Biophysical Journal, Volume *112*

Supplemental Information

Stability and Conformation of a Chemoreceptor HAMP Domain Chimera Correlates with Signaling Properties

Nattakan Sukomon, Joanne Widom, Peter P. Borbat, Jack H. Freed, and Brian R. Crane

Table S1 Mean distances and width at half height of PDS distance distributions (P(r)) of the chimeric Tsr-Aer2H1-3 with the WT Tsr HAMP.

MTSL Spin Label Position	Mean Distances (Å)	Width at Half Height of P(r) (Å)
A220	26	9
R224	23	8
R230	24, 26	4, 3
I232	22, 39	11, 7
G250	25	7
E254	24	5
H258	22	9
Q260	19	6
G261	25	6

Table S2 Mean distances and width at half height of PDS distance distributions (P(r)) of the chimeric Tsr-Aer2H1-3 with WT Tsr HAMP and variants. Broad distributions marked with *.

Genotype	MTSL Spin Label Position	Mean Distances (Å)	Width at Half Height of P(r) (Å)
WT	R230	24, 26	4, 3
	I232	22, 39	11, 7
	H258	22	9
	Q260	19	6
E248L	R230	24, 26	7
	I232	18, 23	4, 2
	H258	23	11
	Q260	21	3
M222P	R230	26*	28
	I232	28, 36*	11, 11
	H258	23	9
	Q260	16, 18	2, 3
A233P	R230	23	8
	I232	24	16
	H258	23	9
	Q260	18	6

Table S3 Mean distances and width at half height of PDS distance distributions (P(r)) of the chimeric Tsr-Aer2H1-3 with WT Aer2 linker and Aer2 E61I

Variant	MTSL Spin Label Position	Mean Distances (Å)	Width at Half Height of P(r) (Å)
WT Aer2 Linker	A220	26	9
	H258	22	9
Aer2 E61I	A220	21	9
	H258	23	14

