

Role of the Cation- π Interaction in Therapeutic Proteins: A Comparative Study with Conventional Stabilizing Forces

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Abstract

The cation- π interaction is an important, general force for molecular recognition in biological receptors. In this study, we have analyzed the energy contribution resulting from cation- π interactions in the set of therapeutic proteins. The contribution of cation- π interacting residues in secondary structure involvement, solvent accessibility, stabilization centers, stabilizing residues and conservation score has been evaluated. Secondary structure of the cation- π involving residues shows that, Arg and Lys prefers to be in strand. Among the π residues, Phe prefer to be in coil, Tyr prefers to be in strand and Trp prefer to be in helix. Among the cation- π interacting residues Arg and Lys were in the exposed regions. Phe and Tyr were in the partially buried region and Trp in the fully buried region. Stabilization centers for these proteins showed that all the five residues found in cation- π interactions are important in locating one or more of such centers. The contribution of stabilizing residues in the cation- π interactions was analyzed. Further, the study shows that, 43 percent of the amino acid residues that are involved in cation- π interactions might be conserved in therapeutic proteins. The comparison between the conventional and nonconventional interactions in the data set, clearly depict the significance of cation- π interaction in the stability of therapeutic proteins. On the whole, the results presented in this work will be very useful for understanding the contribution of cation- π interaction to the stability of therapeutic proteins.

Keywords: Cation- π interactions; Secondary structure; Solvent accessibility; Stabilizing residues; Interaction energy; Stabilizing centre

Introduction

The importance of therapeutic proteins has grown rapidly since the emergence of the biotechnology industry more than 30 years ago. There are approximately 140 therapeutic proteins approved in the United States and Europe, and an additional 500 in clinical trials (Walsh, 2003), with an even large number in preclinical development. In recent years, the number of recombinant proteins used for thera-

peutic applications has increased dramatically. This increasing trend has driven the development of a variety of improvements in protein expression and stability analysis. The stability can be determined by several interactions such as salt bridge, di-sulfide bond, conventional hydrogen bonds electrostatic interaction, Van der Waals and hydrophobic interactions in the protein structure. These interactions are

crucial in many areas of modern chemistry, especially in the field of molecular recognition and for structural stability (Hunter et al., 1990; Wintjens et al., 2000). In addition cation- π interaction (Dougherty, 1996; Ma and Dougherty, 1997; Scrutton and Raine, 1996) is increasingly recognized as an important noncovalent binding interaction relevant to structural biology.

Their understanding is essential for rational drug design and lead optimization in medicinal chemistry (Meyer et al., 2003). In proteins, cation- π interactions occur between the cationic side chain of lysine (K) or arginine (R) and the aromatic side chains of phenylalanine (F), tyrosine (Y) and tryptophan (W) (Chakravarty and Varadarajan, 2000). Previous studies on cation- π interactions have focused on various aspects such as their role in ligand recognition (Zacharias and Dougherty, 2002; Zhong et al., 1998; Scrutton and Raine, 1996) and protein drug interactions (Liu et al., 2002). There are several instances where cation- π interactions have shown to play a significant role. For example, the active site of horse radish peroxidase consists of an arginine interacting with the adjacent tyrosine residue to allow aromatic donor binding (Ma and Dougherty, 1997).

The importance of this interaction has been stressed by several investigators for their role in enhancement of the stability of thermophilic proteins (Chakravarty and Varadarajan, 2000; Gromiha et al., 2002), folding of polypeptides (Shi et al., 2002; Burghardt et al., 2002) and the stability of membrane proteins (Mulhern et al., 2000; Gromiha, 2003). Influence of cation- π interactions in protein-DNA complexes is studied by Gromiha et al., (2004). Also there are reports on these kinds of interactions in a set of 62 non-reductant DNA binding proteins by the same author (Gromiha, 2005). Recently, our group published work on cation- π interactions in Interleukins (Anand et al., 2006) and in RNA-binding proteins (Anand et al., 2007).

One of the most commonly cited examples of cation- π interactions is the acetylcholine-binding site of acetylcholinesterase (Scrutton and Raine, 1996). The active site of this enzyme is divided into two subsites: the 'esteratic' site and the 'anionic' site. Access to the active site of the enzyme is via the deep and narrow 'aromatic gorge' which consists of 14 highly conserved aromatic residues. Studies have shown that docking of the substrate acetylcholine, at the base of the gorge, results in the cation- π binding of choline to Trp-84 in the 'anionic' site (Dougherty, 1996).

To the best of the authors' knowledge, such an interactions data in therapeutic protein data set is not yet available. Hence, in this work an effort has been made to collect the information concerning conventional and nonconventional interactions such as traditional hydrogen bond, di-sulfide bond, salt bridge, and cation- π interactions in the therapeutic protein data set. We emphasize that 43 therapeutic proteins in our data set showed significant number of cation- π interactions and hence we emphasize that this investigation is very significant in the sense that, cation- π interactions in therapeutic proteins do play a major role in structural stability of these proteins. The knowledge gained from this study is important in the detection of interplay of conventional and non conventional interaction in the therapeutic protein. This will facilitate the design of more potent, less toxic and personalized drugs using these proteins.

Materials and Methods

Data Set

We have considered a set of 49 therapeutic proteins from the Protein Data Bank (Berman et al., 2000) for our investigation the details of which are given in Table 1. According to the structural classification of proteins, 42% of this protein comes under alpha group, 29% comes under beta 11% comes under alpha and beta and remaining 18% comes under small proteins in the therapeutic protein data set.

Computation of Cation- π Interactions Energy

The cation- π interaction energy in each enzyme has been calculated using the program CaPTURE (Gallivan and Dougherty, 1999). Initially cation- π interactions were identified with approximate distance based criteria. Energetically significant cation- π interactions can be obtained by using the program CaPTURE. This program has meaningful statistics for cation- π interactions for structures within the PDB. Also, simple and unambiguous protocol makes this tool as one of the choicest candidates for the computation of cation- π energies. The percentage composition of a specific amino acid residue contributing to cation- π interactions is obtained by the equation,

$$\text{Comp}_{\text{cat-}\pi}(i) = n_{\text{cat-}\pi}(i) \times [100/n(i)] \quad (1)$$

where i stands for the five residues, Lys, Arg, Phe, Trp and Tyr, $n_{\text{cat-}\pi}$ is the number of residues involved in cation- π interactions and $n(i)$ is the number of residues of type i in the considered protein structures.

PDB Code	%Lys	%Arg	%Phe	%Tyr	%Trp
1BML	5.2	5.2	3.6	2.0	2.4
1BMP	4.8	2.9	3.8	6.7	1.9
1C4P	9.5	2.9	2.9	5.8	0.0
1CA9	6.3	6.3	5.8	3.1	1.0
1CD9	2.3	2.9	3.5	1.8	1.2
1CN4	1.4	8.3	4.1	2.3	2.8
1DDJ	5.3	5.3	3.6	2.0	2.4
1EER	6.6	7.8	2.4	2.4	1.8
1ES7	5.8	1.9	2.9	4.8	1.9
1GNC	2.3	2.9	3.4	1.7	1.1
1HTZ	4.6	6.8	1.9	1.5	1.5
1L4D	5.2	5.2	3.6	2.0	2.4
1L4Z	5.2	5.2	3.6	2.0	2.4
1L6X	8.3	4.1	3.7	4.1	1.7
1M47	8.2	3.3	4.9	2.5	0.8
1M48	8.1	3.3	4.1	2.4	0.8
1M49	8.1	3.3	4.1	2.4	0.8
1M4C	8.7	2.6	4.4	2.6	0.9
1N8Y	3.0	5.9	2.5	2.9	1.2
1N8Z	6.1	3.3	4.2	4.7	0.9
1NBP	8.3	3.3	5.0	1.7	0.8
1PGG	4.4	5.6	6.7	4.9	1.6
1PGR	2.5	3.1	3.7	1.9	1.2
1PW6	8.3	3.3	4.1	2.5	0.8
1PY2	7.7	3.4	5.1	2.6	0.9
1QQR	8.7	4.3	3.6	5.8	0.0
1QRZ	5.3	5.3	3.7	2.0	2.4
1QVN	8.1	3.3	4.1	2.4	0.8
1R46	4.1	4.9	3.8	3.8	4.1
1R47	4.1	4.9	3.8	3.8	4.1
1REU	4.9	1.9	2.9	4.9	1.9
1REW	4.9	1.9	2.9	4.9	1.9
1RHG	2.8	3.4	4.1	2.1	1.4
1RJX	4.9	4.9	3.7	2.5	2.5
1TPG	3.3	7.7	4.4	4.4	2.2
1WAQ	2.9	4.8	3.8	2.9	1.9
1YY8	5.2	3.8	3.8	3.8	1.4
1YY9	6.0	4.7	2.9	2.6	1.0
1Z92	8.3	3.3	4.2	2.5	0.8
2B5I	8.3	3.3	4.2	2.5	0.8
3BMP	5.7	2.8	2.8	4.7	1.9
2ERJ	4.6	6.2	2.3	4.6	2.3

