Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Lectin-conjugated nanotags with high SERS stability: selective probes for glycans

Mohammad Tavakkoli Yaraki [a], Katherine Wongtrakul-Kish [a], Edward S.X. Moh [a,b], Nicolle H. Packer [a,b,*], and Yuling Wang [a,b,*]

[a] School of Natural Sciences, Faculty of Science and Engineering, Macquarie University, NSW 2109, Australia

[b] ARC Centre of Excellence for Nanoscale BioPhotonics (CNBP), School of Natural Sciences, Faculty of Science and Engineering, Macquarie University, NSW 2109, Australia

*Corresponding authors:

Distinguished Professor Nicolle H. Packer, E-mail: nicki.packer@mq.edu.au

Professor Yuling Wang, E-mail: yuling.wang@mq.edu.au

SI.1 Materials and methods

S1.1.1. Chemicals

Sodium citrate, gold (III) chloride trihydrtae, 2,3,5,6-tetrafluoro-4-mercaptobenzoic acid (TFMBA), 4-mercaptobenzoic acid (4-MBA), 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), 2,7-mercapto-4-methylcoumarin) (MMC), Lens Culinaris Agglutinin (LCA), Wheat germ agglutinin (WGA), Sambucus Nigra Lectin (SNA), and Aleuria Aurantia Lectin (AAL), and 3,3'-Dithiobis (sulfosuccinimidyl propionate) (DTSSP) were purchased from Sigma, Australa.

S1.1.2. Synthesis of citrate-capped Au nanoparticles

Au nanoparticles with an average size of 50 nm were synthesized by the Turkevich method. Briefly, 98 mL of Milli Q water was mixed with 1 mL of HAuCl4 (1 % w/v) solution and heated up to boiling while stirring at 550 rpm. Upon first observation of boiling, 0.9 mL of Na-citrate solution (1 % w/v) was added to the solution and the solution was stirred for another 10 min. Then, the heater was stopped and the solution was stirred for 10 min before turning the stirrer off. Finally, the solution of gold nanoparticles was left undisturbed overnight.

S1.1.3. Synthesis of AuNP@Raman-lectin

To conjugate lectins to Au nanoparticles, DTSSP was used as a linker. First, DTSSP (100 ug/mL) was dissolved in sodium citrate buffer (5 mM, pH 5.3) and lectins (100 ug/mL) resolubilised in 0.5 X PBS buffer (5 mM). Then, 100 µL of lectin solution was added to 100 µL of DTSSP solution dropwise. The mixture was incubated for 40 min at room temperature. Finally, the DTSSP-lectin conjugate (10 µL) and Raman molecule (5 µL, 1 mM in ethanol) was added to 1 mL of washed AuNP solution (centrifuged at 5500 rpm for 10 min and removed extra citrate and redispersed in MQ-water). After overnight incubation at 4 ^{o}C, the the mixture was centrifuged (5500 rpm, 4 ^{o}C, 10 min) to remove any unbonded lectins and Raman molecules and redispersed in MQ-water (500 µL).

S1.1.4. Interaction between AuNP@Raman-lectin nanoparticles

AuNP@Raman-lectin nanoparticles containing different lectins (AAL, LCA, SNA and WGA) were prepared using four different Raman molecules (TFMBA, MMC, 4-MBA and DTNB). The

glycan specificity of different lectins is presented in **Table S1**. The binary mixture of AuNP@Raman-lectin nanoparticles containing the same Raman molecule was studied by mixing equal volume (500 µL) of each SERS nanotags followed by incubation at room temperature for 2 h. The UV-Visible spectra and Raman spectra was recorded for each mixture as well as individual lectin-SERS nanotags. All samples were mixed by pipetting before measurements were taken.

