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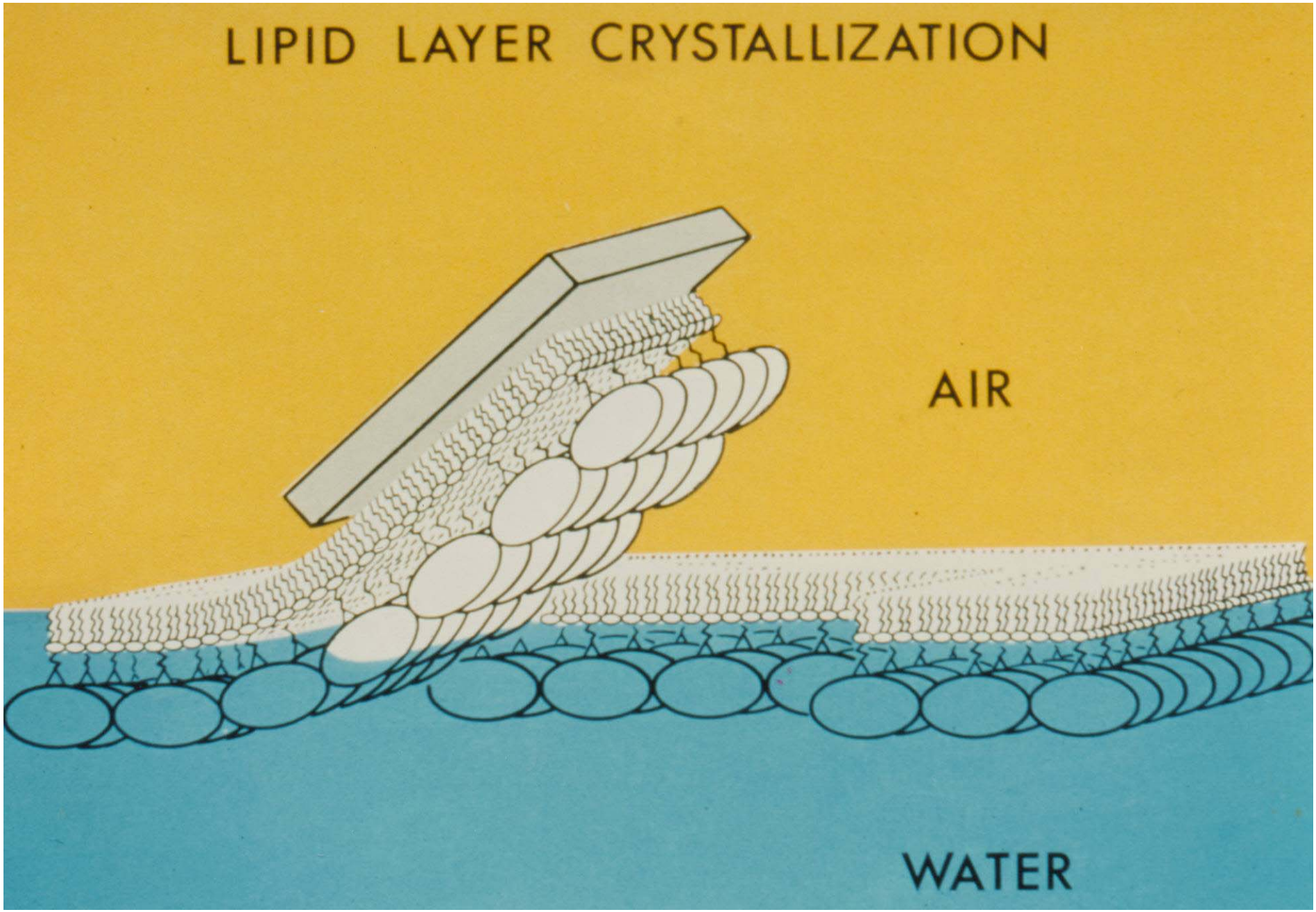

LIPID LAYER CRYSTALLIZATION
AIR
WATER

Advantages of 2-D Crystallization on Lipid-Layers

- **Microgram quantities of protein can suffice for crystallization and structural analysis.**
- **Proteins can be crystallized under physiological conditions.**
- **Provides a more native-like environment for the study of proteins that naturally bind to cell membranes.**
- **Proteins can be adsorbed from solution to the lipid-water interface by specific or non-specific interactions.**

Specific Interactions:

- **Nickel lipids - bind His-tagged proteins**
- **Biotinylated lipids - bind streptavidin, an adaptor molecule**
- **Natural lipid ligands – ex. gangliosides, GM1 and GT1, bind cholera toxin and tetanus toxin respectively**
- **Synthetic lipid ligands – ex. monoclonal anti-DNP IgG binds to DNP-PE**

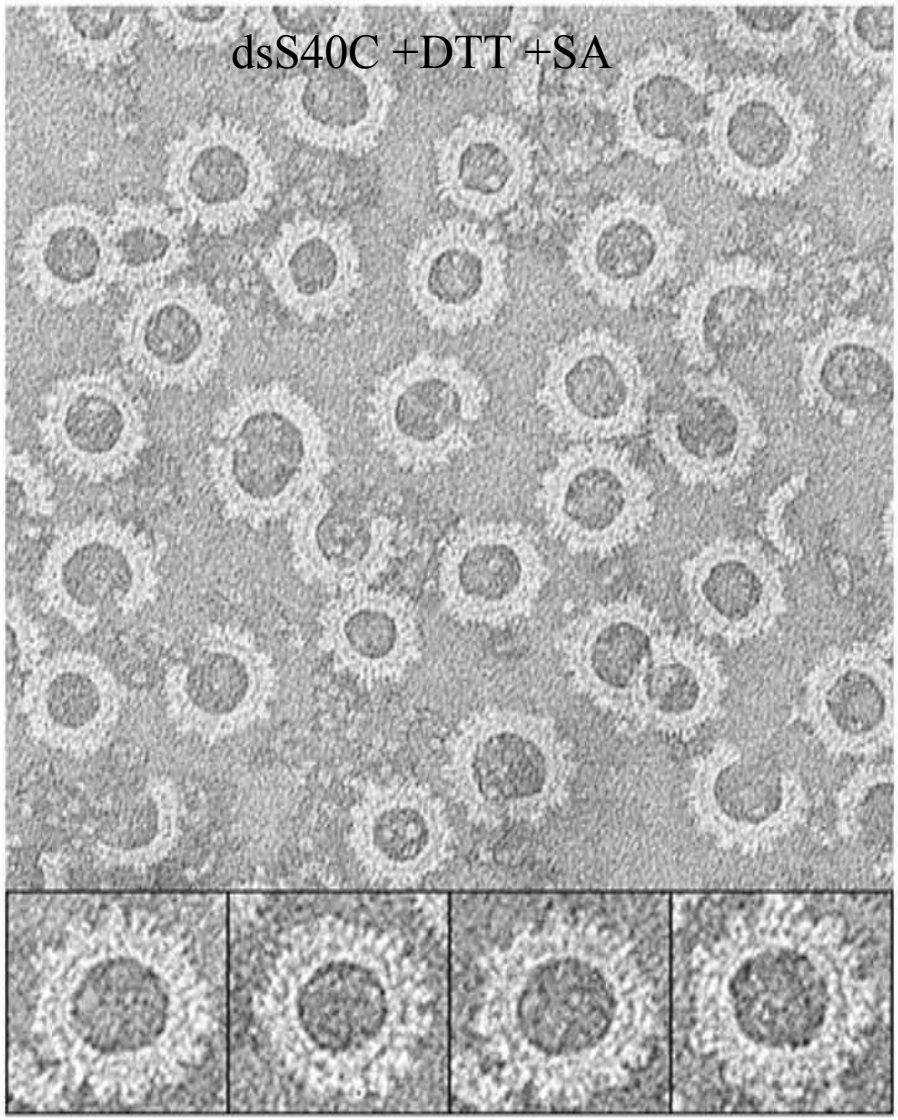
Non-Specific Interactions:

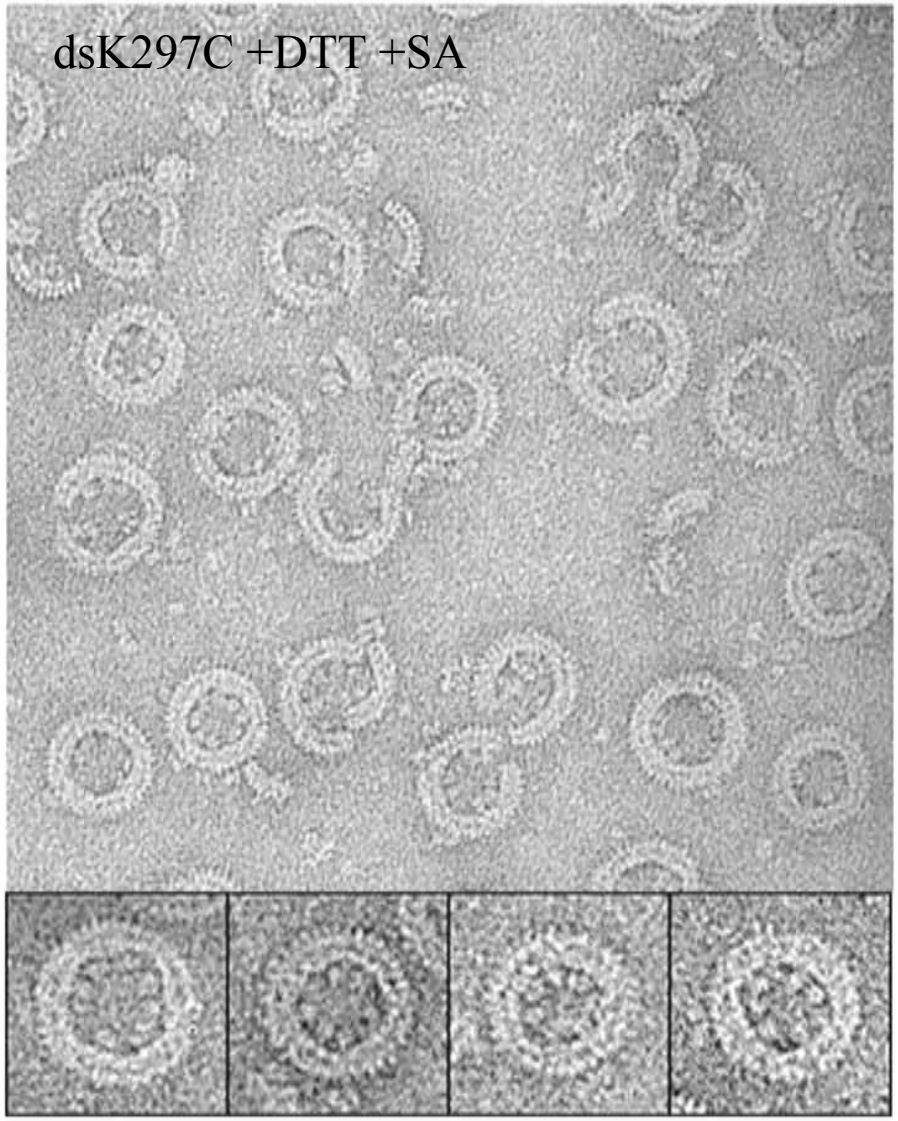
- **Charged lipids – ex. DO-Ethyl-PC, DOTAP, DOPS**

2D Crystallization of Proteins on Monolayers

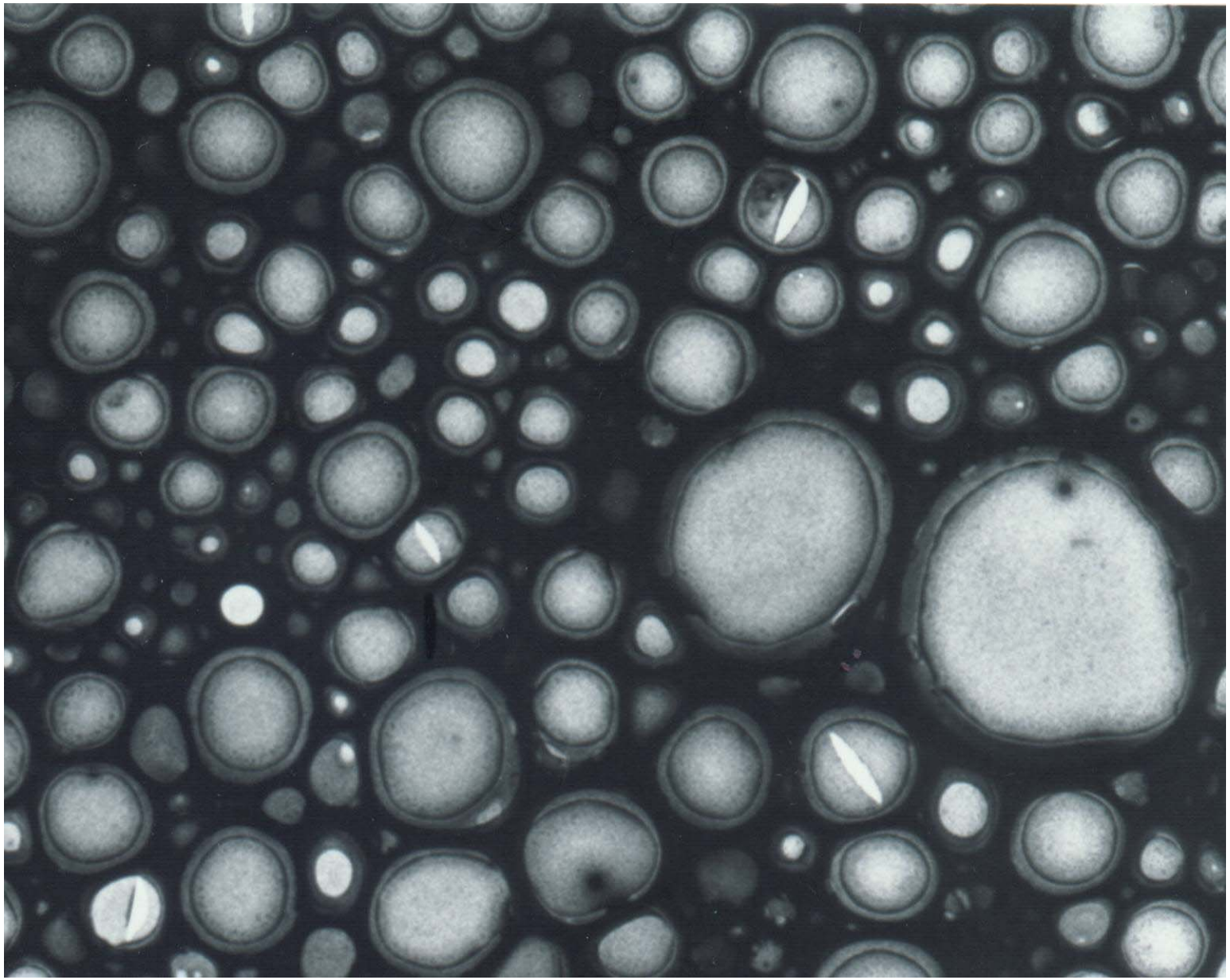
Non-Specific Binding	Specific Binding
Aerolysin	Antibodies
Annexin	Streptavidin
α-toxin	Botulinum toxin
α-actinin	Cholera toxin
Creatine kinase	Coagulation factor
50S ribosomal subunit	DNA gyrase B
F-actin	Reverse transcriptase
RNA polymerase	Tetnus toxin

