

DATA ASSOCIATED TO THE PUBLICATION: Methylene blue not ferrocene: optimal reporters for electrochemical detection of protease activity

Table 1. Data associated to Figure 2A. Percentage signal decrease registered upon trypsin addition (100 nM) for T-Peptide-3 and T-Peptide-4.

Time (min)	T-Peptide-3 (circles)		T-Peptide-4 (triangles)	
	% Signal Decrease (Average)	STDEV	% Signal Decrease (Average)	STDEV
0	0.0	0.0	0.0	0.0
2	-1.1	1.9	-4.0	1.0
4	-0.7	2.3	-1.2	1.5
6	-2.9	3.5	-5.4	2.1
8	-2.5	3.1	-3.4	1.3
10	-2.1	2.3	-4.9	2.5
12	-2.7	3.1	-2.1	2.0
13	-3.9	3.1	-4.2	4.9
15	-5.1	3.8	-6.5	2.0
17	-4.5	4.0	-8.7	2.0
21	-6.8	3.6	-10.1	2.9
23	-7.8	3.5	-10.1	8.7
24	-10.9	2.5	-8.2	7.8
26	-13.3	2.4	-8.1	8.1
28	-17.3	2.4	-8.4	8.5
38	-30.8	1.2	-12.3	5.7
47	-39.4	3.1	-14.8	8.8
57	-45.8	4.7	-17.7	4.7
66	-50.4	5.4	-16.2	4.0
75	-53.5	6.0	-16.7	4.0
85	-57.0	5.9	-21.2	6.2
94	-60.1	5.4	-25.7	5.8

Table 2. Data associated to Figure 2B. Percentage signal decrease registered upon trypsin addition (100 nM) for T-Peptide-5 and T-Peptide-6.

Time (min)	T-Peptide-5 (circles)		T-Peptide-6 (triangles)	
	% Signal Decrease (Average)	STDEV	% Signal Decrease (Average)	STDEV
0	0.0	0.0	0.0	0.0
2	-0.8	2.7	1.3	1.3
4	0.6	3.0	-1.4	3.8
6	1.4	2.9	-0.1	0.7
7	-1.5	1.4	1.3	1.5
9	2.0	2.8	-1.3	3.0
11	1.1	2.1	2.0	1.9
13	1.8	2.1	1.1	2.8
15	2.6	2.1	0.6	0.7
17	2.8	2.5	2.9	0.7
19	-2.6	0.7	-1.4	4.3
20	-10.1	4.8	-3.2	2.2
22	-19.8	2.0	-2.9	1.3
24	-26.6	3.0	-0.7	3.7
26	-30.5	4.7	-1.9	1.2
28	-34.0	6.5	-1.7	3.3
30	-37.5	4.8	0.1	2.2
32	-38.8	7.0	-0.6	2.1
33	-41.2	5.5	0.5	3.3
35	-42.5	7.5	-1.4	1.0
44	-45.9	7.9	0.7	3.7
54	-48.7	7.5	0.4	4.7
63	-50.2	6.5	-1.7	2.9
72	-51.2	6.8	0.7	4.7
81	-52.0	7.3	-0.2	6.7
91	-52.8	5.7	1.1	4.9

Table 3. Data associated to Figure 3A. Percentage signal decrease registered for different T-SAMs prepared for Peptide-5 upon addition of trypsin 100 nM.

[HDT](μM)	% Signal Decrease (Average)	STDEV
75	-29.4	8.7
150	-46.7	6.3
300	-48.5	10.2
600	-52.5	4.8
900	-46.1	8.2

Table 4. Data associated to Figure 3B. Percentage signal decrease registered for different T-SAMs prepared for [Peptide-5] = 40 μM and 600 μM of a dithiol (HDT or PDT).

[HDT]=600 μM							
[Trypsin]=100 nM (black columns)				[BSA]=100 nM (patterned columns)			
T-Peptide-5		T-Peptide-6		T-Peptide-5		T-Peptide-6	
% Signal Decrease (Average)	STDEV	% Signal Decrease (Average)	STDEV	% Signal Decrease (Average)	STDEV	% Signal Decrease (Average)	STDEV
-50.2	9.9	-6.5	4.9	-41.3	10.3	-44.8	9.5

[PDT]=600 μM							
[Trypsin]=100 nM (black columns)				[BSA]=100 nM (patterned columns)			
T-Peptide-5		T-Peptide-6		T-Peptide-5		T-Peptide-6	
% Signal Decrease (Average)	STDEV	% Signal Decrease (Average)	STDEV	% Signal Decrease (Average)	STDEV	% Signal Decrease (Average)	STDEV
-36.6	4.8	-4.7	1.6	-14.3	7.0	-11.9	10.8

Table 5. Data associated to Figure 4A. Percentage decrease registered for T-PDT-Peptide-5 upon incubation with different trypsin concentrations with time.

