Supporting Information

Bunner et al. 10.1073/pnas.0912007107

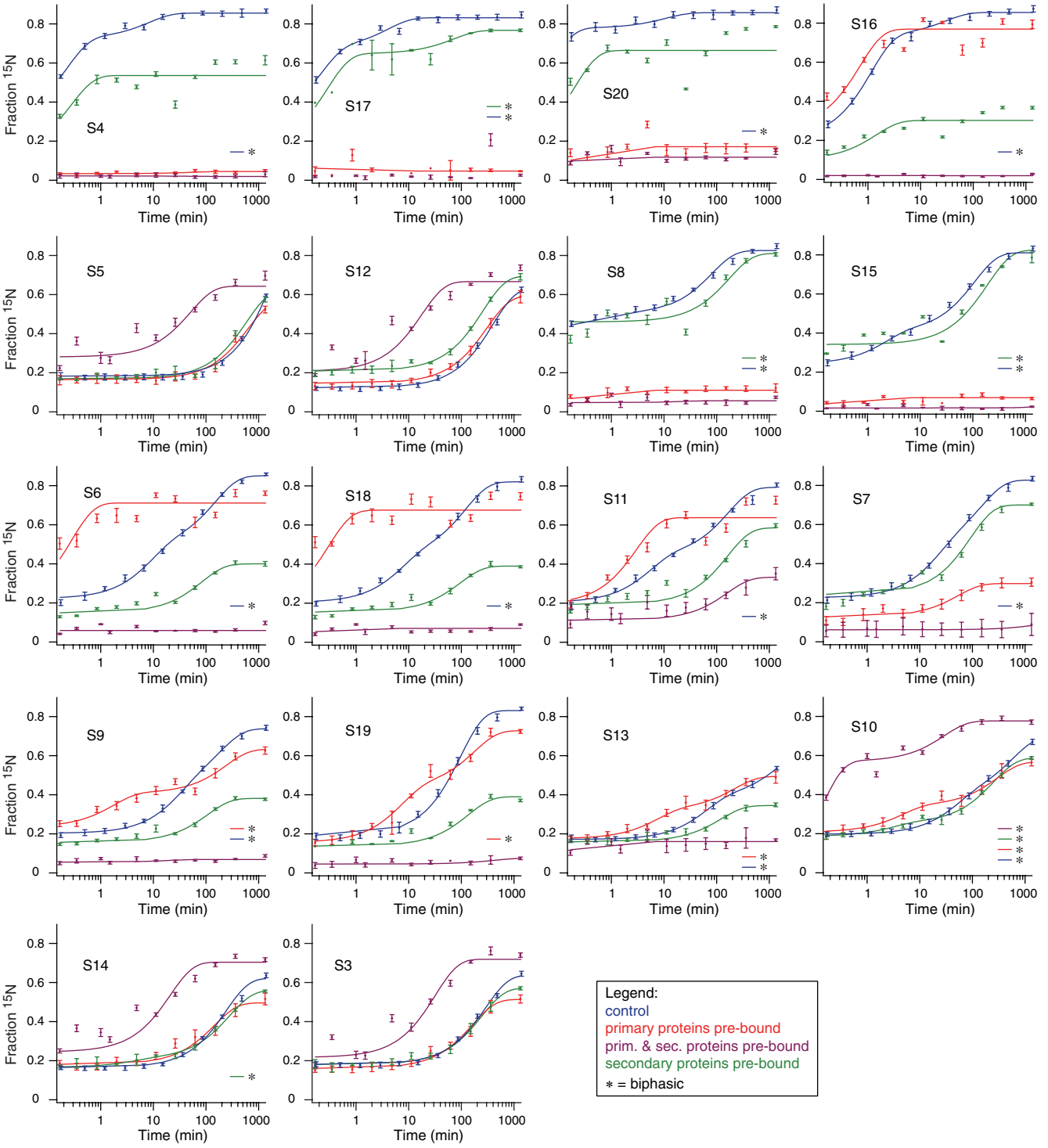

Fig. S1. Protein binding progress curves from experiments prebinding only the primary proteins (*Red*), only the secondary proteins (*Green*), both the primary and secondary proteins (*Purple*), and a control experiment (*Blue*). For these experiments, S11 was included among the secondary proteins. All curves are single exponential fits except those marked with an asterisk, which are double exponential.

