Electronic Supplementary Material (ESI) for Chemical Science.

Supplementary Information

Nascent chain dynamics and ribosome interactions within folded ribosome–nascent chain complexes observed by NMR spectroscopy

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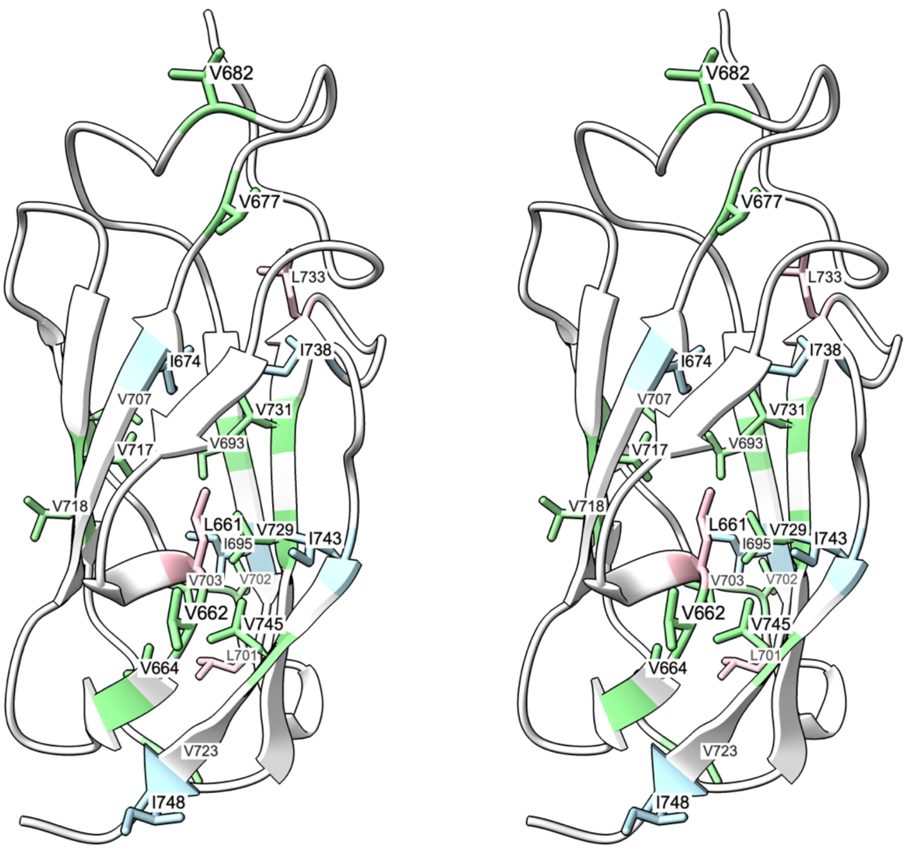
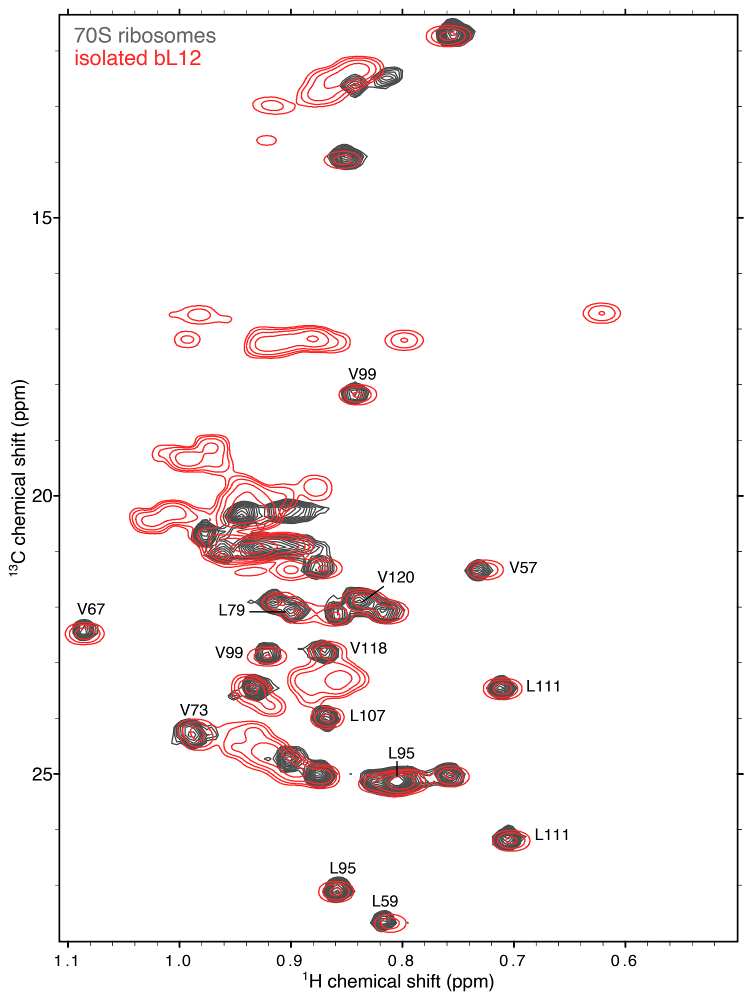
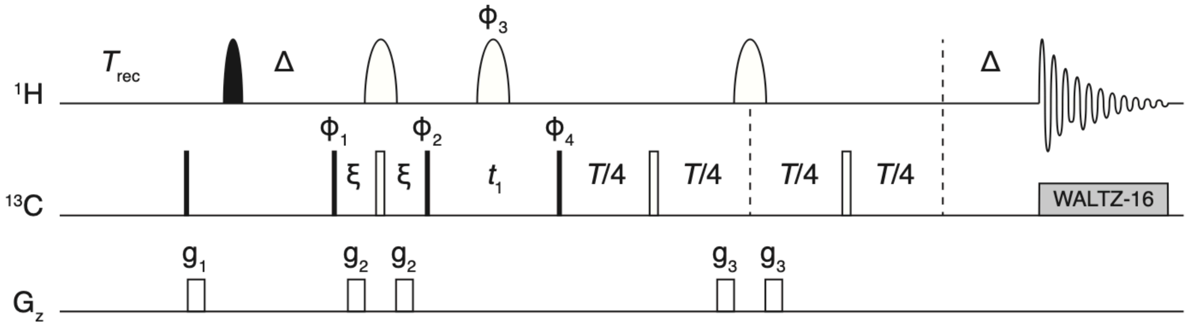
Table of Contents



