

Surface Plasmon Resonance Imaging to Study the Molecular Mechanism of Alzheimer's Disease.

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Abstract We have developed a state-of-the-art surface plasmon resonance imaging (SPRi) apparatus combined with microfluidics and used it to detect the binding of amyloid beta peptides to the lipid membrane, an interaction known to be involved in the molecular mechanism of amyloid toxicity in Alzheimer's disease, and to study protective effect of melatonin. Kinetics of binding was measured, and quantitative analysis was developed in order to detect melatonin partitioning into and amyloid binding to the membrane. We provided for the first time clear experimental evidence that the presence of melatonin in supported lipid membrane prevents the amyloid peptide from binding to the lipid membrane, thus gaining insight into the molecular mechanism of melatonin protection against amyloid toxicity in Alzheimer's disease.

Keywords - SPR; microfluidics; biosensor; Alzheimer's disease; amyloid; melatonin, lipid membrane.

I. INTRODUCTION

Surface plasmon resonance imaging (SPRi) is a well-established methodology for detecting adsorption of biomolecules to surfaces. SPRi has sufficient sensitivity to detect the binding bioaffinity to chemically modified gold thin films at concentrations down to less than 1nM. Surface plasmon resonance (SPR) biosensors are widely used to assess various biomolecular interactions. This label-free technique enables real-time measurements [1][2]. Commercial SPR biosensors are capable of detecting 1pg.mm² of adsorbed analytes with sensitivities of typically 10⁻⁵ refractive index unit (RIU), which works well for large biomolecules. For detecting smaller molecules at low concentrations, commercial SPR instrument is not sensitive enough. We have built a home-made apparatus in which SPR is combined with microfluidics and advanced optical imaging. We developed novel statistical data analysis and achieved a dynamic range of detection from 1 mg/mL down to 10⁻¹⁵ g/mL.

Alzheimer's disease (AD) is a progressive neurodegenerative disease associated with amyloid fibril formation in the brain. Amyloid beta (A β) is a peptide of 36 to 43 amino acids; it is well known as a component of amyloid plaques linked to AD, with the most toxic effect being associated with A β (1-42) peptide and its interaction with cellular membrane [3]. The exact molecular mechanism of amyloid fibril formation and toxicity is not well understood. The A β (1-42) fibrillization, including the

early stages of the aggregation process have been monitored using photonic sensors [4]. Melatonin is an important hormone produced in the pineal gland of the brain. It is responsible for regulating sleep/awake cycle and has been implicated in AD pathology, and has been shown to have protective effect against amyloid toxicity [5]. Although the molecular mechanism of this protection is not known, it is known that melatonin interacts with lipid membrane non-specifically [6] and may affect binding A β to the membrane.

We employed SPR imaging combined with microfluidics to investigate the interactions between the protein A β (1-42) and the 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) lipid bilayers in order to show the protective role played by the melatonin in relation to AD.

II. EXPERIMENTAL DETAILS

Figure 1 shows a schematic of the SPRi set up. The wavelength of the incident light of a halogen lamp was scanned using a monochromator, polarized, expanded and collimated using two lenses with focal lengths of 35 and 70 mm. The SPR chip consisted of SF - 10 glass slides coated on one side with a thin gold film (1nm Cr layer and 45nm Au layer) deposited by thermal evaporation. The expanded beam was coupled to the SPR chip in a Kretschmann configuration, through a high-index prism (RI = 1.72) mounted on a Newport SR50CC rotation stage, allowing the angle of incidence to be precisely controlled. The reflected light was then focused and collimated using a doublet of achromatic 200 mm focal length lenses onto the monochrome digital CMOS camera (1002×1004 pixels, at 8 or 10 bits per pixel and with up to 50 frames per second).

