synthesis of the modified inhibitors and relevant protein crystal structures of these protein-inhibitor complexes.

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Study of Redox Process of Cytochrome C in Yeast under Cold Plasma Irradiation through Raman Micro-spectroscopy

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The production of reactive oxygen species (ROS) through external factors can lead to various microbial processes. As a physical means, cold plasma has a wide range of applications in the areas of bio-engineering and bio-medicine. It can produce ROS and affect microorganisms effectively, resulting in the effects of stimulation, inactivation, apoptosis and necrosis of cells. Cytochrome c is a common globular protein located in the inner mitochondrial membrane of cells, and its function is to transfer electrons in the mitochondrial respiratory chain between cytochrome c reductase (complex III) and cytochrome c oxidase (complex IV). Under oxidative stress, cytochrome c can be released from the inner mitochondrial membrane into the cytoplasm so that it is also regarded as a pro-apoptotic factor. In this work we employed atmospheric room-temperature plasma (ARTP) to treat yeast, and employed Raman micro-spectroscopy to monitor the state changes of yeast cells under plasma irradiation. As a result, we found that the Raman signals attributable to the reduced yeast cytochrome c at 750 cm^{-1}, 1128 cm^{-1}, 1310 cm^{-1}, 1585 cm^{-1} decreased with the increase of discharge time, and at a certain time after the plasma irradiation, the Raman peak at 1636 cm^{-1} which is attributed to oxidation state of cytochrome c became prominent and enhanced. We also examined the apoptosis of plasma irradiated yeast cells by Annexin V-FITC/PI, and found that the number apoptotic cells increased gradually with the increase of plasma irradiation time. This work thus demonstrates that we can utilize Raman micro-spectroscopy to track the vital redox processes of some important biomolecules such as cytochrome c in single yeast cells, which thus may help us to understand the cold plasma irradiation induced biological effects in the microbes.

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Protein Fold Recognition by Circular Dichroism Spectroscopy

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Experimental determination of protein structure has great importance in protein science. CD spectroscopy is a classical method for the study of the secondary structure of polypeptides, especially in the lack of high resolution structure. We constructed a program package based on our recently published CD spectrum analysis algorithm, named Beta Structure Selection (http://bestsel.elte.hu) (Micsonai et al., PNAS 112:E3095, 2015). BeStSel takes into account the parallel-antiparallel orientation and twist of β-sheets providing more detailed information on the secondary structure. Using the extra structural information, we have developed a new algorithm, which is capable of characterizing protein folds using the latest fold database and modern mathematics. Based on this fold prediction from a single CD spectrum at the CATH (Sillitoe et al., Nucleic Acids Res 43:D376, 2015) Homology level, for the first time, a reasonable 3D homology model can be built for the protein structure. This fast and cheap fold determination is valuable for recombinant protein production, functional genomics, protein therapeutics and rational protein design. This work was supported by the Hungarian National Research, Development and Innovation Office (grants K_120391, KH_125597 and TÉT_16-1-2016-0134) and the János Bolyai Research Scholarship program of the Hungarian Academy of Sciences (to A. M.).

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A Coarse-Grained Model of Circular Dichroism of Proteins

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Circular dichroism (CD) is a useful technique to investigate the secondary structure of proteins and some other biomolecules like RNA. There are various theoretical approaches intended to correlate the three-dimensional structure to the corresponding CD spectrum and some of them depend on accurate quantum mechanics calculations. Such approaches, however, require an important computational effort. In this work, we present a computationally tractable model that is based on the classical theory of optical activity. In first stage, we estimate a mean polarizability per residue from experiments of molar absorptivity. Then, we determine the complex polarizability that is used together with a protein structure obtained from the Protein Data Bank to calculate the approximate CD spectrum. Our computed spectra are found to be in good agreement with their experimental counterparts. As a result, this model could be utilized to describe conformational changes of a given protein or peptide.

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Probing Local Protein Environments with a Vibrational Reporter Unnatural Amino Acid

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Vibrational reporter unnatural amino acids have the potential to serve as sensitive, site-specific probes of local protein environments. Here the vibrational reporter unnatural amino acid 4-cyano-L-phenylalanine (pCNF) was utilized to probe multiple distinct sites in the Heme Nitric Oxide and/or Oxygen (H-NOX) binding domain from *Caldanaerobacter subterraneus.* pCNF is an effective reporter of local environment due to the position and sensitivity of the nitrile stretch frequency. pCNF was genetically incorporated into H-NOX into three distinct sites using the amber codon suppression methodology. The sites were selected to represent a myriad of local protein environments. The frequency of the nitrile symmetric stretch of pCNF and the temperature dependence of this frequency was utilized to assess the solvation state of the nitrile group in each of these sites in the protein. This analysis was aided by measuring the frequency and temperature dependence of the nitrile stretch vibration of pCNF in a variety of solvents to mimic different protein environments. These results probing the local environments in H-NOX will be presented.

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Micro- and Nanostructured Surface Architectures for Label-Free Interrogation of Protein Structure and Function

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Most of the complex functions of proteins in cells result from their ability to specifically recognize other biomolecules in a highly regulated manner and to propagate this information via structural reorganization. While screening for drugs that efficiently block binding sites of proteins is well established, probing conformational changes involved in downstream propagation remains challenging. We here aimed to develop surface architectures that enable efficient interrogation of protein interaction and conformational organization by label-free surface-enhanced spectroscopy techniques. To ensure structural integrity and full functionality of immobilized proteins, we developed surface biofunctionalization for site-specific protein capturing into micro- and nanostructured sensor surfaces both in *vitro* and in live cells. Two label-free sensing approaches were explored: Silicon micropillar structures combined with orthogonal surface chemistry were used for high density *in situ* protein capturing from cells cultivated on-chip. Protein interrogation was carried out by FTIR spectroscopy which enables optimal interrogation of IR-active secondary and tertiary protein structures by IR field enhancement via the silicon pillar sidewalls. As a second approach gold nanoparticles (AuNP) immobilized onto a glass surface were employed for LSPR-based reflection spectroscopy and microscopy. Surface functionalization of immobilized AuNP with tris-(nitrilotiacetic acid) (trisNTA) yielded efficient site-specific capturing of his-tagged proteins that allowed real-time monitoring of proteins interaction by LSPR detection. By micropatterning of gold nanoparticles for LSPR-based reflection microscopy, we aim to achieve label-free LSPR detection of interactions with membrane proteins in the plasma membrane of living cells. Moreover, integrating gold nanoparticles on silicon micropillars will allow highly sensitive interrogation of protein conformations by surface-enhanced infrared absorption spectroscopy (SEIRAS).

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Probing Local Solvation Environments in H-NOX Proteins using 4-Cyano-L-Phenylalanine

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Heme Nitric Oxide and/or Oxygen (H-NOX) binding domains are gas-sensing domains found in both eukaryotic and prokaryotic cells and are involved in a variety of functions in the cell. The heme pocket in *Caldanaerobacter subterraneus* H-NOX binds diatomic molecules such as O_2, NO, and CO. Crystal structures of *Cs* H-NOX have provided static structural images of the protein that suggest solvent is inaccessible to the heme pocket. The present study focuses on site-specifically genetically incorporating the unnatural amino acid (UAA) 4-cyano-L-phenylalanine (pCNF) in various distinct sites within the