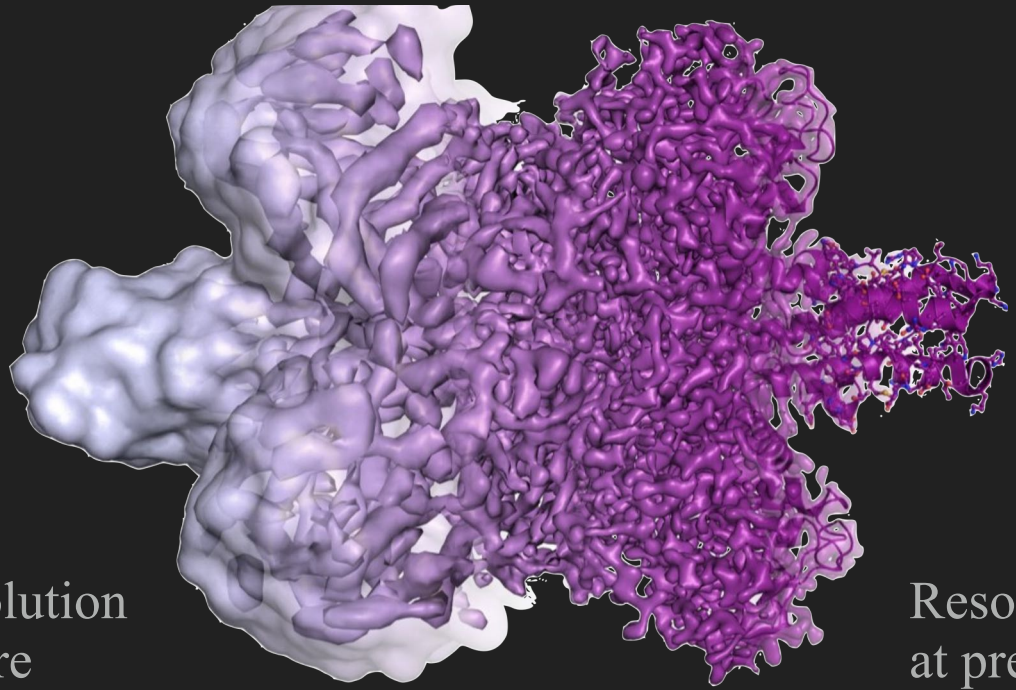
A controlled, automated cryo-EM preparation tool

