University of Zurich[UZH]

Department of Chemistry

Some Tricks for the Single Crystal Growth of Small Molecules

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Updated (9th January 2017) excerpt of a talk held at the 30th European Crystallographic Meeting in Basel, Switzerland on the 29th August 2016

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Importance of single crystal growth

- Prerequisite for single crystal X-ray analysis, a fast analytical method that yields the three dimensional arrangement of the elements within the crystal.
- Despite many technical advances, be it on the instrumental (X-ray beam, detector) or on the theoretical side:

  Still **single** crystals needed, ideally with dimensions of about 0.05*0.05*0.2 mm^3
- Crystal polymorphs also play an extremely crucial role in terms of processing, bioavailability, stability, regulatory affairs, and intellectual property protection.[1]

[1] R. Hilfiker, Ed. *Polymorphism: in the Pharmaceutical Industry*; Wiley-VCH, 2006.

Techniques for single crystal growth

- In liquid phases: Main goal to achieve supersaturation that is followed by nucleation/crystal growth
- Sublimation sometimes also helpful
- Achievement of supersaturation:
  - cooling (from hot oil bath, in fridge or deep freezer)
  - reduction of solvent amount (evaporation, forgotten NMR tubes...)
  - change of solvent (vapor diffusion / layering)
- From pure liquids at low temperature

P. G. Jones *Chem. Brit.* **1981**, *17*, 222;
P. van der Sluis *et al. J. Appl. Crystallogr.* **1989**, *22*, 340;
J. Hulliger *Angew. Chem. Int. Ed.* **1994**, *33*, 143;
A. J. Blake www.nottingham.ac.uk/~pczajb2/growcrys.htm
G. Santiso-Quiniones, I. Krossing *Z. Anorg. Allg. Chem.* **2008**, *634*, 704

Problems for a single crystal analysis

- Chemical purity / identity (e.g. crystals from ^{99m}Tc chemistry)
- Oils / Powders
- Microcrystalline / too small
- Intergrown / twinned
- Not diffracting despite fair size (phase transition?)
- Not solvable
- Not enough data (too weak reflections)
- Residual electron density too high / “unreasonable” electron density

How to overcome the problem of “bad” crystals

- Common situation: crystallographer wants better crystals
- Despite many references, it is not always clear how to optimize the crystals
- Often only a few milligrams available!

⇒ No systematic study of the solubility

Super Nova with Atlas CCD
Mo/**Cu** microfocus

P. G. Jones *Chem. Brit.* **1981**, *17*, 222
P. van der Sluis, *et al. J. Appl. Crystallogr.* **1989**, *22*, 340
J. Hulliger, *Angew. Chem. Int. Ed.* **1994**, *33*, 143
J. Lu and S. Rohani, *Curr. Med. Chem.*, **2009**, *16*, 884
A. J. Blake, www.nottingham.ac.uk/~pczajb2/growcrys.htm
P. D. Boyle, www.xray.ncsu.edu/GrowXtal.html

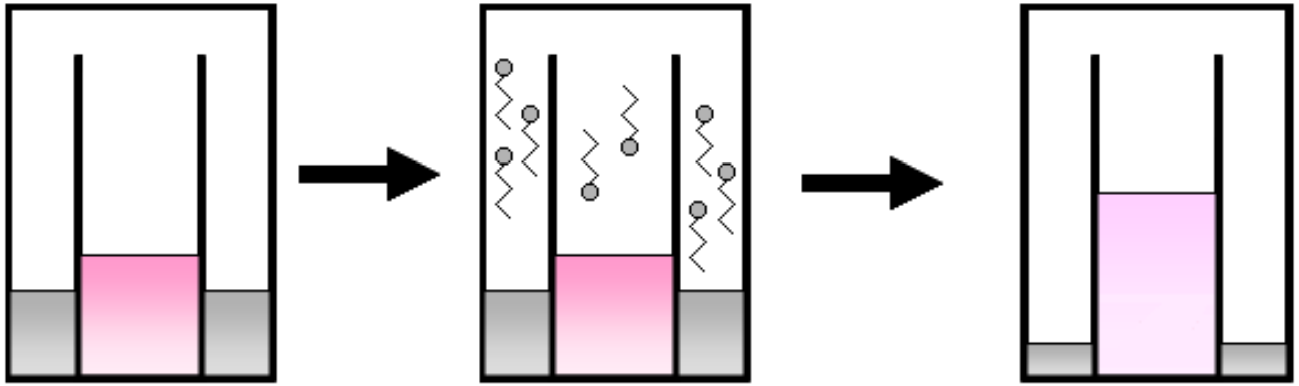
Vapor diffusion

- About 4 mg of substance are dissolved in about 0.5 ml solvent in the inner container.
- About 2.5 ml of antisolvent (normally having a boiling point 5-10 °C **higher** than solvent) are placed in outer container.
- Wait for days, or a few weeks!
- If (anti)solvents have equilibrated and nothing happened, unscrew vial a bit:
  → evaporation experiment from a solvent mixture.

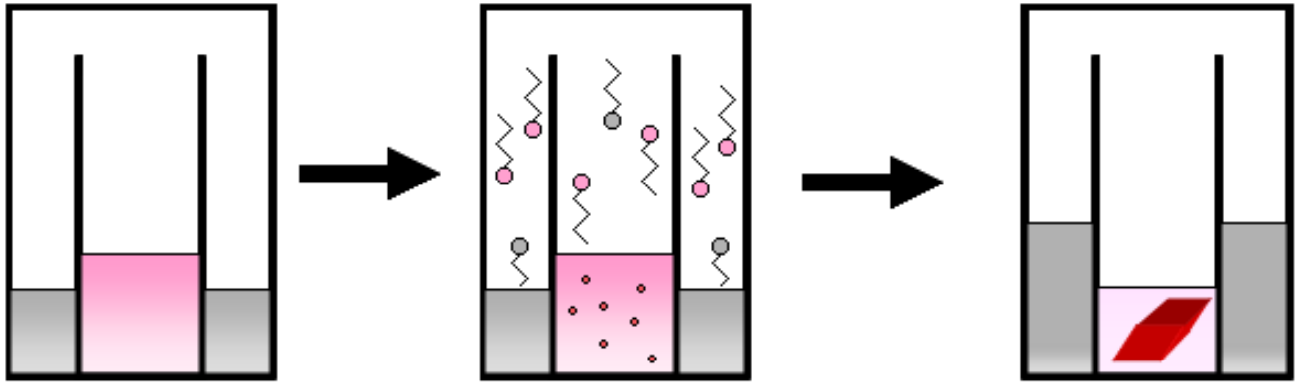
B. Spingler, S. Schnidrig, T. Todorova, F. Wild *CrystEngComm* **2012**, *14*, 751.