2GMF	5.0	5.0	4.1	1.7	1.7
2GOO	4.9	1.9	2.9	4.9	1.9
2H62	5.8	1.9	2.9	4.8	1.9
2H64	6.7	1.9	2.9	4.8	1.9
2IWG	8.7	2.9	3.4	4.3	1.9
2OSL	6.1	2.8	3.3	4.2	1.9
3INK	9.0	3.3	4.1	2.5	0.8
Mean	5.80	4.06	3.71	3.27	1.60

Table 1: Composition of cation- π forming residues in therapeutic proteins.

We have computed the energetic contribution of cation- π interactions for each enzyme in the data set and for all possible pairs of positively charged and aromatic amino acids. The total cation- π interaction energy ($E_{\text{cat-}\pi}$) has been divided into electrostatic (E_{es}) and van der Waals energy (E_{vw}) and was computed using the program CaPTURE, which has implemented a subset of OPLS force field (Jorgensen et al., 1996) to calculate the energies. The electrostatic energy (E_{es}) is calculated using the equation

$$E_{\text{el}} = \sum q_i q_j e^2 / r_{ij}; \quad (2)$$

Where q_i and q_j are the charges for the atoms i and j , respectively, and r_{ij} is the distance between them. The van der Waals energy is given by

$$E_{\text{vw}} = 4\varepsilon_{ij} [(\sigma_{ij}^{12}/r_{ij}^{12}) - (\sigma_{ij}^6/r_{ij}^6)] \quad (3)$$

Where $\sigma_{ij} = (\sigma_{ii}\sigma_{jj})^{1/2}$ and $\varepsilon_{ij} = (\varepsilon_{ii}\varepsilon_{jj})^{1/2}$; σ and ε are the van der Waals radius and well depth, respectively. The electrostatic component of the OPLS binding energies (E_{es}) were compared with the total *ab initio* binding energy. These measurements correlate well. A force field-based method was developed to reproduce the trends in the *ab initio* data. Also the force field-based method was used to select energetically significant cation- π interactions.

Secondary Structure and Solvent Accessibility Studies

Secondary structure and solvent accessibility are considered to be very important to understand the biochemical activity of proteins. Hence a systematic analysis of each cation- π interactions forming residue was performed based on their location in different secondary structures of enzymes and their solvent accessibility. Solvent accessibility

was divided into three classes, buried, partially buried and exposed indicating, respectively, the least, moderate and high accessibility of the amino acid residues to the solvent. We used the program DSSP (Kabsch and Sander, 1983) to obtain the information about secondary structures and solvent accessibility. According to the Science Citation Index (July 1995), the program has been cited in the scientific literature more than 1000 times. Hence in our analysis, we have chosen DSSP for predicting the secondary structure and solvent accessibility.

Computation of Stabilization Center

Stabilization centers are clusters of residues that are involved in medium or long range interactions (Dosztanyi et al., 1997). Residues can be considered part of stabilization centers if they are involved in medium or long range interactions and if two supporting residues can be selected from both of their flanking tetra peptides, which together with the central residues form at least seven out of the nine possible contacts. We used the server which is available at <http://www.enzim.hu/scide> (Dosztanyi et al., 2003) for this purpose.

Stabilizing residues were computed using the parameters such as surrounding hydrophobicity, long-range order, stabilization center and conservation score as described by Gromiha et al., (2004a). We used the server SRide (Gromiha et al., 2004a) for this purpose. Conservation score of ≥ 6 is the cutoff value used to identify the stabilizing residues.

Computation of Short, Medium and Long-range Contacts in Therapeutic Proteins Data Set

The residues coming within a sphere of 8\AA was computed as described earlier (Gromiha et al., 2004b). For a given residue, the comparison of the surrounding residue is analyzed in terms of the location at the sequence level. The contribution from $<\pm 4$ are treated as short-range contacts, $>\pm 4$ to $<\pm 20$ as medium-range contacts and >20 are treated as long-range contacts. This classification enables us to evaluate the contribution of long-range contacts in the formation of cation- π interactions.

Conservation Score

We computed the conservation score of cation- π interacting amino acid residues in each therapeutic protein using the ConSurf server (Glaser et al., 2003). This server computes the conservation based on the comparison of the se-

quence of a PDB chain with the proteins deposited in Swiss-Prot (Boeckman et al., 2003) and finds the ones that are homologous to the PDB sequence. The number of PSI-BLAST iterations and the *E*value cutoff used in all similarity searches were 1 and 0.001, respectively. All the sequences that are evolutionarily related with each one of the proteins in the data set were used in the subsequent multiple alignments. Based on these protein sequence alignments the residues are classified into nine categories from highly variable to highly conserved. Residues with a score of 1 are considered highly variable and residues with a score of 9 are considered highly conserved.

Interplay of Conventional and Nonconventional Interactions in Therapeutic Protein

The conventional interactions such as optimal hydrogen bond, salt bridge (a negative atom (side chain oxygen in Asp or Glu) and a positive atom (side chain nitrogen in Arg, Lys or His with an inter-atomic distance less than 7.0\AA) and di-sulfide (Two cysteine are called a bridged pair if the distance between their sulphur is between 1.5 and 2.5\AA) interactions were computed with the help of WHAT IF (Vriend, 1990). The nonconventional cation- π interaction, as reported earlier in this study is calculated using the program CaPTURE (Gallivan and Dougherty, 1999). The knowledge of these interactions and their comparison with the conventional interactions on a therapeutic protein data set probably, is the first such report available in the literature.

Results and Discussion

Preference of Cationic and Aromatic Residues for Forming Cation- π Interaction in Therapeutic Proteins

The preference of amino acid residues that are involved in cation- π interactions was analyzed and the results are presented in Table 2. We observed that in these proteins, Phe has the highest occurrence among the aromatic residues involving in cation- π interactions. Moreover, only 50% of the Trp residues are involved in these cation- π interactions as compared to Phe and Tyr. Lys is higher than Arg amongst the cationic residues in the set of therapeutic proteins studied. This trend is similar to those observed in transmembrane, globular proteins (Mulhern et al., 2000; Gromiha, 2003) DNA (Gromiha et al., 2004) and RNA binding proteins (Anand et al., 2007).

