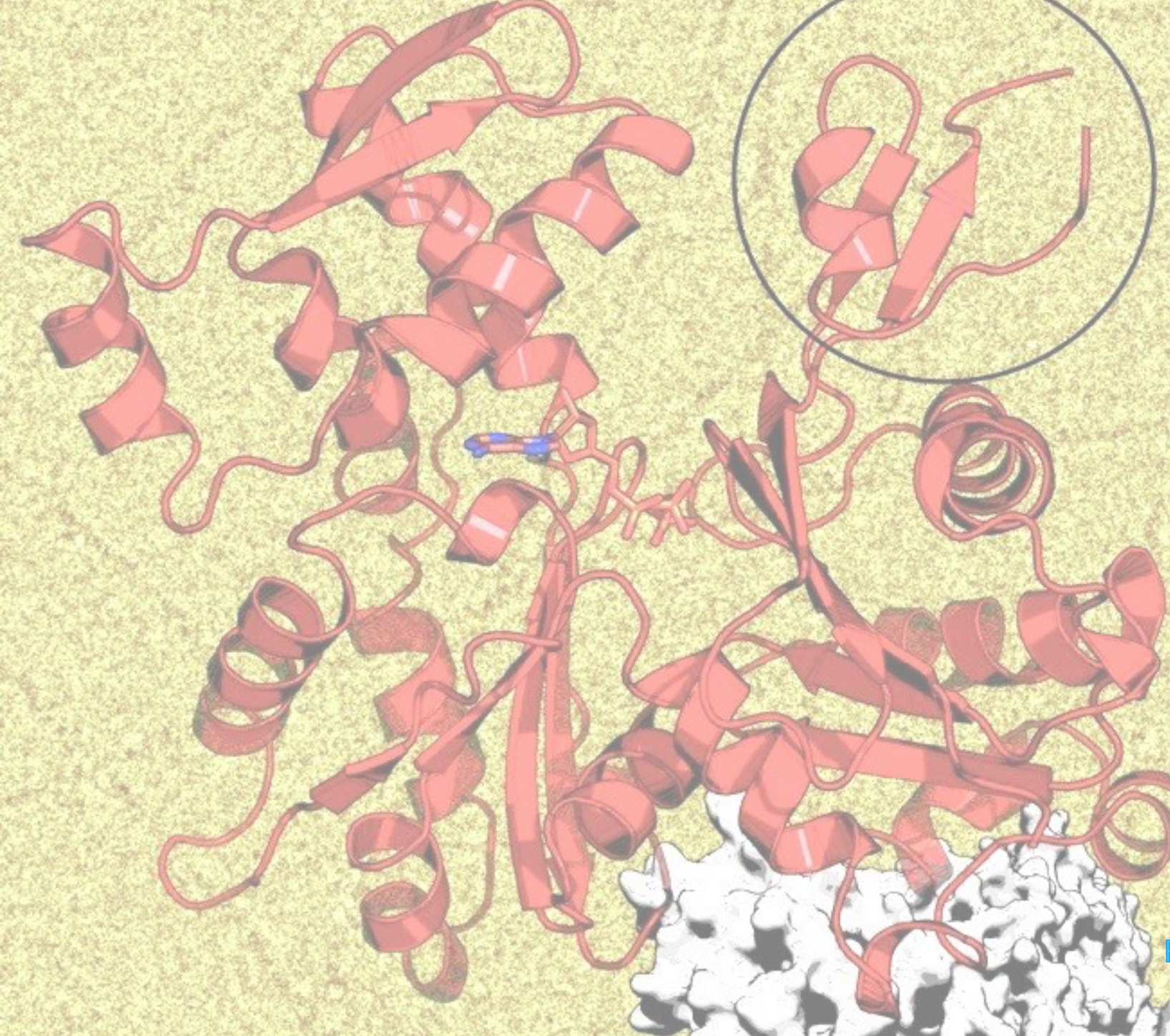

USING PHOTONS AND ELECTRONS TO UNDERSTAND MALARIA PARASITE MOTILITY

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