Cristallisation et Préparation des échantillons

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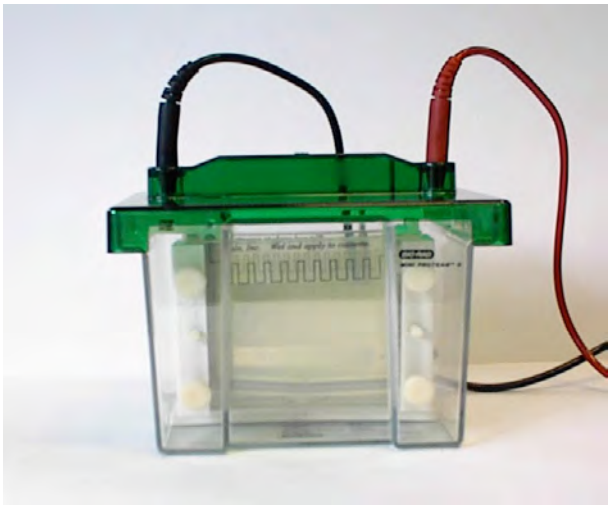
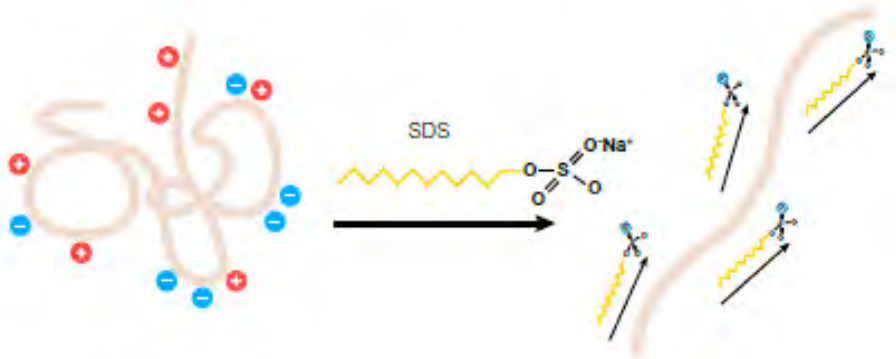
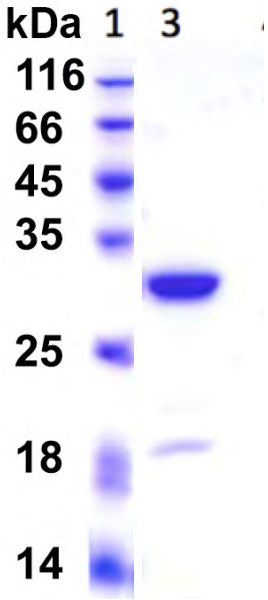
Quality control

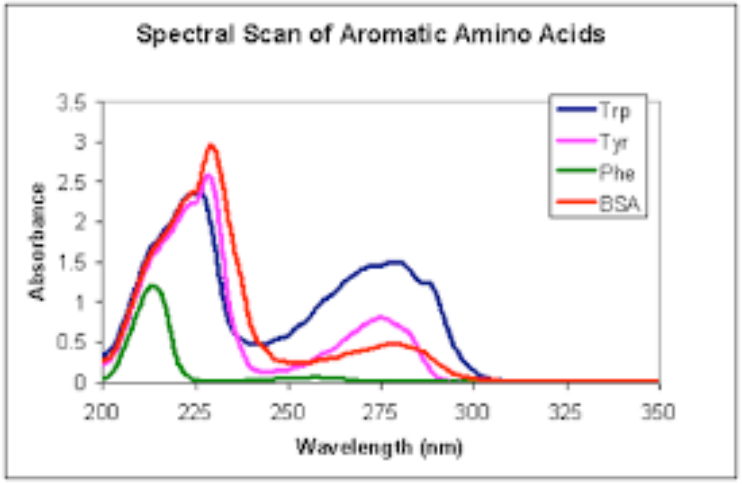
Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

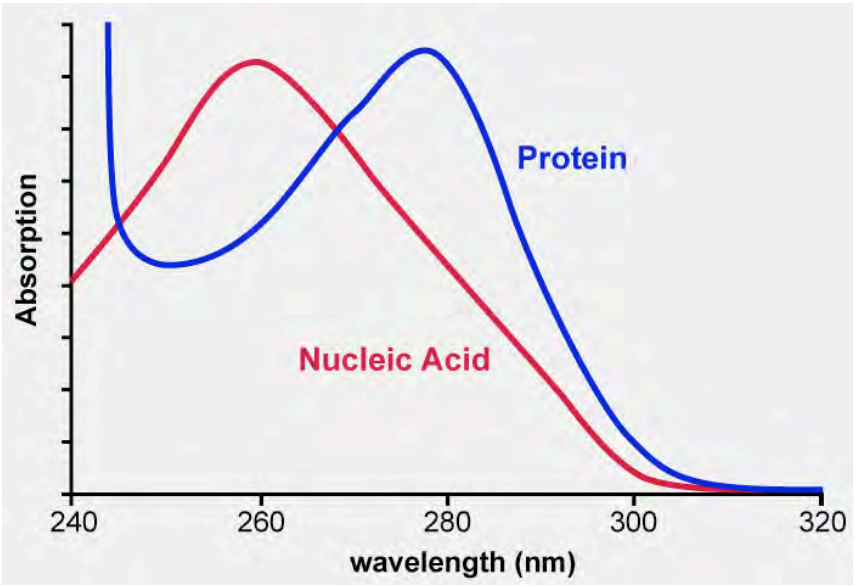




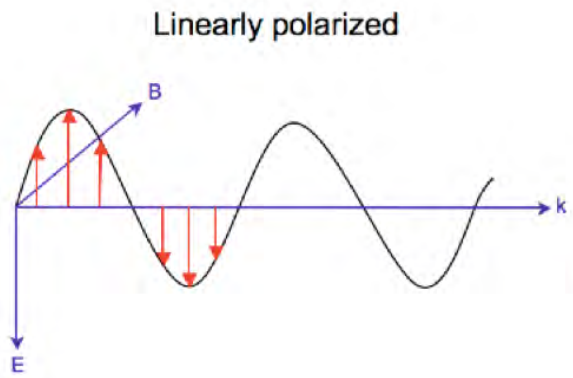


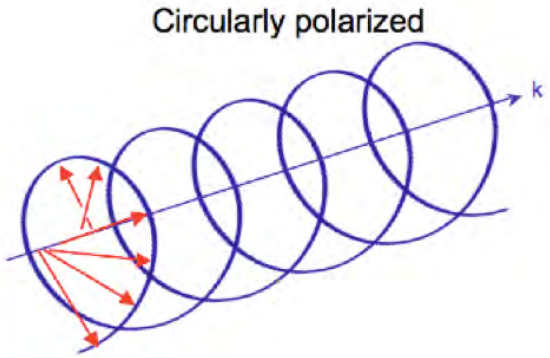
Visible UV absorption

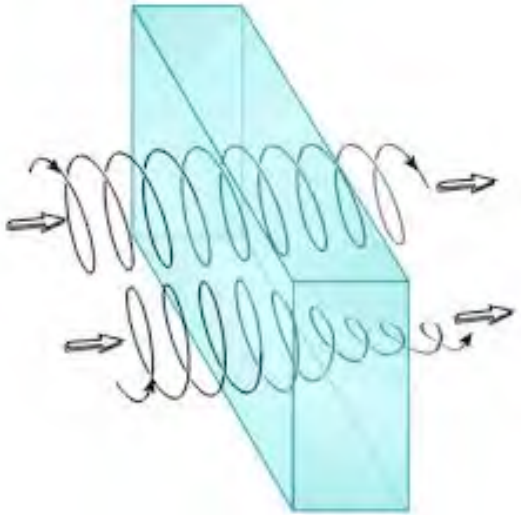




Circular dichroism (in UV spectrum)

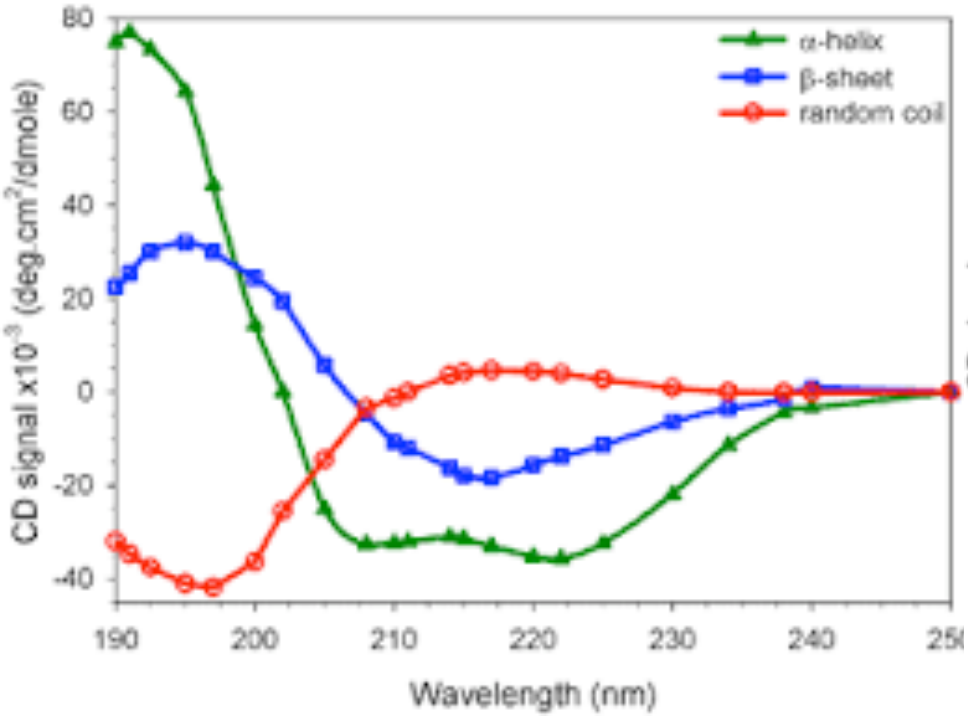


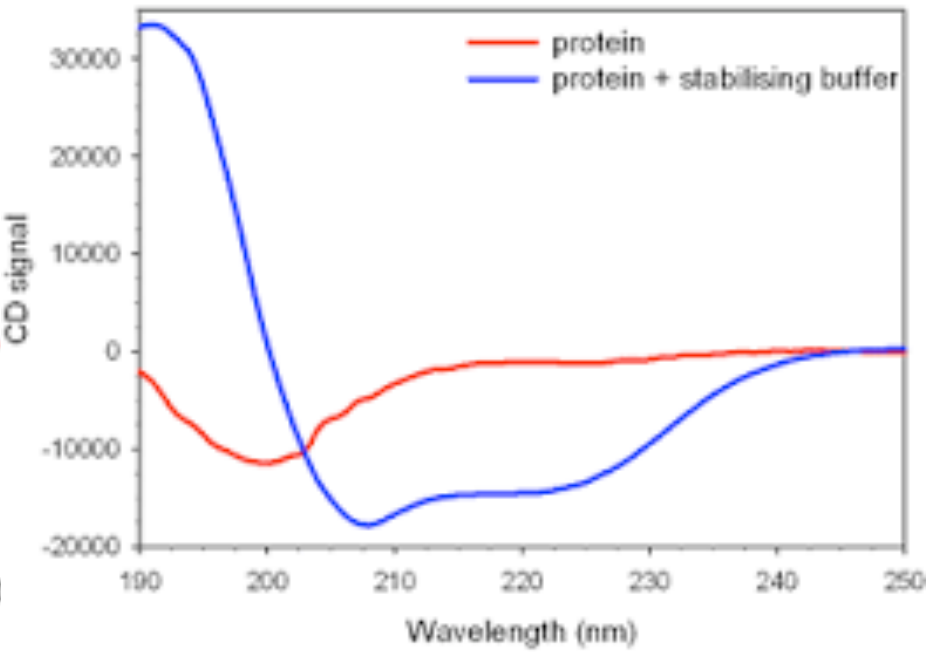




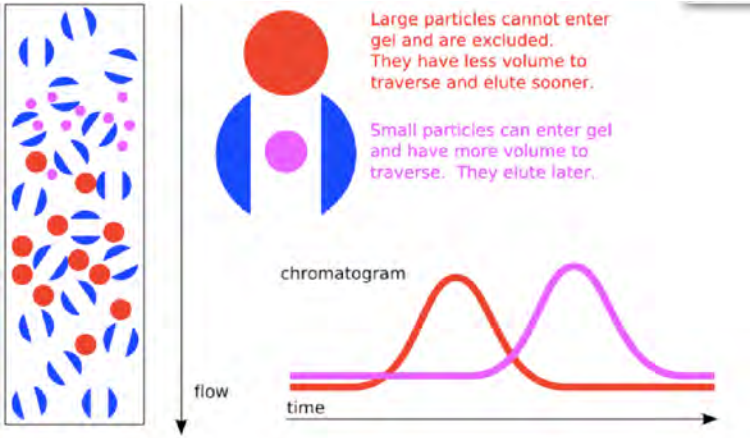
optically active molecule (chiral)

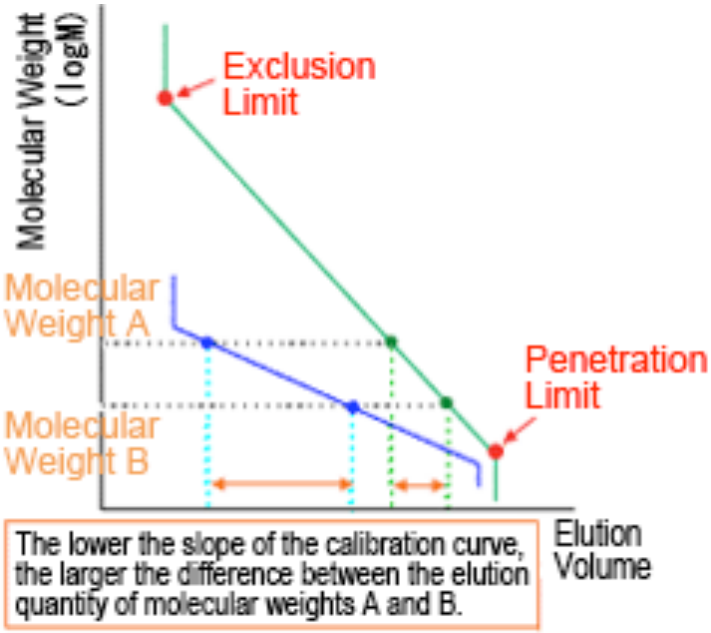
$\Delta\varepsilon \equiv \varepsilon L - \varepsilon R$

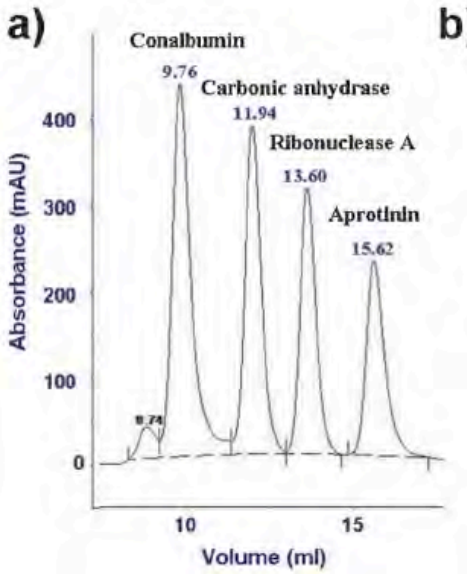


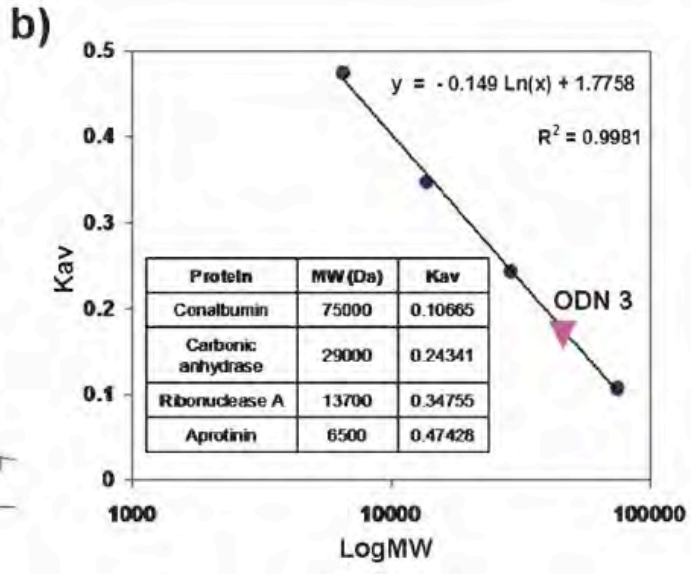


Size exclusion chromatography

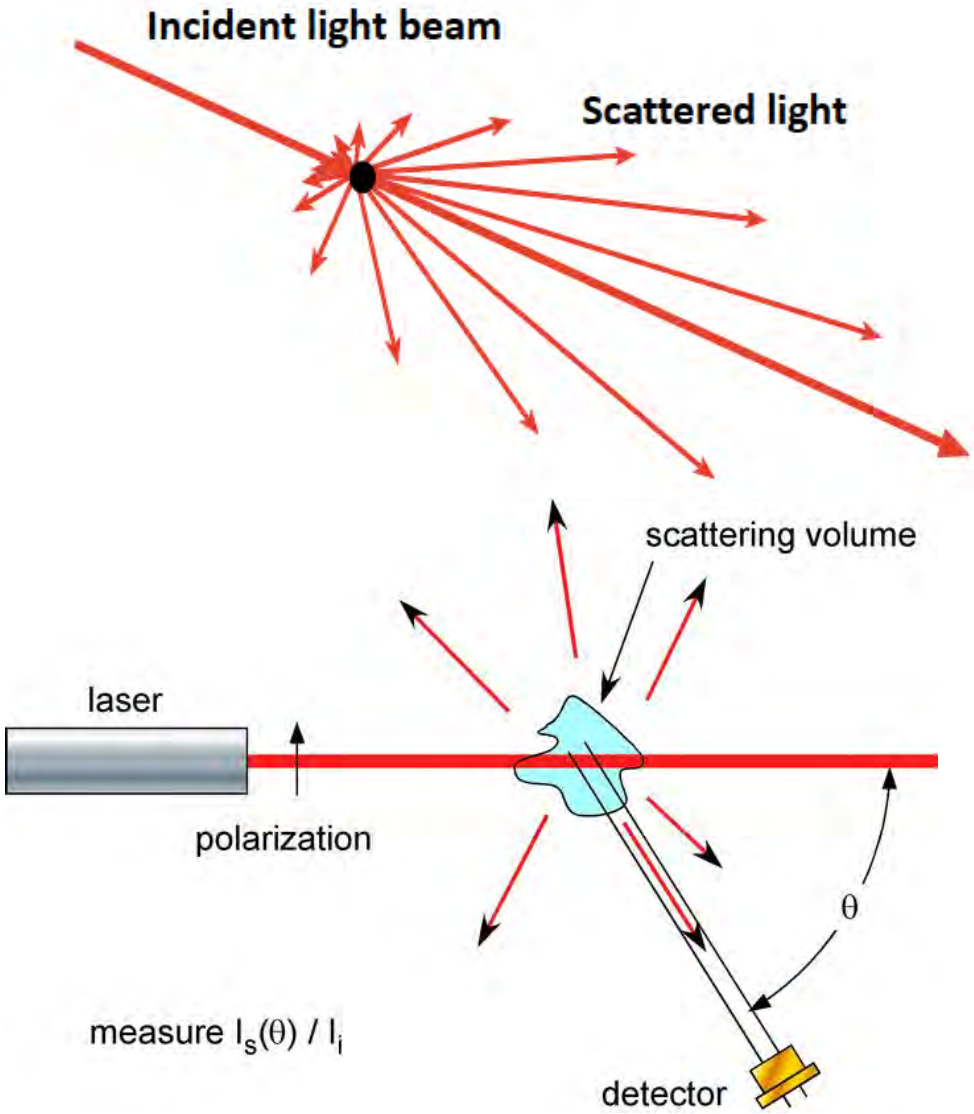








Multi-Angle static Light Scattering (MALS) Detector



WyattQELS DLS Module
Dynamic light scattering

Characterization Via Static Light Scattering

c = Concentration of Solute

Optical constant

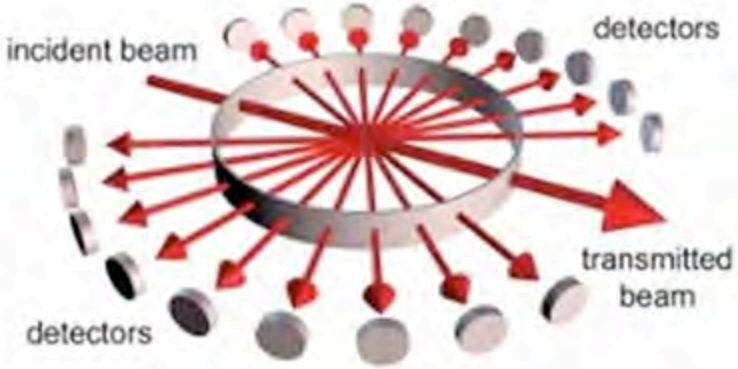
2nd virial coefficient

$$\frac{K^* c}{R(\theta, c)} = \frac{1}{M_w P(\theta)} + 2A_2 c$$

Excess Rayleigh Scattering As Function of Angle and Concentration

Weight Average Molar Mass

Angular Dependence of Scattered Light – Yields RMS Radius of Aggregate



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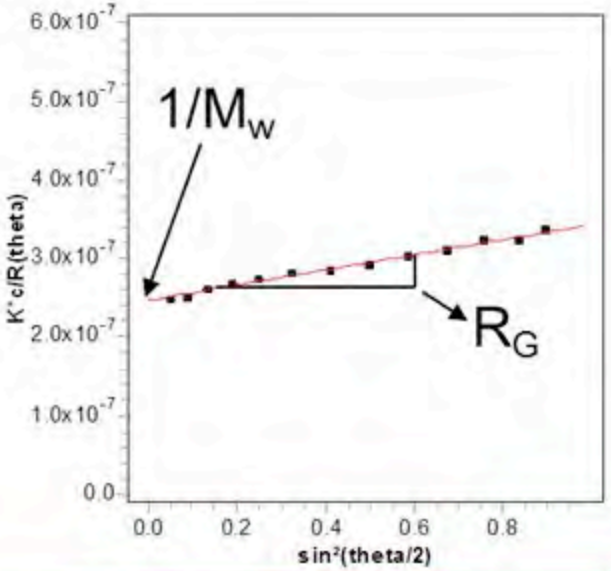


