

Crystallography basics

-

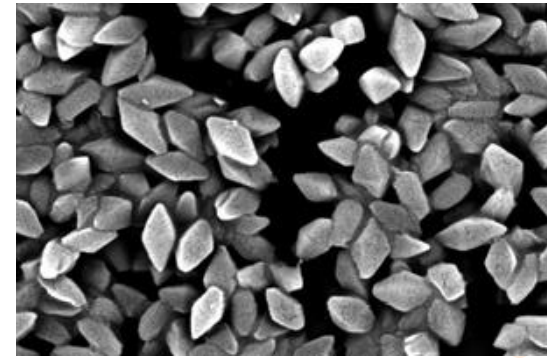
Crystallization

Josef Houser

Autumn 2023

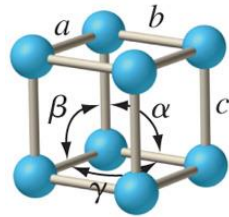
S1004 Methods for structural characterization of biomolecules

Crystal

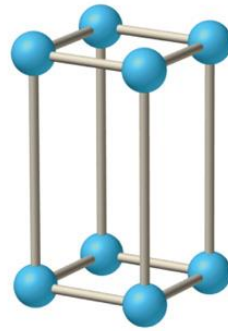


Crystal lattice

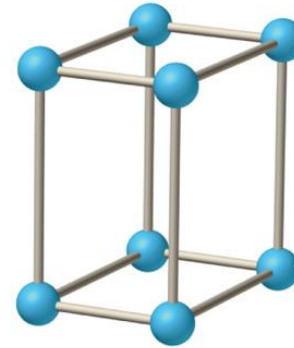
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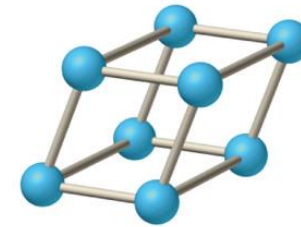
Simple cubic
 $a = b = c$
 $\alpha = \beta = \gamma = 90^\circ$



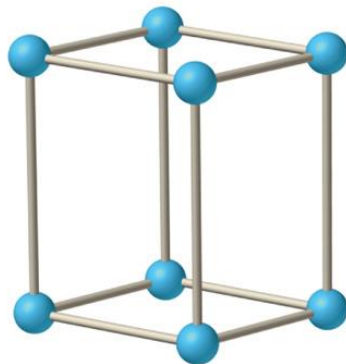
Tetragonal
 $a = b \neq c$
 $\alpha = \beta = \gamma = 90^\circ$



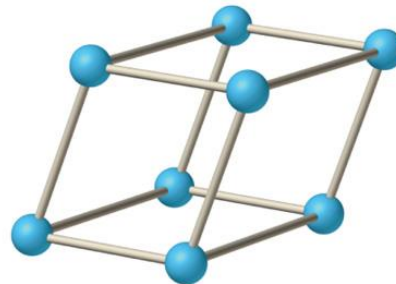
Orthorhombic
 $a \neq b \neq c$
 $\alpha = \beta = \gamma = 90^\circ$



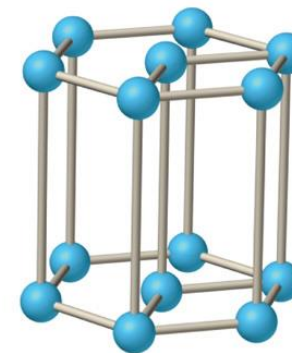
Rhombohedral
 $a = b = c$
 $\alpha = \beta = \gamma \neq 90^\circ$



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



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

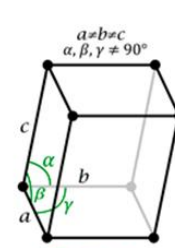


Hexagonal
 $a = b \neq c$
 $\alpha = \beta = 90^\circ, \gamma = 120^\circ$

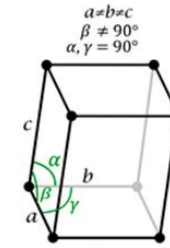
Bravais lattice

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

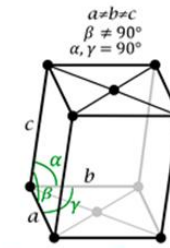
- 14 together



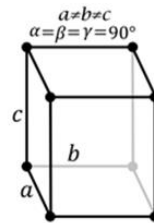
Triclinic



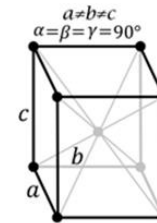
P Monoclinic



C

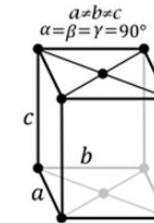


P

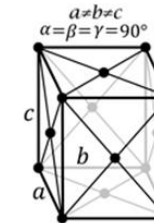


I

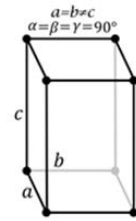
Orthorhombic



C

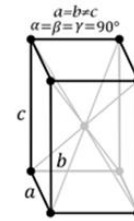


F

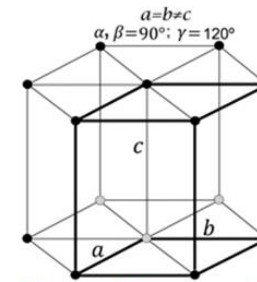


P

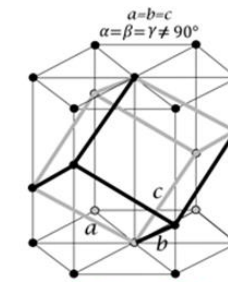
Tetragonal



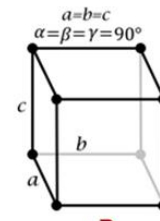
I



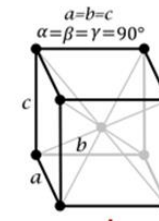
Trigonal / Hexagonal P



Trigonal R

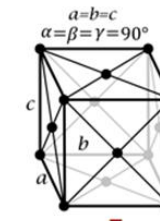


P



I

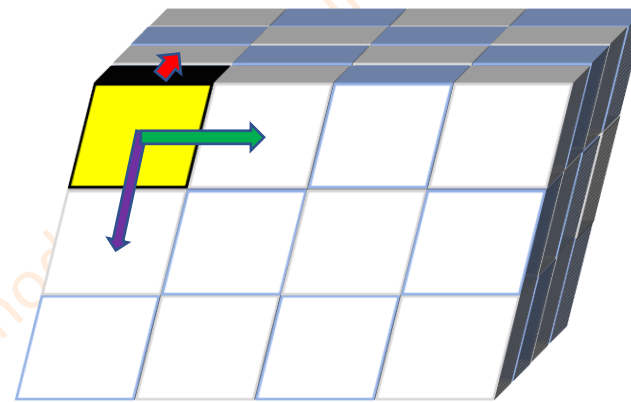
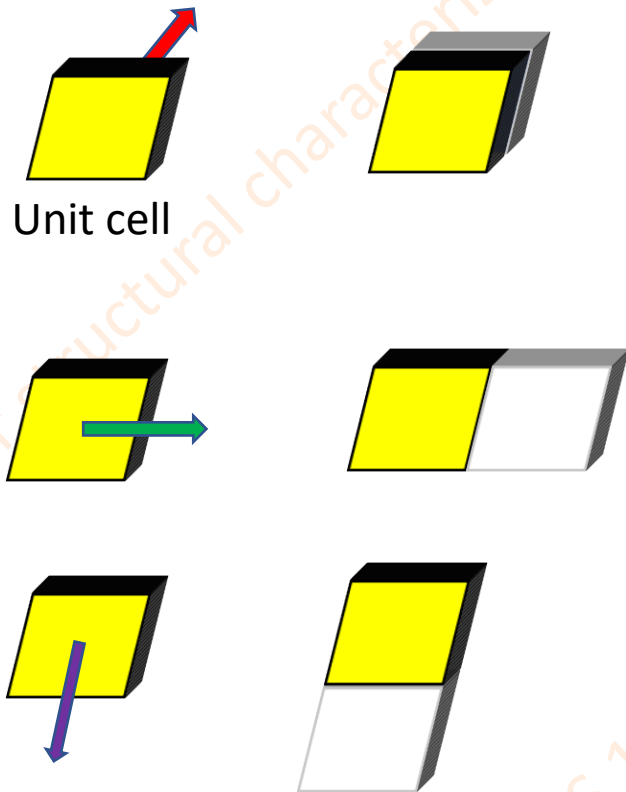
Cubic



F

Translational symmetry

- Essential property of crystal



Symmetry

- Ability of object to stay the same after transformation
- **Symmetry operations:**
 - Translation – vector
 - Reflection – plane
 - Rotation – axis



TRANSLATION

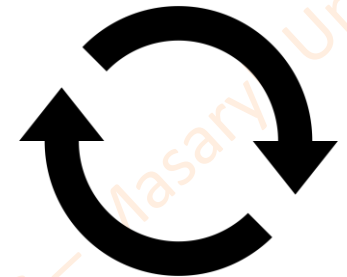
TRANSLATION

REFLECTION



REFLECTION

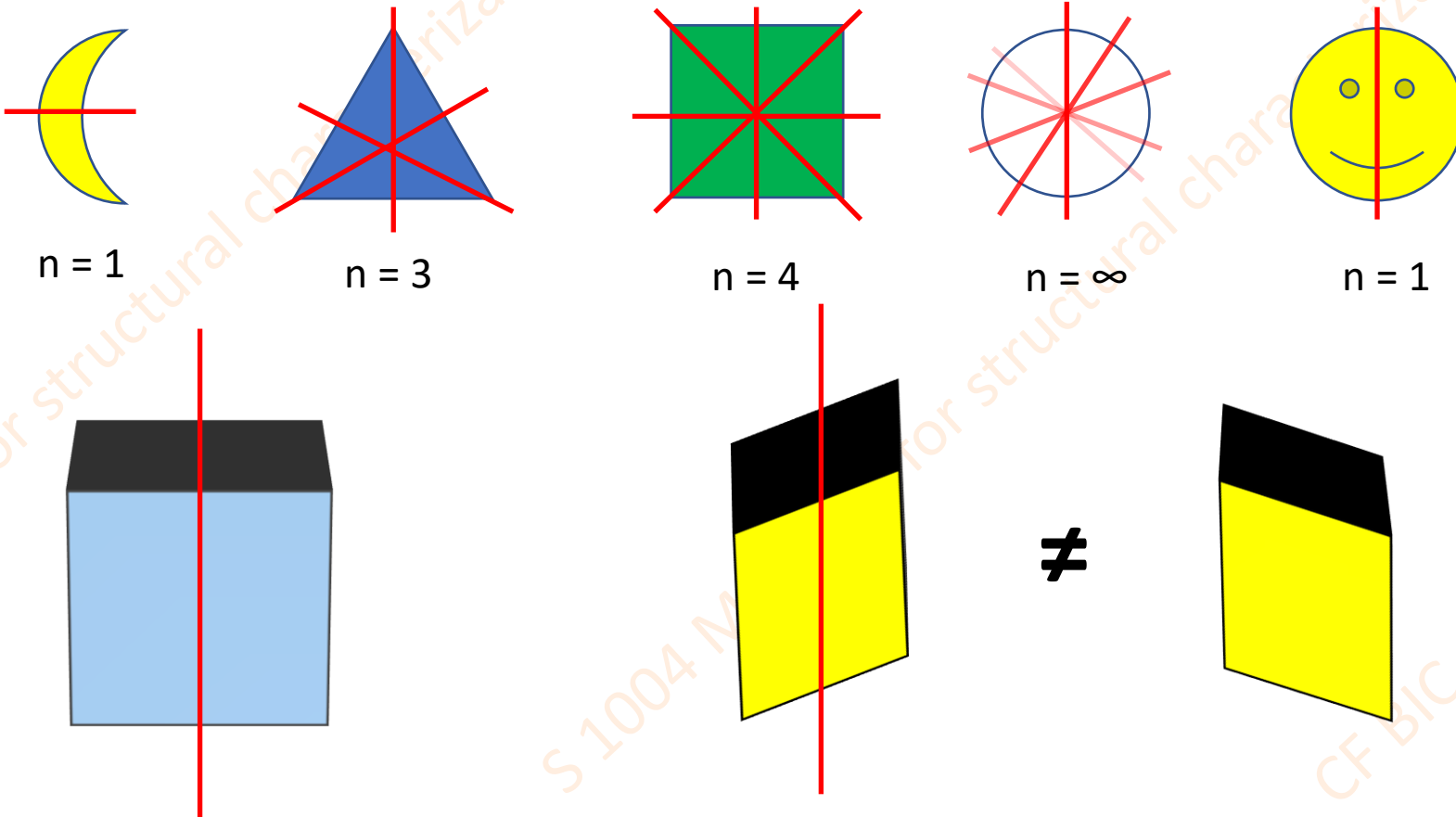
ROTATION



ROTATION

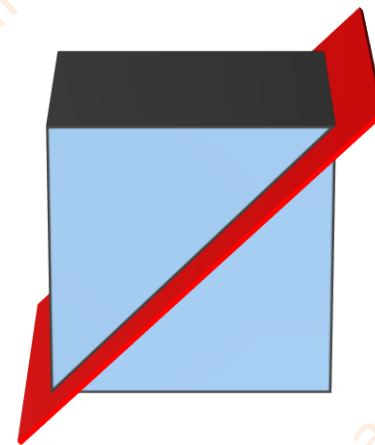
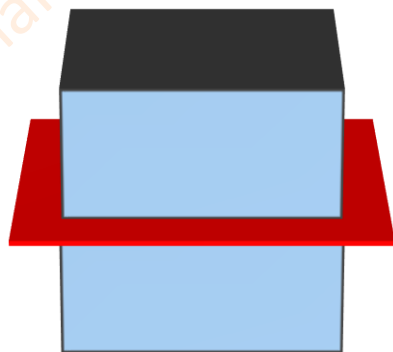
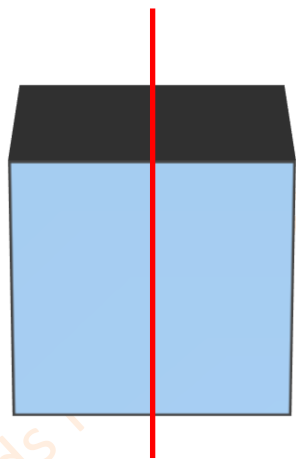
Reflection symmetry

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



Reflection symmetry

- Object may have several reflection symmetries



etc.

