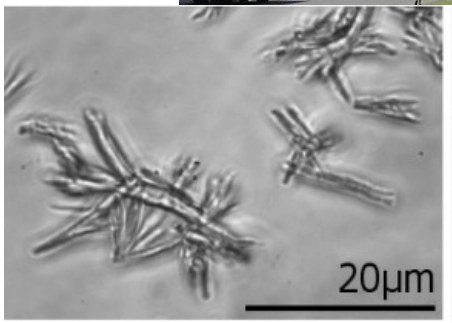
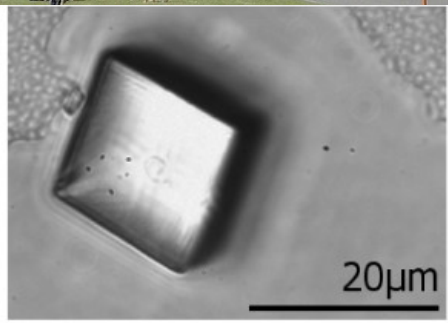
Winter School on Structural Cell Biology

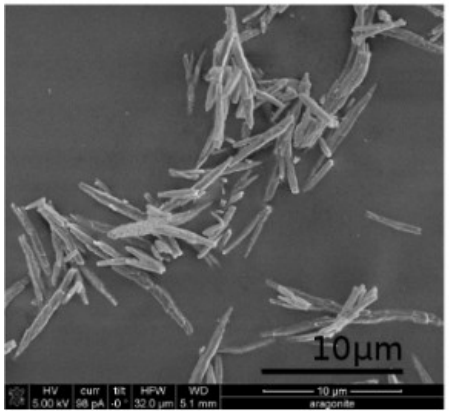
Protein crystallization

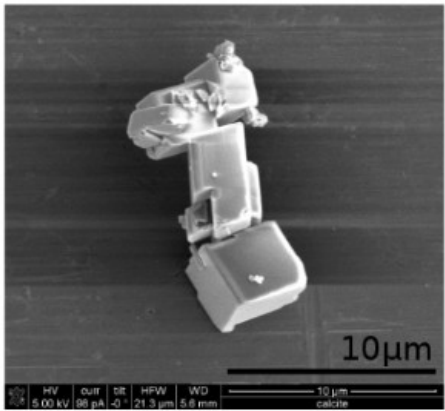
Josef Houser

Crystal

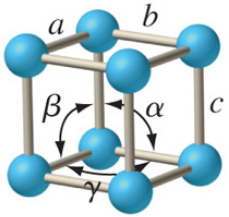




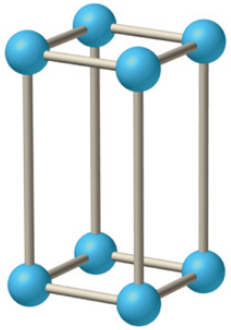




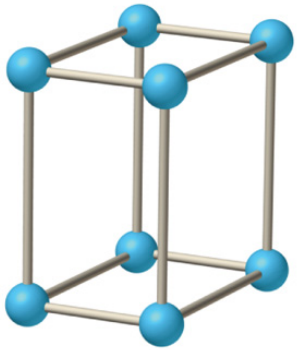
7 Crystal lattices



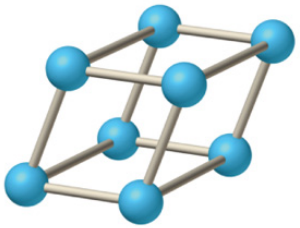
Simple cubic
$a = b = c$
$\alpha = \beta = \gamma = 90^\circ$



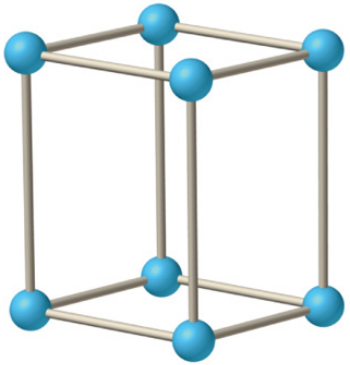
Tetragonal
$a = b \neq c$
$\alpha = \beta = \gamma = 90^\circ$



