Attractive interactions between sidechains of histidine-histidine and histidine-arginine based cationic dipeptides in water

Jan Heyda,[1] Philip E. Mason,[2] and Pavel Jungwirth[1*]

[1]*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, and Center for Biomolecules and Complex Molecular Systems, Flemingovo nám. 2, 16610 Prague 6, Czech Republic*

[2]*Department of Food Sciences, Stocking Hall, Cornell University, Ithaca, NY, 14853, USA.*

Abstract

Molecular dynamics simulations of histidine-based dipeptides in water show that a protonated histidine side chain group has a propensity for forming like-charged contact pairs with another protonated histidine or with arginine. This effect is of similar strenght to that in observed previously for arginine-arginine pairing. Even stronger contact pairs are formed in singly protonated or deprotonated dihistidine, where stacking of aromatic rings is not weakened by Coulomb repulsion between the side chains. Qualitatively the same pairing behavior is also observed in a mixed solution of imidazole and imidazolium chloride.

[*]*Corresponding author: pavel.jungwirth@uochb.cas.cz*

Introduction

Interactions between amino acid side chains are important for the structure and function of proteins in aqueous solutions. Hydrophobic residues are particularly attracted to each other in water, while the tendency for association of polar and charged residues is weaker due to competing strong interactions with solvent molecules.[1,2] For charged residues, attractive interactions are typically considered only between a positively and a negatively charged side chain, leading to a formation of a salt bridge.[3-6] However, close contacts have been observed both experimentally and computationally also between like-charged residues of aqueous proteins and peptides.[7-12] In particular, it has been demonstrated computationally recently, that two positively charged guanidinium moieties of di- and decaarginine can form a contact pair in water, despite the Coulomb repulsion between them.[12] A detailed analysis of a guanidinium pair showed that this net attraction is due to interplay of quadrupole-quadrupole, dispersion, and cavitation forces, which are particularly favorable for pairing of two guanidinium moieties.[12-16] For comparison, it was shown that no such contact pairs are present between two ammonium groups of di- or decalysine.[12] Consequently, a question arises, whether two guanidinium groups are the only cationic moieties forming a like-charge contact pair in aqueous petides and proteins.

Since there are only three cationic amino acids, i.e., lysine, arginine, and (protonated) histidine, the latter is the only choice left. The protonation state of the imidazole group of histidine is the only one in proteins to be significantly pH dependent under physiological conditions. [17-19] The ability of a neutral amino acid side chain to accept a proton, or for the cationic group to lose one has been utilized in Nature in the form of the proton shuttle (PHARMACOLOGY & THERAPEUTICS Volume: 74 Issue: 1 Pages: 1-20 Published: 1997). Histidine is a chemically active amino acid, and is commonly found coordinated to transition

metals like zinc in the active site of enzymes such as carbonic anhydrases [REF]. Directly related to the present study is that fact that it is not uncommon to find histidine-histidine contact pairs in protein structures. For example, in the histidine kinase 2R78 two thirds of histidines exist in stacked pairs (although the structure is, unfortunately, not of a high enough resolution to determine the protonation state of the moieties) [REF].

With this motivation, we investigate here the interactions between imidazolium moieties in dihistidine. As mentioned above, the protonation state of histidine, with pK ~ 6 sensitively depends on pH and on the local environment of the imidazolium side chain.[17-19] At physiological conditions, dihistidine has both or one of the imidazolium groups deprotonated, so that the dipeptide is either neutral or singly positively charged. Only under very acidic conditions could both sidechains become protonated making the dipeptide doubly charged. Here we investigate by means of molecular dynamics (MD) simulation all these three protonation scenarios for aqueous dihistidine. In addition, we study the interactions between arginine or lysine and either protonated or deprotonated histidine in the aqueous dipeptides. These results are compared to those obtained from simulations of an aqueous mixture of imidazolium chloride and imidazole, representing the free side chain groups of hstidine.

Methods

MD simulations of aqueous terminated (capped) dipeptides with the general formula Ace-X-Y-Nme, where the X-Y stands for His-His, His-Hisp, His-Arg, His-Lys, Hisp-Hisp, Hisp-Arg, and Hisp-Lys were performed using the Amber 10 program.[20]

. Here, His denoted deprotonated and Hisp protonated histidine, Arg arginine, Lys lysine, and Ace and Nme the terminal groups, with parameters taken from the parm99 force field.[21] Each of

these dipeptides with 0 - 2 neutralizing chloride anions was surrounded by 700 SPCE water molecules[22] and put in a unit cell with approximate dimensions of 28x28x28 $Å^3$. 3D periodic boundary conditions were applied with long range electrostatic interactions beyond the non-bonded cutoff of 9 Å accounted for using the Particle Mesh Ewald (PME) method.[23] The Berendsen temperature (300 K) and pressure (1 atm) couplings were used[24] and all bonds containing hydrogen were constrained using the SHAKE algorithm.[25] The total simulation time for each run was 100 ns, after equilibration of 1 ns, with a time step of 1 fs and sample collection every 1ps, which yielded to 100 000 frames for further analysis.

The simulated trajectories were analyzed primarily in terms of radial distribution functions (RDF) calculated between centers of masses of imidazole (or imidazolium), guanidinium and ammonium, followed by 2D distributional surfaces, which correlated distance (between above defined groups) and angle between imidazolium and guanidinium planes. Finally 3D spatial distributions between above defined groups of imidazole, imidazolium, guanidinium and ammonium were constructed from the simulations. All results were averaged over the production part of each trajectory.

