

Arraying prostate specific antigen PSA and Fab anti-PSA using light-assisted molecular immobilization technology

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Abstract: We here report for the first time the creation of prostate specific antigen (PSA) and Fab anti-PSA biosensor arrays using UV light-assisted molecular immobilization (LAMI), aiming at the detection and quantification of PSA, a cancer marker. The technology involves formation of free, reactive thiol groups upon UV excitation of protein aromatic residues located in spatial proximity of disulphide bridges, a conserved structural feature in both PSA and Fab molecules. The created thiol groups bind onto thiol reactive surfaces leading to oriented covalent protein immobilization. Protein activity was confirmed carrying out immunoassays: immobilized PSA was recognized by Fab anti-PSA in solution and immobilized Fab anti-PSA cross-reacted with PSA in solution. LAMI technology proved successful in immobilizing biomedically relevant molecules while preserving their activity, highlighting that insight into how light interacts with biomolecules may lead to new biophotonic technologies. Our work focused on the application of our new engineering principles to the design, analysis, construction, and manipulation of biological systems, and on the discovery and application of new engineering principles inspired by the properties of biological systems.

Keywords: cancer biomarker detection; light-assisted molecular immobilization; prostate specific antigen; Fab antibody fragment

Introduction

Prostate specific antigen, PSA, is a valuable biomarker for prostate cancer screening. Quantification of PSA levels has been a common medical practice

Abbreviations: ACT, antichymotrypsin; AF, Alexa fluor; BHP, benign hyperplasia; Cys, cysteine; Fab, fragment of antigen binding; FITC, fluorescein isothiocyanate; IgG, immunoglobulin G; LAMI, light-assisted molecular immobilization; Mab, monoclonal antibody; PSA, prostate specific antigen; Trp, tryptophan; UV, ultraviolet.

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because elevated PSA levels in blood is correlated with the development of prostatic and breast cancer.^{1,2} Several methods for PSA detection are reported.^{3–12} In men, PSA is expressed by prostate gland cells. In women, it is secreted in the periurethral gland¹³ and in the breast.^{14,15} PSA levels in female breast tissue are generally quite low, increasing during the onset of benign diseases. PSA exists in different forms: free or complexed with various proteinase inhibitors, such as alpha-1-antichymotrypsin (ACT) and PSA-2-macroglobulin. PSA-ACT and free PSA are the two dominant forms of PSA contributing to the total PSA serum concentration. Only trace amounts of other PSA complexes are found with intertrypsin inhibitor or 1-protease inhibitor. PSA levels rise during the onset of prostate cancer or other disorders involving PSA secreting organs. The higher a man's

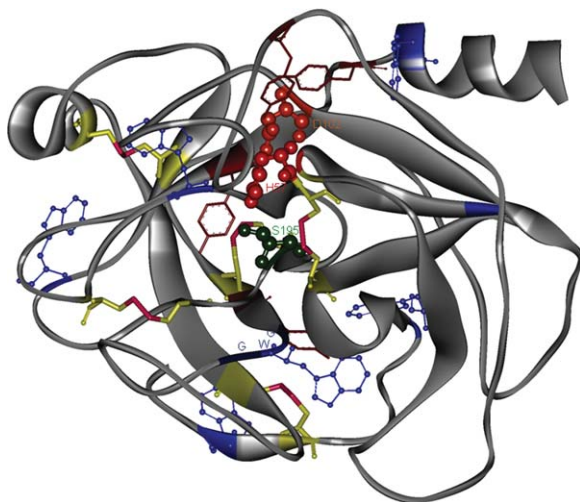


Figure 1. 3D representation of PSA (1pfa.pdb). Catalytic Ser195, His57, Asp102 is highlighted in big ball and stick. Trp are highlighted in small ball and stick. Tyr are highlighted as sticks. Cys are highlighted as sticks.

PSA level is, the more likely prostate cancer is. Currently, a blood test for PSA level measurement is the most effective method for early prostate cancer detection. The review by Healy *et al.*¹⁶ highlights the performance of current and novel technologies used for PSA detection, done using antibodies specific for the different forms of PSA and for the different PSA epitopes.

As the demand for new biosensors for early disease diagnostics has increased, the scientific community has intensified their research into developing new biosensors design, including the development of new immobilization technologies. We have previously shown that one can immobilize proteins onto a thiol reactive surface using UV light-assisted molecular immobilization (LAMI).^{17,18} Our bioinformatic studies reveal that both PSA and Fab fragments have aromatic residues in close spatial proximity of disulphide bridges, making both proteins putative good candidates for LAMI technology. In this article, we demonstrate that light-assisted immobilization of free PSA and Fab anti-PSA onto thiol-functionalized optical flat slides leads to functional protein arrays. Immunoassays confirmed protein activity.

