When Life gives you lemons:  
Cryo-EM of heterogeneous and difficult samples

I will be talking about two separate stories which are considered to be challenging structural targets. The first segment will describe my work to obtain a high-resolution cryo-EM structure of an intrinsically disordered protein called tau. I determined a high-resolution (3.7 Å) cryo-EM structure of microtubule-bound tau and used a combined computational and cryo-EM approach to determine the atomic interactions between tubulin and tau. Our structural analyses indicate that the highly conserved tubulin-binding repeats within tau adopt very similar structures in their interactions with the microtubule.

The second segment will focus on unpublished work on the P element transposase, a DDE transposase which has up until now remained structurally uncharacterized due to the difficulties associated with this class of protein; as with most other transposases, the P element transposase is prone to aggregation and is difficult to work with. Using single-particle cryo-EM, we have obtained an atomic model of the P element transposase strand transfer complex (STC), consisting of a dimeric complex of the P element transposase along with two P element ends covalently linked to the target DNA. Severe preferential orientation was circumvented by collecting data on a tilted (40°) stage. The resulting cryo-EM reconstruction refines to 3.6 Å resolution, and the details of the map are sufficient to interpret atomic details of the nucleic acid substrates and to localize GTP. The RNaseH catalytic domain shares common features with other DDE transposases (so named for the catalytic triad) and the catalytic residues are identifiable. The RNaseH domain is interrupted by a non-canonical GTP-binding insertion domain which exhibits structural similarities to the insertion domain of other transposases, and in our structure makes contacts with target DNA. Surprisingly, we observe that single-stranded DNA (the 17nt single-stranded portion of the terminal inverted repeats) adopts an unusual path in our structure which appears to be stabilized by numerous protein-DNA contacts but ultimately returns to form distorted A-form DNA duplex leading into the active site. GTP is located close to the active site and interacts with the terminal guanosine residue of the P element DNA. We postulate that this serves to position the transposon donor in the active site for attack on the target DNA. Ultimately, the structure of the P element strand transfer complex reveals the molecular basis underlying two of the unusual properties of the P element transposition mechanism.