

The enterobactin siderophore



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Table of Contents

1. Introduction
 2. General references and information
 - 2.1. Siderophores
 - 2.2. Possible theoretical studies for Fe-Enterobactin
 3. Available crystal structures
 - 3.1. FepA - Enterobactin complex
 - 3.2. Enterobactin alone
 4. Modeling enterobactin and its iron complex
 - General electronic configuration
 - Nice pictures (made with Spartan)
 - Related structures (eg to compare Fe-O distances)
 - Force field issues
 - Evaluating a given Fe-enterobactin model
 - Adopted Fe-enterobactin model
 5. Docking enterobactin into FepA
 - 5.1. First tests with docking
 - 5.2. Manual 'docking'
 - 5.3. Setup of an MD simulation from the manually docked structure.
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1. Introduction

Modeling and theoretical investigation of the enterobactin siderophore and its iron complex.

2. General references and information

2.1. Siderophores

Generalities

At physiological pH, free $[Fe^{3+}]$ is limited to 10^{-18} M, [Neilands et al, 1980, High Affinity iron transport in microorganisms ACS Symp Ser 140:264-278], whereas virtually all living microorganisms require a minimum effective concentration of 10^{-8} M for growth. [Braun & N.Hantke, 1997; Klebba et al, 1982 JBact]

There are four groups of siderophores based on the chemical nature of the chelating ligands:

- catecholates
- hydroxamates
- hydroxypyridonates
- aminocarboxylates

The K_d is in the range of 0.1 to 100 nM, whereas ferric-enterobactin binds avidly to FepA with $K_d < 0.1$ nM [Newton et al, 1999]

Ferric enterobactin

- formal formation constant K_f of 10^{49}
- pM value of 35.5 at pH 7.4

The catechoylamide region of the Fe-ent complex is recognized by FepA.

FeEnt-FepA binding reaction:

- NOT stereospecific
- intolerant of modifications to the catechol groups surrounding the metal
- affinity relatively invariant among diverse bacterial species

FeEnt transport: is stereospecific for the right-handed chirality (Δ -cis-FeEnt) coordination propeller. Chiral specificity resides in a subsequent stage of the uptake

process, likely after transport through FepA

The monomer transport rate estimated is

- 1 mol/20 s for chromosomally produced FepA
- 1 mol/min for plasmid-expressed FepA

Iron release may take place via stepwise protonation of FeEnt towards a neutral complex with salicylate binding mode [Cohen et al, JACS, 1998]

2.2. Possible theoretical studies for Fe-Enterobactin

From Xiao et al JOC 1996 100 2345:

- Evaluate (lack of?) preorganization of the ligand by simulating the process protonated free ligand -> chelated ligand by MM
- evaluate intramolecular H-bonding between amide groups and chelating oxygens by replacing the amide hydrogen atom by a methyl group

BUT bear in mind, that when comparing 2 states, appropriate topologies and charges are needed. Especially the charges may be problematic !

3. Available crystal structures

3.1. FepA - Enterobactin complex

Susan Buchanan was kind enough to send the original electron density map from the final refinement and also the anomalous difference density map for the Fe signals. The file (Unix format) should be readable in O, and you can superimpose a C-alpha trace of FepA to identify the two densities attributed to Fe (compare to the figure in my NSB paper).

(Susan Buchanan) The deposited coordinates are actually of the mixed-state structure, as described in the NSB paper. We grew crystals in the absence of Fe-ent and then soaked the ligand in once the crystals were fully grown. With these crystals, we could detect an anomalous Fe signal at the appropriate wavelength but the density was never good enough to build Fe-ent into the map.

If it helps to have the coordinates of the two putative Fe sites from the anomalous difference density, they are 0.33120 0.25000 0.99038 and 0.36808 0.225000 0.42203 (fractional coordinates).

