Electronic Supplementary Information (ESI)

Recognition and Neutralization of Angiotensin I and II with an Artificial Nanogel Receptor Fabricated by Ligand Specificity Determinant Imprinting

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Materials

N-isopropylacrylamide (NIPAm), N,N,N',N'-tetramethylethylenediamine (TEMDEA), acrylic acid (AAc), sodium dodecyl sulfate (SDS), N,N'-methylenebisacrylamide (BIS) and N-t-butylacrylamide (TBAm) were from J&K Scientific Ltd. (Beijing, China). Ammonium persulfate (APS) was from GuangzhouGanhua Chemical Industry Corporation (Guangzhou, China). Angiotensin II was purchased from Sigma (Shanghai, China). Angiotensin I, [Sar1, Val5, Ala8] angiotensin II (SVA), angiotensin II (5–8) (Ang II (5–8)) and Asp-Arg-Val-NHC_2H_5 (DRV- NHC_2H_5) were from GL Biochem. (Shanghai, China). Trifluoroacetic acid (TFA) was obtained from Alfa Aesar (Tianjin, China). Dulbecco's Modified Eagle Medium (DMEM) was from Thermo Fisher Scientific (Beijing, China). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo (Beijing, China). Adult male Wistar rats (250-300g, 10 week-old) were provided by the Animal Center of Sun Yat-Sen University (Certificate number: SCXK (Yue) 2011-0029).

Apparatus

The HPLC system was consisted of a Shimadzu LC-10AD pump (Kyoto, Japan) and a Shimadzu SPD-10A UV-Vis detector. All determinations were performed on an analytical, reverse-phased Symmetry C18 (4.6 mm I.D. × 15 cm long, 5 mm) column (Dikema, Thermo Fisher). HPLC mobile phase was acetonitrile and water (4 : 6, v/v) containing 0.1% TFA. Flow-rate was 1 mL/min and detection wavelength was 220 nm.

Fourier transform infrared (FTIR) spectra were recorded with IRPrestige-21 using a Spectrum One FTIR spectrometer (PerkinElmer Company, USA). The morphology of MIP-nanogels was characterized by transmission electron microscopy (TEM) on a JEM-2100HR (JEOL, Japan). Absorbance in cell viability assays was measured by Multiskan Ascent (Thermo, America).

Preparation of nanogel particles

Nanogels were prepared according to the method reported with modification.[1] Various sets of nanogels with designed composition shown in Table S1 were prepared. The imprinted nanogel particles were synthesized as follows NIPAm ((68-X) mol %), AAc (30 mol %), TBAm (X mol %), BIS (2 mol %), SDS (10 mg) and template were dissolved in water (50 mL). TBAm was dissolved in 1 mL of ethanol before addition to the monomer solution, resulting in a total monomer concentration of 6.5 mM. The reactants were mixed in a sonication bath for 10 min and were shaken at room temperature for 4 h to ensure sufficient template-functional monomer pre-interaction. After the reaction solution was degassed with N_2 for 30min, APS (30 mg) and N,N,N',N'-tetramethylethylenediamine (15 μL) were added and the polymerization was carried out at 23-25 °C for 24 hours under a nitrogen atmosphere. To remove the remanent monomers and template, the nanogels were purified by dialysis against excess amount of pure water (more than twice a day changes) for > 4 days. The purified solution was then lyophilized for 3 days. The non-imprinted nanogels (NIP-nanogels) were synthesized under the same conditions, but in the absence of the template.

Binding assays

2 mL of 2.5 mg/mL MIP-nanogels or NIP-nanogels solution with 1 mg/mL SDS was pipetted into a 5 mL centrifuge tube. Certain volume (<100 μL) of analyte solution with known concentration was added. The mixture was incubated on a horizontal shaker for 12 h, and then was centrifuged at 16,000 rpm for 30 min. The concentration of analyte in the supernatant was measured by HPLC. The amount of analyte bound to MIP-nanogels or NIP-nanogels was calculated by subtracting the amount of free analyte from the initial concentration. The quantity (Q) of the analyte bound to MIP-nanogels or NIP-nanogels was calculated according to the following equation: $Q = (C_0 - C_t) \times V/W$, where C_0 and C_t (mg/L) are the initial concentration and the residual concentration of analyte, respectively; V (L) is the initial volume of the solution, and W (g) is the weight of the MIP-nanogels or NIP-nanogels. Imprinted factor was defined as: $IF = Q_{MIPs} / Q_{NIPs}$. All experiments were carried out in triplicate.