Biotin Labeled PFO Mutants Plus Streptavidin

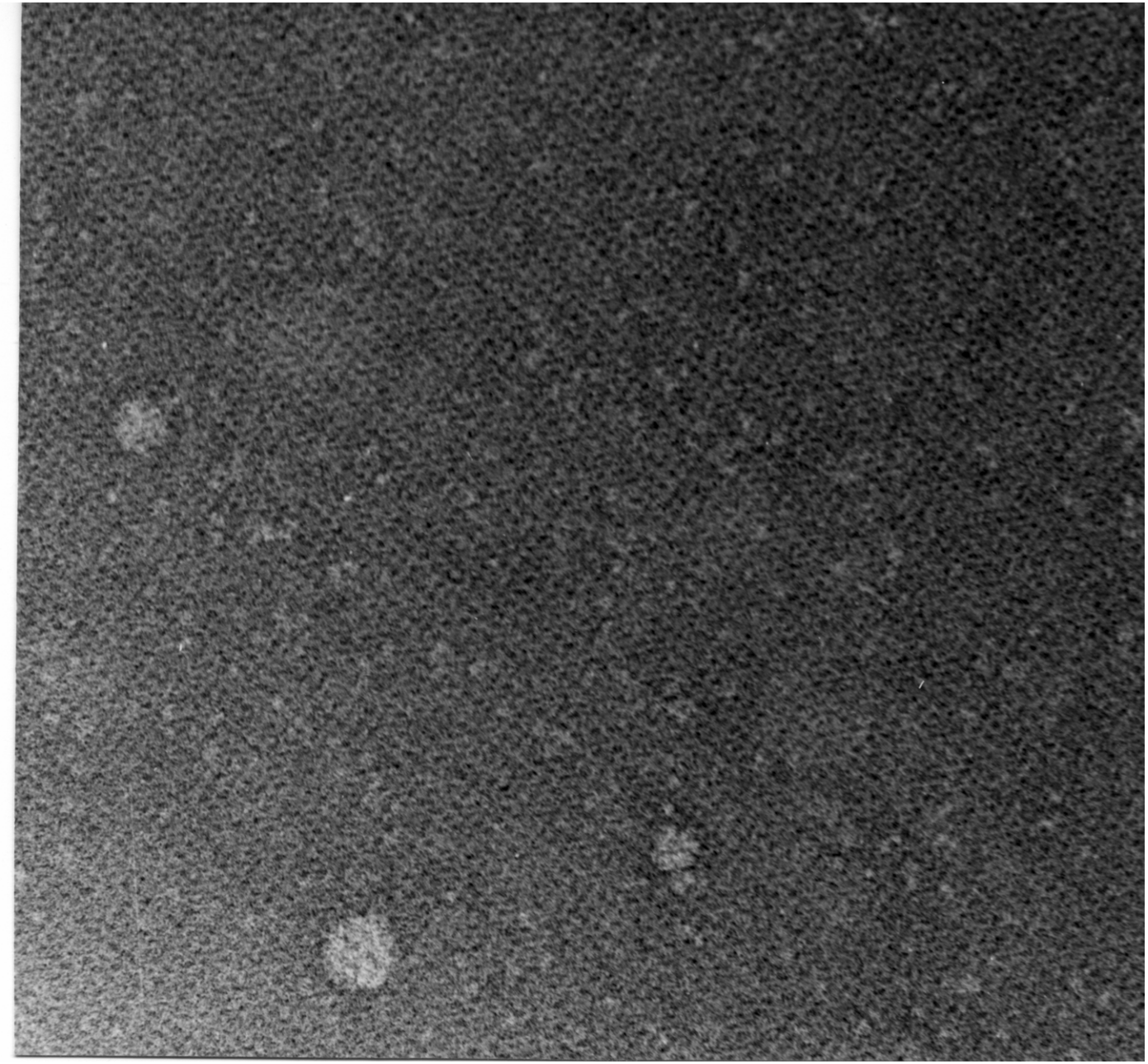




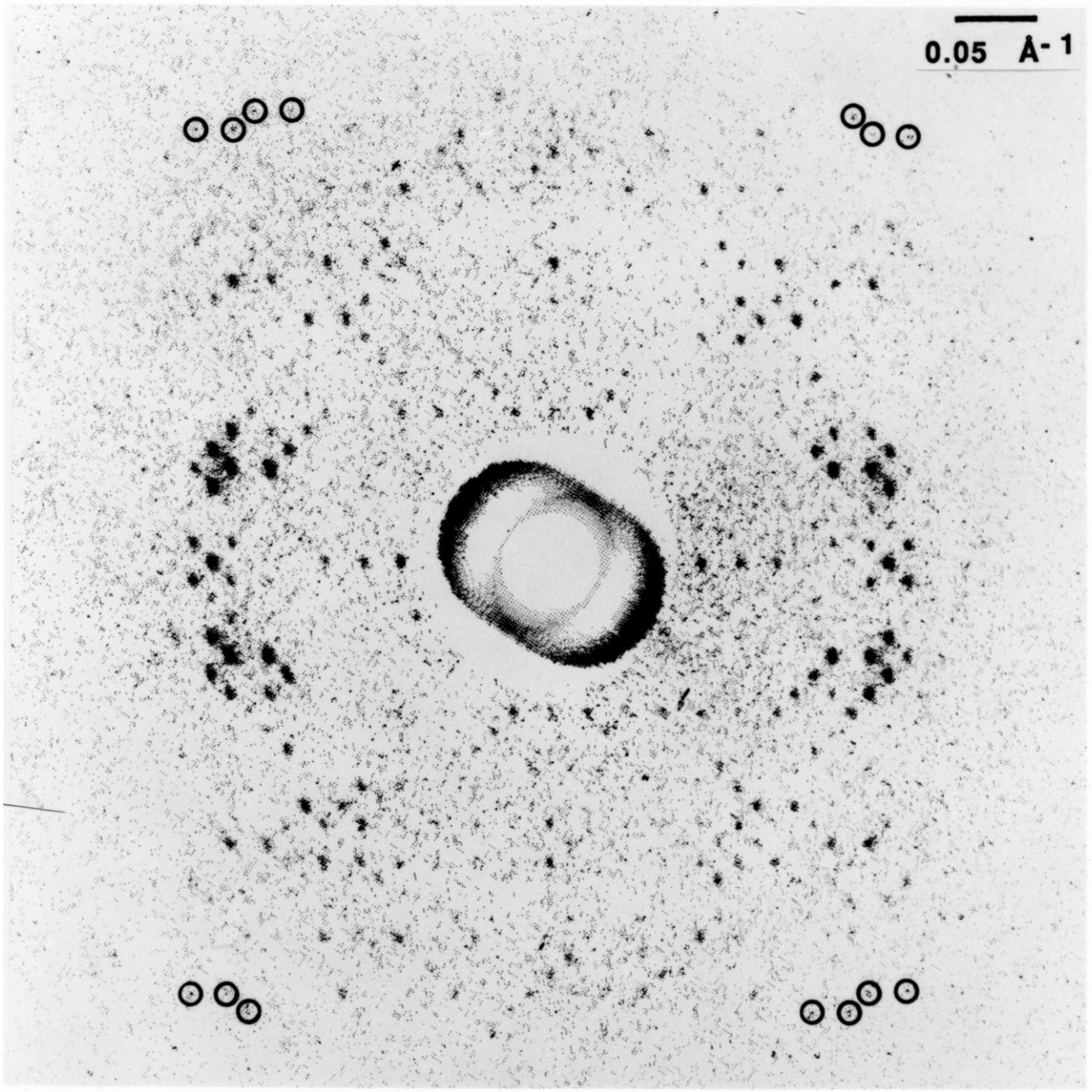
Streptavidin Crystals on Holey Film



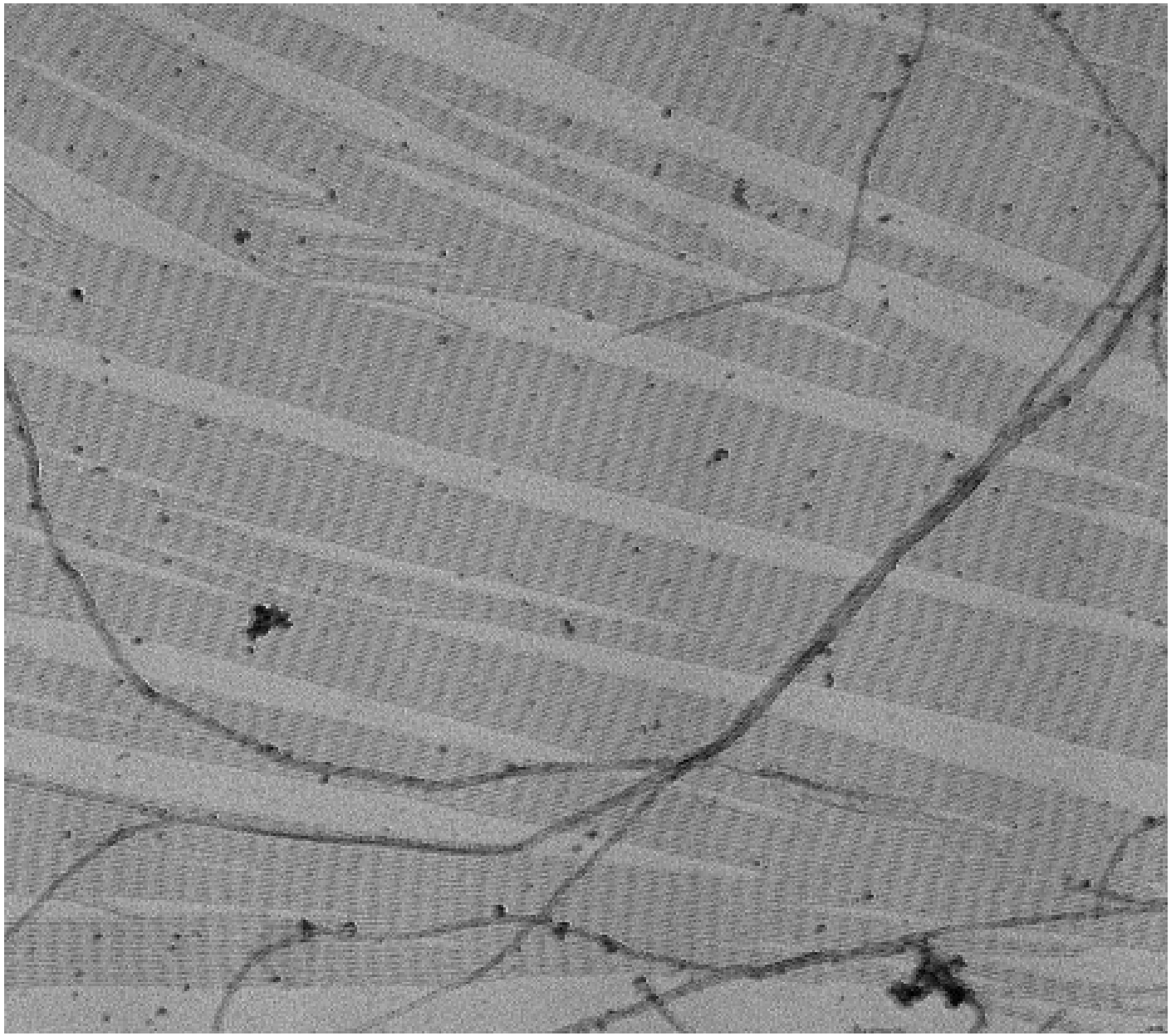
Streptavidin Crystal in Negative Stain



Diffraction Pattern of Streptavidin Crystals in Vitreous Ice

