Probing Nanoscale Self-Assembly of Non-Fluorescent Small Molecules inside Live Mammalian Cells

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Supporting Information

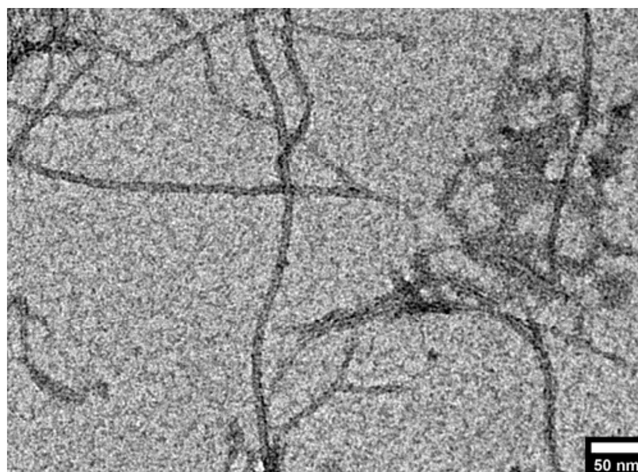

Figure S1. Typical TEM image of 235 μM of **1a** treated with 6 U/mL of ALP. The width of the fibers in this low concentration of **1b** is 11.0 ± 1.6 nm which is close to that of nanofibers from other gel samples.

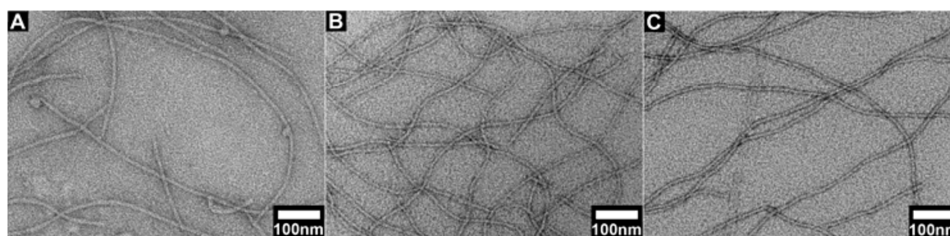

Figure S2. Typical TEM images of hydrogel (A) 0.6 wt% **2b**, (B) 0.6 wt% **1b** and (C) 2 μM of **2b** in 5 mM of **1b** (TEM images are negatively stained by 2% UA in aqueous solution). The fibers are long and thin with a uniform diameter of 11 ± 2 nm which was calculated by image-J. The morphology of the fibers is almost identical, indicating that **2b** and **1b** can co-self assemble into nanofibers.

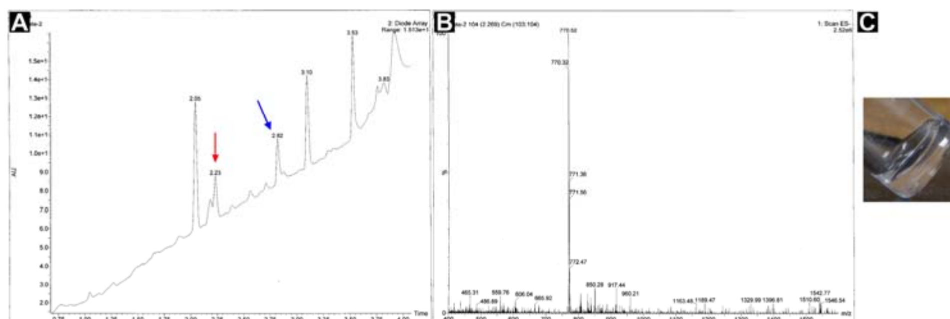

Figure S3. LC-MS result of cell lysate. After taken confocal images, the cells were lysed immediately by replacing culture media with DI water and freeze–drying the sample in vacuum. The freeze dried powder was re-dissolved in water/acetonitrile (1:1 v/v) and filtered before injection into LC-MS. (A) After mapping **1a** and **1b** by mass ESI$^-$, we found that the retention time of **1a** and **1b** was around 2.23 min in the LC spectrum (red arrow). (B) The ESI$^-$ mass spectrum of the peak marked 2.23 in (A) showed most of them are **1b** anion (MW = 770.32 Da) with a small amount of **1a** anion (MW = 850.28 Da) (<10% by intensity). We also found the existence of at least one degradation product from **1b** which was embedded in the peak indicated by a blue arrow which had a molecule weight of 480.20 Da. (C) The optical image of **1b** hydrogel which formed at 4 ^{o}C (cold room) after mixing the ALP enzyme (10 U/mL) and **1a** solution (2 mg/mL or 2.35 mM in PBS buffer) for 2 hours. Both the ALP and **1a** were stored in cold room for the temperature 4°C before mixing.

Tabel 1. The determination of the concentration of gelator inside HeLa cells by LC-MS.

