

Journal Club

黄振 11/21/2023

Article

Structure of the native myosin filament in the relaxed cardiac sarcomere

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

Received: 3 April 2023

Accepted: 28 September 2023

Published online: 01 November 2023

Open access

 Check for updates

Davide Tamborrini¹, Zhexin Wang^{1,3}, Thorsten Wagner¹, Sebastian Tacke¹, Markus Stabrin¹, Michael Grange^{1,4}, Ay Lin Kho², Martin Rees², Pauline Bennett², Mathias Gautel² & Stefan Raunser¹✉

The thick filament is a key component of sarcomeres, the basic units of striated muscle¹. Alterations in thick filament proteins are associated with familial hypertrophic cardiomyopathy and other heart and muscle diseases². Despite the central importance

Article

Cryo-EM structure of the human cardiac myosin filament

<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

Debabrata Dutta¹✉, Vu Nguyen¹, Kenneth S. Campbell², Raúl Padrón¹✉ & Roger Craig¹✉

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

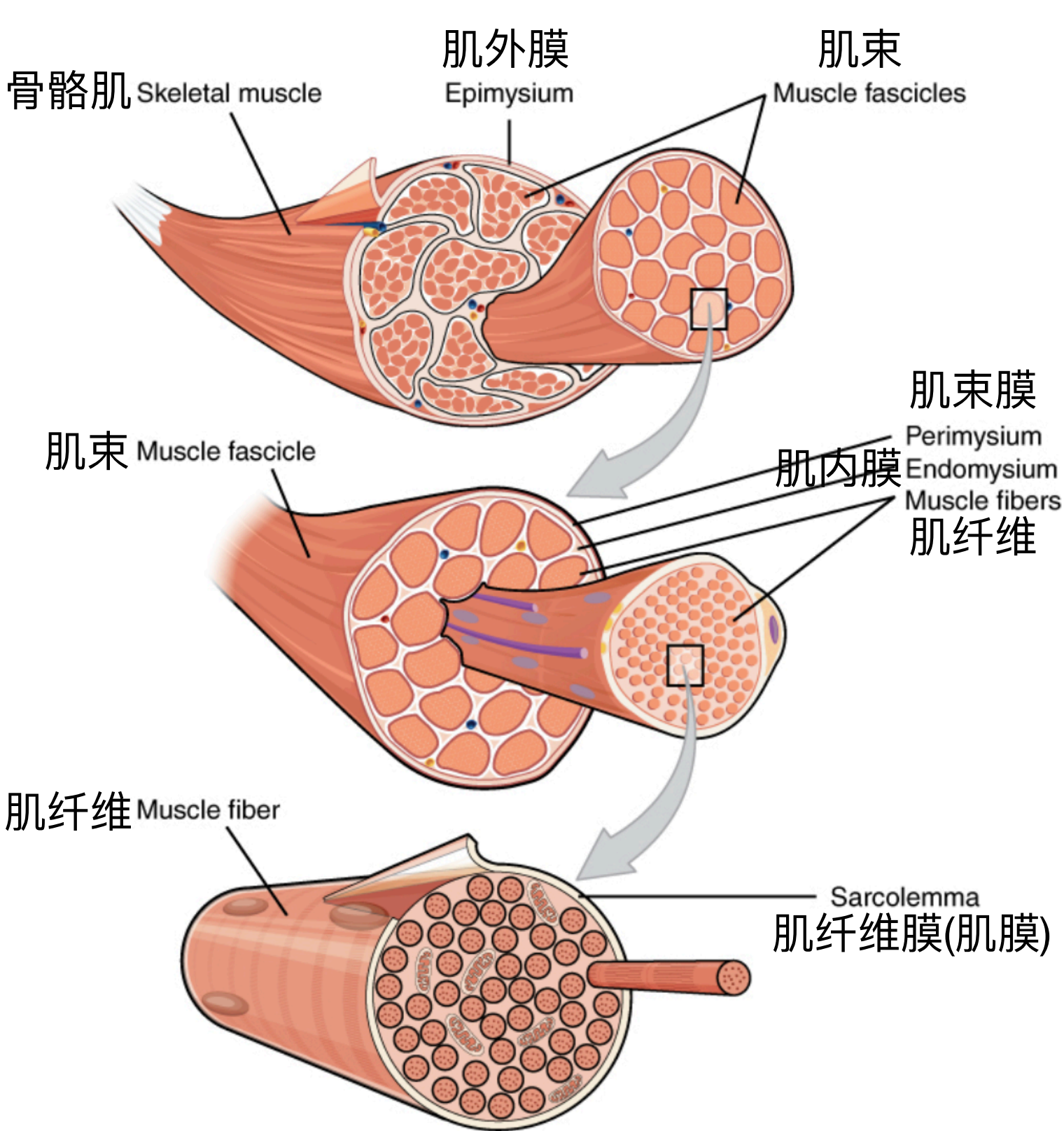


Figure 1. The Three Connective Tissue Layers. Bundles of muscle fibers, called fascicles, are covered by the perimysium. Muscle fibers are covered by the endomysium.

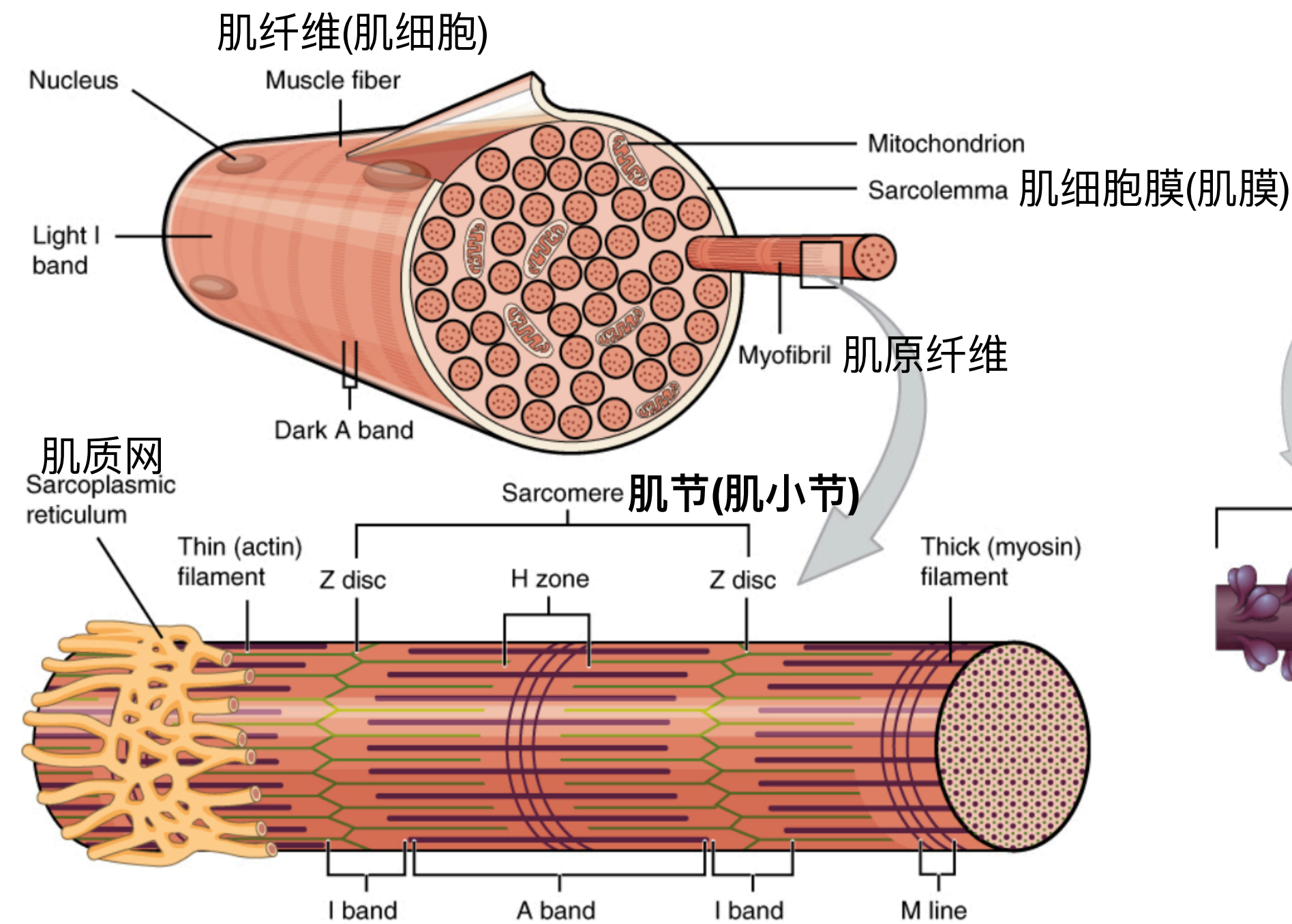


Figure 2. Muscle Fiber. A skeletal muscle fiber is surrounded by a plasma membrane called the sarcolemma, which contains sarcoplasm, the cytoplasm of muscle cells. A muscle fiber is composed of many fibrils, which give the cell its striated appearance.

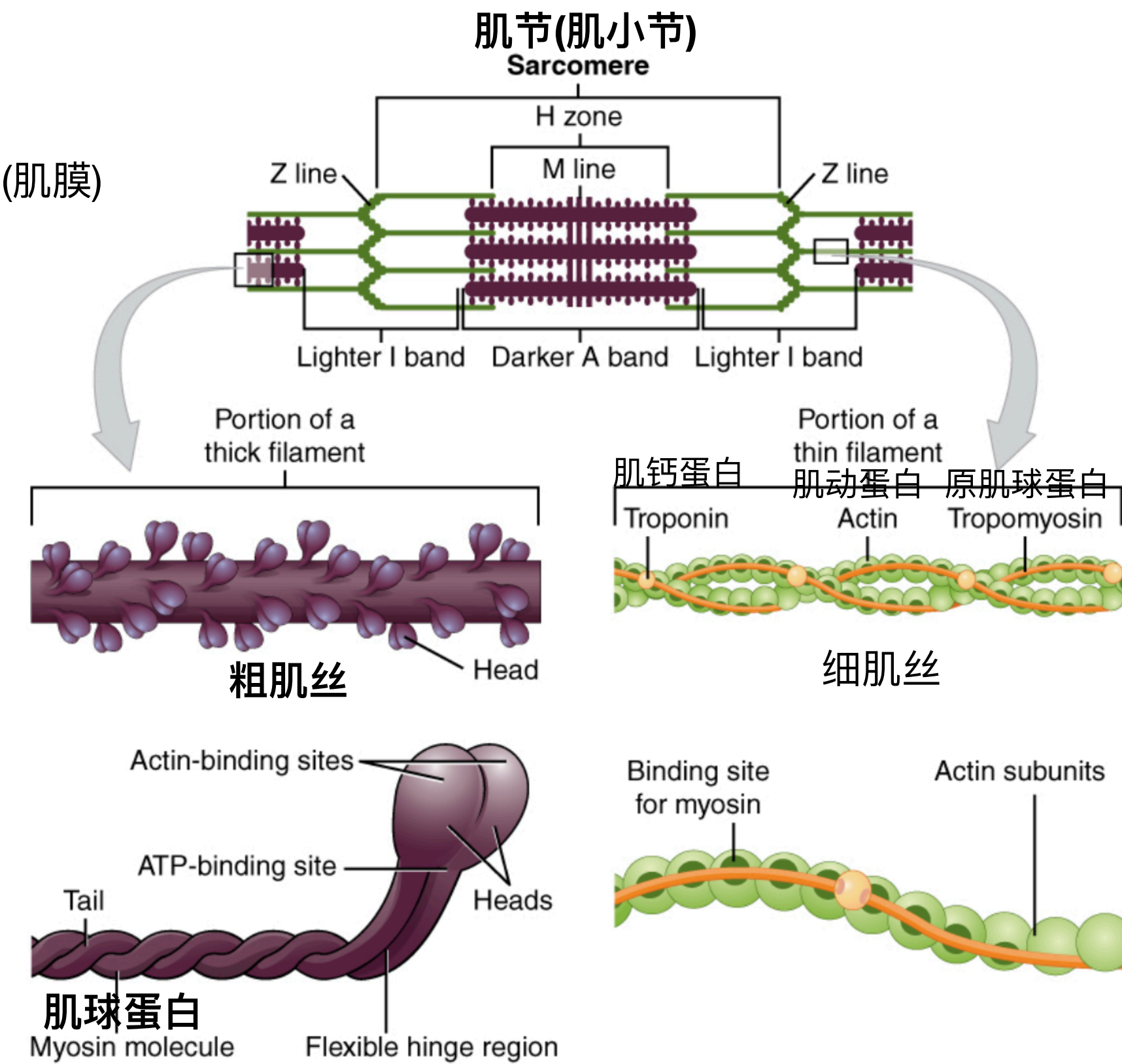
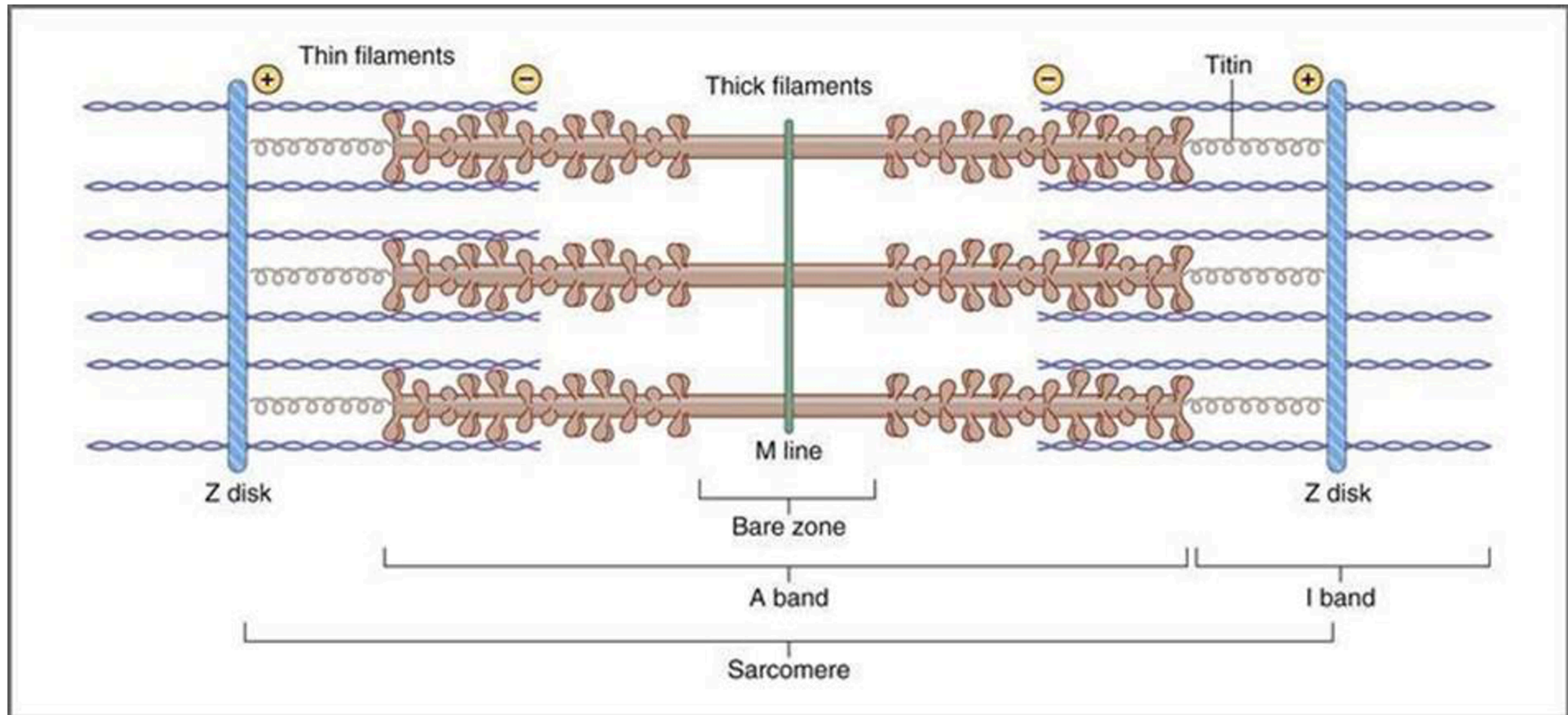


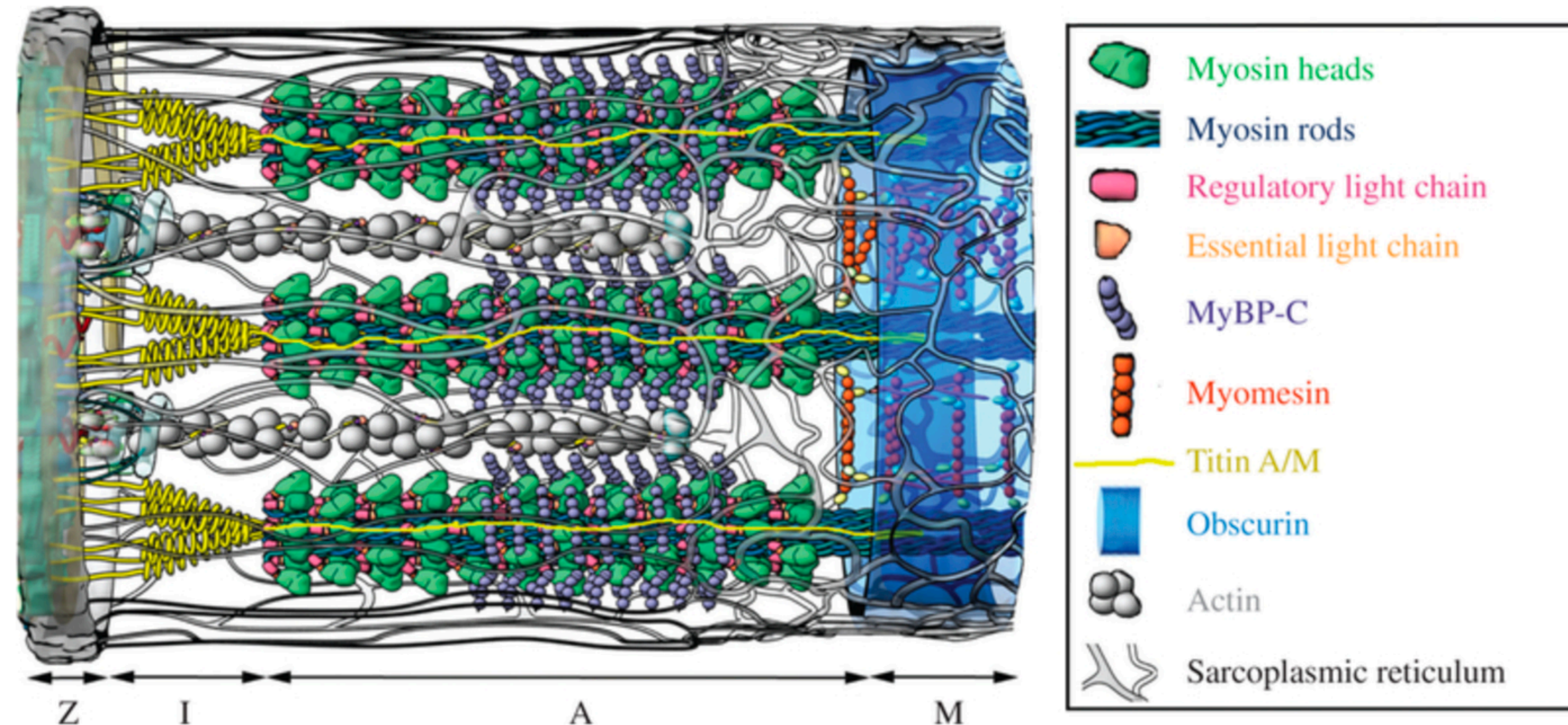
Figure 3. The Sarcomere. The sarcomere, the region from one Z-line to the next Z-line, is the functional unit of a skeletal muscle fiber.

