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Microfluidic Sample Preparation: Opportunities, Challenges and 'Visual Proteomics'

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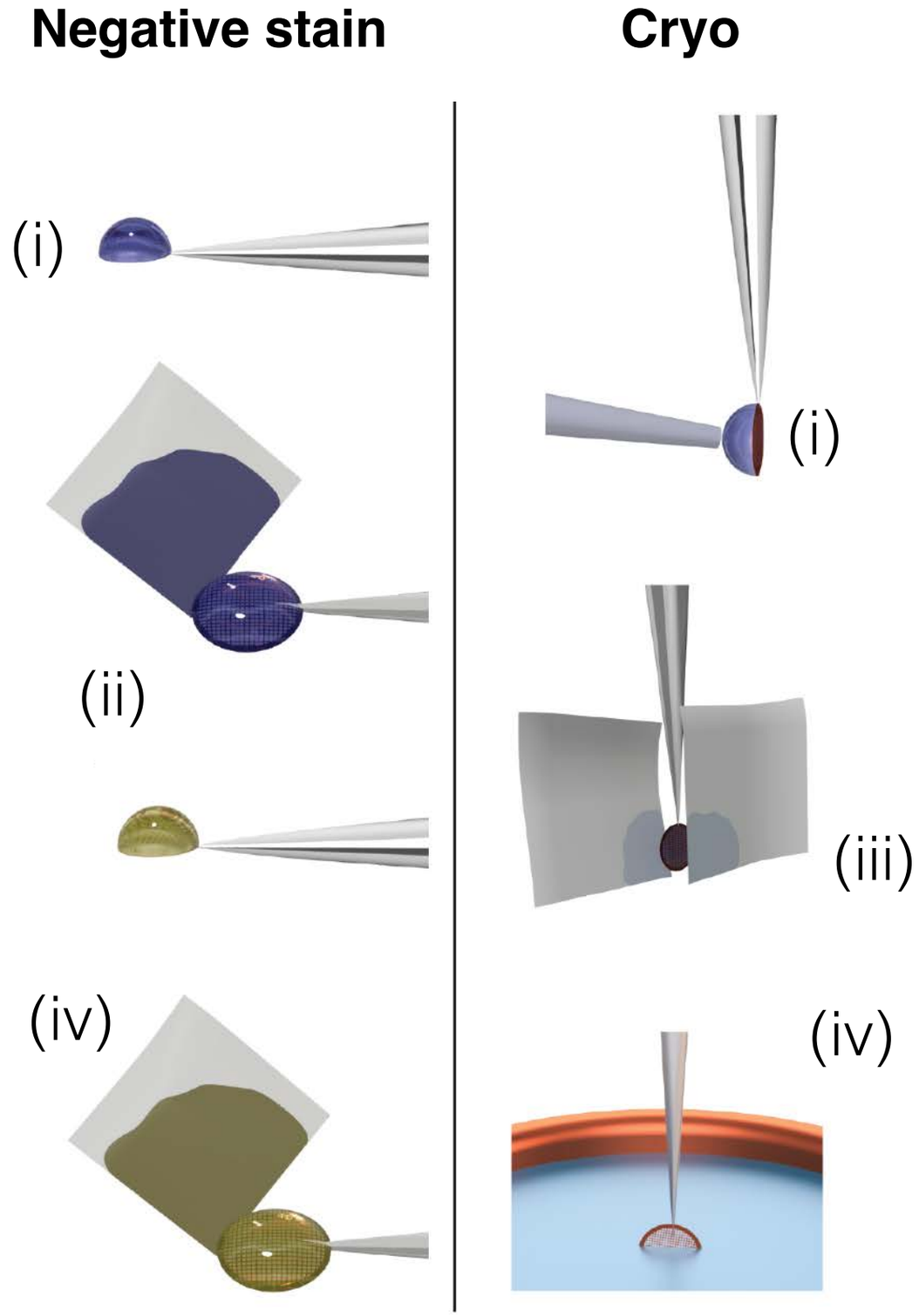
Molecular Life Sciences

TEM grid sample preparation

Main steps

- Sample dispensing (i)
- [Sample conditioning (ii)]
- Sample thinning (iii)
- Post processing, e.g., plunging, drying (iv)



S. Kemmerling *et al.*, “Connecting µ-fluidics to electron microscopy,” J. Struct. Biol., vol. 177, 1,128–134, 2012.

C. Schmidli et al., “Microfluidic sample preparation for transmission electron microscopy”, *in revision*.

Why miniaturisation?

- Minimal sample volumes (nL).
- Minimal sample loss.
- Avoiding harsh conditions (e.g., paper blotting).
- Better control of EM-grid preparation process.
- Minimal time/sample consumption for sample conditioning.
- High through-put applications, e.g., Spotiton.
- New options for biological experiments, e.g., single cell visual proteomics.

Microfluidics

- Behaviour/physics and control of small, geometrically restrained volumes (μL … fL) of a liquid

- Typical characteristics

  - Low Reynold numbers: $Re \equiv \frac{\rho V L}{\eta}$

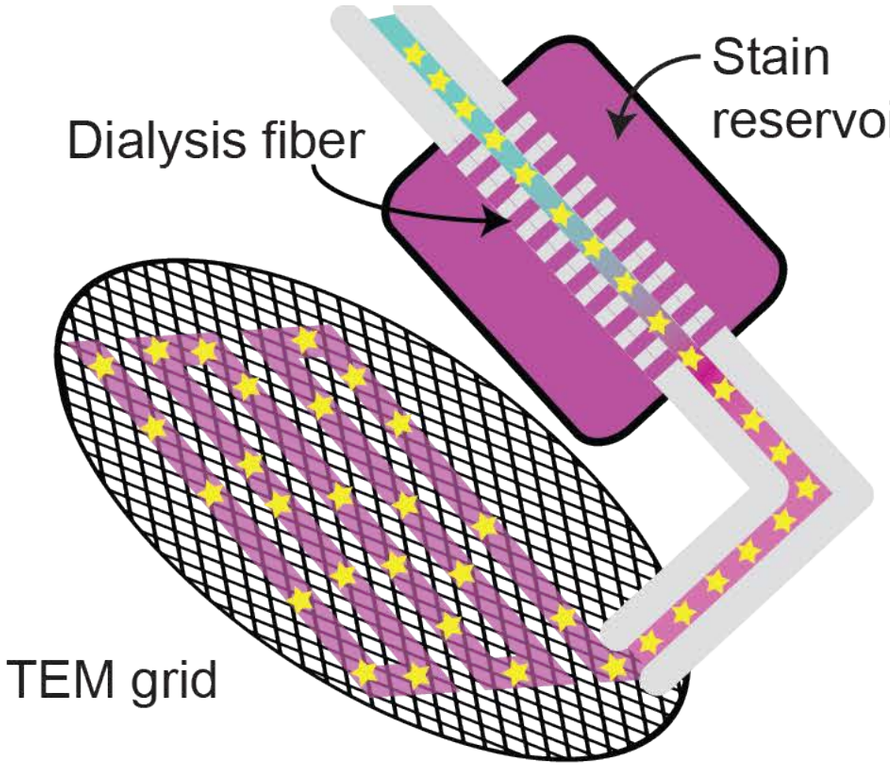
  - Low Péclet numbers: $Pe \equiv \frac{V L}{D}$

  - Capillary number: $Ca \equiv \frac{\eta V}{\gamma}$

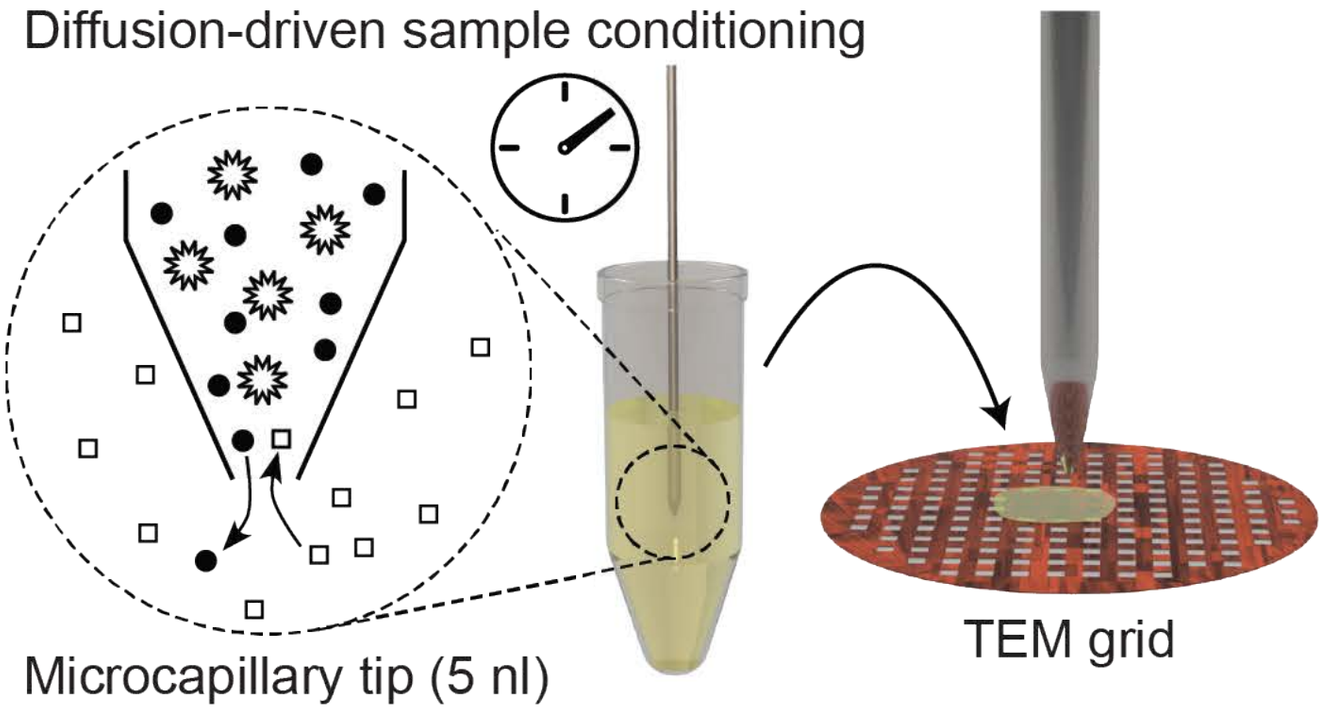
ρ : Mass density γ : Surface stress V: Liquid velocity

η : Viscosity L: Typical length scale D : Diffusion constant

Sample conditioning



Kemmerling *et al.*, 2012

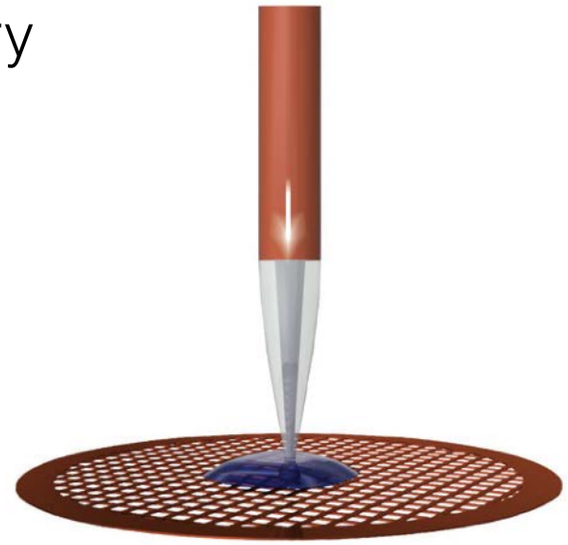


Arnold *et al.*, 2016

Sample dispensing

Micro-capillary writing

Kemmerling *et al.*, 2012
Lee, J. *et al.*, 2012
Arnold, S. A., *et al.*, 2016
Arnold, S. A., *et al.*, 2017

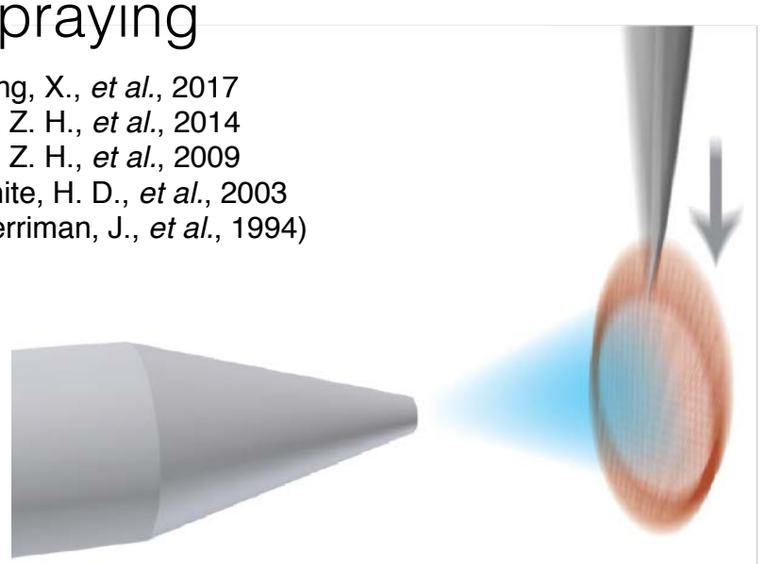


Ink-jet spotting

Jain, T, *et al.*, 2012
Razinkov, I., *et al.*, 2016

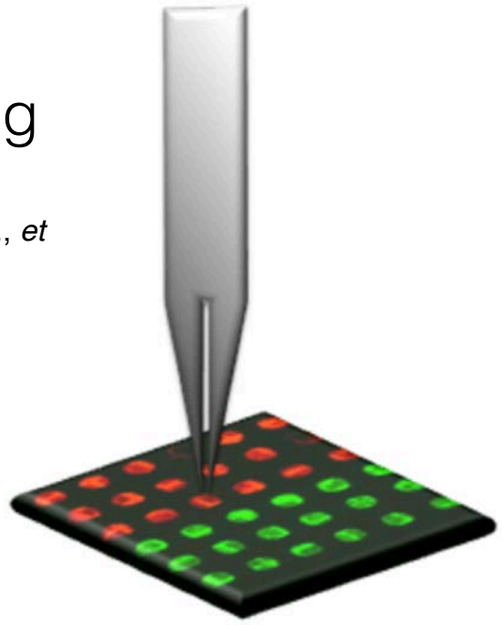
Spraying

Feng, X., *et al.*, 2017
Lu, Z. H., *et al.*, 2014
Lu, Z. H., *et al.*, 2009
White, H. D., *et al.*, 2003
(Berriman, J., *et al.*, 1994)



Contact pin-printing

Castro-Hartmann, P., *et al.*, 2013



Arnold *et al.*, under review

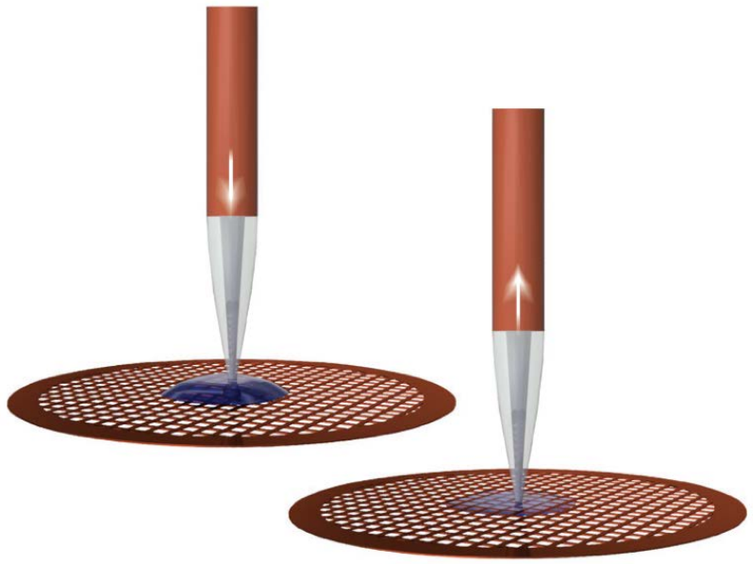
Thin film formation

- Due to surface stress, sample must be thinned.
- Thin films (h_c<100 nm) are inherently unstable/island formation.
  - Polar/aqueous liquids: Destabilised by “polar hydrophobic attraction”.
  - Water evaporation stabilises thin films but may have adverse effects on samples.
  - Dirt helps, especially surface active substances and salts.

A. S. Padmakar, K. Kargupta, and A. Sharma, “Instability and dewetting of evaporating thin water films on partially and completely wettable substrates,” The Journal of Chemical Physics, vol. 110, no. 3, pp. 1735–1744, 1999.
M. Cyrklaff, M. Adrian, and J. Dubochet, “Evaporation during preparation of unsupported thin vitrified aqueous layers for cryo-electron microscopy.,” J Electron Microsc Tech, vol. 16, no. 4, pp. 351–355, Dec. 1990.
R. M. Glaeser, B.-G. Han, R. Csencsits, A. Killilea, A. Pulk, and J. H. D. Cate, “Factors that Influence the Formation and Stability of Thin, Cryo-EM Specimens,” Biophysj, vol. 110, no. 4, pp. 749–755, Feb. 2016.

