Limitations and alternative probes for imaging biological processes

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Immunostaining is a widely employed technique used to image and study biological processes. Typically a primary antibody will target the protein of interest and a secondary antibody carrying a detection element -such as a fluorophore- will bind to the first antibody. However this approach include limitations: distance between the target and the fluorophore (so called linkage error)[1], penetration hindrance of the probes in the sample[2] and induced clustering due to divalency and policlonality of the probes. Those challenges could be overcome by the use of a monovalent, more soluble and smaller probe, such as single domain antibodies (sdAbs, commonly known as *Nanobodies*). However, the availability of the latter for different targets is extremely limited. In this work, we show for the first time the evident clustering created by the secondary antibodies by visualizing B cells receptors. We could observe decrease of this clustering by the use of secondary Nanobodies. We also show how those secondary Nanobodies could be used to overcome the limit of the species origin of the primary antibody. Exploiting those advantages, we coupled those nanobodies to single strand DNA[3] to perform exchange DNA PAINT allowing multiplexed super resolution microscopy, unlimited by the animal species of the antibody, with minimal linkage error and reduced probe induced artifacts.

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