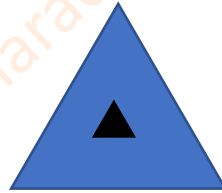
Rotational symmetry

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

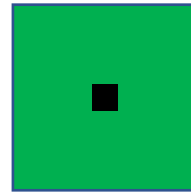


$n = 1$

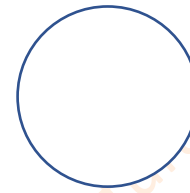
identity



$n = 3$



$n = 4$

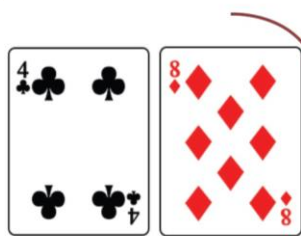


$n = \infty$



$n = 1$

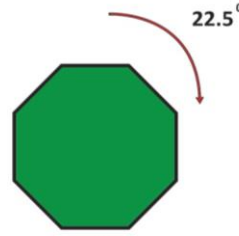
identity



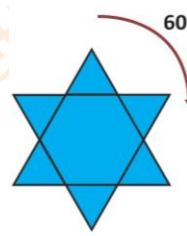
C_2



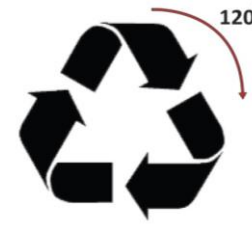
C_4



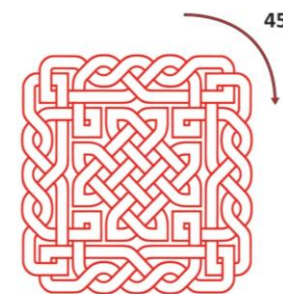
C_8



C_6



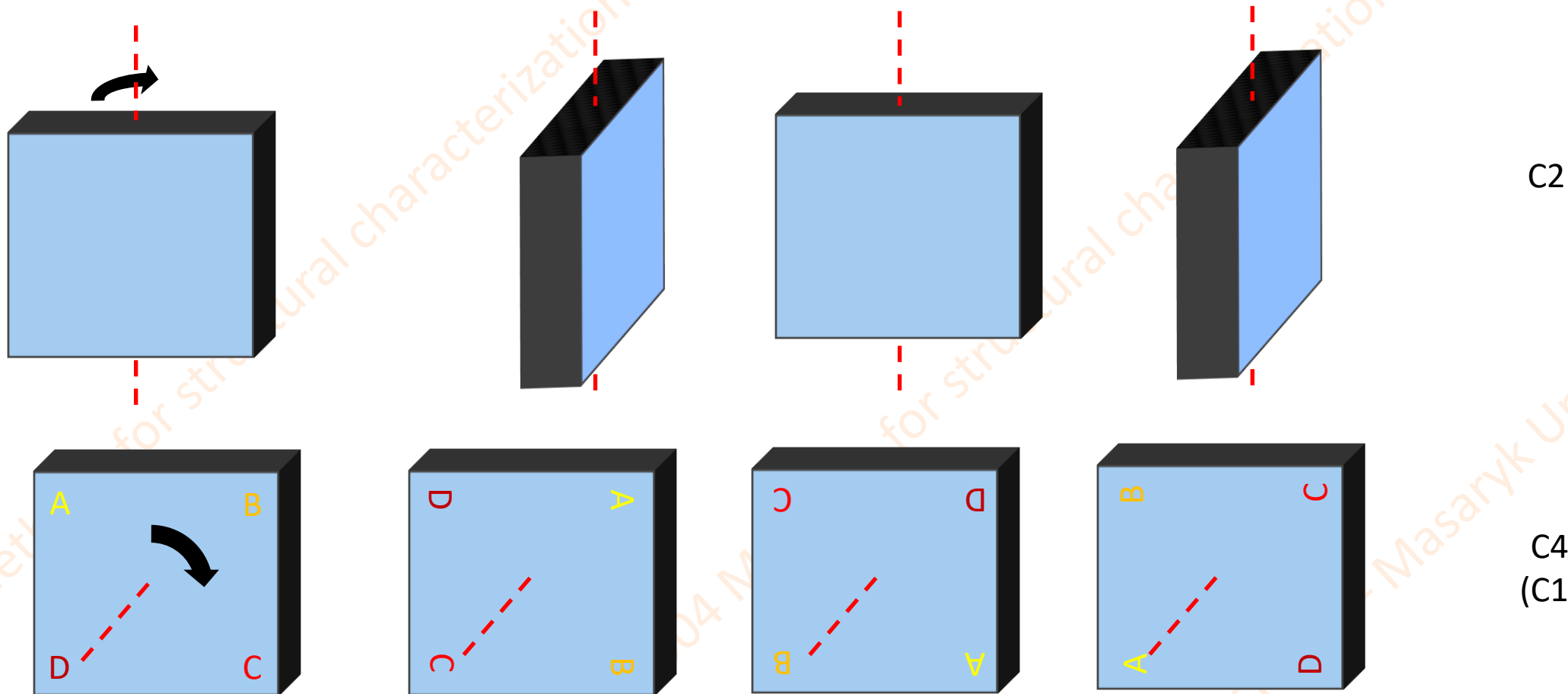
C_3



C_4

Rotational symmetry

- Object may have several axes of symmetry

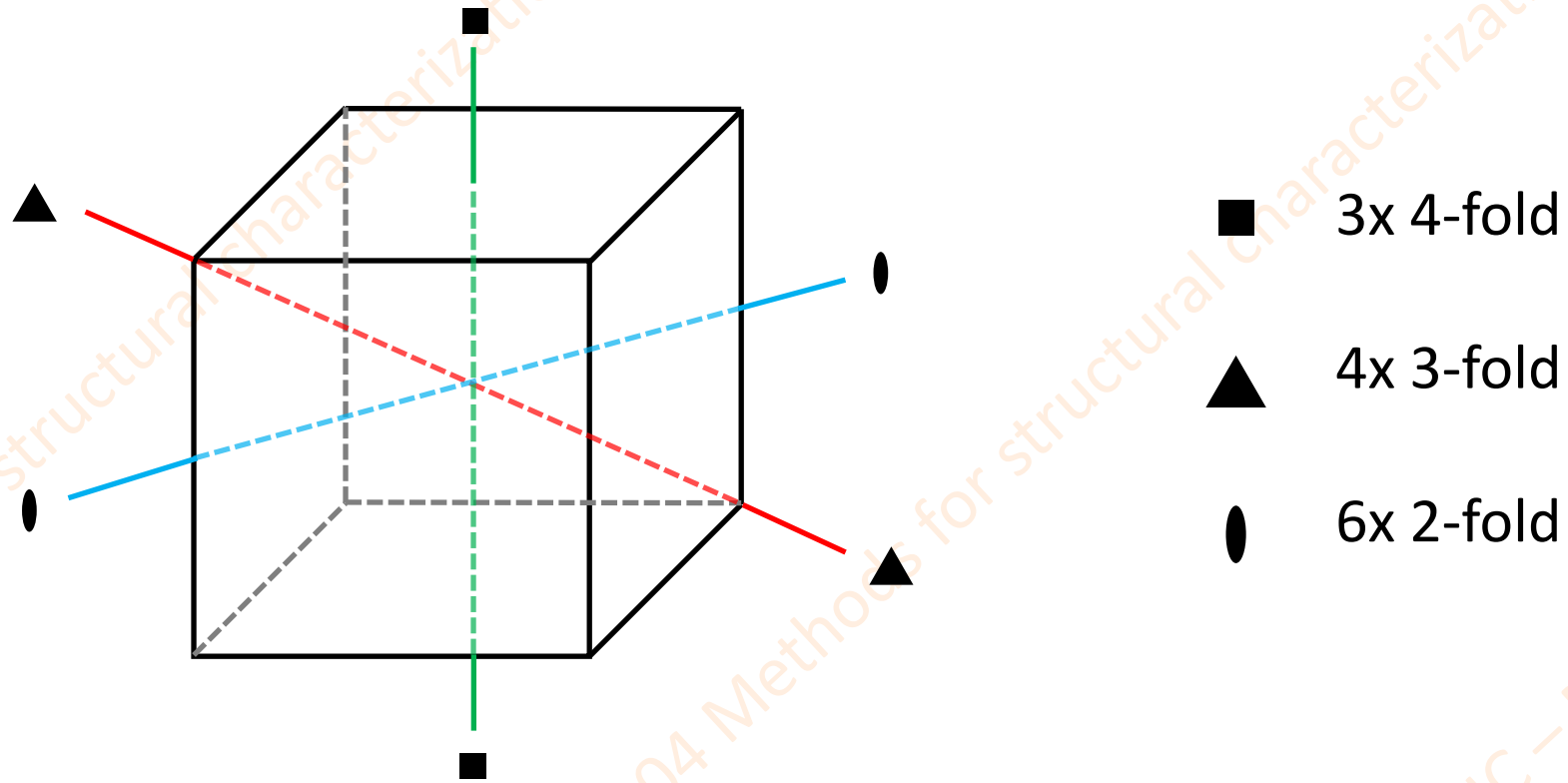


C2

C4
(C1)

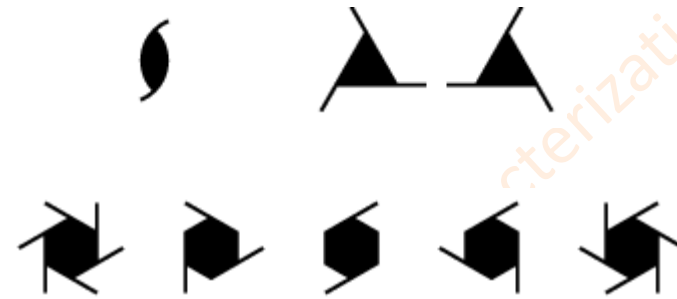
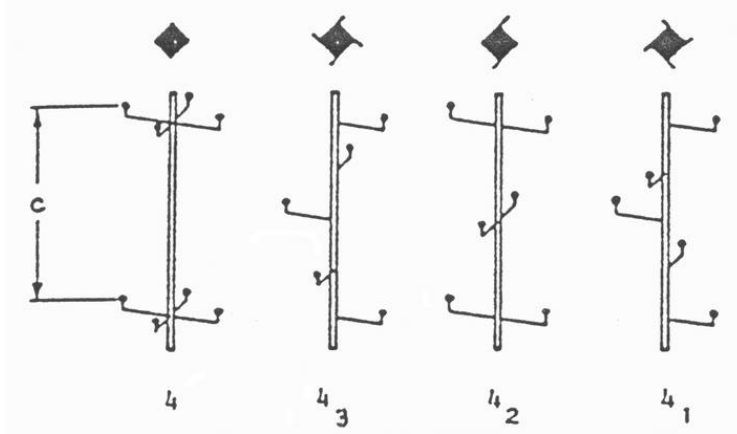
Rotational symmetry

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

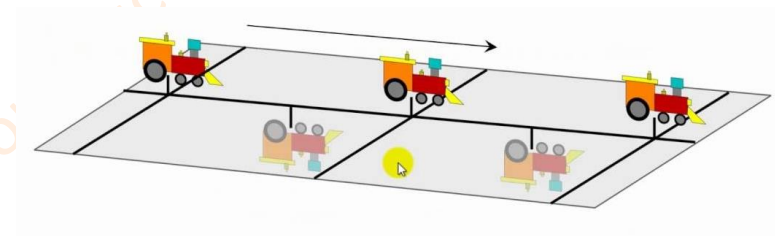


Combined symmetries

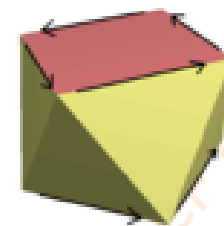
- Rotation + translation = **screw-axes**



- 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	$\bar{6}$	$\frac{6}{m}$	622	6mm	$\bar{6}m2$	$\frac{6}{m}mm$
Trigonal	3	$\bar{3}$		32	3m	$\bar{3}m$	
Tetragonal	4	$\bar{4}$	$\frac{4}{m}$	422	4mm	$\bar{4}2m$	$\frac{4}{m}mm$
Orthorhombic				222		mm2	mmm
Monoclinic	2		$\frac{2}{m}$		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

