

Gulliver  
Multiscale Bioimaging Workshop

Visualizing Life

# Multi-scale imaging

Size (m)

$10^{-8}$

$10^{-7}$

$10^{-6}$

$10^{-5}$

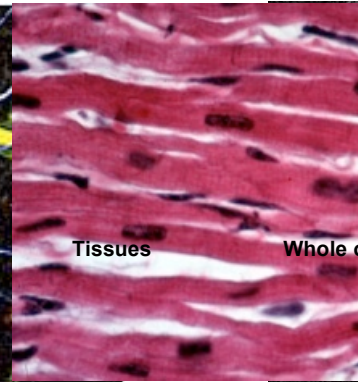
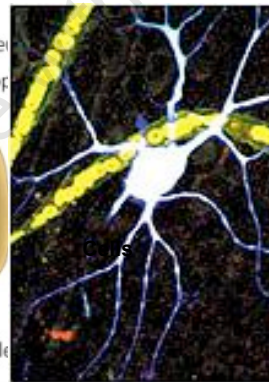
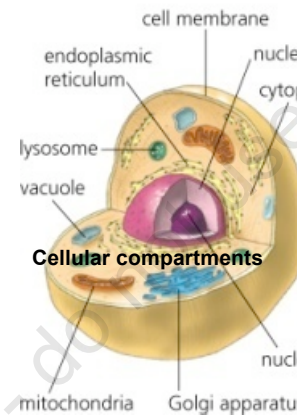
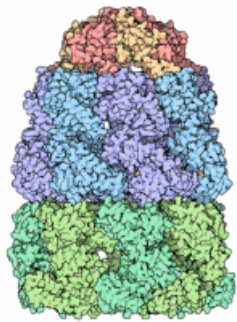
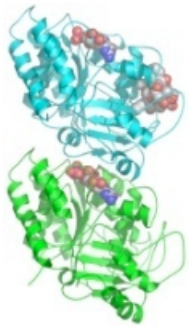
$10^{-4}$

$10^{-3}$

$10^{-2}$

$10^{-1}$

1



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Architecture and Dynamics and  
Interactions  
of Large Macromolecular Complexes:

**Nucleic Acid Transactions**  
and  
**Cytoskeleton in Cell division**

Eva Nogales  
UC Berkeley/HHMI  
Lawrence Berkeley National Lab

# Electron Microscopy and 3-D Reconstruction

- General Applicability

  - No **crystallization** is required

  - Applicable to **very large** complexes

  - Requires very **small amounts** of sample

- Study of Fully Assembled, Functional Complexes

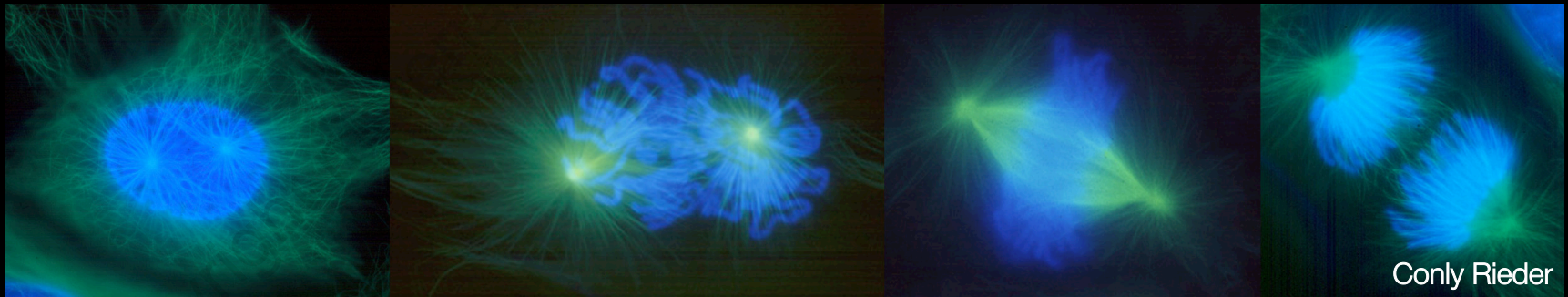
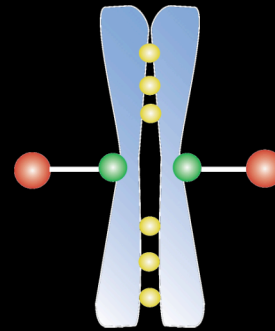
  - In near physiological conditions

