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**Structural Analysis of ROM Mutants Using Crystallographic and NMR Techniques.** Evi B. Struble<sup>1</sup>, Danielle M. Barbazon<sup>2</sup>, Jane E. Ladner<sup>1</sup>, John P. Marino<sup>1</sup>, <sup>1</sup>Center for Advanced Research in Biotechnology of the Univ. of Maryland Biotechnology Inst., and the National Institute of Standards and Technology, Rockville, MD, <sup>2</sup>Loyola College in Maryland, Baltimore, MD.

Solution studies of the ROM protein from ColE1 plasmid have determined that single point mutations at position 14 have deleterious effect on the binding of this protein to kissing loop RNA dimers. We solved the x-ray structures for three of these mutants F14Y, F14W, and F14H. All the three dimensional structures are essentially the same as the native ROM protein, with evidence of increased conformational variability of the amino acid side chain at the mutation site. Surprisingly, solution NMR data, in particular <sup>1</sup>H, <sup>15</sup>N relaxation and residual dipolar coupling (RDC) measurements show significant differences between native and mutant ROM structures. These differences are not limited at the mutation site and suggest that Phe 14 may be important in fine-tuning the presentation of the helical surface of the Rom dimer to the RNA kissing complex. A side-by-side comparison between RDC values predicted from the crystal structures and the ones measured using NMR will be presented and the significance of such comparisons will be discussed.