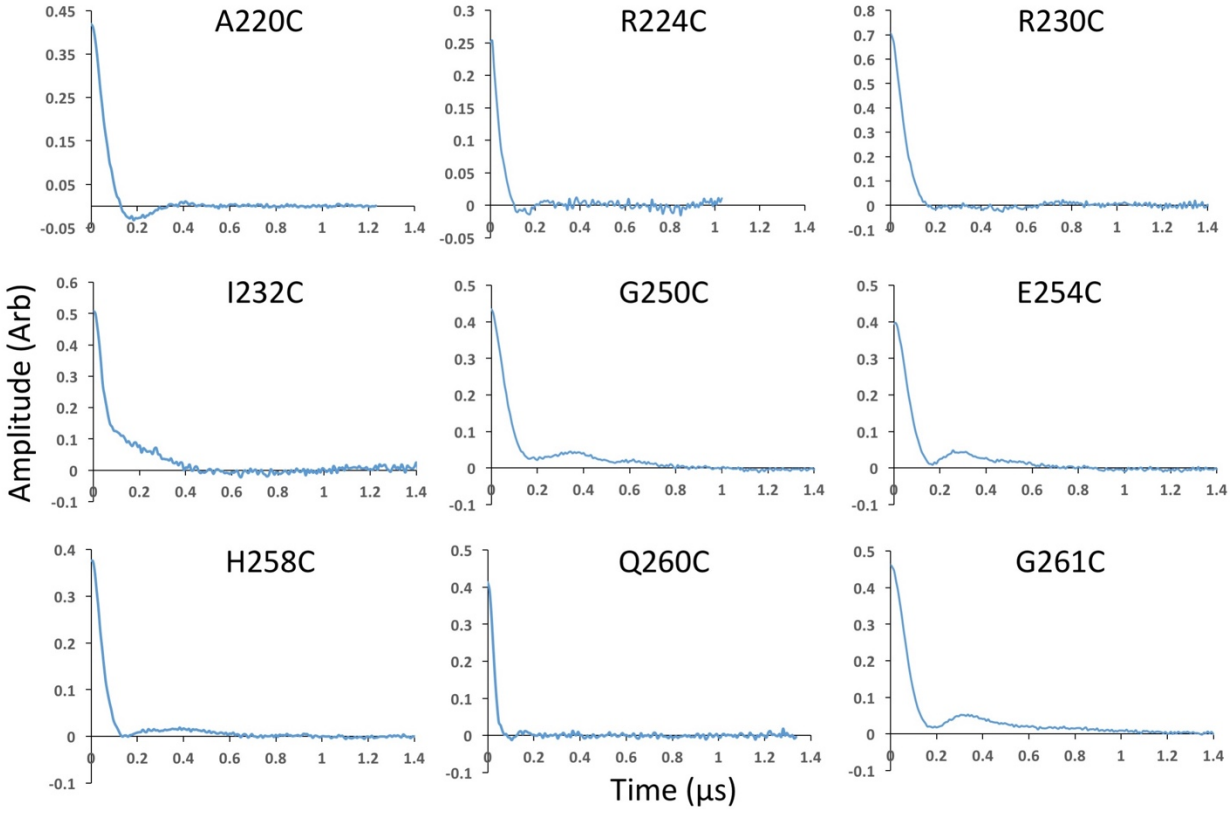

Figure S1 Time domain signals measured by PDS for the chimeric Tsr-Aer2H1-3 with the WT Tsr HAMP. The chosen spin-labeled sites are as indicated.