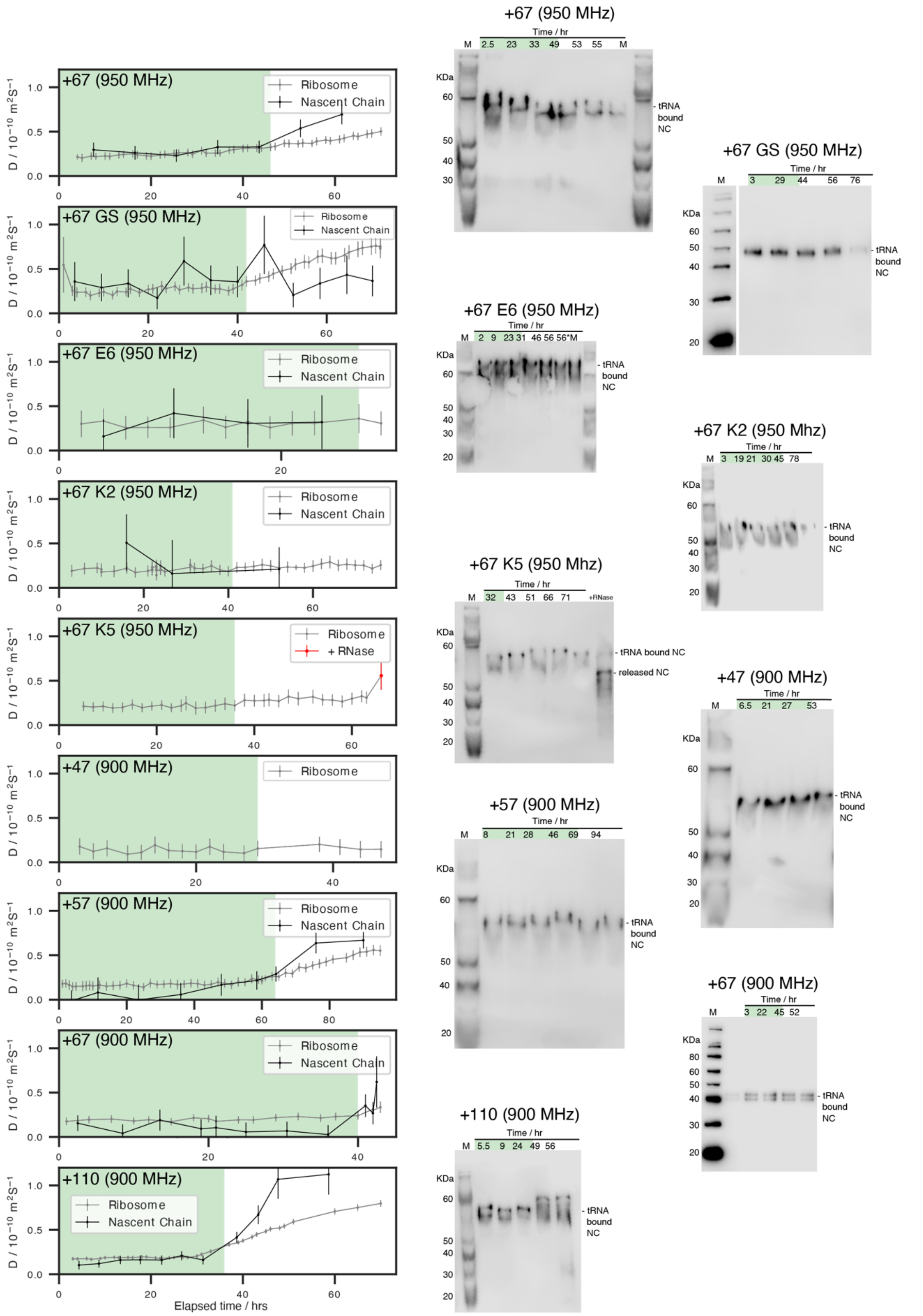
Supplementary Figure 1: Stereoview cartoon representation of the FLN5 crystal structure (pdb: 1qfh) showing the location of isoleucine, leucine and valine residues.



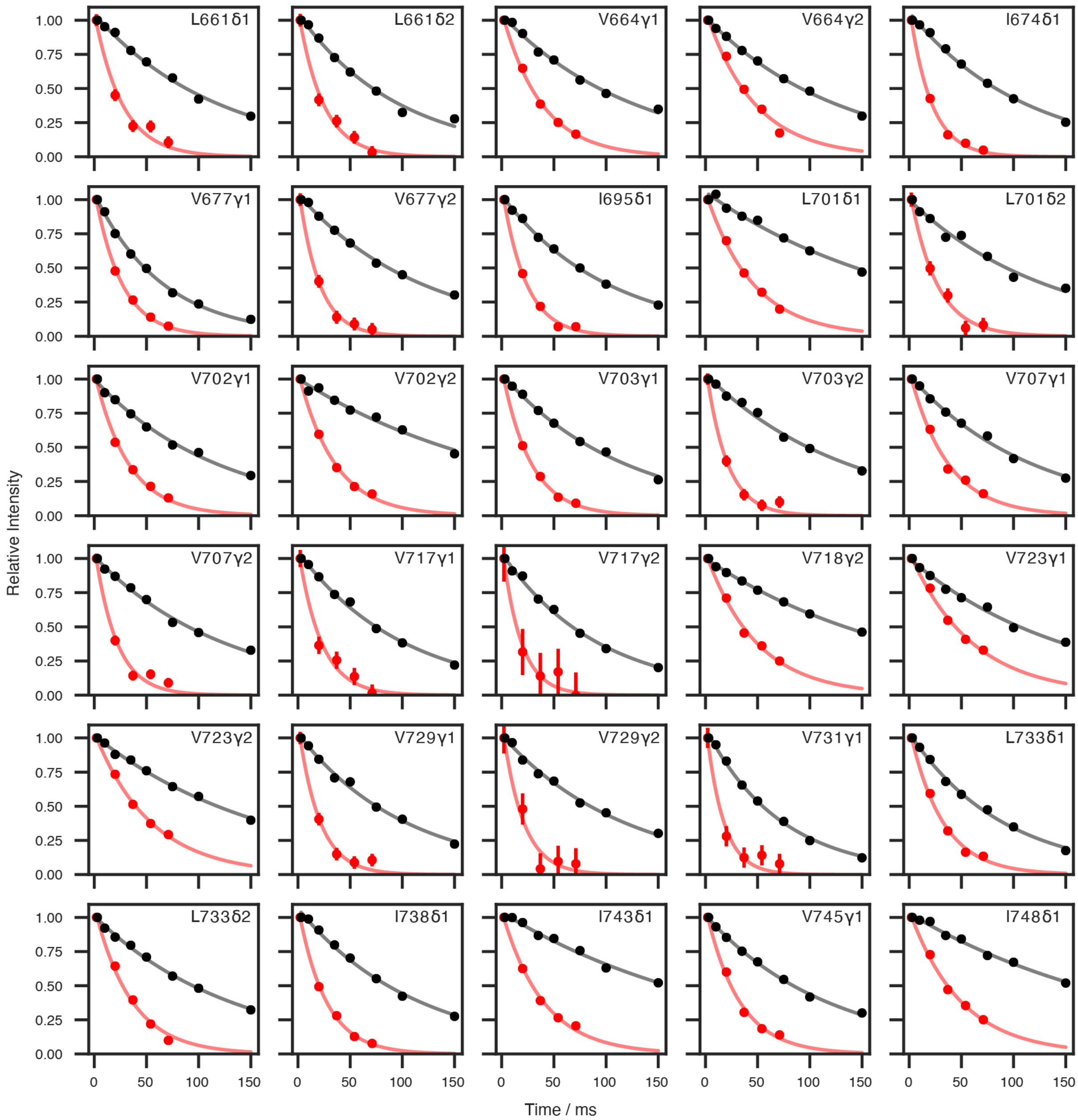
Supplementary Figure 2: ^{1}H,^{13}C HMQC spectra of ILV-labelled unoccupied 70S ribosomes (black, 298 K, 950 MHz) and unlabeled isolated bL12 (red, 298 K, 700 MHz).



