# Supporting Information

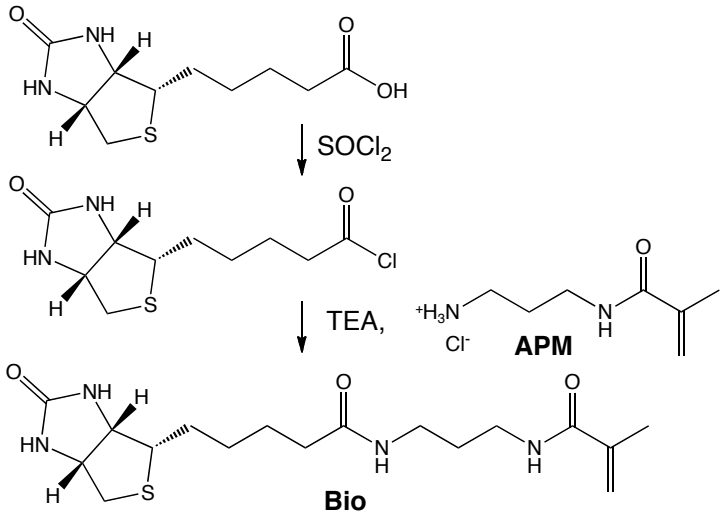
## An ELISA-mimic screen for synthetic polymer nanoparticles with high affinity to target proteins

Yusuke Yonamine[†], Yu Hoshino[‡] and Kenneth J. Shea[*†]

[†]Department of Chemistry, University of California Irvine, Irvine, CA 92697 USA
[‡]Department of Chemical Engineering, Kyushu University, 744 Motooka, Fukuoka 819-0395, Japan

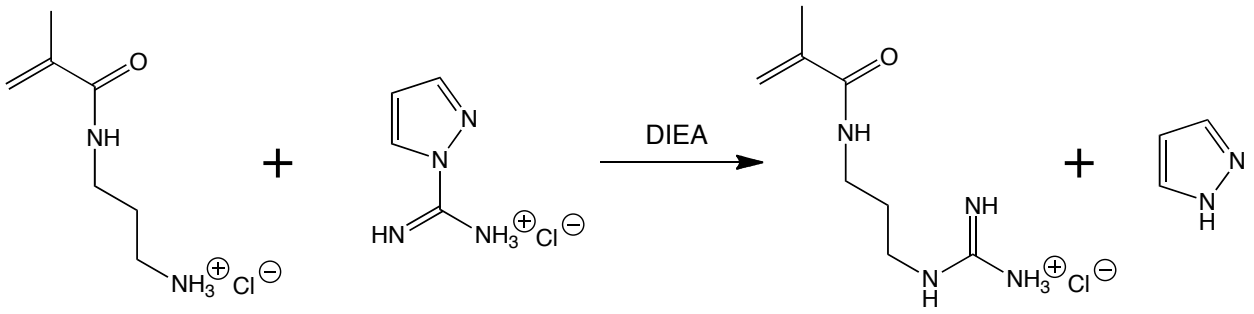
Contents:

**Synthesis of *N*-(3-methacrylamidopropyl)-D-biotinamide (Bio)**




D-biotin (900 mg, 3.68 mmol) was dissoleved in thionyl chloride (15 mL) and the solution was stirred at room temperature for 1 h. The solvent was removed at reduced pressure. The residue was dried and dissolved in dried $CH_2Cl_2$ (25 mL), and the solution was poured into a mixture of *N*-(3-aminopropyl) methacrylamide hydrochloride (APM) (855 mg, 4.78 mmol) and triethylamine (TEA) (1.49 g, 14.7 mmol) in dried $CH_2Cl_2$ (60 mL) at room temperature. The solution was stirred for 2 h and the solvent was removed at reduced pressure. The residue was purified by column chromatography over silica gel eluting with $CH_2Cl_2$/MeOH (9:1, v/v) to give the desired product as a slightly yellow solid (1.05 g, 77% yield). The product was dissolved in DMSO (25 mg/mL) and stored in a freezer. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 7.89 (s, 1H), 7.80 (s, 1H), 6.39 (s, 1H), 6.33 (s, 1H), 5.62 (s, 1H), 5.28 (s, 1H), 4.27 (m, 1H), 4.10 (m, 1H), 3.14-3.02 (m, 5H), 2.80 (m, 1H), 2.54 (m, 1H), 2.03 (t, $J$ = 7.1 Hz, 2H), 1.82 (s, 3H), 1.58-1.44 (m, 4H), 1.29-1.15 (m, 4H); $^{13}$C-NMR (125 MHz, DMSO-$d_6$) δ 172.48, 167.77, 163.11, 140.42, 119.24, 61.44, 59.61, 55.79,45.81, 36.93, 36.55, 35.66, 29.64, 28.60, 28.42, 25.70, 19.03, 8.86; HRMS (ES/MeOH) *m/z* calcd for $C_{17}H_{28}N_4O_3S_1$ $(M+Na)^+$ 391.1774, found 391.1780.