Cation- π Residue Pairs Involved in Therapeutic Proteins

PDB Code	Cation residues	π residues	Total number of cation- π interactions	E(es) kcal/mol	E(vdw) kcal/mol	E _{total} kcal/mol	Total Cat- π energy (kcal/mol)	D seq
1BML	R637	F639	3	-1.40	-1.99	-3.39	-9.04	2
	R580	W685		-2.13	-0.36	-2.49		105
	K651	W597		-2.20	-0.96	-3.16		54
1BMP	K40	Y65	1	-3.34	-0.83	-4.17	-4.17	25
1C4P			0					
1CA9	R393	F377	3	-1.9	-3.25	-5.15	-13.87	16
	R385	Y350		-2.62	-2.76	-5.38		35
	R385	Y386		-2.19	-1.15	-3.34		1
1CD9	R148	Y40	1	-4.43	-4.08	-8.51	-8.51	108
1CN4	R99	W64	4	-3.09	-2.55	-5.64	-20.8	35
	R155	W209		-1.95	-1.36	-3.31		54
	R197	W209		-7.2	-1.64	-8.84		12
	R199	W209		-7.82	4.81	-3.01		10
1DDJ	R582	F583	1	-2.94	-2.66	-5.6	-5.6	1
1EER	R14	Y15	2	-1.66	-1.79	-3.45	-8.12	1
	R53	Y49		-1.77	-2.9	-4.67		4
1ES7	R16	F41	2	-4.69	-3.47	-8.16	-12.51	25
	K101	Y103		-3.88	-0.47	-4.35		2
1GNC	K17	F14	1	-4.65	-0.99	-5.64	-5.64	3
1HTZ	R61	F60	2	-1.11	-2.11	-3.22	-15.56	1
	R159	W290		-7.77	-4.57	-12.34		131
1L4D			0					
1L4Z			0					
1L6X	R292	Y300	2	-4.36	-1.89	-6.25	-9.55	8
	K320	Y278		-2.53	-0.77	-3.3		42
1M47	R120	W121	2	-2.43	-0.41	-2.84	-7.75	1
	K35	Y31		-3.83	-1.08	-4.91		4
1M48			0					
1M49			0					
1M4C	R120	W121	1	-2.00	-0.33	-2.33	-2.33	1
1N8Y	K537	F513	2	-3.43	-0.84	-4.27	-6.47	24
	K89	Y90		-1.1	-1.1	-2.2		1
1N8Z	R142	Y173	2	-1.56	-1.22	-2.78	-6.42	31
	R211	Y186		-2.01	-1.63	-3.64		25
1NBP	R120	W121	2	-2.47	-0.43	-2.9	-6.43	1
	K54	F103		-2.63	-0.9	-3.53		49
1PGG	R245	F329	6	-4.57	-2.61	-7.18	-24.05	84
	R469	F470		-1.85	-1.13	-2.98		1
	R428	Y577		-1.22	-1.21	-2.43		149
	R467	Y475		-2.88	-2.86	-5.74		8
	K532	F371		-2.97	-0.74	-3.71		161
K560	F556	-3.82	1.81	-2.01	4			
1PGR	R148	Y40	1	-4.13	-4.03	-8.16	-8.16	108
1PW6	R120	W121	1	-2.46	-0.41	-2.87	-2.87	1
1PY2	K35	Y31	1	-4.48	-1.4	-5.88	-5.88	4
1QQR	K273	Y275	1	-4.26	-1.32	-5.58	-5.58	2
1QRZ	K557	F748	1	-2.26	-0.51	-2.77	-2.77	191
1QVN	R120	W121	2	-2.12	-0.4	-2.52	-8.87	1
	K35	Y31		-5.25	-1.1	-6.35		4
1R46	R363	F337	2	-2.16	-1.96	-4.12	-13.91	26
	R100	Y151		-5.87	-3.92	-9.79		51
1R47	R49	F50	3	-2.3	-1.35	-3.65	-15.99	1
	R363	F337		-4.07	-0.5	-4.57		26
	R100	Y151		-4.51	-3.26	-7.77		51
1REU	R16	F41	2	-5.06	-3.53	-8.59	-14.93	25
	K101	Y103		-4.98	-1.36	-6.34		2

IREW	R16 K101	F41 Y103	2	-5.46 -2.77	-3.81 -1.22	-9.27 -3.99	-13.26	25 2
1RHG	R22	Y165	1	-4.63	-1.75	-6.38	-6.38	143
1RJX	K556	Y569	1	-2.56	-1.01	-3.57	-3.57	13
1TPG	R55	F71	1	-2.75	-1.20	-3.95	-3.95	16
1WAQ	R21 K107	F46 Y109	2	-5.46 -2.33	-3.86 -0.95	-9.32 -3.28	-12.6	25 2
1YY8	R142 R211	Y173 Y186	2	-2.06 -2.15	-1.69 -1.18	-3.75 -3.33	-7.08	31 25
1YY9	R125	Y93	1	-1.43	-1.31	-2.74	-2.74	32
1Z92			0					
2B5I	R120	W121	1	-2.48	-0.45	-2.93	-2.93	1
3BMP	R16 K101	F41 Y103	2	-4.56 -2.57	-3.34 -0.76	-7.9 -3.33	-11.23	25 2
2ERJ	R117	W110	1	-2.25	-2.38	-4.63	-4.63	7
2GMF	R4	W13	1	-6.75	-4.49	-11.24	-11.24	9
2GOO	R16 K101	F41 Y103	2	-5.29 -2.53	-3.74 -0.99	-9.03 -3.52	-12.55	25 2
2H62	R16 K101	F41 Y103	2	-4.45 -2.89	-2.82 -1.34	-7.27 -4.23	-11.5	25 2
2H64	R16 K101	F41 Y103	2	-5.41 -3.92	-4.03 -1.23	-9.44 -5.15	-14.59	25 2
2IWG	R292	Y300	1	-2.84	-3.43	-6.27	-6.27	8
2OSL	R210	Y185	1	-2.18	-0.98	-3.16	-3.16	26
3INK	R120	W121	1	-2.86	-0.65	-3.51	-3.51	1

Table 2: Cation- π interaction forming residue, total interaction energy, D seq in Therapeutic protein.

Residue	Strand	Coil	Helix
Arg	36.54	34.61	28.84
Lys	52.17	17.39	30.43
Phe	25	58.33	16.66
Tyr	47.22	25	27.77
Trp	6.66	4	53.33

Table 3: Frequency of occurrence of cation- π interaction forming residue in different Secondary structures.

The number of cation- π interaction in therapeutic proteins in the present data set studied ranges from 1 – 6. The study shows that 41, 37 and 10% of the protein had number of interaction of 1, 2 and more than 2 interactions respectively. Almost 10% of the therapeutic protein did not show any cation- π interactions. These results are shown in Table 3. There are six cation- π interacting pairs namely, Arg-Phe, Arg-Tyr, Arg-Trp, Lys-Phe, Lys-Tyr and Lys-Trp pairs. The PyMol view of Arg-Phe, Arg-Trp and Lys-Trp interacting pairs for the protein with a PDB id 1BML in is shown in Fig. 1. It was found that, among the cation- π interactions involving Arg residues Arg-Tyr residues showed the highest percentage of interaction than Arg-Phe and Arg-Trp interactions. Among the cation- π interactions involving Lys residues Lys-Tyr interaction was higher than Lys-Phe and Lys-Trp interactions. These results are shown in Fig. 2. It is interesting to note that even though, individually, Phe and

Lys exhibited higher cation- π interactions, but as pairs, Arg-Tyr and Lys-Tyr were involved in more number of cation- π interactions than the other four pairs. Hence, the Arg-Tyr and Lys-Tyr interactions may be quite important in the stability of these therapeutic proteins. Of the total 49 proteins investigated, 43 proteins had significant cation- π interactions and rest of the 6 proteins did not show any significant interaction at all. The therapeutic protein 1PGG had a maximum of six energetically significant cation- π interactions.