Sarcomere and Thick filaments



Thick Filament Protein Network

Figure 1



- myosin II
- MyBP-C
- Titin
- Others near BZ

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 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

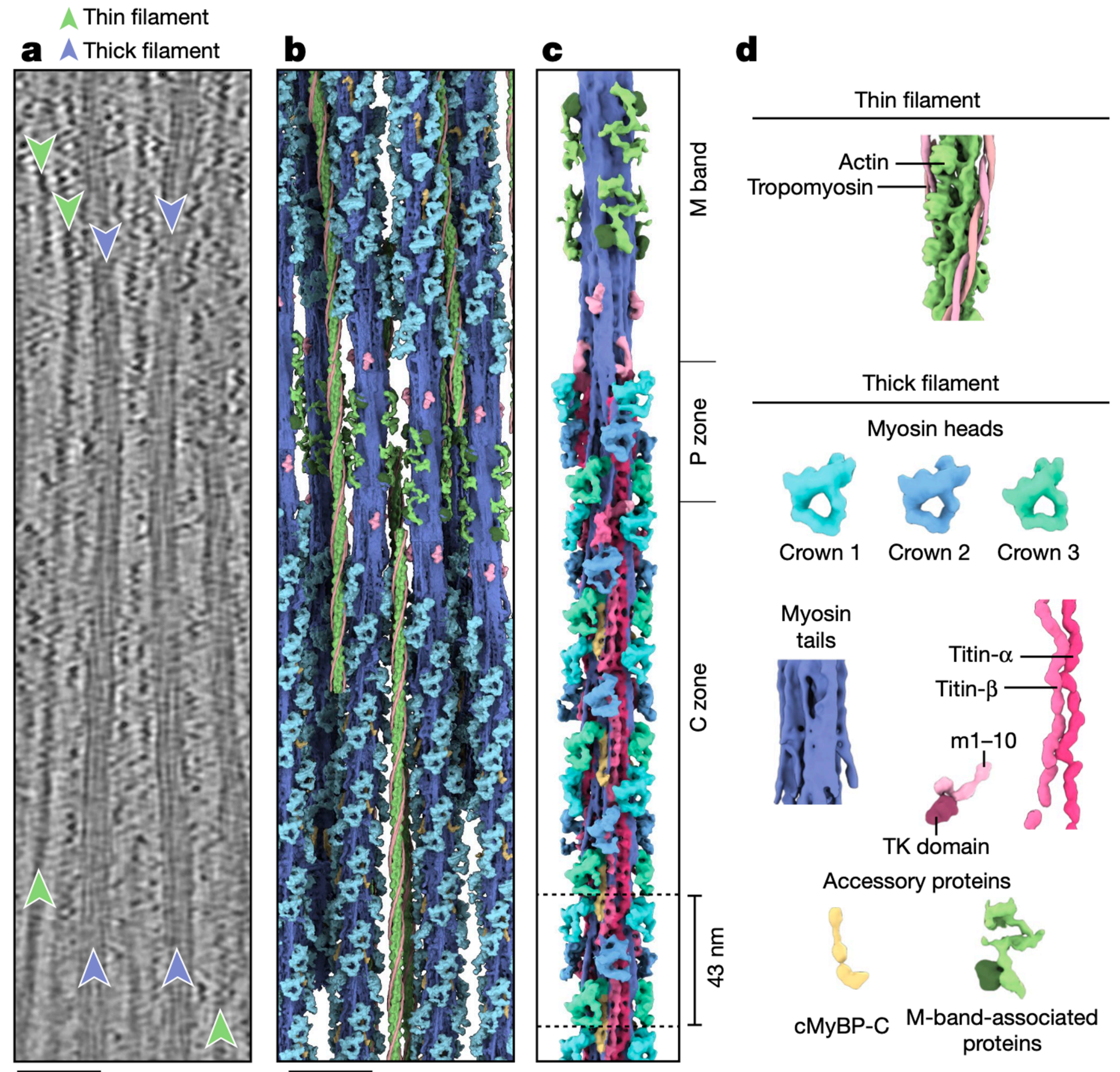
cryo-EM:

- Human cardiac ventricular tissue;
- Mavacamten

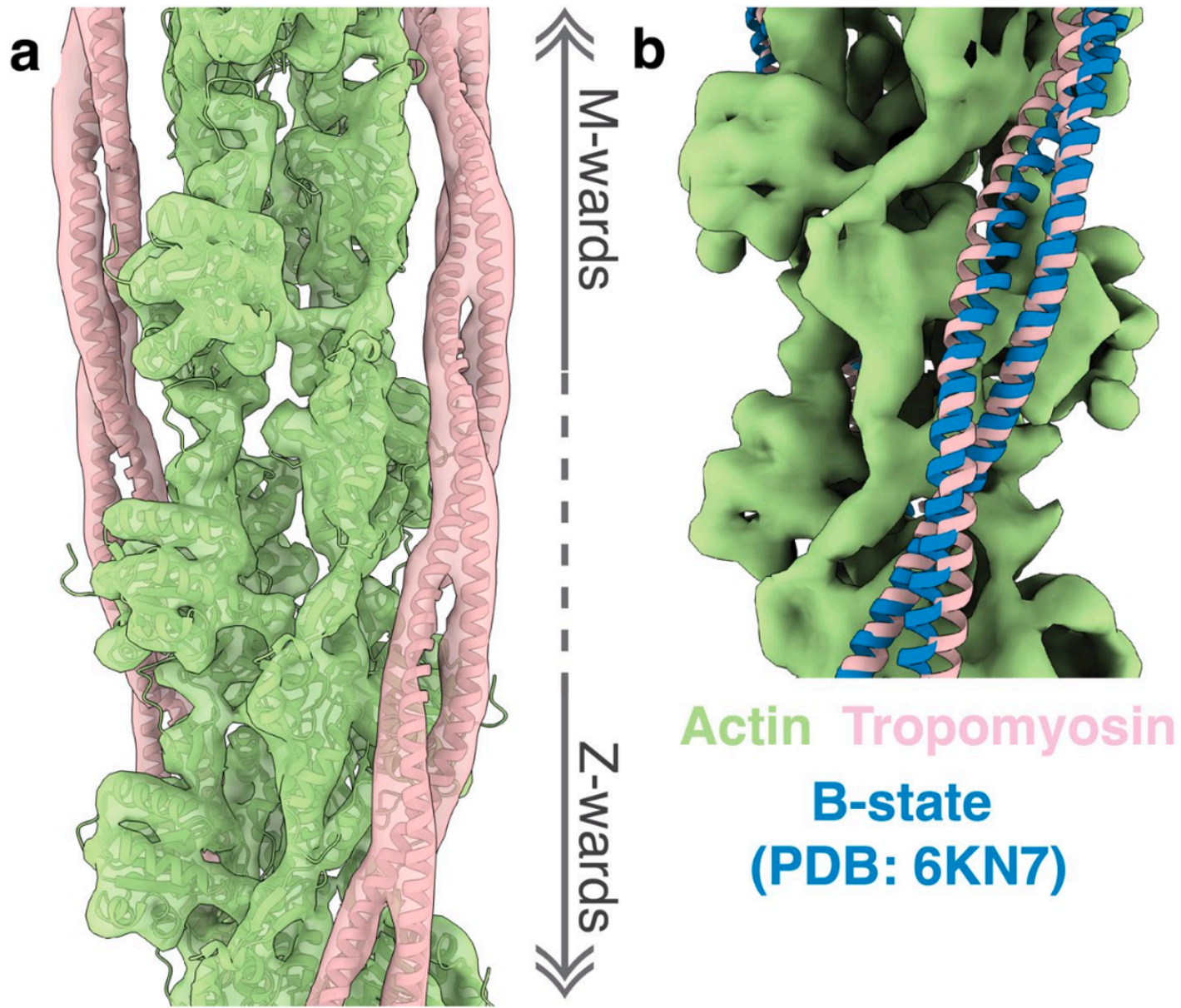
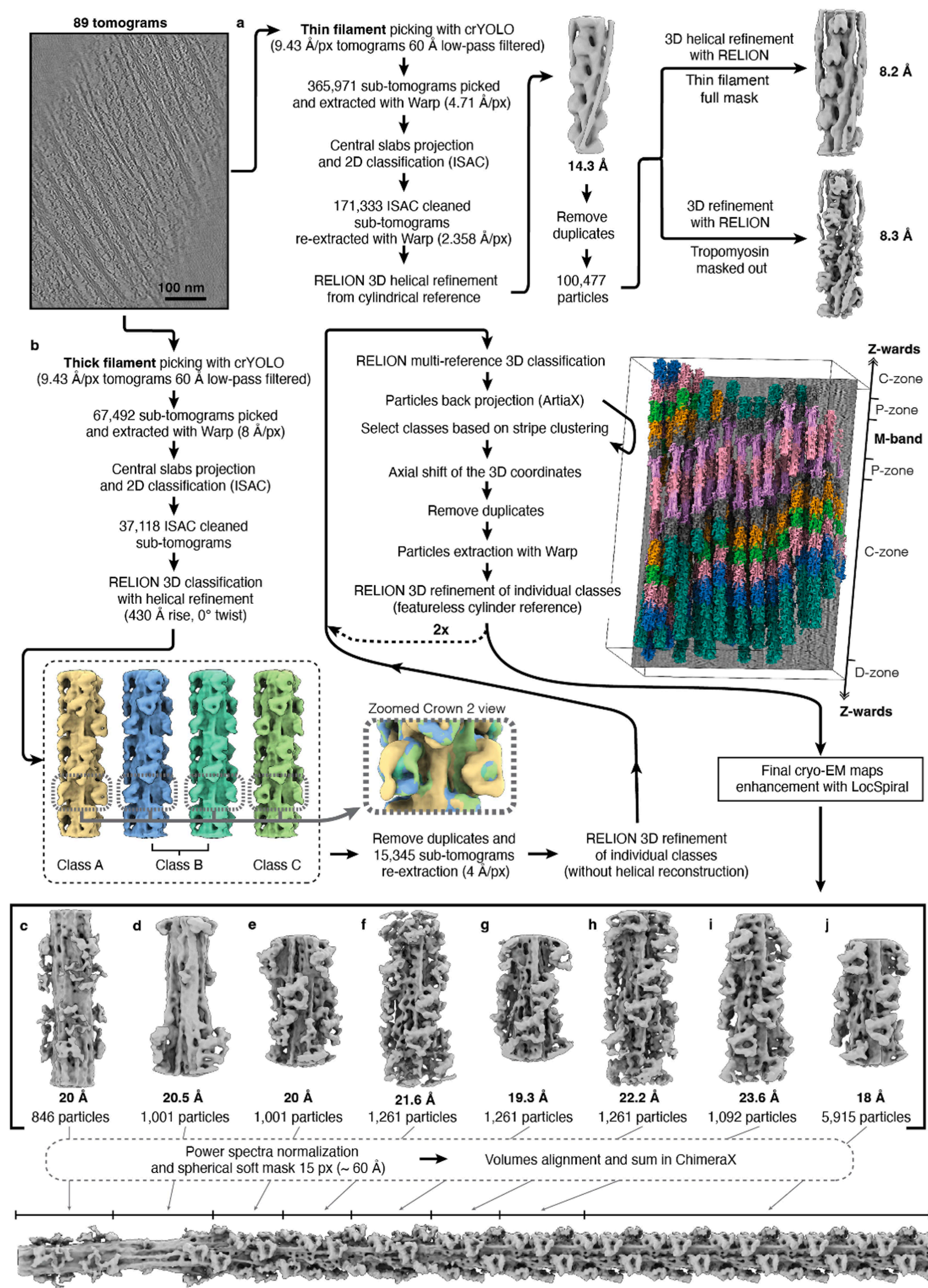
Results in detail

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



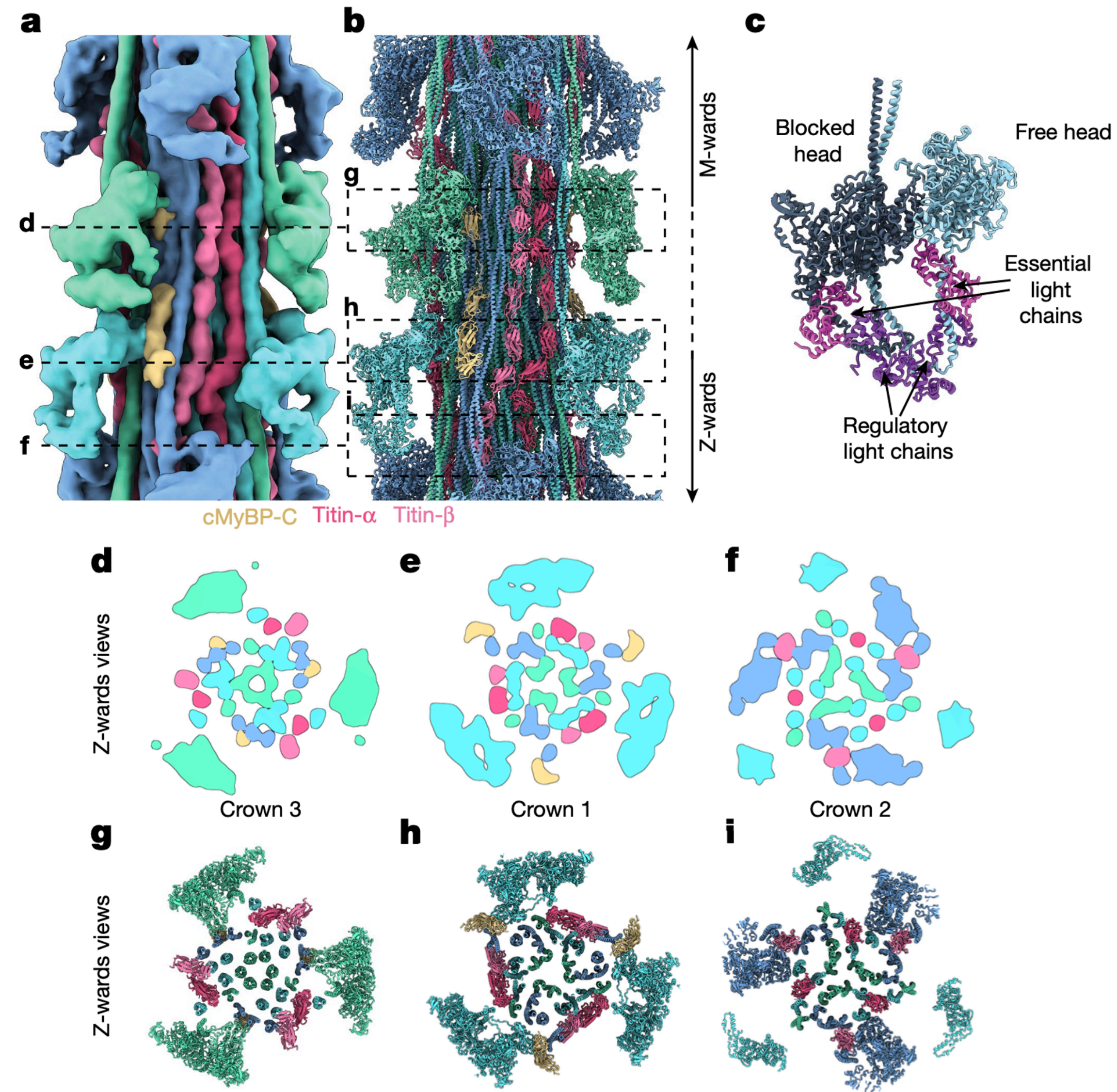
Extended Data Fig. 3 | In situ structure of the thin filament in the Ca^{2+} free state. **a, Subtomogram-averaged structure of the thin filament in the relaxed sarcomere. **b**, Model of the B-state of tropomyosin (PDB: 6KN7) is shown for comparison.**

Extended Data Fig. 1 | Data acquisition and processing pipeline. **a,b, Acquisition and processing of the thin (**a**) and thick filament (**b**). **c-j**, Cryo-EM enhanced maps for: M-band (**c**), A-M transition to crown P1 (**d**), from crown P2 to A1 (**e**), from crown A1 to A5 (**f**), from crown A5 to A7 (**g**), from crown A8 to A12 (**h**), from crown A11 to A15 (**i**), and the combined segments from crown A15 to A29 (**j**).**

