Modelling copper-containing proteins

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Chapter 7

CONCLUDING REMARKS

Summary

It has been shown in the past that Molecular Dynamic (MD) simulations can be very helpful analysing the dynamics of proteins. In order to be able to apply this technique to proteins containing a transition metal ion, a force field should be available that describes the interactions between the metal and the protein. In this thesis, two techniques were developed to obtain a parameter set for various Cu-containing proteins.

7.1 Empirically derived, non-bonded force field

The copper co-ordination sphere may vary considerably for Cu-containing proteins. Different ligands may co-ordinate the Cu-atom at different distances. It is also known that transition metal bonds tend to break much easier than normal covalent bonds. Force fields that bind the copper to its ligands using a harmonic potential are therefore not appropriate. Morse potentials give a better approximation but are hard to parameterise. In this research a non-bonded force field was developed. Azurin was taken as a model to obtain a force field for cupredoxins.

A Potential Energy Surface (PES) was obtained by monitoring the potential energy while the position of the Cu-atom was varied within its binding site. The energy surface shows the position of the Cu-atom where the potential energy is minimal. By varying the charge delocalisation and polarisation of the Cu-atom and co-ordinating residues, the effect of these parameters on the energy profile of the type-I Cu-site was obtained. A parameter set was selected which produced structures that mimicked the crystallographic structures best. This force field was applied to a range of other blue copper proteins. After performing MD-simulations small variations were observed when comparing the Cu-sites of all these proteins which reflect the individual variations in the protein structure and active site properties (charge distribution, local flexibility).

The parameter set was also validated on azurin variants where mutations had been introduced in the ligand sphere. The structure of the site and the stability of the Cu-co-ordination was checked during MD-simulations. The differences between the proteins were reproduced. The application of the force field to the double mutant N42C/H117G azurin appeared very useful to model its structure. Since the latter protein variant was not stable enough to grow crystals or to be studied NMR, the structure of the Cu-site could not be determined experimentally. By pulling Cys 42 towards the Cu-atom using different amounts of Cu-ligand restraints, three structures were obtained. The stability of three different model structures was validated using the non-bonded force field after lifting of the restraints. In Figure 7.1(a) is shown that only two of the four ligands remain stable after removal of the restraints, (c) shows that none of the Cu-ligands remain stable. Only the structure as proposed in (b) could be identified as a stable model since

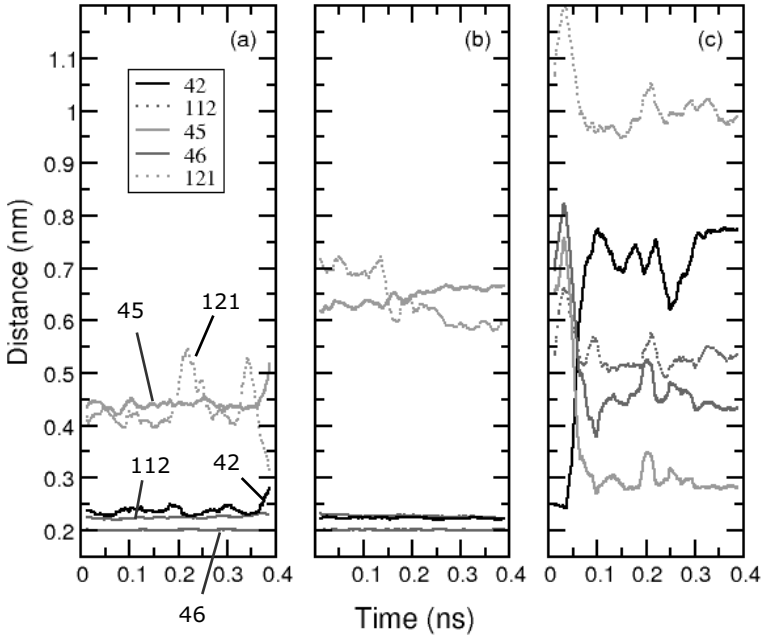

Figure 7.1: Stability analysis of the Cu-site of N42C/H117G azurin after restraining (a) four, (b) three or (c) two ligands to copper.

the three ligands remain stable after removal of the restraints. It shows that the non-bonded force field is useful to obtain information about the structure of a variant. The empirically developed force field was also applied in a project to compare the mobility and flexibility of the apo- and holo form of azurin and two azurin variants where mutations were introduced in the hydrophobic core. The number of dihedral transitions was used to monitor the flexibility of the proteins, see Table 7.1. The non-bonded force field was used in order not to restrict the motion of the Cu-site too severely and to make sure that differences between the apo- and holo form are not due to Cu-ligand constraints.

