Supplementary materials

Table S1. Stereochemical quality assessment of TMPS2 model 1 (5CE1_A template).

Protein Geometry	Poor rotamers	3	1.02%	Goal: <0.3%
	Favoured rotamers	278	94.24%	Goal: >98%
	Ramachandran outliers	3	0.87%	Goal: <0.05%
	Ramachandran favoured	317	92.15%	Goal: >98%
	Rama distribution Z-score	-1.49 ± 0.44		Goal: abs(Z score) < 2
	Cβ deviations >0.25Å	6	1.89%	Goal: 0
	Bad bonds:	0 / 2779	0.00%	Goal: 0%
	Bad angles:	31 / 3779	0.82%	Goal: <0.1%
Peptide Omegas	Cis Prolines:	0 / 16	0.00%	Expected: ≤1 per chain, or ≤5%
Low-resolution Criteria	CaBLAM outliers	7	2.0%	Goal: <1.0%
	CA Geometry outliers	4	1.17%	Goal: <0.5%

Table S2. Stereochemical quality assessment of TMPS2 model 2 (1Z8G_A template).

Protein Geometry	Poor rotamers	4	1.36%	Goal: <0.3%
	Favoured rotamers	283	96.26%	Goal: >98%
	Ramachandran outliers	3	0.87%	Goal: <0.05%
	Ramachandran favoured	323	94.17%	Goal: >98%
	Rama distribution Z-score	-1.31 ± 0.44		Goal: abs(Z score) < 2
	Cβ deviations >0.25Å	2	0.63%	Goal: 0
	Bad bonds:	2 / 2771	0.07%	Goal: 0%
	Bad angles:	22 / 3768	0.58%	Goal: <0.1%
Peptide Omegas	Cis Prolines:	0 / 16	0.00%	Expected: ≤1 per chain, or ≤5%
Low-resolution Criteria	CaBLAM outliers	8	2.3%	Goal: <1.0%
	CA Geometry outliers	5	1.47%	Goal: <0.5%

Table S3. Comparison between TMPS2 and the template 5CE1_A

Secondary structures	Template 5CE1_A		TMPS2 model	
	Residues	**Total**	**Residues**	**Total**
Helices	Ala38-Met48 Ala157-Cys159 Leu167-Ser168 Gln274-Cys277 Val348-Thr361	34 amino acids (9.14%)	Lys83-Met109 Glu178-Tyr103 Arg182-Asp187 Asp220- Lys224 Ala295-Cys297 Thr407-Cys410 Val477-Ala490	62 amino acids (12.60%)
Sheets	Val9-Ser12 Arg17-Asp22 Trp28-Cys32 Leu54-Val61 Ser70-Val75 Gln84-Asp94 Phe100-Cys105 Arg122-Asp123 Gln132-Tyr137 Ala140-Ser149 Trp152-Thr155 Arg169-Ala178 Leu185-Tyr194 Ala214-Leu218 Ile245-Gly250 Gln265-Val269 Ile271-Ile272 Met291-Ala294 Pro311-Glu315 Trp324-Trp332 Gly343-Lys347	129 amino acids (34.68%)	Val149-Tyr152 Ile157-Ser162 Trp168-His169 Lys195-Val201 Phe209-Asn213 Leu225-Tyr226 Val236-Cys241 Ile256-Val257 Glu260-Ser261 Gln270-Val275 Val278-Ile286 Trp290-Thr293 Trp308-Arg316 Gly325-Ser333 Ala347-Leu351 Leu378-Gly383 Asn398-Ile405 Met424-Ala427 Pro444-Lys449 Ile452-Ser460 Gly472-Asn476	116 amino acids (23.58%)
Loops	Ser1-Pro8 Ser13-Ala16 Lys23-Thr27 Ser33-Gln37 Gly45-Ala53 Arg62-Thr69 Asp76-Thr83 Cys95-Arg99 Gln106-Gly121 Thr124-Trp131 Asp138-Gly139 Gly150-Asp151 Ala156 Phe160-Val166 Gln179-Gly184 His195-Ile213 Ser219-Lys244 Trp251-Leu264 Pro270 Ser273 Asn278-Lys290 Gly295-Gly300 Ile301-Gly310 Asp316-Arg323 Gly333-Pro342 His362-Leu372	209 amino acids (56.18%)	Met1-Lys82 Gly110-Cys148 Gly153-Phe156 Ser163-Ser167 Pro170-Asn177 Gly181 Met188-Phe194 Asp202-Ser208 Thr214-Val219 His227-Ala235 Ile242-Arg255 Gly258-Gly259 Ala262-Trp269 Gln276-Asn277 Thr287-Glu289 Ala294 Val298-His307 Gln317-Ala324 His334-Ile346 Gln352-Gln377 Trp385-Leu397 Glu406 Asn411-Ala423 Gly428-Gly443 Asn450-Asn451 Trp461-Pro471 Asp491-Gly492	314 amino acids (63.82%)