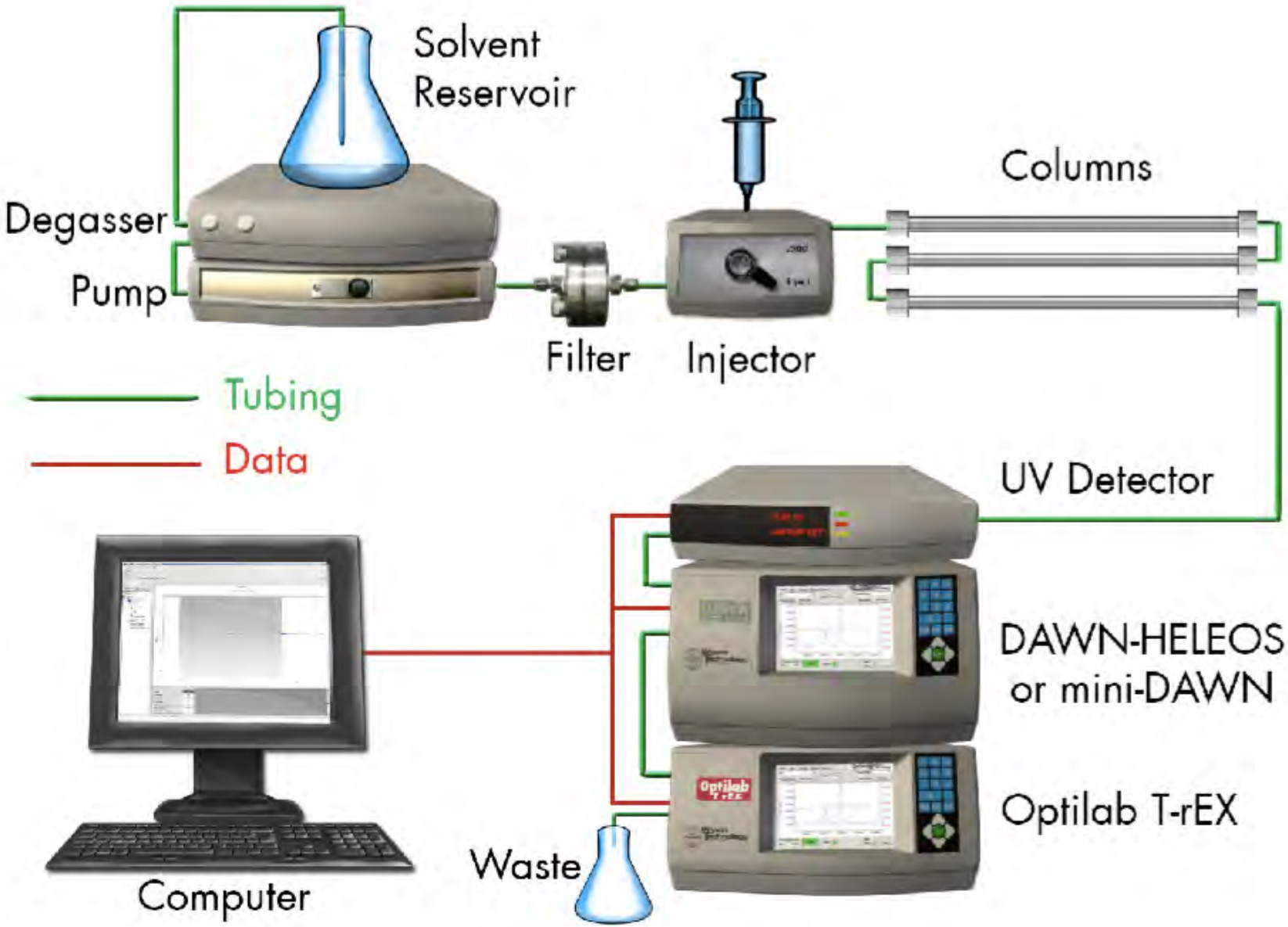
Multi-Angle Light Scattering (MALS) in Batch Mode

Simultaneously obtain:

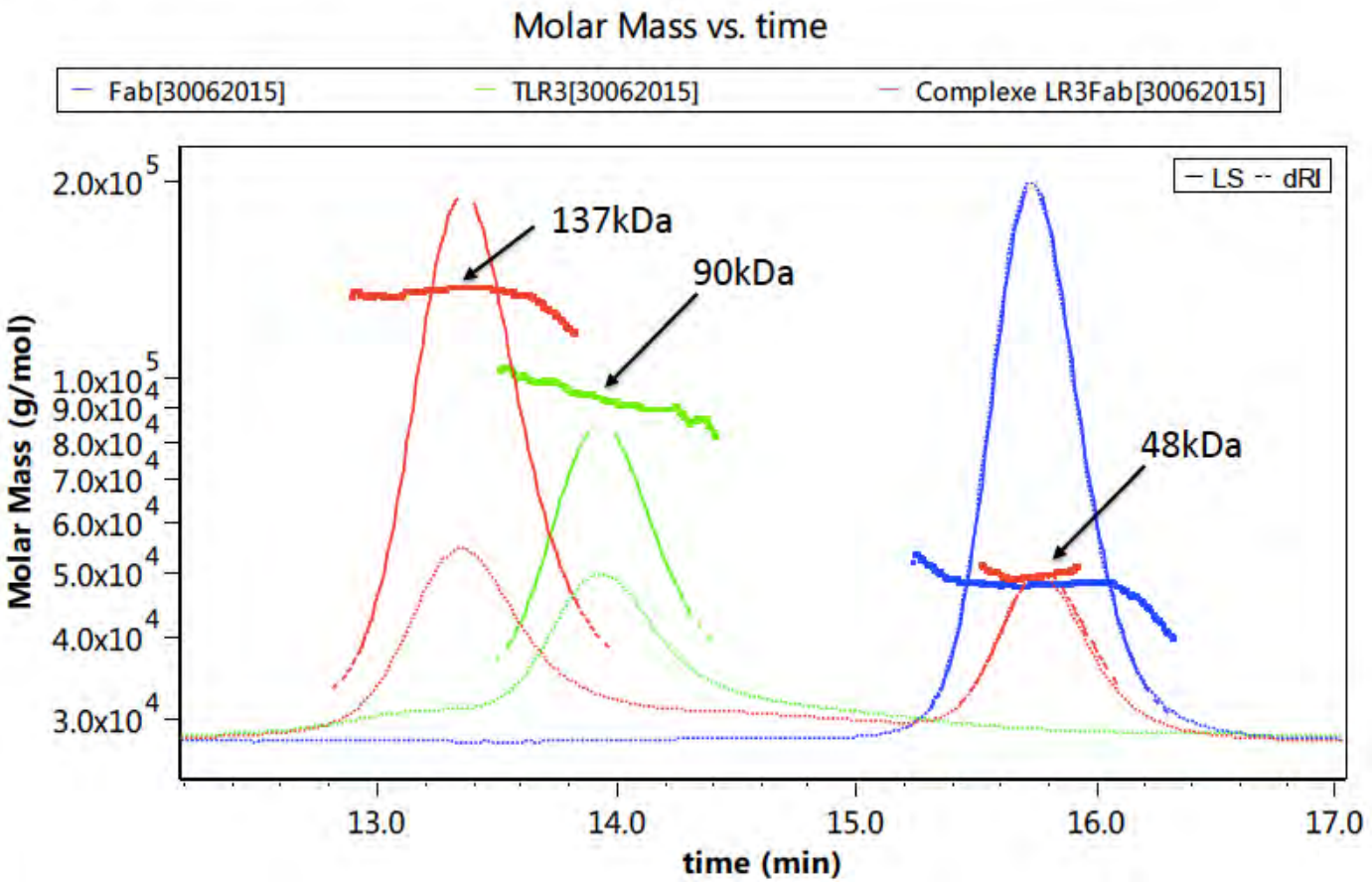
- Absolute Molecular Weight (M_w)
- Second Virial Coefficient (A_2)
- RMS Radius (R_g)

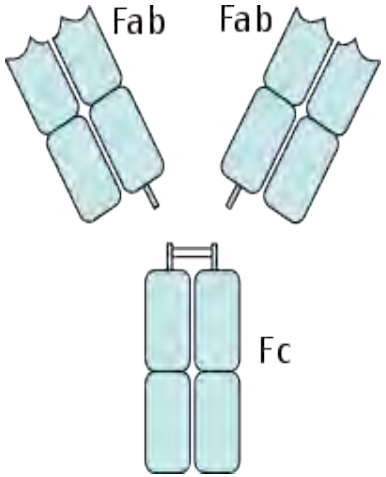
*Wyatt Corp.

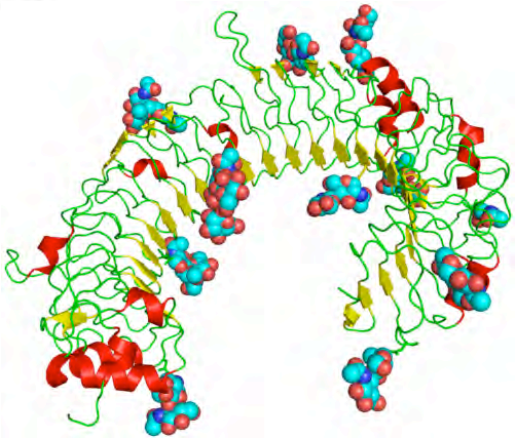
SEC MALS analysis



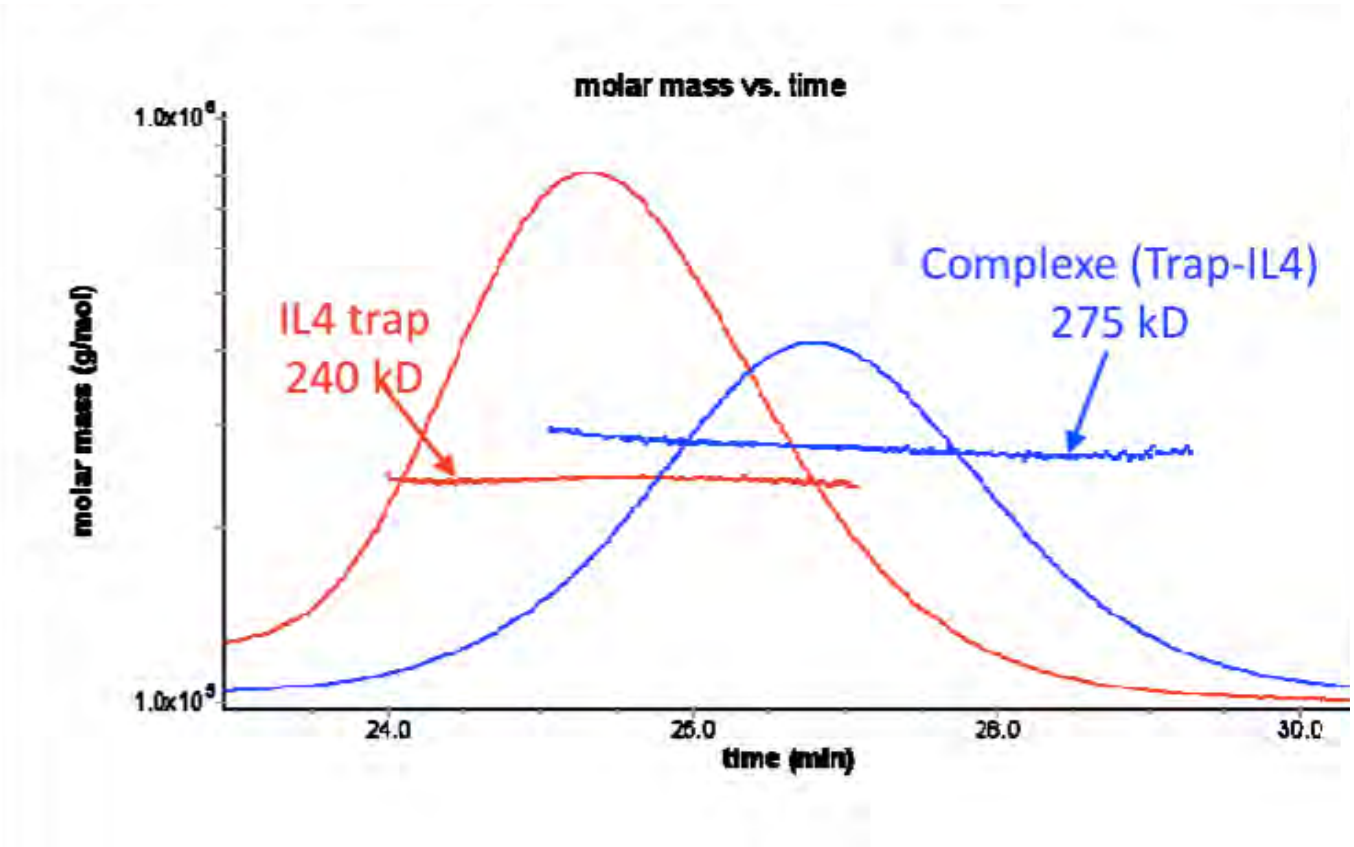
Complex Fab-TLR3 – Molar Masses



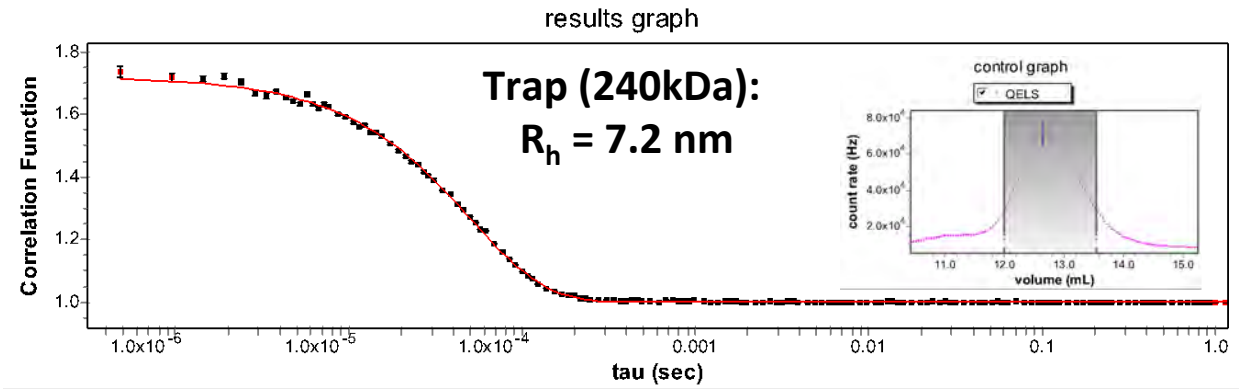


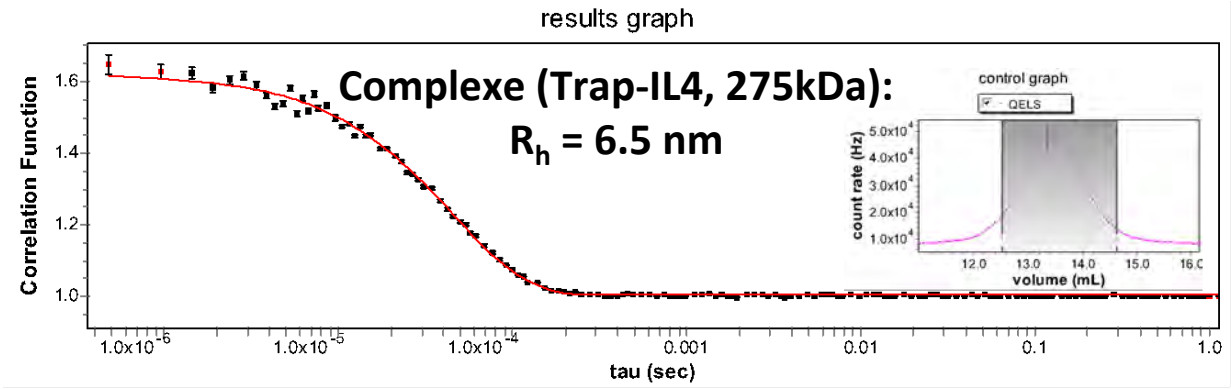


Why such a late elution of the complex Trap-IL4 ?