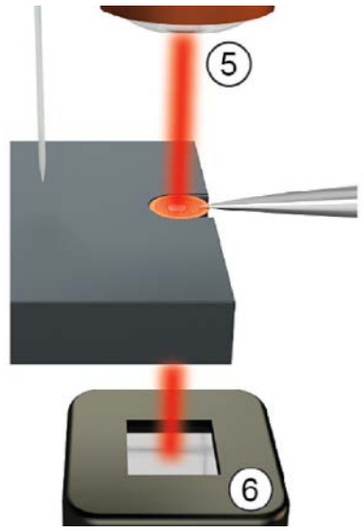
Sample thinning

Sample recovery by respiration



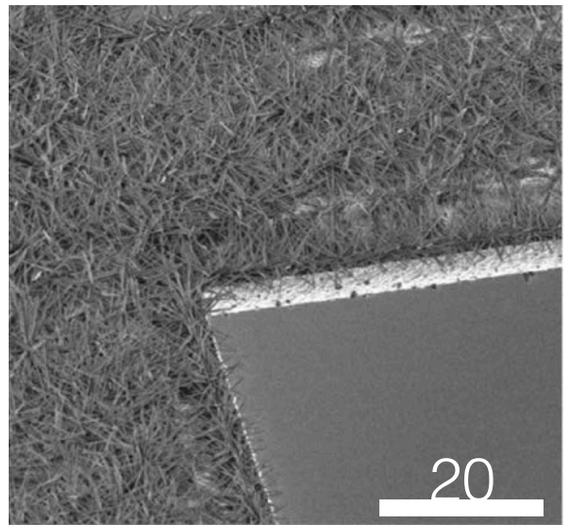
Arnold *et al.*, 2017

Controlled evaporation



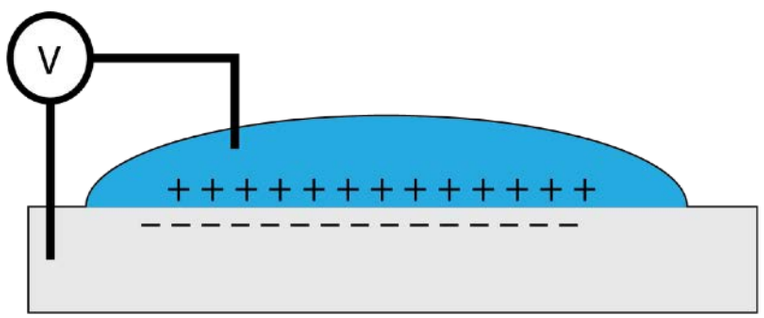
Arnold *et al.*, 2017

Self-blotting nanowire grids

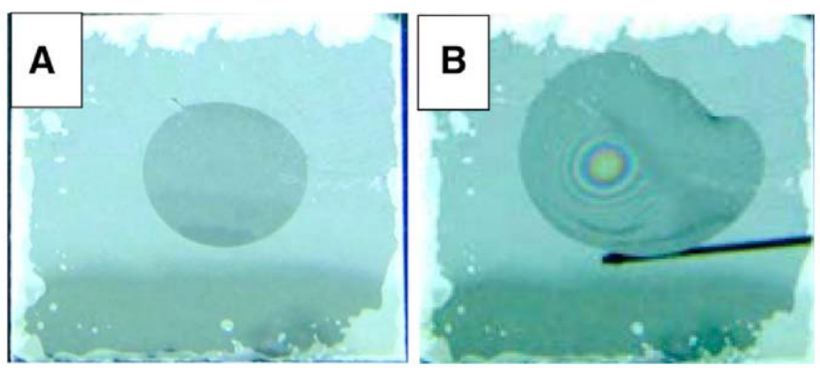


Razinkov *et al.*, 2016

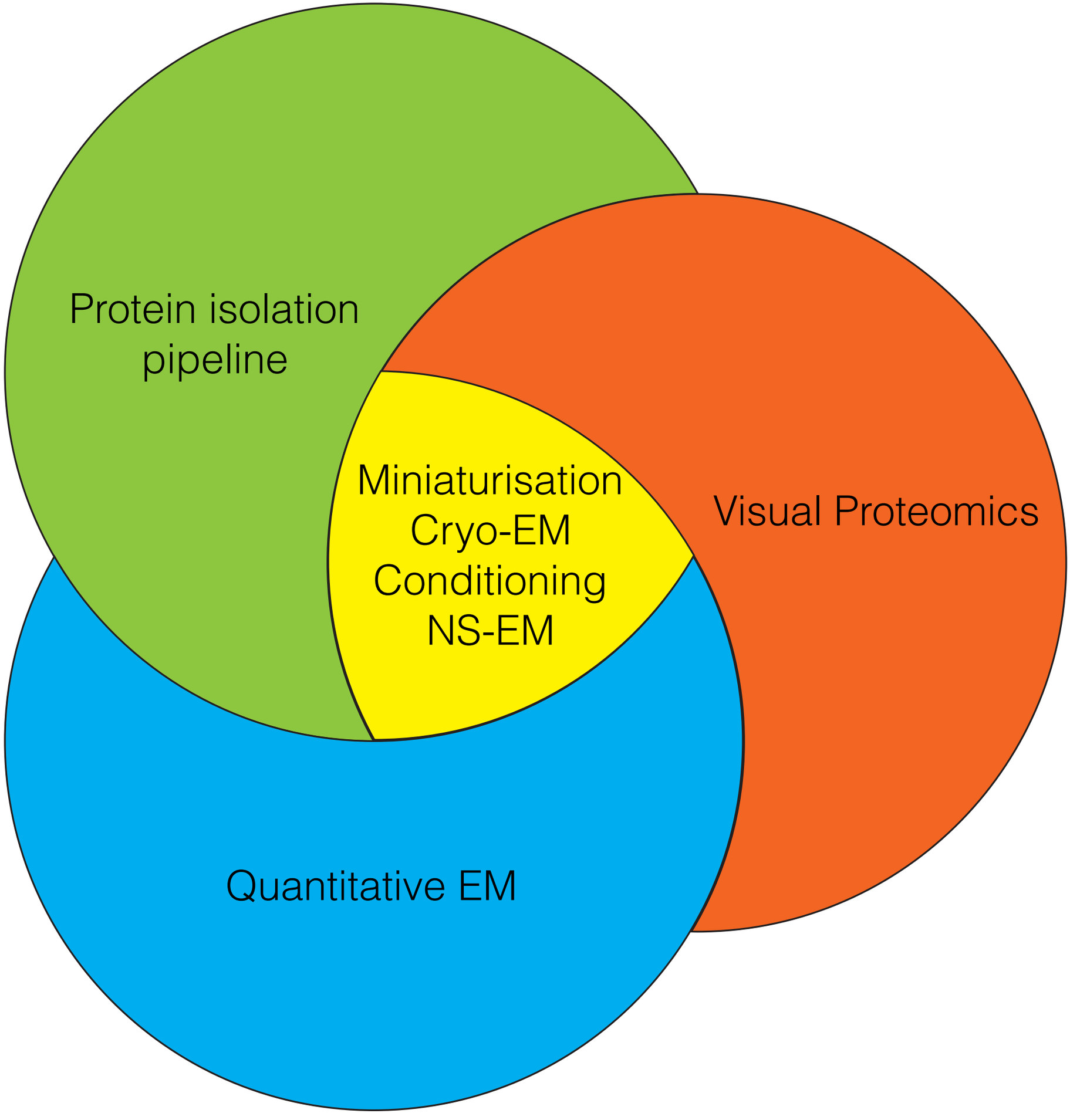
Electrowetting



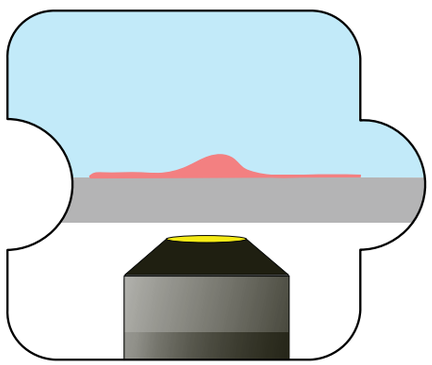
Marangoni flow



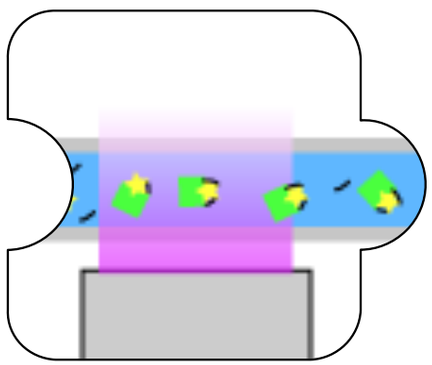
Glaeser *et al.*, 2016


Protein isolation
pipeline
Miniaturisation
Cryo-EM
Conditioning
NS-EM
Visual Proteomics
Quantitative EM

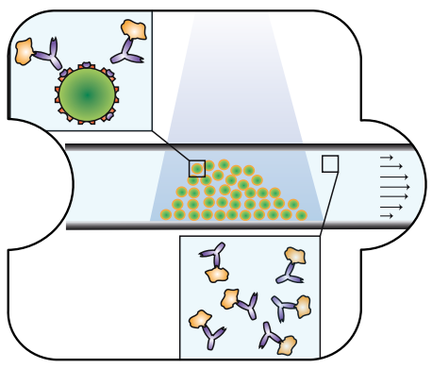
Modular Microfluidics



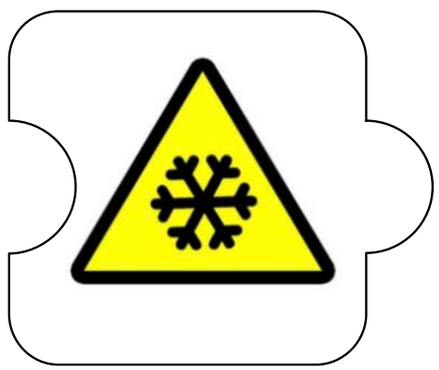
Live cell imaging
Culturing ✅



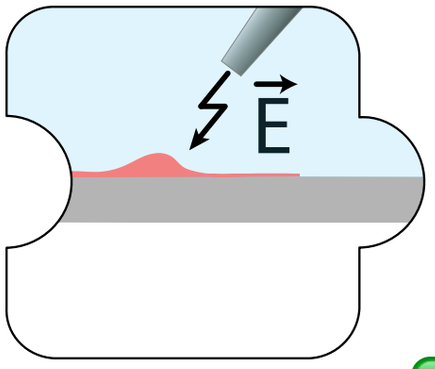
X-linking 🔶



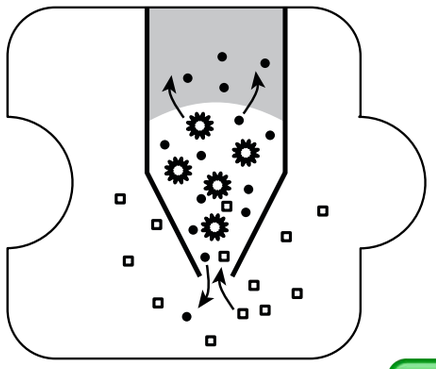
Complex fishing ✅



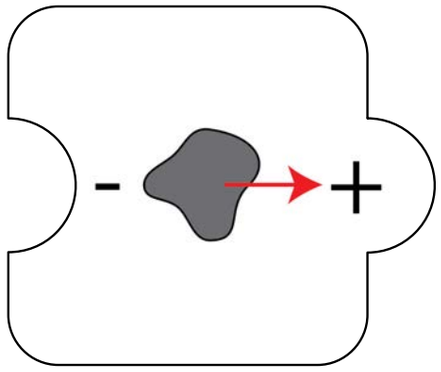
Cryo-plunging ✅



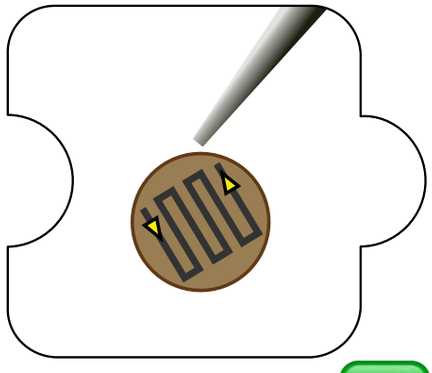
Single cell-lysis ✅



Conditioning ✅



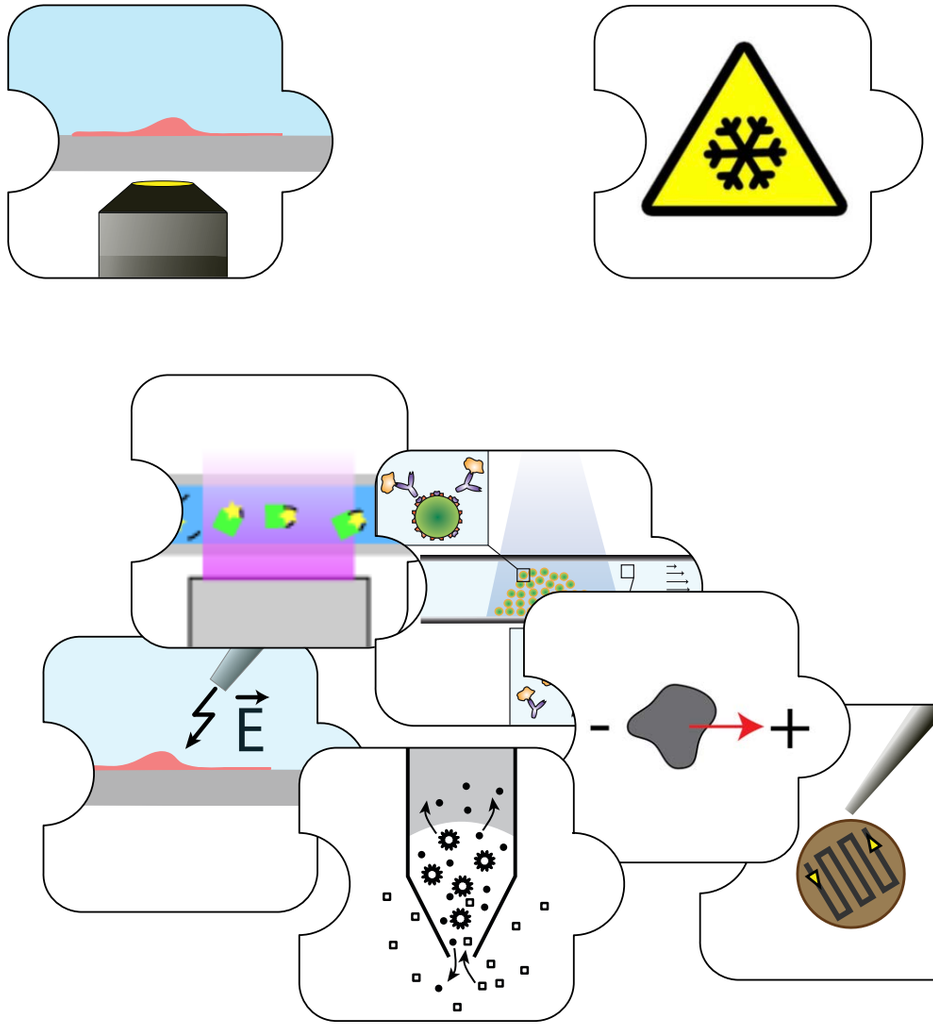
Electrophoresis 🔶

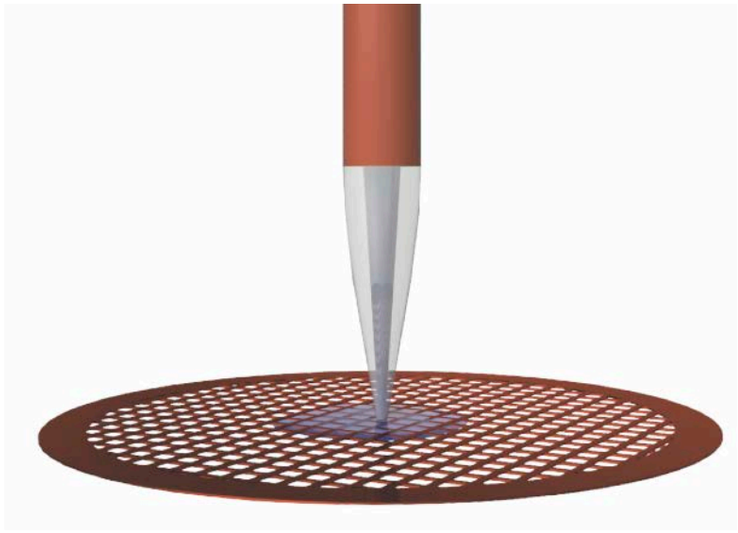


Handover ✅

✅ = Ready 🔶 = Development / preliminary testing

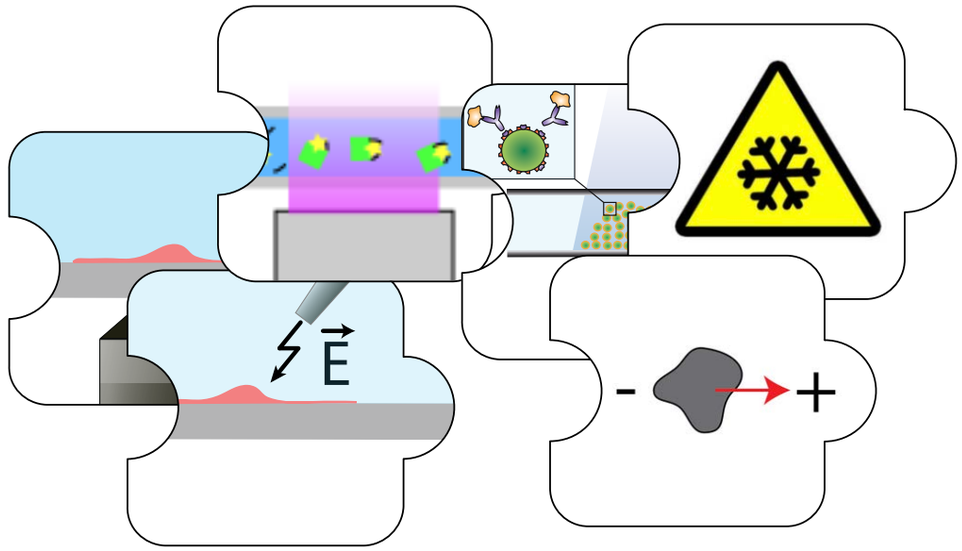
Modular Microfluidics

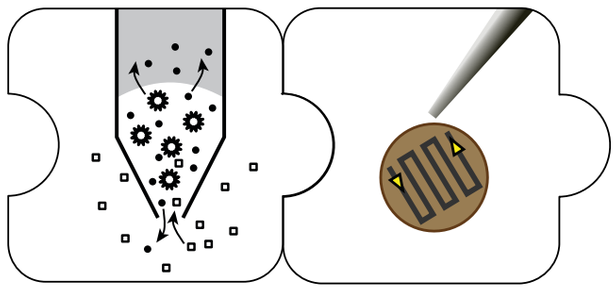




- Integration in processed micro-capillary tips
- Minimises sample-interface contacts
- Minimises loss by unspecific adsorption
- Minimises Tayler dispersion

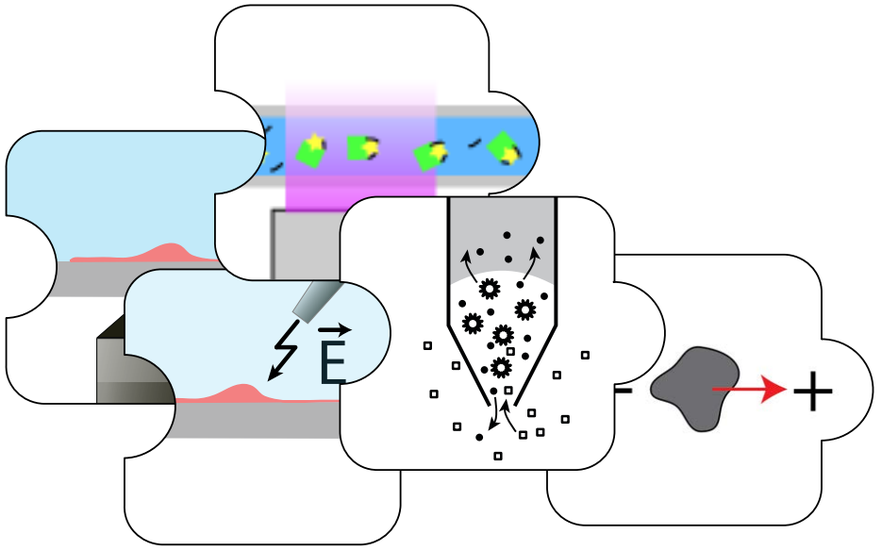
Modular Microfluidics

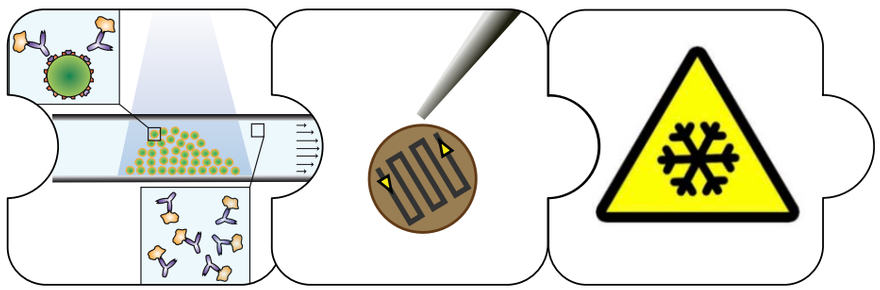




Negative stain TEM

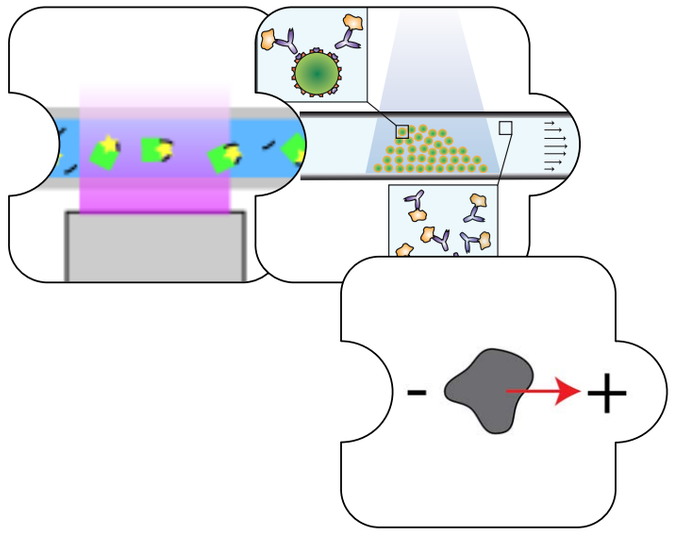
Modular Microfluidics

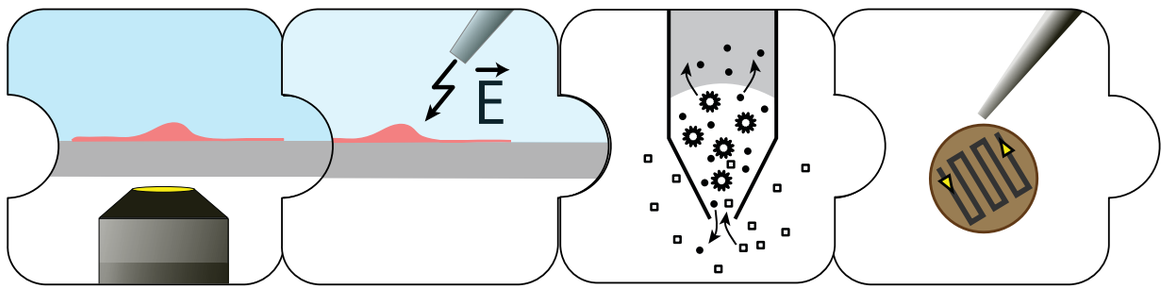




Protein fishing and cryo-EM

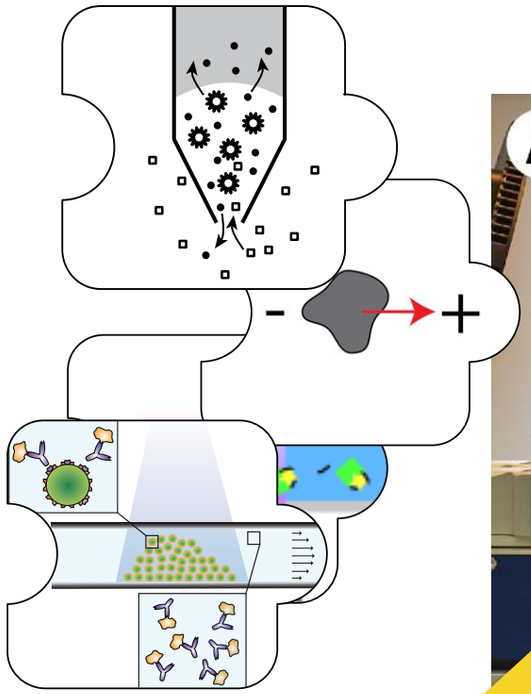
Modular Microfluidics



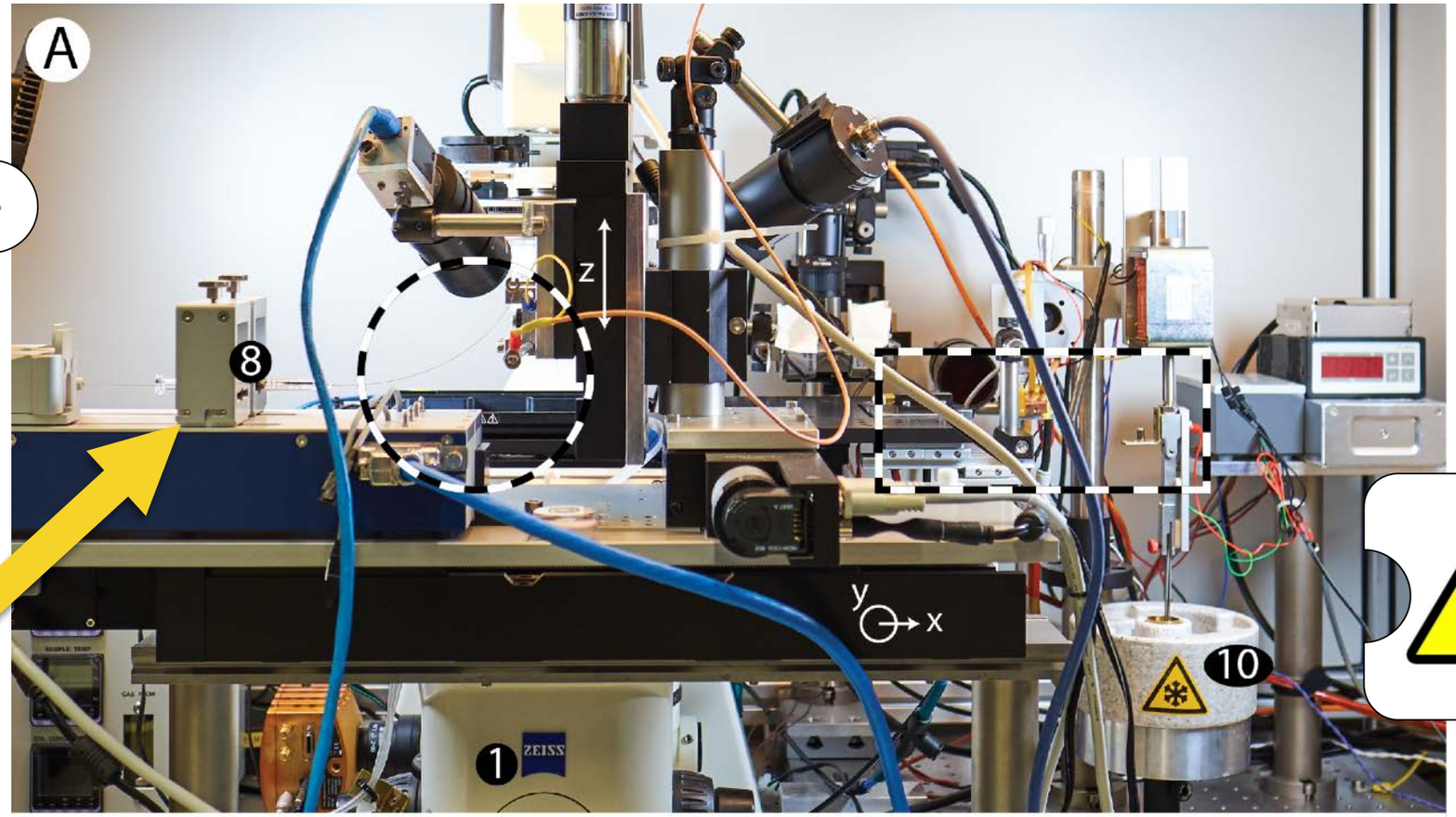


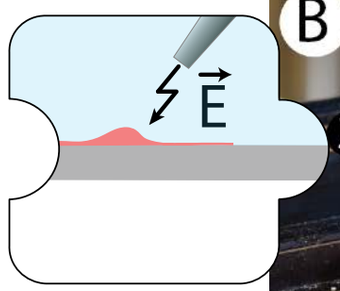
Live cell imaging - single cell lysis - negative stain EM
“Visual proteomics”