Influence of the boiling points

Antisolvent (e.g. diethylether) has a lower boiling



Antisolvent has a higher boiling point than solvent:



B. Spingler, S. Schnidrig, T. Todorova, F. Wild *CrystEngComm* **2012**, *14*, 751.

Initial solvent choice for vapor diffusion

Use information gained during synthesis/purification!

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

B. Spingler, S. Schnidrig, T. Todorova, F. Wild *CrystEngComm* **2012**, *14*, 751.

Optimization of the vapor diffusion

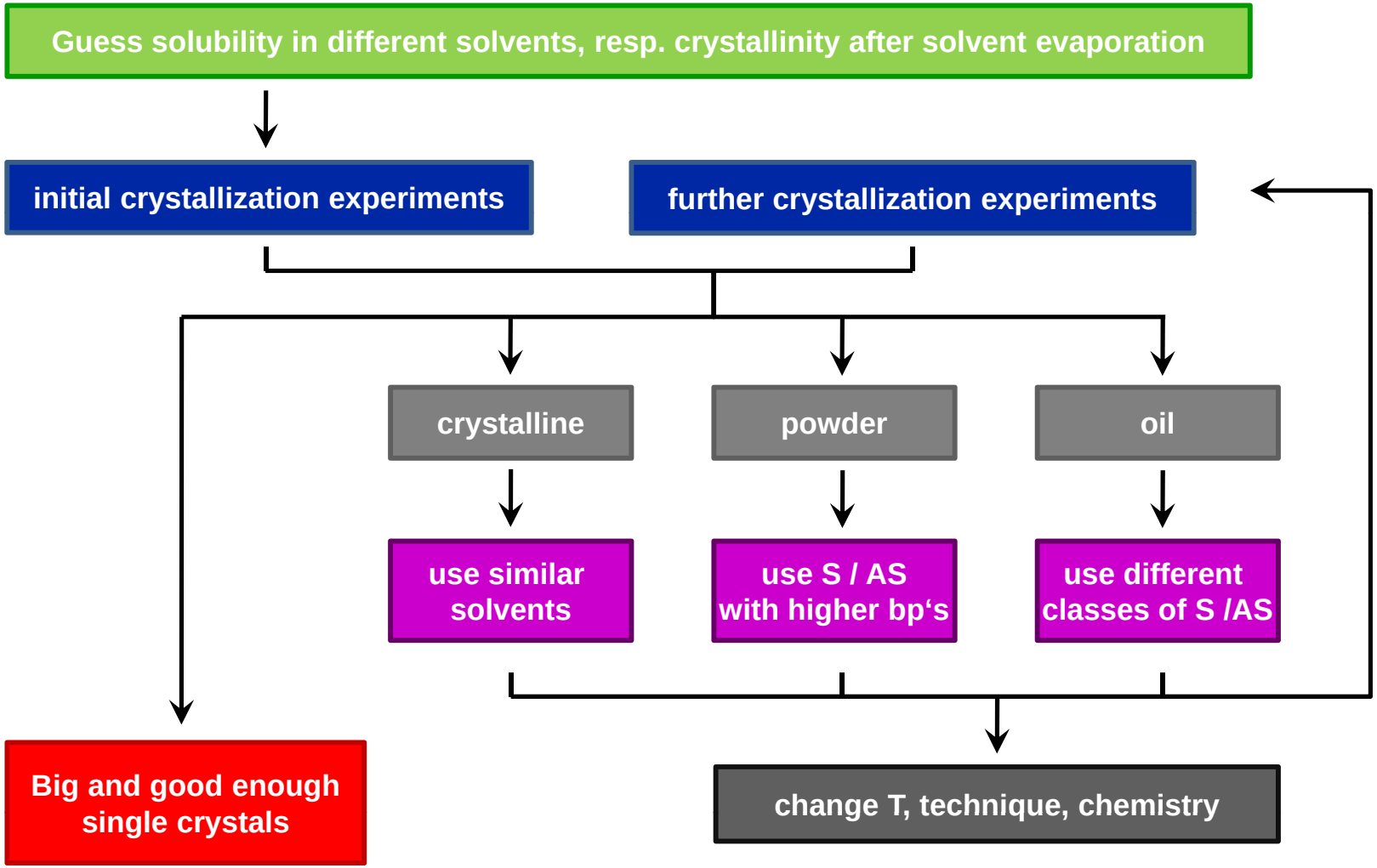
Improving crystal form/size:

Substitute solvent and antisolvent with other solvents of the same class having similar dielectric constants.

For example:

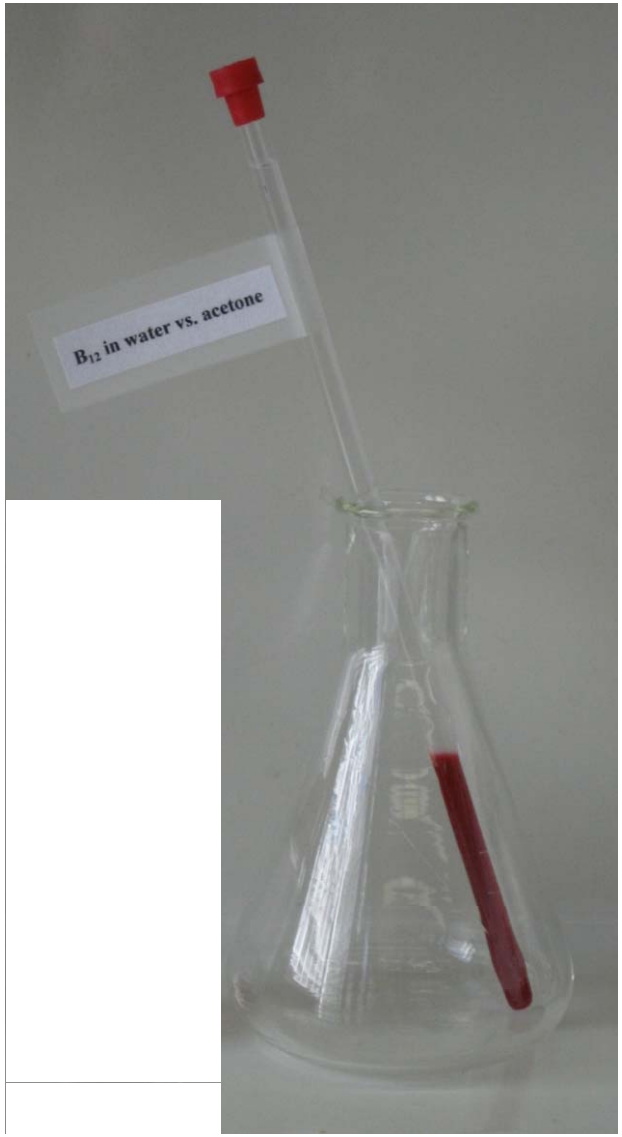
Solvent	B.p.°C / ε		Antisolvent	B.p.°C / ε	
methylene chloride	40	8.93	cyclopentane	49	1.97
1,1,1-trichloro-ethane	74	7.24	cyclohexane	81	2.02
1,2-dichloroethane	84	10.4	methylcyclohexane	101	2.02

An extremely simplified flow chart



Layering I

- About 4 mg of substance are dissolved in ~0.5 ml of a dense solvent and put in a cheap NMR tube.
- With an extra-long Pasteur pipette, a 0.5 cm high protection layer of pure solvent is carefully layered above.
- The lighter antisolvent is carefully layered above, until the NMR tube is full.



