

VISUALISATION OF THE STREPTAVIDIN-BIOTIN INTERACTION USING AFM

Calum S Neish, Robert M Henderson, J Michael Edwardson; University of Cambridge, United Kingdom

The binding of biotin to streptavidin (a 60-kDa tetrameric protein) is the strongest non-covalent interaction known ($K_A \sim 10^{14} \text{M}^{-1}$). We have used AFM to visualise this interaction directly. Biotin was tagged with a 152-basepair DNA rod, incubated with streptavidin and the resulting complexes were imaged. The binding of biotin was found to increase the apparent size of streptavidin, as measured by AFM molecular volume. This result was accompanied by an increase in thermal stability of the protein. Images of streptavidin in various occupancy states were obtained. When two ligands were bound, the angle between the rods was either acute or obtuse, as expected from the relative orientations of the biotin binding sites. However, the ratio of obtuse:acute was 3:1, greater than the expected value of 2:1, demonstrating a degree of steric hindrance. Streptavidin with a single molecule of DNA-biotin bound was used to tag subunits of biotinylated β -galactosidase, a model multimeric protein.

We conclude that using AFM to image the binding of biotin to streptavidin provides useful information about the nature of the interaction, and about the effect of ligand binding had on the structure of the protein. The use of streptavidin-biotin as a tag could provide a means of identifying the architecture of multi-subunit proteins.