MUNI PHARM

Department of Natural Drugs

Supramolecular Pharmacy

6. Crystals and co-crystals

Ondřej Jurček

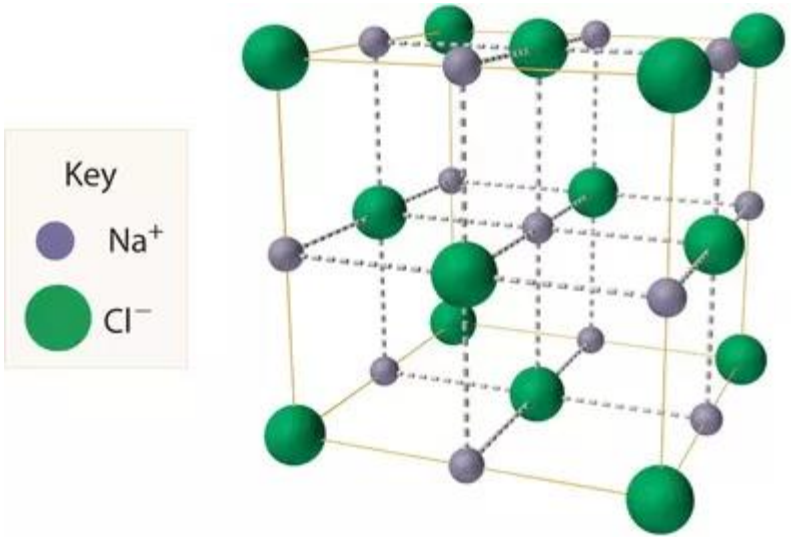
Introduction to Crystallography

- The weak intermolecular interactions form supramolecules or supramolecular self-assemblies
- The same forces also act in the formation of crystals
- Dunitz referred to organic crystals as supramolecular chemistry par excellence
- Crystallography determines arrangement and bonding of atoms in crystalline solids
- Crystallography studies small molecules with ease with weight of the asymmetric unit (W_{au}) 100-700 or with possible difficulties also larger up to 100 000 W_{au}
- Agreement factor, *R*-factor or residual factor, shows the agreement between properly scaled observed structural factors and calculated structure factors (for small W_{au} $R < 0.07$, 7%, large W_{au} $R \sim 15\%$)

Book: Analytical methods in supramolecular chemistry, 2nd edition, Wiley, Kari Rissanen – chapter Crystallography and crystal engineering, book edited by C. Schalley

Crystallization

- Obtaining a single crystal might be the most challenging task
- Solvent evaporation, diffusion, layering method, sublimation, etc.
- 0.05 × 0.05 × 0.05 mm crystal can provide good data for solving the structure, usually 0.1-0.4 mm in at least two dimensions
- Good crystal = good enough periodicity of the crystal lattice in the crystal
- The heavier and the more ordered the atoms are the better will be diffraction
- Importance of crystal stability – keep the crystals in mother liqueur!

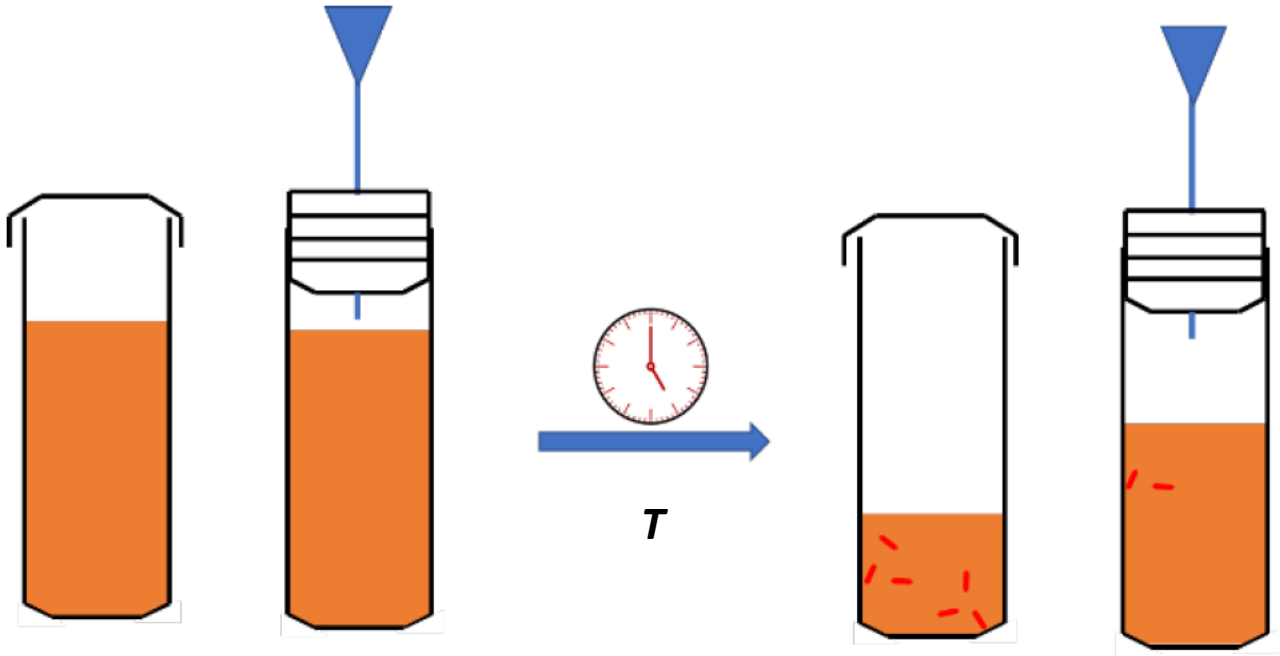


General consideration

- Purify your compound!
- Factors affecting crystallization: solubility of the compound in the solvent used, time, temperature, mechanical agitation, and the number of nucleation sides
- Consider the physicochemical properties of your compounds (thermal stability, sensitivity, etc.)
- Make solubility profile of your compound (mild solvent is good)
- Use clean glassware
- Set up number of crystallizations at the same time under different conditions

Crystallization techniques

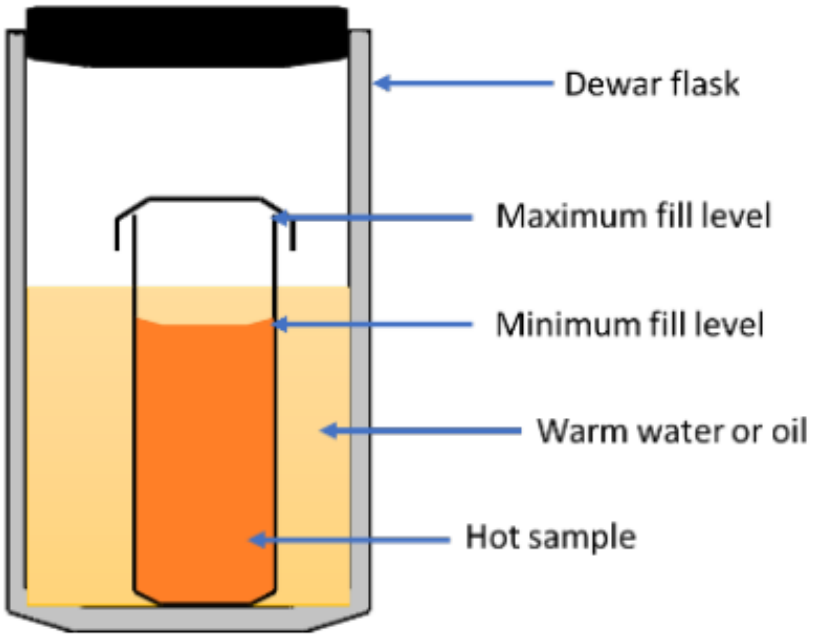
- **Slow solvent evaporation**
  - Controlling evaporation, choice of vessel, single solvent or mixtures
  - Don‘t let it fully dry!



https://www.unifr.ch/chem/de/assets/public/PDFWordDocuments/Guide%20for%20crystallization.pdf

Crystallization techniques

- **Slow cooling**
  - Soluble in hot, insoluble in cold



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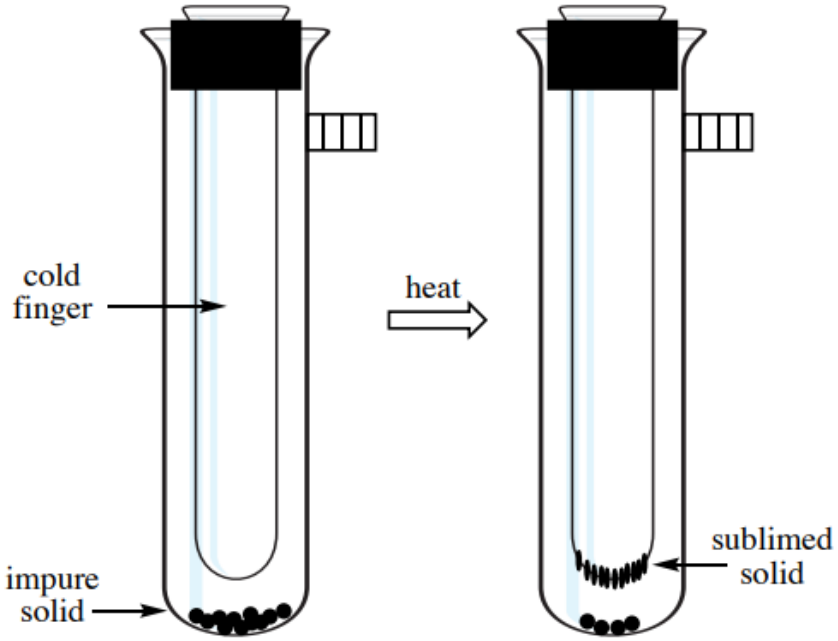
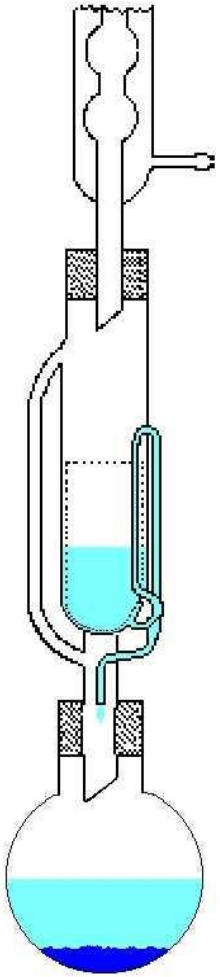
Crystallization techniques

- **Sublimation**
  - The sample is heated under reduced pressure until it vaporizes and then it deposits on cold surface



