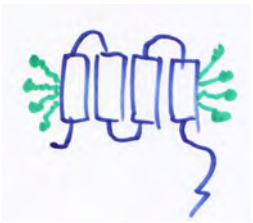
2D Crystallization by Dilution or Cyclodextrin-driven Detergent removal

Andreas Engel

Harsh vs mild solubilization

Typically high-CMC non-ionic detergents are used

Short acyl chains may intercalate between helices

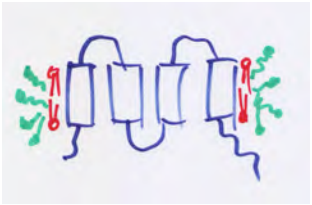


To keep protein in solution 5-10x more detergent is thus required

Often mild detergents maintain lipid environment

Additive lipids during purification may help

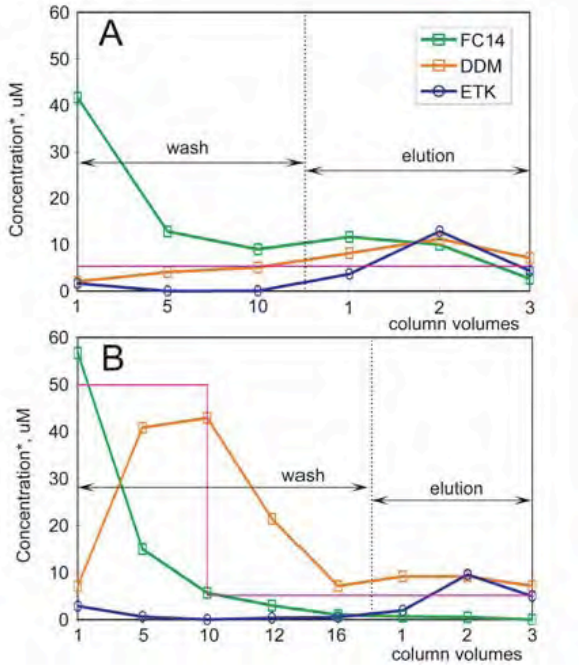
Osmolites (sugars, glycerol may help



NMR and AU of membrane protein detergent complexes

Maslennikov et al., BMC Structural Biology 2007, 7:74

DDM can be exchanged using a high concentration in the first part (B)



The concentration depends on the start

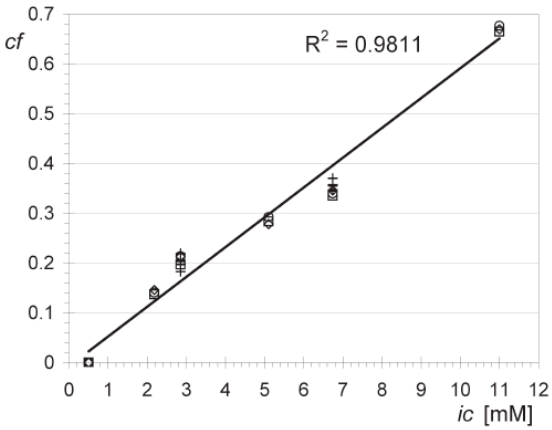
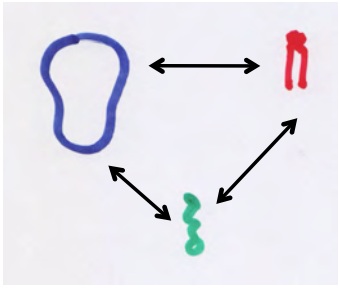
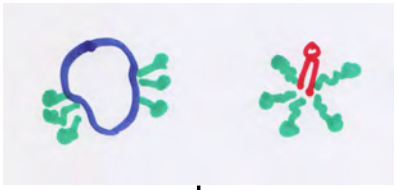


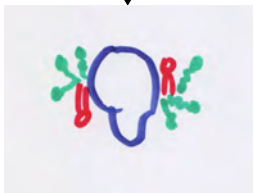
Figure 4
Dependence of the detergent concentration factor on the initial detergent concentration. Dependence of the concentration factor (see Methods) on the initial LDAO concentration. Detergent samples were prepared in 20 mM Tris-HCl buffer, pH 8.0 and concentrated 10× using Vivaspin 30 KDa cutoff ultrafiltration devices.