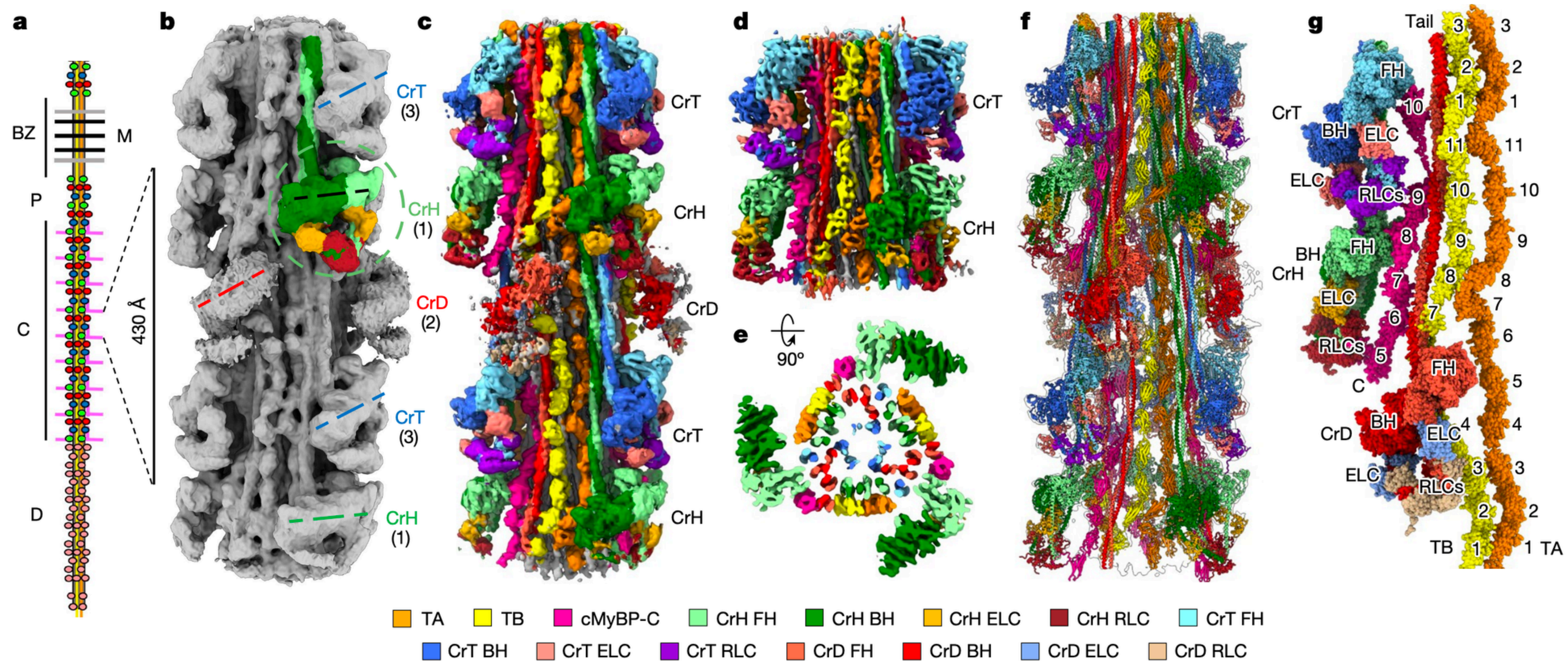
Structures

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

Structure model of the C-zone

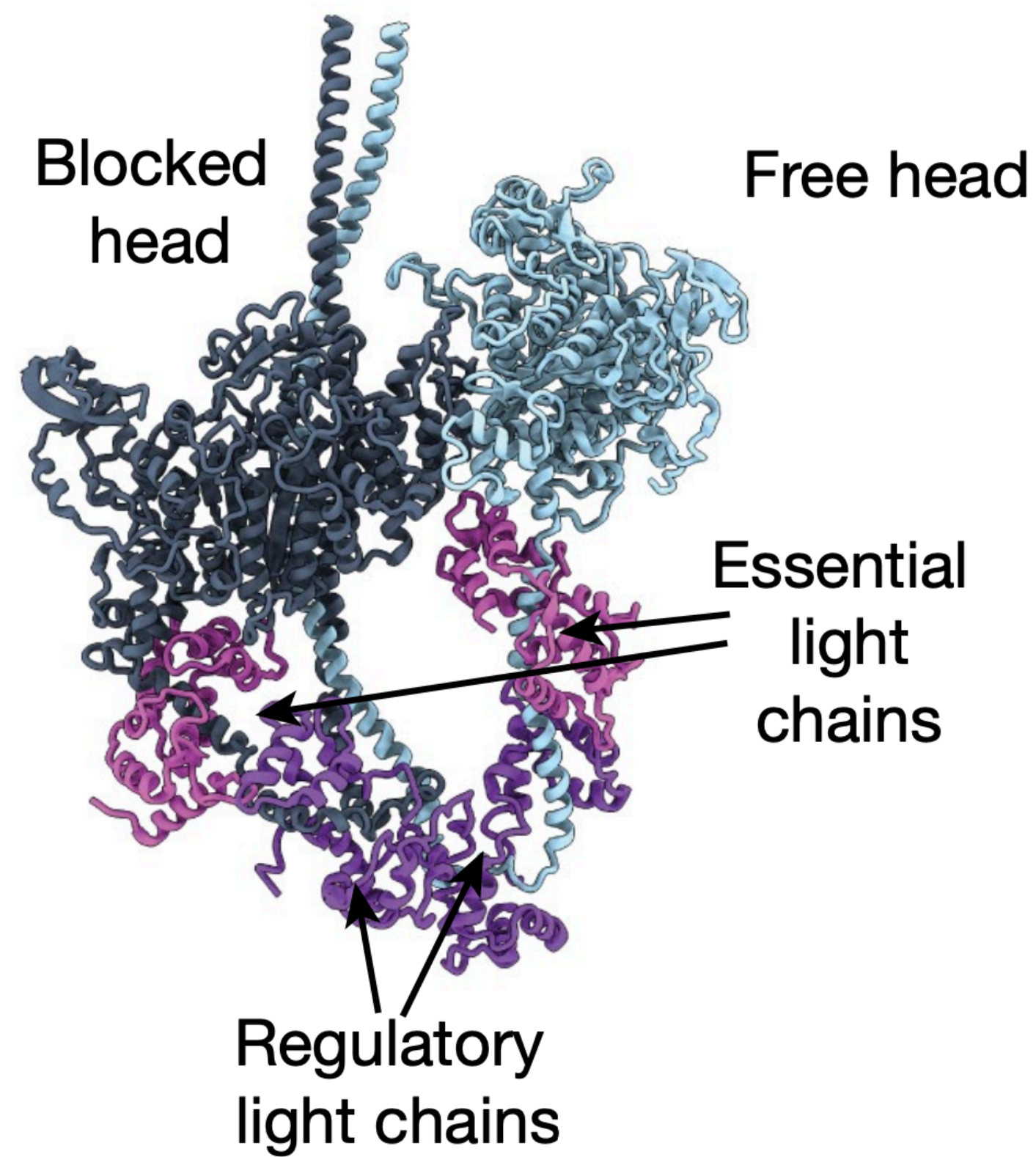


Structure model of the C-zone

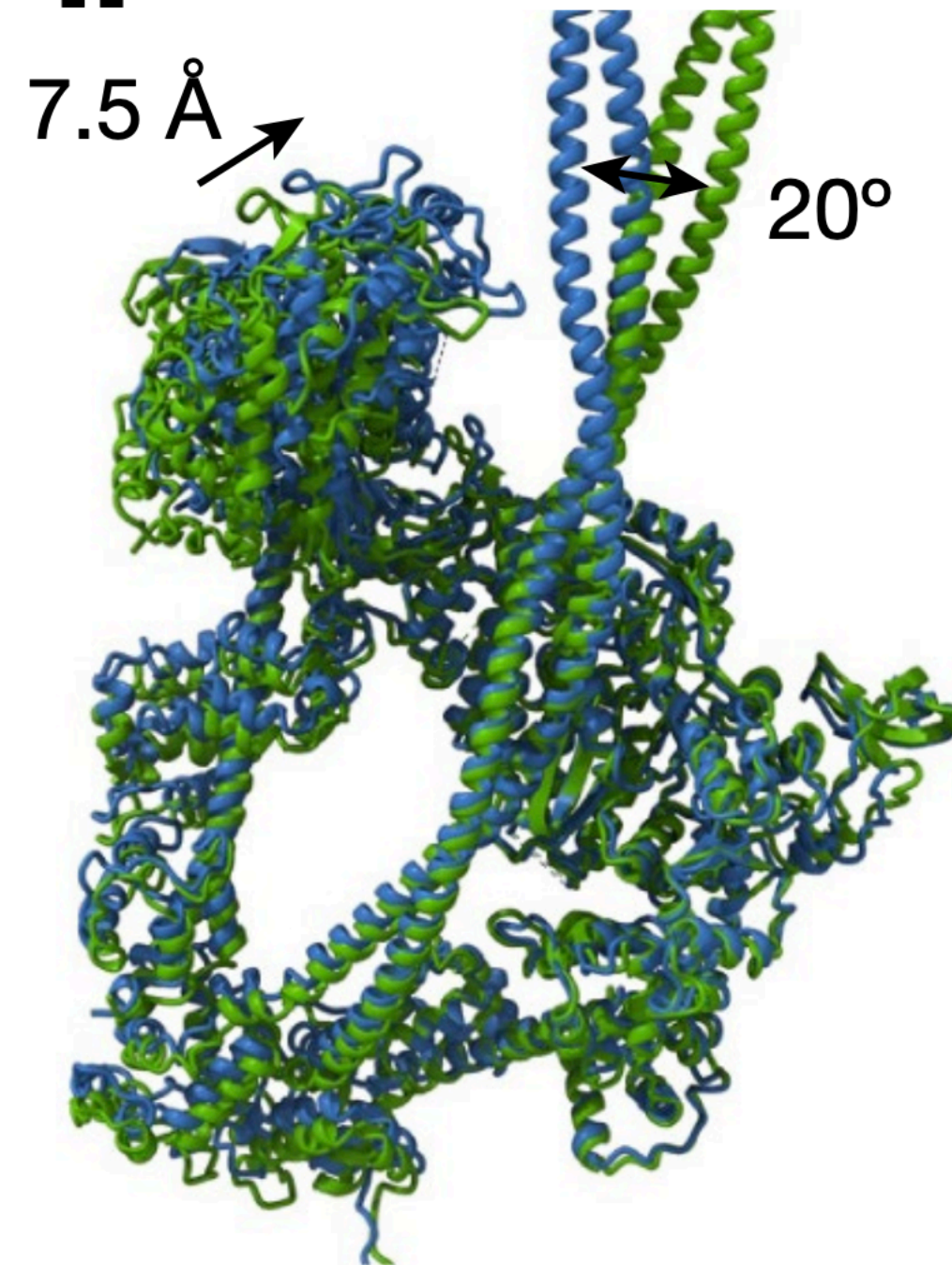


Myosin heads and tails

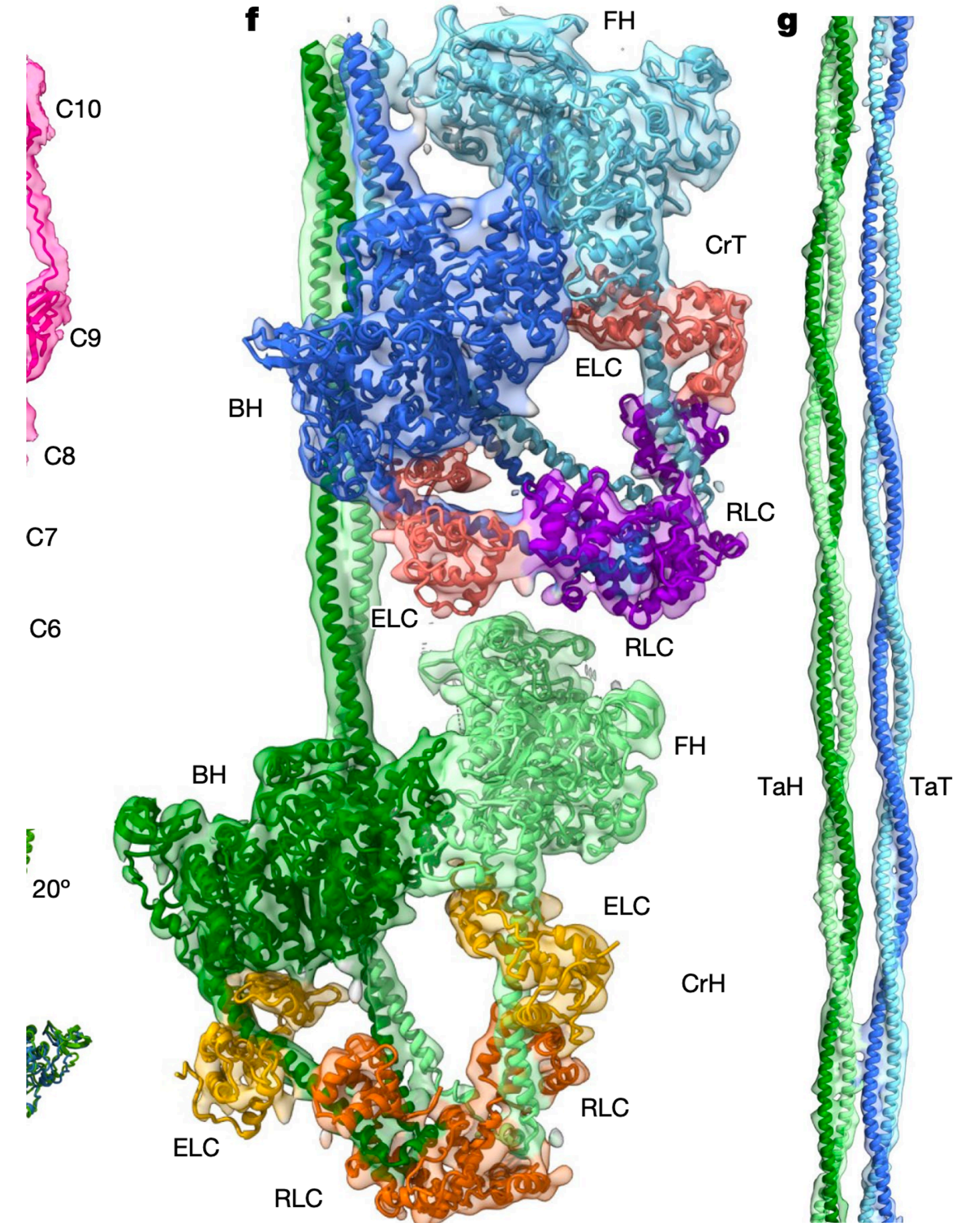
c



h



f

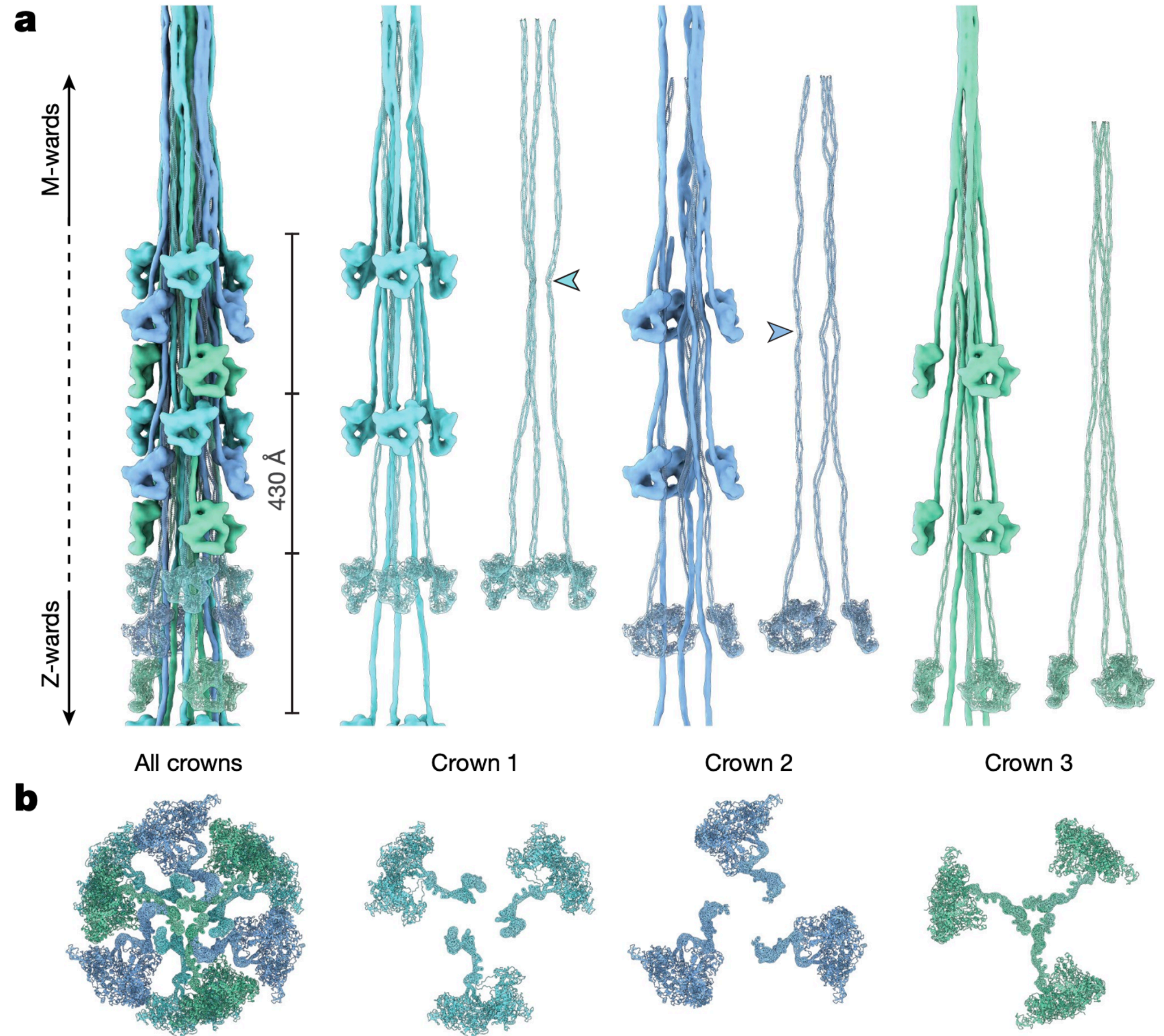
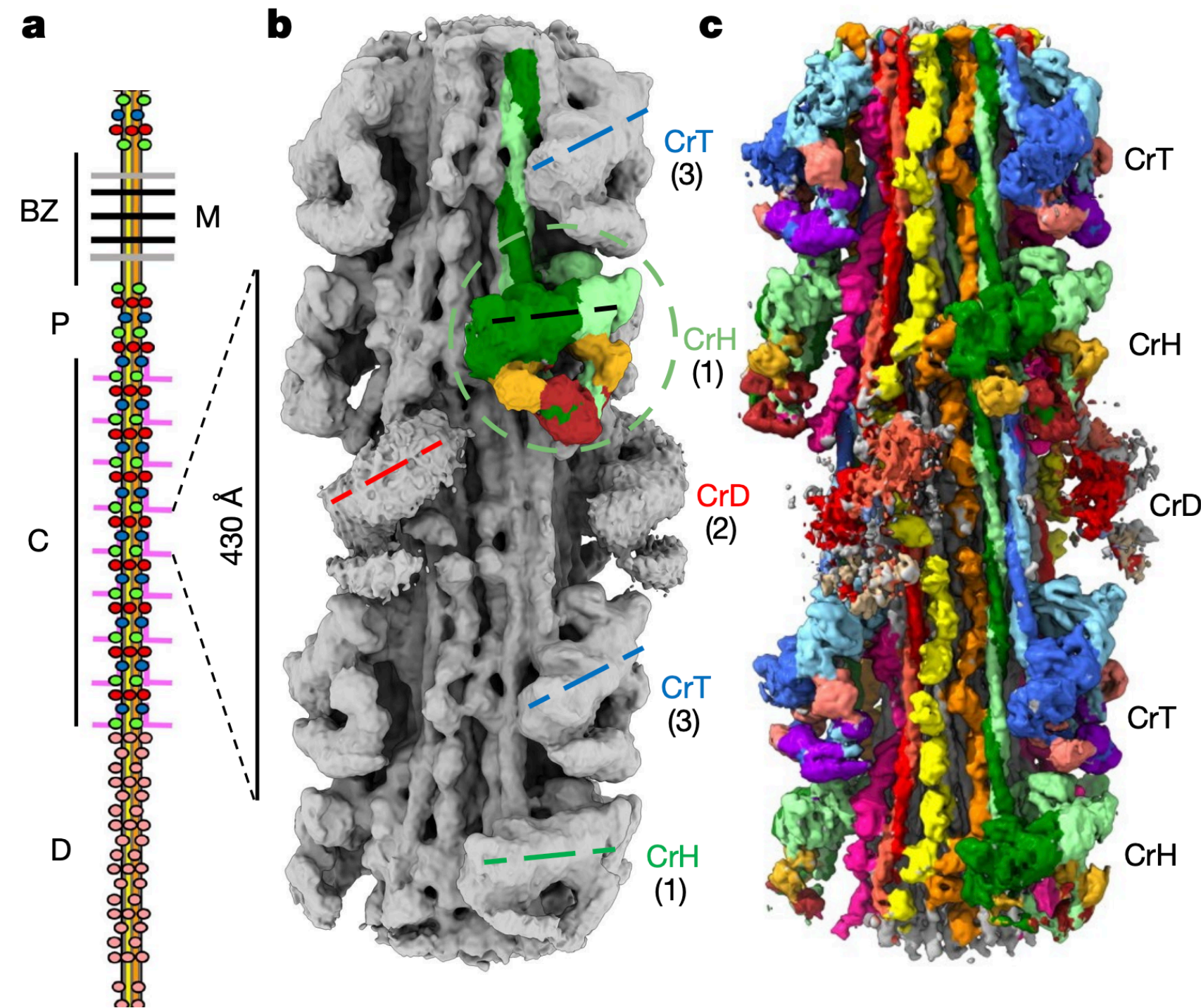


g

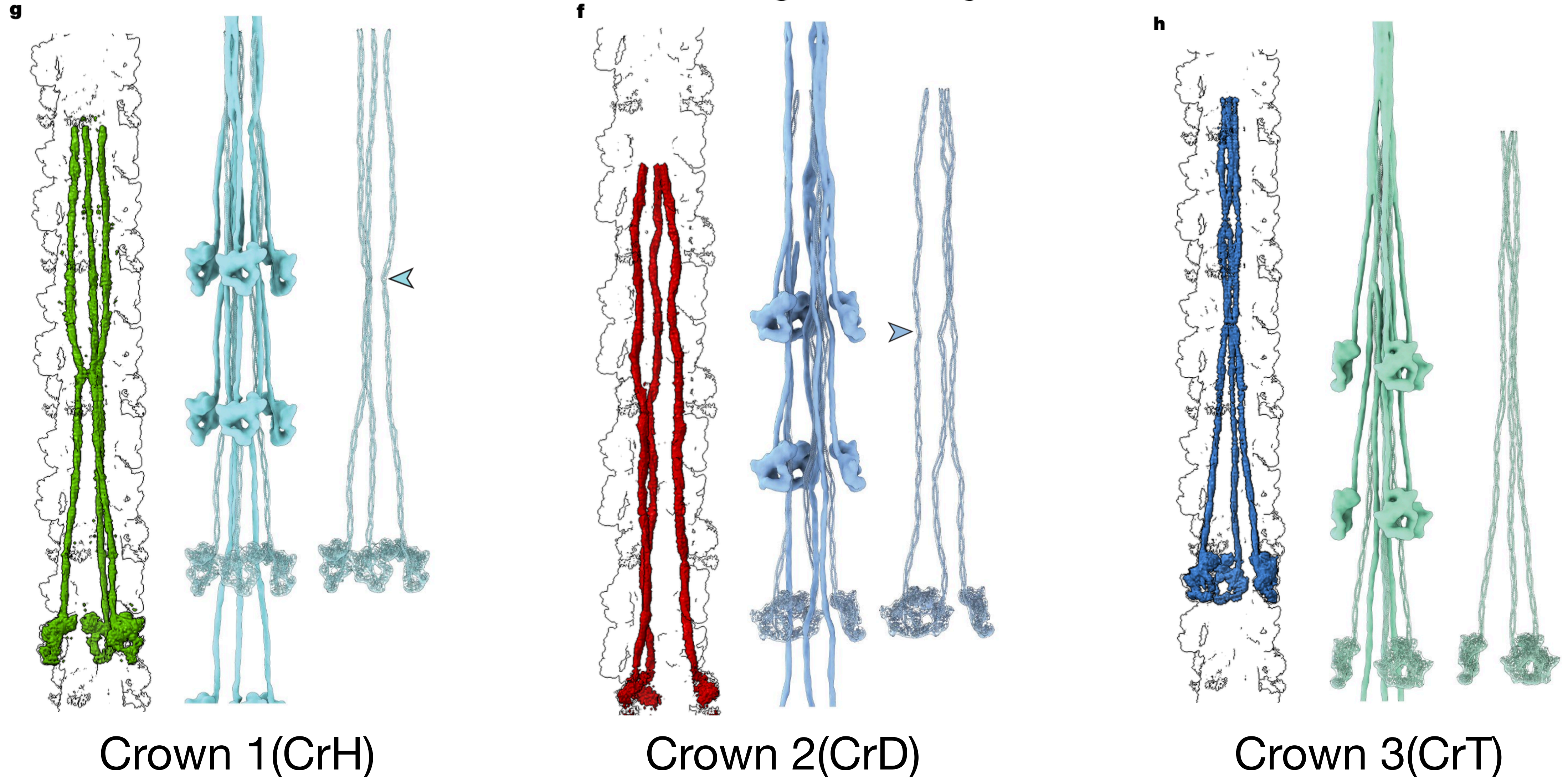
The arrangement of myosin within the C-zone

