Electronic Supplementary Material (ESI) for ChemComm.
This journal is © The Royal Society of Chemistry 2014

Supporting Information

Near-infrared Light-controlled System for Reversible Presentation of Bioactive Ligands Using Polypeptide-engineered Functionalized Gold Nanorods

Jie Yang, Ming-Hao Yao, Ming-Shuo Du, Rui-Mei Jin, Dong-Hui Zhao, Jun Ma, Zhi-Ya Ma, Yuan-Di Zhao*, and Bo Liu*

Britton Chance Center for Biomedical Photonics at Wuhan National Laboratory for Optoelectronics - Hubei Bioinformatics & Molecular Imaging Key Laboratory, Department of Biomedical Engineering, College of Life Science and Technology, Huazhong University of Science and Technology, Hubei, Wuhan 430074, P. R. China

Fax: (+) 86 27-8779-2202
E-mail: zydi@mail.hust.edu.cn (Y.-D. Zhao), lbyang@mail.hust.edu.cn (B. Liu)

Materials and Methods

1 Materials

Restriction endonuclease BamHI, NheI, SpeI, and T4 DNA ligase were obtained from New England Biolabs Inc. (Beijing, China). Tris(2-carboxyethyl)phosphine (TCEP), *β*–mercaptoethanol, isopropyl-*β*-D-thiogalactoside (IPTG), ampicillin, kanamycin, calcein AM, chloroauric acid ($HAuCl_4 \cdot 4H_2O$), cetyltrimethylammonium bromide (CTAB), sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$), ascorbic acid (AA) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Nickelnitrilotriacetic acid (Ni-NTA) separation column was purchased from Qiagen China (Shanghai) Co., Ltd. Polyethylene glycol (PEG, molecular weight, MW: 10 kDa) was obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Acryloyl chloride was obtained from Aladdin Inc. (Shanghai, China). PEG-dimaleimide (MW: 10 kDa) was purchased from Xiamen Sinopeg Biotech，Inc. (Xiamen, China). Ultrapure water (≥18.2 MΩ) purified by the Milli-Q system (Millipore, USA) was used for preparation of all solutions.

2 Synthesis and purification of the coil-coiled polypeptides

$PQE9PC_{10}A$ plasmid was a kind gift from Prof. David Tirrell at the California Institute of Technology, Pasadena, CA. The genes encoding the polypeptides shown in Figure S1 were each constructed in the Qiagen pQE9 expression vector through standard recombinant DNA manipulation. Polypeptides were expressed under control of the bacteriophage T5 promoter in the *E. coli* strain M15 and purified through Ni-NTA metal-affinity chromatography. The purified polypeptides were analyzed on a Bruker Reflex III reflectron MALDI-TOF mass spectrometer. cysA (MS: 9074.6 Da, the theoretical calculation of molecular weight: 9078.2 Da), cysARGD (MS: 10512.1 Da, the theoretical calculation of molecular weight: 10515.6Da),

Bcys (MS: 8794.8 Da, the theoretical calculation of molecular weight: 8796.2 Da).

3 Synthesis of PEG-diacrylate (PEGDA)

PEGDA (MW: 10 kDa) was synthesized according to the literature method.[1] The final yield of PEGDA was more than 85%. High degree of substitution (> 95%) was confirmed by ^{1}H NMR (Varian Unity spectrometer). ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC, TMS): δ = 6.2 (d, 2H), 6.0 (d, 2H), 5.7 (d, 2H), 4.0 (t, 4H), 3.4 ppm (m, 935H).

