Supporting information for: Interplay of Cysteine Exposure and Global Protein Dynamics in Small-molecule Recognition by a Regulator of G-protein Signaling Protein

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Table S1: Naming scheme and details of functional groups for three thiadiazolidinone (TDZD compounds **1**, **2**, and **3**) and two non-TDZD small-molecules (compounds **4** and **5**).[S1,S2]

	compound 1 (CCG-50014)	compound 2 (CCG-203769)	compound 3 (CCG-203920)	compound 4	compound 5
R^1	4-FBn	n-Bu	$MeOCH_2CH_2$	4MeBn	Bn
R^2	4-MePh	Et	Et	4-MePh	4-MePh

Table S2: Details of all classical MD simulations (n denotes number of runs for each system; M1 and M2 denote Model 1 and Model 2, respectively).

	Protein	Ligand	Initial Model	System Size (atoms)	Run time (μs)	n
Set1	RGS4-C95	CCG-50014 (**1**)	M1	31614	1	2
	RGS4-C95	CCG-203769 (**2**)	M1	31600	1	2
	RGS4-C95	CCG-203920 (**3**)	M1	31598	1	2
	RGS4-C95	(**4**)	M1	37644	1	3
	RGS4-C95	(**5**)	M1	37652	1	3
Set2	RGS4-C95	CCG-50014 (**1**)	M2	23498	1	2
	RGS4-C95	CCG-203769 (**2**)	M2	23505	1	2
	RGS4-C95	CCG-203920 (**3**)	M2	23500	1	2
	RGS4-C95	(**4**)	M2	23198	0.051, 0.264, 0.163	3
	RGS4-C95	(**5**)	M2	23191	0.173, 0.087, 0.087	3

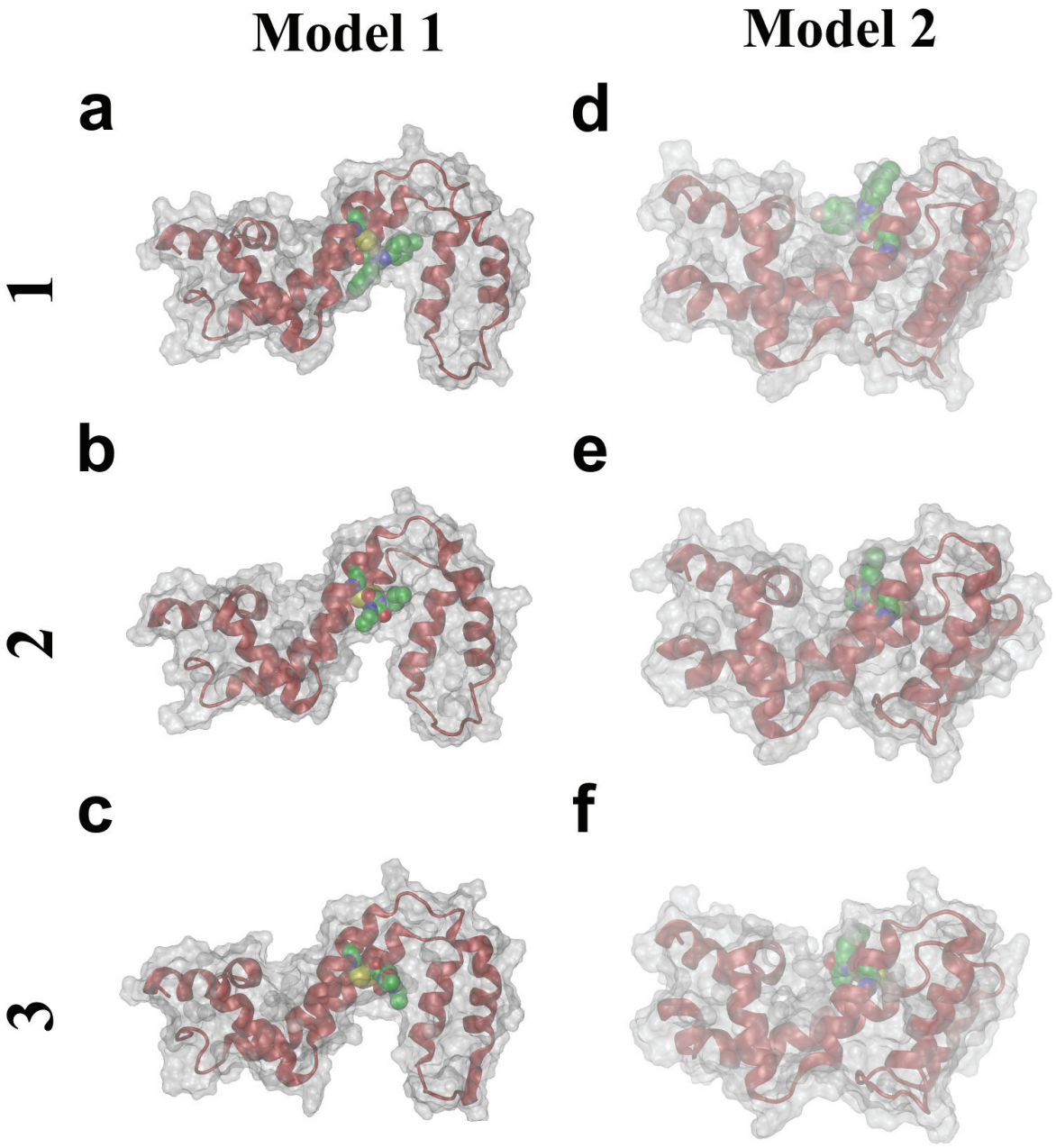

Figure S1: Docked initial conformations of TDZD compounds **1**, **2**, and **3** in Model 1 (panels a, b, and c) and Model 2 (panels d, e, and f) are shown. In all snapshots, the protein backbone is shown in red ribbons as well as white transparent surfaces, while compounds, along with the cysteine residue C95, are shown in green space-filling representations.

