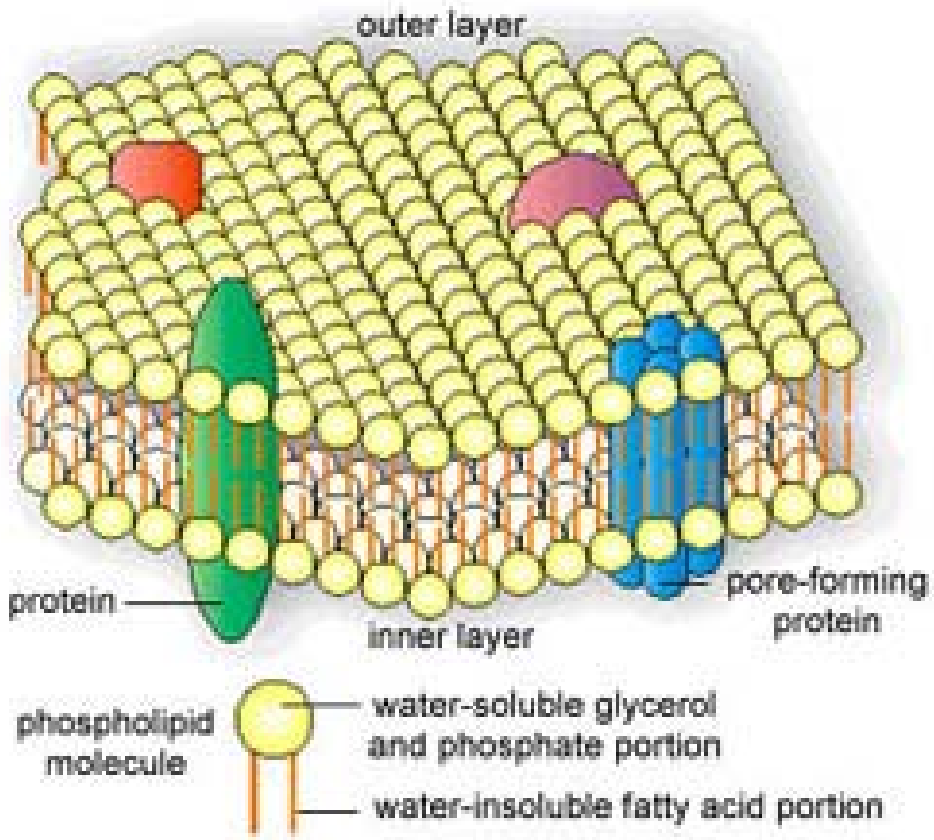
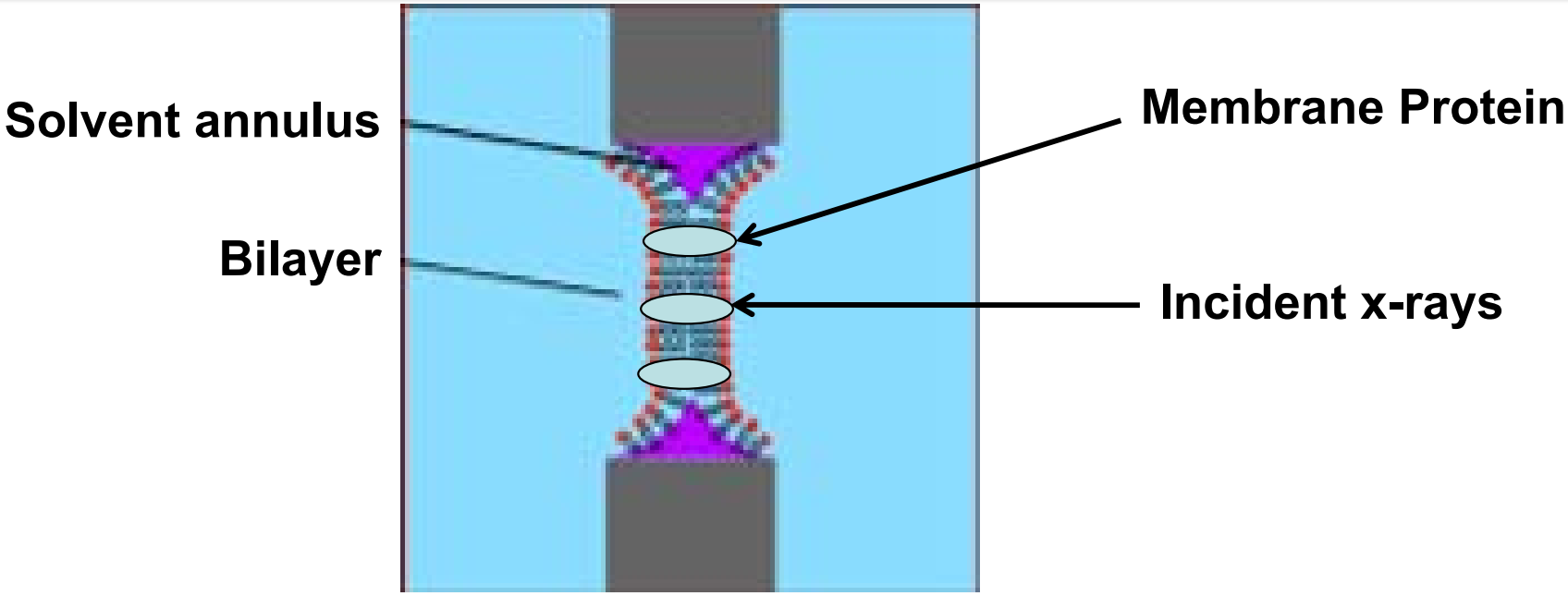
Incidence Parallel to Alignment Axis – E.g. Membrane Proteins *In Situ*



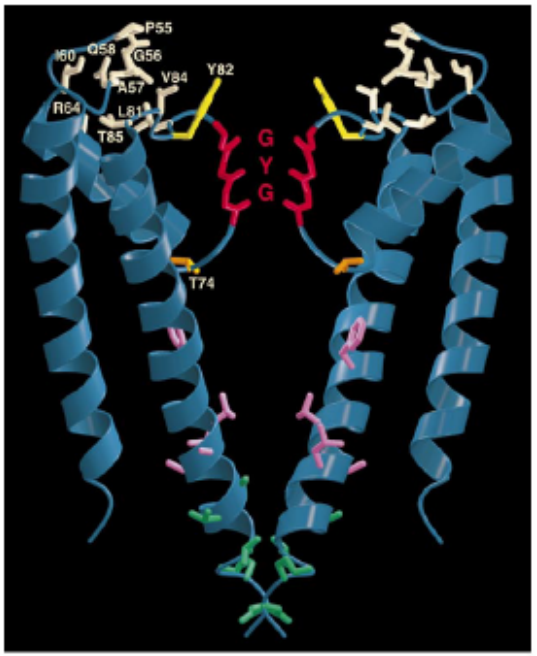
E.g. Ion-channel proteins will be in random locations in membrane and in random orientations about an axis perpendicular to the membrane.

Proposed Experiment: Black Lipid Membrane



Small aperture created in a hydrophobic material such as Teflon. Solution of lipids dissolved in an organic solvent applied by a brush or syringe across aperture.

