Electronic Supplementary Material (ESI) for Integrative Biology

Probing Dynamic Cell-Substrate Interactions using Photochemically Generated Surface-Immobilized Gradients: Application to Selectin-Mediated Leukocyte Rolling

Christine T. Herman,[a] Gregory K. Potts,[a] Madeline C. Michael, [a] Nicole V. Tolan, [a] and Ryan C. Bailey*[a]

[a] *Department of Chemistry, University of Illinois at Urbana-Champaign, 600 South Mathews Avenue, Urbana, IL 61801,USA. Tel: 217-333-0676; E-mail:baileyrc@illinois.edu*

Electronic Supplementary Information (ESI)

Table of Contents:

Table S1 Summary of contact angles following silanization with TESA and functionalization with benzophenone (BP). The contact angles reported for each batch are the average (± standard deviation) of at least four substrates.

	H_2O Contact Angle, Batch 1	H_2O Contact Angle, Batch 2	H_2O Contact Angle, Batch 3	Average of 3 batches
TESA silanization	48.0 (±1.4)	42.7 (±1.1)	45.2 (±2.6)	45.2 (±2.6)
+BP	57.5 (±7.7)	59.6 (±0.9)	57.3 (±3.9)	58.1 (±3.9)

Table S2 Summary of gradient generation conditions used to generate one-component gradients of P-selectin and PSGL-1.

Conditions for generation of one-component gradients used in Jurkat cell flow assays						
Protein	**concentration (µg/mL)**	**power (mW/cm^2)**	**total exposure time (sec)**	**gradient size (mm)**	**size of gradient used in assay (mm)**	**# positions analyzed in assay**
P-selectin	5	14	24	4.8	2.4	8
PSGL-1	10	14	24	4.8	3	7

Conditions for generation of one-component gradients used in HL-60 cell flow assays						
Protein	**concentration (µg/mL)**	**power (mW/cm^2)**	**total exposure time (sec)**	**gradient size (mm)**	**size of gradient used in assay (mm)**	**# positions analyzed in assay**
P-selectin	10	14	30	4.8	4.2	10
PSGL-1	10	14	24	4.8	4.2	7

Table S3 Summary of data and associated approximations from one-component gradients of P-selectin and PSGL-1. Protein site density was determined by converting data from fluorescence assays to site density using data from fluorescence-radioactivity correlation studies (see **Fig. 2** in text).

Substrates for Jurkat cell flow assays		
P-selectin site density	standard deviation	approximation
(molecules/μm^2)		
2392.03	364.52	2400
2180.13	502.35	2200
1782.28	525.53	1800
1384.13	396.81	1400
1026.76	311.67	1000
669.1	92.73	650
425.96	28.84	450
182.81	12.38	180

Substrates for HL-60 cell flow assays		
P-selectin site density	standard deviation	approximation
(molecules/μm^2)		
958.43	87.69	1000
770.42	152.76	800
657.79	130.43	650
545.16	102.76	550
448.74	84.59	450
352.32	61.69	350
286.76	50.21	280
221.19	0.73	220
182.78	22.78	180
144.37	17.99	140

PSGL-1 site density	standard deviation	approximation
(molecules/μm^2)		
5899.34	1313.66	6000
4646.76	777.97	5000
3927.47	712.62	4000
3424.61	721.77	3500
2581.96	544.16	2500
1739.31	365.04	1800
836.41	175.5436	800

PSGL-1 site density	standard deviation	approximation
(molecules/μm^2)		
4339.32	1032.91	4500
3860.19	971.11	4000
3470.98	767.19	3500
2697.33	738.54	2500
1718.97	516.5	1500
1125.06	397.79	1000
123.08	84.66	100

Fig. S1 Linescans for one-component gradient substrates prepared for flow assays with HL-60 cells. BP-modified substrates present immobilized (a) P-selectin and (b) PSGL-1 on BP-modified substrates. Gradients were characterized in a fluorescence assay and fluorescence data was converted to site density using the results from the fluorescence-radioactivity correlation studies. Three representative gradient lines scans are shown for each immobilized protein gradient.

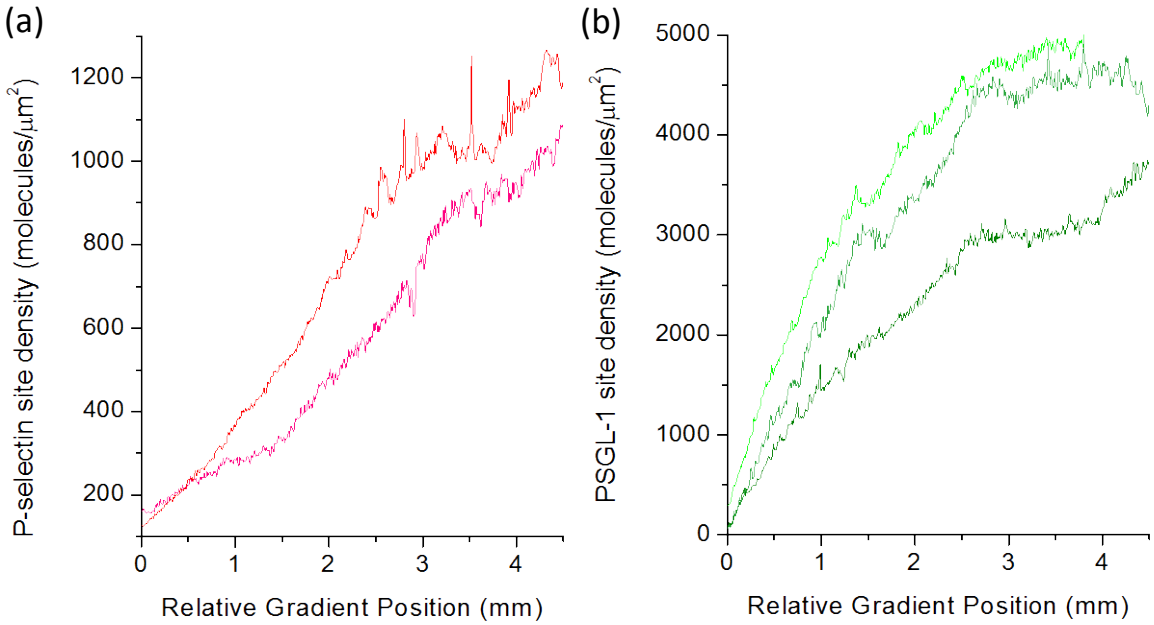

Table S4 The critical velocity (v_{crit}) was determined by analyzing non-interacting cells along the wall of the flow chamber and determining their average velocity at each shear stress. The values and associated error are reported above for *n*=8+ cells. At flow rates greater 250 µL/min, non-interacting cells were traveling to be analyzed as they appeared as streaks in the images.

Flow rate (µL/min)	Shear stress (dyn/cm^2)	*n*	Critical velocity (µm/sec)	Standard deviation	95% C.I.
10	0.062	9	21.7	3.5	2.3
25	0.155	7	43.4	5.7	4.2
50	0.31	8	97.6	21.9	15.2
75	0.465	23	139	32.7	13.4
100	0.62	10	340.9	99	61.3
150	0.93	20	417.3	102.3	44.9
250	1.55	n/a	Not determined	n/a	n/a