Electronic Supplementary Information

Functionalized Gold Nanoparticle-Enhanced Competitive Assay for Sensitive Small-Molecule Metabolite Detection Using Surface Plasmon Resonance

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Complete amino-acid sequence for polyhistidine-tagged folic acid binding protein (FBP):
MGSSHHHHHH SSGLVPRGS HMAFTPFPPT QPTASARPL TLMTLDWAL ATITGADSEK
YMQGQVTADV SQMAEDQHLL AAHCDAGKGM WSNRLFRDG DGFAWIERRS
VREPQLTELK KYAVFSKVTI APDDERVLLG VAGFQARAAAL ANLFSELPSK EKVQVEGAT
TLLWFHEPATE RLFLVDEAT ANMLTDKLRE EAEINFSQQW LALNIEAGFP VIDAANSGQF
IPQATNLQAL GGISFFKGCC TGQEMVARAK FRGANKRALW LLAGASRLEP EAGEDLEKLK
GENWRRGTGV LAAVKLEDGQ VVQVVMNND MEPDSIRVR DDANTLHIEP LPYSLEE

Complete amino-acid sequence for polyhistidine-tagged periplasmic binding protein for glutamine (GBP):
MGSSHHHHHH SSGLVPRGS HMAFTPFPPT QPTASARPL TLMTLDWAL ATITGADSEK
YMQGQVTADV SQMAEDQHLL AAHCDAGKGM WSNRLFRDG DGFAWIERRS
VREPQLTELK KYAVFSKVTI APDDERVLLG VAGFQARAAAL ANLFSELPSK EKVQVEGAT
TLLWFHEPATE RLFLVDEAT ANMLTDKLRE EAEINFSQQW LALNIEAGFP VIDAANSGQF
IPQATNLQAL GGISFFKGCC TGQEMVARAK FRGANKRALW LLAGASRLEP EAGEDLEKLK
GENWRRGTGV LAAVKLEDGQ VVQVVMNND MEPDSIRVR DDANTLHIEP LPYSLEE

Bacterial strains, growth conditions and chemicals
E. coli strains XL10-Gold (Stratagene, USA) and BL21 (DE3) (Novagen Inc, USA) were used for all plasmid construction and protein expression studies described in this work. For plasmid construction, E. coli cells were grown in Luria-Bertani (LB) medium at 37 °C and selected using 50 mg/L ampicillin. For protein expression and purification, E. coli cells were grown in LB medium at 25 °C. Ampicillin and IPTG were procured from Gold Biotechnology (St.Louis, MO, USA), while LB medium was prepared using Difco™ LB broth Miller (BD Biosciences, Canada). Restriction enzymes were obtained from New England Biolabs (Pickering ON, Canada) and primer sequences were commercially synthesized from Integrated DNA Technologies (San Diego, USA). All molecular cloning manipulations were performed according to standard protocols described in the Molecular Cloning Manual (Sambrook and Russell 2001).

Plasmid construction
The DNA sequences coding for the Folate Binding Protein (ygfZ, EcoGene Accession Number: EG12685) and the Glutamine Binding Protein (glnH, EcoGene Accession Number: EG10386) were cloned into the protein expression plasmid pET15b (obtained from Novagen Inc, USA) in a sequential manner. The ygfZ and glnH coding sequences were PCR amplified using E. coli genomic DNA as a template using the primer pairs, ygfZ-FW1, -RW1 and glnH-FW1, -RW1, ligated into PCR 2.1-TOPO (Thermofisher Scientific, K45002), and appropriate number of clones were sequence verified to obtain plasmids PCR 2.1-ygfZ and PCR 2.1-glnH. These plasmids were double-digested using NdeI and BamHI and the DNA fragments corresponding to ygfZ and glnH were inserted into NdeI-BamHI digested pET15b. The ligation mixture was transformed into chemically-competent XL10-Gold cells and selected on LB medium which was supplemented with 50 mg/L ampicillin to identify the recombinant plasmids harboring the ygfZ and glnH coding sequences. Selected colonies harbouring the recombinant plasmid were confirmed via plasmid isolation followed by an NdeI-BamHI digest. These plasmids are hitherto referred to as pET15b-ygfZ and pET15b- glnH.