4 Preparation of B-PEG

B-PEG was synthesized by conjugating polypeptide Bcys (having a C-terminal cysteine residue) with PEGDA or PEG-dimaleimide through the Michael-type addition reaction. Bcys (237 mg, 27 μmol) was dissolved in 7.7 mL 8 M (pH = 8) urea buffer followed by the addition of 300 μL TCEP (150 mM). After incubation at room temperature for 1.5 h, 270 μmol PEGDA or PEG-dimaleimide and 22 mL 8 M (pH = 7) urea buffer were added. The pH of the mixture was adjusted to a desired value (8.0 for conjugation with PEGDA and 6.5 for conjugation with PEG-dimaleimide). The mixture was stirred at room temperature for 24 h under dark condition. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was used to monitor the degree of reaction. The excess PEGDA or PEG-dimaleimide was removed by Ni-NTA affinity column. The purified product B-PEG was dialyzed against ultrapure water for 3 days under dark condition, frozen, and lyophilized. To examine whether the B domain of the B-PEG retained its coiled-coil structure and ability to heterodimerize with the A domain, a mixture of 100 μM cysARGD and 200 μM B-PEG was prepared in PBS (pH 7.4) and incubated at room temperature for 2 h. Polypeptides cysARGD, B-PEG, and their mixture were measured by native polyacrylamide gel electrophoresis.

5 Synthesis and characterization of CTAB-stabilized gold nanorods (GNRs)

GNRs were synthesized according to a seed-mediated growth method and our previous reports.[2, 3] Briefly, a gold seed solution was synthesized by adding $HAuCl_4$ (0.5 mM, 5 mL) to CTAB (0.2 M, 5 mL). Freshly prepared ice-cold $NaBH_4$ (0.01M, 0.6 mL) was quickly added under vigorous stirring. After stirring for 5 min, the solution was stored at room temperature for 2 h. In the meantime, $HAuCl_4$ (1 mM, 30 mL), $AgNO_3$ (0.04M, 132 μL), and H_2SO_4 (0.5 M, 120 μL) were added in CTAB (0.2 M, 30 mL), and the mixture was reduced by AA (0.0788 M, 420 μL) to yield a growth solution. Seed solution (72 μL) was added into the growth solution. After stirring for 10 s, the mixture was left undisturbed at 28 ºC for 12 h. The UV-visible absorbance spectra of the GNRs were measured by using a Lambda 950 UV/VIS spectrometer (PerkinElmer, USA) at room temperature. The sizes and the aspect ratios of GNRs were determined by a Tecnai G^2 20 U-Twin TEM.

6 Preparation of GNRs-polypeptide

For the conjugation of polypeptides (cysA or cysARGD) onto GNRs, cysA or cysARGD (1 μmol) was dissolved in 2 mL ultrapure water followed by the addition of 20 μL TCEP (200 mM). After incubation at room temperature for 1.5 h, the pH of the polypeptide solution was adjusted to 7.4. GNRs (5×10^{-8} M, 2 mL) were centrifuged twice at 10000 rpm for 10 min to remove excess CTAB. GNRs were resuspended with the reduced polypeptide solution, and the mixture was stirred at room temperature to react for 72 h. Unreacted polypeptide was removed by centrifugation at 10000 rpm for 10 min.

7 Coimmobilization and removing of B-PEG

B-PEG (1 μmol) was dissolved in 1 mL ultrapure water, and the pH of B-PEG solution was adjusted to 7.4. The B-PEG solution was added in 1 mL GNRs-polypeptide (GNRs-A or GNRs-ARGD, the concentration was about 5×10^{-8} M), and the mixture was stirred at room temperature for 2 h. Excess B-PEG was removed by centrifugation at 10000 rpm for 10 min.

To examine the ability to remove the coimmobilized B-PEG from GNRs-ARGD-B-PEG nanocomplexes with NIR light irradiation, 200 μL GNRs-ARGD-B-PEG solution was exposed to a continuous wave (cw) NIR laser (λ = 810 nm, spot size: 5 × 8 mm) at an output power of 3.0 W cm^{-2} for 2 min (Changchun New Industries Optoelectronics Technology, Changchun, China). The zeta potentials were determined on a ZS90 Nanosizer (Malvern, UK) at 25 ºC by laser doppler electrophoresis methods.

8 Photothermal Effects GNRs-ARGD-B-PEG

To measure the temperature changes in response to NIR light absorption by GNRs-ARGD-B-PEG, 100 μg mL^{-1} GNRs-ARGD-B-PEG was diluted to 80, 40, 20 or 5 μg mL^{-1} with ultrapure water. Aliquots (200 μL) were deposited into a 0.5 mL PE tube. The NIR laser was placed 5 cm over the test tube. Tubes with different concentrations of GNRs-ARGD-B-PEG were directly exposed for 5 min to continuous NIR light laser. Temperature changes were monitored using a thermocouple. Different laser powers were used.

