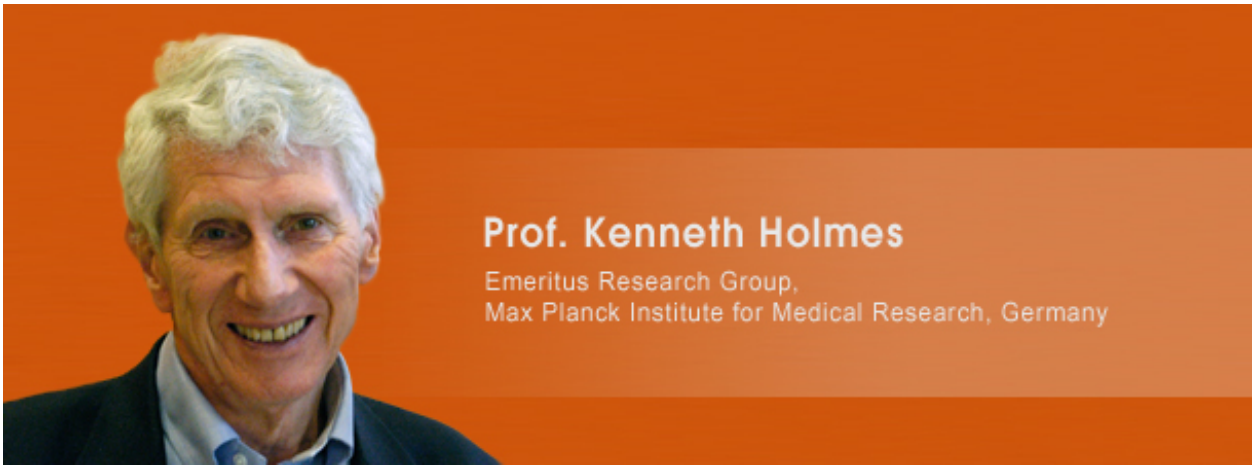
Institute of Molecular and Cell Biology
A*STAR

SEMINAR ANNOUNCEMENT

DATE: 14 March 2012, Wednesday
TIME / VENUE: 11:00AM @ Level 3, IMCB Seminar Room 3-46, Proteos, Biopolis
SPEAKER: Prof. Kenneth Holmes
TITLE OF SEMINAR: **The structural basis of muscle contraction**



Understanding muscle contraction has resulted from the synergy of a number of approaches for which structure has provided an integrating framework. Nearly 60 years ago interference and phase contrast light microscopy established the sliding filament model of muscle contraction(1, 2) that marked the beginning of modern muscle research. A little later Hanson and H.E. Huxley combines biochemistry with electron microscopy to establish the macromolecular architecture of the sarcomere: the thick filaments consist mainly of myosin and the thin filaments mostly of actin (3). The connecting cross-bridges are formed from the N-terminus of the myosin molecule. On the basis of these observations H.E. Huxley outlined a structural basis for muscle contraction. The cross-bridges, which are part of the myosin molecule, protrude from the thick filament. They bind strongly to actin but can be released by binding ATP. A rowing-like movement of the myosin cross-bridges leads to a progression on the myosin filaments passed the actin filaments (4). X-ray fibre diffraction data and EM studies of insect flight muscle with and without ATP demonstrated that the cross-bridges could indeed take up two configurations that might represent the ends of an active rowing-stroke (5). Later X-ray diffraction from intact muscles using intense synchrotron radiation allowed the recording of the movements of the cross-bridges during a contraction with high precision (6-8).

The myosin cross-bridge is an ATPase whereby actin and myosin together are a much better ATPase than myosin alone (see (9) for historical review). Kinetic studies by Lymn and Taylor (10) elucidated the nature of the actin – myosin ATPase. Myosin is product-inhibited. Product release is greatly accelerated by binding to filamentous-actin. These observations led Lymn and Taylor to outline the cross-bridge cycle: in the absence of ATP the myosin cross-bridge binds strongly to actin (rigor); ATP binding to the cross-bridge leads to rapid release from actin; a conformational change of the cross-bridge (the recovery stroke) permits hydrolysis; the cross-bridge then rebinds to actin (allowing phosphate release) and subsequently undergoes a conformational change that drives actin passed myosin (the power stroke); at the end of the power stroke ADP is released, ATP can rebind to repeat the cycle.

In 1993 Rayments's group ushered in a much more detailed understanding of myosin function by solving the structure of the myosin cross-bridge by X-ray crystallography. It showed that the cross-bridge consists of a large catalytic domain, often called the motor domain, based on a central 7-stranded beta-sheet that carries the ATP binding site and the actin binding site. A long lever arm is connected to the C-terminus of the motor domain. The catalytic mechanism of the cross-bridge is similar to the G-proteins: the active site contains a P-loop and switch 1 and switch 2 elements. The lever arm was later found in two different conformations (so called pre-power-stroke and post-rigor) allowing one to see how switch 2 movement was coupled to a swing of the lever arm. The swinging lever arm is the basis of the rowing-like movement of myosin along the actin filament. This mechanism appears to be common to all myosins.

