Defining accurate macromolecular structures, conformations, & assemblies in solution

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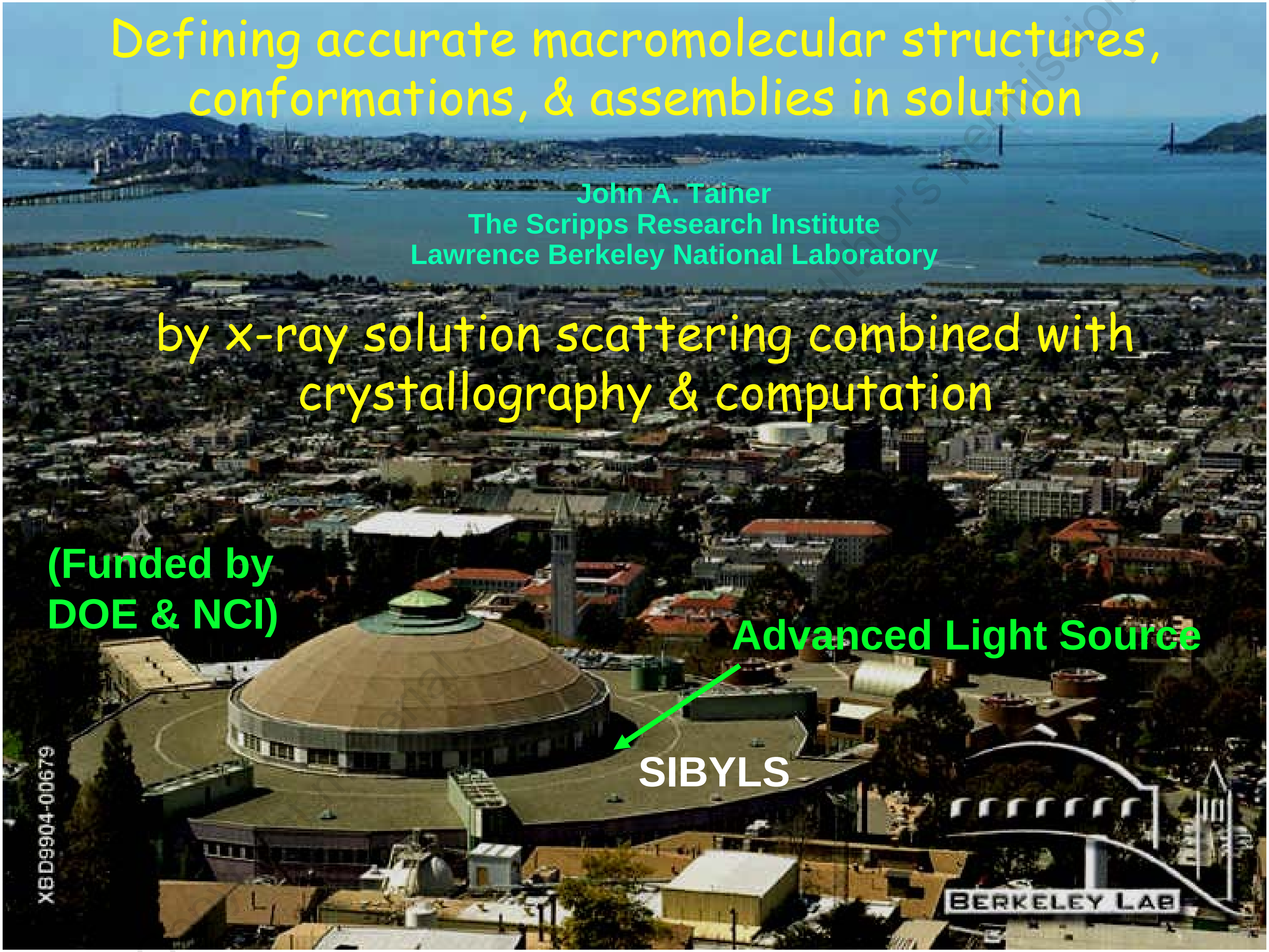
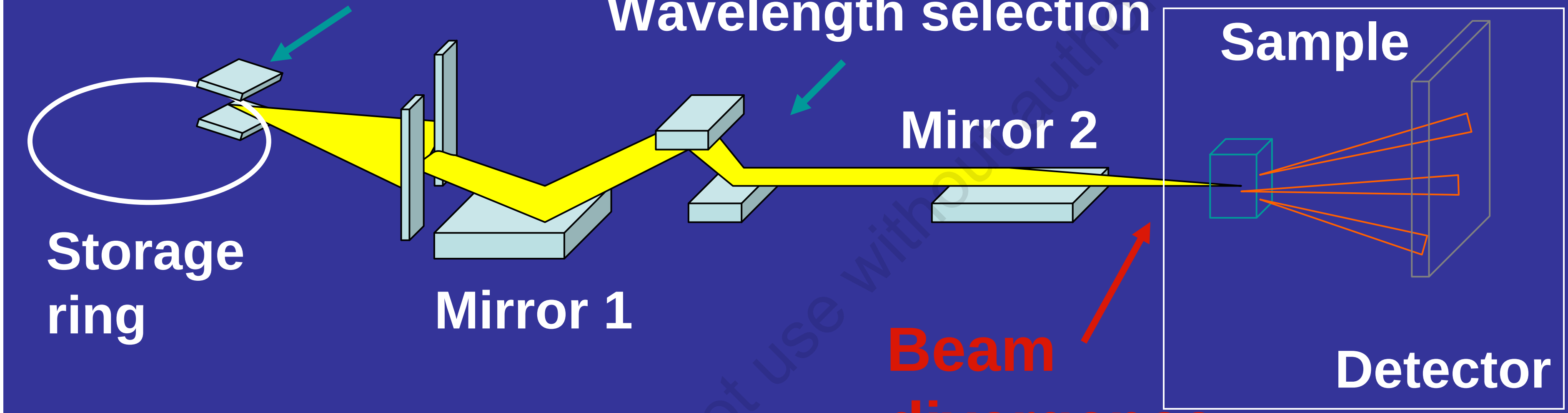
by x-ray solution scattering combined with crystallography & computation

(Funded by DOE & NCI)



What is a Beamline & Why have one?



Insertion Device - “super bend”

Monochromator - Wavelength selection

Sample

Mirror 2

Storage ring

Mirror 1

Beam divergence

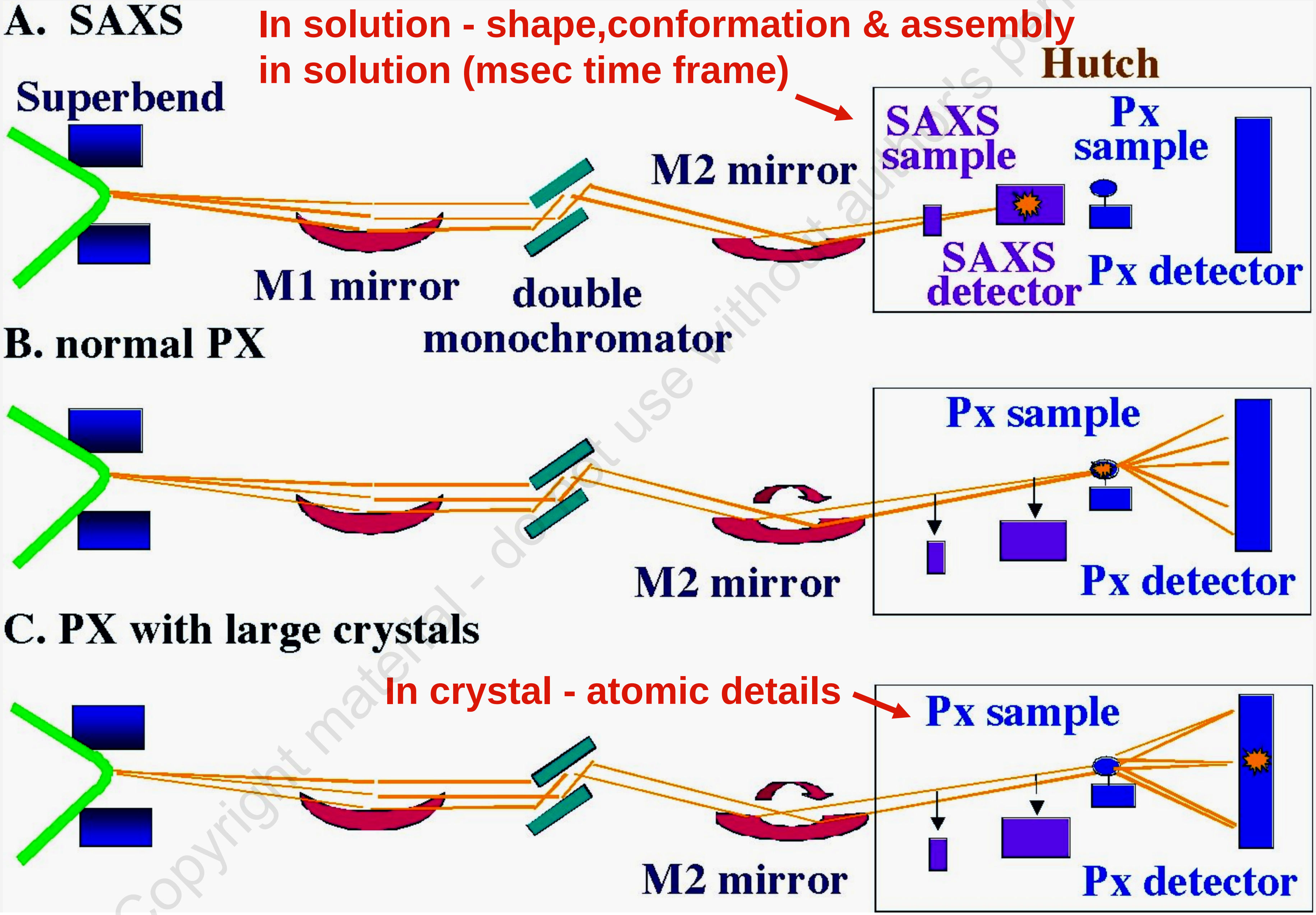
Detector

Hutch

- **Huge increases in X-ray Flux, Brightness and Brilliance vs lab X-ray source**
- **Control of key parameters (Beam divergence, Spectral band path, Wavelength and energy resolution)**

Structurally Integrated BiologY for Life Sciences (SIBYLS):

Goal - combine Solution and Crystal structures



In solution - shape,conformation & assembly in solution (msec time frame)

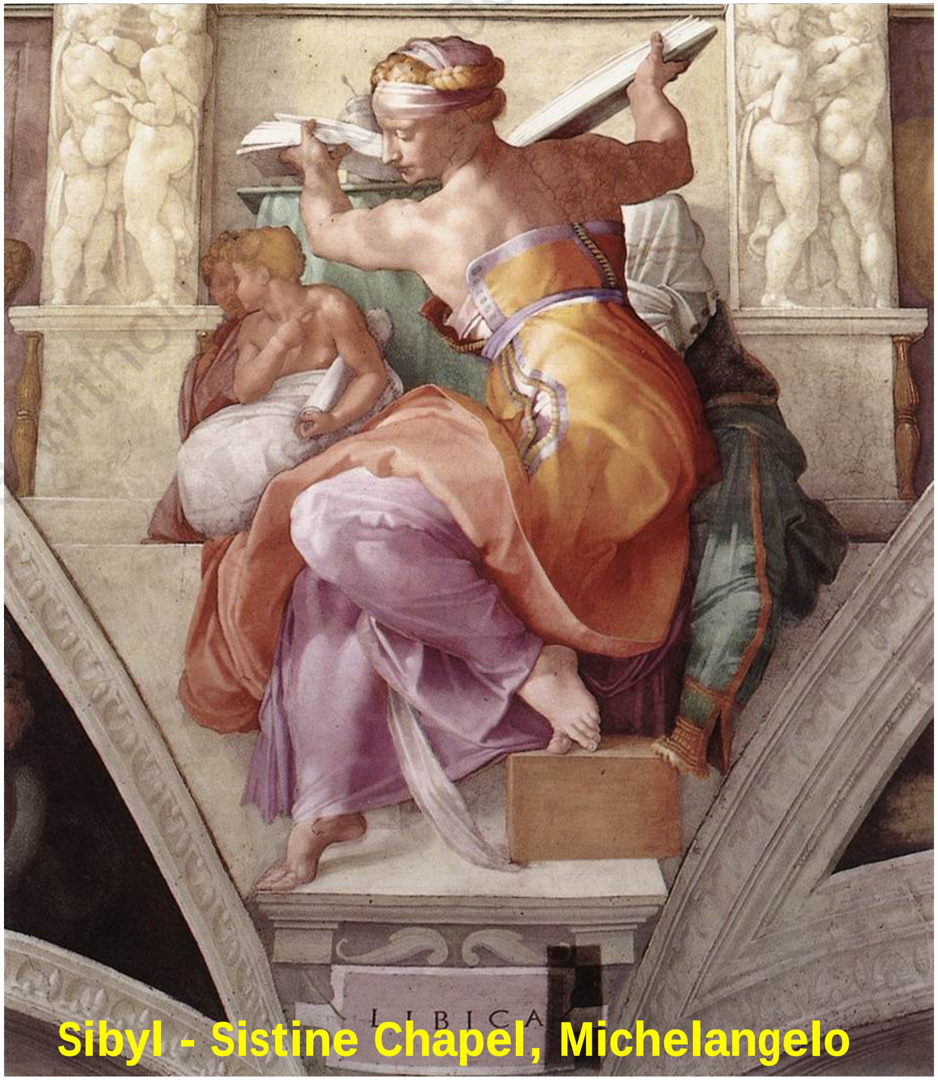
In crystal - atomic details

Idea - cell decision points: ***use reversible complexes, modified molecules, & molecular machines***

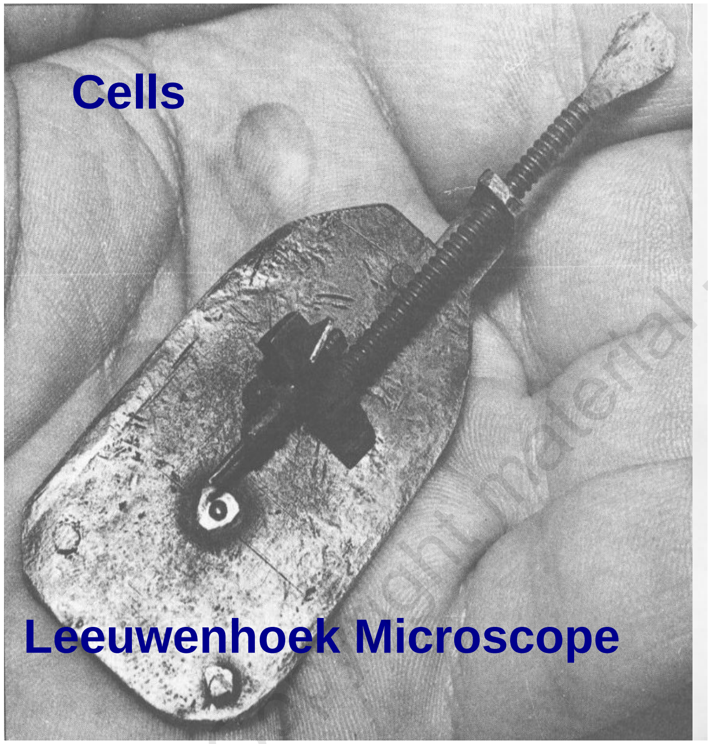
So use combined SAXS & MX

- ***Check conformation of full-length molecules***
- ***Characterize changes allowing super-efficiency***
- ***Clarify solution architectures***

SIBYLS-BL: Small Angle X-ray Scattering & Crystallography Berkeley Lab ALS beamline



Sibyl - Sistine Chapel, Michelangelo



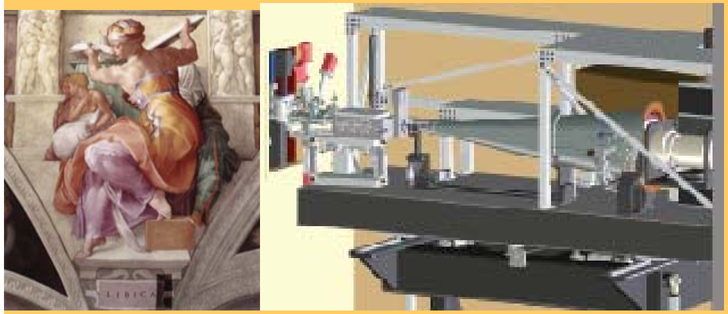
Leeuwenhoek Microscope

SIBYLS robust tool to unite cell biology & protein machines

Bridge from sequences and mutations to functions, phenotypes & cellular outcomes

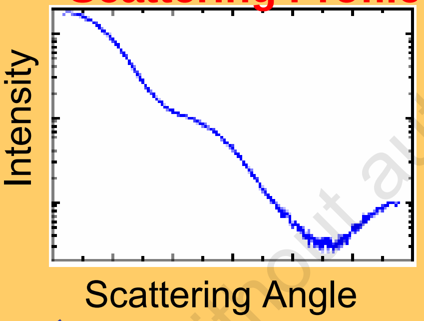
Synchrotron x-rays for solution structures
Small Angle X-ray Scattering (SAXS)

Data Collection: SIBYLS at the Advanced Light Source



Data Processing

Scattering Profile



Match scattering curve to calculated scattering from PDB

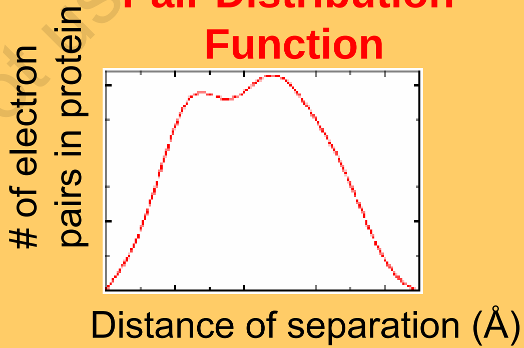
DARA: DAtabase for RApid search of structural neighbors

RCSB PDB PROTEIN DATA BANK

Identify
Structural homologs

Mass

Pair Distribution Function



Radius of Gyration

Maximum Dimension

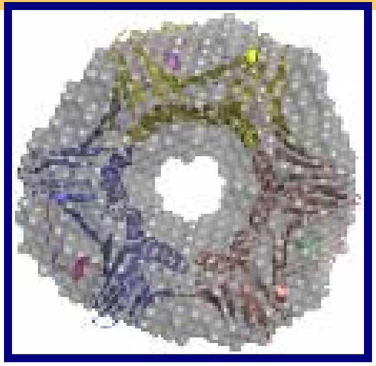
15-30 min. Processing

Start: Samples

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

PX, Mass Spec, EM, NMR

Shape Refinement Programs
ab initio or with added information



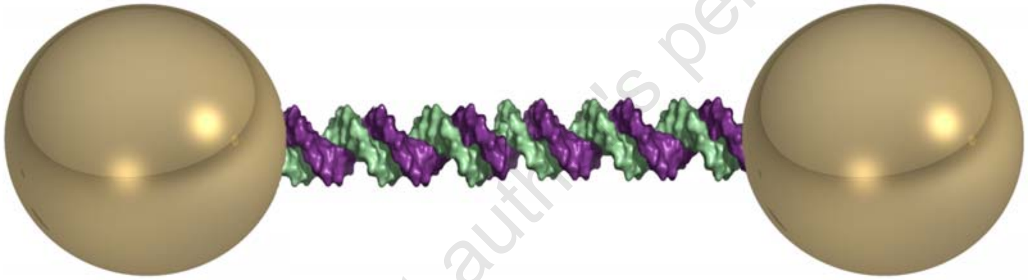
3-D shape

SIBYLS & SAXS - Robust Robot Tools

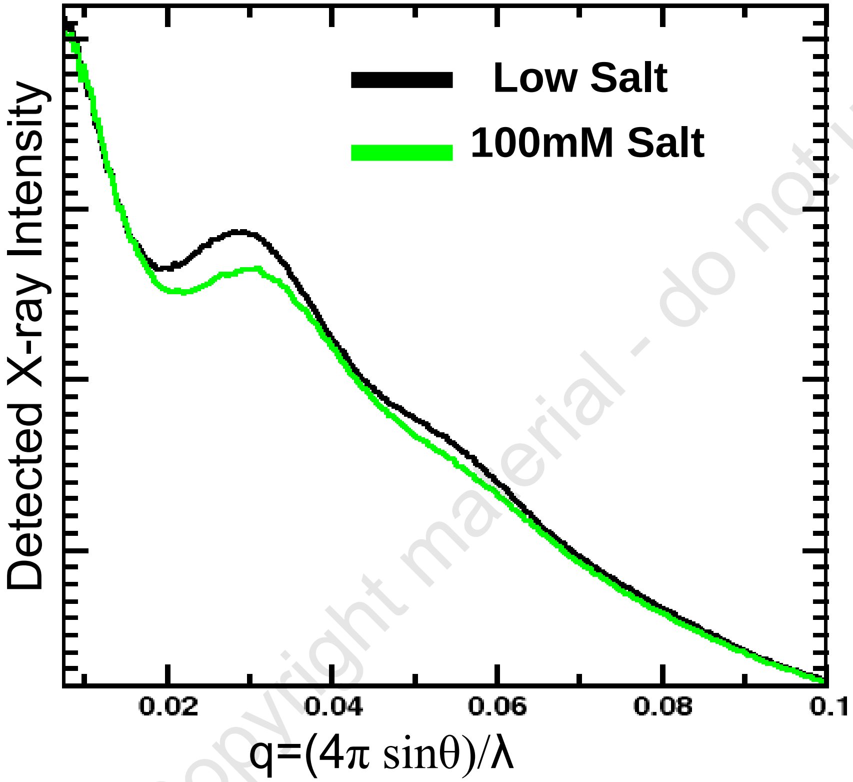
QuickTime™ and a
YUV420 codec decompressor
are needed to see this picture.

