UV/Visible Light Imaging and BioSAXS

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Automated Drop Imaging

• Resolution: What is the smallest size crystal I can detect?
• Detection: Can I see crystals and any other important events even under difficult conditions (precipitate, membrane screens, etc)?
• Image analysis: How much time will I have to spend to score all my images?
New approach to combined UV/visible imaging

Single optical train – Directly compare images
Monochromatic camera – Highest resolution
Integrated polarisation
Integrated UV source
Microscope objectives
Our Solution: High Resolution and Automated Imaging

- We implemented 3 types of objectives in our new imager:
The Result

2 micron thick needle
Introducing Fluor-Score: UV based auto-scoring

• Issue with conventional image analysis approaches: False negatives (missed crystals)
• Flour-Score focuses on detecting clear drops
• Score = Regional variations of fluorescence signal
BioSAXS
A Complementary Technique
High Throughput Structure Success

Northeast Structural Genomics Consortium
High Throughput SAXS Success

Northeast Structural Genomics Consortium
Profile conversion

- **SAXS pattern**

  - Log($I$) vs. $q$ [$\text{Å}^{-1}$]

- **Guinier plot**

  - $\ln(I)$ vs. $q^2$ [$\text{Å}^{-2}$]

- **Kratky plot**

  - $q^2 I$ vs. $q$ [$\text{Å}^{-1}$]

- **Pair distribution function**

  - $P(r)$ vs. $r$ [Å]
Biological applications of SAXS

- Predictor for crystallizability
- *Ab initio* shape determination of native protein state
- Monitoring conformational changes
- Ligand binding studies
- Characterization of oligomeric states and missing loops/residues
- Differentiation of monodisperse and aggregated proteins in solution
- Differentiation of folded and unfolded protein in solution
- Long distance constraint in NMR protein structure refinement
Predictor of Protein Crystallizability

- Too Soluble
- “Crystallization Slot”
- Amorphous Precipitate
BioSAXS-1000

- Pilatus 100K detector
- Sample
- Kratky block
- Source
BioSAXS-1000 optic design

X-ray Source -> 3 pin holes -> Sample -> Detector

Pin hole SAXS

2D Kratky

BioSAXS-1000 and Synchrotron Data Quality Comparison

Comparison data: BL4-2 at SSRL(red) and BioSAXS-1000 Blue
SSRL data courtesy of T. Grant, J. Luft and E. Snell (HWI)
SAXS data collected prior to setting up crystallization experiments can tell you something about the probability of success.

SAXS following structure determination can be useful for many situations.

Crystallization and X-ray analysis can be an iterative process.
Thank You

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