By this means can create a bilipid membrane with membrane proteins inserted. The proteins will be in random positions in the plane of the membrane and at random orientations about the membrane normal. The aim is to determine the structure of each individual protein from the resulting multi-protein diffraction pattern



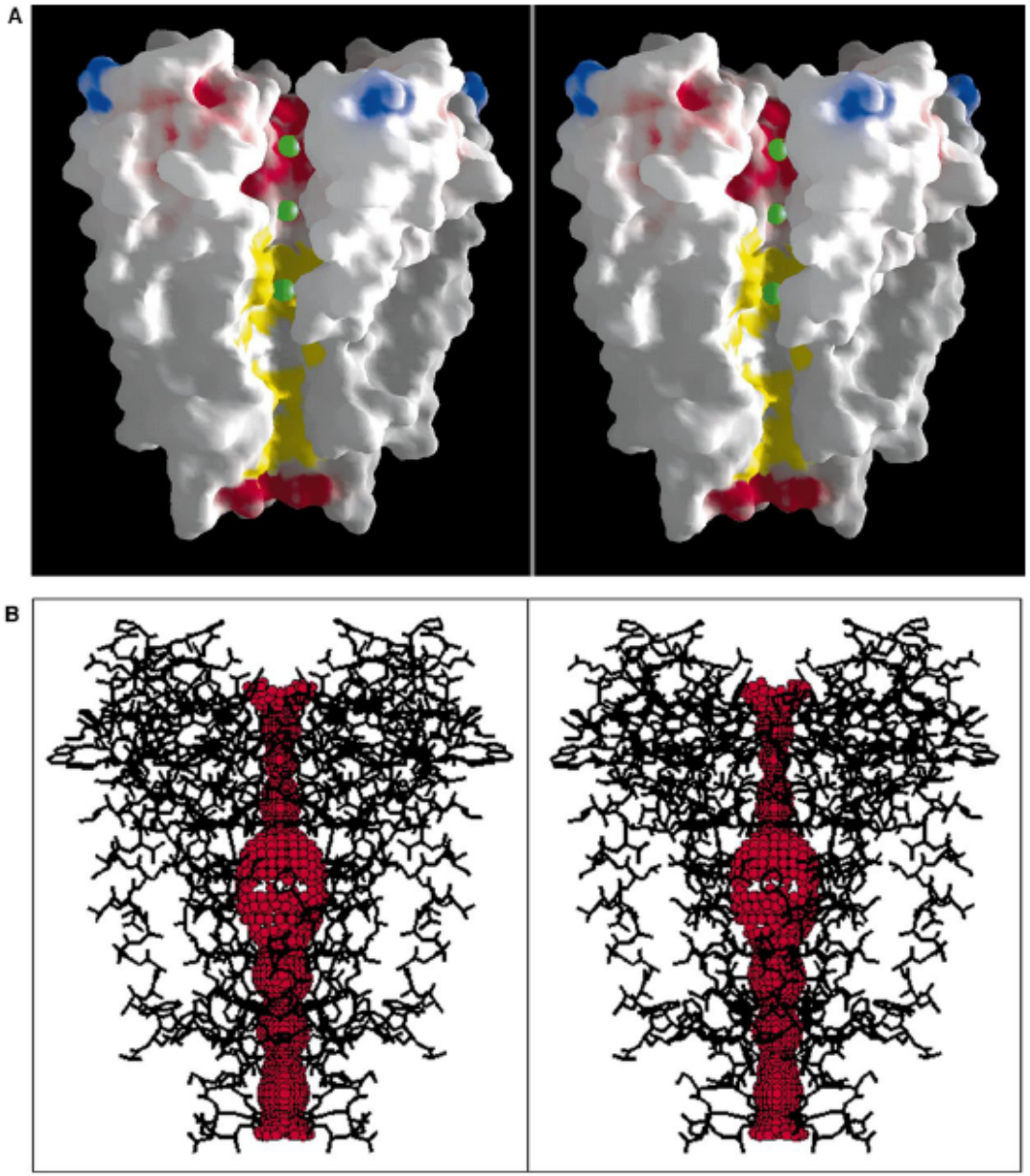
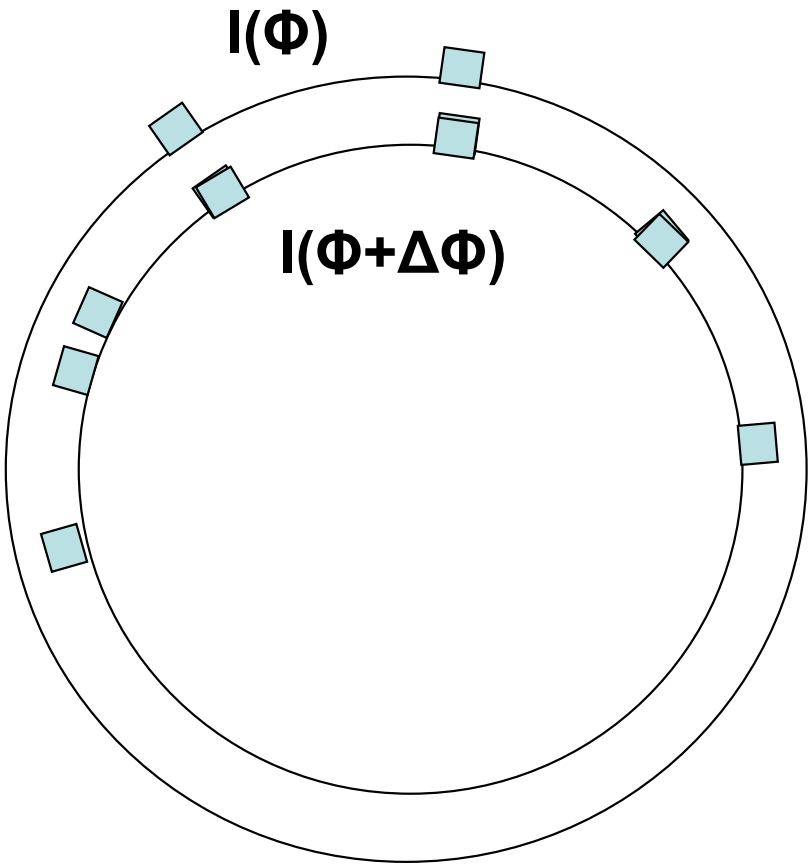
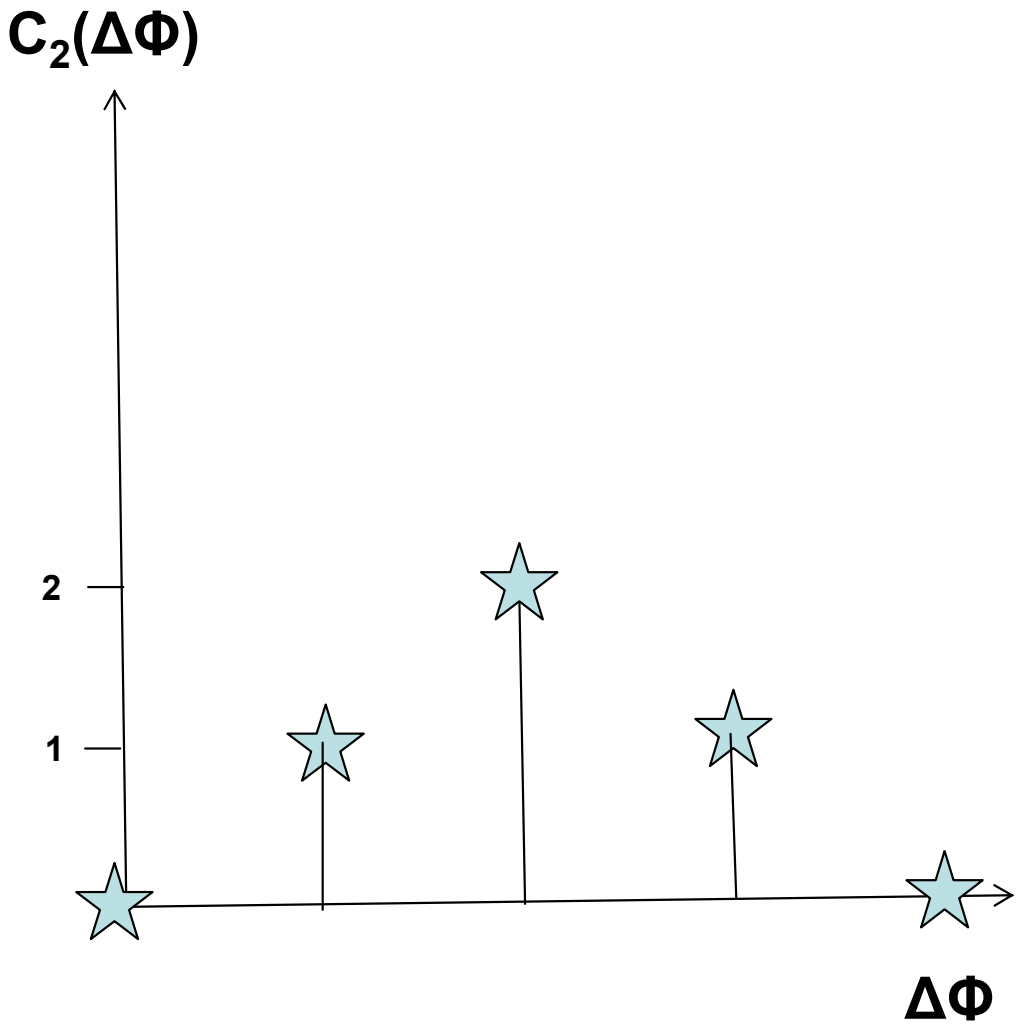


