Pentratricopeptide repeat (PPR) proteins are modular single-stranded RNA-binding proteins. They consist of an alpha-solenoid structure composed of repeating 35 amino acid alpha hairpins, which form an extensive superhelix. Modification of just two amino acids per repeat, according to a derived code, can alter the specificity of a protein to a different target RNA sequence. Studies of wild-type, and consensus PPR proteins demonstrate a conformational change on RNA binding. For example, our crystallographic studies of idealised consensus (“designer”) PPR proteins in the presence and absence of RNA show that they have a superhelical structure of 9, or 10 repeats per superhelical turn in the presence or absence of RNA. Helical averaging of molecules in the superhelix in both crystal structures produced challenges for refinement, but ultimately produced molecular models that effectively represent infinitely long proteins binding to infinitely long RNA (Figure 1). The conformational change on RNA binding results in a contraction of the superhelical pitch from 85 Å to 43 Å, a change that is compatible with the Foerster distance of commonly used FRET fluorophores. We thus built a protein-based RNA FRET sensor by introducing two cysteine residues at appropriate spacing in the structure, and chemically labelling them with Cy3 and Alexafluor647 fluorophores. Having established a plate-based FRET ssRNA-binding assay which yields comparable dissociation constant to alternative methods, we then built a biotinylated version of the protein which can be immobilised suitably for single molecule FRET measurements. In these experiments we explore the conformational repertoire of populations of individual PPR proteins in the presence of variant RNA sequences thus providing insights into the mechanism and kinetics of RNA-binding by this class of biotechnologically useful proteins.

Figure 1. Crystal structures of apo- and RNA-bound designer PPR proteins demonstrate suitability for design of a FRET-based RNA sensor