Crystallography basics
-
Crystallization

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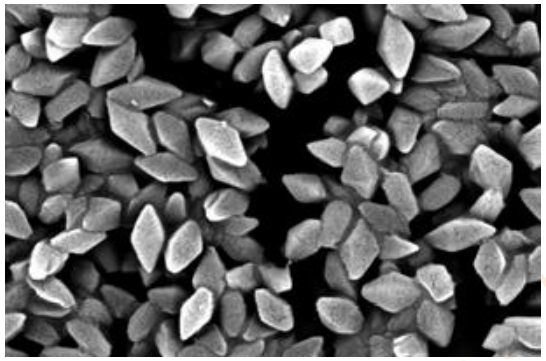
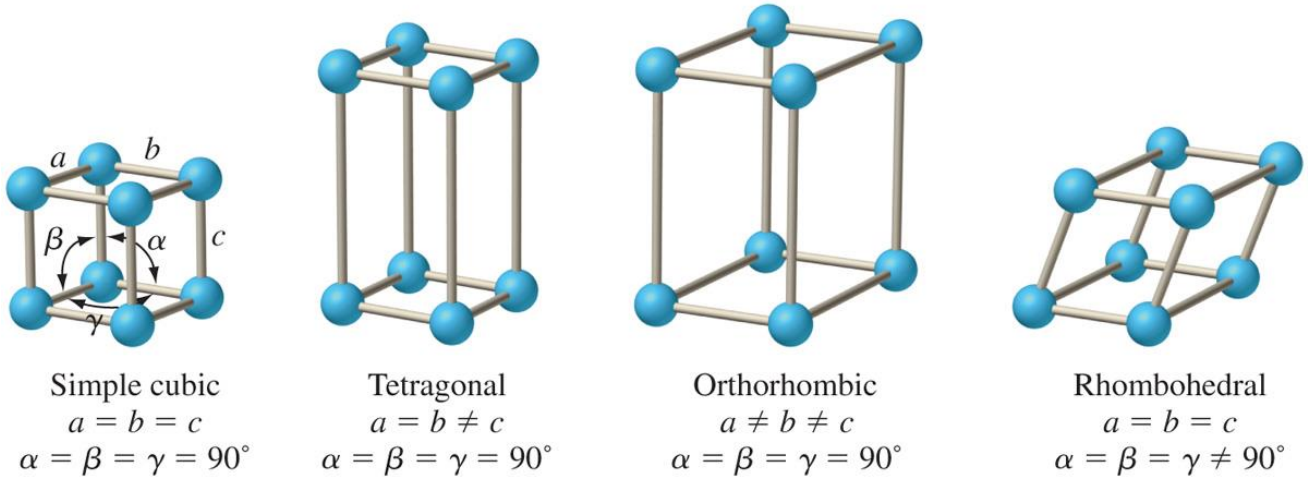
S1004 Methods for structural characterization of biomolecules

Crystal



Crystal lattice

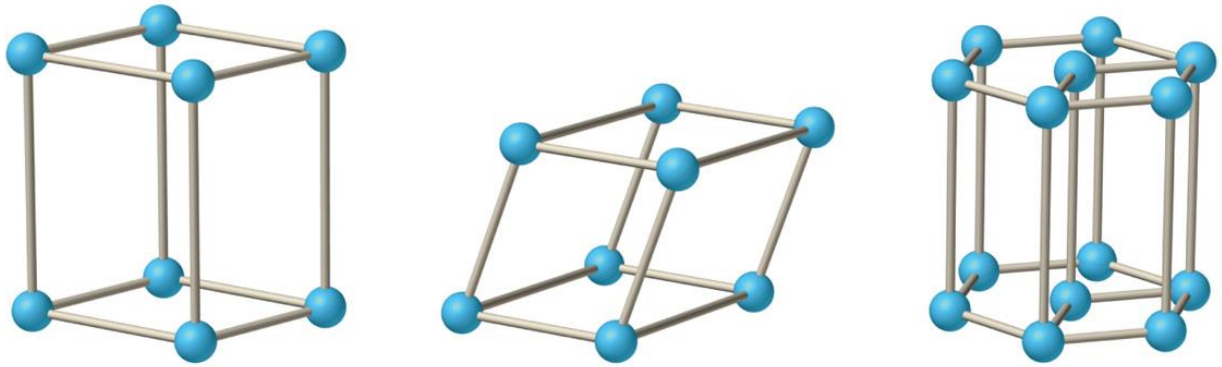


Simple cubic
$a = b = c$
$\alpha = \beta = \gamma = 90^\circ$

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

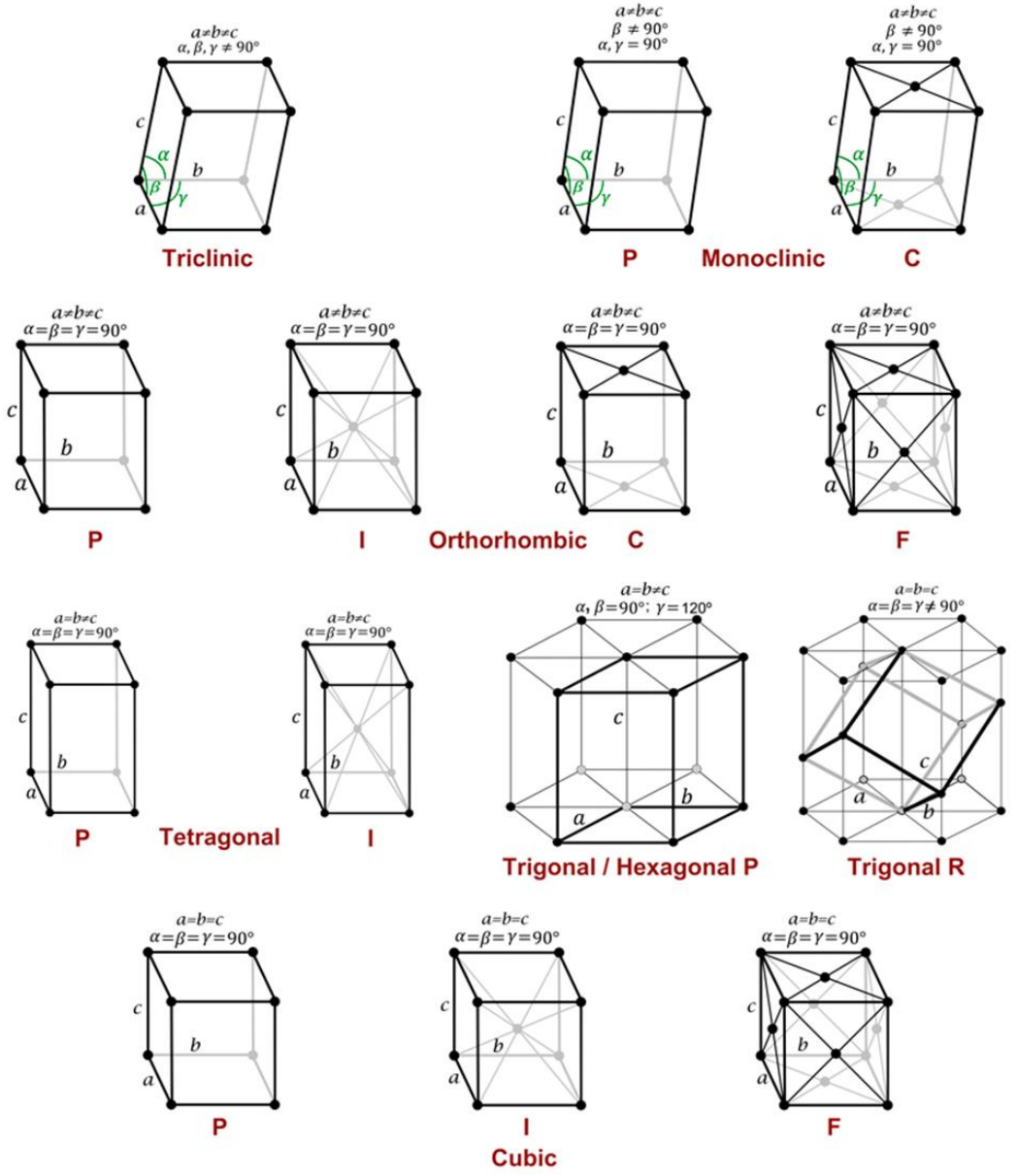
Triclinic
$a \neq b \neq c$
$\alpha \neq \beta \neq \gamma \neq 90^\circ$

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

Bravais lattice

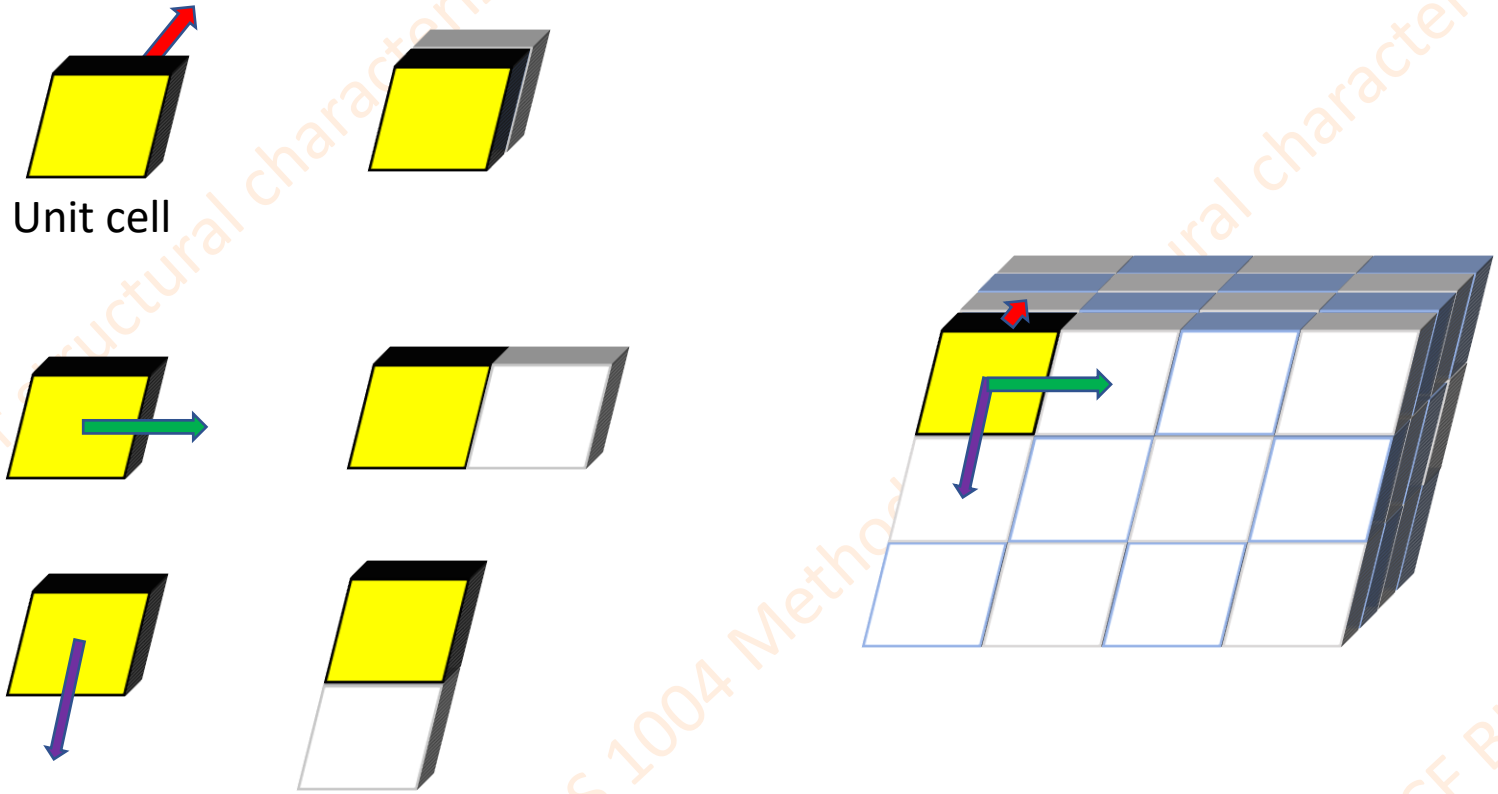
- P – primitive
- I – body centered
- C – base centered
- F – face centered

- 14 together



Translational symmetry

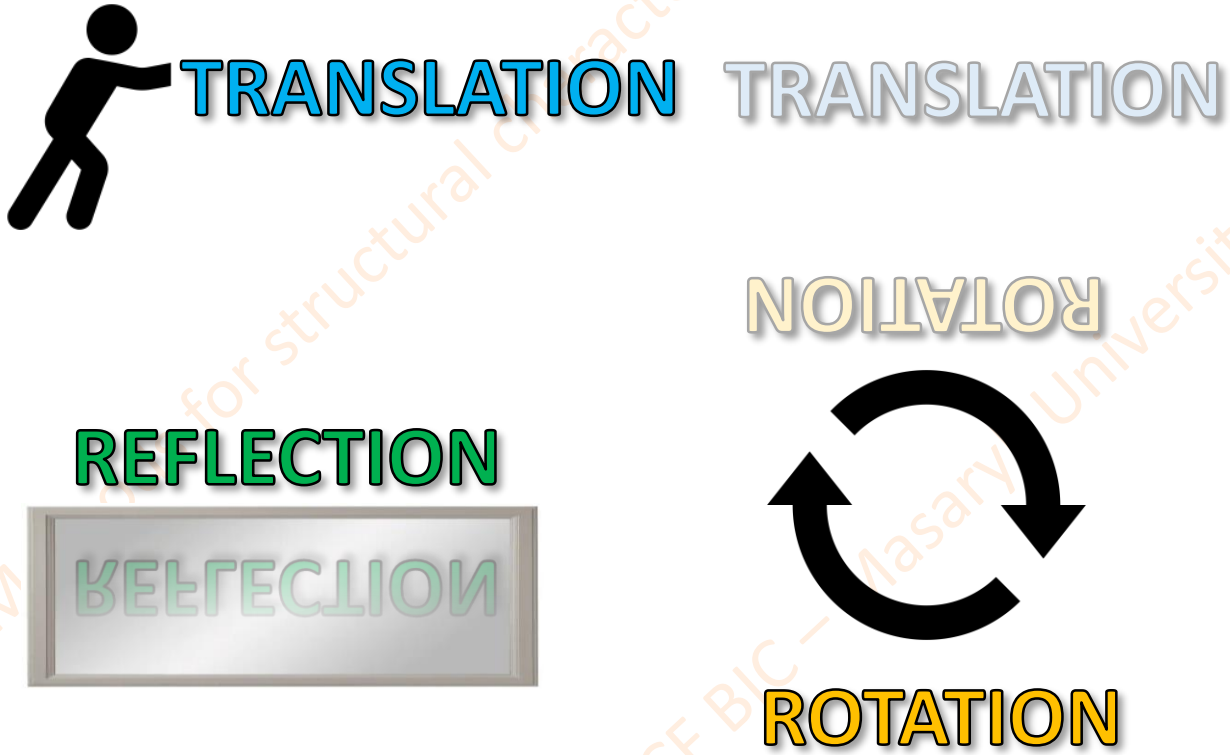
- Essential property of crystal



Symmetry

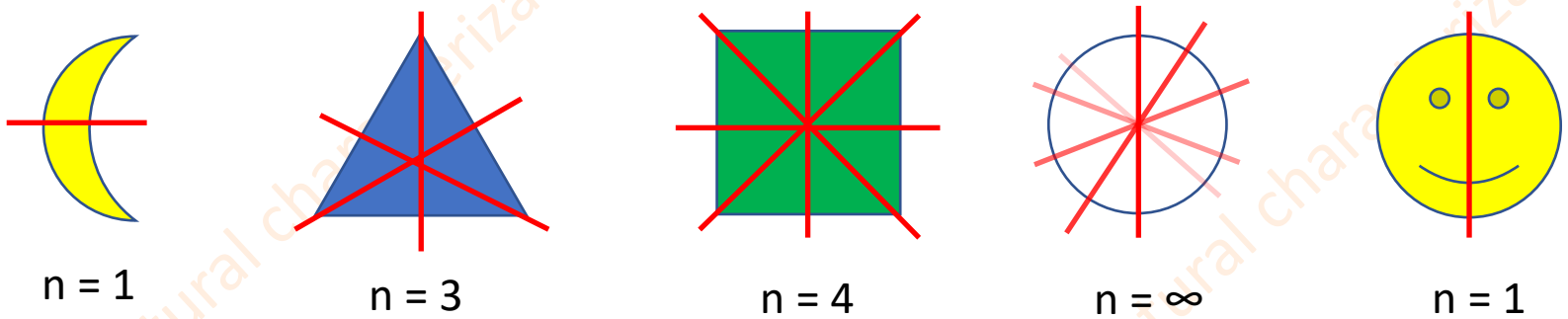
- Ability of object to stay the same after transformation

