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Carbonic anhydrase binding site parameterization in OPLS-AA force field

Guillaume Bernadat,^{a,1} Claudiu T. Supuran^b and Bogdan I. Iorga^{a,*}

^a Institut de Chimie des Substances Naturelles, CNRS UPR 2301, Labex LERMIT, Centre de Recherche de Gif, 1 Avenue de la Terrasse, F-91198 Gif-sur-Yvette, France

^b Dipartimento di Chimica, Laboratorio di Chimica Bioinorganica, Università degli Studi di Firenze, Rm 188, Via della Lastruccia 3, Polo Scientifico, I-50019 Sesto Fiorentino (Firenze), Italy

Abstract

The parameterization of carbonic anhydrase binding site in OPLS-AA force field was performed using quantum chemistry calculations. Both OH₂ and OH[−] forms of the binding site were considered, showing important differences in terms of atomic partial charges. Three different parameterization protocols were used, and the results obtained highlighted the importance of including an extended binding site in the charge calculation. The force field parameters were subsequently validated using standard molecular dynamics simulations. The results presented in this work should greatly facilitate the use of molecular dynamics simulations for studying the carbonic anhydrase, and more generally, the metallo-enzymes.

Keywords

Carbonic anhydrase ; Molecular dynamics ; Force field parameterization ; OPLS-AA force field ; Quantum chemistry

Introduction

Carbonic anhydrase (CA, EC 4.2.1.1) is a widespread enzyme in all organisms, which catalyzes the hydration of CO₂ to bicarbonate and protons. (Scheme 1). Carbonic anhydrase inhibitors are available or in advanced stages of development for the treatment of various diseases e.g. glaucoma and ocular hypertension,^{1,2} cancer,³⁻⁷ obesity,^{8,9} epilepsy,¹⁰ etc. Possible applications of carbonic anhydrase activators in Alzheimer's disease and other memory therapies are also currently under evaluation.¹¹ All these data evidence the importance of this enzyme, for mechanistic studies or drug design of selective inhibitors or activators.

Scheme 1. Chemical reaction catalyzed by carbonic anhydrase.

Molecular dynamics studies of metallo-enzymes in general, and of carbonic anhydrase in particular, were hampered by the lack of appropriate force field parameters within the commonly used software. These difficulties were overcome over the years, but presently the molecular modeling of carbonic anhydrase is somewhat underdeveloped compared to the explosive development that the carbonic anhydrase field has known during the last decades.^{[12-16](#)}

A number of force fields were used in the past for studies involving molecular dynamics simulations of carbonic anhydrase: GROMOS,^{[17-19](#)} CHARMM^{[20-24](#)} and AMBER.^{[25-35](#)} A specific Zn AMBER force field was recently developed,^{[36](#)} with direct applications for the carbonic anhydrase. The same force fields were used in the context of QM/MM studies,^{[24,37-39](#)} to take into account the formation and breaking of bonds during the enzymatic reaction. A general review of molecular dynamics simulations of metallo-proteins is also available.^{[40](#)}

A notable force field missing from the list presented above is OPLS-AA.^{[41,42](#)} This force field was developed with a strong emphasis on reproducing condensed-phase properties of model compounds, is currently implemented in most of the commonly used molecular dynamics software packages and has been recently shown to give particularly good results in free energy calculations.^{[43-46](#)} In these conditions, we decided to undertake the parameterization of CA binding site in the OPLS-AA force field and this paper describes the procedure that we followed, along with the topologies and parameters required to perform molecular dynamics simulations using Gromacs software.^{[47](#)}

Results and discussion

The procedure used in this work involves three steps: i) charge calculation using quantum methods, ii) generation of bonding parameters, and iii) validation of these force field parameters using molecular dynamics simulations.

Charge calculation

For the charge calculations, the neutron structure of carbonic anhydrase II (PDB code 3KKX)⁴⁸ was used. The choice of this structure was prompted by three criteria: the high quality of the atomic coordinates, the presence of the representative water molecules in the binding site and the unambiguous assignment of the protonation states for all residues. Two different representations of the binding site were considered: i) a minimal binding site (Figure 1), containing the side chains of three histidine residues (His94, His96 and His119), the zinc ion and the zinc-bound water molecule (either OH₂ or OH⁻ forms) and ii) an extended binding site (Figure 2), containing the residues from the minimal binding site and the neighbors of the zinc-bound water (the side chain of Thr199 and the DW and W1 water molecules). The charge calculation was performed with or without geometry optimization, and three different protocols can be distinguished:

Protocol A: minimal binding site, with geometry optimization, CHelpG charge calculation, two-stage RESP charge fitting.

Protocol B: minimal binding site, without geometry optimization, CHelpG charge calculation, two-stage RESP charge fitting.

Protocol C: extended binding site, without geometry optimization, CHelpG charge calculation, two-stage RESP charge fitting.

For each protocol and for the two forms of the binding site (OH₂ or OH⁻), the charge calculation (and in the case of Protocol A, the geometry optimization) was performed with three different methods (HF, B3LYP and MP2) with either the 6-31G(d) or 6-311++G(2d,p) basis sets. Whereas the first basis set is generally used for charge calculations and force field parameterization of simple organic molecules, the second one is probably necessary for a better description of the zinc ion influence within this system. The charges were calculated for all binding site atoms, including the imidazole rings of His94, His96 and His119. The results for selected, representative atoms are presented in Tables 1 and 2, where the calculated charges are compared with those already present in the OPLS-AA force field for the same residues (see the Supplementary Data file for the complete charge data set).

