s8b.m5.o3 **On the growth of macromolecular crystals in gels.** J.M. García-Ruiz. *Laboratorio de Estudios Cristalográficos. IACT. CSIC-Universidad de Granada. Campus de Fuentenueva. 18002-Granada. Spain.*
Keywords: methods crystallography, genesis, perfection of biomacromolecular.

Fluid motion triggered by density and/or thermal gradients, as well as sedimentation are processes that breaks the symmetry of the accretion of growth units during crystal growth. They alter the homogeneity of the accretion process and detriment the quality of the growing crystal. Among the techniques to prevent these gravity-dependent processes, gels have been used since the last century as crystallisation media [1-3]. I will present in this communication an overview of the use of different types of gels in protein crystallisation. Different crystallisation techniques, such as bath and hanging drops, and particularly counter-diffusion techniques, will be discussed. Direct use of electrophoretic gels for crystallisation screening will be also presented. Physical and chemical properties of gels relevant to their use as crystallisation media, such as mass transport, mechanical and rheological properties will be discussed. The use of polymers at concentrations lower than the critical concentration to form percolation structures will be also discussed. Adimensional numbers, i.e. Grashof number, will be used to define the window of polymer concentration and reactor's size that prevent convective fluid motion. Finally, recipes to perform crystallisation experiments using gels will be provided.

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s8b.m5.o4 **The Crystallization of BPTI at acidic pH: a Decamer Story**. C. Hamiaux[1], J. Pérez[1], T. Prangé[1,2], M. Riès-Kautt[2], S. Veesler[3] and P. Vachette[1], *[1]LURE, Centre Universitaire Paris-Sud, Bât 209 D, B.P. 34, 91898 Orsay Cedex, France, [2]Laboratoire de Cristallographie et RMN Biologiques, Faculté de Pharmacie, 4, Av. de l'Observatoire, 75006 Paris, France, [3]Centre de Recherche sur les Mécanismes de la Croissance Cristalline (CRMC2) CNRS, Campus de Luminy, Case 913, 13288 Marseille Cedex 09, France.*
Keywords: BPTI, self-association, SAXS.

Bovine Pancreatic Trypsin Inhibitor (BPTI) crystallizes at acidic pH in the presence of thiocyanate, chloride and sulfate ions [1] yielding three different polymorphs in $P2_1$, $P6_422$ and $P6_322$ space groups respectively. In all three crystal forms, the same decamer is found in the packing (ten BPTI molecules organized through two perpendicular 2-fold and 5-fold axes as a well defined and compact object) in contrast to the monomeric crystal forms observed at basic pH [2,3].

The crystallization of BPTI under acidic conditions (pH=4.5) was investigated by Small Angle X-ray Scattering (SAXS) with both under- and supersaturated BPTI solutions. Data showed the oligomerization of BPTI molecules under all investigated conditions. Accordingly, various mixtures of discrete oligomers (n = 1 to 10) were considered. Calculated scattering curves were obtained using models based on the crystallographic structures and the experimental patterns were analyzed as a linear combination of the model curves using a non-linear curve fitting procedure.

The results, confirmed by gel filtration experiments, unambiguously demonstrate the co-existence of two different BPTI particles in solution: a monomer and a decamer, without any evidence for other intermediates [4]. Moreover, using both approaches, the fraction of decamers was found to increase with increasing salt concentration, even beyond the solubility curve. We therefore propose that at acidic pH, BPTI crystallizes following a two step process: decamers are first built in under- and supersaturated solutions, upon which crystal growth proceeds by decamer stacking. Indeed, those BPTI crystals should best be described as "BPTI decamer" crystals.

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