Ternary Mixtures & 2D crystallization

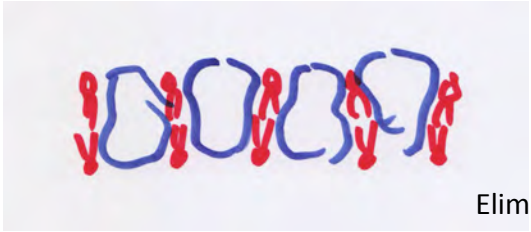


Ternary interactions





Incubate ?



Eliminate Detergent

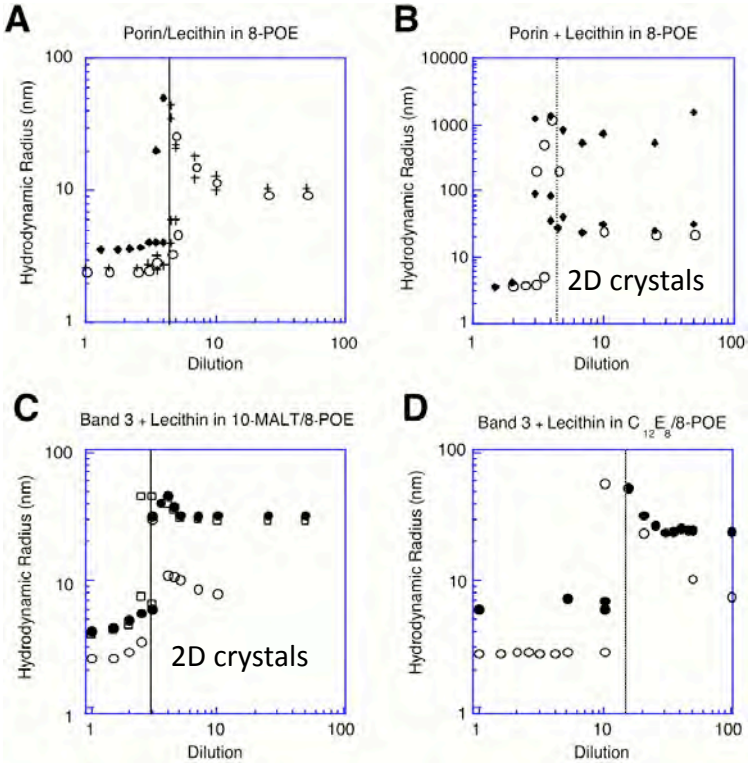
How to make this reproducible?

Accurate starting conditions, materials, concentrations
Accurate detergent removal (rate)
Accurate temperature control