The analysis of the results from Table 1 (OH₂ form of the binding site) shows that i) the geometry optimization (comparison of protocols A and B) has very little influence on the charges of the zinc ion and zinc-bound water, ii) the inclusion of the second sphere of coordination in the charge calculation (extended

binding site, comparison of protocols B and C) leads to sensibly different charges, especially for the zinc-bound water, iii) the use of the 6-311++G(2d,p) basis set provides smaller absolute values for all charges and thus a smoother polarization of the binding site, iv) the B3LYP method provides significantly lower absolute values for the charges compared with HF and MP2, but this behavior is probably due to a method-specific artifact⁴⁹ and v) very important differences are observed between the calculated charges and the OPLS-AA native charges for the same residues. Considering all these elements, the charges calculated at the HF/6-311++G(2d,p) level with the protocol C were selected as representative for this system (line highlighted in red in Table 1).

Figure 1. Minimal representation of the carbonic anhydrase binding site (OH₂ form), containing the zinc ion, side chains of three histidine residues (His94, His96 and His119) and zinc-bound water.

Table 1. QM charges calculated with different protocols and methods for the OH₂ form of carbonic anhydrase binding site.

Protocol	Method	HIS 94	HIS 96	HIS 119	ZN	ZS (O)	ZS (H1)	ZS (H2)
		(ND)	(ND)	(NE)				
A	HF/6-31G(d)	-0.291	-0.291	-0.285	0.745	-0.762	0.459	0.459
A	HF/6-311++g(2d,p)	-0.236	-0.236	-0.216	0.676	-0.715	0.433	0.433
A	B3LYP/6-31G(d)	-0.197	-0.197	-0.164	0.533	-0.670	0.432	0.432

A	B3LYP/6-							
	311++g(2d,p)	-0.162	-0.162	-0.120	0.487	-0.640	0.406	0.406
A	MP2/6-31G(d)	-0.259	-0.259	-0.246	0.691	-0.750	0.456	0.456
B	HF/6-31G(d)	-0.183	-0.183	-0.415	0.745	-0.784	0.483	0.483
B	HF/6-311++g(2d,p)	-0.111	-0.111	-0.357	0.651	-0.703	0.453	0.453
B	B3LYP/6-31G(d)	-0.120	-0.120	-0.345	0.565	-0.691	0.460	0.460
B	B3LYP/6-							
	311++g(2d,p)	-0.065	-0.065	-0.306	0.490	-0.626	0.432	0.432
B	MP2/6-31G(d)	-0.183	-0.183	-0.415	0.745	-0.784	0.483	0.483
C	HF/6-31G(d)	-0.208	-0.208	-0.407	0.802	-0.925	0.480	0.480
C	HF/6-311++g(2d,p)	-0.123	-0.123	-0.326	0.691	-0.868	0.454	0.454
C	B3LYP/6-31G(d)	-0.159	-0.159	-0.341	0.632	-0.877	0.464	0.464
C	B3LYP/6-							
	311++g(2d,p)	-0.090	-0.090	-0.286	0.551	-0.831	0.441	0.441
C	MP2/6-31G(d)	-0.208	-0.208	-0.407	0.802	-0.925	0.480	0.480
–	OPLS-AA^a	-0.564	-0.564	-0.564	2.000	-1.040	0.520	0.520

^a Generic OPLS-AA charges as implemented in Gromacs⁴⁷

Similarly, the analysis of the results from Table 2 (OH[−] form of the binding site) shows analogous trends, and the charges calculated at the HF/6-311++G(2d,p) level with the protocol C were also selected in this case as representative for the carbonic anhydrase binding site (line highlighted in red in Table 2).

Figure 2. Extended representation of the carbonic anhydrase binding site (OH_2 form), containing the zinc ion, side chains of three histidine residues (His94, His96 and His119), zinc-bound water and the residues interacting with the latter: side chain of Thr199 residue and DW and W1 waters.

Table 2. QM charges calculated with different protocols and methods for the OH^- form of carbonic anhydrase binding site.

Protocol	Method	HIS 94 (ND)	HIS 96 (ND)	HIS 119 (NE)	ZN	ZS (O)	ZS (H1)	ZS (H2)
A	HF/6-31G(d)	-0.162	-0.162	-0.328	0.624	-0.916	0.374	–
A	HF/6-311++g(2d,p)	-0.105	-0.105	-0.291	0.600	-0.902	0.351	–
A	B3LYP/6-31G(d)	-0.079	-0.079	-0.243	0.407	-0.762	0.336	–
A	B3LYP/6-311++g(2d,p)	-0.053	-0.053	-0.237	0.426	-0.798	0.331	–
A	MP2/6-31G(d)	-0.165	-0.165	-0.323	0.613	-0.888	0.356	–
B	HF/6-31G(d)	-0.088	-0.088	-0.243	0.576	-0.973	0.361	–
B	HF/6-311++g(2d,p)	-0.041	-0.041	-0.189	0.566	-0.948	0.327	–
B	B3LYP/6-31G(d)	-0.017	-0.017	-0.169	0.346	-0.828	0.340	–

B	B3LYP/6-311++g(2d,p)	0.003	0.003	-0.144	0.388	-0.836	0.306	–
B	MP2/6-31G(d)	-0.088	-0.088	-0.243	0.576	-0.973	0.361	–
C	HF/6-31G(d)	0.047	0.047	-0.228	0.430	-0.849	0.304	–
C	HF/6-311++g(2d,p)	0.114	0.114	-0.152	0.376	-0.778	0.240	–
C	B3LYP/6-31G(d)	0.097	0.097	-0.160	0.230	-0.716	0.275	–
C	B3LYP/6-311++g(2d,p)	0.148	0.148	-0.113	0.214	-0.670	0.220	–
C	MP2/6-31G(d)	0.047	0.047	-0.228	0.430	-0.849	0.304	–
–	OPLS-AA^a	-0.564	-0.564	-0.564	2.000	-1.300	0.300	–

^a Generic OPLS-AA charges as implemented in Gromacs⁴⁷

A comparison of the selected charges for the two systems (OH₂ or OH[–] forms of the binding site) points out very important differences in charge distribution, especially for the histidine residues and for the hydrogen atoms belonging to the zinc-bound water. The charge difference for the zinc atom is important (~0.3), but most of the supplementary charge arising from the zinc-bound water deprotonation seems to be redistributed over the whole binding site.