Results

In Figure 1, the 3D structure of PSA is displayed. The protein consists of two 6-strand antiparallel β -barrels and three α -helices. The catalytic site (Ser 195, His 57, and Asp 102) is conserved and located in a cleft between two β -barrels.¹⁹ Multiple sequence alignments show conservation of the five disulphide bridges. The sequence also contains the GWG motif, which is a typical pattern present in many proteins with proteolytic activity.²⁰ This motif contains a Trp located in the β -barrel nearby disulphide bond Cys 157-Cys 22. The shortest distance between an atom

in Trp 20 and Cys 157 is 5.3 Å. The close proximity of disulphide bridges to the aromatic residues is a feature that makes this protein a good candidate for LAMI technology. Degree of labeling (DOL) in PSA-AF555 and PSA-AF647 was 3.7 and 1.2, respectively. The concentration of labeled Fab-AF647 5A10 anti-PSA was 0.9 μ M and the DOL was 7.

Microarrays visualization

Full PSA and Fab microarrays were imaged with the scanner [Fig. 2(A,B), respectively]. The spatial resolution in the microscope images was $\sim 1 \mu$ m, allowing to image the immobilization pattern achieved in each array spot [Fig. 3, 7(A,B)]. Figure 3 shows that each spot is $\sim 25 \mu$ m in diameter, pitch is $\sim 42 \mu$ m and that using a tighter focused UV beam resulted in a 5–6 μ m spot size.

In Figure 2(A), the intensity of light scattered by 4×5 arrayed PSA-FITC is displayed. Uniform intensity profiles along both axes are observed. Spots are well resolved since nonilluminated protein has been washed away from regions of the slide that have not been illuminated. No signal was detected in the red channel (data not shown). The histogram of the spots' mean fluorescent intensity was well fitted by a normal probability distribution function. The minimum PSA-FITC concentration arrayed (100 ms exposure) visualized in the scattered mode of the scanner was 0.53 μ M. Arraying with tighter focused UV-light and 100 ms exposure allowed to detect the fluorescence of 7.1 pM immobilized PSA-AF555.

Immobilized Fab-AF647 5A10 anti-PSA has been imaged with the scanner in both channels and in a fluorescence microscope. Figure 2(B) clearly confirms light-induced immobilization of Fab fragments: a 7×7 array of immobilized Fab is displayed. The horizontal and vertical fluorescence emission intensity profiles are uniform and the spots are well resolved. The histogram of the spots' mean fluorescent intensity was well fitted by a Weibull probability distribution function. LAMI technology achieved uniform distribution of the spots' area, calculated by the mean number of pixel/spot (not shown). The lowest concentration of Fab-AF647 visualized was 0.9 nM, when immobilized with 100 ms exposure.

Fab-AF488 anti-PSA purified in our laboratory was also arrayed. A well resolved 5×5 array could be seen in the green channel of the scanner and no fluorescence signal was detected in the red channel (not shown). In Figure 4, a confocal image displaying the fluorescence profile of a single spot in the Fab-AF488 array is shown. Fluorescence emitted from the spot area is uniform, confirming homogeneous protein immobilization. The vertical and horizontal fluorescence intensity profiles across the central part of the fluorescent spot are displayed [Fig. 4(A)]. The full width at half maximum is less than or equal to 1 μ m.