Table 7.1: Total number of dihedral transitions per ns for 74 selected dihedral angles.

wt		I7S		F110S	
apo	holo	apo	holo	apo	holo
481	399	524	423	501	413

In conclusion it can be said that the non-bonding force field is suitable to describe blue copper proteins. The stability and structure of Cu-ligand spheres were modelled without performing extensive quantum calculations. Since 40% of the proteins contain one or more metal ions, application of non-bonded force fields gives numerous prospects for

the near future. Emp ff can be used to get a first idea of the stability of the mutant to be made.The non-bonded force field presented in this thesis is not able to distinguish between the oxidised and reduced state of the copper ion. A next step might be the development of a non-bonded force field for each of the redox states of a co-ordinated metal.

7.2 Quantum-chemically derived, bonded force field

Density Functional Theory (DFT) calculations were performed to determine an accurate charge distribution of the copper site. Since these calculations are time-consuming, a selection of atoms to be incorporated in the calculation had to be made. In this research a relatively large number of atoms were included: copper plus the sidechains of the co-ordinating residues and the backbone atoms between the two adjacent ligands, Gly45 and His 46. LJ-values were taken from the standard GROMOS96 library.

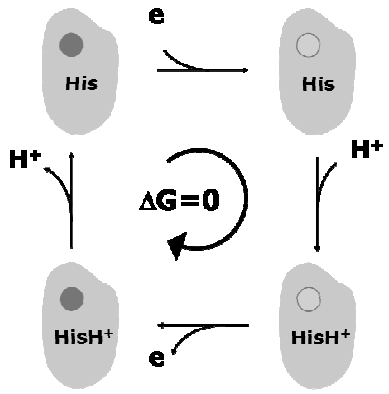

Figure 7.2: Schematic presentation of the free energy calculations.

The charge distribution for both the reduced and oxidised state of azurin was obtained. Performing free energy calculations of the protein and mutants in aqueous solution at different pH values, see Figure 7.2, redox potential differences were computed. The precision of the free energy calculations was evaluated as a function of the sampling time, the sequence of the sampling points and the initial conditions. It was found that the precision is critically dependent on the relaxation of hydrogen bonding networks when changing the atomic charge distribution to mimick a change of redox state or pH. Only qualitative estimates of the change in redox potential due to protein mutation can be obtained.

DFT calculations have also been performed to determine the charge distribution of the type-II Cu-site of the enzyme quercetinase. This was done in the presence and absence of the substrate. Using these charge distributions, the effect of substrate binding was

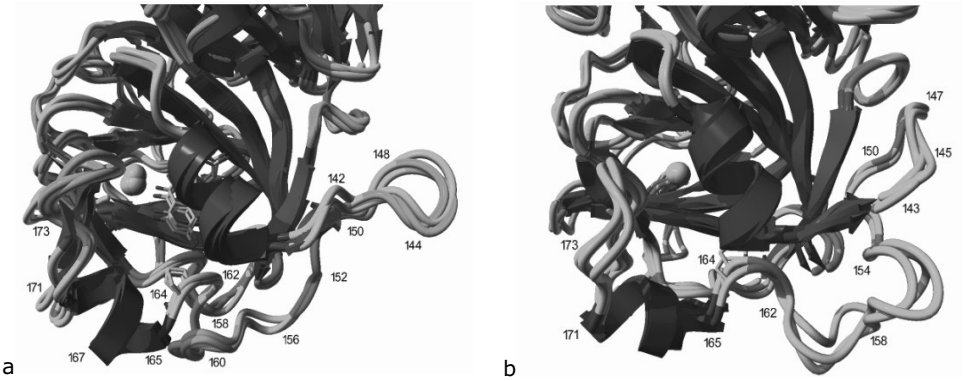

Figure 7.3 Three superimposed snapshots after 6 ns (a) in the presence and (b) in the absence of KMP.

investigated, see Figure 7.3. When the substrate binds into the cavity a loop, containing residues 154-176, orders remarkably although mobility is retained by residues 155–158. Some regions of the loop (residues 154–160 and 164–176) move over a considerable distance and approach the substrate closely, so that they lock the substrate in the substrate cavity. After removal of the substrate the MD-simulation of the free enzyme, shows strongly enhanced mobility in the loop region.

