Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supplementary Text

Comparison of tetrameric structures of Anthranilate Synthase in three states from *S. solfataricus, S. marcescens* and *S. typhimurium*

Anthranilate Synthase shows similar subunit or domain organization in bacteria like *Serratia marcescens*, *Salmonella typhimurium* and *Sulfolobus solfataricus*. However the oligomeric structure of all these enzymes show considerable difference [1–3]. In the substrate and inhibitor bound form of the AS in *S.marcescens* and inhibitor bound form of the AS from *S. typhimurium*, the heterotetrameric interface is formed by TrpE chains whereas in the unbound form of the AS from *S. solfataricus* it is formed by the TrpG chains. These differences in the quaternary structures have been attributed to the differences in molecular binding. AS from *S. marcescens* and *S. typhimurium* show cooperative Chorismate and Tryptophan binding, whereas the one from *S. solfataricus* does not show cooperative binding [1–4]. Another member of the Chorismate utilizing enzyme family, 2-Amino-2-deoxyisochorismate (ADIC) Synthase from *Burkholderia lata* shows very similar domain organization but very different oligomeric (in this case dimeric) structure [5]. Figure I shows all these different structures highlighting varied multimeric structures. Interestingly, given the variability among the AS from different species, there is no structural information available for AS in unbound, substrate-bound, and inhibitor-bound states in a single organism, so that a clear comparison can be made about its structure-function relationships.
In a recent study [6] the structure of AS from *Mycobacterium tuberculosis* has been determined. Although, the structure of the AS I (TrpE) in this was similar to the already determined TrpE structure in *S. marcescens* and *S. typhimurium* the AS II (TrpG) chain was not resolved. Interestingly the oligomeric structure is very different from those observed in other AS structures. The heterotetramer interface is formed by AS I residues but of different region as compared to that in *S. marcescens.* The heterodimer interface however is assumed to be similar to the other AS structures determined till now. This further suggests the similarity in the heterodimer functionality between AS from different organisms.

Equivalence of contact analysis in two dimers of AS

Heterotetramer structures of substrate bound and inhibitor bound AS from S.marcescens were modelled as PCNs. Total 364 contacts were lost and 251 contacts were made in the inhibitor-bound complex (AS-Inhibitor) as compared to substrate-bound complex (AS-Substrate). Out of total 364 contacts lost 174 contacts belonged to heterodimer formed by chains A and B (dimer-AB), 188 contacts belonged to heterodimer formed by chains C and D (dimer-CD) and two contacts were at the heterotetramer interface. Similarly, out of 251 contacts gained in the heterotetramer, 122 contacts belonged to dimer-AB, 127 belonged to dimer-CD and two contacts belonged to heterotetramer interface. Most of the contacts lost (151 out of 174 in dimer-AB and 188 in dimer-CD) and gained (91 out of 122 in dimer AB and 127 in dimer-CD) in two heterodimers are equivalent. Since the contacts lost and gained were similar in the two heterodimers except at the heterotetramer interface, the detailed analysis has been presented only for one heterodimer comprising of chains A and B in both the structures.

