

# COMMENTS

## Comment on “From Subtle to Substantial: Role of Metal Ions on $\pi$ – $\pi$ Interactions”

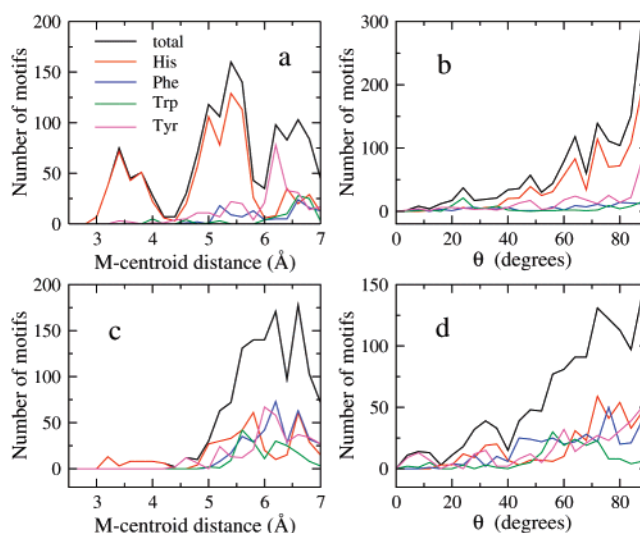
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In a recent letter,<sup>1</sup> Reddy, Vijay, G. M. Sastry, and G. N. Sastry (from now on RVSS) presented the results of an analysis of M– $\pi$ – $\pi$  complexes where M = Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup>, and Li<sup>+</sup> and  $\pi$  = benzene, indole, phenol, and imidazole. In particular, using quantum mechanical calculations, RVSS showed that the strength of the  $\pi$ – $\pi$  interaction (with  $\pi$  = benzene), usually on the order of a few kilocalories per mole, increases by more than 10 kcal mol<sup>–1</sup> when a cation is present. The other result of ref 1 is the statistical analysis performed on a number of biomolecules taken from the Cambridge Structural Database and Brookhaven Protein Data Bank (PDB). According to RVSS, such statistical study revealed the existence of a high affinity of the cations toward  $\pi$ – $\pi$  dimers, which led to claim the importance of M– $\pi$ – $\pi$  interactions in determining the three-dimensional structure of biomolecules. In this comment, we shall address this second aspect of the conclusions of ref 1, by providing an extended statistical analysis on the biomolecules found in the PDB. We will show that the M– $\pi$ – $\pi$  motifs in which the  $\pi$  electronic cloud of the arene ring directly interacts with the cation (see Scheme 1 of ref 1) are rare in the PDB. In particular, we found no sign of a significant occurrence of the PD-M and TB-M orientations indicated in ref 1 as the most preferred in proteins. Among the five cations taken into account by RVSS, we chose to focus on the two most abundant in the PDB, i.e., Mg<sup>2+</sup> and Ca<sup>2+</sup> (see Figure 1 and Table S1 of ref 1). We considered the PDB structures reported in Table S3 of ref 1, but removed the molecules/files for which not all the heavy atoms of the aromatic amino acid side chains (His, Tyr, Trp, and Phe) were provided. This arbitrary constraint, adopted for computational convenience, leads to the unbiased elimination of about 10% of the analyzed proteins, with no significant impact on the quality of the statistics. Indeed, we analyze 302 Mg– $\pi$ – $\pi$  and 352 Ca– $\pi$ – $\pi$  motifs, that are 92% and 89%, respectively, of the original motifs.<sup>1</sup>

In Table 1, we report the number of occurrences of Mg– $\pi$ – $\pi$  and Ca– $\pi$ – $\pi$  motifs for all possible pairs of amino acid aromatic residues. The criterion used for selecting the M– $\pi$ – $\pi$  motifs has been taken from ref 1. In Figure 1a, we report the number of Mg– $\pi$ – $\pi$  motifs as a function of the distance between the Mg cation and the nearest centroid to it. The



**Figure 1.** Panel a: number of Mg– $\pi$ – $\pi$  motifs as a function of the distance between the cation and the nearest centroid to it. Panel b: number of Mg– $\pi$ – $\pi$  motifs as a function of the angle  $\theta$  formed by the M-centroid vector and the normal to the arene ring nearest to the cation. Panel c contains the same information as panel a, but for Ca– $\pi$ – $\pi$  motifs. Panel d contains the same information as panel b, but for Ca– $\pi$ – $\pi$  motifs. The separate contributions from the various aromatic side chains are also shown.

