Research article

Shaoxiang Duan, Bo Wang, Mingqiang Qiao*, Xu Zhang*, Bo Liu, Hao Zhang, Binbin Song and Jixuan Wu

Hydrophobin HGFI–based fibre-optic biosensor for detection of antigen–antibody interaction

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Abstract: A compact S-tapered fibre biosensor functionalised with class I hydrophobin HGFI found in *Grifola frondosa* has been proposed for the first time to accrue label-free detection of reaction between goat–anti-rabbit immunoglobulin G (IgG) (GAR, antibody) and rabbit–anti-hemagglutinin IgG (R, antigen). The HGFI nanolayer self-assembled on fibre surface provides a distinguished analytical platform to implement biocompatible binding owing to its prominent amphiphilicity and remarkable optical as well as biochemical properties. Water contact angle measurements, atomic force microscopy, and immunofluorescence assay are utilised to characterize the modification properties of siliconised substrate surface with HGFI. Functionalisation of the S-tapered fibre is achieved by exploiting self-assembly of HGFI and immobilisation of GAR for specific R detection. HGFI with a concentration of 300 µg/ml allows the formation of a self-assembled

amphipathic film on fibre surface, which can adsorb antibody smoothly with such desirable merits as ease of operation, fast response, good stability, good repeatability, and no side effects. Immunoassay experiments are conducted based on dip wavelengths interrogation of the interferometric transmission spectrum. In this study, we propose a novel antigen detection scheme, which could be extended for the detection of other biomolecules owing to its high integration, good specificity, real-time detection, and simple detection scheme.

Keywords: S-tapered fibre; hydrophobin; fibre surface functionalisation; antigen–antibody interaction.

1 Introduction

Optical biosensors have attracted great attention due to their various applications in medical and healthcare diagnostic, biomolecular interaction detection, environmental monitoring, and so on [1]. Optical fibre sensors based on different sensing mechanisms such as fibre grating sensors [2], interferometers [3], evanescent-field fibre sensors [4], and surface plasmon resonance (SPR) sensors [5, 6] could be used for biological detection owing to their desirable advantages such as small and flexible shape, low sample consumption, good specificity, low-cost fabrication, and rapid and sensitive detection in real time [7, 8].

In particular, optical fibre sensors have also been used as immunosensors to detect antibody–antigen reaction [9–11]. Liu et al. [9] proposed a graphene-oxide nanosheets-functionalised long-period grating sensor for monitoring the antibody–antigen reaction between immunoglobulin G (IgG) and anti-IgG. Lu et al. [11] measured the concentration of infliximab (IFX) in 100-fold diluted serum, plasma, and whole blood by employing an anti-IFX modified fibre-optic surface plasmon resonance biosensor. The S-tapered fibre interferometers have been used for the measurement of temperature [12], strain [13], refractive index (RI) [14], and humidity

***Corresponding authors: Mingqiang Qiao**, Key Laboratory of Molecular Microbiology and Technology, Ministry of Education, College of Life Sciences, Nankai University, Tianjin 300071, China, e-mail: qiaomq@nankai.edu.cn; and **Xu Zhang**, Tianjin Key Laboratory of Optoelectronic Sensor and Sensing Network Technology, College of Electronic Information and Optical Engineering, Nankai University, Tianjin 300350, China, e-mail: nkzhangxu@nankai.edu.cn. <https://orcid.org/0000-0003-0122-0753>

Shaoxiang Duan, Bo Liu and Hao Zhang: Tianjin Key Laboratory of Optoelectronic Sensor and Sensing Network Technology, College of Electronic Information and Optical Engineering, Nankai University, Tianjin 300350, China

Bo Wang: Key Laboratory of Molecular Microbiology and Technology, Ministry of Education, College of Life Sciences, Nankai University, Tianjin 300071, China

Binbin Song: Key Laboratory of Computer Vision and Systems, Ministry of Education, School of Computer Science and Engineering, Tianjin University of Technology, Tianjin 300384, China

Jixuan Wu: Tianjin Key Laboratory of Optoelectronic Detection Technology and Systems, College of Electronic and Information Engineering, Tianjin Polytechnic University, Tianjin 300387, China

[15], as well as other physical parameters. And owing to their compact structure, ease of fabrication, and low cost, S-tapered fibres have great potential in monitoring antibody–antigen interactions.

