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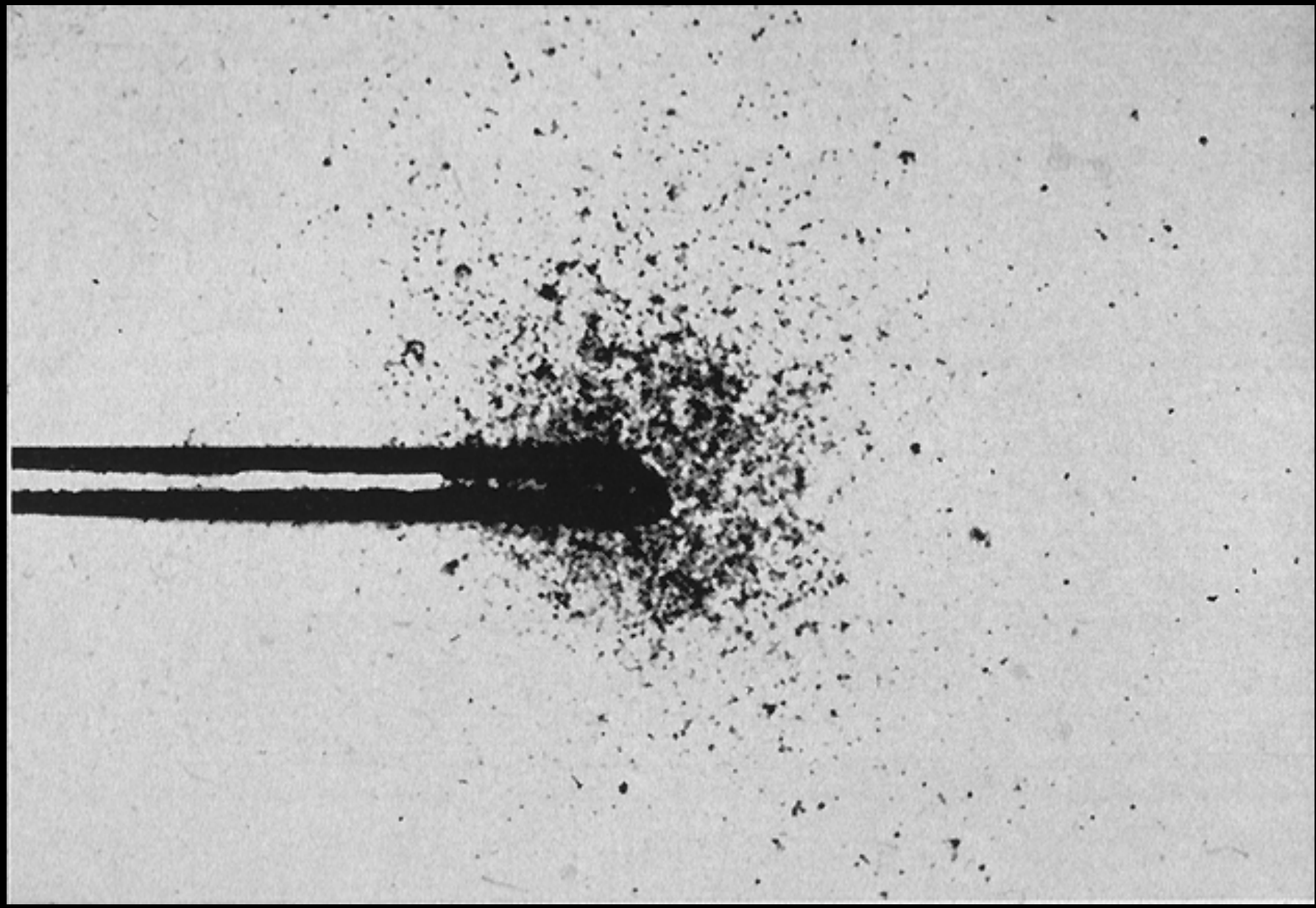
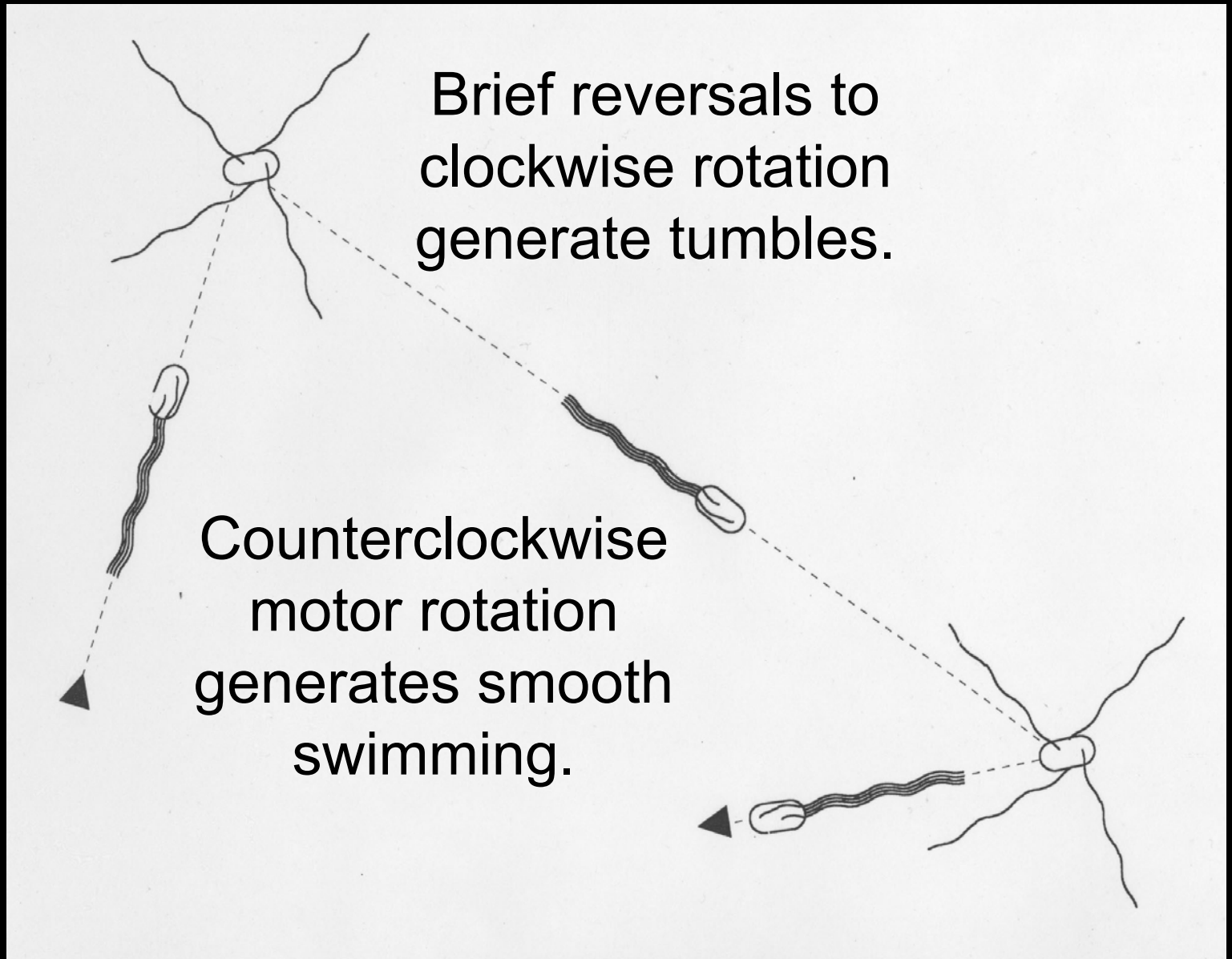
The Structure of the Chemotaxis Machinery

- *Noreen Francis*
- *David Gene Morgan*
- *Tapu Shaikh*
- *Dennis Thomas*
- *James Chen & Niko Grigorieff*
- *Jeff Stock, Mischa Levin, Peter Wolanin*
- *Bob Macnab, May Kihara*
- *Keiichi Namba, K. Yonekura and F. Samatey*
- *Shahid Khan*

Adler: a tube of aspartate attracts E. coli.

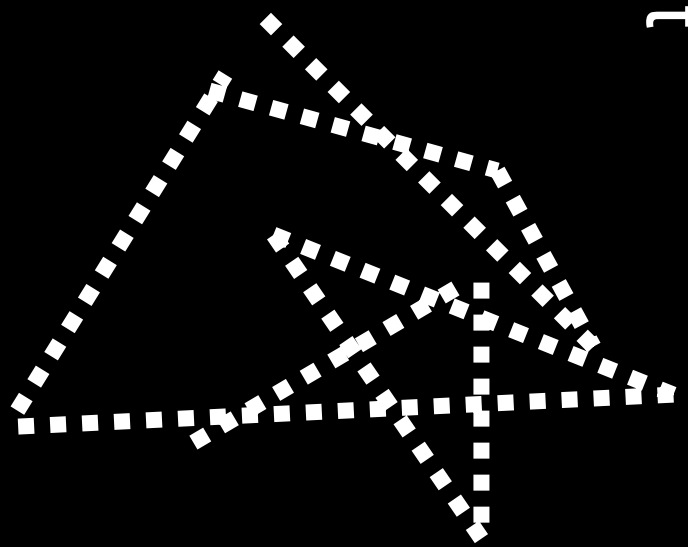


Cells execute a random walk of runs and tumbles.



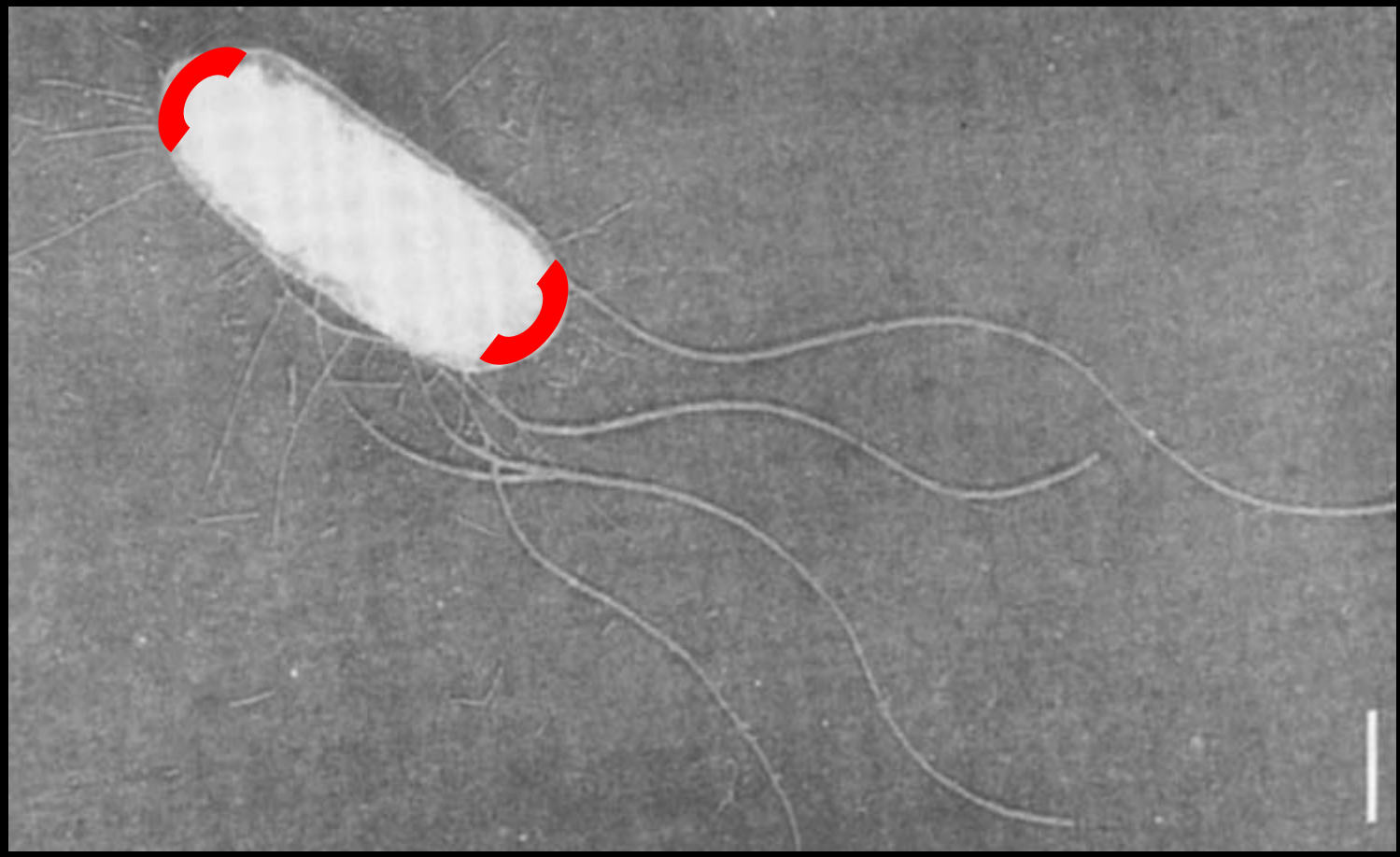
Random walk

No gradient



Biased random walk

Gradient



The chemosensory molecules (CheA, CheW, receptors,CheR,CheB,CheY, CheZ) are localized to cell poles (Maddock and Shapiro).

Signaling

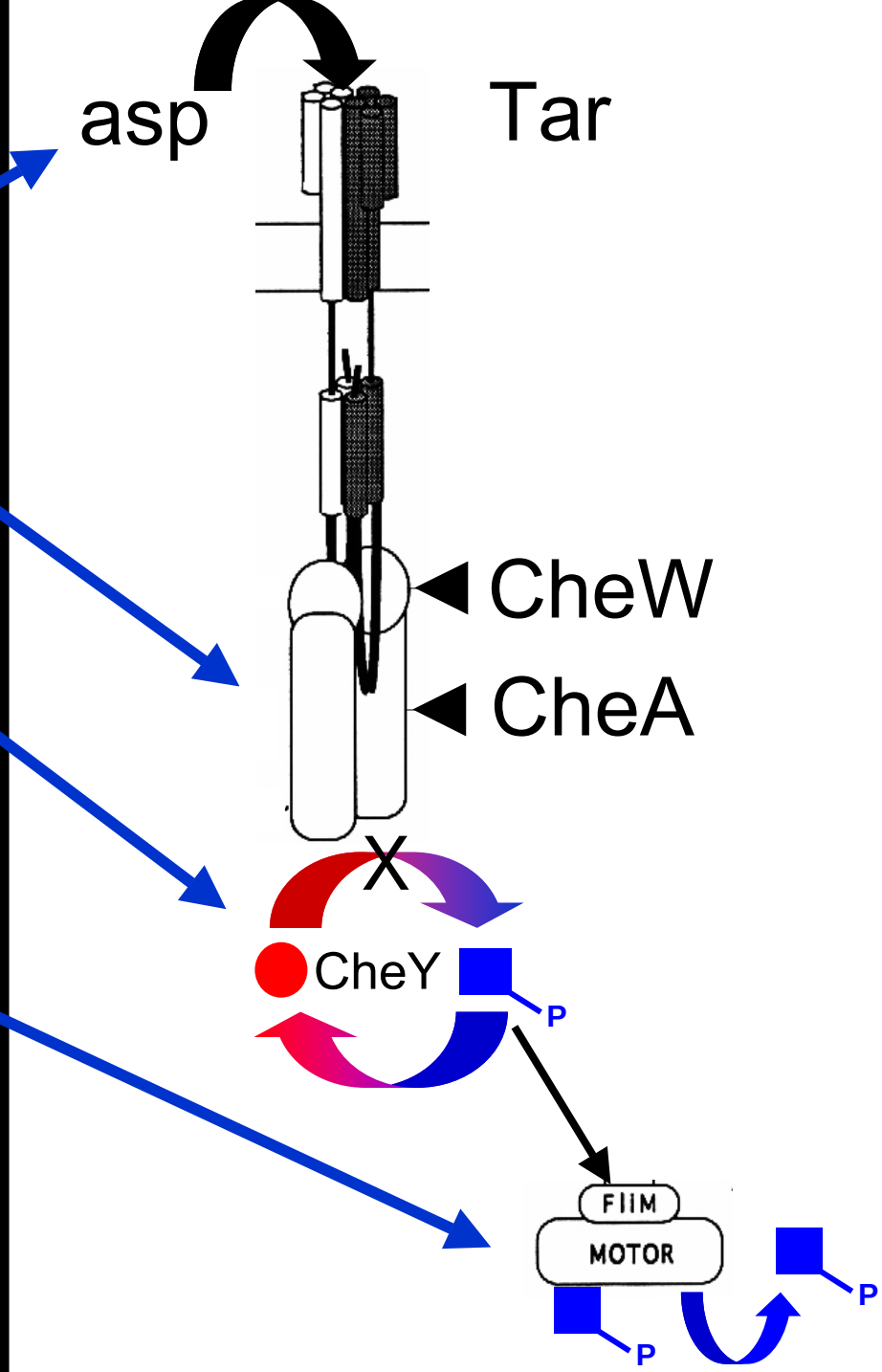
1. Tar binds aspartate.

2. Activity of CheA kinase decreases.

3. Amount of CheY-P decreases.

4. Probability of a tumble decreases.

5. Cell swims longer.



Adaptation

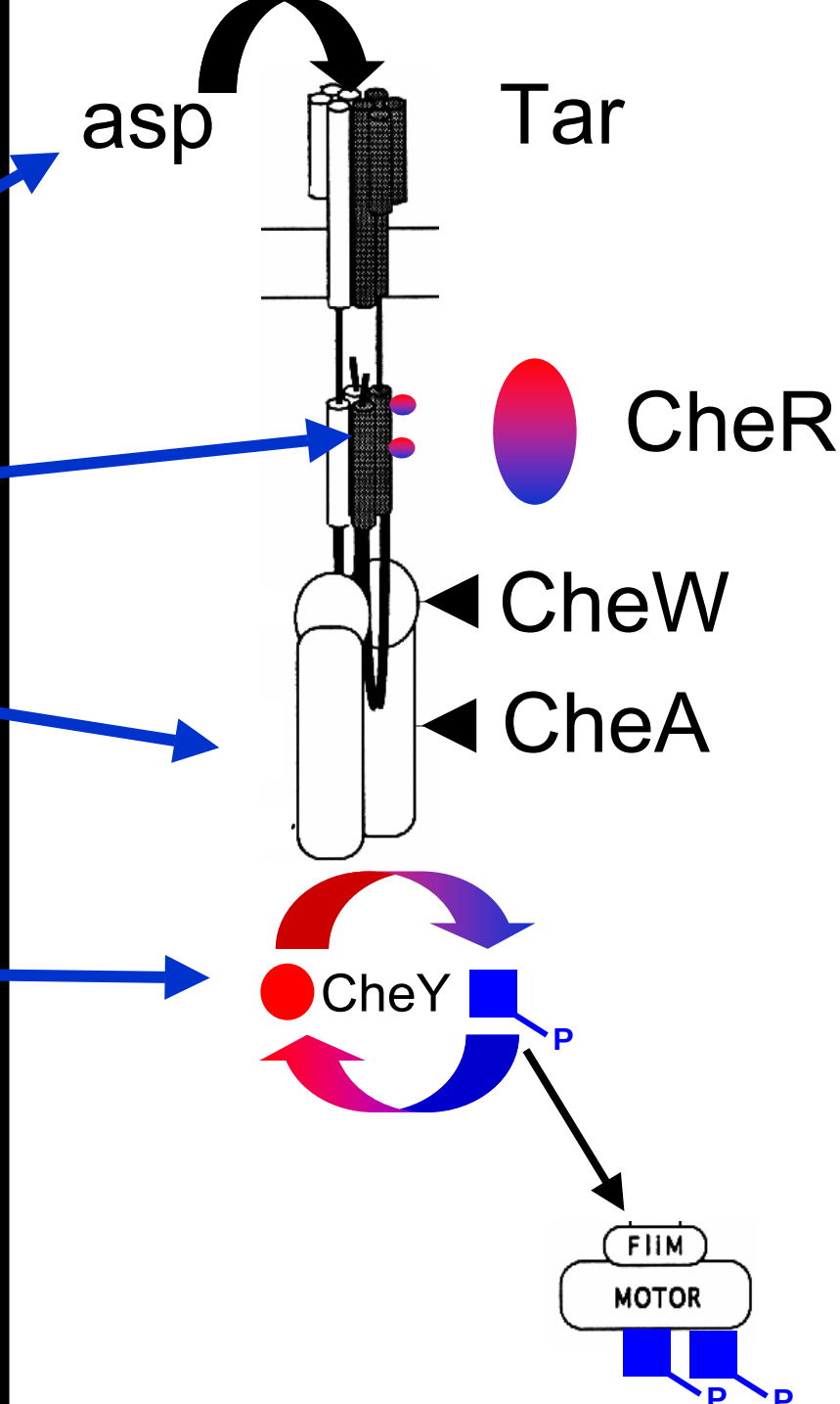
1. Tar binds aspartate.

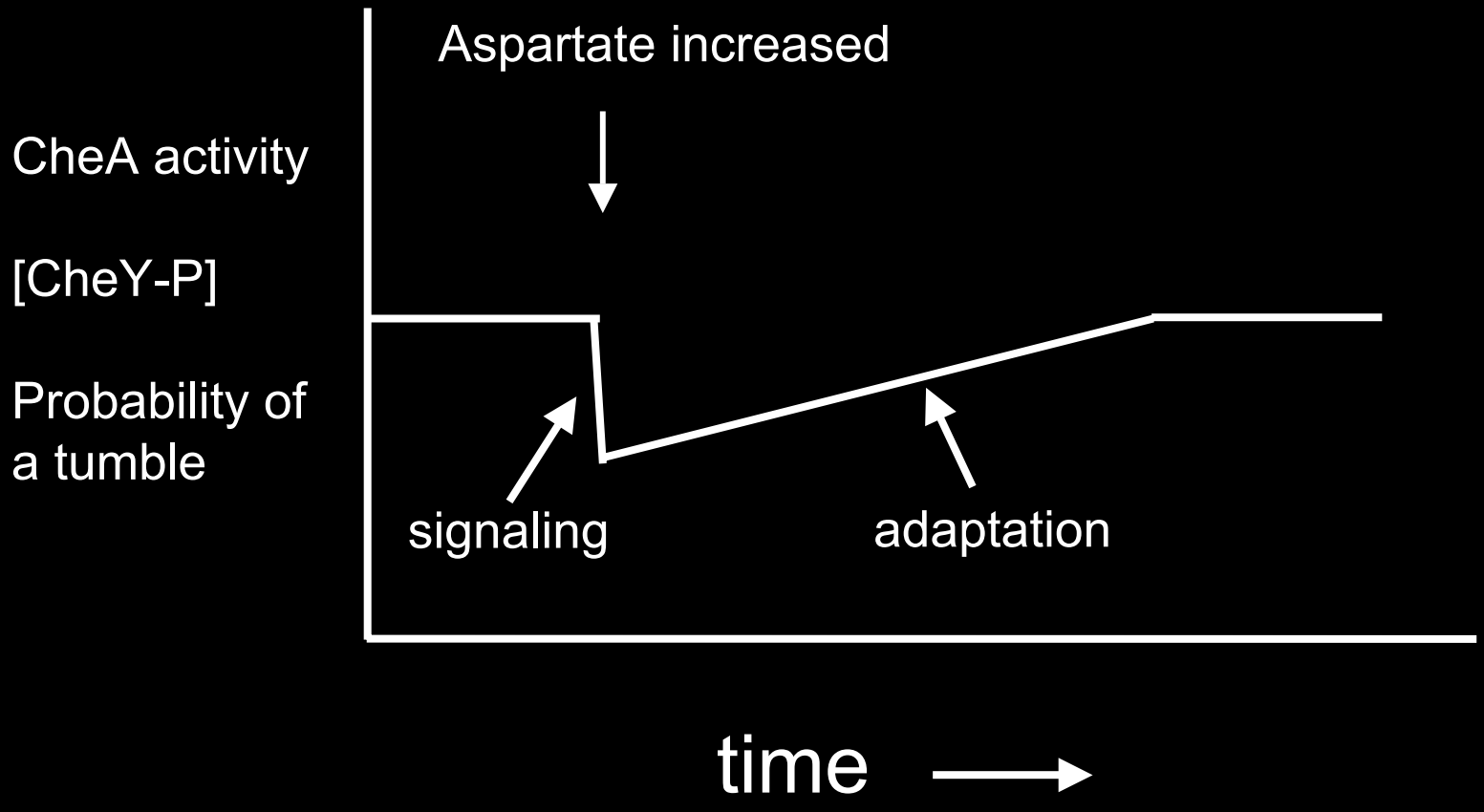
2. CheR methylates Tar.

