PAUL SCHERRER INSTITUT

PSI

NanED Workshop Mainz, Germany

Universität Basel

H2020-MSCA ITN
Grant n. 956099

NanED

Optimal data acquisition of biological samples

Boosting data quality of electron diffraction through novel technologies

BIOZENTRUM
Universität Basel
The Center for Molecular Life Sciences

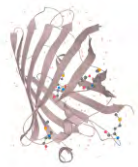
Fluorescent proteins: a way to quantify electron radiation damage

University of Basel

Structural damage vs functional damage

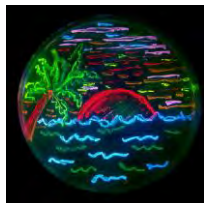
(Decay of Bragg peaks intensity) (fluorescence)

- wtGFP
- mScarlet
- muGFP
- eGFP
- Aquamarine
- YFP



GFP from Aequorea Victoria [1]

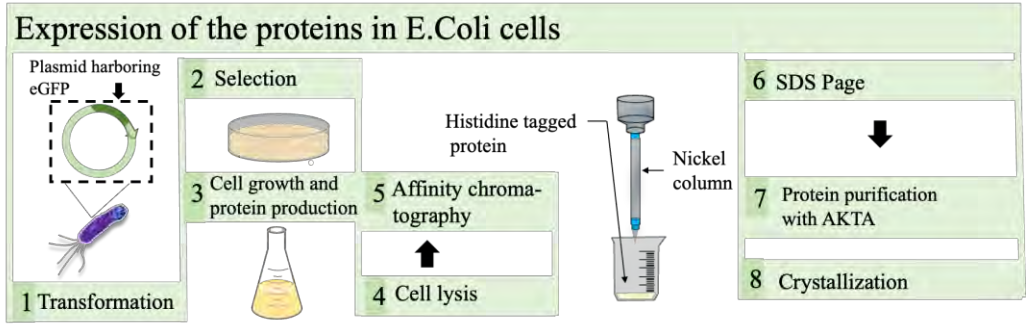
Mice with GFP inserted into their genomes for neurology studies



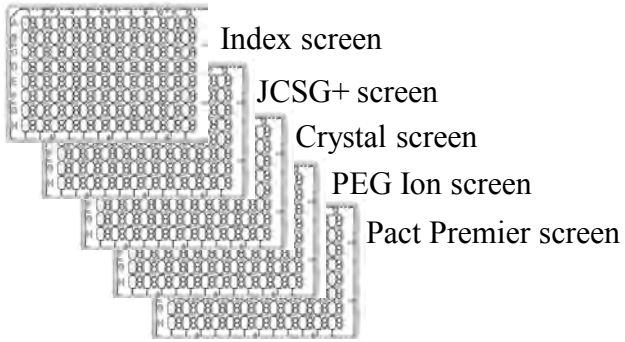
Fluorescent E. coli *on agar* [2]

Need for good crystal quality

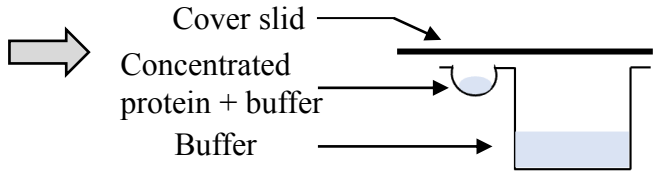
eGFP crystals preparation:



Commercial screening kits



Sitting drop vapor diffusion method



[1] Deposited: Aug 1996, Ormo, M. et al. [2] Fluorescent E. coli on agar, Nathan Shaner, photography by Paul Steinbach, created in the lab of Nobel Prize winner Roger Tsien

Nice rode like eGFP crystals...

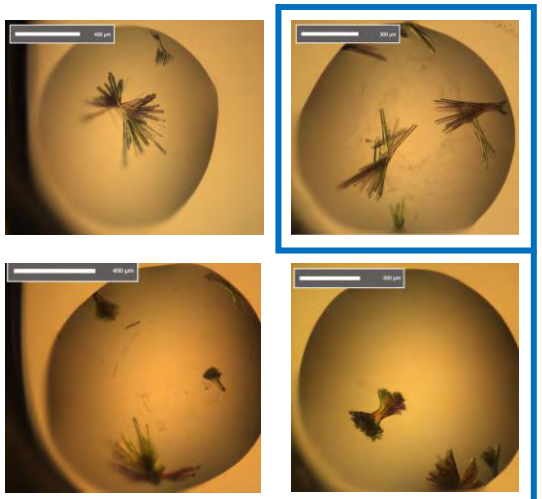


Figure 1: eGFP crystals images taken with the RockImager

…but too big

« If you can see them with the light microcope, they are already too big »

How to reduce their size without breaking or slicing them?

