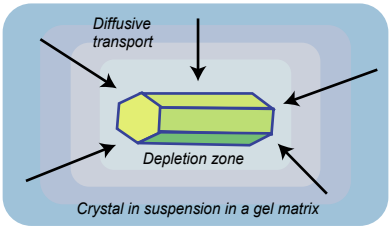
Gels in crystallization of biological macromolecules: additives with numerous properties

Christian Biertümpfel , Jérôme Basquin, Claude Sauter & Dietrich Suck

Gels and crystallization

Gels have been known for long to promote the growth of high-quality crystals of small molecules. During the last decade different types of gels - agarose, silica or polyacrylamide gels - have been tested on the crystallization of biological macromolecules. They are still seldom used, although they provide many advantages from the crystal production to the crystal handling [1].

When a crystal is growing, it takes up and incorporates molecules from the surrounding solution. Thus, the crystal creates a *depletion zone* in the mother liquor. In usual conditions, the resulting concentration gradient and the mass transport are permanently disturbed by convection currents. On the other hand, when a gel is introduced in the crystallization medium, the convection is reduced and the molecule transport towards the crystal is more regular and operates in a diffusive mode.



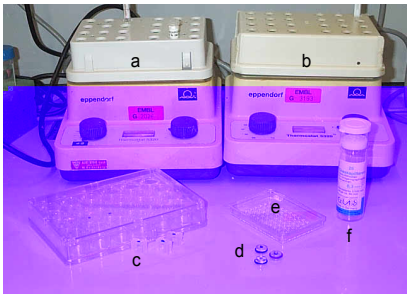
This leads to a crystalline material of improved quality. Indeed, recent comparative studies have shown that crystals grown in gel present a lower mosaicity and produce a more intense diffraction signal [2,3].

Other practical advantages of gels [1]:
- they keep the crystals in suspension in their mother liquor
- they prevent sedimentation
- they promote a growth in 3 dimensions
- they provide a mechanical protection (during soaking, mounting, transport...)

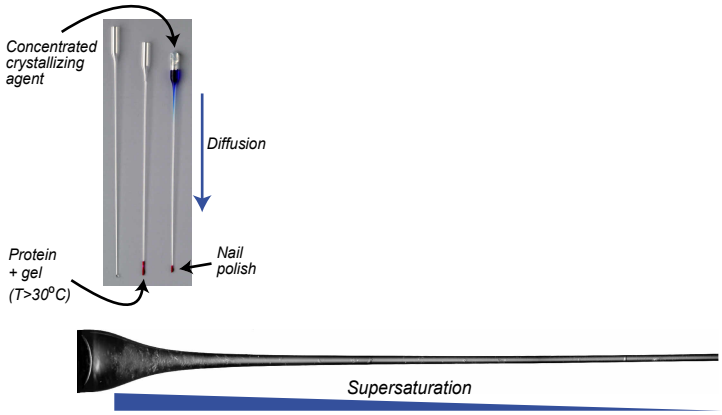
In addition gels are compatible with most common crystallization methods and offer new possibilities like counter-diffusion in capillaries.

Crystallization in agarose gel

Agarose is a very convenient gel to work with. It is even easier when using a gel with a low gelling temperature (~28ºC) which is more compatible with the presence of heat-sensitive macromolecules. A 2% (w/v) agarose stock solution is stored at 4ºC. Before setting-up crystallization trials, this solution is melted at 85-90ºC (a) and kept at 35-45ºC (b). Then it is added to the crystallizing agent or to the macromolecule solution to a final concentration of 0.1-0.2% (w/v). This mixture can either be used in vapor diffusion (c), dialysis (d) or batch (e) assays, and furthermore it can be utilized to perform counter-diffusion experiments in capillaries (f).