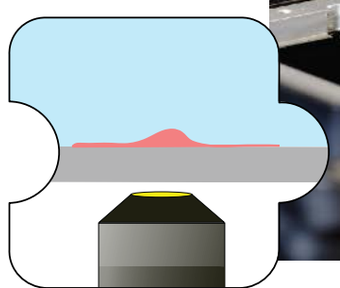
CryoWriter Set-up

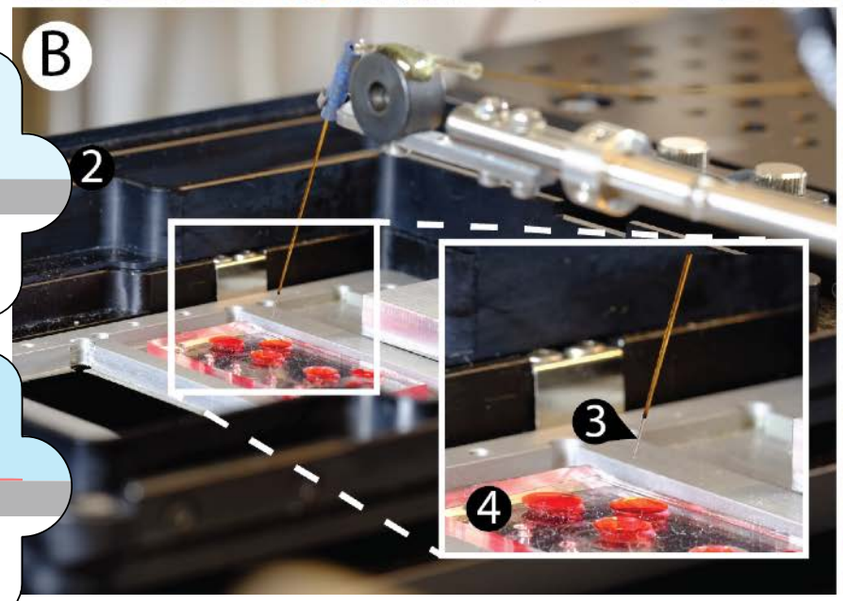


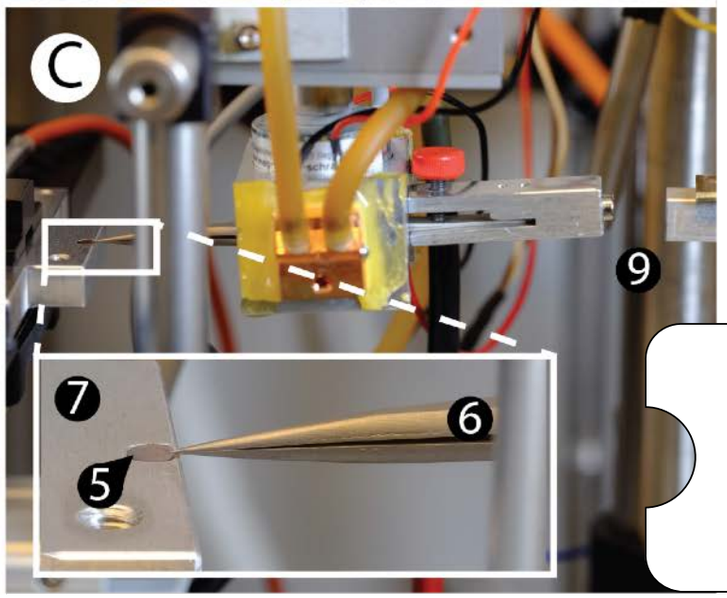
High precision pump system

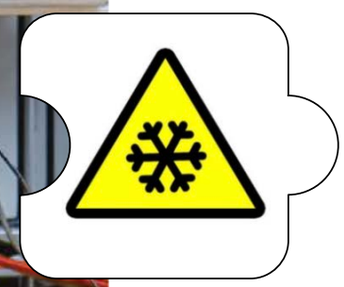


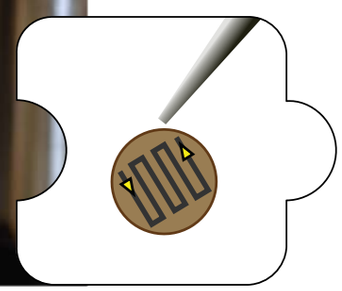


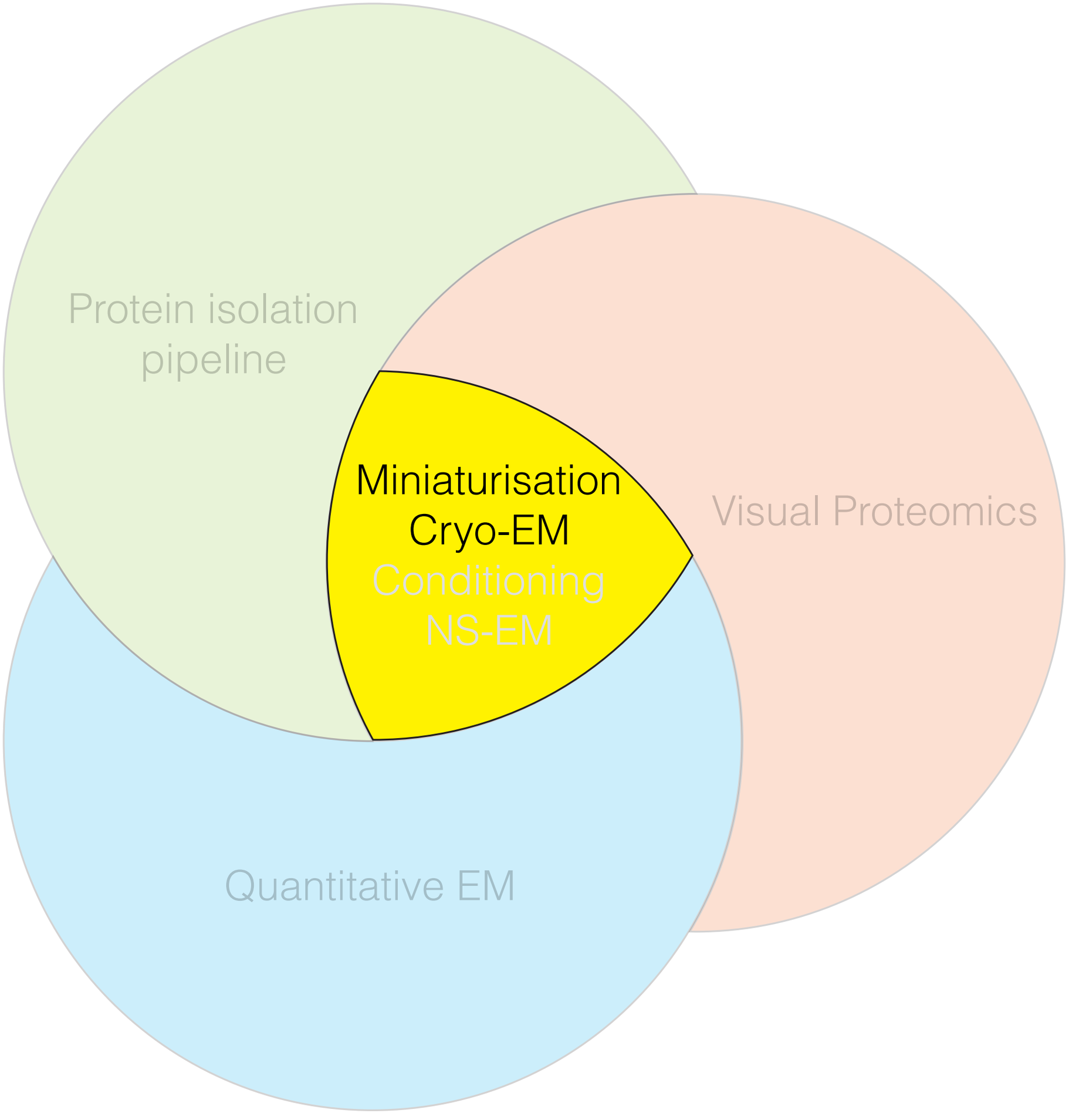











Protein isolation
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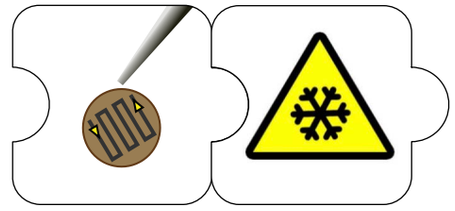
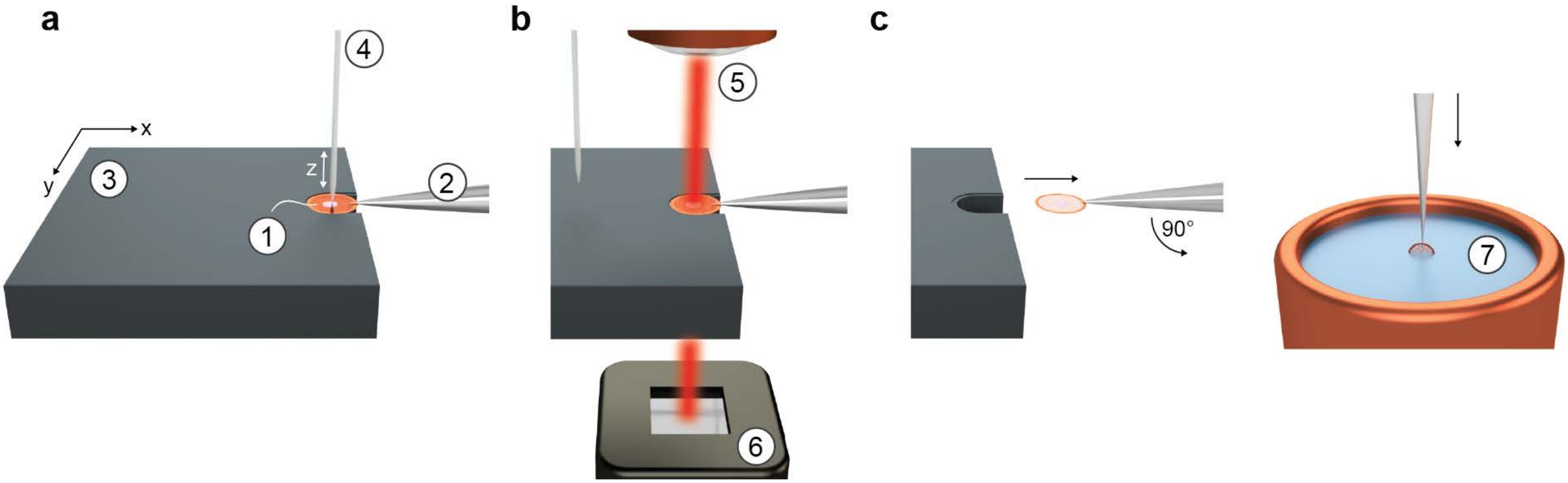
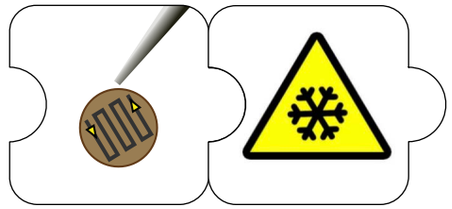


Handover: Cryo-EM

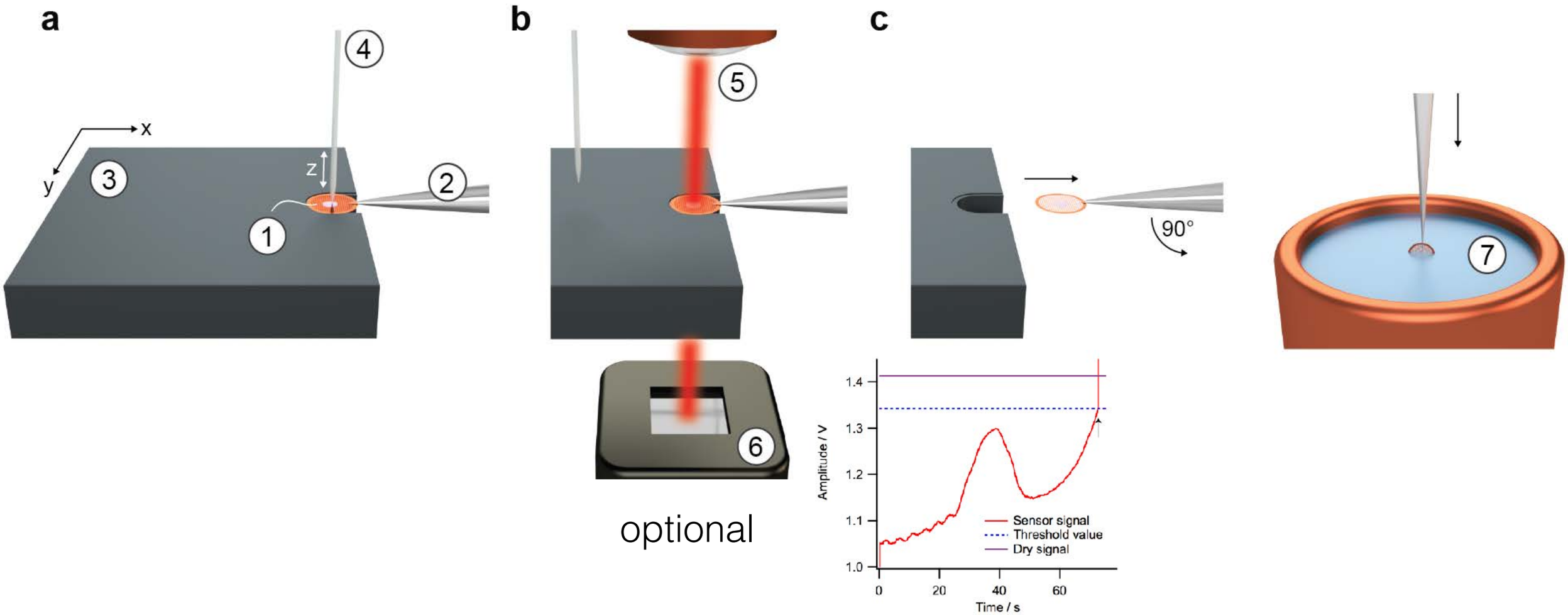


optional

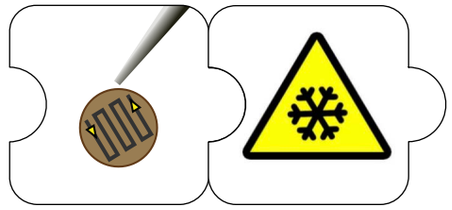
S. A. Arnold, S. Albiez, A. Bieri, A. Syntychaki, R. Adaixo, R. A. McLeod, K. N. Goldie, H. Stahlberg, and T. Braun, “Blotting-free and lossless cryo-electron microscopy grid preparation from nanoliter-sized protein samples and single-cell extracts.,” Journal of Structural Biology, vol. 197, no. 3, pp. 220–226, 2017.