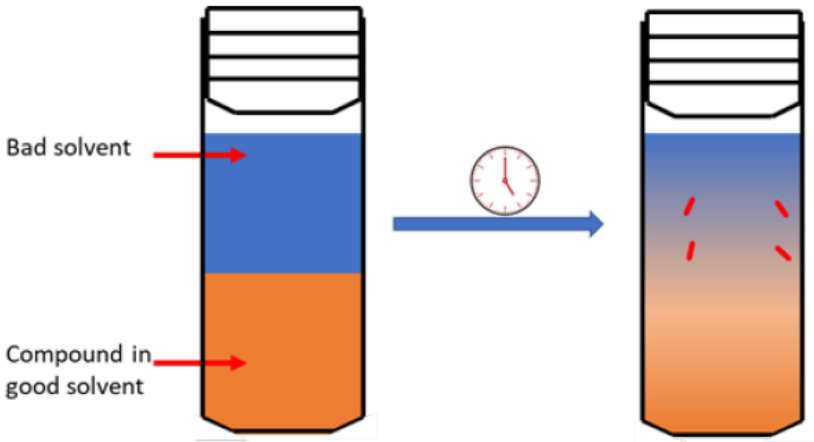
Crystallization techniques

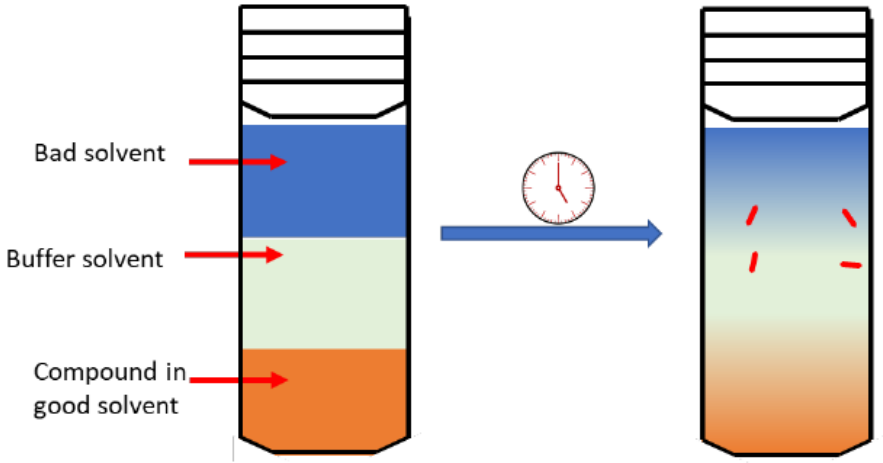
- **Soxhlet extraction**
  - Crystals can appear during extraction or more often later during cooling

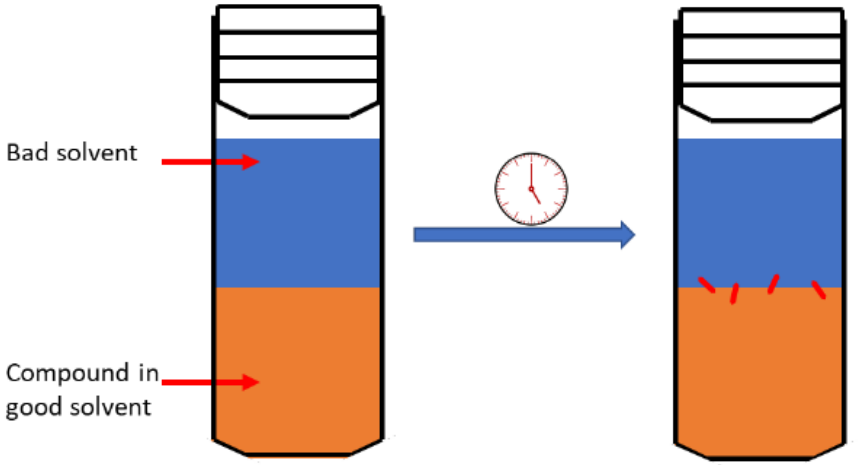


Crystallization techniques

- **Solvent diffusion**
  - Solvent layering method
  - Good for small amounts of sample







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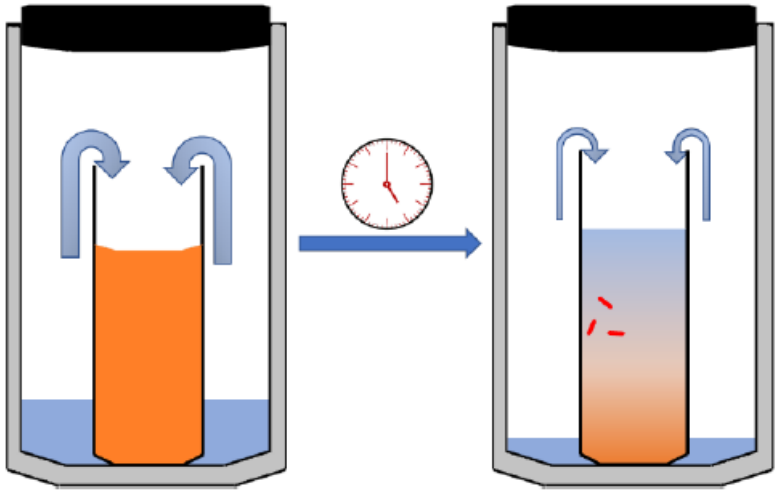
Crystallization techniques

- **Vapor diffusion**
  - Good for small amounts of sample



Solvent	Antisolvent
Tetrahydrofuran	Cyclohexane
Methylformate	Cyclopentane or hexane (dries out)
Methylene chloride	Cyclopentane
Ethanol	Cyclohexane
Methanol	Hexane or tetrahydrofuran
Acetonitrile	Tetrahydropyran
Acetone	Chloroform
Water	Dioxane
Benzene	Diethyl Ether
Toluene	THF

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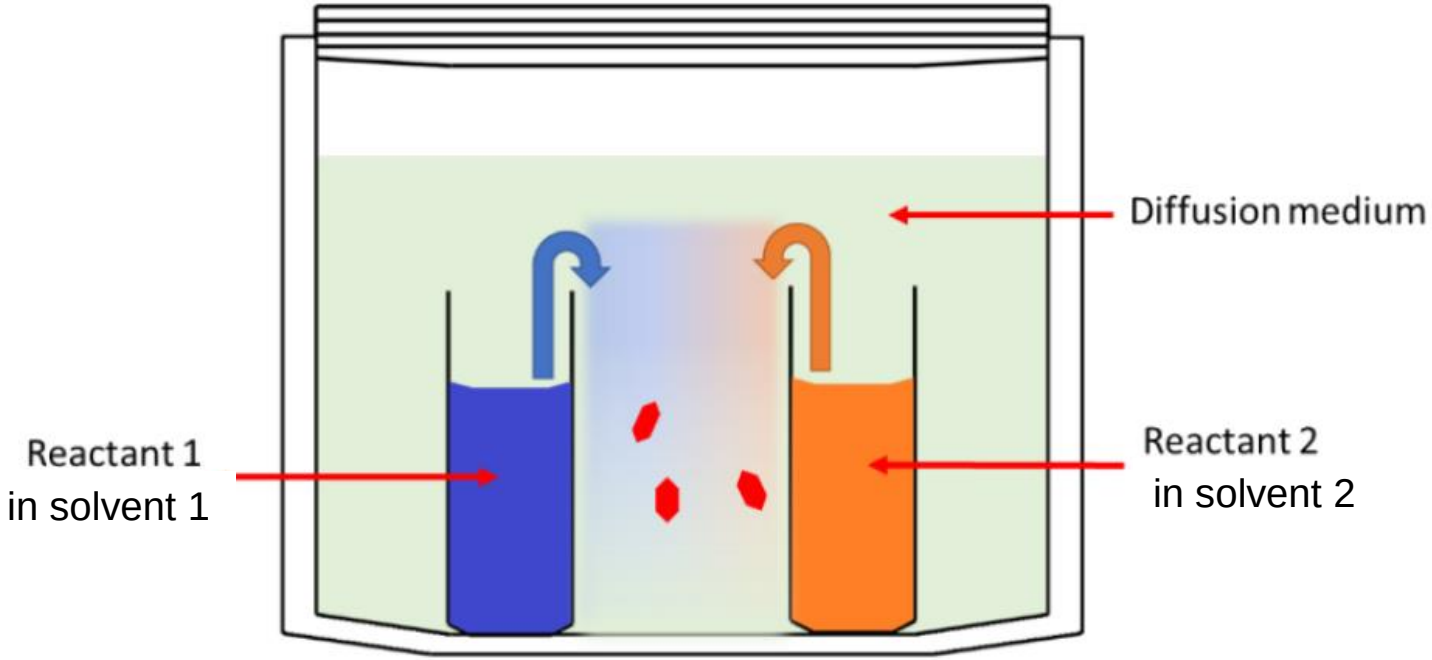
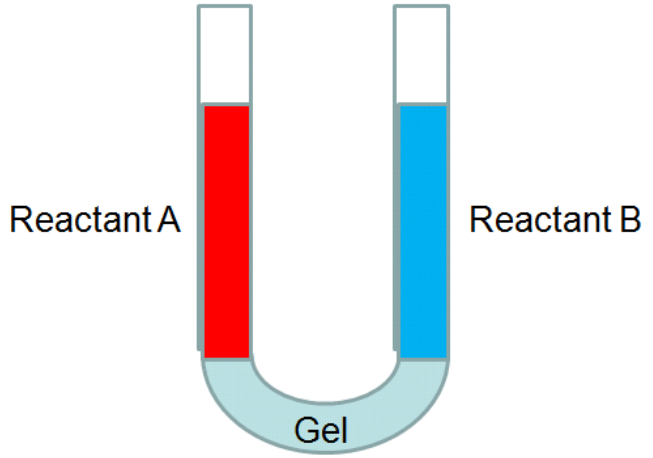
Crystallization techniques

- **Reactant diffusion**





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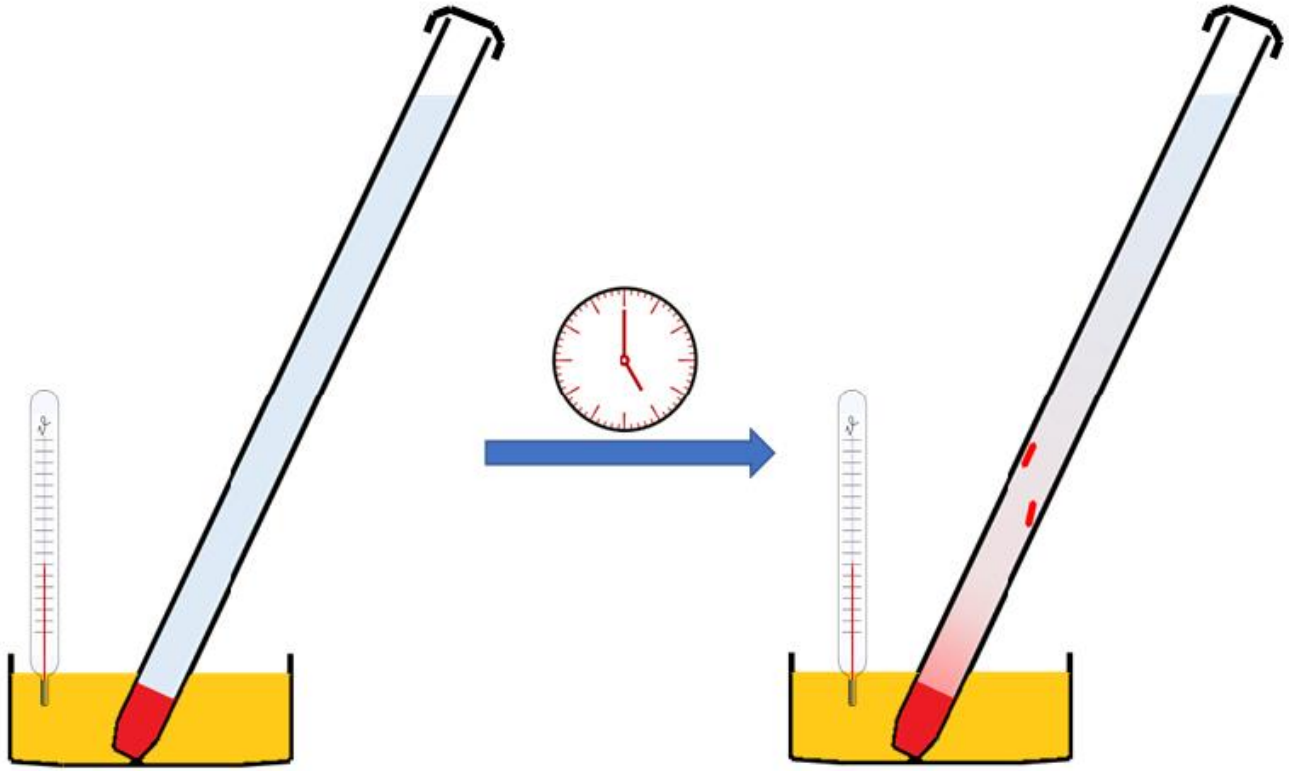
Crystallization techniques