Table S1. Glycan specificity for different lectins

Lectin	**Glycan specificity**
Wheat Germ Agglutinin (WGA)	GlcNAc and Sialic acid
Lens Culinaris Agglutinin (LCA)	core fucose and mannose
Sambucus Nigra Lectin (SNA)	α-2,6 sialic acid
Aleuria Aurantia Lectin (AAL)	α-linked fucose
Cholera Toxin B subunit (CTB)	GM1 ganglioside and Lewis epitope

If we consider that there is no interaction between the lectins, the mixture of two different lectin-SERS nanotags should have a SERS intensity equal to the average SERS intensity of each lectin-SERS nanotags. Therefore, any deviation from this theoretical value in the SERS intensity of the binary mixture could be attributed to lectin-lectin interaction. Therefore, the relative SERS intensity was calculated using the following equation:

$$Relative\ SERS\ Intensity = \frac{SERS\ Intenisty\ for\ binary\ mixture}{(SERS\ Intensity\ of\ AuNP@Raman_{lectin}1 + SERS\ Intensity\ of\ AuNP@Raman_{lectin}2)/2}$$

eq. S1

S1.1.5. Interaction between AuNP@TFMBA-lectin and glycoproteins

To investigate the effect of IgM, Fetuin and RNase B on the binding capability of the AuNP@TFMBA-WGA and AuNP@TFMBA-CTB lectin-SERS nanotags, the tags were mixed with various concentrations of each glycoprotein. For IgM, 500 µL of AuNP@TFMBA-WGA or AuNP@TFMBA-CTB was mixed with 400 µL of MQ-water, followed by addition of 100 µL of

various concentrations of IgM solution in PBS (0.5 X, or 5 mM). For Fetuin, 277 µL of AuNP@TFMBA-WGA or 500 µL of AuNP@TFMBA-CTB was topped up with deionized-water to 900 µL, followed by the addition of 100 µL of various concentrations of Fetuin solution in PBS (0.5 X, or 5 mM). Finally for RNase B, 1 mL of AuNP@TFMBA-lectin was mixed with RNase B, 500 µL of AuNP@TFMBA-WGA or 500 µL of AuNP@TFMBA-CTB was mixed with 400 µL of MQ-water, followed by the addition of 100 µL of various concentrations of RNase B solution in PBS (0.5 X, 5mM). All glycoprotein-nanotag mixtures were were shaken a few times and left undisturbed for 2 h. In all experiments, we did not remove the free SERS nanotags in order to keep the number of nanoparticles the same in all samples. Therefore, the fluctuation in SERS intensity would be attributable to the changes in intraparticle distance only.

S1.1.6. Finite-element method (FEM) simulation

COMSOL Multiphysics software package (see www.comsol.com) was used to calculate the enhanced electric field around Au nanoparticles by solving the governing equations based on Mie theory using finite-element method (FEM). The refractive index of Au was taken from Johnson and Christy data[1]. The surrounding environment was assumed to be water (n=1.33) and 1.6 for proteins[2]. A plane wave linearly polarizing along the z-axis and propagating in x-direction at wavelength of 785 nm was set as the source.

S1.1.7. Statistical analysis

The statistical software GraphPad Prism version 10.1.1 for macOS, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com, was used for analysis. One-Way ANOVA followed by Turkey's multiple comparisons test was implemented to determine the difference between individual groups of data.

S1.1.8. Instrumentation

A Jasco UV-Vis Spectrometer V-760 (Tokyo, Japan) was used to obtain the UV-Vis spectrum of samples. The concentrations and hydrodynamic sizes of AuNPs before and after conjugation with Raman reporter molecules were obtained by nanoparticle tracking analysis (NTA) on a Nanosight NS300 (Malvern Panalytical, Malvern, United Kingdom). Each sample was diluted 100 times before measuring via NTA. The surface charge of the samples were determined using a Zetasizer ZS instrument (Malvern Panalytica, Malvern, United Kingdom). Raman spectroscopy was recorded using a portable IM-52 Raman microscope (Snowy Range

Instrument, Laramie, the United States of America) and under 100 mW laser power. The integration time was set at 2s and all measurements were performed for 100 μL sample in a quartz cuvette with 1 cm path length. The size and morphology of AuNPs were obtained with transmission electron microscopy (TEM; (Philips CM10 TEM, Eindhoven, The Netherlands).

