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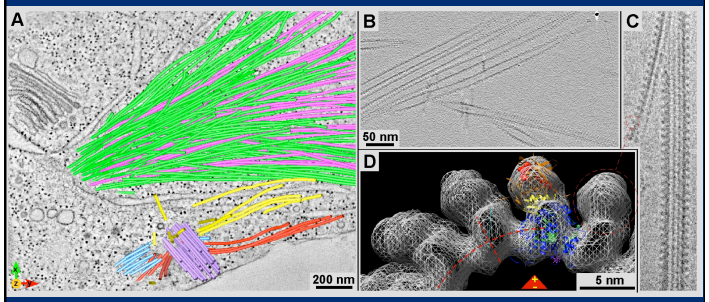
Dick McIntosh (Director emeritus)
Erin White (now Univ. of Chicago)
Jason Pierson (now Univ. of Amsterdam)
Mark Ladinsky (now CalTec)

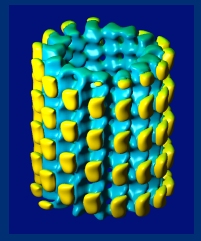
MCDB / NIH/NIGMS:

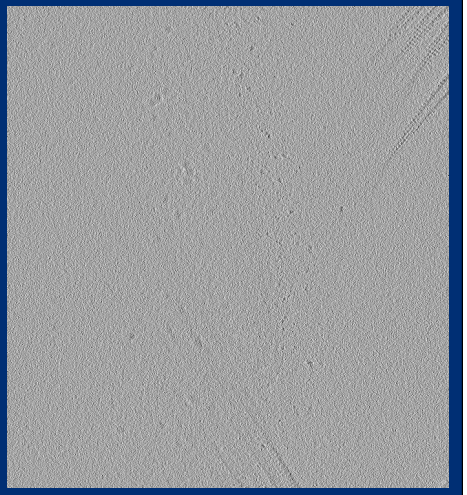
Cynthia Page
Maria Pagratis
Julia Cope
Cedric Bouchet-Marquis
Robert Kirmse
Sacha DeCarlo

NCRR: P41RR000592 to A.H.
NIH: R01GM080993 to A.H.
Biophysics Training Grant to J.C. and A.H.



Tomography of Eg5-motors bound to microtubules



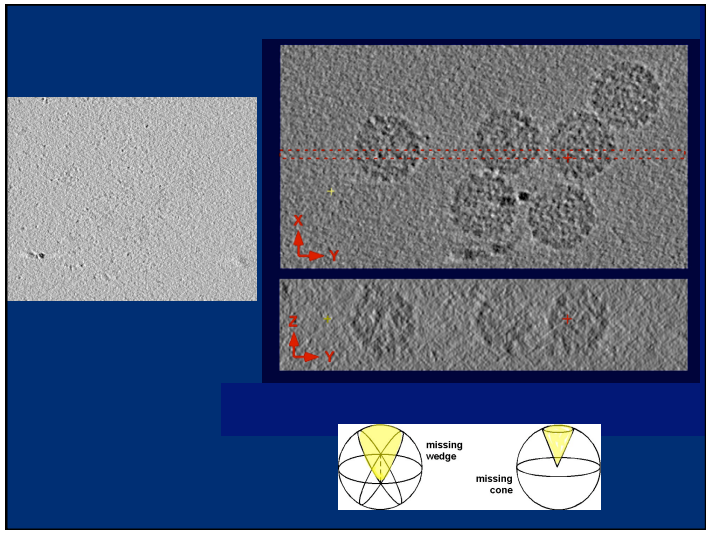


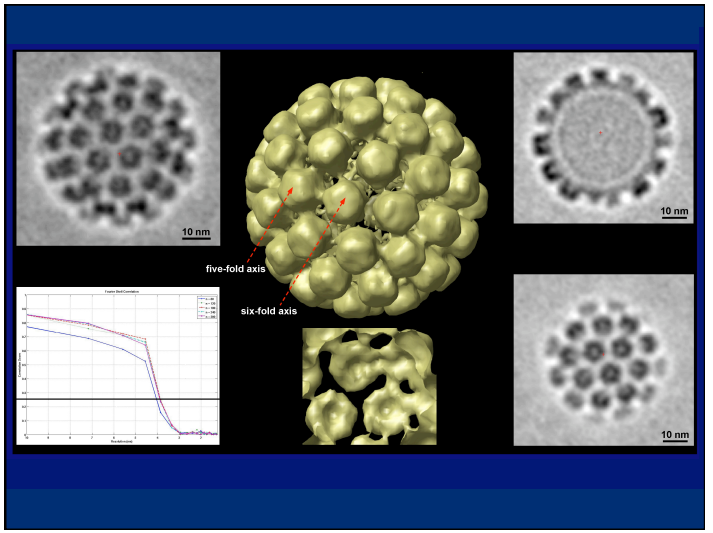
PEET
(Particle Estimation for Electron Tomography): a New Software Package for 3D Alignment and Averaging of Volumes

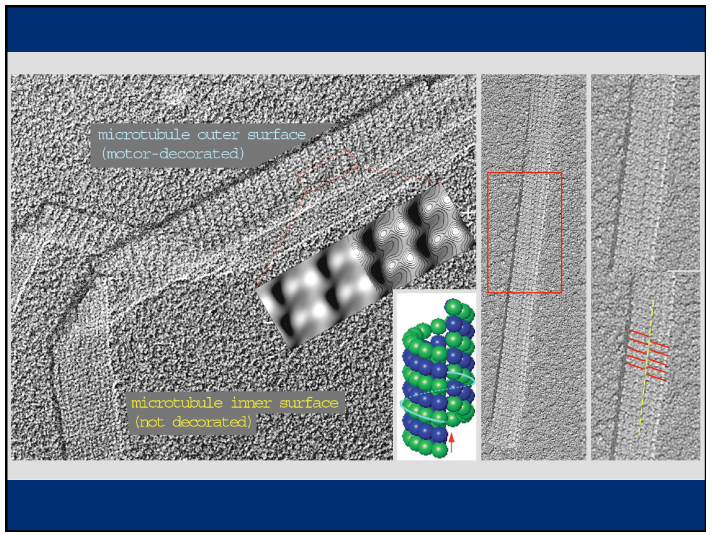
Q. Xiong*, R. Gaudette*, S. Held*, C. Schwartz*,
D. Nicastro#, J. Heumann, A. Hoenger*, and D. Mastronarde*

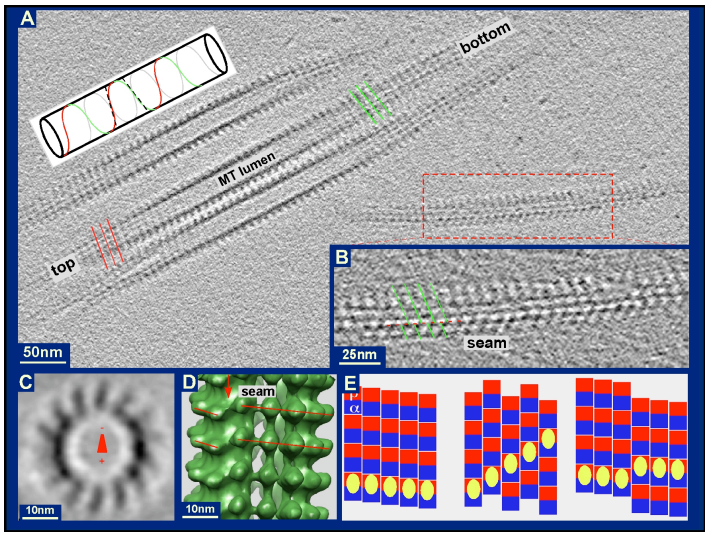
* Boulder Laboratory for 3D EM of Cells, Dept. of MCD Biology, University of Colorado, Boulder, CO

