

	pH	Occupancy (%)		
		High FRET	Inter. FRET	Low FRET
HA*	7	75±3%	13±2%	12±3%
HA*	6.4	58±6%	18±3%	24±4%
HA*	6.1	23±4%	20±2%	57±5%
HA*	5.8	22±4%	15±3%	63±5%
HA*	5.6	10±2%	12±2%	78±3%
HA* + CR9114	6.1	37±5%	26±4%	37±3%
HA* + KB2	6.1	33±6%	31±5%	36±4%
HA* + liposome/GD1a	7.5	53±4%	19±3%	28±5%
HA* + liposome/GD1a	7	40±3%	17±3%	43±4%
HA* + liposome/GD1a	6.4	30±2%	18±4%	52±3%
HA* + liposome/GD1a	6.1	22±2%	19±2%	59±2%
HA* + liposome/GD1a	5.8	19±1%	16±3%	65±2%
HA* + liposome/GD1a	5.6	18±2%	12±3%	70±1%
HA* + LSTa	7.5	52±5%	22±3%	26±3%
HA* + LSTa	7	50±2%	16±2%	34±4%
HA* + LSTa	6.4	39±4%	22±4%	39±4%
HA* + LSTa	6.1	33±2%	20±2%	47±5%
HA* + LSTa	5.8	23±3%	27±4%	50±2%
HA* + LSTa	5.6	13±2%	23±3%	64±1%
HA* + liposome	7.5	60±5%	26±3%	14±4%
HA* + liposome	7	59±6%	21±4%	20±5%
HA* + liposome	6.4	46±3%	25±2%	29±3%
HA* + liposome	6.1	19±4%	13±2%	68±2%
HA* + liposome	5.8	12±2%	17±2%	71±3%
HA* + liposome	5.6	13±1%	12±3%	75±3%
HA0*	7	65±5%	19±6%	16±4%
HA0*	6.1	41±6%	28±5%	31±3%
HA0*	5.3	30±4%	35±4%	35±6%
HA* G4E	7.5	13±4%	16±5%	71±6%
HA* G4E	5.6	12±4%	25±7%	63±5%
<b>pH 5.6 → 7.5</b>				
HA*	5 min	61±4%	19±5%	20±4%
HA*	15 min	52±3%	19±2%	29±6%
HA*	30 min	32±4%	20±3%	48±2%
<b>pH 5.3 → 7.5</b>				
HA*	5 min	72±6%	15±5%	13±3%
HA*	15 min	57±5%	18±4%	25±5%
HA*	30 min	36±6%	23±2%	41±4%
<b>pH 5.6 → 7.5</b>				
HA*	5 min	68±4%	16±4%	16±6%
HA*	15 min	45±4%	23±6%	32±5%
HA*	30 min	21±5%	18±5%	61±4%
<b>pH 5.6 → 7.5</b>				
HA* + liposome/GD1a	5 min	27±2%	23±3%	50±4%

<b>HA* + liposome/GD1a</b>	30 min	22±3%	15±3%	63±2%
<b>pH 5.6 → 7.5</b>				
<b>HA* + LSTa</b>	5 min	41±6%	19±2%	40±4%
<b>HA* + LSTa</b>	30 min	39±5%	18±2%	43±5%
<b>pH 5.6 → 7.5</b>				
<b>HA* + liposome</b>	5 min	15±2%	16±1%	59±4%
<b>HA* + liposome</b>	30 min	13±1%	19±2%	68±6%

**Table S1 | FRET state occupancies determined through HMM analysis, Related to Figures 2, 4, 5, and 6.**

	<b>pH</b>	<b>Lifetime (ns)</b>	<b>Anisotropy</b>
<b>Cy3</b>	7	0.72 ± 0.003	0.19 ± 0.01
<b>Cy3-HA*</b>	7	1.05 ± 0.004	0.13 ± 0.02
<b>Cy5</b>	7	1.05 ± 0.005	0.07 ± 0.01
<b>Cy5-HA*</b>	7	1.57 ± 0.005	0.08 ± 0.01
<b>Cy3</b>	5.6	0.70 ± 0.003	0.19 ± 0.02
<b>Cy3-HA*</b>	5.6	1.15 ± 0.004	0.12 ± 0.02
<b>Cy5</b>	5.6	1.09 ± 0.004	0.08 ± 0.01
<b>Cy5-HA*</b>	5.6	1.62 ± 0.006	0.08 ± 0.01

**Table S2 | Fluorescence lifetime and anisotropy of Cy3 and Cy5, Related to Figure 2.**

Virions were produced as described in **STAR Methods**, and labeled with either the Cy3 or Cy5 fluorophore. Measurements were made on a QuantaMaster 400 fluorimeter equipped with a time-correlated single photon counting module (PTI).

	pH	$k_1$ (sec <sup>-1</sup> )	$k_{-1}$ (sec <sup>-1</sup> )	$k_2$ (sec <sup>-1</sup> )	$k_{-2}$ (sec <sup>-1</sup> )
<b>HA*</b>	7	4.78±0.51	15.33±1.24	5.19±0.73	5.25±0.77
	6.4	6.39±0.58	10.32±0.49	6.19±0.51	4.99±0.26
	6.1	7.95±1.11	8.30±0.44	8.55±0.31	4.87±0.51
	5.8	10.76±2.12	4.87±0.46	11.44±0.81	4.51±0.56
	5.6	11.19±0.37	4.34±0.54	10.73±0.58	4.52±0.27
<b>HA* + liposome/GD1a</b>					
	7	5.20±0.56	10.30±0.95	9.58±0.96	4.87±0.75
	6.4	7.97±0.84	8.36±0.85	8.34±1.02	4.71±0.68
	6.1	9.24±1.03	7.87±1.23	18.47±1.56	4.34±0.48
	5.8	11.24±1.02	7.34±1.03	19.60±1.65	4.23±0.51
	5.6	13.03±0.95	4.45±0.54	20.50±2.05	4.51±0.53

**Table S3 | Rates of transition between the observed FRET states, Related to Figure 7.**

Rate constants were determined by fitting of dwell time histograms to an exponential function (**STAR Methods**). Given the time resolution of imaging of 25 frames/s, according to the Shannon-Nyquist sampling theorem rates greater than 12.5 s<sup>-1</sup> may not have been determined with high accuracy. Errors represent 95% confidence intervals determined during fitting. Rates are defined as in **Figure 7**.