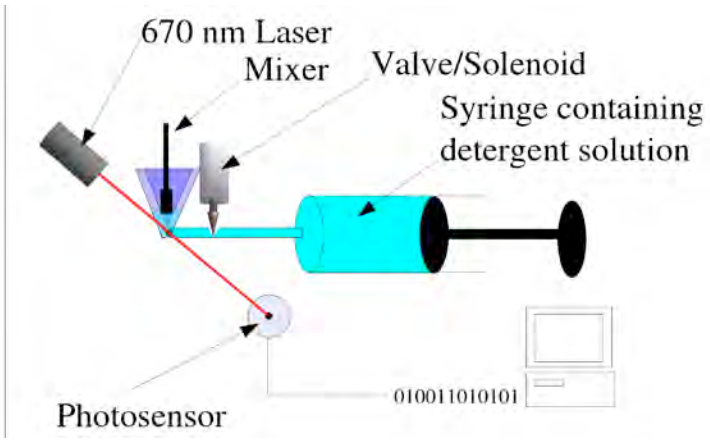
Dialysis
Dilution
Cyclodextrin

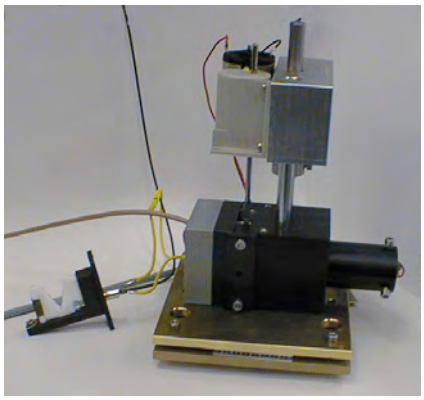
How dilution started

Dolder M, et al, **The micelle to vesicle transition of lipids and detergents in the presence of a membrane protein: towards a rationale for 2D crystallization**. *FEBS Letters* 1996, **382**:203-208.



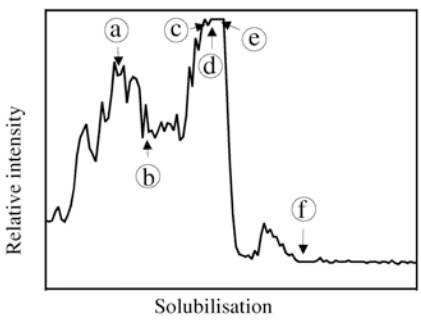
Dilution robot: Hervé Rémigy's first one

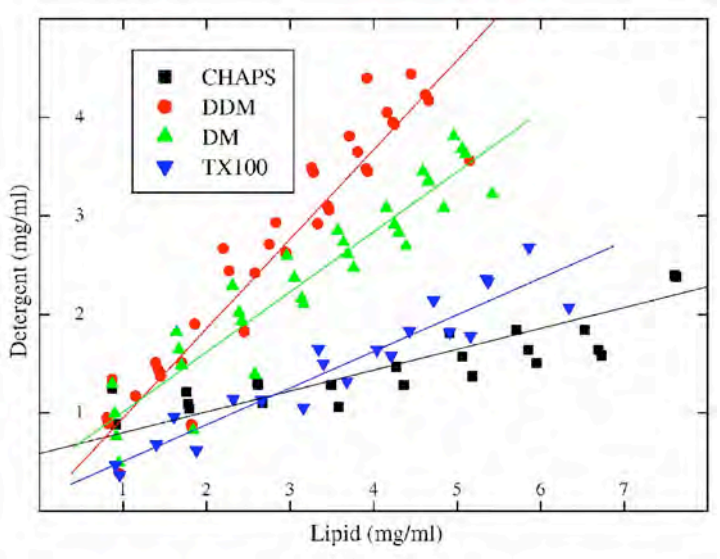




Rémigy et al., Membrane protein reconstitution and crystallization by controlled dilution. FEBSLetters 555 (2003) 160-169

