Holo-Ni^{2+} *Helicobacter pylori* NikR is a symmetric tetramer containing four square-planar nickel-binding sites at physiological pH

Stefano Benini[1], Michele Cianci[2], Stefano Ciurli[3*]

[1]*Faculty of Science and Technology, Free University of Bolzano, Bolzano, Italy*
[2]*European Molecular Biology Laboratory, Hamburg Outstation, Hamburg, Germany*
[3]*Laboratory of Bioinorganic Chemistry, University of Bologna, Bologna Italy*
*E-mail: stefano.ciurli@unibo.it

ELECTRONIC SUPPLEMENTARY INFORMATION

SECTION ESI-1: Protein crystallization

*Hp*NikR was expressed and purified in its apo-form as previously described,[1] with an additional hydrophobic interaction chromatography step. A 70 μM solution of purified apo-*Hp*NikR in 20 mM HEPES at pH 7.6 was incubated for ca. one hour with six equivalents of $NiSO_4$ in the same buffer. Excess nickel was removed by three cycles of concentration and dilution in the same buffer using Vivaspin 15 (MWCO 10 KDa) ultrafiltration cells. The protein was concentrated to 10 mg/mL and used for crystallization trials. Protein crystallization was carried out by microbatch under oil using 96 wells MRC plates (Cambridge, UK) and volatile oil. The crystallization wells were protected from drying using adhesive ClearView sheets (Molecular Dimensions). Drops of 1 μL holo-*Hp*NikR were added to 20 μL of volatile oil, immediately followed by 1 μL of precipitant from the crystallization kits (PACT premier HT-96 and JCSG-plus HT-96, Molecular Dimensions). The best crystals appeared after 24 hours in condition E9 of the PACT screen and were reproduced, using the same crystallization setup, by adding 1 μL of 20% PEG 3350 and 0.2 M K/Na-tartrate to 1 μL protein. Crystals of about 0.2 mm^3 were cryoprotected by adding 2 μL of a solution containing 40% PEG 3350 and 0.2 M K/Na-tartrate to the crystallization drop. After equilibration the crystals were fished out from the mother liquor by cryoloops and flash cooled into liquid nitrogen for storage, transport and data collection. The pH of the crystallization mixture (7.3) was measured on a larger volume of the solution.

SECTION ESI-2: X-ray diffraction data collection

Diffraction data were collected on two single crystals of native *Hp*NikR at 100 K using synchrotron radiation from the X12 beam line, equipped with MAR-CCD 225 detector, at the DORIS storage ring at the EMBL outstation, c/o DESY, Hamburg (Germany). The wavelength of 0.1459 nm was chosen to enhance the anomalous signal from the four putative Ni ions. Each single crystal, of dimensions 0.1 x 0.1 x 0.5 mm^3, was cryo-protected, scooped up in a cryo-loop and rapidly exposed to a cold nitrogen stream at 100 K. The data were processed using the program XDS [2] and scaled with SCALA [3]. Crystals diffracted to 2.39 Å resolution with a unit cell dimensions a = 72.97 Å, b = 72.97 Å, and c = 116.73 Å and space group $P4_3$. The asymmetric unit consists of four *Hp*NikR molecules giving a solvent content of 68.68 %. The data showed a strong anomalous signal with Δ_{anom} correlation between half-sets of 0.655 overall and mid-slope of anomalous normal probability of 1.440. The data collection statistics are reported in Table 1ESI.

SECTION ESI-3: Structure solution

The phasing steps were streamlined using the HKL2MAP package [4] for SHELX program suite [5]. Unmerged data (OUTPUT POLISH UNMERGED option from SCALA) were prepared with SHELXC, which conformed the strong anomalous signal (<d''/sig> 1 till 2.6 Å resolution). SHELXD promptly localized four Ni atoms with a final correlation coefficient for the best solution (All/weak) of 40.45/23.09. The final Patterson figure of merit was 51.04. Initial phases were computed with SHELXE with 20 cycles of density modification to values of contrast 0.963, connectivity 0.940 and a map correlation coefficient of 0.839.