SAXS with nano-gold labeled DNA

50 base pair DNA
labeled at both ends
with 66Å gold



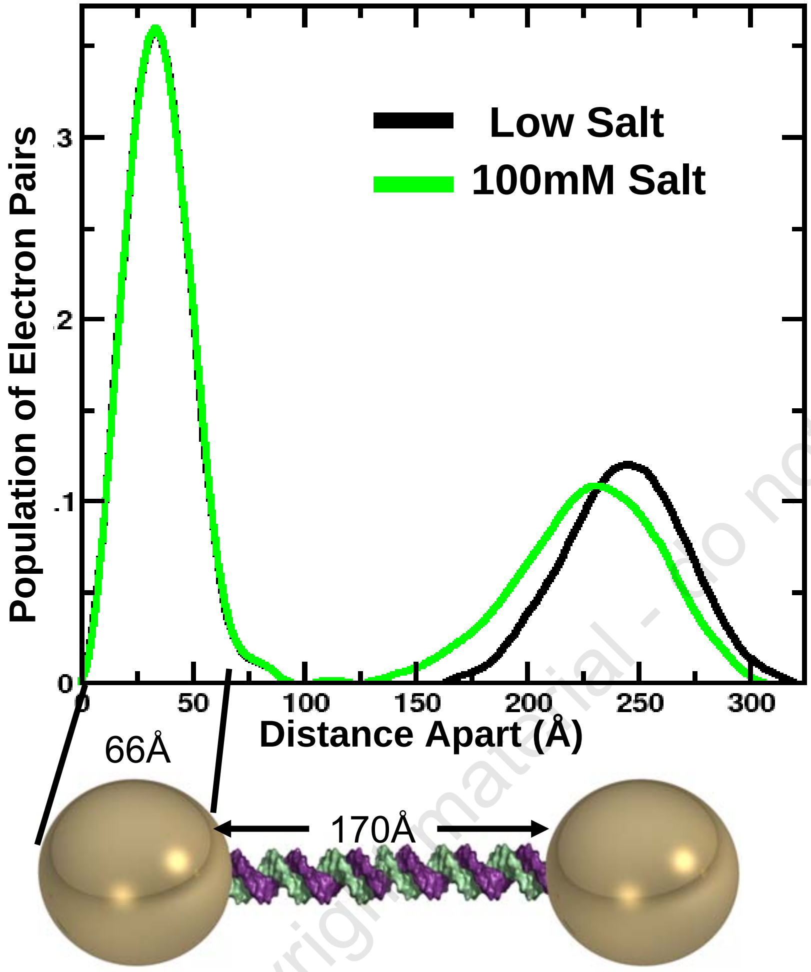
Measured Signals varying NaCl



- Gold labeled DNA provides excellent signal for time resolved work (~1ms)
- Excellent tool for deciphering macromolecular manipulation of DNA
- A variety of functionalized gold surfaces enable protein labeling as well
- Collaboration with Alivisatos Lab: Shelley Claridge

SAXS with nano-gold labeled DNA

Fourier transform of measured SAXS data

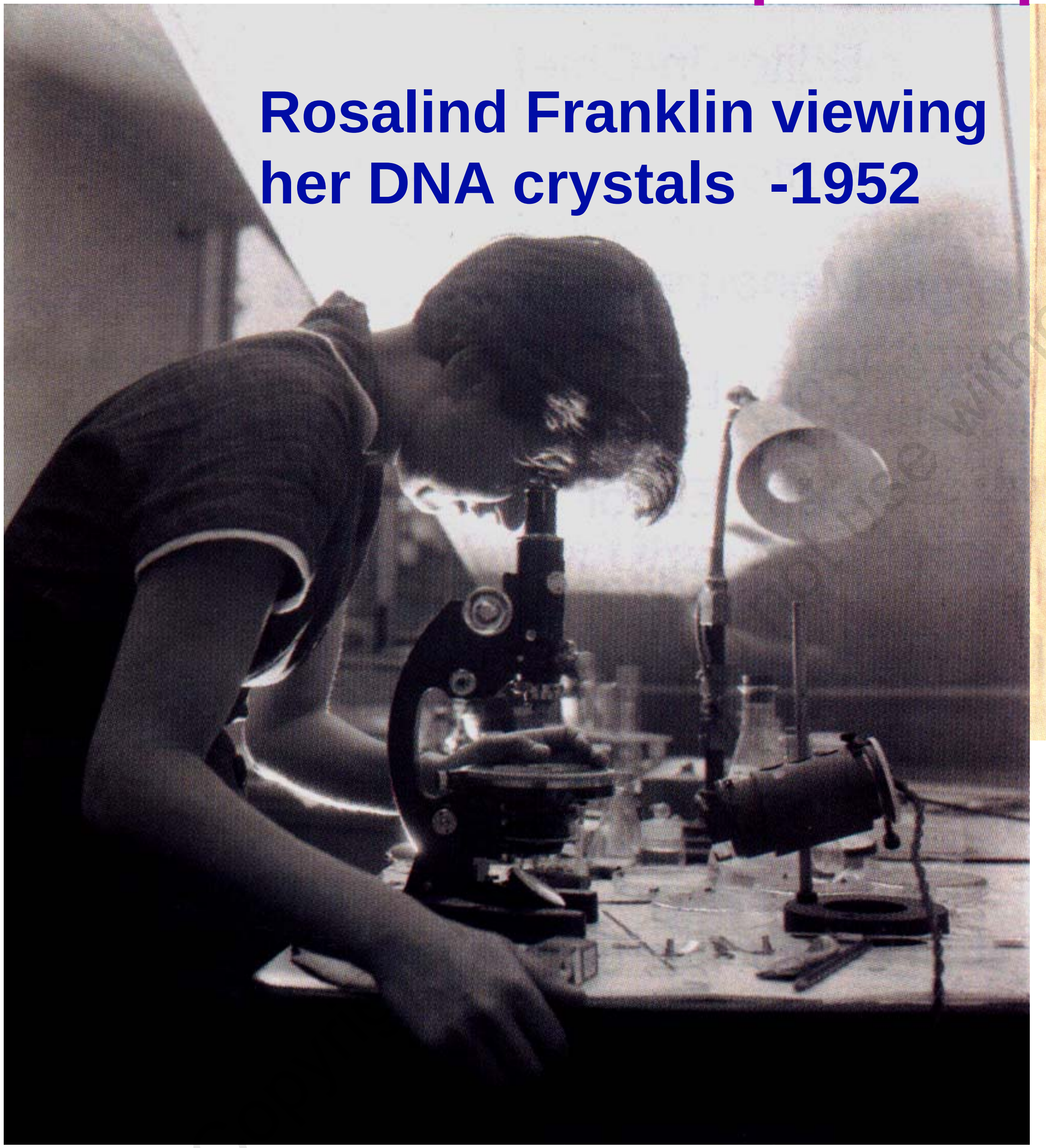


With 100mM NaCl:

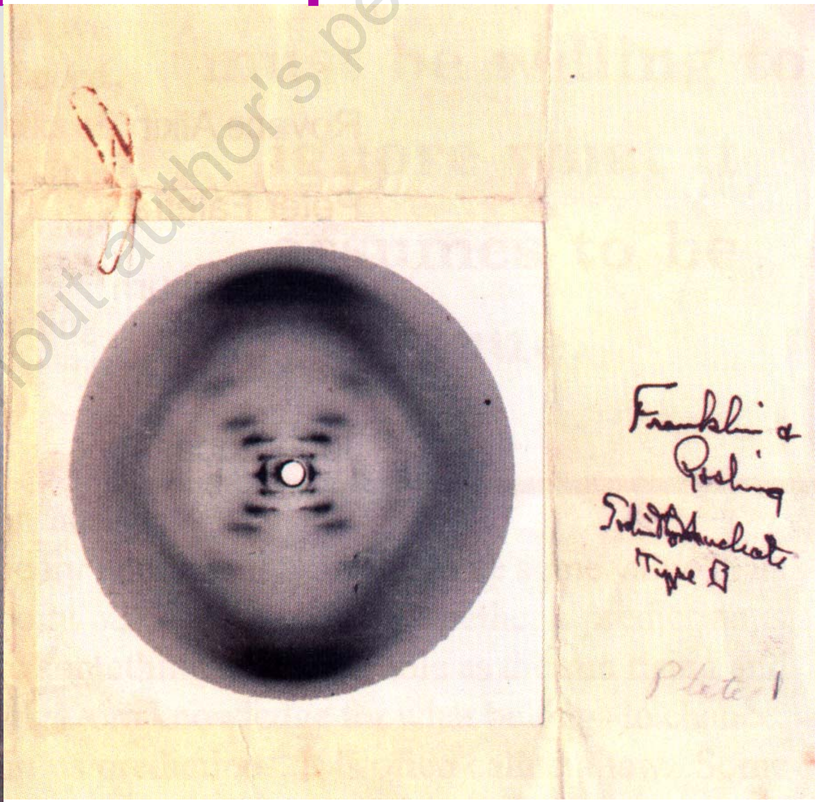
- Gold-Gold distance shifts by 12Å (0.24Å/base)
- Broader 2nd peak indicating a larger variety of gold distances: flexing of DNA

What is the structure of DNA? Rosalind Franklin

Useful resolution - depends upon the question

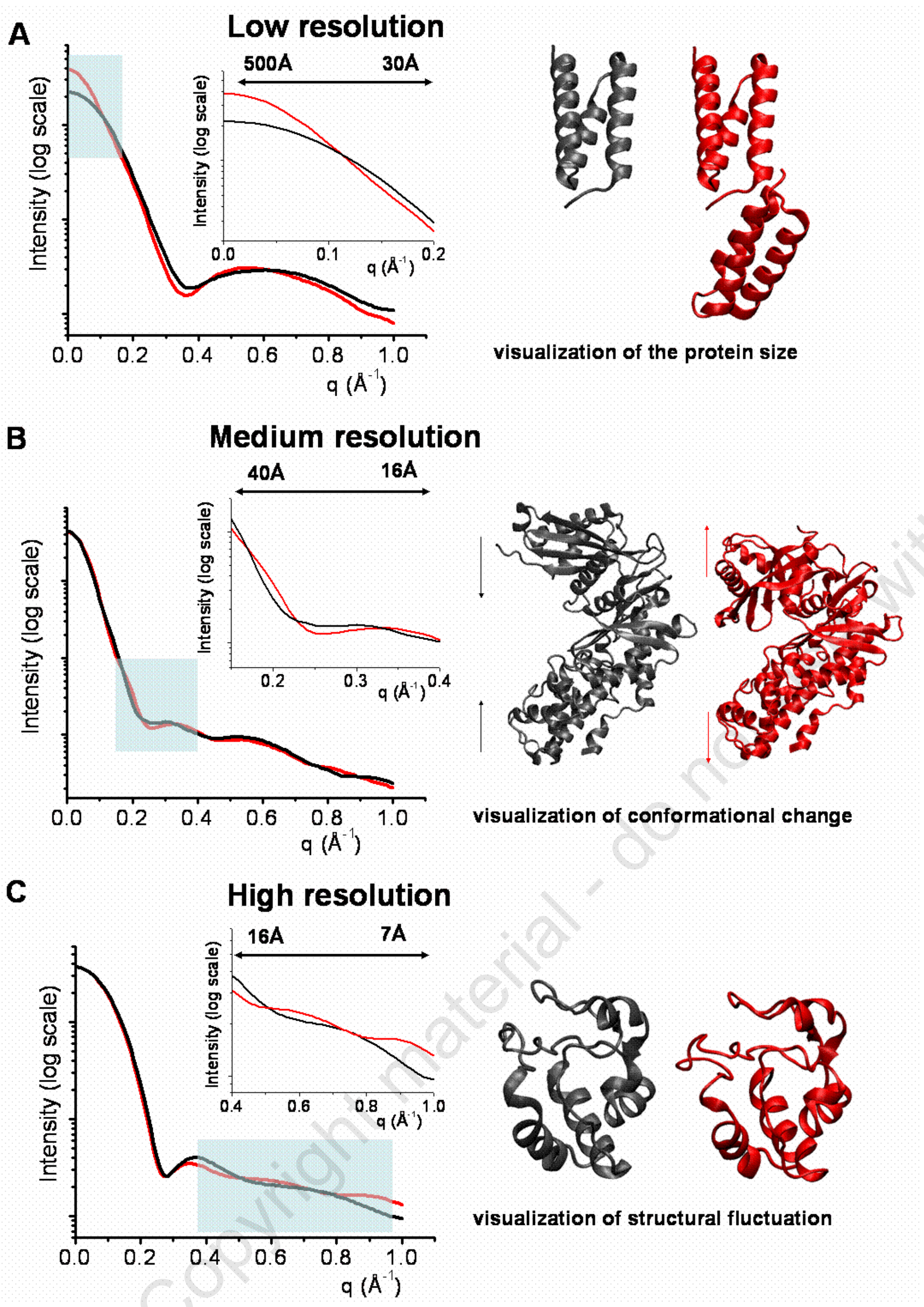


Rosalind Franklin viewing her DNA crystals -1952



Diffraction photo by R. E. Franklin and R. G. Gosling of the Sodium salt of calf-thymus B-DNA

SAXS resolution & structural features



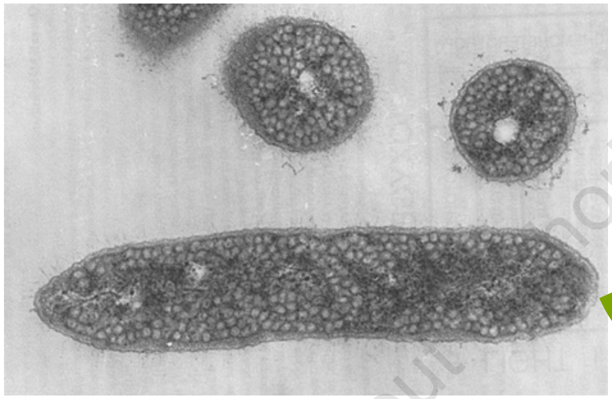
Oligomerization states?

Domain conformations?

Structural fluctuations?

