Detection of Human and Avian Flu Viruses Using Graphenated Infrared Screen

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ABSTRACT

Targeted drug delivery and screening of drugs with specific affinity to membrane proteins require a good monitoring method. Here we monitor the binding of hemagglutinin (HA) to a model membrane- lipid bilayer- by using Infrared (IR) spectroscopy. IR spectroscopy is a useful spectroscopic tool to assess bio-molecular vibrations and provides with molecular fingerprinting. Yet, in order to achieve such goal, one needs to devise a bio-compatible platform that enhances the respective IR signals. We use graphenated IR (G-IR) screens as substrates and demonstrate their usefulness in monitoring the attachment of HA from influenza virus to proper receptors.

Keywords: Graphene, IR Screen, IR spectroscopy, H5N1, H1N1.

1 INTRODUCTION

Metallo-dielectric screens have been investigated from the visible to the THz spectral region for astronomy and remote sensing applications [1]. These screens are made of periodic structure aim to invoke surface plasmon polariton (SPP) modes. These surface modes enable a better coupling between the incident beam and the molecules under test. Yet, in order to achieve sensitive monitoring of bio-species, one needs to devise a bio-compatible platform which, at the same time, enables the propagation of surface electromagnetic modes. IR screena are typically made of metals, which in general, inflict sample oxidation and compromise the integrity of biological samples.

Graphene is a monolayer thick crystal of carbon. Thereby, graphene is conductive and thus may enable propagation of SPP modes. It is chemically inert and bio-compatible, thermodynamically stable and mechanically strong. Recently, we were able to fabricate mono and a few-layers graphene on solid and perforated substrates [2, 3]. Suspended graphene on perforated substrates were obtained for holes whose diameter ranges from a few tens of nanometers to tens of micometers (see Fig. 1) .

One may hypothesize that by combining the bio-compatible graphene with the SPP sustaining IR screens, novel bio-platforms could be emerged. Indeed, we demonstrate here that proper receptors, imbedded in a model membrane and deposited on graphene coated IR screens, enable the monitoring of bound proteins thus enabling the distinction between between bound Avian and Swine flu viruses.

2 THEORY

As mentioned before, surface plasmon polariton (SPP) waves are electromagnetic modes which are confined to a metallic substrate's surface. The intensity of an SPP mode decays quickly in a direction perpendicular to the substrate surface. The wave propagates along the substrate's surface with a wavelength that is very close to the free-space wavelength. That means that resonance conditions will occur when the wavelength of interrogation (the IR wavelength) is on the order of the pitch of the hole-array. When the surface is perforated, namely, contains an array of holes, these waves are scattered back and forth by the periodic hole pattern. Two conditions need to be maintained: (1) the condition for efficient coupling between the intrrogating electromagnetic beam (the IR beam) and an SPP mode and (2) the condition which dictates an efficent scattering process. Polarization of the incident beam also plays a role in the coupling process between the incident beam and the surface wave. Incident beam are denoted as p-polarized (electrical oscillations occur in the plane of incidence) and s-polarized (electrical vector of oscillations is normal to the plane of incidence). When the coupling between the incident beam and the periodic array of holes is co-linear, dispersion relations dictate that the propagation constant of the surface wave k_s is related to the periodicity g of the hole-array and the propagation constant of the incident beam k_0 by, $(k_{sx})^{+}=k_0\sin(\theta)+q2\pi/g$ or $(k_{sx})^{-}=k_0\sin(\theta)-q2\pi/g$. Here q – positive integer. For metals, $k_s=k_0(\varepsilon_m\varepsilon_0/\varepsilon_m+\varepsilon_0)^{1/2}$ with ε_m, the dielectric constant of metal and ε_0, the dielectric constant of air. Therefore, when we consider an ideal metal, the permittivity has a large negative real component, $\varepsilon_m<<0$, implying $k_s\sim k_0$: the surface wave has a wavelength similar to the wavelength of the incident beam. Resonance occurs whenever the two counter-propagating surface components interfere and their standing wave pattern matches that of the screen. For normal incidence ($\theta=0$), $\mathbf{k}_s=(k_{sx})^{+}+(k_{sx})^{-}=0$.

