Site-directed spin labeling (SDSL) studies of large RNA molecules.

We show here that the 4-thio-U scheme[1] can be applied to probe base dynamics and structure in large RNA molecules. SDSL studies were carried out in a 56-nucleotide, 18 kD RNA derived from the P5 domain of group I intron *Tetrahymena* (Fig. 1)[2]. The spin label Rb was attached to six uridine sites within the P5 molecule using the 4-thio-U scheme (Fig. 1). The overall tumbling of the molecule ($\tau_R$), estimated to be $\sim$10 ns under these conditions, is sufficiently slow so that the EPR spectra reflect nitroxide motions that are linked to the local RNA environment. Structural perturbation due to the spin label is minimal, as judged by thermal melting studies (Fig. 2).

Fig. 2 shows Rb spectra between U42 (loop) and U30 (base paired). The spectrum of U30 shows very low mobility, with broad central linewidth ($\Delta H_{pp}$) and large hyperfine splitting (2A). This is consistent with NMR structure showing that U30 is hydrogen bonded and stacked within the helix. On the other hand, the U42 spectrum shows mobile features, with narrow central linewidth and small hyperfine splitting. This is consistent with fast motion of a structurally unrestricted base.

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Fig. 1: (A) The 4-thio-U labeling scheme. (B) The P5 RNA and the corresponding secondary (right) and tertiary structure of the P5 RNA in the presence of Na$^+$.  

Fig. 2: Comparison of EPR spectra at a loop site (U42) and a base-paired site (U30).
Spectra of all six labeled sites are shown in Fig. 3. The inverse central linewidth \( ((\Delta H_{pp})^{-1}) \) and the inverse second moment \( (\langle H^2 \rangle^{-1}) \), were used to characterize the mobility of the nitroxide[1, 3]. The resulting mobility plot is shown in Fig. 3C. Sites that are hydrogen bonded and stacked within the helix again exhibit lower mobility than those in the structurally unrestricted loop regions (Fig. 3C).

**In summary**, data reported here is consistent with studies in another RNA system[1]. It demonstrates that the SDSL, particularly the 4-thio-U scheme, can be employed to studied large RNA molecules.

Reference