  - In **different functional states**

  - Structural Basis of Function and Regulation

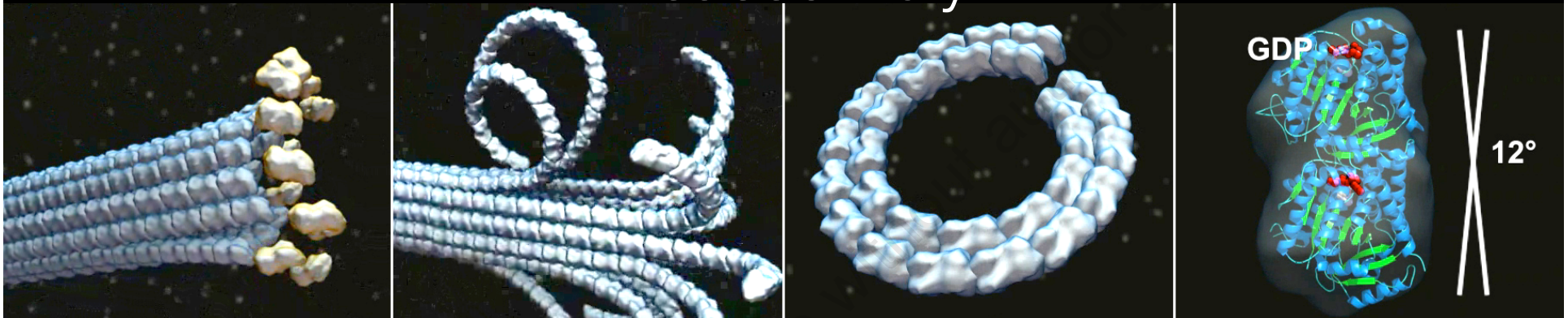


# Dynamic Coupling of Self-Assembly Processes in Chromosome Segregation

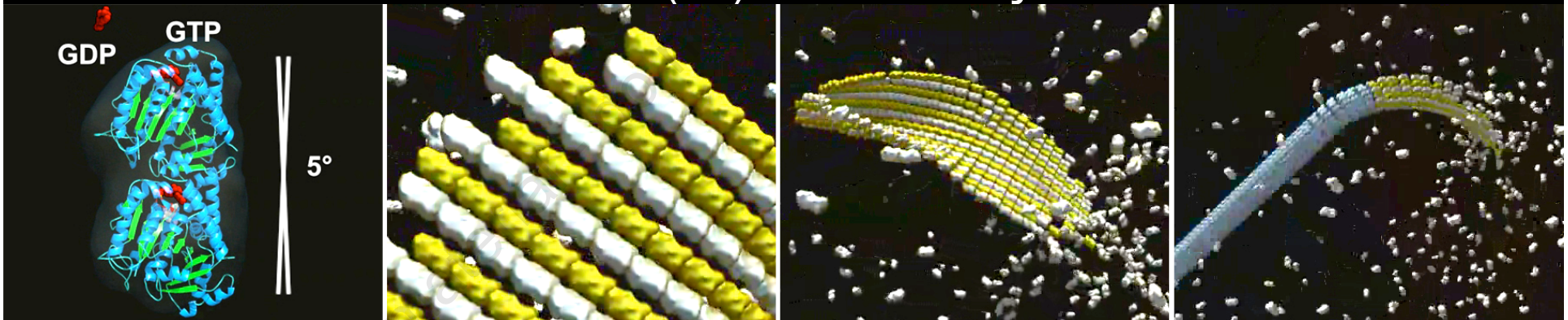


# Structural Intermediates in Microtubule

## Disassembly



## and (re)Assembly



**WHY?**

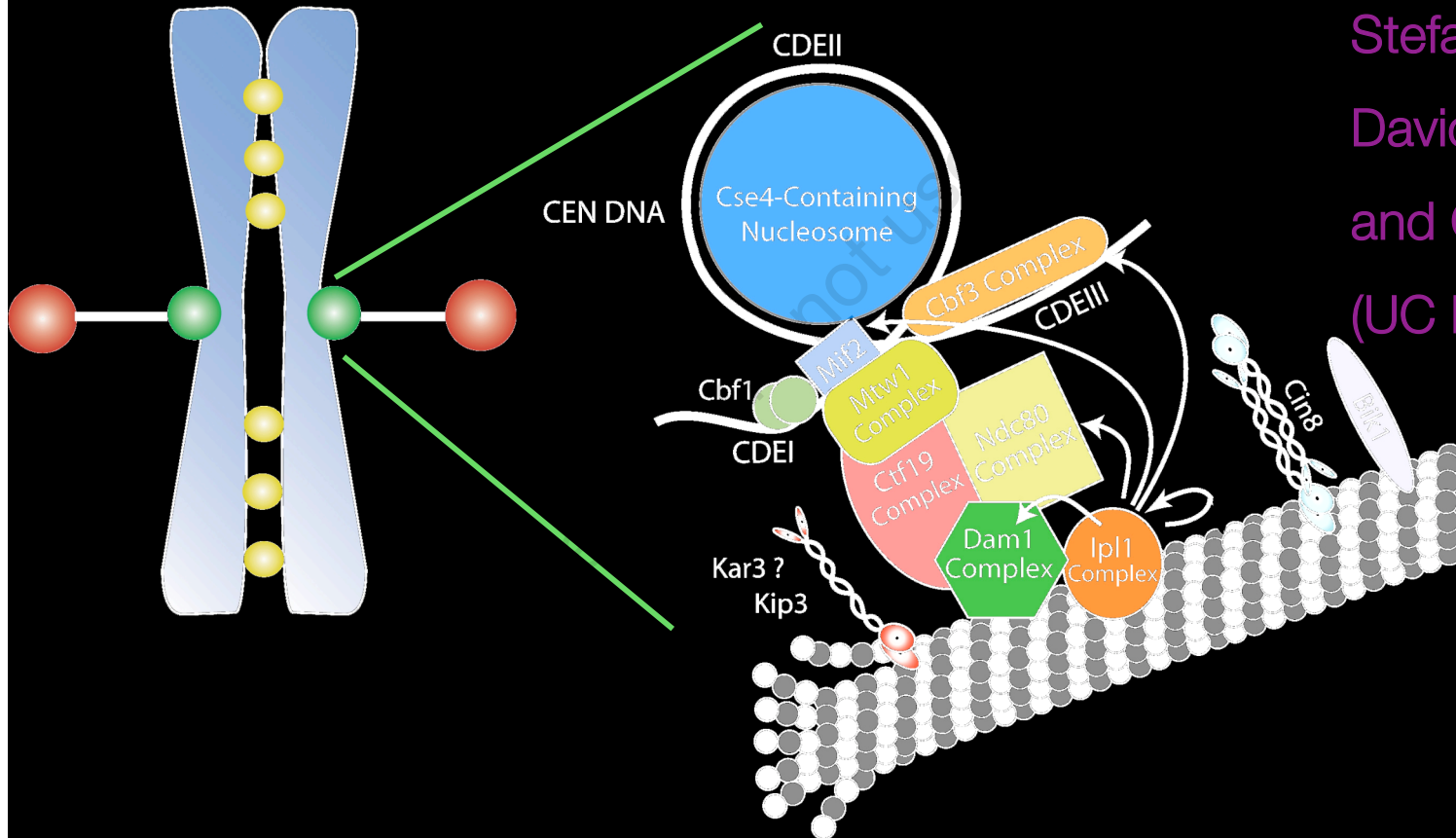
Disassembly Intermediates:  
Coupling of Depolymerization to Movement