Another conclusion from the above discusion is that one out to obtain varying absorption by tilting and rotating the samples. The reason is that the resonance of coupling between the incident IR beam and the surface modes depend on the tilt angle θ.

3 EXPERIMENT AND METHODS

Freestanding, square shaped, electroformed copper screens were purchased from Buckbee-Mears. Those screens were 5 microns thick with periodicity of 15 microns and opening of 8 microns. The deposition of graphene on theses perforated structures is detailed elsewhere.

We used the graphene-coated IR screens as new biosensing platforms to enhance IR absorption of bio materials. For this purpose, we used receptor imbedded lipid bilayer to help protein docking to the membrane surface. Synthetic zwitterionic phospholipid 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) was used for the bilayer [4]. Films were deposited by drop casting the DMPC on the graphene coated screens. The DMPC were washed with PBS buffer solution prior to administering the hemagglutinin of either infuenza virus.

Hemagglutinin (HA1) of H1N1 (HA1 (A/California/06/09) (H1N1) (SWINE FLU 2009) (aa 18-344)) and H5N1 (HA1 (H5N1), 6xHis tagged Hemagglutinin (A/Vietnam/1203/2004) (aa 1-345)) viruses were used: 10 μL of the HA1 protein was mixed with 240 μL of phosphate buffer saline, PBS (pH 7.4). The molar concentration of HA1 in the solution was 1 μM. The amount of bound HA1 to its receptor is unknown. We note that the sensitivity of our approach is better than known analytical techniques by a factor four [5].

Tri-saccharides served as receptors [6,7] and were received from the Functional Glycomics Center, Core D (SCRIPP Inst, San Diego). 1 mg of either obtained saccharide was dissolved in 1 mL of water. The acquired molar concentration of either saccharide was 1.3 mM.

4 RESULTS AND DISCUSSION

SEM picture of graphene-coated IR screen is shown in Fig. 1. Shown is the back side of the screen alluding to the ability of the graphene to withstand stress without a puncture. The experiment may be made on either side of the screens yet one may prefer the coated side.

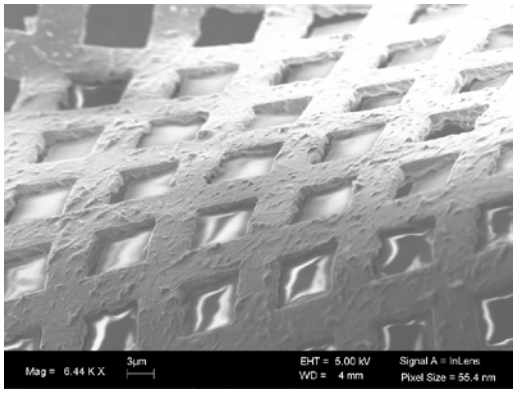


Figure 1: Graphenated infrared(IR) screen.

The schematics of our novel platform is shown in Fig. 2. The DMPC membrane contains receptors aimed at binding the hemagglutinin (HA1) area of the influenza virus. Some of the receptors will be close enough to the membrane surface and will be able to dock the protein.

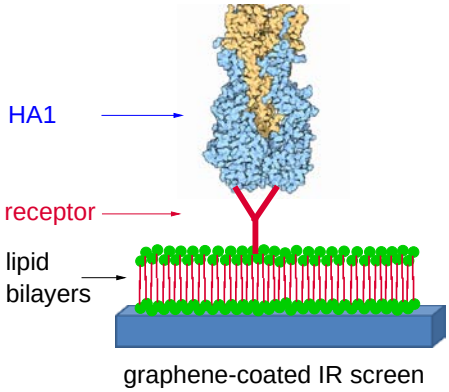
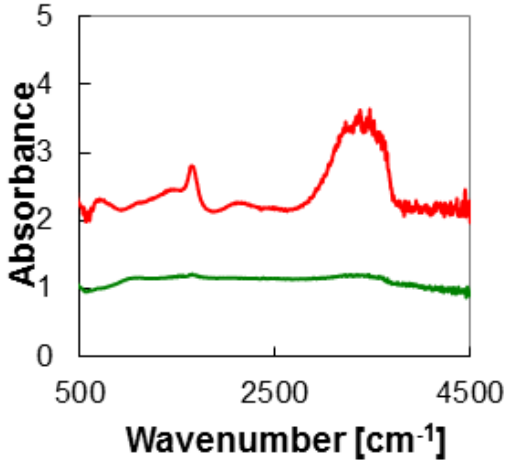