The microfluidic cell was fabricated by molding the SU8 master with polydimethylsiloxan (PDMS) cured and baked at 100°C for 30 min. It was coupled to the chip by exerting a gentle pressure. The microfluidic cell was then functionalized with PEG-silane to passivate the inner surfaces of the microfluidic cell and all tubings, in order to reduce adsorption. The flow was generated using a peristaltic pump operating at constant flow rate of 15 μ L/min.

Figure 1 shows the raw data acquired in reflection mode using the CDD camera. The reflectance R is obtained by dividing the intensities arising from the pixels inside the

channel by the reference area representative of the spectral and temporal fluctuations of the light source.

Since the microfluidic cell in PDMS can swell by 1% in volume when water is flown through the channels, the reference area was taken in a bare gold area, left in the air ($R = 0.9$). One pixel on the camera corresponds to an area of $3\mu\text{m} \times 3\mu\text{m}$ on the SPR chip. In each channel, the data of the five central pixels were averaged to provide one value for a given length x . Fig 1C illustrates the colored image of R for three channels of $100\mu\text{m}$ width and 2mm long filled with distilled water (in blue). The surface of the bare gold film (in blue) appears to be homogeneous. This technique enables real-time measurements. Figures 1B and D show changes in wavelength with time when vesicle solution of DPPC was injected into the channel and lipid bilayer was depositing onto the gold surface.

Supported lipid membranes were formed on the surface of the SPR sensor by method of vesicle fusion as previously described [7][8]. Lipid vesicle solutions were prepared by solubilizing dry powdered lipid (Avanti Polar Lipid, Alabaster, AL) in nanopure water and subjecting the solutions to cycles of sonication and mixing. Melatonin (Sigma) solution in water was added to the supported membrane at concentration of 0.4mM. Solution of $A\beta(1-42)$ (rPeptide, Bogart GA), pretreated to ensure monomeric form [9], was solubilized in HEPES buffer for a final concentration 500 $\mu\text{g}/\text{ml}$ and was added to the supported membrane with and without melatonin.

III. RESULTS AND DISCUSSION

Preliminary experiments have demonstrated that SPRi device is a promising sensor strategy for detecting melatonin and the binding of $A\beta$ to the lipid membrane. Microfluidics allows for parallel experiments at the same time. Four microfluidic channels were used to detect adsorption of a DPPC bilayer, adsorption of melatonin to the bilayer, and adsorption of $A\beta$ to the bilayer with and without melatonin. One channel with distilled and deionised water was used as a reference channel.

Figure 2A shows that the DPPC membrane absorbs the melatonin reaching a saturation point at around 3 hours. The kinetics of melatonin partitioning into the membrane was measured for the first time using this approach, Figure 2B. Melatonin saturation time is important for the next experiments when binding of amyloid to the supported membrane is measured.

Comparison of results is shown on Figures 2 A-F. Figure 2C and D show the adsorption of $A\beta$ on the surface of lipid membrane, and Figure 2E and F show adsorption of $A\beta$ into the lipid membrane, which was saturated with melatonin. We can clearly see that when melatonin is present in the membrane amyloid binding is significantly reduced.

IV. CONCLUSIONS

These results provided for the first time clear experimental evidence that the presence of melatonin in supported lipid membrane prevents $A\beta$ peptide from binding to the lipid membrane, thus clarifying the molecular mechanism of melatonin preventive role in amyloid toxicity.