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

System	Bravais Lattice	Point Group	Space Group							Fraction
Triclinic	P	1	P1							1/2
		$\bar{1}$	P $\bar{1}$							
Monoclinic	P C	2	P2	P2 ₁	C2				1/4	
		$2/m$	P2/m	P2 ₁ /m	C2/m	Cc	P2 ₁ /c	C2/c		
Orthorhombic	P C F I	222	P222	P222 ₁	P2 ₁ 2 ₁ 2	P2 ₁ 2 ₁ 2 ₁	C222 ₁	C222	1/8	
		$mm2$	Pmm2	Pmc2 ₁	Pcc2	Pma2	Pca2 ₁	Pnc2		
			Pmn2 ₁	Pba2	Pna2 ₁	Pnn2	Cmm2	Cmc2 ₁		
			Ccc2	Amm2	Abm2	Ama2	Aba2	Fmm2		
			Fdd2	Imm2	Iba2	Ima2				
		mmm	Pmmm	Pnmm	Pccm	Pbam	Pmma	Pnna		
			Pmna	Pcca	Pbam	Pccn	Pnma	Pnmm		
			Pmnn	Pbcn	Pbca	Pnma	Cmcm	Cmca		
			Cmmm	Cccm	Cmma	Ccca	Fmmm	Fddd		
			Immm	Ibam	Ibca	Imma				
Tetragonal	P I	4	P4	P4 ₁	P4 ₂	P4 ₃	I4	I4 ₁	1/8	
		$\bar{4}$	P4	I4						
		$4/m$	P4/m	P4 ₂ /m	P4/n	P4 ₂ /n	I4/m	I4 ₁ /a		
		422	P422	P4 ₂ 2	P4 ₁ 22	P4 ₃ 22	P4 ₂ 22	P4 ₂ 2 ₂	1/16	
		$4mm$	P4mm	P4bm	P4 ₂ cm	P4 ₂ nm	P4cc	P4nc		
			P4 ₂ mc	P4 ₂ bc	I4mm	I4cm	I4 ₂ md	I4 ₂ cd		
		$\bar{4}2m$	P4 ₂ m	P4 ₂ c	P4 ₂ m	P4 ₂ c	P4 ₂ m	P4 ₂ c		
			P4 ₂ b2	P4 ₂ n2	I4m2	I4c2	I4 ₂ m	I4 ₂ d		
		$4/mmm$	P4/mmm	P4/mcc	P4/nbm	P4/nnc	P4/mbm	P4/mnc		
			P4/nmm	P4/ncc	P4 ₂ /mmc	P4 ₂ /mcc	P4 ₂ /nbc	P4 ₂ /nnc		
	P4 ₂ /mbc	P4 ₂ /mnm	P4 ₂ /nmc	P4 ₂ /ncc	I4/mmm	I4/mcm				
	I4 ₁ /amd	I4 ₁ /acd								
Trigonal/ rhombohedral	P R	3	P3	P3 ₁	P3 ₂	R3			1/6	
		$\bar{3}$	P $\bar{3}$	R $\bar{3}$						
		32	P312	P321	P3 ₁ 12	P3 ₂ 12	P3 ₁ 12	P3 ₂ 12	1/12	
		$R\bar{3}2$	R32							
$3m$	P3m1	P31m	P3c1	P31c	R3m	R3c				
$\bar{3}m$	P31m	P31c	P3m1	P3c1	R3m	R3c				
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 ₂ 22	P6 ₄ 22	P6 ₂ 22	P6 ₃ 22	P6 ₂ 22	1/24	
		$6mm$	P6mm	P6cc	P6 ₃ cm	P6 ₂ mc				
		$\bar{6}m\bar{2}$	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 ₂ /mmc						
Cubic	P I F	23	P23	F23	I23	P2 ₁ 3	I2 ₁ 3	1/24		
		$m\bar{3}$	Pm3	Pn3	Fm3	Fd3	Im3	Pa3		
		$ia\bar{3}$	Ia3							
		432	P432	P4 ₃ 2	F432	F4 ₃ 2	I432	P4 ₃ 2	1/48	
$\bar{4}3m$	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	Pn3n	Fm3m	Fm3c				
	Fd3m	Fd3c	Im3m	Ia3d						

*The 11 Laue symmetries are separated by horizontal lines.

$Cmm2$

No. 35

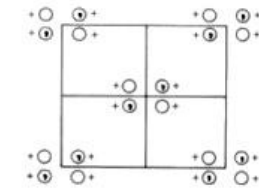
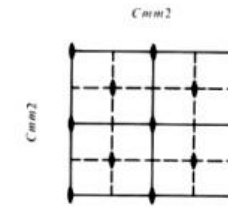
C_{2v}^{11}

$Cmm2$

$mmm2$

Orthorhombic

Patterson symmetry $Cmmm$



Positions

Multiplicity,
Wyckoff letter,
Site symmetry

Coordinates

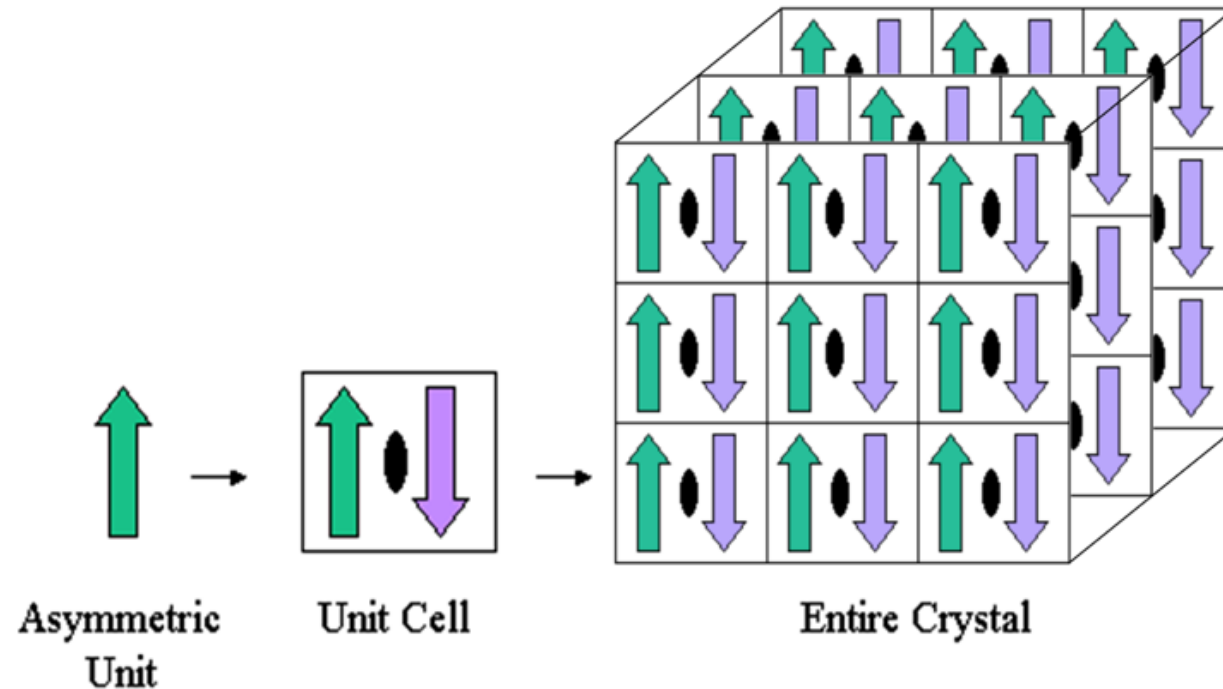
(0,0,0)+ (1/2, 1/2, 0)+

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**



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

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

BRAVAIS- TYPE	POSSIBLE SPACE-GROUPS FOR PROTEIN CRYSTALS [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]

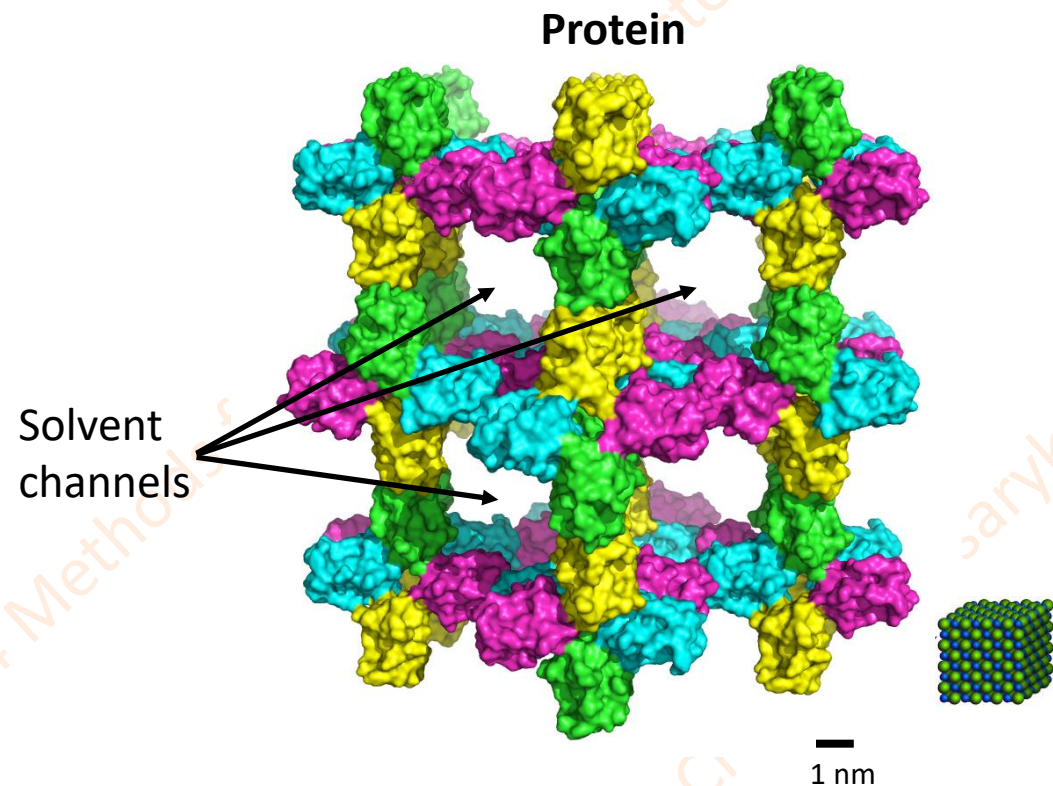
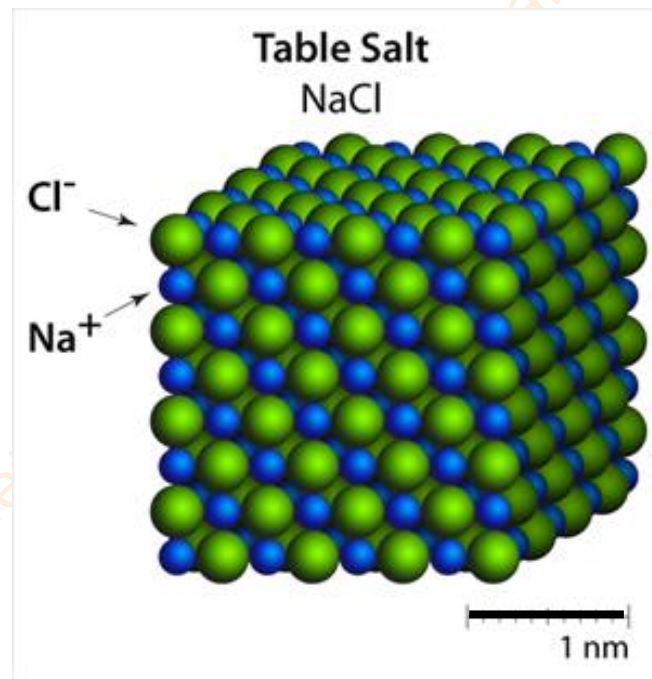
Biomacromolecular crystal

- 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

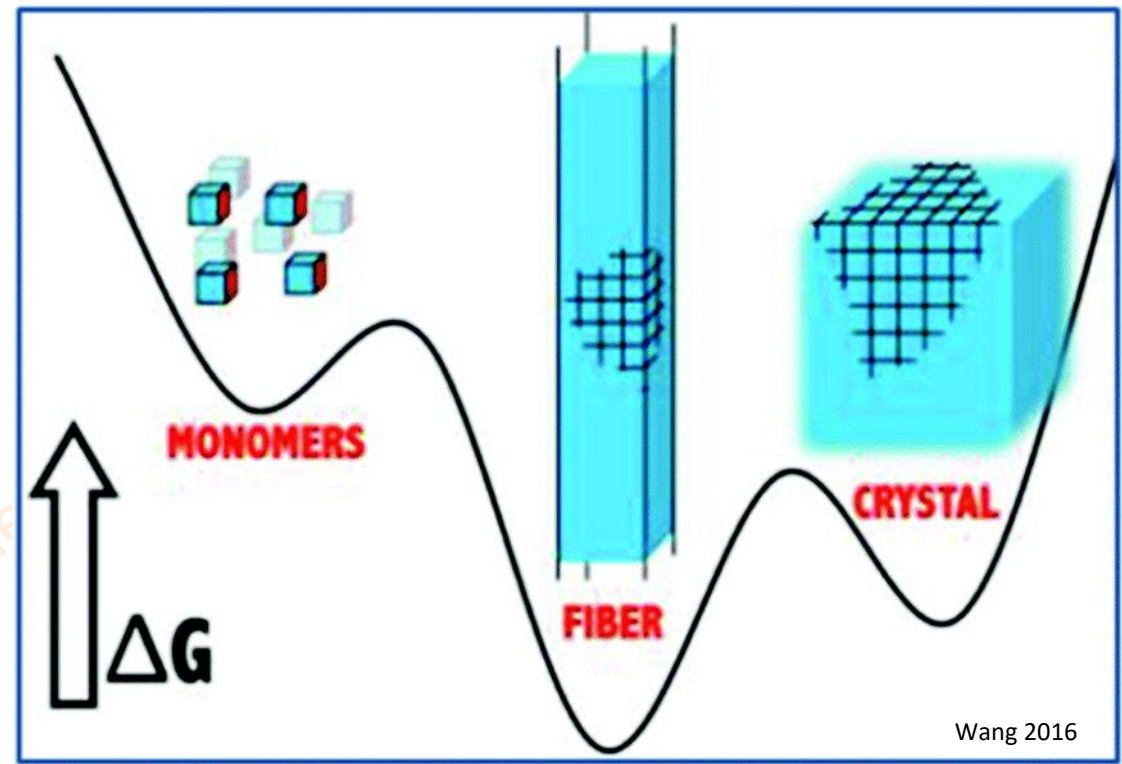
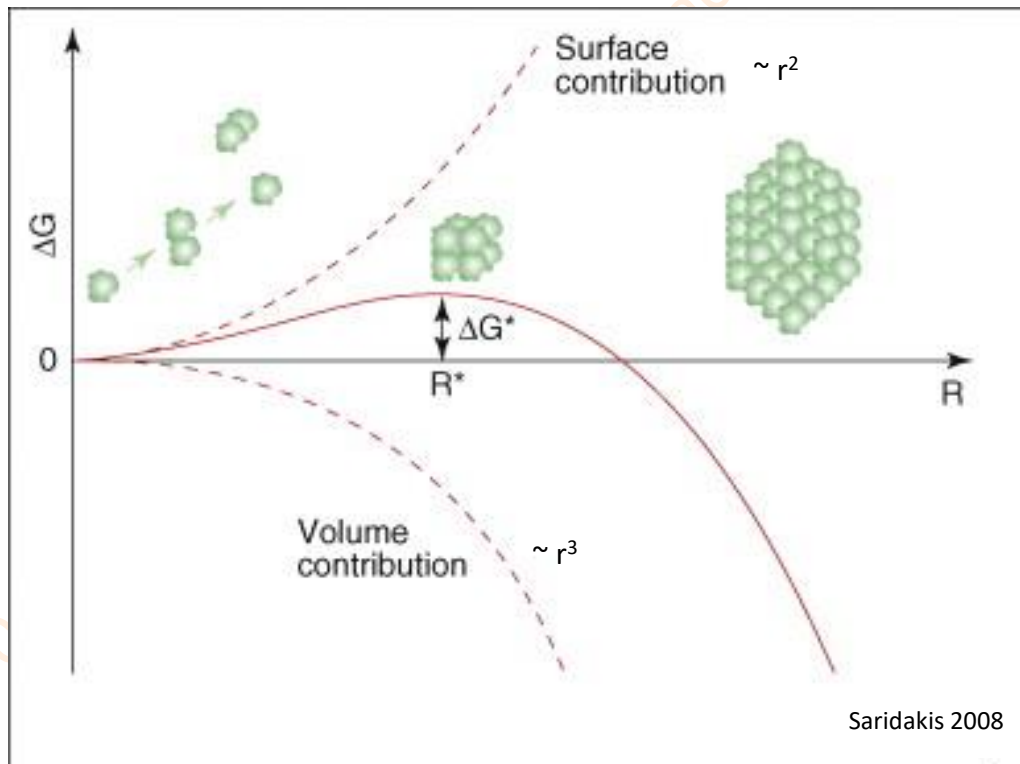
Biomacromolecular crystal