- **Convection**



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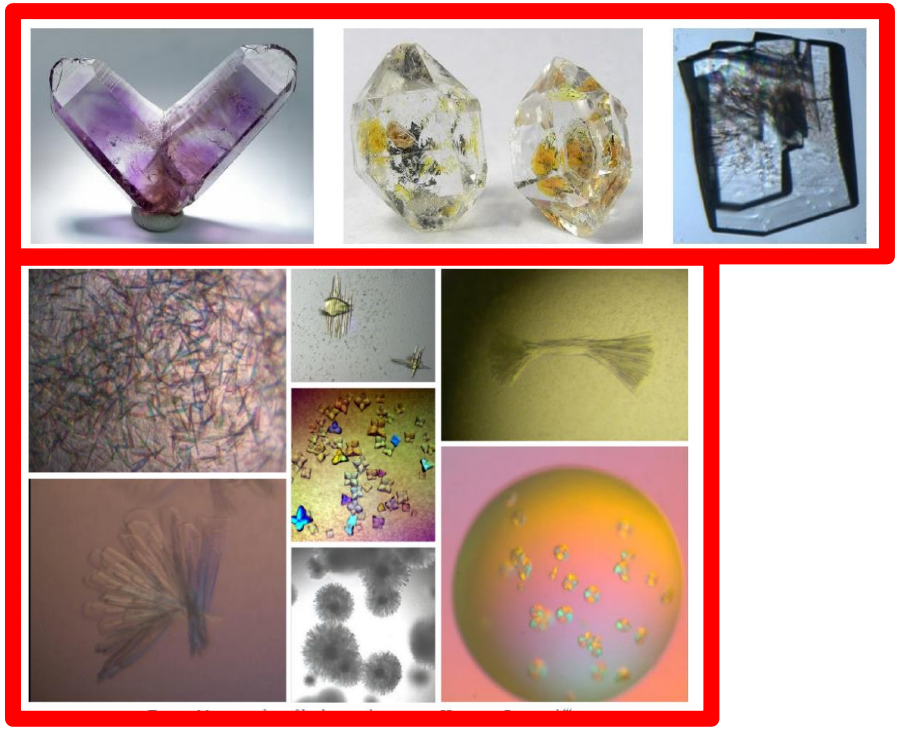
Crystallization techniques

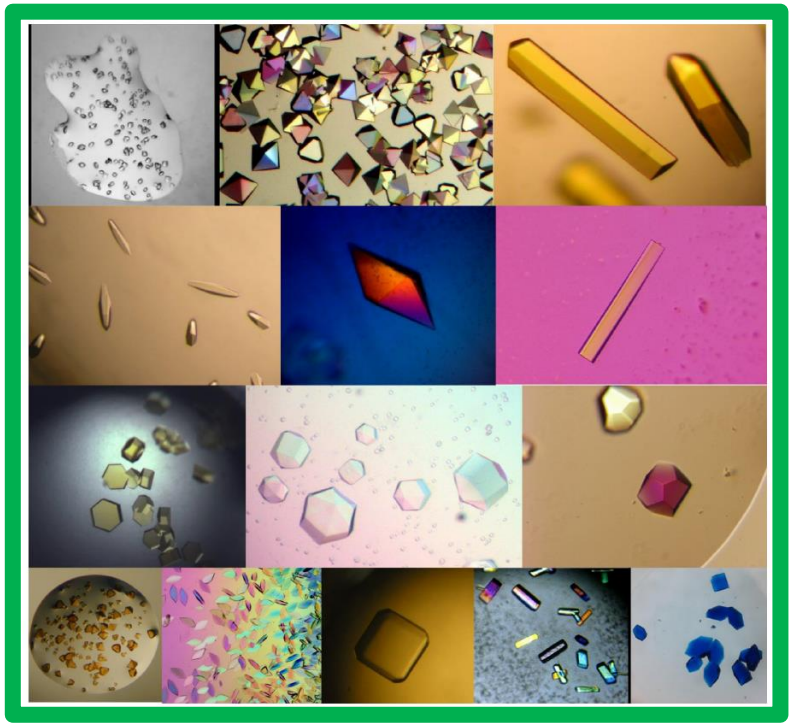
- **Hydrothermal (solvothermal) method**

https://www.unifr.ch/chem/de/assets/public/PDFWordDocuments/Guide%20for%20crystallization.pdf

Crystallization techniques

- **Alternative methods**
  - Counterions – problematic Et_4N^+, Bu_4N^+, BF_4^-, and PF_6^-, by counter ions that are usually ordered are triflate, BPh_4^-, Me_4N^+, $(Ph_4P)_2N^+$, and Ph_4As^+
  - Co-crystals
  - Seeding
  - Melting
  - Gel





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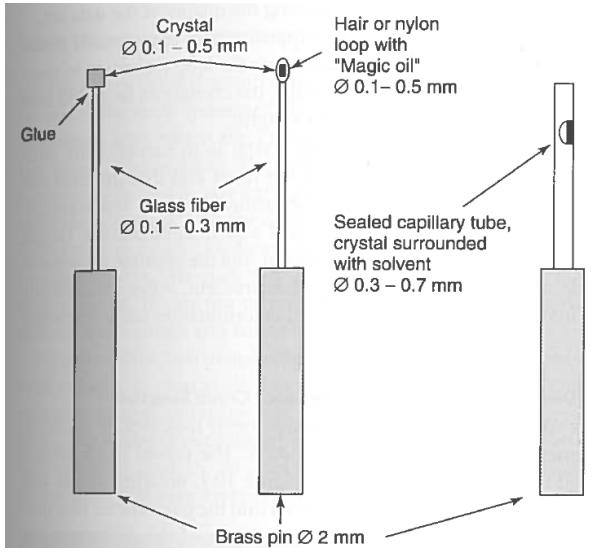
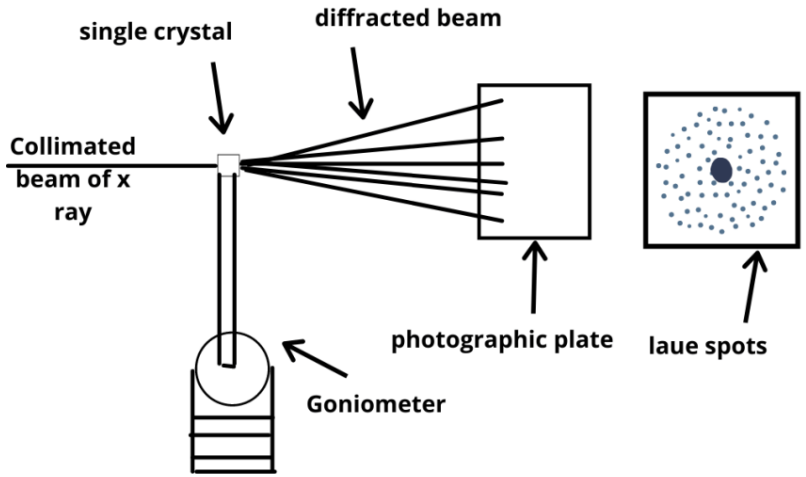
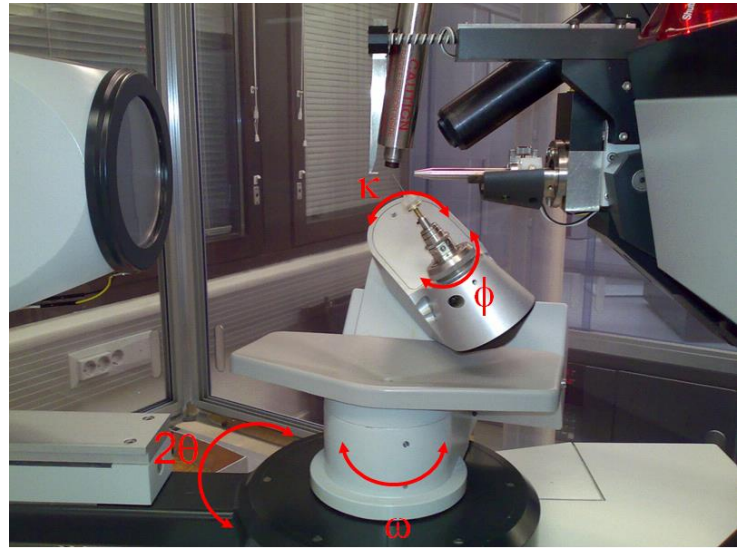
Crystal Structure Determination

- Crystal → selecting and mounting crystal → unit cell determination and preliminary space group selection → data collection (measurement of the intensity data), data processing, and final space determination → data reduction, structure solution, and refinement → analysis of the structure

- Obtaining a single crystal, placing in magic oil in nylon loop
- Mounting the crystal on brass pin into goniometer in diffractometer



