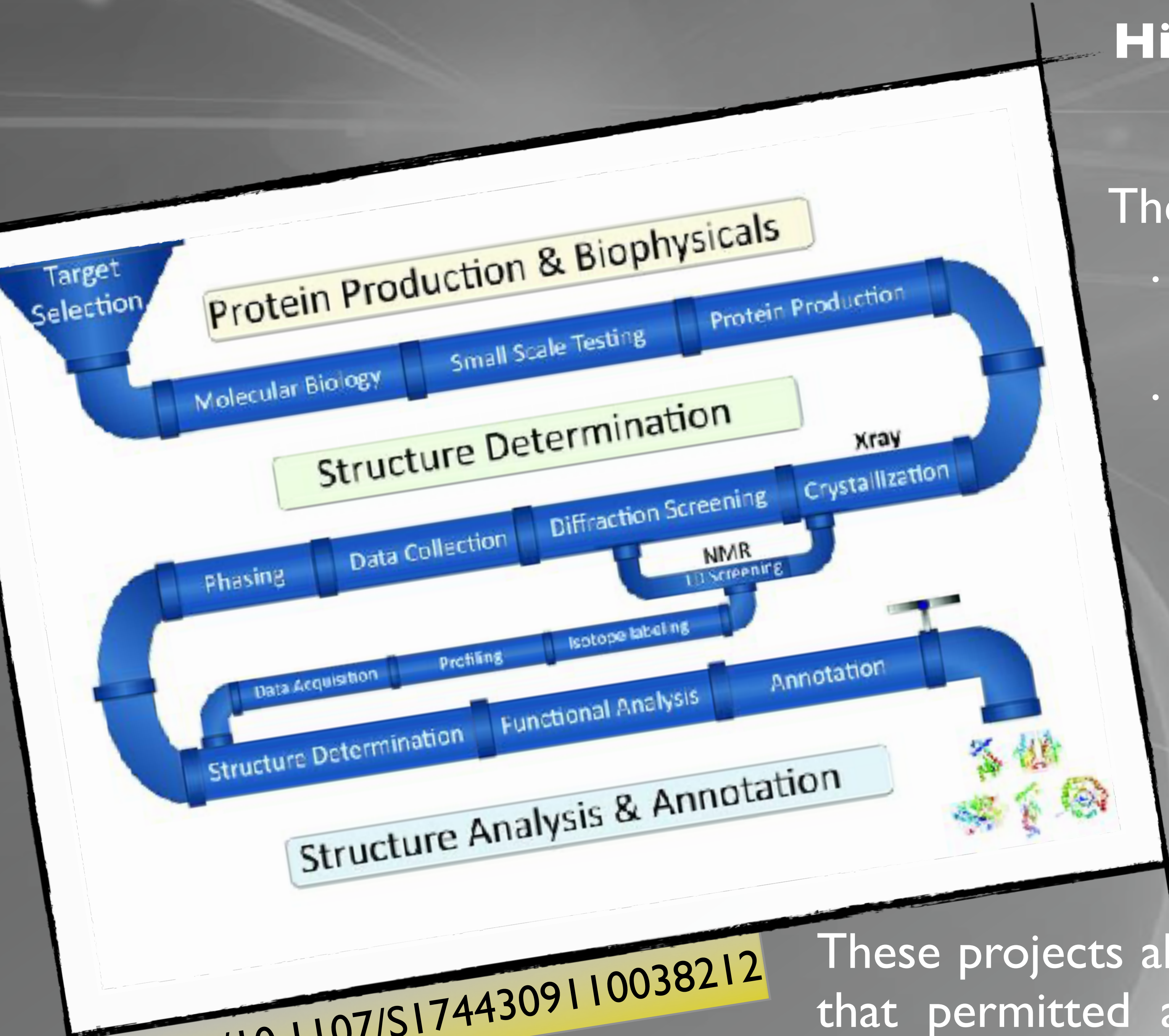
The *in vivo* macromolecular crystallography platform at Nagoya University

For the complete story:
Poster 3P-070

Chavas Leo

Nagoya University, Japan

Why are we here today?



doi.org/10.1107/S1744309110038212

High throughput protein production and crystallization

The obvious:

. production of large quantities of proteins

. production of large quantities of crystals

The hidden secret …

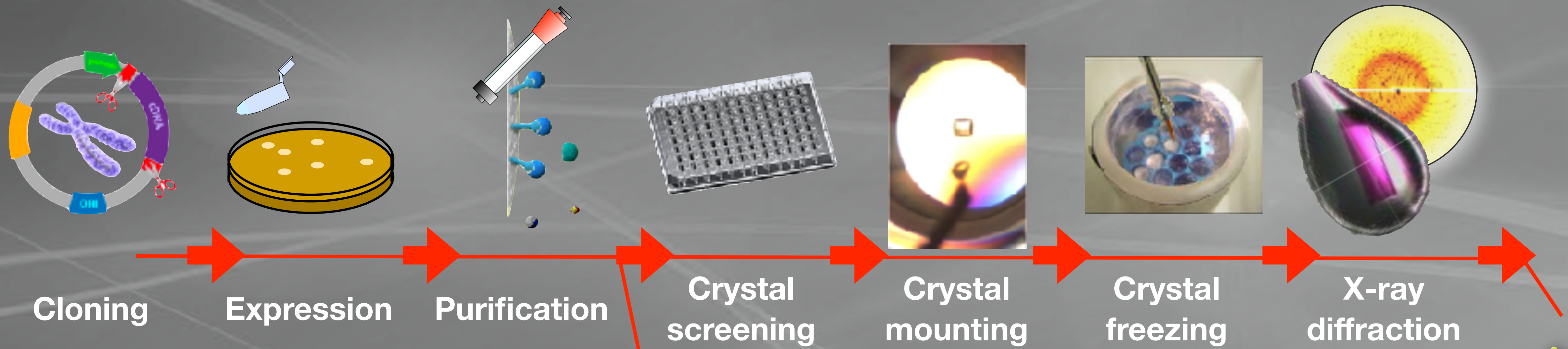
… solve quantities of structures

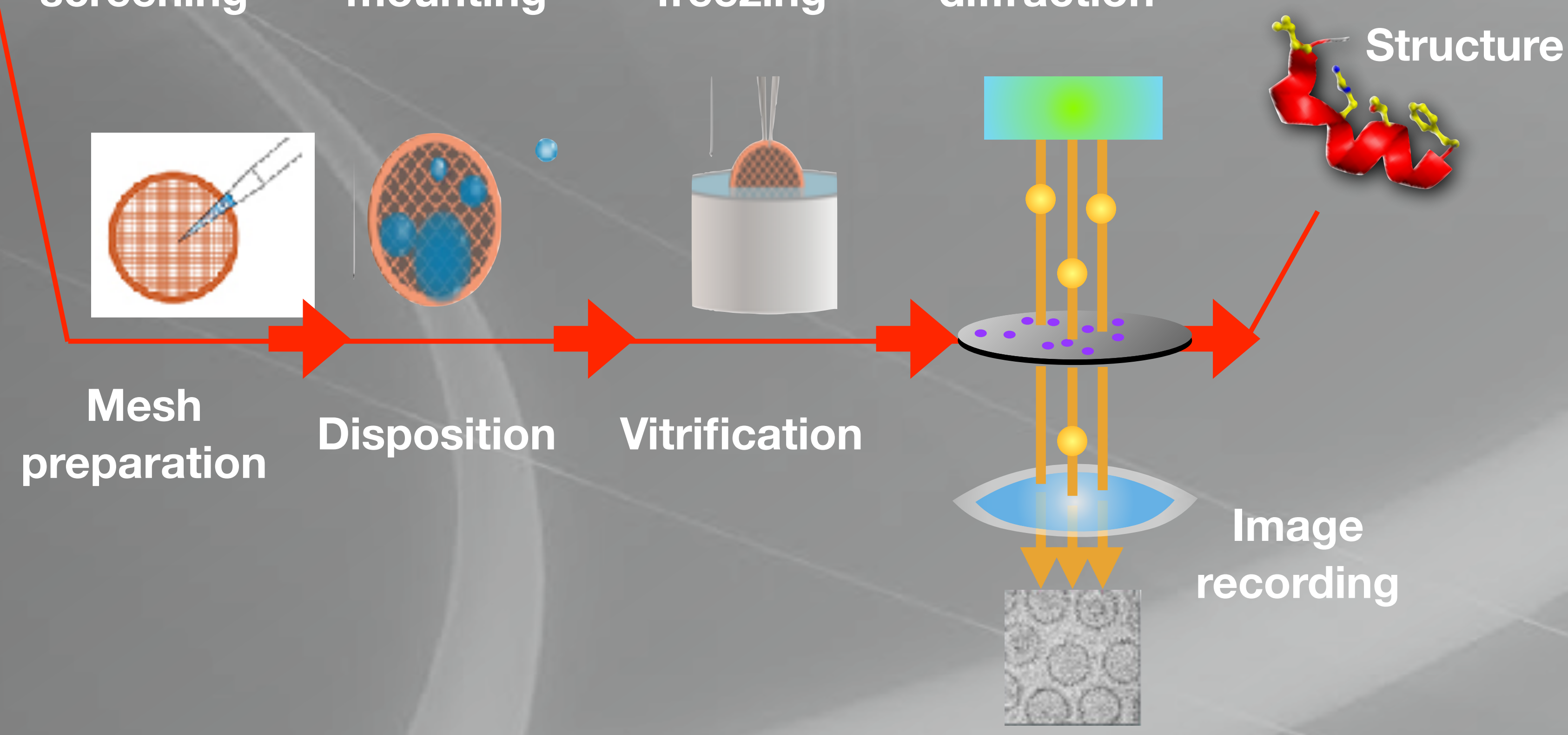
The general concept was proposed nearly 20 years ago, with the emergence of structural genomics projects.

These projects allowed for key technological developments that permitted automation and standardization of these approaches.

Most modern structural biology laboratories own or have access to these facilities.

The accepted workflow for MX





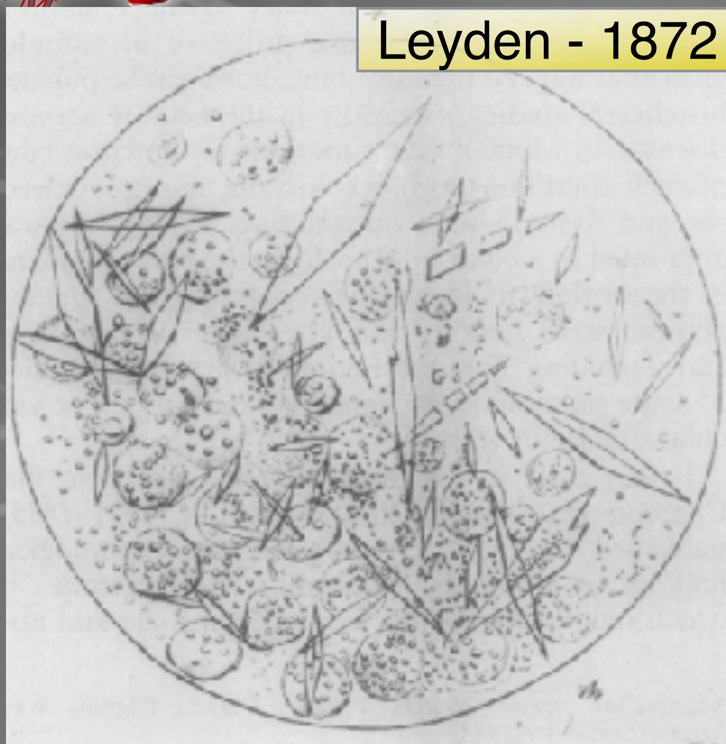
Most (if not all) of the measurement methods in the field of structural biology share common practices for sample preparation, which include Cloning, Production, and Purification.

Biophysical characterization of the purified samples will be carried out in ways adapted to the measurement protocols.