Layering II

- Rather high boiling solvents are used (e.g. dibutyl ether than diethyl ether).
- Different densities of the solvent and the antisolvent are needed. Take change of density due to solute into account!
- Experiment takes more time to equilibrate (several weeks!).
- Once started the experiment is difficult to modify.
- Difficult optical crystal evaluation, if they grow at the bottom of the NMR tube (or when falling down during retrieval attempts).

Excerpt from a table with 107 solvents

Sum formula	Name	b.p. °C	δ	ε
$C_6H_{12}O_2$	t-Butyl acetate	95	0.867	5.67
C_6H_5Cl	Chlorobenzene	132	1.106	5.69
$C_5H_{10}O_2$	Ethyl propanoate	99	0.892	5.76
$C_4H_8O_2$	Ethyl acetate	77	0.900	6.08
$C_5H_{10}O_2$	Butyl formate	106	0.889	6.10
$C_4H_8O_2$	Methyl propanoate	80	0.915	6.20
$C_2H_4O_2$	Acetic acid	118	1.045	6.20
$C_4H_8O_2$	Propyl formate	81	0.906	6.92
$C_5H_{10}O$	2-Methyltetrahydrofuran	78	0.855	6.97
$C_3H_6O_2$	Methyl acetate	57	0.934	7.07
$C_6H_{14}O_3$	Diethylene glycol dimethyl ether	162	0.943	7.23
$C_2H_3Cl_3$	1,1,1-Trichloroethane	74	1.339	7.24
$C_4H_{10}O_2$	Ethylene glycol dimethyl ether	85	0.869	7.30
C_4H_8O	Tetrahydrofurane	65	0.889	7.52
CH_2Br_2	Dibromomethane	97	2.497	7.77
$C_2H_2Cl_8$	1,1,2,2-Tetrachloroethane	131	1.541	8.50
$C_3H_6O_2$	Ethyl formate	54	0.917	8.57

selection criteria for these solvents:

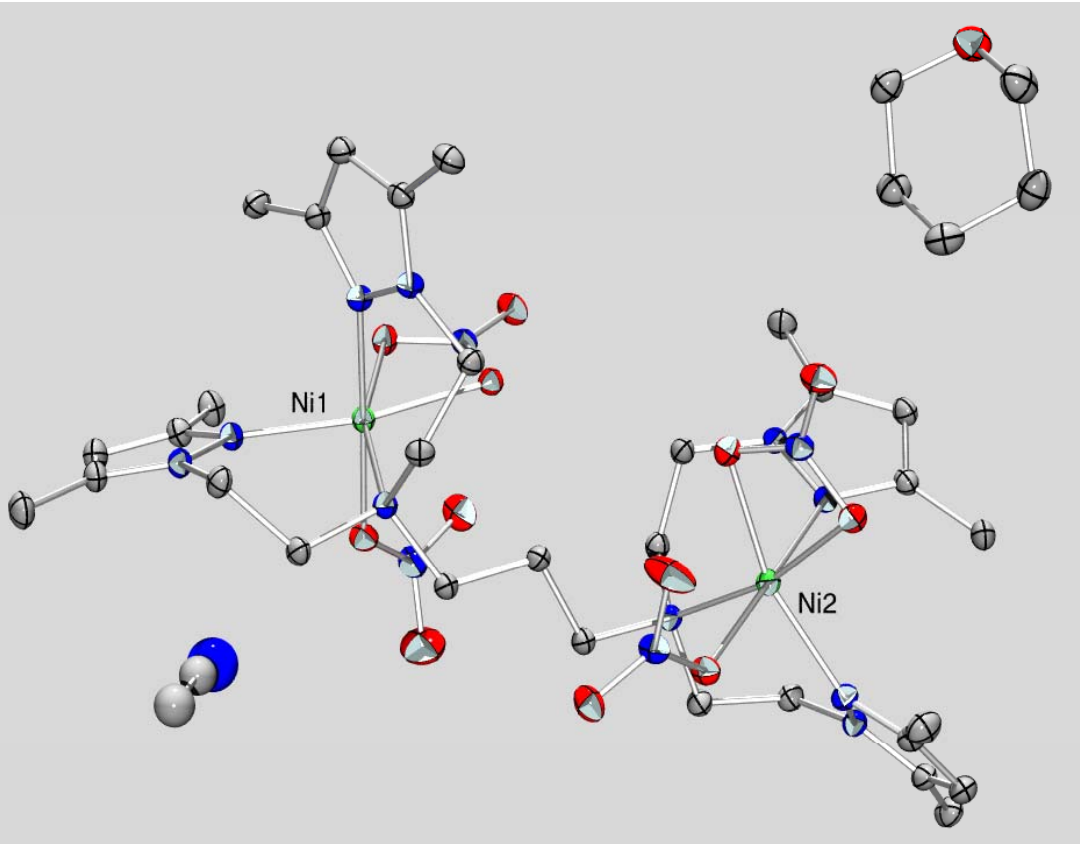
- m.p. < 20° C
- b.p. > 30° C
- few with b.p. > 150°C
- stability
- toxicity
- cost

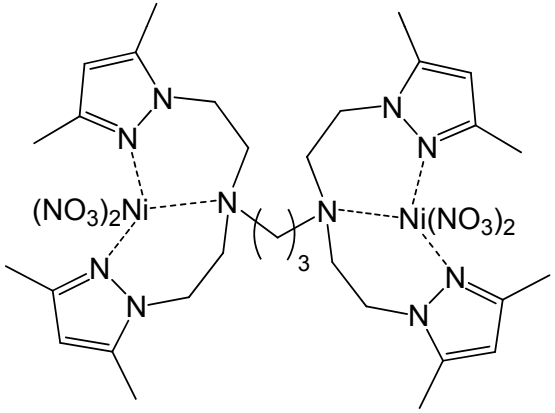
B. Spingler, S. Schnidrig, T. Todorova, F. Wild *CrystEngComm* **2012**, *14*, 751.

