Re-engineering enzymes as dSTORM detection agents

Davies, A.[1], Harkins, M.[1] and Rickman, C.[1]

[1] Institute of Biological Chemistry, Biophysics and Bioengineering, Heriot-Watt University, United Kingdom

In GSDIM and dSTORM microscopy, endogenous proteins are usually fluorescently labelled via immunolablleing or specific marker molecules, such as the actin label, phalloidin. Antibody labelling involves the use of primary antibodies to recognise the protein of interest and a secondary antibody providing the fluorescent label. Each antibody is 12-15 nm in size so the fluorescent label can be 24-30 nm away from the protein of interest[1]. Additionally, antibody generation can be time consuming and uses animals[2]. Finally, there is a reproducibility issue with antibody usage leading towards a drive to standardise antibodies used in research[3, 4]. Several strategies are being pursued to in order to develop other methods to label proteins, including the much smaller nanobodies (4 nm)[5], but are limited in availability. We decided to use naturally occurring proteins - enzymes, as labels, therefore reducing animals used in research and improving reproducibility because the labels are produced recombinantly. Additional advantages include; they already exist and in a range of sizes and, most relevantly, have evolved substrate selectivity over millions of years.

Clostridium botulinum neurotoxins (BoNT) are the most lethal substances known to man and can result in botulism due to BoNT cleavage of SNARE proteins and subsequent inhibition of neuronal exocytosis[6]. There are seven strains of BoNT, labelled BoNT/A-G, each able to bind to and cleave different SNARE proteins. BoNT/A is able to bind to and cleave SNAP-25. However, two point mutations in BoNT/A have been shown to drastically reduce the rate of cleavage of SNAP-25[7]. These mutations could be exploited to inactivate BoNT/A thus utilising its binding ability to label SNAP-25.

To determine if enzymes can be used as labels, we use a proteolytically inactive mutant of the light chain of BoNT/A to bind to and label SNAP-25. We first demonstrate that the mutant BoNT/A is able to bind to SNAP-25 *in vitro* and, unlike the wild type enzyme, does not cleave SNAP-25 under labelling conditions. Furthermore, fluorescently labelled BoNT/A is able to bind to SNAP-25 in Pheochromocytoma (PC12) cells, consequently enabling dSTORM without the need for antibodies.

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This work was supported by the European Research Council (ERC) through the EU's Seventh Framework Programme (FP/2007 - 2013)/ERC under Grant 339747. In addition, we gratefully acknowledge the expertise and infrastructure offered by the MRC-funded Edinburgh Super-Resolution Imaging Consortium (MR/K01563X/1).