Preliminary Data

Negative stain data of this ~20 subunit complex shows that the sample is biochemically pure and suitable for single particle EM (Figure 1). While we see different orientations of the particle in negative stain (Figure 1), we have encountered a severe problem with preferred orientations under cryo conditions (Figure 2). We hope to screen different sample preparation methods, including Chameleon / spotiton to overcome these problems.

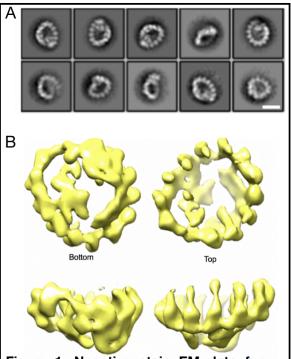


Figure 1. Negative stain EM data for γ-TuRC. A. 2D class averages and B. 3D reconstructions. Several orientations of the complex were obtained, leading to a reliable 3D reconstruction. Scale bar = 20 nm

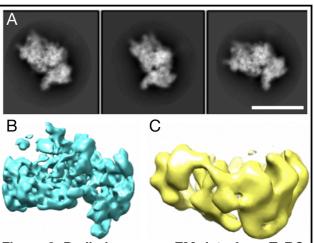


Figure 2. Preliminary cryo EM data for γ-TuRC. A. 2D class averages B. 3D reconstruction C. 3D reconstruction from negative stain data for reference, shown in the same orientation as B. Only the orientation of the sample shown in A was obtained for this sample. The "3D" reconstruction in B is shown only to orient the reviewers with respect to the low resolution reconstruction obtained from negative stain data (C), since the particles do not intuitively compare to the distribution of orientations observed on negative stain grids. Scale bar = 20 nm