Interactions of Androgen Receptor and Nuclear Co-Repressor 1.

Background: Prostate cancer (PC) is the second most deadly cancer in men[1]. PC is almost always dependent on the androgen axis. While there are multiple effective androgen receptor (*AR*) antagonists, including bicalutamide and enzalutamide[2], inevitably all tumors develop resistance, often due to mutations in the ligand binding domain (LBD)[3]. New ways to therapeutically target *AR* are vitally needed.

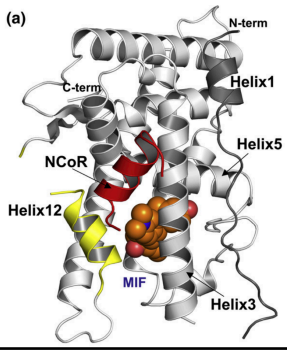

Figure 1: Glucocorticoid receptor (*AR* homology; grey) LBD bound with *NCOR1*(red). Mifepristone (antagonist; orange) stabilizing the complex. Based on binding site sequence similarities, it is thought *AR-NCOR1* bind in the same confirmation.

AR functions in concert with many coregulators. When an agonist, including testosterone or dihydrotestosterone (DHT) bind, *AR* goes through a conformation change, releasing corepressors, binding coactivators, and translocating into the nucleus[4]. One of the key *AR* corepressors is nuclear co-repressor 1 (*NCOR1*)[5]. *NCOR1* regulates transcriptional repression by recruiting histone deacetylates to DNA. *AR-NCOR1* are thought to interact when *AR* is bound to antagonist, however no structure has been solved with both proteins together (**Figure 1**). Multiple studies have demonstrated that *NCOR1* is necessary for *AR* inhibition[6,7]. Additionally, increased *NCOR1* expression is associated with worse PC prognosis, and *NCOR1* nonsense mutations are observed in 2.5% of advanced PC cases[7,8]. It is hypothesized that *AR-NCOR1* interactions change with the most common *AR* treatment resistance mutations(**Table 1**)[9]. It is critical to understand how the interactions change in the presence of well-known resistance mutations,[4] as a novel therapeutic will likely be used in the presence of existing resistance mutations. *By elucidating the interactions and dynamics of the AR-NCOR1 interactions, we will be able to develop novel AR therapies that work in the presence of resistance mutations. I hypothesize that in the presence of antagonist bicalutamide, LBD treatment resistant mutations will reduce the affinity, kinetics, and number of binding residues of AR-NCOR1 compared to WT AR-NCOR1 interactions.*

Aim 1: Perform surface plasmon resonance (SPR) to determine the thermodynamics and kinetics of *AR-NCOR1* interactions under various conditions. *AR* interacts with many coregulators with varying affinities and kinetics depending on the conditions. Previous data has suggested that co-regulators have similar affinities with varying kinetics to allow for transcriptional complexes to form, raising the question if treatment resistant mutations change the kinetics of AR-NCOR1 interactions, with or without changing the affinities[10,11]. Therefore, I will perform SPR to understand both the affinities and kinetics of *AR-NCOR1* under varying conditions (**Table 1**). *AR* LBD variants, stabilized by DHT, will be attached via a biotin tag. DHT or bicalutamide will be flowed in at a high concentration (5 nM) to keep the association of the ligand with *AR* in equilibrium. *NCOR1* will then be flowed in six concentrations (3uM, 5.4uM, 9uM, 15um, and 25uM). SPR measurements will be taken over 10 seconds. As a comparison, the same experiment will be performed with *PAK*, an AR coactivator that binds at the same AR motif, to determine if the results observed are *NCOR1* specific or observed for all coregulators. Experiments will be performed in triplicate. Outputs of these experiments will be K_d, K_a, and $t_{1/2}$, with the expected results displayed in **Table 1**. A potential caveat is that *AR-NCOR1* interaction maybe very transient, and thus under the detection time of our SPR experiment, preventing the collections of the kinetics, but I will still be able to determine the affinity under these different conditions. Overall, I am expecting the functional LBD mutations to prevent or shorten the time of AR-NCOR1 binding, potentially due to conformational changes, which will be explored further in Aims 2 and 3.

	AR Variant Description	**DHT(Agonist)**	**Bicalutamide (Antagonist)**
AR WT	Wild Type	Low affinity/ short kinetics	High affinity/ long kinetics
AR F876L	Bicalutamide Resistant	Low affinity/ short kinetics	Low affinity/ short kinetics
AR T877A	Enzalutamide Resistance	Low affinity/ short kinetics	Medium affinity/ short kinetics
AR V716M	Mutation of unknown significance in LBD	Low affinity/ short kinetics	High affinity/ long kinetics

Table 1: AR variants mutations/conditions examined along with hypothesized NCOR1 affinity (Kd) and kinetics ($t_{1/2}$).