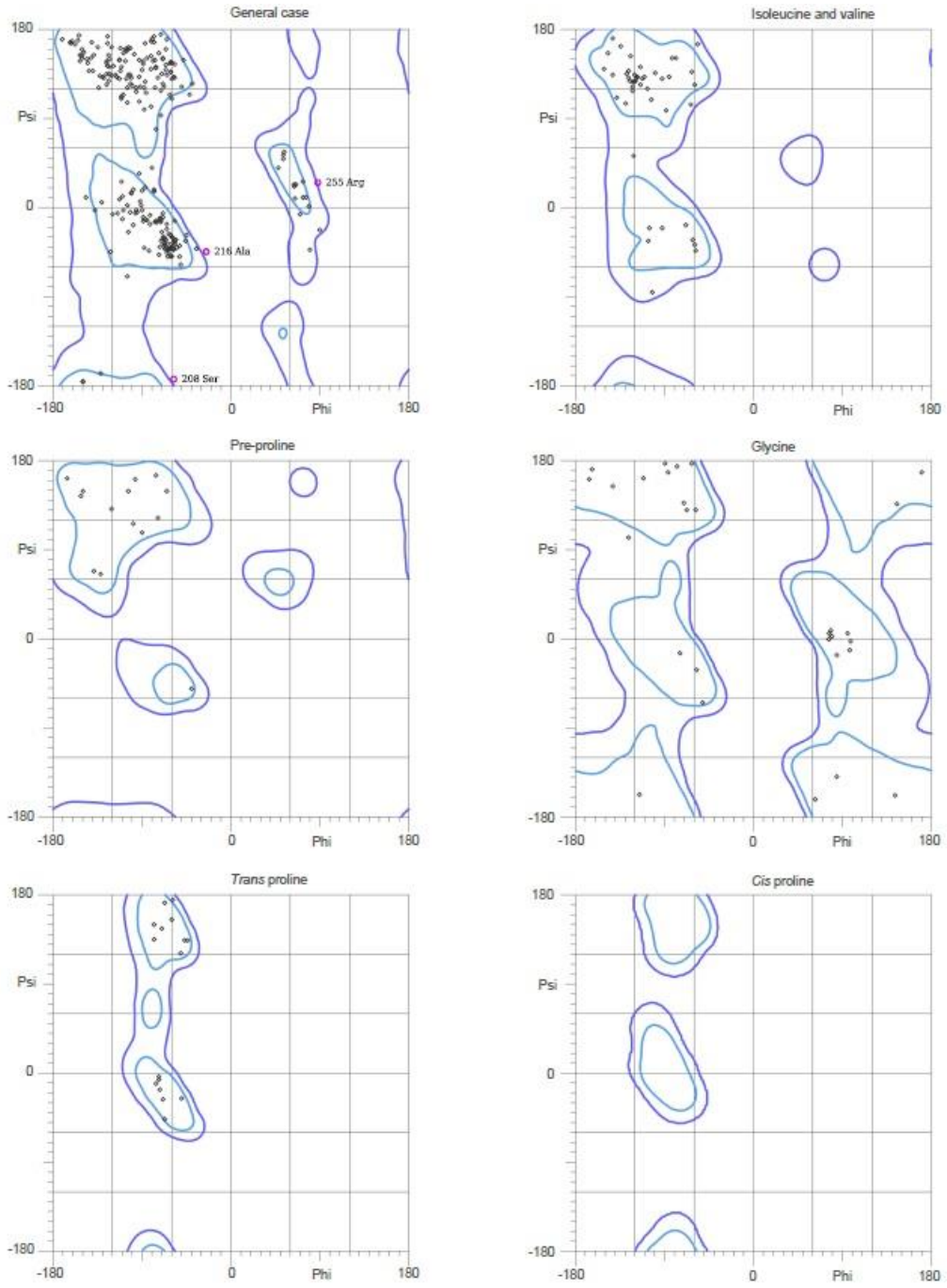

Figure S1. Ramachandran plot for TMPS2 model 1 (5CE1_A template); 92.2% (317/344) of all residues were in favoured (98%) regions, 99.1% (341/344) of all residues were in allowed (>99.8%) regions. There were 3 outliers (phi, psi): 208 Ser (-58.3, -174.6), 216 Ala (-25.8, -45.7) and 255 Arg (88.3, 25.7).