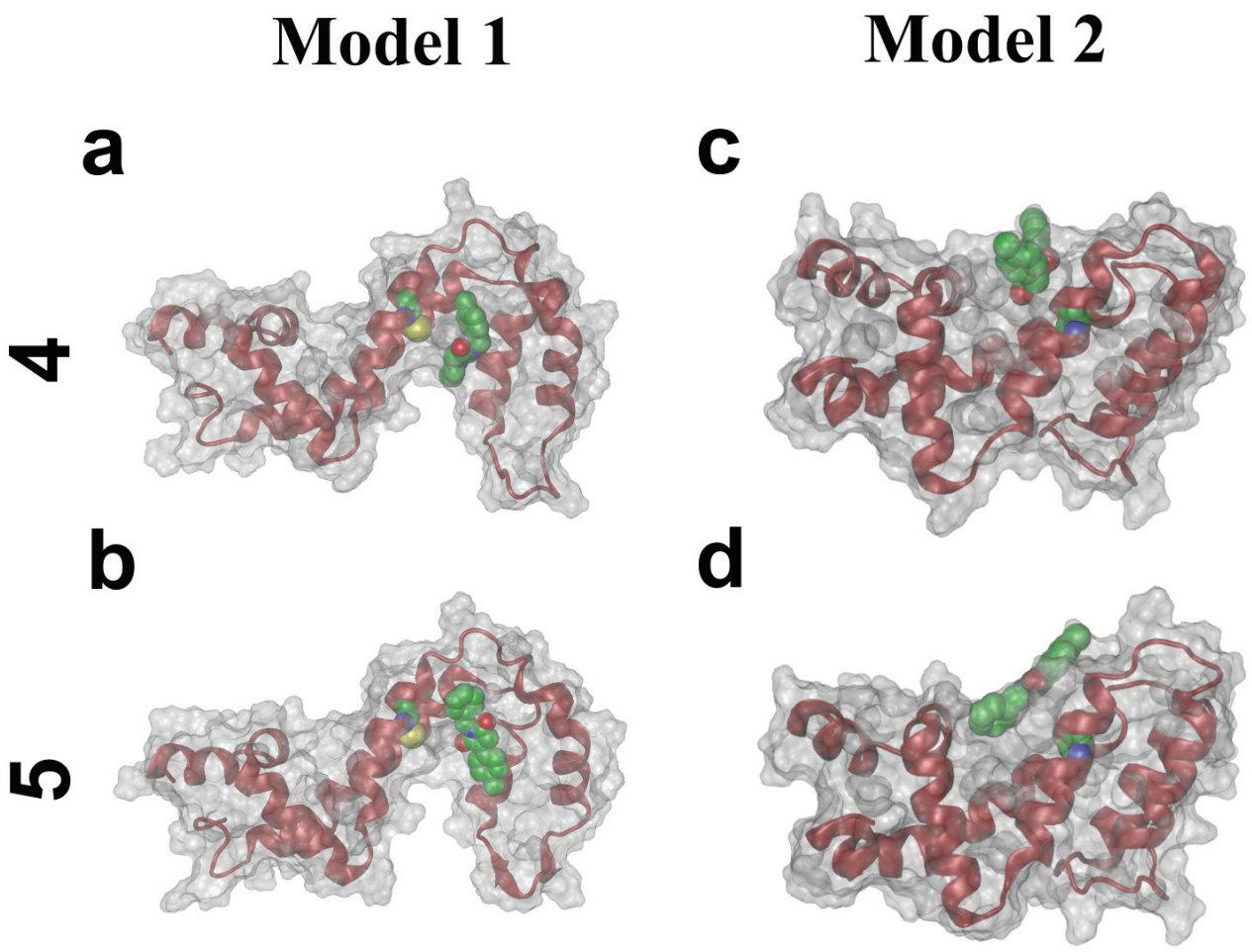

Figure S2: Docked initial conformations of non-TDZD compounds **4** and **5** in Model 1 (panels a and b) and Model 2 (panels c and d) are shown. Coloring and labeling schemes are identical to Fig. S1.

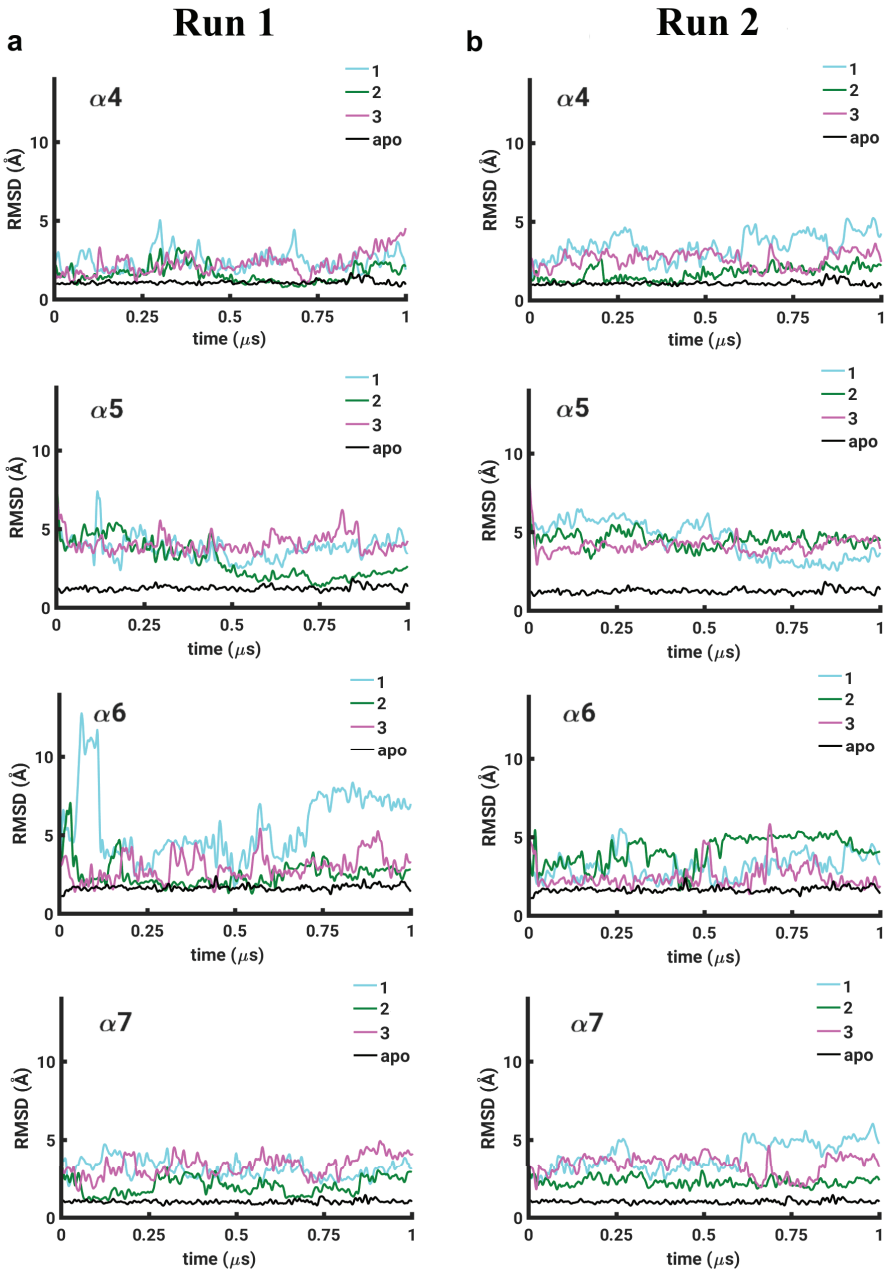

Figure S3: The traces of root-mean-squared-deviation (RMSD) *vs.* simulation time (μs) for 4 helices in the α_4-α_7 helical bundle of RGS4 are shown from two independent simulation runs (Run1, panel a; Run2, panel b) for complexes of RGS4 with TDZD compounds **1** (cyan trace), **2** (green trace), and **3** (magenta trace). The black traces show data for an apo-RGS4 simulation from our previous work.[S3]

