Supplemental content for: Development of nucleic acid probes capable of direct and selective homocysteine detection in human serum

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S1.1 Preparation of homocysteine modified sepharose

L-homocysteine was prepared from homocysteine thiolactone as previously described[1]. 35 mg of L-HcyT was reacted with 250 µL of 5 M NaOH for 15 minutes at room temperature. The reaction was then heated to 100°C for 25 minutes and 1.25 mL of 1 M NaH_2PO_4 was added to the reaction followed by storage at 4°C for 3-4 days to allow precipitation of excess NaCl. Following centrifugation at 10,000 *g* for 10 minutes, the supernatant was removed and filtered through 0.22 µm Spin-X filter tubes. Solvent was removed *in vacuo* and the product was stored under argon at 4°C until further use. Product was verified in D_2O by NMR spectroscopy using a Bruker 300 MHz Spectrometer. L-Hcy: ^{1}H NMR (300 MHz D_2O): δ 3.80 (m, 1H, C*H*NH_2), 2.55 (m, 2H, C*H*$_2$SH), 2.10 (m, 2H, C*H*$_2$$CH_2$S-) and L-HcyT: ^{1}H NMR (300 MHz D_2O): δ 4.25 (dd, 1H, C*H*NH_2), 3.45 (m, 2H, C*H*S), 2.78 (m, 1H, C*H*$_2$$CH_2$S-), 2.20 (m, 1H, C*H*$_2$$CH_2$S-).

L-Hcy was coupled to pre-activated carboxyl-NHS-Sepharose. 10 mg of L-Hcy was dissolved in 0.5 mL coupling buffer containing 0.1 M 2-(*N*-morpholino)ethanesulfonic acid (MES) and 0.5 M NaCl. Drained NHS-Sepharose (1 mL) was mixed and allowed to react for 2 hours with mild shaking. The supernatant was removed following a 5 minute centrifugation at 10,000 *g*. The sepharose was washed 5 times by incubation with 1 mL of 50 mM Tris, pH 10.4 for 30 minutes to quench unreacted amines. The newly modified sepharose was stored at 4°C until further use. Sepharose modified with L-cysteine and L-methionine was prepared in the same way.

Coupling efficiency of L-Hcy was determined using the Ellman's test. A 40 µM DTNB solution was prepared in 10 mM phosphate buffer, pH 8.0. 15 mg of drained L-Hcy sepharose was reacted for 5 minutes with 0.5 mL of the prepared DTNB solution. The supernatant was removed by filtration through Spin-X filter tubes. Coupling efficiency was assessed by measuring the concentration of the produced thiolate ion in solution at 412 nm[2].

S1.4 Cloning and sequencing

S1.2 Preparation of magnetic beads derivatized with homocysteine or counter targets

ProMag 3 Series Carboxylic acid Surfactant Free Bangs were covalently modified with either ≥98% L-homocysteine, L-methionine, L-cysteine and L-serine according to the instructions from the beads manufacturer. Aliquots of 500 µL (approximately $5x10^8$ beads) were washed 5 times with coupling buffer (0.1 M K_2HPO_4, 0.15 M NaCl, pH 5.5) and magnetically separated from the supernatant using a Dynal MPC-S, 6 x 1.5 mL tube supermagnet (Invitrogen). 500 µL of a $5x10^{-5}$ M solution of target and 5 mM EDC was reacted with the beads for 60 minutes, followed by 5 washes with 10 mM Tris-HCl pH 7.4 to remove excess target and EDC.

Coupling efficiency of L-homocysteine and L-cysteine was verified using the Ellman's test as described in section 2.2 using 500 µL of derivatized beads.

S1.3 Preparation of AuNPs

All glassware used for AuNP synthesis was cleaned by soaking in aqua regia (3:1 mixture of concentrated HCl/HNO_3) for 15 minutes followed by thorough rinsing with deionized water. A 250 mL Erlenmeyer flask was used to mix 98 mL of deionized water and 2 mL of 50 mM $HAuCl_4$ for a final concentration of 1 mM $HAuCl_4$. The solution was heated to boiling with magnetic stirring. Upon boiling, 10 mL of 38.8 mM sodium citrate was added. Heating was continued for an additional 20 minutes following a change in suspension color to red. The flask was removed from the heat and allowed to cool to room temperature with continued stirring.