Bonding parameters

The standard OPLS-AA bonding parameters implemented in Gromacs⁴⁷ were used in this work, except for those involving the zinc ion and the zinc-bound water, which were not available. In this latter case, the values for bonds, angles and dihedrals were extracted from the neutron structure⁴⁸ (OH₂ form) or from quantum chemistry calculations (OH[–] form). The other parameters were adapted from those previously published by Maupin *et al.*,²⁸ with the exception of the angle N-Zn-O for which the force constant was too weak to keep the binding site's geometry stable (data not shown). We used then a ~5-fold stronger force constant for this angle (see the Supplementary Data file) which led to stable simulations, as shown below. Free rotation was allowed for dihedral angles involving Zn (X-Zn-N-X and X-Zn-O-X).

Molecular dynamics

Molecular dynamics simulations in explicit solvent were performed in order to evaluate the validity of the OPLS-AA force field parameters generated in this study. Two simulations, of 10 ns each, were carried out

using the neutron structure of carbonic anhydrase II (PDB code 3KKX)⁴⁸ with both OH₂ and OH⁻ forms of the binding site.

The structure proved to be stable for the simulation time, with RMSD values of 1.5-1.9 Å for the whole protein and 0.25-0.35 Å for the binding site, which includes the three histidine residues (His94, His96 and His119), the zinc ion and the zinc-bound water. The distances between the zinc ion and its immediate neighbors are also very stable, with values of 1.80 Å for the Zn-O bond in the OH⁻ form and 2.30 Å in the OH₂ form. The Zn-N bonds have a mean value of 2.05 Å in both forms. The angles N-Zn-O are also stable using the new force constant, the angle average and angle distribution showing equilibrium values of ~117° and ~113° for the OH₂ and OH⁻ forms of the binding site, respectively. All these plots can be found in the Supplementary Data file.

Conclusions

In this work, the carbonic anhydrase binding site parameterization was performed using quantum chemistry calculations and these parameters were subsequently validated using molecular dynamics simulations. Important differences in terms of atomic partial charges were obtained for the OH₂ and OH⁻ forms of the binding site, showing that the supplementary charge induced by the zinc-bound water deprotonation has a significant influence on residues from the entire binding site. The force field parameters and topologies generated in this work will be useful for future molecular dynamics studies of carbonic anhydrase using the OPLS-AA force field.

Materials and methods

Charge calculation

The geometry of each system was optimized in gas-phase using the Gaussian 09 package⁵⁰ using the Hartree-Fock method,⁵¹ the Becke's three-parameter hybrid exchange functional (B3LYP)^{52,53} or the Møller-Plesset perturbation method (MP2),⁵⁴ with either the 6-31G(d) or 6-311++G(2d,p) basis sets. CHelpG charges were calculated at the same level, then a two-stage RESP fitting was carried out using AmberTools.⁵⁵ This methodology has been previously used for heterocycles parameterization in the

OPLS-AA force field^{56,57} and found to be the most appropriate for the OPLS-AA parameterization of the SAMPL3 dataset.⁴⁵

Molecular dynamics

Molecular dynamics simulations were performed using GROMACS version 4.5.4⁴⁷ with the OPLS-AA^{41,42} force field. The system was constructed by positioning the protein in a cubic periodic box of TIP4P waters, with a minimum distance of 1.0 nm on each side. The simulation box, obtained after hydration and neutralization with Na⁺ and Cl⁻ ions to physiological ionic strength (150 mM), contained about 62,000 atoms (protein, water and ions). The system was energy-minimized until convergence using a steepest descents algorithm to remove close contacts of water molecules with the solute. Molecular dynamics simulation with position restraints on the solute atoms was then performed for 200 ps to relax the water molecules around the solute, followed by the production run of 10 ns. During the position restraints and production runs, the Berendsen method⁵⁸ was used for pressure and temperature coupling. Electrostatics were calculated with the particle mesh Ewald method.^{59,60} The P-LINCS algorithm⁶¹ was used to constrain bond lengths, and a time step of 2 fs was used throughout. All simulations were performed using constant temperature, pressure, and number of particles (NPT) and periodic boundary conditions. The cut-off distance for Lennard-Jones interactions and electrostatic interactions were both set at 1.0 nm.

OPLS-AA force field topologies for the binding site residues were generated with an in-house developed script, using the charges presented in Tables 1 and 2 and the parameters described in the Supplementary Data file.

Miscellaneous

Structure images were rendered using Chimera.⁶² Molecular dynamics analysis plots were generated with XmGrace.⁶³

All calculations were performed using the high-performance computing (HPC) facilities at the Institut de Chimie des Substances Naturelles, Gif sur Yvette, France.

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Supplementary Material

Supplementary data are available online, containing i) a detailed analysis of the molecular dynamics simulations and ii) the OPLS-AA force field parameters in the appropriate format for use with the Gromacs software.