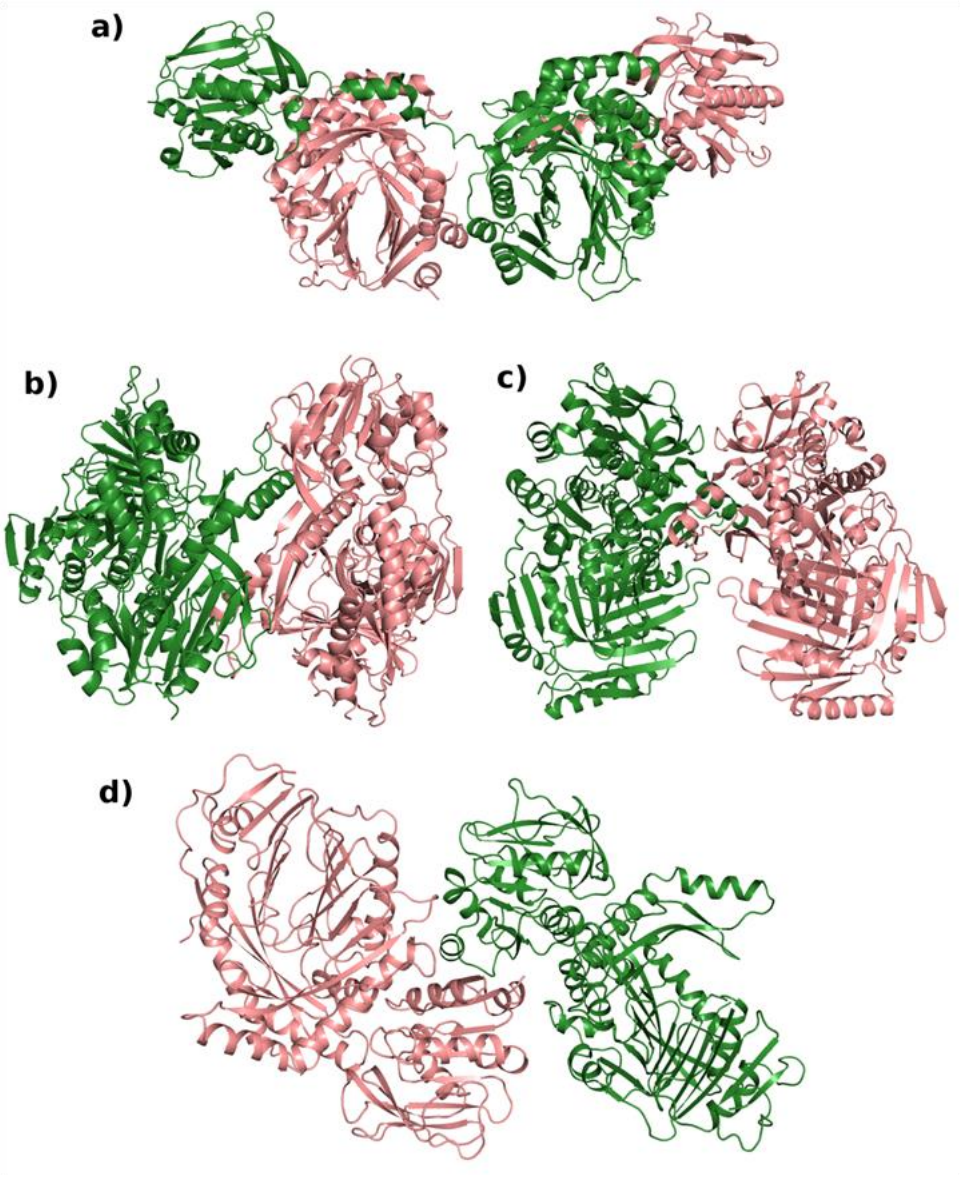

Figure I Anthranilate Synthase structures from a) *Burkholderia lata* (PDB ID 3R76), b) *Salmonella typhimurium* (PDB ID 1I1Q), c) *Serratia marcescens* (PDB ID 1I7Q), and d) *Sulfolobus solfataricus* (PDB ID 1QDL). The two components of the functional bienzyme (TrpE and TrpG) are shown in single color (Pink and Green, respectively)

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Changes in the Root Mean Square Fluctuations in AS residues on Inhibitor Binding

Root Mean Square Fluctuation represents the flexibility of the residues. Generally, the RMSF in both the proteins, do not show much difference, but few residues exhibit marked difference in flexibility. Residue number 330-360, which forms the hetero-tetramer interface, showed higher fluctuations in case of AS-inhibitor (see Figure S6). Some of the residues (106, 107 and 108) at

the heterodimer interface also show elevated RMSF values. These residues lose several contacts with residues from both TrpE and TrpG which might be causing the increase in flexibility.

