Dimerization Interfaces of the GPCR TGR5 Revealed by Integrative Modeling

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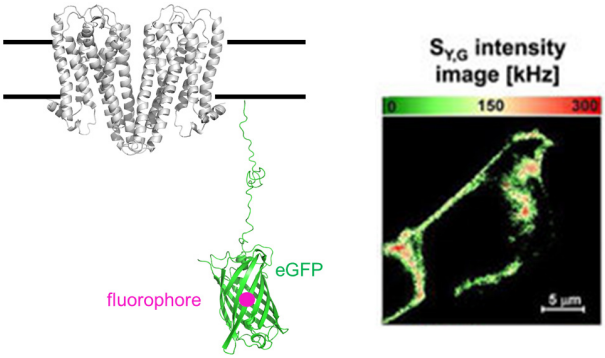
The bile acid sensing G-protein coupled receptor TGR5 is a very interesting drug target[1, 2]. Apart from regulating blood glucose levels, increasing metabolism, and reducing inflammation, it is known to foster the development of various types of cancer if overexpressed[3]. Hence, both enhancing and decreasing the signaling of TGR5 is highly interesting in the treatment of metabolic diseases and cancer, respectively. An emerging factor to influence the activity and signaling of GPCRs is targeting homo- and heterodimers and their formation[4]. Influencing dimers of GPCRs requires knowledge of their dimerization interfaces. However, the di- and oligomerization interfaces of TGR5 are unknown. Here, we present an integrative modeling study, which revealed the primary dimerization and putative oligomerization interfaces of TGR5 [5] based on known GPCR interfaces. We combined homology modeling, molecular dynamics (MD) simulations, and MM-PBSA calculations with live cell multi-parameter MFIS-FRET measurements to determine these interfaces. To measure distances between TGR5 protomers in live cells, the C-termini were labeled with different fluorophores, so that the apparent distance distribution could be used to identify dimerization interfaces. The structures for these interfaces were created via homology modeling of TGR5, based on three interfaces known from X-ray structures of other GPCRs. To calculate the expected FRET distance distributions to be compared to experiment, the TGR5 dimer structures were combined with MD simulations of the linker and fluorophore.



Subsequently, we calculated the effective energies of the snapshots combined with TGR5 dimers via MM-PBSA in an implicit membrane environment. To improve the calculation of the fluorescent dyes' probability distribution with respect to the TGR5 dimers, we estimated the entropic contribution of each snapshot in the ensemble and Boltzmann-weighted the distributions of the fluorophores. Relating the experimental measurements to the calculated distributions allowed us to detect, that the primary dimerization interface of TGR5 utilizes transmembrane helix (TM) 1 and helix 8. This knowledge can be exploited to synthesize bivalent ligands of TGR5 or to alter its dimerization state to influence its function.

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