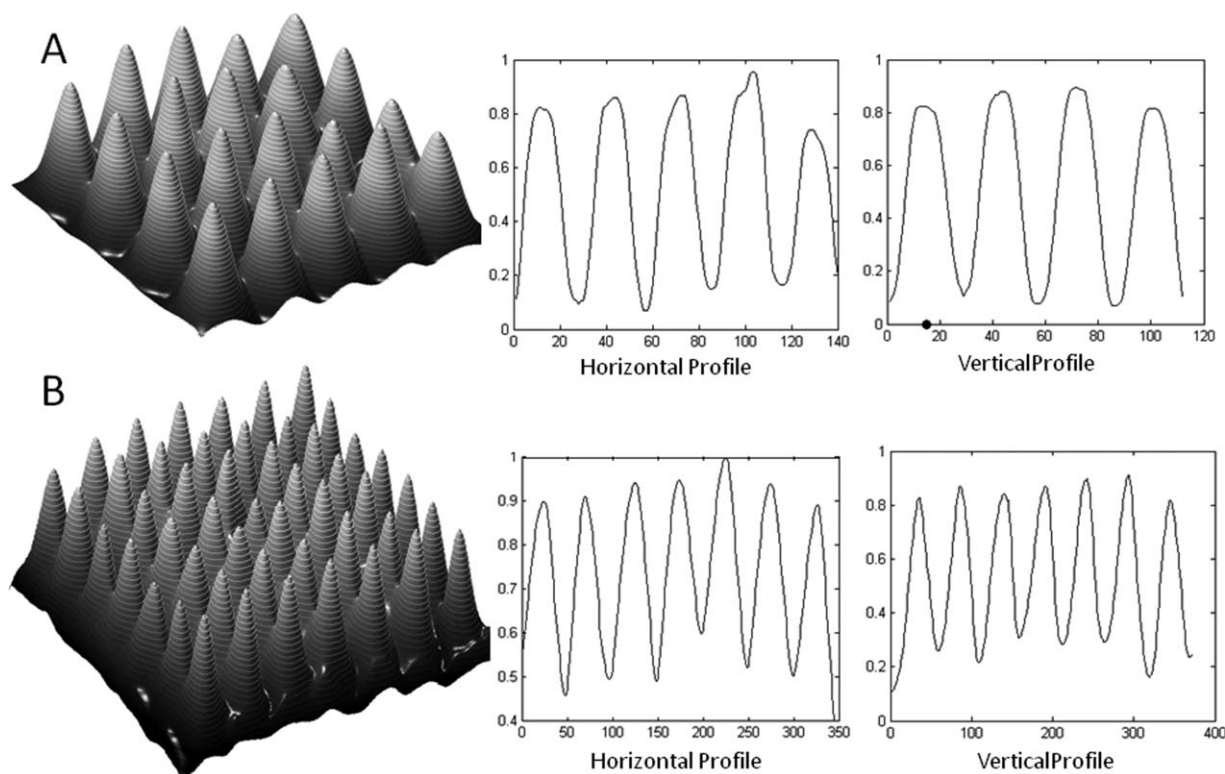


Figure 2. (A) 3D image analysis of arrayed PSA-FITC with vertical and horizontal fluorescence intensity profiles; (B) 3D image analysis of arrayed Fab-AF647 5A10 anti-PSA with a vertical and horizontal fluorescence intensity profiles.

Immunoassays

In Figure 5(A,B), the strategy for Fab immobilization followed by recognition of PSA and the strategy for PSA immobilization followed by Fab recognition is depicted, respectively. In Figure 6, the fluorescence emission intensity of Fab-AF647 5A10 after cross-reacting with immobilized PSA-FITC is displayed. The PSA concentration used to create the array was 1 μM . This array has been washed before

incubation with Fab, thus the exact concentration of immobilized PSA is not known. It can only be inferred from the concentration of Fab detected. Before carrying out the cross-reaction, the extrinsic PSA-FITC fluorescence was imaged in the red channel, as a negative control. No signal is observed in the red channel (not shown).

Immunoreactivity of immobilized PSA-AF647 has also been tested upon incubation with Fab-AF488 anti-PSA. After incubation, slides were washed and imaged in the microscope. Green fluorescence is observed in each array spot, confirming the cross-reaction between Fab and immobilized PSA [Fig. 7(A,B)]. BNIP image analysis of Figure 7(B) demonstrates colocalization of the two fluorophores, one linked to PSA and the other to the Fab molecule. As a negative control, labeled BSA was immobilized onto the same slide. It was incubated using the same conditions as PSA- and Fab-arrayed proteins. The scanner and the fluorescence microscope images showed no detectable signal.

The activity of immobilized Fab-AF647 5A10 anti-PSA is shown in Figure 7(C,D), where immobilized Fab is detected by PSA-AF555. The original Fab concentration used to create the Fab array has been 0.5 μM . This array has been washed before incubation with PSA. Also in this case, the exact concentration of immobilized Fab is not known and can only be inferred from the concentration of PSA detected through the immunoassay.

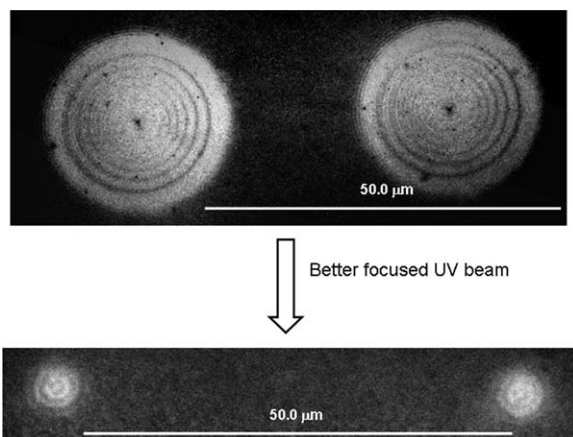


Figure 3. Dimensions of a typical array spot imaged with fluorescence microscope: after tighter focus of the UV light used to immobilize the protein (PSA-AF555), the spot dimensions decrease from 25 to 5 micrometer.