3 crowns:

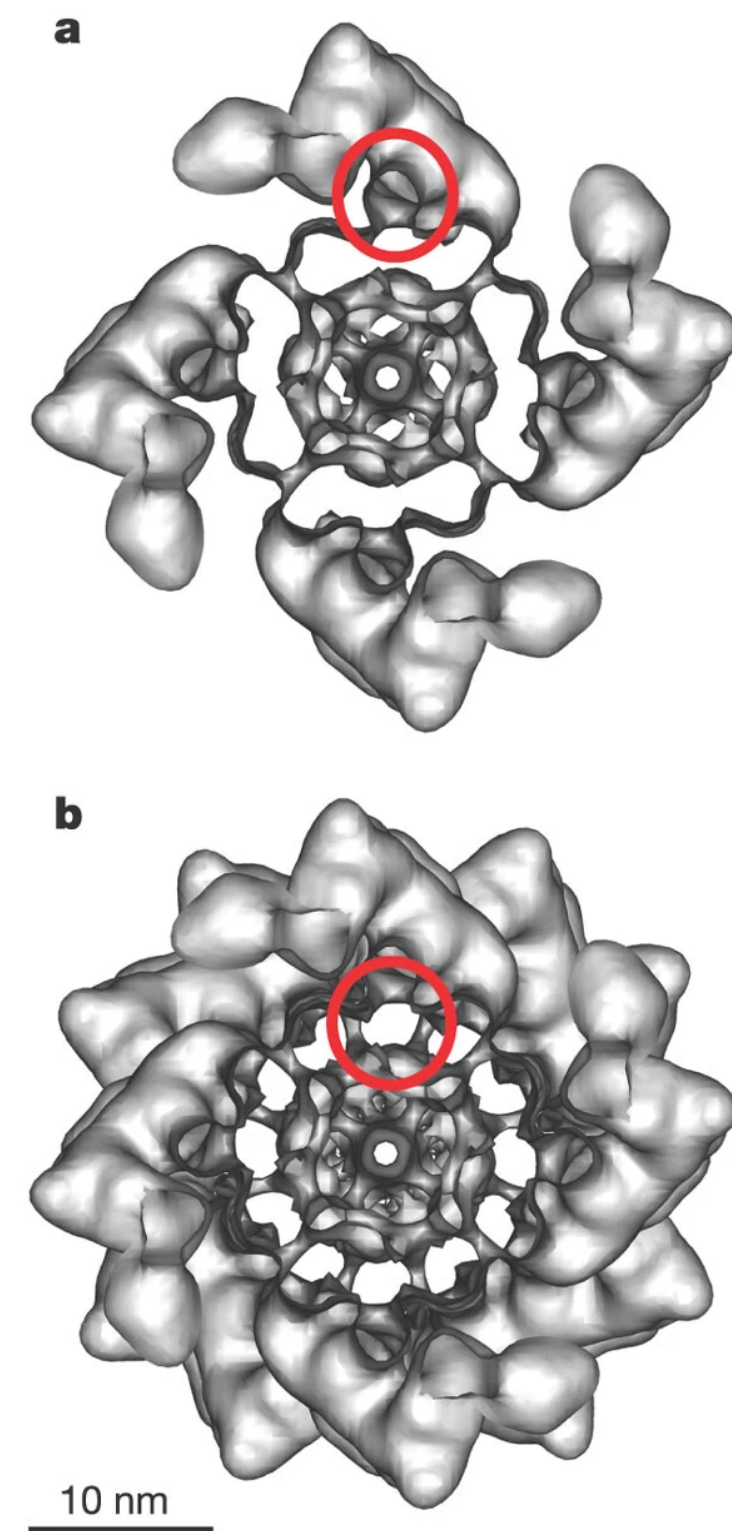
- CrD: crown 2, disordered
- CrT: crown 3, tilted
- CrH: crown 1, horizontal



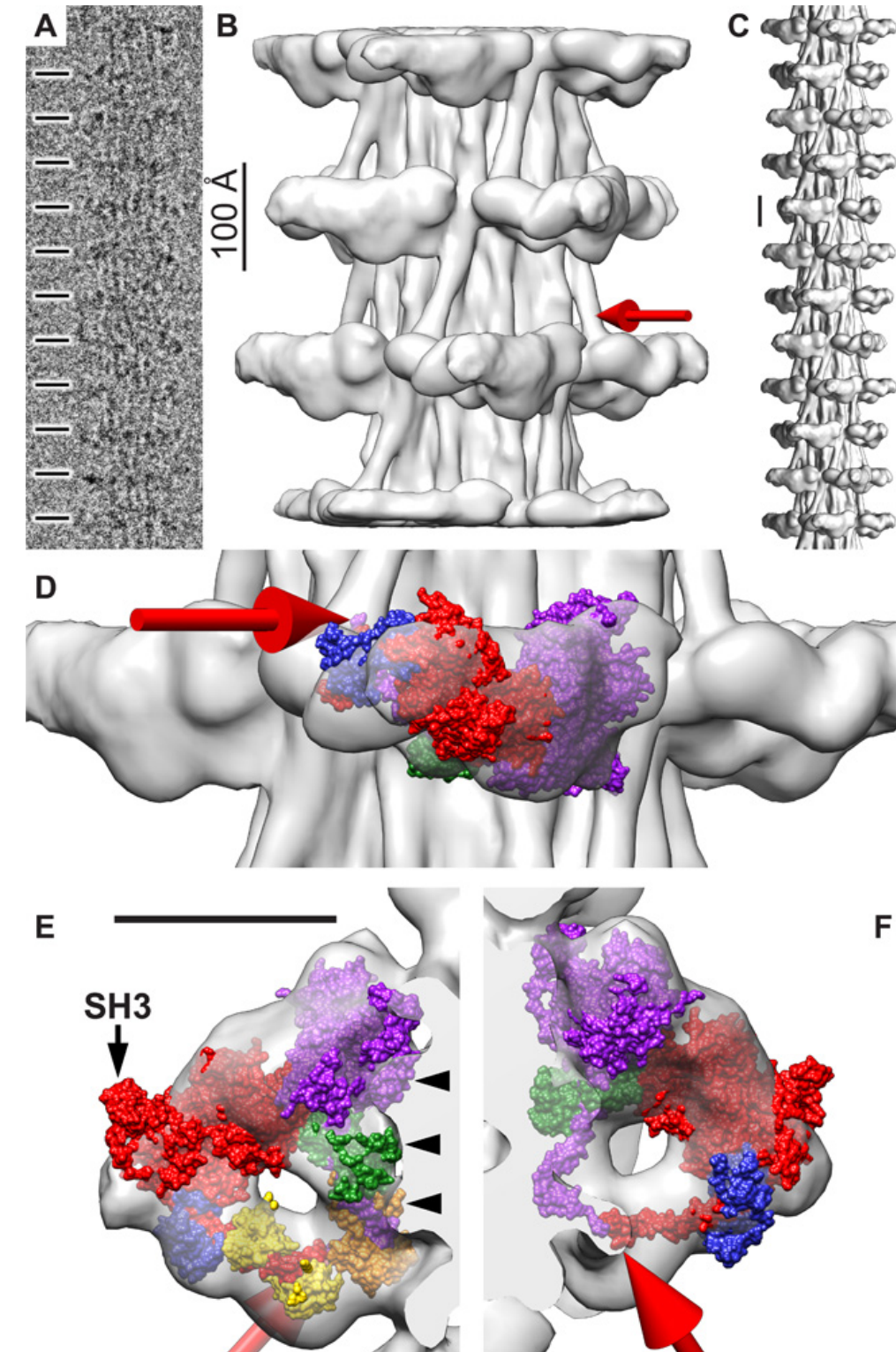
The arrangement of myosin within the C-zone (left: SPA, right: cryo-ET)



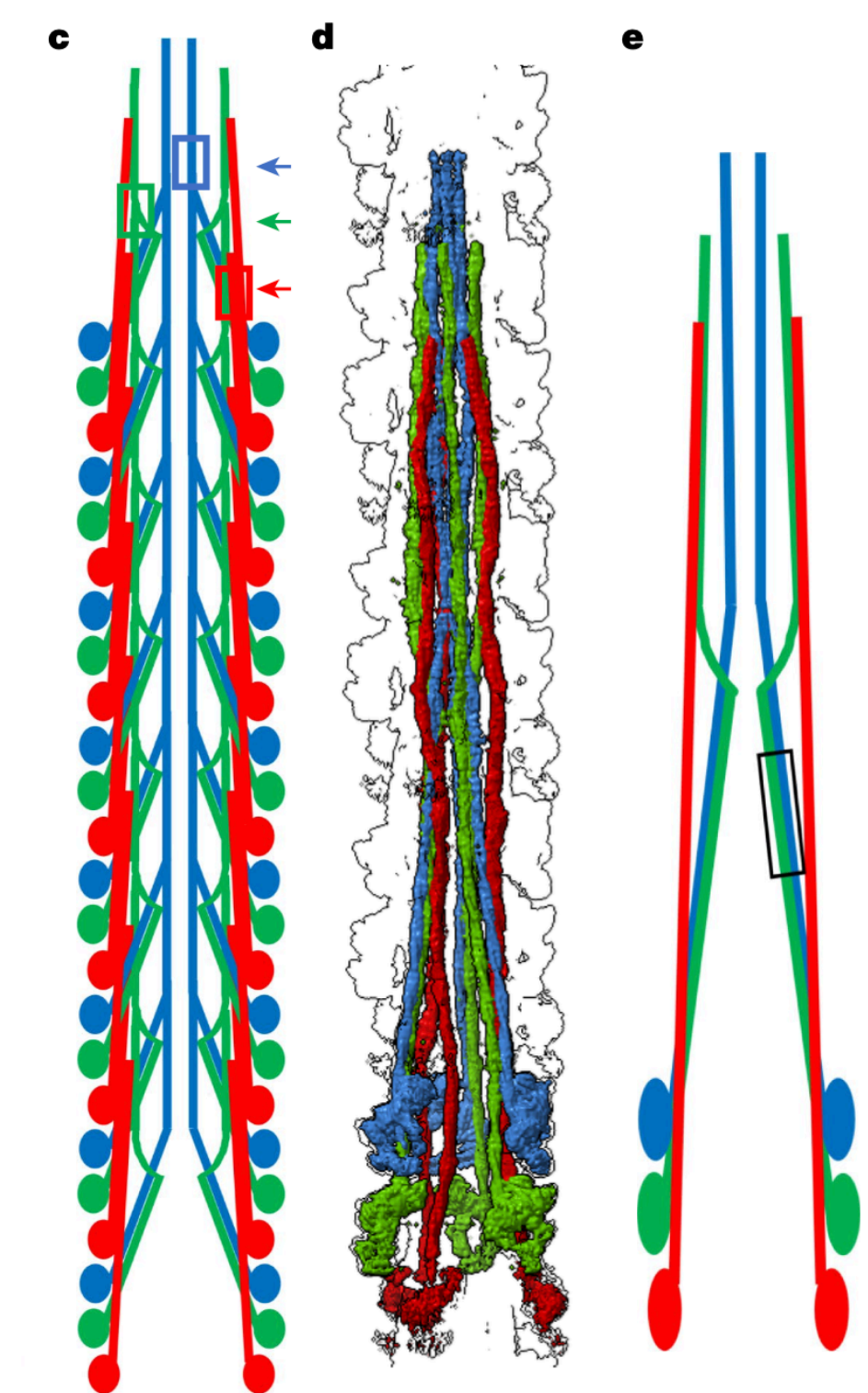
Different arrangement of Myosin in vertebrate and invertebrate