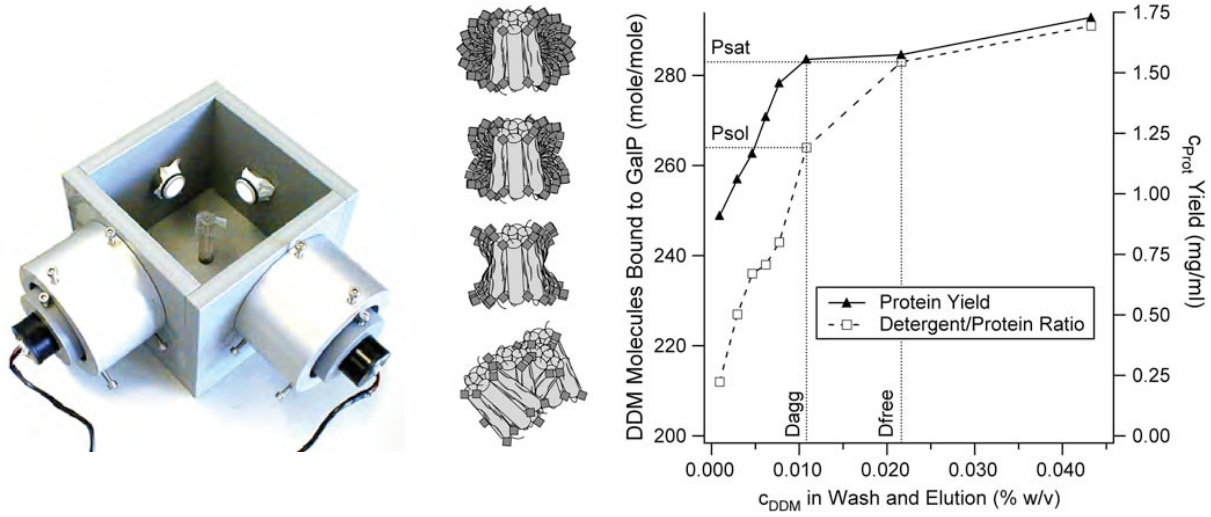
Lipid solubilization capacity





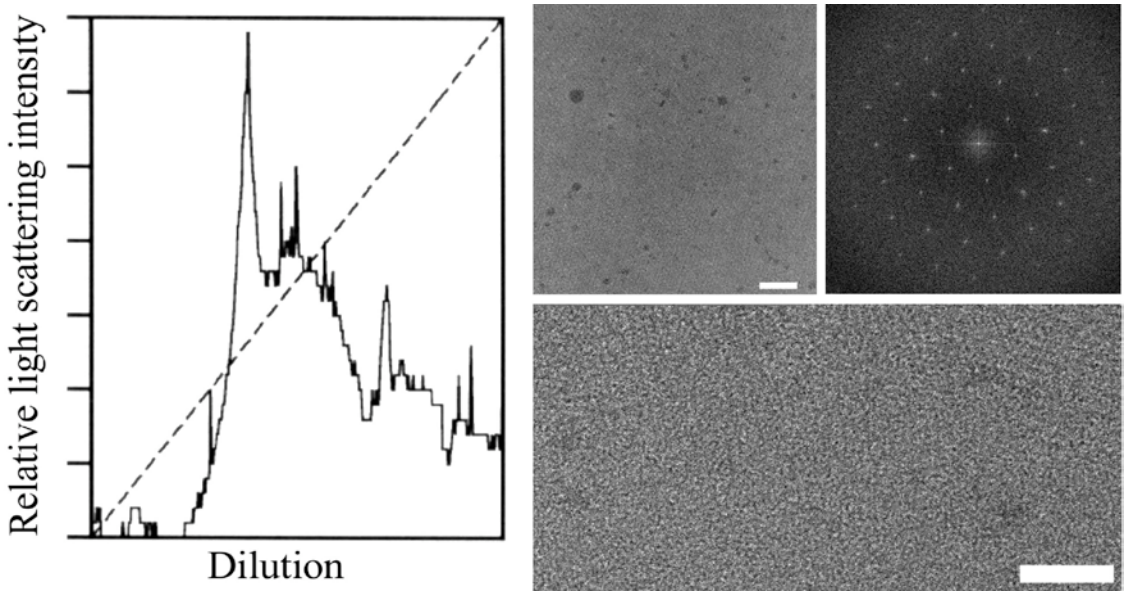
Rémigy et al., Membrane protein reconstitution and crystallization by controlled dilution. FEBSLetters 555 (2003) 160-169

Drop box: Hervé's second one



Kaufmann et al. : Novel Detergent Concentration Determination Method for Assessing Membrane Protein Aggregation Biophys. Journal 90: 310–317

