Diffusion and partitioning of small molecules in protein crystals

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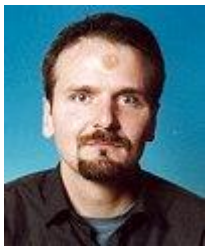


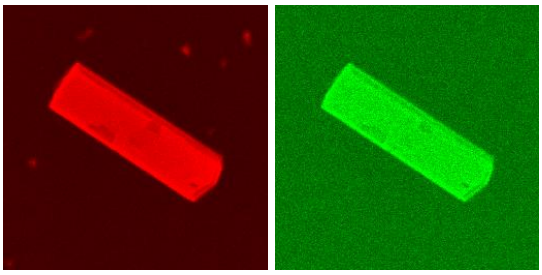
Description

In recent years the number of industrial enzymes being produced as crystalline proteins has been increasing. Protein crystals are open mesoporic structures, similar to zeolites, with porosities ranging from 50-80% and pore diameters from 0.5 to 10 nm. Commonly, protein crystals are used to study the mechanisms by which protein molecules bind to ligands and catalyze reactions, and to study transport processes that might cause differences between the properties of crystalline and dissolved proteins. Cross-linked protein crystals may find a broad range of applications in biocatalytic processes, separation processes such as chromatography, and as biosensors. The understanding of transport rates in the pores is crucial for these applications.

The objective was to gain a qualitative and quantitative understanding of the partitioning and diffusion behavior of small molecules in protein crystals. The following studies were conducted:

- experimental visualization and calculation of the partitioning and diffusion behavior in the crystals,
- correlating such direct experimental data with indirect data obtained from bulk phase measurements
- description and prediction of partitioning and diffusion using theoretical models

We have developed an experimental method applying confocal laser scanning microscopy (CLSM), which, for the first time, can describe the solute diffusion in protein crystals simultaneously as function of space and time without damaging the crystal. It was found with CLSM that the diffusion depends on the protein crystal structure and is anisotropic.



rhodamine B fluorescein

Dissertation

A. Cvetkovic Diffusion and partitioning of small molecules in protein crystals, PhD thesis, Delft University of Technology, 2005. http://resolver.tudelft.nl/uuid:cdaa05b8-55b6-4e6d-a7a9-9caa4f4f8cd4

Publications from the dissertation

1. A. Cvetkovic, M. Zomerdijk, A.J.J. Straathof, R. Krishna, and L.A.M. van der Wielen, Adsorption of fluorescein by protein crystals, Biotechnol. Bioeng. **87** (2004) 658-668.
2. A. Cvetkovic, C. Picioreanu, A.J.J. Straathof, R. Krishna, and L.A.M. van der Wielen, Relation between pore sizes of protein crystals and anisotropic solute diffusivities, J. Am. Chem. Soc. **127** (2005) 875-879.
3. A. Cvetkovic, A.J.J. Straathof, R. Krishna, and L.A.M. van der Wielen, Adsorption of xanthene dyes by lysozyme crystals, Langmuir **21** (2005) 1475-1480.
4. A. Cvetkovic, C. Picioreanu, A.J.J. Straathof, R. Krishna, and L.A.M. van der Wielen, Quantification of binary diffusion in protein crystals, J. Phys. Chem. B **109** (2005) 10561-10566.
5. R. Archipov, A. Cvetkovic, F. Stallmach and A.J.J. Straathof, Measurement of anisotropy of pore diffusion in protein crystals by PFG NMR and by CLSM. Micropor. Mesopor. Mat.112 (2008) 471-480