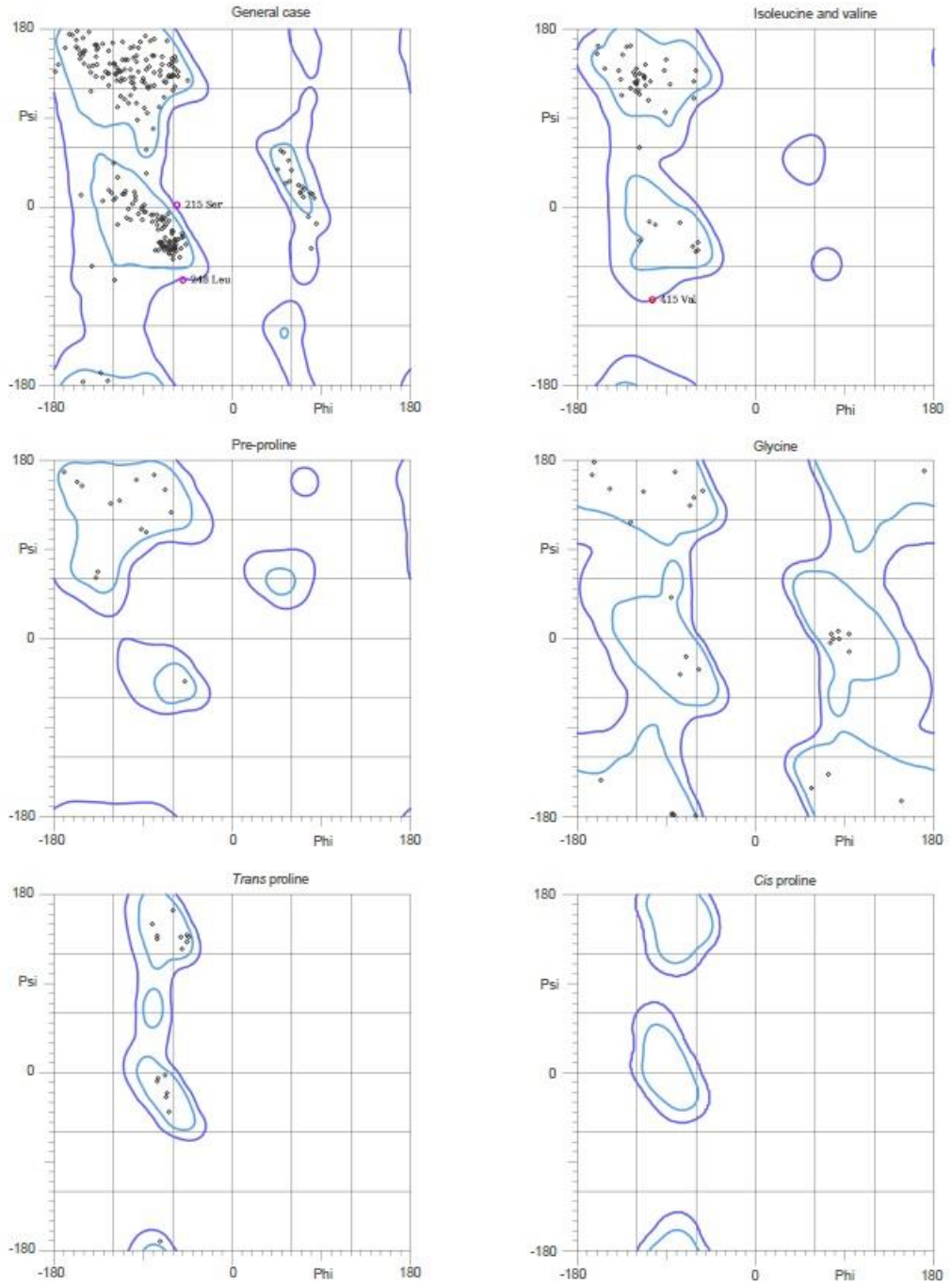

Figure S2. Ramachandran plot for TMPS2 model 2 (1Z8G_A template); 94.2% (323/343) of all residues were in favoured (98%) regions, 99.1% (340/343) of all residues were in allowed (>99.8%) regions. There were 3 outliers (phi, psi): 215 Ser (-56.0, 2.4), 248 Leu (-50.9, -74.7) and 415 Val (-104.8, -94.8).

