Novel Photonic Technique Creates Micrometer Resolution Multi-sensor Arrays and Provides a New Approach to Coupling of Genes, Nucleic Acids, Peptide Hormones and Drugs to Nanoparticle Carriers

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ABSTRACT

Photonic induced immobilisation is a novel technology that results in spatially oriented and localised covalent coupling of biomolecules onto thiol reactive surfaces. The reaction mechanism behind the new technology involves light induced breakage of disulphide bridges upon UV illumination of aromatic amino acids, resulting in the formation of free, reactive thiol groups that will form covalent bonds with thiol reactive surfaces. The new precision immobilisation technique allows for micrometer immobilisation of pharmaceutically relevant biomolecules, allowing for the creation of dense multi-sensor arrays. It provides spatially controlled molecular immobilisation since the immobilisation of each biomolecule can be limited to the focal point of illumination with dimensions as small as a few micrometers. This new technology is ideal to couple drugs, proteins, peptides and other molecules to nanoparticles such as gold nanospheres which can be used as molecular carriers into cells for therapeutical purposes.

Keywords: biosensor, drug carrier, light induced immobilization, immunoglobulins, microarrays

1 INTRODUCTION

In the area of photosynthesis an impressive amount of knowledge has been obtained on how photons from the visual part of the electromagnetic spectrum are converted into high energy metabolites. In contrast, comparatively little is known at the molecular level about the molecular effects of UV photons on proteins. The possibility of engineering the structure and function of molecules with light is a fascinating field of science since ultimately it will allow for the control of chemical reaction pathways. The present paper presents our present understanding of a protein based system that actively is harvesting photons in the mid-UV range (260-300 nm), as well as the applications of such a reaction for immobilizing proteins potentially with micrometer precision. A new and rather interesting effect of UV-light interaction with biomolecules is that UV-radiation absorbed by aromatic residues induces disruption of nearby disulphide bridges [1-3]. The molecule is left with free, reactive thiol groups that can enter chemical reactions with a vast range of applications. The light induced mechanisms leads to a new way of immobilizing biomolecules onto surfaces leading to a new technology for the creation of sensor microarrays [4-7].

The value of immobilisation techniques is demonstrated by the recent boost in the development of DNA and protein microarrays' technologies and is needed for the development of drug/gene delivery technologies for therapeutical purposes (e.g. cancer therapy, gene therapy). Common for most of the described immobilisation methods is their use of one or more thermochemical/chemical steps, sometimes involving reagents which are likely to have a degrading effect on the structure and/or function of the bound protein [8-12]. Also, commonly used protein immobilisation methods lead to a random orientation of the proteins immobilised on a carrier, with the significant risk of lower biological activity and raised detection limits. There is a clear need in the science of protein immobilisation to improve the immobilisation method, where the structural and functional properties of the immobilised component are retained and the orientation of the biomolecule can be controlled.

We here report applications of the new photonic induced method for immobilisation of proteins and other biomolecules onto a carrier via disulphide bonds. The light induced effect is has been observed with a vast range of proteins, from hydrolytic enzymes (lipases/esterases, lysozyme), proteases (human plasminogen), alkaline phosphatase, immunoglobulins' Fab fragment (e.g. antibody against PSA, prostate specific antigen), Major Histocompability Complex Class I protein [13], Pepsin and Trypsin. Bioinformatic studies show that the constellation of amino acid residues needed for the light induced mechanism is present in many protein families such as hydrolases, oxidoreductases, transferases, lyases, membrane receptor proteins and is present in all members of the immunoglobulin superfamily [14], making these

proteins candidates for photonic induced protein immobilisation. Immunoglobulins have a great biomedical relevance of in disease prognostic.

The new technology and its applications merge biotechnology, molecular biology, bioinformatics, nanotechnology, surface science, biophysics and physics. It has a large potential for the development of multipotent dense biosensors, bioelectronics, bioactive nanoparticles, surface chemistry – design of surfaces, and as a technology that will allow the creation of nanosized drug/gene carriers into the cells.

2 LIGHT INDUCED PROTEIN IMMOBILISATION

The reported new methodology results in precise knowledge of the proteins' attachment point to the surface (Figure 1) while preserving the native structural and functional properties of the immobilised protein, avoiding the use of one or more chemical/thermal steps. This new patented technology makes use of the spatial proximity between aromatic residues and disulphide bridges in proteins [15]. The molecular mechanistic aspects of light mediated SS-disruption has been investigated by Neves-Petersen et al., and involves an electron transfer mechanism between the aromatic residues and the disulphide bridge [16].

