

## 4260 Quantification of Binary Diffusion in Protein Crystals

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The combination of protein crystals' open structure (a high porosity and pore surface area) with the wide variety of molecular topologies and with the proteins' ability of regio- and stereo-selective recognition makes protein crystals a novel class of nanoporous materials of high interest for many industrial fields: in separation processes such as chromatography, in enzymatic production processes, in medical formulations for pharmaceutical delivery, in biosensors and in detergents.

Despite the progress made in improving the characteristics of protein crystals, comprehension of solute transport phenomena in the crystal pores is still modest. In the case of single solute uptake, significant progress has been achieved [1]. The following step, required for dealing with actual processes, is a quantitative comprehension of multicomponent phenomena, which is the subject of this abstract.

The use of confocal laser scanning microscopy (CLSM) for visualization and quantification of binary diffusion within anisotropic porous material is described here for the first time. The dynamics of adsorption profiles of di-anionic fluorescein, zwitter-ionic rhodamine B, and their mixture, in the cationic native orthorhombic lysozyme crystal were subsequently analyzed. All data could be described by a classical pore diffusion model. There was no change in the adsorption characteristics, but diffusion decreased with the introduction of a second solute in the solution. It was found that diffusion is determined by the combination of steric and electrostatic interactions, whilst adsorption is dependent on electrostatic and hydrophobic interactions. Thus, it was established that the outcome of binary transport depends on the solute, protein and crystal characteristics.

Figure 1. Confocal images of simultaneous diffusion of fluorescein (left) and rhodamine B (right) into native orthorhombic lysozyme crystals

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[1] Cvetkovic A., Picioreanu C., Straathof A.J.J., Krishna R. and van der Wielen L.A.M. 2005. *J. Am. Chem. Soc.* 127, 875.

