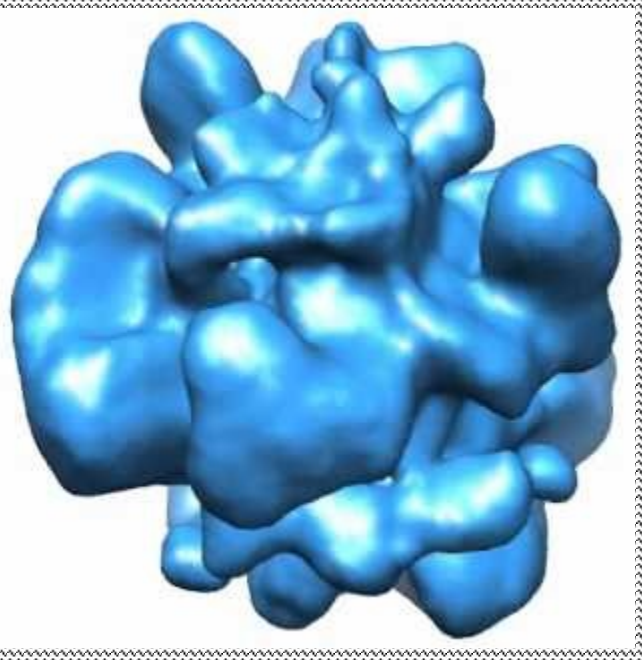
Domain/Subunit Identification and Labeling Strategies:

Answers to the six thought-provoking questions posed in the meeting agenda:

1. Your guess is as good as mine.
2. Maybe yes, maybe no.
3. I have absolutely no idea.
4. No one said electron cryomicroscopy would be easy.
5. I'm not sure we'll ever know the answer to that.
6. The answer to this question is left as an exercise for the student.

3D map of complex



Known protein components

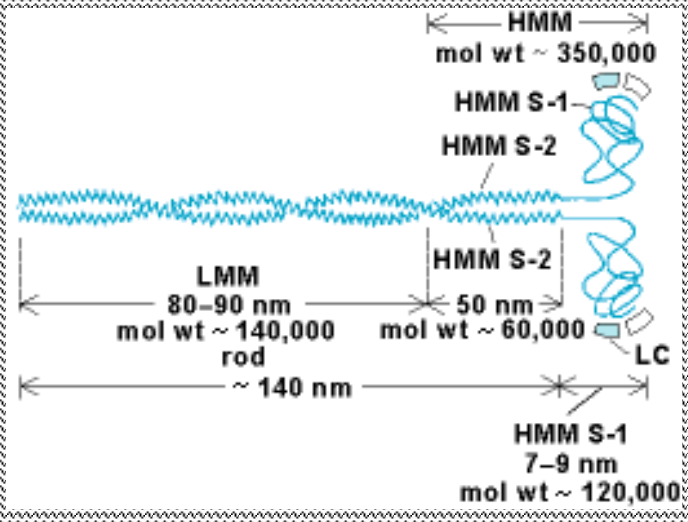
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10

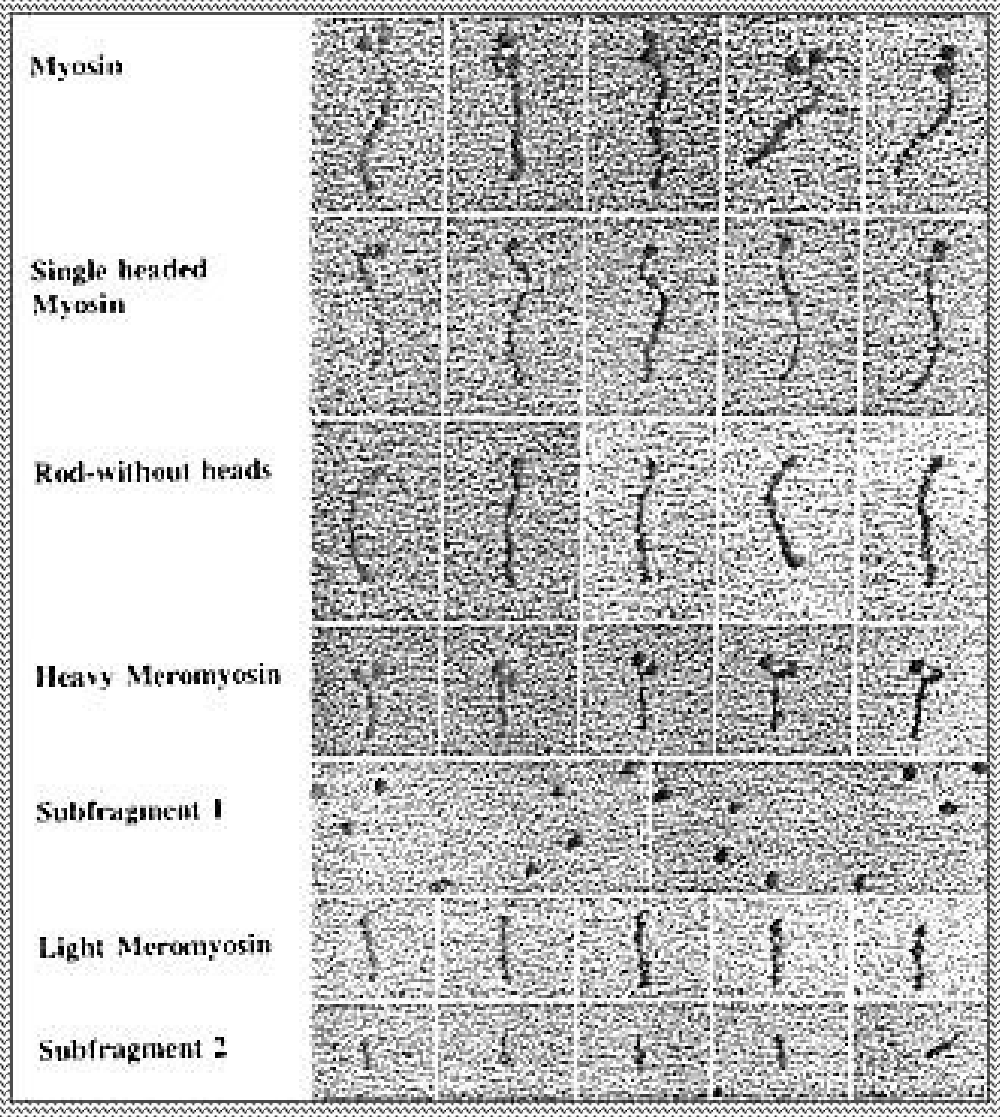
Methods to identify domains/subunits:

1. Addition/subtraction of components
2. Tagging of components
3. Size/Shape/Charge

1. Addition/subtraction of components
2. Tagging of components
3. Size/Shape/Charge

Subtraction by enzymic digestion



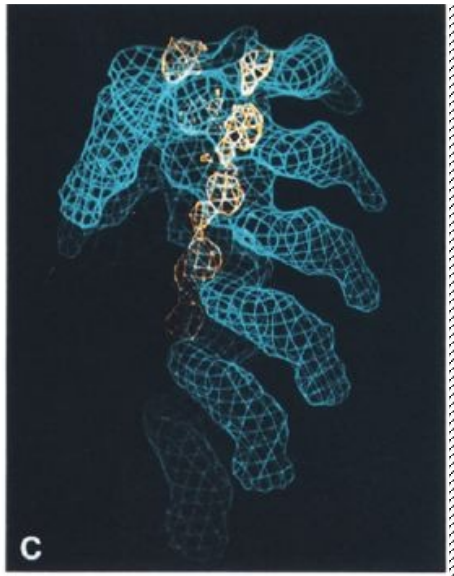


Meromyosins, the subunits of myosin
Szent-Gyorgyi, AG
Arch Biochem Biophys 42, 305-320, 1953

Substructure of the myosin molecule I. Subfragments of myosin by enzymic degradation.
Lowey, S, Slater, HS, Weeds, AG & Baker, H.
J Mol Biol 42, 1-20, 1969.

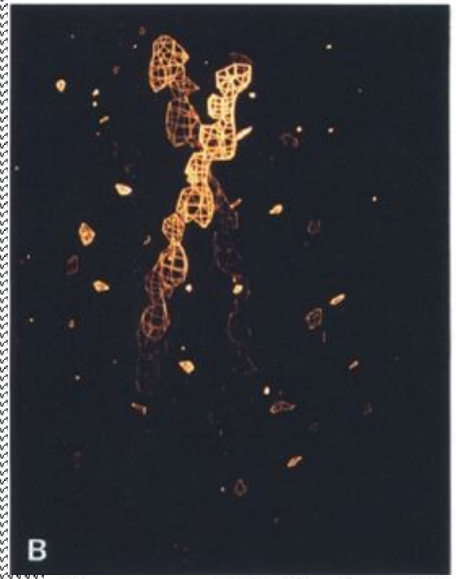
Removing/adding a protein (*in vitro)*

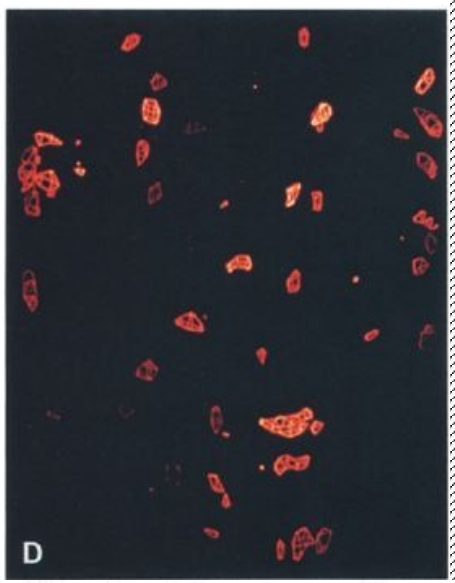
Difference map:
Actin+Tm+S1
Minus
Actin+S1



Difference map plotted on top of map Actin+S1

t-test map of difference at (<0.5% confidence)





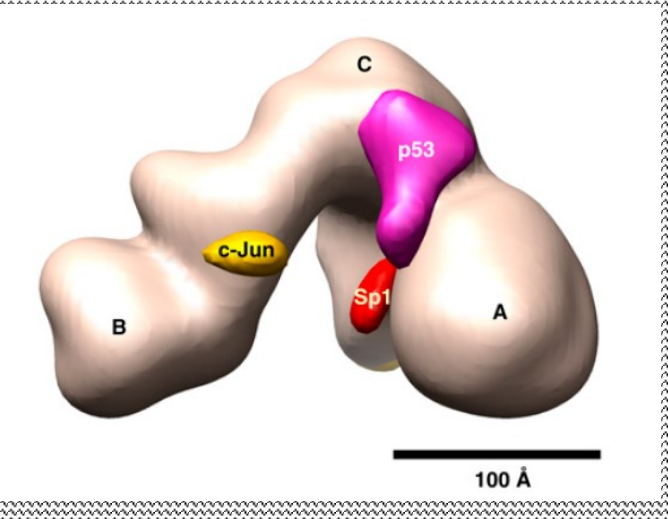
Control t-test map:
Actin+Tm+S1
minus
Actin+Tm+S1
(1% confidence)

Structural Relationships of Actin, Myosin, and Tropomyosin Revealed by Cryo-Electron Microscopy

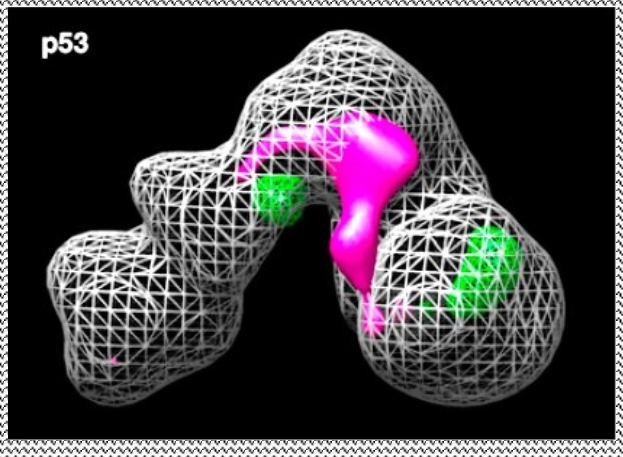
R. A. Milligan and P. F. Flicker JCellBio *105*, 29-39, 1987

Removing/adding a protein – detecting conformational chang

TFIID +/- P53, c-Jun, and Sp1



TFIID*P53 – TFIID =



Magenta = significant positive differences
Green = significant negative differences
Extra magenta and green are interpreted as conformational changes.

