Enzyme mechanisms studied by single molecule optical sensing

Supervisory team:
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Project description:
Ubiquitination is an important post-translational modification involved in modulation and regulation of protein function in many processes in most, if not all, eukaryotic cell types; ubiquitination goes awry in numerous diseases. Ubiquitination involves the covalent attachment to a target protein (the "substrate") of one molecule, or multiple molecules in chains, of a small protein called ubiquitin (Figure 1). As described below, different types of ubiquitin chains can be assembled; this is important because chain type determines the biological outcome of ubiquitination (e.g. whether the substrate protein is degraded, or its function or location is affected), but currently it is difficult to determine chain type. The aim is to develop a rapid, accurate and user-friendly method to identify the type of ubiquitin chain assembled by any E3 ubiquitin ligase, at the same time providing new insights into ubiquitination mechanism and kinetics.

Ubiquitination, catalysed by ubiquitin ligases, involves covalent conjugation of ubiquitin to the protein substrate via formation of an isopeptide bond between ubiquitin's C-terminal carboxylate and the substrate's N-terminal amine or e-amino group of a lysine residue. In many cases a chain of ubiquitin molecules is assembled on a substrate protein (Figure 1) whereby a ubiquitin monomer is linked to the chain via any one of seven lysines or N-terminus. Ubiquitin chains can involve a single type of lysine linkage or mixed linkages, with each linkage producing a different degree of flexibility and repertoire of conformational states. Since chain type determines biological outcome, this project will provide detailed new insights into the relationships between E3 ligase function and resulting phenotype, and will therefore potentially advance understanding of the relationships between particular ubiquitin ligases and health and disease.

The project will involve a combination of cutting edge physical and biochemical methods. Ubiquitination reactions will be studied using plasmonically enhanced whispering gallery mode (WGM) microcavity sensing (Figure 1). This is the first optical technique capable of directly monitoring structural changes within individual biomolecules such as proteins. A major aim will be establishing whether each type of ubiquitin linkage has a unique WGM signature. Biochemical and chemical biology experiments with Drs Bagby and Whitley at Bath will include ubiquitination assays, and production of ubiquitin and enzymes (ligases and deubiquitinases) modified for immobilisation on gold nanoparticles that are used in WGM sensing. Single molecule WGM sensing studies of ubiquitin chain assembly will be conducted at Exeter with Professor Vollmer's group.

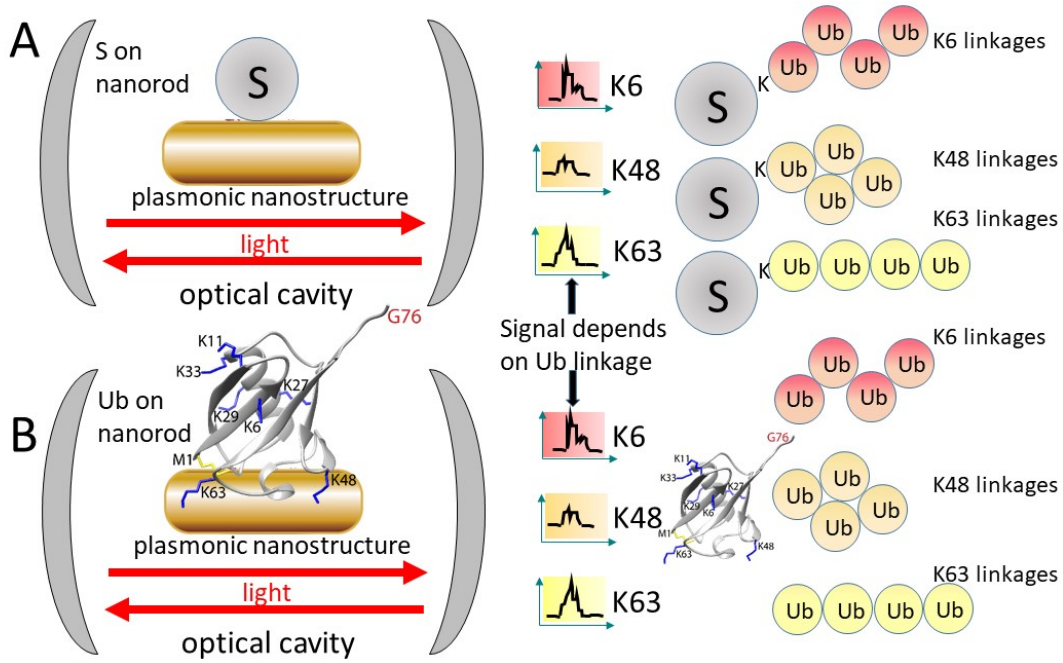

Figure 1 Overall concept. Signal upon ubiquitin (Ub) conjugation to (A) substrate (S) or (B) Ub immobilised on a gold plasmonic nanorod should vary depending on which Lys of Ub forms the new linkage (K6-, K48-, K63-linked Ub chains as examples).