Model 2

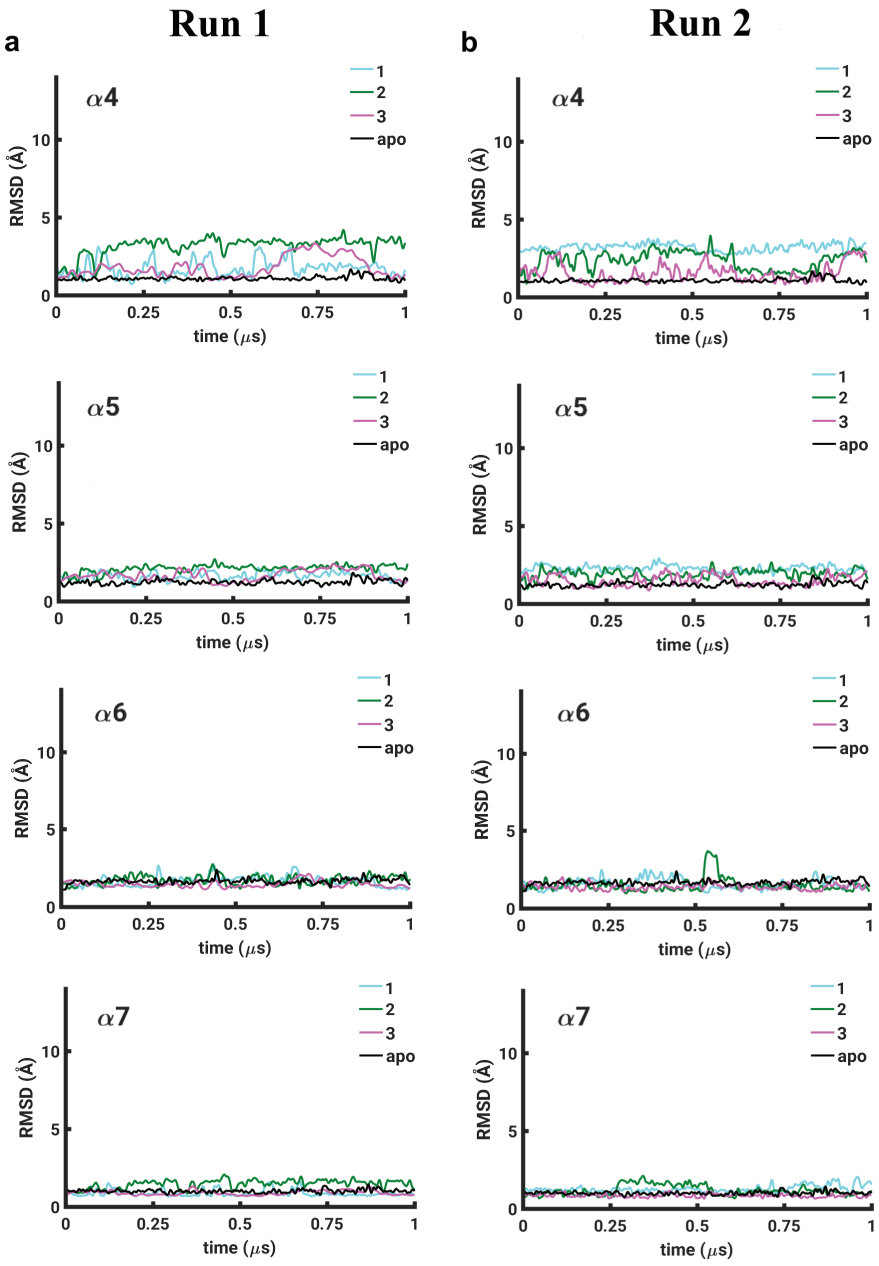

Figure S4: Same data as in Fig. S3 are shown for Model 2.

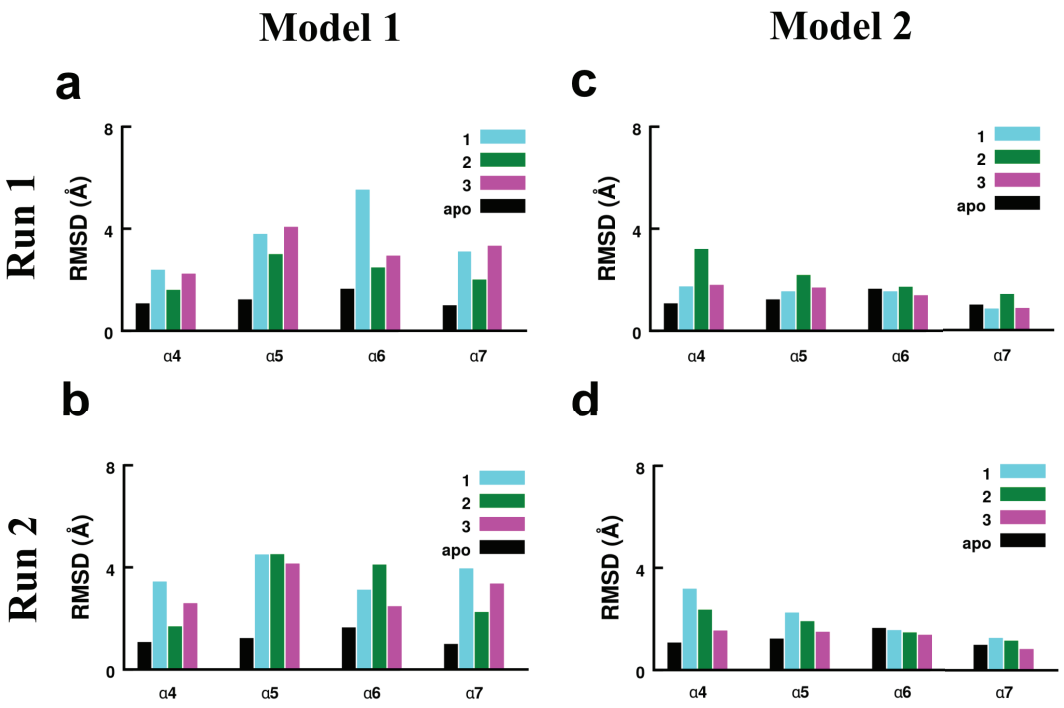

Figure S5: The histograms of RMSD-averages computed based upon data in Fig. S3 (Model 1) and Fig. S4 (Model 2) are shown. Panels a and b show data from two independent runs of Model 1, and panels c and d show data from two independent simulations of Model 2.