The final goal of protein **pur**ification is to get a **pure** sample, known to be much easier to handle for crystallization purposes.


doi.org/10.1107/S2052252516008903

Concept of *in vivo* crystallography



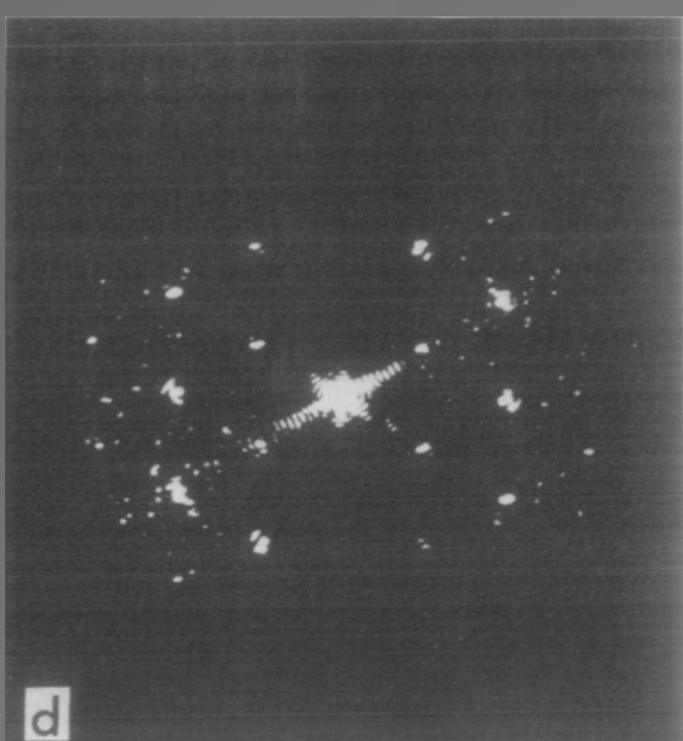
Leyden - 1872

Charcot-Leyden crystals

Early identification of *in vivo* grown crystals

Not only random isolated cases

Plenty of ‘medical’ reports

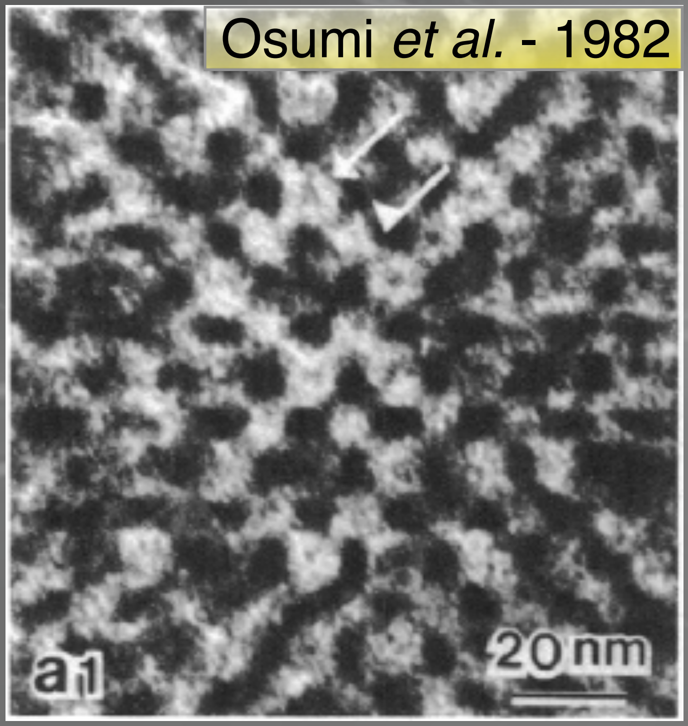


Osumi *et al.* - 1979

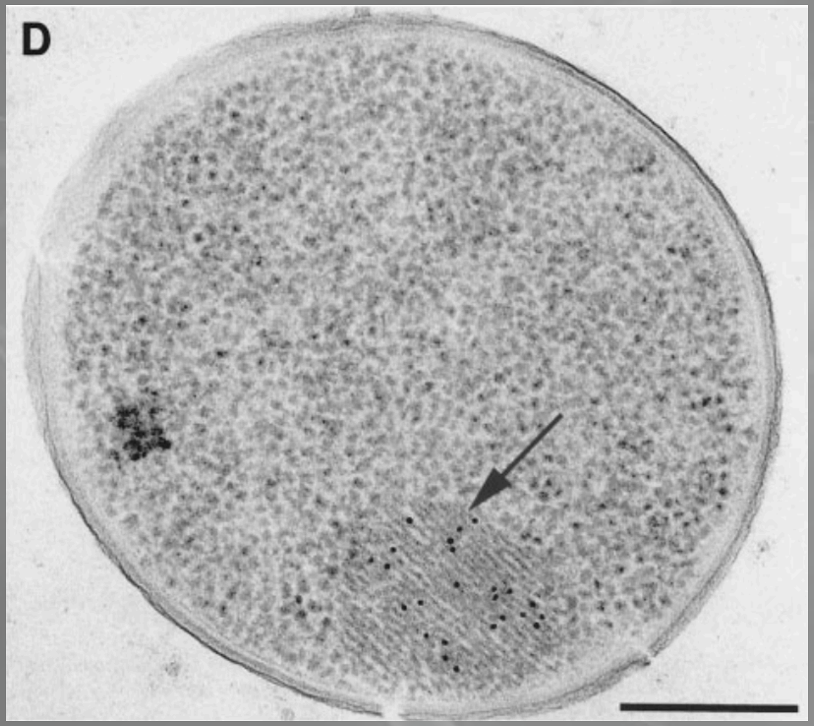
Early electron micrograph

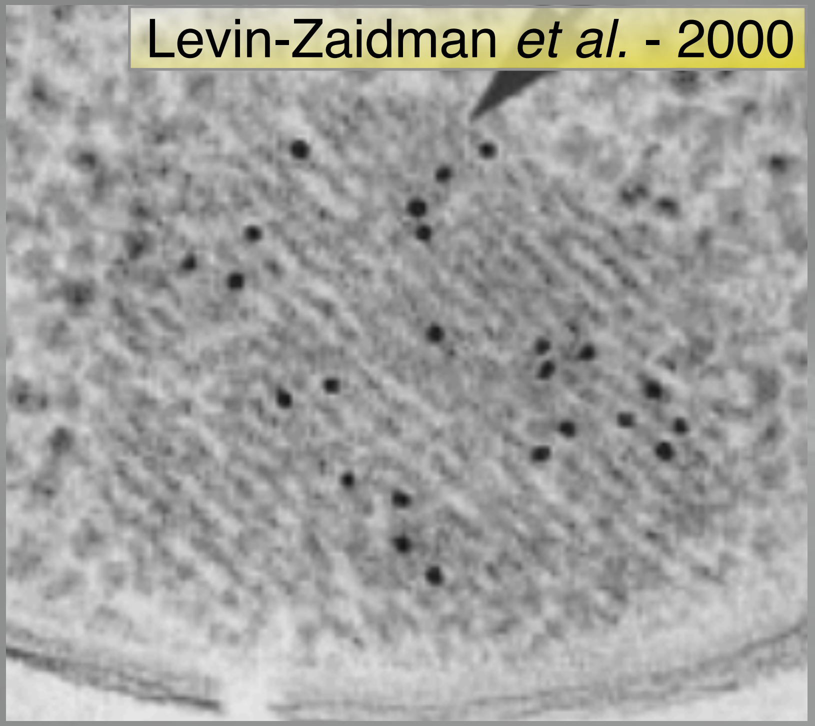
© David Goodsell

Concept of in vivo crystallography



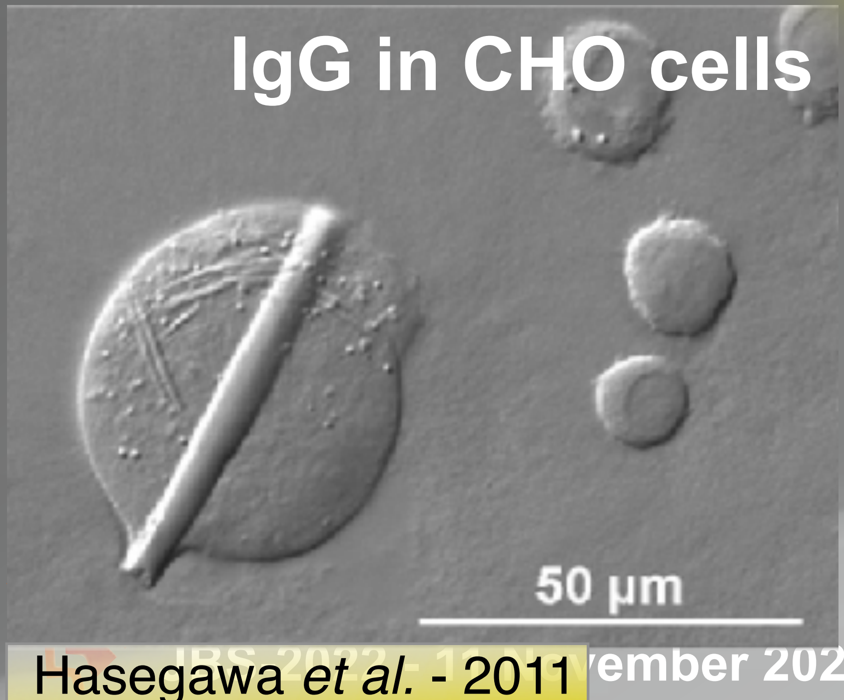
Crystalloid lattice arrangement in cells