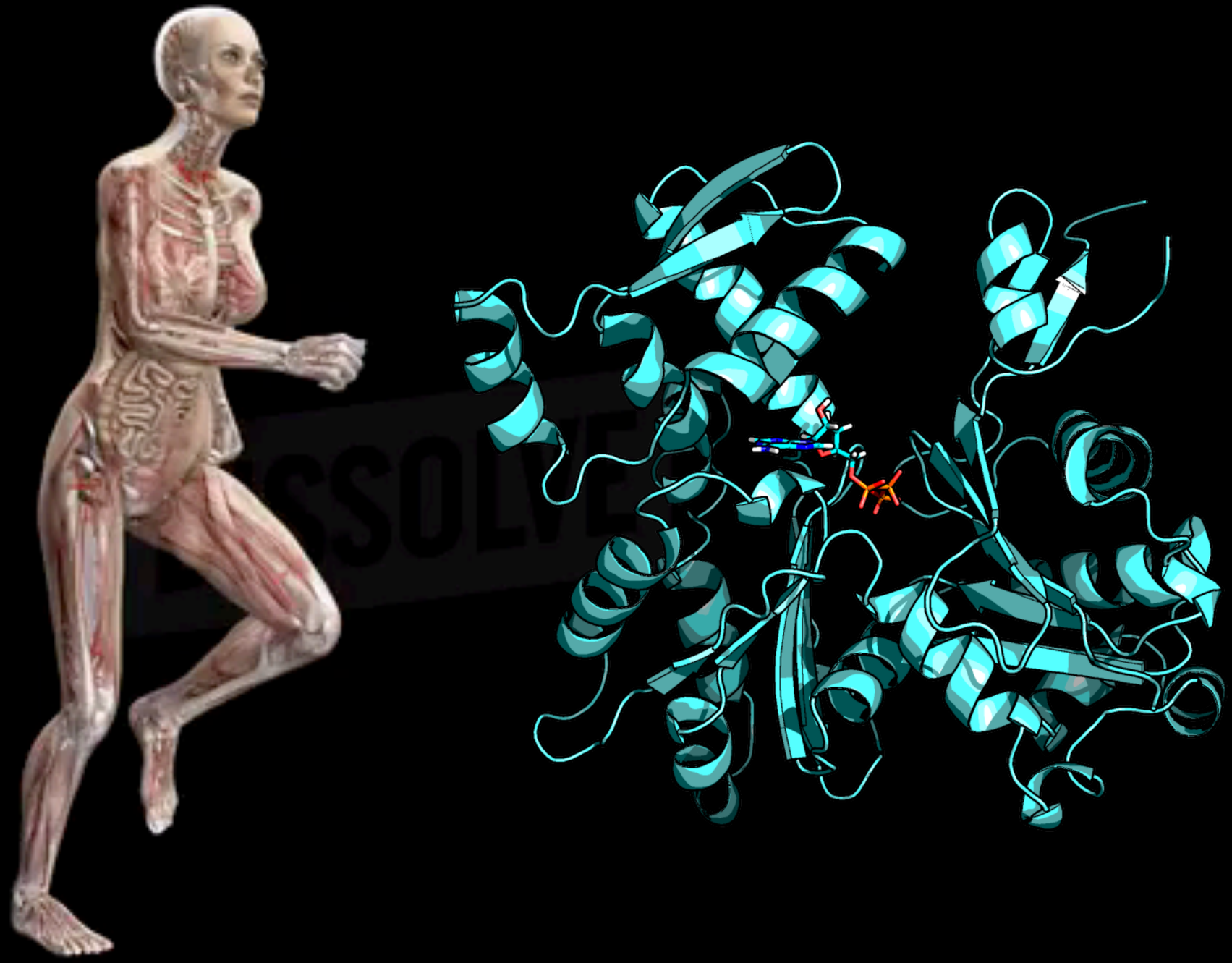
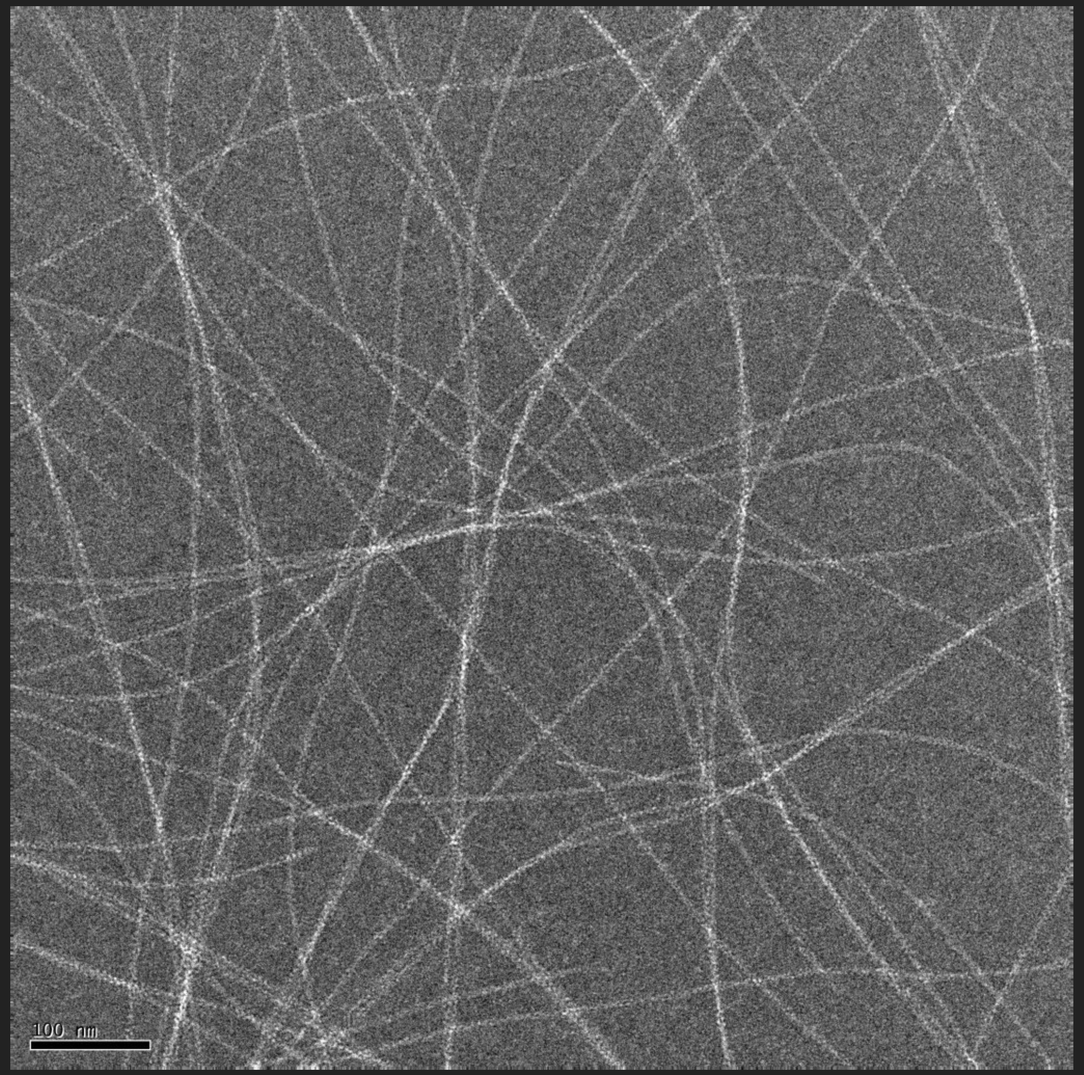
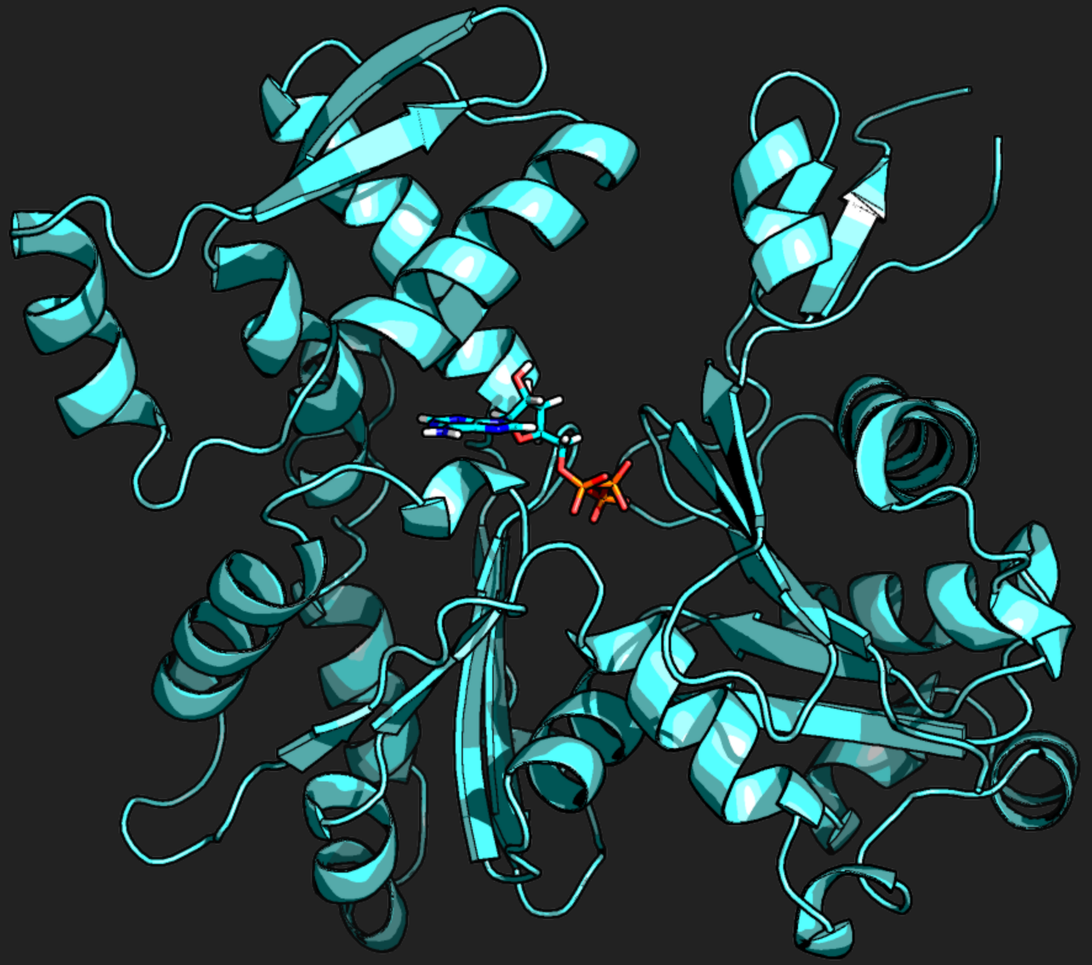
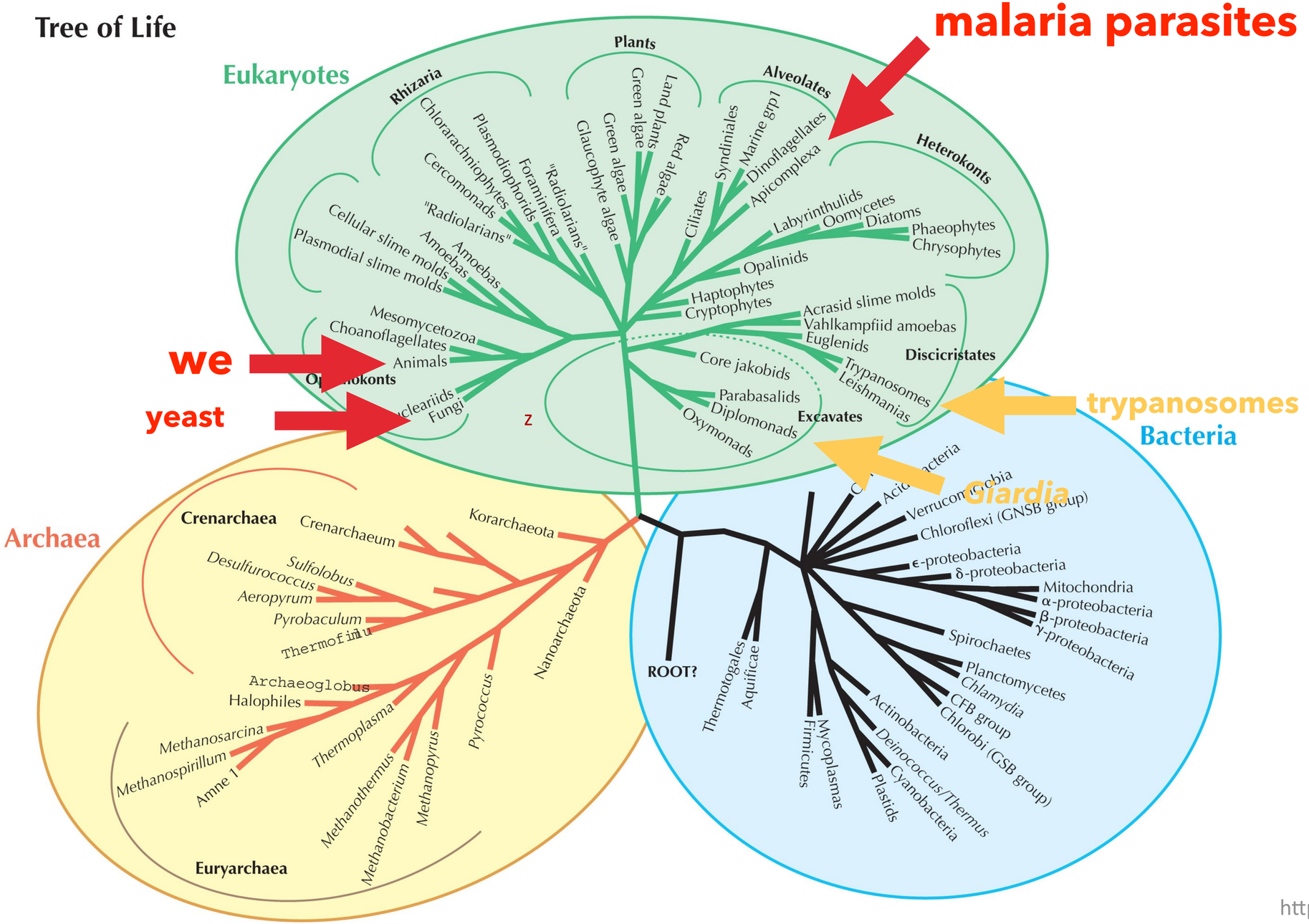


