Dimerization interfaces of the GPCR TGR5

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Introduction & Aims

TGR5 is the first known **bile acid-sensing G-protein-coupled receptor** (GPCR)[1]. Besides its various physiological roles, it is an emerging target for the treatment of the **cholangiocarcinoma**. Thus, targeting TGR5 dimers with bivalent ligands could reduce the risk of side effects [2]. However, the **dimerization interfaces of TGR5 are not known**. The knowledge about these interfaces is crucial for the rational design of bivalent ligands. Here, we present the primary dimerization interface and possible oligomerization interfaces of TGR5. We used **an integrative approach** combining structural modeling, molecular dynamics (MD) simulations, potential of mean force (PMF), and MM-PBSA calculations with live cell multi-parameter MFIS-FRET measurements to determine these interfaces [3]. For this, the apparent distance distributions between fluorescently labeled C-termini of TGR5 were compared to the expected distributions calculated from our TGR5 dimer models. To build these models, we exploited the recently **identified X-ray crystal structures of GPCR dimers (Fig. 1)**.

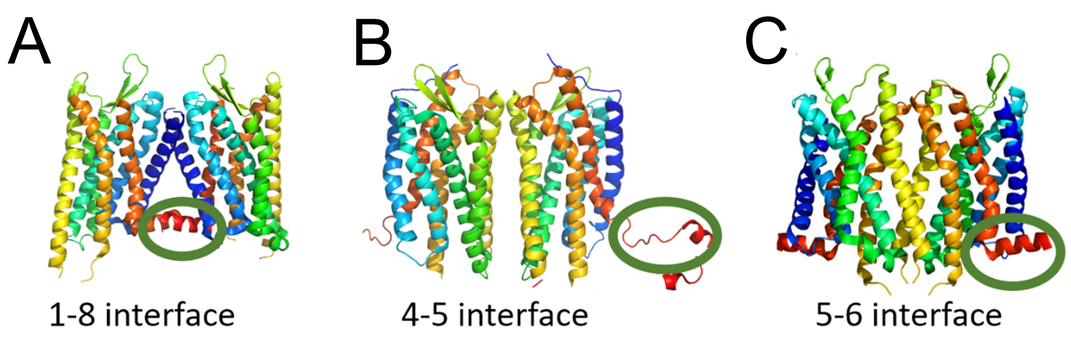

Fig. 1: Dimerization models of TGR5 based on X-ray crystal structures of GPCR dimers with different interfaces: **A** 1-8 interface; **B** 4-5 interface; **C** 5-6 interface. TGR5 monomer chains are **rainbow** colored starting with **TM1** to **Helix 8**.

Methods

The starting point for the integrative modeling were dimer models of TGR5 based on known dimerization interfaces from GPCR X-ray crystal structures. These interfaces utilize transmembrane helix (TM) 1 and helix 8 (**1-8 interface**), TM 4 and 5 (**4-5 interface**), and TM 5 and 6 (**5-6 interface**) (**Fig. 1**). To discriminate between the models, fluorescent probes were fused to the C-terminus of TGR5 via a linker of 42 residues length. Then, **MFIS-FRET** was used to measure the apparent distances between the fluorophores in live cells (**Fig. 2**). To compute the expected distance distributions of the fluorescent probes in an efficient manner, we pursued a step-wise strategy. Initially, we performed **MD simulations of the linker with a fused eGFP in explicit solvent**. Finally, we combined the snapshots of the simulations with the TGR5 dimer models to calculate the conformational free energy with an **implicit membrane MM-PBSA approach**. This energy was then used to Boltzmann-weight the configurations:

$$P_{\text{Boltzmann}} = e^{-\frac{\Delta G}{RT}}$$

Here, we accounted for **configurational entropy** by using a random energy model to describe the energy landscape of the linker heteropolymer [4]:

$$S = R\ln(\Omega P) \quad \text{with} \quad P = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left(\frac{-\left(E_{effective,conf.}-\mu\right)^2}{2\sigma^2}\right)$$

The weighted conformations were finally used to obtain the expected distance distributions between the fluorophores for each dimer interface (**Fig. 3**).