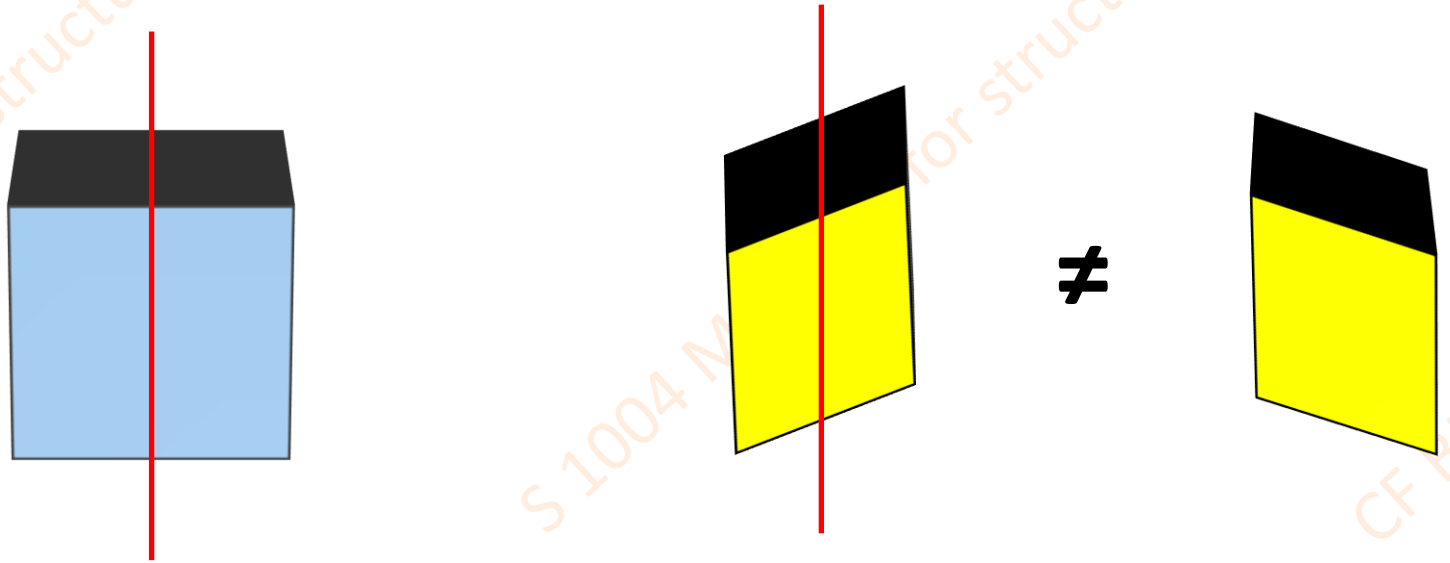
- **Symmetry operations**:
  - Translation – vector
  - Reflection – plane
  - Rotation – axis



Reflection symmetry

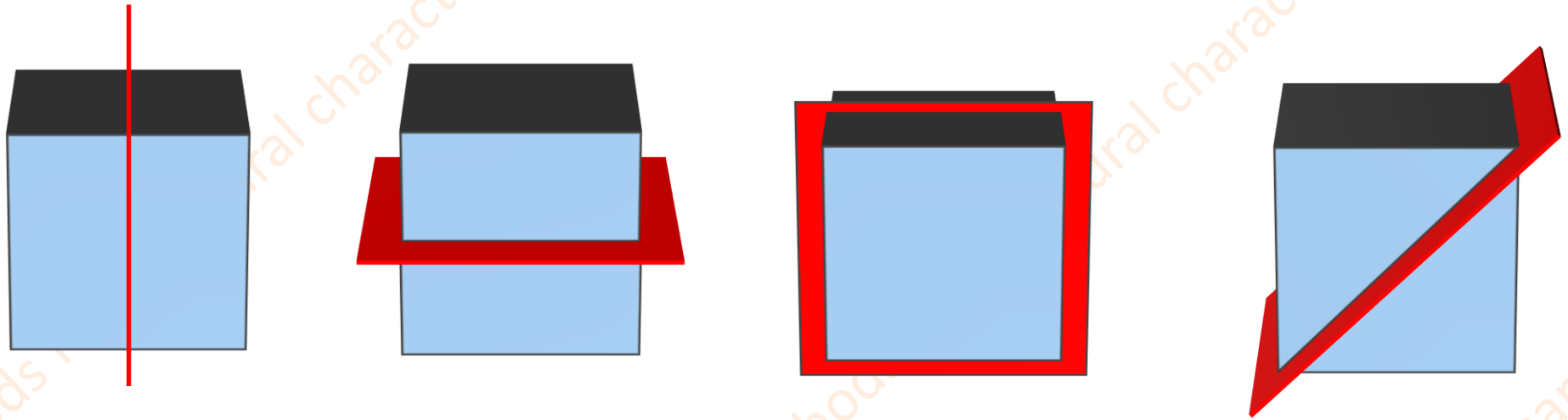
- One half of object is **mirror image** of the other
- **Plane** of symmetry





Reflection symmetry

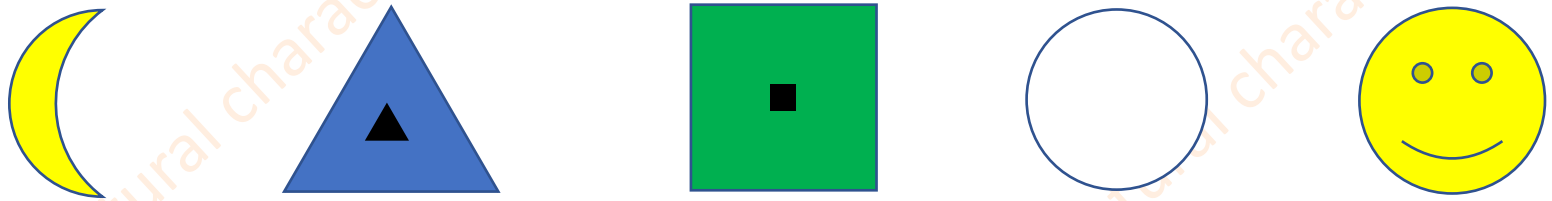
- Object may have several reflection symmetries



etc.

Rotational symmetry

- Degree of symmetry is the number of distinct orientations in which it looks exactly the same
- **Axes** of symmetry



n = 1

identity

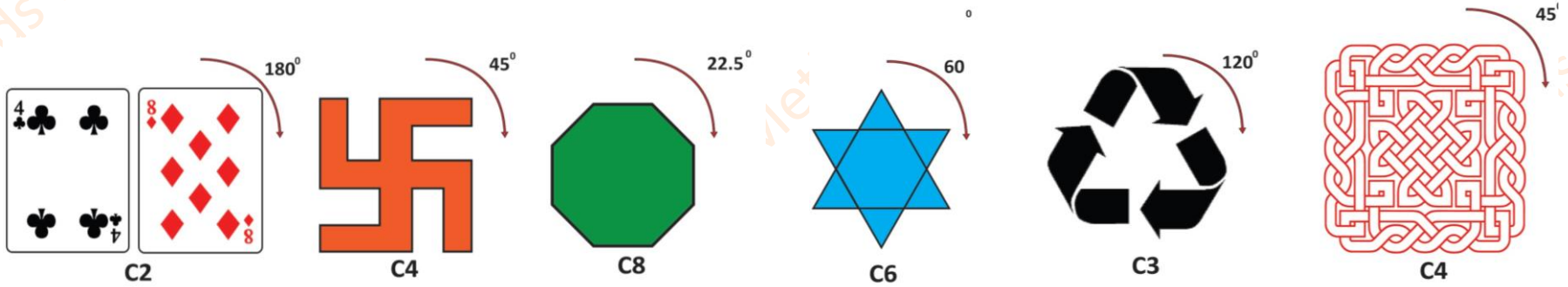
n = 3

n = 4

n = ∞

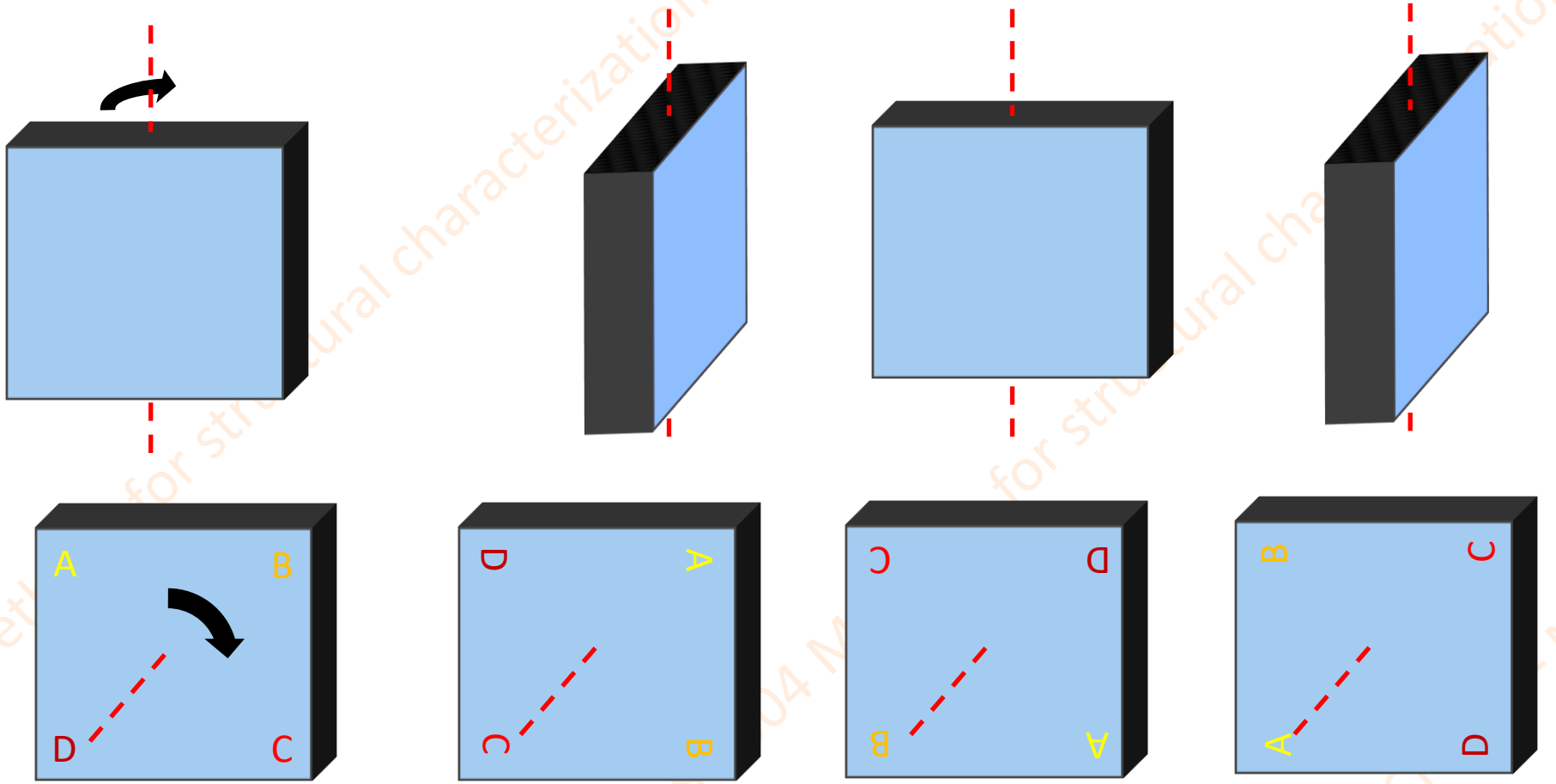
n = 1

identity



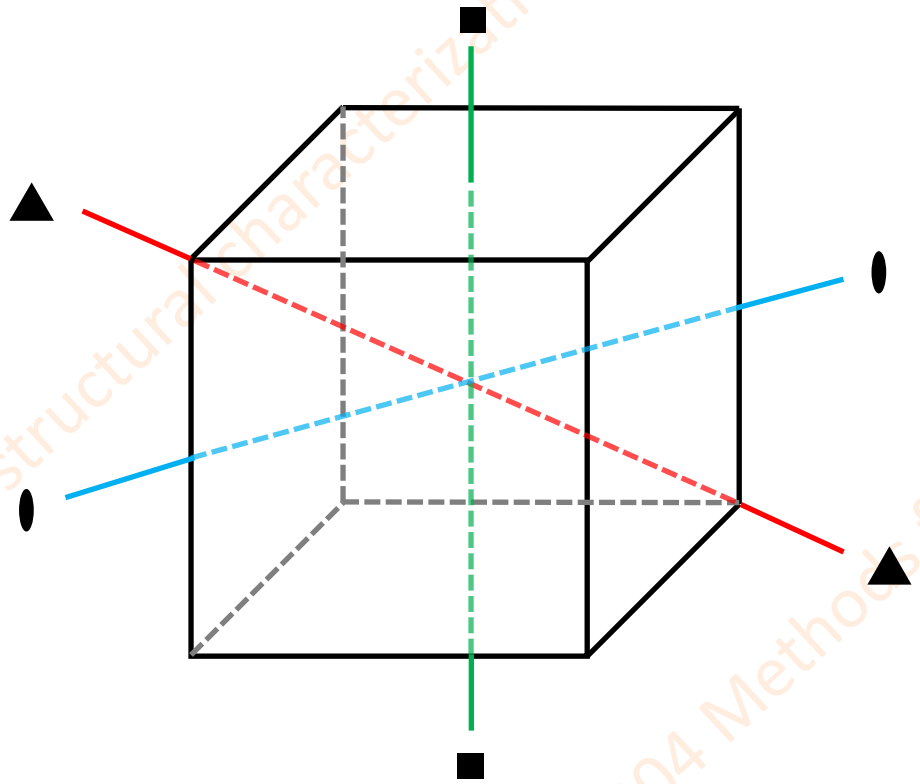
Rotational symmetry

- Object may have several axes of symmetry



Rotational symmetry

- How many symmetry axes (and which) has cube?



