

# Journal Club

黃振 11/21/2023

**Article**

# Structure of the native myosin filament in the relaxed cardiac sarcomere

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<https://doi.org/10.1038/s41586-023-06690-5>

Received: 3 April 2023

Accepted: 28 September 2023

Published online: 01 November 2023

Open access

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The thick filament is a key component of sarcomeres, the basic units of striated muscle<sup>1</sup>. Alterations in thick filament proteins are associated with familial hypertrophic cardiomyopathy and other heart and muscle diseases<sup>2</sup>. Despite the central importance

**Article**

# Cryo-EM structure of the human cardiac myosin filament

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<https://doi.org/10.1038/s41586-023-06691-4>

Received: 22 March 2023

Accepted: 28 September 2023

Published online: 01 November 2023

 Check for updates

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Pumping of the heart is powered by filaments of the motor protein myosin that pull on actin filaments to generate cardiac contraction. In addition to myosin, the filaments contain cardiac myosin-binding protein C (cMyBP-C), which modulates contractility in response to physiological stimuli, and titin, which functions as a scaffold for

# Background

# Overall structure of muscle

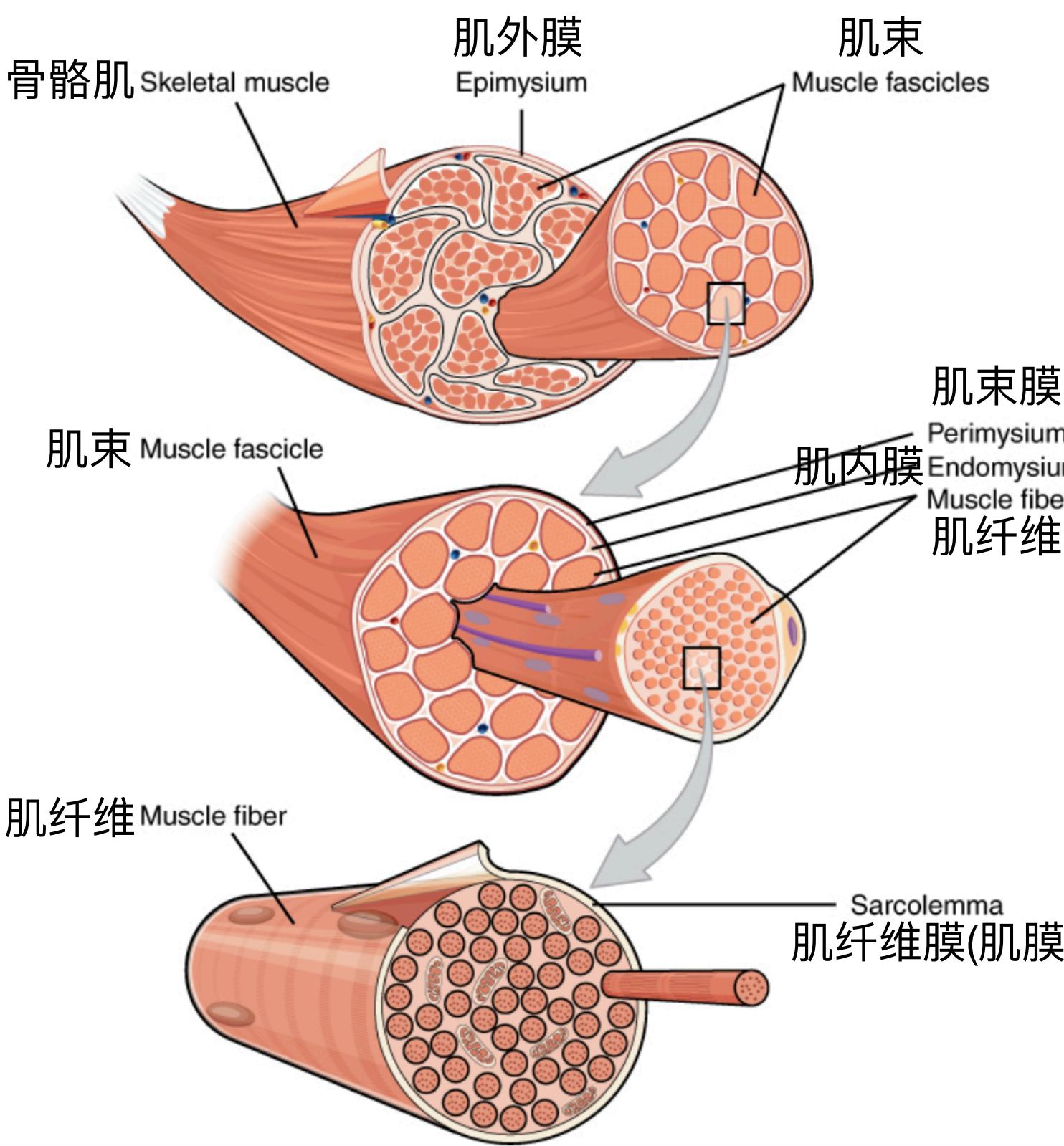


Figure 1. The Three Connective Tissue Layers. Bundles of muscle fibers, called fascicles, are covered by the perimysium. Muscle fibers are covered by the endomysium.

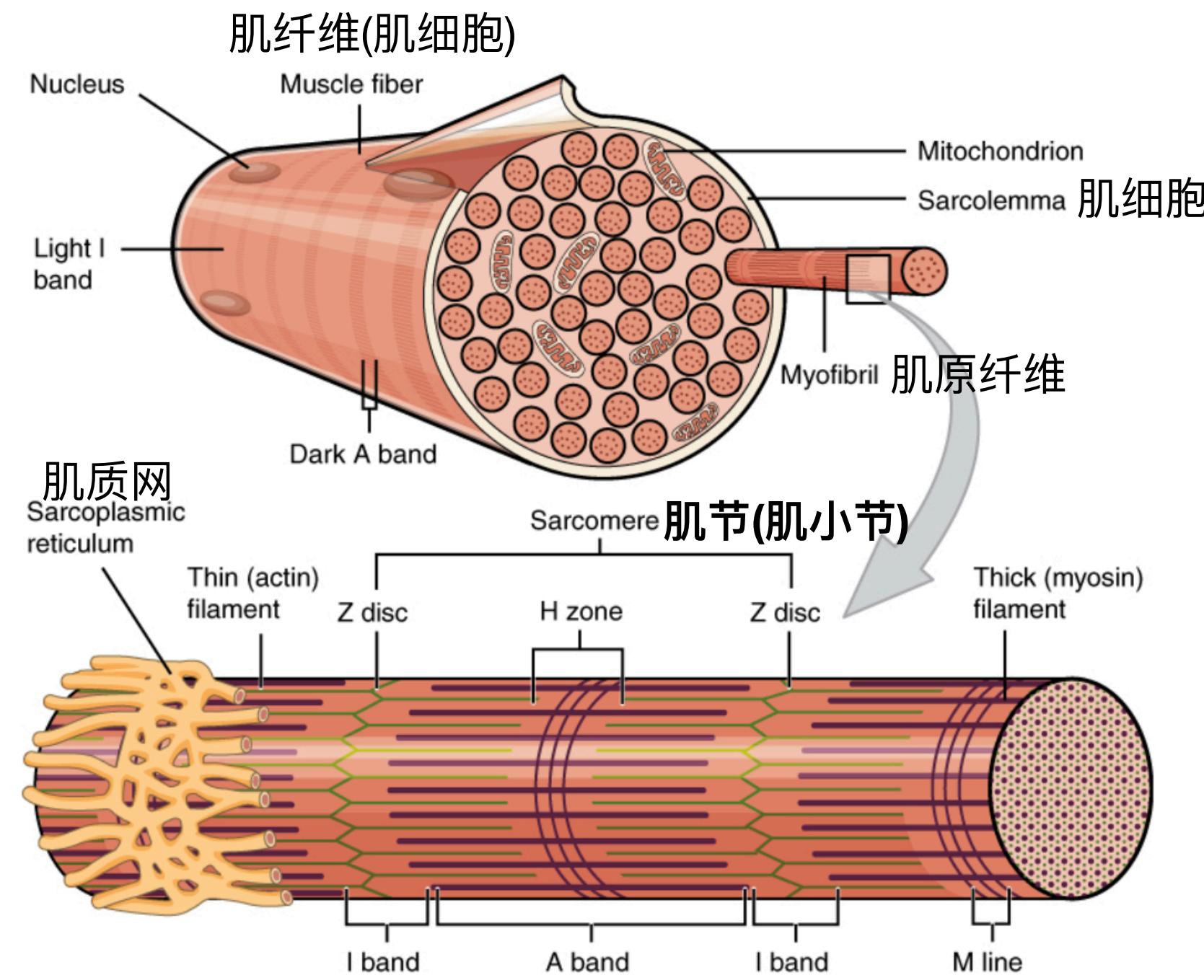


Figure 2. Muscle Fiber. A skeletal muscle fiber is surrounded by a plasma membrane called the sarcolemma, which contains sarcoplasm, the cytoplasm of muscle cells. A muscle fiber is composed of many fibrils, which give the cell its striated appearance.

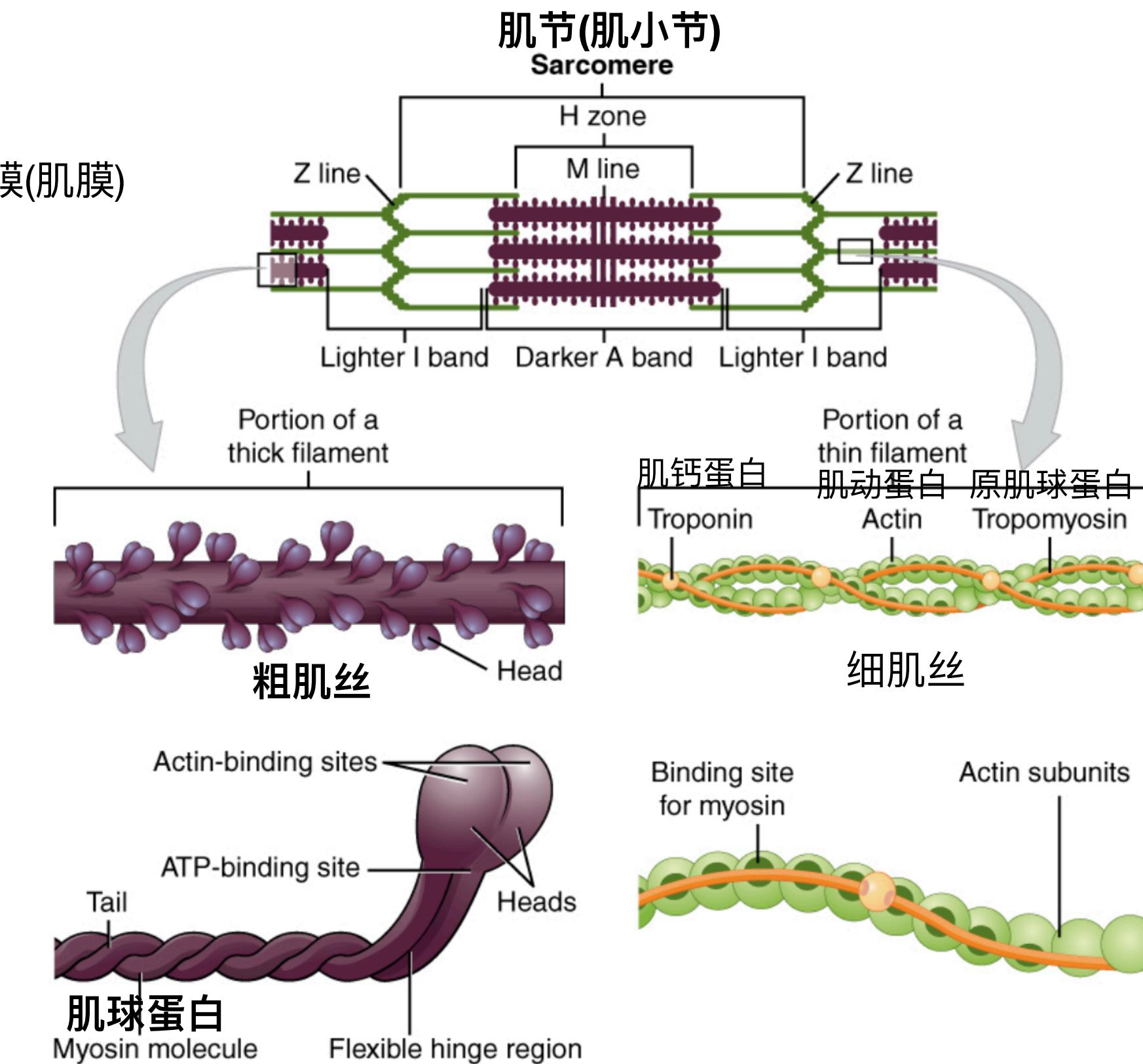
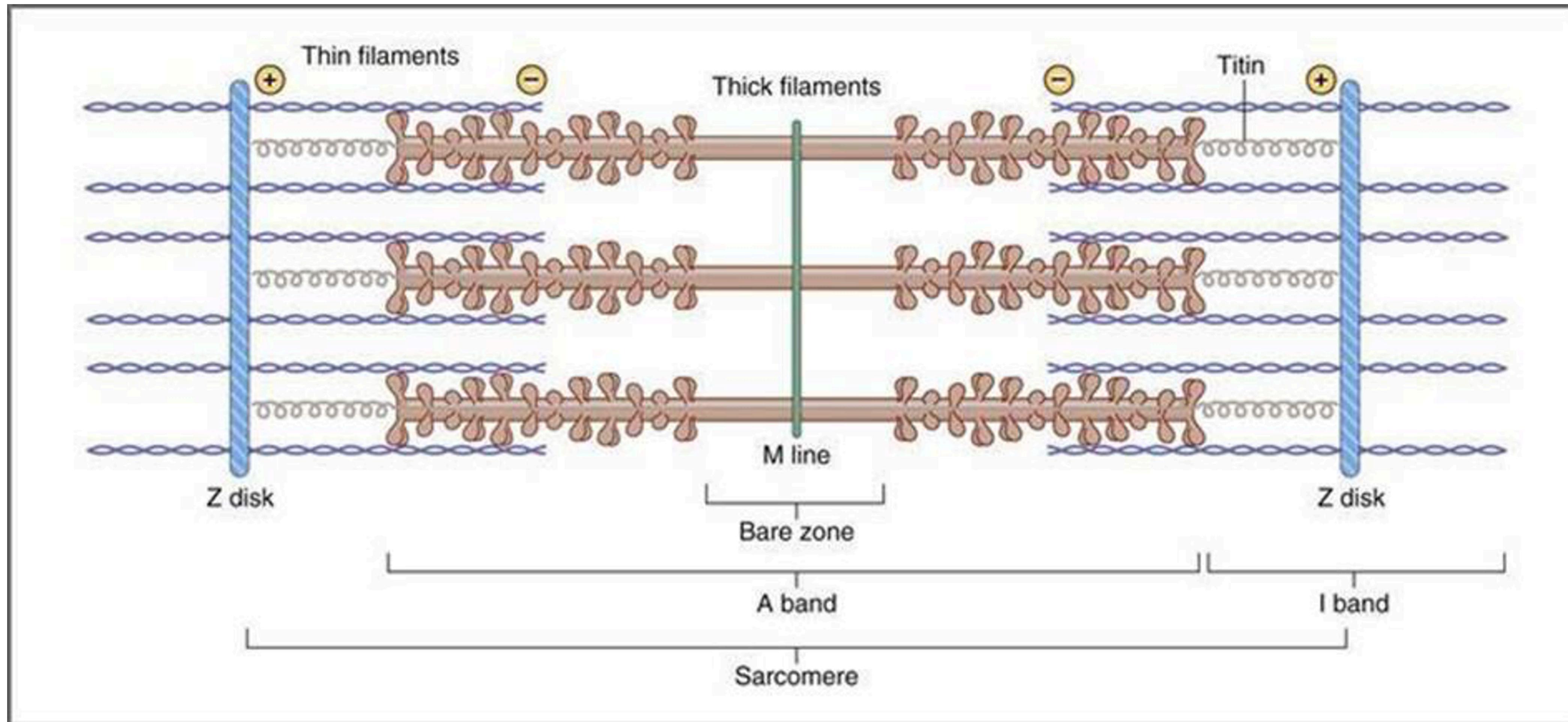


Figure 3. The Sarcomere. The sarcomere, the region from one Z-line to the next Z-line, is the functional unit of a skeletal muscle fiber.

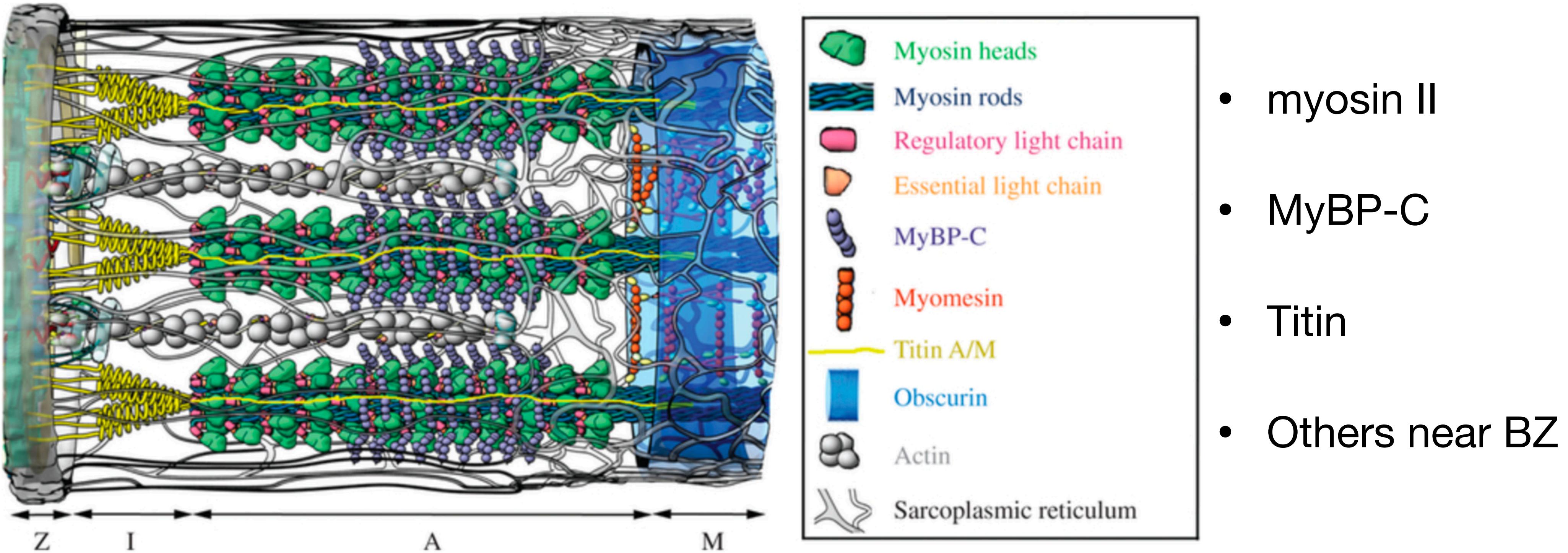
# Sarcomere and Thick filaments



*Thick filament and bare zone. SKELETAL MUSCLE - Cellular Physiology - Physiology 5th Ed.*

# Thick Filament Protein Network

Figure 1



Schematic representation of a half sarcomere depicting the position of the Z-disk, I-band, A-band, and M-band. Myosin thick filaments and associated proteins are shown in color including myosin heads (green), myosin rods (petrol), regulatory light chains (magenta), essential light chains (peach), MyBP-C (purple), myomesin (orange), titin (yellow), and obscurin (light blue), while actin thin filaments and the surrounding sarcoplasmic reticulum are shown in different shades of grey; the structure of the half sarcomere was generated by [e-heart.org](http://e-heart.org) bearing minor modifications.

# **Questions to be addressed**

# Remaining doubts from previous studies

- The detailed structures of **thick filaments in vertebrates**:
  - perfectly helical as in invertebrates?
  - how about titin and MyBP-C?
- How proteins, esp. **myosin, titin and MyBP-C**, carry out their highly regulated functions within the confined space of the thick filament:
  - previous predicted model?
  - how those observations map onto the thick filaments *in situ*?

# Main results

# Main results

- The 3D structure of thick filament *in situ* in vertebrates;
- Molecular details of the complex organization of myosin heads, tails, titin and MyBP-C;
- Visualizing critical interactions between myosin, titin and MyBP-C.

# Methods

# Methods

cryo-ET:

- Demembranated mouse cardiac myofibrils;
- Mavacamten, 一种治疗心肌肥大病dilated cardiomyopathy (DCM) or hypertrophic cardiomyopathy (HCM) 的药物，可以稳定myosin的OFF/ relaxed松弛结构

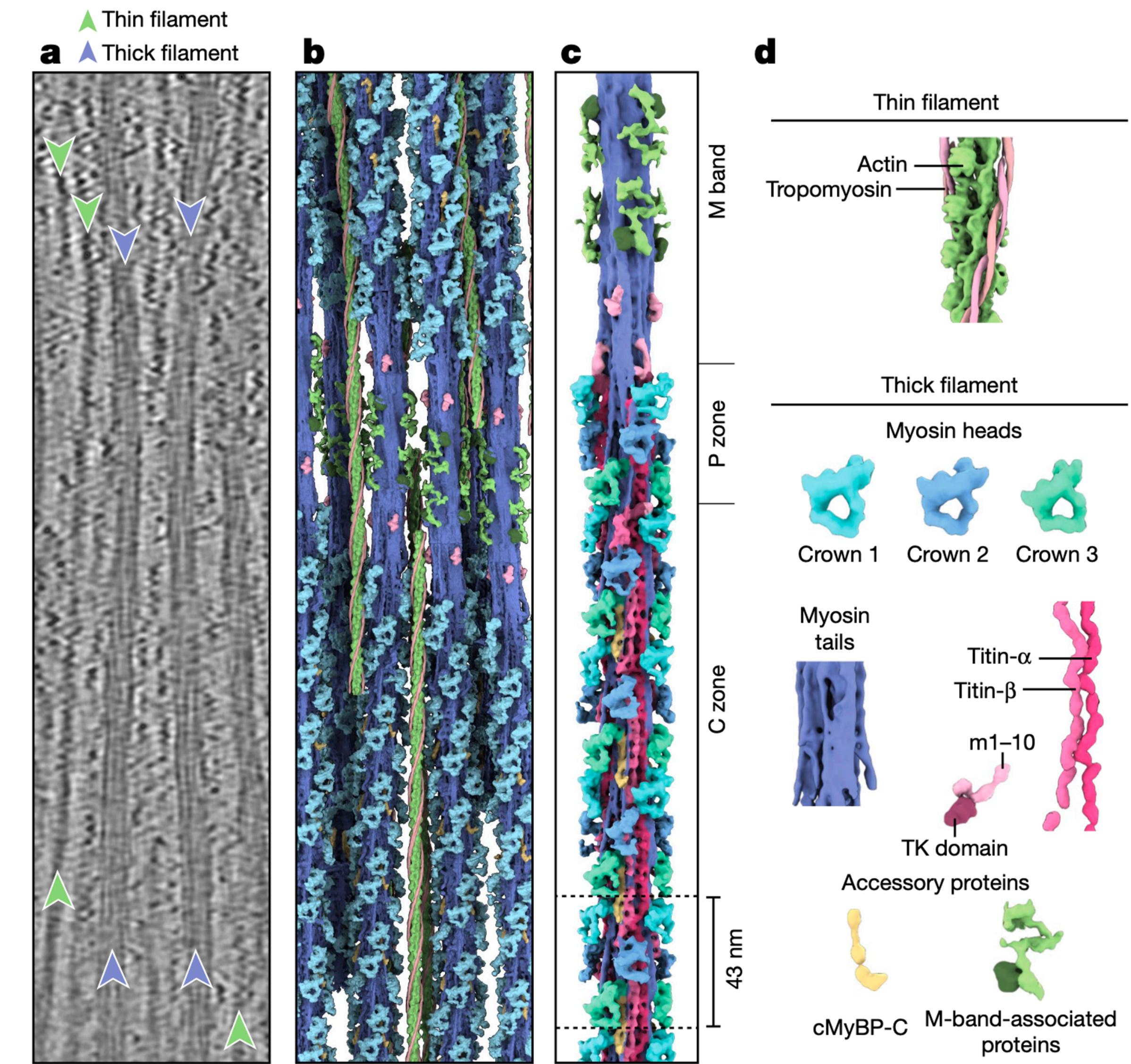
cryo-EM:

- Human cardiac ventricular tissue;
- Mavacamten

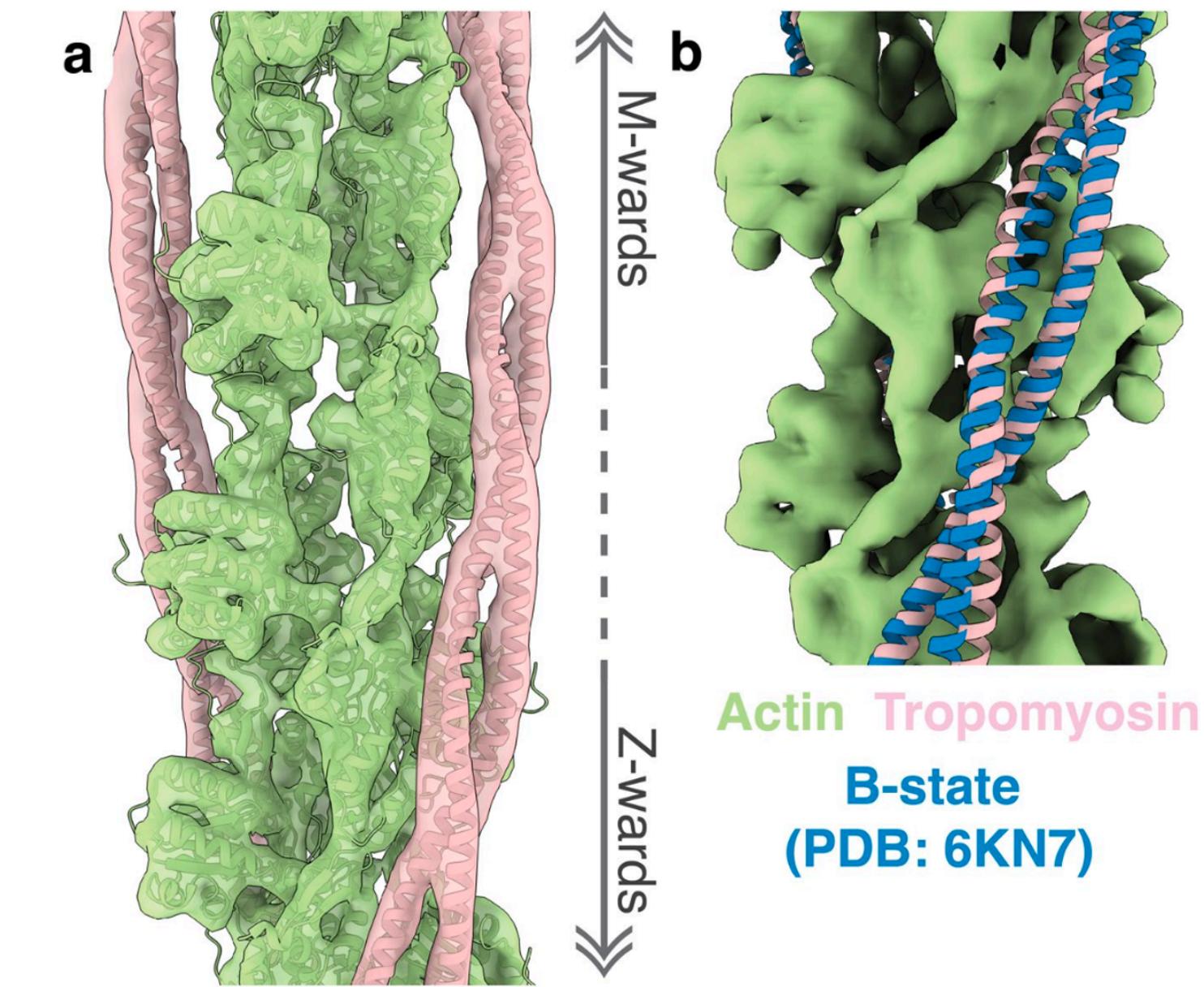
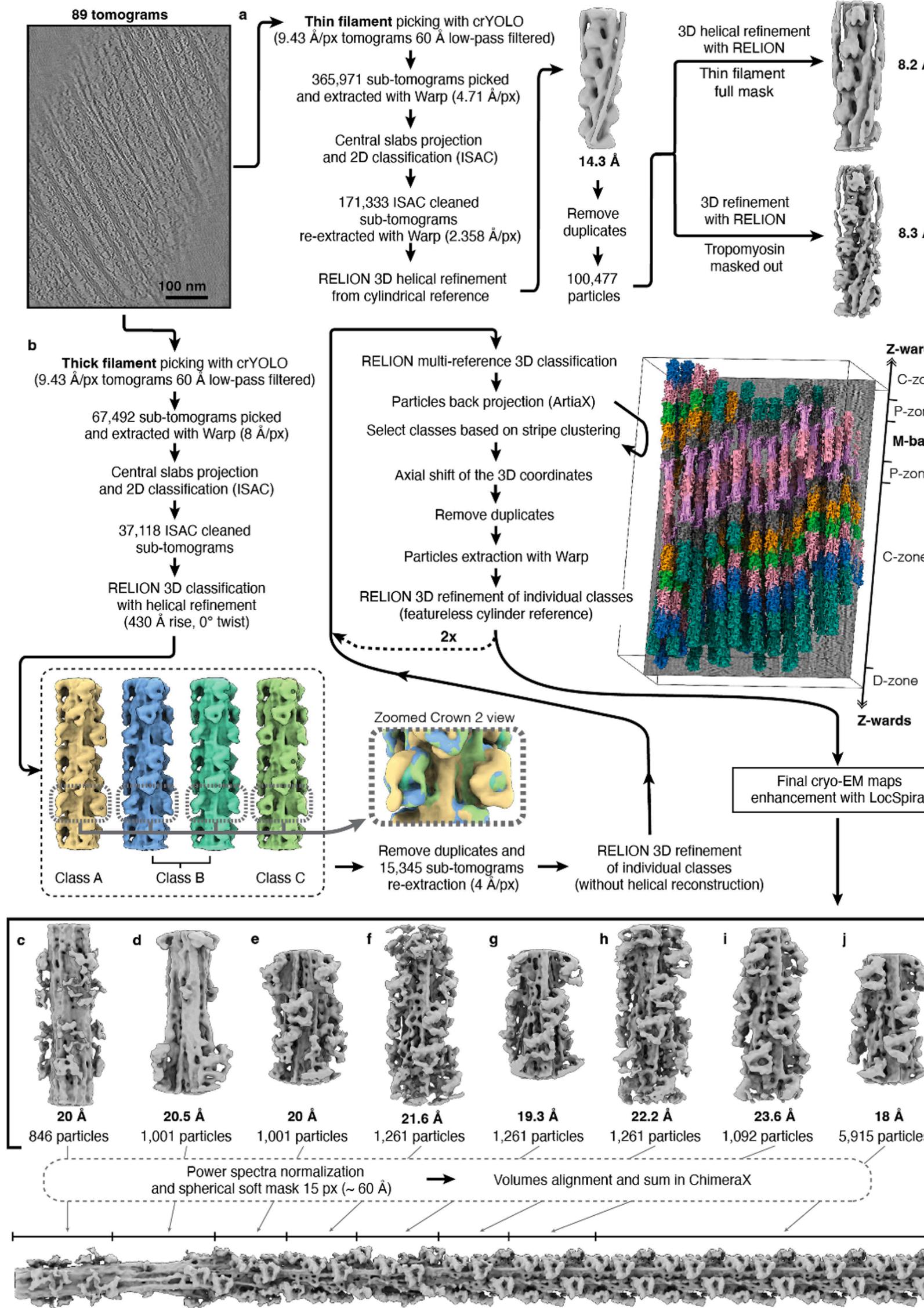
# **Results in detail**

# Structure of relaxed cardiac sarcomere

- P zone: proximal
- C zone: cMyBP-C-containing
- D zone: distal zones
- Bare zone(BZ): (M band) lacking myosin heads



# Structure of relaxed cardiac sarcomere

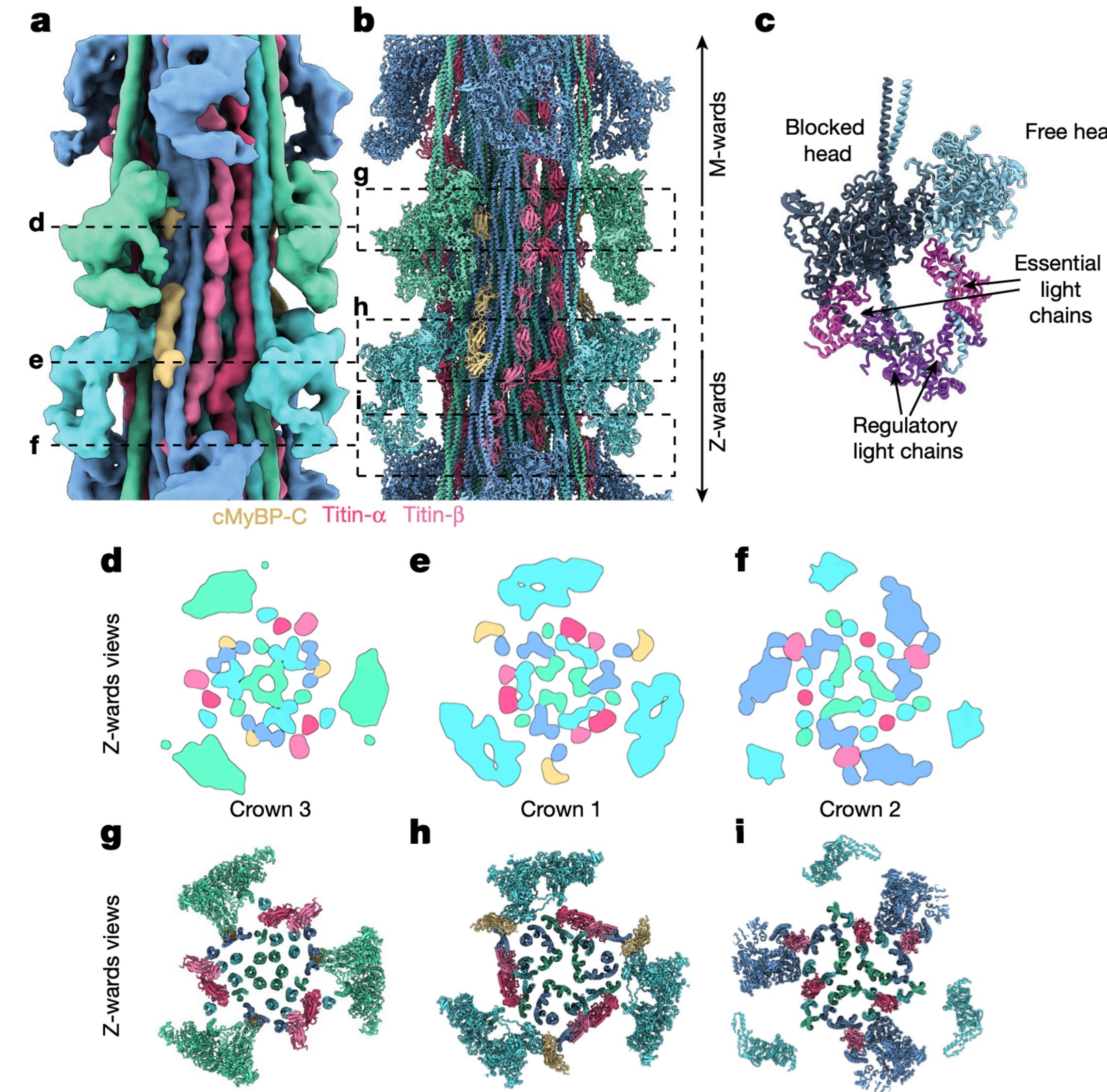


**Extended Data Fig. 3 | In situ structure of the thin filament in the  $\text{Ca}^{2+}$  free state.** **a**, Subtomogram-averaged structure of the thin filament in the relaxed sarcomere. **b**, Model of the B-state of tropomyosin (PDB: 6KN7) is shown for comparison.

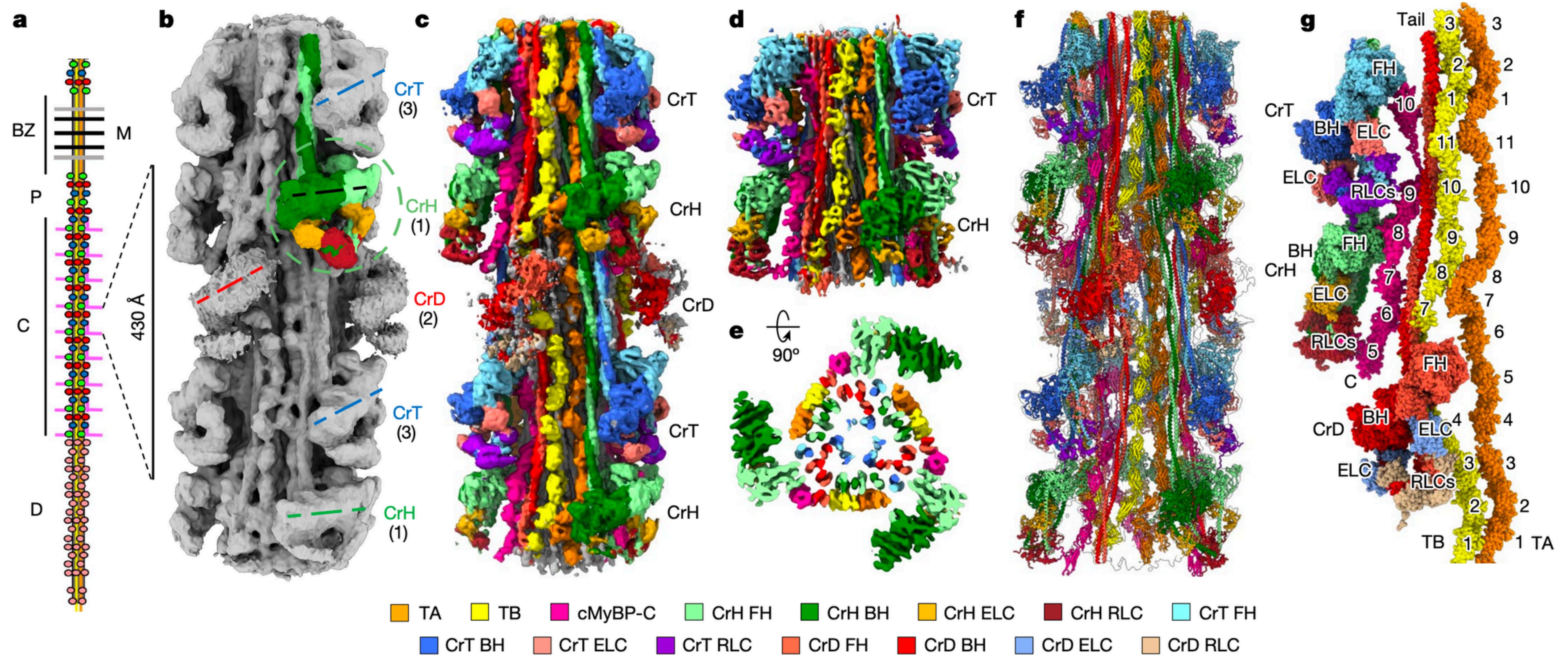
# Structures

- C-zone:
  - Myosin:
    - heads;
    - tails.
  - Two myosin binding proteins:
    - Titin(Titin- $\alpha$  & Titin- $\beta$ );
    - myosin-binding protein C(MyBP-C).
- P-zone;
- M-band(Bare zone):
  - M-band-associated proteins;
  - Titin(Titin- $\alpha$ ).

# Structure model of the C-zone

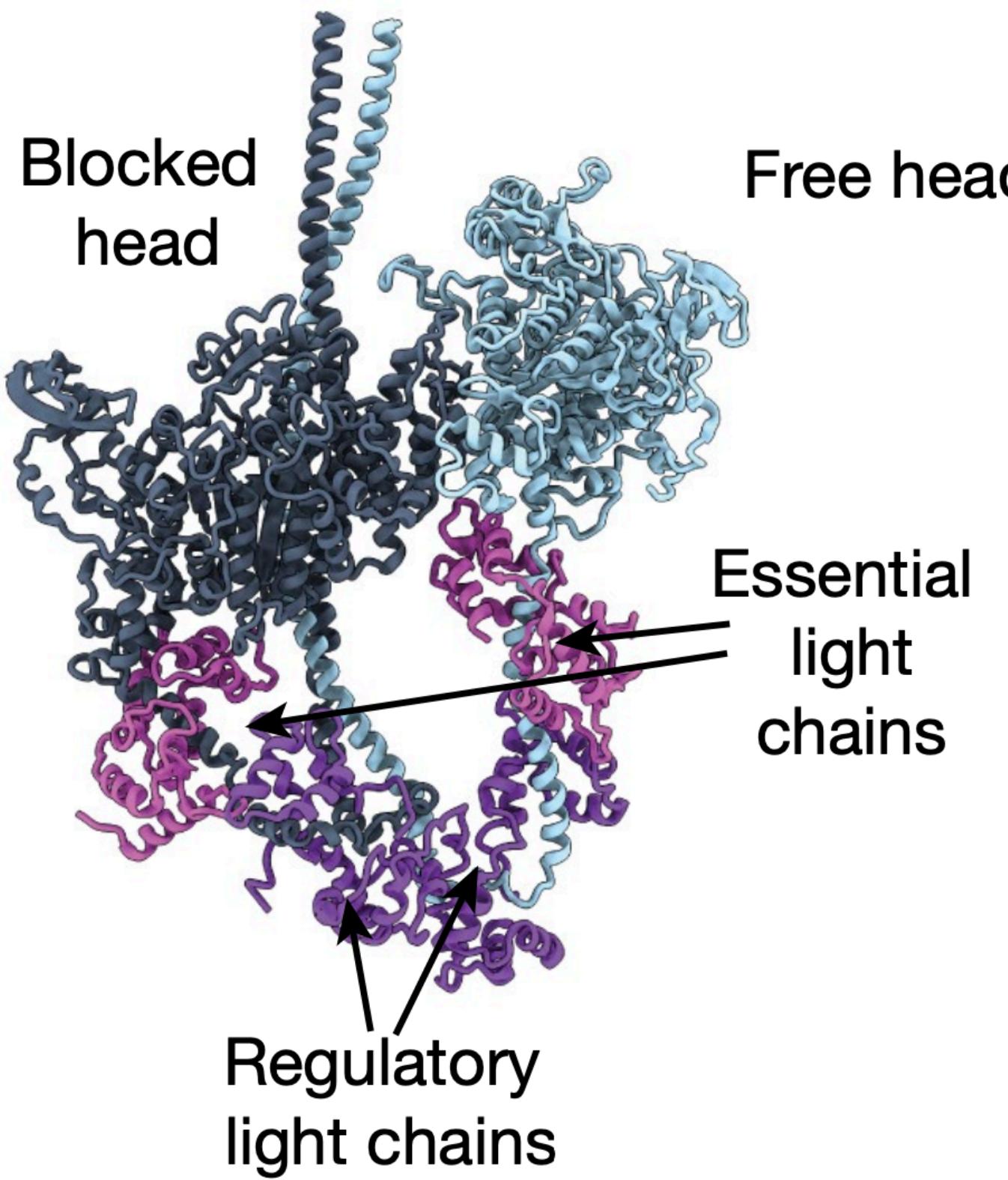


# Structure model of the C-zone

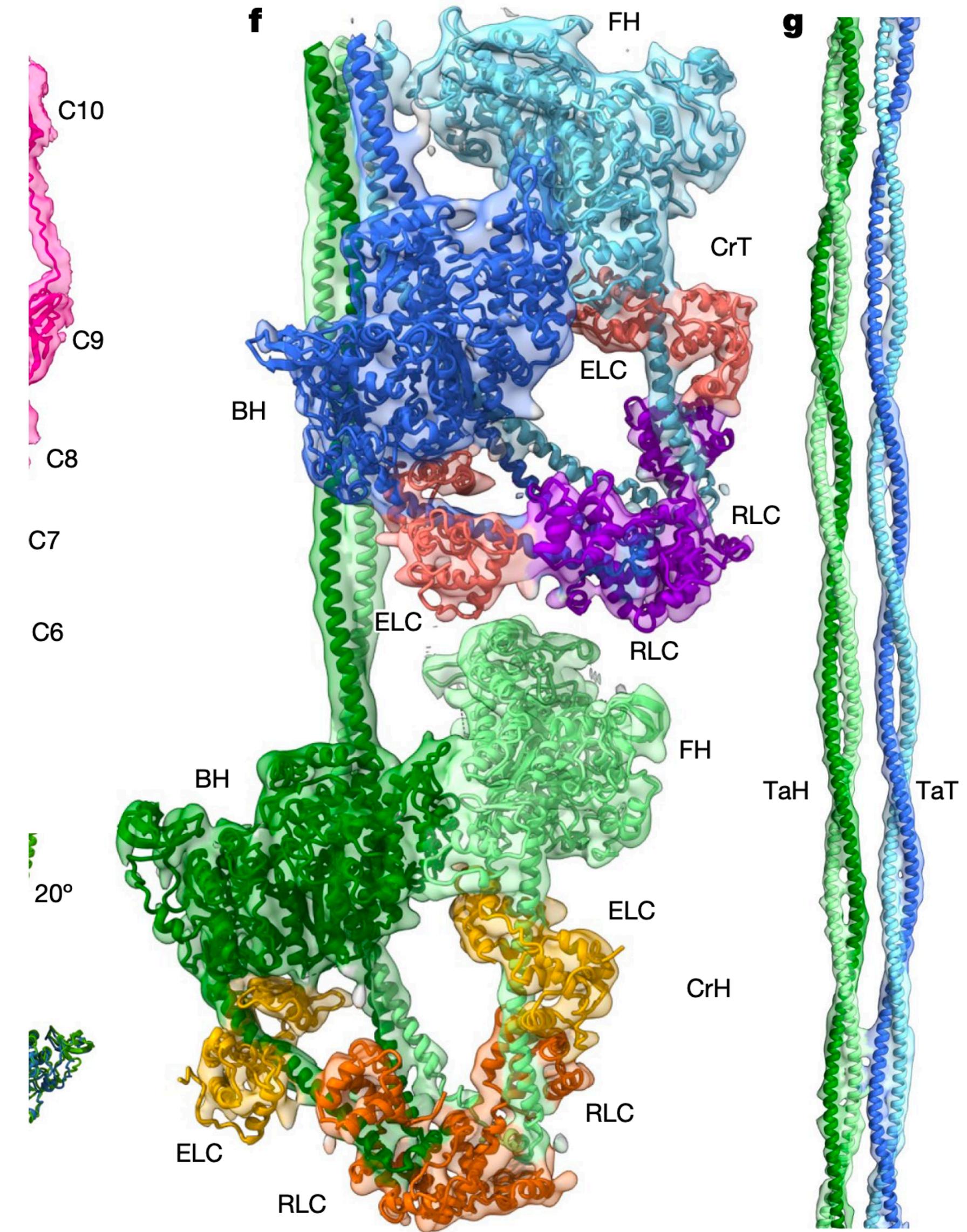
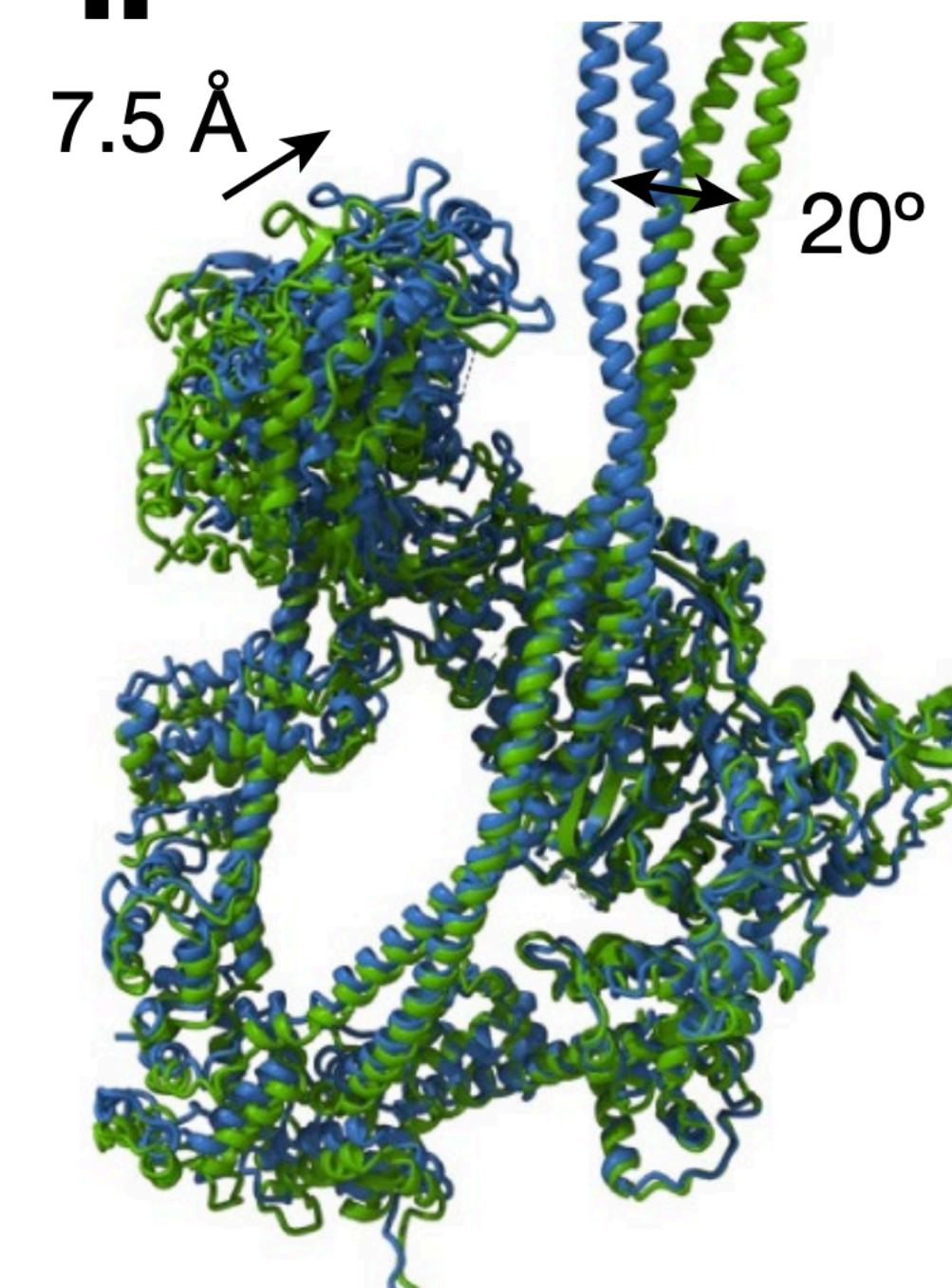


# Myosin heads and tails

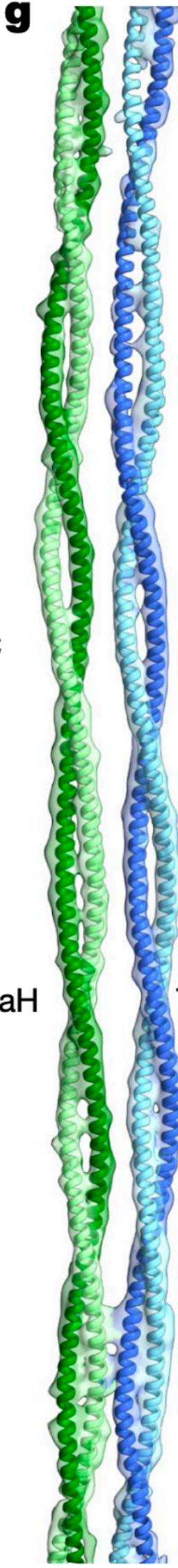
c



h



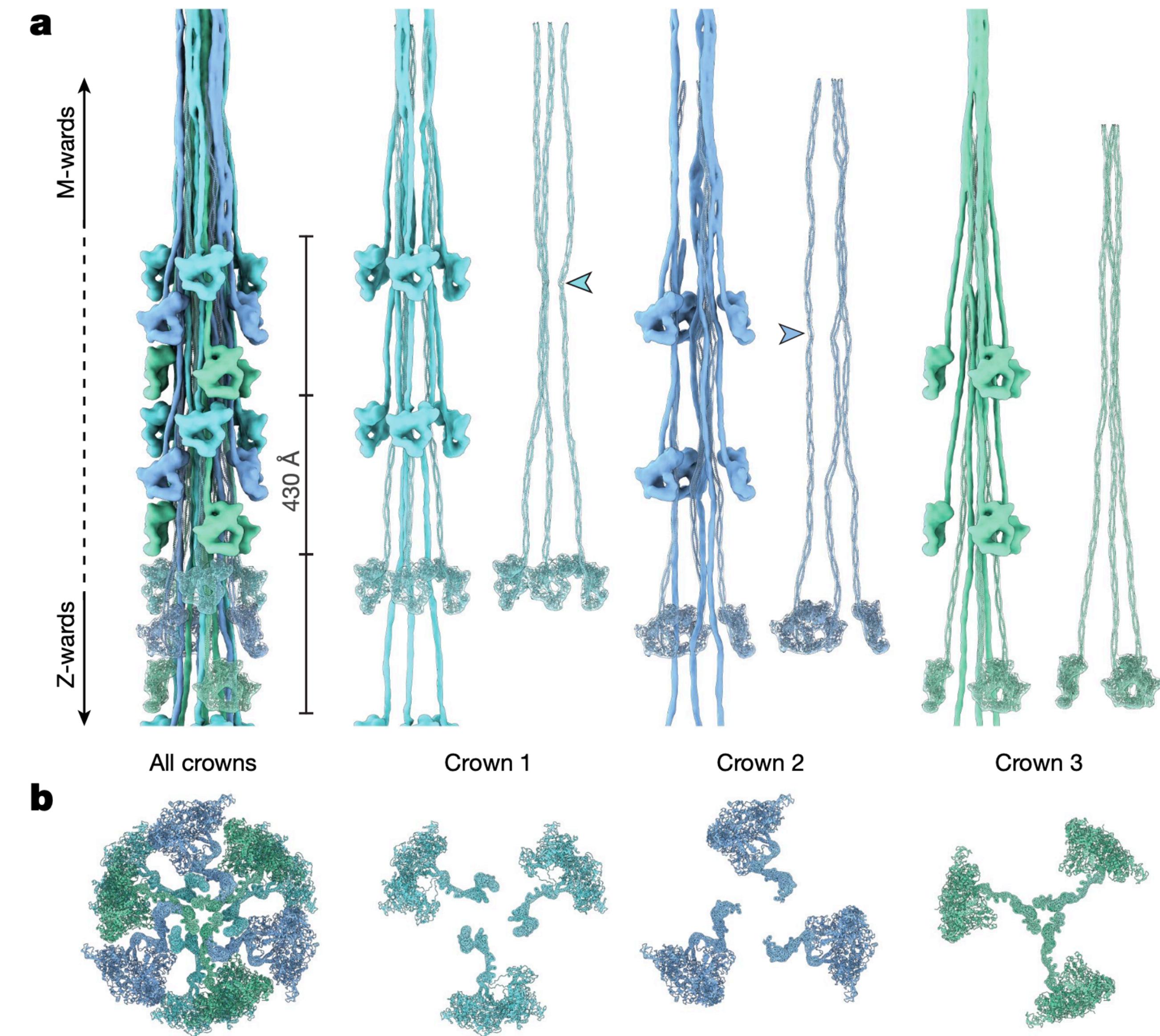
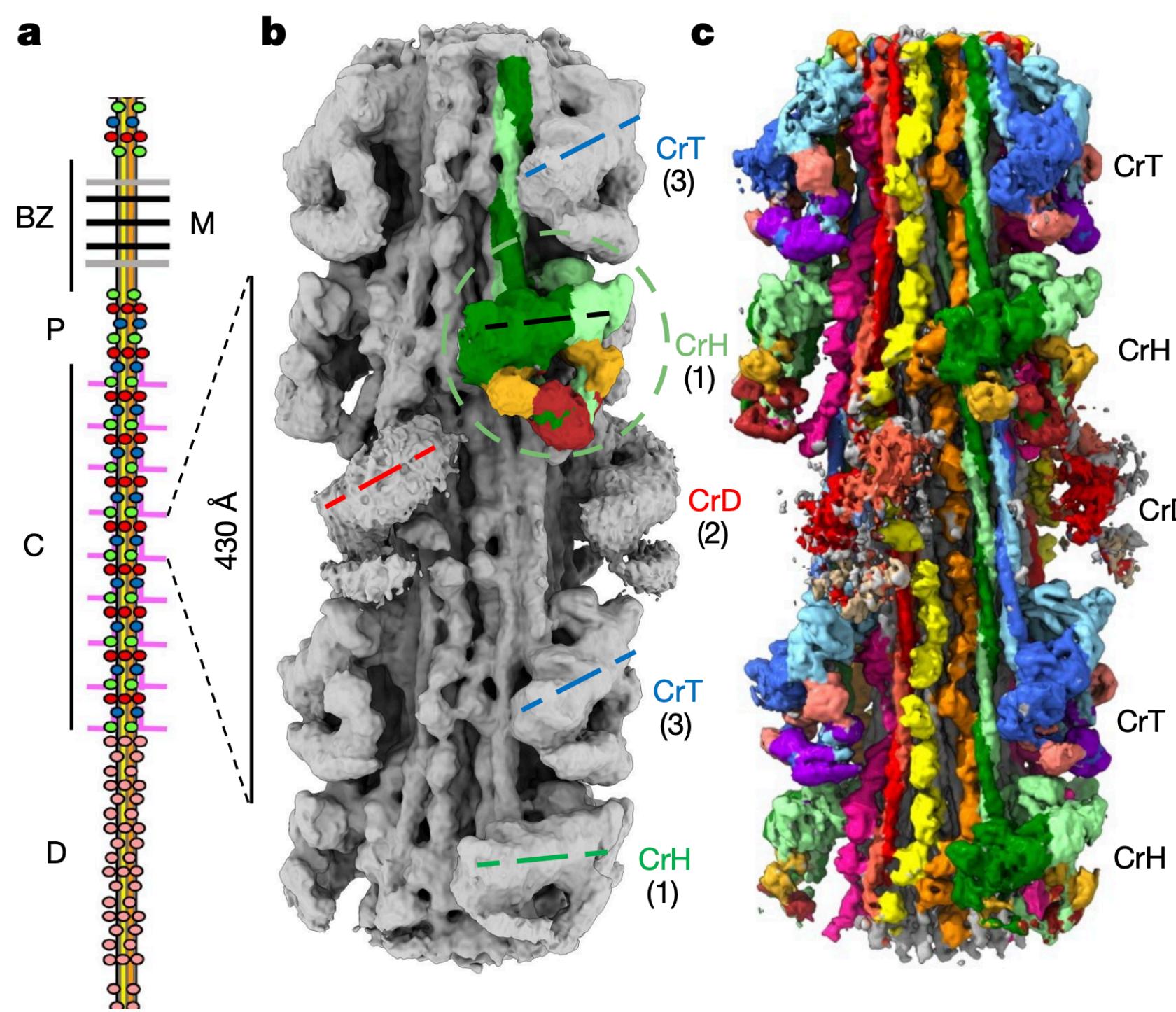
g



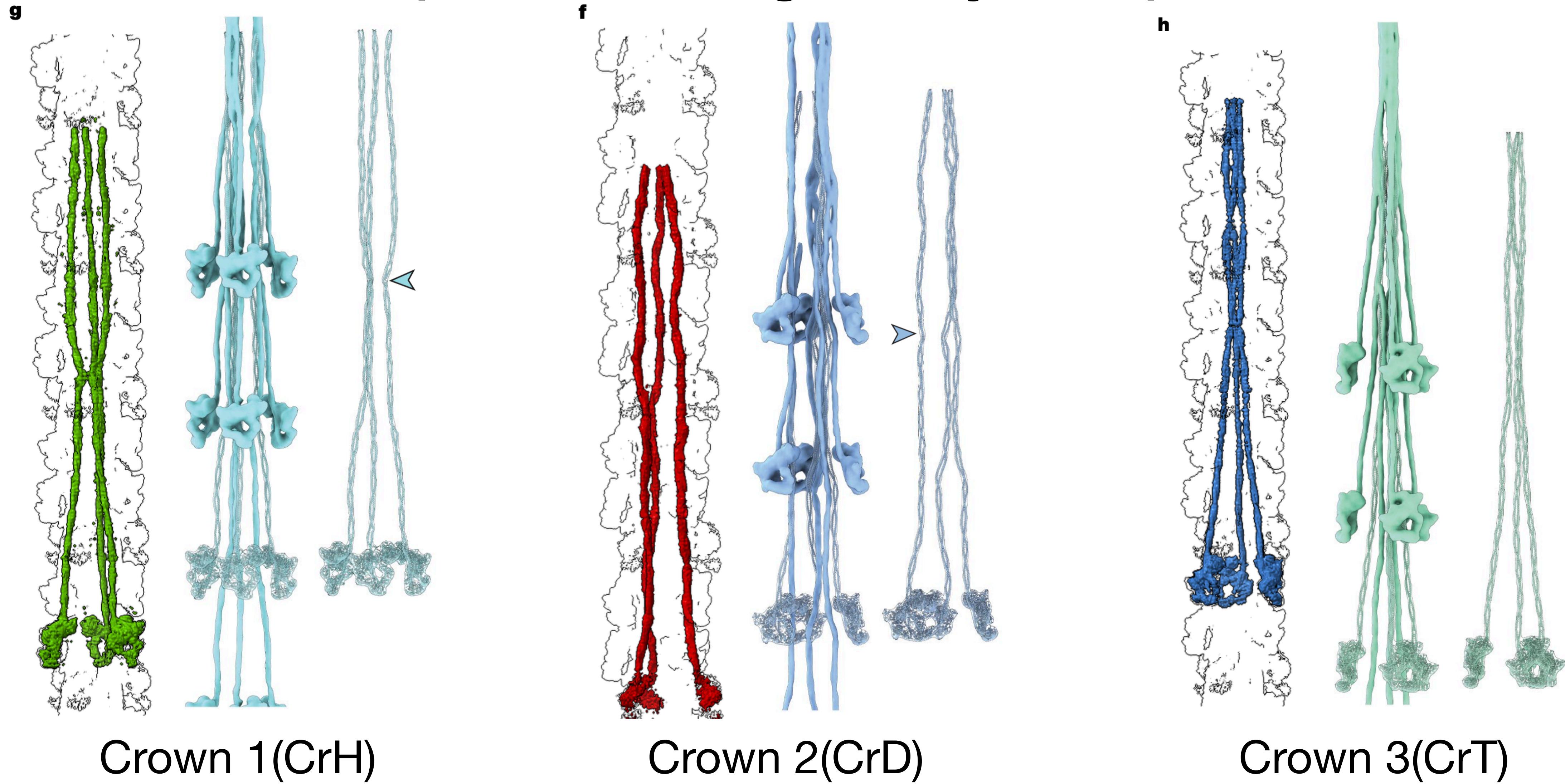
# The arrangement of myosin within the C-zone

3 crowns:

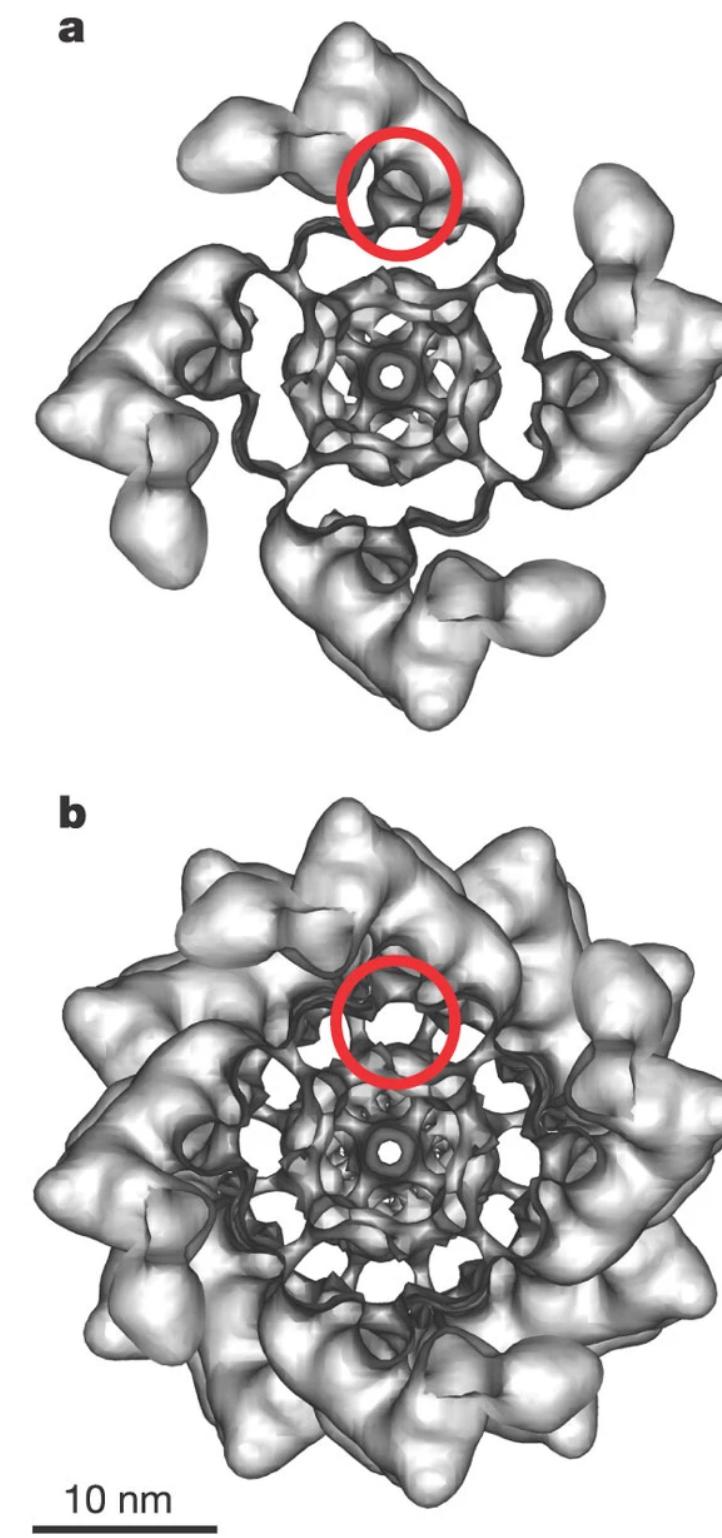
- CrD: crown 2, disordered
- CrT: crown 3, tilted
- CrH: crown 1, horizontal