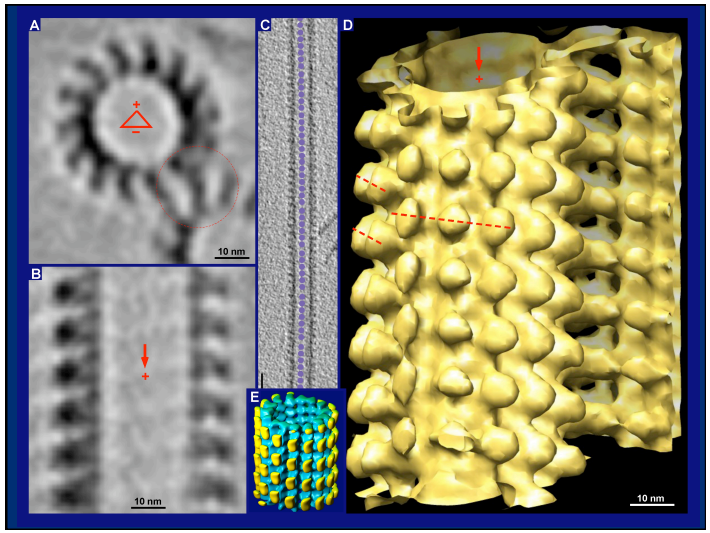
\# now at Dept. of Biology, Brandeis University, Boston, MA

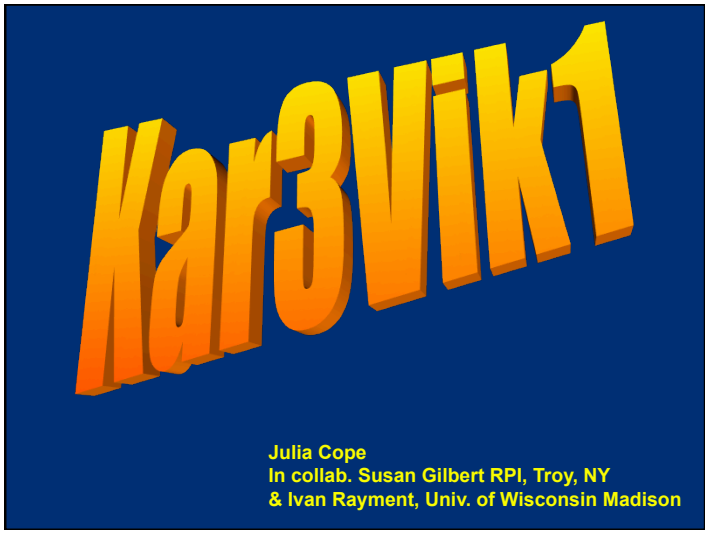

missing
wedge
missing
cone

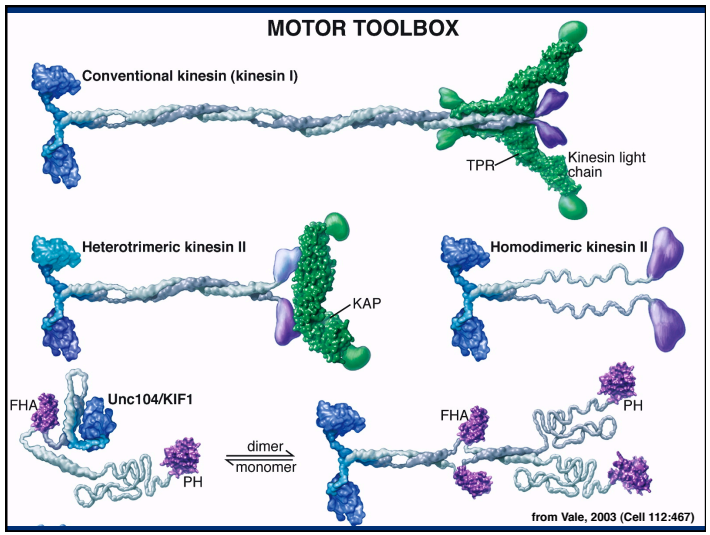

10 nm
five-fold axis
six-fold axis
10 nm
10 nm


microtubule outer surface
(motor-decorated)
microtubule inner surface
(not decorated)


