P041 Novel protease responsive nanosensors

Katharina Welser, Weng C. Chan and Jonathan Aylott
School of Pharmacy, University of Nottingham

Optical nanosensors have proven to be a versatile tool to monitor chemical changes in the intracellular matrix with a minimal disturbance of the cellular environment. They consist of a sphere of a biocompatible polymer matrix, in which fluorescent molecules sensitive to the species of interest are physically entrapped. The most beneficial characteristic of these sensors is the protection offered by the sensor matrix against the inherent cell toxicity of some fluorophors. So far, optical nanosensor have been mainly fabricated for the real time measurement of ions (H^+, Ca^{2+}) and small molecules such as glucose.

To increase the range of analytes, we are aiming to develop nanosensors which allow the detection of proteases - a family of enzymes which play a critical role in many biological processes - within the intracellular domain. With this in mind, fluorogenic peptide substrates containing the bifunctional coumarin derivative 7-amino-4-carboxymethylcoumarin (ACC) are prepared using standard Fmoc solid-phase-peptide synthesis. Protease substrates are obtained by conjugating N-peptidyl ACC to amine-functionalized polyacrylamide nanoparticles. In the presence of a protease the peptide-ACC amide bond is cleaved and a change in the fluorescence is observed. These novel nanosensors not only allow the rapid detection of proteases with a minimal disturbance of the cellular environment but may subsequently also provide the means of studying enzymatic processes in living cells.