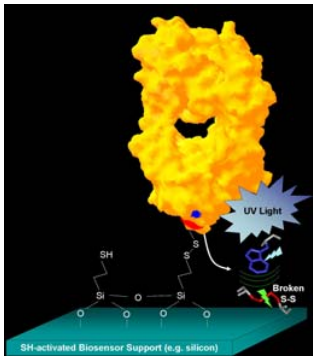

Figure 1: The principle of light induced immobilization sketched with tryptophan (blue) near a disulphide bridge (red) in a protein molecule (yellow). The surface can be gold or – as illustrated– a thiol-derivatized surface that results in the formation of a new disulphide bond between the surface and the protein.

3 CREATION OF PRECISION MICROARRAYS

This new technology provides spatially controlled molecular immobilisation since the UV-light induced immobilisation of each biomolecule to a support surface can be limited to the focal point of illumination with dimensions as small as a few micrometer, as confirmed by the protein microarray displayed in Figure 2.

The presented new technology allows for an extremely dense packing of identifiable and different molecules on a support surface ideal for charging microarrays with molecules aiming at the creation of protein nanobiosensors (see figure 2, 3) [4-7]. Since the density of immobilised sensor proteins in a small area is extremely high, the development of very advanced high density multipotent biosensors for human diagnostic is within reach.

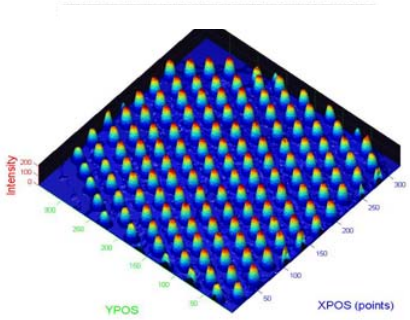

Figure 2: Fluorescence emission of light induced immobilised labelled cutinase (Alexa fluor 488) arrayed in a 15x15 protein microarray. Each spot has a diameter of around 140 µm and their centers are approximately 300 µm apart. Cutinase has been deposited with an arrayer. The whole protein array was illuminated at the same time by a 10x12 mm expanded 280nm light beam for 2 min, washed, and scanned. The control array that has not been UV illuminated showed no fluorescence emission.

4 EARLY DISEASE DIAGNOSTICS

We this new technology we can precisely immobilise a range of medically relevant sensors that can detect, e.g., the concentration of cancer markers in a bio fluid. We have successfully both prostate specific antigen (PSA) (see Figure 3) and the immunoglobulin specifically recognizing prostate specific antigen (PSA) (data not shown). The micrometer sized spot allow for a virtually unlimited number of protein spots. Given that suitable protein markers exists for all relevant diseases it is entirely feasible to test for a range of disease indicators in a single test.

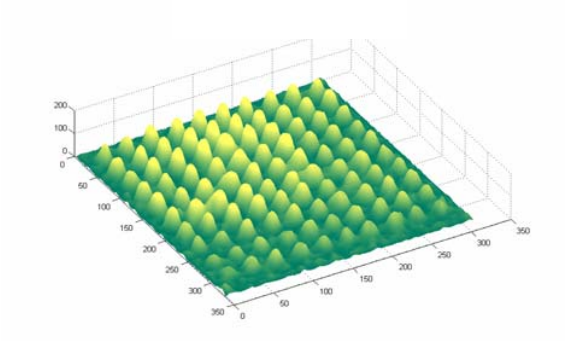

Figure 3: Fluorescence emission of light induced immobilised labelled prostate specific antigen (FITC) arrayed in a 10x10 protein microarray. The control array that has not been UV illuminated showed no fluorescence emission.

Due to the biomedical relevance of immunoblobulins in disease prognostic, our lab will now proceed with the construction of multiple densely packed Fab sensor surfaces. The Fab fragments from IgGs' are structurally identical, the variation lying mainly in the epitope region (the antigene recognitioin site). The "binding site" for UV-light induced immobilisation is fairly conserved and we have already shown that we can induce with light the immobilisation of Fab fragments. Once immobilised the different Fab fragments could detect a multitude of different proteins.

5 COUPLING BIOMOLECULES TO NANOPARTICLES AND BUILDING NEW BIOMATERIALS

This new technology opens exciting perspectives in nanomedicine, for the coupling of drugs, proteins or bioactive peptides, nucleic acids and other molecules to nano-sized materials, like nanoparticles. Possibly, two of the most promising fields of technological applications concern drug-delivery systems and molecular nanomotors. Nanoparticles are used successfully for the delivery of diverse drugs to mononuclear phagocytes, dendritic cells, endothelial cells, and cancer cells [17], to treat a wide range of diseases. These nanoparticles consist generally of biopolymers of diverse chemical nature, like lipids, peptides, polyethylene glycol and lactic acid. Their common properties being that they should be well-defined structurally, biocompatible and biodegradable [18]. In addition, these nanoparticles should be multi-functional: they should at least carry the drug and the chemical signals necessary for proper targeting, and proper delivery. This generally involves the simultaneous presence of ligands binding specific cell-receptors.