Visualizing the difference map with o

```
s_a_i 1FEP.pdb 1fep
mol 1FEP
object 1fep zone ; end
object ca ca_zone ; end
cen_xyz 10 10 10
cen_atom a142 ca
cen_atom a483 ca
fm_file fe.map fediff
fm_setup fediff 30 solid 1 1.0 slate_blue
fm_draw fediff
```

Add a water as fake iron and place it according to the density. Then save as new pdb.

```
wat_init fe 5
wat_add
move_atom yes yes
s_a_o 1fe.pdb fe
save_db
```

Other O commands

```
dele_obj
cen_next atom_mol fe
cen_atom $501 o
object fe zone $500 $501
sym_setup
symm_obj ca
```

3.2. Enterobactin alone

Using the CDS database (ssh cqxb@cds) we search for all enterobactin related structures. Startup procedure is

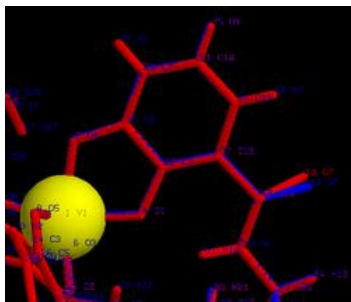
```
ssh cqxb@cds
setenv DISPLAY fepa.biop.ox.ac.uk:1.0
conquest
```

JOSLOS JOSLOS01

Dipotassium enterobactin-vanadium(iv) dimethylformamide solvate
C30[H21]N3[O15]V2-, 2(K+), 3(C3[H7]N[O])

1. T.B.Karpishin, K.N.Raymond, Angew.Chem.,Int.Ed.Engl., 1992, 31, 466
2. T.B.Karpishin, T.M.Dewey, K.N.Raymond, J.Am.Chem.Soc., 1993, 115, 1842

There is a difference in the position of oxygen number 10 ! Due to symmetry the structure from JOSLOS.ebact.pdb (red) should be preferred. Both can be distinguished via the O-C-N angle, which is 121.8 in the 'better' structure and around 133 in JOSLOS01 (blue).



4. Modeling enterobactin and its iron complex

We use the JOSLOS crystal structure of the Dipotassium enterobactin-vanadium(iv) dimethylformamide solvate from the CDS database. The dimethylformamide and dipotassium are deleted and vanadium is replaced by iron.

The complex has a -3 charge. It is a high-spin complex ($S=5/2$) as indicated by EPR data (Feix, Biochem. 37, 1998, 9016 and formerly Pecoraro, JACS, 105, 1983,4617). It thus has a multiplicity of 6. Initially we were not sure about the multiplicity, but there had to be at least one unpaired electron. We thus tested the energies for doublet, quartet or sextuplet with a single point calculation at STO-3G level.

Note: There was a bug in Spartan, now corrected, which made the job crash (related to UHF).

General electronic configuration

atom	number	configuration	valence	allval	total	allel
C	30	[He] 2s2 2p2	30 x 4	120	30 x 6	180
N	3	[He] 2s2 2p3	3 x 5	15	3 x 7	21
O	15	[He] 2s2 2p4	15 x 6	90	15 x 8	120
H	21	1s	21 x 1	21	21 x 1	21
Fe3+	1	[Ar] 3d5 4s0	1 x 5	5	1 x 23	23
6 e-	-	-	6 x 1	6	6 x 1	6
all	70	-	-	251	-	371

Electronic configuration of iron

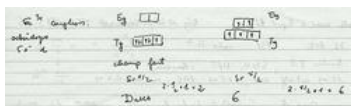
Iron has 26 electrons

Fe $1s^2 2s^2 2p^6 3s^2 3p^6 3d^6 4s^2$
 Fe (II) " " " " " " $3d^6$
 Fe (III) " " " " " " $3d^5$