Cation- π Interaction Energies in Therapeutic Protein

The specific pair wise residue involved in cation- π interaction and their position for all the therapeutic proteins studied are given in Table 3. It could be seen from the table that the therapeutic protein with PDB code 1PGG had a maximum energy of -24.05 (kcal/mol). The pair wise cation- π inter-

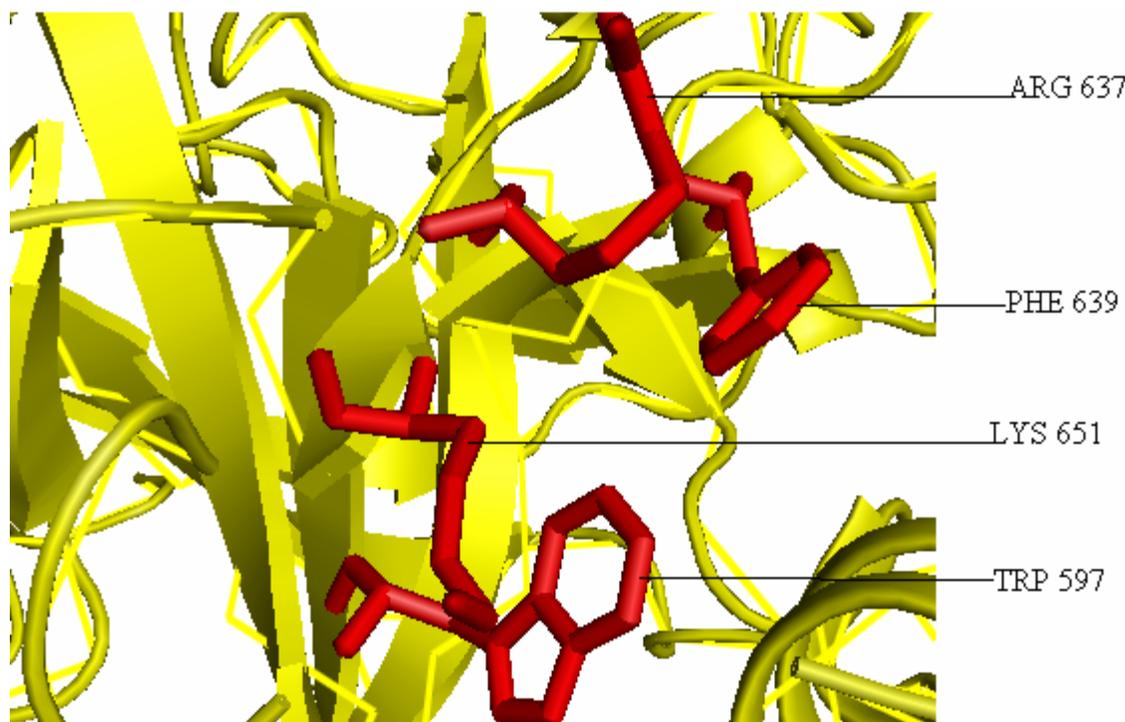


Figure 1: Pymol view of Arg-Phe and Lys-Trp interacting pairs in 1BML.

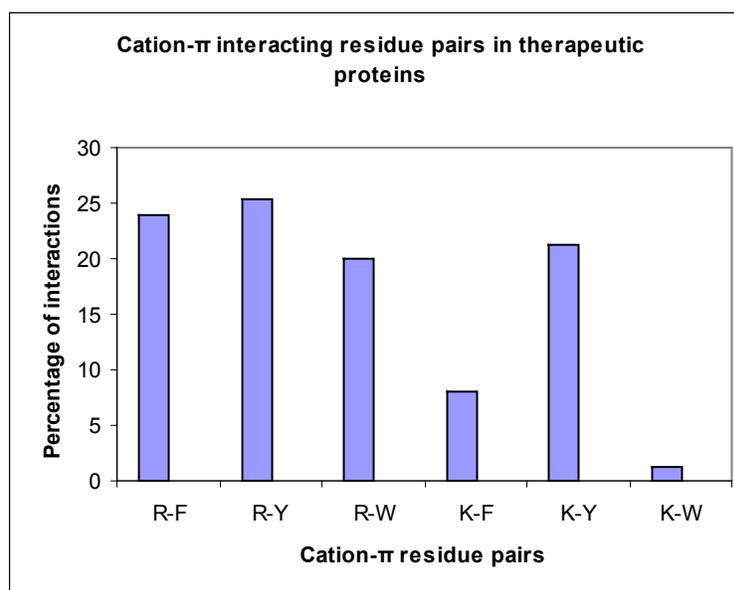


Figure 2: Cation- π interacting residues pairs in Therapeutic proteins.

action energy between the cationic and aromatic residues shows that Arg-Phe energy is the strongest and Lys-Trp is the lowest among the six possible pairs as shown in Fig. 3. The strength of cation- π interaction energy differs significantly in the therapeutic protein. For instance, for 1PGG it was -24.05 (kcal/mol) and in 1M4C it was -2.33 (kcal/mol). Of the 49 proteins investigated, it was found that 69 % showed a cation- π energy less than -10 kcal/mole, 27 %, -

10 to -20 kcal/mol and 4 % of them showed a cation- π interaction energy greater than -20 (kcal/mol) respectively. We observed an average energetic contribution of -4.53 (kcal/mol) in the group of therapeutic protein investigated in this work. The composition of cation- π interaction energy into electrostatic and Van der Waals energy terms showed that, among the 49 therapeutic protein, 43 protein had stronger electrostatic energy than Van der Waals energy.

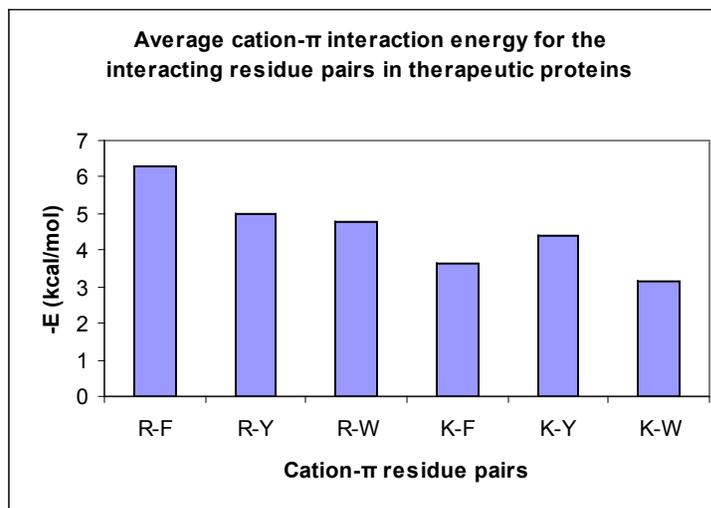


Figure 3: Average cation- π interaction energy for the interacting residue pairs.

Experimental Reports of Cation- π Interaction Analysis

Proteins such as LIVBP, MBP, RBP, and Trx had been used as model systems for studying the magnitude of cation- π interactions to protein stability (Prajapati et al., 2006), because these proteins can be expressed to high levels in *E. coli*. In a separate series of experiments, the aromatic amino acid in each cation- π pair was replaced by Leucine. Stabilities of wild-type (WT) and mutant proteins were characterized by both thermal and chemical denaturation. The experimental results suggest that cation- π interactions can make a significant contribution to the structural stability of proteins.

Secondary Structure Prediction of Amino Acid Residues in the Therapeutic Proteins

The propensities of the amino acid residues to favor a particular conformation are well known. Such conformational preference is not only dependent on the amino acid alone but is also dependent on the local amino acid sequence. We have computed the preference of cation- π interaction forming residues in different secondary structures and the results are shown in Table 4. It was found that, cationic residues such as Arg and Lys preferred to be in strand. In the aromatic group it was found that, Phe prefer to be in coil Tyr preferred to be in strand and Trp prefers to be in helix.

Solvent Sccessibility of the Cation- π Interacting Residues in Therapeutic Proteins

We used DSSP (Kabsch and Sander, 1983) to estimate the solvent accessibility of the residues involved in cation- π

interactions. The average solvent accessibility of the residues Arg, Lys, Phe, Tyr and Trp which are involved in cation- π interactions are 52.13, 74.47, 37.44, 34.86 and 13.72 respectively, as shown in Fig. 4. The solvent accessibility of Arg and Lys residues are significantly higher than other cation- π forming residues. The normalized ASA has been divided into three categories, buried, partially buried and exposed for different ranges of ASA; <20, 20–50 and >50, respectively (Gromiha et al., 1999; Gilis and Rooman, 1996; Gilis and Rooman, 1997). From this classification, we observed that Arg and Lys preferred to be in exposed region. Among the aromatic residues, it was observed that Phe and Tyr preferred to be in partially buried region, while Trp preferred to be in the fully buried regions. This observation is quite reasonable in the sense that, the aromatic residues are in principle, non polar residues, and tend to be buried. Since Arg and Lys are polar in nature they tend to be exposed to the solvent surface.