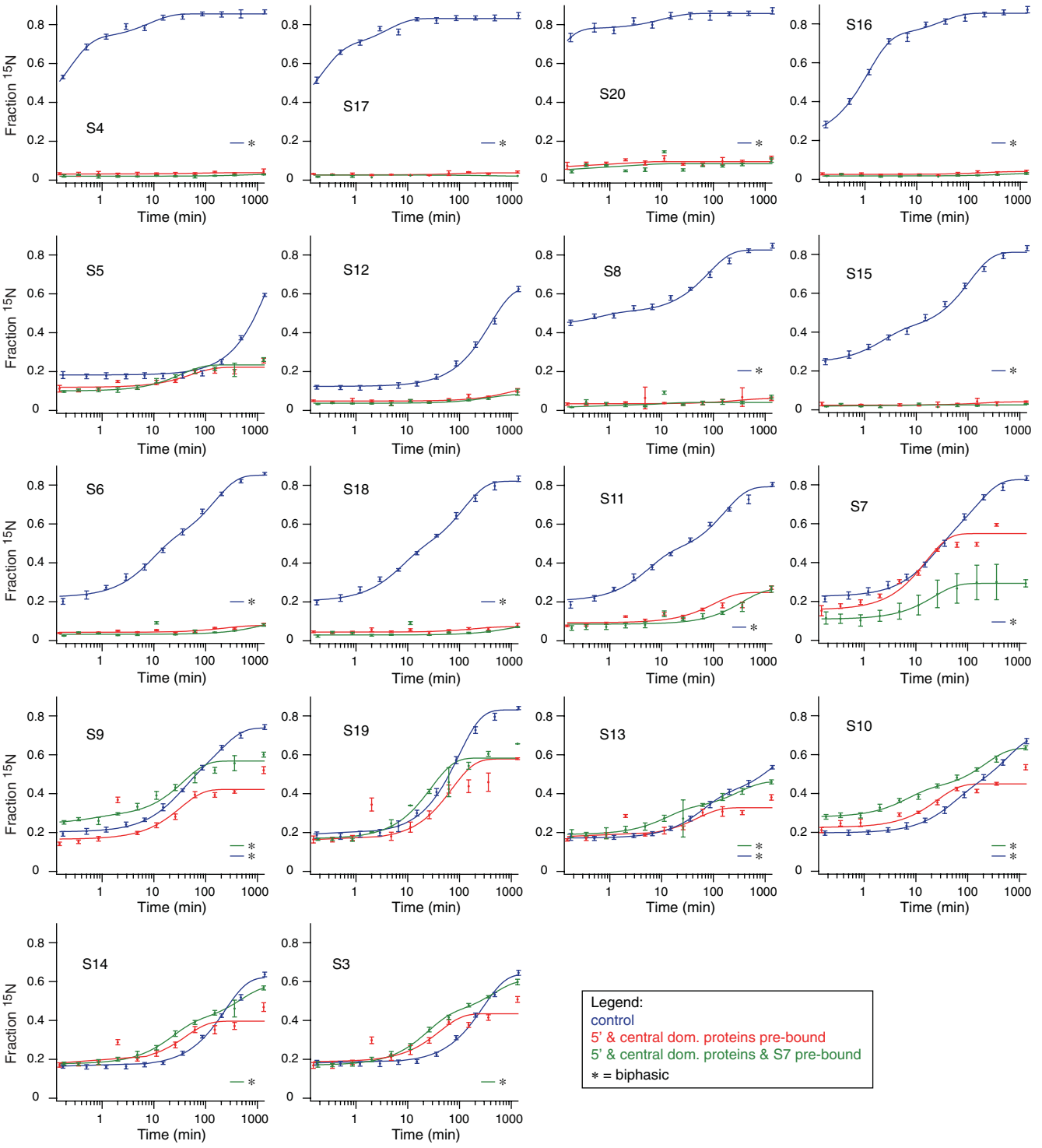

Fig. S2. Protein binding progress curves from experiments prebinding the 5′ and central domain proteins (*Red*), the 5′ and central domain proteins plus S7 (*Green*), and a control experiment (*Blue*). Most 5′ and central domain proteins prebound tightly and have a low fraction ^{15}N for all time points. All curves are single exponential fits except those marked with an asterisk, which are double exponential.

