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NPS for Surface Adsorption Behaviour of Proteins

Introduction

The interaction of proteins with surfaces plays an important role for a wide range of applications, from tissue engineering and implants, to nanomedicine for pharmaceuticals and drug delivery, to biosensors and diagnostic assays. Depending on the protein's sequence and its surrounding environment, it will adopt different structures in solution, and consequently have different behaviour when interacting with a surface; for example amount adsorbed, packing density, number of layers, etc. For things that go inside of live organisms (implants, drug delivery vehicles, etc.), this has a direct impact on whether the body accepts or rejects the material. For sensors and assays, the efficacy of the surface passivation step has consequences on the method's accuracy (false positive, false negative).

Here, the results from three publications [1,2,3] investigating three factors that impact protein conformation and subsequent surface adsorption are reported. In all three cases Nanoplasmonic Sensing (NPS) was used to follow the adsorption in real time, and aid in determining the amount or density of the protein on the sensor surface. All three made use of Insplorion's XNano, and the standard SiO2 coated sensors. Two used several other characterisation techniques (Fluorescence microscopy, DLS, Circular Dichroism Spectroscopy, QCM-D, ATR FTIR) in addition to NPS, while the third focused on evaluating surface adsorption using QCM-D and NPS.

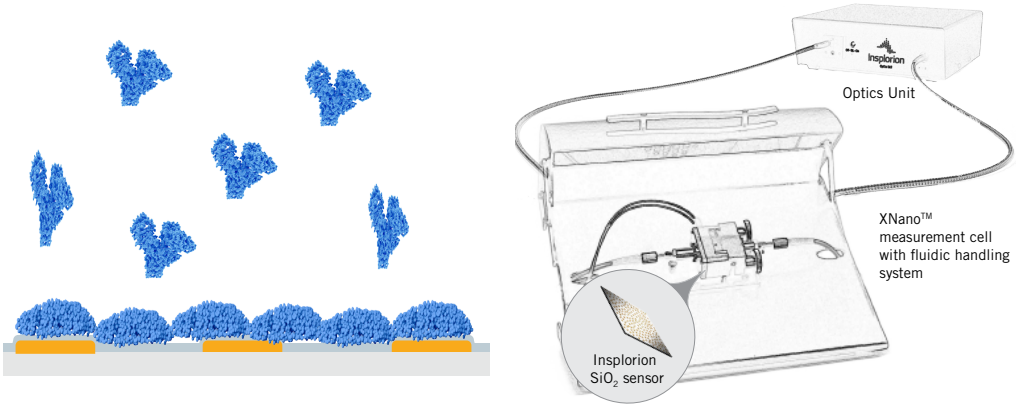

Figure 1: Schematic of BSA protein adsorption on sensor surface (Left) and Insplorion XNano instrument (Right) with inset of SiO_2 sensor.

Conclusions

The results showed that sequence variation, stabiliser molecules, and the polarity of the solvent have a great impact on BSA stability in solution and surface adsorption. At the same time, they showed that NPS is a powerful tool that can be used to monitor surface adsorption and provide unique information. For example, conformational changes in which molecules collapse and become closer to the surface can be detected. The fact that NPS is optically based and does not sense solvent's mass allows working with different solvents while providing unique insights into the dynamics between protein, solvent and surface. Whether more or fewer molecules are then able to interact with the surface is also easier to assess because the probe depth for NPS is 20-30nm. A more detailed summary of the experiments and their results are found below.

