

# Using supramolecular hydrogel to discover the interactions between proteins and molecular nanofibers of small molecules

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## Supporting Information

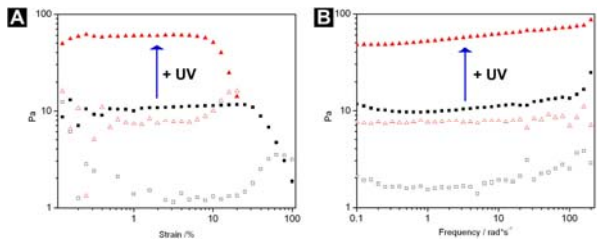
### A) Supplementary methods:

Hydrogel and protein mixture incubation and washing conditions are described as below:

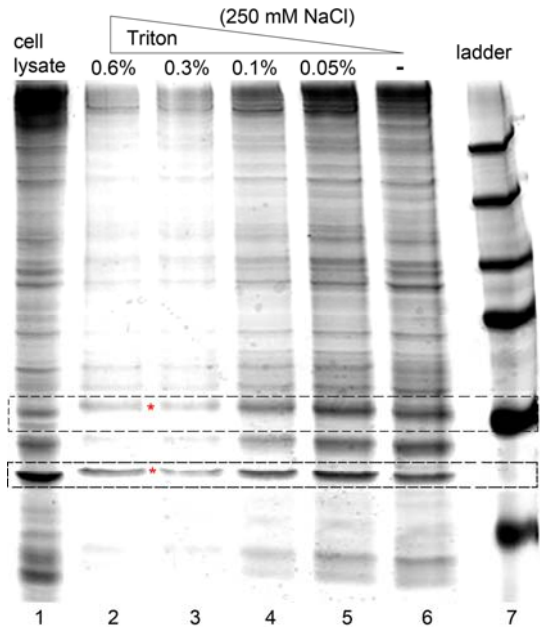
- 1) Preparation of hydrogel **1**: After dissolving the precursor in water at the concentration of 0.6 wt% (pH 7.4, adjusted by 1M NaOH), we add 10 U/mL of alkaline phosphatase (ALP) to the precursor solution and obtain a transparent hydrogel of **1** after two hours of dephosphorylation.
- 2) Hydrogel (0.3 mL) was prepared in an eppendorf. Cos-1 cells were grown to confluence, trypsinized, pelleted (720 g), and washed two times with Dulbecco's PBS. The cell pellet was resuspended with phosphate buffer (100 mM, pH 7.6, 150 mM NaCl) then snapped frozen and thawed for 3 cycles to lyse the cells. The cell lysate was clarified by centrifugation at 12,000 g for 20 min at 4 °C and made to a concentration of 1.1 mg/mL. 50 µL of the supernatant lysate was gently mixed with the hydrogel on a rotator at RT for 30min. Five pieces of the hydrogels were firstly incubated with cell lysate for 30 min and separately washed at different conditions as indicated on the top of the electrophoresis gel to remove non-specific binding proteins. The hydrogel was collected by centrifugation (12,000 g, 5 min, RT) and the supernatant was collected and placed on ice before analysis. The hydrogel was applied to UV irradiation (365nm, 5 min) on ice before washed three times by gently mixing with 50 µL washing buffer (as described in text) on rotator at RT for 10min followed by separation on centrifuge (12,000 g, 5 min, RT). 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.

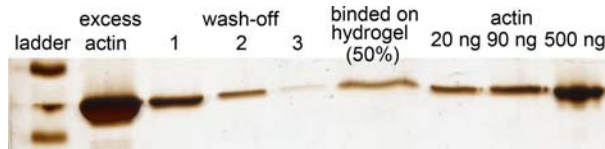
**B) Supplementary figures and schemes:**