**Synthesis of *N*-(3-methacrylamidopropyl) guanidinium chloride (Gua).**




**APM** **Gua**

GUA was synthesized according to the reported procedure[1] with slight modification. APM (1.00 g, 5.60 mmol), 1*H*-pyrazole-1-carboxamidine monohydrochloride (0.82 g, 5.60 mmol) and *N*,*N*-diisopropylethylamine (1.59g, 12.3 mmol) were dissolved in DMF (16 mL). Hydroquinone (10mg) was added as a polymerization inhibitor. The mixture was stirred at room temperature for 24 h under nitrogen atmosphere, and poured into diethyl ether (100 mL). The resulting oil was precipitated and the supernatant was removed. The precipitation was washed with the solution of acetonitrile (20 mL) and triethylamine (1 mL) two times. After washing the resultant solid with dichloromethane (30 mL), the pale yellow sticky solid of GUA was dried in vacuo to yield 480 mg (39%). The product was dissolved in DMSO (50 mg/mL) and stored in a freezer. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 8.09 (s, 1H), 7.91 (s, 1H), 7.70-6.90 (br. 4H), 5.70 (s, 1H), 5.33 (s, 1H), 3.16 (m, 4H), 1.87 (s, 3H), 1.65 (quin, $J$ = 6.8 Hz, 3H); $^{13}$C-NMR (125 MHz, DMSO-$d_6$) δ 168.62, 158.04, 140.80, 120.03, 39.34, 37.15, 29.53, 19.64; HRMS (ES/MeOH) *m/z* calcd for $C_8H_{17}N_4O_1$ $(M–Cl)^+$ 185.1397, found 185.1402.