# The arrangement of myosin within the C-zone (left: SPA, right: cryo-ET)

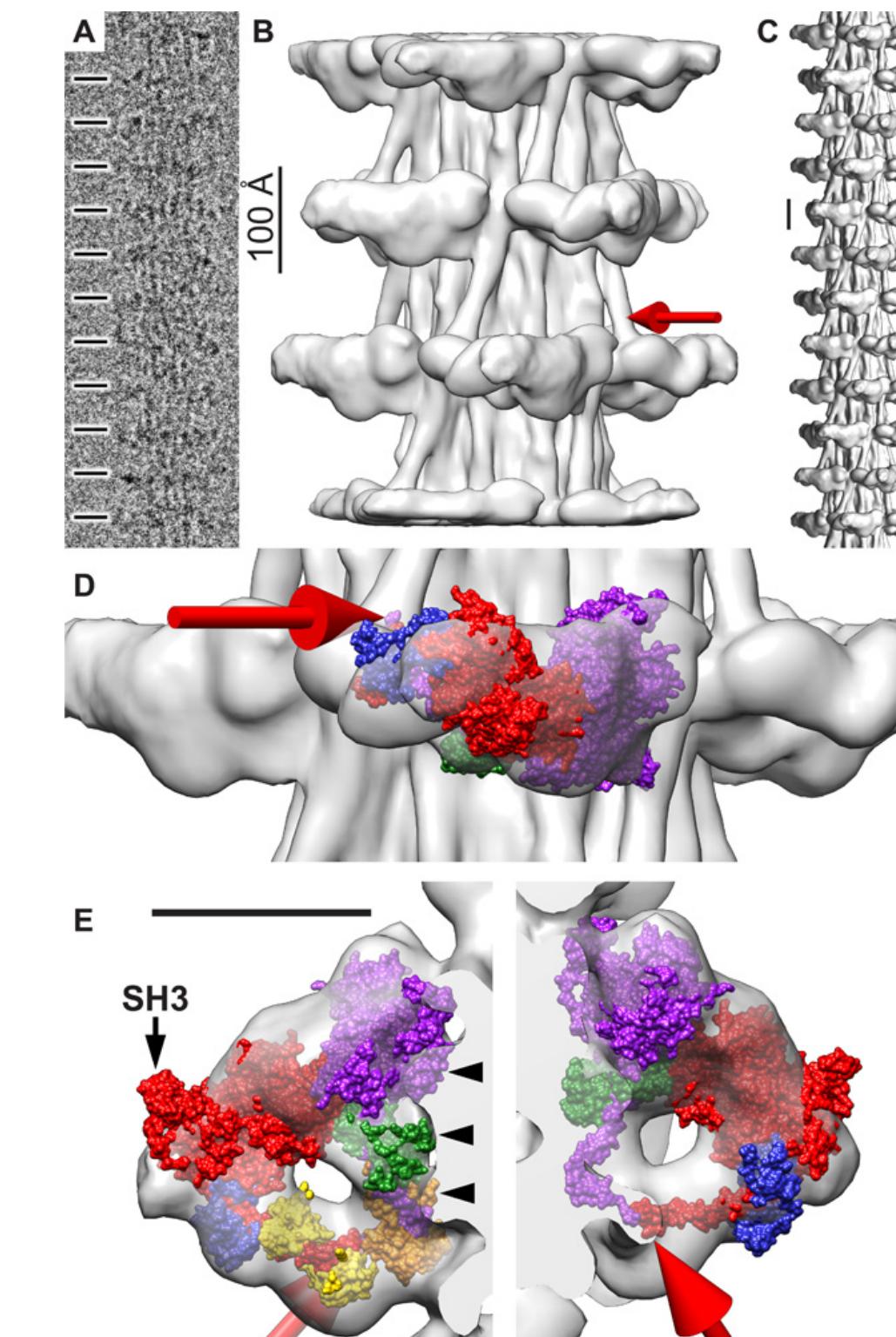


# Different arrangement of Myosin in vertebrate and invertebrate

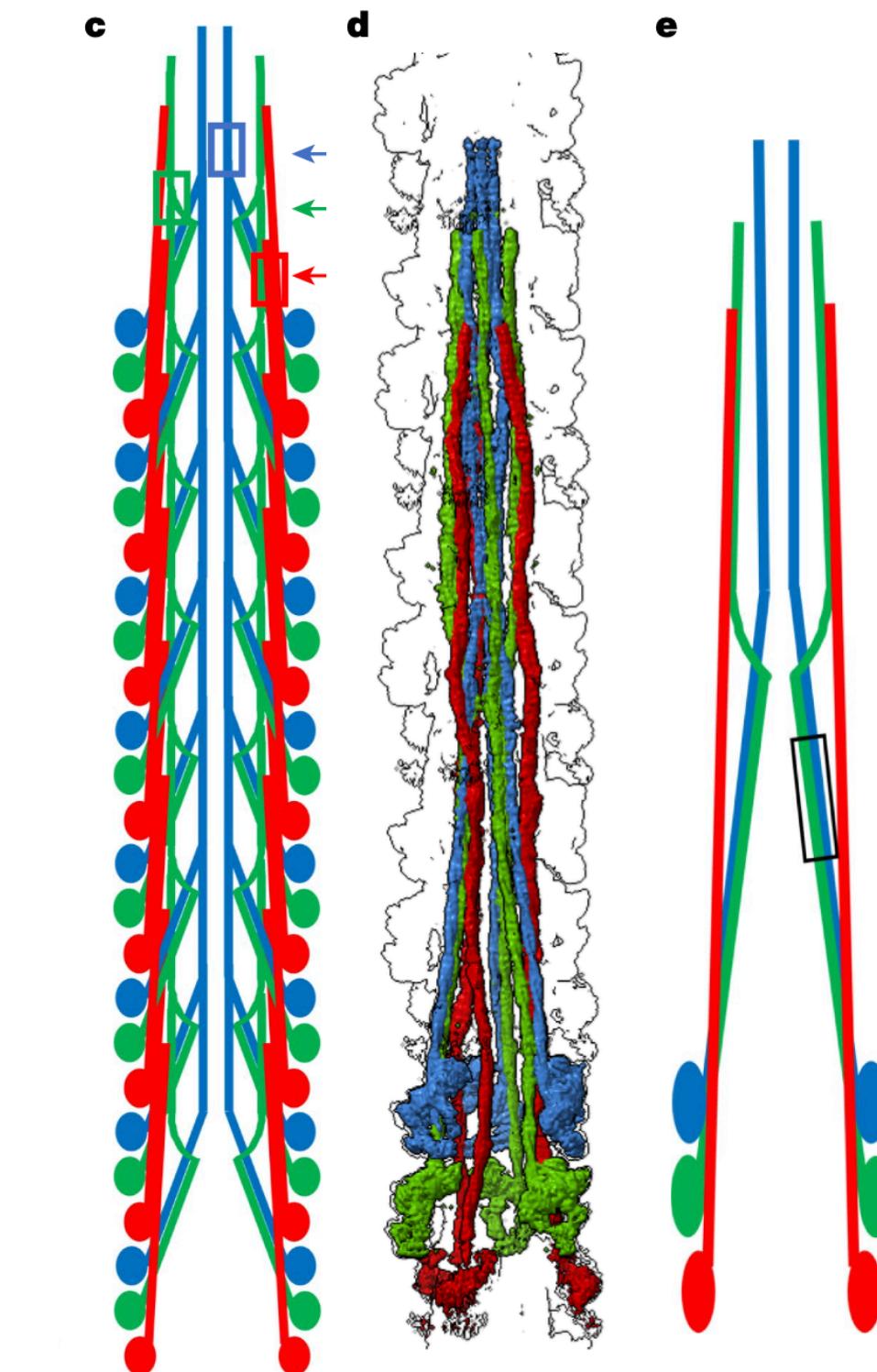


Tarantula(狼蛛), *Nature* 2005

← Perfectly helical →



Giant water bug(负子蝽), *Science* 2016

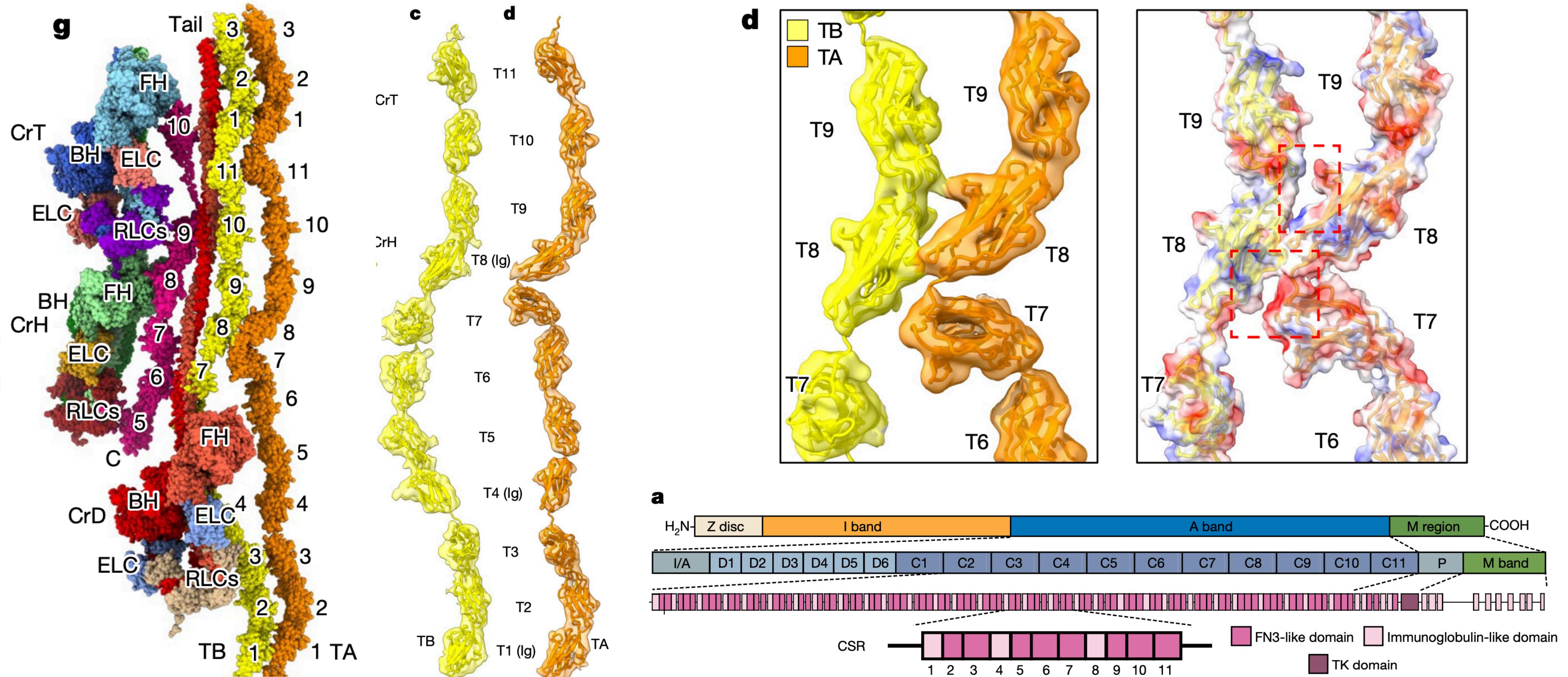


Human, *Nature* 2023

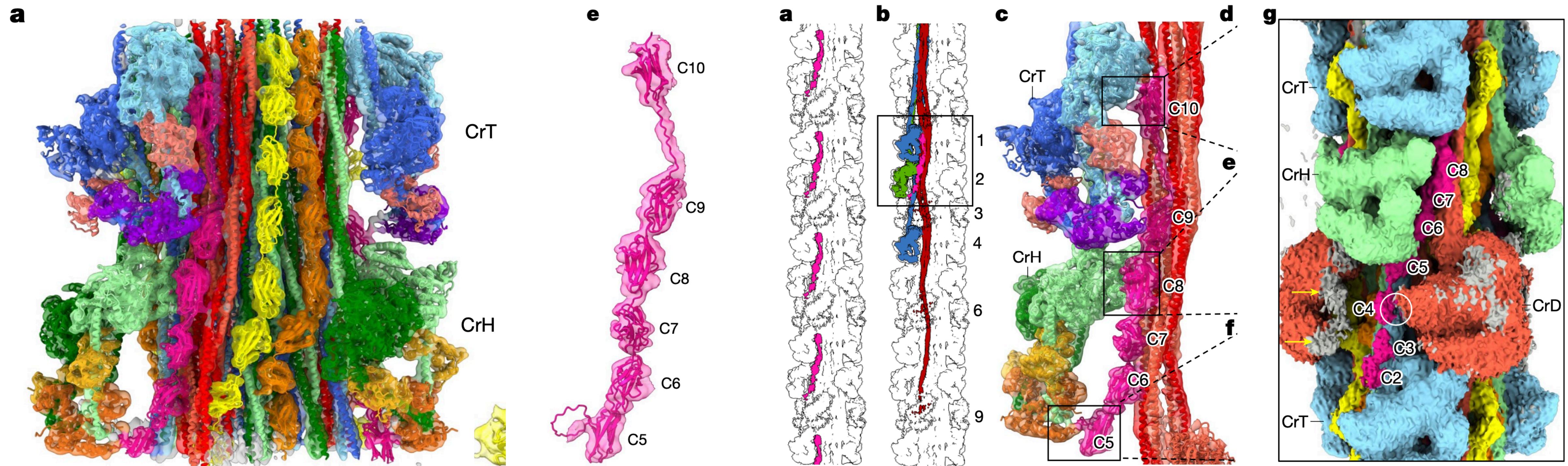
Quasi-helical

Such functional specialization represents an evolutionary increase in complexity

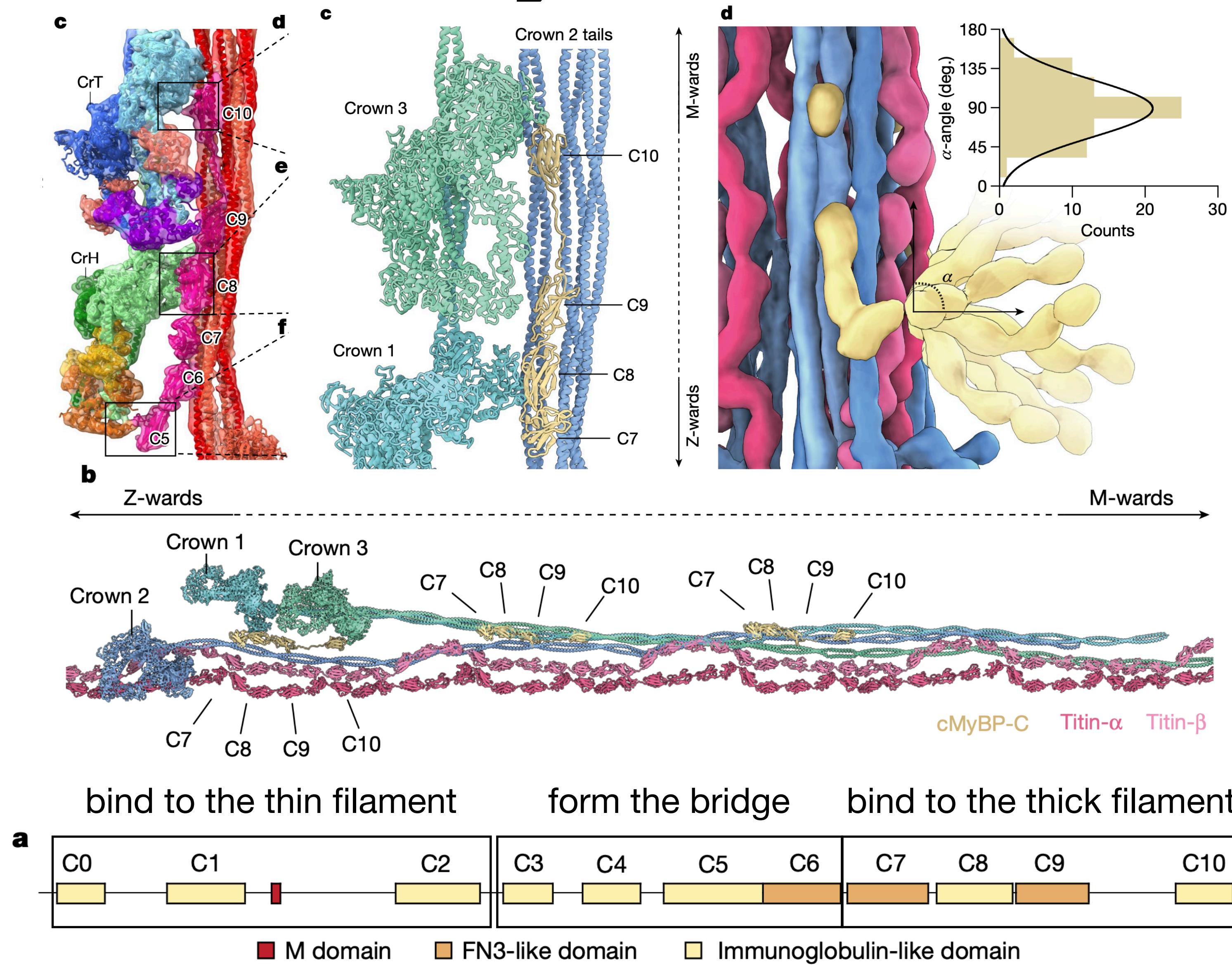
# Titin(C-zone)



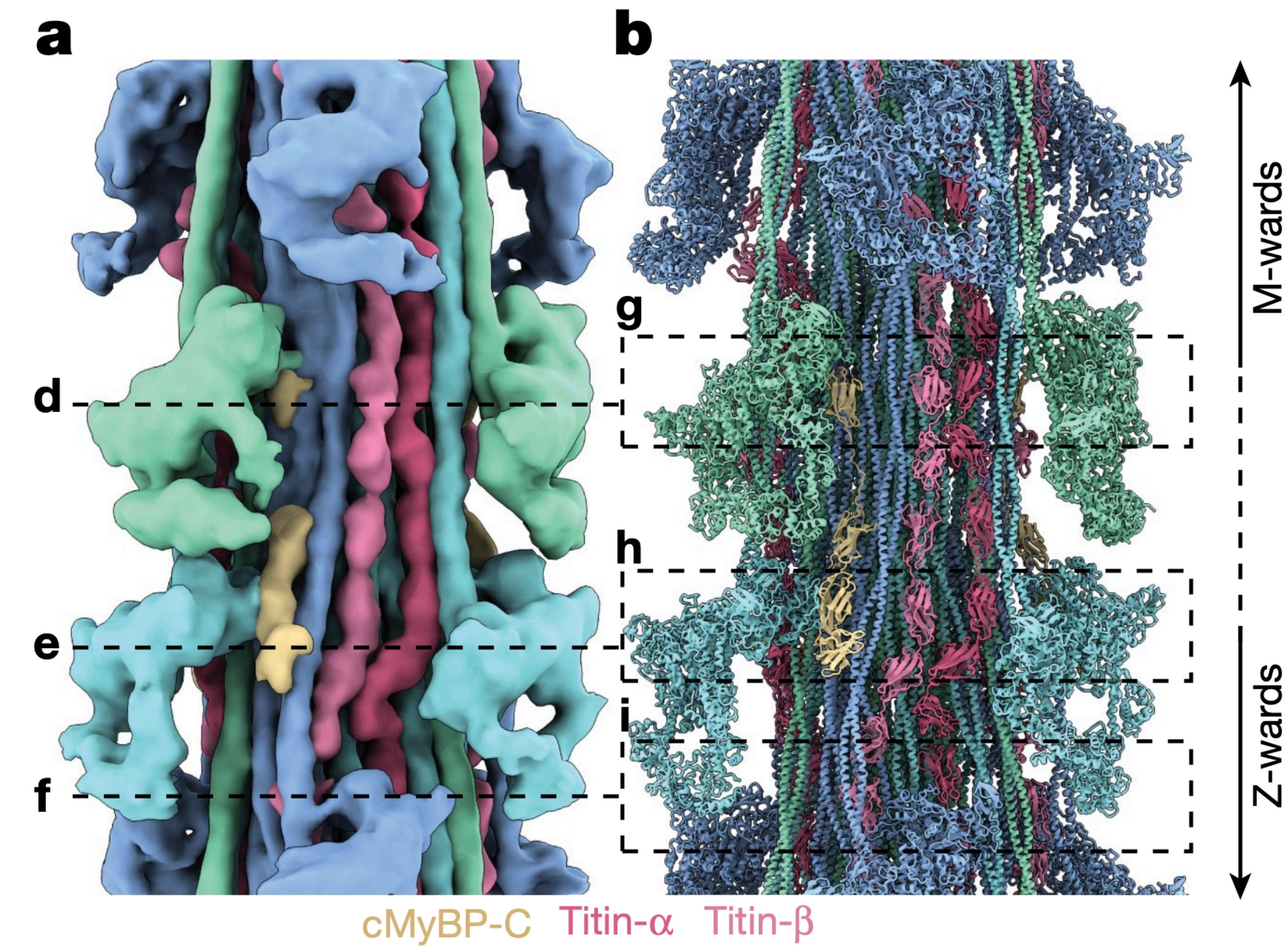
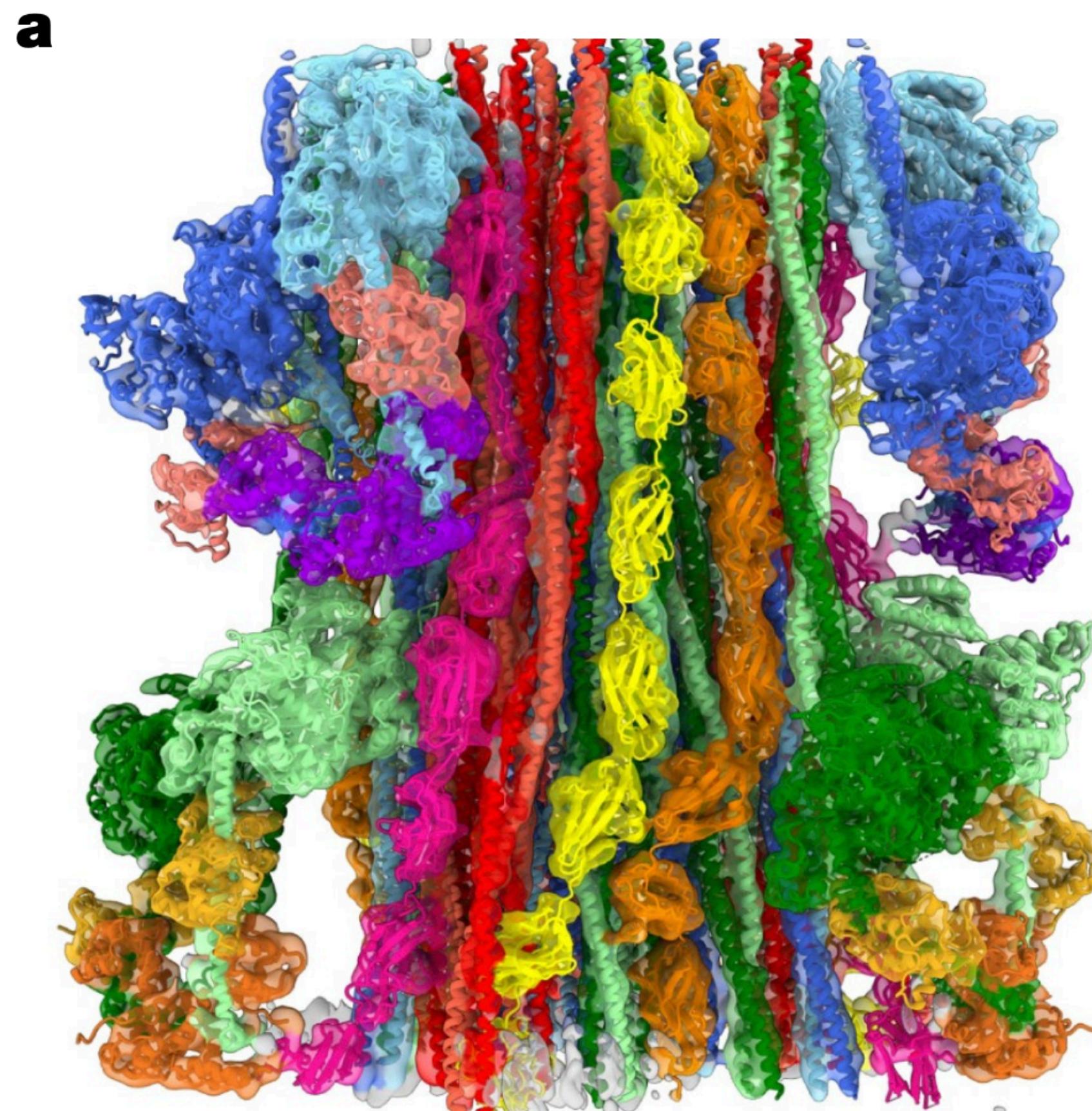
# MyBP-C



