Electronic Supplementary Information

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

Yong Cao[a,b], Bethany Griffith[b], Prasanna Bhomkar[b], David S. Wishart[b,c,d], and Mark T. McDermott*[a,b]

[a]Department of Chemistry, 11227 Saskatchewan Drive, University of Alberta, Edmonton, Alberta T6G 2G2, Canada

[b]National Institute for Nanotechnology, 11421 Saskatchewan Drive, Edmonton, Alberta T6G 2M9, Canada

[c]Department of Computing Science, 2-21 Athabasca Hall, University of Alberta, Edmonton, Alberta T6G 2E8, Canada

[d]Department of Biological Sciences, CW 405, Biological Sciences Building, University of Alberta, Edmonton, Alberta T6G 2E9, Canada

E-mail: mark.mcdermott@ualberta.ca

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Complete amino-acid sequence for polyhistidine-tagged folic acid binding protein (FBP):
MGSS**HHHHHH** SSGLVVPRGS HMAFTPFPPR QPTASARLPL TLMTLDDWAL ATITGADSEK YMQGQVTADV SQMAEDQHLL AAHCDAKGKM WSNLRLFRDG DGFAWIERRS VREPQLTELK KYAVFSKVTI APDDERVLLG VAGFQARAAL ANLFSELPSK EKQVVKEGAT TLLWFEHPAE RFLIVTDEAT ANMLTDKLRG EAELNNSQQW LALNIEAGFP VIDAANSGQF IPQATNLQAL GGISFKKGCY TGQEMVARAK FRGANKRALW LLAGSASRLP EAGEDLELKM GENWRRTGTV LAAVKLEDGQ VVVQVVMNND MEPDSIFRVR DDANTLHIEP LPYSLEE

Complete amino-acid sequence for polyhistidine-tagged periplasmic binding protein for glutamine (GBP): MGSS**HHHHHH** SSGLVPRGSH MADKKLVVAT DTAFVPFEFK QGDKYVGFDV DLWAAIAKEL KLDYELKPMD FSGIIPALQT KNVDLALAGI TITDERKKAI DFSDGYYKSG LLVMVKANNN DVKSVKDLDG KVVAVKSGTG SVDYAKANIK TKDLRQFPNI DNAYMELGTN RADAVLHDTP NILYFIKTAG NGQFKAVGDS LEAQQYGIAF PKGSDELRDK VNGALKTLRE NGTYNEIYKK WFGTEPKGS

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 *Nde*I and *BamH*I and the DNA fragments corresponding to *ygfZ* and *glnH* were inserted into *Nde*I-*BamH*I 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 *Nde*I-*BamH*I 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 amp_{50}). The starter culture was initiated by inoculating 25 mL of LB amp_{100} 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 $amp_{100,}$ and the cells were shaken as before for 4-5 h (OD_{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 beads 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).

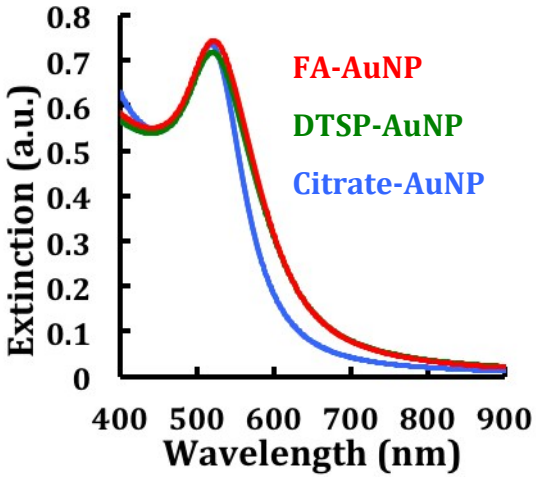

Fig. S1 Extinction spectra for as-purchased 10 nm citrate capped AuNP (blue), the AuNP after modification with DTSP-AuNP (green), and FA-AuNP (red).

