



# Temperature in macromolecular crystallisation

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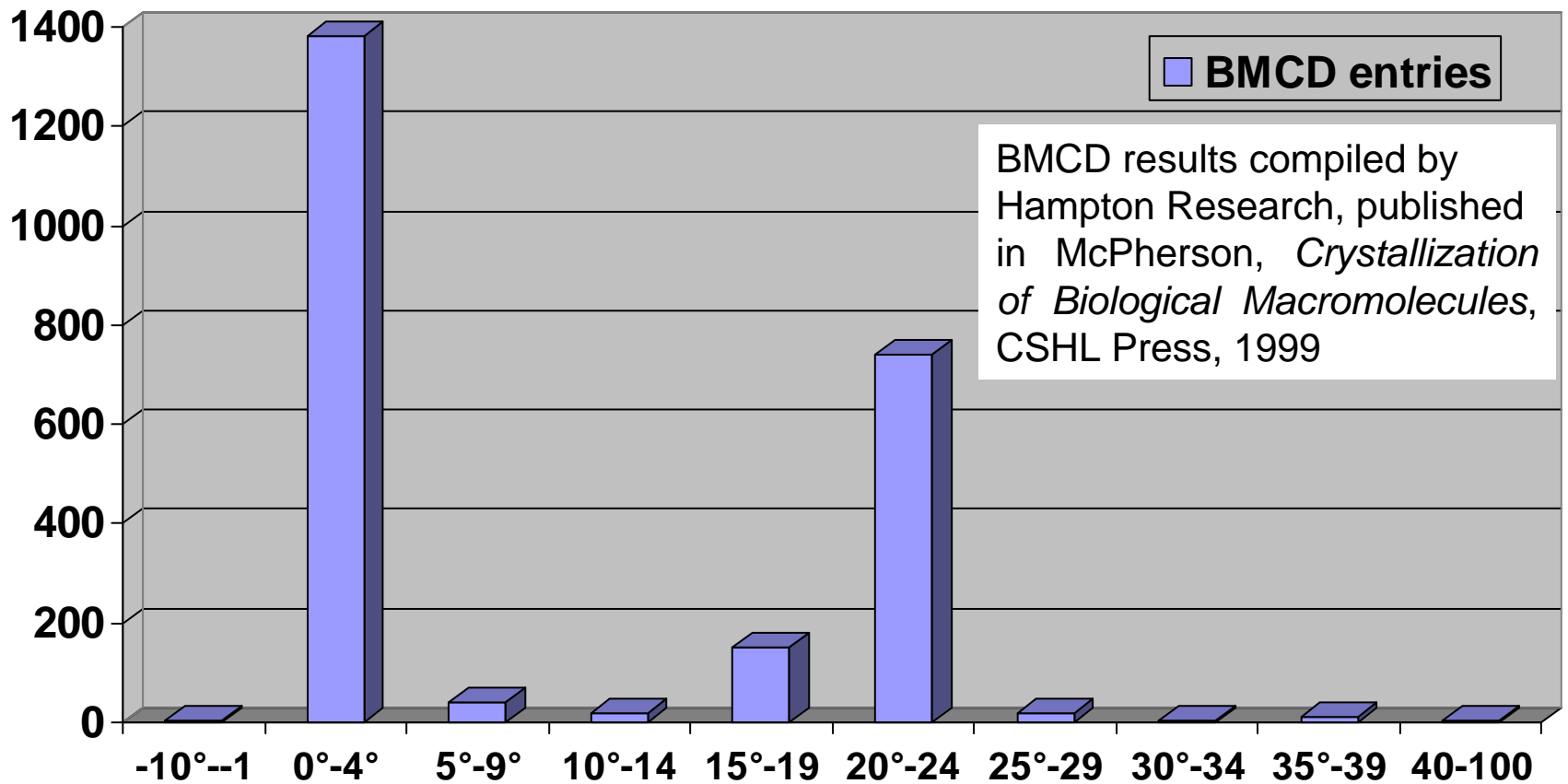
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# Three aspects of the use of temperature