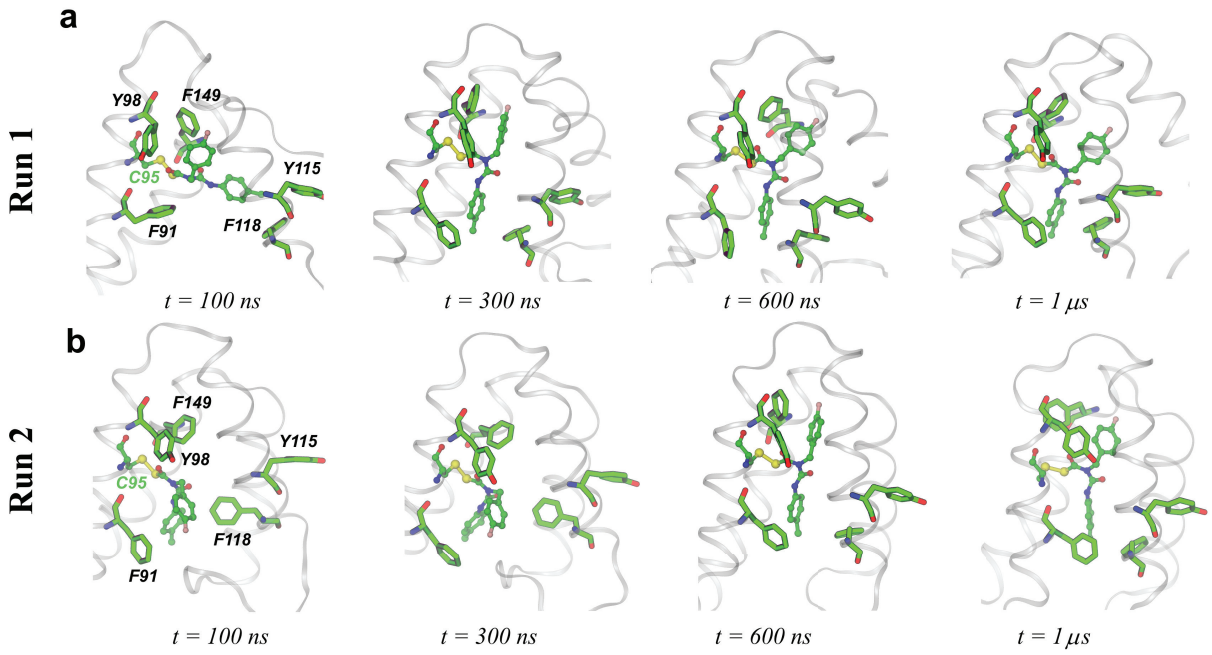

Figure S6. The side-chains of aromatic residues in the vicinity of covalently-docked compound **1** are shown at various time-points from two independent simulations of Model 1 (panels a and b). The compound **1** is covalently-linked to residue C95, and neighboring residues are labeled and shown in green sticks. The protein backbone in all snapshots is shown in a white transparent cartoon.

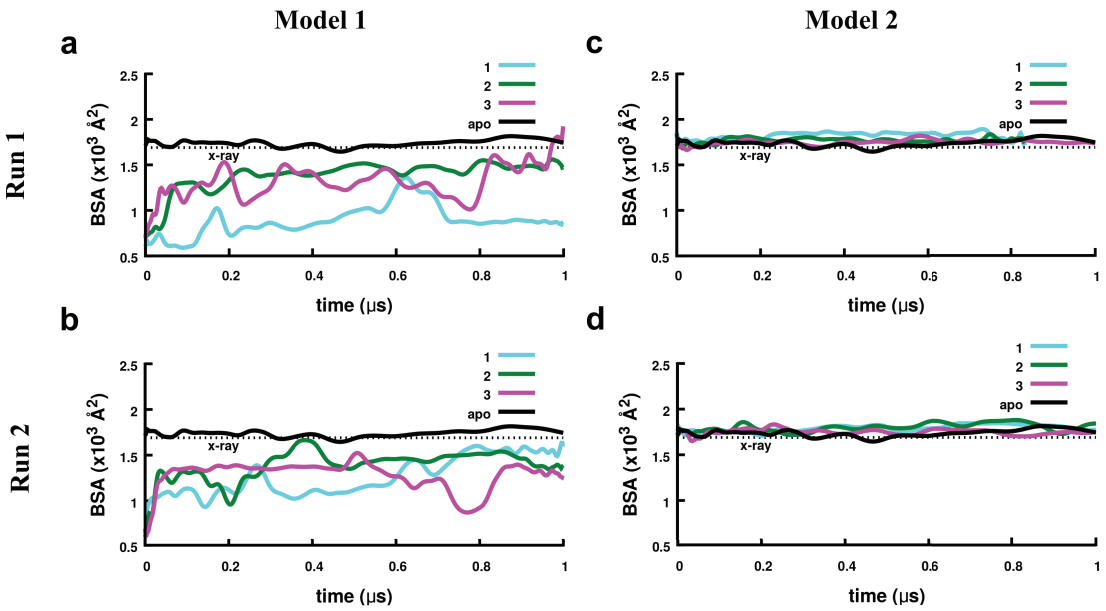

Figure S7: The traces of buried surface area (BSA) between the α_5-α_6 helices and the rest of RGS4 *vs.* simulation time (μs) are shown from two independent simulation runs for each Model (Models 1 and 2). The BSA traces are shown for three TDZD compounds (cyan, green, and magenta traces) and from a simulation of apo-RGS4 (black traces). The dotted horizontal line in each panel highlights the BSA-value in the crystal structure of RGS4 (PDB: 1AGR).