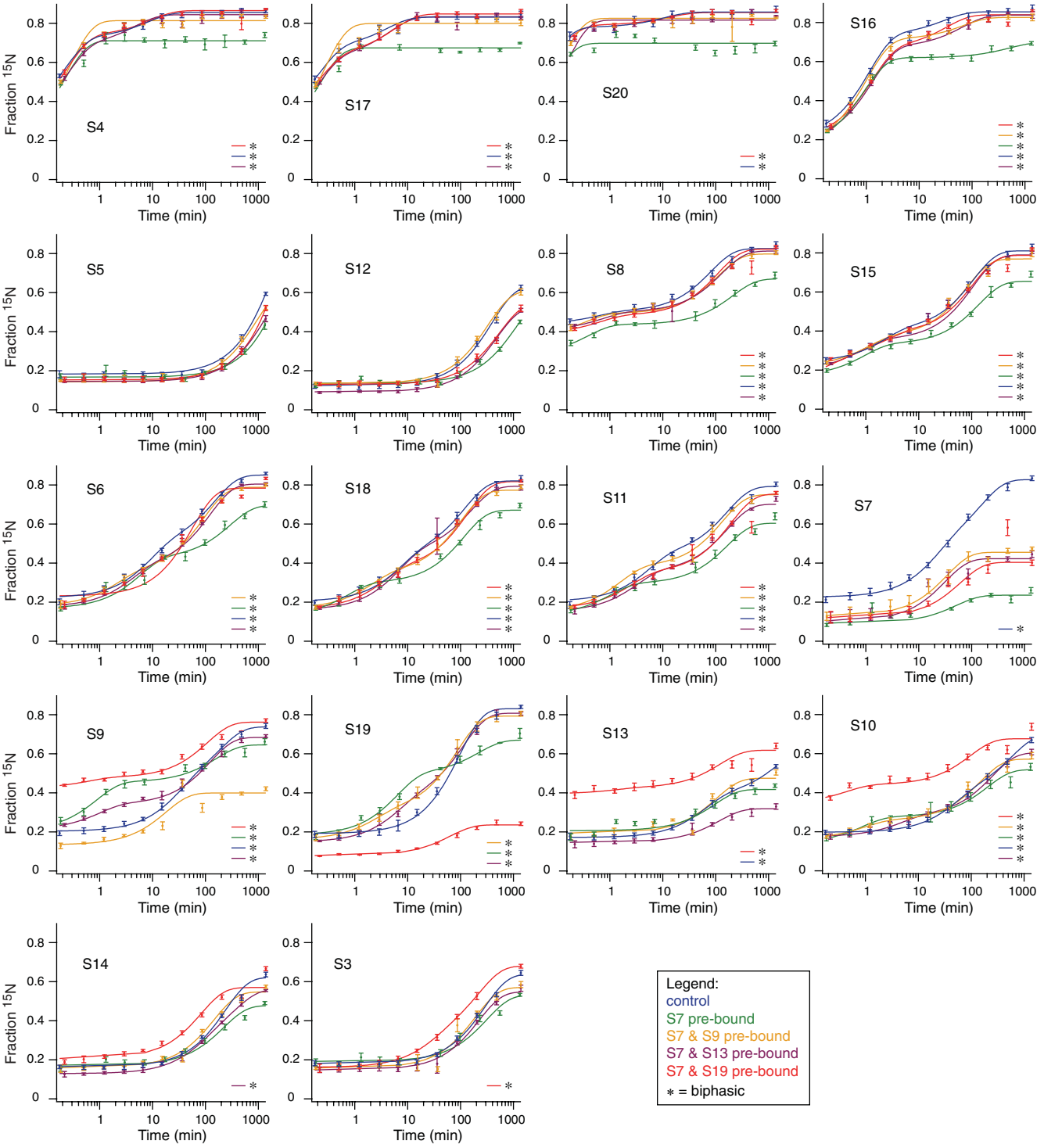

Fig. S3. Protein binding progress curves from experiments prebinding S7 alone (*Green*), S7 and S13 (*Purple*), S7 and S19 (*Red*), S7 and S9 (*Orange*), and a control experiment (*Blue*). The binding rates of 5′ and central domain proteins are not affected by the prebinding of 3′ domain proteins. All curves are single exponential fits except those marked with an asterisk, which are double exponential.

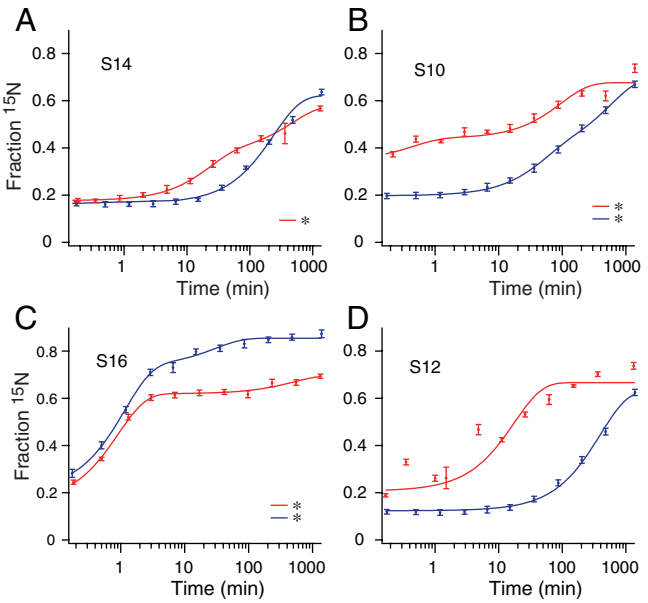

Fig. S4. Select protein binding progress curves demonstrating kinetic and extent changes that occur in prebinding experiments. Curves marked with an asterisk were fit using a double exponential curve. (*A*) S14 binding progress curves from a control experiment (*Blue*) and the prebinding of 5′ domain and central domain proteins plus S7 (*Red*), showing the appearance of an additional kinetic phase in the