# Kinetochore Structure and Function

Hong-Wei Wang

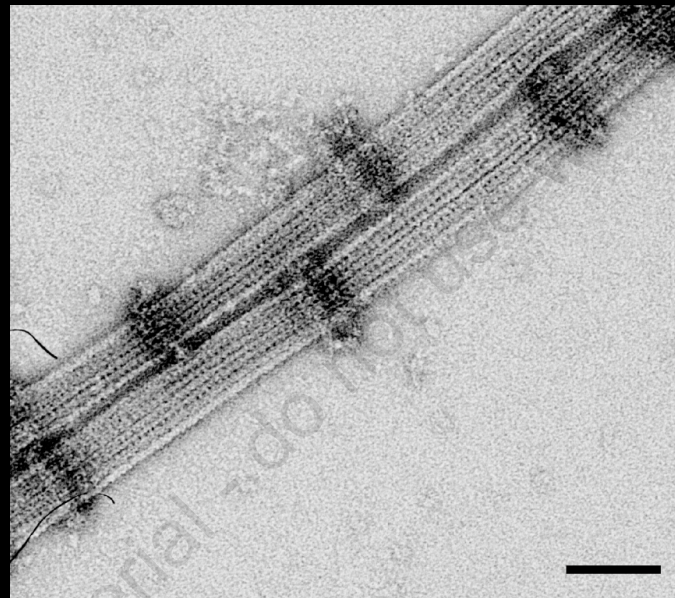
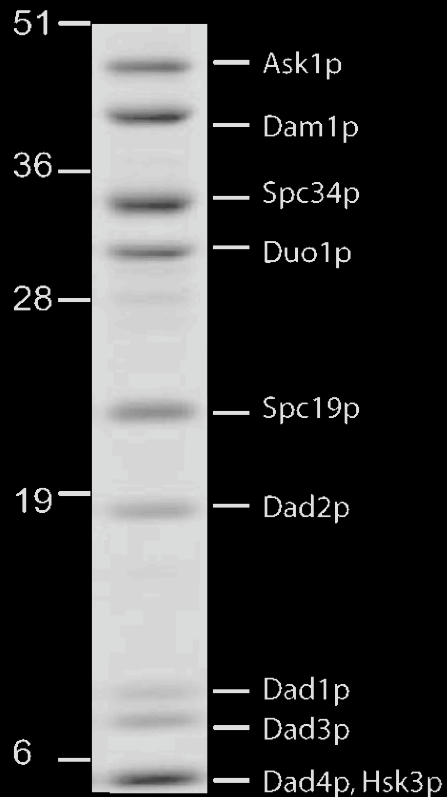
Vincent Ramey

Stefan Westermann  
David Drubin  
and Georjana Barnes  
(UC Berkeley)



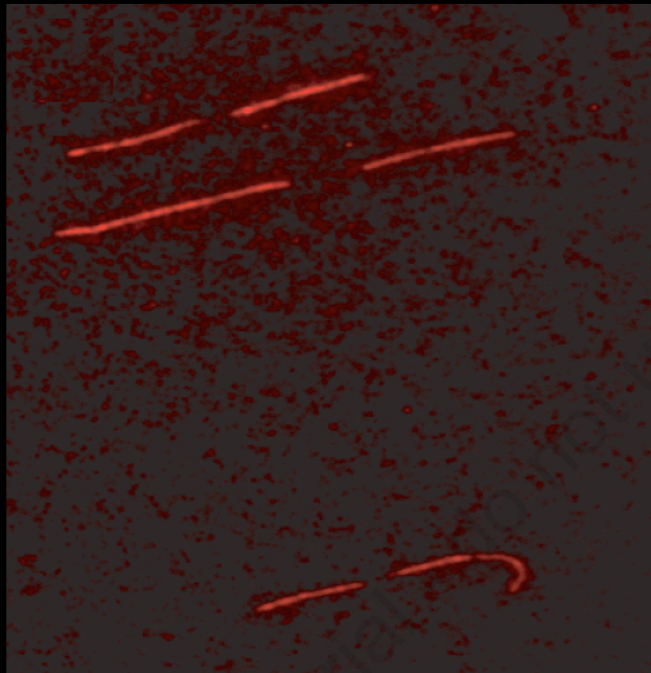


# The Yeast Kinetochores Complex Dam1



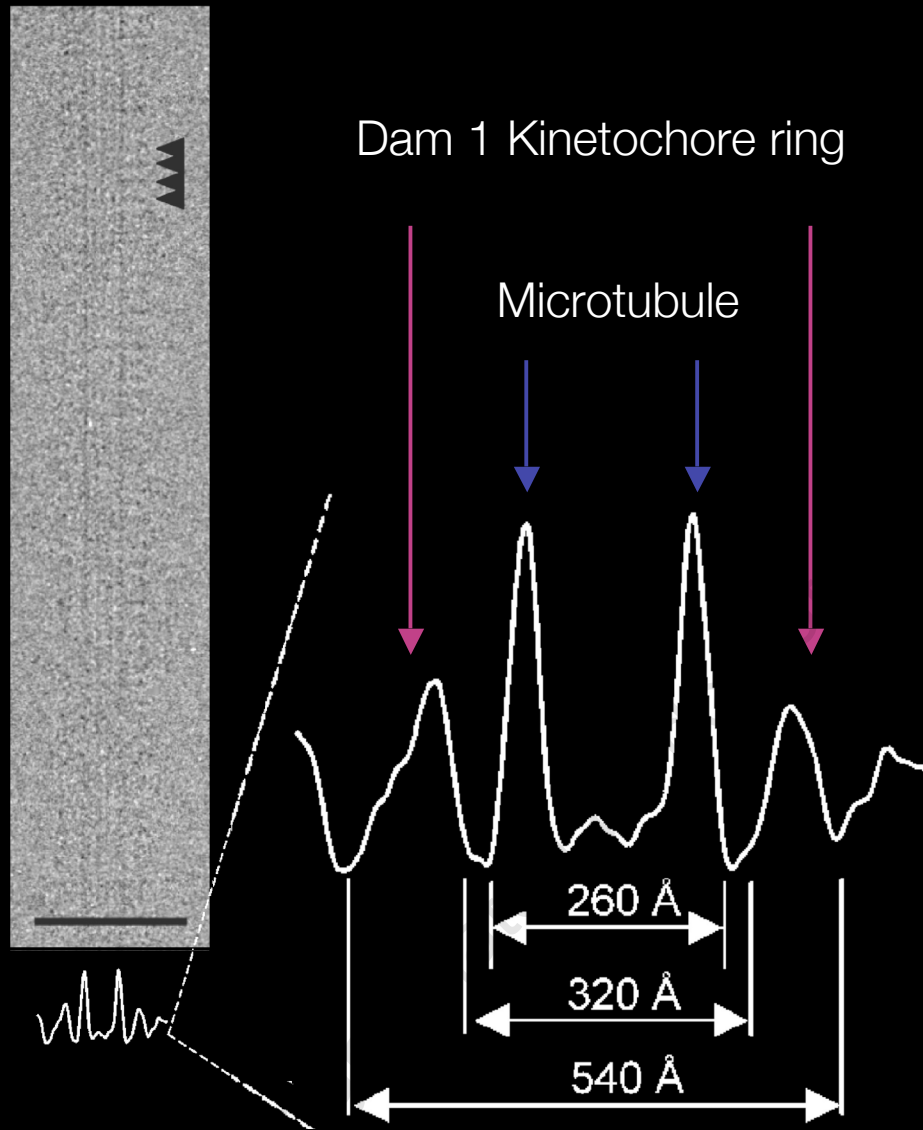
The Dam-1 complex self-assembles into Rings and Spirals in a Microtubule-dependent manner