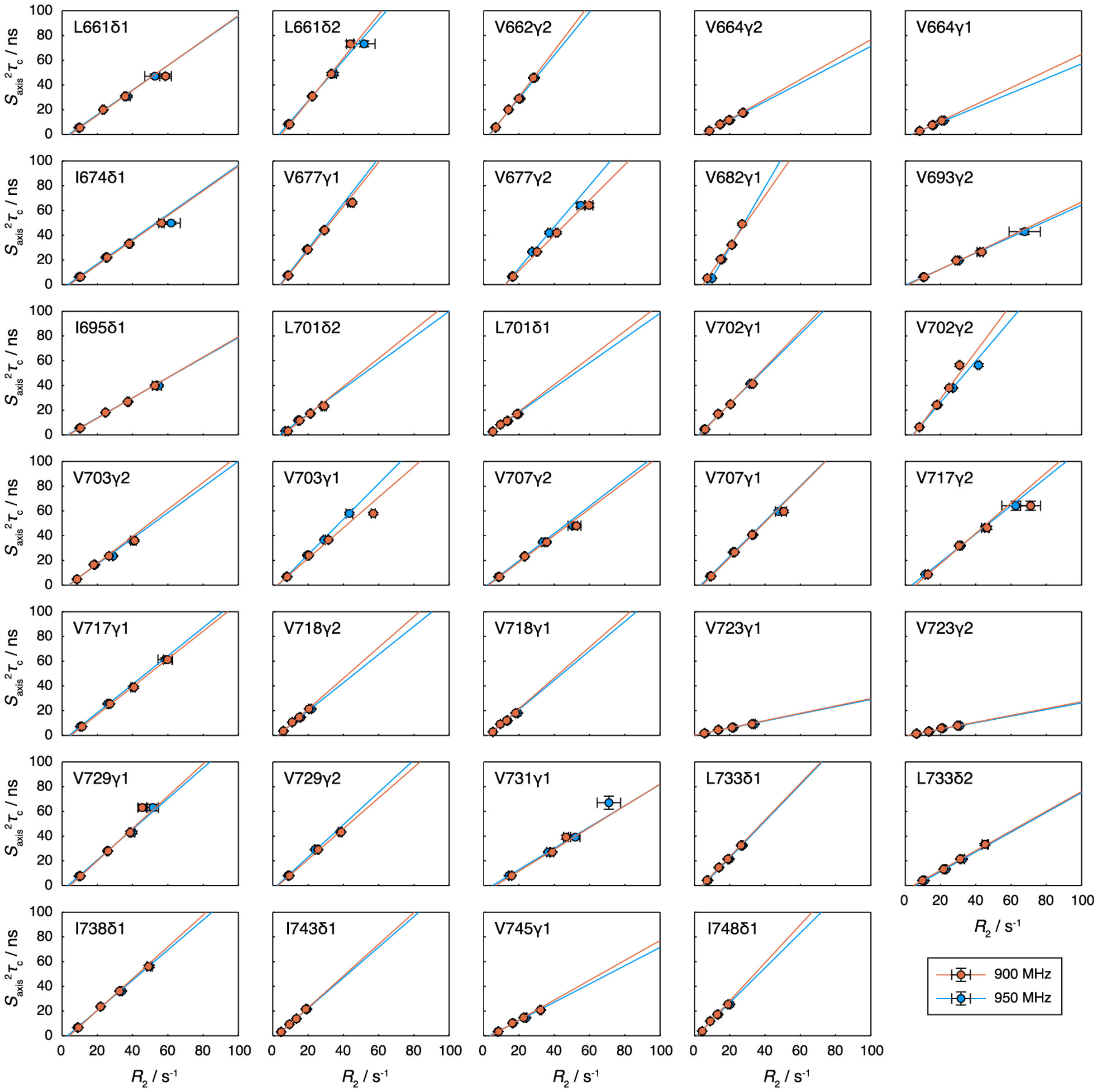
Supplementary Figure 3: Pulse program for the measurement of methyl ^{1}H R_2 relaxation rates, based on a methyl SOFAST-HMQC experiment incorporating a filter for slowly relaxing inner transitions. T is the relaxation time, $\Delta = 1/2J_{CH} = 4$ ms, and $\xi = 1/8J_{CH} = 1$ ms. ^{1}H shaped pulses were applied at 0.5 ppm; the solid pulse represents a 120º Pc9 excitation pulse (1120 µs at 950 MHz) and the hollow pulse represents a 180º Rsnob pulse (564 µs at 950 MHz). Phase cycles: ϕ_1 = 0°,180°; ϕ_2 = $(0°)_2$, $(180°)_2$; ϕ_3 = $(0°)_8$, $(120°)_8$, $(240°)_8$ ϕ_4 = $(0°)_4$, $(180°)_4$ and ϕ_{rx} = 0°, 180°, 0°, 180°, 180°, 0°, 180°, 0°, 120°, 300°, 120°, 300°, 300°, 120°, 300°, 120°, 240°, 60°, 240°, 60°, 60°, 240°, 60°, 240°, with ϕ_4 incremented for States-TPPI quadrature detection. Gradient length, shape and power are: g_1 = 1 ms (SMSQ10.100, 31%); g_2 = 50 µs (SINE.10, –40%); g_3 = 100 µs (SINE.10, 11%).



