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Supporting Information for:

Rapid transfer of an IgG to a biosensor using antibody binding protein-based probe

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Methods

Materials. The AGL gene was synthesised by Shanghai Sango Biotechnology Co. Ltd. (Shanghai, China). *Escherichia coli* DH5α was used for gene cloning (Novagen, USA), and *E. coli* SHuffle T7 express lysY was used for protein expression (New England Biolabs, Ipswich, MA, USA). Restriction endonucleases *Age*I and *Not*I were purchased from New England Biolabs, and Ligation High Ver 2 and polymerases KOD-Plus-Neo and Blend Taq were purchased from Toyobo Biochemicals (Osaka, Japan). The primers were synthesised by Shanghai Sango Biotechnology Co., Ltd. Agarose, ampicillin, and isopropyl β-d-thiogalactoside (IPTG) were purchased from Beijing Solarbio Biotechnology Co. Ltd. (Beijing, China). The antibodies used in the experiment including Mouse anti-Testosterone monoclonal antibody (CSB-DA0051CmN①), Ractopamine monoclonal antibody (CSB-MA00181110m), Mouse anti-Estradiol monoclonal antibody (CSB-DA0061CmN①), Dehydroeplandroaterone (DHEA)Monoclonal Antibody (CSB-MA00651110m), Mouse anti-Human Serum amyloid A protein monoclonal antibody (CSB-DA1188mN②), Mouse anti-human Interleukin-6 (IL-6) monoclonal antibody (CSB-DA436EmN④), PD-L1 Monoclonal Antibody (CSB-MA878942A0m), and the metabolite of furazolldone Monoclonal Antibody (CSB-MA00251110m) were purchased from Cusabio Technology LLC (Wuhan, China).

Construction of AGL expression vector. The AGE gene was amplified via PCR using the synthesised AGL gene as a PCR template and primers M13+

(AGGGTTTTCCCAGTCACG) and NotProAGLrev (ACCCGCGGCCGCGCCCGCGAAGCGGATGTTAA). Approximately 50 μL of polymerase chain reaction (PCR) solution contains 5 μL of 10×PCR Buffer for KOD-Plus-Neo, 5 μL of 2 mM dNTPs, 3 μL of 25 mM MgSO4, 1 μL each of primer M13+ and NotProAGLrev, 0.5 μL of DNA template, 1 μL of KOD-Plus-Neo, and 33.5 μL of deionised water. PCR conditions were as follows: pre-denaturation at 94 °C for 2 min, denaturation at 98 °C for 30 s, annealing at 55 °C for 30 s, and extension at 68 °C for 1 min for 30 cycles, after which there is a final extension at 68 °C for 10 min. PCR products were verified by agarose gel electrophoresis and recovered with a DNA gel recovery kit (Vazyme Biotech Co., Ltd., Nanjing, China). Restriction endonucleases *Not*I and *Age*I were used to digest the PCR product and pCysHF-diL2k. Recovered DNA fragments and vector were ligated at a molecular ratio of 5:1 at 16 °C for 2 h and used to transform competent XL10-Gold cells, following which they were evenly spread on LB containing ampicillin at 100 μg/mL and incubated at 37 °C overnight. Single colonies were picked up and cultured to extract the plasmid, and DNA sequencing after the plasmid in the colony was confirmed using the T7 promoter (TAATACGACTCACTATAGGG) and T7 terminator (GCTAGTTATTGCTCAGCGG).

Expression and purification of AGL. The successfully sequenced plasmid was transferred into Shuffle T7 competent cells and cultured overnight at 37 °C in LB plates containing 100 μg/mL ampicillin. A single colony was picked into 4 mL of liquid

medium containing ampicillin and cultivated overnight at 37 °C. The culture was then expanded to 300 mL and cultured at 37 °C until the OD_{600} reached 0.5, and then, IPTG at a final concentration of 1 mM was added. Afterward, the culture was incubated at 16 °C at 250 rpm for 20 h and then centrifuged at 8,000×g for 20 min at 4 °C. The soluble protein solution was then obtained after the bacterial cells were broken and purified using Ni-NTA Sefinose Resin (Shanghai Sango Biotechnology, Co. Ltd.). To determine the quantity and purity of the purified protein, 10 μL of each sample was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and then stained with Coomassie brilliant blue.