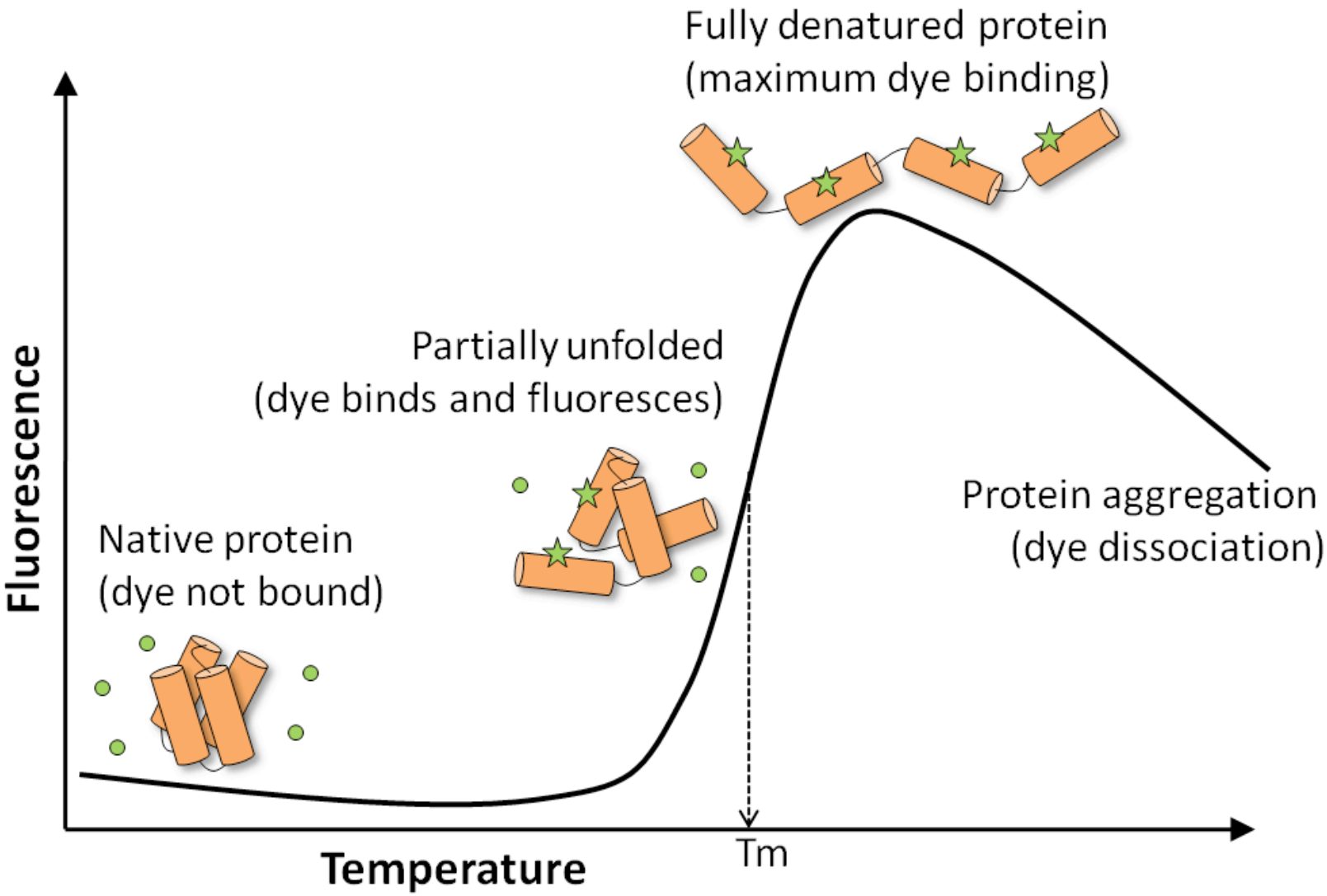


Hydrodynamic radius Rh

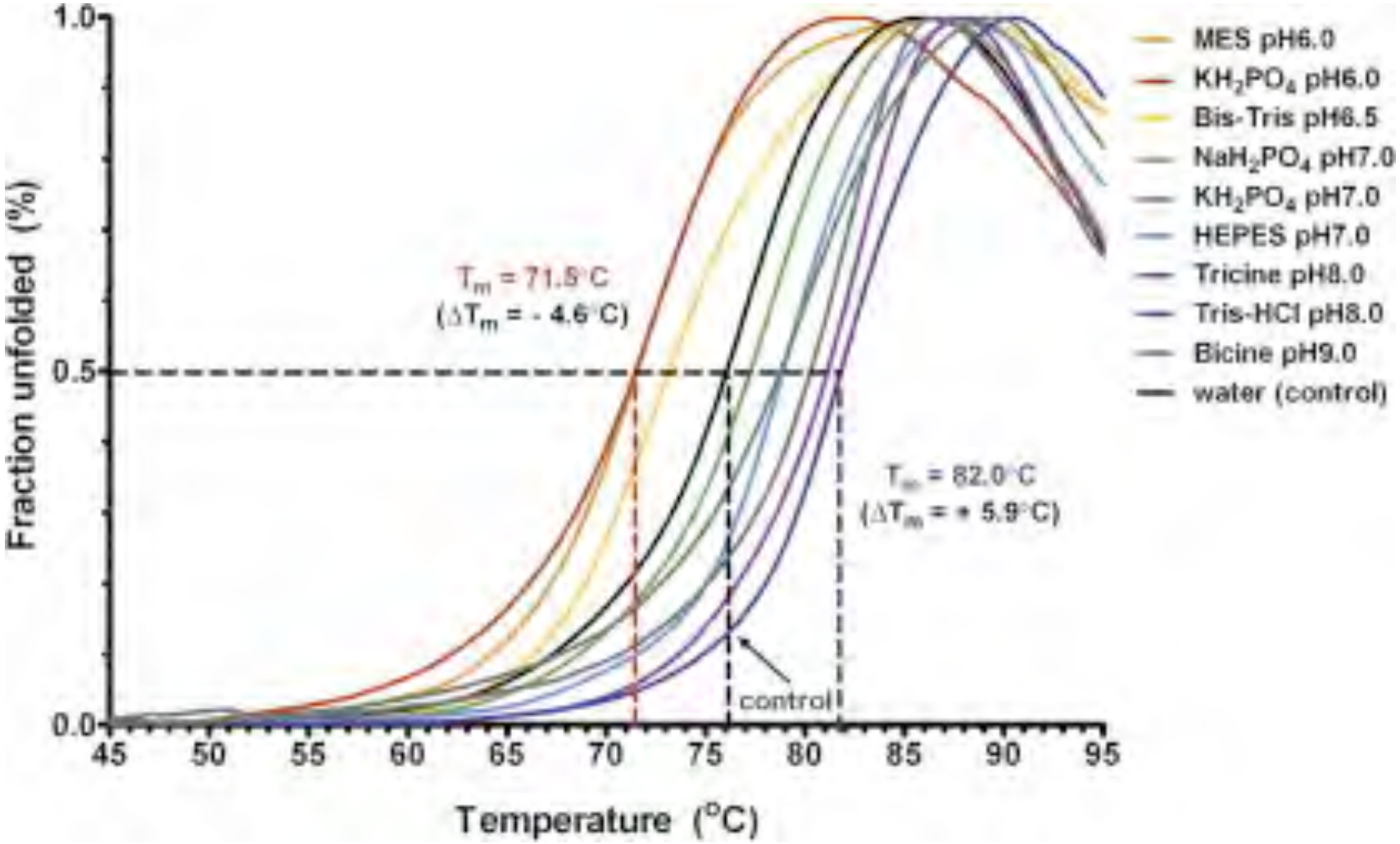




Thermal Shift Assay (TSA)



Thermal Shift Assay (TSA)



When protein stability matters.

Dr. Pierre Soule

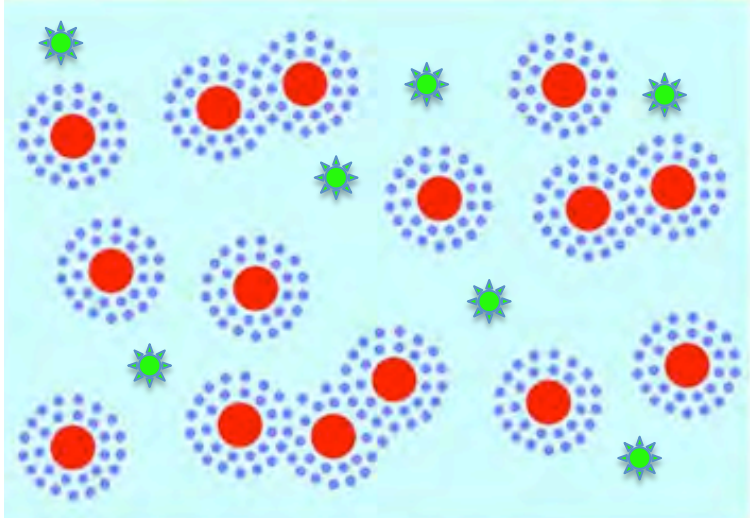
NANOTEMPER

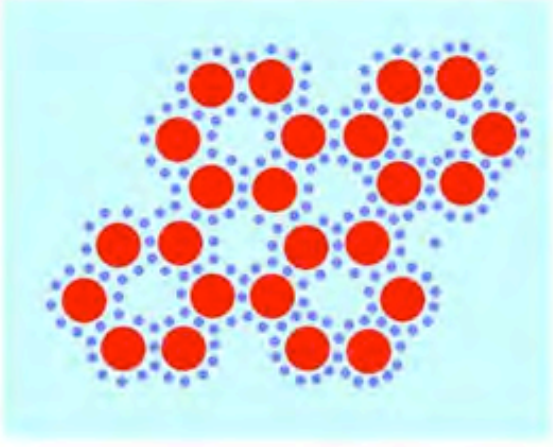
Visualizing the crystallization process

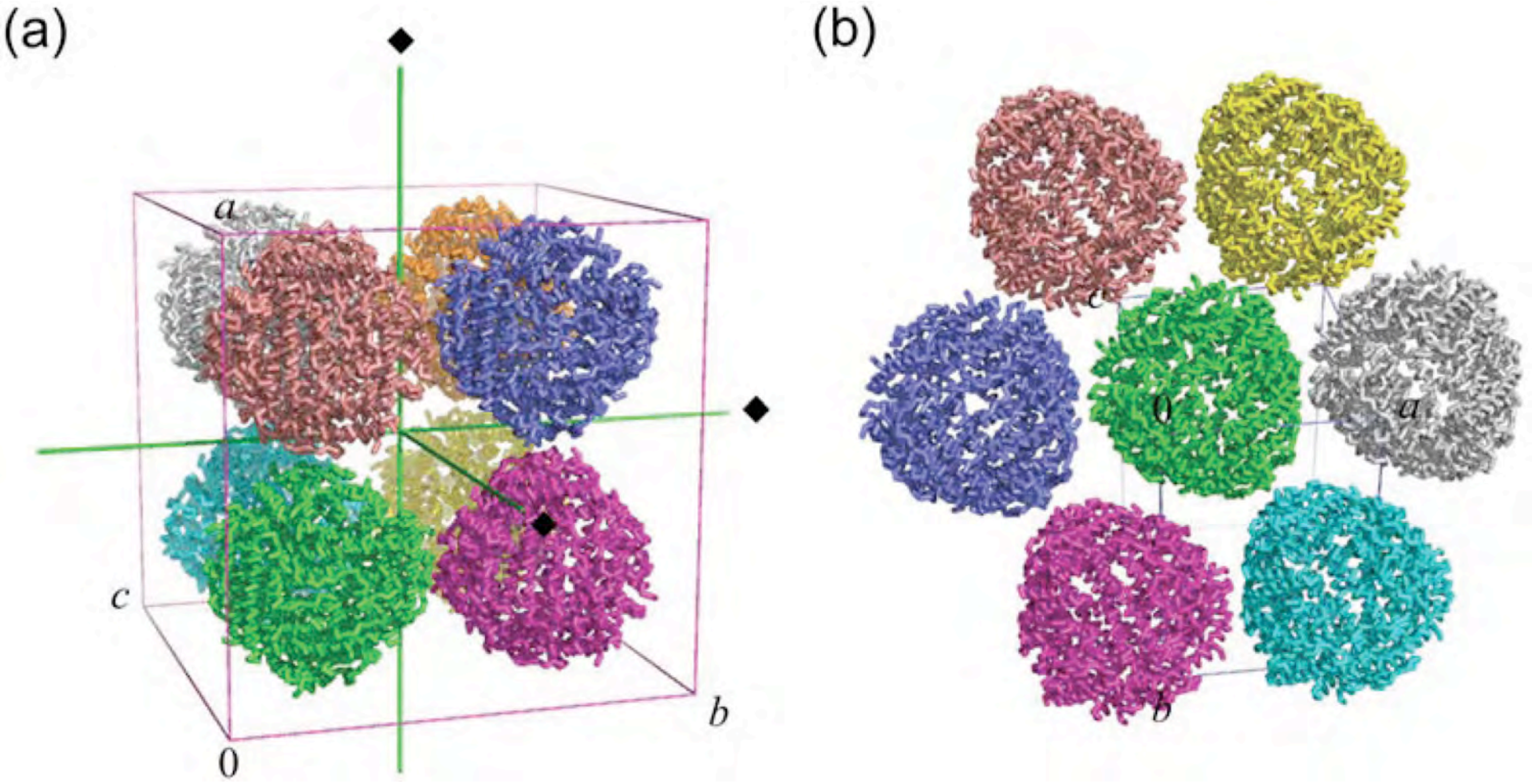
Principle: the release of water molecule from protein surface induces change in protein solubility which may lead either to crystallisation or to precipitation

- The release of water molecule can be achieved by adding to the protein solution molecules called **precipitants** (salts, ploymer, organic solvent) which compete for water molecules.

- By increasing precipitant concentration more and more, water molecules are withdrawn from the protein surface and protein molecules becomes increasingly dehydrated and start to self-associate in order to satisfy their electrostatic requirements.

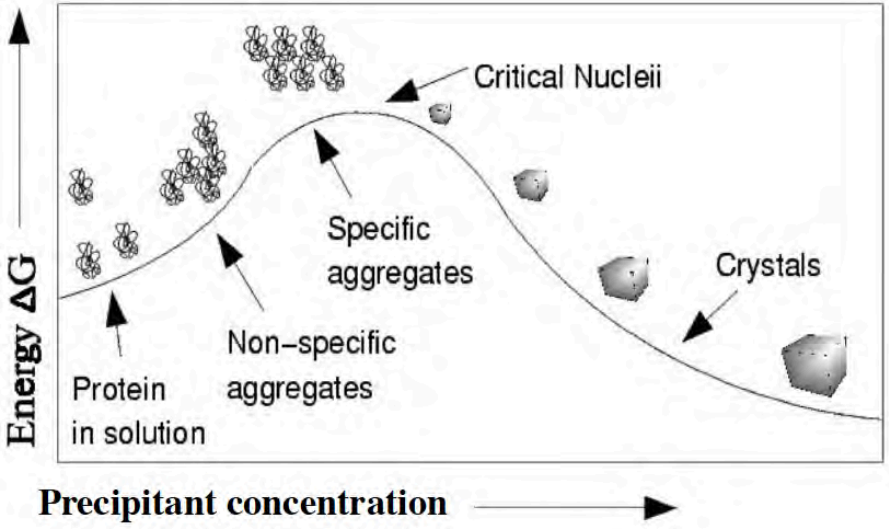


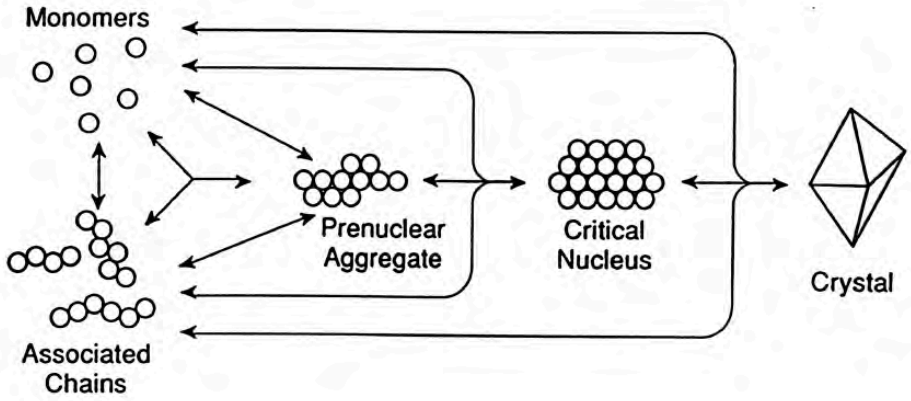



(a)
a
c
0
b
(b)
c
0
a
b

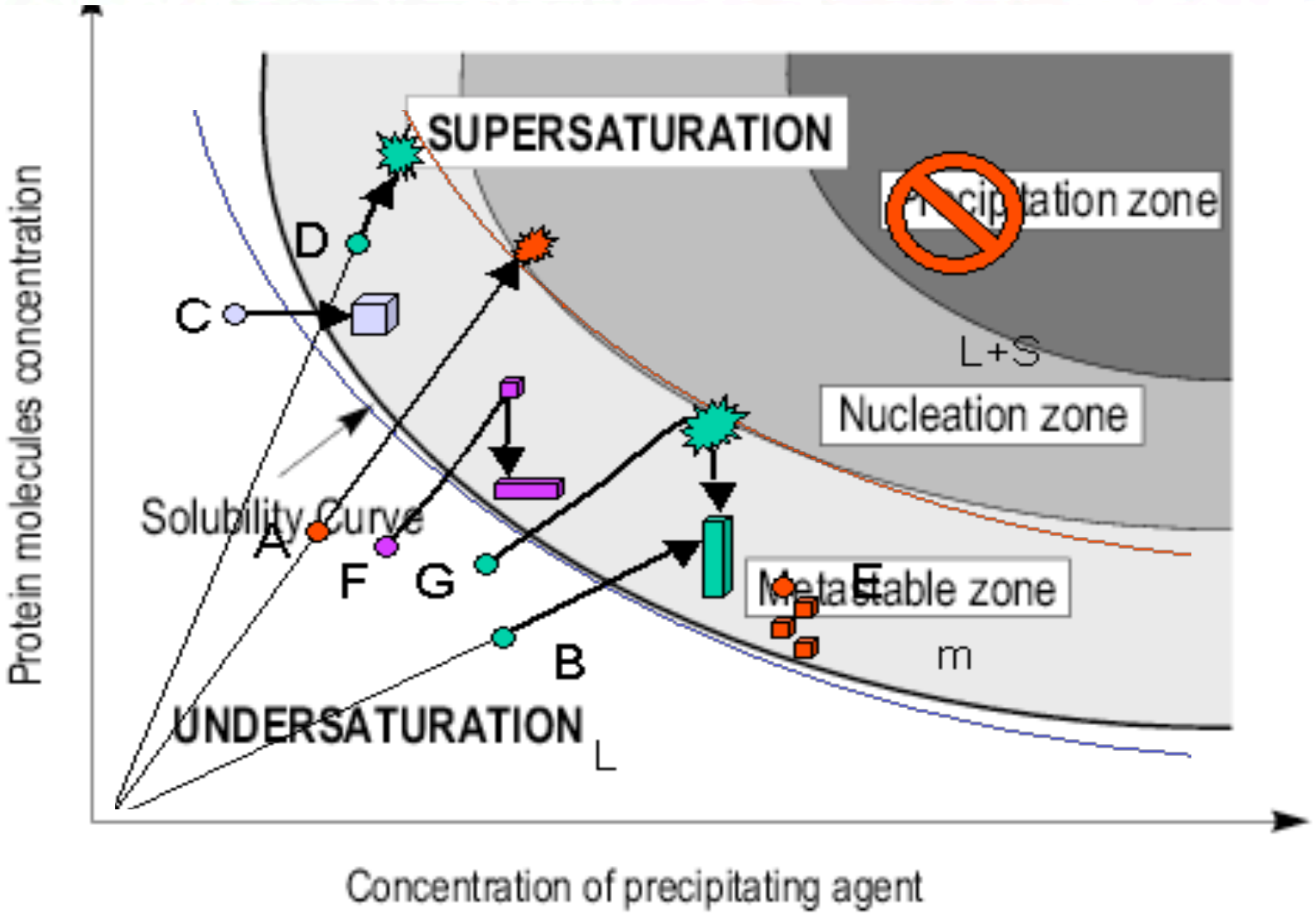
Protein Crystallization – Three major steps

- **Nucleation**: specific interactions necessary for crystal formation are established
- **Crystal growth**: ordered addition of single molecules or ordered aggregates
- **Cessation of growth**: solution is depleted of protein molecules or crystal surfaces become covered by impurities or denatured protein



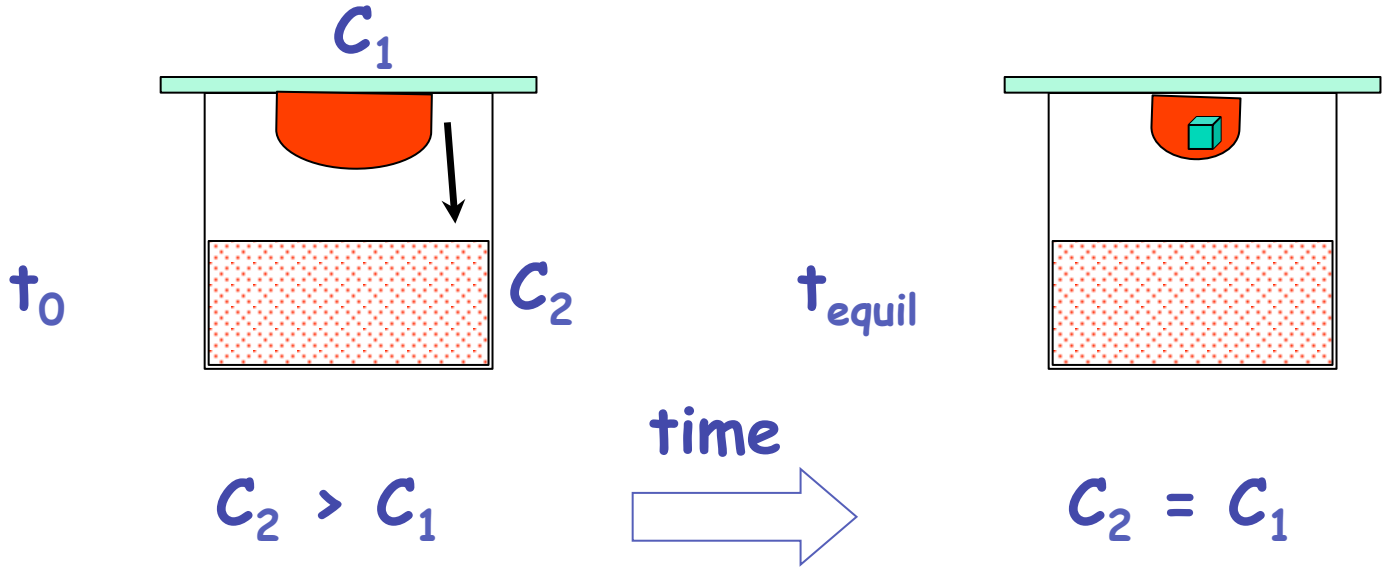


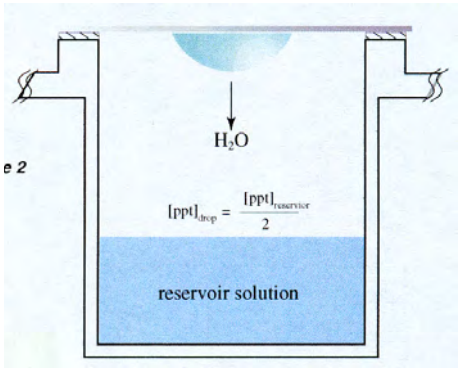
Visualizing the crystallization process – The Phase diagram

