Supporting Information for

Construction of nano receptors for ubiquitin and ubiquitinated proteins based on the region-specific interactions between ubiquitin and polydopamine

Zezhou Li[†a], Xinyi Li[†a], Wei Xian[†b], Huaisyuan Xie[a], Ying Sun[a], Yuxuan Zhang[a], Jiayu Wang[a], Hongwei Li[a], Changwen Jin[a], Xiaoyun Liu*[b], Zhiwei Zhu*[a], Meiping Zhao*[a]

[a] Beijing National Laboratory for Molecular Sciences, MOE Key Laboratory of Bioorganic Chemistry and Molecular Engineering, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China.
[b] Department of Microbiology and Infectious Disease Center, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China.
* Corresponding authors. Email: mpzhao@pku.edu.cn; zwzhu@pku.edu.cn; xiaoyun.liu@bjmu.edu.cn

Experimental Section

Materials: Tris(hydroxymethyl)aminomethane (Tris), human serum albumin (HSA), Tween 20, and Triton X-100 were purchased from Sigma Chemical Co. (St. Louis. MO). Ethanol (EtOH), NaCl, HAc, sodium dodecyl sulfonate (SDS) and isopropanol (*i*PrOH) were purchased from J&K Chemicals Ltd. (Shanghai, China).

Morphology characterization by SEM and XPS

The morphology of the MIP NPs was characterized by scanning electron microscopy-energy dispersive spectrometer (SEM-EDS) and X-ray photoelectron spectroscopy (XPS). The MIP NPs were washed with deionized water for three times and then dispersed in ethanol. After ultrasonic dispersion for 10 min, a small amount of the resultant solution was dropped on the silicon wafer covered with carbon film. SEM-EDS analysis was carried out after drying with an infrared lamp at a voltage of 6-12 kV for 2 h. The elemental composition of the MIP NPs was characterized using XPS. The radiation voltage of Al Kα ray is 1487 eV. The data were analyzed with CASA XPS software, and all XPS

spectra were baselined with the C 1s peak at 284.8 eV. The atomic proportions were calculated with the XPS sensitivity factor of the element.

Zeta potential measurement

Zeta potential measurements were performed on a zeta PALS (Brookhaven Instruments, U.S.A.). First, 0.5 mg/mL NPs were dispersed in 10 mM PBS by ultrasonication. Then, the samples were loaded into a quartz cuvette for instrument measurement.

Cell culture and preparation of cell extracts

The 293T cell line was cultured in Dulbecco's modified Eagle medium (DMEM; HyClone, USA) supplemented with 10% fetal bovine serum (FBS; PAN-Biotech, Germany) under 5% CO_2 conditions at 37 °C. Cells were washed three times with PBS and then lysed by using 20 mM Tris-HCl buffer (pH 7.6) containing 150 mM NaCl and 0.1% Triton X-100. Cell lysates were then centrifuged at 600 × g for 5 min to remove nuclei and cell debris.

Recovery test of ubiquitin from spiked cell lysates

To 1 mL of the 293T cell lysates after removal of the nuclei and cell debris, 20 nmol of ubiquitin was added. The cell lysates containing ubiquitin were incubated with 10 mg of MIP NPs at 4 °C for 6 h. The bound ubiquitin was eluted from the MIP NPs by 0.5% Triton X-100. The quantification of collected ubiquitin was performed by isotope labeled LC-MS/MS.

Supplemental results

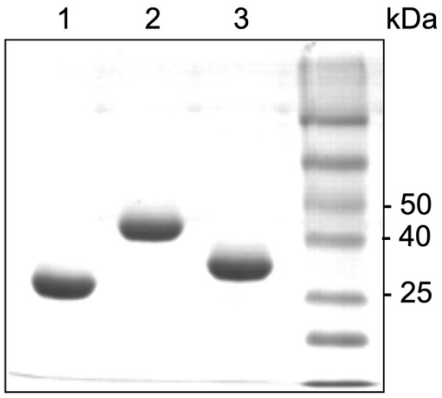

Figure S1. SDS-PAGE analysis of the GST fusion UBDs. Lane 1, UBA; Lane 2, ZnF-UBP; Lane 3, A20-ZnF.