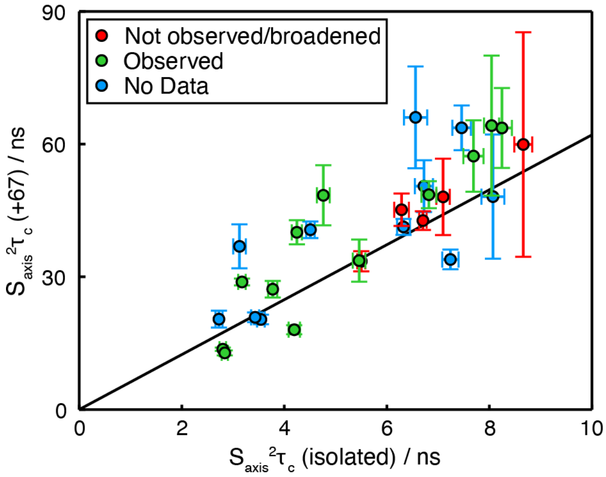
Supplementary Figure 4: Time courses for quality control of RNC samples. (**A**) Time course of nascent chain (I674, I738, I695 methyl resonances) and bL12 diffusion coefficients during data acquisition, shaded green area indicates measurement time used for analysis (**B**) Time course of nascent chain attachment by western blot (anti-histidine), using 2 pmol RNC aliquots taken periodically from a sample incubated in parallel with NMR data acquisition. The tRNA-bound nascent chain and released NC is indicated.



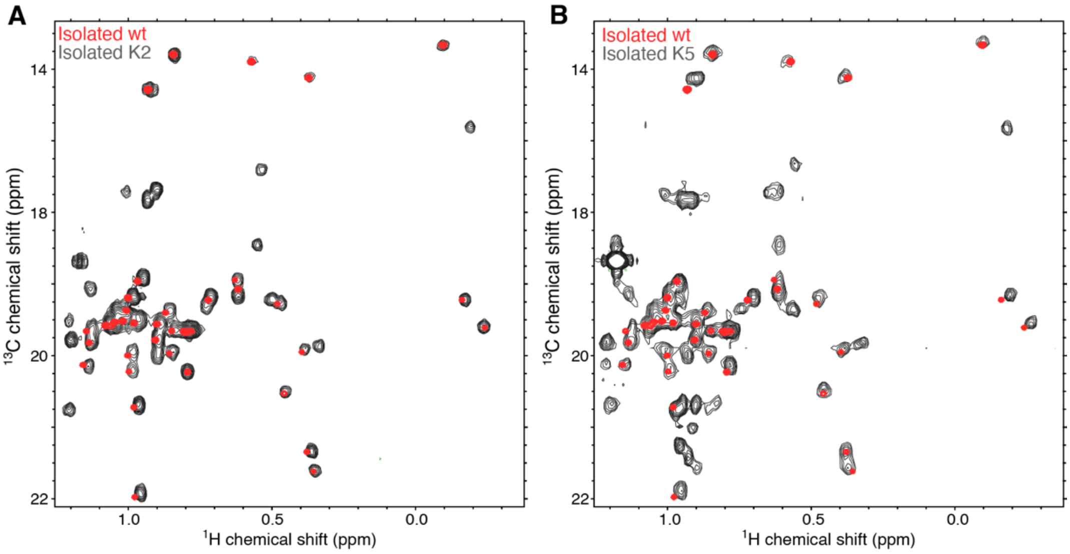
Supplementary Figure 5: Methyl ^{1}H R_2 measurements for isolated FLN5 (black) and FLN5+67 RNC (red) (298 K, 950 MHz).



Supplementary Figure 6: Correlation between [1]H R2 rates and $S^2_{axis}\tau_c$ values for isoleucine, leucine and valine methyls in isolated FLN5 at varying concentrations of d_8-glycerol at 298 K, with [1]H R2 rates acquired at 900 MHz (orange) or 950 MHz (blue).



Supplementary Figure 7: Correlation between $S_{axis}^2\tau_c$ values measured for isolated FLN5, and $S_{axis}^2\tau_c$ values determined from ^1H R_2 rates for the FLN5+67 RNC as shown in Fig. 2D, colored by observability of resonances previously reported for a uniformly ^1H,^{13}C-labelled FLN5 RNC[1].



Supplementary Figure 8: Comparison of $^{1}H,^{13}C$ HMQC spectra of ILV-labelled FLN5 (298 K, 950 MHz) with isolated unlabeled (**A**) K2 FLN and (**B**) K5 FLN (298 K, 500 MHz).