Tarantula(狼蛛), *Nature* 2005



Giant water bug(负子蝽), *Science* 2016



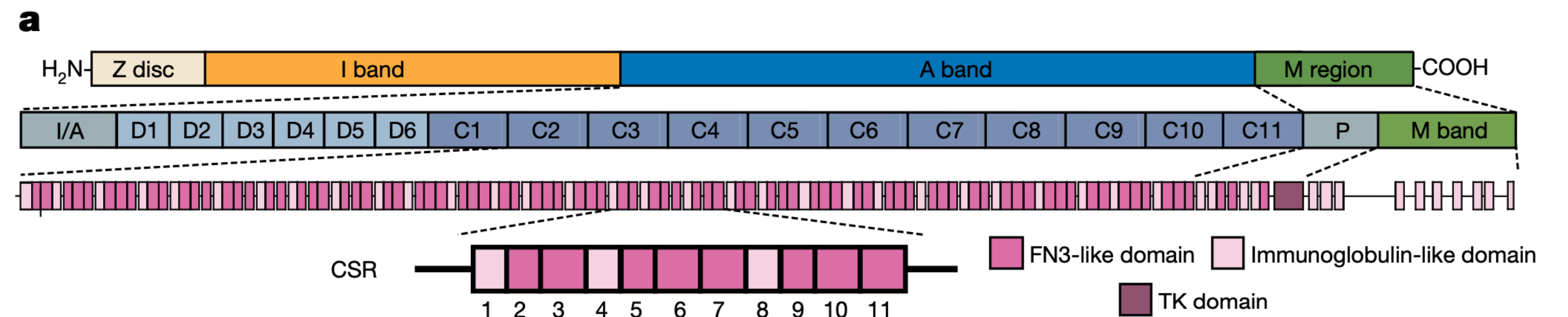
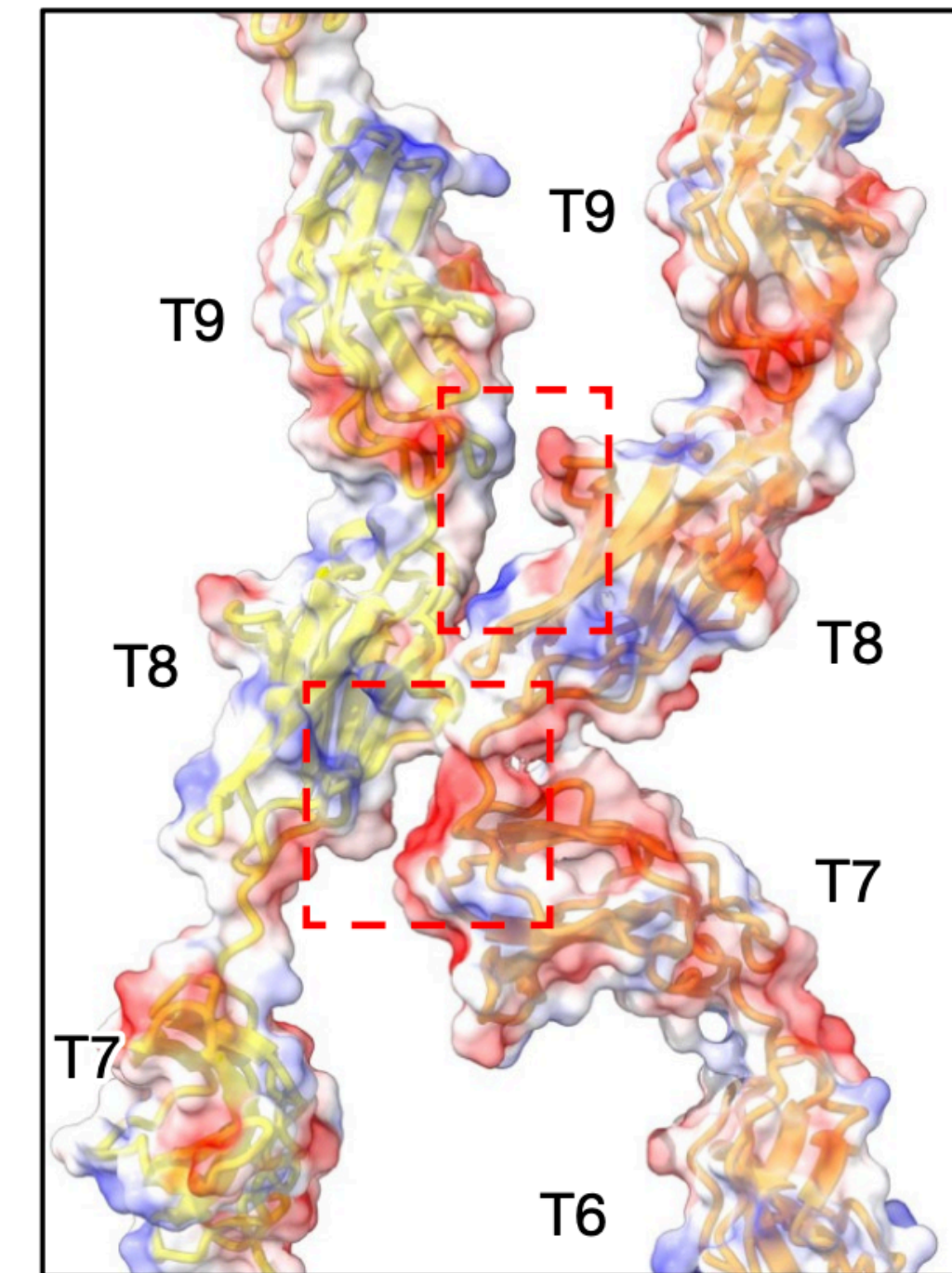
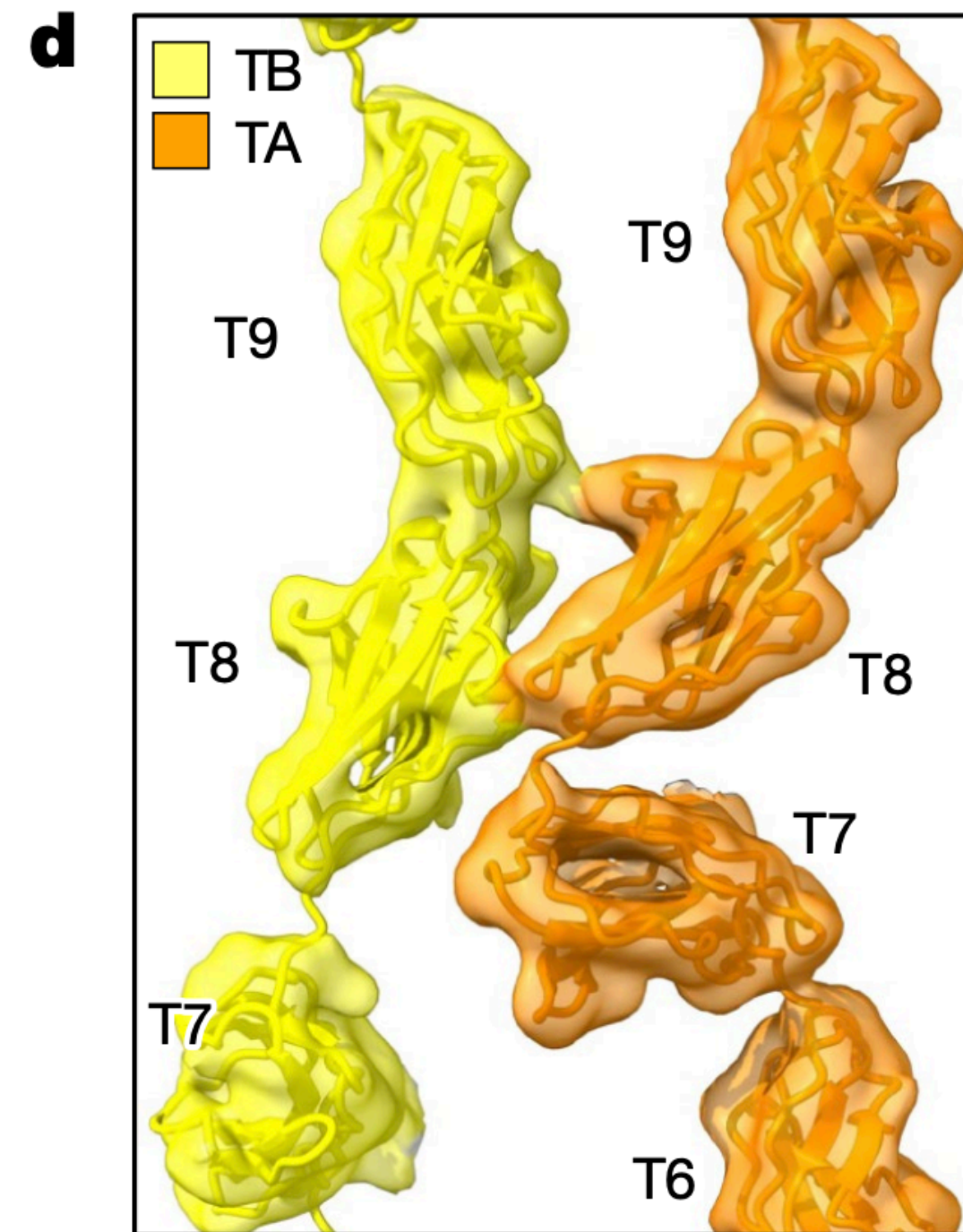
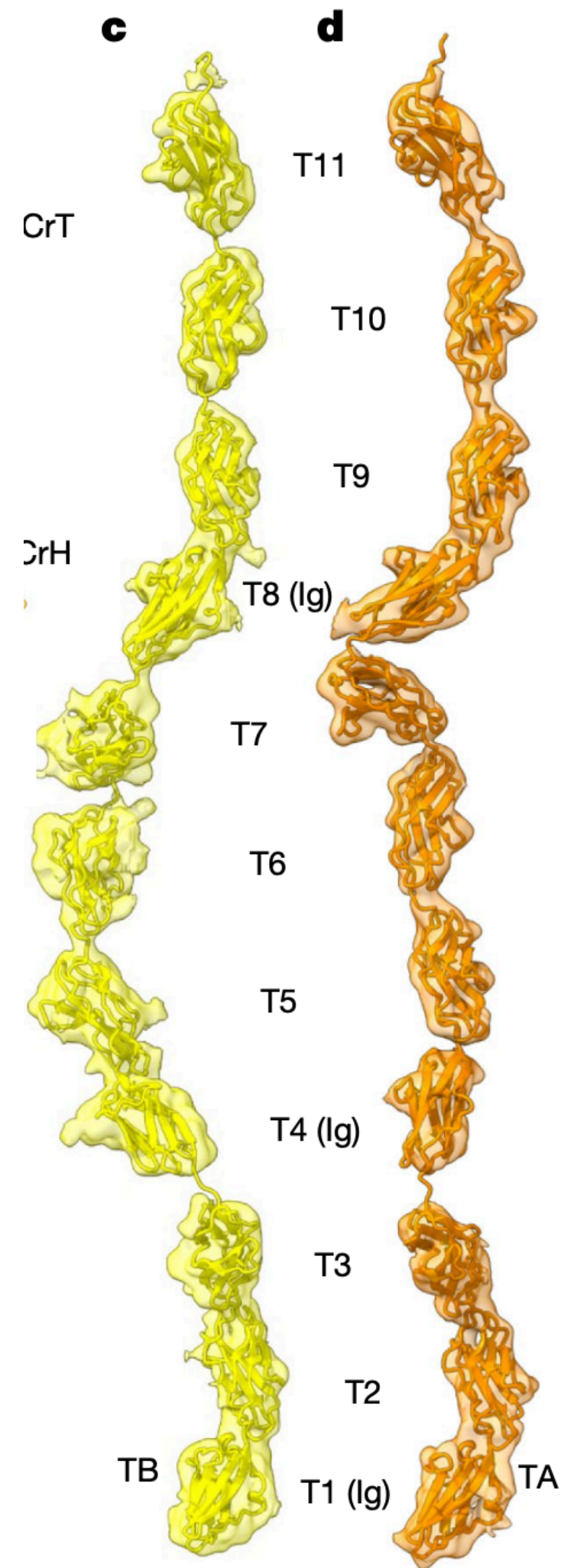
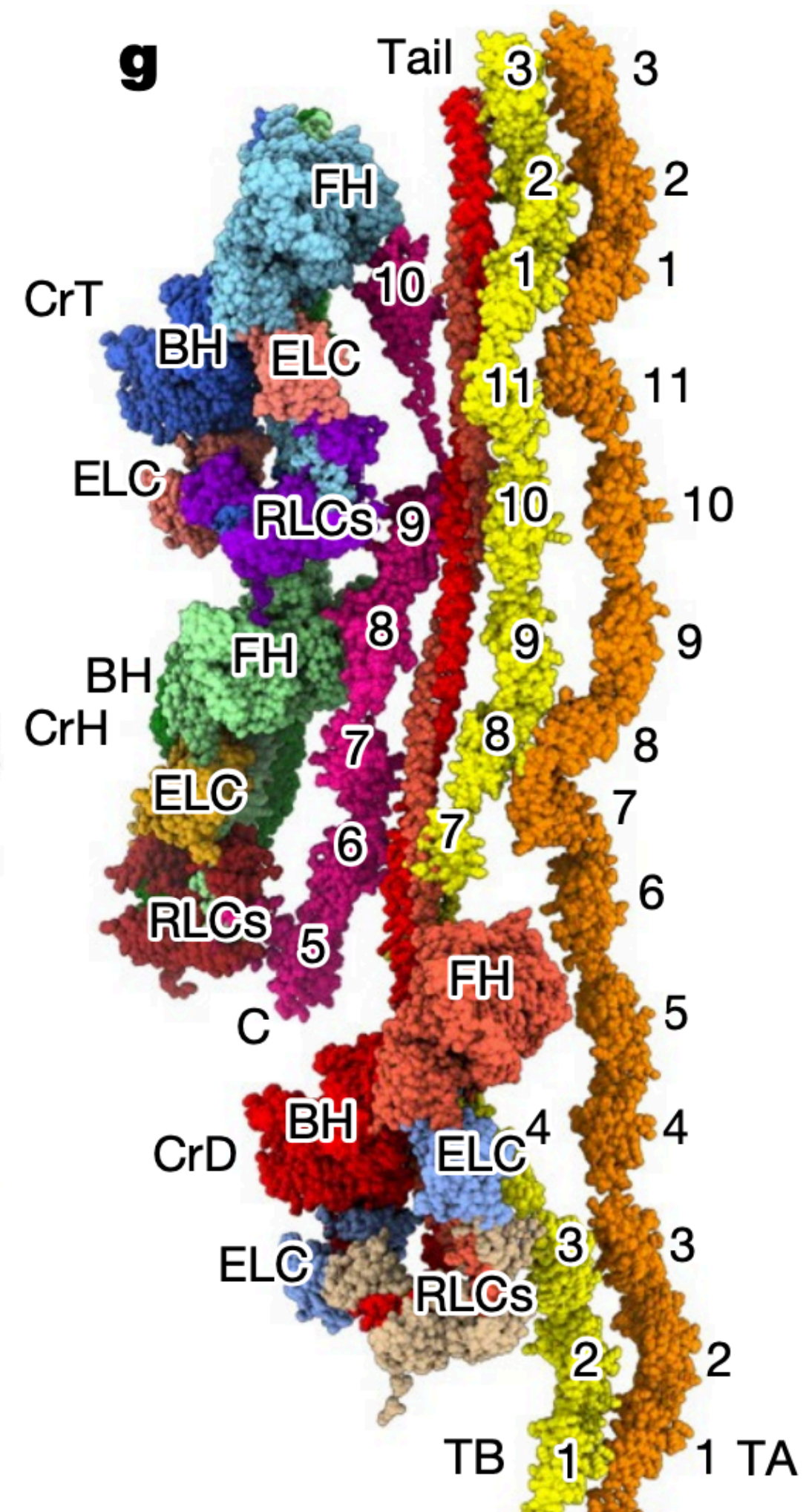
Human, *Nature* 2023

← Perfectly helical →

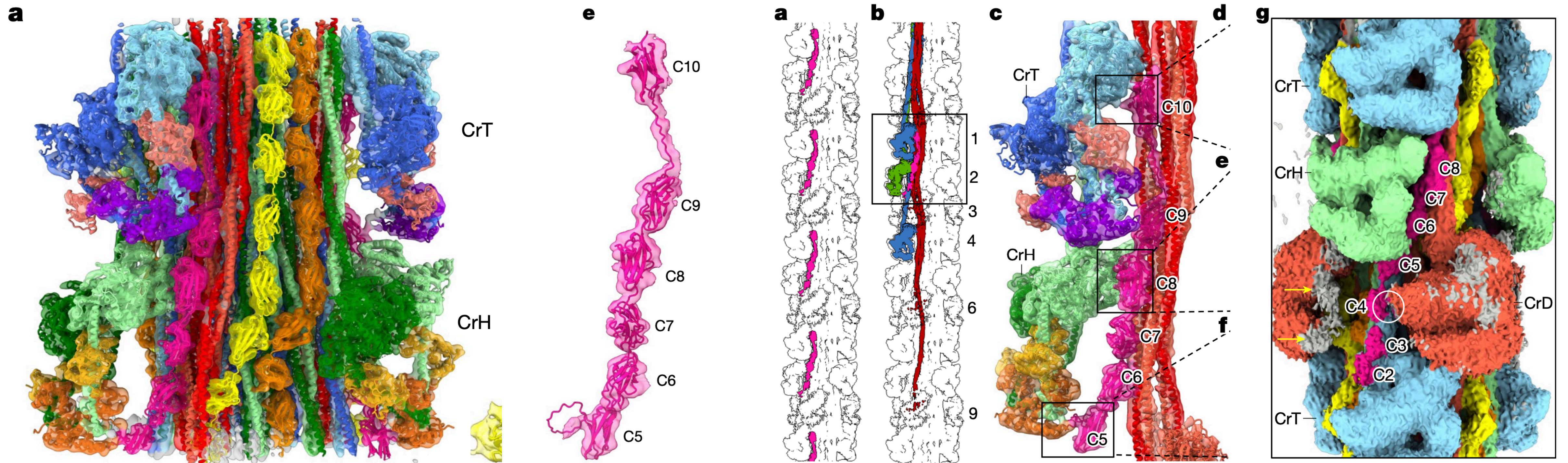
Quasi-helical

Such functional specialization represents an evolutionary increase in complexity

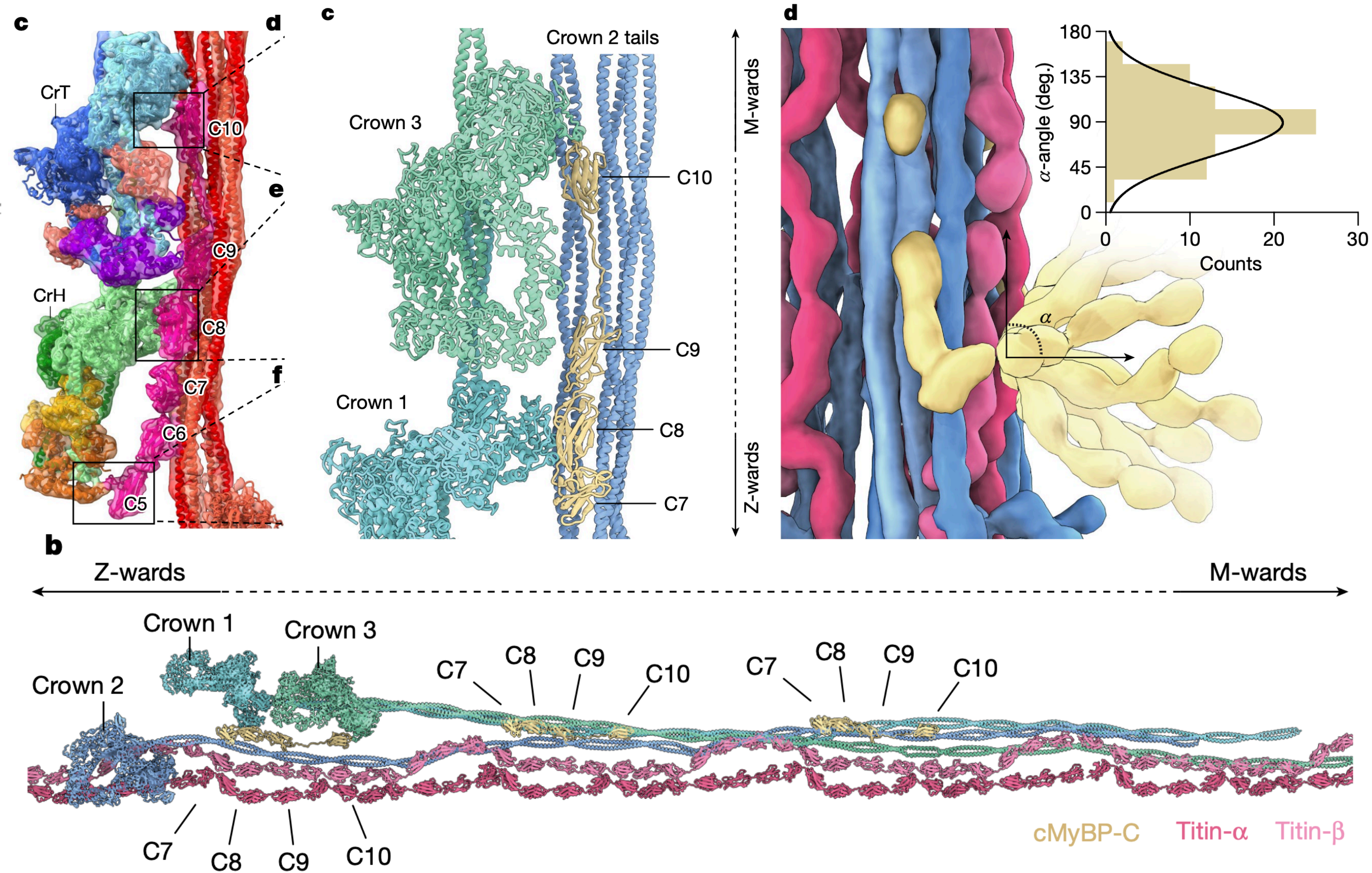
Titin(C-zone)