Structures of three distinct activator–TFIID complexes
Liu, W *et al*,
Genes and Dev *23*, 1510-1521, 2009

Subtraction using mutations

Dissecting the Nexin-Dynein Regulatory Complex

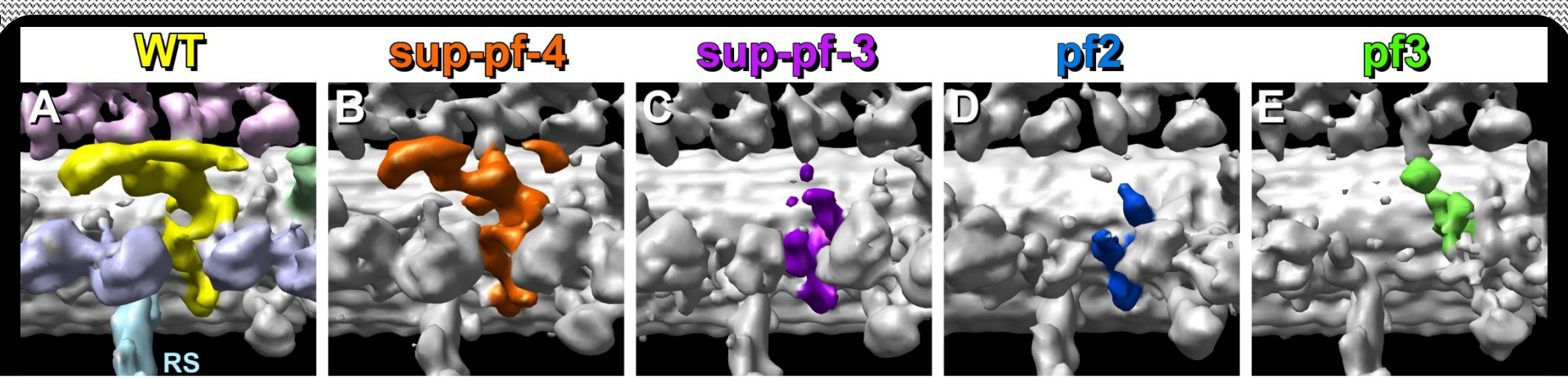
ermine which proteins are present in the mutant flagella by 2D gels and mass sp

	Previous DRC components							Novel DRC candidates				
mutant	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**FAP 61**	**FAP 206**	**FAP 230**	**FAP 252**	**Spot 11**
sup-pf4	+	+	+	+	-	-	+	+/-	+	+	+	+
sup-pf3	+	+	+/-	+/-	+/-	+/-	+/-	+/-	+	+	+	+/-
pf2	+	+	-	-	-	-	-	+/-	+	+	+	+/-
pf3	-	-	+/-	+/-	+/-	-	+/-	+/-	+	+	+	+/-

Note: one deleted/mutant gene leads to many lost proteins!

Song *et al.* (in preparation) Nicastro Lab

oteins lost	-2	+/- 6	-5, +/-2	-2,+



Heuser *et al.* 2009 JCB

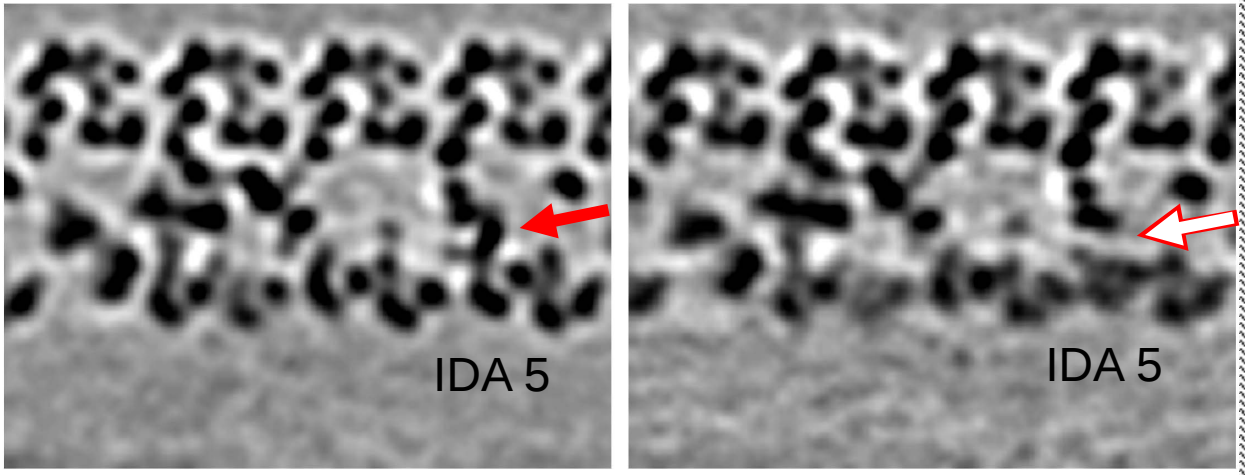
This deletion of regulatory gene 3 shows only one (known) missing protein.

	Previous DRC components							Novel DRC candidates				
mutant	1	2	3	4	5	6	7	FAP 61	FAP 206	FAP 230	FAP 252	Spot 11
Δdrc3	+	+	-	+	+	+	+	+	+	+	+	+

Song *et al.* (in preparation) Nicastro Lab

WT

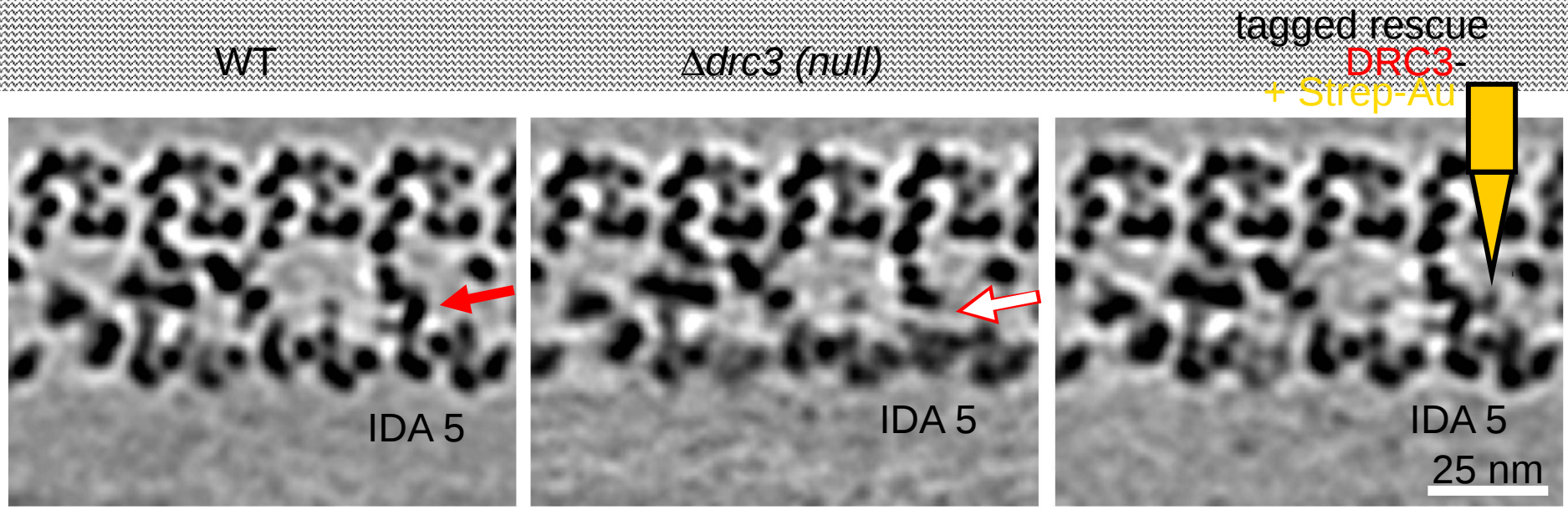
Δ*drc3 (null)*



How do you determine that the difference is not confused by a rearrangement within the complex?

Song *et al.* (in preparation) Nicastro Lab

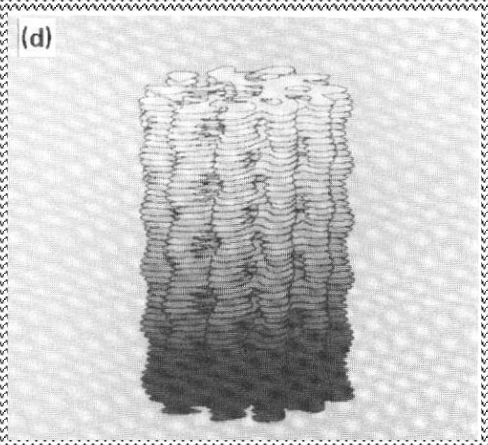
escue with a tagged gene argues that there is not rearrangement on the loss of