Additional simulations of an aqueous mixture of imidazole and imidazolium chloride were performed in order to gain further insight into the observed ion-pairing phenomena. Also, the robustness of the simulations was tested by using a somewhat different force field than for the dipeptides. In these MD simulations a neutral periodic cubic system was created at 1.5 molal concentration of both imidazole and imidazolium chloride with independent molecules being surrounded by explicit water molecules. The simulations employed a solute potential energy function based on the parameters from the CHARMM (version35b2) forcefield.[26] Water molecules were represented using the TIP3P model.[27] These simulations were performed using

the CHARMM program[28], with chemical bonds to hydrogen atoms kept fixed using SHAKE[29] and a time step of 1 fs. Starting coordinates were generated by randomly placing and orienting 24 imidazole molecules and 24 imidazolium and 24 chloride in a cubic box with sides of 34 Å. These coordinates were superimposed on a box of 1296 water molecules and the appropriate cutoff was chosen to affect the correct concentration. By design this procedure produced a ~1.5 molal solution of both imidazole and imidazolium chloride. Finally the box length was rescaled to 31.7736 Å, which this yielded the correct physical number density (0.0982 atoms $Å^{-3}$). Van der Waals interactions were smoothly truncated on an atom-by-atom basis using switching functions from 10.5 to 11.5 Å[28], while electrostatic interactions were treated using the Ewald method.[30] Initial velocities were assigned from a Boltzmann distribution (300 K) followed by 5 ps of dynamics with velocities being reassigned every 0.1 ps. The simulation was then run for 2 ns with no further velocity reassignment. The first 0.5 ns of this were taken as equilibration and the remaining 1.5 ns was used for analysis.

Results

The principal results from the present simulations of histidine-containing dipeptides in water, i.e., the density distributions of one side chain group around the other one, are graphically depicted in Figure 1. In each case, the central side chain group is shown in space-filling representation, the distribution of the other group is shaded in brown, and the licorice representation represents a selected geometry of the dipeptide from the analyzed 100 ns trajectory. For each sub-figure, the distance is that from the central group to the center of the part of the distribution closest to it. We see that the two side chain groups typically acquire

several possible arrangements, as represented by the individual lobes of the density distributions. The lobes closest to the central group represent, with the exception of the lysine containing dipeptides, contact pairing between the two side chain groups, while the other lobes correspond to separated side chains. From the point of view of the present study the former are the most interesting. Contact pairs are particularly well developed for neutral and singly charged histidine-containing dipeptides, with the other group being another histidine or arginine. This is hardly surprising, since these pairs are typical representatives of stacking of aromatic rings in water, which is known to be strong [REF]. Some pairs, like deprotonated and protonated histidine also form T-shaped structures, also known from the literature for aromatic pairs [REF]. Side chain interactions are expectedly weaker between deprotonated histidine and the non-aromatic lysine side chain. What may, however, come as a surprise is that there is still appreciable pairing between protonated histidine and another protonated histidine or arginine despite the fact, that both side chain groups now bear a positive charge. Thus, not only two guanidinium groups attract each other in water, but there are also favorable imidazolium-imidazolium and imidazoloium-guanidinium side chain pairs, despite the unfavorable Coulomb interaction between them. No such pair is, however, formed between imidazolium and ammonium side chain groups. In the prtonated histidine-lysine dipeptides there is instead a favorable interaction between the imidazolium ring on one side and the alkyl chain on the other side.

In order to further quantify these side chain interactions, we plotted in Figures 2 and 3 the radial distribution functions (RDF) between the two side chain groups. As points of reference we considered the center of mass of the imidazolium ring of histidine, the carbon atom of the guanidinium group of arginine, and the nitrogen atom of the ammonium group of lysine.

We see from the sizable first peaks of RDFs around 4 Å (Figure 2), that all the investigated neutral or singly charged dipeptides containing two histidines or histidine and arginine form strong contact pairs in water, while the interaction between deprotonated histidine and lysine is weak, as discussed above. The RDFs between two positively charged side chains are presented in Figure 3. We see that there remains an appreciable amount of contact pairs between two protonated histidines and between protonated histidine and arginine, although due to Coulomb repulsion pairing is weaker than in the above neutral or singly charged dipeptides. As a matter of fact, protonated histidine-protonated histidene and protonated histidine-arginine pairing is of comparable strength as that previously observed for two arginines.[12] In contrast, there is no contact pairing between protonated histidine and lysine (Figure 3).

The geometry of the contact pairs of side chains in histidine-containing dipeptides in water was further analyzed in terms of 2D plots of the distance and orientation between the planes of the aromatic groups (Figure 4). All histidine pairs prefer parallel or only slightly off-parallel orientations of the two aromatic rings in the contact pair, with secondary T-shaped minima when at least one of the histidines is protonated. For histidine-arginine pairs, the co-planarity of the two side chain groups is weaker and the distribution of orientations and distances is broader, nevertheless roughly parallel or weakly tilted geometries are still preferred.