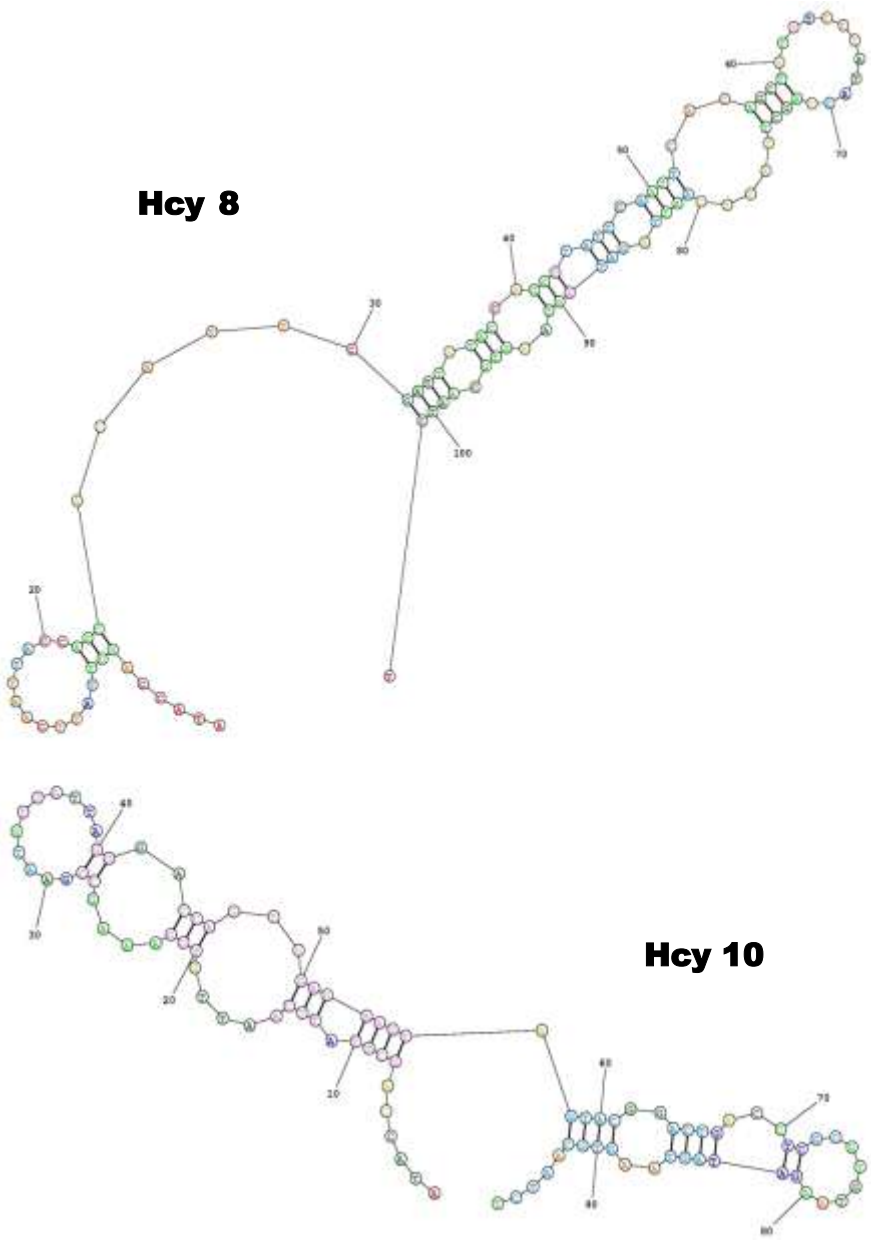

Figure S1 : Secondary structures of putative L-Hcy aptamer sequences predicted by RNAstructure folding software[3].