Preparation of AGL-probe. After the purified AGL was dialysed with PBST, 100 μL was added to a final concentration of 0.5 mM tris (2-carboxyethyl) phosphine hydrochloride (TCEP), protected from light, and rotated for 20 min. A solution of 4-azidobenzoic acid with a final concentration of 2 mM was added to the reaction mixture and allowed to stand on ice for 10 min. Afterward, 1 μL of carboxytetramethylrhodamine (TAMRA-C5-mal) was added, and the mixture was rotated and shaken for 2 h in the dark. Anti-DYKDDDDK G1 Affinity Resin was used to purify the labelled protein, and the 50 μL of resin and protein was mixed. The mixture was then rotated and shaken for 90 min at 4 °C in the dark, following which the resin was washed 15 times with TBST to remove free fluorescence. It was then incubated with 100 μL glycine–HCl buffer (pH 2.8) for 5 min and eluted, and 10 μL Tris–HCl buffer (pH 8.8) was added to adjust the pH to 7.4. Afterward, 10 μL of the purified

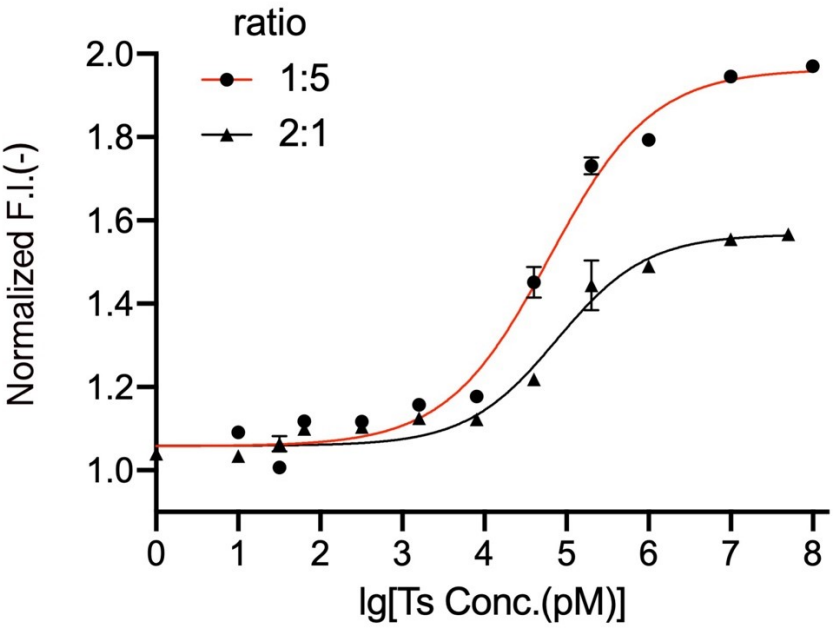
sample was taken for SDS–PAGE, was used to observe the fluorescence of AGL Q-Probe (AGL-p) in a fluorescence imager (GELmieru) (Wako, Osaka, Japan), and was stained with Coomassie Brilliant Blue for visible light imaging.

Enzyme-linked immunosorbent assay. ELISA was used to detect the ability to bind to antibodies. Briefly, 100 ml each of BSA, AGL, and AGL-pat (10 μg/mL) was added to the wells of a 96-well microtiter plate and incubated overnight at 4 °C. After removing the protein solution, the plate was blocked with 2% skim milk for 2 h at 25 °C. After washing three times with PBST, 100 μL of 4000-fold diluted horseradish peroxidase-conjugated rabbit anti-mouse antibody was added and incubated at 25 °C for 1 h. The wells were washed three times with PBST, and 100 μL of substrate solution (100 μg/mL 3,3',5,5'-tetramethylbenzidine and 0.04 μL/mL hydrogen peroxide in 100 mM sodium acetate for colour development, pH 6.0) was added. After incubation for 5 min, the reaction was stopped by adding 50 μL of 10% sulfuric acid per well, and the absorbance was measured at 450 nm and 630 nm using a microplate reader.

Kinetic measurement. AGL and AGL-p were coated on Ni-NTA probe and murine testosterone monoclonal antibody at 25, 50, 100, 200, and 400 nM to detect the kinetics of binding and dissociation between the recombinant protein and monoclonal antibody. The K_D value was calculated by global fitting using Data Analysis 8.1 HD software (Pall ForteBio).

Fluorescence measurements. AGL-p was diluted with PBST to a final concentration of 5 nM. After standing for 5 min at 25 °C, a monoclonal antibody was added to the AGL-p solution at a final concentration of 25 nM or 2.5 nM (Only for TS detection). After reacting for 10 min at 25 °C, the fluorescence was measured on a spectrophotometer at 585 nm with excitation at 546 nm. To draw dose–response curve, the antigen was added at different final concentrations to the solution, and the fluorescence intensity was measured. For detection of TS in urine sample, it was four-fold diluted without changing the amount of AGL-p and antibody in working solution. The four-parameter logistic equation of the dose–response curve was fitted using GraphPad Prism 8 (GraphPad Software, San Diego, California). LOD was calculated as the corresponding concentration of the average blank value plus three times the standard deviation.

Fig. S1 Comparison of the dose-response curves for detection of TS in the probe/antibody ratio at 2:1 and 1:5.


ratio
1:5
2:1
Normalized F.I.(-)
2.0
1.8
1.6
1.4
1.2
1.0
0
1
2
3
4
5
6
7
8
lg[Ts Conc.(pM)]