We further investigated interactions between the functional groups of histidine, i.e., imidazole and imidazolium, via simulations of a mixed aqueous solution of ~1.5 m imidazole and ~1.5 m imidazolium chloride. In order to check the robustness of the results with respect to the adopted force field, we employed a slightly different water and solute parameters than for the above dipeptides (for details see Methods). Figure 5 shows density distributions of imidazole

around imidazole, imidazolium around imidazole, imidazole around imidazolium, and imidazolium around imidazolium. The distributions are similar to those in the corresponding dipeptides, save the steric constraints of the latter. Namely, parallel orientations of the two aromatic rings in the contact geometry are preferred in al cases, being most strictly imposed in the doubly protonated pair. For the singly protonated pair, T-shaped geometries correlating with the extra proton pointing to the neutral ring are also populated. In the deprotonated pair, the mutual orientations are the least constraint, with both coplanar and tilted geometries present. Figure 6 depicts the corresponding imidazole-imidazole, imidazol-imidazolium, and imidazolium-imidazolium RDF, which also demonstrate close contacts at about 4 Å for all these three pairs. As for the dipeptides, due to Coulomb repulsion, pairing is the weakest for the cation-cation pair. One the whole, these free solutes tend to pair less at the investigated ~1.5 m imidazole and ~1.5 m imidazolium chloride concentrations than the corresponding side chain groups in dipeptides due to a lack of a covalent linkage between them (which restricts the extent of the separation between the groups). Nevertheless, the absolute degree of pairing is concentration dependent and qualitatively the pairing behavior is the same as for the corresponding dipeptides.

Discussion and Conclusions

By means of molecular dynamics simulations of histidine containing dipeptides in water we have shown that the previously observed arginine-arginine pairing[12] is not the only case of formation of contact pairs between like-charged side chain groups. Namely, the propensity of protonated histidine side chains to form a contact pair with another one or with arginine is of comparable strength to that found in diarginine. Since the two side chain groups of histidine in

the contact pair both remain protonated only under strongly acidic conditions, we have also investigated singly protonated and deprotonated pairs more pertinent to physiological conditions. These pairs are even stronger since the attractive interaction between the aromatic rings is not compromised by Coulomb repulsion as in the doubly protonated case. Simulations of a mixed aqueous solution of imidazole and imidazolium chloride show that the same pairing mechanisms are operative also for free solutes. In these cases, however, the solute pairs lack a covalent linkage, which restricts the phase space for the dipeptide side chain groups. The absolute amount of contact pairs is then a function of concentration of solutes.

The present computational study showing side chain pairing in histidine-containing dipeptides is consistent with the frequent occurence of histidine-histidine contact pairs in protein structures. Here, we newly predict that a histidine-histidine or histidine-arginine pair could be stabilized in water even if both moieties are positively charged, similarly as for the previously observed argining-arginine pairing.[12] Such histidine-containing cationic pairs, if confirmed by structural studies, may a role for protein stability and protein-protein interactions under acidic conditions.

Acknowledgment

Support from the the Czech Science Foundation (grants 203/08/0114) and the Czech Ministry of Education (grant LC512) is gratefully acknowledged. JH thanks the International Max-Planck Research School for support. Part of the work in Prague was supported via Project Z40550506.

Figure Captions

Figure 1: Density distributions of the center of the side chain group of the second residue around the first one for eight terminated histidine containing dipeptides.

Figure 2: Radial distribution functions of the centers of the side chain groups of terminated dipeptides containing deprotonated histidine and deprotonated histidine, protonated histidine, arginine, or lysine.

Figure 3: Radial distribution functions of the centers of the side chain groups of terminated dipeptides containing protonated histidine and protonated histidine, arginine, or lysine. For comparision, we also show the result for diarginine.

Figure 4: Two dimensional plots of the distance and angular distributions of the centers of the two side chains of histidine-histidine and histidine-ariginine dipeptides, considering both protonation states of the histidine side chain.

Figure 5: Density distributions of imidazole around imidazole (blue), imidazolium around imidazole (green), imidazole around imidazolium (yellow), and imidazolium around imidazolium (red) from a simulation of a mixed aqueous solution of imidazole and imidazolium chloride.

Figure 6: Imidazole-imidazole, imidazole-imidazolium, and imidazolium-imidazolium radial distribution functions from a simulation of a mixed aqueous solution of imidazole and imidazolium chloride.

