Labeling particles with heavy atom clusters

Brian Gibbons
Kornberg Lab
Stanford

- Localization / identification
  - Tissue / cells
  - Tomograms
  - Cryo-EM
  - Negative stain

Alignment - HEAVEM Proposal

Proc. Natl. Acad. Sci. USA Vol. 95, pp. 9262–9267, August 1998 Biophysics

Single-particle selection and alignment with heavy atom cluster-antibody conjugates

GRANT J. JENSEN AND ROGER D. KORNBERG*

- (i) Synthesis of appropriate organothiolate monolayer-protected gold clusters (MPCs)
- (ii) selection, generation and labeling of adaptor molecules
  - linkers between MPCs and targeted particles
- (iii) labeling of particles, removal of excess label
- (iv) data collection and processing.

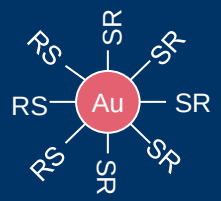
Maia Azubel

Monolayer Protected Cluster (MPC) synthesis

$$Au(III) + 3RSH \rightarrow (-Au(I)/SR-)_n + RS\text{-}SR$$

$$(-Au(I)/SR-)_n + BH_4^- \rightarrow Au_x(SR)_y$$

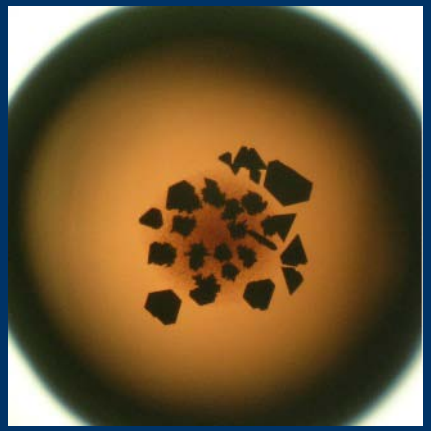
Parameters:
Ligand type
Ligand to gold ratio
Amount of BH_4^-



Maia Azubel

Organothiolate monolayer-protected gold culsters (MPCs)

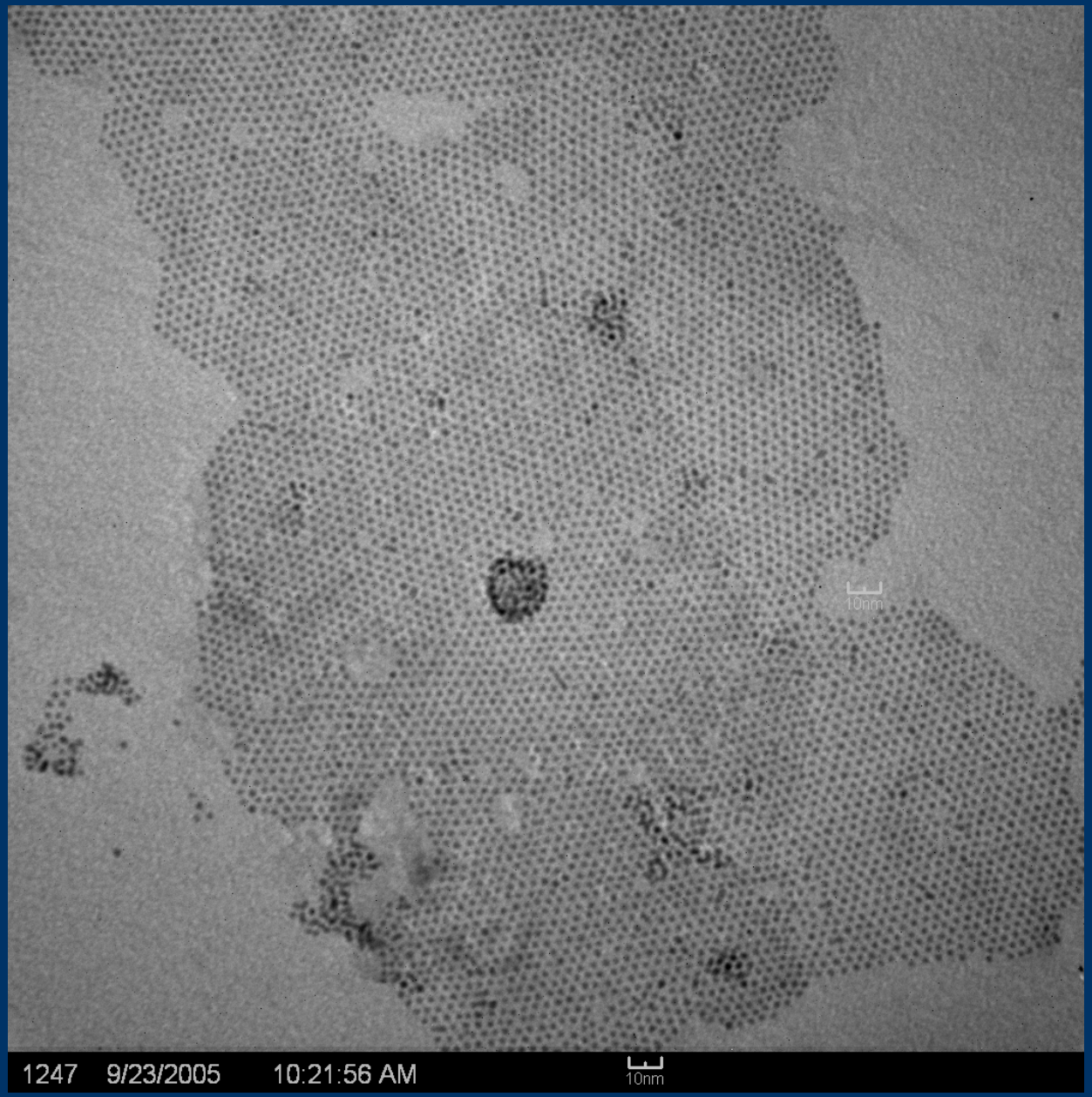
3MBA/Au 3



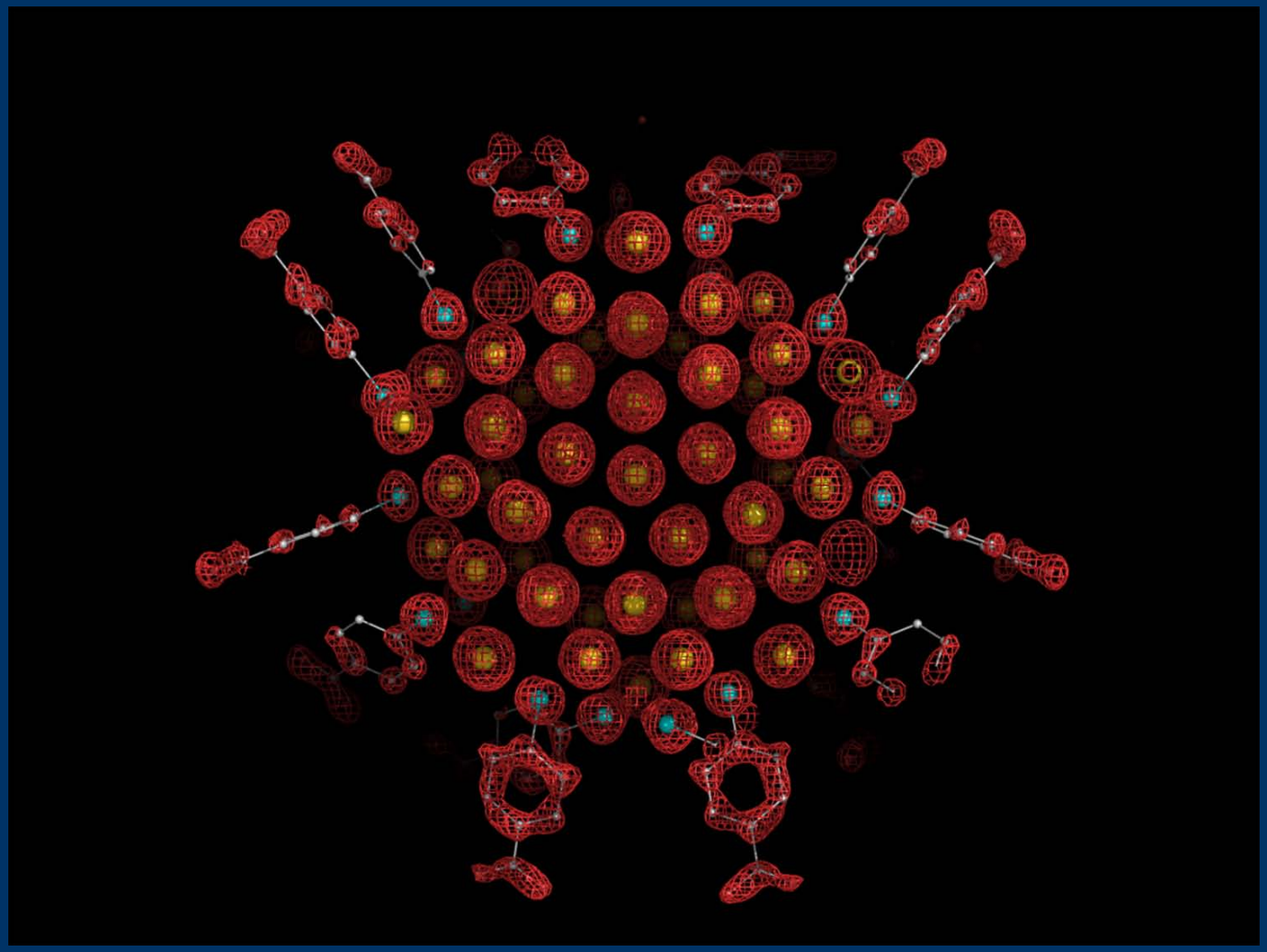
- Stable
- Water soluble
- Reactive (on - off)

Uniformity of MPCs as synthesized

Chris Ackerson

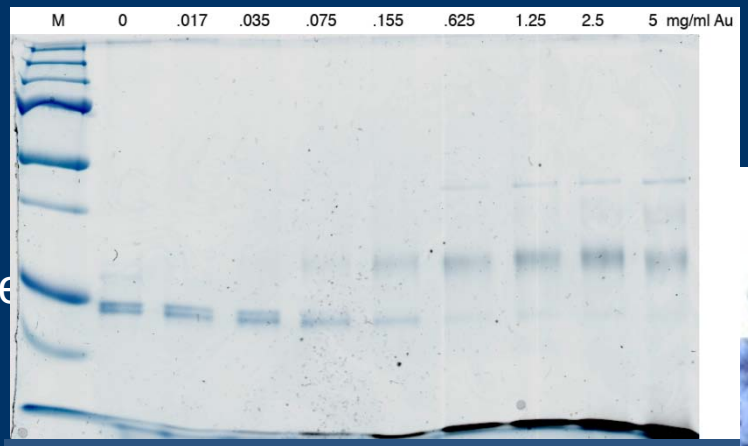


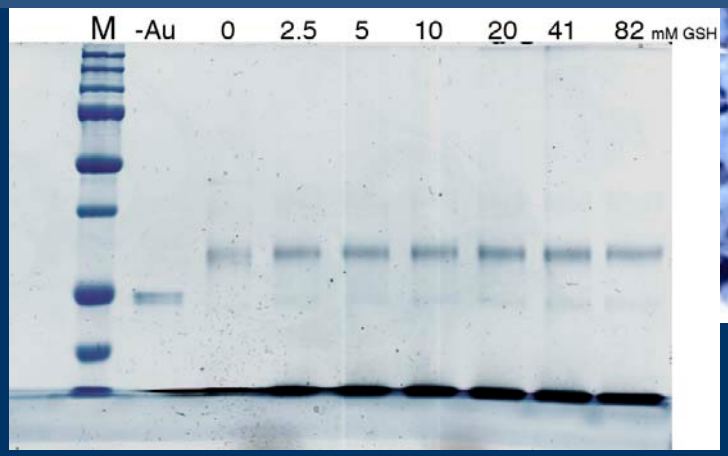
P.D. Jadzinski, G. Calero, C.J. Ackerson, D.A. Bushnell and R.D. Kornberg, *Science* **318**, 430 (2007).