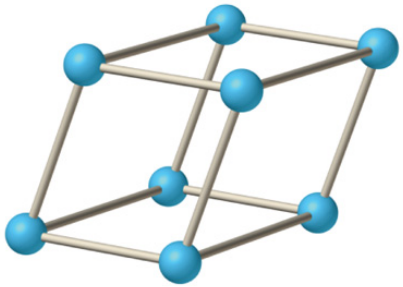
Orthorhombic
$a \neq b \neq c$
$\alpha = \beta = \gamma = 90^\circ$



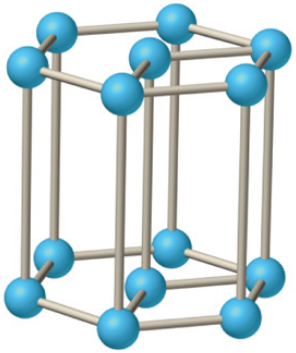
Rhombohedral
$a = b = c$
$\alpha = \beta = \gamma \neq 90^\circ$



Monoclinic
$a \neq b \neq c$
$\gamma \neq \alpha = \beta = 90^\circ$

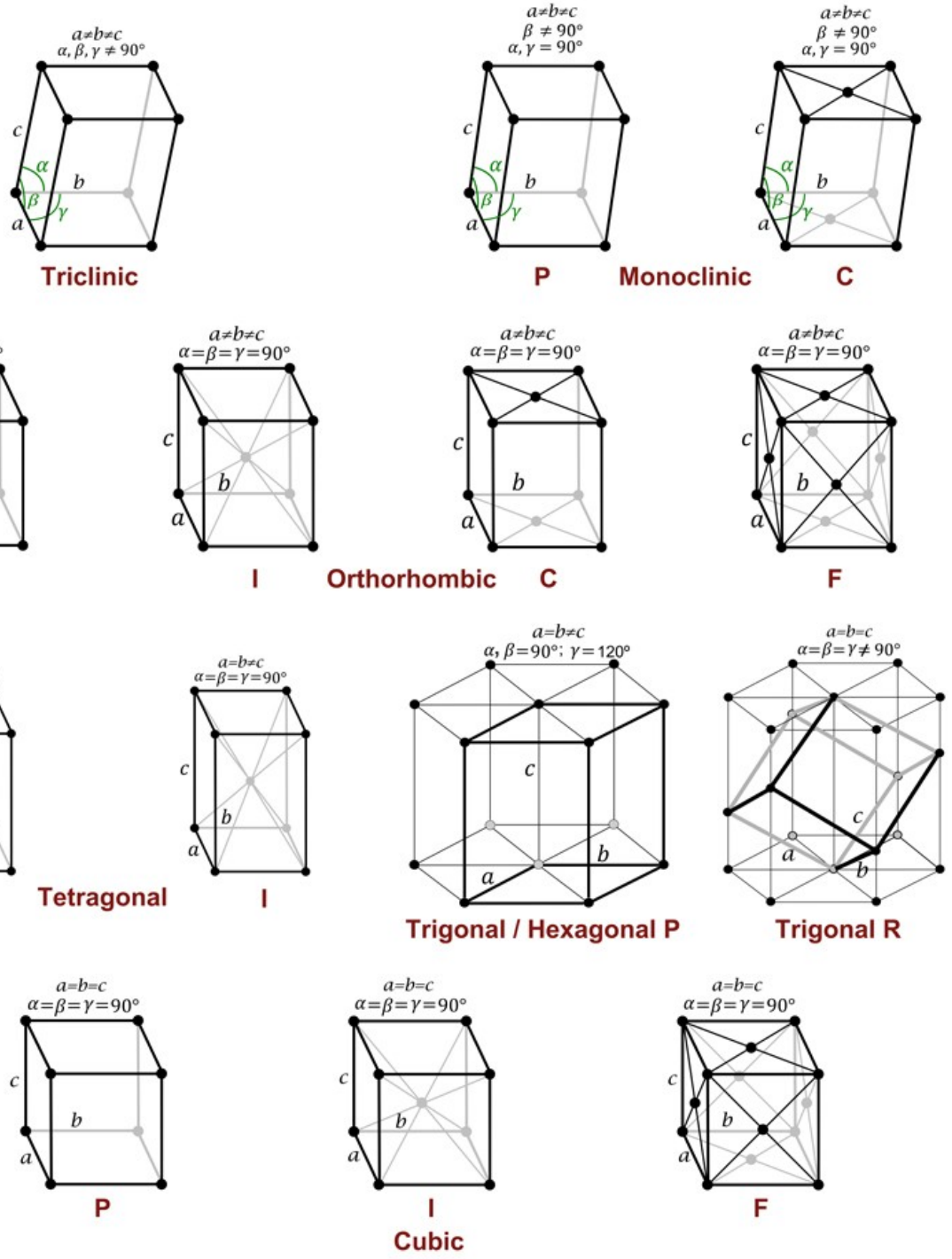


Triclinic
$a \neq b \neq c$
$\alpha \neq \beta \neq \gamma \neq 90^\circ$



Hexagonal
$a = b \neq c$