Deoxy Hb Fe (II) high spin: spin 2

Oxy Hb Fe (II) low spin: spin 0

And this is a draft made after discussion with former colleagues



(Alain CHAUMONT) Si tu as un complexe de Fe^{3+} octaédrique t'as 5e d. Tes niveau orbitaire sont Tg et Eg. Maintenant il s'agit de savoir si ton ligand entraine un champ faible ou un champ fort, si il est a champ fort l'energie d'appariement sera plus faible que l'energie entre le niveau Tg et Eg et tu placera tes electrons dans les trois orbitales de symetrie T, ce qui te fera $S=1/2$, si ton ligand et a champ faible tu placeras trois electrons dans les orbitales Tg et 2 electrons dans les orbitales Eg, ce qui te fera une multiplicité de spin de 5/2. Donc tout consiste de savoir l'effet de ton ligand.

There is experimental evidence for FeEnt being a high-spin complex ($S=5/2$), see eg Klug et al, Biochemistry, 37, 1998, 9018 (EPR/ESR measurements) and also Pecoraro et al, JACS 1983, 105, 4617.

Our system is an OPEN-SHELL system !

(Leach, p108) The Roothaan-Hall equations are not applicable to open-shell systems, which contain one or more unpaired electrons ! Two approaches have been devised to treat open-shell systems:

- **spin-restricted Hartree-Fock (RHF)** theory uses combinations of singly and doubly occupied molecular orbitals
- **spin-unrestricted Hartree-Fock (UHF)** theory of Pople and Nesbet using two disting sets of molecular orbitals for electrons of alpha/ beta spin.

To find out which multiplicity is preferred we calculate doublet and sextuplet and compare the energies:

Calculation	struct ?	xray struct
UHF_STO-3G_m2	-3651.27719057	-3651.18998984
UHF_STO-3G_m4	-3651.36701408	-3651.44279417
UHF_STO-3G_m6	-3651.49580410	-3640.56803363
UHF_631Gs_m6	-	-3696.57316255

Well, difficult to say wether this confirms or not the preference for the high-spin state. But I think that this kind of problem strongly depends on the basis set and thus STO-3G is not a good choice. But calculation time and convergence problems preclude a higher level of theory. So let's use the xray structure and look at the high-spin complex.

Some remarks for ESP calculations with Gaussian

Fe charges cannot be calculated by default, as a van der Waals radius needed for this is missing.

GetVDW: no radius for atom 1 atomic number 26.

3 sets of radii are present in Gaussian 98: Merz-Kollman atomic radii, Francl (CHELP) atomic radii and Breneman (CHELPG) radii

Atomnb	AtTyp	Merz-Kollman	Francl (CHELP)	Breneman (CHELPG)
0	-	0.00	0.00	0.00
1	H	1.20	1.19	1.45
2	He	1.20	1.19	1.45
3	Li	1.37		1.50
4	Be	1.45		1.50
5	B	1.45		1.50
6	C	1.50	1.81	1.50
7	N	1.50	1.67	1.70
8	O	1.40	1.55	1.70
9	F	1.35	1.42	1.70
10	Ne	1.30		1.70
11	Na	1.57		2.00
12	Mg	1.36		2.00
13	Al	1.24		2.00
14	Si	1.17	2.00	2.00
15	P	1.80	2.05	2.00

16	S	1.75	2.11	2.00
17	Cl	1.70	1.93	2.00
18	Ar			2.00
19	K			
20	Ca			
21	Sc			
22	Ti			
23	V			
24	Cr			
25	Mn			
26	Fe			
27	Co			
28	Ni			
29	Cu			
30	Zn	1.00		
31	Ga			
32	Ge			
33	As			
34	Se			
35	Br	2.30		
36	Kr			

For iron there are several ionic radii. Let's use radii from <http://www.webelements.com/webelements/elements/text/Fe/radii.html>.