A
bottom
MT lumen
top
50nm
B
seam
25nm
C
10nm
D
seam
10nm
E

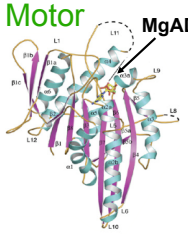






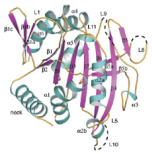
The Kar3/Vik1 Heterodimer

Motor



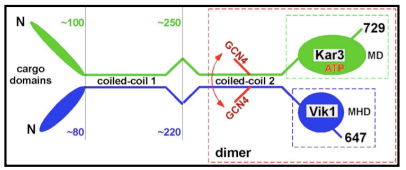
Kar3MD

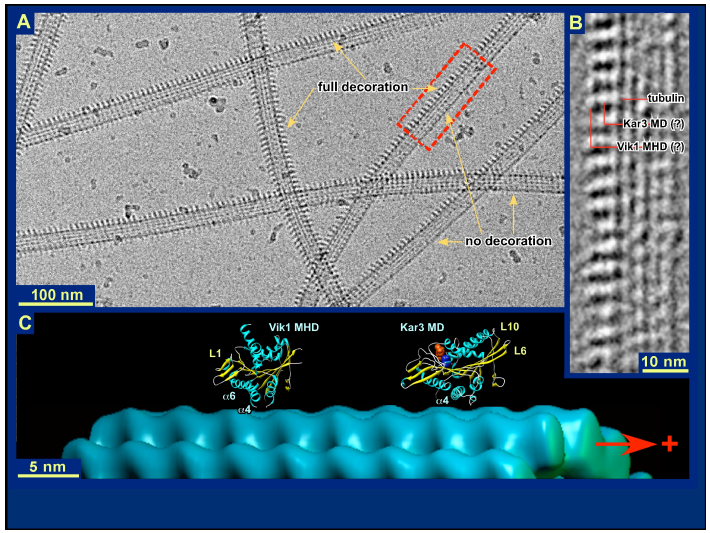
Non-Motor

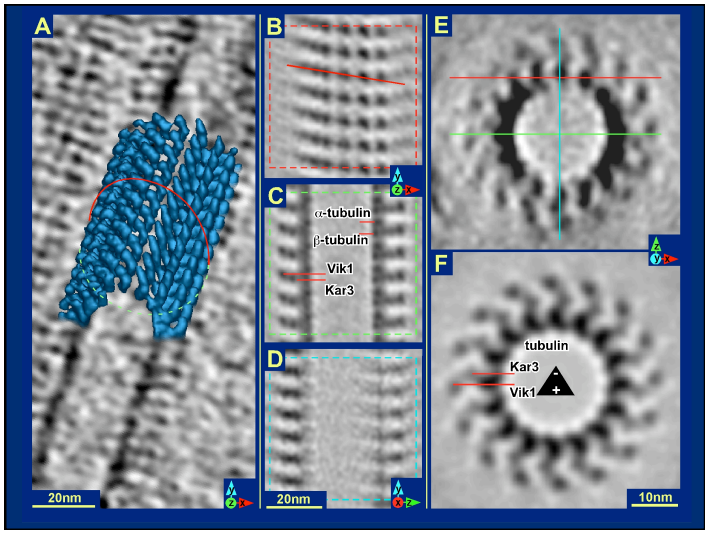


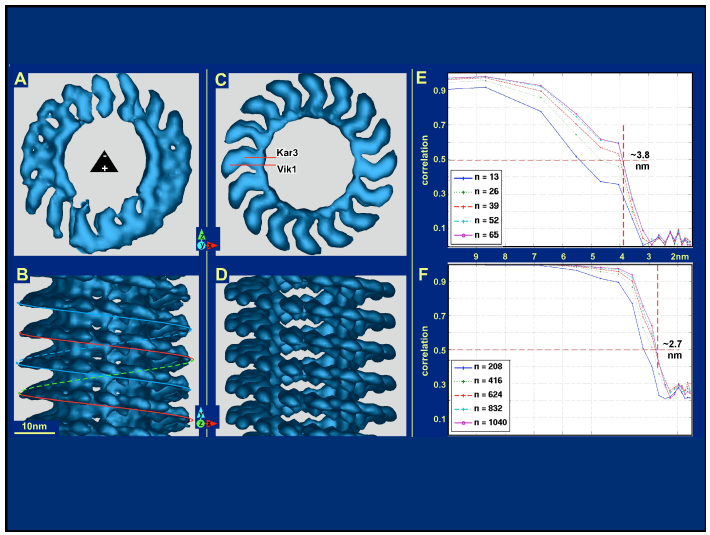
Vik1MHD

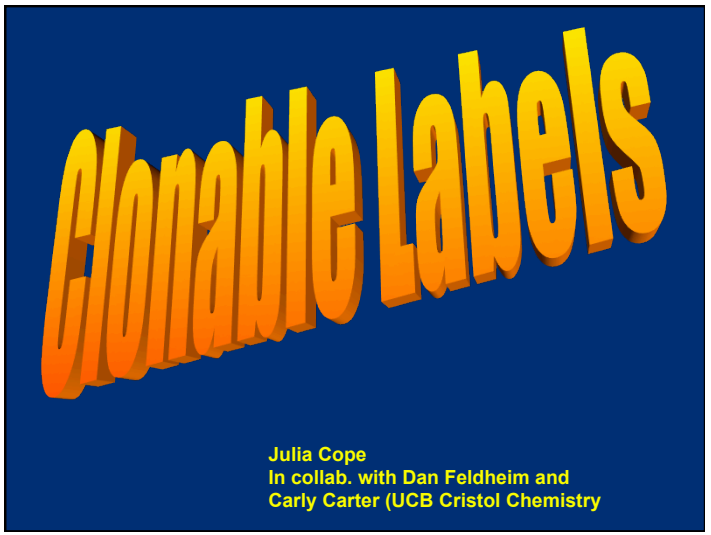
Figure adapted from Allingham *et al* (2007)




A
full decoration
no decoration
100 nm
B
tubulin
Kar3 MD (?)
Vik1 MHD (?)
10 nm
C
Vik1 MHD
Kar3 MD
L1
L10
L6
α6
α4
α4
5 nm
+


A
B
C
α-tubulin
β-tubulin
Vik1
Kar3
D
E
F
tubulin
Kar3
Vik1
+
20nm
20nm
10nm


A
C
Kar3
Vik1
B
D
10nm
E
correlation
n = 13
n = 26
n = 39
n = 52
n = 65
~3.8 nm
2nm
F
correlation
n = 208
n = 416
n = 624
n = 832
n = 1040
~2.7 nm


Clonable Labels
Julia Cope
In collab. with Dan Feldheim and
Carly Carter (UCB Cristol Chemistry

Genetically Clonable Materials Enzymes and Ribozymes for Tomographic Imaging of Cellular Biomolecules

Department of Chemistry and Biochemistry


Dr. Dan Feldheim


Dr. Chris Ackerson


Ms. Carly Carter

Funded by the NIH (Development of High Resolution Probes for Cellular Imaging)

National Center for Research Resources

The Identification Problem

- Electron tomography can visualize biomolecules in their native cellular context with molecular resolution.
- Electron tomography typically *cannot* tell which molecule is which.

The identification problem in electron tomography is analogous to that in fluorescence microscopy, for which green fluorescent protein and other genetically encoded fluorescent protein tags ignited a revolution in biological imaging.

A set of genetically clonable tags for electron tomography would enable the localization and identification of essentially any protein or RNA within complex milieu, such as a whole cell or purified multi-biomolecule complex.

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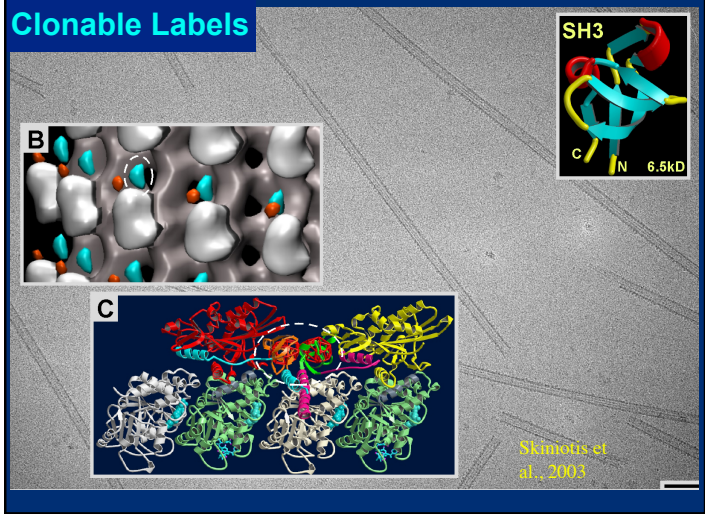
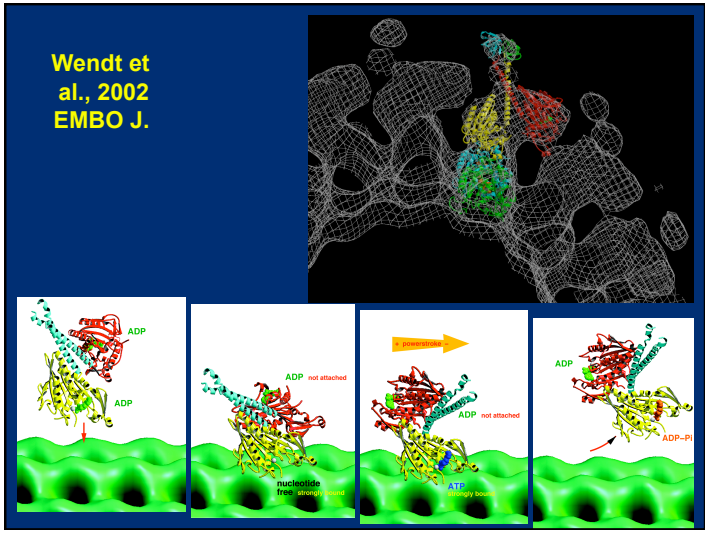
Clonable Labels