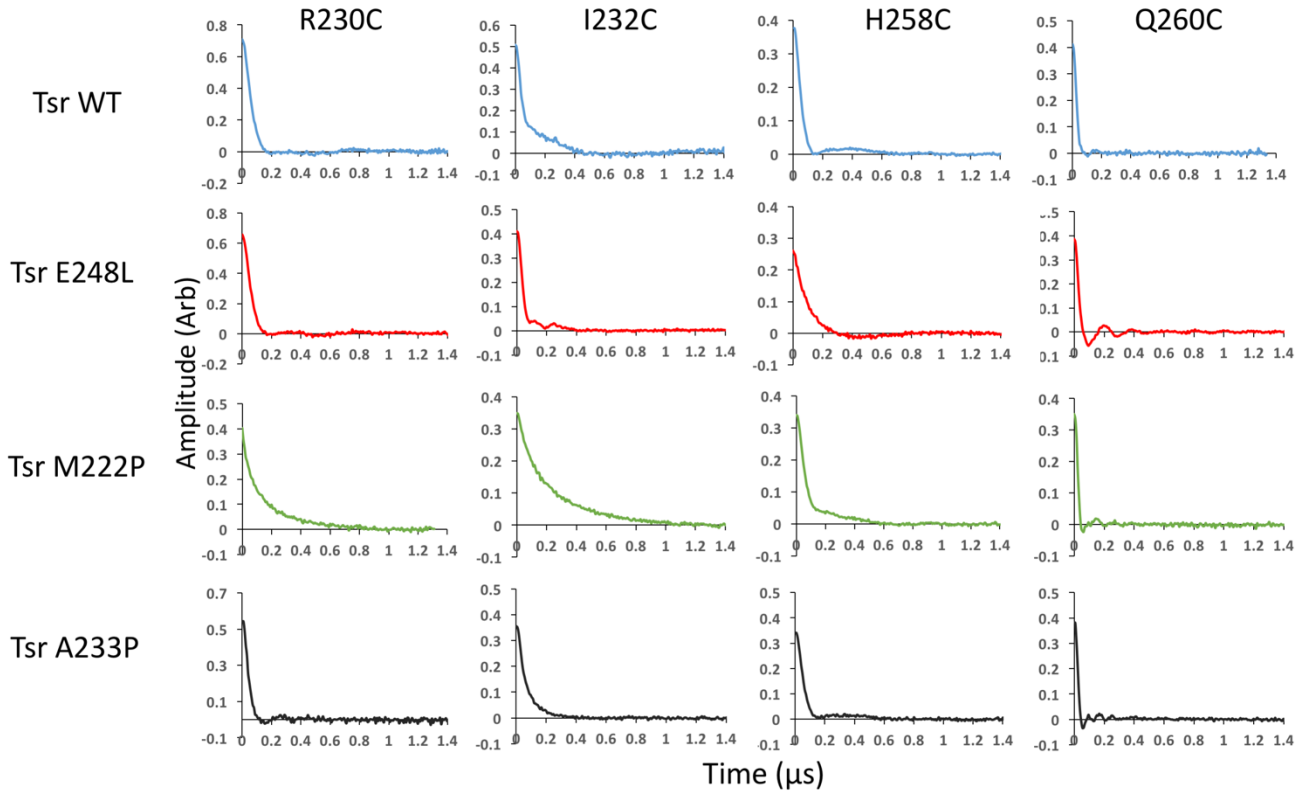

Figure S2 Time domain signals measured by PDS for the chimeric Tsr-Aer2H1-3 with the WT Tsr HAMP and variants. The chosen spin-labeled sites are as indicated.

