Seeing the forest, tree by tree - Single molecule superresolution microscopy of multi protein complexes

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Single molecule localization-based superresolution microscopy methods such as PALM or STORM, have been breakthrough techniques of the last years. Until now however, they require special fluorescent proteins to be cloned or high-affinity antibodies to be generated for specific labeling. On the other hand, many laboratories will have most of their constructs in GFP form and entire genomes are available as functional GFP-fusion proteins.
Here, we report a method that makes all these constructs available for superresolution microscopy by targeting GFP with tiny, high-affinity antibodies coupled to blinking dyes. It thus combines the molecular specificity of genetic tagging with the high photon yield of organic dyes and minimal linkage error. Direct STORM on microtubules labeled with our novel antibodies showed that indeed the linkage error was minimal, whereas the large size of standard antibodies resulted in an additional error of >10 nm in immunolabeling. The brightness of our labels enabled us to perform rapid time-lapse dSTORM and sptPALM on living neurons expressing the outer membrane protein GPI-GFP. Three-dimensional dSTORM on microtubules using the biplane approach allowed us to distinguish overlapping microtubules with an axial separation of ~100 nm. Using a budding yeast GFP-tag genomic library we could readily image several GFPtagged proteins targeted to specific intracellular locations.
In summary, targeting of GFP-labeled constructs with tiny antibodies provides fast and simple access to superresolution microscopy of virtually any known protein in cells. Since for several organisms the entire genome is available as GFP-tagged constructs, all these proteins are immediately accessible without the requirement for cloning or the generation of antibodies. Finally, due to a simple one-step labeling protocol, our technique opens the door to highthroughput localization analysis of entire genomes at the nanoscopic level in cells.