Electronic Supplementary Information

Fluorogenic Displacement Biosensors for PSA Detection Using Antibody-Functionalised Quantum Dot Nanoparticles

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S.1 Peptide Synthesis

The peptides (Figure 1a and 1b) were manually synthesized employing standard Fmoc-Solid Phase Peptide Synthesis (SPPS) strategy using empty fritted polypropylene tubes. The amide linker-resin was swelled in anhydrous DMF for 30 minutes before the first coupling reaction. The acylation steps were conducted using four equivalents of the FMOC amino acids, which were preactivated with HCTU and DIPEA in DMF in a molar ratio of 1:0.95:2 of amino acid, HCTU and DIPEA, respectively. Coupling reactions were conducted for at least 1 hour and monitored by the picrylsulphonic acid test. FMOC deprotection of the coupled amino acids was accomplished by treatment with 20% (v/v) piperidine in DMF (2 x 10 minutes). Successful deprotection was confirmed by picrylsulfonic acid test. Excess of reagents and impurities were removed by extensive washing with DMF, methanol and dichlorometane. The cleavage of the peptides from the solid support, along with the simultaneous removal of the acid-labile side-chain protecting groups, was achieved by treating the peptide-resin with a 95:0.5:0.5:0.5:0.25 solution of trifluoroacetic acid (TFA): water: phenol: thioanisole (TA): triisopropylsilane (TIPS) for 3 hours at room temperature. Next, the resin was filtered off, washed with TFA, and the filtrate concentrated under a stream of nitrogen at 40 °C. Ice cold diethyl ether was added to precipitate the peptides, and the organic solvent was removed by centrifugation. The obtained peptide were washed several time with diethyl ether to remove any residual scavenger traces, and finally dissolved in 50% acetonitrile (acn) in water containing 0.1% TFA and freeze-dried for 24 hours. Finally, the peptides were purified by reverse-phase high-performance liquid chromatography (RP-HPLC) using water/acetonitrile gradients containing 0.1% TFA. The purity and correct mass of the final peptides were confirmed by analytical RP-HPLC and ESI-MS, respectively.

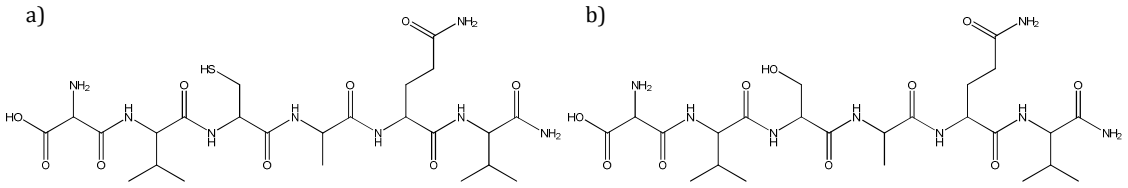

Figure S1 - Structures of peptide a) H-DVCAQV-NH_2 and b) H-DVSAQV-NH_2

S1.1 BHQ-3 Conjugation

Conjugation of peptide DVCAQV to Black Hole Quencher-3 (BHQ-3 Biosearch Technologies, California, USA) was accomplished following the reported procedure in Stefflova *et al*[1]. Briefly, DVCAQV was dissolved in DMSO with 1% DIPEA before reacting for 2 hours with equimolar BHQ-3-NHS in DMSO to give H-DVCAQV-BHQ-3. The reaction was precipitated in ether and dried under vacuum. Final ligand structure was confirmed by MALDI-TOF mass spectrometry (Fig.2 Inset).