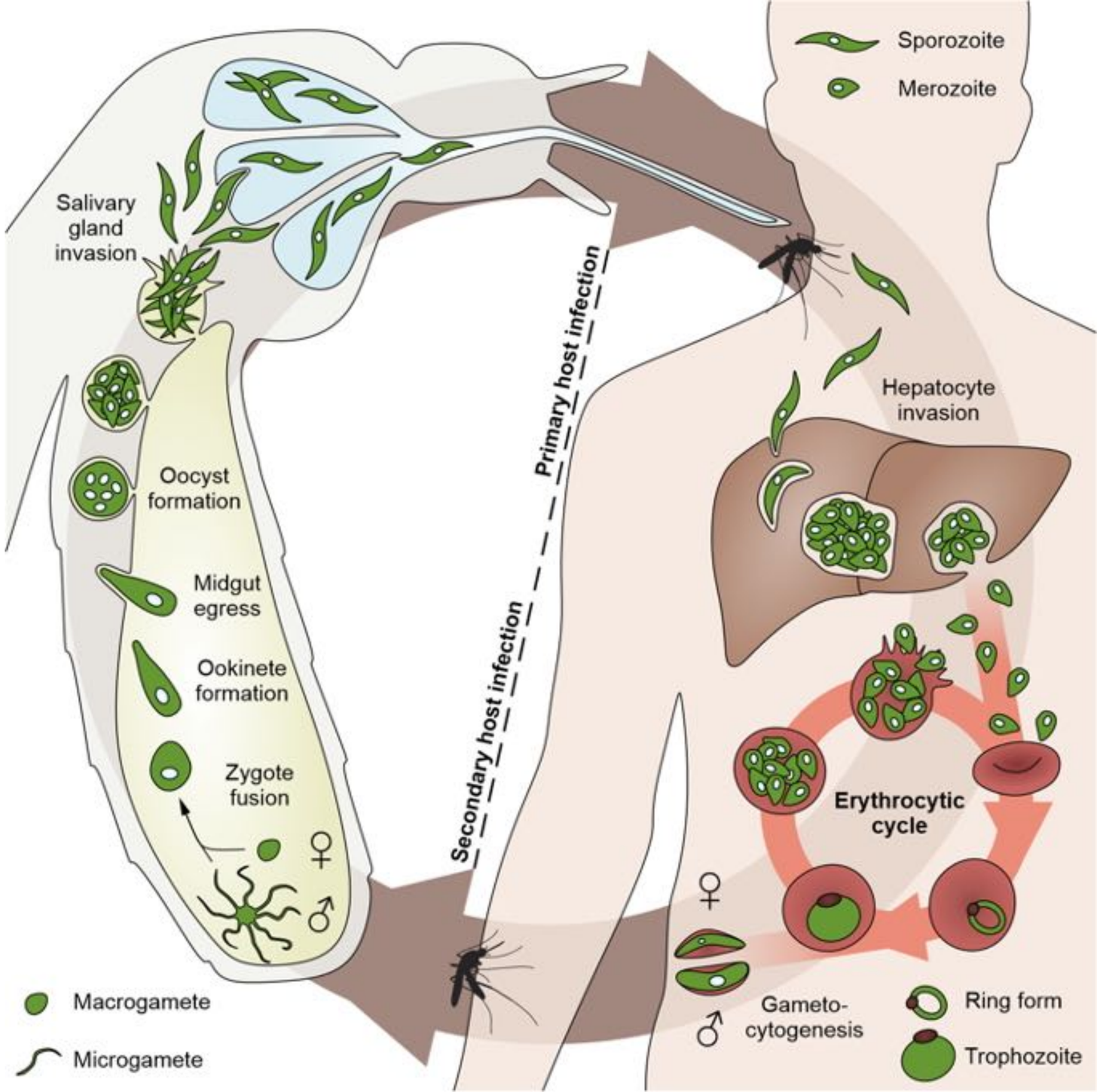

100 nm



CHOICE OF MODEL SYSTEM

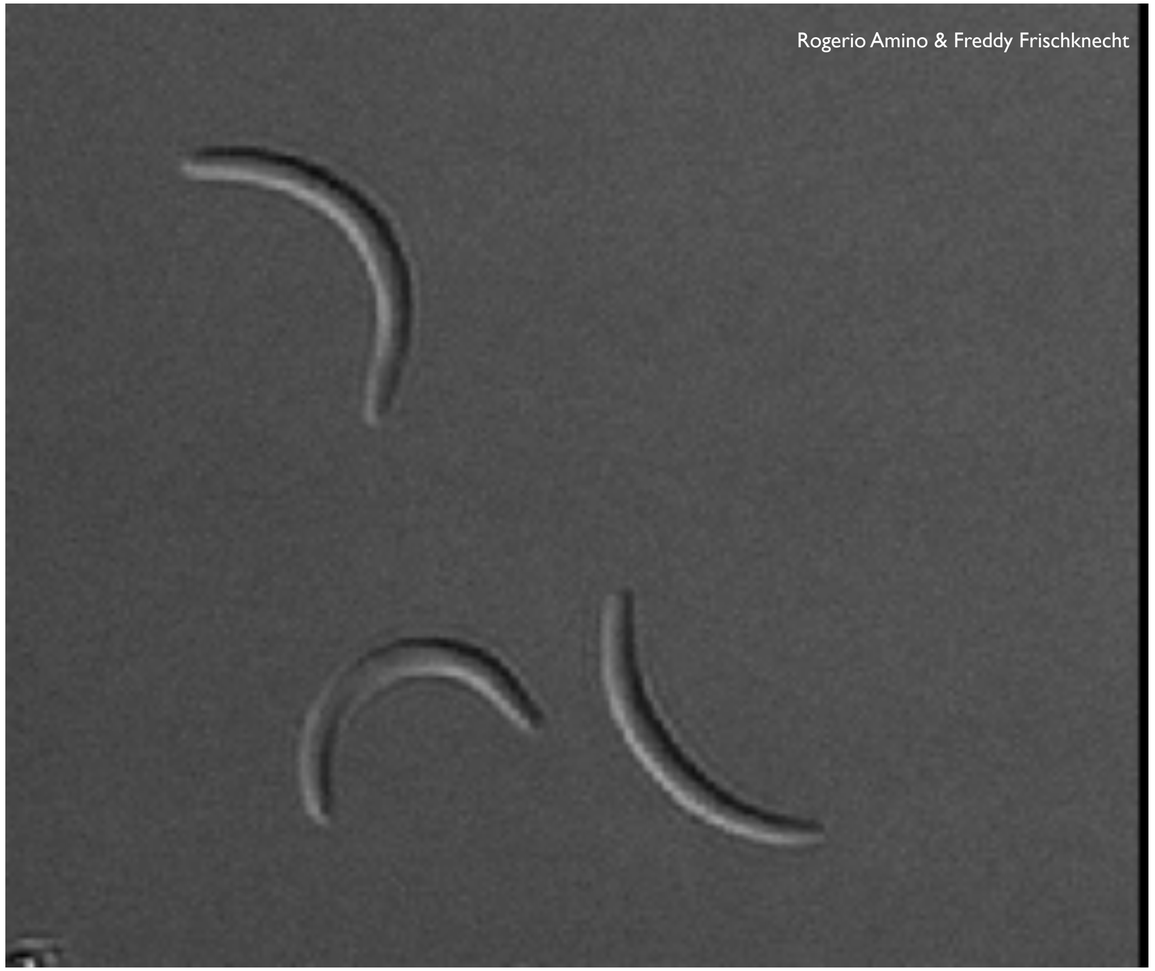


THE PARASITE LIFE CYCLE

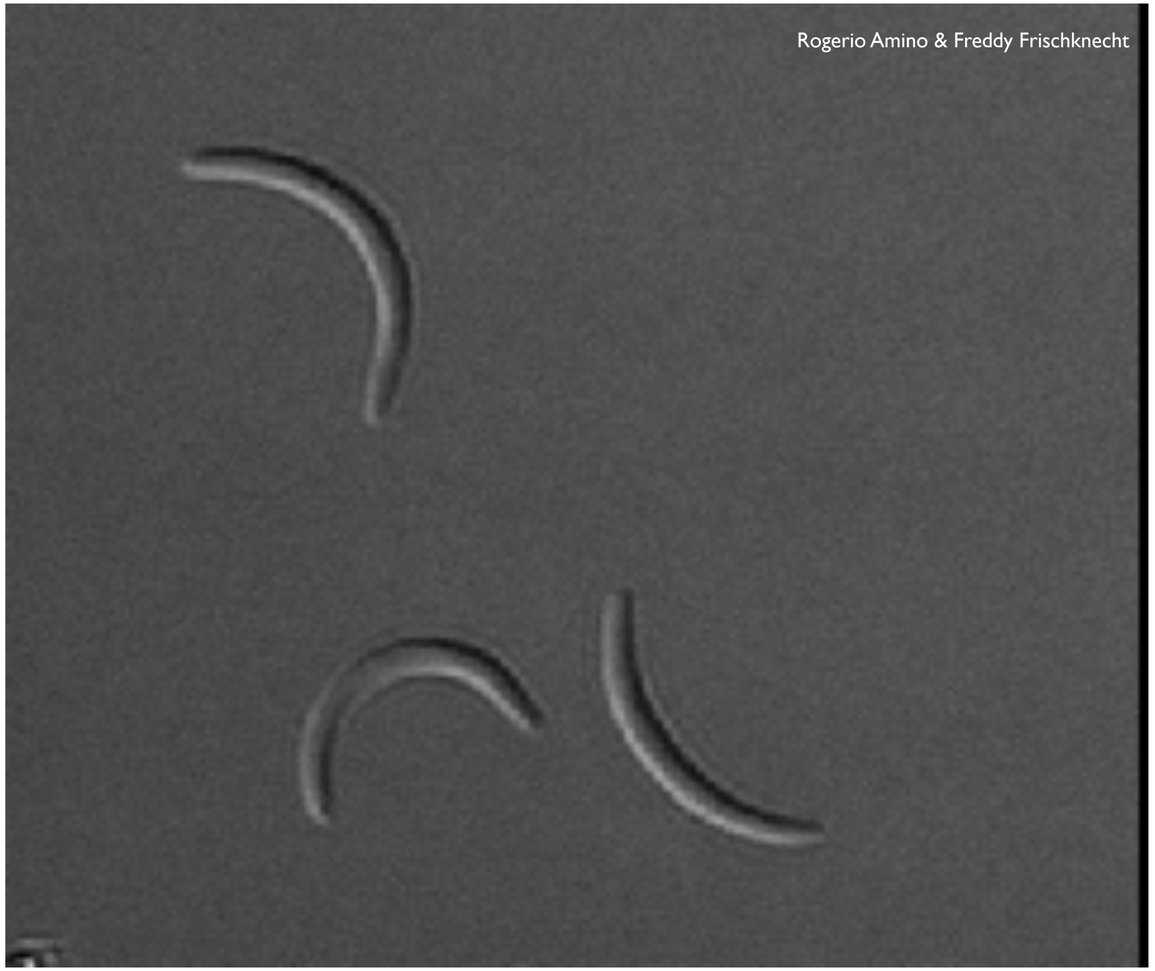


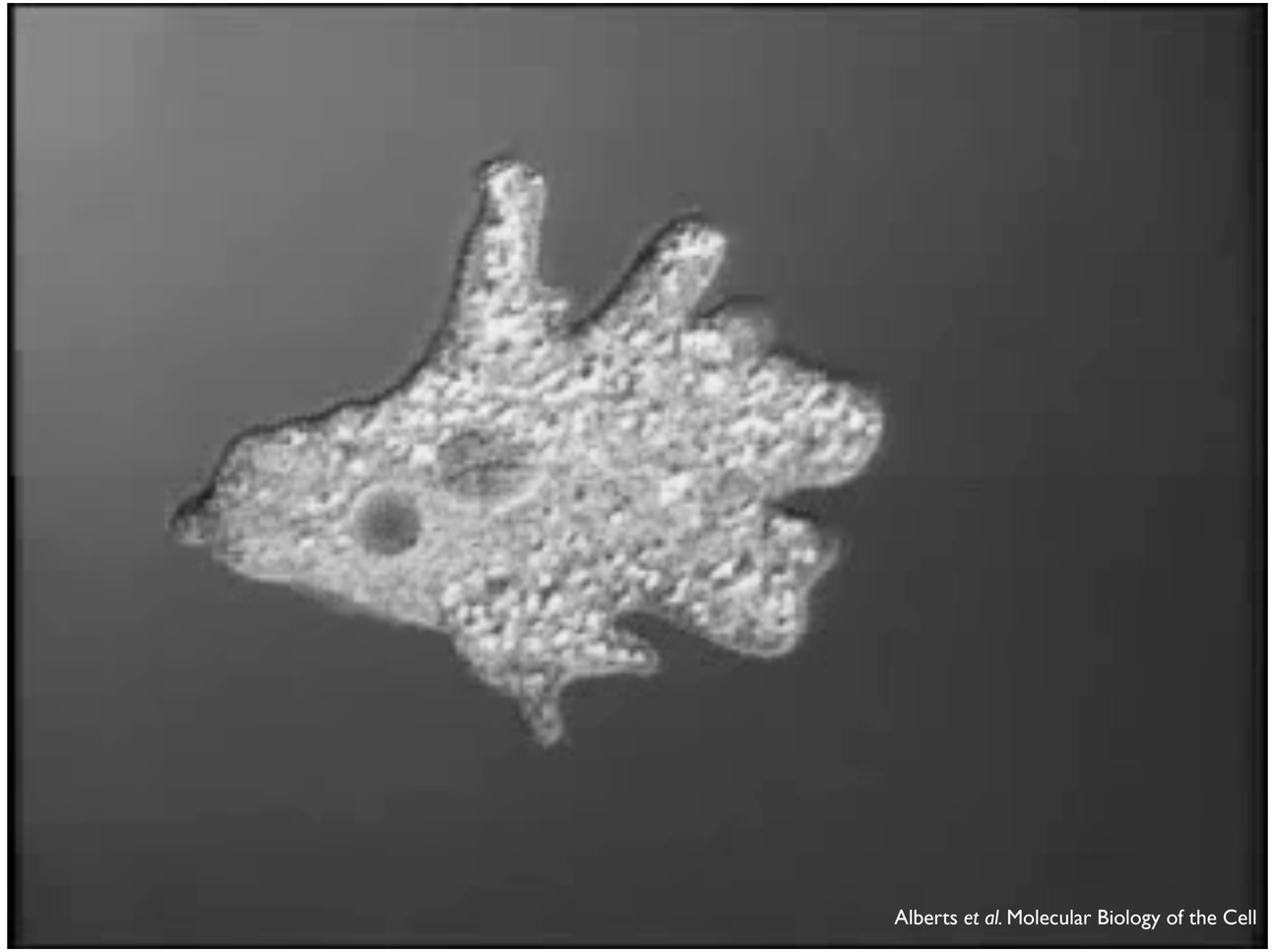
Ábris Bendes (2020)