3. CheA activity is restored.

4. CheY-P is restored.

5. Cell returns to non-signaling state..




Aspartate increased
CheA activity
[CheY-P]
Probability of a tumble
signaling
adaptation
time

Signaling

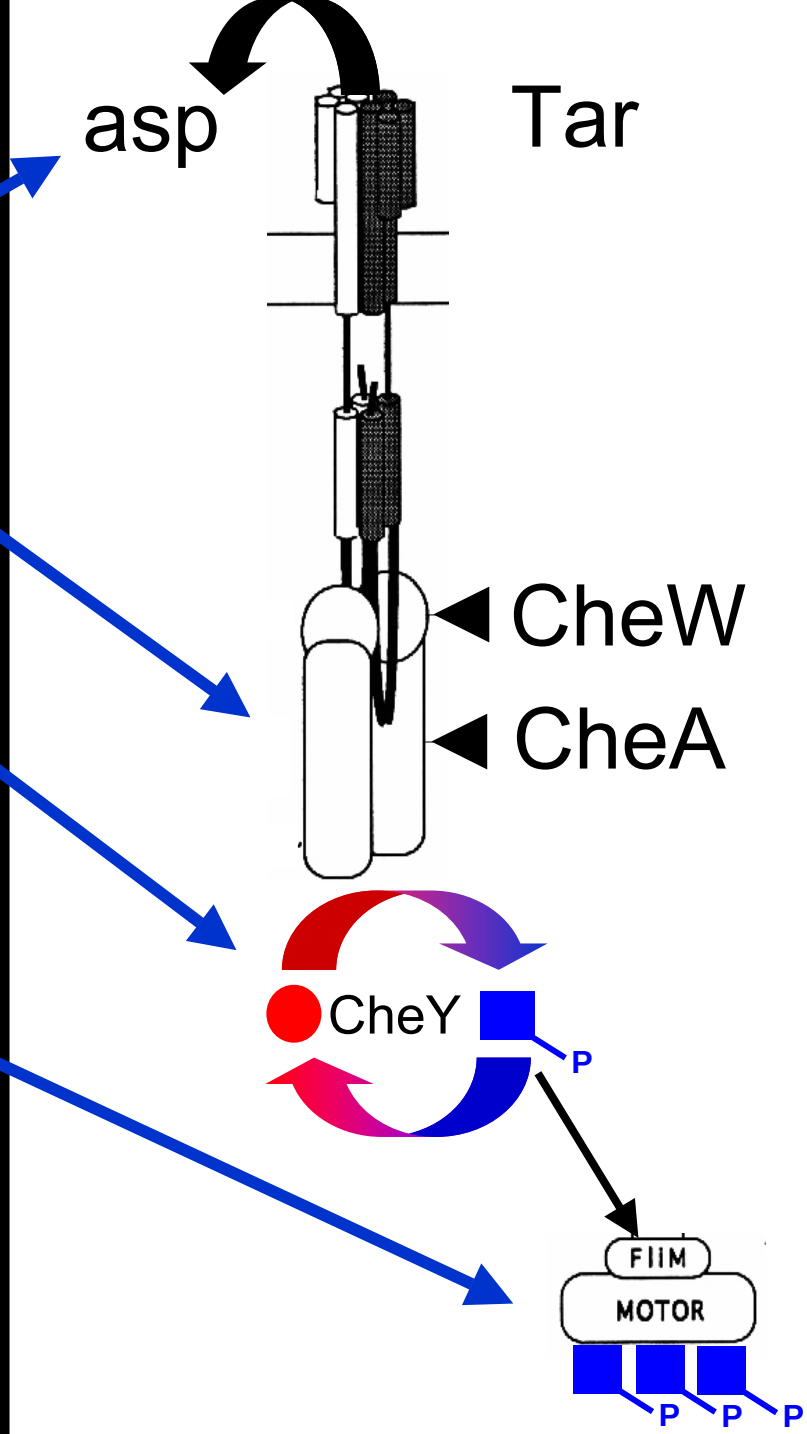
1. Tar loses aspartate.

2. Activity of CheA kinase increases.

3. Amount of CheY-P increases.

4. Probability of a tumble increases.

5. Cell changes direction.



Adaptation

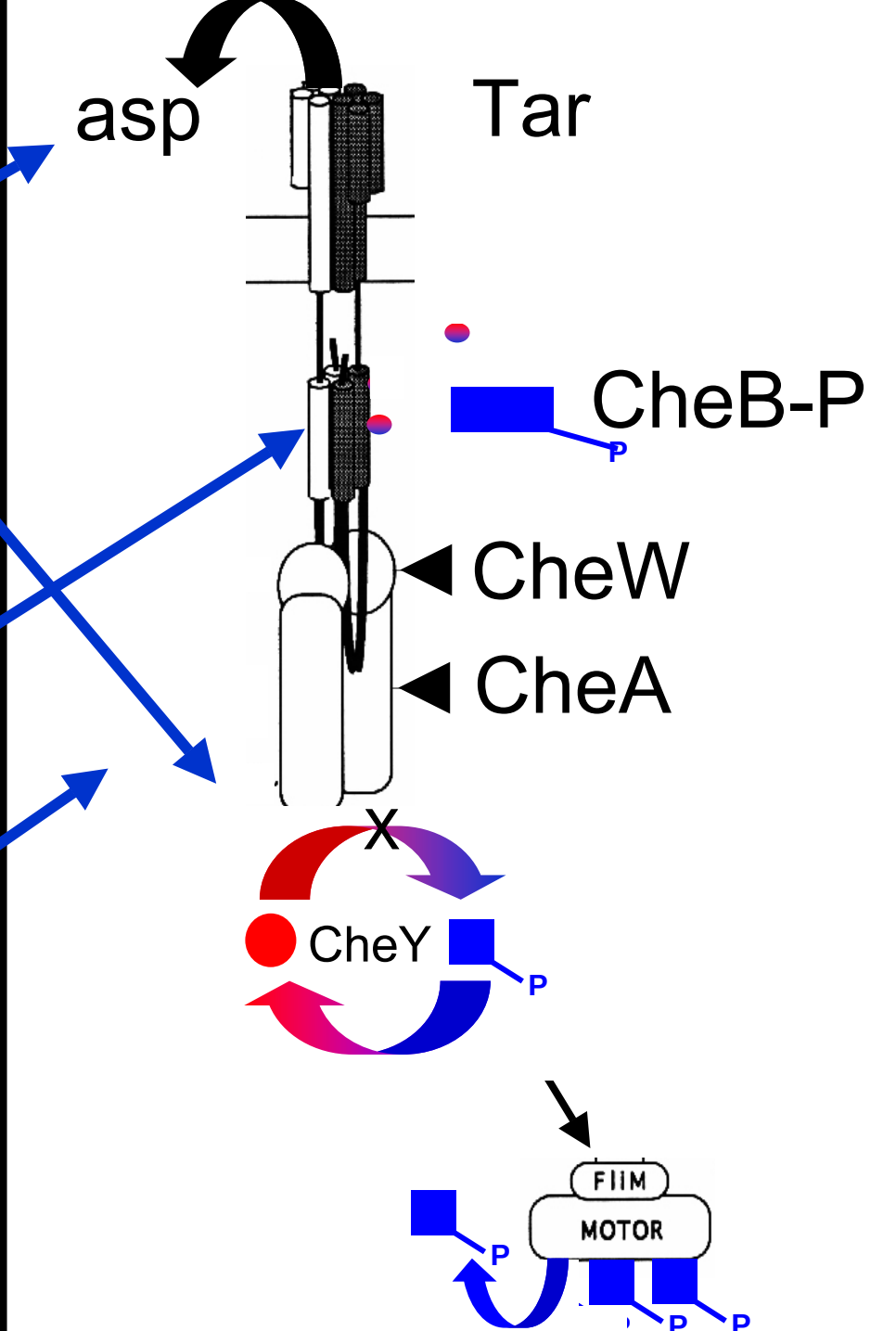
1. Tar loses aspartate.

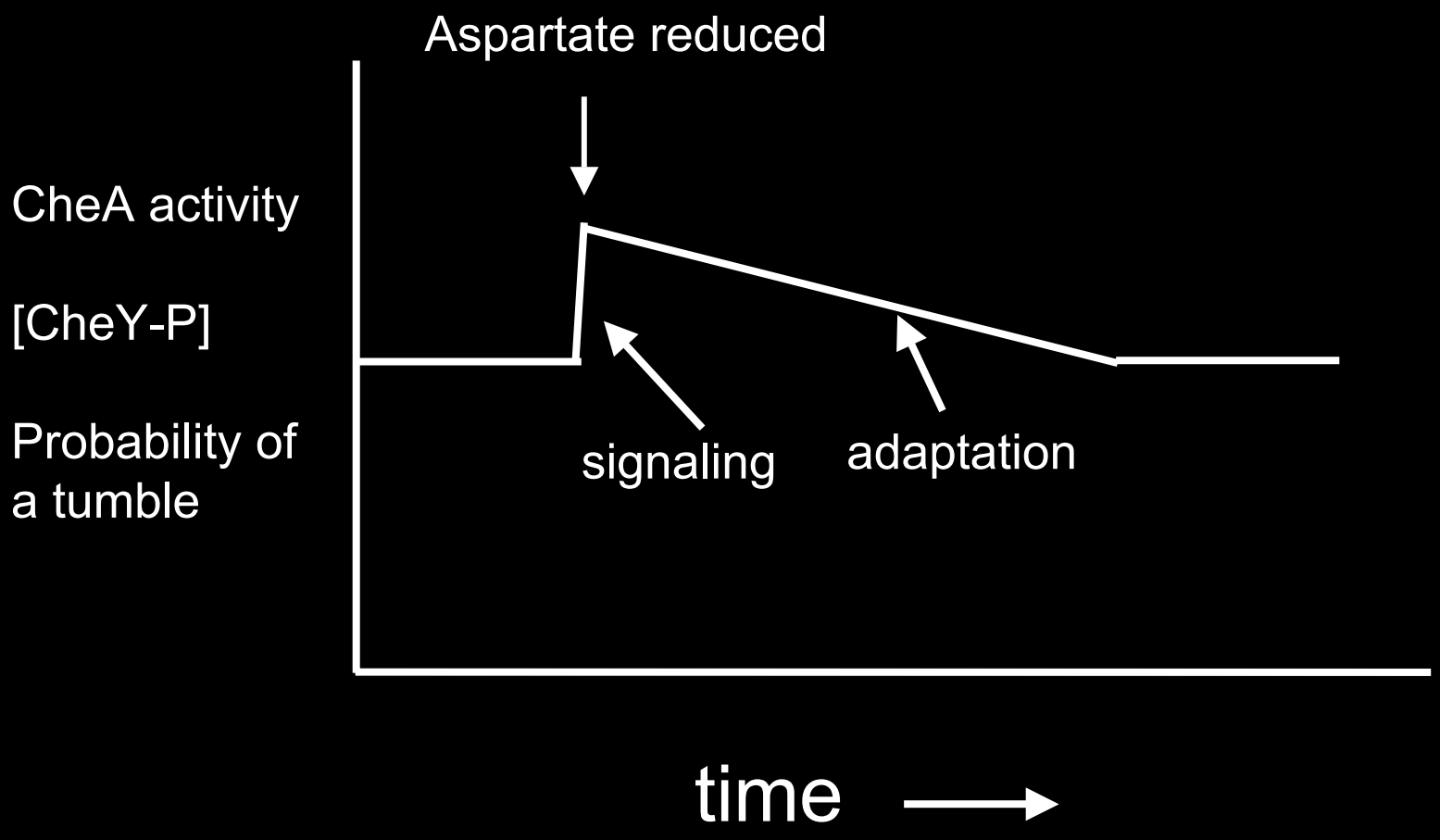
2. CheA phosphorylates CheB

3. CheB-P removes methyl groups from Tar.

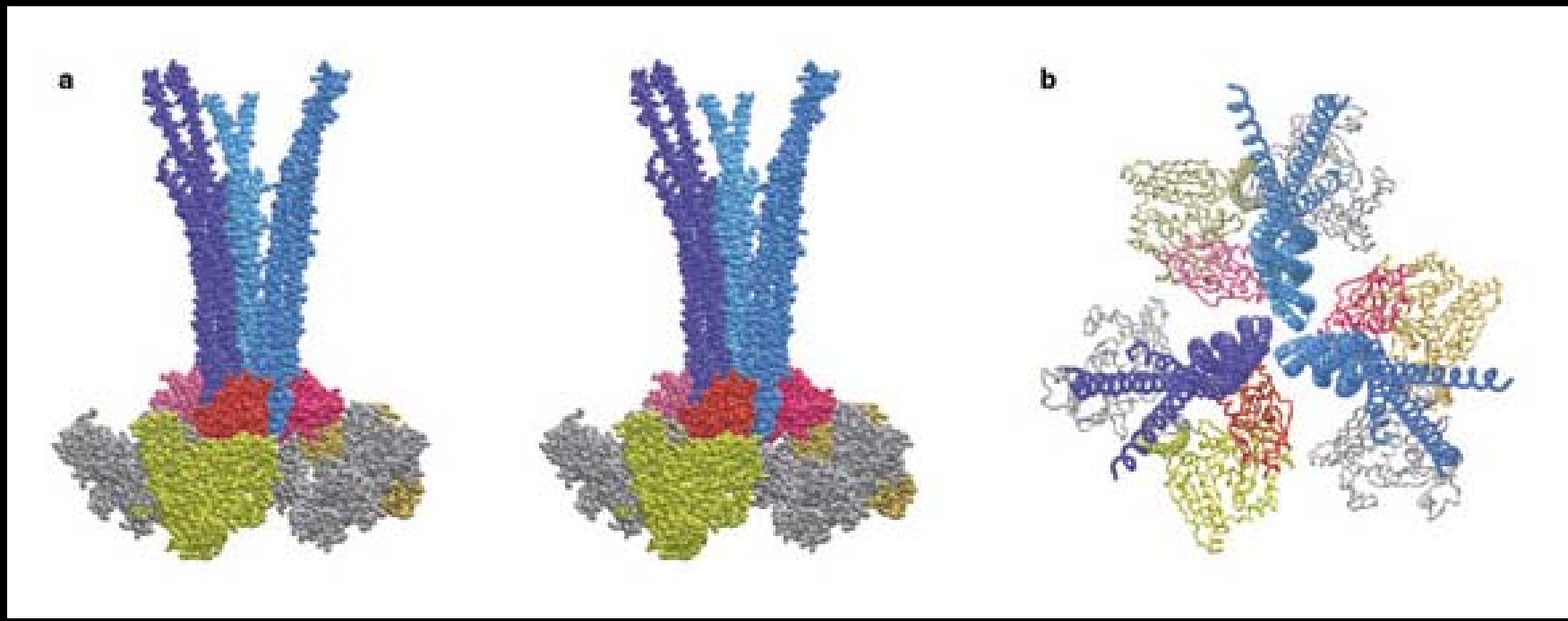
4. CheA activity lowered.

5. Cell returns to non-signaling state..

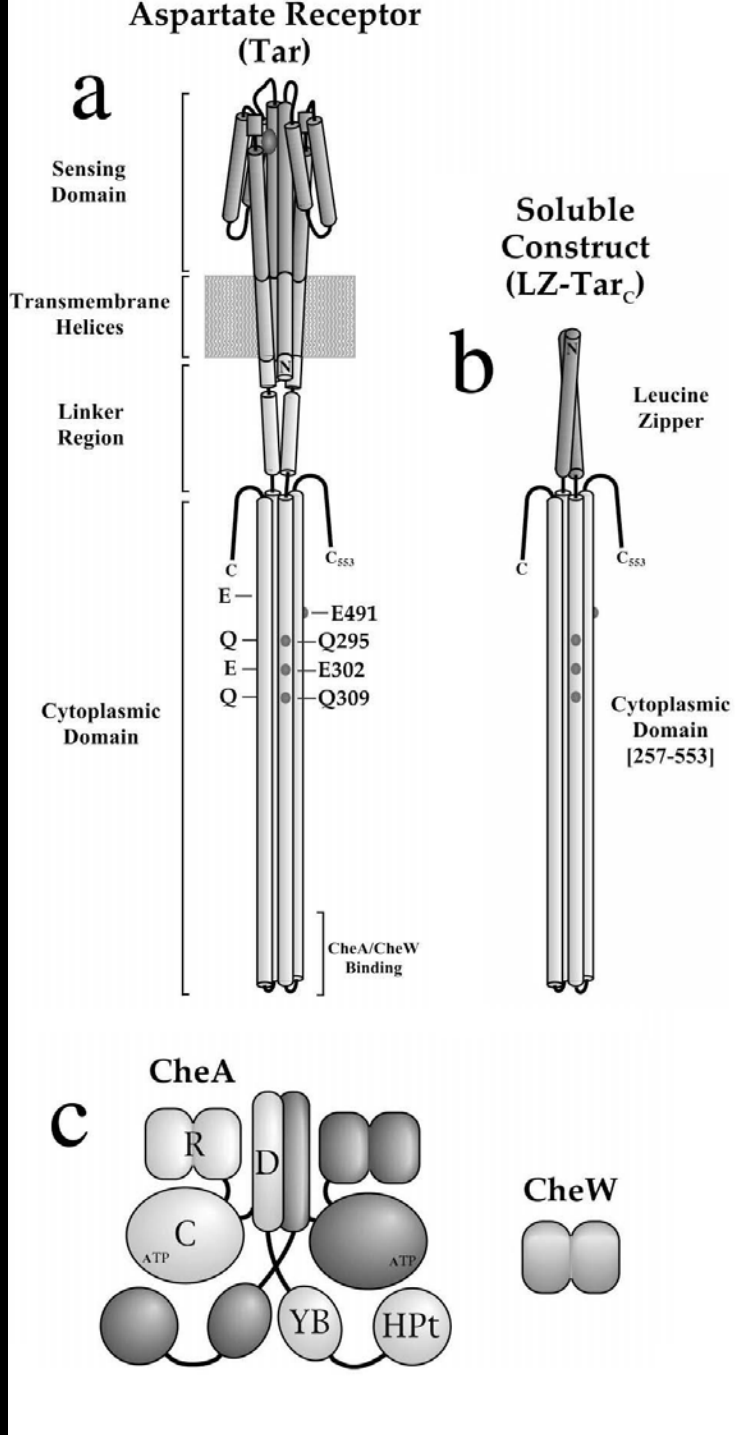


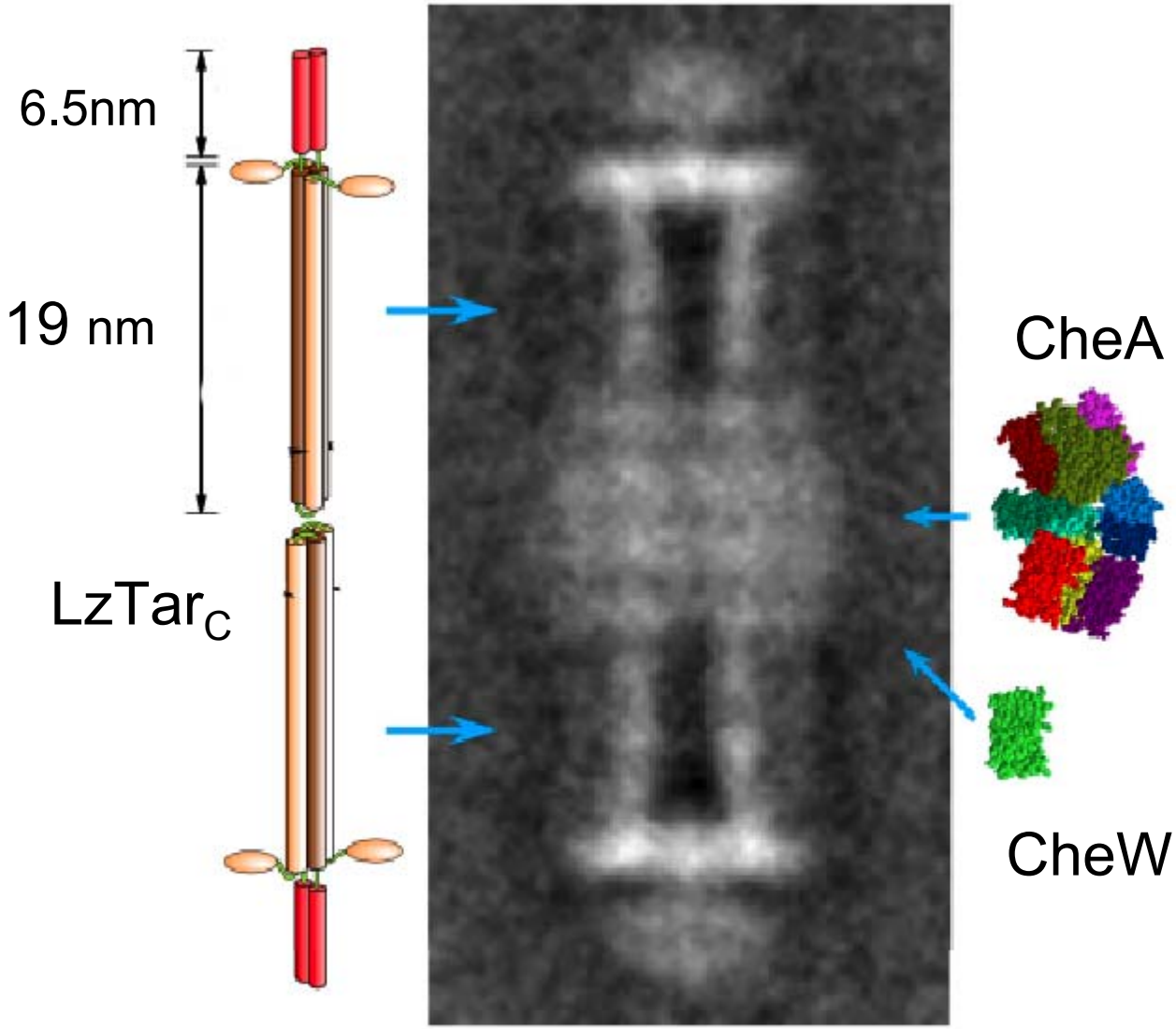

Aspartate reduced
CheA activity
[CheY-P]
Probability of a tumble
signaling
adaptation
time

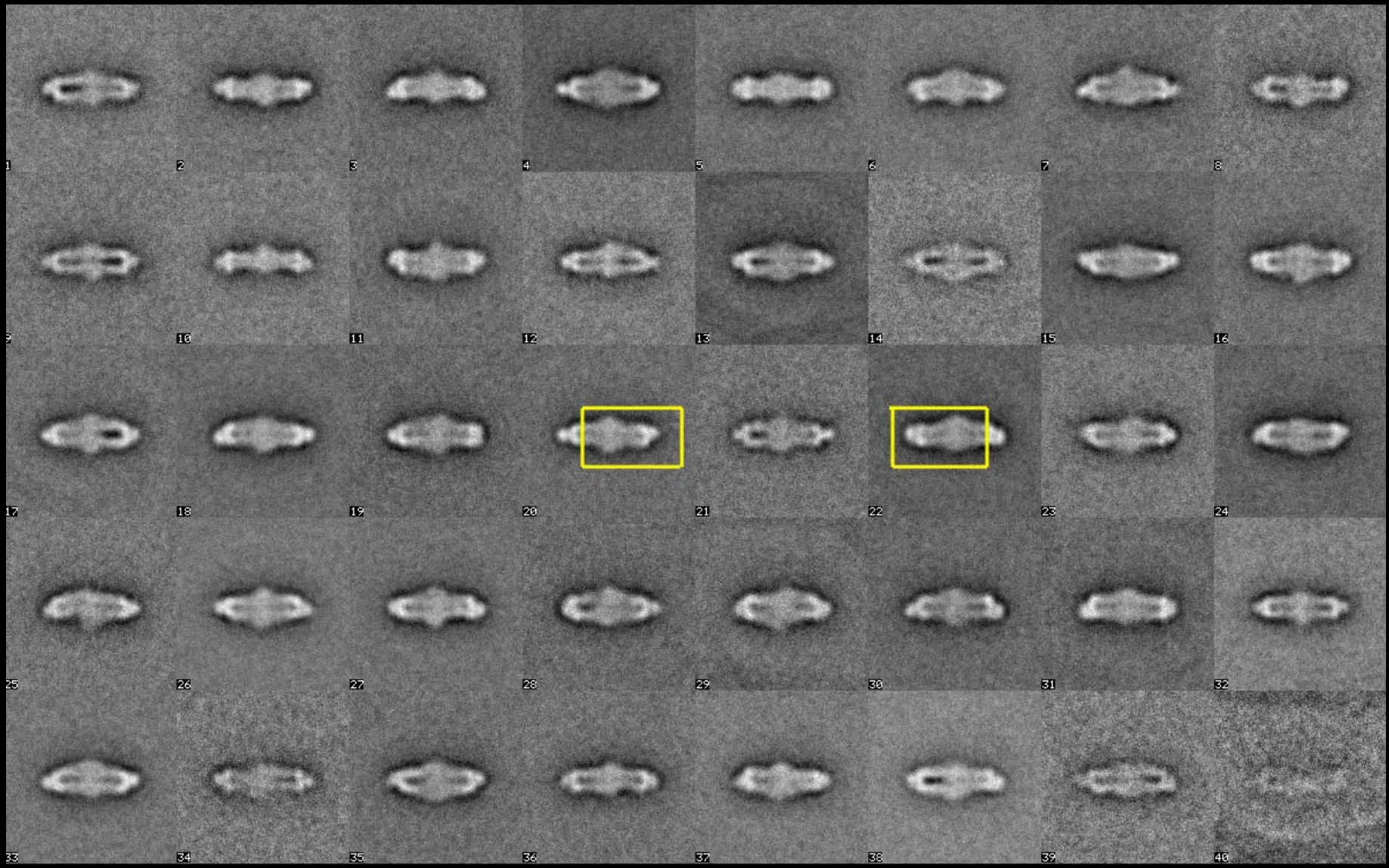
Model with 1:1:1 stoichiometry
Tsr cytoplasmic domain:CheW:CheA (lab of Dennis Bray)