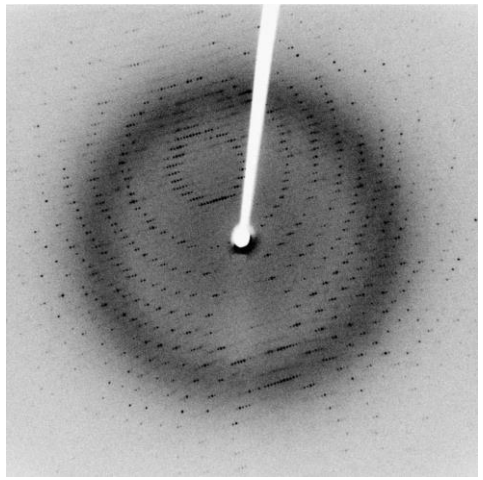


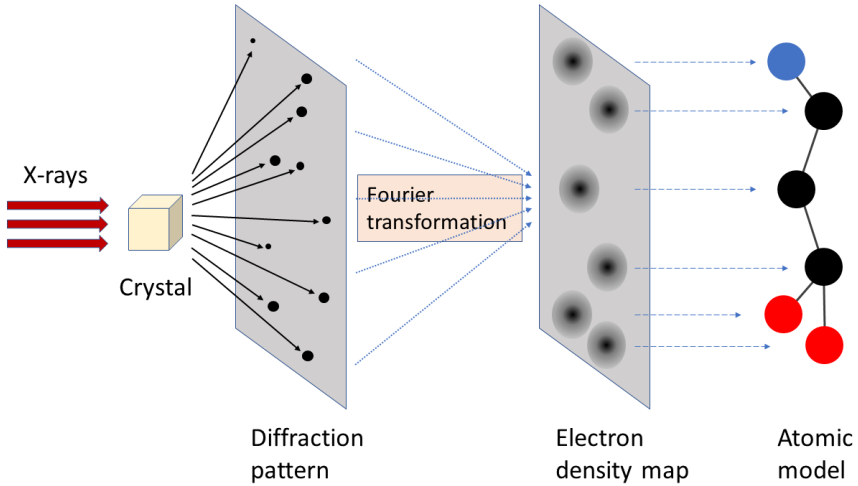


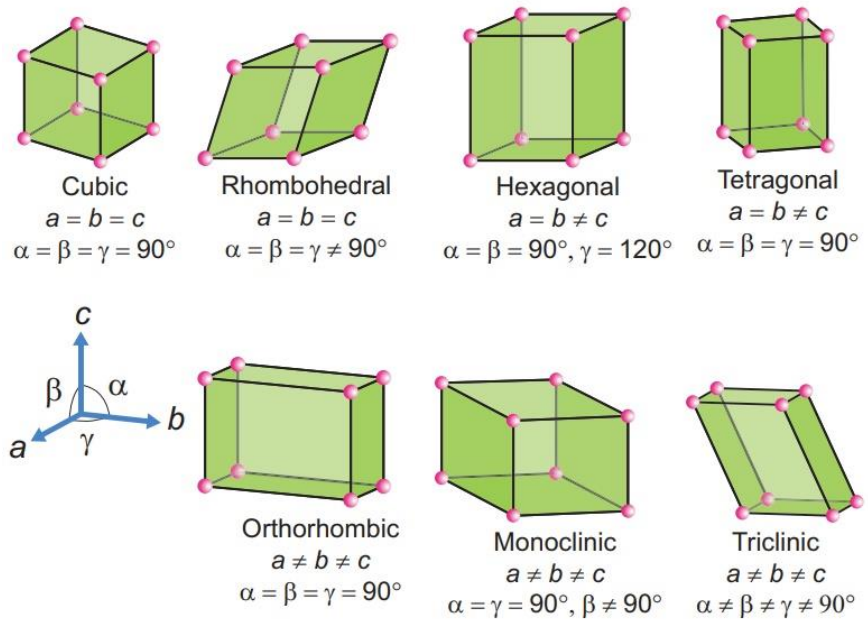
Book: Analytical methods in supramolecular chemistry, 2nd edition, Wiley, Kari Rissanen – chapter Crystallography and crystal engineering, book edited by C. Schalley

Crystal Structure Determination

- Does sample diffract? Does the diffraction pattern indicate twinning? How high is the resolution of diffraction?
- Unit cell determination, the smallest imaginary 3D unit in which the atoms are placed and multiplying this unit will build the whole macroscopic crystal
- Unit cell is defined by axes a, b, c, and angles α, β, γ – volume of unit cell
- Due to crystal symmetry seven types of primitive unit cells can exist
- Bravais lattices







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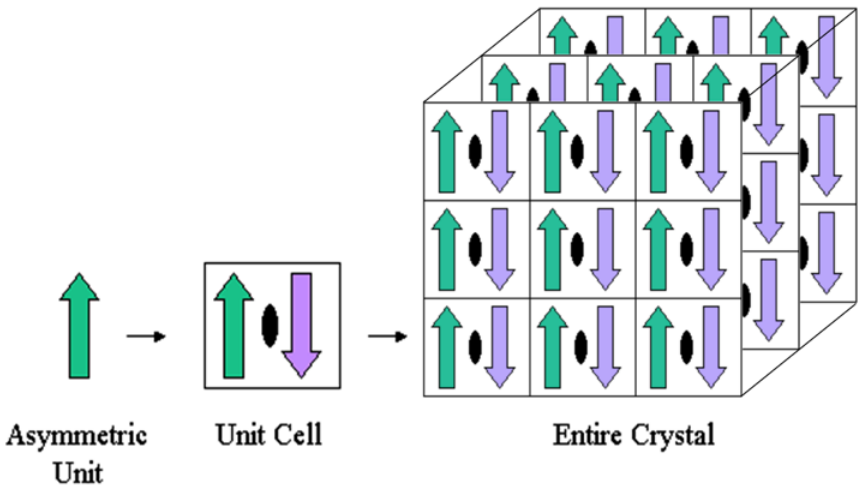
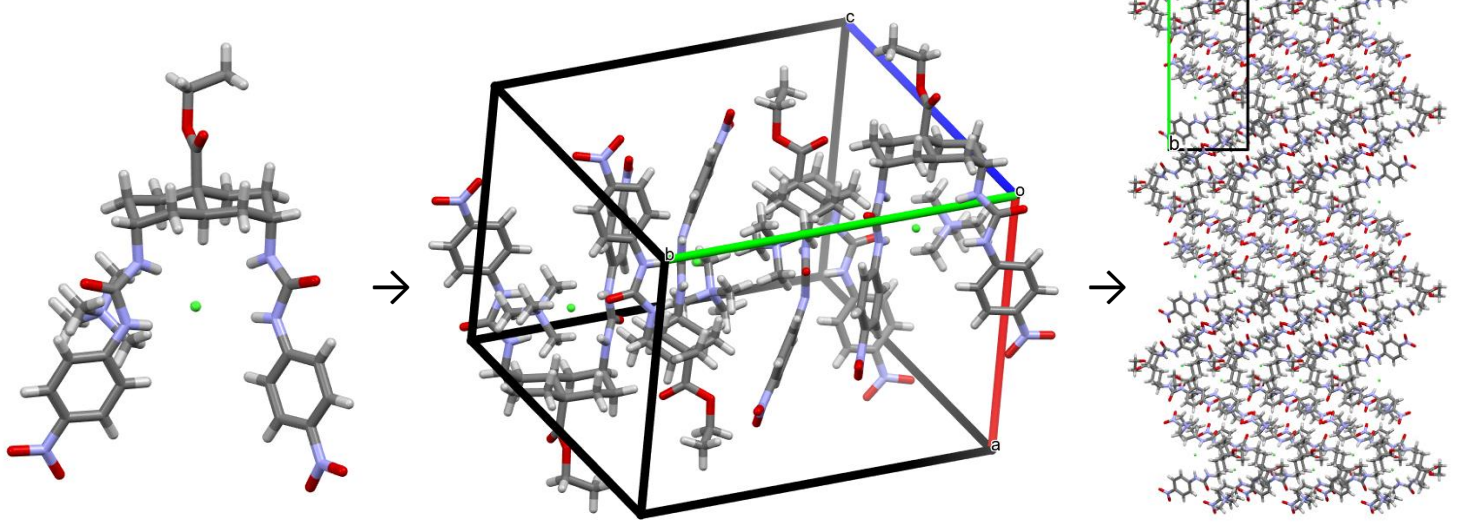
Crystal Structure Determination

- Measurement – data collection – X-ray beam is diffracted by electrons of atoms
- Reflection planes are described by integers *h, k, l* so called Miller indices
- Goniometer enables to collect many reflections (thousands, measured in hours) which are then computationally combined and converted into format for space group determination
- Space group = mathematically derived set of symmetry operation within given unit cell (crystal lattice), there are 230 space groups
- Asymmetric unit

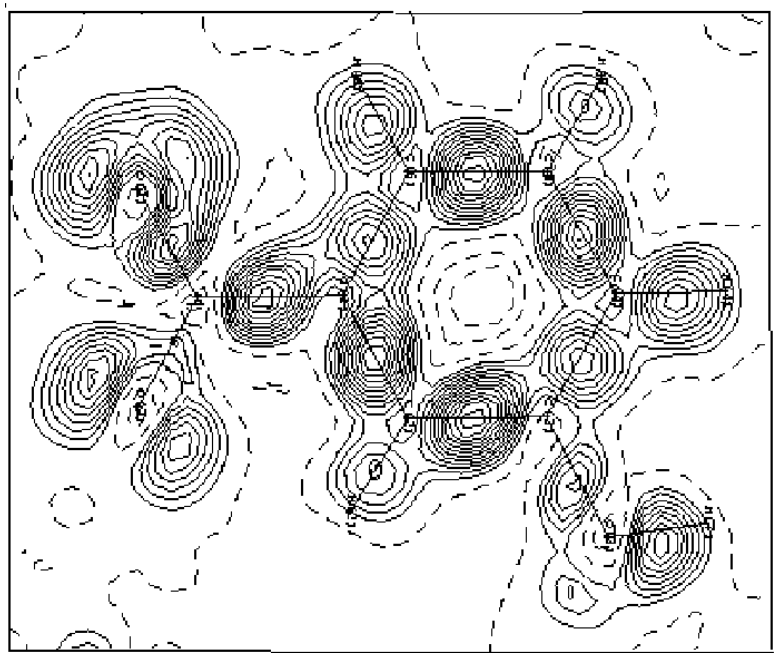




A. P. Davis et al.: *Nature Chemistry* 2016, 8, 24–32.

Crystal Structure Determination

- Data reduction – obtaining structure factor
- Structure solution, refinement



- Supramolecular structures are challenging:
  - Poor crystal quality
  - Large supramolecules do not form well-ordered crystal lattices (periodicity)
  - Crystal density is low
  - W_{au} is large
  - Unit cell usually contains large amount of (different) solvent molecules, which are disordered (further decreasing the quality of single crystals)

- Output file is Crystallographic information file (cif) – save to database Cambridge Crystallographic Data Centre

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2.2. A walkthrough of a single-crystal X-ray experiment

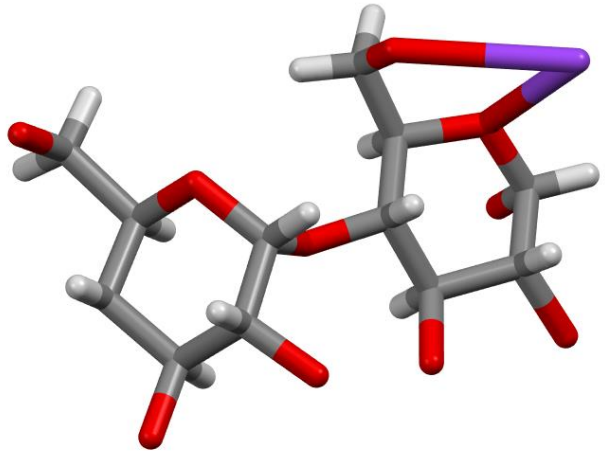
Time taken		Information
?	1. Select a crystal and mount it for X-ray study - preliminary diffraction test	crystalline or not
minutes/ hours	2. Determine unit cell geometry and preliminary symmetry	*a*, *b*, *c*, α, β, γ, *cryst. system,* *space group?* *other symmetry* *information?*
hours/days	3. Measure the intensity data	List of: *h k l*, *I*, $\sigma(I)$
minutes/ hours	4. Carry out data reduction and necessary corrections - LP-correction - Absorption correction etc. Determine the space group	List of: *h k l*, *F*, $\sigma(F)$ or *h k l*, F^2, $\sigma(F^2)$ Space group
from minutes upwards	5. Solve the structure - Direct methods - Patterson methods - Other methods	some of all non-H positions (approximate)