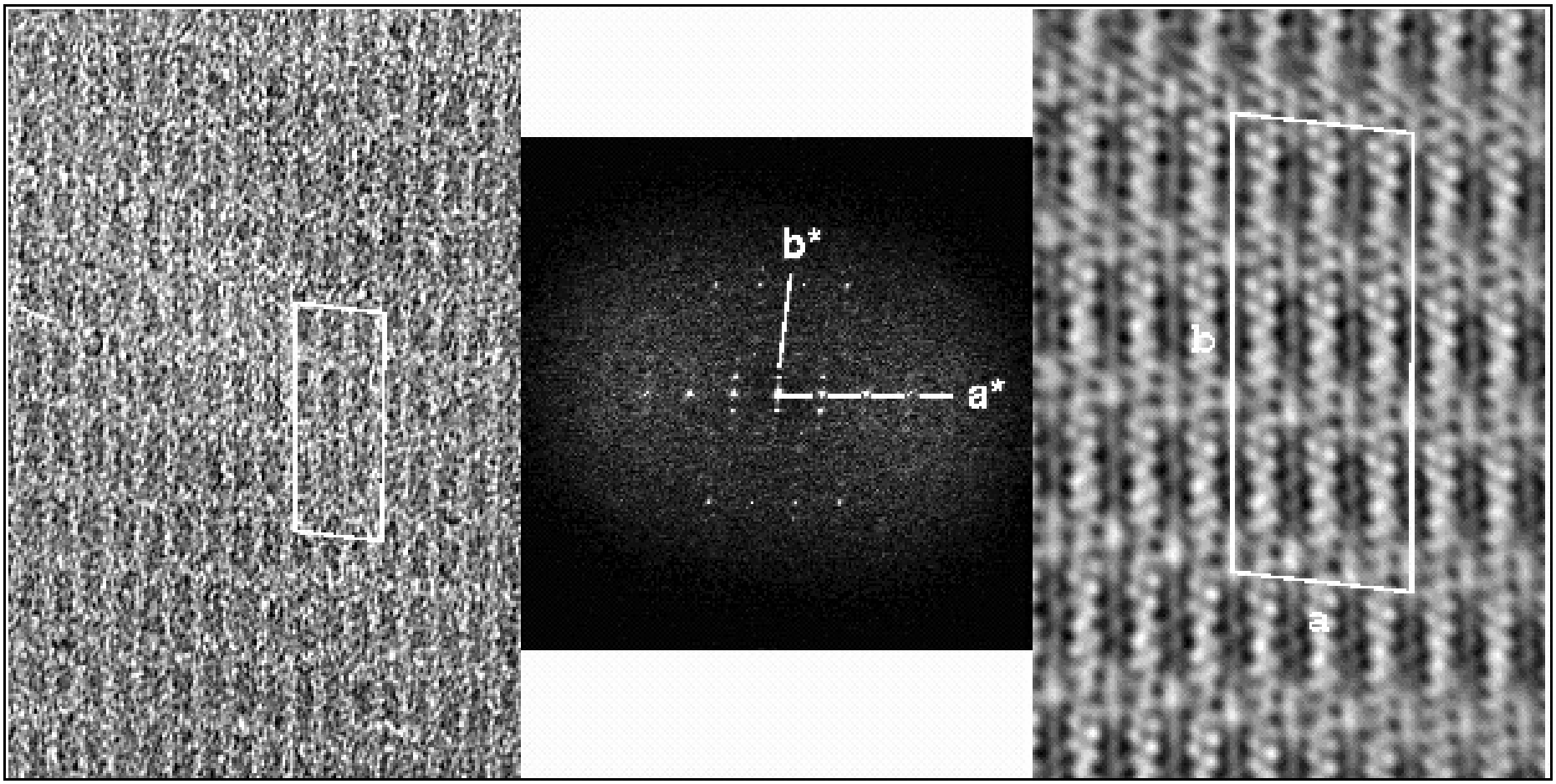


Actin Rafts on Positively Charged Lipid Monolayer



Dianne Taylor

2D Array of Actin-Aldolase

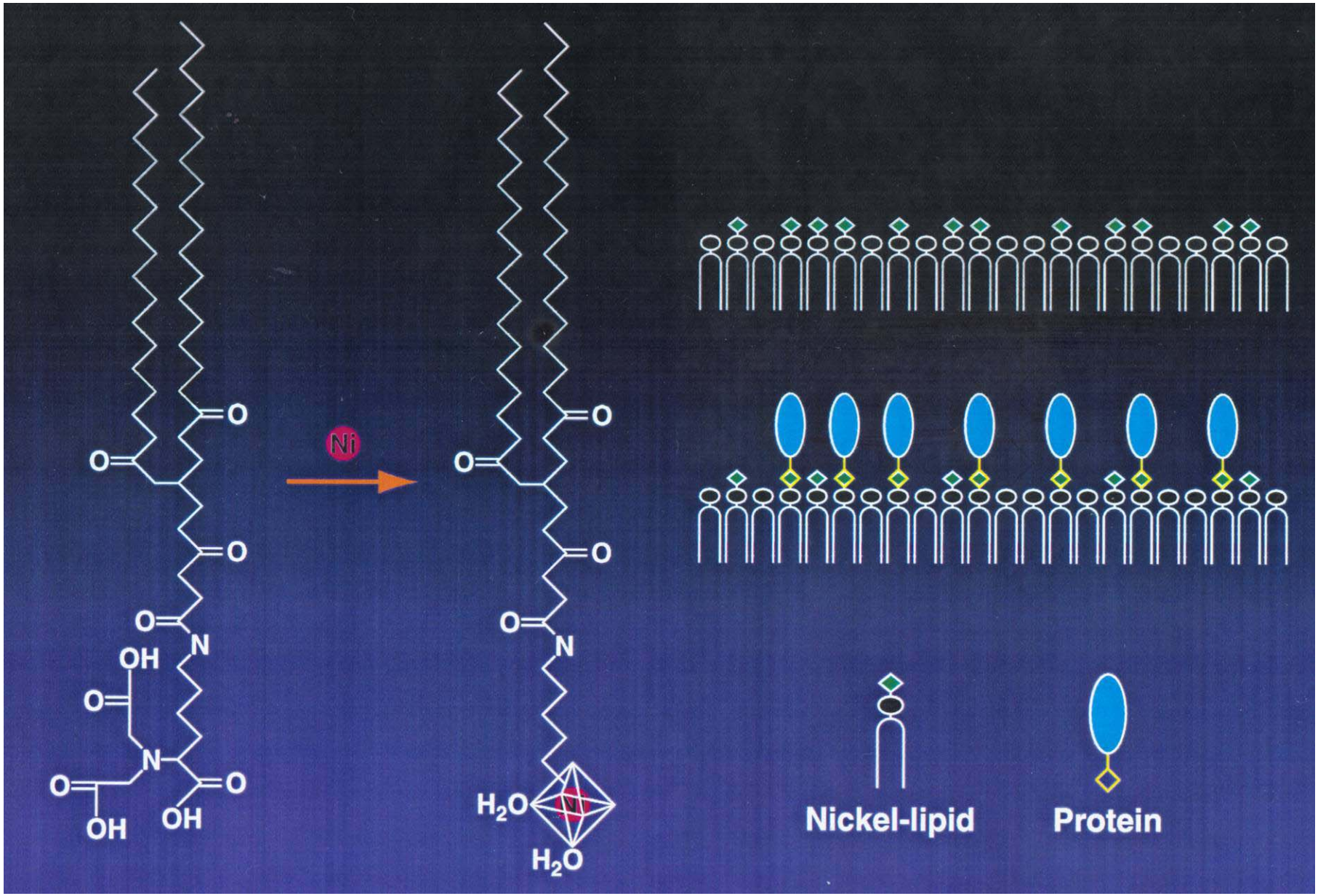


Dianne Taylor

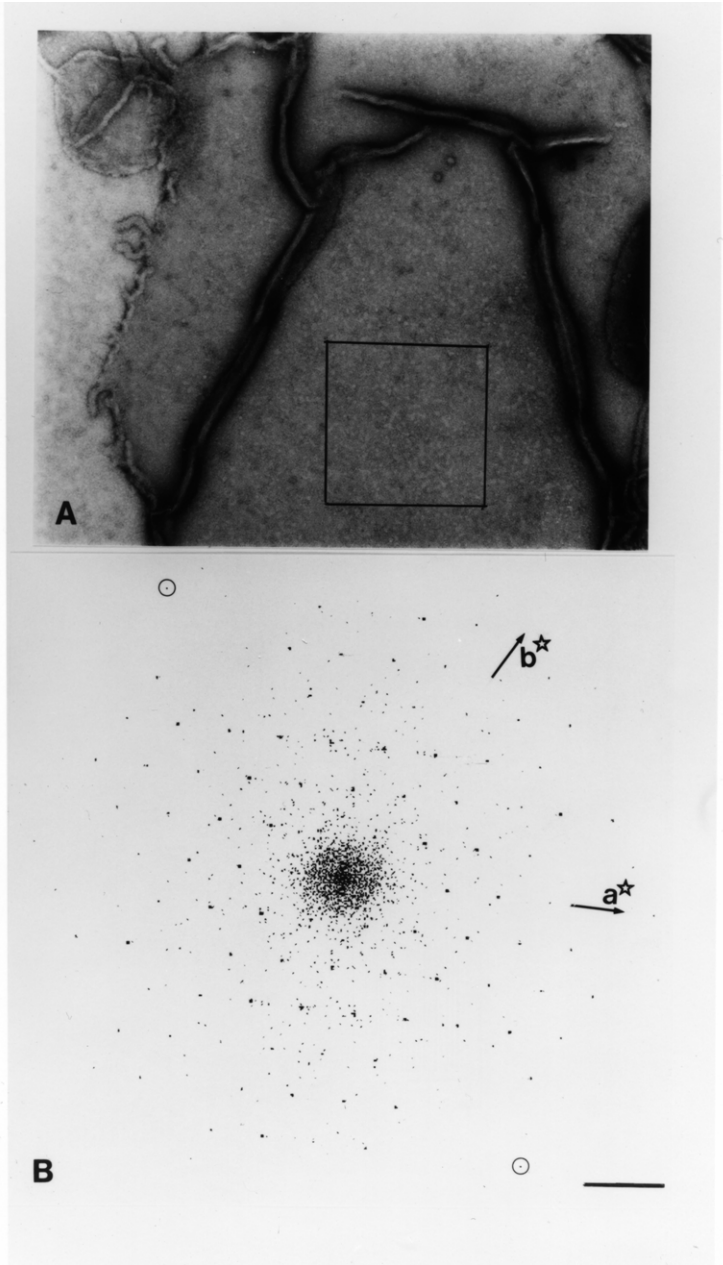
Advantages of using Metal Chelating Lipids