Shimizu et al., Nature Structure Biology,vol 2 Nov. 2000


Aspartate Receptor
(Tar)
a
Sensing
Domain
Transmembrane
Helices
Linker
Region
Cytoplasmic
Domain
N
C
C_{553}
E
Q
E
Q
E491
Q295
E302
Q309
CheA/CheW
Binding
Soluble
Construct
(LZ-Tar_C)
b
Leucine
Zipper
N
C
C_{553}
Cytoplasmic
Domain
[257-553]
c
CheA
R
D
C
ATP
ATP
YB
HPt
CheW


6.5nm
19 nm
LzTar$_C$
CheA
CheW

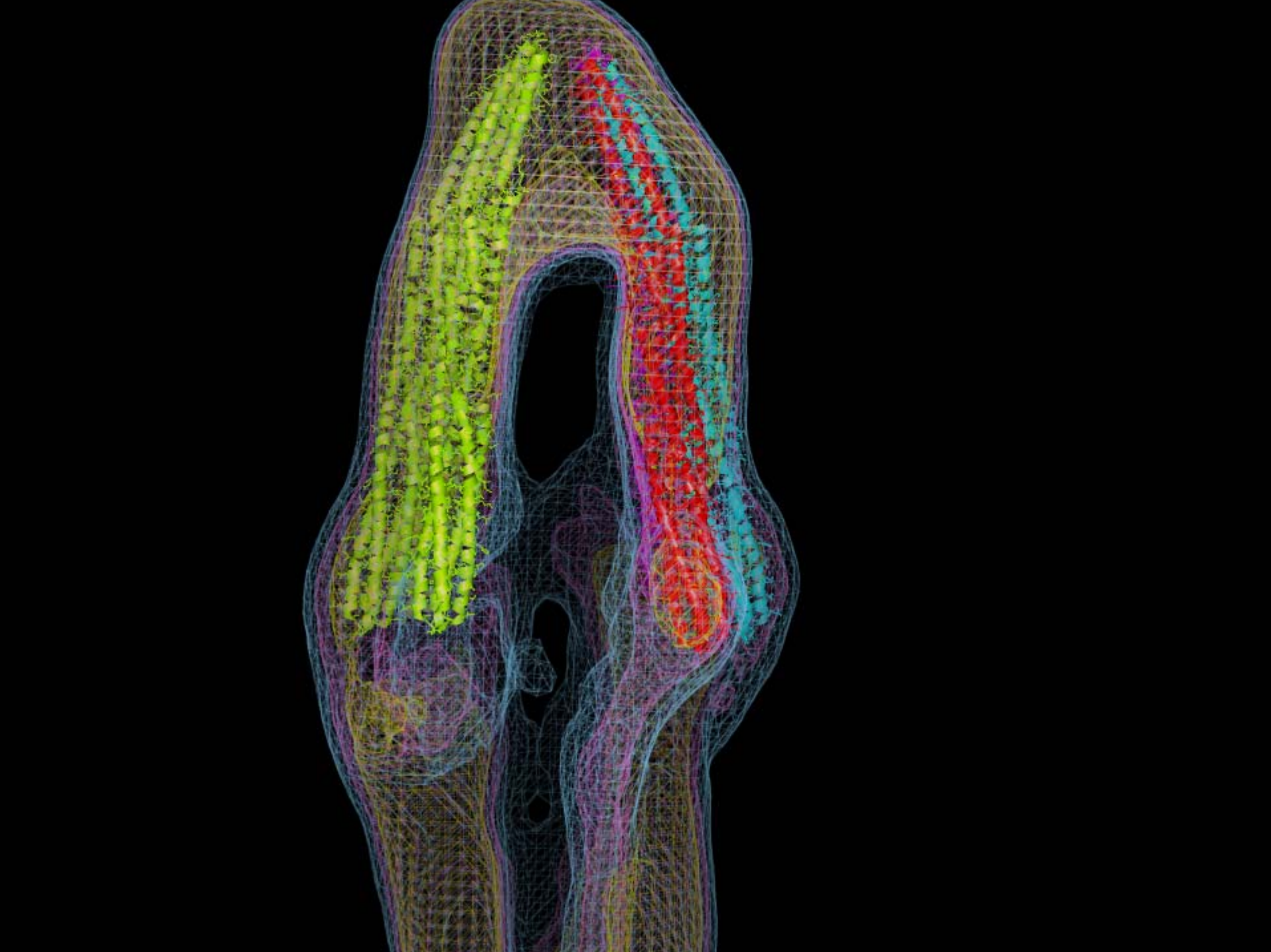


LzTarc:CheW:CheA complex class averages

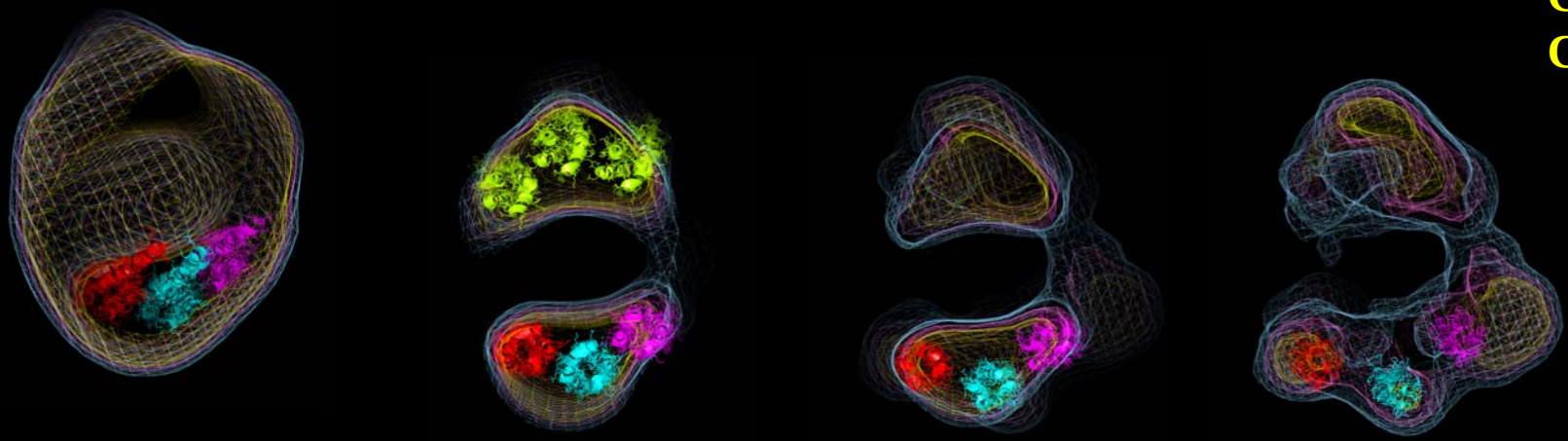
LzTar:CheA:CheW complex

~9800 images

Cytoplasmic
Domain of receptor
(Kim and collaborators)



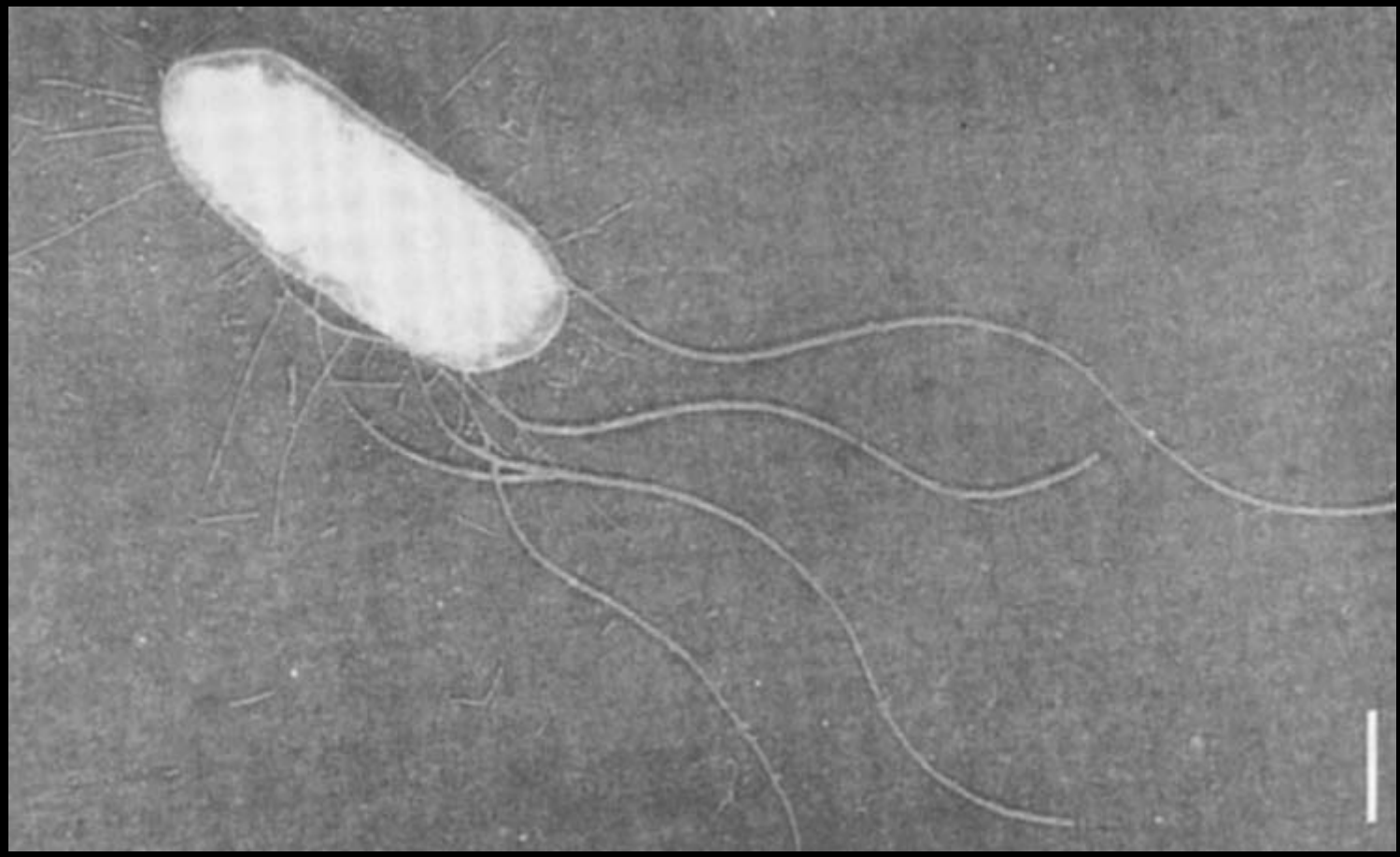
Axial sections



End

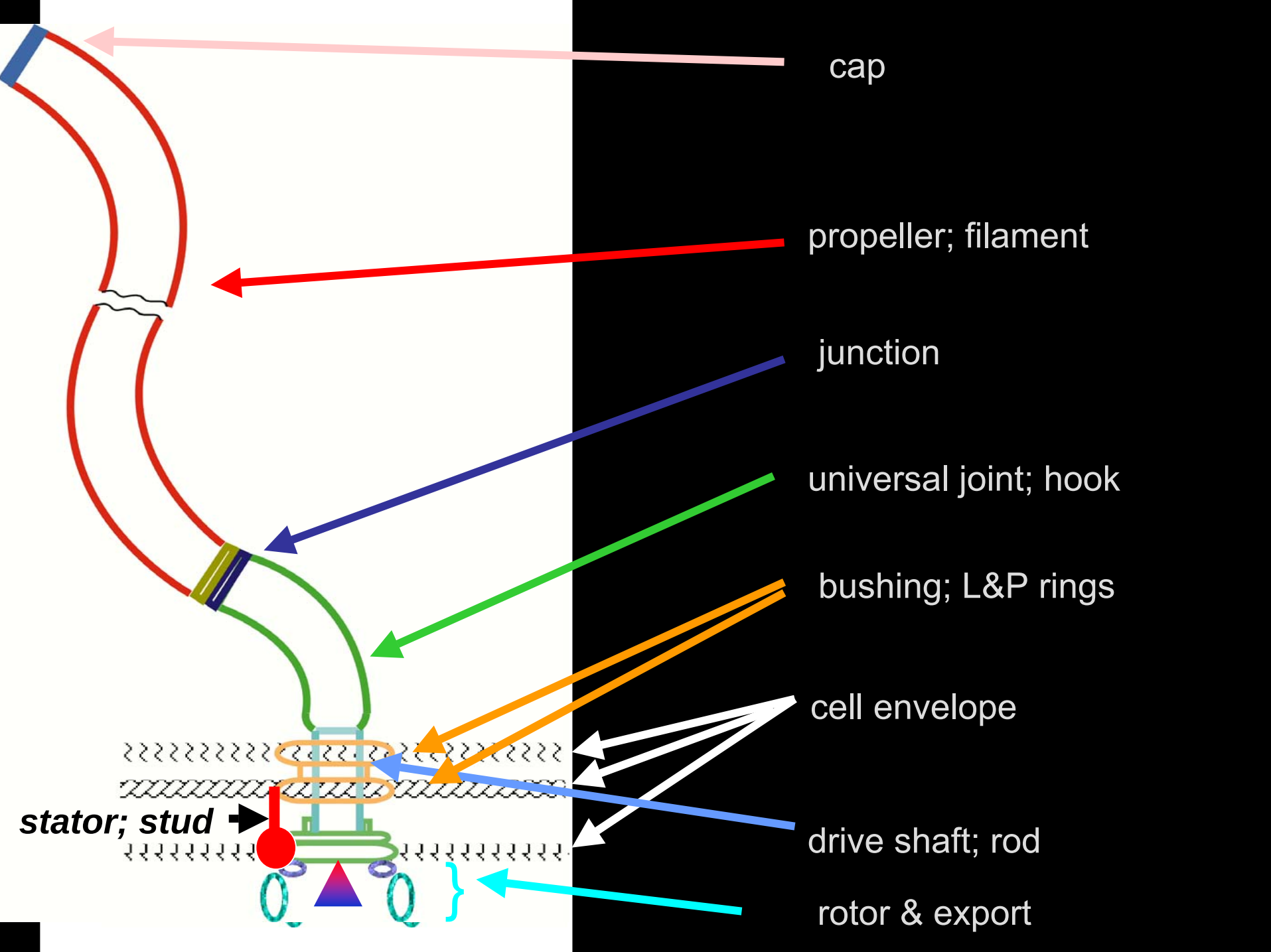
Middle

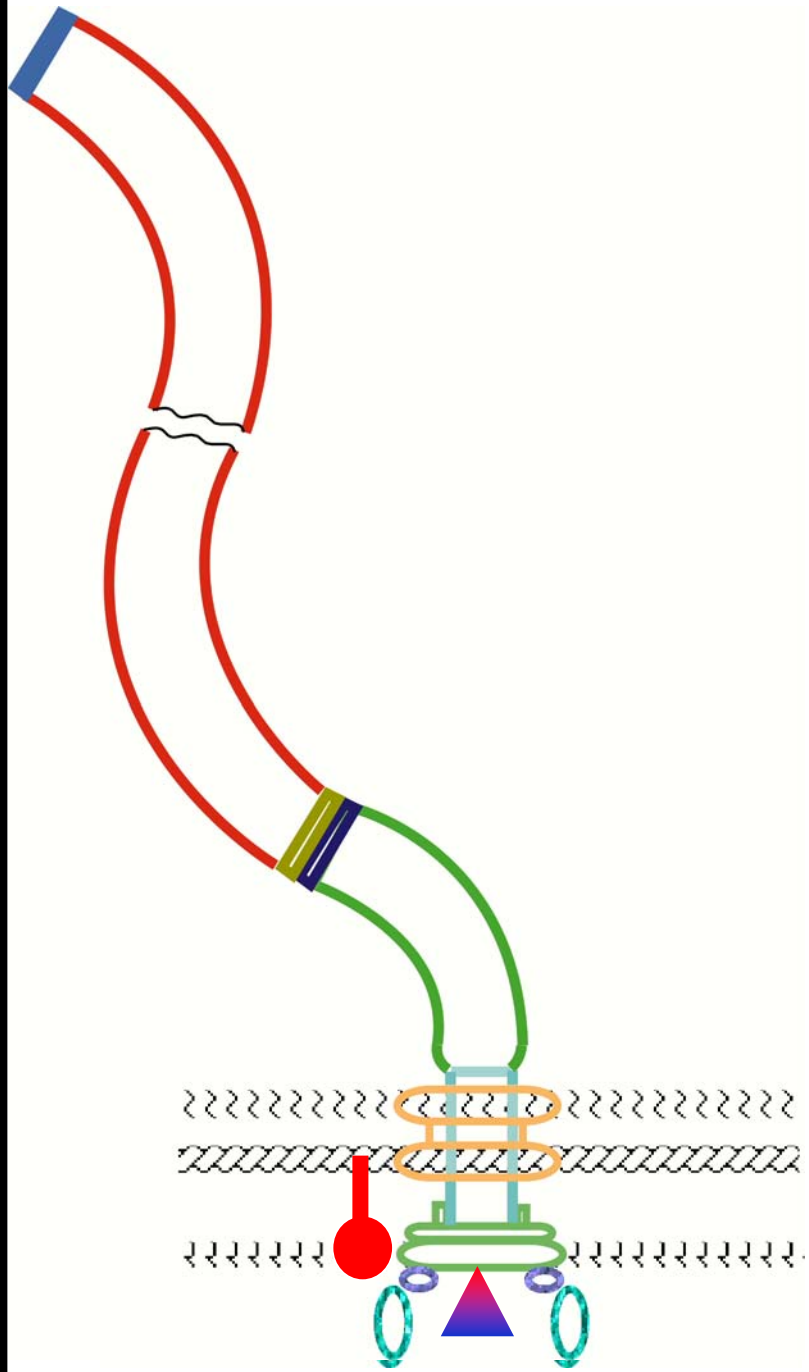
24 ~~28~~ LzTarc; 6 CheW; 4 CheA
6:3:2



Electron micrograph of E. coli showing the peritrichous arrangement of flagella (H. Berg). Bar=0.5um.

Eukaryotic flagellum ≠ prokaryotic flagellum

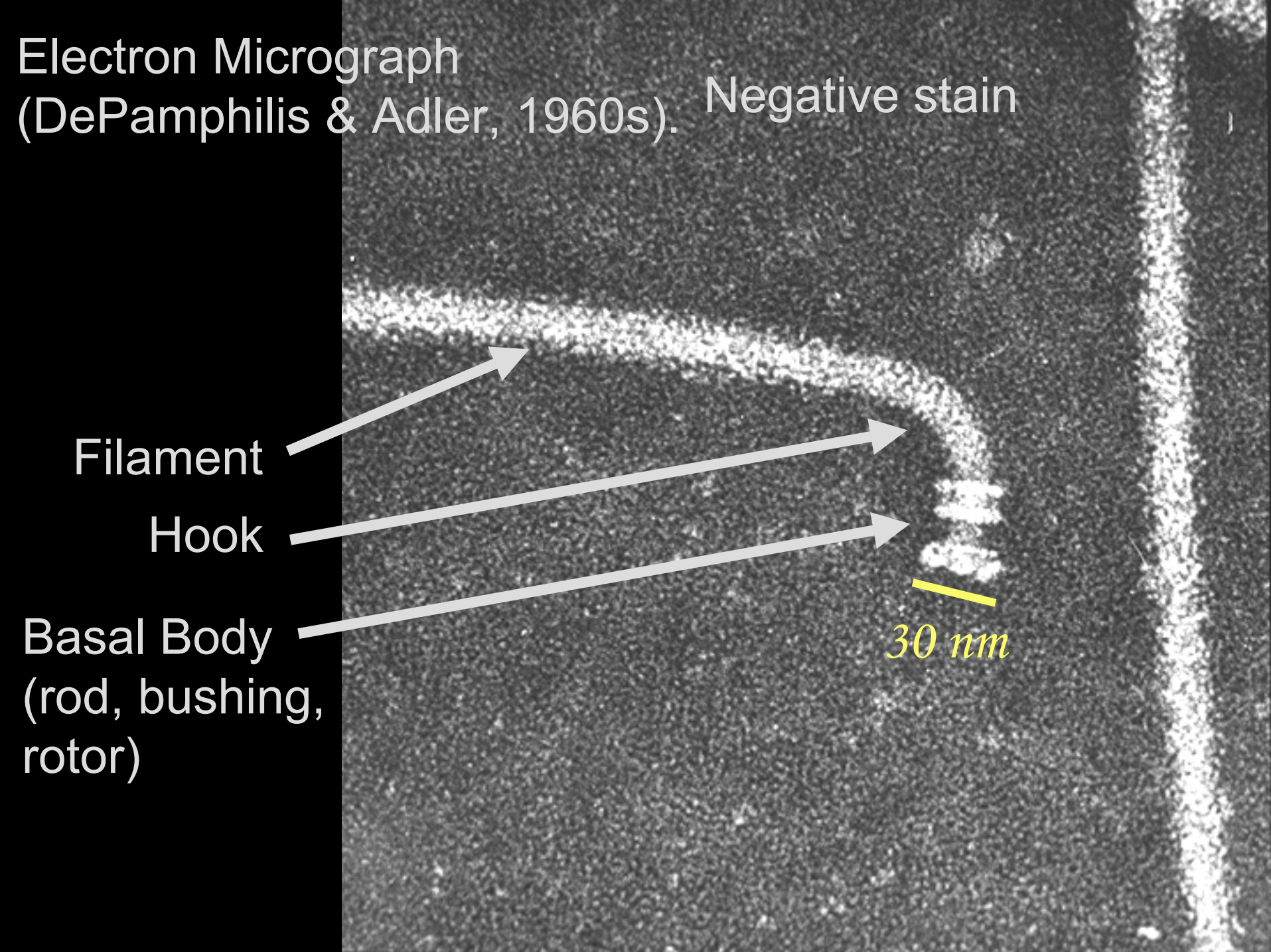

cap
propeller; filament
junction
universal joint; hook
bushing; L&P rings
cell envelope
stator; stud
drive shaft; rod
rotor & export



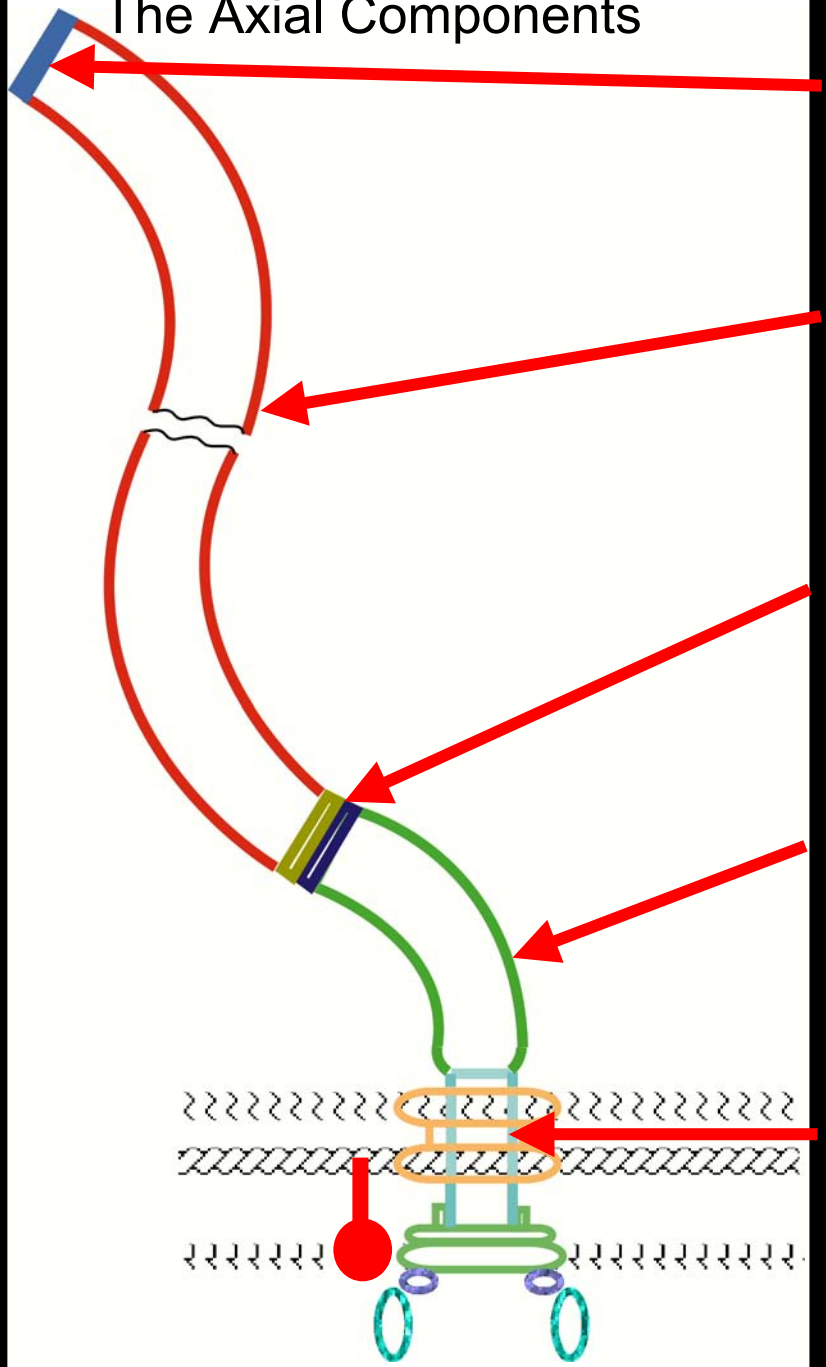
It takes about 40 genes to make a flagellum.

