A Thermal Stability Assay That Can Help Predict Crystallization Likelihood

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Recent Advances in Macromolecular Crystallization
Le Bischenberg, 11-14 Sept. 2011
The High Throughput Crystallization Platform at EMBL Grenoble

Some stats

- 500 Registered Users
- 1,200 samples processed per year
- 3,936,360 Crystallisation Experiments set up

European Infrastructure

Access to crystallization services for Scientists working in academic research Institutions Funded through the E.C. PCUBE project

www.pcube-EU.org
https://embl.fr/htxlab
What The HTX Lab does ...

- Crystallization Services
- Data Management: CRIMS
- New Crystallization Technologies
- Research in Signalling
Data Management: Extracting Useful Information

Protein Production

HTX => Large numbers of experiments are performed under very constant experimental conditions
Data Management: Extracting Useful Information

What’s the likelihood that a certain construct produces crystals?

Concentrate efforts on certain constructs
QC & Crystallization

- Gel Filtration
- Mass Spec
- DLS
- MALLS
- SAXS
- UAC
- NMR
- ...

“Samples that are…
  ✓ stable,
  ✓ monodisperse
  ✓ lack unfolded regions

  … show a higher tendency to crystallize”

Zulauf and D’Arcy (1992) J. Cryst Growth

Zulauf and D’Arcy (1992) J. Cryst Growth
The Crystallization Pipeline

Crystallization

LIMS DB
Biophysical Characterization & QC

![Crystallization](image1.png)

![Thermofluor](image2.png)

LIMS DB

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EXTRACT REFLECTIVE INDEX (x10^8)

150 200 250 300

CUTTING VOLUME (ml)

14 15 16 17 18 19

EXCESS REFLECTIVE INDEX

0 50 100

ELECTRICAL FIELD STRENGTH:

14000 16000 18000

20000

THRESHOLD:

0 25 45 65 85

TEMPERATURE:

25 45 65 85

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EMBL
The Fluorimetric Thermal Denaturation Assay (Thermofluor assay)

Ericsson et al, 2006, Analytical Biochem, 357
Biophysical Characterization & QC

Can the Tm of a protein inform about its crystallization likelihood?

1,534 Thermofluor experiments
657 unique samples

20mM Hepes pH7.5, 150mM NaCl
HT Crystallisation in Grenoble

Protein Production

Tecan Platform
Preparation of crystallisation screens

Web access
138 Registered users among the five institutes
https://embl.fr/htxlab

Nanovolume Crystallisation
Experiment size 100 + 100 nl
90 μl are enough for extensive screening (576 conditions).
6,000 Exp. Per week.
More than 700,000 exp. since start

Automated imaging
Capacity 144,000 exp.

Refinement

Data Collection

20 deg
5 deg
tomated imaging
Capacity 144,000 exp.
The High Throughput Crystallization Platform at the PSB

https://embl.fr/htxlab
www.pcube-EU.org

Web application
External Evaluation
FedEx samples to HTX lab
Results through Web (CRIMS)
Nanovolume VD Crystallization Experiments

100 nl + 100 nl drops
9 samples in parallel
576 exp /sample
IMAGING

FORMULATRIX RI500

- Capacity 500 plates (upgradeable to 1000)
- working Temp 4 deg
- 5 mp camera
- fully integrated in own LIMS
- Very reliable 2x Robodesign Minstrel III

- Capacity 500 plates
- working Temp 20 deg
- 5 mp camera
The Crystallization Information Management System (CRIMS)

User Interfaces
• Project/target/sample trees
• Drop visualization
  (Scoring, annotation, drop History, plate navigation)
• Summary

Operator Interfaces
• Request & Sample management
• Quality control
• Hit Tracking

✔ Currently operating in 4 laboratories in Europe

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Data Management: Extracting Useful Information

• High throughput approaches in crystallization generates new opportunities.
  • Large numbers of experiments are performed under very constant experimental conditions

But…

• No automatic reading of crystallization experiments..
• Large number of users
• The use of HT resources and Data management systems is discontinuous.

… feedback is not for granted
The CRIMS Hit Tracking Module

Requested 3 months after sample run

User has 4 weeks to answer (3 email reminders)

If no answer is received user is inactivated

Systematic Feedback on Results of Crystallization Assays

Systematic Feedback on Results of Crystallization Assays
Biophysical Characterization & QC

- Crystallization
- Thermofluor

1.534 Thermofluor experiments
657 unique samples

20mM Hepes pH7.5, 150mM NaCl

25 45 65 85

Temperature

0 2000 4000 6000 8000 10000 12000 14000 16000 18000 20000

20mM Hepes pH7.5, 150mM NaCl
The Crystallization Pipeline

Protein Production

Crystallization

LIMS DB
Biophysical Characterization & QC

- **Single sigmoid curve**
  - Crystallization rate: 42.7%
  - 437 samples (66.5%)

- **Non interpretable curve**
  - Crystallization rate: 36.6%
  - 196 samples (29.8%)

- **Complex curve**
  - Crystallization rate: 38.1%
  - 24 samples (3.7%)
Biophysical Characterization & QC

- **Tm Histogram**

  ![Tm Histogram](image)

  - Proportion of samples in class (%) for different Tm ranges:
    - [25,34]: 9
    - [35,40]: 33
    - [41,45]: 78
    - [46,50]: 116
    - [51,55]: 85
    - [56,60]: 33
    - [61,65]: 29
    - [66,70]: 21
    - [71,75]: 18
    - [76,95]: 15
Crystallization Success Rate & Tm

“Thermophilic proteins contain a lower proportion of unstructured regions”
Crystallization Success Rate & Tm


A simplified thermofluor experiment may help estimate the crystallization likelihood of a sample.

- consumes very low amounts of sample (0.4nmol)
- It is easy to carry out,
- rapid and inexpensive
- compatible with 96-well format

Results may be used to:

- Determine optimal incubation temperature
- Prioritize certain constructs
- decide when to carry experiments to improve sample properties

Average crystallization rate

26.8%  49.1%

Extremophiles

Tm classes (ºC)
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Acknowledgements

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