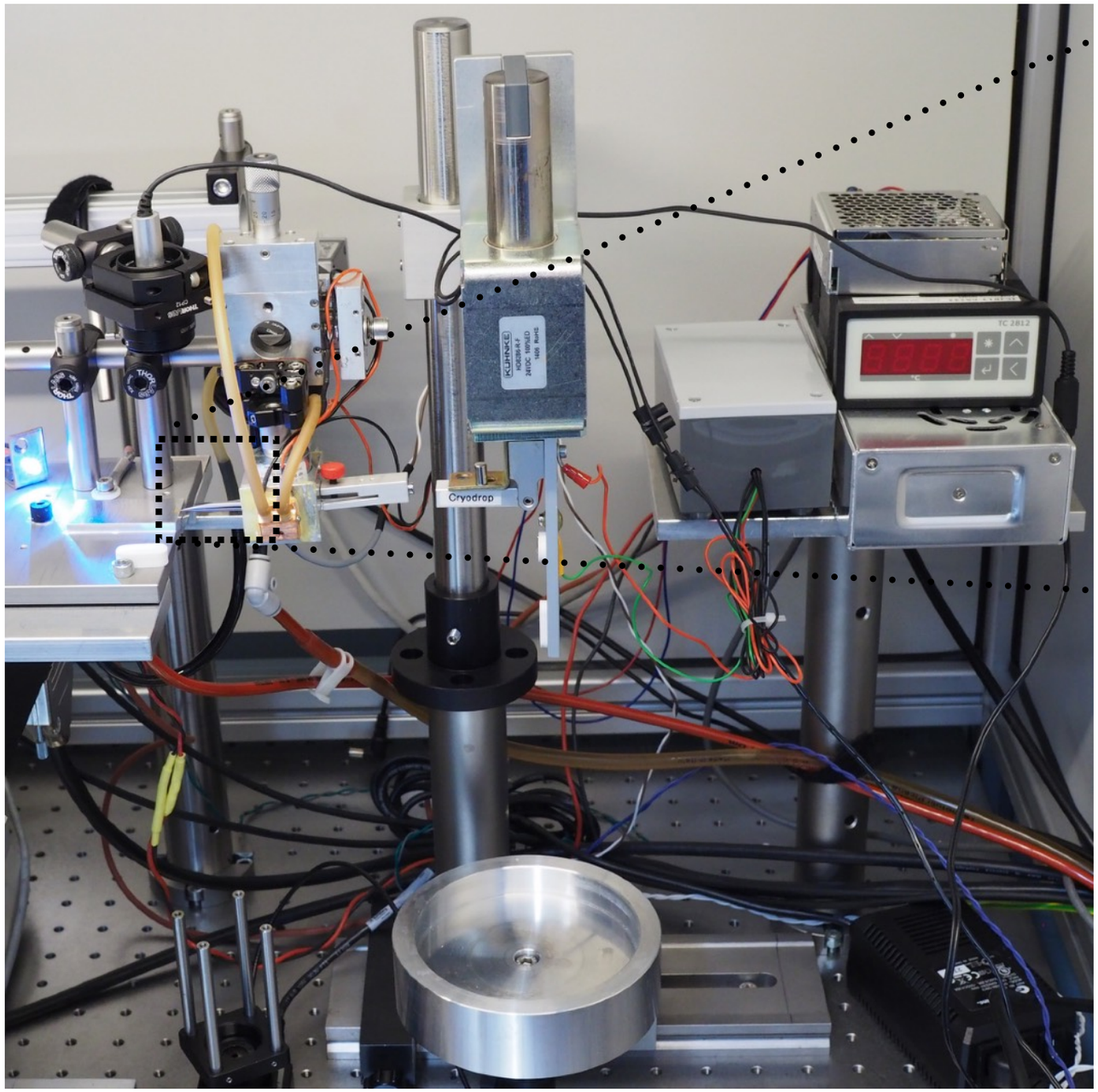
Handover: Cryo-EM

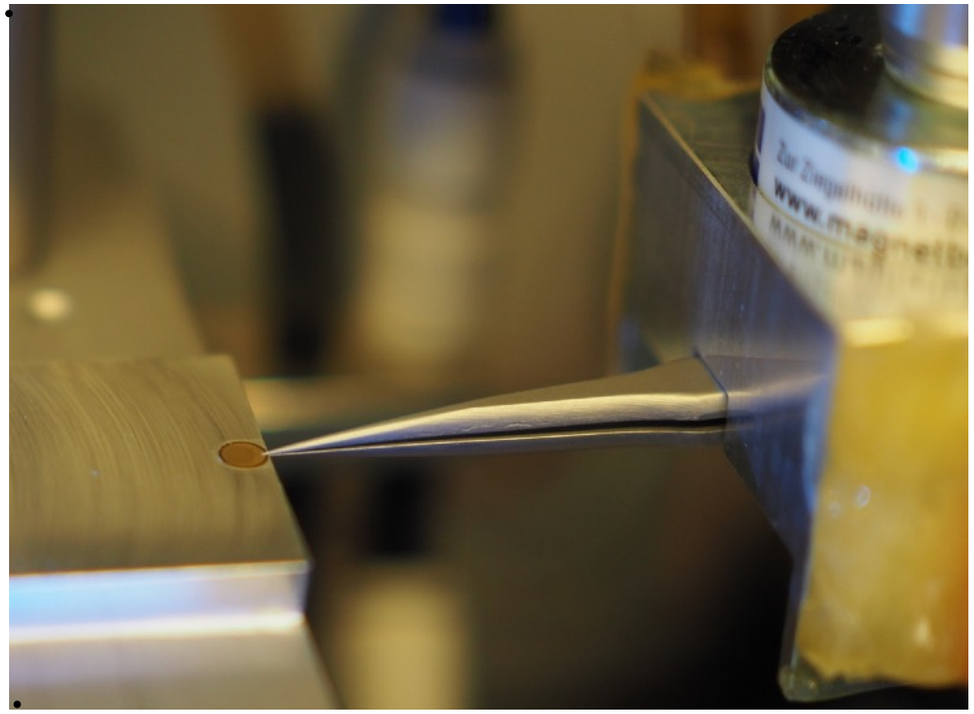


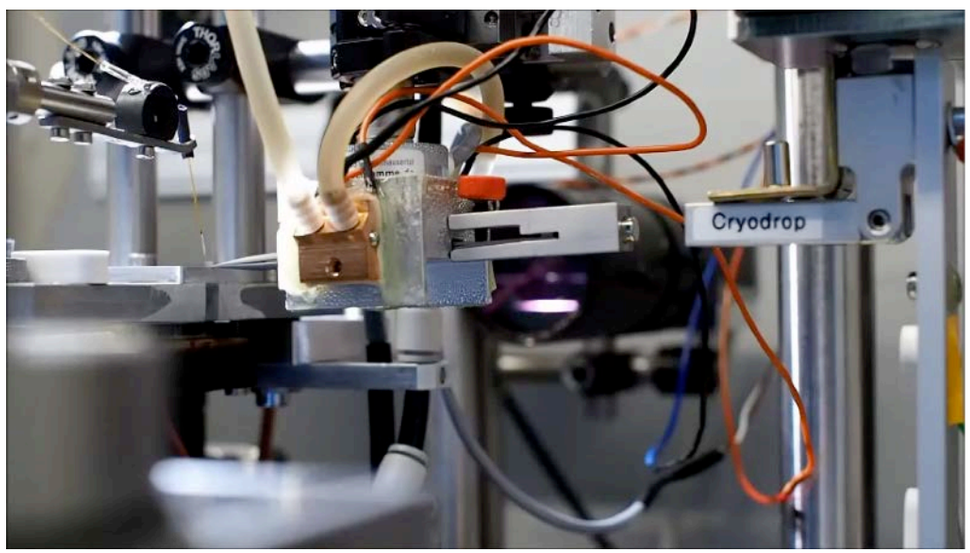
S. A. Arnold, S. Albiez, A. Bieri, A. Syntychaki, R. Adaixo, R. A. McLeod, K. N. Goldie, H. Stahlberg, and T. Braun, “Blotting-free and lossless cryo-electron microscopy grid preparation from nanoliter-sized protein samples and single-cell extracts,” J. Struct. Biol., pp. 1–7, Nov. 2016.

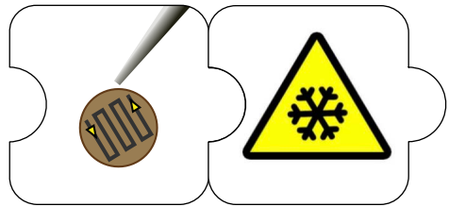
CryoWriter (v. 2)





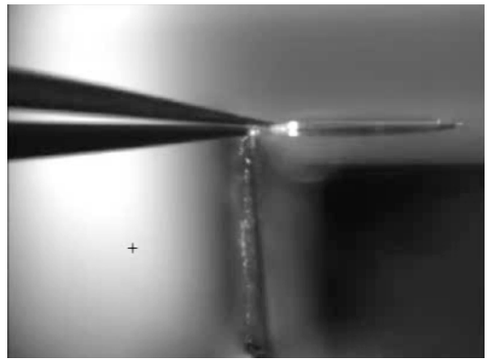
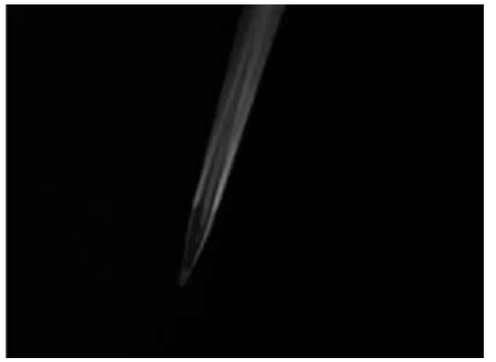



Sample application protocols



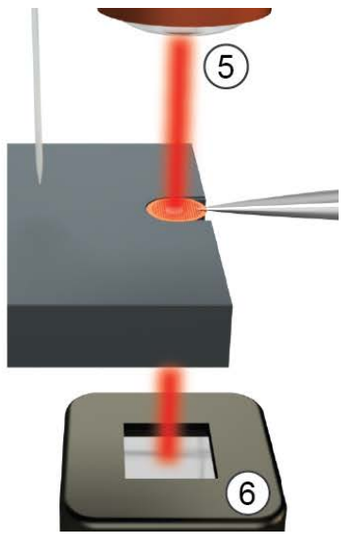
Protocol 1:

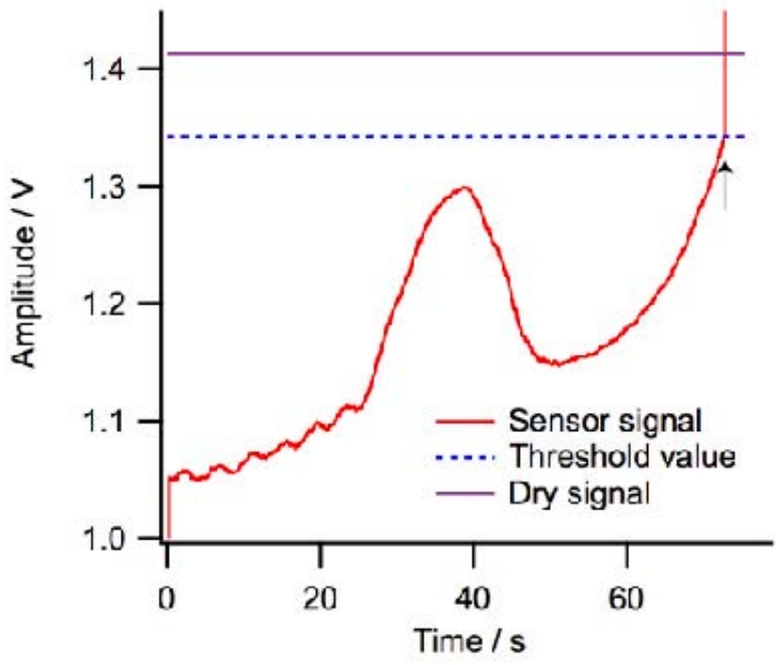
- Approx. 15 nL
- With re-aspiration and sample recovery
- Stage temperature above dew point
- With or without waiting time

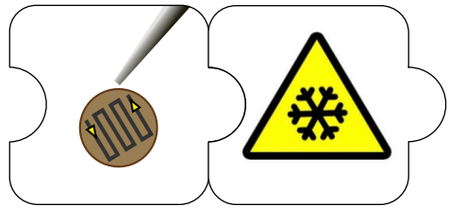



Protocol 2:

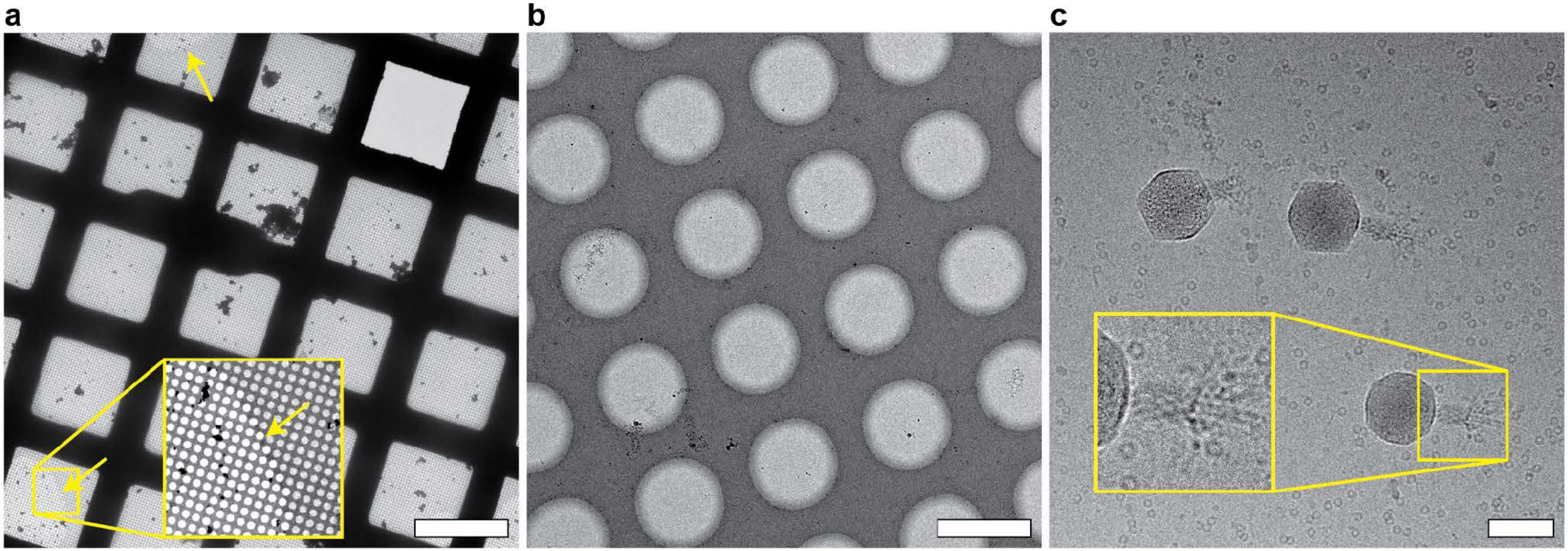
- Total nL sample application at dew point temperature
- Linear increase of stage-temperature
- Controlled evaporation of liquid using sensor
- Single cell lysate analysis



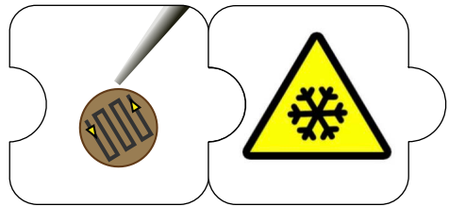




Handover: Cryo-EM



S. A. Arnold, S. Albiez, A. Bieri, A. Syntychaki, R. Adaixo, R. A. McLeod, K. N. Goldie, H. Stahlberg, and T. Braun, “Blotting-free and lossless cryo-electron microscopy grid preparation from nanoliter-sized protein samples and single-cell extracts,” J. Struct. Biol., pp. 1–7, Nov. 2017.

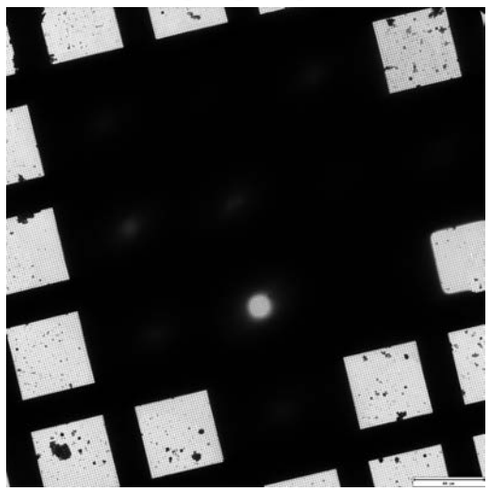


Prescreening of freezing conditions

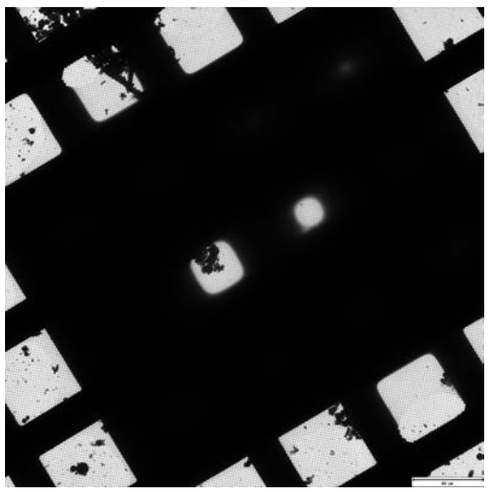
Buffer and dummy protein, e.g., apo-ferritin

Offset temperature screen, constant gap-time (~0.1 s)

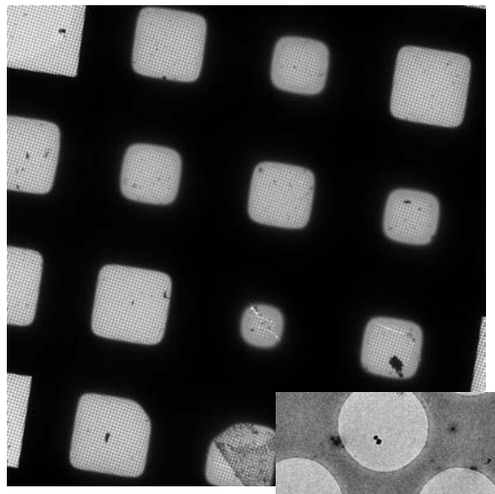
OS 8



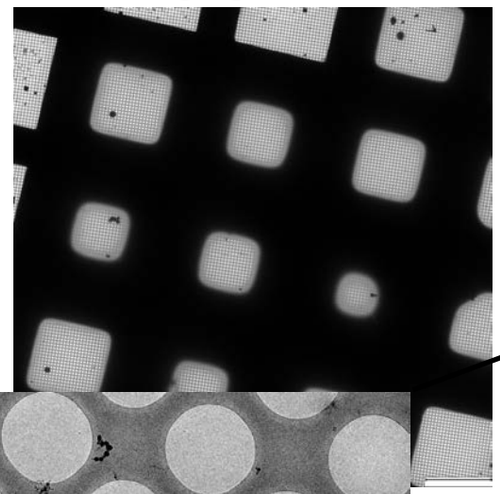
OS 9



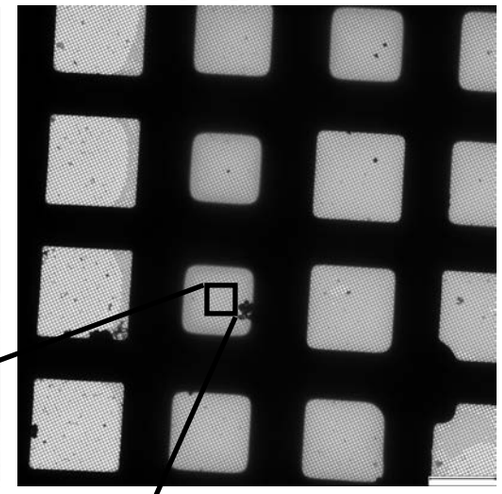
OS 10

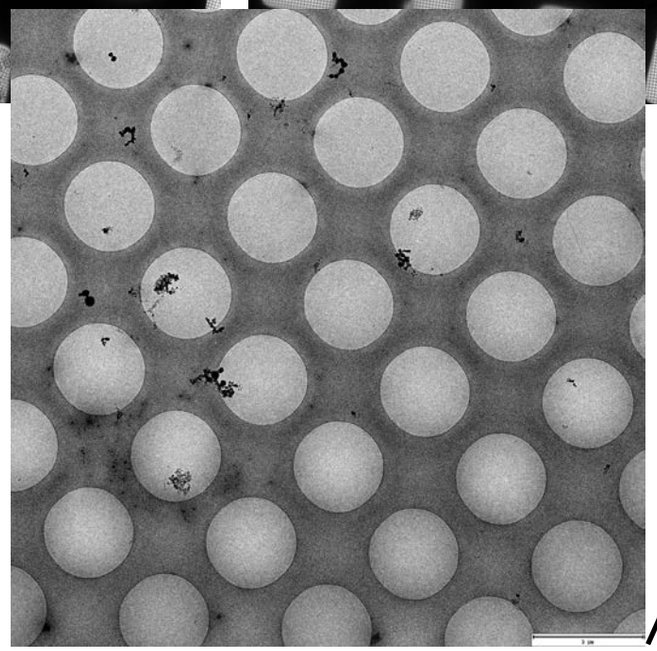


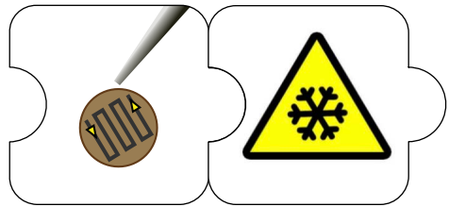
OS 11



OS 12





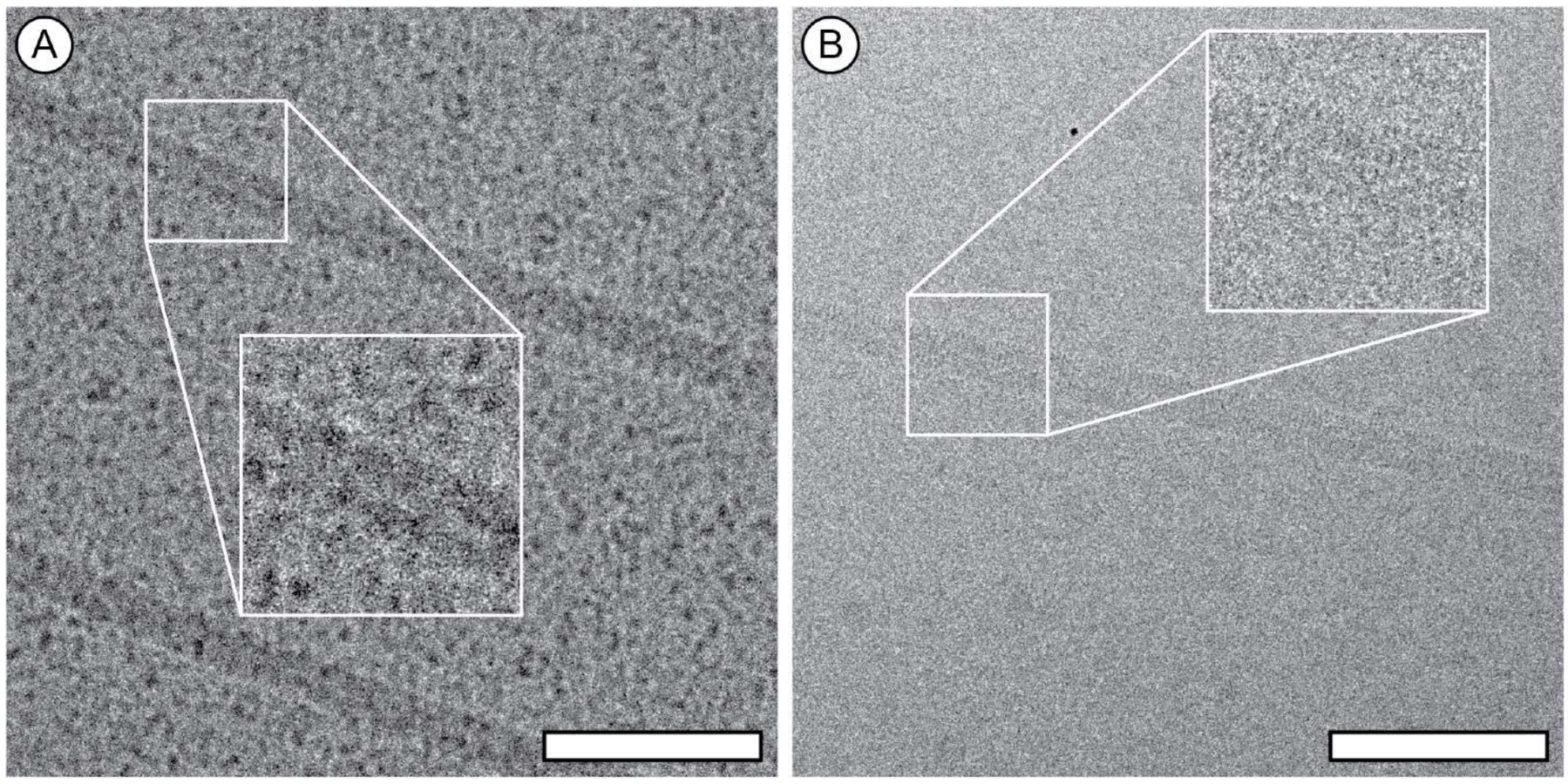


Sample “thinning”

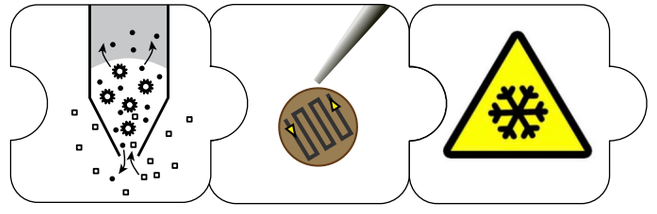
TMV in PBS containing 0.1% DM

Incorrect: Salt effect (too much evaporation)