$\alpha = \beta = 90^\circ, \gamma = 120^\circ$

14 Bravais lattices

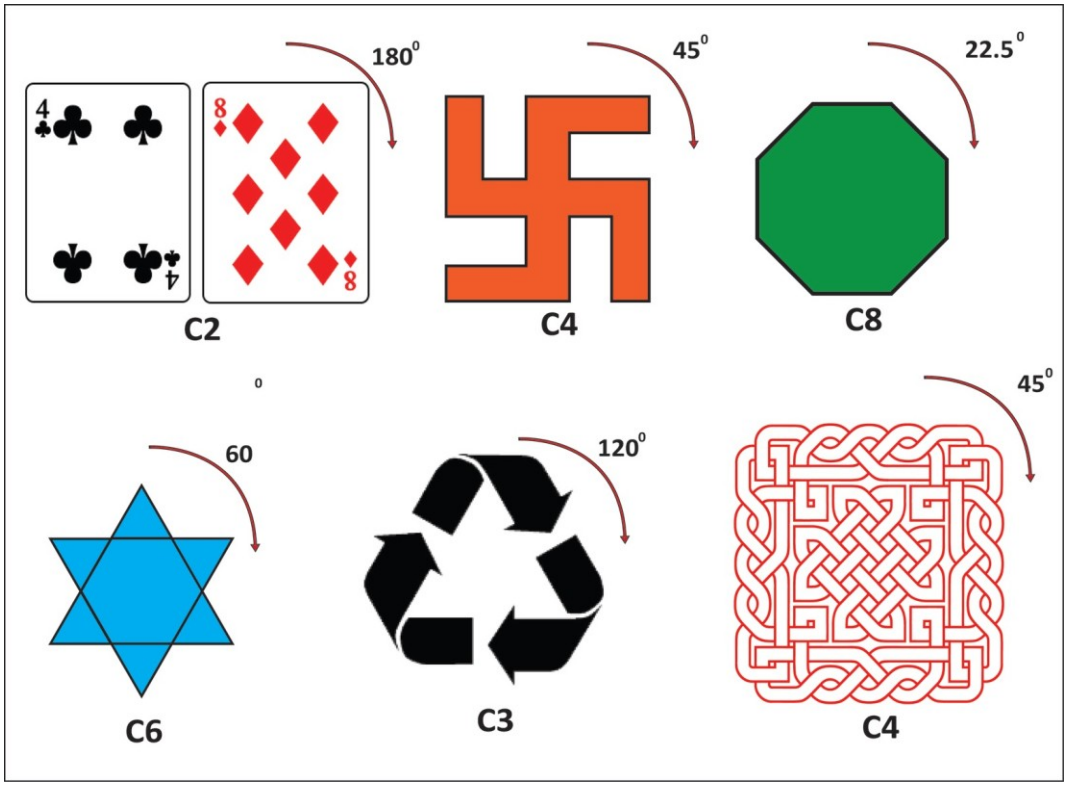


Symmetry – Space groups

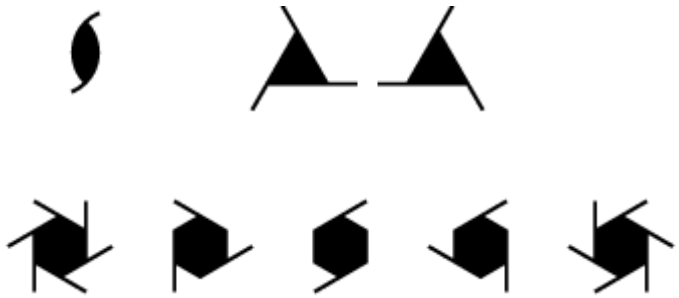
2D symmetry

3D symmetry

Proteins are chiral – no mirror symmetry



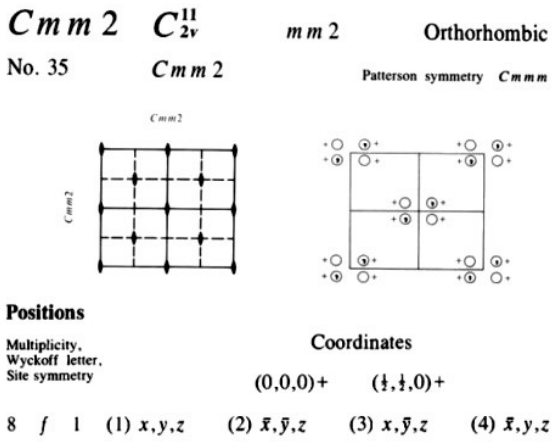
Screw axis



Combination of Bravais lattices and symmetry operations leads to **230 possible space groups** (not all for proteins).

