Supplementary information for

Interaction of D-Amino Acid Oxidase with Carbon Nanotubes: Implications in the Design of Biosensors

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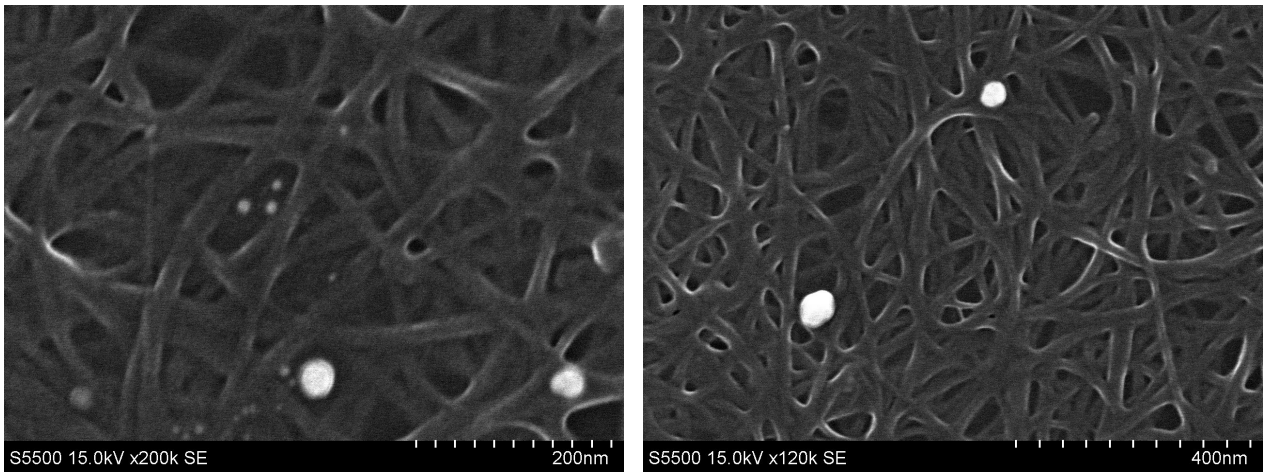
Protein Penetration

In order to verify that the structure of the CNT layer does not allow the proteins to get in contact with the silicon substrate, gold nanoparticles (~5 nm) were deposited on the surface. These nanoparticles were selected because they have similar size to the protein and they can be visualized by SEM. Inspection of such Au-CNT surfaces by SEM shows that the nanoparticles remain on top of the CNT layer.

Other proteins (such as BSA) were also found to remain on the surface of the CNT layer.[1]



SEM pictures of the CNT substrates showing Au nanoparticles on the surface.

Protein Charge

In order to explain the results included in Figure 5 and further understand the interaction of DAAO with CNT, the charge of DAAO as function of pH was computed using the most recent sequence registered in the Protein Data Bank (http://www.rcsb.org/, 1ve9).[2] The calculation was performed using the software package CLC Main Workbench ver 4 (CLC Bio, Aarhus, Denmark) (http://www.clcbio.com/).

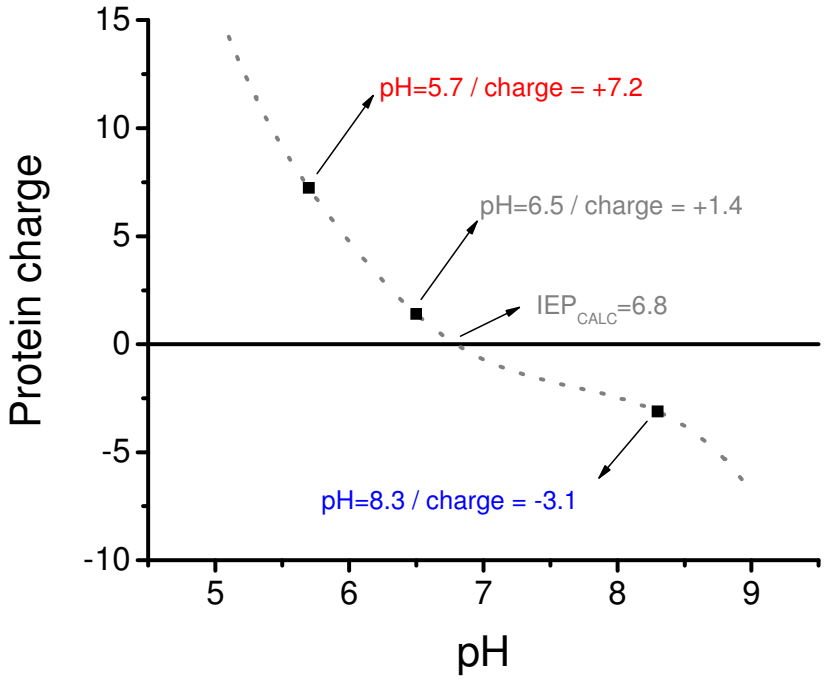


Figure SI-1: Total protein charge as function of pH.