Correct: Smooth background



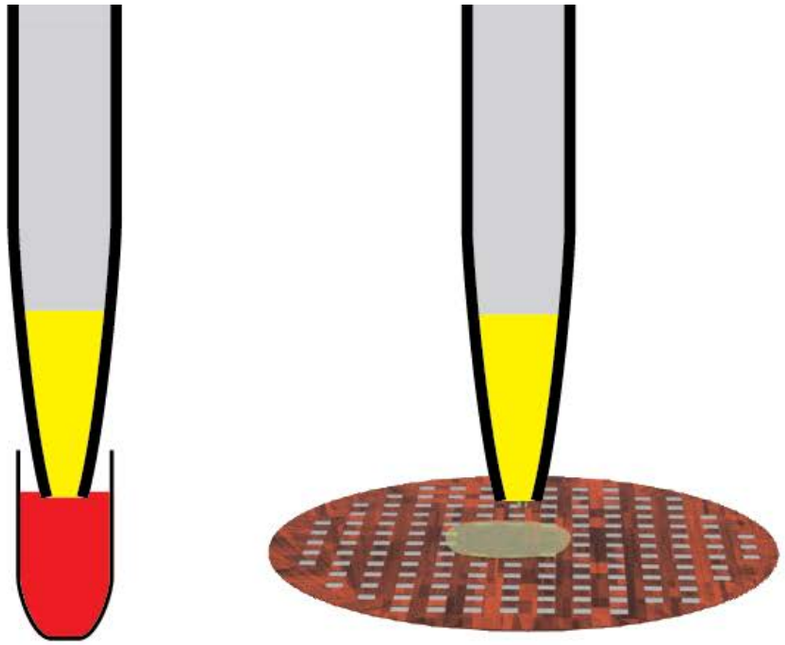
80 nm



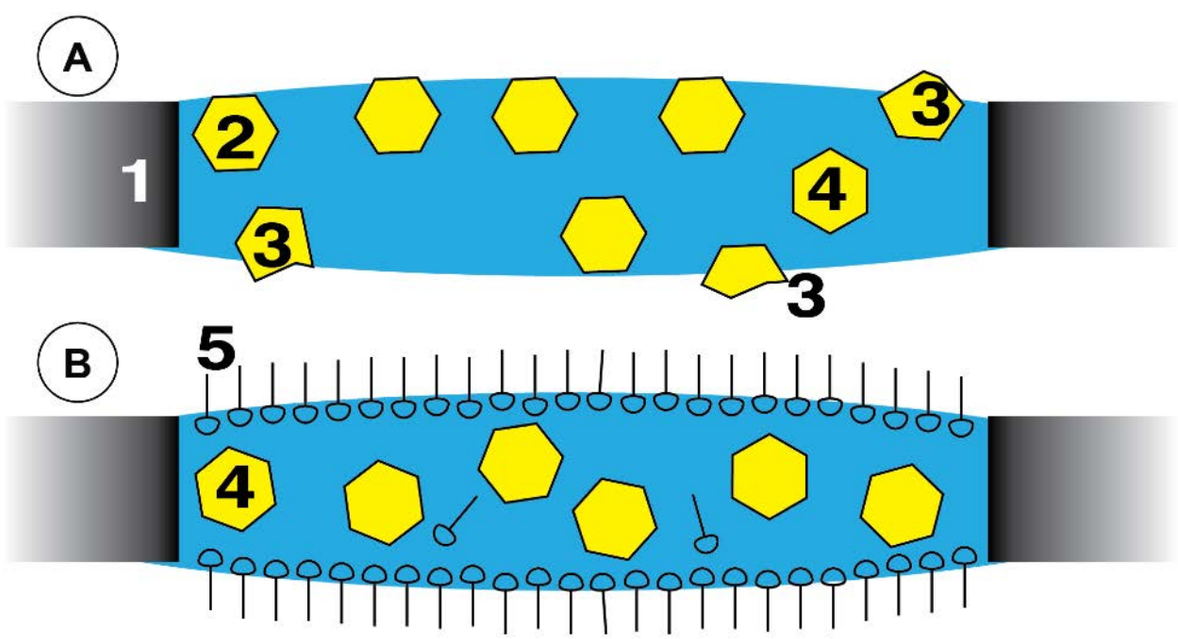
Pre-conditioning

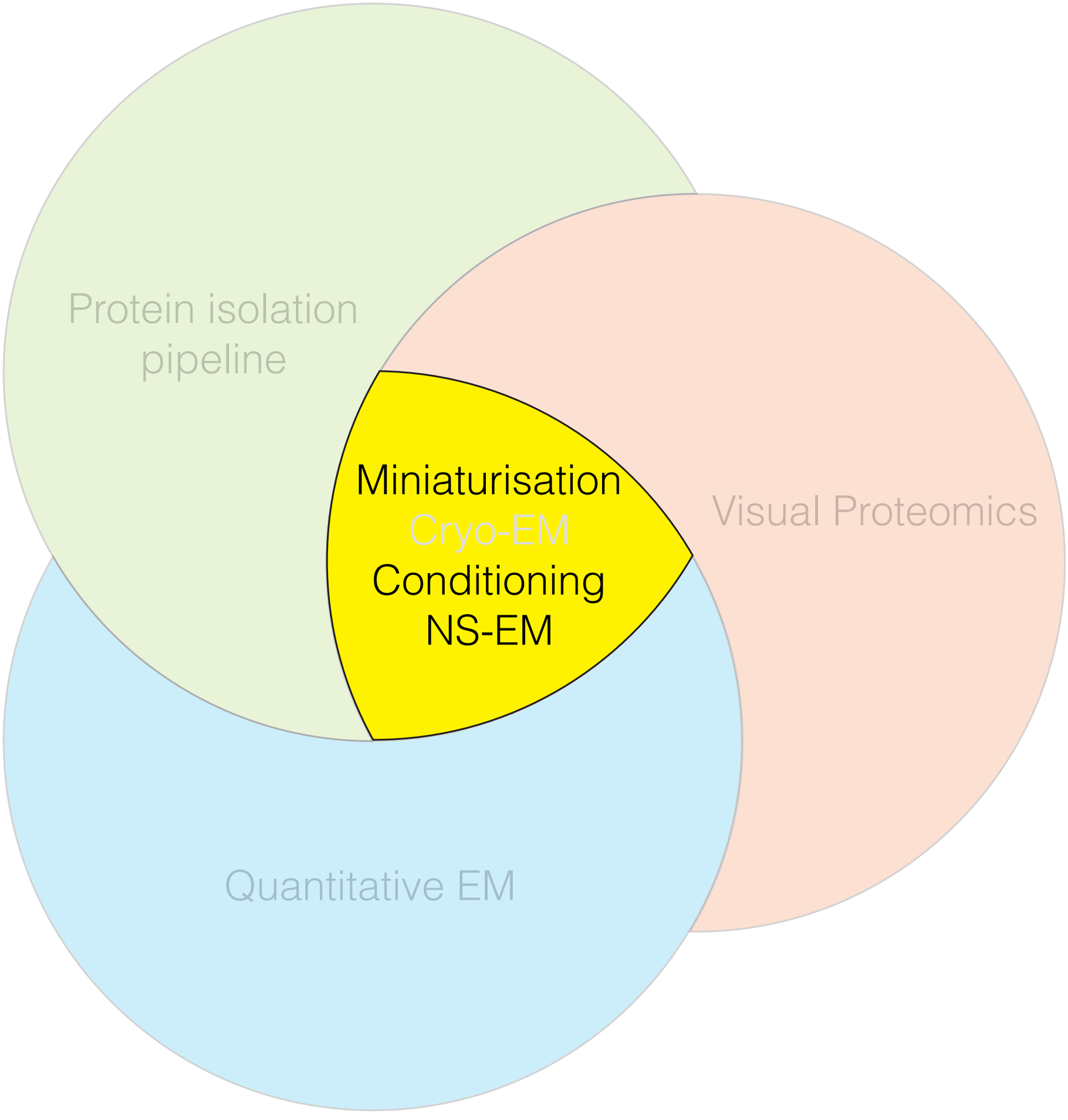
Removal or addition of low MW compounds.

Diffusion driven conditioning



Detergent for air/water interface protection

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Miniaturisation
Cryo-EM
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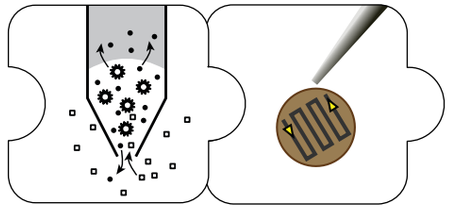
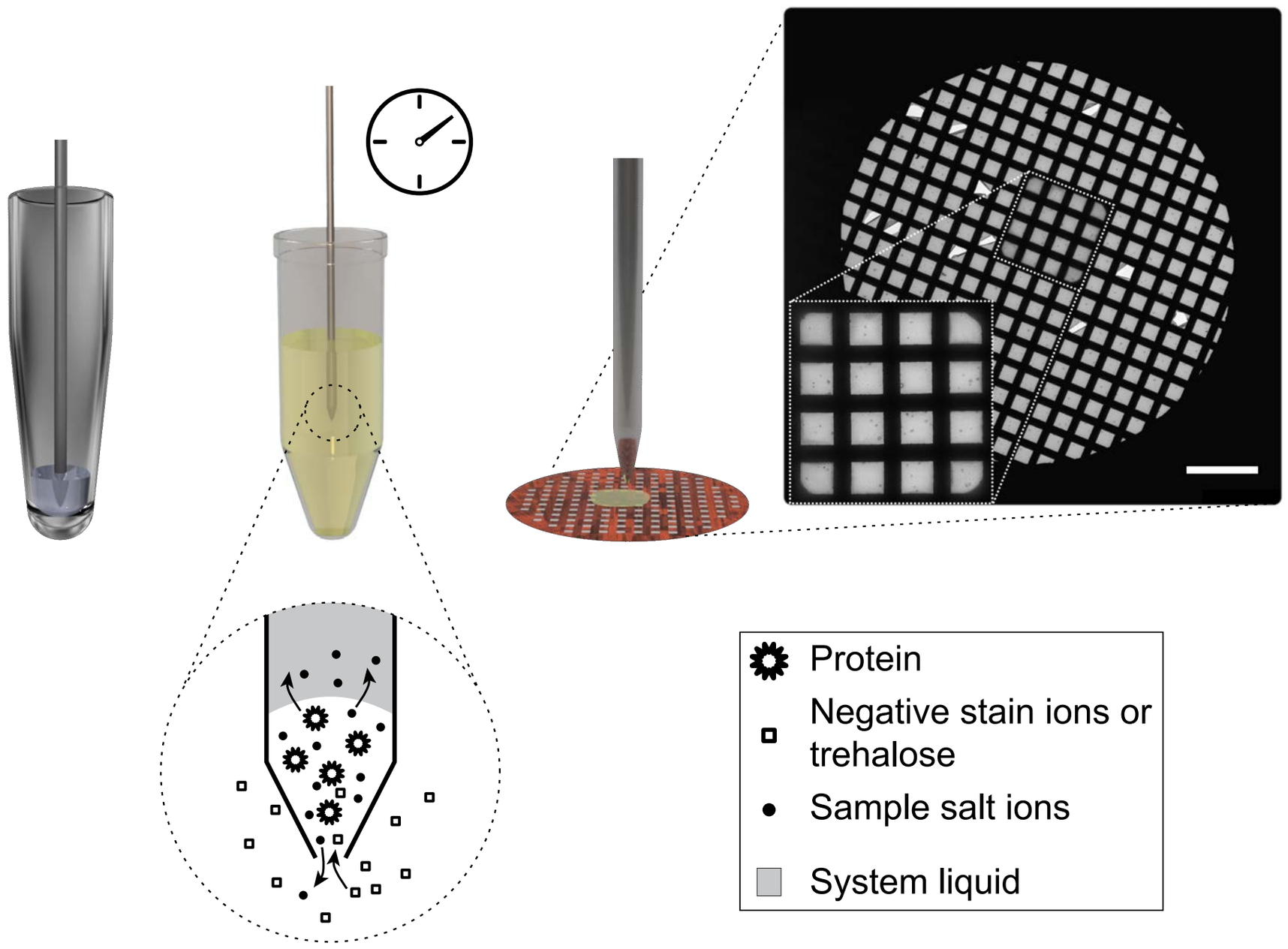
NS-EM
Visual Proteomics
Quantitative EM

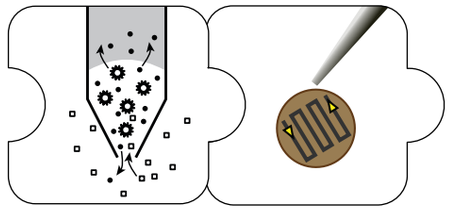


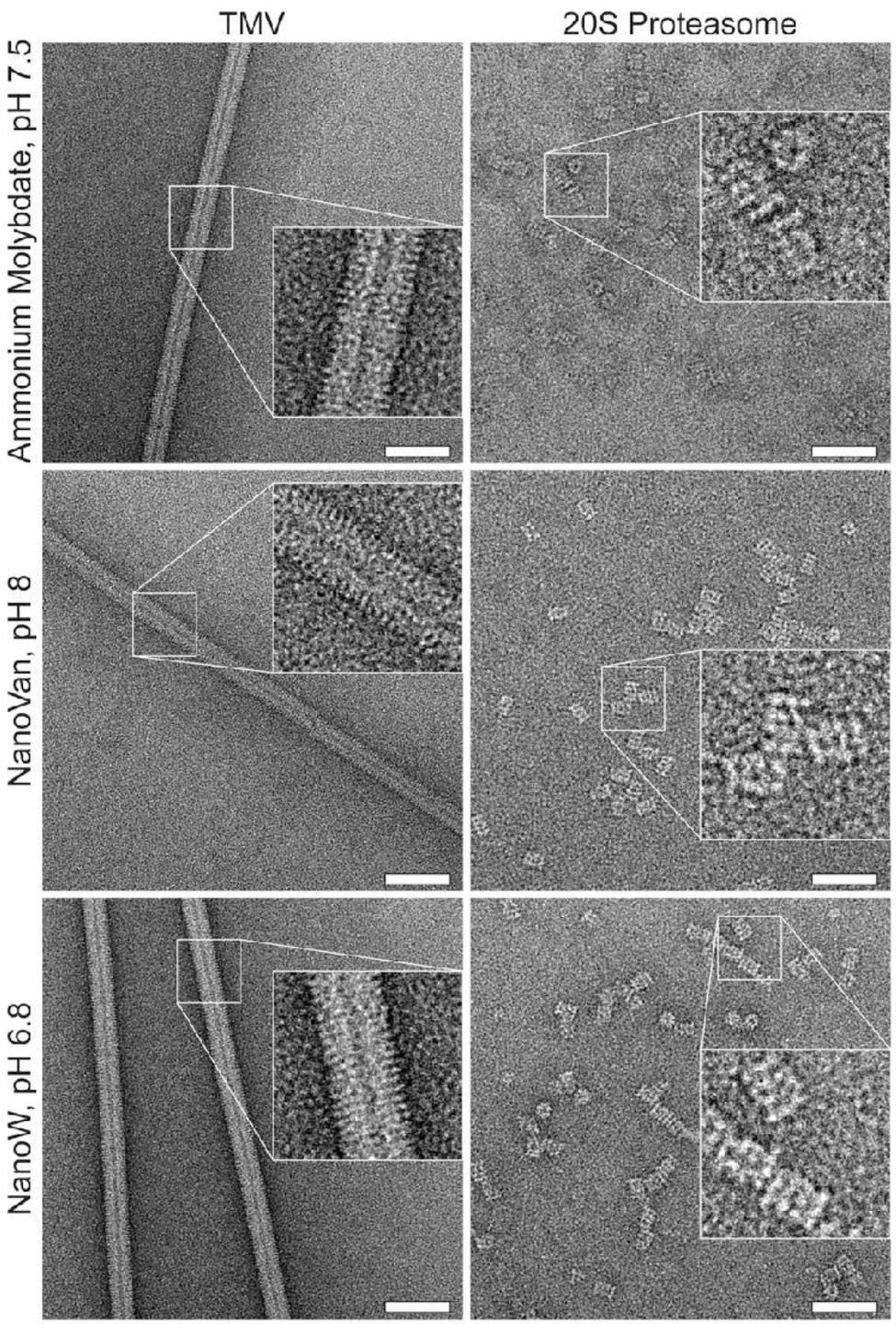
Handover: Negative stain



S. A. Arnold, S. Albiez, N. Opara, M. Chami, C. Schmidli, A. Bieri, C. Padeste, H. Stahlberg, and T. Braun, “Total Sample Conditioning and Preparation of Nanoliter Volumes for Electron Microscopy.,” ACS Nano, vol. 10, no. 5, pp. 4981–4988, 2016.

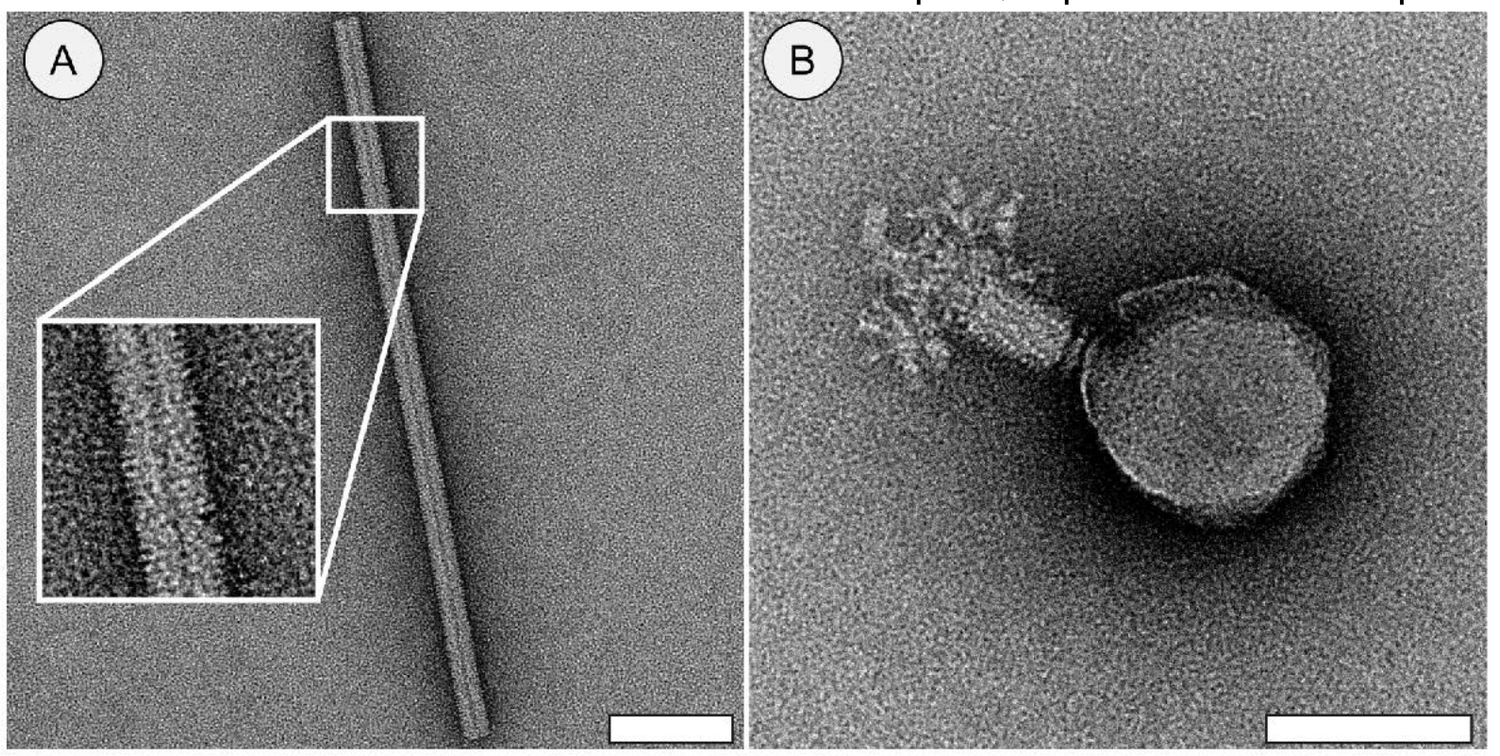
Negative stain





Sample in PBS buffer (7 min)
ID: 250 µm, tip orifice: 40 µm

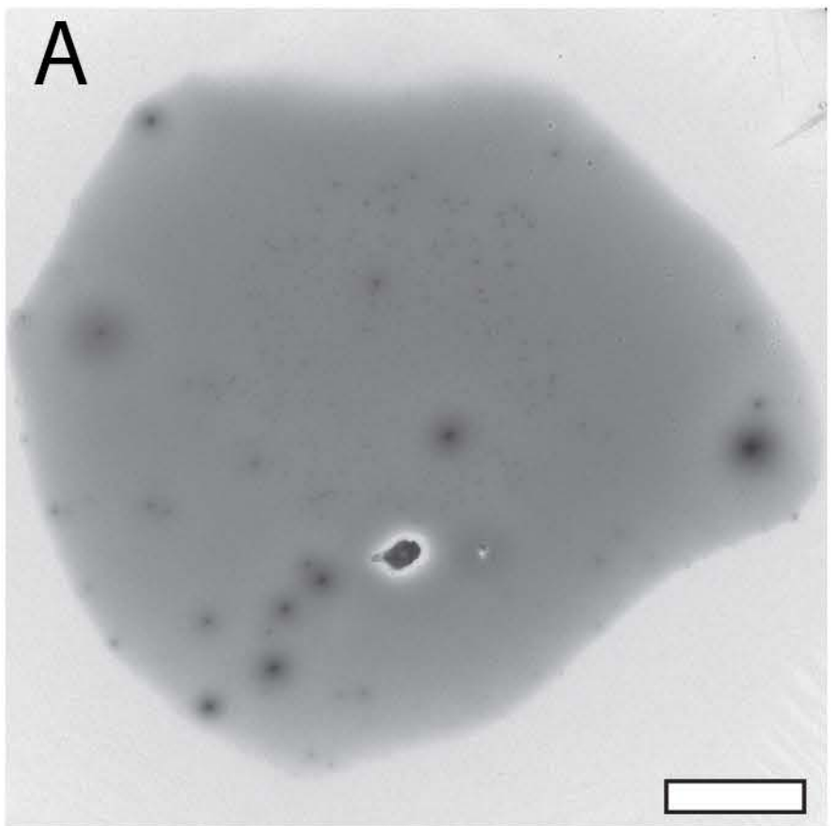
Sample in TRIS buffer (3min)
ID: 100 µm, tip orifice: 30 µm



S. A. Arnold, S. Albiez, N. Opara, M. Chami, C. Schmidli, A. Bieri, C. Padeste, H. Stahlberg, and T. Braun, “Total Sample Conditioning and Preparation of Nanoliter Volumes for Electron Microscopy.,” ACS Nano, vol. 10, no. 5, pp. 4981–4988, 2016.

