Structural elements in the Gαs and Gαq C-termini that mediate selective GPCR signaling

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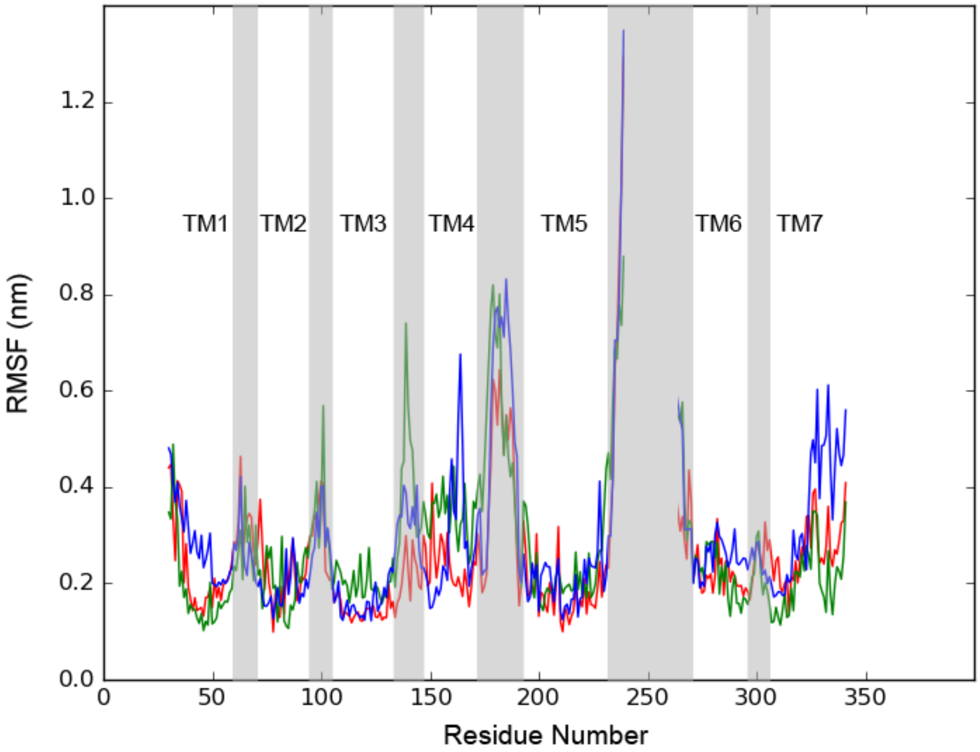
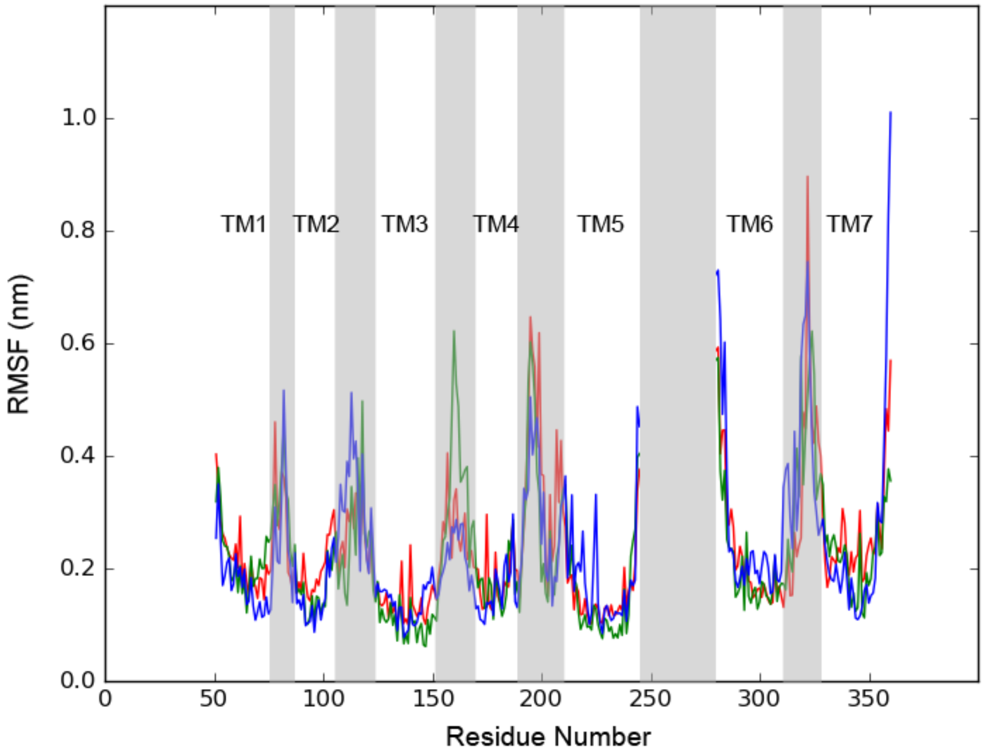
Supplemental Figure 1. *RMSF of β2-AR and V_{1A}R with s, i, and q peptides.* RMSF analysis of total 100 ns ensemble from each receptor-peptide complex. Average structure for RMSF fitting identified by lease-squares fit of entire ensemble. The average RMSF (y-axis) is plotted for each residue in the receptor (x-axis). Extracellular and intracellular loops are denoted with grey shading to distinguish RMSF from transmembrane/helix and loop regions of the receptor. RMSF plots are colored according to the peptide bound: s (red), i (green), q (blue). *(a)* RMSF of β2-AR with s, i, and q peptides. *(b)* RMSF of V_{1A}R with s, i, and q peptides.

a. RMSF of β2-AR with s, i, q peptides





b. RMSF of V_{1A}R with s, i, q peptides

Supplemental Table 1. *Analysis of variance (ANOVA) of ΔFRET for each receptor-peptide pair compared per receptor*. Statistical analysis of ΔFRET values from Figure 1 including the F values and significant *p* values of all receptor-peptide pairings compared per receptor.