References and notes

1. Mincione, F.; Scozzafava, A.; Supuran, C. T. *Curr. Pharm. Des.* **2008**, *14*, 649.
2. Gugleta, K. *Curr. Med. Res. Opin.* **2010**, *26*, 1255.
3. Pastorekova, S.; Zatovicova, M.; Pastorek, J. *Curr. Pharm. Des.* **2008**, *14*, 685.
4. Thiry, A.; Supuran, C. T.; Masereel, B.; Dogne, J. M. *J. Med. Chem.* **2008**, *51*, 3051.
5. Winum, J. Y.; Scozzafava, A.; Montero, J. L.; Supuran, C. T. *Anticancer Agents Med. Chem.* **2009**, *9*, 693.
6. Guler, O. O.; De Simone, G.; Supuran, C. T. *Curr. Med. Chem.* **2010**, *17*, 1516.
7. Poulsen, S. A. *Expert Opin. Ther. Pat.* **2010**, *20*, 795.
8. De Simone, G.; Di Fiore, A.; Supuran, C. T. *Curr. Pharm. Des.* **2008**, *14*, 655.
9. Supuran, C. T.; Di Fiore, A.; De Simone, G. *Expert Opin. Emerg. Drugs* **2008**, *13*, 383.
10. Thiry, A.; Dogne, J. M.; Supuran, C. T.; Masereel, B. *Curr. Pharm. Des.* **2008**, *14*, 661.
11. Temperini, C.; Scozzafava, A.; Supuran, C. T. *Curr. Pharm. Des.* **2008**, *14*, 708.
12. Supuran, C. T. *Curr. Pharm. Des.* **2008**, *14*, 603.
13. Supuran, C. T. *Nat. Rev. Drug Discov.* **2008**, *7*, 168.
14. Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3467.
15. Gilmour, K. M. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **2010**, *157*, 193.
16. Supuran, C. T. *Future Med. Chem.* **2011**, *3*, 1165.
17. Åqvist, J.; Warshel, A. *J. Mol. Biol.* **1992**, *224*, 7.

18. Housaindokht, M. R.; Bozorgmehr, M. R.; Bahrololoom, M. *J. Theor. Biol.* **2008**, *254*, 294.
19. Bozorgmehr, M. R.; Morsali, A.; Beyramabadi, S. A. *Int. J. Phys. Sci.* **2012**, *7*.
20. Stote, R. H.; Karplus, M. *Proteins* **1995**, *23*, 12.
21. Lu, D.; Voth, G. A. *Proteins* **1998**, *33*, 119.
22. Roy, A.; Taraphder, S. *J. Phys. Chem. B* **2009**, *113*, 12555.
23. Zhang, J.-L.; Zheng, Q.-C.; Zhang, H.-X. *Comput. Biol. Chem.* **2011**, *35*, 50.
24. Schmid, M.; Nogueira, E. S.; Monnard, F. W.; Ward, T. R.; Meuwly, M. *Chem. Sci.* **2012**.
25. Ohta, S.; Alam, M. T.; Arakawa, H.; Ikai, A. *Biophys. J.* **2004**, *87*, 4007.
26. Vitale, R. M.; Pedone, C.; Amodeo, P.; Antel, J.; Wurl, M.; Scozzafava, A.; Supuran, C. T.; Simone, G. D. *Bioorg. Med. Chem.* **2007**, *15*, 4152.
27. Fisher, S. Z.; Maupin, C. M.; Budayova-Spano, M.; Govindasamy, L.; Tu, C.; Agbandje-McKenna, M.; Silverman, D. N.; Voth, G. A.; McKenna, R. *Biochemistry* **2007**, *46*, 2930.
28. Maupin, C. M.; Voth, G. A. *Biochemistry* **2007**, *46*, 2938.
29. Maupin, C. M.; Saunders, M. G.; Thorpe, I. F.; McKenna, R.; Silverman, D. N.; Voth, G. A. *J. Am. Chem. Soc.* **2008**, *130*, 11399.
30. Král, V.; Mader, P.; Collard, R.; Fábry, M.; Hořejší, M.; Řezáčová, P.; Kožíšek, M.; Závada, J.; Sedláček, J.; Rulíšek, L.; Brynda, J. *Proteins* **2008**, *71*, 1275.
31. Maupin, C. M.; McKenna, R.; Silverman, D. N.; Voth, G. A. *J. Am. Chem. Soc.* **2009**, *131*, 7598.
32. Maupin, C. M.; Voth, G. A. *Biochim. Biophys. Acta* **2010**, *1804*, 332.
33. Alterio, V.; Vitale, R. M.; Monti, S. M.; Pedone, C.; Scozzafava, A.; Cecchi, A.; De Simone, G.; Supuran, C. T. *J. Am. Chem. Soc.* **2006**, *128*, 8329.
34. D'Ambrosio, K.; Vitale, R.-M.; Dogné, J.-M.; Masereel, B.; Innocenti, A.; Scozzafava, A.; De Simone, G.; Supuran, C. T. *J. Med. Chem.* **2008**, *51*, 3230.
35. Vitale, R. M.; Alterio, V.; Innocenti, A.; Winum, J.-Y.; Monti, S. M.; De Simone, G.; Supuran, C. T. *J. Med. Chem.* **2009**, *52*, 5990.
36. Peters, M. B.; Yang, Y.; Wang, B.; Füsti-Molnár, L.; Weaver, M. N.; Merz Jr, K. M. *J. Chem. Theory Comput.* **2010**, *6*, 2935.