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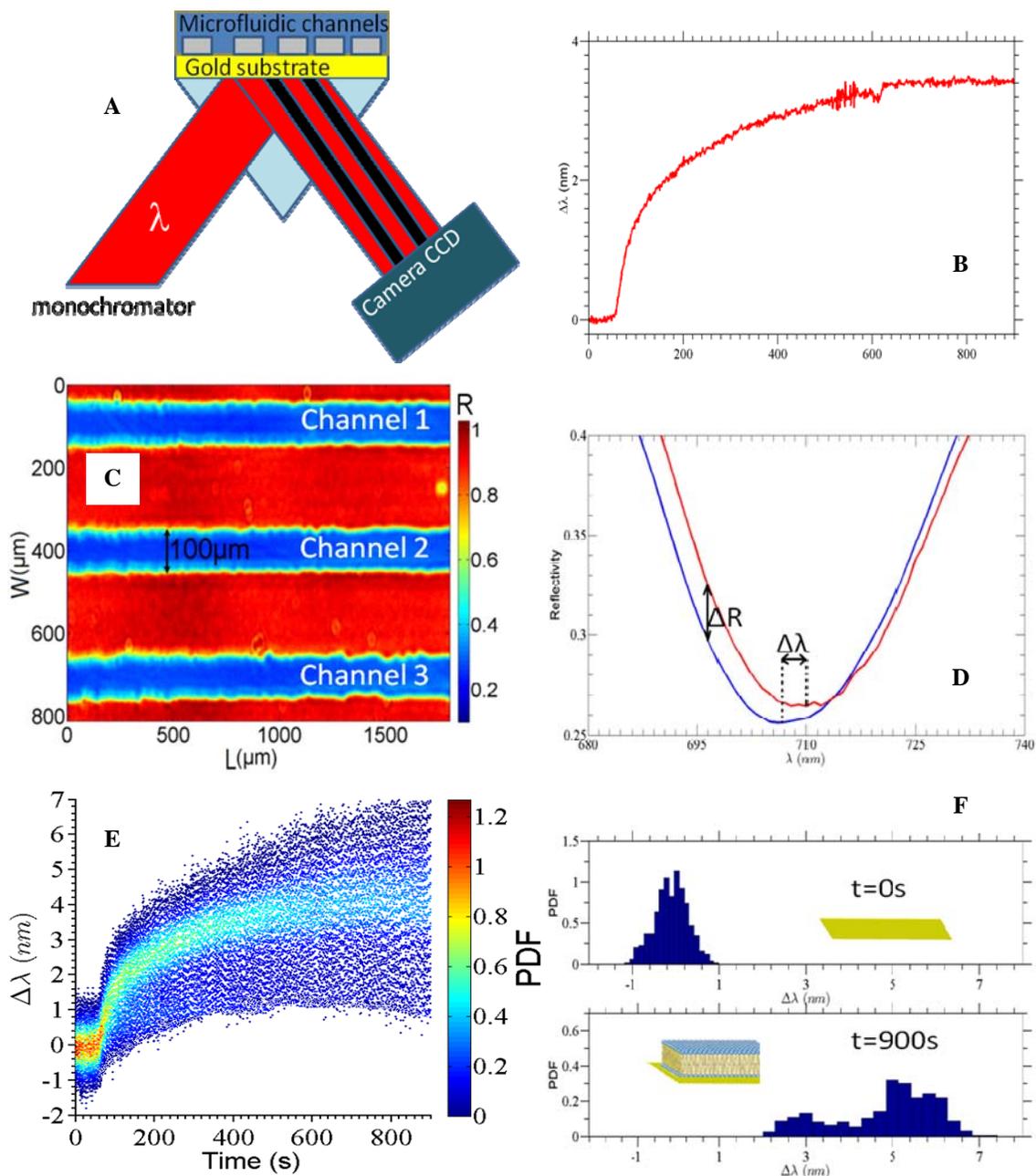


Figure 1. A- SPR set up combined with microfluidic; B- Kinetics of the adsorption of DPPC to gold substrate ; C- SPR image of three channels, blue- gold sensor surface, red- covered with polymer ; D- Spectral shift recorded before and after the adsorption ; E- Kinetics of the spectral distribution showing the probability density function (PDF) of adsorption of DPPC vesicles on gold ; E- Distribution of the shift in SPR wavelength before and after the adsorption.