Fig. 4 (above). Mutagenesis studies on *Shaker*: Mapping onto the KcsA structure. Mutations in the voltage-gated *Shaker* K^+ channel that affect function are mapped to the equivalent positions in KcsA based on the sequence alignment. Two subunits of KcsA are shown. Mutation of any of the white side chains significantly alters the affinity of agitoxin2 or charybdotoxin for the *Shaker* K^+ channel (*12*). Changing the yellow side chain affects both agitoxin2 and TEA binding from the extracellular solution (*14*). This residue is the external TEA site. The mustard-colored side chain at the base of the selectivity filter affects TEA binding from the intracellular solution [the internal TEA site (*15*)]. The side chains colored green, when mutated to cysteine, are modified by cysteine-reactive agents whether or not the channel gate is open, whereas those colored pink react only when the channel is open (*16*). Finally, the residues colored red (GYG, main chain only) are absolutely required for K^+ selectivity (*4*). This figure was prepared with MOLSCRIPT and RASTER-3D. **Fig. 5 (right).** Molecular surface of KcsA and contour of the pore. (**A**) A cutaway stereoview displaying the solvent-accessible surface of the K^+ channel colored according to physical properties. Electrostatic potential was calculated with the program GRASP, assuming an ionic strength equivalent to 150 mM KCl and dielectric constants of 2 and 80 for protein and solvent, respectively. Side chains of Lys, Arg, Glu, and Asp residues were assigned single positive or negative charges as appropriate, and the surface coloration varies smoothly from blue in areas of high positive charge through white to red in negatively charged regions. The yellow areas of the surface are colored according to carbon atoms of the hydrophobic (or partly so) side chains of several semi-conserved residues in the inner vestibule (Thr^{75}, Ile^{100}, Phe^{103}, Thr^{107}, Ala^{108}, Ala^{111}, Val^{115}). The green CPK spheres represent K^+ ion positions in the conduction pathway. (**B**) Stereoview of the entire internal pore. Within a stick model of the channel structure is a three-dimensional representation of the minimum radial distance from the center of the channel pore to the nearest van der Waals protein contact. The display was created with the program HOLE (*34*).

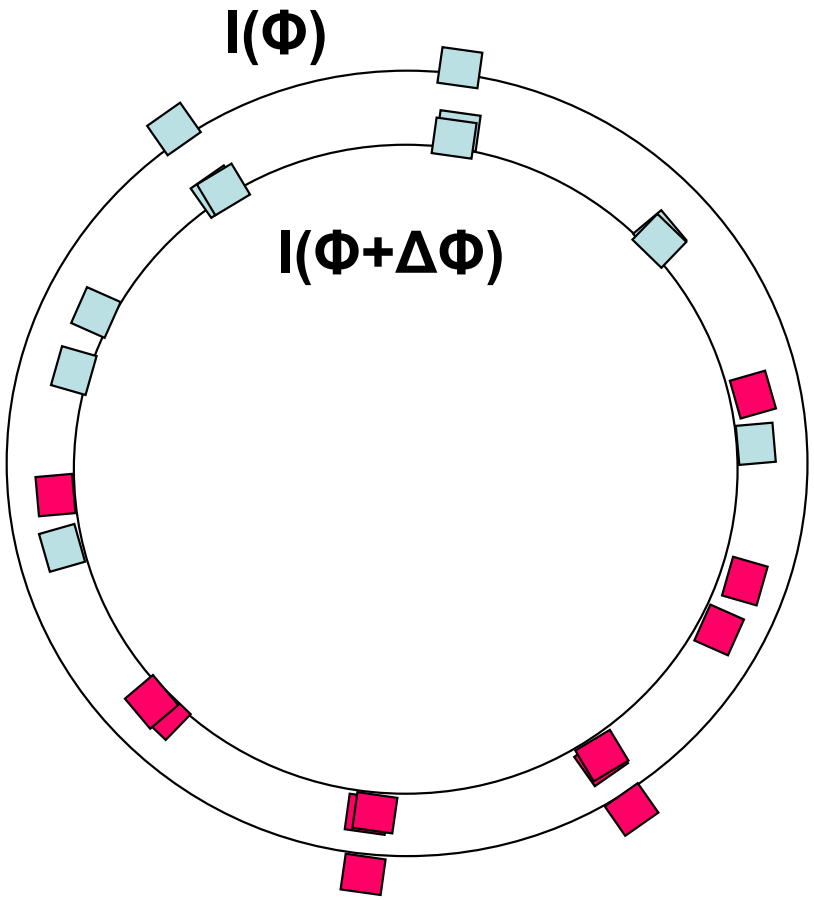
Autocorrelation of DP of a single particle

