**W-Band EPR Study of Local RNA Base Dynamics in the Spin Labeled**

**Glycine Riboswitch Kink-Turn Motif Using HiPER**

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**Introduction**

RNA riboswitches are regions of mRNA transcripts that exhibit the ability to independently and selectively bind a cognate ligand to induce genetic regulation. Binding of ligand induces RNA secondary and tertiary structural rearrangement that results in regulation of downstream genes. Investigation of changes in local dynamics upon conformational rearrangement is well suited for study by site-directed spin labeling (SDSL) and electron paramagnetic resonance (EPR) spectroscopy. Continuous wave (CW) EPR at both X-Band (9.5 GHz) and W-band (94 GHz) are complementary experiments that provide sensitivity to a broad range of motional time scales [1]. W-band spectra have greater sensitivity to dynamic motions in the “fast-motion” regime (rotational correlation times <2 ns) and X-band spectra are more sensitive to varying rotational correlation times greater than 1 ns. In the current study, X-Band EPR was utilized to probe dynamics within an RNA structural motif of the glycine riboswitch known as, the kink-turn motif. The resultant spectra suggest fast motion dynamics where W-band EPR is expected to exhibit greater sensitivity. To further complement the characterization of local dynamic changes upon RNA folding in the glycine riboswitch, experiments utilizing W-band HiPER were performed.



**Experimental**

The kink-turn motif of the glycine riboswitch is shown in **Fig. 1A**. Two nucleobases, U(2) and U(3) (also indicated in **Fig. 1A**), are expected to exhibit differential dynamics. The former is predicted to participate in base stacking and the latter to be solvent exposed upon RNA folding in the presence of salts and glycine ligand. To characterize differences in base dynamics, SDSL, shown in **Fig. 1B**, was performed for each independent site and CW EPR spectra were collected at both X- and W-band. **Fig. 1C** shows W-band spectra collected using HiPER for the U(2) site and similar experiments for the U(3) site are forthcoming. To monitor changes in local dynamics, four RNA folding states were probed by the presence or absence of 100 mM KCl, 5 mM MgCl2, and 5 mM glycine.

**Fig. 1.** (A) RNA glycine riboswitch secondary structure with kink-turn motif boxed and labeled sites indicated with blue circles; (B) RNA site-directed spin labeling strategy (C) X-band and W-band HiPER spectra for the U(2) site shown in panel A.

C

B

A

**Results and Discussion**

 Decreased mobility, due to base stacking at site U(2) is supported by the X-band and W-band (HiPER) spectra which show line shape broadening upon RNA folding in the presence of salts and ligand. Complementary characterization of site U(3) at X-band shows increased mobility relative to U(2) and further characterization with HiPER is in progress. To improve the quality of the collected spectra, efforts are being made to ensure efficient spin labeling and therefore spin concentration for samples that will be used for HiPER measurements.

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**References**

[1] Casey, T.M., *et al*., Biochem. Bioph. Res. Co., **450**, 723-728 (2014).