prebinding experiment. (*B*) S10 binding progress curves from a control experiment (*Blue*) and the prebinding of S7 and S19 (*Red*), showing a burst phase. (*C*) S16 binding progress curves from a control experiment (*Blue*) and the prebinding of S7 (*Red*), showing a decreased binding extent. (*D*) S12 binding progress curves from a control experiment (*Blue*) and the prebinding of primary and secondary proteins (*Red*), showing an increase in the binding rate.

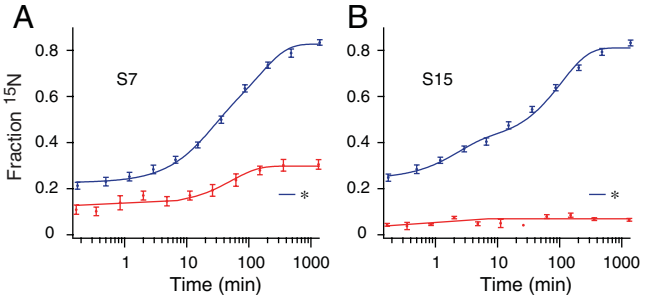

Fig. S5. Prebinding efficiency. Protein binding progress curves for primary binding proteins S7 (*A*) and S15 (*B*) showing a control experiment (*Blue*) and a prebinding experiment in which all primary binding proteins were prebound (*Red*). S15 binds tightly during the prebinding step, whereas S7 binds transiently. Curves marked with an asterisk were fit using a double exponential curve.

