

# Humidity Control -

the key to successful preparation of  
cells and suspensions for 3D cryo-EM



peter frederik

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Colloid  
Chemistry  
=  
Cryo-EM ?



**SDS film thinning**

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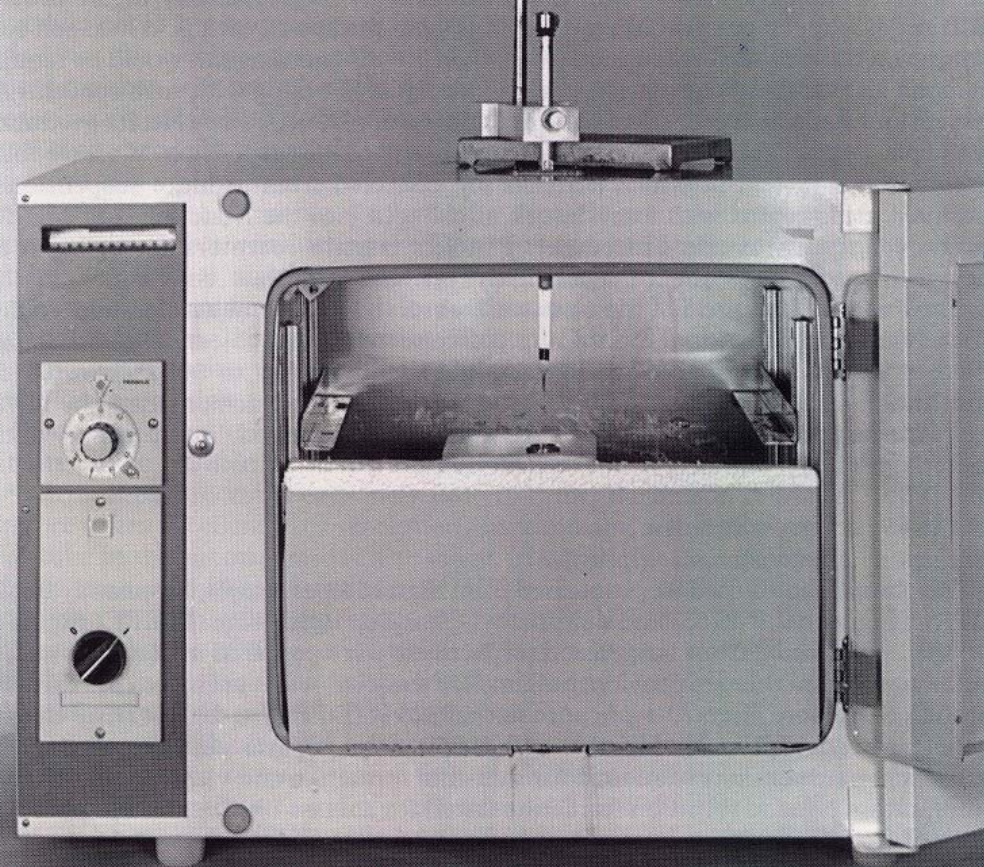
**First prototype (1984) studying “thin” water  
1985 CAMBRIDGE/UK**

**“Fully Hydrated” CRYO-EM is the study of drying thin films**

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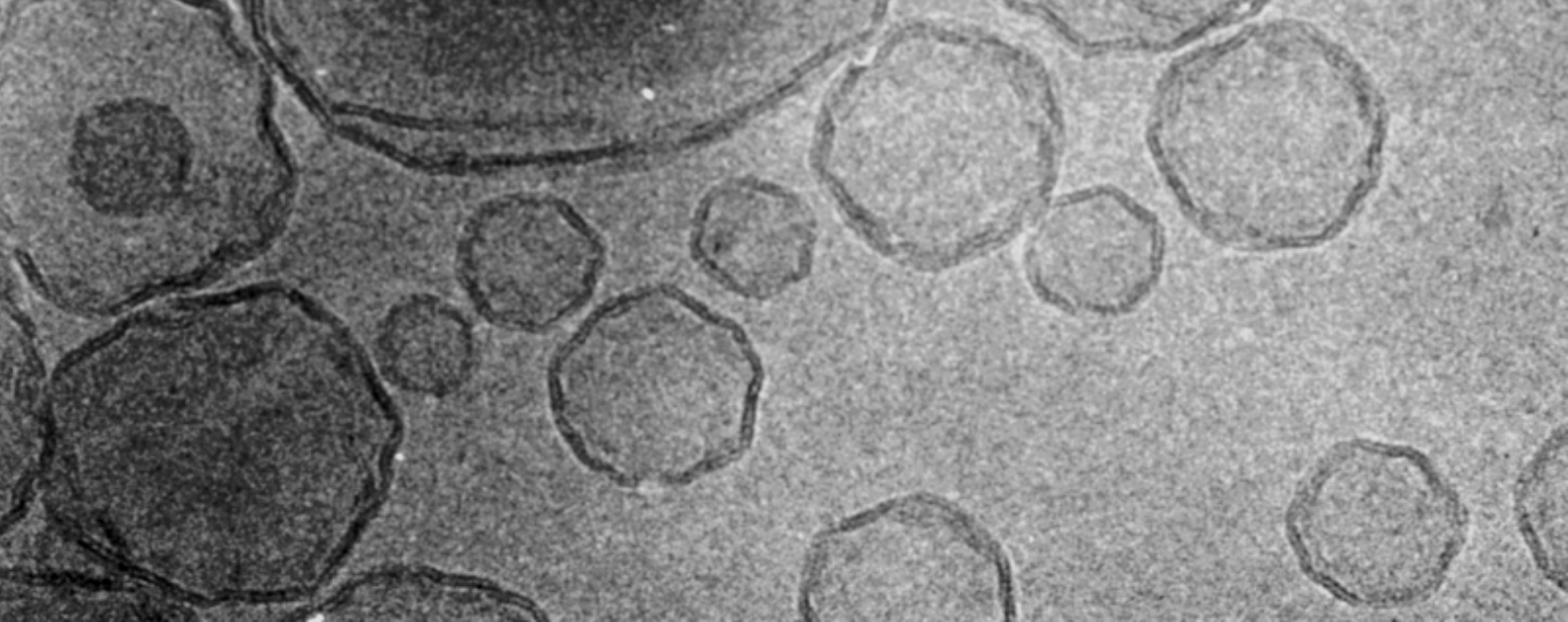
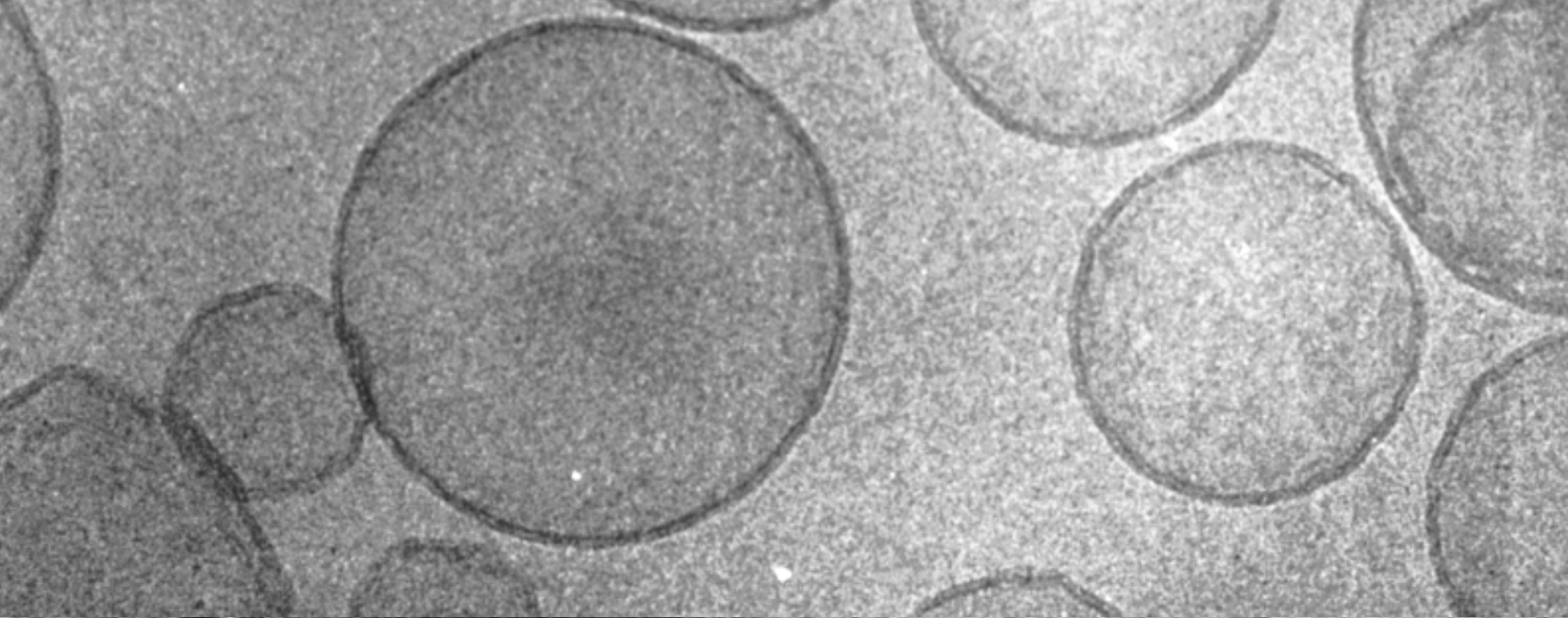
1990



1997

Evolution & revolution

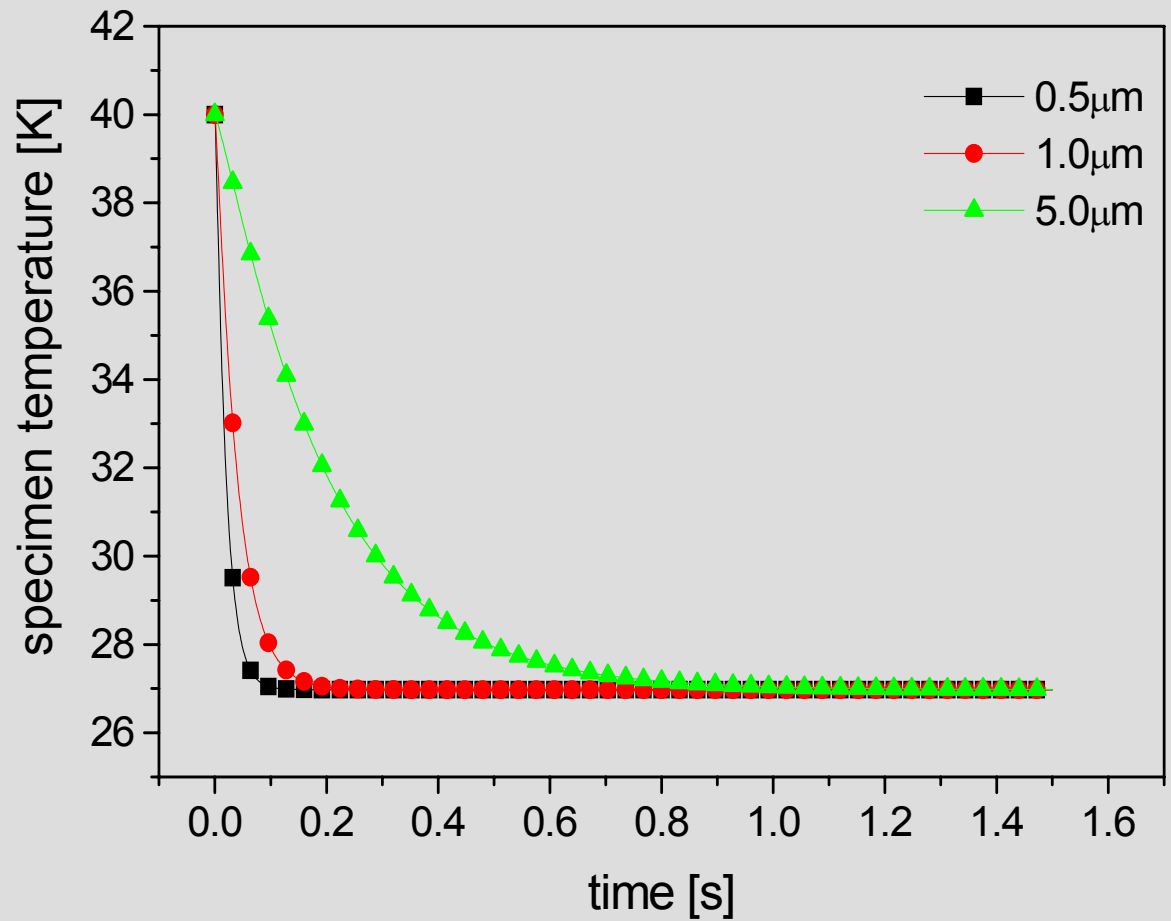




**DPPC 50 °C/40 °C**

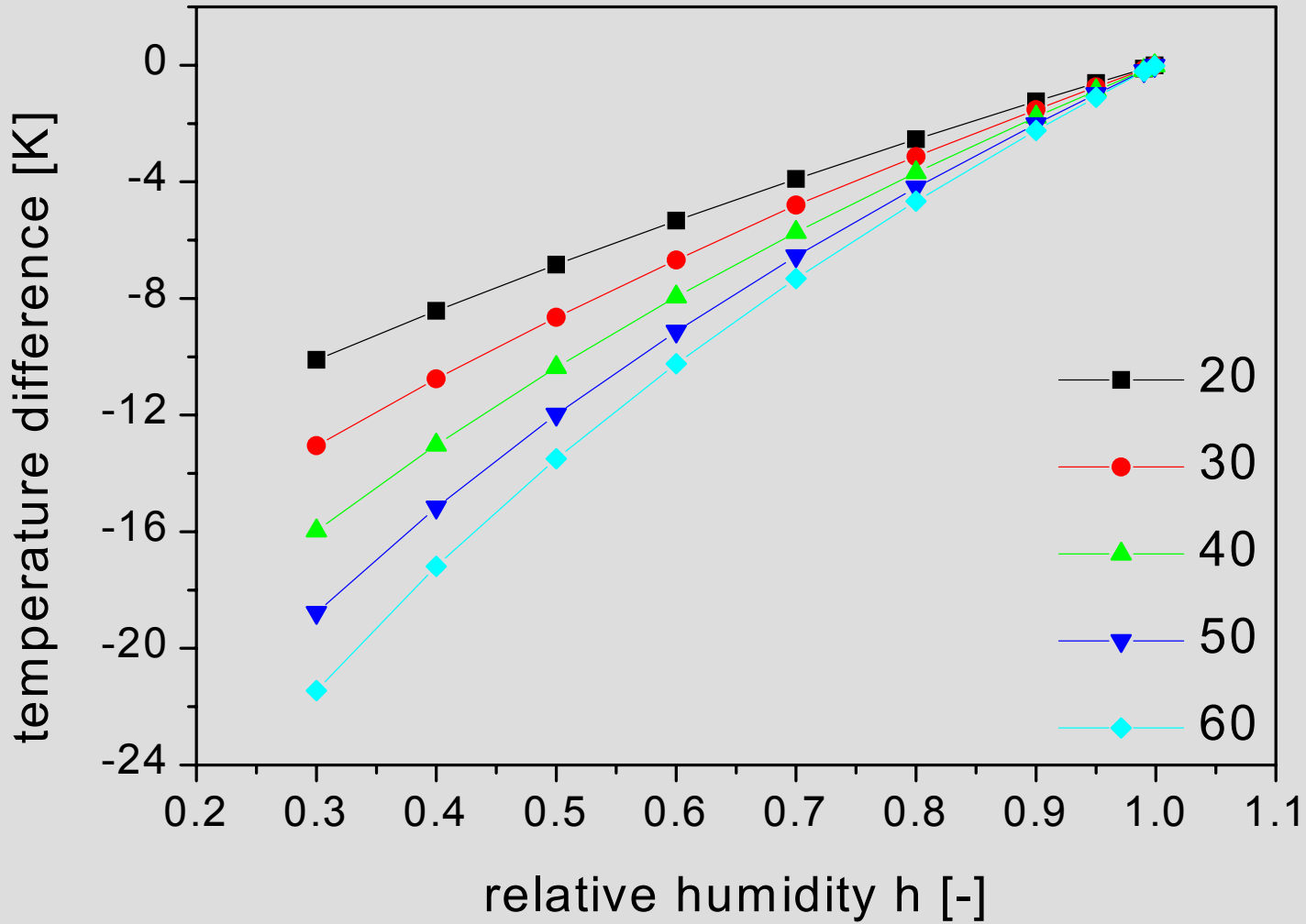
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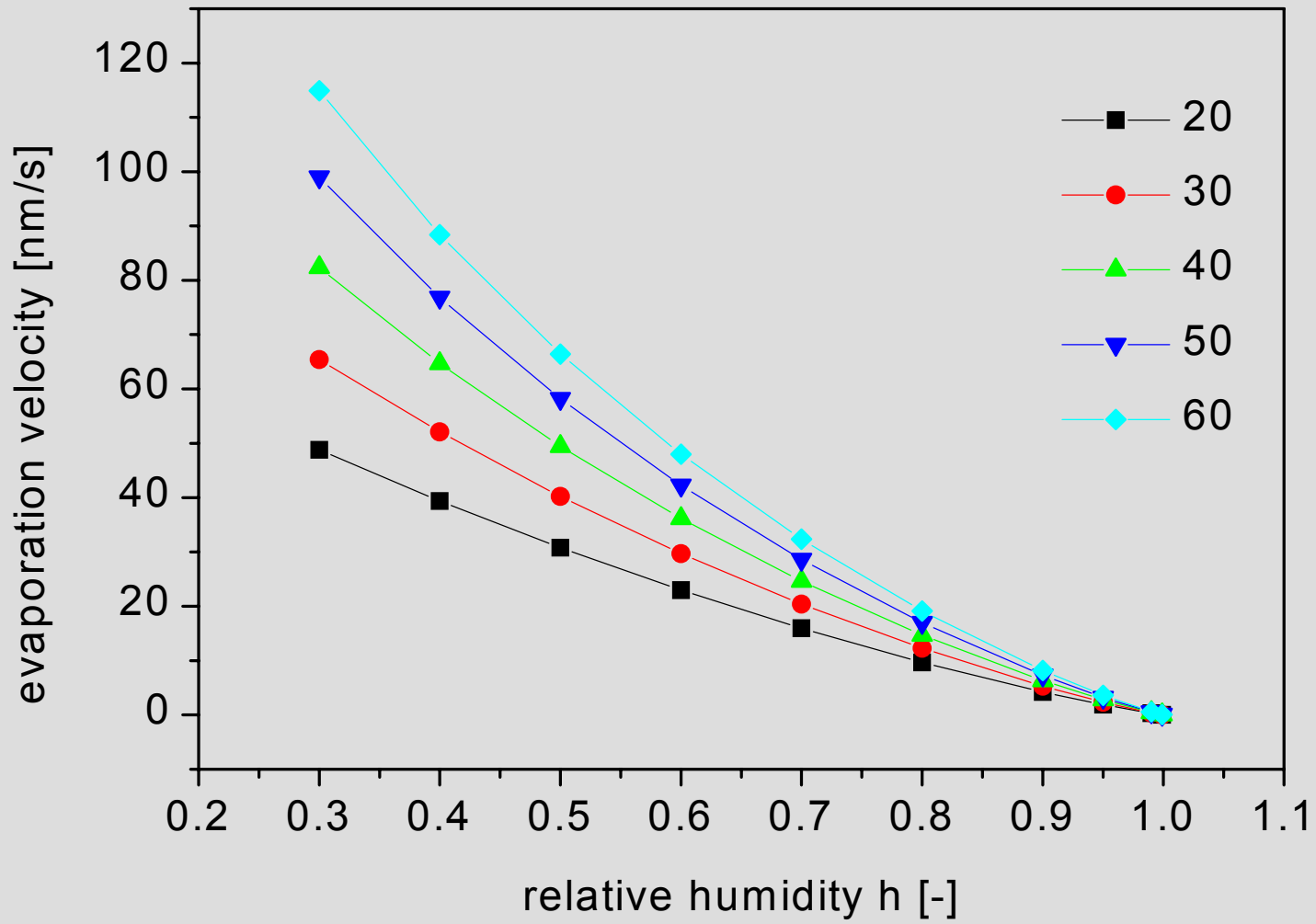