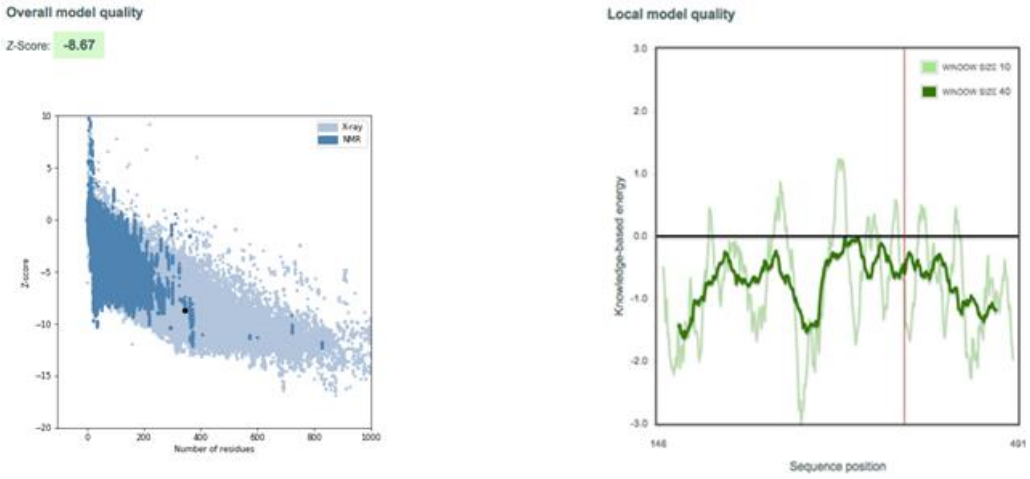

Figure S3. Z-scores of TMPS2 model 1 (5CE1_A template) determined by X-ray crystallography (light blue) or NMR spectroscopy (dark blue), and local model quality by plotting energies as a function of amino acid sequence position, a positive value indicates erroneous parts; most of the amino acids gave negative values.