Example I

Recrystallization from acetonitrile versus tetrahydropyran

Single crystals from methanol versus tetrahydropyran

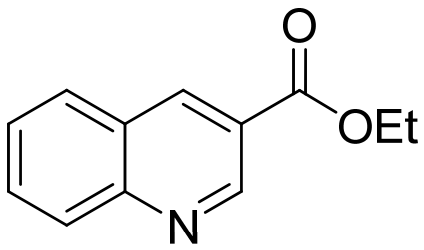




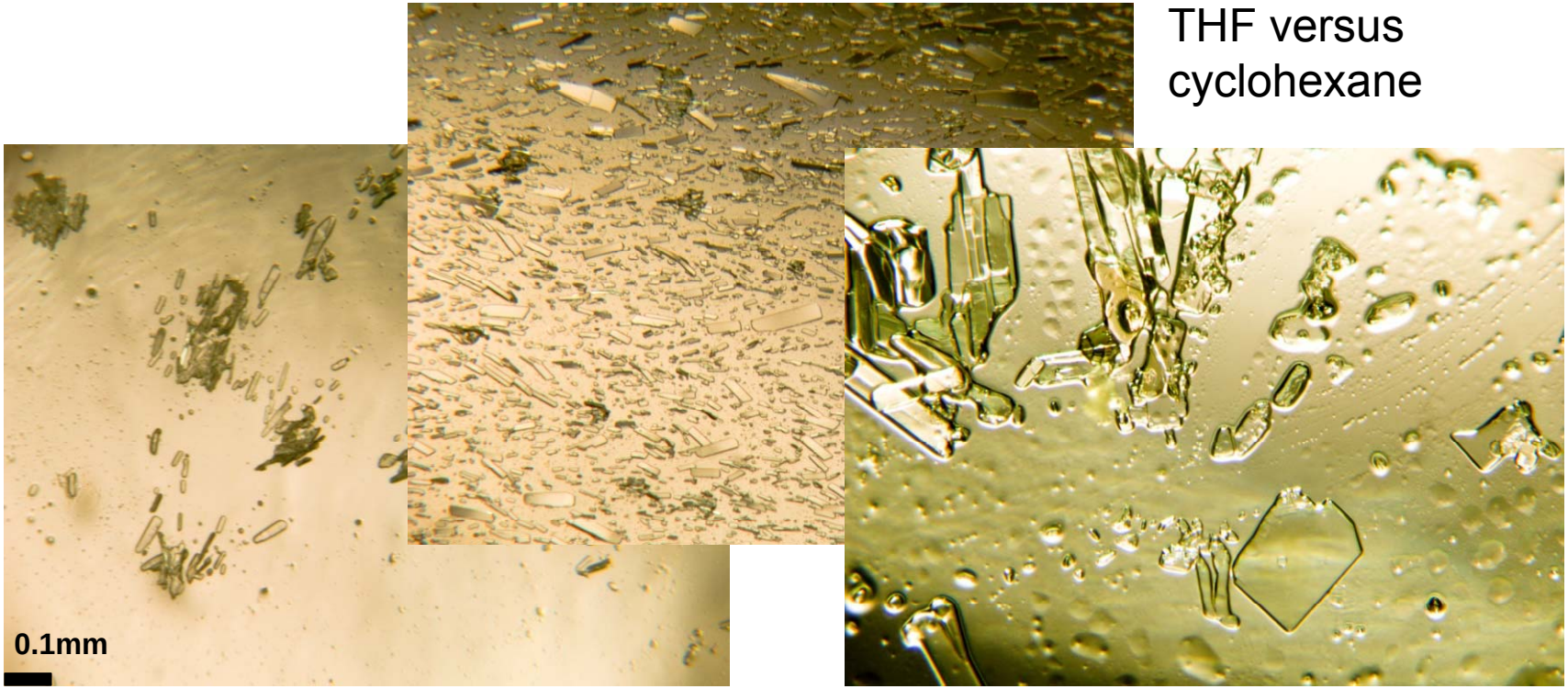
B. Spingler, P. M. Antoni
Chem. Eur. J. **2007**, *13*, 6617

Example II

3-carbethoxyquinoline (vapor diffusion):



