Labeling biomolecules in living cells – are there general rules for probe design?

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A large toolbox of fluorescent dyes and proteins enables highlighting cellular structures of interest for following their dynamics in living cells or deciphering fine structural details in the fixed samples. Small fluorescent probes come in particularly handy as they are very easy to use and, most importantly, do not require genetic modification of the cell. These probes are constructed by linking a desired fluorophore to a targeting moiety – a well characterized high affinity drug or inhibitor binding to a particular protein, structure or organelle. Theoretically, this modular design can provide almost endless colour palette of the probes. In practice, finding an optimal fluorophore, ligand and linker combination can make all the difference between useless and top-performing probes. The probes are influenced by multiple factors in the real-world conditions: aggregation, metabolism, off-target interactions, efflux and binding to extracellular structures or proteins (Fig. 1). This is exemplified by examining several series of the probes targeting DNA and cytoskeletal proteins. The optimized probes yield images of outstanding quality in stimulated emission depletion (STED) and single molecule localization (SML) nanoscopy of living and fixed cells.

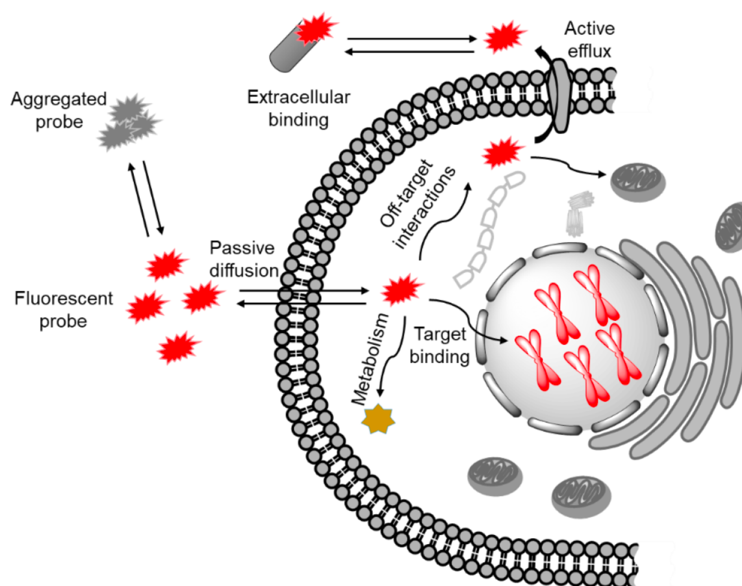

Fig. 1. The possible interactions which are influencing behaviour of the cell membrane permeable fluorescent probes.