- **Provides a common moiety for the binding of a large variety of His-tagged proteins.**
- **Unique orientation of the protein or macromolecular complex.**
- **Histidine clusters can be inserted at alternative sites within a molecule.**
- **Can be used to crystallize His-tagged membrane proteins.**
- **Proteins can be studied in physiological conditions or under high salt conditions.**

His-Tagged Proteins Bind to Chelated Nickel

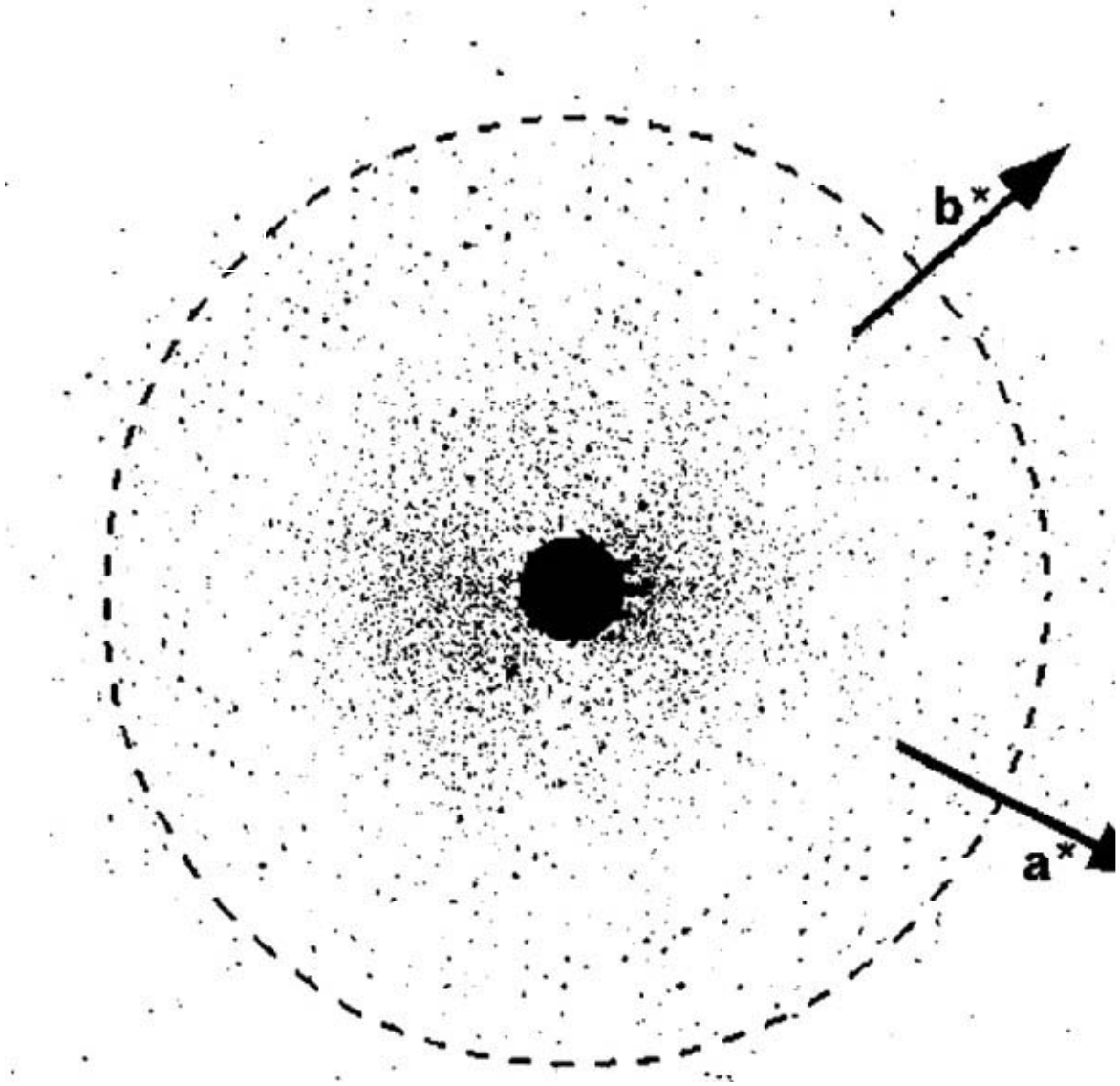


2-D Crystal of Reverse Transcriptase on Nickel Lipid Layer



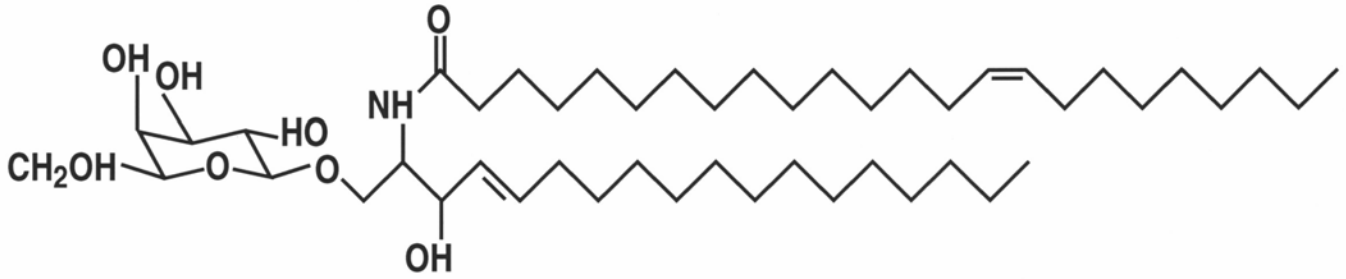
E Wilson-Kubalek

Diffraction Pattern of MHC on Nickel Lipid

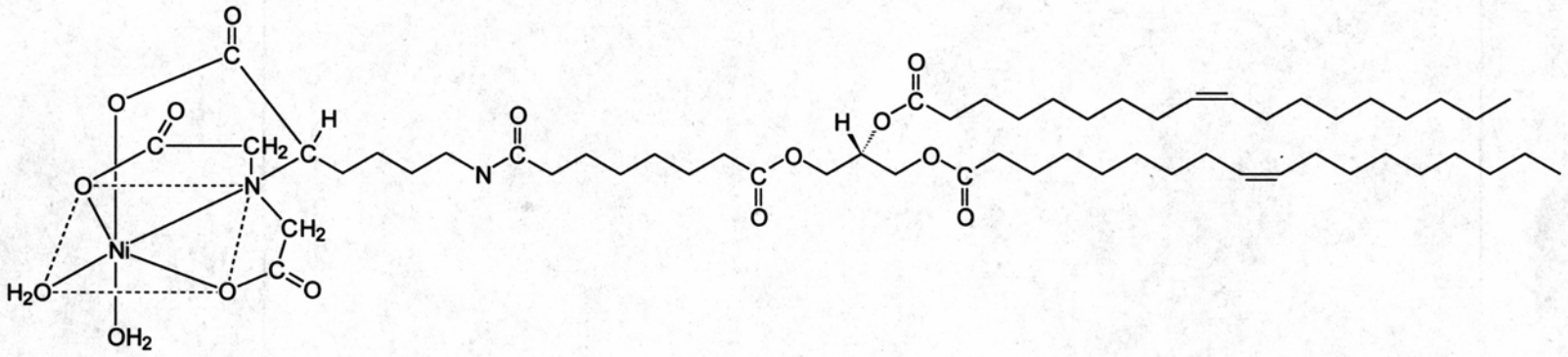


Herve Celia

Galactosylceramide



Nickel lipid (DOGS-NTA-Ni)



E Wilson-Kubalek

Lipid Tube Preparation

Mix Galactosyl cerebroside with synthetic or natural lipids at varying ratios in organic solvents