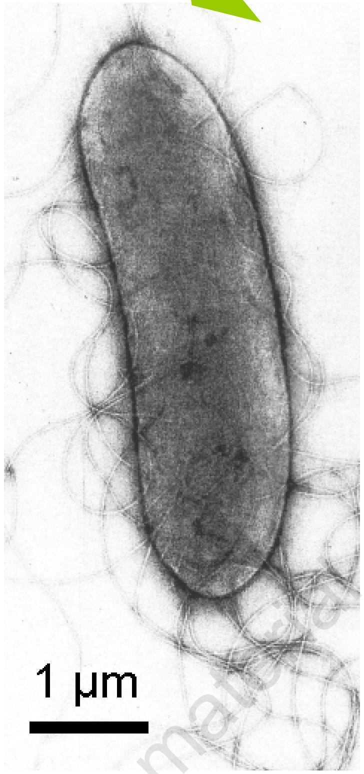
Bio**E**nergy **T**echnologies & **S**cience **I**ntegrated **E**fficiently (BETSIE)

CO_2

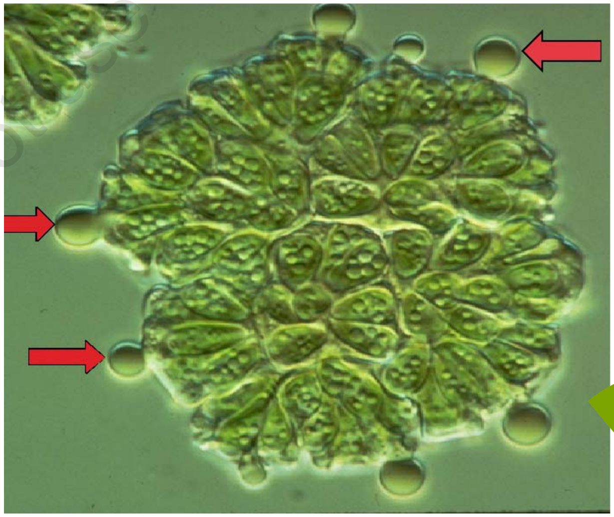


Rhotobacter capsulatus

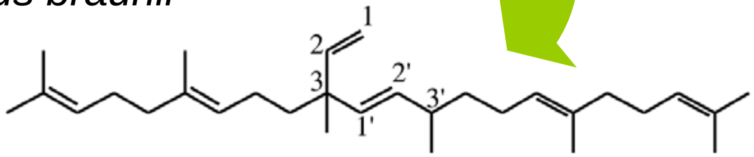
H_2



Clostridium thermoaceticum



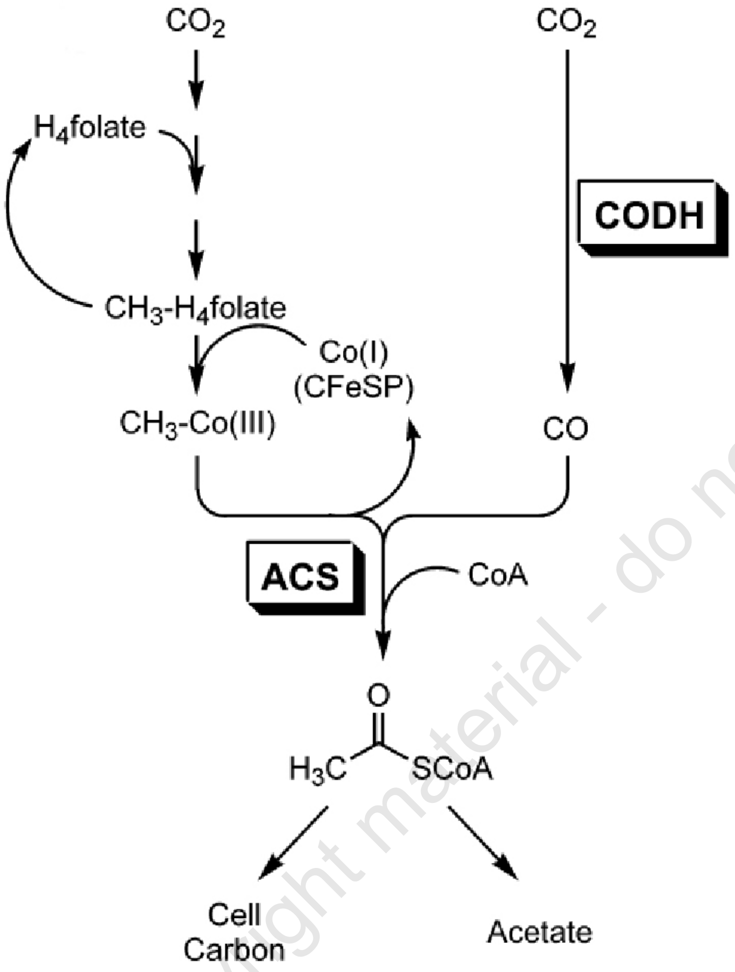
Botryococcus braunii



C_{30} botryococcene

carbon fixation, hydrogen, & hydrocarbons

Wood-Ljungdahl pathway: reduce greenhouse gases by converting carbon dioxide to acetyl-CoA



- ancient pathway-use CO and H_2 as energy source and CO_2 as electron acceptor 1 billion years before O_2
- catalytic efficiencies up to 40,000 mol CO per mol enzyme per second.
- 100 million tons of CO removed from lower atmosphere by bacterial oxidation every year.

Define protein-protein interactions & conformational changes

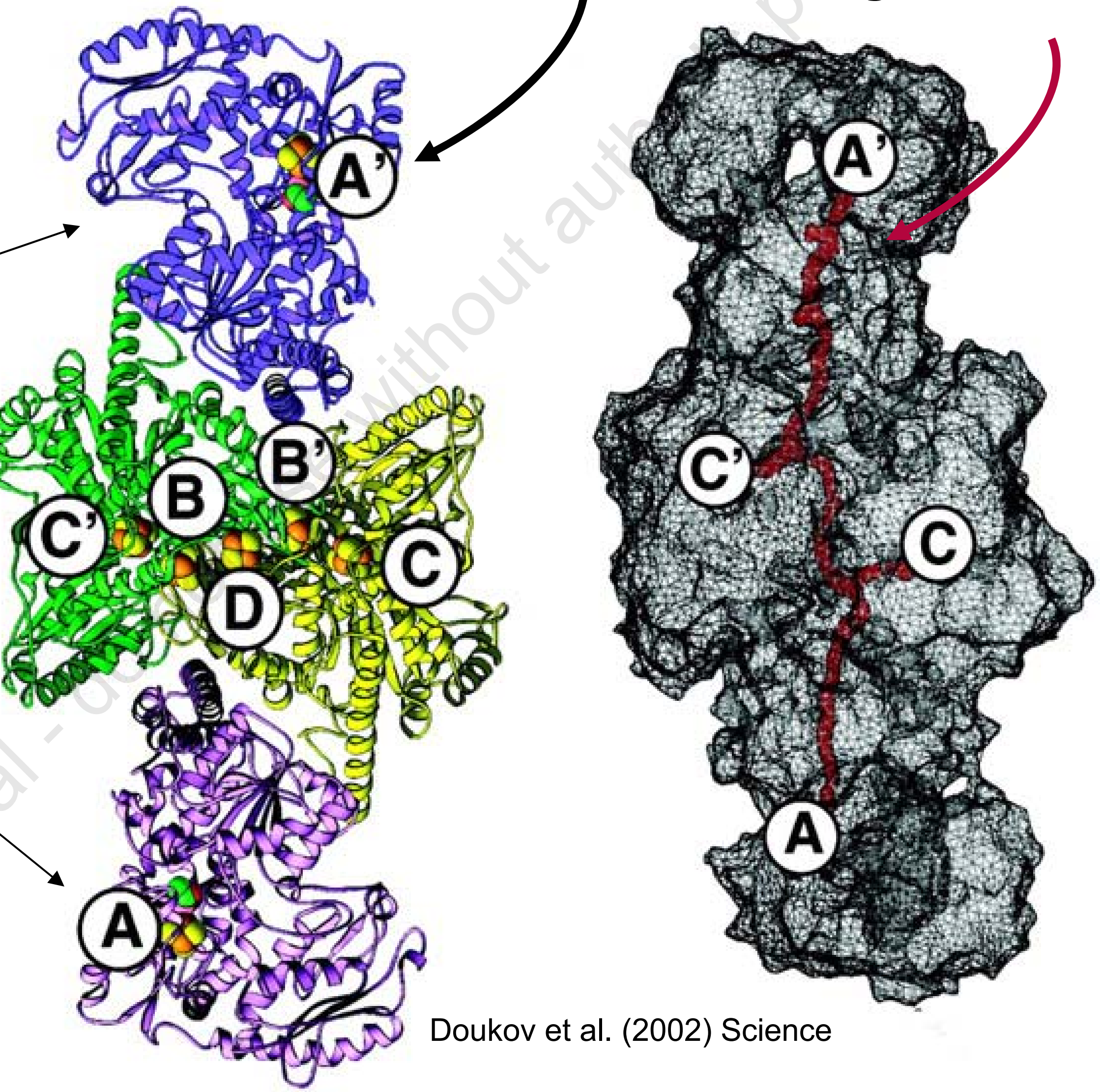
CODH/ACS
metalloenzyme

ACS
acetyl CoA
synthase

CODH
CO_2--CO
chemistry

$[Fe_4S_4]$-X-Cu-X-Ni
cluster

CO gas channel



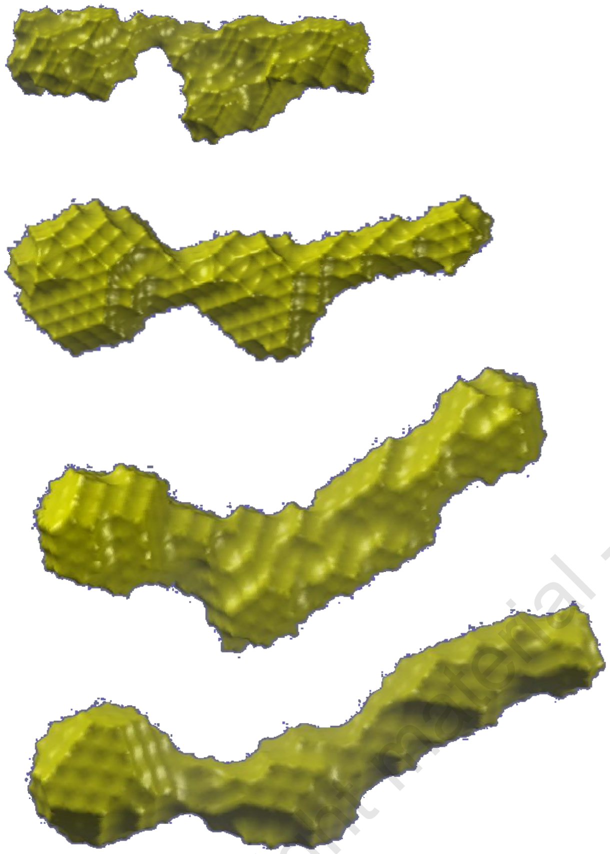
Doukov et al. (2002) Science

**Assemblies with
CFeSP & ferrodoxin
not known**

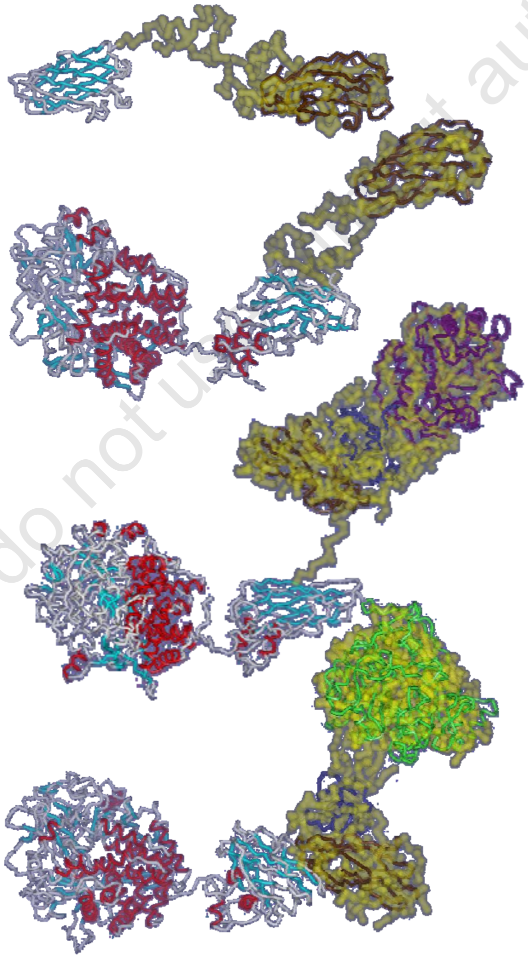
CO_2 recapture - impact multi-billion $ cost reductions

Solution structure modeling of mini-cellulosome - Cellulosic biomass to biofuels

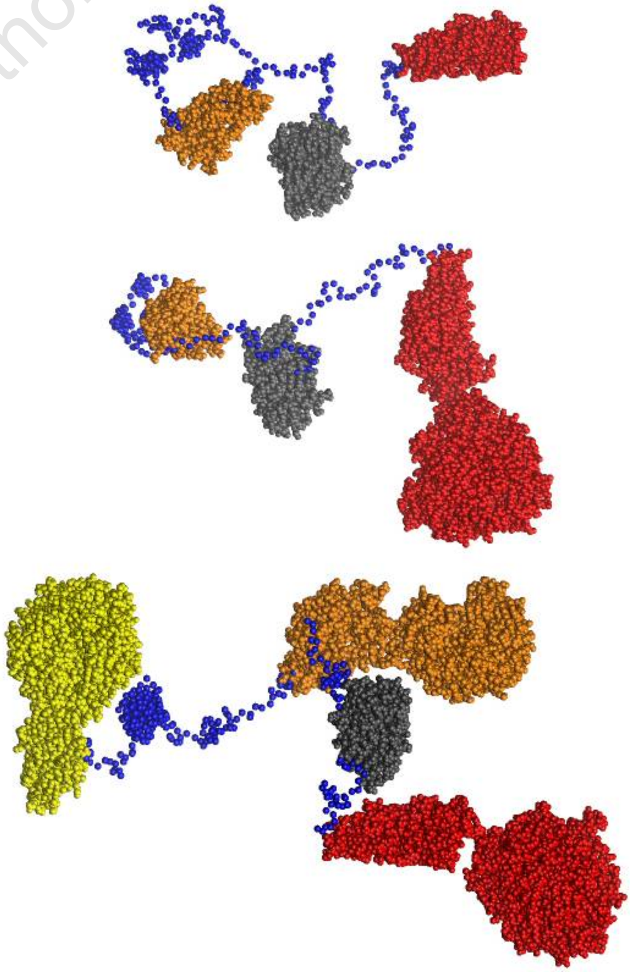
Overall shapes



Partial shapes



Combined MX-SAXS models



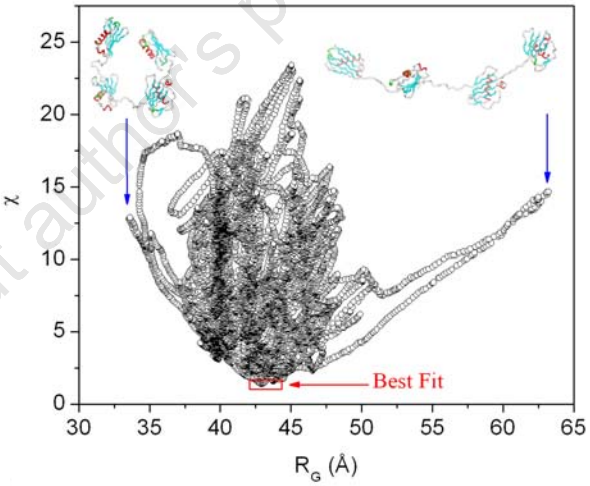
Hammel et al., 2005

Solution structure modeling of minicellulosome

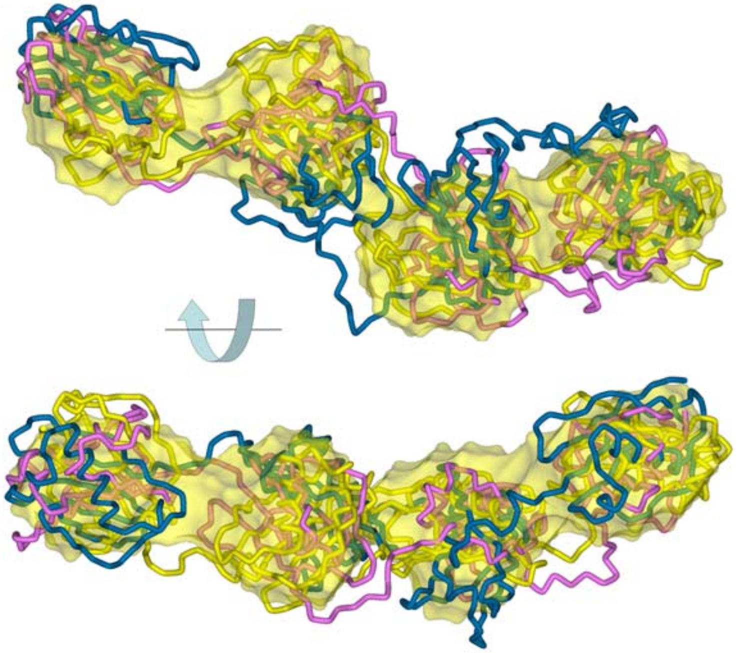
QuickTime™ and a
YUV420 codec decompressor
are needed to see this picture.

Rigid body modeling using molecular dynamics

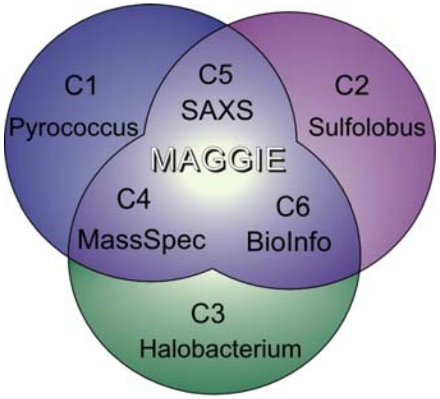
Molecular dynamics is used for exploring of the conformational space of the subdomains protein-complex.



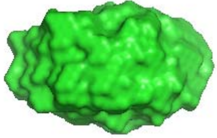
QuickTime™ and a
YUV420 codec decompressor
are needed to see this picture.



MAGGIE - Native PCs, MPs, metabolites



Pf1674

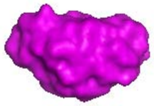


20KD matches homolog PX structure

Pf1282

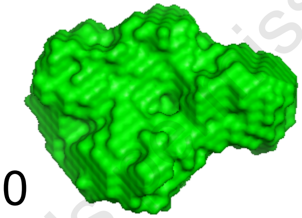
6kD Matches PX structure

Pf1909



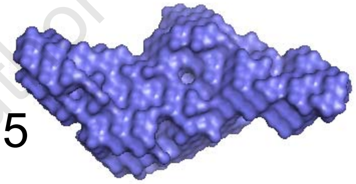
7kD Matches PX Structure

Pf0380



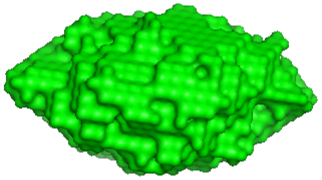
27kD matches PX structure

Pf1205



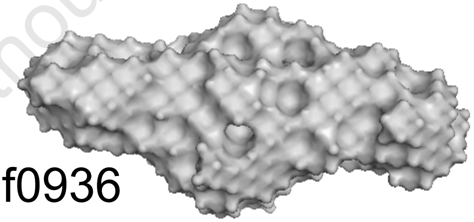
24kD Flexible and poor crystal target

Pf0715



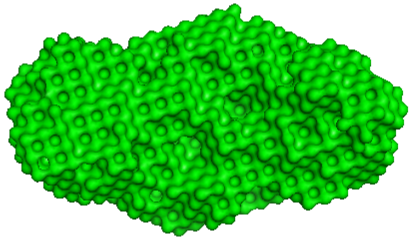
42 KD, dimer matches homolog PX structure

Pf0936



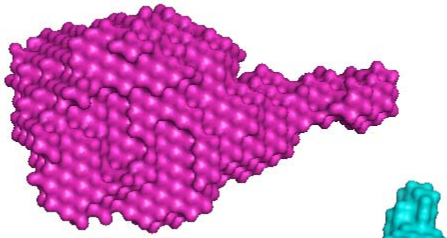
Different dimer organization than in homolog PX structure