APICOMPLEXAN GLIDING MOTILITY

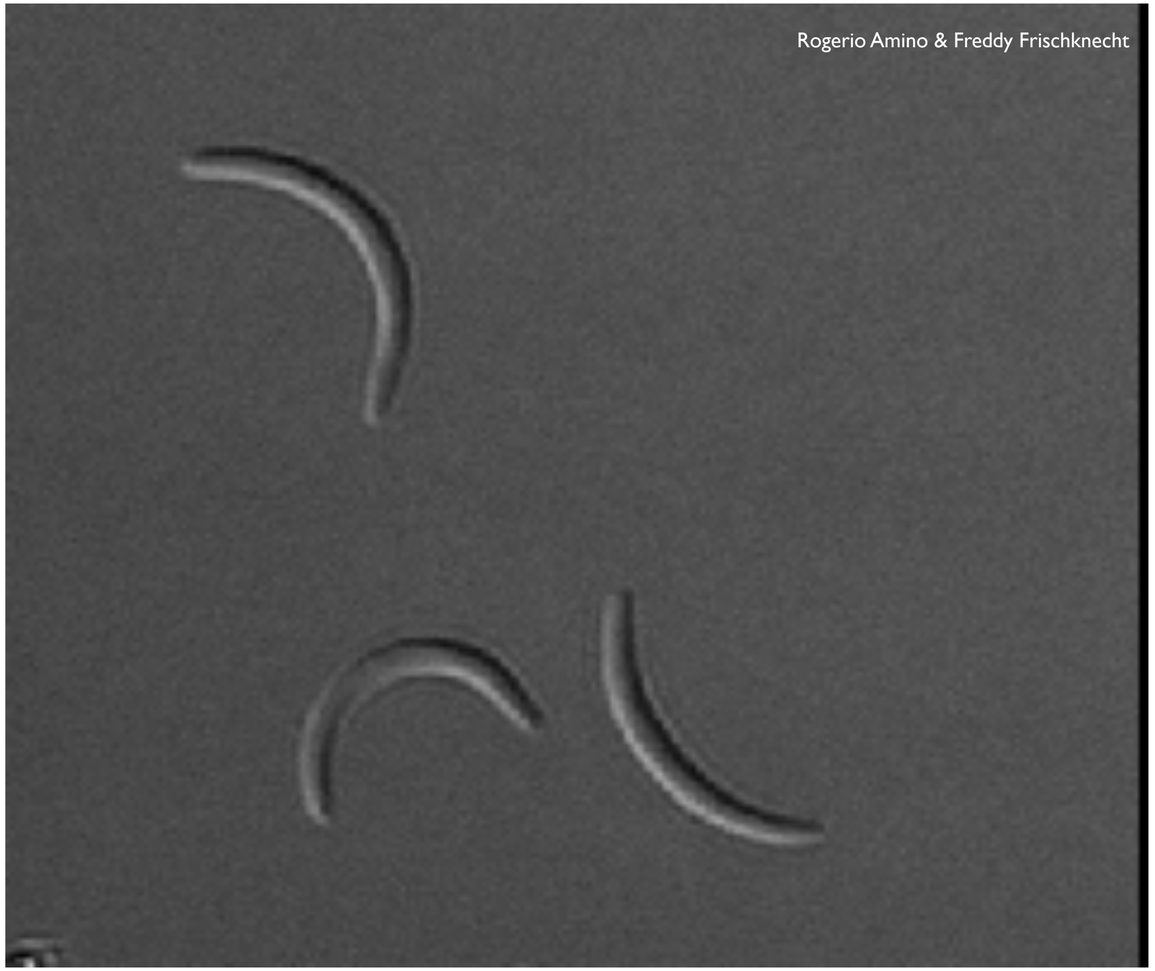


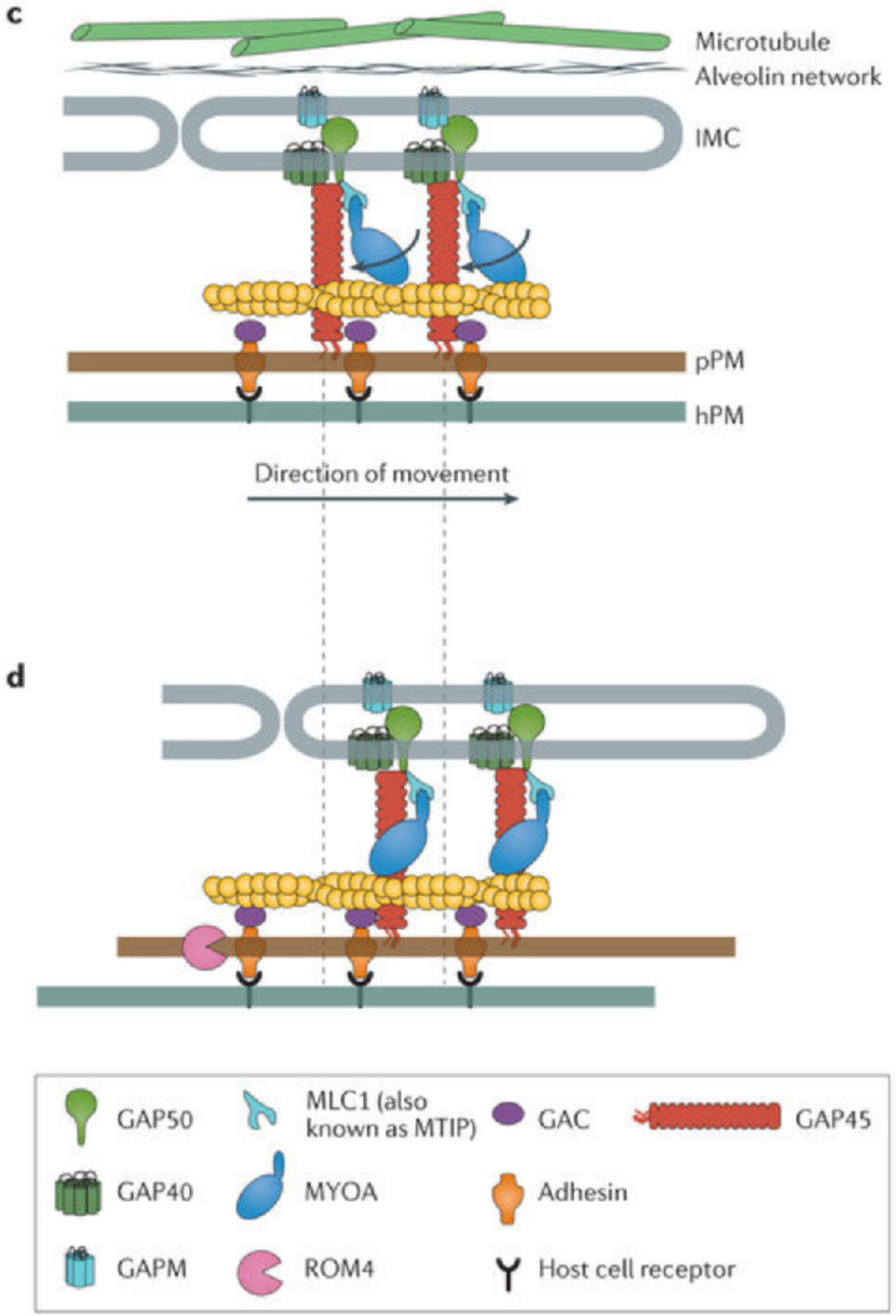
APICOMPLEXAN GLIDING MOTILITY





APICOMPLEXAN GLIDING MOTILITY





Frénal *et al.* (2017) *Nat Rev Microbiol*

AIMS OF OUR WORK

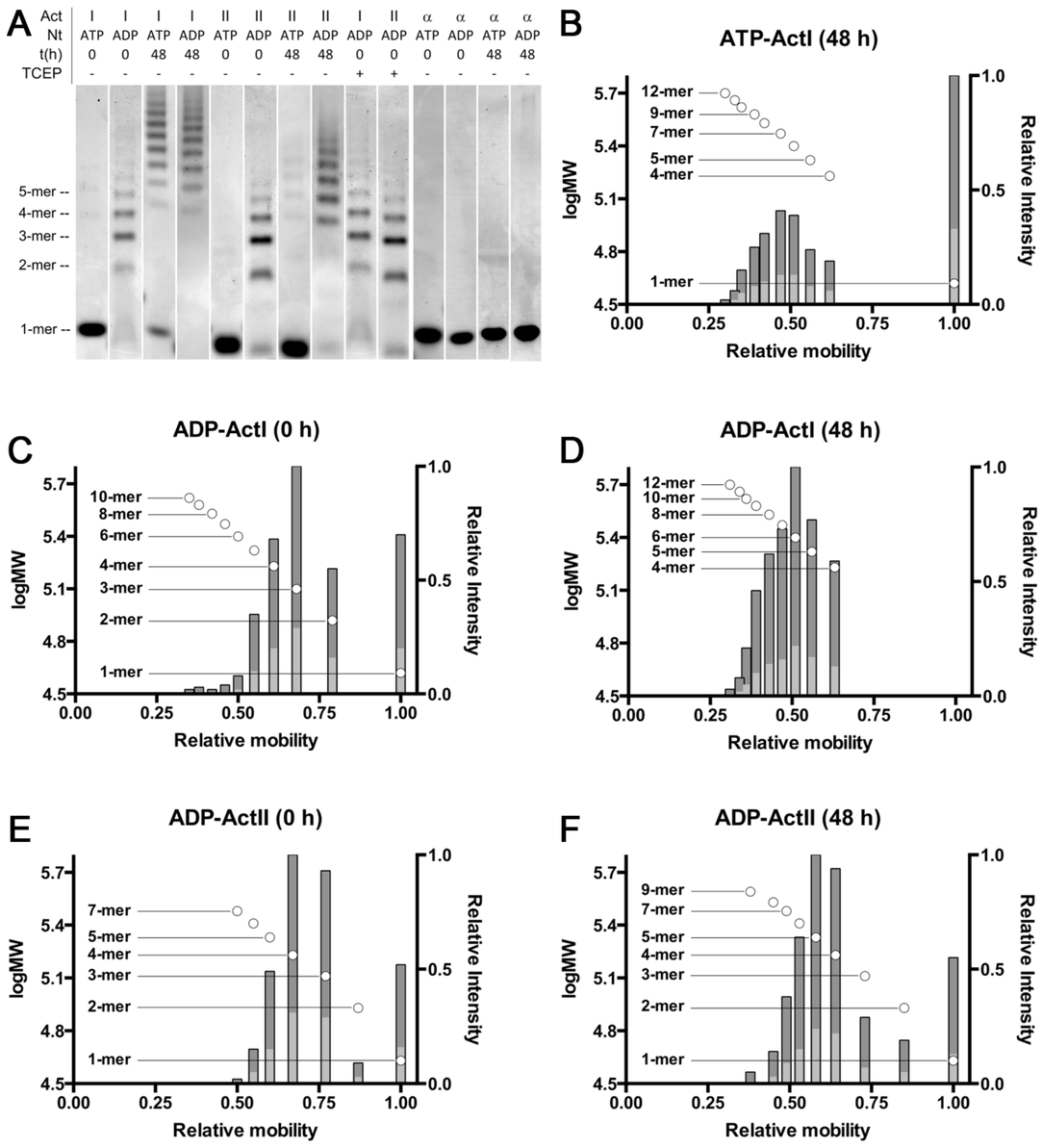
- to understand the ***mechanism of actin polymerization*** in the malaria parasite and ***how actin and its regulatory components have evolved*** in eukaryotes in general
- to draw a ***complete molecular picture*** of the entire glideosome
- to understand how ***force is generated and transmitted*** in gliding motility
- to evaluate the glideosome and actin regulatory components as ***drug and vaccine targets*** for fighting malaria

WHAT IS SO SPECIAL ABOUT PLASMODIUM ACTINS?

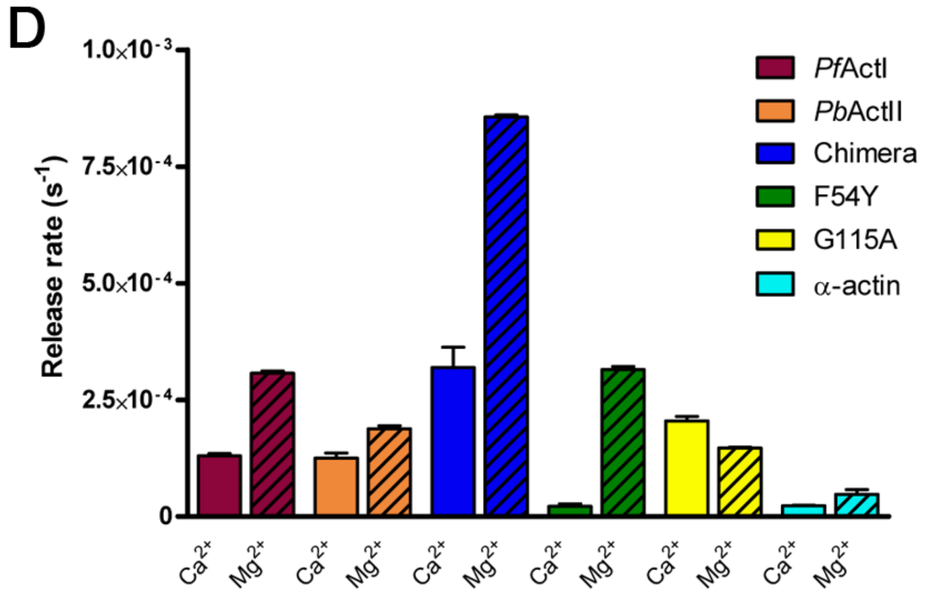
- low sequence identity between them and canonical actins - and also between the two isoforms (<80%)
- **form mainly very short filaments (~100 nm)**
- **ATP hydrolysis/phosphate release pathway and its link to polymerization seems different**
- polymerization not governed by ionic strength in the same way as canonical actins
- stable dimers are a significant species

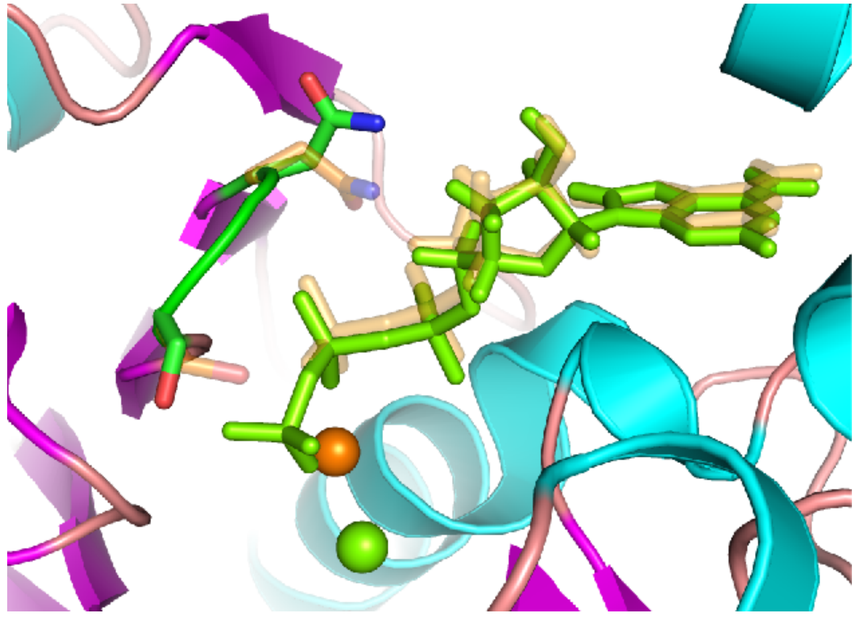
MECHANISM OF ACTIN POLYMERIZATION – IS IT DIFFERENT?