In the current fibre biosensor designs, almost all of the fibres need to be functionalised before the specific biomolecule detection in order to immobilize the probe molecules on the fibre surface. Various methods have been proposed, such as electrostatic incorporation method using poly-L-lysine with the positive charges of amino group [3, 16], physisorption method by gold nanoparticles [17, 18], covalent binding by silanisation [9, 19], and so on.

Hydrophobins are small-molecular-weight proteins with unique properties produced by filamentous fungi [20]. They are known as one group of surface-active protein that have both hydrophobic and hydrophilic parts as amphiphiles [21]. Distinguished by the hydrophobicity patterns and biophysical properties, hydrophobins can be divided into class I and class II [22]. In general, films formed by class I hydrophobins are more stable and insoluble compared with class II [23]. An 8-kDa HGFI was first identified in the edible mushroom *Grifola frondosa*, showing remarkable surface activity and stability. These proteins could form a self-assembled amphipathic film at hydrophilic/hydrophobic interfaces and change the wettability of different surfaces where they are attached. Hydrophobin assemblies offer a universal and straightforward approach for immobilisation of enzyme [24], cell [25, 26], and proteins [27] in diversified electrochemical sensors [28, 29] and optical biosensor [30, 31]. Due to their unique features such as self-assembly, amphipathy, biocompatibility, stability, and homogeneity, hydrophobins are promising for applications in the fields of drug delivery [32], biological sensing [24, 33], food sector [34], especially in the modification of various materials [27], and so on. Therefore, the self-assembly HGFI nanofilm could be utilised as a potential biochemistry platform for detection of biomolecules.

In this article, we present an S-tapered fibre-optic biosensor with HGFI coating as the linking intermedium for real-time label-free detection of reaction between goat–anti-rabbit IgG (GAR) antibody and rabbit–anti-hemagglutinin IgG (R) antigen. It is the first time that HGFI has been combined with optical fibres and used as one new kind of functional reagent with the advantages of ease of operation, fast reaction, strong bonding force, and no side effects to the biological samples. Both of the surface functionalisation and antigen–antibody reaction processes have been experimentally investigated based on wavelength interrogation of the interferometric spectrum in real time.

2 Experimental setup

2.1 Strains, plasmid, and reagents

The *Pichia pastoris* strain GS115 and plasmid pPIC9K are purchased from Invitrogen (Beijing, China). All restriction enzymes, DNA polymerase, DNA marker, and protein markers are purchased from TaKaRa (Dalian, China). Expression plasmid pPIC9K-*hgfi* is prepared in our laboratory, which is constructed to promote the cloning of HGFI gene. Goat–anti-rabbit IgG fluorescein isothiocyanate (FITC) that serves as antibody is obtained from Bioss, and R that serves as antigen is acquired from Santa Cruz Biotechnology. Besides, goat–anti-mouse IgG FITC (GAM) employed as mismatched antigen to evaluate the specificity feature of the proposed biosensor is produced by TransGen Biotech. Goat–anti-rabbit IgG, R, and GAM are all dissolved in phosphate-buffered saline (PBS) solutions (pH 7.4), and their respective concentrations are 4, 1, and 1 µg/ml.

2.2 Protein production, purification, and identification

Figure 1 depicts the key steps in expression, purification, and identification of HGFI. Specifically, the recombinant plasmid pPIC9K-*hgfi* is constructed by inserting HGFI gene into the pPIC9K cloning vector, as shown in step (A). In step (B), recombinant plasmid is transformed into *P. pastoris* GS115. Afterwards, the gene containing HGFI is integrated into *P. pastoris* genome DNA, as shown in step (C). Then, transformed colonies are isolated by histidine-deficient minimal dextrose medium. Step (D) indicates that positive transformed colonies are selected and cultured in buffered minimal methanol medium by shaking flask for scale-up. Next, the final products are purified by ultrafiltration and reverse-phase high-performance liquid chromatography as described by Wang et al. [35]. The expression of HGFI was determined by using 12% tricine–sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis, as shown in step (E). Finally, the purified HGFI was dissolved in distilled water to reach a concentration of 300 µg/ml for successive experiments.