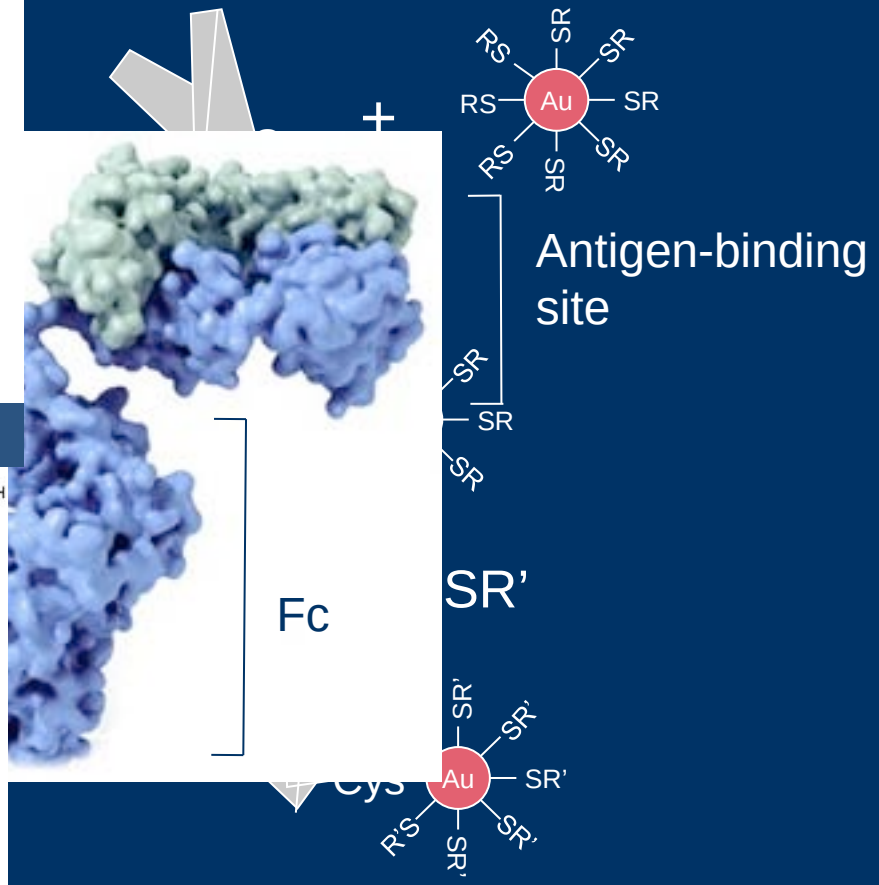
$Au_{102}(p\text{-MBA})_{44}$ at 1.1 Å

Adaptor molecule





Antige
site



Antigen-binding
site

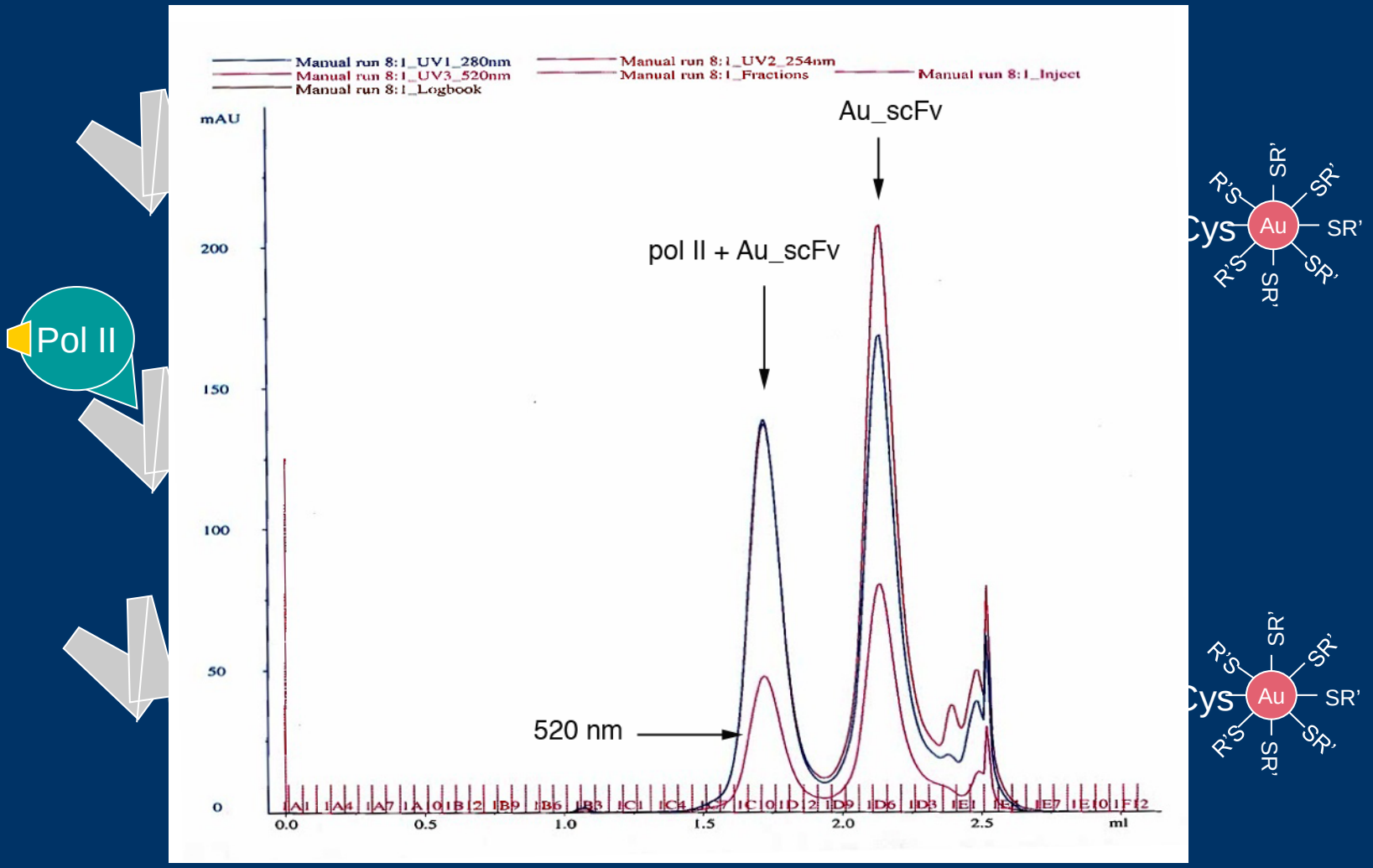
Murray place exchange

Adaptor molecule

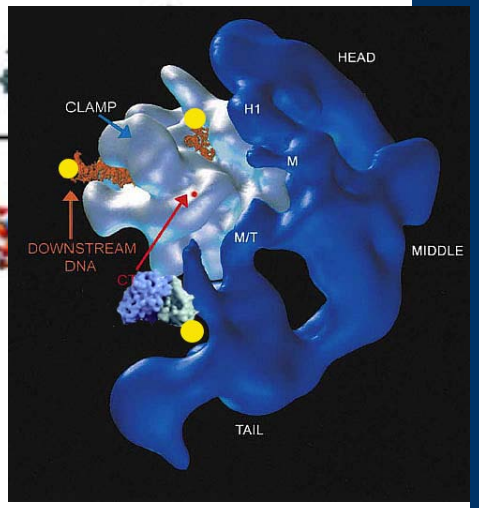
Maia Azubel

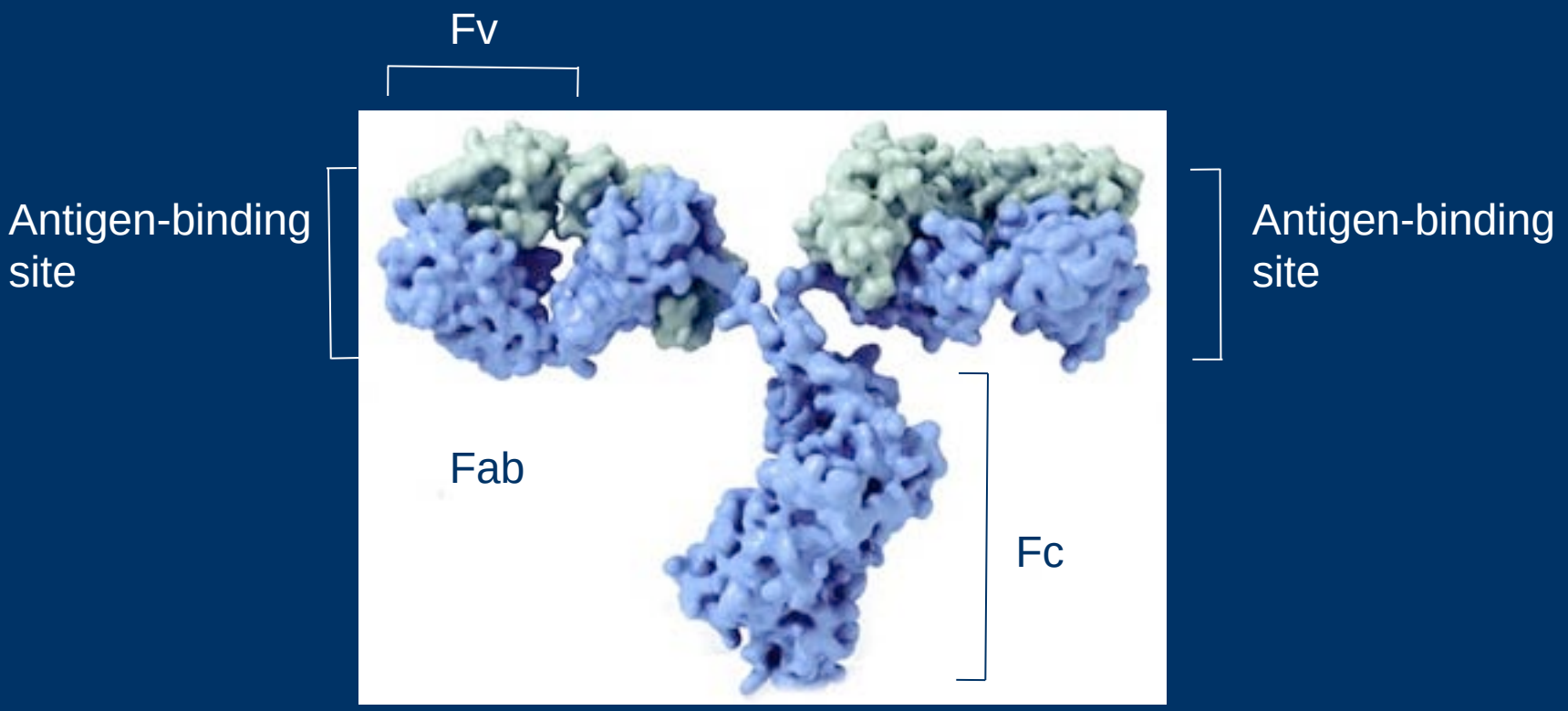
Maia Azubel