REFERENCES

Adman ET (1991) *Advances in Protein Chemistry* **42**, 145.

Adman ET & LH Jensen (1981) *Israel Journal of Chemistry* **21**, 8.

Allured VS, CM Kelly & CR Landis (1991) *Journal of the American Chemical Society* **113**, 1.

Alvarez ML, JY Ai, W Zumft, J Sanders-Loehr & DM Dooley (2001) *Journal of the American Chemical Society* **123**, 576.

Andrew CR, R Fraczkiewicz, RS Czernuszewicz, P Lappalainen, M Saraste & J Sanders-Loehr (1996) *Journal of the American Chemical Society* **118**, 10436.

Andrew CR, J Han, T den Blaauwen, G van Pouderoyen, E Vijgenboom, GW Canters, TM Loehr & J Sanders-Loehr (1997) *Journal of Biological Inorganic Chemistry* **2**, 98.

Andrew CR, H Yeom, JS Valentine, BG Karlsson, N Bonander, G van Pouderoyen, GW Canters, TM Loehr & J Sanders-Loehr (1994) *Journal of the American Chemical Society* **116**, 11489.

Aqvist J (1990) *Journal of Physical Chemistry* **94**, 8021.

Arcangeli C, AR Bizzari & S Cannistraro (1999) *Biophysical Chemistry* **78**, 247.

Arcangeli C, AR Bizzarri & S Cannistraro (2001) *Biophysical Chemistry* **90**, 45.

Badger RM (1934) *Journal of Chemical Physics* **2**, 128.

Baerends EJ, J Autschbach, A Berces, C Bo, PM Boerrigter & L Cavallo (2000) *ADF 2002.01*, SCM, Theoretical Chemistry. Vrije Universiteit, Amsterdam.

Banci L, P Carloni, G Lapenna & PL Orioli (1992) *Journal of the American Chemical Society* **114**, 6994.

Barrow DA & RA Aziz (1988) *Journal of Chemical Physics* **89**, 6189.

Bartolotti LJ, LG Pedersen & PS Charifson (1991) *Journal of Computational Chemistry* **12**, 1125.

Bashford D, M Karplus & GW Canters (1988) *Journal of Molecular Biology* **203**, 507.

Battistuzzi G, M Borsari, L Loschi, F Righi & M Sola (1999) *Journal of the American Chemical Society* **121**, 501.

Beck BW, JB Koerner & T Ichiye (1999) *Journal of Physical Chemistry B* **103**, 8006.

Becke AD (1988) *Physical Review A* **38**, 3098.

Berendsen HJC, JPM Postma, WF van Gunsteren, A DiNola & JR Haak (1984) *Journal of Chemical Physics* **81**, 3684–3690.

Berendsen HJC, JPM Postma, WF van Gunsteren & J Hermans (1981) Interaction models for water in relation to protein hydration. In *Intermolecular forces* (Pullman B, ed), D. Reidel Publishing Company, Dordrecht, pp 331–342.

Berendsen HJC, D van der Spoel & R van Drunen (1995) *Computer Physics Communications* **91**, 43.

Berman HM, J Westbrook, Z Feng, G Gilliland, TN Bhat, H Weissig, IN Shindyalov & PE Bourne (2000) *Nucleic Acids Research* **28**, 235.

Bizzarri AR (2004) *Journal of Physics-Condensed Matter* **16**, R83.

Bol JE, C Buning, P Comba, J Reedijk & M Strohle (1998) *Journal of Computational Chemistry* **19**, 512.

Bond CS, DS Bendall, HC Freeman, JM Guss, CJ Howe, MJ Wagner & MC Wilce (1999) *Acta Crystallographica Section D-Biological Crystallography*, 414.

Bond CS, RE Blankenship, HC Freeman, JM Guss, M Maher, F Selvaraj, MCJ Wilce & K Willingham (2001) *Journal of Molecular Biology* **306**, 47.

