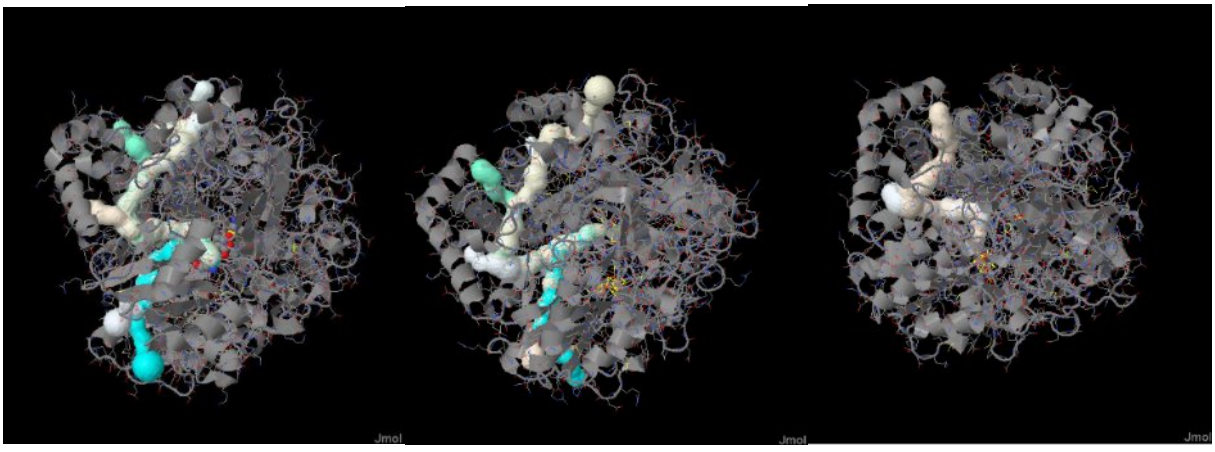
Mapping the molecular tunnels in three nickel-iron hydrogenases

Abdullatypov A.V., Tsygankov A. A.
Institute of basic biological problems RAS, ul. Institutskaya, 2, Puschino, Moscow oblast, Russia
e-mail: azatik888@yandex.ru

HydSL, a nickel-iron hydrogenase from *Thiocapsa roseopersicina*, is a thermostable and oxygen-tolerant enzyme capable of hydrogen oxidation, which makes it a good candidate for application in hydrogen fuel cells [1]. Unfortunately, the crystal structure of this enzyme is not available, which impedes its study and possible site-directed modification. We developed a homology model of this two-subunit enzyme using MODELLER package [2, 3] with the nickel-iron hydrogenase from *Allochromatium vinosum* (PDB-entry 3MYR) as a template.

To determine possible pathways of inhibitors such as oxygen and carbon monoxide, mapping of tunnels in the model was carried out using MOLE2.0 online program [4]. The minimal radius of tunnels and the bottleneck radius were set to 1.2 Å, which is the van der Waals radius of the oxygen molecule. Other parameters were set to default values; two other hydrogenases, i.e. the template and the nickel-iron hydrogenase from *Desulfovibrio vulgaris* Miyazaki F (PDB-entry 1H2A), were also explored in MOLE 2.0. The tunnels which did not lead to the active site were removed.

The picture of the tunnels in *Desulfovibrio* hydrogenase was quite similar to that described in literature. The hydrogenase from *Allochromatium vinosum* had principally the same structure of tunnels, except that they were longer and narrower, whereas HydSL hydrogenase from *Thiocapsa roseopersicina* had no tunnels in the small subunit, thus having less possible pathways for oxygen molecules.



a **b** **c**

Fig. 1. Comparison of tunnel structure in three hydrogenases: **a** – HydAB hydrogenase from *Desulfovibrio vulgaris* Miyazaki F; **b** – HydSL hydrogenase from *Allochromatium vinosum*; **c** – HydSL hydrogenase from *Thiocapsa roseopersicina* BBS. Pictures were made as screenshots from Jmol; red-and-yellow sticks represent iron-sulphur clusters of the small subunit. The large subunit is positioned on top, and the small on the bottom of the images.

We should note that no tunnels could be found after energy minimization procedure on YASARA energy minimization server [5]. It means that these tunnels should be closed during a large time in solution and are opened stochastically with a rather large period of oscillation between close and open state, so these results should be proved by molecular dynamics simulations on supercomputers with oxygen as a probe molecule, so that the tunnels could be represented as the tracks of oxygen molecules inside the enzyme to the active site.

It is interesting that the obtained data show a novel position for possible determinants of oxygen tolerance. The data from literature showed the determinants of oxygen tolerance in the large subunit: the substitution of two amino acid residues in oxygen-sensitive hydrogenase lead to oxygen tolerance [6], and a reverse substitution in oxygen-tolerant hydrogenases lead to oxygen sensitivity [7]. However, the HydSL hydrogenase from *Thiocapsa roseopersicina* has the same amino acids as the oxygen-sensitive hydrogenase from *Desulfovibrio vulgaris* in corresponding positions. Thus, there is another determinant of oxygen tolerance situated in the small subunit.

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REFERENCES

1. I.N. Gogotov, N.A. Zorin, L.T. Serebryakova, E.N. Kondratieva. The properties of hydrogenase from *Thiocapsa roseopersicina* (1978). *Biochim. Biophys. Acta*, **523**: 335-343.

2. Sali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints (1993*) J Mol Biol.*, **234(3)**:779-815.

3. Shen MY, Sali A. Statistical potential for assessment and prediction of protein structures (2006) *Protein Sci.*,**15(11):**2507-24.

4. Berka K, Hanák O, Sehnal D, Banás P, Navrátilová V, Jaiswal D, Ionescu CM, Svobodová Vareková R, Koca J, Otyepka M. MOLEonline 2.0: interactive web-based analysis of biomacromolecular channels (2012). *Nucleic Acids Res.*, **40(W1):** W222-W227

5. Krieger E, Joo K, Lee J, Lee J, Raman S, Thompson J, Tyka M, Baker D, Karplus K. Improving physical realism, stereochemistry, and side-chain accuracy in homology modeling: Four approaches that performed well in CASP8 (2009) *Proteins*, **77 Suppl 9**:114-22

6. Leroux F, Dementin S, Burlat B, Cournac L, Volbeda A, Champ S, Martin L, Guigliarelli B, Bertrand P, Fontecilla-Camps J, Rousset M, Léger C. Experimental approaches to kinetics of gas diffusion in hydrogenase (2008) *Proc Natl Acad Sci U S A*, **105(32):**11188-93

7. Buhrke T, Lenz O, Krauss N, Friedrich B. Oxygen tolerance of the H2-sensing [NiFe] hydrogenase from *Ralstonia eutropha* H16 is based on limited access of oxygen to the active site (2005) *J Biol Chem*, **280(25):**23791-6.