Electronic Supplementary Material (ESI) for RSC Advances

Interactions between cellular proteins and morphologically different nanoscale aggregates of small molecules

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Supplementary Information

This file contains:

S1. Preparation methods of the nanoscale aggregates of small molecules 2

S2. Procedure of hydrogel based protein pull-down assay 3

S3. Optical images of the nanoscale aggregates of small molecules 4

S4. Extended characterization of **2**-f-A and **2**-f-B 5

S1. Preparation methods of the nanoscale aggregates of small molecules

*Preparation of **1**-f*: 0.48 mg of compound **1** was added into 100 µL PBS buffer, then 2 µL of 1 N NaOH was added. The solution was heated to 60 °C for 3 min to completely dissolve the compound. 30 µL of the hot solution was quickly aliquotted into 1.5 mL eppendorf tube, and the tube was placed upright at RT. Stable hydrogel forms after 1 h.

*Preparation of **1**-p*: 0.14 mg of compound **1** was added into 30 µL PBS buffer in 1.5 mL eppendorf tube, then 2 µL of 1 N NaOH was added to dissolve the compound. 2 µL of 1 N HCl was added under stirring in to precipitate **1**-p and centrifuged to separate **1**-p from solution.

*Preparation of **2**-f-A*: 1.3 mg of compound **2** was added into 100 µL PBS buffer. The solution was heated to 60 °C for 5 min to completely dissolve the compound. 30 µL the hot solution was quickly aliquotted into 1.5 mL eppendorf tube, and the tube was placed upright at RT. Stable hydrogel forms overnight.

*Preparation of **2**-f-B*: 1.3 mg of compound **2** was added into 80 µL PBS buffer. 2 µL of 1 N NaOH was added to dissolve the compound. Then 20 µL of 1 N HCl was added under slow stirring. 30 µL the solution was quickly aliquotted into 1.5 mL eppendorf tube, and the tube was placed upright at RT. Stable hydrogel forms overnight.

S2. Procedure of hydrogel based protein pull-down assay

4 x10^7 of HeLa cells were scrapped from petri dish and washed with PBS buffer for 3 times then centrifuged at 300 g for 5 minutes. The collected cell pellet was mixed with phosphate buffer (100 mM) then snap freeze and thaw for 3 cycles to lyse the cells. The cell lysate was clarified by centrifugation at 12,000 g for 20 min at 4 ºC to remove the whole cells, nuclei and large mitochondria. 50 µL of the supernatant lysate was gently mixed with 30 µL hydrogel of **1** (10 mM in PBS buffer, pH 7.6) on rotator at RT for 30min. The hydrogel was collected by centrifugation (12,000 g, 5 min, 4 ºC) and the supernatant was collected and placed on ice before analysis. The hydrogel was washed three times by gently mixing with 50 µL of the washing buffer (50 mM phosphate buffer pH 7.6 supplemented with 150 mM NaCl) on rotator at RT for 10 min followed by separation on centrifuge (12,000 g, 5 min, 4 ºC). Supernatants were collected and placed on ice before analysis. Finally, the remaining hydrogel was dissolved using 1:1 washing buffer and 5X Laemeli buffer. All other samples were mixed with 5X Laemeli buffer (final concentration 2X) before SDS-PAGE.

S3. Optical images of the nanoscale aggregates of small molecules

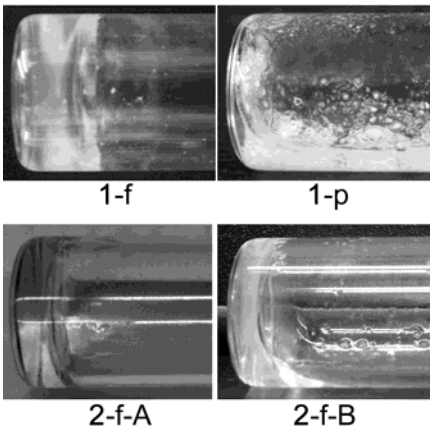

Figure S1. Optical images of **1**-f, **1**-p, **2**-f-A, and **2**-f-B.

S4. Extended characterization of 2-f-A and 2-f-B

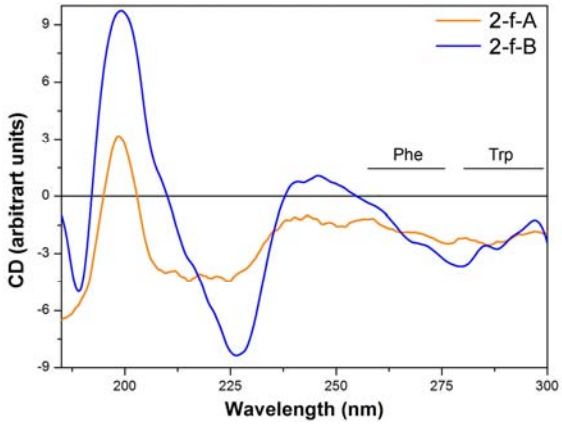

Figure S2. Far to near UV circular dichroism spectra of **2**-f-A and **2**-f-B. The wavelength ranges corresponding to signals from Phe and Trp side chains are marked accordingly.