Buning C & P Comba (2000) *European Journal of Inorganic Chemistry*, 1267.

Burton VJ & RJ Deeth (1995) *J CHEM SOC CHEM COMM* **5**, 573.

Burton VJ, RJ Deeth, CM Kemp & PJ Gilbert (1995) *Journal of the American Chemical Society* **117**, 8407.

Caldwell JW & PA Kollman (1995) *Journal of Physical Chemistry* **99**, 6208.

Carlsson AE & S Zapata (2001) *Biophysical Journal* **81**, 1.

Chowdhury A, LA Peteanu, MA Webb & GR Loppnow (2001) *Journal of Physical Chemistry B* **105**, 527.

Ciocchetti A, AR Bizzarri & S Cannistraro (1997) *Biophysical Chemistry* **69**, 185.

Cioni P, E de Waal, GW Canters & GB Strambini (2004) *Biophysical Journal* **86**, 1149.

Comba P & R Remenyi (2002) *Journal of Computational Chemistry* **23**, 697.

Coremans JWA, OG Poluektov, EJJ Groenen, GW Canters, H Nar & A Messerschmidt (1994) *Journal of the American Chemical Society* **116**, 3097.

Costa J, R Delgado, MGB Drew & V Felix (1998) *Journal of the Chemical Society-Dalton Transactions*, 1063.

Costa J, R Delgado, MGB Drew, V Felix, RT Henriques & JC Waerenborgh (1999) *Journal of the Chemical Society-Dalton Transactions*, 3253.

Czernuszewicz RS, J Legall, I Moura & TG Spiro (1986) *Inorganic Chemistry* **25**, 696.

Daura X, AE Mark & WF van Gunsteren (1998) *Journal of Computational Chemistry* **19**, 535.

de Kerpel JOA, K Pierloot & U Ryde (1999) *Journal of Physical Chemistry B* **103**, 8375.

de Kerpel JOA & U Ryde (1999) *Proteins-Structure Function and Genetics* **36**, 157.

De Rienzo F, RR Gabdoulline, MC Menziani & RC Wade (2000) *Protein Science* **9**, 1439.

Deeth RJ & MA Hitchman (1986) *Inorganic Chemistry* **25**, 1225.

den Blaauwen T & GW Canters (1993) *Journal of the American Chemical Society* **115**, 1121.

den Blaauwen T, CWG Hoitink, GW Canters, J Han, TM Loehr & J Sanders-Loehr (1993) *Biochemistry* **32**, 12455.

den Blaauwen T, M van de Kamp & GW Canters (1991) *Journal of the American Chemical Society* **113**, 5050.

Dorigo AE & KN Houk (1988) *Journal of Organic Chemistry* **53**, 1650.

Durley R, L Chen, LW Lim, FS Mathews & VL Davidson (1993) *Protein Science* **2**, 739.

Falconi M, ME Stroppolo, P Cioni, G Strambini, A Sergi, M Ferrario & A Desideri (2001) *Biophysical Journal* **80**, 2556.

Farver O, LJC Jeuken, GW Canters & I Pecht (2000) *European Journal of Biochemistry* **267**, 3123.

Feenstra KA, B Hess & HJC Berendsen (1999) *Journal of Computational Chemistry* **20**, 786.

Fields BA, JM Guss & HC Freeman (1991) *Journal of Molecular Biology* **222**, 1053.

Formaneck MS, G Li, X Zhang & Q Cui (2002) *Journal of Theoretical and Computational Chemistry* **1**, 53.

Fusetti F, KH Schroter, RA Steiner, PI van Noort, T Pijning, HJ Rozeboom, KH Kalk, MR Egmond & BW Dijkstra (2002) *Structure* **10**, 259.

Gajewski JJ, KE Gilbert & TW Kreek (1998) *Journal of Computational Chemistry* **19**, 1167.

Gerloch M, JH Harding & RG Woolley (1981) *The context and application of ligand field theory*, Berlin.

Gilardi G, G Mei, N Rosato, GW Canters & A Finazzi-Agro (1994) *Biochemistry* **33**, 1425.

Groeneveld CM, MC Feiters, SS Hasnain, J van Rijn, J Reedijk & GW Canters (1986) *Biochimica et Biophysica Acta* **873**, 214.

Gross EL & DC Pearson (2003) *Biophysical Journal* **85**, 2055.