Figure 1

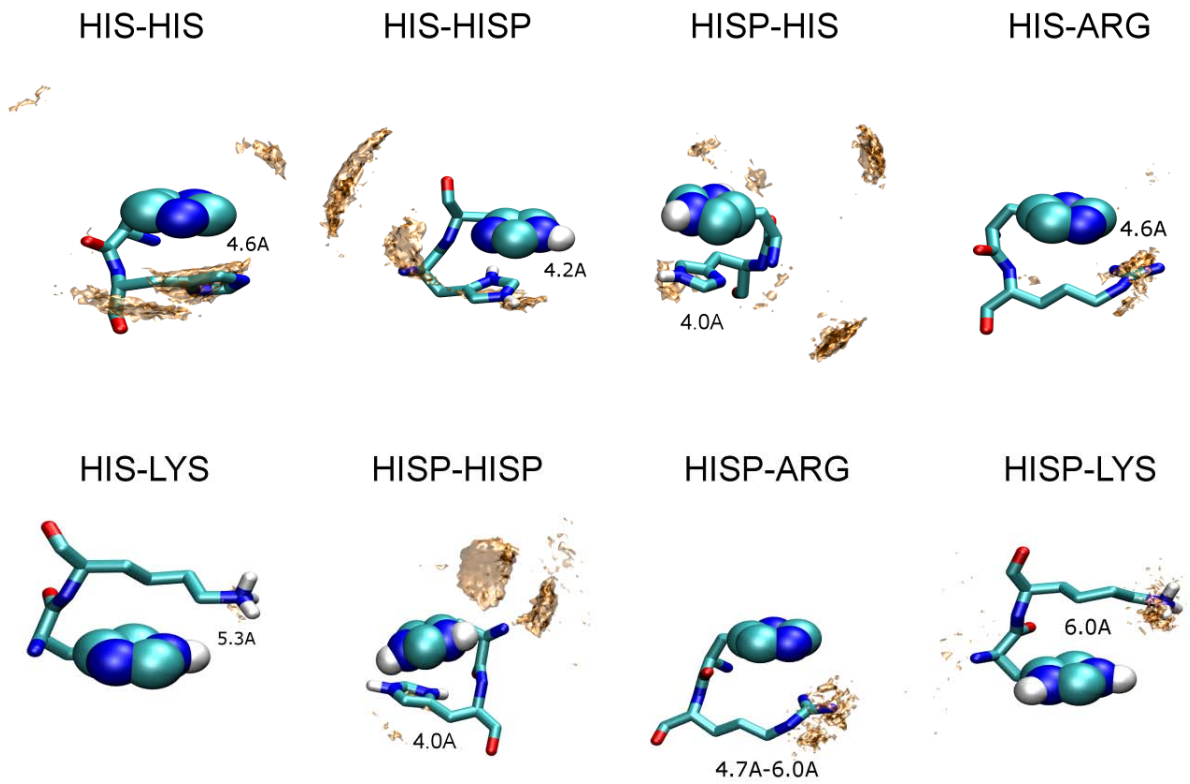

HIS-HIS
4.6A
HIS-HISP
4.2A
HISP-HIS
4.0A
HIS-ARG
4.6A
HIS-LYS
5.3A
HISP-HISP
4.0A
HISP-ARG
4.7A-6.0A
HISP-LYS
6.0A

Figure 2

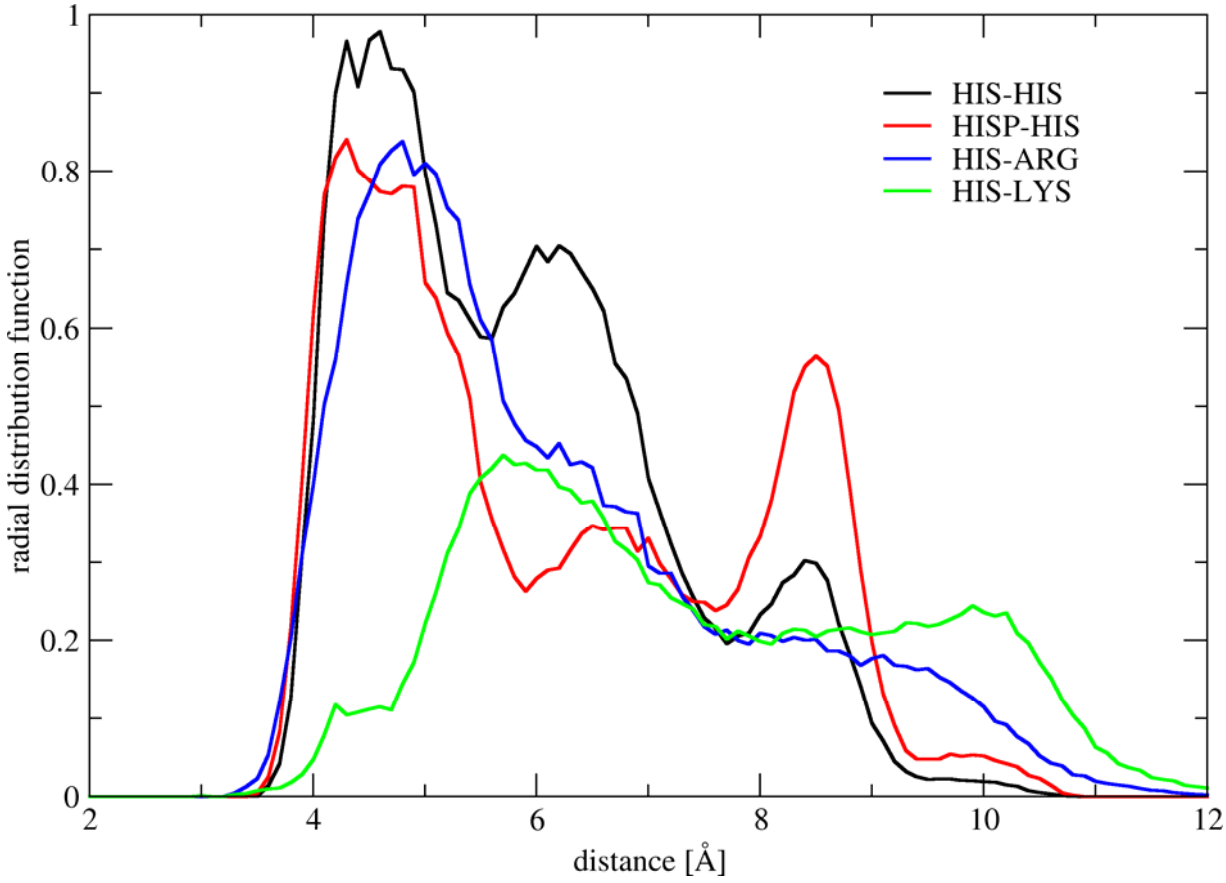

HIS-HIS
HISP-HIS
HIS-ARG
HIS-LYS
radial distribution function
distance [Å]
0
0.2
0.4
0.6
0.8
1
2
4
6
8
10
12

