

Supporting information

Surface enhanced Raman spectroscopy of Aurora kinases: direct, ultrasensitive detection of autophosphorylation

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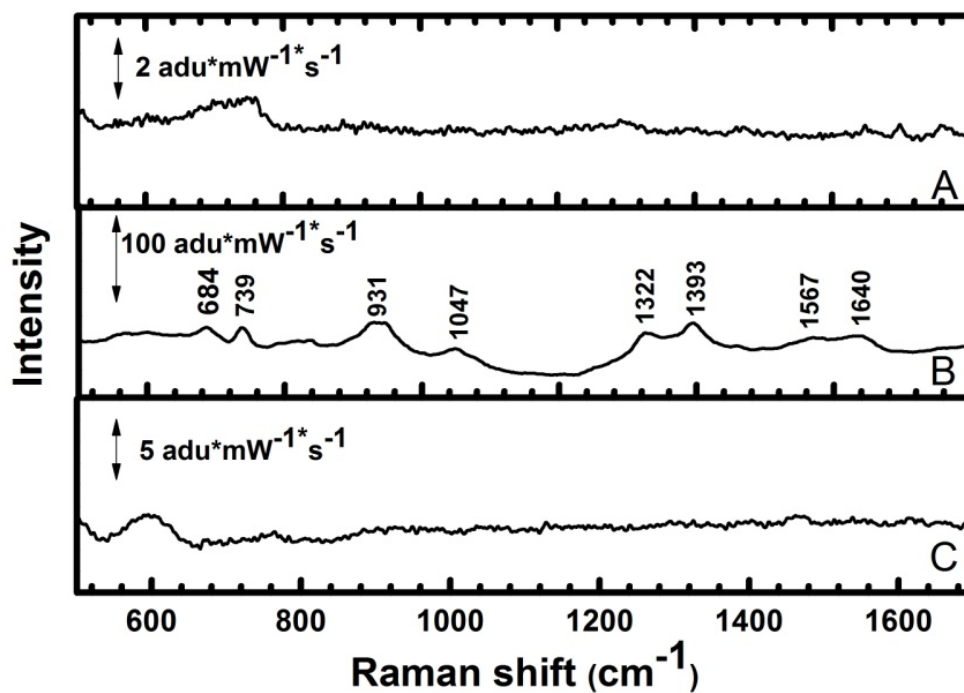


Figure S1. A) SERS spectrum of Ag nanoparticles, B) SERS spectrum of 1 mM ATP solution and C) SERS spectrum of protein buffer

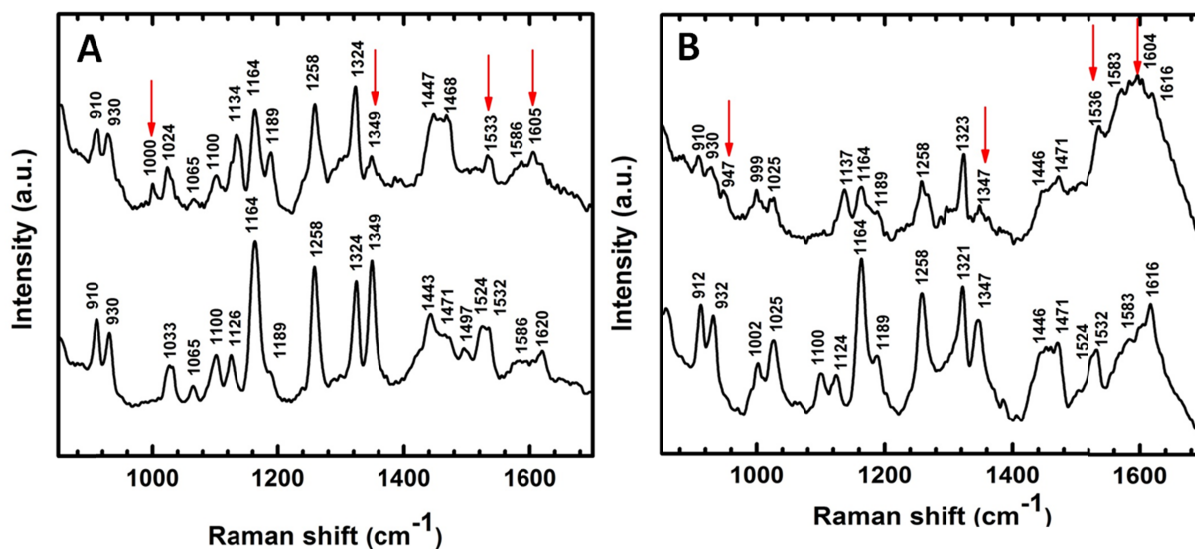


Figure S2 SERS spectra of (A) Aurora A kinase (below) and deuterated Aurora A kinase (top) and (B) Aurora B kinase (below) and deuterated Aurora B kinase (top). The red arrows show changes in amide bands of the proteins and also appearance of new bands.