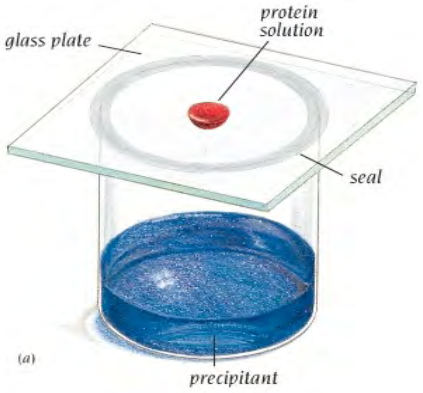


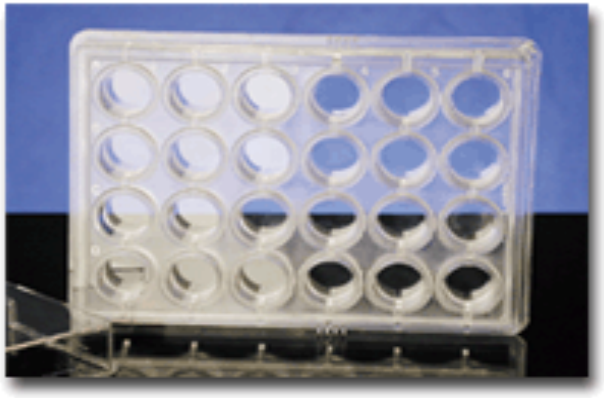
Crystallisation Principle:

Concentration of crystallisation agent in the well C_2 and in the drop C_1. Vapour diffusion









Crystal screen

Tube Number	Salt	Buffer †	Precipitant
1.	0.02 M Calcium Chloride dihydrate	0.1 M Sodium Acetate trihydrate pH 4.6	30% v/v 2-Methyl-2,4-pentanediol
2.	None	None	0.4 M Potassium Sodium Tartrate tetrahydrate
3.	None	None	0.4 M mono-Ammonium dihydrogen Phosphate
4.	None	0.1 M Tris Hydrochloride pH 8.5	2.0 M Ammonium Sulfate
5.	0.2 M tri-Sodium Citrate dihydrate	0.1 M HEPES - Na pH 7.5	30% v/v 2-Methyl-2,4-pentanediol
6.	0.2 M Magnesium Chloride hexahydrate	0.1 M Tris Hydrochloride pH 8.5	30% w/v Polyethylene Glycol 4000
7.	None	0.1 M Sodium Cacodylate pH 6.5	1.4 M Sodium Acetate trihydrate
8.	0.2 M tri-Sodium Citrate dihydrate	0.1 M Sodium Cacodylate pH 6.5	30% v/v iso-Propanol
9.	0.2 M Ammonium Acetate	0.1 M tri-Sodium Citrate dihydrate pH 5.6	30% w/v Polyethylene Glycol 4000
10.	0.2 M Ammonium Acetate	0.1 M Sodium Acetate trihydrate pH 4.6	30% w/v Polyethylene Glycol 4000
11.	None	0.1 M tri-Sodium Citrate dihydrate pH 5.6	1.0 M mono-Ammonium dihydrogen Phosphate
12.	0.2 M Magnesium Chloride hexahydrate	0.1 M HEPES - Na pH 7.5	30% v/v iso-Propanol
13.	0.2 M tri-Sodium Citrate dihydrate	0.1 M Tris Hydrochloride pH 8.5	30% v/v Polyethylene Glycol 400
14.	0.2 M Calcium Chloride dihydrate	0.1 M HEPES - Na pH 7.5	28% v/v Polyethylene Glycol 400
15.	0.2 M Ammonium Sulfate	0.1 M Sodium Cacodylate pH 6.5	30% w/v Polyethylene Glycol 8000
16.	None	0.1 M HEPES - Na pH 7.5	1.5 M Lithium Sulfate monohydrate
17.	0.2 M Lithium Sulfate monohydrate	0.1 M Tris Hydrochloride pH 8.5	30% Polyethylene Glycol 4000
18.	0.2 M Magnesium Acetate tetrahydrate	0.1 M Sodium Cacodylate pH 6.5	20% Polyethylene Glycol 8000
19.	0.2 M Ammonium Acetate	0.1 M Tris Hydrochloride pH 8.5	30% v/v iso-Propanol
20.	0.2 M Ammonium Sulfate	0.1 M Sodium Acetate trihydrate pH 4.6	25% w/v Polyethylene Glycol 4000
21.	0.2 M Magnesium Acetate tetrahydrate	0.1 M Sodium Cacodylate pH 6.5	30% v/v 2-Methyl-2,4-pentanediol
22.	0.2 M Sodium Acetate trihydrate	0.1 M Tris Hydrochloride pH 8.5	30% w/v Polyethylene Glycol 4000
23.	0.2 M Magnesium chloride hexahydrate	0.1 M HEPES - Na pH 7.5	30% v/v Polyethylene Glycol 400
24.	0.2 M Calcium Chloride dihydrate	0.1 M Sodium Acetate trihydrate pH 4.6	20% v/v iso-Propanol
25.	None	0.1 M Imidazole pH 6.5	1.0 M Sodium Acetate trihydrate
26.	0.2 M Ammonium Acetate	0.1 M tri-Sodium Citrate dihydrate pH 5.6	30 % v/v 2-Methyl-2,4-pentanediol
27.	0.2 M tri-Sodium Citrate dihydrate	0.1 M HEPES - Na pH 7.5	20% v/v iso-Propanol
28.	0.2 M Sodium Acetate trihydrate	0.1 M Sodium Cacodylate pH 6.5	30% w/v Polyethylene Glycol 8000
29.	None	0.1 M HEPES - Na pH 7.5	0.8 M Potassium Sodium Tartrate tetrahydrate
30.	0.2 M Ammonium Sulfate	None	30% w/v Polyethylene Glycol 8000
31.	0.2 M Ammonium Sulfate	None	30% w/v Polyethylene Glycol 4000
32.	None	None	2.0 M Ammonium Sulfate
33.	None	None	4.0 M Sodium Formate
34.	None	0.1 M Sodium Acetate trihydrate pH 4.6	2.0 M Sodium Formate
35.	None	0.1 M HEPES - Na pH 7.5	0.8 M mono-Sodium dihydrogen phosphate 0.8 M mono-Potassium dihydrogen phosphate
36.	None	0.1 M Tris Hydrochloride pH 8.5	8% w/v Polyethylene Glycol 8000
37.	None	0.1 M Sodium Acetate trihydrate pH 4.6	8% w/v Polyethylene Glycol 4000
38.	None	0.1 M HEPES - Na pH 7.5	1.4 M tri-Sodium Citrate dihydrate
39.	None	0.1 M HEPES - Na pH 7.5	2% v/v Polyethylene Glycol 400, 2.0 M Ammonium Sulfate
40.	None	0.1 M tri-Sodium Citrate dihydrate pH 5.6	20% v/v iso-Propanol, 20% w/v Polyethylene Glycol 4000
41.	None	0.1 M HEPES - Na pH 7.5	10% v/v iso-Propanol, 20% w/v Polyethylene Glycol 4000
42.	0.05 M mono-Potassium dihydrogen Phosphate	None	20% w/v Polyethylene Glycol 8000
43.	None	None	30% w/v Polyethylene Glycol 1500
44.	None	None	0.2 M Magnesium Formate
45.	0.2 M Zinc Acetate dihydrate	0.1 M Sodium Cacodylate pH 6.5	18% w/v Polyethylene Glycol 8000
46.	0.2 M Calcium Acetate hydrate	0.1 M Sodium Cacodylate pH 6.5	18% w/v Polyethylene Glycol 8000
47.	None	0.1 M Sodium Acetate trihydrate pH 4.6	2.0 M Ammonium Sulfate
48.	None	0.1 M Tris Hydrochloride pH 8.5	2.0 M mono-Ammonium dihydrogen Phosphate
49.	1.0 M Lithium Sulfate monohydrate	None	2% w/v Polyethylene Glycol 8000
50.	0.5 M Lithium Sulfate monohydrate	None	15% w/v Polyethylene Glycol 8000

Table 1

Selected screens commonly used at the SGC

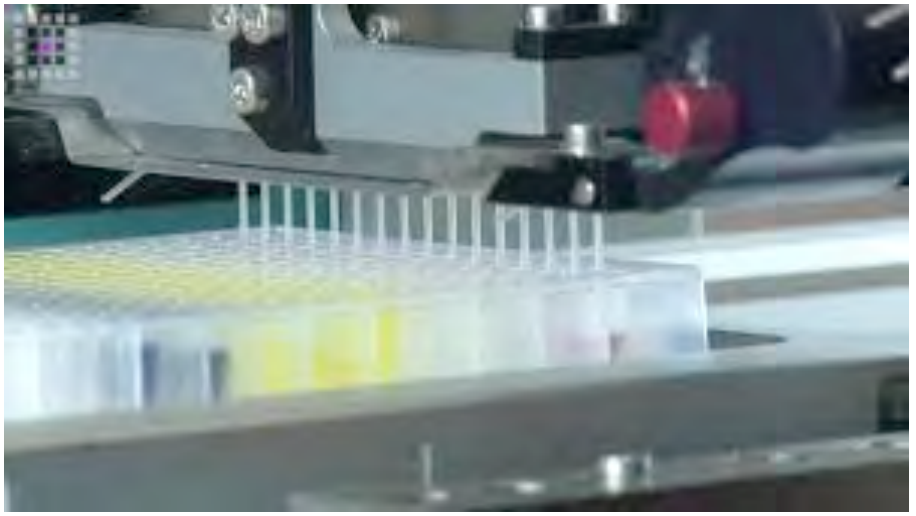
Screen	Abbreviation	Vendor†	Product code
JCSG+	JCSG	MD (modified)	MD1-40
Ligand Friend Screen	LFS	In-house‡	
Crystal Screen HT	HCS	HR	HR2-130
Index	HIN	HR	HR2-134
Basic ChemSpace	BCS	In-house‡	
Modern Intelligent Dynamic Alternative Screen	MIDAS	MD	MD1-60
(Emerald Bio) Precipitant Synergy Screen	EPS	JB	CS-EB-PS-B
Morpheus	MORPHEUS	MD	MD1-47
SaltRX HT	SaltRx	HR	HR2-136
MemGold	MemGold	MD	MD1-41
MemGold2	MemGold2	MD	MD1-64

†MD, Molecular Dimensions; HR, Hampton Research; JB, Jena Bioscience.

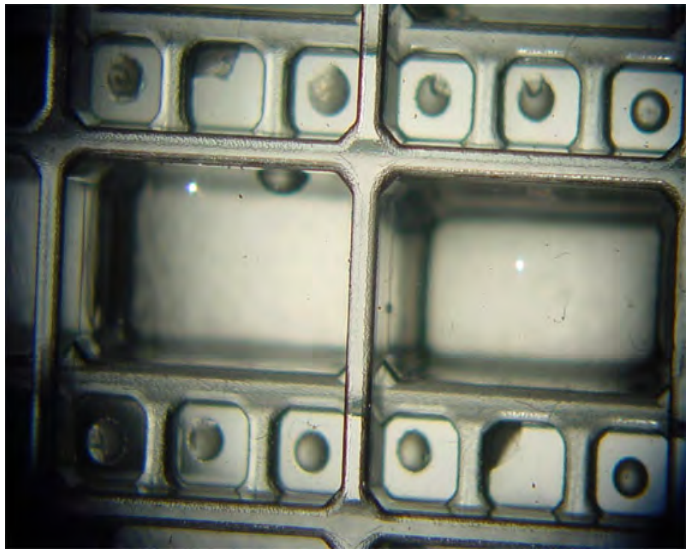
‡In-house indicates design by SGC and formulation by MD. See Supplementary Tables S1 and S2 for the full list of conditions for our modified versions of JCSG+ and LFS.

Automatization of the screen

Nano drop dispensing robot

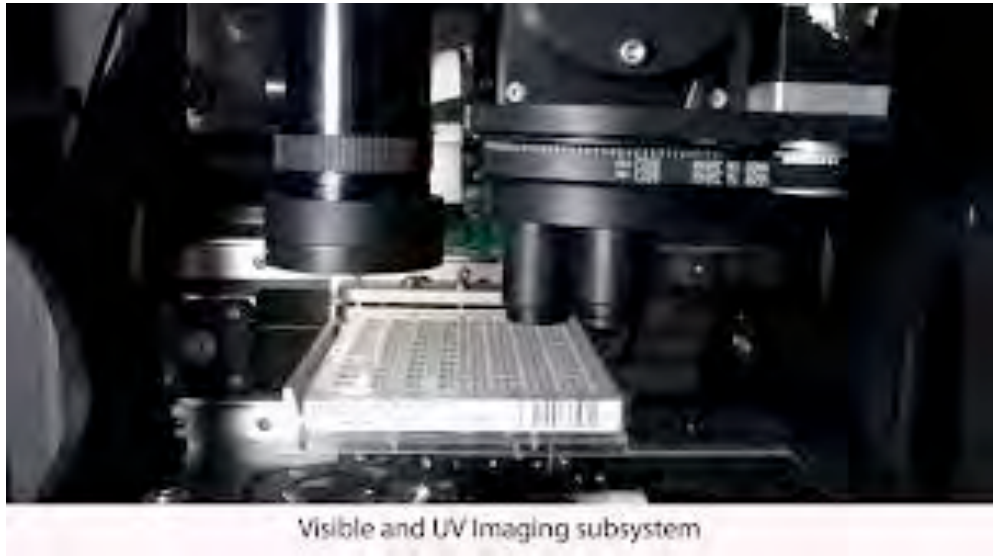


Screen 3 x 96 conditions

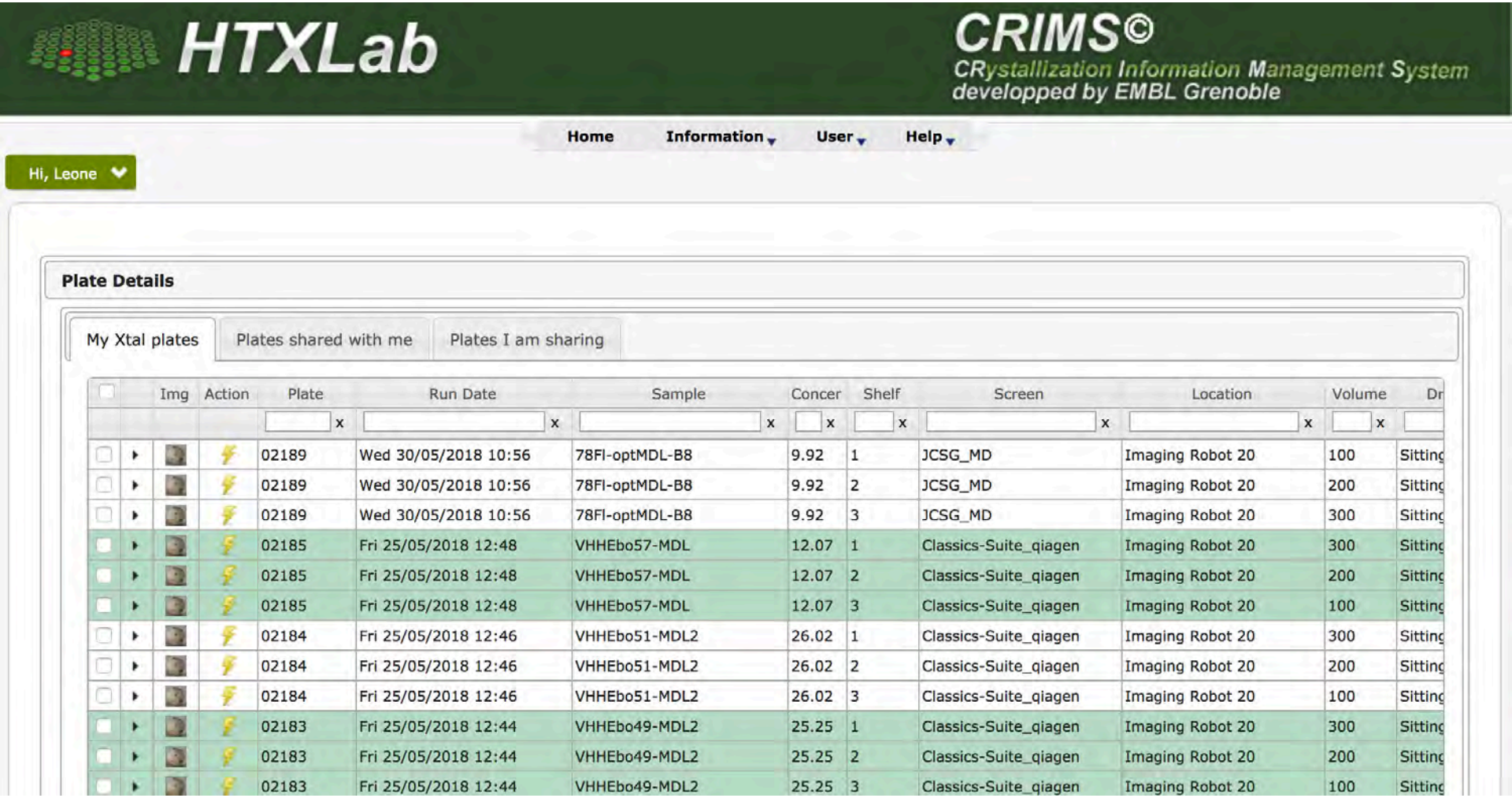


reduction of the size of the drops :
- smaller amount of protein needed
- more conditions tested
- crystallization more rapid (small volume)

