FRET characterization for cross-bridge dynamics in single skinned rigor muscle fibres.

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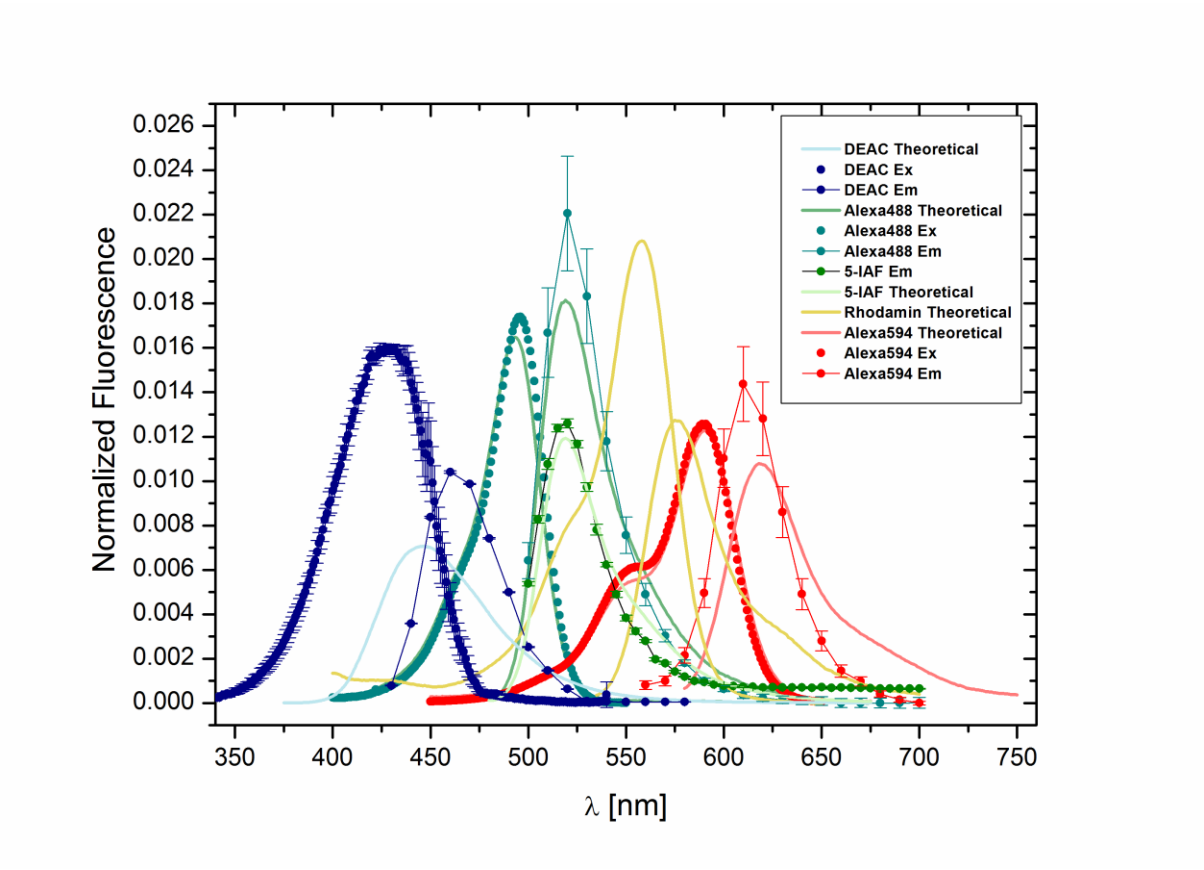

Fig.ESM1 Excitation and emission spectra for the fluorophores used. Emission spectra were evaluated directly on single rigor muscle fibres. The comparison with the theoretical spectra is shown