# Binding of Dam 1 to GMPCPP-microtubules

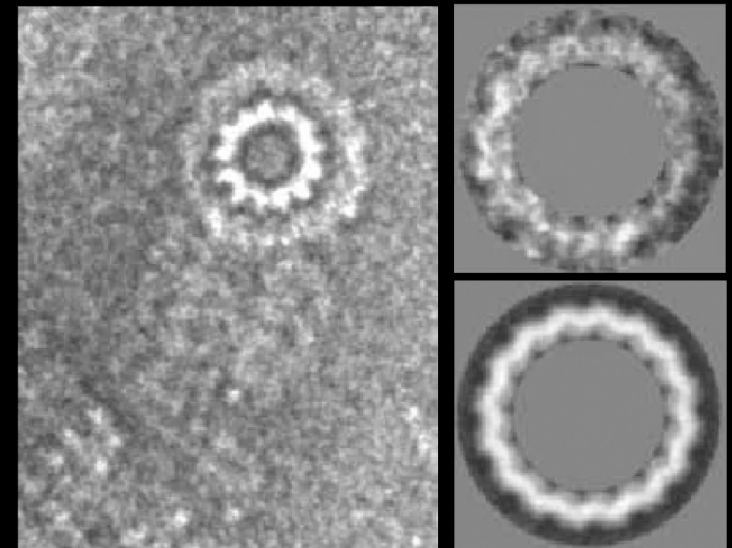


segmented Microtubules

# Dam1 Ring - Microtubule Interaction

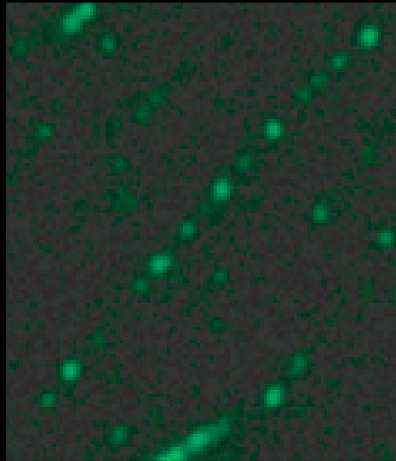


## Microtubule and Dam1 ring lattice mismatch

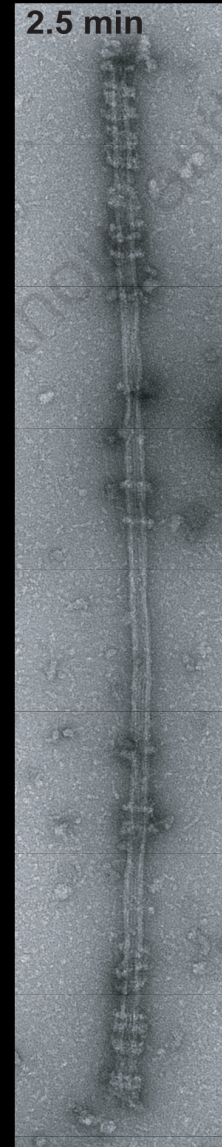


13-fold MT  
16-fold Ring

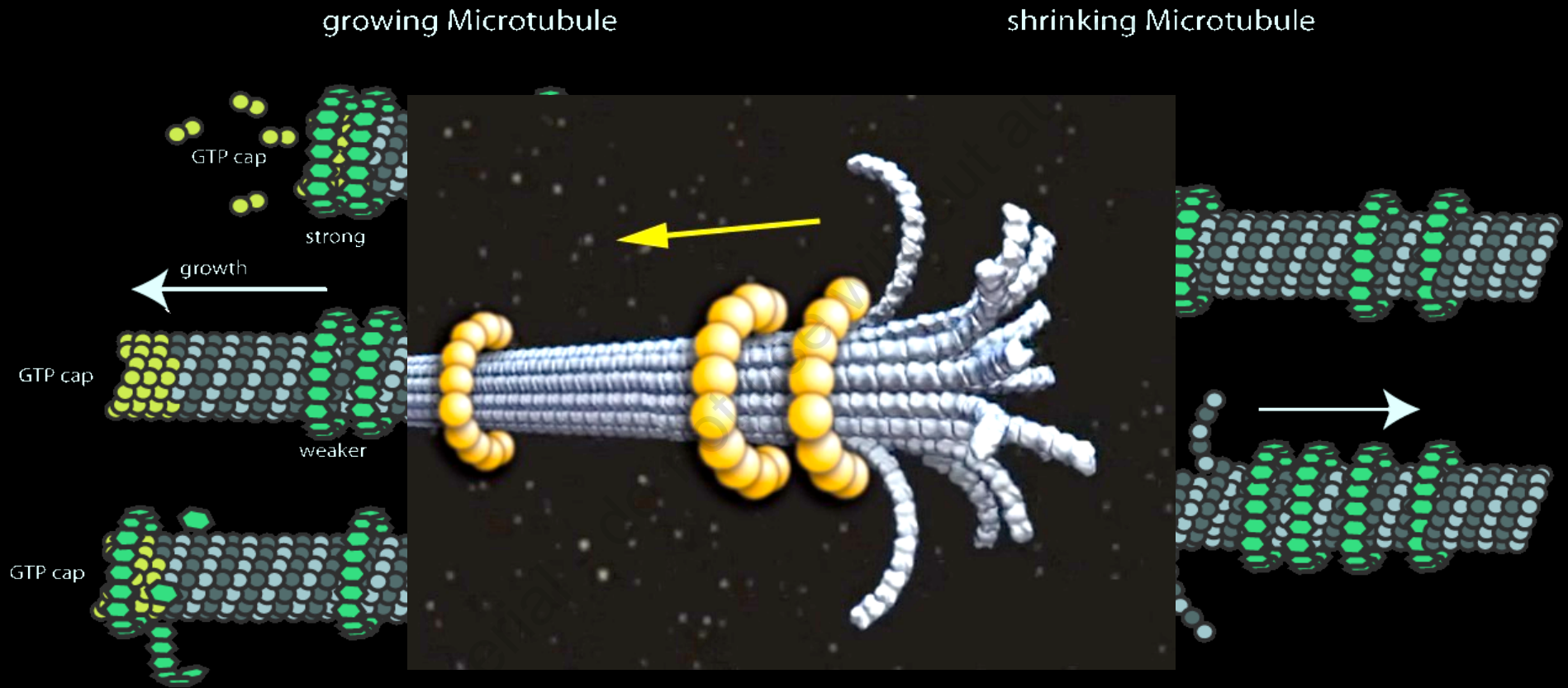
# Directional Dam1-Ring Diffusion During Microtubule Depolymerization