Figure 3

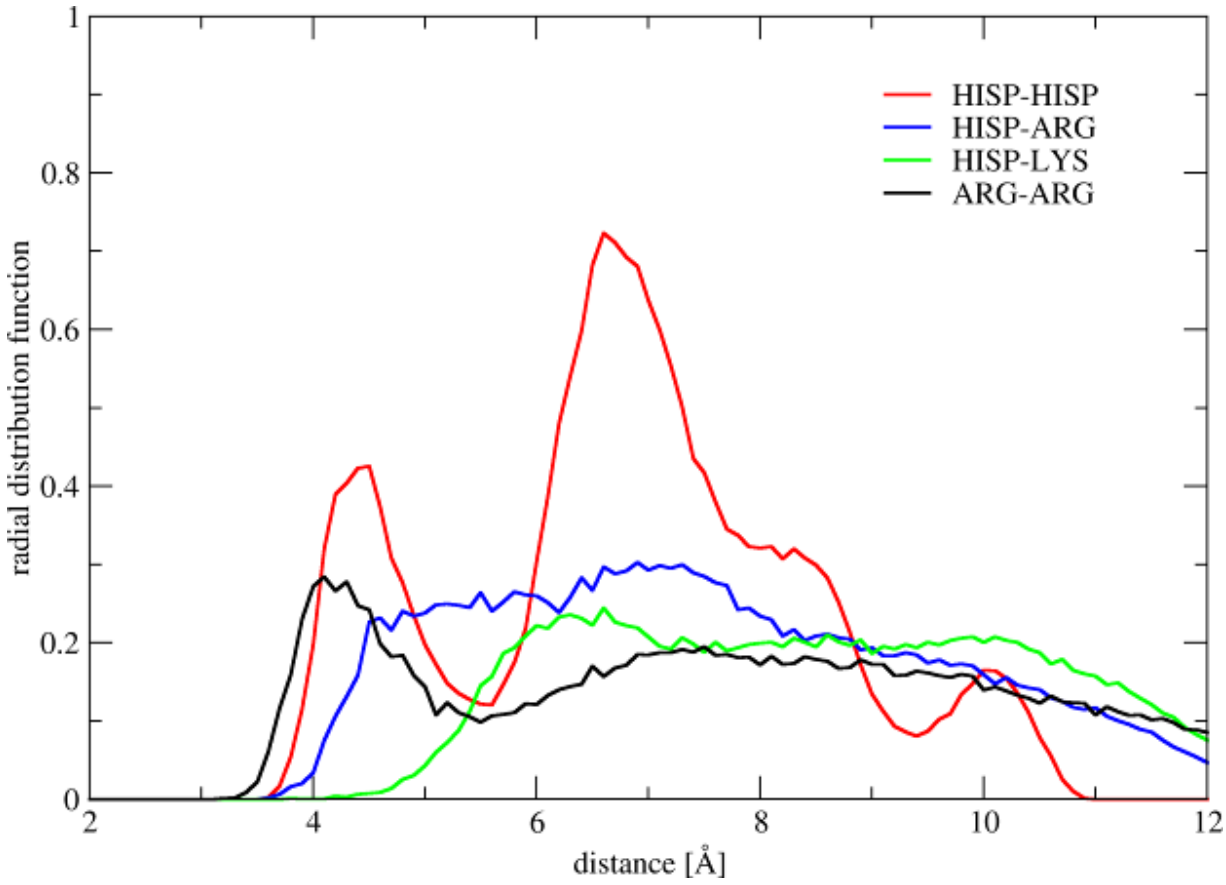

HISP-HISP
HISP-ARG
HISP-LYS
ARG-ARG
radial distribution function
distance [Å]
0
0.2
0.4
0.6
0.8
1
2
4
6
8
10
12

