Synthetic DNA Holliday junctions as controllable nanoswitch systems

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We have recently shown a class of DNA nanoswitches to be a highly novel detector, capable of specific nucleic acid recognition[1]. The detection principle (Figure 1) relies on monitoring the conformational transition, mediated by switching ions such as Mg^{2+}, between open and closed states of a Holliday Junction (HJ) formed from Watson-Crick base hybridization of probe and target strands.

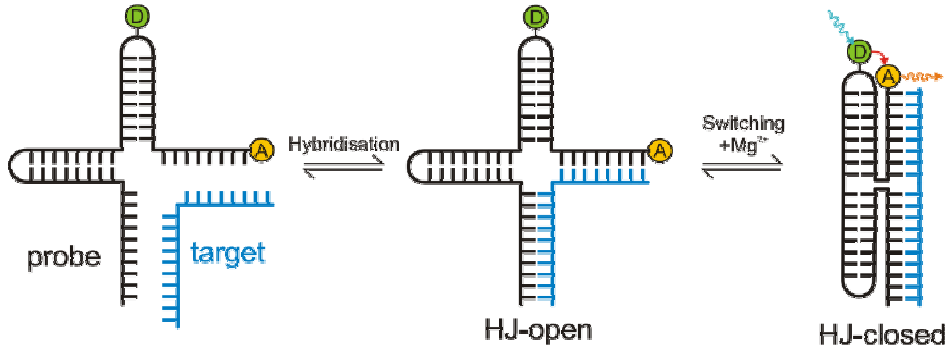

Figure 1 The detection principle for DNA HJ nanoswitches assembled from labelled probe and unlabelled target

Mutations in the unlabelled target located near the junction branch point influence the Fluorescence Resonance Energy Transfer (FRET) output of the dyes (D and A) in the labelled probe and thereby allow for spectroscopic resolution of single base target mismatches. Time-resolved donor fluorescence decays have been used to characterize these nanoswitch biosensors[2]. The fully matched target shows single peak distributions corresponding to the open and closed conformations, reflecting the binary nature of the nanoswitch. Single base target mutations lead to distinct bimodal distributions, in which both open and closed states are observed under ionic conditions where the perfectly matched junction is completely closed. This behaviour is consistent with mutation causing from modest (subtle structural perturbations) to marked (folding inhibition) effects. We have demonstrated the principle that selective probe target binding, combined with sequence-dependent conformational switching, affords molecular recognition precision beyond the limits imposed by the base-pairing energetics and avoids the need for target labelling. As such, these systems offer significant advantages over current biosensing technologies. The principle of electronic control of the molecular conformation of these synthetic HJs is also demonstrated, through the electrochemical generation and removal of switching ions. The methods developed here and the observations reported provide a basis for integrating electronic circuitry with these nanoscale biosensors. Such coupling is a necessary step towards the development of electronically-addressable biostructures.

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[2] C.P. Mountford, A.H. Buck, C.J. Campbell, P. Dickinson, E.E. Ferapontova, J.G. Terry, J.S. Beattie, A.J. Walton, P. Ghazal, A.R. Mount, J. Crain, *J. Phys. Chem. B* **2008**, *112*, 2439-2444.