Stabilization Centers of Cation- π Interacting Residues in Therapeutic Proteins

We have computed the stabilization center for all cation- π interaction forming residues in therapeutic protein using the program SCide and the results are depicted in Fig. 5. It was found that 32% of cationic residues and 24% of π residues were found to have one or more stabilization centers. Cationic residues were found to have more stabilization centers than π residues. This trend was different with the earlier report on RNA binding proteins (Anand et al., 2007). It was interesting to note that all the five residues found in cation- π interactions are important in locating one or more stabilization centers. These observations strongly reveal that

PDB Code	Stabilizing residues
1BML	Ser572, Val616, Val635, Leu652, Ala665, Cys726, Ala727, Gly728, Leu745, Gln756, Gly757, Ser778
1BMP	Lys40 , Ile56, Ile57, Ala64, Cys67, Leu115, Tyr116, Cys136
1C4P	Val160, Tyr275, Leu277
1CA9	Lys357, Thr383, Leu392, His406, Leu407, Ser408, Leu409, Val412, Phe491, Ile492, Lys493
1CD9	Ala115
1CN4	Cys83, Leu96, Val119, Ala123, Pro124, Arg199 , Gly207
1DDJ	Ser572, Gln576, Trp597, Val598, Val616, Val635, Leu652, Cys680, Ala700, Ala727, Gly728, Leu755, Gly757
1EER	Ile6, Cys7, Ala19, Val41, Leu141, Ser146, Lys152
1ES7	Ile32, Val33, Ala40, Met89, Leu90, Val108
1GNC	His80, Gly88, Leu89, Ala115, Gly126, Gly151
1HTZ	Gly45, Thr71, Ile231, Ile260, Val262, Ile263
1L4D	Ser572, Gln576, Trp597, Val616, Val635, Ala648, Leu652, Thr683, Ala727, Gly728, Leu745, Leu755, Gln756, Gly757, Ser778
1L4Z	Gln576, Trp597, Val598, Val616, Val635, Leu652, Thr683, Ala727, Gly728, Leu755, Gly757, Ser778
1L6X	Val240, Thr260, Cys261, Val263, Cys321, Val323, Val369, Phe372
1M47	Leu17, Leu21, Leu118, Cys125
1M48	Leu21, Leu118, Cys125
1M49	Leu21, Leu118, Cys125
1M4C	Leu21, Leu118, Cys125
1N8Y	Tyr61, Phe455
1N8Z	Ala34, Val115
1NBP	Leu17, Leu21, Leu118, Cys125
1PGG	Thr149, Asn195, Leu196, Ala286, Ser427, Gly432, Arg433
1PGR	Ala115
1PW6	Leu21, Leu118, Cys125
1PY2	Leu21, Val93, Thr113, Leu118, Cys125
1QQR	Val160, Tyr275 , Leu277
1QRZ	Gly549, Gln576, Trp597, Val598, Leu599, Val616, Val635, Leu649, Leu652, Ala656, Pro664, Ala665, Cys680, Ile705, Cys726, Ala727, Gly728, Leu745, Leu755, Gln756, Gly757, Val758, Tyr774, Ser778
1QVN	Leu21, Val93, Leu118, Cys125
1R46	Gly43, Leu45, Gly132, Tyr134, Leu166, Leu167, Lys168, Tyr200, Ser201, Cys202, Asn224, His225, Arg227, Asn263, Ser297, Ala352, Cys382, Val413, Leu417
1R47	Gly43, Trp44, Leu45, Gly132, Tyr134, Val137, Leu166, Leu167, Lys168, Ser201, Cys202, Asn224, Arg227, Asn263, Leu294, Met296, Ser297, Ala352, Cys382, Val413, Leu417
1REU	Ile32, Val33, Ala40, Thr65, Met89, Leu90, Tyr91, Val108
1REW	Ile32, Val33, Ala40, Cys43, Met89, Leu90, Tyr91, Val108
1RHG	Tyr85, Ala114
1RJX	Trp597, Val598, Val616, Val635, Leu652, Ala656, Thr683, Cys726, Ala727, Gly728, Leu745, Gln756, Gly757, Ser778
1TPG	Ser20, Val31, Glu32, Cys34
1WAQ	Ile37, Ile38, Ile95, Leu96, Phe97
1YY8	Val115, Cys194
1YY9	Tyr64, Phe380
1Z92	Leu17, Leu21, Cys58, Thr113, Cys125
2B5I	Leu17, Leu21, Thr113, Cys125
3BMP	Ile32, Val33, Ala40, Cys43, Met89, Leu90, Tyr91, Val108
2ERJ	Thr24, Met44, Ser145
2GMF	Val42
2GOO	Ile32, Val33, Ala40, Met89, Leu90, Tyr91, Val108
2H62	Ile32, Val33, Ala40, Met89, Leu90, Tyr91, Val108
2H64	Ile32, Val33, Ala40, Cys43, Met89, Leu90, Tyr91, Val108,
2IWG	Val259, Cys261, Val263, Val302, Cys321, Val323, Val369, Phe372
2OSL	Trp36, Ile48
3INK	Nil

Bolded residues are amino acid residues involved in cation- π interactions.

Table 4: List of stabilizing residues.

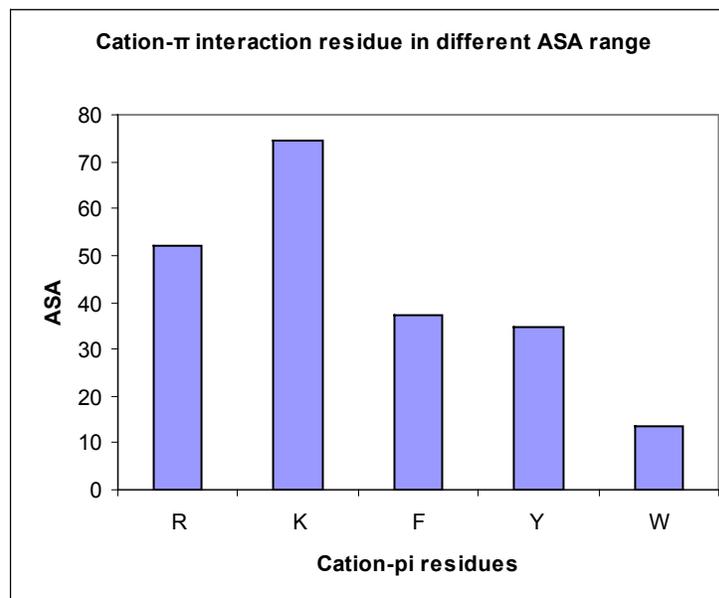


Figure 4: Cation- π interaction residues in different ASA range.

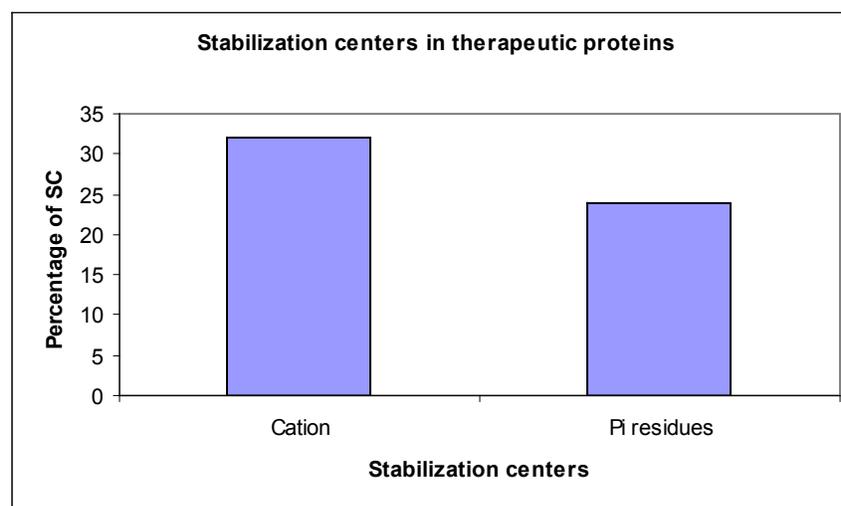


Figure 5: Stabilization centers in Therapeutic protein.

these residues may contribute significantly to the structural stability of these proteins in addition to participating in cation- π interactions.