LUCA RIMA, 10.09.2024

The cryoWriter

Resolution Revolution



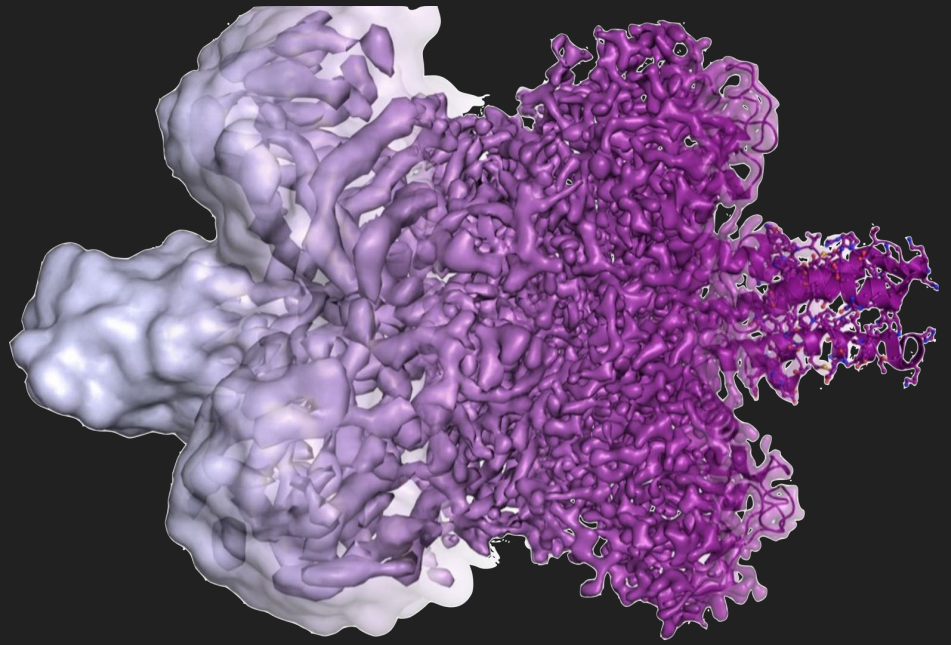
Resolution before 2013

Resolution at present

Martin Hoegbom/The Royal Swedish Academy of Sciences

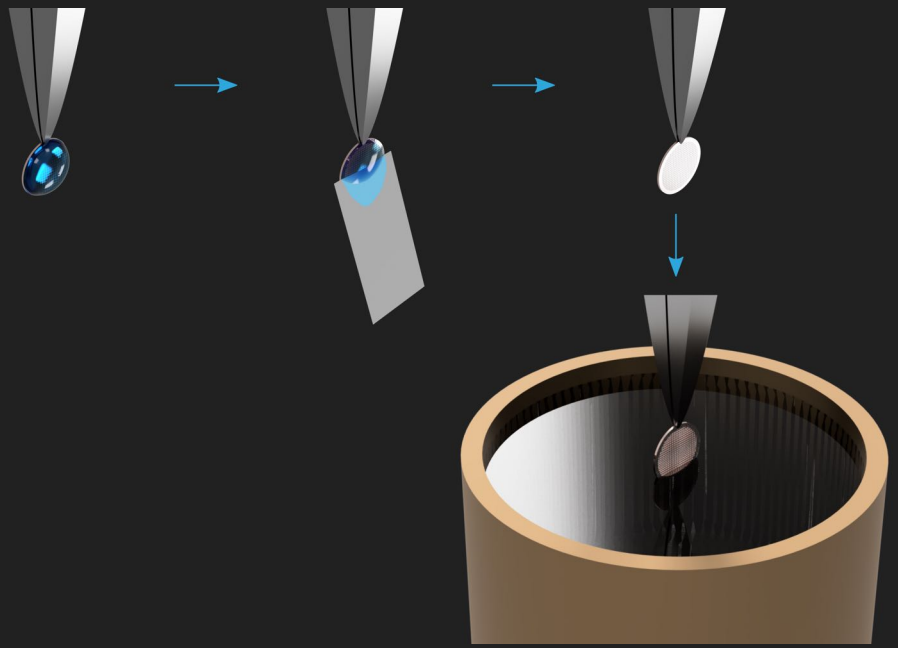
Resolution Revolution

- Drastic improvement in achievable resolution over the last 11 years
- Advances in instrumentation, electron detection as well as data processing
- Advantages over other methods (e.g. no crystallization, lower quantities, lower concentrations)
- Nobel Prize in Chemistry to J.Dubochet, R.Henderson and J.Frank in 2017
- Today: cryo-EM has become one of the most important methods for protein structure elucidation



Martin Hoegbom/The Royal Swedish Academy of Sciences

Sample Grid Preparation cryo-EM



- ✔ Sample dispensing (2-3 µl)
- ✔ Blotting / Thinning
- ✔ Plunging / Vitrification

- ✗ 99.99 % of sample lost
- ✗ Harsh treatment
- ✗ Uncontrolled evaporation
- ✗ Trial and error

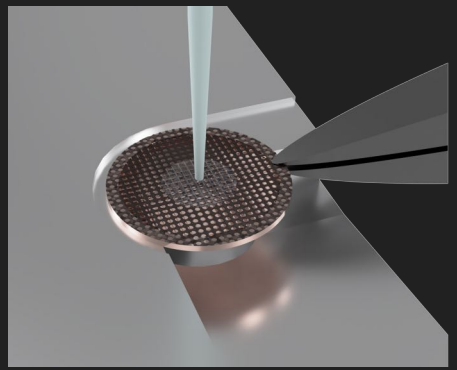
Reasons for a New Approach

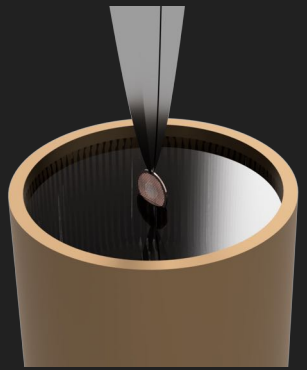
- Goal: Vitrified layer of approx. 100 nm thickness
- Required volume on a 3 mm wide sample grid → only ≈ 700 pl
  - ➔ Microfluidic systems are ideal for such small volumes

Advantages the New Approach

- Access to new, sparse samples
- Required starting volumes during protein expression and purification also become significantly smaller
- Offers the possibility of microfluidic sample purification
- Enables single cell preparation for TEM investigation