**ELISA-mimic screen using NPs without biotin groups.**

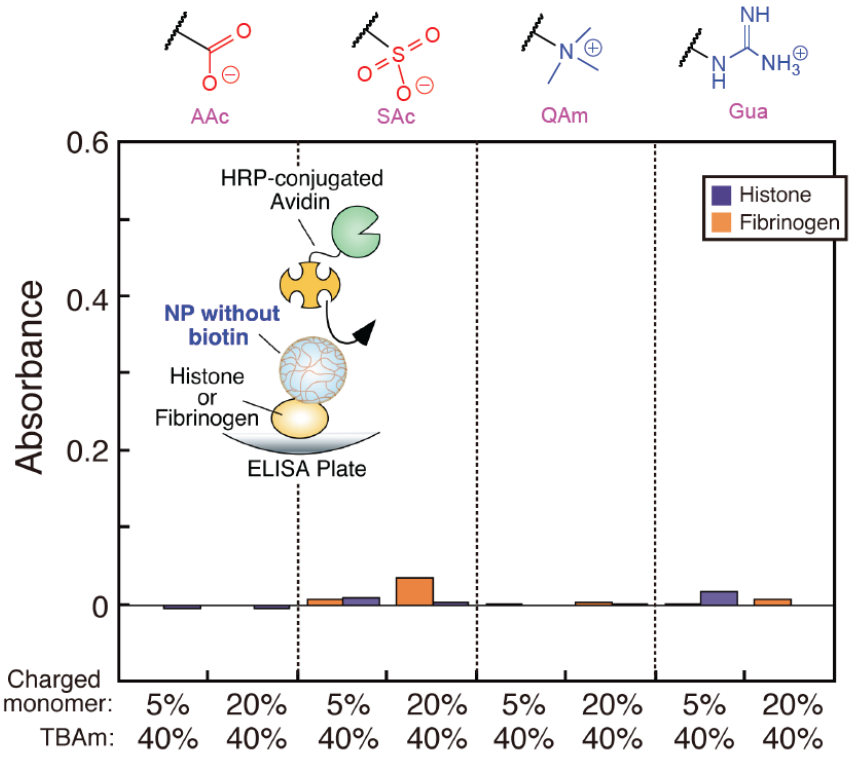



Figure S1. Results of ELISA-mimic screen using NPs without biotin groups (purple: histone, orange: fibrinogen). The absorbance of the product (at 492 nm) was quantified by UV-Vis spectroscopy. The charged groups of NPs are indicated on the top. The absorbance was a mean value of two wells (n = 2) under the same condition on the same plate. The molar ratios of charged and TBAm monomers are indicated at the bottom, respectively.

**Characterization of NPs by dynamic light scattering.**

The hydrodynamic diameter of the NPs was determined in aqueous solution by dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern Instruments Ltd.). The temperature of the NP sample was controlled via Peltier device at 25 ± 0.1 °C. The refractive index of polystyrene latex beads was used as standard. All DLS data meets quality criteria set by Malvern. The diameter sizes of negatively and positively charged NPs are listed in Table S1 and Table S2, respectively. The concentration of NPs was determined by measuring the volume of obtained NP solutions and weighing an aliquot of the solution of NPs after lyophilization.

Table S1. Monomer composition ratio and diameter of negatively charged NPs.

| Entry | Monomer feed ratio (mol %) | | | | | | Diameter (nm) |
|---|---|---|---|---|---|---|---|
| | AAc | SAc | TBAm | Bio | NIPAm | BIS | |
| AAc 5/40 | 5 | – | 40 | 1 | 52 | 2 | 109 |
| AAc 5/0 | 5 | – | – | 1 | 92 | 2 | 629[a] |
| AAc 20/40 | 20 | – | 40 | 1 | 37 | 2 | 102 |
| SAc 5/40 | – | 5 | 40 | 1 | 52 | 2 | 112 |
| SAc 5/0 | – | 5 | – | 1 | 92 | 2 | 303[a] |
| SAc 20/40 | – | 20 | 40 | 1 | 37 | 2 | 125 |
| AAc 5/40 w/o | 5 | – | 40 | – | 53 | 2 | 110 |
| AAc 20/40 w/o | 20 | – | 40 | – | 38 | 2 | 95 |
| SAc 5/40 w/o | – | 5 | 40 | – | 53 | 2 | 85 |
| SAc 20/40 w/o | – | 20 | 40 | – | 38 | 2 | 88 |

[a] The lower critical solution temperature (LCST) of the NP was above 25 °C because of a lack of hydrophobicity and the NP was swollen[1,2].

Table S2. Monomer composition ratio and diameter of positively charged NPs.

| Entry | Monomer feed ratio (mol %) | | | | | | Diameter (nm) |
|---|---|---|---|---|---|---|---|
| | QAm | Gua | TBAm | Bio | NIPAm | BIS | |
| QAm 5/40 | 5 | – | 40 | 1 | 52 | 2 | 52 |
| QAm 5/0 | 5 | – | – | 1 | 92 | 2 | 140[a] |
| QAm 20/40 | 20 | – | 40 | 1 | 37 | 2 | 43 |
| Gua 5/40 | – | 5 | 40 | 1 | 52 | 2 | 58 |
| Gua 5/0 | – | 5 | – | 1 | 92 | 2 | 126[a] |
| Gua 20/40 | – | 20 | 40 | 1 | 37 | 2 | 18 |
| QAm 5/40 w/o | 5 | – | 40 | – | 53 | 2 | 47 |
| QAm 20/40 w/o | 20 | – | 40 | – | 38 | 2 | 41 |
| Gua 5/40 w/o | – | 5 | 40 | – | 53 | 2 | 26 |
| Gua 20/40 w/o | – | 20 | 40 | – | 38 | 2 | 27 |

[a] The lower critical solution temperature (LCST) of the NP was above 25 °C because of a lack of hydrophobicity and the NP was swollen[1,2].

**References:**

1) Debord, J. D.; Lyon L. A. *Langmuir* **2003,** *19,* 7662-7664.

2) Ito, S.; Ogawa, K.; Suzuki, H.; Wang, B.; Yoshida, R.; Kokufuta, E. *Langmuir* **1999,** *15,* 4289-4294.