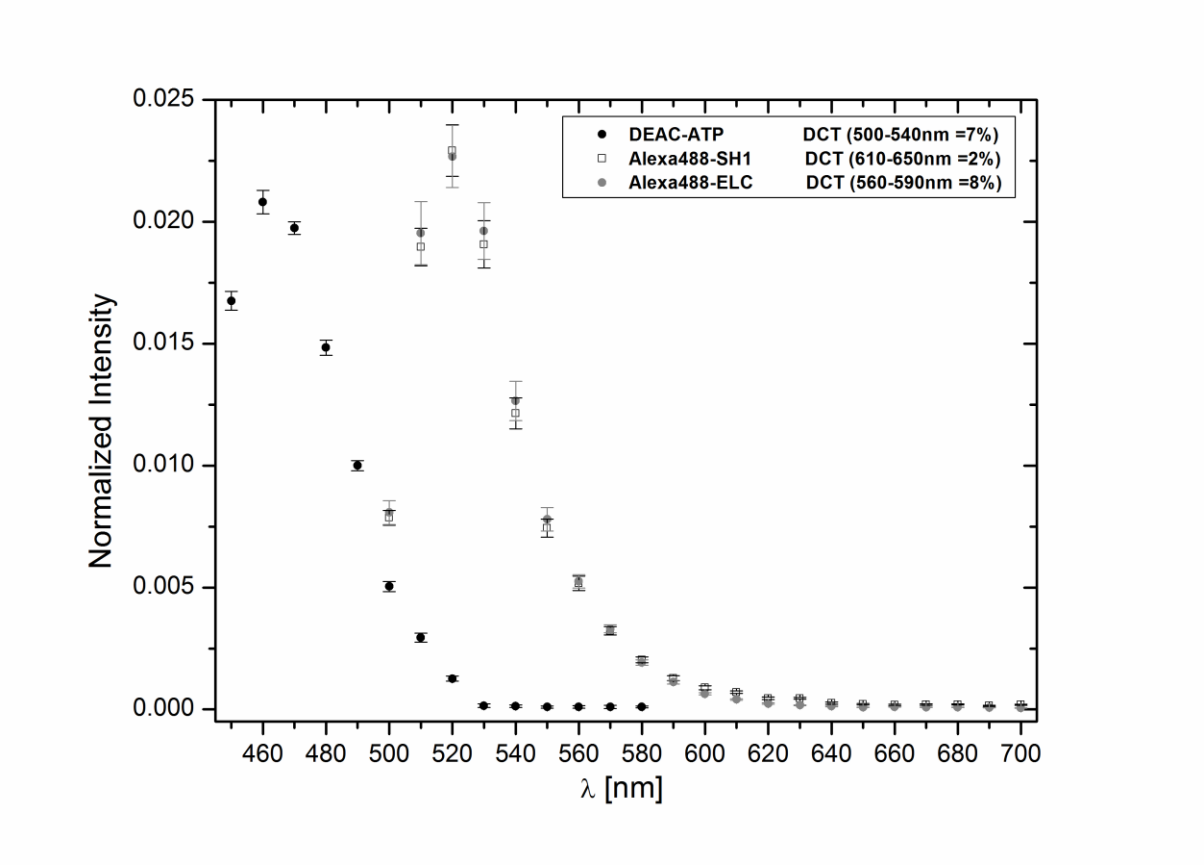

Fig.ESM2 Donor Cross Talk (DCT) characterization for the three FRET couples measured in rigor muscle fibres. The emission spectra are normalized so that the integral over the entire spectrum is equal to one; the percentage of donor cross talk in the acceptor channel is shown in the inset; the donor excitation wavelength was set to 790nm for DEAC-ATP, 488nm for Alexa488-SH1 and Alexa488-ELC