At least 20 of them are present in the completed structure.

Others are involved in regulation of expression,export, or assembly.


Electron Micrograph
(DePamphilis & Adler, 1960s).
Negative stain
Filament
Hook
Basal Body
(rod, bushing,
rotor)
30 nm

The Axial Components



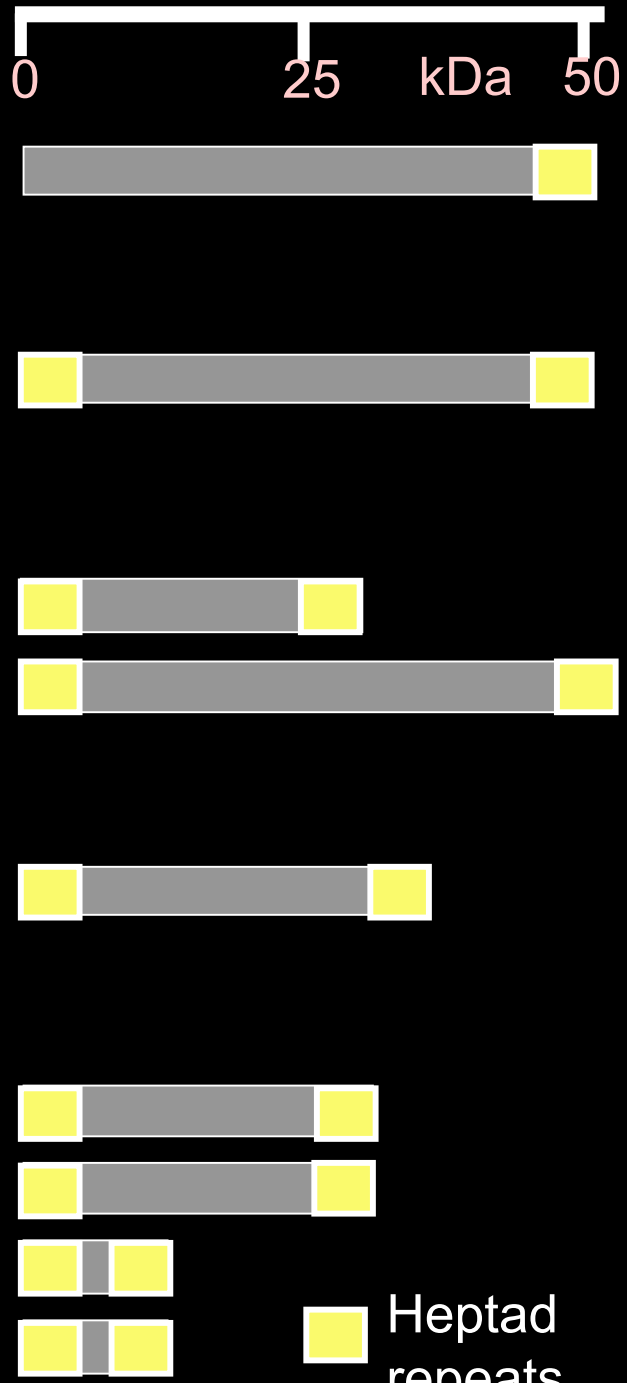
Cap-
FliD

Filament-
FliC

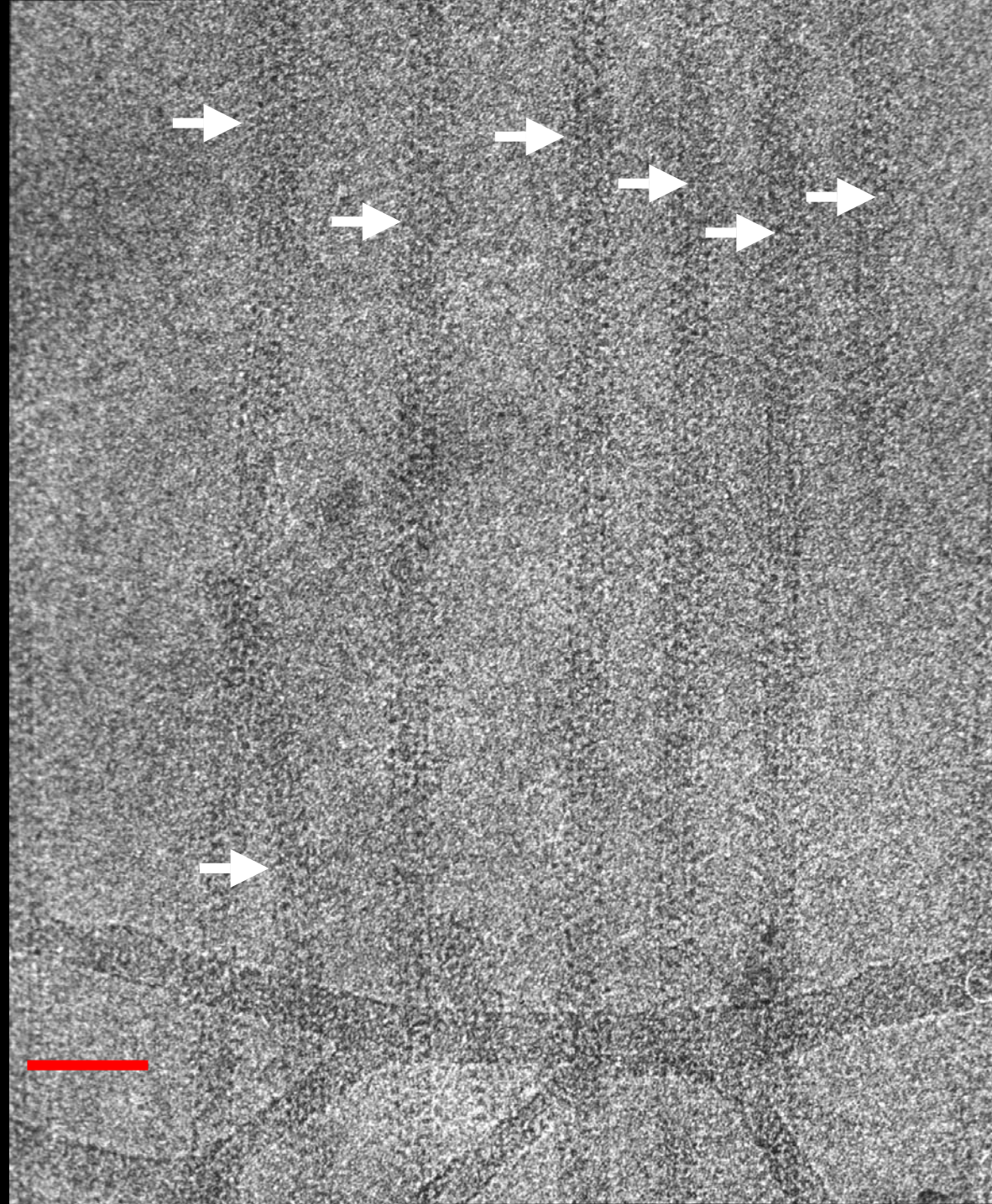
Junction-
FlgL
FlgK

Hook-
FlgE

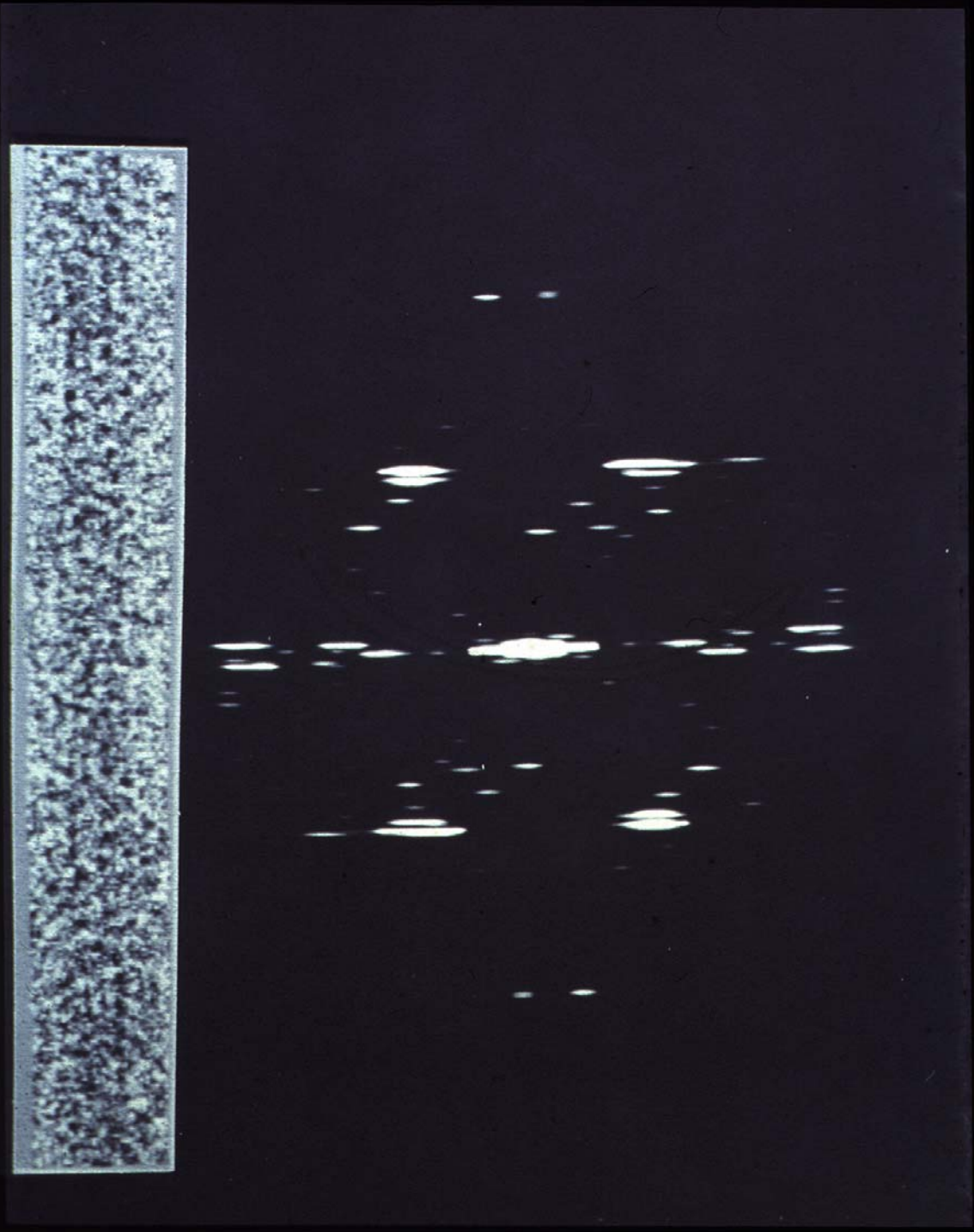
Rod-
FlgG
FlgF
FlgC
FlgB

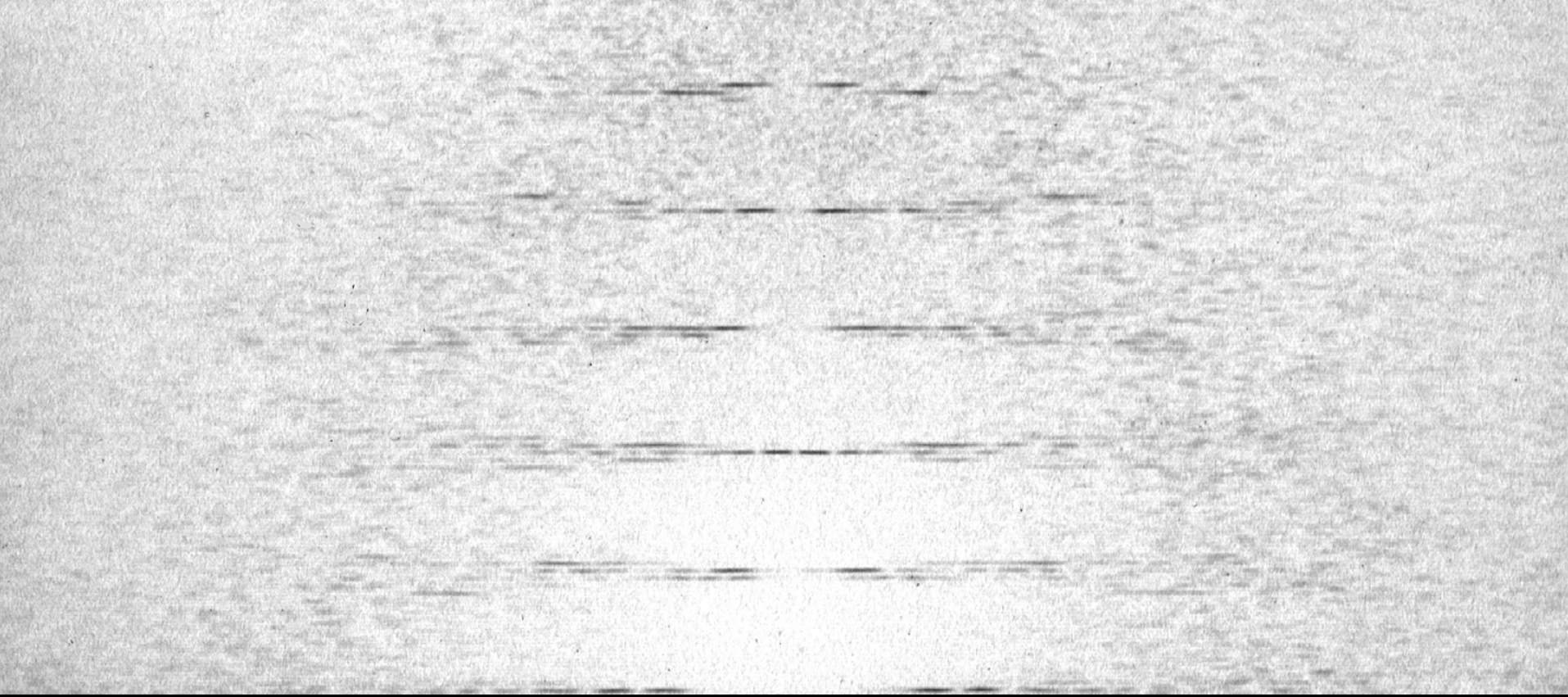


Filaments seen in frozen hydrated preparations.



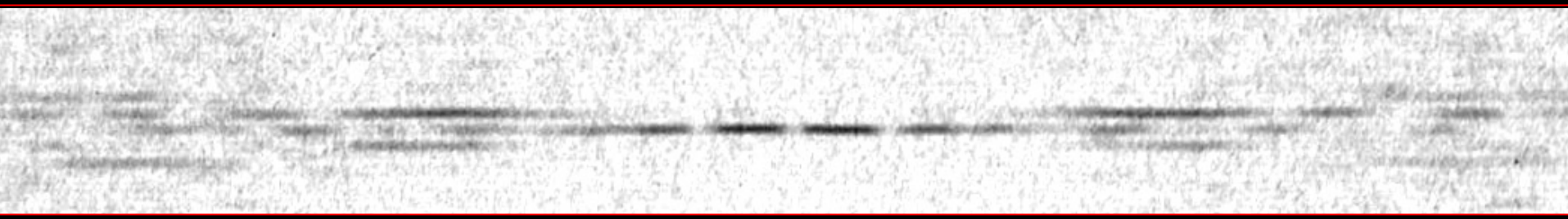
A single filament and its Fourier transform.



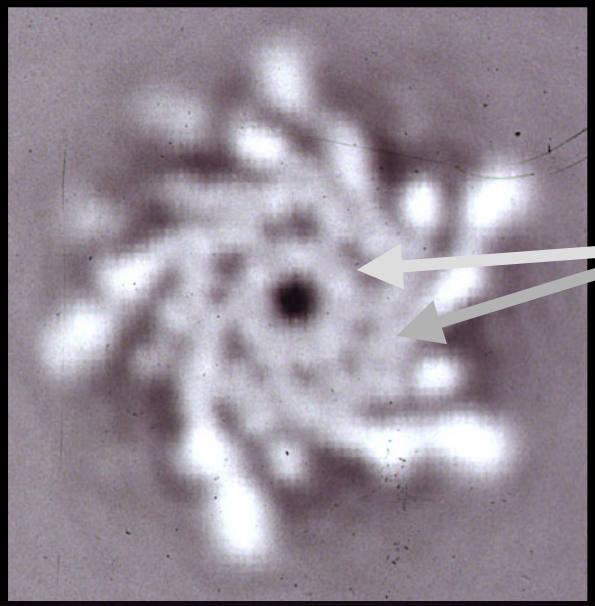


The Fourier transform from the image of a single filament.

The Fourier transform averaged from many filaments.



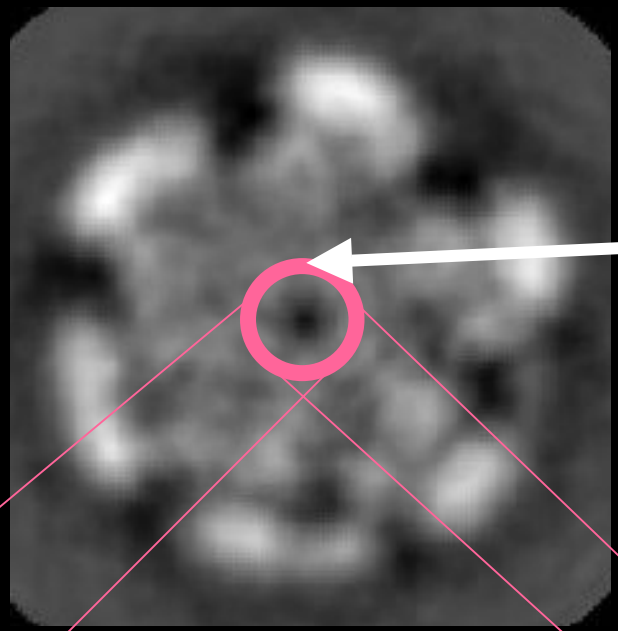
A detail showing the presence of 8 or 9 layer lines .



The filament or propeller (FliC).

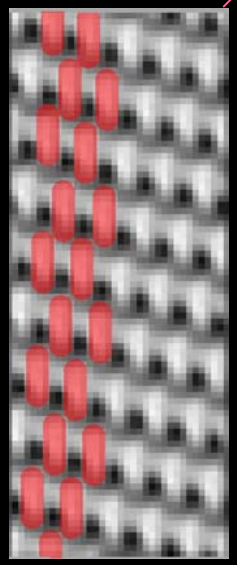
Alpha helical bundles.

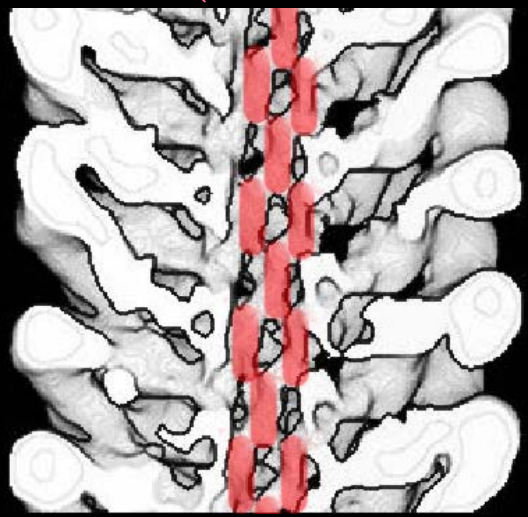
F Samatey, K Imada, S Nagashima, F Vondervist, T Kumasaka, K Namba

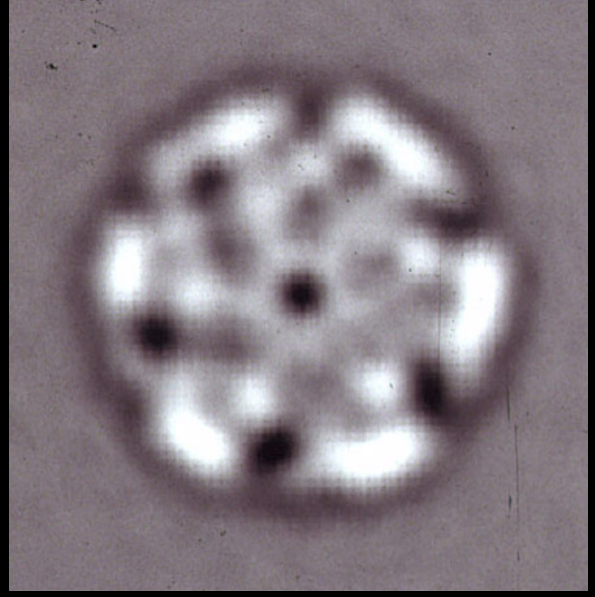


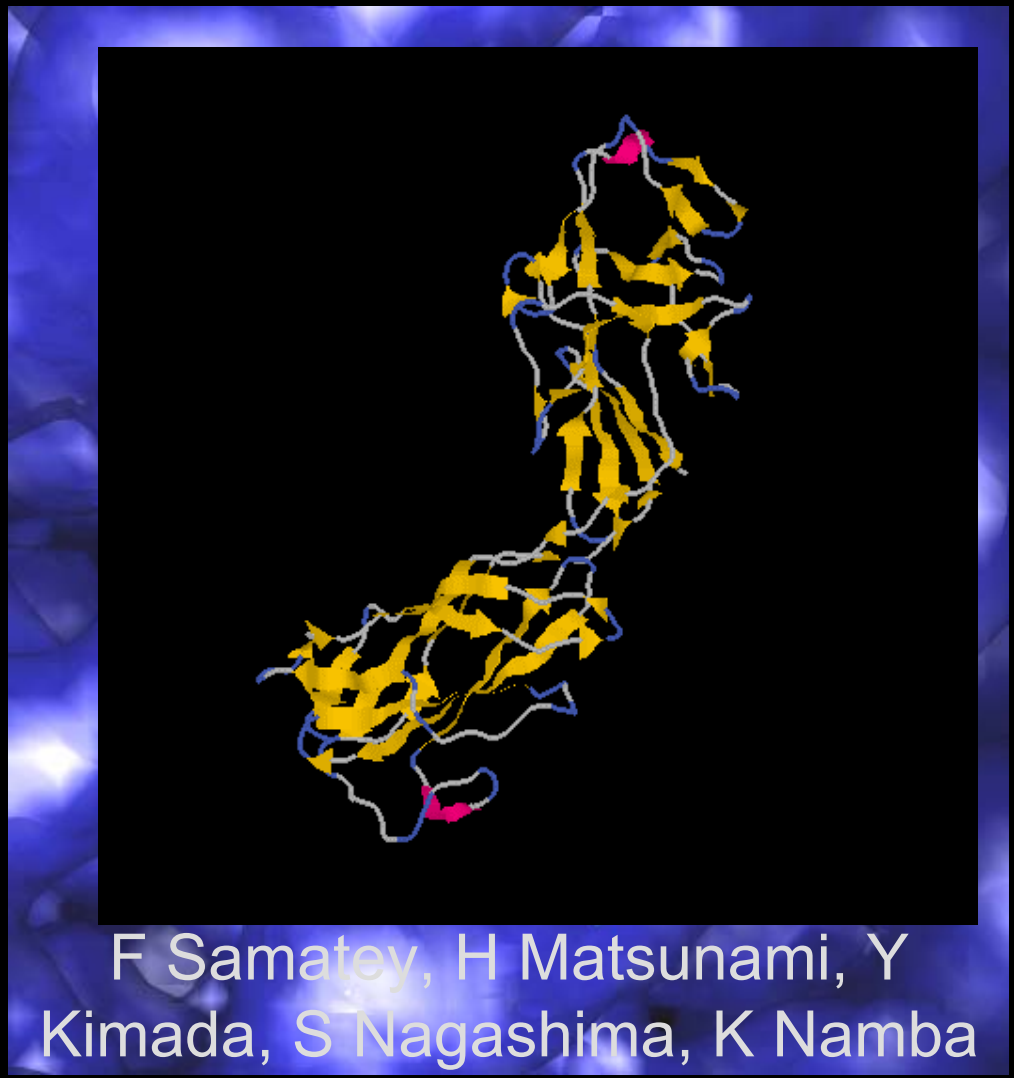
Tube of axial alpha helices like the filament.