	Cell lysate			
	37 °C, 48h Cell No=2*10^5;	37 °C, 20h Cell No=1*10^6;	4 °C, 20h Cell No=1*10^6;	
Sample Volume / μL	200	300	300	
Injection Volume / μL	40	20	20	
Integration Area / μV*sec	3397876 [a] (**1b**)	2759608 [a] (**1b**)	21321266 (**1b**)	32191816 (**1a**)
Calculated conc. inside cells [b, c]	0.26-0.94 mg/mL	0.13-0.46 mg/mL	0.99-3.55 mg/mL	1.49-5.37 mg/mL

[a] Another smaller peak indicated the digestion of **1b** can also be found in LC trace.

[b] The volume of a single HeLa cell ranges 760-2730 μm^3.

[C] Method to calculate the concentration:

We inject 0.1 μL of the standard sample **1b** at the concentration of 0.905 mg/mL and the corresponding integration area is 10746884 μV*sec. That means the *absorbance intensity* of **1b** is 1.19 * 108 μV*sec/μg.

For each cell lysate sample, the *total integration area* = integration area * (sample V / injection V) and the *calculated concentration* = (*total integration area* / *absorbance intensity*) / (cell number * single cell volume).

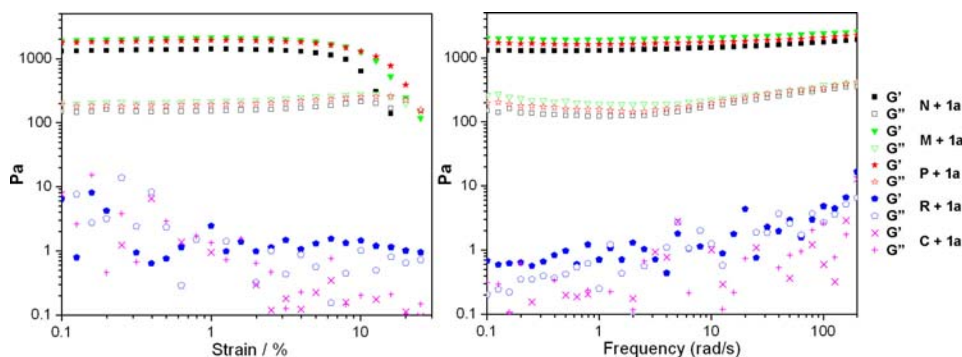

Figure S4. Rheological test of the mixture of each cell fraction with **1a** (final conc. =6mg/mL) 48 hrs after mixing. For the three self-supported hydrogel **N** + **1a**, **M** + **1a** and **P** + **1a**, the value of G'/G" was around 10 which is the indication of a stable hydrogel. The curve of **R** + **1a** flattened at higher strain percentage or lower frequency range, indicating it was a weak gel. G' and G" of **C** + **1a** showed both strain and frequency dependent which suggested it behaved as a solution.

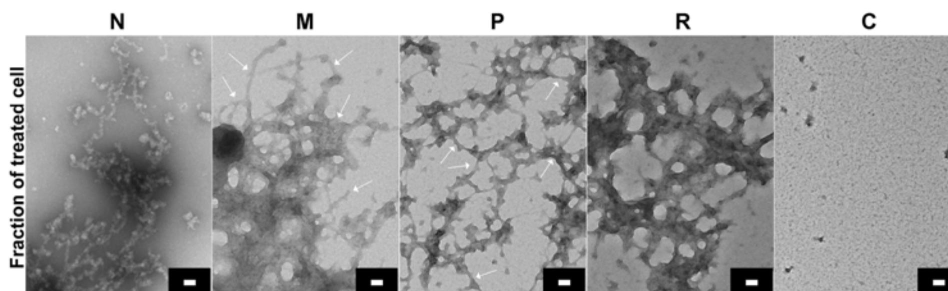

Figure S5. TEM images show the morphology of each fraction of HeLa cells which were pre-incubated with **1a** (500 μM or 0.42 mg/mL) for 2 days. The typical nanofibers structure (indicated by white arrows, around 10±2 nm in width) present in the fractions **M** and **P**. (Scale bars: 50 nm)

Figure S6. (A) Z-slices of a confocal image stack showing HeLa cells incubated with **2a** at 200 nM. Physical dimensions of each frame: 111 μm x 111 μm. (B) Z-slices of a confocal image stack showing HeLa cells incubated with both **2a** at 200 nM and **1a** at 500 μM. Physical dimensions of each frame: 111 μm x 111 μm.