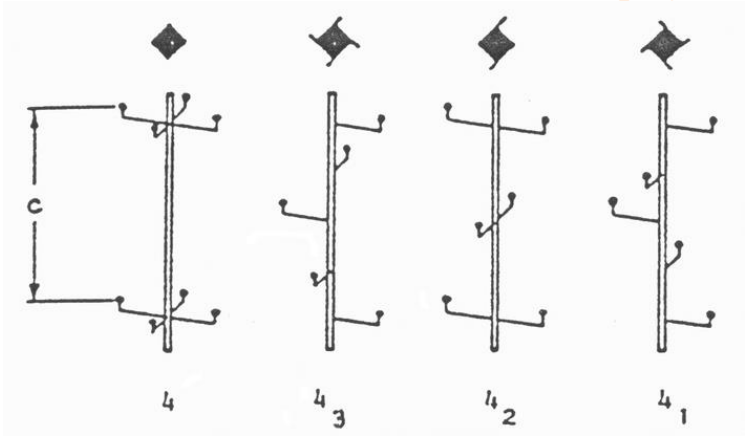
3x 4-fold

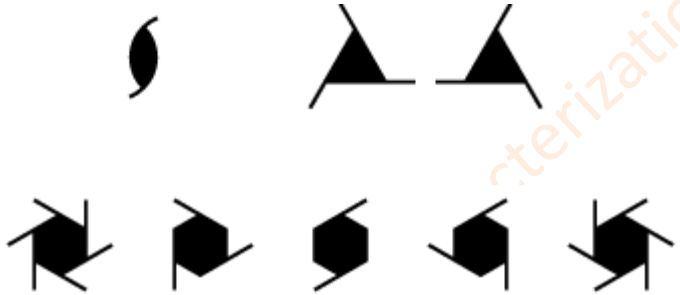
4x 3-fold

6x 2-fold

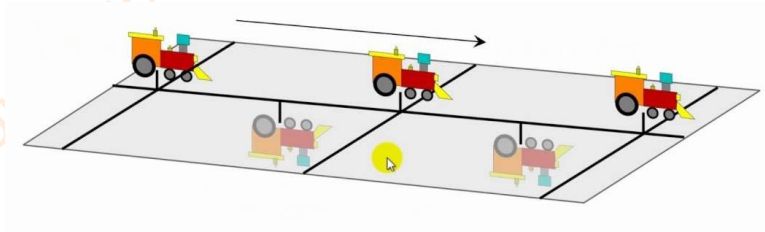
Combined symmetries

- Rotation + translation = **screw-axes**

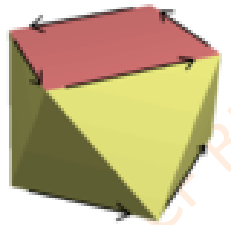




- Reflection + translation = glide plane



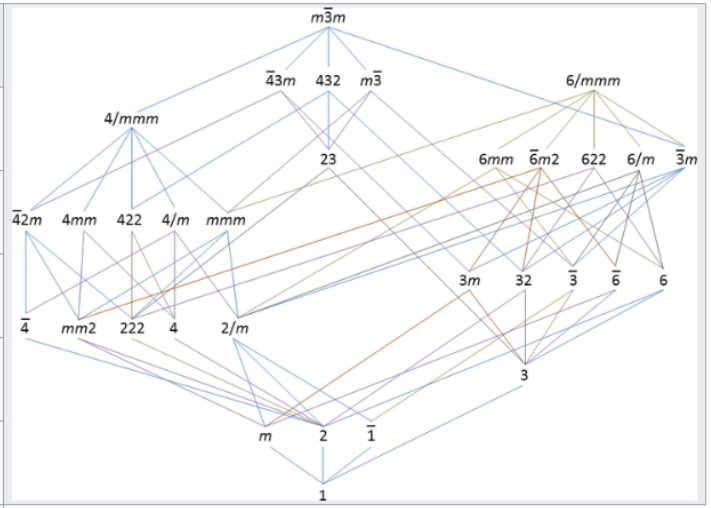
- Reflection + rotation = rotoinversion



Point groups

- Set of symmetry operations that can be applied to the crystal with the fixed initial point
- 32 existing point groups for crystals

Class	Group names						
Cubic	23	$m\bar{3}$		432	$\bar{4}3m$	$m\bar{3}m$	
Hexagonal	6	$\bar{6}$	$^{6}/_{m}$	622	6mm	$\bar{6}m2$	$^{6}/_{m}mm$
Trigonal	3	$\bar{3}$		32	3m	$\bar{3}m$	
Tetragonal	4	$\bar{4}$	$^{4}/_{m}$	422	4mm	$\bar{4}2m$	$^{4}/_{m}mm$
Orthorhombic				222		mm2	mmm
Monoclinic	2		$^{2}/_{m}$		m		
Triclinic	1	$\bar{1}$					



Subgroup relations of the 32 crystallographic point groups
(rows represent group orders from bottom to top as: 1,2,3,4,6,8,12,16,24, and 48.)

Space groups

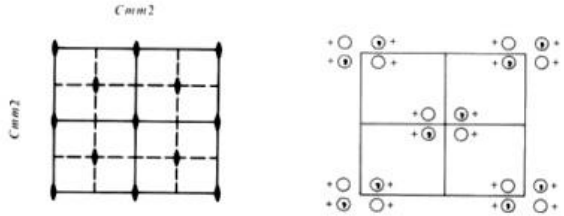
System	Bravais Lattice	Point Group	Space Group						Fraction
Triclinic	P	1	P1						1/2
		$\bar{1}$	$P\bar{1}$						
Monoclinic	P	2	P2	$P2_1$	C2				1/4
	C	m	Pm	Pc	Cm	Cc			
		2/m	P2/m	$P2_1/m$	C2/m	P2/c	$P2_1/c$	C2/c	
Orthorhombic	P	222	P222	$P222_1$	$P2_12_12$	$P2_12_12_1$	$C222_1$	C222	1/8
	C		F222	I222	$I2_12_12_1$				
	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 trig-onal	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	$Fm\bar{3}m$	Fm3c	
			Fd3m	Fd3c	Im3m	Ia3d			

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

Combination of Bravais lattices and symmetry operations leads to **230 possible space groups**.

$Cmm2$ C_{2v}^{11} $mm2$ Orthorhombic

No. 35 $Cmm2$ Patterson symmetry $Cmmm$



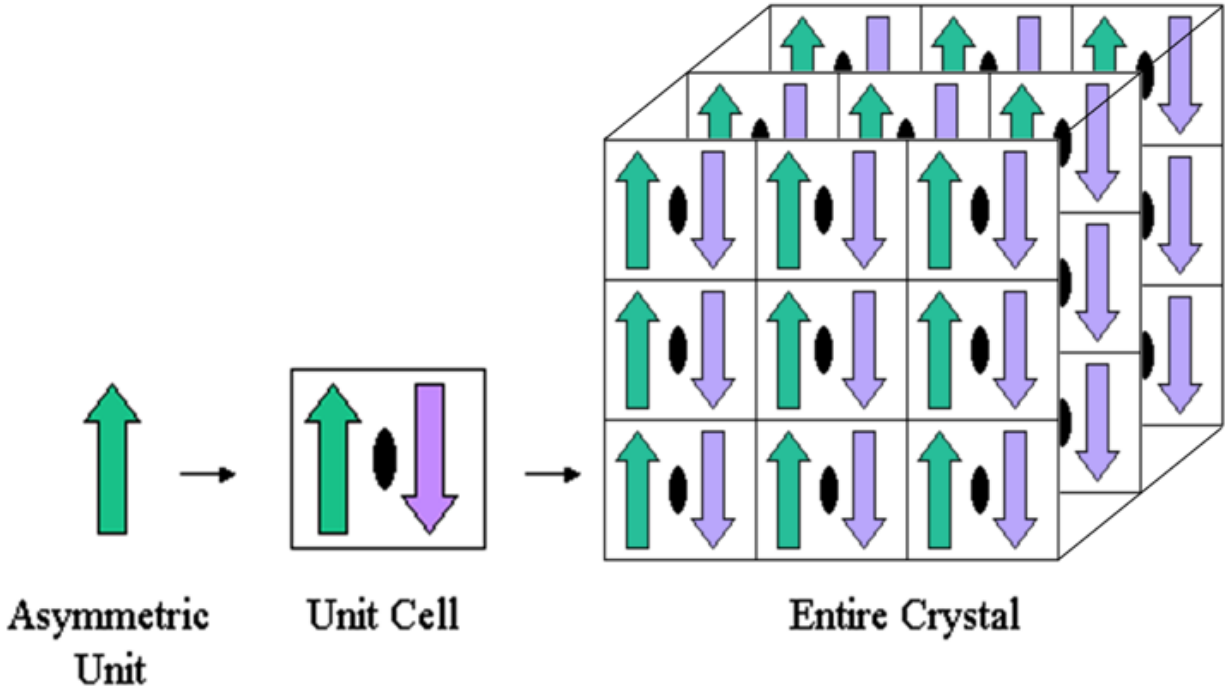
Positions

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**

Asymmetric unit

- Smallest part of crystal that can be used to reconstruct crystal based on **crystal symmetry** and **translation**



pdb.rcsb.org

Biomacromolecular crystal

- Not all symmetry operations possible

Amino acids
Carbohydrates
(and Nucleic acids)