The hook or universal joint; FlgE

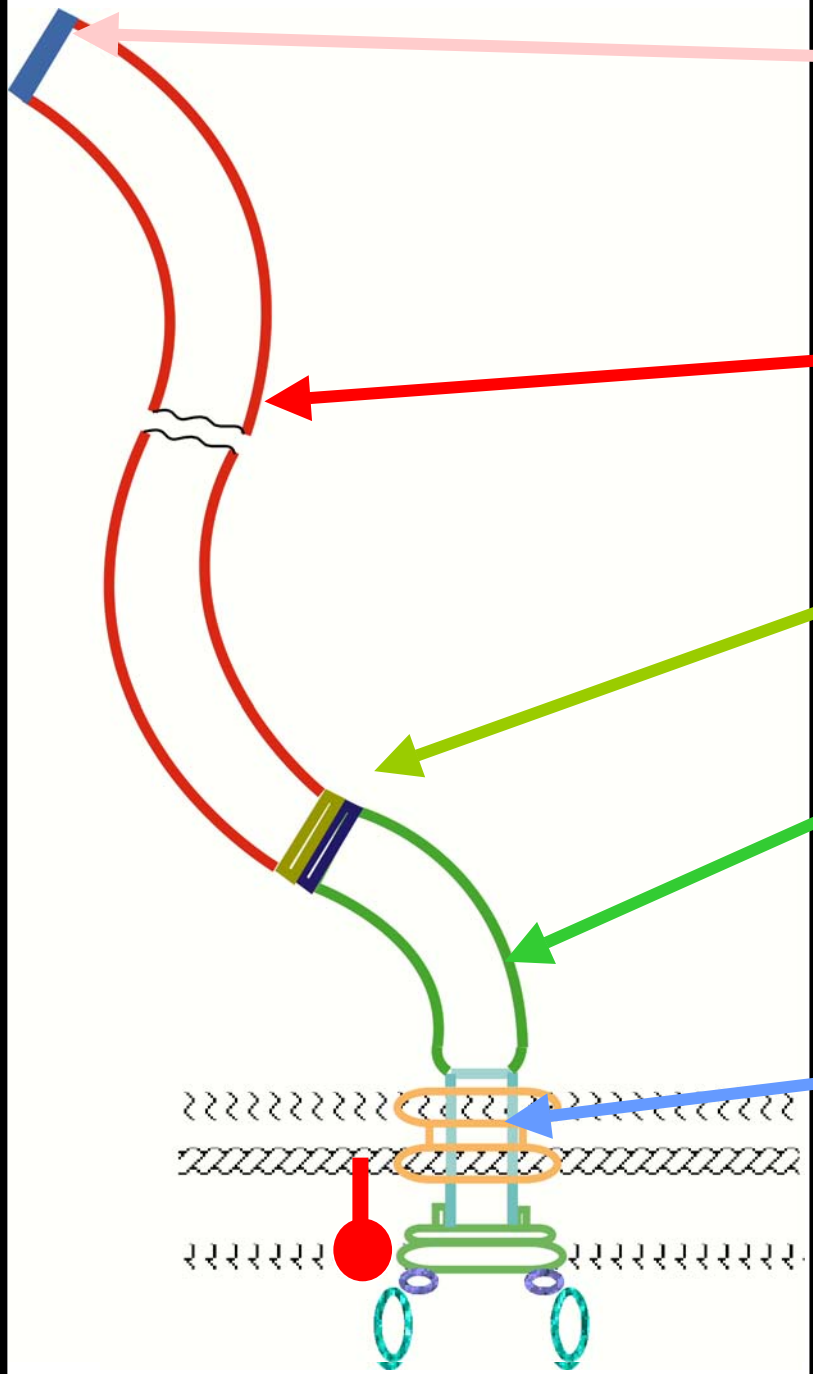








F Samatey, H Matsunami, Y Kimada, S Nagashima, K Namba



Cap

Filament

Junction

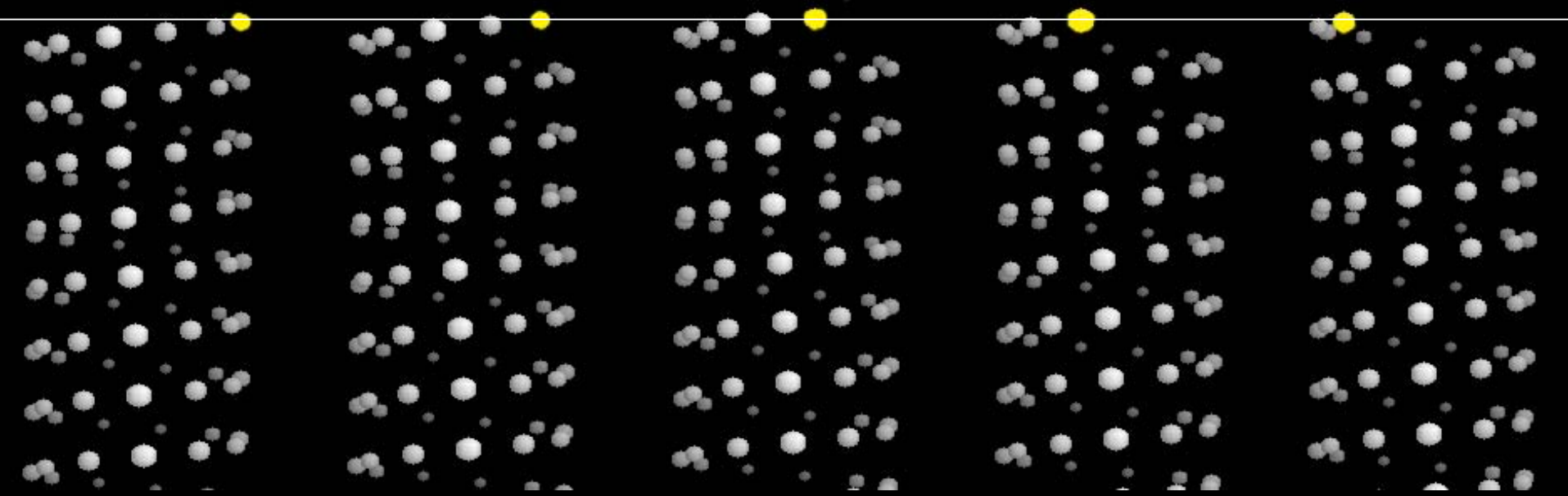
Hook

Rod

The axial components

The hybrid method

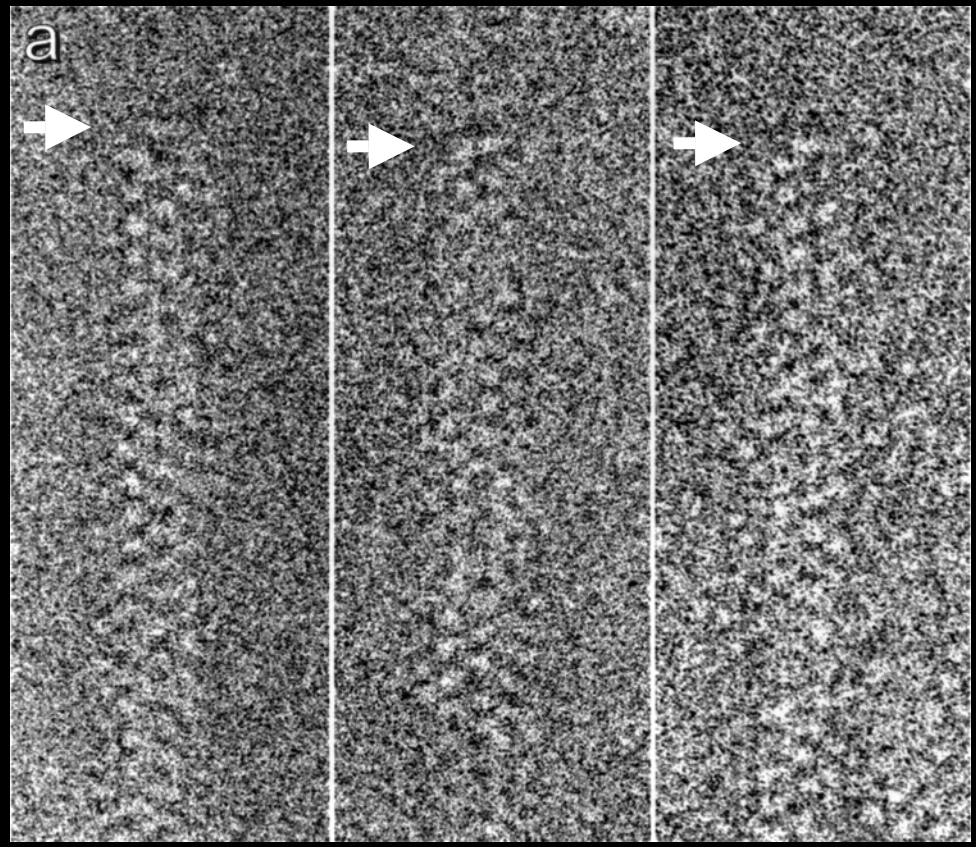
1. The ends and the helical axes are aligned.



2. The rotational angles between the helices (i.e., the orientations of ends) are determined using the helical body.

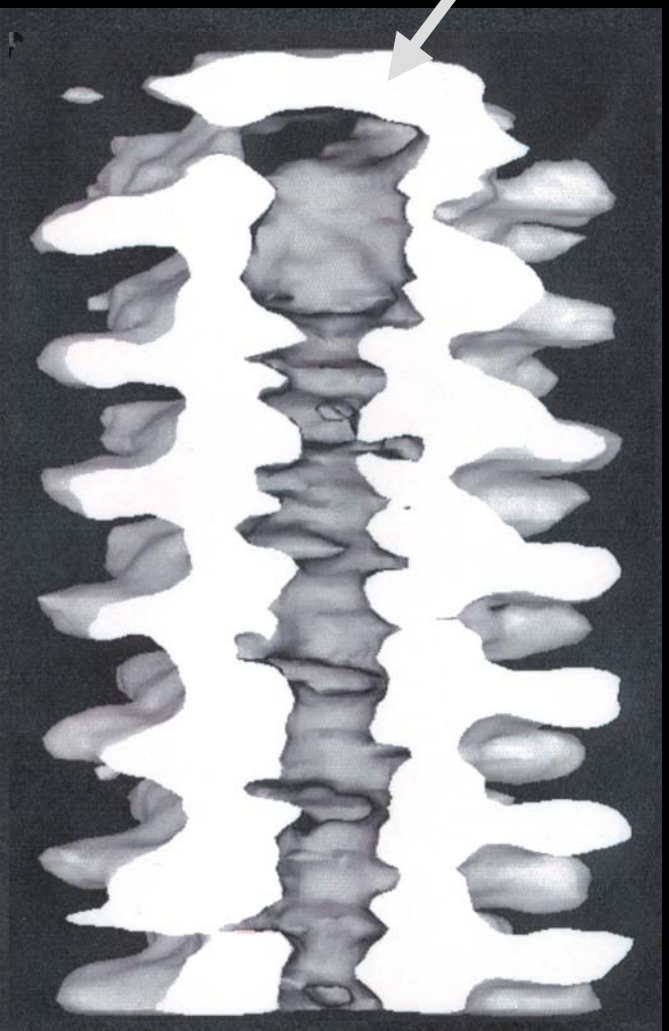
3. The different views are then combined to generate a 3D map of the helix and its end (rod, junction or cap).

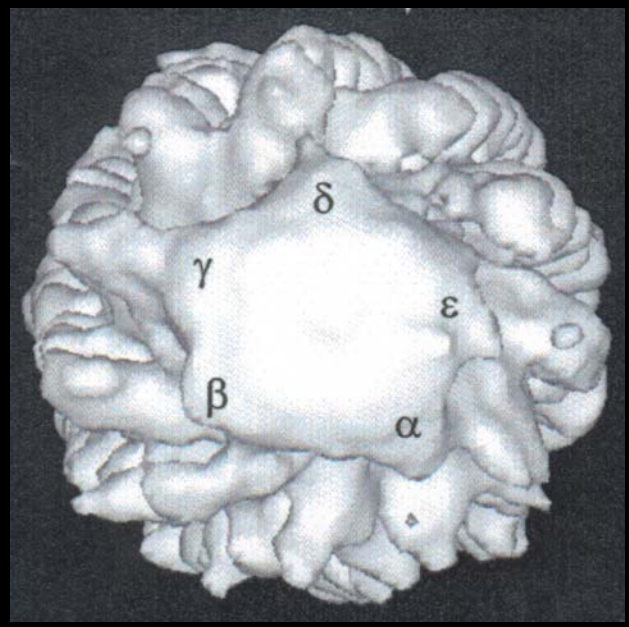
The FliD capped end of the filament



Yonekura et al.

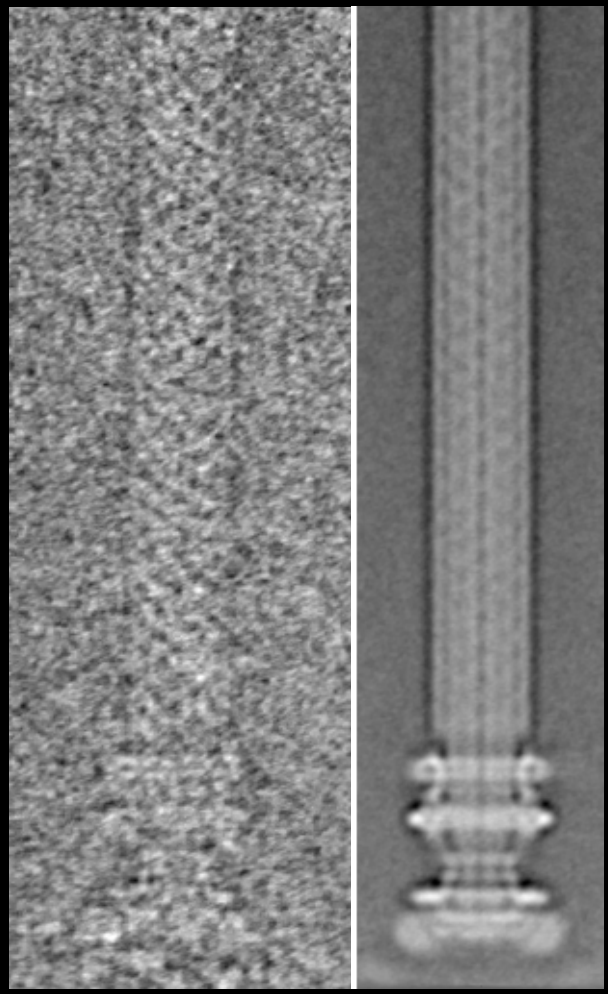
Cap, FliD



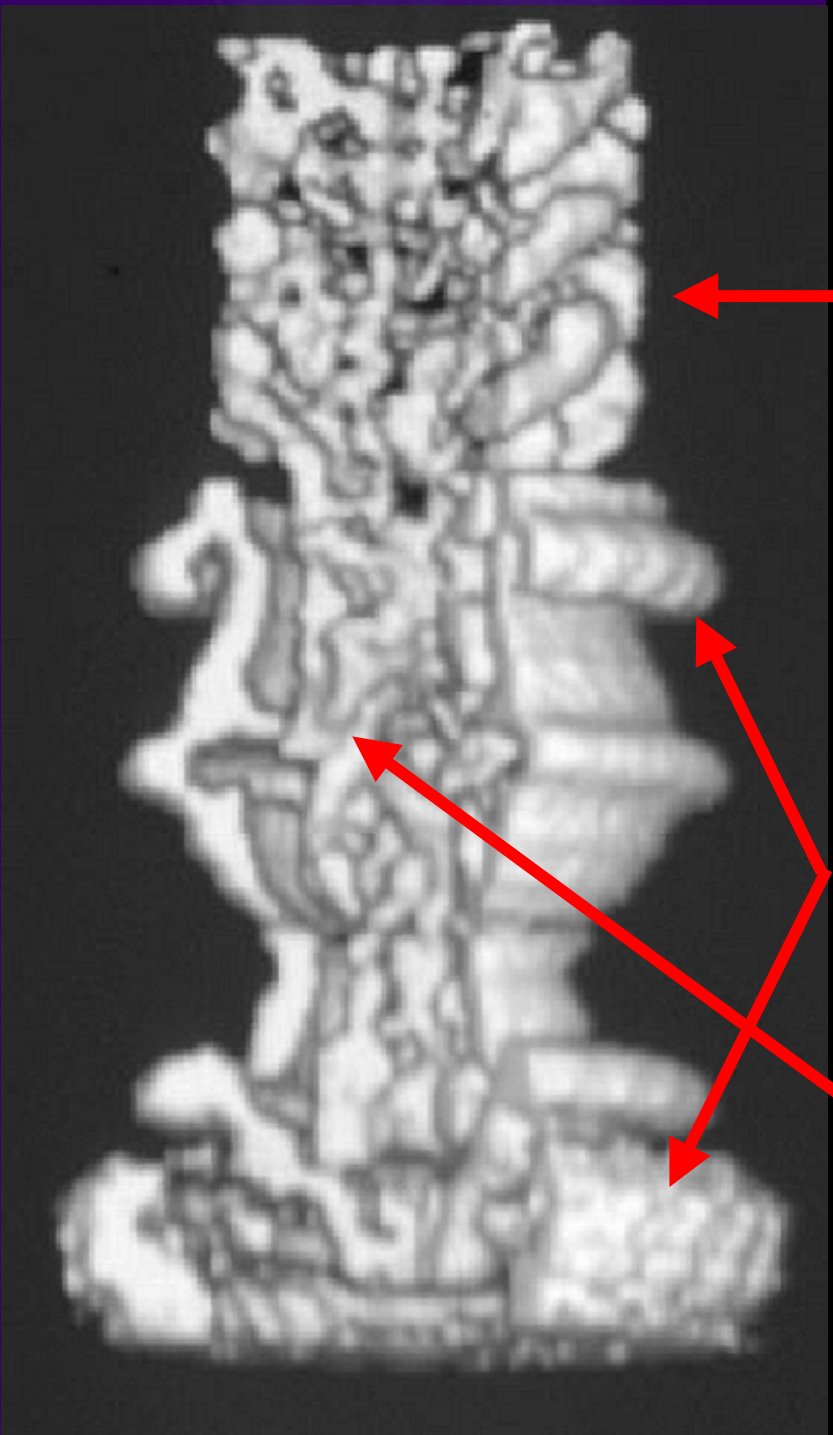


The pentameric cap covers a 6x9 nm chamber .
Chamber thought to be refolding site.

The rod-rings end of the hook.



Single particle Average



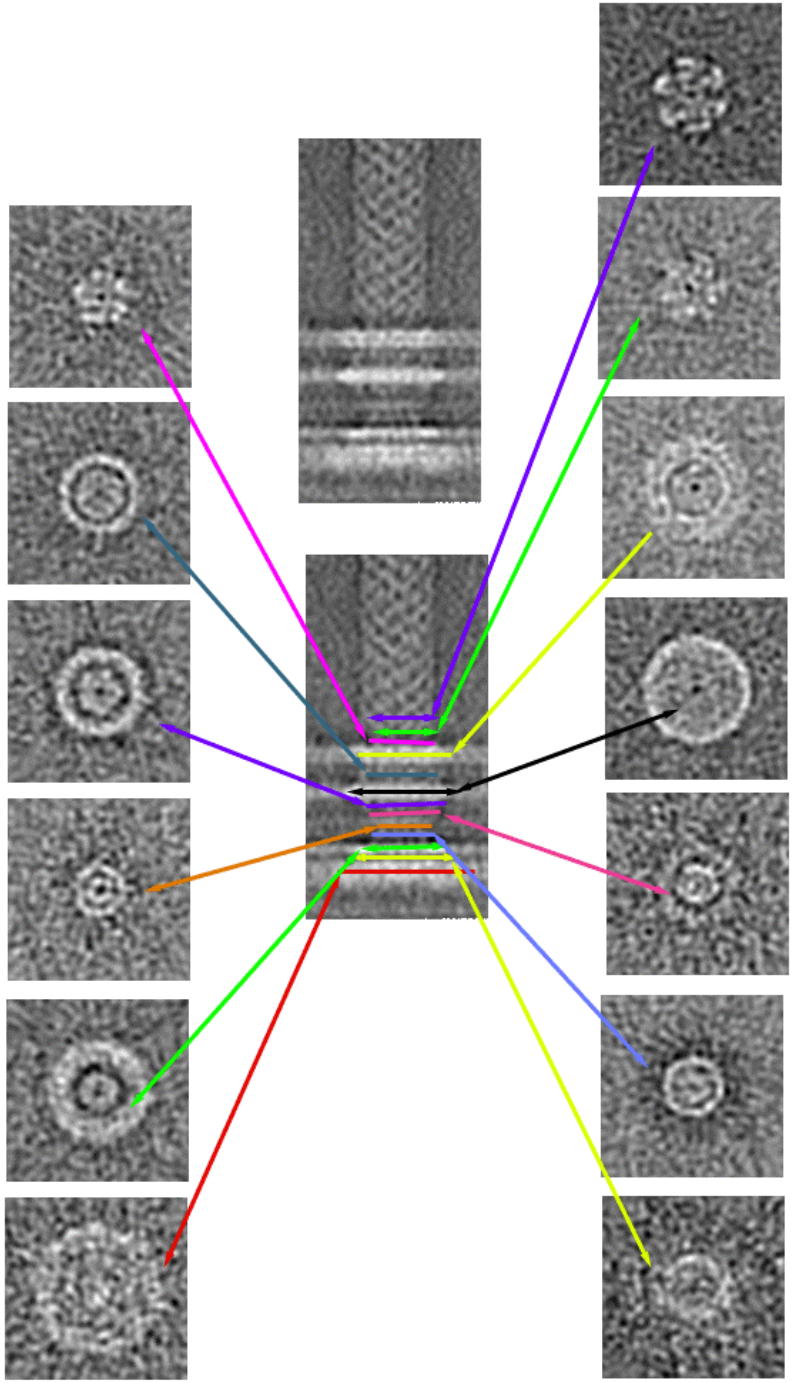
Surface view of 3D map generated using hybrid method

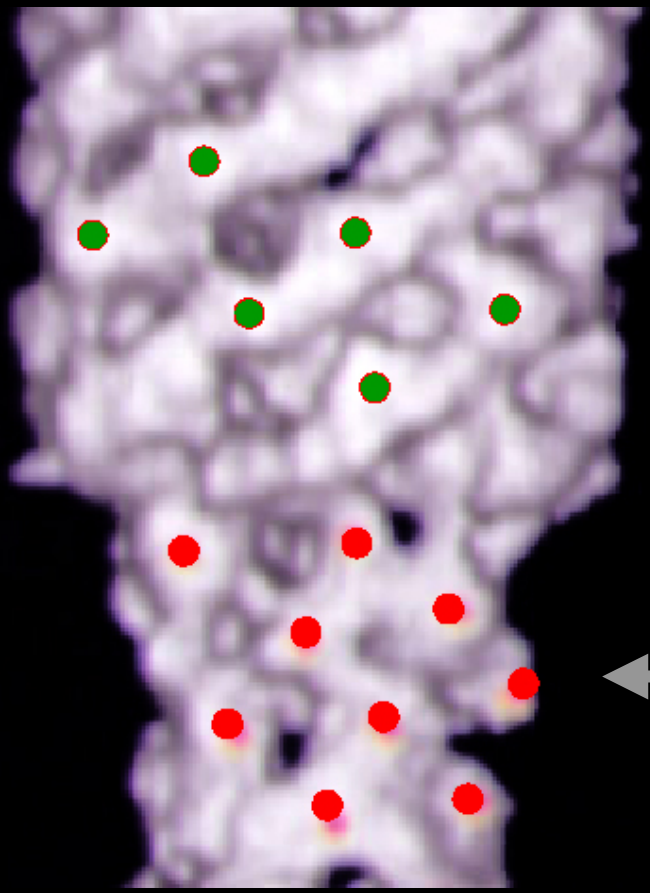
Hook portion.

The ring structures appear to be cylindrically symmetrized by this procedure.

The rod, on the other hand, revealed subunit detail.