Construct	Sequence
+47	MHHHHHHASKPAPSAEHSYAEGEGLVKVFDNAPAEFTIFAVDTKGVARTDGGDPFEVAINGPDGLVVDAKVTDNNDGTYG VVYDAPVEGNYNVNVTLRGNPIKNMPIDVKCIEGANGEDSSFGSFTFTVAAKNKKGEVKTYGELFSTPVWIWWWPRIRGPP
+57	MHHHHHHASKPAPSAEHSYAEGEGLVKVFDNAPAEFTIFAVDTKGVARTDGGDPFEVAINGPDGLVVDAKVTDNNDGTYG VVYDAPVEGNYNVNVTLRGNPIKNMPIDVKCIEGANGEDSSFGSFTFTVAAKNKKGEVKTYGGDKFEVSITGELFSTPVW IWWWPRIRGPP
+67	MHHHHHHASKPAPSAEHSYAEGEGLVKVFDNAPAEFTIFAVDTKGVARTDGGDPFEVAINGPDGLVVDAKVTDNNDGTYG VVYDAPVEGNYNVNVTLRGNPIKNMPIDVKCIEGANGEDSSFGSFTFTVAAKNKKGEVKTYGGDKFEVSITGPAEEITLD AIELFSTPVWIWWWPRIRGPP
+110	MHHHHHHASKPAPSAEHSYAEGEGLVKVFDNAPAEFTIFAVDTKGVARTDGGDPFEVAINGPDGLVVDAKVTDNNDGTYG VVYDAPVEGNYNVNVTLRGNPIKNMPIDVKCIEGANGEDSSFGSFTFTVAAKNKKGEVKTYGGDKFEVSITGPAEEITLD AIDNQDGTYTAAYSLVGNGRFSTGVKLNGKHIEGSPFKQVLGNTSEFFSTPVWIWWWPRIRGPP
+67 GS	MHHHHHHASKPAPSAEHSYAEGEGLVKVFDNAPAEFTIFAVDTKGVARTDGGDPFEVAINGPDGLVVDAKVTDNNDGTYG VVYDAPVEGNYNVNVTLRGNPIKNMPIDVKCIEGGGGSGGGGSGGSGGGSGGGGSGGGSGGSGGGSGGGGSGGGSGGGSG GGELFSTPVWIWWWPRIRGPP
+67 K2	MHHHHHHASKPAPSAEHSYAKGKGLVKVFDNAPAEFTIFAVDTKGVARTDGGDPFEVAINGPDGLVVDAKVTDNNDGTYG VVYDAPVEGNYNVNVTLRGNPIKNMPIDVKCIEGANGEDSSFGSFTFTVAAKNKKGEVKTYGGDKFEVSITGPAEEITLD AIELFSTPVWIWWWPRIRGPP
+67 K5	MHHHHHHASKPAPSAEHSYAKGKGLVKVFDNAPAKFKIFAVDTKGVARTDGGDPFEVAINGPDGLVVKAKVTDNNDGTYG VVYDAPVEGNYNVNVTLRGNPIKNMPIDVKCIEGANGEDSSFGSFTFTVAAKNKKGEVKTYGGDKFEVSITGPAEEITLD AIELFSTPVWIWWWPRIRGPP
+67 E6	MHHHHHHASKPAPSAEHSYAEGEGLVKVFDNAPAEFTIFAVDTKGVARTDGGDPFEVAINGPDGLVVDAKVTDNNDGTYG VVYDAPVEGNYNVEVTLEGEPIENMPIEVECIEGANGEDSSFGSFTFTVAAKNKKGEVKTYGGDKFEVSITGPAEEITLD AIELFSTPVWIWWWPRIRGPP

Supplementary Table 1: Protein sequences for nascent chains used in this study.

Methyl	0% 950 MHz	0% 900 MHz	40% 950 MHz	40% 900 MHz	50% 950 MHz	50% 900 MHz	60% 950 MHz	60% 900 MHz
L661δ1	8.6 ± 0.1	9.5 ± 0.1	22.2 ± 0.3	22.4 ± 0.4	34 ± 1	33.1 ± 0.5	51 ± 6	43 ± 2
L661δ2	9.3 ± 0.1	10.2 ± 0.1	23.5 ± 0.5	23.3 ± 0.6	37 ± 2	35.8 ± 0.7	52 ± 6	58 ± 3
V662γ2	6.59 ± 0.07	6.94 ± 0.06	14.27 ± 0.08	13.9 ± 0.1	20.8 ± 0.1	19.89 ± 0.06	29.0 ± 0.3	28.1 ± 0.3
V664γ1	8.24 ± 0.06	8.53 ± 0.06	14.84 ± 0.09	14.4 ± 0.1	20.3 ± 0.1	19.42 ± 0.06	27.9 ± 0.2	27.3 ± 0.3
V664γ2	8.03 ± 0.08	8.24 ± 0.07	16.1 ± 0.1	15.3 ± 0.1	22.1 ± 0.2	20.62 ± 0.09	-	-
I674δ1	9.52 ± 0.06	10.57 ± 0.07	24.7 ± 0.2	25.6 ± 0.3	38.3 ± 0.9	38.0 ± 0.4	61 ± 5	56 ± 2
V677γ1	16.1 ± 0.1	16.8 ± 0.2	27.5 ± 0.4	30.3 ± 0.5	37.2 ± 0.8	41.5 ± 0.6	54 ± 2	59 ± 2
V677γ2	8.28 ± 0.08	8.84 ± 0.07	19.2 ± 0.1	19.9 ± 0.2	29.0 ± 0.4	29.4 ± 0.2	43 ± 1	45 ± 1
V682γ1	10.0 ± 0.1	7.22 ± 0.06	15.66 ± 0.08	14.8 ± 0.1	21.4 ± 0.1	21.05 ± 0.06	-	27.0 ± 0.2
V693γ2	10.1 ± 0.1	10.8 ± 0.1	30.4 ± 0.6	28.9 ± 0.6	42 ± 2	43 ± 1	67 ± 9	-
I695δ1	9.83 ± 0.07	10.39 ± 0.06	24.6 ± 0.2	24.6 ± 0.3	37.6 ± 0.7	37.2 ± 0.3	54 ± 1	52 ± 1
L701δ1	5.09 ± 0.09	5.22 ± 0.05	9.49 ± 0.07	9.5 ± 0.07	13.9 ± 0.1	13.19 ± 0.04	19.8 ± 0.2	18.9 ± 0.2
L701δ2	7.1 ± 0.2	8.6 ± 0.2	14.3 ± 0.3	15.2 ± 0.3	21.4 ± 0.5	21.2 ± 0.2	28 ± 1	29 ± 1
V702γ1	8.13 ± 0.06	7.93 ± 0.06	18.4 ± 0.1	17.8 ± 0.2	27.1 ± 0.3	24.9 ± 0.1	41.5 ± 0.7	30.8 ± 0.3
V702γ2	5.54 ± 0.07	6.16 ± 0.05	13.77 ± 0.09	13.28 ± 0.09	20.5 ± 0.1	20.47 ± 0.07	31.7 ± 0.5	33.1 ± 0.4
V703γ1	8.34 ± 0.08	8.53 ± 0.07	18.7 ± 0.1	17.9 ± 0.2	28.9 ± 0.4	26.6 ± 0.2	39 ± 1	41.1 ± 0.9
V703γ2	7.66 ± 0.09	8.2 ± 0.08	19.4 ± 0.2	20.4 ± 0.3	29.2 ± 0.6	31.5 ± 0.3	43 ± 2	56 ± 1
V707γ1	8.79 ± 0.07	9.56 ± 0.08	21.8 ± 0.2	22.8 ± 0.3	33.3 ± 0.5	32.6 ± 0.3	47 ± 2	50 ± 1
V707γ2	8.23 ± 0.08	8.99 ± 0.09	23.1 ± 0.3	23.3 ± 0.4	33.5 ± 0.8	35.8 ± 0.5	50 ± 3	52 ± 2
V717γ1	10.22 ± 0.08	11.4 ± 0.1	25.8 ± 0.4	27.2 ± 0.4	40 ± 1	40.8 ± 0.6	58 ± 4	59 ± 2
V717γ2	11.3 ± 0.1	12.9 ± 0.1	31.5 ± 0.6	30.4 ± 0.7	45 ± 2	46 ± 1	62 ± 8	71 ± 6
V718γ1	6.05 ± 0.06	5.94 ± 0.04	11.06 ± 0.05	10.91 ± 0.05	15.9 ± 0.09	14.93 ± 0.03	21.9 ± 0.1	20.4 ± 0.2
V718γ2	5.34 ± 0.06	5.13 ± 0.03	9.44 ± 0.04	9.41 ± 0.04	13.67 ± 0.08	12.96 ± 0.02	19.4 ± 0.2	18.1 ± 0.1
V723γ1	6.67 ± 0.06	6.15 ± 0.04	13.8 ± 0.07	13.13 ± 0.07	21.4 ± 0.1	20.33 ± 0.06	31.0 ± 0.3	29.6 ± 0.3
V723γ2	6.06 ± 0.06	5.49 ± 0.03	13.8 ± 0.08	13.35 ± 0.08	22.3 ± 0.2	21.24 ± 0.07	34.1 ± 0.5	32.6 ± 0.4
V729γ1	9.51 ± 0.09	10.5 ± 0.1	25.6 ± 0.4	26.1 ± 0.5	39 ± 1	38.6 ± 0.6	51 ± 3	45 ± 2
V729γ2	8.52 ± 0.09	9.4 ± 0.1	23.9 ± 0.3	25.7 ± 0.5	38 ± 1	38.7 ± 0.6	-	-
V731γ1	14.3 ± 0.1	15.9 ± 0.1	36.5 ± 0.9	38.9 ± 0.9	51 ± 3	46 ± 1	71 ± 7	-
L733δ1	11.0 ± 0.2	9.7 ± 0.1	23.1 ± 0.4	21.7 ± 0.4	32.6 ± 0.9	31.1 ± 0.4	45 ± 1	45 ± 2
L733δ2	7.77 ± 0.09	7.05 ± 0.08	14.0 ± 0.1	13.8 ± 0.1	19.8 ± 0.2	18.86 ± 0.07	27.4 ± 0.3	26.4 ± 0.4
I738δ1	8.63 ± 0.05	9.3 ± 0.06	21.9 ± 0.1	21.8 ± 0.2	33.9 ± 0.4	32.6 ± 0.2	49 ± 1	49 ± 1
I743δ1	4.6 ± 0.05	4.73 ± 0.03	9.38 ± 0.05	9.4 ± 0.04	13.45 ± 0.09	13.31 ± 0.03	19.4 ± 0.2	18.6 ± 0.1
V745γ1	8.0 ± 0.06	8.46 ± 0.06	16.8 ± 0.1	16.2 ± 0.1	24.1 ± 0.3	22.65 ± 0.09	32.4 ± 0.4	32.2 ± 0.5
I748δ1	4.36 ± 0.05	4.43 ± 0.03	8.85 ± 0.03	8.94 ± 0.04	13.55 ± 0.07	12.9 ± 0.02	20.3 ± 0.1	19.0 ± 0.1