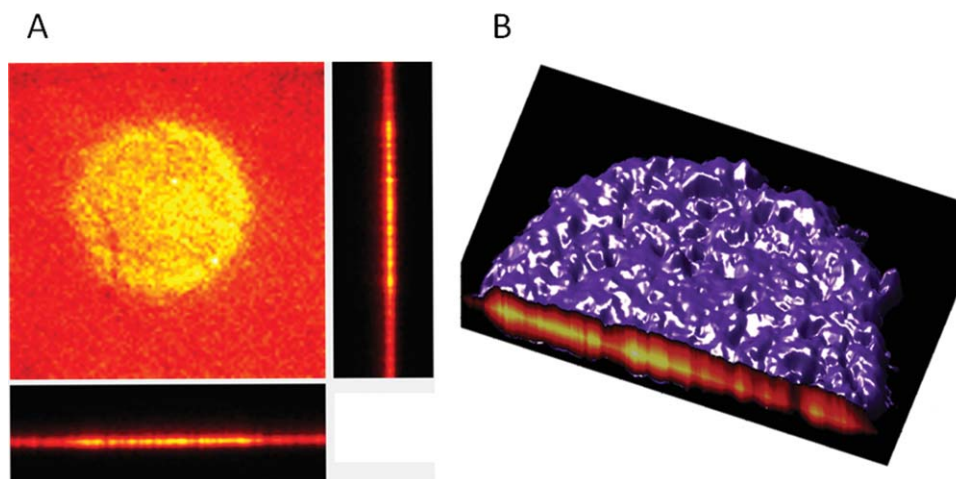


Figure 4. (A) Confocal fluorescence microscope image of a single spot in a Fab anti-PSA array showing the fluorescence in a particular z-plane, with vertical and horizontal fluorescence intensity profiles across the central part of the peak. (B) Confocal fluorescence microscope image of a single spot in a Fab anti-PSA array showing the fluorescence in the whole 3D volume of the spot.

Discussion

We have presented that LAMI is a powerful technique for the creation of PSA and Fab microarrays, allowing for rapid, covalent immobilization of proteins onto sensor surfaces. Arrays show a high level of reproducibility and uniformity [Figs. 2(A,B)]. In this study, the spot thickness has an upper limit of 1 μm (Fig. 4). In a previous study, we have reported that LAMI has achieved uniform distribution of a monolayer of Fab molecules.¹⁸ Among the different immobilization strategies for generating protein biochips, LAMI is considered to be the one offering uniform orientation and covalent binding to the surface.²¹ LAMI requires the presence of aromatic residues and disulphide bridges in the protein to be

immobilized. The 3D structure of PSA shows that this protein has aromatic residues in close spatial proximity of disulphide bridges (Fig. 1). It has also been reported that in Fab fragments, two Cys residues that form an intradomain disulphide bridge between the two faces of the β -sheet are located nearby a Trp residue. The average distance between these residues is 6.9 Å. It has been shown that these residues are conserved in more than 60 different Fabs,²² which makes Fab fragments excellent candidate for LAMI as well.

Fluorescence immunoassays confirmed the binding capacity of immobilized PSA and Fab fragments after LAMI (Fig. 6). This proves that after LAMI, the conformation of PSA's epitope and the Fab anti-

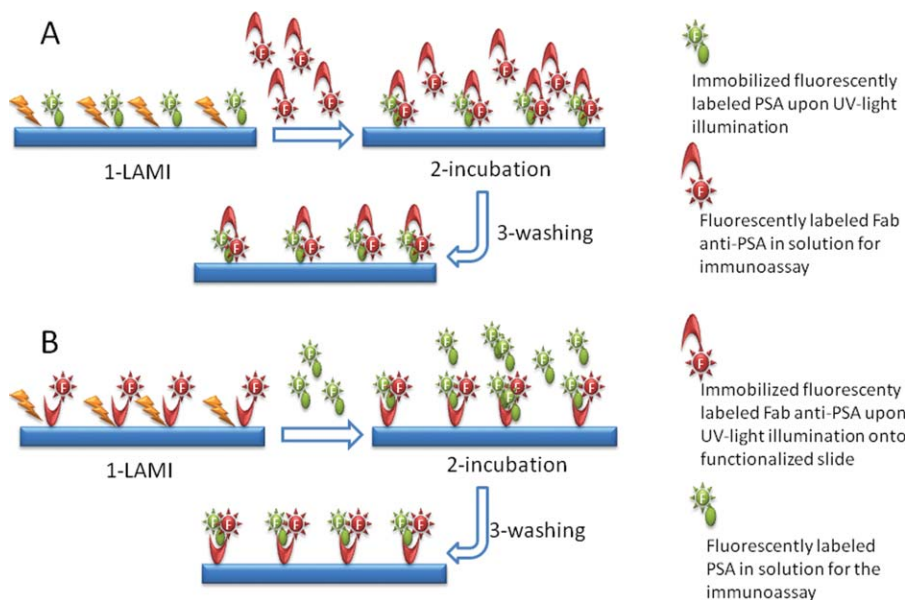


Figure 5. (A) Light-induced immobilization of PSA followed by immunoreaction with Fab anti-PSA; (B) Light-induced immobilization of Fab anti-PSA followed by immunoreaction with PSA.

