

# Agarose gel as a medium for growing and tailoring protein crystals

*Fiora Artusio<sup>1</sup>, Albert Castellví<sup>2,3</sup>, Anabel Sacristán<sup>3</sup>, Roberto Pisano<sup>1</sup>,*

*José A. Gavira<sup>4\*</sup>*

<sup>1</sup> Department of Applied Science and Technology, Politecnico di Torino, 24 corso Duca degli  
Abruzzi, 10129 Torino, Italy

<sup>2</sup> Molecular Biology Institute of Barcelona, Carrer Baldri Reixac 4-8 (Science Park), 08028  
Barcelona, Spain

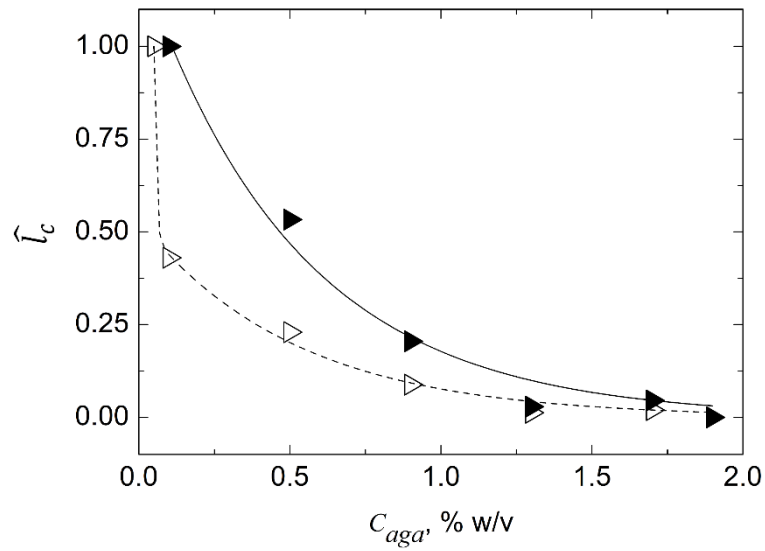
<sup>3</sup> ALBA Synchrotron, Carrer de la Llum 2-26, 08290 Cerdanyola del Vallès, Barcelona, Spain

<sup>4</sup> Laboratorio de Estudios Cristalográficos, Instituto Andaluz de Ciencias de la Tierra (Consejo  
Superior de Investigaciones Científicas-Universidad de Granada), Avenida de las Palmeras 4,  
18100 Armilla, Granada, Spain

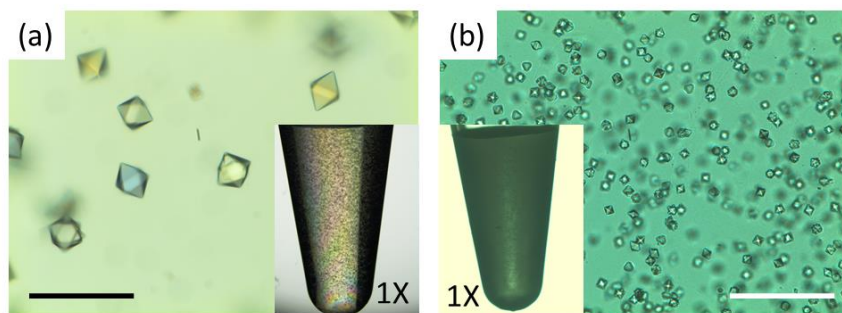
\*Correspondence to: [jgavira@iact.ugr-csic.es](mailto:jgavira@iact.ugr-csic.es)

**Table S1.** The fitting parameters of the single exponential decay law used to fit the experimental data of the crystal size vs. agarose gel trend. A is the pre-exponential factor, and B the decay constant. The respective standard error is also reported.