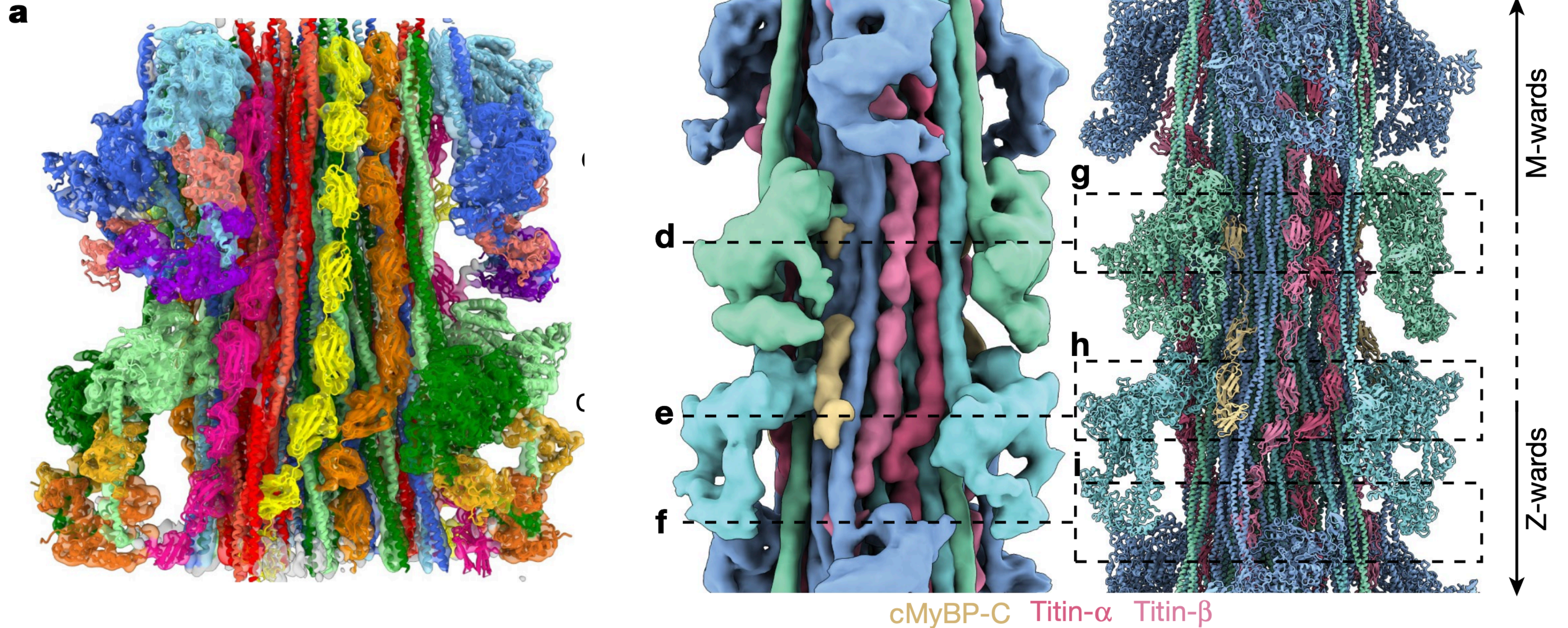
MyBP-C



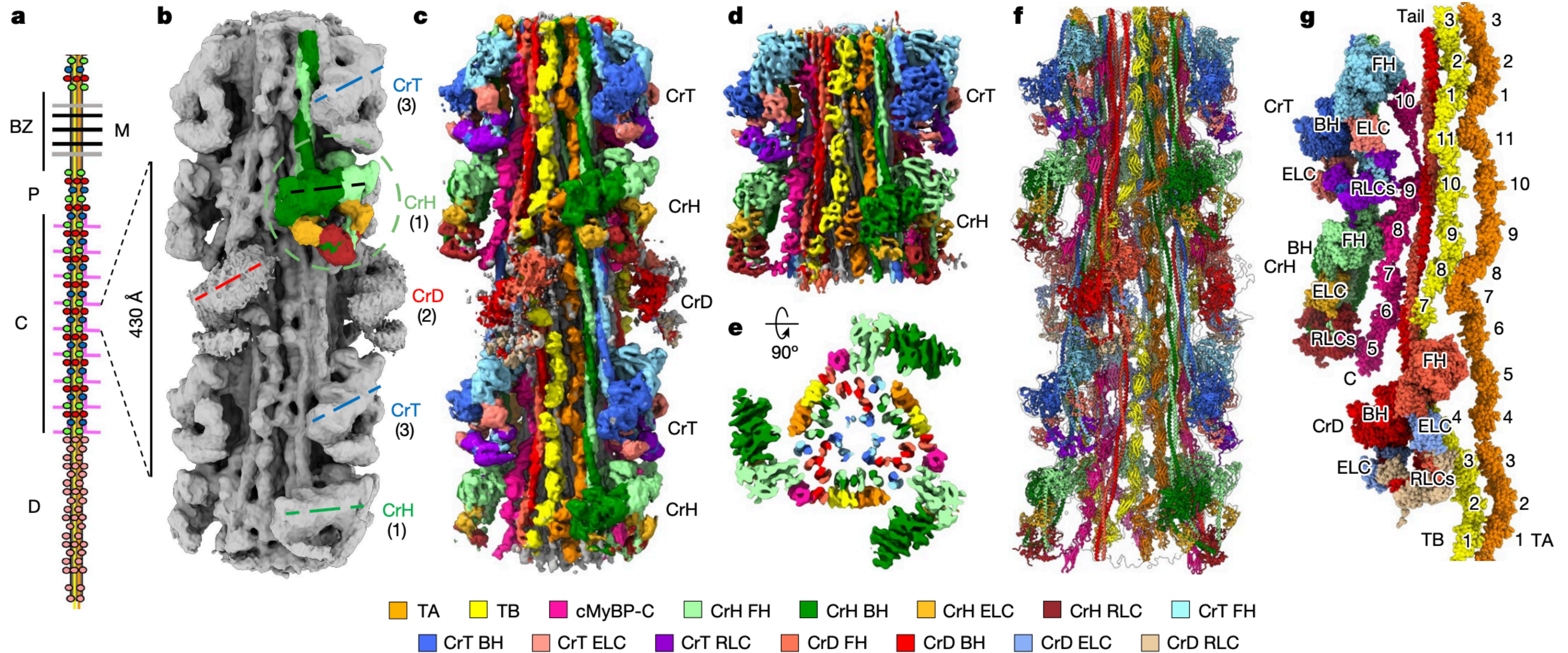
MyBP-C



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



3D model of the C-zone



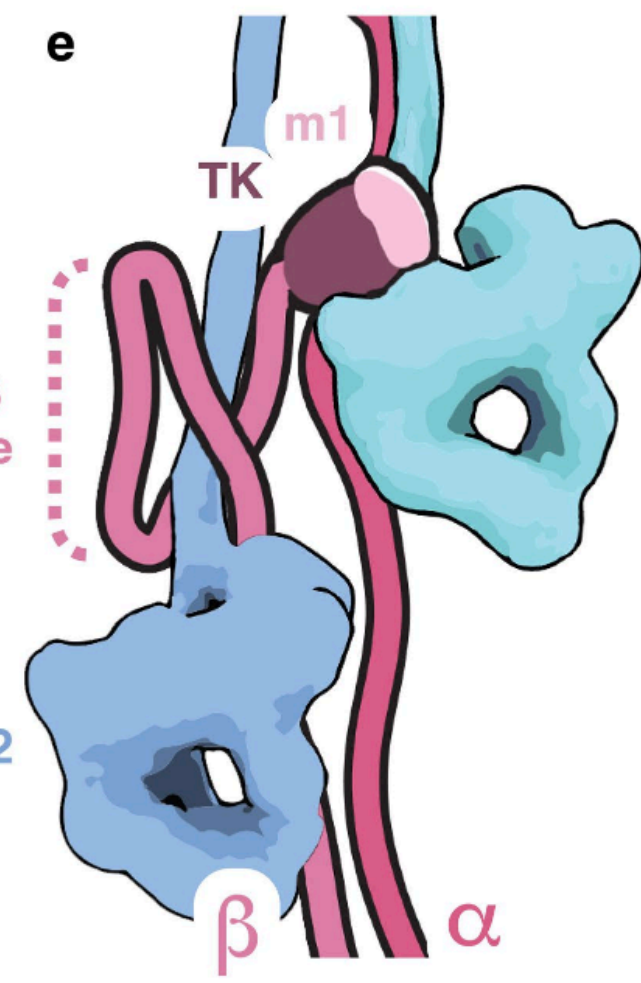
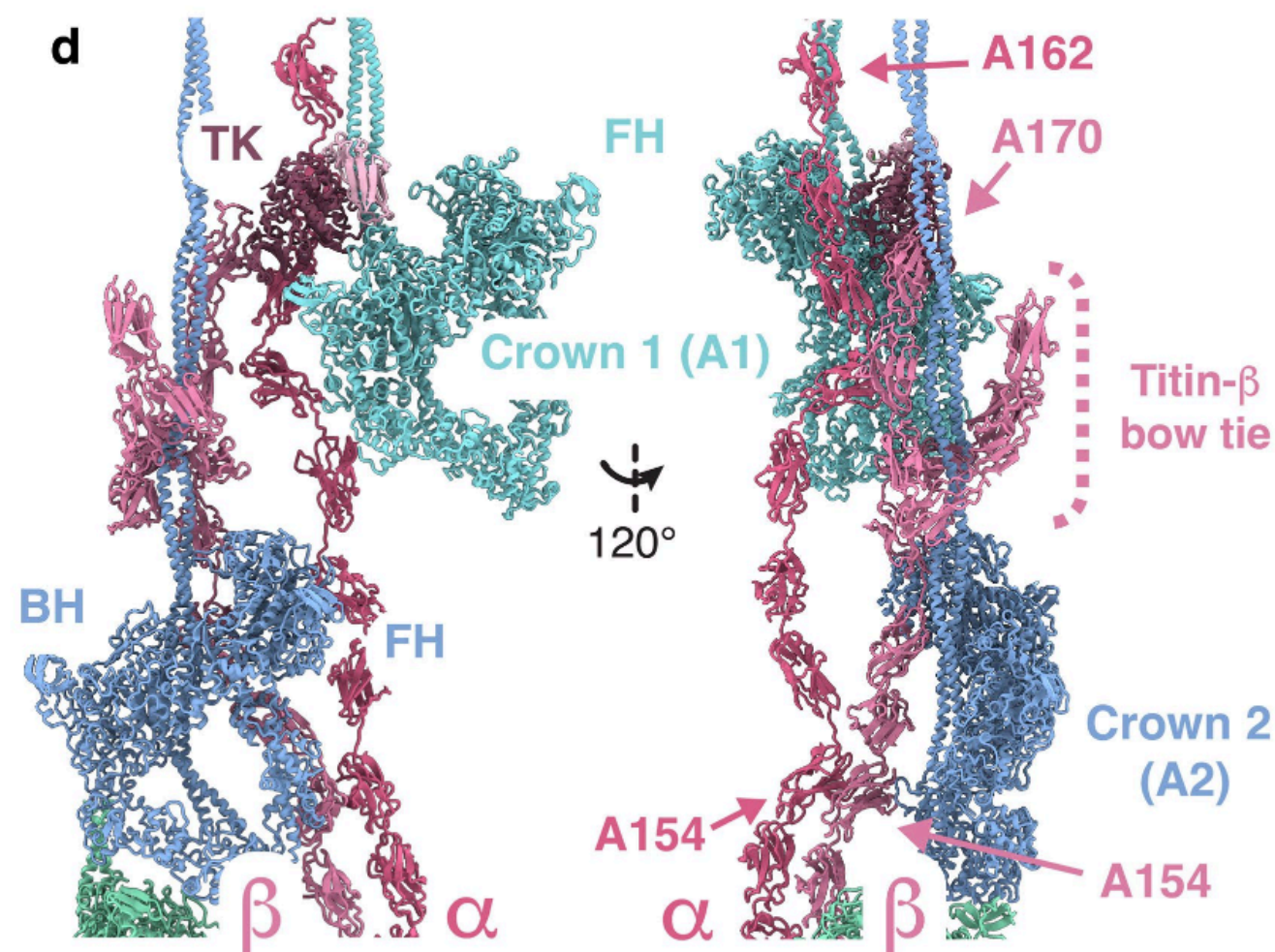
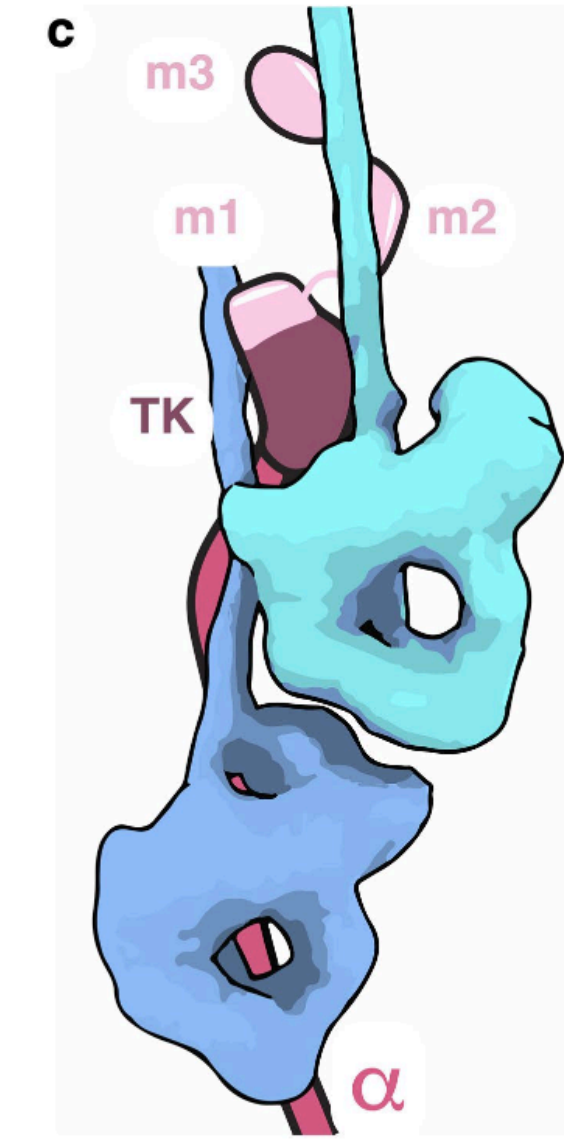
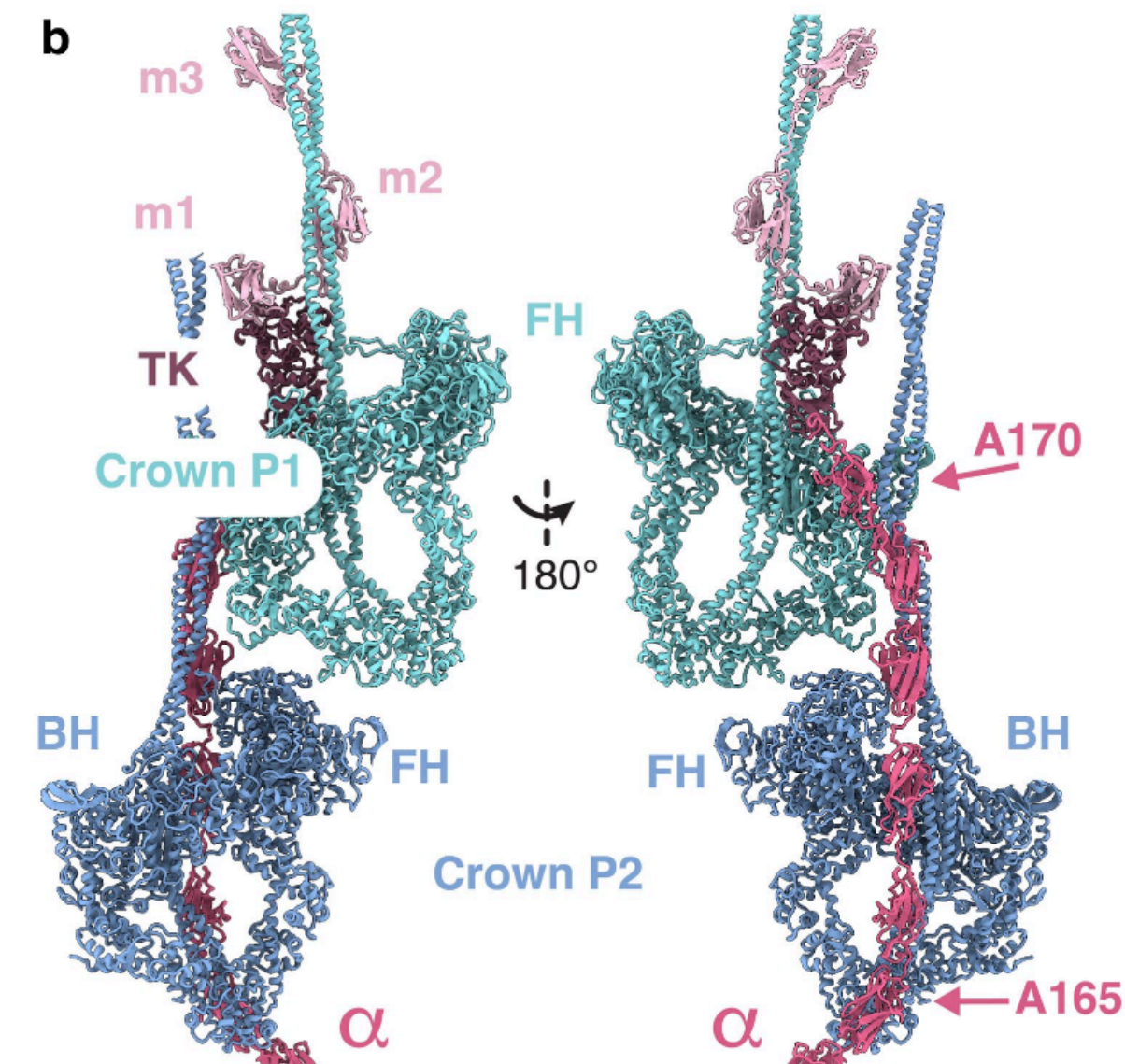
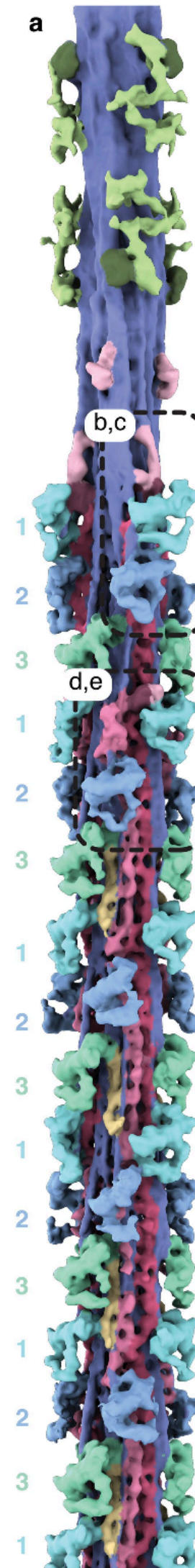
3D model of the P-zone