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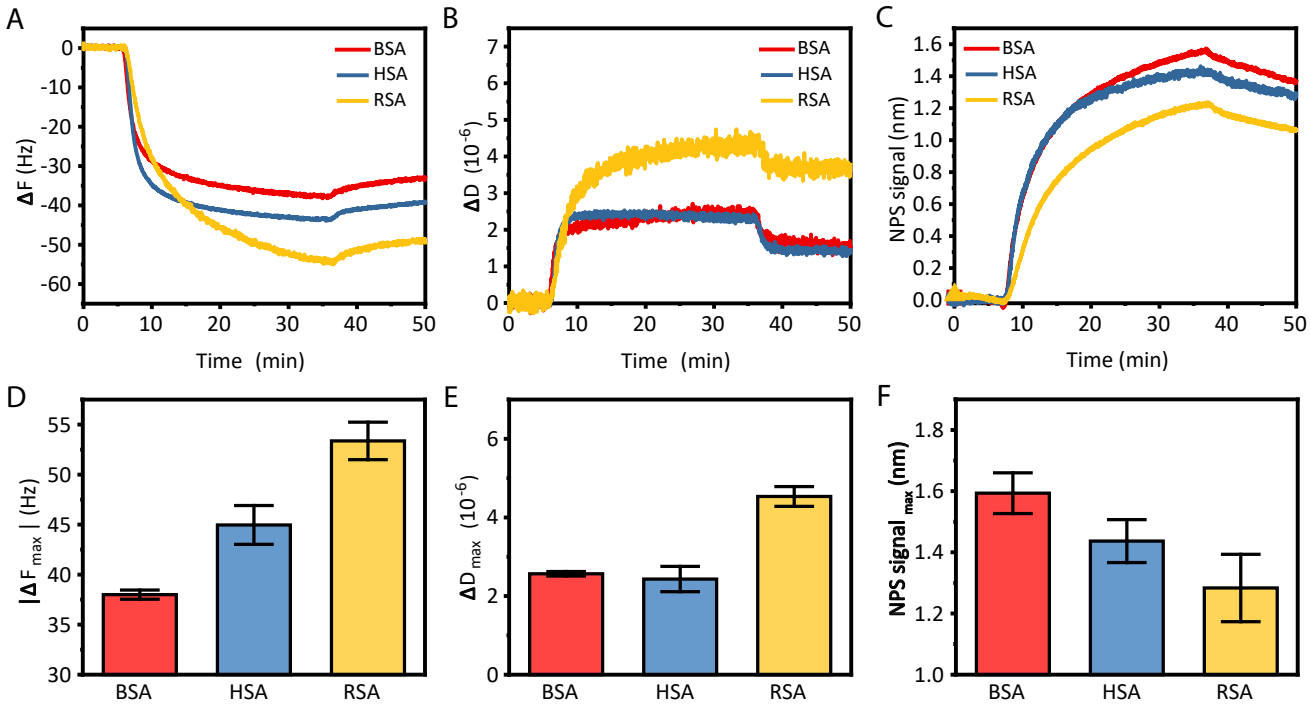

Figure 2: (A,B,C) Surface adsorption of BSA, HSA, RSA over time with (A) showing change in QCM-D frequency, (B) showing change in QCM-D dissipation, and (C) showing NPS signal. (D,E,F) Corresponding maximum values at saturation with (D) as the maximum frequncy change, (E) maximum dissipation change, and (F) the maximum NPS signal.

In the first paper [1] serum albumin, a commonly used protein, from three mammalian species (human, bovine, rat) were compared in their stability in solution and surface adsorption behaviour. Although it is the same protein family with similar functions in each of the organisms, the proteins' sequences are different due to amino acid substitutions. These lead to HSA and BSA forming monomers in solution, while RSA forms multimers. BSA was shown to have lower conformational stability in solution, as it suffered temperature-induced oligomerization at a lower threshold and greater temperature-induced conformation changes. It also had the greatest loss of α-helicity after adsorption, meaning it denatured to a greater extent. This agrees with results obtained by comparing NPS and QCM-D, which showed that BSA is closer to the sensor surface, with greater surface induced spreading, and thus a larger adsorption footprint. In other words, there were fewer BSA molecules adsorbed in total as compared to HSA.

Looking more closely at the results, the change in frequency, which is analogous to mass adsorption, shows BSA<HSA<RSA, whereas the signal change for NPS shows BSA>HSA>RSA. While at first glance these seem contradictory, they reveal a key point in understanding the system. NPS is much less bulk sensitive, meaning that while heavier RSA multimers have a greater signal for QCM-D frequency, only the molecules touching the surface are detected by NPS, and hence a lower signal. Similarly, BSA spreading out and covering more of the surface creates the highest NPS peak shift. The authors also used the NPS derivative, which gives information on the rate of adsorption, to estimate surface-induced denaturation (these have previously been shown to correlate).

In conclusion minor changes in the sequence of amino acids effects solution stability, adsorption and conformation changes upon adsorption. NPS helps detect conformation changes close to the surface, as well as the rate of adsorption. In this particular case, BSA denatured the most, followed by HSA and RSA. RSA adsorbed the most sparsely due to the multimer formation.