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



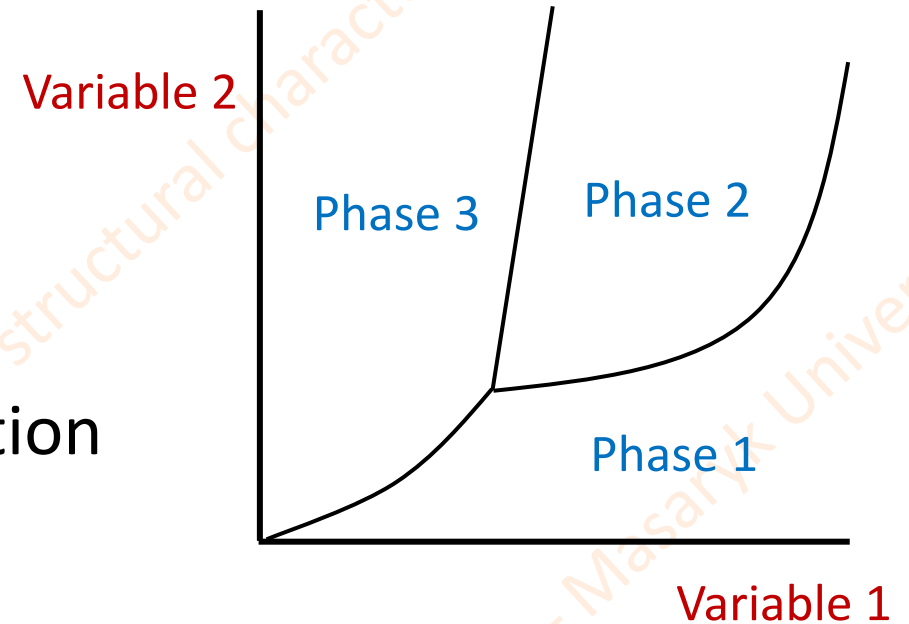
Crystallization

- Process of self-arranging of molecules into crystal lattice
- **Thermodynamics** – enthalpy, entropy, kinetics

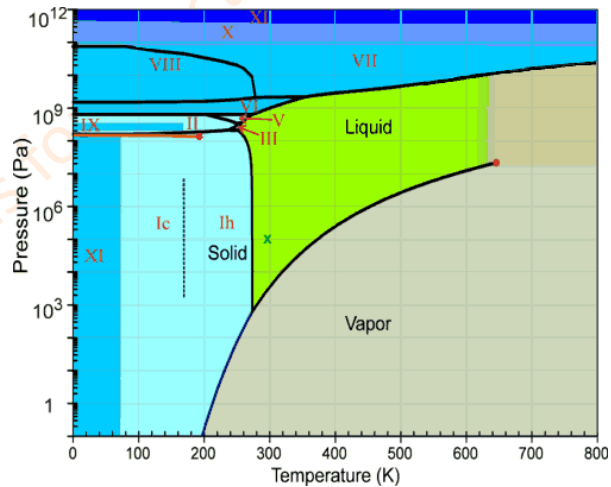
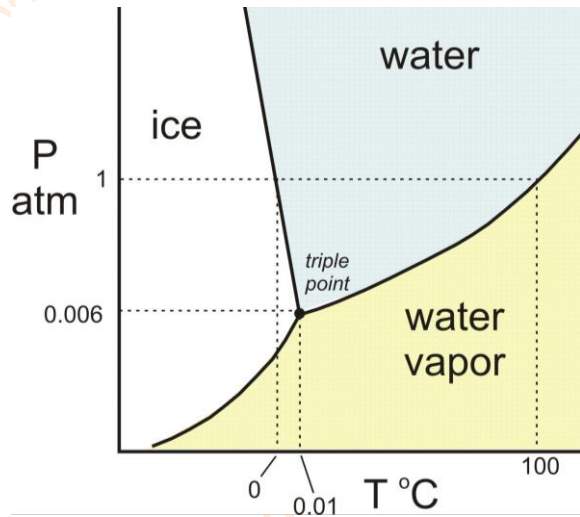


Phase diagram

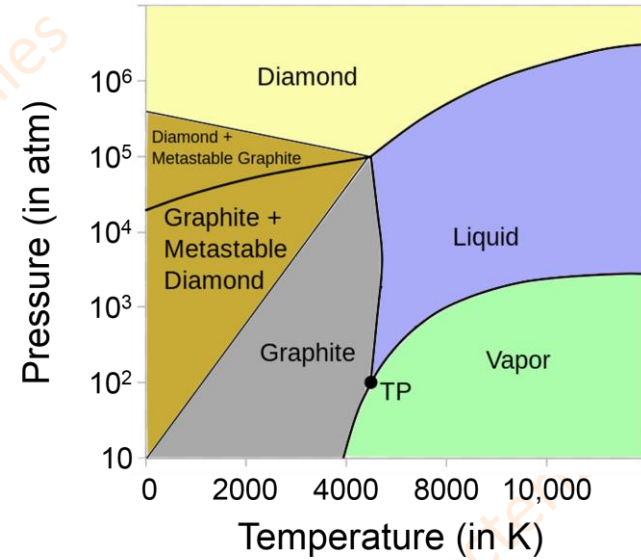
- Existence of various phases in the system depending on variables
- **Phase:** gas, liquid, solid
- **Variable:** temperature, pressure, composition



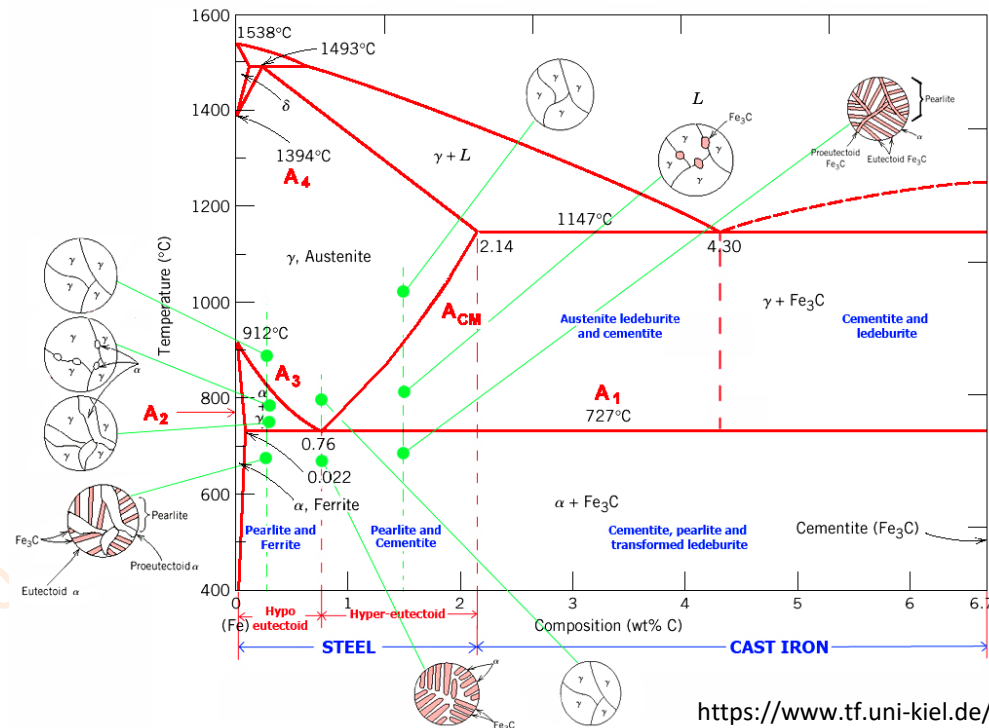
Phase diagram



<http://ergodic.ugr.es/>



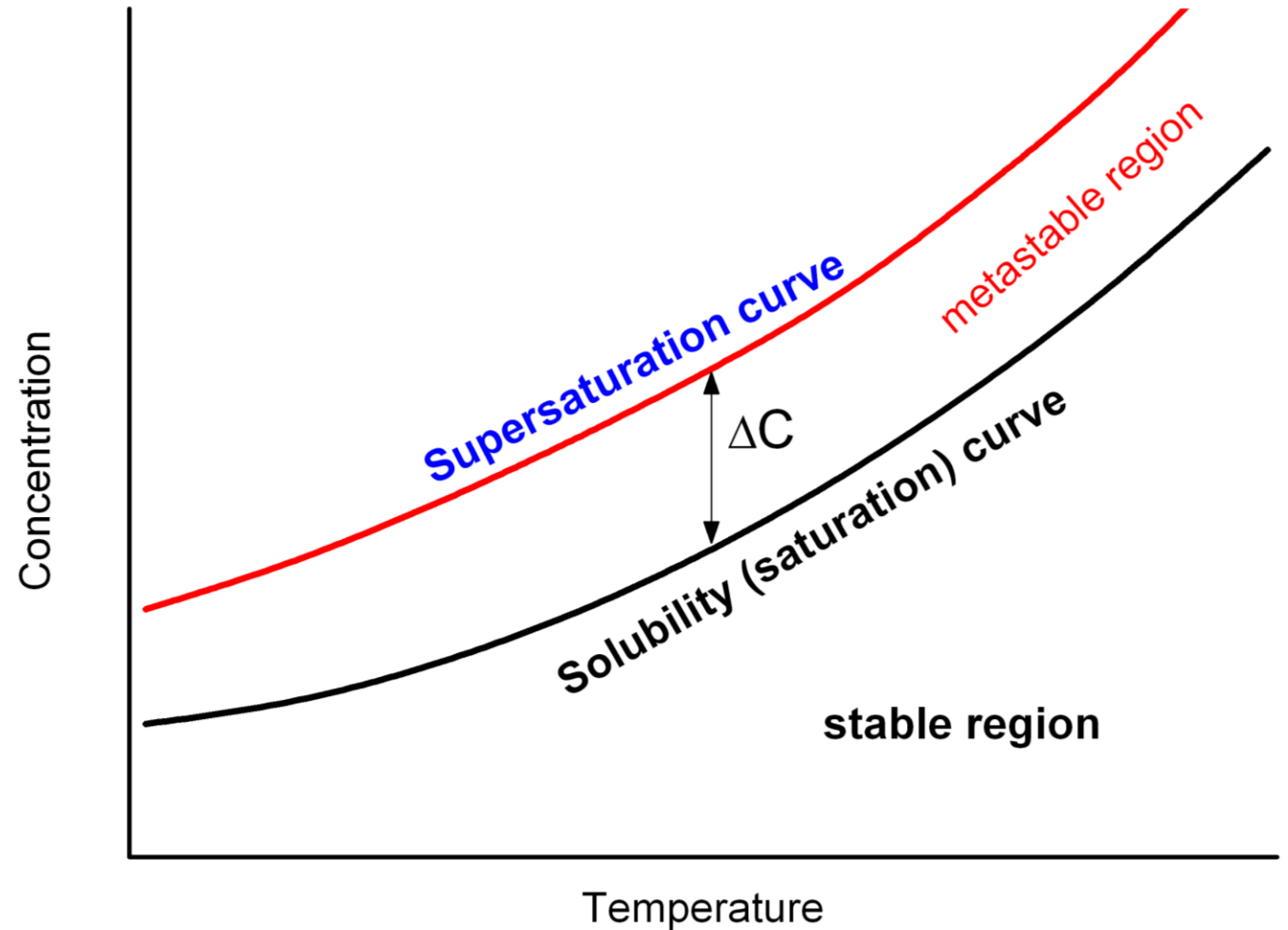
<https://www.chegg.com/>



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

Protein phase diagram – T vs. conc.

