Supporting Information for manuscript:

Measuring Surface Binding Thermodynamics and Kinetics by using Total Internal Reflection with Fluorescence Correlation Spectroscopy: Practical Considerations

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SUPPLEMENTARY MATERIAL

This material contains four Tables. Equilibrium constants K are in units of M^{-1}, concentrations A are in units of nM, and surface site densities are in units of molecules/μm^2. $G_s(0)$ values, $G_a(0)$ values, X = KA values, and rebinding probabilities P are unit-less. In all cases, it has been assumed that d = 0.1 μm, h = 0.5 μm, D = 50 $\mu m^2 s^{-1}$ and $k_a = 10^6$ $M^{-1}s^{-1}$. Data are given for the four cases in which the minimum tolerable value of $G_s(0)$, denoted by $[G_s(0)]_{min}$, is 0.05, 0.01, 0.005 or 0.001.

S_{max} values denote the surface site densities for which $G_s(0)$ is maximized; depend on d, X and K; and were calculated from Eq. 13. The S_{max} values give an expression for the maximum value of $G_s(0)$ which also depends on d, X and K (Eq. 14). By setting this expression equal to $[G_s(0)]_{min}$, one finds a maximum value of X, denoted by X_{max}, which equals

$$X_{\max} = \frac{1}{2}[-1 + \sqrt{1 + \frac{K}{\pi h^2 d[G_s(0)]_{\min}}}]$$

In the tables, for a given value of K and $[G_s(0)]_{min}$, the minimum value of X considered was 0.0001 and the maximum value of X was calculated from the above equation. Surface site densities S_1 and S_2 denote the minimum and maximum values for which $G_s(0) \geq [G_s(0)]_{min}$, depend on K, X, d and h, and were calculated from the following generalization of Eq. 16:

$$S_{1,2} = \frac{K - 2\pi h^2 dX(1+X)[G_s(0)]_{\min} \mp \sqrt{K^2 - 4\pi h^2 dKX(1+X)[G_s(0)]_{\min}}}{2\pi h^2 KX[G_s(0)]_{\min}}$$

Surface site densities S_3 denote values below which $G_s(0)/G_a(0)$ exceeds 0.1, depend on d, X and K, and were calculated from Eq. 18. Surface site densities S_4 denote values above which $P \geq 0.05$, depend on S and X, and were found by numerically integrating Eq. 19 with Eq. 20. The range of allowable surface site densities was calculated as the maximum of S_1 and S_3 to the minimum of S_2 and S_4. This range reflects the constraints that $G_s(0) \geq [G_s(0)]_{min}$, $[G_s(0)/G_a(0)] \geq 0.1$, and $P \leq 0.05$, and is not meant as an absolute requirement for successful TIR-FCS measurements. Using surface site densities outside of this range will have the consequence of violating one of these three constraints and the violation of these constraints may require analysis with a more sophisticated model than that shown in Eqs. 6 and 9-11.

All four tables show calculated quantities for the four considered values of $[G_s(0)]_{min}$, six considered values of K, and X = 0.0001 or $X = X_{max}$. Table S1 shows S_1, S_2, S_3, S_4, S_{max} and the S range. Tables S2, S3 and S4 show predicted values of $G_s(0)$, $G_s(0)/G_a(0)$, and P, respectively, for the five surface site densities and the S range.

