Fig. 1S. *Image of cardiac myofibril. A: Lifetime image. Scale to the right is in ns. Intensity scales in* ***B*** *(total fluorescence intensity. Scale bar is 5 µm,* ***C*** *(parallel component of fluorescence intensity) and* ***D*** *(perpendicular component of fluorescence intensity) are in B/W scale, 0 corresponding to black, 255 corresponding to white. Red circle in A pointed to by the red arrow is a projection of the confocal aperture on the image plane.*

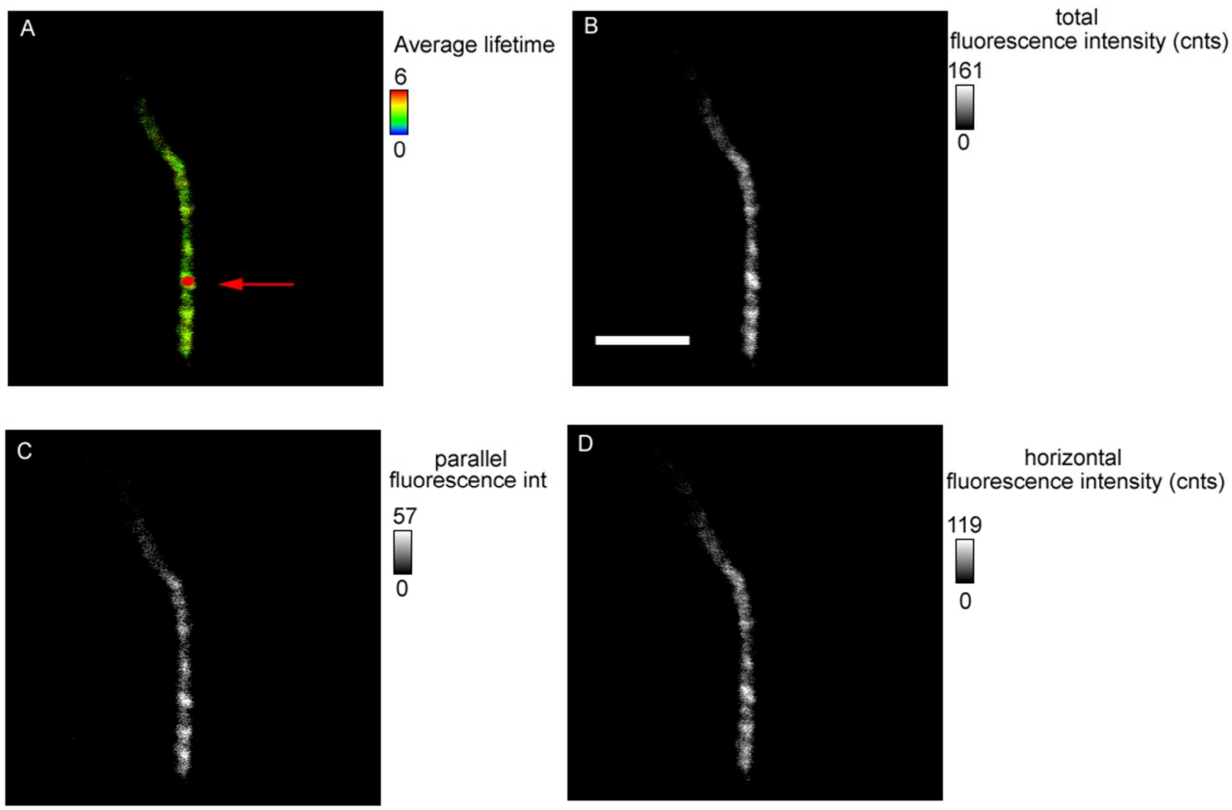

Fig. 2S. *Typical time-trace of intensity of the LV. The mean intensity of perpendicular channel (red) was 0.8580± 0.3914 (SD), mean intensity of parallel channel (blue) was 2.0544± 0.6479 and the mean polarization of fluorescence (black) was -0.4179± 0.1759.In this example the total intensity was 2.05+2*0.85=3.76 counts/ ms and the number of detected molecules was 3760/1200≈3. Typically the total fluorescence intensity was between 4,000 and 7,000 counts/s, i.e. the number of detected molecules was between 3 and 6.*