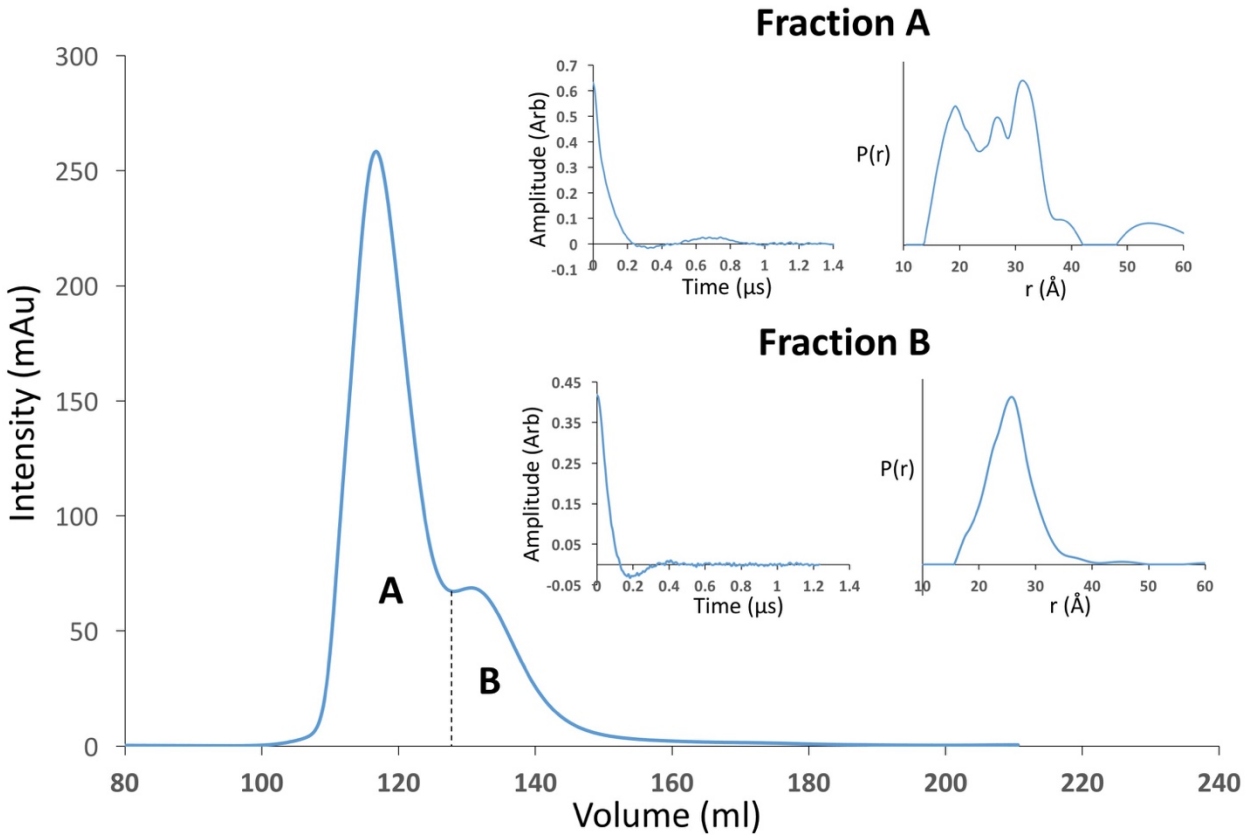

Figure S3 Oligomerization properties of Tsr-Aer2H1-3 spin-labeled at the A220 position (A220C). The SEC profile for A220C-SL displays a bimodal peak with larger (fraction A), and smaller (fraction B) species. The time domain signals and distance distributions [P(r)] for each fraction are shown as insets. The time-domain DEER spectrum got fraction A indicates the formation of tetramer, which is consistent with its P(r) distribution. The oligomer dissociated in the trailing fraction B, whose time domain signals and P(r) indicate dimerization of the protein.

