

Supplementary Information

The effect of ribosome assembly cofactors on *in vitro* 30S subunit reconstitution

Anne E. Bunner¹, Stefan Nord², P. Mikael Wikström², James R. Williamson¹

1, Departments of Molecular Biology and Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, California, 92037

2, Department of Molecular Biology, Umeå University, SE-901 87 Umeå, Sweden

Contents

Supplementary Table 1. Protein binding rates with Era

Supplementary Table 2. Protein binding rates with RimM

Supplementary Table 3. Protein binding rates with RimP

Supplementary Table 4. Protein binding rates without cofactors

Supplementary Figure 1. Protein binding progress curves from an experiment with Era and a control experiment.

Supplementary Figure 2. Protein binding progress curves from an experiment with RimM and a control experiment.

Supplementary Figure 3. Protein binding progress curves from an experiment with RimP and a control experiment.

Supplementary Figure 4. Overlaid protein binding progress curves from experiments with Era, RimM, RimP and a control experiment.

Supplementary Tables

Table 1. Protein binding rates with Era. 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.

Protein	First order kinetics	Biphasic kinetics				F-test value
	k_{obs} (min^{-1})	A_1	$k_{\text{obs},1}$ (min^{-1})	A_2	$k_{\text{obs},2}$ (min^{-1})	
S3	0.32 ± 0.01					1.4
S4	[9.8 ± 0.7]	0.05	0.4	0.61	18	11
S5	0.37 ± 0.01					1.3
S6	[3.31 ± 0.09]	0.2	0.8	0.48	7	7.6
S7	[3.0 ± 0.1]	0.13	0.1	0.54	4.0	89
S8	[4.8 ± 0.2]	0.2	2	0.42	16	12
S9	[1.25 ± 0.05]	0.20	0.1	0.43	2.2	48
S10	[0.80 ± 0.03]	0.24	0.09	0.42	1	63
S11	[1.61 ± 0.05]	0.2	0.2	0.46	2.6	17
S12	0.28 ± 0.01					0.9
S13	[0.75 ± 0.04]	0.2	0.09	0.34	1.2	11
S14	[0.46 ± 0.01]	0.2	0.07	0.45	0.69	4.0
S15	[3.8 ± 0.1]	0.2	0.9	0.41	10	17
S16	[6.0 ± 0.2]	0.1	0.9	0.57	10	10
S17	[7.3 ± 0.4]	0.1	0.8	0.53	12	5.7
S18	[3.5 ± 0.1]	0.2	0.7	0.48	7	6.5
S19	[1.02 ± 0.03]	0.2	0.1	0.53	1.3	9.0
S20	[8.1 ± 0.3]	0.07	0.6	0.60	12	7.8

Table 2. Protein binding rates with RimM. 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.

Protein	First order kinetics	Biphasic kinetics				F-test value
	k_{obs} (min^{-1})	A_1	$k_{\text{obs},1}$ (min^{-1})	A_2	$k_{\text{obs},2}$ (min^{-1})	
S3	0.35 ± 0.02					1.0
S4	[15 ± 1.7]	0.1	0.2	0.67	24	4.0
S5	0.31 ± 0.02					0.8
S6	[4.1 ± 0.1]	0.24	0.9	0.48	8.8	5.8
S7	[2.9 ± 0.1]	0.1	0.3	0.57	4.6	7.1
S8	[6.5 ± 0.3]	0.2	1	0.51	17	4.4
S9	[1.6 ± 0.1]	0.2	0.3	0.45	3	7.0
S10	[0.95 ± 0.04]	0.2	0.2	0.44	2	6.7
S11	[0.82 ± 0.04]	0.3	0.2	0.38	2	5.9
S12	0.23 ± 0.01					0.7
S13	1.0 ± 0.1					1.7
S14	0.38 ± 0.01					1.8
S15	[4.5 ± 0.1]	0.18	0.5	0.53	8.5	8.6
S16	[7.0 ± 0.3]	0.1	0.7	0.63	10	4.3
S17	[6.9 ± 0.6]	0.2	0.9	0.5	16	7.7
S18	[4.3 ± 0.1]	0.3	0.9	0.45	9	15
S19	[2.5 ± 0.1]	0.2	0.4	0.55	4.2	9.8
S20	[9.3 ± 0.4]	0.1	0.4	0.65	14	5.4