Supplementary References

1 A. A. Morollo and M. J. Eck, *Nat. Struct. Biol.*, 2001, **8**, 243–247.

2 T. Knöchel, A. Ivens, G. Hester, A. Gonzalez, R. Bauerle, M. Wilmanns, K. Kirschner and J. N. Jansonius, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 9479–84.

3 G. Spraggon, C. Kim, X. Nguyen-Huu, M. C. Yee, C. Yanofsky and S. E. Mills, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 6021–6.

4 M. G. Caligiuris and R. Bauerles, *J. Biol. Chem.*, 1991, **266**, 8328–8335.

5 Q.-A. Li, D. V Mavrodi, L. S. Thomashow, M. Roessle and W. Blankenfeldt, *J. Biol. Chem.*, 2011, **286**, 18213–21.

6 G. Bashiri, J. M. Johnston, G. L. Evans, E. M. M. Bulloch, D. C. Goldstone, E. N. M. Jirgis, S. Kleinboelting, A. Castell, R. J. Ramsay, A. Manos-Turvey, R. J. Payne, J. S. Lott and E. N. Baker, *Acta Crystallogr. Sect. D Biol. Crystallogr.*, 2015, **71**, 2297–2308.

Supplementary Figures

Figure S1- Structure of Anthranilate Synthase heterodimer with all the contacts lost (red lines) and gained (blue lines)

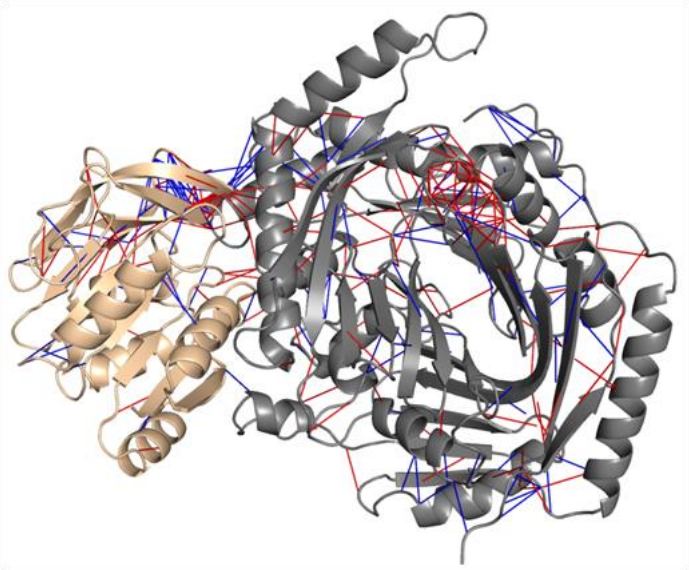


Figure S2 The cavities between two active sites become solvent exposed on inhibitor binding. Cavities between the active sites of TrpE and TrpG, determined by CASTp in a) AS-substrate and b) AS-inhibitor. The cavities have been shown in red surface representation and the substrate binding residues have been shown in red stick representation

