Intramolecular proton transfer of hypoxanthine, induced by application of a laser on the surface of a noble nanomaterial, was monitored in real time using surface-enhanced Raman spectroscopy (SERS). This monitoring demonstrated the dependence of the reaction on the identity of the nanomaterial and on the laser power density. The results pave the way for monitoring the proton transfer reaction in various relevant fields. In addition, we observed the presence of the proton transfer phenomenon of hypoxanthine in serum, providing a way to avoid the effect of proton transfer and hence achieve more reliable spectra of sera for clinical diagnosis.

In general, a vast amount of specific information is present in serum, and this information can be utilized for therapeutic or diagnostic purposes. Therefore, the differences between the spectra of the sera of different individuals and between the serum spectra of an individual taken at different times are tremendously important for achieving early and final disease diagnosis. Surface-enhanced Raman spectroscopy displays the advantages of providing a unique spectroscopic fingerprint and high sensitivity, as well as weak Raman scattering for water, and has therefore been used to investigate and monitor serum for cancer diagnosis and drug metabolism. However, there have been many reports indicating that changes in the critical experimental parameters of laser power and substrate identity may lead to significant changes in serum spectral bands during SERS measurements. Consequently, understanding and monitoring the impacts of the experimental conditions on the spectrum of serum is conducive to obtaining more accurate and stable serum spectra, and providing reliable diagnostic information.

Hypoxanthine, an intermediate of the metabolism of nucleic acids in living systems, is the primary contributor to the SERS signal of serum and can participate in proton transfer because of its intrinsic features in different environments. The enolic form (EF) and ketonic form (KF) of hypoxanthine are considered to be its two main prototropic tautomers. What is more, these tautomers of hypoxanthine can be distinguished by their Raman spectra, with the Raman peak at 744 cm$^{-1}$ ascribed to the EF and that at 724 cm$^{-1}$ ascribed to the KF. Analysis of previous work suggested the proton transfer of the hypoxanthine to only depend on pH (Fig. S1†). However, this conclusion cannot offer a reasonable explanation for the changes in the results of serum SERS resulting from using different laser power levels and using different substrates. It is particularly worth mentioning that SERS needs metal nanostructures to amplify the scattering signal of molecules, but the physical properties and high chemical activity of the metal nanostructures may cause some unpredictable phenomena that may not occur in other spectroscopic techniques. For example, plasmon nanostructures can drive the conversion of 4-nitrothiophenol (4-NTP) to 4,4′-dimercaptoazobenzene (DMAB). Another example is that the molecule can be converted to other species under the catalytic effect of metal nanoparticles. Whether the proton transfer of the hypoxanthine can occur under the different laser power levels and substrates during SERS measurements, it is important to understand the differences between serum spectra. Different experimental conditions, such as different substrates and laser power densities, have been used to monitor the proton transfer of hypoxanthine as shown in Scheme 1. Our research showed proton transfer of hypoxanthine to possibly depend on whether it is plasmon-induced. Such induction was shown to play a crucial role in the differences of the SERS bands of serum under different experimental conditions.

As shown in Fig. 1a, we monitored the time-resolved surface-enhanced Raman spectrum (SERS) of hypoxanthine under a 633 nm-wavelength laser exposure. The SERS spectrum was obtained upon addition of a hypoxanthine solution (pH = 5) to a concentrated Au nanoparticles (Au NPs) colloid in a 1 : 1
volume ratio and dried to form thin solid films on a Si wafer. Au NP colloids yielded a UV-Vis absorption band at a wavelength of about 534 nm (Fig. S2†). It is worth emphasizing that the surface plasmon resonance (SPR) band of Au NPs would be expected to be red-shifted due to the plasmonic coupling between adjacent Au NPs in a closely packed array in the process of transforming the Au NPs colloids to thin solid films.22,23 What is more, while the surface plasmon resonance of Au NPs was observed in the range of 600–650 nm, the Au NPs showed the most enhanced SERS performance. In the initial Raman spectra, a Raman peak at 724 cm$^{-1}$ (KF) was obvious while the obtained Raman band at 744 cm$^{-1}$ (EF) was weak. With continued laser exposure, the band at 744 cm$^{-1}$ gradually strengthened, indicating the proton transfer of hypoxanthine. A more visual display of the proton transfer process was made by producing a 2D SERS time-mapping (Fig. 1b). It is unclear why hypoxanthine can effect proton transfer under a stable pH condition between its EF and KF structures, which always appear under different pH conditions. We wondered whether the surface plasmon was sensitive enough to induce the proton transfer.

To help resolve this confusion, we carried out a series of control experiments. As indicated by Fig. S3†, a continuous laser exposure or an inclusion of noble metal nanoparticles did not apparently induce the occurrence of proton transfer. On the basis of the above results, one can propose that the laser and noble metal nanoparticles should jointly influence the proton transfer reaction. More importantly, the proton transfer of hypoxanthine was indicated to also be dependent on the excitation wavelength (Fig. S4†). Therefore, the surface plasmon has an important role in this transfer process.