2.3 Contact angle assay

The capacity of HGFI to alter surface hydrophobicity is confirmed by measuring water contact angle (WCA).

In this work, the WCA of bare and HGFI-coated ordinary glasses are measured by using the KSV Contact Angle Measurement System with a 5 μ l water droplet on the protein-coated side of glass slide.

2.4 Atomic force microscopy measurement

Freshly cleaved mica substrate is used to observe the self-assembled characteristic of HGFI by using an atomic force microscopy (AFM) (Bruker, Germany). Mica is coated with 50 μ l of low-concentration HGFI solutions (100 μ g/ml) and incubated overnight under room temperature. Dry sample is imaged by the AFM operating in tapping mode.

2.5 Immunofluorescence assay

To prove the adhesion capacity of HGFI and the feasibility of the modification procedure, a verification test is carried out. Goat-anti-rabbit IgG antibody labelled with FITC is employed to form a fluorescence assay based on the single-mode fibres (SMFs) with or without HGFI modification.

2.6 Fibre-optic sensor configuration and principle

Figure 2A shows the schematic experiment setup of the GAR–R interaction detection system based on S-tapered fibre. The S-tapered fibre sensing region is packed in a microfluidic capillary channel, and all the samples are injected by syringes. Light from a broadband source (wavelength ranges from 1250 to 1640 nm) is launched into the S-tapered fibre, and the transmission spectrum is monitored by an optical spectrum analyser (Yokogawa AQ6370C, operation wavelength ranges from 600 to 1700 nm with a resolution of 0.05 nm) in real time.

The S-tapered fibre is fabricated by off-axis tapering the SMFs using a commercial fusion splicer. The microscopic image of the S-taper fibre is shown in Figure 2B. The waist diameter, axial offset, and total length of the S-tapered fibre are 51.53, 50.73, and 510.08 μ m, respectively. As shown in Figure 2A, cladding modes sensitive to external environment will be excited at the first coupling region of the S-tapered fibre on account of the presence of bending sections. The input light would propagate along different paths until they arrive at the output port taper; thus, interference between cladding modes and

Figure 1: Illustration of HGFI expression, purification, and identification.

(A) Insert the gene containing HGFI into plasmid DNA. (B) Transform recombinant plasmid into *P. Pastoris*. (C) Plasmid integration. (D) Selection of positive transformed colonies and expression of HGFI. (E) Purification and identification of HGFI.

Figure 2: Experimental setup.

(A) Schematic diagram of experiment setup for GAR–R interaction detection system. (B) Microscopic image of fabricated S-taper fibre.

core mode would occur. The output light intensity could be expressed as follows [15]:

$$I_{\text{out}}(\lambda) = I_{\text{co}}(\lambda) + I_{\text{cl}}(\lambda) + 2\sqrt{I_{\text{co}}(\lambda)I_{\text{cl}}(\lambda)}\cos(2\pi\Delta n_{\text{eff}}L/\lambda) \quad (1)$$

where I_{co} and I_{cl} refer to optical intensity of the core mode and high-order modes, respectively; λ is the optical wavelength; Δn_{eff} is effective RI difference between the fundamental core mode and the cladding modes; and L is the length of the S-tapered fibre. The m th attenuation dip wavelength λ_m could be expressed as

$$\lambda_m = 2\Delta n_{\text{eff}}L/(2m+1) \quad (2)$$

where m is the interference order.

Figure 3A and B give the simulation results based on beam propagation theory to describe the light beam characteristics of the S-tapered fibre in air and biological solution environments, respectively, which are in good agreement with the above theoretical analysis. Moreover, it is obvious that the light confinement capability of the fibre cladding gradually weakens with the increment of surrounding RI. Figure 3C shows the simulated as well as experimentally acquired transmission spectra of the S-tapered fibre. It could be seen that the dip wavelengths of simulated transmission spectrum are basically in accordance with the experimental measurement results. The transmission loss difference between the simulation and experimental results is mainly due to experimental loss and the deviation of simulation parameters from actual geometrical values, which is not that important for our proposed sensor based on intensity interrogation.