SECTION ESI-4: Structure refinement

Initial unbiased phases and data allowed ArpWarp[6] to trace successfully the model. After ten building cycles the structure consisted of four chains and 317 residues for an estimated model correctness of 96.9%. Automatic solvent building was performed using the program ArpWarp, keeping only those water molecules having density greater than 1.0 s in the 2Fo-Fc electron density map, and that after visual inspection resulted to be in good density regions. The structure was refined using Refmac5 [7]. The protein regions displaying different conformations were manually rebuilt with the program COOT[8]. Randomly selected reflections (5 % of the total) were used as an Rfree set for cross validation. No restraints were imposed on the Ni-ligand distances. Using isotropic temperature factors the refinement converged to a final R_{factor} and R_{free} were 22.9% and 27.8%. The stereochemistry of the final model was routinely checked using COOT [8] and PROCHECK [3]. The refinement statistics are reported in Table 1ESI. The refined crystallographic coordinates and structure factor amplitudes have been deposited in the Protein Data Bank under the accession code 2Y3Y.

REFERENCES

1 B. Zambelli, M. Bellucci, A. Danielli, V. Scarlato and S. Ciurli, *Chem. Commun.*, 2007, 3649-3651.
2 W. Kabsch, *Acta Crystallogr D Biol Crystallogr*, 2010, **66**, 125-132.
3 N. Collaborative Computational Project, *Acta Cryst.*, 1994, **D50**, 760-763.
4 T. Pape and T. R. Schneider, *J. Appl. Cryst.*, 2004, **37**, 843-844.
5 G. M. Sheldrick, *Acta Cryst.*, 2008, **A64**, 112-122.
6 G. Langer, S. X. Cohen, V. S. Lamzin and A. Perrakis, *Nat Protoc*, 2008, **3**, 1171-1179.
7 G. N. Murshudov, A. A. Vagin and E. J. Dodson, *Acta Crystallogr D Biol Crystallogr*, 1997, **53**, 240-255.
8 P. Emsley, B. Lohkamp, W. G. Scott and K. Cowtan, *Acta Crystallogr D Biol Crystallogr*, 2010, **66**, 486-501.

Table 1ESI Data Collection and Model Refinement Statistics

Data collection

Wavelength (Å)	1.459
Space group	$P4_3$
Unit cell (*a*, *b*, *c*, Å)	72.97, 72.97, 116.73
Resolution range (Å)	19.94 – 2.39
Redundancy[1]	14.0 (8.2)
Total reflections	339536
Unique reflections	24202
Completeness[1] (%)	99.8 (100.0)
R_{sym}[1,2] (%)	6.6 (64.2)
Mean I/σ(I)[1]	26.8 (3.5)
Anomalous completeness[1]	99.8 (99.9)
Anomalous multiplicity[1]	6.6 (3.8)
Δ_{anom} correlation between half-sets[1]	0.655 (0.031)
Mid-Slope of Anom Normal Probability	1.440

Phasing

Number of trials[3]	100
Correlation coefficient best solution (All/weak) [3]	40.45/23.09
Patterson figure of merit[3]	51.04
Number of cycles of density modification	20
Contrast[3]	0.963
Connectivity[3]	0.940
Map Correlation coefficient[3,1]	0.84 (0.59)
Weight[3,1]	0.65 (0.36)

Refinement statistics

Number of building cycles[4]	10
Estimated correctness of the model[4] (%)	96.9
Number of Chains[4]	4
Number of residues[4]	317
R-factor[5] (%)	22.9
R_{free}[5] (%)	27.8
Cruickshanks DPI for coordinate error (Å) [5]	0.3
Average B-factors	
Main chain atoms[6]	40.9
Side chain atoms and waters[6]	44.5
Average RMS B-factor	
Main chain atoms[6]	1.1
Side chain atoms and waters[6]	3.2
Total number of atoms[7]	2804
Total number of water molecules[7]	71
Solvent content (%) [7]	68.6
Ramachandran plot[7] :	
Core	88.7%
Allowed	10.7%
Generously allowed	0.0%
Disallowed	0.3%