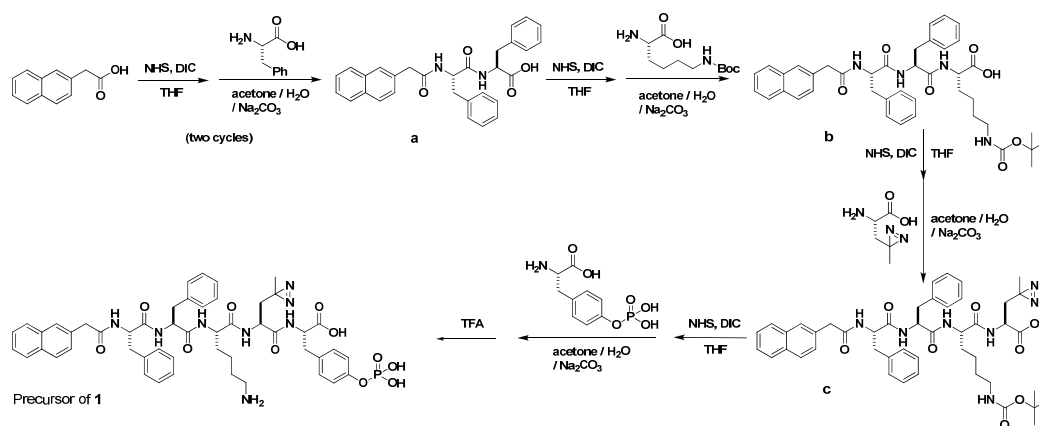
**Figure S1.** The rheological test of the two hydrogels as shown in Fig. 1A by (A) straining sweep and (B) frequency sweep. After UV irradiation, the hydrogel shows a less critical strain which decreases from 28.3% to 8.0% but gets a 6 times higher storage moduli.



**Figure S2.** The full scope of the SDS-PAGE gel showing the silver staining result of the cell lysate pull down assay.

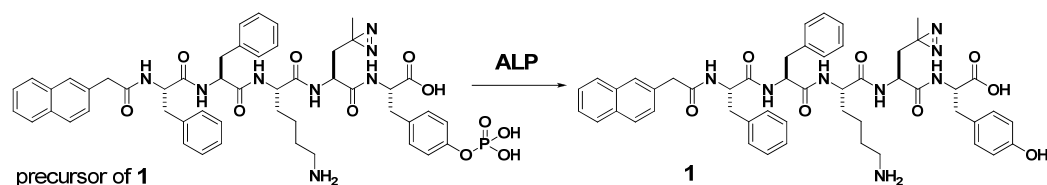


**Figure S3.** The calibration of the loading capacity of actin on hydrogel **1**. We start with 0.3 mL of hydrogel **1** being incubated with excess actin. After thoroughly washing (3 times), half volume of the hydrogel protein mixture is applied to electrophoresis. According to the bands on the right side, we calculate that the loading capacity of actin on hydrogel **1** is 1.13  $\mu\text{g/mL}$ .



**Scheme S1.** Synthetic route of the precursor of **1**.

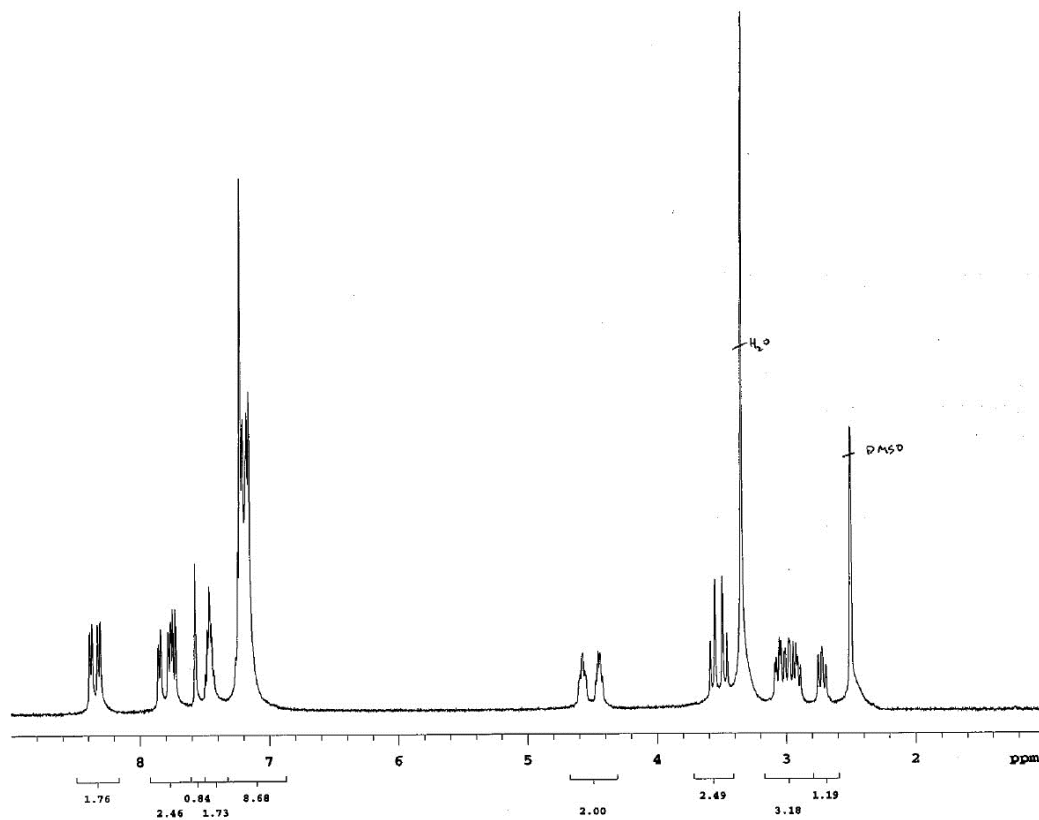
ESI+ mass results: precursor of **1**, cal.  $M^+$ : 976.39, found: 977.43  $[M+H]^+$ , 949.42  $[M+H-N_2]^+$ .