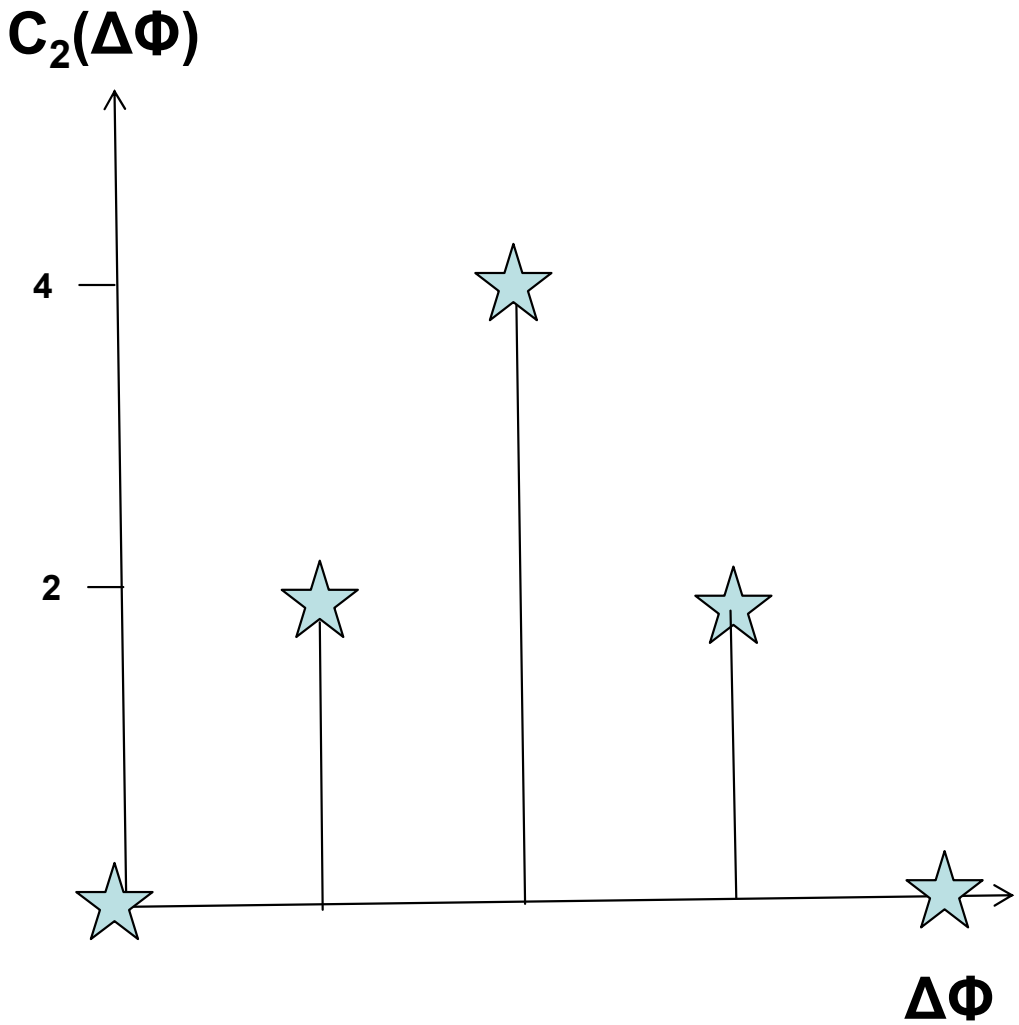




$$C_2(\Delta\Phi) = \sum_{\Phi} I(\Phi) I(\Phi + \Delta\Phi)$$

Autocorrelation of superposed DPs of N=2 particles in different orientations





$$C_2(\Delta\Phi) = \sum_{\Phi} I(\Phi) I(\Phi + \Delta\Phi)$$

$C_2 \propto N$ **but otherwise identical**

Phases of Expansion Coeffs.:
Positivity Constraint

For a single resolution ring

$I_M(q)=|I_M(q)|_{corr}\exp(2\pi i.rand)$

Evaluate
$I(q,\theta)=\sum_M I_M(q)\exp(im\theta)$

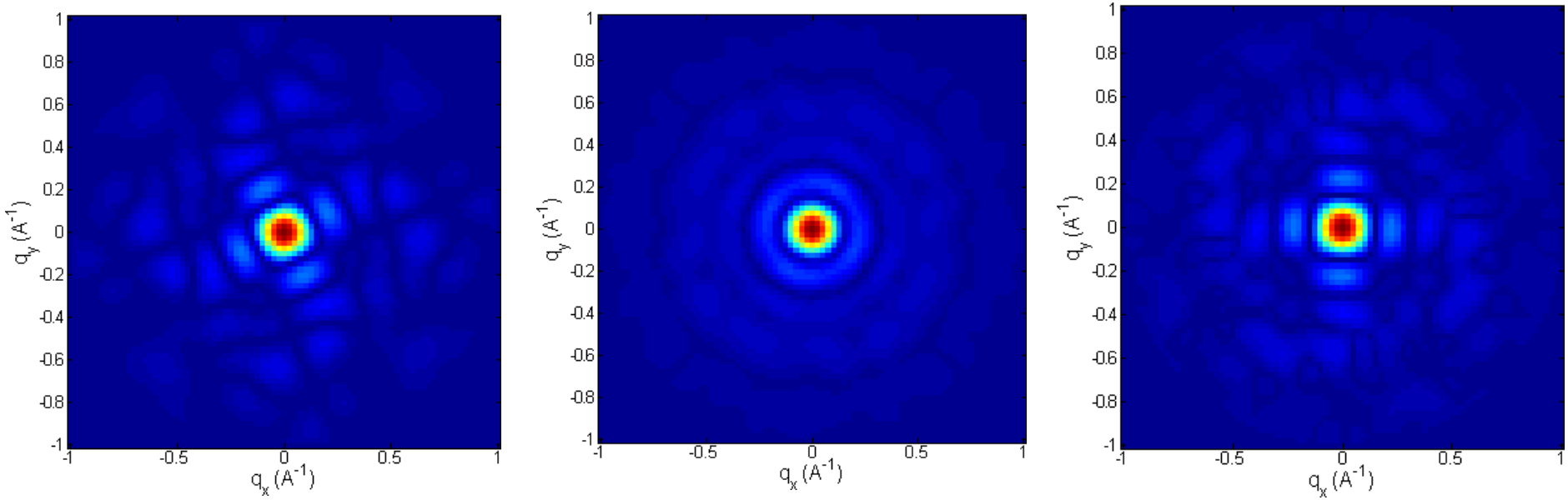
Positivity constraint
$I'(q,\theta)=0$ if $I(q,\theta)<0$
$=I(q,\theta)$, otherwise

$I'_M(q)=\int I'(q,\theta)\exp(-iM\theta)d\theta$

$I_M(q)=|I_M(q)|_{corr}\exp(i.\arg(I'_M(q))$

Single-Particle DP from Multiparticle DPs
Particles Frozen in Space or Time

Use radiation with pulse length shorter than rotational diffusion time, or freeze the particles in e.g. an ice sheet.
Application to ion-channel membrane protein in situ



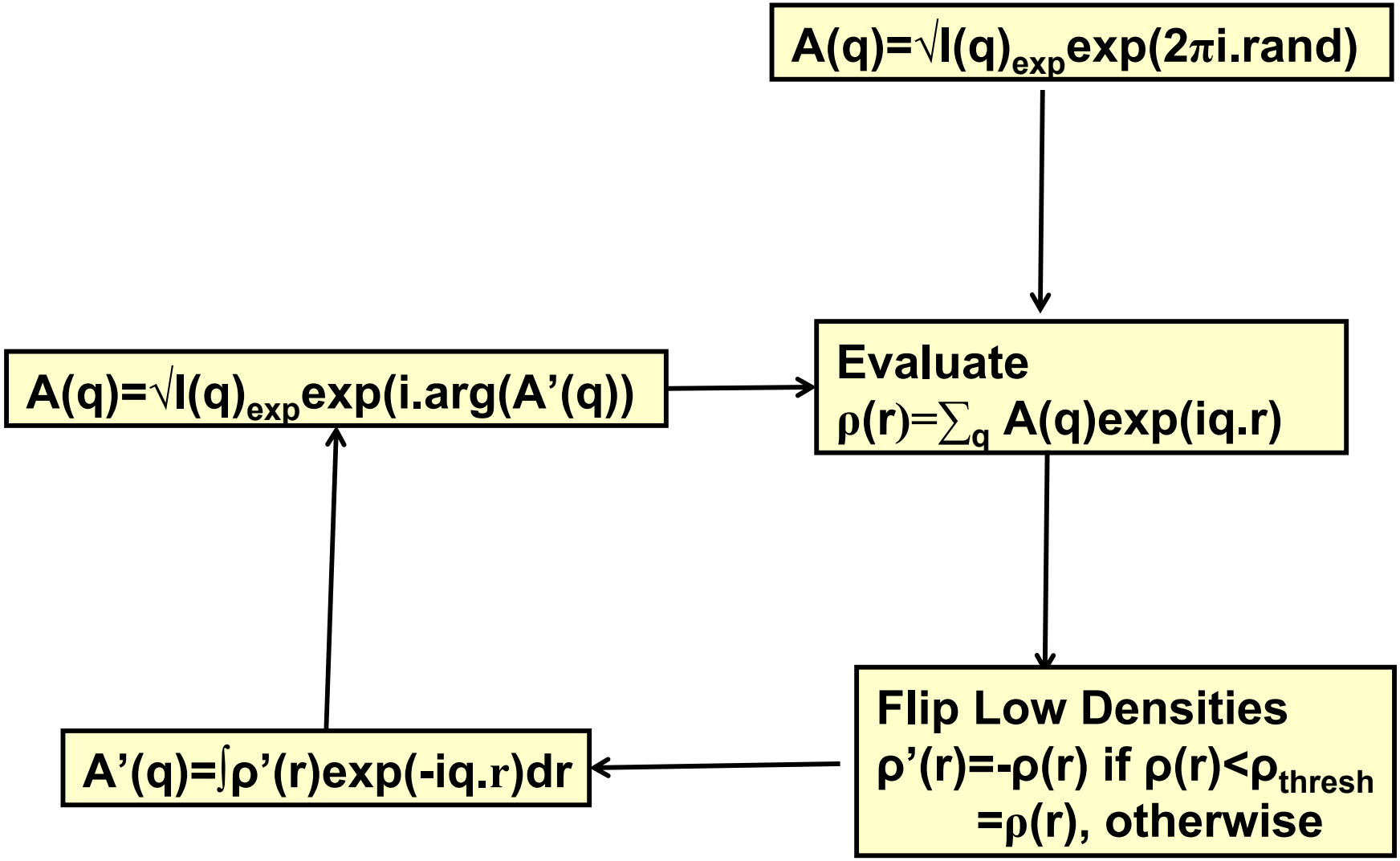
Original DP **10-Particle DP** **Reconstructed Single-Particle DP**

Like unscrambling an egg

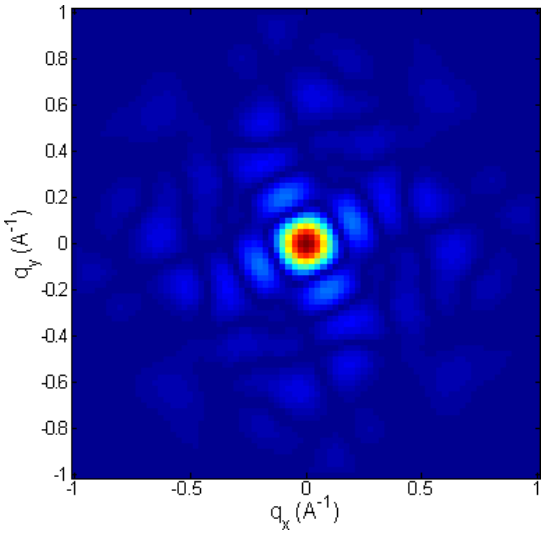
Saldin et al., Phys. Rev. B 81, 174105 (2010)