Pf1911



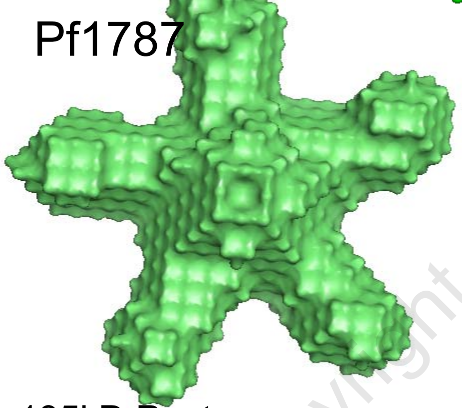
67KD, dimer

Pf0230



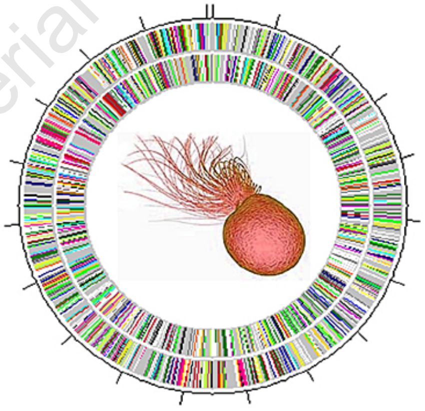
24kD poor match to homolog PX structure

Pf1787

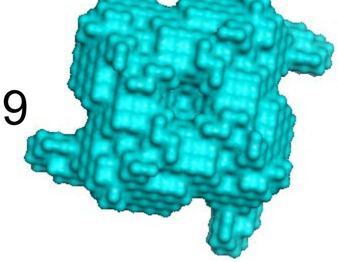


135kD Pentamer
Excellent crystal target

Pyrococcus furiosus



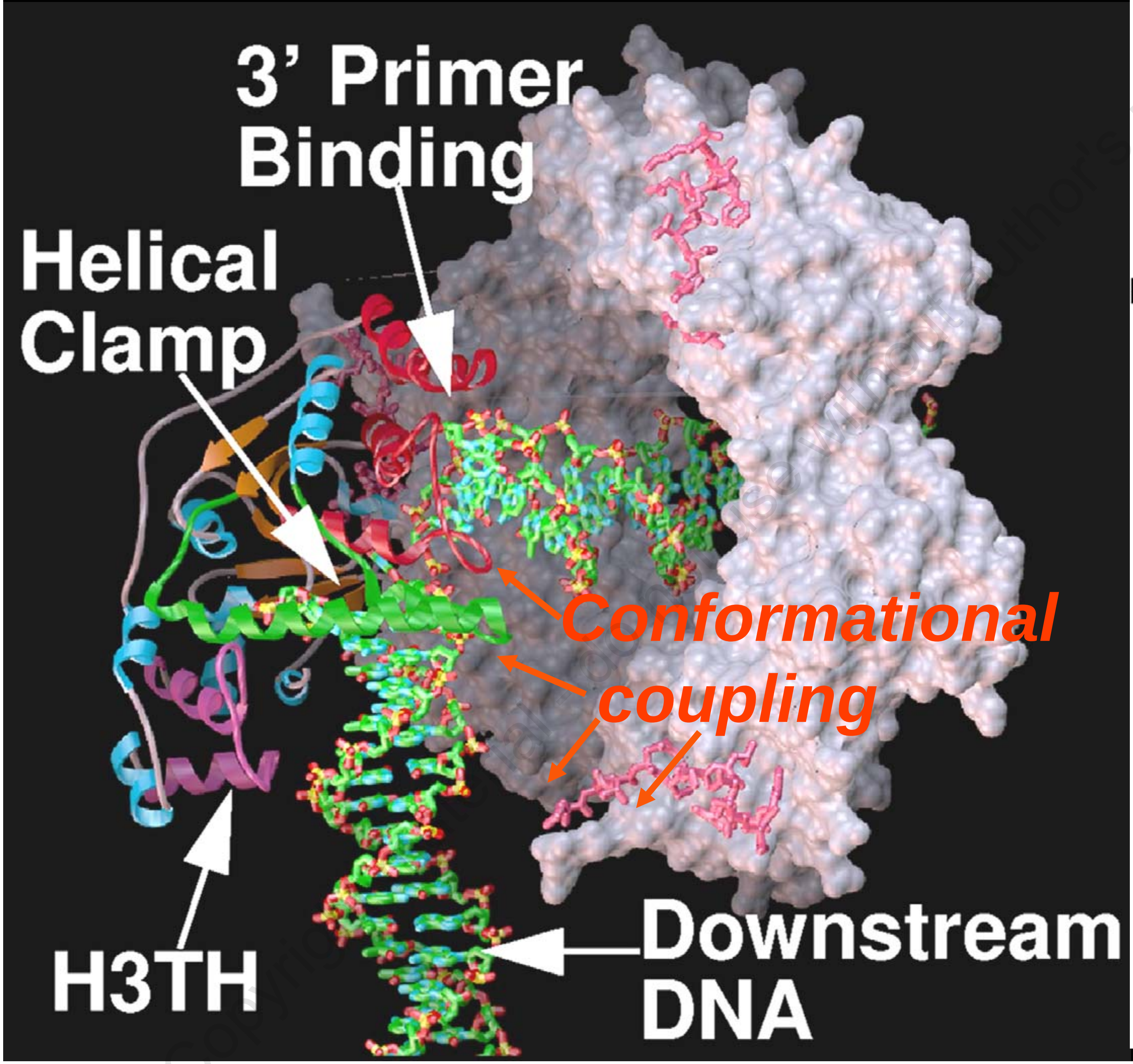
Pf0259

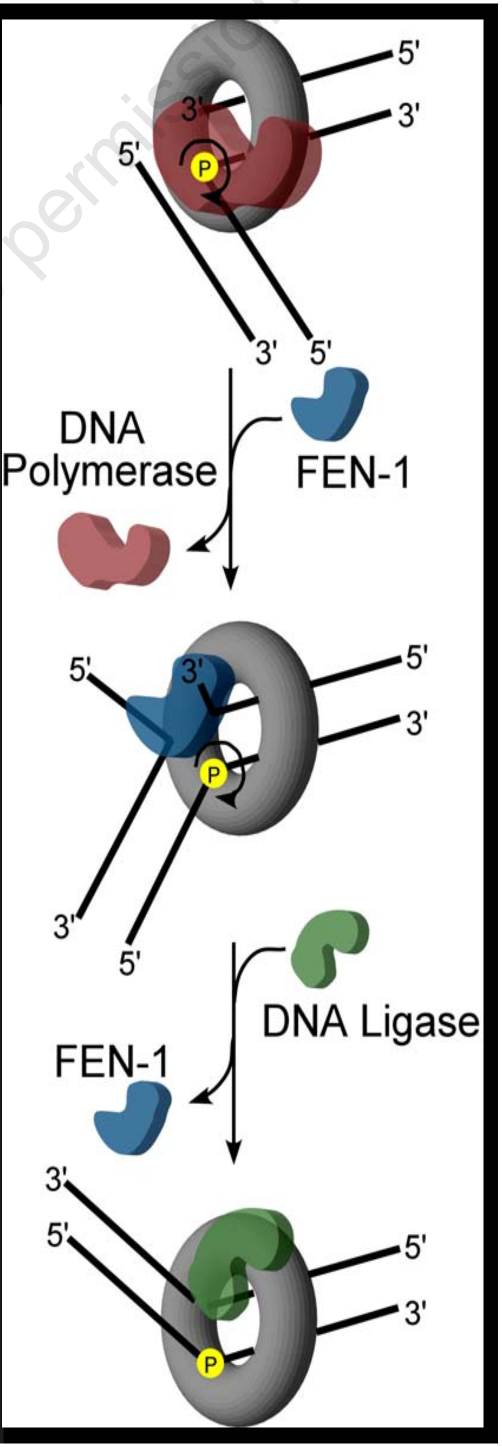


48kD tetramer

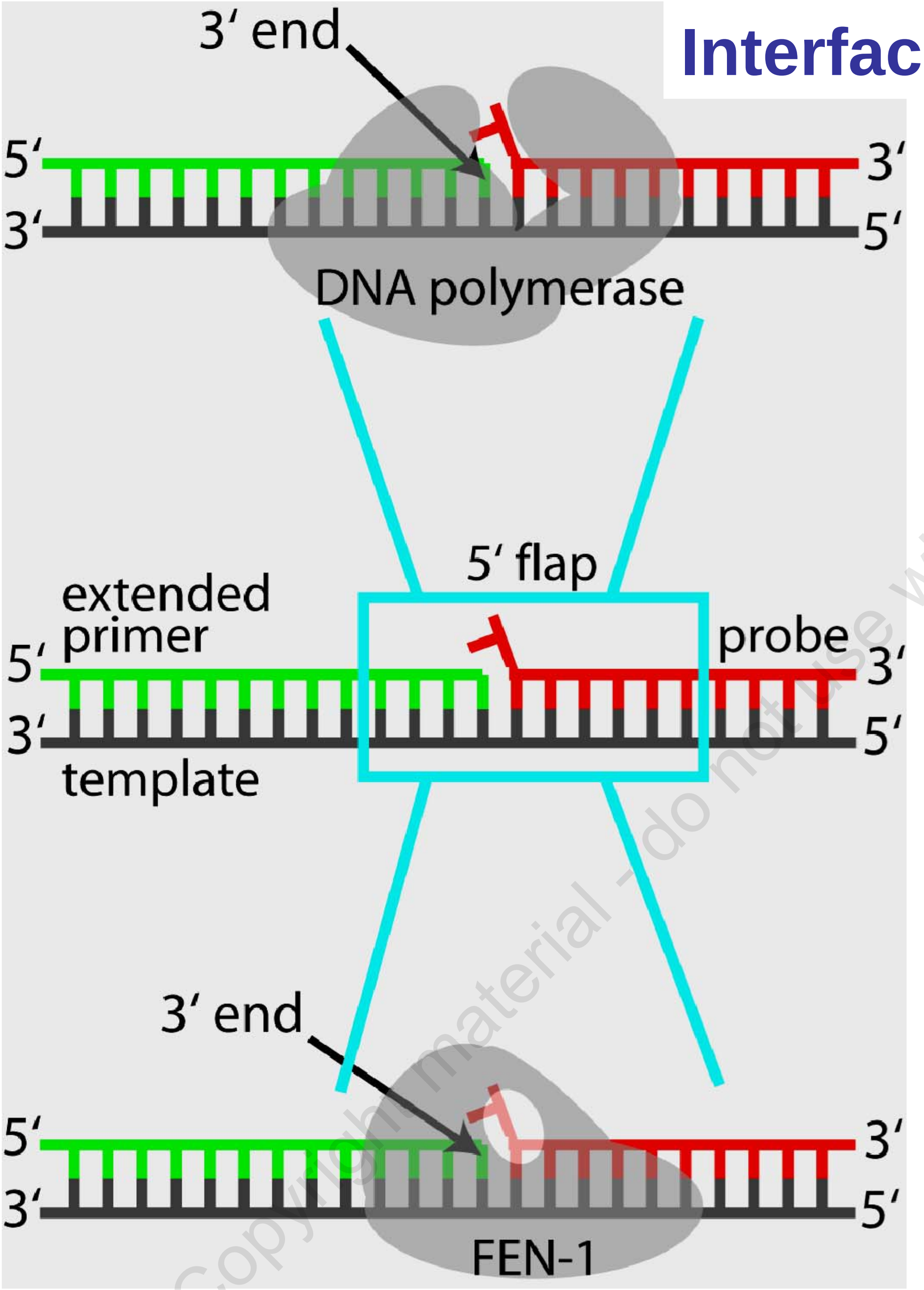
Interface exchanges & assembly machinery?

Tethering & Handoffs for PCNA - test?

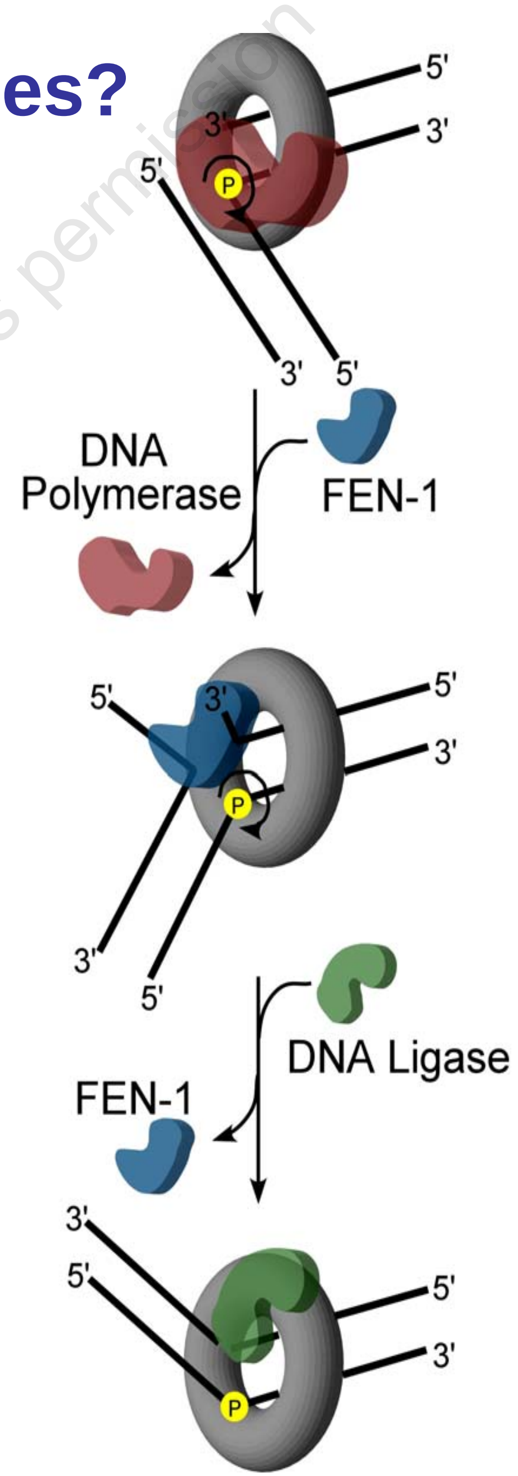




Interface exchanges?



PCNA is a hub for interface exchanges



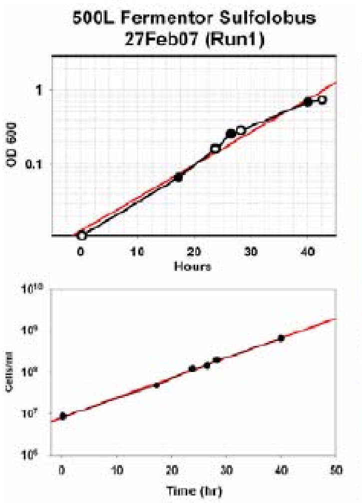
Chapados et al., Cell 2004

How do PCNA & ligase engage DNA?

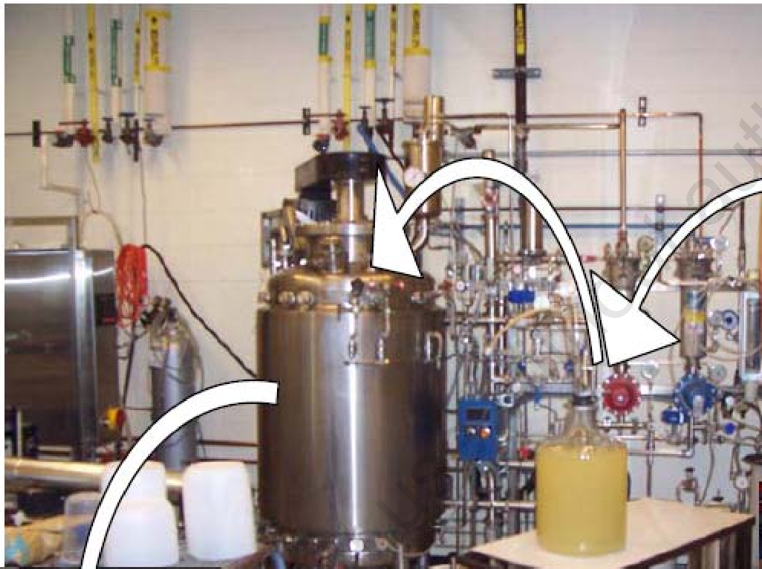
MAGGIE - Native PCs, MPs, metabolites

2x4L MC Grown in **Berkeley**
Live Pellets sent to GA
20L Culture Grown In GA

4 x 500L Fermentors Grown in Athens



~5.5 hour doublings



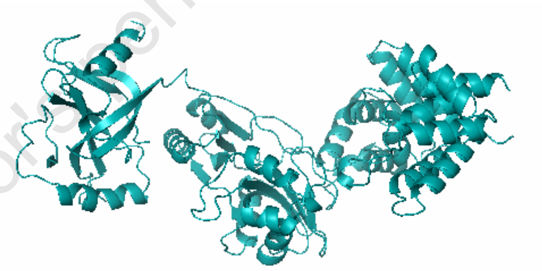
1.5 Kilograms Sulfolobus 3x 500L

Rob Rambo
(Turning Knobs)

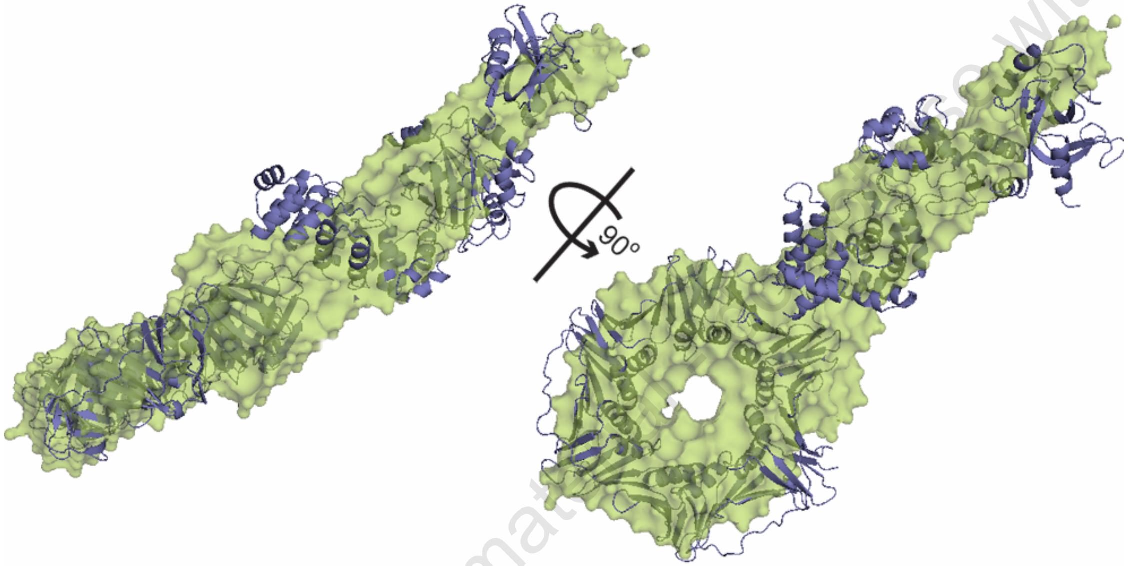
PCNA & ligase SAXS - interface exchange & conformation