Receptor	**F (DFn, DFd)**	***p***
β3-AR	F (3, 64) = 24.02	< 0.0001
D_1R	F (3, 118) = 22.67	< 0.0001
α_2-AR	F (3, 111) = 83.25	< 0.0001
CB_1	F (3, 63) = 101.6	< 0.0001
α_1-AR	F (3, 69) = 8.852	< 0.0001
$V_{1A}R$	F (3, 78) = 58.95	< 0.0001

Supplemental Table 2. *Tukey's Multiple Comparison Test*. Pairwise statistical analysis of ΔFRET values from Figure 1 comparing all means of peptides tested per receptor. Mean difference is the difference between the means of the tested peptide comparison. A significant difference between the tested peptides are represented with the following legend: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$.

Receptor	Peptide comparison	Mean Difference	S.E. of Difference	Sig.
β3-AR	NP vs. s	-0.02467	0.003208	****
	NP vs. i	-0.009918	0.003563	*
	NP vs. q	-0.001885	0.003799	ns
	s vs. i	0.01475	0.003383	***
	s vs. q	0.02278	0.00363	****
	i vs. q	0.008032	0.003948	ns
D$_1$R	NP vs. s	-0.01881	0.002723	****
	NP vs. i	-0.006621	0.0031	ns
	NP vs. q	0.001141	0.003472	ns
	s vs. i	0.01219	0.002755	***
	s vs. q	0.01995	0.003168	****
	i vs. q	0.007762	0.003498	ns
α$_2$-AR	NP vs. s	-0.002332	0.003516	ns
	NP vs. i	-0.03187	0.00308	****
	NP vs. q	0.0006149	0.003581	ns
	s vs. i	-0.02953	0.002624	****
	s vs. q	0.002946	0.003197	ns
	i vs. q	0.03248	0.002711	****
CB$_1$	NP vs. s	0.008413	0.003084	*
	NP vs. i	-0.03256	0.002812	****
	NP vs. q	0.01266	0.003026	***
	s vs. i	-0.04097	0.003016	****
	s vs. q	0.004245	0.003216	ns
	i vs. q	0.04521	0.002956	****
α$_1$-AR	NP vs. s	-0.008844	0.004879	ns
	NP vs. i	-0.01126	0.004762	ns
	NP vs. q	-0.02061	0.004346	****
	s vs. i	-0.002415	0.004233	ns
	s vs. q	-0.01176	0.003759	*
	i vs. q	-0.009348	0.003606	ns
V$_{1A}$R	NP vs. s	-0.001396	0.004833	ns
	NP vs. i	-0.004346	0.004833	ns
	NP vs. q	-0.0429	0.004029	****
	s vs. i	-0.002951	0.005048	ns
	s vs. q	-0.04151	0.004285	****
	i vs. q	-0.03856	0.004285	****

Supplemental Table 3. *Measures of central tendency*. Measures of central tendency are provided for distributions of the TM3 to TM6 distance from simulations of each receptor-peptide complex indicated in the table, and shown in Figs. 2, *b*, and 5, *f*. The mean and median distance are given as an indication of the average TM3 to TM6 distance sampled for each complex, and standard deviation (S.D.) is provided to show the overall variance for this distance across difference pairs of receptor-peptide.

Receptor	Peptide	mean	median	S.D.
β2-AR	s	18.5986	18.5377	0.6708
	i	18.9454	19.1708	1.1618
	q	17.6702	17.6769	0.9922
	q-L349Q/E355Q	19.4268	19.3480	0.8172
$V_{1A}R$	s	15.0258	15.524	1.2769
	i	18.0478	18.245	0.7241
	q	14.8066	14.6131	0.5479

Supplemental Table 4. *Two-sample Kolmogorov-Smirnov Test*. Statistical analysis which assesses the equality of two distributions, with the null hypothesis that both distributions are the same. Each comparison in the table below shows rejection of the null hypothesis at the 95% confidence level. The test-statistic is a measure of the difference between the cumulative density function (cdf) plotted for each distribution in the comparison. The *p*-value indicates the significance of the test result: ****, $p < 0.0001$.

Receptor	Peptide comparison	Test statistic	p	Sig.
β2-AR	s vs. q	0.4673	9.70×10^{-61}	****
	s vs. i	0.3349	2.08×10^{-31}	****
	q vs. i	0.5694	5.75×10^{-90}	****
$V_{1A}R$	s vs. q	0.4045	1.20×10^{-10}	****
	s vs. i	0.9036	8.20×10^{-74}	****
	q vs. i	0.9387	1.44×10^{-82}	****
β2-AR	s vs. q	0.4673	9.70×10^{-61}	****
	s vs. q-L349Q/E355Q	0.4354	8.72×10^{-49}	****
	q vs. q-L349Q/E355Q	0.7110	1.05×10^{-124}	****