

Bioc585 2008 Exercise 5 – Molecular Replacement
Homework due Tuesday, April 29

For this exercise, we will use three nitrophorins for molecular replacement structure solution. The nitrophorins from *Rhodnius prolixus* (the kissing bug) store nitric oxide (NO) at the low pH of the insect saliva, and release NO after injection into the tissue of an unsuspecting victim, in part in response to the higher pH. The NO leads to vasodilation and interferes with blood coagulation, which help the insect acquire a satisfying meal. The nitrophorins use a ferric heme and a large change in protein conformation for NO binding and release. The conformational change is centered on two loops, the A-B loop (residues 31-37) and the G-H loop (residues 125-133). Once in the victim, the proteins bind histamine at the site previously occupied by the NO molecule.

Shown below is a sequence alignment for the four *Rhodnius* nitrophorins, called NP1-4. We will attempt to solve the T121V NP4 mutant structure, with NO bound to the heme, using two different starting models. The first model is the histamine complex with NP1, PDB file 1NP1, which contains two subunits in the asymmetric unit and histamine bound to heme. NP1 and NP4 are 90% identical. The second model is NP2 with imidazole bound, PDB file 1PEE. NP2 and NP4 are 42% identical.

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      1          11          21          31          41          51          61          71          81
NP1 KCTKNALAQT GFNKDKYFNG DWVYVTDYLD LEPDDVPKRY CAALAAGTAS GKLKEALYHY DPKTQDTFYD VSELQEESPG -KYTANFKKV
NP4 A*TK*ALIAQT *FN*DD**N* DV****DY** LE*DD*PKR* *AALAAGTAS *KL*****Y DPKTQDT**D VS*LQVE*L* -K***NFKK*
NP2 D*ST*ISPKQ *LD*AK**S* -K****HF** KD*-Q*TDQ* *SSFTPRESD *TV*****Y NANKKTS**N IG*GKLE*S* LQ***KYKT*
NP3 D*ST*ISPKK *LD*AK**S* -T****HY** KD*-Q*TDP* *SSFTPKESG *TV*****F NSKKKTS**N IG*GKLG*L* VQ***KYNT*

      90          100          110          120          130          140          150          160          170          180
NP1 EKNGNVKVDV TSGNYTFTV MYADSSALI HTCLHKGKND LGDLYAVLNR NKDTNAGDKV KGAVTAASLK FSDFISTKDN KCEYDNVSLK SLLTK
NP4 D*NGNVKVAV TAGNY**F** MY*****I *T**HK*N** *****A**NR N*DAAAGDK* *SA*SA*TLE FSK*IS**EN N*A**NDSLK SLLTK
NP2 D*KKAVLKEA DEKNS**L** LE*****V *I**RE*S** *****T**TH Q*DAEPSAK* *SA*TQ*GLQ LSQ*VG**DL G*Q**DQFTS L
NP3 D*KRKEIEPA DPKDS**L** LE*****V *I**RE*P** *****T**SH Q*TGEPSAT* *NP*AQ*GLK LND*VD**TL S*T**DQFTS M

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Fig. 1. Sequence comparison of NP1–4. Numbering is for NP1 and NP4. Certain residues of functional interest are in bold type and identical residues are indicated with an *.

1. Initial Setup. COPY (don't drag!) the folder called MR from the Shared directory into your home directory. Start CCP4i and run 'Directories&ProjectDir'. Add a new project ('Add Project') called MR that uses your new MR directory in your home directory. Be sure to select the MR project for this session, and run 'Apply&Exit'.

2. Cell Content Analysis. Select 'Molecular Replacement' from the menu on the left of the CCP4i main window. Run 'Cell Content Analysis' using file NP4.mtz. The approximate molecular weight is 20,000 Da (184 x 107 Da/amino acid). Note the possible number of molecules per asymmetric unit (only one possibility listed in this case).

3. Molecular replacement with NP1. Edit 1NP1.pdb for use in molecular replacement, using a text editor such as gedit (Accessories > text editor). Be sure to include only one molecule for searching and remove all of the solvent atoms (why?).

4. Molrep. Run 'Molrep – auto MR'. We will use only the low resolution data for this (the high resolution data are only effective once the model is correctly placed). Select NP4.mtz and your edited model file. **SELECT 'Experimental Data' and limit the maximum resolution to 3.0.** Run the job, which should take 3-4 minutes, and examine the log file. Notice the top 10 rotation

solutions, and top translation solutions. Is there a single rotation solution highlighted, or are many potential solutions indicated in the output? (Examine 'Rf/sigma', which indicates the value of the rotation function at the given angles, divided by the error.) Is the R-factor for the top translation solution significantly better than the others listed?

5. Refinement of the initial model. Refine the output model against NP4.mtz, using Refmac. Limit the resolution to 1.5 Å so that the initial refinement is more robust. Examine the log graphs paying particular attention to the graphs for 'Rfactor vs cycle' and 'Geometry vs cycle'.

6. Model building. View the resulting model and electron density maps using Coot. Examine the mobile loops, the residues that differ between model and crystal, and the ligand binding site. Change one or more incorrect amino acids to the correct type, using the sequence alignment shown above, and refine the new model and examine the resulting map.

Homework (10 pts)

1. How many molecules are in the asymmetric unit of the NP4 crystal? What is the solvent content?
2. Show density for an NP1 incorrect amino acid, fix the residue, refine the new model with Refmac, and show the new density.
3. Examine the distal pocket. Is NO bound?
4. Examine the loops near the heme distal pocket. Are they the same or different in NP4? Justify your answer.
5. Extra credit question: 5 points. If you would like to experience a more difficult molecular replacement problem (but still relatively straightforward), repeat the assignment but using NP2 (1PEE.pdb) as a starting model. The lower homology makes the initial model less reliable, but by trimming away portions of the search model that disagree (e.g. mobile loops, incompatible amino acids, etc.) a molecular replacement solution can be found. For full credit, try one or more strategies and describe your results.