[1] Ma, G.J., et al (2020) Colloids and Surfaces B: Biointerfaces **DOI: 10.1016/j.colsurfb.2020.111194**

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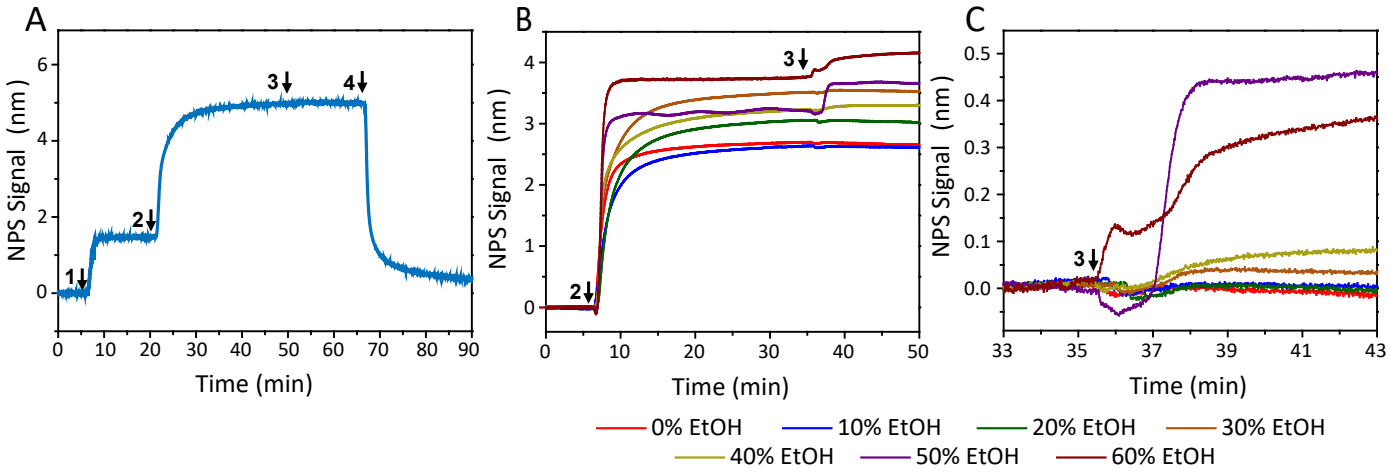

Figure 3: (A) Example of the measurement protocol; with 1 indicating the change from aqueous buffer to the ethanol-water mixture, 2 indicating the change to the water-ethanol mixture with BSA, 3 indicating a return to the ethanol-water mixture (without BSA), and 4 a return to the aqueous buffer. (B) NPS Signal during the addition of BSA for each water-ethanol mixture from 0-60% ethanol. (C) Detailed view of (B) at step 3 where the BSA solution is changed by to the respective ethanol-water mixture. The same numerical indicators shown in (A) are applied in (B) and (C).

The second article [2] also used Bovine Serum Albumin (BSA) and investigated how changes in its environment, namely the solvent, impact surface adsorption behaviour using QCM-D and NPS. The solvent was varied by creating six mixtures of water and ethanol between 0-60% v/v ethanol. It has previously been shown that more ethanol means a greater loss of α-helices, in other words more denaturing of the protein, and ultimately changes in conformation. The experiment was conducted by establishing a baseline in aqueous buffer solution, then changing to the water-ethanol solution, and subsequently the same water-ethanol solution with BSA protein. This was followed by switching back to the corresponding water-ethanol solution (without protein), and finally back to the aqueous buffer solution. QCM-D and NPS signals were used for monitoring surface adsorption, determining wet versus dry mass, and calculating surface coverage to determine the packing density trend.

For solvent fractions with 0-30% ethanol, the behaviour is as expected; increasing ethanol led to more denaturing, which in turn led to more adsorption and greater packing density as there are more protein-protein interactions.

A comparison of acoustic (QCM-D) and optical techniques (NPS) showed that at 40% ethanol, the BSA molecules had high wet mass, but a lower than expected dry mass. It is proposed that this is due to the more elongated conformation of the BSA molecules at this mixture ratio, causing a smaller adsorption footprint and having the molecule extend further from the surface. Moreover, the weaker surface interactions meant that more molecules became detached during the washing step.

Conversely, for the final two fraction (50 and 60%) the non-polar environment of the solvent leads to partial recovery of the α-helices and a more globular conformation. While a larger footprint meant less adsorption, the molecules were closer to the sensor surface, leading to a larger peak shift for NPS. For these, a further (positive) shift is observed after the washing step, indicating collapse of the BSA molecules and densification of the adsorbed layer.