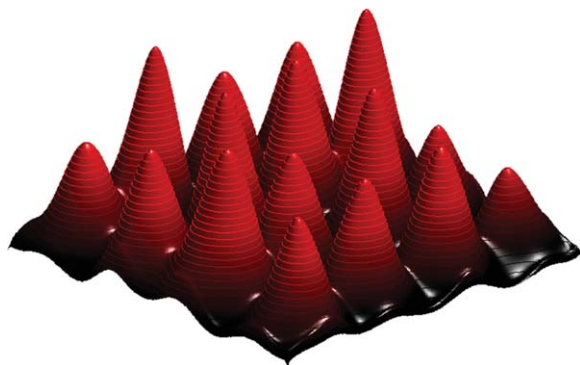


Figure 6. 3D visualization of the fluorescence intensity of Fab-AF647 anti-PSA-5A10 after cross-reaction with immobilized PSA.

PSA's binding site remains intact, rendering them active. Negative controls confirmed the specificity of the immunoreactions. Fluorescence colocalization analysis of Figure 7(D) reveals the presence of a red fluorescence plane, suggesting possible energy transfer between AF555 attached to PSA molecules and AF647 attached to Fab anti-PSA. Energy transfer is made possible by the immunoreaction between these

molecules. When observing the fluorescence patterns at the single spot level [Figs. 7(A,C)], concentric fluorescent rings are observed. Any optical system has a finite aperture, leading to the appearance of Airy diffraction patterns around any objects visible in the optical field. Proteins were hereby immobilized according to the light diffraction pattern with which they were illuminated. We have previously reported protein immobilization according to the diffraction patterns of UV light.^{23,24}

Black *et al.* have reported the development and characterization, in terms of cross-reactivity and antigen affinity, of 11 anti-PSA monoclonal antibodies for the development of a sensitive immunofluorimetric assay for serum PSA detection.^{25,26} In this study, we have worked with two Fab fragments: Fab fragment of Mab 5A10 that specifically recognizes an epitope in free PSA and a Fab fragment purified in our laboratory from IgG. The smaller size of Fabs compared with antibodies can be advantageous because smaller MW is likely to lead to an increase in the number of immobilized molecules per square area. The recombinant Fab fragments are very specific against the antigen and their use can reduce