Song *et al.* (in preparation) Nicastro Lab

Subtraction by comparing orthologs with missing amino acids

Flagellar filament
C. crescentus

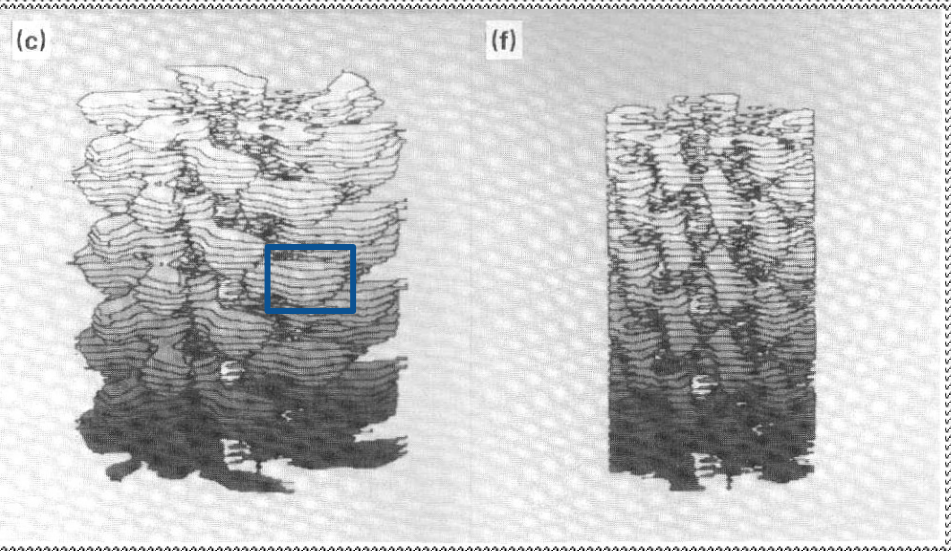


aligned proteins sequences

25kDa

54 kDa

Flagellar filament
S. typhimurium



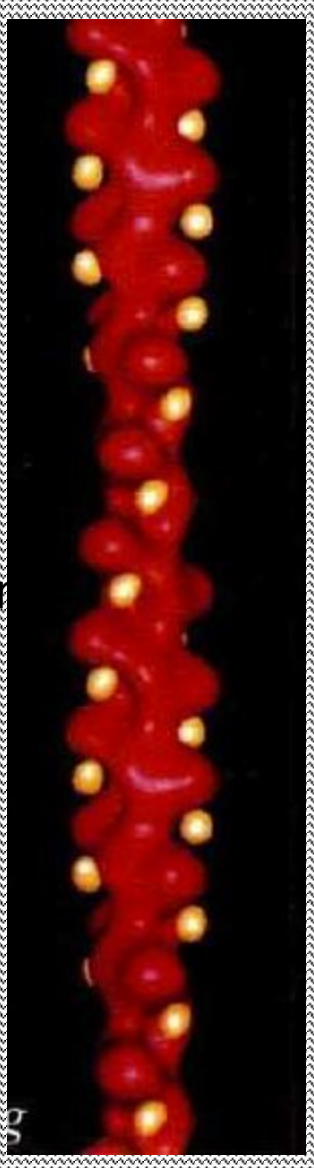
Outermost lobe removed in computer

1. Addition/subtraction of components
2. Tagging of components
3. Size/Shape/Charge

By gold tag attached to Cysteine

mposite t-test difference map

cation of undecagold attached to C-terminal cysteine 375 of acti



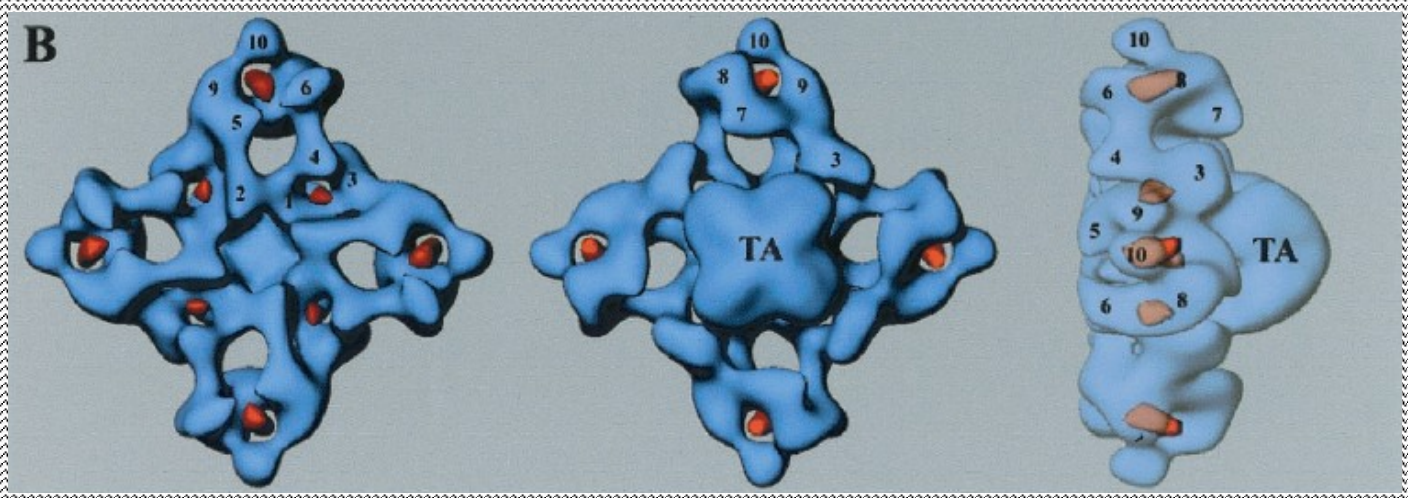
Molecular structure of F-actin and location of surface binding sites

R. A. Milligan, M. Whittaker & D. Safer* Nature *348*, 217-221, 1990.

By domain addition at one end

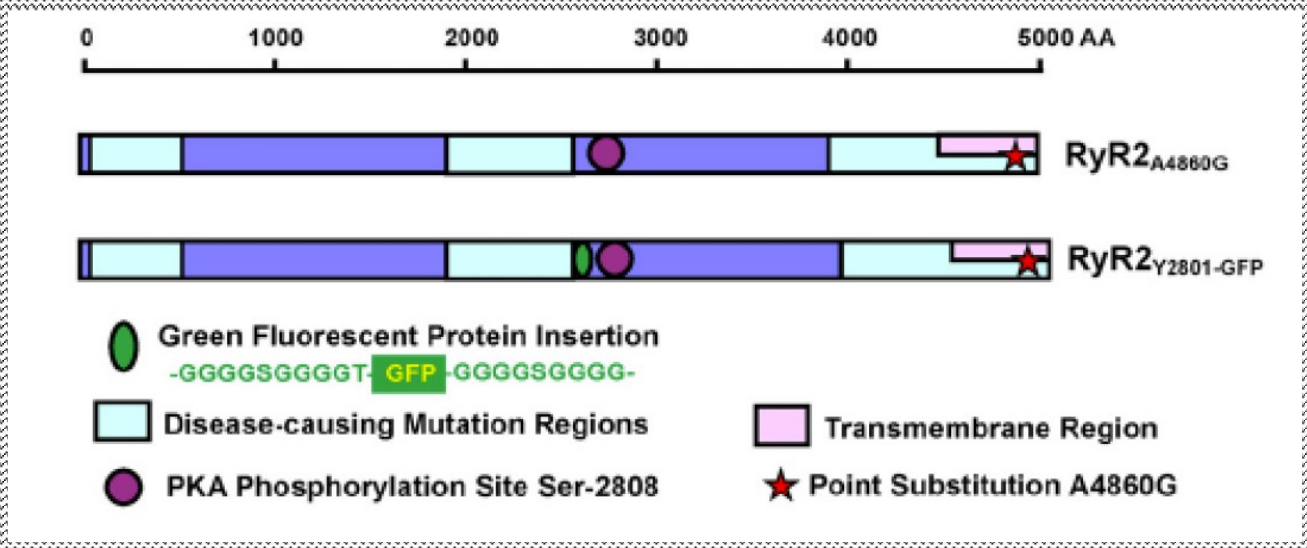
Tag – GST at N-terminus of ryanodine receptor prote

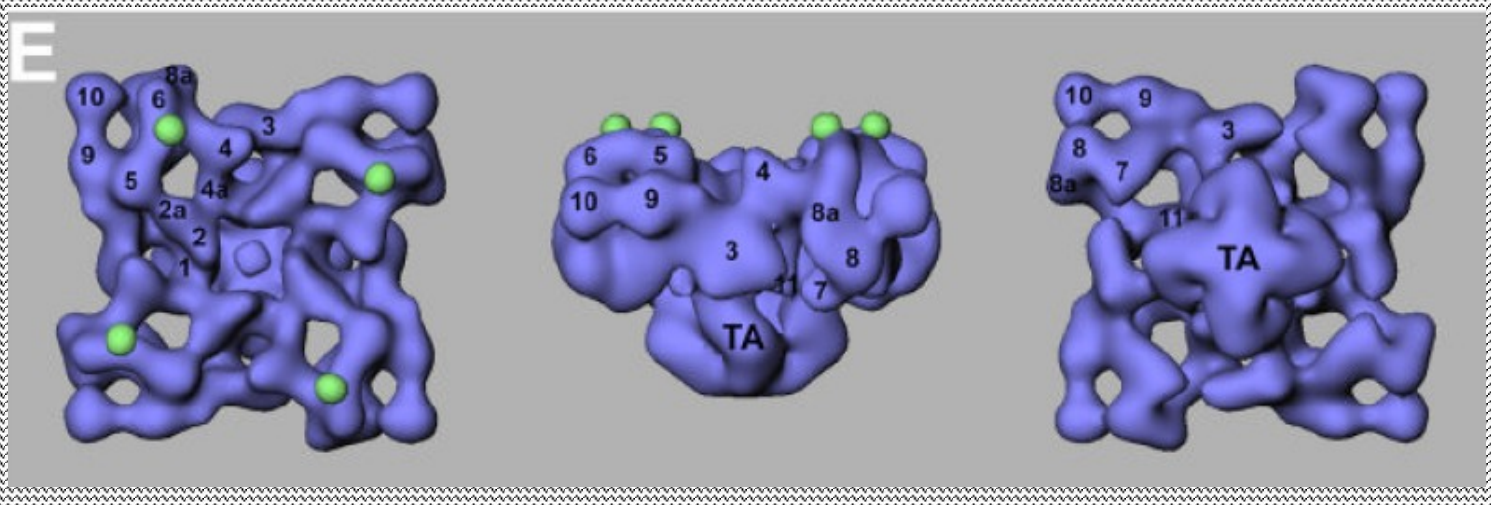
Difference map (red) plotted with WT



Three-dimensional reconstruction of the recombinant type 3 ryanodine receptor and its localization of its amino terminus
Liu *et al.*
PNAS *98*, 6104-6109, 2001