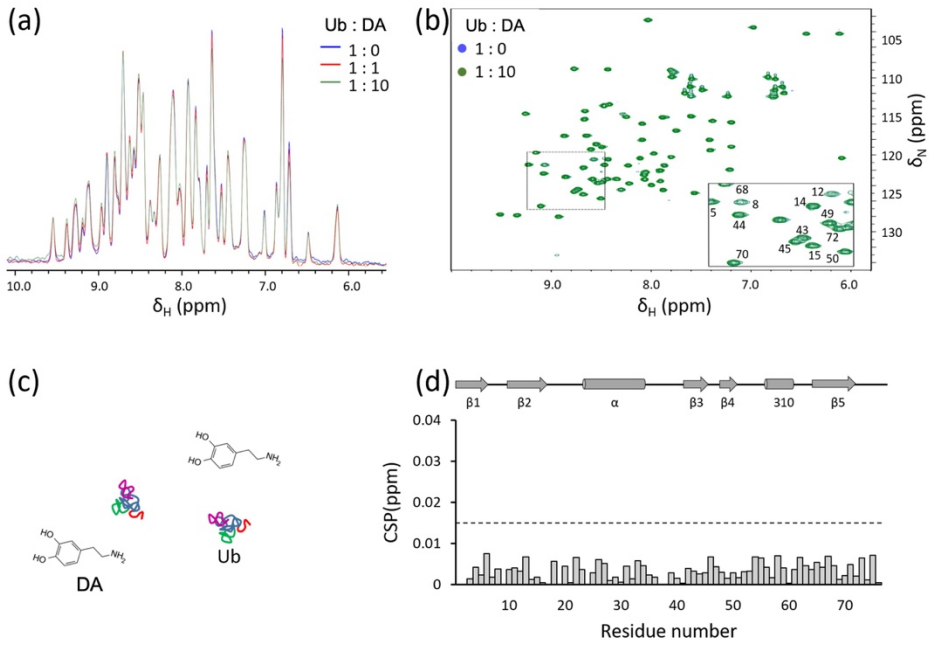

Figure S2. (a) ^{1}H-NMR spectra of ubiquitin during the titration with dopamine. (b) ^{1}H-^{15}N HSQC spectra of ubiquitin with or without the addition of dopamine at a molar ratio of Ub:DA=1:10. (c) Schematic diagram of the negligible interactions between dopamine and ubiquitin. (d) Chemical shift perturbation of ^{1}H-^{15}N HSQC spectra of ubiquitin residues with the addition of dopamine at a molar ratio of Ub : DA=1:10.

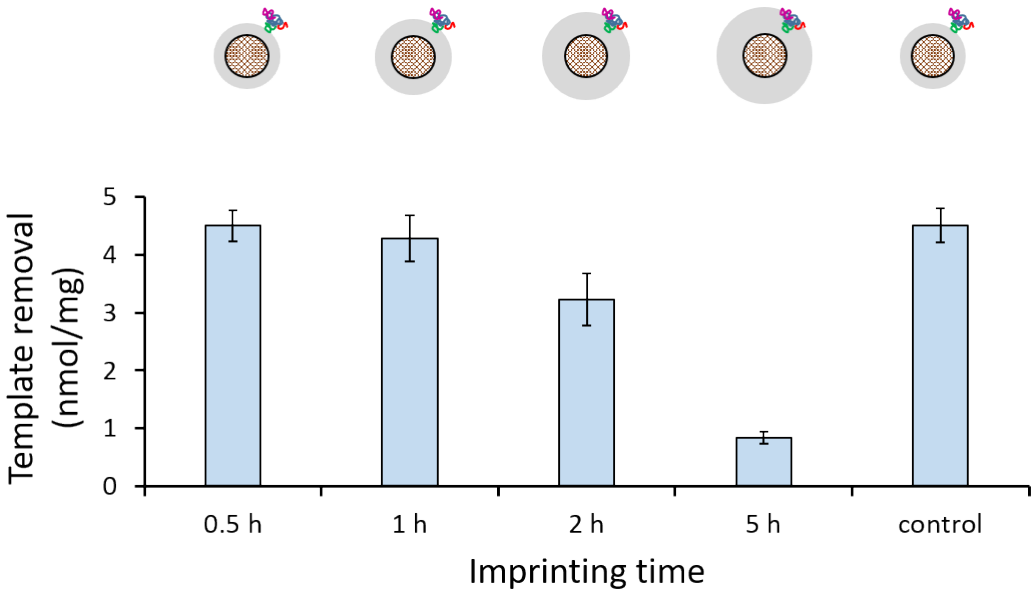

Figure S3. The amounts of ubiquitin templates that could be removed from the MIP NPs by trypsin digestion after imprinting with dopamine for different polymerization time.