For evaluation of the synthesized nanogels to find the optimal MIP-nanogels, experimental conditions: 2 mL of 2.5 mg/mL MIP-nanogels or NIP-nanogels solution with 1 mg/mL SDS contained 1 mM template.

For investigating pH effect on adsorption capacity of MIP-nanogels, experimental conditions: The pH of 2.5 mg/mL MIP-nanogels or NIP-nanogels solution with 1 mg/mL SDS was adjusted by 0.1M HCl or 0.1 M NaOH. The concentration of Ang II was 0.125 mg/mL.

For investigating salt-induced response of the nanogels, experimental conditions: 2 mL of 2.5 mg/mL MIP-nanogels or NIP-nanogels solution contained 1 mg/mL SDS and specified NaCl concentration at pH 7.4. The concentration of Ang II was 0.125 mg/mL.

For investigating the adsorption capacity of nanogels for different peptides, experimental conditions: 2 mL of 2.5 mg/mL MIP-nanogels or NIP-nanogels solution contained 1 mg/mL SDS and 130 mM NaCl at pH 7.4; the concentration of tested peptide was 1.2×10^{-4}M.

For investigating adsorption isotherms of MIP-nanogels and NIP-nanogels to Ang I and Ang II, experimental conditions: 2 mL of 2.5 mg/mL MIP-nanogels or NIP-nanogels solution contained 1 mg/mL SDS and 130 mM NaCl at pH 7.4.

Molecular simulation

Binding energies of the complexes between designed template and the functional monomer were calculated with the Gaussian 03 software package. [2] Their molecular geometries were optimized by using B3LYP/6-31G (d, p).[3-4] All of the stable structures were further confirmed by analytical computations of harmonic vibrational frequencies. The binding energies (ΔE) of complexes between designed template and the functional monomer were evaluated by the following equation:

$\Delta E = E(\text{complex}) - E(\text{functional monomer}) - E(\text{template})$.

Cytocompatibility Studies

Ealy926 cells were cultured in DMEM containing 10% fetal bovine serum at 37 ºC. The cells were seeded onto 96-well plate (4×10^4 cells/well). After the cells were cultured for 24 h, various concentrations of the MIP-nanogels were added to the culture, and incubated for 48 h. Then, CCK-8 (100 μL/ well) was added and incubated for 4 h. The absorbance was measured at 450 nm by a microplate reader. Blank (culture medium), negative (cells in culture medium), positive (cells in culture medium with cisplatin) and culture medium containing various concentrations of MIP-nanogels (without cells) controls were also prepared, and the absorbance was measured. All experiments were carried out in triplicate. The neutralization of cytotoxicity was calculated as the percentage of negative control cell viability.

In vivo tests

All animal experiments were reviewed, approved, and supervised by the Animal Center of Sun Yat-Sen University. Experiments were performed in two groups of adult male Wistar rats. Rats were anesthetized with pentobarbital sodium (45 mg/kg body weight, Merck) and placed on an operating table. Following tracheostomy, the right

external jugular vein was catheterized (PE-50) for administration of experimental solutions. The right common carotid artery was catheterized (PE-50) for monitoring blood pressure via a biological function recording system (BL-420S, Taimeng, Chengdu, China). After the blood pressure had stabilized for 15 min, the anesthetized rats in group 1 (n = 8 Rats) received intravenous injection of 0.2 mL of 0.9% NaCl containing 0.125 mg/mL Ang II. And the anesthetized rats in group 2 (n = 8 Rats) received intravenous injection of 0.2 mL of 0.9% NaCl containing 0.125 mg/mL Ang II along with 3 mg/mL artificial receptor (Ang II-receptor). Anesthetized rats in group 3 (n = 8 Rats) received injections of 0.2 mL of 0.9% NaCl containing 3 mg/mL MIP-nanogels and in group 4 (n = 8 Rats) anesthetized rats received injections of 0.2 mL of 0.9% NaCl containing 3 mg/mL NIP-nanogels. After the experimental solutions were administered, 0.4 mL of 0.9% NaCl was intravenously infused slowly to ensure all experimental solutions injected into the rats. Then blood pressure was monitored continuously for the next 15 min. Results were expressed as means ± SD. Statistical significance was evaluated using t-test. Significance is defined as $P < 0.05$.

References

[1]Y. Hoshino, T. Kodama, Y. Okahata, and K. J. Shea, *J. Am. Chem. Soc*. 2008, **130**, 15242.

[2] M.J. Frisch, et al., Gaussian 03, Revision E. 01, Gaussian, Inc., Wallingford, CT, 2004.

