

High pressure experiments

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We performed experiments with two different crystal forms of myosin. This was our first attempts to study this molecular motor with HPMX experiments. The crystals studied diffracted around 3 Å only while we are usually able to collect beyond 2 Å resolution data when the crystals are frozen. The crystals apparently suffered from the transport to Grenoble and it was clear that the improvement in the buffer used to soak the crystals was necessary since they also suffered from the high pressure experiment. Most of them died after the collection of a few images only (whereas other protein crystals already studied allow to collect 30 to 80 images).

Since then, we have improved not only the size and diffraction of the crystals themselves but we have also determined a mother liquor solution that maintain and stabilize the crystals for diffraction at 20°C temperature. The crystals will also be grown at Grenoble so that any problem during their transportation will be avoided.

We are proposing to collect on four different crystal forms. In particular a new crystal form of myosin VI is particularly interesting since it likely corresponds to the Pi release state which allows us to describe for the first time the beginning of the powerstroke of myosin.

Interestingly, this myosin VI crystal form is related to that found for the state that precedes this Pi release state. It would be extremely interesting to see whether pressure on these crystals would allow to populate a High energy conformer of myosin that would describe the structural state populates by the motor upon force generation.