Complex formation and purification



Maia Azubel

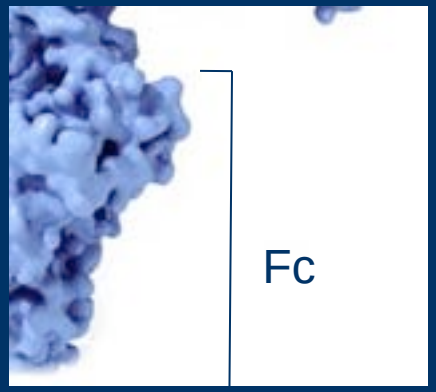



Fv
Antigen-binding site
Antigen-binding site
Fab
Fc

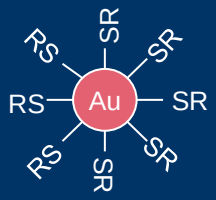
trypsin

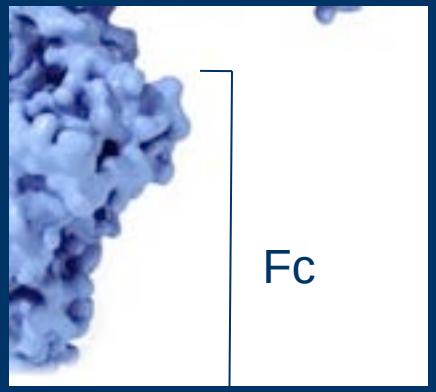
TCEP

HS-

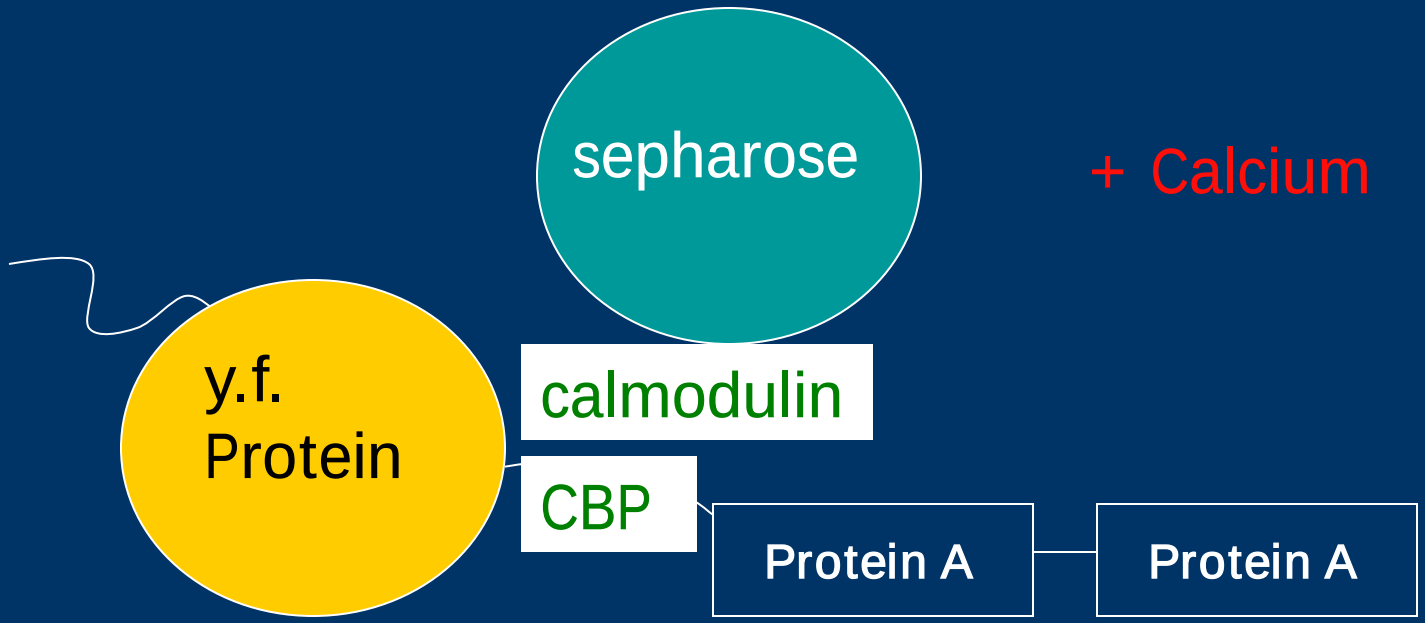


Fc-Au

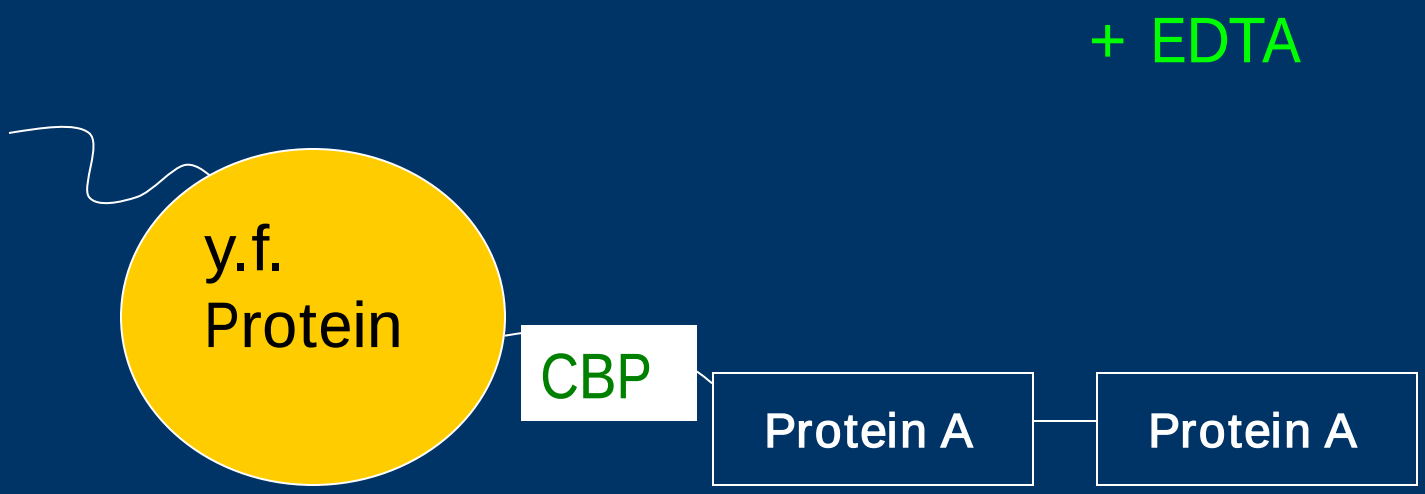