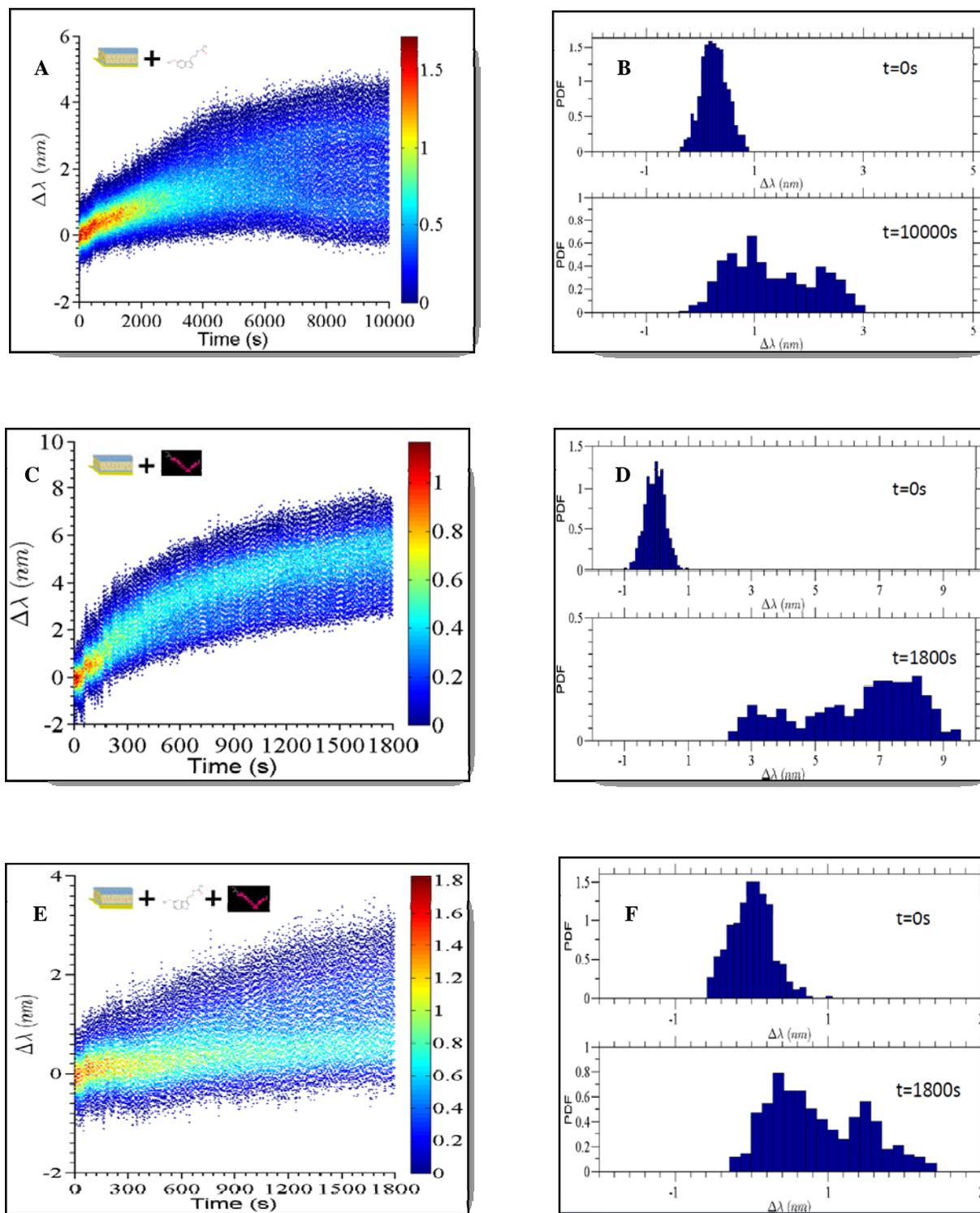


Figure 2. A and B - Kinetics of melatonin (2mM) partition into the DPPC membrane; C and D – adsorption of $A\beta$ to the lipid membrane without melatonin; D and F – adsorption of $A\beta$ to the lipid membrane saturated with melatonin