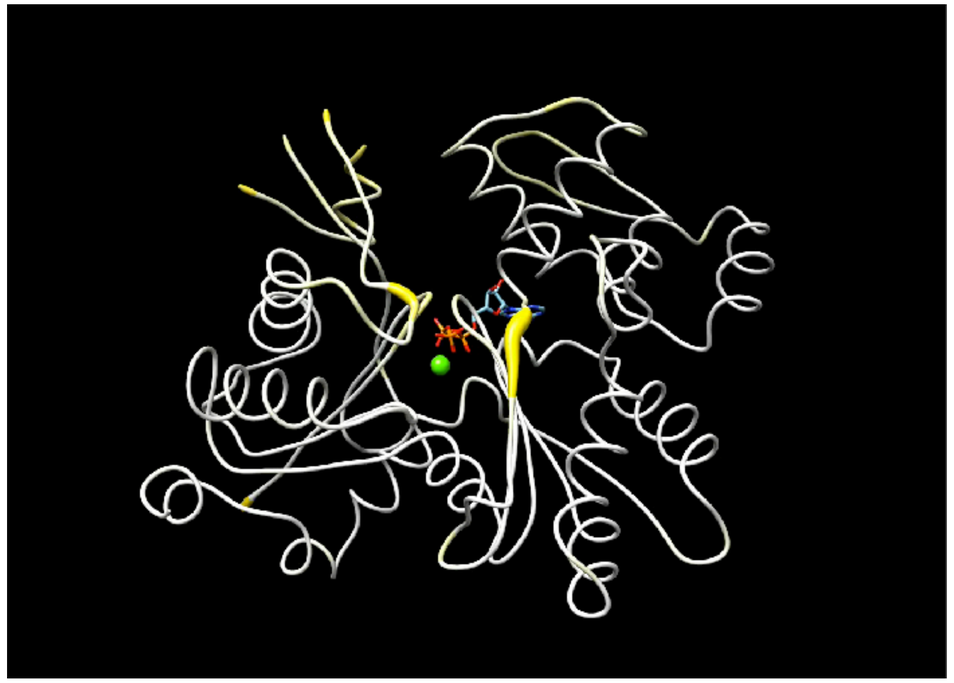
ATP HYDROLYSIS IN PLASMODIUM ACTIN

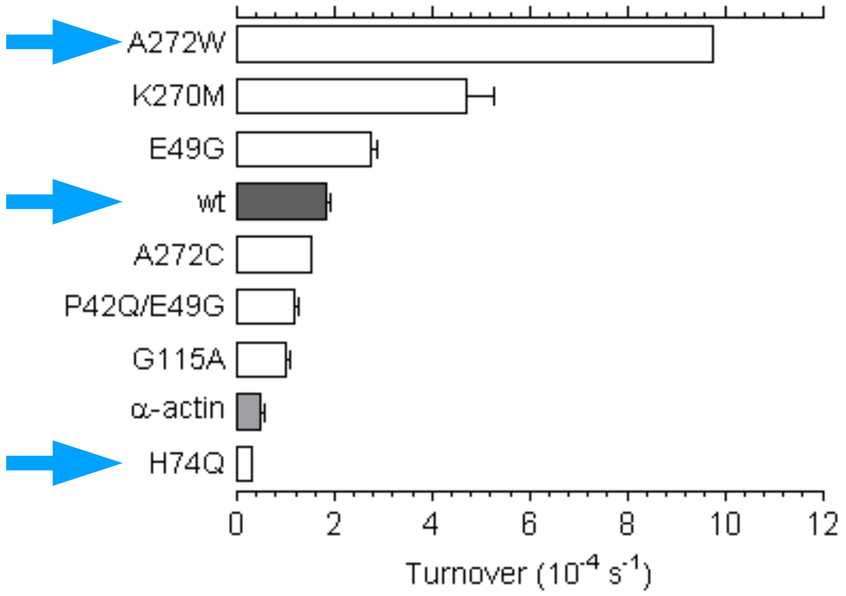


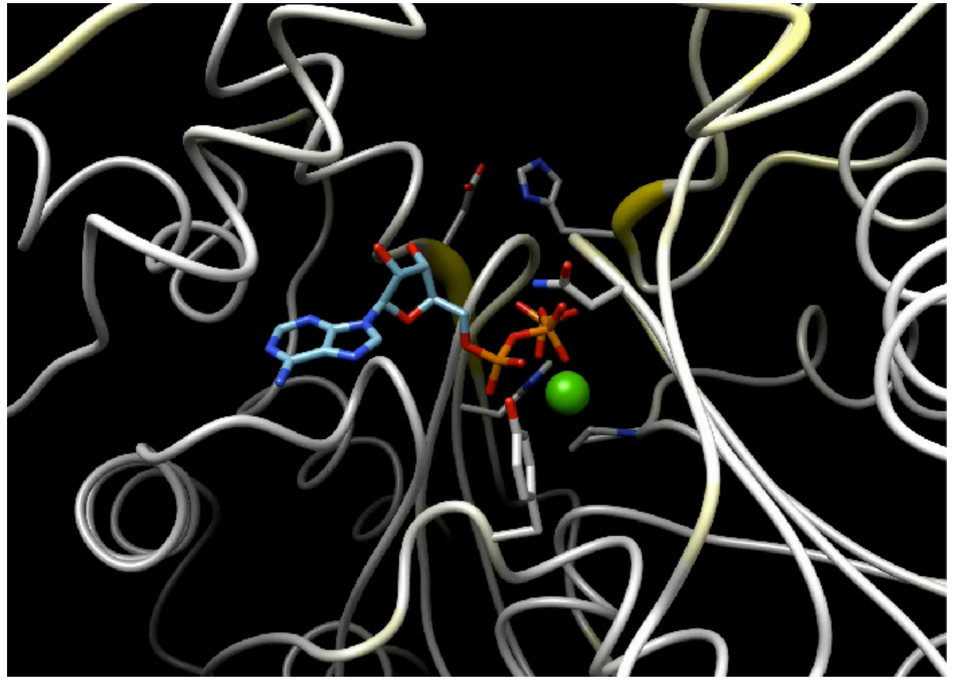
Vahokoski *et al.* (2014) *PLOS Pathog*

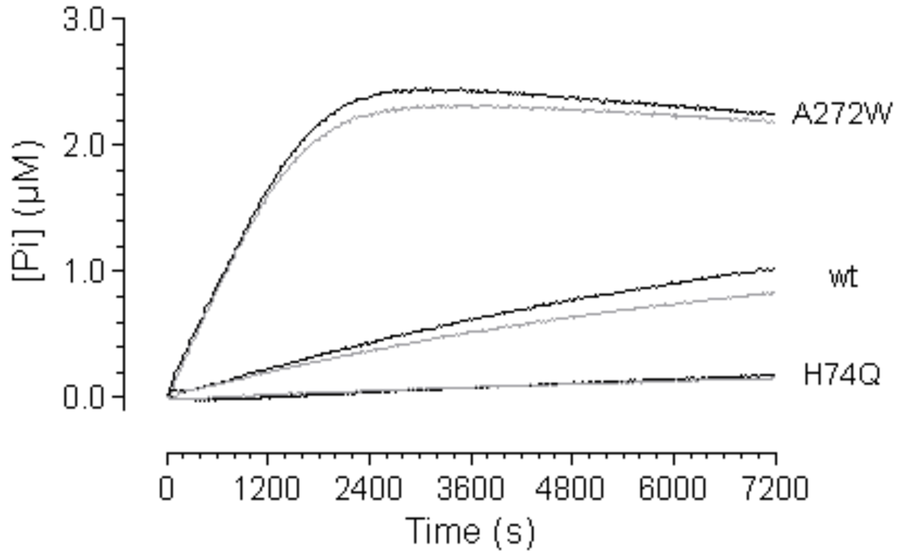




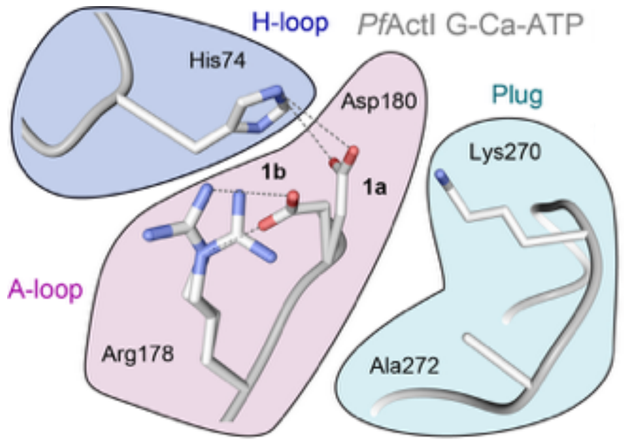



A272W
K270M
E49G
wt
A272C
P42Q/E49G
G115A
α-actin
H74Q
0
2
4
6
8
10
12
Turnover (10^{-4} s^{-1})

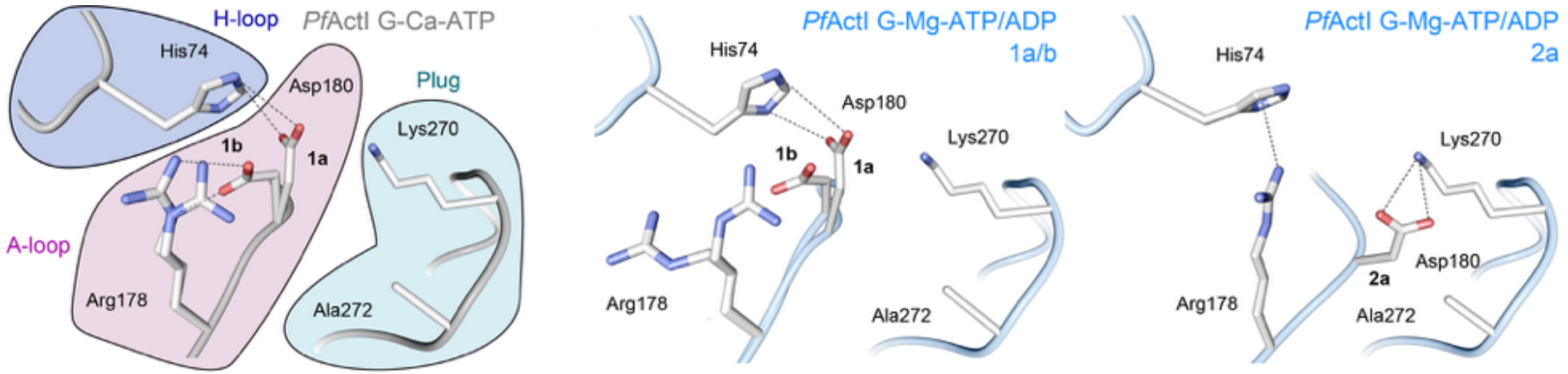



3.0
2.0
1.0
0.0
[Pi] (μM)
A272W
wt
H74Q
0
1200
2400
3600
4800
6000
7200
Time (s)

