SUPPLEMENTARY INFORMATION

Membrane Driven Spatial Organization of GPCRs

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Movie S1 Oligomerization of β_2AR in POPC lipid bilayer. The movie shows key events in how β_2AR oligomers emerge in the course of the simulation. The trajectory is centered at one of the protomers, and shown top-down in the movie. The orientation of the protein is indicated by coloring TM4 (red) and TM5 (blue). For clarity, the lipid beads are depicted as dots, and only shown for the simulation box (and not in the periodic cells). The movie shows four segments in the trajectory, highlighting some of the key events in how the oligomers emerge. The switch from one segment to another is marked by a change in the zoom level, and the corresponding.

Segment 1 (1 – 15s): GPCRs begin to form initial dimeric contacts, locally exploring interfaces.

Segment 2 (16-28s): Dimers of GPCRs

Segment 3 (29-42s): Two neighboring dimers associate to form tetramers.

Segment 4 (43-60s): A fifth GPCR transiently associates with the tetramer to form a pentamer. The interaction is short-lived, and the pentamer dissociates into a tetramer and a monomer.

Table S1: List of CGMD simulations

	Brief Description	**Simulation Length (μs)**	**Lipid type**
1	**Spontaneous oligomerization of eight β_2AR molecules, from monomers to oligomers**	~18	POPC
2	**β_2AR monomer**	2	POPC/10% Chol
3	**β_1AR monomer**	2	POPC/10% Chol
4	**β_1AR dimer with TM1-TM1 interface**	2	POPC
5	**β_1AR dimer with TM5-TM5 interface**	2	POPC
6	**β_1AR pair, facing each other at the TM1 region**	2	POPC
7	**β_1AR pair, facing each other at the TM4/5 region**	2	POPC

Table S2: Residual Hydrophobic Mismatch energy per protomer (in k_BT) calculated for β_1AR dimer constructs.

TM	**TM1-TM1 interface**	**TM5-TM5 interface**	**TM1 region (separated)**	**TM4/5 region (separated)**
1	6.8	21.9	2.8	21.6
2	0	0	0	0
3	0	<1	<1	<1
4	<1	<1	<1	<1
5	<1	<1	<1	<1
6	0	0	0	0
7	0	0	0	0