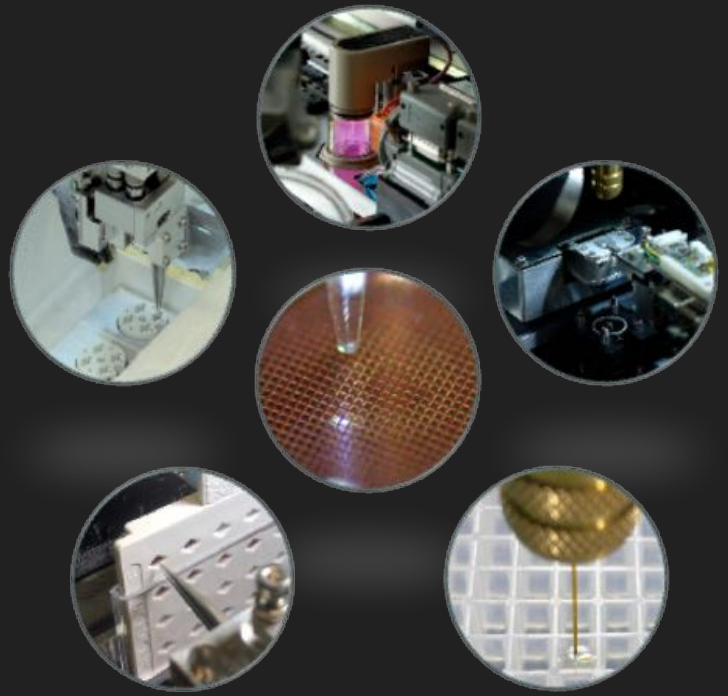
µ-fluidic Sample Preparation cryo-EM





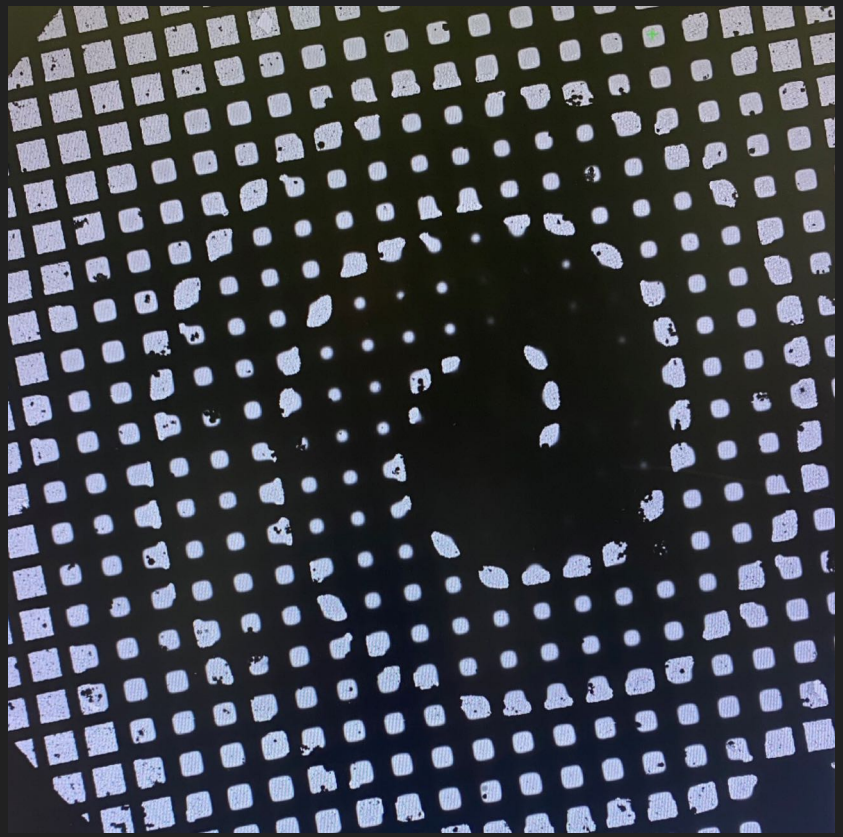
- ✔ Sample dispensing (1-3 nl)
- ✔ Plunging / Vitrification
- ✔ No paper-blotting
- ✔ Precise control of process

Next generation sample preparation robot

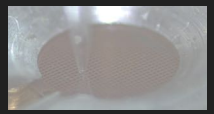
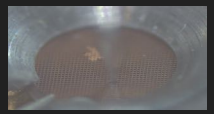
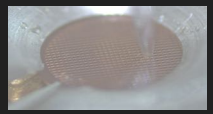
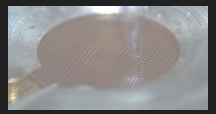
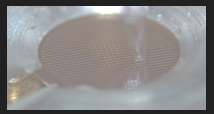


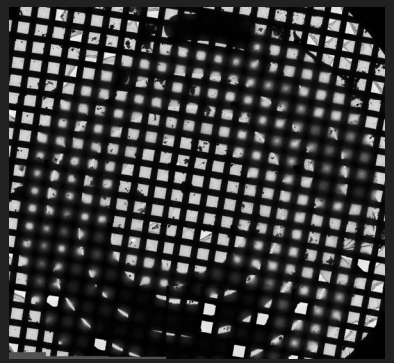
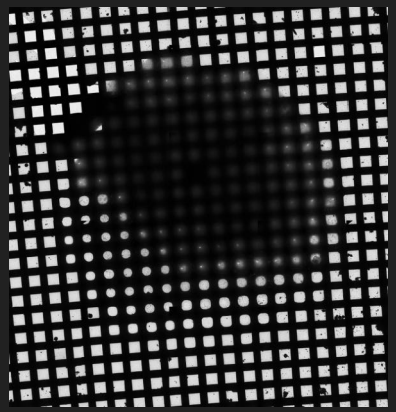
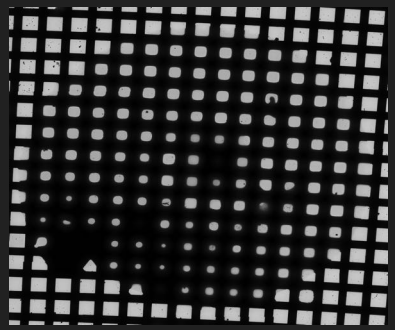
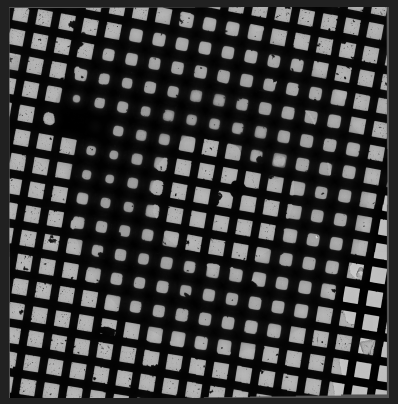
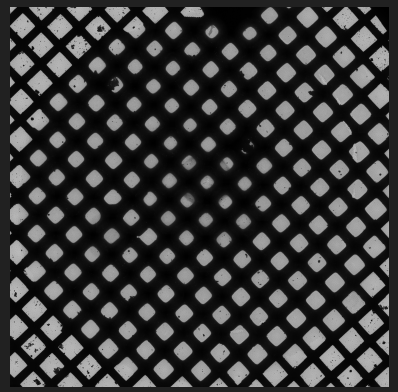
New Writing Method