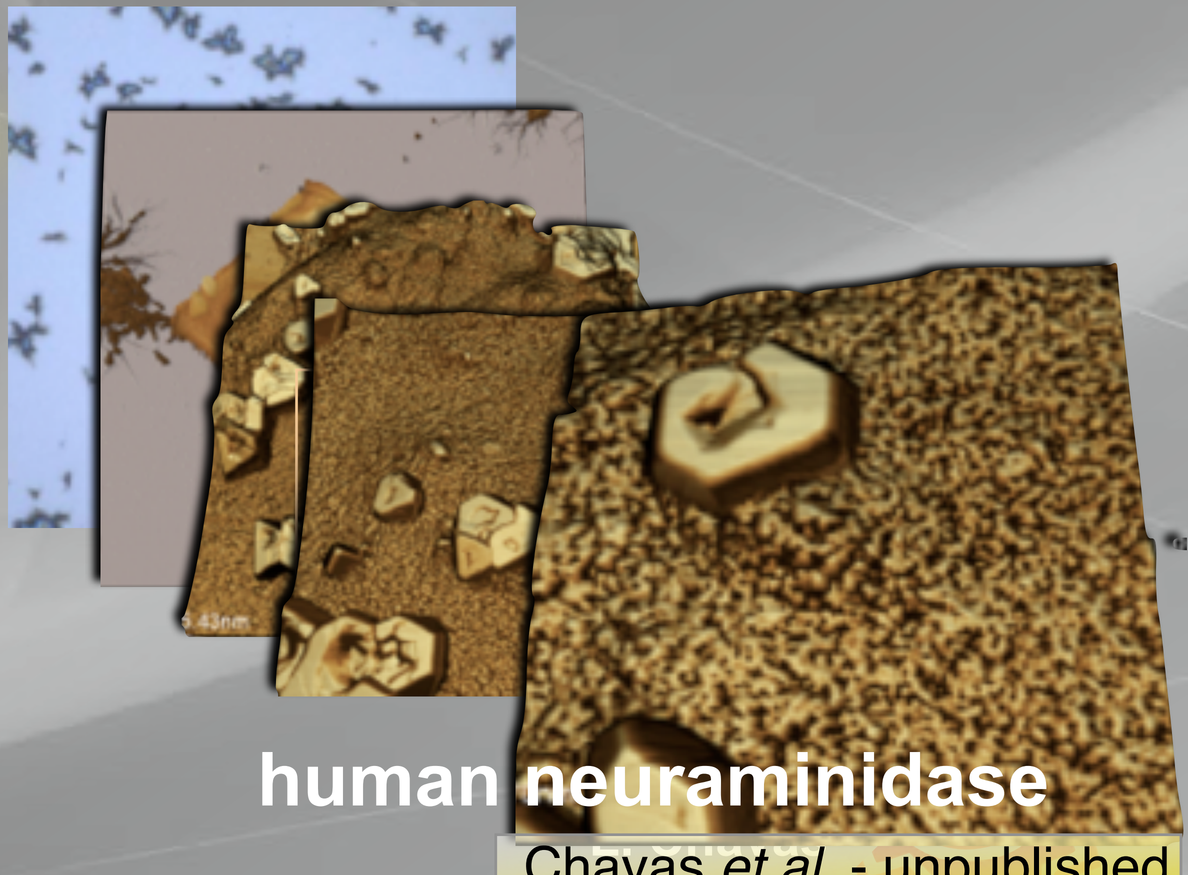
RecA-DNA assemblies

Early identification of *in vivo* grown crystals



Plenty of 'medical' reports

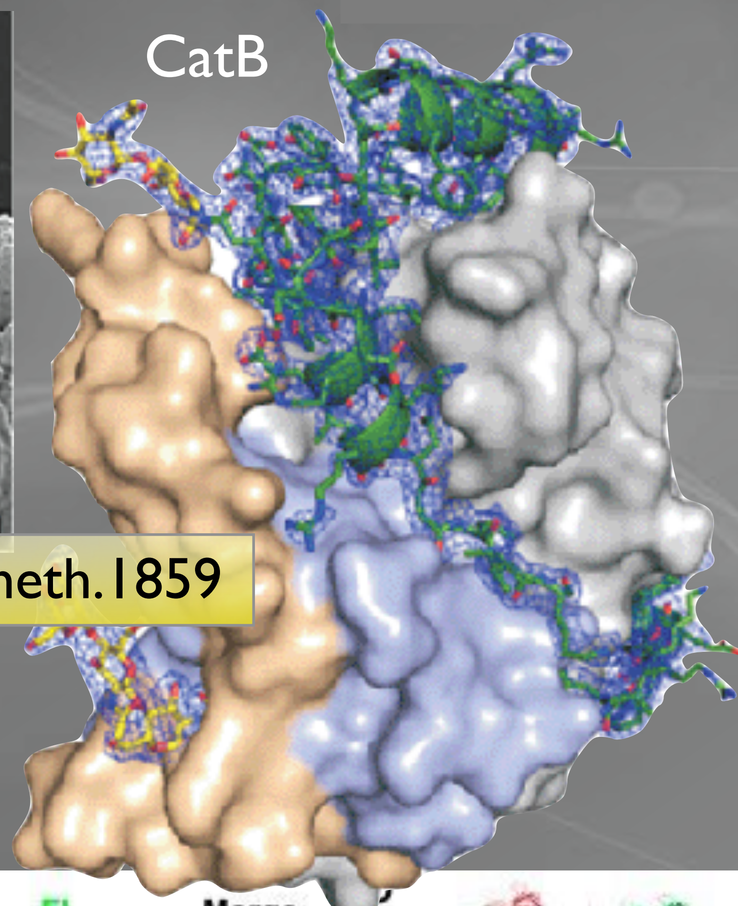
No systematic studies until late 2000's

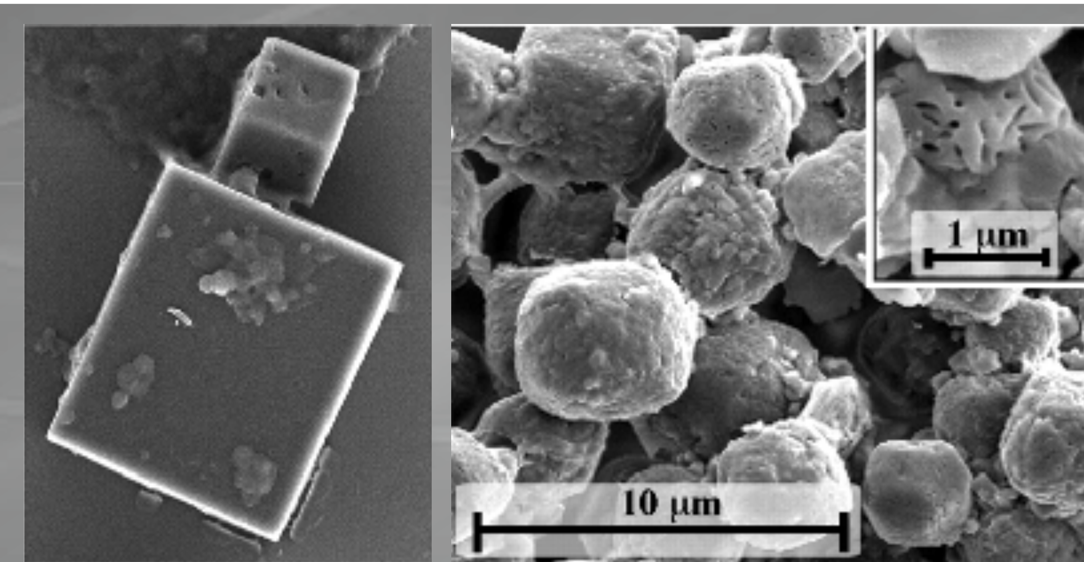


Concept of *in vivo* crystallography

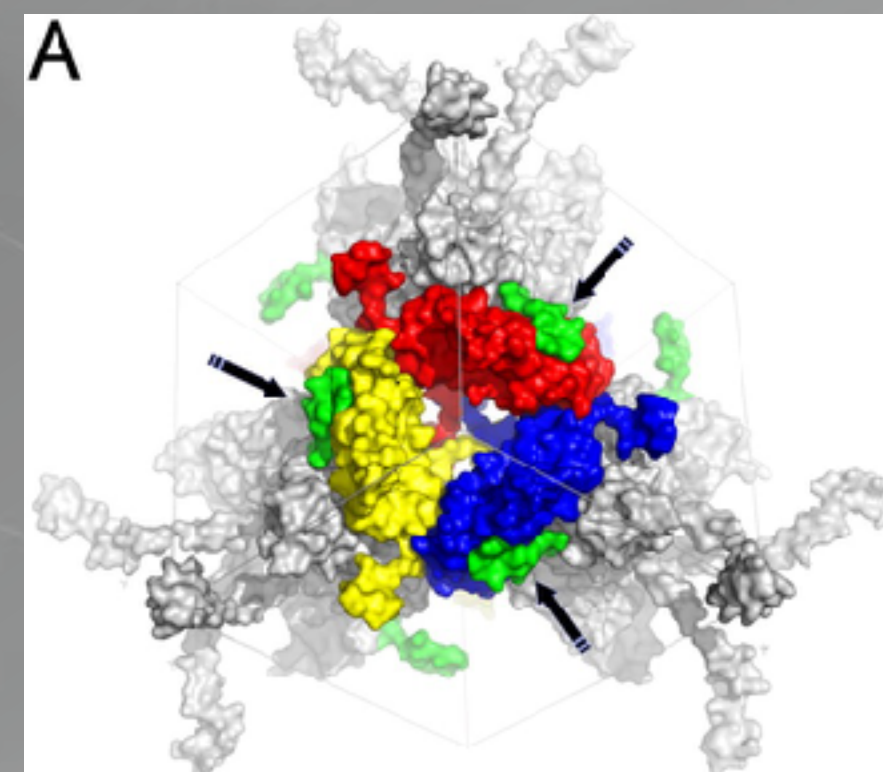
doi.org/10.1038/nmeth.1859

CatB

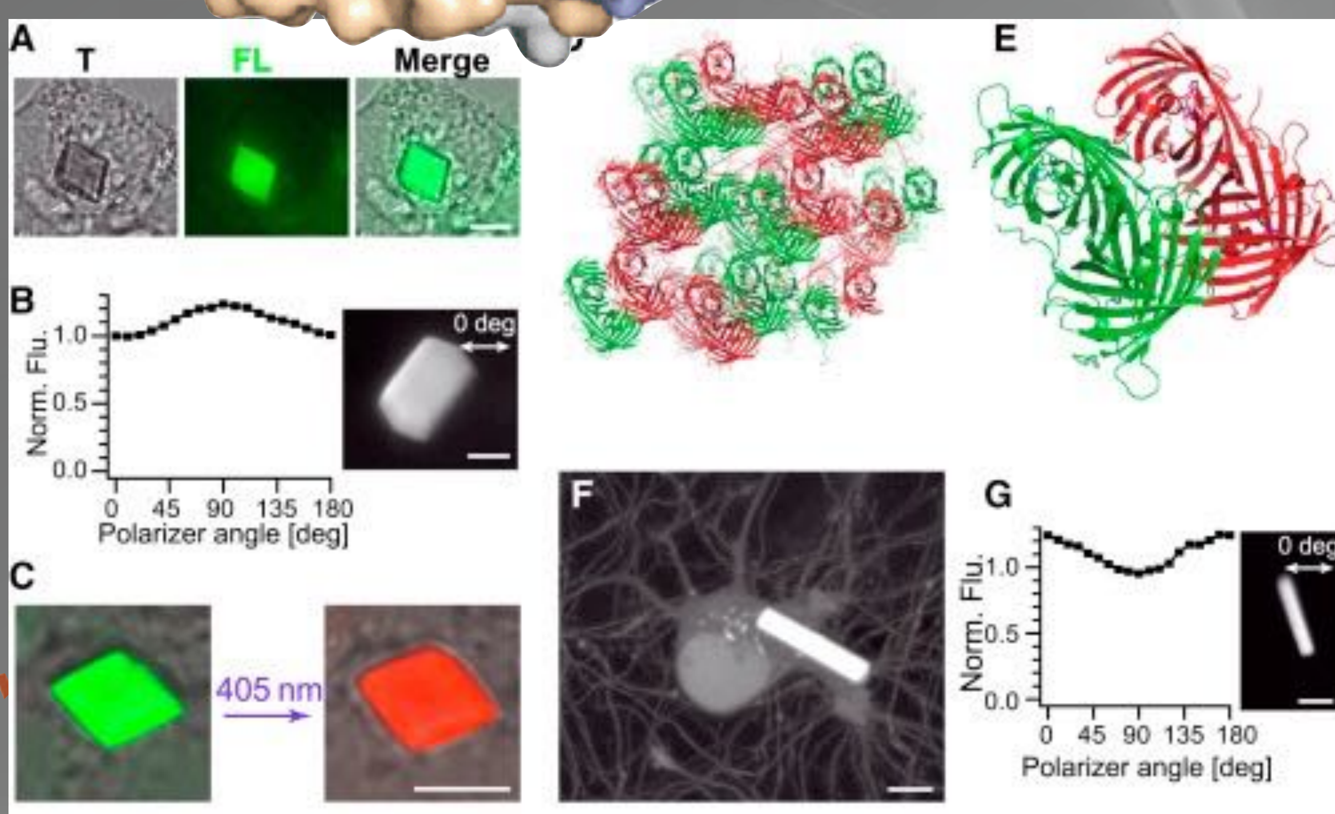




Polyhedra



doi.org/10.1073/pnas.0910686106



Xpa

doi.org/10.1016/j.molcel.2015.02.007

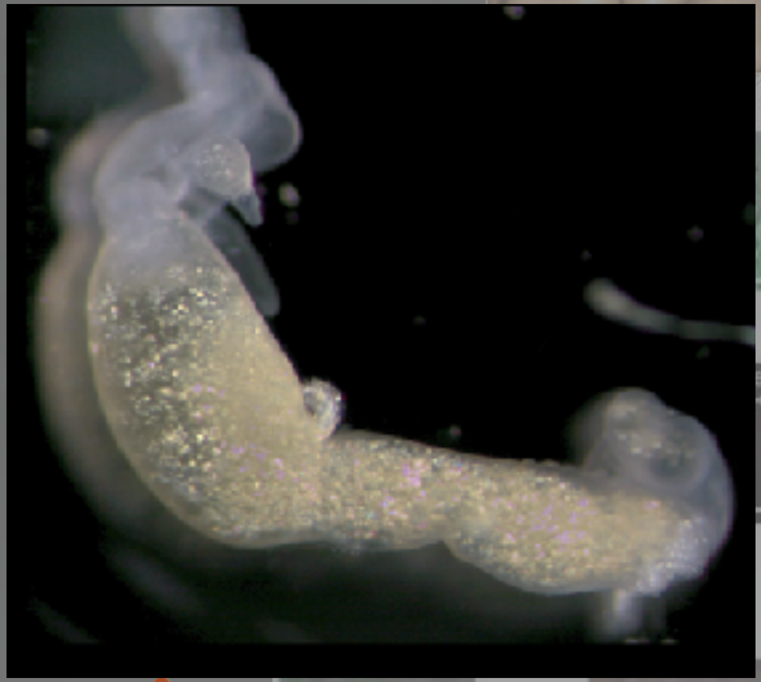
Concept of *in vivo* crystallography

in vivo / in cellulo macromolecular crystallography

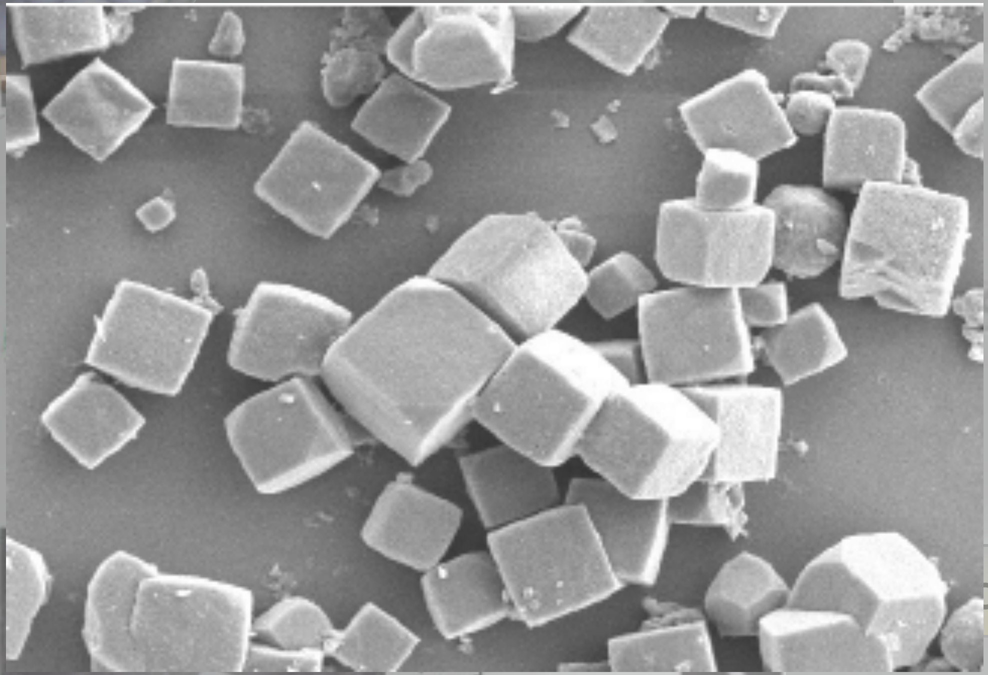
Protein storage
- Plant seeds
- Secretory granules (insulin, EMBP…)
- Bacterial toxins
- Cockroach milk proteins

Encapsulation
- Polyhedrin
- Spheroidin
- Granulin

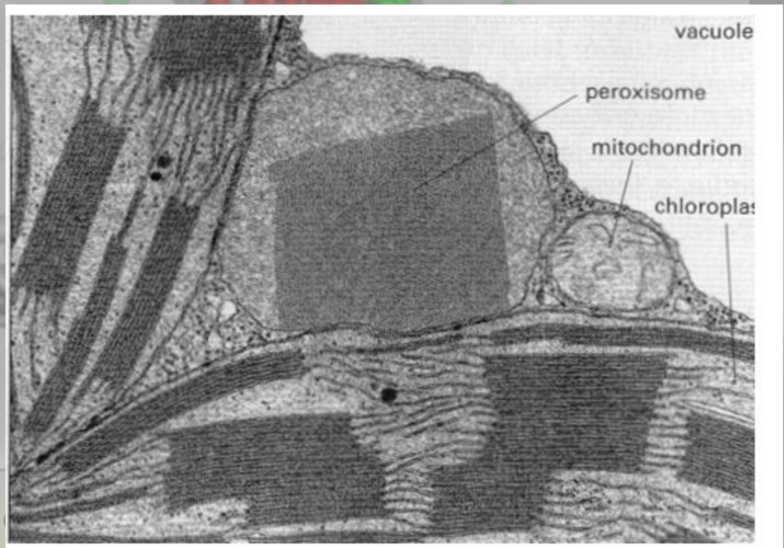
Solid state catalyst
- Peroxysome enzymes (Catalase, alcohol oxidase…)