With thanks to Kari Rissanen and Matti Haukka

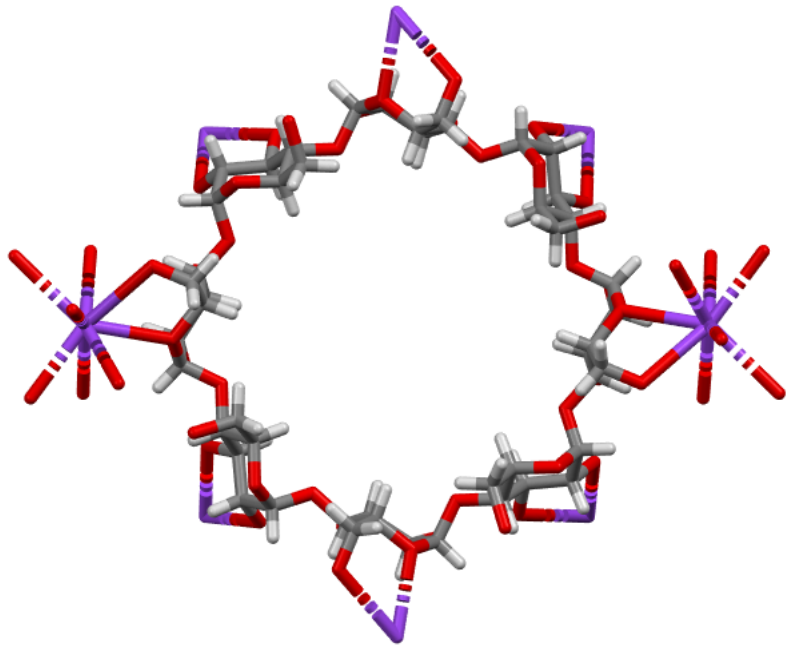
Time taken		Information
minutes/ hours	6. Completing the structure - Finding all atoms: Fourier and diffrerence Fourier synthesis	all atom positions (approximate)
minutes/ days	7. Refinement of the structure model	atom positions and displacement parameters
minutes/ hours	8. Estimation of the structure quality	quality of the structure
?	9. Interpret the results	molecular geometry, packing arrangement, etc.

Crystal Structure Analysis

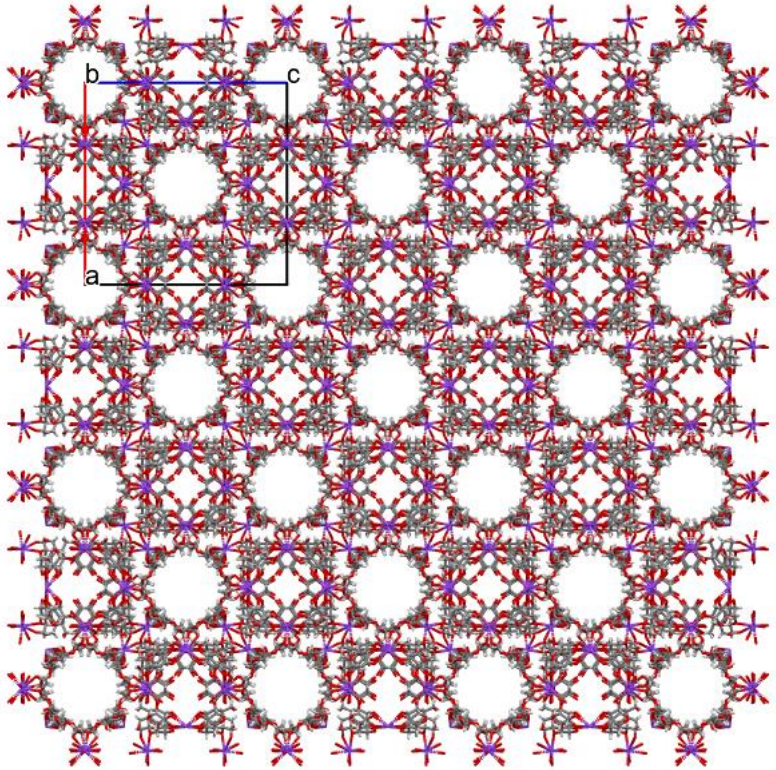
- Detailed analysis of intra- and intermolecular interactions
- Plot of data with their van der Waals radii (CPK model)
- Crystal preparation



Asymmetric unit



Unit cell



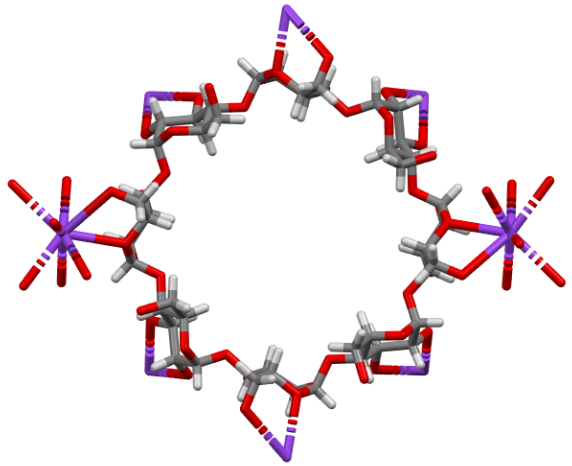
Crystal packing

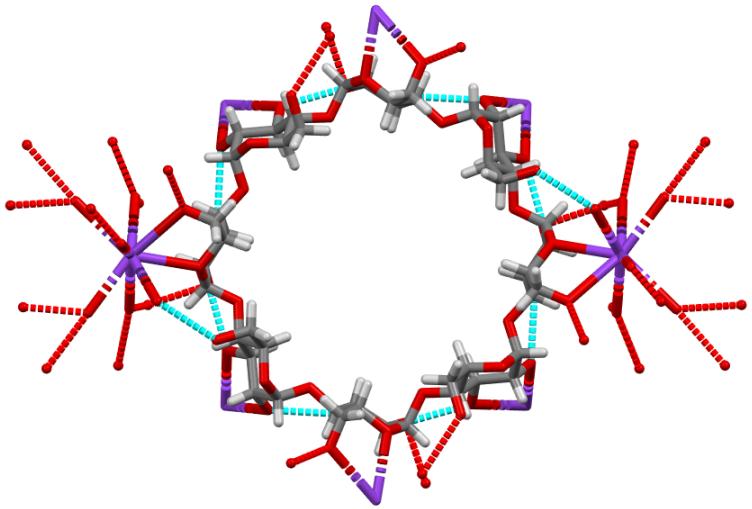
CD-MOF-1

J. F. Stoddart: *J. Am. Chem. Soc.* **2012**, *134*, 406–417.

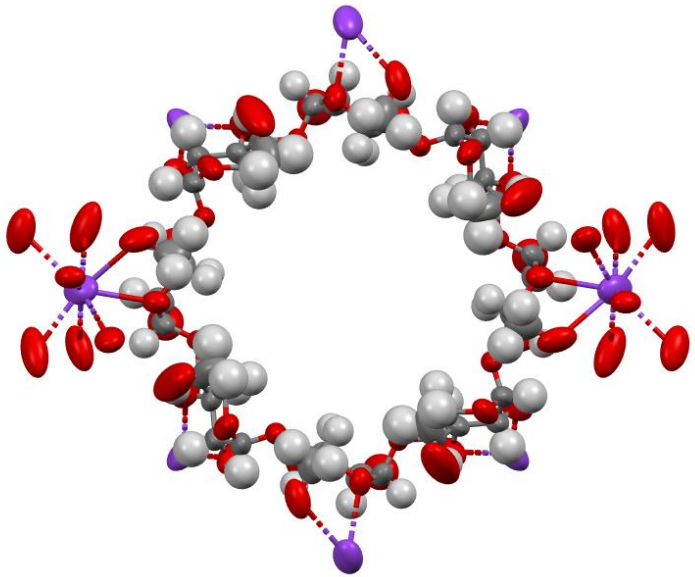
Crystal Structure Analysis

- Detailed analysis of intra- and intermolecular interactions
- Plot of data with their van der Waals radii (CPK model)