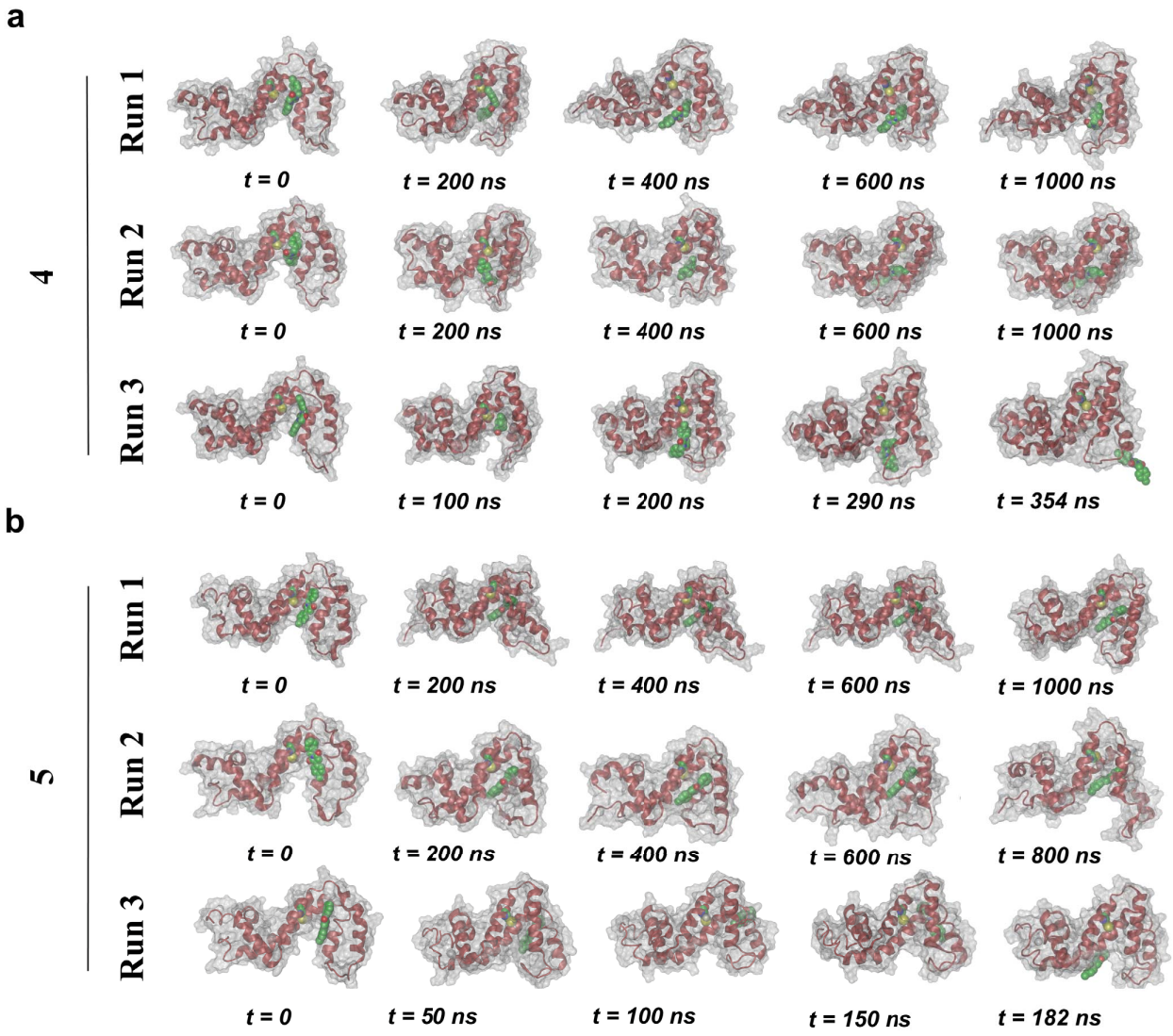

Figure S8: Snapshots at various time-points for conformational evolution of complexes of non-TDZD compounds **4** (panel a) and **5** (panel b) with RGS4 (Model 1). In each panel, snapshots from three independent simulation runs are shown for each compound. Coloring and labeling schemes are identical to initial states shown in Fig. S2.