+ XMCAK1  
t=0



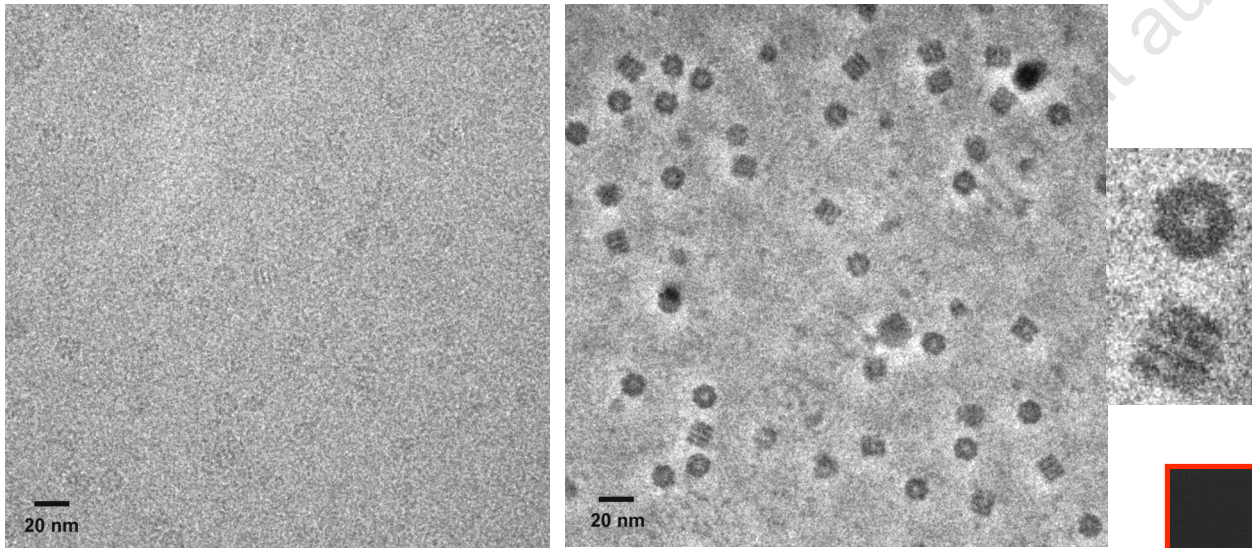
# Model for Plus End Attachment and Tracking



**Microtubule Depolymerization does the Work!!**

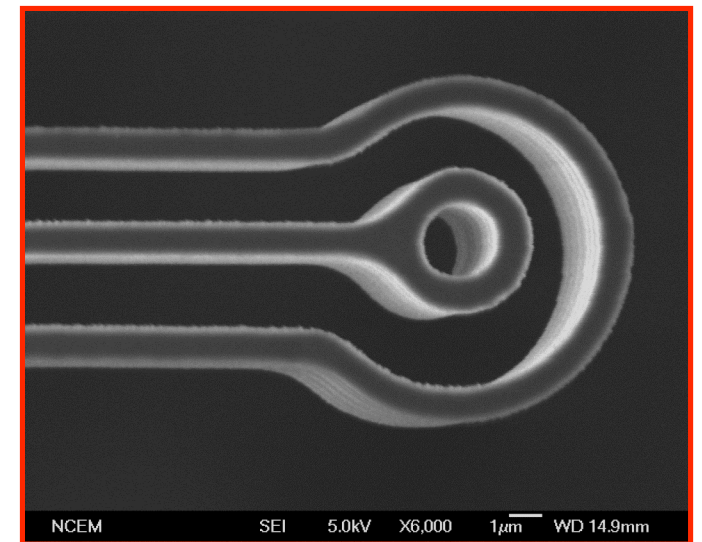


# IN-FOCUS PHASE CONTRAST FOR EM Will Bring Electron Microscopy of Multiprotein Complexes to a New Level



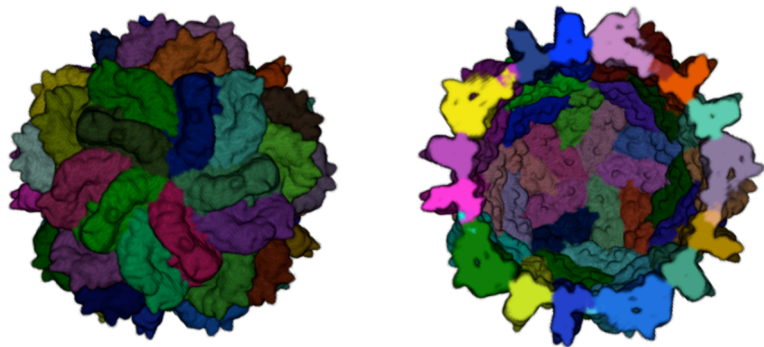
Jian Jin  
Rosanna Cambie

K. Nagayama & R. Danev  
Okazaki Center for Integrative Bioscience



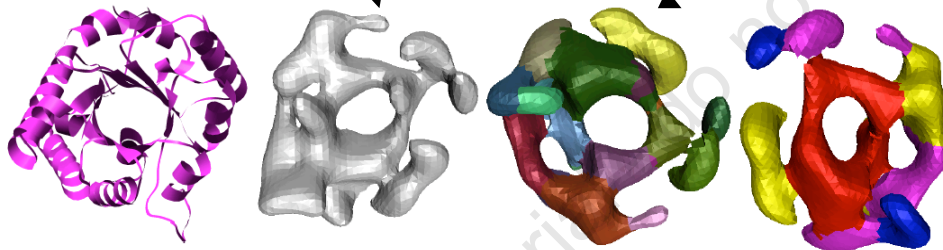
Robert M. Glaeser

# Computational analysis of 3D-EM Structures



**Segmentation** of 3D Reconstructed PSV into asymmetric subunits

$\alpha/\beta$ -barrel



**Secondary and Tertiary Structure Identification**

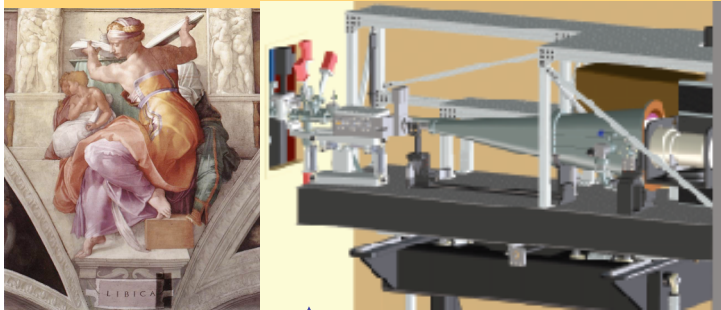


Center for Computational Visualization  
Institute of Computational and Engineering Sciences  
Department of Computer Sciences