Spiral on Grid



cryoWriting with µm-accuracy

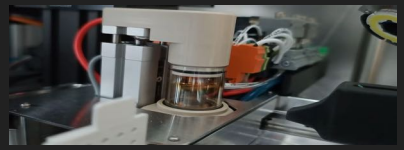






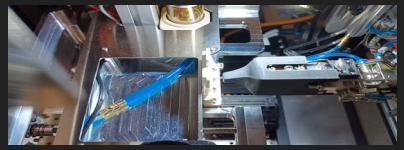
Workflow Cryo Grid Writing

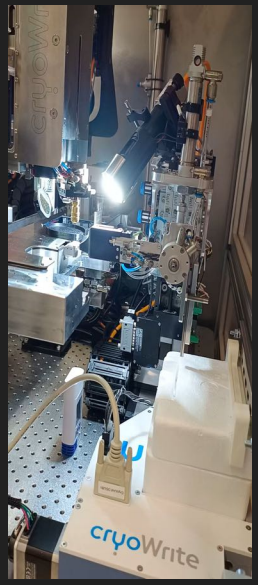
Position

Glow discharge



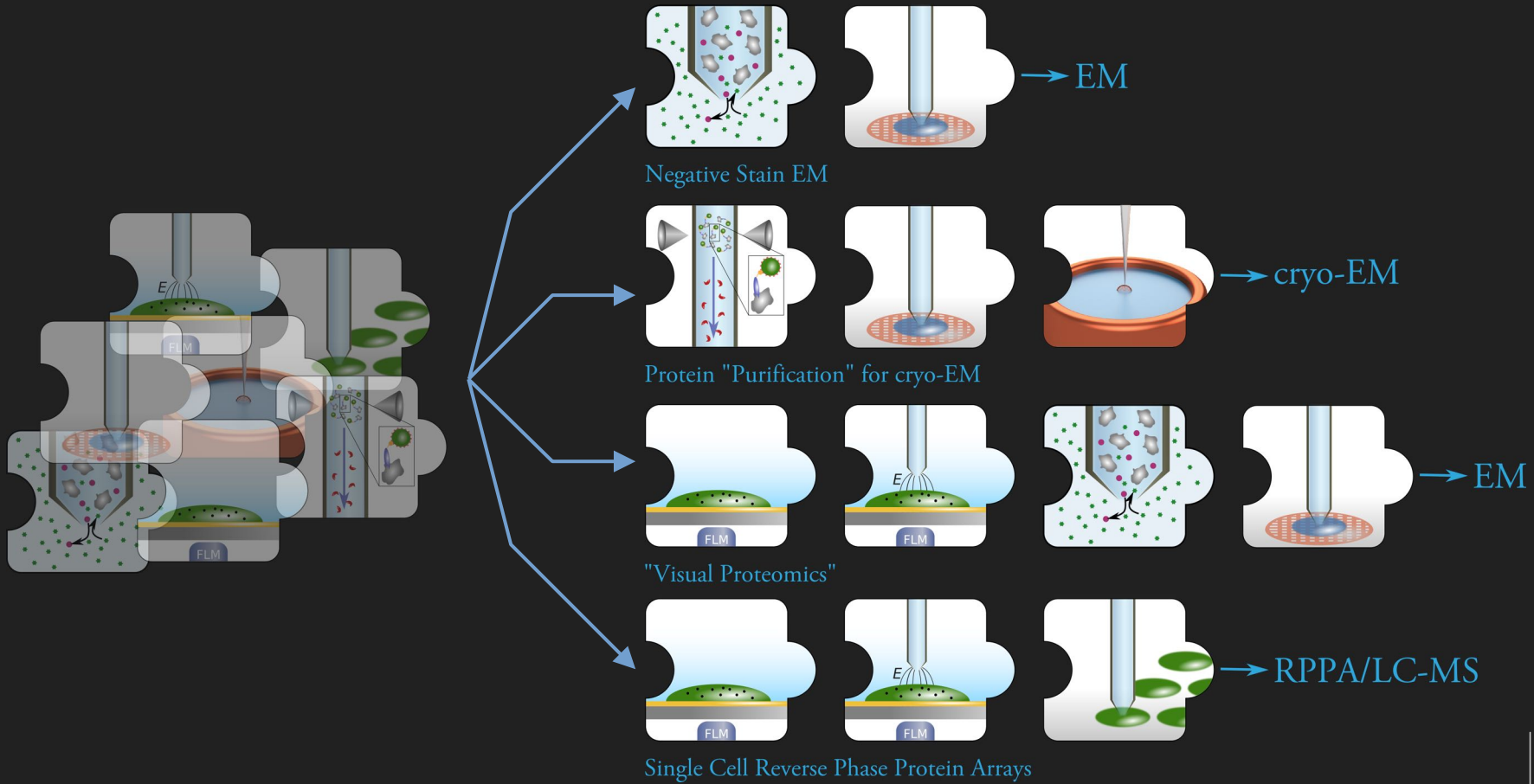
Fetch grid





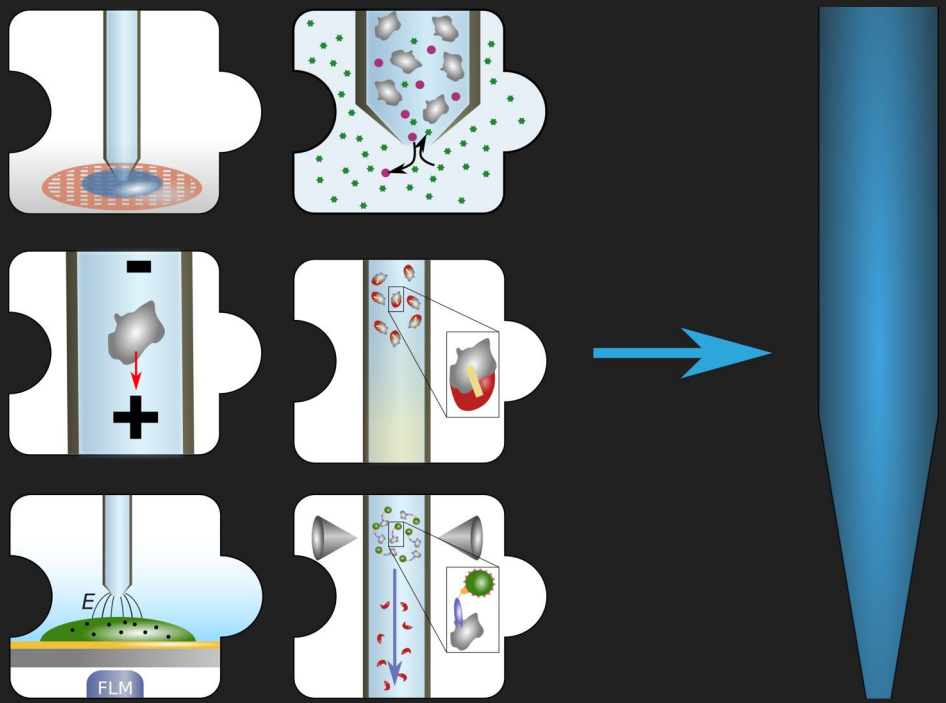
Write, plunge