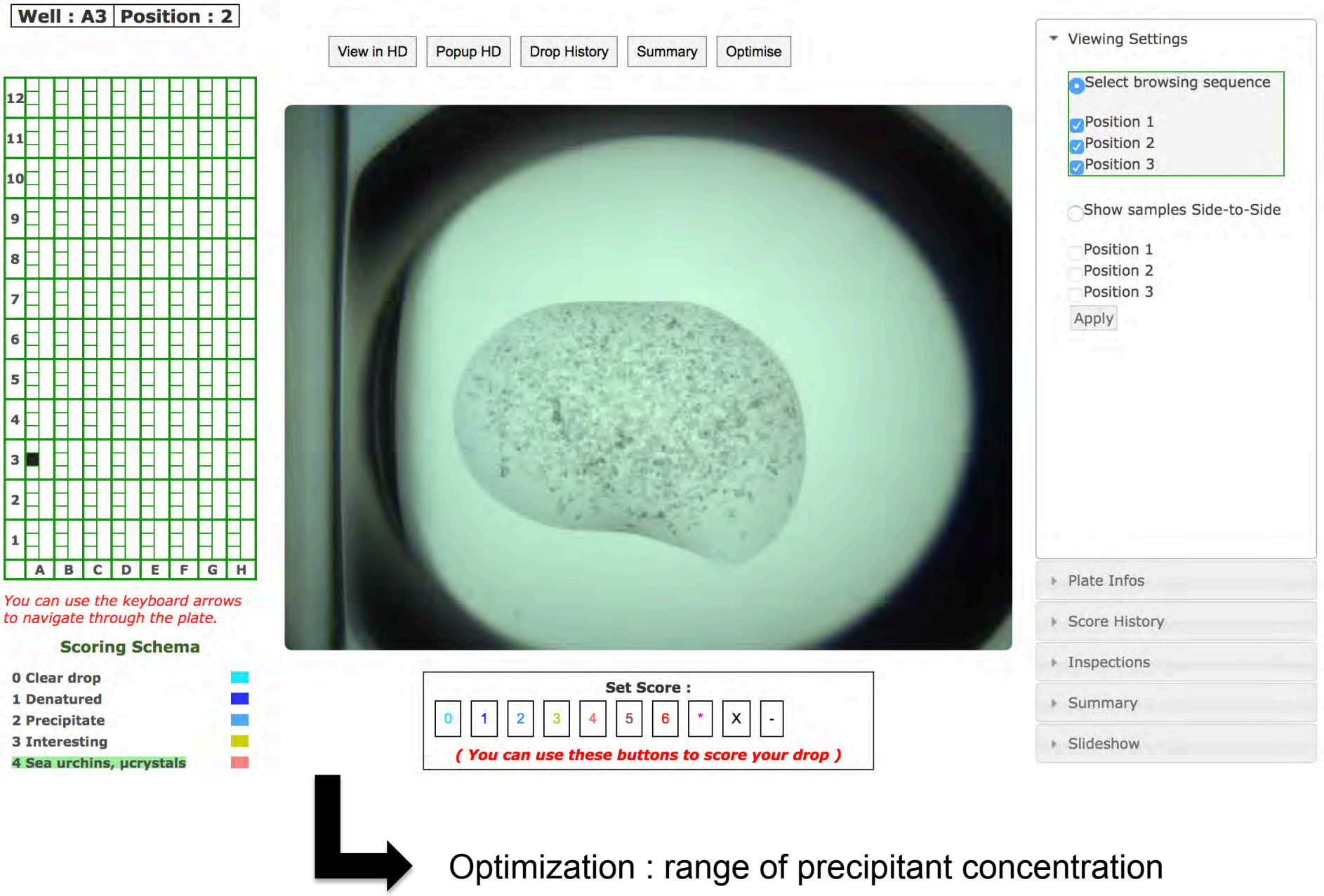
Visualization robot



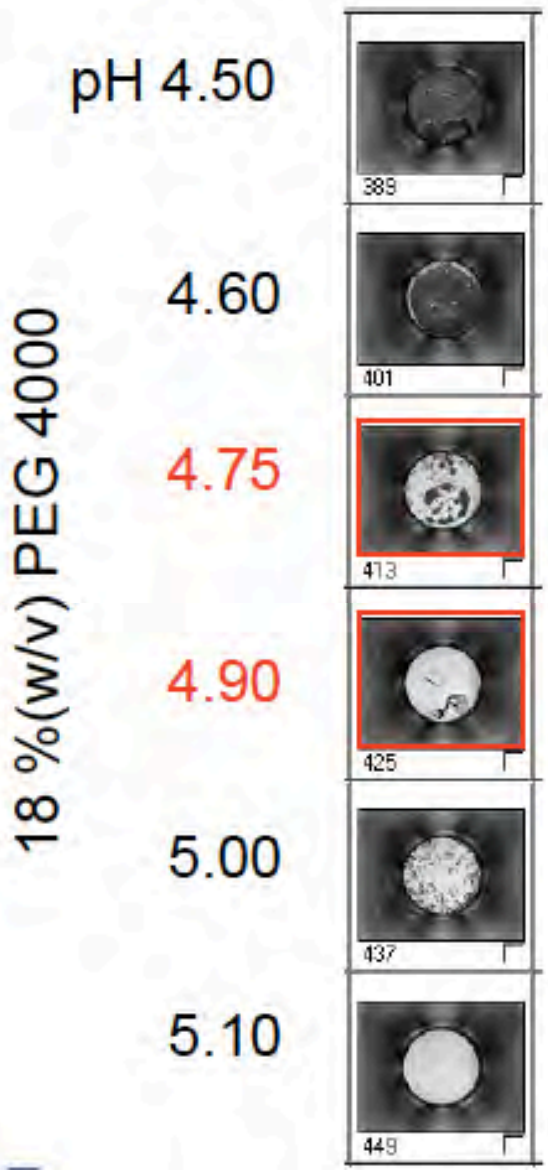
Visible and UV Imaging subsystem



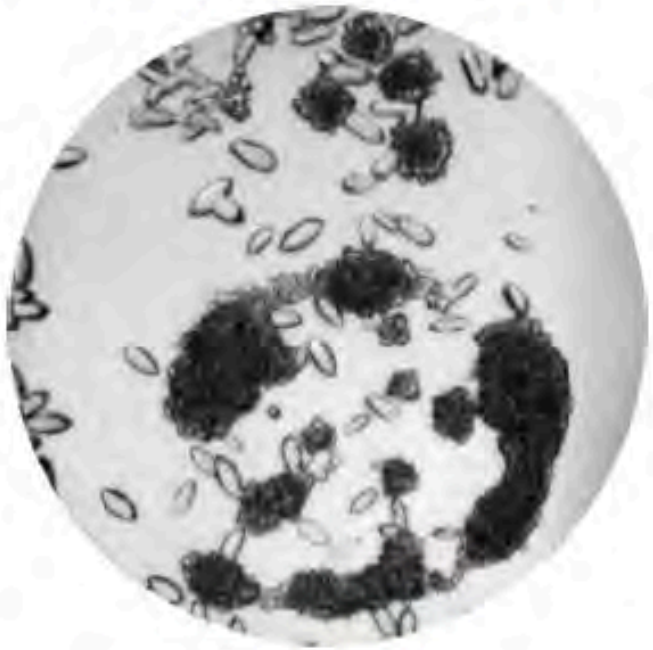
Img	Action	Plate	Run Date	Sample	Concer	Shelf	Screen	Location	Volume	Dr
		02189	Wed 30/05/2018 10:56	78FI-optMDL-B8	9.92	1	JCSG_MD	Imaging Robot 20	100	Sitting
		02189	Wed 30/05/2018 10:56	78FI-optMDL-B8	9.92	2	JCSG_MD	Imaging Robot 20	200	Sitting
		02189	Wed 30/05/2018 10:56	78FI-optMDL-B8	9.92	3	JCSG_MD	Imaging Robot 20	300	Sitting
		02185	Fri 25/05/2018 12:48	VHHEbo57-MDL	12.07	1	Classics-Suite_qiagen	Imaging Robot 20	300	Sitting
		02185	Fri 25/05/2018 12:48	VHHEbo57-MDL	12.07	2	Classics-Suite_qiagen	Imaging Robot 20	200	Sitting
		02185	Fri 25/05/2018 12:48	VHHEbo57-MDL	12.07	3	Classics-Suite_qiagen	Imaging Robot 20	100	Sitting
		02184	Fri 25/05/2018 12:46	VHHEbo51-MDL2	26.02	1	Classics-Suite_qiagen	Imaging Robot 20	300	Sitting
		02184	Fri 25/05/2018 12:46	VHHEbo51-MDL2	26.02	2	Classics-Suite_qiagen	Imaging Robot 20	200	Sitting
		02184	Fri 25/05/2018 12:46	VHHEbo51-MDL2	26.02	3	Classics-Suite_qiagen	Imaging Robot 20	100	Sitting
		02183	Fri 25/05/2018 12:44	VHHEbo49-MDL2	25.25	1	Classics-Suite_qiagen	Imaging Robot 20	300	Sitting
		02183	Fri 25/05/2018 12:44	VHHEbo49-MDL2	25.25	2	Classics-Suite_qiagen	Imaging Robot 20	200	Sitting
		02183	Fri 25/05/2018 12:44	VHHEbo49-MDL2	25.25	3	Classics-Suite_qiagen	Imaging Robot 20	100	Sitting


Well : A3 | Position : 2
View in HD
Popup HD
Drop History
Summary
Optimise
12
11
10
9
8
7
6
5
4
3
2
1
A B C D E F G H
You can use the keyboard arrows to navigate through the plate.
Scoring Schema
0 Clear drop
1 Denatured
2 Precipitate
3 Interesting
4 Sea urchins, µcrystals
Set Score :
0 1 2 3 4 5 6 * X -
(You can use these buttons to score your drop)
Viewing Settings
Select browsing sequence
Position 1
Position 2
Position 3
Show samples Side-to-Side
Position 1
Position 2
Position 3
Apply
Plate Infos
Score History
Inspections
Summary
Slideshow
Optimization : range of precipitant concentration

Fine-sampling of pH

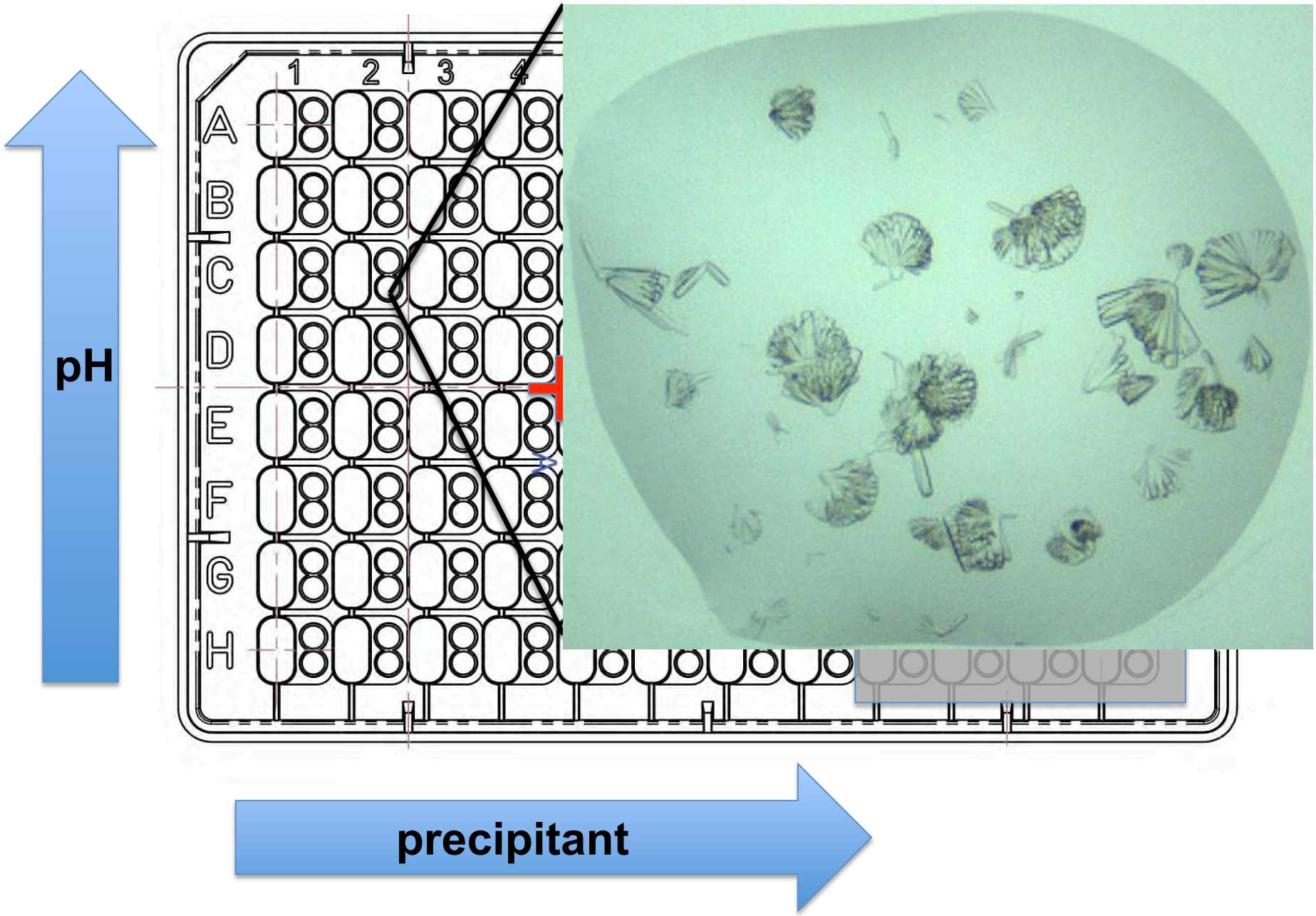


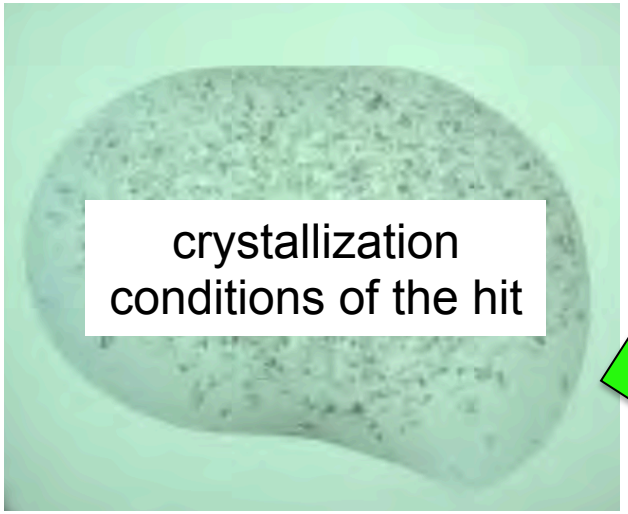
pH 4.75



pH 4.90

8x8 optimization matrix

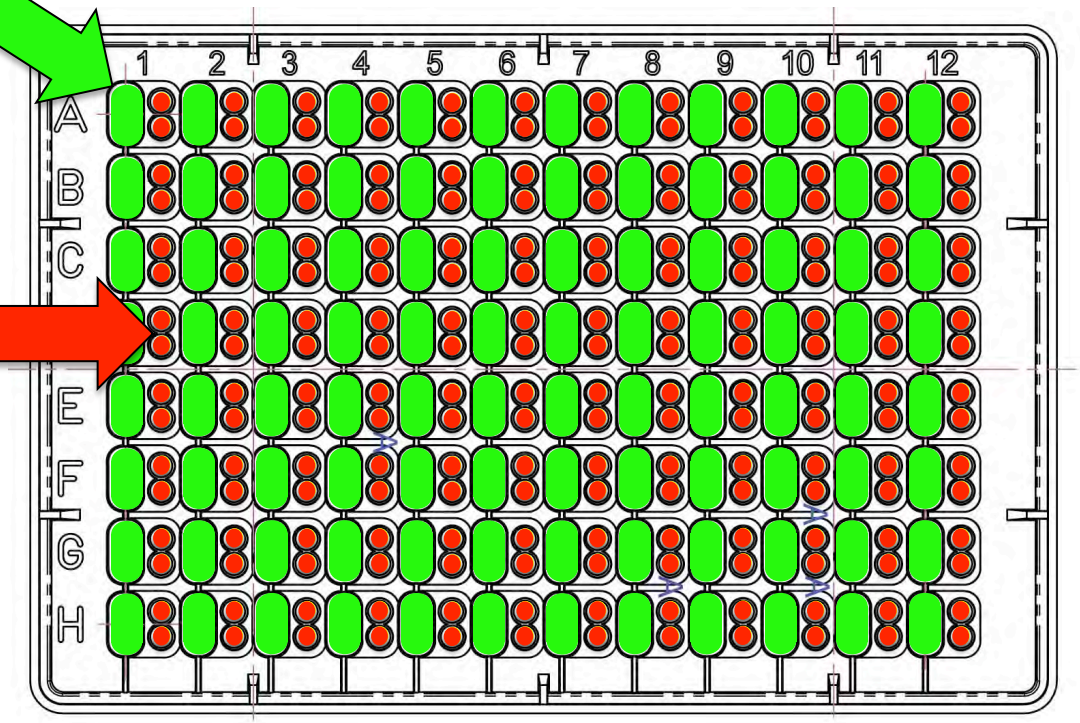


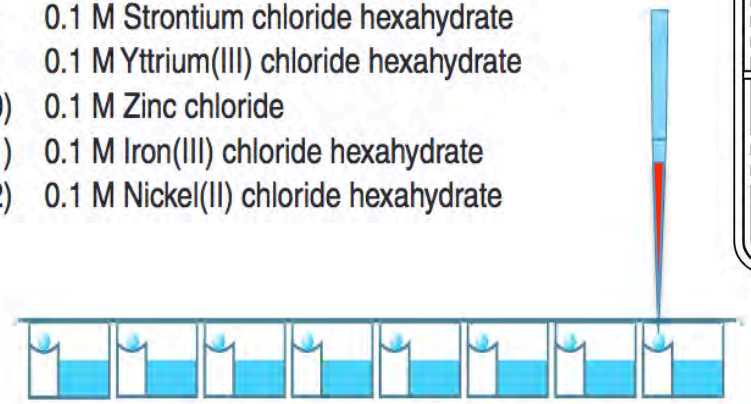


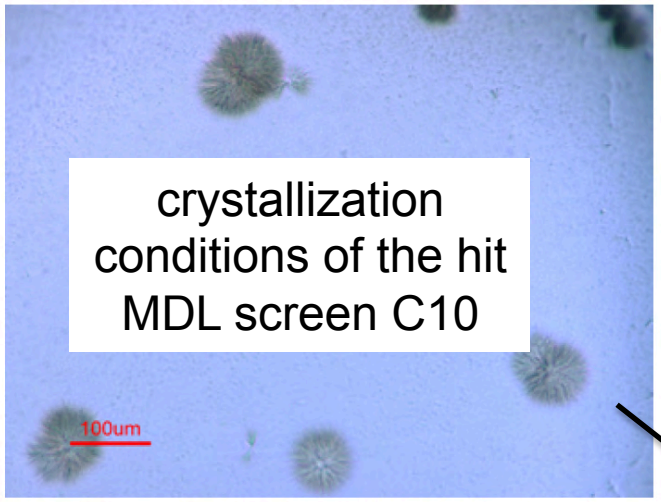
additive screen

Additive Screen HT is a kit designed to allow rapid and convenient evaluation of 96 unique additives and their ability to influence the crystallization of the sample.

1. (A1) 0.1 M Barium chloride dihydrate
2. (A2) 0.1 M Cadmium chloride hydrate
3. (A3) 0.1 M Calcium chloride dihydrate
4. (A4) 0.1 M Cobalt(II) chloride hexahydrate
5. (A5) 0.1 M Copper(II) chloride dihydrate
6. (A6) 0.1 M Magnesium chloride hexahydrate
7. (A7) 0.1 M Manganese(II) chloride tetrahydrate
8. (A8) 0.1 M Strontium chloride hexahydrate
9. (A9) 0.1 M Yttrium(III) chloride hexahydrate
10. (A10) 0.1 M Zinc chloride
11. (A11) 0.1 M Iron(III) chloride hexahydrate
12. (A12) 0.1 M Nickel(II) chloride hexahydrate