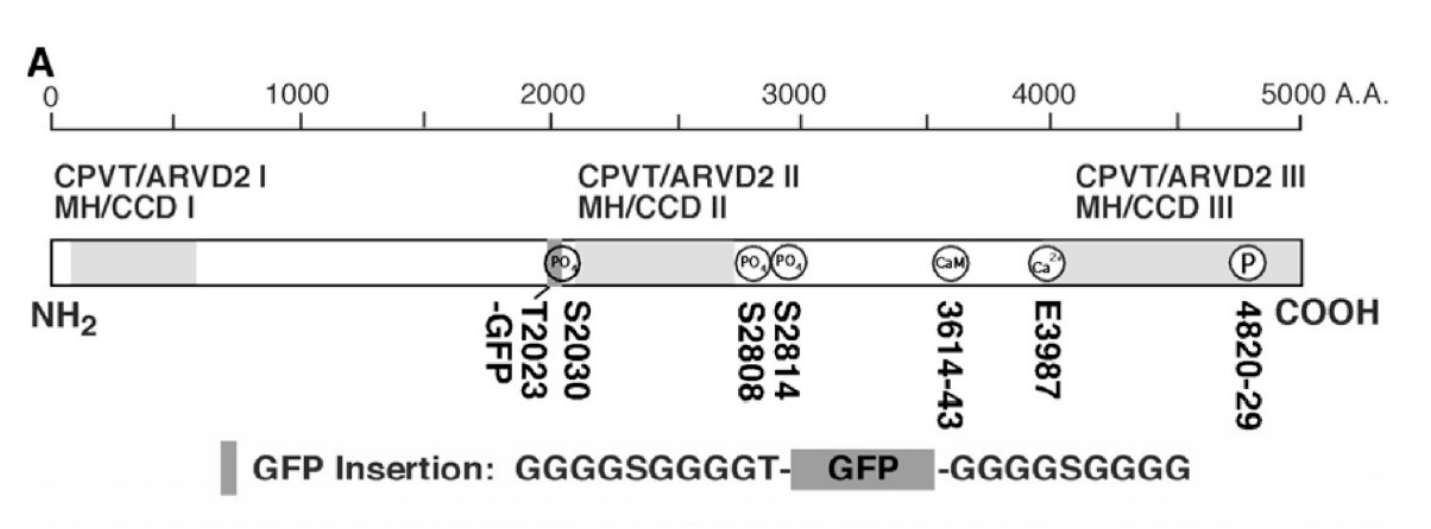
By domain insertion

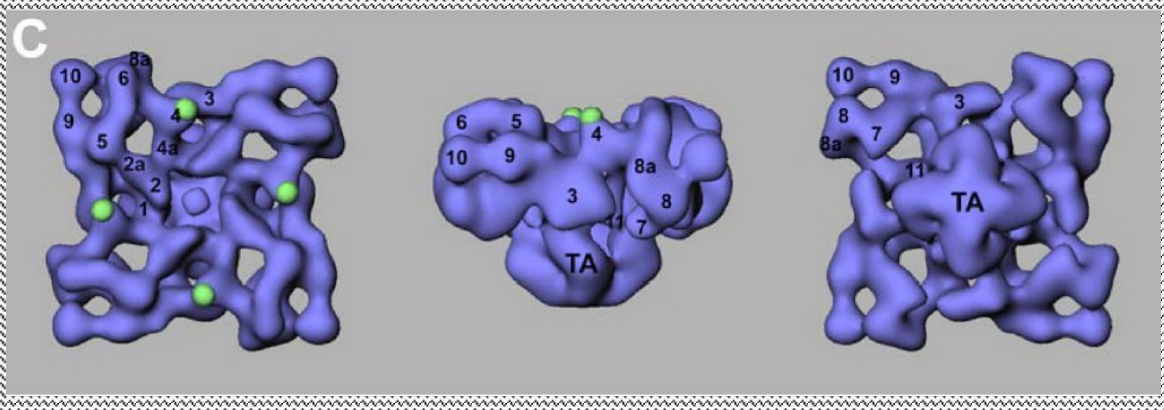




Three-dimensional localization of serine 2808, a phosphorylation site in cardiac ryanodine receptor
Meng et al. J Biol Chem 282, 25929-25939, 2007.

By domain insertion



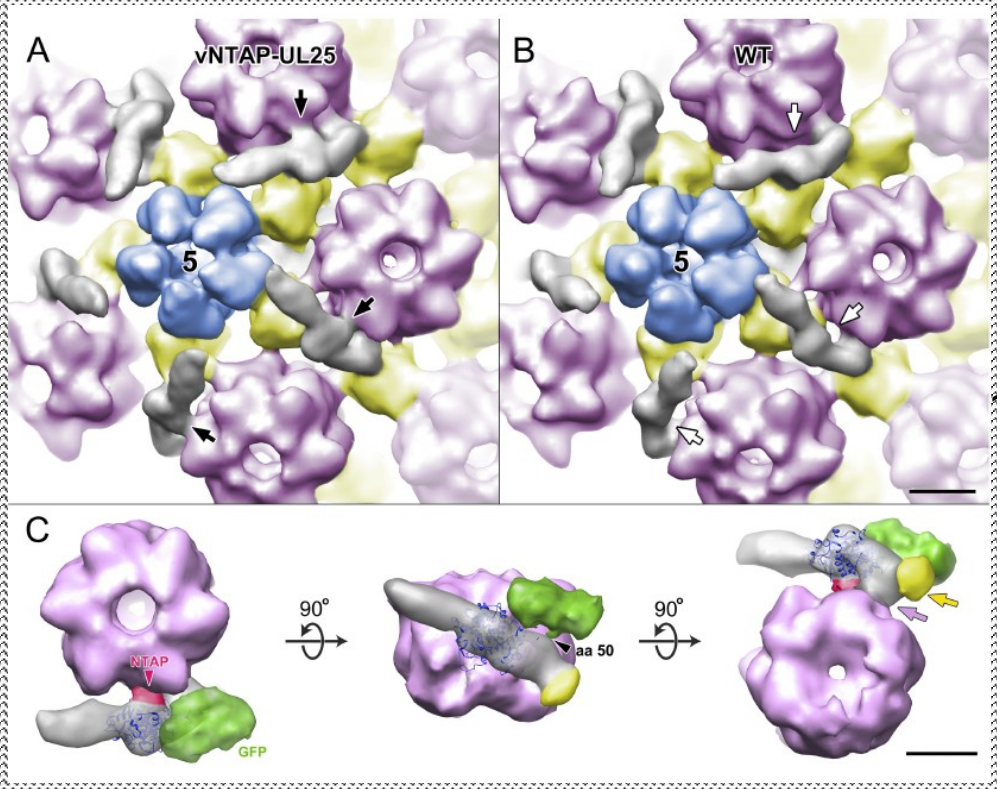


Localization of PKA Phosphorylation Site, Serine-2030, in the Three-Dimensional Structure of Cardiac Ryanodine Rec
ones *et al.*
Biochem J *410*, 261-271, 2008.

By domain insertion

HSV protein UL25 (580 aa):
WT, WT + TAP tag (~5K), and WT + GFP inserted between resides 50 and 51.

UL25 is grey
TAP is red
GFP is green.



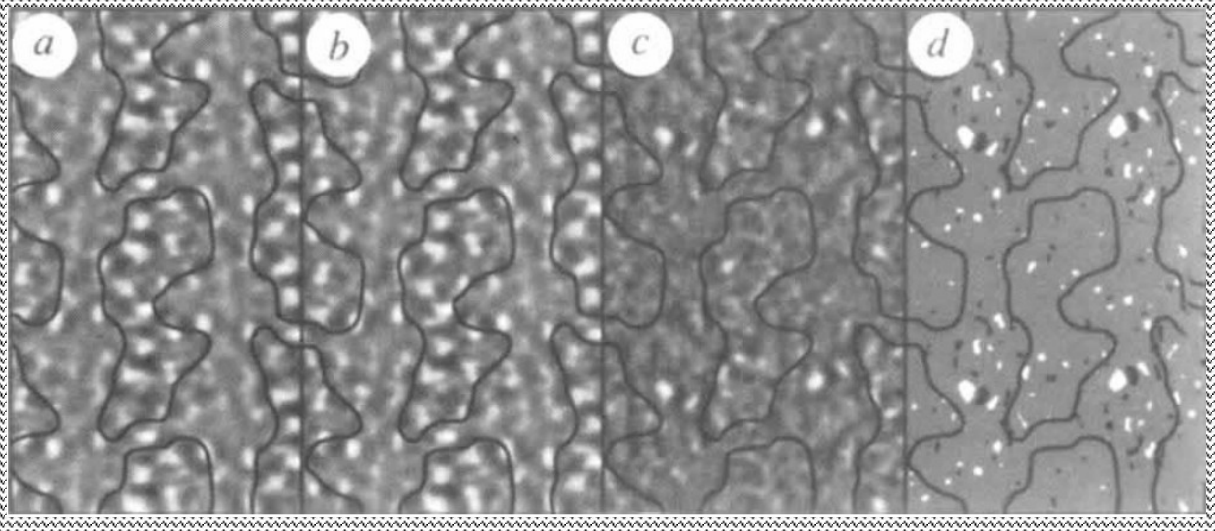
~2.5 nm resolution

Residues of the UL25 Protein of Herpes Simplex Virus That Are Required for Its Stable Interaction with Capsids
Cockrell, SK *et al.*
J Virol *85*, 4875-4887, 2011.

Ligand binding

Tubulin zinc sheets +/- taxol (MW=850 Da)

-taxol +taxol difference t-test map 0.5% confidence



Structure of tubulin at 6.5 A and location of the taxol-binding site
Nogales *et al.*
Nature *375*, 424-427, 1995

By peptide binding

GSLLGRMKGA binds to Glu77 and Asp78 region of HBV

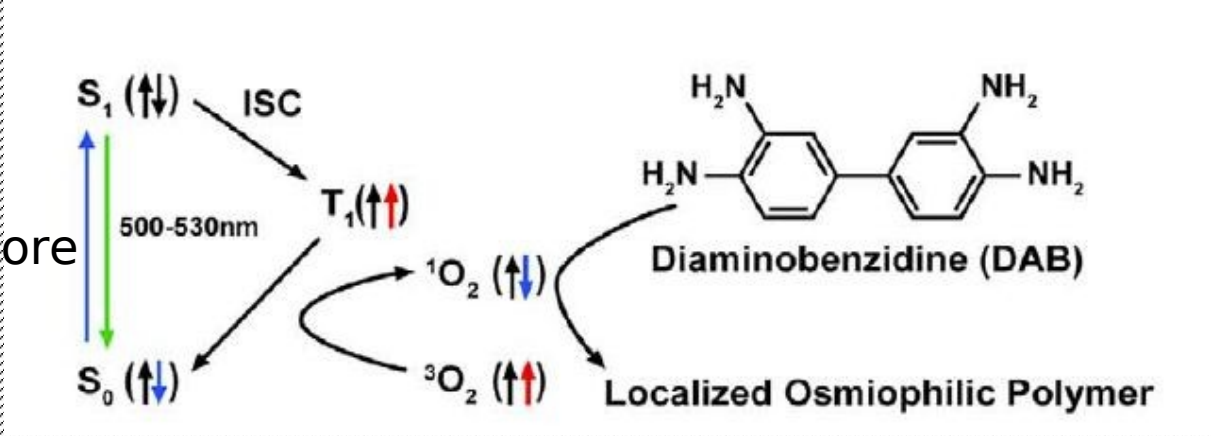
Difference map plotted on virus to show peptide

Peptides that block hepatitis B virus assembly: analysis by cryomicroscopy, mutagenesis and transfectio
Bottcher, B, Tsuji, N, *et al.*
EMBO J 17,6839-6845, 1998.

