# Protein Adsorption to Biomaterials - Atomic Force Microscopy & Radioactive Labeling Analysis

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In this study protein adsorption onto biomaterials is investigated using Atomic Force Microscopy (AFM) and radioactive labeling. Biomaterials are defined as artificial materials that interact with a biological system and are used in implants, biosensors, biomedical devices etc. Protein adsorption onto these biomaterials can result in dysfunctional or less efficient devices and can evoke unwanted response from the biological system the material is introduced onto [1-5].

By labeling proteins with different radioactive isotopes that emit gamma radiation with different energies we have developed a unique protocol for detection of several proteins onto a surface simultaneously [6-8]. Combining this quantitative technique with AFM measurements in liquid makes it possible to monitor formation of protein layers onto biomaterials in an environment similar to that, the materials are designed to function in. The setup combining AFM and radioactive labeling gives an opportunity to study interaction between artificial materials and biomolecules on nano-, micro- and macro-scale.

Figure 1 shows results from radioactive labeling where three different blood proteins (albumin, IgG and fibrinogen) are detected simultaneously on a polyethylene terephthalate (PET) polymer surface as a function of adsorption time. It is observed that the protein adsorption is increasing with adsorption time and that different proteins dominate the surface at different times. The concentrations used correspond to a protein level of 0.25 % blood and the amount protein adsorbed in Figure 1 indicates monolayer adsorption at this concentration level.

Figure 2 shows AFM Tapping Mode images (5x5 µm) obtained in cPBS (citrated Phosphate Buffered Saline) buffer of a) a clean PET surface, b) the same PET surface after introduction of 1 mg/ml albumin and c) the same PET surface after performing a scratching experiment. The roughness obtained before and after proteins are introduced is rather similar, indicating that the adsorbed albumin is homogeneously distributed over the surface. After performing a scratching experiment, where contact mode imaging with a rather high force is performed in a restricted area of the surface (700x700 nm), one can clearly observe that material is removed from the scanned area (see arrow in Figure 2c).

Figure 3 shows an AFM Tapping Mode image (10x10 µm) of a PET surface to which 10 mg/ml albumin has been introduced. The image is obtained in cPBS buffer and the different areas on the surface have had more or less contact with the scanning tip. Most scans and scratching experiments have been performed in area A, while area B only has been scanned once or twice. Area C is an area on the surface that never has been scanned before obtaining the image. The degree of impact on the protein layer from scanning gives valuable information about strength and character of interaction between protein and surface.

## References

[1] B.D. Ratner, S.J. Bryant, Annu. Rev. Biomed. Eng., **6** (2004) 41.
[2] D.F. Williams, Biomaterials, **30** (2009) 5897
[3] N. Huebsch, D. Mooney, Nature, **vol 462** (2009) 426.
[4] J.M. Anderson, A. Rodriguez, D.T. Chang, Seminars in Immunology, **20** (2008) 80.
[5] T.S. Tsapikouni, Y.F. Missirlis, Material Science and Engineering B, **152** (2008) 2
[6] M. Holmberg, K.B. Stibius, N.B. Larsen, X.L. Hou, J. Mat. Science. Mat. in Med., **19** (2008) 2179
[7] M. Holmberg, X.L. Hou, Langmuir, **25** (2009) 2081
[8] M. Holmberg, X.L. Hou, Langmuir, **26** (2010) 938

**Figures**

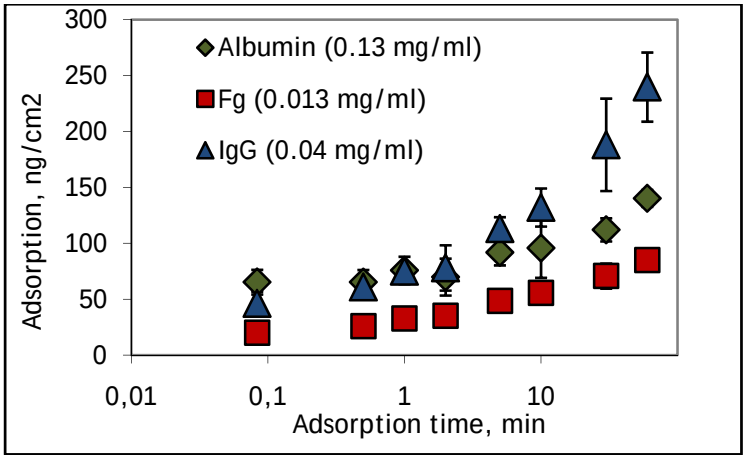


**Figure 1:** Albumin, fibrinogen (Fg) and IgG adsorption onto PET surface as a function of adsorption time. Proteins are added to the surface simultaneously with the concentrations shown in the figure and which corresponds approximately to the concentrations in 0.25 % blood.