Lipocalin



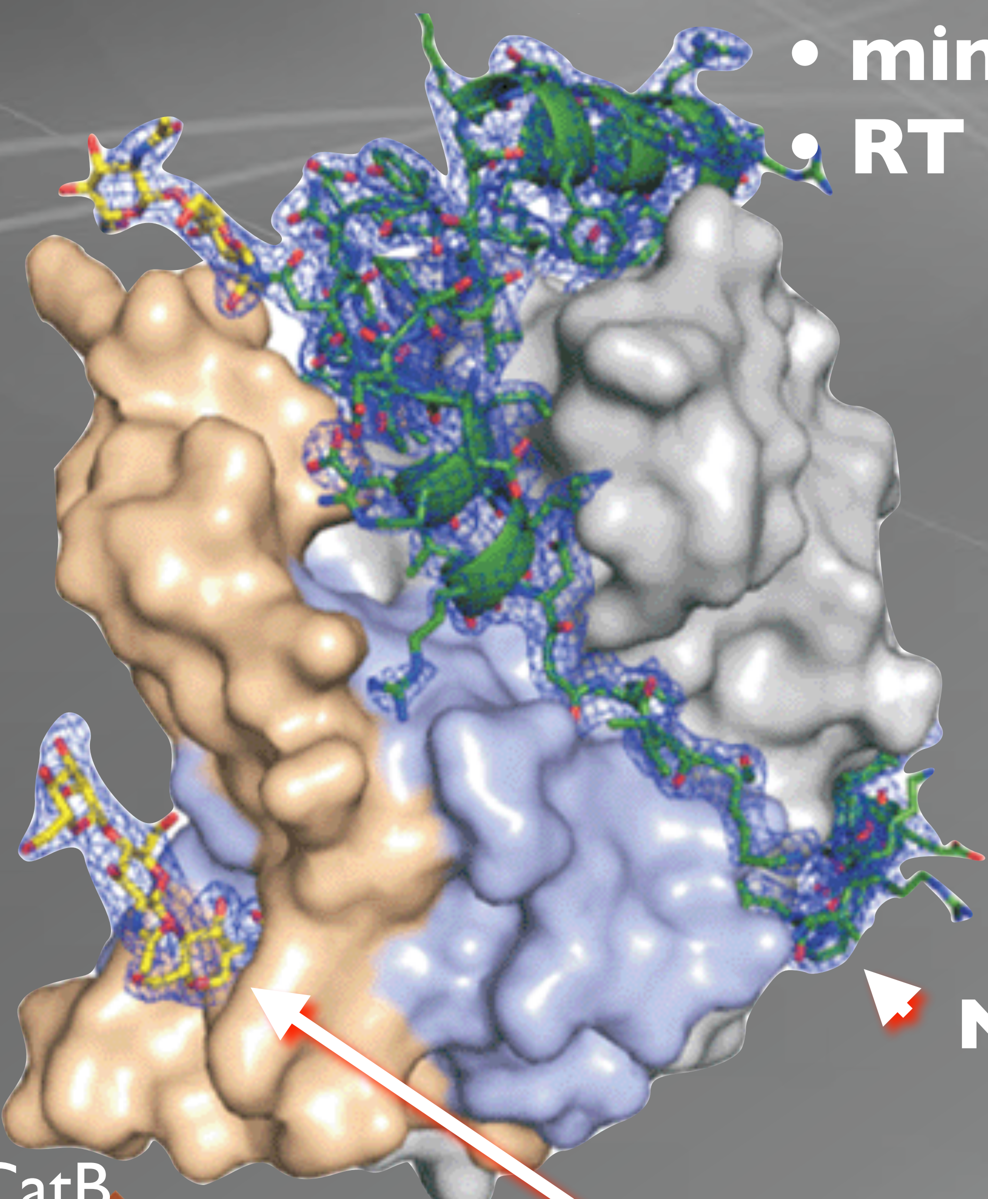
Polyhedrin

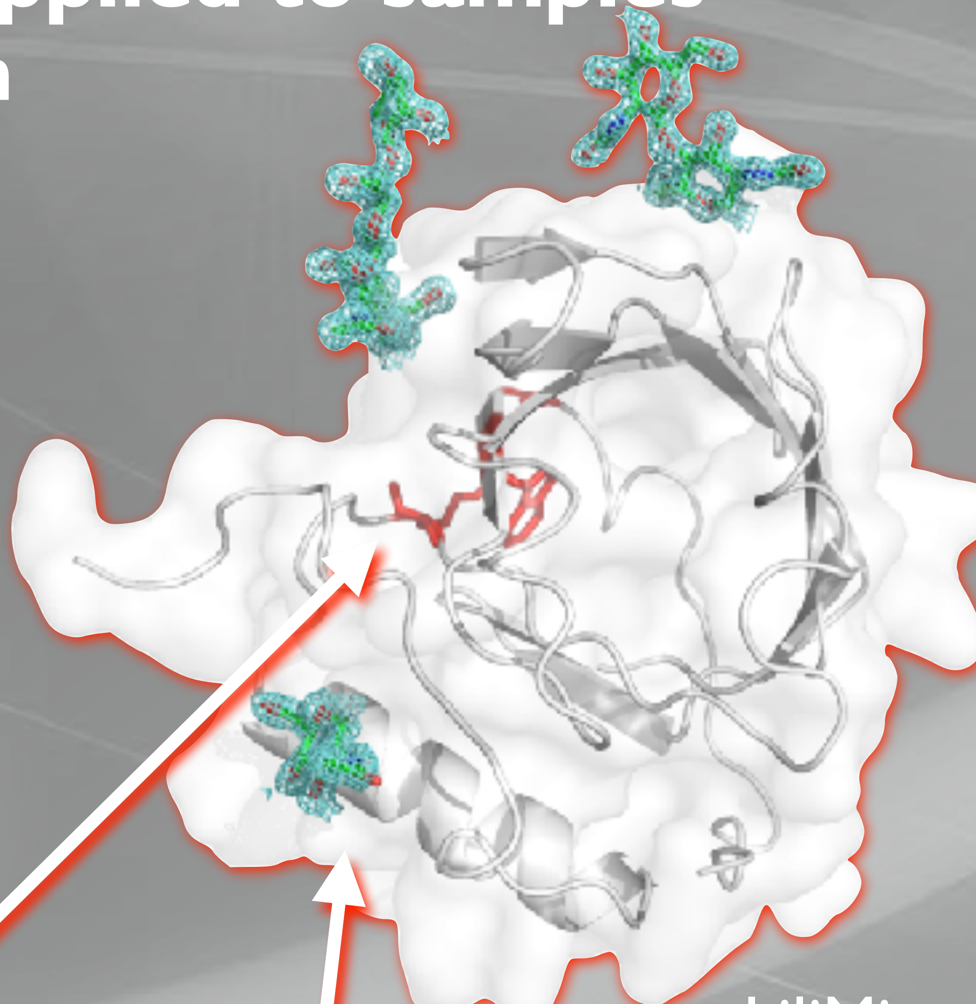


Catalase

Why is ivMX of any interest ?

- **sample preparation simplified**
- **natural environment (! free lunch !)**
- **unassisted crystallization**
- **minimum stress applied to samples**
- **RT data collection**





Natural ligands

CatB

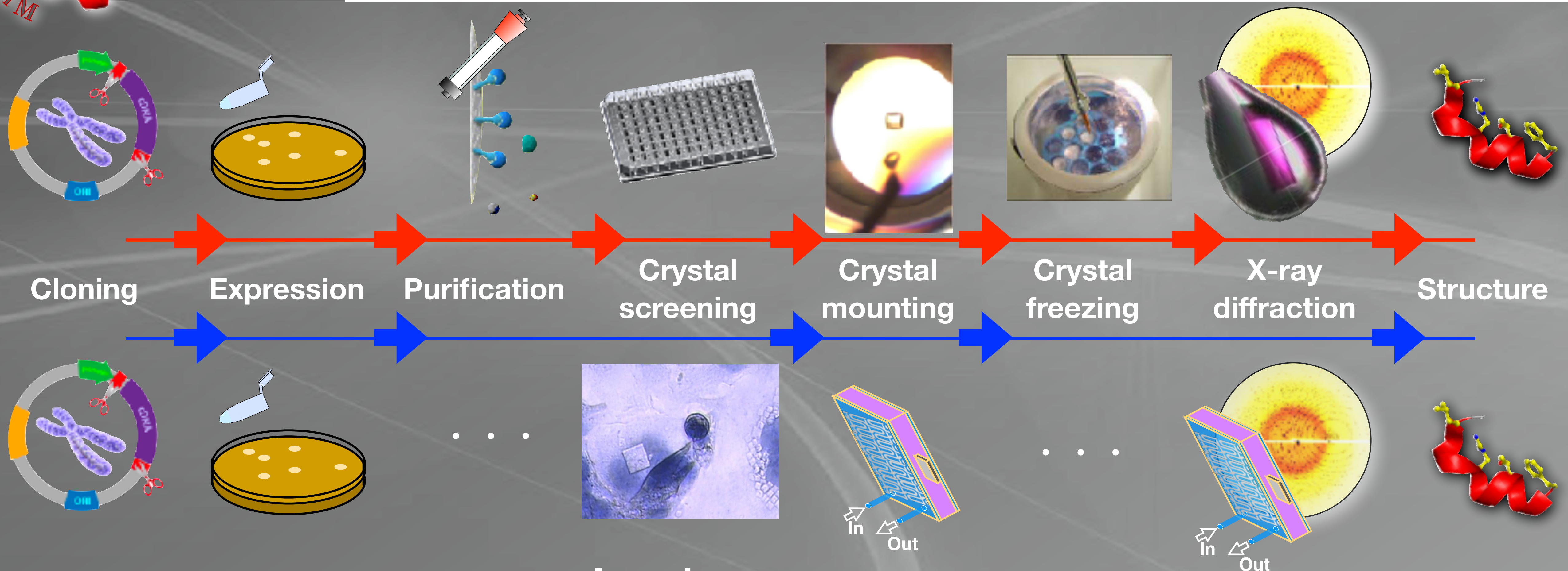
doi.org/10.1038/nmeth.1859

LiliMip

doi.org/10.1107/S2052252516008903

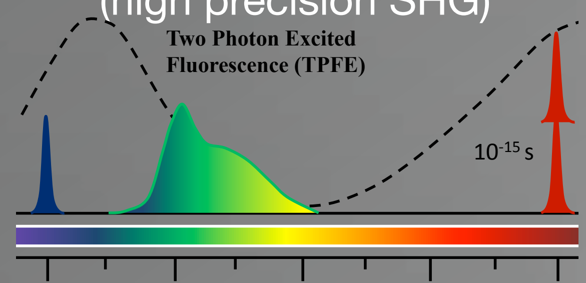
Post-translational modifications

What do we need prior to master ivMX



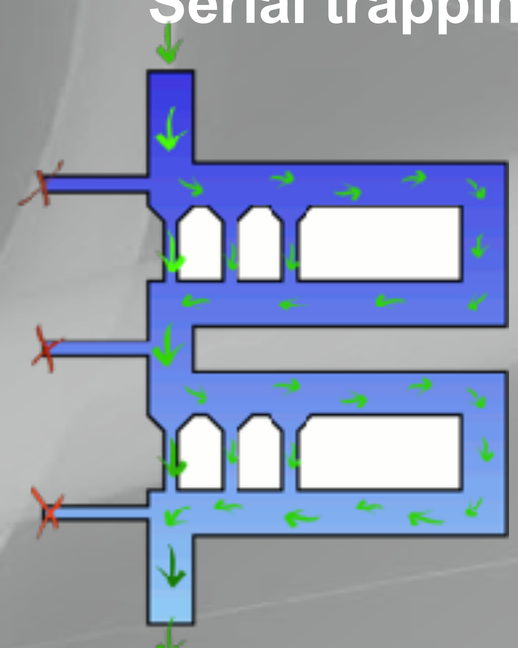
Cloning
(*iv*MX plasmid library)

Imaging
(high precision SHG)

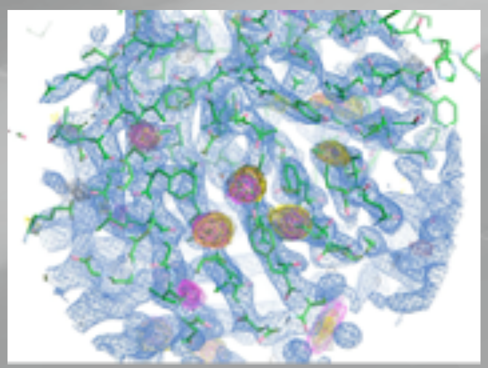


Cell biology
(expression facility)