1. Antigen-specific antibody
2. Actin nucleating peptide (Stroupe, Grigorieff)

Photoconversion to generate heavy metal (osmium) label

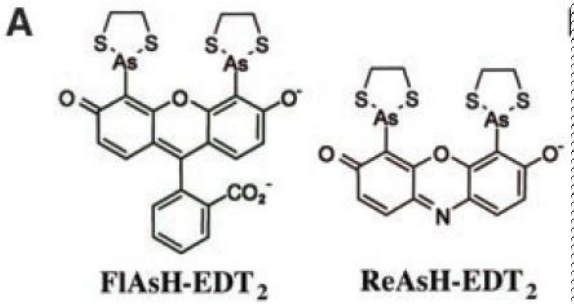
fluorophore

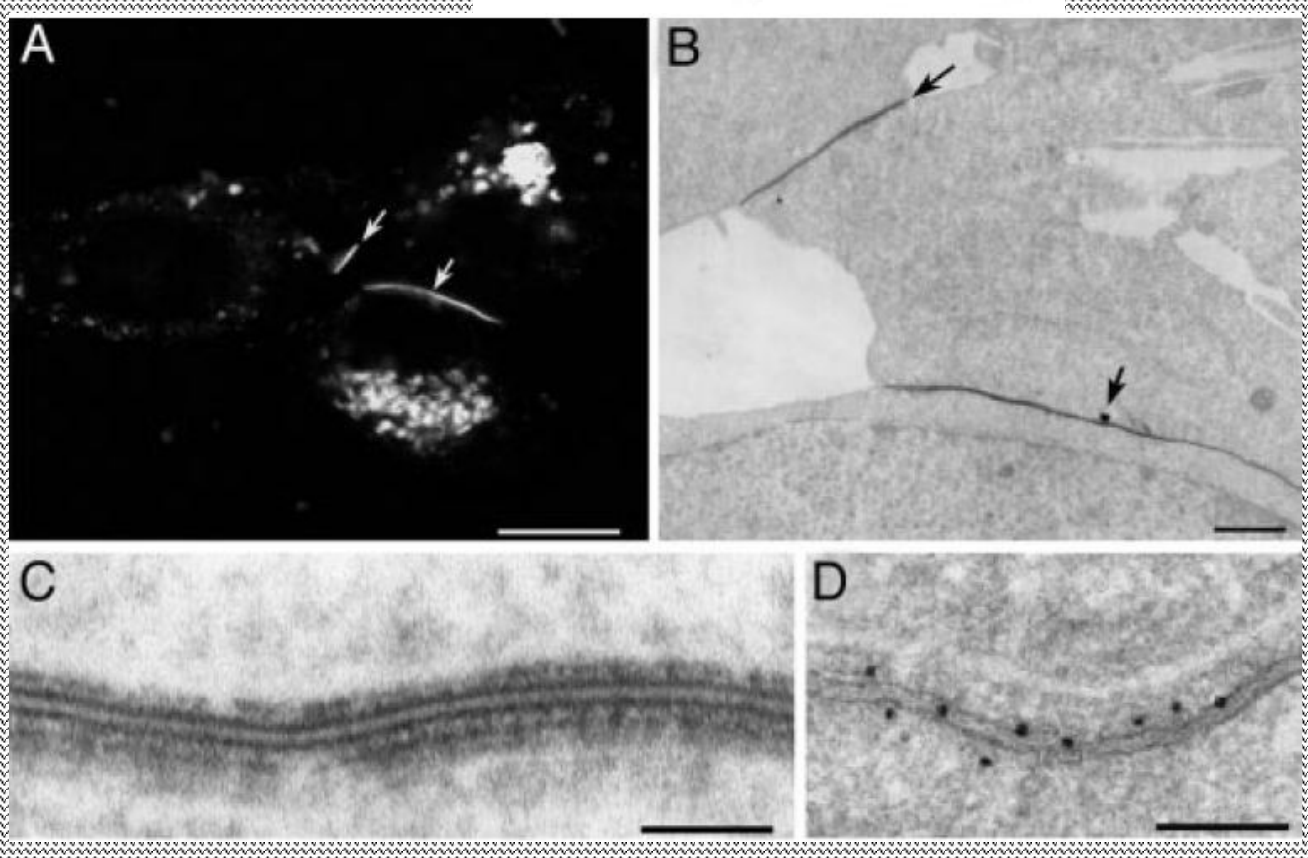


A Genetically Encoded Tag for Correlated Light and Electron Microscopy of Intact Cells, Tissues, and Organisms
Shu, X *et al.*
PLOS Biology 9, 1-10, 2011

FlAsH (green) and ReAsH (red)

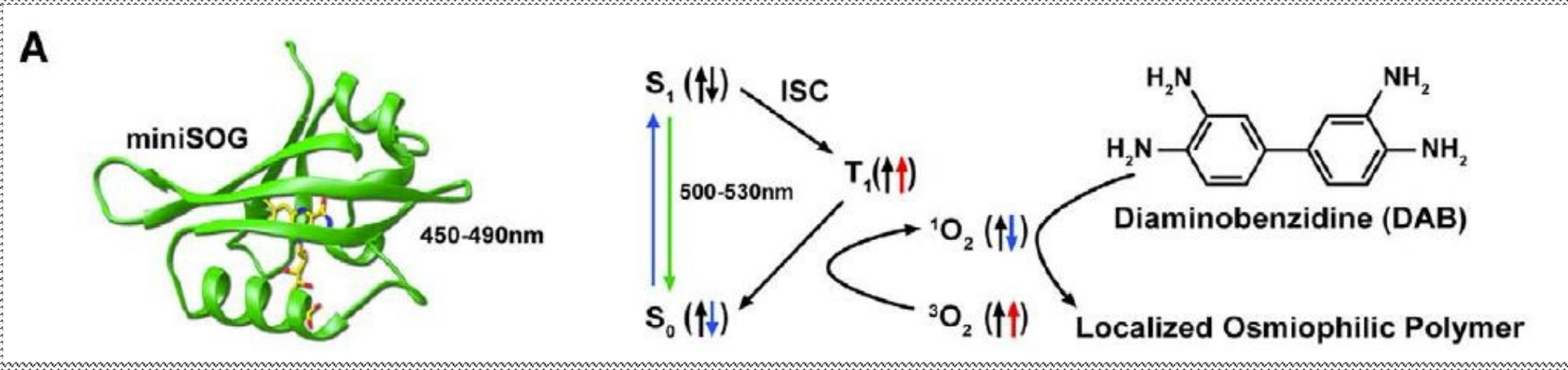
--CCXXCC--





Multicolor and Electron Microscopic Imaging of Connexin Trafficking
Gaietta, G. *et al.*
Science *296*, 503-507, 2002.

Singlet Oxygen Generator



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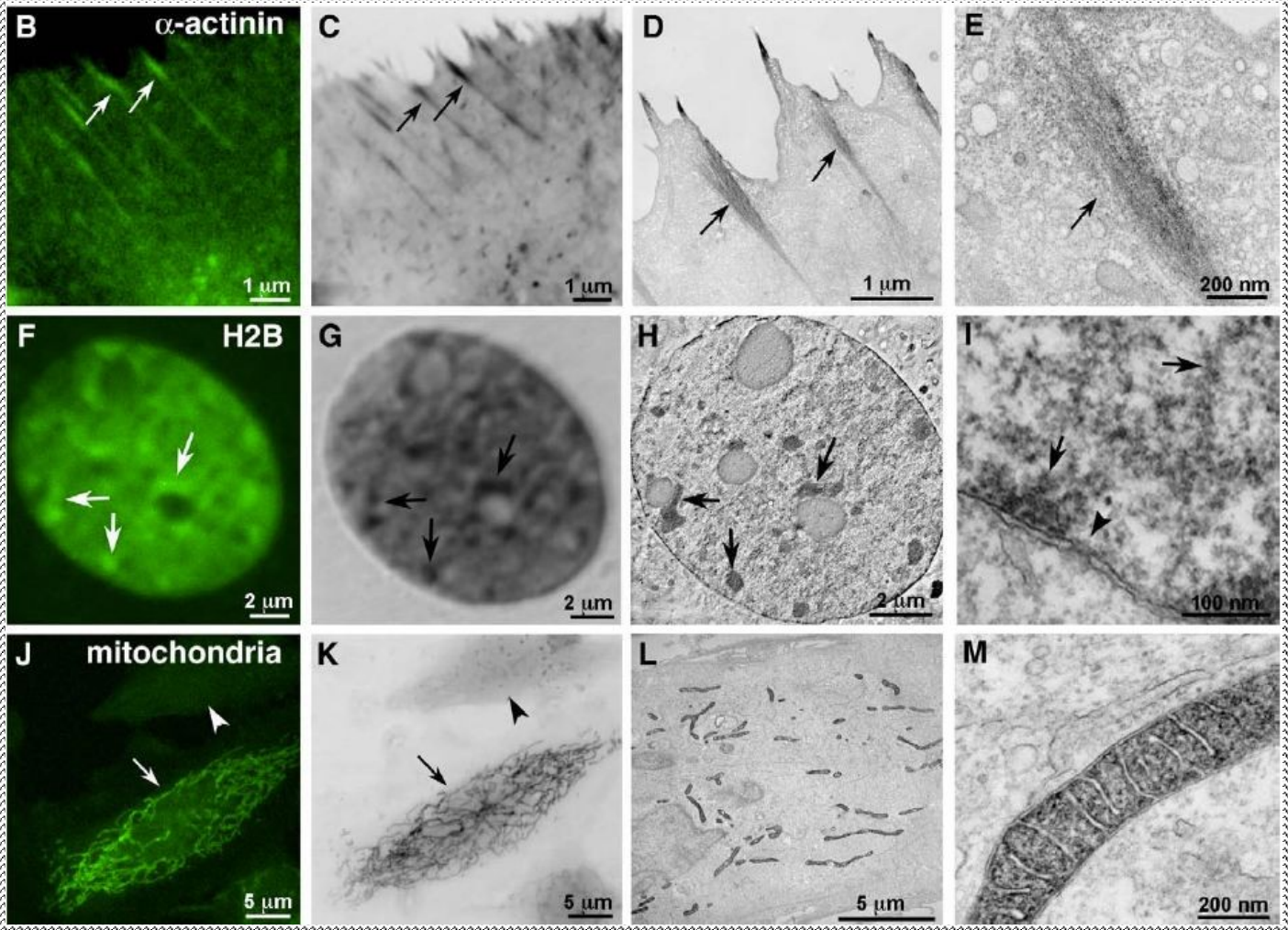
PLOS Biology 9, 1-10, 2011



A Genetically Encoded Tag for Correlated Light and Electron Microscopy of Intact Cells, Tissues, and Organisms
Shu, X *et al.*
PLOS Biology 9, 1-10, 2011