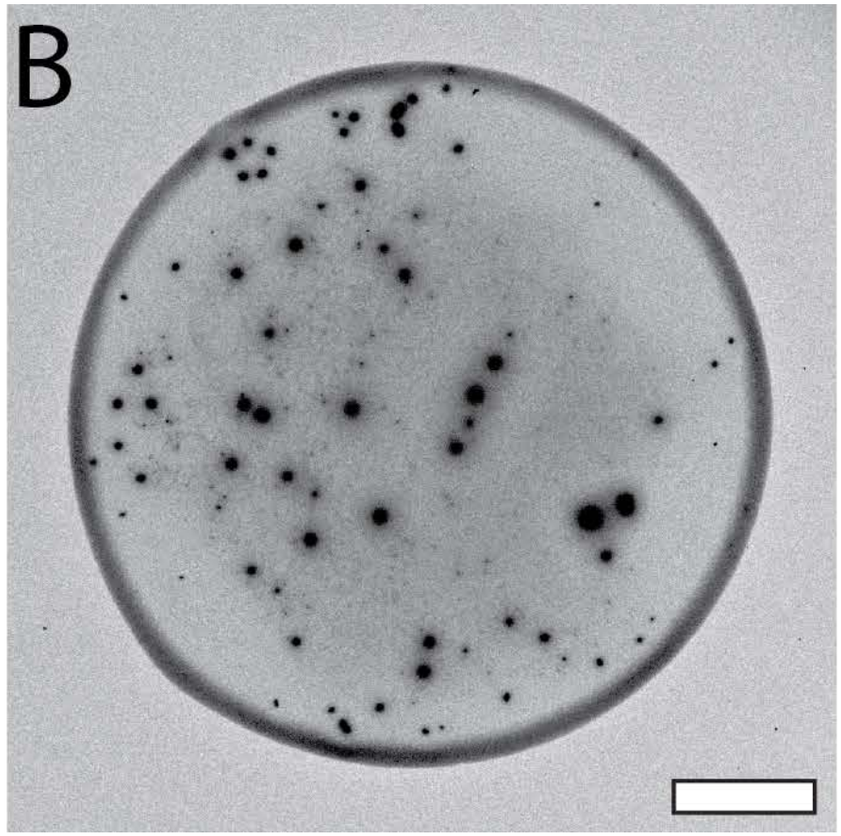
Negative stain artefacts

Slow drying:
Homogeneous stain



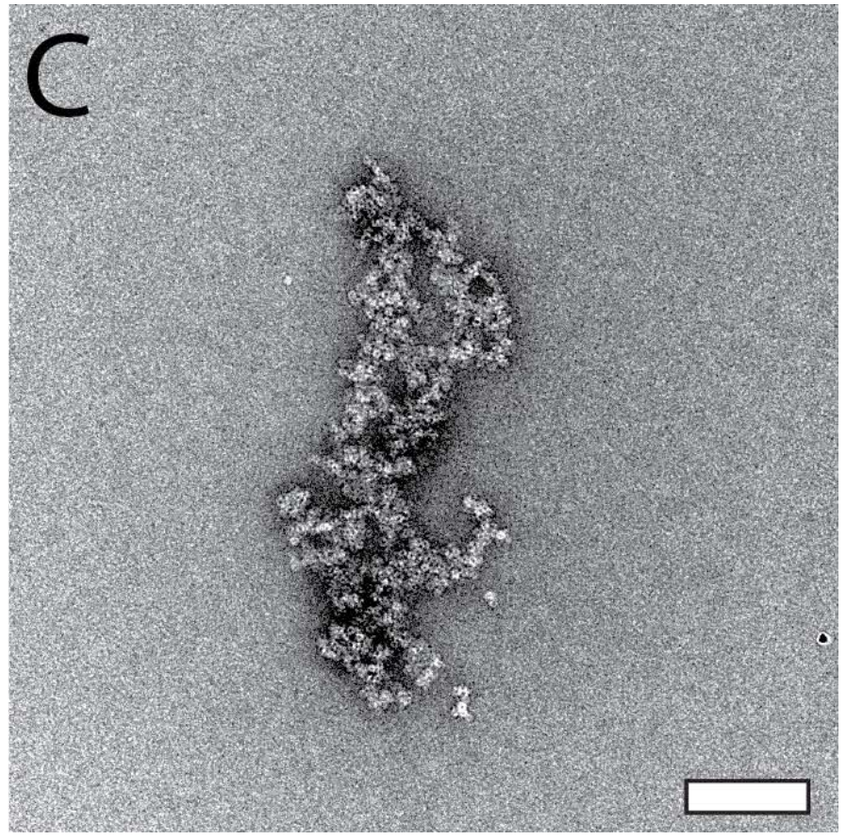
✅ 100 µm

Fast drying:
Coffee ring effect



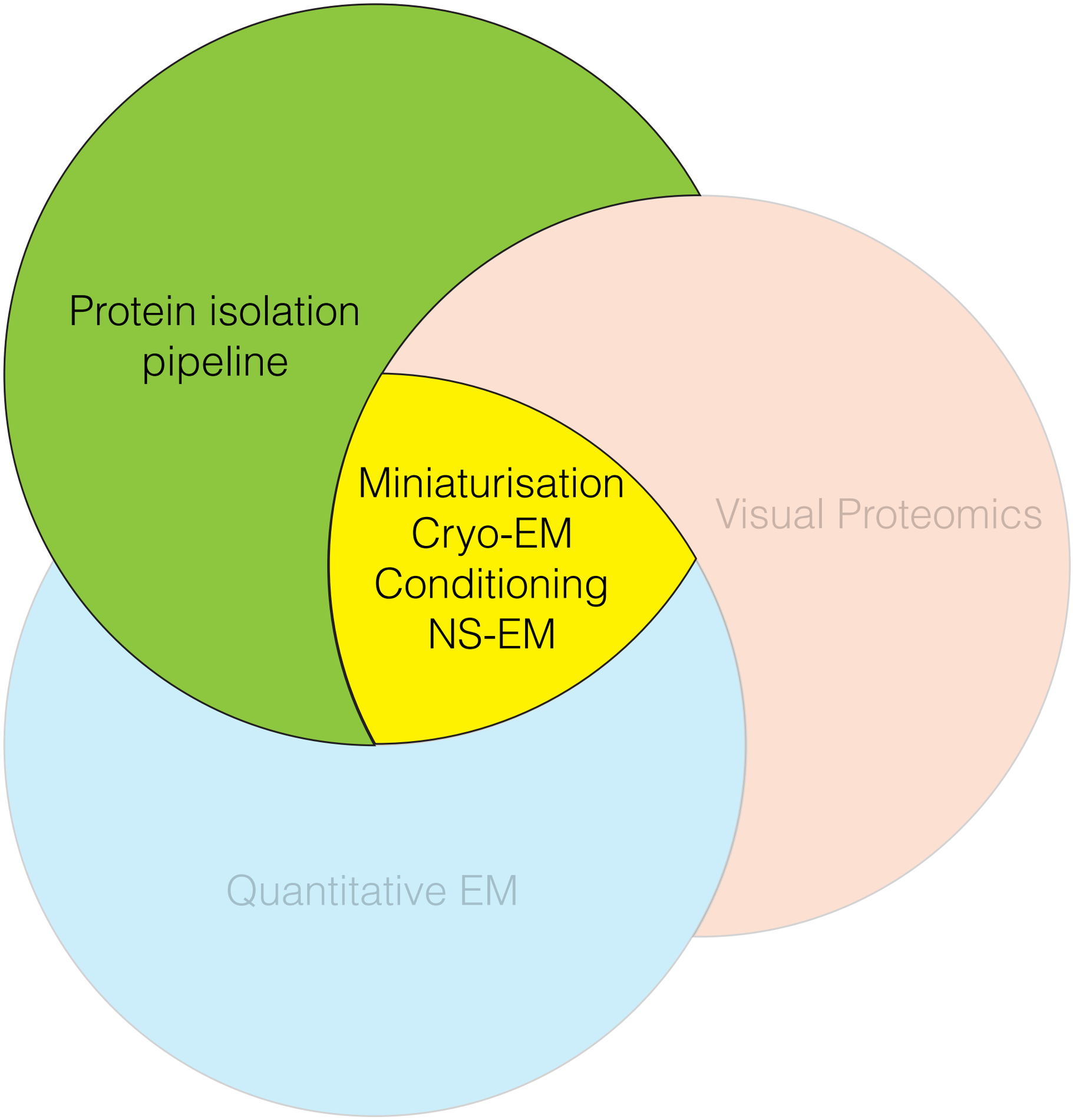
⚠️ 80 µm

Cross-linking by
Uranyl acetate



🎃 200 nm

Schmidli, Rima, Arnold *et al.*, in revision
Kemmerling et al., 2012


Protein isolation
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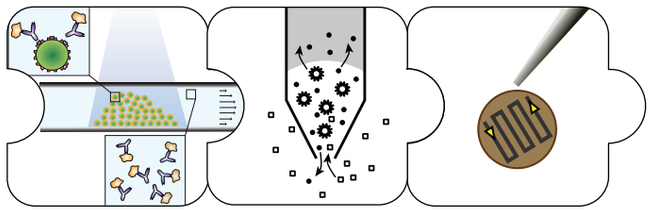


Protein fishing & labelling

Super paramagnetic particle

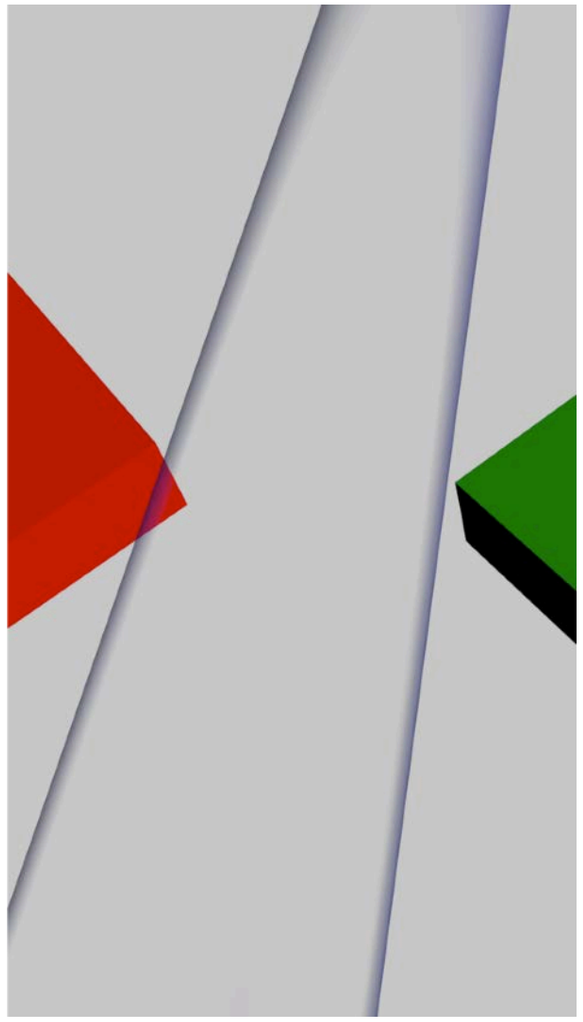
Photo-cleavable linker

AB

Target protein

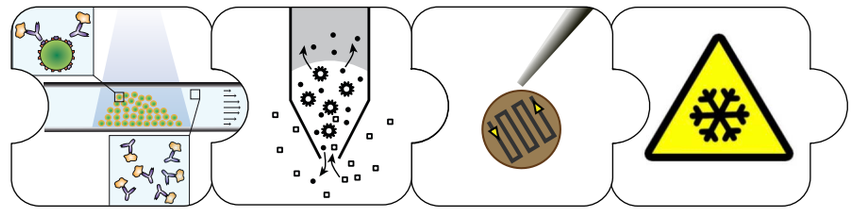
Magnetic trap

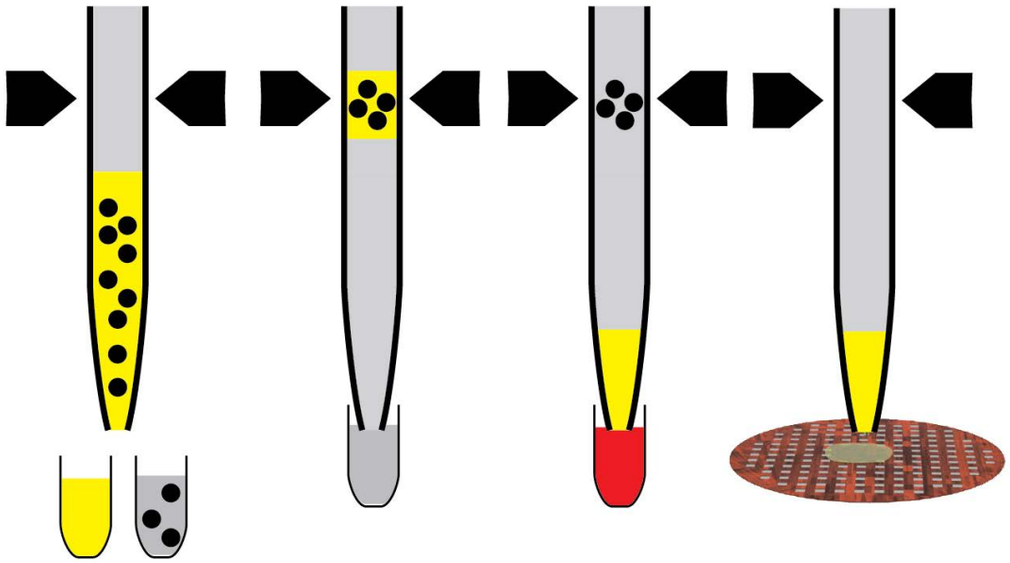
“Photo-elution”



D. Giss, S. Kemmerling, V. Dandey, H. Stahlberg, and T. Braun, “Exploring the interactome: microfluidic isolation of proteins and interacting partners for quantitative analysis by electron microscopy.,” Anal. Chem.,86 (10), 4680–4687, 2014.