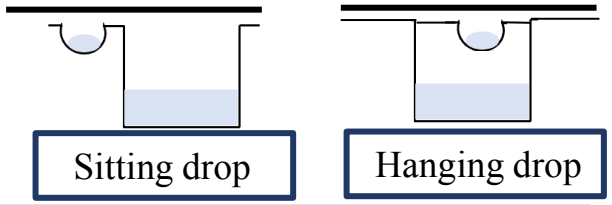
Try to grow crystals with suitable size in the first place. Avoid as much as possible the viscosity of the growth environment to produce better quality grids.

~~PolyEthylene Glycol (PEG)~~

How ?

Add salt (NaCl) to a hit with big crystals and reduce PEG concentration.

REF Condition
[Prot] = 17mg/mL
25% PEG 1500
MMT 1M pH5



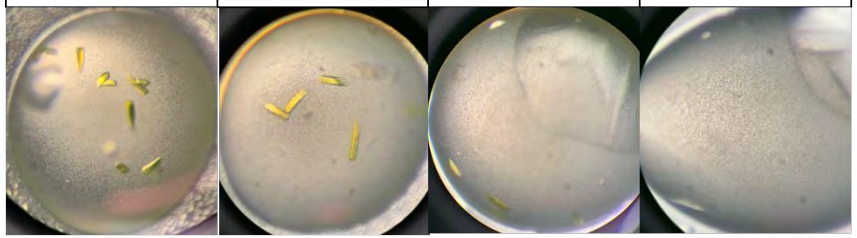
- ❑ Increase the concentration of proteine
- ❑ Add salt at different concentration in the droplet only and keep the ref buffer
- ❑ Add salt at different concentration in the reservoir and not in the droplet
- ❑ Add salt at different concentrations in the droplet and in the reservoir
- ❑ Change the ratio protein:buffer in the droplet
- ❑ Increase the droplet volume without changing the volume of the reservoir

…..

Effect of adding salt in the droplet only

Well 400 µL Buffer B1
Fixed eGFP concentration: [19.820 mg/mL]

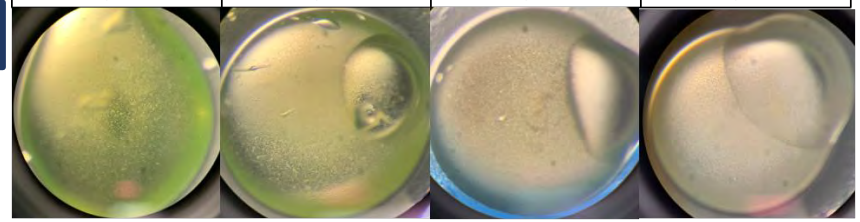
Droplet: 1µL protéine + 1 µL B1 Well 400 µL B1	Droplet: 1µL protéine + 1µL e1 Well 400 µL B1	Droplet: 1 µL protéine + 1 µL e2 Well 400 µL B1	Droplet: 1 µL protéine + 1 µL e3 Well 400 µL B1



Effect of adding salt in the droplet, and change the ratio prot:buffer in the droplet

Well 400 µL Buffer B1
Fixed eGFP concentration: [19.820 mg/mL]

Droplet: 1.5 µL protéine + 0.5 µL B1 Well 400 µL e3	Droplet: 1.5 µL protéine + 0.5 µL e1 Well 400 µL e2	Droplet: 1.5 µL protéine + 0.5 µL e2 Well 400 µL b1	Droplet: 1.5 µL protéine + 0.5 µL e3 Well 400 µL e1