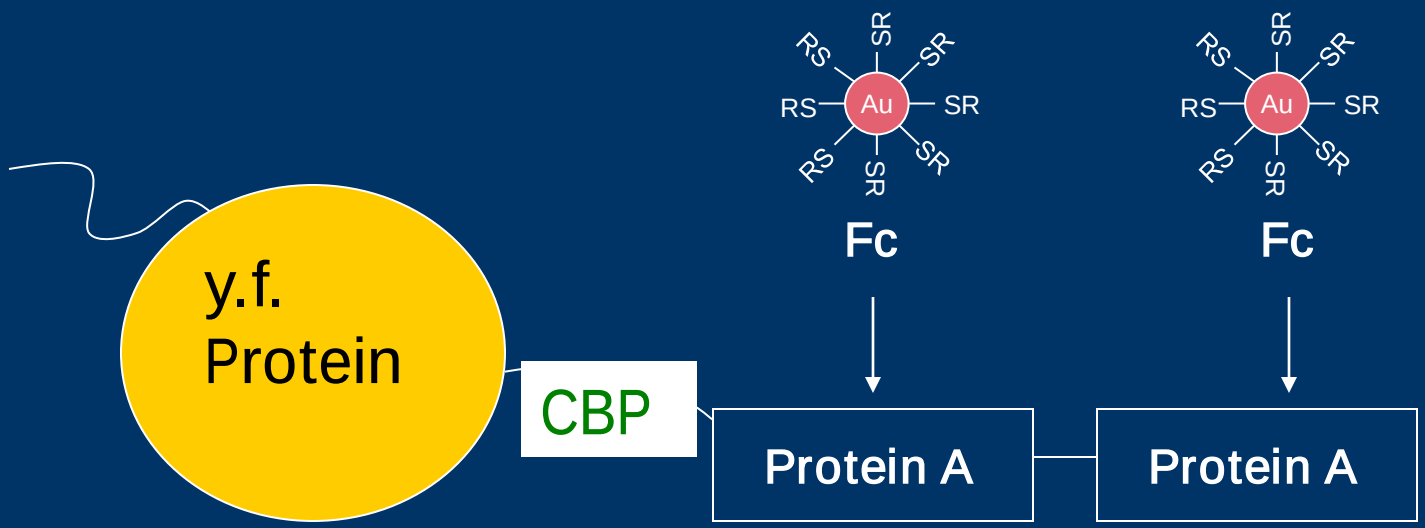
CBP affinity purification



CBP affinity purification

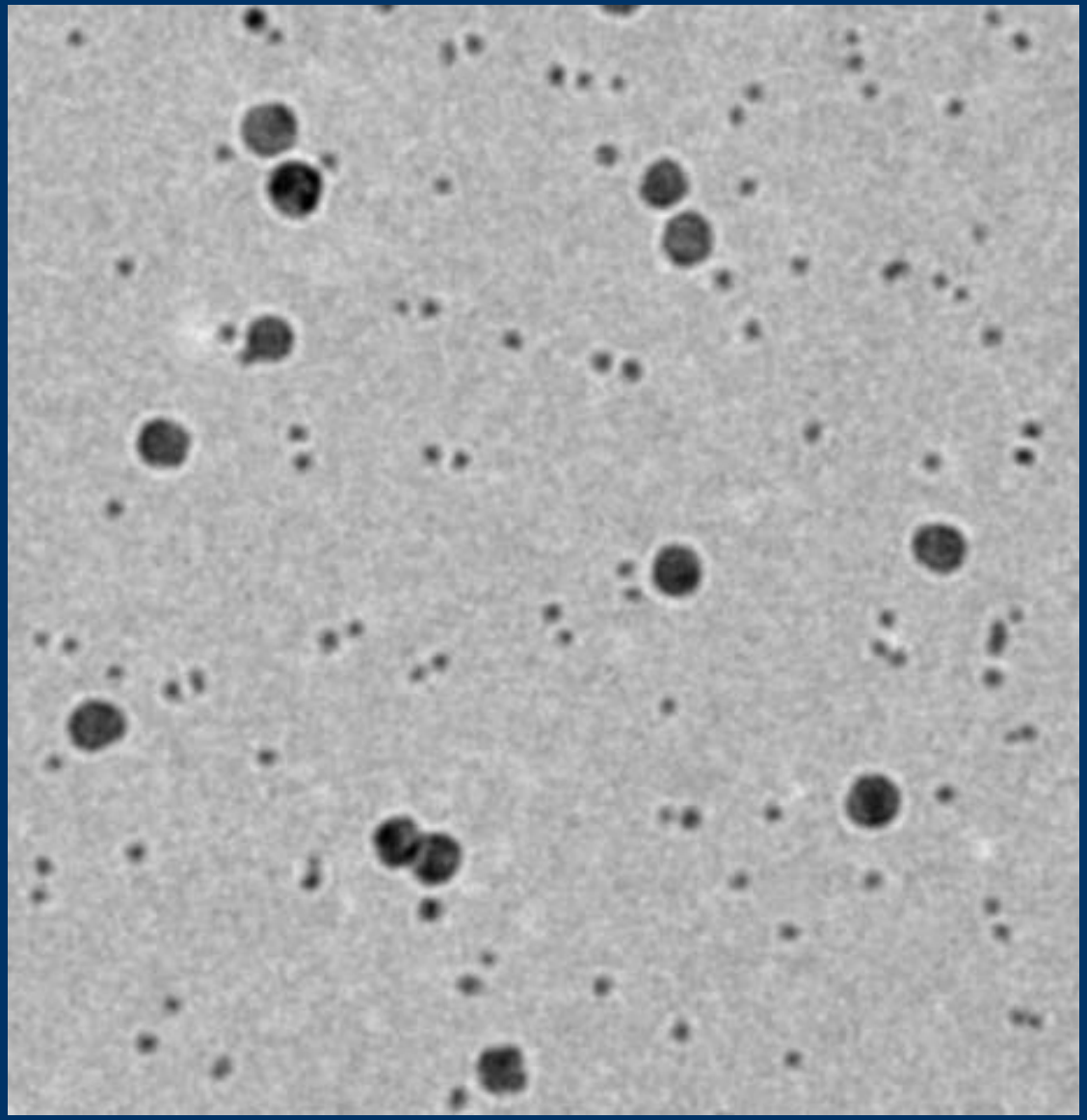


TAP tag - Fc interaction

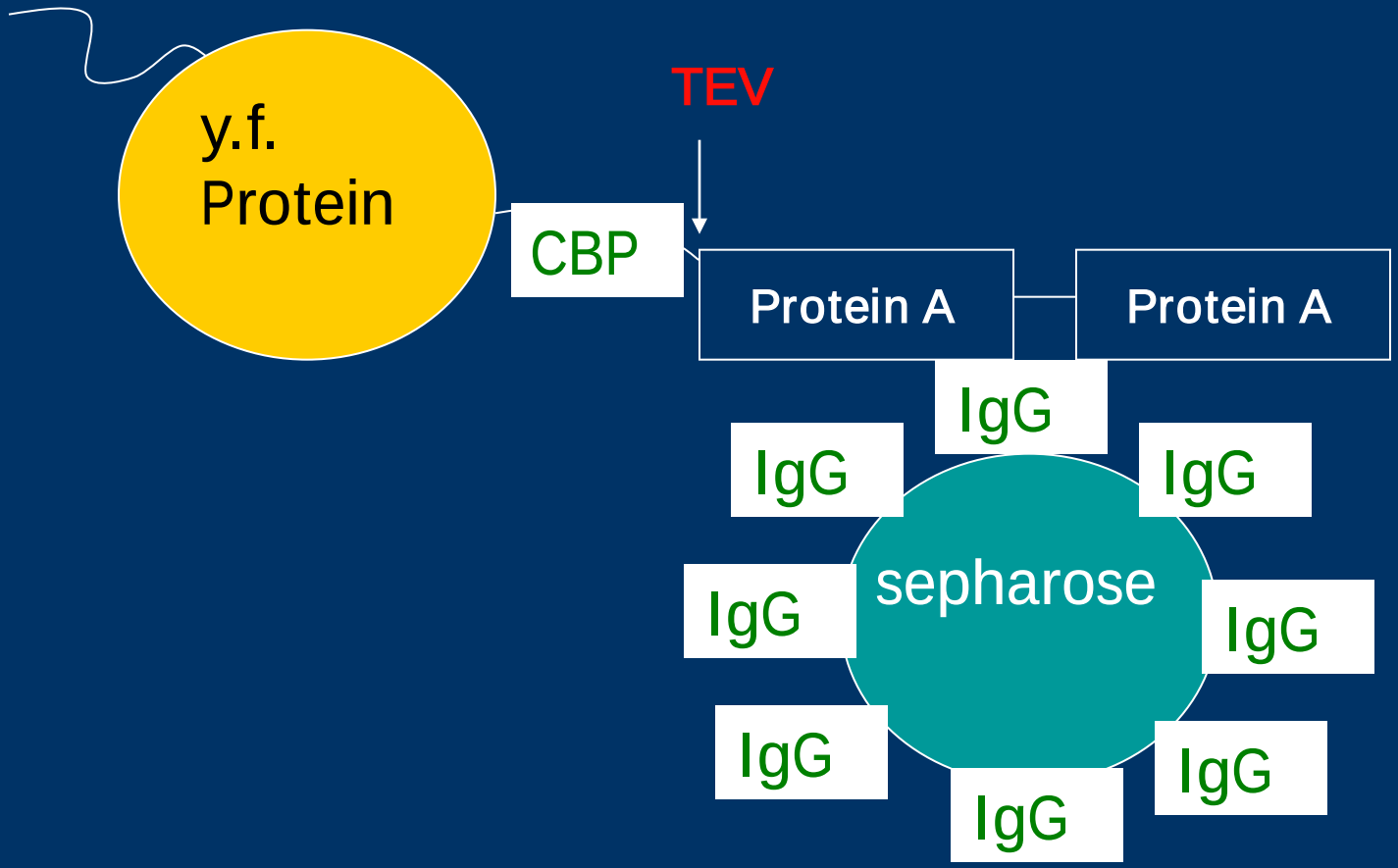


Chris Ackerson

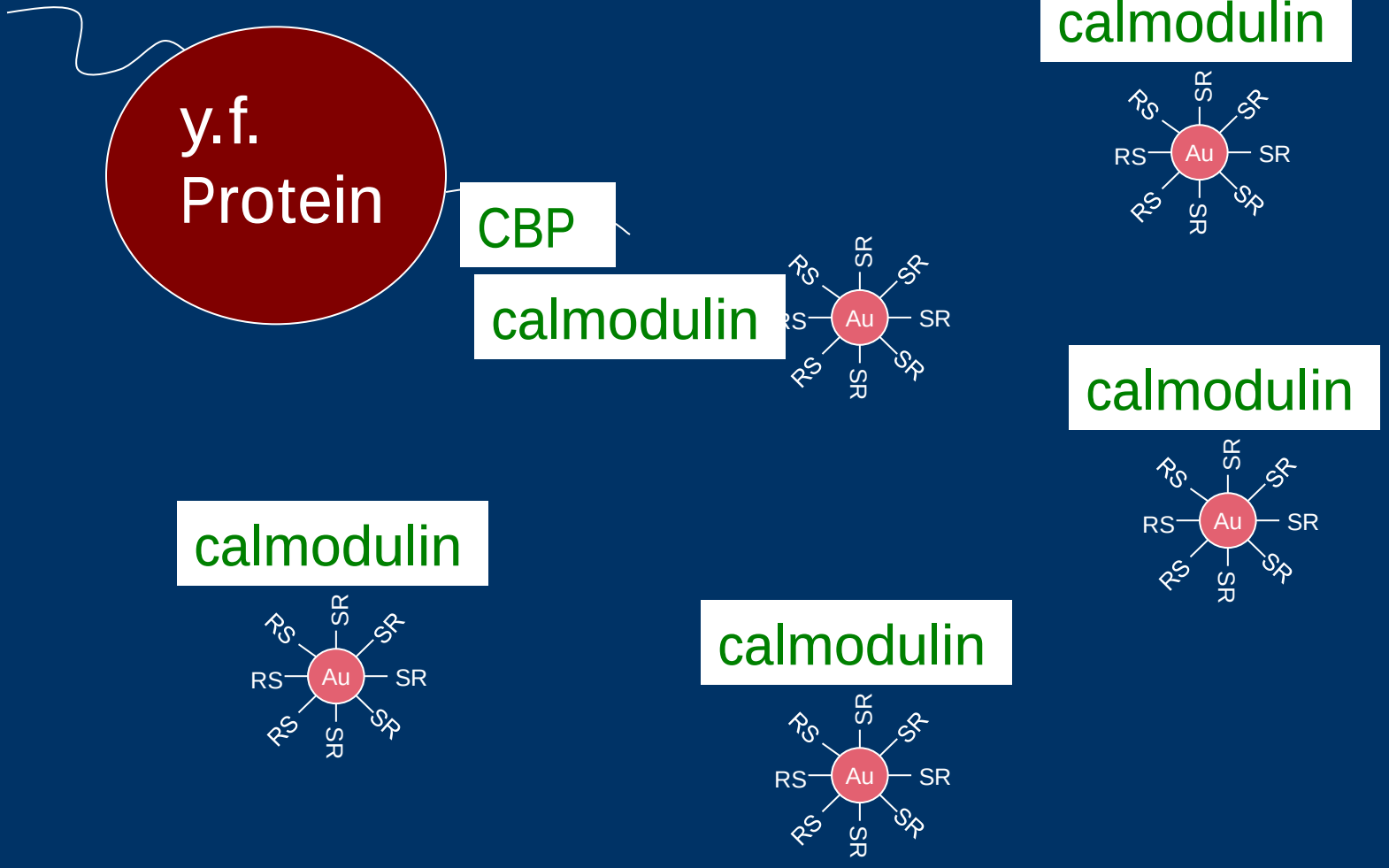
Cryo-EM of TAP- RNA polymerase II - F_c - gold



