Electronic Supporting Information for

The use of an engineered single chain variable fragment in a localized surface plasmon resonance method for analysis of the C-reactive protein

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Materials and methods

Materials. Recombinant human CRP (r-CRP) was purchased from Oriental Yeast (Osaka, Japan). A polyclonal mouse anti-human CRP antibody was obtained from Abcam (Cambridge, UK). All other reagents were purchased from Sigma and used without purification. The ATP, GTP, UTP, CTP, creatine phosphate (CP), and *E. coli* total tRNA mixture were purchased from Roche Applied Science (Indianapolis, IN). T7 RNA polymerase and S30 extract were prepared using a previously described protocol.[1]

Cell-free synthesis of scFv. An open reading frame (ORF) of scFv cloned into plasmid pET21a was used as the template for PCR.[2] Cysteine fused-gene construct was prepared via overlap PCR (Figure S1), and a P3 primer containing a CAT5 sequence (5'-ctttaagaaggagat-3') for enhanced cell-free expression from pK7CAT (chloramphenicol acetyltransferase) was used.[3] In the first round, primary PCRs were conducted using standard conditions, in which ORF was amplified using the following primers: P2 and P4(Cys-VL), P3 and P5(VH-Cys) (Table S1). To construct the cystein in the linker, the V_H region was amplified with P3 and P5 (CAT5-V_H-Cys), and the V_L region was amplified with P4 and P2 (Cys-V_L). PCR products were purified by employing gel extraction and used for the second round of PCR, in which full-length expression templates were synthesized using P1 and P2 primers. After amplification, the PCR products were purified using the PCR-clean up kit (Promega, Madison, USA) before their use in protein expression.

The standard reaction mixture for cell-free protein synthesis consisted of the following components in a total volume of 15 μL: 57 mM Hepes-KOH (pH 8.2), 1.2 mM ATP, 0.85 mM each of CTP, GTP, and UTP, 0.17 mg/mL E. coli total tRNA mixture (strain MRE600), 0.64 mM cAMP, 90 mM potassium glutamate, 80 mM ammonium acetate, 12 mM magnesium acetate, 34 μg/mL L-5-formyl-5,6,7,8-tetrahydrofolic acid (folinic acid), 1 mM each of 20 amino acids, 2% PEG (8000), 67 mM CP,

3.2 μg/mL creatine kinase, 10 μg/mL of PCR-amplified genes, and 27% (v/v) S30 extract. Glutathione buffer (5 mM) was added to the mixture giving varying ratios of oxidized (GSSG) and reduced (GSH) to provide oxidizing conditions to form disulfide bonds within the cell-free expressed scFvs.[4] The reaction mixture was incubated in a water bath at 30 °C for 3 h. The molecular weight of the expressed scFv was determined using 12% Tricine–SDS–polyacrylamide gels and western blot analysis.

Preparation of purified scFv. Upon completion of the cell-free synthesis reaction, 1 mL of the reaction mixture was added to 1 mL of buffer (50 mM NaH_2PO_4, 300 mM NaCl, pH 8.0) and mixed with 0.1 mL of Ni– NTA agarose beads (Qiagen, Hilden, Germany). After 1 h of gentle mixing at 4 °C, the recovered beads were washed three times with 10 mL of washing buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, 0.05% Tween-20, pH 8.0), and the bound protein was eluted with 200 mM imidazole. The final yield of purified soluble scFvs was estimated by using the Bradford protein assay (Bio-Rad, USA).

Synthesis of Gold nanorods. Gold nanorods (GNRs) were prepared using a seed-mediated method.[5,6] A seed solution was prepared by adding the following reagents to a conical tube in the following order and then gently mixing for 2 min and then incubation for 2 h at 25 °C: 0.3 mL of 0.01 M $NaBH_4$ into the mixture of 3.75 mL of 0.1 M CTAB and 0.125 mL of 0.01 M $HAuCl_4$. The growth solution was prepared by adding the following reagents to a conical tube in the following order and then gently mixing: 4.72 mL of 0.1 M CTAB, 0.2 mL of 10mM $HAuCl_4$, and 0.03 mL of 10 mM $AgNO_3$. To initiate nanorod growth, 0.02 mL of seed solution was added to the growth solution, mixed gently by inversion for 10 s, and left standing for 3 h. During this time, the color changed gradually to dark purple, with most of the change occurring in 1 h. UV/Vis absorption spectra of the gold nanorod solution were obtained by using a UV/Vis spectrophotometer (Beckman Coulter DU800, Brea, CA).

The size and aspect ratios were determined by using high resolution transmission electron microscopy (HR-TEM).