y growing nanoparticles of heavy metals on subunit of intere

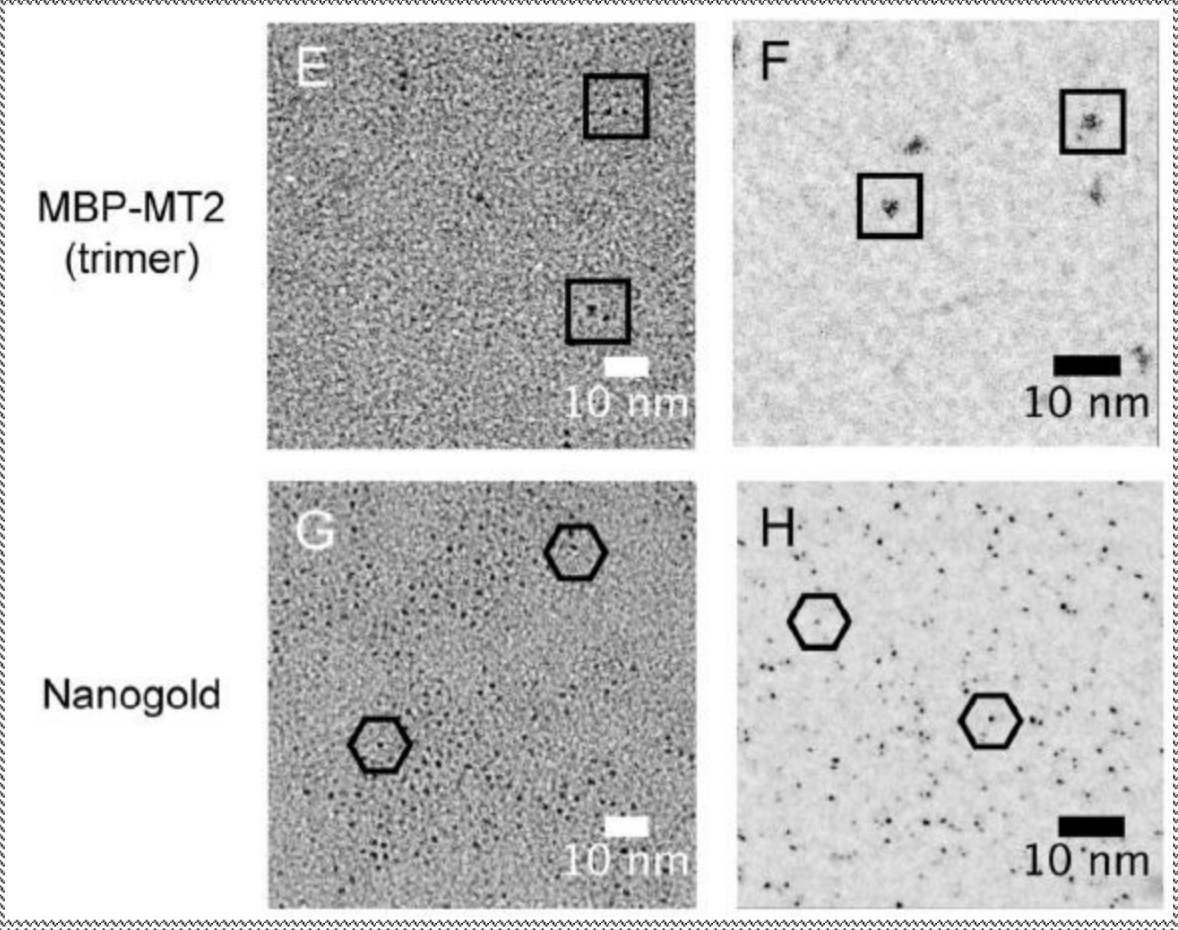
Peptide/RNA + solution of heavy metal salt ⎵ nanoparticle

Example:
Metallothionein (MT) + AuCl ⎵ MT-Aun n~20 to 40

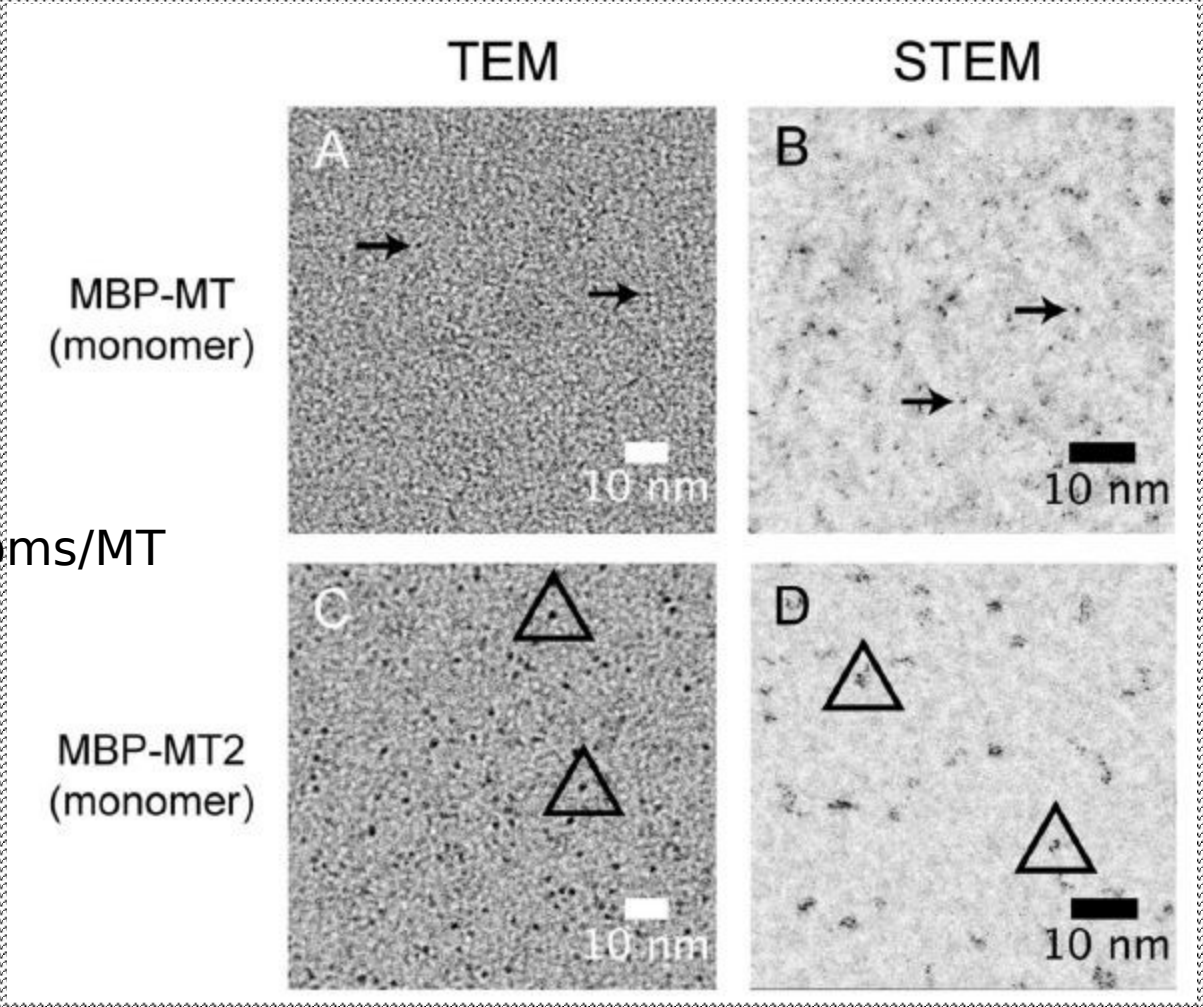
Pt (10Pt/MT), Ag (19Ag/MT), and Cd (6Cd/MT) have also been used.

Enhanced detection efficiency of genetically encoded tag allows the visualization of monomeric proteins by electron Microscopy
Fukunaga, Y *et al.* J Elec Microsc *61*, 229-236, 2012.

Concatenated Metallothionein as a Clonable Gold Label for Electron Microscopy
Mercogliano, C & DeRosier, DJ
J Struct Biol *160*, 70-82, 2007

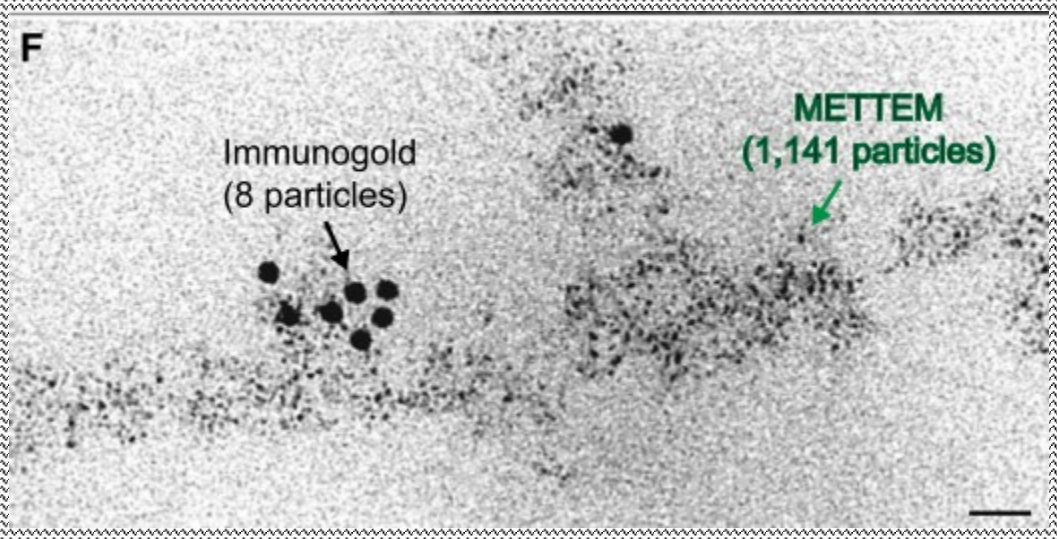


~20 gold atoms/MT



Concatenated Metallothionein as a Clonable Gold Label for Electron Microscopy
Mercogliano, C and DeRosier, DJ
J Struct Biol 160, 70-82, 2007.

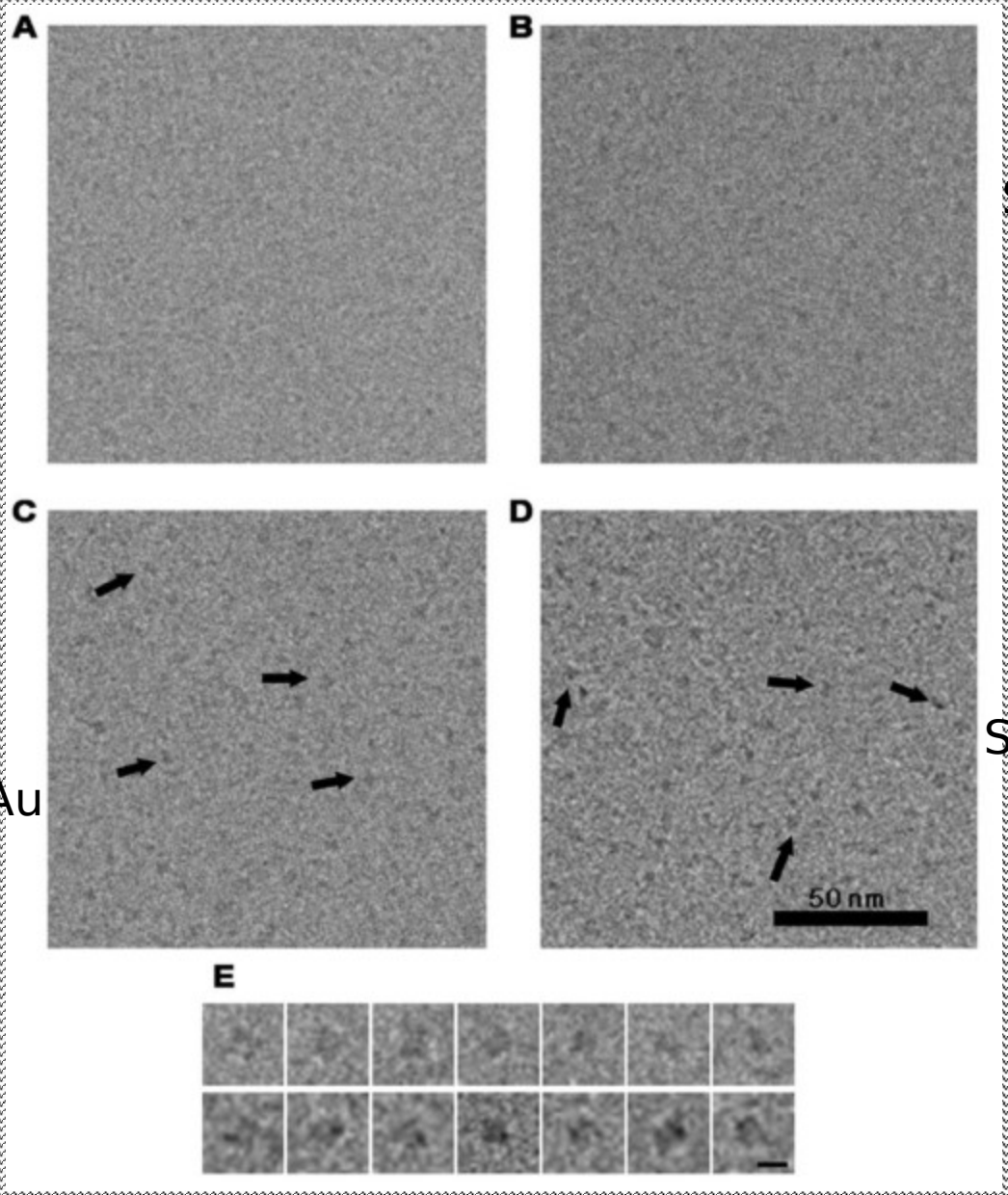
BHK21 cells with viral protein P150-MT-GFP



Cells tolerate 1 mM AuCl for at least 60 min with no obvious ill effects. An incubation of 15 to 30 min is sufficient for labeling.