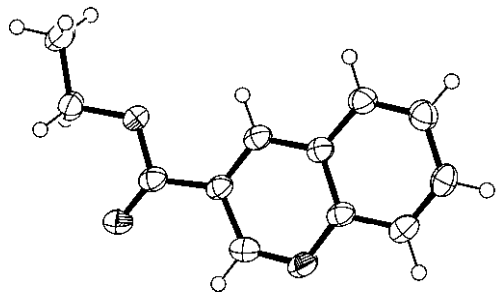
THF versus cyclohexane



Chloroform versus cyclohexane

Trichloroethylene versus **heptane**

Example II

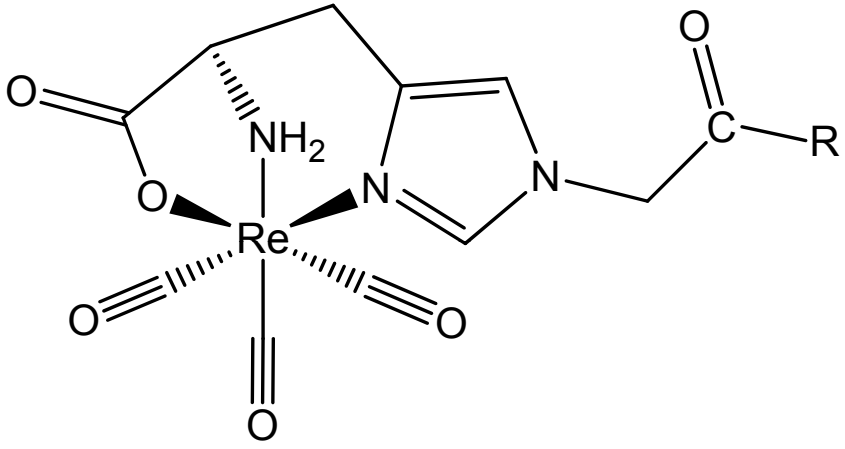


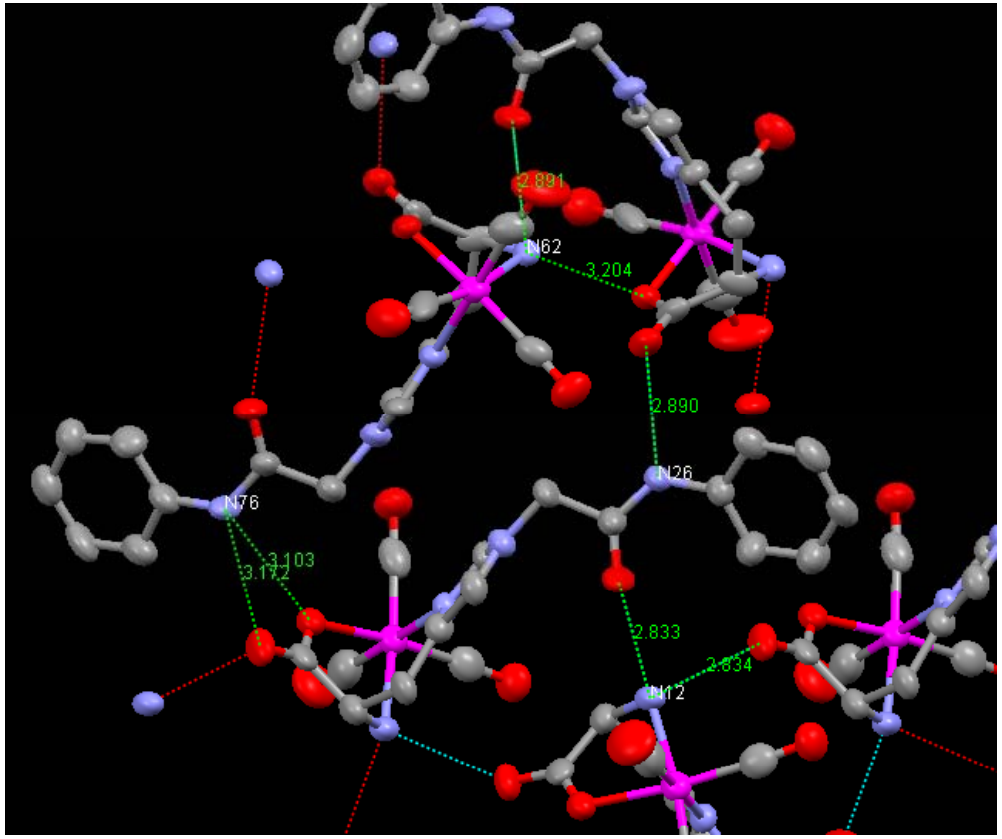
Formula	$C_{12}H_{11}NO_2$
Space group	P-1
a [Å]	7.5975(5)
b [Å]	12.2026(7)
c [Å]	12.8137(8)
α [°]	61.607(6)
β [°]	77.247(5)
γ [°]	78.353(5)
Volume [Å^3]	1012.74(11)
Z	4

Crystal size [mm^3]	**0.21** x **0.13** x **0.08**
Wavelength [Å]	**0.71073**
Independent reflections	5457 [$R_{(int)}$ = **0.0402**]
Reflections observed (>2sigma(I))	2329
Completeness to theta	99.9 % to 29.13°
Max. and min. transmission	0.9928 and 0.8558
Data / restraints / parameters	5457 / 0 / 273
Goodness-of-fit on F^2	0.810
Final R indices (I>2sigma(I))	R_1 = **0.0479**, wR_2 = 0.0782
Largest diff. peak and hole [e.Å^{-3}]	0.163 and -0.268

B. Spingler, S. Schnidrig, T. Todorova, F. Wild *CrystEngComm* **2012**, *14*, 751.

Example III, different chemistry





R: OH, OEt, O*t*-Bu
all did not crystallize.

But R: NHPh did.

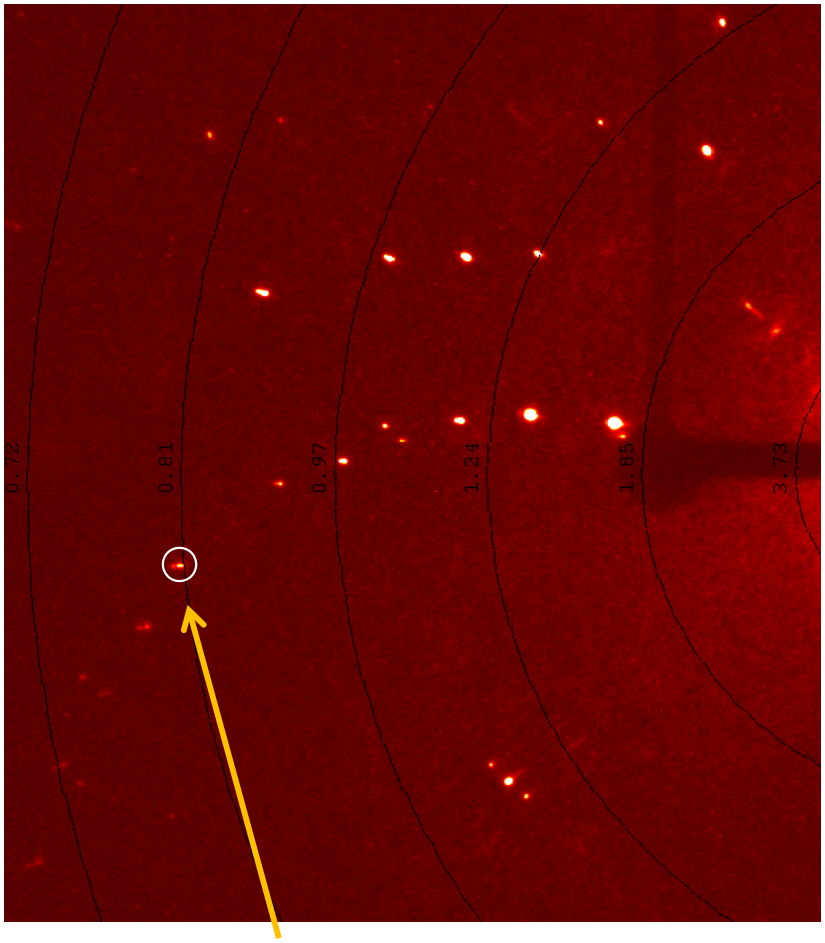
J. K. Pak, P. Benny, B. Spingler, K. Ortner, R. Alberto *Chem. Eur. J.* **2003**, *9*, 2053.

Mo versus Cu radiation?