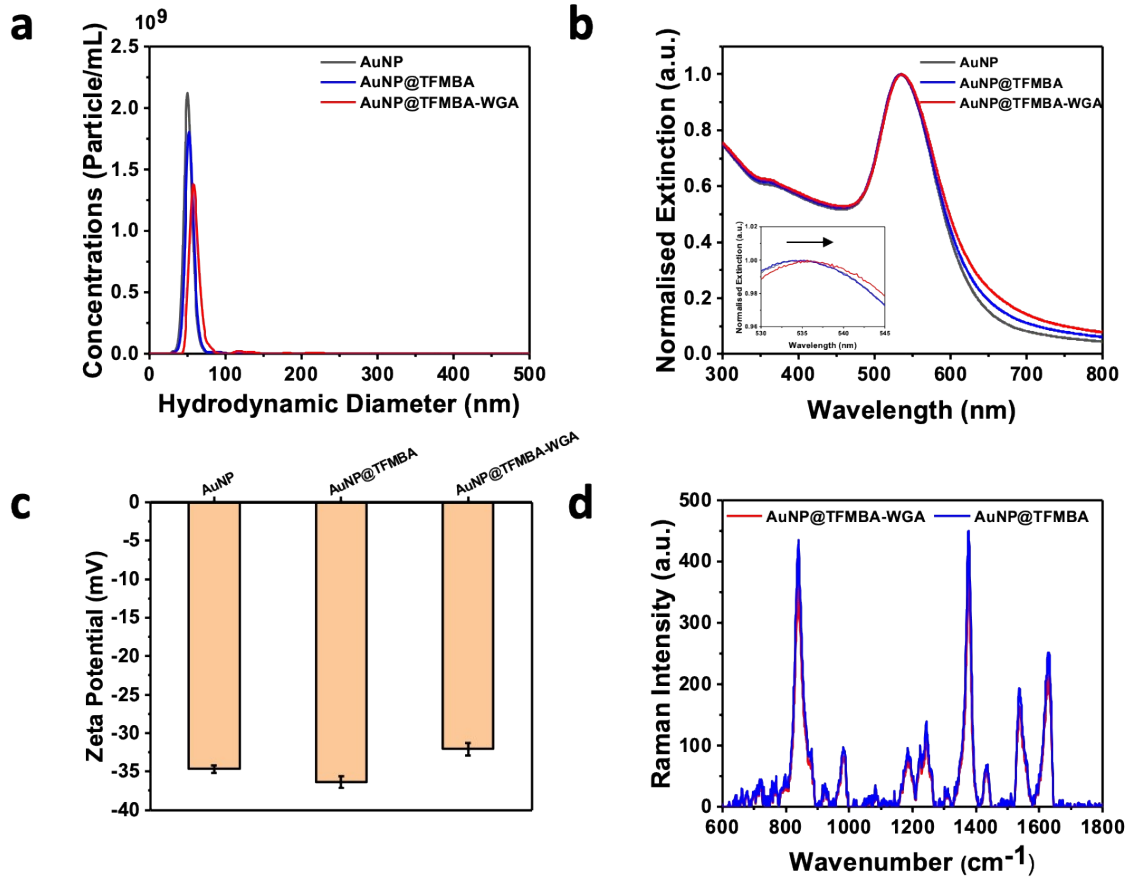

Fig. S1. a) NTA analysis , b) UV-Visible spectra and c) Zeta potential value for AuNPs, AuNP@TFMBA, and AuNP@TFMBA-WGA. d) SERS spectrum for AuNP@TFMBA and AuNP@TFMBA-WGA, peaks at (i) Ring stretch/C–F stretch and (ii) In-phase C═O stretch

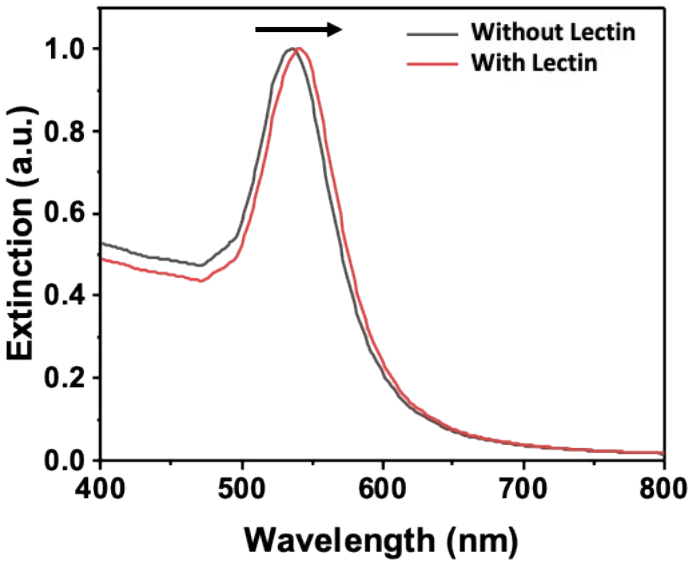

Fig. S2. Simulated UV-Vis spectra for 50 nm AuNPs before and after adsorption of lectin with refractive index of 1.6. The thickness of the lectin was considered 5 nm. The arrow indicates the redshift (~5 nm) in LSPR peak of AuNPs after conjugation of lectin.