2.7 Surface modification of S-tapered fibre

The whole procedure of the fibre surface modification and antigen detection is shown in Figure 4. The fibre surface can be regarded as a hydrophobic surface, where HGFI can form self-assembled nanolayer to realize fibre surface hydrophobic modification. Distilled water is used to remove the redundant HGFI after reaction for 15 min.

2.8 Immobilisation of GAR antibody

The HGFI-modified S-tapered fibre is immersed in GAR antibody solution (4 $\mu\text{g}/\text{ml}$ in PBS) for 2 h to bind the antibody to the fibre surface via hydrophobic interaction. Subsequently, the nonbound GAR antibodies are washed away by PBS solutions.

2.9 R antigen detection

The S-tapered fibre with HGFI and GAR antibody on the surface forms a sandwich-like coating for the detection of R antigen. After the S-tapered fibre is immersed in R antigen solution (1 $\mu\text{g}/\text{ml}$ in PBS) for 90 min, the washing procedure is repeated as described earlier with PBS solutions.

2.10 Sensor specificity and repeatability assessment

To confirm whether the spectral shifts are caused by the interaction between GAR and R for the evaluation of the

Figure 3: Simulation results of S-taper fibre. The simulated light propagation of the S-taper fibre in air (A) and in biological reagent (B), respectively. (C) The simulated as well as experimentally acquired transmission spectra of the proposed S-tapered fibre in air.

sensor specificity, another S-tapered fibre with similar geometrical parameters is fabricated and exposed in air, PBS solution, and GAM antigen solution (1 μg/ml in PBS), respectively.

To investigate the repeatability and reproducibility of the proposed sensor, we have also fabricated an additional

Figure 4: Schematic representation of the fibre surface modification and detection procedure.

S-tapered fibre with similar geometrical parameters. Likewise, immune experiments are performed using this optical fibre sensor.

3 Experimental results and discussion

3.1 Identification of HGFI

As shown in Figure 5A, the purified HGFI was identified through silver-stained SDS-PAGE and Western blotting analysis. Lane 1 in Figure 5A reveals that the purified HGFI mainly possesses a band with a molecular weight of about 16 kDa, as reported in our previous study [36], and the 72-kDa bands are the likely oligomer of this protein. Meanwhile, Western blotting shows that HGFI could specifically bind with anti-HGFI antibody, which indicates that HGFI is pure enough to be used in the surface modification experiments.

Figure 5: Expression, purification and identification of the HGFI.

(A) Identification of purified HGFI based on SDS-PAGE. Lane M, molecular-mass markers. Lane 1, silver stain of HGFI. Lane 2, Western blot of HGFI using HGFI antibody. WCA on (B) an ordinary bare glass substrate and (C) an HGFI-coated ordinary glass substrate. (D) AFM observation of HGFI-modified mica interface.

3.2 WCA measurements

Surface modification properties are among the most important characteristics of HGFI. Changes in the surface hydrophilicity of hydrophobic glass are analysed based on WCA measurements, as shown in Figure 5B and C. The WCA of ordinary bare glass is about 42° that is decreased to 18° after treatment with HGFI, suggesting that HGFI enhances hydrophilicity of the glass surface. In this case, the hydrophobic surface of silica optical fibres tends to be hydrophilic after HGFI modification.

3.3 AFM

A prominent feature of HGFI is its ability to self-assemble into amyloid mosaic rodlets. Figure 5D shows that rodlets are the most typical structure of the formed HGFI nano-film. And the lengths of rodlets are about 100 to 200 nm, which is in accordance with the results reported in a related study [36].

3.4 Fluorescent validation assay

Figure 6A and B show respective fluorescence images of the SMFs modified with HGFI and bare SMFs after they are immersed in GAR antibody labelled with FITC solution. Compared with bare SMFs, it is apparent that there

are much more GAR-FITC antibodies evenly immobilised over the modified fibre surface. Thus, it is proved that HGFI allows for the formation of a self-assembled film on

Figure 6: Fluorescent assay.