are CHIRAL

Reflection symmetry is not applicable – no mirrors

Only **65 space groups** exist for **protein**

Biomacromolecular crystal

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****** LATTICE SYMMETRY IMPLICATED BY SPACE GROUP SYMMETRY ******

BRAVAIS-           POSSIBLE SPACE-GROUPS FOR PROTEIN CRYSTALS
 TYPE                     [SPACE GROUP NUMBER,SYMBOL]
 aP       [1,P1]
 mP       [3,P2] [4,P2(1)]
mC,mI     [5,C2]
 oP       [16,P222] [17,P222(1)] [18,P2(1)2(1)2] [19,P2(1)2(1)2(1)]
 oC       [21,C222] [20,C222(1)]
 oF       [22,F222]
 oI       [23,I222] [24,I2(1)2(1)2(1)]
 tP       [75,P4] [76,P4(1)] [77,P4(2)] [78,P4(3)] [89,P422] [90,P42(1)2]
          [91,P4(1)22] [92,P4(1)2(1)2] [93,P4(2)22] [94,P4(2)2(1)2]
          [95,P4(3)22] [96,P4(3)2(1)2]
 tI       [79,I4] [80,I4(1)] [97,I422] [98,I4(1)22]
 hP       [143,P3] [144,P3(1)] [145,P3(2)] [149,P312] [150,P321] [151,P3(1)12]
          [152,P3(1)21] [153,P3(2)12] [154,P3(2)21] [168,P6] [169,P6(1)]
          [170,P6(5)] [171,P6(2)] [172,P6(4)] [173,P6(3)] [177,P622]
          [178,P6(1)22] [179,P6(5)22] [180,P6(2)22] [181,P6(4)22] [182,P6(3)22]
 hR       [146,R3] [155,R32]
 cP       [195,P23] [198,P2(1)3] [207,P432] [208,P4(2)32] [212,P4(3)32]
          [213,P4(1)32]
 cF       [196,F23] [209,F432] [210,F4(1)32]
 cI       [197,I23] [199,I2(1)3] [211,I432] [214,I4(1)32]
```

XDS Package

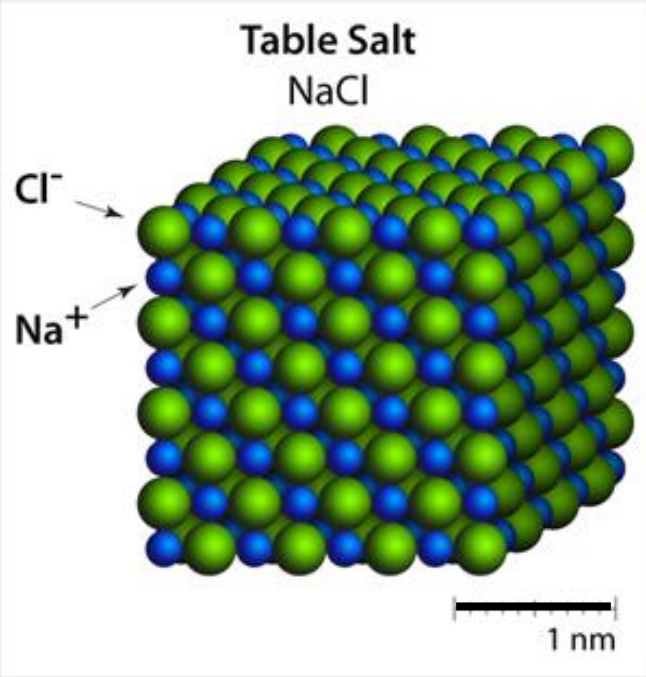
Biomacromolecular crystal

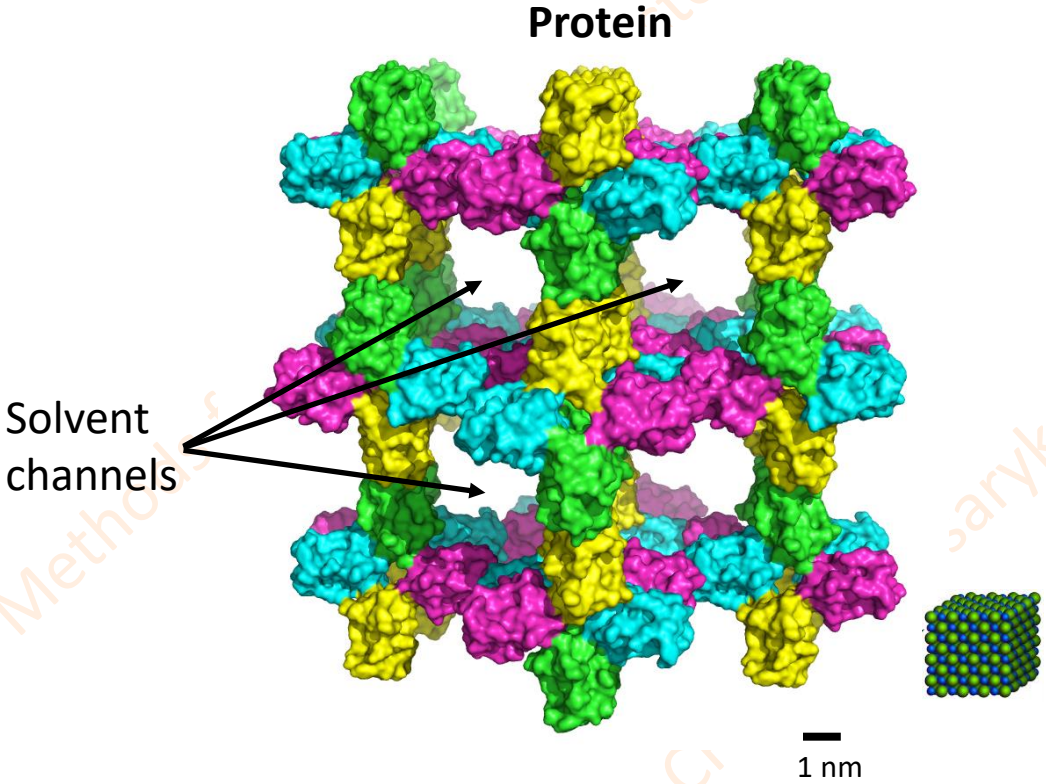
- Properties for crystals of small molecules and biomacromolecules differ

