Bacterially Derived Antibody Binders as Small Adapters for DNA-PAINT Microscopy

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Current optical super-resolution implementations are capable of resolving features spaced just a few nanometers apart. However, translating this spatial resolution to cellular targets is limited by the large size of traditionally employed primary and secondary antibody reagents. Recent advancements in small and efficient protein binders for super-resolution microscopy, such as nanobodies or aptamers, provide an exciting avenue for the future; however, their widespread availability is still limited. To address this issue, here we report the combination of bacterial-derived binders commonly used in antibody purification with DNA-based point accumulation for imaging in nanoscale topography (DNA-PAINT) microscopy. The small sizes of these protein binders, relative to secondary antibodies, make them an attractive labeling alternative for emerging superresolution techniques. We present here a labeling protocol for DNA conjugation of bacterially derived proteins A and G for DNA-PAINT, having assayed their intracellular performance by targeting primary antibodies against tubulin, TOM20, and the epidermal growth factor receptor (EGFR) and quantified the increases in obtainable resolution.

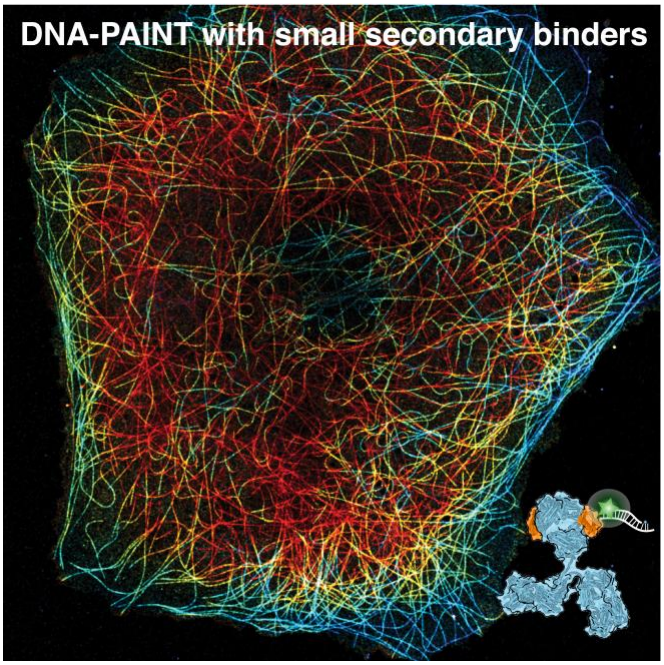

Figure: DNA-PAINT reconstructed image of microtubules obtained by immunostaining with anti-microtubule antibody labeled with protein G. Here the docking for the imaging strand was conjugated to protein G.

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