Crystal Structure of human Ligase wrapped around DNA

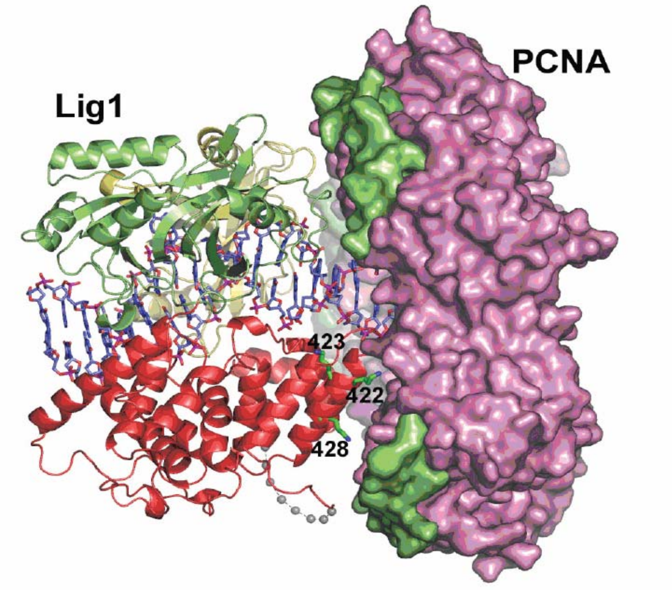
Crystal structure of heterotrimeric S. sulf. sliding clamp



Crystal structure of *S.sulf.* Ligase w/o DNA



Putting the machinery together with SAXS



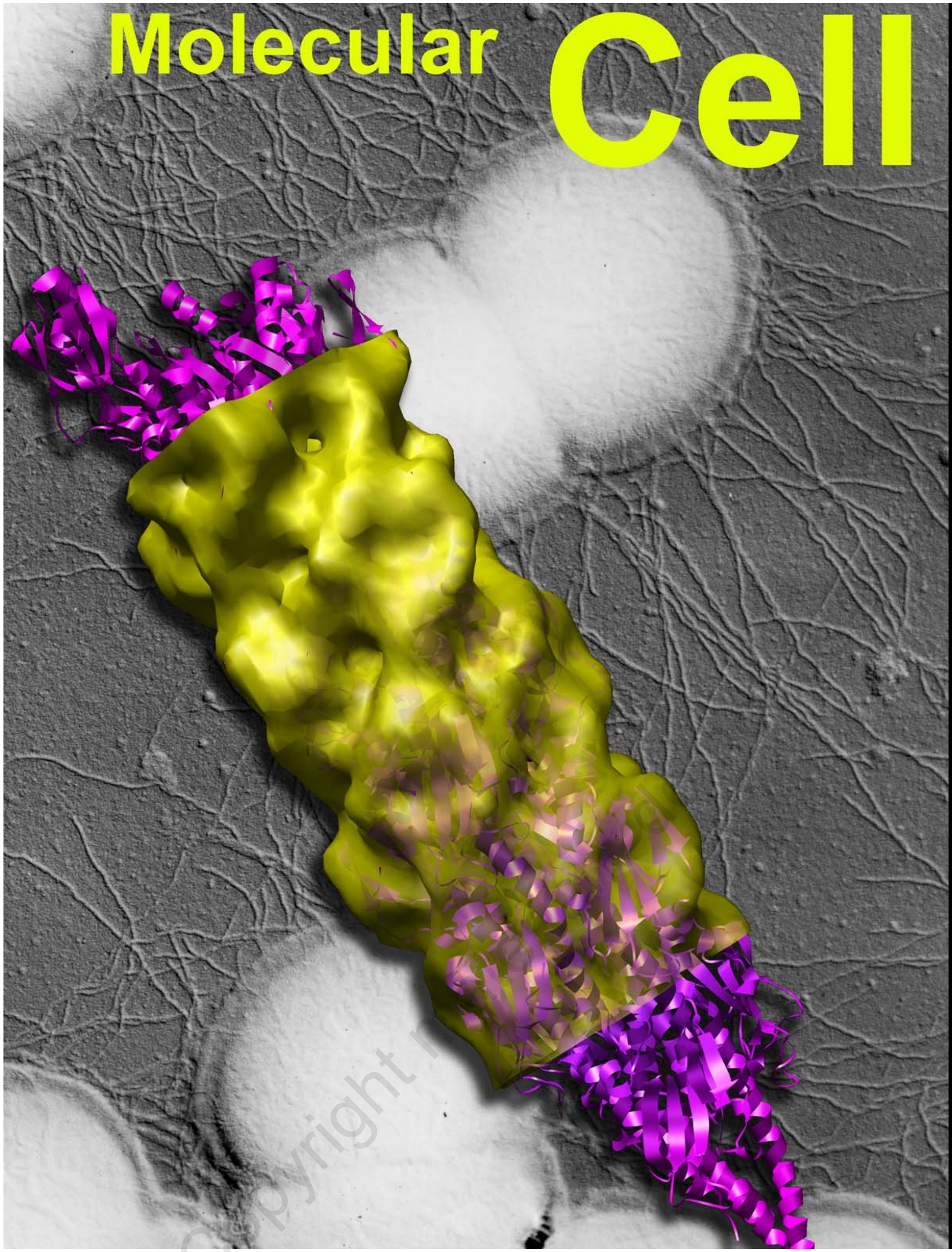
Modeled Complex

A flexible interface between DNA ligase and a heterotrimeric sliding clamp supports conformational switching and efficient ligation of DNA. **Pascal JM, Tsodikov OV, Hura GL, Song W, Cotner EA, Classen S, Tomkinson AE, Tainer JA, and Ellenberger T,***Molecular Cell*, 2006.

Interface Exchange choreography - partners, timing, steps, handoffs, and dynamic movements

- Very high effective molarity by localization
- Scale - mechanical forces but friction & drag insignificant
- Binding energy drives conformational changes between functional states
- Domain rotations plus disorder-order transitions & deformations allow “magical super efficiency” compared to typical machines & tissue

SAXS-MX-Computation - combo for dynamic interfaces & conformations



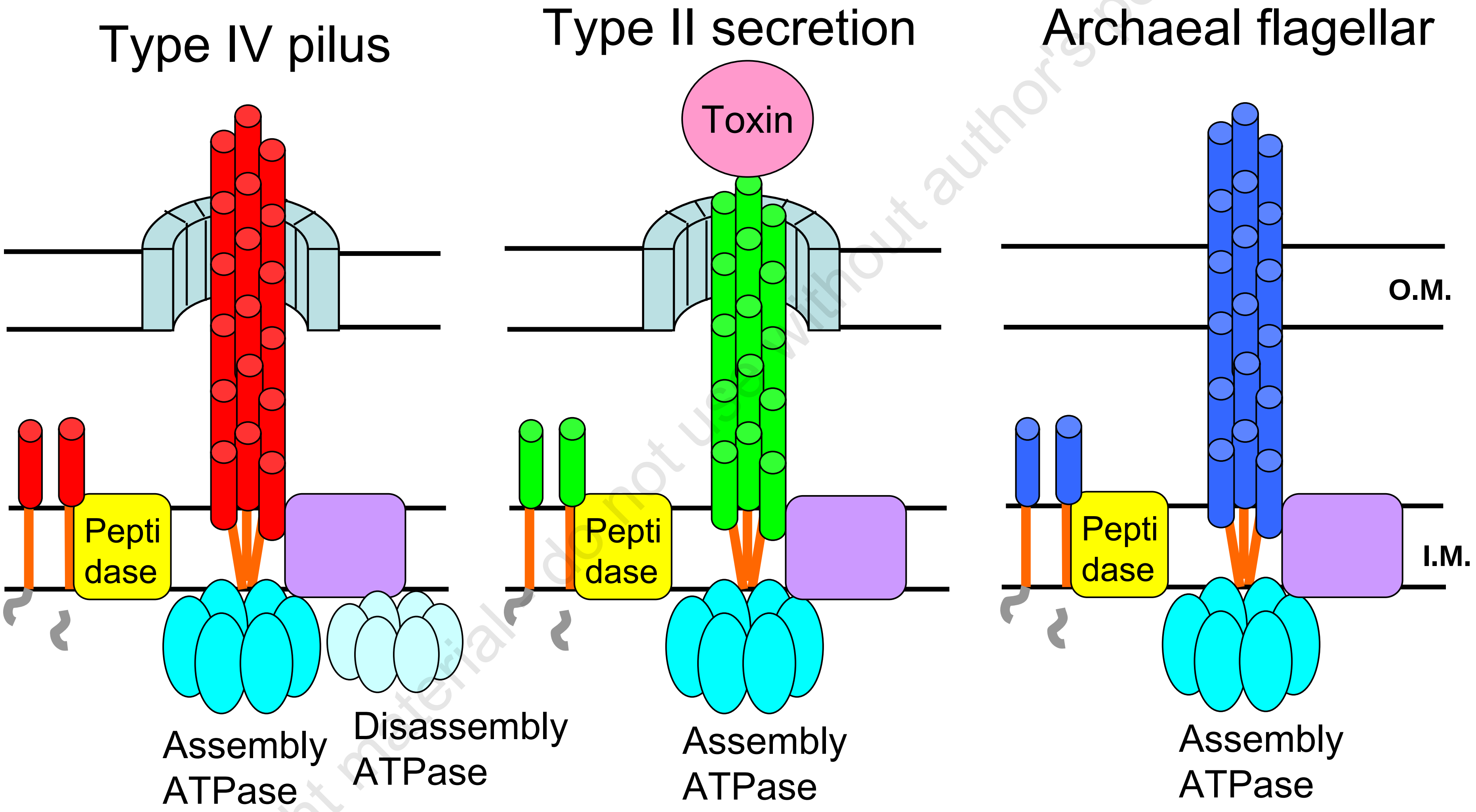
Assembly Machinery?

Type IV Pilus (T4P) Membrane & Fiber Protein Assembly System

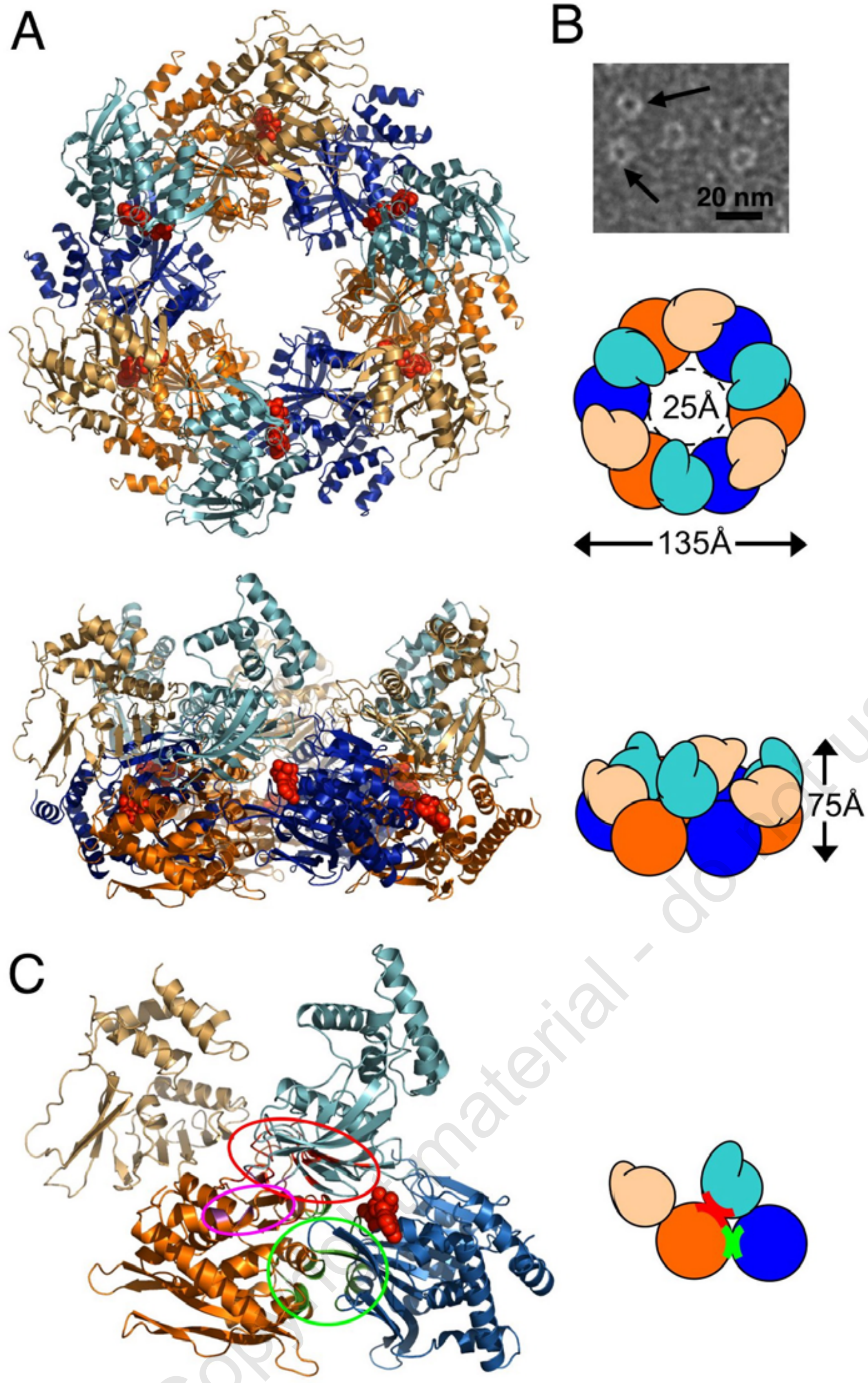
L. Craig et al., (2006)
Molecular Cell 23:651-62.

Testing the Assembly model - how do the secretion super family ATPases function to efficiently assemble and disassemble T4P, secrete toxins & assemble archaeal flagella?

T4P Membrane & Fiber Protein Machine Systems keys to pathogenicity & protein secretion machines



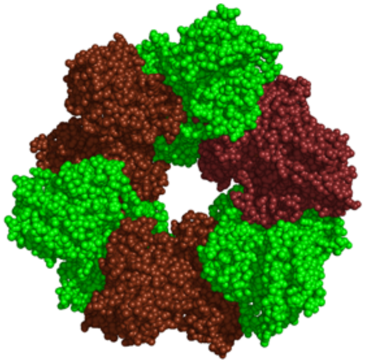
How do secretion super family ATPases act in their secretion & assembly functions?

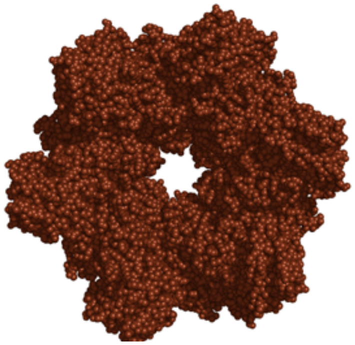


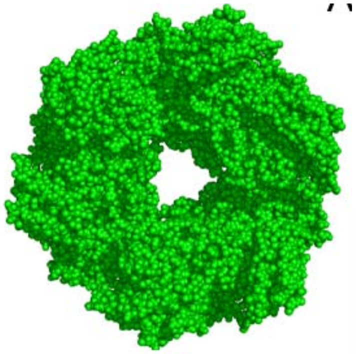
What is the structure of the assembly ATPase hexamer in solution?

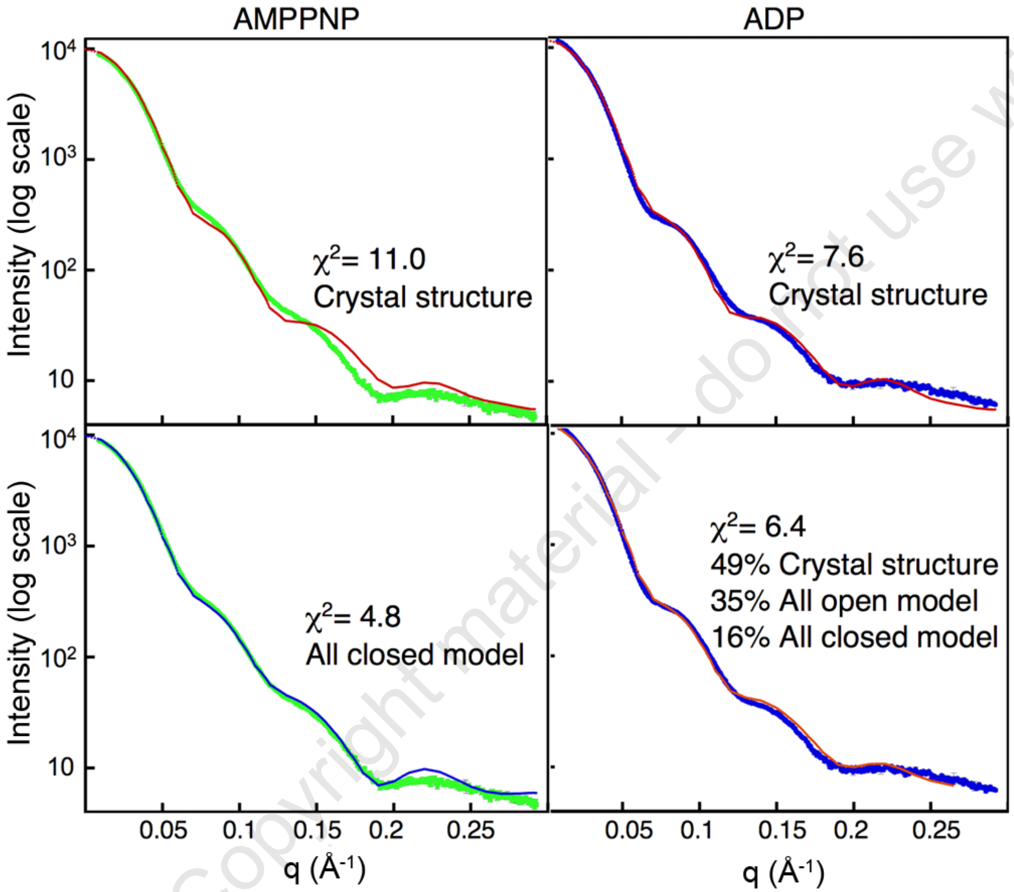
Test by imaging assemblies in solution by SAXS at the SIBYLS beam line at the ALS

