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# Letter to the Editor: NMR assignment of the full-length ribosomal protein L11 from *Thermotoga maritima*

Sergey Ilin[a,b], Aaron Hoskins[b], Harald Schwalbe[a,*] & Jens Wöhnert[a,*]
[a]*Institut für Organische Chemie, Johann Wolfgang Goethe-Universität, Marie-Curie-Strasse 11, D-60439 Frankfurt am Main, Germany;* [b]*Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, U.S.A.*

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## Biological context

Interactions of RNAs with proteins are often characterized by an 'induced fit' conformational adjustment of both the protein and the RNA with respect to their conformation in the unbound state (Leulliot and Varani, 2001). Furthermore, changes in the conformational dynamics are thought to play an important role in the binding process. Such dynamics are important for the function of the ribosomal protein L11 which is a strongly conserved component of the large ribosomal subunit. RNA binding of the C-terminal domain of L11 stabilizes the tertiary structure of a compactly folded RNA domain. The N-terminus is implicated in the binding of the antibiotic thiostrepton (Xing and Draper, 1996). The conformation of RNA-bound L11 and the RNA-protein-complex is structurally well characterized (Wimberly et al., 1999; Conn et al., 1999). In addition, the conformation of the C-terminal domain of L11 from *Bacillus stearothermophilus* has been characterized in its RNA bound and free form by NMR (Hinck et al., 1997; Markus et al., 1997). However, to obtain a more detailed picture of the dynamic processes accompanying RNA-protein interactions it would be interesting to characterize changes in the conformation and the dynamics of the full-length protein between its bound and free states in detail. To this end, information is lacking about the conformation of the full-length protein in the unbound state and the changes in the conformational dynamics during the binding process. Here, we report a nearly complete resonance assignment for the full-length L11 from *Thermotoga maritima* in its free form.

*To whom correspondence should be addressed. E-mail: schwalbe@nmr.uni-frankfurt.de; jewoe@nmr.uni-frankfurt.de

## Methods and experiments

The gene coding for L11 from *Thermotoga maritima* was cloned by PCR using *T. maritima* genomic DNA as a template. It was inserted into the pET11a-overexpression vector and overexpressed in $^{15}$N- or $^{15}$N/$^{13}$C-labeled form in *E. coli* BL21(DE3) growing in M9-minimal medium containing only $^{15}$N-labeled $NH_4Cl$ and $^{13}$C-labeled glucose as the sole nitrogen and carbon source. The purification of the protein was achieved by taking advantage of the thermostability of the protein and consisted of a heat denaturation step followed by a cation-exchange-chromatography step on a SP-Sepharose-column (Pharmacia). Protein purified by this procedure was >95% pure and showed excellent solubility and long term-stability. Samples for NMR-spectroscopy contained ~1.2 mM protein in 20 mM $KHPO_4$, pH 6.2, 50 mM KCl and 5% $^2H_2O$.

Spectra were acquired at 25 °C on Bruker DRX600 and DRX700 and Varian $^{UNITY}$*INOVA* 600 and 750 spectrometers equipped with z-axis gradient $^1$H{$^{13}$C,$^{15}$N} triple resonance probes. Spectra were processed with XWINNMR 2.1 (Bruker) or VNMR (Varian) and analyzed with XEASY (Bartels et al., 1995). Sequential backbone resonance assignments were obtained with HNCACB, CBCA(CO)NH and HNCO experiments. Side chain assignments were based on HCC(CO)NH, CC(CO)NH, HBHA(CO)NH and HCCH-TOCSY experiments (reviewed in Sattler et al., 1999). $^1$H chemical shifts were referenced to TMSP at 0.00 ppm and $^{13}$C and $^{15}$N chemical shifts

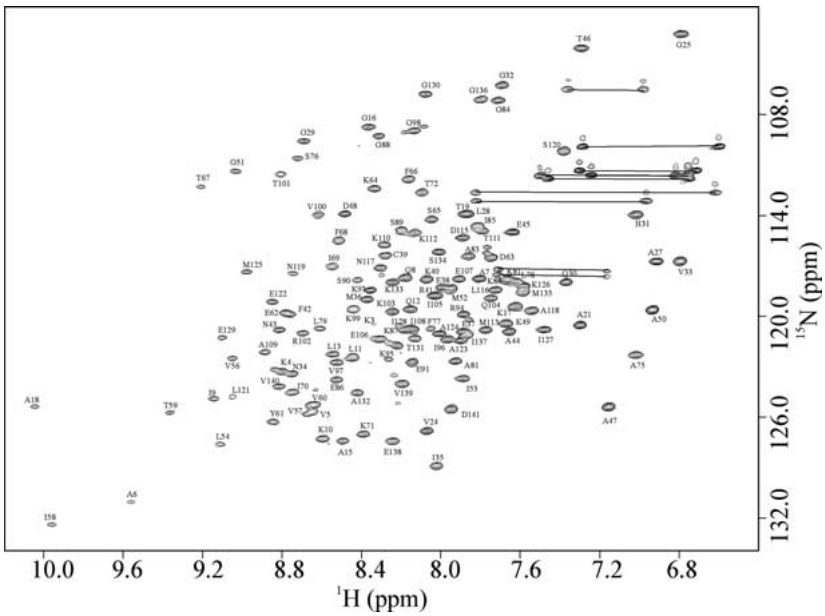


*Figure 1.* $^{1}$H,$^{15}$N-HSQC (600 MHz) spectrum of *Thermotoga maritima* L11 recorded at 298 K with the assignments indicated.

were calculated from the $^{1}$H frequency (Wishart et al., 1995).

Based on the L11 $H_\alpha$, $H_\beta$, $C_\alpha$, $C_\beta$, CO, N, HN chemical shift values the database system TALOS (Cornilescu et al., 1999) predicted a secondary structure similar to the crystal structure of the RNA bound L11 protein.

## Extent of assignment and data deposition

For the 141 residues of *T. maritima* L11 the backbone HN and N assignments are essentially complete with the exception of M1 and A2 and the N of all 10 prolines (Figure 1). Side chain assignments are mostly complete except for the P74-F77 region. In addition, there are no assignments for P22 and P73 both preceding proline residues. From the triple resonance experiments, further backbone and non-aromatic side-chain assignments were made to the following extents: 95% of $C_\alpha$, $H_\alpha$, $C_\beta$, $H_\beta$; 96% of CO, 92% of $C_\gamma$ and $H_\gamma$, 87% of $C_\delta$ and $H_\delta$, and 90% of $C_\varepsilon$ and $H_\varepsilon$ resonances.

The assigned $^{1}$H, $^{15}$N, $^{13}$C chemical shifts of *Thermotoga maritima* L11 have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu/) under accession number BMRB-5513.

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