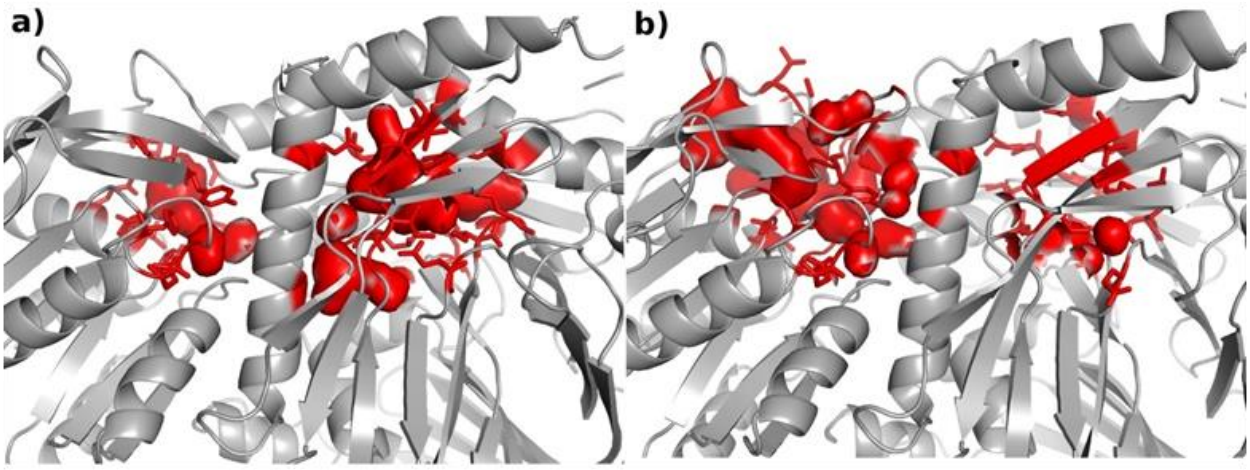


Figure S3- Structure of AS heterodimer with residues colored as per conservation score, obtained from Consurf server, grading from most conserved (Magenta) to least conserved (Teal). The residues participating in the shortest paths between two active sites have been shown as spheres.

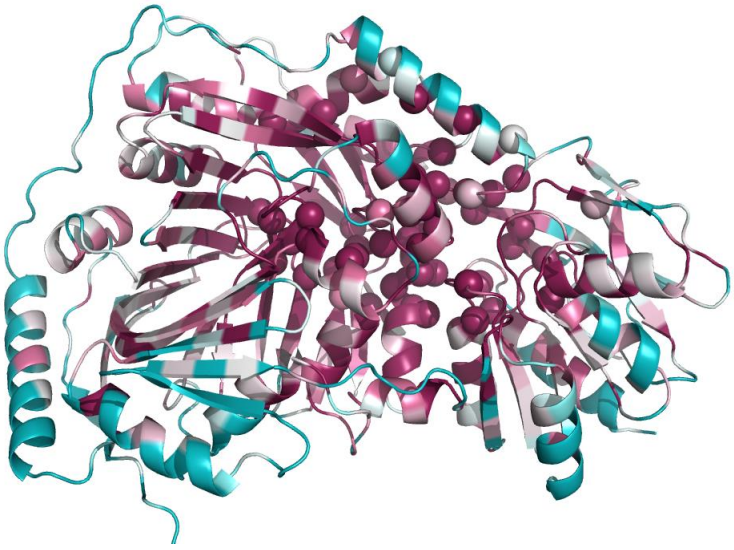


Figure S4 Root mean square deviation of frames of MD simulation trajectories of AS-substrate and AS-inhibitor.