Fluorescence images of the SMFs modified with HGFI (A) and bare SMFs (B) after they are respectively immersed in GAR-FITC antibody solution.

Figure 7: Detection of R antigens.

(A) Initial transmission spectrum of the S-tapered fibre immersed in HGFI. (B) Real-time relative wavelength shift in response to HGFI modification and antibody–antigen reaction. (C–E) show typical spectra of HGFI modification, GAR immobilisation, and R detection, respectively.

the fibre surface, which enables efficient immobilisation of the GAR antibody. Moreover, the adsorption capacity of the fibre surface could be actually neglected.

In addition, we have compared the fibre surface modification method of using HGFI with other investigations. From the comparison results, it could be found that our proposed fibre surface functionalisation method based on HGFI has obvious advantages of being least time consuming and having the simplest operation [7, 9, 16, 17].

3.5 Detection of R antigens in a solution

During the whole experimental process, the optical transmission spectrum of the S-tapered fibre sensor is monitored in real time. Figure 7A shows the initial transmission spectrum of the S-tapered fibre immersed in HGFI, and Figure 7C–E show the typical spectral response evolution around 1495 nm for the HGFI modification and antibody–antigen reaction processes. Equation (2) indicates

Figure 8: Sensor specificity and repeatability.

(A) Temporal evolution of relative wavelength shift for the fabricated fibre taper sensor in air, PBS solution, and GAM antigen solution environments, respectively. (B) Real-time relative wavelength shift of the additional sensor for comparison purpose.

that for the cladding modes Δn_{eff} would decrease with the reduction of surrounding RI, and λ_m will move towards the shorter wavelength region accordingly [37]. Figure 7B shows the real-time relative interference dip shift at the particular wavelength. Due to the self-assembly of HGFI over the fibre surface, the individual transmission dip shows an initial blue shift of about 0.14 nm and then experiences another wavelength shift of about 1.17 nm due to the absorption effect of GAR antibody immobilised on the HGFI nanolayer. After the antibody is bonded with the fibre surface for 100 min, the transmission dip no longer exhibits observable wavelength shift, implying that the immobilisation process reaches a saturation stage. Eventually, R antigen could react with the GAR antibody on the fibre surface, causing the transmission dip to further move towards a shorter wavelength region by 1.02 nm in 90 min.

3.6 Sensor specificity and repeatability

Figure 8A gives the temporal evolution of relative wavelength shift. It could be found that compared with the GAR–R reaction process, the transmission spectrum only exhibits irregular fluctuations in wavelength shift when the fibre taper sensor is respectively exposed in air or immersed in PBS solution and GAM antigen solution,

which indicates our proposed fibre taper sensor has good spectral stability and antigen specificity.

Figure 8B shows the real-time relative interference dip shift as functions of observation time for individual sensors. Similar transmission dip response behaviours indicate that the proposed S-tapered fibre sensor is reproducible for detection of R antigen with good repeatability.

4 Conclusions

We have proposed and experimentally demonstrated a label-free immunosensor based on HGFI-modified S-tapered fibre for monitoring the antigen–antibody interaction process. By applying the S-tapered fibre, both of the surface modification and antigen detection procedures could be monitored in real time. Experiment results indicates that the fabricated S-tapered fibre exhibits a detection limit of less than $1 \mu\text{g/ml}$ for antigens (IgG from rabbit) after being functionalised with HGFI and antibody.

To the best of our knowledge, in this study HGFI is employed for fibre surface functionalisation for the first time. Compared with the conventional methods based on electrostatic incorporation and silanisation, our proposed immobilisation scheme for biocompatible materials possesses such desirable advantages as ease of operation, fast response, good stabilisation, and no side effects. The distinguished capacity of HGFI nanofilm to bind antibodies with glass surfaces provides us with a competitive way to implement biocompatible interface modification. In this article, without the need of complex covalent chemical treatment procedure, IgG could be stably immobilised on the sensitive surface by interacting with HGFI within 100 min. Our further research effort would be put on combining HGFI with fusion proteins [26, 38], which will further shorten the antibody immobilisation time.