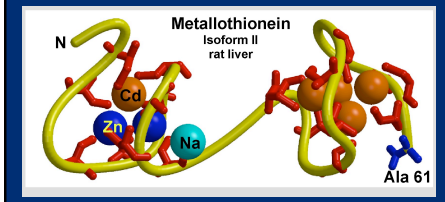


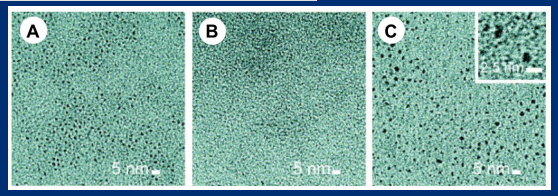
Skiniotis et al., 2003

Wendt et al., 2002 EMBO J.



Metallothionein as a clonable high-density label





Mercogliano & DeRosier, 2006

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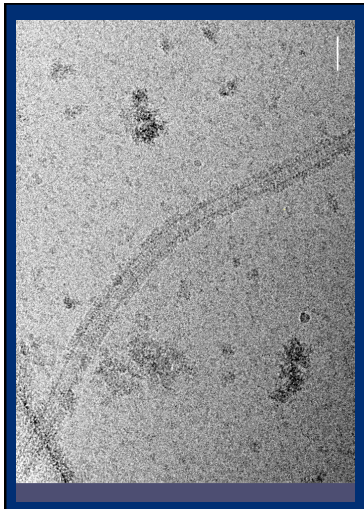
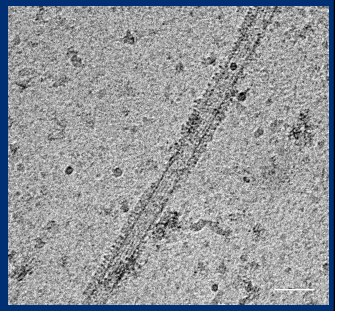
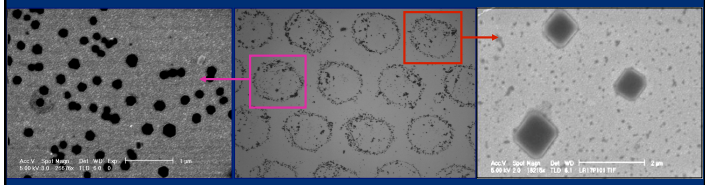




Eg5-367-MTH

1-Pot Synthesis of Shape Distinguishable Labels



150 μm

Pd source

RNA Code For Hexagons

RNA Code For Cubes

Gold

National Center for Research Resources

Peptides that generate metal nanoparticles

SLKMPHWPHLLP

AYSSGAPPMPPF

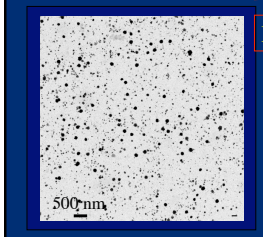
$AuCl_4^{1-}$

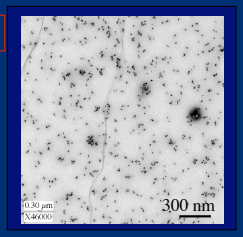
Ag^{1+}

Ag^{1+}

$AuCl_4^{1-}$

No Crosstalk





* Slocik et al. Small, 2005, 11, 1048.

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Current List of Materials Ribozymes/Enzymes

Enzymes

In hand: TiO_2, Au, Ag, GaO

In progress: Fe Oxide

Ribozymes

In hand: Pt (cubes and spheres),
Pd (cubes and hexagons)
Fe Oxide

In progress: ZnO

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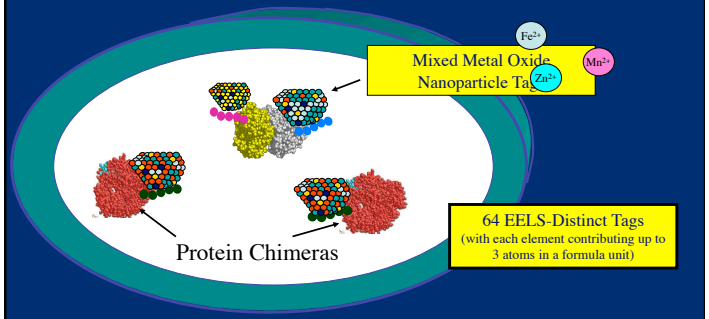
The Potential for Multiplexing

Consider a library of enzymes that combines 3 elements in different proportions



- 25 Tags could visually map ~1/2 of the proteins in the yeast genome in 100 experiments.
- Tags could be fluorescent (ZnO) as well as electron dense—localization on many length scales.