9 Fabrication of 2-D system

Sulfhedryl-functionalizated glass substrate was prepared according to previously reported method. [4, 5]

Prior to immobilization of the GNRs on the sulfhedryl-functionalizated glass substrate, excess CTAB was removed by centrifugation at 10000 rpm for 10 min. The GNRs were resuspended in ultrapure water with equal volume, and the mercaptosilane-modified glass slides were incubated in the GNRs solution for 12 h. After washing twice with PBS, the GNRs-functionalized substrates were incubated in 100 μM reduced cysA or cysARGD overnight. Excess cysA or cysARGD were removed by washing five times with PBS. Solution of B-PEG was prepared in PBS at a concentration of 300 μM. The substrate modified with cysARGD-functionlized GNRs was incubated with the B-PEG solution

overnight, followed by washing with PBS and serum-free Dulbecco's Modified Eagle Medium (DMEM) (three times for each).

Fibroblasts (NIH 3T3) were cultured in DMEM medium supplemented with 10% FBS and 1% penicillin-streptomycin in a cell incubator (5% CO_2, 37 ºC) for 2 days. Fibroblasts were seeded on each surface (substrate modified with cysA-functionalized GNRs, cysARGD-functionalized GNRs, or cysARGD-B-PEG-functionalized GNRs) at a density of 50,000 cells cm^{-2} in serum-free DMEM and allowed to adhere for 2 h at 37 ºC, 5% CO_2 in a cell incubator. For the cell adhesion assay, each sample was washed to remove non-adherent cells, and adherent cells were stained with the calcein AM dye. The cells were imaged with a 10 × objective on an inverted fluorescent microscope (IX71, Olympus, Japan).

To measure the photothermal response of GNRs nanocomplexes, the substrate modified with GNRs-ARGD-B-PEG was irradiated with the 810 nm cw laser light at a power density of 3.0 W cm^{-2} for 4 min and washed with PBS three times. Fibroblasts were seeded on the irradiated substrates at a density of 50,000 cells cm^{-2} in serum-free DMEM and allowed to adhere for 2 h at 37 ºC, 5% CO_2 in a cell incubator. For the cell adhesion assay, samples were washed to remove non-adherent cells, and adherent cells were stained with the calcein AM dye. The cells were imaged with a 10 × objective on the inverted fluorescent microscope.

10 *In vitro* cytotoxicity

HeLa cells and NIH 3T3 cells were seeded in 96-well plates (500 cells per well). After incubation for 24 h, the medium was discarded, and the cells were incubated with CTAB-GNRs, GNRs-ARGD, GNRs-ARGD-B-PEG (40 μg mL^{-1}) and cysARGD (20 μM) in serum-free DMEM supplemented with penicillin-streptomycin (100 units mL^{-1}) for another 24 h. The incubated cells were assayed for cell viability with MTT. The cells were washed twice with PBS, and MTT (20 μL, 5 mg mL^{-1}) solution was added to each well. After incubation for 4 h, the medium was discarded, and the insoluble purple formazan crystals were dissolved

with DMSO (150 μL). The absorbance at 490 nm was recorded with a microplate reader (BioTek ELX808IU, USA). The absorbance was directly correlated with cell quantity, and cell viability was calculated by assuming 100% viability in the control.

References:

(1) M. Yao, J. Yang, M. Du, J. Song, Y. Yu, W. Chen, Y. Zhao and B. Liu, *J. Mater. Chem. B* 2014, **2**, 3123-3132.

(2) B. Nikoobakht and M. A. El-Sayed, *Chem. Mater.* 2003, **15**, 1957-1962.

(3) H. Xia, X. Yang, J. Song, J. Chen, M. Zhang, D. Yan, L. Zhang, M. Qin, L. Bai, Y. Zhao and Z. Ma, *J. Mater. Chem. B* 2014, **2**, 1945-1953.

(4) S. M. Marinakos, S. Chen and A. Chilkoti, *Anal. Chem.* 2007, **79**, 5278-5283.