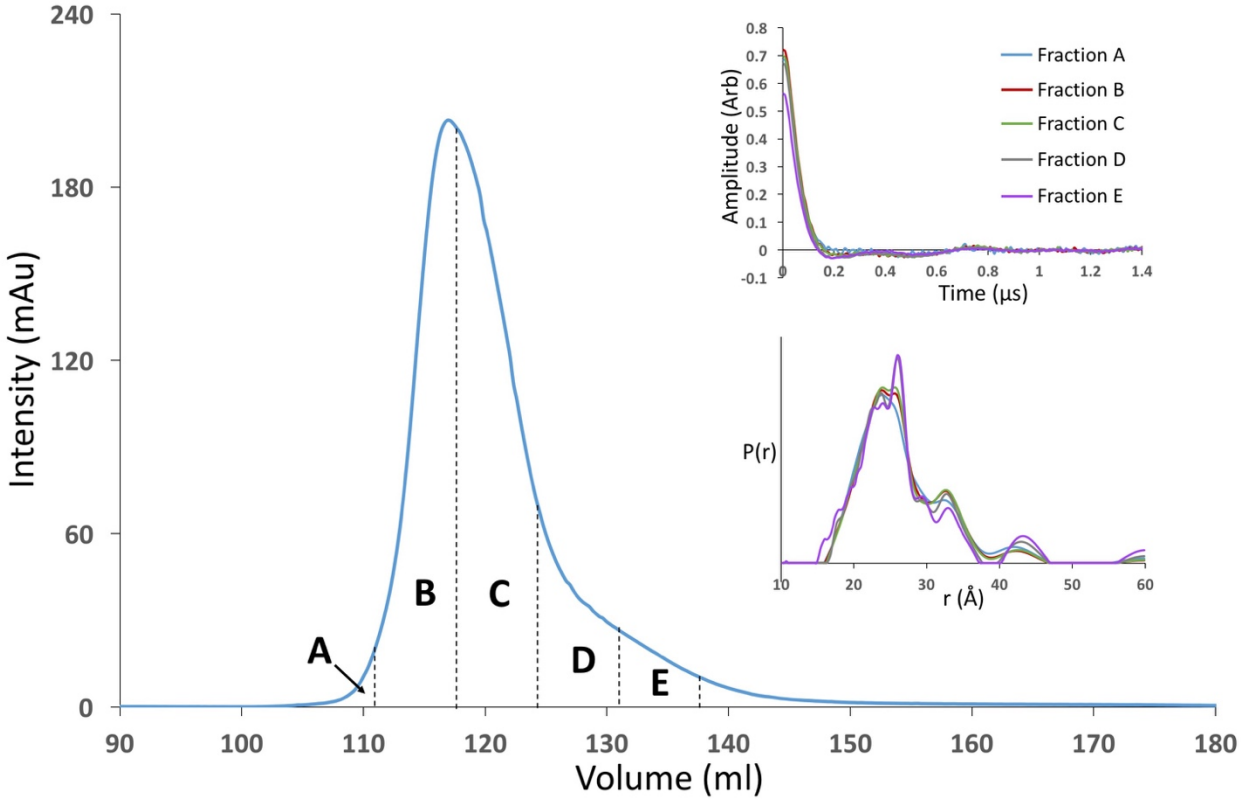

Figure S4 Oligomerization properties of Tsr-Aer2H1-3 spin-labeled at the R230 position (R230C). The SEC profile of R230C-SL does not show distinct species, but does indicate smaller components in the trailing shoulder. The DEER time domain signals and P(r) for each denoted SEC fractions are shown as insets. Only the trailing fraction (E) shows some reduction in oligomeric state with a modulation depth that begins to approach that of a dimer. Dilution of the heavier fractions show little change until concentrations reach (< 5 μM).