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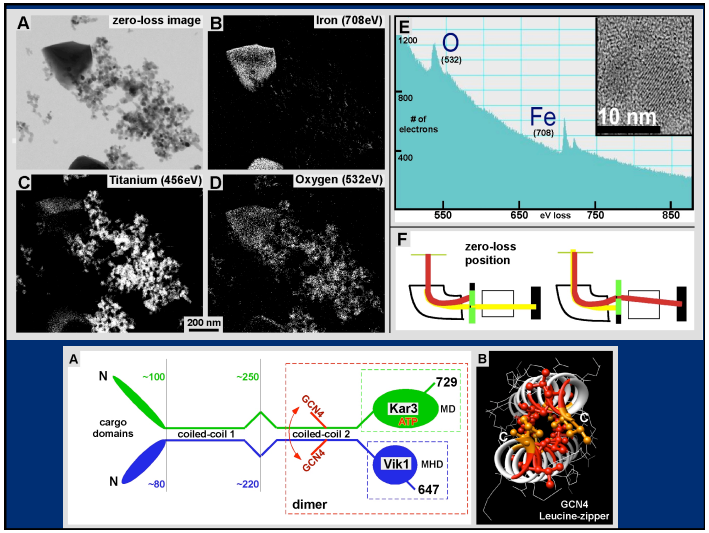
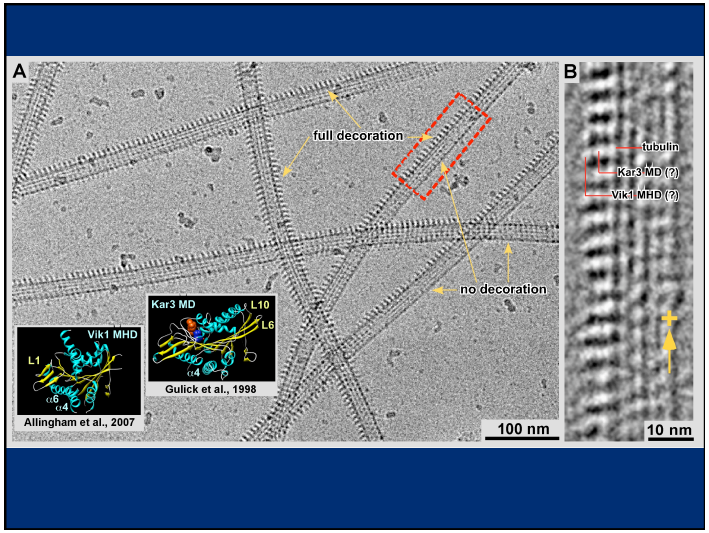
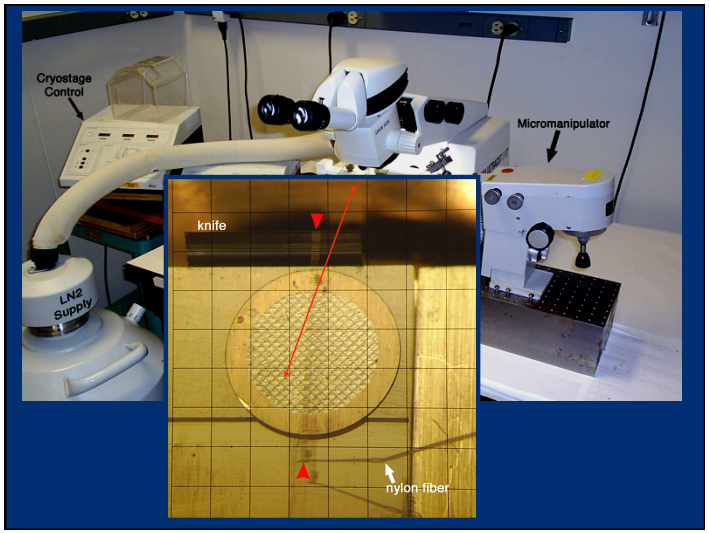
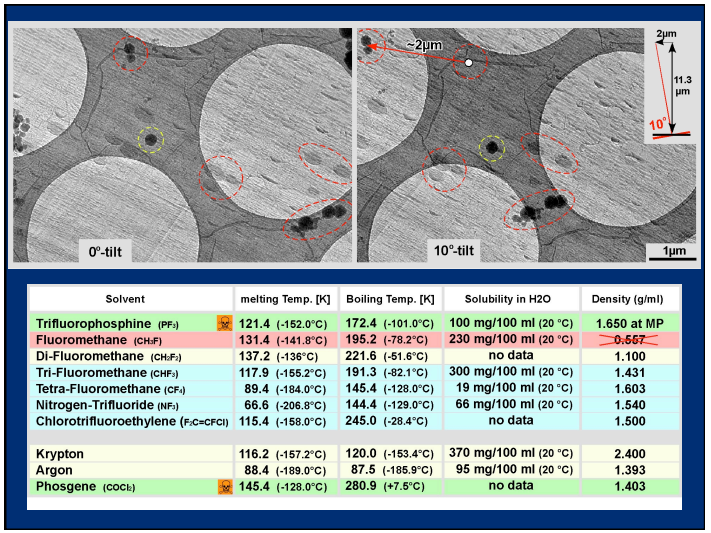
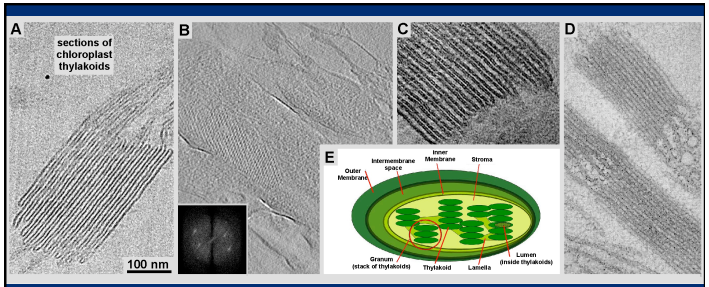






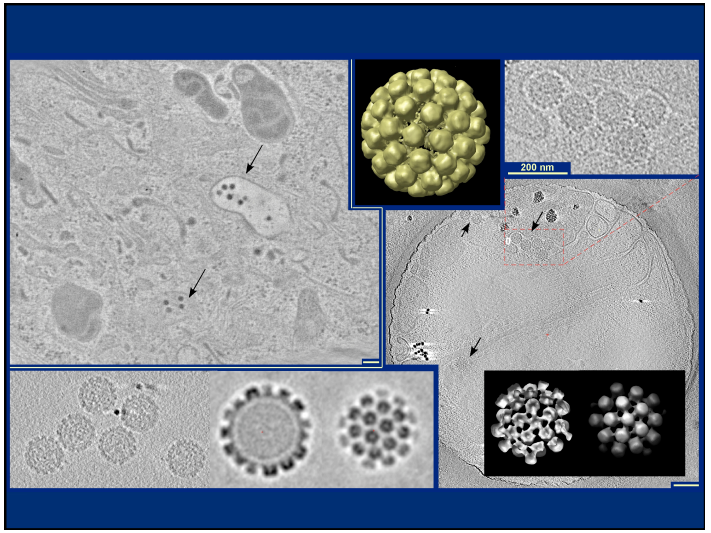


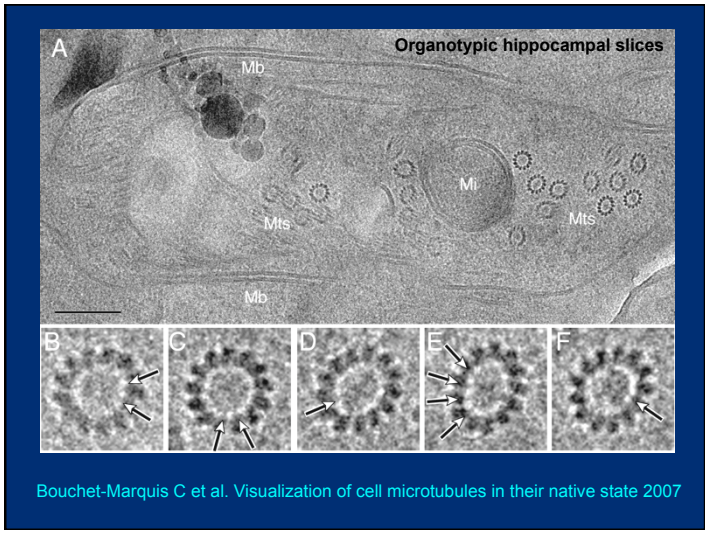
Solvent	melting Temp. [K]	Boiling Temp. [K]	Solubility in H2O	Density (g/ml)
Trifluorophosphine (PF_3)	121.4 (-152.0°C)	172.4 (-101.0°C)	100 mg/100 ml (20 °C)	1.650 at MP
Fluoromethane (CH_3F)	131.4 (-141.8°C)	195.2 (-78.2°C)	230 mg/100 ml (20 °C)	~~0.557~~
Di-Fluoromethane (CH_2F_2)	137.2 (-136°C)	221.6 (-51.6°C)	no data	1.100
Tri-Fluoromethane (CHF_3)	117.9 (-155.2°C)	191.3 (-82.1°C)	300 mg/100 ml (20 °C)	1.431
Tetra-Fluoromethane (CF_4)	89.4 (-184.0°C)	145.4 (-128.0°C)	19 mg/100 ml (20 °C)	1.603
Nitrogen-Trifluoride (NF_3)	66.6 (-206.8°C)	144.4 (-129.0°C)	66 mg/100 ml (20 °C)	1.540
Chlorotrifluoroethylene ($F_2C=CFCl$)	115.4 (-158.0°C)	245.0 (-28.4°C)	no data	1.500
Krypton	116.2 (-157.2°C)	120.0 (-153.4°C)	370 mg/100 ml (20 °C)	2.400
Argon	88.4 (-189.0°C)	87.5 (-185.9°C)	95 mg/100 ml (20 °C)	1.393
Phosgene ($COCl_2$)	145.4 (-128.0°C)	280.9 (+7.5°C)	no data	1.403