Supplementary Table 2: Methyl ^1H R_2 for isolated FLN5 at glycerol concentrations of 0, 40, 50 and 60 % (w/w), acquired at 900 and 950 MHz, 298 K.

Methyl	0%	40%	50%	60%
L661δ1	8.2 ± 0.2	30.9 ± 0.4	48 ± 1	73 ± 2
L661δ2	5.5 ± 0.1	20.0 ± 0.3	30.8 ± 0.6	47 ± 2
V662γ2	5.73 ± 0.09	20.0 ± 0.2	28.9 ± 0.3	45.8 ± 0.5
V664γ1	2.84 ± 0.06	8.2 ± 0.1	11.5 ± 0.1	17.5 ± 0.2
V664γ2	2.8 ± 0.06	7.52 ± 0.07	11.2 ± 0.2	19.2 ± 0.4
I674δ1	6.3 ± 0.1	22.1 ± 0.3	33.1 ± 0.9	49 ± 1
V677γ1	6.6 ± 0.2	26.6 ± 0.4	42.0 ± 0.8	64 ± 1
V677γ2	7.5 ± 0.2	28.5 ± 0.3	44.1 ± 0.6	66.3 ± 0.9
V682γ1	5.19 ± 0.08	20.5 ± 0.3	32.2 ± 0.4	49.0 ± 0.8
V693γ2	6.2 ± 0.1	19.5 ± 0.4	26.4 ± 0.8	42 ± 2
I695δ1	5.5 ± 0.08	18.1 ± 0.2	26.8 ± 0.3	39.8 ± 0.7
L701δ1	2.72 ± 0.07	8.34 ± 0.07	11.2 ± 0.1	16.9 ± 0.2
L701δ2	3.1 ± 0.1	11.7 ± 0.2	17.3 ± 0.4	23.2 ± 0.8
V702γ1	6.3 ± 0.1	24.2 ± 0.2	38.0 ± 0.4	56 ± 1
V702γ2	4.51 ± 0.08	16.9 ± 0.2	24.8 ± 0.3	41.3 ± 0.4
V703γ1	4.8 ± 0.1	16.6 ± 0.2	23.6 ± 0.4	36.0 ± 0.7
V703γ2	6.8 ± 0.1	24.0 ± 0.3	36.7 ± 0.7	58.0 ± 0.9
V707γ1	7.2 ± 0.2	26.6 ± 0.3	40.7 ± 0.4	59 ± 1
V707γ2	6.7 ± 0.2	23.3 ± 0.3	34.8 ± 0.6	47 ± 1
V717γ1	7.1 ± 0.1	25.5 ± 0.3	38.9 ± 0.8	61 ± 1
V717γ2	8.7 ± 0.2	31.9 ± 0.3	46 ± 1	64 ± 4
V718γ1	3.54 ± 0.07	10.6 ± 0.1	14.6 ± 0.2	21.4 ± 0.2
V718γ2	2.85 ± 0.04	9.12 ± 0.05	11.9 ± 0.1	18.0 ± 0.2
V723γ1	1.36 ± 0.03	3.16 ± 0.08	5.8 ± 0.3	7.8 ± 0.1
V723γ2	1.72 ± 0.03	4.47 ± 0.09	6.4 ± 0.2	9.1 ± 0.2
V729γ1	7.7 ± 0.2	28.0 ± 0.3	42 ± 1	63 ± 3
V729γ2	8.0 ± 0.1	29.1 ± 0.4	43.4 ± 0.6	63 ± 2
V731γ1	8.1 ± 0.2	27.0 ± 0.5	39 ± 2	67 ± 5
L733δ1	4.2 ± 0.1	13.2 ± 0.2	21.5 ± 0.4	33.4 ± 0.7
L733δ2	4.2 ± 0.1	14.7 ± 0.2	21.5 ± 0.3	32.5 ± 0.5
I738δ1	6.7 ± 0.07	23.6 ± 0.2	36.2 ± 0.3	56 ± 1
I743δ1	3.17 ± 0.07	9.31 ± 0.04	13.9 ± 0.1	21.6 ± 0.2
V745γ1	3.43 ± 0.08	10.3 ± 0.1	14.7 ± 0.2	20.9 ± 0.4
I748δ1	3.77 ± 0.09	11.85 ± 0.06	17.3 ± 0.1	25.4 ± 0.2

