

Specificity of protein-ligand binding essay



**ASSIGN
BUSTER**

1). One can conclude that Orange G. has the lowest affinity for the albumin. In the experiment the concentration of Orange G that binds to protein was a lot less than either Ponceau S. or Bromophenol blue. The ligand with the highest affinity to BSA was a little more difficult to decipher. The experiment shows that all of the 2 μL of both Ponceau S. and Bromophenol blue bind to the BSA. However, when 5 μL of the ligand is added the BSA is saturated and cannot bind anymore of the ligand.

The data illustrates that Ponceau S. as a higher concentration of extra ligand at 5 μL ; therefore more Bromophenol blue binds to the BSA. With this said one can conclude that Bromophenol blue has the highest affinity to the albumin followed by a close second to Ponceau and Orange G has the lowest affinity to the albumin. 2). The more ligand added to the BSA, the more ligand there will be able to bind to the BSA up to the point of saturation. For instance, when 5.0 μL of Bromophenol blue was added to the albumin; all of the 5.0 μL did not bind to the BSA because it had reached its saturation point.

The concentration of Bromophenol blue it takes to saturate albumin is approximately 2.5 μL . 3). The results clearly show that Bromophenol blue has a much higher binding affinity to the albumin than Ponceau S. The results in experiment I was not as clear. The data illustrates that when albumin has the opportunity to bind to Bromophenol blue or Ponceau S. it will bind to Bromophenol. The results of the competition with the Ponceau S. and Orange G. were a little more surprising.

Experiment I showed Ponceau S. affinity to albumin was a close second to Bromophenol blue, but when Ponceau S. as in competition with Orange G. it did not illustrate such binding affinity. It barely seemed to bind more than Orange G. The color of the band of albumin plus ligand is a slight orangish-red which indicates Ponceau S. binds more but it is not quite clear. It is evident to note the extra ligand of Ponceau S. at the bottom of lanes 8, 9, and 10 are approximately 1/4 shade lighter than the control in lane 4 which indicates more Ponceau S. binds to the albumin.

So Ponceau S. has a higher affinity for albumin than Ponceau S. , but that affinity is diminished in competition with another ligand.). Hemoglobin has zero binding affinity to any of the ligands. Experiment II details that hemoglobin stays where it wants to and the ligands go where they need to go. 5). There was no evidence that the BSA bind to any of the ligands. However, it is what one would predict once the protein was treated with SDS. The SDS will unfold the protein causing the protein to lose its shape and its ability to bind to the substrates it would usually bind. This is why there is no clear evidence of BSA binding to any ligand because the BSA cannot bind because it has been denatured by the SDS.