Supplementary Information for:

Conformational distributions of protease-serpin complexes: a partially translocated complex

Lu Liu[‡]. Nicole Mushero[§], Lizbeth Hedstrom[‖] & Anne Gershenson[‡]

[‡]Department of Chemistry, [§]Graduate Program in Biochemistry & [‖]Department of Biochemistry
Brandeis University, 415 South Street, Waltham, MA, USA 02454

Supplementary Text:

Steady state single molecule anisotropies. Single molecule anisotropies were measured by exciting the sample with linearly polarized light, and replacing the emission dichroic used for spFRET measurements with a polarizing beamsplitter (Newport). In addition, identical bandpass filters, D675/50 (Chroma Technology) for Atto 610, were placed before each detector. Anisotropies, r_{SM}, were calculated from the intensities of the s polarized emission, I_s, and p polarized emission, I_p:

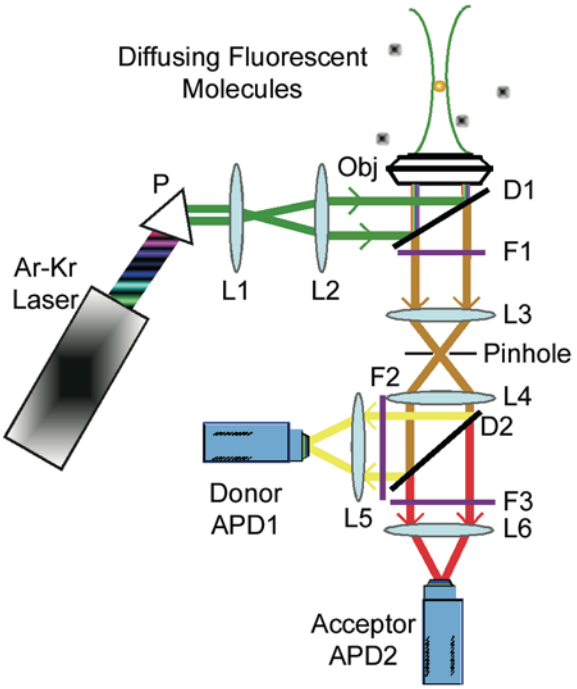
$$r_{SM} = \frac{\left(I_s - I_p\right)}{\left(I_s + 2I_p\right)} \quad (1)$$

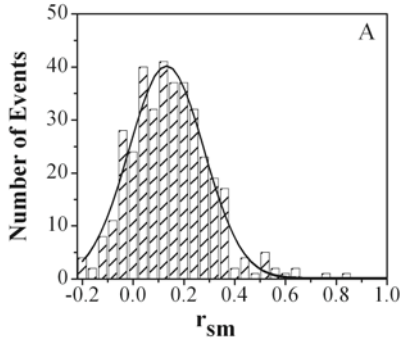
Single molecule anisotropy histogram peaks were fit to a single Gaussian. As can be seen from the single molecule anisotropy histograms for vertically polarized 568 nm excitation, the center of the anisotropy peak is lower, 0.13, for Atto610 maleimide, but the centers, 0.25, and half-widths, 0.13, of the anisotropy histograms are essentially identical for Atto 610 α_1PI, S113C BTryp-Atto 610 α_1PI and K113C RTryp-Atto 610 α_1PI (Figure S2). Thus, changes in orientation of Atto610 are not responsible for the second peak seen in the Alexa Fluor 555 K113C RTryp-Atto 610 α_1PI spFRET histograms.

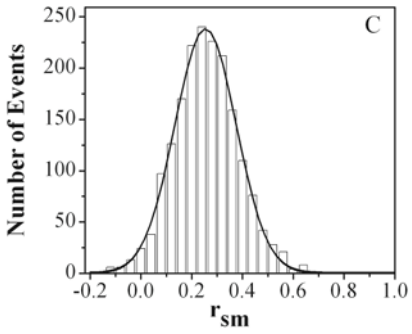
Supplementary Figure Legends.

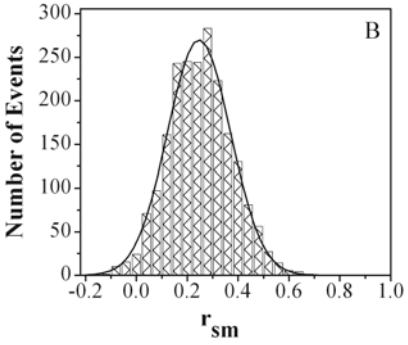
Figure S1. Schematic of the experimental setup. Individual laser lines from an argon-krypton air cooled, multi-line laser (Melles Griot) are separated using a quartz prism, P. The 520 nm (488 nm) laser line is picked off, expanded 5 times using two lenses, L1 & L2, in order to overfill the back aperture of the microscope objective and reflected into the sample using a 535drlp (500drlp) dichroic (Chroma Technology), D1. In the IX-70 inverted microscope (Olympus), the 60X water objective, Obj, with a numerical aperture of 1.2 focuses the laser light into the sample and collects the emission. The emission passes through D1 and any remaining scattered laser light is blocked by a HQ540lp (HQ505lp) long pass filter (Chroma Technology), F1. The emission is then focused onto a 100 μm pinhole by the tube lens, L3, in the microscope to limit the detection region and collimated by lens L4. To differentiate donor and acceptor fluorescence, the emission is spilt by color by a Q605lp (550dcxr) dichroic (Chroma Technology), D2. The greener emission is reflected by D2, passes through a HQ580/30 (HQ520/40) bandpass filter (Chroma Technology), F3 and is focused on the "donor" avalanche photodiode, APD1, by lens L5. The redder emission is transmitted by D2, passes through a D675/50 (HQ645/75) bandpass filter (Chroma Technology), F4 and is focused on the "acceptor" avalanche photodiode, APD2, by lens L6.

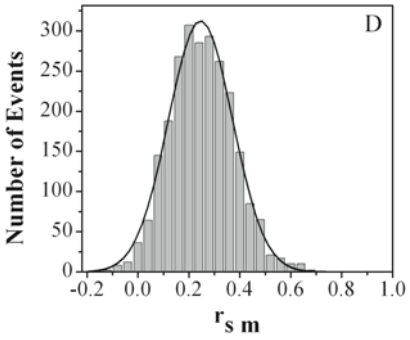
Figure S2. Single molecule anisotropy histograms for A. Atto 610 maleimide; B. Atto 610 α_1PI; C. S113C BTryp-Atto 610 α_1PI and D. K113C RTryp-Atto 610 α_1PI in 50 mM HEPES, pH 7.4, 100 mM NaCl with vertically polarized 568 nm excitation. For 568 nm excitation, a Q585lp (Chroma Technology) and an E590lp (Chroma Technology) are used as the excitation dichroic and filter respectively (D1 and F1 in Figure S1).


Diffusing Fluorescent
Molecules
Obj
D1
P
F1
Ar-Kr
Laser
L1
L2
L3
Pinhole
F2
L4
D2
Donor
APD1
L5
F3
L6
Acceptor
APD2


A
Number of Events
r_{sm}


C
Number of Events
r_{sm}


B
Number of Events
r_{sm}


D
Number of Events
$r_{s\ m}$