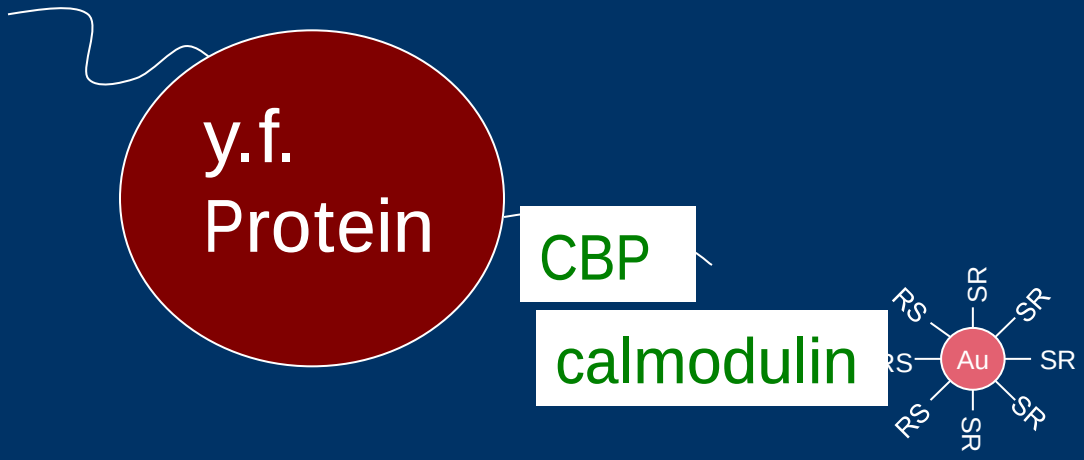
IgG affinity purification



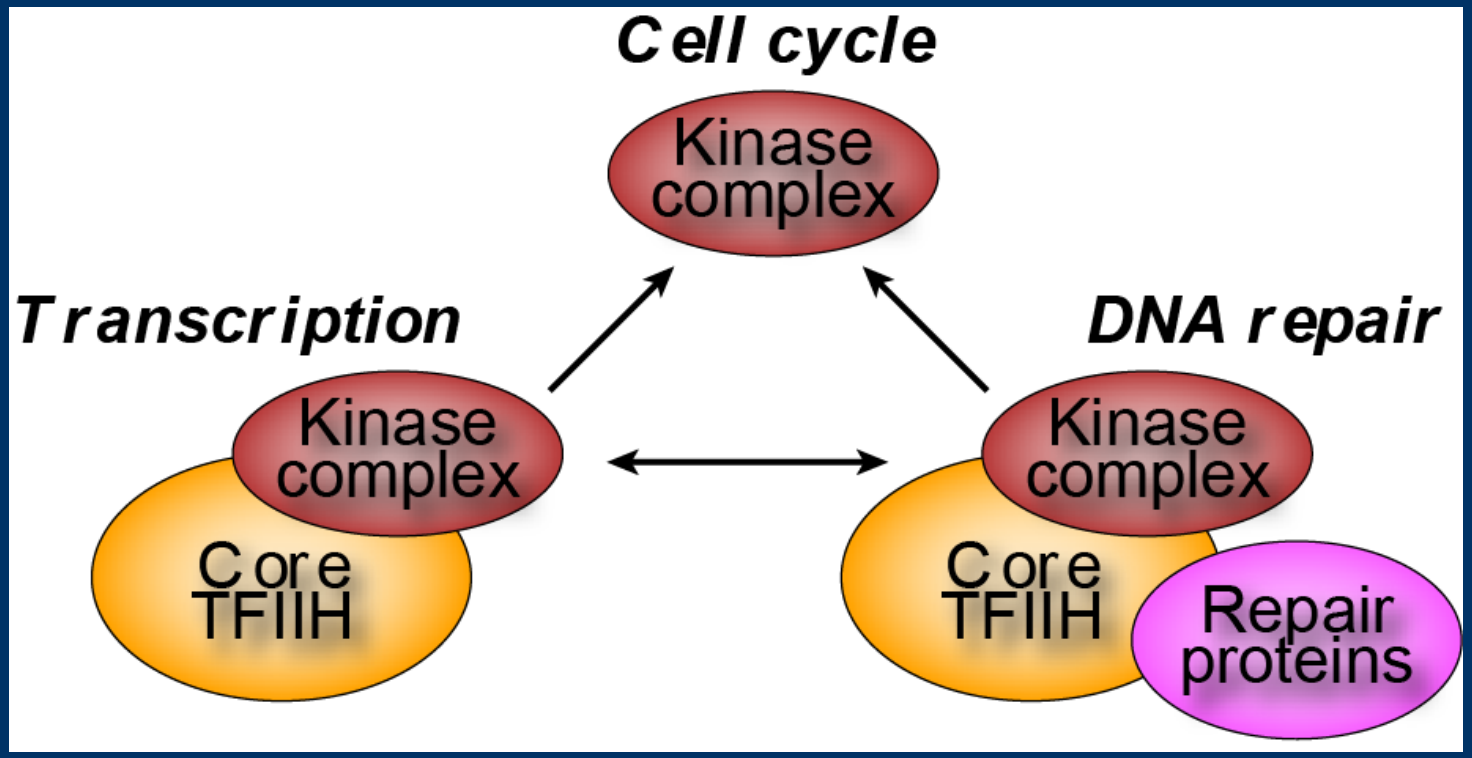
Label with calmodulin-Au



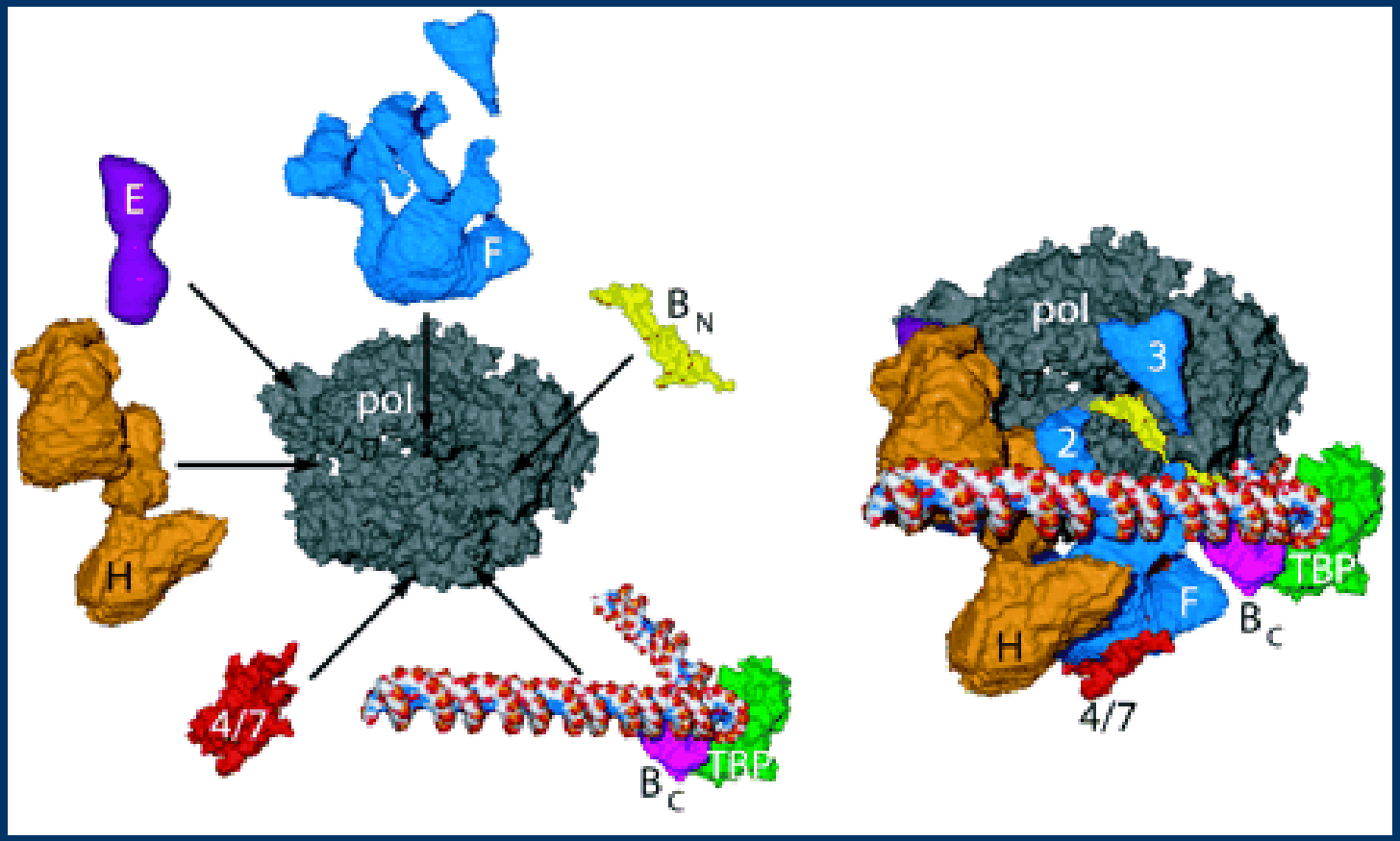
SEC or density gradient



TFIIH is essential for transcription and DNA repair

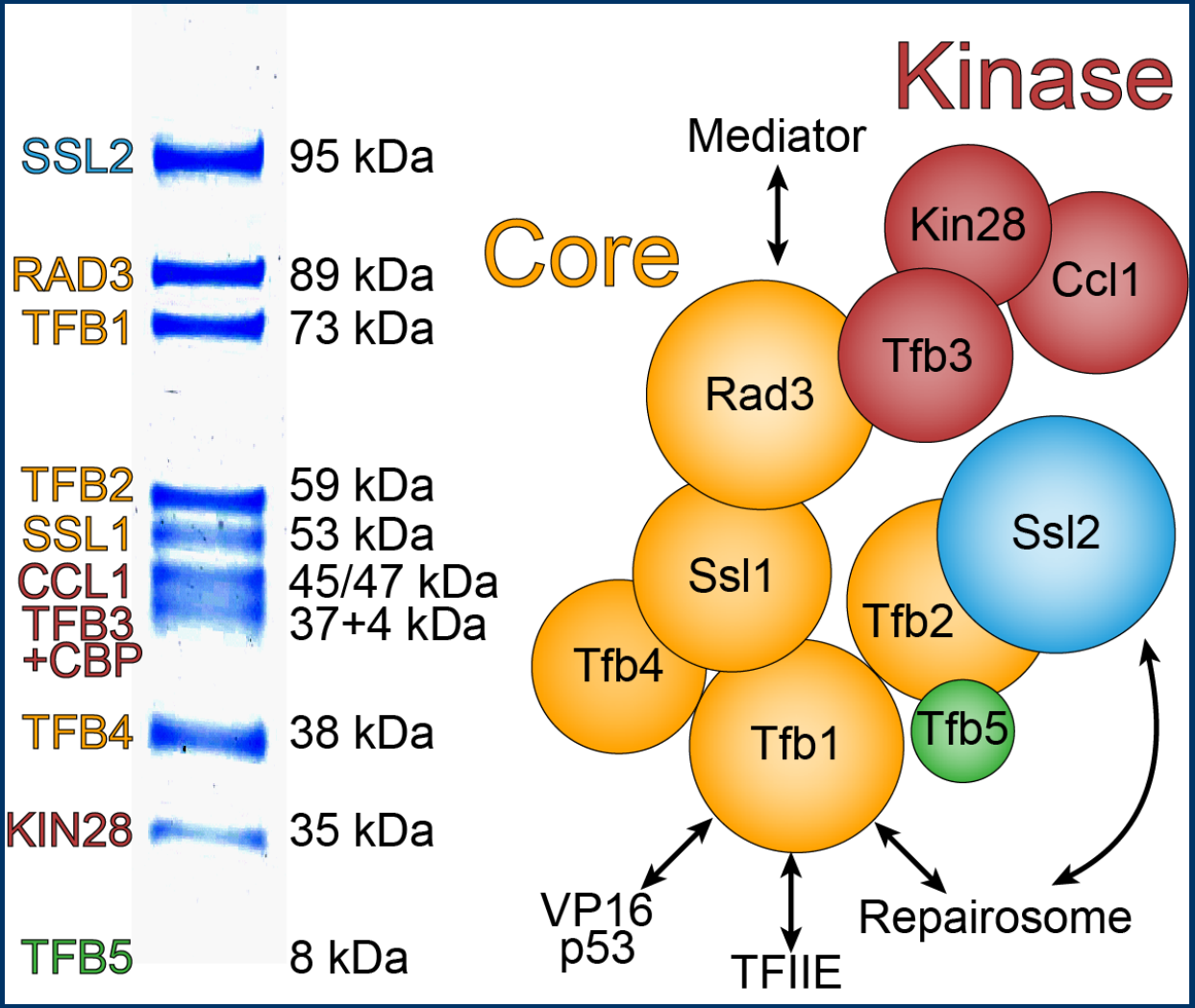


RNA Polymerase II pre-initiation complexes



Bushnell et al. 2004

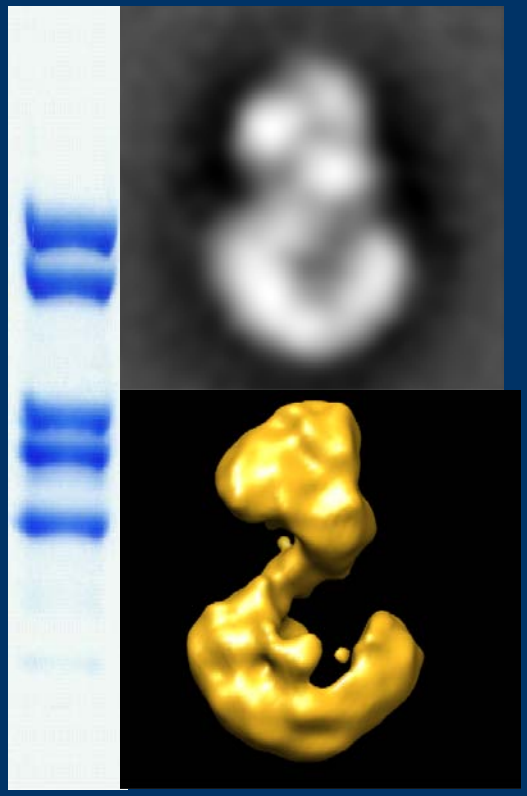
TFIIH holoenzyme – 10 subunits

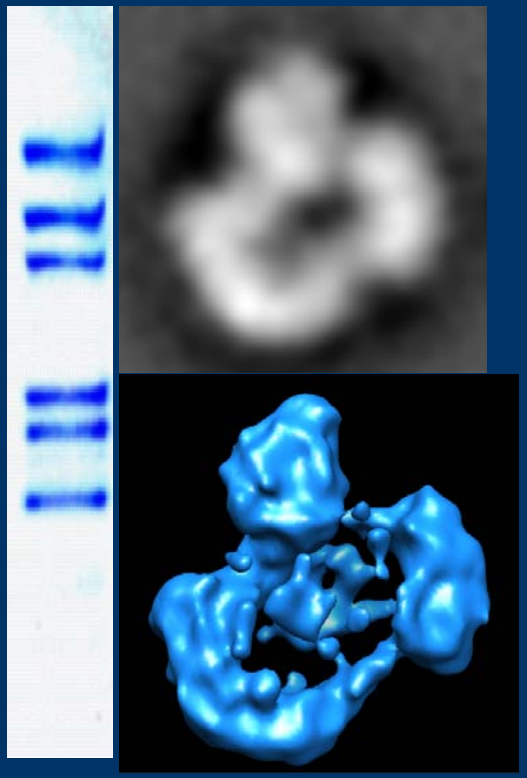