[1]highest resolution bin in parenthesis
[2]$R_{sym} = \Sigma_{hkl} \Sigma_j | I_j - \langle I \rangle | / \Sigma_{hkl} \Sigma_j I_j$ where I is the intensity of a reflection, and <I> is the mean intensity of all symmetry related reflections j.
[3]taken from HKL2MAP/SHELXD/SHELXE
[4]taken from ArpWarp
[5]taken from Refmac5 v5.5.0109 (CCP4)
[6]taken from B-Average (CCP4)
[7]taken from Procheck (CCP4)

Table 2ESI **Distances (Å) and angles (°) for the four Ni^{2+} sites in *H. pylori* NikR, and relative estimated standard deviations (ESD[a])**

$Ni_{AB}1$	His88B Nε	His99A Nε	His101A Nδ	Cys107A Sγ
	1.9±0.6	2.0±0.6	1.9±0.6	2.1±0.6

His88B Nε – $Ni_{AB}1$ – His101A Nδ	92°±17°
His101A Nδ – $Ni_{AB}1$ – His99A Nε	92°±17°
His99A Nε – $Ni_{AB}1$ – Cys107A Sγ	89°±17°
Cys107A Sγ – $Ni_{AB}1$ – His88B Nε	84°±17°

$Ni_{AB}2$	His88A Nε	His99B Nε	His101B Nδ	Cys107B Sγ
	1.9±0.6	1.9±0.6	2.0±0.6	2.2±0.6

His88A Nε – $Ni_{AB}2$ – His101B Nδ	89°±17°
His101B Nδ – $Ni_{AB}2$ – His99B Nε	96°±17°
His99B Nε – $Ni_{AB}2$ – Cys107B Sγ	92°±17°
Cys107B Sγ – $Ni_{AB}2$ – His88A Nε	80°±17°

$Ni_{CD}3$	His88D Nε	His99C Nε	His101C Nδ	Cys107C Sγ
	2.0±0.6	2.0±0.6	1.9±0.6	2.1±0.6

His88D Nε – $Ni_{CD}3$ – His101C Nδ	86°±17°
His101C Nδ – $Ni_{CD}3$ – His99C Nε	90°±17°
His99C Nε – $Ni_{CD}3$ – Cys107C Sγ	90°±17°
Cys107C Sγ – $Ni_{CD}3$ – His88D Nε	93°±17°

$Ni_{CD}4$	His88C Nε	His99D Nε	His101D Nδ	Cys107D Sγ
	1.9±0.6	1.9±0.6	2.0±0.6	2.2±0.6

His88C Nε – $Ni_{CD}4$ – His101D Nδ	84°±17°
His101D Nδ – $Ni_{CD}4$ – His99D Nε	94°±17°
His99D Nε – $Ni_{CD}4$ – Cys107D Sγ	96°±17°
Cys107D Sγ – $Ni_{CD}4$ – His88C Nε	84°±17°

---

[a] ESD were calculated on the basis of the Cruickshanks diffraction precision index (DPI) for coordinate error (Å). Cruickshank's DPI for coordinate error is calculated using R-factor, number of reflections, number of parameters and number of observables. Completeness of the data is also taken into account:

$$DPI = \mathrm{sqrt}(N_{atom}/(N_{refl}-N_{param}))\ R_{factor}\ D_{max}\ C^{-1/3}$$

where N_{atom} is the number of the atoms included in the refinement, N_{refl} is the number of reflections included in the refinement, R_{factor} is the overall R-factor, D_{max} is the maximum resolution of reflections included in the refinement, and C is the completeness of the observed data.
The Ni - ligand atom distance is calculated as a difference between the coordinates of the two atoms. Hence the ESD for such measured distance will be twice the value of the ESD of each atom coordinate. Since Cruickshank's DPI for this structure is 0.3 Å (calculated with Refmac5 (CCP4)), the estimated distance error is 0.6.
The angle value ESD has been estimated as ~ 2 x Tan^{-1}(ESD atom position/inter-atom distance value)= 2 x Tan^{-1}(0.3/2.0) hence 17 degrees.

Electronic Supplementary Material (ESI) for Dalton Transactions

Figure 1ESI

Crystal packing of Holo-Ni^{2+} *Helicobacter pylori* NikR tetramer. Panel A) view along *a* axis; B) view along *b* axis; C) view along *c* axis. Large cavities are visible along the two-fold axis (panels A and B).