



Excerpt of a fluorescence trace (black line) showing the cycling of Cy3-Cy5 switches separated by a contour length of 46 nm on dsDNA (See **Fig. 3a** in the main text). Each switching cycle lasts for 5 seconds and consists of a brief green pulse (underlying green bar) to activate one or two switches followed by a long red exposure (underlying red bar) to excite Cy5 fluorescence and return the switches to the off state. In cycles when one switch is activated (e.g. 225-230 seconds), a single intensity level is apparent before the return to the dark state. In cycles where two switches are activated (e.g. 245-250 seconds), a "staircase" with two decreasing levels corresponding to the sequential switching off of each dye is observed. Regions where only a single switch is on and all of the peak quality criteria (see **Supplementary Methods** online) are satisfied (highlighted in cyan) were used for single-switch localization.