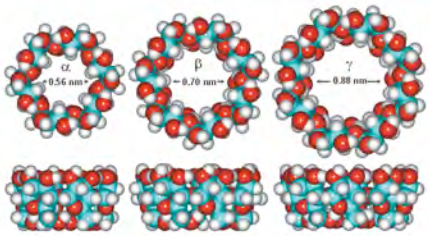
2D crystallization vs light scattering

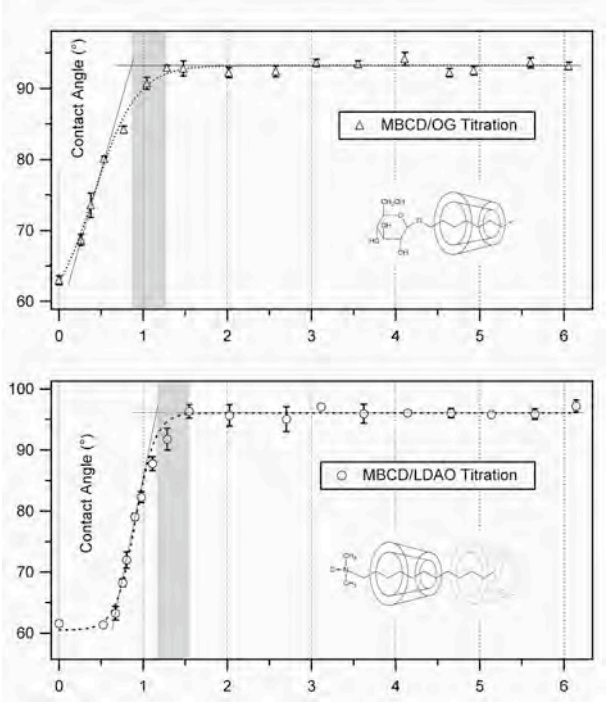


Rémigy et al., Membrane protein reconstitution and crystallization by controlled dilution. FEBSLetters 555 (2003) 160-169