- (1) **a crystallisation parameter**, to be included in the parameter space search alongside the type and concentration of precipitating agent, pH and buffer, protein concentration etc.
- (2) **a means to induce crystallisation** by increasing the supersaturation (commonplace in small molecule crystallisation, too rare for macromolecules)
- (3) **a means to optimise crystal growth**, by separating nucleation from the growth stage

# (1) Temperature as one more parameter to test



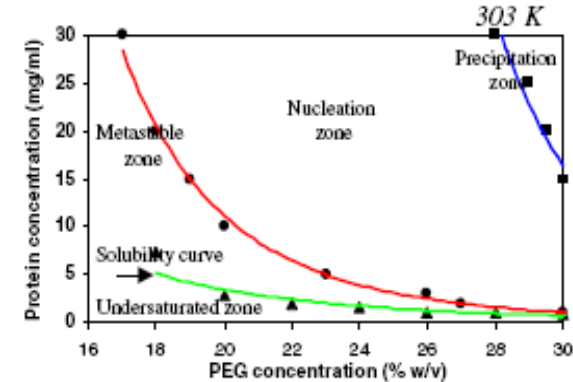
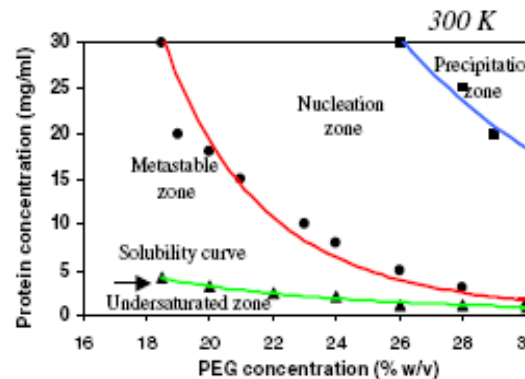
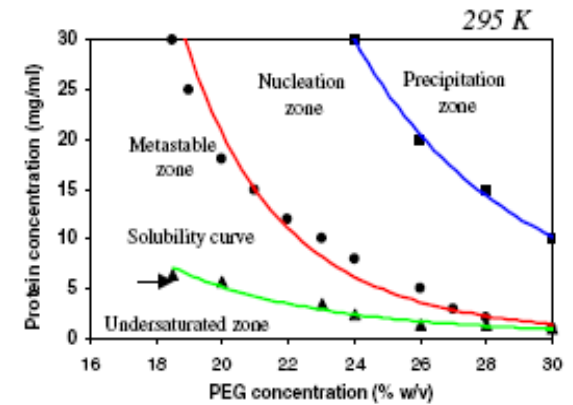
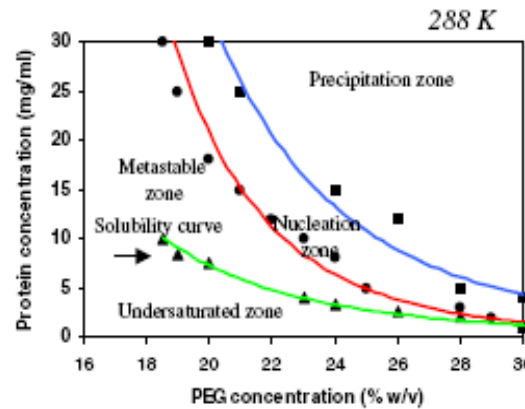
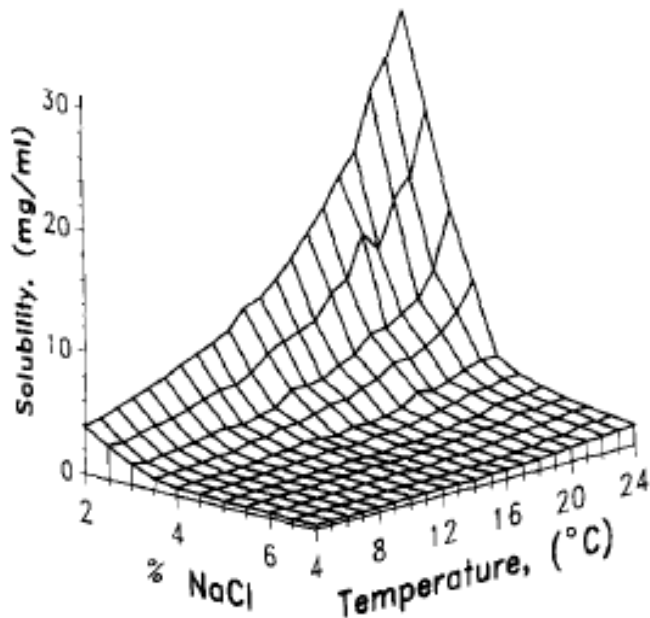
“there is clearly room for more creative use of temperature” (McPherson, 1999)

