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Modelling copper-containing proteins

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Citation
Bosch, M. van den. (2006, January 18). *Modelling copper-containing proteins*. Retrieved from https://hdl.handle.net/1887/4361

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/4361

Note: To cite this publication please use the final published version (if applicable).

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

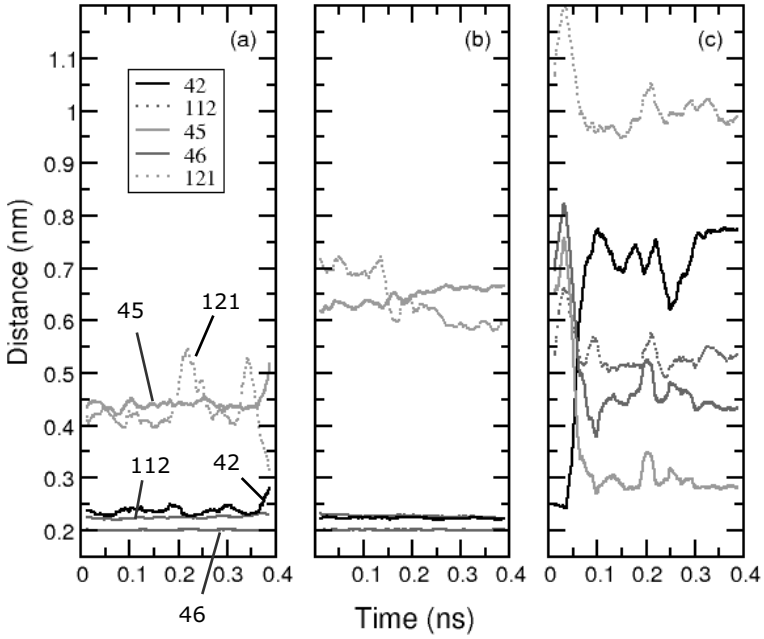

Figure 7.1: Stability analysis of the Cu-site of N42C/H117G azurin after restraining (a) four, (b) three or (c) two ligands to copper.

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.

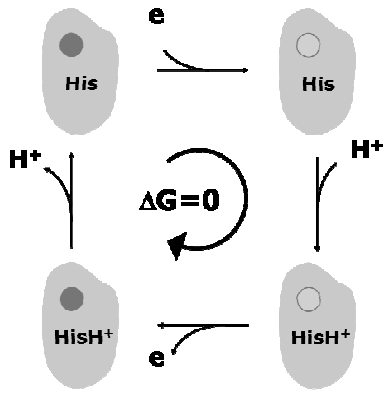

Figure 7.2: Schematic presentation of the free energy calculations.

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

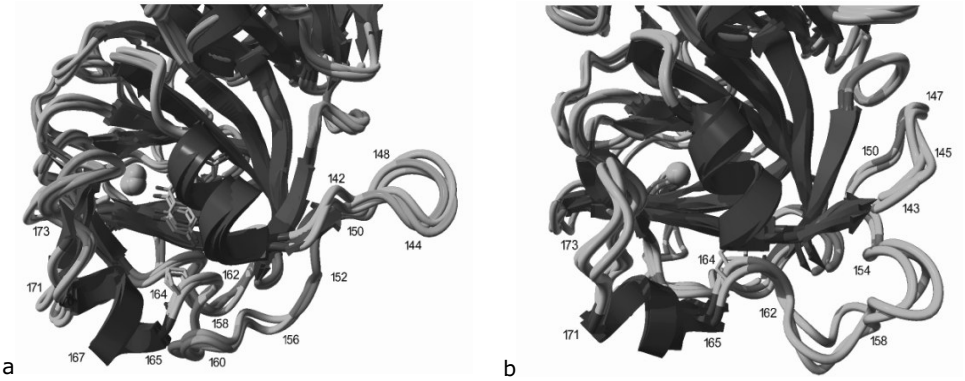

Figure 7.3 Three superimposed snapshots after 6 ns (a) in the presence and (b) in the absence of KMP.

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.

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