37. Loferer, M. J.; Tautermann, C. S.; Loeffler, H. H.; Liedl, K. R. *J. Am. Chem. Soc.* **2003**, *125*, 8921.
38. Braun-Sand, S.; Strajbl, M.; Warshel, A. *Biophys. J.* **2004**, *87*, 2221.
39. Riccardi, D.; Yang, S.; Cui, Q. *Biochim. Biophys. Acta* **2010**, *1804*, 342.
40. Banci, L. *Curr. Opin. Chem. Biol.* **2003**, *7*, 143.
41. Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1996**, *118*, 11225.
42. Kaminski, G. A.; Friesner, R. A.; Tirado-Rives, J.; Jorgensen, W. L. *J. Phys. Chem. B* **2001**, *105*, 6474.
43. Shirts, M. R.; Pitera, J. W.; Swope, W. C.; Pande, V. S. *J. Chem. Phys.* **2003**, *119*, 5740.
44. Shivakumar, D.; Williams, J.; Wu, Y.; Damm, W.; Shelley, J.; Sherman, W. *J. Chem. Theory Comput.* **2010**, *6*, 1509.
45. Beckstein, O.; Iorga, B. I. *J Comput Aided Mol Des* **2012**, *26*, 635.
46. Jorgensen, W. L.; Schyman, P. *J. Chem. Theory Comput.* **2012**, in press (DOI: 10.1021/ct300180w).
47. Hess, B.; Kutzner, C.; van der Spoel, D.; Lindahl, E. *J. Chem. Theory Comput.* **2008**, *4*, 435.
48. Fisher, S. Z.; Kovalevsky, A. Y.; Domsic, J. F.; Mustyakimov, M.; McKenna, R.; Silverman, D. N.; Langan, P. A. *Biochemistry* **2010**, *49*, 415.
49. Frison, G.; Ohanessian, G. *Phys. Chem. Chem. Phys.* **2009**, *11*, 374.
50. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.;

Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian 09, Revision A.2, Gaussian Inc., Wallingford CT, USA (<http://www.gaussian.com>).

51. Slater, J. C. *Phys. Rev.* **1951**, *81*, 385.
52. Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
53. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
54. Møller, C.; Plesset, M. S. *Phys. Rev.* **1934**, *46*, 618.
55. Iorga, B.; Eymery, F.; Savignac, P. *Tetrahedron Lett* **1998**, *39*, 4477.
56. Jorgensen, W. L.; McDonald, N. A. *J. Mol. Struct. THEOCHEM* **1998**, *424*, 145.
57. McDonald, N. A.; Jorgensen, W. L. *J. Phys. Chem. B* **1998**, *102*, 8049.
58. Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; DiNola, A.; Haak, J. R. *J. Chem. Phys.* **1984**, *81*, 3684.
59. Darden, T.; York, D.; Pedersen, L. G. *J. Chem. Phys.* **1993**, *98*, 10089.
60. Essmann, U.; Perera, L.; Berkowitz, M.; Darden, T.; Lee, H.; Pedersen, L. *J. Chem. Phys.* **1995**, *103*, 8577.
61. Hess, B. *J. Chem. Theory Comput.* **2007**, *4*, 116.
62. Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. *J. Comput. Chem.* **2004**, *25*, 1605.
63. Scozzafava, A.; Iorga, B.; Supuran, C. T. *J. Enz. Inhib.* **2000**, *15*, 139.

Supporting information

Carbonic anhydrase binding site parameterization in OPLS-AA force field

Guillaume Bernadat,^a Claudiu T. Supuran^b and Bogdan I. Iorga^{a,*}

^a Institut de Chimie des Substances Naturelles, CNRS UPR 2301, Labex LERMIT, Centre de Recherche de Gif, 1 Avenue de la Terrasse, 91198 Gif-sur-Yvette, France

^b Dipartimento di Chimica, Laboratorio di Chimica Bioinorganica, Università degli Studi di Firenze, Rm 188, Via della Lastruccia 3, Polo Scientifico, I-50019 Sesto Fiorentino (Firenze), Italy

Validation of carbonic anhydrase binding site parameterization in OPLS-AA force field, using molecular dynamics, for both OH⁻ and OH₂ forms

Plots of:

- root mean square distance (RMSD) for protein and binding site
- distance between Zn and its coordinating atoms
- angle average and distribution for OW-Zn-N(HIS)

RMS Distance for Protein and Binding Site

Distance between Zn and its coordinating atoms

Angle average for OW-Zn-N(HIS)

Angle distribution for OW-Zn-N(HIS)

Modifications required for the PDB input files and for OPLS-AA files as implemented in Gromacs 4.5.4

PDB input files

Residues corresponding to HIS94, HIS96 and HIS119 should be renamed as following (example for the 3KKX structure). The zinc atom and the zinc-bound water should belong to the HIS119 residue.