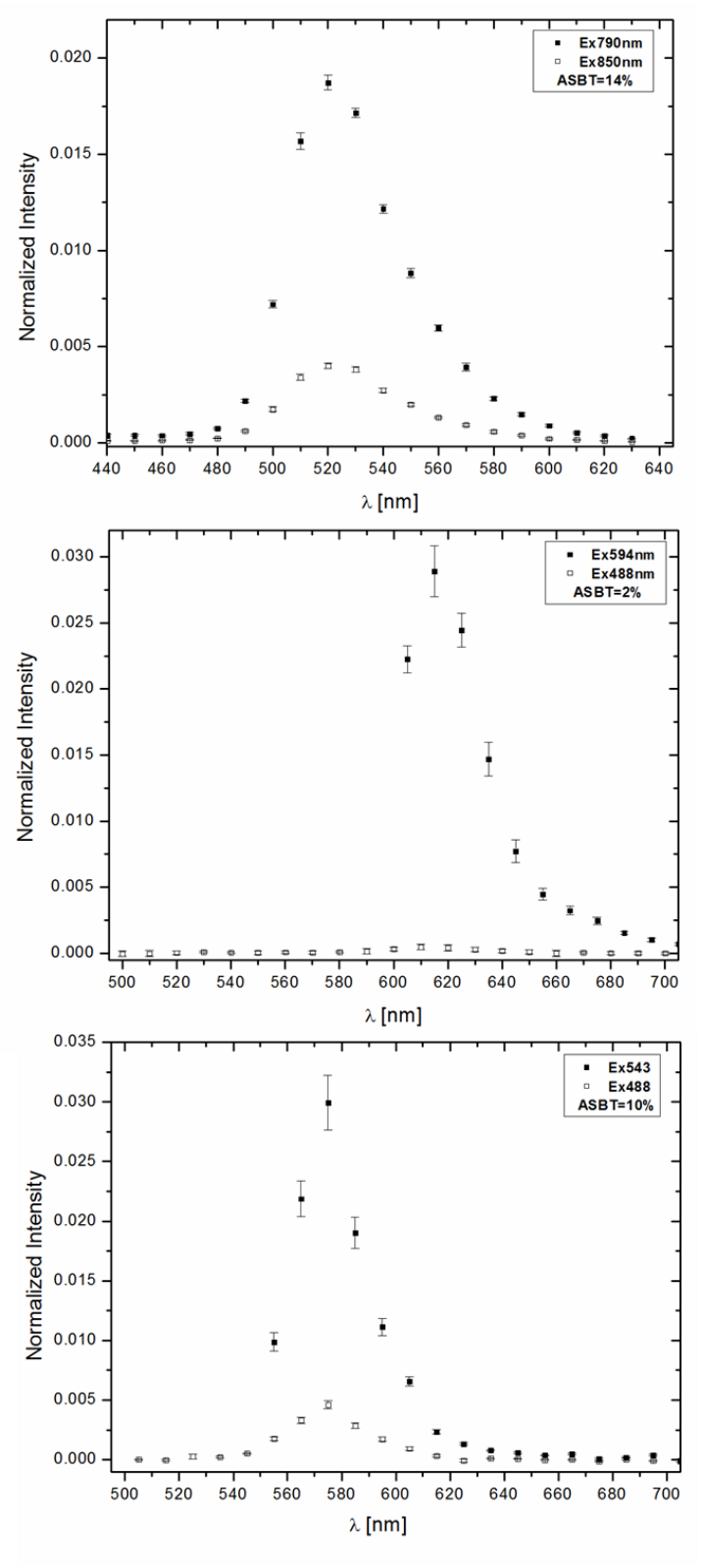

Fig.ESM3 Acceptor Spectral Bleed Through (ASBT) characterization for the three FRET couples measured in rigor muscle fibres: DEAC-ATP-ELC (top), SH1-ELC (middle), Actin-ELC (bottom). The emission spectra obtained at the maximum acceptor excitation wavelength (790nm TPE for DEAC-ATP, 594nm for Alexa594-ELC, 543nm for Rhodamine Phalloidin-Actin) are normalized so that the integral over the entire spectrum is equal to one, in this way the integral under the spectra at the donor excitation wavelength represent the percentage of ASBT, shown in the inset