	Small molecule crystal	Biomacromolecular crystal
Size	< 1 dm	< 1 mm
Composition	Localized molecules/ions (Coordinated water molecules)	Localized macromolecules Free ions/water molecules
Stiffness	Moderate – Hard	Soft
Temperature stability	Frequently up to melting	Frequently only within few degrees
Growth	Usually quick and spontaneous	Usually slow or problematic

Biomacromolecular crystal

- Biomolecular crystals contain 25 – 80 % of **solvent**
- **Matthews coefficient** $V_M = V_{(cryst)} / 1\ Da_{(prot)}$
- “Oriented gel”-like

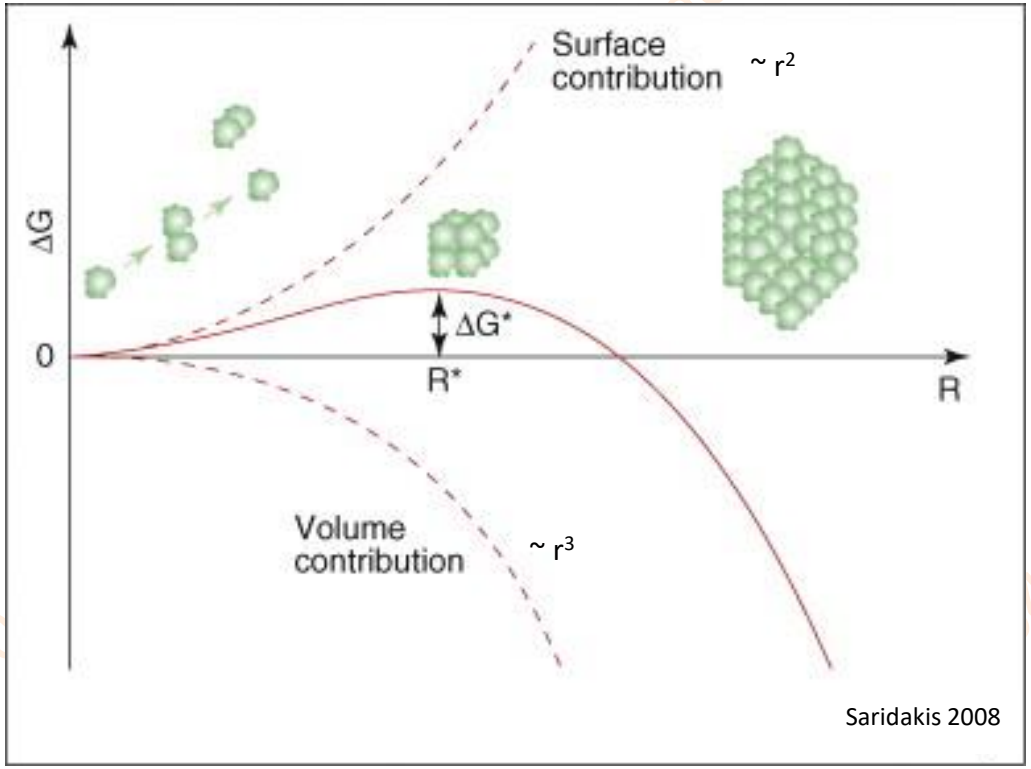




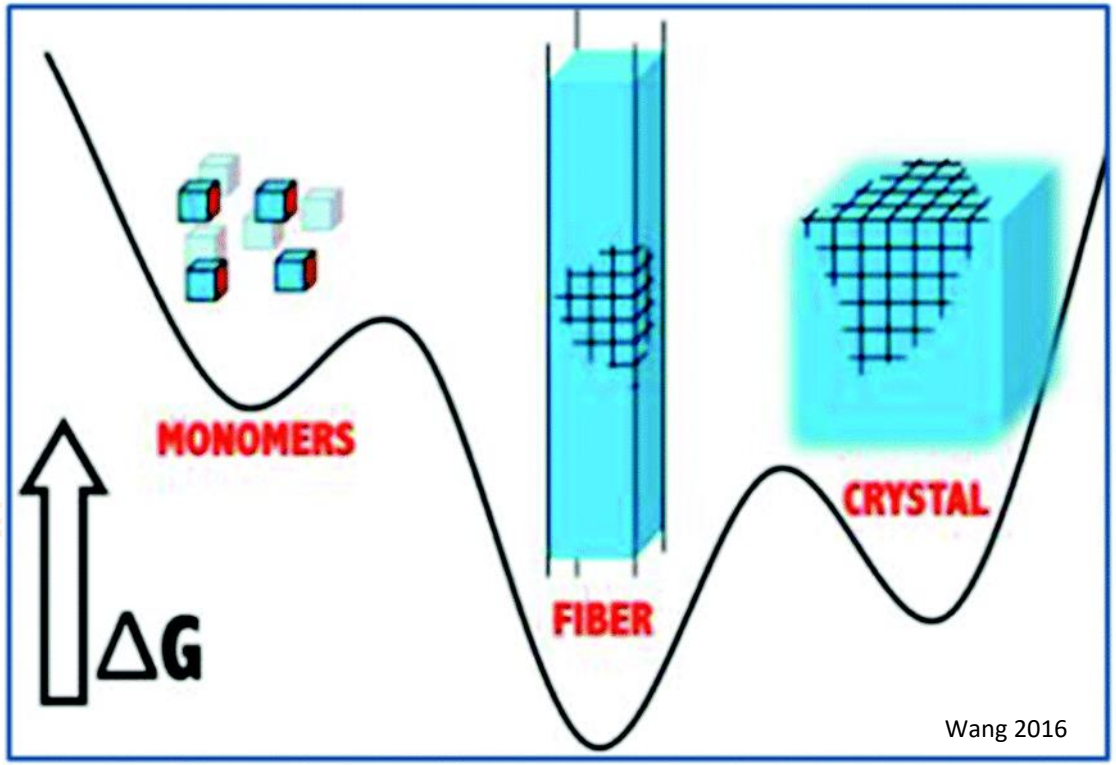
Crystallization

- Process of self-arranging of molecules into crystal lattice

- **Thermodynamics** – enthalpy, entropy, kinetics



Saridakis 2008



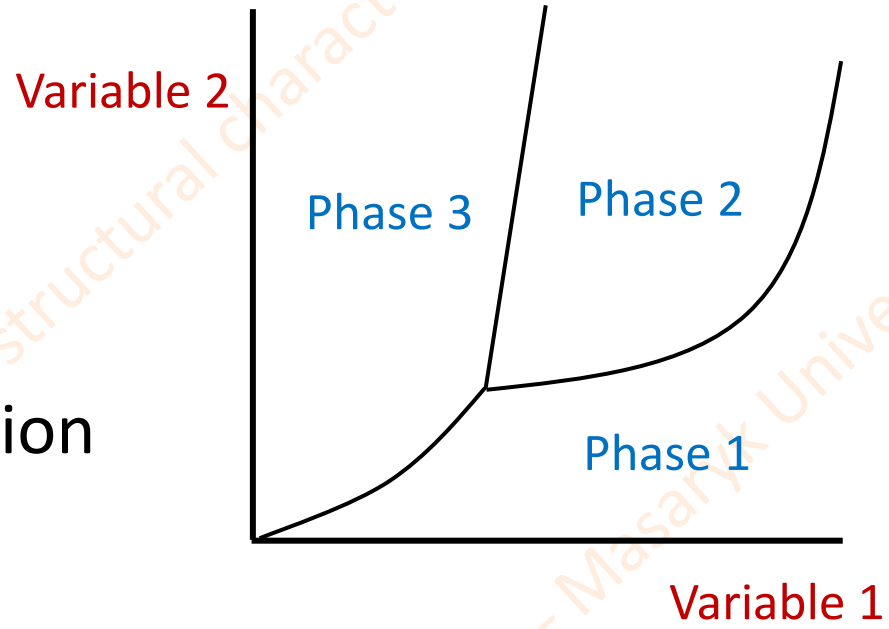
Wang 2016

Phase diagram

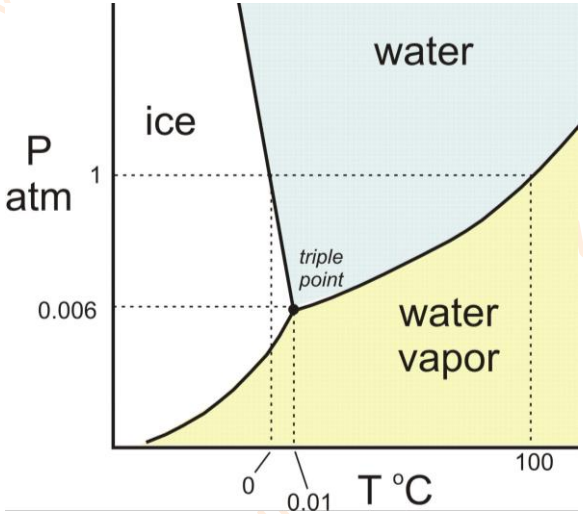
- Existence of various phases in the system depending on variables

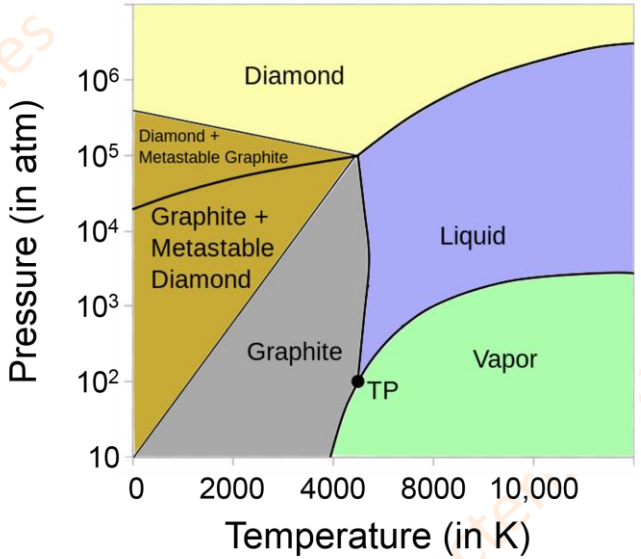
- **Phase**: gas, liquid, solid

- **Variable**: temperature, pressure, composition

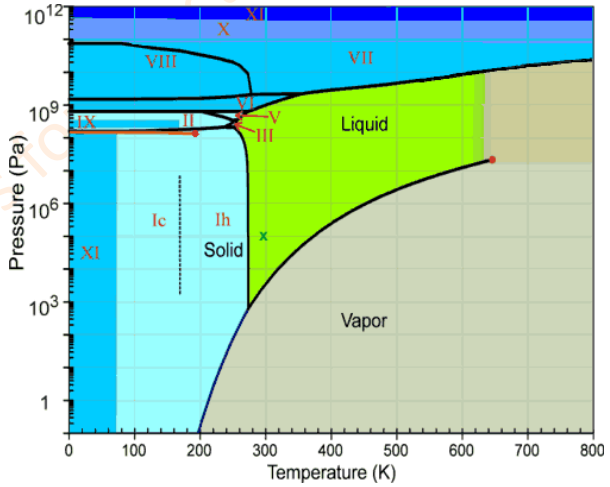


Phase diagram

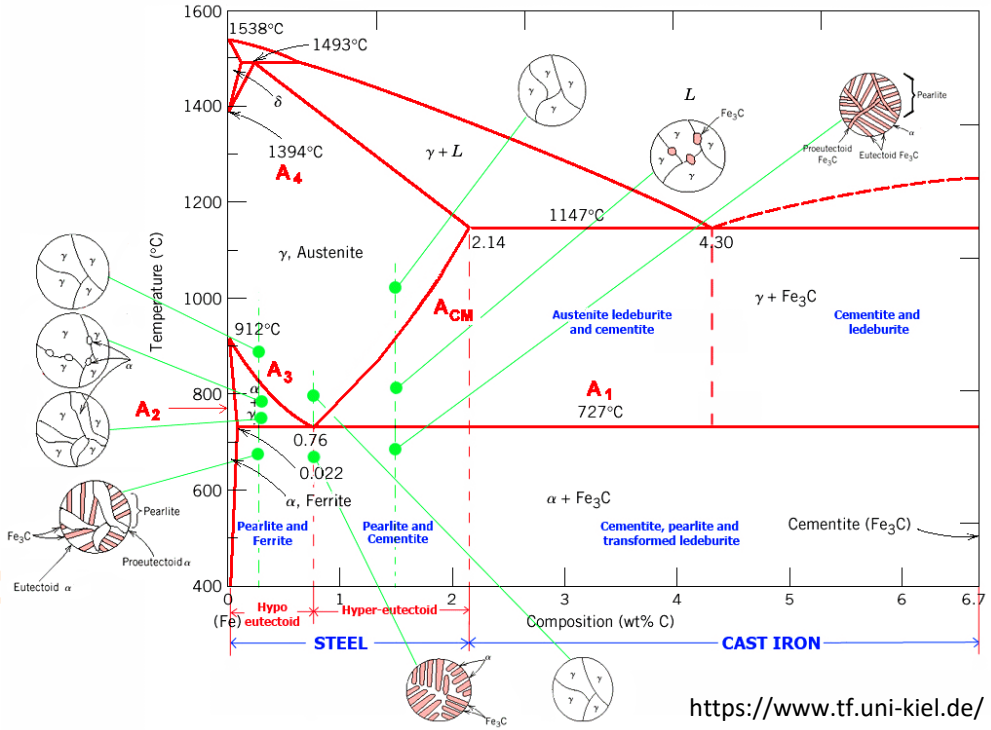




https://www.chegg.com/



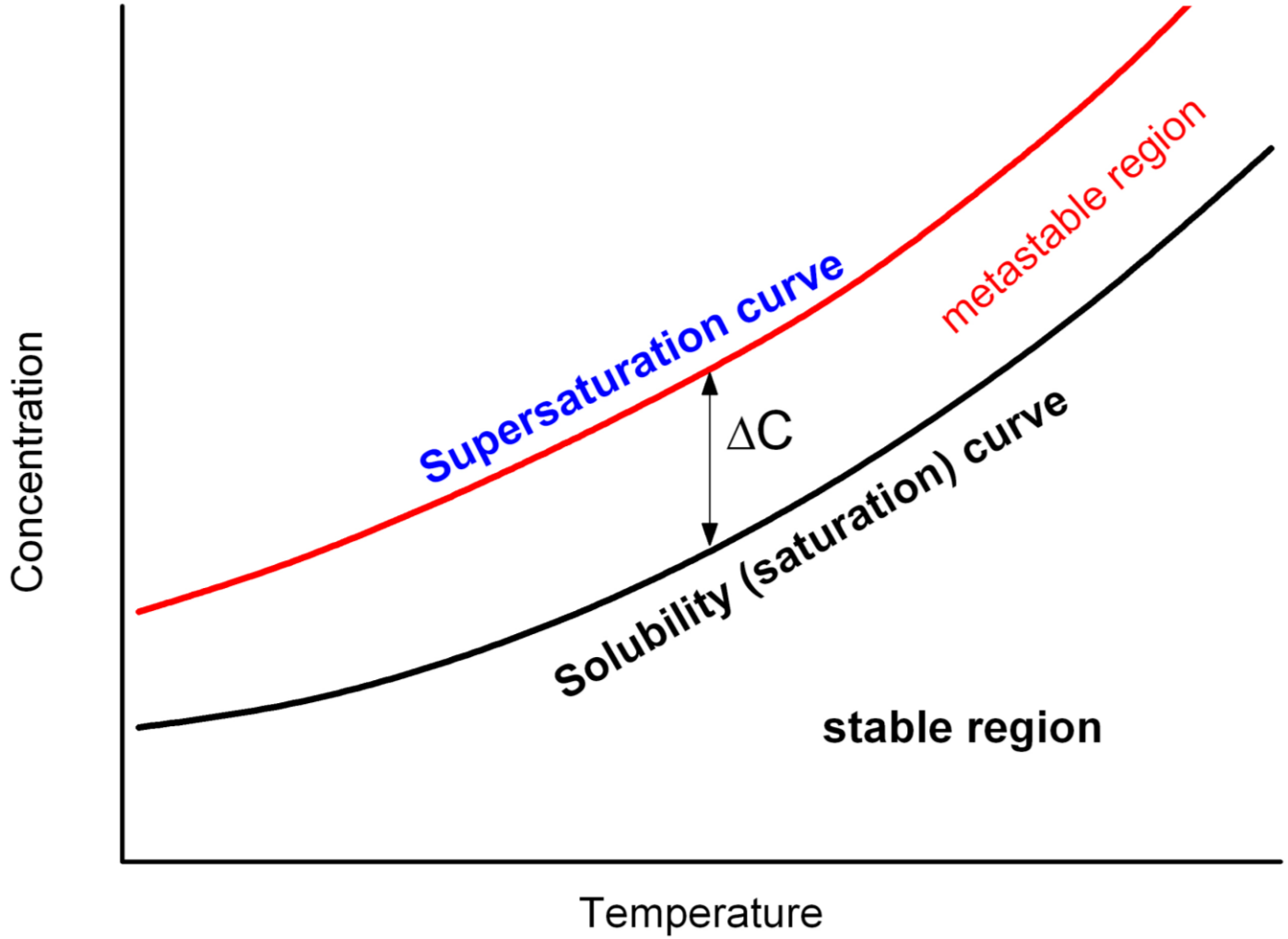
http://ergodic.ugr.es/



https://www.tf.uni-kiel.de/

Protein phase diagram – T vs. conc.

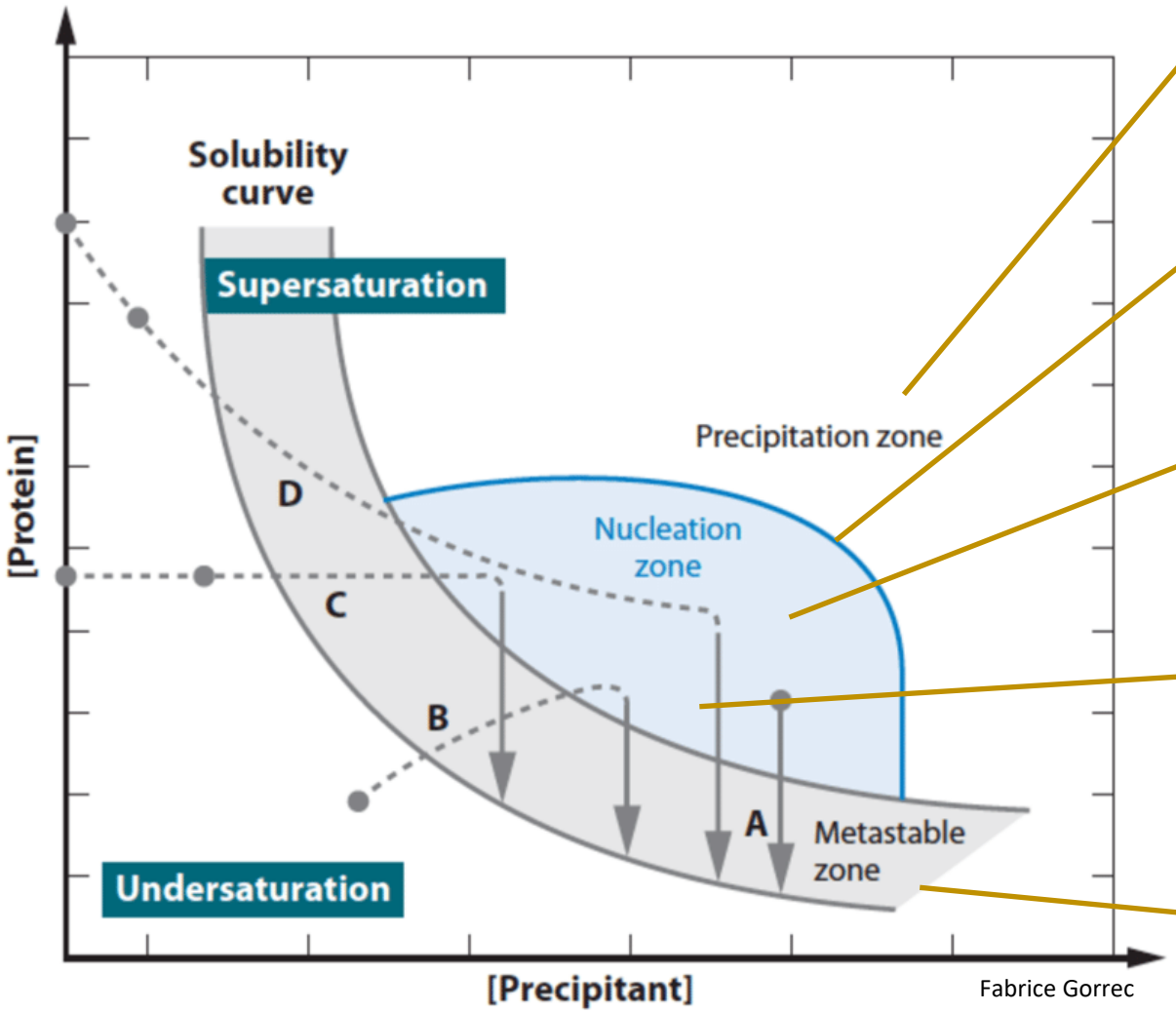
- Solubility increase with temperature
- Limited application due to protein conformational instability at higher T



http://ppl.ippt.gov.pl/solution-crystallization

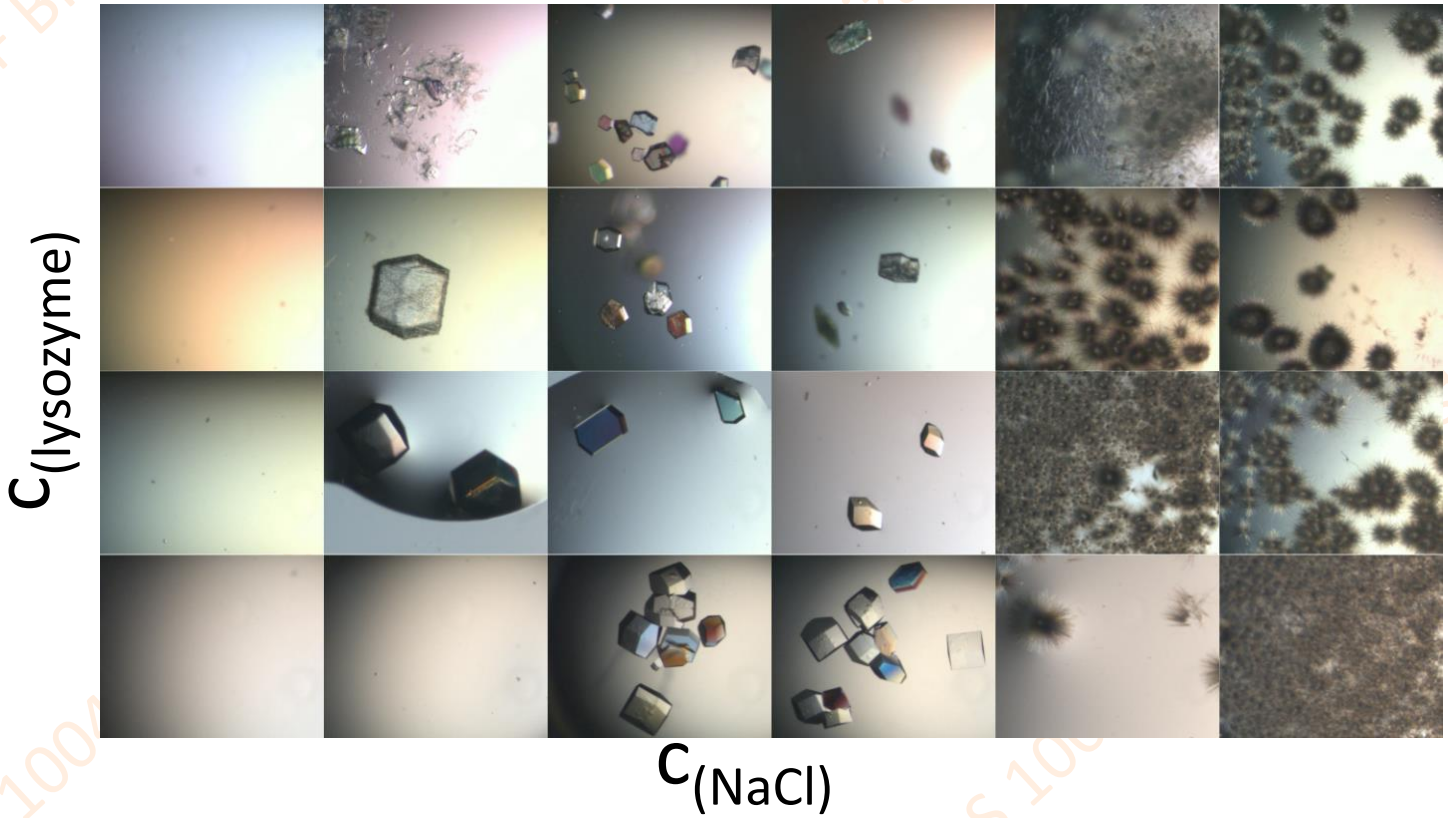
