# The cytoskeleton and molecular motors, from molecular mechanisms to super-resolution imaging

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## Introduction

All cells need a cytoskeleton to maintain shape, to move, and for movement of intracellular vesicles and proteins. In skeletal and cardiac muscles, the cytoskeleton is highly organised to facilitate the main function of these muscles, which is to contract and either generate movement or force. The two main proteins that interact to generate contraction are actin, which is organised into thin filaments, and myosin, which is organised into thick filaments.. The organisation of these cytoskeletal proteins is so precise that each thick filament has exactly 296 molecules of myosin in each thick filament. Thick and thin filaments inter-digitate and are organised into an almost crystalline array. Along the length of the muscle fibre, the filaments are organised into a repeating pattern of units, about 2 microns long, called the muscle sarcomere, and each muscle sarcomere is identical to the next. Building these precise structures in muscle, and precisely regulating the lengths and organisation of contractile proteins is a major feat, and still one that is only poorly understood.

In non-muscle cells, myosins and actin are less organised, but still interact to enable cells to crawl on a substrate, generate force, or to move substances around inside the cells. There is still much to learn about the 39 different types of myosin in humans and how they are specialised for their functions either in muscle, or in non-muscle cells.

## Investigating mutations in cardiac myosin that cause disease

Mutations in sarcomeric proteins cause the disease hypertrophic cardiomyopathy. About 40% of these mutations occur in the β-cardiac myosin heavy chain (bMHC), the major component of the myosin molecule. Some of these mutations are found in the filament-forming tail domain of this myosin, and we have been investigating how these mutations affect the structure of this region of the myosin (funded by the British Heart Foundation). Intriguingly, we've discovered through this work that while myosin has a well-characterised coiled coil that dimerises this type of myosin, shorter peptide fragments up to 105 residues long do not necessarily dimerise by themselves, unless they contain a specific region of the coiled coil.

## Novel 'single alpha-helical' domains

While that tail of cardiac myosin contains a well-characterised coiled coil, which is responsible for dimerising the myosin heavy chain, we recently showed that many other unconventional myosins originally thought to contain a region of coiled coil, actually contain a stable single alpha helix. We've also shown that this type of structure is stiff enough to contribute to the movement of a myosin lever. We have just recently begun to study this domain in more detail (funded by BBSRC), to try to understand why it is so stable, and how the residues found in this type of structure contribute to its stability.

## Coiled coil or single alpha helix?

None of the prediction programs available are able to convincingly predict whether a sequence is coiled coil or single alpha helix. Programs that detect coiled coils such as Pepcoil or Coils, commonly fail to distinguish between these two distinct structures. Regions of coiled coil can show features of single alpha helices that may help in triggering formation of a region of alpha helix that helps to seed coiled-coil formation.

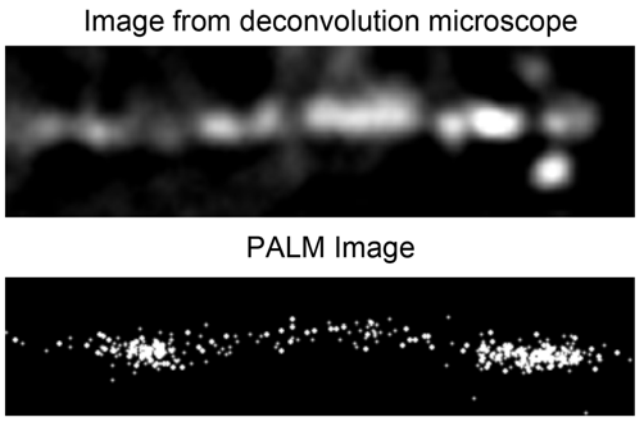


**Figure 1:** comparison of a typical high resolution light microscope image, obtained by deconvolution microscopy, of alpha-actinin in the Z-disc, with a PALM image of a Z-disc obtained using mEos2-tagged alpha-actinin.

Notice the large improvement in resolution in the PALM image.

**Super-resolution imaging**

Normally, the amount of detail that can be observed in a specimen in a light microscope is limited by the resolution of the instrument (~200nm). Various techniques have been developed in the last few years to overcome this limit, and I have been learning one of these; Photo-activated Light Microscopy (PALM) through visits to Hari Shroff's lab at NIH (USA), funded by a Wellcome Trust flexible travel award. I obtained high-resolution images of alpha-actinin in the Z-disc of adult cardiomyocytes (Figure 1), with a resolution of 20nm. Money has now been raised to build this type of microscope in Leeds in the next year.

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