dewpoint

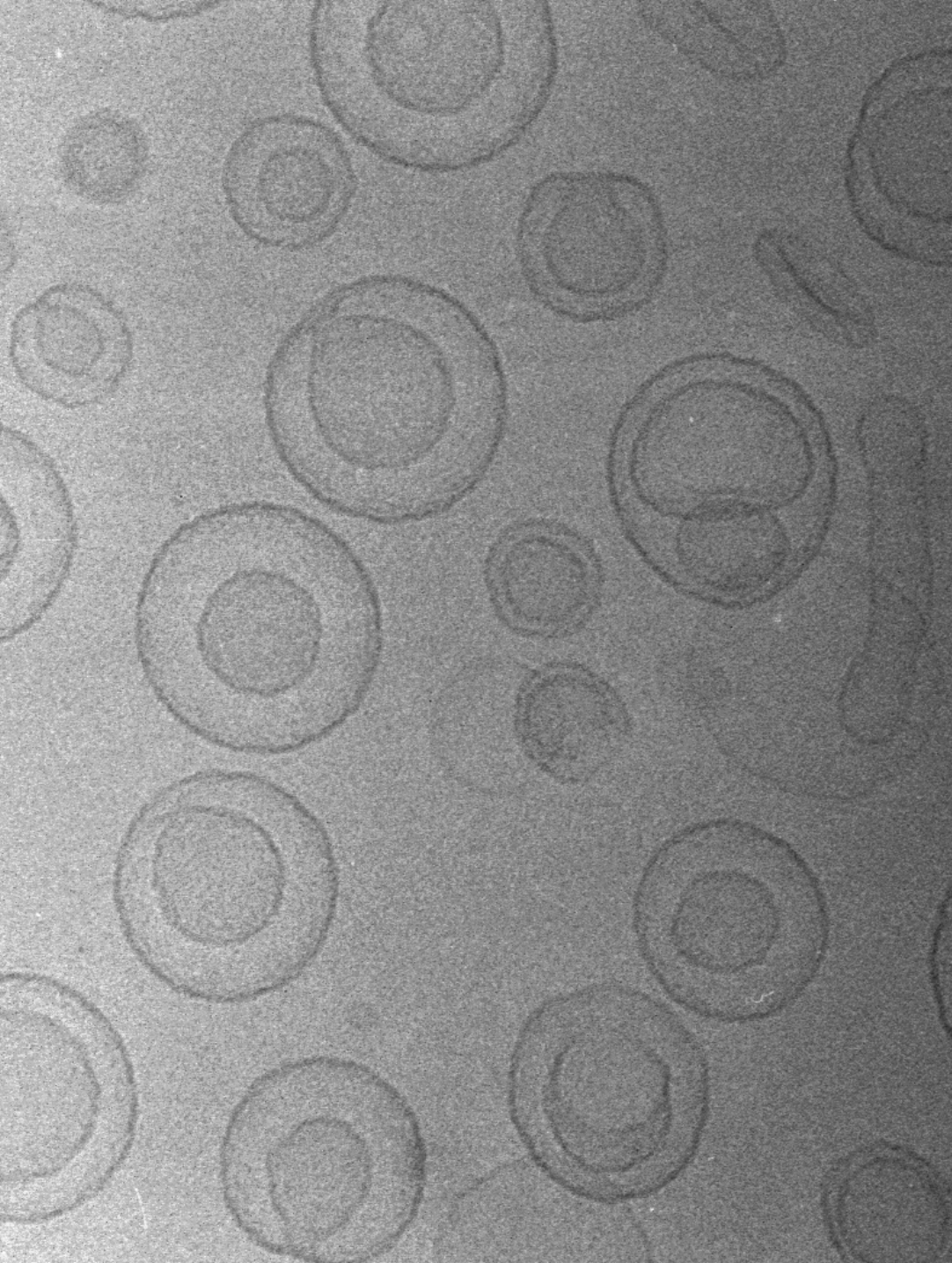




Dew point DT





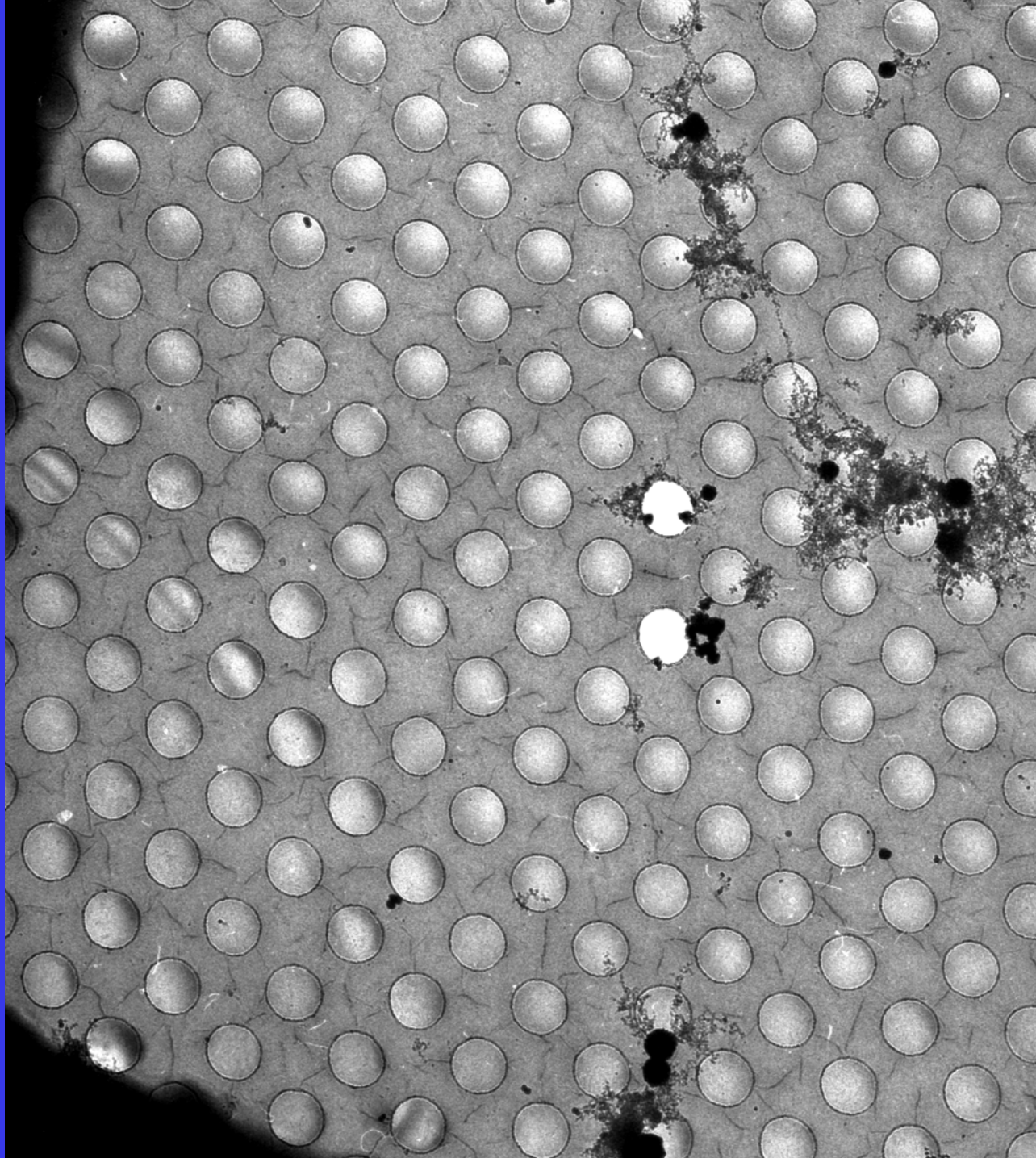
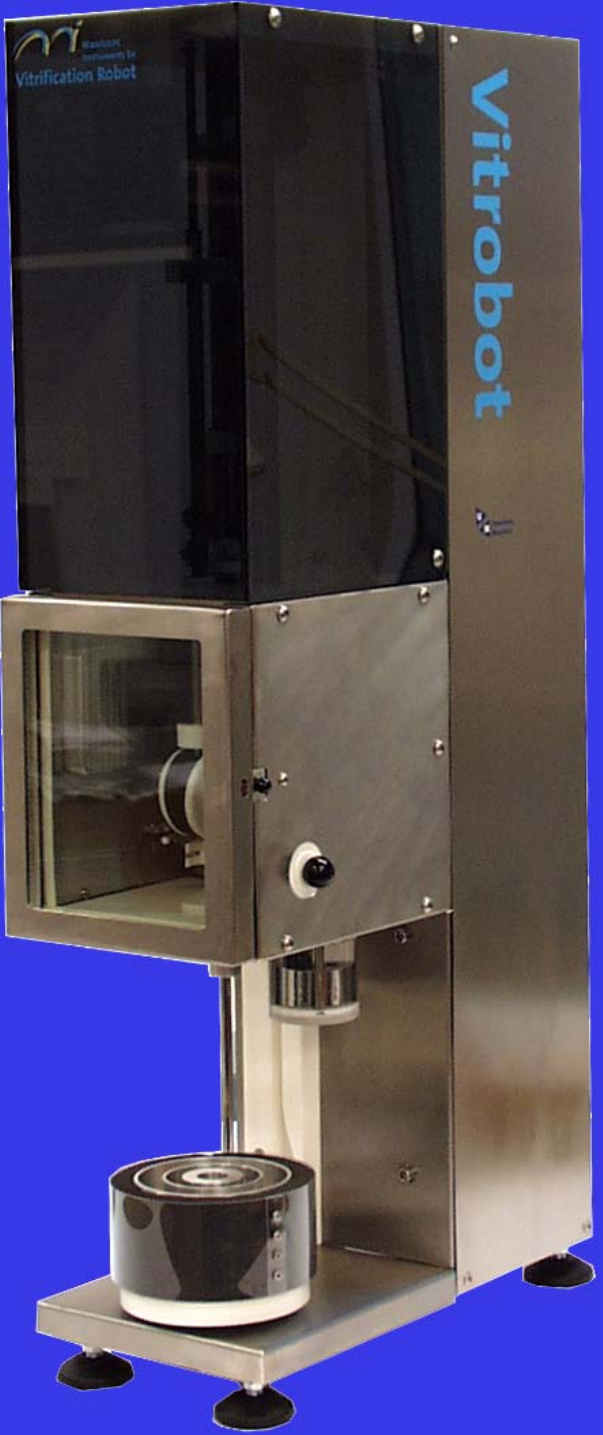


# Osmotic collapse



 Milestone  
Instruments by  
Vitrification Robot

Vitrobot







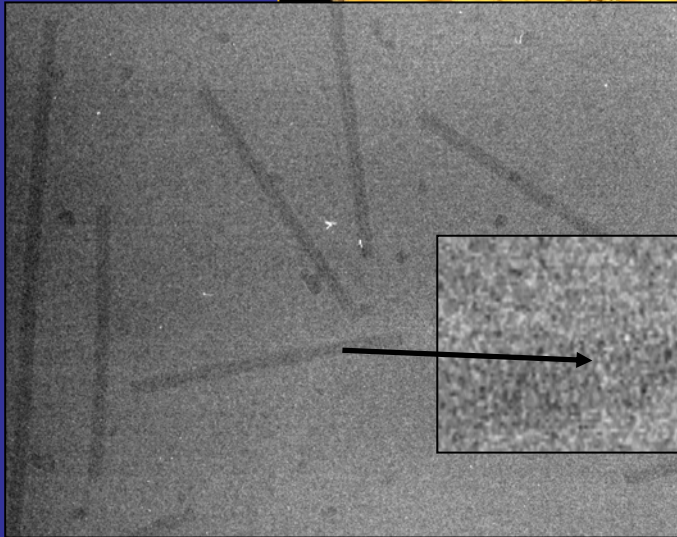
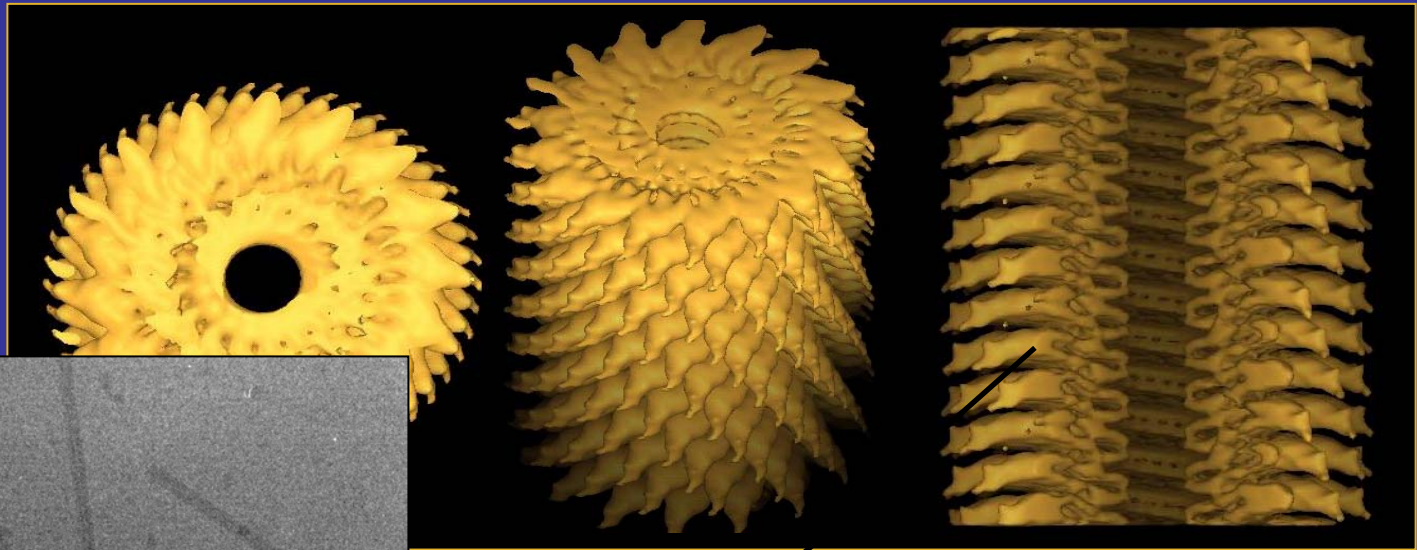
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# Vitrobot™: Applications in Cryo-TEM