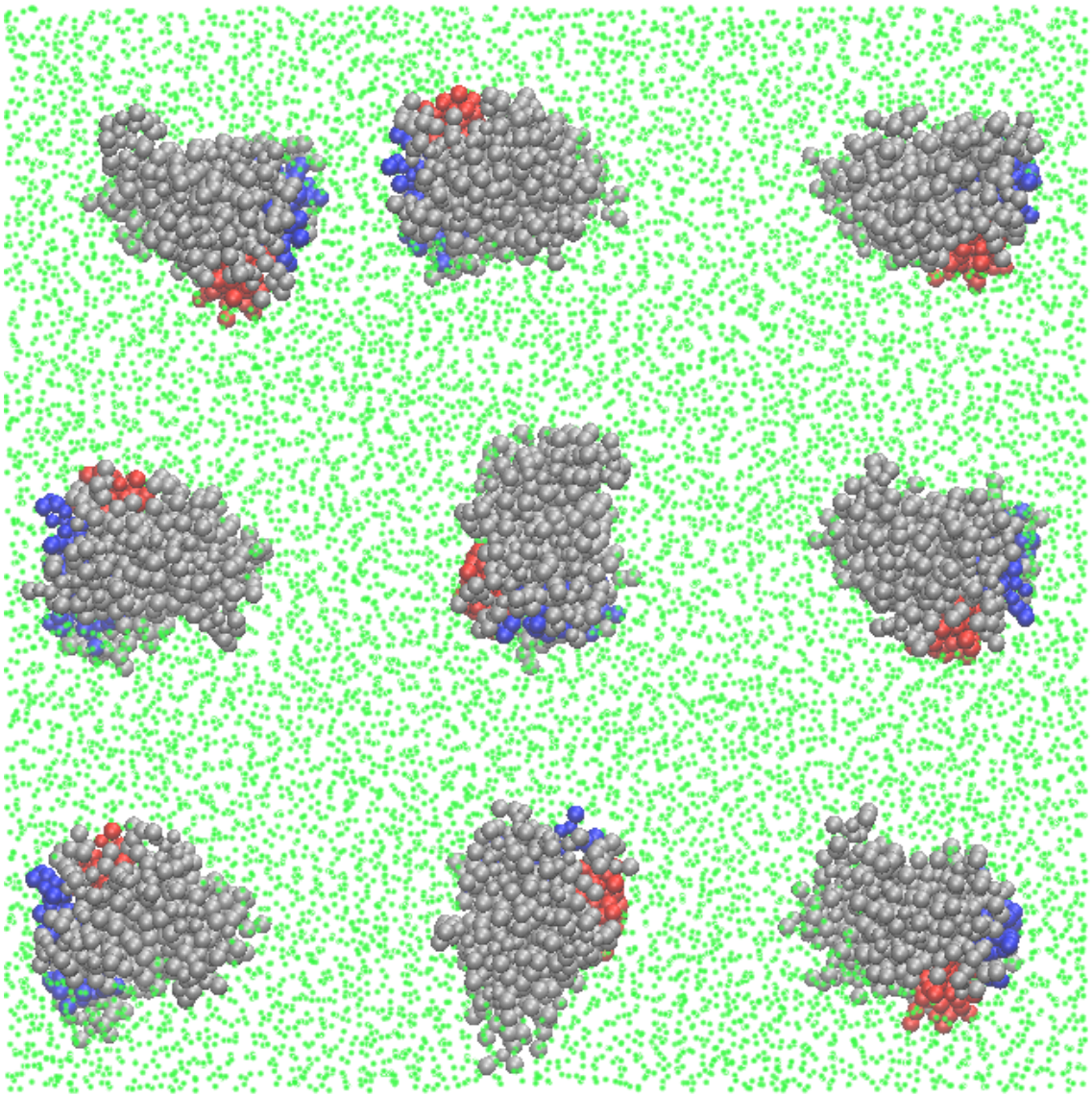

Figure S1 A snapshot from the intial phase of the simulation, presented in a top-down view. The orientation of the protein is indicated by coloring TM4 (red) and TM5 (blue), as also in Movie S1. For clarity, the lipid beads are depicted as green dots, and water is not shown. Near the beginning of the simulation, the GPCRs are far from each other, and they diffusionally rotate to different relative configurations by the time they encounter each other via translational diffusion. (See Supplementary Movie)

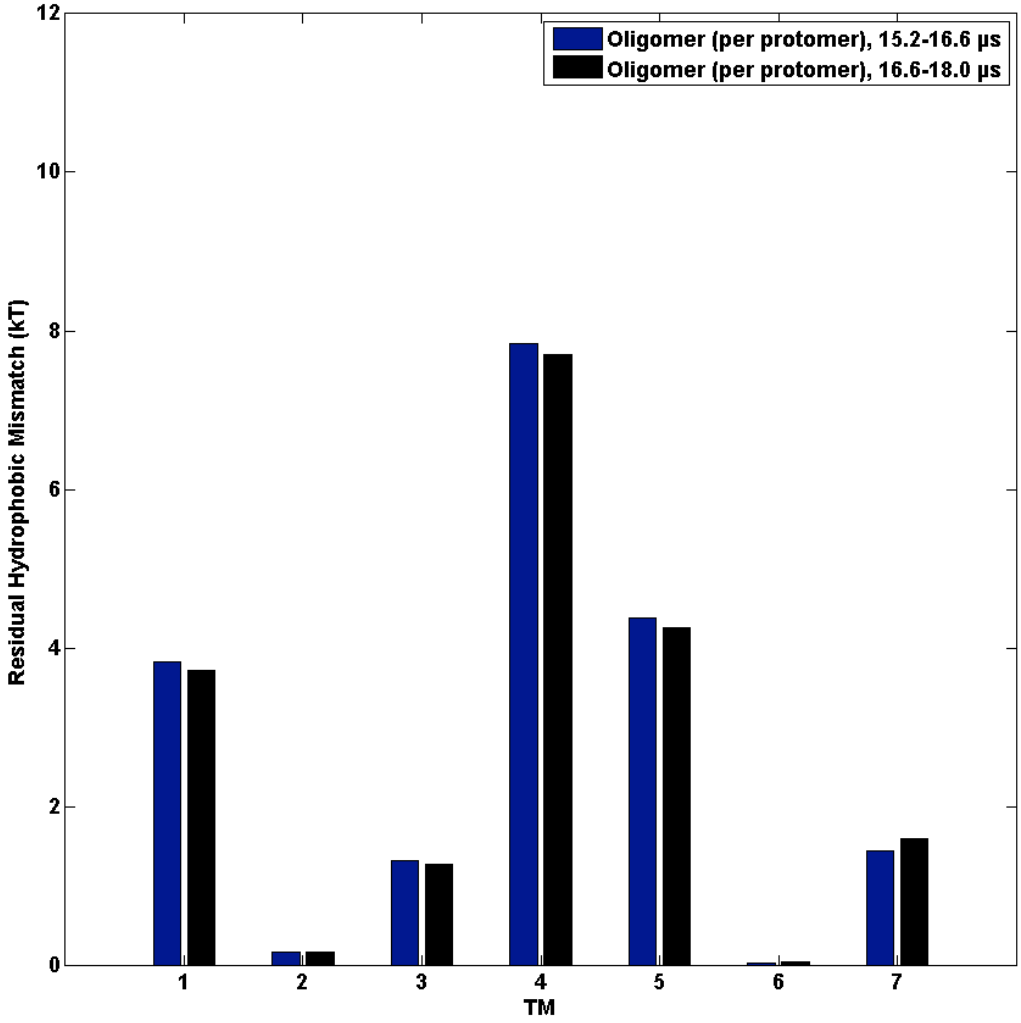

Figure S2 The energy cost due to residual hydrophobic mismatch (RHM) in the TM-bundle of β_2AR, in the last 1.4 μs and the preceding 1.4 μs of the oligomerization simulation. These results show that the RHM at each TM is approximately the same in the two time segments, indicating that the spontaneously emerged oligomerization interfaces result in a RHM that remains robust with respect to time.