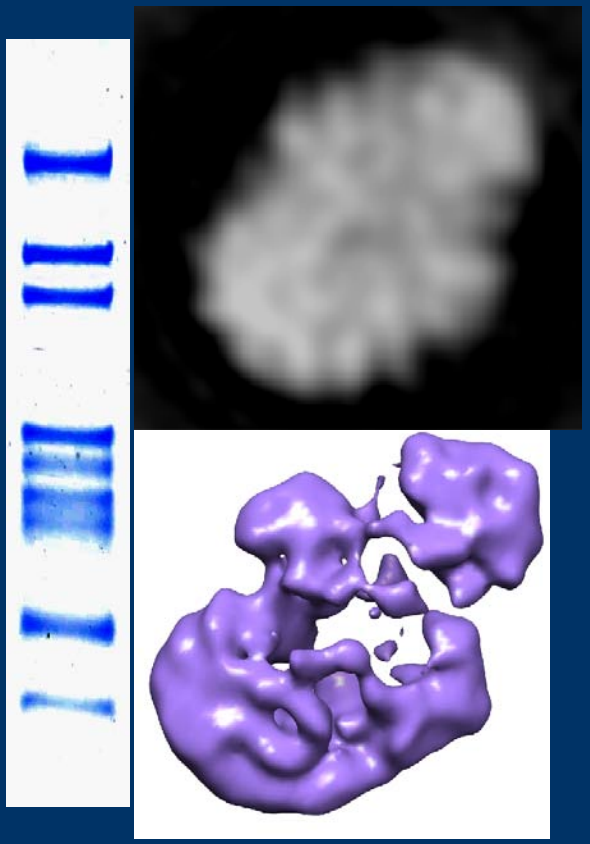
core

core + Ssl2

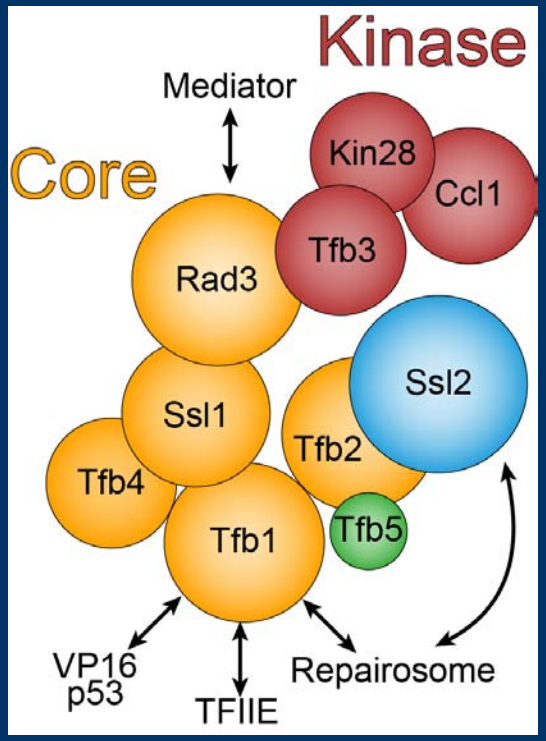
holo TFIIH

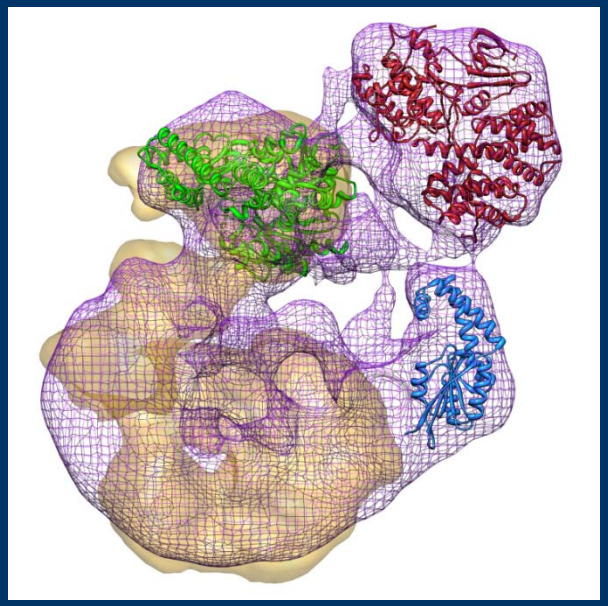


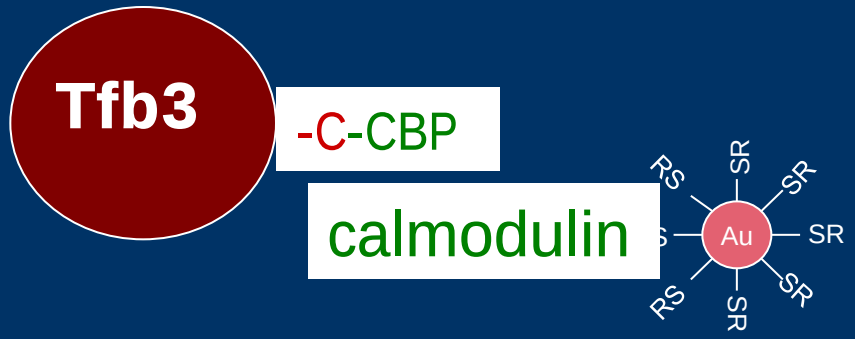




Localization of Tfb3 with calm-Au







Acknowledgments

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Pablo Jadzinski

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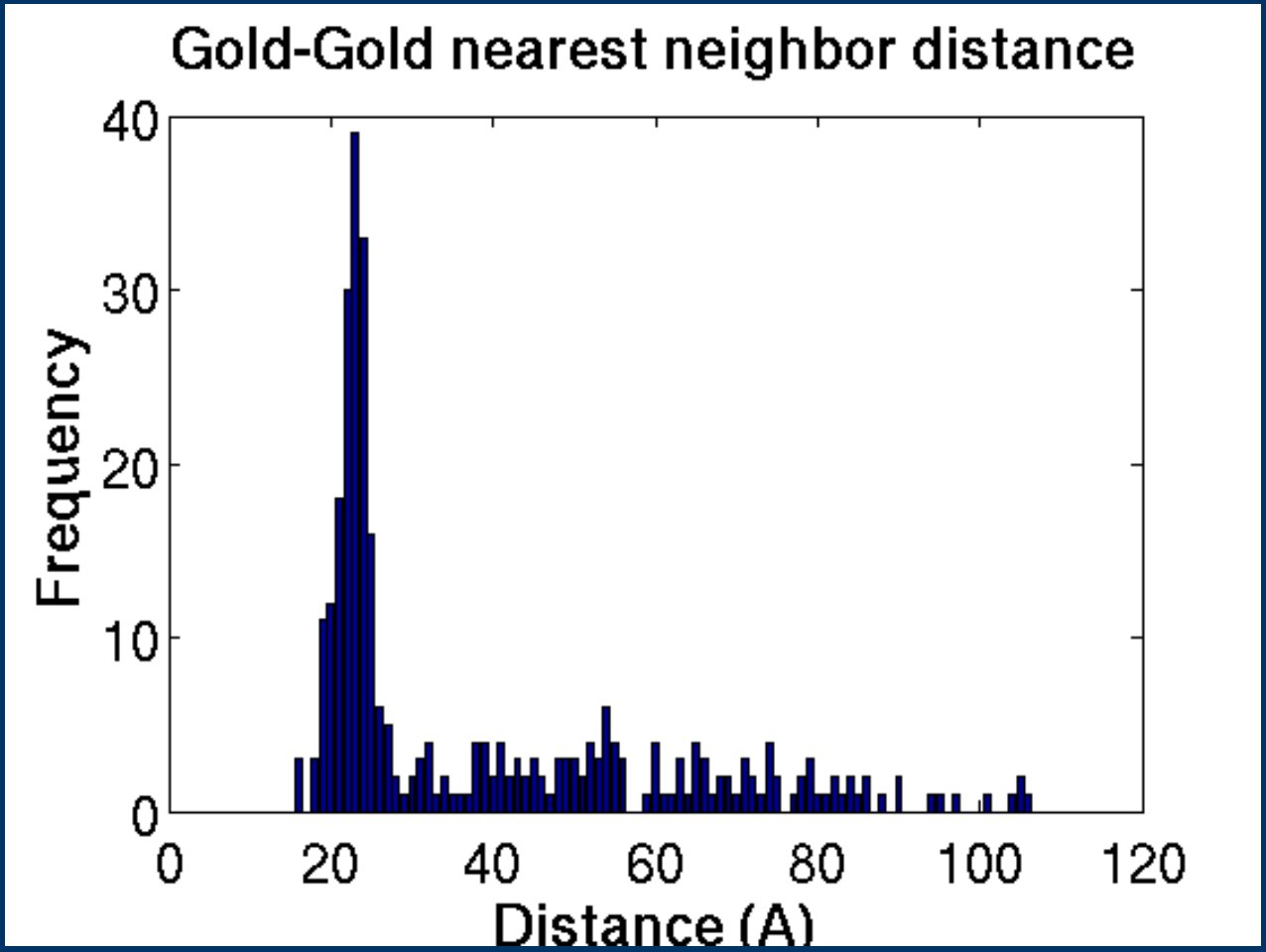
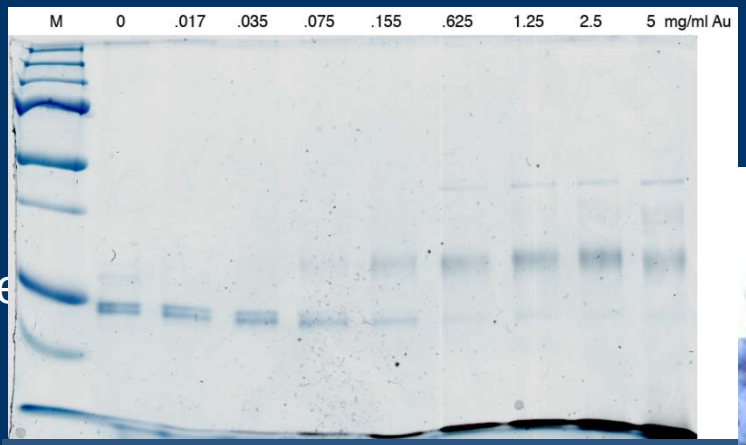
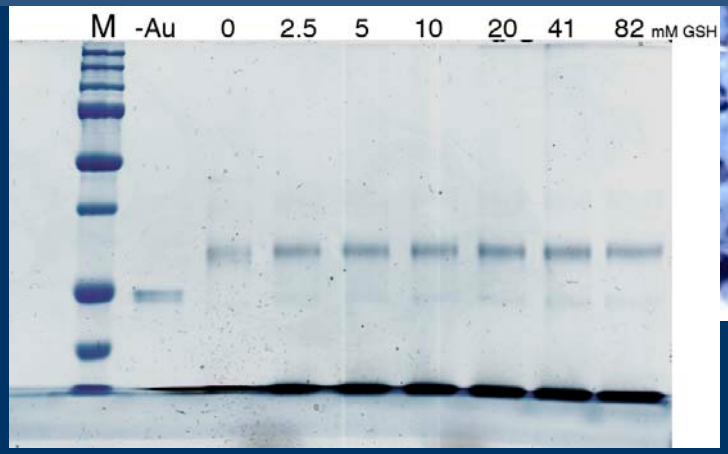