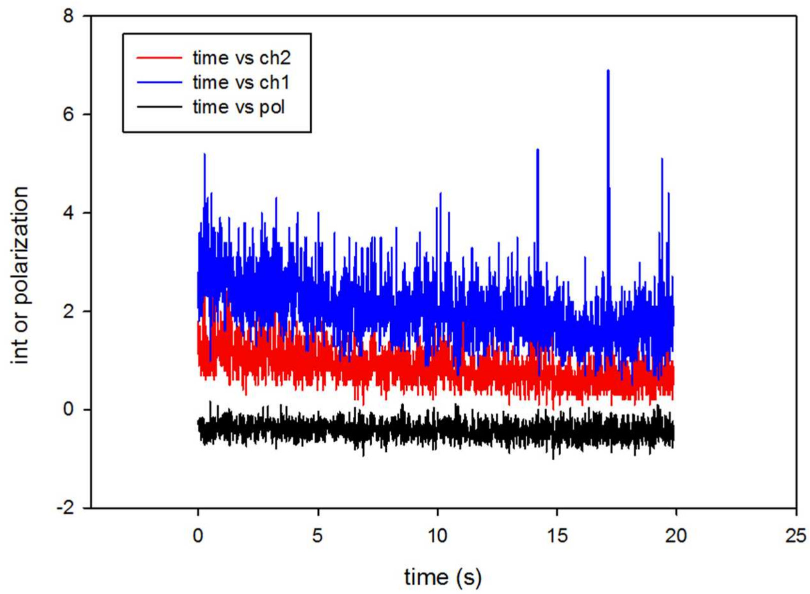

Fig. 3S*. Calculation of the rate constants. Experimental ACF curves (col 1) were fitted by the equation shown as Fig. 5S of this Supplement. The resulting hyperbolic fit was (arbitrarily) divided into slow (col 2) and fast (col 3) decaying part, re-plotted using linear horizontal scale and fit to a straight line. First and last two rows are the data from LVs and RV's, respectively. Although fits appear similar, RV and LV decay with different rates.*

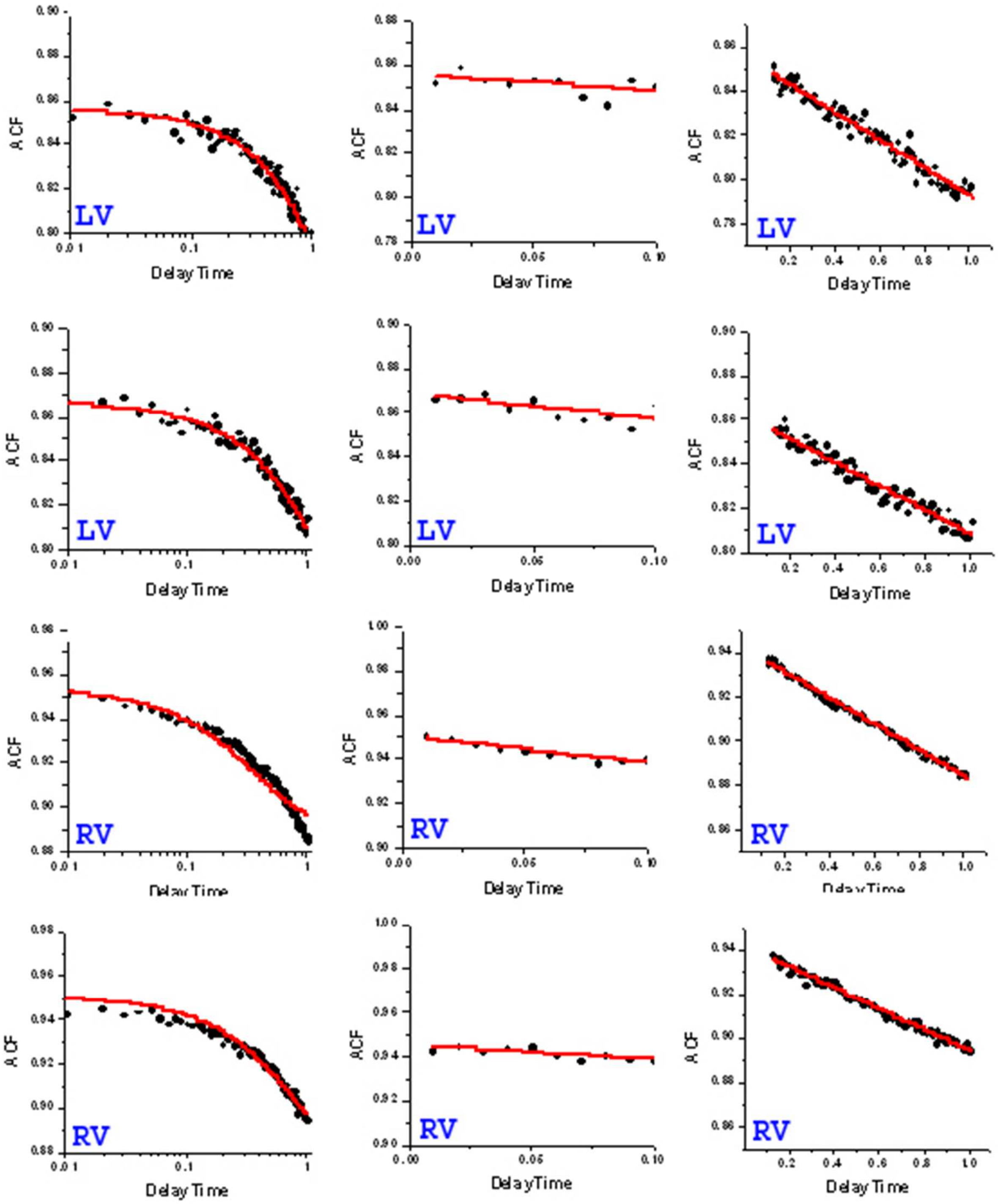

Fig. 4S. *Examples of distribution of polarization values of* ***RELAXED*** *LV (top panels) and RV (bottom panels). The statistical significance of the differences are in text Table 3.*