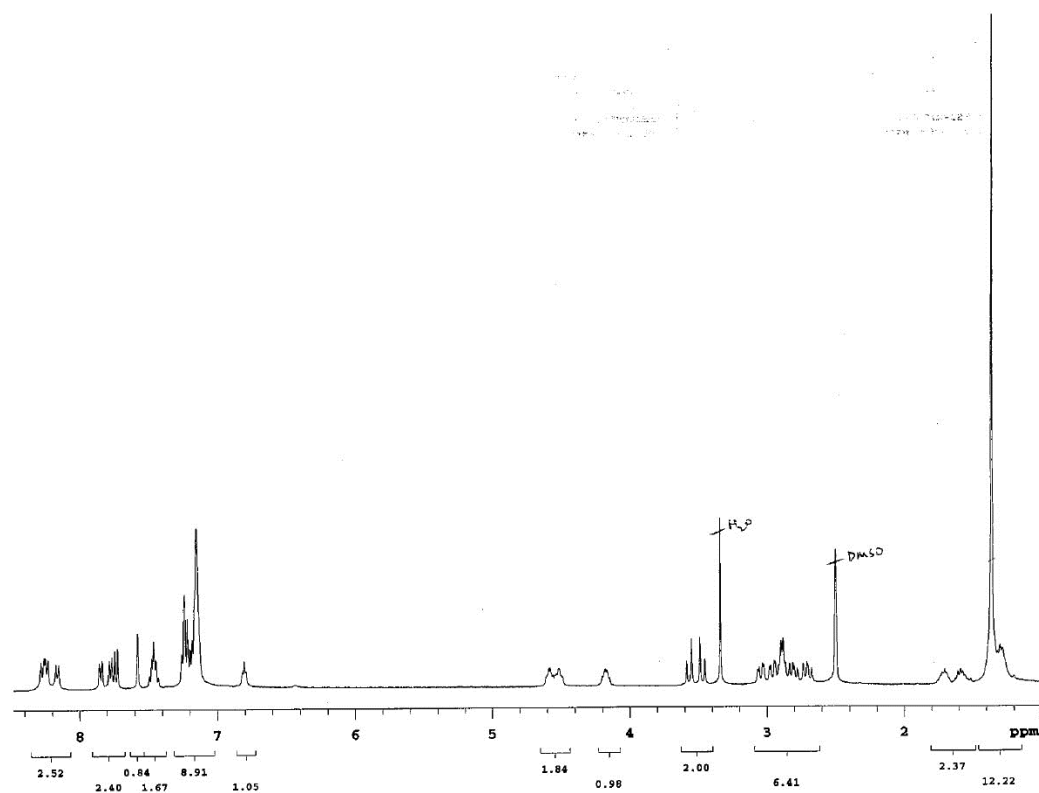
**Scheme S2.** The generation of **1** via the dephosphorylation catalyzed by ALP.

$^1\text{H}$  NMR of **a**, **b**, and **c**:

**a:**



**b:**



C:

