Structural biology of the thin filament

The sarcomere is the basic unit of contraction in striated muscle, which includes skeletal muscle and cardiac muscle. The sliding filament model, proposed in 1954, explains how muscle contraction results from the sliding of actin thin filaments against myosin thick filaments.

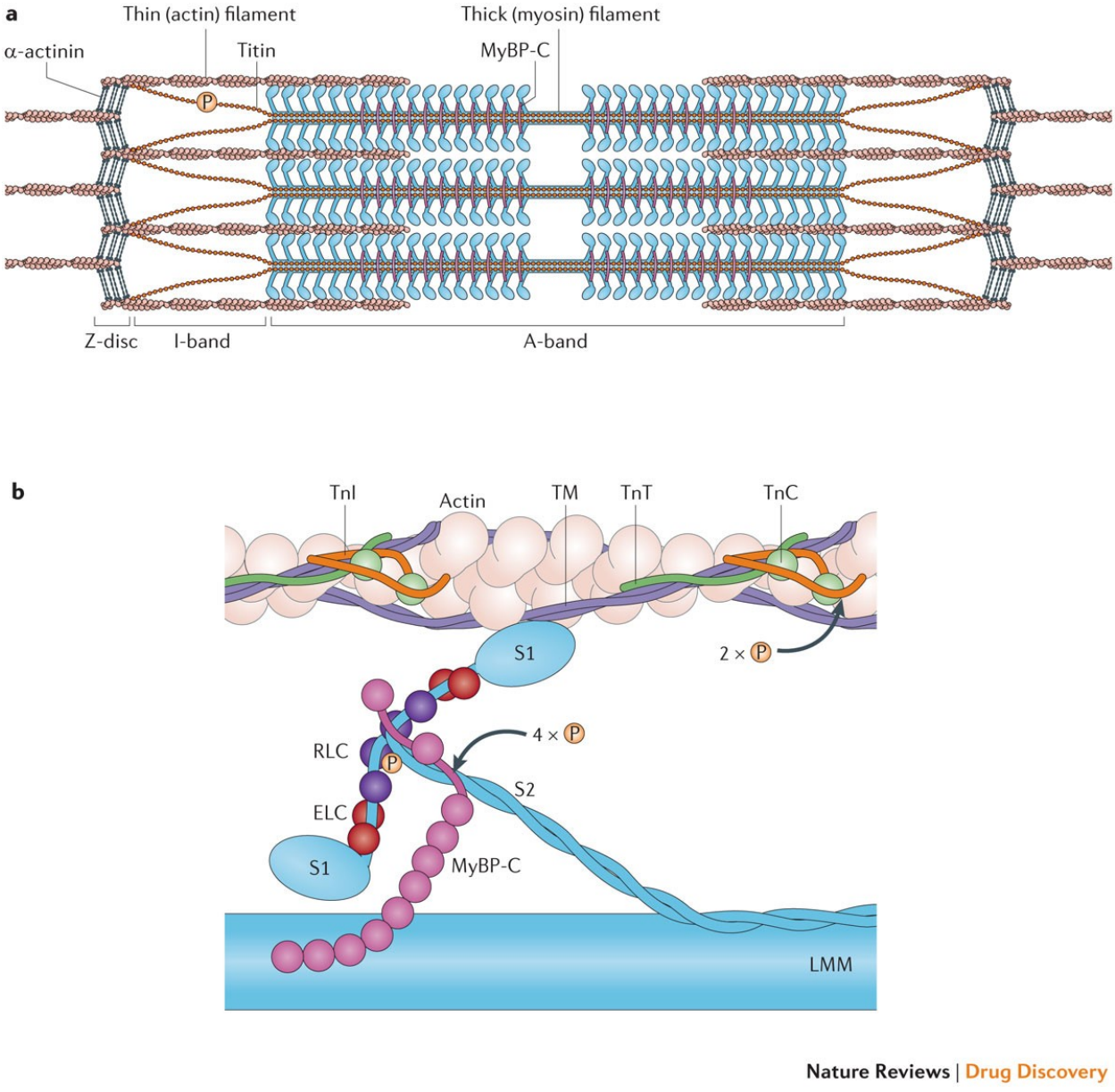

Figure 1. a) Basic structure of the sarcomere. **b)** Structure of the thin and thick filaments.