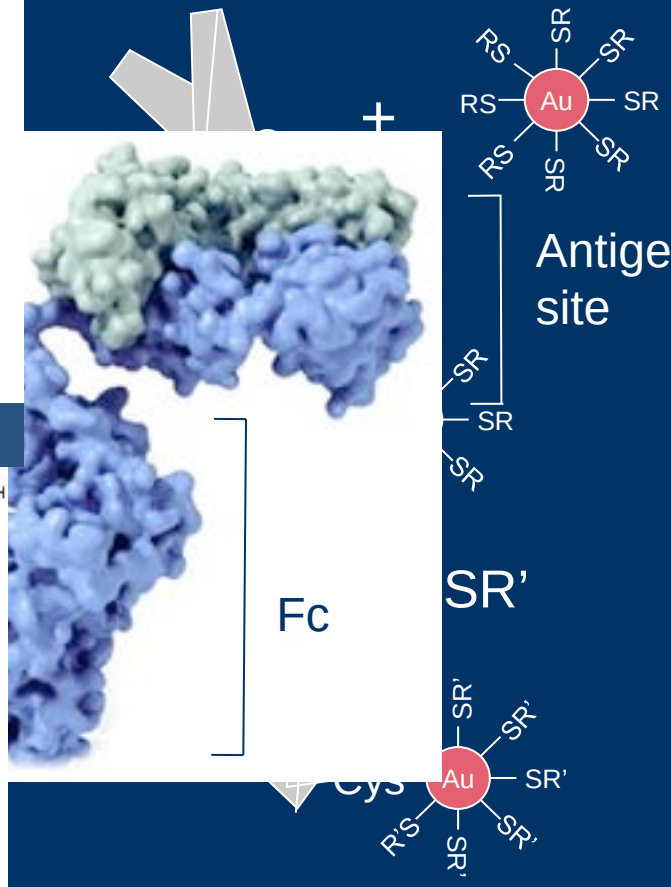
Adaptor molecule

Maia Azubel





Antige
site



Antigen-binding
site

Murray place exchange

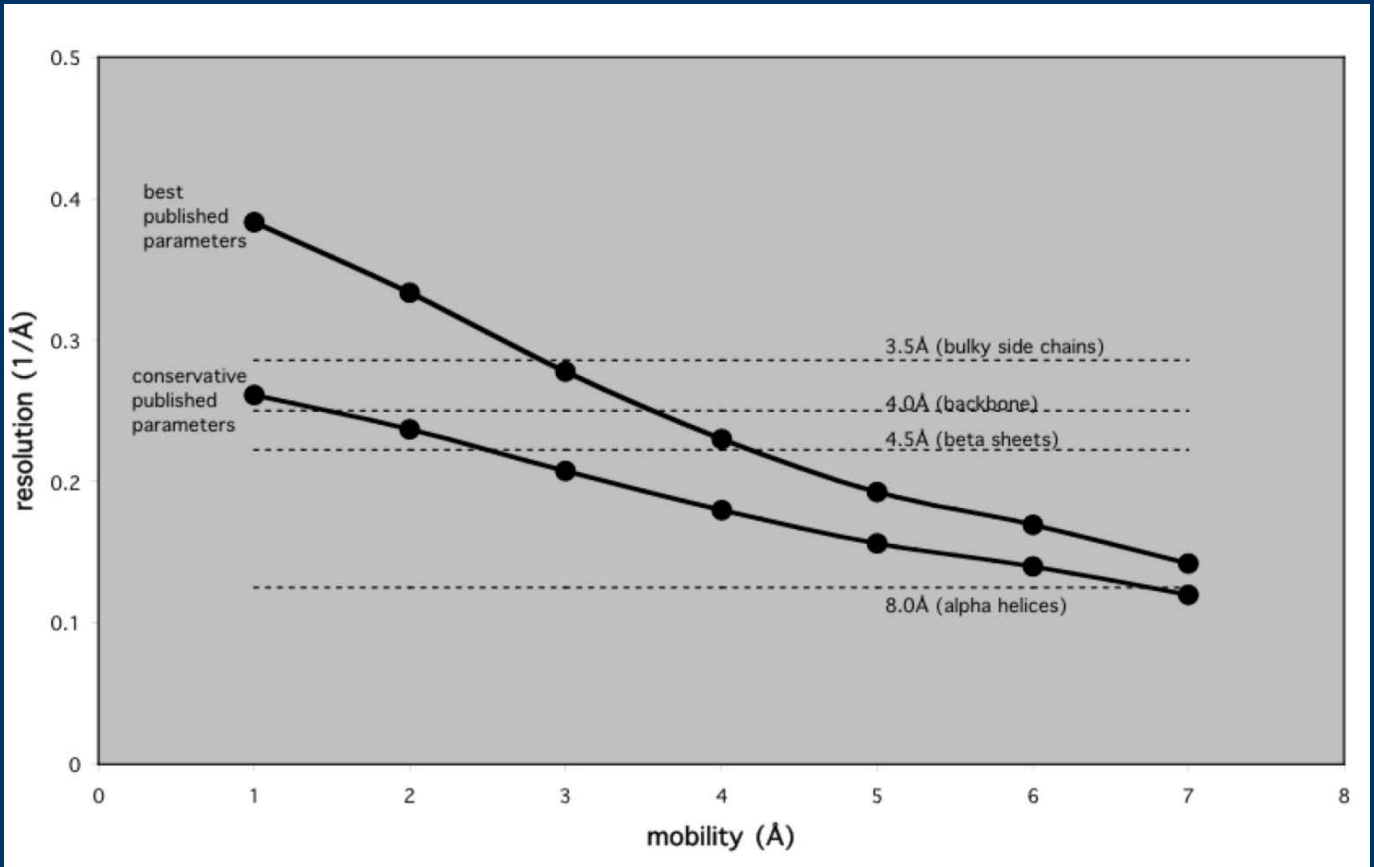
HEAVEM Proposal

- Relies on derivatization of biological sample with heavy atom clusters

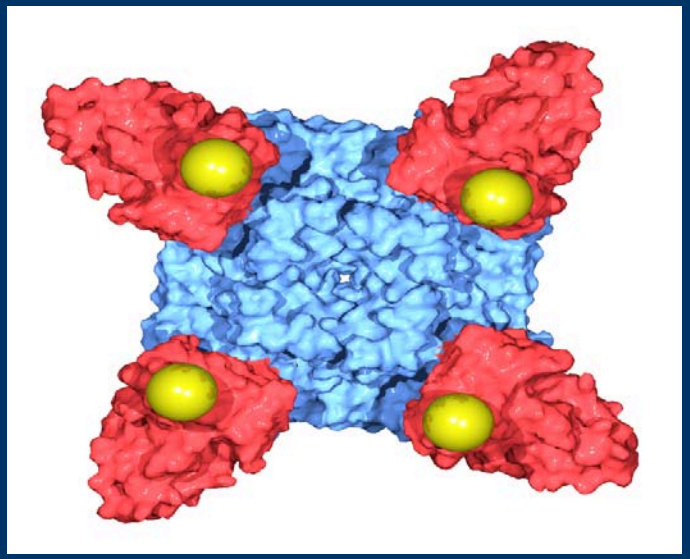
scatter electron strongly

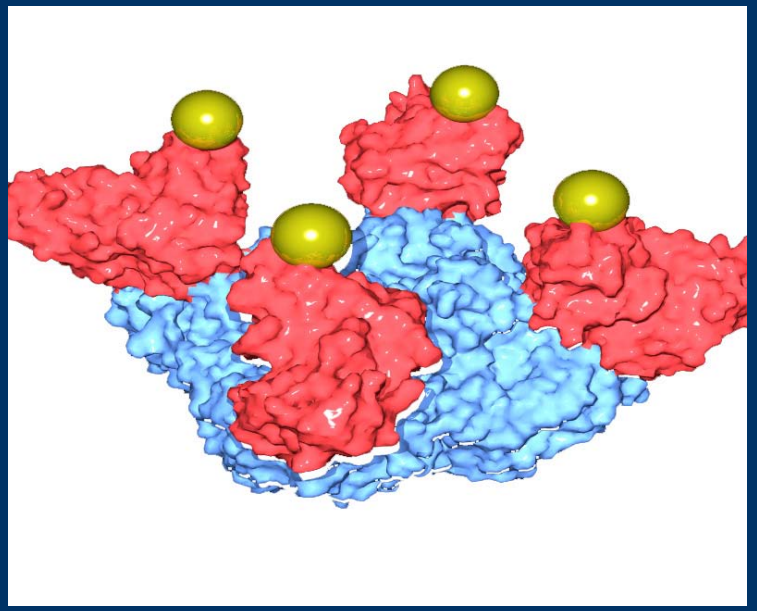
- Requires discrete particles
  specific attachment
  rigid attachment

- Imaging under conditions required for high resolution
  - low dose
  - close to focus
- Overcome technical limitations
  - detection of drift, astigmatism & magnification variation (microscope aberrations)
  - CTF determination
  - Resolve particle heterogeneity
- Allows faster & automated data collection and particle picking

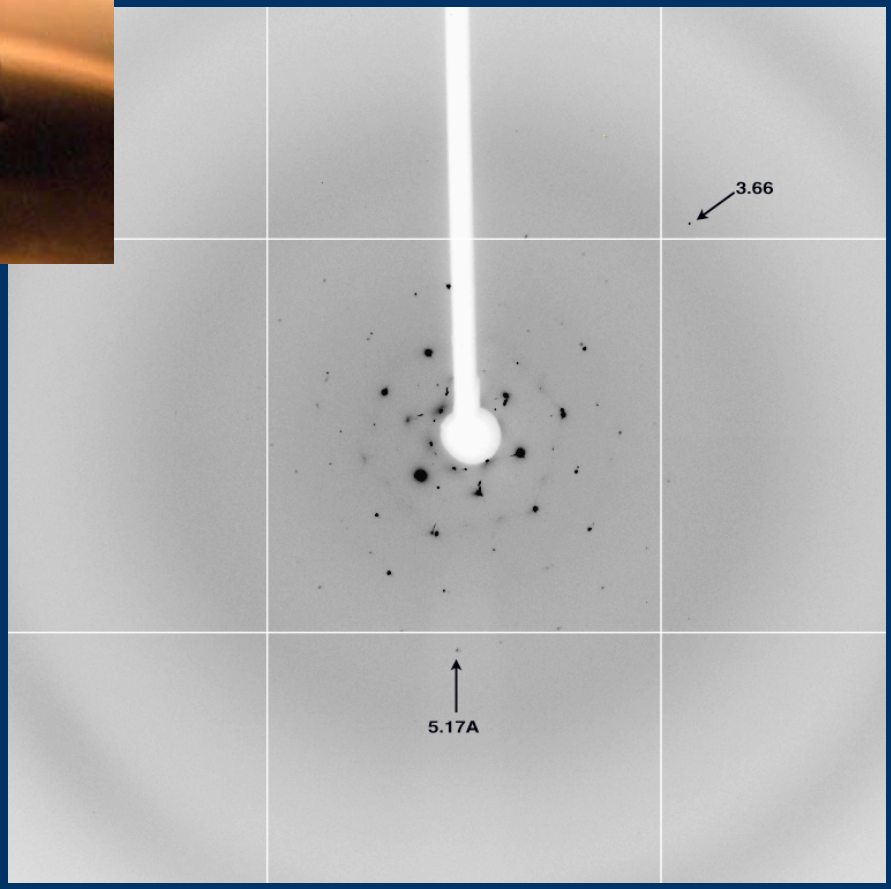

0.5
0.4
0.3
0.2
0.1
0
resolution (1/Å)
best
published
parameters
conservative
published
parameters
3.5Å (bulky side chains)
4.0Å (backbone)
4.5Å (beta sheets)
8.0Å (alpha helices)
0
1
2
3
4
5
6
7
8
mobility (Å)