and store

Modular Microfluidics: Workflows



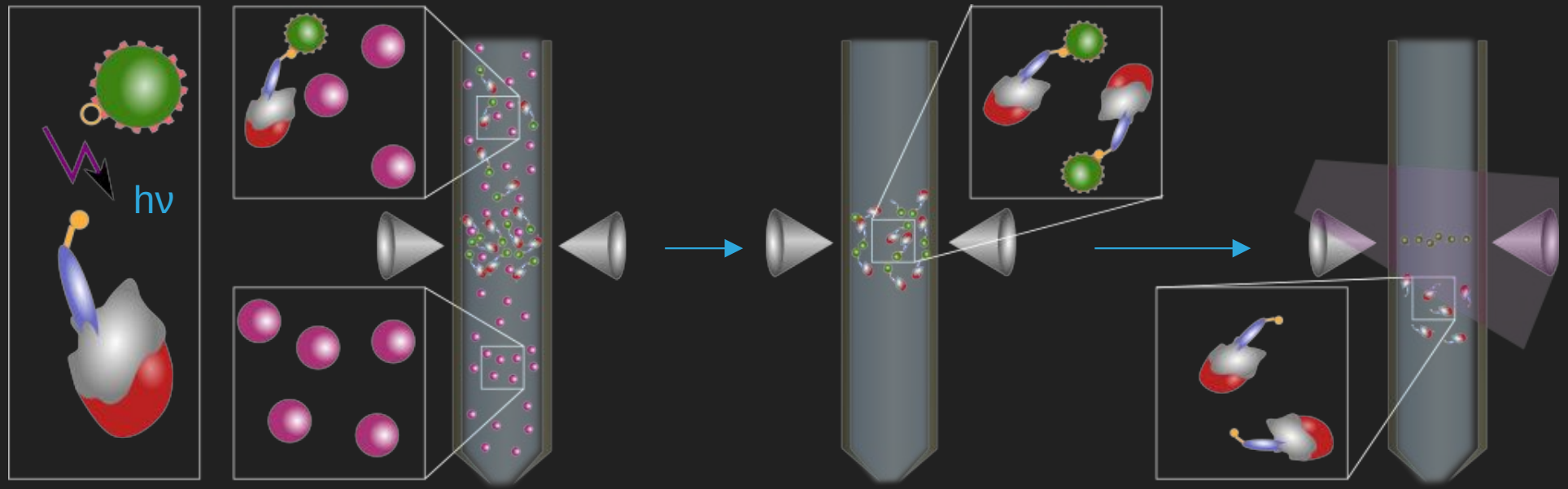
Kemmerling, 2013; Giss, 2014; Syntychaki, 2019; Arnold, 2016; Rima, 2024

Modular Microfluidics: Microcapillary Integragion



- One Nozzle
- Minimize sample-interface contacts
- Minimize loss by unspecific adsorption
- Minimize Taylor dispersion

