Probing side-chain dynamics of a ribosome-bound nascent chain using methyl NMR spectroscopy

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Material and Methods

The Ig_2-RNC samples (70S ribosome concentrations of 10 μM as determined by the absorbance at 260 nm; 1 A_{260} = 24 picomole) were prepared in binding buffer (10 mM HEPES, pH 7.6, 140 mM NH_4Cl. 6 mM $MgCl_2$, 0.05 mM spermine, 2 mM spermidine and 2 mM DTT) as described previously[1]. NMR data were recorded using Bruker 700MHz and 900MHz spectrometers, both equipped with triple resonance cryogenic probeheads. 10% D_2O was present in all NMR samples as a lock solvent. All NMR data were acquired at 283 K with a variable temperature control unit to maintain the sample temperature. The acquisition parameters for 1H-X SOFAST-HMQC[2] are detailed in Table S1. All spectra were processed using NMR Pipe[3] and analyzed using Sparky (http://www.cgl.ucsf.edu/home/sparky).

References

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Table S1. Data acquisition parameters for ^{1}H-X SOFAST-HMQC spectroscopy

X-nucleus	Spectral width (Hz)		X-nucleus carrier frequency (Hz)	Relaxation delay (ms)	^{1}H excitation shape pulse (PC9)[1]		^{1}H inversion shape pulse (R-SNOB)		Number of complex points		Number of transients per increment	Total experimental time (hours)
	F2 (^{1}H)	F1 (X)			Pulse length (μs)	Offset (Hz)	Pulse length (μs)	Offset (Hz)	F2 (^{1}H)	F1 (X)		
^{15}N (amide)	10776	2918	10762	300	1900	3300	650	3300	1024	32	1024	7.5
^{13}C (methyl)	12626	4979	3961	400	2200	-4250	480	-4250	1024	16	800	3.5
^{13}C (aromatic)[2]	8091	5659	29423	400	2200	2700	650	2700	800	12	512	

[1] The total flip angle of the ^{1}H excitation pulse (PC9) was optimized to be 120º using an isolated ddFLN5 sample. The setting has been used throughout the whole measurements as the limited sample concentrations and life -time of the RNC samples prohibited detailed optimization procedures.

[2] Only applied to [13C/15N-A/F]-70S ribosome

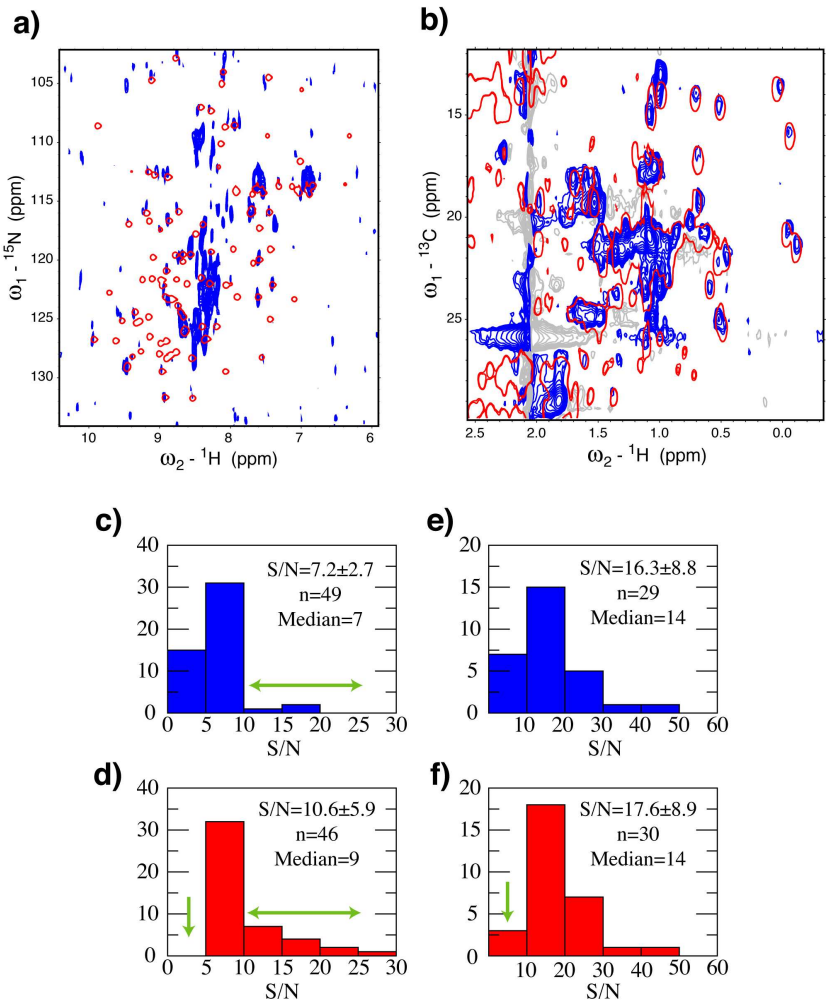

Figure S1. Comparison of ribosome-bound nascent chain spectra at the backbone and side-chain levels. Overlaid ^{1}H-^{15}N correlation spectra of the backbone amide region (**a**) and ^{1}H-^{13}C correlation spectra in the methyl region (**b**) of ddFLN5 as a ribosome-bound nascent chain (blue) and in isolation (red). Histograms of the signal-to-noise ratio (S/N) of resolve amide cross-peaks in the ^{1}H-^{15}N correlation spectra of ddFLN5 as a ribosome-bound nascent chain (**c**) and released by incubating the RNCs with puromycin (**d**). Histograms of the S/N of resolved side-chain methyl cross-peaks in the ^{1}H-^{13}C correlation spectra of ddFLN5 as a ribosome-bound nascent chain (**e**) and released by incubating the RNCs with puromycin (**f**). Changes in the distributions of S/N ratio are highlighted by green arrows.