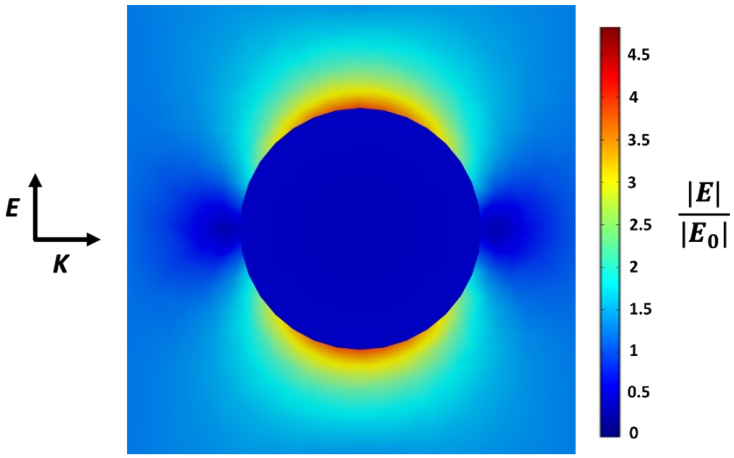

Fig. S3. Simulated enhanced electric field around 50 nm Au nanoparticle. Excitation wavelength is 785 nm and surrounding medium is water.

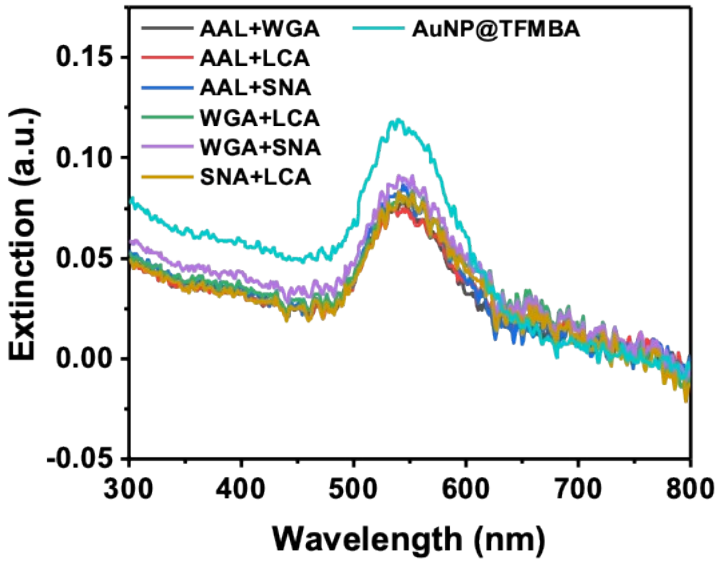

Fig. S4. Extinction spectra of different binary mixtures of AuNP@TFMBA-lectin SERS nanotags. No redshift in LSPR peak and also no peak at longer wavelengths (>600 nm) indicates the stability of these SERS nanotags in their binary mixture.

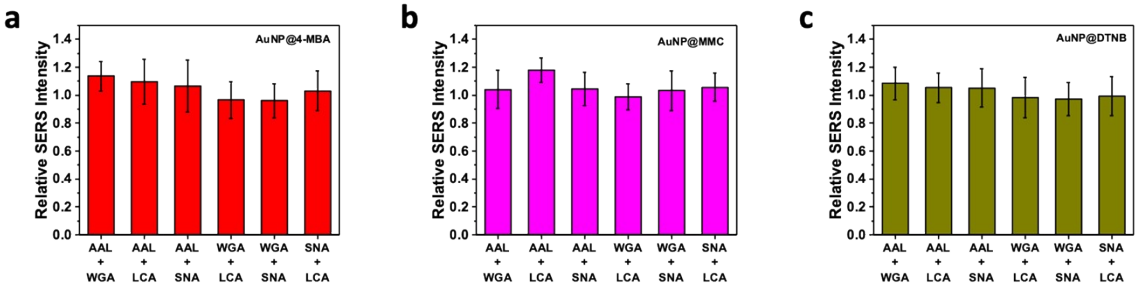

Fig. S5. Relative SERS intensity of different binary mixtures of AuNP@MBA-lectin (a), AuNP@MMC-lectin (b), and AuNP@DTNB-lectin (c) SERS nanotags compared to predicted value based on SERS intensity of individual samples. ANOVA statistical analysis did not show any significant difference.