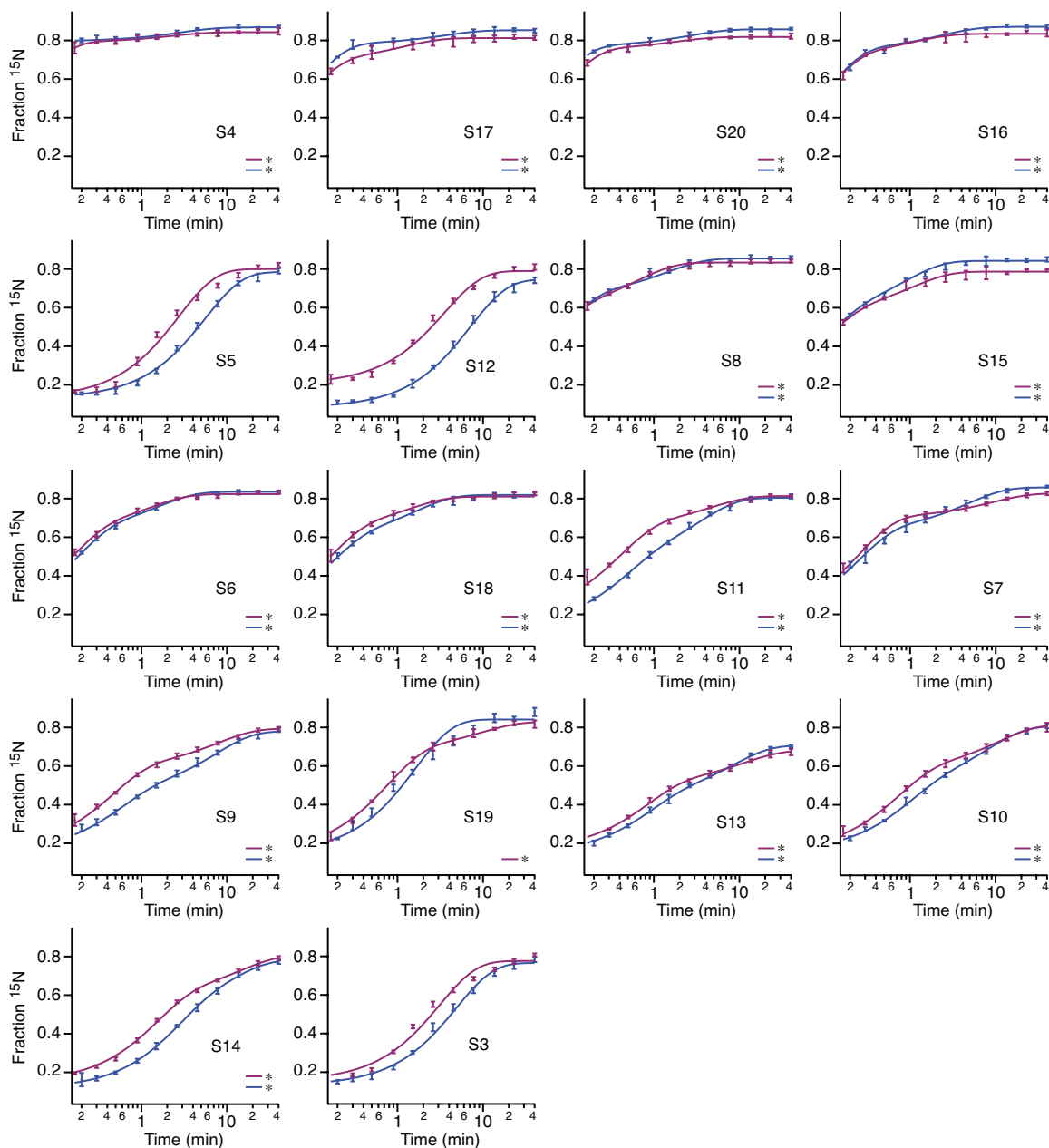
Table 3. Protein binding rates with RimP. Bracketed rates indicate multiphasic kinetics as determined by F-tests. Biphasic rates and phase amplitudes are given for biphasic datasets. Amplitudes given are unitless fraction ¹⁵N values.