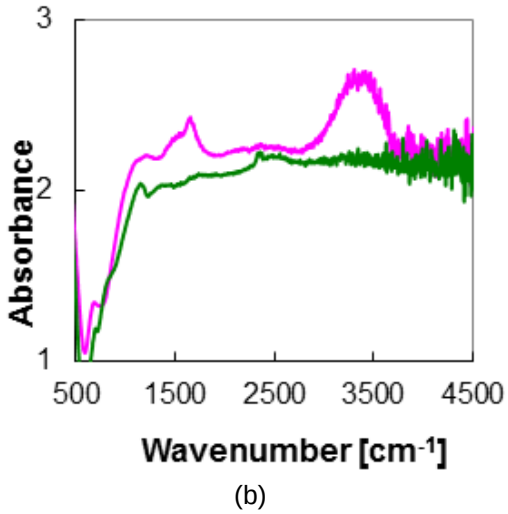
Figure 2: Graphenated infrared(IR) screen.

Our hypothesis is that upon binding new IR absorption peaks will emerge whereas, in the event of no-binding between receptor and protein, no peak will be observed.

Indeed in Fig. 3a we show the IR absorption spectra of HA1 of H1N1 and H5N1 on human receptor (containing α2-6). Clearly, the HA1(H1N1) (swine) interacts with the human receptor while the HA1(H1N5) (avian) is not: newly formed IR absorption bands at 1500 cm^{-1} and at 3400 cm^{-1} appear for H1N1. The picture is reversed for the avian receptor (containing α2-3): these peaks albeit shifted, appear only for HA1(H1N5) (avian). As shown below, most of the IR absorption peaks, associated with the binding events may be assigned to stretching modes of H-bonds. We also note a small but clear affinity of the HA1(H5N1) to the human receptor , indicating some degree of weak binding. Figure 4 shows the angular dependency of tr40 bound HA1 (H5N1). Signal to noise ratio was calculated as a function of tilt angle, θ in degree.



(a)



(b)

Figure 3: IR absorption spectra of HA1 with receptors. (a) human receptor: a large IR absorption (top red curve) exhibits the binding between HA1(swine) to the human receptor. Almost flat response is the result of avian flu interacting with the human receptor (bottom curve). (b)Avian receptor: a large IR absorption (top pink curve) exhibits the binding between HA1(avian) and the avian receptor. The flat bottom curve is the response for swine flu and the avian receptor.

The signal-to-noise ratio (SNR) as a function of the sample's tilt angle with respect to the direction of the incident IR beam is shown in Fig. 4. As expected, there is an optimal angle (~8 degrees) which maximizes the IR absorption. SNR was calculated by taking the ratio between the peak absorption value at 3400 cm^{-1} and a reference value at 810 cm^{-1}. Note that this curve was obtained for a different sample than the one shown in Fig. 3.

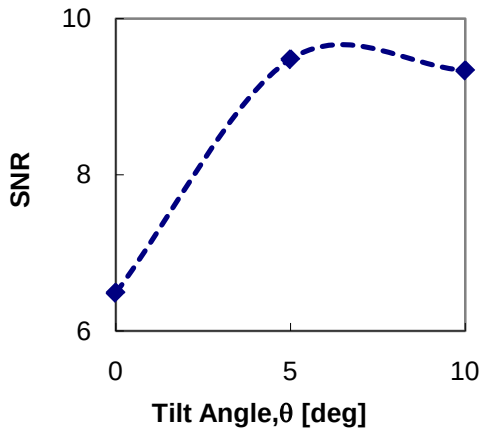


Fig. 4: Angular dependency of HA1 of H5N1, bound to avian receptor. Shown is the signal-to-noise ratio of as a function of tilt angle, θ (deg).