Table S1: Surface site densities for lower values of $G_s(0)$

$[G_s(0)]_{min}$	K	X	A	S_1	S_2	S_3	S_4	S_{max}	S range
0.05	10^7	0.0001	0.01	1.4×10^{-4}	2.5×10^5	0.30	3300	6.0	0.30-3300
		0.643	64.3	9.9	9.9	0.81	5400	9.9	9.9
	3×10^6	0.0001	0.0333	0.0016	2.5×10^5	1.0	3300	20	1.0-3300
		0.253	84.3	25	25	1.6	4100	25	25
	10^6	0.0001	0.1	0.014	2.5×10^5	3.0	3300	60	3.0-3300
		0.0964	96.4	66	66	3.6	3600	66	66
	3×10^5	0.0001	0.333	0.16	2.5×10^5	10	3300	200	10-3300
		0.0308	103	210	210	11	3400	210	210
	10^5	0.0001	1	1.4	2.5×10^5	30	3300	600	30-3300
		0.0105	105	610	610	31	3300	610	610
	3×10^4	0.0001	3.33	16	2.5×10^5	100	3300	2000	100-3300
		0.00316	105	2000	2000	100	3300	2000	2000
0.01	10^7	0.0001	0.01	2.9×10^{-5}	1.3×10^6	0.30	3300	6.0	0.30-3300
		1.85	185	17	17	2.4	9400	17	17
	3×10^6	0.0001	0.0333	3.2×10^{-4}	1.3×10^6	1.0	3300	20	1.0-3300
		0.855	285	37	37	3.5	6100	37	37
	10^6	0.0001	0.1	0.0029	1.3×10^6	3.0	3300	60	3.0-3300
		0.382	382	83	83	5.8	4500	83	83
	3×10^5	0.0001	0.333	0.032	1.3×10^6	10	3300	200	10-3300
		0.139	463	230	230	13	3700	230	230
	10^5	0.0001	1	0.29	1.3×10^6	30	3300	600	30-3300
		0.0503	503	630	630	33	3400	630	630
	3×10^4	0.0001	3.33	3.2	1.3×10^6	100	3300	2000	100-3300
		0.0156	520	2000	2000	100	3300	2000	2000
0.005	10^7	0.0001	0.01	1.4×10^{-5}	2.5×10^6	0.30	3300	6.0	0.30-3300
		2.79	279	23	23	4.3	12500	23	23
	3×10^6	0.0001	0.0333	1.6×10^{-4}	2.5×10^6	1.0	3300	20	1.0-3300
		1.35	450	47	47	5.5	7700	47	47
	10^6	0.0001	0.1	0.0014	2.5×10^6	3.0	3300	60	3.0-3300
		0.643	643	99	99	8.1	5400	99	99
	3×10^5	0.0001	0.333	0.016	2.5×10^6	10	3300	200	10-3300
		0.253	843	250	250	16	4100	250	250
	10^5	0.0001	1	0.14	2.5×10^6	30	3300	600	30-3300
		0.0964	964	660	660	36	3600	660	660
	3×10^4	0.0001	3.33	1.6	2.5×10^6	100	3300	2000	100-3300
		0.0308	1030	2100	2100	110	3400	2100	2100
0.001	10^7	0.0001	0.01	2.9×10^{-6}	1.3×10^7	0.30	3300	6.0	0.30-3300
		6.79	679	47	47	18	25000	47	47
	3×10^6	0.0001	0.0333	3.2×10^{-5}	1.3×10^7	1.0	3300	20	1.0-3300
		3.51	1170	91	91	20	15000	91	91
	10^6	0.0001	0.1	2.9×10^{-4}	1.3×10^7	3.0	3300	60	3.0-3300
		1.85	1850	170	170	24	9400	170	170
	3×10^5	0.0001	0.333	0.0032	1.3×10^7	10	3300	200	10-3300
		0.854	2840	370	370	35	6100	370	370
	10^5	0.0001	1	0.029	1.3×10^7	30	3300	600	30-3300
		0.382	3820	830	830	58	4500	830	830
	3×10^4	0.0001	3.33	0.32	1.3×10^7	100	3300	2000	100-3300
		0.139	4630	2300	2300	130	3700	2300	2300