Guckert JA, MD Lowery & EI Solomon (1995) *Journal of the American Chemical Society* **117**, 2817.

Guex N & MC Peitsch (1997) *Electrophoresis* **18**, 2714.

Guss JM, EA Merritt, RP Phizackerley & HC Freeman (1996) *Journal of Molecular Biology* **262**, 686.

Halgren TA (1990) *Journal of the Chemical Society* **112**, 4710.

Hammann C, A Messerschmidt, R Huber, H Nar, G Gilardi & GW Canters (1996) *Journal of Molecular Biology* **255**, 362.

Hammann C, G van Pouderoyen, H Nar, FXG Ruth, A Messerschmidt, R Huber, T den Blaauwen & GW Canters (1997) *Journal of Molecular Biology* **266**, 357.

Han S, RS Czernuszewicz, T Kimura, MWW Adams & TG Spiro (1989) *Journal of the American Chemical Society* **111**, 3505.

Hart PJ, AM Nersissian, RG Herrmann, RM Nalbandyan, JS Valentine & D Eisenberg (1996) *Protein Science* **5**, 2175.

Hashimoto K & K Morokuma (1994) *Journal of the American Chemical Society* **116**, 11436.

Heinz TN, WF van Gunsteren & PH Hunenberger (2001) *Journal of Chemical Physics* **115**, 1125.

Hendrix DA & C Jarzynski (2001) *Journal of Chemical Physics* **114**, 5974.

Hess B, H Bekker, HJC Berendsen & JGEM Fraaije (1997) *Journal of Computational Chemistry* **18**, 1463.

Hogervorst W (1971) *Physica* **51**, 77.

Hoitink CWG & GW Canters (1992) *Journal of Biological Chemistry* **267**, 13836.

Hoitink CWG, PC Driscoll, HAO Hill & GW Canters (1994) *Biochemistry* **33**, 3560.

Holm RH, P Kennepohl & EI Solomon (1996) *Chemical Reviews* **96**, 2239.

Hunenberger PH, AE Mark & WF van Gunsteren (1995) *Journal of Molecular Biology* **252**, 492.

Hush NS (1967) *Prog Inorg Chem* **8**, 391.

Jensen F (1999) *Introduction to Computational Chemistry*, Wiley & Sons, New York.

Jeuken LJC, M Ubbink, JH Bitter, P van Vliet, W Meyer-Klaucke & GW Canters (2000a) *Journal Molecular Biology* **199**, 737.

Jeuken LJC, P van Vliet, MP Verbeet, R Camba, JP McEvoy, FK Armstrong & GW Canters (2000b) *Journal of the American Chemical Society* **122**, 12186.

Kabsch W & C Sander (1983) *Biopolymers* **22**, 2577.

Kaitner B, N Paulic, G Pavlovic & J Sabolovic (1999) *Polyhedron* **18**, 2301.

Kalverda AP, M Ubbink, G Gilardi, SS Wijmenga, A Crawford, LJC Jeuken & GW Canters (1999) *Biochemistry* **38**, 12690.

Karlsson BG, LC Tsai, H Nar, J SandersLoehr, N Bonander, V Langer & L Sjolin (1997) *Biochemistry* **36**, 4089.

Kirkwood JG (1935) *Journal of Chemical Physics* **3**, 300.

Kisternmacher H, H Popkie & E Clementi (1973) *Journal of Chemical Physics* **59**, 5842.

Koch W & MC Holthausen (2001) *A chemist's guide to density functional theory*, Wiley-VHC, Weinhem.

Kolczak U, C Dennison, A Messerschmidt & GW Canters (2001) *Handbook of Metalloproteins* (Messerschmidt A, R Huber, T Poulos & K Wieghardt eds), Wiley, Chichester.

Konecny R, J Li, CL Fisher, V Dillet, B D. & L Noodleman (1998) *Inorganic Chemistry* **38**, 940.

Kroes SJ, GW Canters, G Gilardi, A van Hoek & AJWG Visser (1998) *Biophysical Journal* **75**, 2441.

Li GH, XD Zhang & Q Cui (2003) *Journal of Physical Chemistry B* **107**, 8643.

Li H, SP Webb, J Ivanic & JH Jensen (2004) *Journal of the American Chemical Society* **126**, 8010.