- Traditional knowledge [1]:
  - Mo for crystals with heavy elements
  - Cu for organic crystals (absorption challenges with heavy elements)
- However with new diffractometer and software systems, Cu became an important rescue option for weakly diffracting crystals of high quality containing only a few heavy elements and mainly light elements

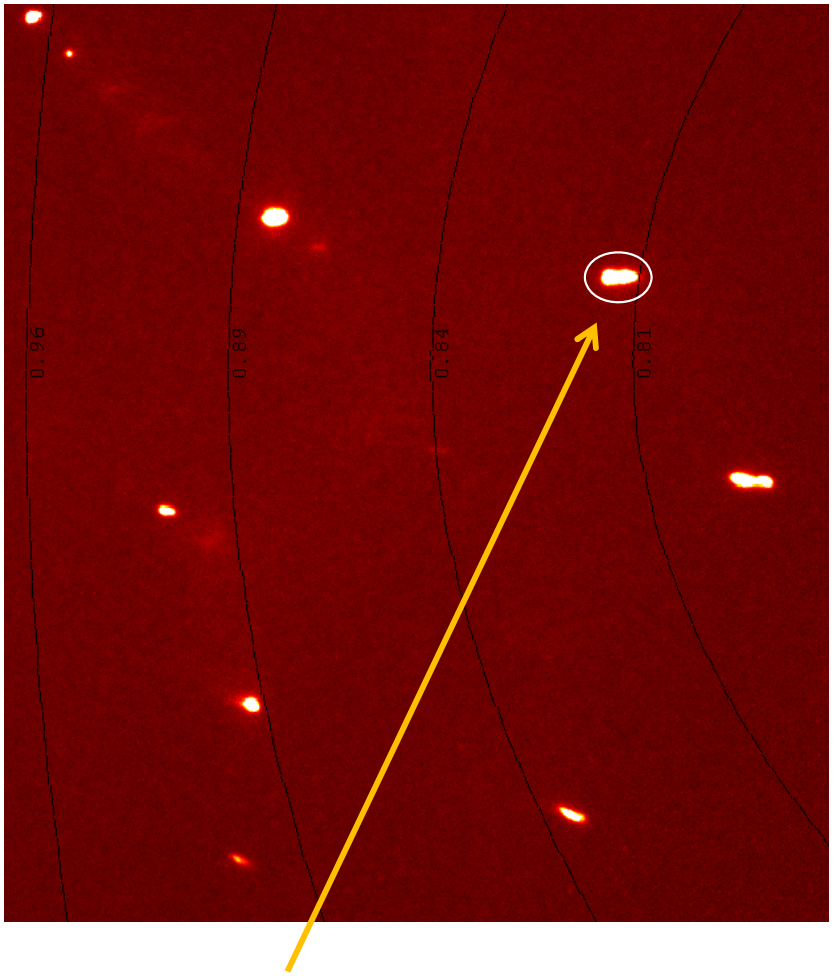
[1] A. J. Blake, J. M. Cole, J. S. O. Evans, P. Main, S. Parsons, D. J. Watkin *Crystal Structure Analysis, Principles and Practice*; Oxford University Press, **2009**, pp. 352

Mo: 60 s exposure



hkl: 1 -10 -21; I/σ: 13

Cu: 16 s exposure



hkl: 1 -10 -21; I/σ: 105

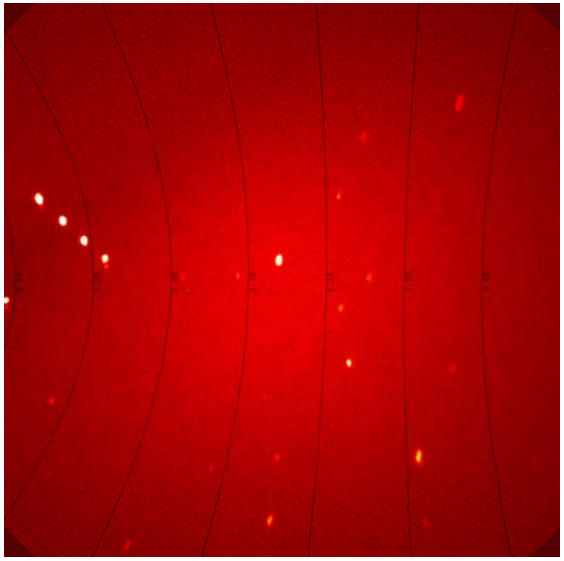
Two examples of heavy metal containing structure measured with Cu radiation

Empirical formula	$C_{39}H_{33.25}Cl_2F_6N_8O_{7.13}$P**Ru** [1]	$C_{44}H_{58}$**Br_2Co**N_6O_8 [2]
Diffractometer	SuperNova dual radiation CCD	SuperNova dual radiation CCD
Space group	*P*-1	*P*-1
Abs. coeff. (mm^{-1})	**5.465**	**5.664**
Crystal size (mm^3)	0.33 x 0.08 x 0.04	0.18 x 0.04 x 0.02
Indep. reflections	15295 [R_{int} = 0.0338]	4532 [R_{int} = 0.0218]
Completeness to θ	95.0 % to 66.97°	99.4 % to 66.97°
Absorption corr.	Semi-empirical from equiv.	Gaussian + Semi-empirical from equiv.
Max. and min. transm.	0.8110 and 0.6223	0.918 and 0.617
Fin. R ind. [I > 2 σ(I)]	R1 = 0.0760, wR2 = 0.2128	R1 = 0.0235, wR2 = 0.0586
Fin. diff. ρ_{max} ($e^-/Å^{-3}$)	**1.356** and **-1.533**	**0.544** and **-0.324**

[1] C. Mari, *et al. Chem. Eur. J.* **2014**, *20*, 14421
[2] E. Joliat, *et al. Dalton Trans*. **2016**, *45*, 1737

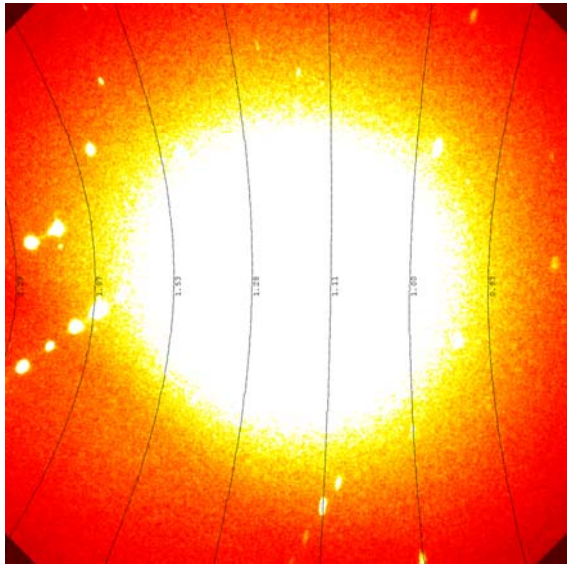
Disadvantages of Cu radiation

- Only till 0.8 Å resolution
- Big theta values to be covered mean 3 series of scans (→ longer measurement times)
- For cobalt containing compounds and weekly diffracting crystals: observation of X-ray fluorescence



