Exploring the Role of Structure and Dynamics in the Function of Chymotrypsin Inhibitor 2
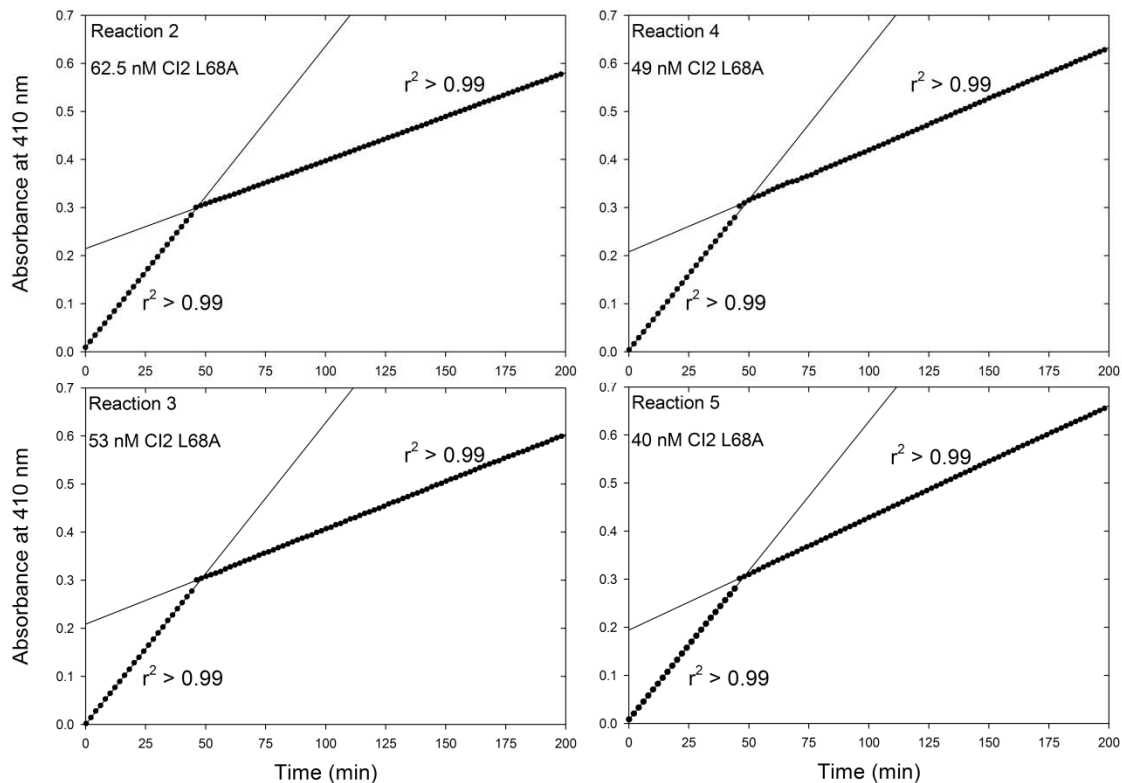
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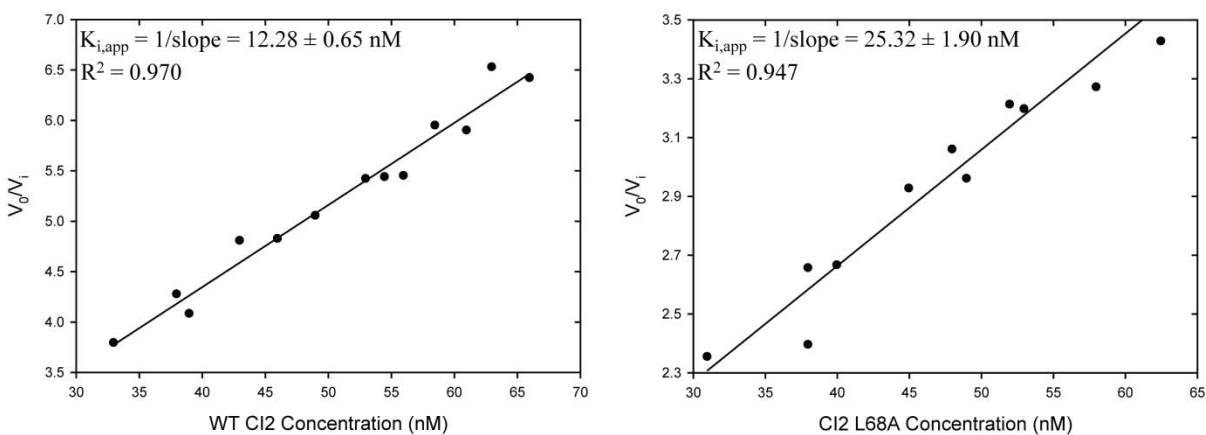University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Supplementary Information

Supplementary Figure 1

This figure displays individually the experimental kinetics data for reactions 2-5 shown in Figure 2. In each panel, the experimental data points are shown as solid black circles. The thin black lines represent linear regressions of the portions of the data before (time < 45 min) and after (time > 45 min) the addition of inhibitor.