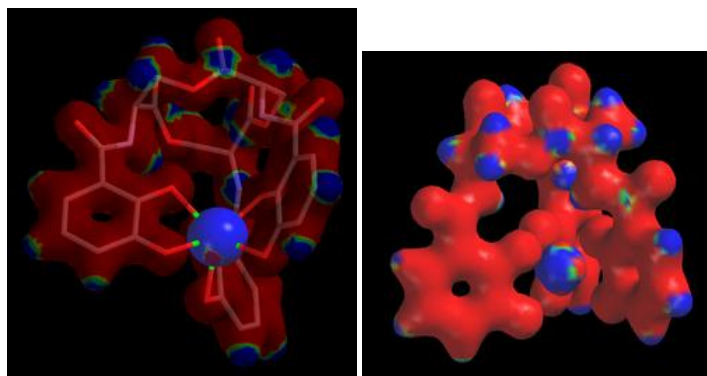
- Fe(III) 6-coordinate, octahedral 69 pm
- Fe(III) 6-coordinate, octahedral, high spin 78.5 pm

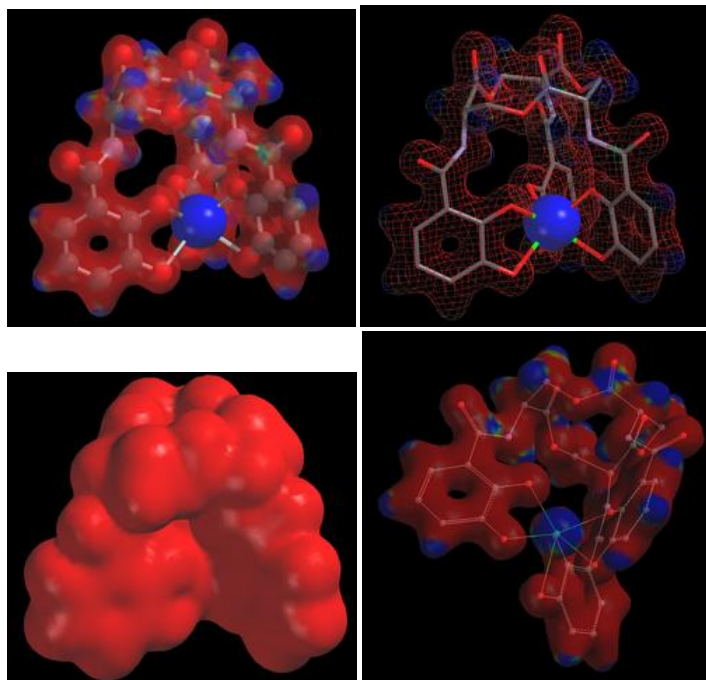
Note: The "effective ionic radii" quoted here assume that the ionic radius of F⁻ is 133 pm and that of O²⁻ is 140 pm. The values for iron thus correspond quite well to the tabulated Gaussian Merz-Kollman radii, and will be scaled for Francl and Breneman, with a mean factor for F⁻ and O²⁻. Values depend upon the coordination number and for d-block metals on the fact whether or not the metal is in a high or low spin state.

Atomnb	AtTyp	Merz-Kollman	Francl (CHELP)	Breneman (CHELPG)
26	Fe 6-coordinate, octahedral	0.69	0.75	0.85
26	Fe 6-coordinate, octahedral, high spin	0.785	0.85	0.97

Nice pictures (made with Spartan)

- the electrostatic potential units are in kcal/mol, how does that compare to kT/e ? It goes $1 \text{ kT} / e == 10.4 \text{ kcal/mol} / e$ (at 300 K)





Related structures (eg to compare Fe-O distances)

1	2	3
DAGZUG	DAGZOA	SABKIP PEXJUX

Structure	Reference	Fe-O dist
DAGZUG	R. C. Scarrow P. E. Riley K. Abu-Dari D. L. White K. N. Raymond , Inorganic Chemistry, 24 (1985) p954	0.20160 +- 0.00003
DAGZOA	R. C. Scarrow P. E. Riley K. Abu-Dari D. L. White K. N. Raymond , Inorganic Chemistry, 24 (1985) p954	0.20088 +- 0.00003
SABKIP	R. C. Scarrow K. N. Raymond , Inorganic Chemistry, 27 (1988) p4140	0.202 +- 0.003
PEXJUX	P. S. Dobbin R. C. Hider A. D. Hall P. D. Taylor P. Sarpong J. B. Porter G. Xiao D. van der Helm , Journal of Medicinal Chemistry, 36 (1993) p2448	0.202 +- 0.002

