proceedings

MDPI

Abstract

Highly Sensitive Plasmon-Enhanced Spectroscopic Detection of Peptide-Antibody Interactions †

Aruna Chandra Singh [1], Divya Balakrishnan [1], Hugo Payen [1], Clara Sidhoum [1], Thomas Østerbye [2] and Sivashankar Krishnamoorthy [1,*]

[1] Materials Research and Technology, Luxembourg Institute of Science and Technology, 41, rue du Brill, L-4422 Belvaux, Luxembourg; aruna.singh@hahn-schickard.de (A.C.S.); divya.balakrishnan@list.lu (D.B.); hugo.payen@list.lu (H.P.); clsidhoum@gmail.com (C.S.)

[2] Laboratory of Experimental Immunology, Faculty of Health Sciences, University of Copenhagen, 2200 Copenhagen, Denmark; thos@sund.ku.dk

* Correspondence: sivashankar.krishnamoorthy@list.lu; Tel.: +352-621700467

† Presented at the XXXV EUROSENSORS Conference, Lecce, Italy, 10–13 September 2023.

Abstract: We demonstrate a highly sensitive plasmon-enhanced fluorescence sensor to detect antibodies to Cytomegalovirus (CMV), using their specific interaction with a peptide identified through in silico methods. The results show high promise for sensor miniaturization, ease of spatial multiplexing, high sensitivity, and quick response times. The developments are readily applicable to detect antibodies to range of other viruses (e.g., SARS-CoV-2 virus, Bird and Swine Flu).

Keywords: nanoplasmonics; metal-enhanced fluorescence; nanoarrays; immunoassays; surface-plasmon resonance; peptide-protein interactions; biosensor

Citation: Singh, A.C.; Balakrishnan, D.; Payen, H.; Sidhoum, C.; Østerbye, T.; Krishnamoorthy, S. Highly Sensitive Plasmon-Enhanced Spectroscopic Detection of Peptide-Antibody Interactions. *Proceedings* **2024**, *97*, 217. https://doi.org/10.3390/proceedings2024097217

Academic Editors: Pietro Siciliano and Luca Francioso

Published: 16 May 2024

1. Introduction

Sensors to monitor the immune status of an individual play a crucial role in understanding acquired immunity or signs of a latent infection. Such sensors can be an effective tool in the identification and spread of infectious diseases, not only in humans, but also from animals to human populations. In this work, we report a sensor that detects the acquired immunity of a patient to infection by cytomegalovirus (CMV). CMV is caused by beta-herpesvirus, also known as HHV-5. CMV is most active in older people, young people and in people with low immunity. CMV, the most common virus, is also reported to be a reason for infectious mental diseases in newborns. According to reports, the prevalence of this virus is estimated to be 50% among the population, and it can lead to fatal complications in some cases. This study showcases the application of a plasmon-enhanced fluorescence sensor with high sensitivity in detecting human antibodies against Cytomegalovirus (anti-CMV).

2. Materials and Methods

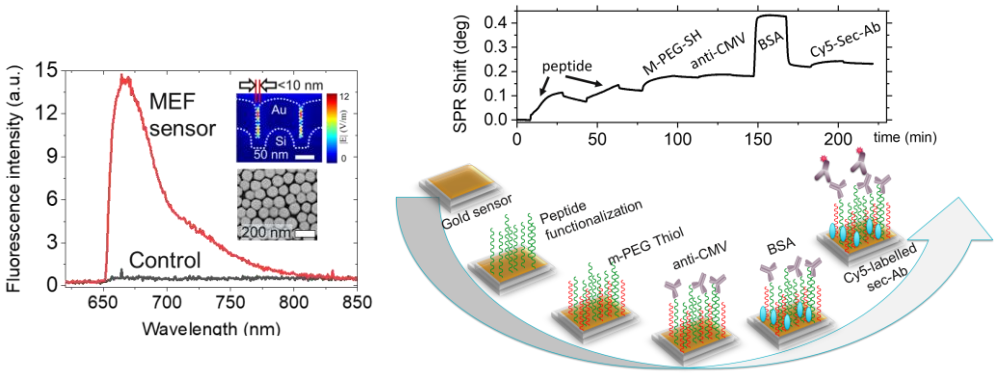
Gold nanoarrays were fabricated and used as metal-enhanced fluorescence (MEF) sensors. Gold nanoarrays were fabricated using macromolecular self-assembly and pattern-transfer approaches, as reported earlier [1,2]. The peptide with a 16 amino acid sequence, identified as exercising strong affinity to the anti-CMV antibodies, was received as lyophilized powder from the University of Copenhagen, Denmark. The peptide solution was dissolved in DMSO to prepare a stock solution that were subsequently diluted to 1 µg/mL in PBS prior to use. Gold sensor surfaces (MEF sensor, or SPR) were functionalized with the peptide solution enabled by thiol-gold interactions between the cysteine amino acid at the peptide's C-terminal and the sensor surface. Peptide-functionalized sensors were subsequently exposed to methoxy PEG thiols to fill any non-specific binding sites prior to exposure to samples containing anti-CMV antibodies (100 ng/mL–50 µg/mL in PBS). The surfaces were flown with BSA to additionally

block any non-specific binding pockets, followed by exposure to Cy5 labelled anti-human IgG. The surfaces were characterized using SPR and fluorescence microscopy.

