Supporting Material

Computational and functional analyses of a small-molecule binding site in ROMK

Daniel R. Swale[1,2] , Jonathan H. Sheehan[3,4] , Sreedatta Banerjee[1], Afeef S. Husni[1], Thuy T. Nguyen[1], Jens Meiler[2,3], Jerod S. Denton[1,2,5,6]

Department of Anesthesiology[1], Department of Pharmacology[2], Center for Structural Biology[3], Department of Biochemistry[4], Institute of Chemical Biology[5], Institute for Global Health[6], Vanderbilt University Medical Center, Nashville, TN 37232, United States

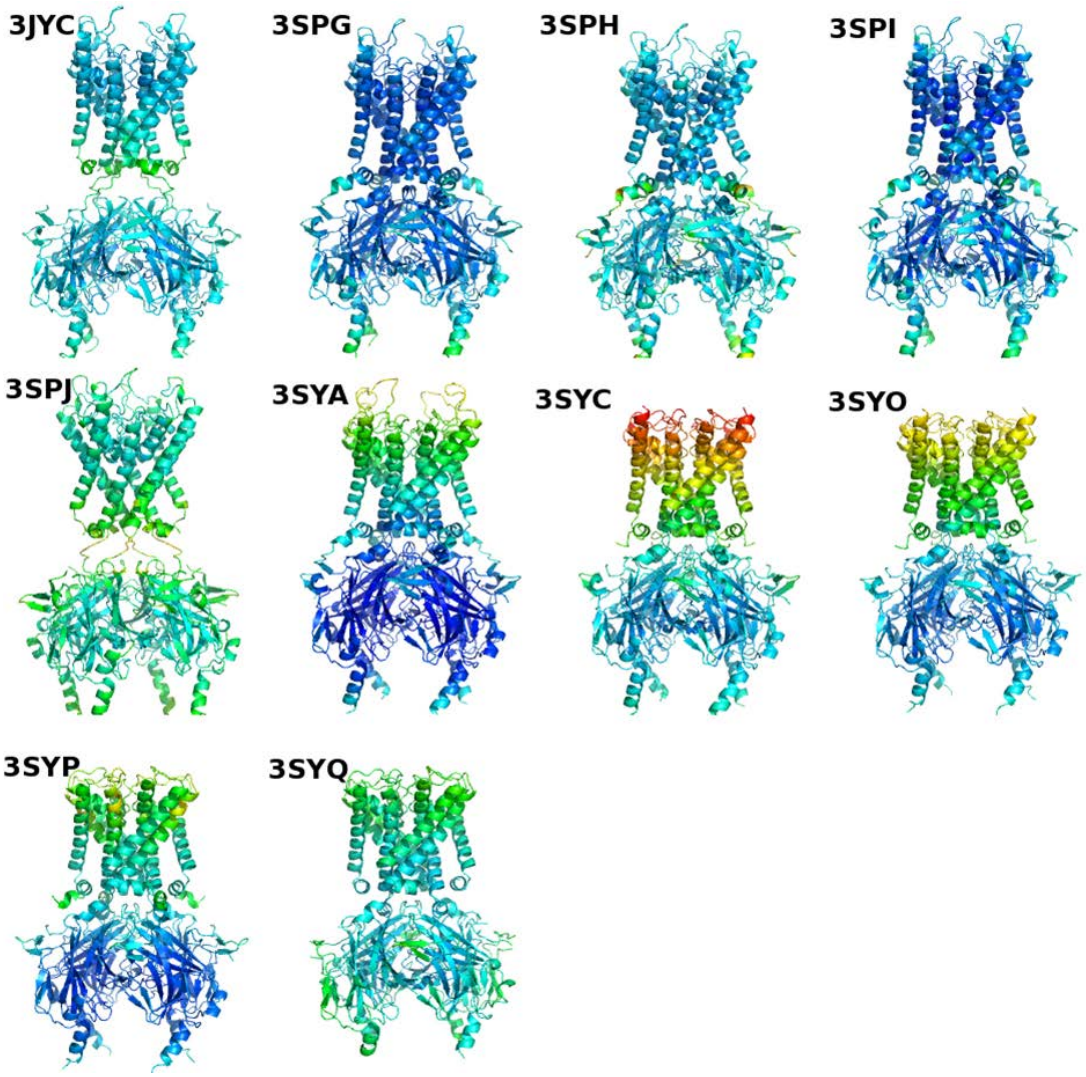

Fig. S1. Structural diversity of the templates used for constructing the ensemble of ROMK models. Ten structures representing Kir 2.2 and Kir 3.2 in the presence or absence of different ligands, ions, and functionally important mutations (19-21) are shown in cartoon mode, and labeled with their respective PDB codes. Structures are colored by temperature factor from blue (low B values) to red (high B values).