**TABLE 1: Number of Mg– $\pi$ – $\pi$  and Ca– $\pi$ – $\pi$  Motifs for All Possible Pairs of Aromatic Side Chains**

	Mg– $\pi$ – $\pi$	Ca– $\pi$ – $\pi$
PheTyr	192	208
Phe Phe	19	115
Phe Trp	66	133
Phe His	150	170
Tyr Tyr	66	146
Tyr Trp	48	65
Tyr His	279	170
Trp Trp	14	30
Trp His	99	93
His His	440	137

separate contributions from the four aromatic residues are also shown. In Figure 1b, we report the statistics calculated by considering the angle  $\theta$  formed by the normal to the arene ring and the vector from the centroid to the cation. The same quantities for the Ca– $\pi$ – $\pi$  motifs are shown in Figure 1c,d.

By inspecting Table 1, we note that the Mg– $\pi$ – $\pi$  motifs containing at least one His residue occur much more frequently than the other types of motifs. This feature becomes even more evident if we consider the number of Mg– $\pi$ – $\pi$  motifs where the His residue is the nearest neighbor to the cation. Such number (in percentage with respect to the total number of Mg– $\pi$ – $\pi$  motifs) is 64%, against 21%, 8%, and 7% obtained for Tyr, Phe, and Trp, respectively. This markedly different behavior for the various aromatic amino acid residues cannot clearly be ascribed to the different occurrence of His in the PDB with respect to the other residues.<sup>2</sup> It must be rather due to a certain “affinity” occurring between His and Mg<sup>2+</sup>, perhaps reminiscent of the capability of Mg<sup>2+</sup> to form covalent complexes with His.

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Such affinity is also evident in Figure 1a. We notice in fact that the His related curve shows three well-defined peaks. The peak at the shortest distances ( $d < 4.5$  Å) originates from a covalent or quasi-covalent bond, involving a strong and short-ranged Mg–N(His) interaction, with Mg lying on the plane of the imidazole ring. Hence, in this case, the M– $\pi$  interaction cannot be considered as such (the  $\pi$  electronic cloud is not directly involved). Also the most intense peak at  $4.5 < d < 6$  Å can be ascribed, rather than to Mg– $\pi$  complexes, to a sort of weak Mg–N(His) interaction. In fact, in these labile configurations, the cation is still placed along the direction connecting the centroid of the His ring to one of the two nitrogen atoms (data not shown). On the other hand, such a type of orientational behavior can be clearly inferred from Figure 1b, from which we note that, in the case of His, the cation lies preferentially on the plane of the nearest arene irrespective of the centroid-cation distance. Considering that these configurations characterized by a relevant Mg–N(His) affinity are about 770, the truly interesting Mg– $\pi$ – $\pi$  motifs become only 44% of the initial ensemble.

In the case of the Ca– $\pi$ – $\pi$  motifs, the special affinity between Ca and His residue seems to be less important (see Table 1 and Figure 1c), though as observed for the Mg– $\pi$ – $\pi$  motifs, the cation lies preferentially on the plane of the imidazole ring (Figure 1d). Only 46 Ca– $\pi$ – $\pi$  motifs, against 242 for the Mg– $\pi$ – $\pi$  case, have an M-centroid distance smaller than 4.5 Å.

From Figure 1, other important and general observations can be made:

(1) Even if only the curves of Figure 1b,d related to Trp, Tyr, and Phe are accounted for, we notice that the cation lies preferentially far away from the normal to the arene ring passing from the centroid. As noted for the His case, in the most probable orientations the angle  $\theta$  is significantly larger than  $0^\circ$ ,

corresponding to the ideal M– $\pi$ – $\pi$  complex. This implies that none of the candidate orientations proposed in ref 1 are actually representative of the M– $\pi$ – $\pi$  motifs in the PDB.

(2) The average distance between the cation and its nearest neighboring centroid (Figure 1a,c) is much larger than the typical M– $\pi$  distances at which the effect of the cation on the  $\pi$ – $\pi$  interaction energy becomes important. In fact, from the figure we realize that rarely this average distance is smaller than 5 Å, against values of about 2–3 Å obtained after energy minimization (see Table S6 of ref 1).

In conclusion, the data presented here indicate that, for the M–arene distances and orientations commonly found in the PDB, the stabilizing effect of the cations is very likely negligible. At variance with ref 1, we found a dominant presence of orientations where the cation lies on the plane of the nearest arene ring far away from the centroid, therefore ruling out the existence of a significant M– $\pi$  interaction in proteins. The observed configurational behavior of the M– $\pi$ – $\pi$  complexes, with the probability of occurrence growing with the angle  $\theta$ , is probably correlated to an entropy-related effect (apart from the motifs involving His for which the M–N interaction surely plays a fundamental role), consistent with an almost stochastic distribution of the cations with respect to the centroids of the aromatic side chains.

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## References and Notes

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- (2) Chelli, R.; Gervasio, F. L.; Procacci, P.; Schettino, V. *Proteins: Struct., Funct., Genet.* **2004**, *55*, 139.