Table S2: $G_s(0)$ values for different surface site densities

$[G_s(0)]_{min}$	K	X	$G_s(0)$					
			S_1	S_2	S_3	S_4	S_{max}	S range
0.05	10^7	0.0001	0.05	0.05	95	3.8	530	3.8-95
		0.643	0.05	0.05	0.014	3.7×10^{-4}	0.05	0.05
	3×10^6	0.0001	0.05	0.05	29	3.8	160	3.8-29
		0.253	0.05	0.05	0.011	0.0012	0.05	0.05
	10^6	0.0001	0.05	0.05	9.5	3.7	53	3.7-9.5
		0.0964	0.05	0.05	0.0099	0.0035	0.05	0.05
	3×10^5	0.0001	0.05	0.05	2.9	3.4	16	2.9-3.4
		0.0308	0.05	0.05	0.0093	0.011	0.05	0.05
	10^5	0.0001	0.05	0.05	0.95	2.8	5.3	0.95-2.8
		0.0105	0.05	0.05	0.0092	0.026	0.05	0.05
	3×10^4	0.0001	0.05	0.05	0.29	1.5	1.6	0.29-1.5
		0.00316	0.05	0.05	0.0091	0.047	0.05	0.05
0.01	10^7	0.0001	0.01	0.01	95	3.8	530	3.8-95
		1.85	0.01	0.01	0.0044	7.3×10^{-5}	0.01	0.01
	3×10^6	0.0001	0.01	0.01	29	3.8	160	3.8-29
		0.855	0.01	0.01	0.0031	2.4×10^{-4}	0.01	0.01
	10^6	0.0001	0.01	0.01	9.5	3.7	53	3.7-9.5
		0.382	0.01	0.01	0.0024	7.1×10^{-4}	0.01	0.01
	3×10^5	0.0001	0.01	0.01	2.9	3.4	16	2.9-3.4
		0.139	0.01	0.01	0.0020	0.0022	0.01	0.01
	10^5	0.0001	0.01	0.01	0.95	2.8	5.3	0.95-2.8
		0.0503	0.01	0.01	0.0019	0.0053	0.01	0.01
	3×10^4	0.0001	0.01	0.01	0.29	1.5	1.6	0.29-1.5
		0.0156	0.01	0.01	0.0018	0.0095	0.01	0.01
0.005	10^7	0.0001	0.005	0.005	95	3.8	530	3.8-95
		2.79	0.005	0.005	0.0027	3.7×10^{-5}	0.005	0.005
	3×10^6	0.0001	0.005	0.005	29	3.8	160	3.8-29
		1.35	0.005	0.005	0.0019	1.2×10^{-4}	0.005	0.005
	10^6	0.0001	0.005	0.005	9.5	3.7	53	3.7-9.5
		0.643	0.005	0.005	0.0014	3.5×10^{-4}	0.005	0.005
	3×10^5	0.0001	0.005	0.005	2.9	3.4	16	2.9-3.4
		0.253	0.005	0.005	0.0011	0.0011	0.005	0.005
	10^5	0.0001	0.005	0.005	0.95	2.8	5.3	0.95-2.8
		0.0964	0.005	0.005	9.9×10^{-4}	0.0026	0.005	0.005
	3×10^4	0.0001	0.005	0.005	0.29	1.5	1.6	0.29-1.5
		0.0308	0.005	0.005	9.3×10^{-4}	0.0047	0.005	0.005
0.001	10^7	0.0001	0.001	0.001	95	3.8	530	3.8-95
		6.79	0.001	0.001	8.1×10^{-4}	7.5×10^{-6}	0.001	0.001
	3×10^6	0.0001	0.001	0.001	29	3.8	160	3.8-29
		3.51	0.001	0.001	6.0×10^{-4}	2.4×10^{-5}	0.001	0.001
	10^6	0.0001	0.001	0.001	9.5	3.7	53	3.7-9.5
		1.85	0.001	0.001	4.4×10^{-4}	7.1×10^{-5}	0.001	0.001
	3×10^5	0.0001	0.001	0.001	2.9	3.4	16	2.9-3.4
		0.854	0.001	0.001	3.1×10^{-4}	3.2×10^{-4}	0.001	0.001
	10^5	0.0001	0.001	0.001	0.95	2.8	5.3	0.95-2.8
		0.382	0.001	0.001	2.4×10^{-4}	5.3×10^{-4}	0.001	0.001
	3×10^4	0.0001	0.001	0.001	0.29	1.5	1.6	0.29-1.5
		0.139	0.001	0.001	2.0×10^{-4}	9.5×10^{-4}	0.001	0.001