System	Bravais Lattice	Point Group	Space Group						Fraction
Triclinic	P	1	$P1$						1/2
		$\bar{1}$	$P\bar{1}$						
Monoclinic	P	2	$P2$	$P2_1$	$C2$				1/4
	C	m	Pm	Pc	Cm	Cc			
		$2/m$	$P2/m$	$P2_1/m$	$C2/m$	$P2/c$	$P2_1/c$	$C2/c$	
Orthorhombic	P	222	$P222$	$P222_1$	$P2_12_12$	$P2_12_12_1$	$C222_1$	$C222$	1/8
	C		$F222$	$I222$	$I2_12_12_1$				
	F	$mm2$	$Pmm2$	$Pmc2_1$	$Pcc2$	$Pma2$	$Pca2_1$	$Pnc2$	
	I		$Pmn2_1$	$Pba2$	$Pna2_1$	$Pnn2$	$Cmm2$	$Cmc2_1$	
			$Ccc2$	$Amm2$	$Abm2$	$Ama2$	$Aba2$	$Fmm2$	
			$Fdd2$	$Imm2$	$Iba2$	$Ima2$			
		mmm	$Pmmm$	$Pnnn$	$Pccm$	$Pban$	$Pmma$	$Pnna$	
			$Pmna$	$Pcca$	$Pbam$	$Pccn$	$Pbcm$	$Pnnm$	
			$Pmmn$	$Pbcn$	$Pbca$	$Pnma$	$Cmcm$	$Cmca$	
			$Cmmm$	$Cccm$	$Cmma$	$Ccca$	$Fmmm$	$Fddd$	
			$Immm$	$Ibam$	$Ibca$	$Imma$			
Tetragonal	P	4	$P4$	$P4_1$	$P4_2$	$P4_3$	$I4$	$I4_1$	1/8
	I	$\bar{4}$	$P\bar{4}$	$I\bar{4}$					
		$4/m$	$P4/m$	$P4_2/m$	$P4/n$	$P4_2/n$	$I4/m$	$I4_1/a$	
		422	$P422$	$P42_12$	$P4_122$	$P4_12_12$	$P4_222$	$P4_22_12$	1/16
			$P4_322$	$P4_32_12$	$I422$	$I4_122$			
		$4mm$	$P4mm$	$P4bm$	$P4_2cm$	$P4_2nm$	$P4cc$	$P4nc$	
			$P4_2mc$	$P4_2bc$	$I4mm$	$I4cm$	$I4_1md$	$I4_1cd$	
		$\bar{4}2m$	$P\bar{4}2m$	$P\bar{4}2c$	$P\bar{4}2_1m$	$P\bar{4}2_1c$	$P\bar{4}m2$	$P\bar{4}c2$	
			$P\bar{4}b2$	$P\bar{4}n2$	$I\bar{4}m2$	$I\bar{4}c2$	$I\bar{4}2m$	$I\bar{4}2d$	
		$4/mmm$	$P4/mmm$	$P4/mcc$	$P4/nbm$	$P4/nnc$	$P4/mbm$	$P4/mnc$	
			$P4/nmm$	$P4/ncc$	$P4_2/mmc$	$P4_2/mcm$	$P4_2/nbc$	$P4_2/nnm$	
			$P4_2/mbc$	$P4_2/mnm$	$P4_2/nmc$	$P4_2/ncm$	$I4/mmm$	$I4/mcm$	
