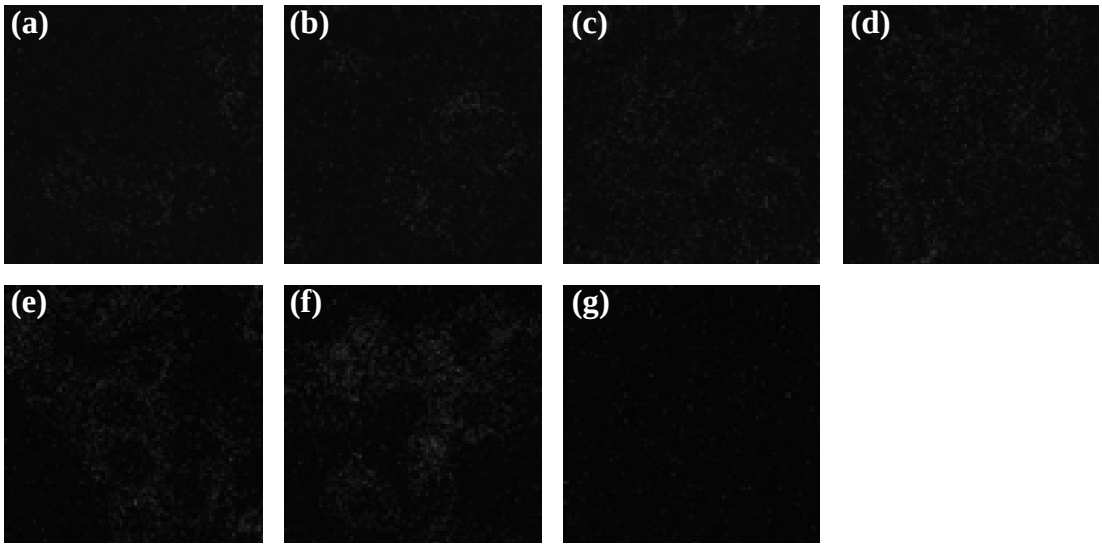

Figure S14. Time dependent fluorescence images of MCF-7 cells incubated with 25 μM of **1**. Time points (a) 1 min, (b) 5 min, (c) 10 min, (d) 30 min, (e) 120 min, (f) 180 min, (g) control.

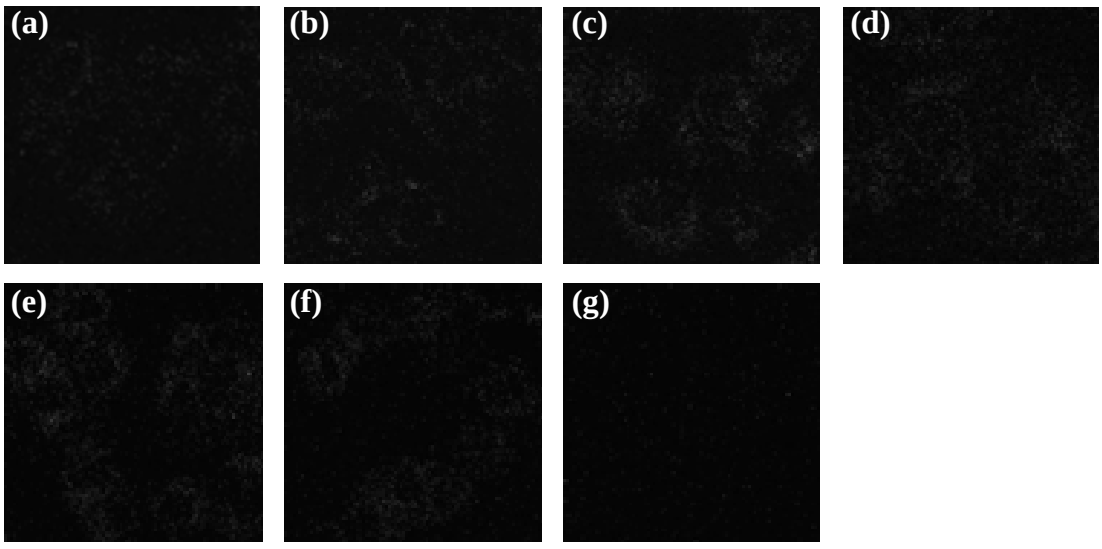

Figure S15. Time dependent fluorescence images of MCF-7 cells incubated with 50 μM of **1**. Time points (a) 1 min, (b) 5 min, (c) 10 min, (d) 30 min, (e) 120 min, (f) 180 min, (g) control.