Table S3: $G_s(0)/G_a(0)$ values for different surface site densities

$[G_s(0)]_{min}$	K	X	$G_s(0)/G_a(0)$					
			S_1	S_2	S_3	S_4	S_{max}	S range
0.05	10^7	0.0001	4.7×10^{-5}	8.5×10^4	0.1	1100	2.0	0.1-1100
		0.643	1.2	1.2	0.1	660	1.2	1.2
	3×10^6	0.0001	1.6×10^{-4}	2.5×10^4	0.1	330	2.0	0.1-330
		0.253	1.6	1.6	0.1	260	1.6	1.6
	10^6	0.0001	4.7×10^{-4}	8500	0.1	110	2.0	0.1-110
		0.0964	1.8	1.8	0.1	99	1.8	1.8
	3×10^5	0.0001	0.0016	2500	0.1	33	2.0	0.1-33
		0.0308	1.9	1.9	0.1	32	1.9	1.9
	10^5	0.0001	0.0048	840	0.1	11	2.0	0.1-11
		0.0105	2.0	2.0	0.1	11	2.0	2.0
	3×10^4	0.0001	0.016	250	0.1	3.3	2.0	0.1-3.3
		0.00316	2.0	2.0	0.1	3.3	2.0	2.0
0.01	10^7	0.0001	9.5×10^{-6}	4.2×10^5	0.1	1100	2.0	0.1-1100
		1.85	0.70	0.70	0.1	380	0.70	0.70
	3×10^6	0.0001	3.2×10^{-5}	1.3×10^5	0.1	330	2.0	0.1-330
		0.855	1.1	1.1	0.1	180	1.1	1.1
	10^6	0.0001	9.5×10^{-5}	4.2×10^4	0.1	110	2.0	0.1-110
		0.382	1.5	1.5	0.1	78	1.5	1.5
	3×10^5	0.0001	3.2×10^{-4}	1.3×10^4	0.1	33	2.0	0.1-33
		0.139	1.8	1.8	0.1	28	1.8	1.8
	10^5	0.0001	9.5×10^{-4}	4200	0.1	11	2.0	0.1-11
		0.0503	1.9	1.9	0.1	10	1.9	1.9
	3×10^4	0.0001	0.0032	1300	0.1	3.3	2.0	0.1-3.3
		0.0156	2.0	2.0	0.1	3.2	2.0	2.0
0.005	10^7	0.0001	4.7×10^{-6}	8.5×10^5	0.1	1100	2.0	0.1-1100
		2.79	0.53	0.53	0.1	290	0.53	0.53
	3×10^6	0.0001	1.6×10^{-5}	2.5×10^5	0.1	330	2.0	0.1-330
		1.35	0.86	0.86	0.1	140	0.86	0.86
	10^6	0.0001	4.7×10^{-5}	8.5×10^4	0.1	110	2.0	0.1-110
		0.643	1.2	1.2	0.1	66	1.2	1.2
	3×10^5	0.0001	1.6×10^{-4}	2.5×10^4	0.1	33	2.0	0.1-33
		0.253	1.6	1.6	0.1	26	1.6	1.6
	10^5	0.0001	4.7×10^{-4}	8500	0.1	11	2.0	0.1-11
		0.0964	1.8	1.8	0.1	9.9	1.8	1.8
	3×10^4	0.0001	0.0016	2500	0.1	3.3	2.0	0.1-3.3
		0.0308	1.9	1.9	0.1	3.2	1.9	1.9
0.001	10^7	0.0001	9.5×10^{-7}	4.2×10^6	0.1	1100	2.0	0.1-1100
		6.79	0.26	0.26	0.1	140	0.26	0.26
	3×10^6	0.0001	3.2×10^{-6}	1.3×10^6	0.1	330	2.0	0.1-330
		3.51	0.44	0.44	0.1	73	0.44	0.44
	10^6	0.0001	9.5×10^{-6}	4.2×10^5	0.1	110	2.0	0.1-110
		1.85	0.70	0.70	0.1	38	0.70	0.70
	3×10^5	0.0001	3.2×10^{-5}	1.3×10^5	0.1	33	2.0	0.1-33
		0.854	1.1	1.1	0.1	18	1.1	1.1
	10^5	0.0001	9.5×10^{-5}	4.2×10^4	0.1	11	2.0	0.1-11
		0.382	1.5	1.5	0.1	7.8	1.5	1.5
	3×10^4	0.0001	3.2×10^{-4}	1.3×10^4	0.1	3.3	2.0	0.1-3.3
		0.139	1.8	1.8	0.1	2.8	1.8	1.8