[trypsin]=0 nM			[trypsin]=0.1 nM			[trypsin]=0.5 nM		
Time (min)	% Signal Decrease (Average)	STDEV	Time (min)	% Signal Decrease (Average)	STDEV	Time (min)	% Signal Decrease (Average)	STDEV
0	0.0	0.0	0	0.0	0.0	0	0.0	0.0
1	-0.5	0.3	2	-0.7	0.3	2	-0.5	0.1
3	-0.3	0.1	4	-0.9	0.4	4	-0.7	0.4
4	-0.5	0.1	6	-0.9	0.5	6	-1.0	0.3
8	-0.7	0.2	9	-1.2	0.6	9	-1.4	0.5
17	-1.3	0.3	18	-2.5	0.5	18	-2.5	0.5
26	-1.8	0.3	27	-3.3	0.9	27	-3.5	0.6
35	-2.5	0.4	36	-4.1	0.8	36	-4.6	0.7
52	-3.3	0.4	54	-5.7	1.2	54	-6.6	0.6
70	-4.1	0.5	72	-7.4	1.1	72	-8.4	0.5

[trypsin]=1 nM			[trypsin]=5 nM			[trypsin]=10 nM		
Time (min)	% Signal Decrease (Average)	STDEV	Time (min)	% Signal Decrease (Average)	STDEV	Time (min)	% Signal Decrease (Average)	STDEV
0	0.0	0.0	0	0.0	0.0	0	0.0	0.0
2	-0.4	0.2	3	-0.7	0.7	2	-0.4	0.6
4	-0.7	0.2	5	-1.8	0.6	3	-1.3	0.9
5	-0.8	0.3	6	-2.8	0.7	5	-2.5	0.6
9	-1.4	0.2	10	-4.7	1.1	9	-5.5	0.5
18	-2.7	0.2	19	-9.3	1.7	18	-12.5	0.7
27	-4.1	0.1	28	-13.1	1.8	26	-17.6	1.1
36	-5.7	0.1	37	-16.2	1.9	35	-21.2	1.5
53	-9.0	0.4	54	-20.6	1.1	53	-25.4	2.3
71	-12.0	0.5	72	-23.7	0.5	71	-28.0	2.5

[trypsin]=25 nM			[trypsin]=50 nM			[trypsin]=100 nM		
Time (min)	% Signal Decrease (Average)	STDEV	Time (min)	% Signal Decrease (Average)	STDEV	Time (min)	% Signal Decrease (Average)	STDEV
0	0.0	0.0	0	0.0	0.0	0	0.0	0.0
2	-0.5	0.5	2	-0.8	0.3	2	-1.1	0.1
3	-2.7	1.1	4	-4.2	1.9	4	-6.8	1.9
5	-5.6	1.5	5	-8.2	2.9	5	-11.9	3.7
9	-11.4	2.5	9	-15.1	3.5	9	-18.7	5.5
18	-21.5	5.0	18	-24.0	3.5	18	-25.1	6.5
27	-26.0	6.3	27	-27.9	3.6	27	-28.1	6.6
35	-28.5	6.9	36	-30.1	3.6	36	-30.3	6.6
53	-31.8	7.5	53	-33.2	3.1	53	-32.9	6.0
71	-33.5	7.9	71	-35.0	2.7	71	-35.0	5.3

T-PDT-Peptide-6 with [trypsin]=100 nM		
Time (min)	% Signal Decrease (Average)	STDEV
0	0.0	0.0
2	-0.5	0.3
4	-0.7	0.5
5	-0.9	0.5
9	-1.1	0.6
18	-1.9	0.8
27	-2.4	0.9
36	-2.8	1.2
53	-3.4	1.6
71	-4.1	1.8

Table 6. Data associated to Figure 4A, Inset. Percentage signal after 70 min of incubation vs the logarithm of the concentration of trypsin.

log([Trypsin]/ nM)	% Signal	STDEV
-1.0	78.9	3.2
-0.3	76.1	1.3
0.0	65.9	1.4
0.7	32.2	7.1
1.0	20.1	1.5
1.4	4.4	22.7
1.7	0.0	7.8
2.0	0.0	15.0

Table 7. Data associated to Figure 4B. Current (μA) vs potential registered by SWV for T-PDT-Peptide-5 upon incubation with trypsin 25 nM for different incubation times.

