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Visualization of the mechanochemical coupling in myosin V using deac-aminoATP TAKESHI SAKAOTO, Wayne State University, MARTIN WEBB, MRC National Institute for Medical Research, UK, EVA FORGACS, HOWARD WHITE, Eastern Virginia Medical School, JIM SELLERS, National Heart, Lung, and Blood Institute — MyosinV is a twoheaded motor, which moves processively along an actin with ADP-release as the rate limiting step. The kinetic cycles of the two heads are gated by the internal strain each places on the other. Mechanical studies suggest that there is tight coupling (i.e., one ATP is hydrolyzed per power stroke). We investigated the coordination between the ATPase mechanism of the two heads-myosin Va and directly visualized the binding and dissociation of nucleotide molecules, while simultaneously observing the stepping motion of the myosin V as it moved along an actin filament. To do this, we used a fluorescent labeled ATP analog, deac-aminoATP, which shows a 20fold increase in fluorescent intensity when bound to the active site of myosinV. I directly demonstrate tight coupling between myosin V movement and the binding and dissociation of nucleotide by simultaneously imaging with near nanometre precision. Supported by K99/R00 NIH grant.

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