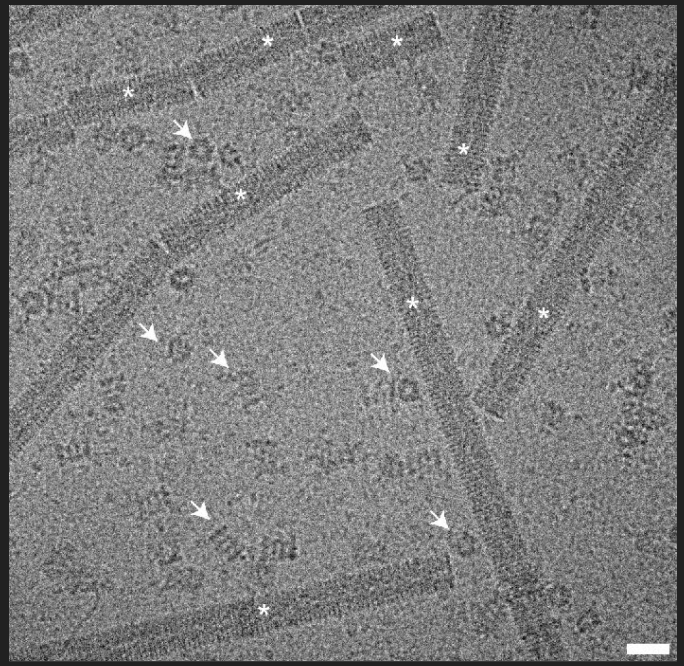
Microfluidic Protein Isolation



Giss, 2014; Schmidli, 2019

Structural Analysis of Human Proteasome 20S

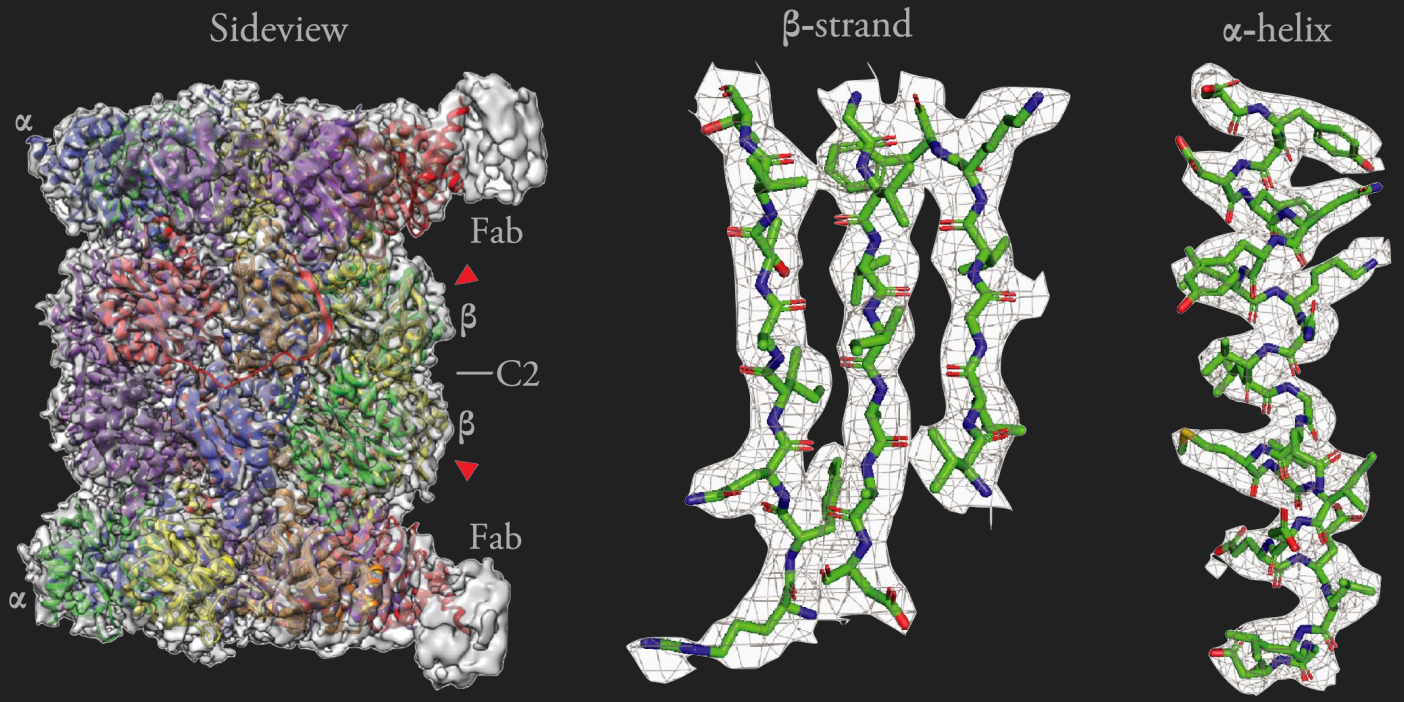
- <900 nL HeLa cell lysate
- Fab-fragments
- Human Proteasome 20S: 14 different subunits
- Tobacco mosaic virus (TMV) added (“Resolution control”)