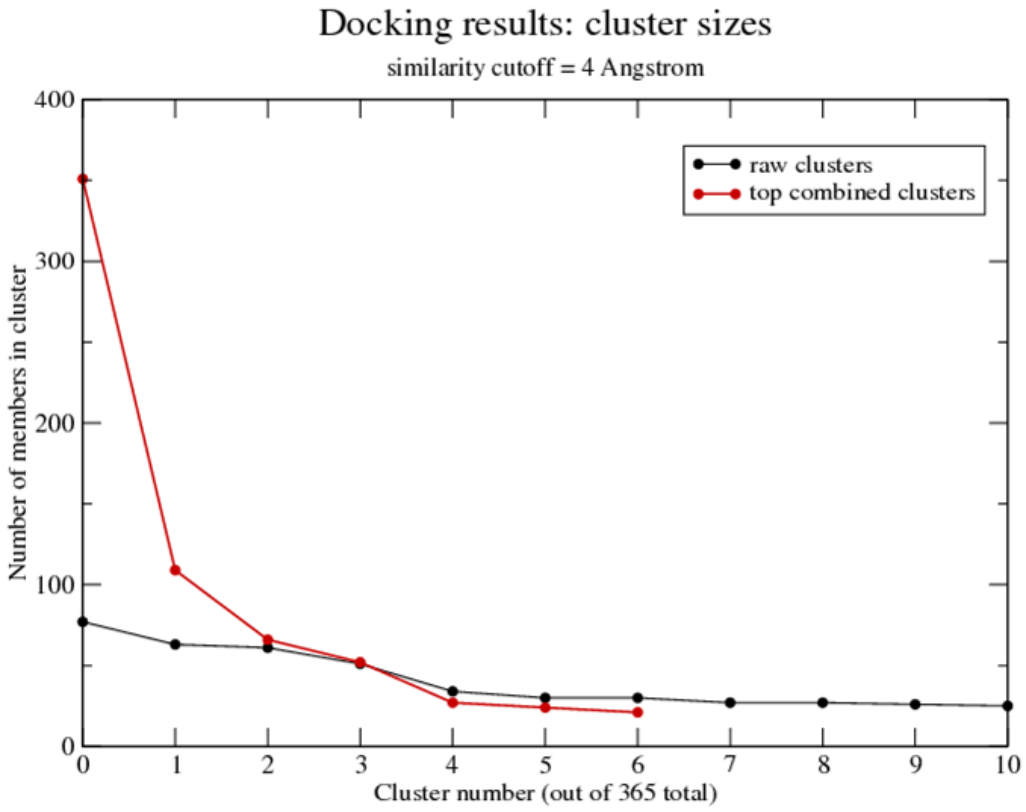

Fig. S2. Discrimination is improved by combining symmetry-related clusters. Clustering the docked poses by a similarity metric (RMSD) helps identify the most native-like results, by reducing the effects of noise and uncertainty due to the comparative modeling procedure. We also took advantage of the 4-fold symmetry of the tetrameric channel, combining symmetry-related conformations to produce a smaller number of clusters, in which the two largest stood out from the others.

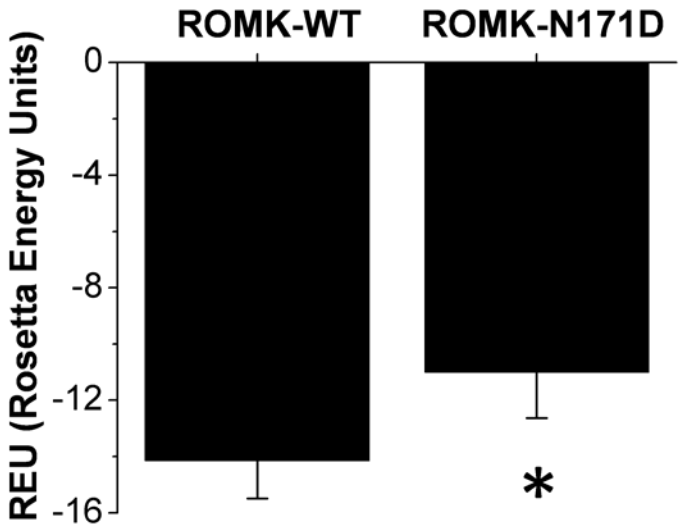

Fig. S3. In silico prediction of the effect of mutation (N171D) upon inhibitor binding. The best 10 models of VU591 docked into ROMK were mutated using Rosetta. The models were then minimized and rescored to predict the binding energy. The process was repeated 100 times for each mutated model, and compared to the wild-type models. *P = 1.8^{-9}.

Table S1. Clusters of top docking poses combined by similarity and symmetry relations to increase discriminatory power.

merged cluster	*cluster rank*	*cluster number*	*size of cluster*	*representative ddG*
m1 (middle)		**351**		
	1	4399	77	697	-10.9698
	2	4455	63	354	-13.0003
	3	4440	61	124	-11.2989
	4	4418	51	2316	-11.4673
	6	4450	30	554	-11.1488
	11	4412	25	1219	-11.0008
	15	4275	23	1996	-10.6223
	16	4485	21	569	-10.1381
m2 (upper)		**109**		
	5	4369	34	602	-10.238
	7	4303	30	636	-10.3281
	10	4374	26	1362	-10.8271
	20	4453	19	600	-12.0512
m3 (middle2)		**66**		
	14	4404	24	1750	-10.2767
	17	4482	21	1359	-10.7613
	19	4347	21	1655	-11.1465
m4 (lower)		**52**		
	8	4473	27	426	-10.8572
	12	4336	25	2058	-10.0712
others:				
	9	4402	27	1580	-10.2737
	13	4490	24	2481	-12.4143
	18	4472	21	390	-11.9424

Table S2. The distribution of the top 2498 docking results depends on the conformation of the ROMK model.

template PDB code	*# in Lower cavity*	*# in Middle cavity*	*#_in Upper cavity*
3SPG	6	73	46
3SPH	32	105	73
3SPI	20	102	80
3SPJ	3	79	142
3SYA	0	151	49
3SYC	0	311	13
3SYO	0	169	92
3SYP	0	250	67
3SYQ	2	157	78
3SYC	16	258	124