Protein phase diagram – composition

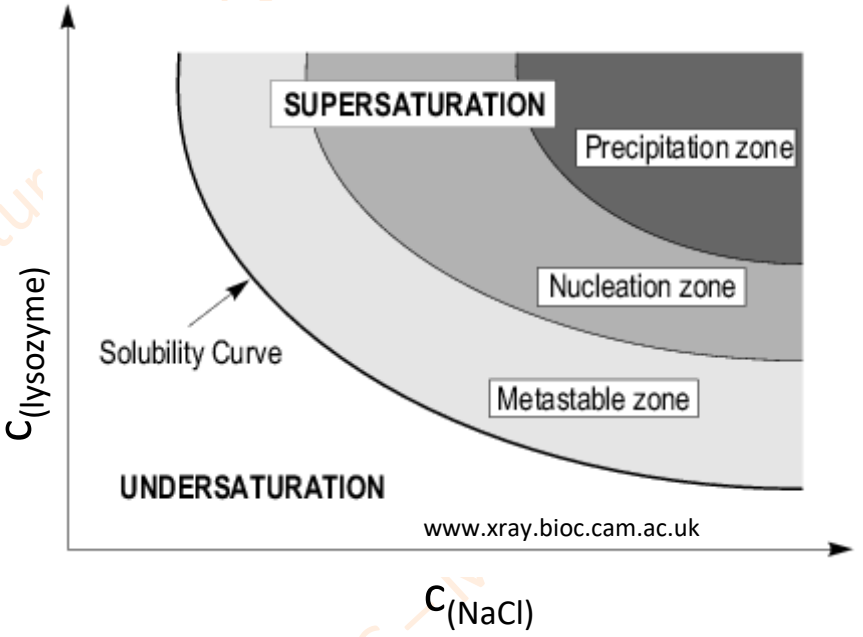
- Precipitant:
  - Buffer
  - Salt
  - Small organics
  - Mixtures



Real phase diagram

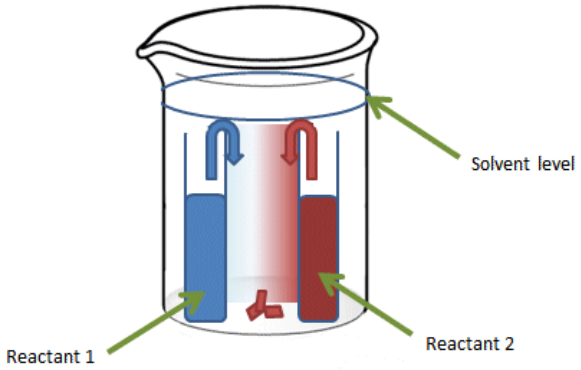
- Lysozyme vs. NaCl in acetate buffer



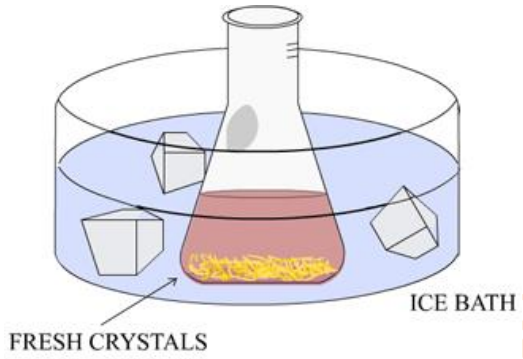


Crystallization techniques

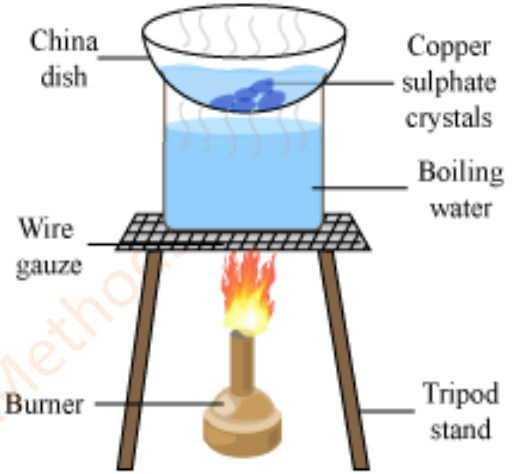
- **Small molecules**
  - *In situ* crystallization
  - Cooling
  - Solvent evaporation (free, under vacuum)
  - Solvent exchange



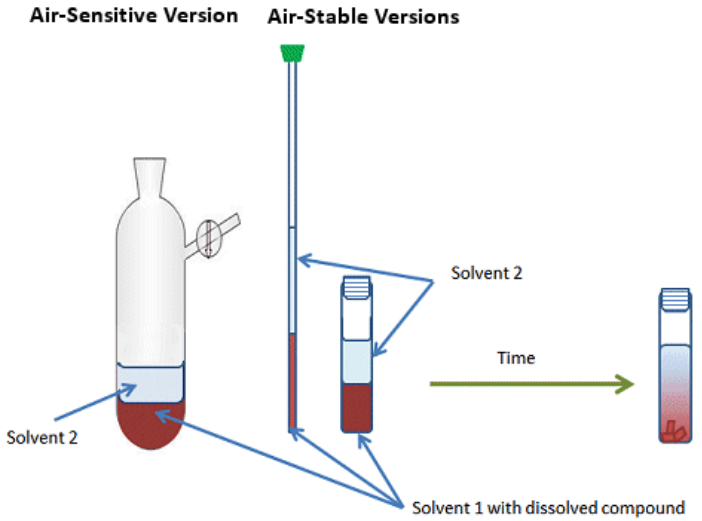
chemistryviews.com



chemhelper.com



jinachem.blogspot.com

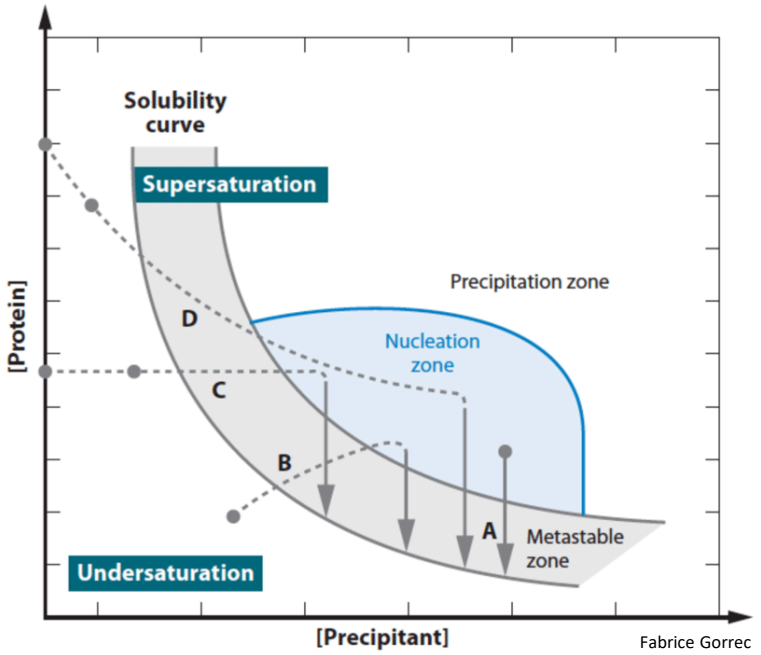


chemistryviews.com

Crystallization techniques

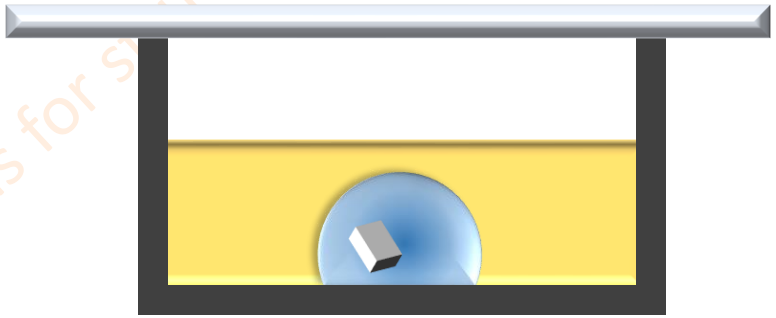
- Small molecules
  - *In situ* crystallization
  - Cooling
  - Solvent evaporation (free, under vacuum)
  - Solvent exchange

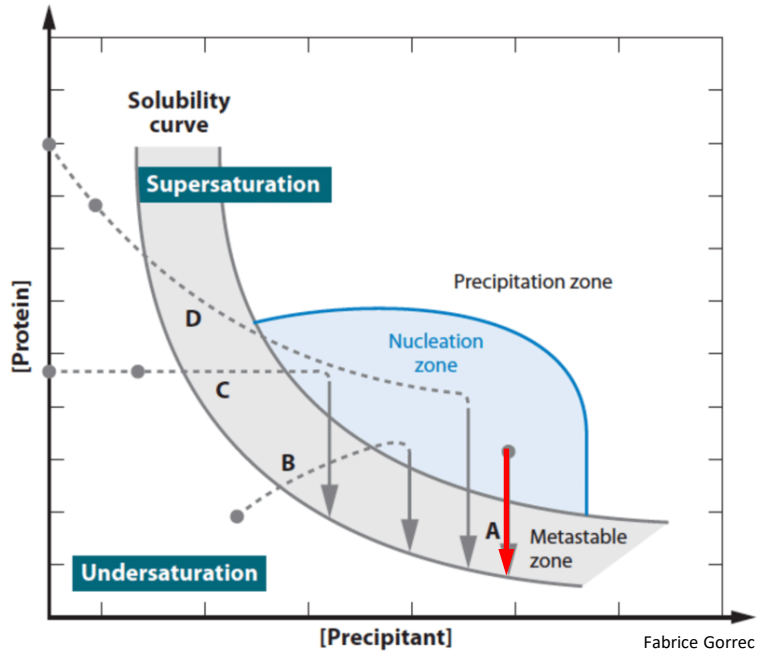
- **Macromolecules**
  - Batch
  - Vapor diffusion
  - Dialysis
  - Free interface diffusion



Batch method

- **Direct mixing** of protein and precipitant
- Drop **under paraffine oil** – prevention of evaporation



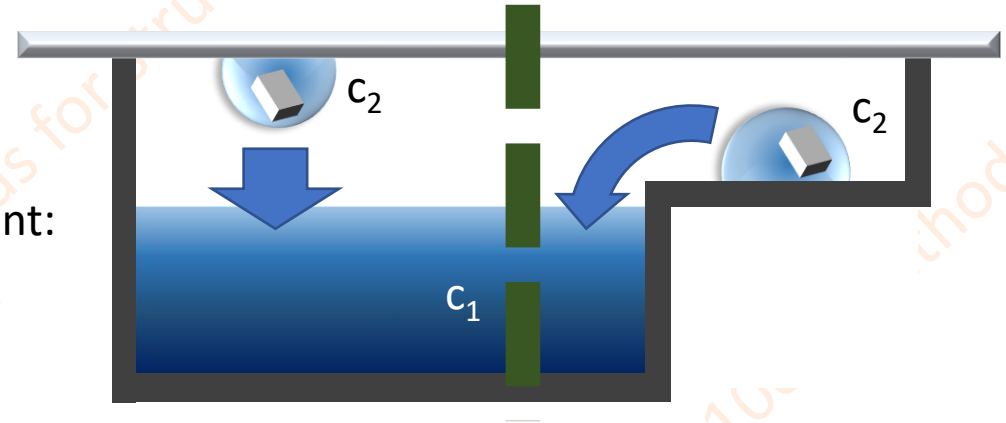


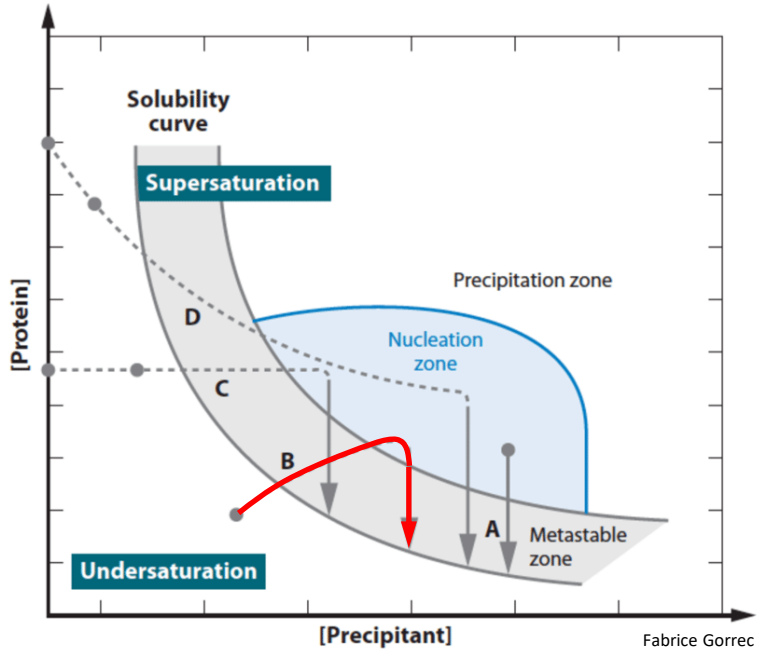
Vapor diffusion

- Increasing of protein and precipitant concentrations due to **H_2O evaporation**

- **Sitting drop** vs **Hanging drop** vs Sandwich

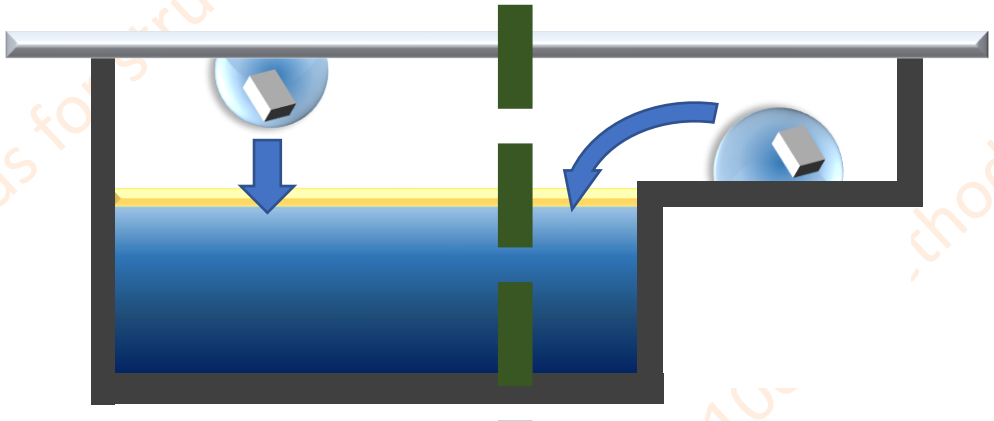
Precipitant:
$c_1 > c_2$

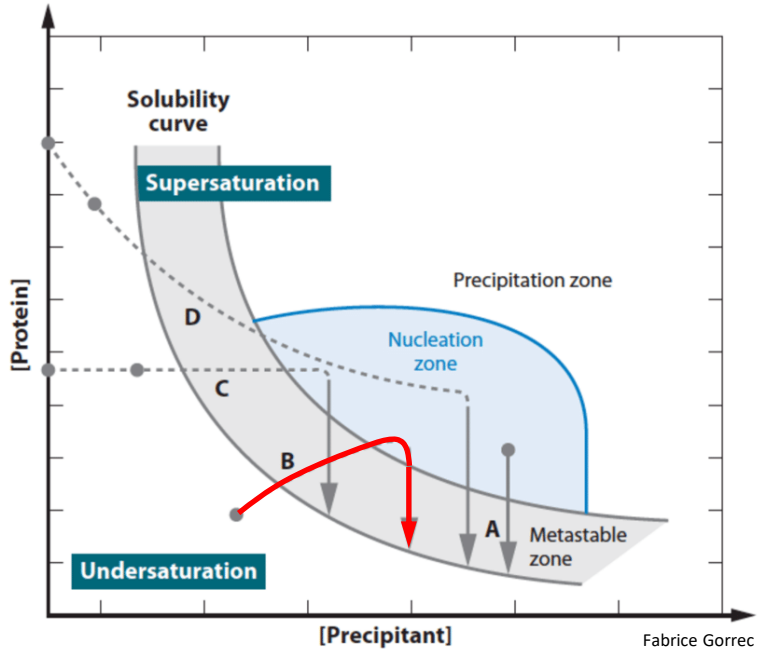




Under oil vapor diffusion

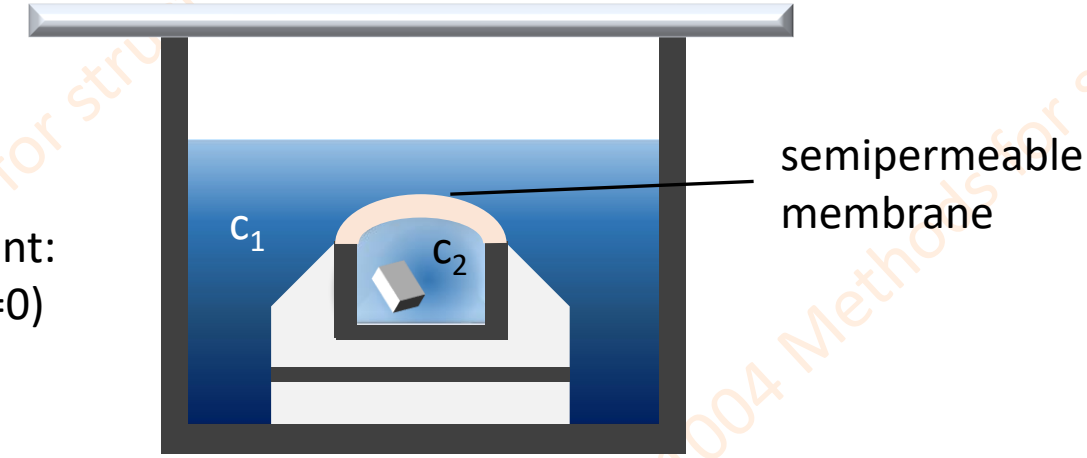
- Modification of vapor diffusion
- **Slowing down** the process