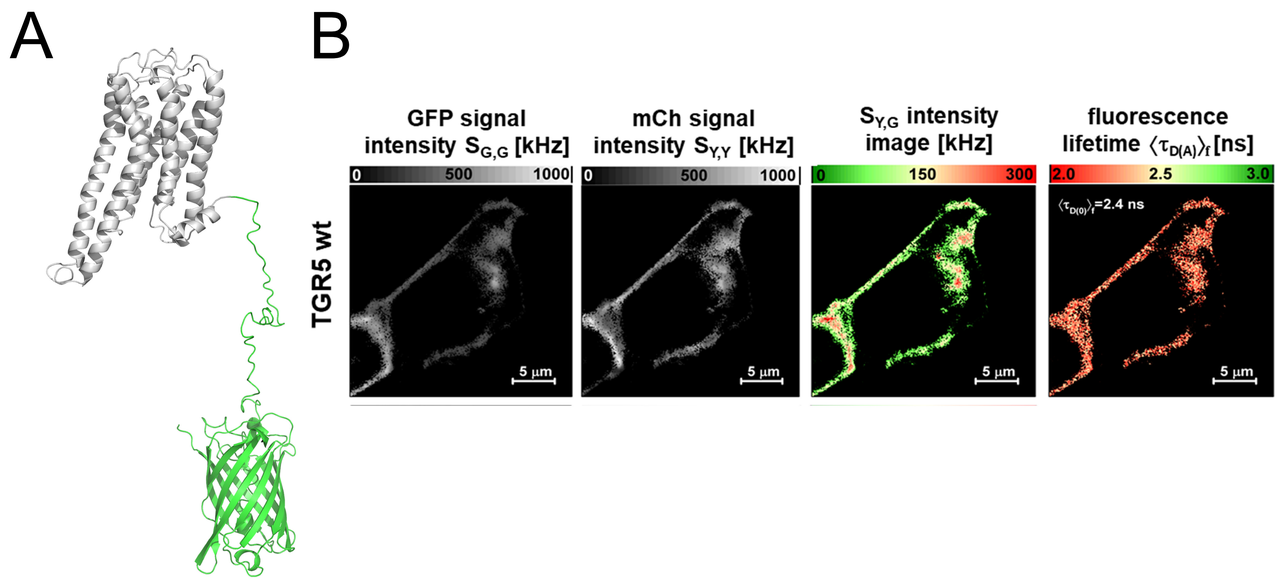

Fig. 2: A Model of TGR5 fused to the **linker** with **eGFP**. **B** Different fluorescence properties measured in live cells with MFIS-FRET.