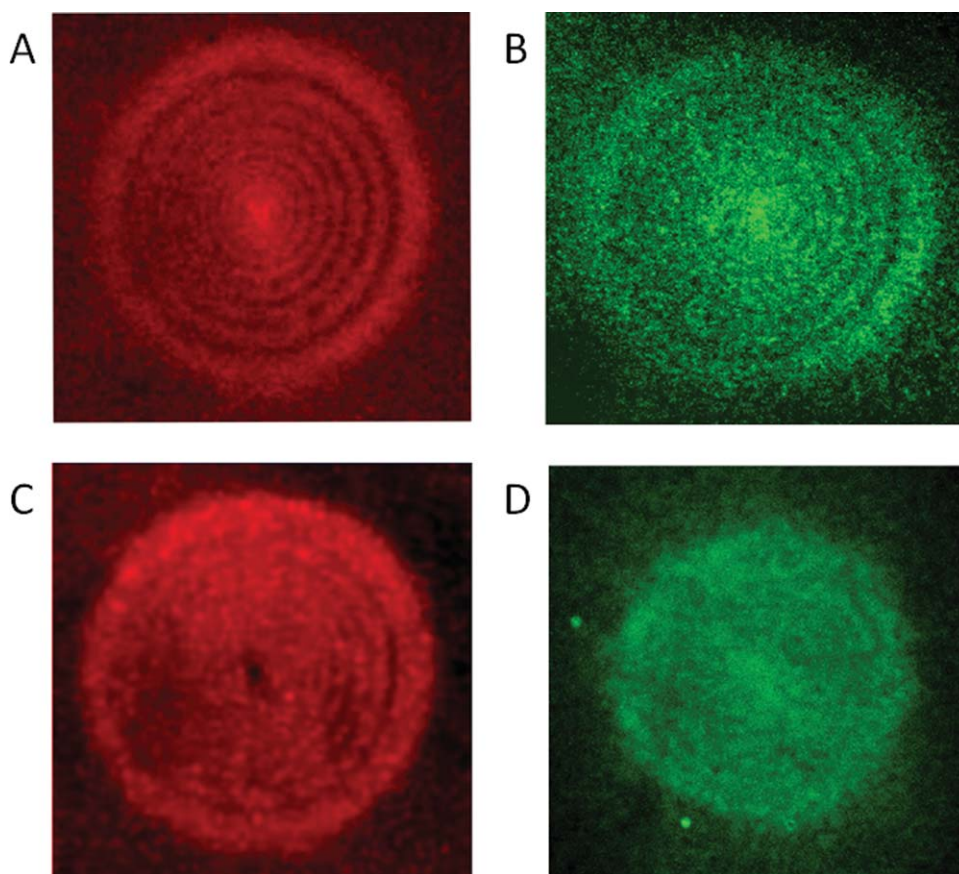


Figure 7. (A) Fluorescence microscope image showing red fluorescence from immobilized PSA-AF647; (B) Fluorescence microscope image showing green fluorescence from Fab-AF488 anti-PSA after incubation with immobilized PSA-AF647; (C) Fluorescence microscope image of immobilized Fab-AF647 5A10 anti-PSA; (D) Fluorescence microscope image showing green fluorescence from PSA-AF555 after incubation with immobilized Fab-AF647 5A10 anti-PSA.

false-positive and negative results by eliminating the effects of heterophilic part of antibodies.²⁶

Another important factor when designing an array is to optimize the number of sensor molecules that have the correct orientation once immobilized. This can be obtained by introducing a site-specific group, such as a thiol group, on the Fab fragments which can be done by genetic engineering²⁶ or using LAMI technology because UV excitation of aromatic residues induces disruption of nearby disulphide bridges, leading to free thiol groups in the molecule.^{17,27} Immobilization yield is a function of protein concentration and illumination time. Increasing illumination time allows for arraying proteins using less concentrated protein stock solutions. This is an advantage when the biomolecules are expensive or scarce. On the other hand, the tighter we focus the UV beam used for immobilization the smaller each array spot will be (Fig. 3), allowing us to engineer the density of the array. The initial 25–30 μm spot size was achieved when the illumination setup was $\sim 2\text{--}4\text{ }\mu\text{m}$ out of focus. Tighter focusing lead to a spot size of 5–6 μm . Smaller spots have larger fluorescence intensity signal per unit area because a larger number of proteins will be immobilized in a smaller area. This will improve the signal to noise level significantly. Currently, we are able to detect 7 pM (0.23 ng/mL) PSA concentration. LAMI technology can provide the immobilization of very low amount of proteins and the creation of several different arrays on the same slide.

Materials and Methods

Materials

PSA and PSA-FITC were purchased from Research Diagnostics (RDI, Fitzgerald Industries International, MA). Recombinant Fab 5A10 anti-PSA was supplied by Turku University. The parent monoclonal antibody 5A10 was raised against seminal plasma PSA and recognizes an epitope accessible only in the free form of the antigen not in complex to any protease inhibitor.¹³ The other reported Fab (Fab-AF488) was prepared by the Nanobio group from full IgG anti-PSA purchased from RDI using Pierce's Fab preparation kit (product 44885, Thermo Fisher Scientific, IL). Optical flat quartz slides (2 nm average flatness) were purchased from ArrayIt (Arrayit Corporation, CA). Fluorophores were purchased from Invitrogen. 3-mercaptopropyl-trimethoxysilane and *m*-xylene (99%) were obtained respectively from Merck 63800 (Merck, NJ) and Acros Organics 1808600100 (Acros Organics BVBA, Janssen Pharmaceutica, Belgium). Potassium persulphate (99% of $\text{K}_2\text{S}_2\text{O}_8$) was purchased from Acros Organics 20201.

Protein labeling

Ten microliters of 3.3 mg/mL PSA solution was labeled with AF555 and AF647, and 25 μL of 2 mg/mL Fab 5A10 anti-PSA was labeled with AF647 following the Invitrogen's protocol (Invitrogen, Paisley, UK). The same procedure was followed to label 100 μL of 1 mg/mL Fab anti-PSA purified in our laboratory with AF488.

Absorption and fluorescence spectroscopy

Thermo scientific UV–visible spectrophotometer (VWK International UV1 v4.60) was used to characterize protein's concentration and DOL. Cuvette path length was 1 cm. Absorbance spectra were acquired between 220 and 700 nm. Molar extinction coefficients of AF555, AF647, and AF488 used were $150,000\text{ cm}^{-1}\text{ M}^{-1}$ (at 555 nm), $239,000\text{ cm}^{-1}\text{ M}^{-1}$ (at 647 nm), and $71,000\text{ cm}^{-1}\text{ M}^{-1}$ (at 488 nm), respectively.