Figure reproduced from Hwang PM and Sykes BD. 2015. Targeting the sarcomere to correct muscle function. *Nat. Rev. Drug Discov.* **14**, 313-328.

It is important to characterize the structural biology of the sarcomere at the atomic level, because changes at this level can have a pronounced effect on muscle function. Mutations in sarcomere proteins give rise to heritable skeletal myopathies and cardiomyopathies. Drugs that bind to sarcomere proteins can potentially be used to treat these diseases, as well as a number of more common conditions in which muscle function is compromised, like heart failure.

Atomic resolution structures for many proteins of the sarcomere are still lacking. Solution NMR is the ideal method for characterizing the mobile proteins that constantly switch between contracting and relaxing states. We are using solution NMR to characterize the three-dimensional structures and motional dynamics of the major thin filament proteins bound to actin: troponin C, I, T, and tropomyosin.

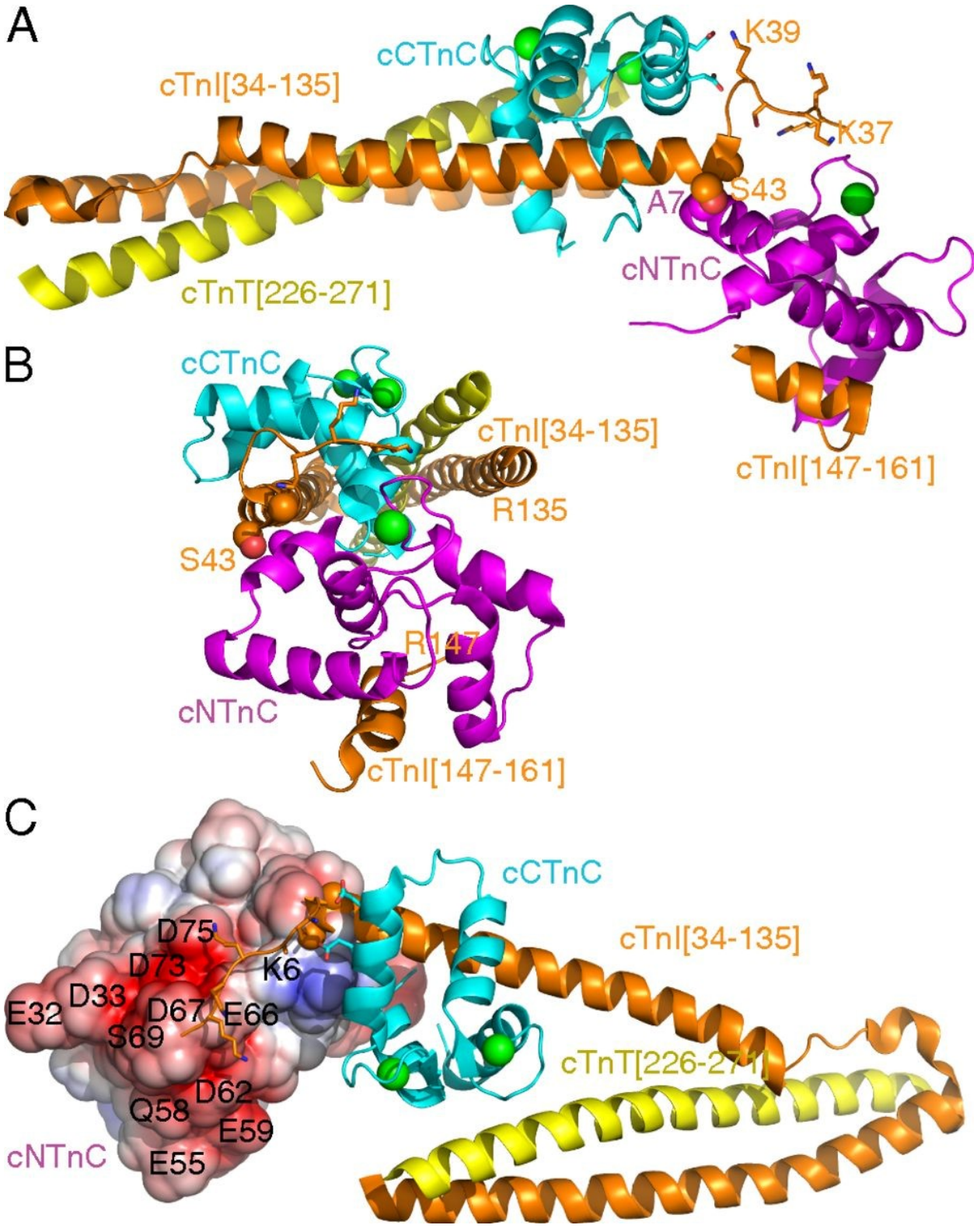
A
cCTnC
cTnI[34-135]
K39
K37
S43
A7
cNTnC
cTnT[226-271]
B
cCTnC
cTnI[34-135]
R135
S43
R147
cNTnC
cTnI[147-161]
cTnI[147-161]
C
cCTnC
cTnI[34-135]
D75
D73
K6
D33
E32
D67
E66
S69
cTnT[226-271]
D62
Q58
E59
cNTnC
E55