University of Texas at Austin

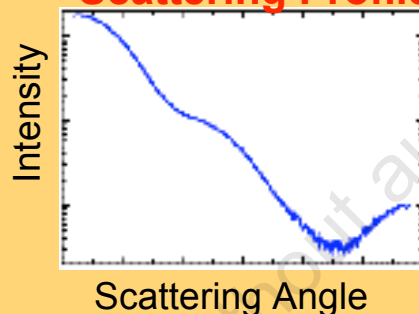
# High-Throughput Architecture and Oligomerization state by SAXS with MX & computation

**Data Collection: SIBYLS at the Advanced Light Source**



**Data Processing**

**Scattering Profile**



Match scattering to calculated from PDB

DARA: **D**atabase for **R**apid search of structural neighbors



Identify **Structural homologs**

**15-30 min. Processing**

**Start: Samples**

Proteins in solution  
30-450 ug protein  
2-10 ug/ul  
MW: 10-1000 kD  
Medium throughput

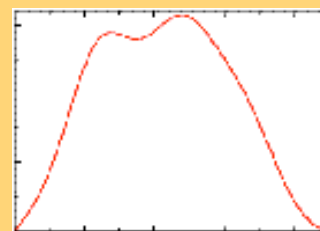
**Mass**

**Radius of Gyration**

**Maximum Dimension**

**Pair Distribution Function**

# of electron pairs in protein

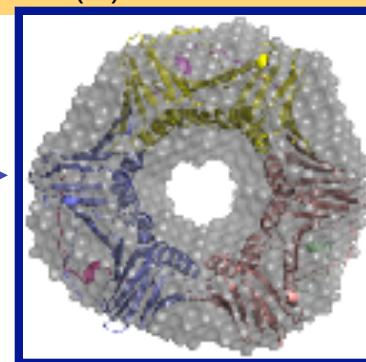


Distance of separation (Å)

Accuracy - by combining known structures & computational restraints

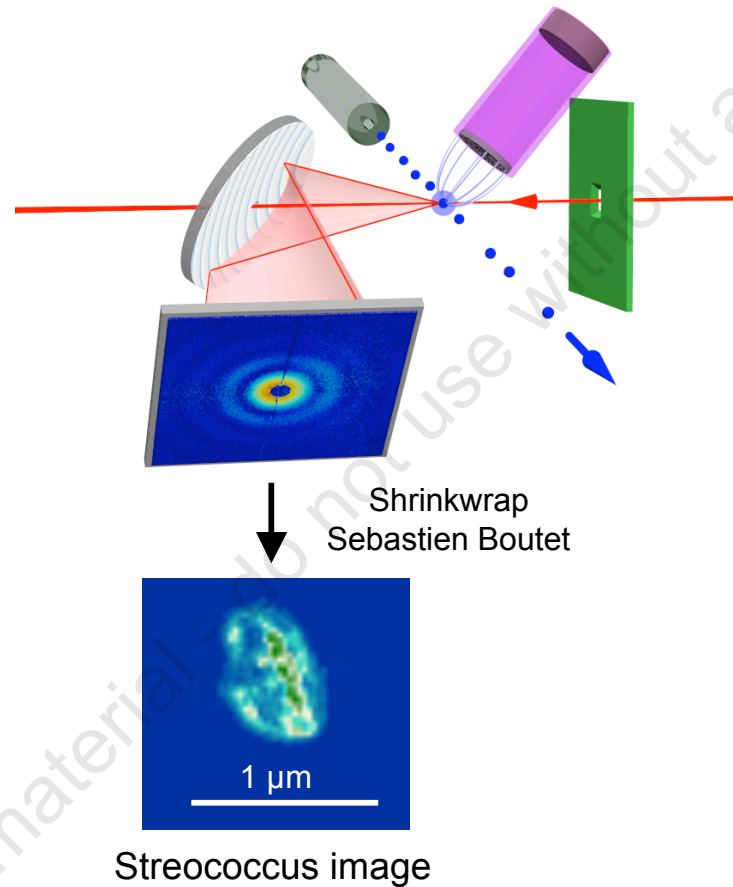
PX, Mass Spec, EM, NMR

**Shape Refinement Programs**  
ab initio or with added information



**3-D shape**

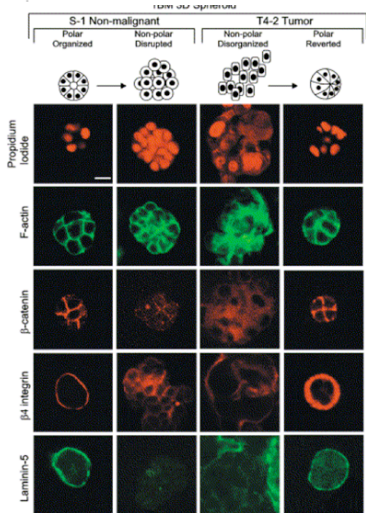
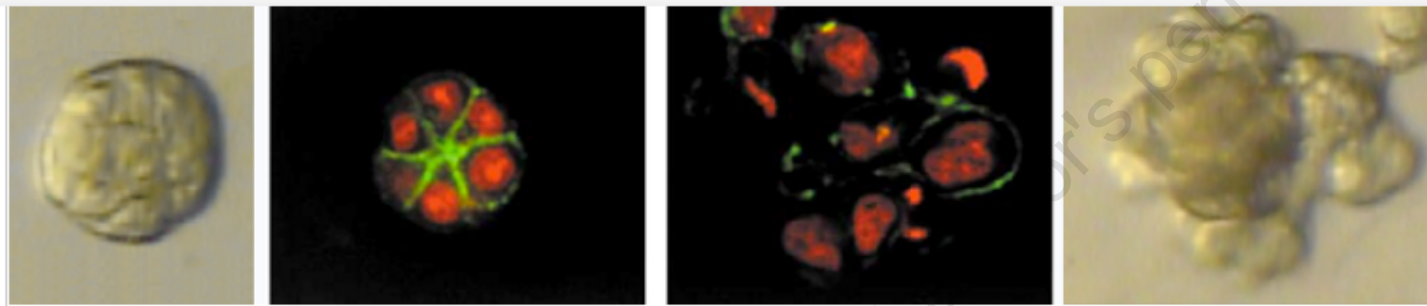
# X-ray diffraction imaging of proteins and biological cells with ultrafast FEL pulses