## Proteomics / Structural Biology

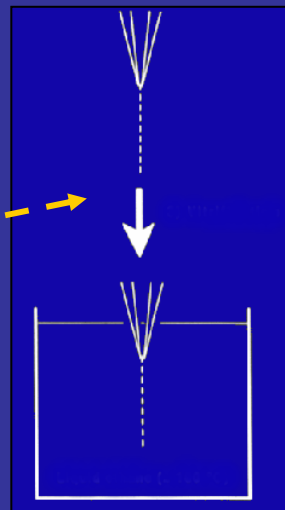
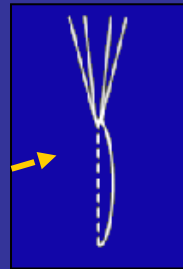
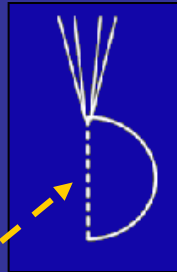
Tobacco Mosaic Virus reconstructed in 3D down to 10Å resolution from one data collection session (<24hrs)



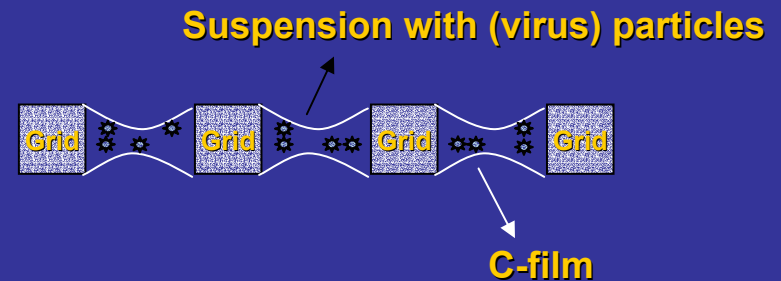
Courtesy of Dr. Bridget Carragher,  
Scripps Institute, La Jolla (CA, USA)

# Vitrobot™

## Vitrification Robot



- Incubation of suspension on grid (holey carbon, lacey film or quantifoils) at constant temperature and humidity
- Automated Blotting



- Plunging in liquid ethane (propane, N<sub>2</sub>)

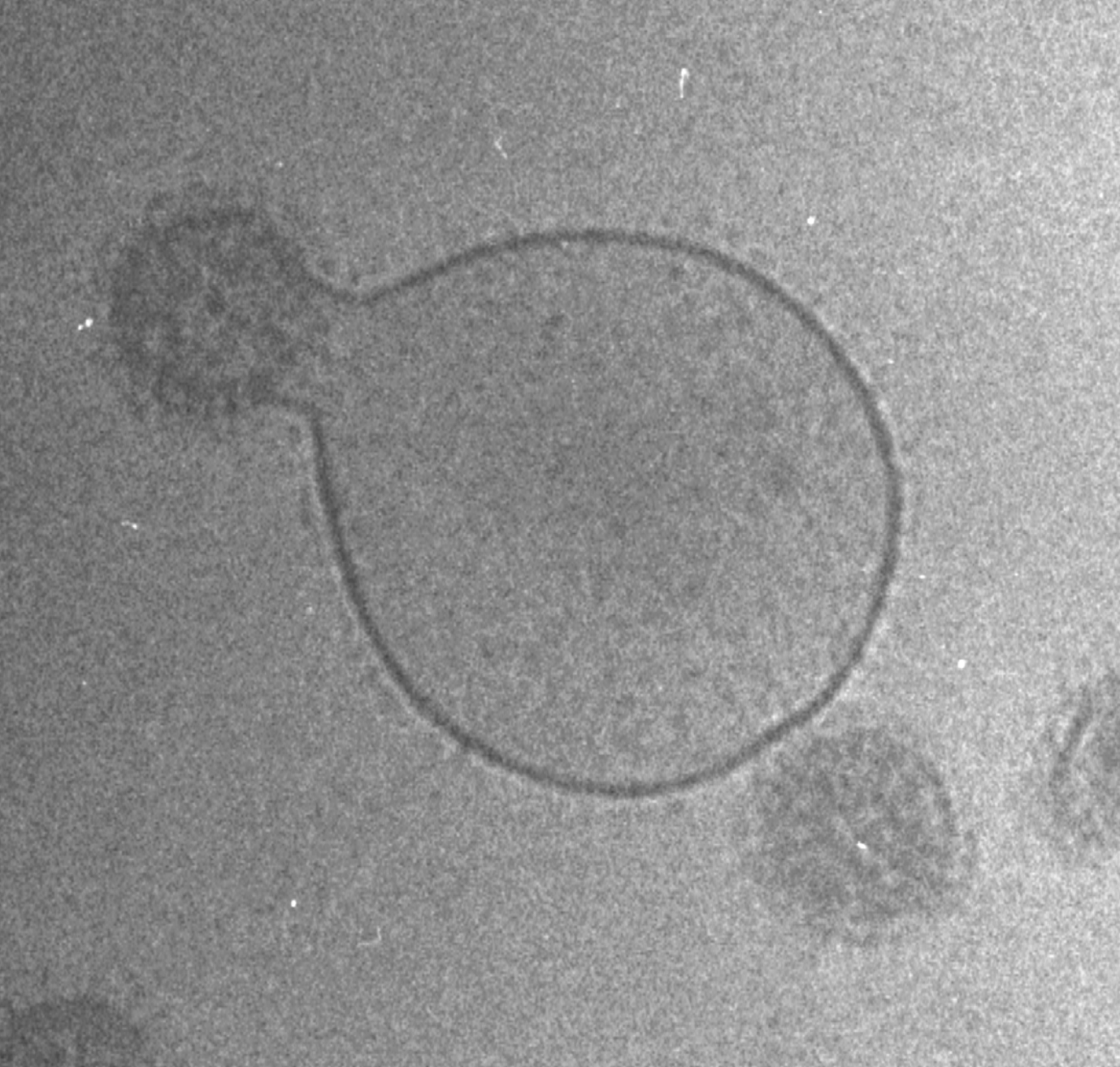
***Ready for investigation  
in vitrified matrix***



# Vitrification

## Prerequisites

- Speed of Vitrification →  $1 \cdot 10^7$  °C / sec (liquid coolant dependant)
- Thickness and Quality of Ice Layer
  - Constant thickness - impossible when blotting manually
  - Amorphous Ice
- Temperature and Relative Humidity (RH) during vitrification
  - At 100% RH → no evaporation, constant ice layer thickness
  - Repetitive conditions for optimal results



**K.Burger**  
**UU**

**Influenza-membrane fusion**

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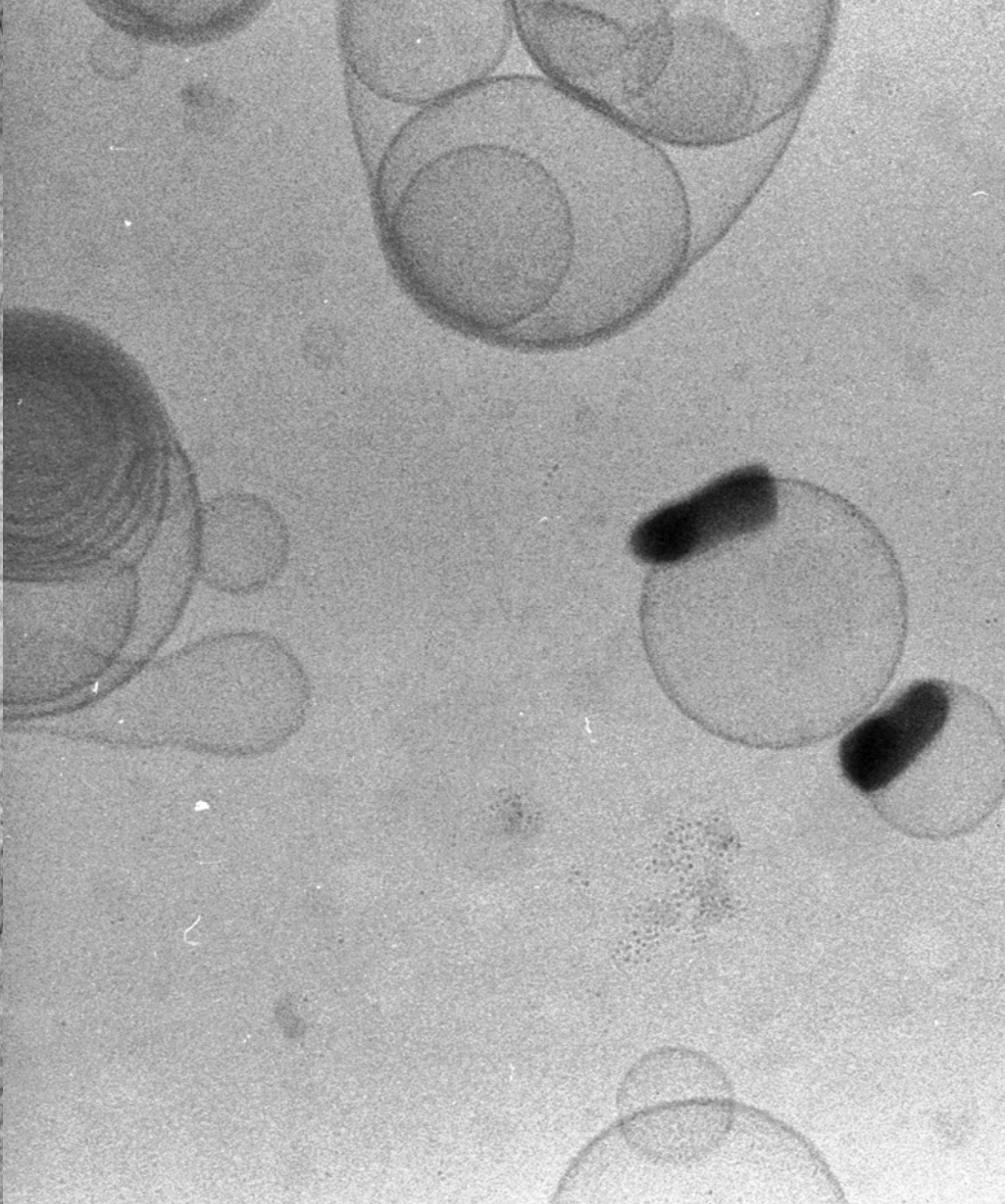
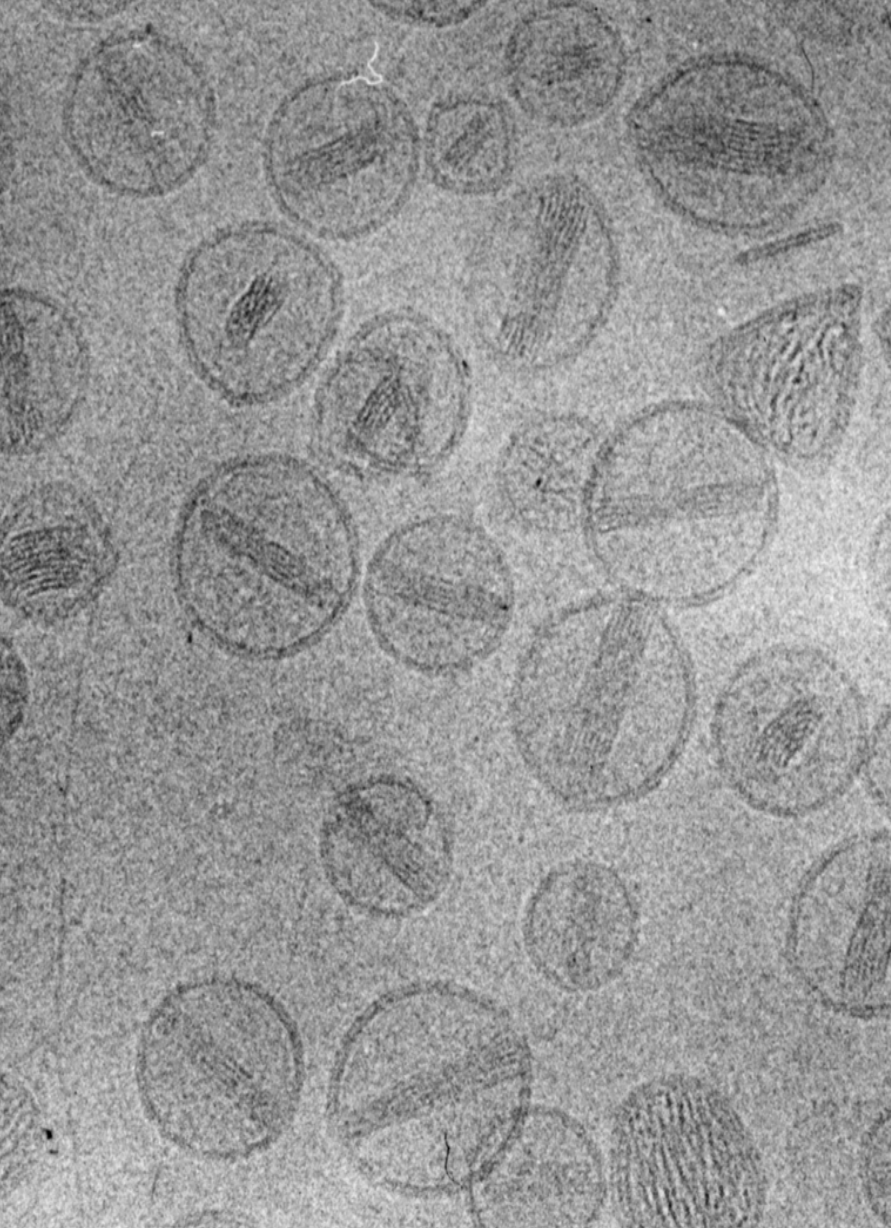
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# **Virus membrane interaction**

