**Harnessing the power of Bertozzi reaction for capturing flexible parts in cryo-EM structures**

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**Abstract** Despite that single particle cryo-EM has become a powerful tool for structural biology as it offers maps with resolutions comparable to those from X-ray crystallography but without the need of crystal, this technique fails to capture flexible parts in a macromolecule since it is an ensemble method. In the seminar, I use RNA polymerase III (Pol III), which synthesizes structural and non-translated RNAs, as an example to illustrate the problem and our approach that utilized the famous Bertozzi reaction to achieve biorthogonal chemistry to enable selective labeling of a flexible part. Specifically, we targeted the tandem winged-helix domain (tWH) in the Rpc34 subunit of Pol III’s TFIIE-related sub-complex exerts multiple roles central to Pol III transcription. We deployed fluorescence resonance energy transfer (FRET) to probe Rpc34 tWH in a transcribing Pol III. By site-specifically incorporating an unnatural amino-acid (UAA) and developing a protocol for its selective labeling using strain-promoted azide-alkyne cycloaddition, we measured single-molecule FRET (smFRET) between an acceptor in the tWH and a donor in DNA. Our smFRET results of various FRET states along with inter-converting transitions demonstrate that Rpc34 tWH is a dynamic structural module, with multiple docking sites identified by smFRET-based nano-positioning. Our findings of tWH dynamical positioning in a transcribing Pol III may provide a structural basis underpinning its multiple-functions during a transcription. If time permits, I will cover our recent structural findings on a metalloenzyme that coverts methane to methanol where identifying the copper centers turned out to be very challenging.