5 MODELING

Modeling indicated a strong interaction between the avian receptor and the avian flu virus but no interaction between the avian flu virus and the human receptor. Similarly, the avian flu virus has very strong interaction with avian receptor yet a weaker interaction with human receptor (Fig. 5,6). These modeling corroborates the experimental data of Fig. 3.

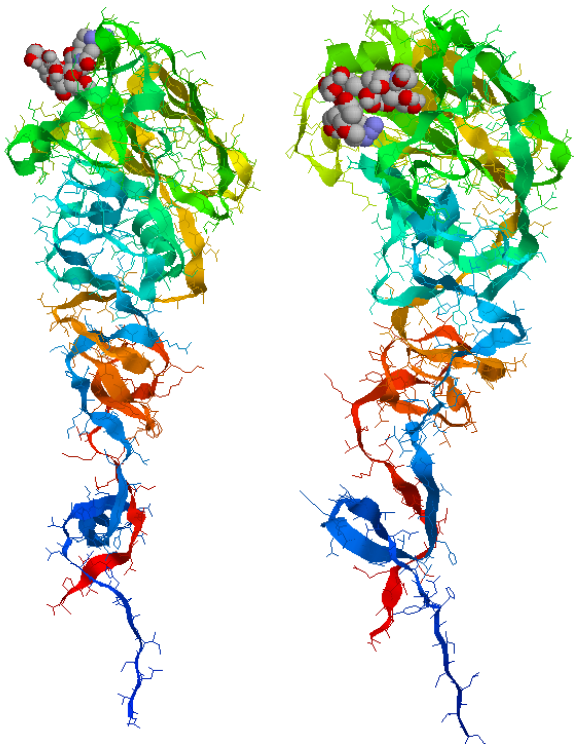


Figure 5: HA1 (H1N1) (swine) bound to (a) avian receptor (b) to human receptors.

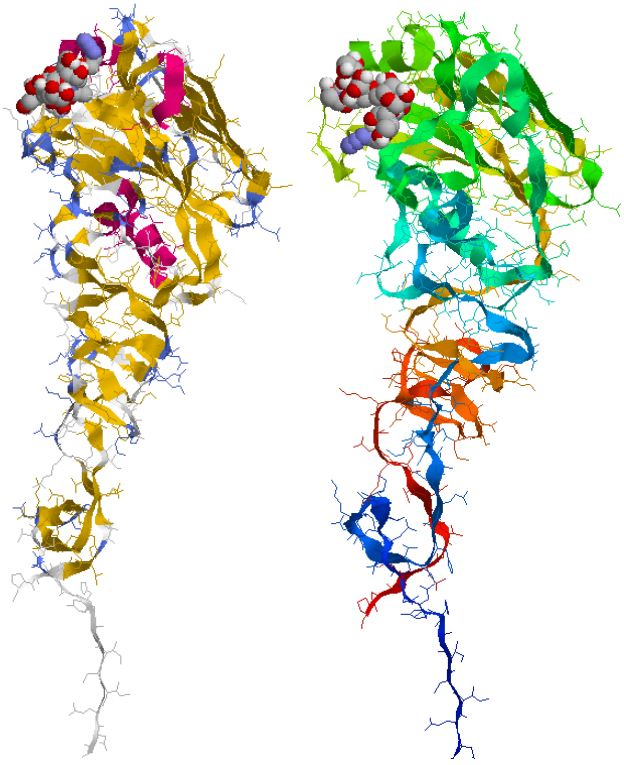


Figure 6: HA1 (H5N1) (avian) bound to (a) avian receptor (b) human receptor.

6 CONCLUSION

A powerful rapid, non-invasive spectroscopic tool has been employed in the monitoring of selective binding events between the hemagglutinin area, HA1 of influenza viruses to the human and avian receptor molecules. Such approach may enable on-site sensitive monitoring of these viruses.

7 ACKNOWLEDGEMENT

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