Stabilizing Residues

We thought it would be useful to identify any patterns of correlation between the Cation- π interactions in a given therapeutic proteins and the theoretically predicted stabilizing residues (Gromiha et al., 2004a). Stabilizing residues were computed using the parameters such as surrounding hydrophobicity, long-range order, stabilization center and conservation score. We used the server SRide for this purpose. Stabilizing residues information was available for 48 out of 49 therapeutic proteins and the results are presented in Table

5. It shows that, 0.93% of these stabilizing residues were also involved in cation- π interactions. From these we infer that, these residues also might contribute to additional stability to therapeutic proteins.

Sequential Separation between Residues that are Forming the Cation- π Interaction

The sequential distance was calculated between the cationic and the aromatic residues for each cation- π interaction and results are depicted in Fig 6. The contribution from $<\pm 4$ are treated as short-range contacts, $>\pm 4$ to $<\pm 20$ as medium-range contacts and >20 are treated as long-range contacts. In our study group 40, 13 and 47% of the therapeutic proteins exhibited short, medium and long-range in-

PDB Code	Hydrogen bond (H)	Salt bridge (S)	Di Sulfide (DS)	Cation- π interaction	Total number of interactions H+S+DS+Cat- π
1BML	114	61	6	3	184
1BMP	38	8	3	1	50
1C4P	64	40	0	0	104
1CA9	93	146	0	3	242
1CD9	85	17	2	1	105
1CN4	74	91	2	4	171
1DDJ	121	69	6	1	197
1EER	82	43	2	2	129
1ES7	47	8	3	2	60
1GNC	68	10	2	1	81
1HTZ	167	120	1	2	290
1L4D	126	67	6	0	199
1L4Z	118	63	6	0	187
1L6X	478	73	3	2	556
1M47	111	20	1	2	134
1M48	68	23	1	0	92
1M49	68	25	1	0	94
1M4C	115	48	2	1	166
1N8Y	262	207	22	2	493
1N8Z	109	50	2	2	163
1NBP	110	21	1	2	134
1PGG	323	182	5	6	516
1PGR	88	18	2	1	109
1PW6	57	22	1	1	81
1PY2	48	29	1	1	79
1QQR	73	42	0	1	116
1QRZ	120	74	6	1	201
1QVN	60	26	1	2	89
1R46	202	143	5	2	352
1R47	199	133	5	3	340
1REU	59	12	3	2	76
1REW	47	5	3	2	57
1RHG	86	21	1	1	109
1RJX	294	61	6	1	362
1TPG	22	13	5	1	41
1WAQ	77	26	3	2	108
1YY8	116	45	2	2	165
1YY9	272	303	25	1	601
1Z92	67	20	1	0	88

2B5I	64	29	1	1	95
3BMP	71	11	3	2	87
2ERJ	45	11	5	1	62
2GMF	61	21	2	1	85
2GOO	47	5	3	2	57
2H62	50	10	3	2	65
2H64	50	9	3	2	64
2IWG	95	61	2	1	159
2OSL	110	41	2	1	154
3INK	71	29	1	1	102
Total	5392	2612	172	75	8251

Table 5: Conventional and nonconventional interactions in therapeutic protein.

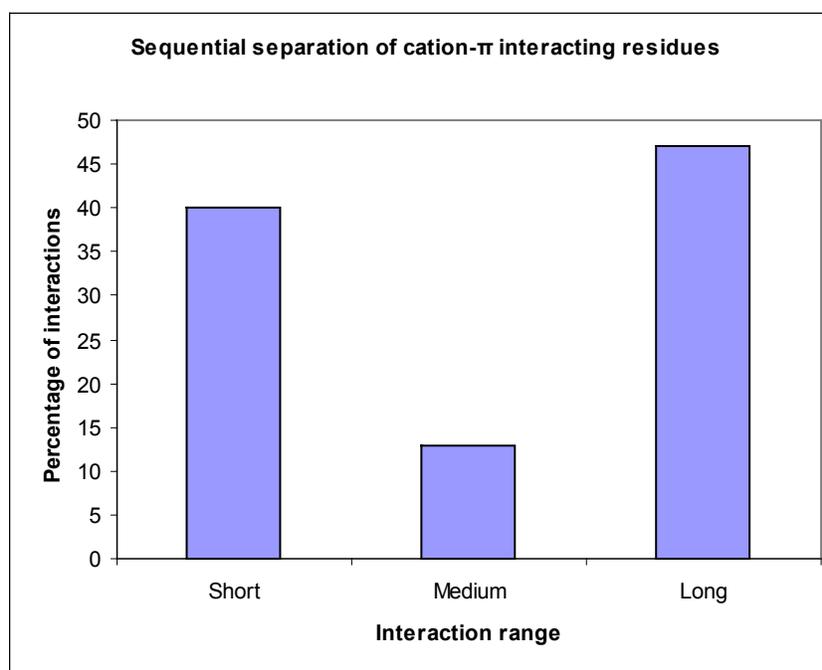


Figure 6: Sequential separation of cation – π interacting residues.

teractions respectively. Long-range cation- π interactions are the predominant type of interactions in therapeutic proteins.

Conservation Score

We used the ConSurf server to compute the conservation score of amino acid residues involved in cation- π interactions in therapeutic proteins, and the results are shown in Fig. 7. 57 percent of the amino acid residues had a conservation score, in the range of below 5, while 43 percent of the amino acid residues had a conservation score 6-9. Conservation score of 6 is the cutoff value used to identify the

stabilizing residues. From these observations, we were able to infer that, 43 percent of the amino acid residues that are involved in cation- π interactions might be conserved in therapeutic proteins.

Interplay of Conventional and Nonconventional Interaction in the Stability of Therapeutic Proteins

The conventional interactions studied in this work were computed with the help of WHAT IF (Vriend, 1990). We undertook these studies to infer the role of conventional and the cation- π interactions in individual proteins and in the whole data set as well. Table 6 shows the number of hydro-

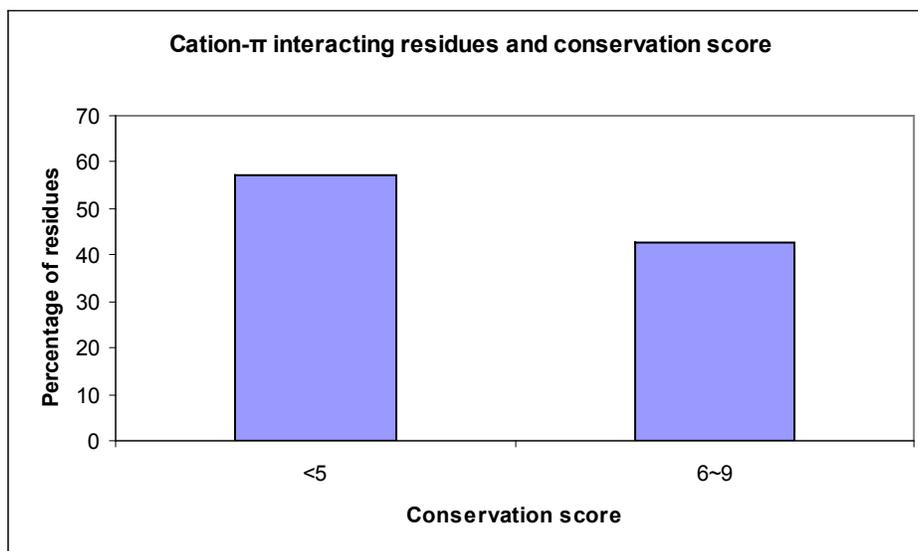


Figure 7: Cation- π interacting residues and conservation score.