M-band

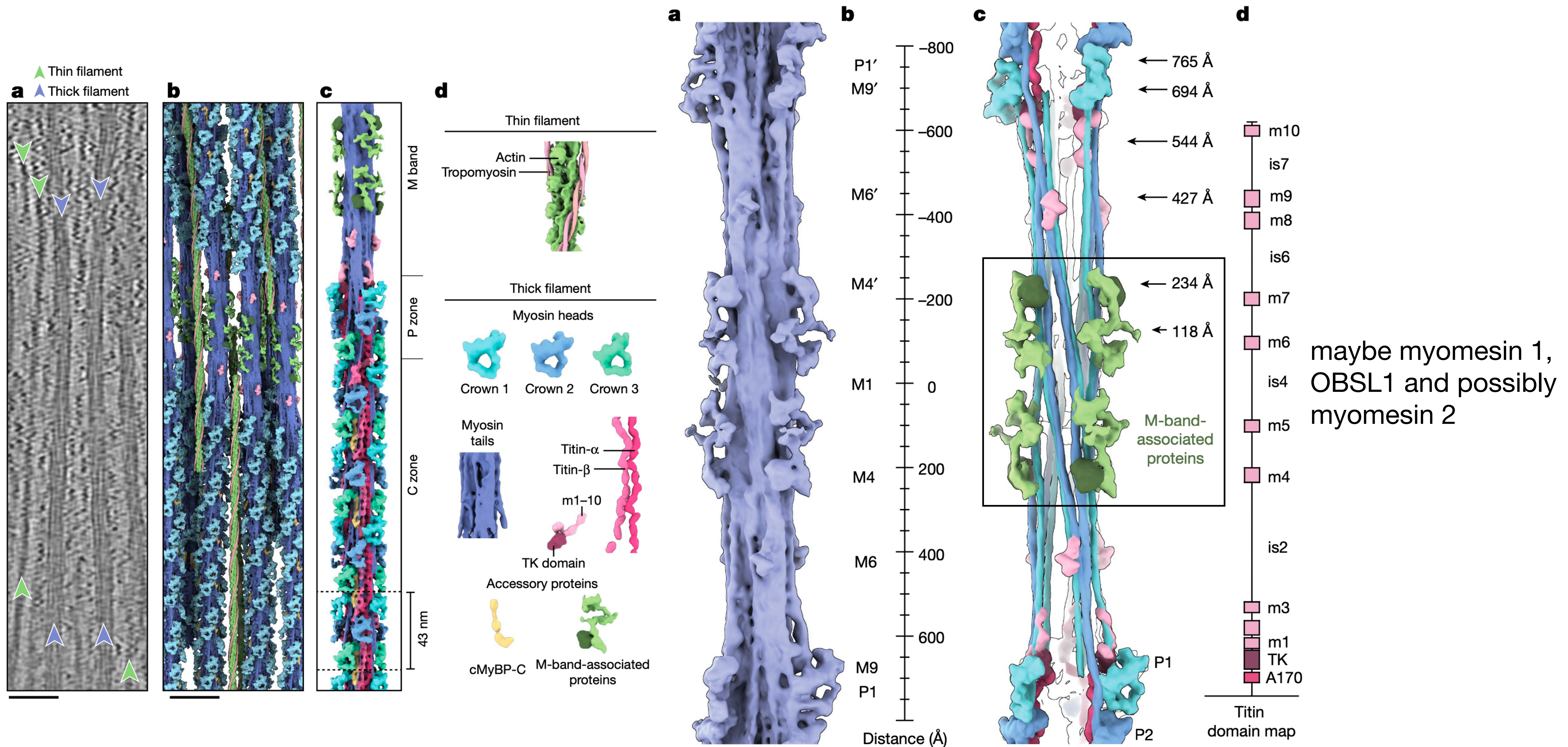
P-zone

A-band

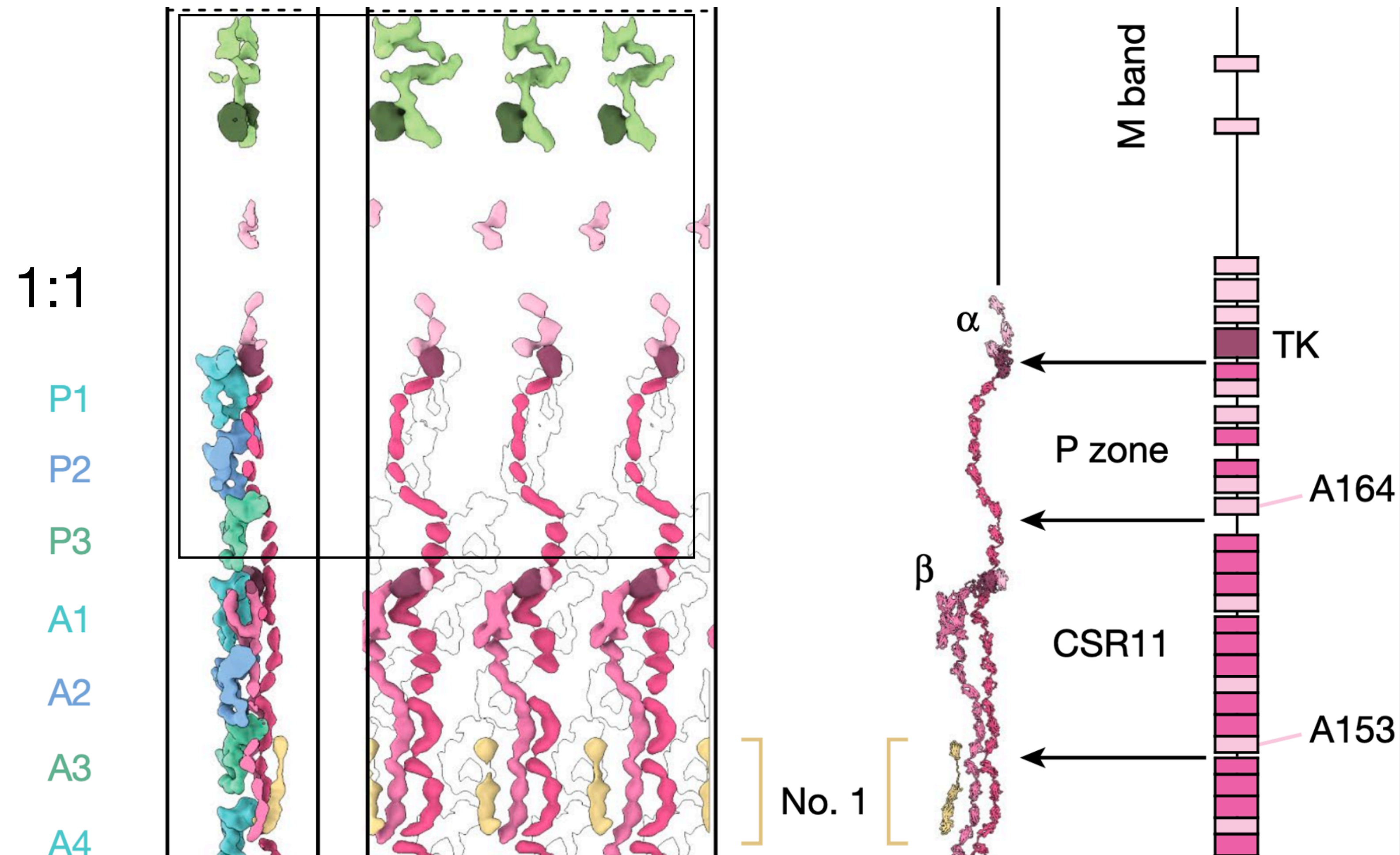
C-zone



3D reconstruction of M-band

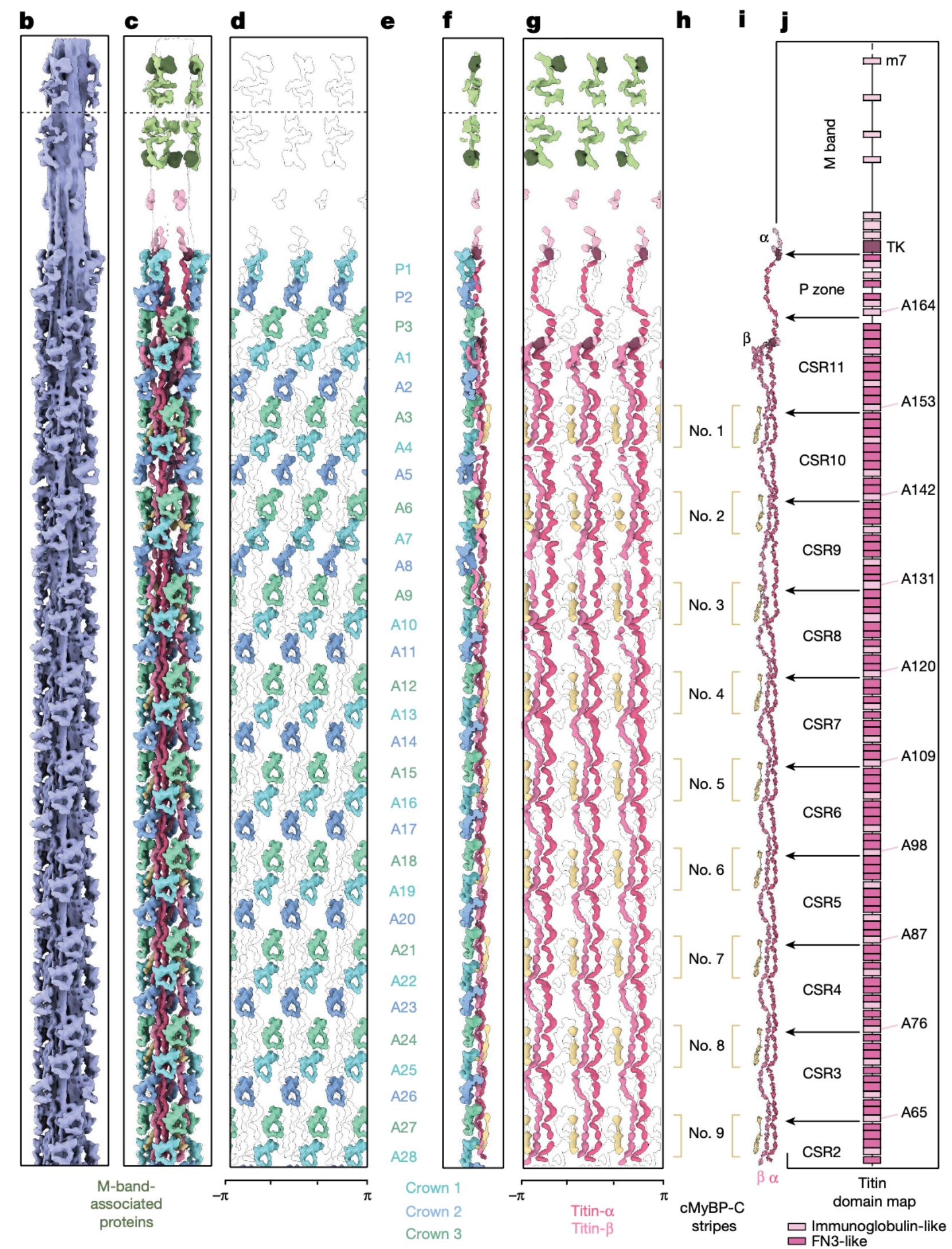


3D reconstruction of M-band



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

Structures

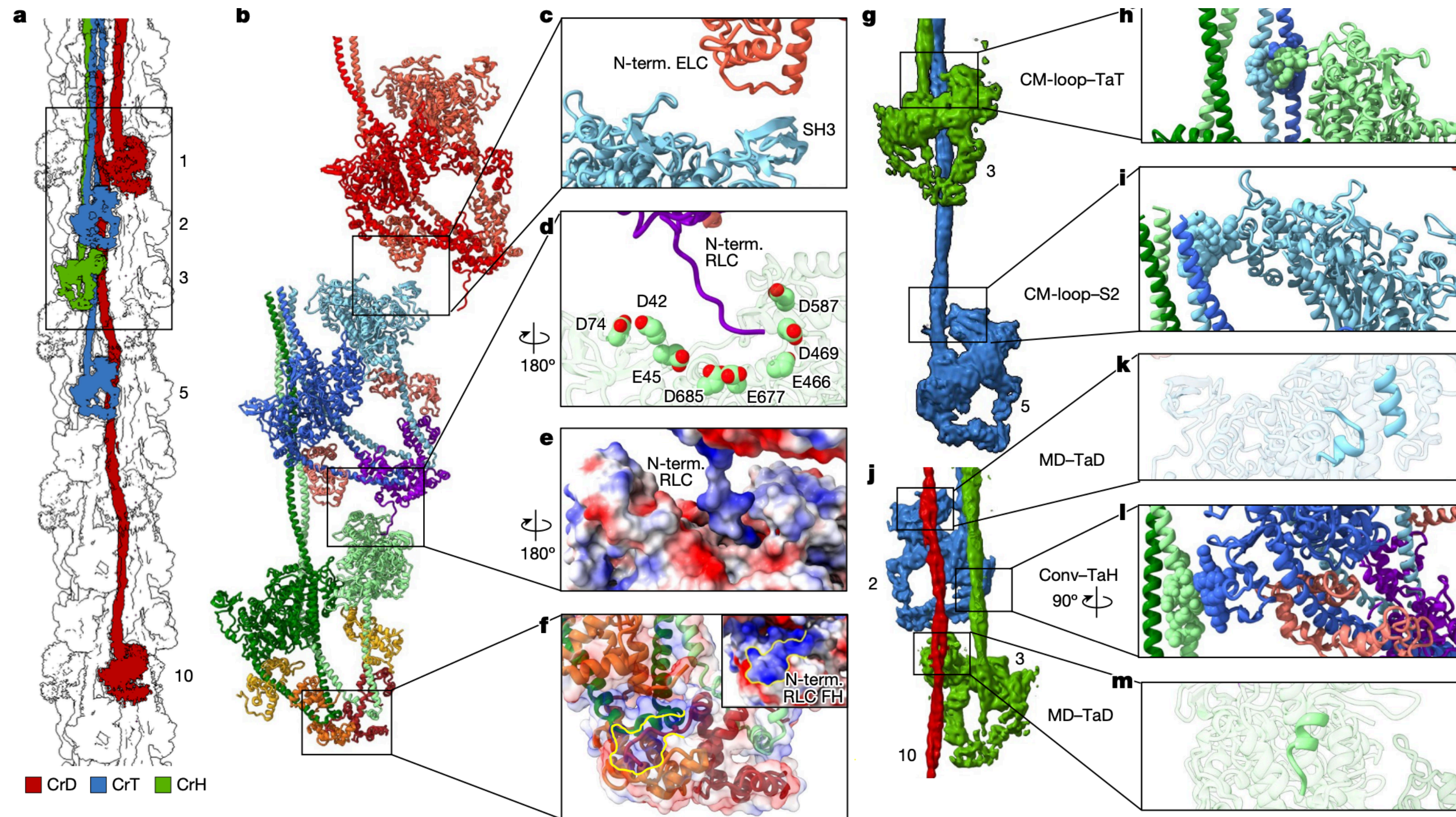


Interactions

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

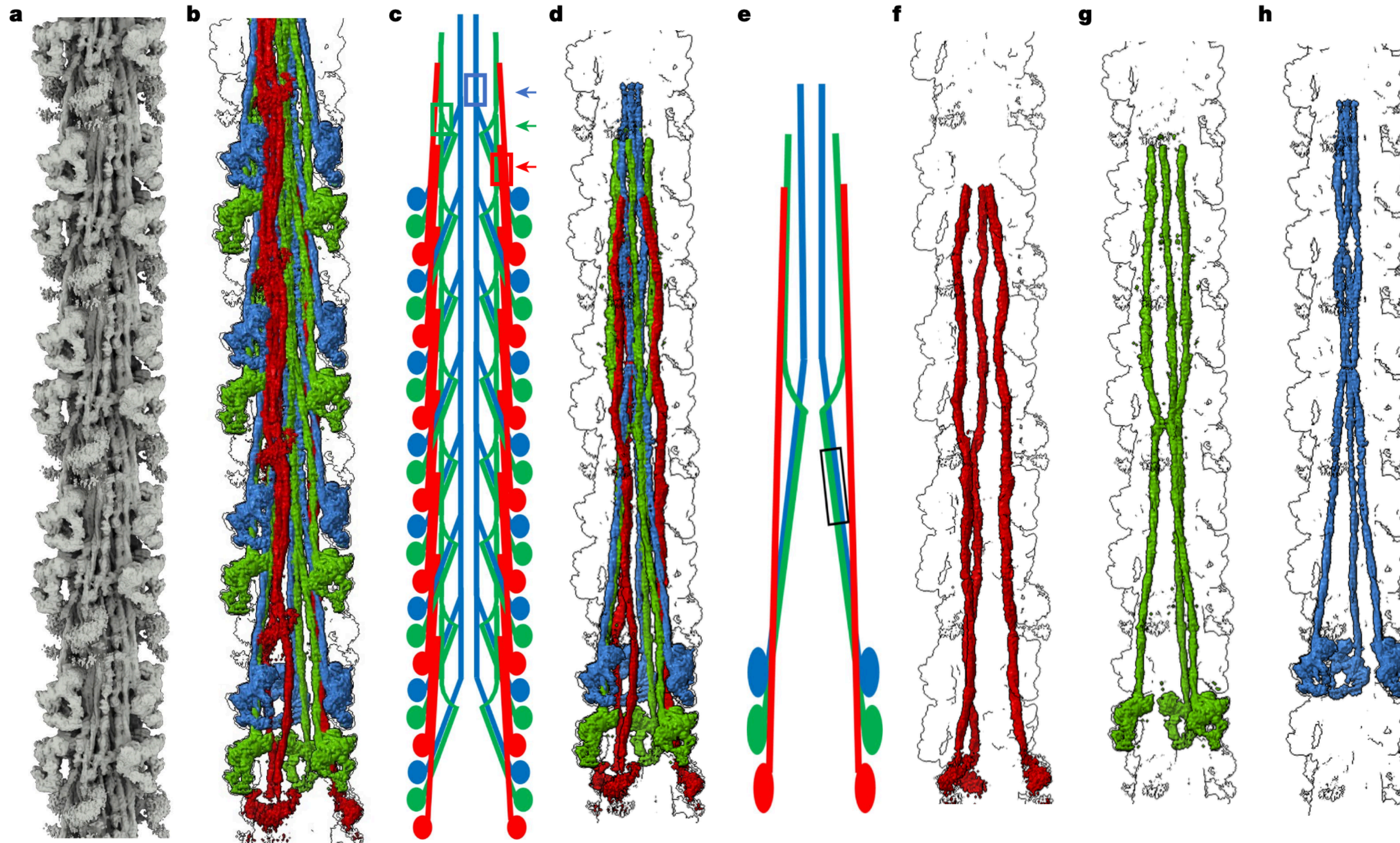
但是，titin和cMyBP-C以及titin和myosin heads之间都是没有互作的

Myosin head-head



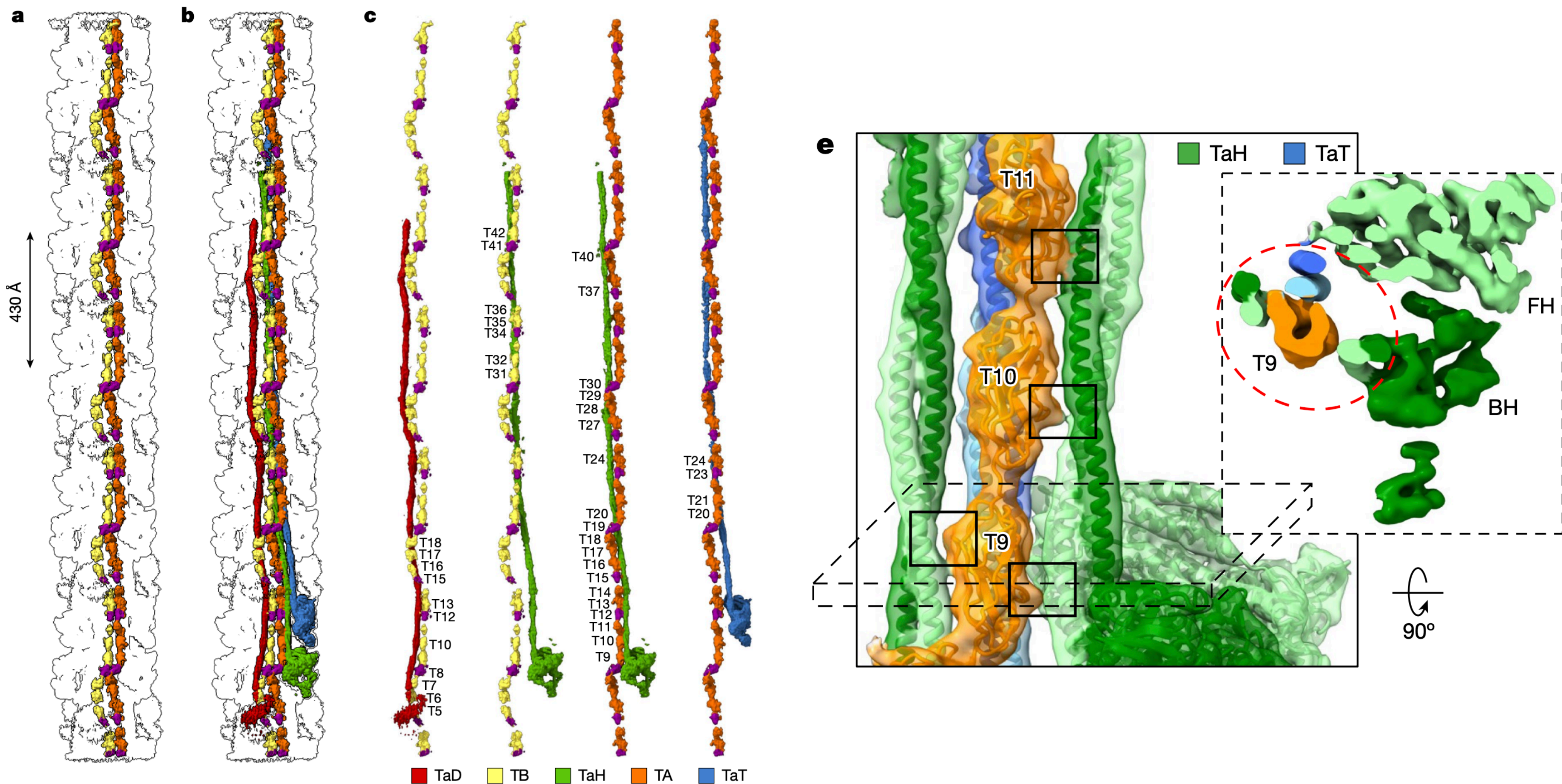
IHM interactions may stabilize the relaxed state