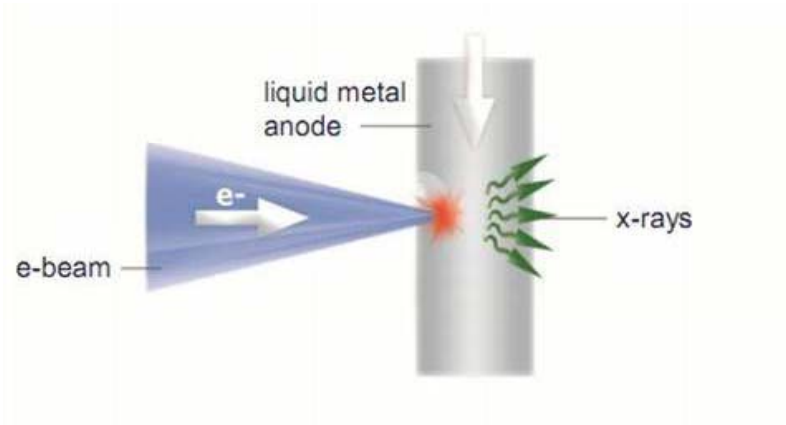
Left: 6s exposure
2.55-0.95 Å

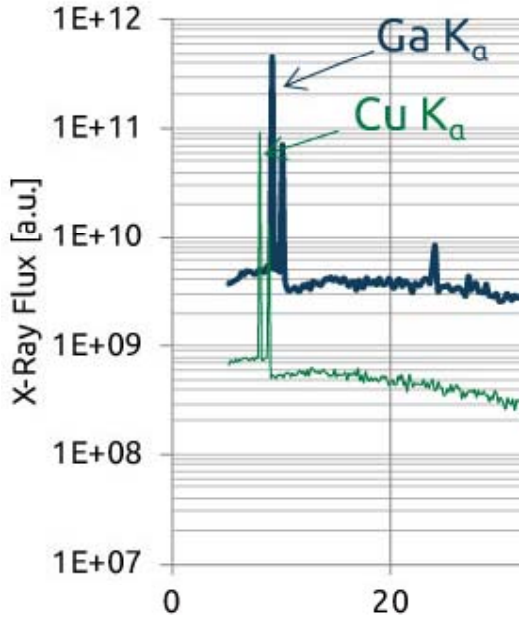
Right: 24s exposure
2.29-0.93 Å



What about Ga radiation?

- Melts at 29.8°C, but actually Ga rich alloys being used
- Liquid metal dissipates heat much quicker than solid one
- Most intensive microsource
- Wavelength 1.34 Å
- No cobalt fluorescence observed
- Air-conditioning needed for diffractometer room, as Ga source most stable for room temperature not varying more than 0.2°C





[1] Pictures taken from www.excillum.com/technology/metal-jet-technology.html
[2] M. Otendal, T. Tuohimaa, U. Vogt, H. M. Hertz *Rev. Sci. Inst.* **2008**, *79*, 016102-3

Ga jet with a Pixel detector

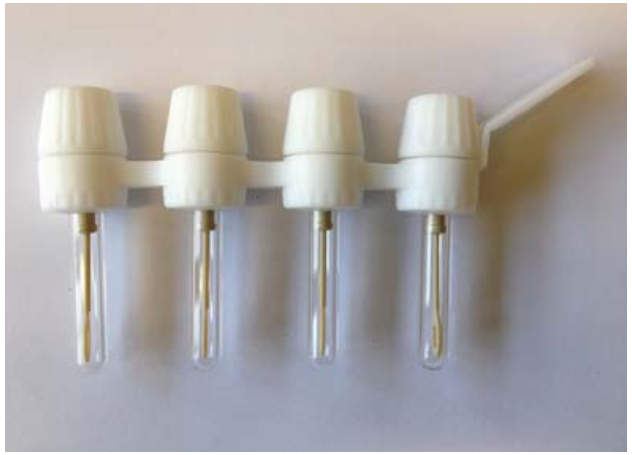
Setup at the University of Basel, Switzerland

See: L. Prieto *et al. Org. Lett.* **2016**, *18*, 5292

What about non-isothermal crystallization?

CrystalBreeder

- Working volume about 0.1 ml
- Temperature range from -15°C up to 150°C
- 8 independently temperature controlled blocks each holding 4 vials
- Transmissivity detection
- Overhead stirring

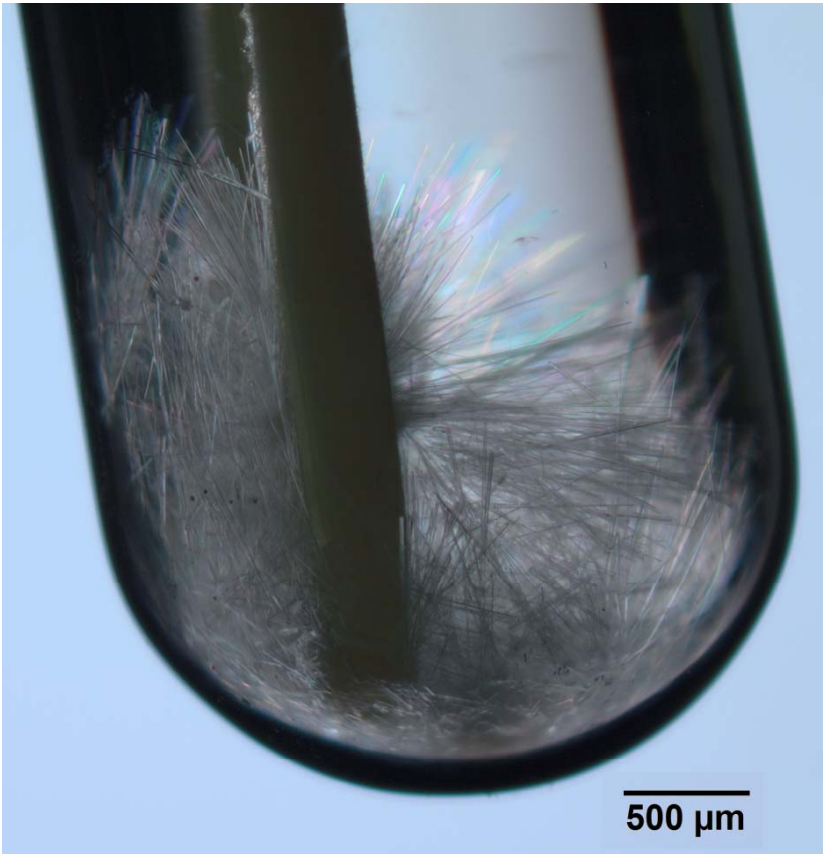


Experimental considerations for the thermal recrystallization of CBZ [1]