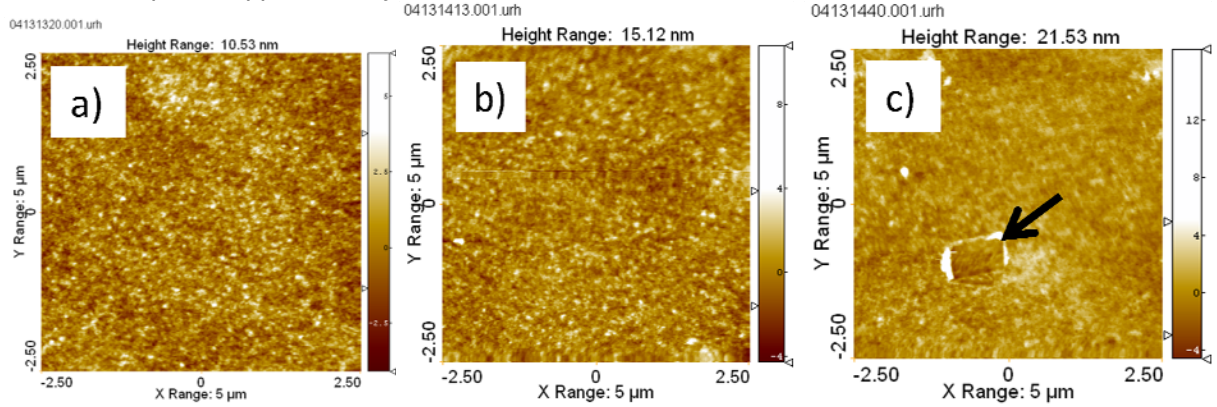


**Figure 2:** AFM Tapping Mode images (5x5 µm) of a) clean PET in cPBS, b) the same PET surface after introduction of 1 mg/ml albumin and c) the same PET surface after performing a scratching experiment.

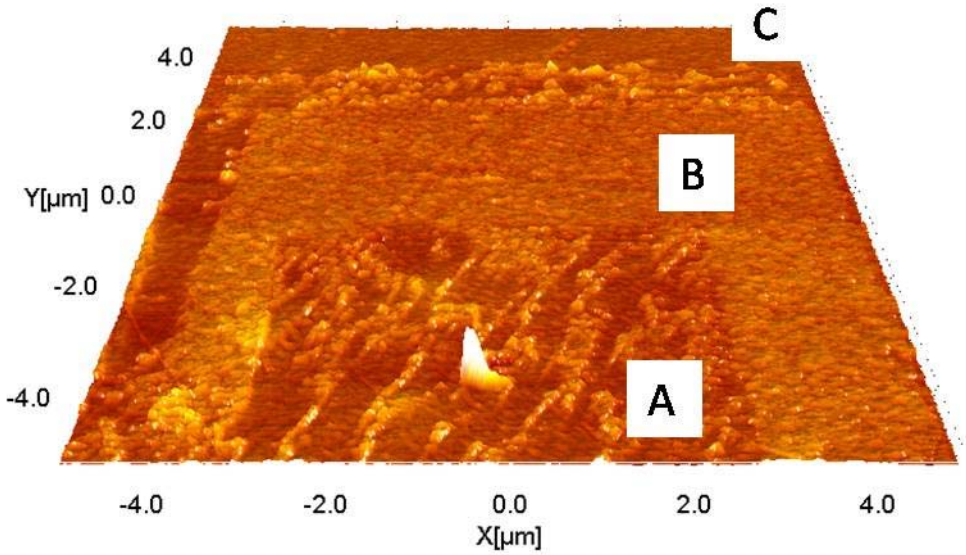


**Figure 3:** AFM Tapping Mode image of a 10x10 µmeter large area where a protein layer (10 mg/ml albumin) has been more or less disturbed by the scanning cantilever and tip. Most scans and scratching experiments have been performed in area A, while area B only has been scanned once or twice. Area C is an area on the surface that has never been scanned before.