			$I4_1/amd$	$I4_1/acd$					
Trigonal/ rhombohedral	P	3	$P3$	$P3_1$	$P3_2$	$R3$			1/6
	R	$\bar{3}$	$P\bar{3}$	$R\bar{3}$					
		32	$P312$	$P321$	$P3_112$	$P3_121$	$P3_212$	$P3_221$	1/12
			$R32$						
		$3m$	$P3m1$	$P31m$	$P3c1$	$P31c$	$R3m$	$R3c$	
		$\bar{3}m$	$P\bar{3}1m$	$P\bar{3}1c$	$P\bar{3}m1$	$P\bar{3}c1$	$R\bar{3}m$	$R\bar{3}c$	
Hexagonal	P same as trigonal	6	$P6$	$P6_1$	$P6_5$	$P6_2$	$P6_4$	$P6_3$	1/12
		$\bar{6}$	$P\bar{6}$						
		$6/m$	$P6/m$	$P6_3/m$					
		622	$P622$	$P6_122$	$P6_522$	$P6_222$	$P6_422$	$P6_322$	1/24
		$6mm$	$P6mm$	$P6cc$	$P6_3cm$	$P6_3mc$			
		$\bar{6}m\bar{2}$	$P\bar{6}m2$	$P\bar{6}c2$	$P\bar{6}2m$	$P\bar{6}2c$			
		$6/mmm$	$P6/mmm$	$P6/mcc$	$P6_3/mcm$	$P6_3/mmc$			
Cubic	P	23	$P23$	$F23$	$I23$	$P2_13$	$I2_13$		1/24
	I	$m3$	$Pm3$	$Pn3$	$Fm3$	$Fd3$	$Im3$	$Pa3$	
	F		$Ia3$						
		432	$P432$	$P4_232$	$F432$	$F4_132$	$I432$	$P4_332$	1/48
			$P4_132$	$I4_132$					
		$\bar{4}3m$	$P\bar{4}3m$	$F\bar{4}3m$	$I\bar{4}3m$	$P\bar{4}3n$	$F\bar{4}3c$	$I\bar{4}3d$	
		$m3m$	$Pm3m$	$Pn3n$	$Pm3n$	$Pn3m$	$Fm3m$	$Fm3c$	
			$Fd3m$	$Fd3c$	$Im3m$	$Ia3d$			

[a] The 11 Laue symmetries are separated by horizontal lines.



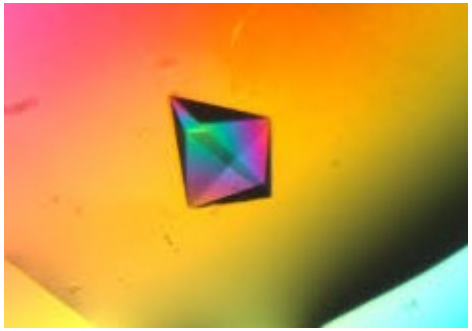
$C\,m\,m\,2$ C_{2v}^{11} $m\,m\,2$ Orthorhombic

No. 35 $C\,m\,m\,2$ Patterson symmetry $C\,m\,m\,m$

Positions

Multiplicity, Wyckoff letter, Site symmetry	Coordinates (0,0,0)+	(½,½,0)+
8 f 1	(1) x,y,z	(2) $\bar{x},\bar{y},z$ (3) $x,\bar{y},z$ (4) $\bar{x},y,z$