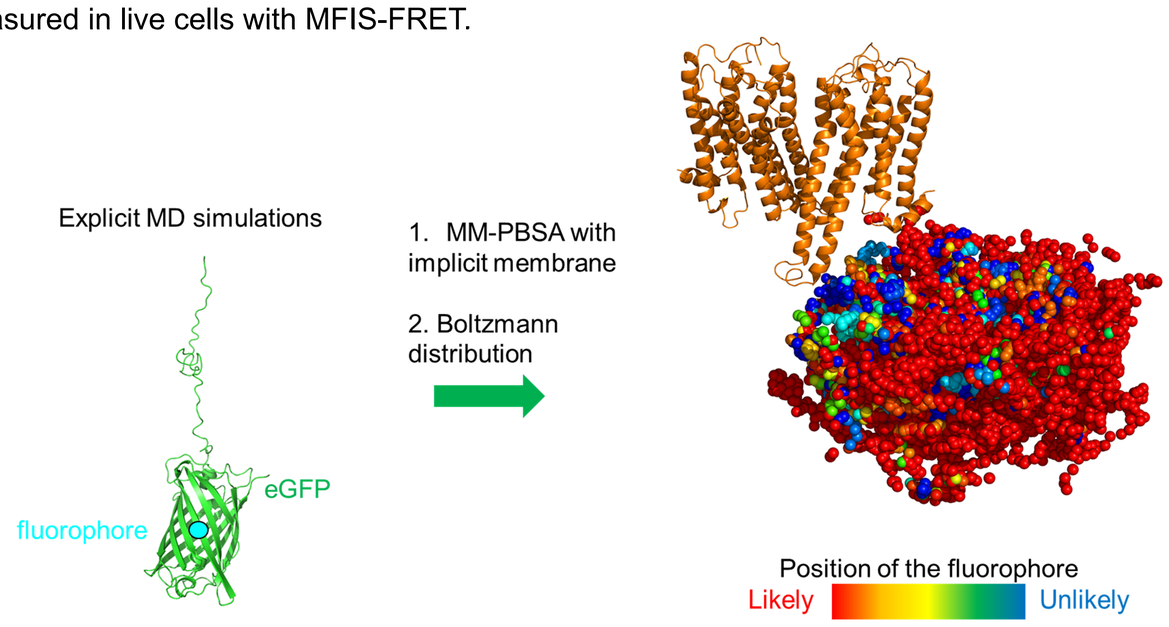

Fig. 3: Schematic of the computational procedure. First, explicit all-atom MD simulations of the linker and fluorophore were conducted. Then, the conformational free energy was calculated via implicit membrane MM-PBSA calculations. Subsequently, these energies were corrected for their entropy and used to weight the likelihood of the presence of the fluorophore (dots on the right) via a Boltzmann distribution.

Results & Discussion

The computed distance distributions of our dimer models **(Fig. 4A)** show that if TGR5 adopts the 1-8 interface, FRET can be measured without interference from the other interfaces. Indeed, the measured and expected distance distributions show a very good correlation, showing that **the 1-8 interface is the primary dimerization interface of TGR5**. Additionally, we modeled the interfaces in their active state and found that due to severe clashes of TM6, TGR5 can not be activated while adopting the 5-6 interface. PMF calculations of the dimerization of the 1-8 and 4-5 interfaces in a membrane showed a favorable energy for the formation of the interfaces with 1-8 being more favorable (**Fig. 4B**). Hence, TGR5 dimerizes via the 1-8 interface and adopts **oligomers via the 4-5 interface** constructing rows of higher order oligomers (**Fig. 4C**).

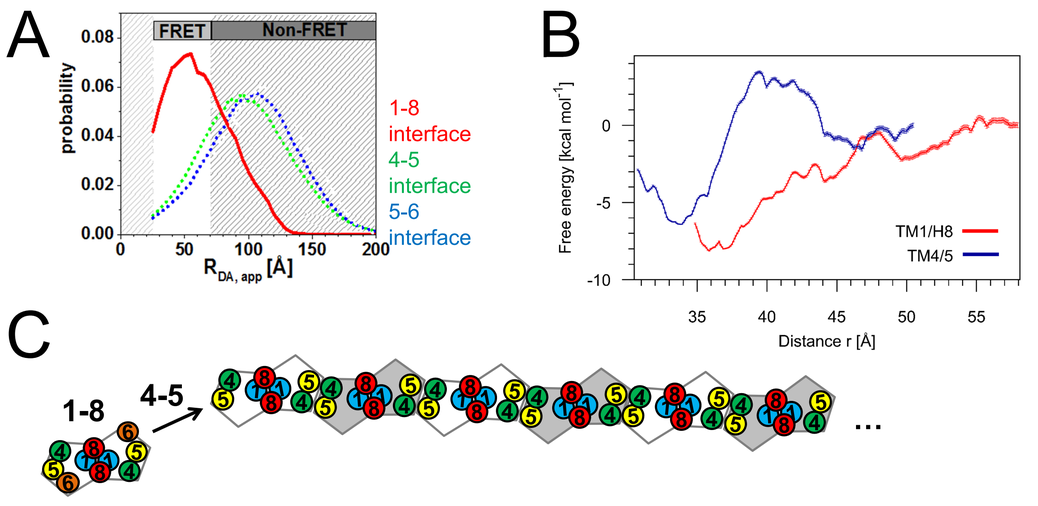

Fig. 4: A Computed distance distributions for our TGR5 dimer models. **B** PMFs of the dimerization of the 1-8 and 4-5 interface.. **C** Possible oligomerization states with 1-8 as the primary dimerization interface, forming higher order dimers of dimers with the 4-5 interface.

Conclusions

We showed that, while TGR5 **dimerizes via the 1-8 interface**, it forms higher order oligomers involving the 4-5 interface. This is the **first tim**e that a distance distribution of fluorophores was simulated in all-atom MD simulations while the entropic contribution of the linker was considered in the conformational free energies. Obtaining these results was only possible by **tightly integrating advanced MFIS-FRET experiments in live cells** with comprehensive computations of the thermodynamic ensembles of fluorophore locations. Our results might aid in the development of novel TGR5 ligands with reduced side-effects.

Acknowledgement

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References

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