Figure 2. Multiple views depicting the delicate positioning of the regulatory N-domain of cardiac troponin C (cNTnC) relative to the rest of the troponin complex.

Figure reproduced from Hwang PM, Cai F, Pineda-Sanabria, SE, Corson DC, Sykes BD. 2014. The cardiac-specific N-terminal region of troponin I positions the regulatory domain of troponin C. *Proc. Natl. Acad. Sci. USA* **111**, 14412-14417.

One common bottleneck in NMR is obtaining adequate quantities of isotope-enriched protein. Our lab has developed methods to overexpress recombinant proteins in the inclusion bodies of *E. coli* by using the membrane protein, PagP, as a fusion tag. This strategy is effective in protecting intrinsically disordered proteins from proteolytic degradation.

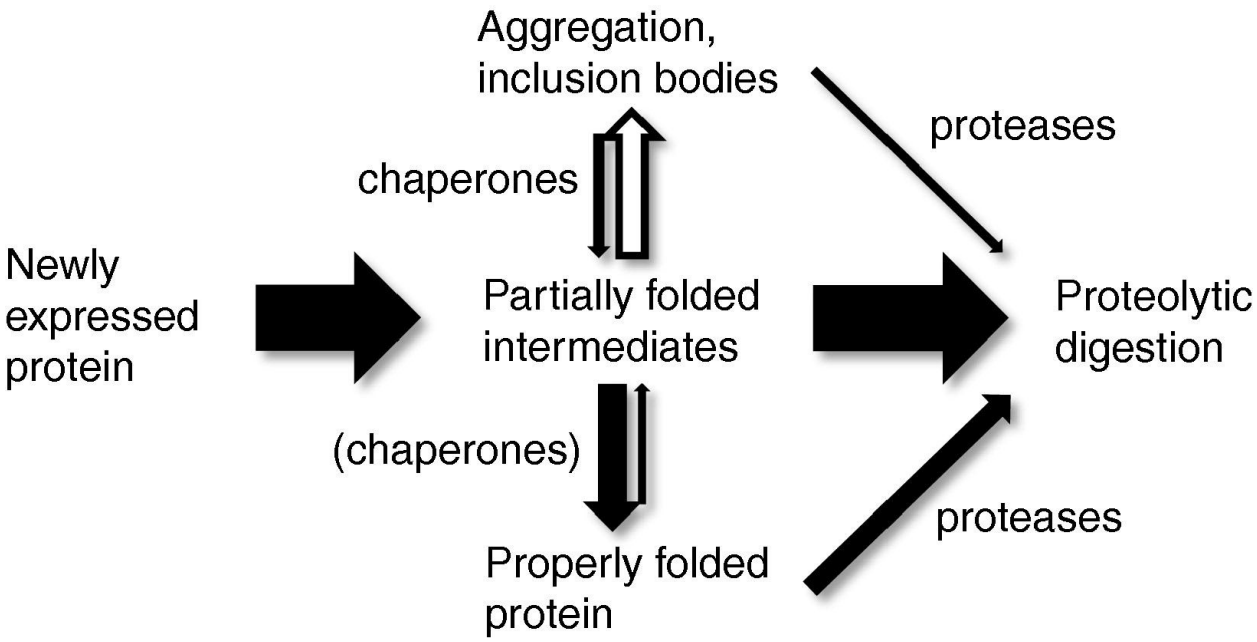

Figure 3. Targeting of proteins to inclusion bodies protects them from degradation.

Figure reproduced from Hwang PM, Pan JS, Sykes BD. 2014. Targeted expression, purification, and cleavage of fusion proteins from inclusion bodies in *Escherichia coli. FEBS Lett* **587**, 247-252.