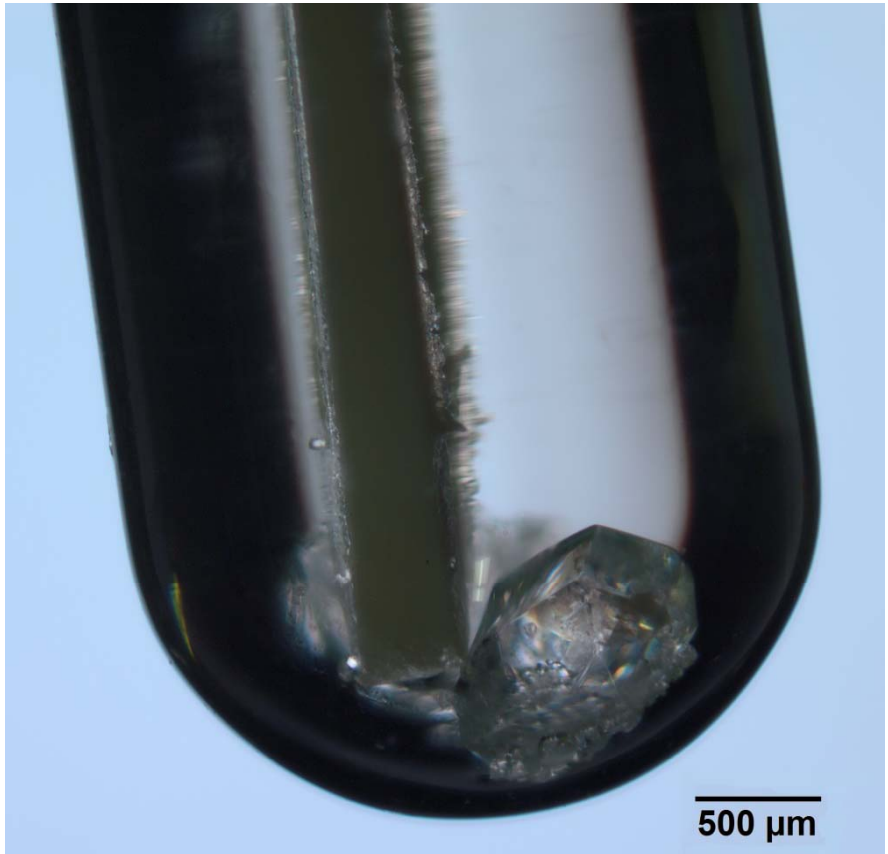
Solvent	Solubility of CBZ in that solvent	Melting point	Boiling point	Mass (in mg) of CBZ for 0.1ml of a saturated solution
1-propanol	18.1 g/l (25°C) [2]	-126°C	97°C	0.91
2-propanol	9.11 g/l (25°C) [2]	-88°C	82°C	1.8
2-butanol	~8 g/l (25°C) [3]	-115°C	99°C	0.8
THF	45.2 g/l (22°C) [4]	-108°C	66°C	4.5
cyclohexane	0.055 g/l (25°C) [2]	6.7°C	81°C	0.005

[1] P. Nievergelt, B. Spingler, *CrystEngComm* **2017**, *19*, 142
[2] R. C. Kelly, N. Rodriguez-Hornedo, *Org. Process Res. Dev.* **2009**, *13*, 1291
[3] estimated from 2-propanol
[4] W. J. Liu, L. P. Dang, S. Black, H, Y. Wei, *J. Chem. Eng. Data* **2008**, *53*, 2204

Carbamazepine crystals after 6 hours



5 mg **CBZ** / 0.1 ml THF



6 mg **CBZ** / 0.1 ml THF

P. Nievergelt, B. Spingler, *CrystEngComm* **2017**, *19*, 142

Crystal data of CBZ and *p*ABA

Compound	**CBZ**	**CBZ*1/6(H_2O)**	***p*ABA**
grown from	2-propanol at a concentration of 15 g/l	2-propanol at a concentration of 15 g/l	Ethyl acetate at a concentration of 80 g/l
Space group	$P2_1/n$	*R*-3	$P2_1/n$
a [Å]	7.49441(10)	35.2832(9)	18.5413(3)
b [Å]	11.06430(14)	35.2832(9)	3.77083(7)
c [Å]	13.80360(15)	5.20165(13)	18.5858(3)
α [°]	90	90	90
β [°]	92.9142(11)	90	93.6727(17)
γ [°]	90	120	90
Crystal size [mm^3]	0.37 • 0.33 • 0.17	0.17 • 0.05 • 0.05	0.34 • 0.09 • 0.07
X-ray wavelength [Å]	0.71073	1.54184	0.71073
Independent reflections	5891 [R_{int} = 0.0244]	2484 [**R_{int} = 0.0086**]	4512 [R_{int} = 0.0201]
Refl. obs. [I>2σ(I)]	4768	2279	3791
Completeness	99.9 % to **0.6 Å**	99.9 % to 0.84 Å	99.9 % to 0.7 Å
Final R indices [I>2σ(I)]	R1 = 0.0426, wR2 = 0.1200	R1 = 0.0457, wR2 = 0.1466	R1 = 0.0505, wR2 = 0.1313
Largest diff. peak and hole [e.$Å^{-3}$]	0.459 and -0.219	0.509 and -0.209	0.368 and -0.190

P. Nievergelt, B. Spingler, *CrystEngComm* **2017**, *19*, 142

Summary

- Stay with your system as long as it works!
- For optimization of unsatisfactory crystals:
  - Systematically explore the crystallization properties of a solvent class with a similar dielectric constant
  - use an anti-/solvent pair with an inversed polarity
  - change the technique
  - change the anions, add additives
- Do not be afraid of copper radiation, even if you have some heavy elements present!
- Consider thermal recrystallization with careful control of T

Acknowledgements

– Philipp Nievergelt (University of Zurich)
– Prof. Dr. Dongwhan Lee (Seoul National University)
– Dr. Philipp M. Antoni (University of Zurich)
– Dr. Tonya Todorova (University of Zurich)
– Stefan Schnidrig (University of Zurich)
– Dr. Markus Neuburger (University of Basel)
– All the colleagues that have been discussing crystallization issues with me over the last 15 years

– University of Zürich
– Swiss National Science Foundation, BNF