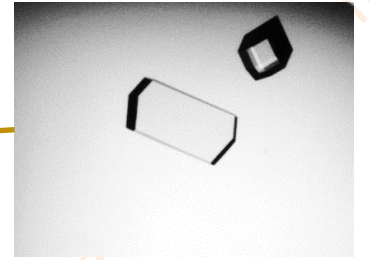
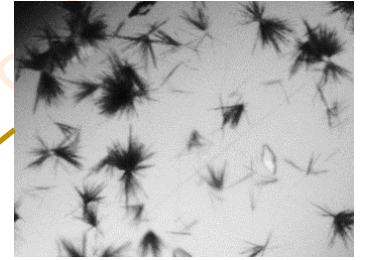
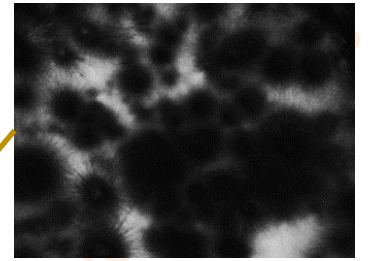
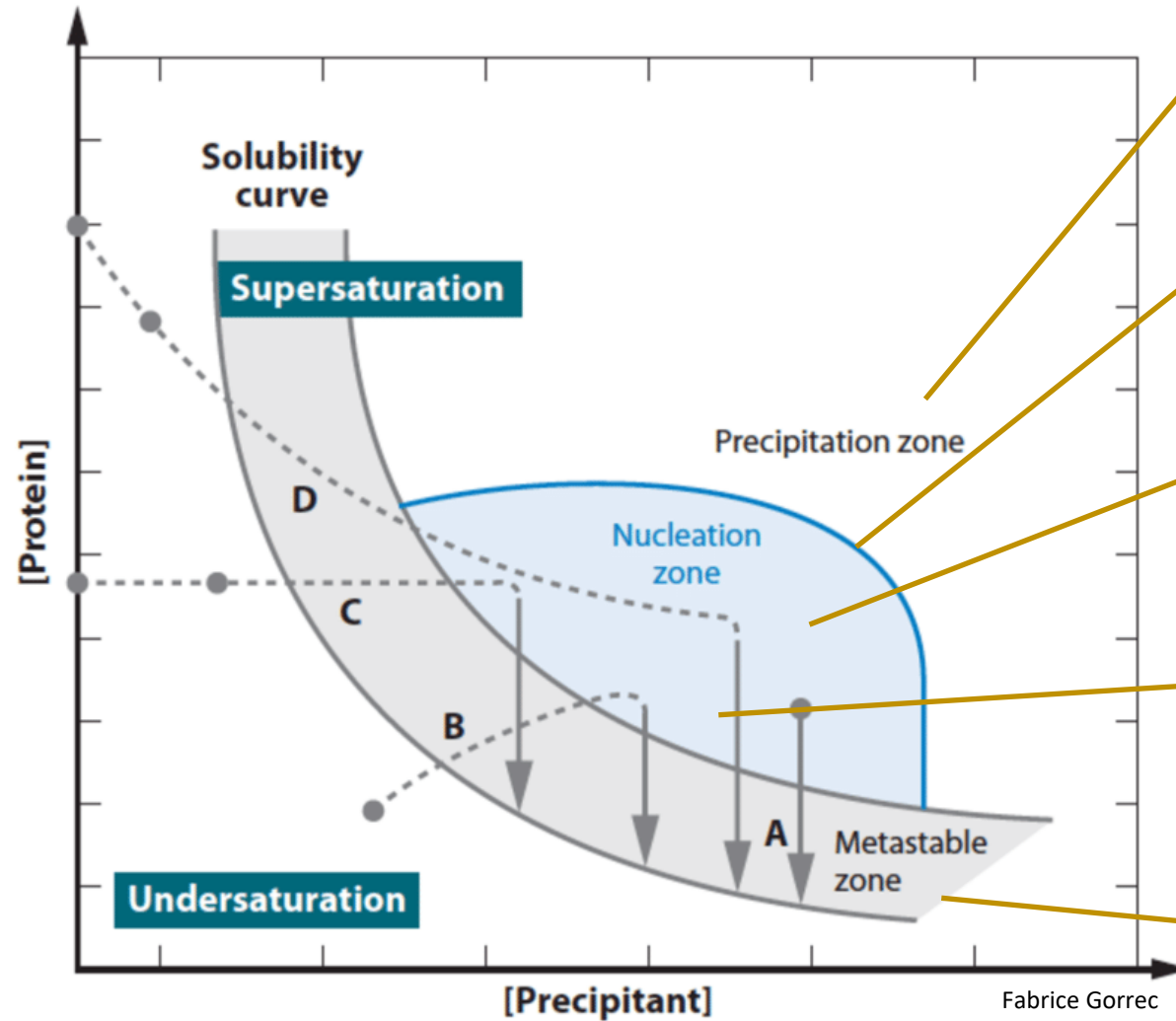
- Solubility increase with temperature
- Limited application due to protein conformational instability at higher T



Protein phase diagram – composition

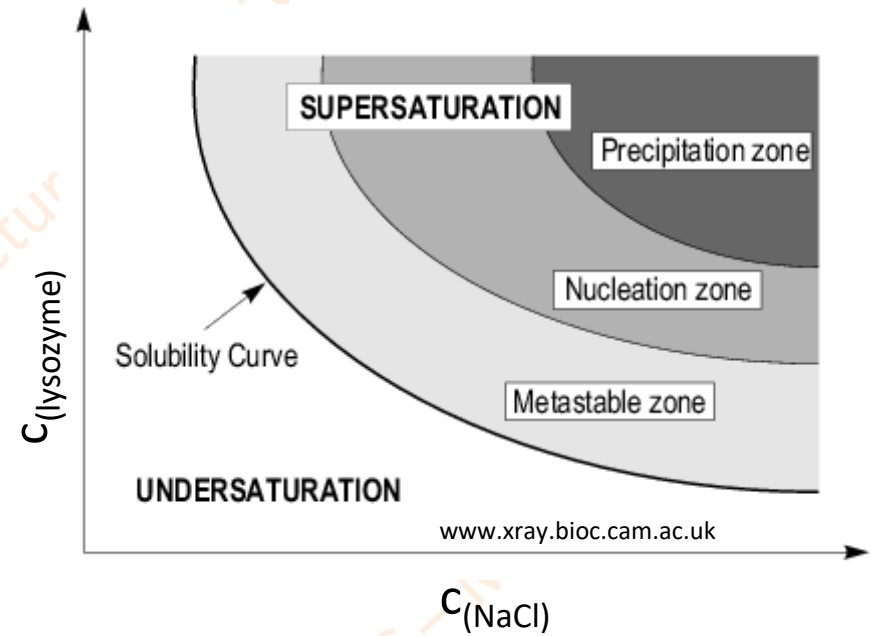
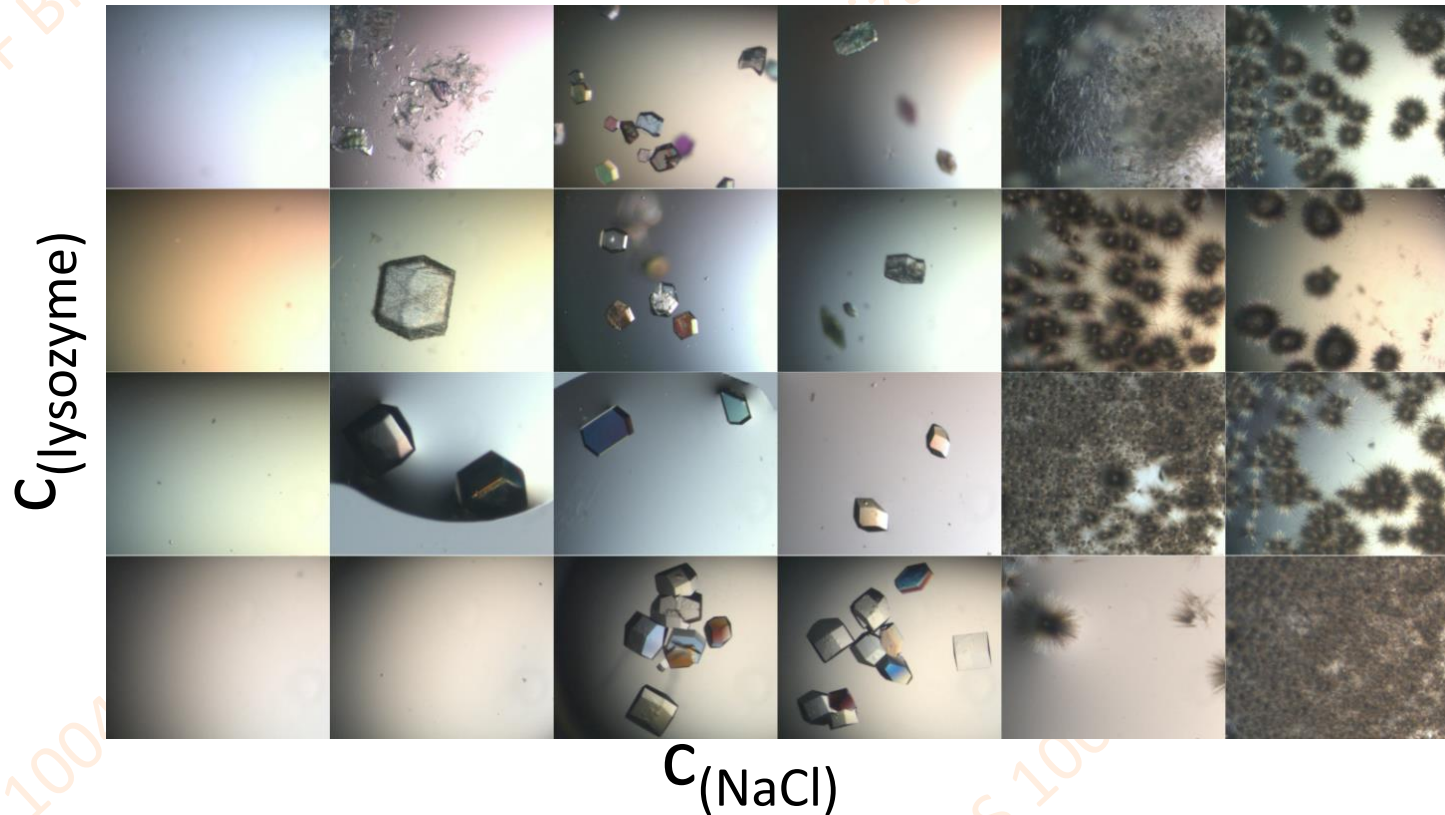
- Precipitant:

- Buffer
- Salt
- Small organics
- Mixtures



Real phase diagram

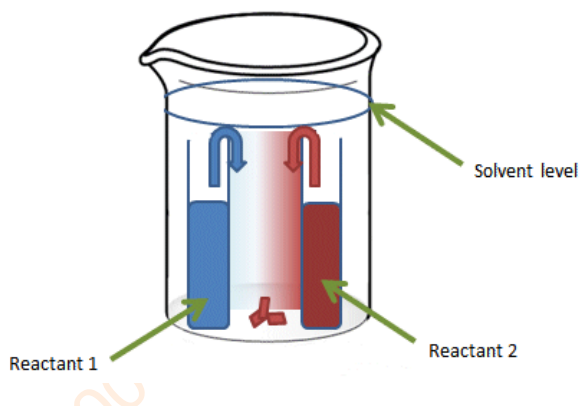
- Lysozyme vs. NaCl in acetate buffer



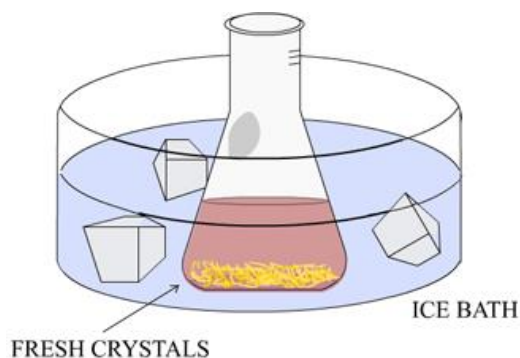
Crystallization techniques

- **Small molecules**

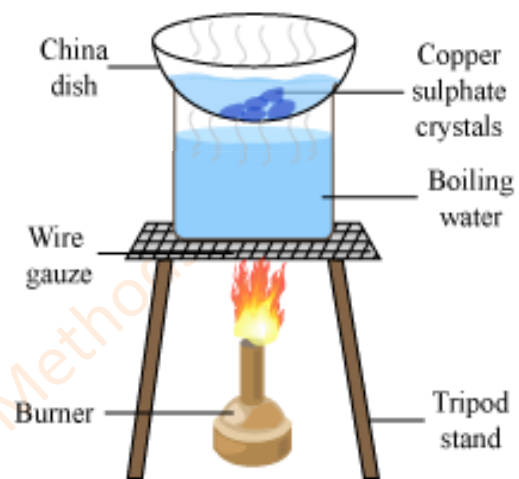
- *In situ* crystallization
- Cooling
- Solvent evaporation (free, under vacuum)
- Solvent exchange



