Folded Polypeptide Scaffolds for Biosensor and Biochip Applications – Design, Synthesis, Functionalisation and Characterisation

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Abstract

This thesis describes the design, synthesis and evaluation of functional molecular units intended for use in biosensor and microarray applications. A flexible, synthetic helix-loop-helix polypeptide that dimerises to form four-helix bundles was used as a scaffold and was modified with affinity ligands and fluorescent probes to specifically bind a target biomolecule and report on this event in an integrated process. The well-characterised binding of carbonic anhydrase by its benzenesulphonamide inhibitor was employed as a model interaction, and the emission intensity of the probe(s) was found to correlate with carbonic anhydrase concentration. A molecular array, spanning two orders of magnitude in affinity and useful for one-step target quantification, was designed by varying the spacer of the benzenesulphonamide derivative. The scaffold itself was found to contribute to binding, expanding the parameters available for affinity modulation. In a separate study focused on the interaction model system, it was revealed that a destabilising point mutation distant from the carbonic anhydrase active site resulted in faster dissociation rates of the benzenesulphonamide ligand, and that this effect was mediated by increased molecular dynamics caused by destabilisation.

The fluorescence intensity difference displayed by free and target-bound peptides was found to be critically dependent on the position of the probe(s) in the scaffold, showing that the polypeptide fold, providing directionality of incorporated moieties, contributed considerably to peptide function. Dual labelling of the scaffold with different probes in positions where they displayed increased intensity in the corresponding single-probe peptides resulted in a synergistic emission increase upon target protein binding, significantly enhancing sensitivity. The peptides were shown to bind the target protein as monomers, and the molecular basis for sensing was a combination of specific peptide-protein interactions and dimer dissociation. The photochemical crosstalk between the probes was interrupted upon expulsion of one of the monomers upon binding.

Strategies for thiol-dependent attachment of the peptides to modified gold surfaces were explored, and folding of immobilised scaffolds was demonstrated in the case of a model system with controllable dimerisation properties. Results indicating that the sensing ability was retained upon peptide immobilisation were encouraging and prompted future studies on the relation between peptide structure and function, aiming at successful sensor surface and microarray designs for the identification, quantification and characterisation of a wide variety of target biomolecules.

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