Table S4: Rebinding probabilities P for different surface site densities

$[G_s(0)]_{min}$	K	X	P					
			S_1	S_2	S_3	S_4	S_{max}	S range
0.05	10^7	0.0001	2.4×10^{-9}	0.79	5.0×10^{-6}	0.050	1.0×10^{-4}	5.0×10^{-6}-0.050
		0.643	1.0×10^{-4}	1.0×10^{-4}	8.2×10^{-6}	0.050	1.0×10^{-4}	1.0×10^{-4}
	3×10^6	0.0001	2.6×10^{-8}	0.79	1.7×10^{-5}	0.050	3.3×10^{-4}	1.7×10^{-5}-0.050
		0.253	3.3×10^{-4}	3.3×10^{-4}	2.1×10^{-5}	0.050	3.3×10^{-4}	3.3×10^{-4}
	10^6	0.0001	2.4×10^{-7}	0.79	5.0×10^{-5}	0.050	0.0010	5.0×10^{-5}-0.050
		0.0964	0.0010	0.0010	5.5×10^{-5}	0.050	0.0010	0.0010
	3×10^5	0.0001	2.6×10^{-6}	0.79	1.7×10^{-4}	0.050	0.0033	1.7×10^{-4}-0.050
		0.0308	0.0033	0.0033	1.7×10^{-4}	0.050	0.0033	0.0033
	10^5	0.0001	2.4×10^{-5}	0.79	5.0×10^{-4}	0.050	0.0098	5.0×10^{-4}-0.050
		0.0105	0.0098	0.0098	5.1×10^{-4}	0.050	0.0098	0.0098
	3×10^4	0.0001	2.7×10^{-4}	0.79	0.0017	0.050	0.031	0.0017-0.050
		0.00316	0.031	0.031	0.0017	0.050	0.031	0.031
0.01	10^7	0.0001	4.8×10^{-10}	0.95	5.0×10^{-6}	0.050	1.0×10^{-4}	5.0×10^{-6}-0.050
		1.85	1.0×10^{-4}	1.0×10^{-4}	1.4×10^{-5}	0.050	1.0×10^{-4}	1.0×10^{-4}
	3×10^6	0.0001	5.3×10^{-9}	0.95	1.7×10^{-5}	0.050	3.3×10^{-4}	1.7×10^{-5}-0.050
		0.855	3.3×10^{-4}	3.3×10^{-4}	3.1×10^{-5}	0.050	3.3×10^{-4}	3.3×10^{-4}
	10^6	0.0001	4.8×10^{-8}	0.95	5.0×10^{-5}	0.050	0.0010	5.0×10^{-5}-0.050
		0.382	0.0010	0.0010	6.9×10^{-5}	0.050	0.0010	0.0010
	3×10^5	0.0001	5.3×10^{-7}	0.95	1.7×10^{-4}	0.050	0.0033	1.7×10^{-4}-0.050
		0.139	0.0033	0.0033	1.9×10^{-4}	0.050	0.0033	0.0033
	10^5	0.0001	4.8×10^{-6}	0.95	5.0×10^{-4}	0.050	0.0098	5.0×10^{-4}-0.050
		0.0503	0.0098	0.0098	5.3×10^{-4}	0.050	0.0098	0.0098
	3×10^4	0.0001	5.3×10^{-5}	0.95	0.0017	0.050	0.031	0.0017-0.050
