Cover Page

Universiteit Leiden

The handle http://hdl.handle.net/1887/20273 holds various files of this Leiden University dissertation.

Author: Elmalk, Abdalmohsen
Title: Exposing biomolecular properties one molecule at a time
Date: 2012-12-13

Summary

Proteins are responsible for many different functions in the living cell. They are involved in enzymatic catalysis, transport and storage, coordinated motion, mechanical support, immune protection, generation and transmission of nerve impulses, control of growth and cell differentiation, etc. Our understanding of biological systems is bounded by our knowledge of the structure and function of these proteins. Over the past decade increasingly sophisticated techniques have been developed to visualize the distribution, the dynamics, and the interactions of proteins and other components in living cells with single-molecule sensitivity. The rapidly increasing knowledge of biological processes in various organisms and the understanding of genetic functions spurred a revolution in biotechnology, i.e., the use of biological processes to create useful products. Biotechnology is now being applied across a huge range of industries with a rapidly increasing expansion in the scope of its applications.

One of the exciting developments has been the study of enzyme activity and turn-over at the single-molecule level. Observing a single molecule removes the usual ensemble average, allowing the exploration of hidden heterogeneity in complex condensed phases as well as direct observation of dynamic changes without synchronization. Single-molecule studies of enzymes have been limited until now to only a few examples, most of which relied on the detection of light from fluorogenic substrates or products. This has been a severe limitation since it required the design and synthesis of special substrates that would fit the substrate profile of the enzyme under study. In this thesis we describe the application of a new method that removes this limitation. Moreover, it has potential for the construction of a new generation of biosensors with high sensitivity.

We have specifically targeted redox protein and enzymes, i.e., proteins that are capable of exchanging electrons with suitable reaction partners, and implemented a novel fluorescence scheme (FluRedox, see Chapter 1) which allows single-molecule observation of redox events with enhanced sensitivity. Of interest is the combination of fluorescent redox state detection and electronic control, for which the protein together with its fluorescent label needs to be placed close to an electrode, usually a metal surface. In bionanotechnology, such protein–surface interactions are pivotal for the assembly of interfacial protein constructs, such as (bio)sensors. A detailed mechanistic understanding of the protein–surface interaction would be of value to these fields, and the ability to tailor specific protein–surface interactions would benefit nanoscale materials and bionano-assembly technologies.

In chapter 1 a brief overview of single-molecule fluorescence detection is presented including a general introduction about the experimental techniques. The salient features of the various biomolecules that were studied are briefly described.

In chapter 2, real-time spectral detection at the single-molecule level allows us to characterize the dynamic fluorescence behavior of individual phycobilisome (PBsomes) antenna complexes in response to intense light. Our data reveal that strong green light can induce the fluorescence decrease of the phycobiliprotein allophycocyanin (APC), as well as the simultaneous fluorescence increase of phyoerythrin (PE) at the first stage of photobleaching. It strongly indicates an energetic decoupling occurring between PE and its APC neighbor. The fluorescence of PE was subsequently observed to further decrease, showing that PE could be photobleached when energy transfer in the PBsomes was disrupted. In contrast, the energetic decoupling was not observed in either the PBsomes fixed with gluteraldehyde, or in mutant PBsomes that lacked PE. It was concluded that the energetic decoupling of the PBsomes occurs at the specific association between two PE moieties within the PBsome rod. In addition, this process was demonstrated to be power- and oxygen-dependent.

In Chapter 3, redox activity of individual azurin was observed on gold surfaces using FRET-based fluorescent redox state detection. In this experiment we show how specific immobilization allows us to characterize the photophysical properties of individual, labeled azurine molecules positioned in close proximity to the gold films, and to link these properties to a defined distance and orientation. Fluorescence quenching starts at distances below 2.5 nm from the gold surface. At shorter distances the quenching may increase to 80% for direct attachment of the protein to bare gold. Outside of the quenching range, a four-fold enhancement of the fluorescence is observed with increasing roughness of the gold layer. Fluorescence-detected redox activity of individual azurin molecules, with a lifetime switching ratio of 0.4, is demonstrated for the first time close to a gold surface. These results highlight the importance of single-molecule fluorescence intensity and lifetime measurements for understanding the interaction between labeled proteins and metallic surfaces.

Chapter 4 describes the results of a study of the interaction between a fluorescently labeled copper protein (i.e. azurin (CuAz), from *Pseudomonas aeruginosa*) and gold nanoparticles (AuNPs) at the single-molecule level, where the fluorescence intensity reflects the redox state of the protein's active site. The labeled CuAz is immobilized on AuNPs of varying size (1.4-80 nm). The Zn substituted variant is used as a control. Intensity and life time variations were analyzed quantitatively by taking into account the influence of the AuNP

on the fluorophore excitation rate and quantum yield. We find that attachment of fluorescently labeled CuAz to a AuNP may lead to a tenfold enhancement of the sensitivity for detecting a redox change of the protein. These findings open new ways to study hitherto unexplored mechanistic details of oxido-reductases. They are also of relevance for the design of biosensors and devices for electronic data storage.

In Chapter 5 the newly developed FluRedox detection scheme (see Chapter 1, Introduction) was used to monitor the kinetics and mechanism of enzyme activity. In particular, this method allows the turn-over of the so-called T1-type redox centers of copper-containing oxido-reductases to be observed in real time and with single-molecule sensitivity. In this study we have used the blue nitrite reductase (bNiR) from *Alcaligenes xylosoxidans* whose T1 copper site exhibits a pronounced absorbance around 600 nm, but only when oxidized. This results in an enhanced FRET contrast of the attached fluorophore, between the reduced and oxidized forms of the T1 copper site of bNiR. Two populations of single NiR molecules can be distinguished experimentally, exhibiting different turn-over rates. The relative size of the populations varies with substrate (nitrite) concentration. The two populations are tentatively connected with two enzymatic routes: the ‘reduction first’ and the ‘binding first’ pathway.