Protein	First order kinetics	Biphasic kinetics				F-test value
	k_{obs} (min ⁻¹)	A ₁	$k_{obs,1}$ (min ⁻¹)	A ₂	$k_{obs,2}$ (min ⁻¹)	
S3	0.39 ± 0.02	0.1	0.04	0.5	0.5	1.3
S4	[16 ± 2]	0.05	0.3	0.6	24	4.8
S5	0.43 ± 0.02					1.7
S6	[3.8 ± 0.1]	0.2	0.7	0.5	8	41
S7	[3.5 ± 0.2]	0.2	0.3	0.5	6	16
S8	[7.1 ± 0.3]	0.1	0.6	0.5	12	38
S9	[1.32 ± 0.06]	0.3	0.3	0.3	3	8.8
S10	[0.90 ± 0.03]	0.2	0.1	0.4	1	35
S11	[0.86 ± 0.03]	0.4	0.4	0.3	2	4.6
S12	[0.78 ± 0.03]	0.1	0.1	0.4	1	11
S13	[0.68 ± 0.03]	0.2	0.08	0.4	1	7.2
S14	0.44 ± 0.01					2.5
S15	[5.0 ± 0.2]	0.2	0.6	0.5	9	29
S16	[7.4 ± 0.3]	0.07	0.2	0.6	9	54
S17	[8.1 ± 0.6]	0.06	0.3	0.6	9	6.7
S18	[3.8 ± 0.1]	0.2	0.6	0.5	8.3	43
S19	0.86 ± 0.04					1.6
S20	[11.1 ± 0.3]	0.06	0.3	0.6	13	16

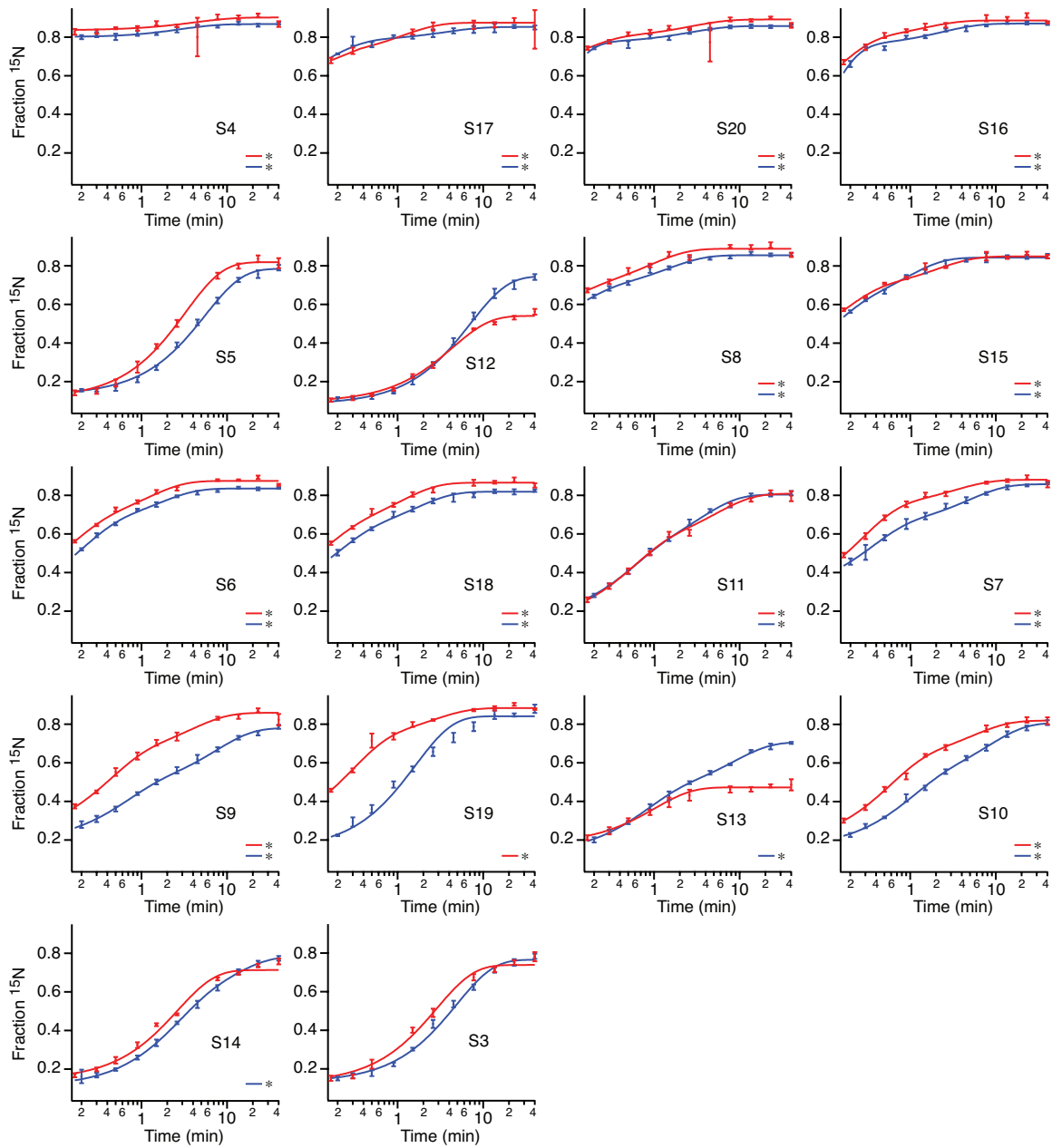
Table 4. Protein binding rates without cofactors.¹ Bracketed rates indicate multiphasic kinetics as determined by F-tests. Biphasic rates and phase amplitudes are given for biphasic datasets from control expt. 1. Amplitudes given are unitless fraction ¹⁵N values. No F-test was performed on the data from control expt. 2.