Figure 4

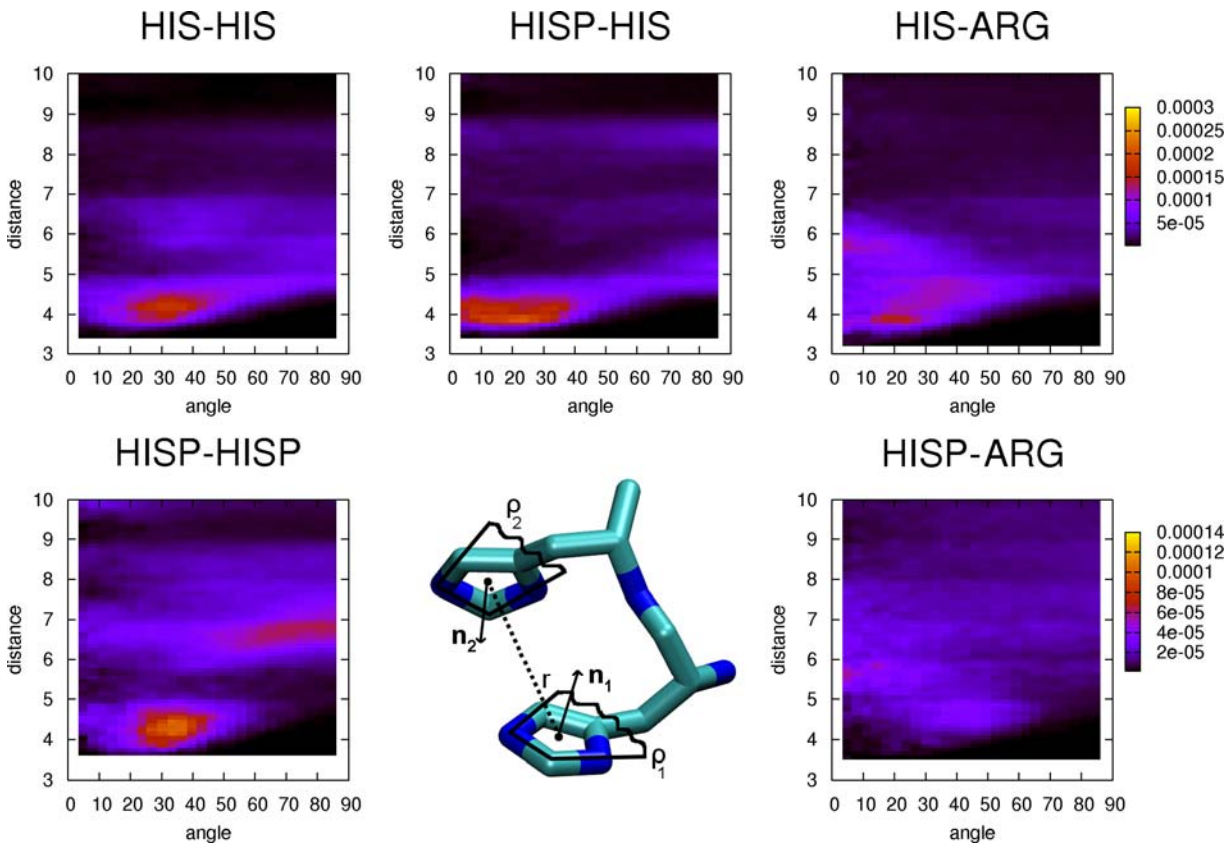

HIS-HIS
HISP-HIS
HIS-ARG
HISP-HISP
HISP-ARG
distance
angle
0.0003
0.00025
0.0002
0.00015
0.0001
5e-05
0.00014
0.00012
0.0001
8e-05
6e-05
4e-05
2e-05
ρ2
n2
r
n1
ρ1