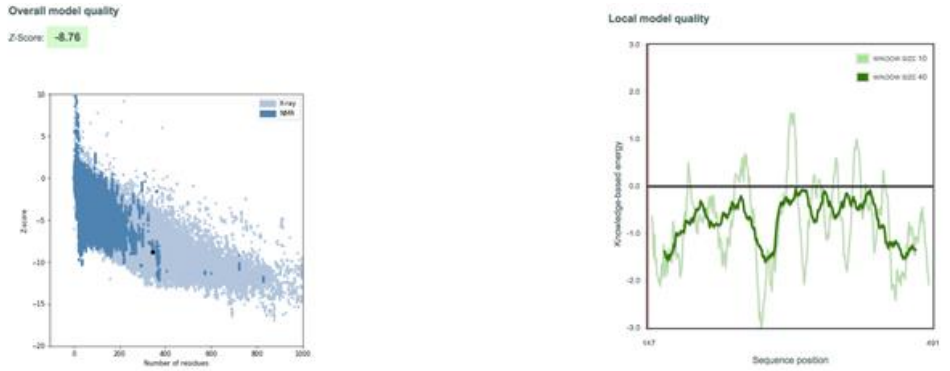

Figure S4. Z-scores of TMPS2 model 2 (1Z8G_A template) determined by X-ray crystallography (light blue) or NMR spectroscopy (dark blue), and local model quality by plotting energies as a function of amino acid sequence position, a positive value indicates erroneous parts; most of the amino acids gave negative values.

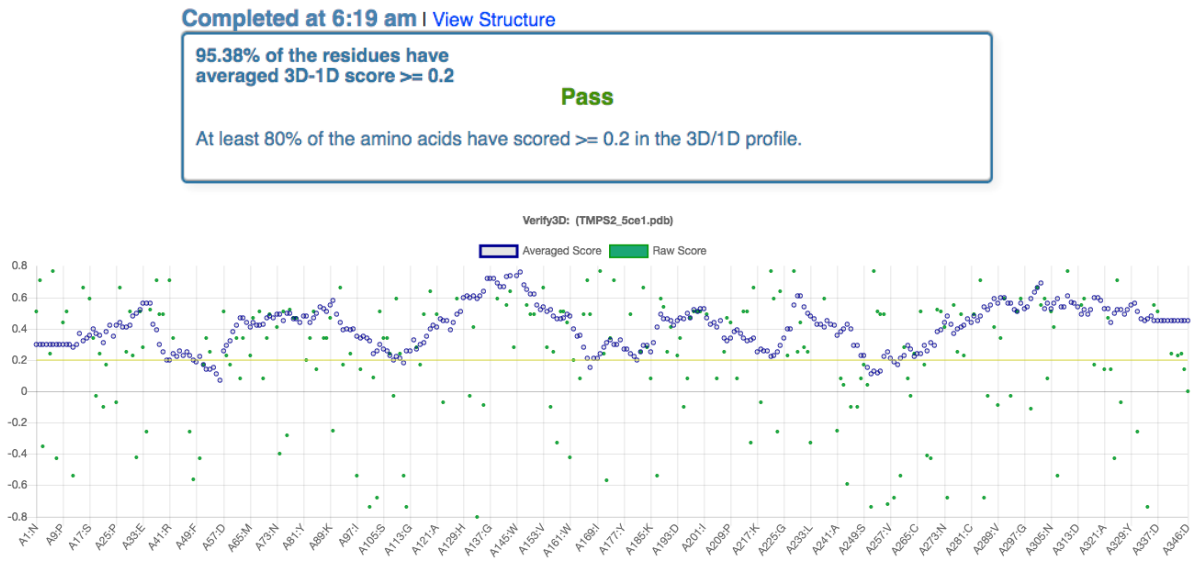

Figure S5. Verify 3D results for model 1 (5CE1_A template) showing that the model passes in the 3D/1D profile.