Protein	First order kinetics		Biphasic kinetics - Control expt. 1				F-test value
	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)	
S3	0.21 ± 0.01	0.22 ± 0.01					1.0
S4	[12.3 ± 0.9]	16 ± 2	0.1	0.4	0.64	260	8.5
S5	0.18 ± 0.01	0.20 ± 0.01					0.8
S6	[3.23 ± 0.08]	3.0 ± 0.2	0.20	0.6	0.48	6.3	23
S7	[1.88 ± 0.08]	2.5 ± 0.1	0.22	0.2	0.48	4	21
S8	[5.4 ± 0.2]	5.5 ± 0.2	0.2	0.7	0.51	12	14
S9	[0.67 ± 0.03]	0.62 ± 0.04	0.32	0.1	0.31	2	24
S10	[0.50 ± 0.02]	0.41 ± 0.02	0.32	0.1	0.3	1	31
S11	[0.83 ± 0.03]	0.85 ± 0.04	0.3	0.3	0.3	2	13
S12	0.14 ± 0.01	0.14 ± 0.01					0.8
S13	[0.31 ± 0.01]	0.47 ± 0.03	0.27	0.1	0.29	1	33
S14	[0.25 ± 0.01]	0.23 ± 0.01	0.3	0.1	0.4	0.4	5.3
S15	[3.9 ± 0.1]	3.3 ± 0.2	0.3	1	0.42	10	19
S16	[5.8 ± 0.2]	6.7 ± 0.2	0.1	0.4	0.60	10	9.4
S17	[9.1 ± 0.2]	10 ± 1	0.07	0.3	0.63	11	7.8
S18	[2.92 ± 0.09]	2.7 ± 0.3	0.2	0.6	0.44	7	14
S19	0.62 ± 0.03	0.56 ± 0.02					2.1
S20	[9.0 ± 0.3]	11 ± 3	0.1	0.4	0.61	15	17