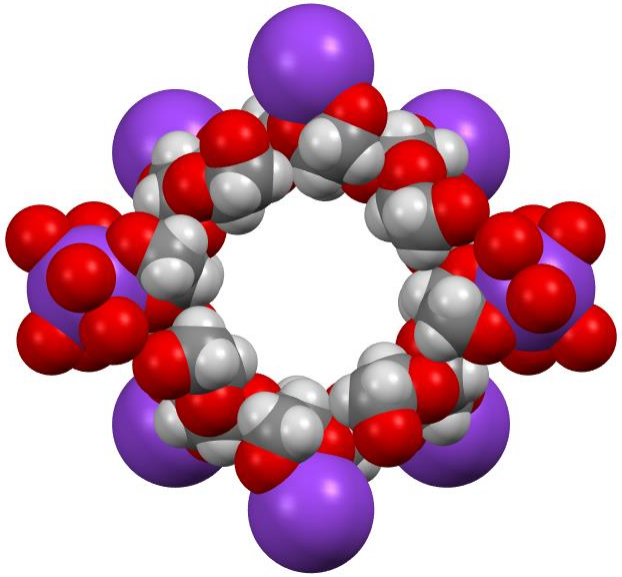




Non-covalent interactions



Thermal elipsoids

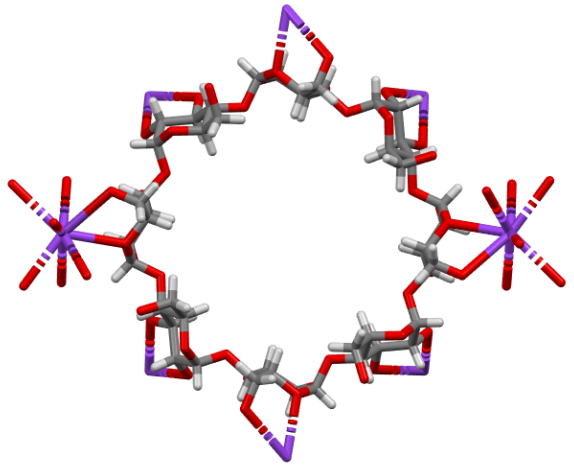


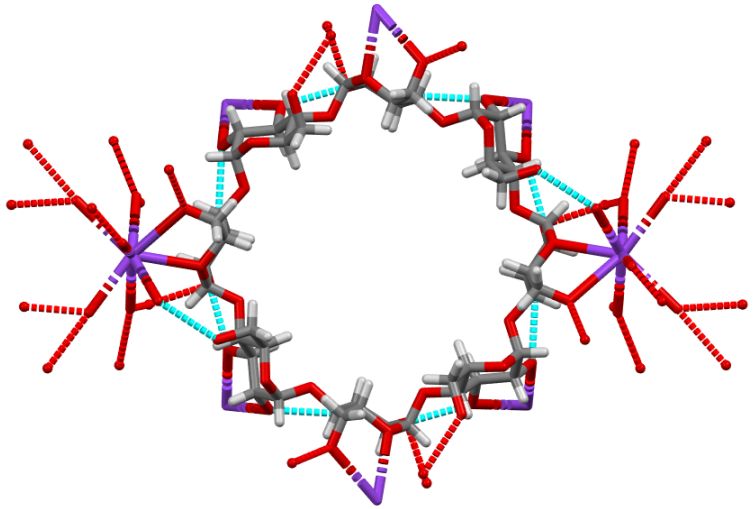
CPK (space filling) model

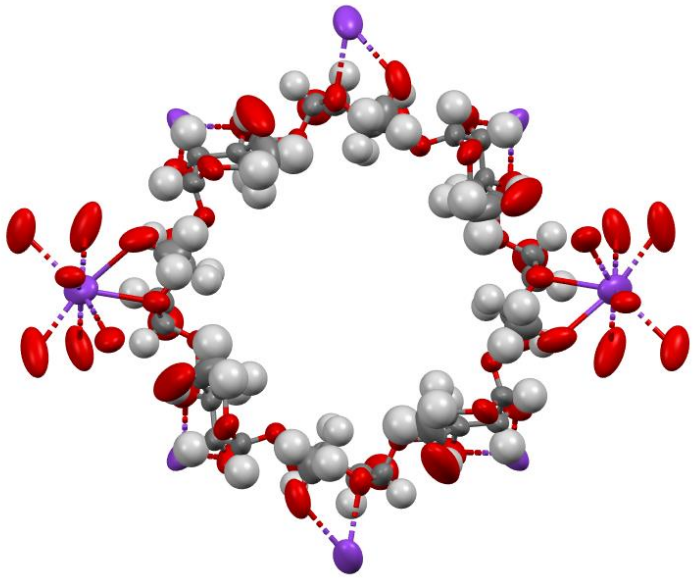
J. F. Stoddart: *J. Am. Chem. Soc.* **2012**, *134*, 406–417.

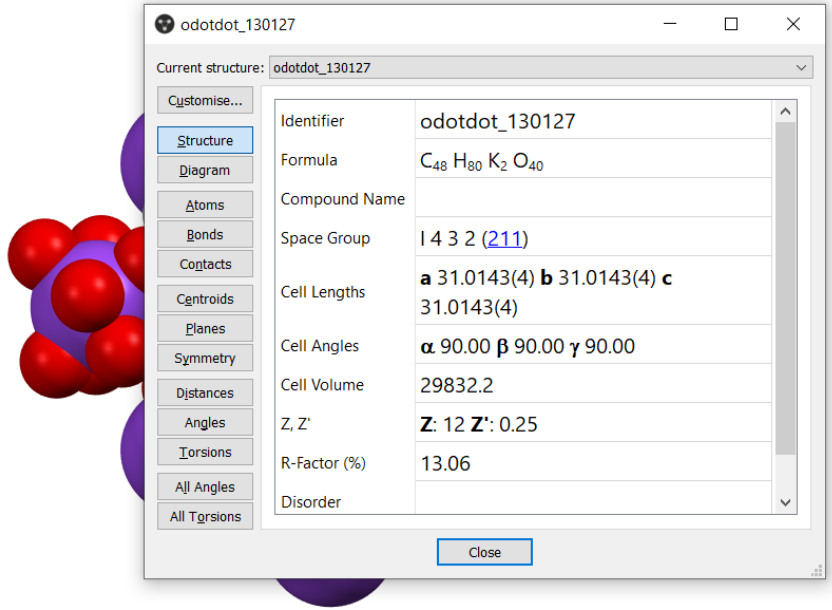
Crystal Structure Analysis

- Detailed analysis of intra- and intermolecular interactions
- Plot of data with their van der Waals radii (CPK model)









J. F. Stoddart: *J. Am. Chem. Soc.* **2012**, *134*, 406–417.

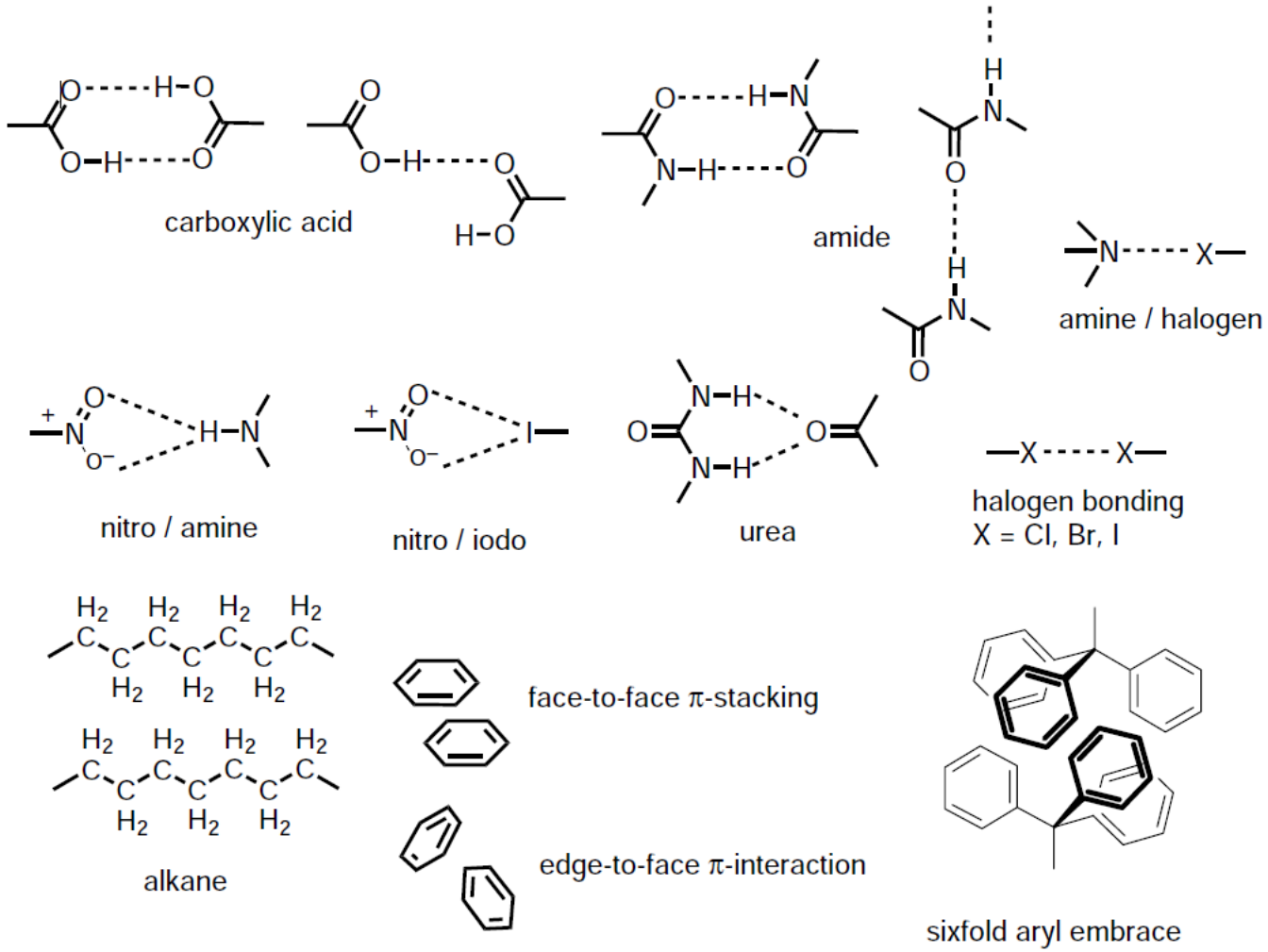
Crystal Engineering

- A set of designed processes which aim to form a crystalline material utilizing the supramolecular interactions and self-assembly of the components

- Simultaneous development of supramolecular chemistry and crystallographic techniques led to development of novel field Crystal Engineering
- Crystal engineering term mentioned already in 1955, but in article by Schmidt (1971) was used as explicit term – the article also postulated, that under suitable conditions, the molecular recognition events, viz. self-assembly, could be the major factor leading to crystal formation
- Journals dealing with this phenomena Crystal Engineering Communication or Crystal Growth and Design, etc.
- Over 39 000 publications on Web of Knowledge by now

Crystal Engineering

- Using supramolecular synthons and good knowledge of non-covalent interactions



Co-crystals

- Co-crystal is a multicomponent molecular crystal; that is, a crystalline substance comprising two or more chemically different molecules (although includes solvates, hydrates, and both stoichiometric and non-stoichiometric lattice inclusion compounds)

- Crystalline, structurally homogeneous materials in which the two or more component molecules are present in a well-defined stoichiometric ratio