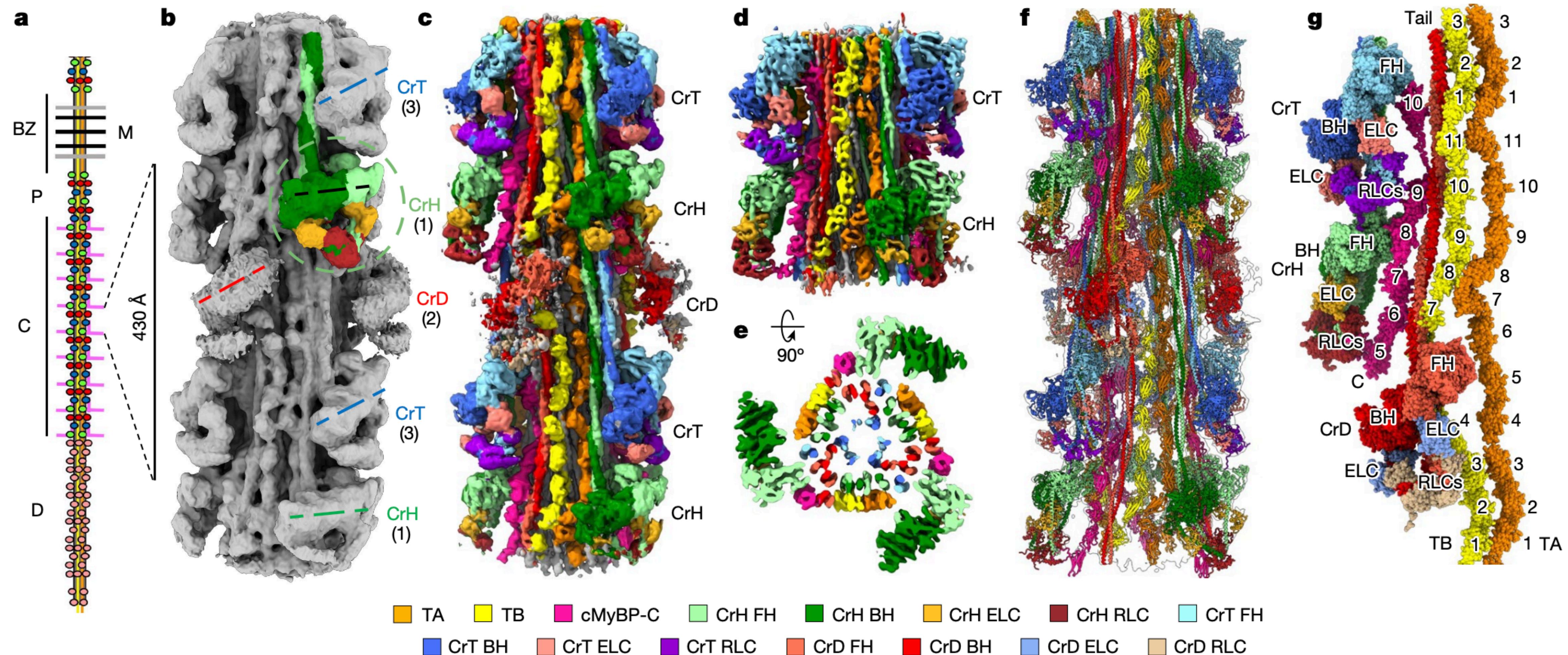
# MyBP-C



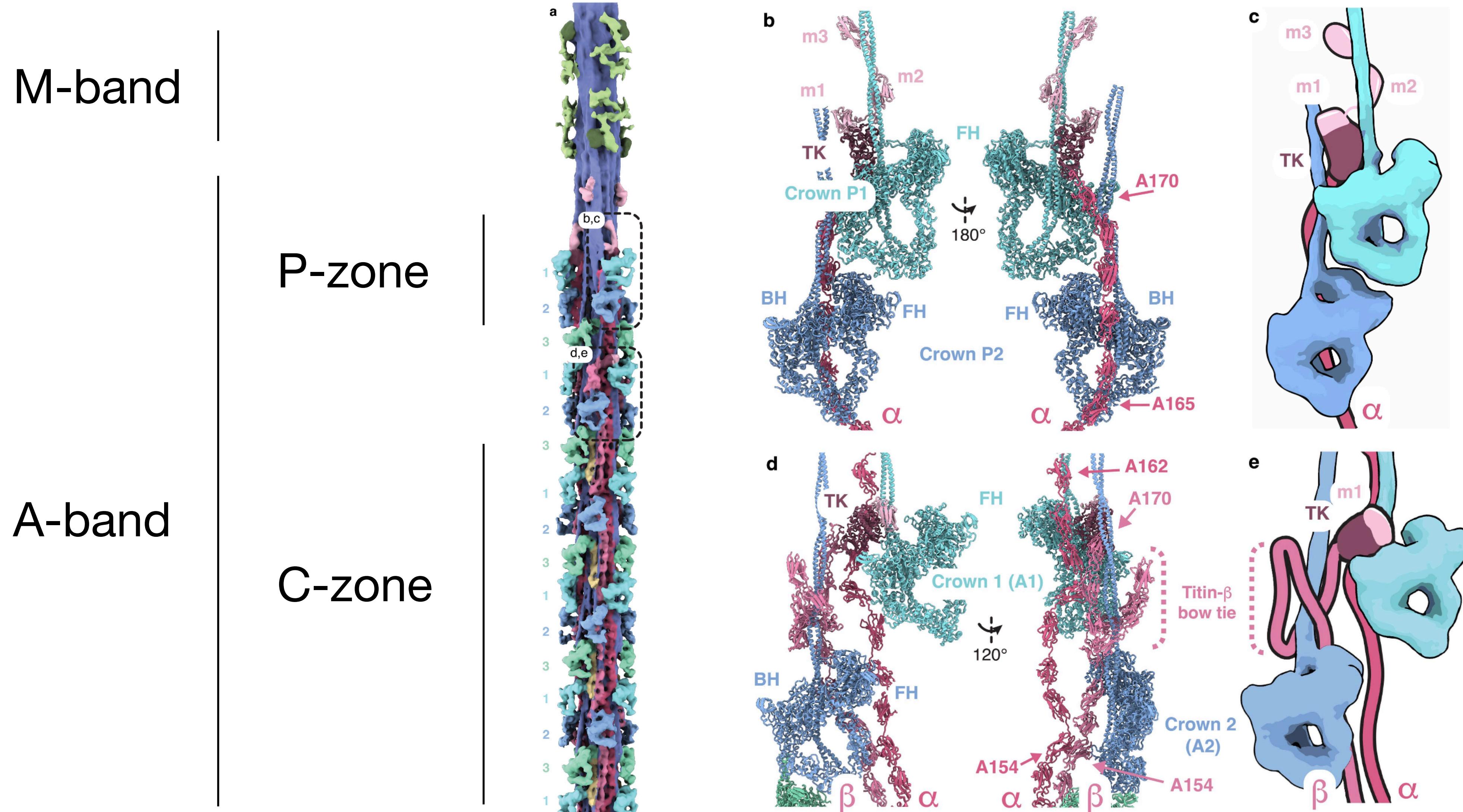
# 3D model of the C-zone (left: SPA, right: cryo-ET)



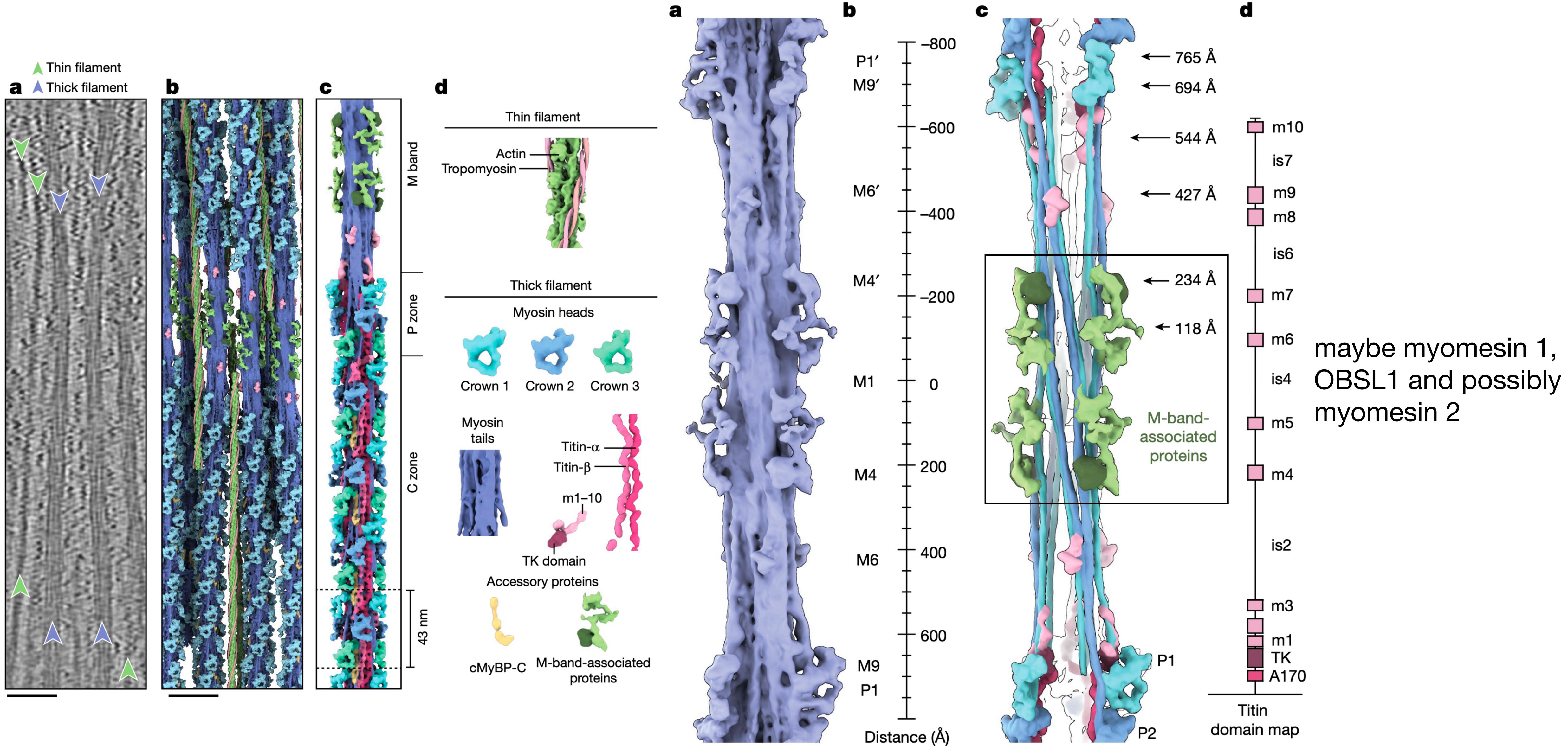
# 3D model of the C-zone



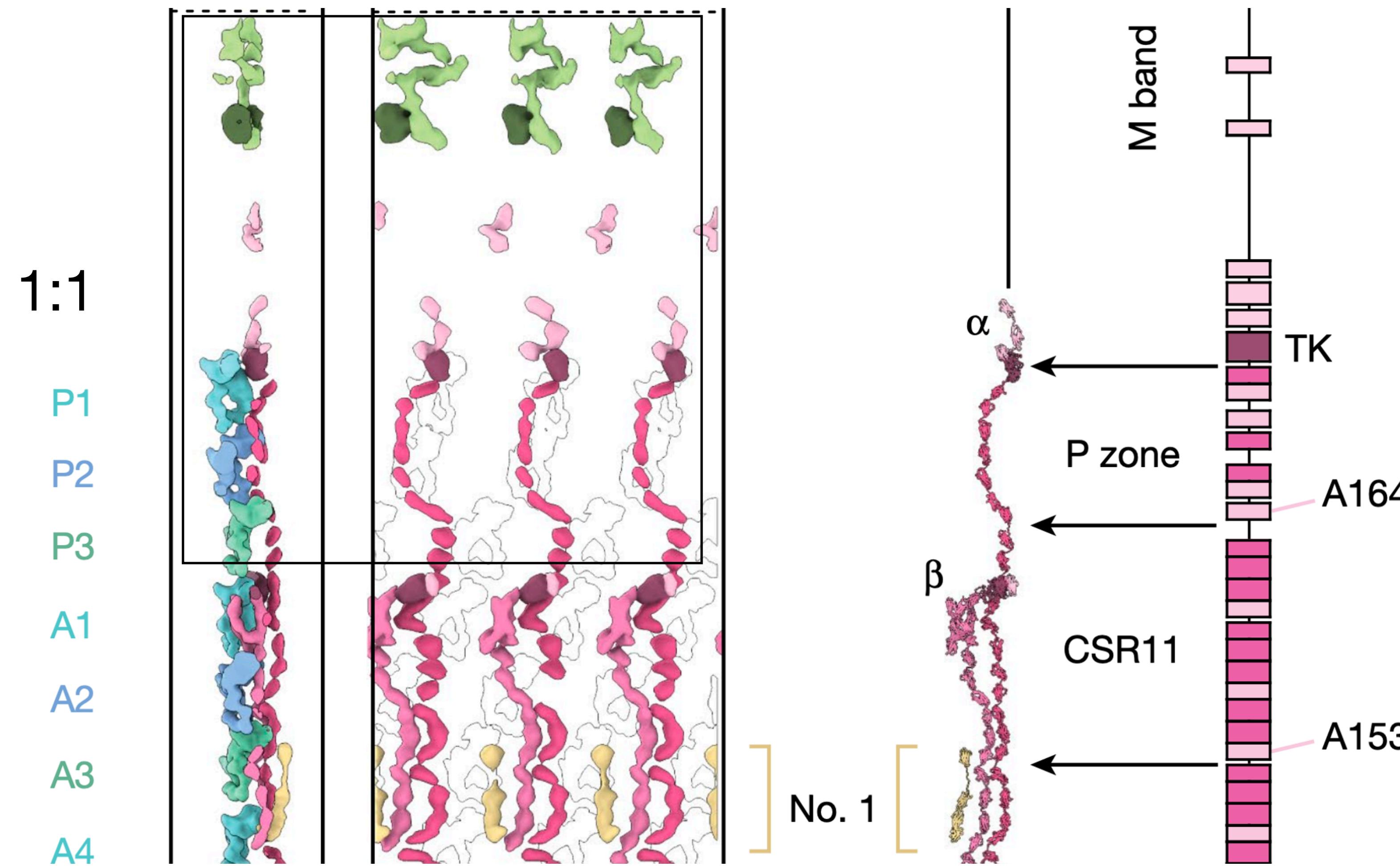
# 3D model of the P-zone



# 3D reconstruction of M-band

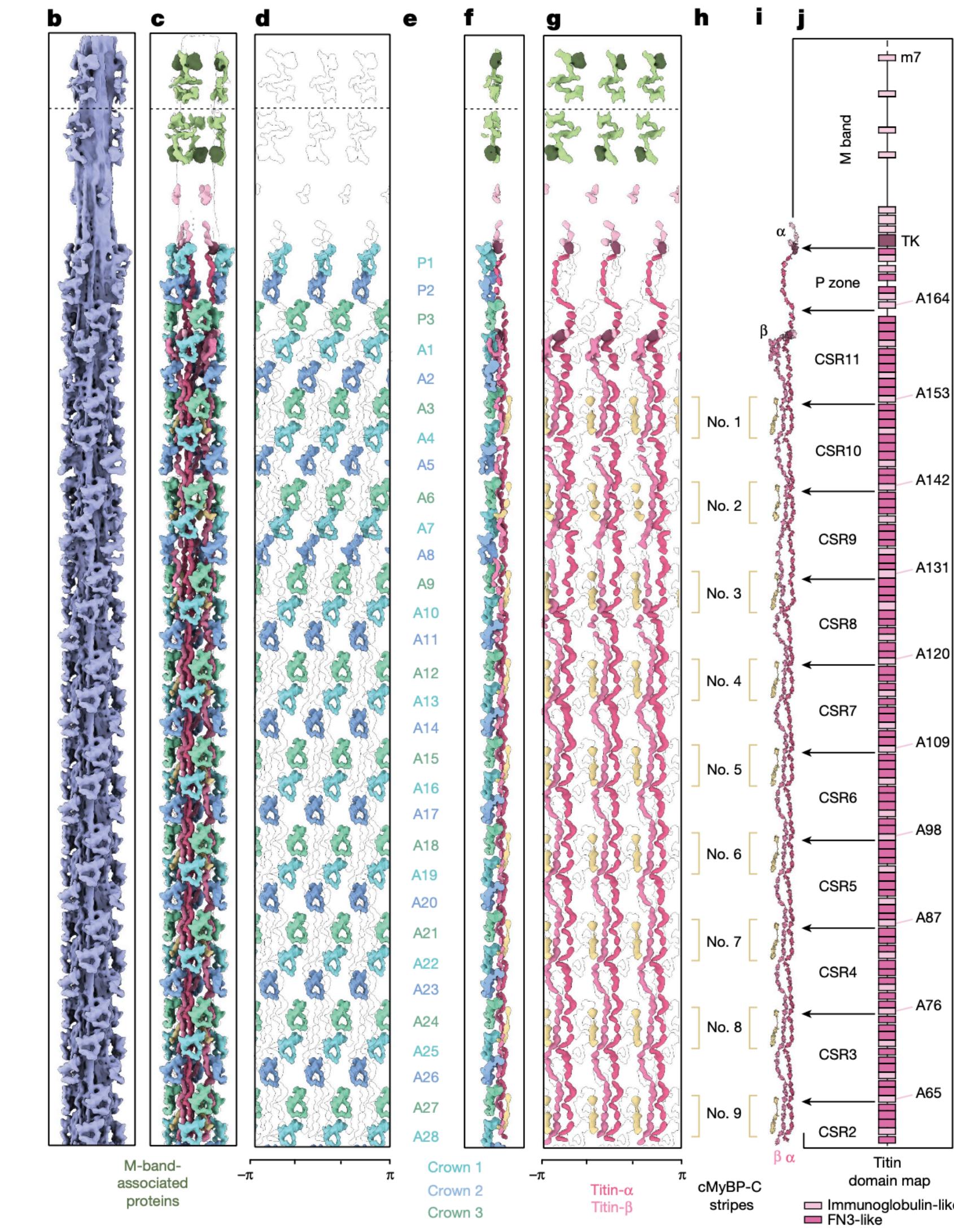


# 3D reconstruction of M-band



blueprint for titin m-domain localization and structural rationalization, initial model

# Structures

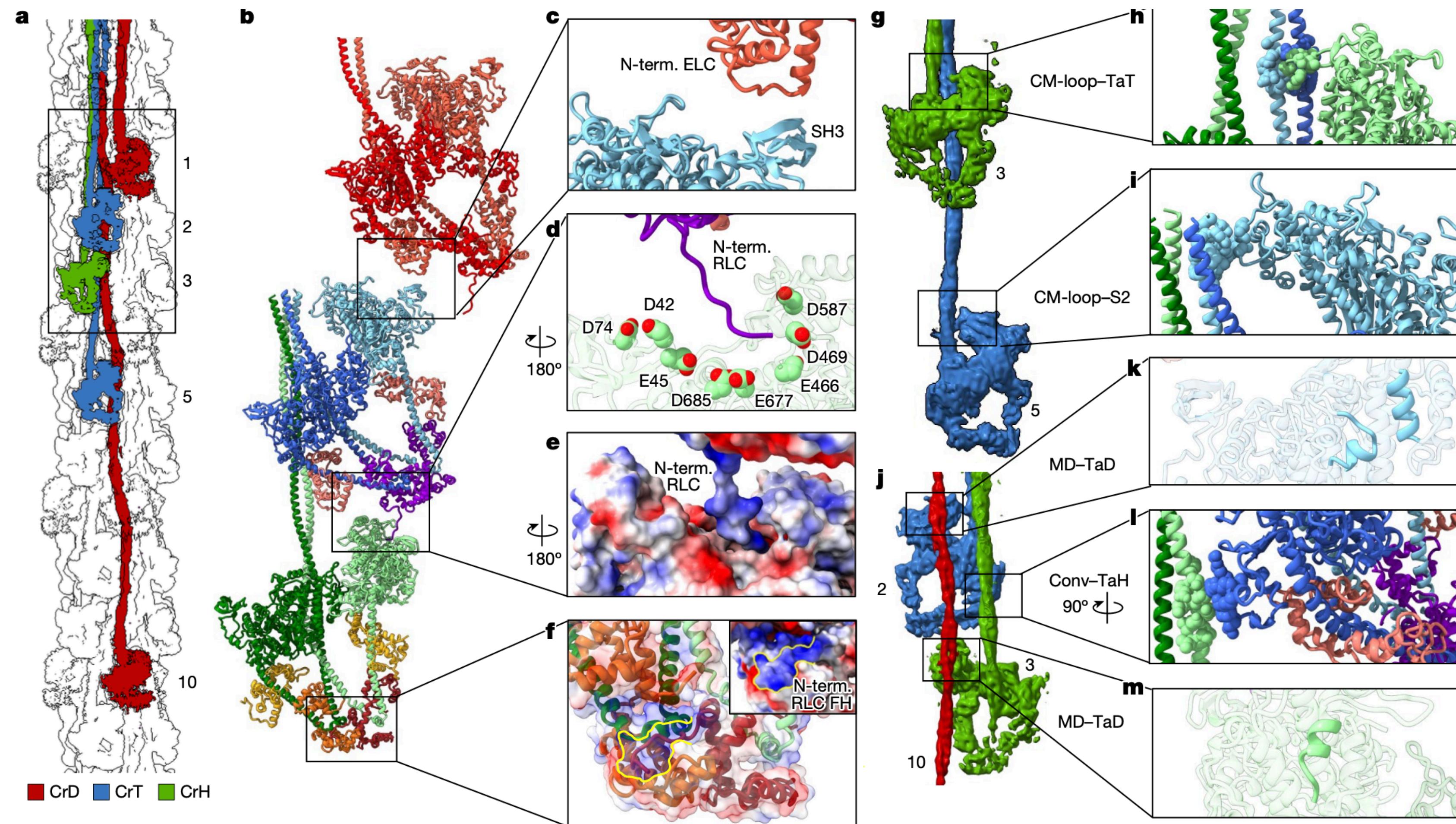


# Interactions

- myosin heads with heads
- myosin tails with tails
- titin with myosin tails
- MyBP-C with myosin heads
- MyBP-C with myosin tails