scFv-gold for heavy atom electron microscopy

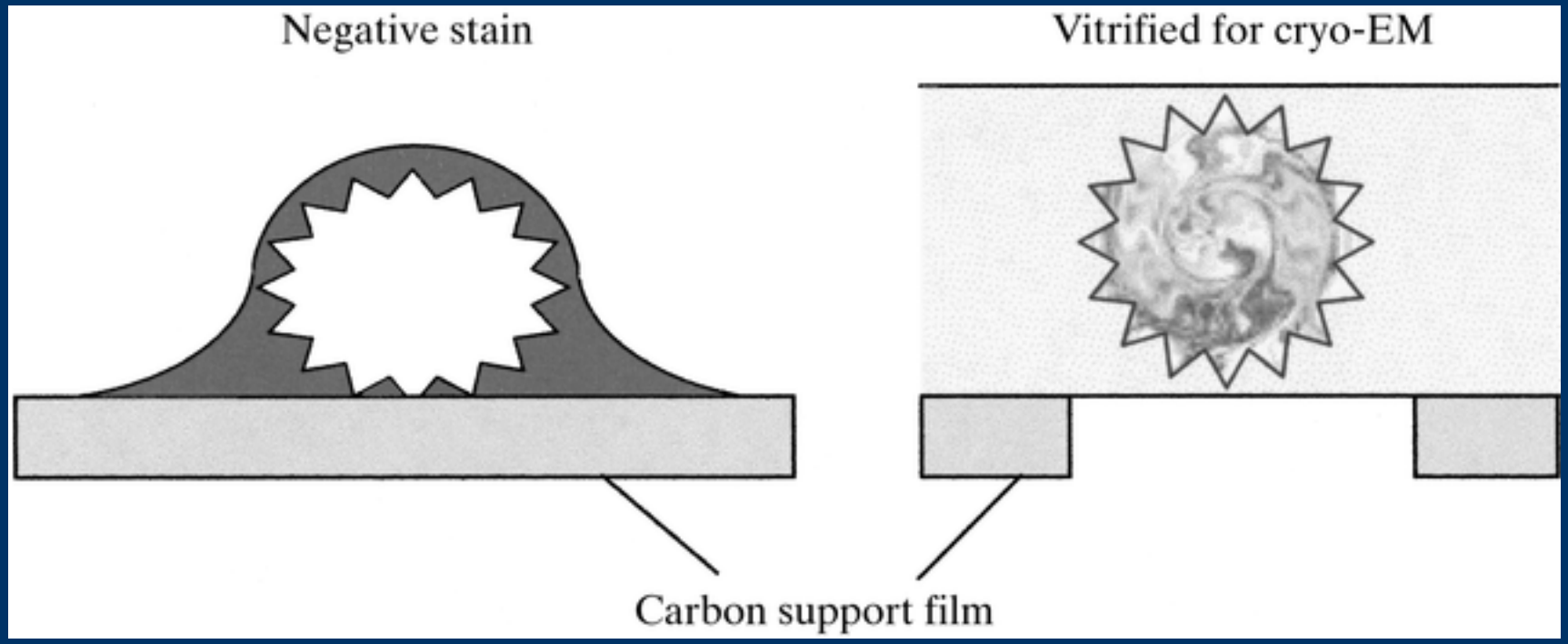




3-D crystals of 2 nm 4-mercaptobenzoic acid MPCs

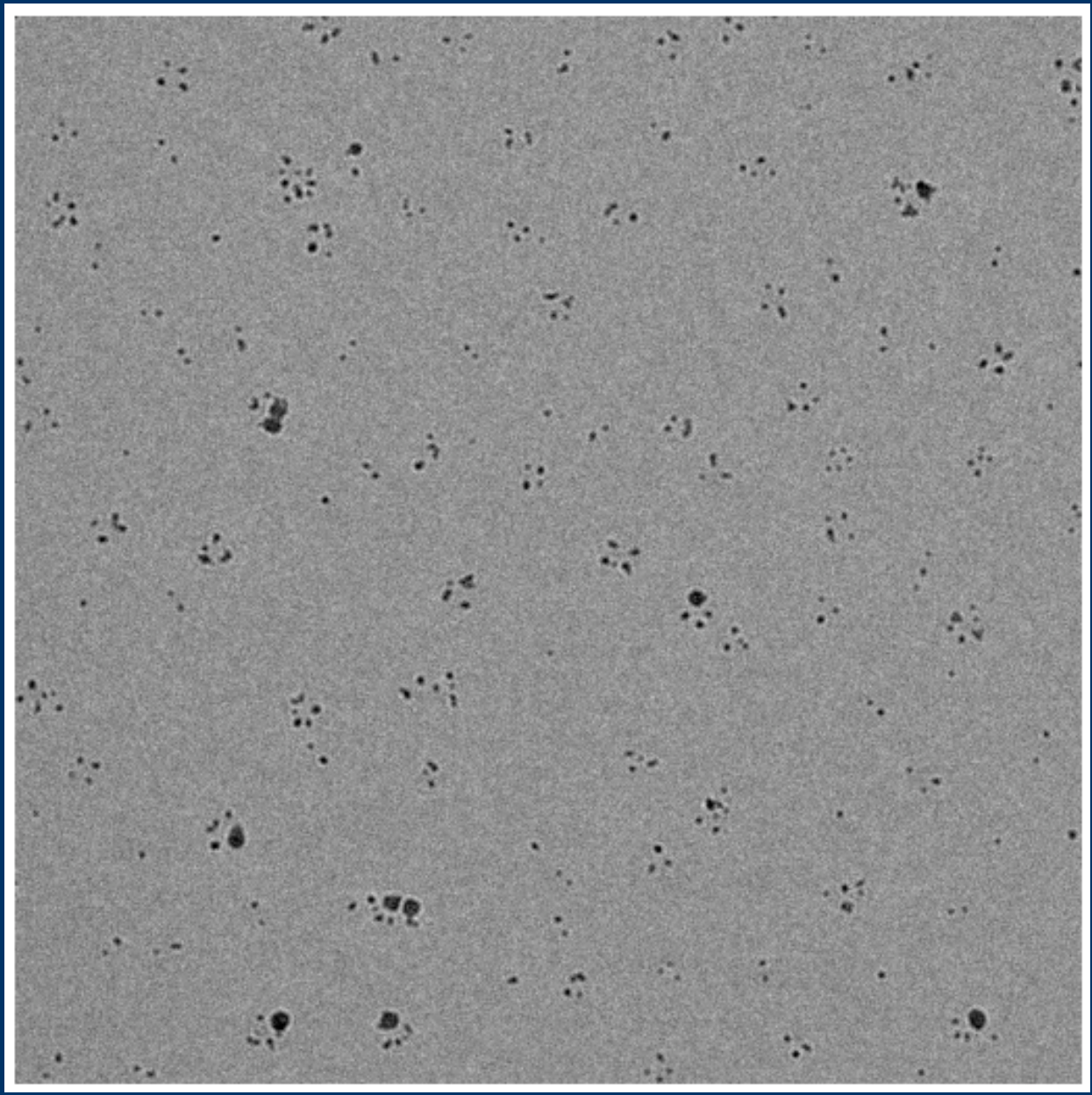


Sample preparation

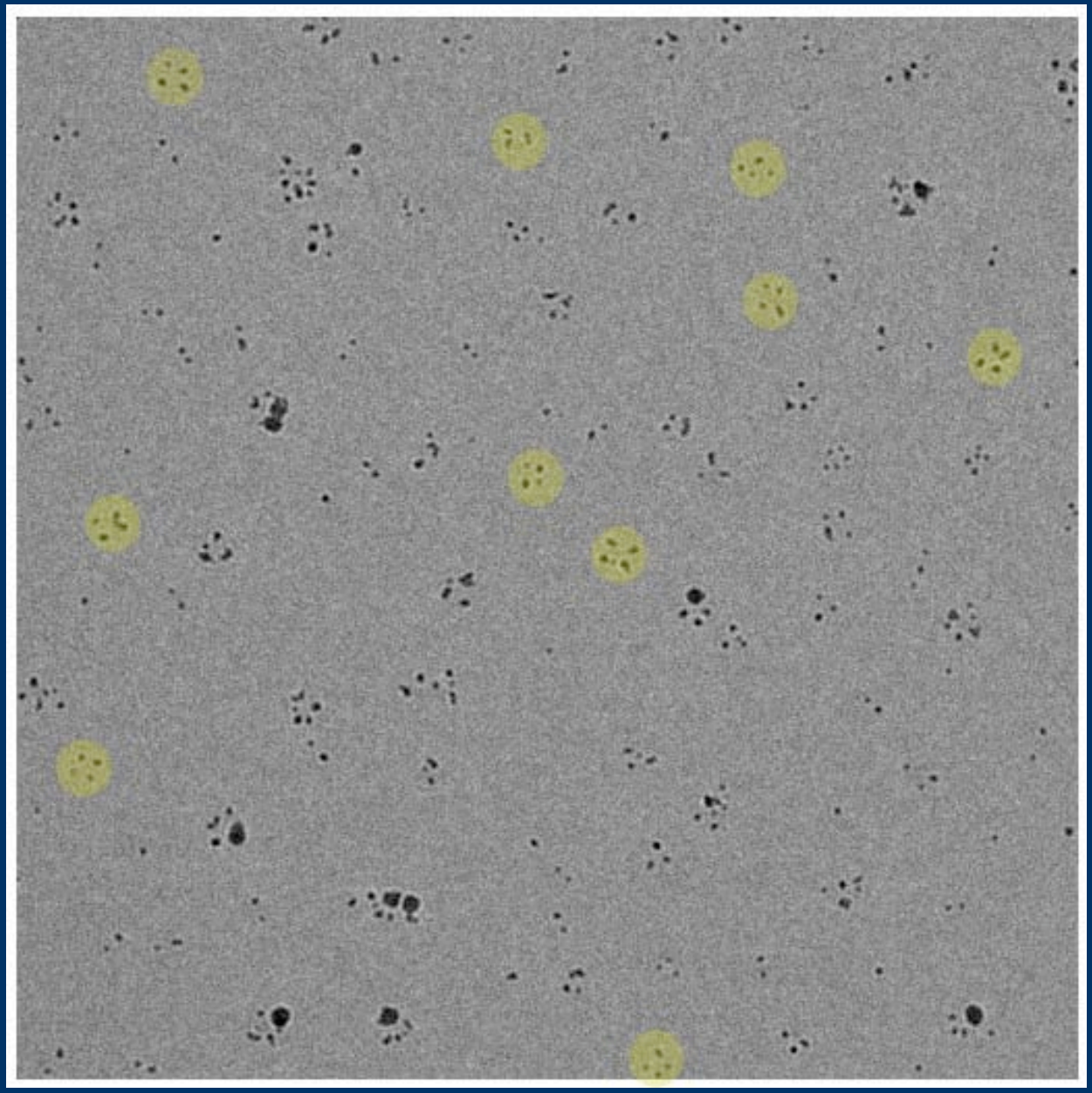


H.R. Saibil 2000

Cryo-EM of neuraminadase-scFv-gold



Cryo-EM of neuraminadase-scFv-gold



Summary

- ✓ Synthesis of defined Gold MPC
- ✓ Selection and generation of adaptor molecule-cojugates that provide Specificity & Rigidity
- ✓ Feasibility, Stability and visualization of complexes

Stage is set for empirical test of the HEAVEM Proposal.

Acknowledgments

Roger Kornberg

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