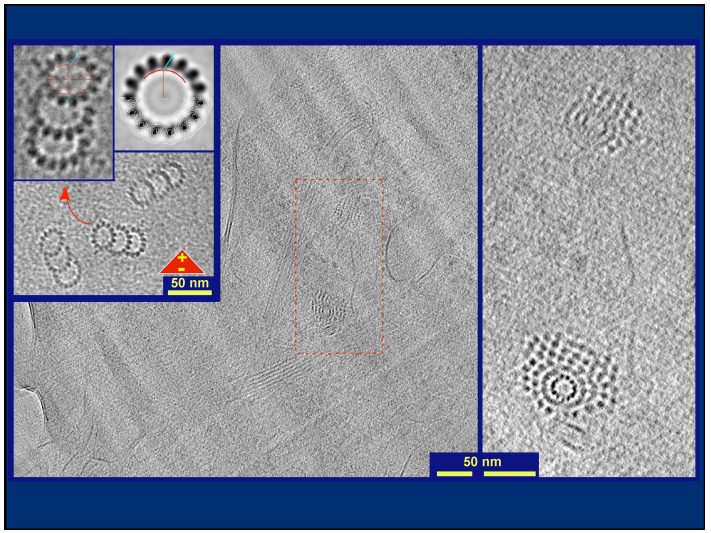
Preserving Vitrification during Freeze-Substitution

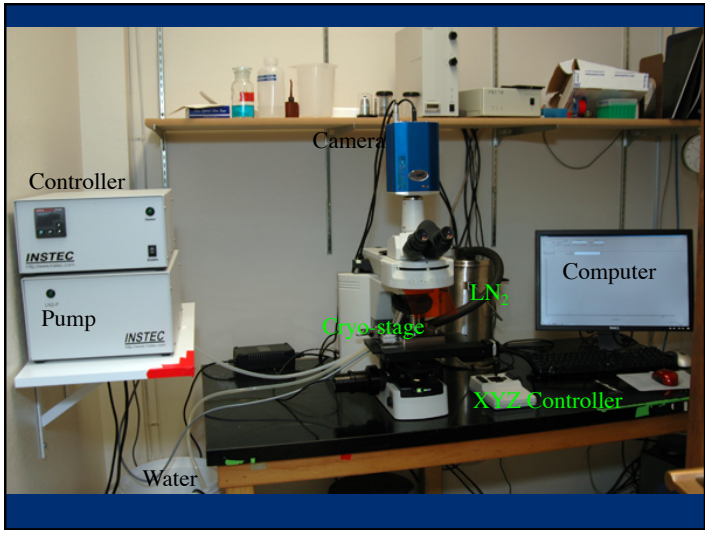
Group	Solvent	melting Temp. [K]	Boiling Temp. [K]	Solubility in H2O	Density (g/ml)
Alkohols:	Methanol	176.0 (-97.0 °C)	337.8 (+64.7 °C)	fully miscible	0.792 (+25°C)
	Ethanol	158.8 (-114.3°C)	351.6 (+78.4°C)	fully miscible	0.789 (+25°C)
	1-Propanol	146.7 (-126.5°C)	370.3 (+97.1°C)	fully miscible	0.804 (+25°C)
	Isopropanol	185.0 (-89.0°C)	355.0 (+82.3°C)	fully miscible	0.786 (+25°C)
Alkanes:	Butane	135.0 (-138.2°C)	272.6 (-0.5°C)	6.1 mg/100 ml (20 °C)	n/a
	Isobuthane	113.6 (-159.6°C)	261.5 (-11.7°C)	insoluble	0.593 (-20°C)
Ketones:	Acetone	178.2 (-94.9°C)	329.4 (+56.5°C)	fully miscible	0.790 (+25°C)
	Butanone	187.0 (-86.2°C)	353.0 (+80.2°C)	29 g/100 ml (20 °C)	0.805 (+25°C)
Ethers:	Di-methylether	134.6 (-138.5°C)	254.2 (+23.1°C)	328 mg/100 ml (20°C)	0.713 (+25°C)
Haloalkanes:	Fluoroform	117.9 (-155.2°C)	191.0 (-82.1°C)	100 mg/100 ml (20 °C)	1.520 (-100°C)
	Di-Fluoromethane	137.2 (-136°C)	221.6 (-51.6°C)	no data	1.100 (-100°C)
	Fluoromethane	131.4 (-141.8°C)	195.0 (-78.2°C)	230 mg/100 ml (20 °C)	0.557 (+25°C)