↓

Dry down under argon

↓

Resuspend in desired buffer solution

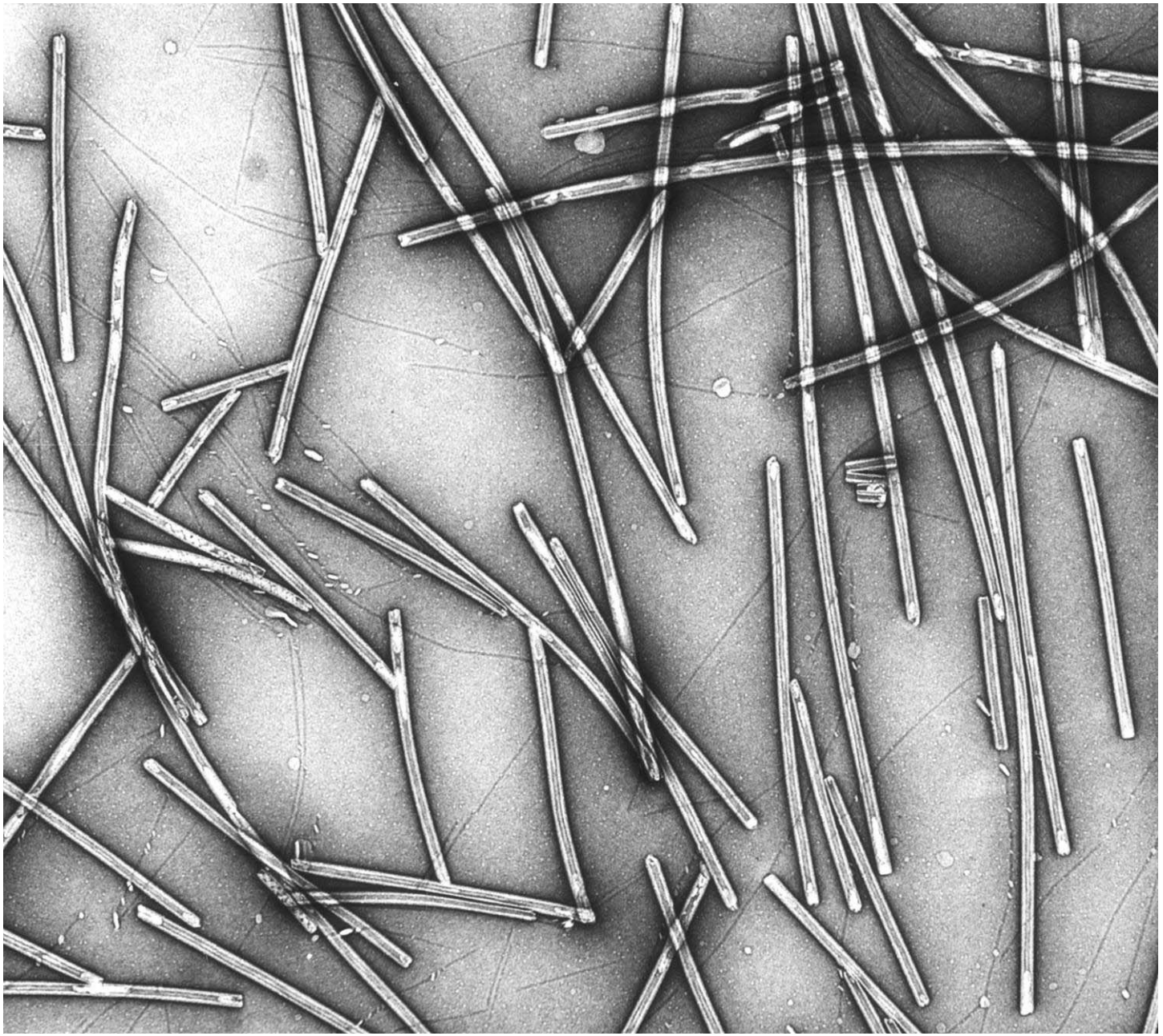
↓

Sonicate or vortex

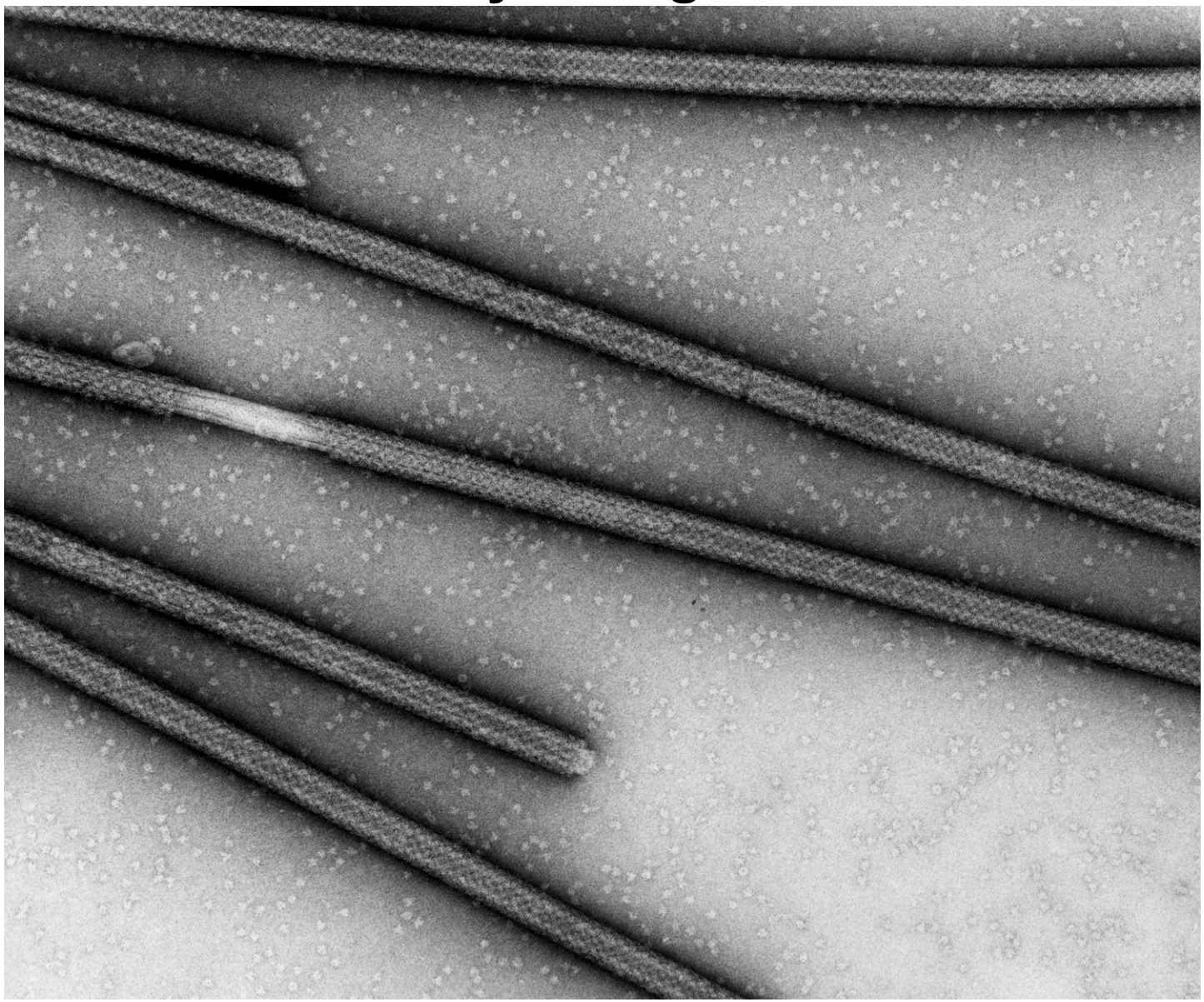
↓

Check tube formation by EM

Negative Stained Nickel Tubes

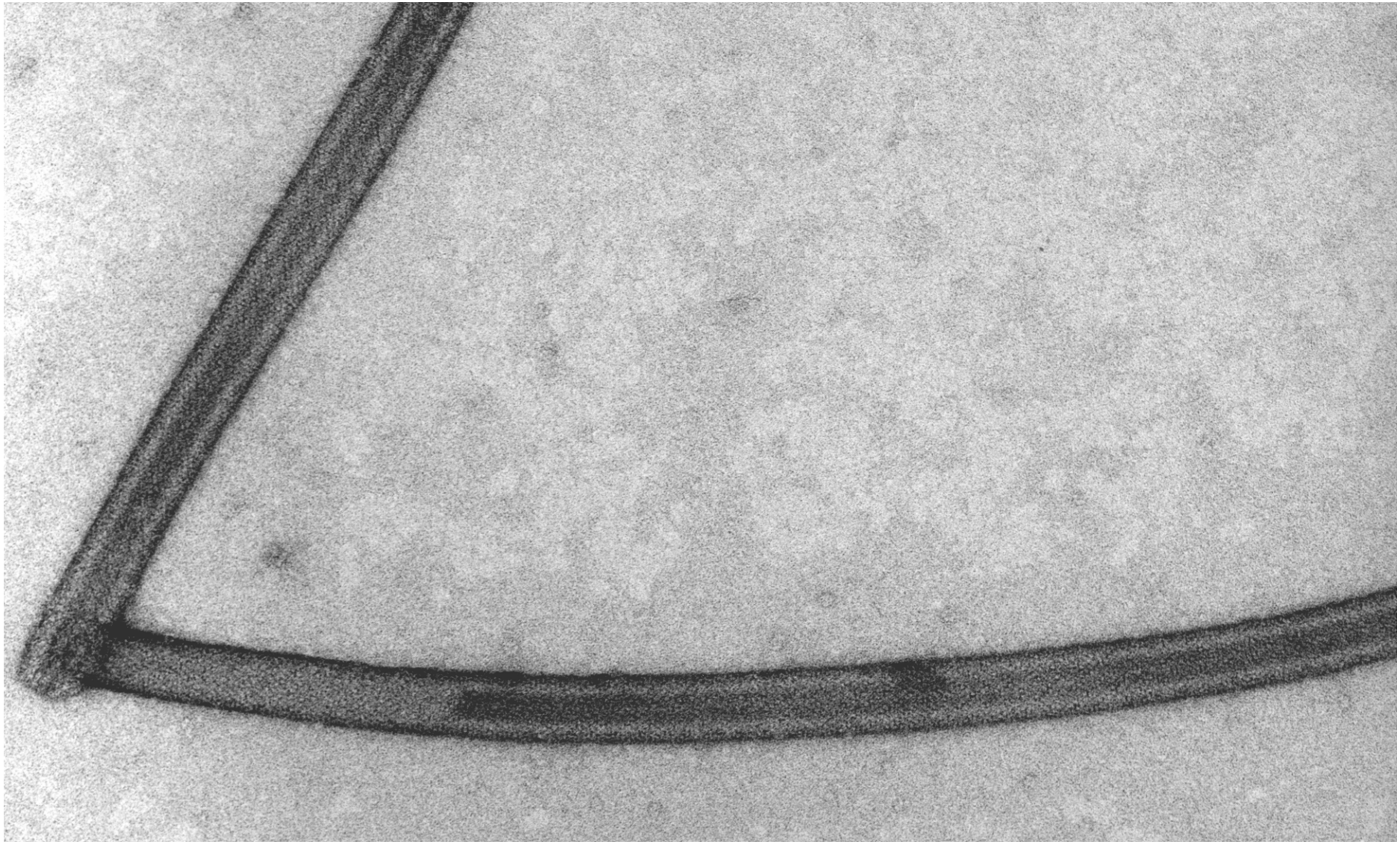


Helical Arrays of RNA polymerase Molecules on Positively Charged Tubes



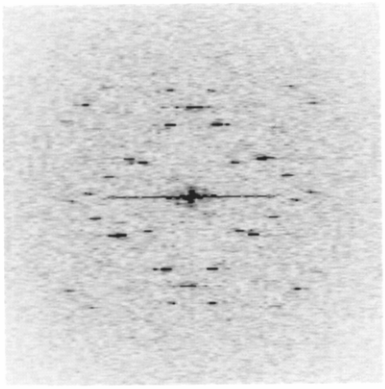
Helical Arrays of PA63 on Positively Charged Tubes