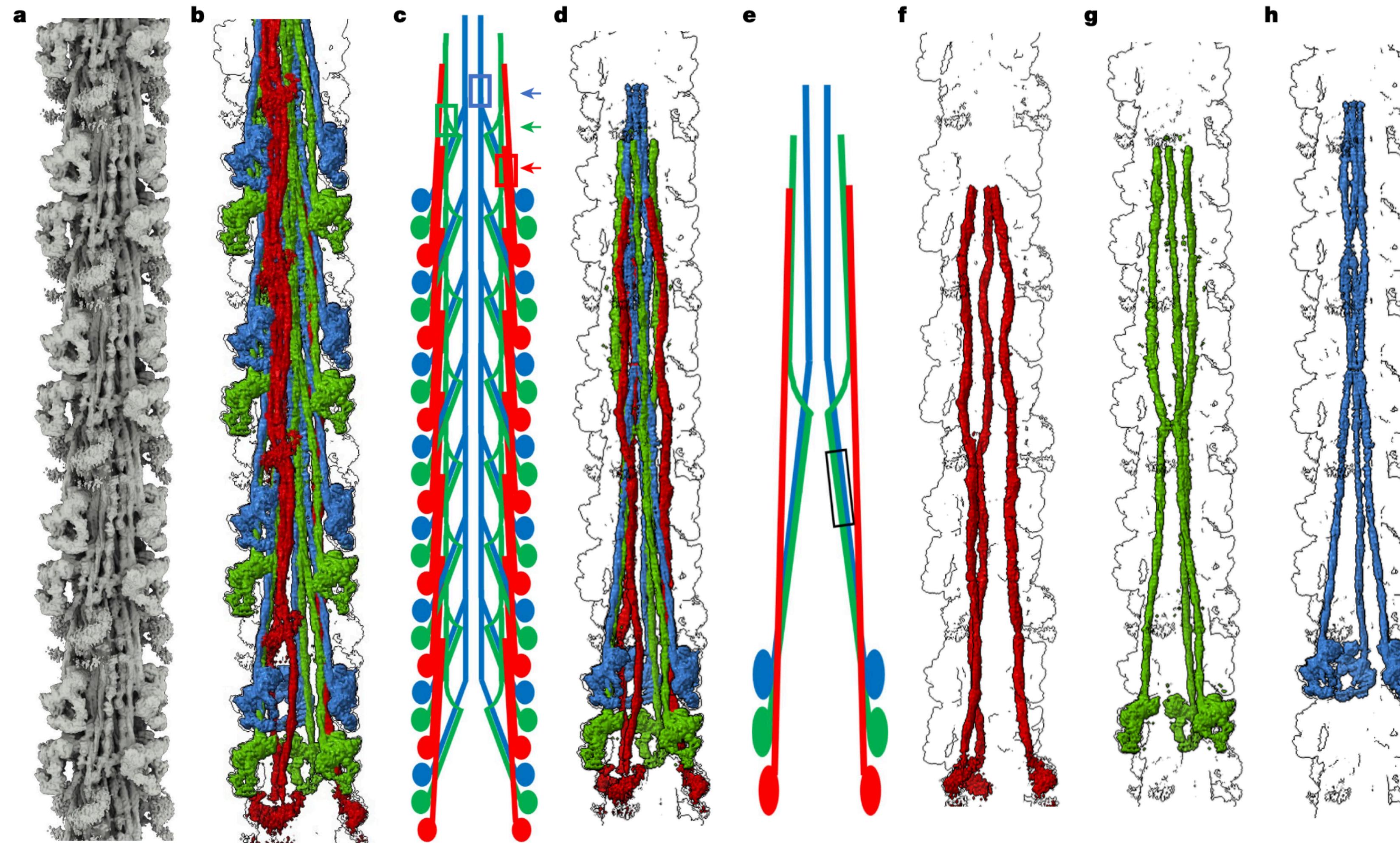
但是， titin和cMyBP-C以及titin和myosin heads之间都是没有互作的

# Myosin head-head



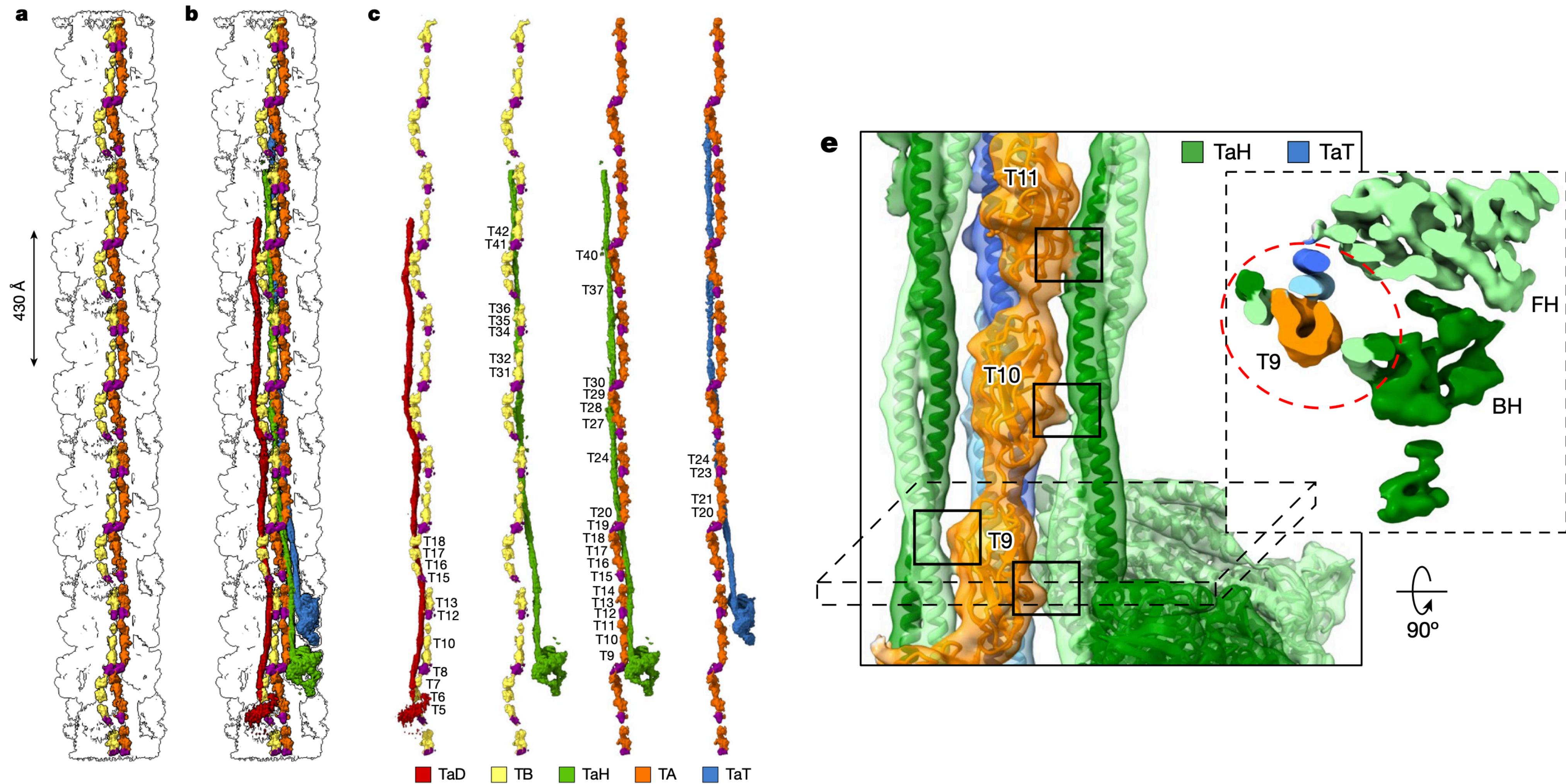
IHM interactions may stabilize the relaxed state

# Myosin tail-tail

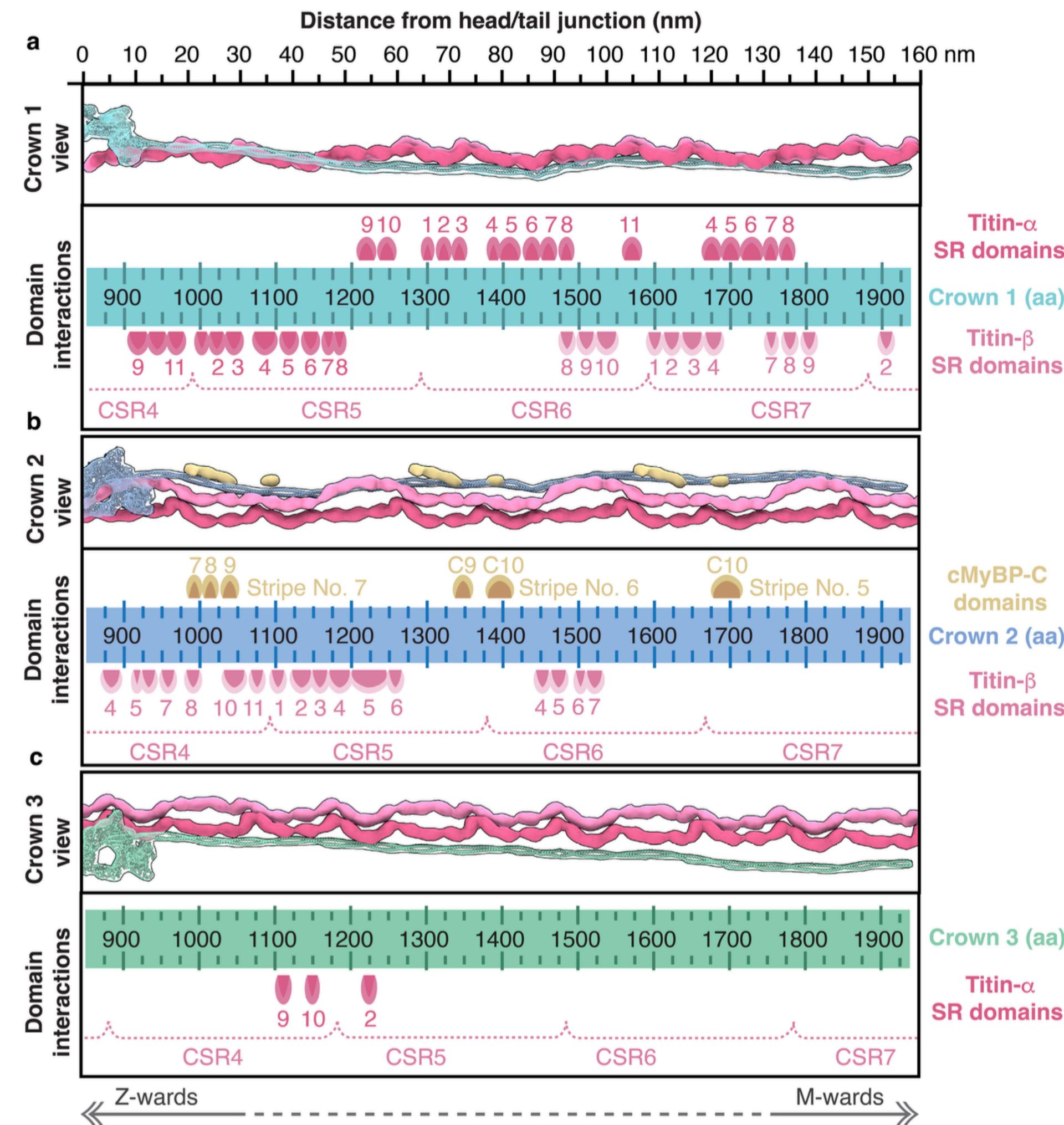


Myosin tails form an interconnected network in the filament backbone

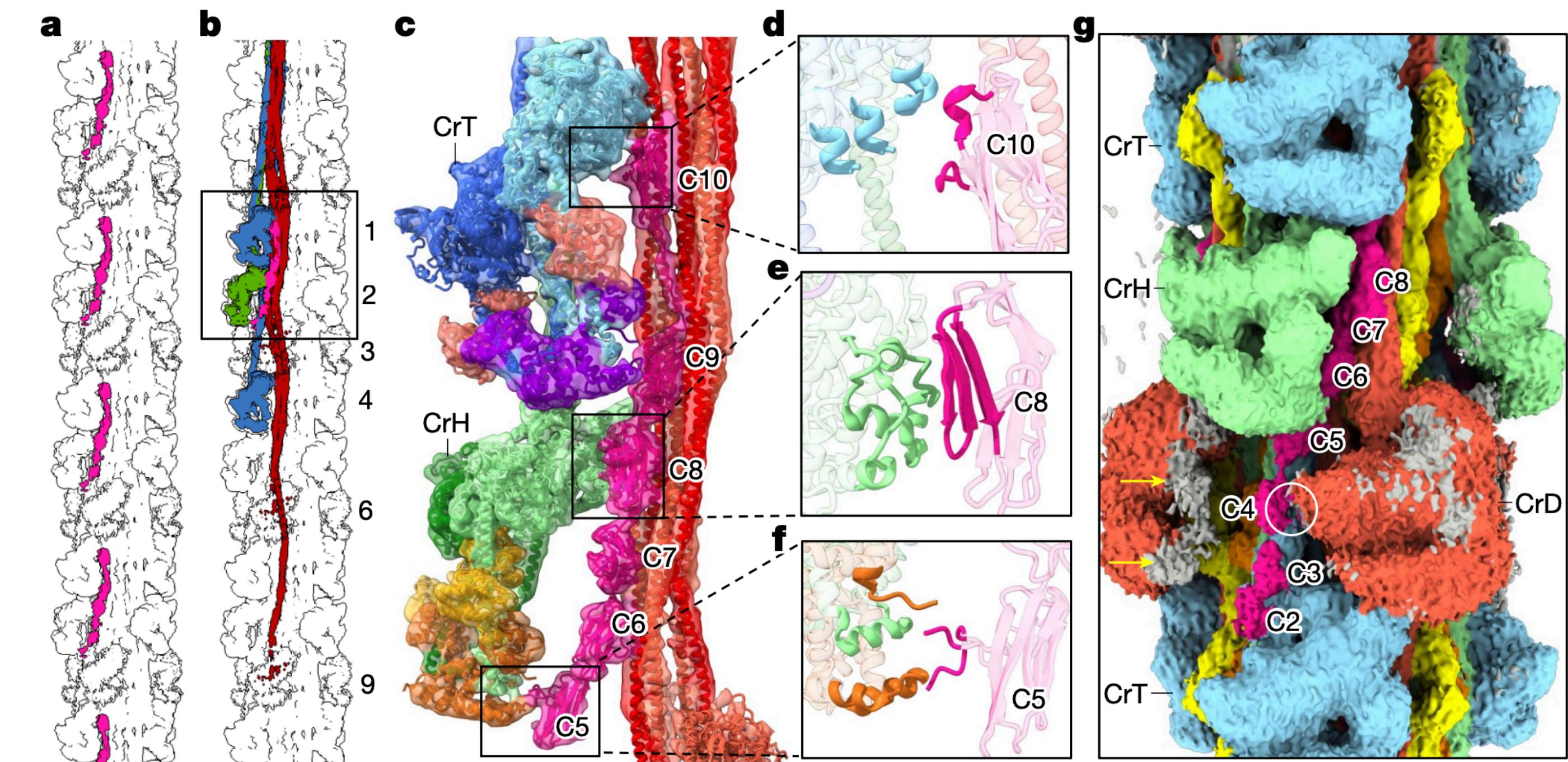
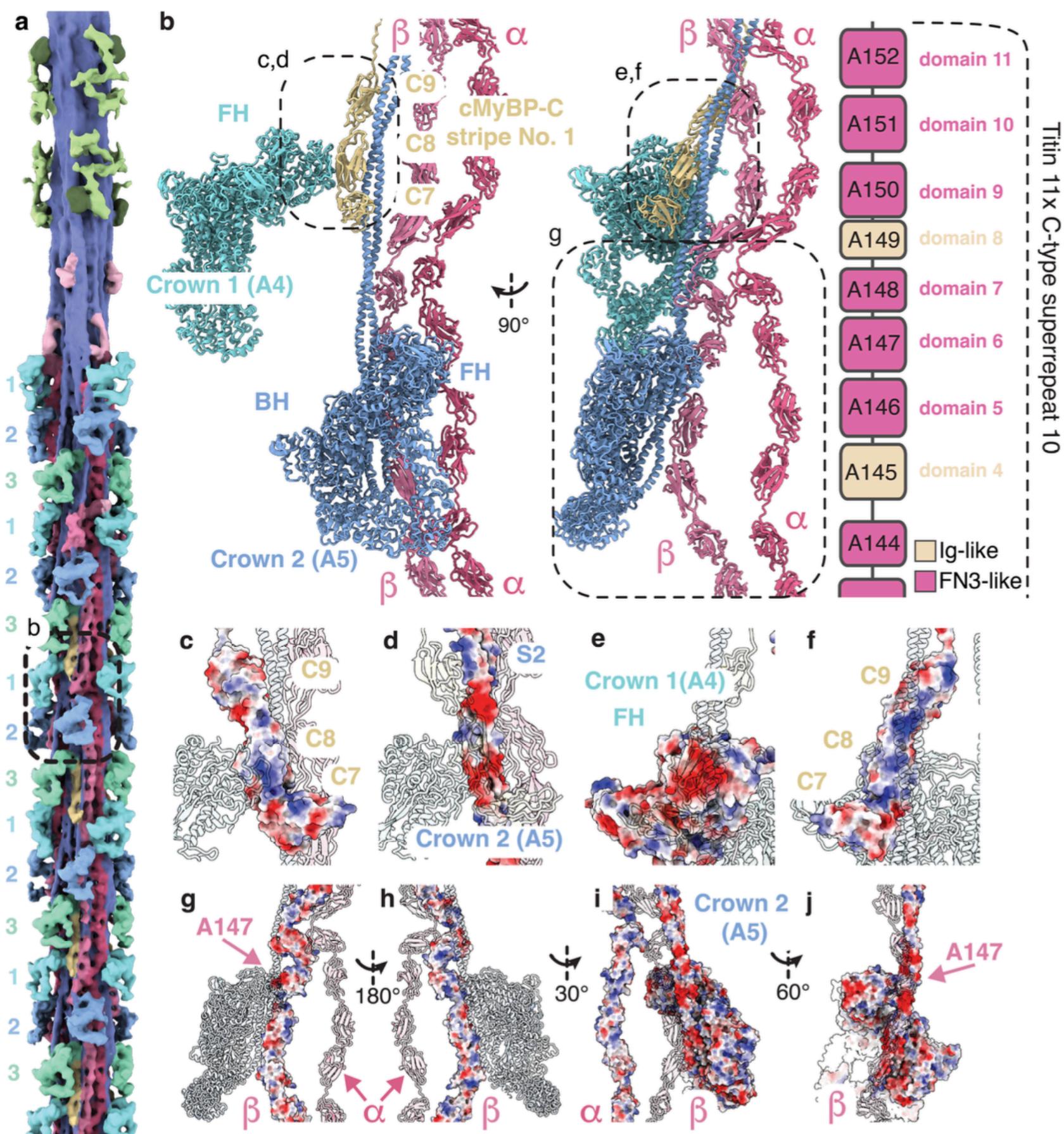
# Titin - Myosin tails