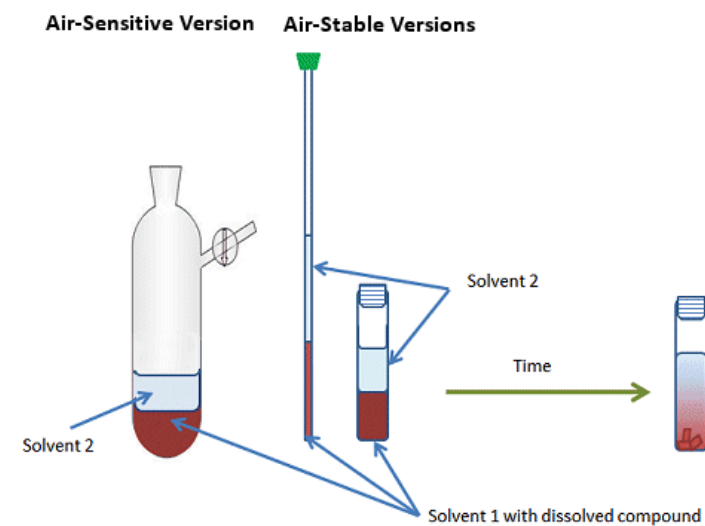
chemistryviews.com



chemhelper.com



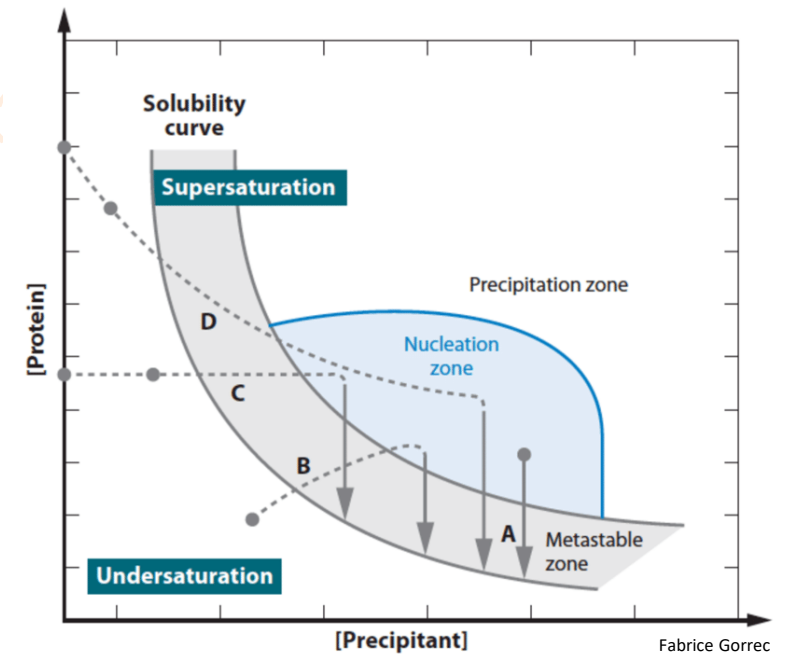
jinachem.blogspot.com



chemistryviews.com

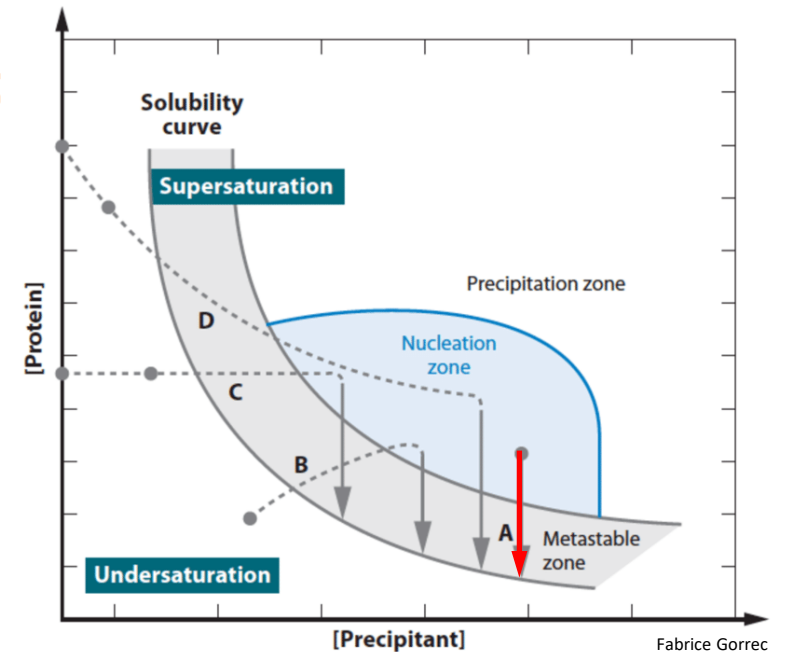
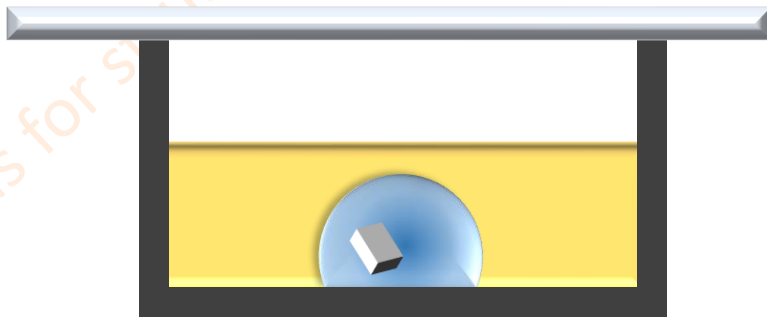
Crystallization techniques

- Small molecules
 - *In situ* crystallization
 - Cooling
 - Solvent evaporation (free, under vacuum)
 - Solvent exchange
- Macromolecules
 - Batch
 - Vapor diffusion
 - Dialysis
 - Free interface diffusion



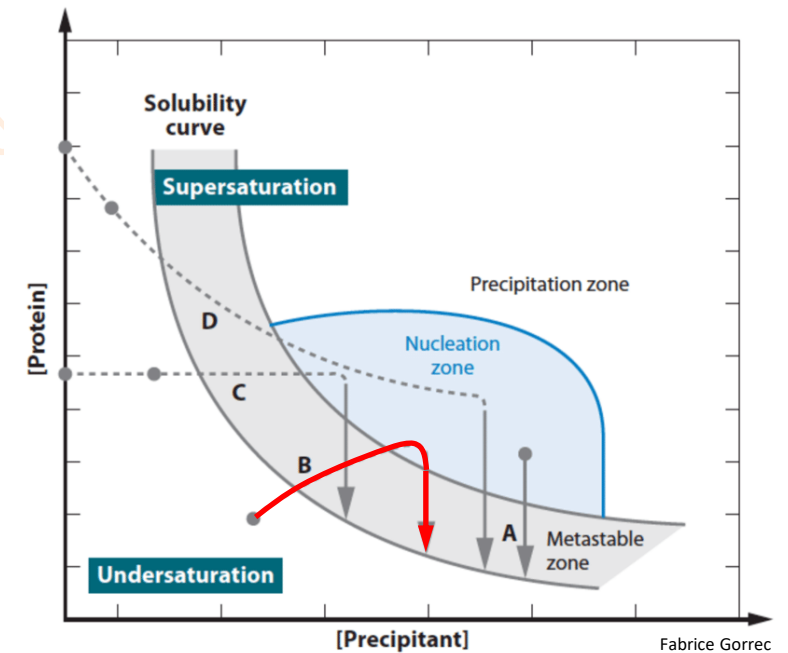
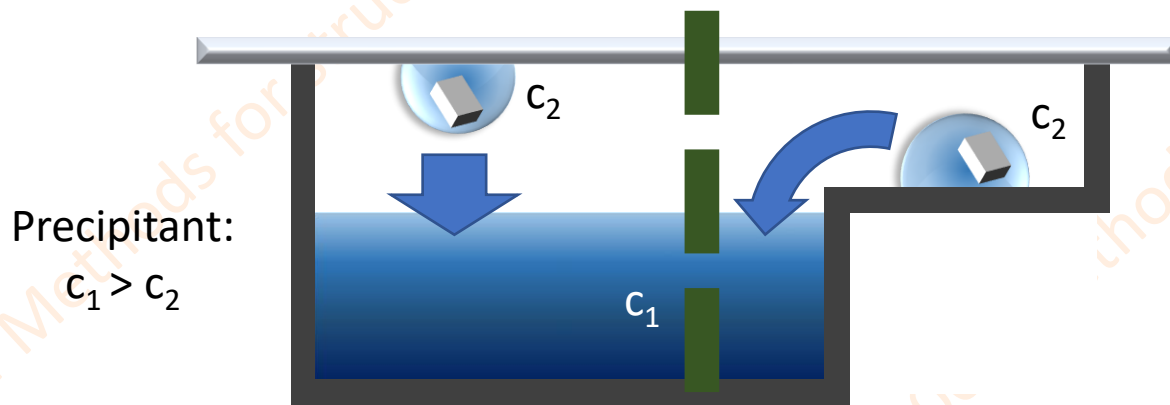
Batch method

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



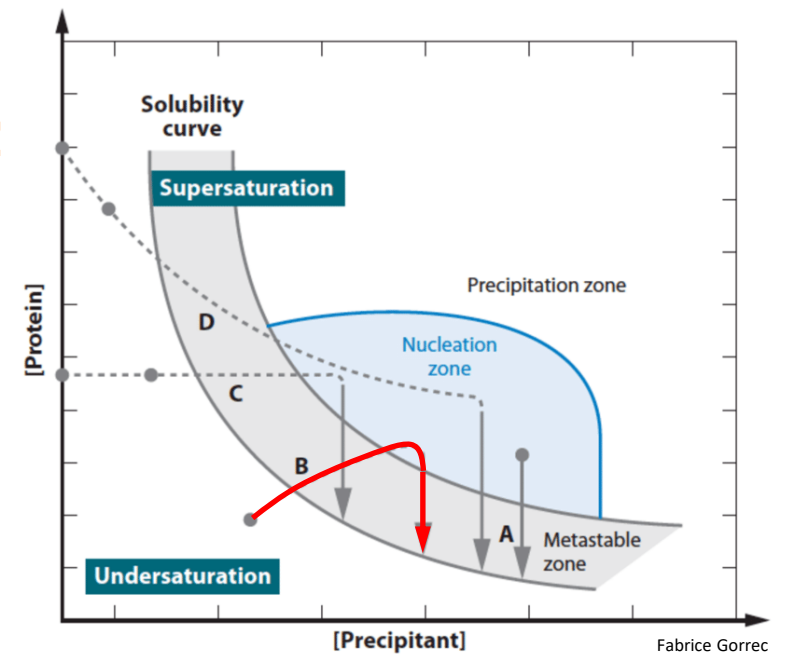
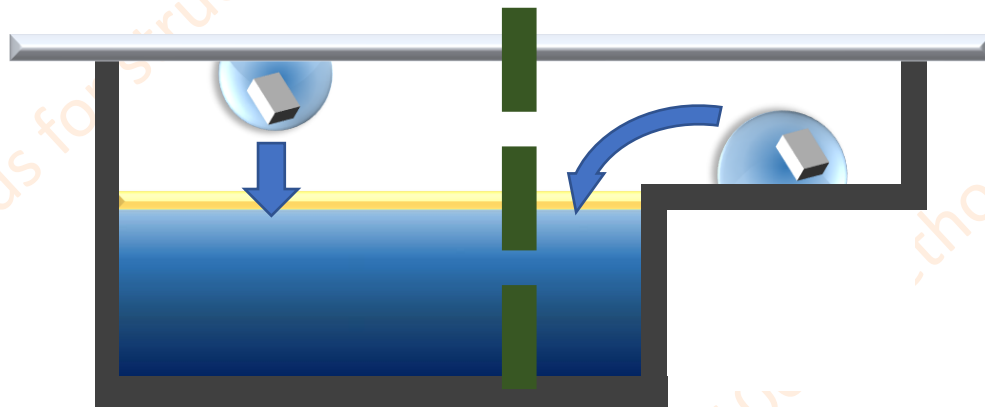
Vapor diffusion

- Increasing of protein and precipitant concentrations due to H_2O evaporation
- **Sitting drop vs Hanging drop vs Sandwich**



Under oil vapor diffusion

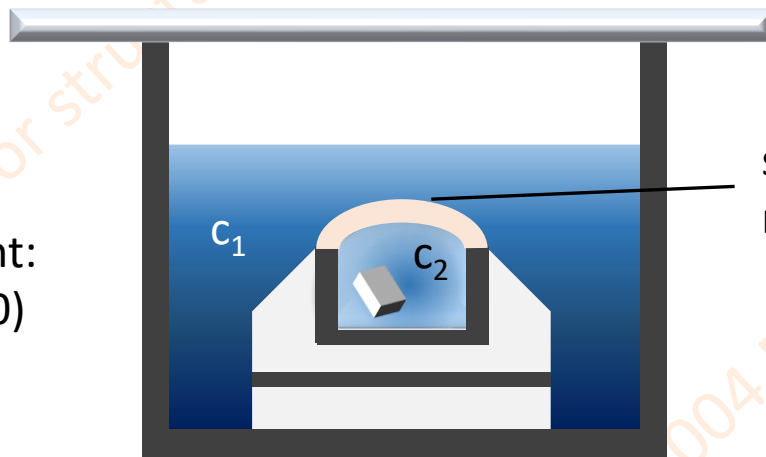
- Modification of vapor diffusion
- **Slowing down** the process
- **Paraffin oil vs. Silicon** oil
- Speed adjustment by paraffin oil : silicon oil ratio