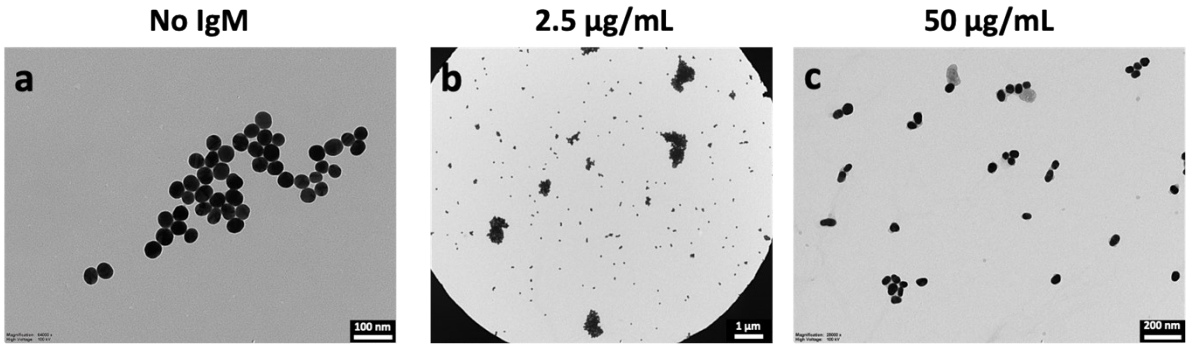

Fig. S6. TEM image of AuNP@TFMBA-WGA nanoparticles after incubation of IgM with different concentrations : a) 0 µg/mL, b) 2.5 µg/mL, and c) 50 µg/mL.

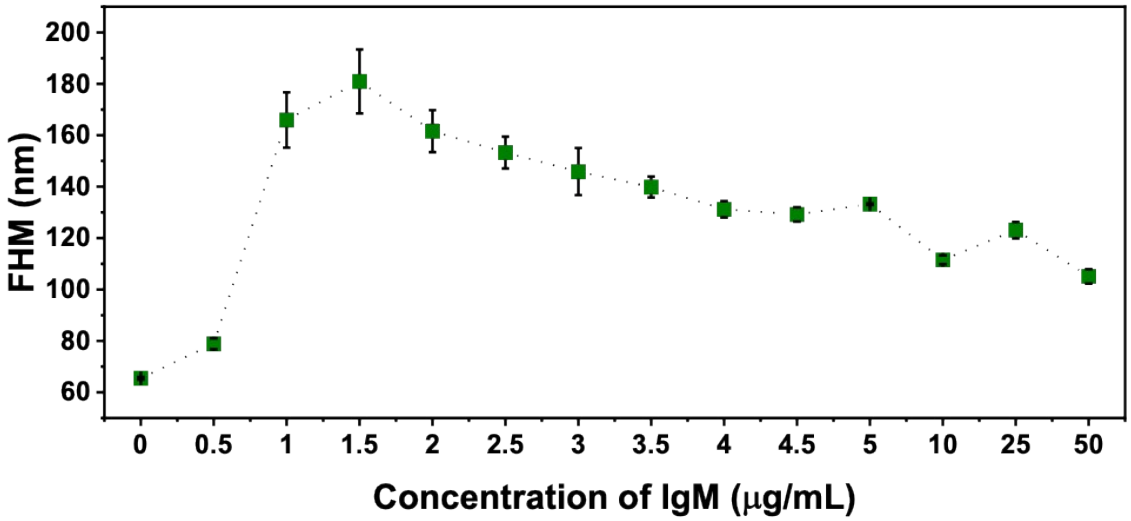

Fig. S7. Full width at half maximum (FWHM) of AuNP@TFMBA-WGA nanoparticles after incubation with different concentrations of IgM.