100 nm

Schmidli et al., 2019

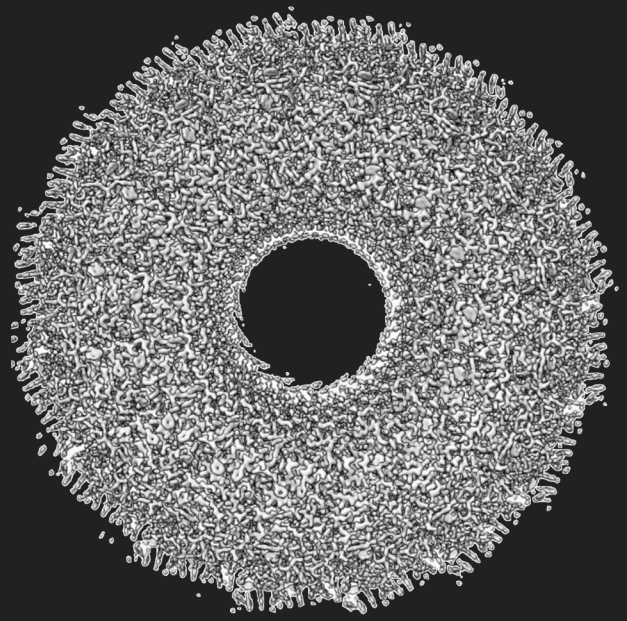
Human Proteasome 20S at 3.5 Å



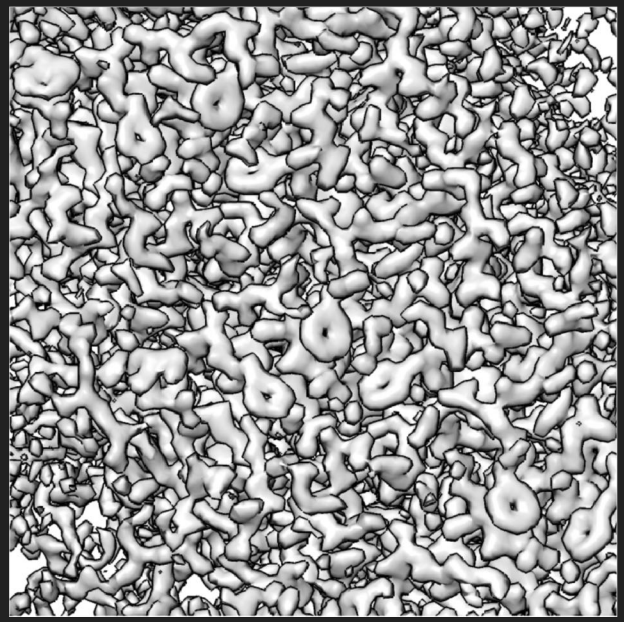
Schmidli et al., 2019

TMV at 1.9 Å

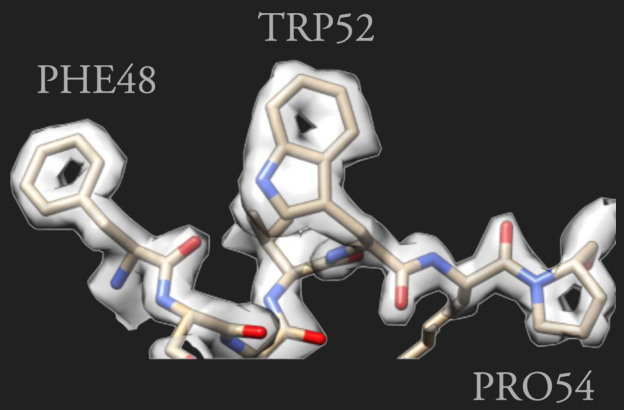
Overview



Zoom-in



Aromatic amino acids



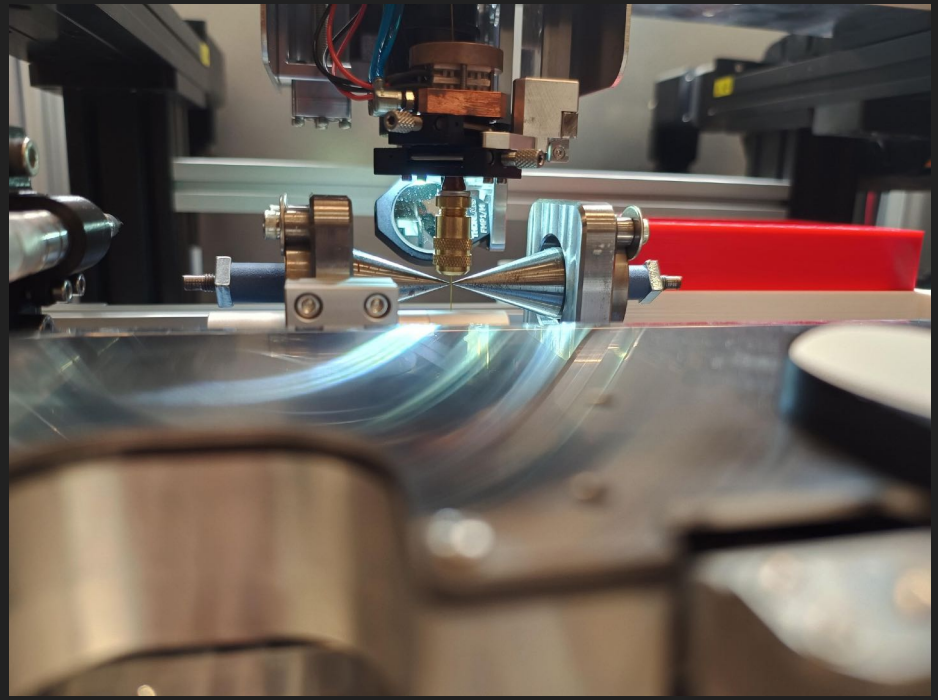
Schmidli et al., 2019

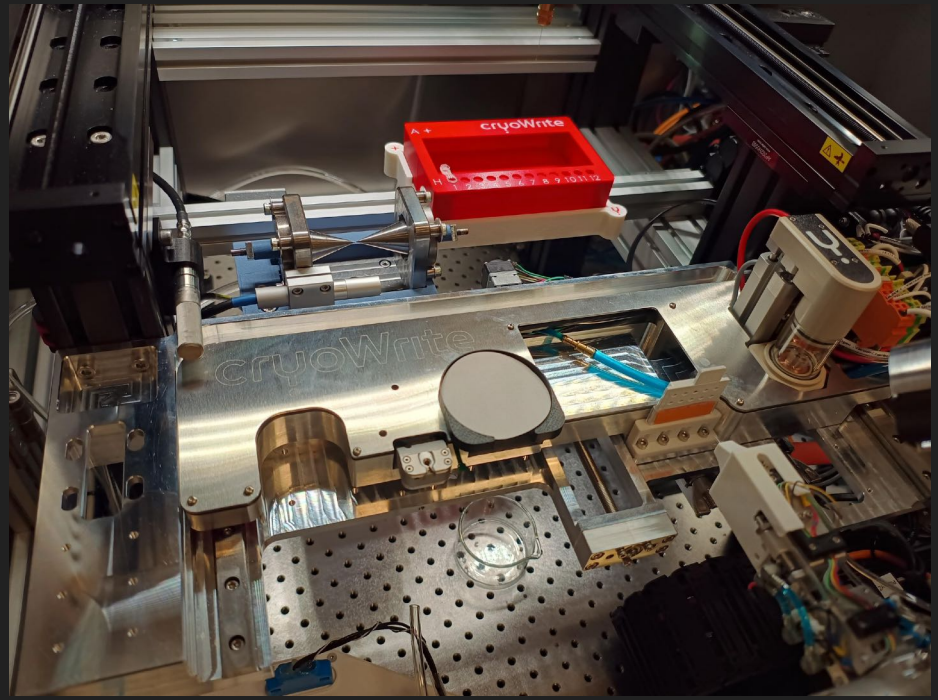
Advantages of Microfluidic Protein Isolation

Standard procedure		Microfluidic approach
Start with (milli)liters of cell lysate and milligrams of proteins	⟷	Start with microliters of cell lysate and micrograms of proteins