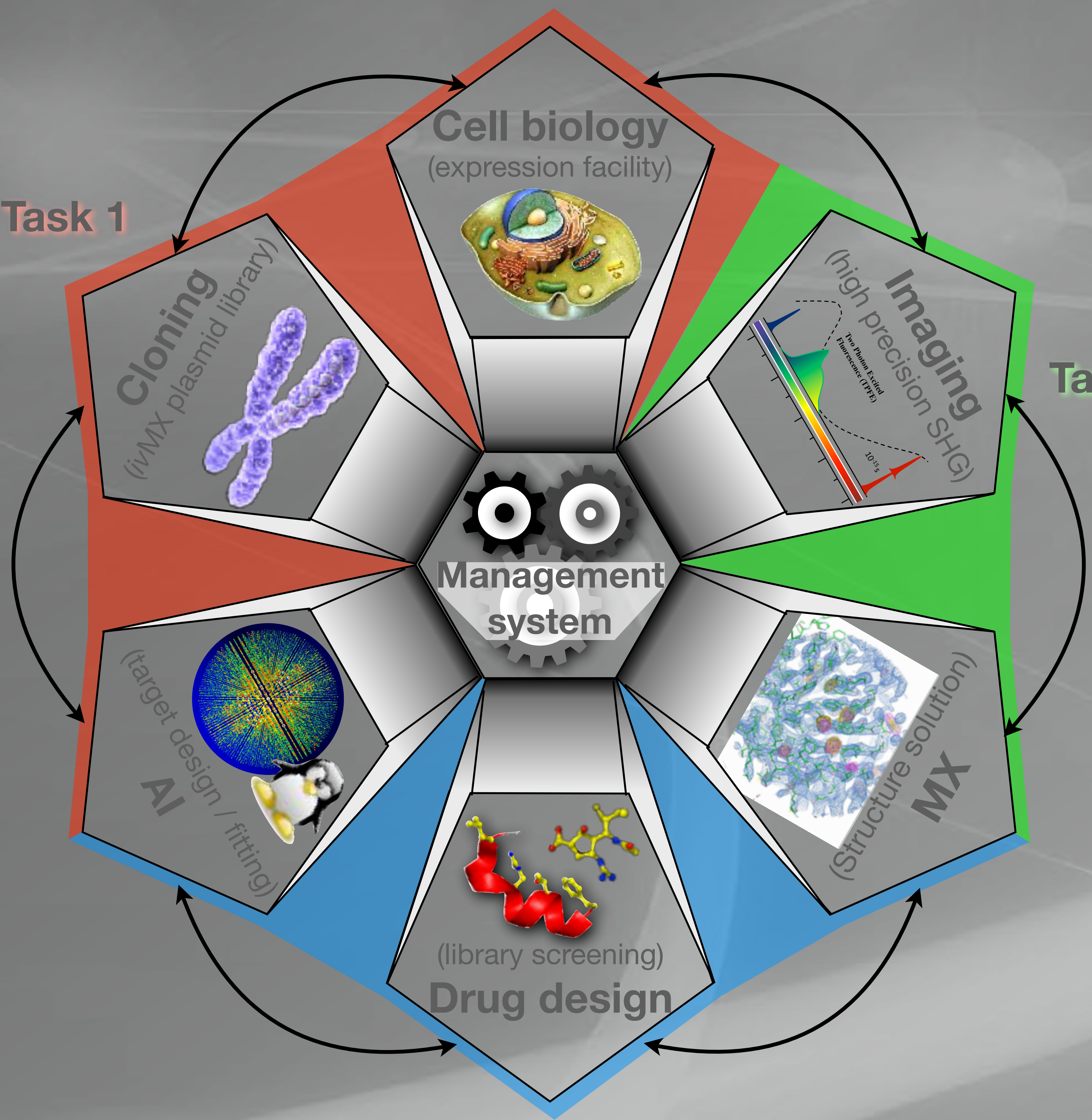
Serial trapping



(Structure solution)
MX

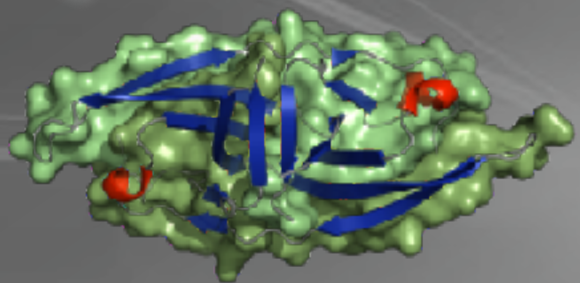


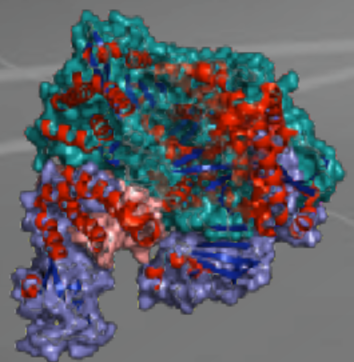
What do we need prior to master ivMX

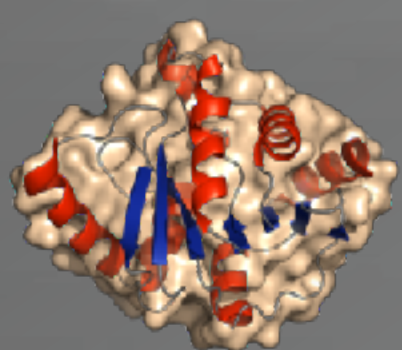


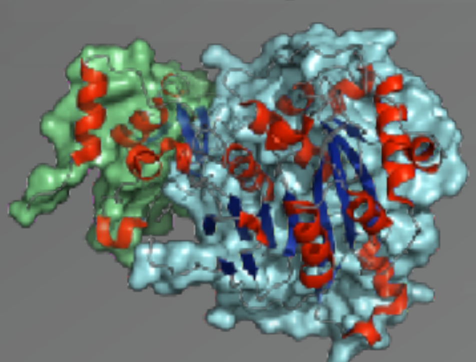
Leymarie - *accepted*

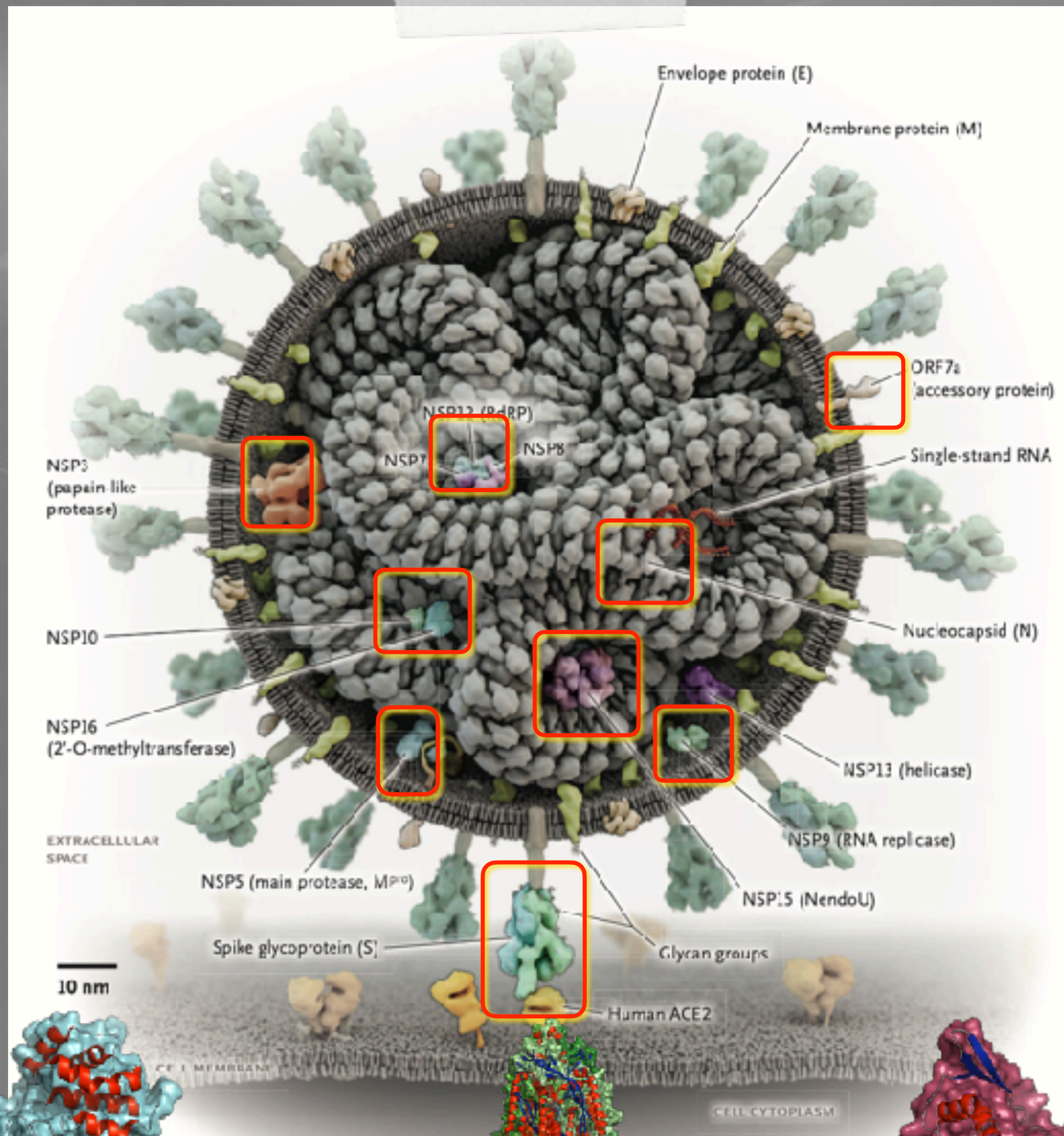
ivMX : the brute force approach

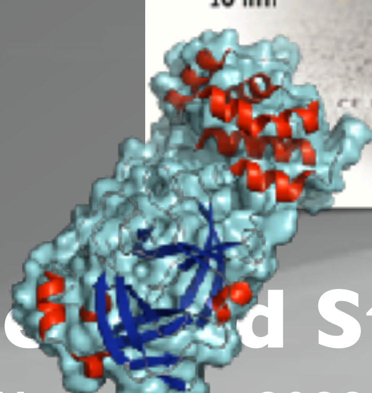


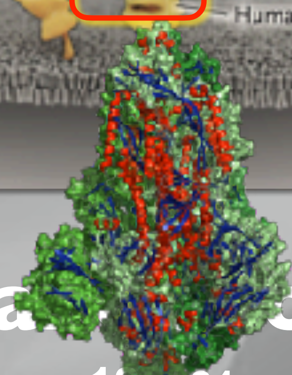


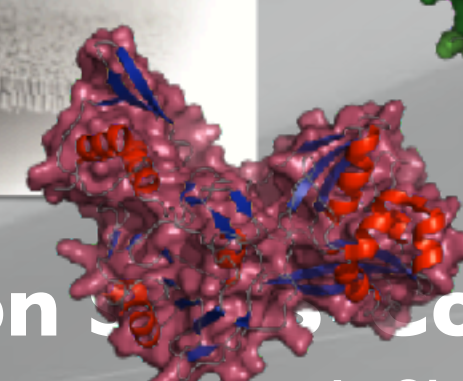


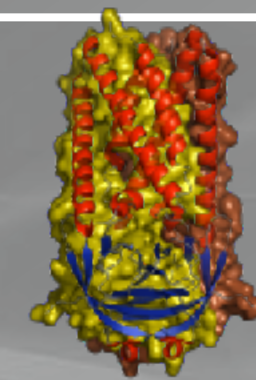


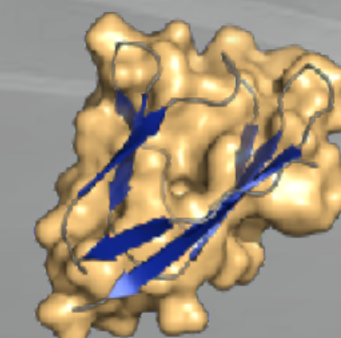


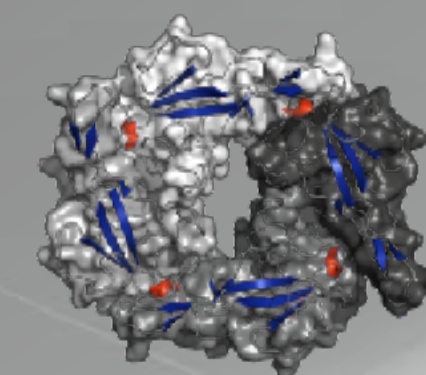


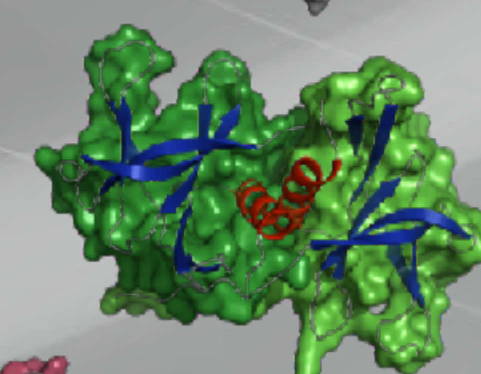








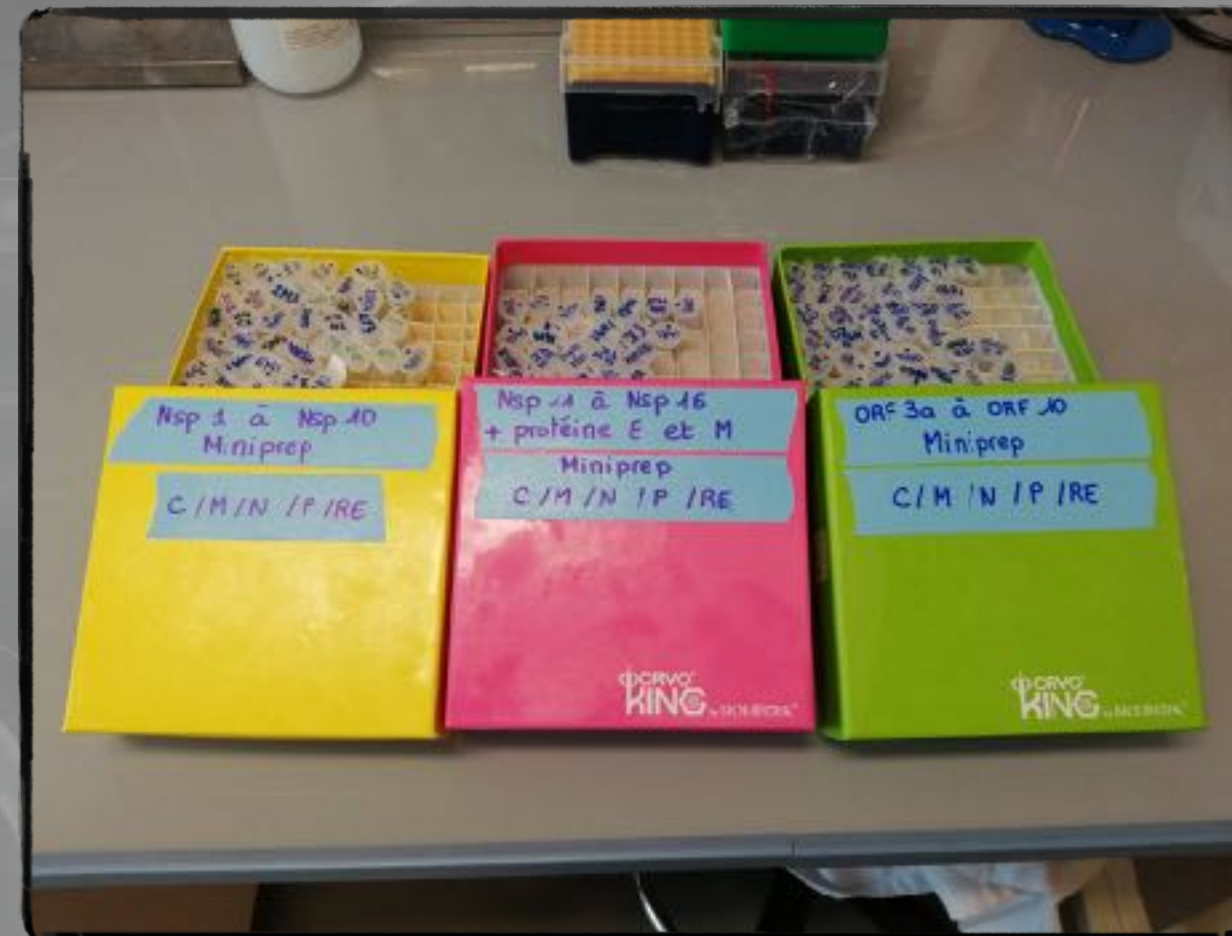