Figure 5

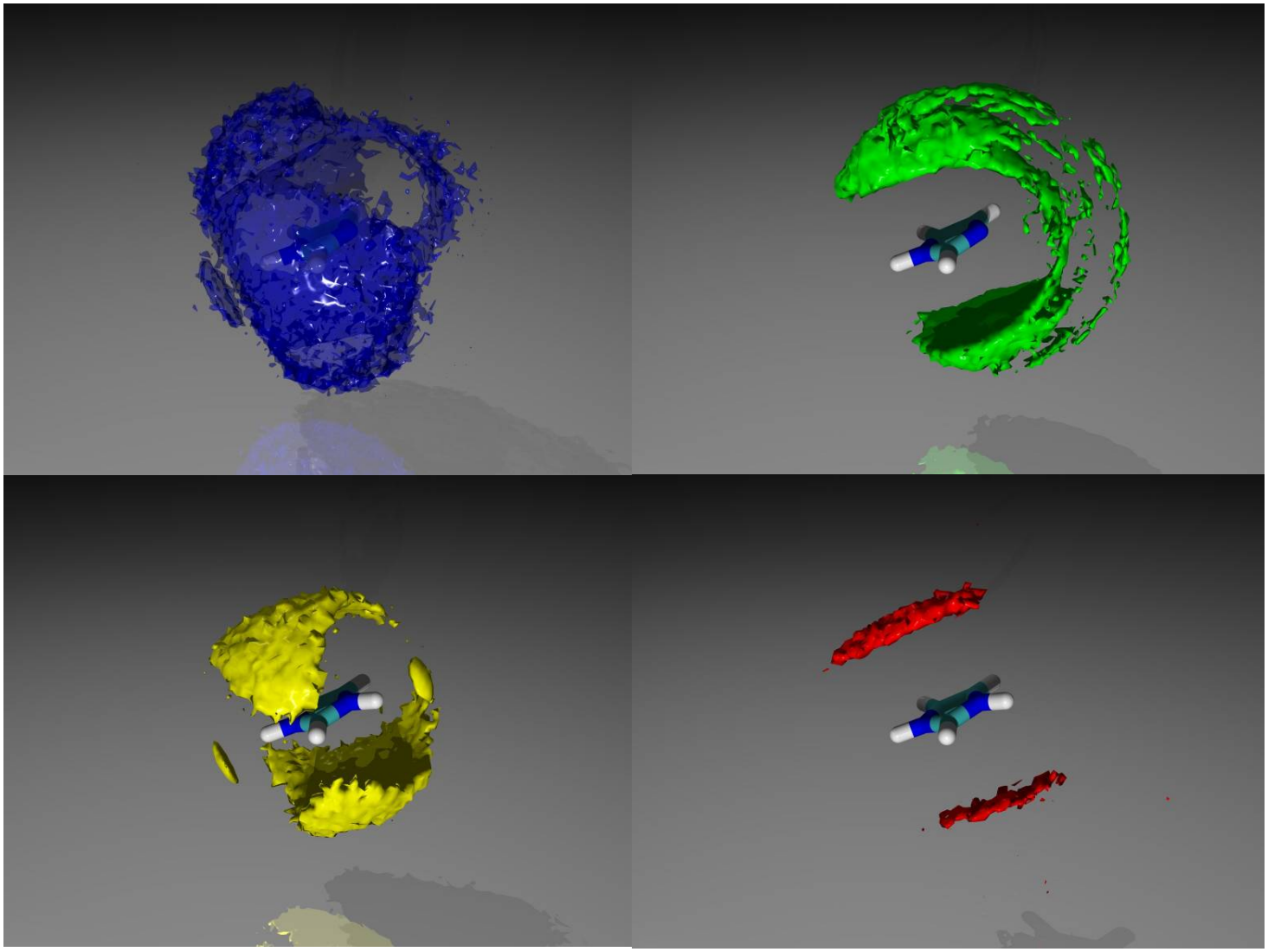
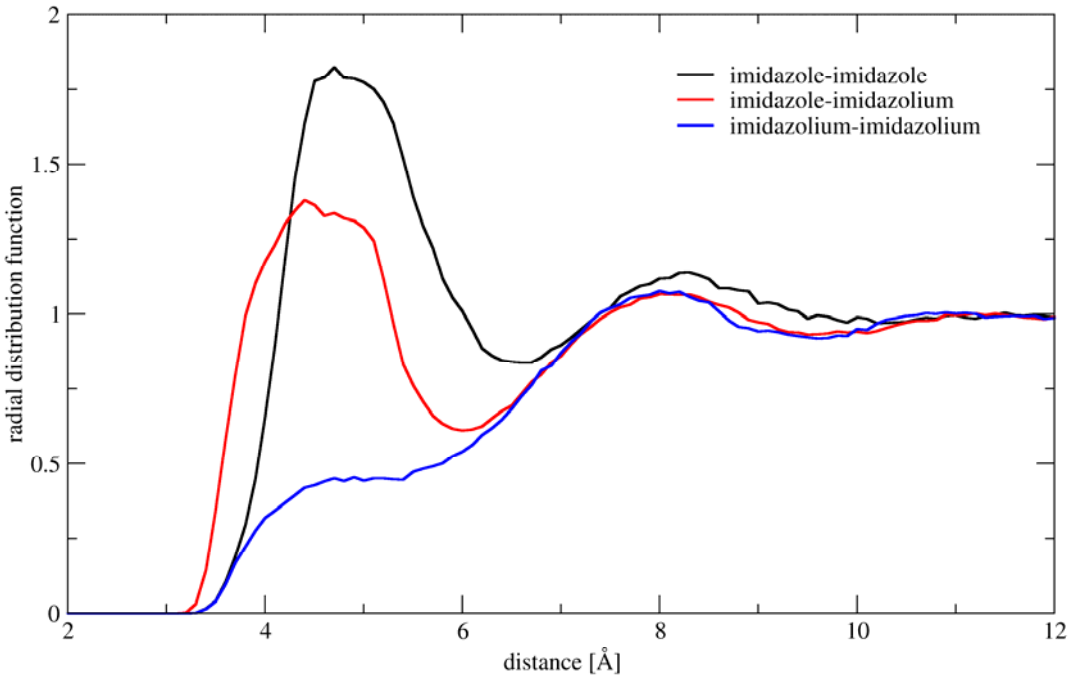
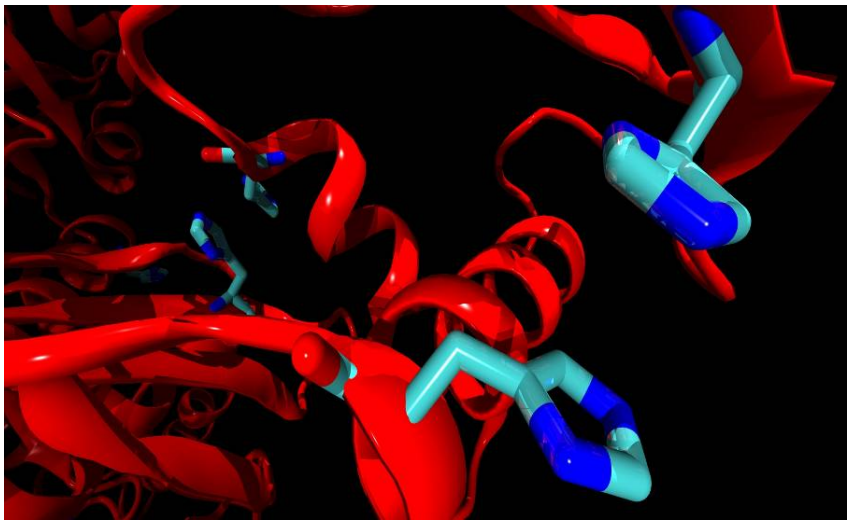


Figure 6


imidazole-imidazole
imidazole-imidazolium
imidazolium-imidazolium
radial distribution function
distance [Å]
0
0.5
1
1.5
2
2
4
6
8
10
12

TOC Histidine stacking is rather common in protein structures (protein 2R78).



References

(1) Thomas, P. D.; Dill, K. A. *Journal of Molecular Biology* **1996**, *257*, 457.

(2) Hendsch, Z. S.; Tidor, B. *Protein Science* **1994**, *3*, 211.