Libeu CAP, M Kukimoto, M Nishiyama, S Horinouchi & ET Adman (1997) *Biochemistry* **36**, 13160.

Lindahl E, B Hess & D van der Spoel (2001) *Journal of Molecular Modeling* **7**, 306.

Liu HB, HN Zhu, DK Eggers, AM Nersissian, KF Faull, JJ Goto, JY Ai, J Sanders-Loehr, EB Gralla & JS Valentine (2000) *Biochemistry* **39**, 8125.

Lu N, DA Kofke & TB Woolf (2003) *Journal of Physical Chemistry B* **107**, 5598.

Lu ND & DA Kofke (2001a) *Journal of Chemical Physics* **114**, 7303.

Lu ND & DA Kofke (2001b) *Journal of Chemical Physics* **115**, 6866.

Lu ND, DA Kofke & TB Woolf (2004) *Journal of Computational Chemistry* **25**, 28.

Lybrand TP, I Ghosh & JA McCammon (1985) *Journal of the American Chemical Society* **107**, 7793.

Lybrand TP & PA Kollman (1985) *Journal of Chemical Physics* **83**, 2923.

Maret W, AK Shiemke, WD Wheeler, TM Loehr & J Sandersloehr (1986) *Journal of the American Chemical Society* **108**, 6351.

Mark AE & WF van Gunsteren (1994) *Journal of Molecular Biology* **240**, 167.

Mark AE, WF van Gunsteren & HJC Berendsen (1991) *Journal of Chemical Physics* **94**, 3808.

Marrone TJ & KM Merz (1992) *Journal of the American Chemical Society* **114**, 7542.

Marrone TJ & KM Merz (1993) *Journal of Physical Chemistry* **97**, 6524.

Mayo SL, BD Olafson & WAI Goddard (1990) *Journal of Physical Chemistry* **94**, 8897.

Mei G, A Di Venere, FM Campeggi, G Gilardi, N Rosato, F De Matteis & A Finazzi-Agro (1999) *European Journal of Biochemistry* **265**, 619.

Mei G, G Gilardi, M Venanzi, N Rosato, GW Canters & AF Agro (1996) *Protein Science* **5**, 2248.

Melnikov VL & IV Pletnev (1997) *Russian Chemical Bulletin* **46**, 1221.

Merz KM (1991) *Journal of the American Chemical Society* **113**, 406.

Merz KM, MA Murcko & PA Kollman (1991) *Journal of the American Chemical Society* **113**, 4484.

Messerschmidt A, L Prade, SJ Kroes, J Sanders-Loehr, R Huber & GW Canters (1998) *PNAS* **95**, 3443.

Mezei M & DL Beveridge (1986) *Annals of the New York Academy of Science* **482**, 1.

Miyamoto S & PA Kollman (1992) *Journal of Computational Chemistry* **13**, 952–962.

Moon S, S Patchkovskii & DR Salahub (2003) *Journal of Molecular Structure-Theochem* **632**, 287.

Murphy ME, S Turley, M Kukimoto, M Nishiyama, S Horinouchi, H Sasaki, M Tanokura & ET Adman (1995) *Biochemistry* **34**, 12107.

Nar H, A Messerschmidt, R Huber, Mvd Kamp & GW Canters (1991) *Journal of Molecular Biology* **221**, 765.

Nar H, A Messerschmidt, R Huber, M van de Kamp & GW Canters (1992) *Federation of European Biochemical Societies Letters* **306**, 119.

Norrby PO & P Brandt (2001) *Coordination Chemistry Reviews* **212**, 79.

O'Keeffe M & NE Brese (1991) *Journal of the American Chemical Society* **113**, 3226.

Oka T, FJ Simpson & HG Krishnam (1972) *Canadian Journal Of Microbiology* **18**, 493.

Olsson MHM, GY Hong & A Warshel (2003) *Journal of the American Chemical Society* **125**, 5025.

Olsson MHM, U Ryde, BO Roos & K Pierloot (1998) *Journal of Biological Inorganic Chemistry* **3**, 109.

Oostenbrink BC, JW Pitera, MMH van Lipzig, JHN Meerman & WF van Gunsteren (2000) *Journal of Medicinal Chemistry* **43**, 4594.