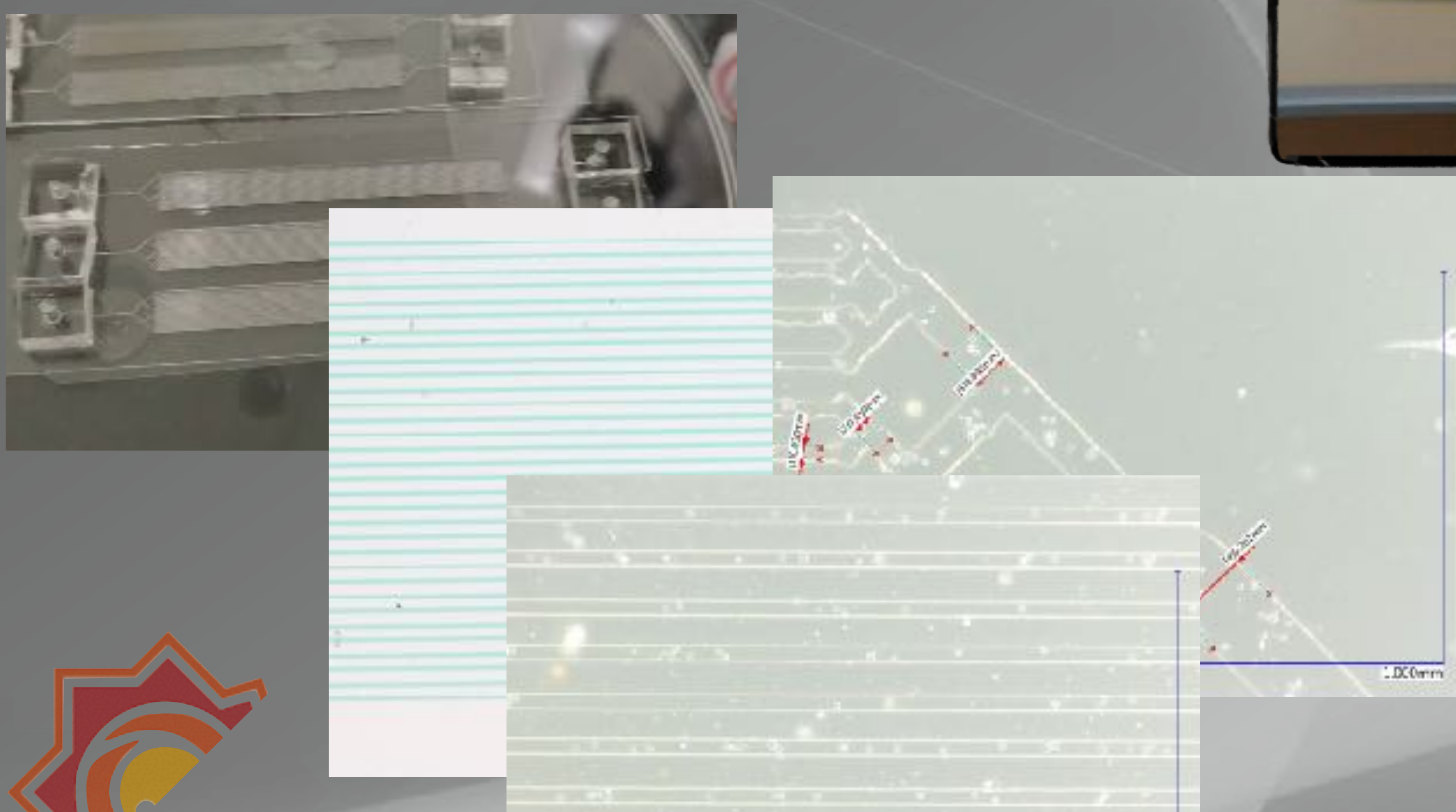


ivMX : the brute force approach

very large-scale cloning of :
- 26 proteins (out of 27)
- in 2 sets of 5 plasmids for 5 sub-cellular localizations

~ 250 different constructs for over expression in human cells





efficient microfluidic handling of cells for :
- crystal identification
- cell isolation
- x-ray diffraction experiments

ivMX : the brute force approach

very large-scale clonin
• 26 proteins (out
• in 2 sets of 5 pla
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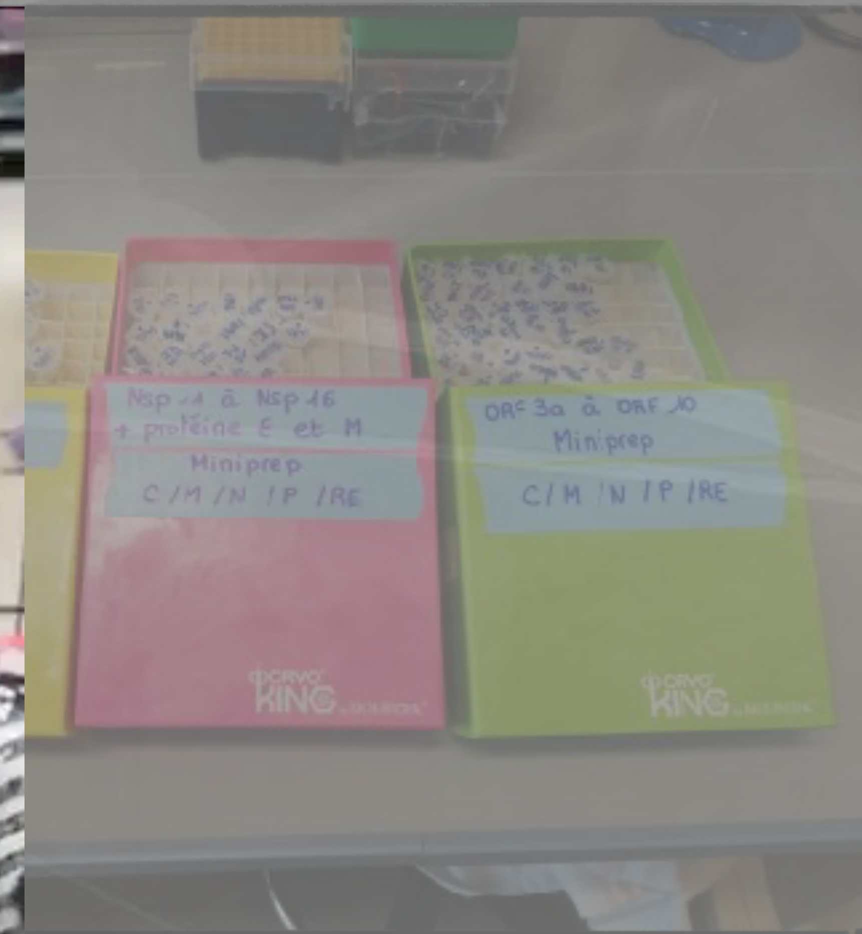

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• crystal identification
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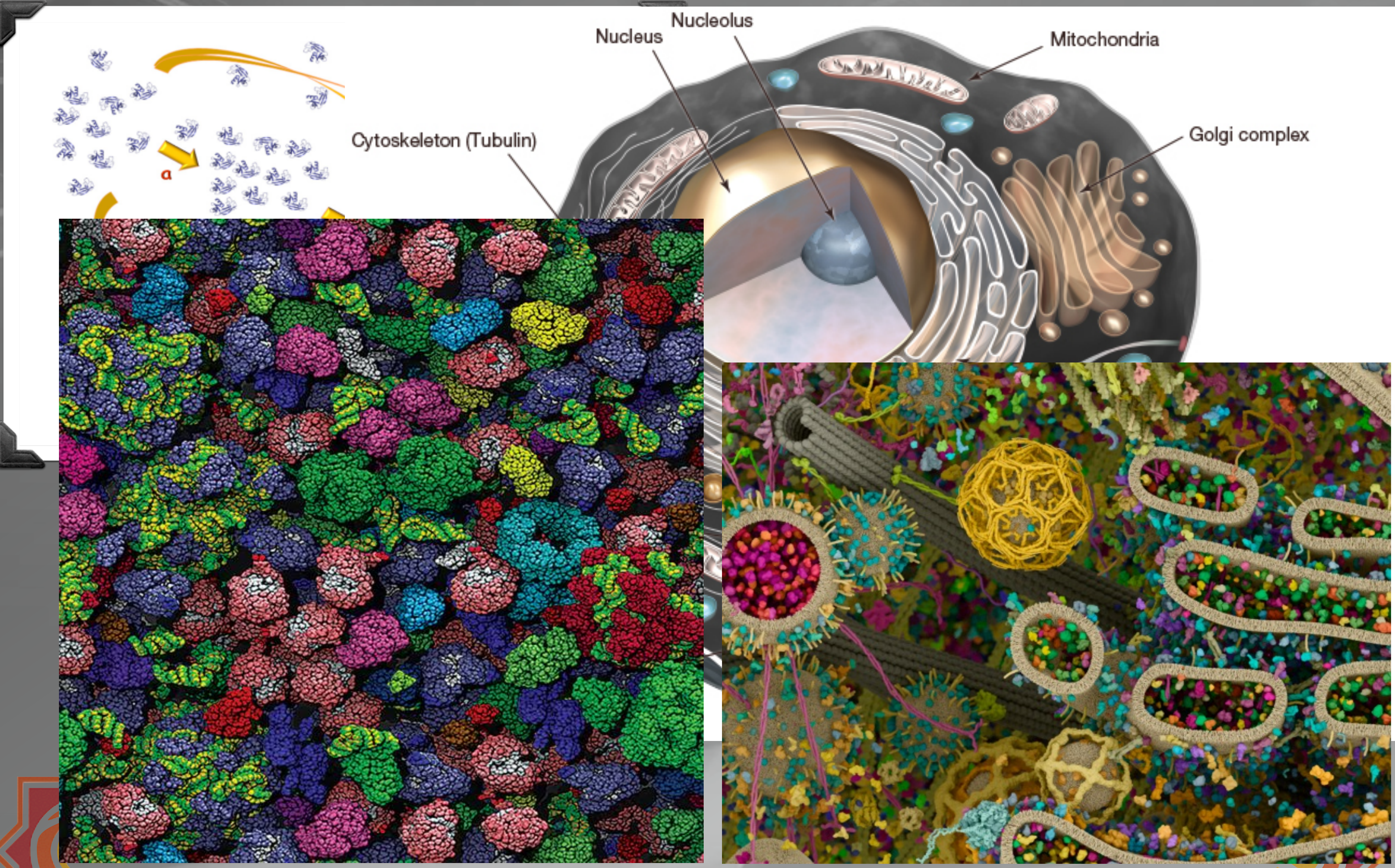
experiments