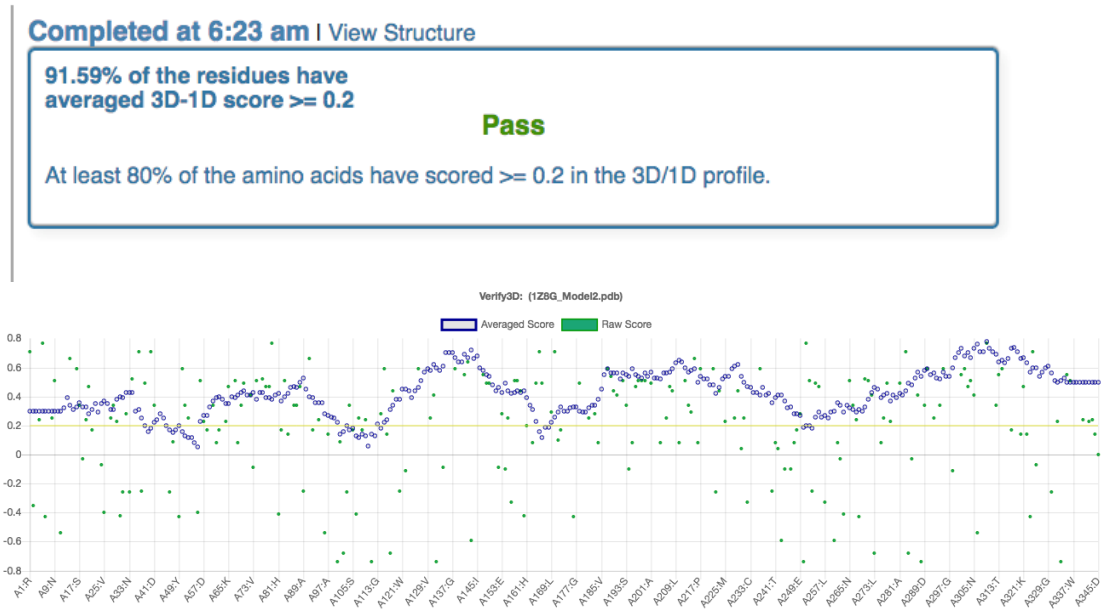

Figure S6. Verify 3D results for model 2 (1Z8G_A template) showing that the model passes in the 3D/1D profile.

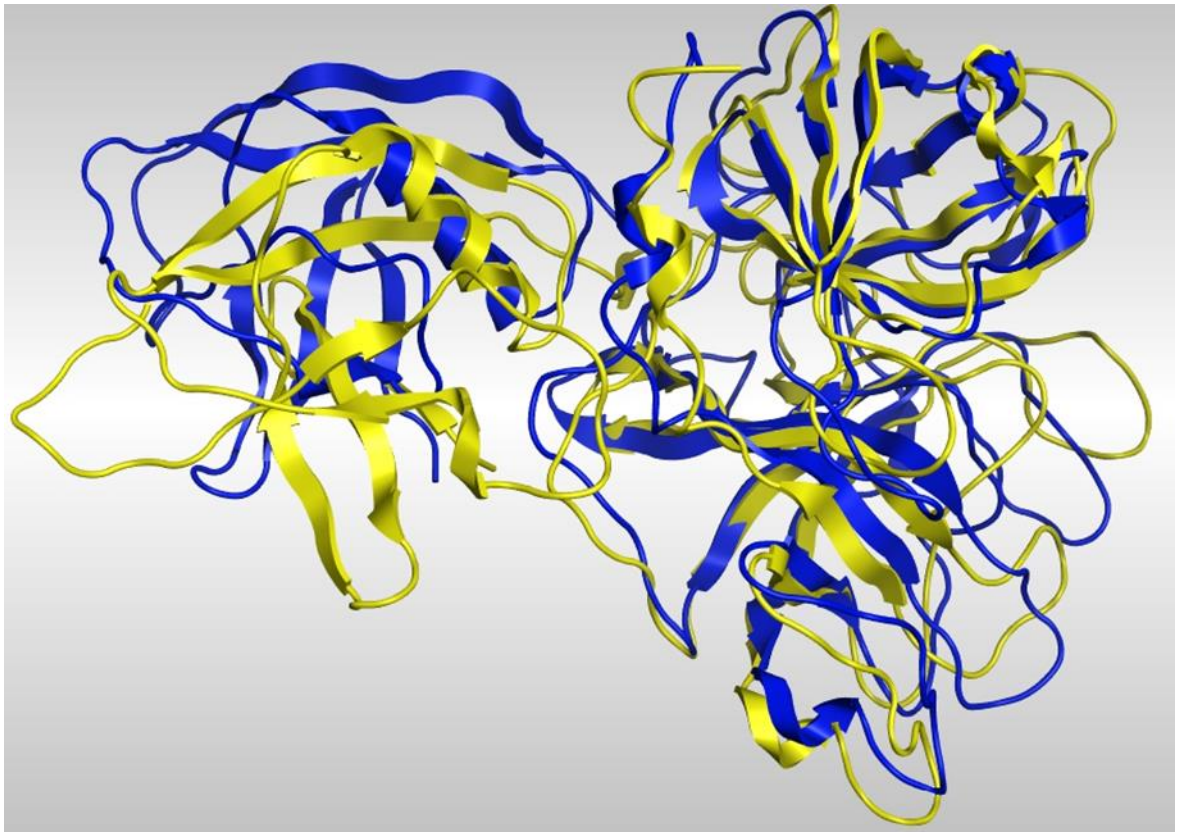

Figure S7. Overlap of 5CE1_A (blue) and 1Z8G_A (yellow) after MD simulation.