Specific, Sensitive, High-Resolution Detection of Protein Molecules in Eukaryotic Cells Using Metal-Tagging Transmission Electron Microscopy
Cristina Risco, Eva Sanmarti´n-Conesa, Wen-Pin Tzeng, Teryl K. Frey, Volker Seybold, and Raoul J. de Groot
Structure *20*, 759-766,2012

SecB/OmpA
110 kDa



SecB/OmpA-MT-Cd

~6Cd/MT

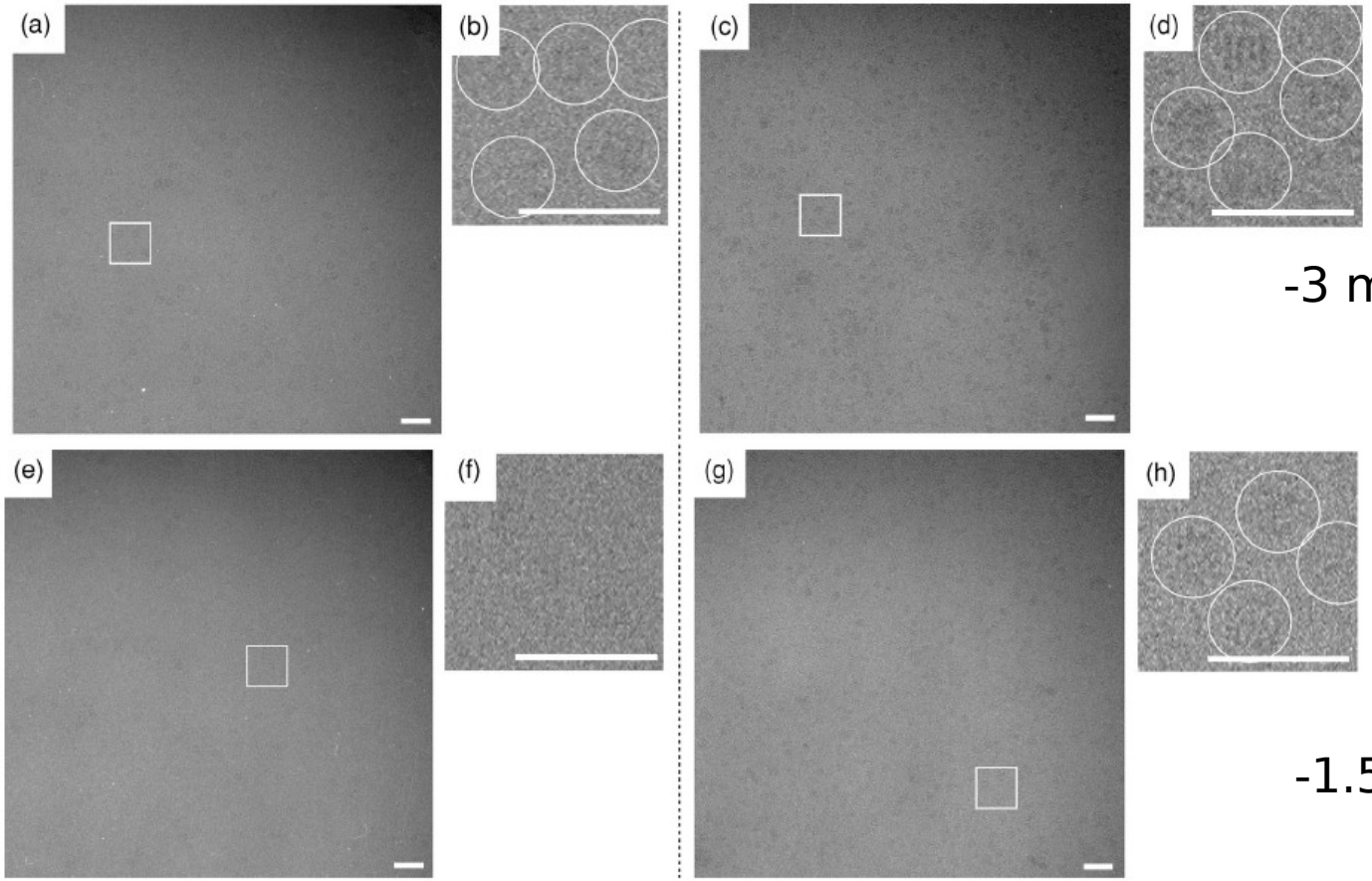
SecB/OmpA-MT-Au

SecB/OmpA-2MT-Au

~40Au/MT

Structural Characterization of the Complex of SecB and Metallothionein-Labeled proOmpA by Cryo-Electron Microscopy
Qiang Zhou1, Shan Sun1, Phang Tai2, Sen-Fang Sui1*
PLoS ONE 7, 1-10, 2012.

GroEL GroEL+3MT+Cd



-3 microns

-1.5 microns

Fig. 7. Detection of Cd^{2+}-bound 3MT-tagged GroEL by cryo-TEM. Frozen GroEL and Cd^{2+}-bound GroEL-14(3MT) particles were imaged with a cryoelectron microscope at 200 kV. Typical images of GroEL (a, b, e, and f) and Cd^{2+}-bound GroEL-14(3MT) (c, d, g, and h) at 3 μm underfocus (a–d) and at 1.5 μm underfocus (e–h) are shown. The indicated boxes are enlarged in (b), (d), (f,) and (h). The circles indicate each particle of GroEL (b) or Cd^{2+}-bound GroEL-14(3MT) (d and h), and the scale bars represent 500 Å.

A genetically encoded metallothionein tag enabling efficient protein detection by electron microsco
Yuri Nishino Takuo Yasunaga and Atsuo Miyazawa
Journal of Electron Microscopy **56(3)**: 93–101 (2007)

Pd_017 5′-CCCUUUCUAUCCUCAAUGUACCAACAAAAAAUGUAUUCC-3′

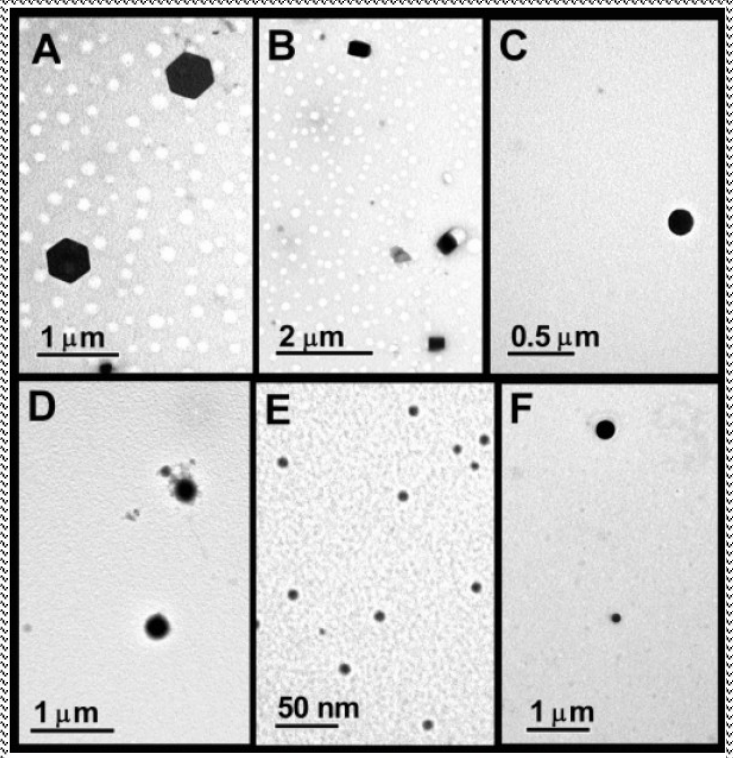
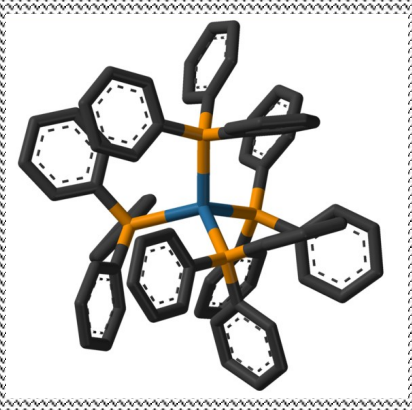


Figure 4. TEM images of particles formed with Pdase 017 and (A−C) $[Pt_2(DBA)_3]$, (D) $[Pd(PPh_3)_4]$, (E) $[Pt(PPh_3)_4]$, and (F) $[Ni(PPh_3)_4]$.