3. Discussion

Metal-Enhanced Fluorescence (MEF) Detection

MEF sensors leverage the intense electromagnetic field enhancements on plasmonic nanostructures to enhance fluorescence signals from fluorophores in close vicinity to the sensor surface, thereby contributing to higher sensitivity of fluorescence immunoassays [3,4]. Here, we use MEF sensors consisting of gold nanoarrays with sub-10 nm electromagnetic hotspots (Figure 1a, inset) that were used to detect anti-CMV antibodies using the sequence, as illustrated in Figure 1b. The fluorescence intensities follow the concentration of Cy5-labelled detection antibodies. The immunoassay sequence was performed on both the MEF and SPR under equivalent conditions. This enables the use of SPR as means to quantify the areal density of the detection antibody on a planar gold SPR chip. SPR measurements show very low binding (~17 ng/cm^2) of sec-Antibody. (Figure 1b) This is equivalent to what can be expected for the bare Au controls (black curve Figure 1a). The analyte densities are expected to be even lower on the MEF sensors, due to the reduced surface available for binding arising from steric hindrance to binding on closely separated pillar arrays. Based on the binding densities of the sec-Antibody measured using SPR, and the available surface on the nanopillar arrays, the MEF sensor is expected to exhibit a sensitivity equivalent to few attograms of sec-Ab/μm^2. This would mean sensitivity of the sensor to only a few tens of molecules/μm^2. The strong enhancement in fluorescence intensities (red curve, Figure 1a) despite the low densities of sec-Ab, makes the MEF sensors quite attractive for fluorescence immunoassays. Besides high sensitivity, MEF offers significant opportunities for miniaturizing the sensor footprints to few square microns, and thus enabling facile multiplexing in fluorescence microarray formats. SPR is especially useful for optimization of assay conditions prior to implementing them on the MEF sensors.



(**a**) (**b**)

Figure 1. Plasmonic detection of peptide–protein interactions (**a**) Gold nanoarrays (SEM-top view), and numerically simulated electromagnetic field profiles showing sub-10 nm electromagnetic hotspots; plot compares fluorescence intensities on MEF sensors and on control surface (equivalent to the gold SPR chips used in (**b**)) (**b**) Illustration of the assay steps and the corresponding experimentally measured SPR shifts.

Author Contributions: Conceptualization, S.K., A.C.S. and T.Ø.; methodology, A.C.S., H.P., C.S. and D.B.; validation, A.C.S., H.P. and C.S.; writing—review and editing, A.C.S., S.K. and T.Ø.; Supervision, S.K.; Visualization, S.K. and A.C.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by H2020 Future Emerging Technlogies of the European Commission under the grant No 862539—Electromed-FET-Open, Luxembourg Institute of Science and

Technology—Internal funding MICROSPOT and Fonds National de la Recherche (FNR) Luxembourg FNR, CORE-PLASENS, NANOSENS.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rastogi, R.; Arianfard, H.; Moss, D.; Juodkazis, S.; Adam, P.M.; Krishnamoorthy, S. Analyte Co-localization at Electromagnetic Gap Hot-Spots for Highly Sensitive (Bio)molecular Detection by Plasmon Enhanced Spectroscopies. *ACS Appl. Mater. Interfaces* **2021**, *13*, 9113–9121. [CrossRef] [PubMed]
2. Dinda, S.; Suresh, V.; Thoniyot, P.; Balčytis, A.; Juodkazis, S.; Krishnamoorthy, S. Engineering 3D Nanoplasmonic Assemblies for High Performance Spectroscopic Sensing. *ACS Appl. Mater. Interfaces* **2015**, *7*, 27661–27666. [CrossRef]
3. Jeong, Y.; Kook, Y.-M.; Lee, K.; Koh, W.-G. Metal Enhanced Fluorescence (MEF) for Biosensors: General Approaches and a Review of Recent Developments. *Biosens. Bioelectron.* **2018**, *111*, 102–116. [CrossRef] [PubMed]
4. Bauch, M.; Toma, K.; Toma, M.; Zhang, Q.; Dostalek, J. Plasmon-Enhanced Fluorescence Biosensors: A Review. *Plasmonics* **2014**, *9*, 781–799. [CrossRef]