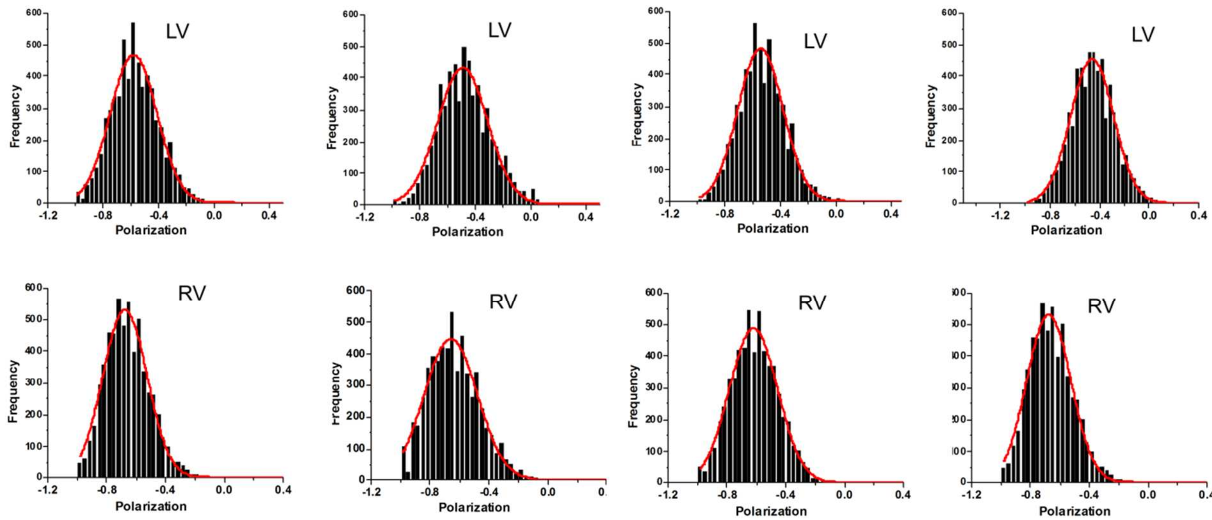

Fig. 5S. *The constant a_1 is the anisotropy of state MT and MDP. The constant a_2 is the anisotropy of state AMDP. The constant a_3 is the anisotropy of state AM. The values are determined by the computer.*

$$
\begin{aligned}
R_3(t) = {} & \frac{\left(a_1k_2k_3 + a_2k_3k_1 + a_3k_1k_2\right)^2}{\left(k_2k_3 + k_3k_1 + k_1k_2\right)^2} + \\
& + \frac{k_1k_2k_3\left(A\kappa + B\right)}{2\kappa\left(k_2k_3 + k_3k_1 + k_1k_2\right)^2}\exp\left(-\frac{1}{2}\left(k_1 + k_2 + k_3 - \kappa\right)t\right) + \\
& + \frac{k_1k_2k_3\left(A\kappa - B\right)}{2\kappa\left(k_2k_3 + k_3k_1 + k_1k_2\right)^2}\exp\left(-\frac{1}{2}\left(k_1 + k_2 + k_3 + \kappa\right)t\right)
\end{aligned}
\tag{1}
$$

$$\kappa = \sqrt{k_1^2 + k_2^2 + k_3^2 - 2k_2k_3 - 2k_3k_1 - 2k_1k_2} \tag{2}$$

$$A = a_1^2\left(k_2 + k_3\right) + a_2^2\left(k_3 + k_1\right) + a_3^2\left(k_1 + k_2\right) - 2\left(k_1a_2a_3 + k_2a_3a_1 + k_3a_1a_2\right) \tag{3}$$

$$
\begin{aligned}
B = {} & a_1^2\left(k_2^2 + k_3^2 - k_1\left(k_2 + k_3\right)\right) + a_2^2\left(k_3^2 + k_1^2 - k_2\left(k_3 + k_1\right)\right) + \\
& + a_3^2\left(k_1^2 + k_2^2 - k_3\left(k_1 + k_2\right)\right) + 2\left(k_2k_3 - k_1^2\right)a_2a_3 + \\
& + 2\left(k_3k_1 - k_2^2\right)a_3a_1 + 2\left(k_1k_2 - k_3^2\right)a_1a_2
\end{aligned}
$$