- OH⁻ form

ATOM	733	N	H2D	A	94	-11.270	-0.912	10.982	1.00	13.80	N
ATOM	734	CA	H2D	A	94	-10.338	-1.868	10.387	1.00	14.21	C
ATOM	735	C	H2D	A	94	-10.428	-3.168	11.181	1.00	14.70	C
ATOM	736	O	H2D	A	94	-11.030	-3.183	12.237	1.00	14.38	O
ATOM	737	CB	H2D	A	94	-8.880	-1.336	10.333	1.00	14.21	C
ATOM	738	CG	H2D	A	94	-8.350	-1.070	11.697	1.00	13.64	C
ATOM	739	ND1	H2D	A	94	-8.429	0.175	12.301	1.00	15.01	N
ATOM	740	CD2	H2D	A	94	-7.757	-1.906	12.598	1.00	13.69	C
ATOM	741	CE1	H2D	A	94	-7.892	0.088	13.524	1.00	14.16	C
ATOM	742	NE2	H2D	A	94	-7.483	-1.169	13.705	1.00	12.81	N
...											
ATOM	754	N	H2D	A	96	-8.103	-6.883	12.469	1.00	13.66	N
ATOM	755	CA	H2D	A	96	-6.895	-7.669	12.656	1.00	14.25	C
ATOM	756	C	H2D	A	96	-7.334	-9.133	12.494	1.00	14.61	C
ATOM	757	O	H2D	A	96	-8.426	-9.505	12.965	1.00	15.41	O
ATOM	758	CB	H2D	A	96	-6.290	-7.457	14.042	1.00	14.92	C
ATOM	759	CG	H2D	A	96	-6.025	-6.011	14.337	1.00	13.72	C
ATOM	760	ND1	H2D	A	96	-4.794	-5.461	14.134	1.00	15.69	N
ATOM	761	CD2	H2D	A	96	-6.871	-5.021	14.766	1.00	15.31	C
ATOM	762	CE1	H2D	A	96	-4.879	-4.148	14.467	1.00	12.87	C
ATOM	763	NE2	H2D	A	96	-6.117	-3.878	14.841	1.00	14.53	N
...											
ATOM	934	N	H2E	A	119	-11.996	-3.099	14.903	1.00	13.18	N
ATOM	935	CA	H2E	A	119	-11.779	-1.864	15.644	1.00	12.59	C
ATOM	936	C	H2E	A	119	-12.488	-0.684	14.964	1.00	13.77	C
ATOM	937	O	H2E	A	119	-12.120	-0.370	13.831	1.00	14.33	O
ATOM	938	CB	H2E	A	119	-10.263	-1.553	15.732	1.00	13.09	C
ATOM	939	CG	H2E	A	119	-9.561	-2.520	16.621	1.00	13.90	C
ATOM	940	ND1	H2E	A	119	-8.209	-2.718	16.674	1.00	15.24	N
ATOM	941	CD2	H2E	A	119	-10.105	-3.367	17.544	1.00	13.82	C
ATOM	942	CE1	H2E	A	119	-7.901	-3.630	17.567	1.00	14.62	C
ATOM	943	NE2	H2E	A	119	-9.042	-4.049	18.131	1.00	14.93	N
ATOM	2060	ZN	H2E	A	119	-6.735	-2.066	15.380	1.00	14.83	Zn
ATOM	2061	OW	H2E	A	119	-5.366	-0.838	16.084	1.00	17.01	O

- OH₂ form

ATOM	733	N	H1D	A	94	-11.270	-0.912	10.982	1.00	13.80	N
ATOM	734	CA	H1D	A	94	-10.338	-1.868	10.387	1.00	14.21	C
ATOM	735	C	H1D	A	94	-10.428	-3.168	11.181	1.00	14.70	C
ATOM	736	O	H1D	A	94	-11.030	-3.183	12.237	1.00	14.38	O
ATOM	737	CB	H1D	A	94	-8.880	-1.336	10.333	1.00	14.21	C
ATOM	738	CG	H1D	A	94	-8.350	-1.070	11.697	1.00	13.64	C
ATOM	739	ND1	H1D	A	94	-8.429	0.175	12.301	1.00	15.01	N
ATOM	740	CD2	H1D	A	94	-7.757	-1.906	12.598	1.00	13.69	C
ATOM	741	CE1	H1D	A	94	-7.892	0.088	13.524	1.00	14.16	C
ATOM	742	NE2	H1D	A	94	-7.483	-1.169	13.705	1.00	12.81	N
...											
ATOM	754	N	H1D	A	96	-8.103	-6.883	12.469	1.00	13.66	N
ATOM	755	CA	H1D	A	96	-6.895	-7.669	12.656	1.00	14.25	C
ATOM	756	C	H1D	A	96	-7.334	-9.133	12.494	1.00	14.61	C
ATOM	757	O	H1D	A	96	-8.426	-9.505	12.965	1.00	15.41	O

ATOM	758	CB	H1D	A	96	-6.290	-7.457	14.042	1.00	14.92	C
ATOM	759	CG	H1D	A	96	-6.025	-6.011	14.337	1.00	13.72	C
ATOM	760	ND1	H1D	A	96	-4.794	-5.461	14.134	1.00	15.69	N
ATOM	761	CD2	H1D	A	96	-6.871	-5.021	14.766	1.00	15.31	C
ATOM	762	CE1	H1D	A	96	-4.879	-4.148	14.467	1.00	12.87	C
ATOM	763	NE2	H1D	A	96	-6.117	-3.878	14.841	1.00	14.53	N
...											
ATOM	934	N	H1E	A	119	-11.996	-3.099	14.903	1.00	13.18	N
ATOM	935	CA	H1E	A	119	-11.779	-1.864	15.644	1.00	12.59	C
ATOM	936	C	H1E	A	119	-12.488	-0.684	14.964	1.00	13.77	C
ATOM	937	O	H1E	A	119	-12.120	-0.370	13.831	1.00	14.33	O
ATOM	938	CB	H1E	A	119	-10.263	-1.553	15.732	1.00	13.09	C
ATOM	939	CG	H1E	A	119	-9.561	-2.520	16.621	1.00	13.90	C
ATOM	940	ND1	H1E	A	119	-8.209	-2.718	16.674	1.00	15.24	N
ATOM	941	CD2	H1E	A	119	-10.105	-3.367	17.544	1.00	13.82	C
ATOM	942	CE1	H1E	A	119	-7.901	-3.630	17.567	1.00	14.62	C
ATOM	943	NE2	H1E	A	119	-9.042	-4.049	18.131	1.00	14.93	N
ATOM	2060	ZN	H1E	A	119	-6.735	-2.066	15.380	1.00	14.83	Zn
ATOM	2061	OW	H1E	A	119	-5.366	-0.838	16.084	1.00	17.01	O

OPLS-AA Gromacs files

The following lines should be added to the files mentioned below, in the corresponding sections.

