

Acknowledgments



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Executive Summary: Results



- **Succeeded in orienting dp's down to $\sim 10^{-2}$ ph/pixel**
 - Extreme shot noise limit, with and without “background”
 - In presence of “background”
 - Critical threshold for biomolecules in XFEL
- **Using only $\leq 10^5$ photons**
 - XFEL delivers 10^9 photons in minutes
- **Single-molecule crystallography now possible in principle**
 - Based on analysis of simulated data
- **Possible to sort molecular species & conformations**
 - Mitigate purification problems (major bottleneck)
 - Molecular conformation key in reactions

- **Orders of magnitude**
 - “Canonical” 500kD biomolecule scatters $\sim 4 \cdot 10^{-2}$ ph/pixel
- **Manifold-embedding route to snapshot orientation**
 - Common-line needs 100 – 1000x more flux than available
 - Manifold embedding uses all photons at same time
 - Recovered structure of small protein
- **Sorting molecular species & conformations**
 - Sorted closed & open conformation of adenylate kinase
- **Future prospects**
 - What *might* be possible

Single-Molecule X-ray Scattering: Orders of Magnitude



- **Assumptions:**
 - a. Macromolecule with N atoms scatters as N carbon atoms
 - b. Pixel area: $(1/2L)^2$
 - c. Need 10^3 scattered photons per pixel
 - d. Scattered amplitude: low-angle $\sim N^2$; high-angle $\sim N$
 - e. 0.1nm radiation (12.4 keV)
 - f. 500 kDa (globular) molecule
 - Yeast proteins: ~ 50 kDa
 - Largest known proteins (titins) ~ 3000 kDa
- **Number of scattered photons/pulse/pixel:**

$$n \approx \Omega_{\text{pixel}} W \sigma_C N_{\text{atoms}} = \frac{\lambda^2}{4a^2} W \sigma_C N_{\text{atoms}}^{1/3}$$

Single-Molecule X-ray Scattering: Orders of Magnitude



X-ray Beam		Flux per mm ²	Counts per pulse per pixel		No. of Pulses for 10 ³ scattered photons/pixel		Time (sec) for 10 ³ scattered photons/pixel	
Source	Ø (µm)	per pulse	Small Angle	Large Angle	Small Angle	Large Angle	Small Angle	Large Angle
XFEL	0.1	3.10 ²⁰	10 ⁴	4.10 ⁻²	0.1	2.10 ⁴	10 ⁻³	250
APS	0.01	10 ¹⁵	4.10 ⁻²	2.10 ⁻⁷	3.10 ⁴	6.10 ⁹	3.10 ²	6.10 ⁷

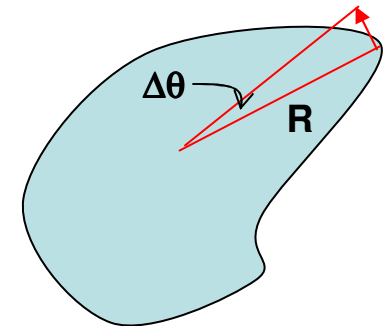
1. XFEL scatters 10⁹ photons from a 500 kDa protein in minutes
2. PLENTY of scattered photons; VERY FEW scattered per shot
3. Orienting Diffraction patterns is KEY

Alignment Requirements



- Object diameter D , imaged to resolution d
- Required orientational accuracy:

$$\Delta\theta_{shannon}^{orient} = \frac{1}{2} \frac{d}{R} = \frac{d}{D}$$



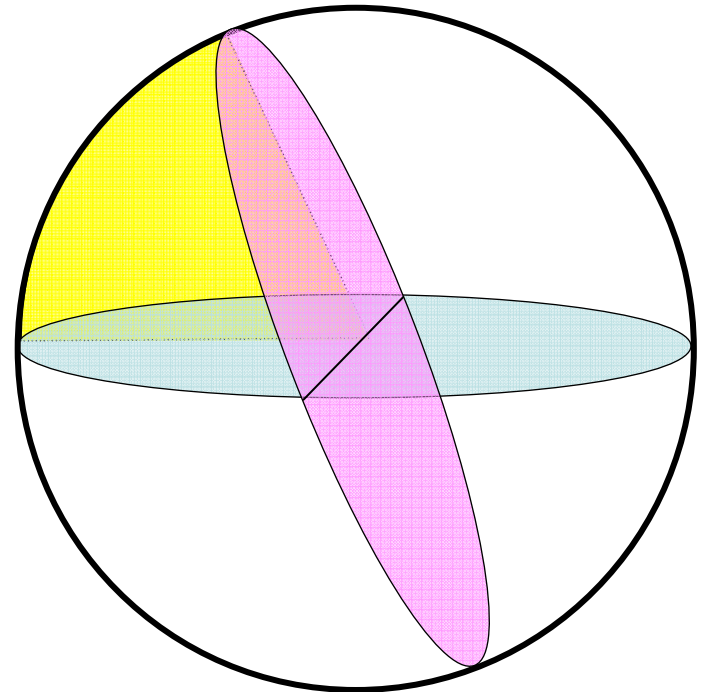
- No. of orientational classes:

$$\begin{aligned} N_{nodes} &= \frac{1}{2} \cdot \left(\frac{\text{Area of 4-sphere}}{\text{Shannon element on 4-sphere}} \right) \cdot \frac{1}{\text{symmetry}} \\ &= \frac{8\pi^2}{S(\Delta\theta_{shannon}^{orient})^3} \end{aligned}$$

Orienting 2-D Snapshots: Common-Line Approach



- Diffraction patterns of same object share “common line” of diffracted intensity
 - “Central Section Theorem”
 - Three planes fix relative orientations
 - Two with Ewald-sphere curvature
- Needs ~10 photons/pixel
 - 1000x more than in $0.1\mu\text{m}\varnothing$ XFEL spot
 - Acta Cryst. A64, 303–315 (2008)
 - Reachable by classification & averaging?



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