Intensities to Electron Density

Phasing Algorithm (Oszlànyi and Süto, 2004)

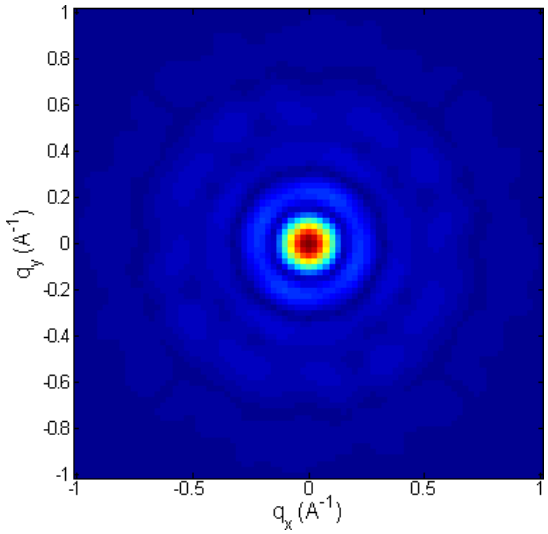


From Diffraction Patterns to Projected Electron Density



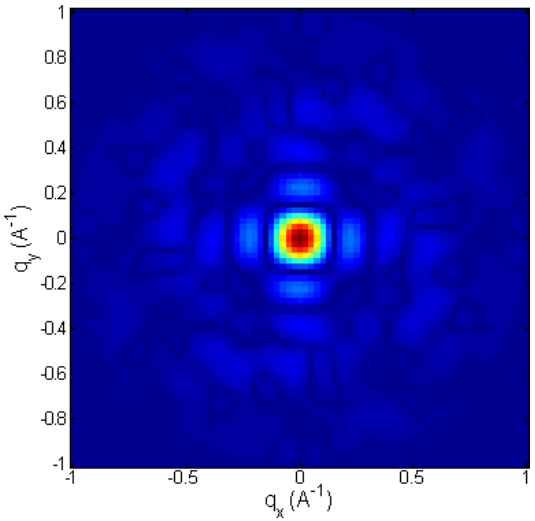
Single Particle DP

»

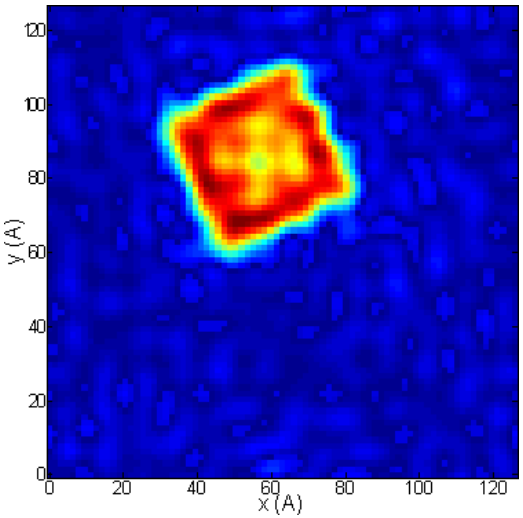


Scrambled Egg

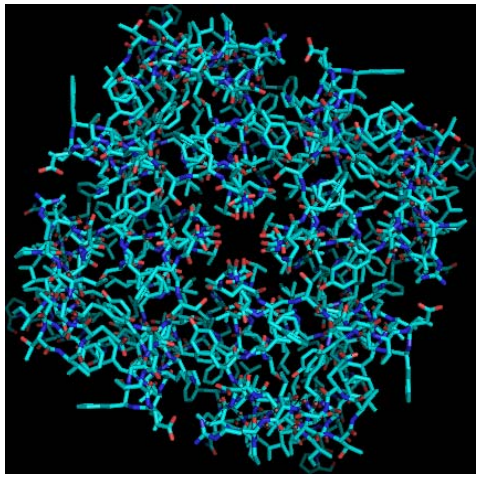
»