Effect of deuteration of protein on SERS:

Hydrogen deuteration exchange is a way to study the stability and flexibility of proteins.¹ It is also used as a method to assign Raman bands to proteins, especially in the case of the amide bands.² The deuteration of the NH group brings about a change in the position of the amide I band. The amide I vibration is independent of the nature of the side chains but are influenced by the changes in the secondary structure of the backbone.^{1,3} Therefore it is important to deduce the position of these bands for the understanding of their secondary structure.

On deuteration these peaks shifted to lower wave numbers suggesting that our assignment is correct. In case of Aurora A (see Figure S2), the most intense peak in the deuterated sample was observed at 1605 cm^{-1} , nearly 15 cm^{-1} shift. In the case of Aurora B the amide I band shifted from 1616 cm^{-1} to 1604 cm^{-1} on deuteration. This confirms our amide I vibrations assignments.

Interestingly, upon deuteration, there is a change observed in the way Aurora B binds to the nanoparticle, suggesting that this could be an important clue to study of the subtle difference in the structures of Aurora A and Aurora B.

The amide II has large contributions from N-H bend. Therefore N-deuteration results in the conversion of the mode to a CN stretching mode at around 1460 cm^{-1} . Based on the deuteration studies, we have assigned the band at 1524 cm^{-1} in both Aurora A and Aurora B to be amide II band. This band lies very near to the 1532 cm^{-1} band arising from Trp W3 and could have some influence from the Trp residue. On deuteration the amide II band indeed disappear. The expected new band at 1460 cm^{-1} is not directly observed as there is an overlap with $\delta(\text{CH}_2)/\delta(\text{CH})$ modes in that region. Similar to amide II, upon deuteration, the amide III peaks also shift to lower wave number but to a larger extent around $960\text{-}1000\text{ cm}^{-1}$. We see a reduction in the intensity of the band at 1258 cm^{-1} in both Aurora A and Aurora B and emergence of new band at 1000 cm^{-1} for Aurora A and 947 cm^{-1} for Aurora B respectively. On deuteration we also observe a change in the intensities of the stretching vibrations of C-COO^- bands in both Aurora A and Aurora B. New bands at 1134 cm^{-1} and 1137 cm^{-1} arise for Aurora A and Aurora B respectively which may be assigned to $\nu_{\text{as}}(\text{C}_\alpha\text{CN})$. Prior to deuteration the attachment was mainly through the carboxylate groups, but on deuteration the attachment is mainly through the N containing groups as interpreted from our results. This kind of behavior has also been observed in case of homopeptides, where the mode of attachment to the nanoparticle surface changes over time.⁴ This confirms our earlier suggestion that deuteration changes the conformation of the protein.

One way to look at this is that the hydrogen-deuterium exchange requires temporal breakage of hydrogen bonds⁵ which may result in partial structure opening, thus exposing other groups to the nanoparticles surface. Since the surface groups and side chains are mostly enhanced in SERS

experiments^{6,7} and these groups in the present case are more exposed to the solvent than to the interior of the protein, a greater change in spectra of deuterated proteins is seen.

The modes corresponding to the ring vibrations of phe and tyr also undergo intensity changes, which again suggests the orientation of the rings to the surface of the nanoparticles has changed. The phe and tyr residues have modes A_1 and A_g that have polarizability components in the x,y and z directions.^{7,8} The enhancement of these modes does not depend on the orientation of the ring to the nanoparticle surface. These modes can be seen at 1024 cm^{-1} and 1189 cm^{-1} for Aurora A and at 1002 , 1025 and 1189 cm^{-1} for Aurora B. These modes do not show any change in intensity on deuteration. The group of modes corresponding to B_1 and B_{2g} will be enhanced if the face of the ring is tilted towards the nanoparticle surface since their polarizabilities are in the x and z direction of the ring. The A_2 and the B_{1g} modes will be enhanced if the edge of the ring is tilted towards the nanoparticles surface as their polarizabilities are in the y, z direction. These bands are seen at 1164 and 1586 cm^{-1} in case of Aurora A and 1164 and 1583 cm^{-1} in case of Aurora B. On deuteration due to the change in orientation of the rings to the nanoparticle surface, we do see a change in these modes.

References

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