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Direct Experimental Evidence of Surface-induced Protein Unfolding at the Single-molecule Level

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Protein unfolding, i.e. the loss of its native tertiary (quaternary) structure, is a part of many important biological processes [1,2] and plays a big role in various biomedical and biotechnological applications [3,4]. Protein unfolding may be induced by a number of physicochemical factors including addition of chemical substances (such as different salts, acids, alcohols, detergents), mechanical stress, change of pH, heating and adsorption on a surface [5,6]. Protein unfolding upon adsorption on a surface may be responsible for activating an immune system response (e.g., foreign body reaction) and other biochemical reactions in an organism [7]. The aim of this work was to obtain direct insight into surface-induced protein unfolding at the level of an individual protein molecule using atomic force microscopy (AFM). Several proteins such as ferritin, fibrinogen and RNA polymerases (RNAP) have been used in this study.

Graphitic materials are very promising for utilization in biotechnology and medicine [8] and, therefore, understanding of their interaction with single protein molecules represents a big interest. Using time-lapsed AFM in air and in aqueous solution we have visualized single protein molecules adsorbed on a surface of bare or modified (with an organic layer made from oligoglycine-hydrocarbon graphite modifier GM, stearyl amine or stearic acid) highly oriented pyrolytic graphite (HOPG) during different time periods varying from ~0.1 s to ~2 h.

We have visualized and characterized the unfolded states of single protein molecules on a bare HOPG surface (typical AFM images of the unfolded states of SP6 bacteriophage RNAP and fibrinogen molecules are shown in Figure 1). Protein unfolding (denaturation) on this surface usually takes place within 1 s after protein deposition and may be accompanied by aggregation of a denatured protein and formation of a denatured protein layer.

We have demonstrated that modified HOPG surfaces slow down protein unfolding (as compared to bare HOPG): the characteristic time usually increases to 3-10 minutes and the extent of protein unfolding is significantly less than on a bare HOPG surface. Moreover, we have visualized unfolding of several proteins on a GM-HOPG surface using time-lapsed AFM in real time (three successive AFM images of *E.coli* RNAP molecules adsorbed on a GM-HOPG surface are shown Figure 2).

In conclusion, we provide direct evidence of surface-induced protein unfolding at the single-molecule level and contribute to the understanding of this process. The obtained results may also be interesting for the development of new biocompatible graphitic materials [9].

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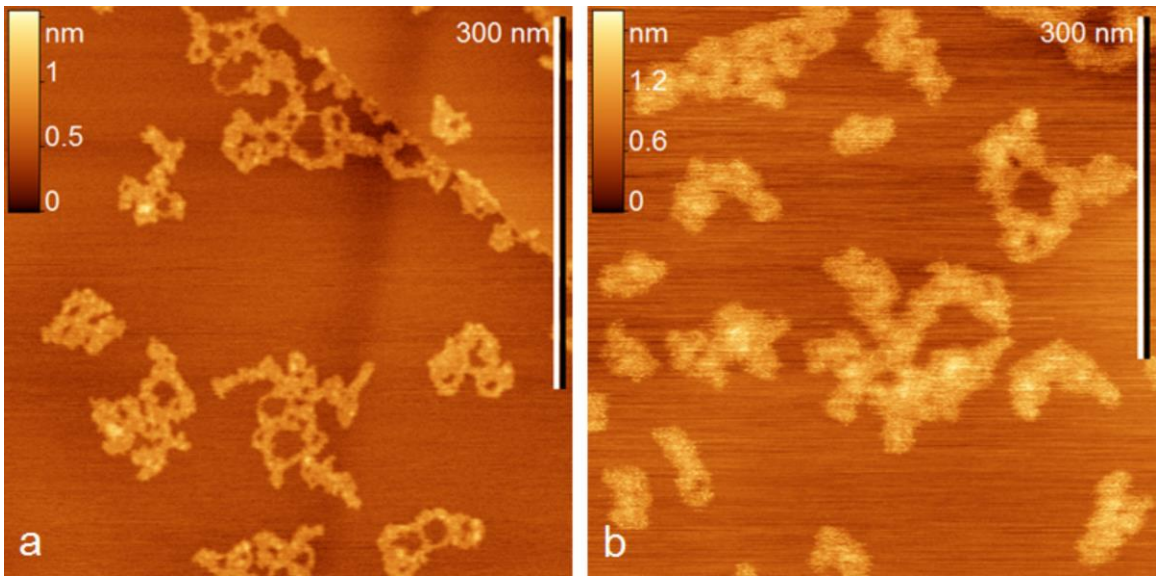

Figure 1. AFM image of (a) SP6 bacteriophage RNA polymerase and (b) human fibrinogen adsorbed onto freshly cleaved HOPG for 60 s (after drying).

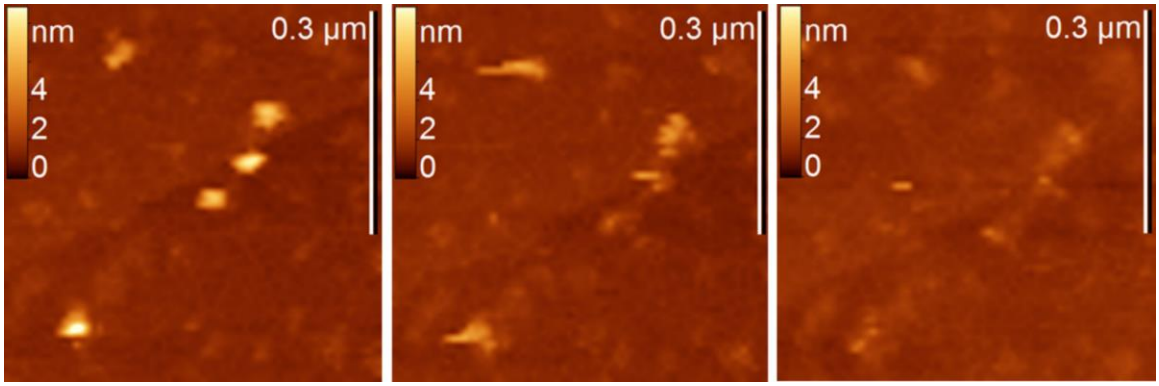

Figure 2. Three subsequent AFM images demonstrating unfolding of E.coli RNAP on a GM-HOPG surface in water in real time. The time interval between the frames is 9 min. Frame size is 0.5×0.5 μm.

References

[1] A. Matouschek, *Current Opinion in Structural Biology*, 2003, **13**, 98–109.
[2] N. Gehde, C. Hinrichs, I. Montilla, S. Charpian, K. Lingelbach and J. M. Przyborski, *Molecular Microbiology*, 2009, **71**, 613–628.
[3] G. Marx, X. Mou, A. Hotovely-Salomon, L. Levdansky, E. Gaberman, D. Belenky and R. Gorodetsky, *J. Biomed. Mater. Res.*, 2008, **84B**, 49–57.
[4] B. Sivaraman and R. A. Latour, *Biomaterials*, 2010, **31**, 832–839.
[5] S. Chodankar, V. K. Aswal, J. Kohlbrecher, R. Vavrin and A. G. Wagh, *Phys. Rev. E*, 2008, **77**, 031901.
[6] D. Coglitore, J.-M. Janot and S. Balme, *Advances in Colloid and Interface Science*, 2019, **270**, 278–292.
[7] T. A. Horbett, *Journal of Biomedical Materials Research Part A*, 2018, **106**, 2777–2788.
[8] A. C. Ferrari, et al., *Nanoscale*, 2015, **7**, 4598–4810.
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