Preparation of GNR substrates. Glass slides (2.5 cm×1.2 cm) were cleaned in a piranha solution at 70 °C for 15 min. Subsequently, the slides were rinsed repeatedly with ethanol and then incubated in an ethanol solution of 2% (3-aminopropyl) trimethoxysilane (Aldrich, Milwaukee, WI, USA) at room temperature for 4 h. The slides were then rinsed with ethanol and air dried. After amine functional groups formed, the slide was immersed in 1 M succinic anhydride in DMF for 4 h at 37 °C, rinsed with DW, and dried. Coupling reaction between the carboxylate groups on the surface and the amine groups was promoted using a solution of 100 mM EDC and 25 mM NHS in DW for 15 min, followed by the immersion in 100 mM cysteamine in DW. Finally, the thiol-modified glass slides were incubated in the GNR suspension for 12 h to prepare the GNR substrates.

Label-free detection of CRP on GNR substrates. In order to attach scFv and full-length antibody on the GNR surface, the glass slides with gold nanorods were incubated in 100 μg/mL scFv and antibody solution. After incubation for 30 min, the glass slides were rinsed with 10 mM phosphate buffer saline (PBS) and then the unreacted gold nanorod surface was blocked by adding 1 mM mercaptoundecanol (MUOH) for 30 min to prohibit possible nonspecific adsorption in the subsequent analytical process. CRP spiked serum, in the concentration range of 10 ng/mL – 100 μg/mL, was 10-fold diluted with PBS and incubated at room temperature for 30 min. The modified glass slide was inserted into the cuvette for measurements. UV visible spectrometry (Beckman Coulter DU800, Brea, CA) was used for LSPR signal acquisition and data evaluation.

Table S1 Oligonucleotide primers used in this study

Primer	Sequence (5'- 3')
P1(Forward)	TCGATCCCGCGAAATTAATACGACTCACTATAGGGAGACCACAACGGT TTCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATG
P2(Reverse)	CAAAAAACCCCTCAAGACCCGTTTA
P3(CAT-DB) (Forward)	CTTTAAGAAGGAGATATACATATGGAGAAAAAAATCACTGCTAGCCAG GTCCAGC
P4(Cys-VL) (Forward)	GGCGGTTCAGGTGGATGTTGTTGTGGAGGAGGAGGGTCGACCGGTG AAATTGTGCTCACCC
P5(VH-Cys) (Reverse)	GGTCGACCCTCCTCCTCCACAACAACATCCACCTGAACCGCCTCCG CCTGCAGAGACA

