| | | |
|---|---|---|
| ESRF | **Experiment title:**<br>bio-SAXS analysis of solution shape of hydrogenosomal processing peptidase with or without substrate and screening for favourable crystallization conditions | **Experiment number**:<br>MX-1441 |
| **Beamline**:<br>BM29 | **Date of experiment**:<br>from: 29. July 2012 to: 30. July 2012 | **Date of report**:<br>25. September 2012 |
| **Shifts:**<br>3 | **Local contact(s)**:<br>Louiza Zerrad (louiza.zerrad@esrf.fr) | *Received at ESRF:* |


**Names and affiliations of applicants** (* indicates experimentalists):

**M.Sc. Tomas Kucera * -** Academy of Sciences of the Czech Republic, Institute of Microbiology

**Dr. Jiri Janata -** Academy of Sciences of the Czech Republic, Institute of Microbiology

**Dr. Eva Kutejova -** Slovak Academy of Sciences, Dep. of Biochemistry, Institute of Molecular Biology


## Report:

*Hydrogenosomal processing peptidase is designated as HPP.*
*Mitochondrial processing peptidase is designated as MPP.*

## 1) Investigation of conformation changes of HPP

In order to investigate the conformation changes upon addition of substrate, we used two different systems:

1. Wild-type HPP without peptide substrate
2. HPP mutated in catalytic β-subunit (mutation E56Q) with peptide substrate

From the scattering curves we could conclude that there is no change in the shape and size of the mutant HPP when a substrate is added to the protein. The sample with the substrate is less aggregated than the mutated enzyme alone (Figure 1).

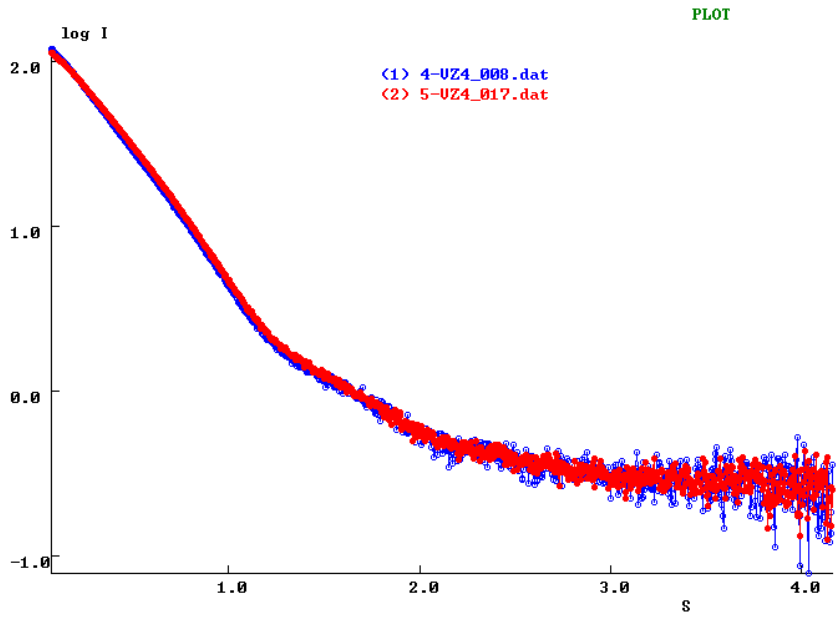


***Figure 1.*** *Comparison between the scattering curves of mutant enzyme without substrate (4-VZ4) and with substrate (5-VZ4).*

We however observe an important difference between the scattering curves of the mutant enzyme and the scattering curve of the wild-type enzyme, both without substrate. The two enzymes have roughly the same molecular weight (MW~70-75 kDa) since the zero intensity I(0) for the two enzymes are the same. However, both proteins have different shapes because a discrepancy around q=1.5 nm-1 is observed (Figure 2). The combination of "same

molecular weight" and "different scattering profile" may let think of a different conformation between the two enzymes. It is not due to aggregation since a potential aggregation would have increased the zero intensity (and the molecular weights) of the enzyme.