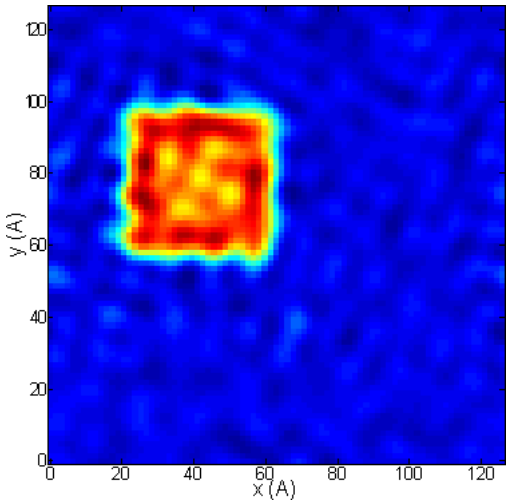
Unscrambled Egg



Proj. Electron Density

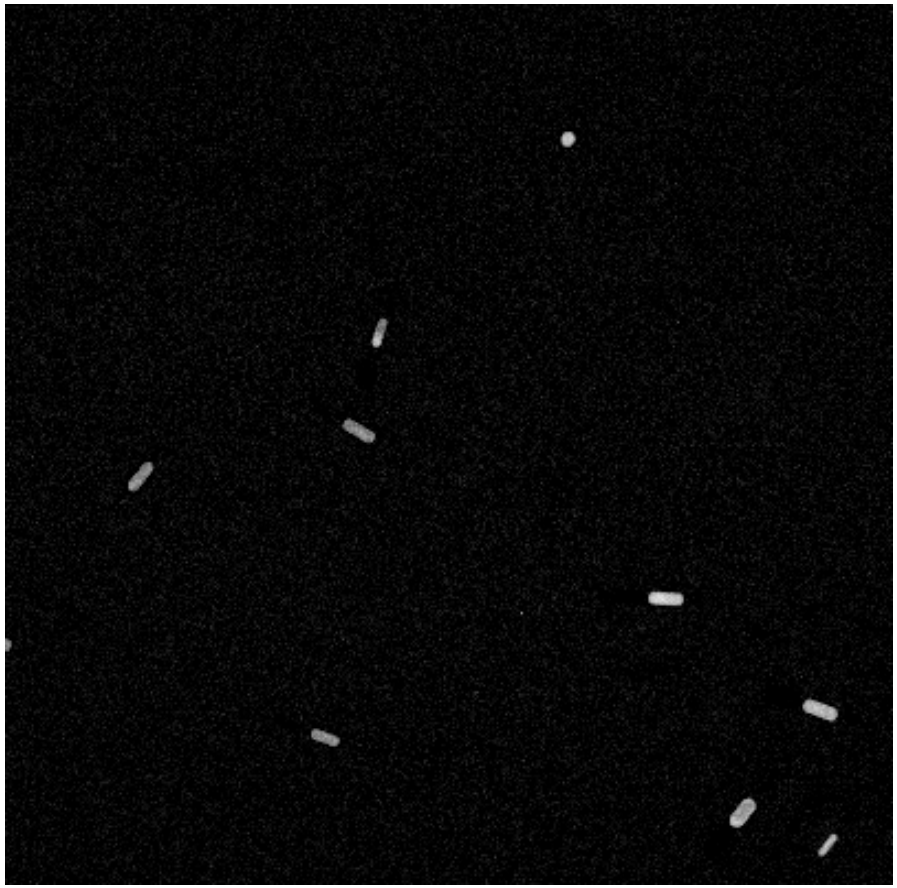


PDB structure



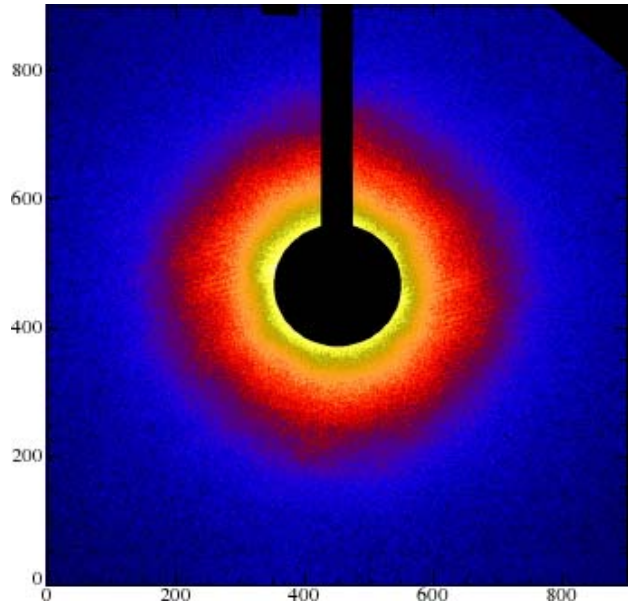
Proj. Electron Density

EM Image of Sample for Experiment



Mainly ~ 80 nm x 20 nm randomly oriented metal rods on a SiN substrate

Information In Angular Correlations

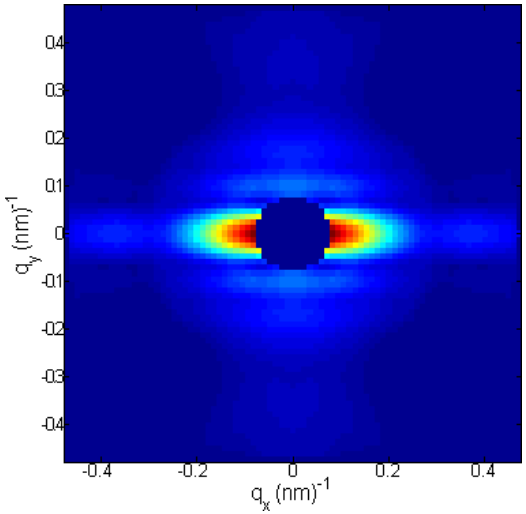


Diffraction pattern from disordered subunits Appears to have no angular structure, only radial variation, studied by SAXS. However there is untapped information in the angular correlations, revealed by evaluating

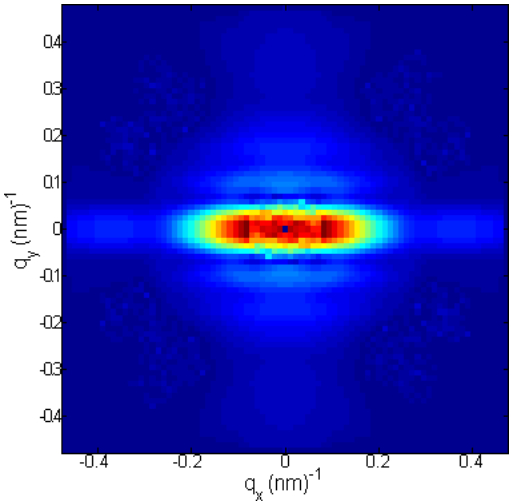
$$C_2(q;q',\Delta\varphi) = \left\langle \frac{1}{N_\varphi} \sum_j \left\{ I(q,\varphi_j) - I_{saxs}(q) \right\} \left\{ I(q',\varphi_j + \Delta\varphi) - I_{saxs}(q') \right\} \right\rangle_{DP}$$

DP and Image Reconstruction from Simulated Correlations

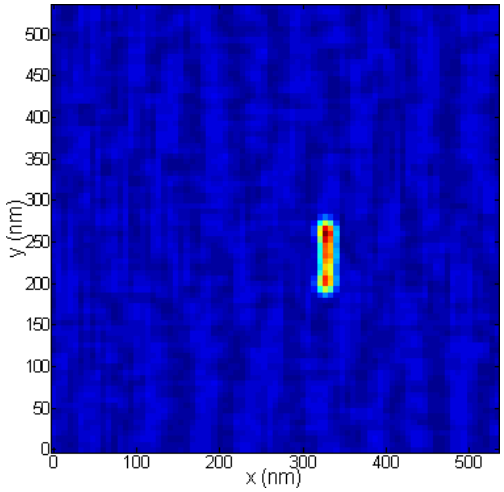
Identical Rods



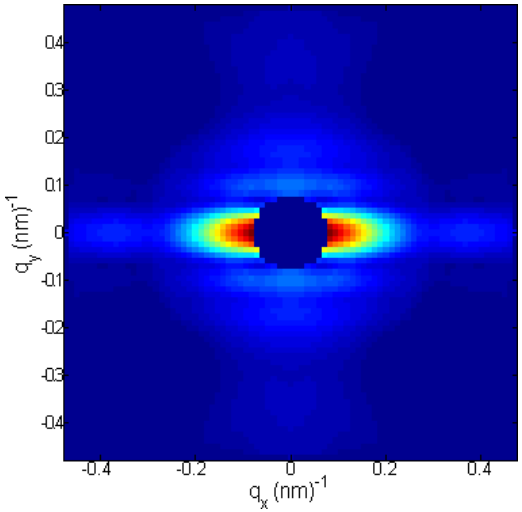
With beam stop

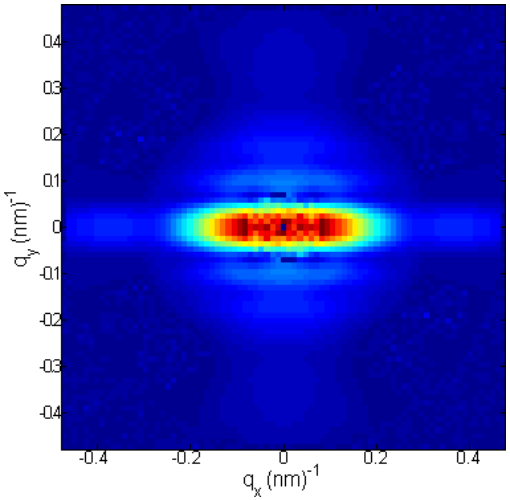


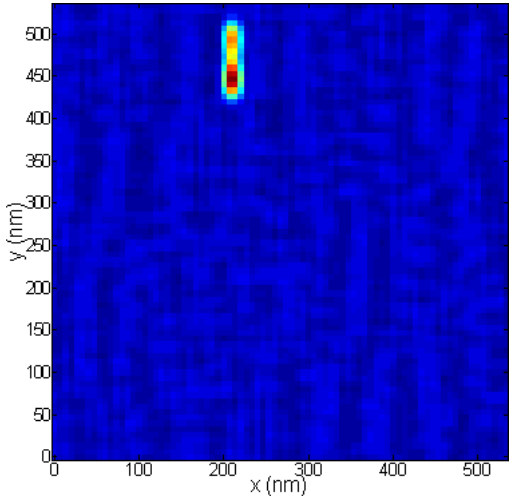
Beam stop filled by phasing alg.