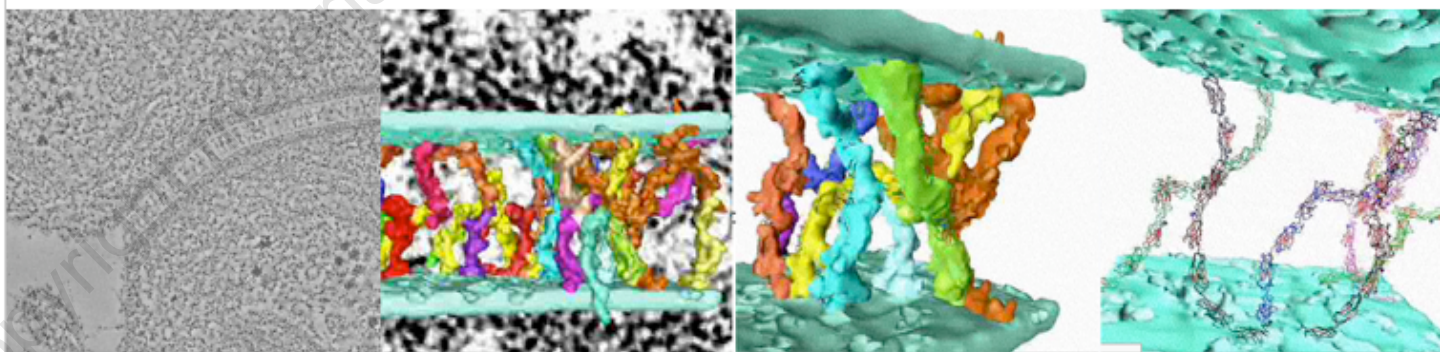
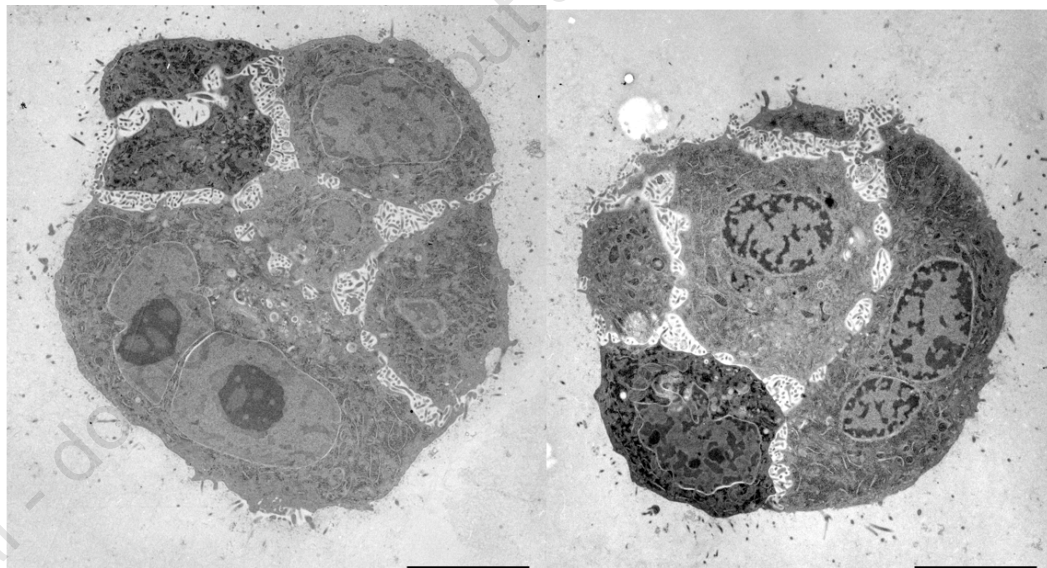
Henry Chapman, Janos Hajdu et al.