Supplementary Figure 2

These panels show V_0/V_i plotted versus inhibitor concentration for WT CI2 (left) and CI2 L68A (right) assayed against porcine pancreatic elastase. When the primary data are plotted in this manner, the slope of the regression line is equal to $1/K_{i,app}$. These inhibitory constants, calculated via linear regression of all the experimental data for each variant, are identical within error to the values given in Table I that were calculated simply by averaging the results of the individual measurements.

Supplementary Table I

WT Residue	$R_{2,\text{effective}}$ (s^{-1}) τ^{-1} = 200 s^{-1}	$R_{2,\text{effective}}$ (s^{-1}) τ^{-1} = 2000 s^{-1}	$\Delta R_{2,\text{effective}}$ ± Error (s^{-1})		
22	8.98	8.98	0.00	±	0.20
23	7.50	7.05	0.45	±	0.08
24	9.01	8.32	0.70	±	0.12
26	9.56	8.81	0.74	±	0.11
27	8.27	7.51	0.76	±	0.10
28	9.10	8.92	0.18	±	0.07
29	8.54	8.44	0.10	±	0.10
30	8.01	7.33	0.68	±	0.07
31	6.95	6.83	0.12	±	0.06
32	9.11	8.85	0.26	±	0.08
33	9.62	8.95	0.67	±	0.07
34	7.58	7.08	0.50	±	0.06
35	9.30	8.85	0.46	±	0.08
36	8.58	7.68	0.90	±	0.07
37	6.44	6.27	0.17	±	0.05
38	7.05	6.95	0.11	±	0.06
39	8.70	8.40	0.30	±	0.08
40	9.89	9.28	0.62	±	0.10
41	8.27	7.78	0.49	±	0.07
42	8.07	7.57	0.50	±	0.09
43	9.38	9.43	-0.04	±	0.13
45	10.38	9.83	0.55	±	0.16
46	8.95	8.63	0.31	±	0.09
47	8.91	8.47	0.44	±	0.14
48	8.07	8.12	-0.04	±	0.08
49	7.60	6.98	0.62	±	0.09
50	7.56	7.41	0.14	±	0.08
51	9.00	8.16	0.85	±	0.09
53	8.86	8.48	0.38	±	0.07
54	9.50	9.40	0.10	±	0.11
55	7.89	7.64	0.26	±	0.06
56	7.56	7.51	0.04	±	0.07
57	7.32	6.53	0.78	±	0.08
58	9.86	9.51	0.35	±	0.14
59	18.27	17.40	0.86	±	1.88
60	7.41	7.22	0.19	±	0.09
61	7.17	6.81	0.35	±	0.10

62	8.03	7.11	0.92	± 0.19
63	16.02	14.95	1.07	± 0.40
64	11.46	10.75	0.71	± 0.18
65	8.90	8.80	0.10	± 0.11
66	9.51	9.14	0.37	± 0.15
67	10.16	9.62	0.54	± 0.14
68	8.93	8.48	0.45	± 0.10
69	9.30	8.69	0.61	± 0.11
70	7.79	7.39	0.40	± 0.09
71	8.86	8.47	0.39	± 0.10
72	11.13	10.86	0.27	± 0.16
73	7.59	7.06	0.53	± 0.08
74	7.93	7.48	0.45	± 0.08
75	6.78	6.26	0.52	± 0.07
76	9.68	9.40	0.28	± 0.10
77	8.72	8.46	0.26	± 0.12
78	8.66	7.85	0.81	± 0.08
79	9.50	9.33	0.17	± 0.08
81	8.00	7.72	0.28	± 0.09
82	8.54	8.57	-0.03	± 0.10
83	7.42	7.25	0.17	± 0.16