Integration



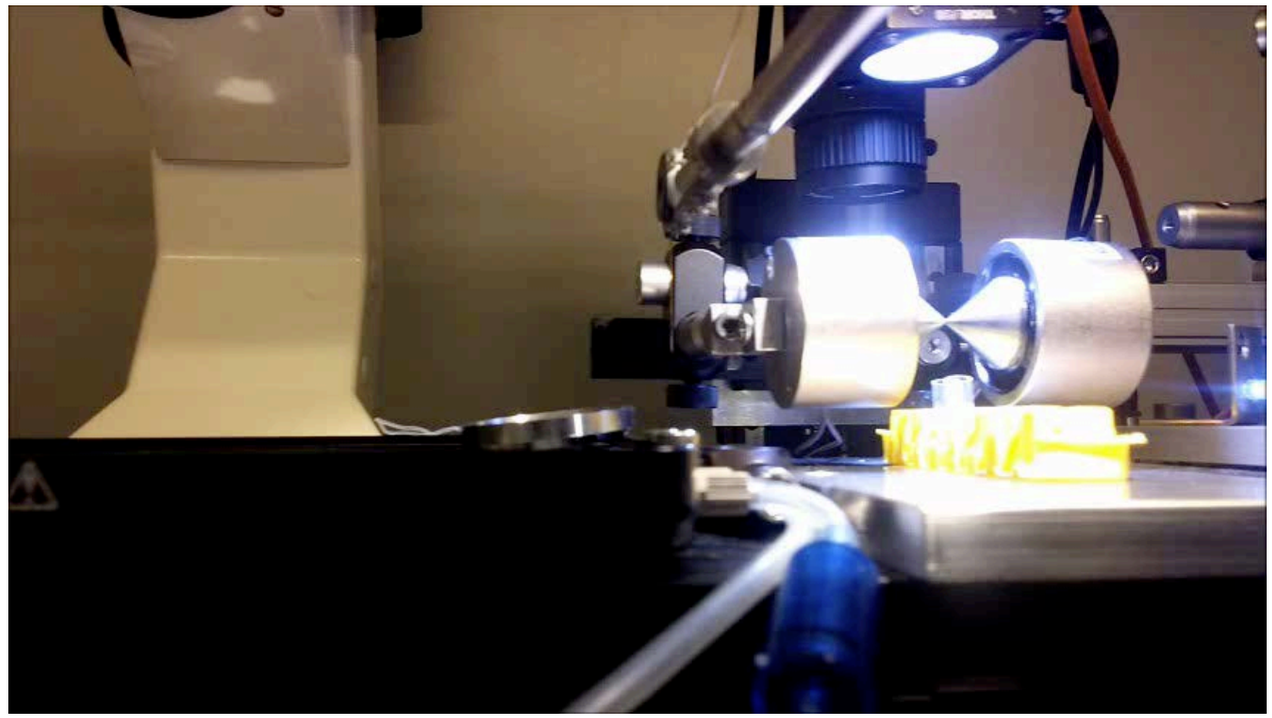


Uptake cell-lysate

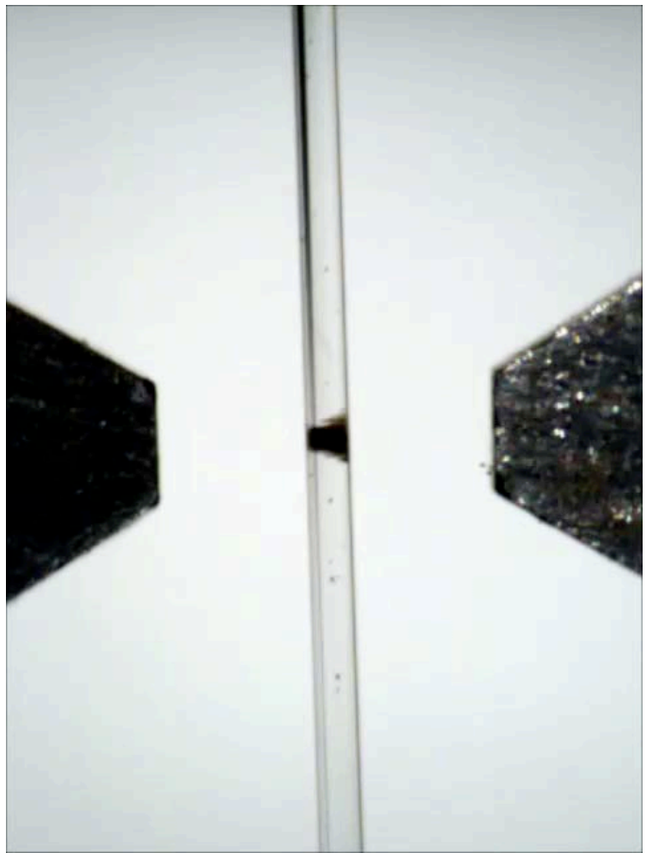
Protein isolation

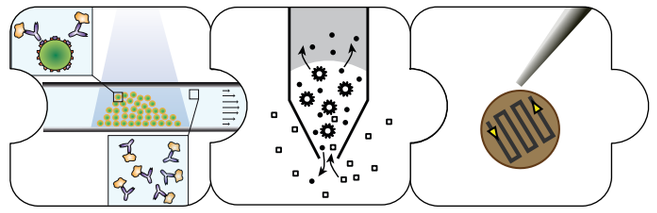
Conditioning

EM-grid preparation



Trap allows mixing

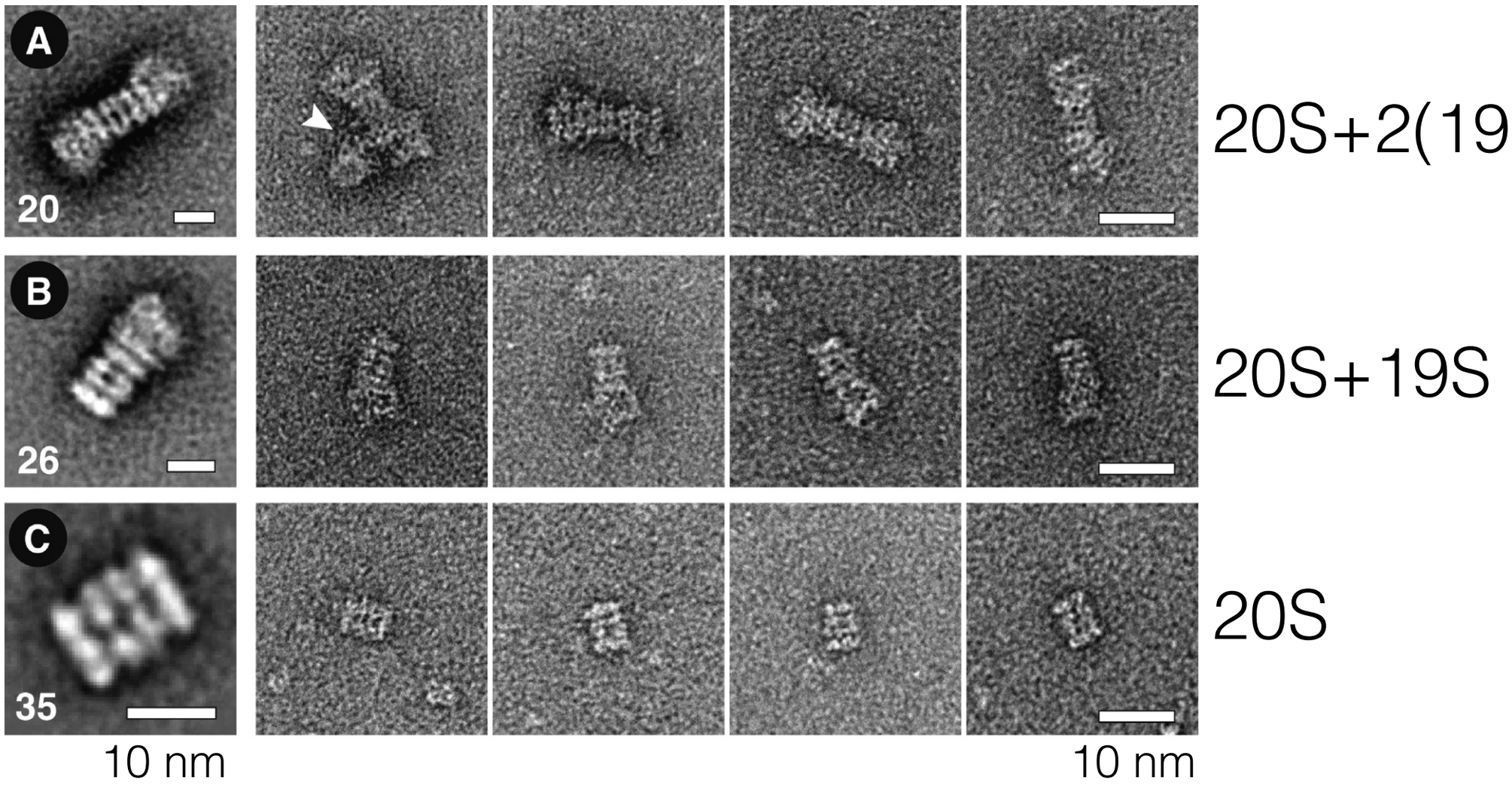




20S proteasome fishing

Endogenous 20S proteasome from 30’000 HEK cells

> 2h total experimental time

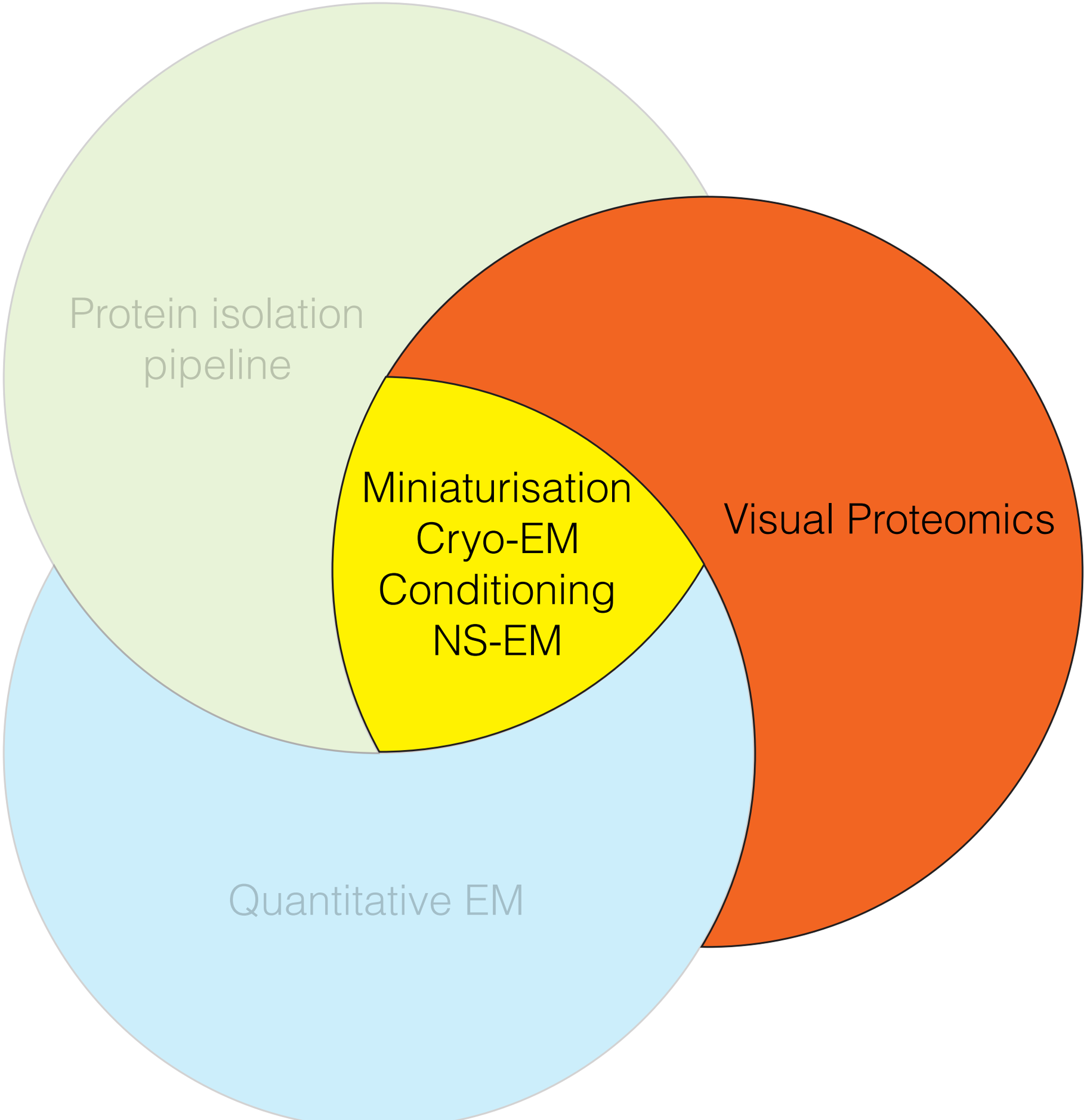


20S+2(19S)

20S+19S

20S

D. Giss, S. Kemmerling, V. Dandey, H. Stahlberg, and T. Braun, “Exploring the interactome: microfluidic isolation of proteins and interacting partners for quantitative analysis by electron microscopy.,” Anal. Chem.,86 (10), 4680–4687, 2014.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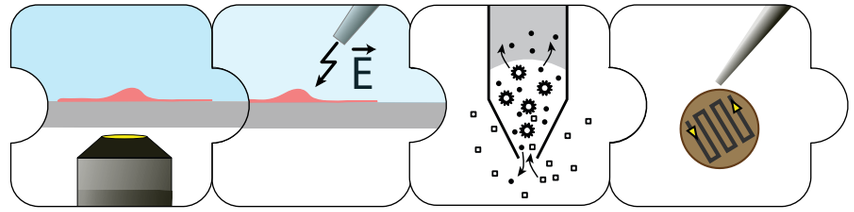
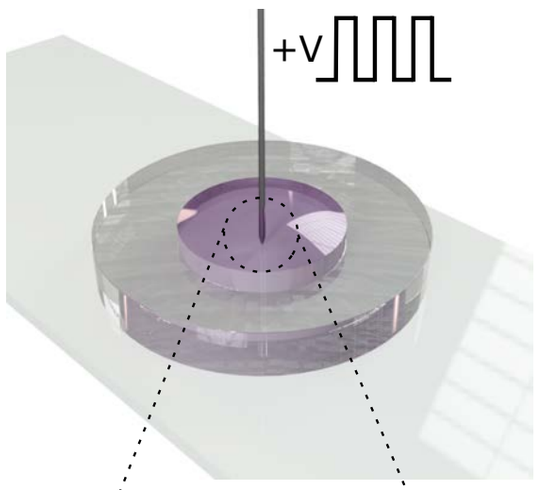
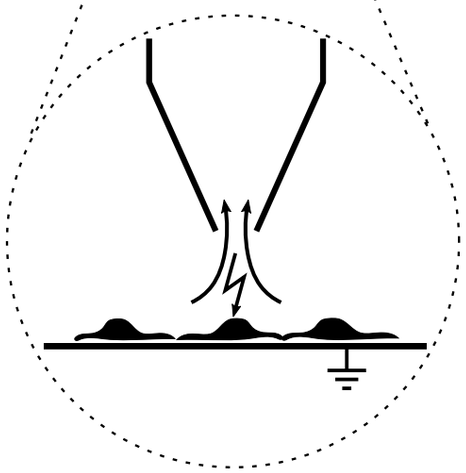
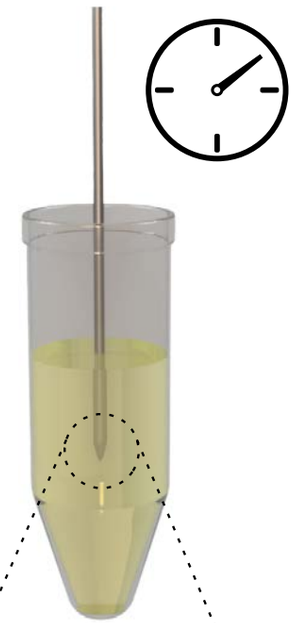
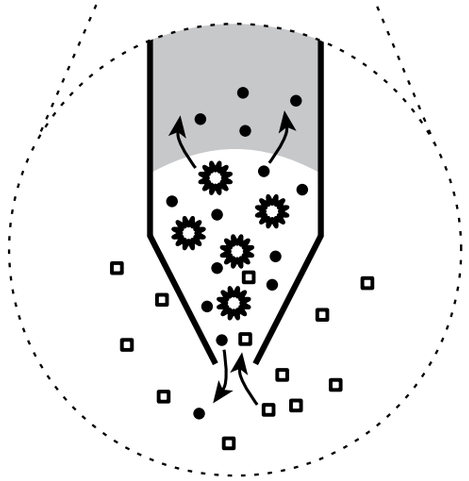


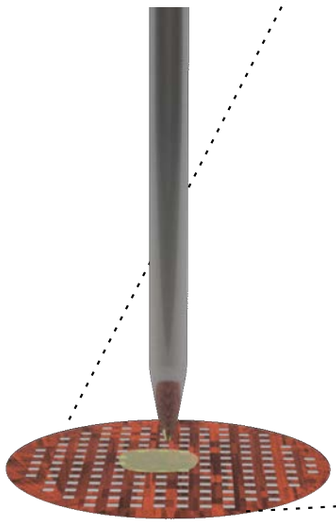
Visual proteomics

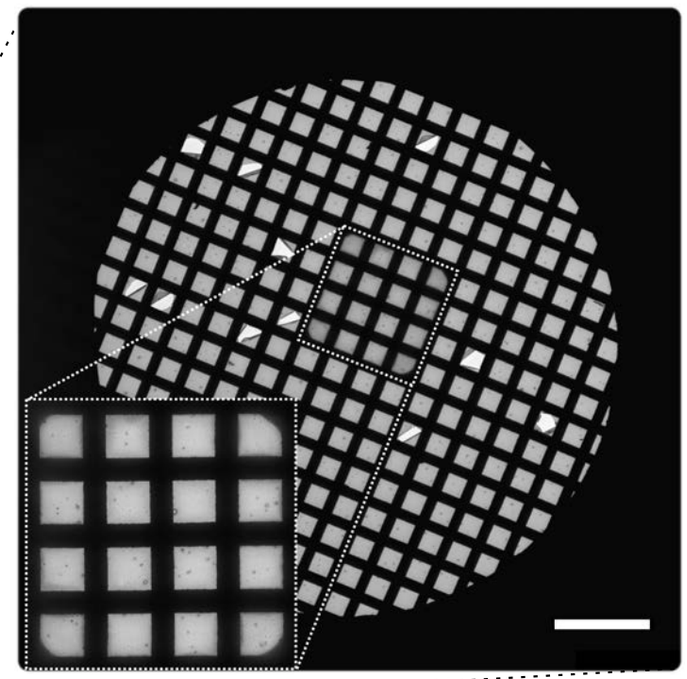




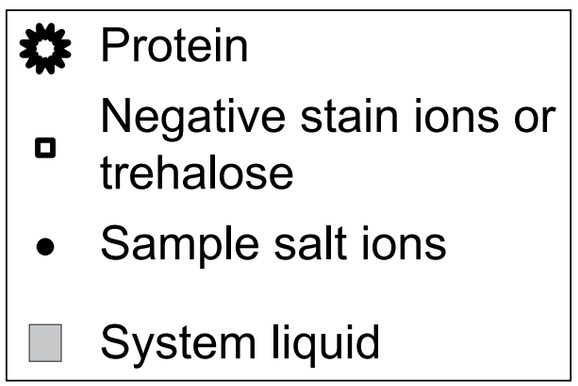








Quantitative EM



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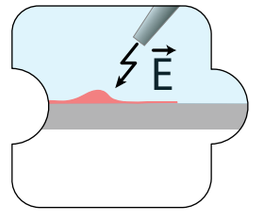
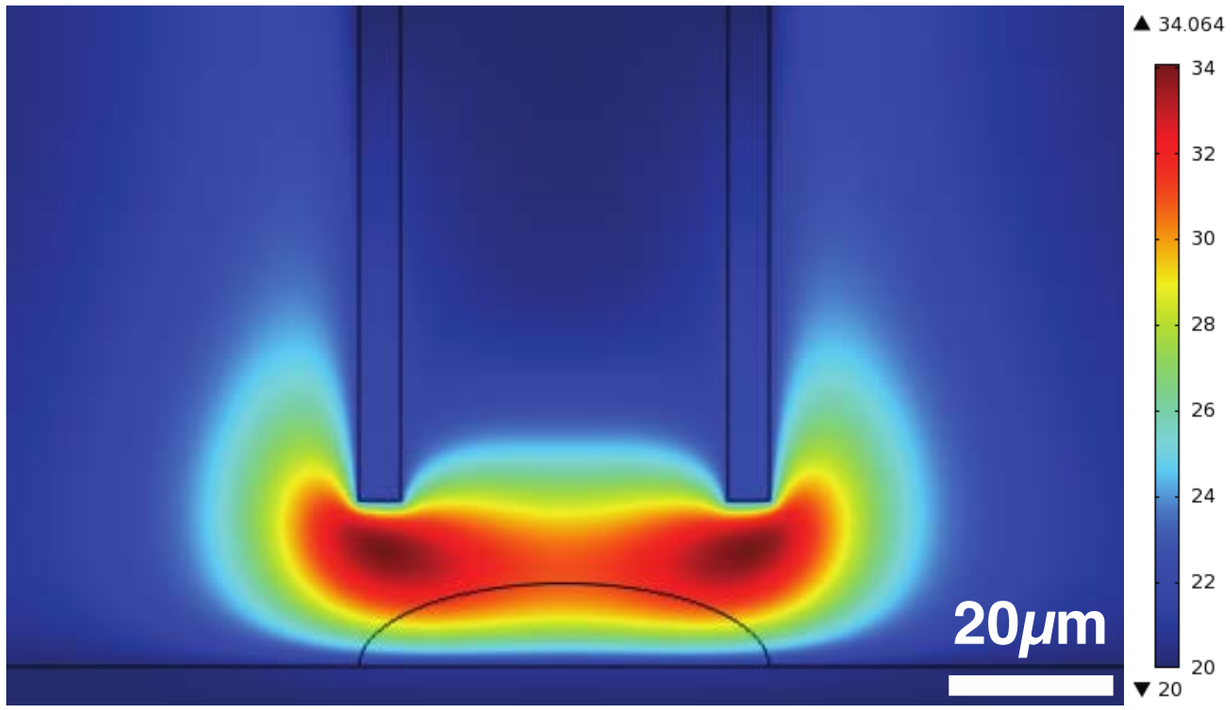
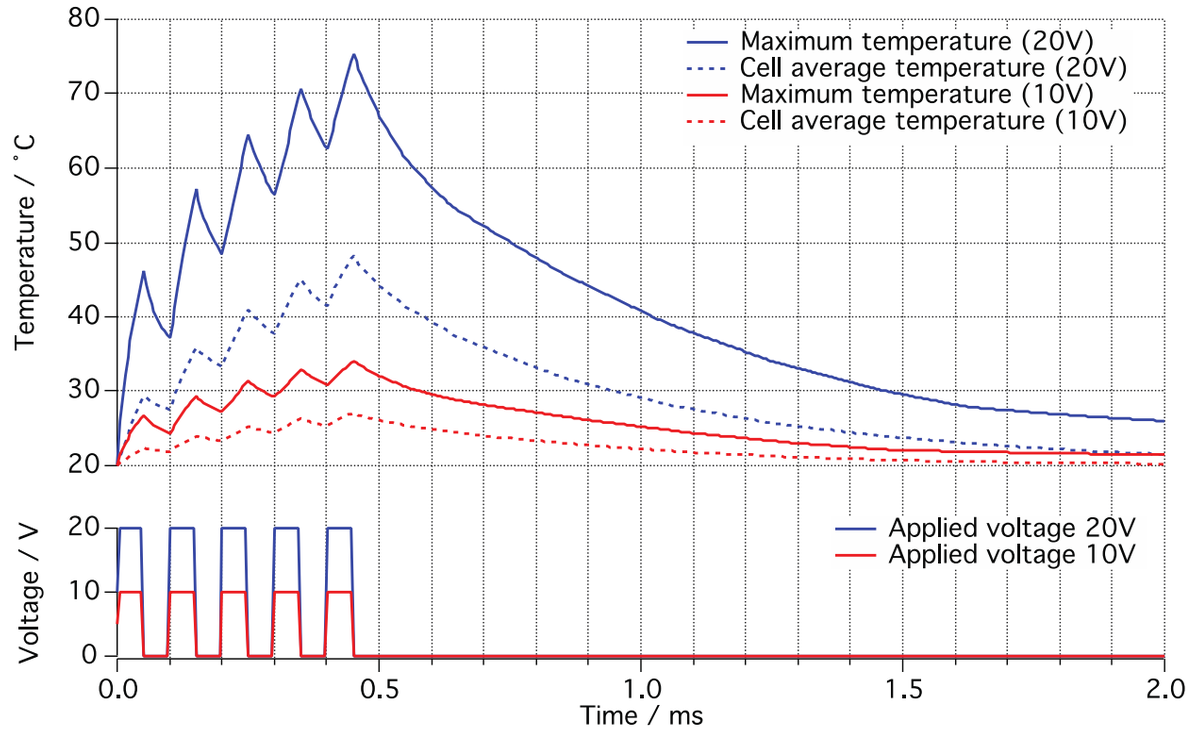


Does it harm proteins?

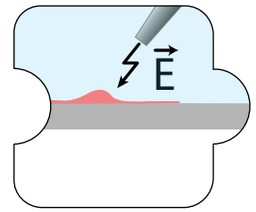
Temperature





FEM analysis: 5x10μs 10V DC pulses

S. Kemmerling, S. A. Arnold, B. A. Bircher, N. Sauter, C. Escobedo, G. Dernick, A. Hierlemann, H. Stahlberg, and T. Braun, “Single-cell lysis for visual analysis by electron microscopy.,” Journal of Structural Biology, vol. 183, no. 3, pp. 467–473, 2013.