Supplementary Table 3: Methyl $S^2_{axis}\tau_c$ values for isolated FLN5 at glycerol concentrations of 0, 40, 50 and 60 % (w/w) recorded at 900 and 950 MHz, 298 K.

Methyl	^{1}H R_2 (s^{-1})							
	+67 950 MHz	+67 GS 950 MHz	+67 E6 950 MHz	+67 K2 950 MHz	+47 900 MHz	+57 900 MHz	+67 900 MHz	+110 900 MHz
L661δ1	37.7 ± 4.7	35.0 ± 11.0	35.0 ± 8.1	-	-	70.2 ± 9.9	50.0 ± 7.2	37.3 ± 6.1
L661δ2	42.3 ± 5.5	42.0 ± 11.0	49.0 ± 20.0	-	-	88.0 ± 18.0	41.7 ± 6.8	41.5 ± 7.1
V664γ1	26.4 ± 0.61	28.5 ± 2.3	28.0 ± 1.4	46.8 ± 6.5	46.6 ± 7.7	42.7 ± 2.3	27.2 ± 1.2	20.58 ± 0.61
V664γ2	21.54 ± 0.65	-	-	-	-	-	-	20.86 ± 0.69
I674δ1	48.4 ± 3.3	45.5 ± 8.9	40.1 ± 5.3	-	-	57.3 ± 6.2	51.9 ± 4.6	35.5 ± 2.1
V677γ1	38.9 ± 2.5	43.4 ± 8.9	38.7 ± 2.8	-	-	58.0 ± 5.3	41.0 ± 5.9	36.6 ± 3.8
V677γ2	51.6 ± 6.7	-	42.0 ± 13.0	-	-	-	-	42.7 ± 6.4
I695δ1	44.3 ± 2.5	55.7 ± 8.6	49.3 ± 6.2	146.0 ± 30.0	-	70.1 ± 6.1	45.7 ± 3.4	33.7 ± 1.8
L701δ1	22.26 ± 0.76	21.1 ± 1.8	24.1 ± 2.9	82.0 ± 13.0	-	34.4 ± 2.3	20.8 ± 1.4	15.93 ± 0.62
L701δ2	39.3 ± 4.1	43.0 ± 17.0	-	-	-	57.7 ± 9.9	37.1 ± 6.5	27.5 ± 2.2
V702γ1	31.0 ± 1.0	-	27.2 ± 1.2	-	39.8 ± 2.5	-	-	-
V702γ2	28.98 ± 0.74	29.3 ± 2.3	18.6 ± 1.5	84.0 ± 10.0	-	50.7 ± 1.7	37.73 ± 0.91	21.43 ± 0.83
V703γ1	36.7 ± 2.1	31.9 ± 5.3	31.3 ± 2.4	83.0 ± 20.0	-	52.6 ± 5.8	37.6 ± 3.4	29.1 ± 2.3
V703γ2	50.2 ± 5.7	49.0 ± 15.0	33.9 ± 3.2	-	-	70.8 ± 6.2	43.8 ± 8.7	31.1 ± 2.8
V707γ1	27.5 ± 1.5	27.4 ± 4.1	-	-	-	-	-	-
V707γ2	48.0 ± 5.2	35.4 ± 9.3	34.4 ± 3.7	-	-	-	-	-
V717γ1	45.9 ± 7.4	27.0 ± 18.0	-	-	-	-	-	42.1 ± 5.0
V717γ2	56.0 ± 22.0	-	-	-	-	-	-	65.0 ± 21.0
V718γ2	20.52 ± 0.32	-	18.9 ± 1.7	-	-	-	20.84 ± 0.83	15.13 ± 0.47
V723γ1	16.76 ± 0.26	19.43 ± 0.62	15.45 ± 0.73	44.6 ± 2.3	34.6 ± 2.4	30.03 ± 0.71	17.42 ± 0.55	13.92 ± 0.15
V723γ2	18.52 ± 0.33	20.77 ± 0.96	20.3 ± 1.0	37.8 ± 3.3	36.8 ± 3.8	32.6 ± 1.2	18.94 ± 0.67	14.27 ± 0.37
V729γ1	49.5 ± 6.4	-	52.0 ± 10.0	-	-	77.0 ± 12.0	44.2 ± 10.0	32.4 ± 4.2
V729γ2	51.0 ± 12.0	-	-	-	-	-	-	49.0 ± 10.0
V731γ1	61.0 ± 16.0	-	-	-	-	-	-	41.7 ± 6.9
L733δ1	31.9 ± 1.4	35.7 ± 7.3	30.7 ± 2.6	85.0 ± 14.0	39.0 ± 23.0	65.1 ± 4.5	32.9 ± 2.6	24.6 ± 1.7
L733δ2	28.4 ± 1.1	36.3 ± 8.9	24.4 ± 2.8	64.0 ± 29.0	-	50.7 ± 5.4	32.4 ± 2.3	21.8 ± 1.0
I738δ1	38.1 ± 1.5	46.2 ± 6.3	38.0 ± 2.4	-	-	63.7 ± 3.5	41.3 ± 2.1	35.0 ± 1.6
I743δ1	25.29 ± 0.42	32.0 ± 2.0	22.2 ± 1.0	63.9 ± 3.9	50.0 ± 14.0	44.1 ± 1.5	25.07 ± 0.2	16.96 ± 0.33
V745γ1	31.5 ± 1.2	29.9 ± 4.2	31.8 ± 2.0	47.3 ± 9.8	-	45.5 ± 4.1	29.7 ± 1.8	22.6 ± 0.91
I748δ1	20.32 ± 0.31	21.06 ± 0.79	22.9 ± 1.1	81.0 ± 18.0	33.1 ± 4.2	37.1 ± 1.4	21.06 ± 0.64	14.77 ± 0.33

Supplementary Table 4: Methyl ^{1}H R_2 measurements for RNCs.