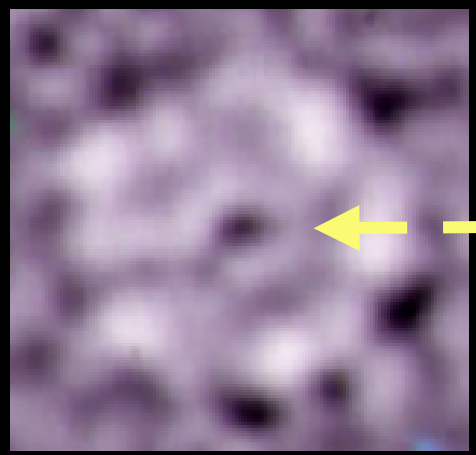
Tranverse sections through 3D map





hook

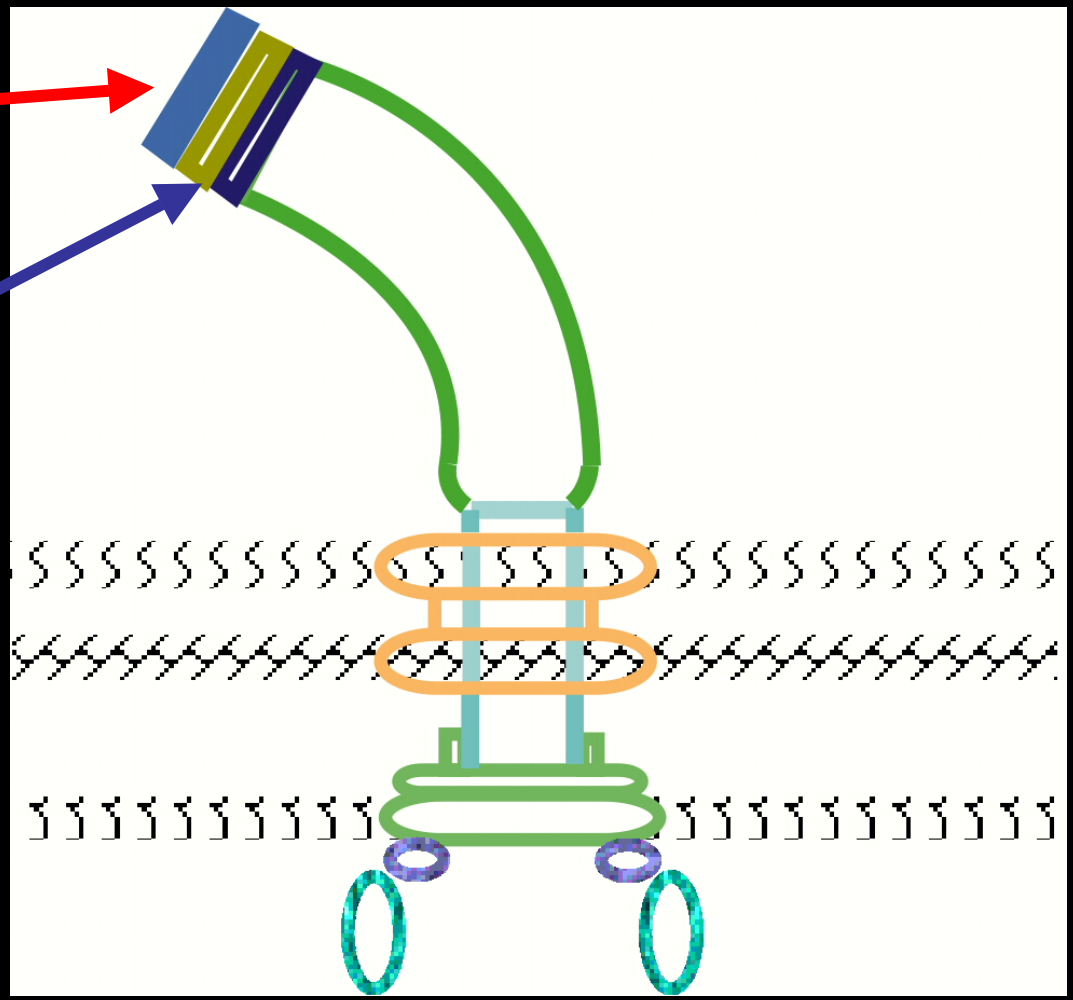
upper rod;
FlgB, FlgC, FlgF, FlgG

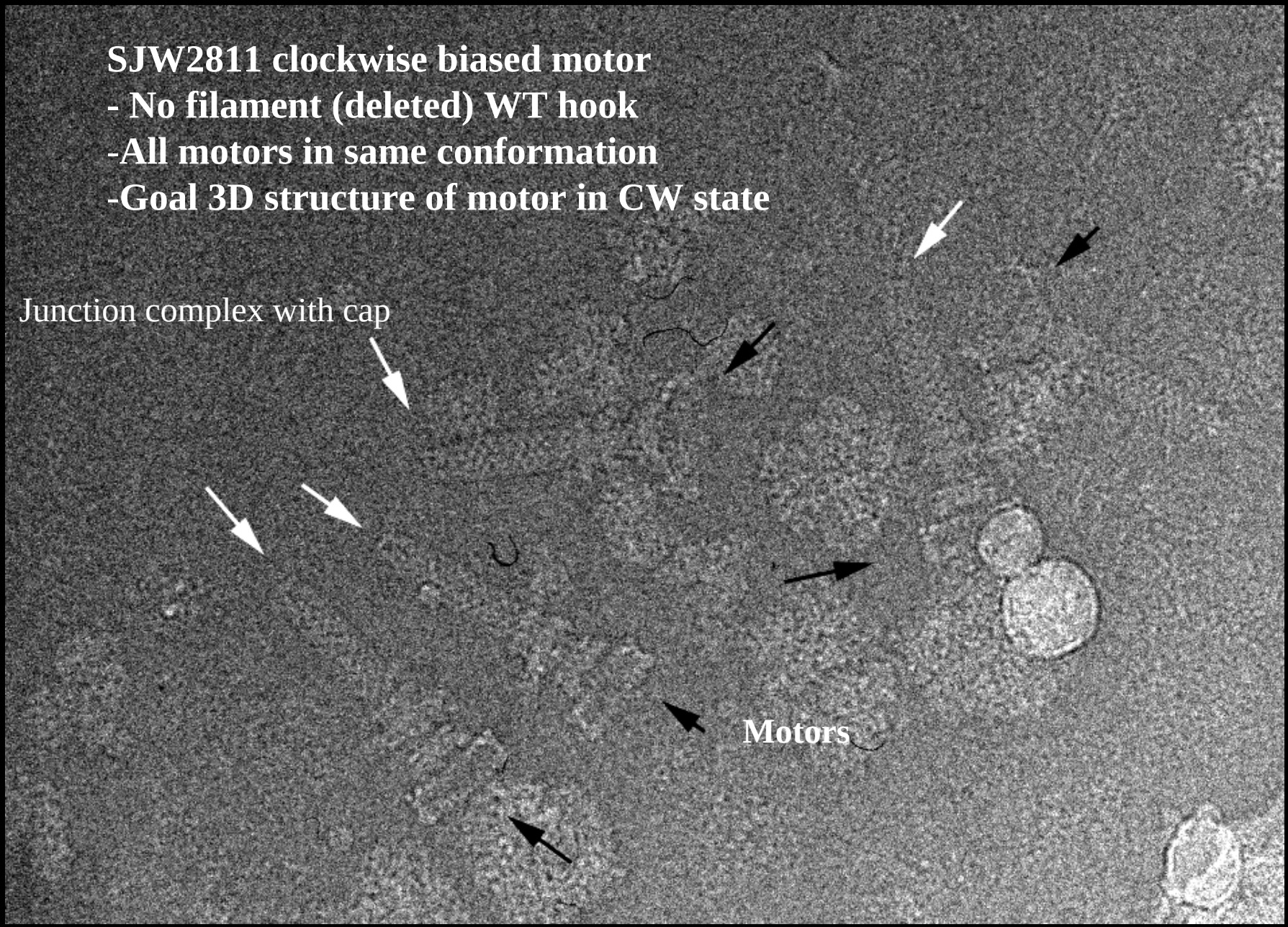


tube of alpha helices like
the filament and hook?

Cap
HAP2

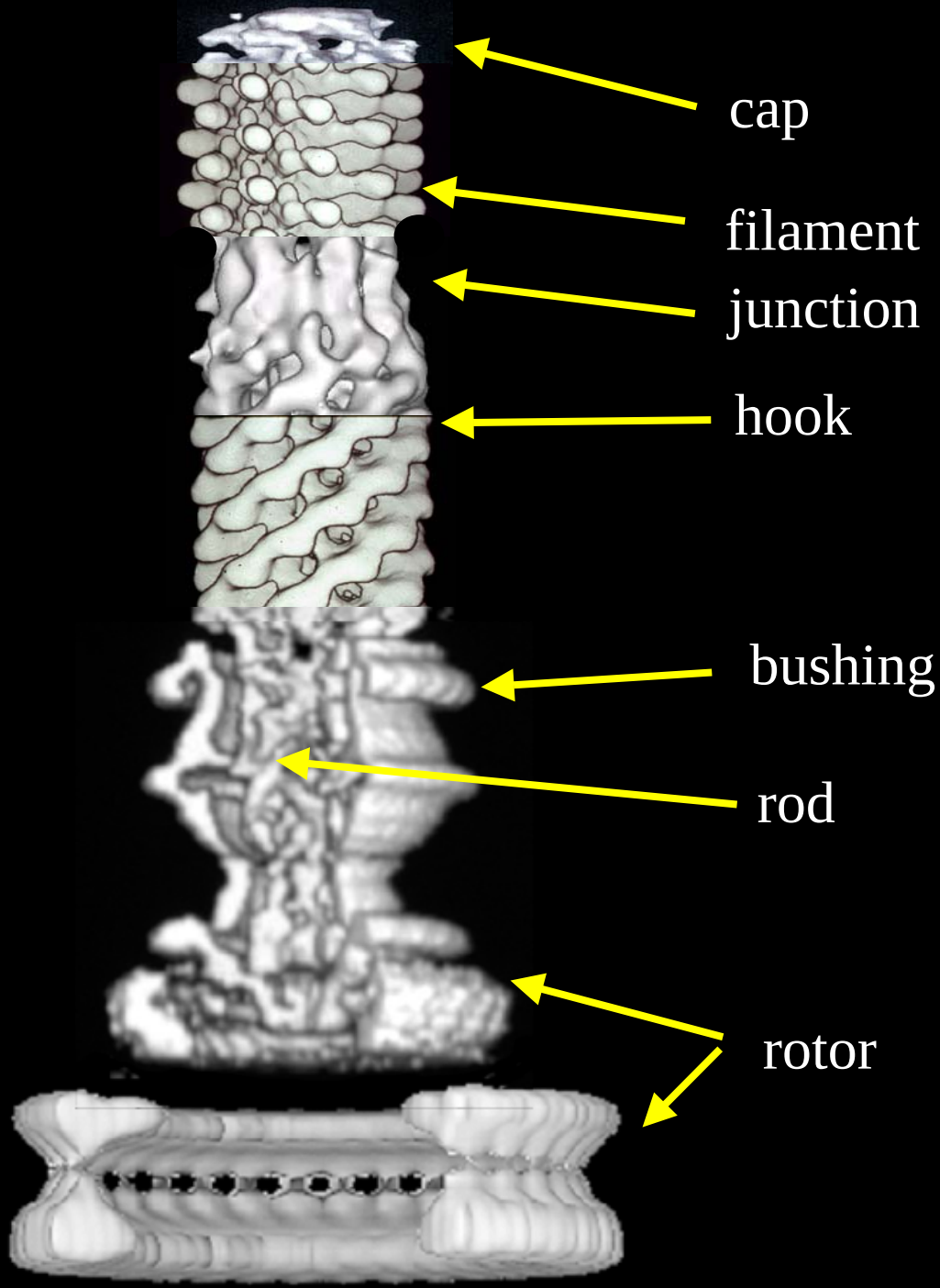
Junction
HAP1 + HAP3




SJW2811 clockwise biased motor
- No filament (deleted) WT hook
-All motors in same conformation
-Goal 3D structure of motor in CW state
Junction complex with cap
Motors

SJW2811 Hook-HAPs complex
Reconstructed from 218 images

A promising start!!


cap
filament
junction
hook
bushing
rod
rotor

Axial components – Conclusions:

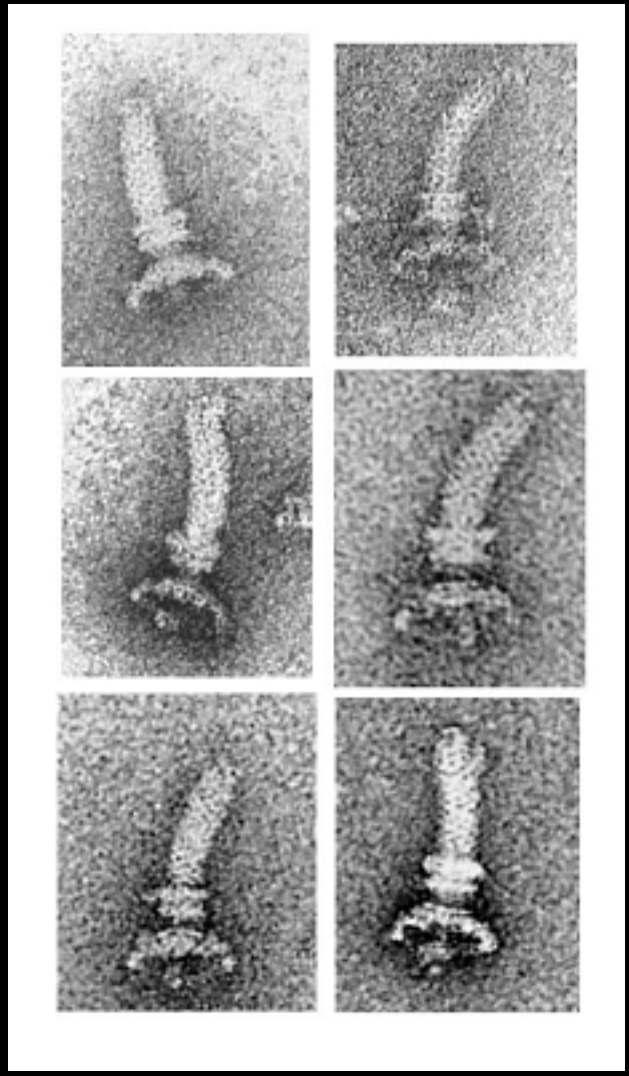
The filament, rod and hook share a common helical symmetry.

The rod, hook junction and filament all have a central tube with a ~2-3nm (export) channel .

In the hook and filament, the heptad repeats generate the packed alpha helices that make up the walls of the tube.

Movie

Hook, rod, rotor (with export apparatus?)



The rotary motor

CW and CCW rotation.

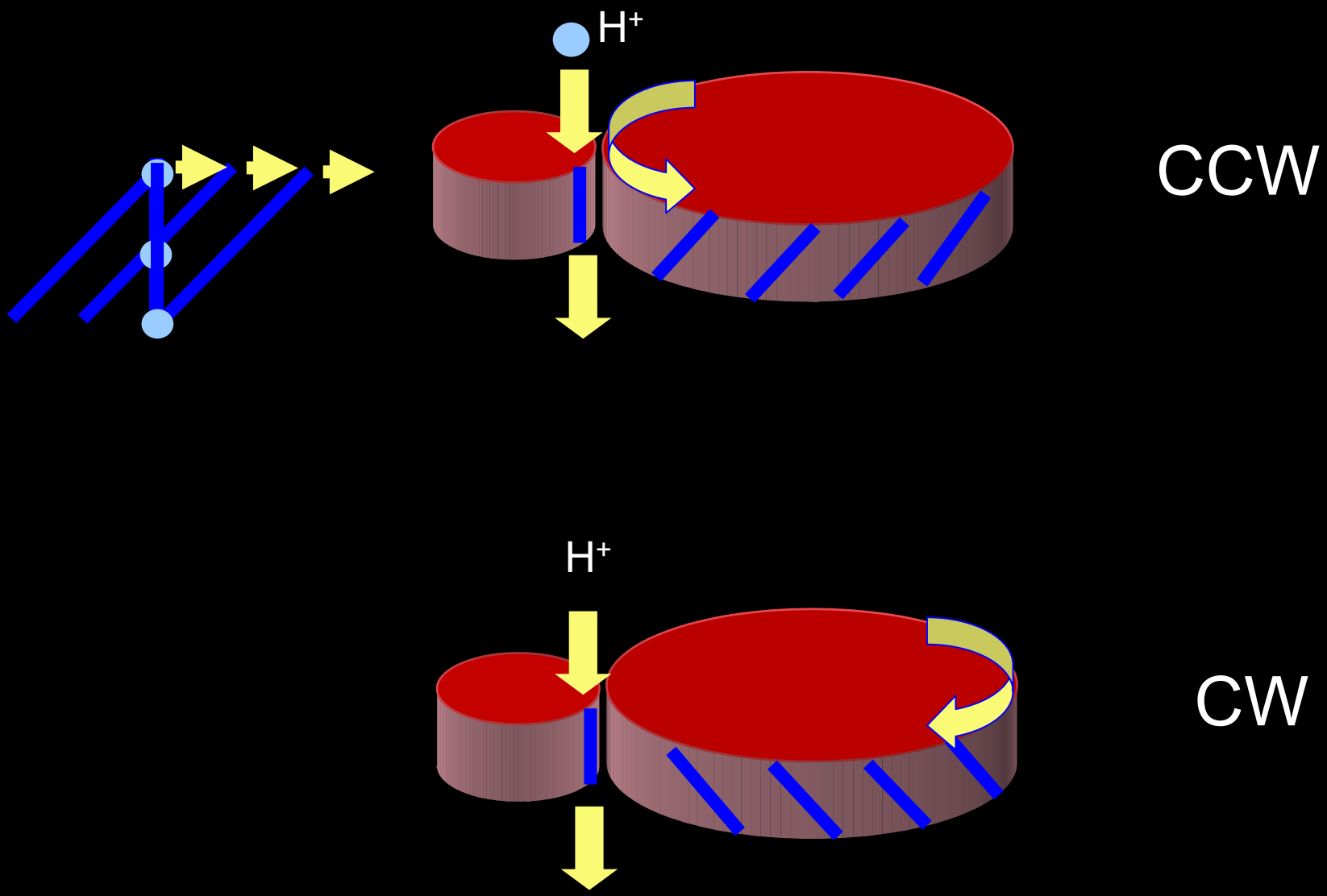
18,000 rpm.

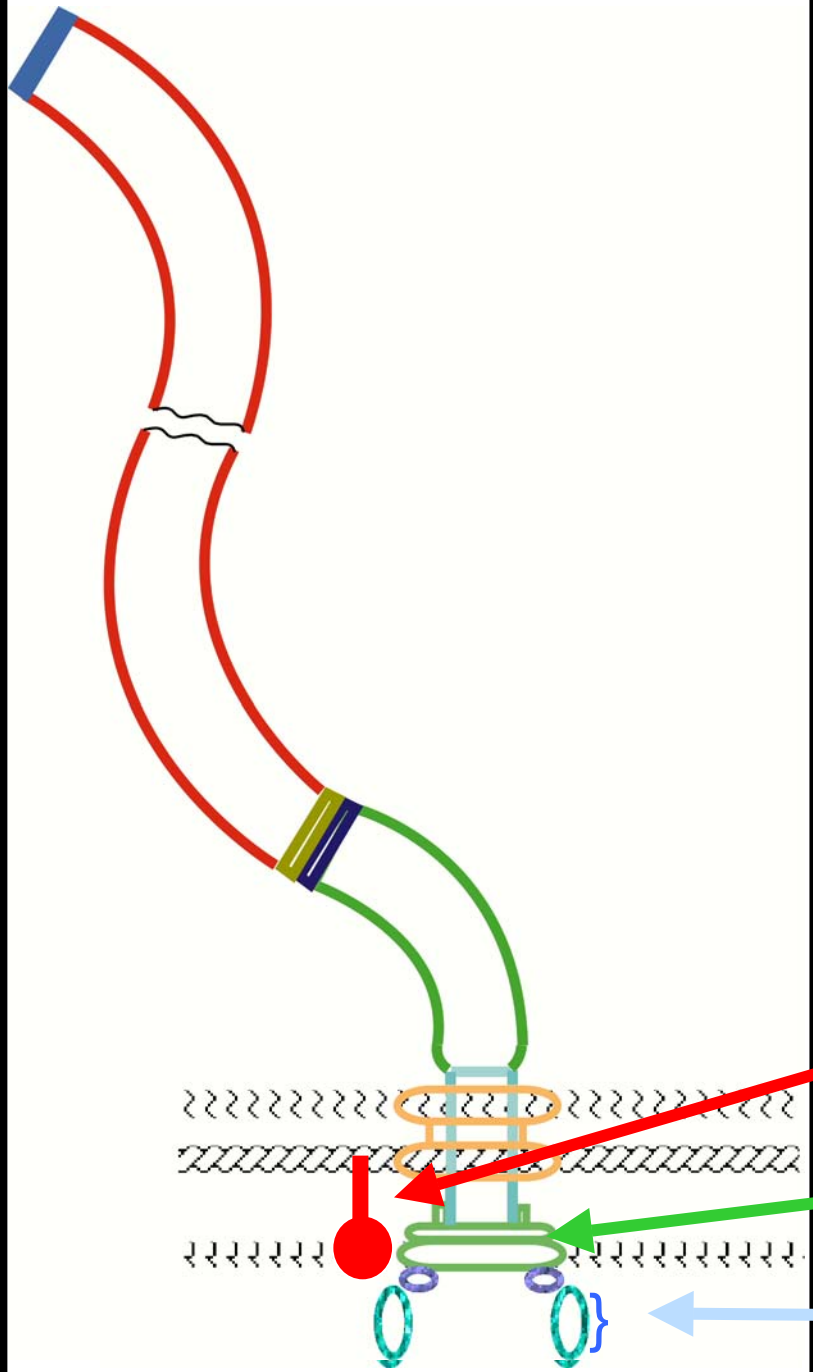
~1000 protons/revolution.

400 steps/revolution.

10^{-19} horsepower; 10^{-16} watts

8 independent torque generators

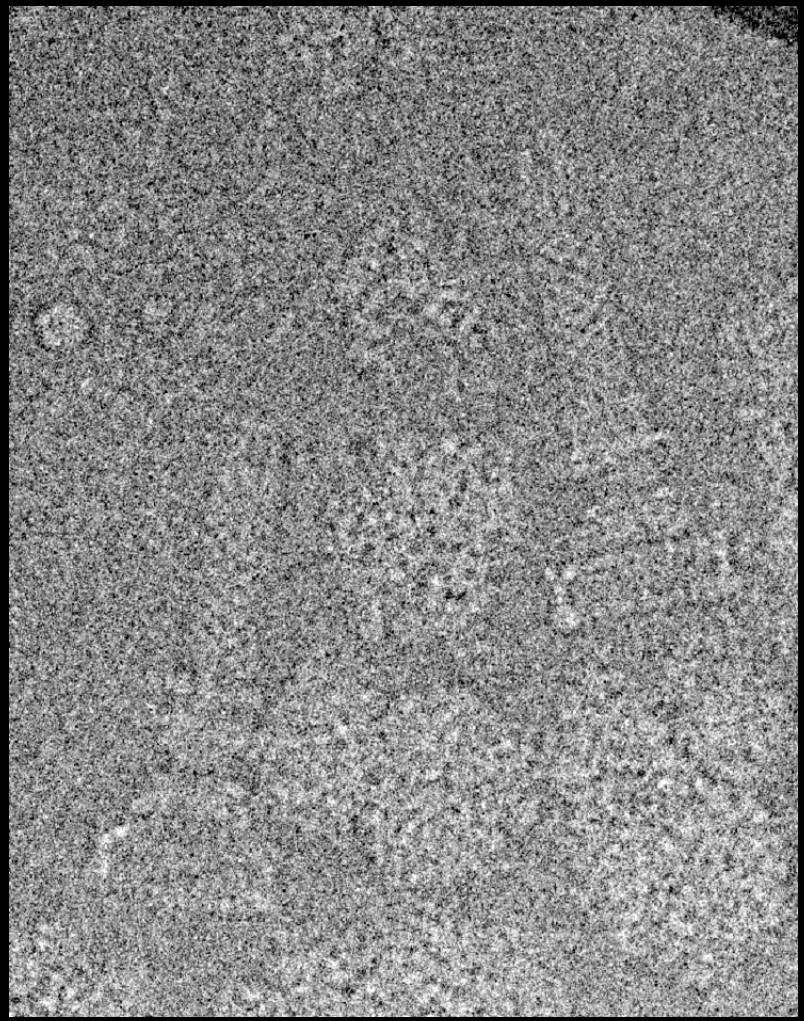

H+
CCW
H+
CW
stator
rotor



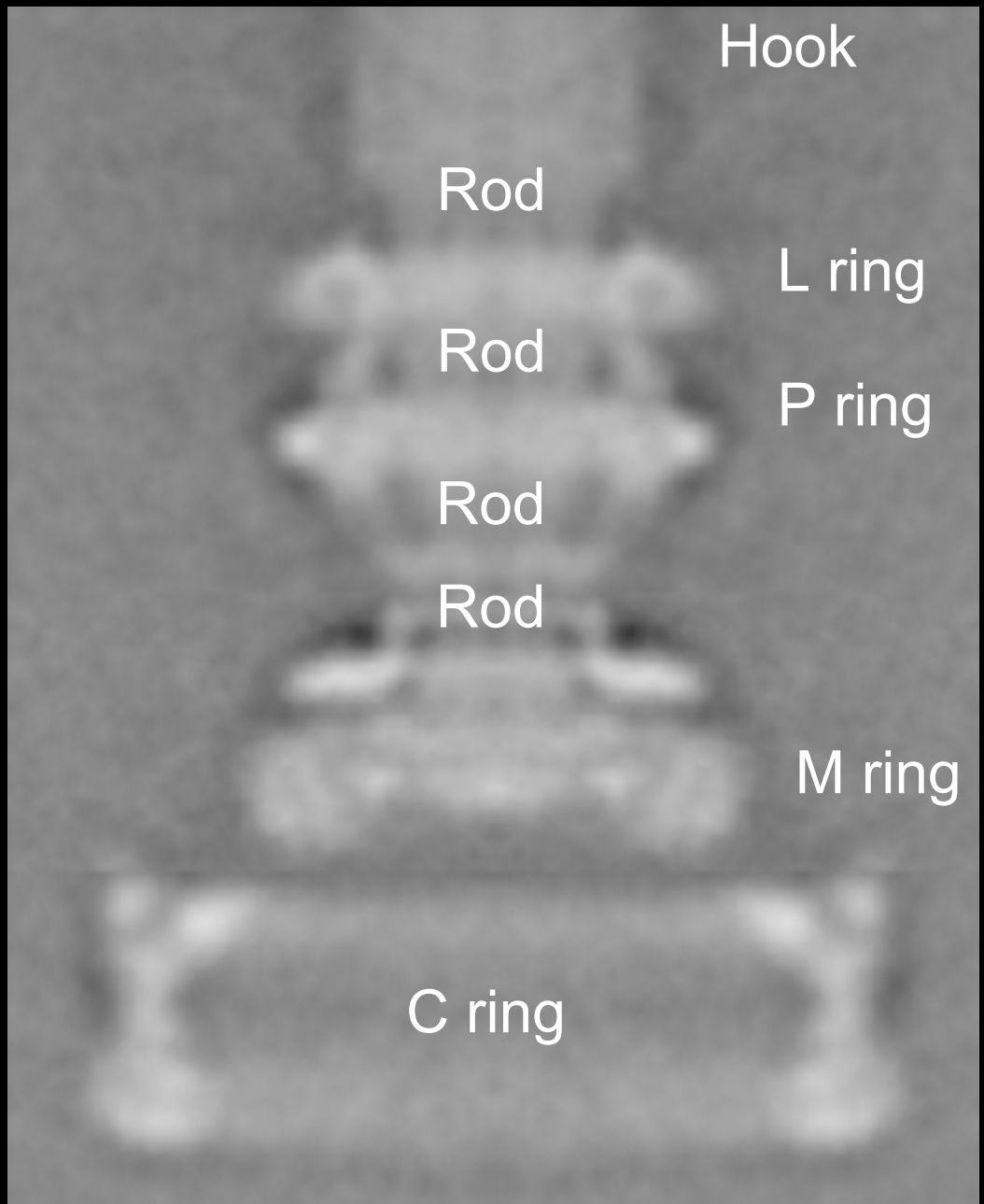
MotA
MotB

FliF

FliG,FliM,FliN



Averaged image of purified flagella.