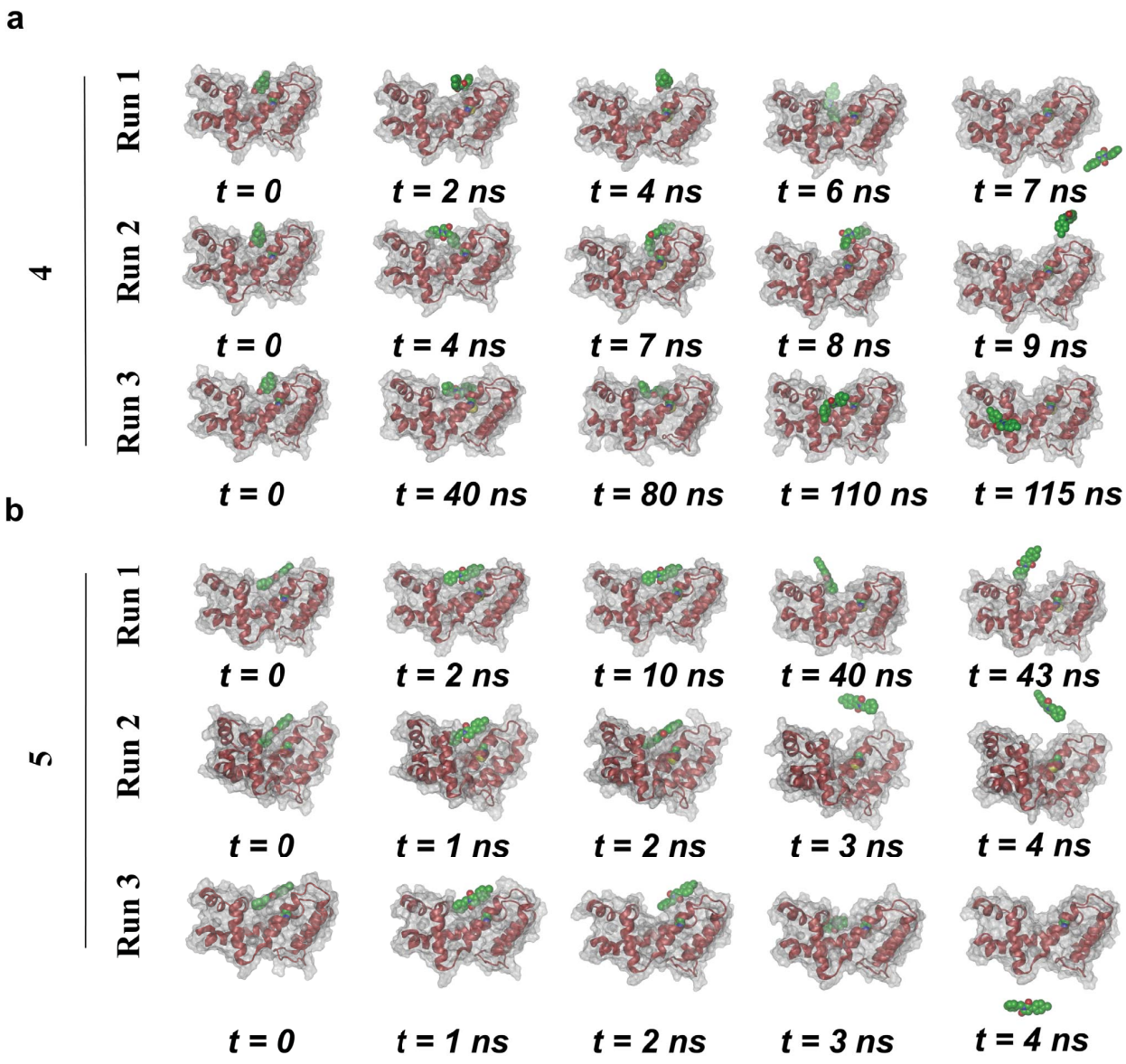

Figure S9: Snapshots at various time-points for conformational evolution of complexes of non-TDZD compounds **4** (panel a) and **5** (panel b) with RGS4 (Model 2). Coloring and labeling schemes are similar to Fig. S8.

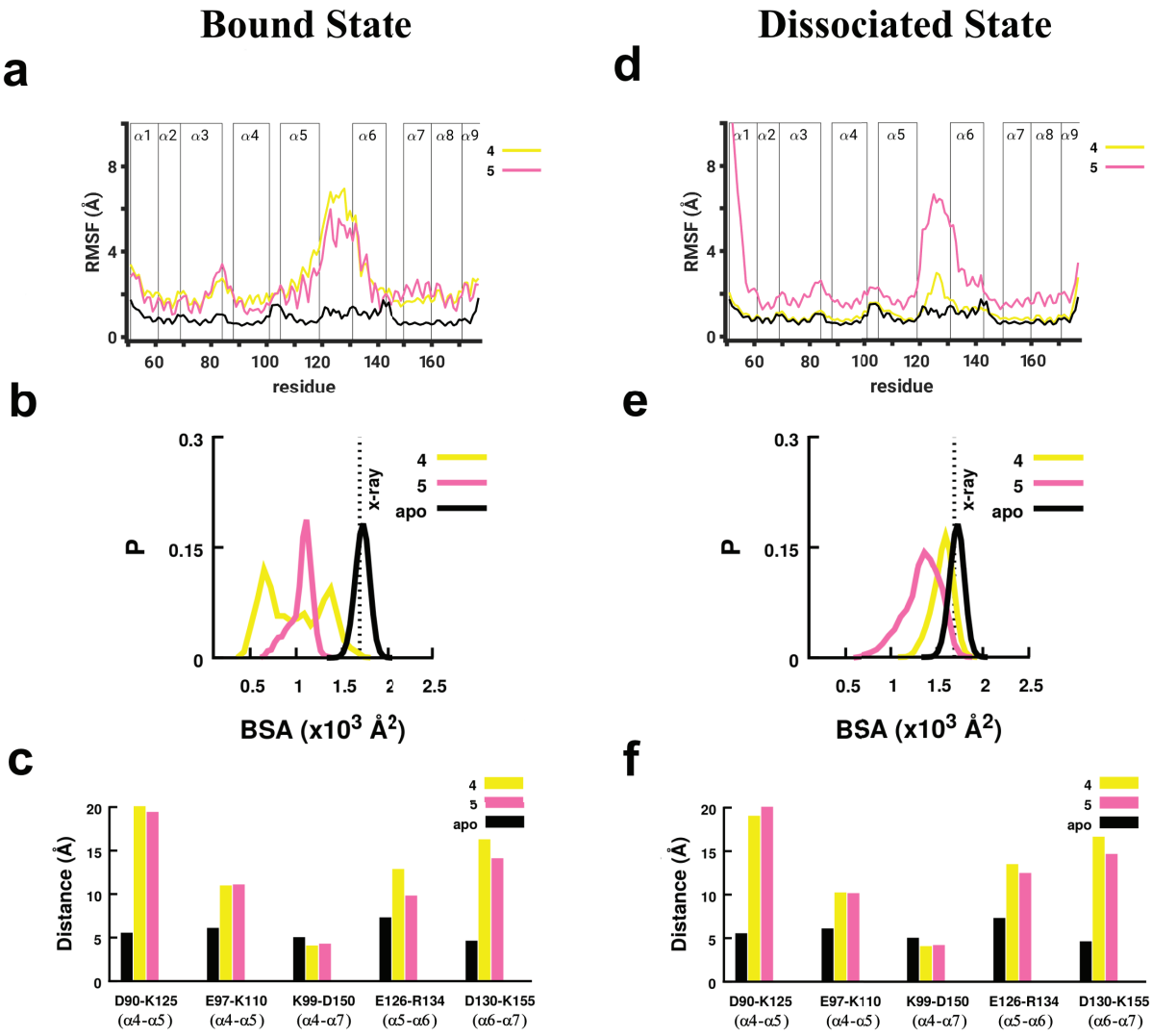

Figure S10: Data similar to those presented in Fig. 5 are shown from Run3 for compounds **4** and **5** in which diffusion of each compound out of the protein pocket was observed. The left-panels show data for parts of trajectories when compounds still reside within the protein, and the right-panels show data for the remaining parts of trajectories when compounds have diffused out of the pocket.