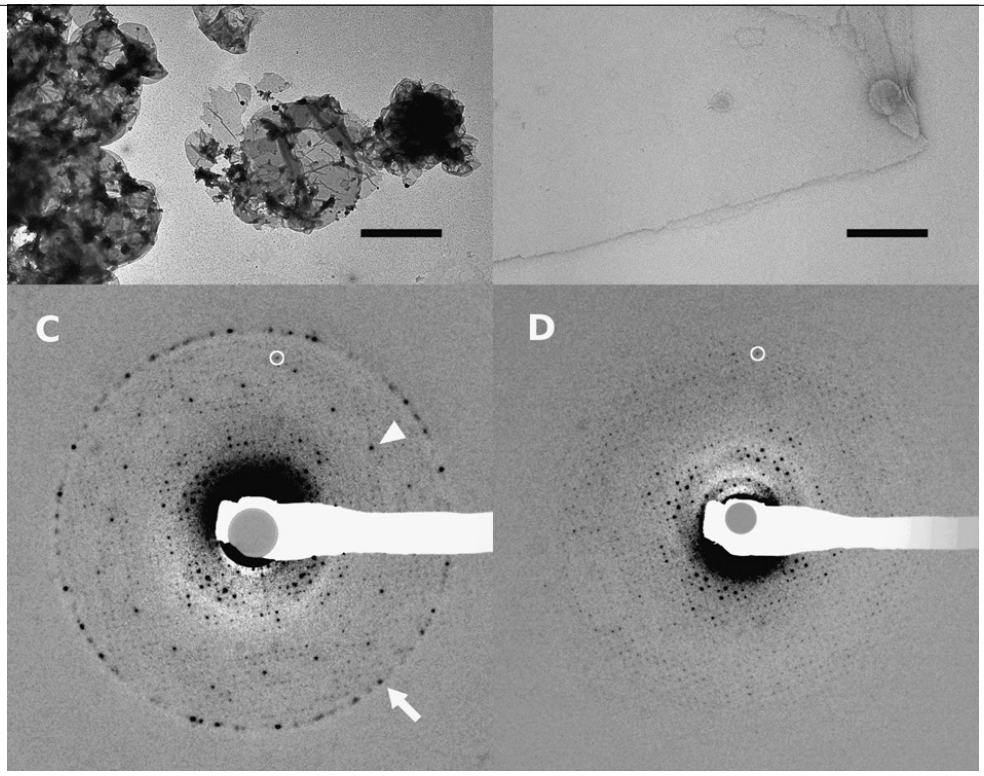
Cyclodextrin for detergent removal

Key is the stoichiometric relation



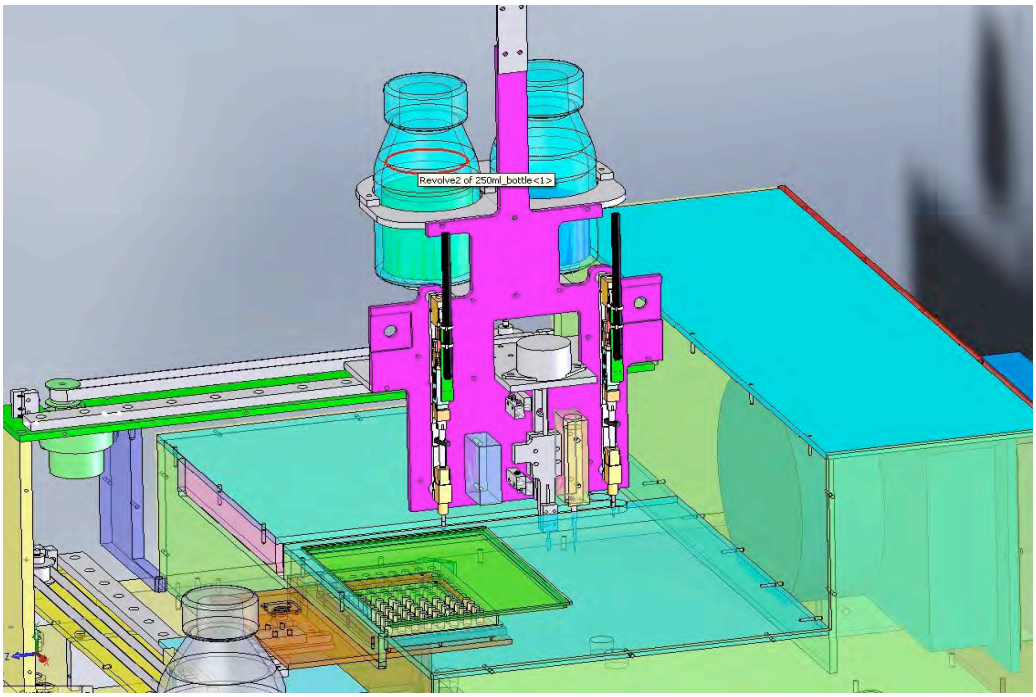


Signorell et al., Controlled 2D crystallization of membrane proteins using methyl—cyclodextrin, JSB 157 (2007) 321–328