Supplementary Table I lists per-residue effective R_2 relaxation rate constants calculated for WT CI2 backbone amide position. The data were obtained from CPMG-based relaxation-dispersion experiments conducted using two different effective CPMG field strengths, noted in the table as $1/\tau$. When the difference ($\Delta R_{2,\text{effective}}$) is calculated from effective R_2 values measured in the presence of "slow" and "fast" CPMG pulsing ($1/\tau$ = 200 and 2000 s^{-1}, respectively) during the relaxation period, the value of $\Delta R_{2,\text{ effective}}$ can be used as a metric for the possible presence of dynamics on the μs-ms time scale. Typically, only a $\Delta R_{2,\text{ effective}}$ value ≥ 2 s^{-1} is held to indicate possible motions on the μs-ms time scale. Of the 58 backbone amide positions for which data could be obtained, no amide in WT CI2 meets this criterion.

Supplementary Table II

L68A Residue	$R_{2,effective}$ (s^{-1}) τ^{-1} = 67 s^{-1}	$R_{2,effective}$ (s^{-1}) τ^{-1} = 2200 s^{-1}	$\Delta R_{2,effective}$ ± Error (s^{-1})		
22	9.52	9.54	-0.03	±	0.31
23	7.65	7.42	0.23	±	0.10
24	8.28	8.18	0.09	±	0.12
26	7.81	7.79	0.02	±	0.12
27	8.25	8.13	0.12	±	0.13
28	7.91	7.89	0.02	±	0.08
29	8.79	8.56	0.23	±	0.18
30	7.77	7.64	0.13	±	0.10
31	7.69	7.34	0.35	±	0.10
32	8.48	8.59	-0.11	±	0.10
33	7.86	7.54	0.32	±	0.09
34	7.96	8.05	-0.09	±	0.09
35	8.08	7.91	0.16	±	0.09
36	8.44	7.92	0.52	±	0.10
37	7.92	8.06	-0.14	±	0.08
38	8.11	8.12	-0.01	±	0.08
39	8.94	9.12	-0.18	±	0.11
40	8.39	8.13	0.25	±	0.11
41	7.84	7.46	0.38	±	0.08
42	8.13	7.86	0.26	±	0.13
43	9.25	8.34	0.90	±	0.14
45	11.27	9.61	1.66	±	0.26
46	8.17	7.91	0.26	±	0.11
47	8.38	8.22	0.16	±	0.17
48	7.48	7.73	-0.25	±	0.11
49	8.37	8.37	0.00	±	0.14
50	7.53	8.09	-0.57	±	0.12
51	8.11	8.52	-0.41	±	0.11
53	8.32	8.38	-0.06	±	0.10
54	9.60	9.87	-0.27	±	0.23
55	7.50	7.50	0.00	±	0.10
56	7.77	8.57	-0.80	±	0.12
57	7.52	7.01	0.52	±	0.10
58	8.86	8.79	0.07	±	0.21
59	18.34	18.04	0.30	±	2.09
60	7.71	7.36	0.35	±	0.15
61	8.29	8.49	-0.20	±	0.16

62	8.06	7.29	0.77	±	0.31
63	17.10	11.40	5.70	±	0.48
64	12.19	9.50	2.69	±	0.24
65	7.72	7.77	-0.06	±	0.12
66	10.55	9.09	1.46	±	0.21
67	8.69	8.34	0.35	±	0.17
68	7.86	7.48	0.39	±	0.13
69	7.76	7.78	-0.02	±	0.13
70	8.42	8.65	-0.23	±	0.14
71	8.53	8.07	0.46	±	0.13
72	10.38	10.08	0.30	±	0.26
73	7.42	7.52	-0.11	±	0.12
74	8.10	7.83	0.27	±	0.11
75	7.51	7.79	-0.28	±	0.09
76	8.70	8.88	-0.17	±	0.12
77	8.63	8.68	-0.05	±	0.16
78	6.96	6.50	0.46	±	0.07
79	8.54	8.68	-0.14	±	0.13
81	8.01	7.91	0.10	±	0.12
82	8.36	8.58	-0.21	±	0.11
83	8.45	8.78	-0.33	±	0.25

Supplementary Table II lists per-residue effective R_2 relaxation rate constants calculated for L68A CI2 backbone amide position. The data were obtained from CPMG-based relaxation-dispersion experiments in a similar manner to the data presented in Supplementary Table I. Of the 58 backbone amide positions for which data could be obtained, only two (residues 63 and 64) have a $\Delta R_{2,\,\text{effective}}$ value ≥ 2 s^{-1} and thus meet the criterion for possible chemical exchange.