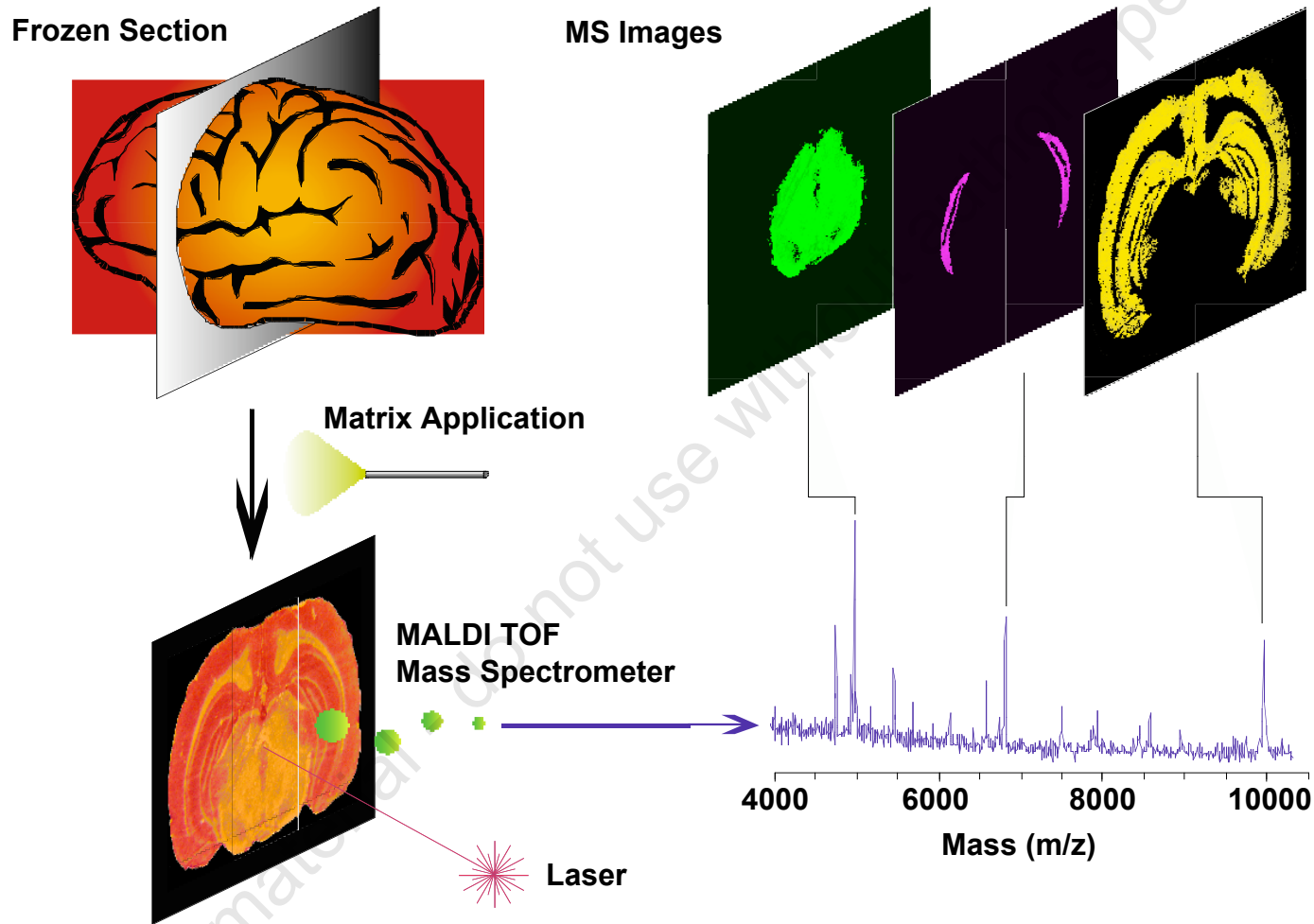
# From Tissue (or biofilm) to Molecules



Weaver et al. & Bissell Cancer Cell 2002

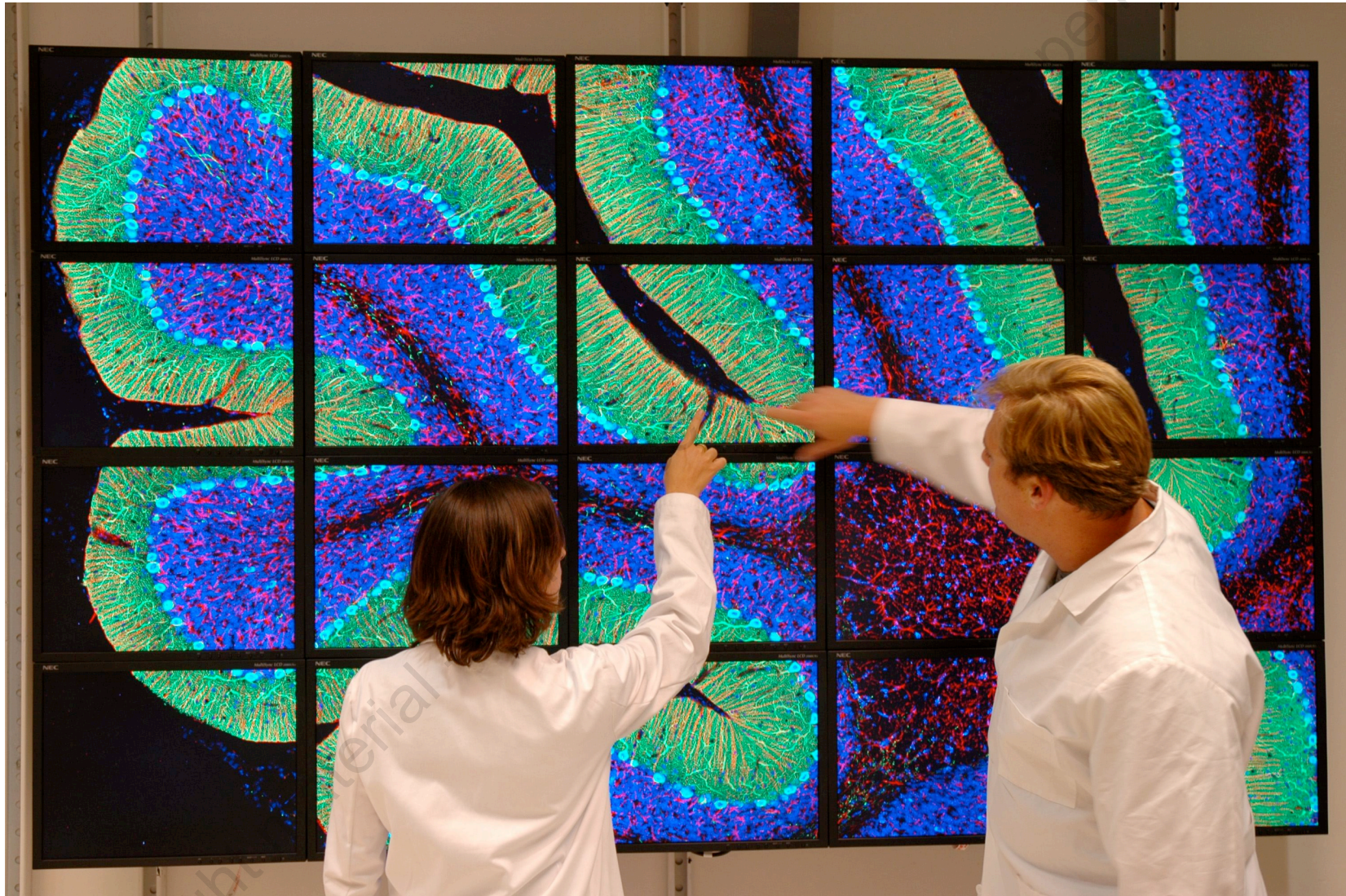


# Principle of MALDI MS Imaging



Pierre Chaurand  
Vanderbilt University





Maryann Martone

UCSD

# Gulliver

## DOE-GTL (JBEI)

Bioremediation  
Cellulose degradation  
Biofuel cells  
Carbon sequestration

## Low dose

Damage response  
Cellular interactions

## Imaging technologies

**EM:** phase contrast, large area

**X-ray:** tomography, diffraction, detectors

**Mass spec:** Ion beam, SELDI

**Light:** structured illumination, selective plane, dynamic

PET: detectors, CT/MRI

**Chem:** mol. tags, reporters, immuno, in situ hybe, radiopharm, tracers

**Comp:** multi-scale overlay, pattern recog, atlas dev, quant.

## Pathophysiology

Cancer  
Neurophysiology

## Cell biology

Signaling biology  
DNA repair  
Chromatin structure