# The solubility of many proteins depends on temperature

- 86% of proteins tested by Christopher *et al.* (1998) *J. Cryst. Growth* **191**,820, out of which 54% have direct temperature dependence and the rest have retrograde dependence. 9 of 13 retrograde solubility cases were in high salt, 4 of 13 in low salt, none from PEG.
- Results corroborated by Zhu *et al.* (2006) *J. Struct. Biol.* 154,297: **80% of proteins tested displayed temperature dependence.**
- Temperature dependence is often shallow and can become virtually insignificant at high ionic strength, but becomes much steeper at low ionic strength, with PEG, MPD and organic solvents. Also retrograde solubility is more frequent at high ionic strength, in the cases where there still is temperature dependence (see Lloyd Haire in Bergfors (*ed*) *Protein Crystallization*, IUL 1999).
- Thus the temperature - solubility function is not a property of the protein itself but of the protein-salt system.

# Examples:

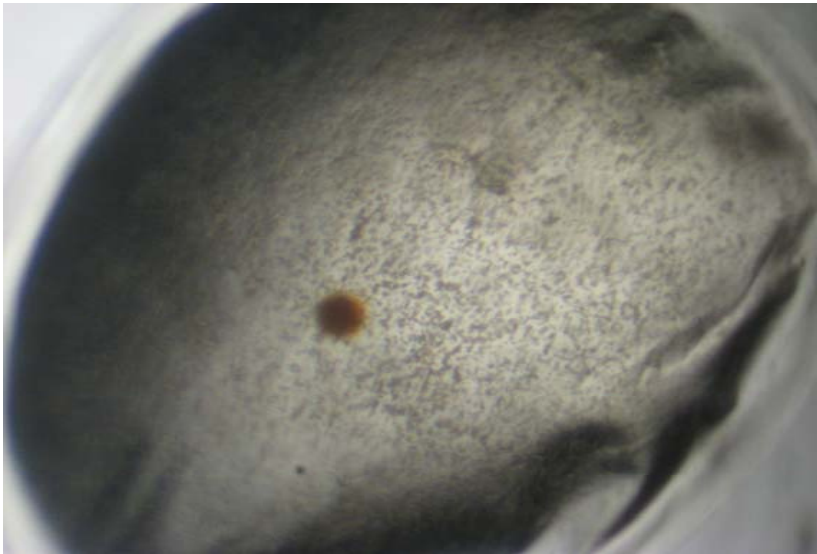
Lysozyme at pH 4.6, from Cacciopo & Pusey (1991) *J. Cryst. Growth* **114**,286