Further crystallographic studies coupled with high resolution EM reconstructions of decorated actin (the rigor complex) showed how ATP binding sequesters switch 1 to open the large cleft in the motor domain thereby breaking actin binding site and weakening the binding to actin. Conversely, the strong binding to actin, together with the subsequent power-stroke, causes a movement of switch 1 with respect to the P-loop that opens the nucleotide binding site, bringing about the release of nucleotide (for review see (11).

The structure of globular actin (G-actin) was solved by Kabsch et al. (see (12)). Placing this structure into the actin helix so as to fit the X-ray fibre diffraction pattern resulted in an approximate atomic model of filamentous actin (F-actin). However, actin undergoes a conformational change from G- to F-actin. A more detailed study of the X-ray fibre diagram showed the nature of the G- to F- conformational change (Oda et al). Subsequently, a high resolution cryo-EM reconstruction of F-actin (Fujii et al) has yielded a more precise atomic model of F-actin.

Decorated actin (the rigor complex) allows one to study the strong F-actin-myosin interaction. Most recently high resolution cryo-EM reconstructions of decorated actin by Raunser et al and Fujii et al (in press) have produced atomic coordinates of the actin myosin interaction in the strong-binding state.

A number of crystallographic studies, notably by Anne Houdusse, (for review see (13)) have given insight into the function of myosin as a molecular machine – interacting domains much like cogs in a gear chain that are linked to a twisting of the central beta-sheet. The dynamics of these processes have been graphically demonstrated by Stefan Fischer - (14, 15) and unpublished. Nevertheless, for a complete structural description of the cross-bridge cycle one structure of seminal importance is lacking: the start the power stroke. During the cross-bridge cycle the cross-bridge in the pre-power stroke form carrying ADP and phosphate rebinds to actin. The presumption is that rebinding leads to a specific conformational change in the cross-bridge that closes the actin-binding cleft (completes the actin binding site) in a way that enables phosphate release but without initially altering the switch 1 element of the nucleotide binding site. Sweeney and Houdusse propose that the key is a coupled opening of switch 2 to allow phosphate release but without moving switch 1. The power stroke that follows is coupled with an opening of switch 1 to enable ADP release. On this basis we are now able to give a (nearly complete) description of the conformational changes in myosin that accompany the cross-bridge cycle.

[Related publications]

1. Huxley AF & Niedergerke RM (1954) Structural changes in muscle during contraction. Interference microscopy of living muscle fibres. *Nature* 173:971-973.
2. Huxley H & Hanson J (1954) Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature* 173:973-976.
3. Hanson J & Huxley HE (1957) Quantitative studies on the structure of cross-striated myofibrils. II. Investigations by biochemical techniques. *Biochim Biophys Acta* 23:250-260.

4. Huxley HE (1958) The contraction of muscle. *Sci Am* 199:67-82.
5. Reedy MK, Holmes KC, & Tregear RT (1965) Induced changes in orientation of the cross-bridges of glycerinated insect flight muscle. *Nature* 207:1276-1280.
6. Huxley HE, et al. (1981) Millisecond time-resolved changes in x-ray reflections from contracting muscle during rapid mechanical transients, recorded using synchrotron radiation. *Proc Natl Acad Sci U S A* 78:2297-2301.
7. Irving M, Lombardi V, Piazzesi G, & Ferenczi MA (1992) Myosin head movements are synchronous with the elementary force-generating process in muscle. *Nature* 357:156-158.
8. Piazzesi G, et al. (2007) Skeletal muscle performance determined by modulation of number of myosin motors rather than motor force or stroke size. *Cell* 131:784-795.
9. Szent-Gyorgyi AG (2004) The early history of the biochemistry of muscle contraction. *J Gen Physiol* 123:631-641.
10. Lymn RW & Taylor EW (1971) Mechanism of adenosine triphosphate hydrolysis by actomyosin. *Biochemistry* 10: 4617-4624.
11. Geeves MA & Holmes KC (2005) The molecular mechanism of muscle contraction. *Adv Protein Chem* 71:161-193.
12. Dominguez R & Holmes KC (2011) Actin Structure and Function. *Annual Review of Biophysics* 40:169-186.
13. Sweeney HL & Houdusse A (2010) Structural and functional insights into the Myosin motor mechanism. *Annu Rev Biophys* 39:539-557 .
14. Fischer S, Windshugel B, Horak D, Holmes KC, & Smith JC (2005) Structural mechanism of the recovery stroke in the myosin molecular motor. *Proc Natl Acad Sci U S A* 102:6873-6878.
15. Koppole S, Smith JC, & Fischer S (2007) The structural coupling between ATPase activation and recovery stroke in the myosin II motor. *Structure* 15:825-837.

Host: Prof. Robert Robinson

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