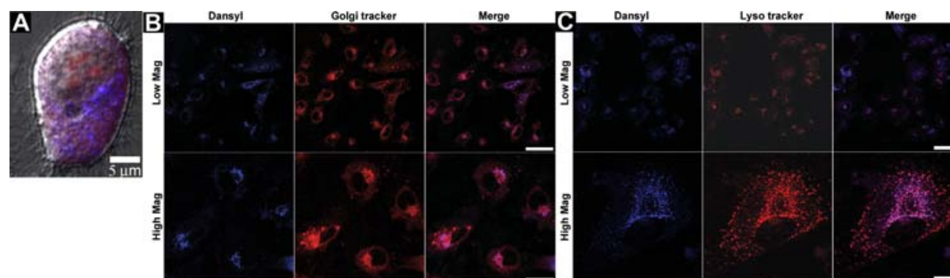

Figure S7. (A) Confocal image of a HeLa cell incubated under the same condition as in Fig. 3B, *i.e.* with fluorescent (dansyl: blue) **1a**/**2a** (500 μM, 200 nM), and stained with SYTO® 85 fluorescent nucleic acid stain (red). (B) Co-staining with **1a**/**2a**-Dansyl (blue) and Golgi tracker (red). The concentrations of **1a** and **2a** in culture medium are the same as shown in Fig 3D. Scale bars: 50 μm (top row); 20 μm (bottom row). (C) Co-staining with **1a**/**2a**-dansyl (blue) and Lysotracker (red). The concentrations of **1a** and **2a** in culture medium are the same as shown in Fig 3D. Scale bars: 50 μm (top row); 20 μm (bottom row).

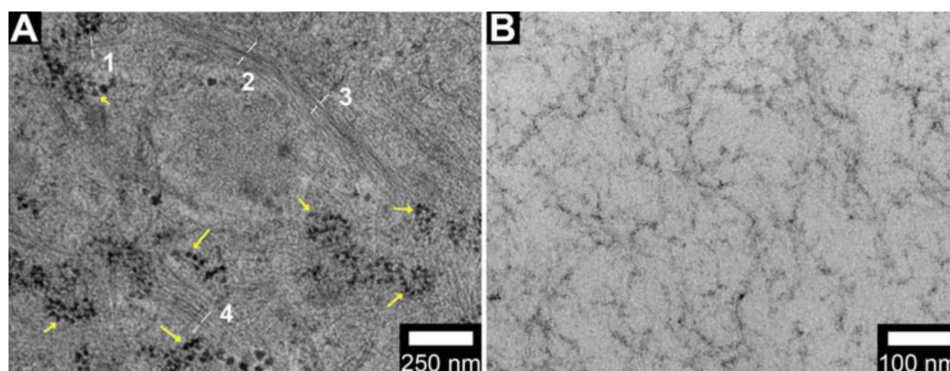

Figure S8. (A) TEM image of microtome section of a HeLa cell treated with 500 μM of **1a** reveals the clusters of nanofibers structure. (Yellow arrows: ribosome; The fiber width measured at randomly picked point “1”, “2”, “3”,and “4”) The diameters of these nanofibers are 12±2 nm, agreeing with the width of the nanofibers in the hydrogels of **1b** *in vitro*. (B) A TEM image showing the typical morphology in the sample of the hydrogel of **1b** (0.6 wt%) mixing with 3.0 wt% agarose solution at the ratio of 1:1 (v/v). The procedure for the TEM sample preparation is the same as CLEM.

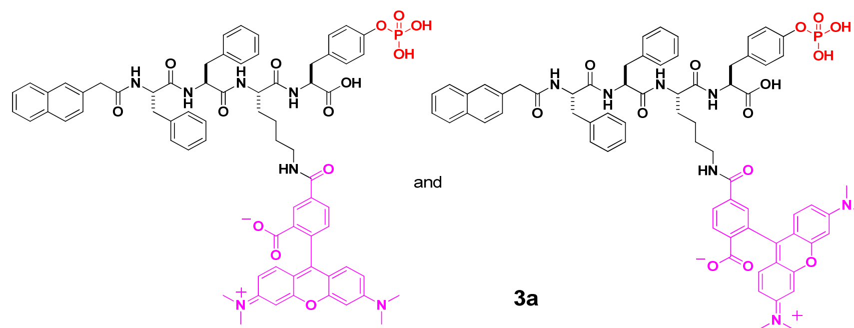

Figure S9. The molecular structure of **3a**. The commercial NHS-Rhodamine (Product No. 46406 from Thermo Scientific) is a mixture of 5-(and 6)-carboxytetramethylrhodamine, succinimidyl ester, which was used without the separation.

Figure S10. The optical image of a solution of **3a** (0.6 wt%, pH 7.4) which fails to form a hydrogel after dephosphorylation by ALP (20 U/mL).

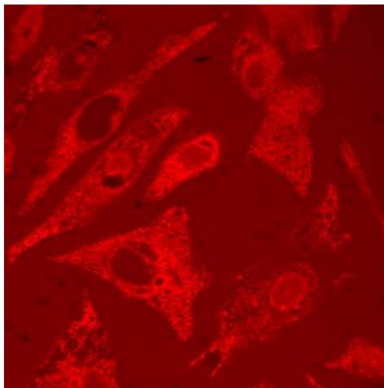

Figure S11. A typical fluorescent image shows that a group of HeLa cells incubated with 500 μM of **3a** are homogeneously shown in red and there is no molecular agglomeration in ER region. (Dimension: 125 μm × 125 μm).