Steady-state Fluorescence Measurements

Steady-state spectra and time courses were detected in a FluoroMax SPEX-320 spectrofluorimeter at 20°C. Excitation wavelength was 296 nm, which enabled us to excite tryptophans specifically without exciting tyrosines. Slits were 2 nm on both the excitation and the emission monochromators. Emission spectra were recorded from 305 nm to 420 nm to avoid detecting the direct light. We used a Hellma 105.250-QS precision cuvette with 10 mm light path. Time courses were recorded with the same settings on the excitation side and the emission monochromator was adjusted to 340 nm where the intensity changes of W501 fluorescence were the largest. Time courses were detected by applying either tryptophan fluorescence or light scattering signal at 340 nm. Actomyosin association and ATP-dependent dissociation was followed by light scattering at 340 nm to check the effects of the mutations on actomyosin interaction. 3 μM MD-s were mixed with 4 μM F-actin at 20 ºC in the absence of nucleotide, after rigor formation (enhanced light scatter signal) ATP was added in 3 molar excess. Intensity decrease referred to actomyosin dissociation and reattachment is detected after the ATP turnover as signal level returned.