E / V	I (μA)				
	Time: 0 min	Time: 5 min	Time: 15 min	Time: 35 min	Time: 60 min
-0.050	-0.66	-0.65	-0.63	-0.58	-0.55
-0.055	-0.66	-0.65	-0.63	-0.58	-0.55
-0.060	-0.67	-0.66	-0.63	-0.60	-0.57
-0.065	-0.68	-0.67	-0.64	-0.60	-0.58
-0.070	-0.70	-0.67	-0.65	-0.61	-0.58
-0.074	-0.71	-0.69	-0.67	-0.62	-0.60
-0.079	-0.73	-0.71	-0.68	-0.64	-0.61
-0.084	-0.74	-0.72	-0.69	-0.65	-0.61
-0.089	-0.75	-0.74	-0.70	-0.66	-0.63
-0.094	-0.77	-0.76	-0.72	-0.67	-0.64
-0.099	-0.81	-0.78	-0.74	-0.69	-0.66
-0.104	-0.84	-0.82	-0.77	-0.71	-0.67
-0.109	-0.90	-0.86	-0.81	-0.74	-0.69
-0.114	-0.96	-0.94	-0.86	-0.76	-0.71
-0.118	-1.05	-1.02	-0.92	-0.81	-0.76
-0.123	-1.18	-1.14	-1.02	-0.87	-0.81
-0.128	-1.35	-1.28	-1.13	-0.95	-0.88
-0.133	-1.55	-1.48	-1.27	-1.05	-0.95
-0.138	-1.79	-1.70	-1.45	-1.18	-1.06
-0.143	-2.07	-1.97	-1.64	-1.31	-1.18
-0.148	-2.39	-2.25	-1.86	-1.48	-1.33
-0.153	-2.71	-2.55	-2.10	-1.65	-1.48
-0.157	-3.03	-2.83	-2.33	-1.85	-1.66
-0.162	-3.35	-3.12	-2.56	-2.03	-1.82

E / V	Time: 0 min	Time: 5 min	Time: 15 min	Time: 35 min	Time: 60 min
-0.167	-3.62	-3.36	-2.76	-2.21	-1.99
-0.172	-3.82	-3.55	-2.92	-2.34	-2.12
-0.177	-3.94	-3.66	-3.03	-2.46	-2.25
-0.182	-3.98	-3.70	-3.08	-2.52	-2.33
-0.187	-3.92	-3.64	-3.07	-2.56	-2.37
-0.192	-3.77	-3.52	-3.00	-2.54	-2.36
-0.197	-3.55	-3.32	-2.88	-2.48	-2.32
-0.201	-3.28	-3.09	-2.71	-2.36	-2.23
-0.206	-2.98	-2.82	-2.51	-2.23	-2.13
-0.211	-2.68	-2.55	-2.30	-2.07	-1.99
-0.216	-2.39	-2.28	-2.10	-1.91	-1.85
-0.221	-2.13	-2.04	-1.90	-1.75	-1.69
-0.226	-1.89	-1.82	-1.71	-1.60	-1.54
-0.231	-1.69	-1.63	-1.55	-1.45	-1.39
-0.236	-1.51	-1.47	-1.40	-1.32	-1.28
-0.240	-1.36	-1.33	-1.27	-1.20	-1.17
-0.245	-1.25	-1.21	-1.17	-1.11	-1.08
-0.250	-1.16	-1.13	-1.09	-1.03	-1.00
-0.255	-1.08	-1.06	-1.03	-0.98	-0.94
-0.260	-1.02	-1.01	-0.98	-0.92	-0.88
-0.265	-0.98	-0.96	-0.93	-0.88	-0.85
-0.270	-0.94	-0.92	-0.90	-0.85	-0.82
-0.275	-0.92	-0.89	-0.87	-0.83	-0.80
-0.280	-0.90	-0.88	-0.86	-0.82	-0.78
-0.284	-0.88	-0.87	-0.84	-0.81	-0.77
-0.289	-0.88	-0.86	-0.84	-0.79	-0.76
-0.294	-0.86	-0.85	-0.82	-0.79	-0.76
-0.299	-0.86	-0.84	-0.82	-0.78	-0.75

Table 8. Data associated to Figure S1. Surface coverage vs concentration of HDT employed for preparation of the T-SAM.