(5) A. Bertin and H. Schlaad, *Chem. Mater*. 2009, **21**, 5698-5700.

cysA: MRGS**[6H]**GSDDDDKASSGSGCSGSGT**[A]**IGDHVAPRDTSW
cysARGD: MRGS**[6H]**GSDDDDKASSGSGCSGSGT**[A]**IGDHVAPRDTSYAVTG**RGD**SPASSTSW
Bcys: MRGS**[6H]**GSDDDDKWASGT**[B]**IGDHVAPRDTSMGGC
Abbreviations for peptide domains:
[6H]: HHHHHH
[A]: SGDLENEVAQLEREVRSLEDEAAELEQKVSRLKNEIEDLKAE
[B]:SGDLKNKVAQLKRKVRSLKDKAAELKQEVSRLENEIEDLKAK

Fig. S1. Amino acid sequences of polypeptides used in this study.

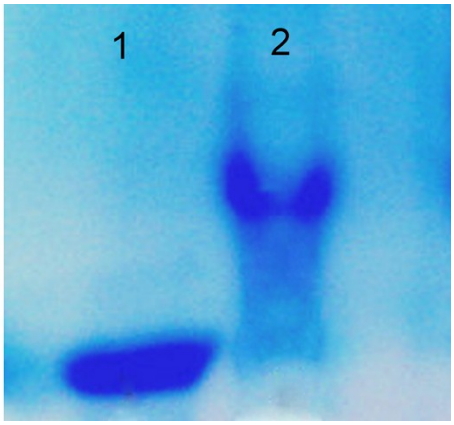

Fig. S2. SDS-PAGE of Bcys (lane 1) and B-PEG (lane 2, PEG: 10kDa).

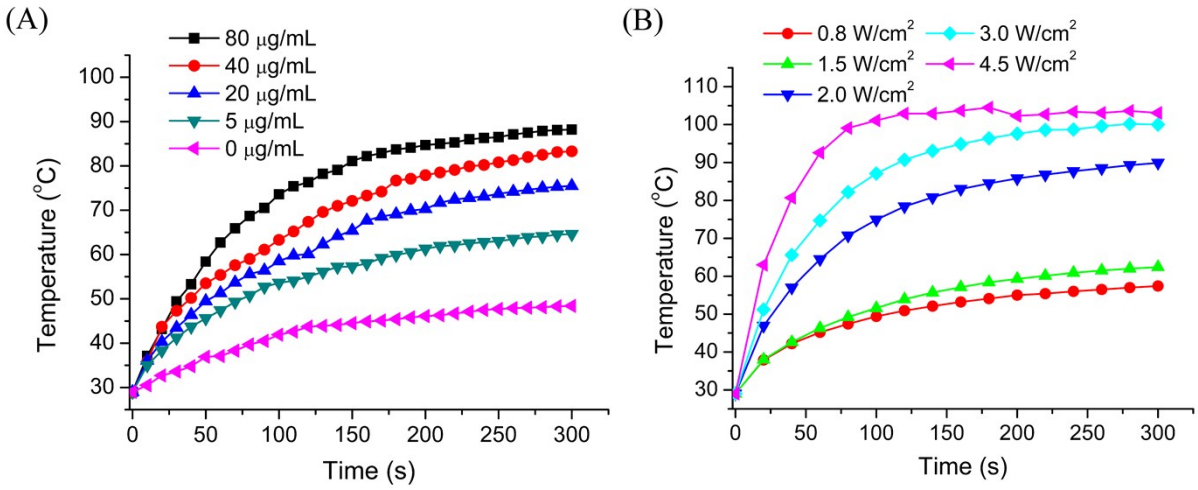

Fig. S3. Photothermal effect of GNRs-ARGD-B-PEG nanocomplexes. (A) Temperature changes of different concentration of GNRs-ARGD-B-PEG were measured over a period of 300 s of exposure to NIR laser with a wavelength of 810 nm at an output power of 2.5 W/cm^2. (B) Temperature changes upon irradiation using a NIR laser (λ = 810 nm) at different laser powers. The concentration of GNRs-ARGD-B-PEG is 20 μg/mL.