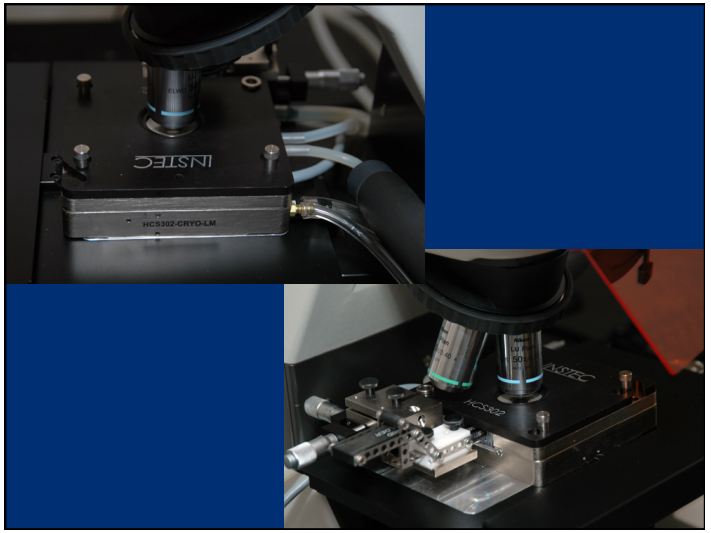


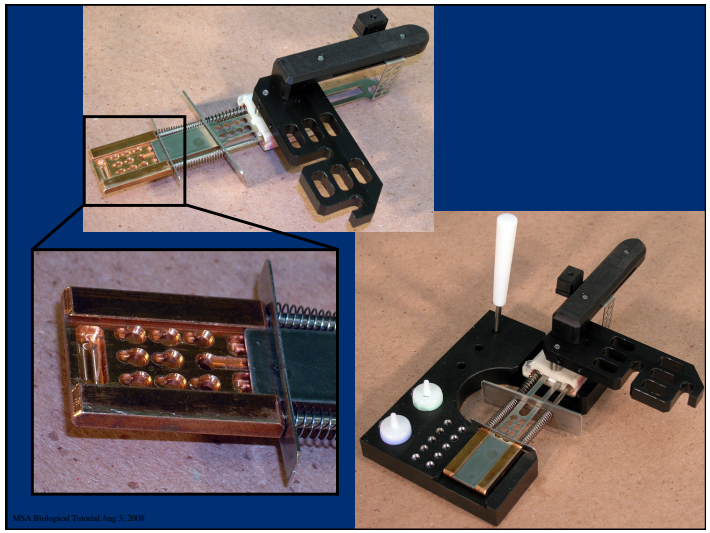


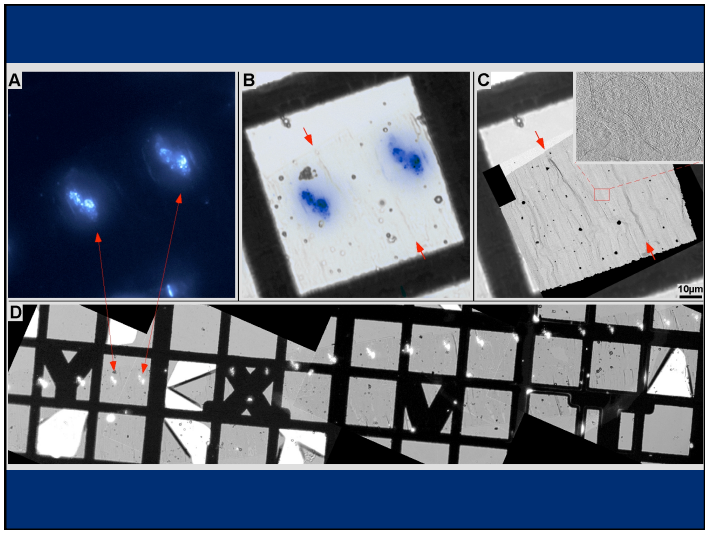
Bouchet-Marquis C et al. Visualization of cell microtubules in their native state 2007











Giardia

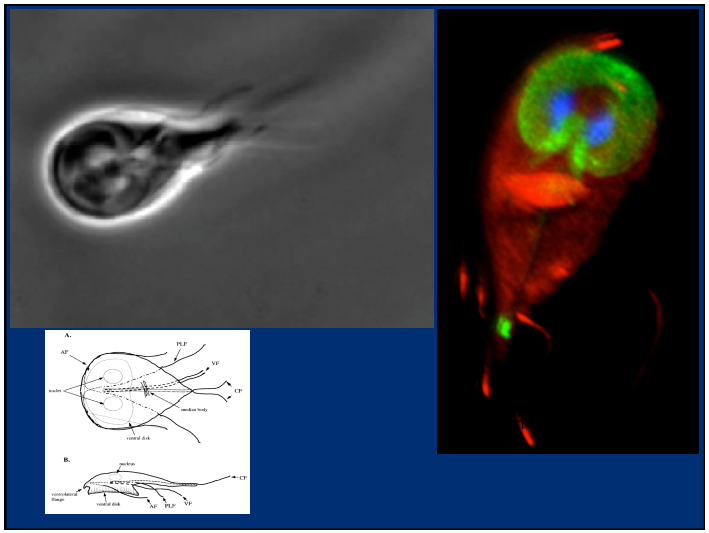
Cindi Schwartz
In collab. With Scott Dawson, UC Davis

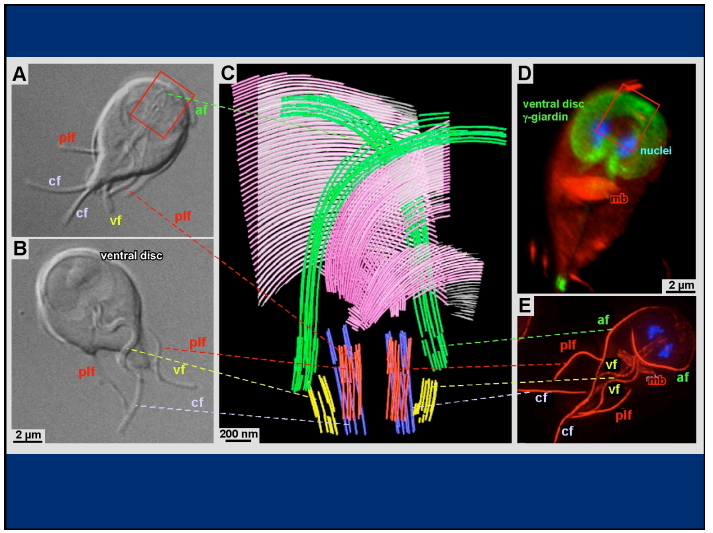
Giardia

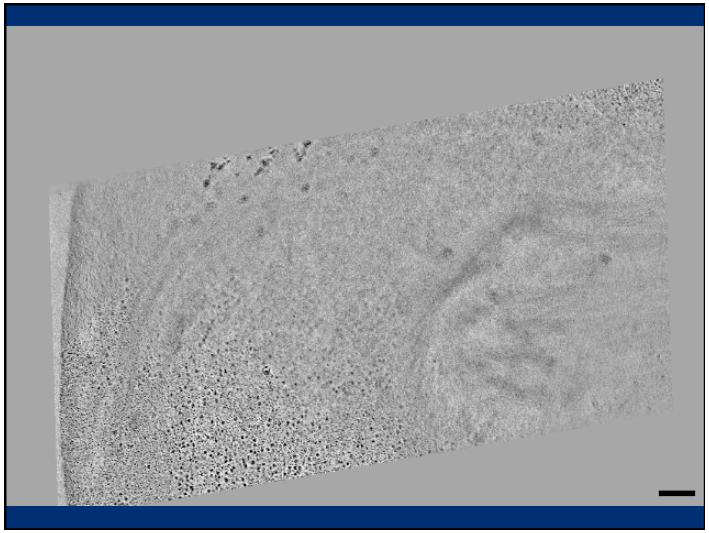
- Intestinal parasite of mammals, birds, and amphibians
- Giardia intestinalis = G. lamlia = G. duodenalis
- First discovered in 1681 by Antoni van Leeuwenhoek