Reconstructed rod image



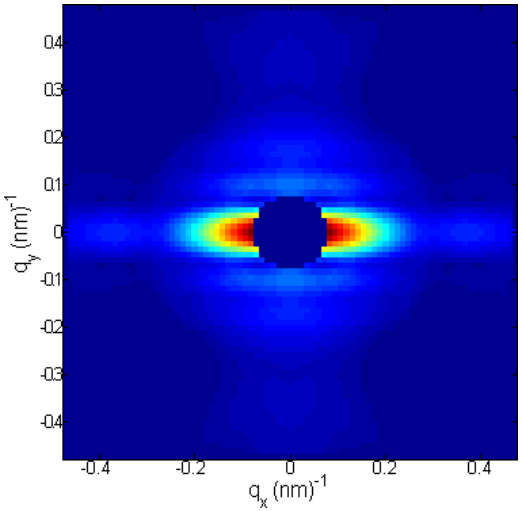




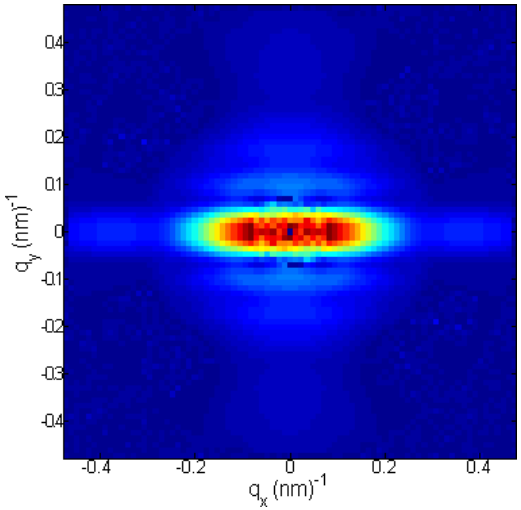
Objects w/ 10% range of sizes

DP and Image Reconstruction
Simulated & Measured Correlations

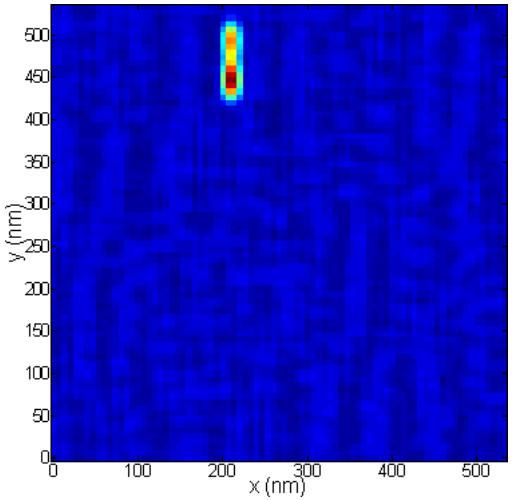
Simulation



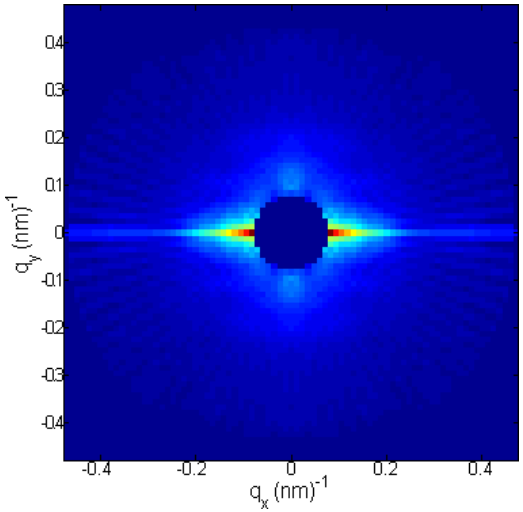
With beam stop

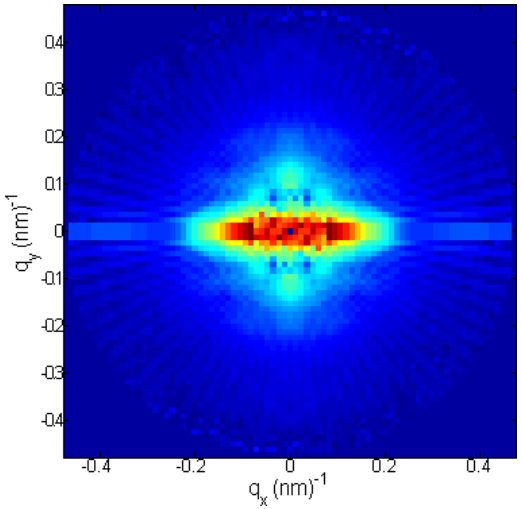


Beam stop filled by phasing alg.

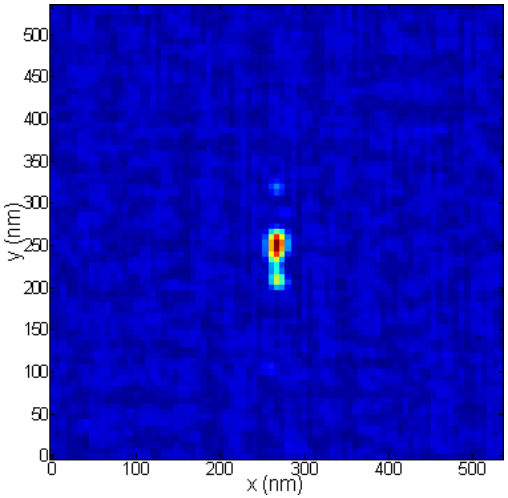


Reconstructed rod image

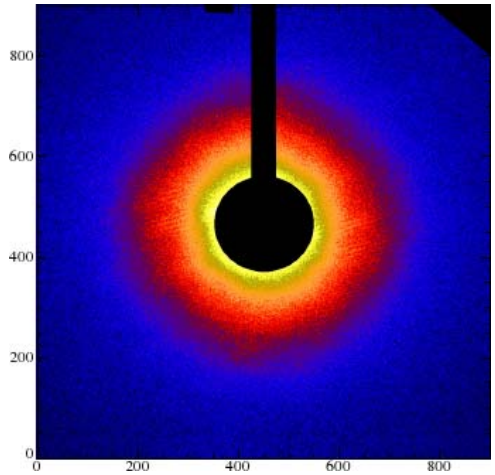




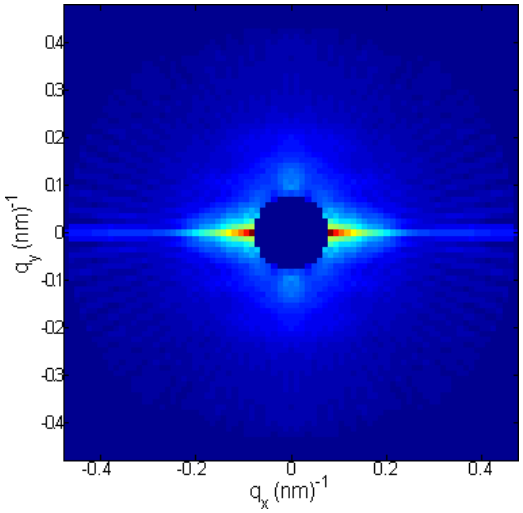
Experiment



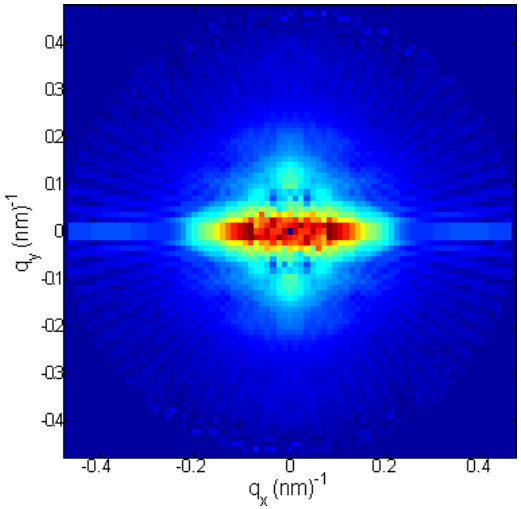
From Measured Multiparticle DP to Single-Particle Image



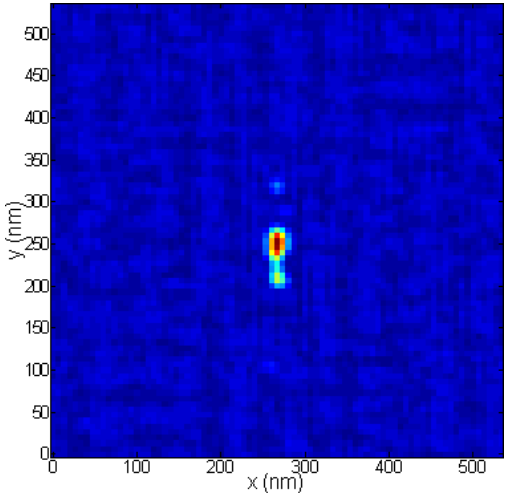
Measured DP



DP reconstructed from correls.



Beam stop filled by phasing alg.