Therefore, to provide additional evidence for our conjecture, we varied factors, such as laser power density and the noble nanomaterial, that influence the surface plasmon. Many previous studies have shown that the laser power plays an important role in the plasmon catalysis reaction.24–26 However, we found laser power density to have a greater influence than laser power (Fig. S5†). Many researchers are not aware of this issue, because the laser power and power density have the same effect on the surface plasmon when using the same laser spot size. In the current work, however, there was a difference between laser power and power density because the size of the laser spot under the same laser wavelength depended on the objective lens (Fig. S6†); therefore, consideration of the laser power density led to a more precise evaluation of the plasmon effect. As shown in Fig. 2a, the KF of hypoxanthine gradually converted into the EF on the surface of the bare Au NPs as the
laser power density was increased. This power-density-dependent spectral variation may have been due to a process induced by the high-power laser. In addition to the laser power density, the noble metal nanomaterial also has an important effect on the surface plasmon. Thus, we prepared the Au/SiO\textsubscript{2} to monitor this proton transfer process (Fig. S7\textsuperscript{†}); the Au core was encapsulated with a thin silica shell according to the concept of shell-isolated nanoparticle-enhanced Raman spectroscopy (SHINERS).\textsuperscript{3} Proton transfer did not occur on the Au/SiO\textsubscript{2} nanostructure when with laser power density was increased (Fig. 2b). This result was consistent with all of the previous studies where the proton transfer process was monitored for hypoxanthine molecules directly exposed to a bare metal surface with a strong plasmon effect.\textsuperscript{28-30} Moreover, to avoid the effects of surface ions coated on the SERS substrates, the time-dependent SERS of samples of a mixture of sodium citrate and hypoxanthine on the Au/SiO\textsubscript{2} substrate were collected (Fig. 2c). Proton transfer of hypoxanthine was not obvious, even when using a high power density of 11.2 mW \textmu m\textsuperscript{-2}. These experiments also confirmed that the proton transfer occurred when the hypoxanthine molecules were directly exposed to bare metal nanoparticles under a strong plasmonic effect. Ag NPs can be also used as a SERS substrate to monitor the proton transfer of hypoxanthine (Fig. S8\textsuperscript{†}). Moreover, the structure of Ag NPs is more efficient than that of Au NPs for the proton transfer of hypoxanthine. Abalde and co-workers reported silver to show a significant effect of plasmon in the proton transfer reaction. We also detected the vibrational data from serum samples is crucial for achieving an accurate diagnosis.\textsuperscript{31} However, the spectral changes caused by proton transfer are easy to ignore. Our study provided a good reference for avoiding the effect of proton transfer to achieve reliable serum spectra for clinical diagnosis.

We have carried out an investigation aimed at determining the proper conditions to apply for monitoring the proton transfer of hypoxanthine using SERS. To verify the practicability of our strategy, proton transfer of hypoxanthine was monitored \textit{in situ} in the serum. In previous studies, the concentration of hypoxanthine in the serum was in the range 0.9 to 12.0 \textmu M.\textsuperscript{32} The concentration of hypoxanthine in the serum was under our control in our study (Fig. S9\textsuperscript{†}). The serum came from the CAS Hefei Cancer Hospital. This serum was added to a concentrated metal colloid in a 1 : 1 volume ratio and the resulting solution was dried on a Si wafer. SERS was obtained from the dried solution. Changes were obvious in the time-dependent SERS mapping of the serum, especially for the bands at 724 cm\textsuperscript{-1} and 744 cm\textsuperscript{-1} (Fig. 3a). We also noticed that some peaks suddenly strengthened with time (Fig. S10\textsuperscript{†}). This result may have been due to competitive adsorption of disrupting chemicals in the serum during the \textit{in situ} detection; we do not further discuss this phenomenon, because it does not affect the main conclusion of our research. Fig. 3b shows the intensity of the EF and KF of hypoxanthine as a function of time. The intensity of the KF peak clearly increased initially and then plateaued. As shown in Fig. 3c, the ratio of the intensity of the EF of hypoxanthine to that of the KF of hypoxanthine gradually decreased initially, and then remained constant, which confirmed that proton transfer of hypoxanthine took place in the serum. In contrast to bare Au NPs, there was no obvious change in the serum spectrum with continuous exposure to a laser on the Au/SiO\textsubscript{2} (Fig. S11\textsuperscript{†}). At present, some diseases are still diagnosed based on the differences between serum spectra.\textsuperscript{3} Therefore, obtaining reliable and accurate vibrational data from serum samples is crucial for achieving an accurate clinical diagnosis.\textsuperscript{33} However, the spectral changes caused by proton transfer are easy to ignore. Our study provided a good reference for avoiding the effect of proton transfer to achieve reliable serum spectra for clinical diagnosis.

### Conclusions

In summary, we successfully monitored proton transfer in real time using hypoxanthine as a model molecule. The experiment was controlled by separately testing the continuous laser radiation and noble metal nanomaterial, and the results of the experiment confirmed the important role of the surface plasmon in the proton transfer reaction. We also detected the proton transfer phenomenon of hypoxanthine in serum. Our study not only offers a new explanation for the differences of the SERS of serum under different experimental conditions, but also provides a good reference for avoiding the proton transfer reaction to achieve reliable spectra of sera for clinical diagnosis. More importantly, our research paves the way for real-time monitoring of the proton transfer reaction in biochemical studies, which may be related to physiological and pathological events.

### Conflicts of interest

There are no conflicts to declare.
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