Pappalardo M, D Milardi, DM Grasso & C La Rosa (2003) *Journal of Computational Chemistry* **24**, 779.

Park S, F Khalili-Araghi, E Tajkhorshid & K Schulten (2003) *Journal of Chemical Physics* **119**, 3559.

Peng ZW, CS Ewig, MJ Hwang, M Waldman & AT Hagler (1997) *Journal of Physical Chemistry A* **101**, 7243.

Perdew JP (1986a) *Physical Review B* **34**, 7406.

Perdew JP (1986b) *Physical Review B* **33**, 8822.

Popovic DM, SD Zaric, B Rabenstein & EW Knapp (2001) *Journal of the American Chemical Society* **123**, 6040.

Postma JPM, HJC Berendsen & JR Haak (1982) *Faraday Symposia of the Chemical Society*, 55.

Qiu D, S Dasgupta, PM Kozlowski, WA Goddard & TG Spiro (1998) *Journal of the American Chemical Society* **120**, 12791.

Rappe AK, CJ Casewit, KS Colwell, WA Goddard & WM Skiff (1992) *Journal of the American Chemical Society* **114**, 10024.

Rappe AK, KS Colwell & CJ Casewit (1993) *Inorganic Chemistry* **32**, 3438.

Rappe AK & WA Goddard (1991) *Journal of Physical Chemistry* **95**, 3358.

Reichert DE, PO Norrby & MJ Welch (2001) *Inorganic Chemistry* **40**, 5223.

Rick SW (2001) *Journal of Chemical Physics* **114**, 2276.

Rizzuti B, M Swart, L Sportelli & R Guzzi (2004) *Journal of Molecular Modeling* **10**, 25.

Romero A, CWG Hoitink, H Nar, R Huber, A Messerschmidt & GW Canters (1993) *Journal of Molecular Biology* **229**, 1007.

Ryckaert JP, G Ciccotti & HJC Berendsen (1977) *Journal Of Computational Physics* **23**, 327.

Ryde U (1995) *Proteins* **21**, 40.

Ryde U (2003) *Current Opinion in Chemical Biology* **7**, 136.

Sanders RT (1983) *Polar Covalence*, Academic Press, New York.

Schuler LD & WF van Gunsteren (2000) *Molecular Simulation* **25**, 301.

Seminario JM (1996) *International Journal of Quantum Chemistry* **60**, 59.

Shen J, CF Wong, S Subramaniam, TA Albright & JA McCammon (1990) *Journal of Computational Chemistry* **11**, 346.

Shepard WEB, BF Anderson, DA Lewandoski, GE Norris & EN Baker (1990) *Journal of the American Chemical Society* **112**, 7817.

Sjolin L, LC Tsai, V Langer, T Pascher, G Karlsson, M Nordling & H Nar (1993) *Acta Crystallographica Section D-Biological Crystallography* **49**, 449.

Solomon EI, MJ Baldwin & MD Lowery (1992) *Chemical Reviews* **92**, 521.

Solomon EI, KW Penfield, AA Gewirth, MD Lowery, SE Shadle, JA Guckert & LB LaCroix (1996) *Inorganica Chimica Acta* **243**, 67.

St Clair CS, WR Ellis & HB Gray (1992) *Inorganica Chimica Acta* **199**, 149.

Steiner RA, KH Kalk & BW Dijkstra (2002) *PNAS* **99**, 16625.

Straatsma TP & HJC Berendsen (1988) *Journal of Chemical Physics* **89**, 5876.

Subramanian V, C Shankaranarayanan, BU Nair, M Kanthimathi, R Manickkavachagam & T Ramasami (1997) *Chemical Physics Letters* **274**, 275.

Swart M, Mvd Bosch, HJC Berendsen, GW Canters & JG Snijders (in preparation).

Swart M, PTv Duijnen & JG Snijders (2001) *Journal of Computational Chemistry* **22**, 79.

te Velde GT, FM Bickelhaupt, EJ Baerends, CF Guerra, SJA Van Gisbergen, JG Snijders & T Ziegler (2001) *Journal of Computational Chemistry* **22**, 931.

Toth G (1996) *Journal of Chemical Physics* **105**, 5518.

Tsai LC, N Bonander, K Harata, G Karlsson, T Vanngard, V Langer & L Sjolin (1996) *Acta Crystallographica Section D-Biological Crystallography* **52**, 950.