**membrane adhesion  
cell entrance / fusion  
change cell functions**

**model for drug targeting  
model for gene targeting**

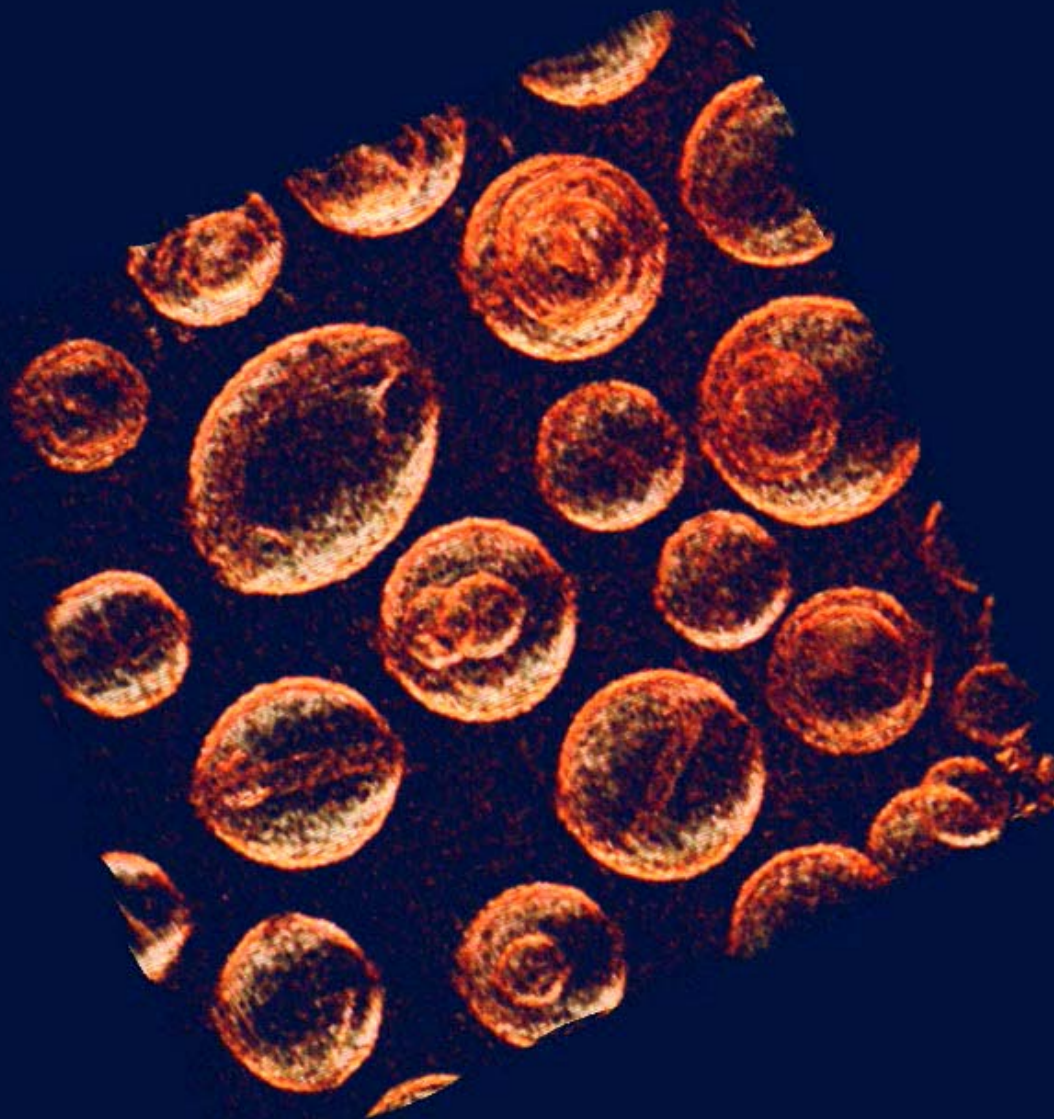




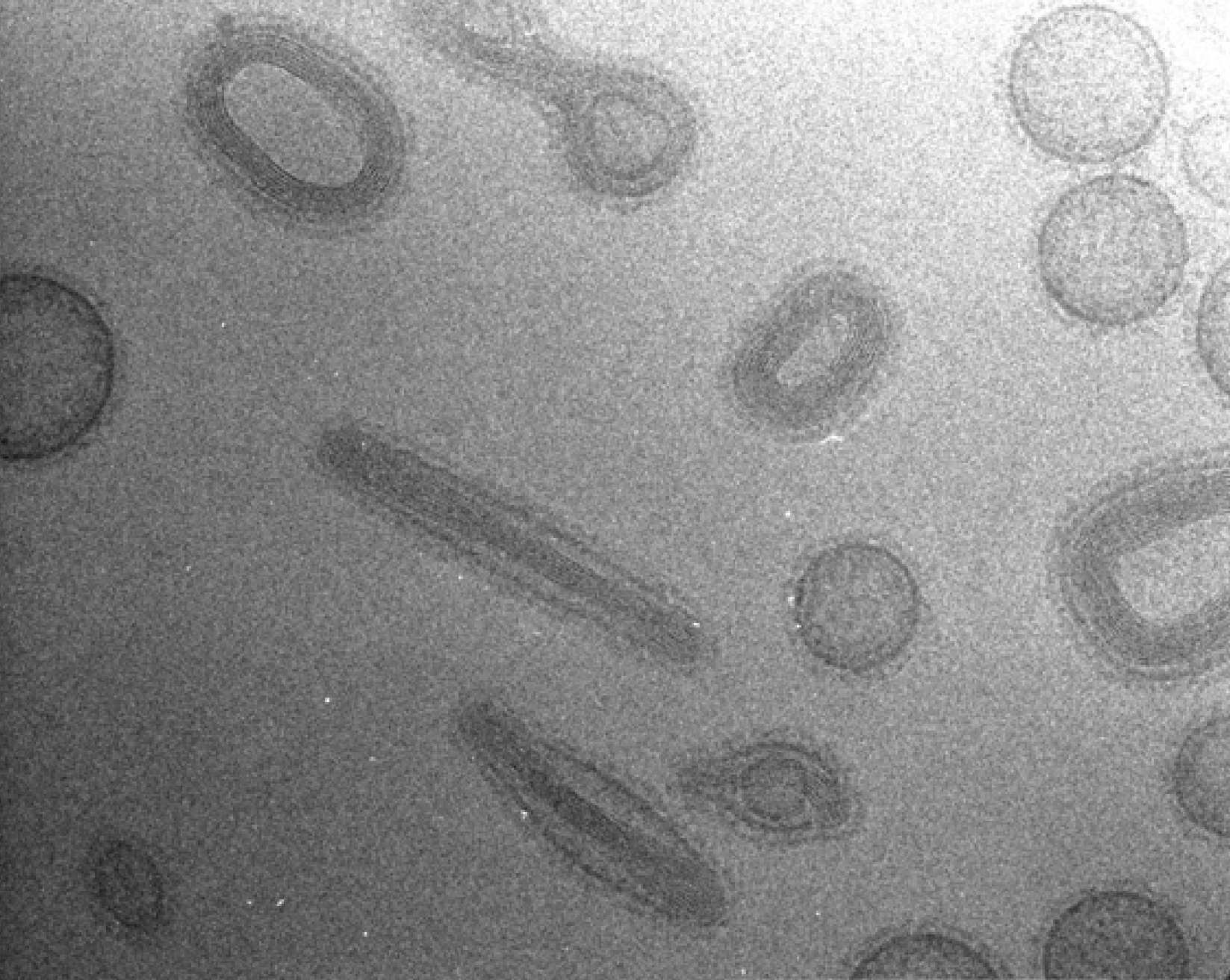
**Doxil** Science 1995

**cisPt** Nature medicine 2002

**Doxil/Caelyx**







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**Targeted Doxorubicine loaded vesicles**



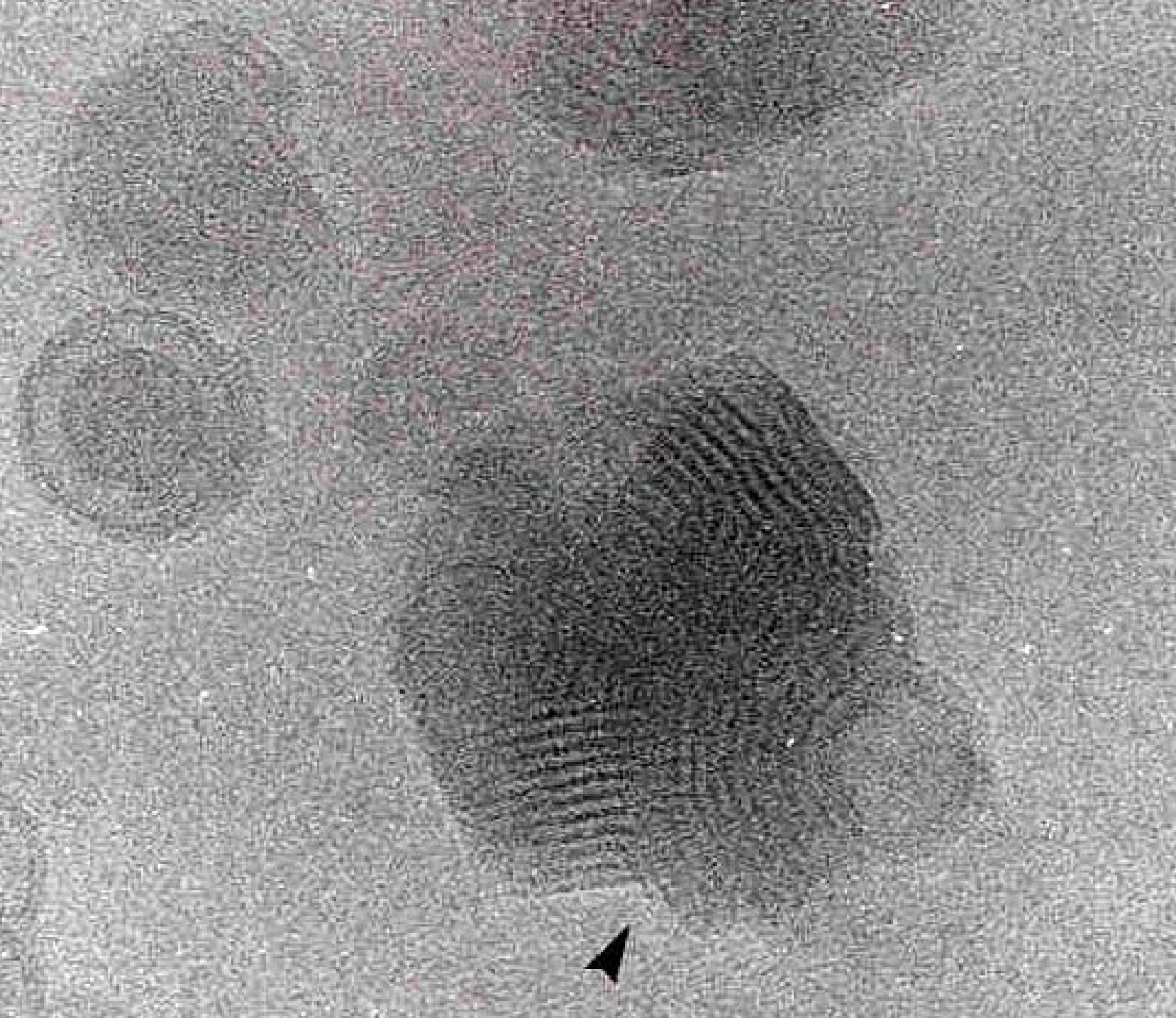
# Vesicles for transfection *in vivo* gene targeting

cationic vesicles + DNA  
charge interaction  
vesicle remodeling

N. Templeton Houston/ D. Lasic Newark / W. Baumeister Martinsried  
BRD/ Y. Perrie London/ L Xu Georgetown & Ann Arbor



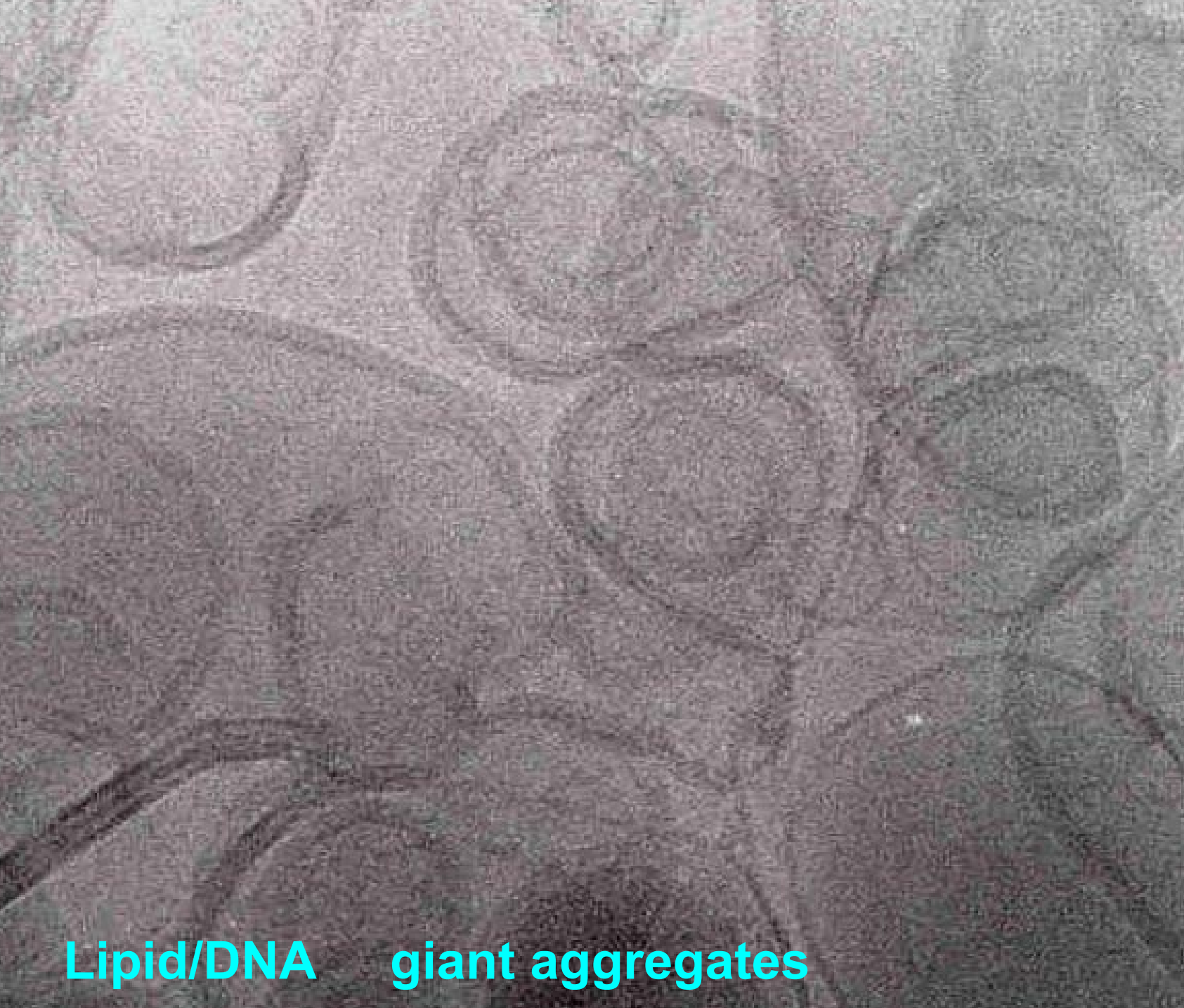
1997 Nature BT gene therapy Hum Gene Ther 2002



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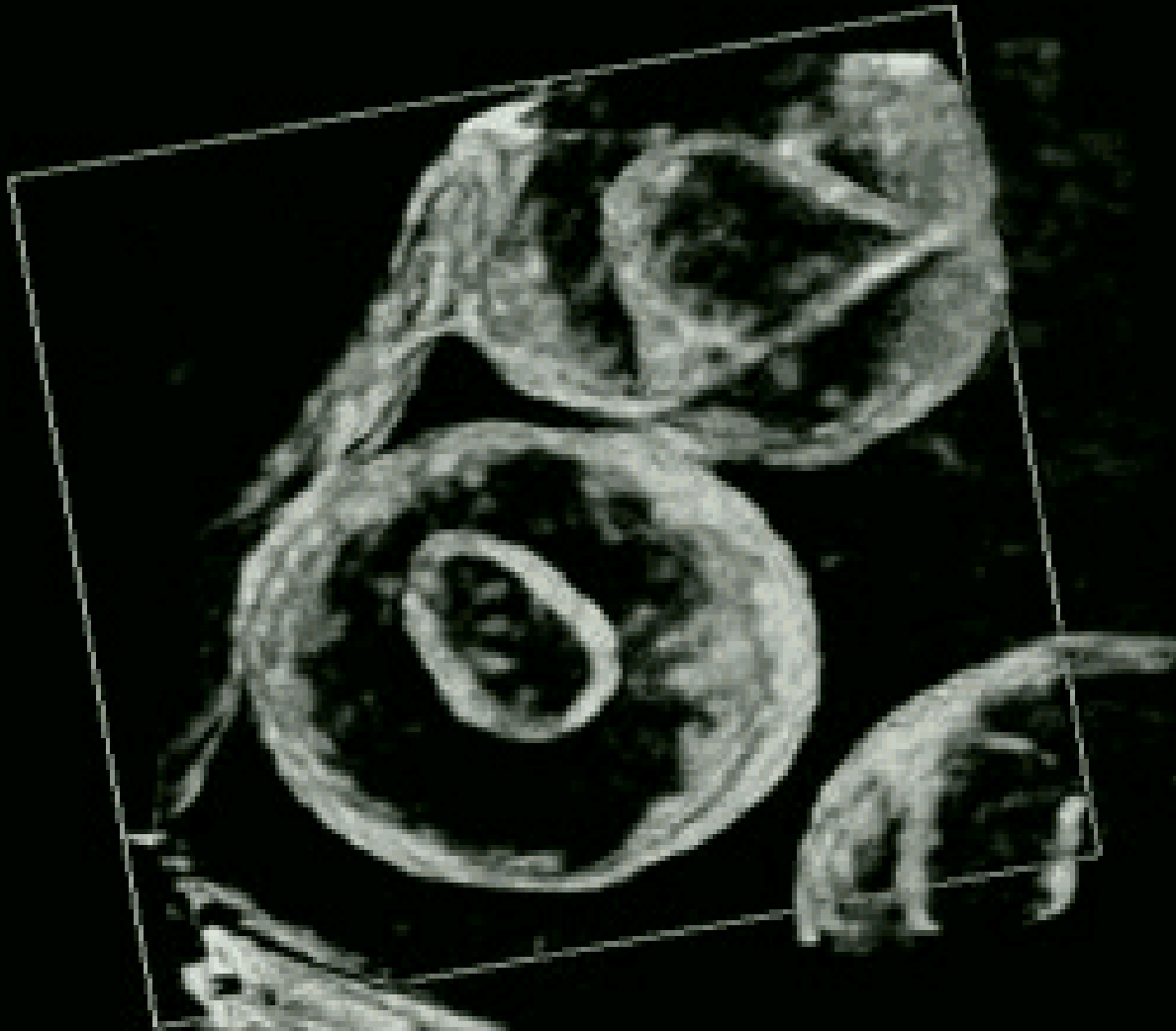


**Lipid/DNA**

**giant aggregates**

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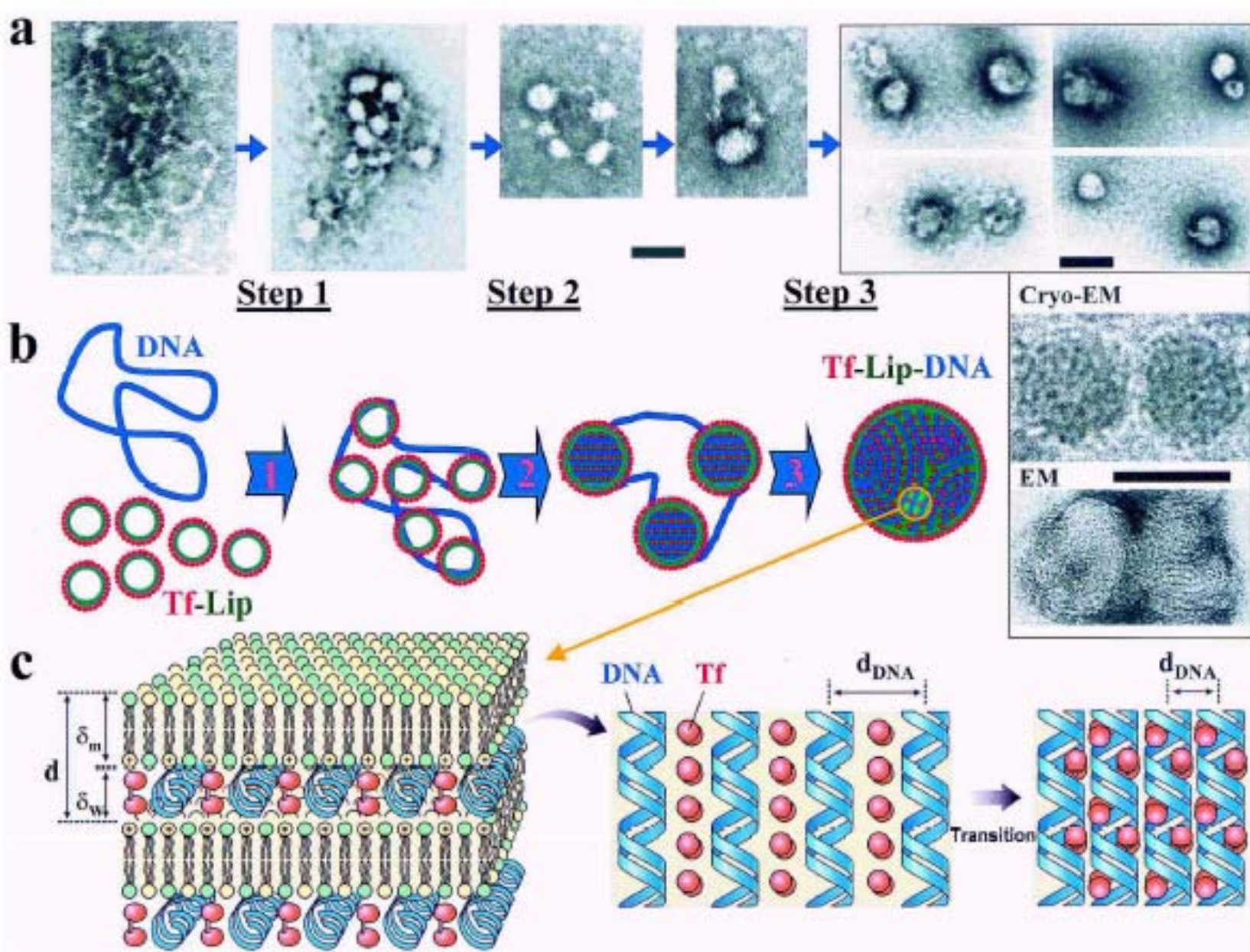
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Tomography/MPI Martinsried