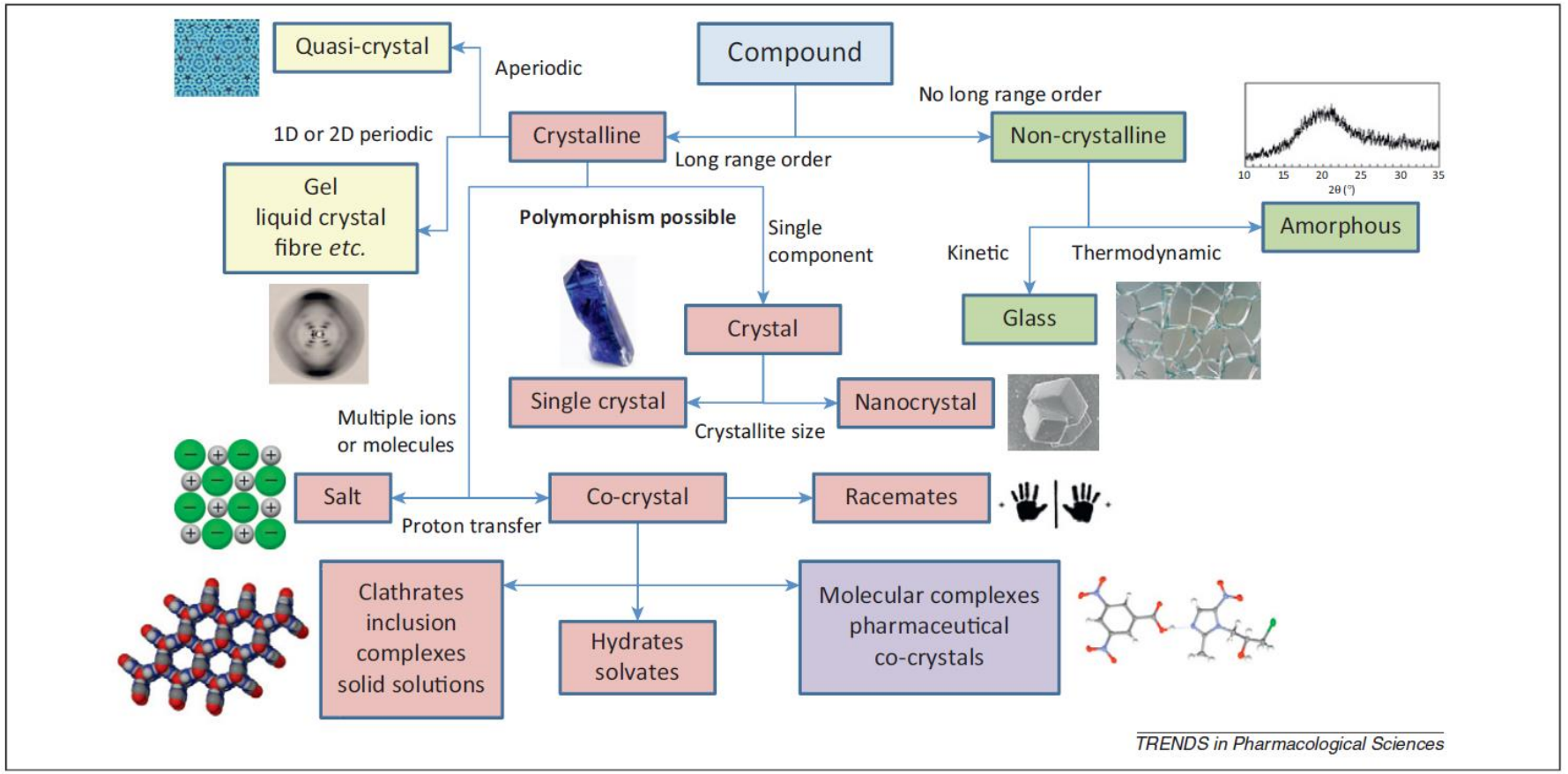
- A pharmaceutical co-crystal is simply a co-crystal in which at least one of the molecular components is an API in conjunction with another type of molecule termed a cocrystal former. Co-crystal former should be included on the US FDA 'The Substances Added to Food' (formerly EAFUS) list, which comprises over 3000 substances that are suitable as food additives.

a) J. Steed: *Trends Pharmacol. Sci.* 2013, 34, No. 3. b) A. S. Myerson et al. *Cryst. Growth Des.* 2011, *11*, 887–895

https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=FoodSubstances

Co-crystals

- Co-crystals have different physical properties such as habit, bulk density, solubility, compressibility, friability, melting point, hygroscopy, and dissolution rate.



J. Steed: *Trends Pharmacol. Sci.* 2013, 34, No. 3.

Benefits of Co-crystals

- Co-crystal formation transform an amorphous or hard-to-crystalize active pharmaceutical ingredient (API) into a readily handled, stable crystalline solid
- Crystalline products are generally preferred because of their easier and more reproducible characterization, lower hygroscopicity, and greater chemical stability
- It is far more likely poor biopharmaceutical characteristics rather than toxicity or lack of efficacy that prevent a candidate active compound from progressing in clinical trials
- The 1:1 co-crystal of a candidate sodium channel blocker and glutaric acid proved to dissolve 18 times faster than the pure drug crystal and the co-crystal has three times the bioavailability
- Although, solubility measurements on highly soluble co-crystals must be treated with caution because the substance can undergo recrystallisation to the most stable pure API form on contact with solvent
- Solvate forms are the least soluble forms in the solvent they contain

a) J. Steed: *Trends Pharmacol. Sci.* 2013, 34, No. 3. b) D. P. McNamara *Pharm. Res.* 2006, 23, 1888–1897.

Co-crystals and Legal Issues

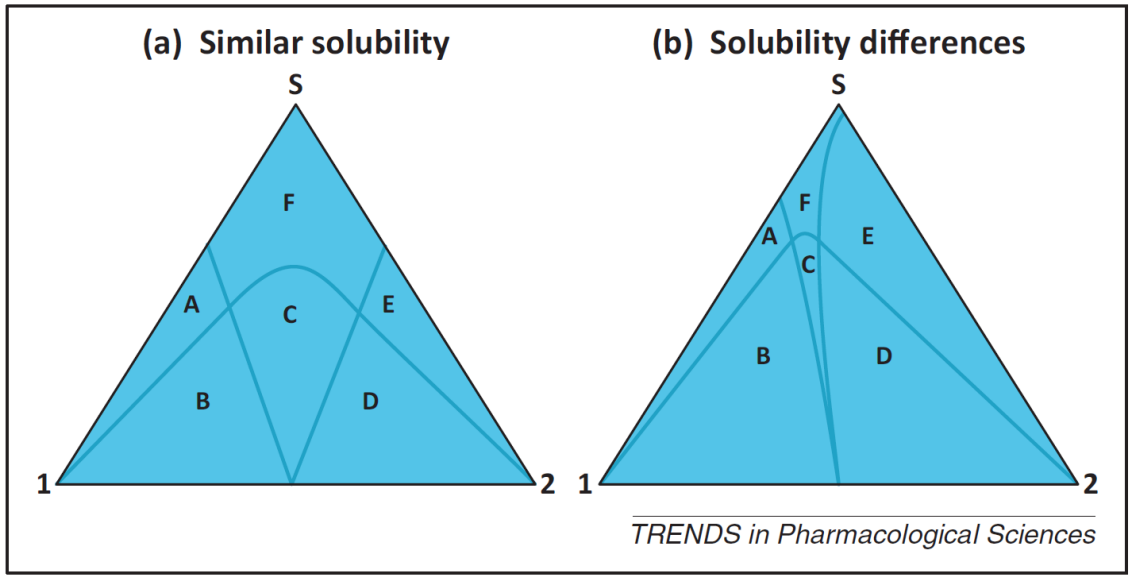
- Useful co-crystal solid form also offers new opportunities for the exploitation of intelectual property (IP) and there are now numerous patents covering co-crystals – novel, useful, non-obvious – new patent.
- In legal terms, patent protection for co-crystals is thought to be significantly easier to enforce than that of other solid-form IP (solvates, salts), because allegations of obviousness are more difficult to establish
- Of particular relevance to co-crystal research is the issue of proton transfer; a stoichiometric crystal containing a neutral acid such as carboxylic acid along with a free-base drug substance is a co-crystal. However, proton transfer from acid to base would give a carboxylate salt, which is not a cocrystal – IP or regulatory relevance.
    - Example: Valproic acid is an approved medication for epilepsy. It exists as an acid form and a sodium salt (sodium valproate) form. The acid form is liquid at ambient conditions and sodium salt is highly hygroscopic. The cocrystal form contains both valproic acid and sodium valproate.

a) J. Steed: *Trends Pharmacol. Sci.* 2013, 34, No. 3. b) A. V. Trask *Mol. Pharm.* 2007, 4 (3), 301-309.

Synthesis of Co-crystals

- Easiest is to crystallise the API from a supersaturated solution in the presence of (varying amounts of) the co-crystal former
- Most commonly (in ~40% of cases), supersaturation is achieved by slowly cooling an undersaturated mixture until the solubility limit is reached
- Key to the rational synthesis of co-crystals is an understanding of the binary or ternary phase diagrams for the equilibria involving the solvent (if present) and two-solute mixture

Region A, component 1 and solvent; B, component 1 + co-crystal; C, co-crystal; D, component 2 + co-crystal; E, component 2 and solvent; F, solution.

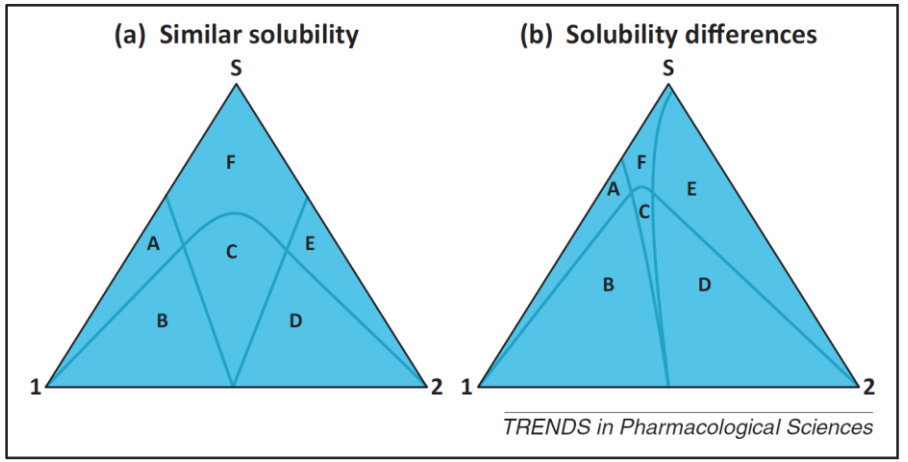


J. Steed: *Trends Pharmacol. Sci.* 2013, 34, No. 3.

Synthesis of Co-crystals

(i) use of an excess of one of the co-crystal components with a consequent reduction in the solubility of the co-crystal in the presence of the excess component;
(ii) slurry crystallisation to access the low percentage solvent region of the phase diagram;
(iii) careful tuning of solvent identity or composition to maximise the pure co-crystal regions in the phase diagram;
(iv) wet milling of the solid components in the presence of just a few drops of solvent;
(v) involving an intermediate phase such as a hydrate or amorphous form as part of a solid-state synthesis;
(vi) use of a metastable polymorph to give an unstable intermediate that can trigger co-crystal growth;
(vii) seeding solutions using co-crystal seeds derived from melt crystallisation using hot-stage microscopy

Region A, component 1 and solvent; B, component 1 + co-crystal; C, co-crystal; D, component 2 + co-crystal; E, component 2 and solvent; F, solution.

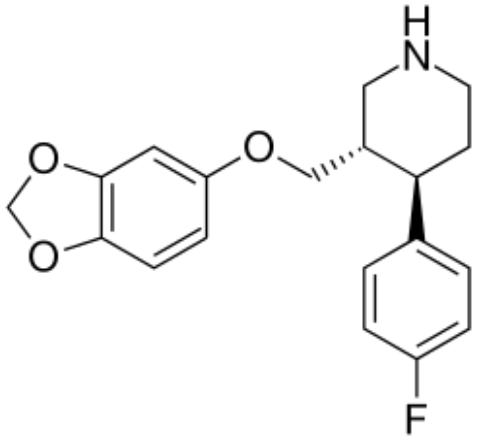


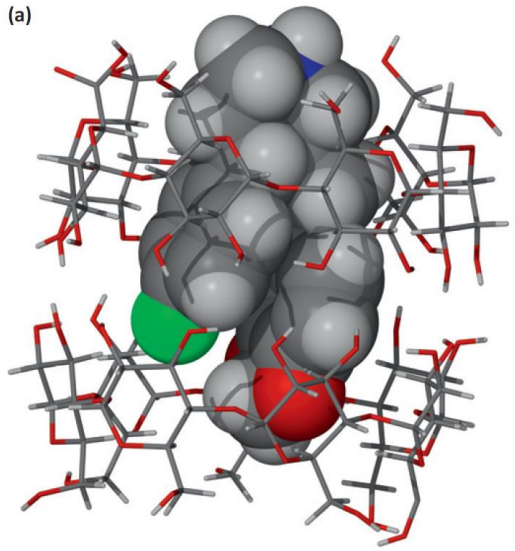
J. Steed: *Trends Pharmacol. Sci.* 2013, 34, No. 3.