- aminoacids.rtp

```
[ H1D ] ; HISD bound to Zn for CA (OH2 form)
[ atoms ]
  N      opl_238      -0.500      1
  H      opl_241       0.300      1
  CA     opl_224B      0.140      1
  HA     opl_140       0.060      1
  CB     opl_505      -0.038      2
  HB1    opl_140       0.099      2
  HB2    opl_140       0.099      2
  CG     opl_508       0.228      3
  ND1    opl_503      -0.332      4
  HD1    opl_504       0.384      4
  CD2    opl_507      -0.311      5
  HD2    opl_146       0.197      5
  CE1    opl_506       0.110      6
  HE1    opl_146       0.140      6
  NE2    opl_511      -0.123      7
  C      opl_235       0.500      8
  O      opl_236      -0.500      8
[ bonds ]
  N      H
  N      CA
  CA     HA
  CA     CB
  CA     C
  CB     HB1
  CB     HB2
  CB     CG
  CG     ND1
  CG     CD2
  ND1    HD1
  ND1    CE1
  CD2    HD2
  CD2    NE2
  CE1    HE1
  CE1    NE2
  C      O
  -C     N
```

```

[ dihedrals ] ; override some with residue-specific ones
  N    CA    CB    CG    dih_HIS_chi1_N_C_C_C
  CG    CB    CA    C    dih_HIS_chi1_C_C_C_CO
  CA    CB    CG    ND1   dih_HIS_chi2_C_C_C_N

[ impropers ]
  -C    CA    N    H    improper_Z_N_X_Y
  CA    +N    C    O    improper_O_C_X_Y
  ND1   CD2    CG    CB    improper_Z_CA_X_Y
  CG    CE1    ND1   HD1   improper_Z_N_X_Y
  CG    NE2    CD2    HD2   improper_Z_CA_X_Y
  ND1   NE2    CE1    HE1   improper_Z_CA_X_Y

[ H1E ] ; HISE bound to Zn for CA (OH2 form)
[ atoms ]
  N    opls_238    -0.500    1
  H    opls_241    0.300    1
  CA    opls_224B   0.140    1
  HA    opls_140    0.060    1
  CB    opls_505    -0.136    2
  HB1   opls_140    0.111    2
  HB2   opls_140    0.111    2
  CG    opls_507    0.226    3
  ND1   opls_511    -0.326    3
  CD2   opls_508    -0.213    4
  HD2   opls_146    0.226    4
  CE1   opls_506    0.110    5
  HE1   opls_146    0.140    5
  NE2   opls_503    -0.248    6
  HE2   opls_504    0.372    6
  C     opls_235    0.500    7
  O     opls_236    -0.500    7
  ZN    Zn2+        0.691    8
  OW    opls_116    -0.87    9
  HW1   opls_117    0.45    9
  HW2   opls_117    0.45    9

[ bonds ]
  N    H
  N    CA
  CA    HA
  CA    CB
  CA    C
  CB    HB1
  CB    HB2
  CB    CG
  CG    ND1
  CG    CD2
  ND1   CE1
  CD2   HD2
  CD2   NE2
  CE1   HE1
  CE1   NE2
  NE2   HE2
  C     O
  -C    N
  ZN    ND1
  OW    HW1
  OW    HW2
  ZN    OW    0.23000    16740.0    ; 418.5*40 (adapted from Maupin,
Biochemistry, 2007, 46, 2938)
[ angles ]
  HW    OW    HW    104.500    418.500    ; 4.185*100 (adapted from Maupin,
Biochemistry, 2007, 46, 2938)
[ dihedrals ] ; override some with residue-specific ones
  N    CA    CB    CG    dih_HIS_chi1_N_C_C_C

```

```

      CG      CB      CA      C      dih_HIS_chi1_C_C_C_CO
      CA      CB      CG      ND1    dih_HIS_chi2_C_C_C_N
[ impropers ]
      -C      CA      N      H      improper_Z_N_X_Y
      CA      +N      C      O      improper_O_C_X_Y
      CE1     CD2     NE2     HE2     improper_Z_N_X_Y
      CG      NE2     CD2     HD2     improper_Z_CA_X_Y
      ND1     NE2     CE1     HE1     improper_Z_CA_X_Y
      ND1     CD2     CG      CB      improper_Z_CA_X_Y

```

[H2D] ; HISD bound to Zn for CA (OH form)

```

[ atoms ]
      N      opls_238      -0.500      1
      H      opls_241      0.300      1
      CA      opls_224B     0.140      1
      HA      opls_140      0.060      1
      CB      opls_505      -0.117     2
      HB1     opls_140      0.079     2
      HB2     opls_140      0.079     2
      CG      opls_508      0.258     3
      ND1     opls_503      -0.402     4
      HD1     opls_504      0.389     4
      CD2     opls_507      -0.422     5
      HD2     opls_146      0.195     5
      CE1     opls_506      0.077     6
      HE1     opls_146      0.169     6
      NE2     opls_511      0.113     7
      C      opls_235      0.500     8
      O      opls_236      -0.500     8

```

[bonds]

```

      N      H
      N      CA
      CA      HA
      CA      CB
      CA      C
      CB      HB1
      CB      HB2
      CB      CG
      CG      ND1
      CG      CD2
      ND1     HD1
      ND1     CE1
      CD2     HD2
      CD2     NE2
      CE1     HE1
      CE1     NE2
      C      O
      -C      N

```

[dihedrals] ; override some with residue-specific ones

```

      N      CA      CB      CG      dih_HIS_chi1_N_C_C_C
      CG      CB      CA      C      dih_HIS_chi1_C_C_C_CO
      CA      CB      CG      ND1    dih_HIS_chi2_C_C_C_N