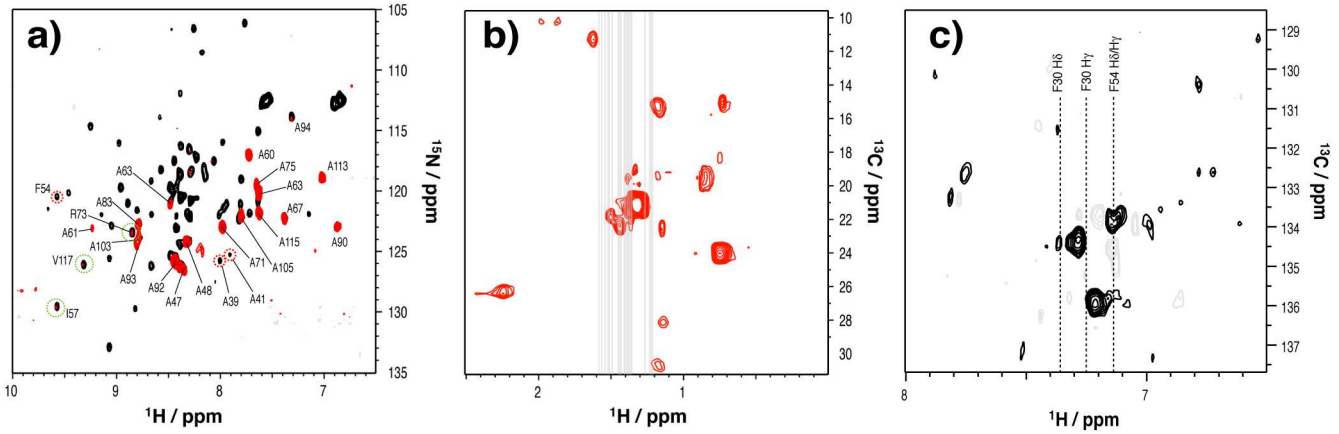

Figure S2. Selective stable isotope labeling of intact ribosomes for NMR analysis. (**a**) Overlaid ^{1}H-^{15}N SOFAST-HMQC spectra of the 70S ribosome from *E. coli* with uniform ^{15}N labeling (black) and selective ^{13}C and ^{15}N labeling for alanine and phenylalanine residues, designated as [^{13}C/^{15}N-A/F]-70S (red). The resolved crosspeaks correspond to the C-terminal domain of the ribosomal protein L7/L12 (residues 40-120, designated as L7/L12-CTD). All 19 expected alanine resonances from the L7/L12-CTD (an additional 9 alanines are present in the N-terminal domain of L7L12, L7/L12-NTD but are invisible due to the direct attachment of the methyl to the ribosome) have been identified in the uniformly labeled sample while those of A39 and A41 are missing in the selectively labeled spectrum (indicated in dashed red circles). Similarly, the crosspeak of F54 is present in the uniformly ^{15}N labeled spectrum but missing in the selectively labelled one. The other phenylalanine residue, F30, is located in the linker region, which has not been identified in the spectrum. Detailed examination reveals that a supplement of ^{13}C/^{15}N labeled amino acids during ribosome biogenesis can lead to very limited amino acid scrambling, leading to the presence of weak ^{15}N resonances for I57, R73 and V117 in the selectively labeled spectrum (indicated in green circles). (**b**) ^{1}H-^{13}C SOFAST-HMQC spectrum of [^{13}C/^{15}N-A/F]-70S with selective excitation proton resonances in the methyl region. The same sample was used to record the ^{1}H-^{15}N correlations as shown in (**a**). The ^{1}H resonances of 28 alanine side-chain Hβ of L7/L12 have been previously assigned using ^{15}N-labeled L7/L12 in isolation, as indicated by grey lines (BMRB entry 4429); assignments of the corresponding ^{13}C resonances, however, are currently unavailable. Assuming that the structure of the L7/L12-CTD is not affected by the attachment to the ribosome and that minimal amino acid scrambling is present in this NMR sample, the additional crosspeaks with much large chemical shift dispersions along both the ^{1}H and ^{13}C dimensions may well correspond to alanine methyl groups in other regions of the 70S ribosome. Methyl NMR in combination with selective labeling therefore affords additional information that cannot be probed at the backbone level. (**b**) ^{1}H-^{13}C SOFAST-HMQC spectrum of [^{13}C/^{15}N-A/F]-70S with selective excitation proton resonances in the aromatic region. Three well-resolved crosspeaks are present with ^{1}H chemical shifts that are similar, but not identical, to the previously assigned values (indicated by dashed line with corresponding assignments). Assuming that the observed crosspeaks correspond to F30 and F54 of L7/L12 as expected from the highly dynamic nature of this part of the ribosome, the results suggest that the chemical shifts of aromatic side-chains are more sensitive to the attachment to the ribosome, in comparison to the corresponding backbone amide ^{1}H-^{15}N correlations (**a**) and alanine methyl ^{1}H-^{13}C correlations (**b**).