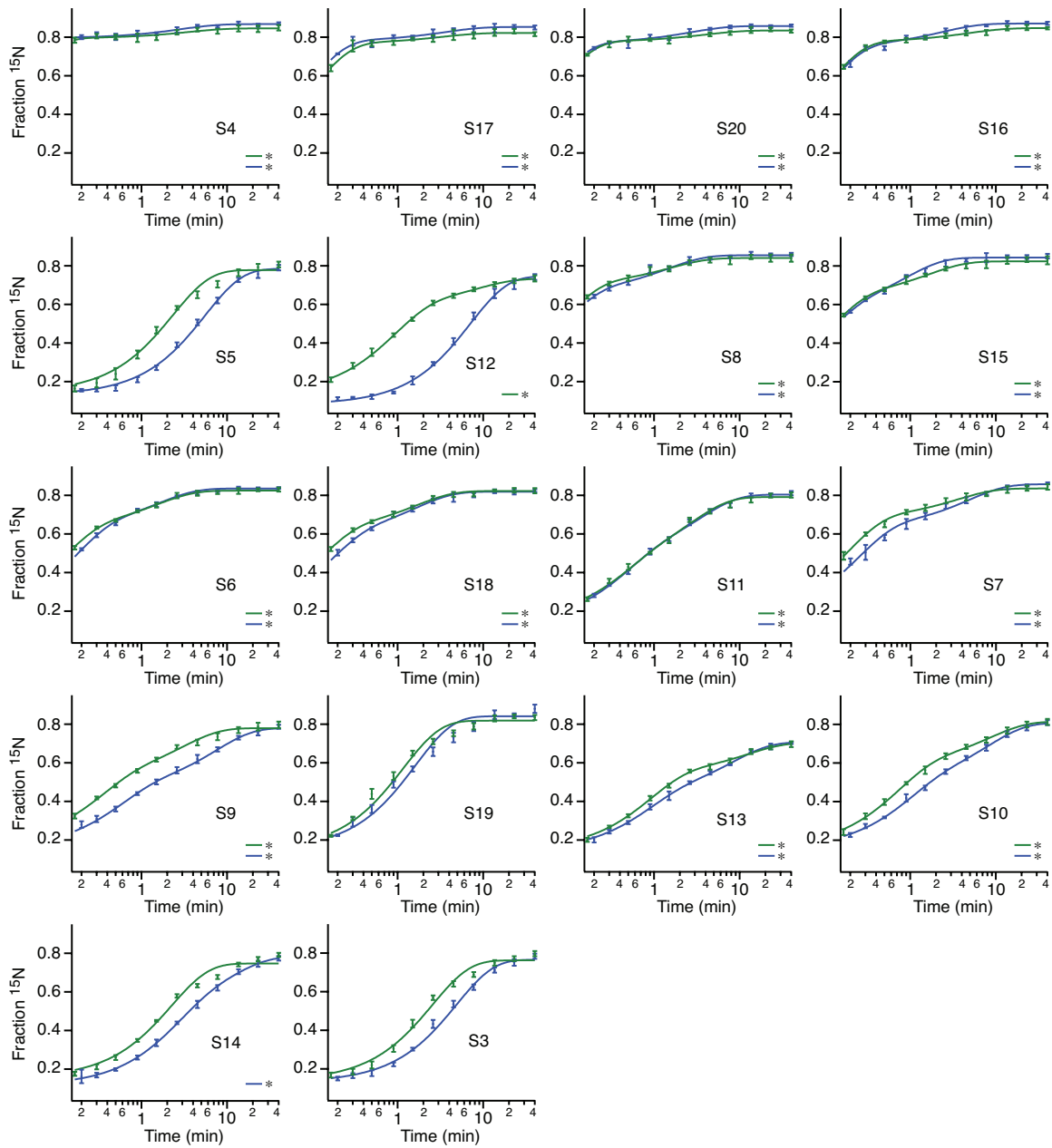
Supplementary Figures



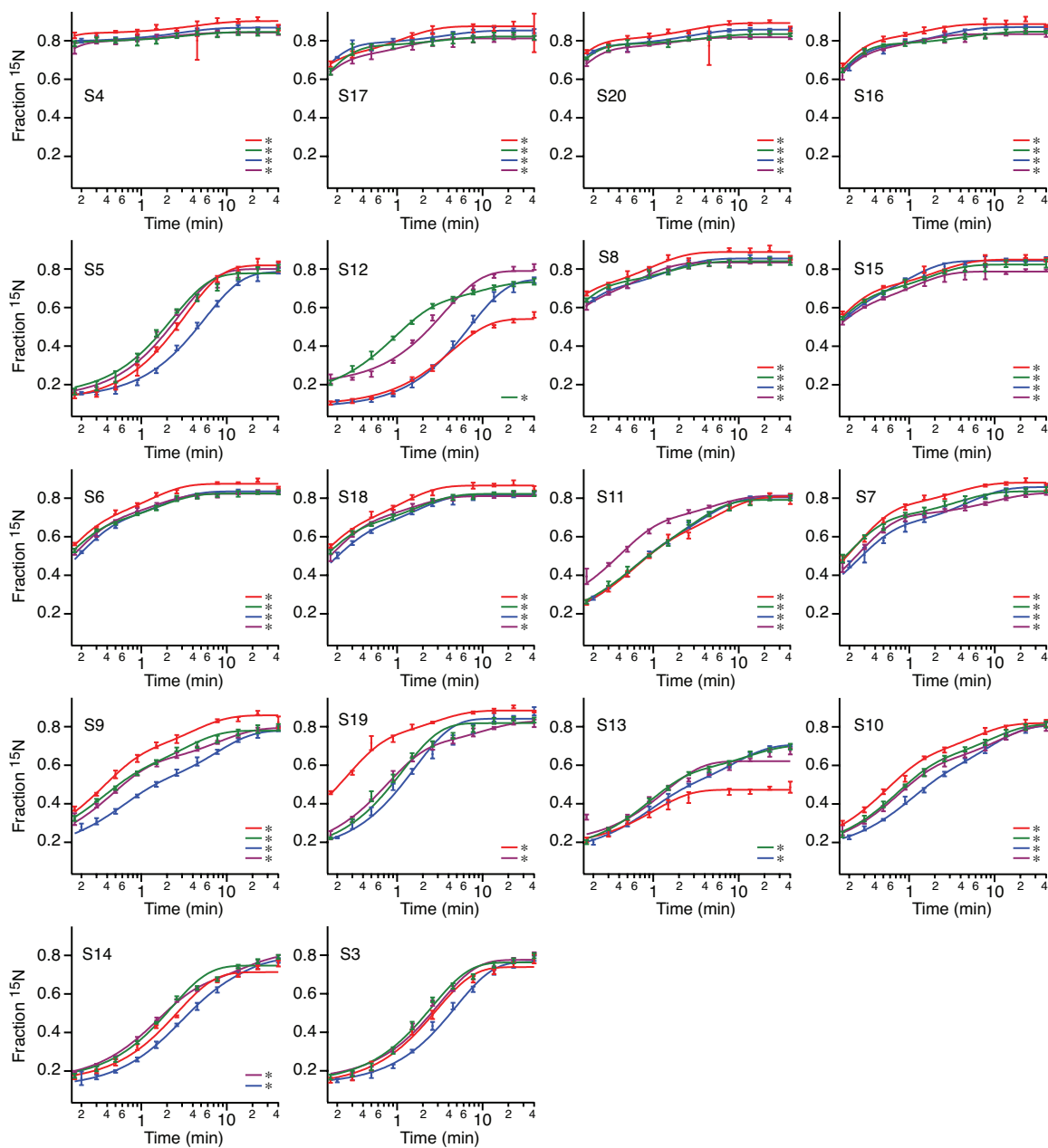
Supplementary Figure 1. Protein binding progress curves with Era (purple) and without¹ (blue). Curves marked with asterisks are fit to a double exponential based on the results of a statistical f-test, using a 95% confidence interval. Changes in the kinetic curve for S13 are very subtle, although the calculated rates suggest that S13 binds significantly faster with Era. The discrepancy between the kinetic curves and the calculated first-order rates is due to imperfect fitting of biphasic kinetics with single exponential curves, and was only observed for S13.



Supplementary Figure 2. Protein binding progress curves with RimM (red) and without¹ (blue). Curves marked with asterisks are fit to a double exponential based on the results of a statistical f-test, using a 95% confidence interval.



Supplementary Figure 3. Protein binding progress curves with RimP (green) and without¹ (blue). Curves marked with asterisks are fit to a double exponential based on the results of a statistical f-test, using a 95% confidence interval. Changes in the kinetic curve for S13 are very subtle, although the calculated rates suggest that S13 binds significantly faster with RimP. The discrepancy between the kinetic curves and the calculated first-order rates is due to imperfect fitting of biphasic kinetics with single exponential curves, and was only observed for S13.



Supplementary Figure 4. Overlaid protein binding progress curves. Data from experiments with Era (purple), RimM (red), RimP (green) and a control experiment¹ (blue) are shown. Curves marked with asterisks are fit to a double exponential based on the results of a statistical f-test, using a 95% confidence interval. A discrepancy between the kinetic curves and the calculated first-order rates was observed for the binding of S13 with Era and RimP, and was due to imperfect fitting of biphasic kinetics with single exponential curves.

Reference

1. Bunner, A. E., Trauger, S. A., Siuzdak, G. & Williamson, J. R. (2008). Quantitative ESI-TOF Analysis of Macromolecular Assembly Kinetics. *Anal. Chem.* **80**, 9379-9386.