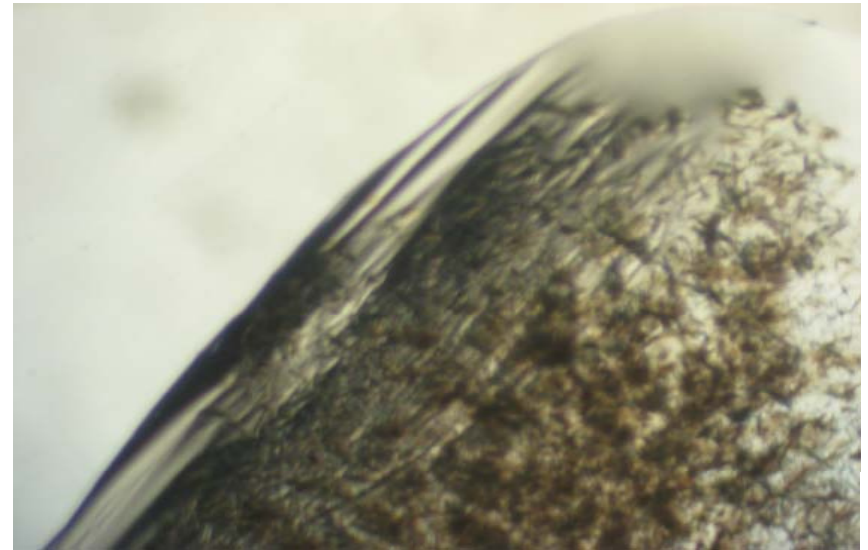
Snake muscle fructose-1,6-bisphosphatase, from Zhu *et al.* (2006) *J. Struct. Biol.* **154**,297

A very interesting example, keep in mind for later !

# Bacterial ferredoxin triple mutant

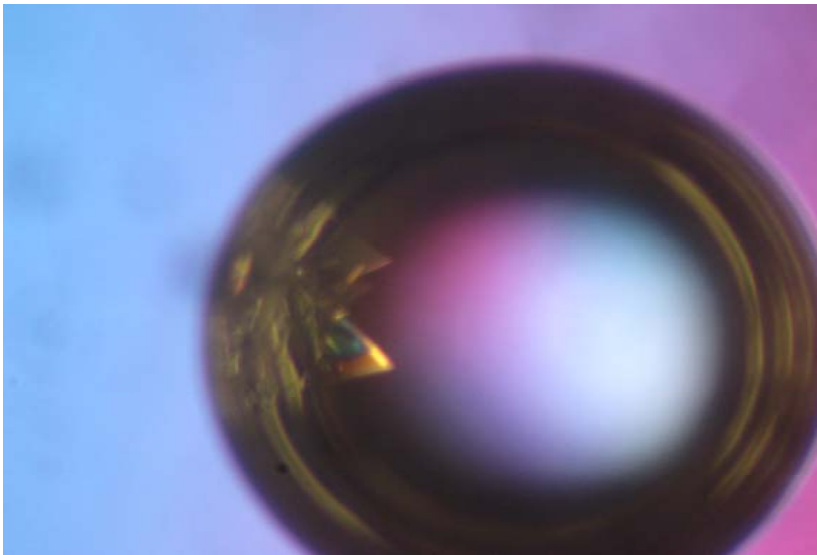


At  $4^{\circ}\text{C}$ , after extensive precipitant concentration and pH screening – precipitation



After 2 weeks at  $4^{\circ}\text{C}$ , trial transferred to  $16^{\circ}\text{C}$  – tiny needles, often single

Nucleic acids can be successfully tested over an even wider temperature range, typically at least 4-37°C