Myosin tail-tail

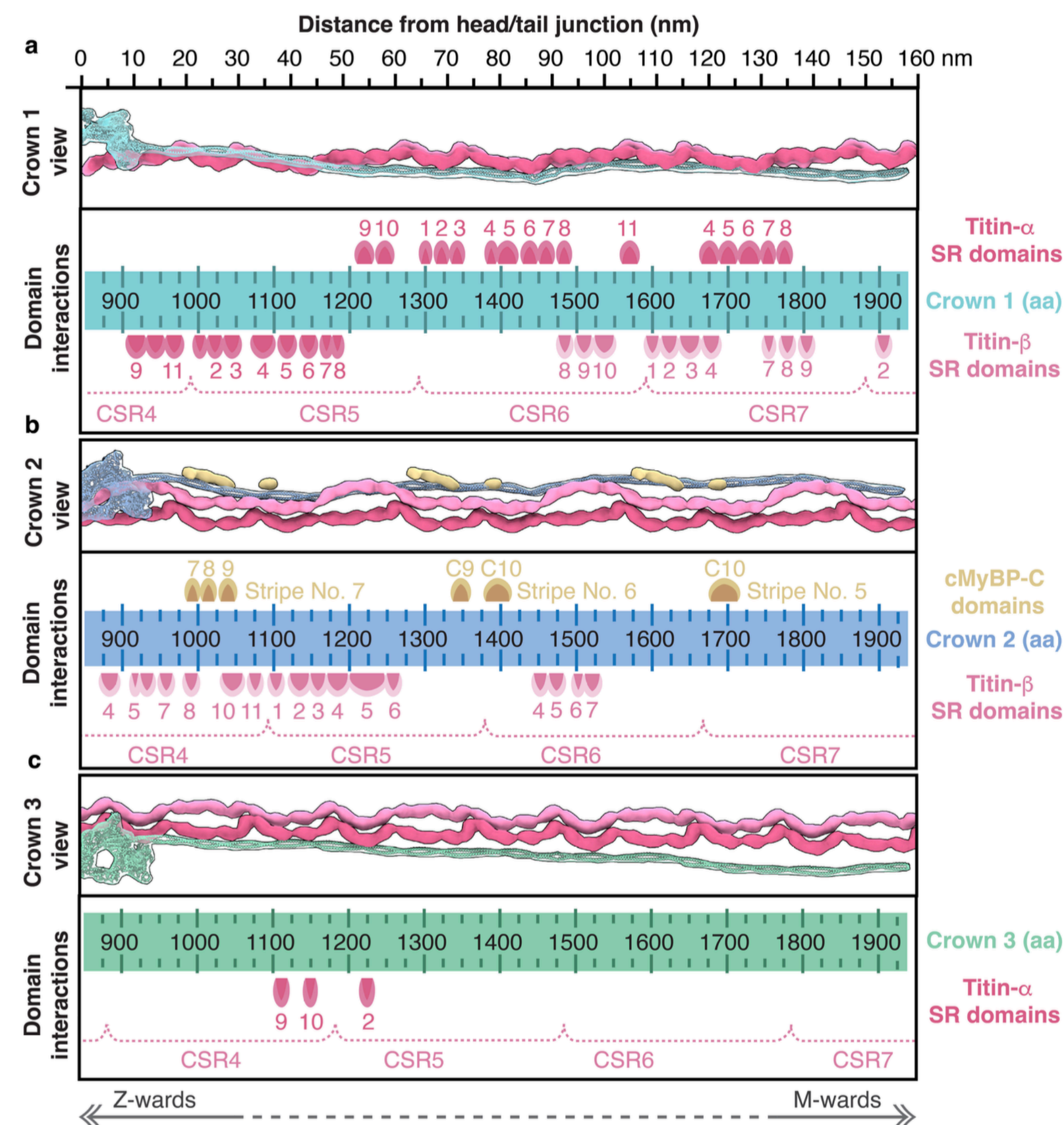


Myosin tails form an interconnected network in the filament backbone

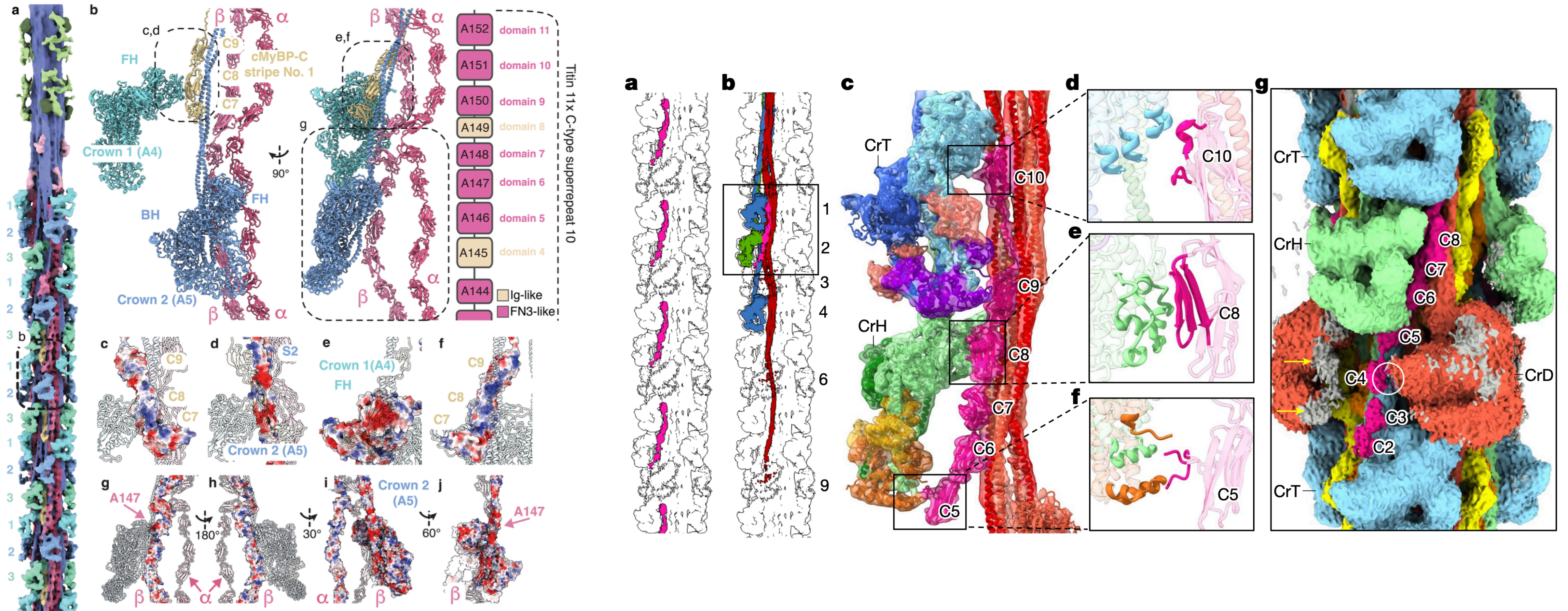
Titin - Myosin tails



Titin - Myosin tails

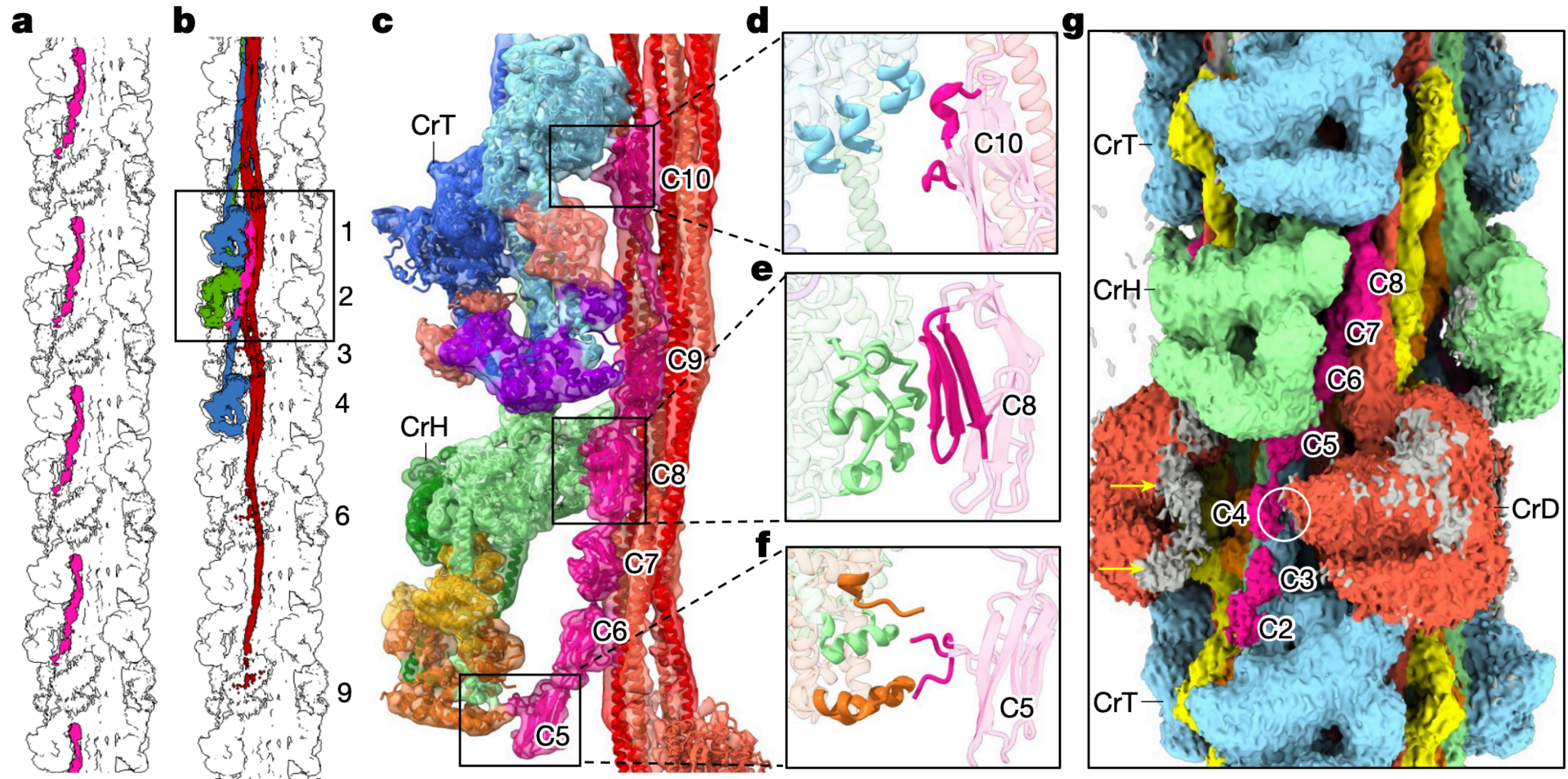


MyBP-C - Myosin heads

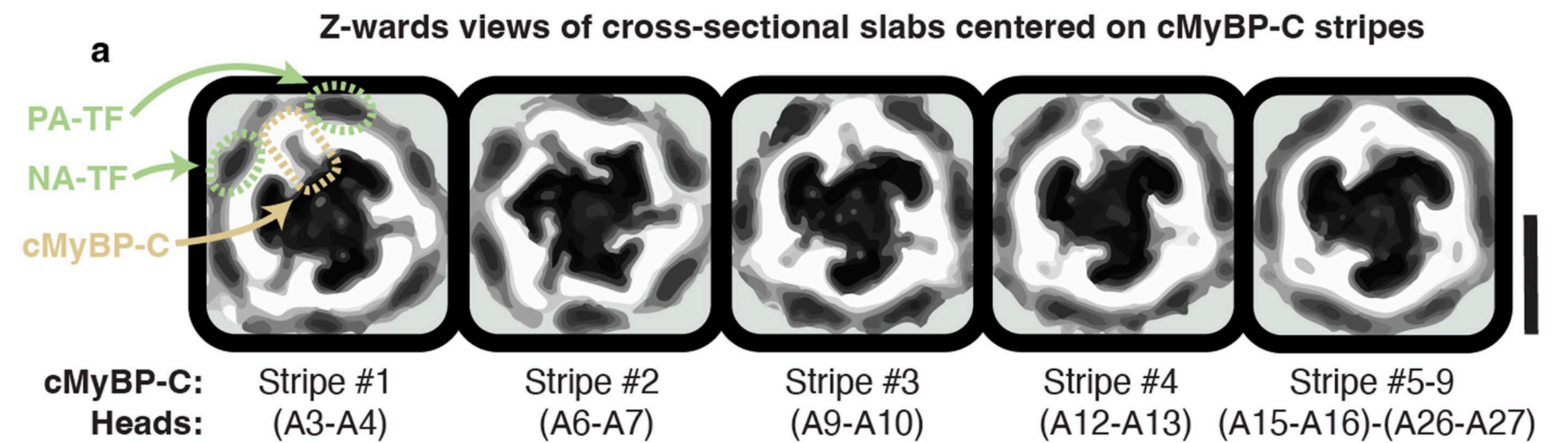
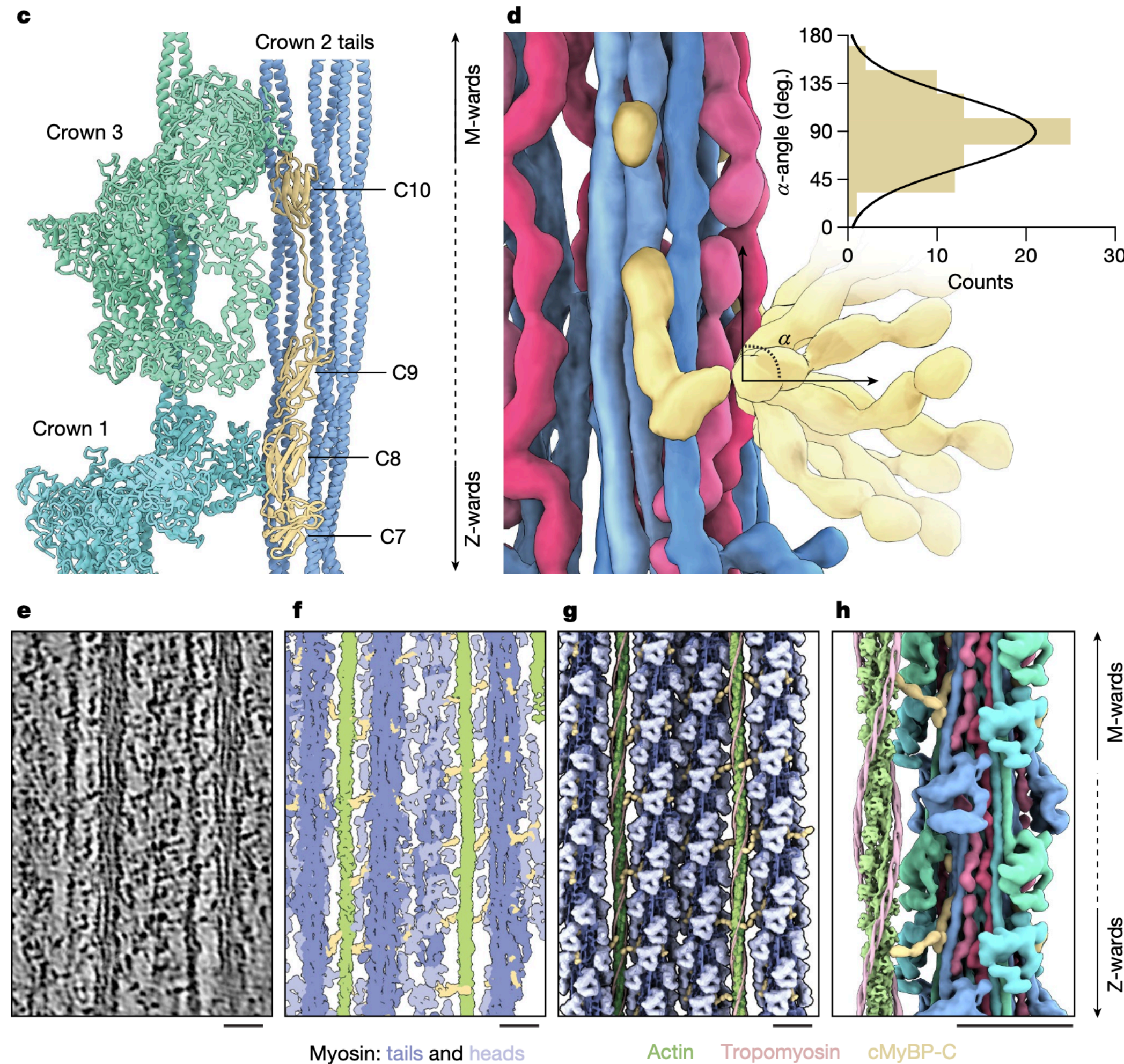


The OFF state of crowns 1 and 2 is stabilized by interactions with cMyBP-C and titin

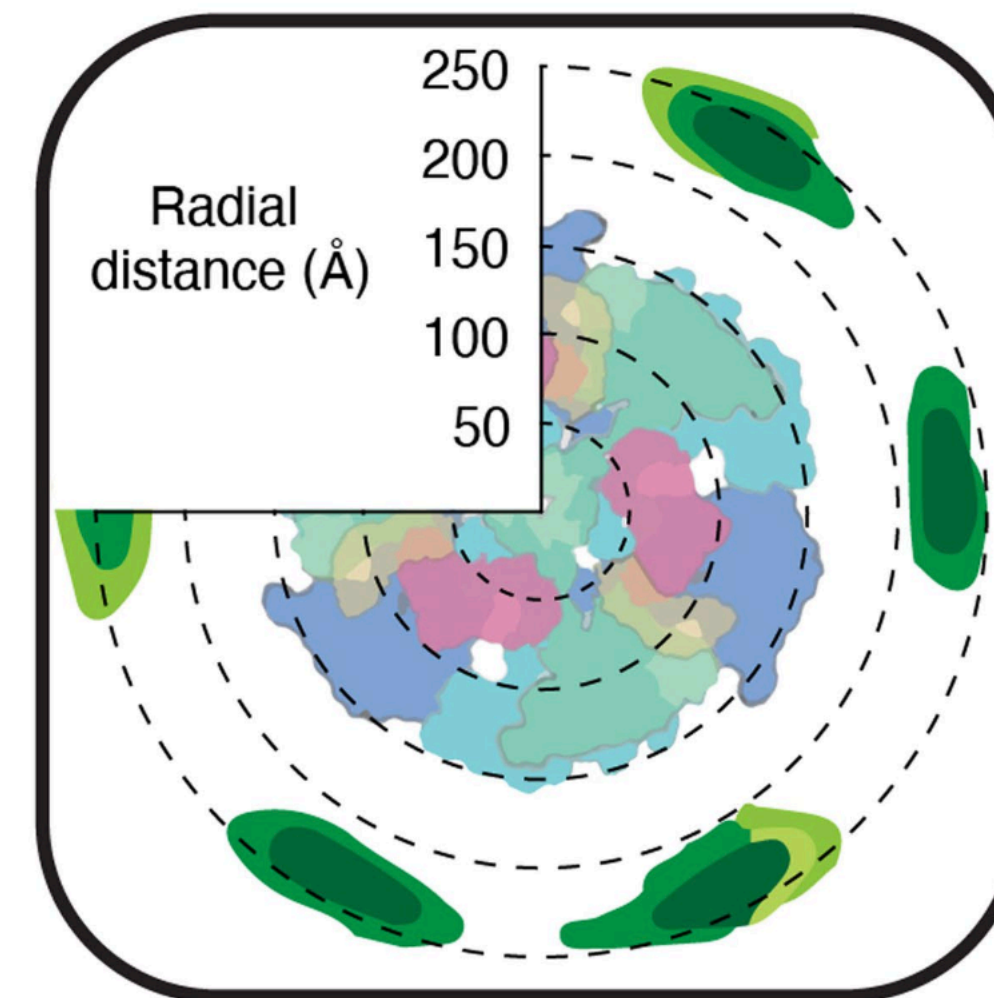
MyBP-C - Myosin tails



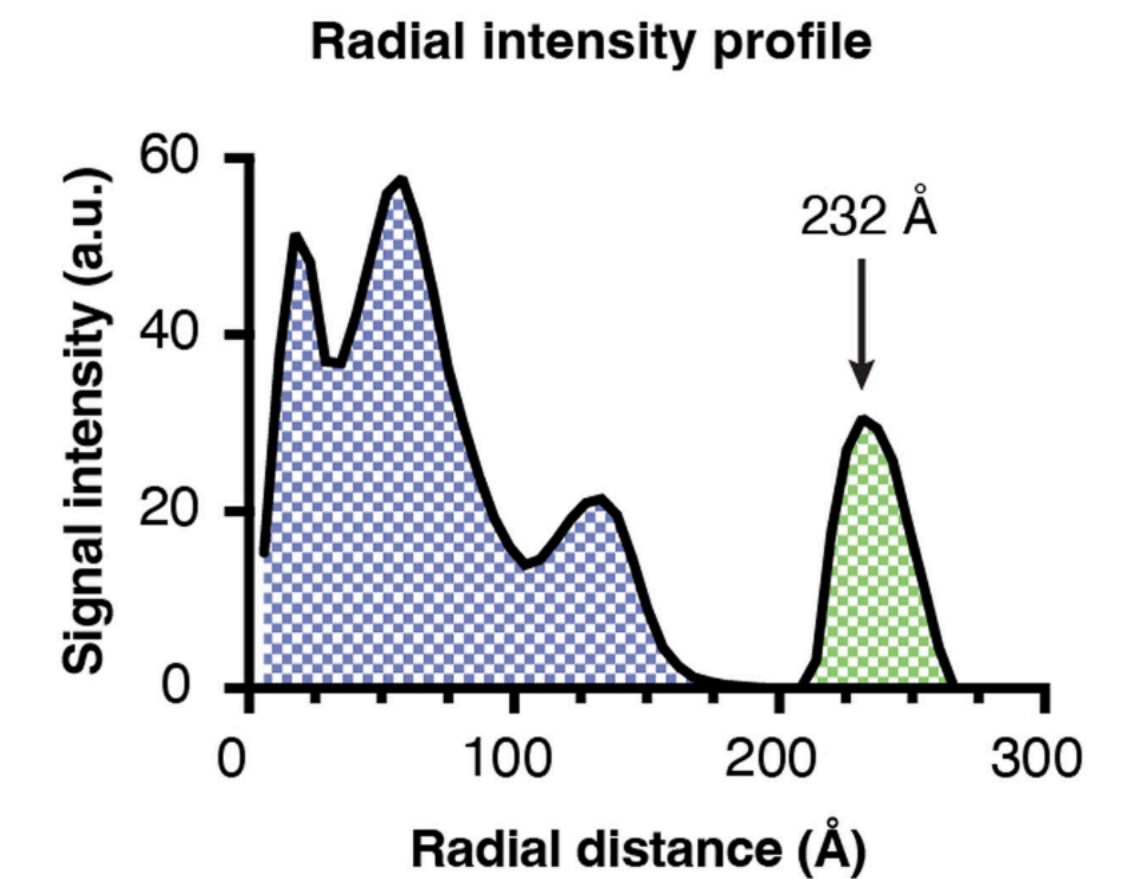
MyBP-C - Thin filaments



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



c



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.