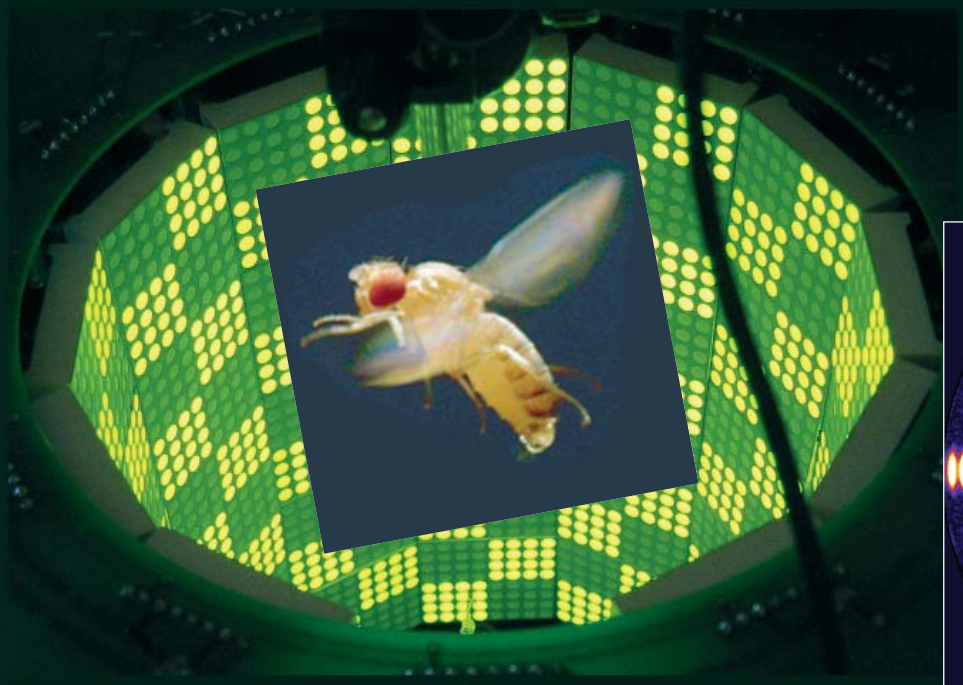
Storing the Power to Fly



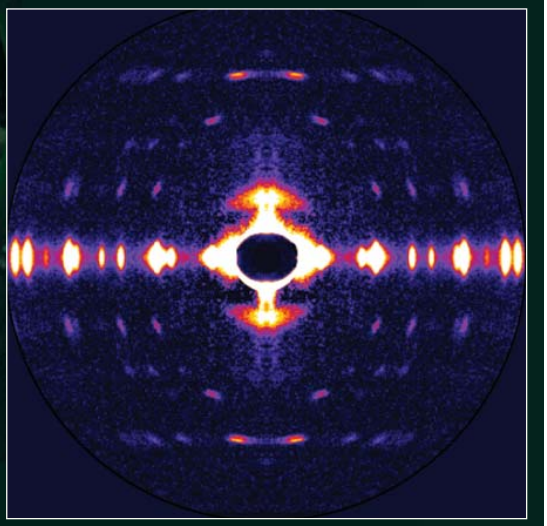


Fig. 1 (above). Smaller than the flashing green lights on its flight simulator, a fruit fly (inset) uses spring-like flight muscle proteins to beat its wings. Fig. 2 (right). All the muscle components diffract high-intensity x-rays that are captured on the detector in colorful patterns.

Fruit flies beat their wings faster than their cellular powerplants can generate the energy needed for flapping. To resolve this energetic discrepancy, researchers from the California Institute of Technology, the Illinois Institute of Technology, and the University of Vermont used the Bio-CAT beamline 18-ID at the APS to obtain a series of x-ray photographs that revealed the flies' secret: A muscle protein used to power wings acts like a spring, storing energy while stretched before snapping back. Not only did this finding surprise researchers who study muscle, but the results might also help scientists better understand the human heart.

Drosophila, the fruit fly, beats its wings up and down once every 5 milliseconds. Two layers of muscle control this action: when one layer contracts, it stretches the other. The stretched muscle is then primed to contract; when it does, it stretches the first layer and completes the cycle. These cycles occur faster than nerve impulses can stimulate them; hence, the muscles themselves must keep the beat going.

To find out how muscles do this, the research team needed to visualize the proteins that make muscle contract. Within the flight muscle cells, two proteins cooperate to do this. Myosin proteins have flexible heads and long tails that bundle together. The heads grab filaments made of the protein actin that ultimately connect at either end to opposite cell walls. Like a bunch of hands pulling on a rope, the myosin heads drag actin inward, making the muscle cell shorter. Methods to visualize what proteins look like often require purifying the protein or killing the organism in which it exists. Not only did the team want to keep the flies alive, but they wanted to watch myosin and actin in action. Short bursts of APS's high-intensity x-rays allowed them to achieve both goals.