[3] A.D. Becke, *J. Chem. Phys.* 1993, **98**, 5648.

[4] C. Lee, W. Yang, G. Parr, *Phys. Rev. B.* 1988, **37**, 785.

Table S1. Functional Monomer Composition and Ratio of Template and Monomer

	Functional monomer composition (mol%)		Template / g	Template :AAc
	AAc	TBAm		
MIP-nanogels 1	30	20	0.0081	1:5
MIP-nanogels 2	30	20	0.0058	1:7
MIP-nanogels 3	30	20	0.0045	1:9
MIP-nanogels 4	30	20	0.0036	1:11
MIP-nanogels 5	30	10	0.0045	1:9
MIP-nanogels 6	30	5	0.0045	1:9
NIP-nanogels 1	30	20	0	
NIP-nanogels 2	30	10	0	
NIP-nanogels 3	30	5	0	

Table S2 Binding sites and energy between AAc and three designed templates

Designed template	A_T	B_T	C_T
Total amount of binding sites	6	4	5
Binding energy (ΔE) (kJ/mol, in vacuum)	-350.0	-223.2	-299.7

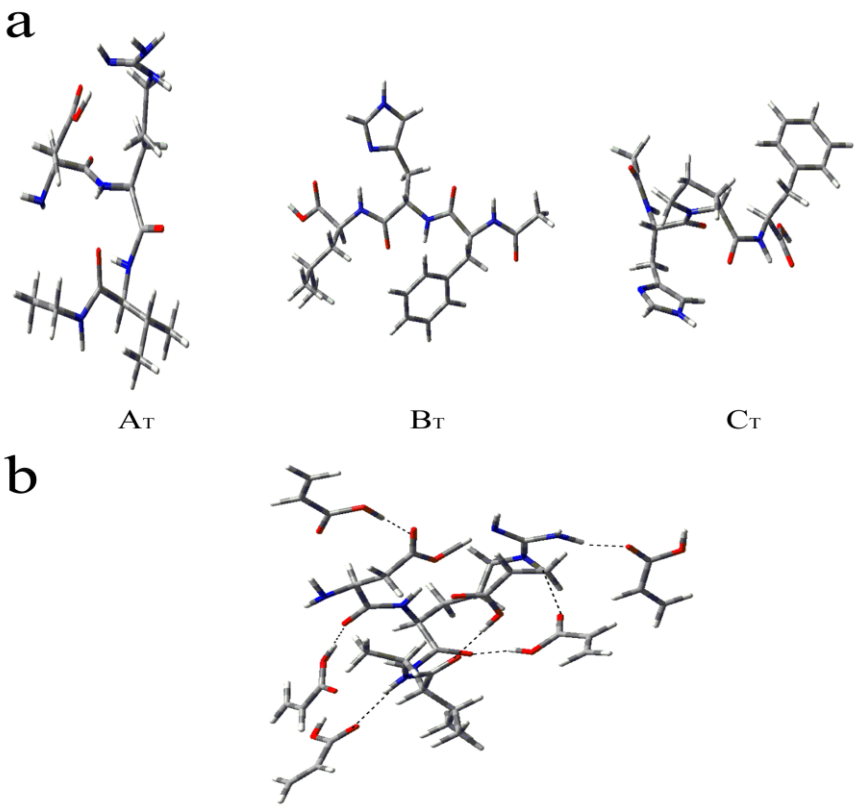

Fig. S1 (a) The optimized configurations of three designed templates；(b) Optimized structures of pre-arrangement between A_T and AAc.