Xu et al Hum Gene Therapy 2002



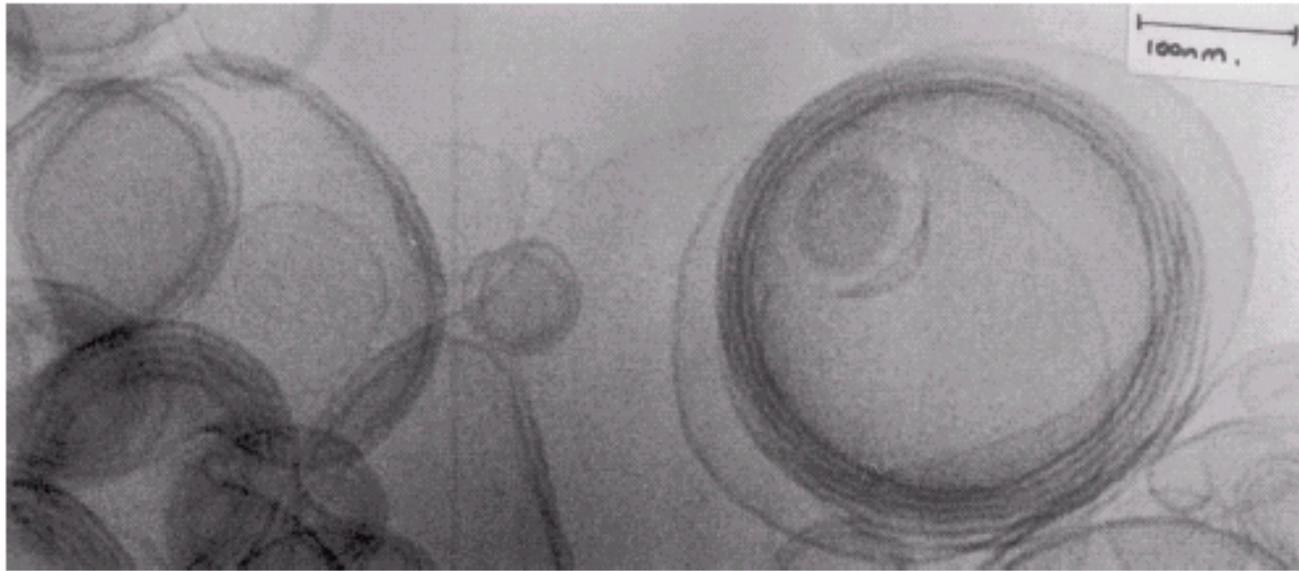


Fig. 1. Cryo-electron microscopy of DRV(DNA) composed of 16  $\mu\text{mol}$  PC, 8  $\mu\text{mol}$  DOPE and 4  $\mu\text{mol}$  DOTAP, and prepared in the presence of 100  $\mu\text{g}$  pRc/CMV HBS.

## Liposome-mediated DNA vaccination: the effect of vesicle composition

Yvonne Perrie <sup>a,b,1</sup>, Peter M. Frederik <sup>c</sup>, Gregory Gregoriadis <sup>a,\*</sup>

<sup>a</sup> Centre for Drug Delivery Research, The School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX, UK

<sup>b</sup> Lipoxen Ltd, 29–39 Brunswick Square, London WC1N 1AX, UK

<sup>c</sup> EM-Untt, University of Maastricht, P.O. Box 616, 6200 MD Maastricht, The Netherlands



**Karin Butter**

et. al.

**UU**

**Nature**

materials

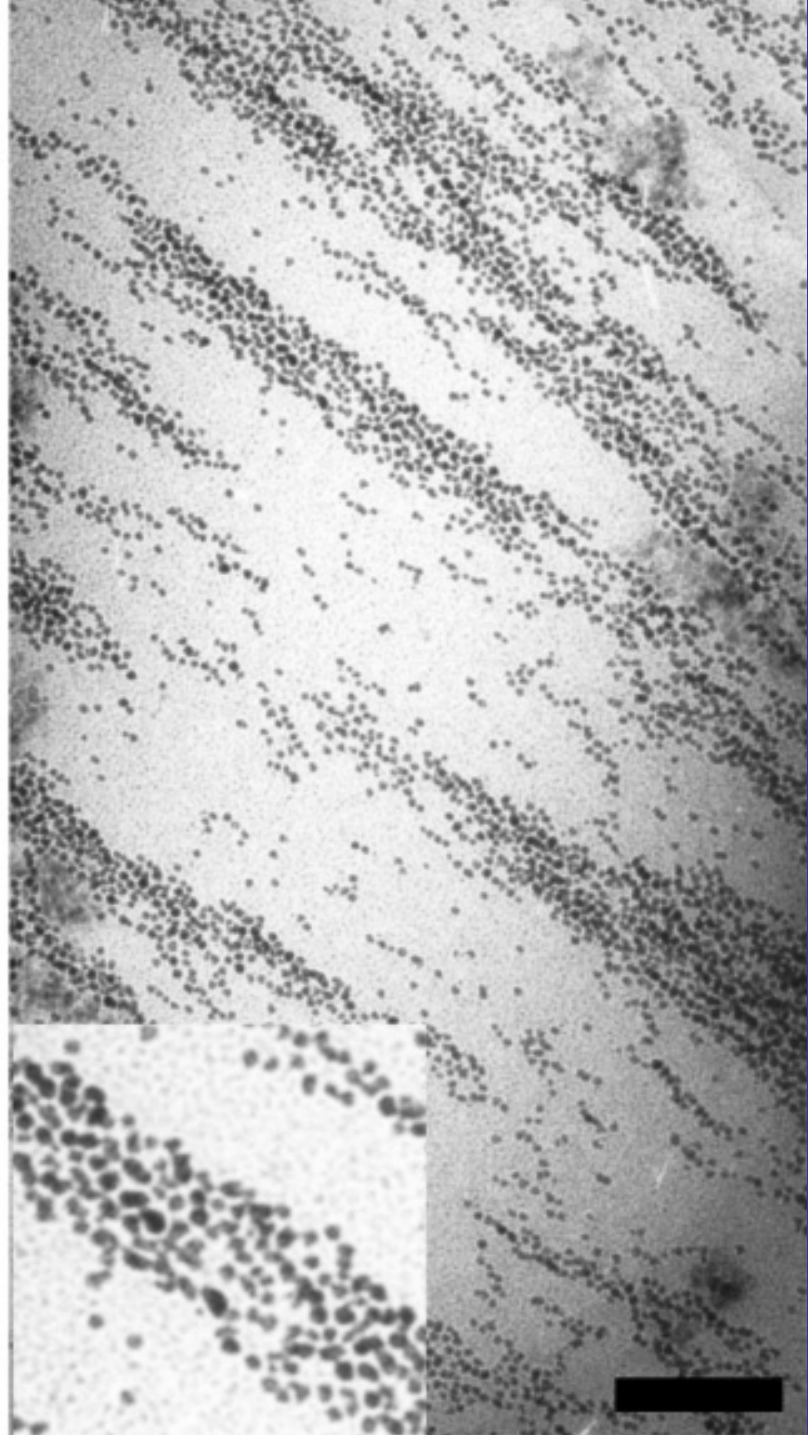
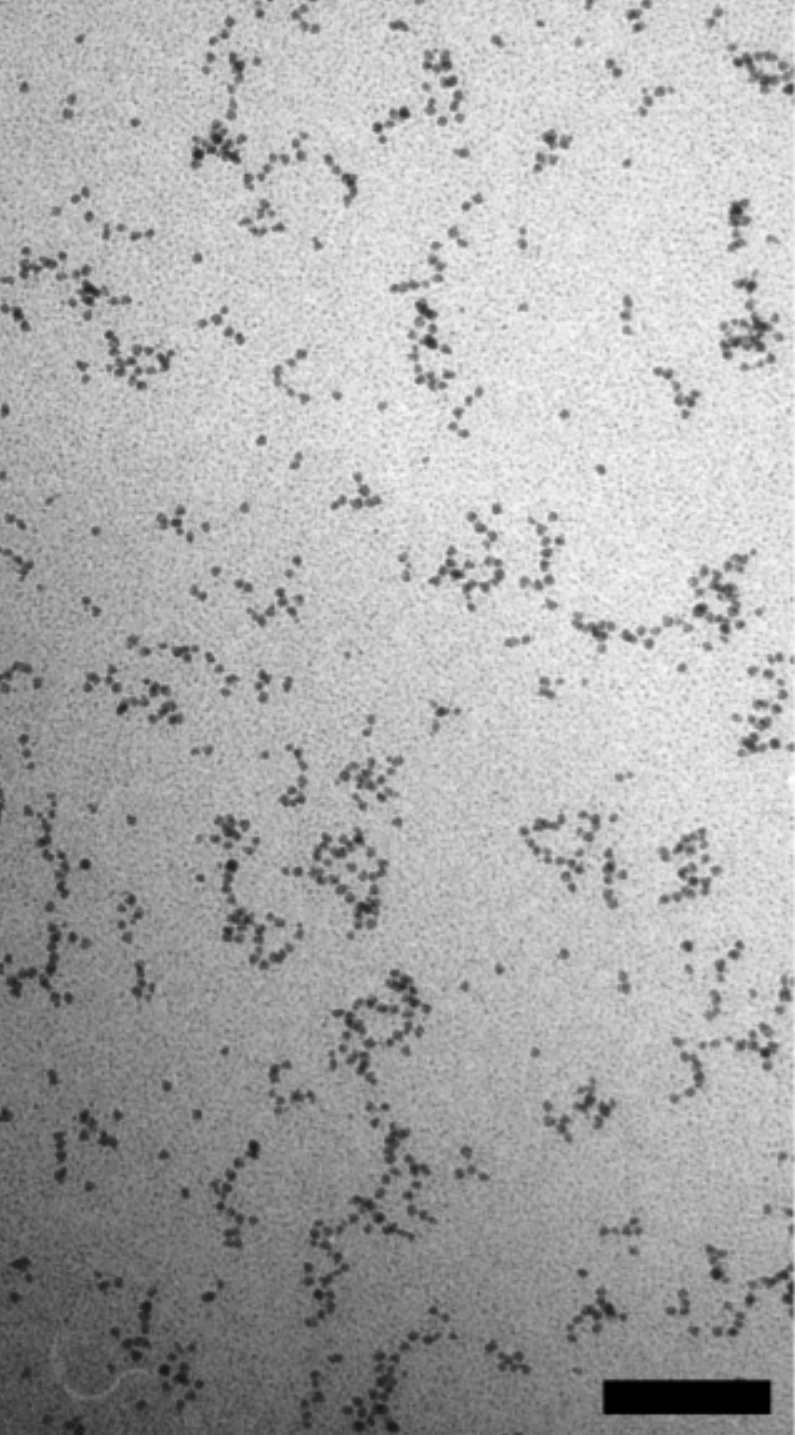
**2003**

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**Iron particles in decalin**