Fluorescence characterization of native and labeled proteins was carried out using a RTC 2000 PTI spectrofluorimeter (Photon Technology International, NJ) using as excitation light the characteristic excitation wavelength of each fluorophore. Cuvette path length was 1 cm. All measurements were carried out at 20°C using 5-nm slit width. Lamp power at 295 nm was 142 nW at the sample holder location.

Antibody digestion and Fab purification

IgG antifree PSA (0.35 mg/mL) were digested with papain following Pierce kit's protocol. Purity of Fab anti-PSA was investigated by 12% SDS-PAGE. After purification, the Fab was dialyzed against PBS 1X pH7.5 overnight at 4°C.

Slides derivatization

Optically flat quartz slides were immersed into sulphuric acid for 1 h, rinsed thoroughly with water, and hydroxylated for 1 h with 5% (w/v) $\text{K}_2\text{S}_2\text{O}_8$. Slides were afterward thiolated upon incubation with 400- μL 0.3% [v/v] 3-mercaptopropyl-trimethoxysilane in *m*-xylene for 30 min at room temperature. The surface was rinsed with pure xylene before flushing thoroughly with ethanol and deionized water and dried using compressed air.

LAMI optical setup

Mode-locked Ti:Sapphire femto-second laser (Tsunami 3960, Spectra-Physics, CA) was pumped by a high-power diode laser (Millennia V, Spectra-Physics). Average power of the 80 fs 840 nm pulses was 910 μW . Pulses were sent through a pulse picker (Model 3980, Spectra-Physics) reducing the repetition rate to 8 MHz and passed through a frequency doubler/tripler (GWU, Spectra-Physics) to generate 280 nm UV pulses (average power 0.9 mW) needed for protein immobilization. UV pulses was

sent through a computer controlled shutter and expanded by two quartz lenses. Afterward the beam passed through an iris diaphragm before being reflected off 90° by a mirror. The beam was focused onto the sample by a 18-mm focal length, half-inch diameter plano-concave quartz lens. The beam has been expanded enough to slightly overfill the back aperture of the focusing lens and focused on the slide surface, inducing protein immobilization. The slide was mounted on a three axis parallel-flexure translation stage equipped with three computer-controlled stepper-motor actuators (Duroux *et al.*, 2007). Shutter and translation stage were controlled by homemade software programmed in LabView 8.0, which could be run in an array mode. The number of rows and columns in the array, the exposure time (ms), and the distance (μm) between successive rows and columns was specified. One microliter of protein solution was deposited on the slide. Arrays were made on a dried film with a distance of 150 μm between spots. Average spot size was 20–30 μm with 75 μm pitch. Tighter focus leads to 5–6 μM spots. Immobilization time was 100/1000 ms per spot. When immobilizing one spot, the translation stage moved the sample into position and the shutter opened to expose the sample for a preset period of time.

Immobilization of PSA and Fab onto slides

One microliter of a 1 micromolar PSA-AF555 solution in 20 mM Tris-HCl pH 8 was illuminated according to an array pattern. One microliter of 0.45 μM of Fab-AF647 5A10 anti-PSA in Tris-HCl 20 mM pH 8 was immobilized with 280 nm light (0.9 mW), 100 ms time exposure. One microliter of 2.5 μM stock solution of Fab-AF488 anti-PSA purified in our laboratory was immobilized onto the thiol-derivatized slide with 280 nm light (0.9 mW) and 100 ms exposure. Each slide carrying immobilized PSA and Fab was washed overnight with CHAPS 0.5% to remove noncovalently immobilized protein.

Once the reactivity between immobilized PSA-FITC and the Fab-AF647 5A10 anti-PSA was confirmed, experiments were repeated lowering PSA concentration. We aimed at finding the lower concentration of immobilized PSA that could be detected with this immunoassay. Experiments were carried out spotting 1 μL PSA solution with concentrations between 10 and 0.053 μM . Exposure time was 100 or 1000 ms.

Arrays visualization

Tecan LS 200 scanner was used to visualize immobilized proteins with 6 μm resolution (Tecan Group, Männedorf, Switzerland). Excitation at 532 nm was done to image AF555 fluorescence emission [cy3 filter, em 575/50 nm (± 20 nm)]. Excitation at 633 nm was used to image AF 647 fluorescence emission [cy5 filter, em 670/25 nm (± 20 nm)]. PMT gain was

set between 100 and 200 depending on sample concentration.

Protein arrays were also imaged with a fluorescence microscope (Olympus IX71, inverted optics, Olympus, PA) through oil-immersion 60x objective (NA = 1.42). Snapshots were recorded with a CCD camera (DP70, Olympus). To image FITC, AF488, and AF555, filter cube 2 was used U-MWIB3 (exc 460–495 nm, em 510–550 nm, and dichroic mirror at 505 nm). For AF647 imaging, filter cube 4 U-MWIY2 was used (exc 545–580 nm, em 610 nm, and dichroic mirror at 600 nm). The arrayed Fab-AF488 anti-PSA was imaged using a confocal Olympus-FV1000 microscope.