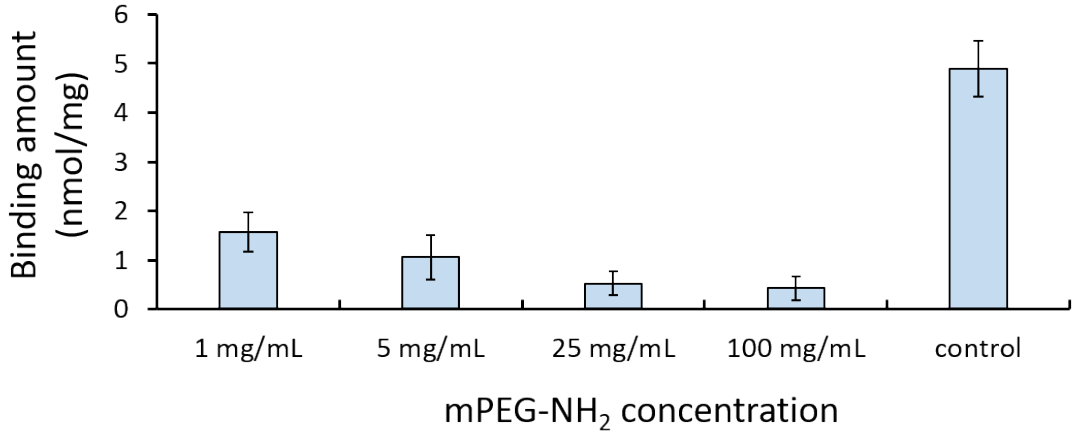

Figure S4. The amounts of ubiquitin that could be adsorbed by polydopamine (PDA) before and after overnight treatment with mPEG-NH_2 at different concentrations.

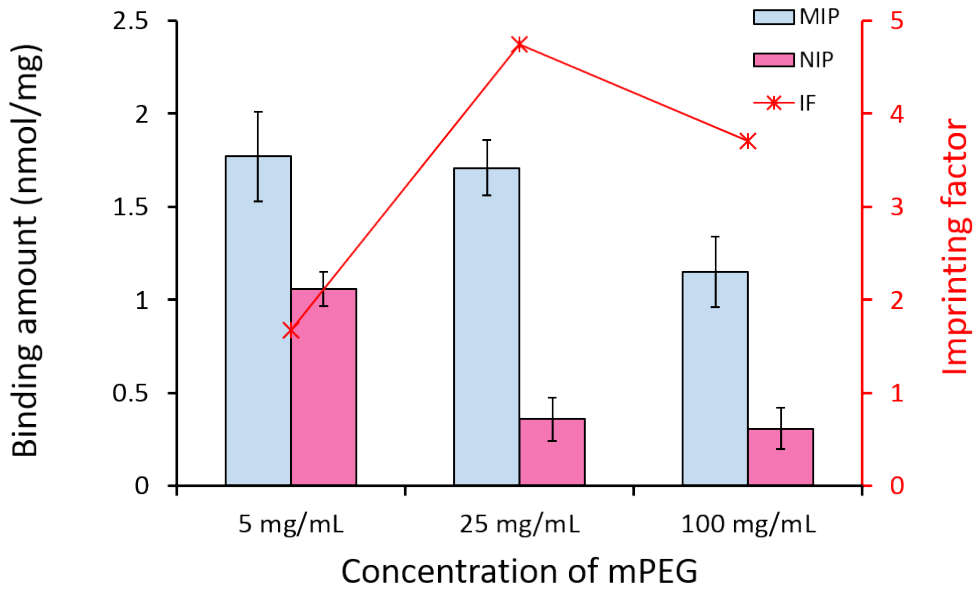

Figure S5. Comparison of the binding amounts of ubiquitin on MIP and NIP NPs modified with different concentrations of mPEG-NH_2. The dopamine imprinting time was fixed at 2 h.

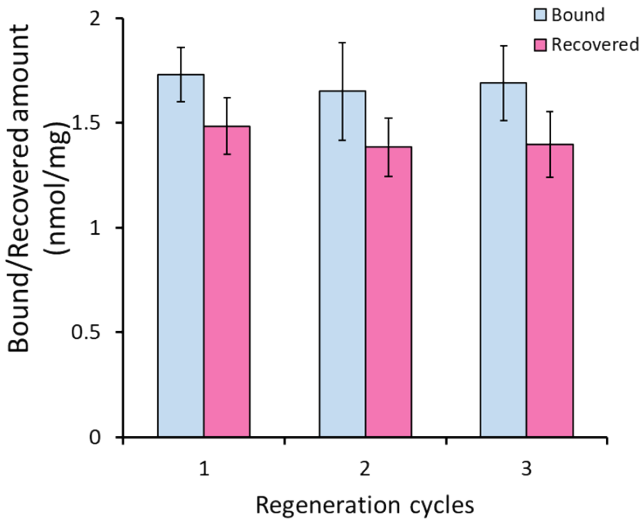

Figure S6. The reusability test results of the MIP NPs.

Table S1. Thermodynamic parameters of different NPs determined by ITC

NPs	K_a ($\times 10^6$ M^{-1})	K_d (μM)	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	ΔS (cal K^{-1} mol^{-1})
MIP	2.6 ± 0.4	0.38 ± 0.04	-9.7	-13.6 ± 0.2	-16.2
NIP	0.022 ± 0.014	46 ± 20	-6.1	-0.41 ± 0.37	19.1

Table S2. Atomic percentages determined by XPS analysis.

At (%)	Fe 2p	O 1s	N 1s	C 1s	Si 2p
Fe_3O_4@SiO_2-COOH	3.4	58.2	0.6	29.1	8.7
MIP	0.3	28.9	6.6	61.6	2.6

Reference

1. Pickart, C. M.; Raasi, S., Controlled synthesis of polyubiquitin chains. *Methods Enzymol.* **2005**, *399*, 21–36.