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Recent Work

Title
The LBNL Crystallization and Structure Determination Pipeline

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The LBNL Crystallization and Structure Determination Pipeline
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PipeLine Work at LBNL.

Experiments Conducted at the Facility
- For purified proteins
  - Crystallization screening and optimization
  - Image acquisition and scoring for crystallization experiments
- Crosslinking
  - Glutaraldehyde crosslinking
- Slow dehydration
- Anomalous signal, crystal decay and etc.; mostly used 1M NaBr, soaking for about 3-5 mins.

An Alternative Crystallization Approach: Modified Microbatch

Advantages:
- Water never stops evaporating
- Easy in-drop cryo and soaking
- Small storage footprint
- Easy to set up by hand for individual experiments or small condition screens

Crystal Screening Automation at ALS

- Biophotonic crystal centering interface
- Crystals are in the taskQ for screening
- Data collection
- Structure determination
- Structure refinement upon request

Post-crystallization Treatment:
- Slow dehydration
  - Air drying in cryoprotectant
- Crosslinking
- Glutaraldehyde crosslinking
- Increasing precipitant concentration
- Heavy atom soaking
  - Bi or other heavy metals soaking
- Crystal annealing
- Back annealing (2A annealing on loop (AL); macromolecular crystal annealing (MCA))

Crystallization from Random Screens to Optimization
- Basic 448 random screen conditions, 544 conditions if complexes (150 d-proteins + 150 e-protein solutions – total 300 d-protein dispersions)
- Hampe1 & D • Wizard II & D • Wizard III PEGs • Mif 
  - (ISFI MCB + Rob-ConSec)
- Initial optimization with coarse gradients to identify most promising component and range for fine optimization
- "g" 3-3 grid optimization: pH value; PEG concn.; salt concn.
- Full hit-parameter space sampling with a knowledge-based random screen generator, CrxTool (after Crystool by Segelke et al.), used in selected cases.
- Optimization reagents generated @150uL with Tecan robot

Cryoprotectant Screening and Crystal Harvesting
- No cryo/RT + various sets of cryoprotectants
- E.g. physical, synthetic glycols, PEGs, oil, (DMP, 1,4-butanediol), sugars, salts, and etc.
- Based on cryoprotectant conditions and crystal supplies, try 1-5 crystals in each category if possible.
- Score diffraction results and refine conditions

RT Diffraction screening used as well
- Harvest crystals with Mitegen capillary tools

Recent New Structures Determined with Data collected at the LBNL facility

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