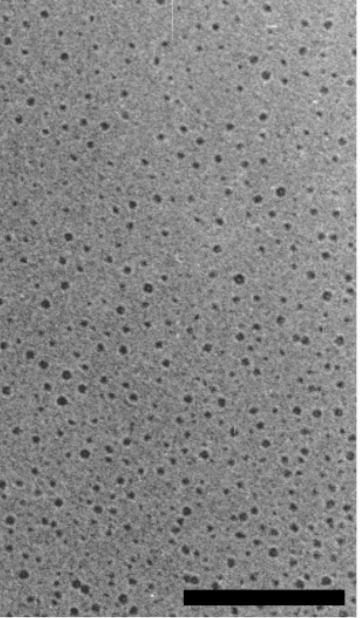




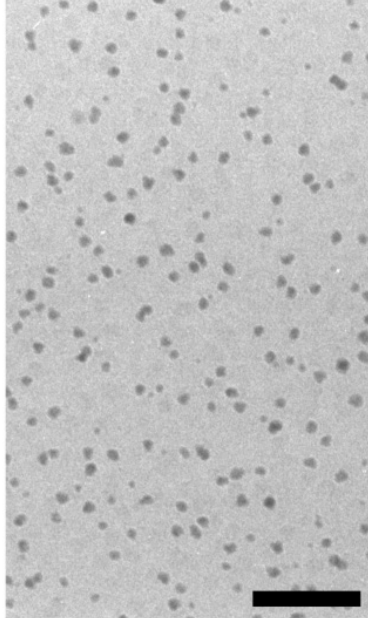


# Fe particle size & interaction

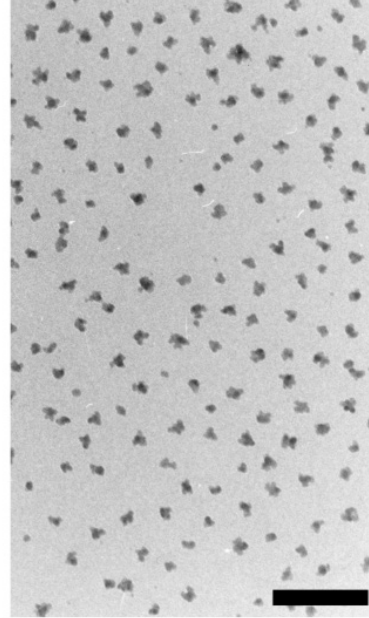
K. Butter UU



**A**



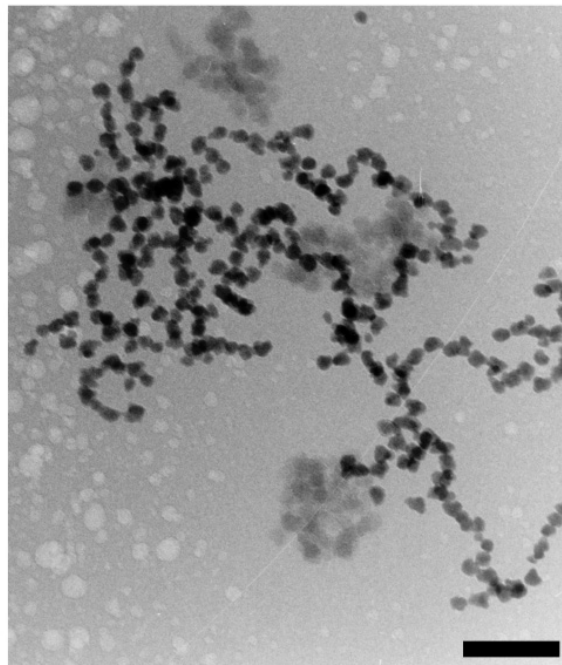
**B**



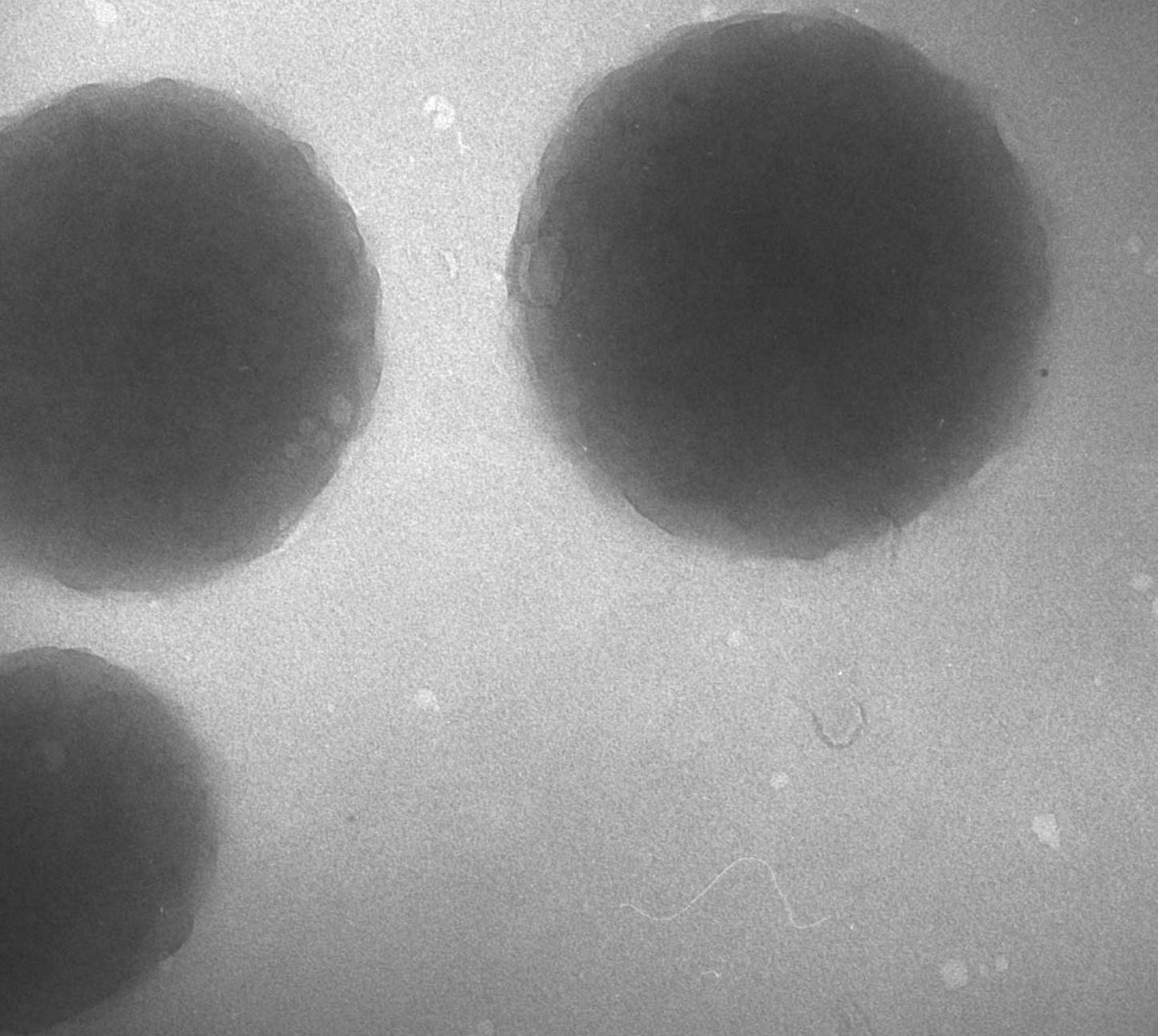
**C**



**D**



**E**



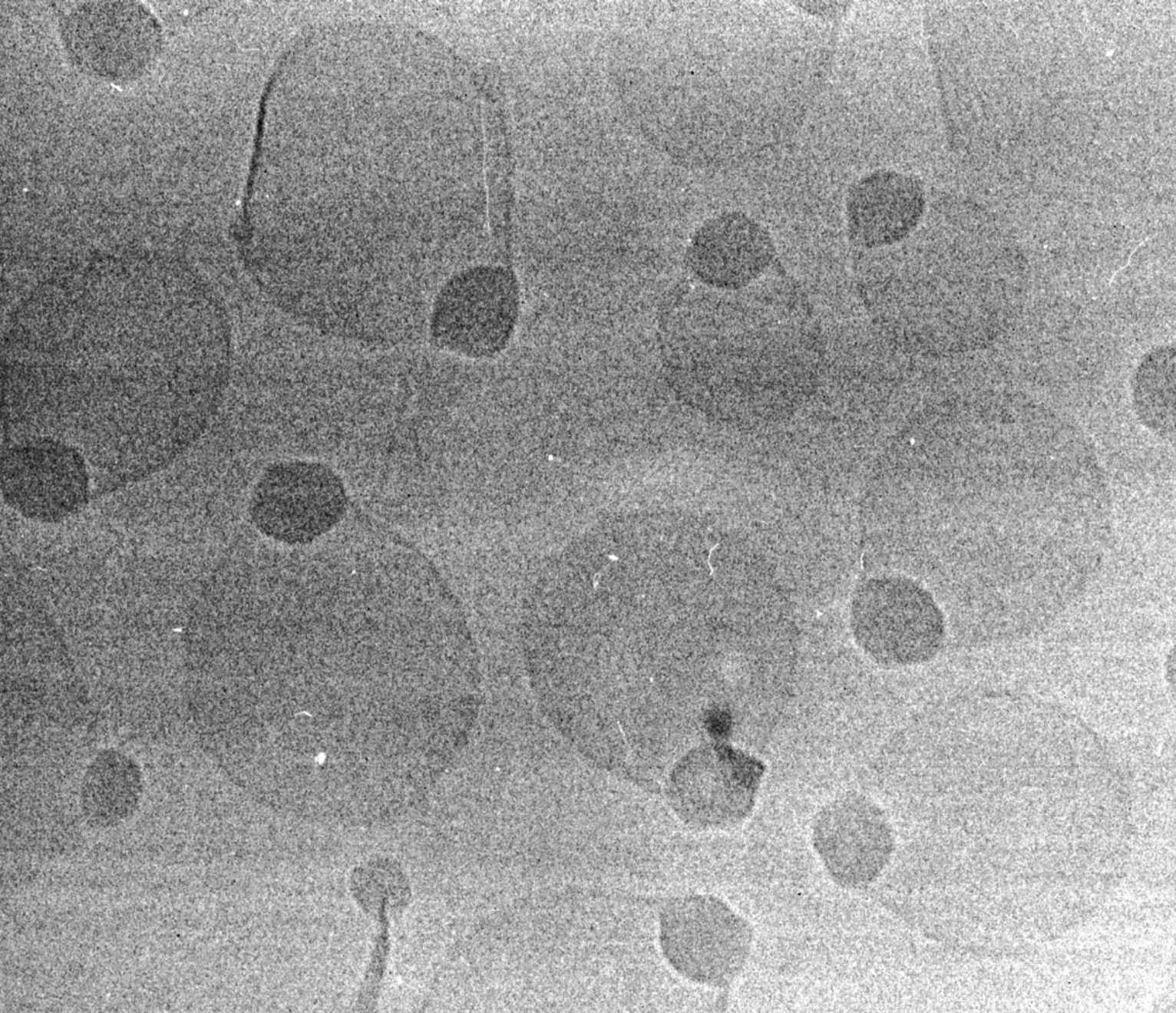
**Catalyst in acetone/UU**

**-Aqueous thin specimens behave like soap films (air/water interfaces)**

**-Thin films can be formed from organic solutes**

**- Heat and mass exchange are fast processes: dew point (time scale 0.1 sec)**





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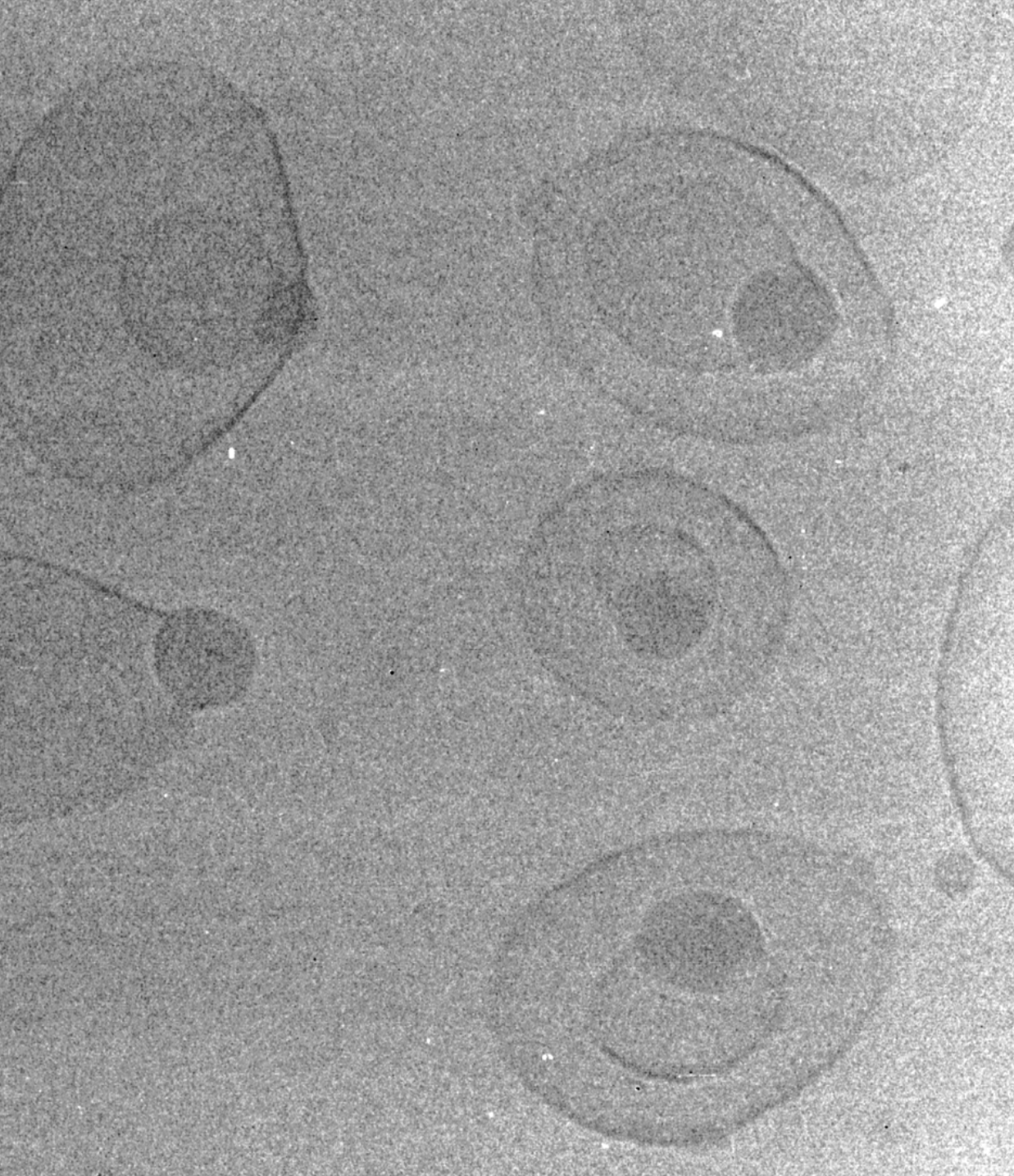
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Parachutes

TUE/UM

styrene-DMPA





**Matrioshka**

**TUE/UM**

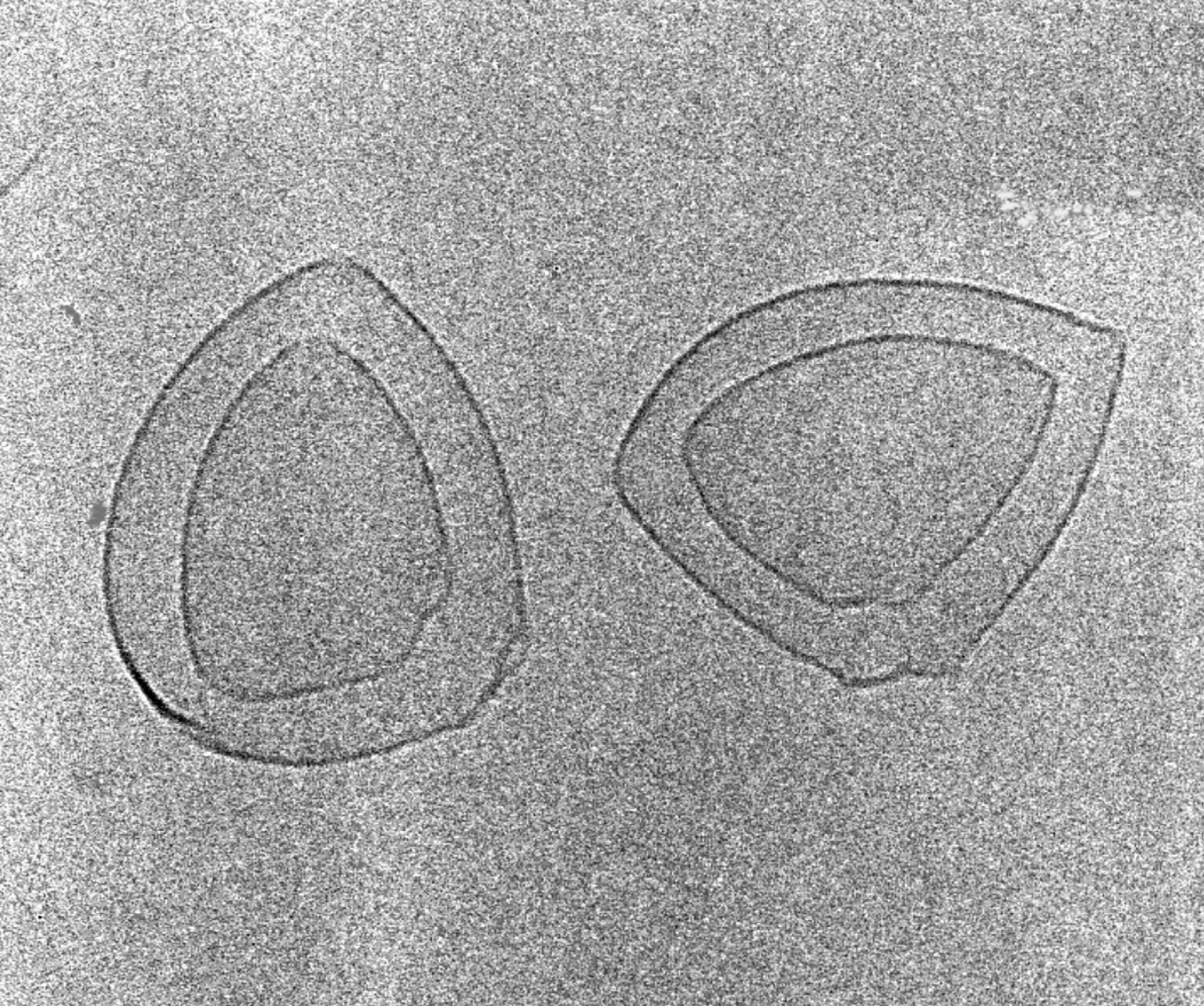
**Styrene  
MAPTMAC**

**DMPA**

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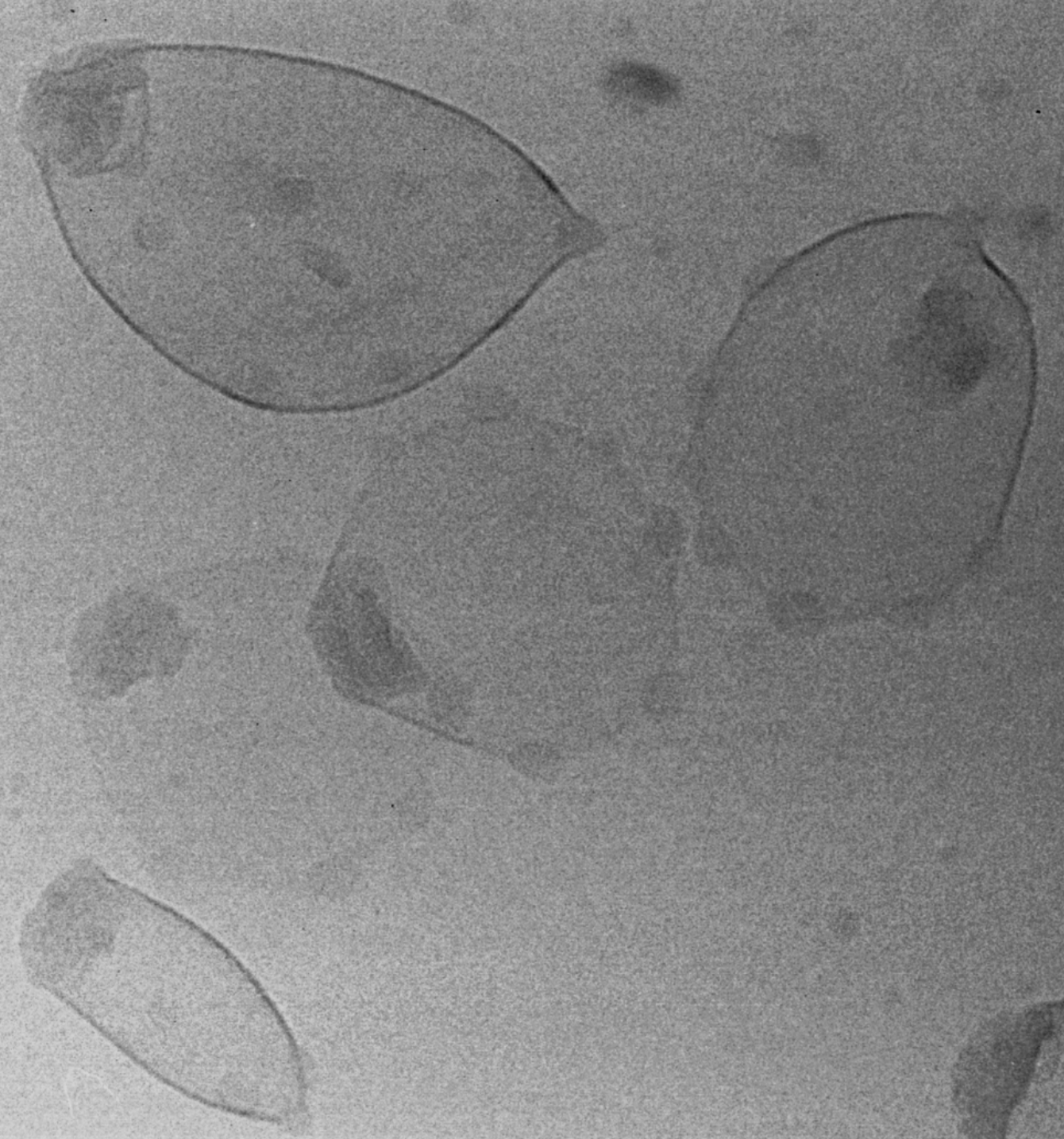