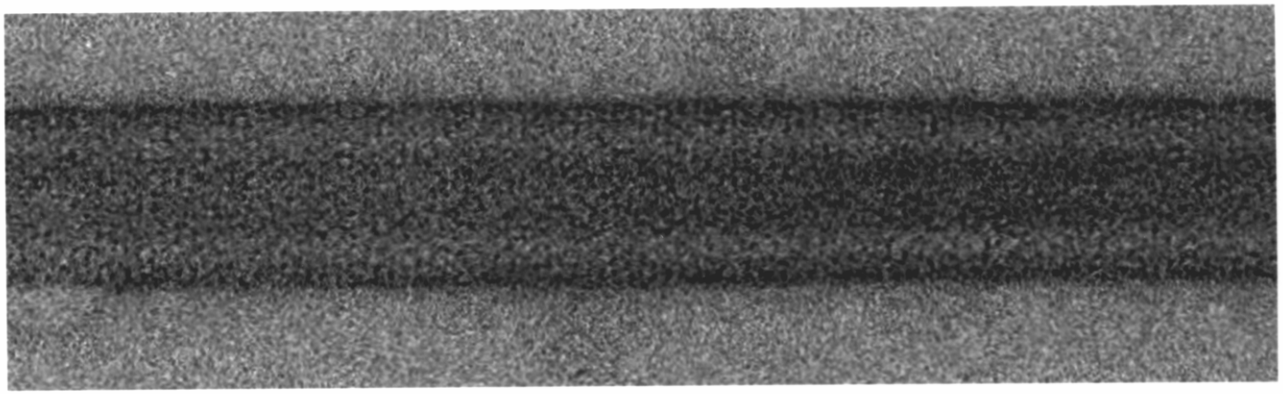
Helical Arrays of PFO on Nickel Lipid Tubes



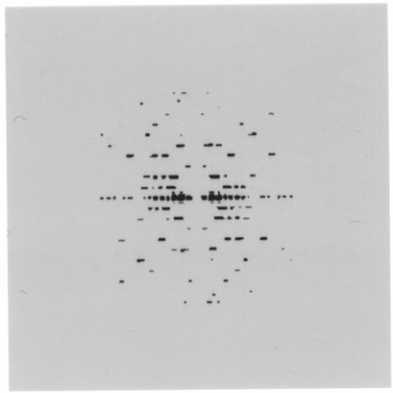
Helical Arrays of PFO on Nickel Lipid Tube and PA63 on Positively Charged Lipid Tube