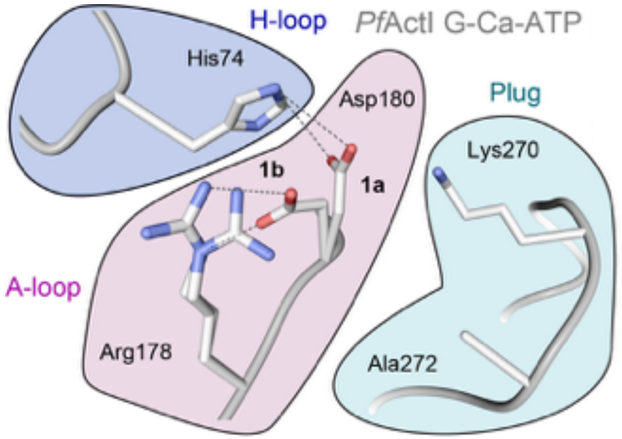
THE A-LOOP IS LINKED TO PHOSPHATE RELEASE

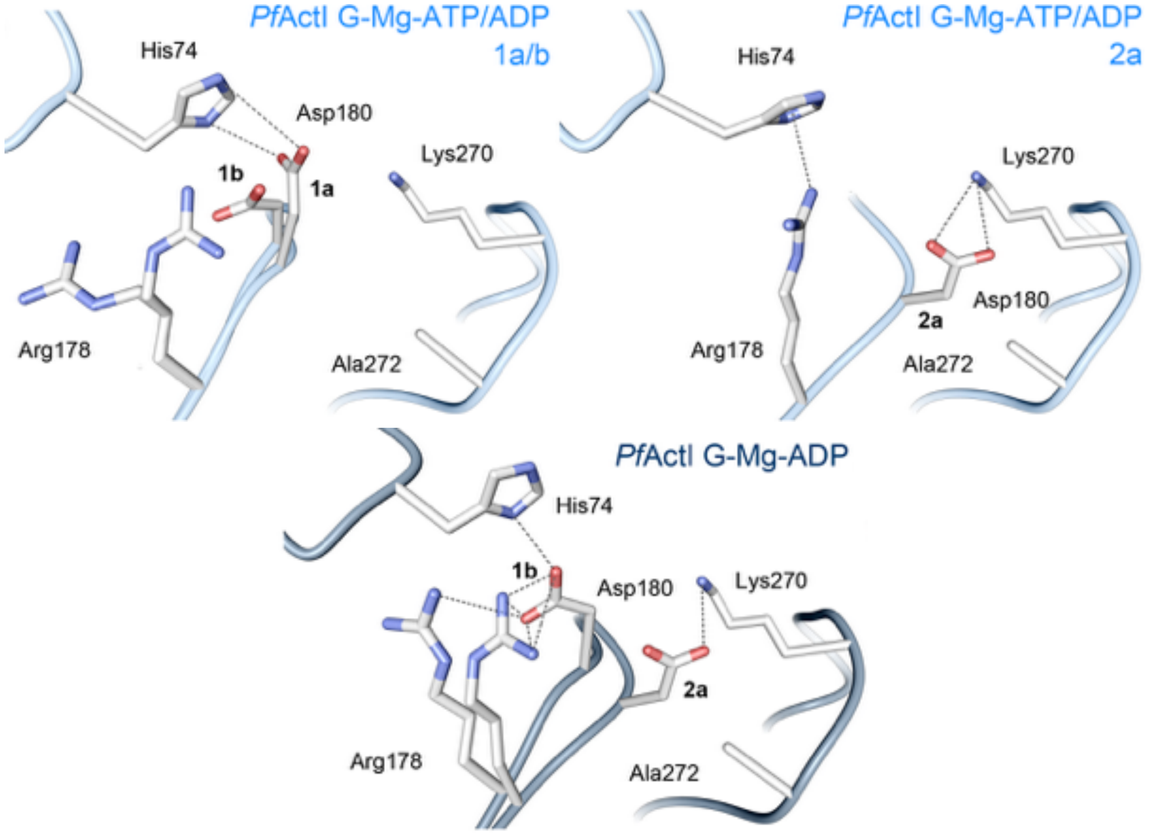


Kumpula *et al.* (2019) *PLOS Biol*

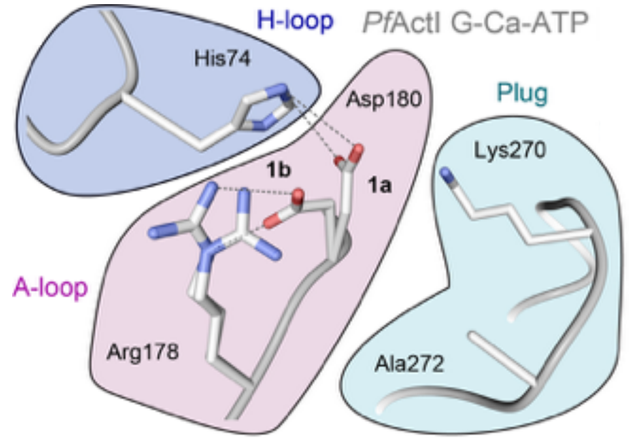


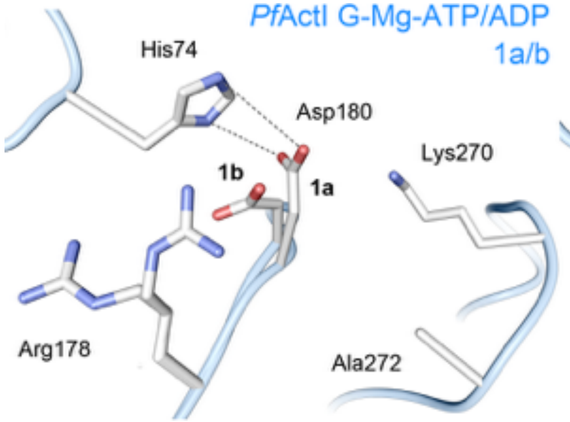
Kumpula *et al.* (2019) *PLOS Biol*

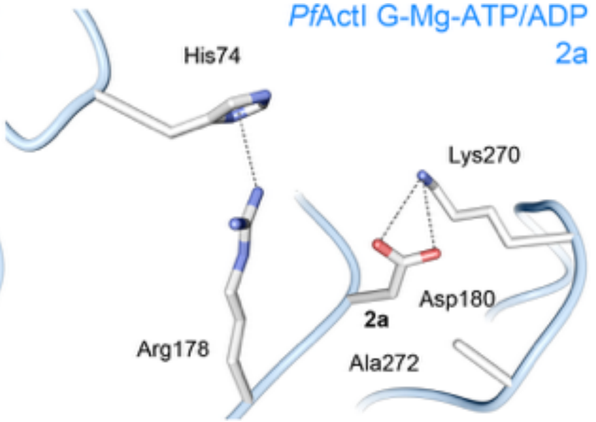


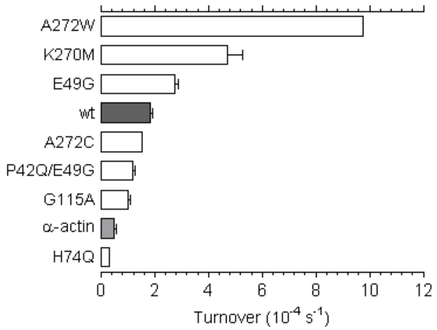


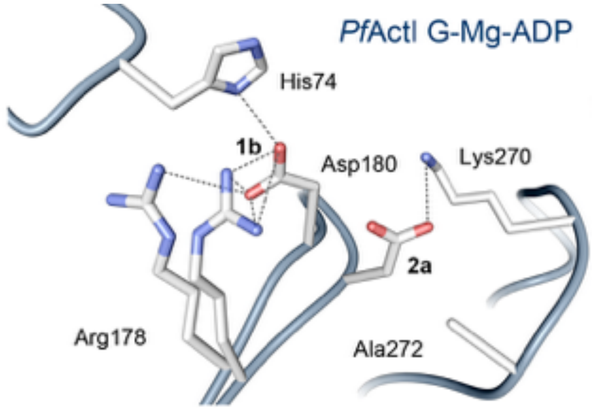
Kumpula *et al.* (2019) *PLOS Biol*



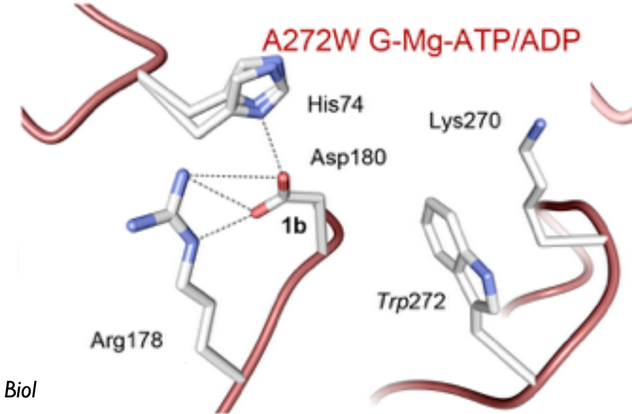


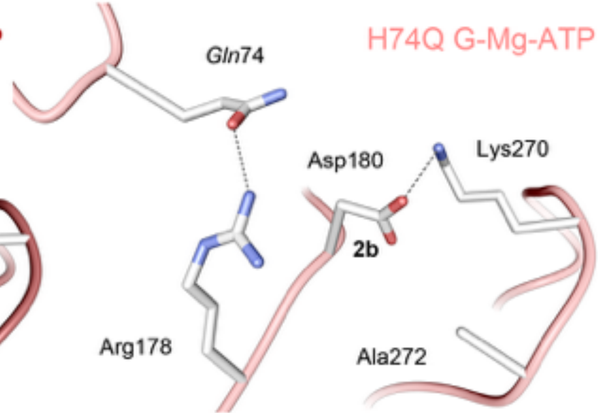




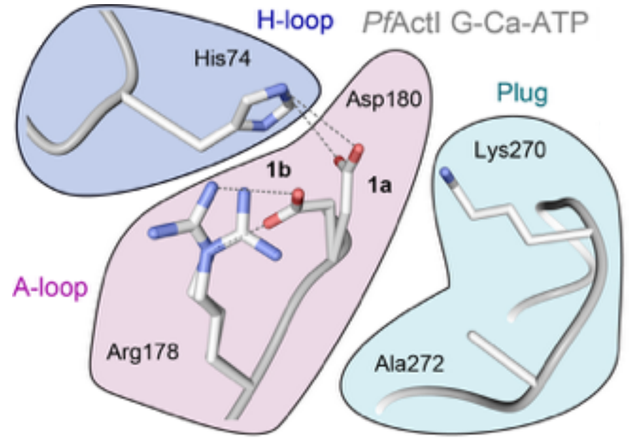


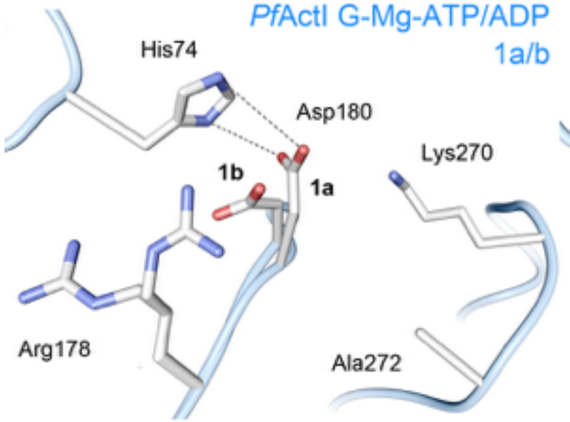
hyperactive **inactive**

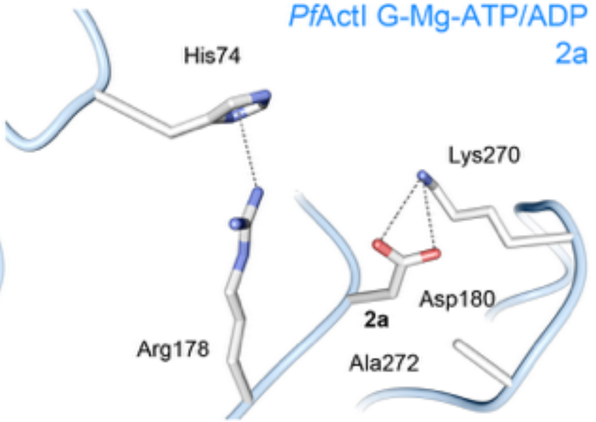


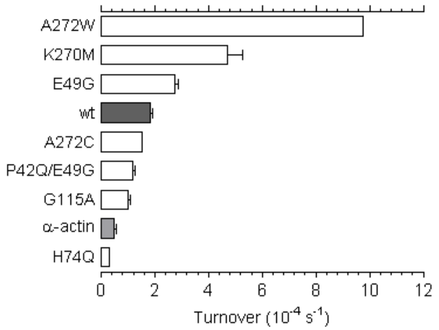


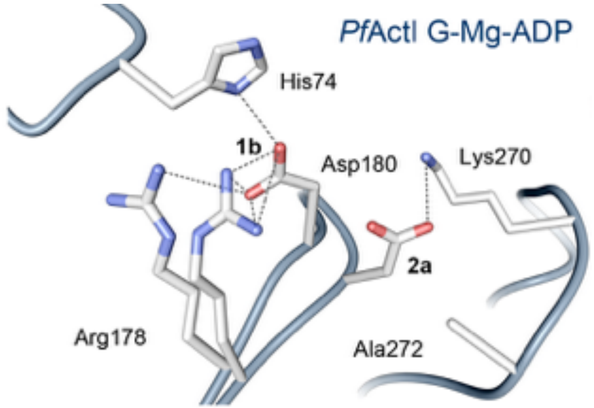
Kumpula *et al.* (2019) *PLOS Biol*