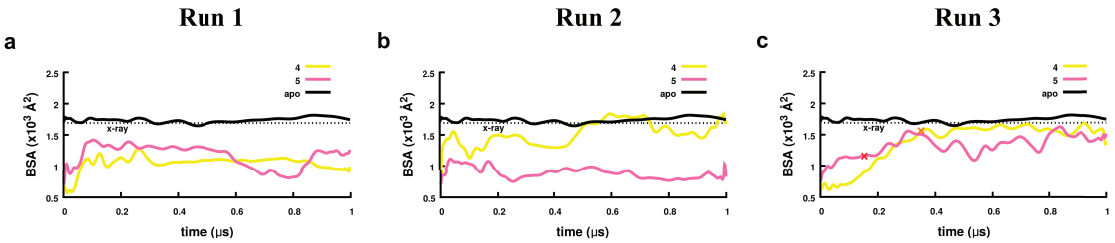

Figure S11: The traces of buried surface area (BSA) between the α_5-α_6 helices and the rest of RGS4 *vs.* simulation time (μs) are shown from three independent simulation runs for Model 1. The BSA traces are shown for two non-TDZD compounds (magenta and yellow traces) and from a simulation of apo-RGS4 (black traces). The dotted horizontal line in each panel highlights the BSA-value in the crystal structure of RGS4 (PDB: 1AGR). The symbols ($\times$) on the BSA traces mark the locations of time-points in Run3 of each compound (panel c) after which compounds diffuse out of the binding pocket

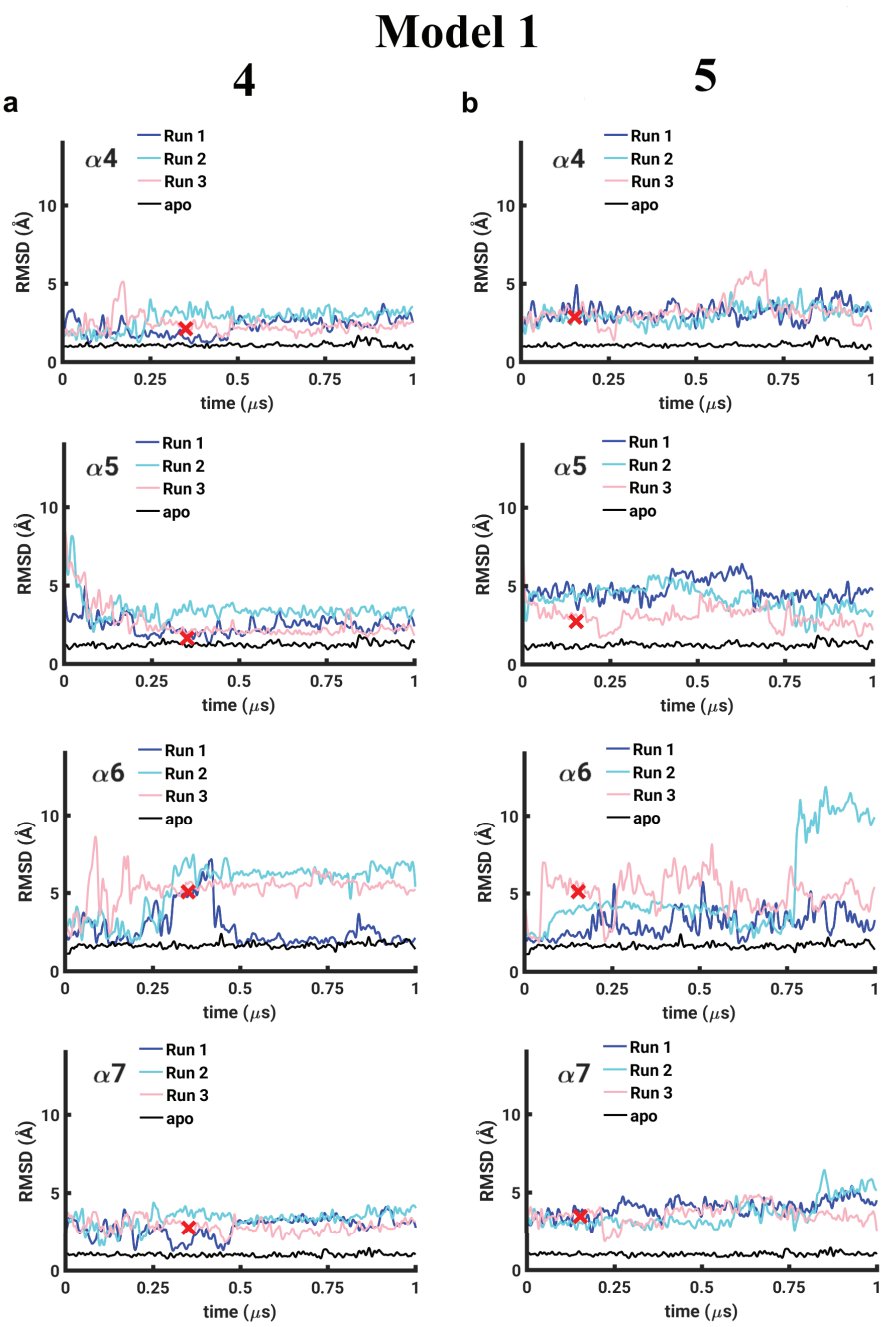

Figure S12: The RMSD data similar to Fig. S3 are shown for non-TDZD compounds **4** and **5** from three independent simulations. The red symbol (×) marks the locations of time-points in Run3 of each compound after which compounds diffuse out of the binding pocket (see snapshots in Fig. S8).

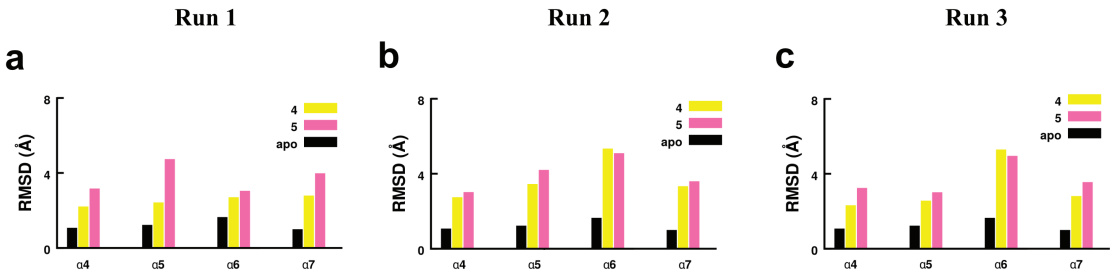

Figure S13: The histograms of RMSD-averages computed based upon data from each run in Fig. S12 are shown.