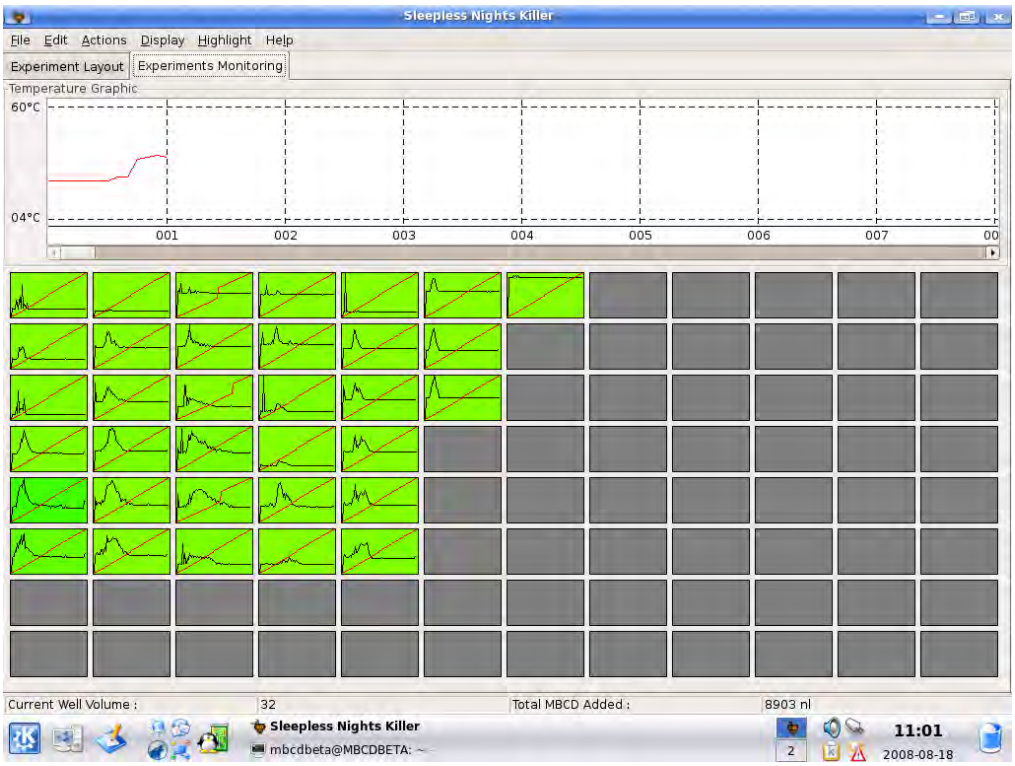


Signorell et al., Controlled 2D crystallization of membrane proteins using methyl—cyclodextrin, JSB 157 (2007) 321–328