M. Jung  
D. Hubert  
TU/e

**V-50 induced fission in DODAB**





**Double  
necklace**

**TUE/UM**

pDVB  
pBA

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**Soft Matter**

**NWO-proposal**

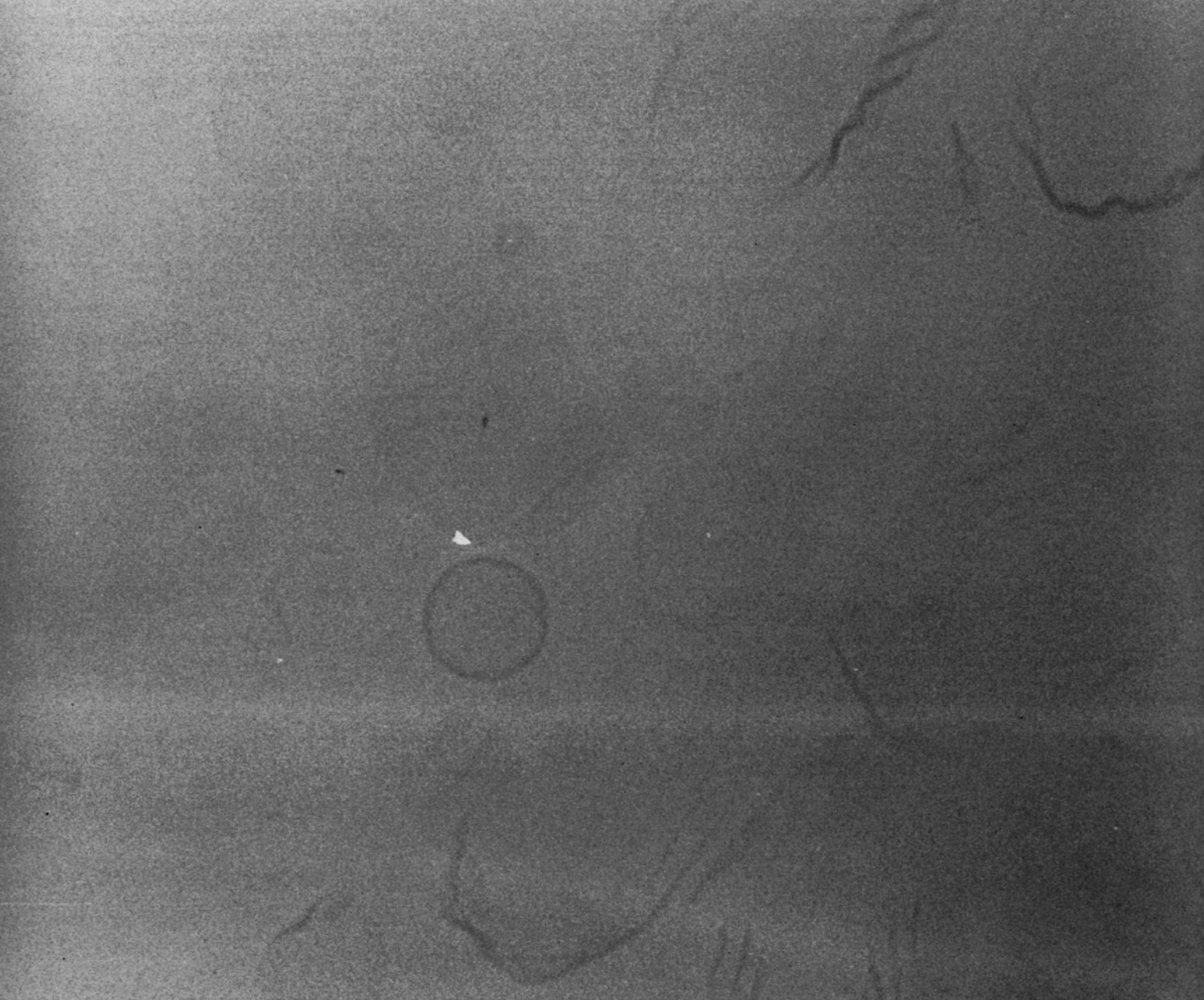
**Cryo-TEM**

**Advanced analysis of nanostructures by  
cryo-transmission electron microscopy**

**TU/e**

**UM**





**DMPC+DSPE-PEG 2000 50'**

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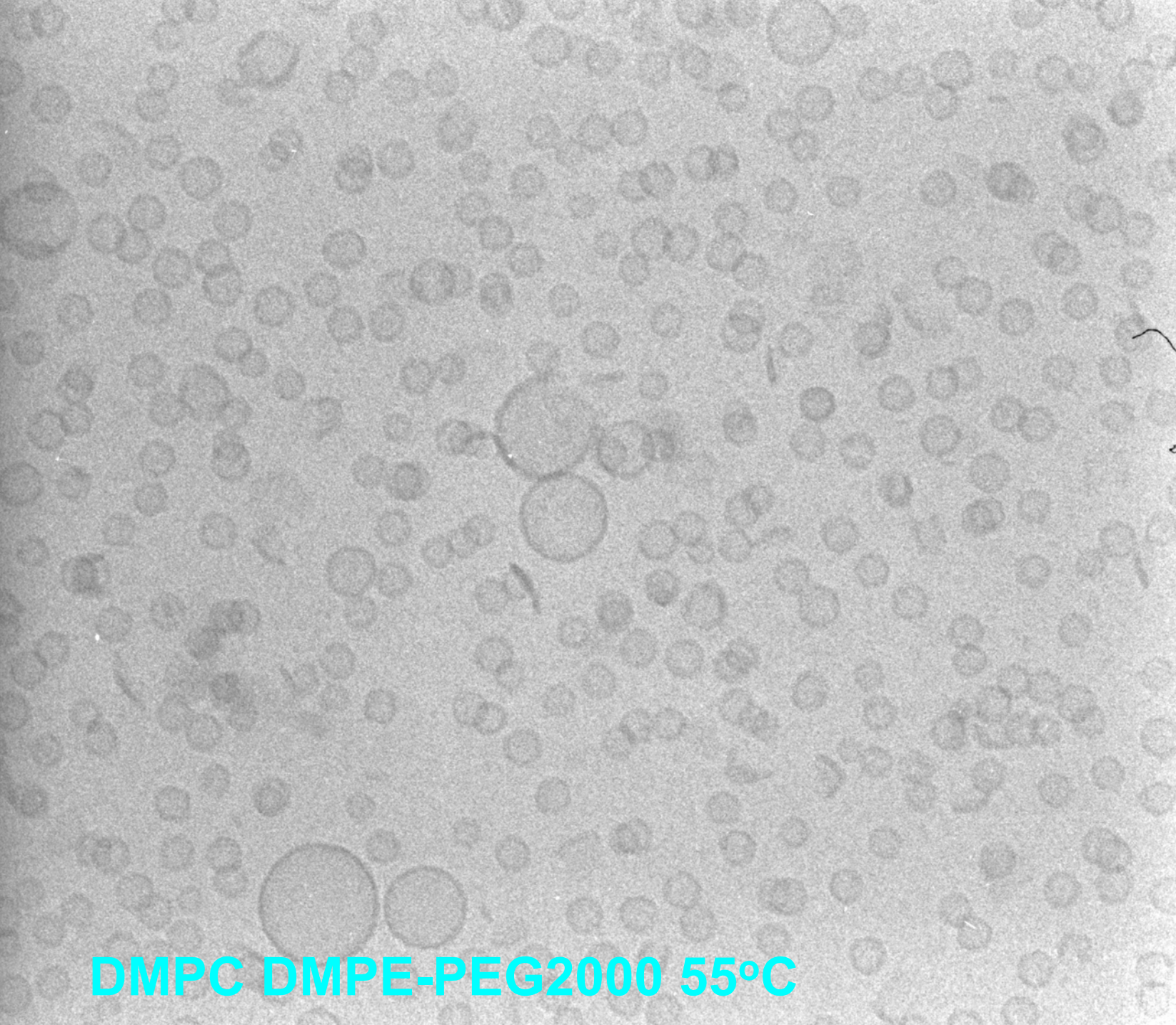


DMPC DMPE-PEG2000 29°C

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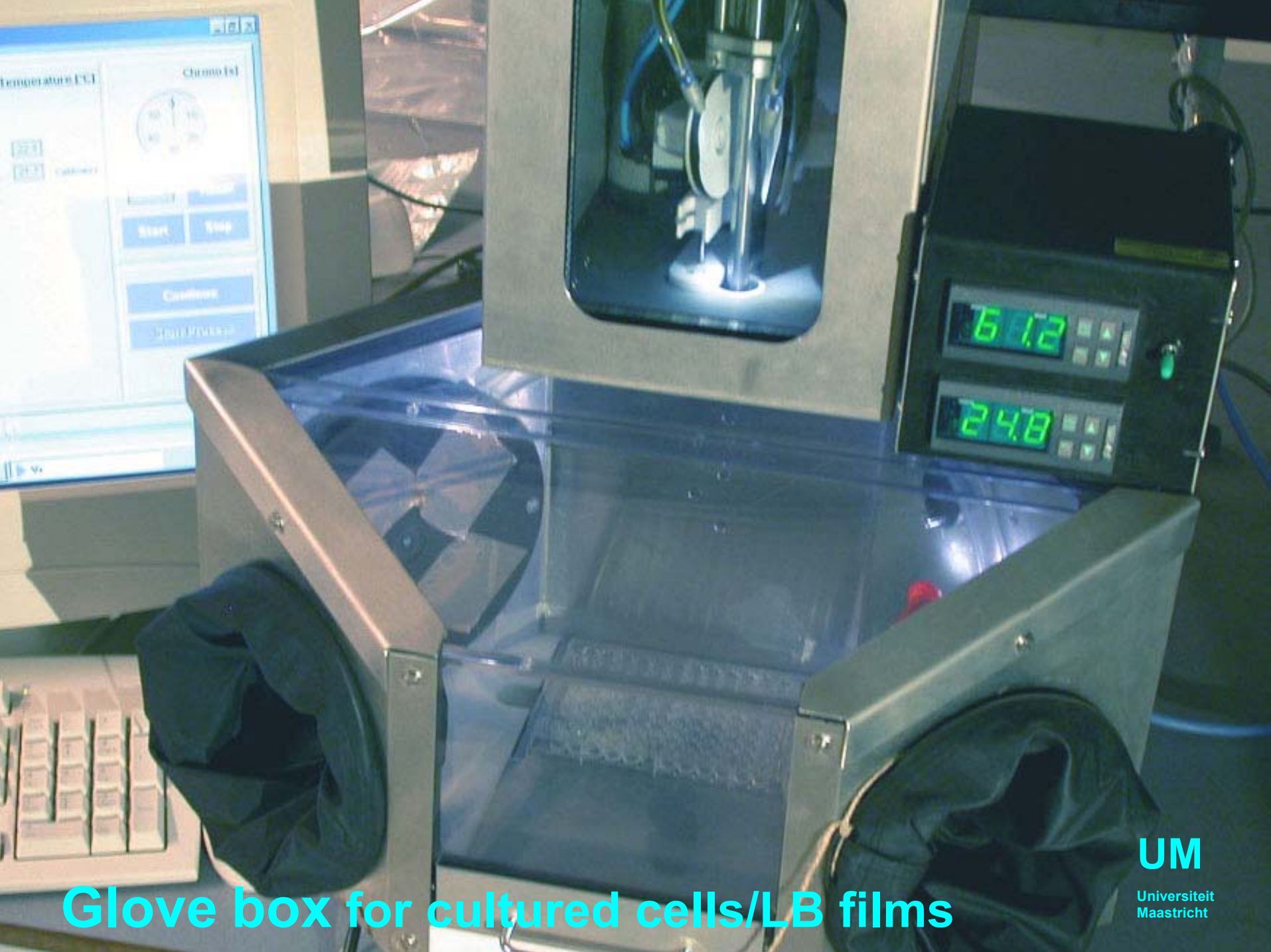


**DMPC DMPE-PEG2000 55°C**

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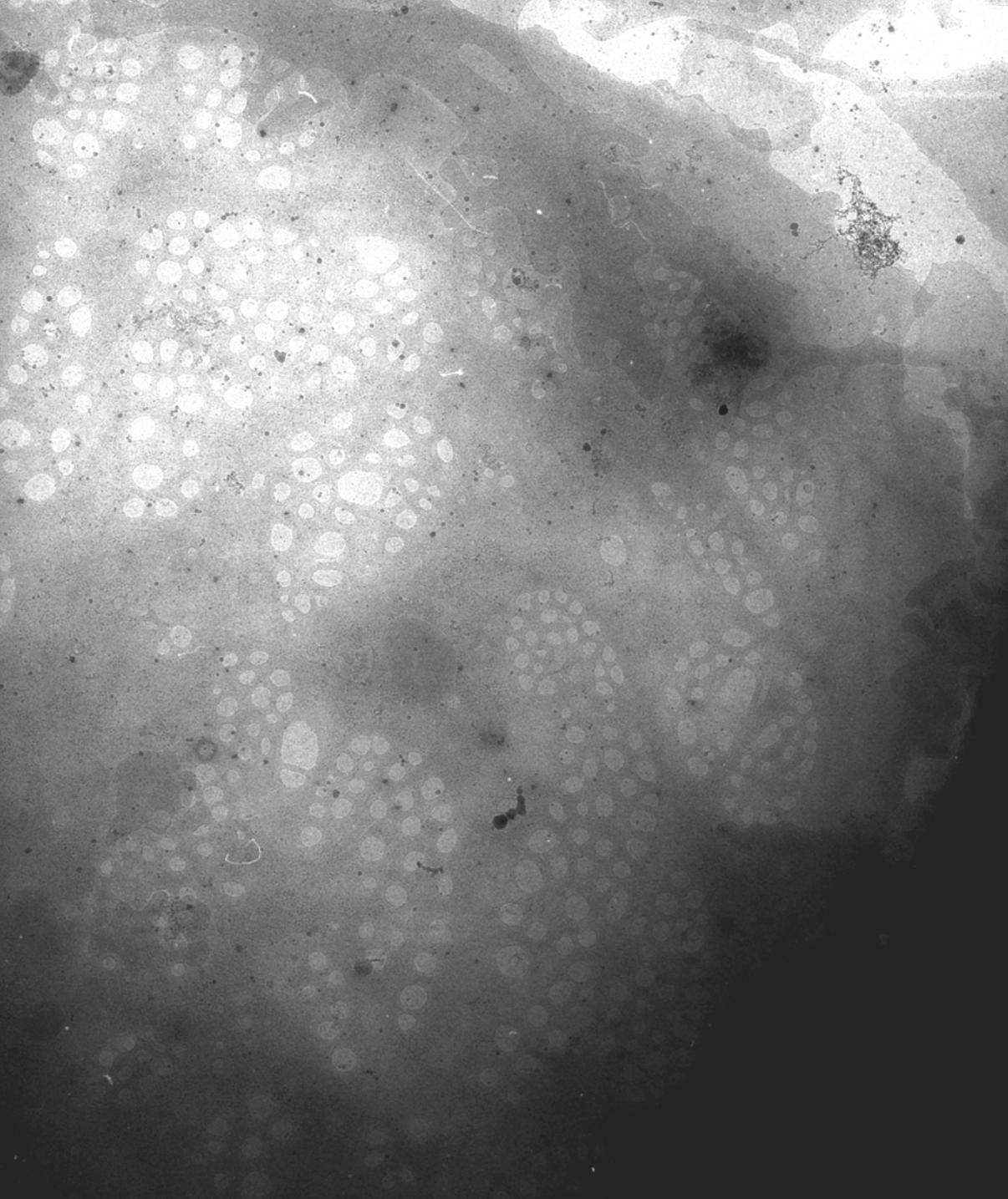


