Rational design of cyanine-based fluorogenic dimers for background-free imaging of GPCRs in living cells

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Résumé

Fluorogenic dimers with polarity-sensitive folding are powerful imaging probes able to switch on their fluorescence only after interacting with their targets, making them promising tools for live cell imaging.(1) Our team previously reported the first near-infrared fluorogenic dimers derived from cyanine 5.5 dyes for the optical detection of G protein-coupled receptors.(2) However, due to their hydrophobic character, these dimers are prone to form non-specific interactions with circulating proteins such as albumin and with the lipid bilayer of the cell membrane, resulting in residual background fluorescence in complex biological media.
In the search for improved probes, we synthesized and studied a series of less hydrophobic cyanine 5 dimers. By modulating the chemical structure of the cyanine units and after evaluation of various parameters, we selected the novel asymmetric cyanine 5.25-based fluorogenic dimer able to form intramolecular H-aggregates and self-quenched in aqueous media. This optimal probe enabled to significantly reduce the non-specific interactions with bovine serum albumin and lipid bilayers (membrane mimics) as compared to the first generation of cyanine 5.5 dimers.

The optimized asymmetric fluorogenic dimer was grafted to carbetocin, an agonist of the oxytocin receptor, for the imaging of this receptor at the cell surface under no-wash conditions. Herein, we report that the optimal cyanine 5.25 conjugate displays a significant improvement of the signal-to-noise ratio compared to the previous generation of dimeric cyanine 5.5 probes. It enables visualization of the oxytocin receptor without any washing step and without any fluorescent background even in the cell growth medium in presence of serum protein.

References: (1) Klymchenko, A. S. *Acc. Chem. Res.* **2017**, *50* (2) 366–375. (2) Esteoulle, L.; Daubeuf, F.; Collot, M.; Riché, S.; Durroux, T.; Brasse, D.; Marchand, P.; Karpenko, J.; Klymchenko, A. S.; Bonnet, D. *Chem. Sci.* **2020**, *11* (26), 6824–6829

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