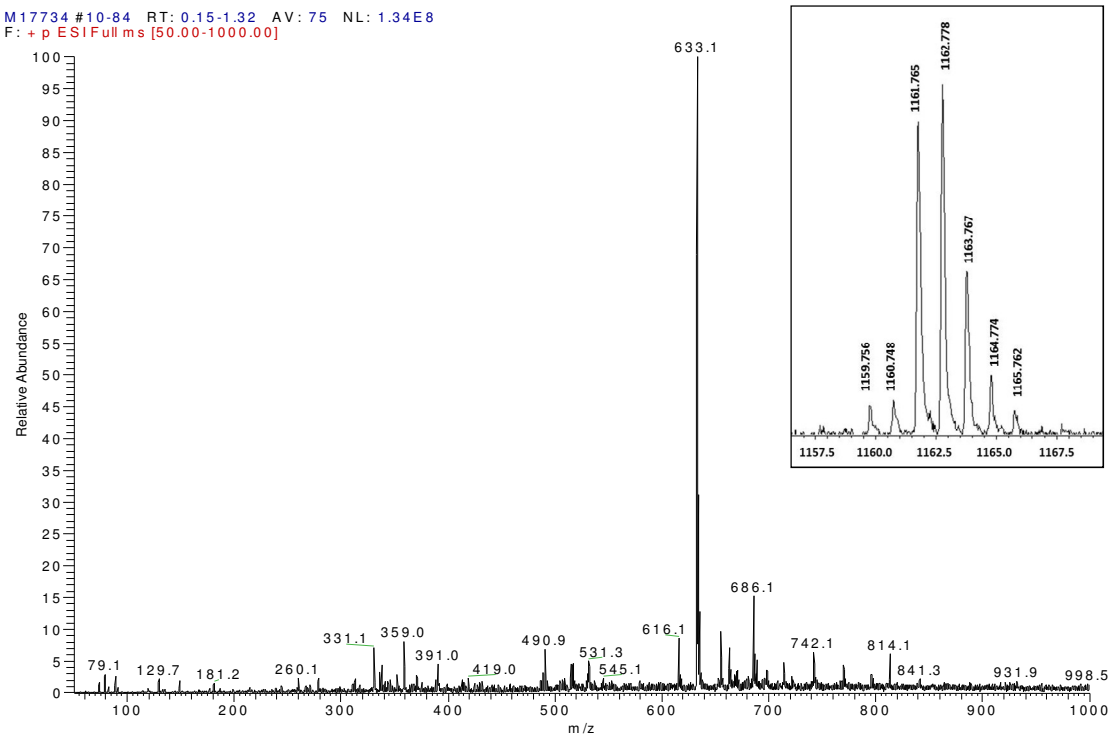

Figure S2 - ESI-MS of peptide DVCAQV (calculated m/z = 632.3). Inset: MALDI-TOF of peptide BHQ3-DVCAQV-NH_2 conjugate (calculated m/z = 1161.5).

S.2 Biacore Affinity Testing

Surface plasmon resonance analysis was conducted on a Biacore T200 (GE Healthcare, Buckinghamshire). Approximately 11,000 response units of antibody were immobilized on a carboxy-methylated dextran matrix CM5 sensor chip after activation with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS). Samples were injected at concentrations of 40 nM in HBS-P running buffer (0.01 M HEPES pH 7.4, 0.15 M NaCl, 0.005% v/v Surfactant P20) and analysed using BIAevaluation software.

S.3 Conjugation of QDots to antibodies

Antibody reduction was achieved by incubation with 1 M aqueous dithiothreitol (DTT), exposing free thiol groups, whilst QDot nanocrystals were simultaneously activated with malemide crosslinkers through the addition of 10 mM succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC). The reduced antibody and activated Qdots were then allowed to react for 1 hour before β-mercaptoethanol was used to block remaining functional groups. Conjugates were finally separated from any unconjugated antibody through a supplied gel filtration column (Invitrogen).

S.3.1 Agarose Gel Electrophoresis

Collected Antibody-QDot625 conjugates, unconjugated nanocrystals and SMCC activated nanocrystals were diluted to a working concentration of 10 nM and loaded onto a 3% agarose gel (Sigma-Aldrich, Poole, UK) in

0.5 X TBE buffer. The gel was run in 0.5 x TBE at 100 V for up to 3 hours.

If an ideal Ab-Nc conjugation ratio of 4:1 is assumed, then approximate conjugate weight will be 800,000 kDa, Conjugates are therefore likely to run slower compared to ~200,000 kDa unconjugated QDots. Activated nanocrystals would also be expected to travel slightly slower due to the addition of SMCC cross-linkers. Fig. 3 illustrates a certain degree of separation that reflects these size differences and therefore suggests successful conjugation.

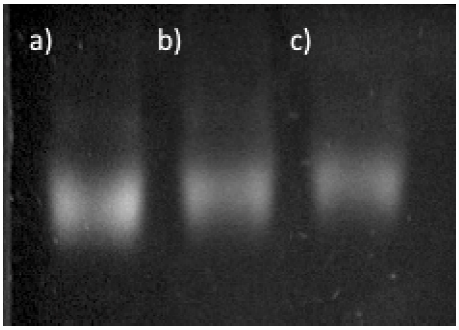

Figure S3 – A 3% agarose gel demonstrating separation of a) Unconjugated Qdot Nanocrystals, b) SMCC Activated QDot Nanocrystals and c) PSA Antibody-Nanocrystal conjugates.

S.4 Spectrofluorimetry

Fluorescence spectra of the final sensor displacement assay were measured on a Biotek Synergy HT microplate spectrofluorimeter. 10 nM Ab-Nanocrystal conjugates were incubated with varying concentrations (5, 10, 20, 40, 80, 160 nM) of quencher-labelled PSA peptide (BHQ3-DVCAQV-NH_2) for 1 hour. Negative PBS controls were used. Emissions were recorded before the addition of 160 nM PSA, whereupon spectra were re-measured.

S.5 DNA Profiling

Blood samples were taken upon informed consent by venipuncture and stored in a BD Vacutainer® Plus tube (Oxford, UK) containing 3.2% sodium citrate coagulation preservative. All tissue samples were stored at 4 °C until analysis. 50 µl of 160 nM assay solution was applied to 150 µl of whole blood before extraction using the QIAmp® DNA Mini kit (Qiagen, Manchester, UK). Quantification was performed by the Quant-iT™ PicoGreen® dsDNA Assay Kit. Samples were diluted to 0.1 ng/µl and amplified with the AmpFlSTR® SGM Plus® PCR Amplification Kit (Applied Biosystems, Paisley, UK) using a Perkin Elmer 9700 thermal (Cambridge, UK). STR amplicons were resolved on an ABI3130 genetic analyser and evaluated using GeneMapper® software.

1. K. Stefflova, J. Chen, H. Li and G. Zheng, *Molecular imaging*, 2006, **5**, 520-532.