Figure S8. Sequence alignment of TMPS enzymes using Clustal O in which "*" means that the residues are identical, ":" means that conserved substitutions have been observed, "." means that semi-conserved substitutions are observed. The SRCR domain is indicated in blue lettering, cysteine residues involved in disulphide bond formation are highlighted in yellow, the catalytic triad residues are highlighted in cyan and the cleavage site is highlighted in pink.

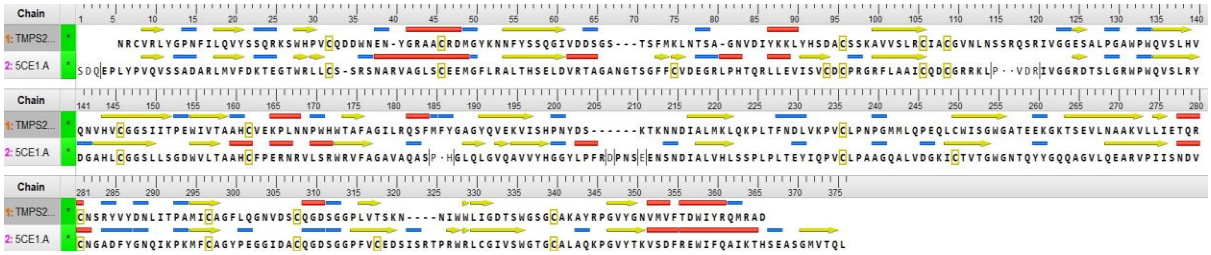

Figure S9. Comparison between the secondary structure of TMPS2 and the template 5CE1_A after alignment and superimposition using MOE software, showing α-helices as red lines, β-sheets as yellow arrows and loops as blue lines.