PDB Code	Percentage of hydrogen bond	Percentage of saltbridge	Percentage of di-sulfide bond	Percentage of cation-π interactions
1BML	61.96	33.15	3.26	1.63
1BMP	76.00	16.00	6.00	2.00
1C4P	61.54	38.46	0.00	0.00
1CA9	38.43	60.33	0.00	1.24
1CD9	80.95	16.19	1.90	0.95
1CN4	43.27	53.22	1.17	2.34
1DDJ	61.42	35.03	3.05	0.51
1EER	63.57	33.33	1.55	1.55
1ES7	78.33	13.33	5.00	3.33
1GNC	83.95	12.35	2.47	1.23
1HTZ	57.59	41.38	0.34	0.69
1L4D	63.32	33.67	3.02	0.00
1L4Z	63.10	33.69	3.21	0.00
1L6X	85.97	13.13	0.54	0.36
1M47	82.84	14.93	0.75	1.49
1M48	73.91	25.00	1.09	0.00
1M49	72.34	26.60	1.06	0.00
1M4C	69.28	28.92	1.20	0.60
1N8Y	53.14	41.99	4.46	0.41
1N8Z	66.87	30.67	1.23	1.23
1NBP	82.09	15.67	0.75	1.49
1PGG	62.60	35.27	0.97	1.16
1PGR	80.73	16.51	1.83	0.92
1PW6	70.37	27.16	1.23	1.23
1PY2	60.76	36.71	1.27	1.27
1QQR	62.93	36.21	0.00	0.86

1QRZ	59.70	36.82	2.99	0.50
1QVN	67.42	29.21	1.12	2.25
1R46	57.39	40.63	1.42	0.57
1R47	58.53	39.12	1.47	0.88
1REU	77.63	15.79	3.95	2.63
1REW	82.46	8.77	5.26	3.51
1RHG	78.90	19.27	0.92	0.92
1RJX	81.22	16.85	1.66	0.28
1TPG	53.66	31.71	12.20	2.44
1WAQ	71.30	24.07	2.78	1.85
1YY8	70.30	27.27	1.21	1.21
1YY9	45.26	50.42	4.16	0.17
1Z92	76.14	22.73	1.14	0.00
2B5I	67.37	30.53	1.05	1.05
3BMP	81.61	12.64	3.45	2.30
2ERJ	72.58	17.74	8.06	1.61
2GMF	71.76	24.71	2.35	1.18
2GOO	82.46	8.77	5.26	3.51
2H62	76.92	15.38	4.62	3.08
2H64	78.13	14.06	4.69	3.13
2IWG	59.75	38.36	1.26	0.63
2OSL	71.43	26.62	1.30	0.65
3INK	69.61	28.43	0.98	0.98

Table 6: Percentage of conventional and nonconventional interaction in individual Therapeutic proteins.

gen bonds, salt bridge, di-sulfide bonds, cation- π interactions and the total number of conventional and non-conventional interactions in individual proteins. It is quite reasonable that the number of hydrogen bonds is much more than salt bridge and di-sulfide bonds on the conventional interaction side and number of cation- π interactions on the non-conventional interaction side, except for one protein (PDB id 1YY9). This protein incidentally also has the highest number of total number of interactions. The protein with PDB id 1PGG shows a total number of 516 interactions out of which 6 of them from cation- π interaction. There were a total of 8251 interactions for the whole data set out of which, 5392 where from hydrogen bond, 2612 from salt bridge, 172 from di-sulfide bond and 75 from cation- π interactions. The individual interaction such as hydrogen bond, salt bridge, di-sulfide bond and cation- π interaction in terms of the percentage are depicted in Table 7. The protein with PDB id 1L6X had the highest percentage of conventional hydrogen bond, which showed a cation- π interaction of 0.36%. How-

ever the highest percentage of cation- π interactions was shown by 2GOO even though it had only 2 cation- π interactions. Hence we could not generalize and come to any conclusion from these individual interactions. Hence we undertook the calculation to find out the relation between hydrogen bond, salt bridge, di-sulfide interactions with cation- π interactions. These are shown in Figure 8 to Figure 10. It is observed that, the significance of cation- π interactions is more than conventional interactions like hydrogen bond, salt bridge, and di-sulfide bond for the whole data set. Hence we calculated the percentage contribution of each of these interactions for the whole data set. This result is shown in Figure 11. It is clear from Fig. 11, that, the percentage of cation- π interactions is higher as compared to all the other conventional interactions like hydrogen bond, salt bridges and di-sulfide bonds for the whole data set of protein studied in this work. Based on all the results, in general, and the results of the interplay between conventional and non-conventional forces in particular, we emphasize that,

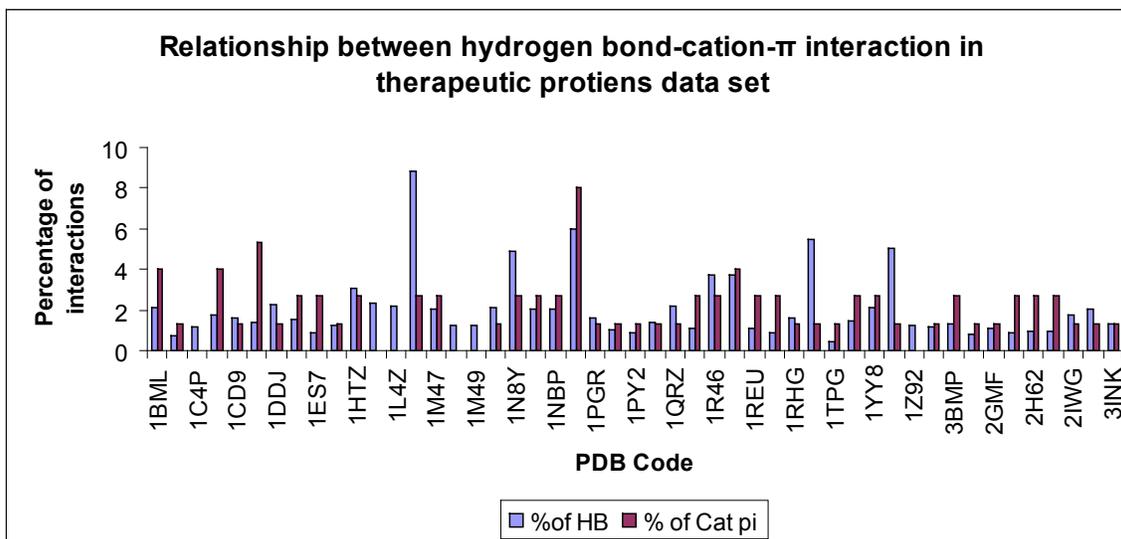


Figure 8: Relationship between hydrogen bond and cation- π interaction in Therapeutic proteins data set.