ATPase hexamer in solution

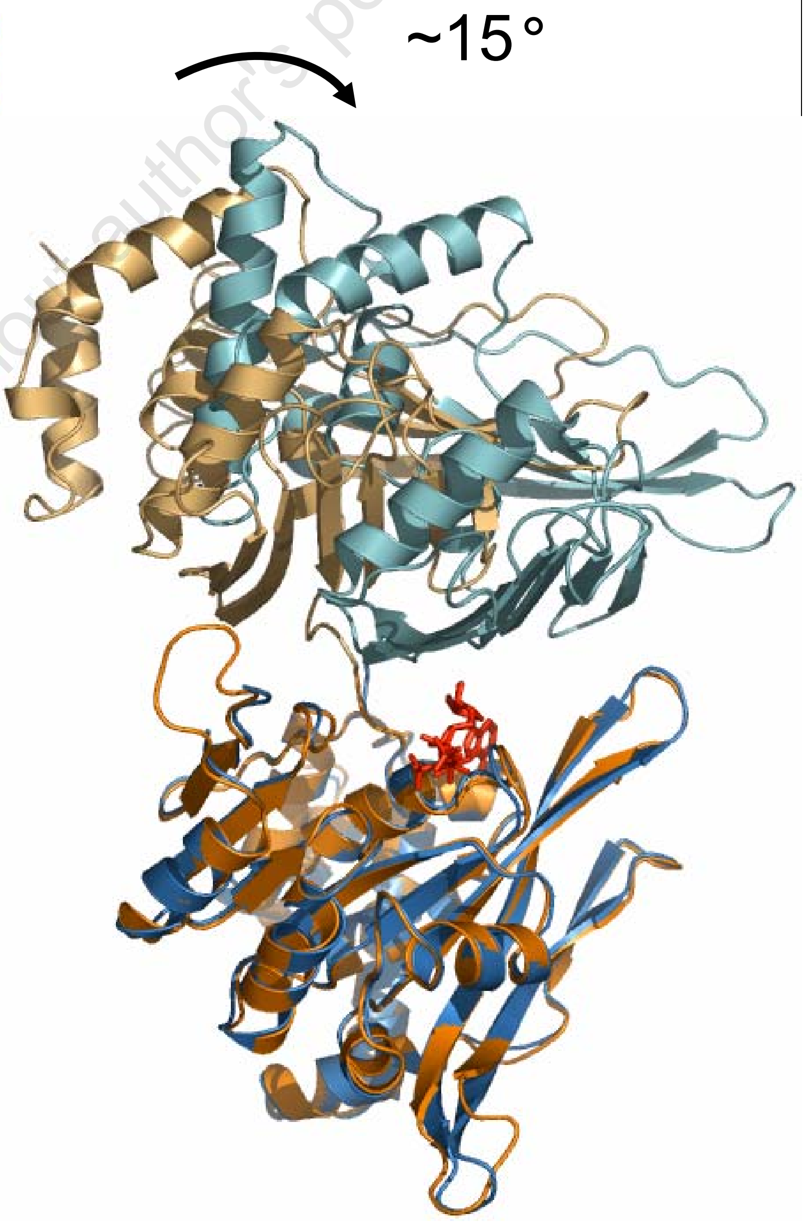

Crystal Structure


All Closed model

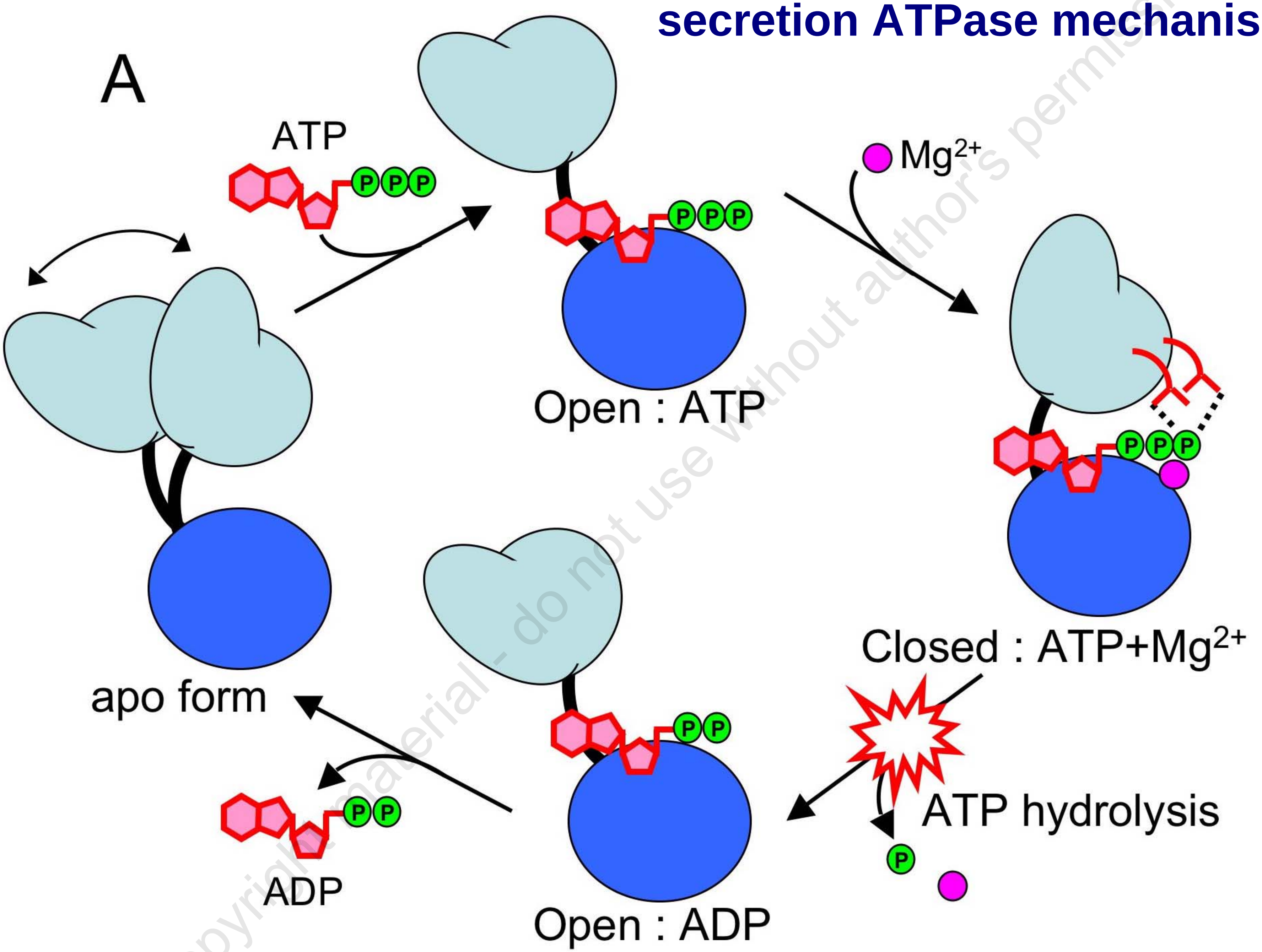

All open model

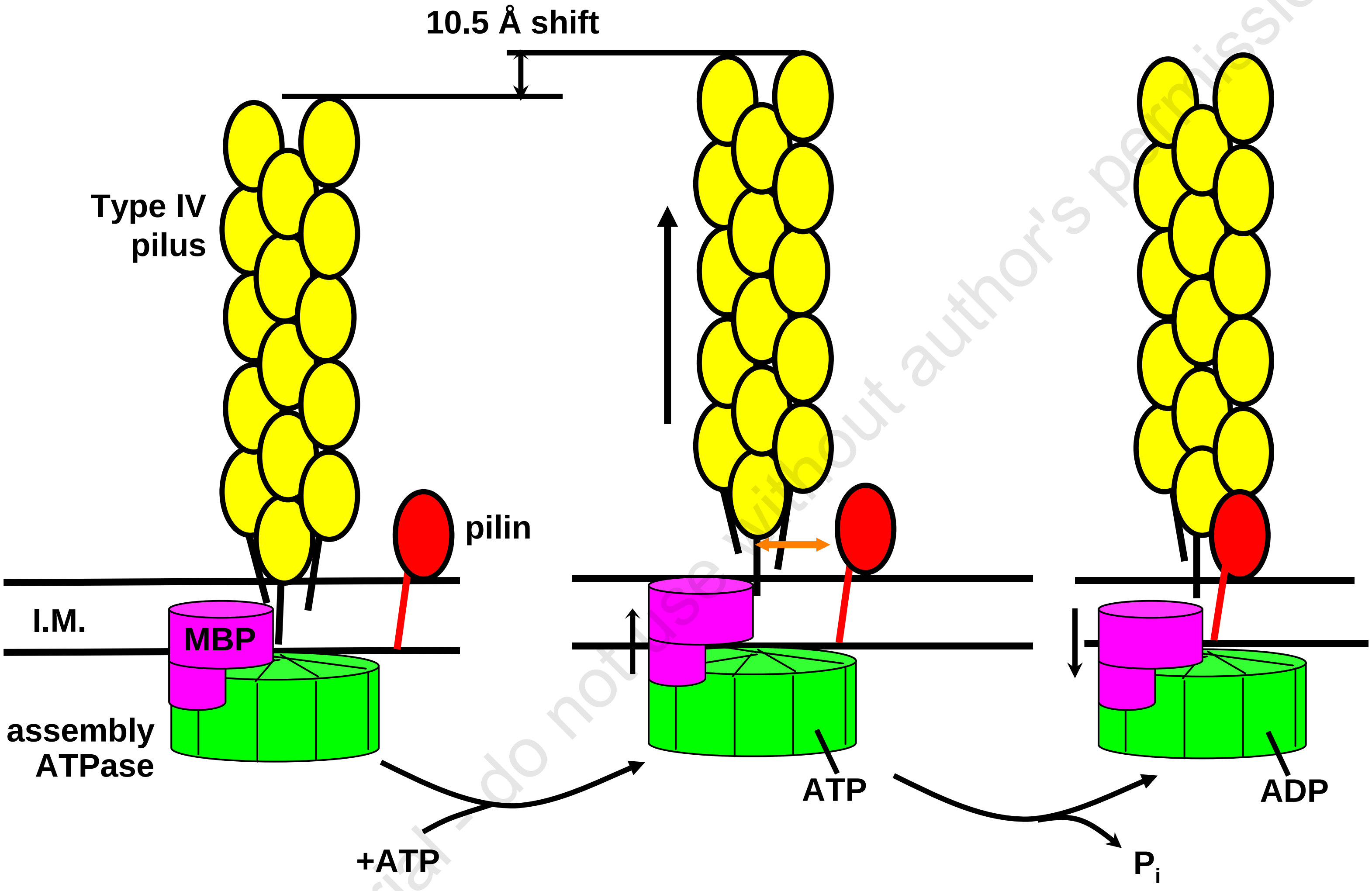


Open-close switch for afGspE2 ATPase



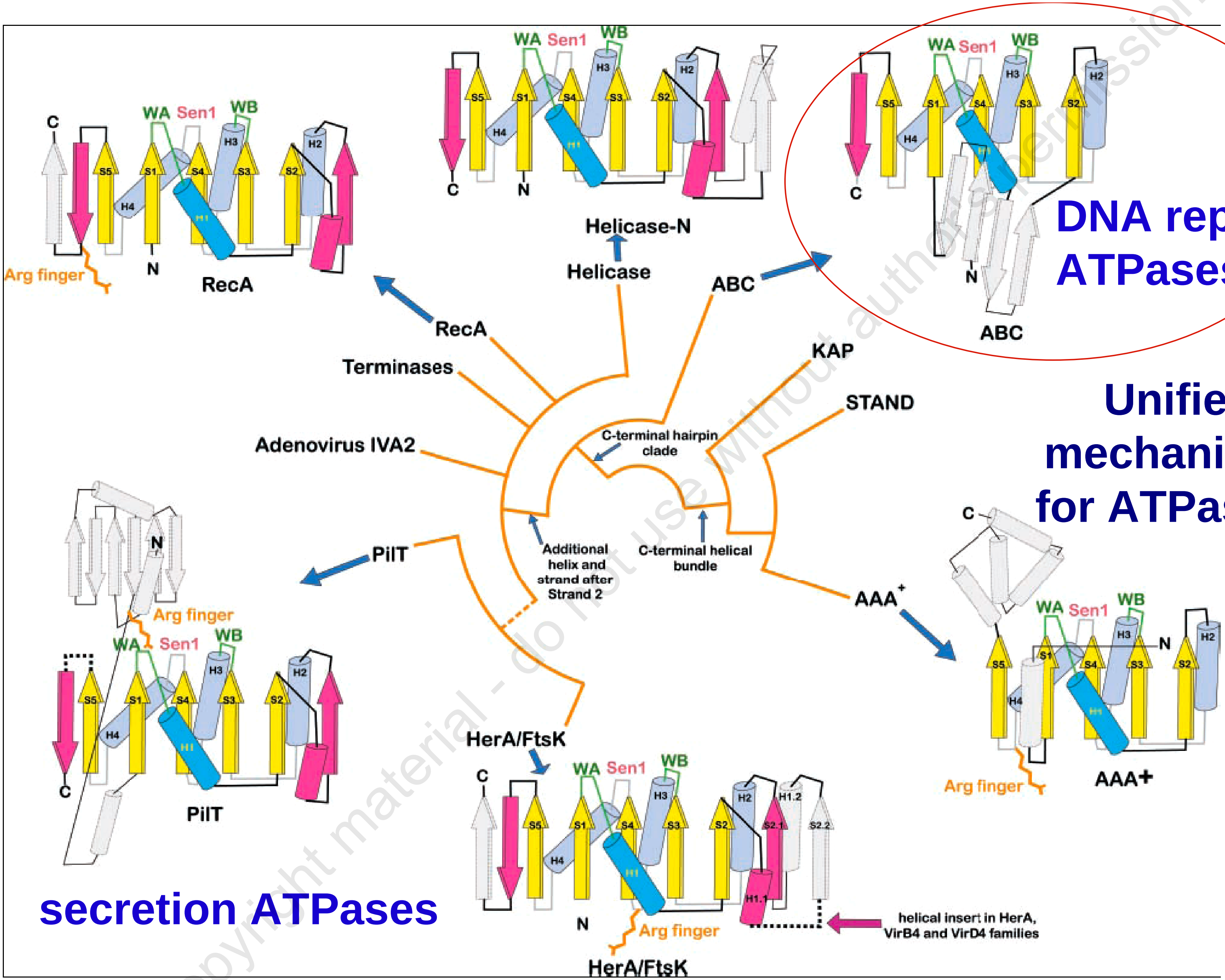
secretion ATPase mechanism





A unified mechanism explains secretion super family ATPase function to efficiently assemble and disassemble T4P

SAXS is useful to define solution conformations & mechanisms for assembly machinery for large complexes

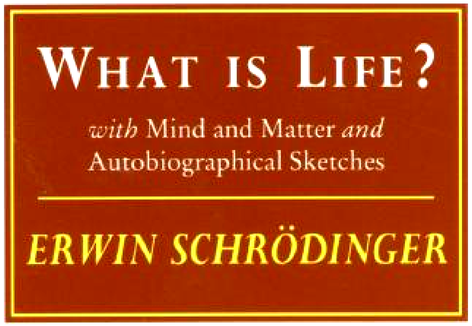


DNA repair ATPases

Unified mechanisms for ATPases?

secretion ATPases

DNA Repair: the major missed aspect of the double helix



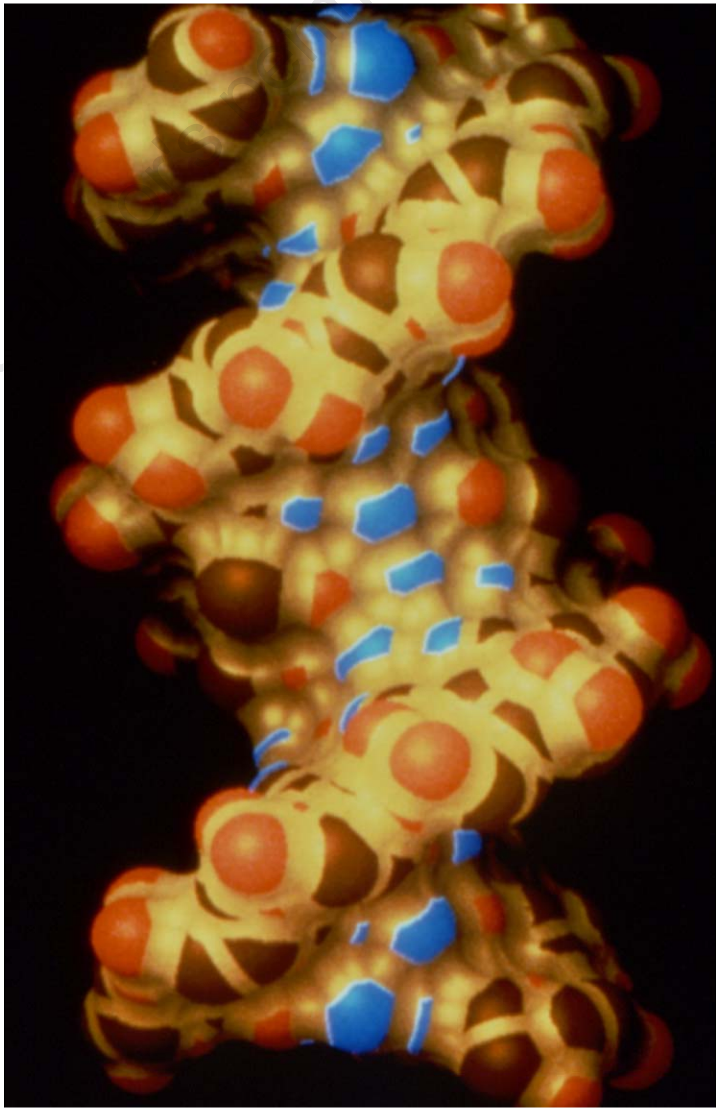
WHAT IS LIFE?

with Mind and Matter *and*
Autobiographical Sketches

ERWIN SCHRÖDINGER

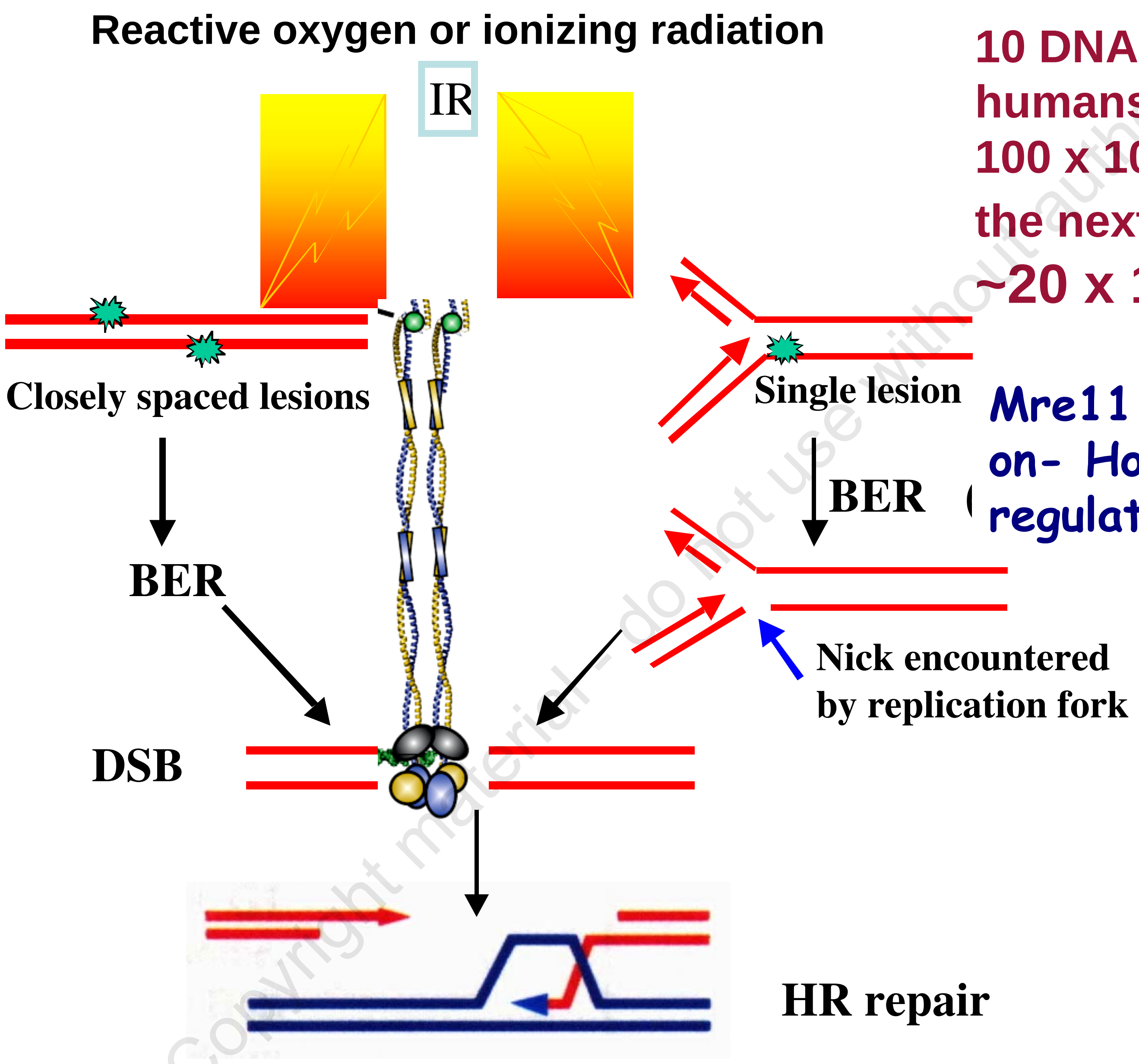
90% of mutations are endogenous - **DNA Repair is essential to life**

