

Figure 1: Representative native polyacrylamide gel for an electrophoretic mobility shift assay with 5'-³²P labeled siRNA incubated with increasing concentrations of Dicer-2•R2D2 +/- 5mM ATP. R111 indicates mutations in the R111 domain to prevent cleavage. K_d of complex binding siRNA is 4nM.

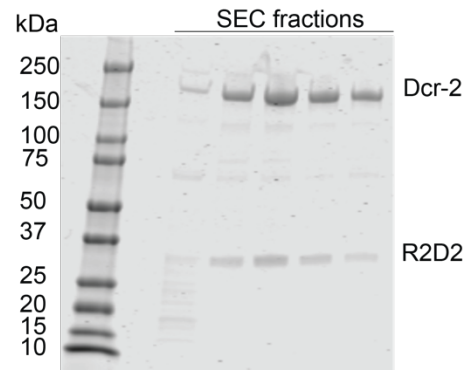


Figure 2: Coomassie stained SDS-PAGE gel for size exclusion chromatography (SEC) fractions after purifying the Dicer-2•R2D2 complex. The fractions with Dicer-2 and R2D2 are pure and the bands are of the correct size. Dicer-2 is 197kDa and R2D2 is 35kDa.

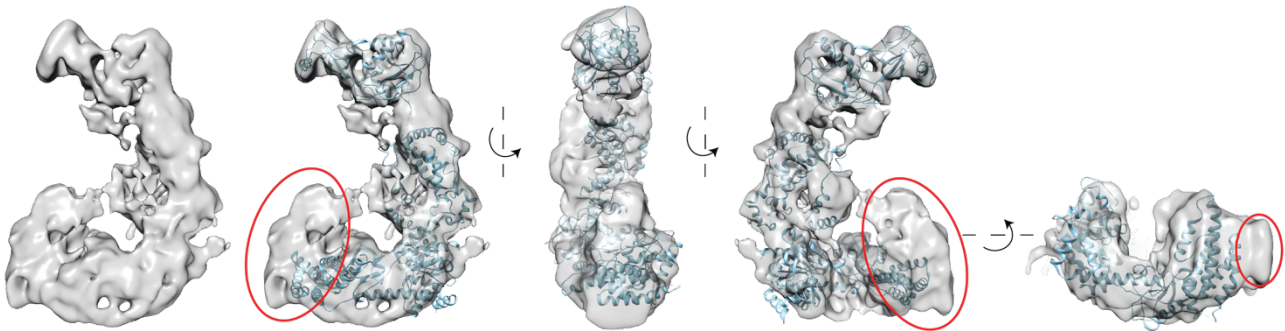


Figure 3: 6.0Å 3D reconstruction of the Dicer-2•R2D2 complex. Reconstruction fit with apo-Dicer-2 (6BUA, Sinha et al. 2018). The red circles indicate the extra density not taken into account by apo-Dicer-2; this extra density is likely R2D2, but may include part of the siRNA as well, but with the current resolution at 6.0Å it is difficult to accurately model this density.

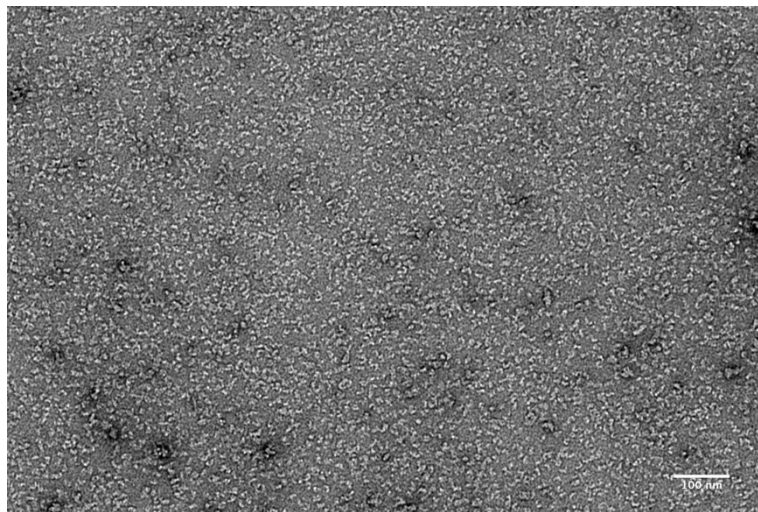


Figure 4: Negative stain image of the Dicer-2•R2D2 complex in the presence of siRNA. The particles are monodispersed and are the correct size, Dicer-2 is approximately 150Å in length.