Force field issues

PRODRG was used to generate the topology for the enterobactin Ent6- chore. Standard gromacs ffgmx parameters were used. Iron parameters in the force field do not include Lennard-Jones terms, so another approach has to be taken if one wants to leave the iron in a semi-ionic state with existing van der Waals interactions.

Iron nonbonded parameters

4 ions are parametrised in the Gromacs forcefield: Na⁺, Zn²⁺, Ca²⁺, Mg²⁺ Those parameters were used in an initial test to assess their quality with respect to the [FeEnt]3- complex. We used the Fe-O distances as a measure.

Distance	Crystal	NA	ZN	CA	MG
Fe-O1a	1.94	2.50	2.50	2.60	2.57
Fe-O1b	1.93	2.48	2.54	2.61	2.47
Fe-O1c	1.94	2.61	2.71	2.62	2.94
Fe-O2a	1.93	2.08	2.03	2.39	1.90
Fe-O2b	1.94	2.08	2.03	2.39	1.90
Fe-O2c	1.94	2.07	2.02	2.39	1.89

It is not very satisfactory that the Fe-O1 distances are 0.2 to 1.0 Angstrom longer than the Fe-O2 ones. The above tests should just be considered as a bad approach. Additional bonded interactions with the iron are needed to properly reproduce its coordination sphere. Still, the Ca parameters can be considered as case of minimum discrepancy between both sets of distances.

Further iron parameters can be obtained from Charmm (parm22), as to M. Mezei's conversion there is rmin=0.6500 and sigma=1.1582. **But this must be wrong !**. The Charmm parameters use epsilon=0 => the LJ term with iron is nil.

It seems that Xiao et al (JPC 1996, 100, 2345) have a set of parameters, too.

Set	original parameters	C6	C12
Xiao1	eps=0.013 kcal/mol r*=1.55A		

The coordinating oxygen parameters in the enterobactin topology are of type OA, with C6=0.22617E-02 and c12=0.15062E-05.

We performed test calculations using **exclusively** a non-bonded term and keeping the Fe3⁺ ionic. This does not well reproduce the specific characteristics of enterobactin (right-handed propeller, twisting of the catechol moieties, ..). In our tests we used the equilibrium Fe-O distance which was set to our average value from the crystal structures: 2.01 Å, and the well depth from Xiao et al is used.

For the final version we reverted to Xiao et al's standard values which are converted to C6=9.661081e-05 C12=4.287123e-08.

N-H .. O hydrogen bond (not modified)

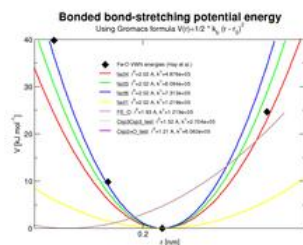
Hay et al give r*=1.68 Å and eps*=10.0 kcal/mol, but we did not modify it.

bonded interactions ?

Hay et al have

- Fe-O bond stretching r0=1.931 kb=2.024 mdyne/Å²
r0 was adjusted to 0.197 Å to better reproduce Fe-O distances
their unit does not make sense ! it should be mdyne/Å as stated in the supporting information. But anyway, trying to reproduce their fit with the VWN Qm energies, it seems that a factor of 5 is missing !! This could be a factor of 10 and then divided by 2 (as compared to Leach, force constants are about 2 * what they should be) - very confusing
- Fe-O-C-C dihedral V2=1.859 kcal/mol
believing Leach, one should have to divide this barrier by 2, but this doesn't yield the correct potential energy surface !