Rad50 ABC ATPase - finds, signals & repairs DNA DSBs



DNA Repair key to metabolism, transcription, replication, & cell cycle

Base damage and ssDNA breaks create DNA double-strand breaks (DSBs) whenever the genome is replicated

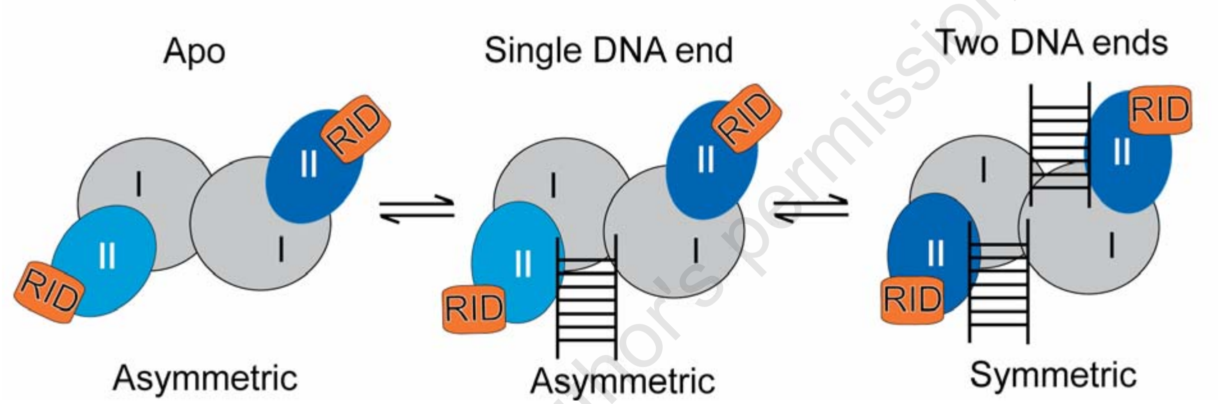


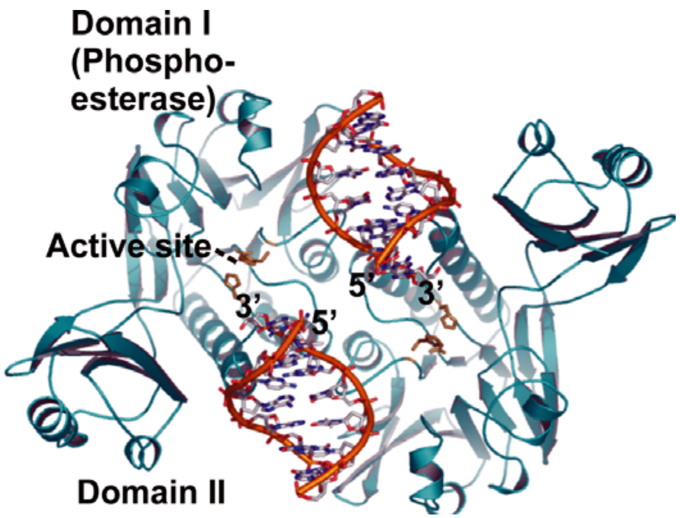
Homologous Recombination

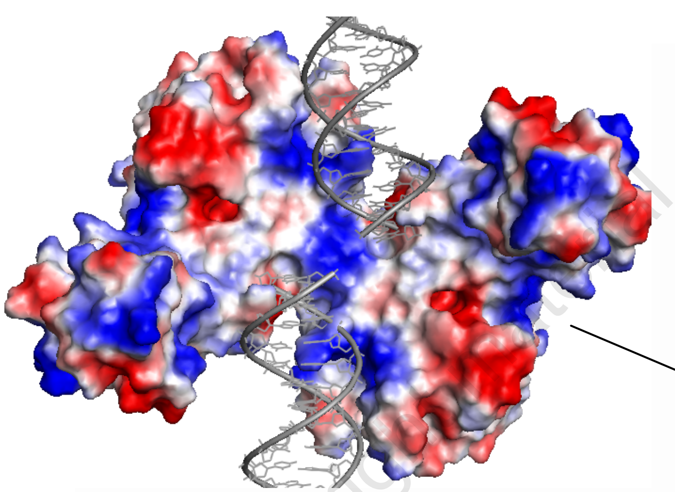
10 DNA DSBs /cell /day in humans
100 x 10^{12} cells/human so the next 30 minutes ~ ~20 x 10^{12} DSB's

Mre11:Rad50 ATPase first on- How does Rad50 find, regulate, & repair DSBs?

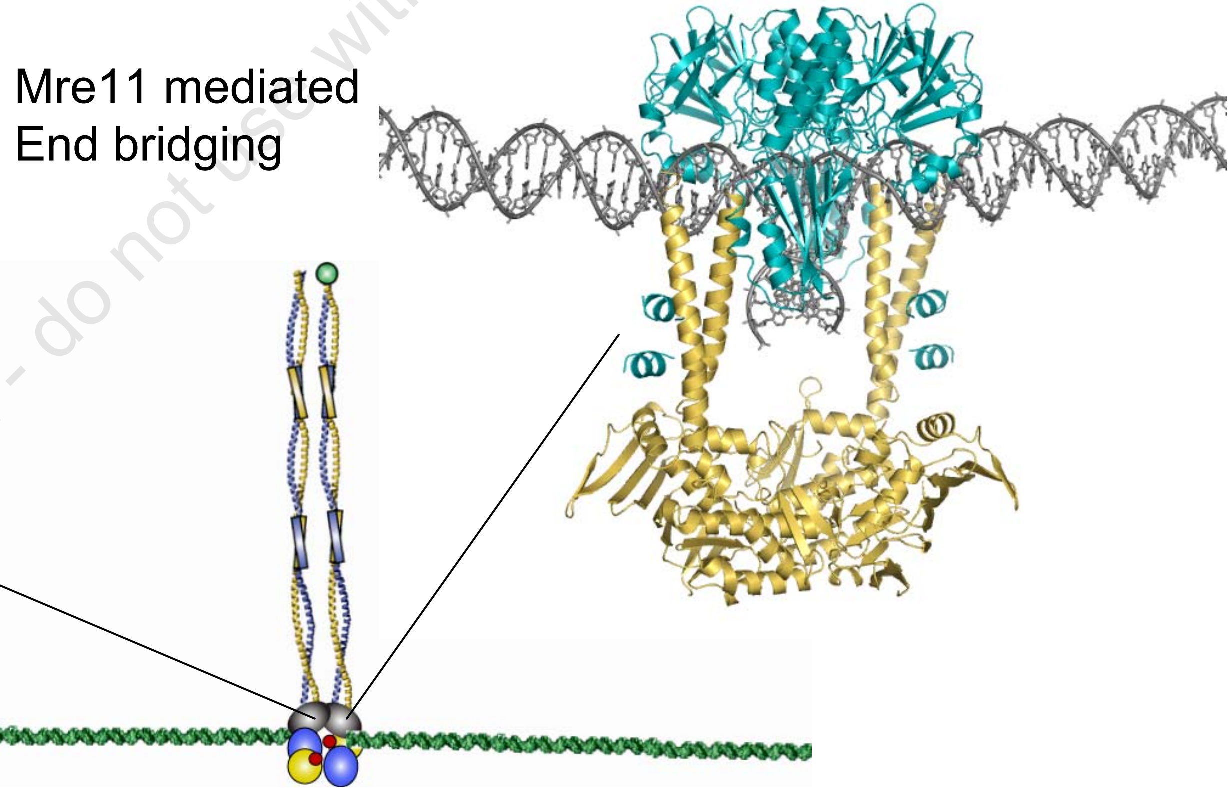
MR(N) DNA end tethering



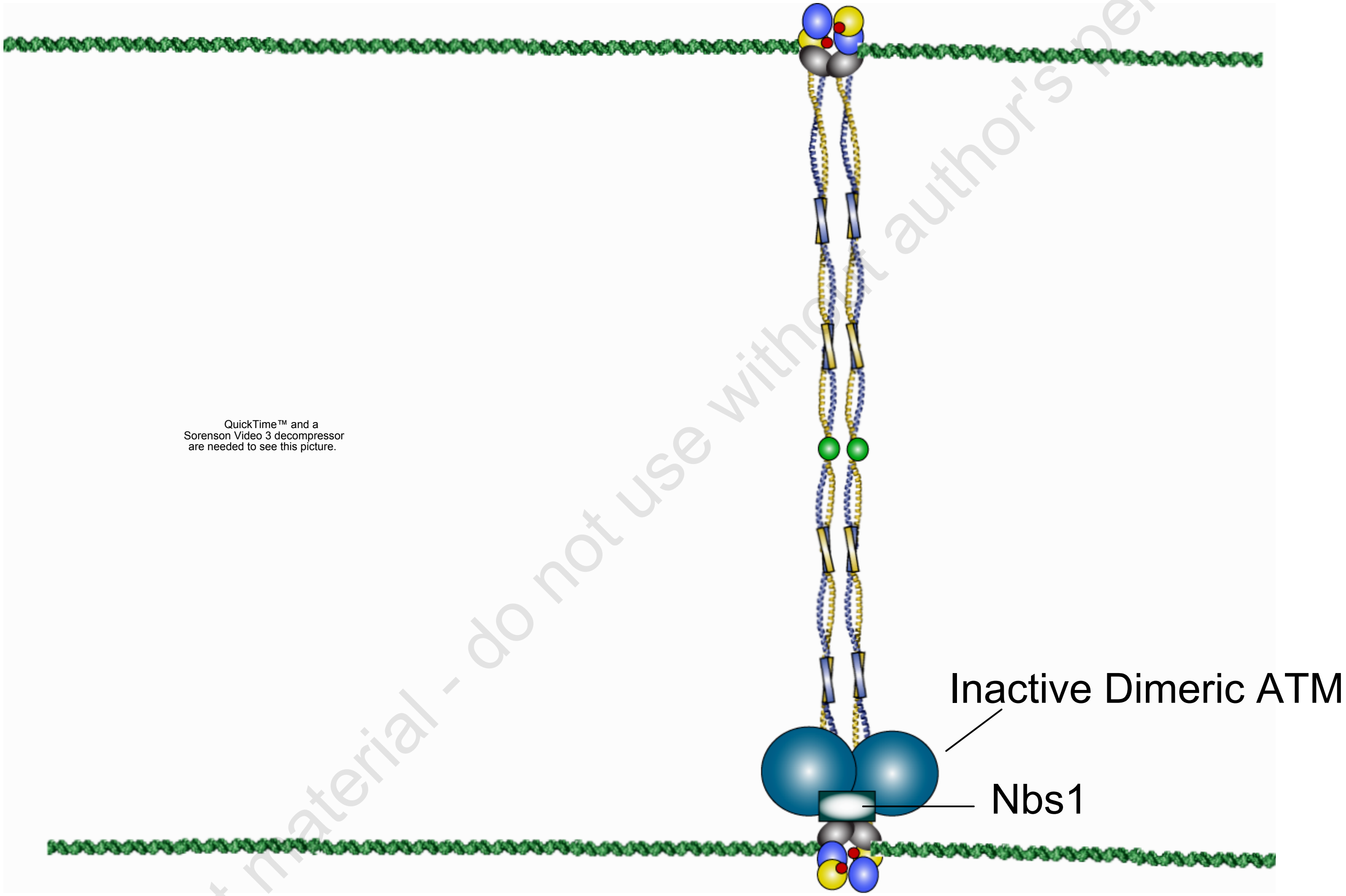




Mre11 mediated
End bridging

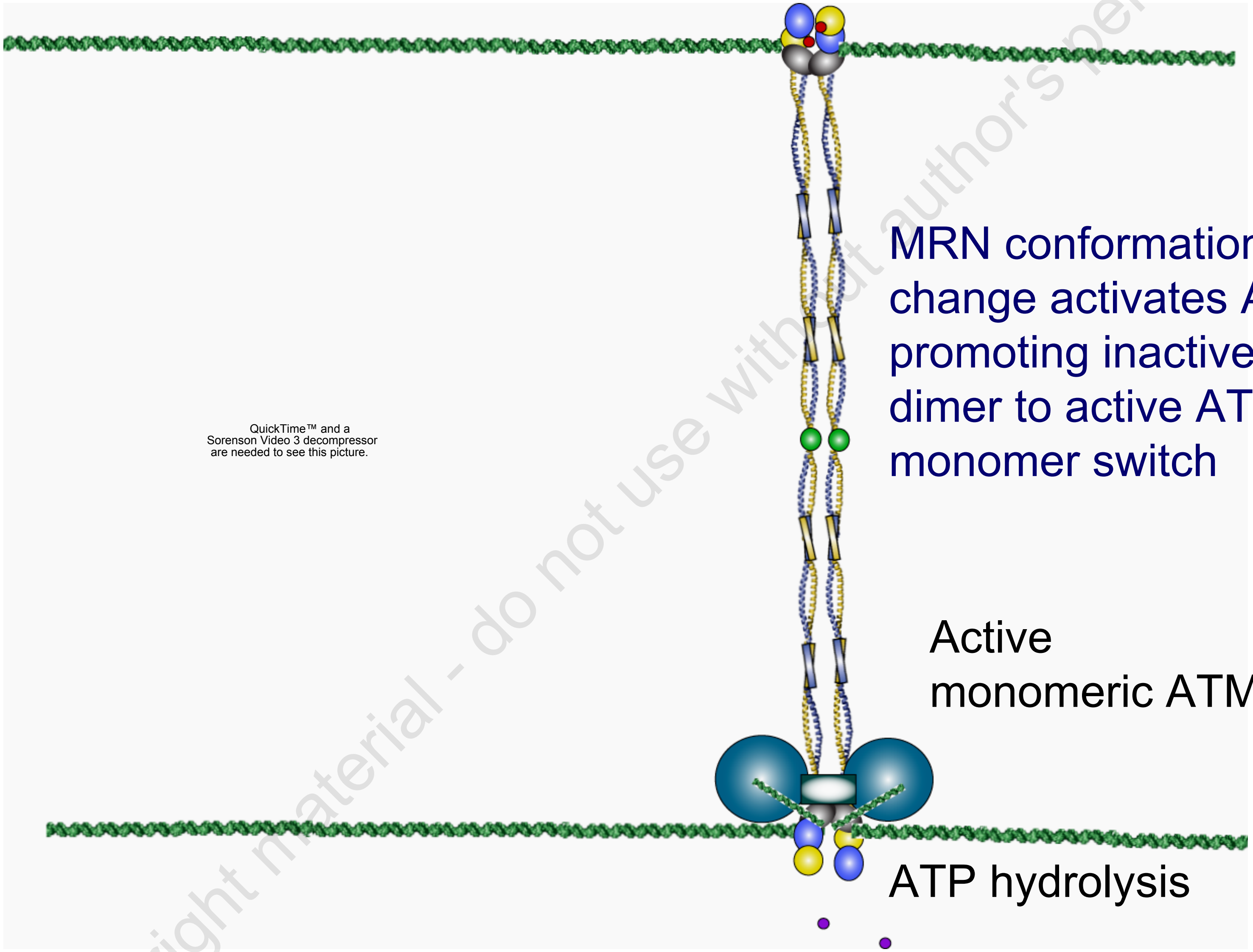


ATM recruitment to MRN via Nbs1



QuickTime™ and a
Sorenson Video 3 decompressor
are needed to see this picture.

ATM activation via MRN conformational change



MRN conformational change activates ATM by promoting inactive ATM dimer to active ATM monomer switch

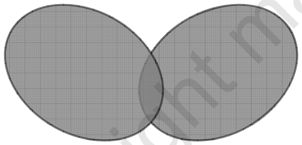
Active
monomeric ATM

ATP hydrolysis

MR hierarchical composition from SAXS and crystallography

Mre11

QuickTime™ and a
Sorenson Video 3 decompressor
are needed to see this picture.



Rad50-ATP complex

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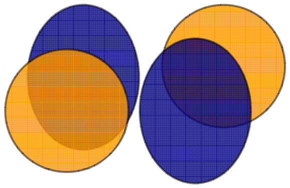
are needed to see this picture.