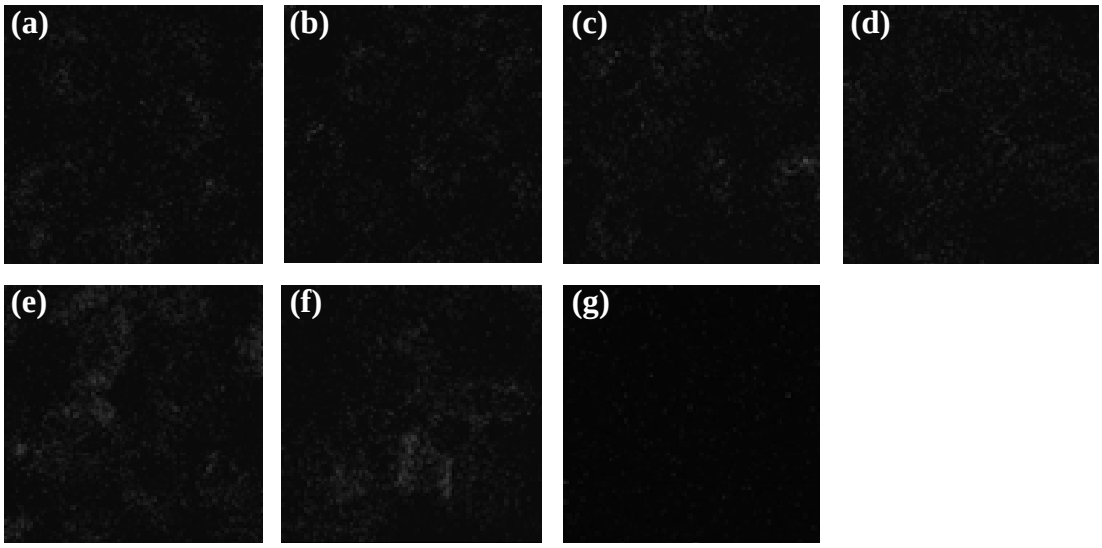

Figure S16. Time dependent fluorescence images of MCF-7 cells incubated with 25 μM of **2**. Time points (a) 1 min, (b) 5 min, (c) 10 min, (d) 30 min, (e) 120 min, (f) 180 min, (g) control.

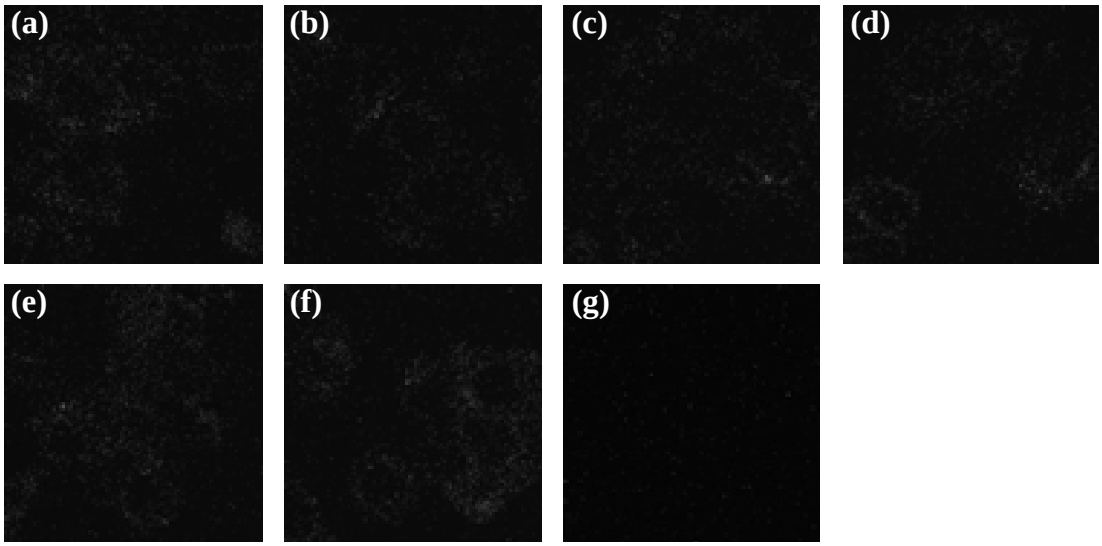

Figure S17. Time dependent fluorescence images of MCF-7 cells incubated with 50 μM of **2**. Time points (a) 1 min, (b) 5 min, (c) 10 min, (d) 30 min, (e) 120 min, (f) 180 min, (g) control.

Table S1. Physical properties of NDI-capped dipeptides under acidic conditions.

Entry	pH	Appearance[a]	G′, G″ (Pa)	Fiber diameter (nm)
1	4.8	OG	$1.8x10^4$, $1.5x10^3$	9±1
2	4.7	SG	$1.3x10^4$, $1.4x10^3$	9±1
3	4.3	OG	$1.1x10^4$, $9.5x10^2$	9±1
4	3.8	OG	$1.7x10^3$, $1.7x10^2$	11±1

[a]*OG: opaque gel; SG: semi-translucent gel. All hydrogels were prepared at 2 wt%.*