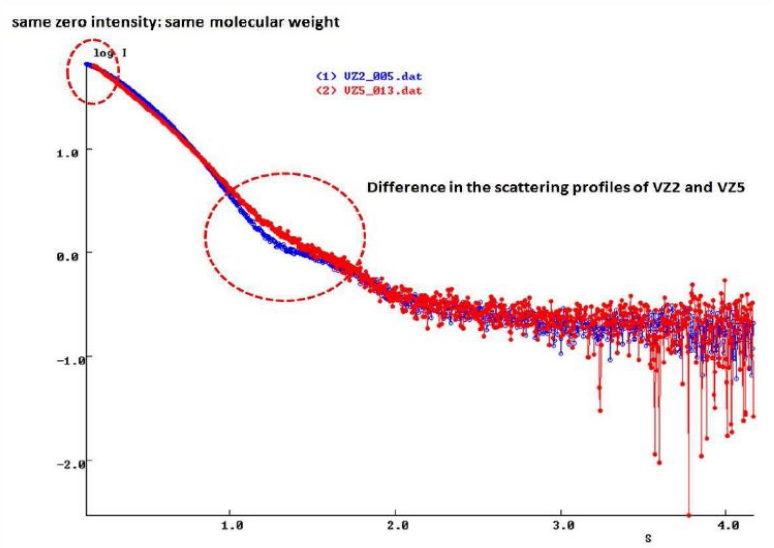


***Figure 2.*** *Comparison between scattering curves of wild-type enzyme (VZ2) and mutant enzyme (VZ5), both without substrate.*

## 2) Investigation of solution shape of HPP

Futher, we used bio-SAXS data to compute theoretical solution shape of wild-type and mutated HPP. From the comparison of the p(r) profiles (Figure 3), we have found that the mutated enzyme shows a more elongated shape than the wild-type enzyme because the mutated enzyme is bigger than wild one (Rg=4.2 nm versus Rg=3.6 nm and Dmax~14 nm versus Dmax~12 nm). The bio-SAXS models obtained are compacted and very similar in shape. Mutated HPP is bigger and has slightly more open conformation than wild-type HPP.

The bio-SAXS models obtained are compacted and very similar in shape. Based on crystal structure of homologous MPP we expected globular shape of MPP and this expectation was confirmed. However, "the tail extension" of HPP model is remarkable and we can just speculate if this is real structural trait of HPP or just artifact of measurements, arisen from sample aggregation. To decide which possibility is more likely we need to repeat bio-SAXS under different conditions and possibly also with sample purified by different procedure.

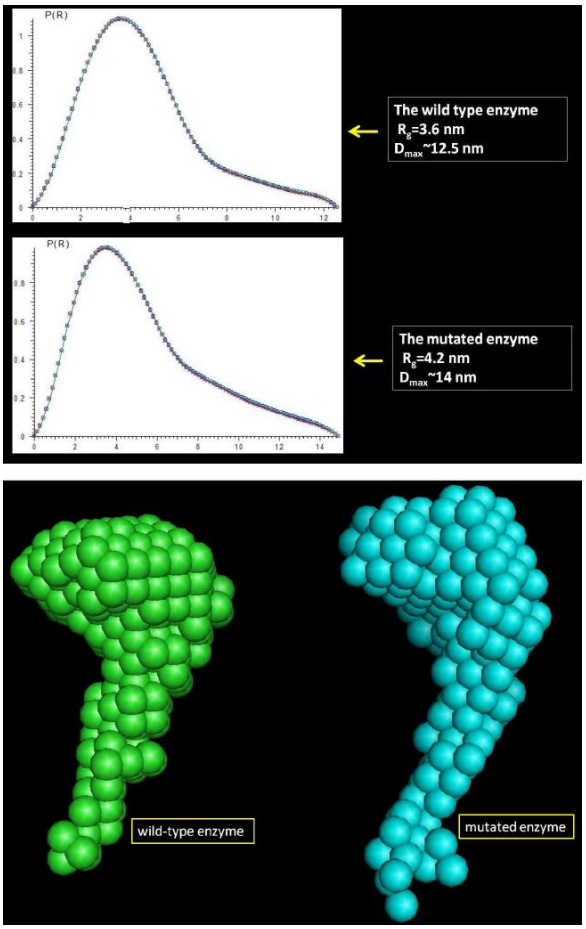


***Figure 3.*** *Pair Distance Distribution Functions, p(r) profiles and the corresponding SAXS DAMMIF models of wild-type and mutant enzymes.*

## 3) Screening for favourable crystallization conditions

Because of the lack of time, we decided to focus on previous two goals and skipped the screening for favourable crystallization conditions.

## 4) Final notes

In order to verify and extend these preliminary observations we plan to perform bio-SAXS experiments on MPP (peptidase with know crystal structure) that is homologous to HPP. For this reason we intend to apply for another beamtime at ESRF in the very near future.

During this pilot experiment we had the first chance to use bio-SAXS method on HPP. We spent considerable amount of time with buffer measurements and sample concentration optimizations. We gained a new experience that will be quite useful to perform more effective bio-SAXS experiments on HPP and MPP under different conditions.