

Advanced Nano 2017: AFM characterization of the physicochemical properties and activity of single protein molecules of CYP 102A1 (BM3) - Yuri Ivanov - Institute of Biomedical Chemistry, Russia**Yuri Ivanov***Institute of Biomedical Chemistry, Russia*

Atomic force microscopy (AFM) is a nano-technological multifunctional molecular platform for measuring of physicochemical and functional properties of single proteins molecules. AFM was used for visualization of oligomeric state, activity, elasticity and electron transfer of single molecules of CYP 102A1 (BM3). It was shown that BM3 in water solution exists as monomer, dimer, trimer, tetramer and oligomers of higher order by use sharp and super sharp AFM probes. Functional activity of single monomers and oligomers of BM3 was measured by AFM. The height BM3 fluctuations amplitude during catalytic cycle is much larger than the height fluctuations amplitude of the enzyme molecules in the resting state. It was found that an average amplitude of height oscillations of P450 BM3 molecule of dimers during catalytic cycle increased up to $5.0 \pm 2 \text{ \AA} \cdot \text{s}^{-1}$ that was 2.5 times larger than an average amplitude of P450 BM3 height oscillations in the resting state. It was obtained that the height fluctuation amplitude of single globule of

cytochrome P450 BM3 depends on temperature, and 22°C is a peak of this temperature profile. Mass spectrometry (MS) measurements were used to obtain a time course of a hydroxylation product of lauric acid oxidation during the enzymatic reaction of P450 BM3 in two cases: when enzyme was solubilized in the volume and when it was immobilized on the AFM chip. In both cases the number of enzyme molecules was $\sim 10^{10}$, and the kinetics was linear during the first 10 minutes. It was shown that in the case of solubilized enzyme $k_{\text{cat}} = 10^{-3} \text{ s}^{-1}$, and in the case of immobilized enzyme $k_{\text{cat}} = 0.4 \cdot 10^{-3} \text{ s}^{-1}$ that was 2.5 times less than the first one. Elasticity of single protein was measured based on deformation of this protein under AFM probes with various radii of curvature. Young's modulus of BM3 molecules depends on AFM modes. Based on the obtained data, the following conclusions may be made: the enzyme catalytic activity of single molecules can be measured as amplitude of enzyme globule fluctuations.