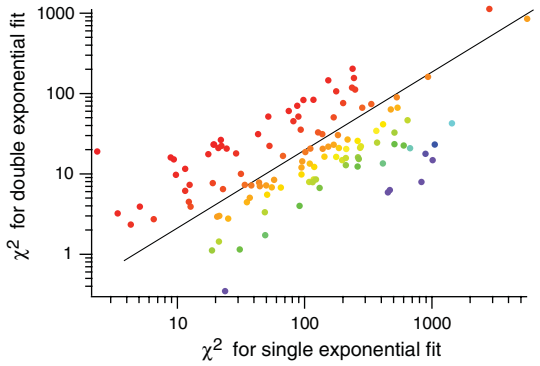

Fig. S6. F-test results for fits to kinetic data using double and single exponential curves. Each kinetic curve is represented by a dot filled with a color corresponding to the F-test value. Lowest values are shown in red and highest values are shown in purple. The black line represents the division between kinetic curves that are monophasic (*Red*, *Above Line*) and those that are multiphasic (*Other Colors*, *Below Line*). There were 124 kinetic curves in the ten experimental datasets in this study, and among those, 71 are multiphasic according to the F-test at a 95% confidence level. The F-test value was calculated according to: $F = (\chi_1^2/v_1)/(\chi_2^2/v_2)$, where χ_1^2 is the χ^2 value for the single exponential fit, v_1 is the degrees of freedom for the single exponential fit, χ_2^2 is the χ^2 value for the double exponential fit, and v_2 is the degrees of freedom for the double exponential fit.

Table S1. Protein binding rates from control experiments

	First order kinetics		Biphasic kinetics—Control expt. 1				
Protein	Control expt. 1 k_{obs} (min^{-1})	Control expt. 2 k_{obs} (min^{-1})	A_1	$k_{obs,1}$ (min^{-1})	A_2	$k_{obs,2}$ (min^{-1})	F-test value
S3	0.007 ± 0.001	0.0035 ± 3.E-04					0.9
S4	[2.30 ± 0.07]	3.9 ± 0.2	0.1	0.1	0.54	5.7	11
S5	9.E-04 ± 6.E+04	5.E-04 ± 2.E-04					0.7
S6	[0.030 ± 0.002]	0.019 ± 0.001	0.36	7E-03	0.28	0.1	6.9
S7	[0.021 ± 0.003]	0.017 ± 0.001	0.4	6E-03	0.3	0.06	4.4
S8	[0.042 ± 0.002]	0.46 ± 0.04	0.32	0.012	0.33	10	39
S9	[0.018 ± 0.002]	0.014 ± 0.001	0.3	5E-03	0.2	0.04	6.2
S10	[0.014 ± 0.002]	0.0054 ± 5.E-04	0.3	2E-03	0.2	0.02	21
S11	[0.032 ± 0.003]	0.028 ± 0.001	0.35	6.0E-03	0.25	0.2	7.9
S12	0.003 ± 0.001	0.0026 ± 2.E-04					1.3
S13	[0.014 ± 0.003]	0.0062 ± 6.E-04	0.2	7E-04	0.2	0.02	53
S14	0.008 ± 0.001	0.0043 ± 3.E-04					1.3
S15	[0.035 ± 0.003]	0.023 ± 0.002	0.41	9.3E-03	0.22	0.8	6.3
S16	[0.81 ± 0.03]	0.64 ± 0.04	0.1	0.03	0.54	0.92	5.4
S17	[3.9 ± 0.4]	2.8 ± 0.2	0.2	0.3	0.49	6	5.2
S18	[0.025 ± 0.003]	0.029 ± 0.001	0.38	8E-03	0.25	0.2	10
S19	0.0188 ± 7.E-04	0.0105 ± 6.E-04					2.5
S20	[5.5 ± 0.2]	10 ± 1	0.08	0.09	0.58	10	5.7

Bracketed rates indicate multiphasic kinetics as determined by F-tests. Biphasic rates and phase amplitudes are given for biphasic datasets from control expt. 1. Amplitudes given are unitless fraction ^{15}N values. No F-test was performed on the data from control expt. 2.