ivMX : the thoughtful method

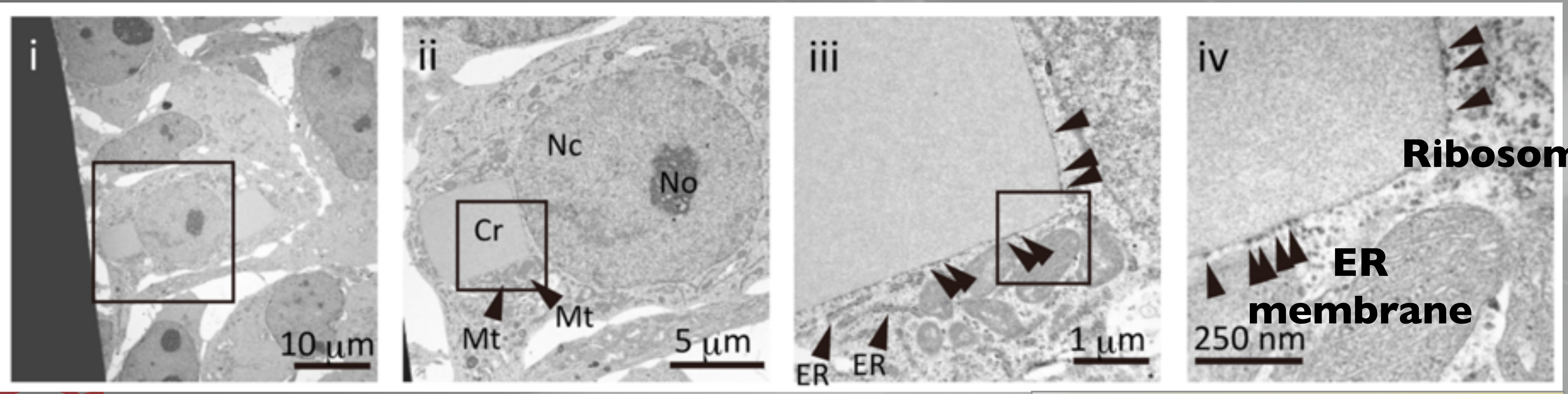


ivMX : the thoughtful method

doi.org/10.1098/rstb.2013.0497

Case of human protein in human cells

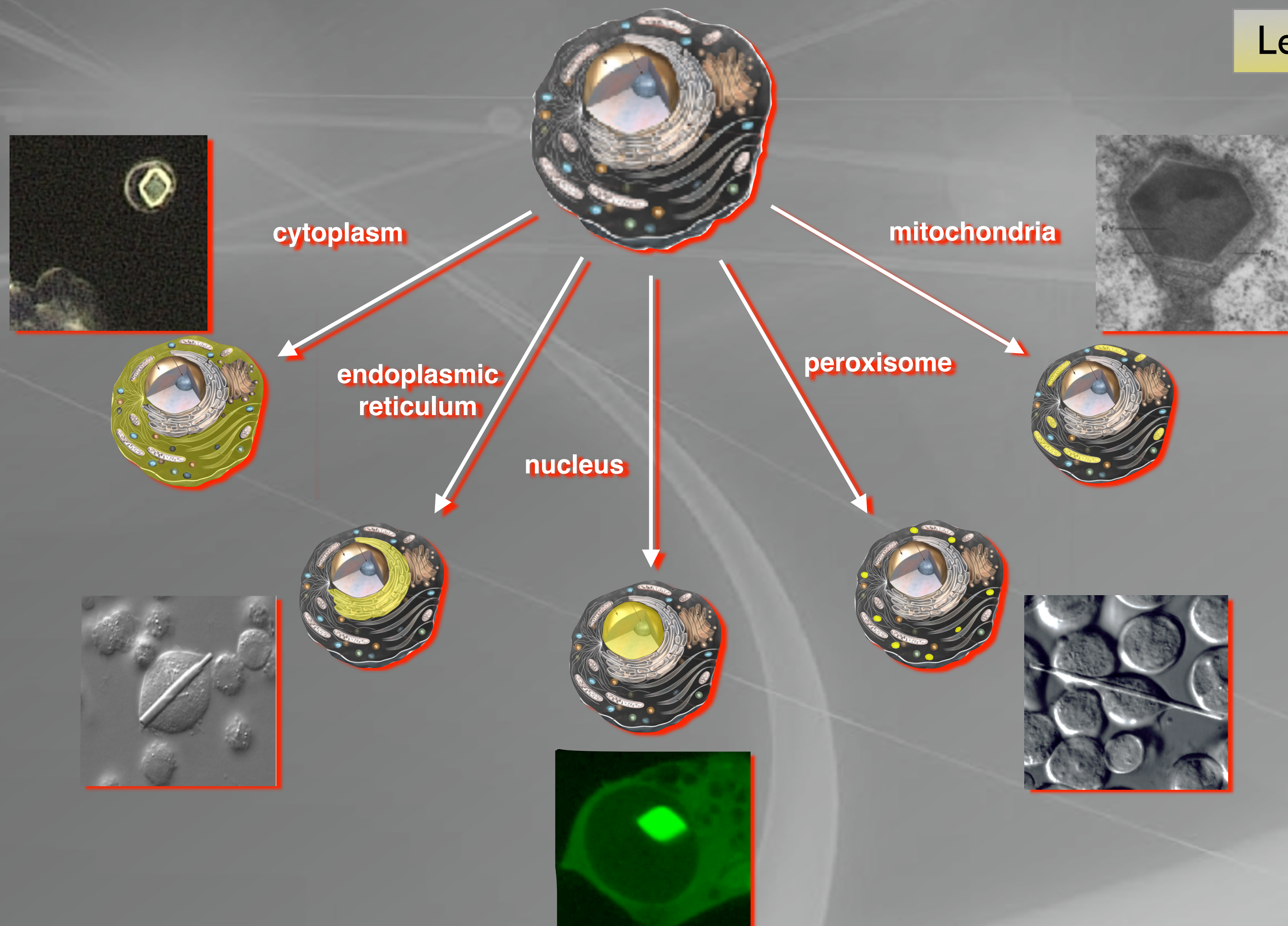
Good approximation of where are the crystals growing, however need to get a better resolution to understand how is the process happening.



doi.org/10.1021/acsabm.9b00686

Step 1 - cloning, cloning, cloning ...

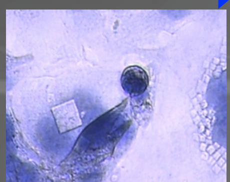
Leymarie - *accepted*



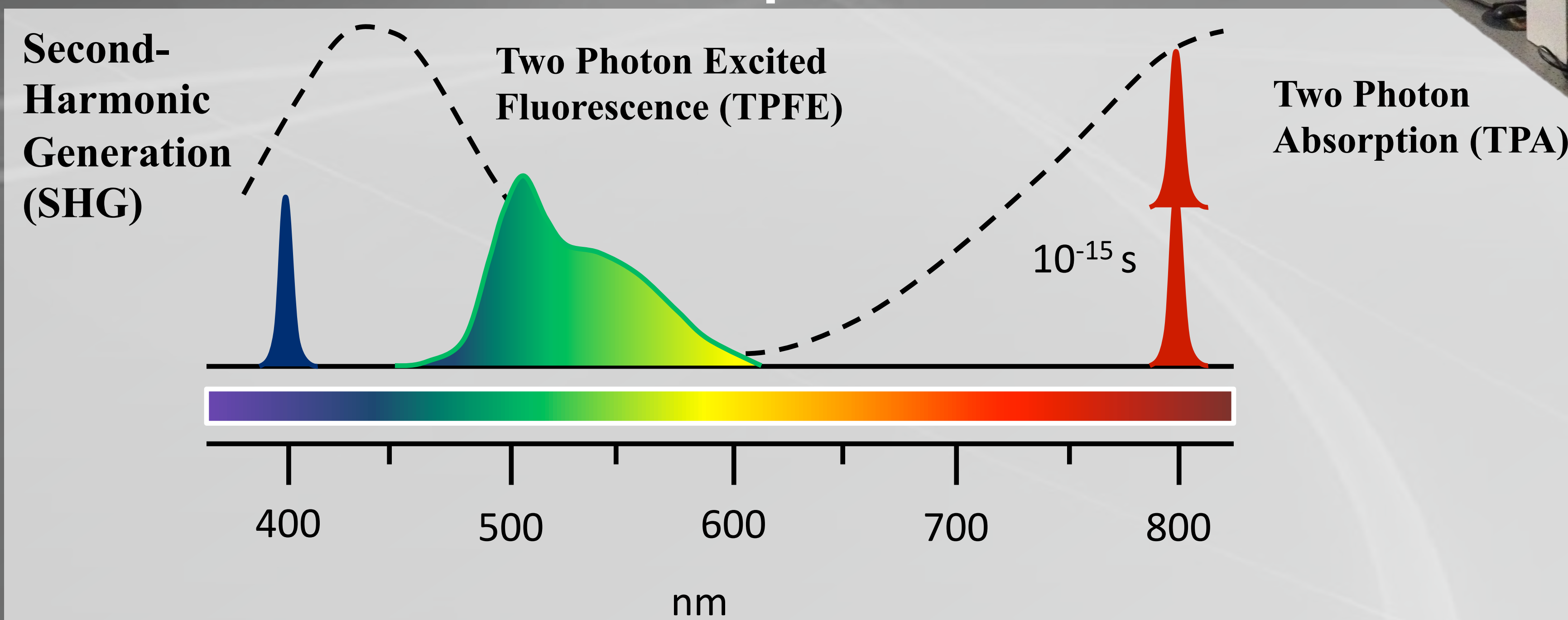
Systematic investigation of *in vivo* crystallization events

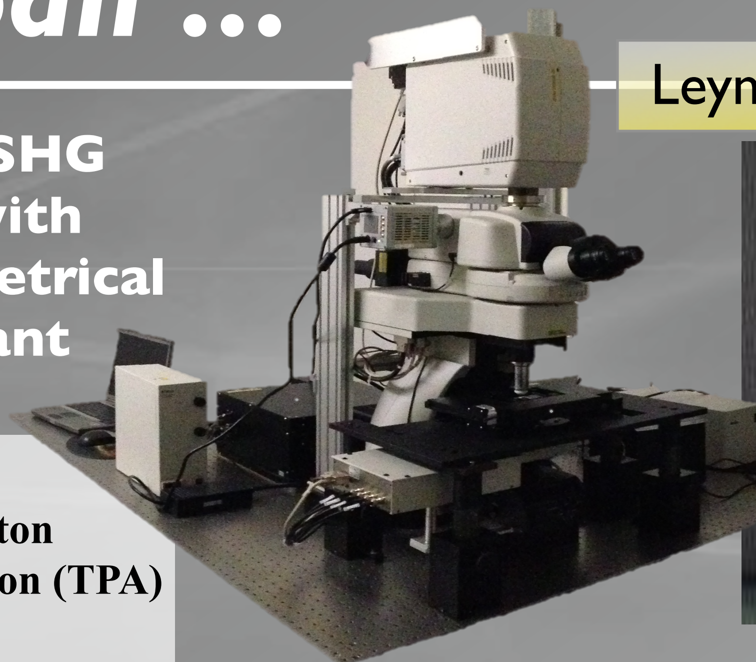
Unravelling the basis for generalizing the approach to larger sets of samples