- **Paraffin** oil vs. **Silicon** oil
- Speed adjustment by
  paraffin oil : silicon oil ratio



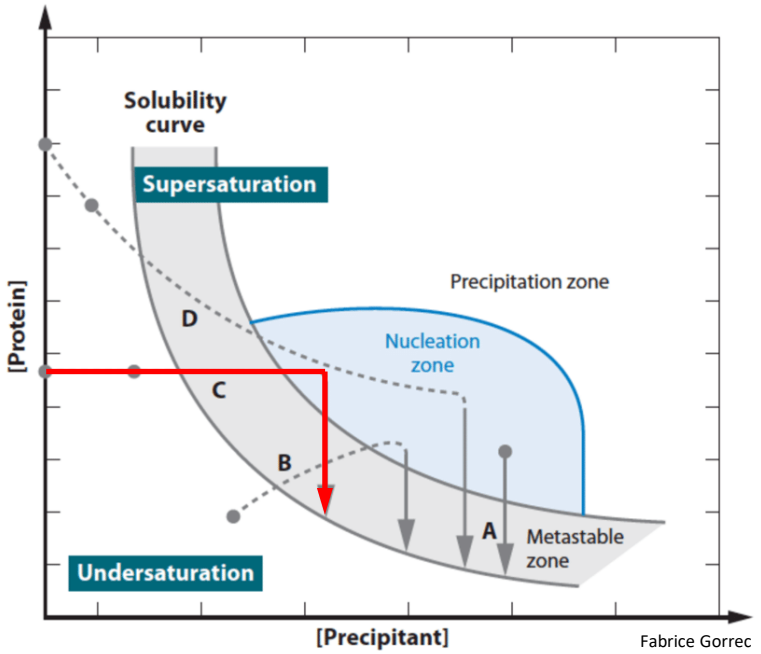


Dialysis

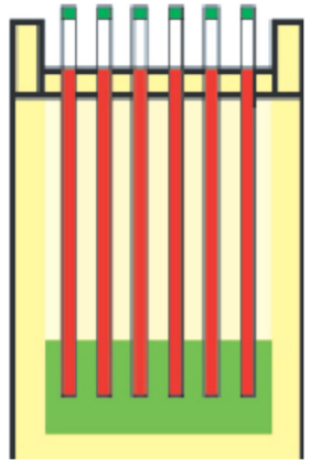
- Increase of precipitant concentration due dialysis through semipermeable **membrane**
- Constant protein concentration



Precipitant:
$c_1 > c_2 (=0)$



Diffusion

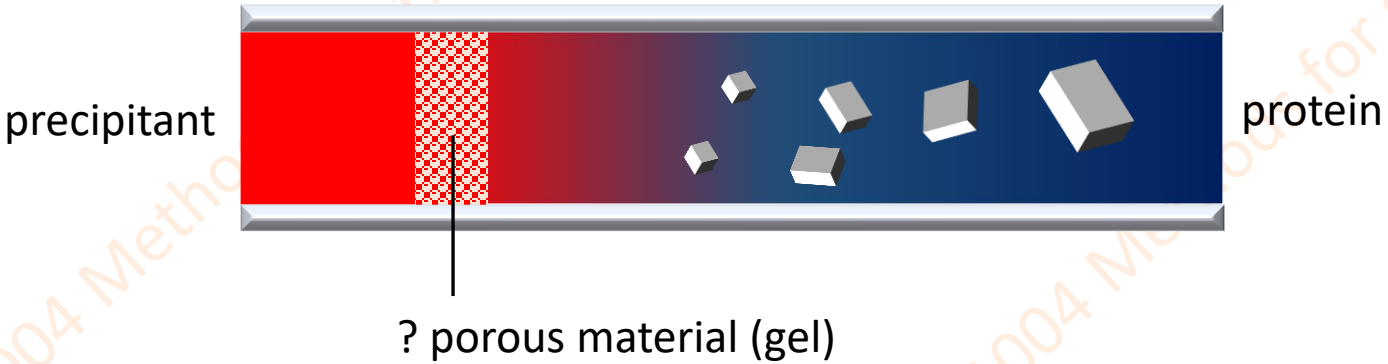


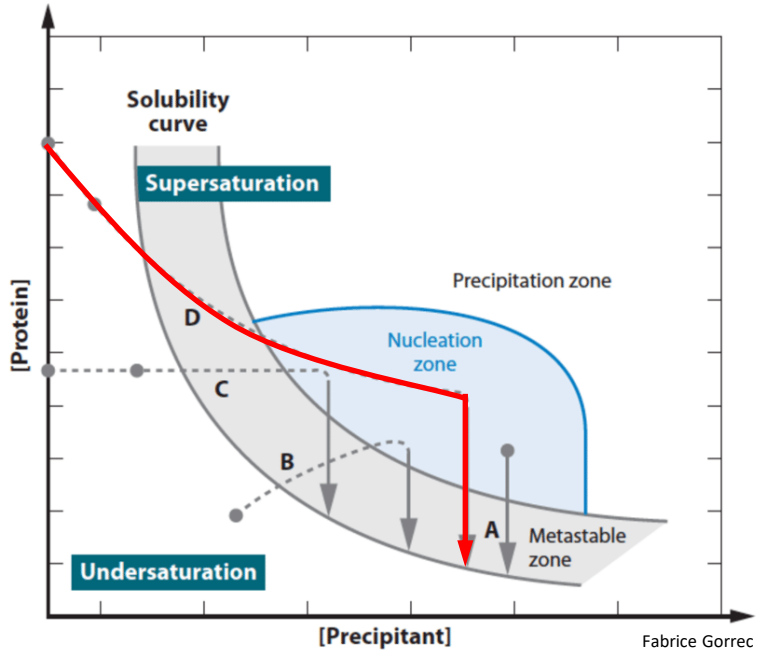
McPherson 2013

- Diffusion of protein and precipitant in opposite direction
- **Free-interface** in capillary

  or

  Unidirectional diffusion – similar to dialysis



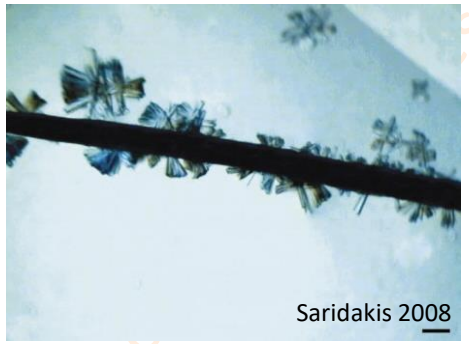


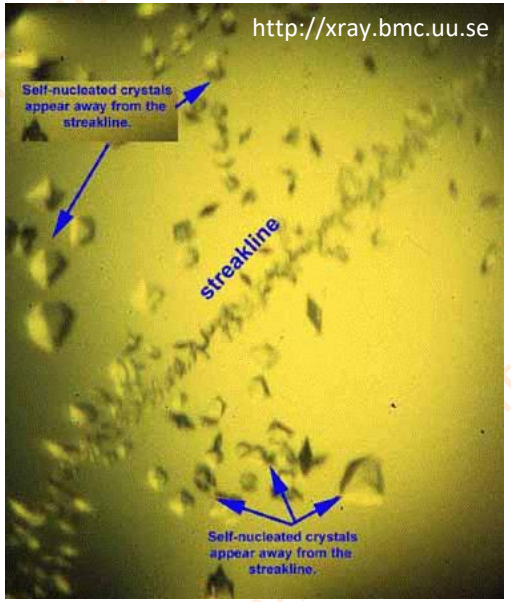
Fabrice Gorrec

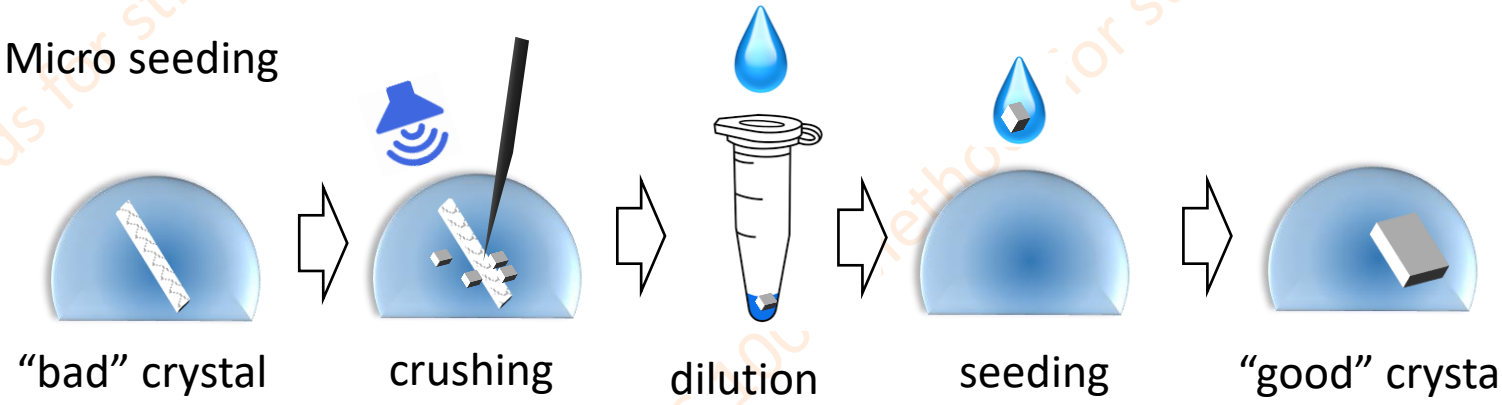
Seeding

Stimulation of crystallization by introducing crystallization **nucleus**

- Macro seeding
- **Micro seeding**
- Streak seeding
- Cross seeding

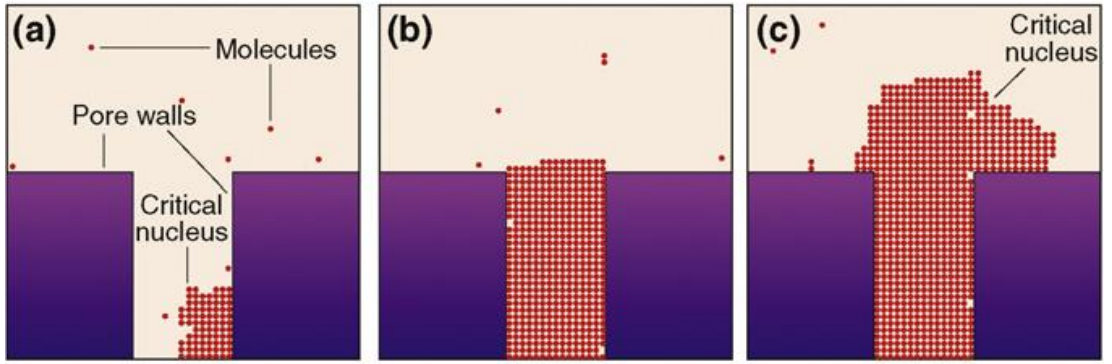


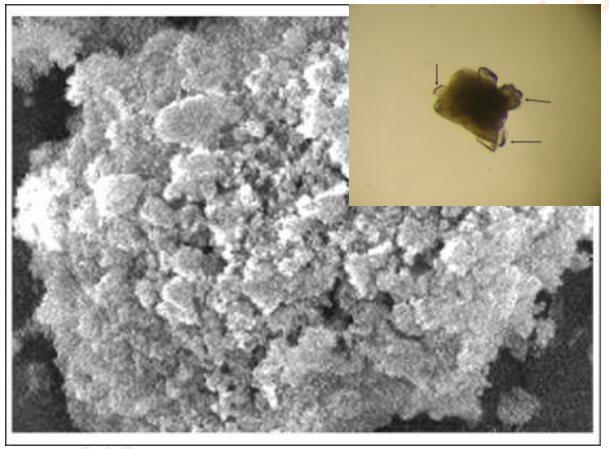




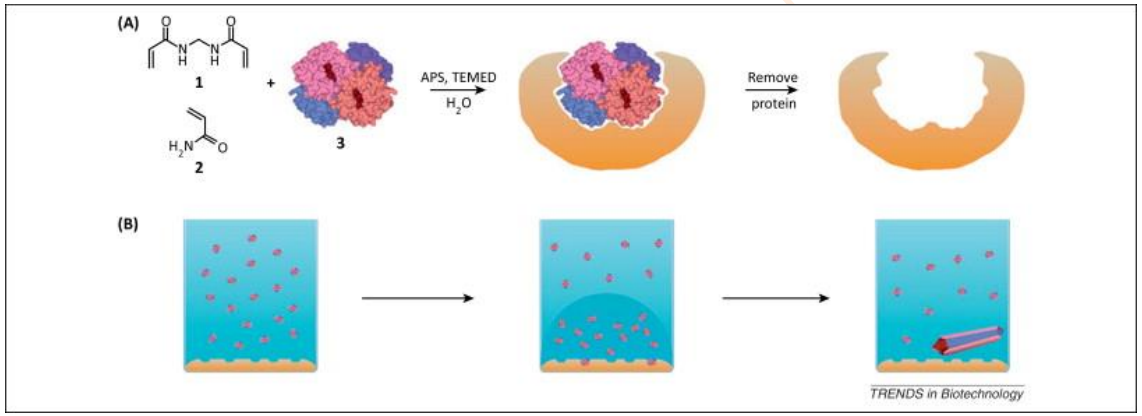
Seeding specials

- **Universal nucleant**





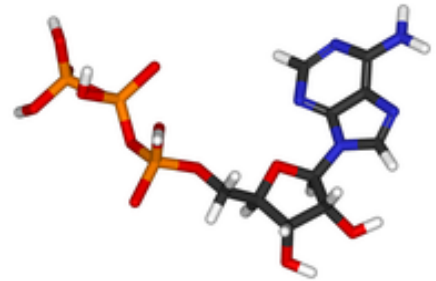
- Imprinted polymers

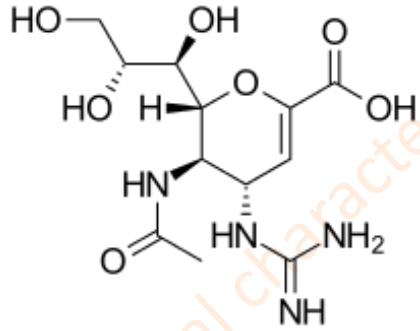


N.Chayen

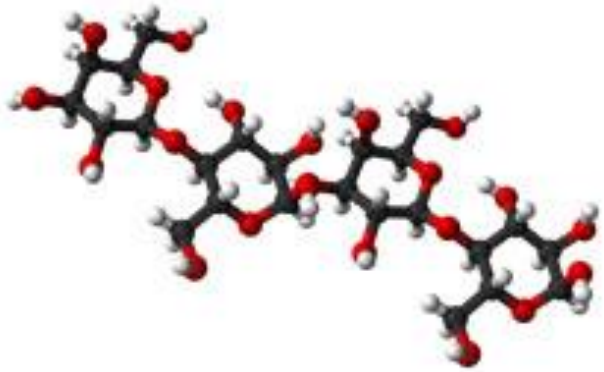
Derivatization

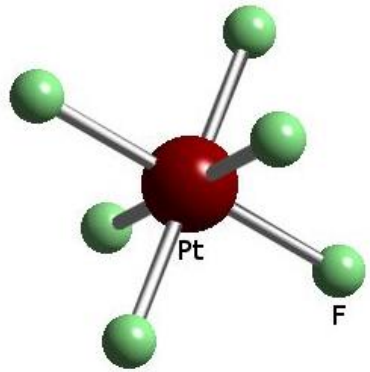
- **Modification** of crystallized protein
  - Ligands/substrates
  - Co-factors
  - Inhibitors
  - Heavy-atom compounds





- **Techniques**
  - Co-crystallization
  - Soaking





Co-crystallization

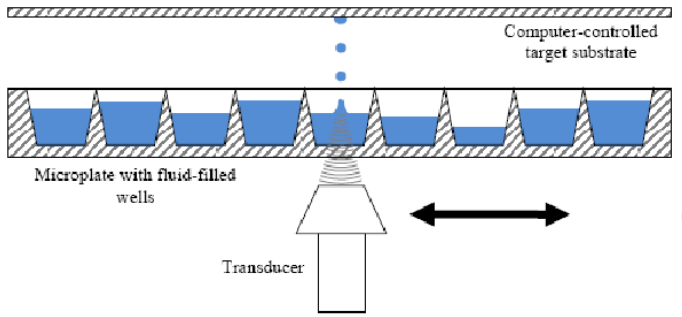
- Adding of ligand **BEFORE** crystallization
