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A FRET-based sensor reveals large ATP hydrolysis-induced conformational changes and three distinct states of the molecular motor myosin.

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The molecular motor myosin is proposed to bind to actin and swing its light-chain binding region through a large angle to produce an approximately 10 nm step in motion coupled to changes in the nucleotide state at the active site. To date, however, direct dynamic measurements have largely failed to show changes of that magnitude. Here, we use a cysteine engineering approach to create a high resolution, FRET-based sensor that reports a large, approximately 70 degree nucleotide-dependent angle change of the light-chain binding region. The combination of steady-state and time-resolved fluorescence resonance energy transfer measurements unexpectedly reveals two distinct prestroke states. The measurements also show that bound Mg.ADP.Pi, and not bound Mg.ATP, induces the myosin to adopt the prestroke states.