Preliminary Results

Capitalizing on advancements in the cryoEM field and our many years of experience in the characterization of NRs, we have carried out significant studies in recent years.

Purification of AR and AR-V7: We successfully purified recombinant AR and it's variant AR-V7 from baculovirus (**Figure 1**). Both AR and AR-V7 form homodimers when bound to DNA in the presence or absence of hormone ligand.

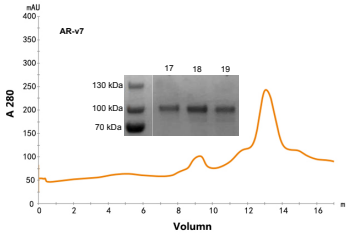

Figure 1. AR-V7 were applied to Superdex 200 10/300 GL column. The SDS-PAGE gel results of each fraction of chromatography are shown accordingly.

Cryo-grid optimization using graphene oxide (GO) support film: A rate-limiting step in our previous NR studies was frequent specimen aggregation and poor distribution on the cryo-EM grid. A key strategy to improve grid quality is to use newly developed sample-support films designed to yield a more even sample distribution, limit aggregation at the air-water interface, and reduce beam-induced movement during data collection[54]. The grids of NRs using our team recently developed new protocol that yields GO grids with high coverage (~90%), which was substantially better than the previously published 30–60% coverage. Using the new preparation method, we successfully solved structures with molecular weights similar to that of DNA–AR complexes to near-atomic resolutions, including rotavirus VP3 (380 kDa at **2.7Å**)[57] and RSV Fusion protein (150 kDa at 3.8Å)[58]. We used the new GO support film in our recent AR structural study (*Molecular Cell, 2020*)[43] and achieved a higher resolution than was possible for previous ER–CoR structures determined using continuous carbon support films [45,46].

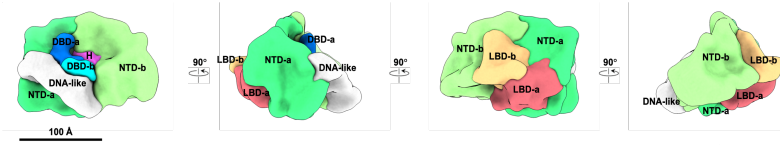

Figure 2. CryoEM density of ARE-DNA/AR at resolution ~12.6 Å with segmentation viewed from different orientation rotating in vertical direction.

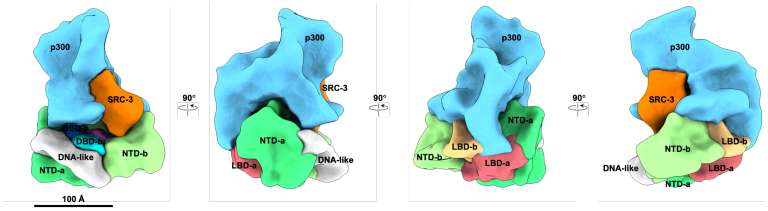

Figure 3. CryoEM density map of the ARE DNA-bound AR/SRC-3/p300 complex at resolution ~20 Å. The p300 density mainly interacts with two NTDs of AR and has a small region interacting with both of the two LBDs.

Breakthrough in DNA–AR structural biology: DNA-bound AR and AR-V7 complexes are large enough to provide sufficient contrast for successful single-particle cryoEM. In a preliminary experiment, we incubated purified Flag-tagged AR with an excess of 32 bp ARE-containing DNA oligonucleotides. Using single-particle cryoEM, we solved a preliminary structure of the full-length DNA–AR complex at ~12 Å resolution. We validated the structure using cryoET with sub-tomogram averaging. Following the same protocol[45,46], we were able to identify each functional domain within the DNA–AR complex (**Figure 2**). Our structure demonstrated for the first time that AR does not form a simple anti-parallel dimer, as proposed by previously[59–61].

The structure of the AR–CoR complex: Using purified recombinant proteins, we performed a preliminary experiment to assemble an androgen-bound AR–SRC-3–p300 complex on a biotinylated ARE derived from a PSA promoter[62]. We then reconstructed a preliminary 20 Å-resolution map of the DNA-bound AR–SRC-3–p300 complex from 16,000 particles collected on a JEM3200FSC microscope (**Figure 3**). AR appears to recruit only one SRC-3 molecule and one p300 molecule to form a complex. We also prepared sample additional monoclonal antibodies that specifically recognize the AR domains and other components in the complexes using the same grid conditions.