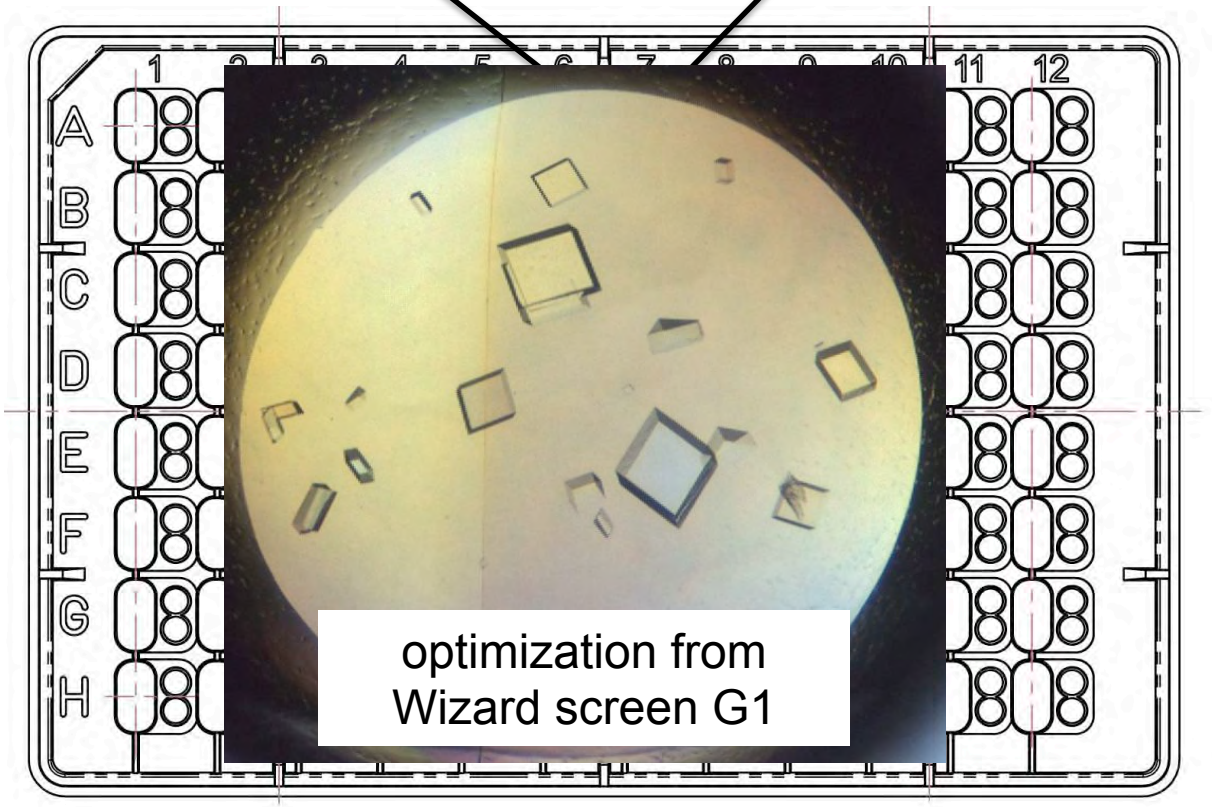


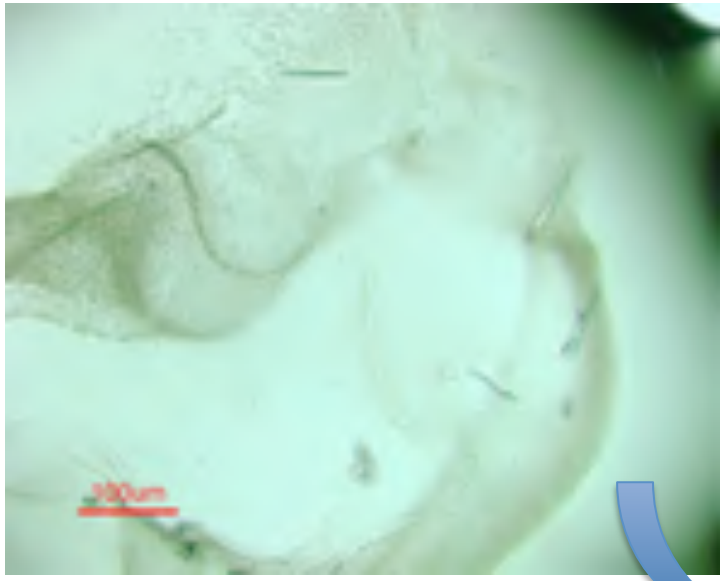


Monalysin

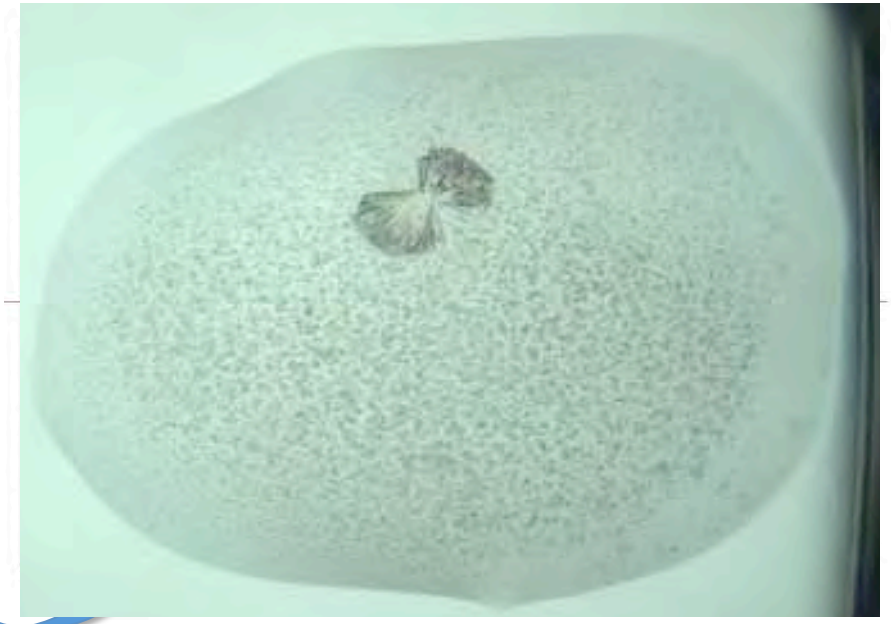
re-screen

2/3 + 1/3





H4 morpheus



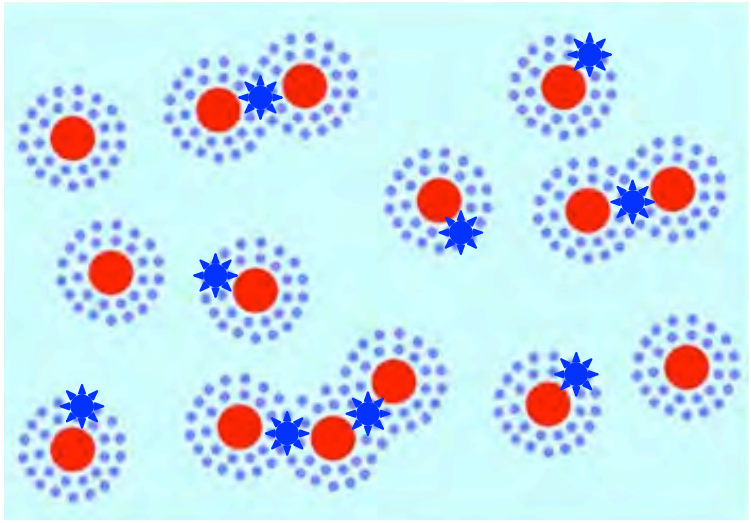
E4 morpheus

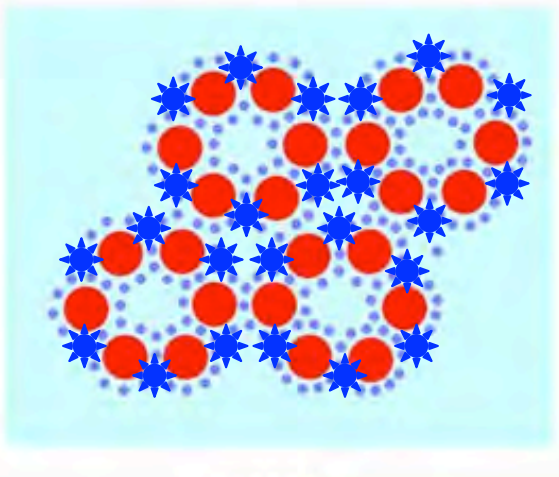
Microseed matrix seeding

The use of seeding has been extended by the microseed matrix screening (MMS) approach, in which seeds are systematically transferred into new conditions to promote crystal growth (Ireton & Stoddard, 2004)

F4 LMB

Crystallization chaperones





Gd-DOTA

Crystallophore

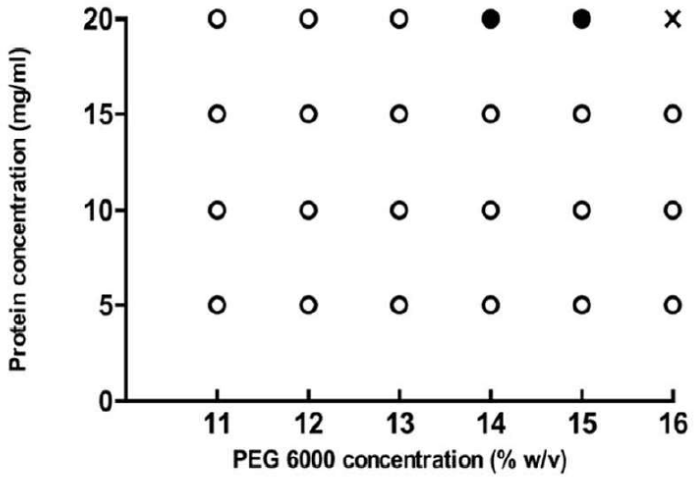
Crystallophore is a cationic lanthanide complex that :

- promotes crystallization
- highly facilitates the structure determination process

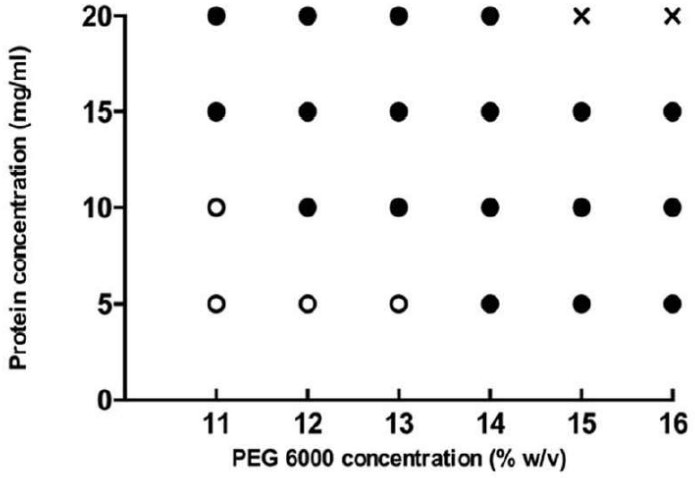
Engilberge et al. (2017) Chemical Science
Vogeli et al. (2018) PNAS

Crystallophore

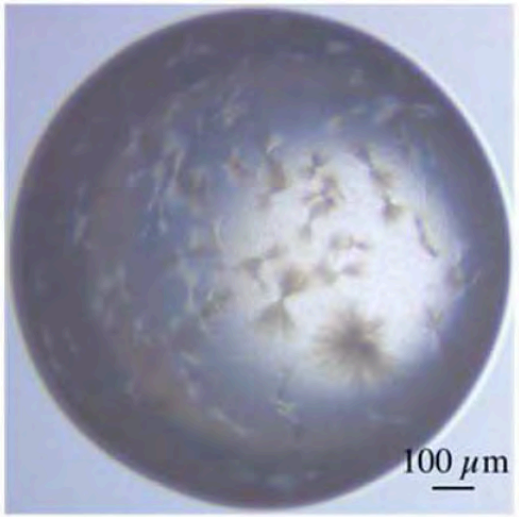
Without Tb-Xo4



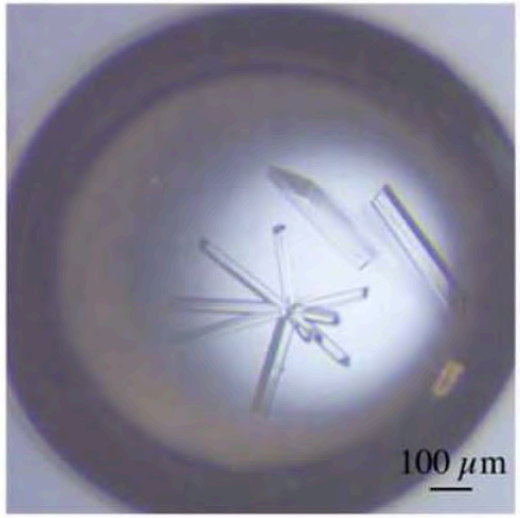
With Tb-Xo4



Native pb6

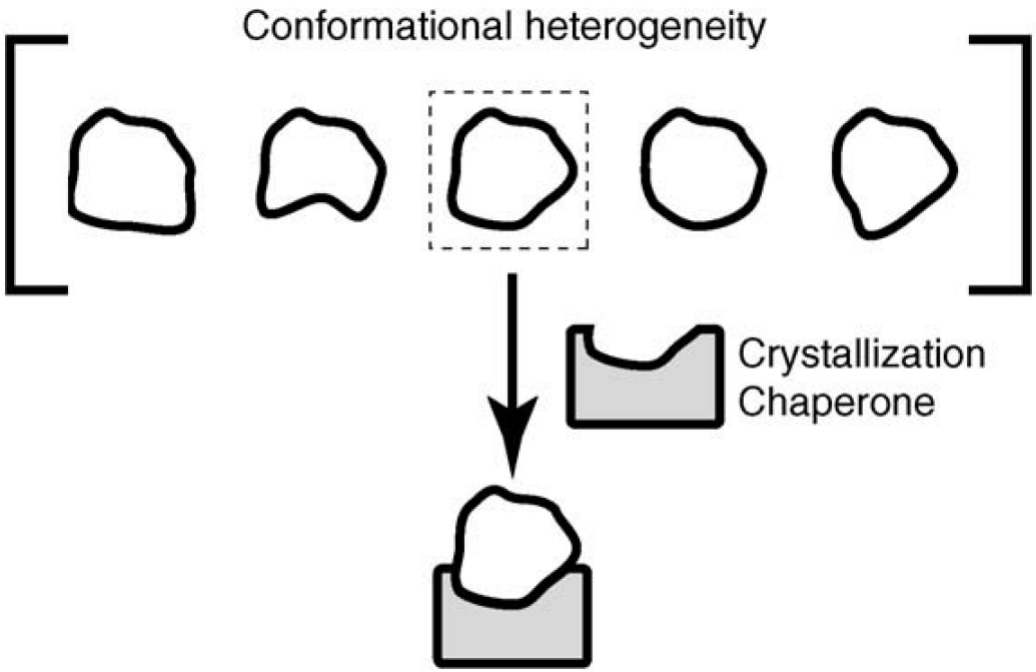


pb6 with Tb-Xo4

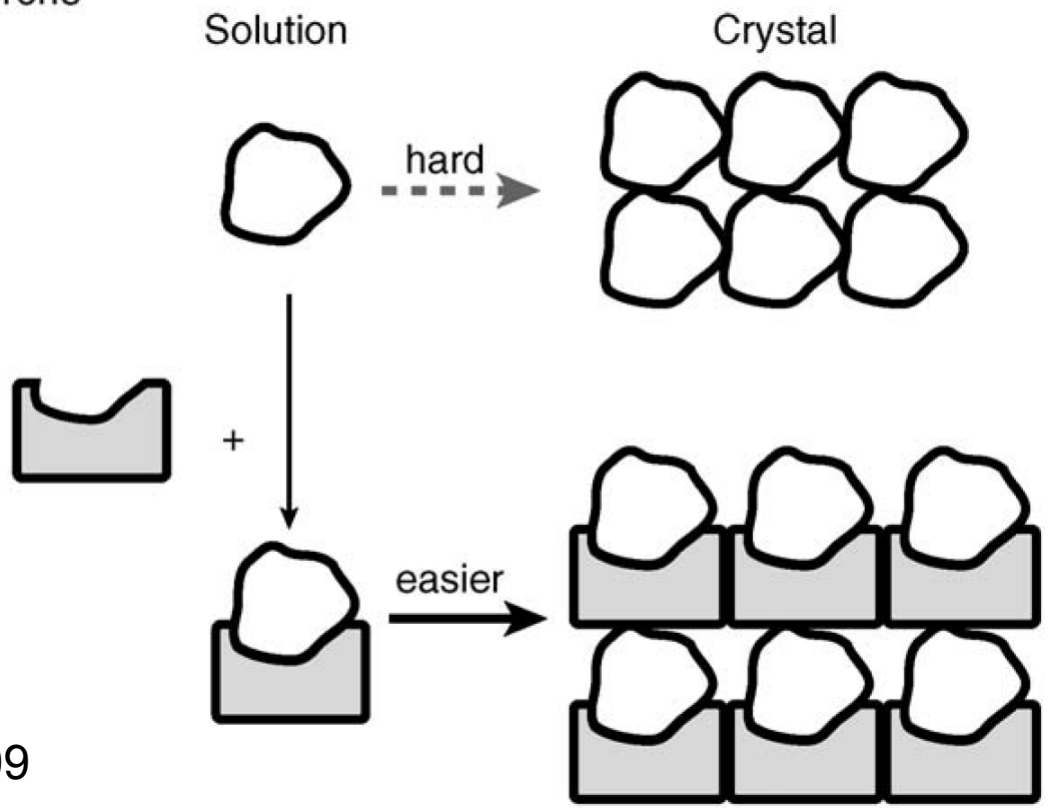


Engilberge et al. (2017) Chemical Science
Vogeli et al. (2018) PNAS

Recombinant crystallization chaperones



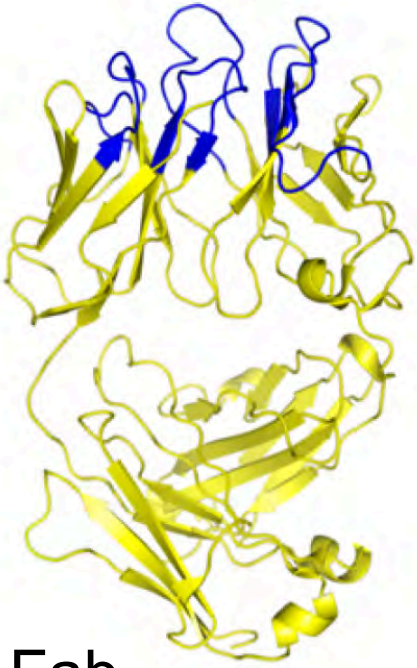
The strategy to increase the probability of obtaining well-ordered crystals is:
first, minimizing the conformational heterogeneity in the target by binding to a specific conformation



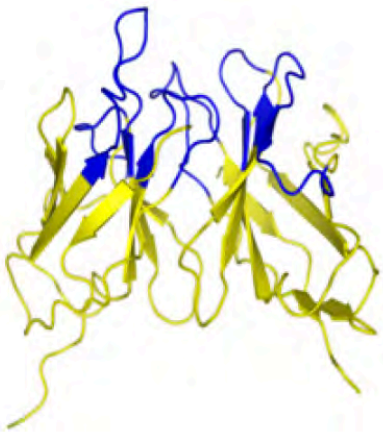
second, supplementing the amount of protein surface that can facilitate primary contacts between molecules in the crystal lattice.