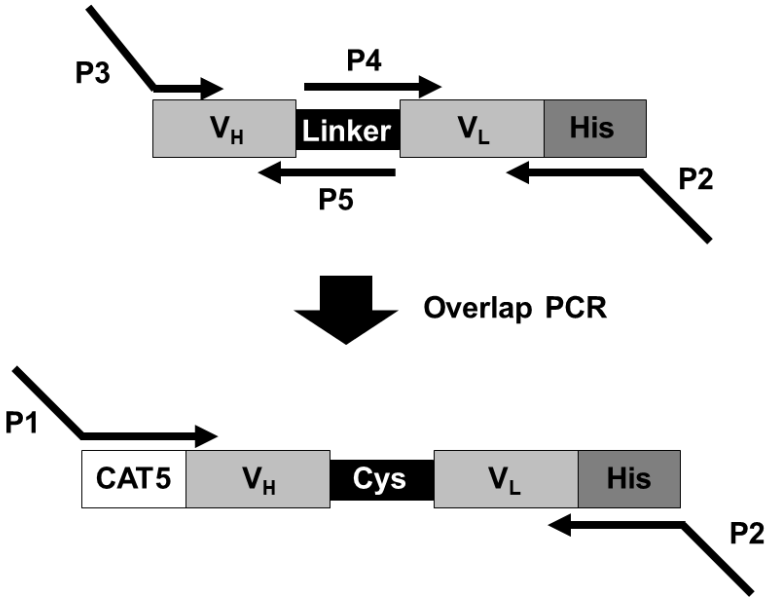


Figure S1 Structure of PCR products for the expression of functionalized scFvs

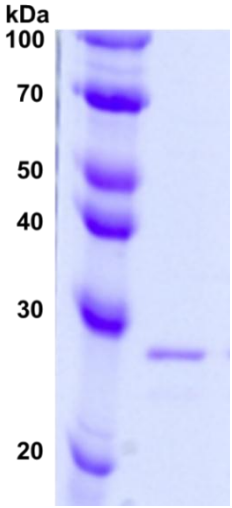
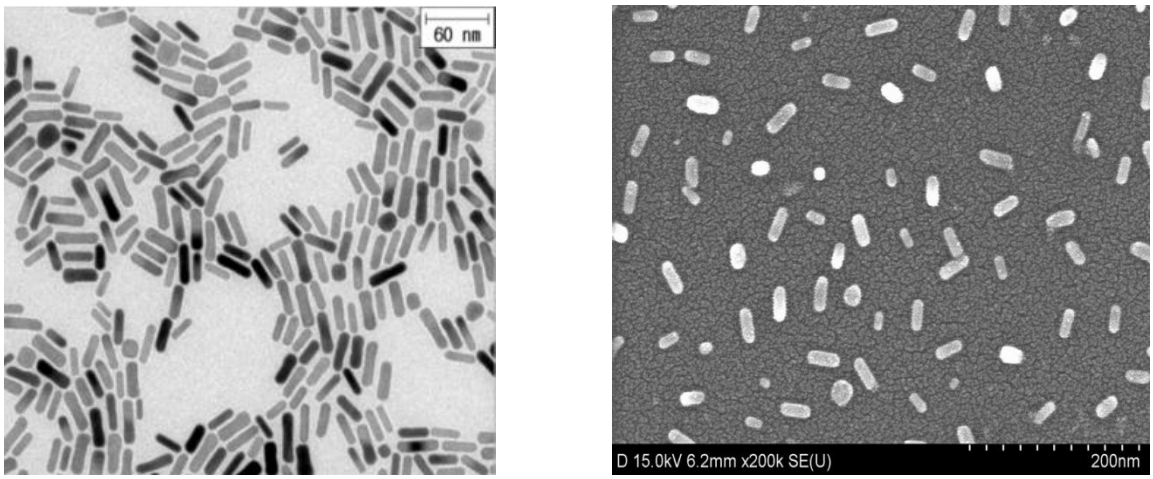


Figure S2 Purification of cysteine tagged scFv. Cell-free synthesized scFv was purified using Ni-NTA agarose beads. Purified scFv was determined using a coomassie blue stained gel. ScFv was detected as the major band (28 kDa).

Expressed Cys-scFvs in the cell-free mixture were found to be nearly completely soluble in the GroEL/ES-enriched cell extract (data not shown). An indirect ELISA assay was used to demonstrate that the expressed Cys-scFvs have a high binding affinity to CRP (data not shown). Prior to immobilization of the scFvs on the GNR substrate, Cys-scFv was purified and then analyzed by using the SDS-PAGE method (Figure S2). The band size was found to agree with the size of scFv, which was calculated to be ca. 28 kDa. Analysis of the image of SDS-PAGE, stained with Coomassie brilliant blue, provided an estimate of the purity of the prepared scFvs to be *ca.* 95%. The results of a Bradford protein assay showed that the final yield of the prepared scFvs was *ca.* 100 μg per mL of the cell-free synthesis reaction mixture.

(a) **(b)**



(c)

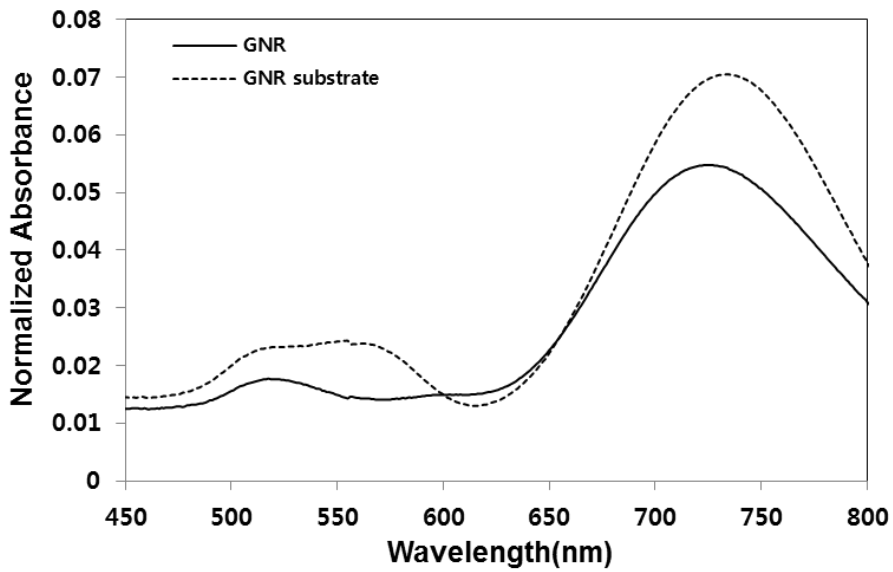


Figure S3 TEM images (a) of synthesized GNR and SEM image (b) of GNR coated surface (GNR substrate) and UV-visible adsorption spectra (c) of GNR and GNR coated surface (GNR substrate).

References for Supporting Information

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