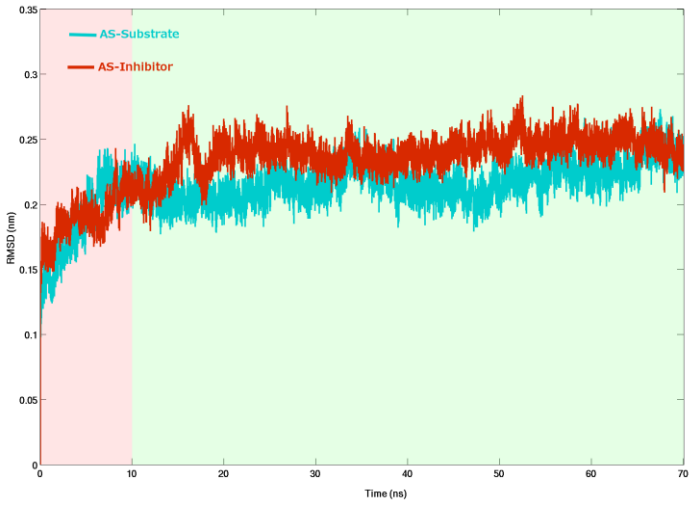


Figure S5 Difference in Root Mean Square Fluctuations of the Cα atoms in AS-substrate and AS-inhibitor show increased fluctuations for certain residues. a) Difference of RMSF for all residues b) Residues showing high increase (green spheres) and high decrease (yellow spheres) in RMSF in inhibitor bound complex. The TrpE subunit is shown in Grey and TrpG subunit in Wheat color. The residues in the top part of helix α7, showing some increase in RMSF are at the hetero-tetramer interface. Since we are considering only the dimer, that helix is solvent exposed and therefore shows high fluctuations in general. Rest of the increases in RMSFs is seen generally in the residues at the termini and loops.

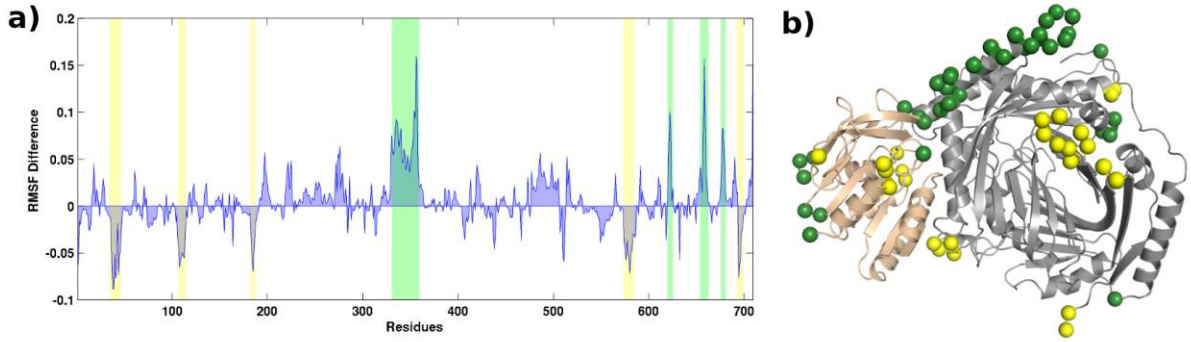
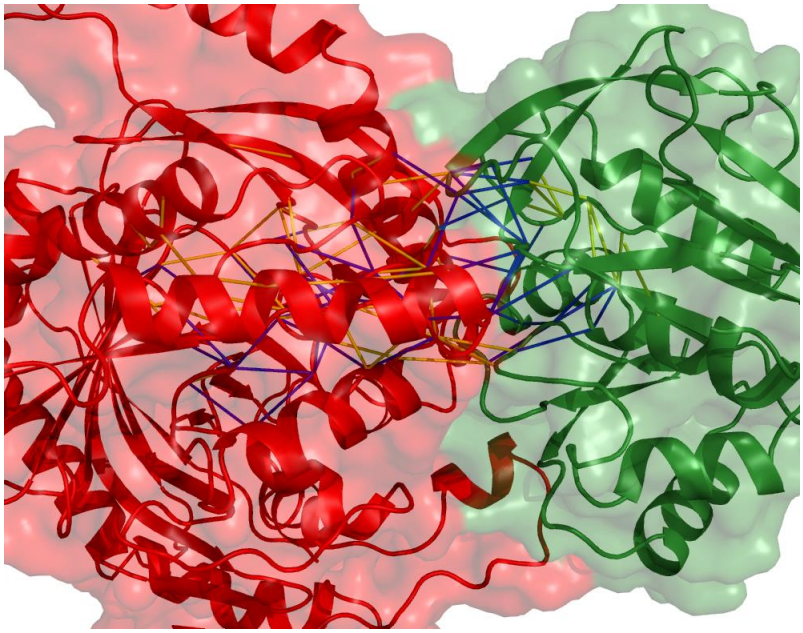


Figure S6. The residue pairs showing increase (shown in orange) and decrease (shown in blue) in dynamic correlations mapped on the crystal structure.



