

m01.p07**TIM: a model protein for the study of crystal growth in space and gels**C. Vanhee¹, C. VandeWeerd², D. Maes¹, L. Wyns¹, and I. Zegers¹¹Department Ultrastructure, VUB. Pleinlaan 2, 1050 Brussels, Belgium²Labo de Biologie Moléculaire et de Génie Génétique, Bât. B6, Allée de la Chimie, 3, 4000 Liège, Belgium**Keywords: phase diagram, nucleation, crystallization of proteins**

The understanding of the basic processes underlying the crystallisation of proteins requires the use of very well-characterised model systems, for which theory and models of crystal growth can be verified experimentally. Triose phosphate isomerase (Tm TIM) [1] can be produced in large amounts, and crystallises reproducibly. It has been used for experiments in microgravity and in gels, and it has been shown to produce crystals of marked improvement in these non-convective environments. It is thus an interesting model protein for studying the effect of convective mixing on protein crystallisation. We present here the phase diagrams for Tm TIM in function of temperature, protein, and precipitant concentration, and relate the results with the observed crystallisation behaviour. Solubility curves and the binodal curve in function of these three parameters show that Tm TIM is a case of 'retrograde solubility'. This phenomenon has been observed in many cases but only been described in detail in the case of human hemoglobin [2]. The phase diagrams form the basis for the understanding of the results from counterdiffusion experiments with Tm TIM, including improved crystal quality, and polymorphic variation in function of growth conditions.

[1] Maes, D., Zeelen, J. P., Thanki, N., Beaucamp, N., Alvarez, M., Thi, M. H., Backmann, J., Martial, J. A., Wyns, L., Jaenicke, R., and Wierenga, R. K. 1999. The crystal structure of triosephosphate isomerase (TIM) from *Thermotoga maritima*: a comparative thermostability structural analysis of ten different TIM structures. *Proteins* 37: 441-453.

[2] Vekilov, P. G., A. R. Feeling-Taylor, D. N. Petsev, O. Galkin, R. L. Nagel, and R. E. Hirsch. 2002. Intermolecular interactions, nucleation and thermodynamics of crystallization of hemoglobin C. *Biophys. J.* 83:1147-1156.

m01.p08**Design and construction of a microfluidic device for studying protein nucleation and crystallisation**R. Willaert^a, C. Vanhee^a, C. De Tandt^b, W. Ranson^b, R. Vounckx^b, L. Wyns^a^a Dept. Ultrastructure, Flanders Interuniversity Institute for Biotechnology, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussel, Belgium; ^b Dept. of Electronics and Informatics, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussel, Belgium.**Keywords: microfluidics, T-mixer, free interface diffusion, PDMS, supersaturation profile modelling, protein nucleation and crystallisation**

The design and construction of a microfluidic T-mixer is described. In a T-mixer, 2 channels with a small width (50 - 100 μm) are merged into a single channel (100 μm width) in which mixing takes place. In this device, the protein solution and precipitant solution are mixed. Due to the small dimensions of the channels, fluid flow is laminar and mass transport in the mixing channel occurs only by diffusion, normal to the flow direction. At proper supersaturation values, nuclei will be formed in the mixing channel. These nuclei are allowed to grow into crystals in a metastable condition.

A 3D-mathematical model is constructed that describes fluid flow and diffusion of the precipitant and protein. Therefore, the convection-diffusion equation is combined with the incompressible Navier-Stokes equation. These equations are solved using the finite element method. Based on the local concentrations and the solubility diagram, supersaturation profiles are calculated. Calculations are performed for two model proteins, i.e. hen egg-white lysozyme and triosephosphate isomerase (TIM) from *Thermotoga maritima*. The influence of the residence time in the T-mixer on the supersaturation profile is assessed. Polydimethylsiloxane (PDMS) microfluidic chips were built using soft lithography. Silicon wafers were coated with Photoresist Shipley AZ 1400-37 by spinning 6 different layers on top of each other at 1000 rpm for 30 s on a Headway Research Inc. spincoater. Each Photoresist layer has been separately soft baked at 90°C for 3 min 30 s. UV exposure through black-and-white transparency masks was performed for 2 min on a 365 nm mask aligner (Karl Suss MJB3). The Photoresist channel mold (total thickness 8.6 - 9 μm) has been developed in Developer 312 (Shipley) in 1:1 ratio for 3min and hard baked on a hot plate at 120°C for 5 min. PDMS (Sylgard 184, Dow Corning) in 10:1 ratio was poured onto the Photoresist mold in a Petri dish and baked in an 80°C oven for 60 min. Glass coverslips were coated with PDMS (Sylgard 184) in 5:1 ratio spin coated on a glass substrate at 5000 rpm for 60 sec and baked at 80°C during 15 min. The thick PMDS chip was sealed to the coverslip by baking at 80°C for 120 min.

The fluid flow of the inlet streams in the main channel is characterised experimentally using coloured solutions. Initial nucleation/crystallisation experiments using lysozyme are presented.