Proteins in the cell are both building blocks and molecular motors of most cellular functions. To perform and regulate complex cellular processes proteins form large molecular assemblies (or multi protein complexes, MPCs) whose components exchange continuously during the lifetime of the process creating more or less well-defined stages. Solving the atomic structures of multi-protein complexes (MPCs) is the biggest challenge that modern-day structural biologists face. MPCs are an enormous and unexplored resource, and in many instances, MPCs will hold the key to comprehend chemistry and biology at atomic level and ultimately human disease. Structural biologists encounter three main bottlenecks when dealing with MPCs. The first one involves generating pure and monodisperse MPCs for structural studies. Biochemical reconstitution of MPCs is a daunting task since they cannot be isolated as a whole from cells and requires expression and purification of individual subunits in a form (mono-dispersed, and properly folded) conducive to its reconstitution. To overcome these problems, we have established highly reproducible biochemical techniques that have allowed us to purify and reconstitute a wide range of MPCs. Second, given the transient nature of their interactions affinities between individual components of multi-protein complexes are usually low. This is problematic for electron microscopy and X-ray studies since sample freezing or crystallization might promote complex disruption. To overcome this difficulty, we have developed matrix-based approaches to cross-link and stabilize MPCs (in milligram amounts) for structural studies. Third, X-ray crystal structures are generally limited by the ability to obtain large, well diffracting crystals. The emergence of X-ray free electron laser (X-FEL) based serial femtosecond crystallography opens the possibility of solving the three-dimensional structures of samples that can only crystallize as nano crystals (NCs). Given the new opportunities that X-FELs offer to the field of crystallography, my laboratory is using transmission electron microscopy (TEM) to identify NCs from granular precipitates observed in crystallization trials. Moreover, since crystalline lattices can be directly visualized with TEM, calculating Fourier transforms from the images allows quantitative evaluation of electron diffraction patterns (Bragg spots). Identifying NCs from granular precipitates opens the possibility of finding NCs with different crystals forms. This is of paramount importance since different NC forms could potentially yield higher resolution X-ray diffraction. In addition to the advantages that TEM brings to the field of nano-crystallography, NC imaging may be beneficial during conventional crystallization trials to improve crystallization conditions and provide useful crystal ‘seeds’ for the production of macro-crystals. This work could uncover the potential of TEM as a fundamental tool for evaluating NC, as essential as brightfield microscopy is for evaluating and optimizing traditional, large crystals.

Using these methodologies we have been able to crystallize and solve the structure of novel MPCs involved in eukaryotic transcription. In addition our nano crystal studies have allowed us to generate a wide variety of samples for X-FEL diffraction experiments.