We have thus performed a trial-and-error fit of the potentials, by re-tracing the points given in Hay's supporting information and overlaying Gromacs potentials for several scaled LJ parameters.



Evaluating a given Fe-enterobactin model

Various

- Delta complex more stable than lambda one !
Hay: 2.06 kcal/mol; Shanzer: 0.5 kcal/mol; Karpishin: 6.93 kcal/mol

Data from Hay et al InorgChem 2001 40 3922

- experimental structural features of chelated 2,3-dioxy-N-alkylbenzamide
- O- - O bite distances
- N-H - O distances

Data analysis from the CSD

Fe-O distances

Analysis of 13 Fe-tri-catecholate crystal structures (39 distances) yields 2.01 +- .03

Searching for 6-coordinated iron(III) by O-donors yields 16 structures and an average distance of 2.005 +- 0.025

2 entries for iron(III)-OH₂ distances (coordinated !) are at 2.032 +- 0.004 Å.

Comparison of a [Fe(CAT)₃]³⁻ fragment ?

- structure
- octahedral inversion barrier

Adopted Fe-enterobactin model

Modification of the internal energy by added terms

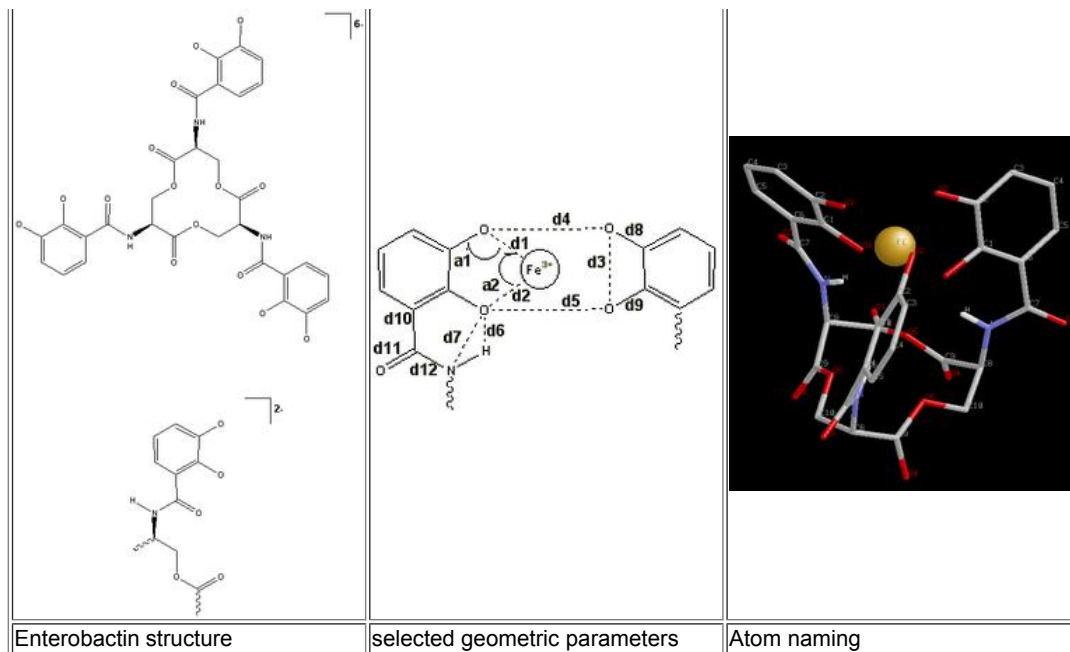
Term	turned off	turned on	Energy type
Fe-O Bond stretching	0.0	1.51890e+03	Harmonic Pot.
Fe-O-C Angle bending	2.46470e+01	6.45733e+01	Angle
Fe-O-C-C torsion	3.10095e+01	4.01679e+01	Proper Dih.
Fe nb (Xiao et al)	-9.15012e+01	-3.88789e+01	LJ (SR)

Note: energies in kJ/mol

Evaluation of the model

Note: energy minimization is problematic for some strange reason. Steepest descent leads to a 1-4 vdW warning and screws up completely. CG works more or less well, but crashes at some point with a strange error (negative sqrt...). Nevertheless no problems are observed for MD. Probably the combination of bonded and non-bonded parameters which keep the iron-coordination rigid are at the origin of this behaviour (?).