As can be observed in Figure SI-1, there is a clear difference in charge at the selected pH values. As stated in the manuscript, these results indicate that electrostatic interactions between the CNT surface and the protein, as well as among the adsorbed molecules could be higher at pH = 5.7, favoring a particular orientation of adsorbed DAAO, which maintains the protein molecules separated and exposes the active site to the solution. When DAAO is adsorbed at pH = 8.3 (charge = -3.1), molecules could adopt a different conformation, be close enough to interact with each other, and block each other's active sites. Figure SI-1 also shows the calculated isoelectric point of the protein (6.8), which is also in good agreement with previously reported experimental values (IEP_{DAAO}= 6.3[3] – 7.0[4]).

Protein-Protein Interactions

In order to support the hypothesis that protein-protein interactions between adsorbed molecules could be the responsible factor for the significant difference in enzymatic activity, the identification of the interaction interface for DAAO was modeled using POLYVIEW 3D (http://polyview.cchmc.org/).[5]

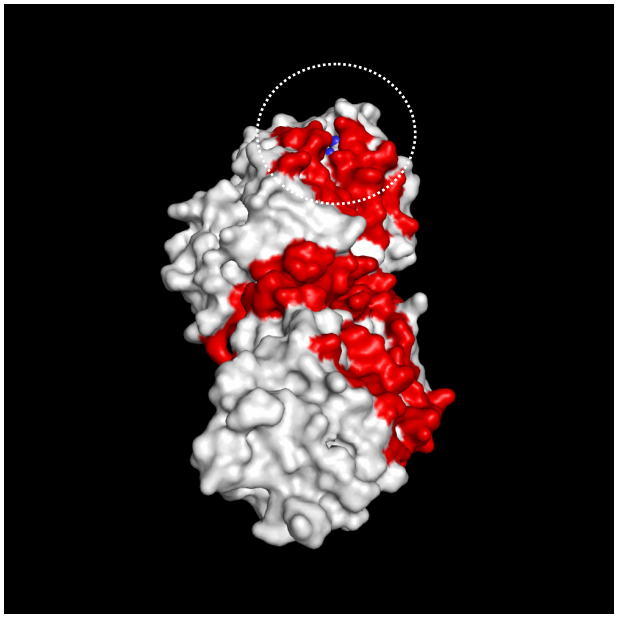


Figure SI-2A: Side view of DAAO. Interaction sites are highlighted in red. Note the dotted circles, which show the active site of the upper monomeric unit of DAAO.

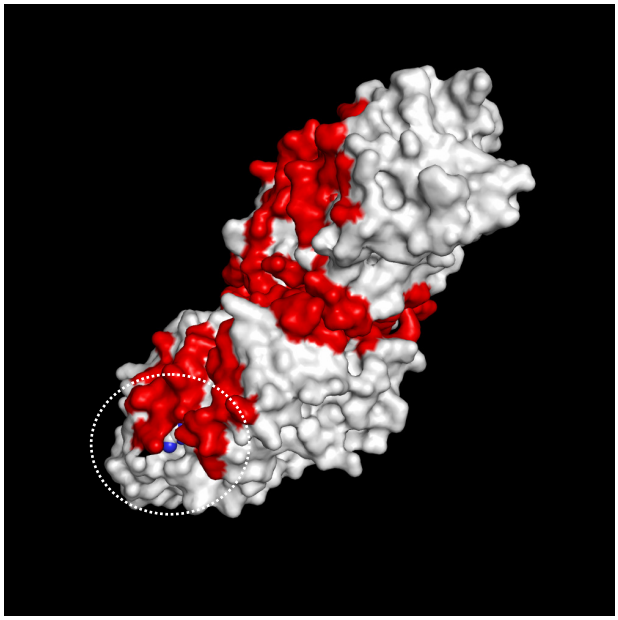


Figure SI-2B: Side view of DAAO. Interaction sites are highlighted in red. Note the dotted circles, which show the active site of the lower monomeric unit of DAAO.

Figures SI-2A and SI-2B were obtained by highlighting interaction sites in red and setting the remaining residues as white. As can be observed, DAAO shows a clear interaction area between the two monomers (top and bottom). DAAO also shows two other interaction sites located in only one side of the protein. These interaction sites, which are located above the active site (inside the dotted circle), could allow DAAO dimmers to interact with other molecules in the surface.

References

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