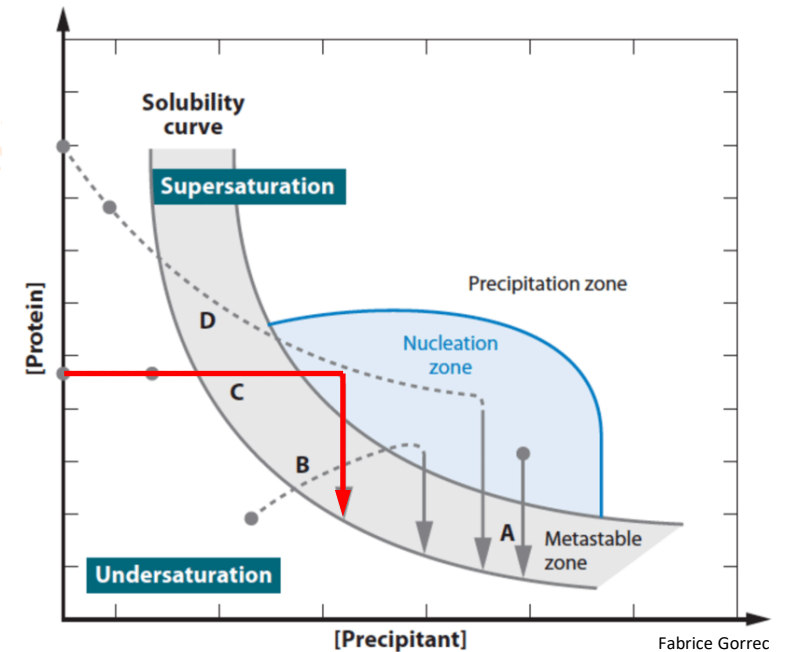
Dialysis

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

Precipitant:
 $c_1 > c_2 (=0)$



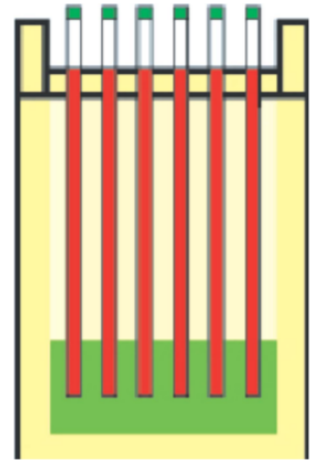
semipermeable
membrane



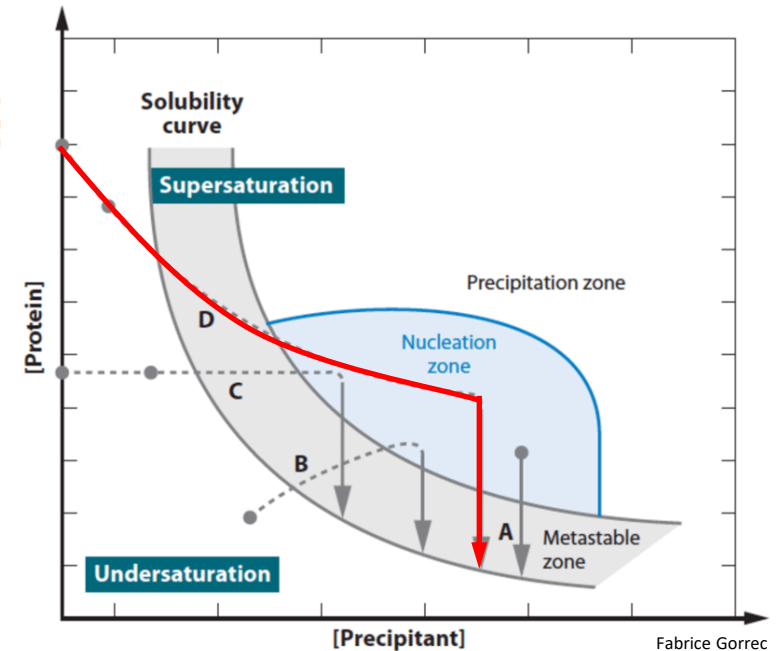
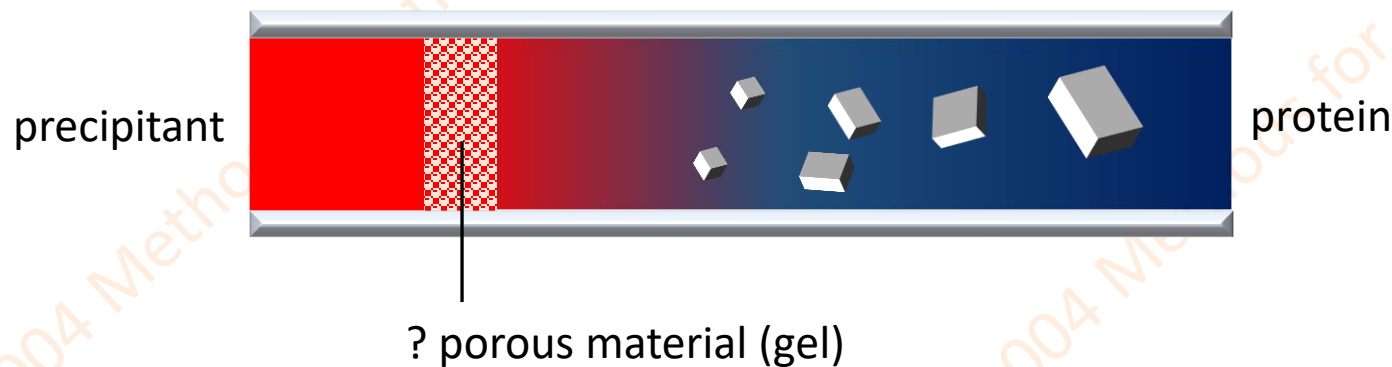
Fabrice Gorrec

Diffusion

- Diffusion of protein and precipitant in opposite direction
- **Free-interface** in capillary
or
Unidirectional diffusion – similar to dialysis



McPherson 2013

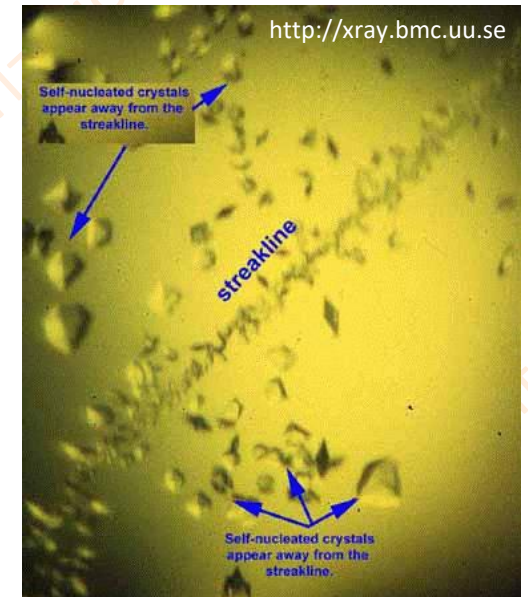
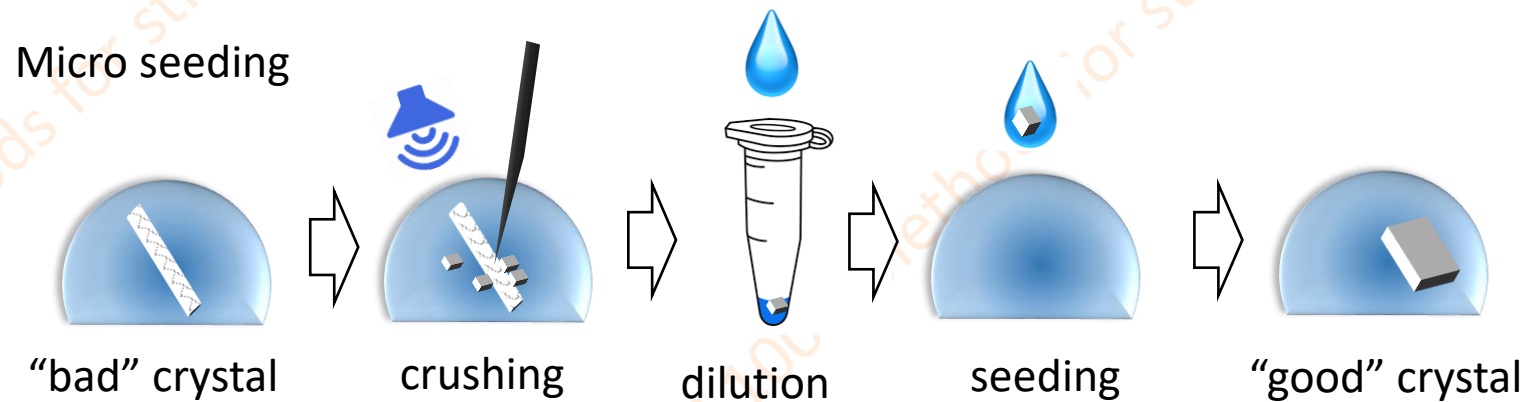


Fabrice Gorrec

Seeding

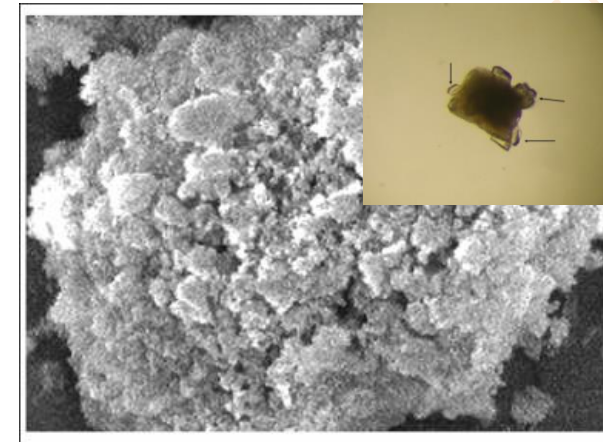
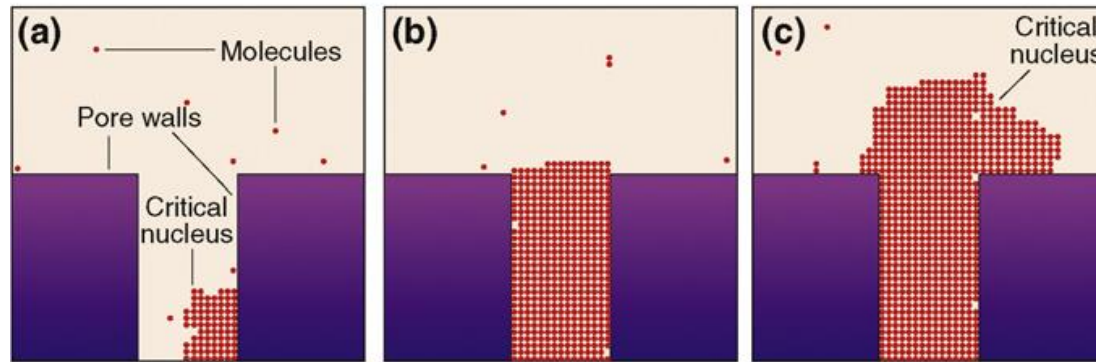
Stimulation of crystallization by introducing crystallization **nucleus**

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

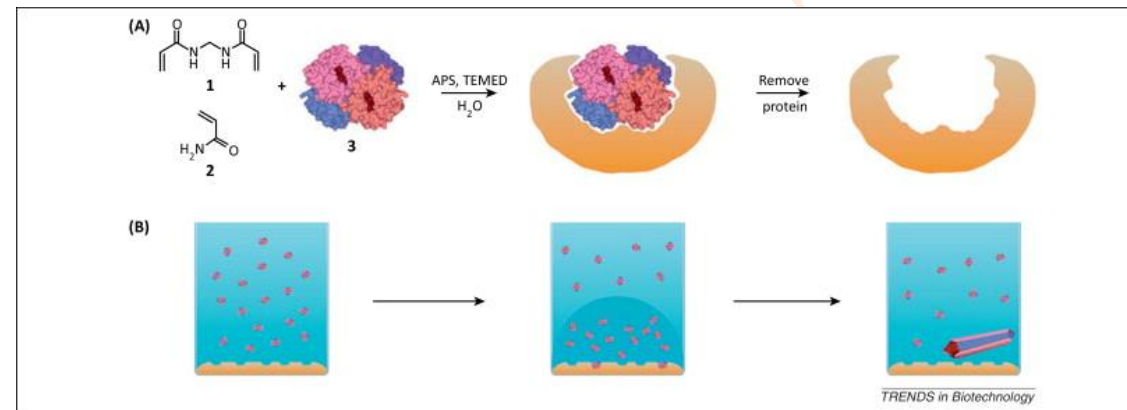


Seeding specials

- Universal nucleant



- Imprinted polymers



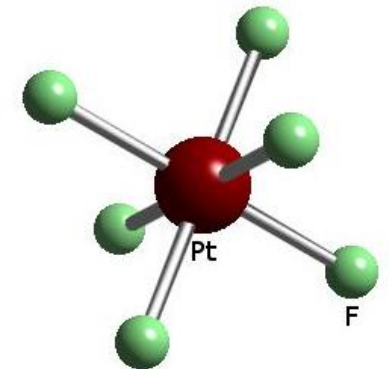
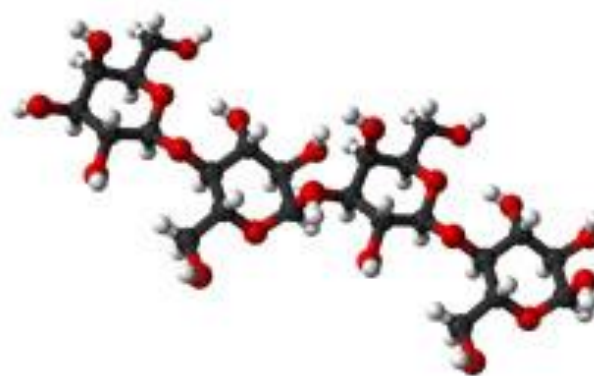
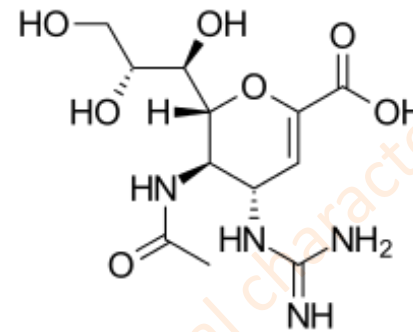
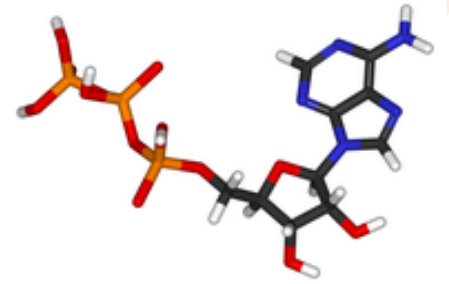
Derivatization