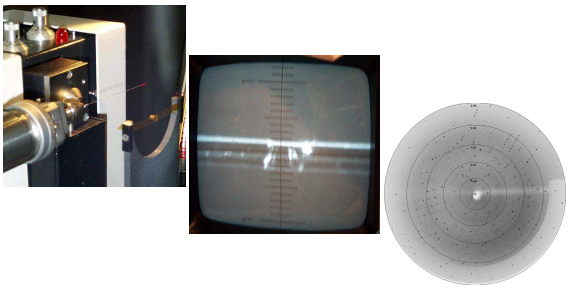


The counter-diffusion method



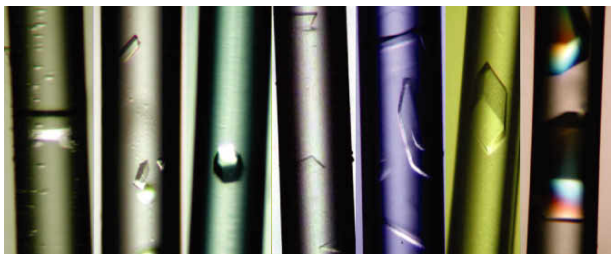
Crystal analysis

Because of the low agarose gel concentration (0.1-0.2 % (w/v)), crystals are easily recovered before mounting in capillaries or in cryo loops. The presence of the gel can even facilitate flash-freezing [7].
As shown below for lysozyme, crystals that were grown in capillaries using the counter-diffusion method can be analyzed *in situ* at room temperature or in cryogenic conditions [6].



A few examples

A Host Factor I (HF-I)	*Escherichia coli*	66 kDa (hexamer)
B Sm-like protein(Sm1)	*Aeropyrym pernix*	59 kDa (heptamer)
C Endonuclease VII (EndoVII)	Bacteriophage T4	36 kDa (dimer)
D EndoVII / DNA cruciform junction	"	36 / 13 kDa
E EndoVII / missmatched DNA duplex	"	36 / 13 kDa
F Lysozyme	*Gallus gallus*	14 kDa (monomer)



A B B C D E F

Capillary diameter: 0.3 mm

Conclusion ...

Crystallization in gel
- is easy to peform
- can be adapted to all current crystallization methods
- makes the use of counter-diffusion techniques possible
- provides better growth conditions
- leads to crystals of enhanced quality

You should definitely try it!

References

[1] M.-C. Robert, O. Vidal, J.-M. Garcia-Ruiz & F. Otalora. Crystallization in gels and related methods In Crystallization of Nucleic Acids and Proteins: A Practical Approach (second edition). A. Ducruix & R. Giegé, eds., IRL Press (1999) 149-175.

[2] B. Lorber, C. Sauter, J.D. Ng, D.-W. Zhu, R. Giegé, O. Vidal, M.-C. Robert & B. Capelle. Characterization of protein and virus crystals by quasi-planar wave topography: A comparison between crystals grown in solution and in agarose gel. J. Crystal Growth 204 (1999) 357-368.

[3] B. Lorber, C. Sauter, M.-C. Robert, B. Capelle & R. Giegé. Crystallization within agarose gel in microgravity improves the quality of thaumatin crystals. Acta Cryst. D55 (1999). 1491-1494.

[4] J.M.Garcia-Ruiz, F. Otalora, M.L. Novella, J.A. Gavira, C. Sauter & O. Vidal. Protein crystallization: A history of a supersaturation wave. J. Crystal Growth (2001) in press.

[5] J.M. Garcia-Ruiz & A. Moreno. Investigations on protein crystal growth by the gel acupuncture method. Acta Cryst. D50 (1994) 484-490.

[6] F.J. Lopez-Jaramillo, J.M. Garcia-Ruiz, J.A. Gavira & F. Otalora. Crystallization and cryocrystallography inside X-ray capillaries. J. Appl. Cryst. 34 (2001) 365-370.

[7] D.-W. Zhu, B. Lorber, C. Sauter, J.D. Ng, P. Bénas, C. Le Grimellec & R. Giegé. Growth kinetics, diffraction properties and effect of agarose on the stability of a novel crystal form of *Thermus thermophilus* aspartyl-tRNA synthetase-1. Acta Cryst. D57 (2001) 552-558.