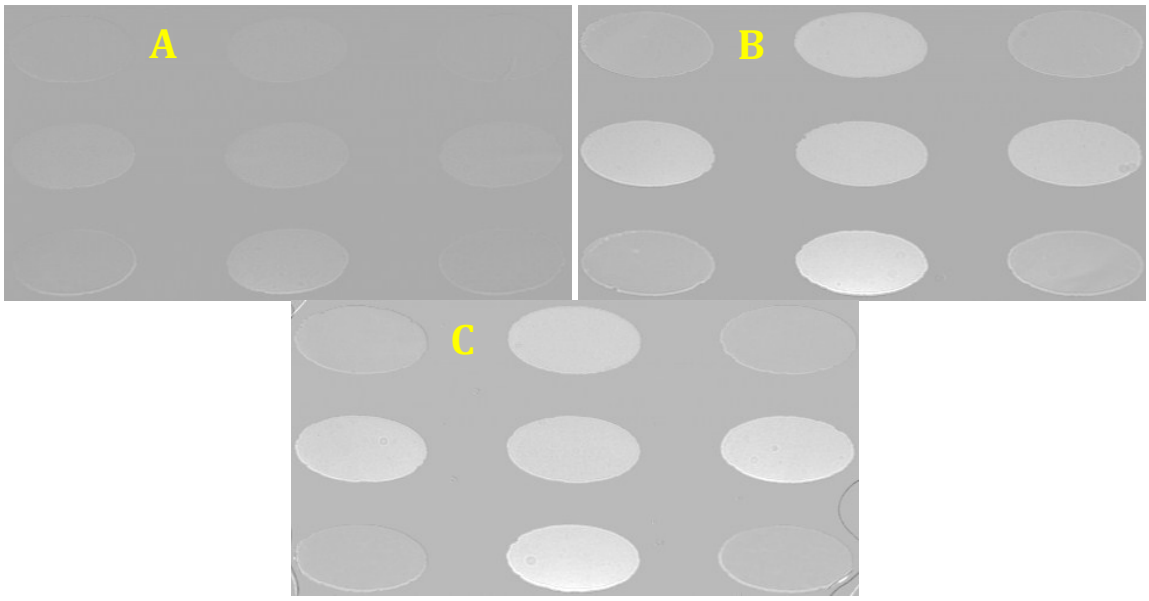

Fig. S2 Representative difference images of three SPRi chip surfaces after exposure to 1 nM (A), 2 nM (B), and 2.5 nM (C) FA-AuNP solutions, respectively. Each chip surface was modified with FBP (five spots in the middle) and GBP (four spots at the corners) before exposure to FA-AuNP solution.

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.