Koide, COSB, 2009

Recombinant crystallization chaperones

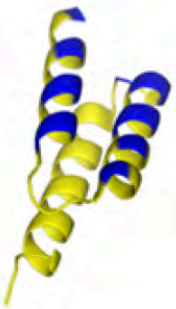


Fab

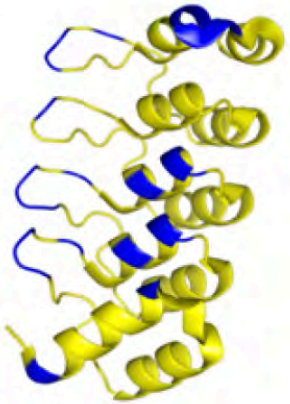


scFv

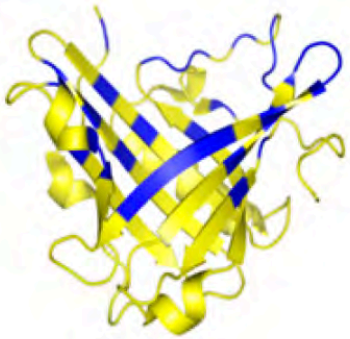
Nanobody



Affibody



DARPIN

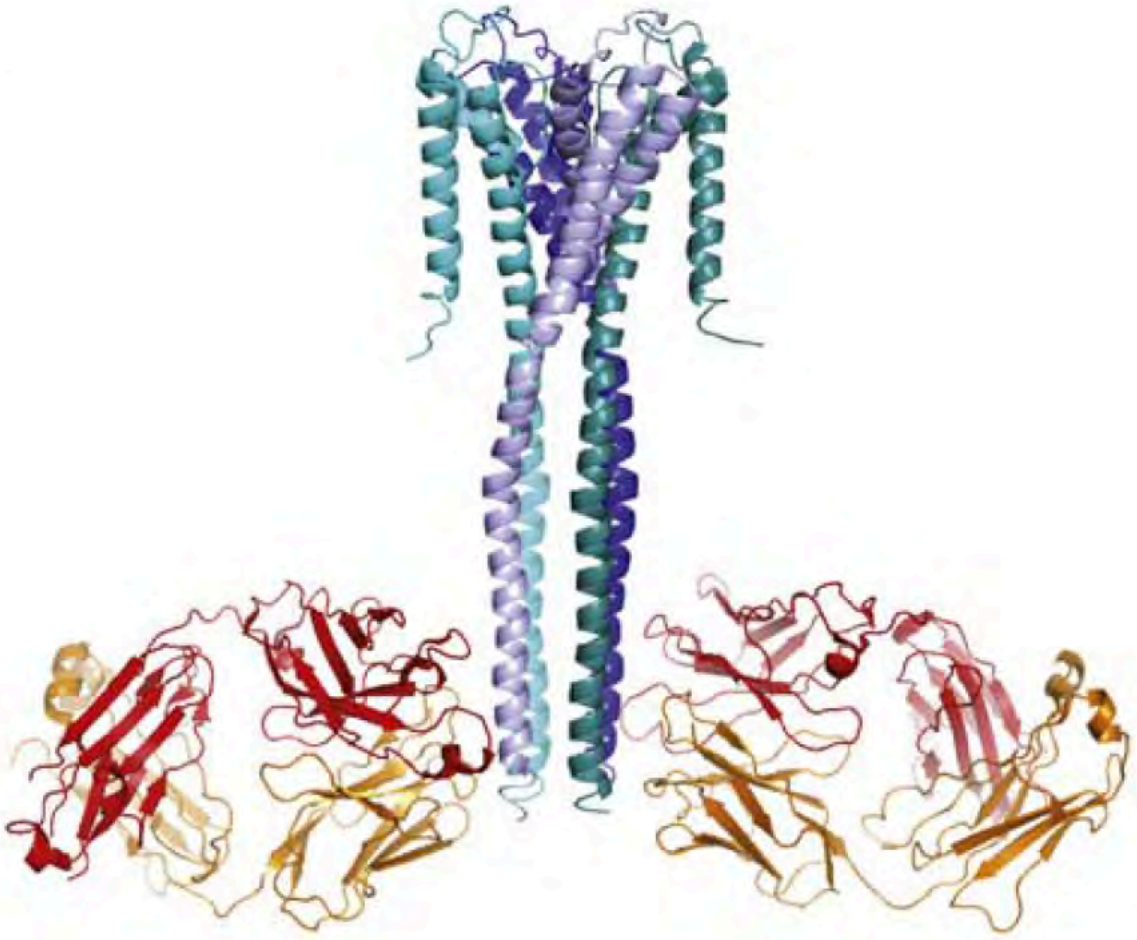


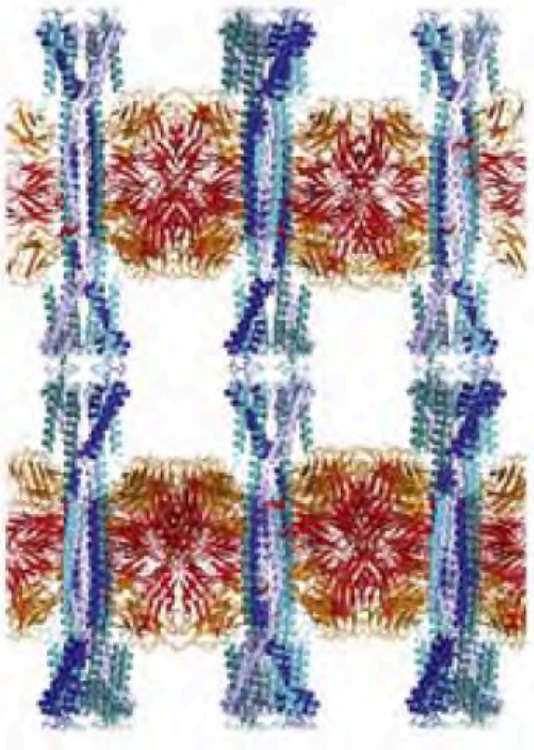
Anticalin

FN3

Lieberman et al, Methods, 2011

Recombinant crystallization chaperones: Fab

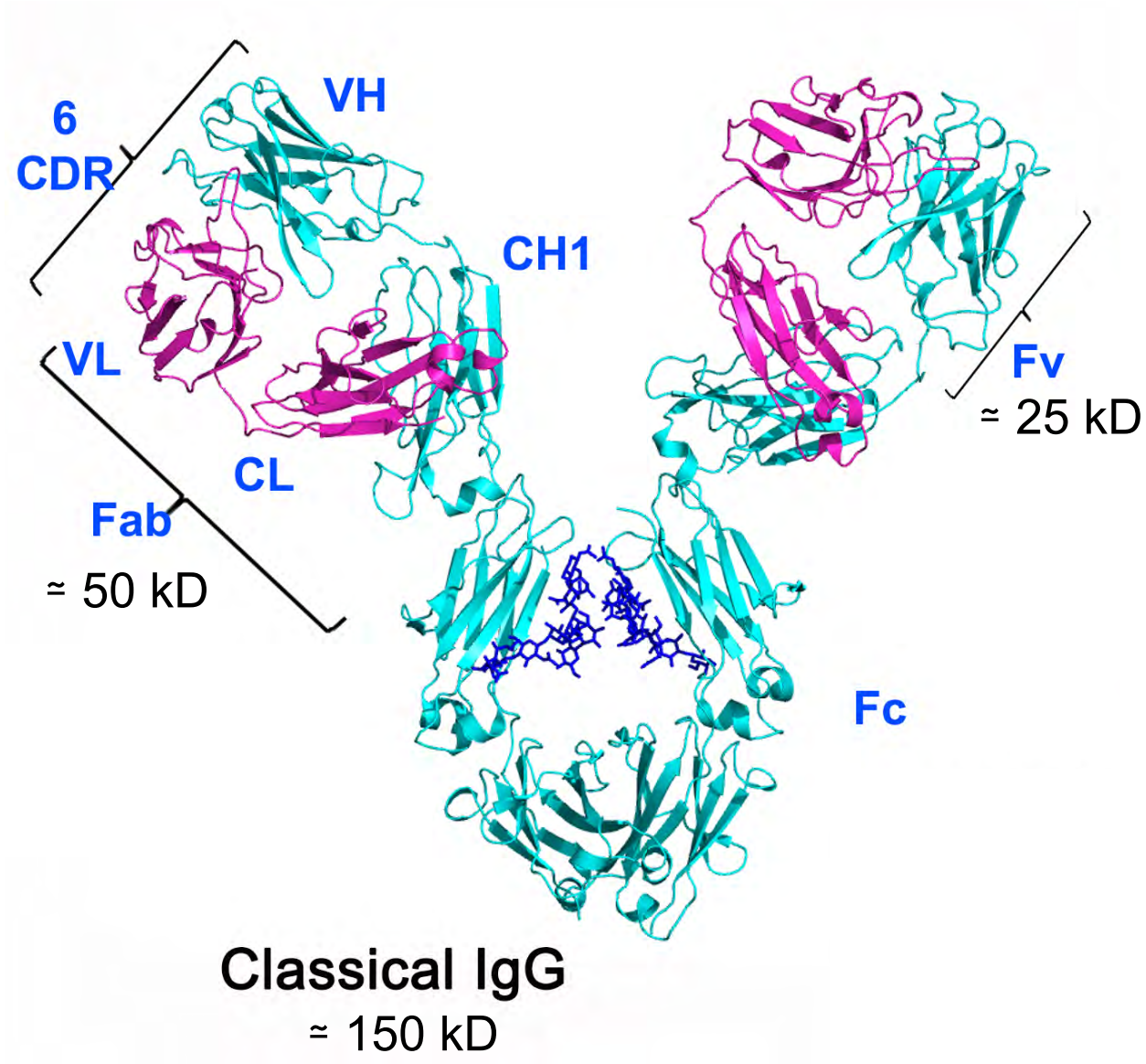




Full-length KcsA with a synthetic Fab (PDB 3EFF)

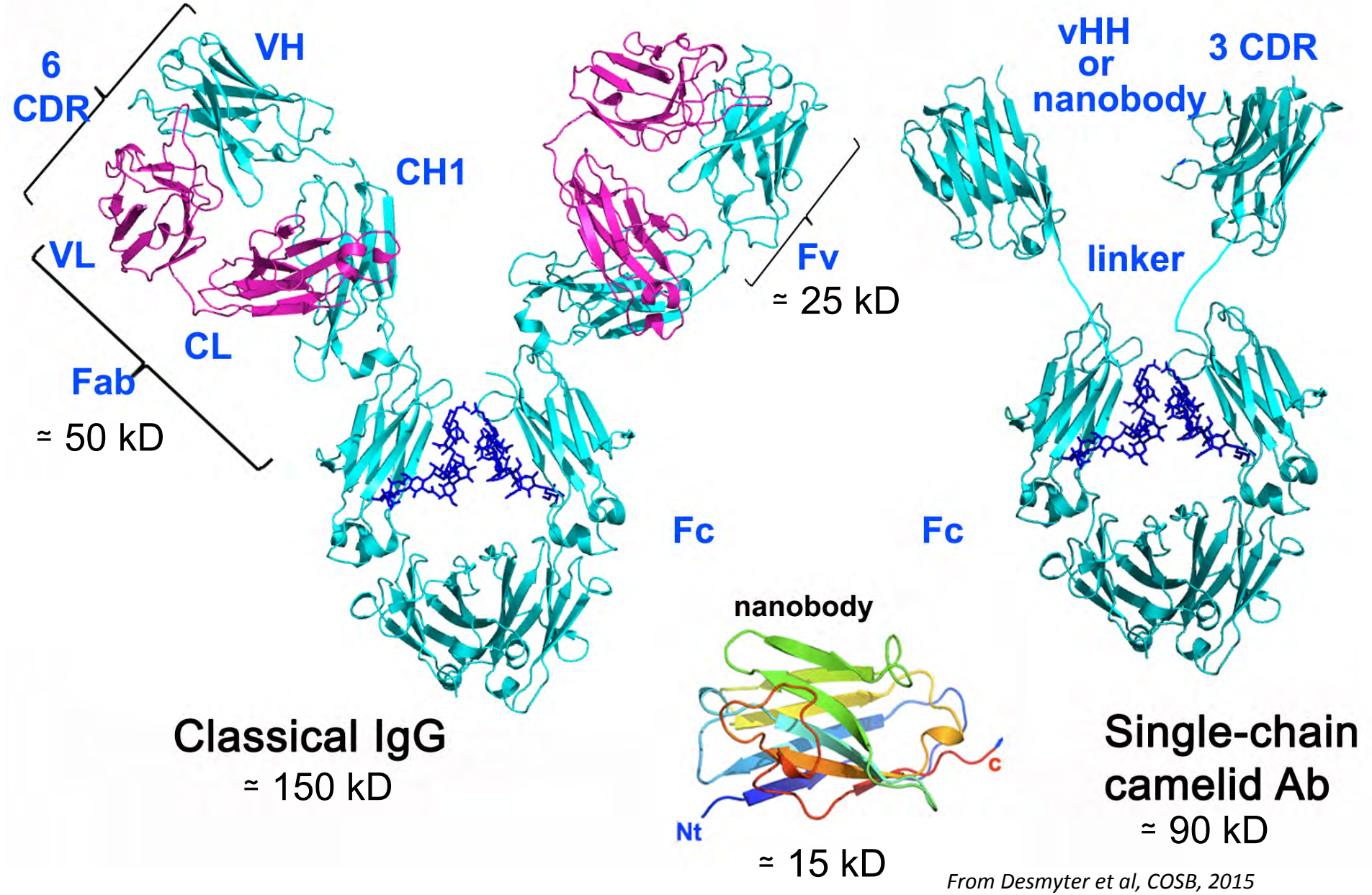
Koide, COSB, 2009

Heavy chain-only antibodies (HcAbs)



From Desmyter et al, COSB, 2015

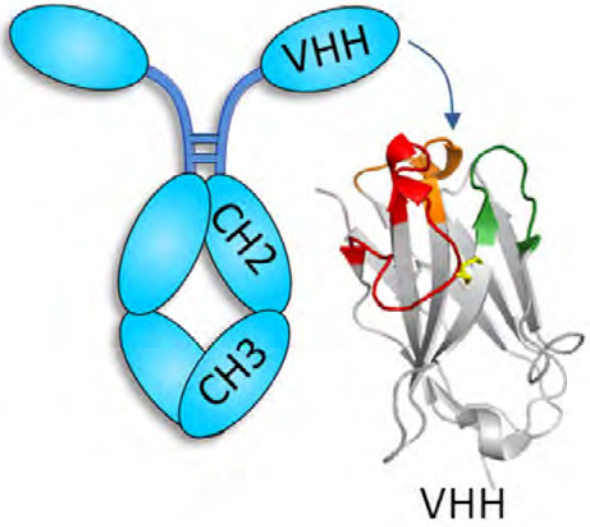
Heavy chain-only antibodies (HcAbs)



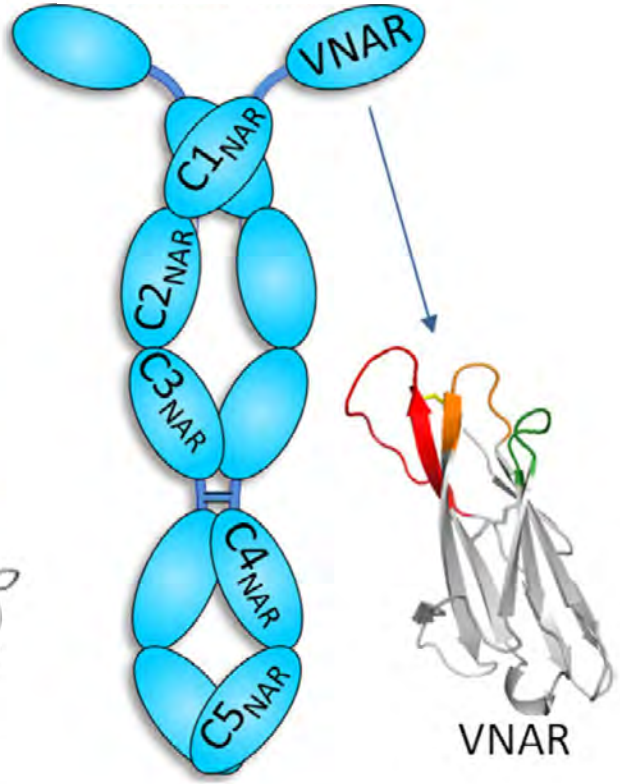
From Desmyter et al, COSB, 2015

Heavy chain-only antibodies (HcAbs)

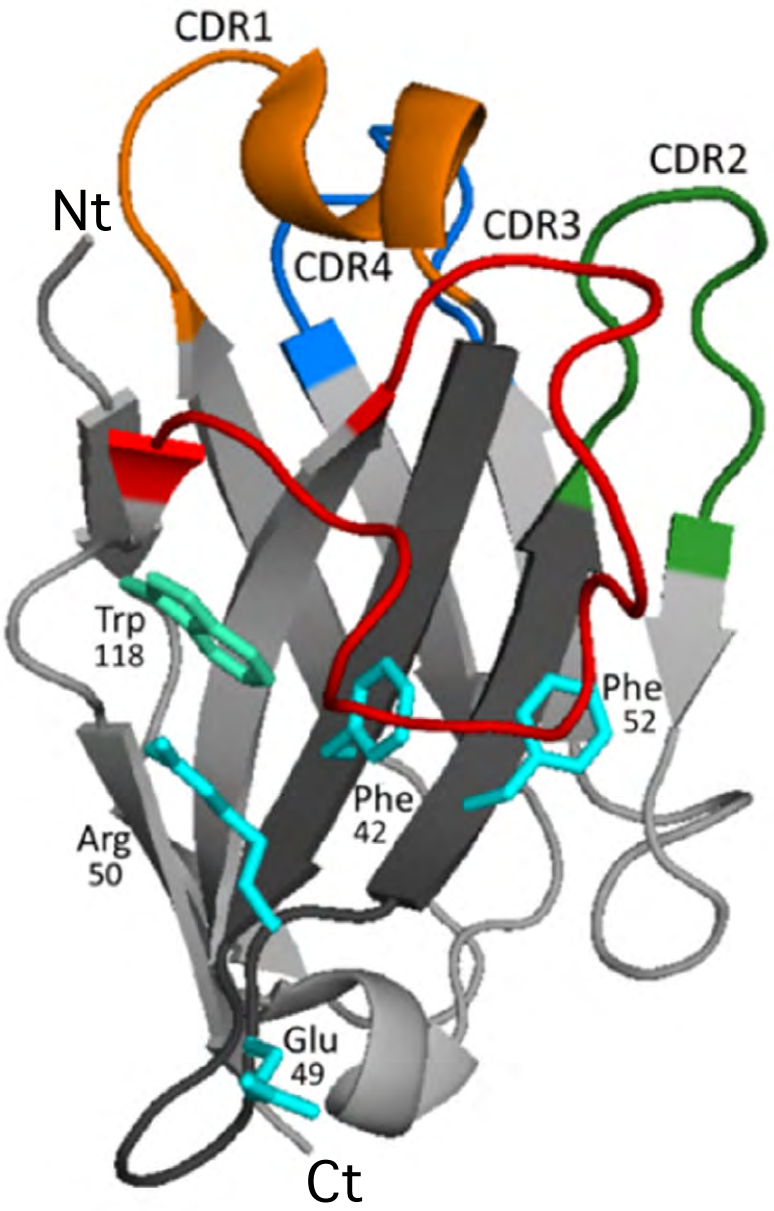
Camelid HcAb



Shark HcAb



Structure and key characteristics of the nanobody domain



Structure of nanobodies:

✔ monomeric Ig domain of ~120 residues
✔ one conserved disulphide bond
✔ framework mutations in residues involved in the VH-VL interaction (G49E, L50R)

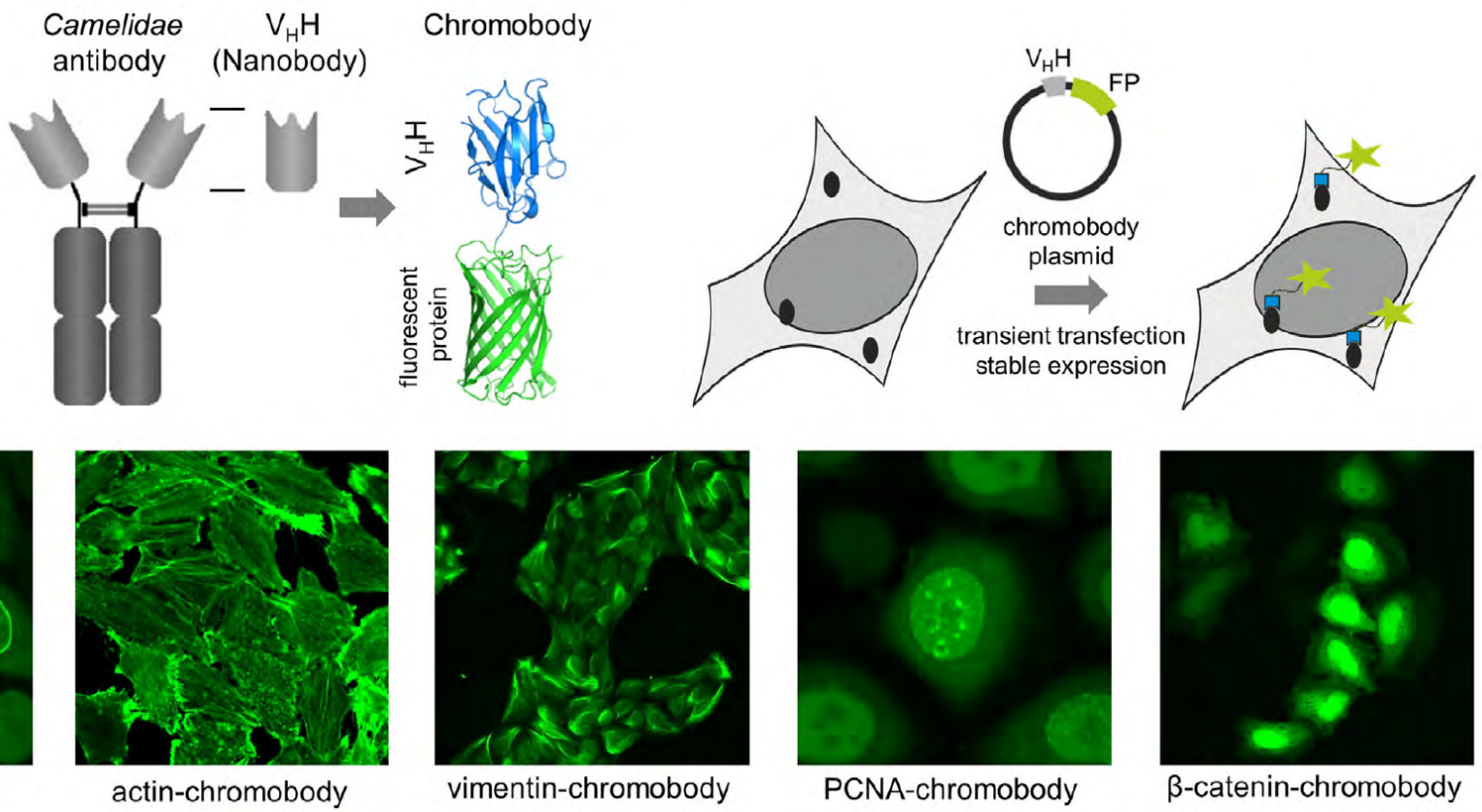
Key characteristics of nanobodies:

✔ high **affinity** and **specificity** (equivalent to conventional antibodies)
✔ high **thermostability**
✔good **solubility** and strictly **monomeric** behavior
✔ **small size** (2.5 nm in diameter and about 4 nm in length; ~15 kDa)
✔ relatively low production cost
✔ ease of genetic engineering, format flexibility or **modularity**
✔ low immunogenicity, and a higher penetration rate into tissues