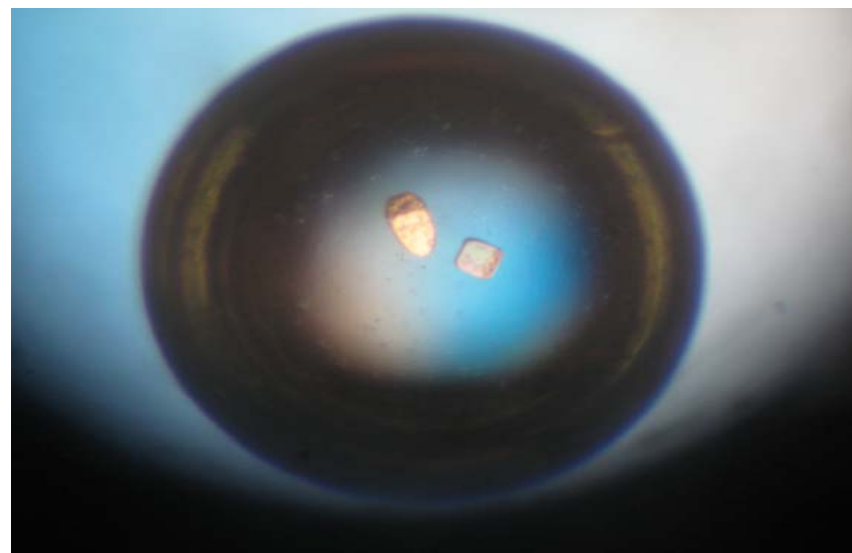
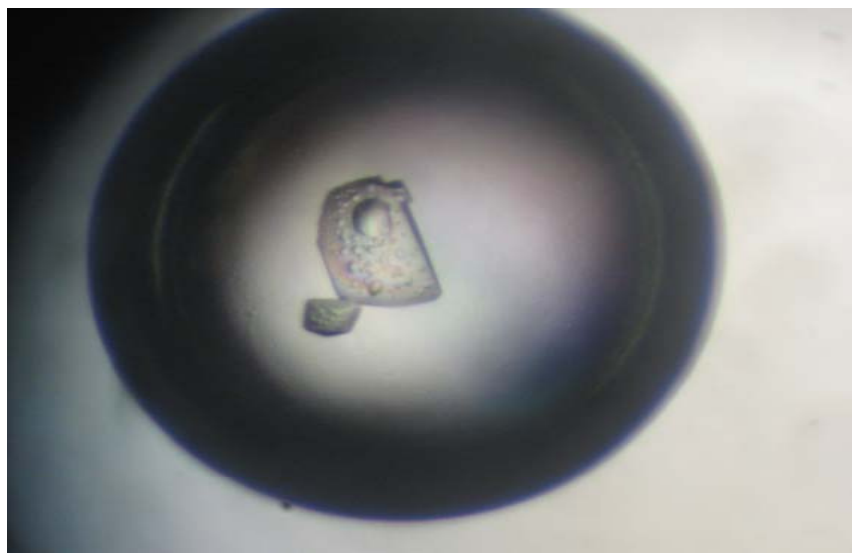


Bacterial RNA A-site 17mer at 16°C, at high salt (A.S.) conditions (optimised). Crystals, rarely single, diffract to 7 Å at best.



Bacterial RNA A-site 17mer at 37°C, at high salt (A.S.) conditions (optimised). Crystals are almost always single, and diffract to 1.6 Å.

More RNA A-site crystals obtained after transfer to 37°C...



# Solubility (a thermodynamic quantity) is not the only route through which temperature can influence crystallisation

- Temperature can also influence the kinetics of crystallisation, by:
  - (1) **changing the speed of mass and heat diffusion** in the crystallisation solution. Low temperature can therefore sometimes increase the rate of nucleation, whilst reducing the rate of growth (see eg. Lorber & Giegé (1992) *J. Cryst. Growth* **122**,168).
  - (2) **changing the rate of equilibration** in vapour diffusion, dialysis, free interface diffusion...