Table S2. Protein binding rates from primary protein prebinding experiment

	First order kinetics	Biphasic kinetics				
Protein	k_{obs} (min^{-1})	A_1	$k_{obs,1}$ (min^{-1})	A_2	$k_{obs,2}$ (min^{-1})	F-test value
S3	0.006 ± 0.001					1.4
S5	0.0016 ± 3.E-04					0.8
S6	3.6 ± 0.4					1.1
S9	[0.022 ± 0.003]	0.23	4.1E-03	0.18	0.67	4.2
S10	[0.045 ± 0.008]	0.2	3E-03	0.1	0.3	5.3
S11	0.32 ± 0.04					0.9
S12	0.0035 ± 6.E-04					0.8
S13	[0.06 ± 0.01]	0.2	5E-03	0.1	0.1	5.5
S14	0.007 ± 0.002					2.1
S16	1.4 ± 0.1					1.7
S18	3.3 ± 0.2					1.0
S19	[0.047 ± 0.003]	0.3	6E-03	0.3	0.1	7.5

Bracketed rates indicate multiphasic kinetics as determined by F-tests. Biphasic rates and phase amplitudes are given for biphasic datasets. Amplitudes given are unitless fraction ^{15}N values.

Table S3. Protein binding rates from primary and secondary protein prebinding experiment

	First order kinetics	Biphasic kinetics				
Protein	k_{obs} (min^{-1})	A_1	$k_{obs,1}$ (min^{-1})	A_2	$k_{obs,2}$ (min^{-1})	F-test value
S3	0.031 ± 0.002					1.3
S5	0.020 ± 0.002					1.3
S10	[0.96 ± 0.08]	0.21	0.03	0.43	5.6	5.8
S12	0.052 ± 0.003					1.5
S14	0.048 ± 0.002					3.3

Bracketed rates indicate multiphasic kinetics as determined by F-tests. Biphasic rates and phase amplitudes are given for biphasic datasets. Amplitudes given are unitless fraction ^{15}N values.

Table S4. Protein binding rates from secondary protein prebinding experiment

	First order kinetics	Biphasic kinetics				
Protein	k_{obs} (min^{-1})	A_1	$k_{obs,1}$ (min^{-1})	A_2	$k_{obs,2}$ (min^{-1})	F-test value
S3	0.0041 ± 5.E-04					0.8
S4	3.6 ± 0.3					2.0
S5	0.0017 ± 2.E-04					0.6
S7	0.0108 ± 7.E-04					3.4
S8	[0.0084 ± 7.E-04]	0.33	5.7E-03	0.31	6	4.6
S10	[0.0068 ± 4.E-04]	0.33	3.9E-03	0.10	0.8	10
S12	0.0038 ± 3.E-04					0.8
S14	[0.0041 ± 3.E-04]	0.35	3.6E-03	0.05	0.3	4.9
S15	[0.0129 ± 4.E-04]	0.46	5.0E-03	0.22	6.7	5.1
S17	[2.2 ± 0.4]	0.12	0.01	0.44	3	58
S20	5 ± 0.2					2.0

Bracketed rates indicate multiphasic kinetics as determined by F-tests. Biphasic rates and phase amplitudes are given for biphasic datasets. Amplitudes given are unitless fraction ^{15}N values.

Table S5. Protein binding rates from 5′ and central domain proteins prebinding experiment

Protein	k_{obs} (min^{-1})	F-test value
S3	0.024 ± 0.003	1.4
S7	0.064 ± 0.004	2.9
S9	0.031 ± 0.004	0.9
S10	0.038 ± 0.004	2.0
S13	0.021 ± 0.004	1.2
S14	0.024 ± 0.004	1.0
S19	0.014 ± 0.002	1.1

First order rates are given. None of these datasets are biphasic according to the F-tests.