Methyl	$S^2_{axis}\ \tau_c$ (ns)							
	+67 950 MHz	+67 GS 950 MHz	+67 E6 950 MHz	+67 K2 950 MHz	+47 900 MHz	+57 900 MHz	+67 900 MHz	+110 900 MHz
L661δ1	33.7 ± 4.8	31.0 ± 11.0	31.0 ± 8.1	-	-	66.0 ± 11.0	45.7 ± 7.6	32.9 ± 6.3
L661δ2	63.7 ± 9.0	63.0 ± 18.0	74.0 ± 33.0	-	-	147.0 ± 33.0	65.0 ± 12.0	64.0 ± 13.0
V664γ1	13.63 ± 0.38	14.9 ± 1.4	14.59 ± 0.82	25.7 ± 3.8	28.7 ± 5.2	26.0 ± 1.6	15.62 ± 0.84	11.12 ± 0.41
V664γ2	12.8 ± 0.56	-	-	-	-	-	-	12.85 ± 0.66
I674δ1	45.2 ± 3.7	42.3 ± 9.0	36.9 ± 5.5	-	-	53.1 ± 6.4	47.7 ± 4.8	31.3 ± 2.2
V677γ1	63.7 ± 5.1	72.0 ± 17.0	63.3 ± 5.6	-	-	95.0 ± 10.0	65.0 ± 11.0	57.2 ± 7.1
V677γ2	66.0 ± 11.0	-	51.0 ± 21.0	-	-	-	-	43.6 ± 9.2
I695δ1	33.5 ± 2.3	42.7 ± 7.1	37.6 ± 5.2	116.0 ± 25.0	-	54.7 ± 5.3	34.7 ± 3.0	24.7 ± 1.6
L701δ1	20.4 ± 1.9	19.2 ± 2.4	22.3 ± 3.5	80.0 ± 16.0	-	34.5 ± 3.6	19.9 ± 2.0	14.6 ± 1.0
L701δ2	36.9 ± 5.0	40.0 ± 18.0	-	-	-	59.0 ± 12.0	35.8 ± 7.7	24.9 ± 2.9
V702γ1	40.7 ± 1.9	-	35.3 ± 2.0	-	54.2 ± 5.3	-	-	-
V702γ2	41.3 ± 1.8	41.8 ± 4.0	23.9 ± 2.6	133.0 ± 18.0	-	87.6 ± 5.0	62.9 ± 3.1	31.9 ± 1.9
V703γ1	48.6 ± 3.1	41.7 ± 7.7	40.8 ± 3.4	115.0 ± 28.0	-	62.0 ± 8.6	43.4 ± 5.2	32.9 ± 3.6
V703γ2	48.4 ± 6.8	47.0 ± 16.0	31.5 ± 3.8	-	-	73.0 ± 8.8	43.0 ± 10.0	29.7 ± 3.6
V707γ1	33.9 ± 2.2	33.8 ± 5.9	-	-	-	-	-	-
V707γ2	50.5 ± 5.9	37.0 ± 10.0	35.6 ± 4.1	-	-	-	-	-
V717γ1	48.1 ± 8.6	26.0 ± 21.0	-	-	-	-	-	41.7 ± 5.6
V717γ2	60.0 ± 25.0	-	-	-	-	-	-	72.0 ± 26.0
V718γ2	20.4 ± 1.1	-	18.6 ± 2.2	-	-	-	22.3 ± 1.3	15.16 ± 0.74
V723γ1	4.87 ± 0.29	5.64 ± 0.39	4.49 ± 0.33	12.9 ± 1.2	10.37 ± 0.95	9.02 ± 0.58	5.29 ± 0.31	4.25 ± 0.2
V723γ2	4.5 ± 0.13	5.1 ± 0.28	4.99 ± 0.3	9.64 ± 0.91	9.8 ± 1.1	8.64 ± 0.41	4.87 ± 0.22	3.58 ± 0.13
V729γ1	57.3 ± 8.1	-	61.0 ± 13.0	-	-	94.0 ± 16.0	52.0 ± 13.0	36.2 ± 5.6
V729γ2	64.0 ± 16.0	-	-	-	-	-	-	58.0 ± 13.0
V731γ1	48.0 ± 14.0	-	-	-	-	-	-	30.7 ± 6.4
L733δ1	40.1 ± 2.7	46.0 ± 11.0	38.3 ± 4.2	118.0 ± 22.0	51.0 ± 34.0	90.2 ± 6.9	42.5 ± 3.9	30.3 ± 2.6
L733δ2	18.0 ± 1.0	24.3 ± 7.2	14.8 ± 2.3	46.0 ± 23.0	-	36.8 ± 4.4	22.3 ± 1.9	13.76 ± 0.83
I738δ1	42.7 ± 2.1	52.6 ± 7.8	42.6 ± 3.1	-	-	76.7 ± 4.8	47.9 ± 2.9	39.8 ± 2.2
I743δ1	28.84 ± 0.77	37.1 ± 2.6	25.1 ± 1.3	76.5 ± 5.1	62.0 ± 18.0	53.7 ± 2.3	29.28 ± 0.7	18.88 ± 0.54
V745γ1	20.8 ± 1.1	19.7 ± 3.2	21.1 ± 1.6	32.6 ± 7.3	-	33.3 ± 3.6	20.6 ± 1.7	14.89 ± 0.91
I748δ1	27.2 ± 1.9	28.2 ± 2.2	30.9 ± 2.7	112.0 ± 27.0	48.4 ± 6.9	54.5 ± 3.5	29.9 ± 1.6	20.19 ± 0.85

Supplementary Table 5: Calculated methyl $S^2_{axis}\tau_c$ values for RNCs.

References

1. Hsu, S.-T. D., Cabrita, L. D., Fucini, P., Christodoulou, J. & Dobson, C. M. Probing side-chain dynamics of a ribosome-bound nascent chain using methyl NMR spectroscopy. *J. Am. Chem. Soc.* **131**, 8366–8367 (2009).