- Ligand in excess – homogenous complex needed

- Binding not affected by crystallization
- Crystallization affected (or blocked) by binding
- Change of space group
- Automation compatible

Soaking

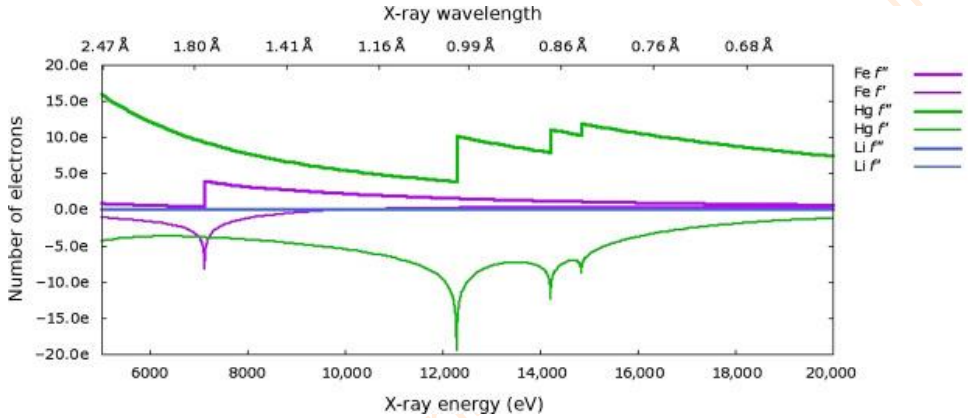
- Adding of ligand **AFTER** crystallization
- Ligand in various concentrations

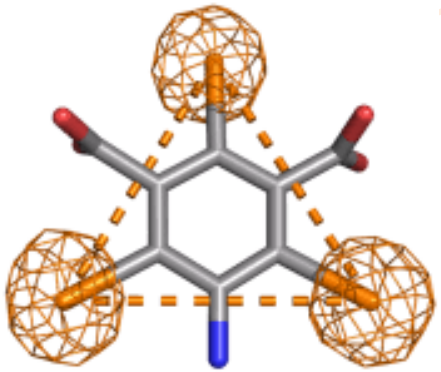
- Crystallization not affected by binding
- Binding affected (or blocked) by crystal formation
- Usually manual
- High-throughput possible

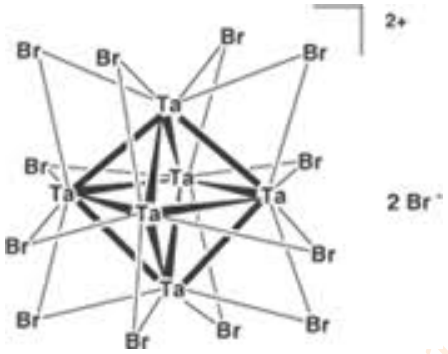


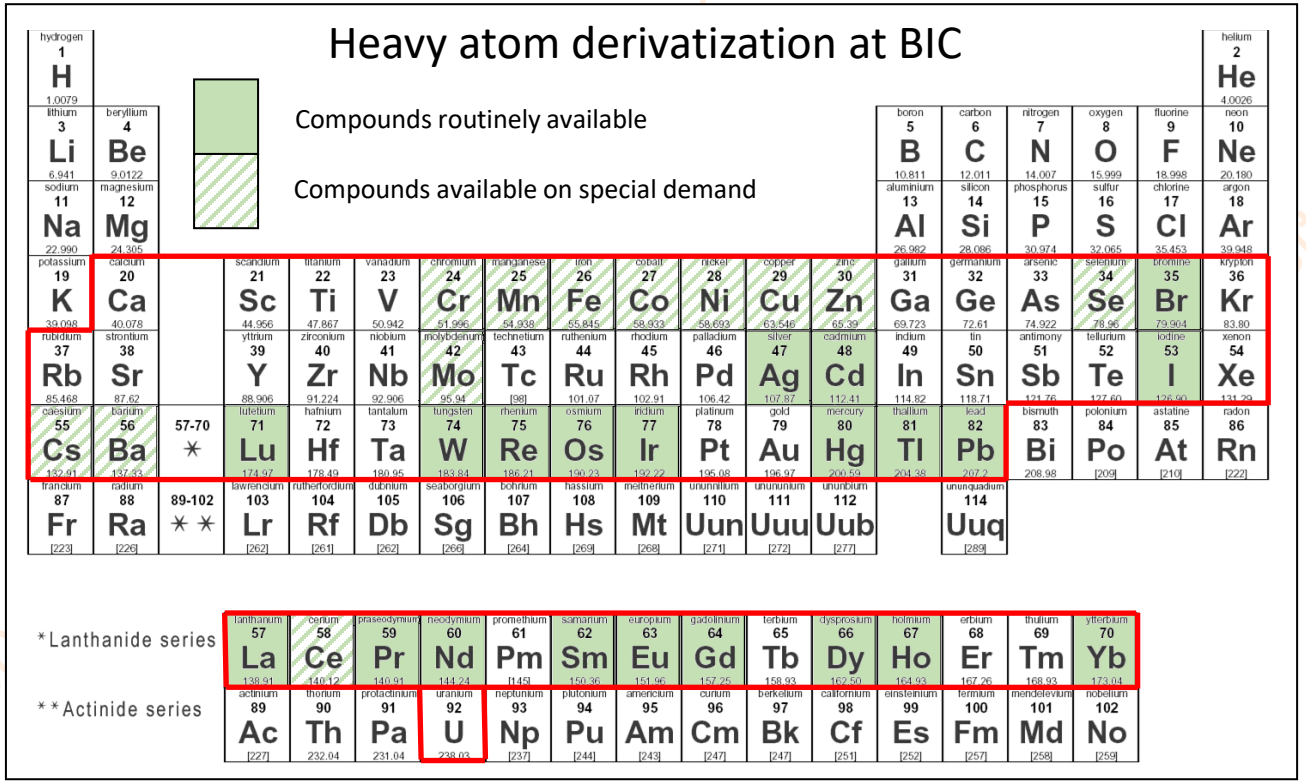
Heavy atoms derivatization

- Introduction of heavy atom for **phasing**



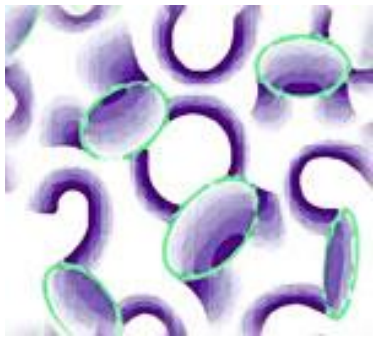


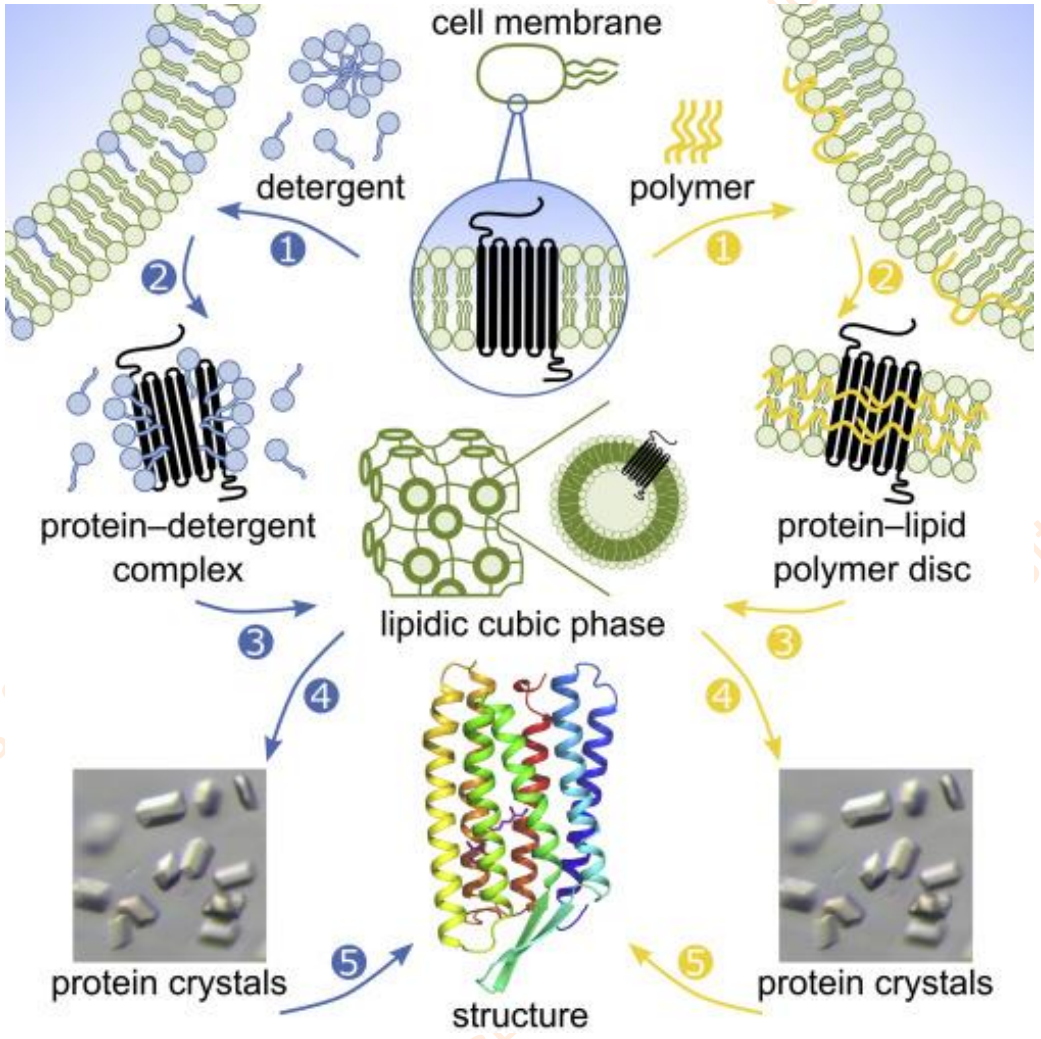




Membrane proteins crystallization

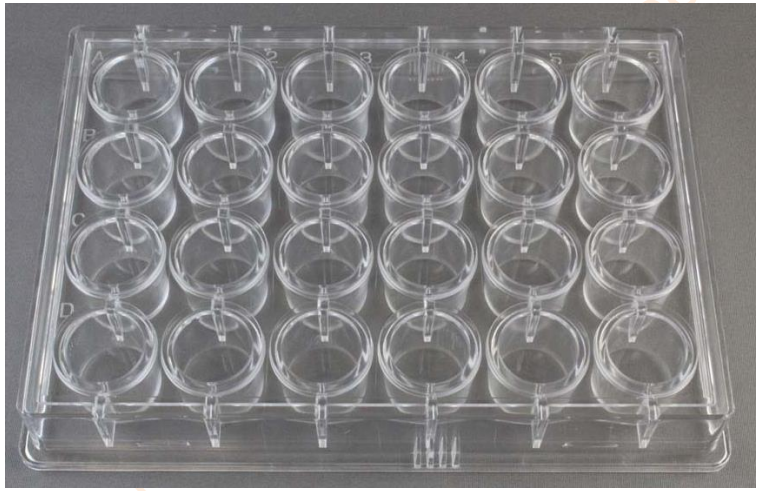
- **Detergents**
- Liquid **cubic phase** (LCP)
- Sponge phase





Crystallization plastics

- **Standardized** footprint formats (SBS, VDX)
  - 24/96 wells
  - high-throughput/automation
- Various layouts
- UV transparent



Commercial screens

- 48 or 96 conditions screens (deep well or tubes)
- Various purposes – sparse screens, systematic screens, optimization screens, additive screens, ...
- Producers:
  - Molecular Dimensions
  - Hampton
  - Jena Biosciences
  - Qiagen
  - …

Robotics for crystallization

- **Precipitant mixtures** preparation
- **Drops** set-up
- Storage and **imaging**
- (Crystal harvesting)

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.

Questions?

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