The researchers tethered flies inside a box (a “flight simulator”) and used air and moving lights to convince the bugs that they were buzzing around. Then the team took x-ray snapshots of the proteins responsible for beating the wings. They synchronized the beam's shutter speed with the wing beat's frequency. Much like a strobe light reveals the status of a dancer instantaneously, synching the wing beat to the synchrotron x-rays allowed the scientists to view the positions of the muscle proteins at one point in time. The team put together a series

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of these images taken at different points of the beat to create a stop-action film of the protein movements within muscle cells.

After exposure in the beam, myosin and the other muscle components diffract x-rays onto a detector in specific patterns. From this pattern, researchers can tell if myosin is grabbing onto the actin filament, pulling, or releasing its grasp. In contracted muscle, the researchers saw myosin dragging the actin filament as expected.

But when the muscles relaxed, the team saw an unanticipated movement. Instead of completely releasing its grasp as myosin does in other relaxed muscles, the myosin briefly let go, but then snagged the filament again. And the bundle of myosin tails—long believed to be stiff for pulling during contractions—stretched a bit, due to the second layer of contracting flight muscle. This finding suggested to the researchers that myosin stores elastic energy in its tail that can be used in the next contraction, much like a stretched spring holds onto energy and releases it when the spring recoils. The stretching also appears to stimulate the myosin and actin filaments to again contract the muscle.

In addition to this novel finding, the technological advancement that allows scientists to view the composition of a living muscle will now let them study muscle contraction in greater detail. Fruit flies are well suited for genetic studies that might eventually illuminate how human heart muscle, which is similar to a fly's muscle, can fail when diseased. — *Mary Beckman*

See: M. Dickinson[1], G. Farman[2], M. Frye[1*], T. Bekyarova[2], D. Gore[2], D. Maughan[3], and T. Irving[2], "Molecular Dynamics of Cyclically Contracting Insect Flight Muscle *In Vivo*," Nature **433**, 330 (20 January 2005)

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IRON ON THE BRAIN

Everyone needs iron, but too much of it in the wrong place in the body can be a very bad thing. Neurodegenerative disorders, such as Alzheimer's, Huntington's, and Parkinson's disease, have all been shown to be closely related to the presence of too much iron in brain tissue, which is the result of disturbances in normal iron metabolism. But discovering how much iron is present in the brain, and in what particular compounds, has proven to be a challenge. The chief methods used at present rely on staining of tissue samples, a technique that provides poor resolution and does not provide any data on the makeup of specific iron compounds. Clearly, a more precise and accurate strategy for locating and identifying iron anomalies in brain tissue is needed. Now, researchers from the University of Florida, the University of Minnesota, Keele University, and Argonne National Laboratory, using the MR-CAT insertion device beamline at the APS, may have found just that strategy.

Using the MR-CAT 10-ID beamline at the APS, the team has developed a technique that combines the technique of x-ray absorption near-edge spectroscopy (XANES) with with iron-edge area scanning. The process not only allows individual, minute iron anomalies to be detected with much greater resolution and sensitivity than ever before, but it also makes possible their identification and specific characterization *in situ*.

The experimenters used tissue samples from the midbrain of a homing pigeon for this initial demonstration of the technique. The samples were fixed and sealed between two sheets of Kapton film containing gridlines of zinc wire for use as an orientation tool. The researchers ensured that the methods and materials used to prepare the samples, including the fixing solutions, Kapton sheets, and slides, were fully cleaned and purified to prevent the detection of extraneous impurities during the experimental procedures. Comparing the x-ray fluorescence of the tissue samples above the iron K absorption edge at 7,112 eV with their fluorescence below the iron absorption edge, the team was able to generate an iron concentration map for each sample (Fig. 1). A detection limit of less than 1 ppm is possible because of the high intensity of the APS microfocused synchrotron x-rays. Because of the extreme sensitivity this affords, a single particle of nanometer scale can be detected in a 500-μm spot.

The tiny areas of iron concentration found were then examined at even higher resolution— down to 5 μm—followed by XANES analysis. The data obtained from the XANES studies were compared with measured standards and published results for magnetite, ferritin, haemosiderin, and haemoglobin. This revealed that the iron anomalies detected in the tissue samples consisted of ferritin, magnetite, and haemoglobin. The researchers also confirmed that the sample preparation tech-