[2] Tan, J.Y.B, et al (2020) Langmuir, **DOI: 10.1021/acs.langmuir.0c01478**

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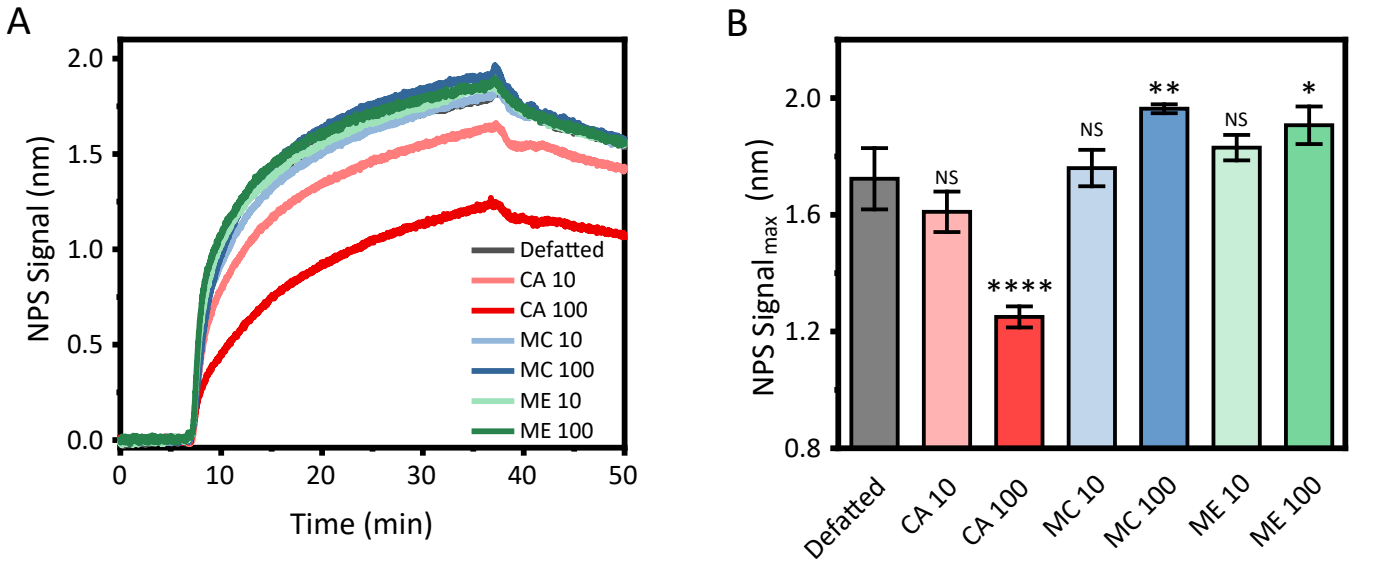

Figure 4: (A) Tracking realtime adsorption of BSA solution with caprylic acid (CA), monocaprylin (MC) and methyl caprylate (ME) at the low (10) and high (100) concentrations, as the stabiliser. Defatted BSA was used as a control. (B) The maximum change in signal at saturation for each solution is shown in (B).

BSA is often stabilised with amphipathic compounds, such as fatty acids. The third study [3] compared three such fatty acids with the same 8-carbon-long chains, but different head groups: caprylic acid (CA) with an anionic head; monocaprylin (MC) with a non-ionic hydrophilic head; and methyl caprylate (ME) with a non-ionic hydrophobic head. The authors used a high and a low concentration solution for each compound, and defatted BSA (DBSA) as the control. First, their solution stability was assessed and compared using the characterisation techniques listed above. While QCM-D and NPS signals were used for determining amount adsorbed and surface coverage, the derivative of the NPS signal and ATR FTIR were used to confirm adsorption related conformational changes.

The results showed that ME had the weakest bonding with BSA in solution, and the least stability. It also had the least impact on adsorption and the most surface induced denaturation, in other words it was the most similar to DBSA.

On the other hand, CA being negatively charged, had the highest binding affinity to BSA, the highest solution stability, and suffered from the least surface induce denaturing. While low denaturing usually means higher packing density, in this case the negatively charged head group led to repulsion of molecules on the surface, in other words low surface coverage. This impact was visible with NPS even at the lower 10:1 concentration.

MC being non-ionic and hydrophilic sat between the two. It had modest enhancement of solution stability (giving conformational stability only), low surface induced conformational changes, and the highest surface packing density and surface coverage. Its impact was best observed at the higher 100:1 concentration.

Taking this all together, the authors suggest that CA would be best for improving the solution stability of BSA, for example during purification processes; while MC would be the best stabilizer when using BSA for surface passivation.

[3] Ma, G.J., et al (2020) Langmuir, **DOI: 10.1021/acs.langmuir.0c02048**

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