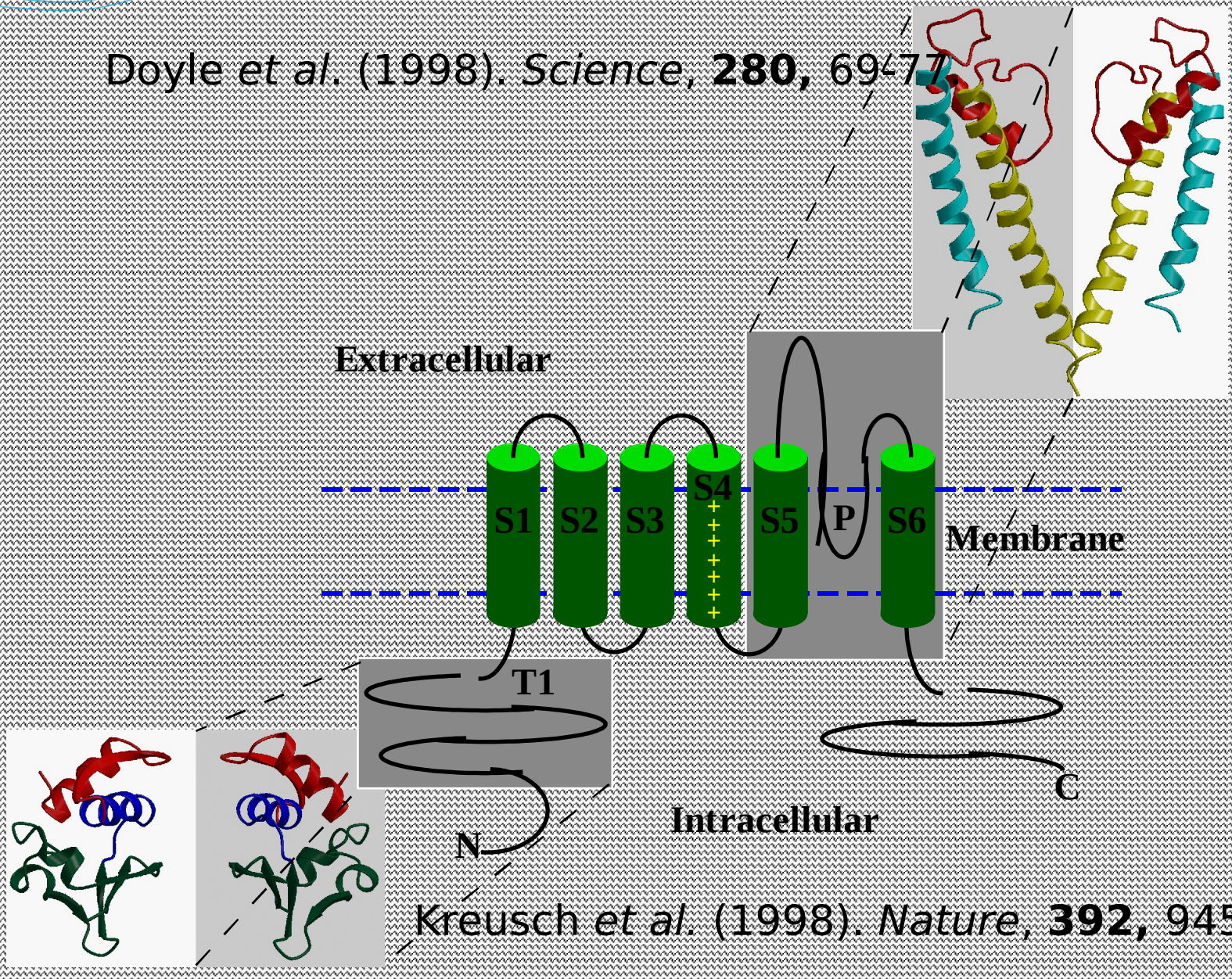


Triphenylphosphine-Pt

RNA-Mediated Control of Metal Nanoparticle Shape
Lina A. Gugliotti, Daniel L. Feldheim, and Bruce E. Eaton
JACS 127, 17814-17818, 2005

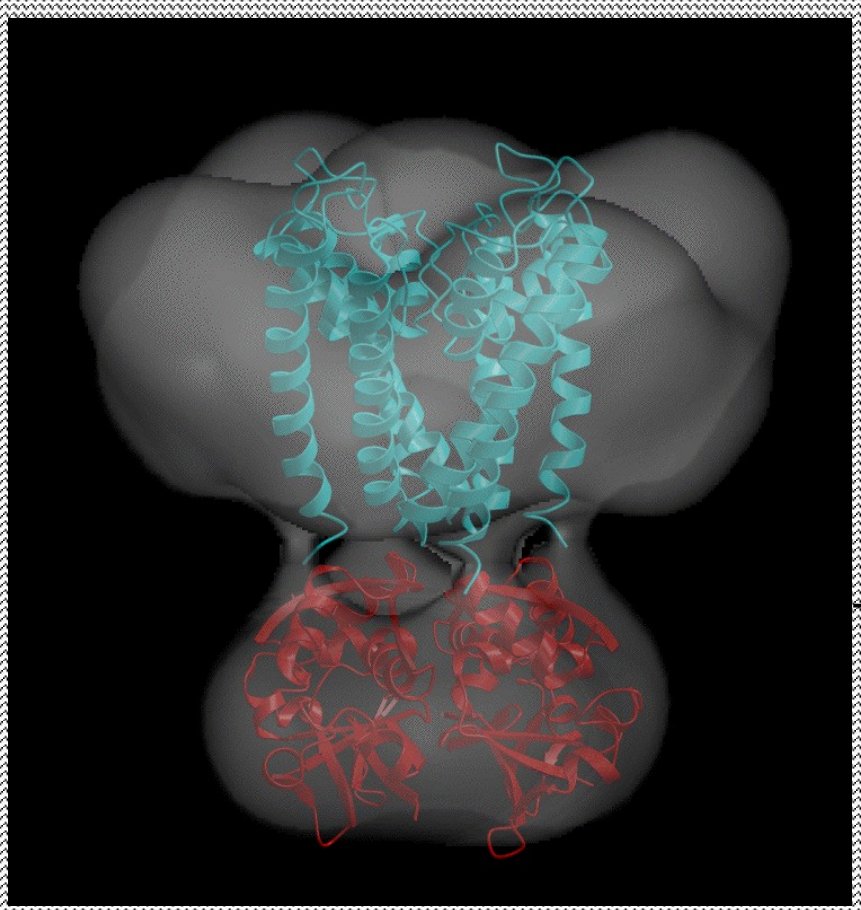
1. Addition/subtraction of components
2. Tagging of components
3. Size/Shape/Charge

Doyle *et al.* (1998). *Science*, **280,** 69-77.



Kreusch *et al.* (1998). *Nature*, **392,** 945-48.

cture of the eukaryotic K channel interpreted using the homologous bacterial cha



Bacterial channel (atomic structure in blue) is transmembrane and too big to fit in lower density.

T1 (soluble) fits nicely into lower density.

Unfilled densities are the missing four helix bundles

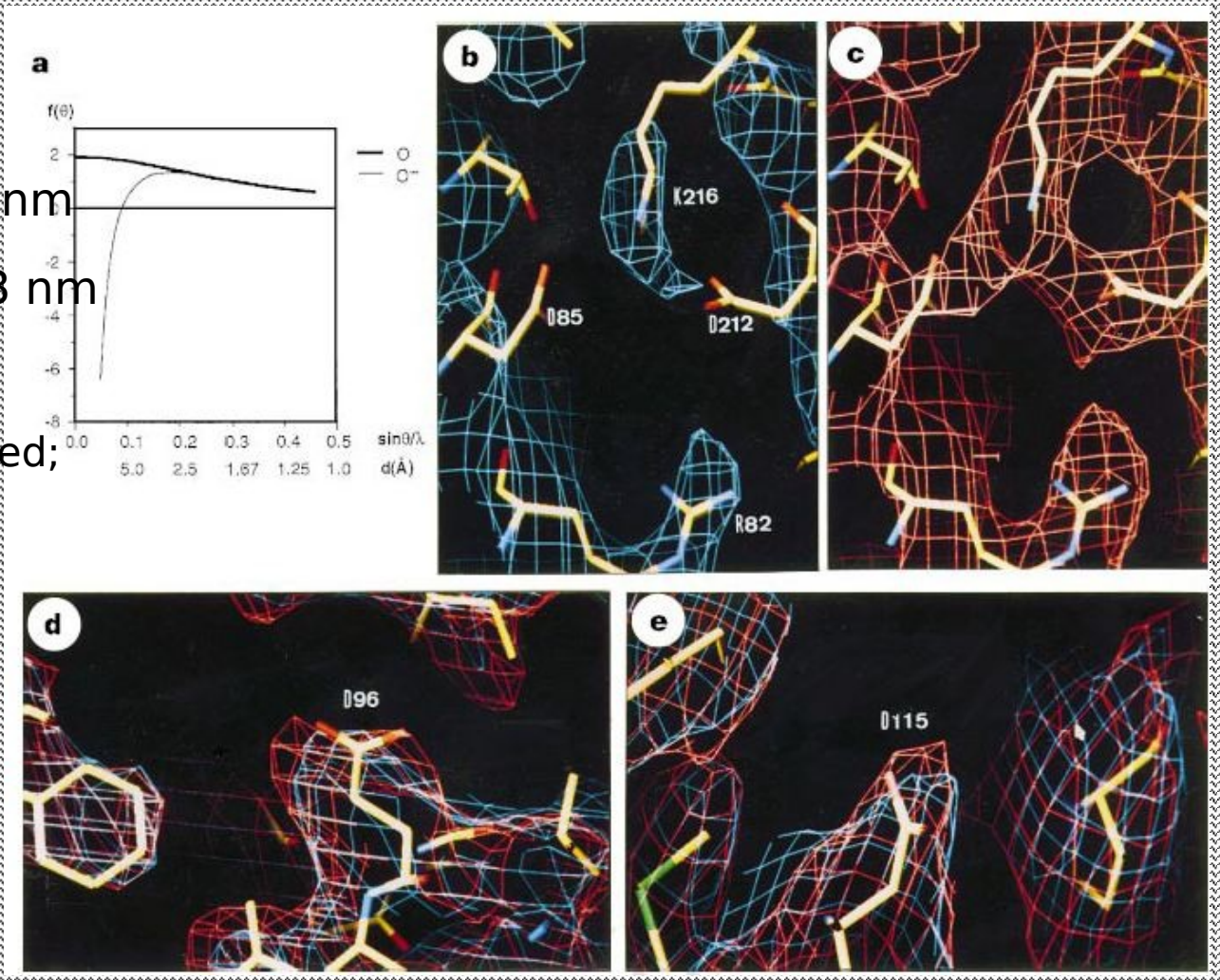
Three-dimensional structure of a voltage-gated potassium channel at 2.5 nm resolution
Sokola O, Kolmakova-Partensky L, Grigorieff N
Structure 9, 215-220, 2001

By charge

yan data from 5.4 to .3 nm

range data from .7 to .3 nm

sp85 and asp212 are charged;
p 96 and asp 115 are not
cording to IR spectroscopy



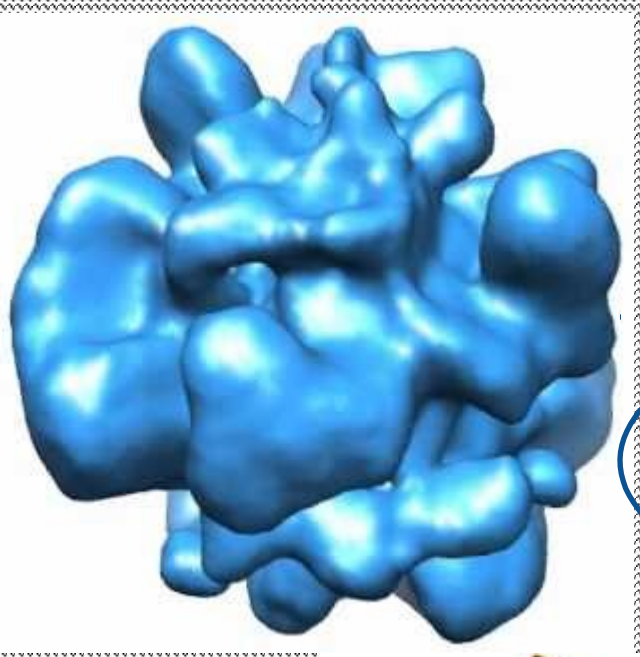
Surface of bacteriorhodopsin revealed by high-resolution electron crystallography
Kimura, Y *et al.*
Nature 389, 206-211, 1997

If none of these works,

1. Addition/subtraction of components
2. Tagging of components
3. Size/Shape/Charge

your last ditch effort is:

4.Divine intervention



That is the feature corresponding to P7!