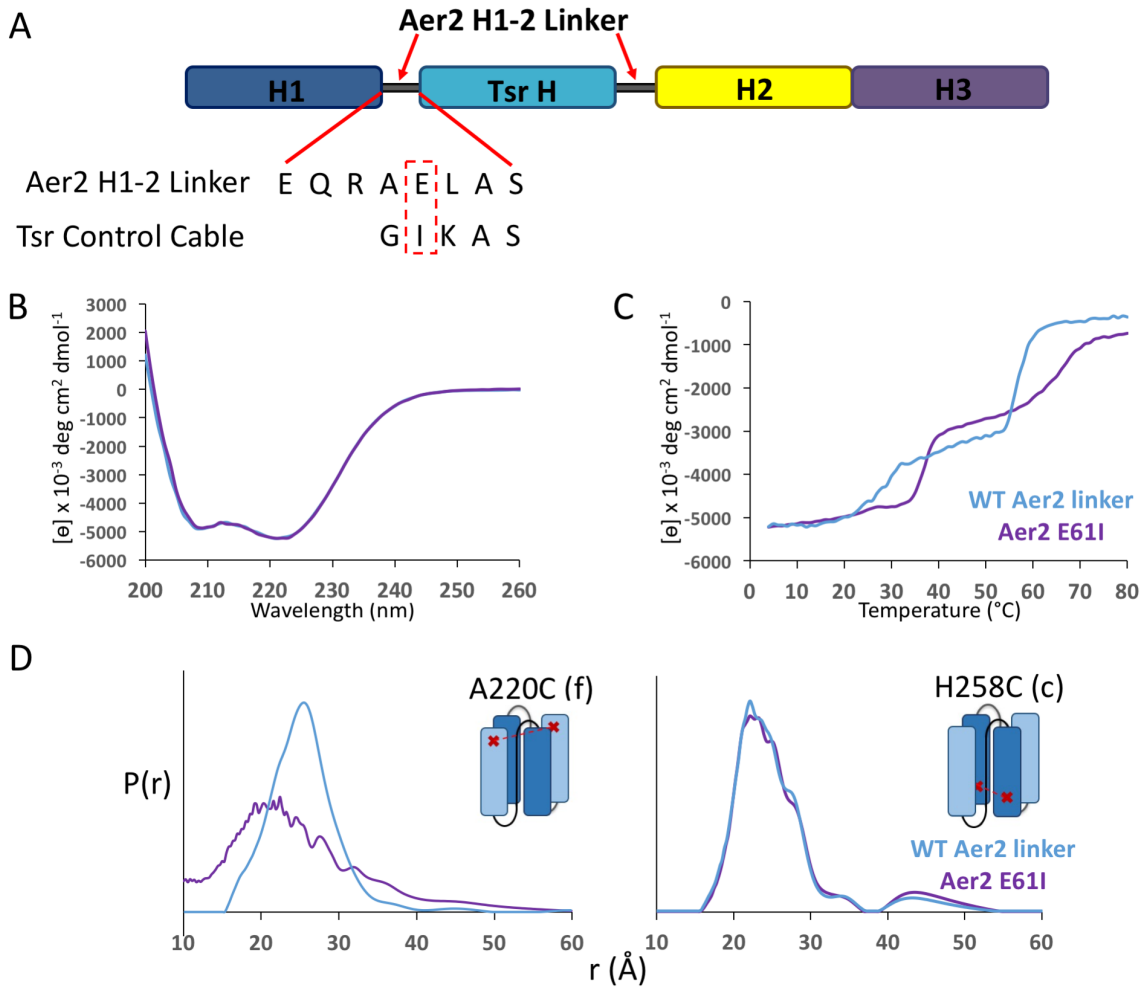

Figure S5 Effects of the linker sequence on the Tsr HAMP domain structure and stability.
(A) Schematic of the chimeric Tsr-Aer2H1-3 domain structure and linker sequence. Sequence for the PaAer2 linker and Tsr control cable are similar but differ in the critical residue Tsr I214 which in PaAer2 is E61 (red box.)
(B) CD spectra showing the secondary structure properties of the chimeric proteins containing either the WT Aer2 linker (blue) or the Aer2 E61I substitution (purple). The two proteins have nearly identical α-helical content.
(C) Thermal stabilities of the chimeric Tsr-Aer2H1-3 proteins with either the WT Aer2 linker or the Aer2 E61I substitution as measured by CD spectroscopy. The E61I substitution increased the melting temperatures for both the Tsr HAMP and the Aer2 poly HAMP domains to 37 and 65°C, respectively.
(D) PDS distance distributions of the Tsr HAMP domain in the recombinant protein with and without the Aer2 E61I substitution. The spin-label sites A220 and H258 are depicted as red stars.

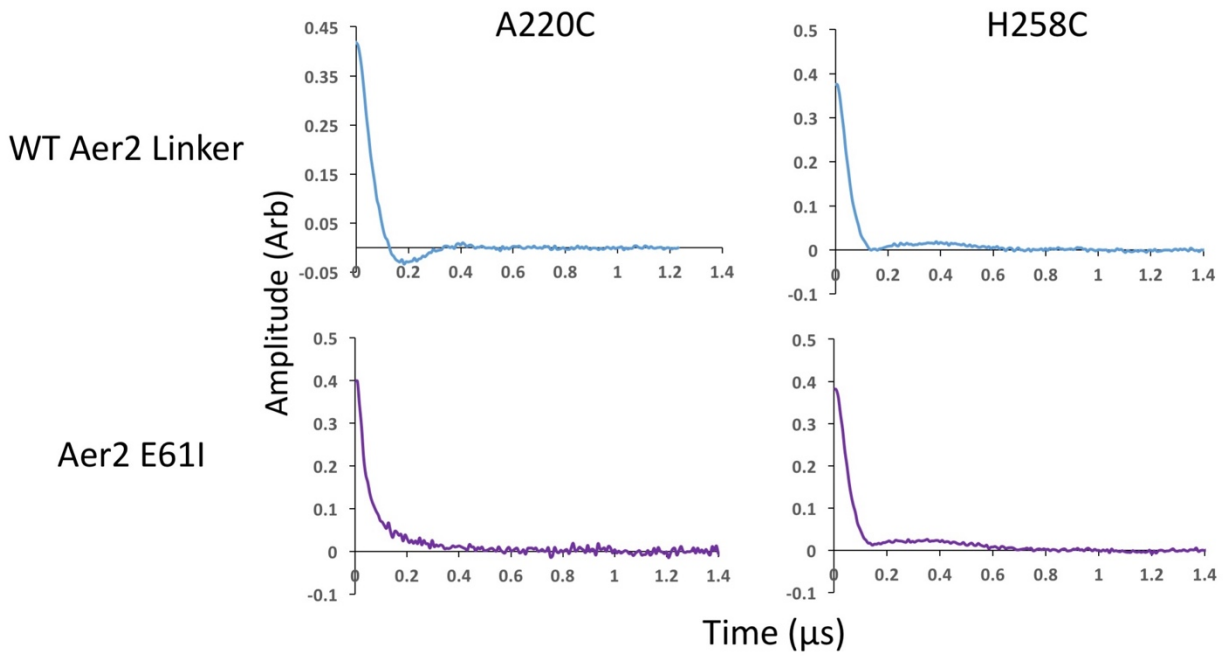

Figure S6 Time domain signals measured by PDS for the chimeric Tsr-Aer2H1-3 with the WT Aer2 linker and the Aer2 E61I mutant. The chosen spin-labeled sites are as indicated.