Reconstructed image

Poisson Noise and Mean of Correlations

Poisson distribution P(n,λ): probability of measured count of n, mean λ.

$$P(n,\lambda) = \lambda^n e^{-\lambda} / n!$$

Mean count

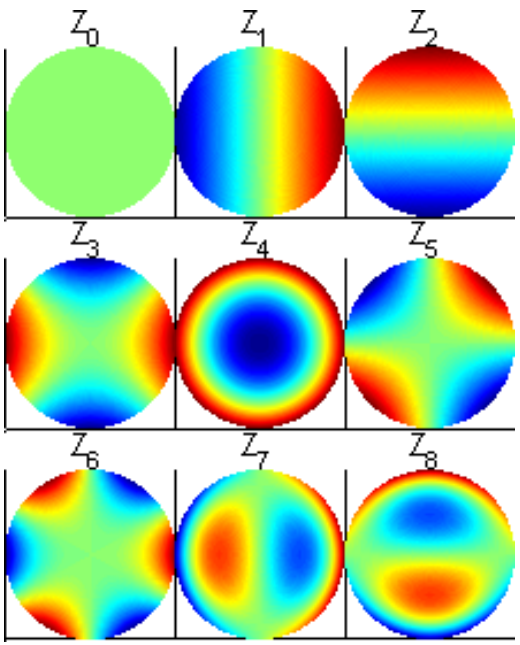
$$\lambda = \sum_{n=0}^{\infty} nP(n,\lambda)$$

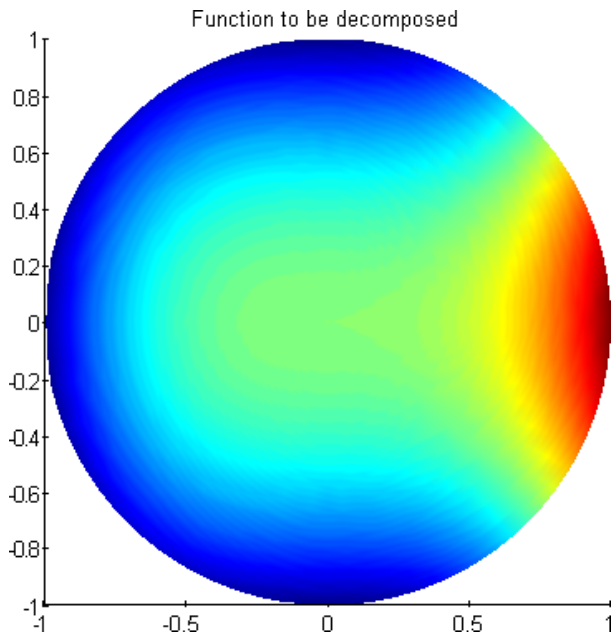
Mean value of product of two variables with individual means λ_1 and λ_2:

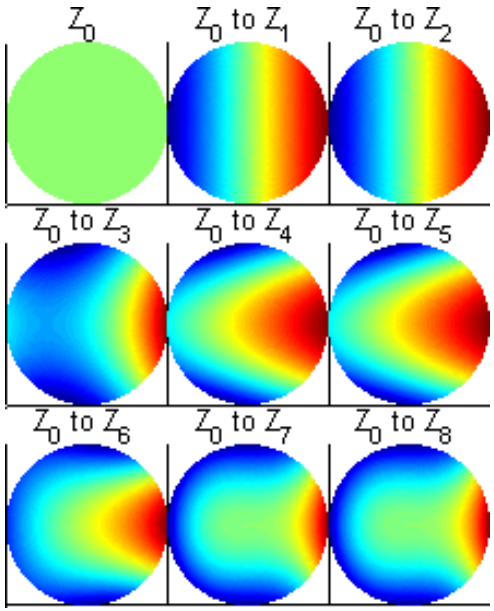
$$\sum_{n=0}^{\infty}\sum_{m=0}^{\infty} nmP(n,\lambda_1)P(m,\lambda_2)$$
$$= \left\{\sum_{n=0}^{\infty} nP(n,\lambda_1)\right\}\left\{\sum_{m=0}^{\infty} mP(m,\lambda_2)\right\}$$
$$= \lambda_1\lambda_2$$

Mean of products of counts, nm = product of individual means, $\lambda_1\lambda_2$.

Zenike Function Expansion

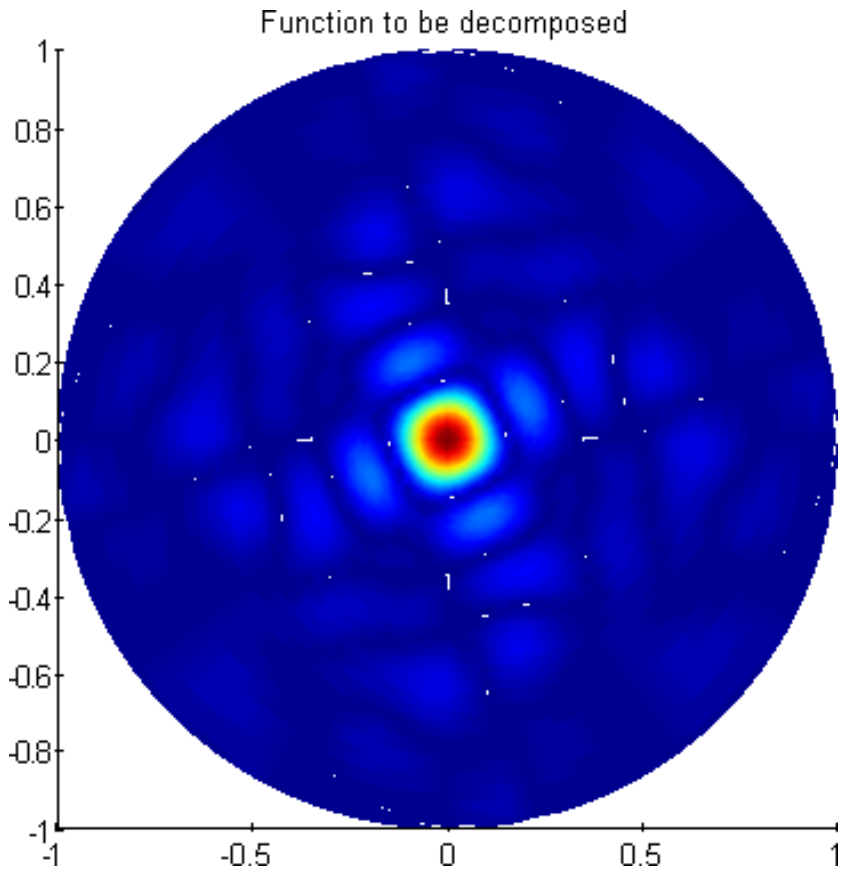


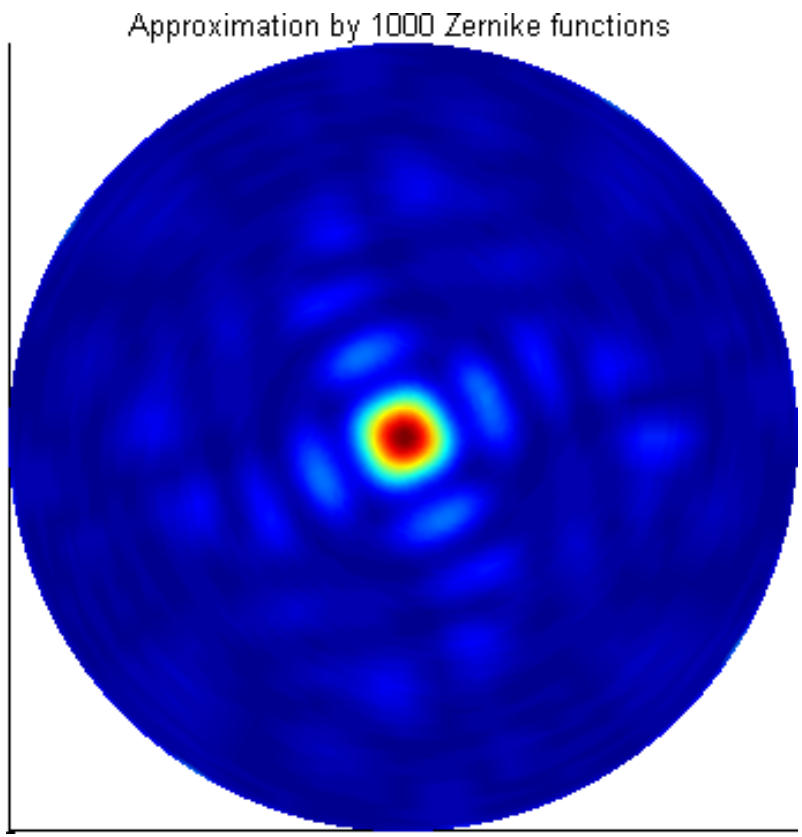




Calculated with MATLAB programs by Paul Fricker
http://www.mathworks.com/matlabcentral/fileexchange/7687-zernike-polynomials

Zernike Function Expansion of K-channel Diffraction Pattern





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http://www.mathworks.com/matlabcentral/fileexchange/7687-zernike-polynomials