Step 2 - catching the ball …

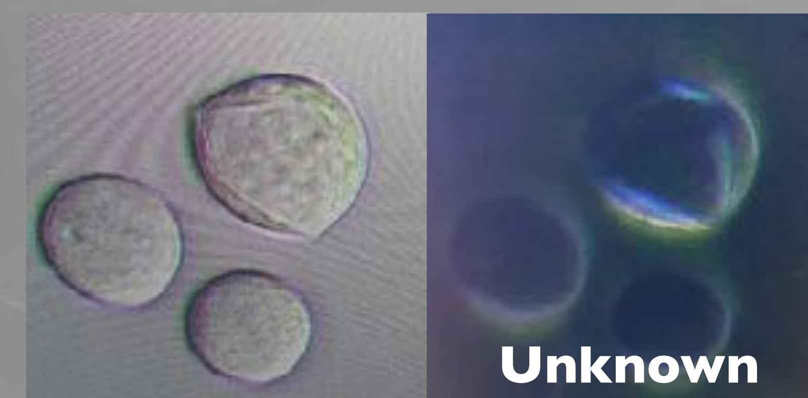


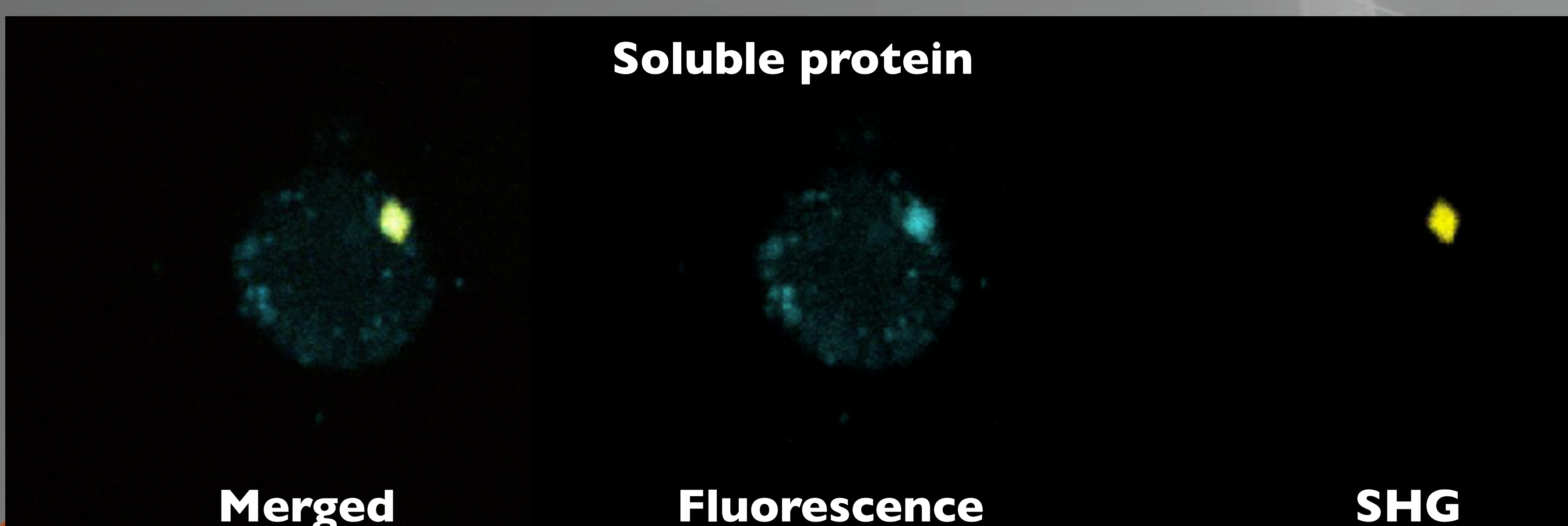
The coherent nature of signal generation in SHG microscopy is one fundamental difference with fluorescence microscopy, as it makes the geometrical structure of the sample become an important parameter !!!

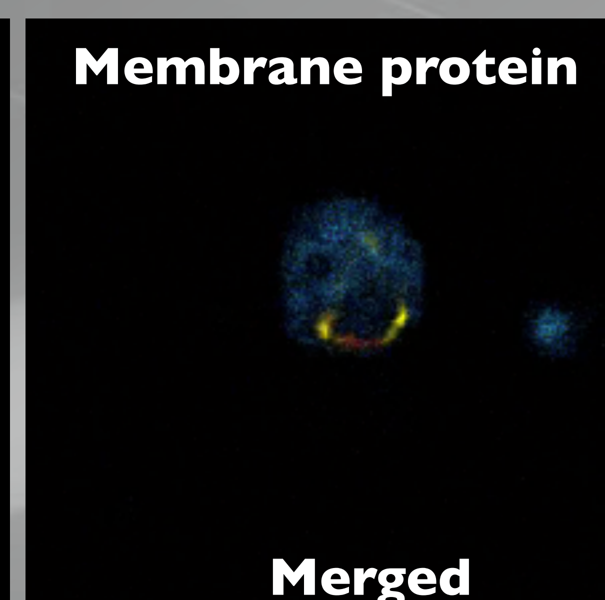




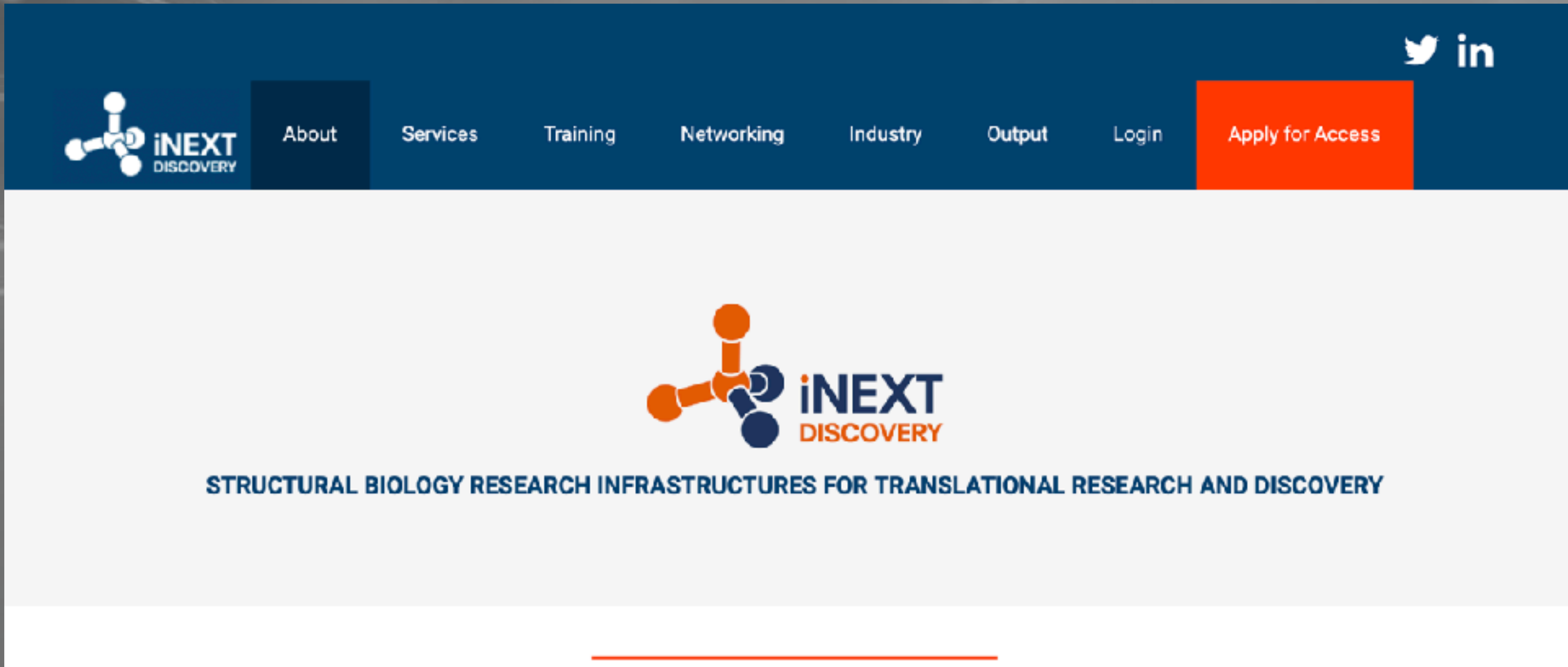
Leymarie - *accepted*







ivMX within iNEXT Discovery (EU)



ivMX within iNEXT Discovery (EU)

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Several **practical workshops** were originally planned to be organized by our partners that want to share their expertise in state-of-the-art technological developments in X-ray technology, NMR spectroscopy, Electron Microscopy and Biophysics. Most of these have been postponed and will take place later in the project, others can be converted into online events. At this time, we are not sure if the 2021 practical workshops can take place.

Our practical workshops are listed below, we will announce and include links to the information/registration sites when they are being prepared!

A list with our **other physical and online events** can be found **here**.

Subject	Organizer	City, Country	Date
Fragment screening	HZB	Berlin, DE	(2021)
Serial crystallography	EMBL-HH	Hamburg, DE	(2021)
In vivo crystallisation	SOLEIL	Paris, FR	(2021-22)
Sample preparation for single particle EM (4X)	DIAMOND	Oxford, UK	(2021-22)
Cryo-electron tomography and cryo soft X-ray tomography	EMBL-HD / ALBA	Online event	April 8-9 2021
Single particle EM data processing (2X)	CSIC	Madrid, ES	(2021-22)
NMR sensitivity enhancement	UU	Utrecht, NL	(2022-23)
Kinetic structural biology in cells	BMRZ	Frankfurt, DE	(2022-23)

ivMX within NextBINDS (Japan)

ivMX within NextBINDS (Japan)

ivMX within NextBINDS (Japan)

トップ | BINDSについて | 支援利用について | BINDSの成果 | お問い合わせ

A1-2 タンパク質細胞内結晶化支援

ユニット名

構造解析ユニット

支援担当者

所属		① 名古屋大学 シンクロトロン光研究センター ② 名古屋大学 シンクロトロン光研究センター ③ 名古屋大学 シンクロトロン光研究センター
氏名		① シャバス レオナルド ② 梅名 泰史 ③ 小野田 浩宜
AMED	課題名	生命科学と創薬研究に向けた相関構造解析プラットフォームによる支援と高度化

タンパク質X線結晶解析支援 +

タンパク質X線自由電子レーザーによる構造解析支援 +

Acknowledgments

For the complete story:
Poster 3P-070

P. MONTAVILLE
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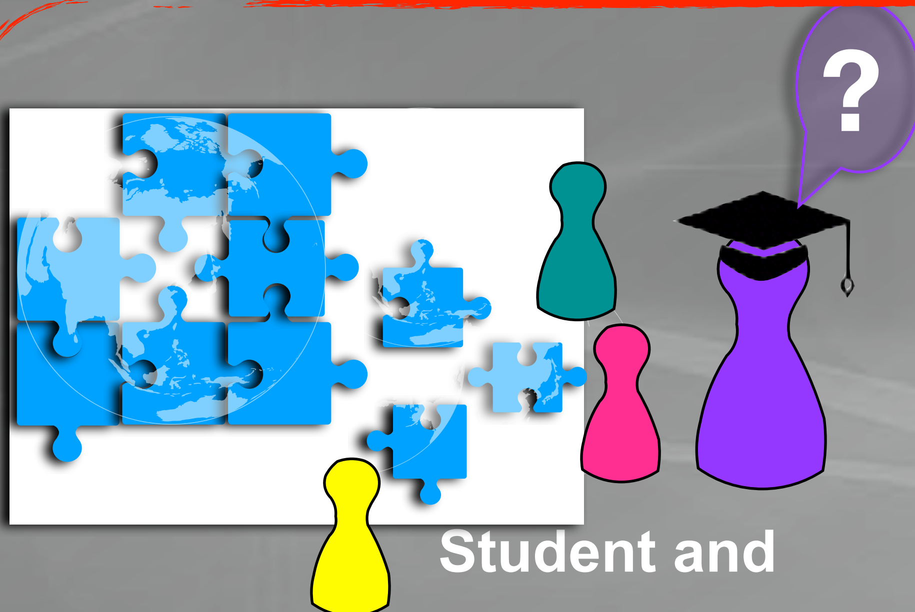
N. COUSSENS

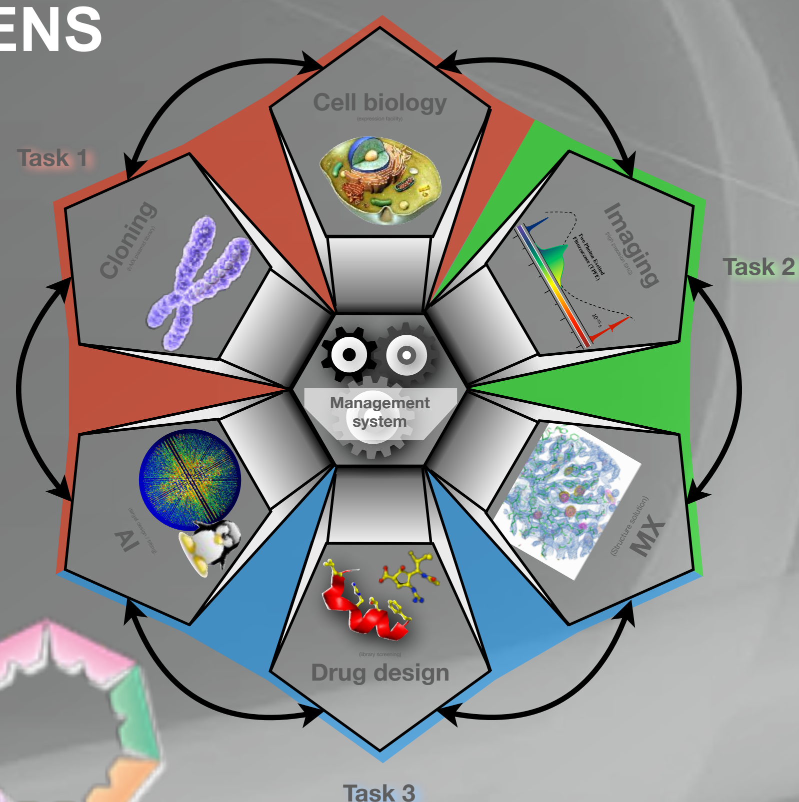
T. ISABET
C. BREWEE
O. LEYMARIE
A. GARDAIS
B. PINEAU
R. VASIREDDI



Student and
post-doctoral positions
available

l.chavas@nusr.nagoya-u.ac.jp

Platform for in vivo crystallization



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S. GHOSH
E. TOKUNAGA

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