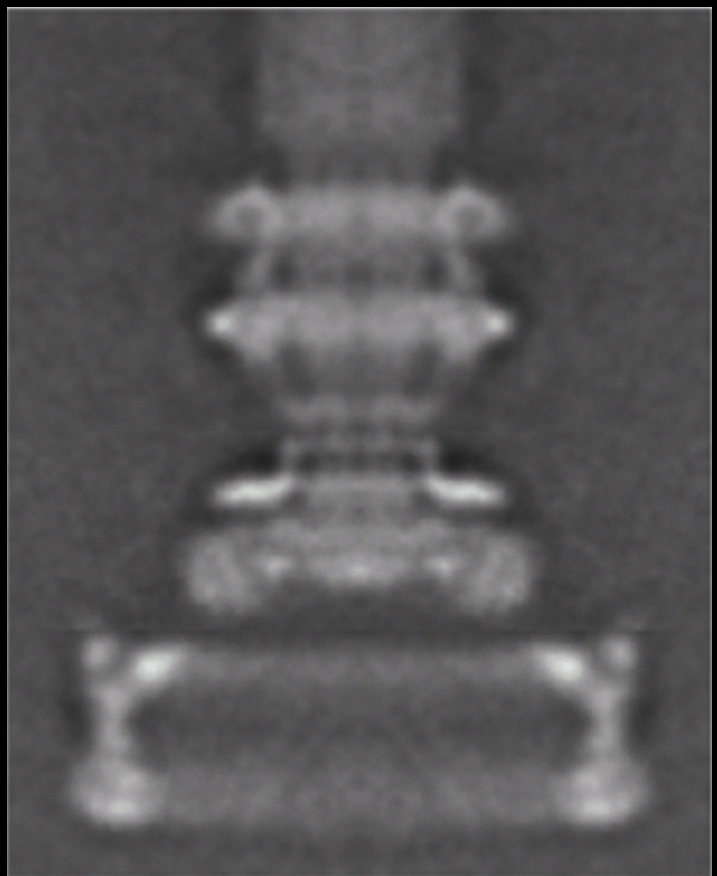


Has FliG, FLiM & FliN but not MotA/MotB

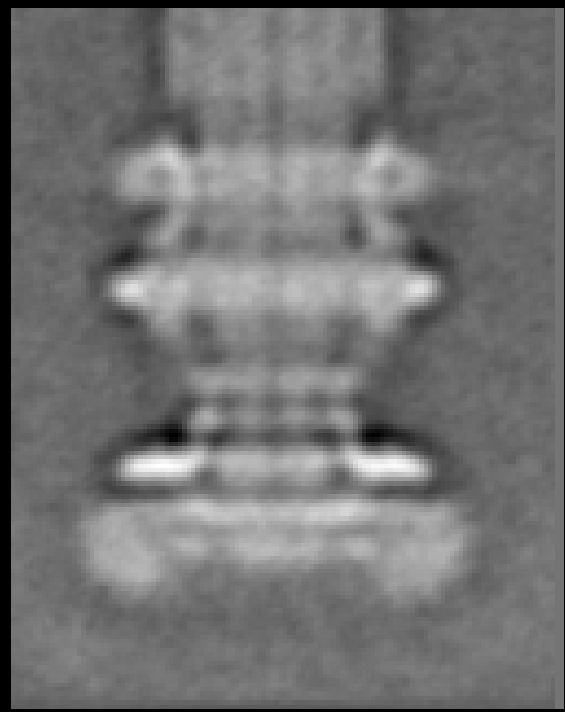
Which features correspond to FliG, FliM and FliN?

FliM and FliN make up the C ring.

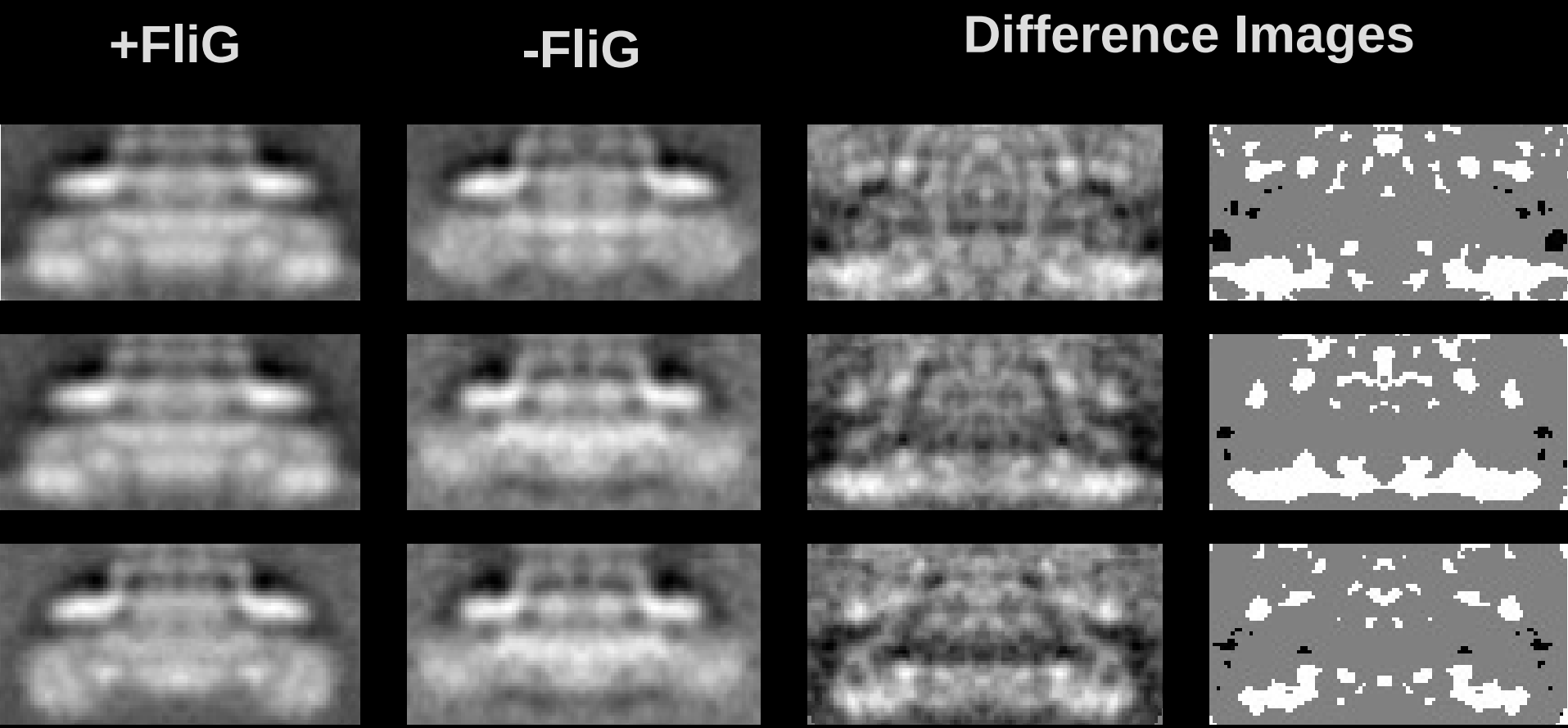
Has FliM and FliN and C ring.

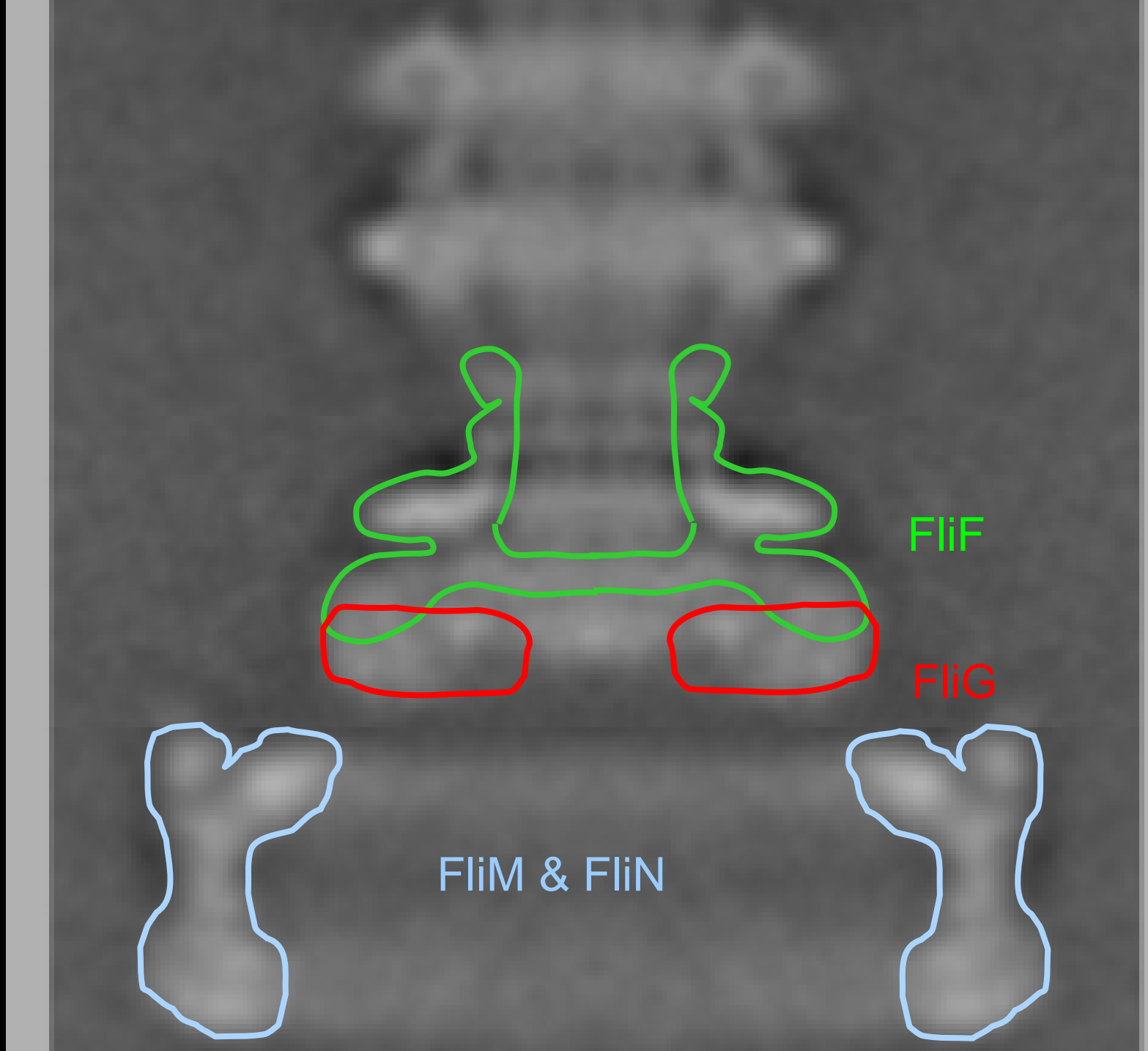


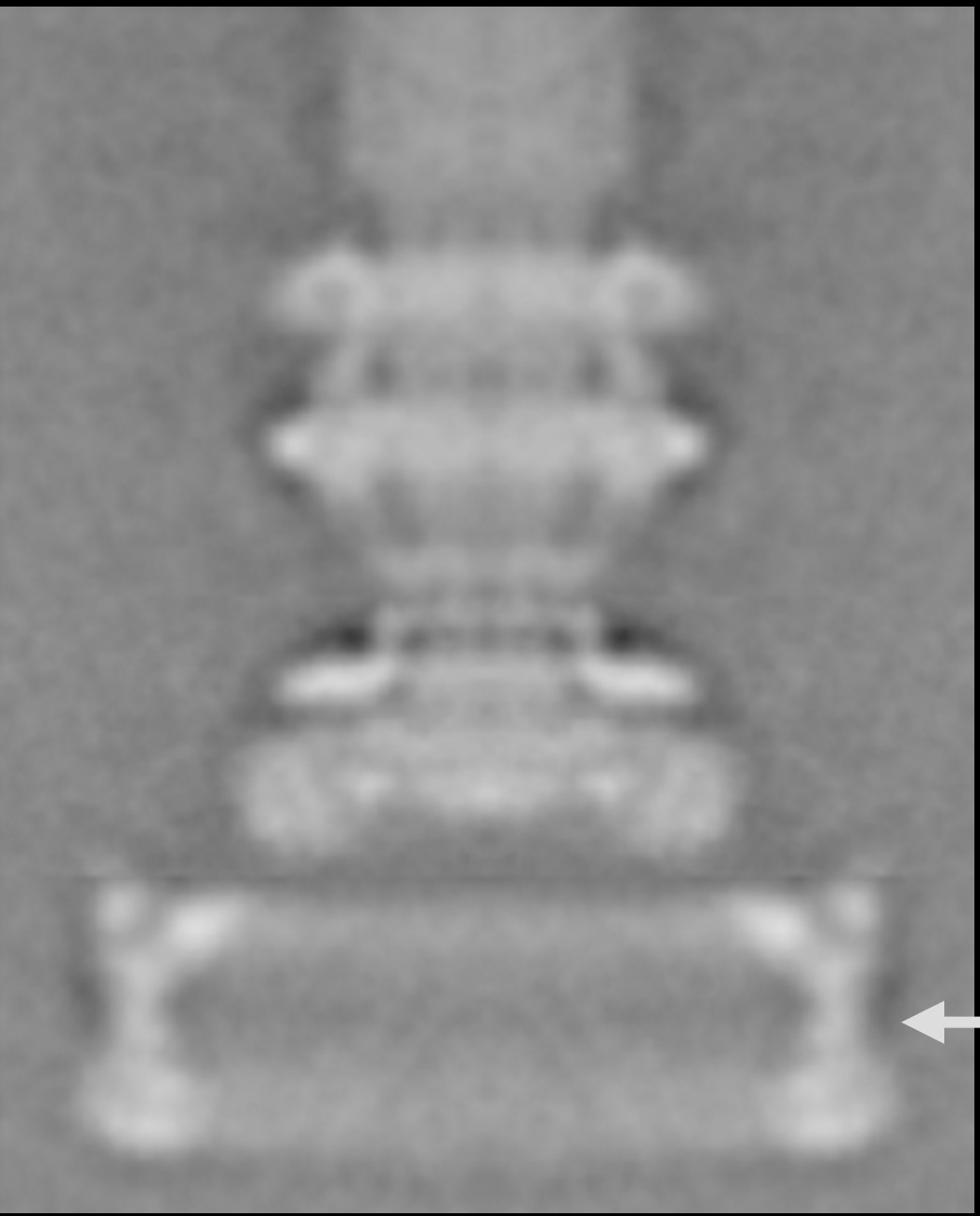
Lacks FliM and FliN and C ring.



FliG occupies the cytoplasmic face of the M ring.

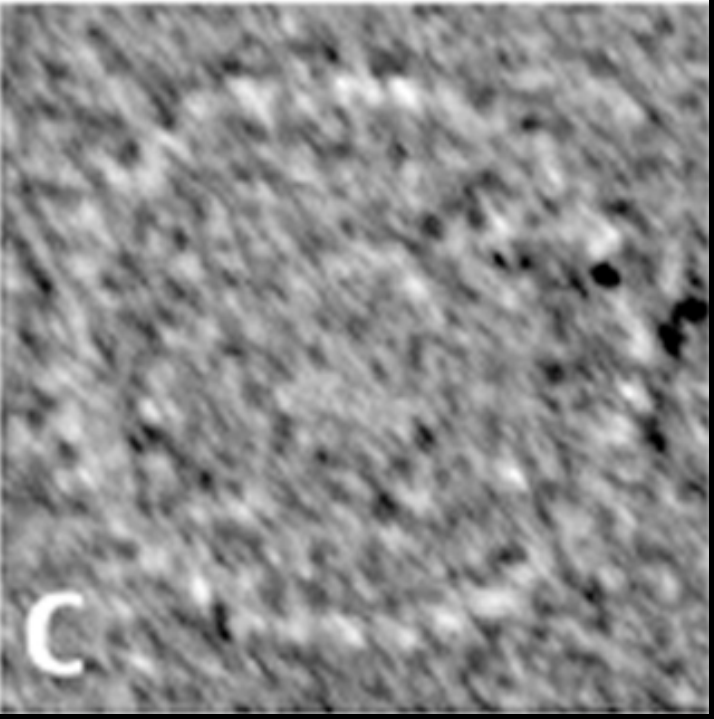


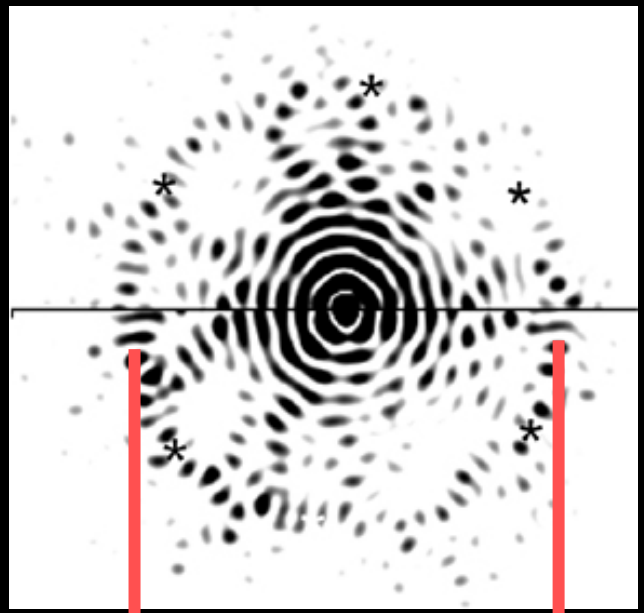

FliF
FliG
FliM & FliN

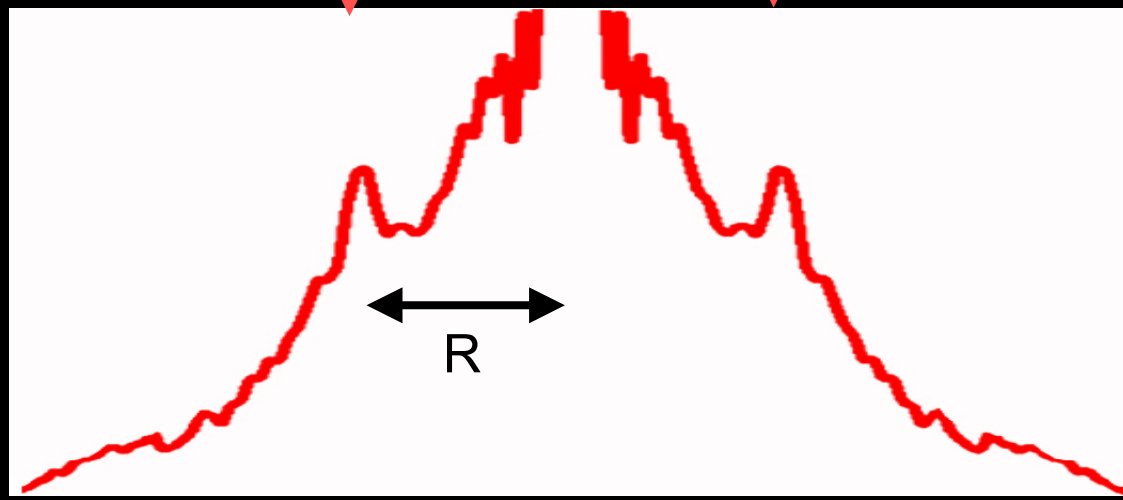


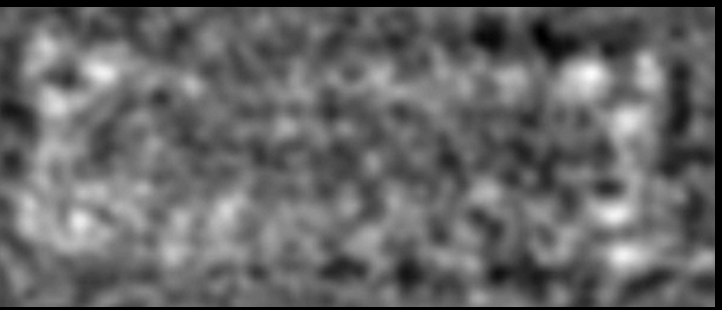
M ring; n=26 subunits

What is the symmetry of the C ring?







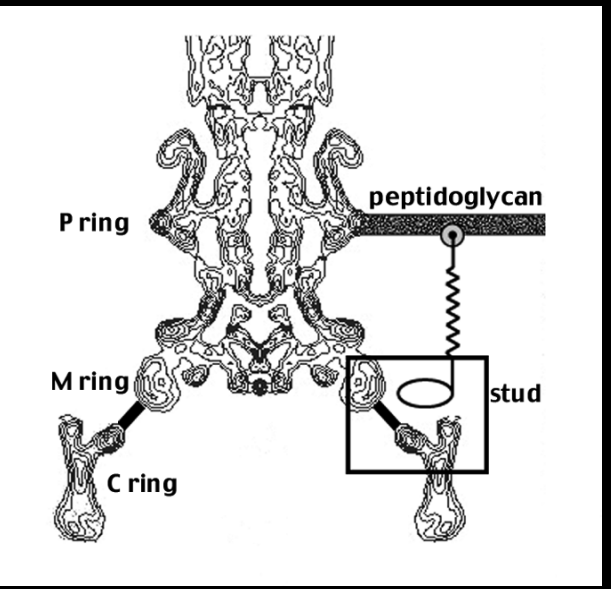


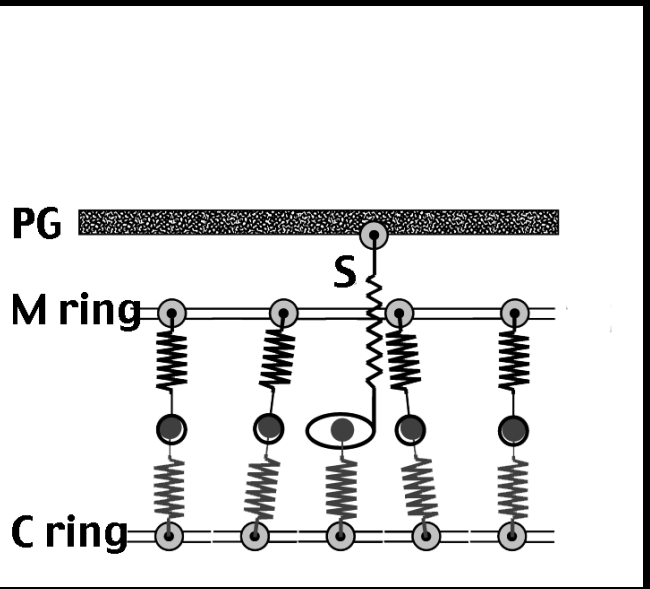
$N \approx \pi * d * R\text{-}2 = 34$

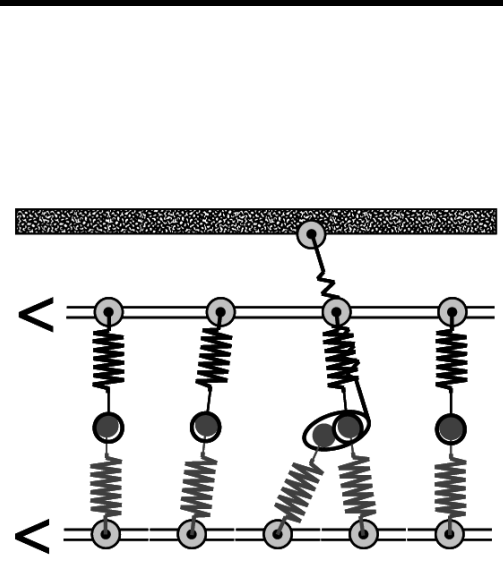
Motor Numerology

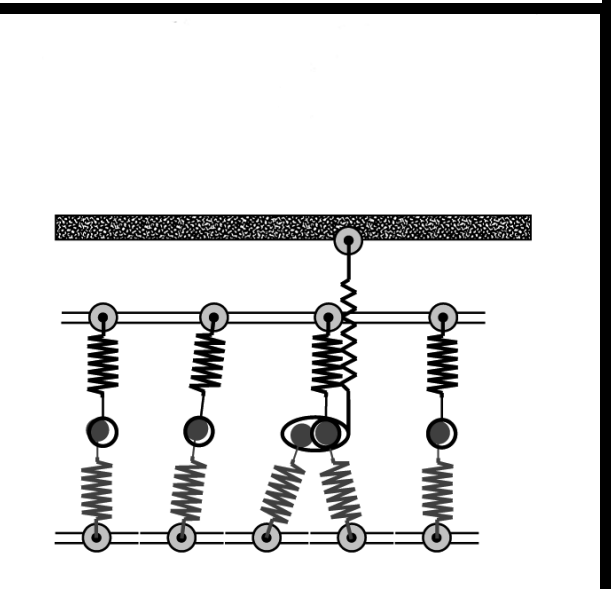
1. Torque generators	8
2. Units in M ring	26
3. Units in C ring	34
4. Steps per revolution	400