Supplementary Tables

Table S1- Residues showing change (≥3) in degree between AS-Inhibitor and AS-Substrate

Residues (Chain)	Increase	Residues	Decrease
103 (TrpG)	8	106 (TrpG)	6
104 (TrpG)	5	108 (TrpG)	5
519 (TrpE)	3	138 (TrpG)	5
488 (TrpE)	3	425(TrpE)	4
257 (TrpE)	3	107 (TrpG)	4
191 (TrpE)	3	198(TrpE)	3
162 (TrpE)	3	262(TrpE)	3
		288(TrpE)	3
		361(TrpE)	3
		422 (TrpE)	3
		430 (TrpE)	3
		505 (TrpE)	3
		306 (TrpE)	3
		136 (TrpG)	3
		156 (TrpG)	3
		135 (TrpG)	3

Table S2- List of all the hubs in AS-Substrate and AS-Inhibitor, residues in bold are unique to corresponding PCN

Hubs in AS-Substrate	Hubs in AS-Inhibitor
TrpE- **35**, 54, **57**, 151, 152, **194**, **196**, **256**, **259**, **261**, **284**, **306**, 307, 371, 374, 377, **398**, 402, 403, **430**, 434, **452**, 457, 464, 465, **466**, 467, 478 TrpG- **5**, 6, 8, 33, 56, 83, 84, 87, 88, **89**, **92**, 130, 131, 168, 178	TrpE- 54, **58**, 151, 152, **178**, **191**, 307, **314**, 371, 374, 377, 402, 403, **405**, 434, 457, 464, 465, 467, 478, **480**, **506**, **509** TrpG- 6, 8, 33, 56, 83, 84 ,**85**, 87, 88, **103**, 130, 131, 168, 178

Table S3- Residues with high betweenness, common ones are in bold

AS-Substrate	AS-Inhibitor
309, **311**, **324**, **326**, 332, 345, **351**, **383**, 389, 395, 401, 505, **179**(TrpG)	310, **311**, **324**, **326**, 328, 346, 348, **351**, 352, 355, 367, **383**, 424, 465, **179**(TrpG)

Table S4- Community membership of all the nodes in two PCNs

AS Substrate		**AS- Inhibitor**	
Community Name	**Nodes**	**Community Name**	**Nodes**
CS1	**TrpE-** 4-106, 122-199, 223, 235-241, 261-275, 288-332, 361-408,420-487 **TrpG-** 11-26	**CI1**	**TrpE-** 4-107, 122-161, 169-199, 234-240, 267-275, 289-332, 355-411, 445-483, 503 **TrpG-** 12-28
CS2	**TrpE-** 107-121, 224-234, 242-260, 276-287, 360, 488-520 **TrpG-**2-10, 27-195	**CI2**	**TrpE-** 108-121, 162, 241-266, 422-443, 484-502, 504 **TrpG-** 2-11, 29-195
CS3	**TrpE-** 200-222	**CI3**	**TrpE-** 163-168, 444
CS4	**TrpE**-409-419	**CI4**	**TrpE-** 200-233, 276-288, 505, 507-520
CS5	**TrpE-** 333-359	**CI5**	**TrpE**-333-354
		CI6	**TrpE-** 412, 414-421

Table S5- SASA values for the residues lining the paths determined by Mole2. Highlighted residues are common in two tunnels.