		0.0156	0.031	0.031	0.0017	0.050	0.031	0.031
0.005	10^7	0.0001	2.4×10^{-10}	0.98	5.0×10^{-6}	0.050	1.0×10^{-4}	5.0×10^{-6}-0.050
		2.79	1.0×10^{-4}	1.0×10^{-4}	1.9×10^{-5}	0.050	1.0×10^{-4}	1.0×10^{-4}
	3×10^6	0.0001	2.6×10^{-9}	0.98	1.7×10^{-5}	0.050	3.3×10^{-4}	1.7×10^{-5}-0.050
		1.35	3.3×10^{-4}	3.3×10^{-4}	3.9×10^{-5}	0.050	3.3×10^{-4}	3.3×10^{-4}
	10^6	0.0001	2.4×10^{-8}	0.98	5.0×10^{-5}	0.050	0.0010	5.0×10^{-5}-0.050
		0.643	0.0010	0.0010	8.2×10^{-5}	0.050	0.0010	0.0010
	3×10^5	0.0001	2.6×10^{-7}	0.98	1.7×10^{-4}	0.050	0.0033	1.7×10^{-4}-0.050
		0.253	0.0033	0.0033	2.1×10^{-4}	0.050	0.0033	0.0033
	10^5	0.0001	2.4×10^{-6}	0.98	5.0×10^{-4}	0.050	0.0098	5.0×10^{-4}-0.050
		0.0964	0.0098	0.0098	5.5×10^{-4}	0.050	0.0098	0.0098
	3×10^4	0.0001	2.6×10^{-5}	0.98	0.0017	0.050	0.031	0.0017-0.050
		0.0308	0.031	0.031	0.0017	0.050	0.031	0.031
0.001	10^7	0.0001	4.8×10^{-11}	1.0	5.0×10^{-6}	0.050	1.0×10^{-4}	5.0×10^{-6}-0.050
		6.79	1.0×10^{-4}	1.0×10^{-4}	3.9×10^{-5}	0.050	1.0×10^{-4}	1.0×10^{-4}
	3×10^6	0.0001	5.3×10^{-10}	1.0	1.7×10^{-5}	0.050	3.3×10^{-4}	1.7×10^{-5}-0.050
		3.51	3.3×10^{-4}	3.3×10^{-4}	7.5×10^{-5}	0.050	3.3×10^{-4}	3.3×10^{-4}
	10^6	0.0001	4.8×10^{-9}	1.0	5.0×10^{-5}	0.050	0.0010	5.0×10^{-5}-0.050
		1.85	0.0010	0.0010	1.4×10^{-4}	0.050	0.0010	0.0010
	3×10^5	0.0001	5.3×10^{-8}	1.0	1.7×10^{-4}	0.050	0.0033	1.7×10^{-4}-0.050
		0.854	0.0033	0.0033	3.1×10^{-4}	0.050	0.0033	0.0033
	10^5	0.0001	4.8×10^{-7}	1.0	5.0×10^{-4}	0.050	0.0098	5.0×10^{-4}-0.050
		0.382	0.0098	0.0098	6.9×10^{-4}	0.050	0.0098	0.0098
	3×10^4	0.0001	5.3×10^{-6}	1.0	0.0017	0.050	0.031	0.0017-0.050
		0.139	0.031	0.031	0.0019	0.050	0.031	0.031