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References

- [1] Fan X, White IM, Shopova SI, et al. Sensitive optical biosensors for unlabeled targets: a review. *Anal Chim Acta* 2008;620:8–26.
- [2] Chiavaioli F, Baldini F, Tombelli S, et al. Biosensing with optical fiber gratings. *Nanophotonics* 2017;6:663–79.
- [3] Song B, Zhang H, Liu B, et al. Label-free in-situ real-time DNA hybridization kinetics detection employing microfiber-assisted Mach-Zehnder interferometer. *Biosens Bioelectron* 2016;81:151–8.
- [4] Jensen JB, Pedersen LH, Hoiby PE, et al. Photonic crystal fiber based evanescent-wave sensor for detection of biomolecules in aqueous solutions. *Opt Lett* 2004;29:1974–6.
- [5] Daems D, Knez K, Delpont F, et al. Real-time PCR melting analysis with fiber optic SPR enables multiplex DNA identification of bacteria. *Analyst* 2016;141:1906–11.
- [6] Kant R, Tabassum R, Gupta BD. Fiber optic SPR-based uric acid biosensor using uricase entrapped polyacrylamide gel. *IEEE Photonic Tech L* 2016;28:2050–3.
- [7] Ribaut C, Voisin V, Malachovská V, et al. Small biomolecule immunosensing with plasmonic optical fiber grating sensor. *Biosens Bioelectron* 2016;77:315–22.
- [8] Huang Y, Tian Z, Sun L-P, et al. High-sensitivity DNA biosensor based on optical fiber taper interferometer coated with conjugated polymer tentacle. *Opt Express* 2015;23:26962–8.
- [9] Liu C, Cai Q, Xu B, et al. Graphene oxide functionalized long period grating for ultrasensitive label-free immunosensing. *Biosens Bioelectron* 2017;94:200–6.
- [10] Zhou W, Li K, Wei Y, et al. Ultrasensitive label-free optical microfiber coupler biosensor for detection of cardiac troponin I based on interference turning point effect. *Biosens Bioelectron* 2018;106:99–104.
- [11] Lu J, Spasic D, Delpont F, et al. Immunoassay for detection of infliximab in whole blood using a fiber-optic surface plasmon resonance biosensor. *Anal Chem* 2017;89:3664–71.
- [12] Yang R, Yu Y, Zhu C, et al. PDMS-coated S-tapered fiber for highly sensitive measurements of transverse load and temperature. *IEEE Sens J* 2015;15:3429–35.
- [13] Tian K, Zhang M, Farrell G, et al. Highly sensitive strain sensor based on composite interference established within S-tapered multimode fiber structure. *Opt Express* 2018;26:33982–92.
- [14] Li Y, Miao Y, Wang F, et al. Serial-tilted-tapered fiber with high sensitivity for low refractive index range. *Opt Express* 2018;26:34776–88.
- [15] Liu H, Miao Y, Liu B, et al. Relative humidity sensor based on S-taper fiber coated with SiO₂ nanoparticles. *IEEE Sens J* 2015;15:3424–8.
- [16] Gonçalves HMR, Moreira L, Pereira L, et al. Biosensor for label-free DNA quantification based on functionalized LPGs. *Biosens Bioelectron* 2016;84:30–6.
- [17] Luo B, Xu Y, Wu S, et al. A novel immunosensor based on excessively tilted fiber grating coated with gold nanospheres improves the detection limit of Newcastle disease virus. *Biosens Bioelectron* 2018;100:169–75.
- [18] Liang G, Zhao Z, Wei Y, et al. Plasma enhanced label-free immunoassay for alpha-fetoprotein based on a U-bend fiber-optic LSPR biosensor. *RSC Adv* 2015;5:23990–8.
- [19] Arghir I, Spasic D, Verlinden BE, et al. Improved surface plasmon resonance biosensing using silanized optical fibers. *Sensor Actuat B-Chem* 2015;216:518–26.