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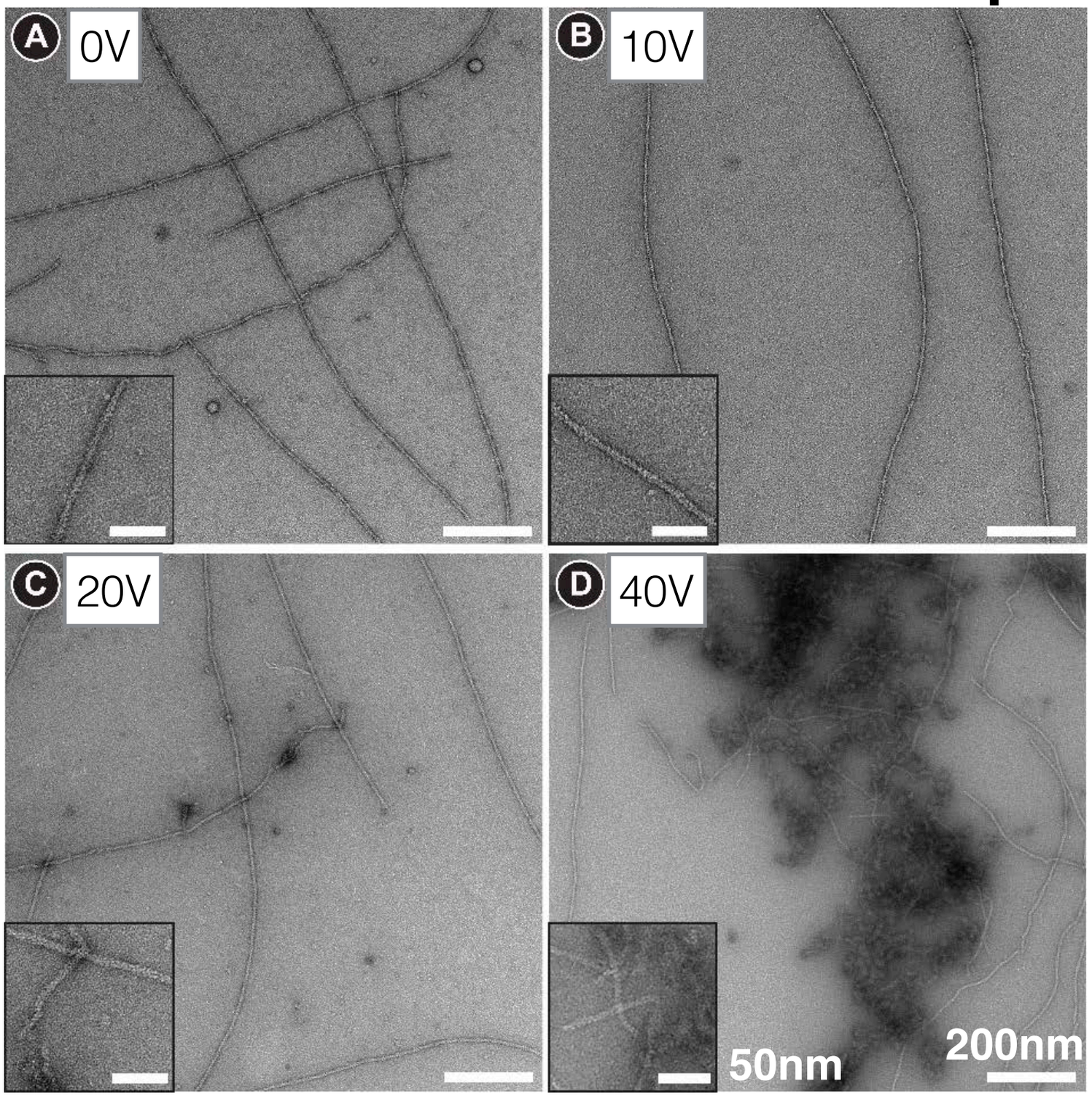

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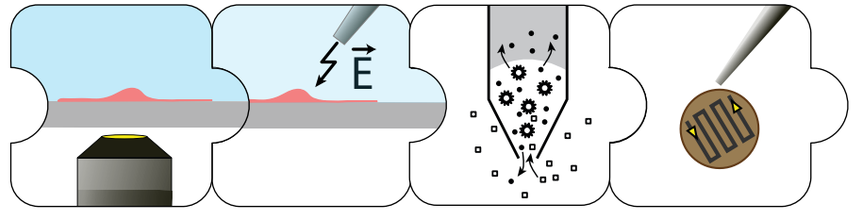
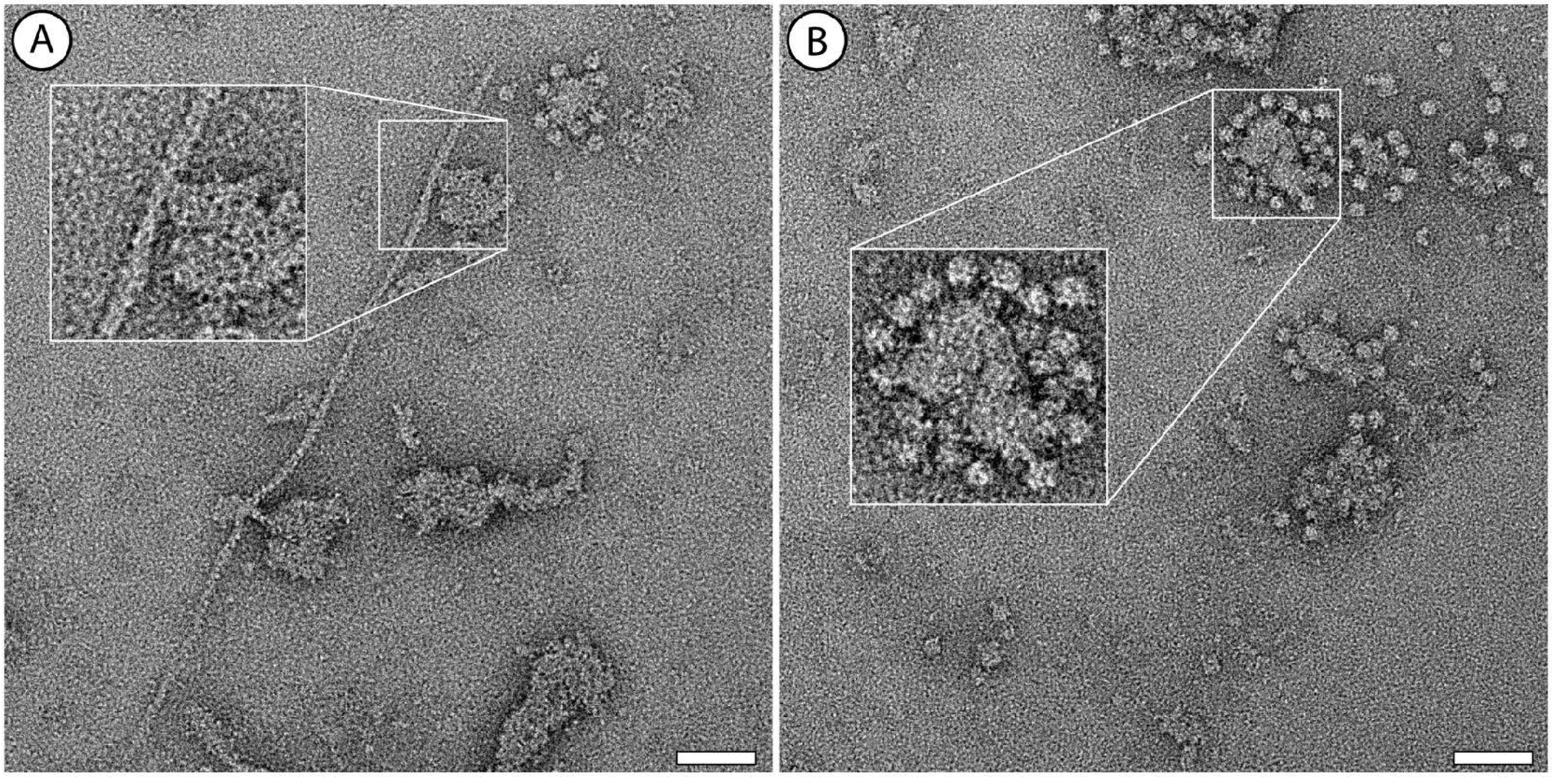


Single cell preparation

negative stain



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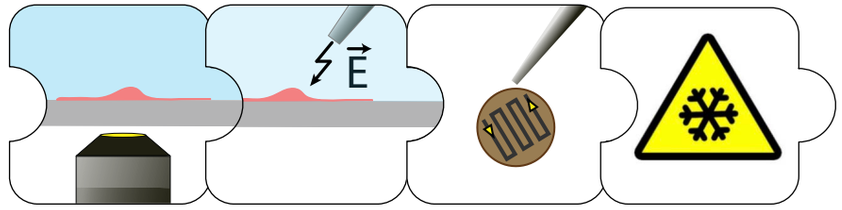
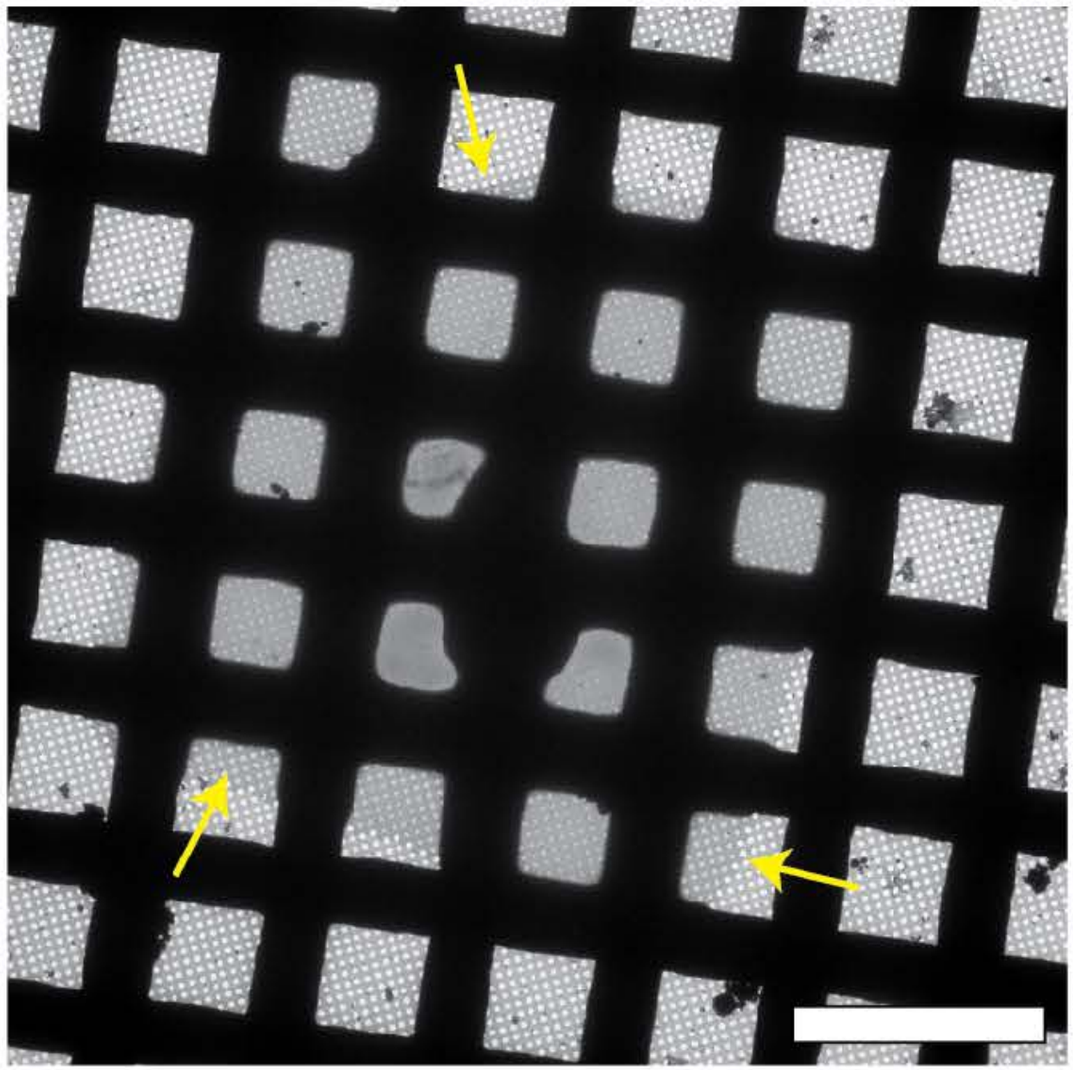
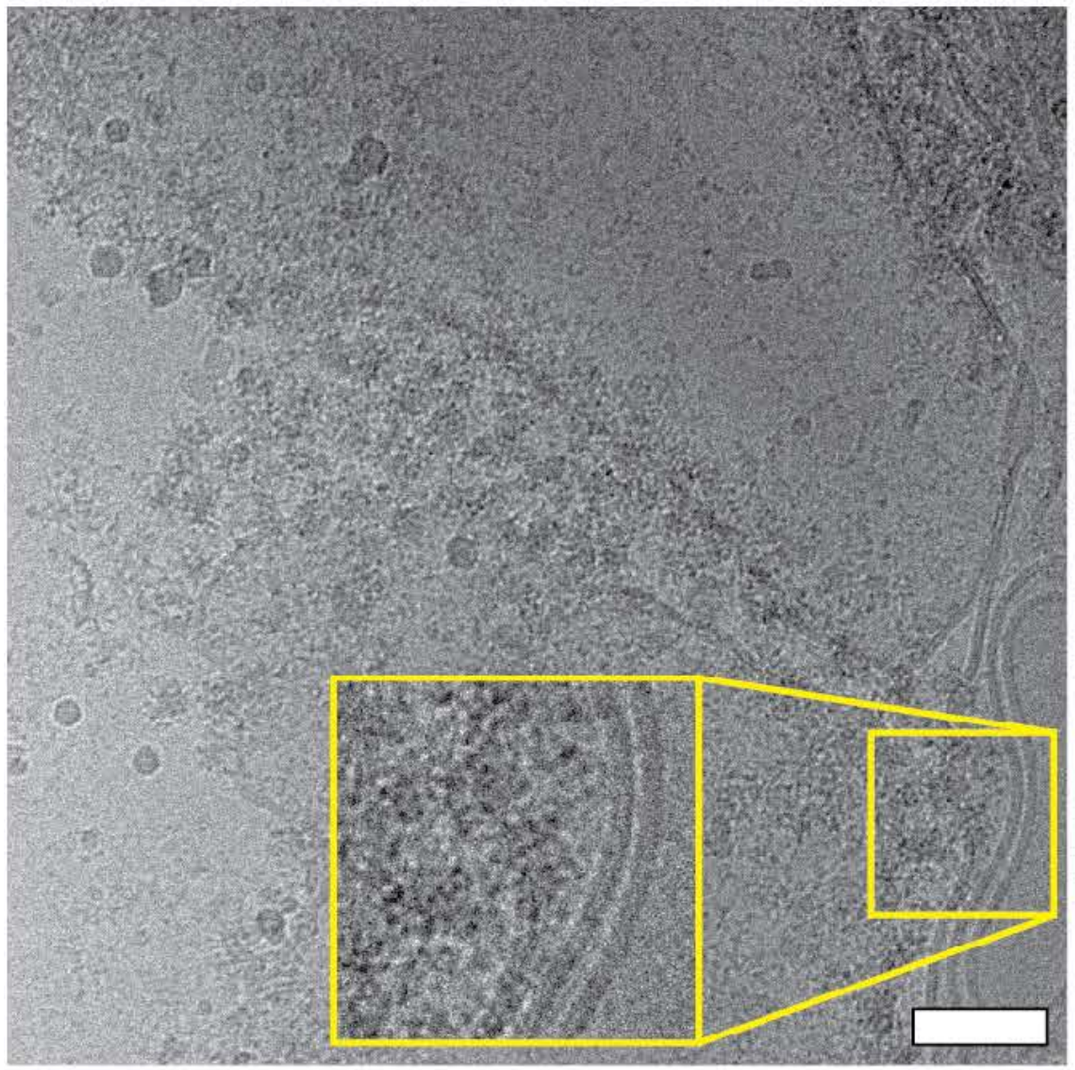


Single cell preparation

cryo-EM





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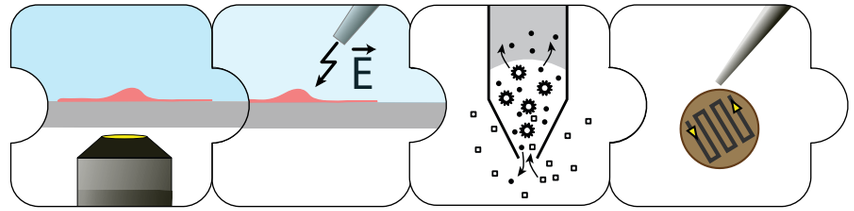
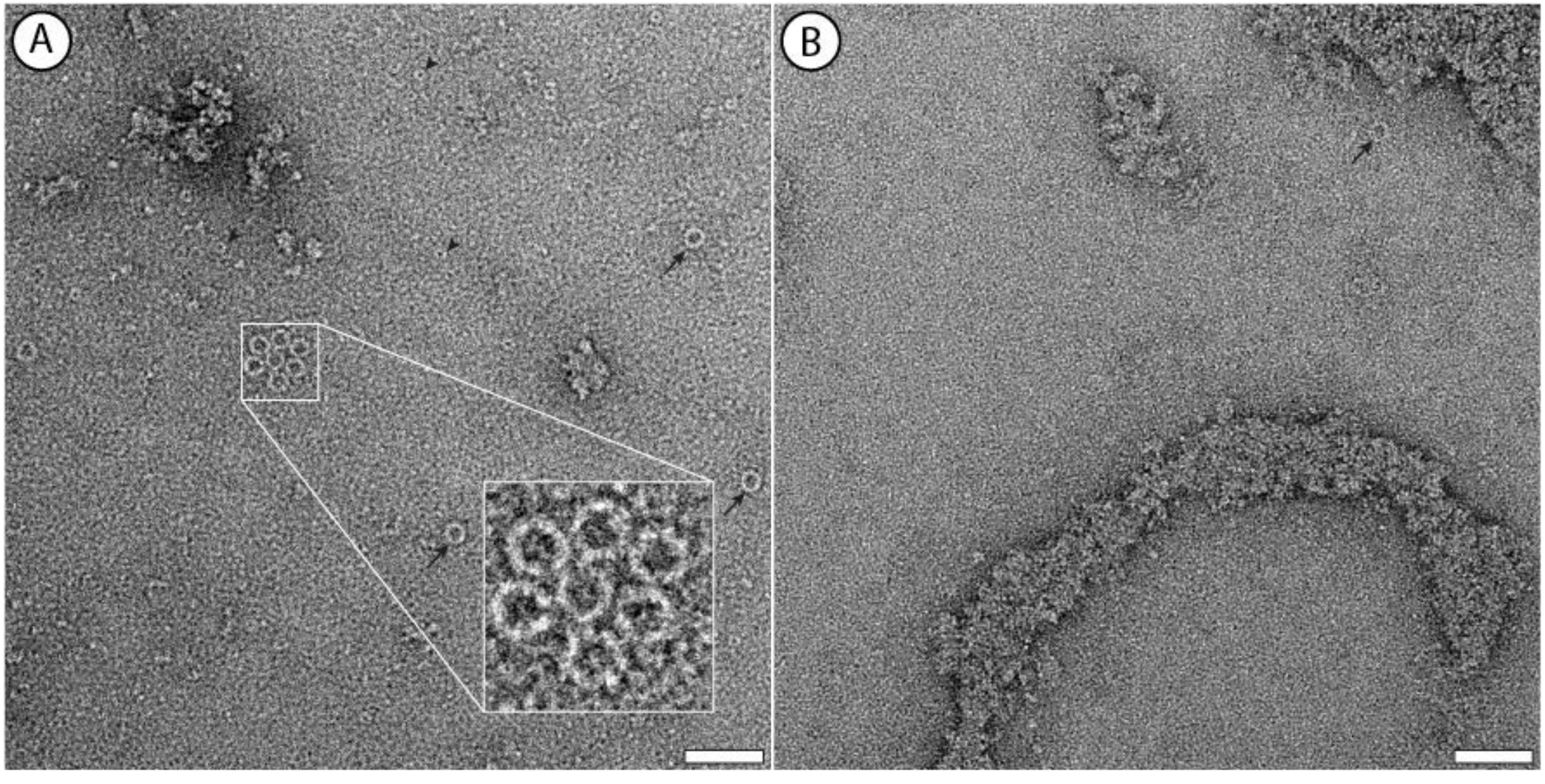


Single cell preparation

negative stain

Heat shock

Negative control



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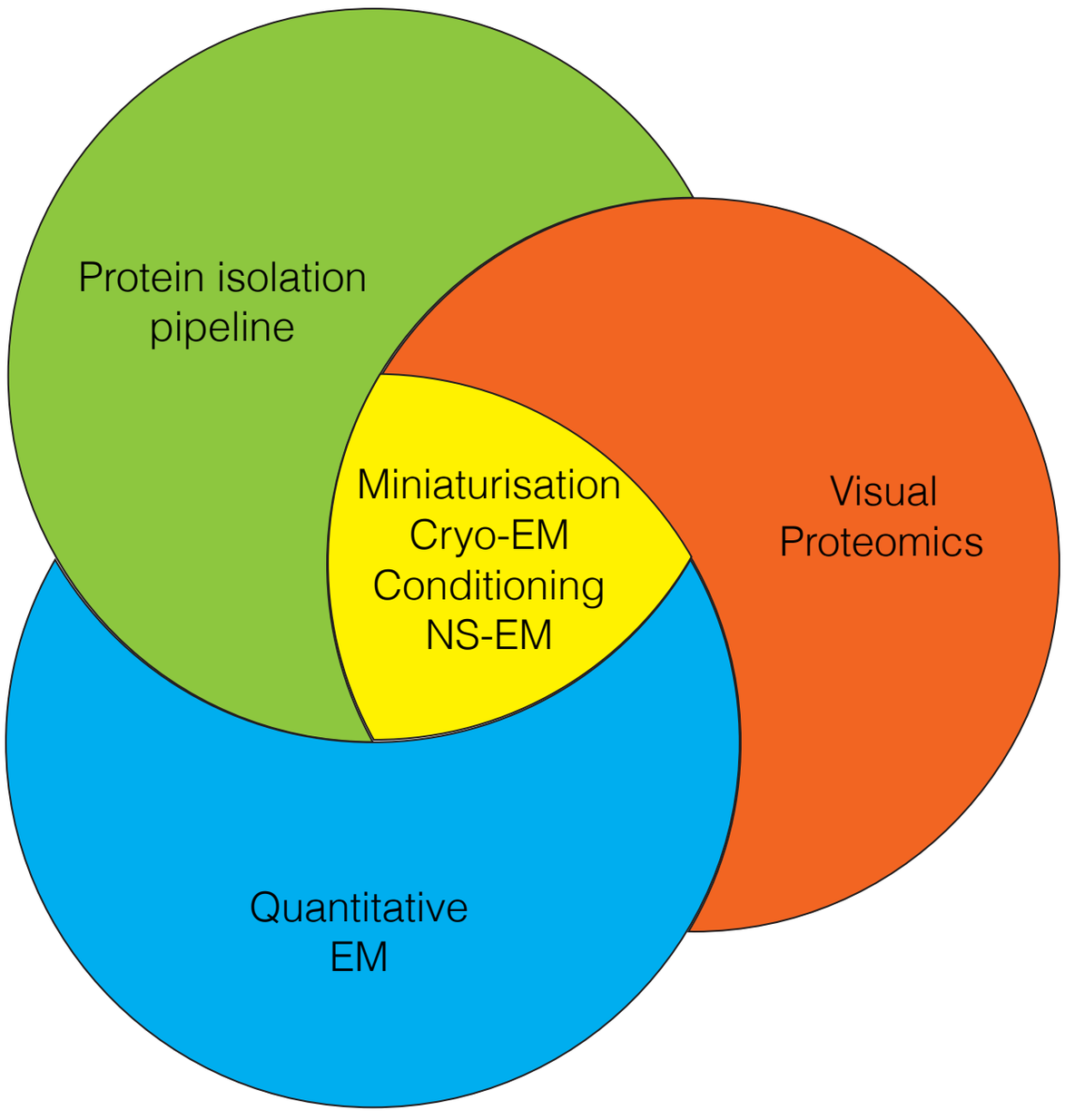
Conclusions



- Overview miniaturised sample preparation
- EM-grid preparation from nL sized volumes:
    - Cryo-EM
    - Negative stain EM
- Sample conditioning
- Protein isolation
- Interaction labelling
- Visual proteomics for quantitative EM

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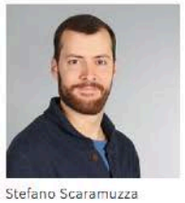
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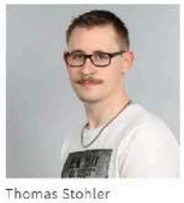
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