Tunnel Comparison					
1I7Q	SASA in 1I7Q	SASA in 1I7S	1I7S	SASA in 1I7Q	SASA in 1I7S
262 PHE A	16.43	34.08	262 PHE A	16.43	34.08
58 GLY B Backbone	12.96	27.71	85 CYS B	5.8	18.56
85 CYS B	5.8	18.56	134 HIS B	21.72	67.88
133 TYR B Backbone	16.79	23.91	172 HIS B	16.18	26.69
57 PRO B	14.44	22.09	58 GLY B Backbone	12.96	27.71
172 HIS B	16.18	26.69	59 PRO B Backbone	32.67	56.15
11 ASP B	12.7	24.26	430 PRO A	16.32	26.73
13 PHE B	15.57	19.24	259 GLY A Backbone	13.26	30.19
430 PRO A	16.32	26.73	260 GLU A Backbone	42.62	68.11
430 PRO A Backbone	16.32	26.73	261 ILE A Backbone	11.71	14.96
12 SER B	12.26	19.18	105 ILE B	28.03	27.36
429 ALA A	14.73	13.6	104 GLU B Backbone	100.25	35.55
368 LEU A	13.84	19.9	256 ILE A	9.77	12.94
364 MET A Backbone	16.35	14.75	262 PHE A Backbone	16.43	34.08
365 LEU A	16.91	33.25	488 GLN A	43.11	40.11
364 MET A	16.35	14.75	105 ILE B Backbone	28.03	27.36
263 GLN A	21.8	39.22	106 LEU B Backbone	28.1	87.58
428 GLY A Backbone	14.46	18.02	106 LEU B	28.1	87.58
429 ALA A Backbone	14.73	13.6	487 VAL A	13.78	37.34
424 GLY A Backbone	12.11	19.92	361 GLU A	17	83.19
265 VAL A	15.45	18.41	489 ASP A	43.93	92.35
425 THR A Backbone	17.2	80.5	357 LYS A	18.13	112.79
327 ALA A	13.22	30.83	490 SER A	14.76	21.03
361 GLU A	17	83.19	495 GLU A	16.74	20.27

326 ILE A Backbone	10.71	53.74	498 GLU A	17.16	68.79
327 ALA A Backbone	13.22	30.83	486 VAL A Backbone	15.38	16.49
485 GLY A Backbone	15.18	31.19	485 GLY A Backbone	15.18	31.19
502 LYS A	17.37	87.39	502 LYS A	17.37	87.39
469 ARG A	32.11	115.08	425 THR A Backbone	17.2	80.5
426 LEU A	20.28	30.85	265 VAL A	15.45	18.41
309 GLU A	13.27	34.7	426 LEU A	20.28	30.85
468 ILE A	10.95	34.04	483 GLY A Backbone	12.8	21.15
			449 TYR A	16.5	26.31

Table S6- Details of the pockets determined by CASTp that exist between the substrate binding site of TrpE and TrpG.

1i7q					
ID	N_mth	Area_sa ($Å^2$)	Area_ms ($Å^2$)	Vol_sa ($Å^3$)	Vol_ms ($Å^3$)
102	0	146	448.82	56.344	453.64
99	0	37	164.22	7.265	141.55
96	0	47	186.31	16.263	164.25
85	0	6.3	64.35	0.721	44.39
63	0	3.4	50.28	0.266	32.08
59	0	1.3	42.13	0.059	24.17
34	0	0.7	45.43	0.016	21.81
1i7s					
ID	N_mth	Area_sa ($Å^2$)	Area_ms ($Å^2$)	Vol_sa ($Å^3$)	Vol_ms ($Å^3$)
112	5	345	826.92	125.55	901.69
96	1	26	87.02	9.587	77.54
91	0	4.9	73.37	0.442	39.99
88	0	3.8	81.47	0.197	42.01
54	1	1.2	25.48	0.032	16.13
51	0	0.9	38.43	0.041	21.37
25	0	0.1	29.98	0.001	14.81
24	0	0	25.2	0	11.88
13	0	0	29.28	0	13.21
6	0	0	30.37	0	12.79

N_mth- Number of mouths
Area_sa- Solvent Accessible Area
Area_ms- Molecular Surface Area
Volume_sa- Solvent Accessible Volume
Volume_ms- Molecular Surface Volume