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Assessing Orthopoxvirus Protein Affinity to Chondroitin Sulfate Using Single Molecule TIRF Analysis

Jesse S. Aaron[1], Jerilyn A. Timlin[1], Masood Z. Hadi[2]
[1]*Dept. Bioenergy and Defense Technologies, Sandia National Laboratories, Albuquerque, NM 87185*
[2]*Biomass Science and Conversion Technology, Sandia National Laboratories, Livermore, CA 94550*

Cell-surface carbohydrate binding is one of the initial, and critical, steps in the interaction of orthpoxviruses with host cells. Chondroitin sulfate (CS) binding proteins present on the surface of vaccinia intracellular mature virus (IMV) greatly enhance cell-surface interactions and infectivity [1]. Understanding the basis of the interaction of these proteins with their cell-surface receptor can assist development of affinity ligands for smallpox detection and improved anti-pox drugs. We constructed homology models, as well as several site-directed mutants of the CS binding protein from variola which was used to identify a CS-binding groove on the protein surface, whose affinity was testing using surface plasmon resonance (SPR) measurements.

However, SPR may be problematic in cases where ligands display relatively weak associations (and thus low surface coverage), or associate to moieties that may extend hundreds of nanometers beyond the SPR substrate [2]. To gain further insight, CS was immobilized to a glass substrate via biotin/Neutravidin linkages such that the CS molecules were oriented perpendicular to the sample plane. We then implemented a novel total internal reflectance fluorescence (TIRF) microscopy assay to visualize viral protein behavior. This approach has several advantages, including high temporal resolution (<20ms), as well as single protein sensitivity. In addition, TIRF microscopy offers an ideal imaging platform for these purposes, as it provides sub-diffraction (*ca.* 100nm) optical resolution in the axial direction. This ability avoids interference from unbound proteins, confining our measurements to CS-interacting molecules. Through this, we are able to probe parameters such as average binding time and possible nanoscale axial motions of the proteins on the CS polymer, by virtue of the evanescent field decay inherent to TIRF [3].

This study investigated the properties of F8L and D8L orthopox viral proteins, including a number of site-directed mutants, which are known to mediate viral entry into cells via membrane-associated CS. Single molecule imaging was first used to determine average binding times (see Figure 1). In general, longer binding times corresponded to those proteins with the highest binding constants. However, exceptions were noted, suggesting possible inaccuracies of SPR measurements (Figure 2).

Single particle analysis reveals that CS-binding proteins do move axially along CS fibers. However, the directionality is random. This data suggests that Orthopoxvirus binding proteins do not impact active transport functionality, but rather confine the viral particle to movement in a single linear dimension perpendicular to the cell membrane.

References:

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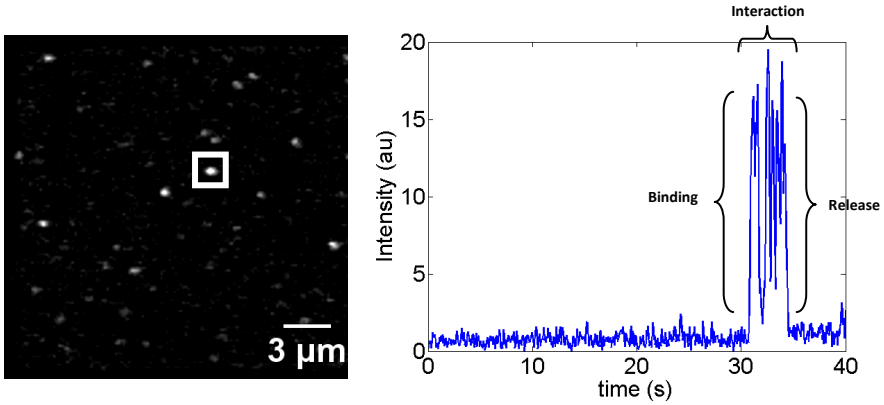

Figure 1. TIRF imaging of viral protein binding to chondroitin sulfate (CS) substrates. At left, a TIRF image is shown of single molecules bound to the ligand-coated surface. At right, a signal trajectory, (taken from the area bounded by the box in the left image), which identifies binding, interaction, and release events from a single protein.

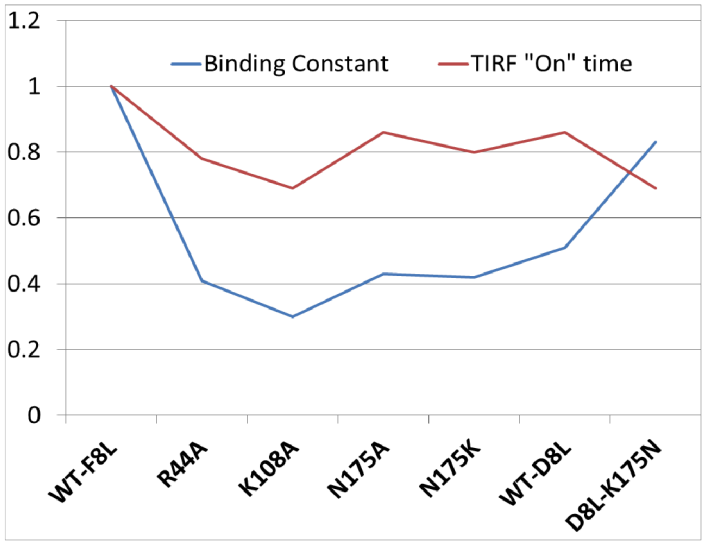

Figure 2. Average binding times determined by TIRF as compared to relative binding constants for F8L, D8L, as well as several site-directed mutants. Overall, longer binding times correspond to higher binding constants, as determined by SPR. However, TIRF and SPR data from the D8L-K175N mutant (far left) did not correspond.