```

[impropers]

```

      -C      CA      N      H      improper_Z_N_X_Y
      CA      +N      C      O      improper_O_C_X_Y
      ND1     CD2     CG      CB      improper_Z_CA_X_Y
      CG      CE1     ND1     HD1     improper_Z_N_X_Y
      CG      NE2     CD2     HD2     improper_Z_CA_X_Y
      ND1     NE2     CE1     HE1     improper_Z_CA_X_Y

```

[H2E] ; HISE bound to Zn for CA (OH form)

```

[ atoms ]
      N      opls_238      -0.500      1

```

```

      H      opls_241      0.300      1
      CA      opls_224B      0.140      1
      HA      opls_140      0.060      1
      CB      opls_505      -0.195      2
      HB1      opls_140      0.094      2
      HB2      opls_140      0.094      2
      CG      opls_507      0.198      3
      ND1      opls_511      -0.152      3
      CD2      opls_508      -0.244      4
      HD2      opls_146      0.214      4
      CE1      opls_506      0.077      5
      HE1      opls_146      0.169      5
      NE2      opls_503      -0.298      6
      HE2      opls_504      0.369      6
      C      opls_235      0.500      7
      O      opls_236      -0.500      7
      ZN      Zn2+      0.376      8
      OW      opls_116      -0.778      9
      HW      opls_117      0.240      9
[ bonds ]
      N      H
      N      CA
      CA      HA
      CA      CB
      CA      C
      CB      HB1
      CB      HB2
      CB      CG
      CG      ND1
      CG      CD2
      ND1      CE1
      CD2      HD2
      CD2      NE2
      CE1      HE1
      CE1      NE2
      NE2      HE2
      C      O
      -C      N
      ZN      ND1
      OW      HW
      ZN      OW      0.18000      39339.0      ; 418.5*94 (adapted from Maupin,
Biochemistry, 2007, 46, 2938)
[ dihedrals ] ; override some with residue-specific ones
      N      CA      CB      CG      dih_HIS_chi1_N_C_C_C
      CG      CB      CA      C      dih_HIS_chi1_C_C_C_CO
      CA      CB      CG      ND1      dih_HIS_chi2_C_C_C_N
[ impropers ]
      -C      CA      N      H      improper_Z_N_X_Y
      CA      +N      C      O      improper_O_C_X_Y
      CE1      CD2      NE2      HE2      improper_Z_N_X_Y
      CG      NE2      CD2      HD2      improper_Z_CA_X_Y
      ND1      NE2      CE1      HE1      improper_Z_CA_X_Y
      ND1      CD2      CG      CB      improper_Z_CA_X_Y

[ ZN1 ]
; in CA (OH2 form)
[ atoms ]
      ZN      Zn2+      0.691      0

[ ZN2 ]
; in CA (OH form)
[ atoms ]
      ZN      Zn2+      2.000      0

```

- aminoacids.hdb

```

H1D      6
1  1      H      N      -C      CA
1  5      HA      CA      N      C      CB
2  6      HB      CB      CG      CA
1  1      HD2     CD2     CG      NE2
1  1      HE1     CE1     ND1     NE2
1  1      HD1     ND1     CG      CE1
H1E      7
1  1      H      N      -C      CA
2  6      HB      CB      CG      CA
1  5      HA      CA      N      C      CB
1  1      HE2     NE2     CE1     CD2
1  1      HD2     CD2     CG      NE2
1  1      HE1     CE1     ND1     NE2
2  7      HW      OW
H2D      6
1  1      H      N      -C      CA
1  5      HA      CA      N      C      CB
2  6      HB      CB      CG      CA
1  1      HD2     CD2     CG      NE2
1  1      HE1     CE1     ND1     NE2
1  1      HD1     ND1     CG      CE1
H2E      7
1  1      H      N      -C      CA
2  6      HB      CB      CG      CA
1  5      HA      CA      N      C      CB
1  1      HE2     NE2     CE1     CD2
1  1      HD2     CD2     CG      NE2
1  1      HE1     CE1     ND1     NE2
1  2      HW      OW      ZN      ND1

```

- ffbonded.itp

```

[ bondtypes ]
  Zn2+  NB      1      0.20500      16740.0      ; 418.5*40 (adapted from Maupin,
Biochemistry, 2007, 46, 2938)

[ angletypes ]
  Zn2+  NB      CR      1      126.000      83.700      ; 4.185*20 (adapted from
Maupin, Biochemistry, 2007, 46, 2938)
  Zn2+  NB      CV      1      126.000      83.700      ; 4.185*20 (adapted from
Maupin, Biochemistry, 2007, 46, 2938)
  Zn2+  NB      CW      1      126.000      83.700      ; 4.185*20 (adapted from
Maupin, Biochemistry, 2007, 46, 2938)
  NB    Zn2+    NB      1      109.500      96.255      ; 4.185*23 (adapted from
Maupin, Biochemistry, 2007, 46, 2938)
  NB    Zn2+    OW      1      109.500      418.500      ; 4.185*100
  Zn2+  OW      HW      1      124.000      83.700      ; 4.185*20 (adapted from
Maupin, Biochemistry, 2007, 46, 2938)

[ dihedraltypes ]
  X      Zn2+  NB      X      3      0.00000      0.00000      0.00000      0.00000
0.00000      0.00000 ; (Maupin, Biochemistry, 2007, 46, 2938)
  X      Zn2+  OW      X      3      0.00000      0.00000      0.00000      0.00000
0.00000      0.00000 ; (Maupin, Biochemistry, 2007, 46, 2938)

```