Example of space group information from International Crystallographic Tables



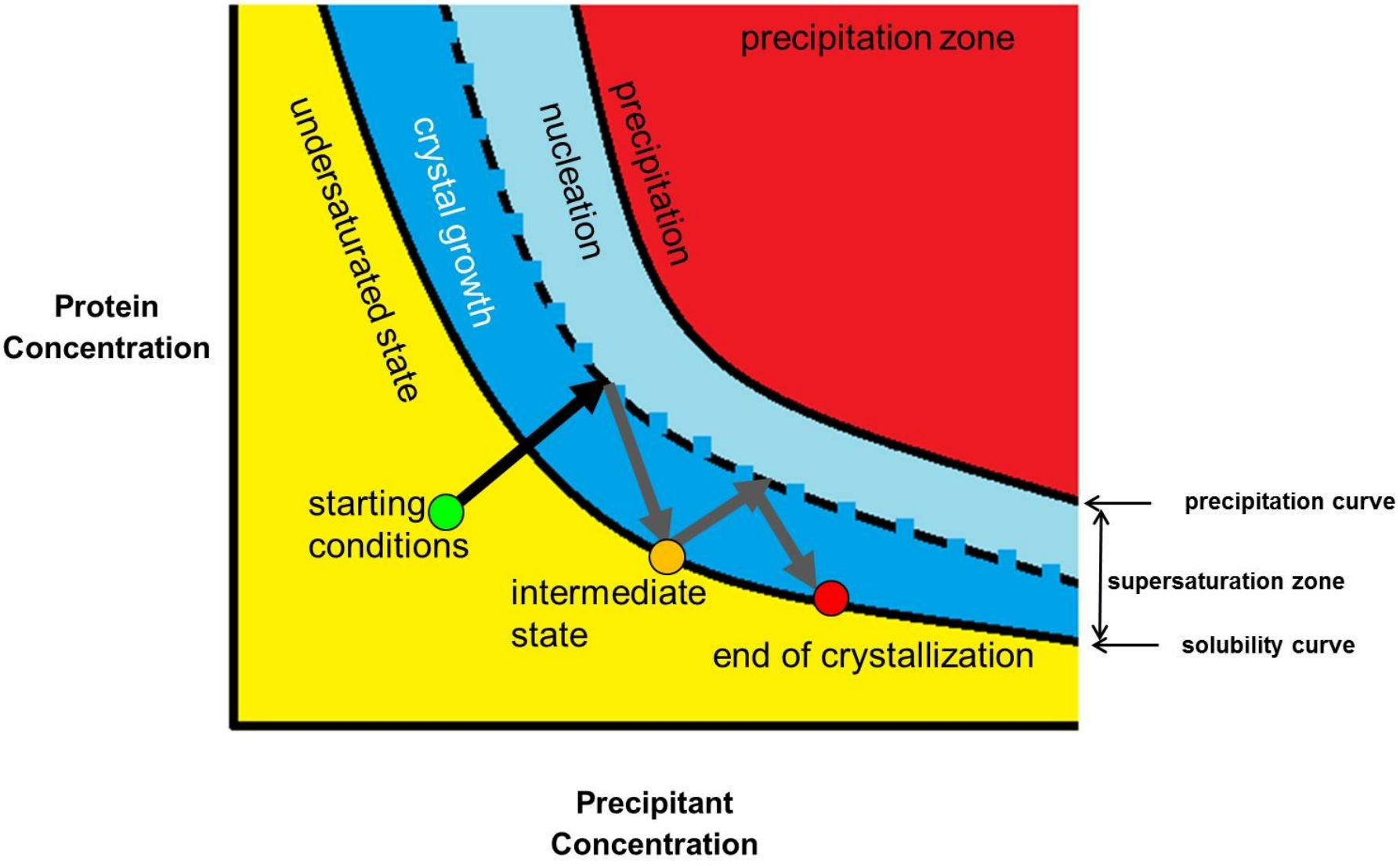
Protein

- Big molecule
- Temperature sensitive
- Hundreds-thousands of rotating bonds
- Weak interactions mostly involved
- High solvent content (30-70 %)
- Fragile crystals