- **Modification** of crystallized protein

- Ligands/substrates
- Co-factors
- Inhibitors
- Heavy-atom compounds

- **Techniques**

- Co-crystallization
- Soaking

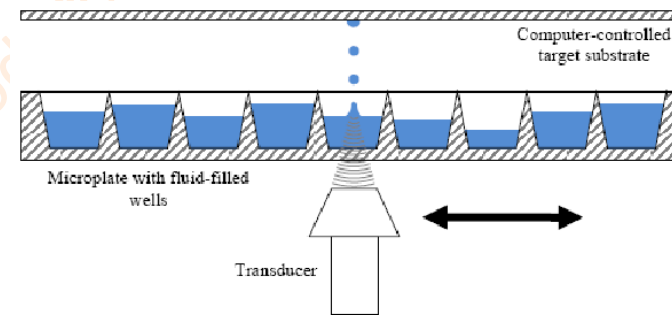


Co-crystallization

- 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

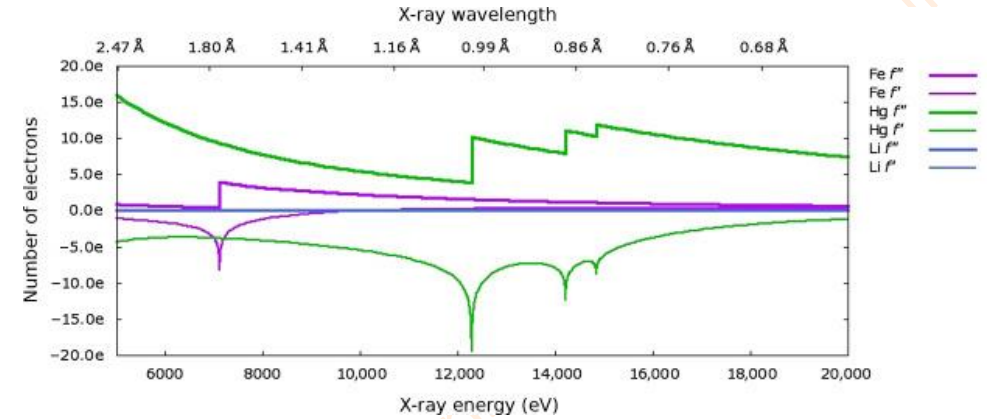
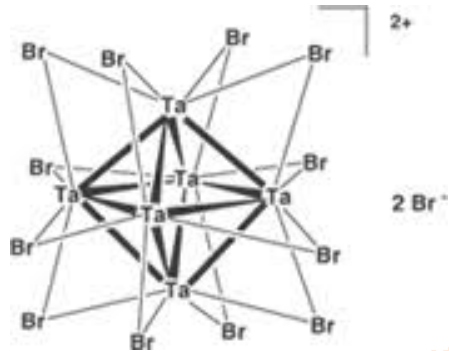
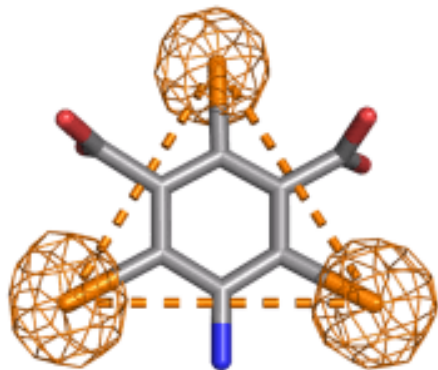
Soaking

- 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**



Heavy atom derivatization at BIC

Compounds routinely available

Compounds available on special demand

hydrogen 1 H 1.0079																	helium 2 He 4.0026	
lithium 3 Li 6.941	beryllium 4 Be 9.0122																	neon 10 Ne 20.180
sodium 11 Na 22.990	magnesium 12 Mg 24.305																	argon 18 Ar 39.948
potassium 19 K 39.098	calcium 20 Ca 40.078	scandium 21 Sc 44.956	titanium 22 Ti 47.867	vanadium 23 V 50.942	chromium 24 Cr 51.996	manganese 25 Mn 54.938	iron 26 Fe 55.845	cobalt 27 Co 58.933	nickel 28 Ni 58.693	copper 29 Cu 63.546	zinc 30 Zn 65.39	gallium 31 Ga 69.723	germanium 32 Ge 72.61	arsenic 33 As 74.922	selecnium 34 Se 78.96	bromine 35 Br 79.904	krypton 36 Kr 83.80	
rubidium 37 Rb 85.468	strontium 38 Sr 87.62	yttrium 39 Y 88.906	zirconium 40 Zr 91.224	niobium 41 Nb 92.906	molybdenum 42 Mo 95.94	technetium 43 Tc [98]	ruthenium 44 Ru 101.07	rhodium 45 Rh 102.91	palladium 46 Pd 106.42	silver 47 Ag 107.87	cadmium 48 Cd 112.41	indium 49 In 114.82	tin 50 Sn 118.71	antimony 51 Sb 121.76	tellurium 52 Te 127.60	iodine 53 I 126.90	xenon 54 Xe 131.29	
cesium 55 Cs 132.91	barium 56 Ba 137.33	* 57-70	lutetium 71 Lu 174.97	hafnium 72 Hf 178.49	tantalum 73 Ta 180.95	tungsten 74 W 183.84	rhenium 75 Re 186.21	osmium 76 Os 190.23	iridium 77 Ir 192.22	platinum 78 Pt 195.08	gold 79 Au 196.97	mercury 80 Hg 200.59	thallium 81 Tl 204.38	lead 82 Pb 207.2	bismuth 83 Bi 208.98	polonium 84 Po [209]	astatine 85 At [210]	radon 86 Rn [222]
francium 87 Fr [223]	radium 88 Ra [226]	** 89-102	lawrencium 103 Lr [262]	rutherfordium 104 Rf [261]	dubnium 105 Db [262]	seaborgium 106 Sg [266]	bohrium 107 Bh [264]	hassium 108 Hs [269]	meitnerium 109 Mt [268]	unnilium 110 Uun [271]	ununium 111 Uuu [272]	unbibium 112 Uub [277]	ununquadium 114 Uuq [289]					

* Lanthanide series

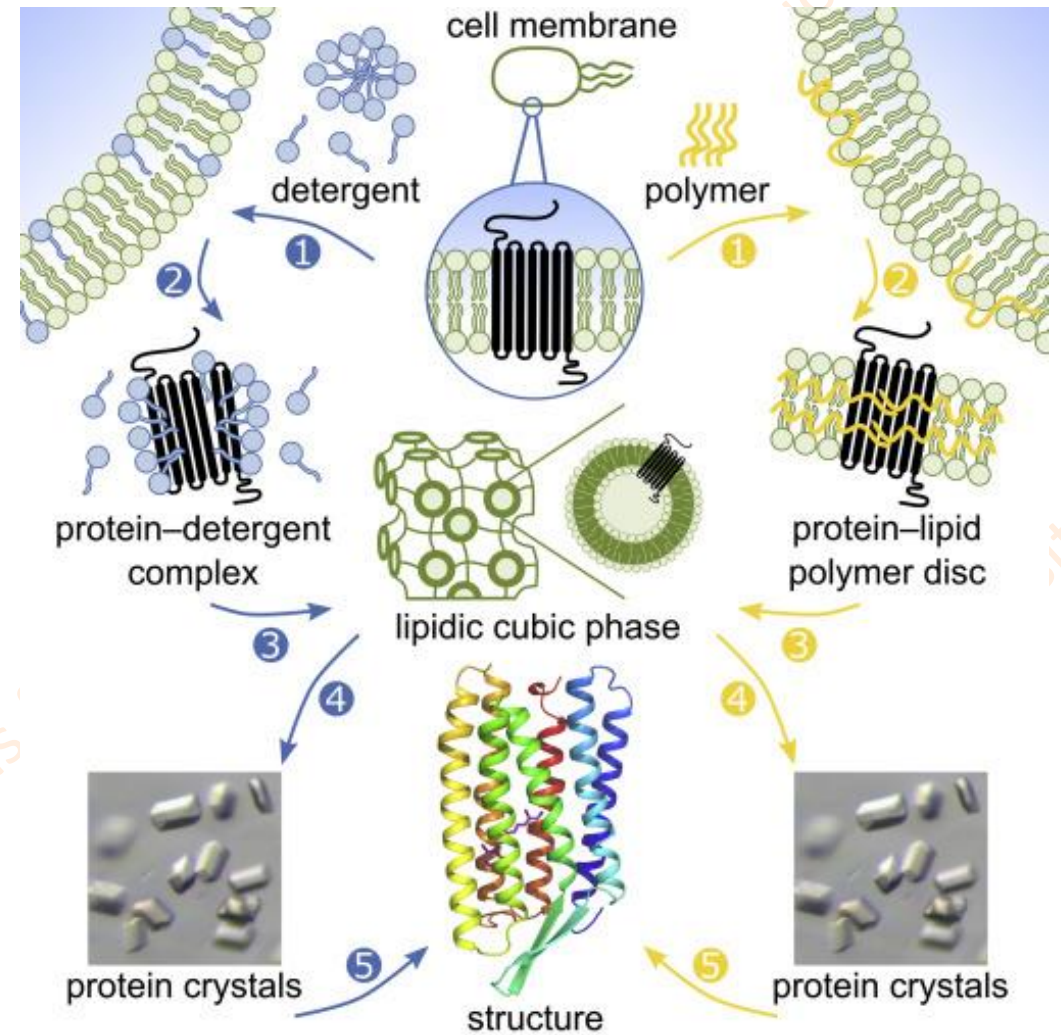
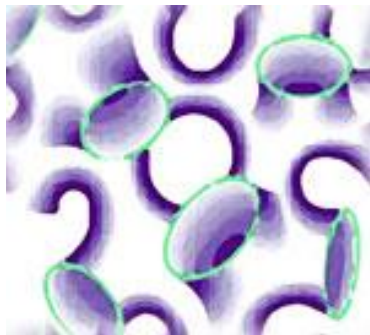
lanthanum 57 La 138.91	cerium 58 Ce 140.12	praseodymium 59 Pr 140.91	neodymium 60 Nd 144.24	promethium 61 Pm [145]	samarium 62 Sm 150.36	europium 63 Eu 151.96	gadolinium 64 Gd 157.25	terbium 65 Tb 158.93	disprosium 66 Dy 162.50	holmium 67 Ho 164.93	erbium 68 Er 167.26	thulium 69 Tm 168.93	ytterbium 70 Yb 173.04
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** Actinide series

actinium 89 Ac [227]	thorium 90 Th 232.04	protactinium 91 Pa 231.04	uranium 92 U 238.03	neptunium 93 Np [237]	plutonium 94 Pu [244]	americium 95 Am [243]	curium 96 Cm [247]	berkelium 97 Bk [247]	californium 98 Cf [251]	einsteinium 99 Es [252]	fermium 100 Fm [257]	mendeleevium 101 Md [258]	nobelium 102 No [259]
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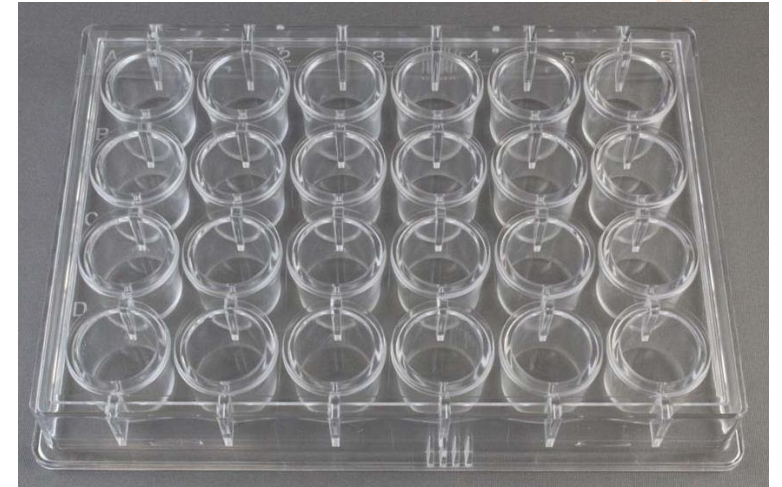
Membrane proteins crystallization

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



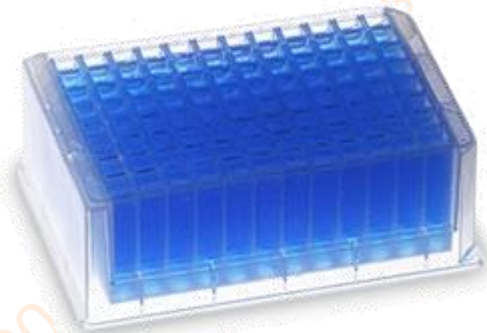
Crystallization plastics

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



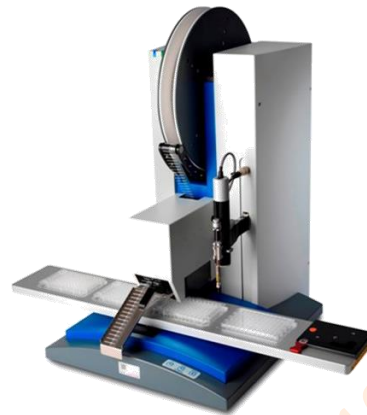
Commercial screens

- 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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