Software to implement binaryFRET Software descriptions:

1. LabView (FPFA v4.vi): The LabView (LV) software tool was developed to communicate with • ProScan III Controller (Prior Scientific): the controller is responsible for movement of HLD117 XY scanning stage, which is used to laterally position a sample on top of the microscope objective. • MFC1 Motorized Microscope Focus Controller (Thorlabs): to vertically position a sample along the z direction. • SC10 Optical Beam Shutter Controller (Thorlabs): this controller control SH1 Optical Shutter SH1 (Thorlabs) that opens/blocks excitation beam. • SPC-130EM card (Becker and Hickl): the LV uses the SC10 to send a TTL signal to the SCP card to initiate data acquisition. The acquisition time was set as a parameter of the SCP card. • The LV tool also serves as timer for the time-lapse binary-FRET experiments. • The LV tool uses LabView drives and libraries (.llb) developed by Prior Scientific, Thorlabs and Becker & Hickl.
2. Igor (Homo and Hetero-FRET fifo.pxp): • Data acquired by SPC-130EM card was saved in hard drive of a PC. For time-lapse binary-FRET, individual measurement was saved as single .sdt file which contains micro-time (and macro-time) of photons detected for both parallel and perpendicular channels. Each .sdt file was converted into a single ASCII .asc file by SPCM software (Becker and Hickl). • The Igor routine was developed to read the micro-time data from multiple ASCII files and correspondingly perform calculations of lifetime and steady state anisotropy. • The calculated lifetime and steady state anisotropy were transferred to Prism 8 (Graph Pad) for graphs and statistical calculations.
3. Igor (SSA-FLIM Images.pxp): Data acquired by SPC-150N card was saved in hard drive of a PC. For Imaging binary-FRET, individual measurement was saved as single .sdt file which contains micro-time (and macro-time) of photons detected for both parallel and perpendicular channels at each X- and Y- image coordinate. Each .sdt file was imported into Becker and Hickl SPCImage software, and a triple exponential decay model fit was performed for the parallel and perpendicular channels at each X- and Y- pixel coordinate. SPCImage software generated intensity image files, amplitude image files, and average lifetime image files for each channel ( all saved as .asc files). • The Igor routine was developed to read the multiple ASCII files and correspondingly perform calculations of lifetime and steady state anisotropy.

In-vitro binary FRET data files: measure\_c1.asc, measure\_c2.asc, measure\_c3.asc Background: dark\_c1.asc Instructions: Steps of using Homo and Hetero-FRET fifo.docx. Note, use the latest version.

Imaging binary FRET data files: data-Ch1-\_a1.asc, data-Ch1-\_a2.asc, data-Ch1-\_a3.asc, data-Ch1-\_color coded value.asc, data-Ch1-\_photons.asc, data-Ch2-\_a1.asc, data-Ch2-\_a2.asc, data-Ch2-\_a3.asc, data-Ch2-\_color coded value.asc, data-Ch2-\_photons.asc. Instructions: Imaging binaryFRET instructions.

Install times on normal Mac desktop are near instntaneous, as are run times. This software should work on user data obtained using Becker and Hickl FLIM software exported as an asc file.