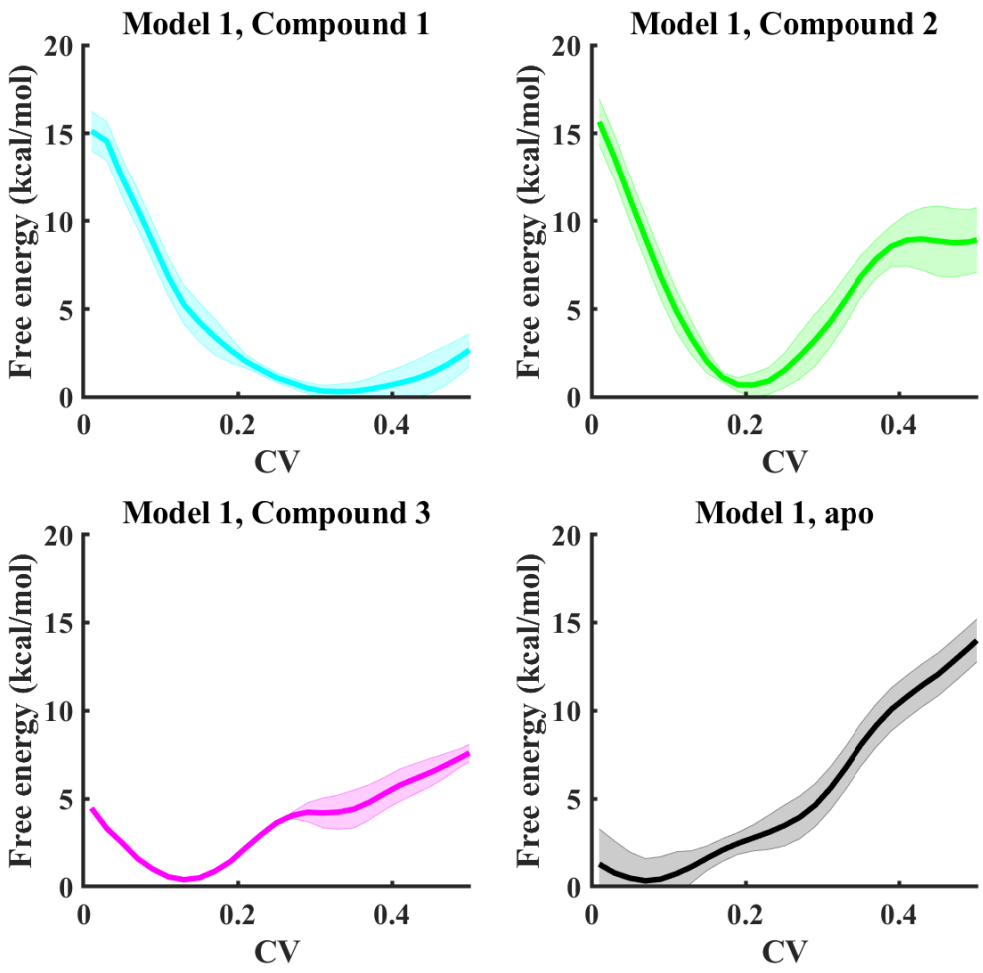

Figure S14: Error bars corresponding to PMF traces shown in Fig. 6a are shown.

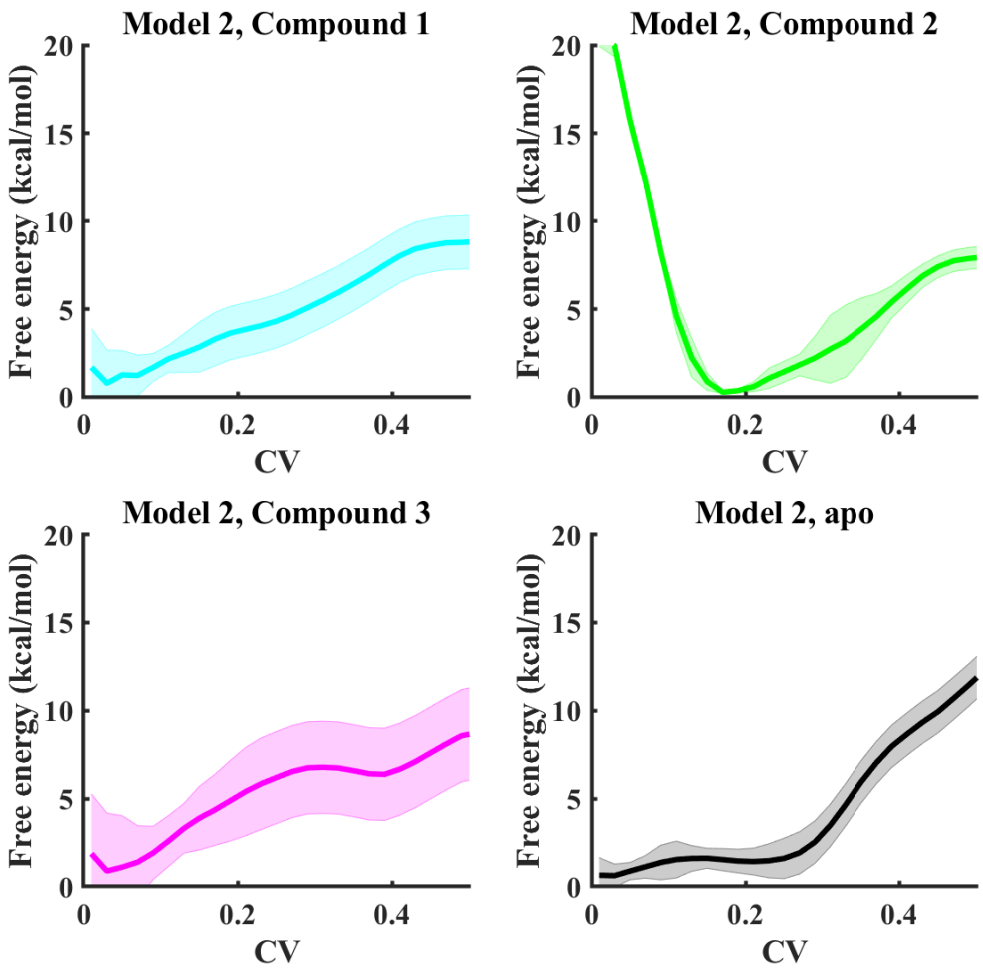

Figure S15: Error bars corresponding to PMF traces shown in Fig. 6b are shown.

References

(S1) Turner, E. M., Blazer, L. L., Neubig, R. R., and Husbands, S. M. (2011) Small molecule inhibitors of regulators of G Protein signaling (RGS) proteins. *ACS Med. Chem. Lett. 3*, 146–150.

(S2) Neubig, R., Blazer, L., Husbands, S., Larsen, S., and Traynor, J. Small molecule inhibitors of RGS proteins. 2014; US Patent 8,865,750.

(S3) Shaw, V. S., Mohammadiarani, H., Vashisth, H., and Neubig, R. R. (2018) Differential Protein Dynamics of Regulators of G-Protein Signaling: Role in Specificity of Small-Molecule Inhibitors. *J. Am. Chem. Soc. 140*, 3454–3460.