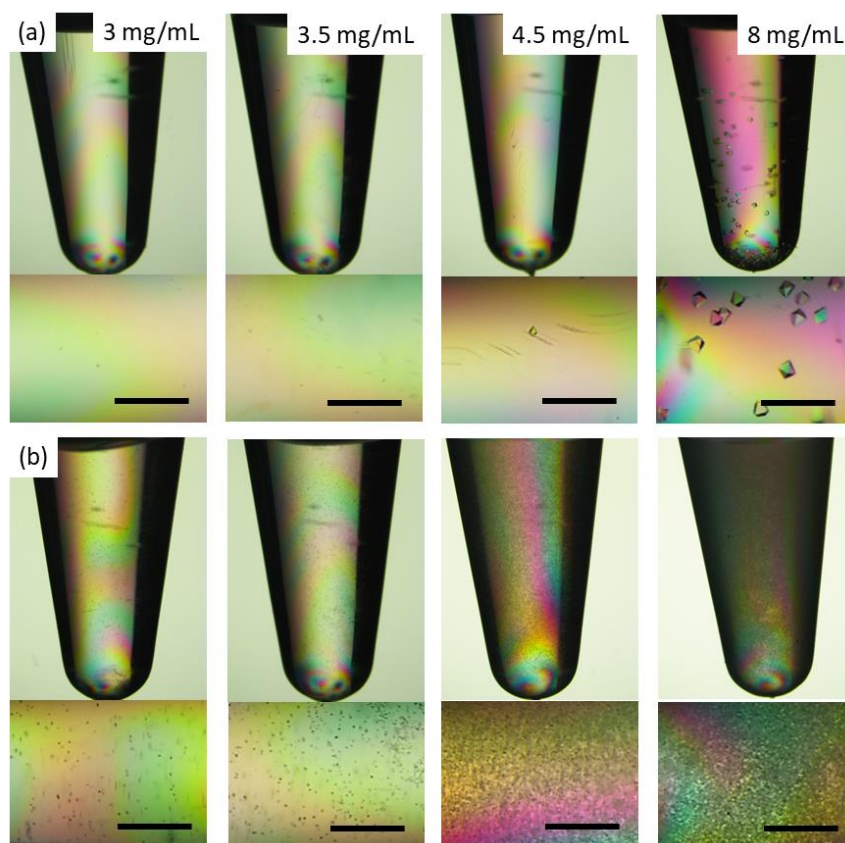
Protein	A	STD error	B	STD error
Proteinase K	1.238	0.074	0.515	0.051
HEWL	1.176	0.058	0.605	0.049
Insulin	1.165	0.060	0.601	0.051



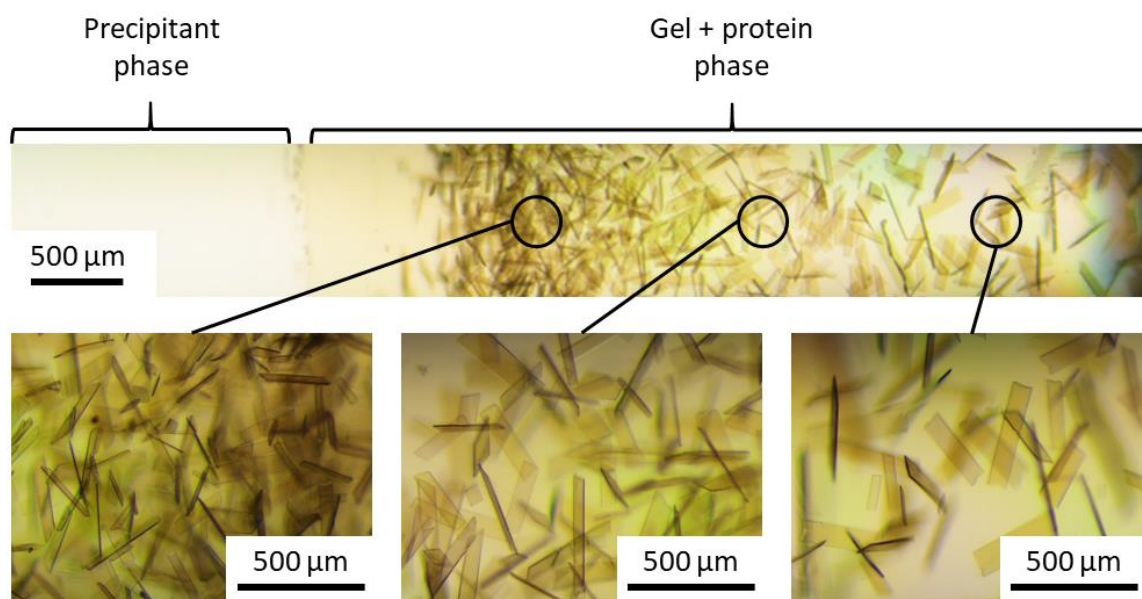
**Figure S1.** The normalized proteinase K crystal size vs. agarose content in the batch is illustrated. Lines refer to fitting and symbols to experimental data. Continuous line and full symbols refer to the data set excluding 0.05% (w/v) gel, whereas the data set of dashed line and empty symbols includes it. In the first case, the trend was well described by a single exponential decay law. In the second case, a double exponential decay law was found to best fit the trend.



**Figure S2.** Representative micrographs of proteinase K crystals grown with protein concentration equal to (a) 6.0 and (b) 9.0 mg/mL. Crystallization conditions involved 0.5% (w/v) agarose gel, 0.5 M NaNO<sub>3</sub>, and 25 mM Na citrate pH 6.5.



**Figure S3.** Batch crystallization of proteinase K carried out with increasing protein concentration (3.0, 3.5, 4.5, 8.0 mg/mL), 0.5 M NaNO<sub>3</sub> and 50 mM Na citrate pH 6.5. Analogous concentrations were implemented in (a) gel-free medium and (b) 0.5% (w/v) agarose gel. Below each vial an enlarged micrograph is reported. Scale bar is 500  $\mu$ m.



**Figure S4.** Counter-diffusion crystallization of catalase in 0.5% (w/v) agarose gel. Enlarged micrographs of highlighted areas are reported. The nucleation density decreased from the top to the bottom of the PCR vial. The yellowy zone of the vial refers to the gel with immobilized protein, the white one refers to the precipitant solution.