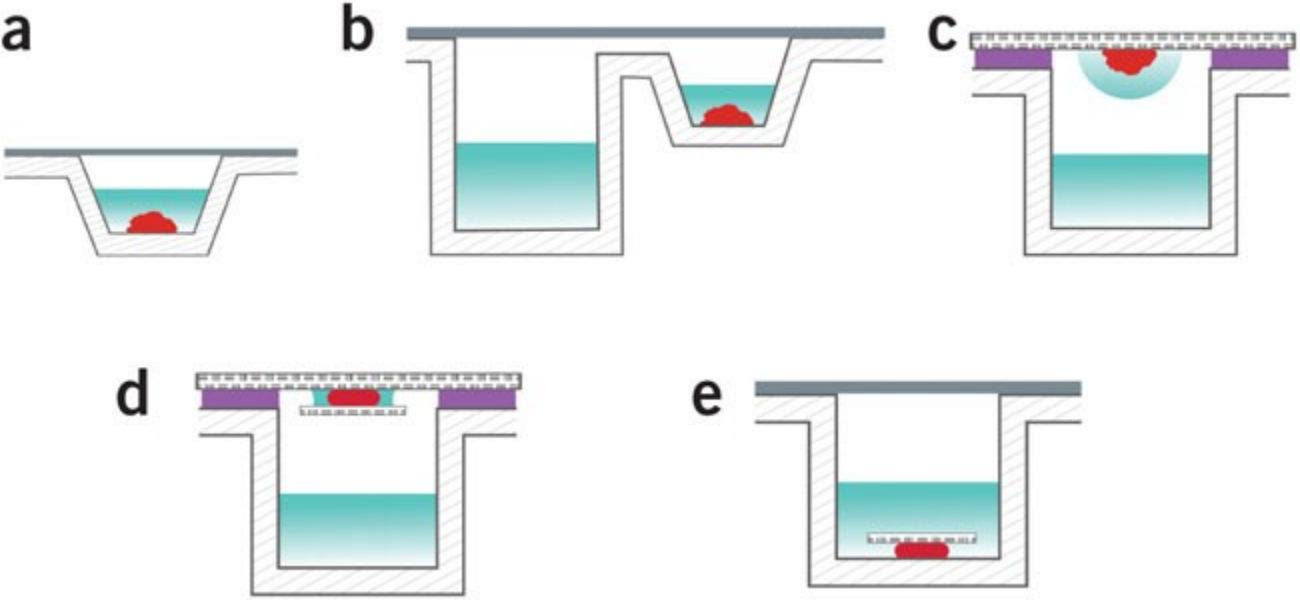
Inorganic salt (ev. organics)

- Small molecule
- Thermostable
- None/few rotating bonds
- Strong (coulombic) interactions frequent
- Low/none solvent content
- Hard crystals

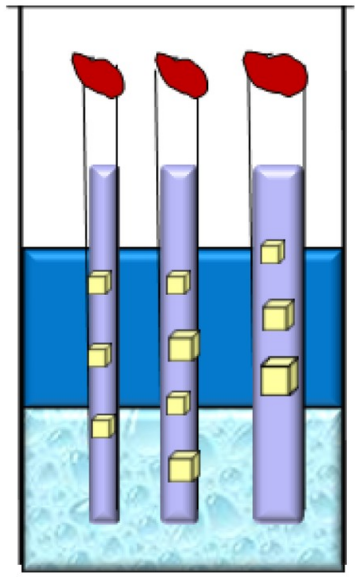
Phase diagram



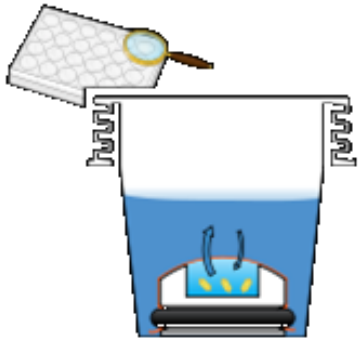
Protein crystallization techniques



Protein crystallization by vapour diffusion: (**a**) microbatch, (**b**) sitting drop and (**c**,**d**) hanging drop. In **d** and **e**, a sandwich is made of the mesophase (red) by placing a small glass coverslip (hatched) (**d**) below or (**e**) above the bolus. *From Nature Protocols*