(3) Barlow, D. J.; Thornton, J. M. *Journal of Molecular Biology* **1983**, *168*, 867.

(4) Sindelar, C. V.; Hendsch, Z. S.; Tidor, B. *Protein Science* **1998**, *7*, 1898.

(5) Kumar, S.; Nussinov, R. *Journal of Molecular Biology* **1999**, *293*, 1241.

(6) Takano, K.; Tsuchimori, K.; Yamagata, Y.; Yutani, K. *Biochemistry* **2000**, *39*, 12375.

(7) Magalhaes, A.; Maigret, B.; Hoflack, J.; Gomes, J. N. F.; Scheraga, H. A. *Journal of Protein Chemistry* **1994**, *13*, 195.

(8) Vila, J. A.; Ripoll, D. R.; Villegas, M. E.; Vorobjev, Y. N.; Scheraga, H. A. *Biophysical Journal* **1998**, *75*, 2637.

(9) Brooks, C. L.; Karplus, M. *Journal of Molecular Biology* **1989**, *208*, 159.

(10) Villarreal, M.; Montich, G. *Protein Science* **2002**, *11*, 2001.

(11) Pednekar, D.; Tendulkar, A.; Durani, S. *Proteins-Structure Function and Bioinformatics* **2009**, *74*, 155.

(12) Vondrasek, J.; Mason, P. E.; Heyda, J.; Collins, K. D.; Jungwirth, P. *Journal of Physical Chemistry B* **2009**, *113*, 9041.

(13) Mason, P. E.; Dempsey, C. E.; Neilson, G. W.; Brady, J. W. *Journal of Physical Chemistry B* **2005**, *109*, 24185.

(14) Mason, P. E.; Neilson, G. W.; Enderby, J. E.; Saboungi, M. L.; Dempsey, C. E.; MacKerell, A. D.; Brady, J. W. *Journal of the American Chemical Society* **2004**, *126*, 11462.

(15) Boudon, S.; Wipff, G.; Maigret, B. *Journal of Physical Chemistry* **1990**, *94*, 6056.

(16) No, K. T.; Nam, K. Y.; Scheraga, H. A. *Journal of the American Chemical Society* **1997**, *119*, 12917.

(17) Browne, C. A.; Campbell, I. D.; Kiener, P. A.; Phillips, D. C.; Waley, S. G.; Wilson, I. A. *Journal of Molecular Biology* **1976**, *100*, 319.

(18) Miyagi, M.; Nakazawa, T. *Analytical Chemistry* **2008**, *80*, 6481.

(19) Khandogin, J.; Brooks, C. L. *Biochemistry* **2006**, *45*, 9363.

(20) Case, D. A. D., T. A.; Cheatham, III, T. E; Simmerling, C. L.; Wang, J.; Duke, R. E.; Luo, R.; Crowley, M.; Walker, R. C.; Zhang, W.; Merz, K. M.;Wang, B.; Hayik, S.; Roitberg, A.; Seabra, G.; Kolossvary, I.;Wong, K. F.; Paesani, F.; Vanicek, J.;Wu, X.; Brozell, S. R.; Steinbrecher, T.; Gohlke, H.; Yang, L.; Tan, C.; Mongan, J.; Hornak, V.; Cui, G.; Mathews, D. H.; Seetin, M. G.; Sagui, C.; Babin, V.; Kollman, P. A. Amber 10; Amber 10, University of California, San Francisco: San Francisco, 2008.

(21) Wang, J. M.; Cieplak, P.; Kollman, P. A. *Journal of Computational Chemistry* **2000**, *21*, 1049.

(22) Berendsen, H. J. C.; Grigera, J. R.; Straatsma, T. P. *Journal of Physical Chemistry* **1987**, *91*, 6269.

(23) Essmann, U.; Perera, L.; Berkowitz, M. L.; Darden, T.; Lee, H.; Pedersen, L. G. *Journal of Chemical Physics* **1995**, *103*, 8577.

(24) Berendsen, H. J. C.; Postma, J. P. M.; Vangunsteren, W. F.; Dinola, A.; Haak, J. R. *Journal of Chemical Physics* **1984**, *81*, 3684.

(25) Ren, P. Y.; Ponder, J. W. *Journal of Physical Chemistry B* **2003**, *107*, 5933.

(26) MacKerell, A. D.; Bashford, D.; Bellott, M.; Dunbrack, R. L.; Evanseck, J. D.; Field, M. J.; Fischer, S.; Gao, J.; Guo, H.; Ha, S.; Joseph-McCarthy, D.; Kuchnir, L.; Kuczera, K.; Lau, F. T. K.; Mattos, C.; Michnick, S.; Ngo, T.; Nguyen, D. T.; Prodhom, B.; Reiher, W. E.; Roux, B.; Schlenkrich, M.; Smith, J. C.; Stote, R.; Straub, J.; Watanabe, M.; Wiorkiewicz-Kuczera, J.; Yin, D.; Karplus, M. *Journal of Physical Chemistry B* **1998**, *102*, 3586.

(27) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *Journal of Chemical Physics* **1983**, *79*, 926.

(28) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; Swaminathan, S.; Karplus, M. *Journal of Computational Chemistry* **1983**, *4*, 187.

(29) Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C. *Journal of Computational Physics* **1977**, *23*, 327.

(30) Darden, T.; York, D.; Pedersen, L. *Journal of Chemical Physics* **1993**, *98*, 10089.