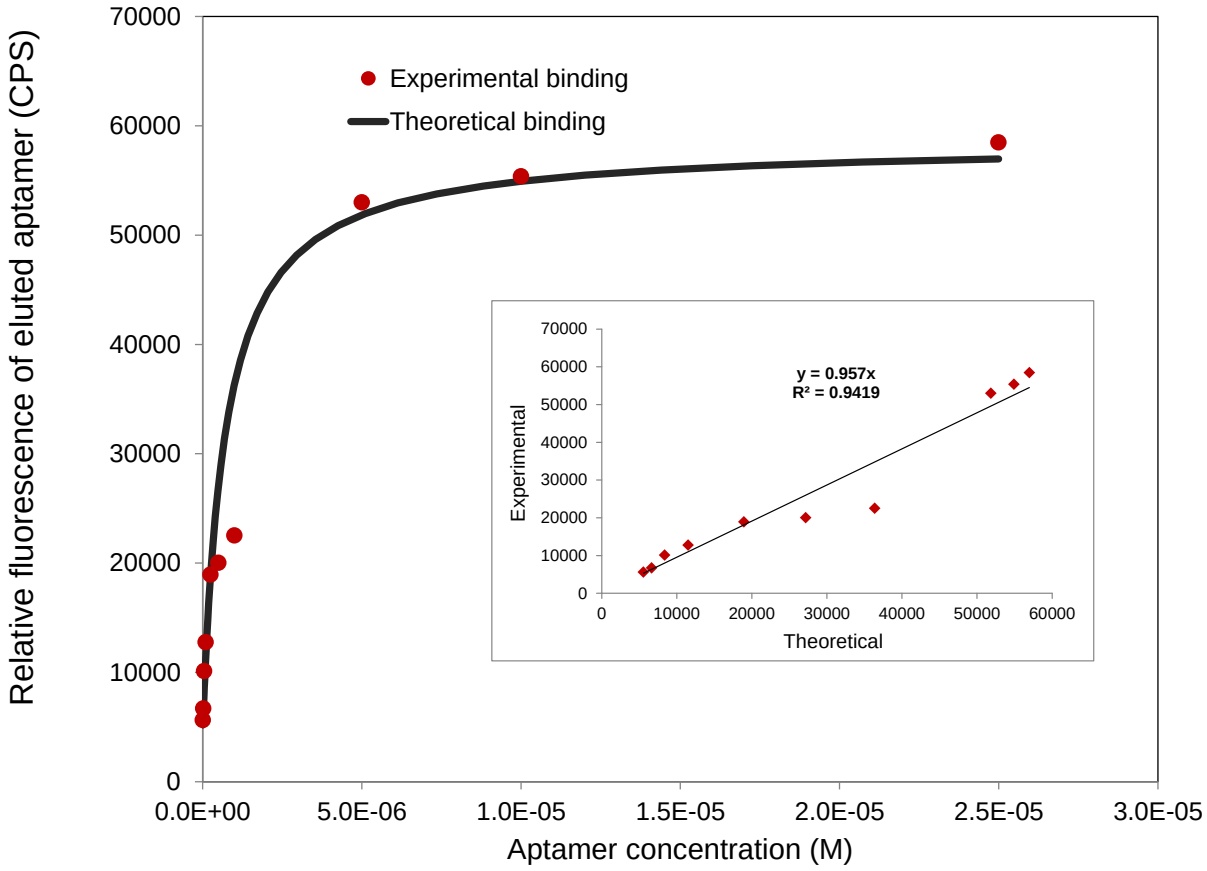

Figure S2: Sample binding isotherm for aptamer Hcy8 with L-Hcy-modified magnetic beads obtained using affinity chromatography. DNA aptamer concentration was varied and incubated with $2x10^8$ L-Hcy beads. A theoretical curve was fit using Microsoft Excel Solver. K_D was determined to be 700 nM for this trial. Inset: Plot of the experimentally determined fluorescence vs. the theoretical fluorescence.

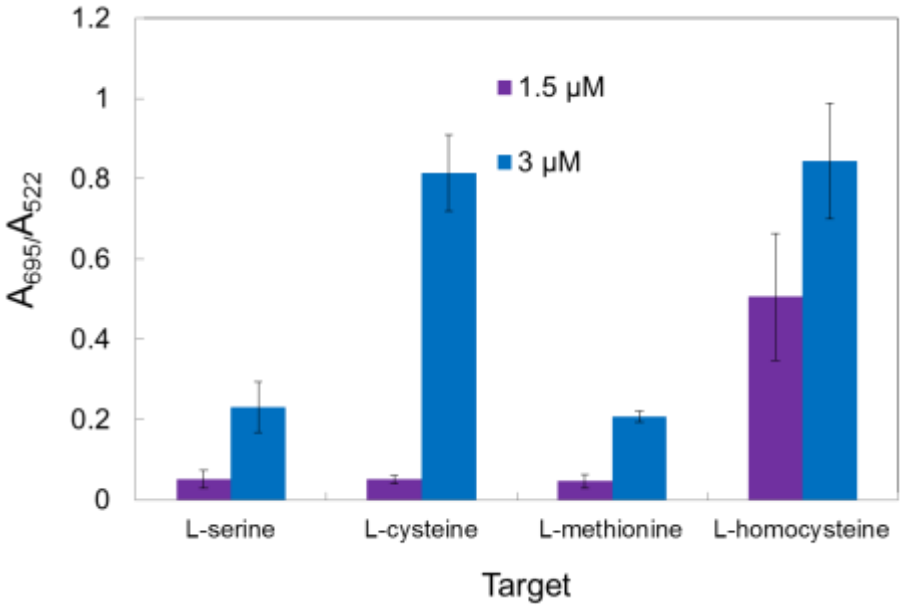

Figure S3: Selectivity of the aptamer-AuNP biosensor biosensor at 1.5 and 3 μM target concentration. At 1.5 μM, the sensor is more responsive to homocysteine than to the other amino acids. At 3 μM, the sensor is more responsive to both homocysteine and cysteine.

1 J. A. Duerre and C. H. Miller, *Anal. Biochem.*, 1966, **17**, 310-315 (DOI:10.1016/0003-2697(66)90209-0).

2 J. Sedlak and R. H. Lindsay, *Anal. Biochem.*, 1968, **25**, 192-205 (DOI:10.1016/0003-2697(68)90092-4).

3 D. H. Mathews, M. D. Disney, J. L. Childs, S. J. Schroeder, M. Zuker and D. H. Turner, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 7287-7292 (DOI:10.1073/pnas.0401799101).