Aim 2: Molecular dynamics (MD) to examine the free binding energies between *AR-NCOR1*. While Aim 1 provides insight into changes in kinetics and affinities between different conditions, it is imperative to understand why these changes are occurring, and how binding patterns change with various treatment resistant mutations. I will perform MD simulations of the AR LBD with the ID1 domain of *NCOR1*, both of which have crystal structures[12,13], under the conditions outlined in **Table 1** to calculate the thermodynamic integration (TI) in the presence of DHT or bicalutamide. I will do this using AMBER Pmemd using their side chain mapping program. I will start with AR-WT with DHT and AR-T877A for bicalutamide based on the crystal structures. For the simulation, the following parameters will be used; AMBER99SB ILDN force field for the proteins, general amber force field for the ligands, TIP3P water molecules, along with counter ions to neutralize the system.[19,20]The proteins will be constrained in all simulations to ensure they are next to each other. After protein preparation, I will use a conjugate gradient method for the minimization, adjusting for pressure and heating up the structure to 300K. The production runs will last 5-us, with snapshots saved every 100 ps. From the MD outputs, I will examine the free energy differences of *NCOR1* and *AR* LBD binding between different states, using the AR-WT as the base state (**Figure 2**). Additionally, I will measure the ligand residue interactions using the native contact analysis (Q), using WT *AR* as the reference structure. Residues, more than 3 apart in linear sequence, that are within 4.5A of each other will be considered interacting. Q values closer to one indicate less deviation from the native structure. I will calculate the number of heavy-atom pairs in contact with each of the *AR-NCOR1* binding residue. Overall, this aim will allow me to understand how the overall binding between *AR* and *NCOR1*, as well as specific residues contacts, change with different *AR* mutations. I would expect treatment resistant mutations to decrease the binding between *AR* and *NCOR1* relative to the *AR*-WT state. One caveat for this aim is that the MD simulation will be occurring in a different environment which may not be applicable to the prostate cancer cellular environment. By understanding these changes, we will have additional information on how a potential *NCOR1* mimic or stabilizing drug will need to be designed to work with various resistant mutations.

$$\Delta F(\mathbf{A} \rightarrow \mathbf{B}) = F_{\mathbf{B}} - F_{\mathbf{A}} = -k_{\mathrm{B}}T \ln \left\langle \exp\left(-\frac{E_{\mathbf{B}} - E_{\mathbf{A}}}{k_{\mathrm{B}}T} \right) \right\rangle_{\mathbf{A}}$$

Figure 2: Zwanzig equation to calculate the free energy difference between AR-WT and AR-variants.

Aim 3: Hydrogen-Deuterium Exchange (HDX), coupled with mass spectrometry, to identify amino acids contributing to *AR-NCOR1* interactions. To further compliment the two previous aims, I will use a solution based HDX system to examine the *AR* LBD and *NCOR1 interactions*, as has been done with other nuclear receptors[16]. In this system, I will examine *AR* and *NCOR1* alone, along with *AR* mutations *(***Table 1)** and *NCOR1*. DHT/bicalutamide will be incubated for 2 hours before the beginning of the HDX experiment. To begin the HDX experiment the proteins will be diluted into deuterated (D2O) buffer at pH 7.0 and allowed to exchange for varying lengths of time (45, 100, 1000 seconds), followed by a urea quenching step[17]. Proteins will then be digested with pepsin and separated via reverse phase chromatography. I will then perform mass spectrometry using electrospray ionization to identify the relative deuterium levels (%D), compared to the %D with each protein alone. The intensity of each peptide will be calculated to determine the %D incorporation. Statistical significance for the differential %D will be determined by a paired t-test for each time point. The %D from overlapping peptides will then be consolidated to individual amino acids via residue averaging. For each residue, the %D values and peptide lengths from all overlapping peptides will be assembled, and weighted based on peptide length. With this data, I will create a map of the different %D in interacting residues between *NCOR1* and AR under various conditions (**Table 1**; **Figure 3**). I would expect conditions with shorter kinetics to have increased %D in the interacting residues of AR and NCOR1, especially compared with WT-*AR* in bicalutamide. One main caveat may be the difficulty of detecting significant variations in %D between residues that have little structural order or those with very transient NCOR1/AR interactions. This aim will provide further experimental validation of interacting residues observed in Aim 2.

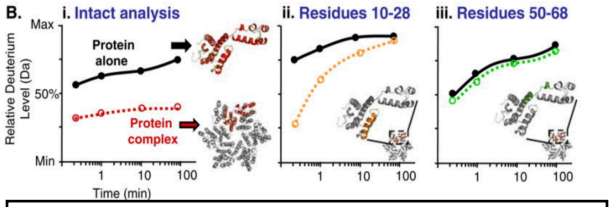

Figure 3: Expected outcome of HDX experiment between *AR-NCOR1*. Specific residues, especially the LXXLL binding motif will be highlighted.

Impact: This proposal will illuminate *AR-NCOR1* interactions providing key information leading to innovative AR inhibitors, potentially including an *NCOR1* mimic. By incorporating three distinct techniques, including experimentally quantitative (aim 1), experimentally qualitative (aim 3), and computationally quantitative (aim 2), I will provide much needed evidence to design novel *AR* coregulatory therapeutics.

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