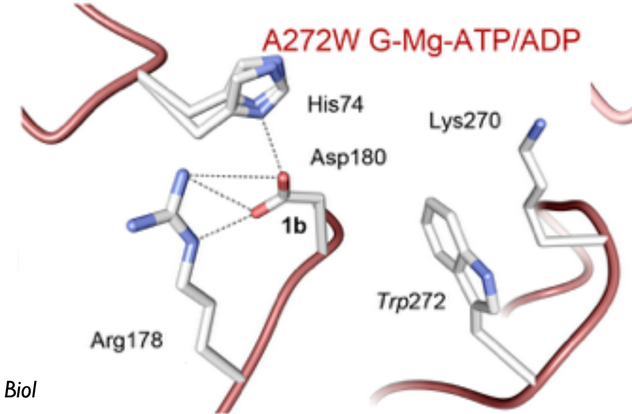


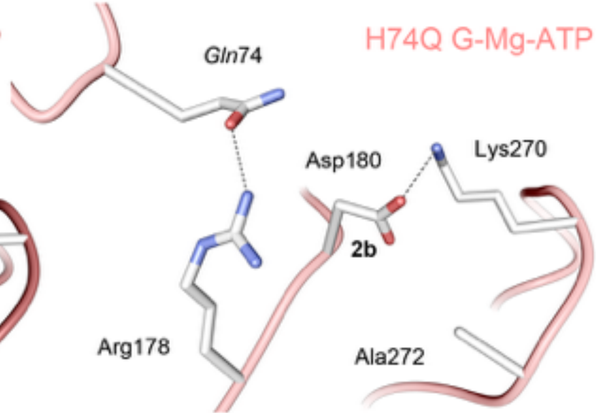


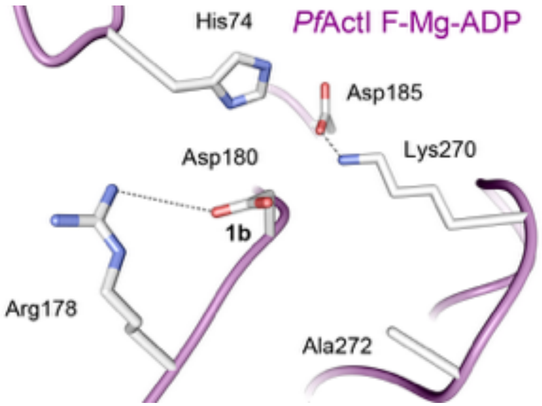




hyperactive **inactive**

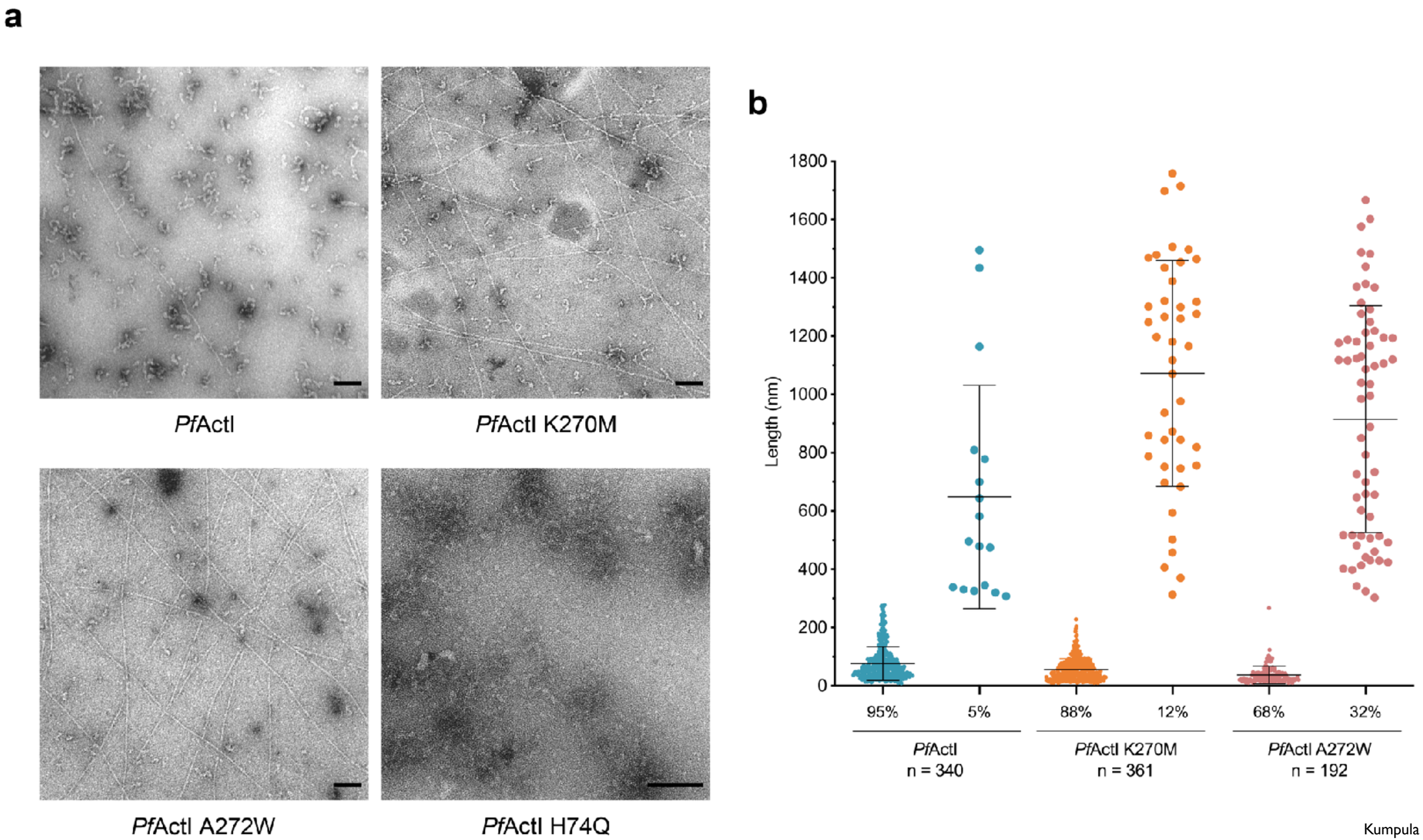






Kumpula *et al.* (2019) *PLOS Biol*

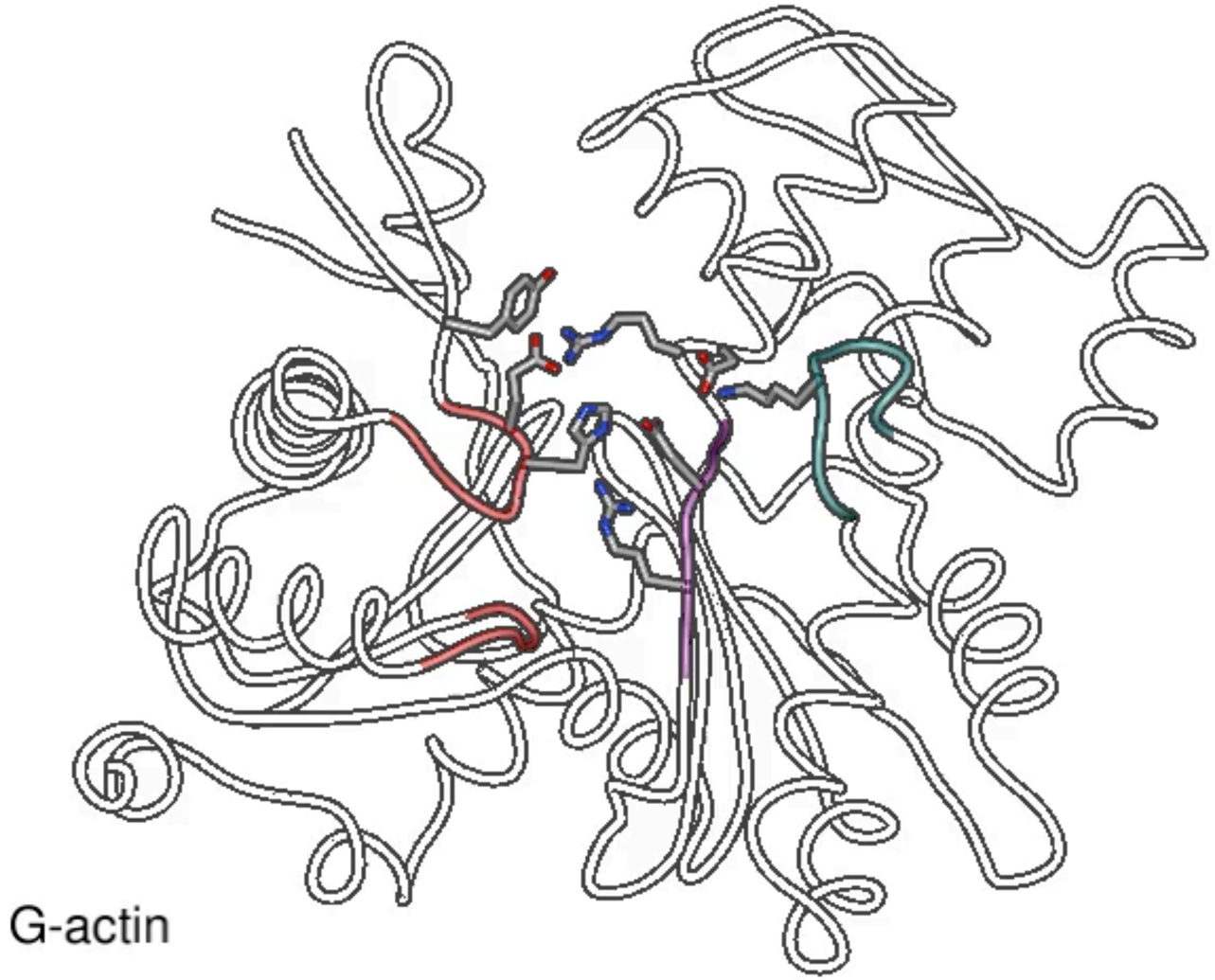
THE A-LOOP GOVERNS ALSO FILAMENT LENGTH



Kumpula *et al.* (2019) *PLOS Biol*

OVERVIEW OF THE STRUCTURAL TRANSITIONS FROM G TO F FORM

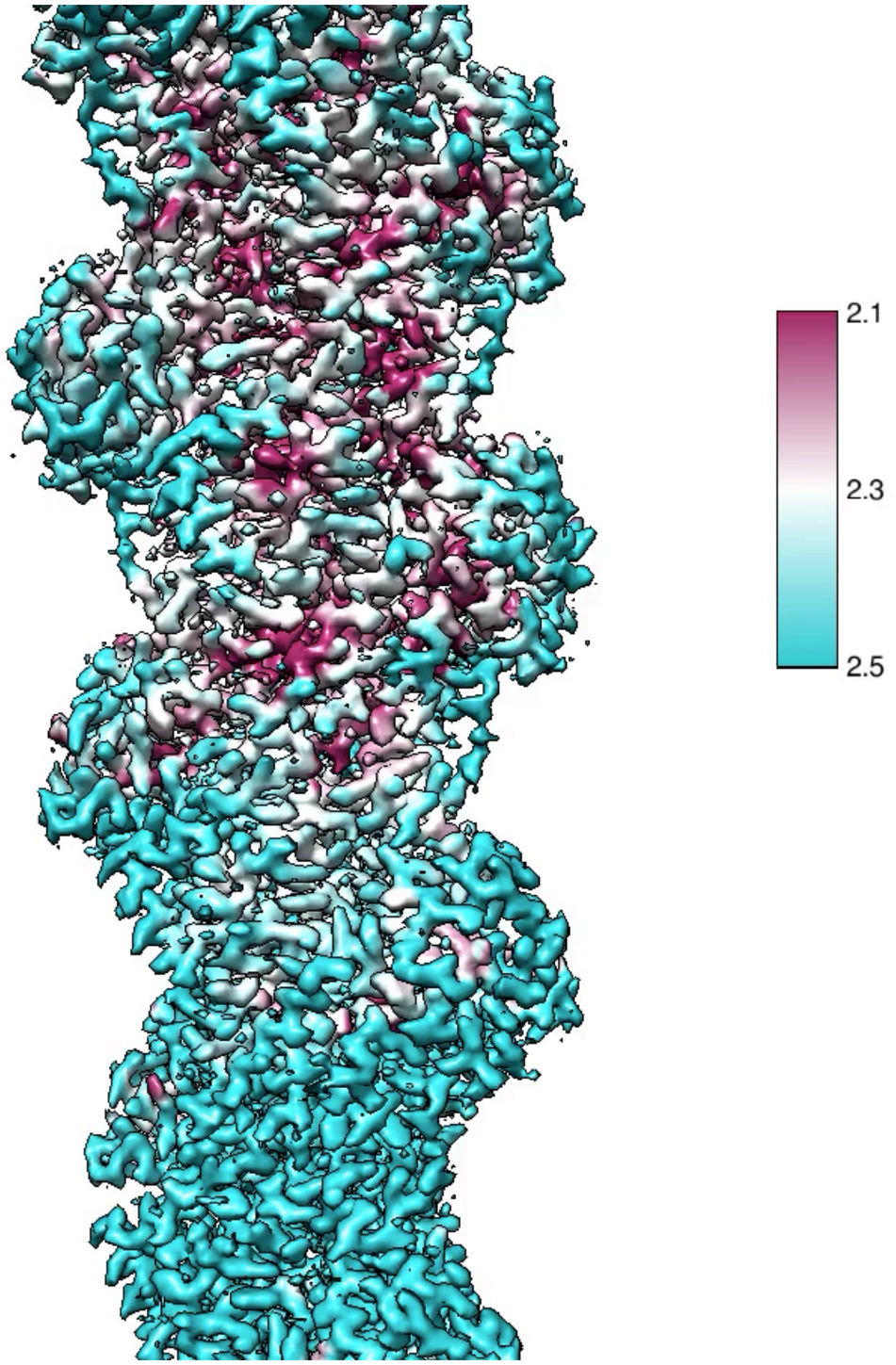
- the movement of the A-loop has not been seen in canonical actins
- it may still happen but not be as favorable due to two small amino acid differences:
  - Lys270 -> Met in canonical actins
  - His74 is methylated in muscle actins
- thus, the A-loop mobility increases the rate of phosphate release and decreases filament stability