Mre11-Rad50
ATP complex

QuickTime™ and a
Sorenson Video 3 decompressor
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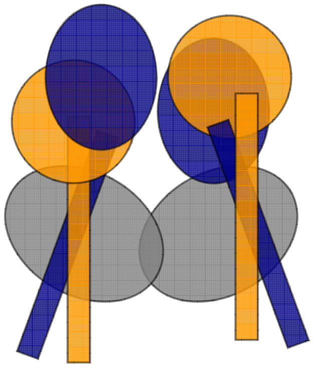
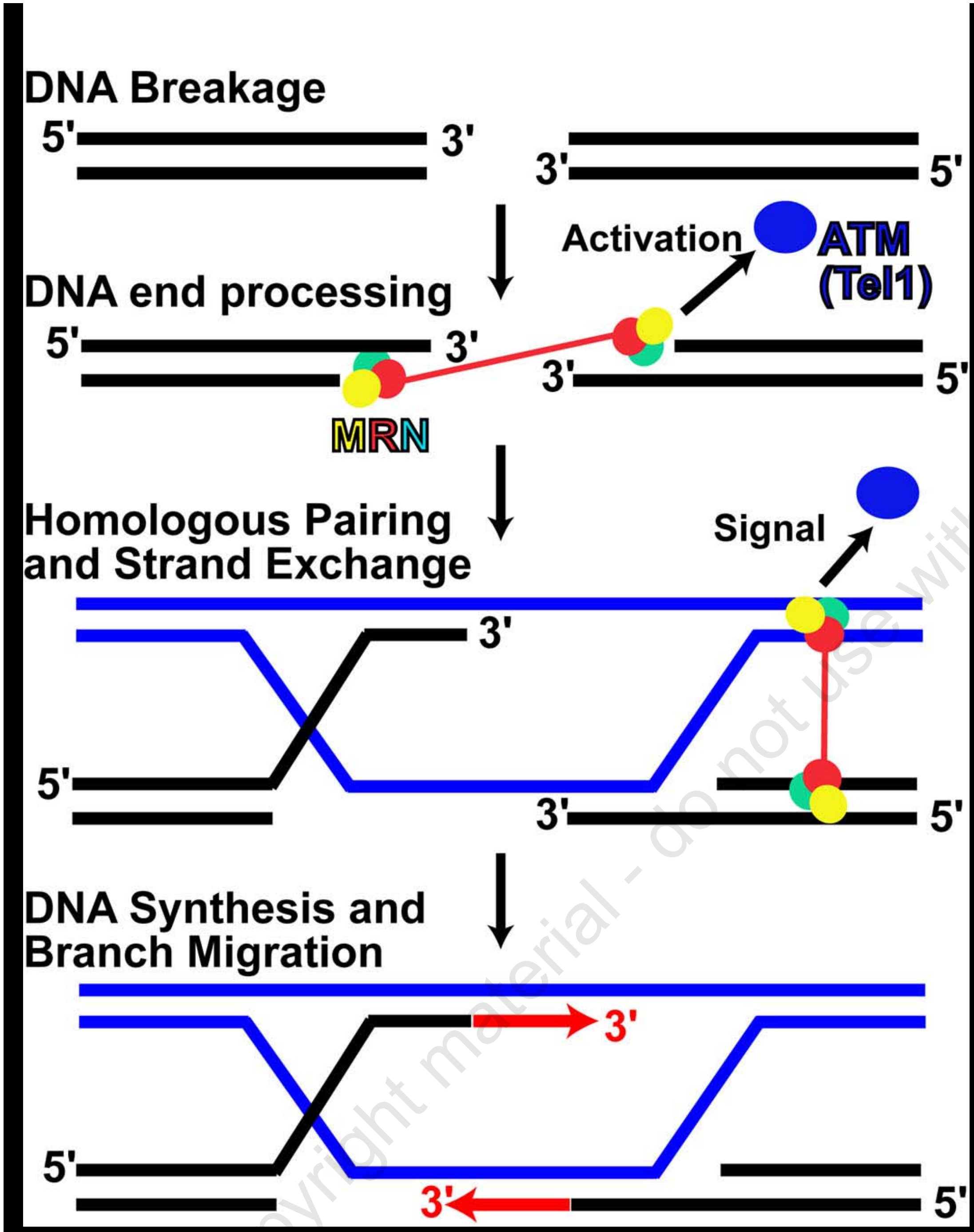


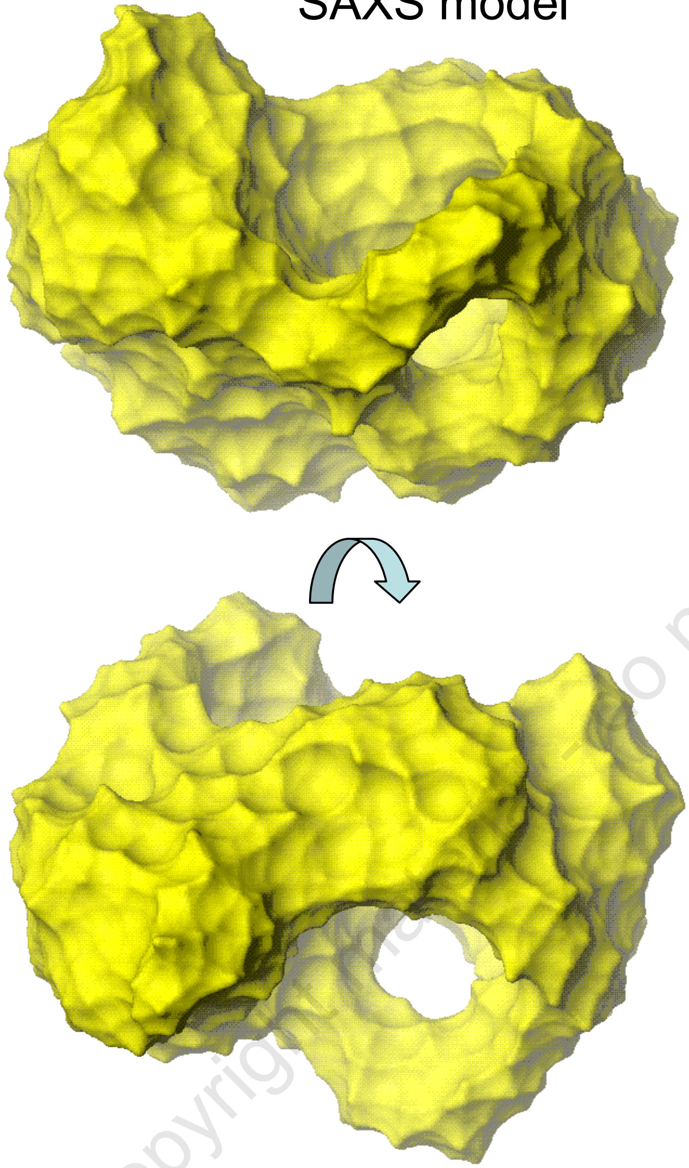


Double-Stand Break repair: MRN on first

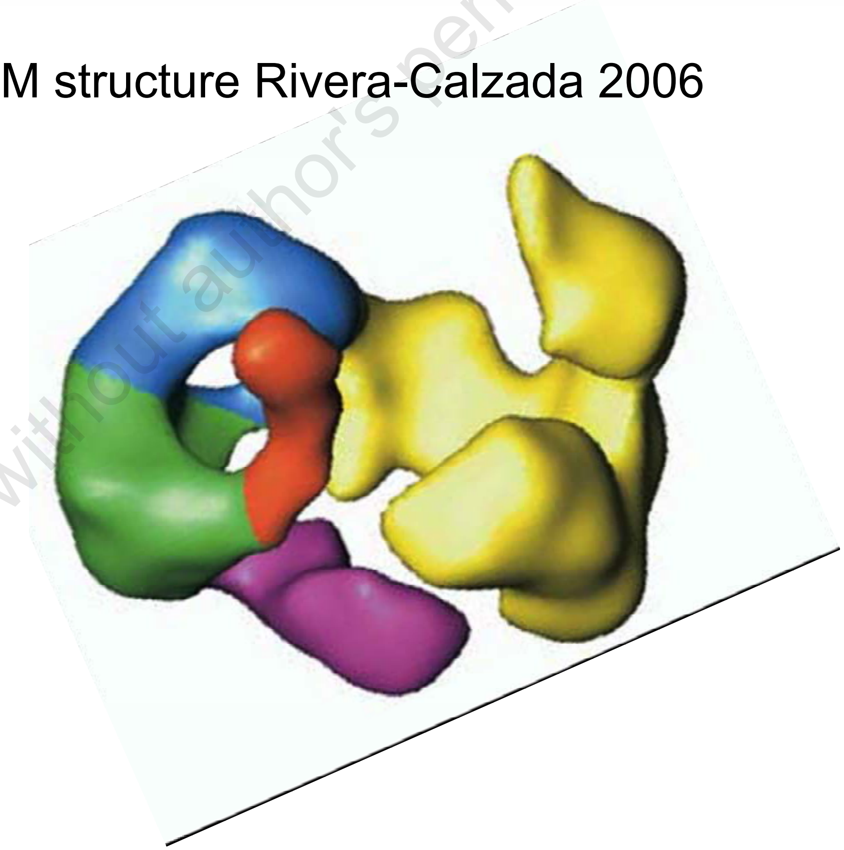
Conformational controls
Interface mimicry
Interface exchange
(Lessons from BER and RCR - apply to HRR)

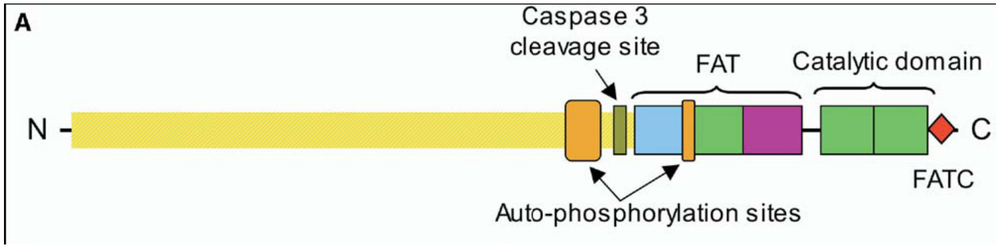
DNA-dependent Protein Kinase (DNA-PKcs)
SAXS solution structure

SAXS model

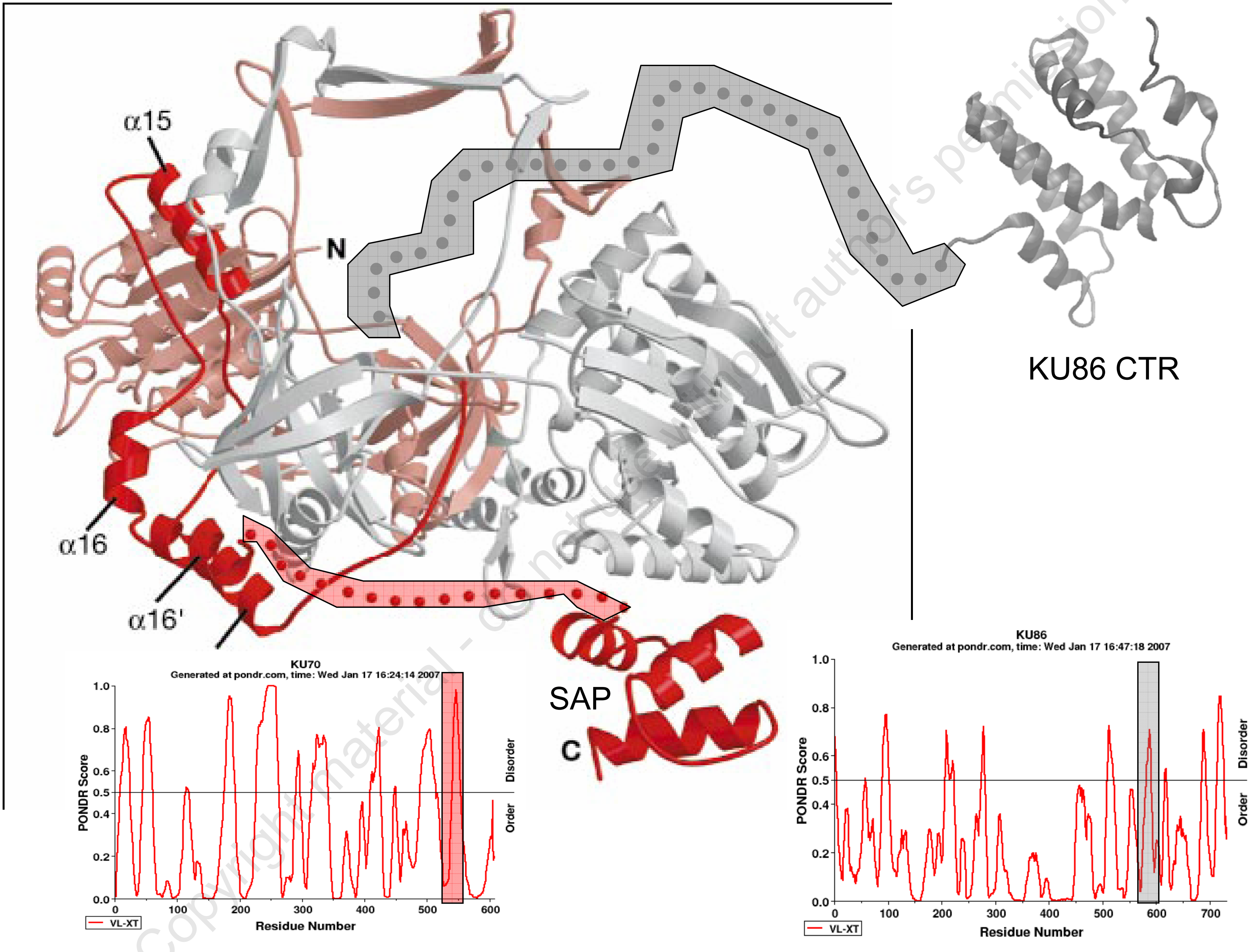


EM structure Rivera-Calzada 2006





SAXS solution structure of DNA-dependent Protein Kinase (DNA-PKcs) vs cryo-EM reconstruction


α15
N
α16
α16'
SAP
C
KU86 CTR
KU70
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Residue Number
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300
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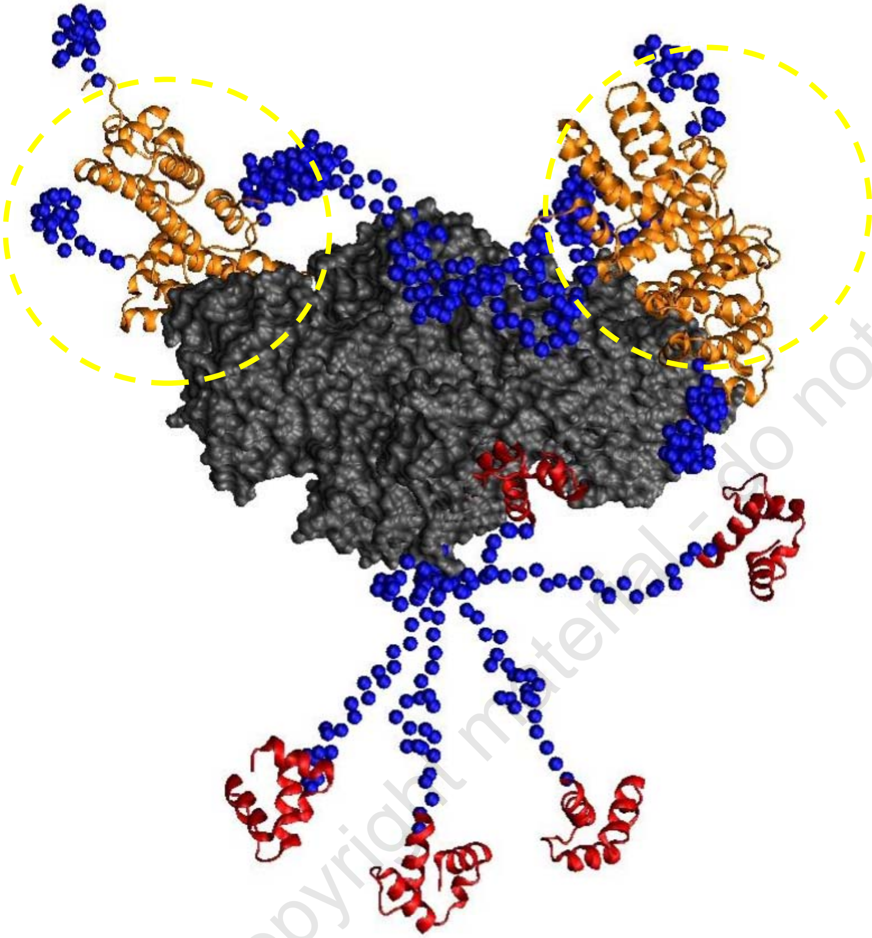
500
600
700
Residue Number
Disorder
Order
VL-XT

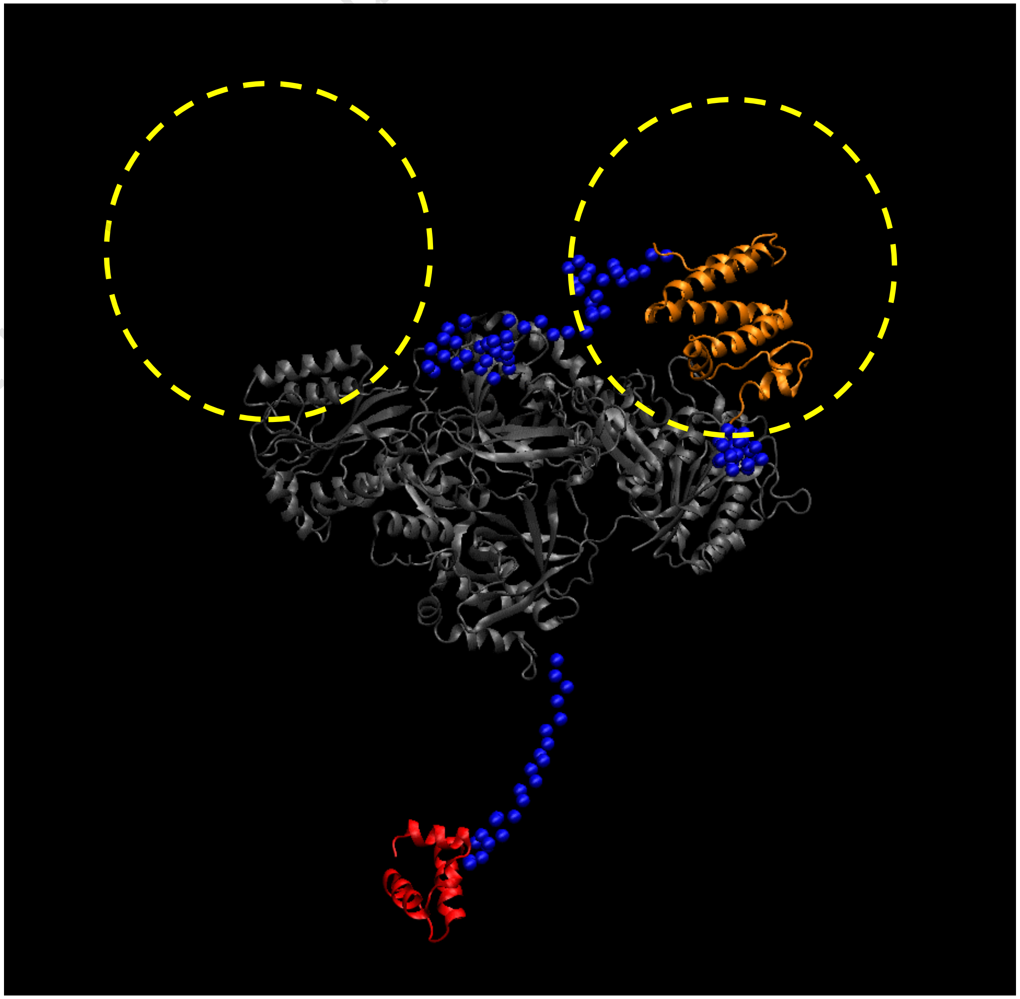
SAXS solution
structure of Ku
dimer) vs cryo-EM
reconstruction

SAXS - Rigid body modeling of KU 70/80 show the extended feature of Ku70 C-terminus (SAP)

→Six rigid body models revealed the possible flexibility of the KU70 CTR (SAP)
→KU80 CTR have been found on the two main location due to symmetrical structure of the KU7080

● KU70 CTR (SAP) ● KU80 CTR ● KU70/80 ● dummy beads - reconstructed linkers





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DNA Replication & Repair PCNA--FEN1-ligase:
Tom Ellenberger, John Pascale, Brian Chapados

Collaborators:
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Clare Wyman

(Funded by
DOE & NCI)

Secretion Superfamily ATPases:
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SAXS: Greg Hura, Michal Hammel, Susan Tsutakawa
MX: Scott Classen, James Holton, Ken Frankel