Rather harsh procedure that involves many steps	⟷	More gentle, as it consists of one main purification step
Days	⟷	Hours

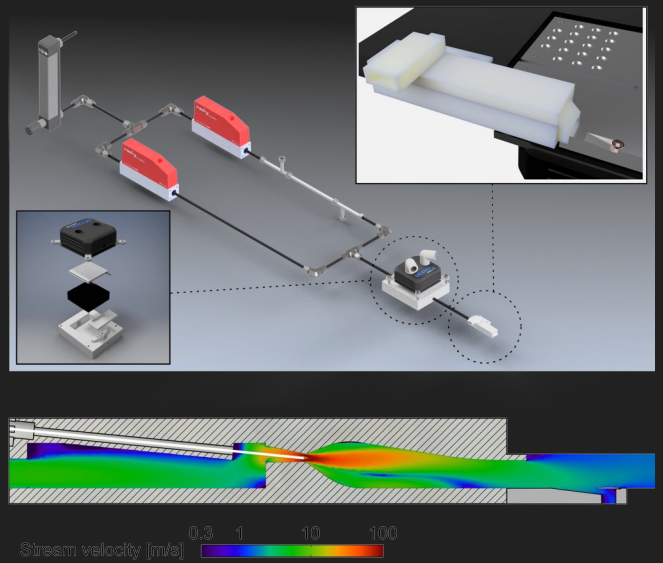
- **Allows working with lower yields or even endogenous proteins**
- **Offers the possibility to address difficult target proteins**
- **No protein engineering required to increase yield or to develop expression and purification methods**
- **Get the structures faster!**

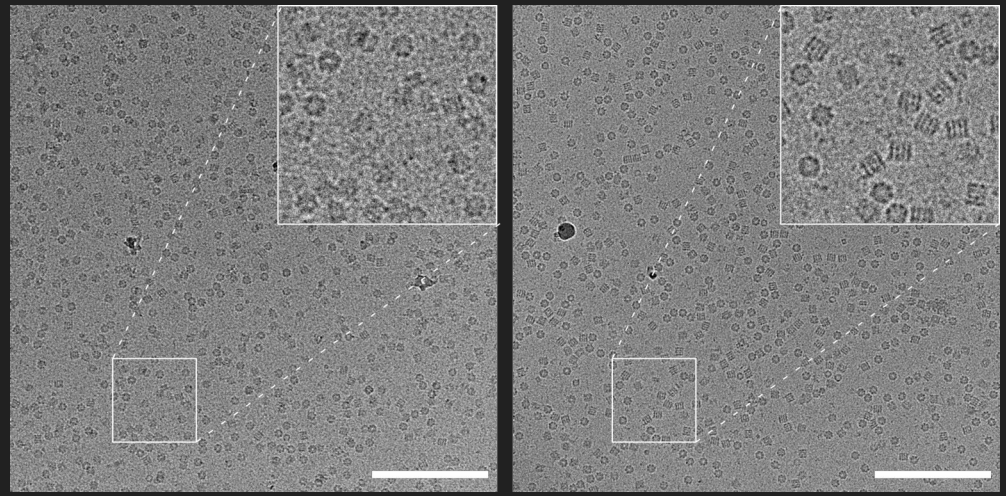
cryoWriter – Interior





Climate Jet and Cover-slip Injector





Rima et al., 2022

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