Ullmann GM (1998) *PhD Thesis: Simulation and analysis of docking and molecular dynamics of electron-transfer protein complexes*, Free University Berlin.

Ungar LW, NF Scherer & GA Voth (1997) *Biophysical Journal* **72**, 5.

Vakoufari E, KS Wilson & K Petratos (1994) *Federation of European Biochemical Societies Letters* **347**, 203.

van Amsterdam IMC, M Ubbink, GW Canters & M Huber (2003) *Angewandte Chemie-International Edition* **42**, 62.

van Amsterdam IMC, M Ubbink, O Einsle, A Messerschmidt, A Merli, D Cavazzini, GL Rossi & GW Canters (2002a) *Nature Structural Biology* **9**, 48.

van Amsterdam IMC, M Ubbink, M van den Bosch, F Rotsaert, J Sanders-Loehr & GW Canters (2002b) *Journal of Biological Chemistry* **277**, 44121.

van de Kamp M, CW Canters, CR Andrew, J Sanders-Loehr, CJ Bender & J Peisach (1993) *European Journal of Biochemistry* **218**, 229.

van de Kamp M, GW Canters, SS Wijmenga, A Lommen, CW Hilbers, H Nar, A Messerschmidt & R Huber (1992) *Biochemistry* **31**, 10194.

van der Spoel D, B Hess, KA Feenstra, E Lindahl & HJC Berendsen (1999a) *GROMACS user manual version 2.0*, Internet: http://www.gromacs.org, Nijenborgh 4, 9747 AG Groningen, The Netherlands.

van der Spoel D, AR van Buuren, A Apol, PJ Meulenhoff, DP Tieleman, ALTM Sijbers, B Hess, KA Feenstra, E Lindahl, R van Drunen *et al.* (1999b) *Gromacs user manual*, Bioson, Groningen.

van Gunsteren WF (1989) *Computer simulation of biomolecular systems, Theoretical and experimental applications.* (van Gunsteren WF & PK Weiner eds), Escom Science Publications, Leiden.

van Gunsteren WF & HJC Berendsen (1977) *Molecular Physics* **34**, 1311.

van Gunsteren WF, TC Beutler, F Fraternali, PM King, AE Mark & PE Smith (1993) *Computer simulation of biomolecular systems, Theoretical and experimental applications.* (van Gunsteren WF, PK Weiner & AJ Wilkinson eds), Escom, Leiden.

van Gunsteren WF, SR Billeter, AA Eising, PH Hünenberger, P Krüger, AE Mark, WRP Scott & IG Tironi (1996) *Biomolecular simulation: The GROMOS96 manual and user guide*, BIOMOS b.v., Zürich.

van Gunsteren WF, X Daura & AE Mark (2002) *Helvetica Chimica Acta* **85**, 3113.

van Pouderoyen G, T den Blaauwen, J Reedijk & GW Canters (1996) *Biochemistry* **35**, 13205.

Vanderkooi JM (1991) *Tryptophan phospshorescence from proteins at room temperature. In:Topics in fluorescence spectroscopy* (Lakowicz JR ed, Plenum publishing, New York.

Wachter RM & BP Branchaud (1996) *Journal of the American Chemical Society* **118**, 2782.

Walter RL, SE Ealick, AM Friedman, RC Blake II, P Proctor & M Shoham (1996) *Journal of Molecular Biology* **263**, 730.

Wang CX, AR Bizzarri, YW Xu & S Cannistraro (1994) *Chemical Physics* **183**, 155.

Werst MM, CE Davoust & BM Hoffman (1991) *Journal of the American Chemical Society* **113**, 1533.

Whitham CJ, H Ozeki & S Saito (2000) *Journal of Chemical Physics* **112**, 641.

Yang W, R Bitetti-Putzer & M Karplus (2004) *Journal of Chemical Physics* **120**, 2618.

Yu HB, T Hansson & WF van Gunsteren (2003) *Journal of Chemical Physics* **118**, 221.

Yunyu S, AE Mark, CX Wang, FH Huang, HJC Berendsen & WF van Gunsteren (1993) *Protein Engineering* **6**, 289.

Zwanzig RW (1954) *Journal of Chemical Physics* **22**, 1420.