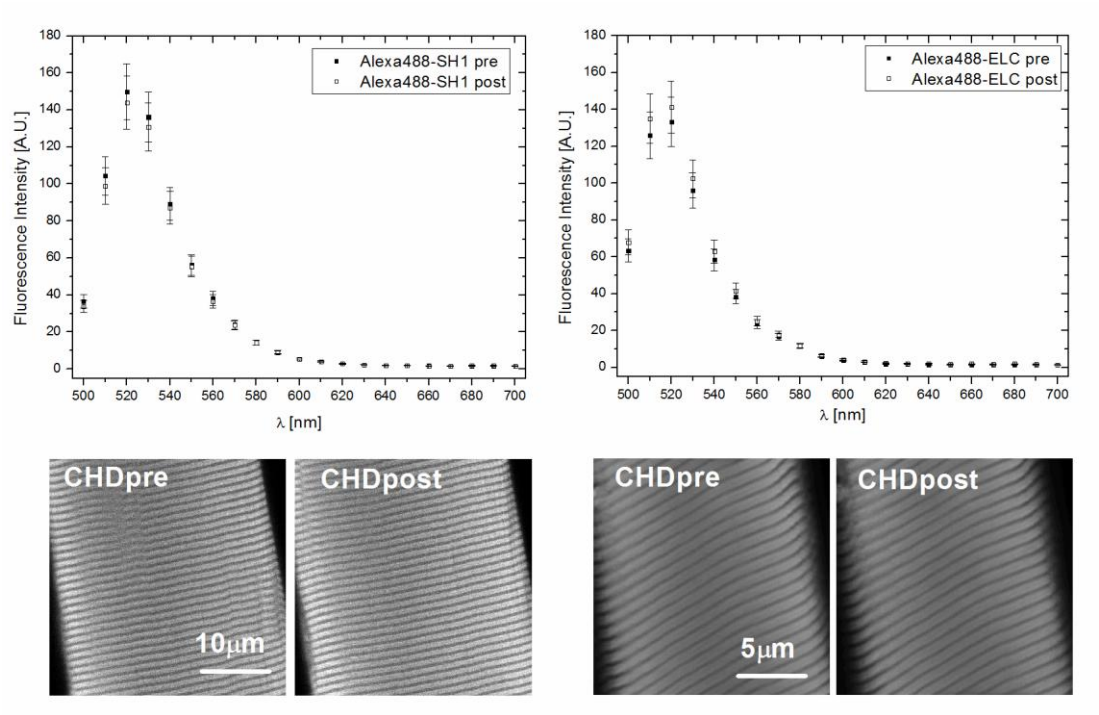

Fig.ESM4 Donor Bleaching Characterization. Acceptor photobleaching experiments performed on donor alone sample (Alexa488-SH1, right, Alexa488-ELC, left) to check possible donor photobleaching by the acceptor excitation wavelength, 594nm and 543nm respectively. As it is clearly shown from both the images and the spectra no significant donor photobleaching is observed

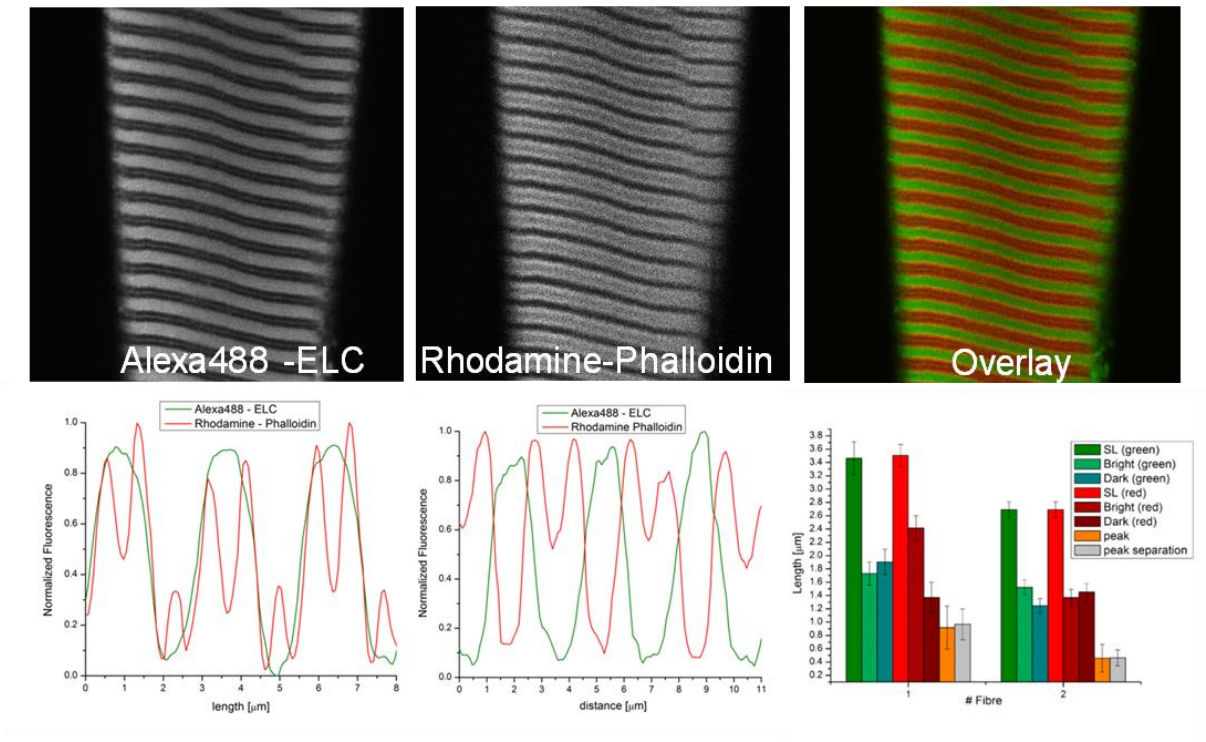

Fig.ESM5 Labelling characterization for the ELC-180 and actin: images showing the fluorescence signal arising from Alexa488-ELC-180 (top, left), Rhodamine Phallodin-Actin (top, centre) and the overlay (top, right); the intensity plot profiles (tracing a line perpendicular to the sarcomeres) are presented at two different sarcomere lengths (green line for the Alexa488-ELC signal, red line for the Rhodamine-Phalloidin-Actin signal); on the right the related lengths histogram is shown, demonstrating that the lengths of the bright or dark bands is in agreement with the theoretical expectations