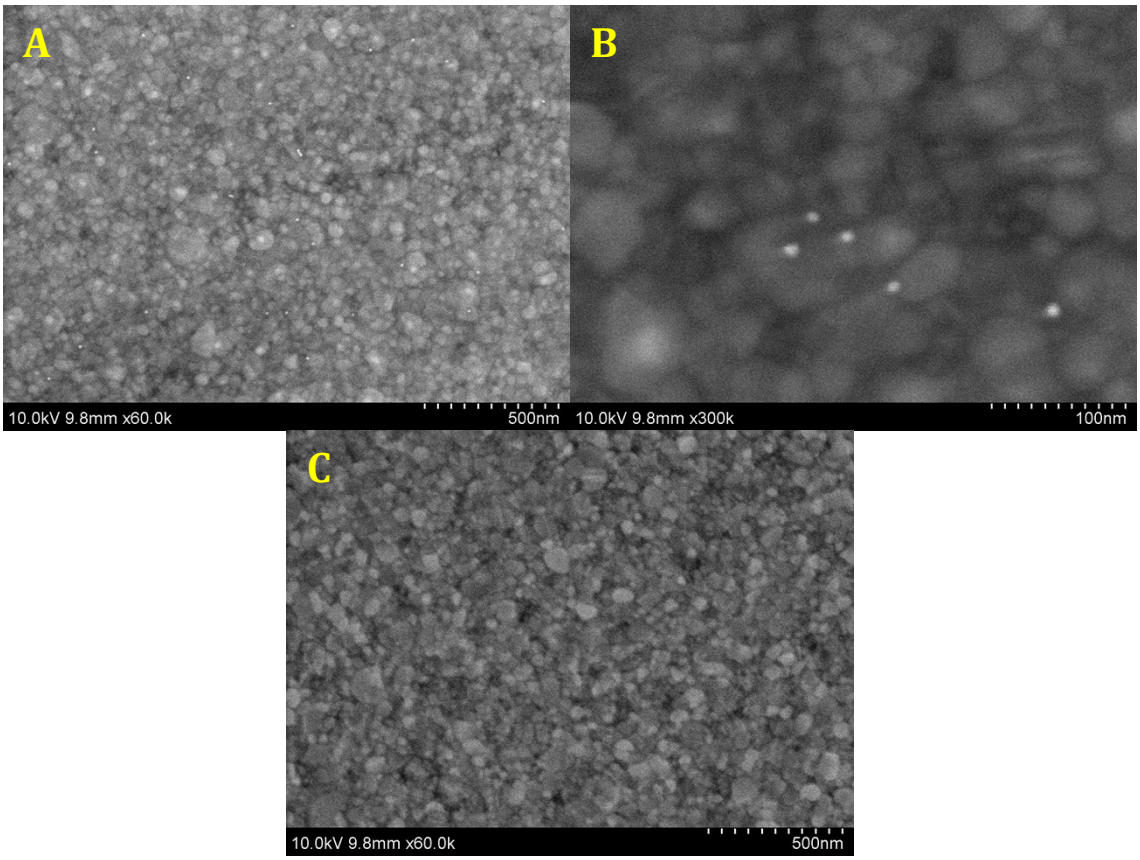

Fig. S3 Scanning electron micrographs of SPRi Au chip surfaces with (A, B) FBP (× 60.0 k, × 300 k) and (C) GBP (× 60.0 k) after exposure to 94.7 pM of the as-prepared 10 nm FA-AuNPs.

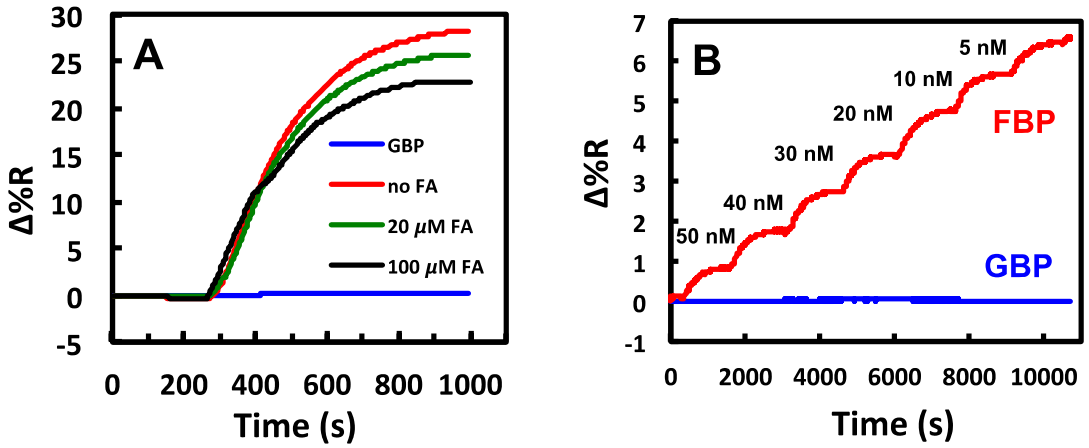

Fig. S4 Representative sensorgrams for analysis of FA in the (A) micromolar (***μ***M) range and (B) nanomolar (nM) range. The concentrations of 10 nm FA-AuNPs in the competitive assays are (A) 4.74 nM and (B) 189 pM.