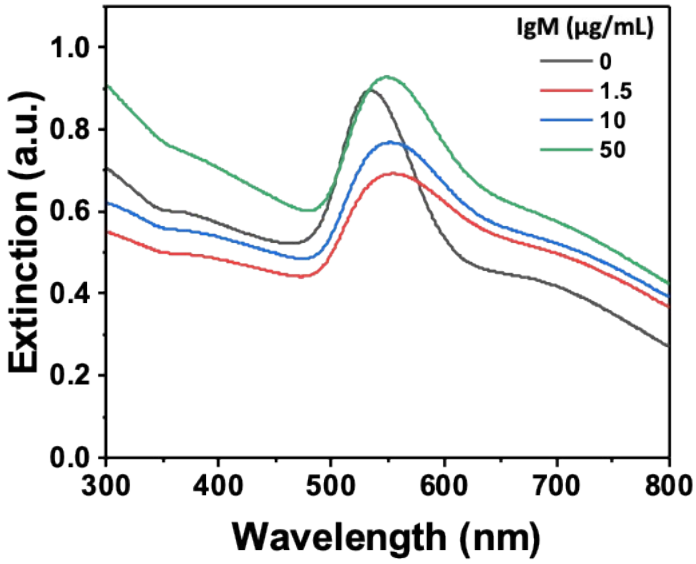

Fig. S8. Effect of concentration of IgM on extinction spectra of AuNP@TFMBA-CTB SERS nanotags.

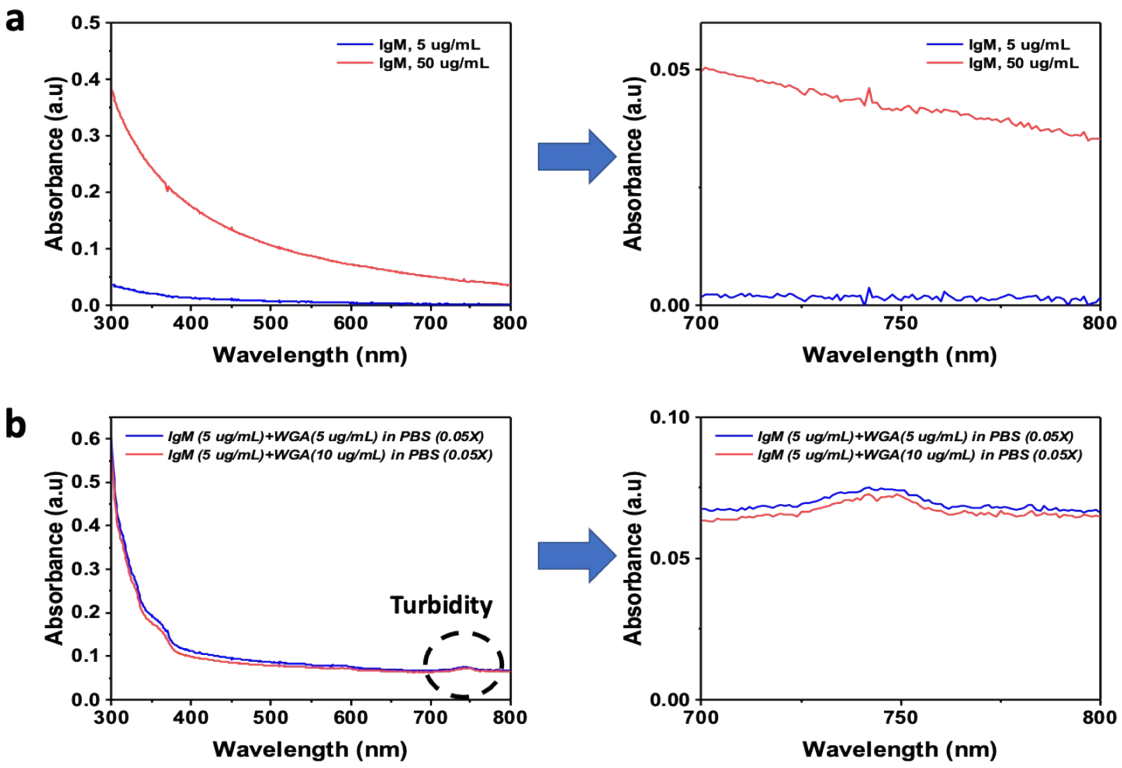

Fig. S9. a) Absorbance spectra for IgM solutions in PBS (0.05X, 0.5 mM), b) Absorbance spectra of the mixtures of IgM and WGA in PBS (0.05X, 0.5 mM).