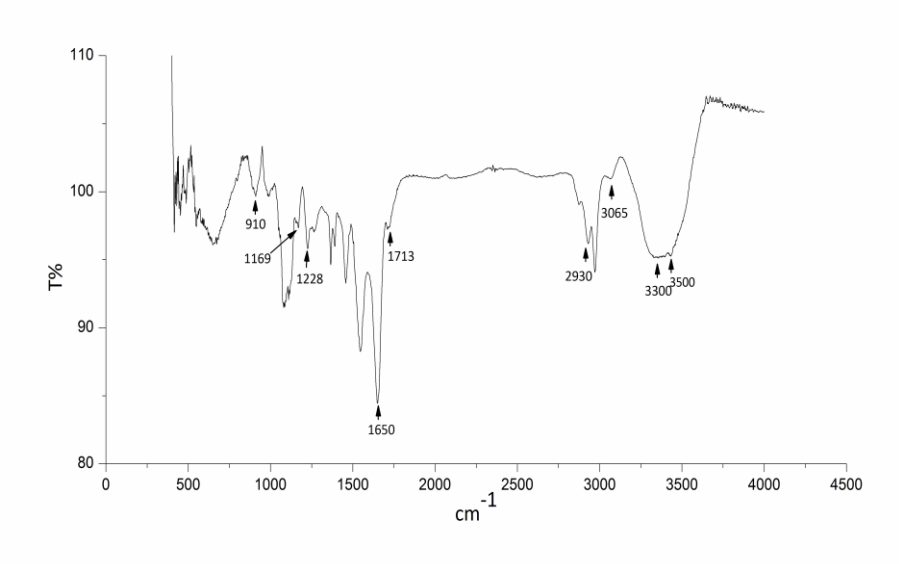

Fig. S2 FT-IR spectra of MIP-nanogels **3**

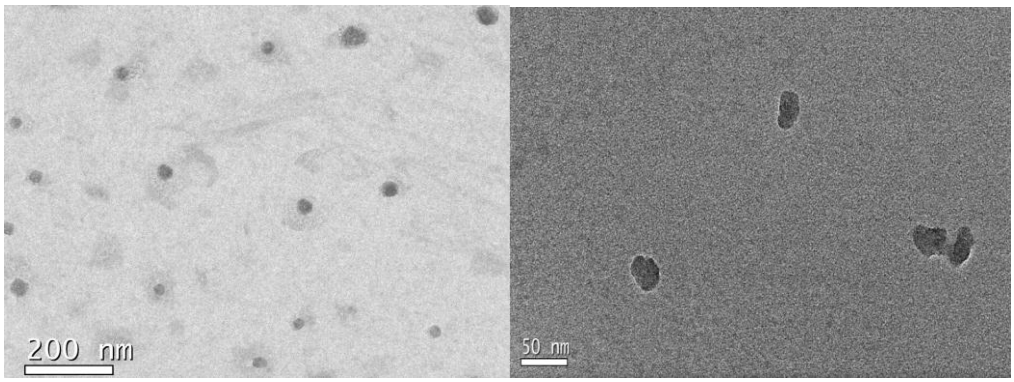

Fig. S3 TEM images of the MIP-nanogels **3** showing small size distribution.

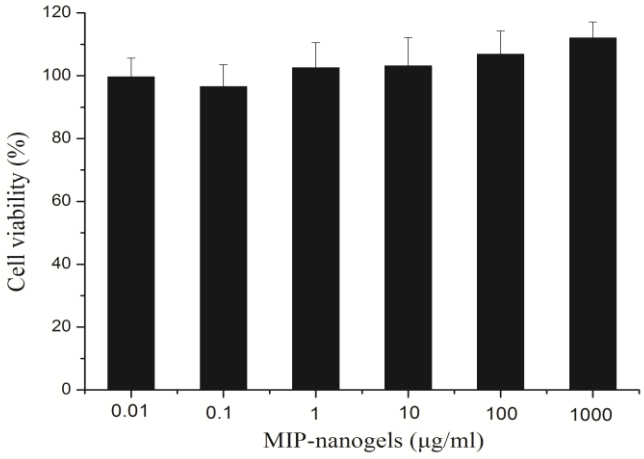

Fig. S4 Cell viability for MIP-nanogels. Mean values and standard deviations (n=3).