Table S6. Protein binding rates from 5′ and central domain proteins plus S7 prebinding experiment

	First order kinetics	Biphasic kinetics				
Protein	k_{obs} (min^{-1})	A_1	$k_{obs,1}$ (min^{-1})	A_2	$k_{obs,2}$ (min^{-1})	F-test value
S3	[0.02 ± 0.002]	0.2	2E-03	0.24	0.04	6.3
S9	[0.12 ± 0.01]	0.29	0.03	0.11	6	5.7
S10	[0.023 ± 0.002]	0.22	4E-03	0.15	0.2	4.2
S13	[0.015 ± 0.002]	0.1	3E-03	0.1	0.09	12
S14	[0.013 ± 0.001]	0.2	2E-03	0.2	0.05	22
S19	0.036 ± 0.003					1.9

Bracketed rates indicate multiphasic kinetics as determined by F-tests. Biphasic rates and phase amplitudes are given for biphasic datasets. Amplitudes given are unitless fraction ^{15}N values.

Table S7. Protein binding rates from S7 prebinding experiment

	First order kinetics	Biphasic kinetics				
Protein	k_{obs} (min^{-1})	A_1	$k_{obs,1}$ (min^{-1})	A_2	$k_{obs,2}$ (min^{-1})	F-test value
S3	0.0032 ± 4.E-04					2.7
S4	4.7 ± 0.3					N/D
S5	3.E-04 ± 3.E-04					0.5
S6	[0.045 ± 0.003]	0.27	3E-03	0.26	0.2	6.8
S8	[0.43 ± 0.02]	0.23	5E-03	0.27	5	23
S9	[0.47 ± 0.05]	0.19	6E-03	0.28	1	10
S10	[0.063 ± 0.008]	0.24	5E-03	0.11	0.8	7.5
S11	[0.011 ± 0.002]	0.31	6E-03	0.14	0.8	7.3
S12	0.0010 ± 2.E-04					0.8
S13	0.011 ± 0.001					4.0
S14	0.0045 ± 5.E-04					2.5
S15	[0.015 ± 0.001]	0.32	7E-03	0.18	1	13
S16	[0.94 ± 0.04]	0.08	2E-03	0.45	1.0	7.2
S17	4.7 ± 0.3					N/D
S18	[0.0122 ± 9.E-04]	0.37	8.6E-03	0.14	1	5.1
S19	[0.030 ± 0.001]	0.18	5E-03	0.31	0.2	5.9
S20	12.3 ± 0.9					N/D

Bracketed rates indicate multiphasic kinetics as determined by F-tests. Biphasic rates and phase amplitudes are given for biphasic datasets. Amplitudes given are unitless fraction ^{15}N values. F-test values could not be determined for S4, S17, and S20.

Table S8. Protein binding rates from S7 and S13 prebinding experiment

	First order kinetics	Biphasic kinetics				
Protein	k_{obs} (min^{-1})	A_1	$k_{obs,1}$ (min^{-1})	A_2	$k_{obs,2}$ (min^{-1})	F-test value
S3	0.0045 ± 4.E-04					1.9
S4	[2.8 ± 0.1]	0.2	0.2	0.53	5.1	4.7
S5	0.0007 ± 2.E-04					0.4
S6	[0.0169 ± 6.E-04]	0.43	8.2E-03	0.22	0.3	21
S8	[0.178 ± 0.009]	0.31	7.7E-03	0.35	7.2	53
S9	[0.082 ± 0.004]	0.34	8.2E-03	0.19	2	17
S10	[0.009 ± 0.001]	0.33	4E-03	0.1	0.2	11
S11	[0.0128 ± 8.E-04]	0.35	6.0E-03	0.21	0.3	14
S12	0.0021 ± 1.E-04					1.5
S14	[0.0049 ± 2.E-04]	0.3	3E-03	0.2	0.01	5.4
S15	[0.0142 ± 6.E-04]	0.44	9.3E-03	0.21	2.2	11
S16	[0.54 ± 0.02]	0.17	0.01	0.52	0.86	9.8
S17	[2.7 ± 0.1]	0.21	0.2	0.47	5.8	5.5
S18	[0.0143 ± 5.E-04]	0.34	6E-03	0.29	0.2	15
S19	[0.0210 ± 8.E-04]	0.45	9E-03	0.2	0.2	11
S20	7.1 ± 0.4					N/D