Before going to the TEM to check for diffraction…

SONICC

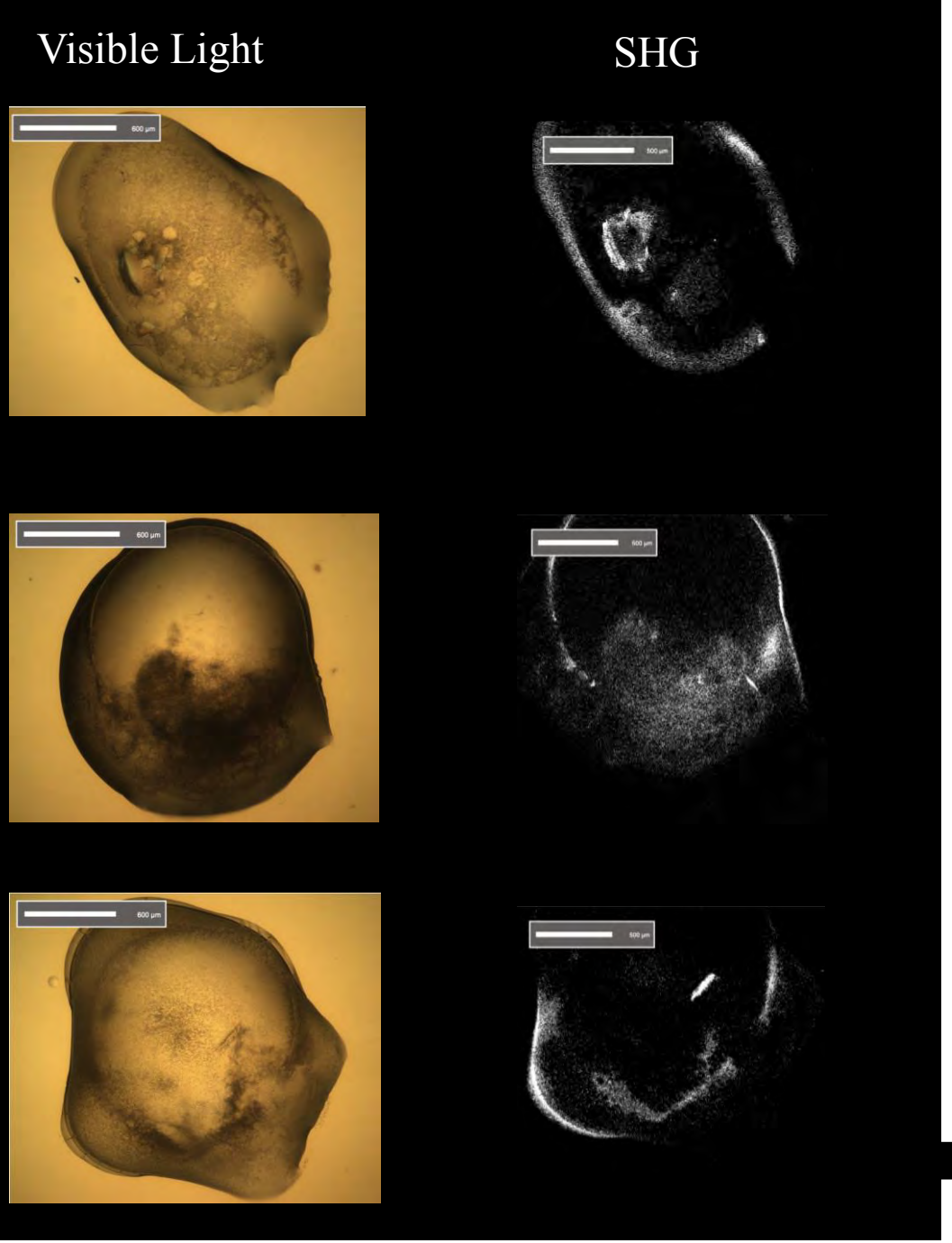
Second order non-linear
Imaging of Chiral crystals

- To find crystals buried in precipitate or detect microcristalinity in the drop.
- Crystals appear white against a stark black background and can therefore be distinguished from amorphous precipitate.