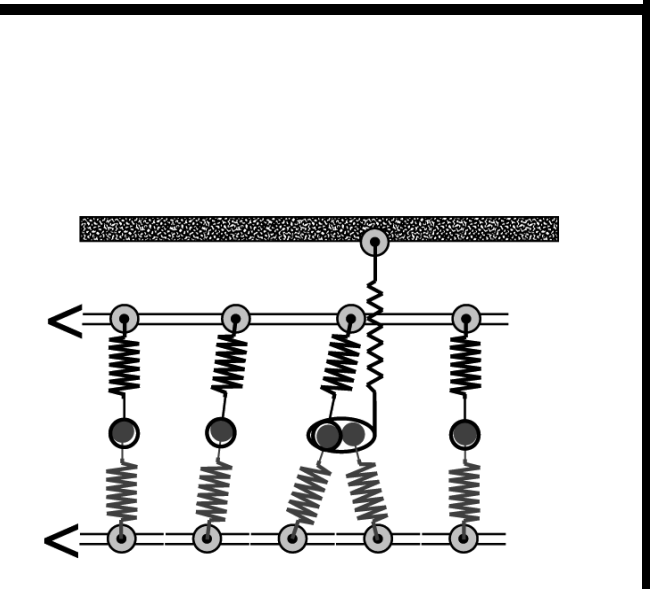
A model for the mechanism of the motor:

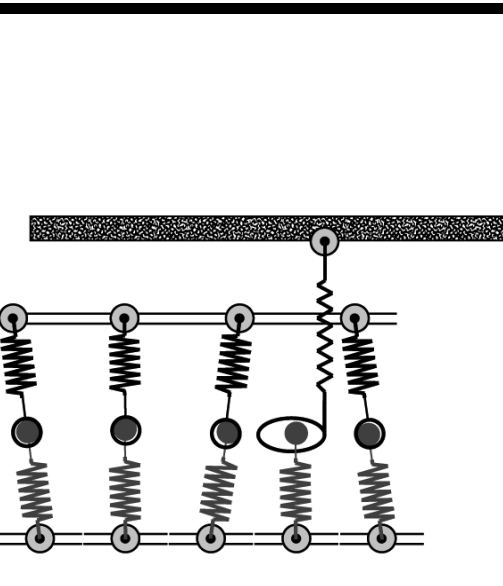


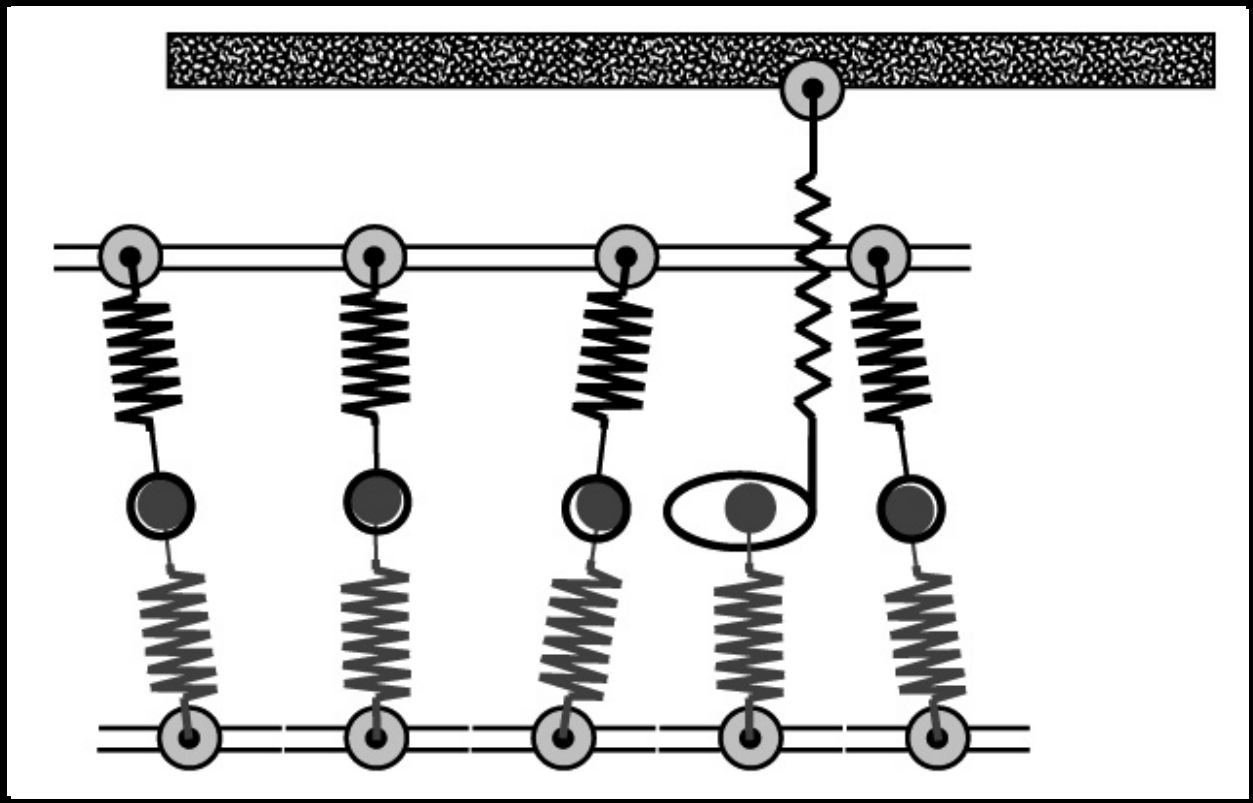


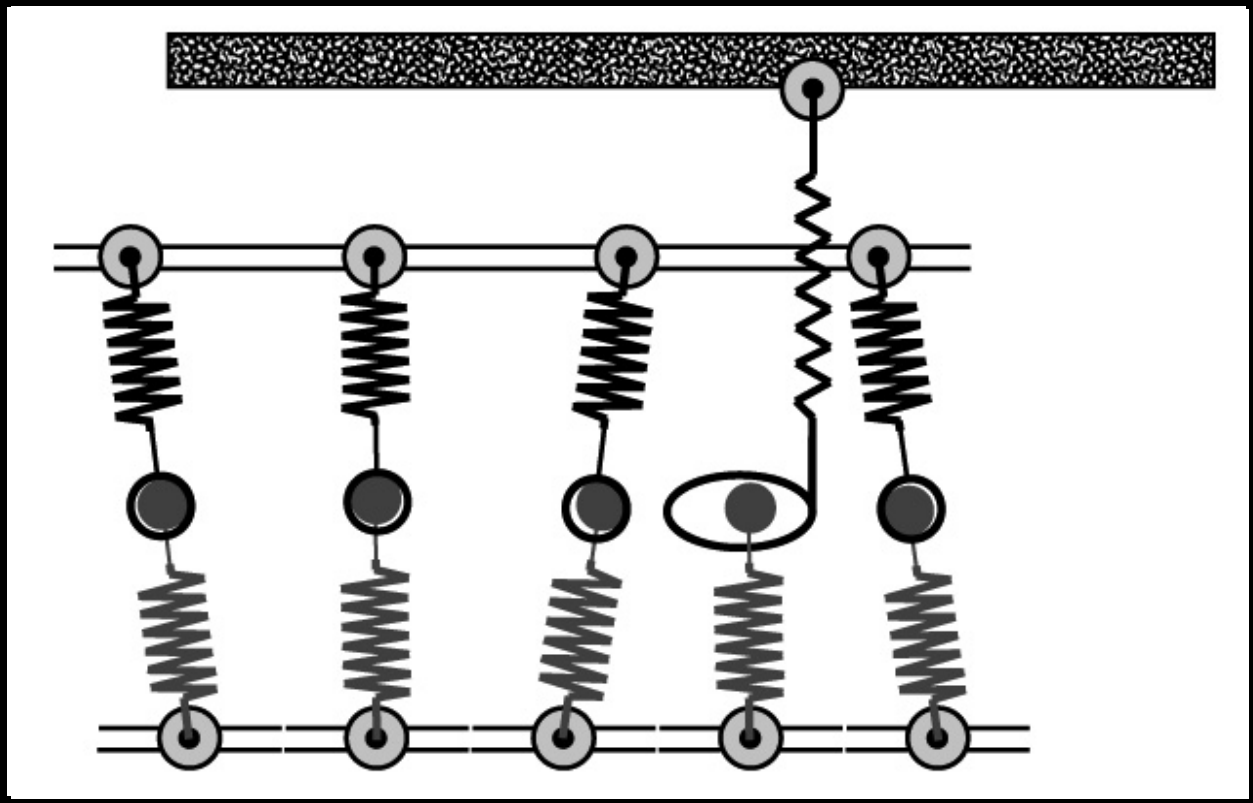




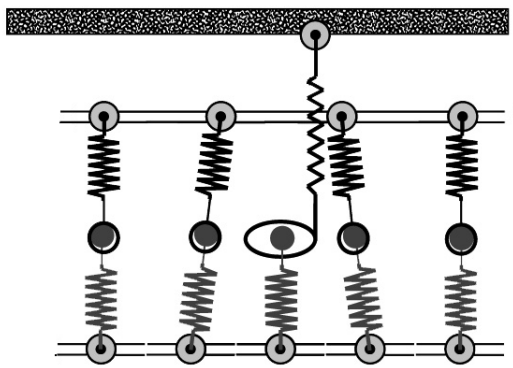




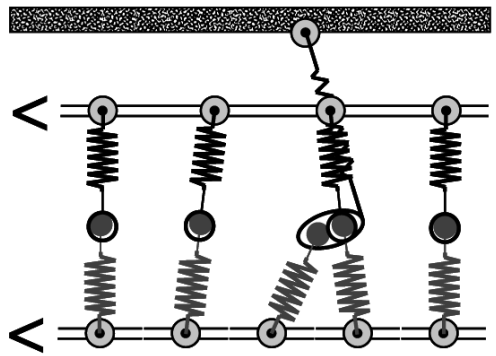


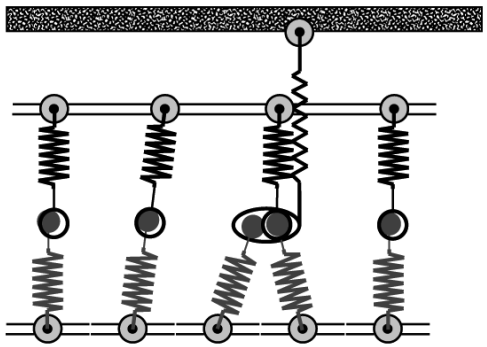


In each cycle, there are two steps.

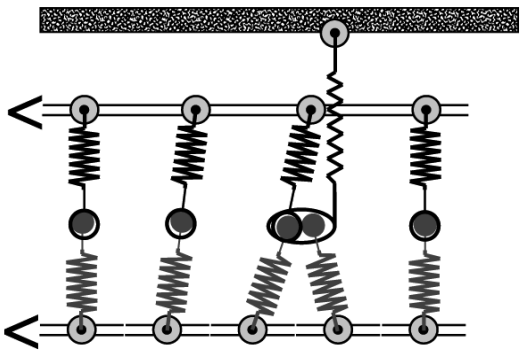


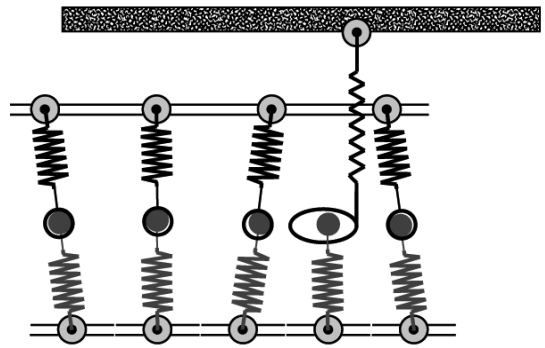
Step 1





Step 2

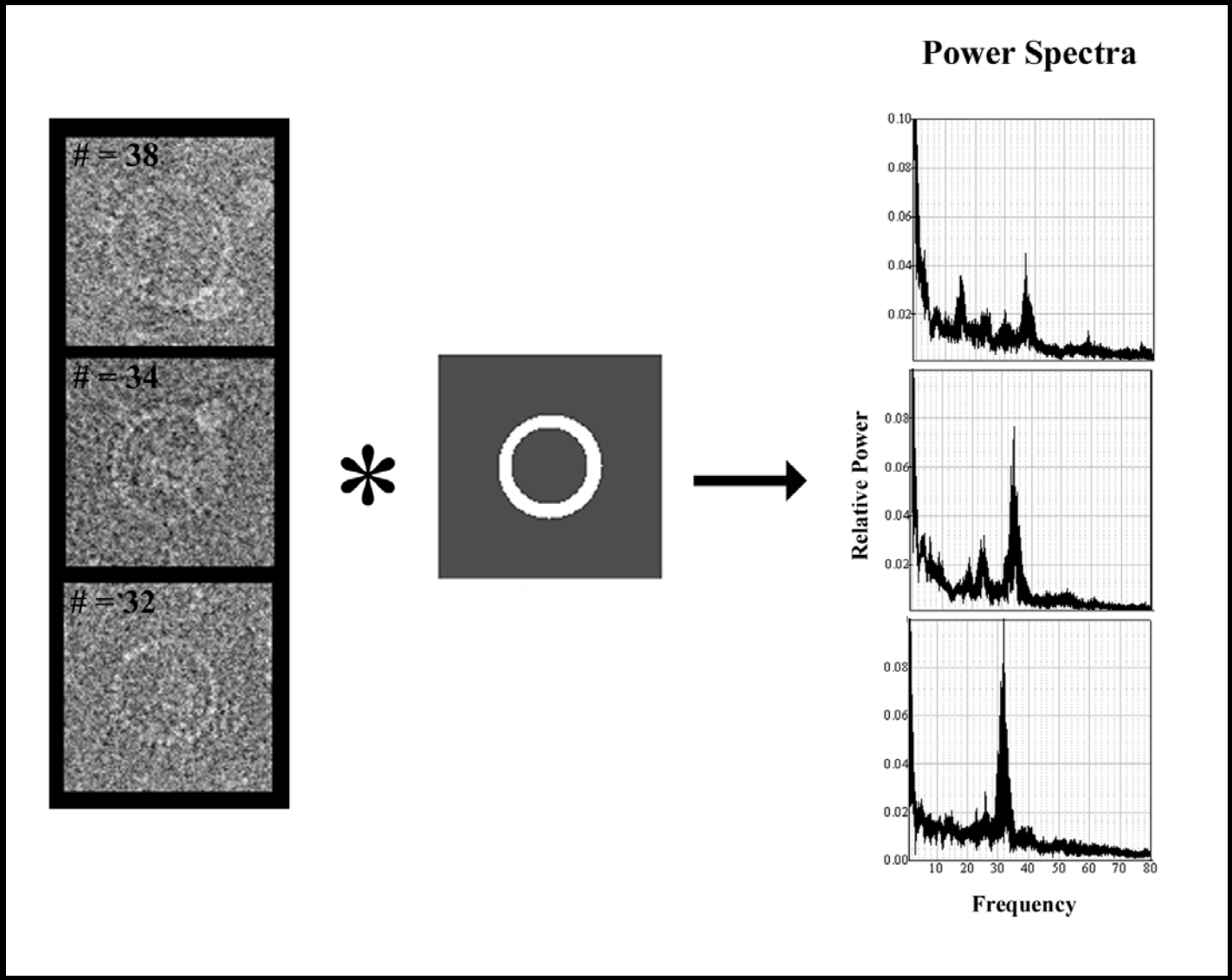




The model explains the number of steps/revolution

cycles/torque generator * revolution	26
steps/cycle	2
steps/torque-generator * revolution = 2*26	52
torque generators	8
steps/revolution = 52 * 8	416

Analysis of C-ring Subunit Periodicity



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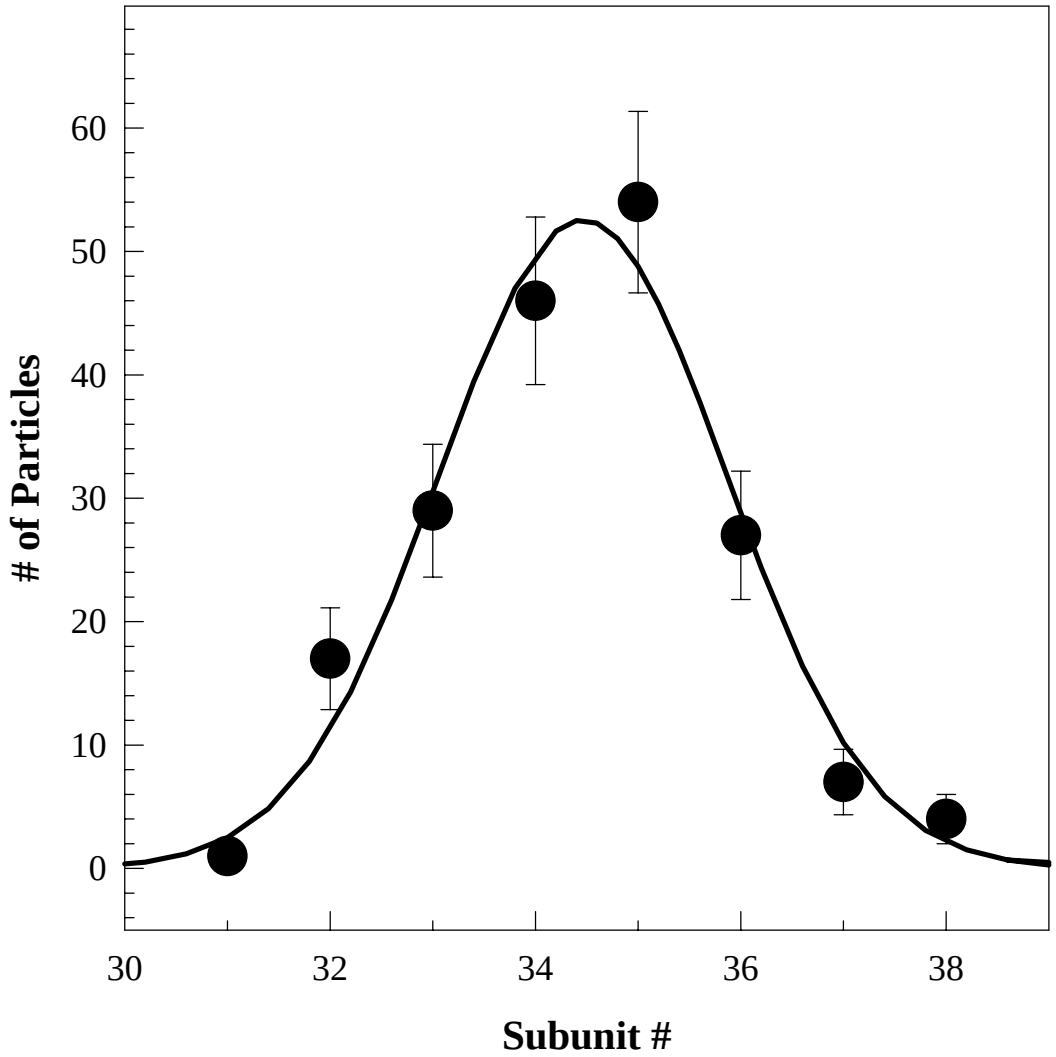
A Gaussian Distribution of Rotor Particles with Different C-Rings



<n> = 34.5± 1.5 subunits.

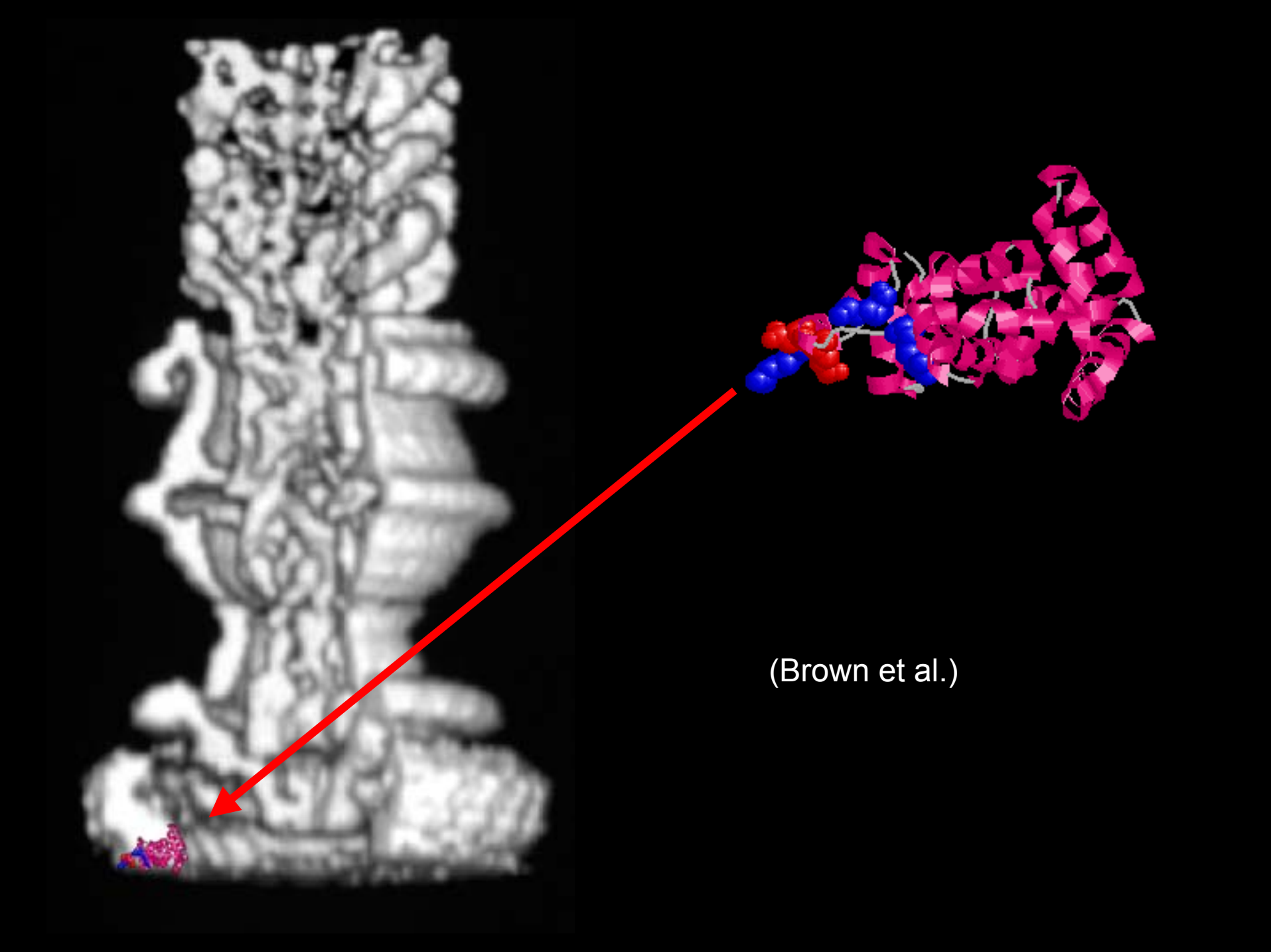
For the future:

Locate by difference mapping the motor domain of FliG.

Locate domains of FliM and FliN by gold labeling.

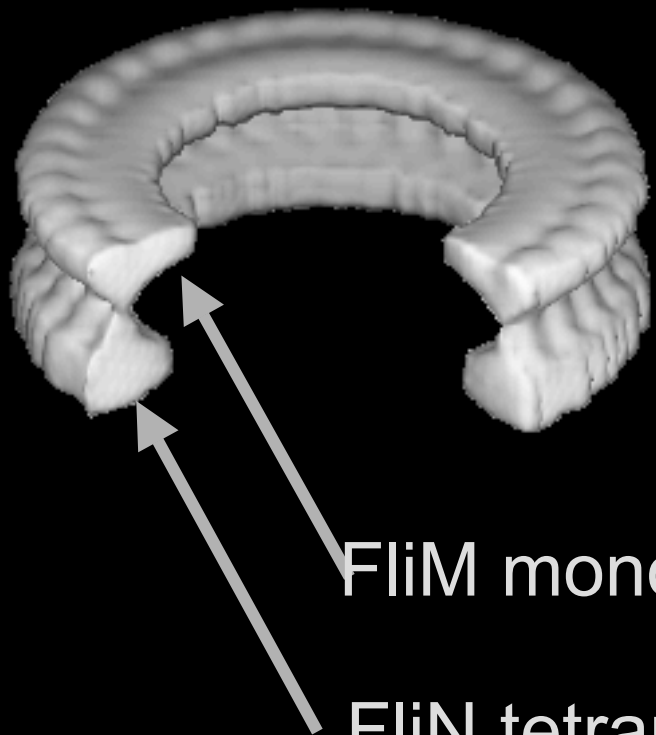
Locate binding site of CheY-P, the response regulator and look for changes in the structure of the motor.

Determine 3D structure of rotor and the export apparatus.



(Brown et al.)

3D reconstruction of the C ring (FliM and FliN) by electron cryomicroscopy



- made by combining different views using single particle methods.

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