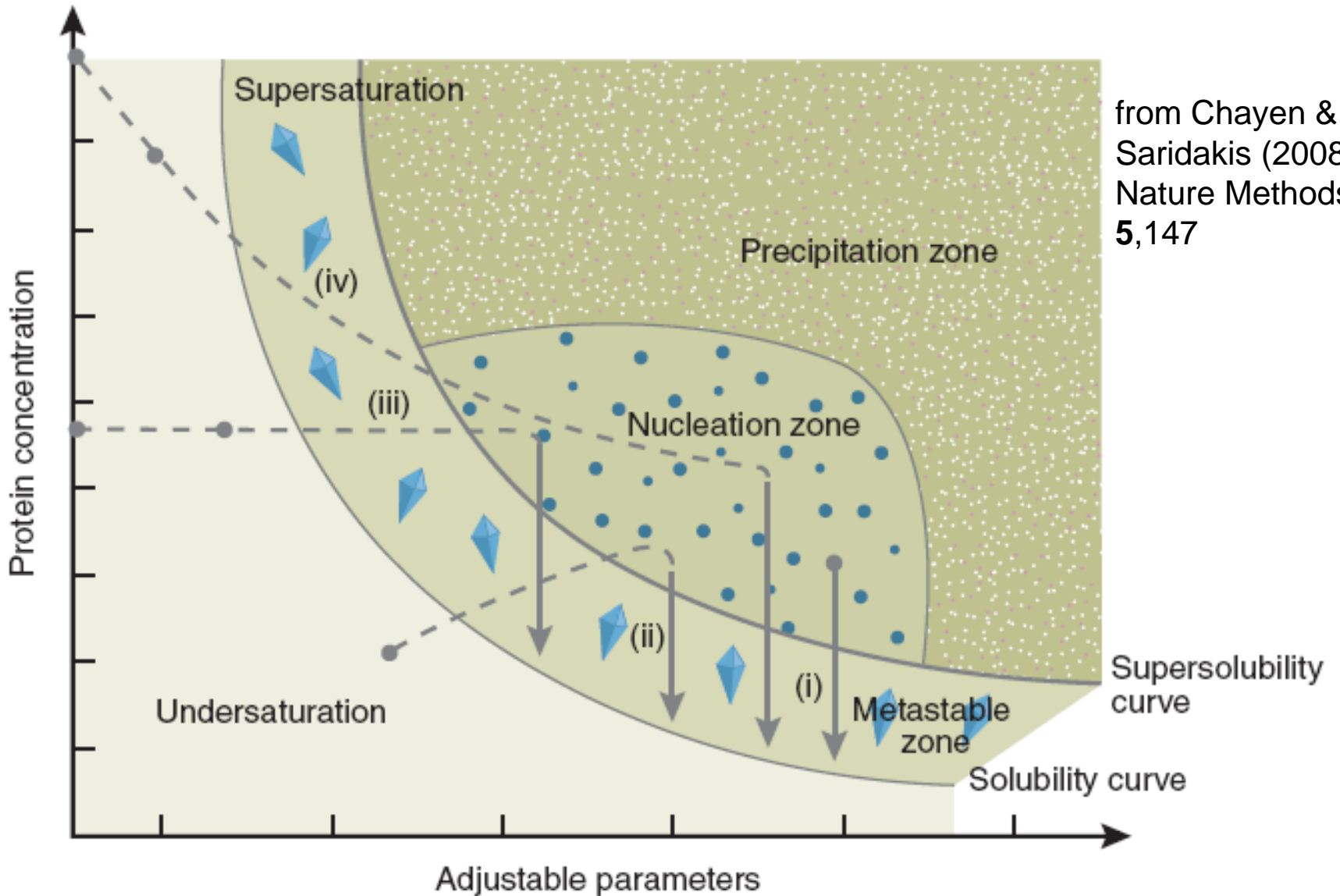
## (2) Temperature as a means for inducing crystallisation

- Small-molecule crystallisers often use a controlled, slow drop in temperature to induce crystallisation
- It is a minimally invasive, reversible method to modify the level of supersaturation
- It is however much less used in the macromolecular crystallisation lab, due in part to the belief that the protein-dependence is too shallow (which is generally true only in high salt) and in part to the lack of dedicated apparatus
- For reports of the use of temperature shift in macromolecular crystallisation, see L. Lloyd Haire, *in* T.M. Bergfors (ed) *Protein Crystallization*, I.U.L. 1999, pp. 65-68 and refs therein.

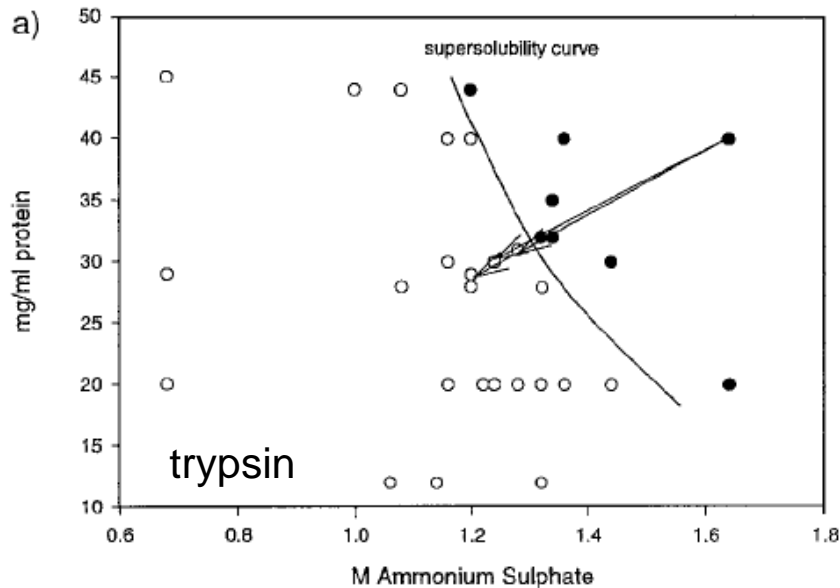
### (3) Temperature as a means to separate the nucleation and growth stages of crystallisation