HDT (μM)	Γ (mol/cm^2) $\times 10^{12}$
75	5.1
150	2.7
300	2.5
600	2.3
900	1.9

Table 9. Data associated to Figure S2. Comparative signal decrease registered for T-SAMs (T-PDT-peptide-5 and TPDT-peptide-6) upon the addition of different interferences at the specified concentrations.

Interferents	T-PDT-Peptide-5		T-PDT-Peptide-6	
	% Signal Decrease (Average)	STDEV	% Signal Decrease (Average)	STDEV
[Trypsin]=100 nM	-36.6	4.8	-4.7	1.6
[BSA]=100 nM	-14.3	7.0	-11.9	10.8
[Casein]=100 nM	-16.0	6.6	-16.3	7.0
[Ca ²⁺]=1 mM	-3.3	4.2	-5.4	0.6
[Dopamine]=1 nM	-4.0	1.9	-1.3	1.3

Table 10. Data associated to Figure S3. Calculated fractional cleavage, θ , vs time for data for T-PDT-Peptide-5 cleavage data from Figure 4.

[trypsin]=0.1 nM		[trypsin]=0.5 nM		[trypsin]=1 nM		[trypsin]=5 nM	
Time (min)	% Signal Decrease (Average)	Time (min)	% Signal Decrease (Average)	Time (min)	% Signal Decrease (Average)	Time (min)	% Signal Decrease (Average)
0	0.00	0	0.00	0	0.00	0	0.00
2	0.02	2	0.01	2	0.01	3	0.02
4	0.02	4	0.02	4	0.01	5	0.05
6	0.02	6	0.02	5	0.02	6	0.08
9	0.02	9	0.03	9	0.03	10	0.13
18	0.05	18	0.05	18	0.05	19	0.27
27	0.06	27	0.06	27	0.08	28	0.37
36	0.07	36	0.08	36	0.12	37	0.45
54	0.08	54	0.11	53	0.19	54	0.57
72	0.11	72	0.14	71	0.25	72	0.63

[trypsin]=10 nM		[trypsin]=25 nM		[trypsin]=50 nM		[trypsin]=100 nM	
Time (min)	% Signal Decrease (Average)	Time (min)	% Signal Decrease (Average)	Time (min)	% Signal Decrease (Average)	Time (min)	% Signal Decrease (Average)
0	0.00	0	0.00	0	0.00	0	0.00
2	0.01	2	0.01	2	0.02	2	0.03
3	0.04	3	0.08	4	0.13	4	0.21
5	0.07	5	0.17	5	0.26	5	0.38
9	0.16	9	0.35	9	0.47	9	0.59
18	0.37	18	0.66	18	0.74	18	0.78
26	0.52	27	0.79	27	0.85	27	0.86
35	0.62	35	0.85	36	0.90	36	0.91
53	0.72	53	0.93	53	0.97	53	0.96
71	0.77	71	0.95	71	1.00	71	1.00

Table 11. Data associated to Figure S3, Inset. θ vs time for T-PDT-Peptide-5 upon addition of trypsin (100 nM) solution as in Figure S3, and upon initial immersion in the same solution, followed by immersion in an inhibited trypsin solution (100 nM trypsin pre-incubated for 30 min with 200 nM of trypsin inhibitor from Glycine max (soybean)) after 10 min.

Time (min)	θ , trypsin 100 nM	Time (min)	θ , trypsin 100 nM + inhibited trypsin
0	0.00	0	0.00
2	0.10	2	0.02
3	0.23	4	0.16
5	0.41	6	0.28
7	0.53	7	0.37
9	0.60	9	0.45
11	0.66	11	0.51
13	0.72	14	0.50
15	0.75	16	0.51
16	0.79	17	0.51
18	0.81	19	0.51
27	0.87	21	0.52
37	0.93	23	0.52
46	0.95	25	0.53
55	0.97	26	0.53
64	0.98	28	0.53
74	1.00	37	0.55
		46	0.56
		55	0.57
		76	0.60

Table 12. Data associated to Figure S4. Variation of the effective constant rate, k_{eff} vs concentration of trypsin.

[Trypsin] (nM)	k_{eff} (min⁻¹)
0.1	0.002
0.5	0.002
1	0.004
5	0.015
10	0.025
25	0.052
50	0.065
100	0.080