# Titin - Myosin tails

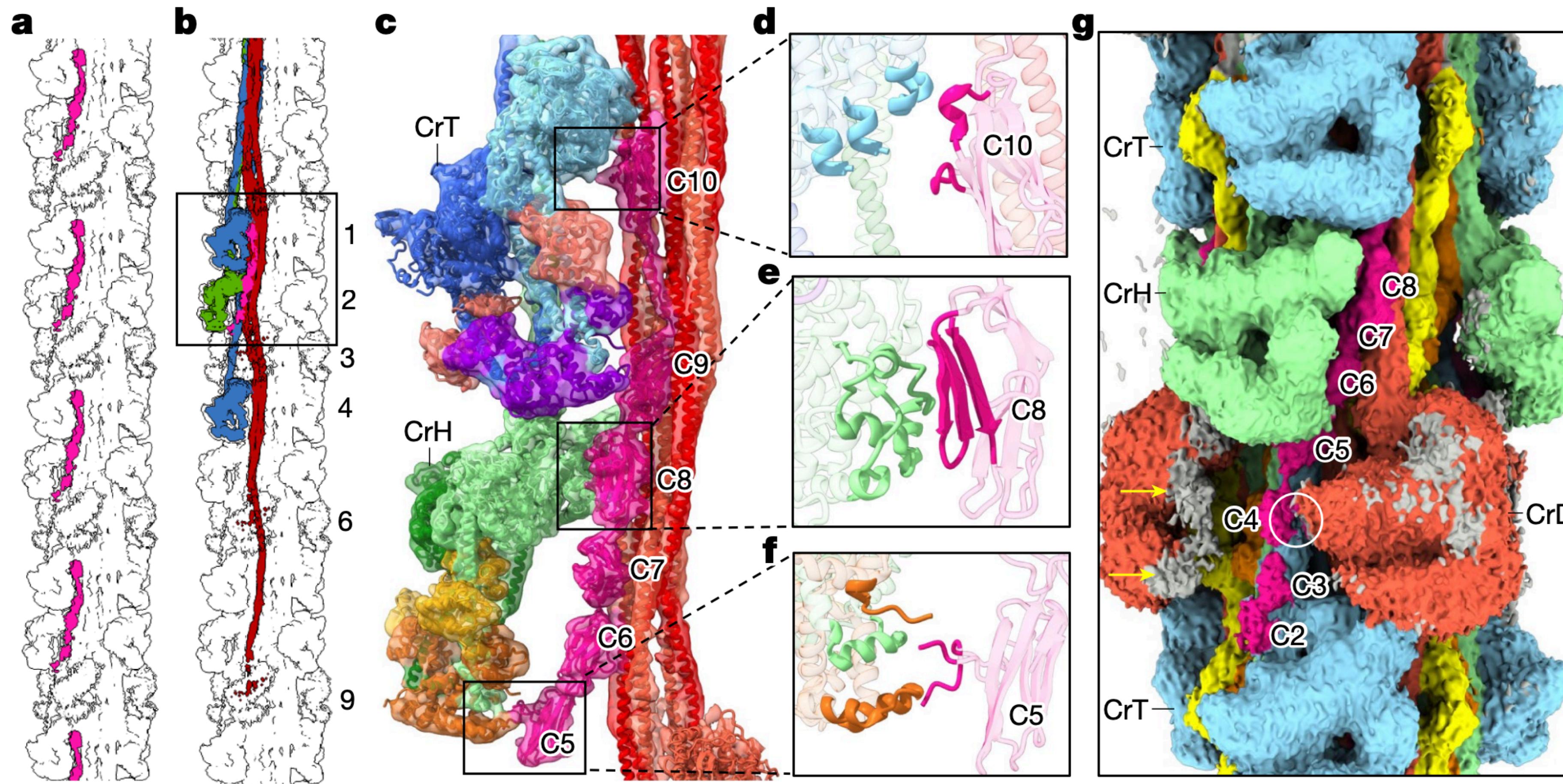


# MyBP-C - Myosin heads

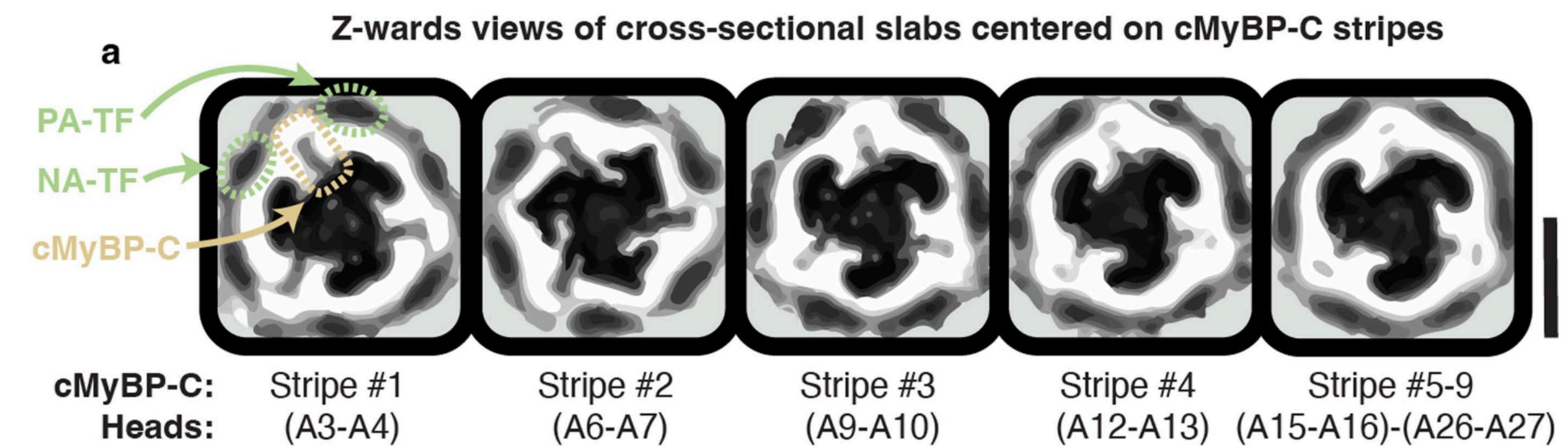
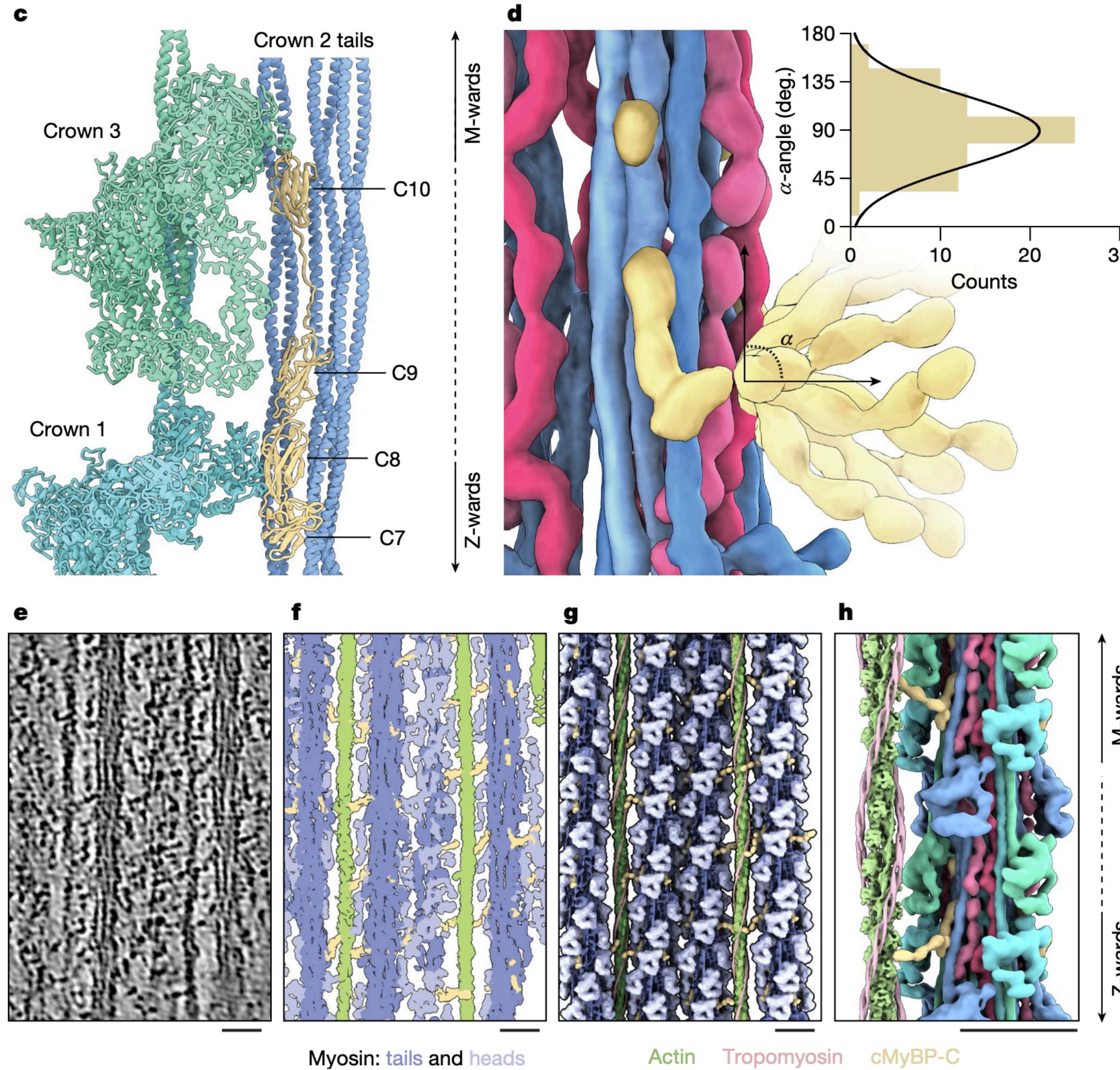


The OFF state of crowns 1 and 2 is stabilized by interactions with cMyBP-C and titin

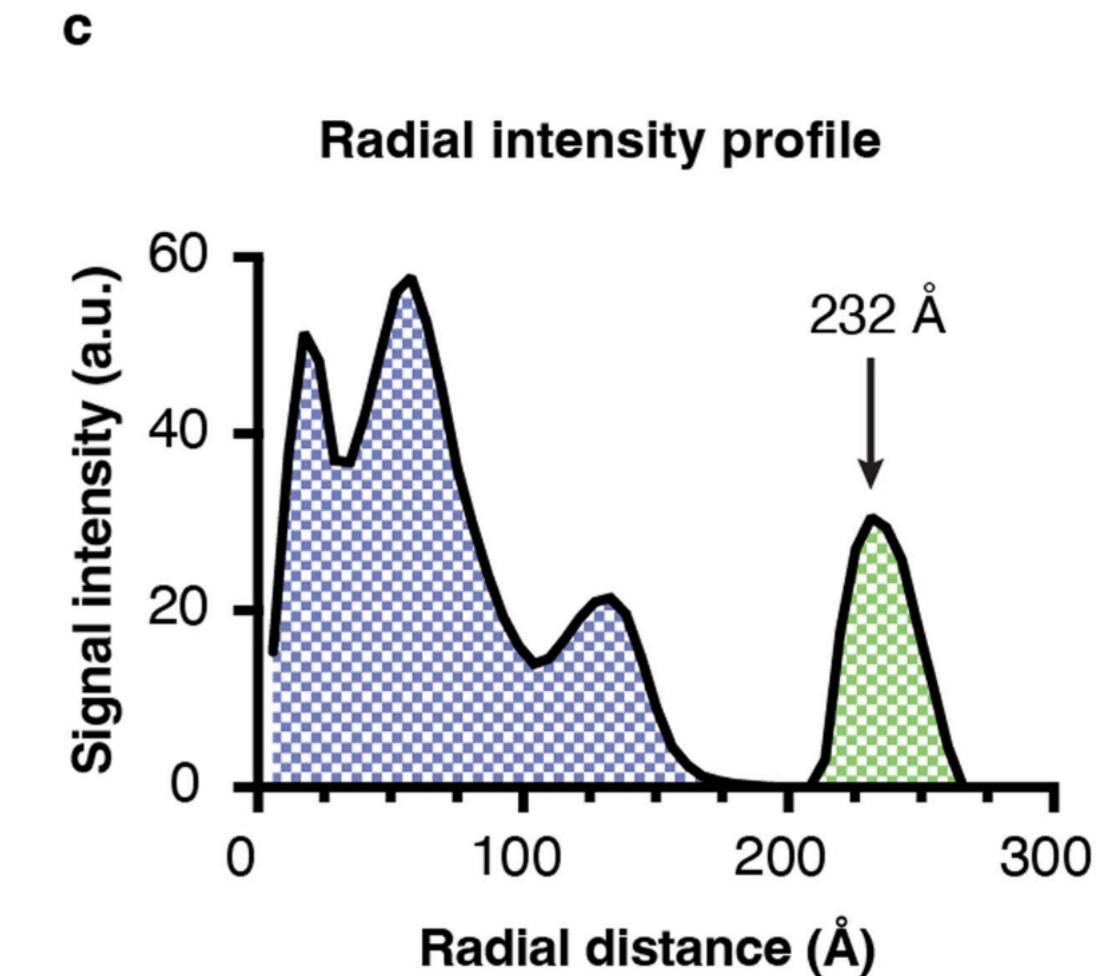
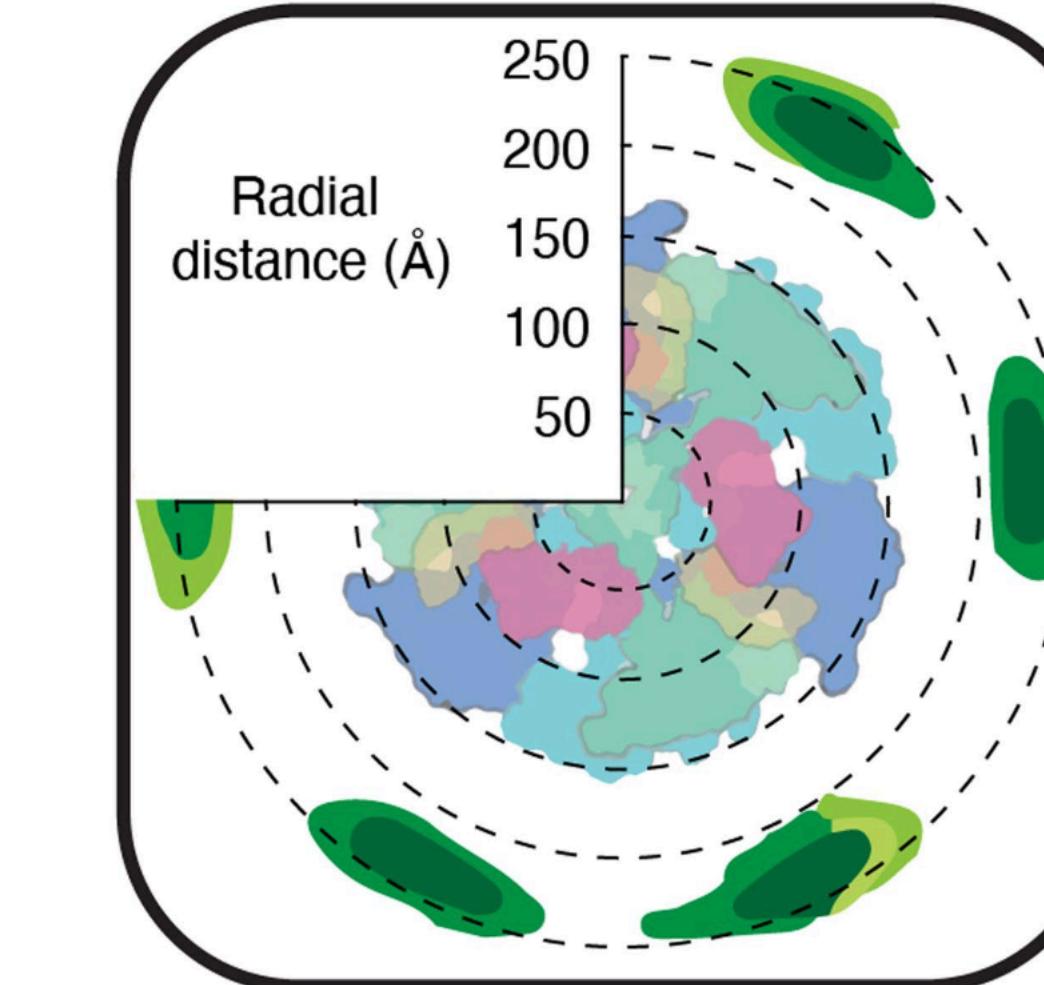
# MyBP-C - Myosin tails



# MyBP-C - Thin filaments



**b** Z-wards view of the average from all 9 cMyBP-C stripes



# Summary

- The 3D structure of thick filament *in situ* in vertebrates;
- Molecular details of the complex organization of myosin heads, tails, titin and MyBP-C;
- Visualizing critical interactions between myosin, titin and MyBP-C.