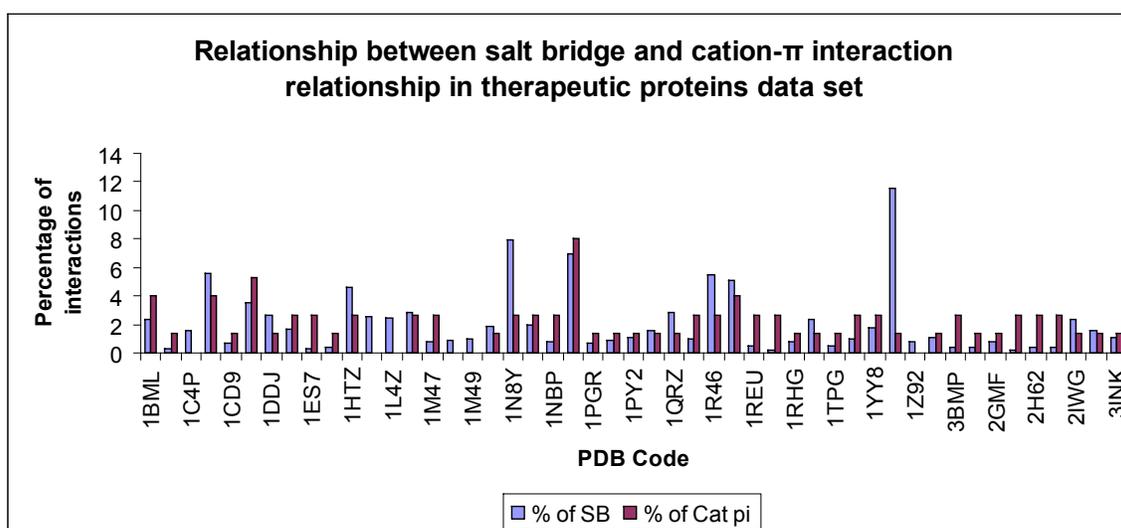


Figure 9: Relationship between salt Bridge and cation- π interaction relationship in Therapeutic proteins.

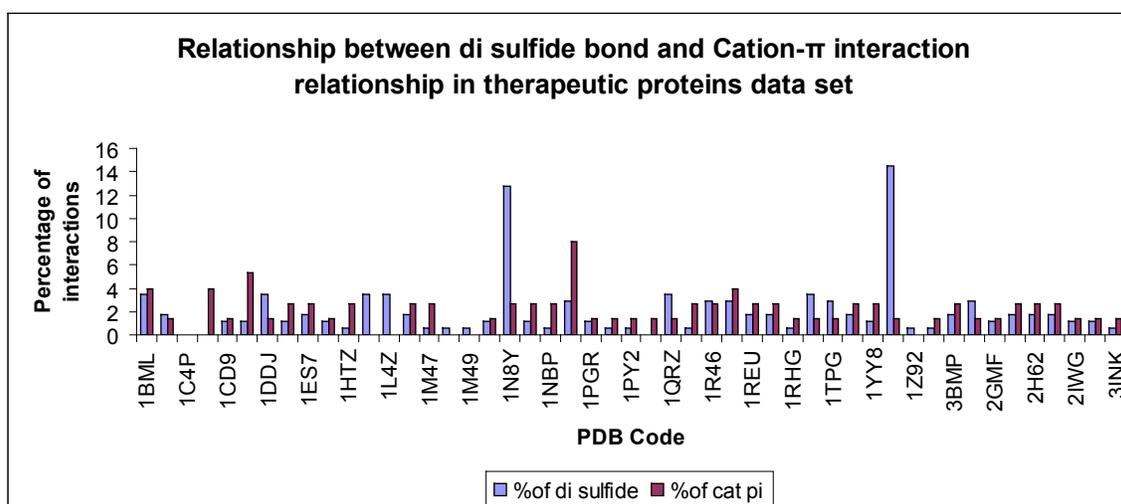


Figure 10: Relationship between di-sulfide bond and cation- π interaction relationship in Therapeutic proteins.

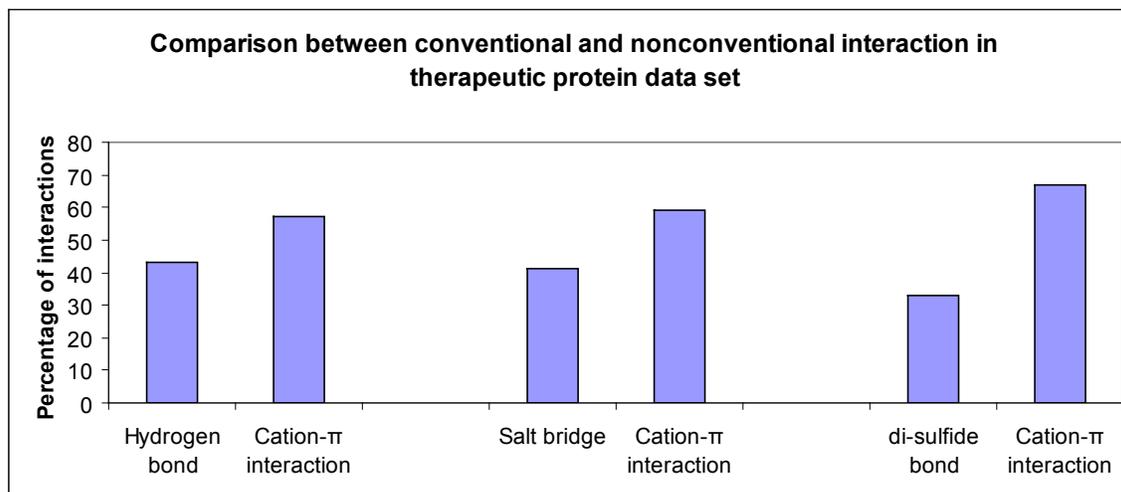


Figure 11: Comparison between conventional and non-conventional interaction in Therapeutic protein data set.

cation- π interactions should be considered as an important contributing factor for the structural stability of the set of therapeutic protein studied in this work.

Conclusions

We have systematically analyzed the influence of cation- π interactions to the stability of therapeutic proteins. The side chain of Lys is more likely to be in cation- π interaction than Arg in the cationic residues. Phe has the highest occurrence in this interaction than the other two π residues such as Tyr and Trp. In the data set 43 therapeutic protein showed significant cation- π interactions in the total of 49. Among the cation- π residue pairs that were involved in this interaction, Arg-Tyr residue pair showed the maximum number of cation- π interaction and Lys-Trp pair showed the minimum number of interaction. The cation- π interaction energy shows that Arg-Phe energy is the strongest and Lys-Trp is the lowest among the six possible pairs in the 49 therapeutic proteins investigated. In the secondary structure arrangement of cationic group, Arg and Lys preferred to be in strand. In the aromatic group it was found that, the Phe prefer to be in coil, Tyr prefers to be in strand and Trp preferred to be in helix. In the cationic residues Lys and Arg preferred to be in exposed region. Among the aromatic residues, Phe and Tyr preferred to be in partially buried region, while Trp preferred to be in the fully buried regions. We found that, all the five residues found in cation- π interactions are important in locating one or more stabilization centers. In the cation- π interacting residues, 43 percent of the amino acid residues that are involved in cation- π interactions might be conserved in therapeutic proteins. These residues might contribute to additional stability to therapeutic

proteins. The contribution of cation- π interaction for the stability for the whole therapeutic protein data set is much higher as compared to the conventional interactions such as hydrogen bond, salt bridge and di-sulfide interaction. More specifically, 57% of the proteins exhibited a higher cation- π interaction than hydrogen bond, almost 59% of the proteins exhibited cation- π interaction than salt bridges and 67% of the proteins showed higher cation- π interaction than the di-sulfide bonds. In all the cases, the contribution of cation- π interaction for the stability of therapeutic protein data set is much higher than the conventional interactions such as hydrogen bond, salt bridge and di-sulfide interaction. Hence we could conclude that, the contribution of cation- π interaction is an important factor for the structural stability of the therapeutic protein studied in this work. On the whole, the results presented in this work will be very useful for further investigations on the specificity and selectivity of therapeutic proteins pharmaceutical applications.

Future Perspectives

Although a great deal of progress has been made in the field of system biology, it is still a long way to understand structural stability of protein and docking studies. This may be possible after getting a better understanding of the various interactions within the protein molecule. Among the different interactions, the reports on cation- π interactions in poly peptides and proteins are scarce. Hence, computation of cation- π interactions energies may be considered significantly important in protein stability, specificity, protein-protein interfaces and potentially useful for protein docking studies. Majority of the protein complexes analyzed contained at least one such interaction. Therefore, the pres-

ence of cation- π interactions could be used as a means of discriminating chemically relevant docking results from false positives. This scrutiny will assist structural biologist and medicinal chemist to design better and safer drugs.

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