Protein expression
Chemically competent cells of the E. coli protein expression strain BL21 (DE3) were transformed with pET15b-ygfZ and pET15b- glnH and transformants were selected on LB medium supplemented with 50 mg/L ampicillin (LB amp₅₀). The starter culture was initiated by inoculating 25 mL of LB amp₁₀₀ with a single colony and the cells were cultured overnight at 37 °C, 225 rpm. After this incubation period, all
of the starter culture was added to 500 ml pre-warmed LB amp\textsubscript{100}, and the cells were shaken as before for 4-5 h (OD\textsubscript{600}=0.4-0.8). At this point, expression of recombinant proteins was induced by addition of 2 mM IPTG and the cells were allowed to grow overnight at 25 °C, 250 rpm. Following overnight incubation, the cells were pelleted by centrifugation at 11,000 g for 30 minutes at 4 °C. The supernatant was discarded and cell pellets stored at -20 °C.

**Protein purification using Ni-NTA Affinity Column**

The cell pellet was thawed, resuspended in 50 mL of Lysis buffer (25 mM Tris, 100 mM NaCl, pH 6.5-7.5) and sonicated on ice (six times for 15 s at 42% amplitude). Following sonication, the cell lysate was centrifuged for 802000 g for 60 minutes at 10 °C and the protein supernatant was transferred to a clean tube. The protein supernatant was carefully loaded onto a Ni-NTA chromatography column (pre-cleaned by washing with lysis buffer) and allowed to flow-through under the influence of gravity. The chromatography column was washed sequentially with five volumes of wash buffer 1 (0.1% Triton X-100 with 25mM Tris 25mM NaCl pH 6.5-7.5) and 5 volumes of wash buffer 2 (25mM Tris 25mM NaCl pH 6.5 to 7.5). The imidazole gradient was re-established by washing the column with approximately 50mL of 25mM Tris 25mM NaCl pH 6.5 - 7.5. Following this final wash, the protein bound to Ni-NTA was eluted by setting up a gradient from 0 to 500mM imidazole in 25mM Tris 25mM NaCl pH 6.5 to 7.5, and running the gradient at a flow rate of 1.0mL/minute for 90 minutes (BioLogic LP, BIORAD). The gradient was run for an additional 15 minutes at a flow rate of 1.0mL/minute of 500mM imidazole 25mM Tris 25mM NaCl pH 6.5 to 7.5 to ensure all protein was eluted off the column. Protein fractions were collected at 1.2 minute intervals (BioLogic Biofrac Fraction Collector, BIORAD). The different fractions were checked on an SDS-PAGE gel (4% stacking, 14 % resolving), the relevant fractions enriched for purified protein were pooled and dialyzed into 100 mM sodium phosphate buffer (pH 7). Protein concentration was determined by the MicroBCA Protein assay kit (product number 23235, Thermo Scientific, Rockford, USA).
Fig. S1 Extinction spectra for as-purchased 10 nm citrate capped AuNP (blue), the AuNP after modification with DTSP-AuNP (green), and FA-AuNP (red).
Fig. S2 Representative difference images of three SPRi chip surfaces after exposure to 1 nM (A), 2 nM (B), and 2.5 nM (C) FA-AuNP solutions, respectively. Each chip surface was modified with FBP (five spots in the middle) and GBP (four spots at the corners) before exposure to FA-AuNP solution.
**Scanning Electron Microscopy (SEM):** SPRi chip surfaces with FBP and GBP after exposure to the least concentrated (94.7pM) 10 nm FA-AuNP solution were air-dried, mounted on specimen stubs and examined under Philip/FEI XL 30 SEM at 10.0 kV.

**Fig. S3** Scanning electron micrographs of SPRi Au chip surfaces with (A, B) FBP (× 60.0 k, × 300 k) and (C) GBP (× 60.0 k) after exposure to 94.7 pM of the as-prepared 10 nm FA-AuNPs.
**Fig. S4** Representative sensorgrams for analysis of FA in the (A) micromolar (µM) range and (B) nanomolar (nM) range. The concentrations of 10 nm FA-AuNPs in the competitive assays are (A) 4.74 nM and (B) 189 pM.