

S008 Probing protein interactions using single molecule fluorescence

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Single molecule methods have been used reveal new details of biological and cellular processes that are undetectable using ensemble methods. However new methods are needed to study biological systems of increasing complexity. Such systems are frequently heterogeneous and the species of interest may be present as a small fraction of all species present.

To address this issue we have developed two colour coincidence detection in order to be able to identify macromolecular complexes, in an excess of the individual components, and determine the stoichiometry of these complexes. Our method uses the experimental data alone to determine the optimum threshold for detection of fluorescence bursts and number of chance coincidence events, without the need to run control experiments, hence can deal with the inherent sample-to-sample variability in complex biological preparations or living cells. This method has been used to study the stoichiometry of human telomerase, the interaction between proteins on the surface of live T cells and to follow the oligomerisation of proteins during amyloid fibril formation. Other more recent applications of this method will also be described.