- [20] Scholtmeijer K, Janssen MI, van Leeuwen MBM, et al. The use of hydrophobins to functionalize surfaces. *Bio-Med Mater Eng* 2004;14:447–54.
- [21] Linder MB, Szilvay GR, Nakari-Setälä T, et al. Hydrophobins: the protein-amphiphiles of filamentous fungi. *Fems Microbiol Rev* 2005;29:877–96.
- [22] Wessels JGH. Developmental regulation of fungal cell wall formation. *Annu Rev Phytopathol* 1994;32:413–37.
- [23] Wessels JGH, De Vries OMH, Asgeirsdóttir SA, et al. Hydrophobin genes involved in formation of aerial hyphae and fruit bodies in schizophyllum. *Plant Cell* 1991;3:793.
- [24] Mirzaei F, Mirzaei M, Torkzadeh-Mahani M. A hydrophobin-based-biosensor layered by an immobilized lactate dehydrogenase enzyme for electrochemical determination of pyruvate. *Bioelectrochemistry* 2019;130:107323.
- [25] Zhang M, Wang Z, Wang Z, et al. Immobilization of anti-CD31 antibody on electrospun poly(ϵ -caprolactone) scaffolds through hydrophobins for specific adhesion of endothelial cells. *Colloid Surface B* 2011;85:32–9.
- [26] Wang X, Mao J, Chen Y, et al. Design of antibacterial biointerfaces by surface modification of poly(ϵ -caprolactone) with fusion protein containing hydrophobin and PA-1. *Colloid Surface B* 2017;151:255–63.
- [27] Wang ZF, Lienemann M, Qiao M, et al. Mechanisms of protein adhesion on surface films of hydrophobin. *Langmuir* 2010;26:8491–6.
- [28] Wang X, Wang H, Huang Y, et al. Noncovalently functionalized multi-wall carbon nanotubes in aqueous solution using the hydrophobin HFBI and their electroanalytical application. *Biosens Bioelectron* 2010;26:1104–8.
- [29] Yamasaki R, Takatsuji Y, Lienemann M, et al. Electrochemical properties of honeycomb-like structured HFBI self-organized membranes on HOPG electrodes. *Colloid Surface B* 2014;123:803–8.
- [30] Gravagnuolo AM, Morales-Narváez E, Matos CRS, et al. On-the-spot immobilization of quantum dots, graphene oxide, and proteins via hydrophobins. *Adv Funct Mater* 2015;25:6084–92.
- [31] Della Ventura B, Rea I, Calì A, et al. Vmh2 hydrophobin layer entraps glucose: a quantitative characterization by label-free optical and gravimetric methods. *Appl Surf Sci* 2016;364:201–7.
- [32] Singh BN, Singh BR, Gupta VK, et al. Coating with microbial hydrophobins: a novel approach to develop smart drug nanoparticles. *Trends Biotechnol* 2018;36:1103–6.
- [33] Wang Z, Huang Y, Li S, et al. Hydrophilic modification of polystyrene with hydrophobin for time-resolved immunofluorometric assay. *Biosens Bioelectron* 2010;26:1074–9.
- [34] Khalesi M, Gebruers K, Derdelinckx G. Recent advances in fungal hydrophobin towards using in industry. *Protein J* 2015;34:243–55.
- [35] Wang Z, Feng S, Huang Y, et al. Expression and characterization of a *Grifola frondosa* hydrophobin in *Pichia pastoris*. *Protein Express Purif* 2010;72:19–25.
- [36] Yu L, Zhang B, Szilvay GR, et al. Protein HGFI from the edible mushroom *Grifola frondosa* is a novel 8 kDa class I

- hydrophobin that forms rodlets in compressed monolayers. *Microbiology* 2008;154:1677–85.
- [37] Yang R, Yu Y-S, Xue Y, et al. Single S-tapered fiber Mach–Zehnder interferometers. *Opt Lett* 2011;36:4482–4.
- [38] Piscitelli A, Pennacchio A, Cicatiello P, et al. Rapid and ultrasensitive detection of active thrombin based on the Vmh2 hydrophobin fused to a green fluorescent protein. *Biosens Bioelectron* 2017;87:816–22.