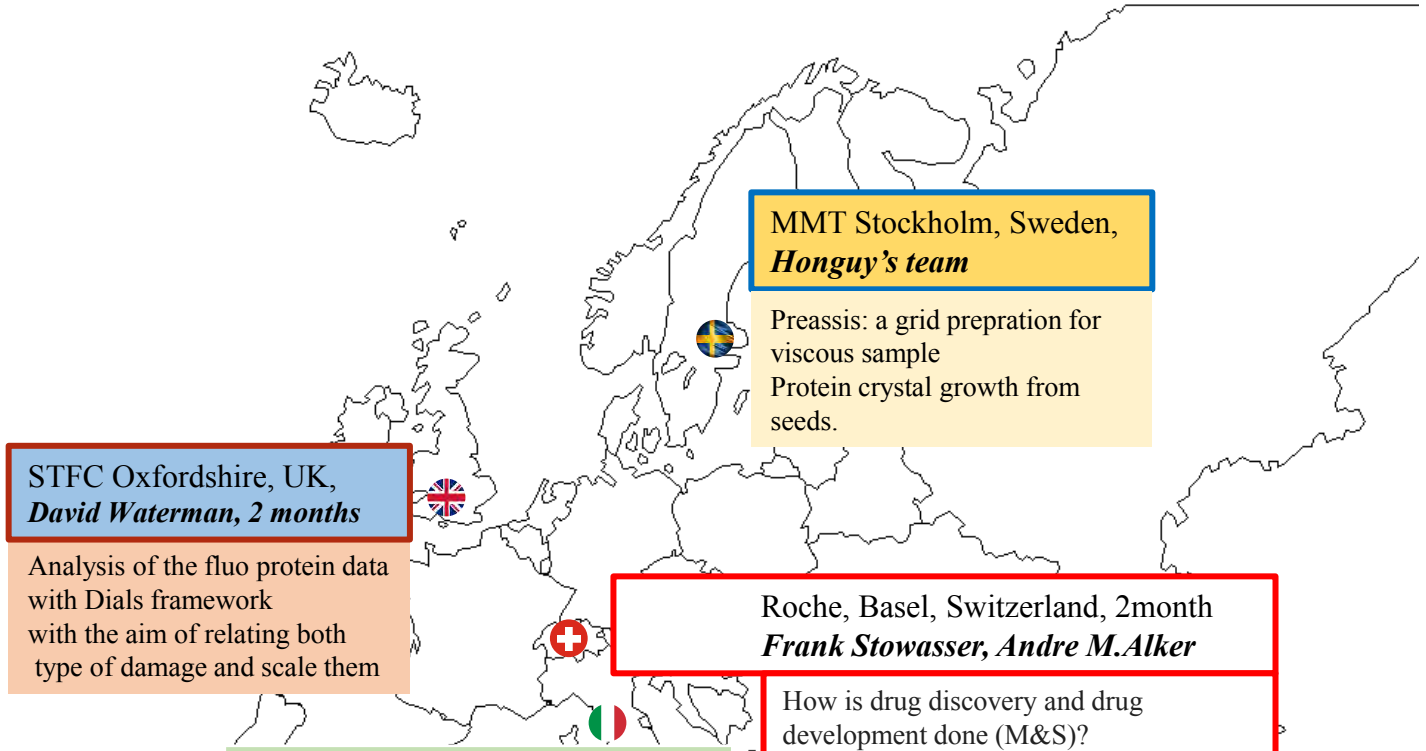
⬇

A hit ?

Unsuccessful too icy grids…



➡ To be followed



MMT Stockholm, Sweden,
Honguy's team

Preassis: a grid prepration for viscous sample
Protein crystal growth from seeds.

STFC Oxfordshire, UK,
David Waterman, 2 months

Analysis of the fluo protein data with Dials framework
with the aim of relating both type of damage and scale them

Roche, Basel, Switzerland, 2month
Frank Stowasser, Andre M.Alker

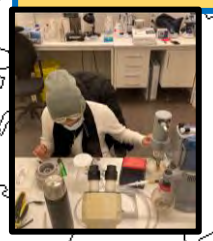
How is drug discovery and drug development done (M&S)?
What is polymorphism ?
What is the role of analytics at a pharmaceutical company and how does small molecule structure science come into play there.
Practical work: Hands on pharmaceutical samples for ED, compare data from eldico EM and Jeol F200 at PSI.

IIT, Pontedera, Italy, 1month
Mauro Gemmi's team

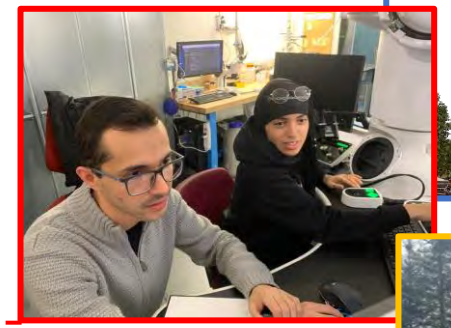
-Handling of the Zeiss Libra, 120keV
-Collection of diffraction data from MOFs, RR3 without cryo-condition, and from a protein (sample from Gratz) so an opportunity to apply knowledges acquired during my first secondment in Stockholm
-Opportunity to assist to a Fib milling session with a post doc from there

Versailles Conference

MMT Stockholm, Sweden,
Honguy's team



Biozentrum, *Basel*, PSI, *Villigen*, Switzerland



THANK YOU

IIT, Pontedera, Italy, 1month
Mauro Gemmi's team