- The temperature-dependence of solubility can be used for modifying the supersaturation level with respect to protein of the solution during the course of the experiment – thus **uncoupling the nucleation from the crystal growth stage**

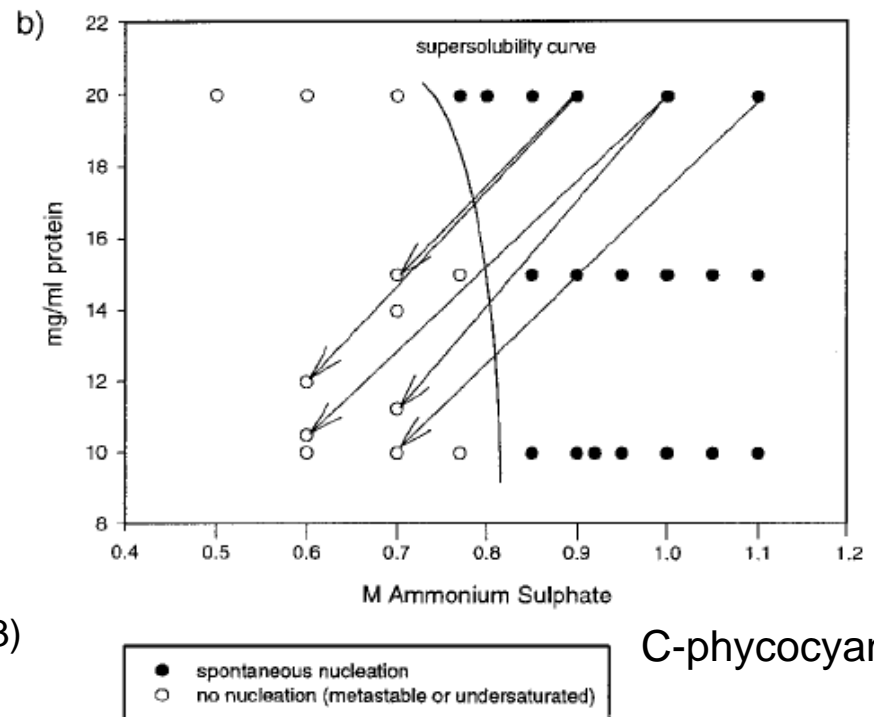
# Crystallisation Phase Diagrams



# Uncoupling nucleation and growth

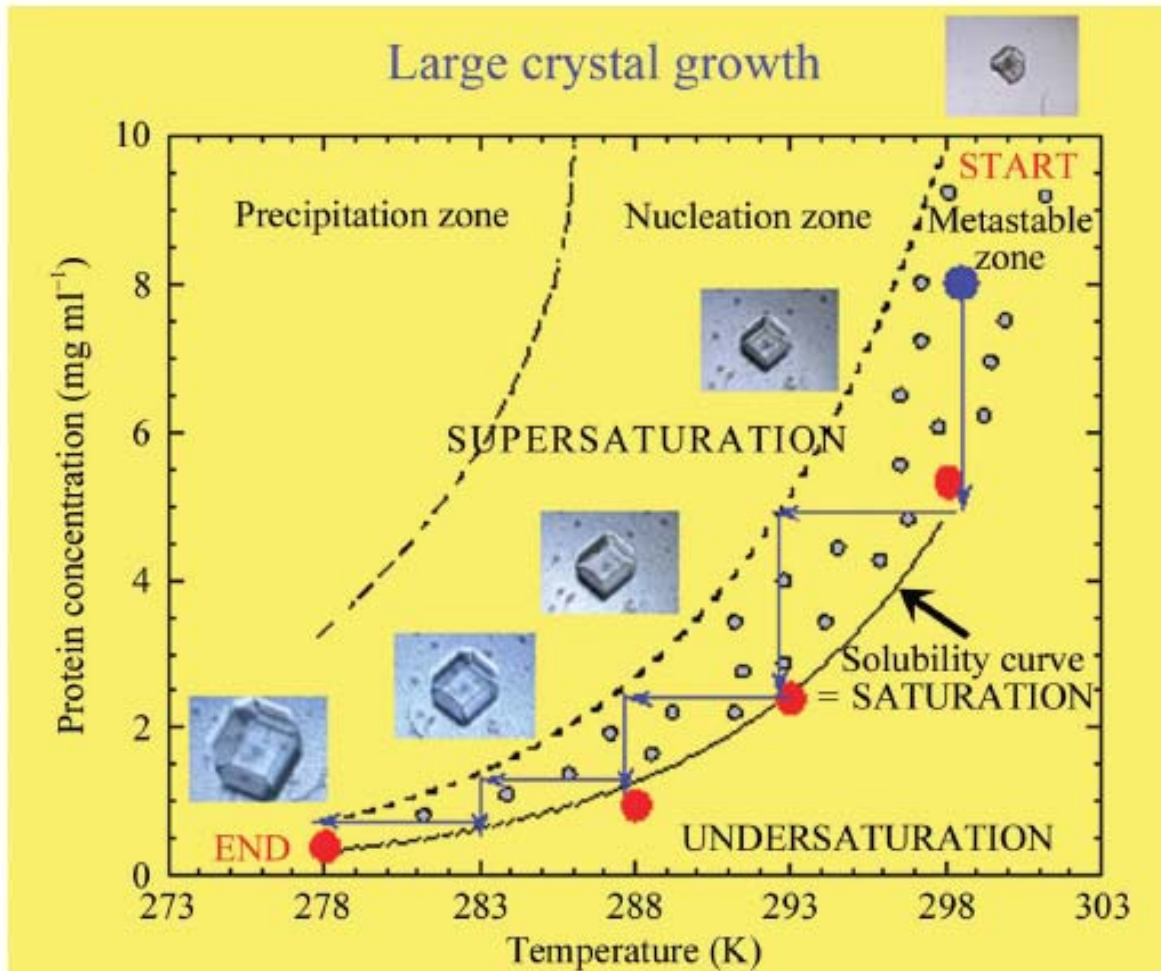


from Saridakis & Chayen (2003)  
*Biophys. J.* **84**,1218)



Such uncoupling can also be done very advantageously using temperature instead of precipitant/ protein concentration as the dynamically varying parameter (Lloyd Haire (1996) *PhD Thesis*, University of London; Penkova *et al.* (2002) *Acta Cryst. D* **58**,1606).

# A more sophisticated approach for nucleation/growth uncoupling: combination of seeding and temperature control



(Budayova-Spano *et al.* (2007) *Acta Cryst. D* **63**, 339)

# Some final remarks

- Temperature control can be more crucial for **membrane proteins** than for other macromolecules. Indeed, **detergent solubility is much more temperature dependent than protein solubility**. The phase behaviour of protein-detergent solutions depends thus crucially on temperature (Garavito & Picot (1991) *J. Cryst. Growth* **110**,89; Lorber *et al.* (1991) *J. Cryst. Growth* **110**,103; Sennoga *et al.* (2003) *Acta Cryst. D***59**,239.)
- Crystal form is one of the most temperature dependent aspects of crystallisation. Thus, if crystallisation has been successfully optimised but structure determination is not forthcoming and the possibility of a different space group is thought to be worth investigating, a new round of optimisation adding temperature in the search space may be useful.



Thank you!