Kumpula *et al.* (2019) *PLOS Biol*

THAT WE LEARNED FROM PHOTONS

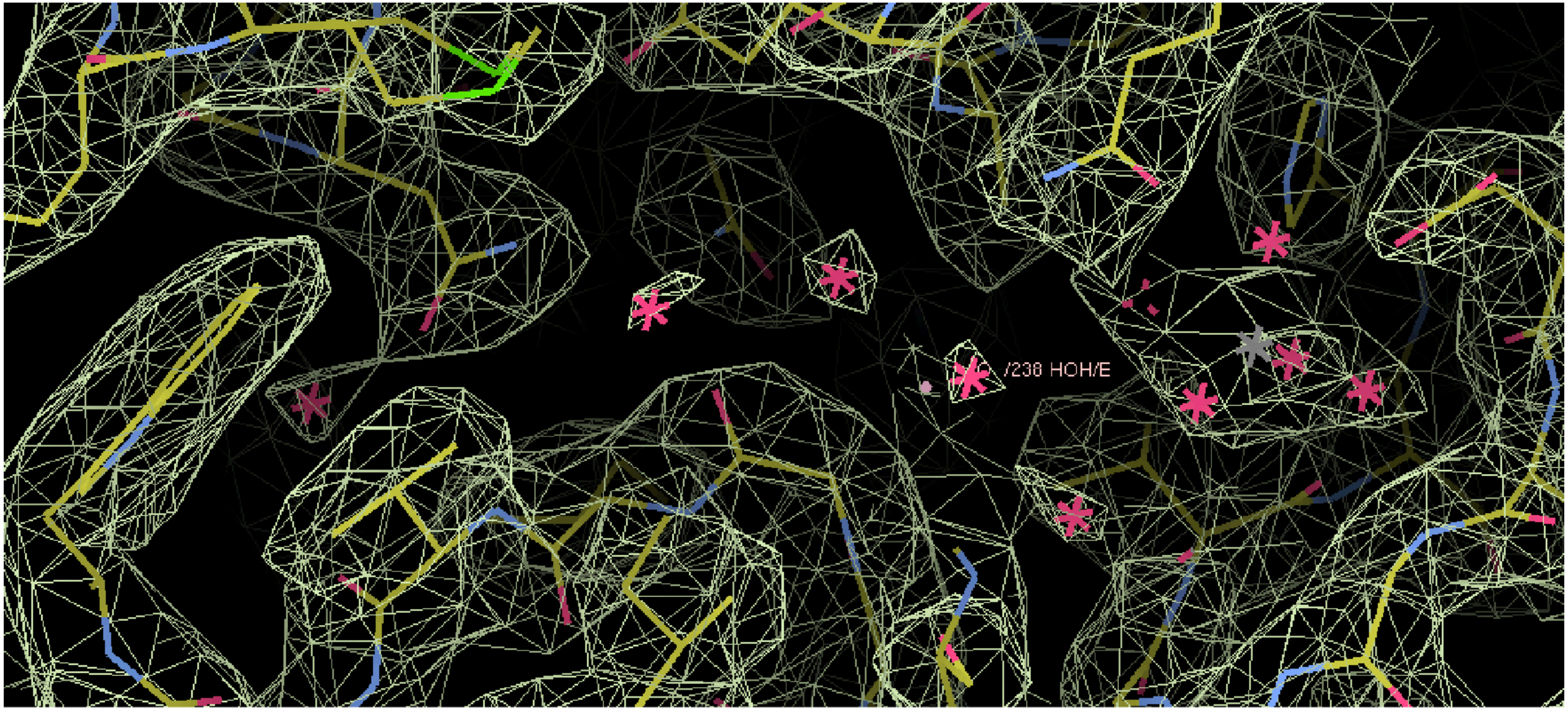
WHAT ABOUT ELECTRONS?



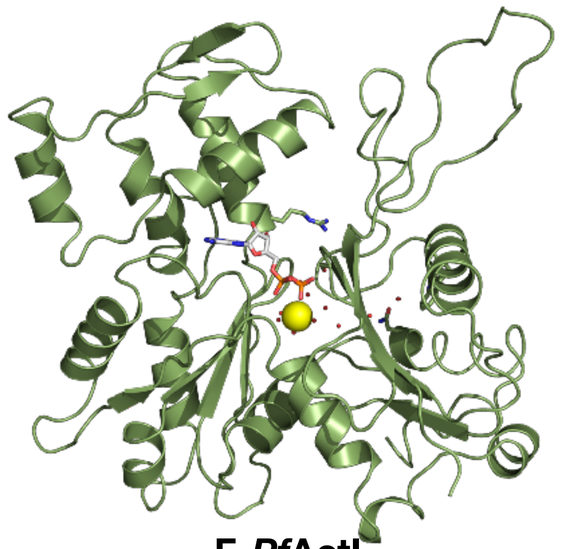
Vahokoski *et al.* (2020) *bioRxiv*

WHAT CAN WE SEE IN ACTIN?

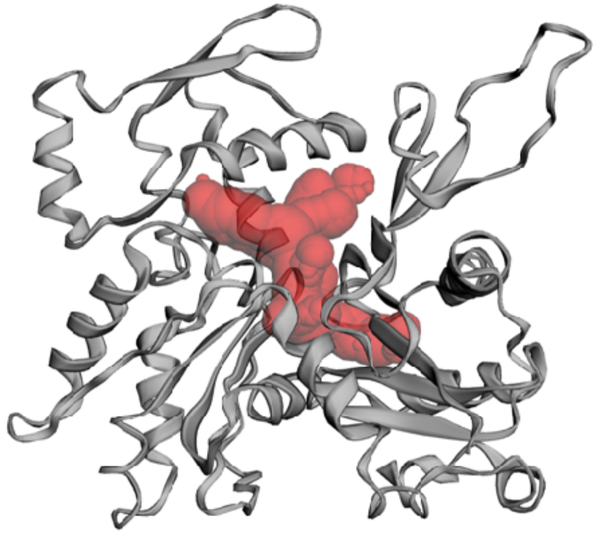
HOW FAR CAN WE PUSH THE RESOLUTION?



ALTERNATIVE PATH OF THE LEAVING PO4?

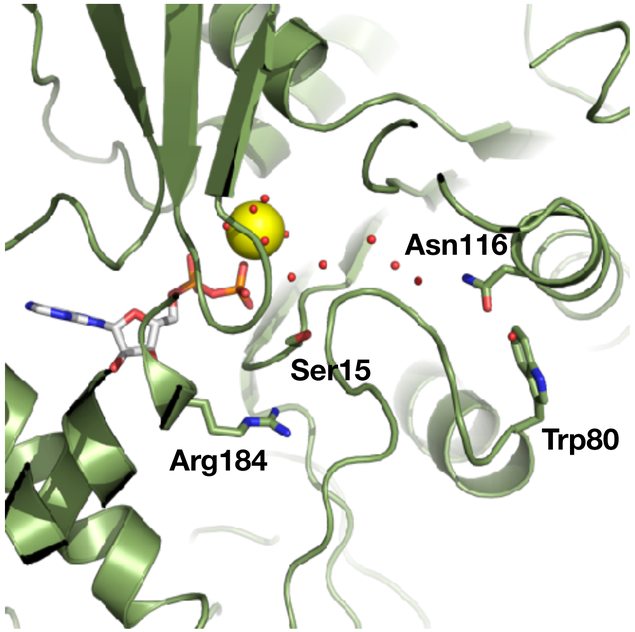


F-*Pf*ActI

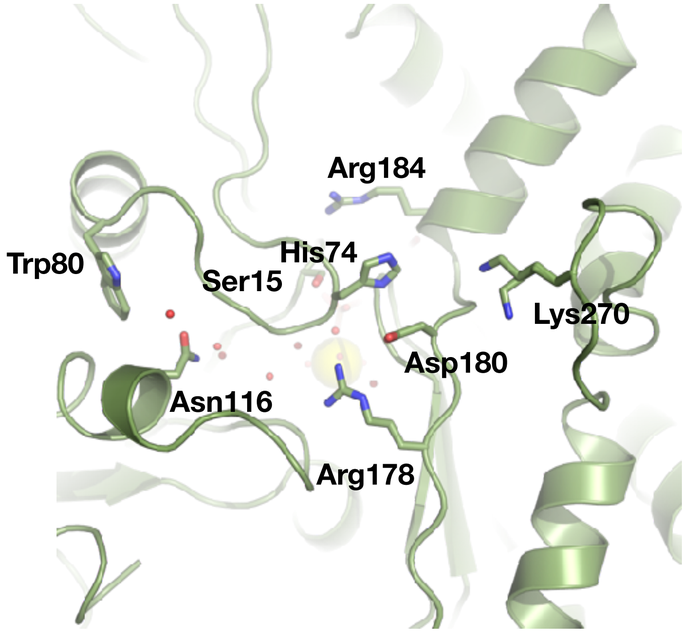


F-*Pf*ActI

G-*Pf*ActI



upside-down view



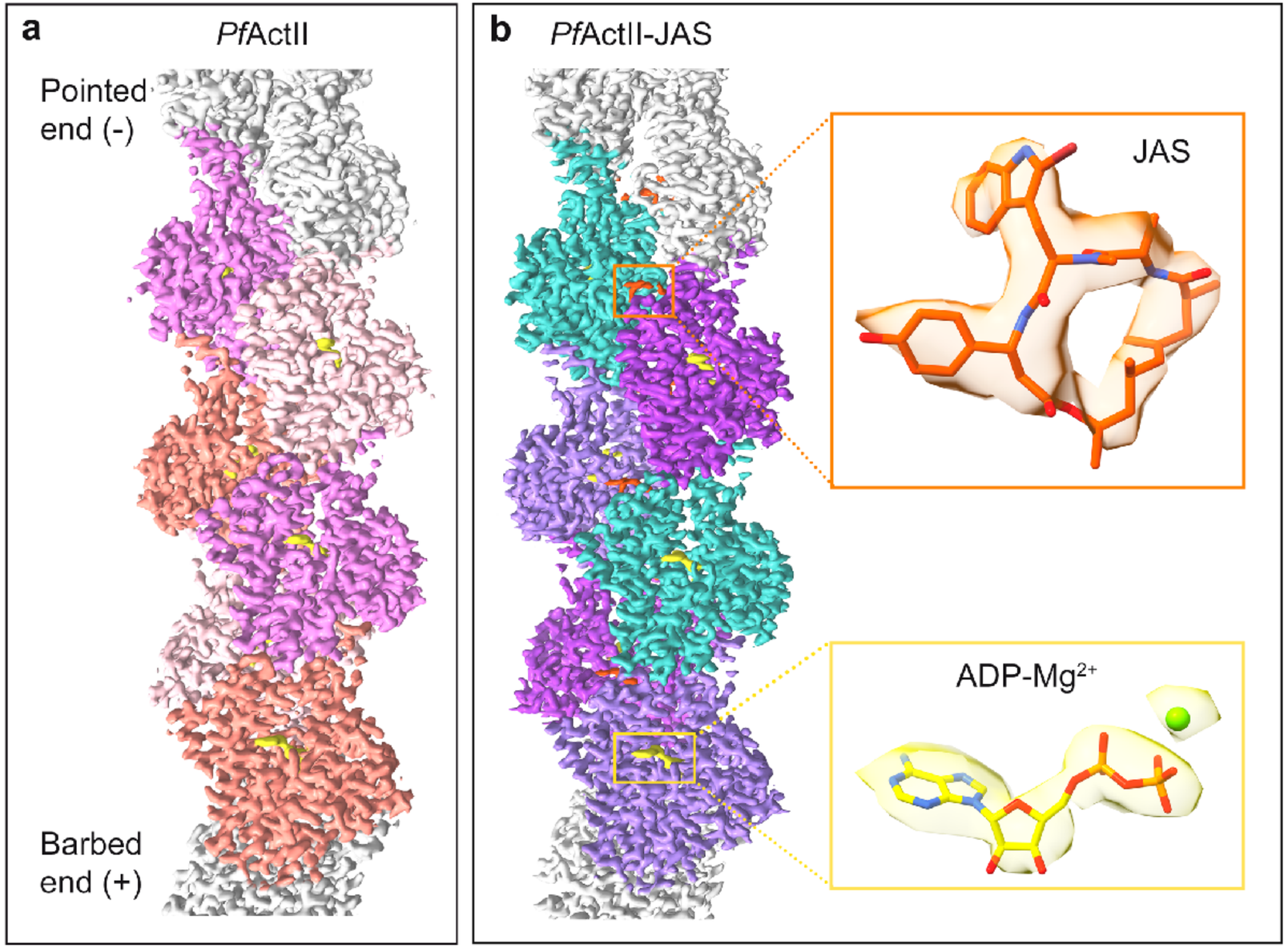
A-loop perspective

SUMMARY

- PfActI polymerization follows the classical nucleation-elongation pathway
- a high fragmentation rate leads to short filament length
- subtle amino acid changes and the lack of methylation of His74 allow the A-loop to act as a fast switch between stable and unstable filament conditions (enforcing and weakening the ID-OD connections)
- a similar mechanism of fragmentation is possible in canonical actins, albeit at a lower frequency
- a channel gated by Asn116 and Trp80 could be the path for the leaving phosphate

NEXT:

- actin II
- mutagenesis in the active site and the possible phosphate leaving routes
- different nucleotide states
- trap leaving phosphate?
- even higher resolution
- look into the catalytic mechanism in more detail



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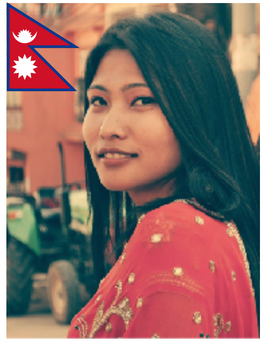

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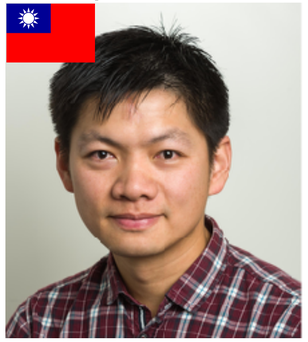

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THANK YOU! QUESTIONS???