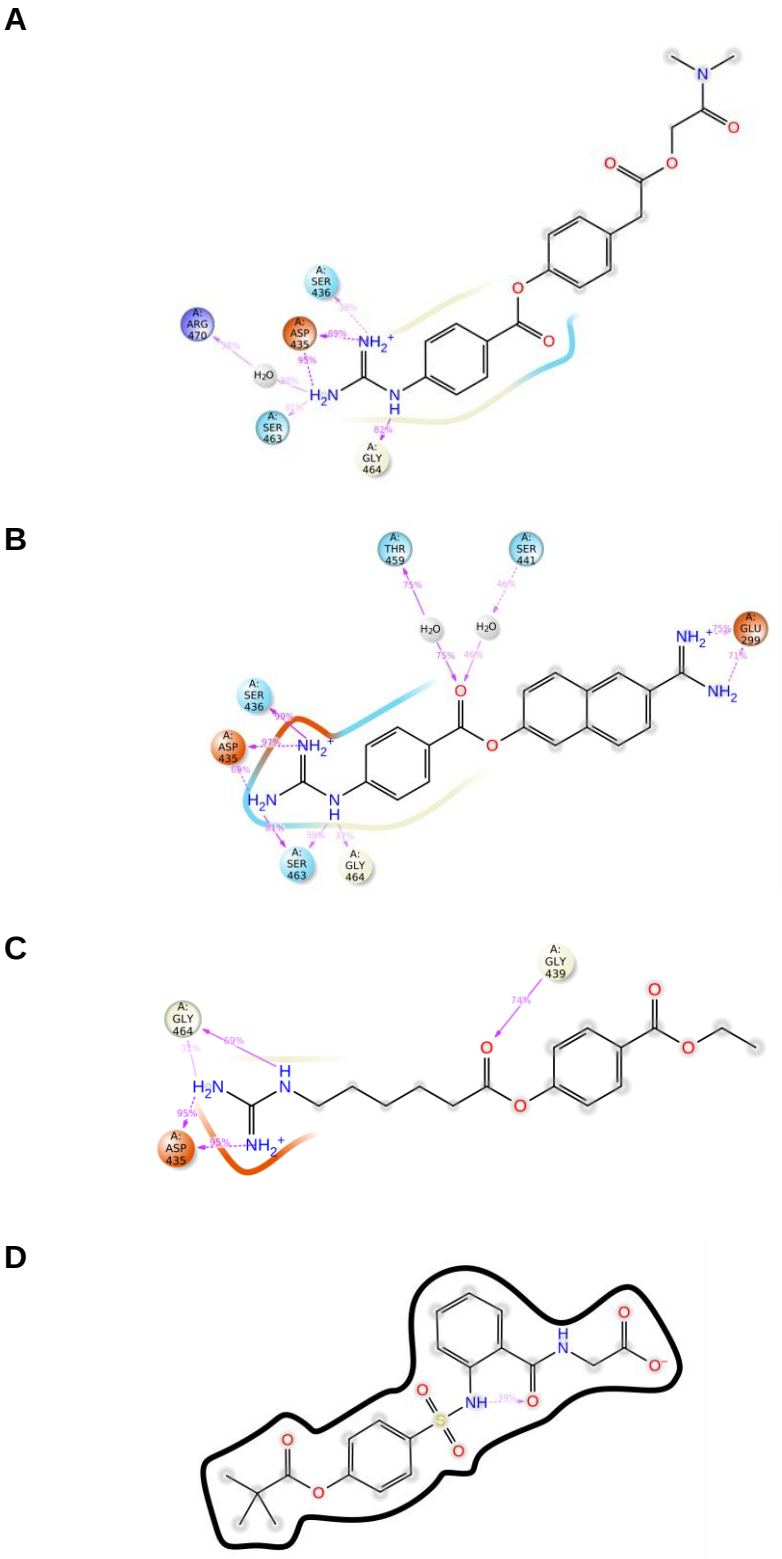

Figure S10. A schematic of detailed ligand atom interactions of (A) camostat, (B) nafamostat, (c) gabexate and (D) sivelestat with the protein residues of TMPS2 protein. Interactions that occur more than 30.0% of the simulation time in the selected trajectory (0 through 200 ns) are shown.

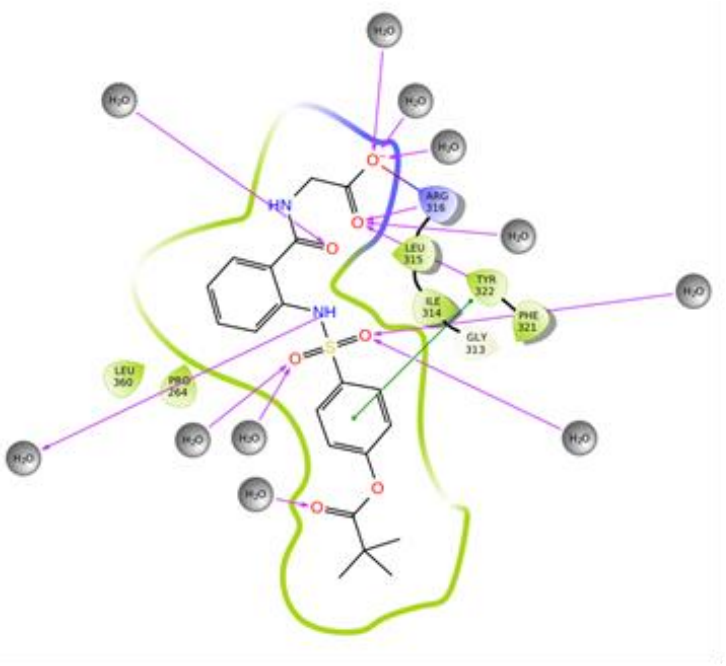

Figure S11. 2D Ligand interactions of sivelestat in TMPS2 protein.