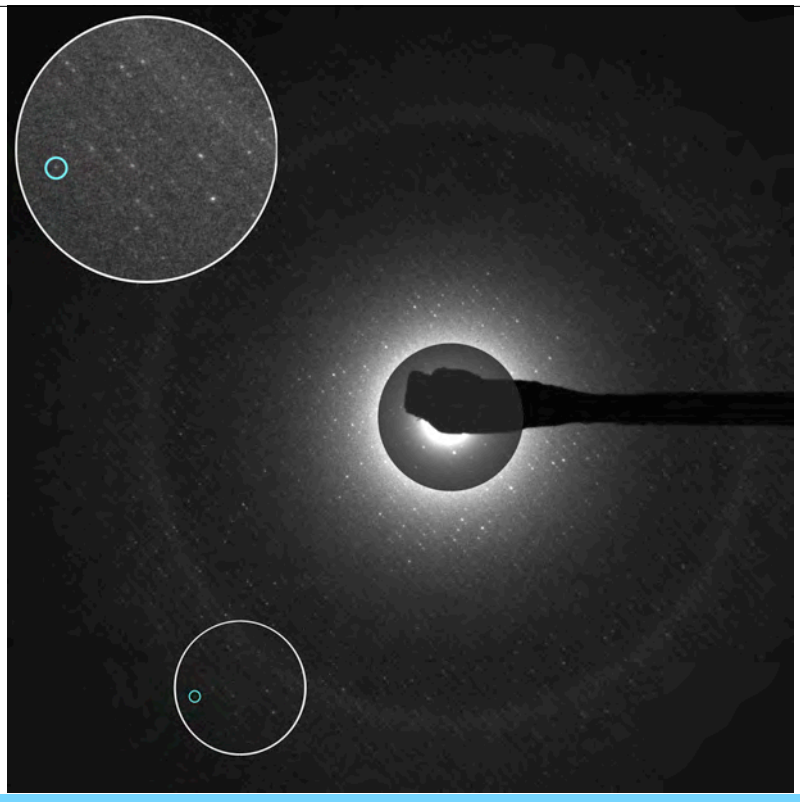
Cyclodextrin robot: Hervé's third one



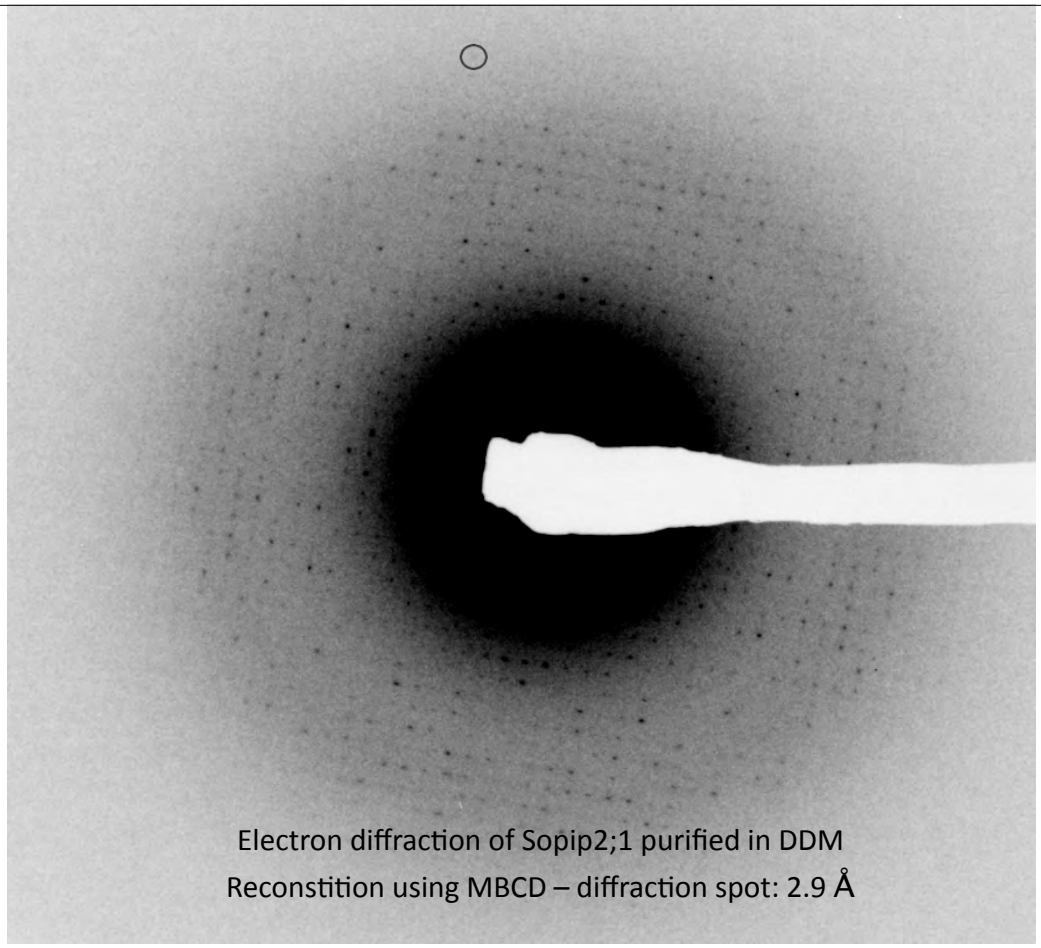
Iacovache et al. The 2DX robot: A membrane protein 2D crystallization Swiss Army knife
J. Structural Biology 169 (2010) 370–378



Iacovache et al. The 2DX robot: A membrane protein 2D crystallization Swiss Army knife
J. Structural Biology 169 (2010) 370–378



***Aeromonas hydrophila* Aerolysin**
Cyclodextrin method



Electron diffraction of Sopip2;1 purified in DDM
Reconstition using MBCD – diffraction spot: 2.9 Å

End