From Gonzales-Sapienza et al, Front. Immun., 2017

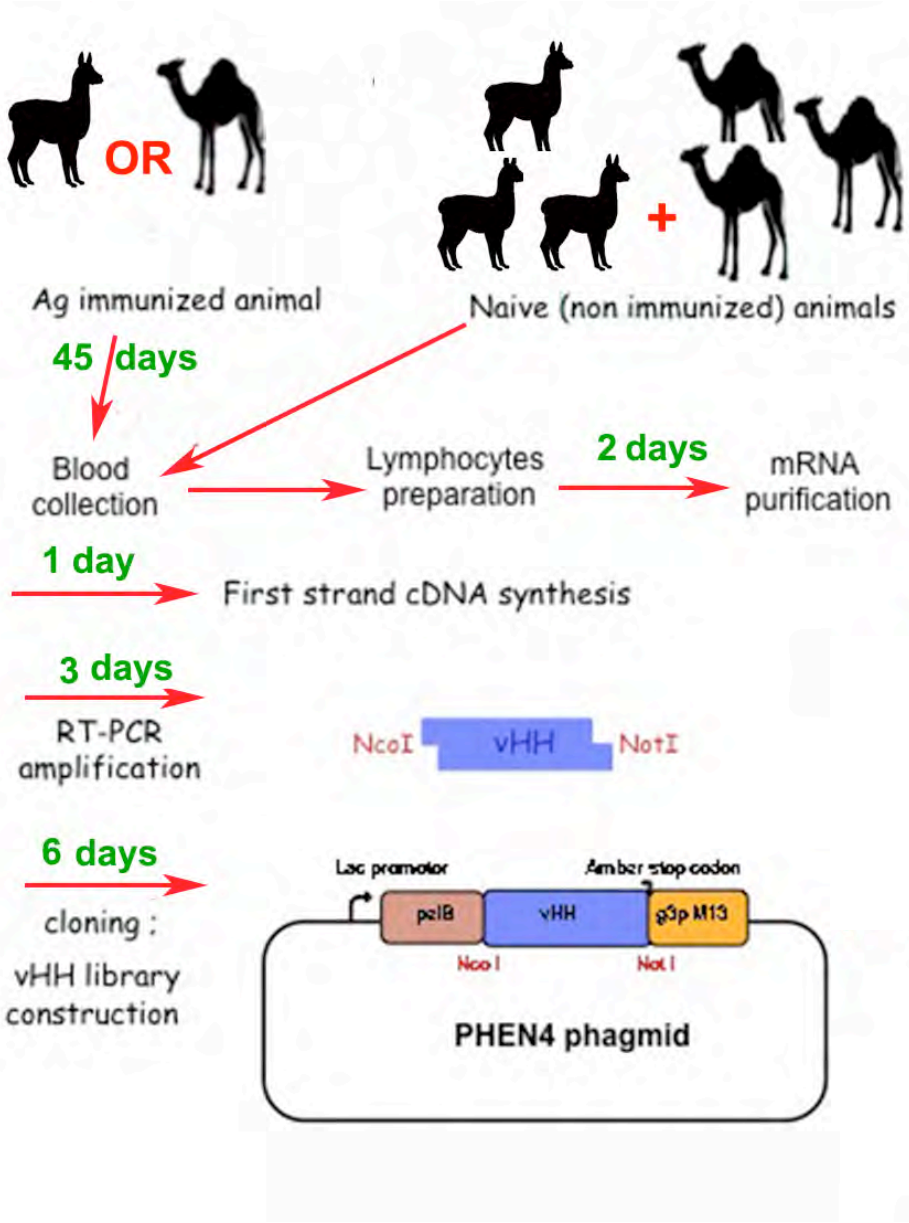
Live-cell imaging

Nanobodies are ideally suited for cytosolic expression due to their ability to fold in the reducing intracellular environment.



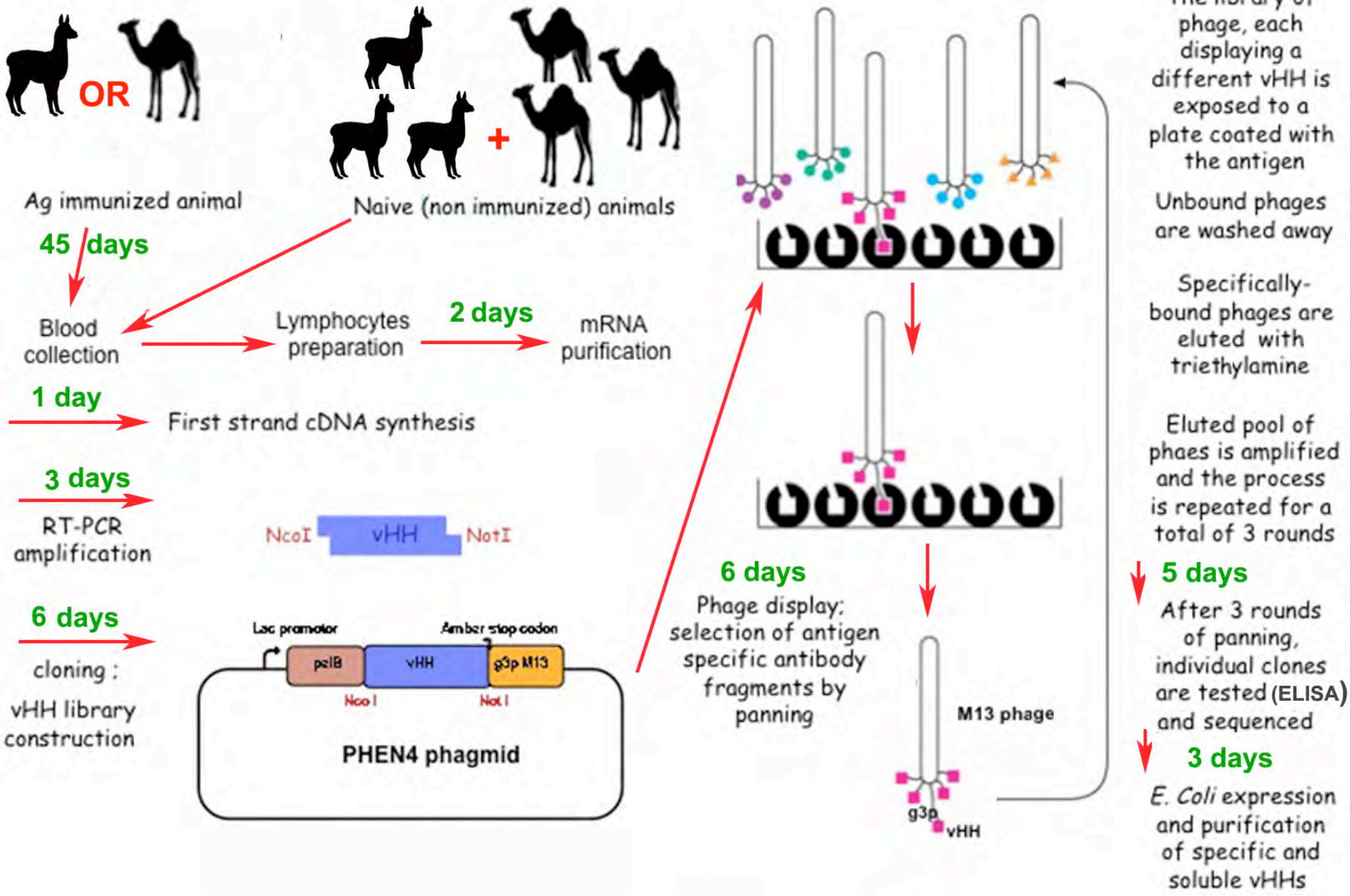
from Traenkle and Rothbauer, Front. Immun., 2017

Selection and production of camelid VHH/nanobodies @AFMB

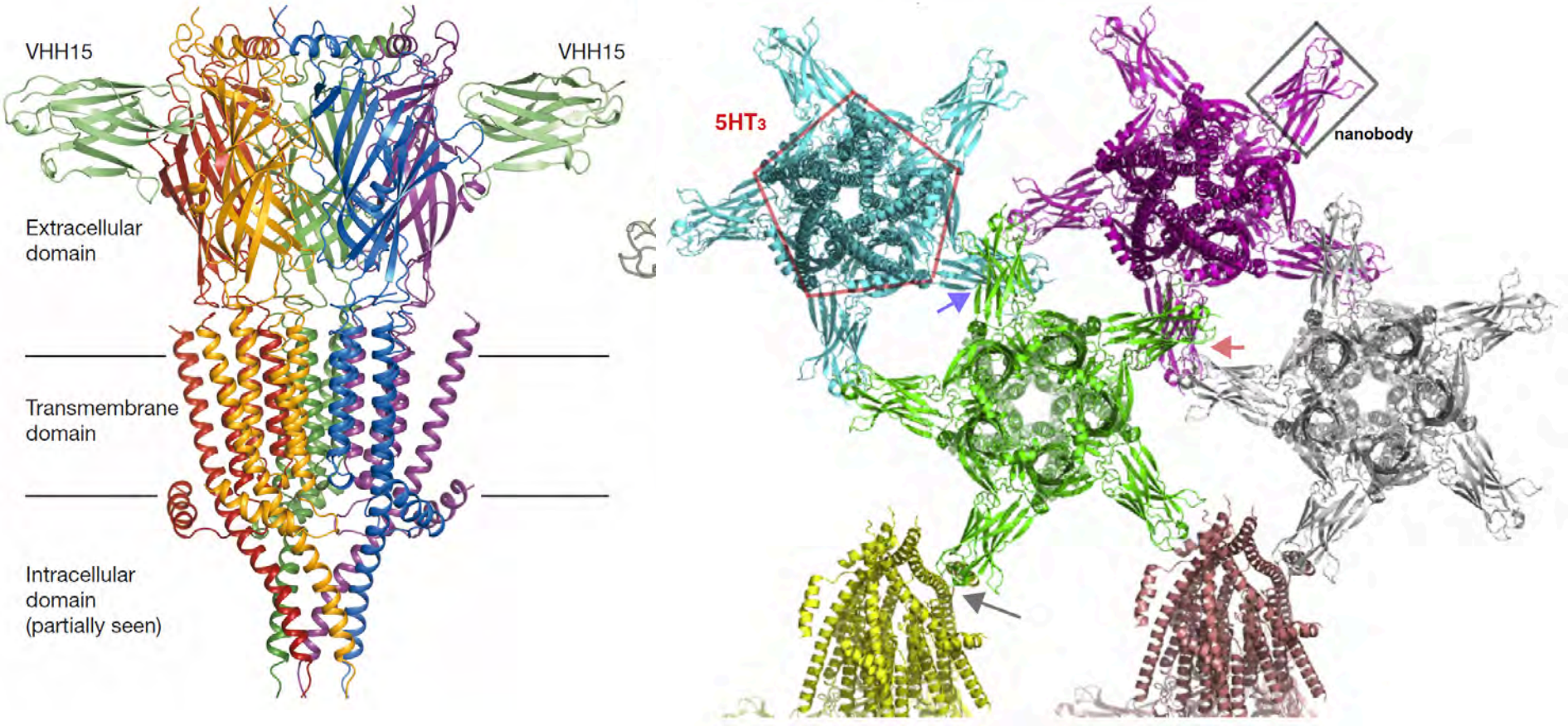


From Desmyter et al, COSB, 2015

Selection and production of camelid VHH/nanobodies @AFMB

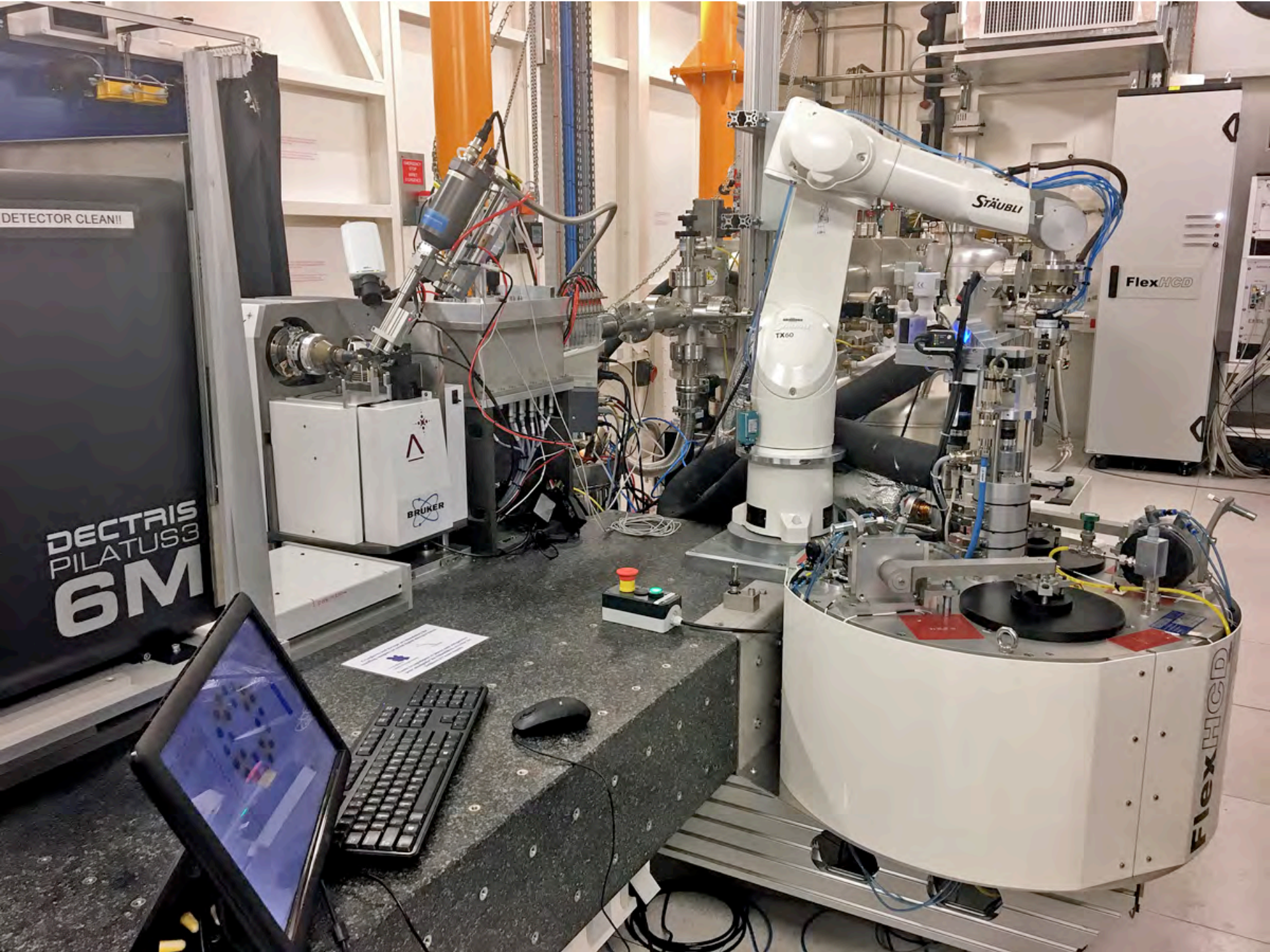


From Desmyter et al, COSB, 2015



Crystal structure of the mouse serotonin 5-HT3 receptor

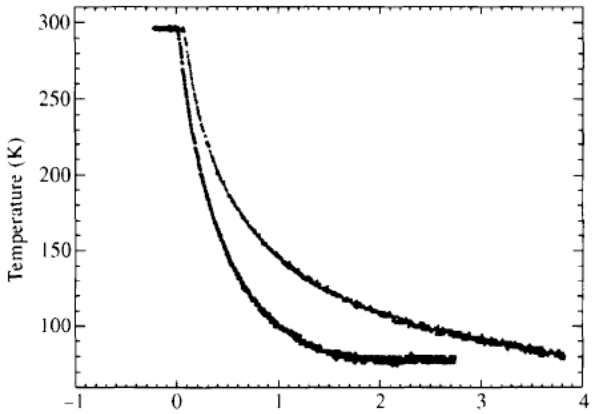
Hassaine et al, Nature, 2014


DETECTOR CLEAN!!
DECTRIS
PILATUS3
6M
BRUKER
STÄUBLI
TX60
FlexHCD
FlexHCD

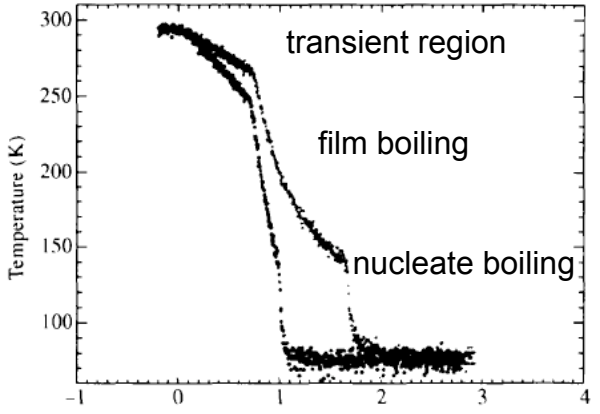
J. Appl. Cryst. (1998). **31**, 252–257

Cooling Rates During Flash Cooling

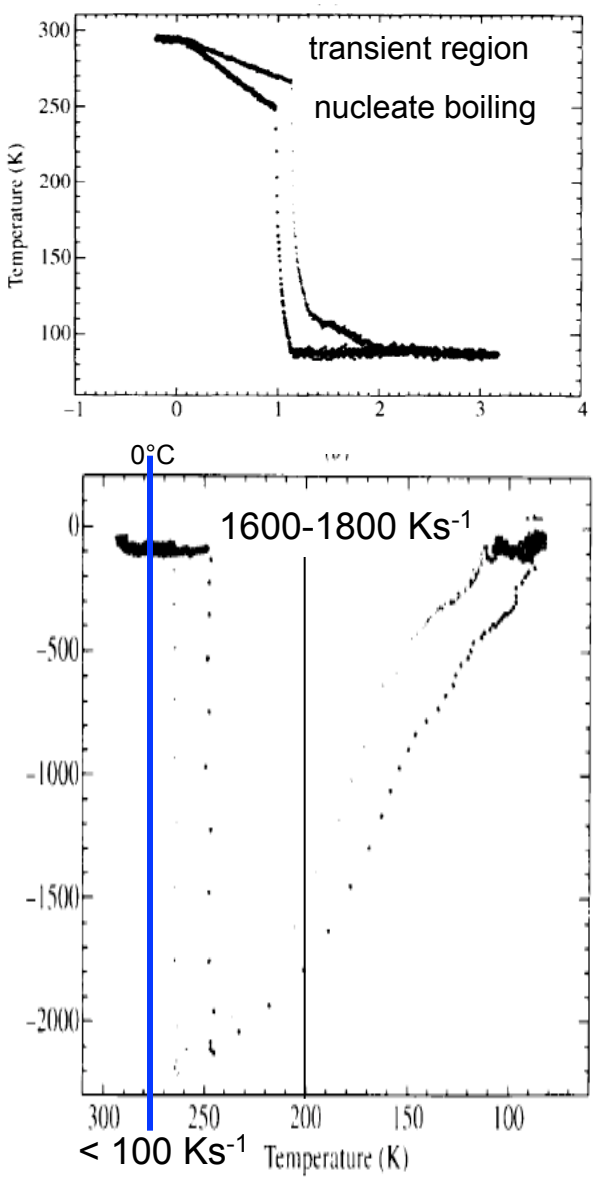
nitrogen gaz



liquid nitrogen

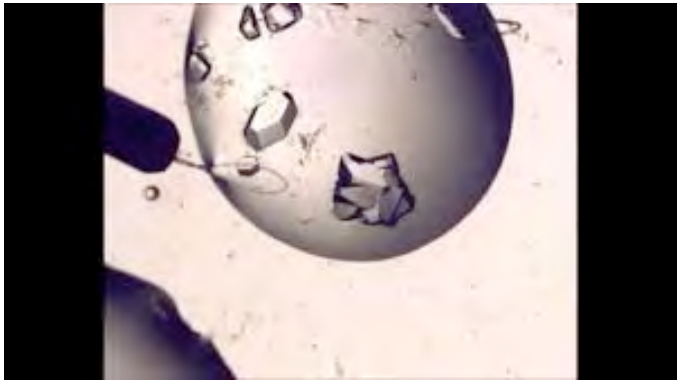


liquid propane



Liquid propane has a boiling point (231 K) and melting point (83 K) which are far apart, unlike liquid nitrogen, for which thc boiling point (77 K) and melting point (63 K) are close. Liquid nitrogen coolingis dominated by **'film boiling'** (temperature range 250 to 150 K) because of its low boiling point.

Crystal cryo cooling



Crystal fishing

Transfert in cryoprotectant solution

Flash freezing in N2 gaz at 100 K

Solving the phase problem with heavy atom derivative

- Incorporation of selenomethione (SeMet)
- Co-crystallization with heavy atom containing solution
- Soaking the crystal in a solution containing heavy atoms

classical heavy atom derivative

Iodine et bromine (quick soak)

Tantalum bromide cluster (very big, green color)