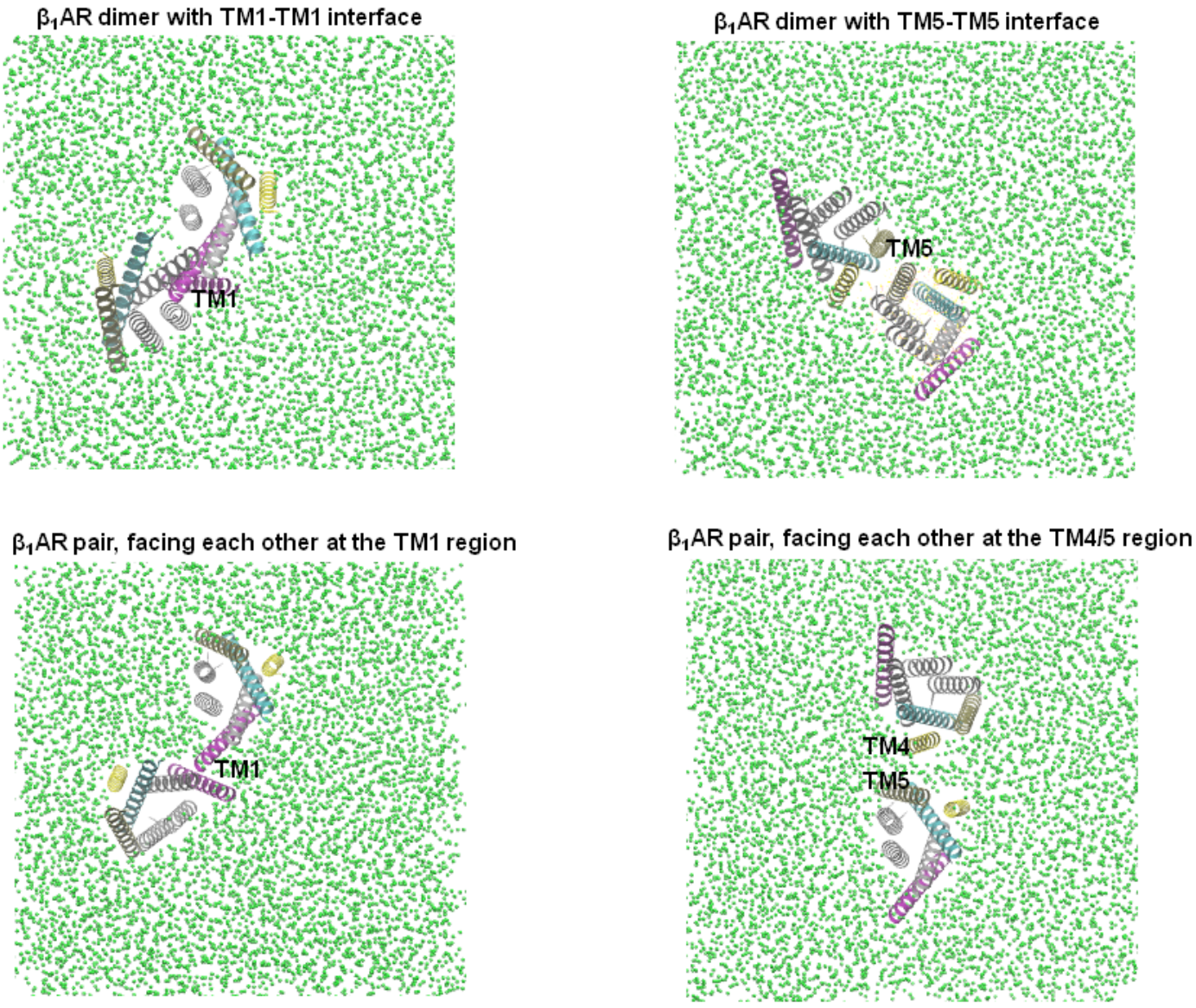

Figure S3 Snapshots from the initial phase of the simulations of β_1AR dimers with different interfaces, shown in a top-down view. The list of simulations is presented in Table S2, and the lipid:protein ratio is ~1:250. The TM-bundle is depicted in cartoon representation, with TM1 shown in purple, TM3 in cyan, TM4 in yellow, TM5 in tan, and TMs 2,6, and 7 in silver colors. The lipids are shown as green dots. For clarity, the lipid beads are depicted as green dots, and water is not shown.