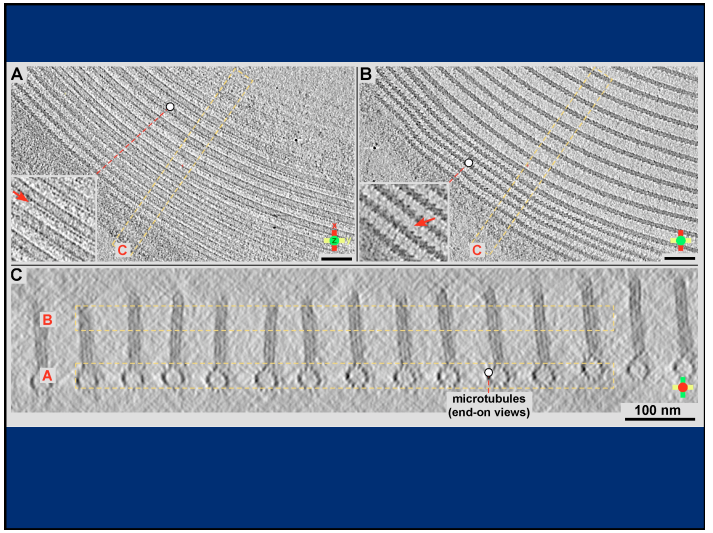
- Lifecycle:
  - cysts are ingested through contaminated water
  - excyst in duodenum
  - encyst as they pass through digestive tract
  - infection can have 14 billion cysts (typical is 300 million)
  - one parasite per epithelial cell
  - lives off of mucus secreted by cell
- Infection eliminated by Flagyl (metronidazole)

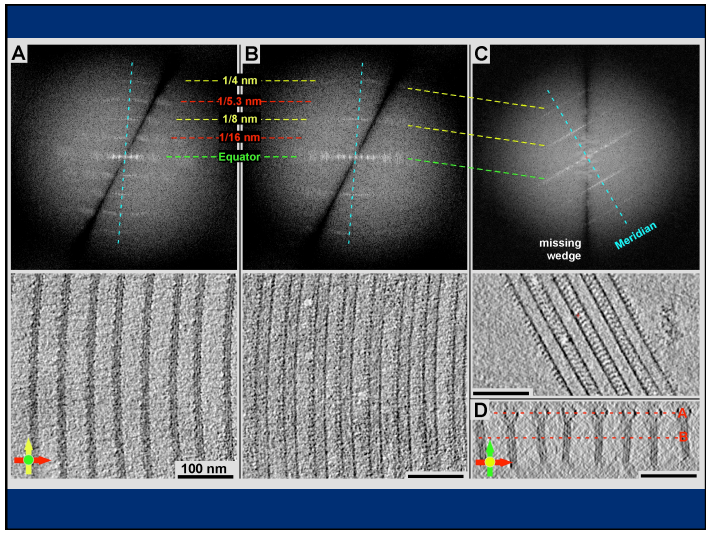
- In some areas of the world > 2/3 of population infected

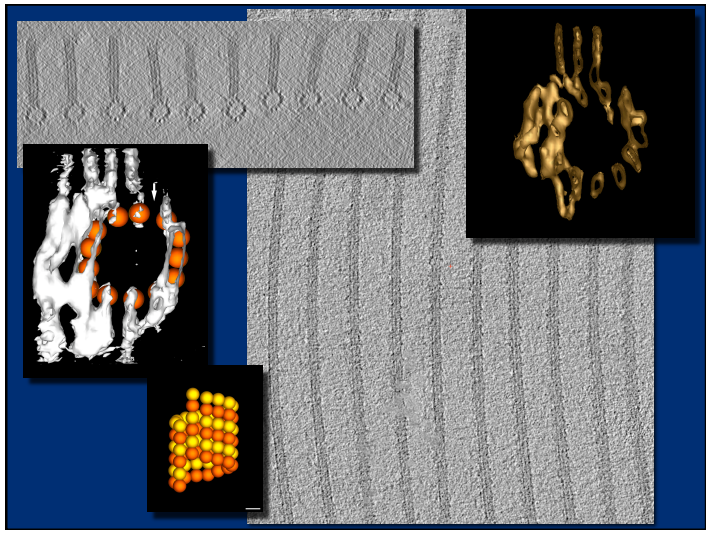

A.
AF
PLF
VF
nuclei
CF
median body
ventral disk
B.
nucleus
CF
ventral disk
AF
PLF
VF

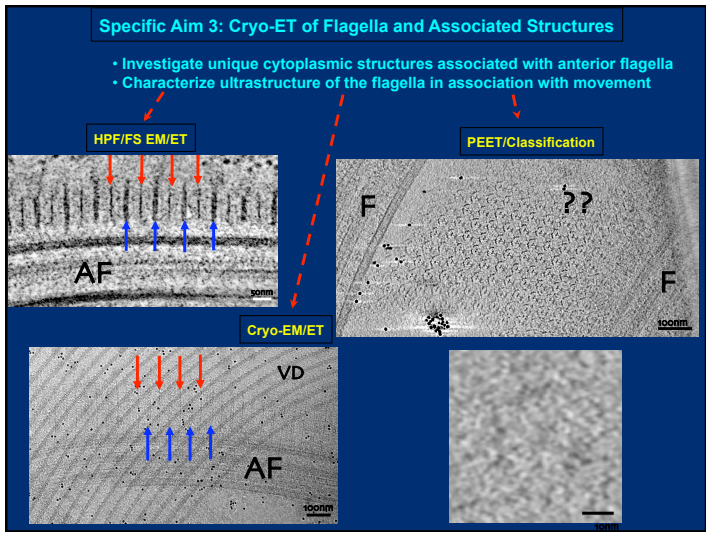

A
af
plf
cf
cf
vf
plf
B
ventral disc
plf
plf
vf
cf
2 µm
C
200 nm
D
ventral disc
γ-giardin
nuclei
mb
2 µm
E
af
plf
vf
mb
af
vf
cf
cf
plf

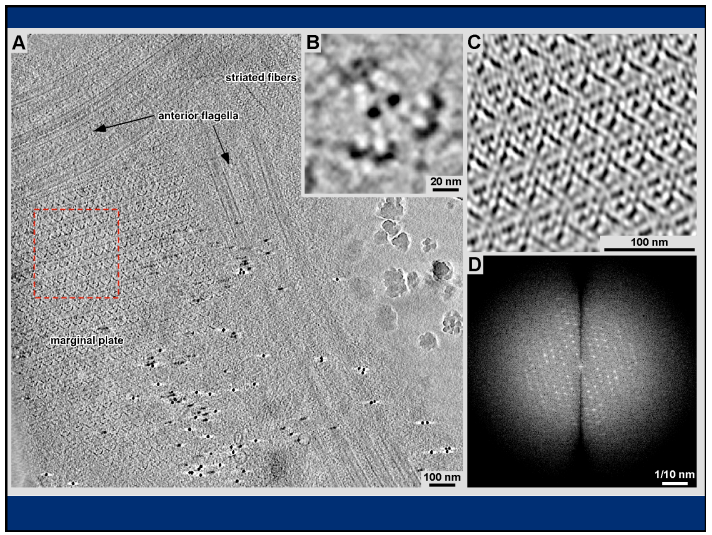



A
C
B
C
C
B
A
microtubules
(end-on views)
100 nm


A
B
C
1/4 nm
1/5.3 nm
1/8 nm
1/16 nm
Equator
missing wedge
Meridian
D
100 nm




Specific Aim 3: Cryo-ET of Flagella and Associated Structures
• Investigate unique cytoplasmic structures associated with anterior flagella
• Characterize ultrastructure of the flagella in association with movement
HPF/FS EM/ET
PEET/Classification
AF
F
??
F
Cryo-EM/ET
VD
AF


A
striated fibers
anterior flagella
marginal plate
100 nm
B
20 nm
C
100 nm
D
1/10 nm


that's it,
Thank You !!!