Bracketed rates indicate multiphasic kinetics as determined by F-tests. Biphasic rates and phase amplitudes are given for biphasic datasets. Amplitudes given are unitless fraction ^{15}N values. An F-test value could not be determined for S20.

Table S9. Protein binding rates from S7 and S9 prebinding experiment

	First order kinetics	Biphasic kinetics				
Protein	k_{obs} (min^{-1})	A_1	$k_{obs,1}$ (min^{-1})	A_2	$k_{obs,2}$ (min^{-1})	F-test value
S3	0.0052 ± 4.E-04					0.8
S4	3.7 ± 0.1					3.1
S5	0.0011 ± 2.E-04					0.6
S6	[0.0217 ± 9.E-04]	0.44	0.011	0.19	0.6	16
S8	[0.61 ± 0.03]	0.31	9E-03	0.34	8	81
S10	[0.013 ± 0.001]	0.32	7E-03	0.10	1	11
S11	[0.016 ± 0.001]	0.37	7.4E-03	0.22	0.64	25
S12	0.0031 ± 3.E-04					0.8
S13	0.0097 ± 9.E-04					0.8
S14	0.0069 ± 4.E-04					1.9
S15	[0.035 ± 0.002]	0.40	0.012	0.19	1	8.3
S16	[0.78 ± 0.03]	0.11	0.01	0.56	0.98	8.4
S17	3.8 ± 0.2					6.0
S18	[0.017 ± 0.001]	0.43	9.8E-03	0.18	0.5	13
S19	[0.0196 ± 9.E-04]	0.49	0.01	0.2	0.5	18
S20	8.9 ± 0.5					3.6

Bracketed rates indicate multiphasic kinetics as determined by F-tests. Biphasic rates and phase amplitudes are given for biphasic datasets. Amplitudes given are unitless fraction ^{15}N values.

Table S10. Protein binding rates from S7 and S19 prebinding experiment

	First order kinetics	Biphasic kinetics				
Protein	k_{obs} (min^{-1})	A_1	$k_{obs,1}$ (min^{-1})	A_2	$k_{obs,2}$ (min^{-1})	F-test value
S3	[0.0080 ± 5.E-04]	0.4	4E-03	0.1	0.04	13
S4	[3.1 ± 0.1]	0.13	0.1	0.58	5.0	8.1
S5	0.0003 ± 8E+04					0.1
S6	0.0175 ± 7.E-04					3.5
S8	[0.024 ± 0.001]	0.34	9.1E-03	0.33	7.0	26
S9	[4.9 ± 0.2]	0.28	9E-03	0.33	9.7	34
S10	[1.26 ± 0.08]	0.24	0.01	0.29	7	12
S11	[0.021 ± 0.001]	0.41	5.1E-03	0.20	0.5	7.8
S12	0.0018 ± 2.E-04					1.0
S13	[3.1 ± 0.4]	0.19	8E-03	0.27	9	10
S14	0.0121 ± 8.E-04					2.6
S15	[0.0242 ± 9.E-04]	0.40	9.4E-03	0.22	1.5	4.5
S16	[0.78 ± 0.03]	0.17	0.03	0.50	0.88	6.3
S17	[1.45 ± 0.06]	0.21	0.2	0.48	6.0	58
S18	[0.022 ± 0.001]	0.45	7.4E-03	0.21	0.3	11
S20	[8.7 ± 0.4]	0.07	0.07	0.64	11	6.4

Bracketed rates indicate multiphasic kinetics as determined by F-tests. Biphasic rates and phase amplitudes are given for biphasic datasets. Amplitudes given are unitless fraction ^{15}N values.