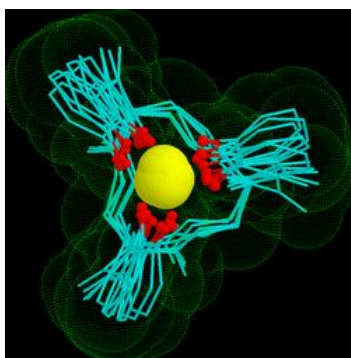
1	2	3
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Compare to experimental data given below

1	2	3	4	5	6	7	8	9	10	11	12	13
<d1,d2>	d3	<d4,d5>	d6	d7	d8	d9	d10	d11	d12	a1	a2	Ref
2.017(16)	2.626(8)	2.88(5)	-	-	-	-	-	-	-	112.2(7)	81.2(6)	Hay et al, [Fe(CAT)3]3-
-	2.55(5)	-	-	2.67(3)	1.34(1)	1.33(1)	1.49(2)	1.24(1)	1.34(2)	-	-	Hay et al, 2,3-dioxy-N-alkylbenzamide

A visual illustration of the enterobactin flexibility is given below.



5. Docking enterobactin into FepA

5.1. First tests with docking

To make some first steps on a related system we started from the FecA structure with the bound iron citrate (1KMP) and tried to reproduce the experimentally evidenced binding site.

Hex

Prepare PDBs for receptor, ligand and complex (remove waters, lipids, check chain ids). Several solutions < 1.4 Å RMS were found in a quick test.

5.2. Manual 'docking'

The electron density of the anomalous difference map from Se and Fe was displayed and the two potential iron sites located by eye. One of these sites is close to a loop and less marked, so we focused on the other site by refining a water at its position. The approximate coordinates are

32.706 15.430 28.649

Then the enterobactin complex was superposed onto the iron position and manually oriented in order to minimize steric constraints. The fepa structure used for this complex was from

[/sansom/gfip/baaden/fepa/002_eq_fepa_ss_cap_in_DMPC/6_prep_equi_pme/md_equil8.gro](#)

which is the starting structure for the PME production run. Then - with the iron atom fixed in space - we minimize first via steepest descent, with the protein frozen as well. Then conjugate gradients minimization is applied and the protein unfrozen. Several other steps are applied, but the system crashes in MD.

The next approach is to start from the same structure, but to re-hydrate it. A first test without neutralizing and caring about crystal waters shows that this leads to a system that can be run in MD. We only performed a very short testrun (16 ps), but it can be seen that the enterobactin moves and locks into place by distorting the protein in its vicinity. So it seems desirable to freeze iron position and protein in a preliminary equilibration.

5.3. Setup of an MD simulation from the manually docked structure.

We start the equilibration from a previous structure (as for PME vs cutoff equilibration, see

[/home/marc/lmb/staffs/005_prep_lipid_slabs/6_insert_in_DMPC/13_fepa_eq_PME](#) for details):

[/home/marc/lmb/staffs/005_prep_lipid_slabs/6_insert_in_DMPC/11_fepa_delwat_eq_prot1/fepa-delwat.gro](#)

- keep crystal waters
- add 3 Na⁺ to neutralize the system
- re-hydrate the system (keep ~ same nb of waters !)
- equilibrate while restraining iron pos, protein and crystal water (100 ps)
- relax crystal water, protein and iron pos stepwise 75 ps @ 1k, 50 ps @ 500, 250, 100 and 10
- start free run