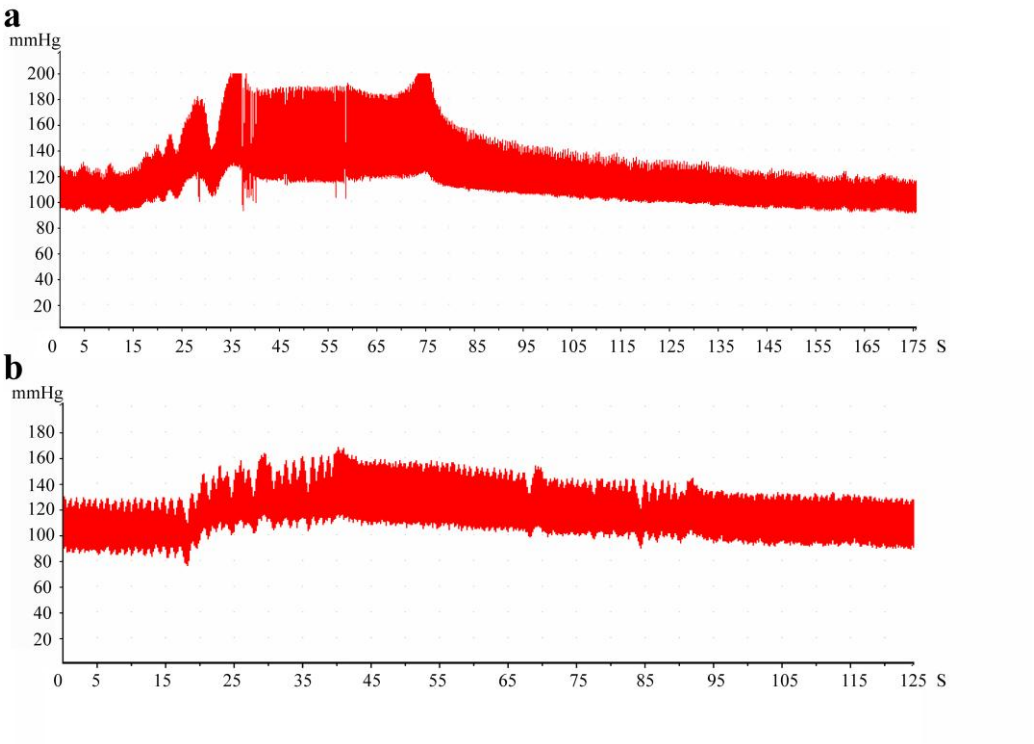

Fig. S5 Effect of Ang II injection (a) and Ang II-receptor injection (b) on blood pressure of anesthetized rats.

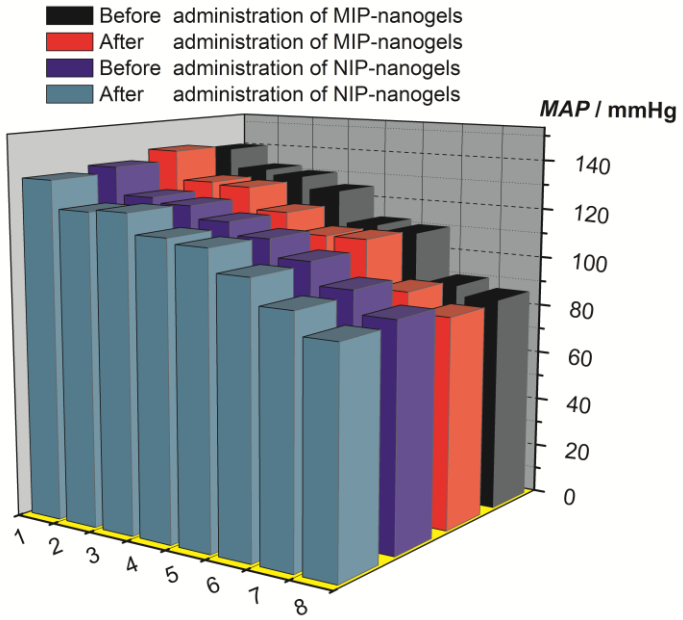

Fig. S6 The MAP of anesthetized rats in group 3 and 4. The numbers one to eight mean different rats in each group.

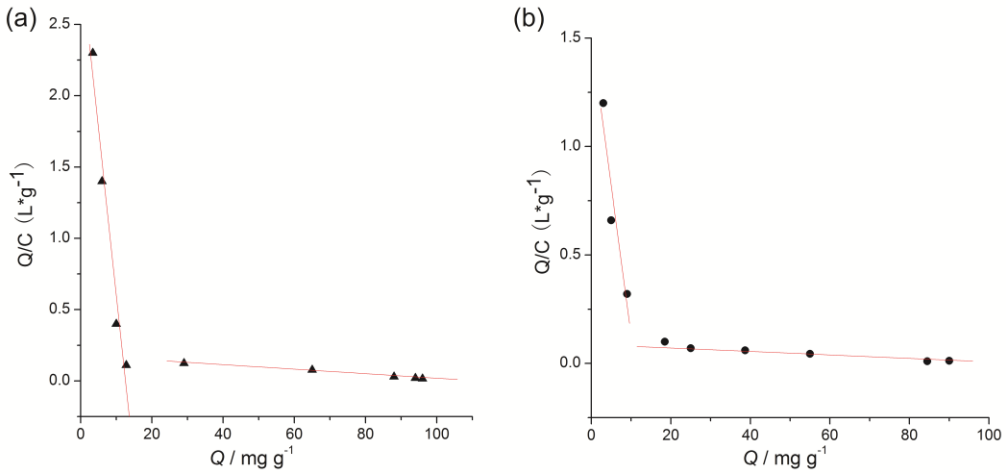

Fig. S7 Scatchard plots of the experimental adsorption isotherm for MIP-nanogels for Ang I (a) and Ang II (b).