

Offer of Topic for a Doctoral Dissertation

Topic:

Fluorescence Solvent Relaxation Technique and Fluorescence Antibunching, Experiments: Defined Applications in Protein Sciences

Téma:

Fluorescenční techniky relaxace rozpouštědla a fluorescenční antibunching, Experimentální aplikace ve výzkumu proteinů

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Annotation:

After the understanding of solvent relaxation (SR) in isotropic solvents, scientists started about 10 years ago to characterise SR in supra- and biomolecular assemblies. It was the group Hof that established the SR method for probing micro-mobilities and -polarities in biomembranes. Recently, we succeeded to apply this technique to the investigation on hydration and mobility close to the active site of enzymes (Journal of the American Chemical Society, 2009, 131 (2), 494-501). Specifically our experiments on haloalkane dehalogenases (DhaA from *Rhodococcus* sp., DbjA from *Bradyrhizobium japonicum* USDA 110) were using a unique labeling procedure introducing a coumarin derivative to the active site of those different dehalogenase enzymes.

In following up studies to be performed by a PhD student different mutants with different catalytic activity (enzymatic studies using different mutants will be performed by the group of Prof. Damborsky in Brno) will be investigated by that SR approach. Comparing the dynamic fluorescence data with the enzymatic data, we will try for the first time to correlate enzymatic activity with the grade of water organisation in the active side of a protein. Connected with those haloalkane dehalogenases the aggregation of those enzymes appear to play a role in their function. Fluorescence antibunching is a new, elegant single molecule fluorescence methods which has been shown to be an ideal toll for the investigation of protein aggregation behaviour. Within the PhD study this method, which is established in our group, will be used for a comprehensive characterisation of haloalkane dehalogenases aggregation.

Anotace česky:

Techniku „solvent relaxation“, jejíž aplikace na zkoumání polarity a mobility různých domén biomembrány byla vyvinuta v naší laboratoři, jsme v současné době použili na sledování hydratace a mobility v blízkosti aktivního místa enzymů (Journal of the American Chemical Society, 2009, 131 (2), 494-501). Pro experimenty na haloalkan dehalogenáze (DhaA z *Rhodococcus* sp., DbjA z *Bradyrhizobium japonicum* USDA 110) byla použita unikátní metoda označení vložení derivátu kumarinu do aktivního místa těchto enzymů.

V následujících studiích, na kterých se bude podílet student PhD, se budou zkoumat různé mutanty s různou katalytickou aktivitou (enzymatické studie se budou provádět v Brně ve skupině Prof. Damborského). Cílem je korelovat enzymatickou aktivitu se stupněm organizace vody v aktivním místě proteinu. Další proces, který hraje roli ve funkci haloalkan dehalogenázy, je pravděpodobně agregace. Charakterize tohoto procesu bude prováděna pomocí „fluorescence antibunching“, nové elegantní „single molecule“ fluorescenční metody.