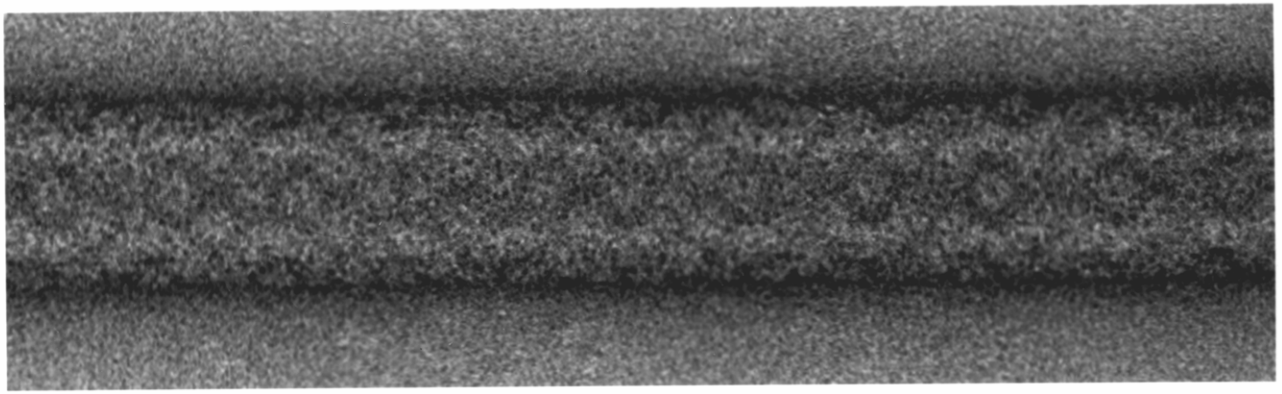
PFO



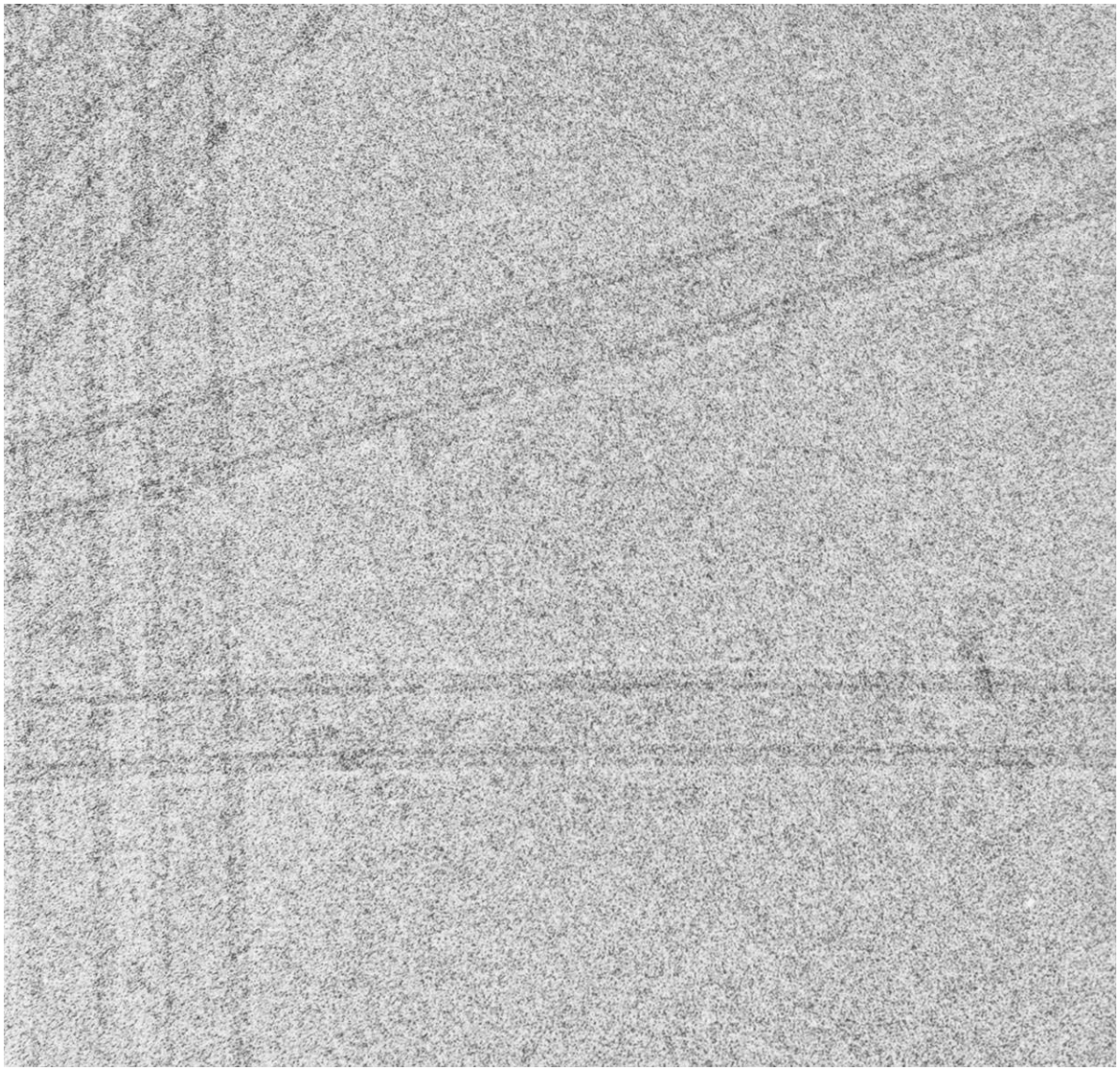


PA63

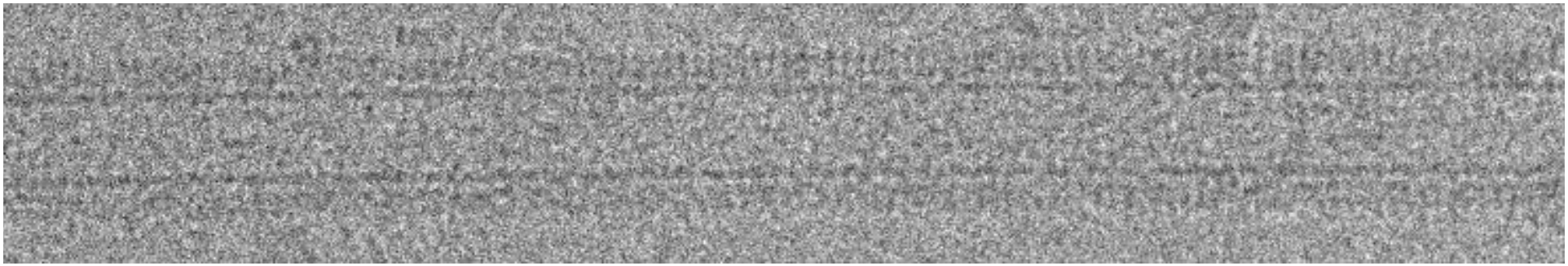


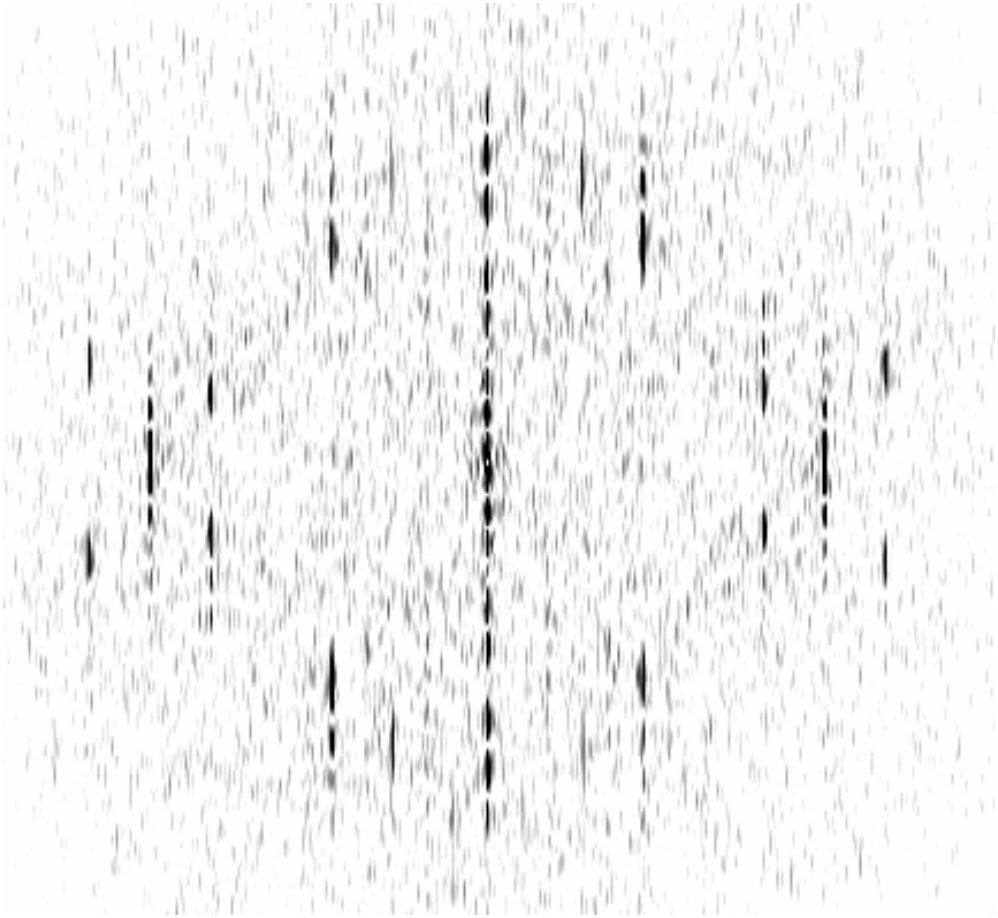


Bilayer Visible in Tubes Preserved in Vitreous Ice

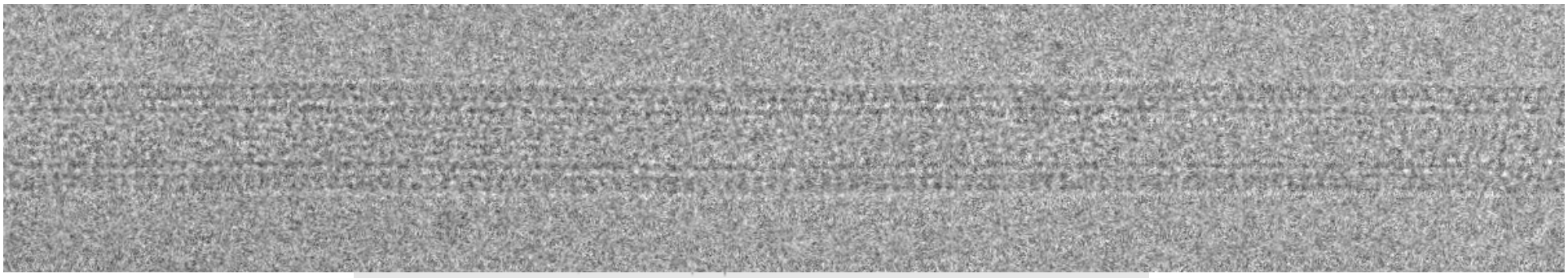


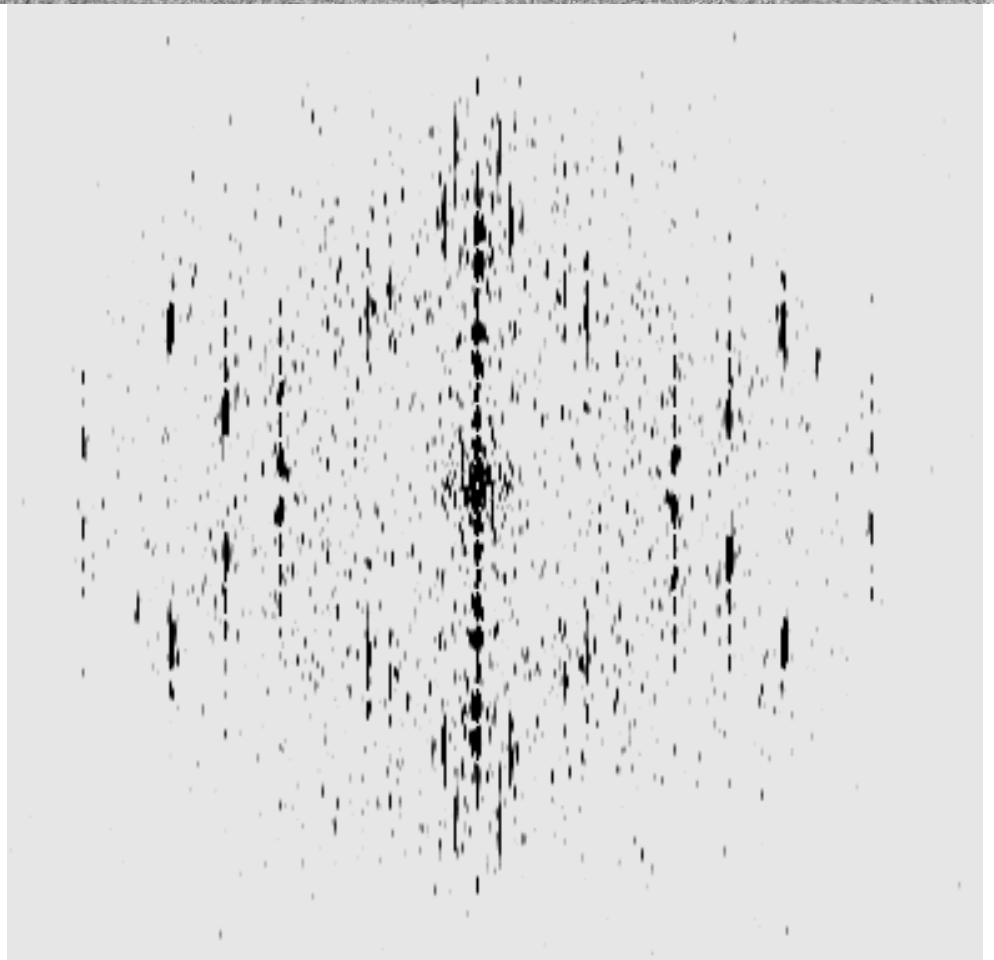
Helical Array of PFO on Nickel Lipid Tube





Helical Array of Streptavidin on Biotin Lipid tube





ADVANTAGES OF HELICAL CRYSTALLIZATION ON LIPID TUBULES

- **Helical arrays have all the required molecular views, no tilting required.**
- **Preparation of unilamellar lipid tubes is simple and rapid.**
- **Unilamellar tubes parallel an in vivo environment for membrane associated proteins.**
- **Samples can be easily manipulated during crystallization trials.**
- **A drop containing lipid tubes can be placed directly on an EM support film without transfer problems.**

Helical Arrays Formed on Lipid Tubule Substrates

Nickel lipid tubes: Fab 3B3, Fab AP7, PFO,

Biotin lipid tubes: Streptavidin

Positively charged lipid tubes: E.coli RNA polymerase g-actin, PA63

Negatively charged lipid tubes: annexin