N. Blagden et al. *New J. Chem.* 2008, 32, 1659–1672.

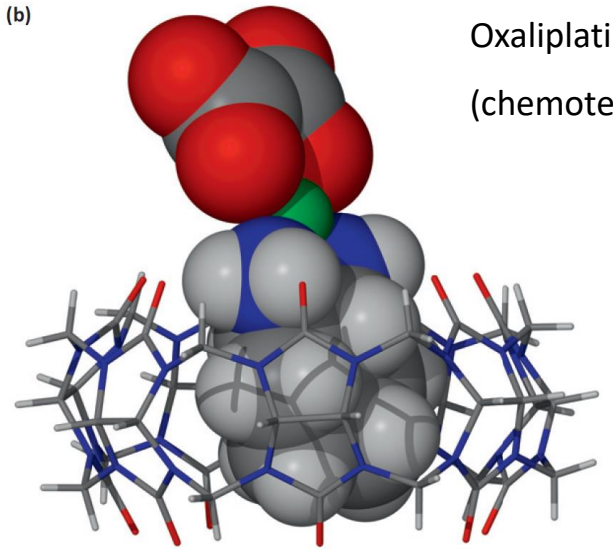
Synthesis of Co-crystals

(viii) mechanical grinding
(ix) supercritical fluid co-crystallization
(x) forming host-guest inclusion complexes, *e.g.*, with cyclodextrins, cucurbiturils
(xi) spray-drying method





Paroxetin (antidepressant)



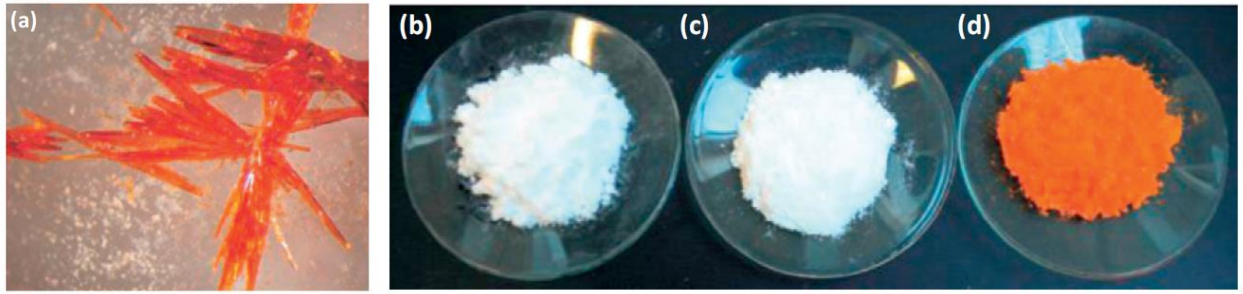
Oxaliplatin

(chemoterapeutics)

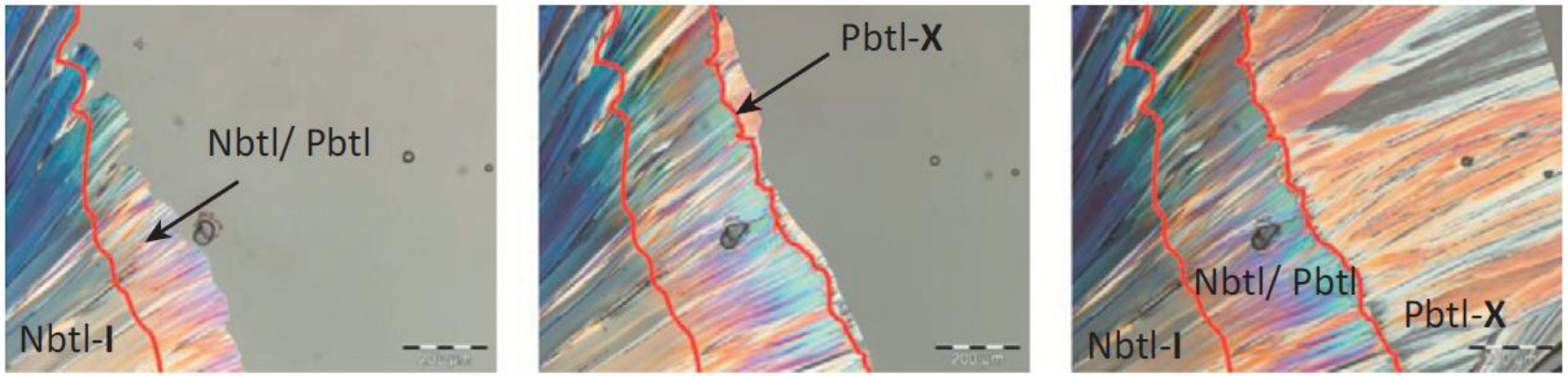
a) J. Steed: *Trends Pharmacol. Sci.* 2013, 34, No. 3. b) R. K. Bandaru et al. *Front. Pharm.* 2021, 12, 780582.

Analysis of Co-crystals

- Obvious change of properties of crystalline materials, *e.g.*, solubility, melting point, etc.
- Visual
- Single crystal X-ray diffraction
- Powder X-ray diffraction
- Differential scanning calorimetry
- Thermogravimetry
- Vibrational spectroscopy (IR, Raman)
- Solid state magic angle spinning NMR
- Polarised optical hot-stage microscopy, *e.g.*, barbiturates – nembutal, phenobarbital
- Neutron diffraction



1:1 acetaminophen (paracetamol) and 2,4-pyridine dicarboxylic acid



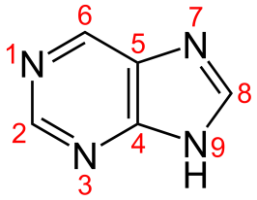
J. Steed: *Trends Pharmacol. Sci.* 2013, 34, No. 3.

TRENDS in Pharmacological Sciences

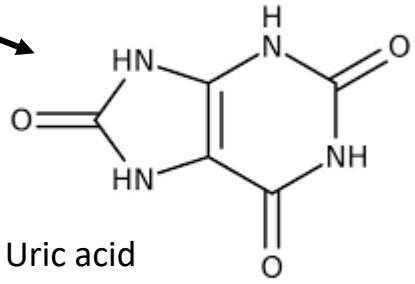
Ternary and Salt Co-crystals

- Usually ternary multicomponent solids are ‘co-crystal hydrates’, in which water is one of three types of molecule present in the material. For example, mechanochemically grinding theophylline results in the formation of a co-crystal hydrate with citric acid.

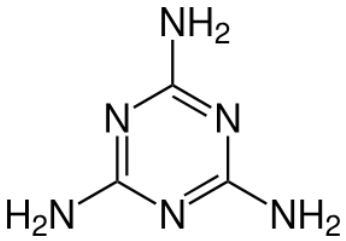
- An interesting related example is the formation of a 1:1:2 ‘co-gel’ of melamine (M), uric acid (U), and water. Sonication of an aqueous suspension of M and U gives a solid gel that was shown by crystal structure calculation and X-ray powder diffraction to comprise co-crystal fibres of composition M:U:2H_2O.



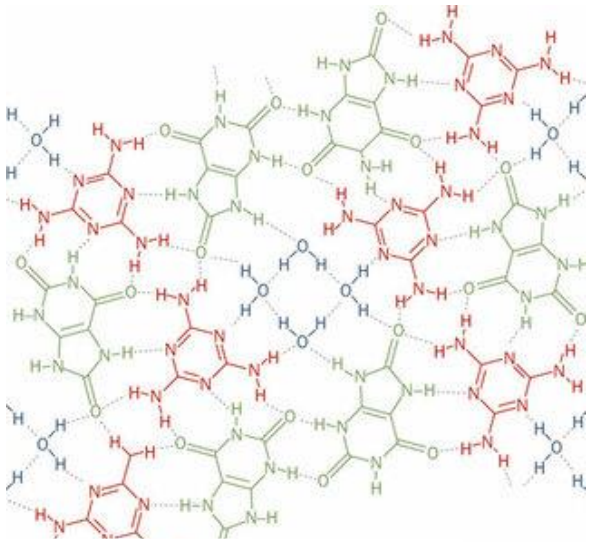
purine

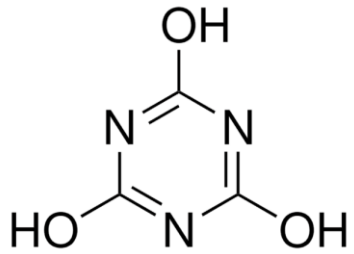


Uric acid



Melamine



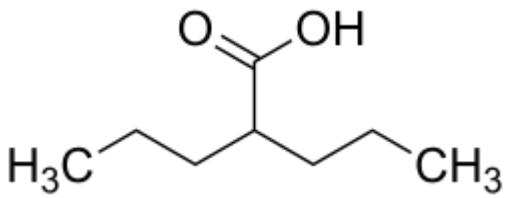


Cyanuric acid

J. Steed: *Trends Pharmacol. Sci.* 2013, 34, No. 3.

Ternary and Salt Co-crystals

- Salt co-crystals: Valproic acid is an approved medication for epilepsy. It exists as an acid form and a sodium salt (sodium valproate) form. The acid form is liquid at ambient conditions and sodium salt is highly hygroscopic. The cocrystal form contains both valproic acid and sodium valproate in 1:1 ratio. This cocrystal form is less hygroscopic than the components. Commercially, it is called by different names, such as Depakote®, Epilim, and divalproex sodium. This structure is considered salt–cocrystal.



R. K. Bandaru et al. Front. Pharm. 2021, 12, 780582.

Co-crystals and Market

- Does it still fall within current guidelines or is this a new chemical entity (safety, toxicology - expensive)?
- Drug-drug co-crystals, *e.g.*, co-crystallization of quercetin (a plant-derived flavonoid, used as a nutritional supplement and reputed to have anticancer properties) with antidiabetic agents such as metformin or tolazamide. The combination drug has been suggested to have physical properties and biological activity that are distinct from the individual properties of the two components

Drug	Components	Indication
Depakote	Valproic acid and valproate sodium	Epilepsy
Entresto	Valsartan sodium and sacubitril sodium	Heart failure
Dimenhydrinate	Diphenhydramine and 8-chlorotheophylline	Motion sickness
Dichloralphenazone	Antipyrin and hypnotic chloral hydrate	Vascular headaches

X. Wang et al. *Asian J. Pharm. Sci.* 2021, *16*, 307–317.

Polymorphism and co-crystallization

- Walter McCrone once famously said ‘every compound has different polymorphic forms, and that, in general, the number of forms known for a given compound is proportional to the time and money spent in research on that compound’

In the next class…

Polymorphism

Thank you for your attention!