**Glove box for cultured cells/LB films**

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**LEC**

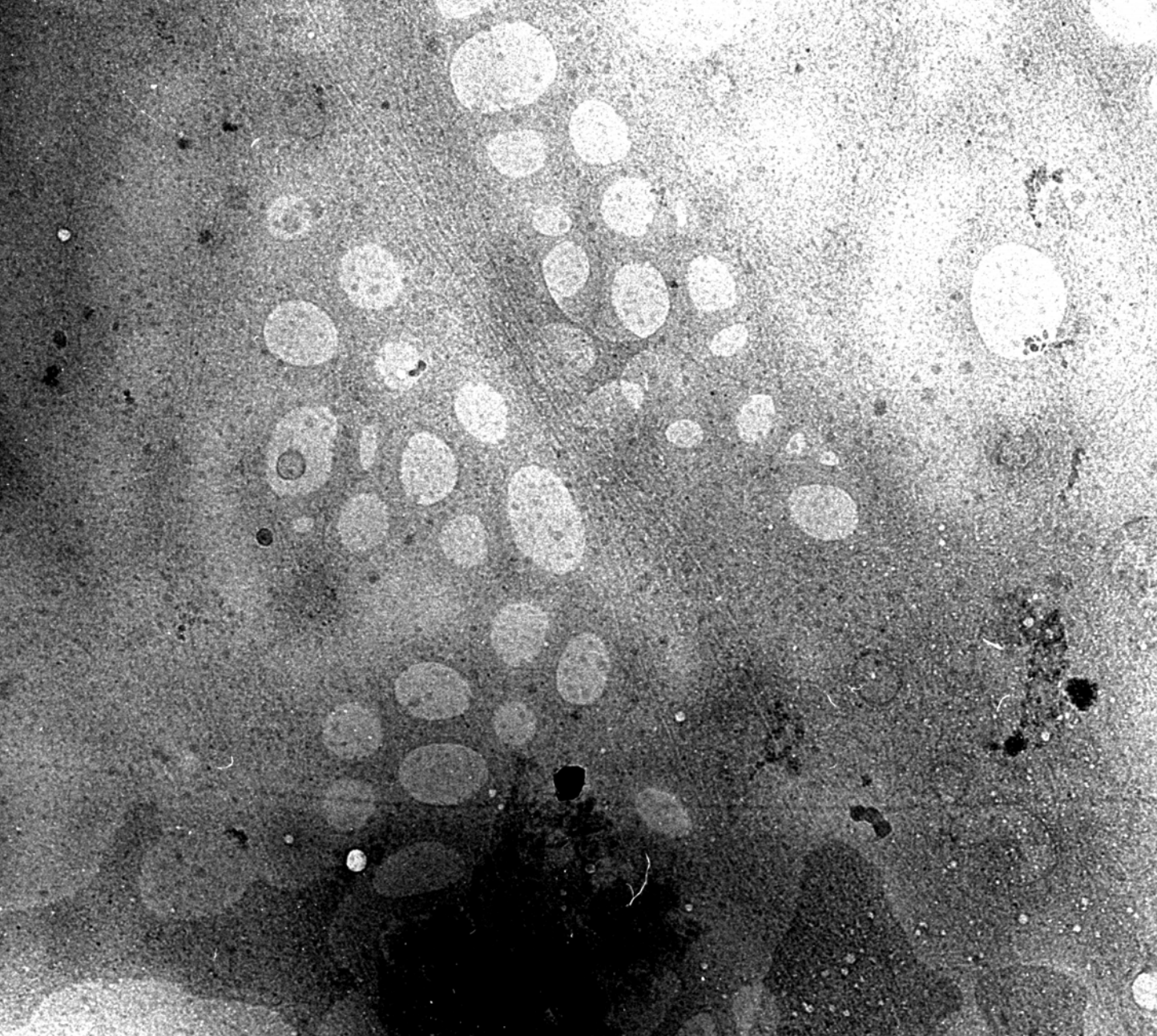
**liver endothelial  
cells**

**F. Braet/VUB  
Brussels**

**UM**

Universiteit  
Maastricht





LEC



**E coli 100% rH**

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**LB film/ DNA**

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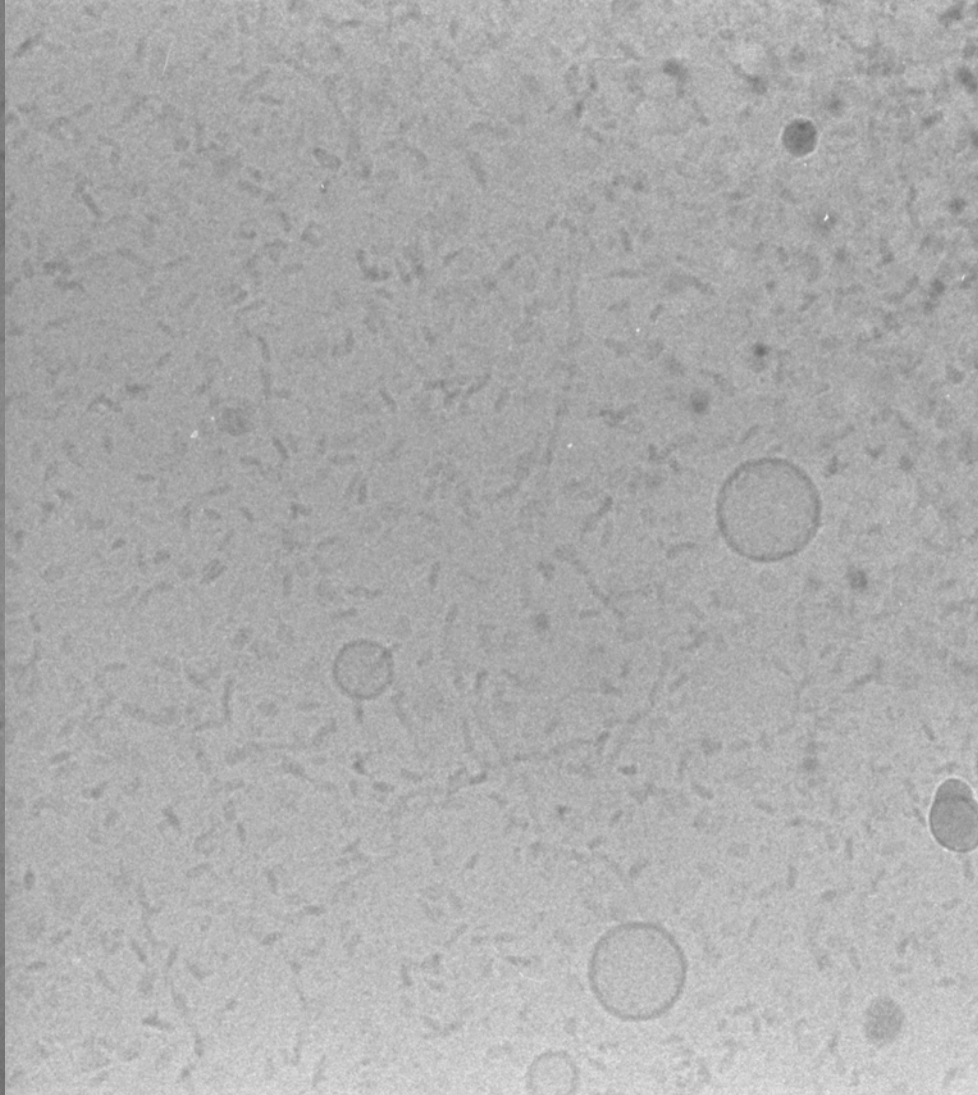
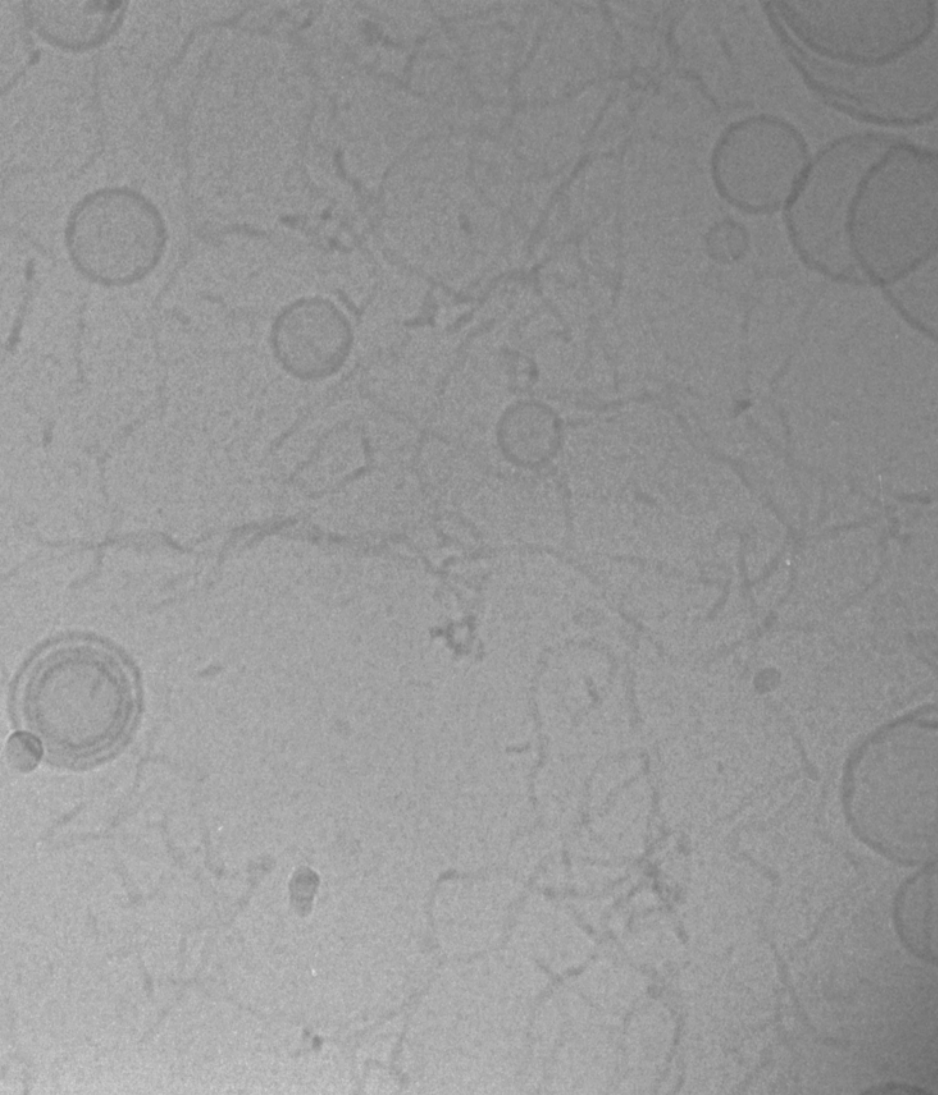
# Nano-structures studied by Cryo-EM

- micelle/bilayer interaction
- membrane fusion & fission
- bilayer & protein/DNA/drug interactions
- macromolecular structure (better than 0.4 nm?)
- nano-technology
- (macro) molecular complexes- cells

## prospects

- time resolution (UV flash 1 msec)
- 3D resolution (tomography 2- 4 nm, cryosections?)
- low magn/ high resolution





5'

DMPC+apo LPIII

30'

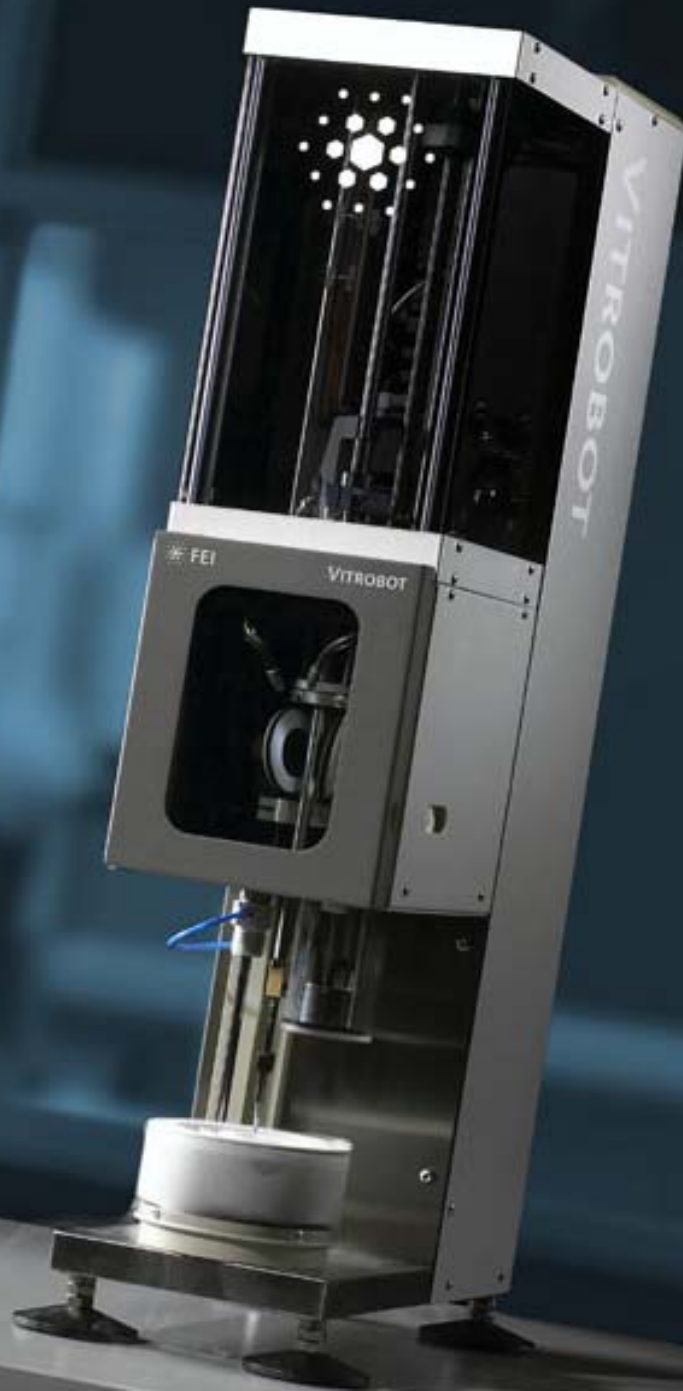
Time resolved Cryo-EM

KNJ Burger/UU

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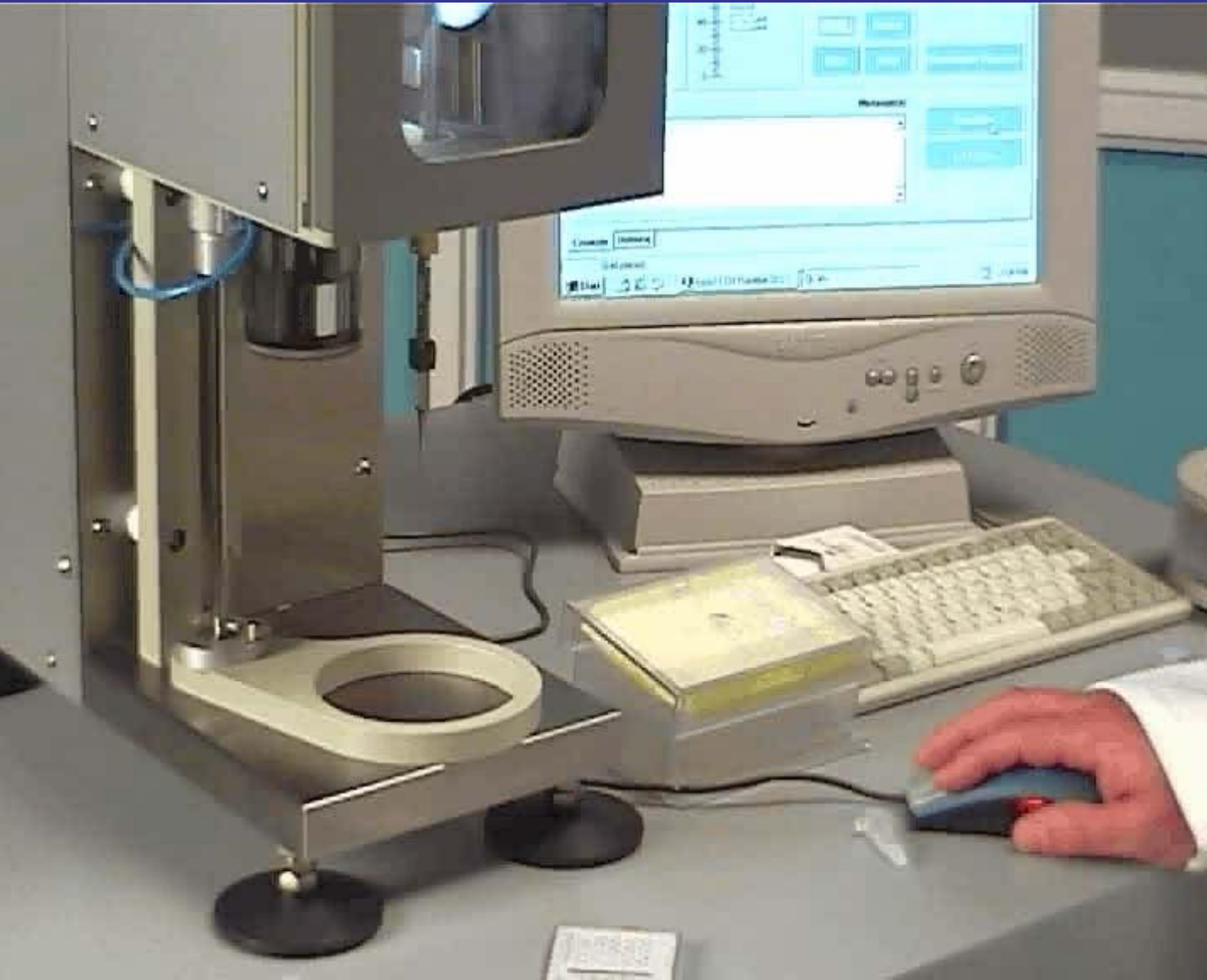


**21th century**

**Liquid slicer**

**Molecular &  
Cellular  
dynamics**

# Vitrobot™ in Motion Part I



Uptake of grid  
into climate  
chamber



# Vitrobot™ in Motion Part II



Uplift of liquid  
coolant container

# Vitrobot™ in Motion Part III



processing



# Vitrobot™ in Motion Part IV



Collecting  
the vitrified  
specimen

# Vitrobot™

The Ultimate Vitrification Solution!



*The  
Vitrobot!*