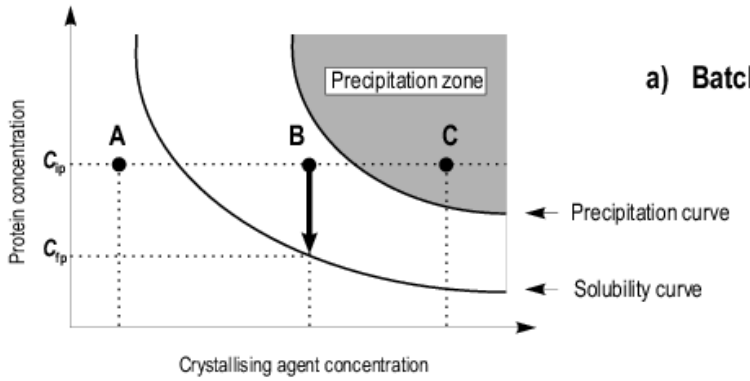


Protein crystallization by counter diffusion in capillaries.

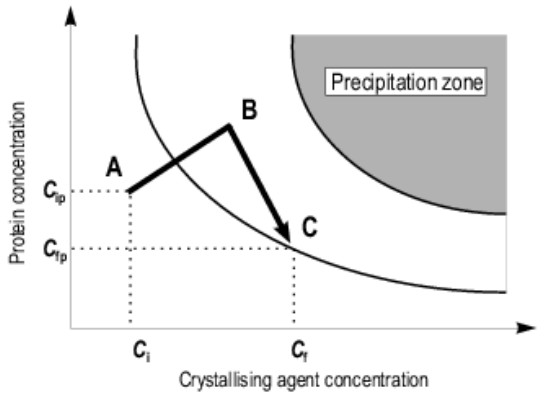


Protein crystallization by dialysis.

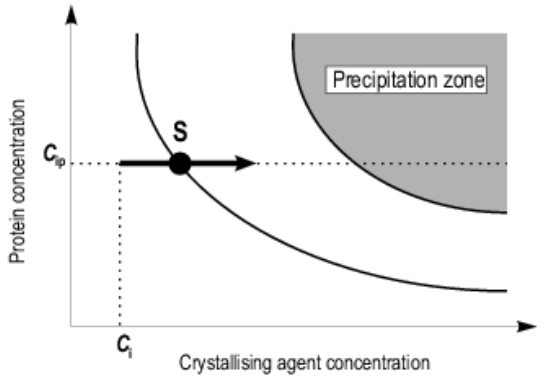
Various techniques = various path in the phase diagram



a) Batch set-up



b) Vapour-diffusion set-up

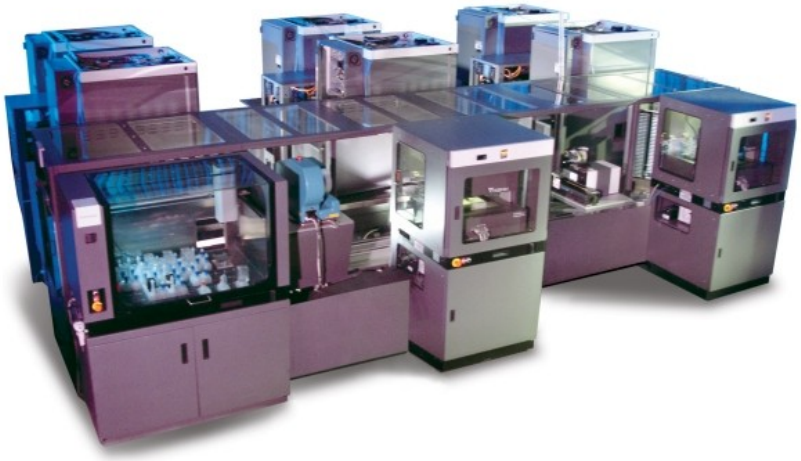


c) Dialysis set-up

Automatization vs. manual work

- ❑ High-throughput
- ❑ Low volumes (20-150 nl)
- ❑ Reproducibility

- ❑ Individual design
- ❑ Immediate visual control
- ❑ Complex sample handling



Further reading

- http://journals.iucr.org/
- Naomi E. Chayen: Protein Crystallization Strategies for Structural Genomics, 2007
- Terese M. Bergfors: Protein Crystallization, 2009
- Alexander McPherson: Introduction to Macromolecular Crystallography, 2011
- etc.