The photonic technology presented here offers the distinctive advantage of being able to precisely and selectively activate chemical groups (thiols), with laser beams, on the surface of nanomaterials. The level of precision is essentially dictated by the size of the beam's focal point. Hence, our photonic technology presents a possibility that no other technology (like nano-lithography, or nano-deposition) may currently offer: that is, to graft several molecules of different natures on the same nanoparticle, with some level of control about their respective spatial arrangement.

Another potential field of application lies in the design and fabrication of molecular nanomotors [19]. Biomolecular motors, in particular motor proteins, are ideally suited to introduce chemically powered movement of selected components into devices engineered at the micro- and nanoscale level [20]. Again, the intrinsic ability of our technology to precisely arrange molecules of different nature on sub-micron sized materials, like kinesin or myosin, looks particularly promising.

Also, the presented technology enables the creation of new light synthesized biopolymers. The polymerisation reaction can be induced upon light excitation of each monomer. Illumination will render the monomer reactive and able to polymerise via the thiol groups that it acquired upon illumination.

This new technology is ideal to couple drugs, proteins, peptides and other molecules to nanoparticles such as gold nanospheres which can subsequently be used as molecular carriers into cells for therapeutical purposes.

6 LIGHT SURPASSES PRESENT NANO- MICRO-ARRAY DISPENSING TECHNOLOGIES

Interestingly this technology may bypass the use of microdispensors broadly applied in microarray technology since the spatial resolution of this technique will be defined by the area of the sensor surface that is illuminated and not by the physical size of the dispensed droplets of sensor molecule. Nowadays, in order to create micro or nanoarrays, small droplets are dispensed by micro, nano-injection systems in order to create very tiny droplets that would immobilise on the sensor surface. Our new technology may bypass the use of microdispensors broadly applied in microarray technology since the spatial resolution of this technique will be defined by the area of the sensor surface that is illuminated and not by the physical size of the dispensed droplets of sensor molecules. Our technology makes no use of micro-fluid systems (pumps, valves) that easily get clogged.

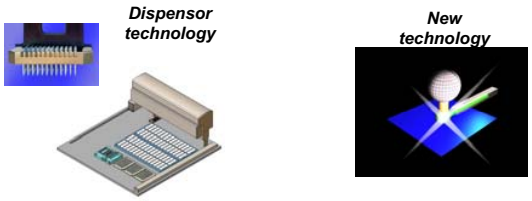

Figure 4: Light induced molecular immobilisation technology may bypass the use of classical microdispensors broadly applied in microarray technology.

7 POTENTIAL FOR PLASMONIC ENHANCEMENT

The use of plasmon assisted immobilization on, e.g., gold surfaces will increase the sensitivity of the biosensor through surface plasmon coupled emission (SPCE). Also, the area onto which the biosensor is immobilized onto could further be reduced since we would not be limited by the optical resolution of the excitation light (approximately half wavelength) but by the resolution of the plasmon wave (almost an order of magnitude shorter).

Biomolecules bound to the immobilized sensor molecules on the biosensor chip can be excited using surface plasmon resonances (SPR) in a thin metal film. In this case the fluorophores are excited by the evanescent field from the surface plasmons in the metal film that only penetrates about $\lambda/3$ into the sample. As a result only fluorophores close to the metal surface (i.e. those close to the sensor surface) will be excited thereby greatly reducing the background emission from the bulk solution above. When using visible light the metal film is typically a gold or silver coating with a thickness of 20-50 nm, but by using a thin film of aluminum instead allows this technique to be used in the UV too [21].

Upon excitation the fluorophores can couple back to the plasmons in the metal surface and result in so-called surface plasmon coupled emission (SPCE), which is both highly directional and polarized. The highly directional emission makes it easy to collect with high efficiency and the fact that the emission is highly polarized makes it possible to reduce the background signal from e.g. auto-fluorescence by inserting a polarizer in front of the detector. The angle at which the light is emitted depends on wavelength, so emission at different wavelengths will be spatially resolved without any additional optics. Increases in sensitivity of up to 1000 fold have been reported for this kind of surface plasmon assisted excitation and detection [22]. Recently it has been shown that SPCE based detection of fluorescence also can be used in optically dense media like whole blood [23].

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