Binding of Fab anti-PSA to immobilized PSA

The reactivity of immobilized PSA-FITC against Fab-AF647 5A10 anti-PSA was tested. Before incubation, immobilized PSA was imaged by looking at the light scattered by the PSA array illuminated in the scanner (green channel) and by looking at the FITC fluorescence with the fluorescence microscope ($\lambda_{\text{ex}} = 532$ nm, $\lambda_{\text{em}} = 570$ nm). Immobilized PSA was incubated overnight at 4°C upon constant agitation with 0.88 nM Fab-AF647 5A10 anti-PSA diluted 1000x in milk 5%/PBS1X from the mother solution (0.88 μM). To minimize the incubation time while still achieving proof-of-concept regarding the reactivity of the immobilized PSA, 1- μM range Fab solution was used. Afterward, the slide was washed four times with CHAPS 0.5%, changing buffer every 10 min to eliminate non reacted Fab. Cross-reaction was verified upon imaging the fluorescence of Fab-AF647 5A10 anti-PSA (exc 633 nm and em 670 nm).

Binding of PSA to immobilized Fab anti-PSA

The reactivity of the immobilized Fab 5A10 anti-PSA against PSA was tested. Seven micromolar of PSA-AF555 was diluted 1:1000 in milk 5%/PBS1X and incubated overnight at 4°C upon constant agitation with immobilized Fab. The slide was washed with CHAPS 0.5% four times each 10 min. Cross-reaction was verified upon imaging the array in the scanner (green channel, $\lambda_{\text{ex}} = 532$ nm, $\lambda_{\text{em}} = 570$ nm) and imaging AF555 fluorescence with the microscope (filter cube 4, $\lambda_{\text{ex}} = 545$ –580 nm, and $\lambda_{\text{em}} = 610$ nm).

After immobilization of Fab and slide washing (as previously described), PSA-AF647 was incubated with immobilized Fab. PSA solution (0.1 $\mu\text{g/mL}$) was diluted 1000x in milk 5%/PBS1X (0.1 ng/mL, 4 pM), to minimize the incubation time while still achieving proof-of-concept regarding the activity of the immobilized Fab fragments. The slide was incubated overnight with constant agitation at 4°C. Afterward, the slide was washed with CHAPS 0.5% four times changing the wash solution every 10 min. Binding between PSA and Fab was verified upon imaging the slide with the scanner ($\lambda_{\text{ex}} = 650$ nm and $\lambda_{\text{em}} =$

670nm) and imaging the arrays with a fluorescence microscope using filter cube 2 ($\lambda_{\text{ex}} = 460\text{--}495\text{ nm}$, $\lambda_{\text{em}} = 510\text{--}550\text{ nm}$, and dichroic mirror at 505 nm).

Negative control

As negative control, the arrays with immobilized proteins labeled with FITC, AF488, and AF555 were imaged in the red channel. These fluorophores have characteristic fluorescence emission in the green and their fluorescence is blocked in the red channel. Furthermore, slides with immobilized protein labeled with AF647, which emits red light, were imaged in the green channel. Another negative control was carried out by immobilizing BSA onto functionalized slides and cross-react BSA with the anti-PSA Fab or with PSA in the conditions above described. Negative controls were imaged in the scanner and in the microscope.

Image processing

MATLAB 2009b was used to develop a software package (BNIP-Pro) allowing for advanced analysis of microarray images. Confocal images have been further analyzed with BNIP Confocal Viewer. Stacks of confocal images were loaded into the program. After selecting the region of interest, as well as a threshold value for surface definition, a 3D-volume was displayed. The number of slices contributing to the image can be selected, as well as which region of interest in each slice. If the 3D volume was truncated in the final selection, the program will display the actual fluorescence intensity across that surface.

Colocalization analysis: the RGB image [Fig. 7(A)] was split into its three color planes. In the green plane, the spots were well defined. In the red plane, no clear spot were observable. However, after removing the zero frequency point in the image obtained after the red plane image was exposed to a 2D Fourier transform, we retained the high frequency component of the image. This component revealed weak, but clear definition of the same spots seen in the green image.

Conclusions

The goal of this article was to demonstrate that LAMI technology allows for the creation of microarrays of biomarkers of pharmaceutical importance such as PSA and Fab anti-PSA, remaining the immobilized proteins active. pM concentration of immobilized PSA has been detected with immunoassays. LAMI has is a competitive technology in terms of sensitivity, specificity, density, high signal-to-noise, and reproducibility.¹⁸

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