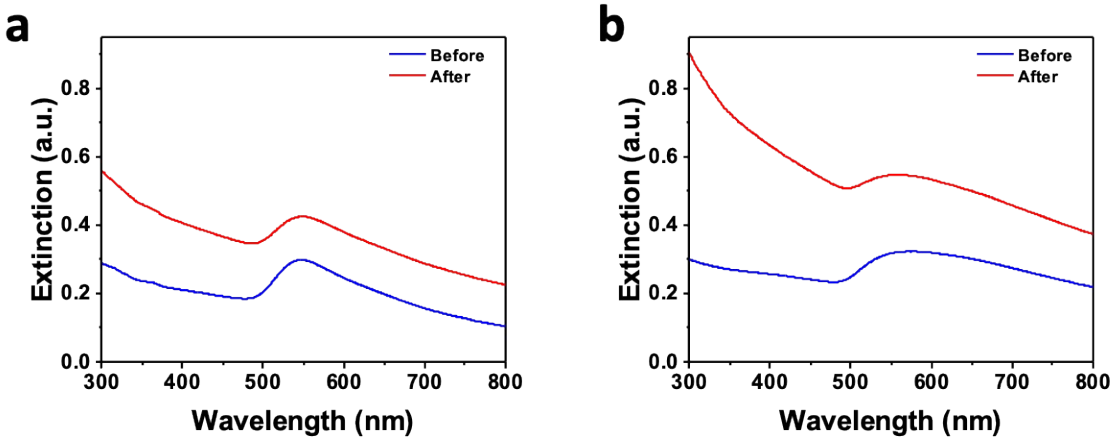

Fig. S10. UV-Vis spectra for AuNP@TFMBA-WGA incubated with a) 10 µg/mL or b) 2.5 µg/mL IgM (blue), and after incubation with an additional 50 µg/mL IgM (red).

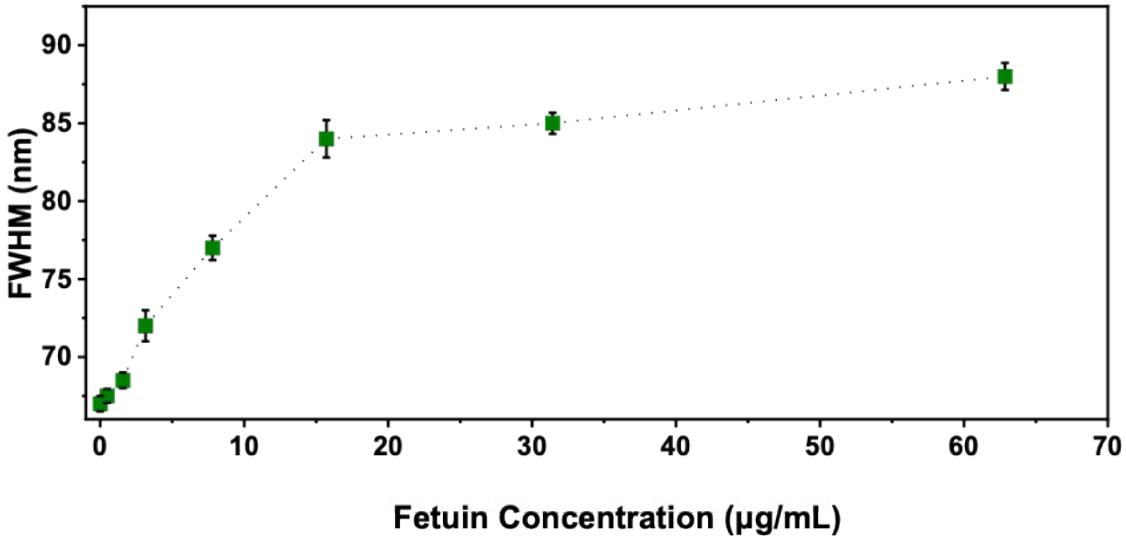

Fig. S11. Full width at half maximum (FWHM) of AuNP@TFMBA-WGA nanoparticles after incubation with different concentrations of Fetuin.

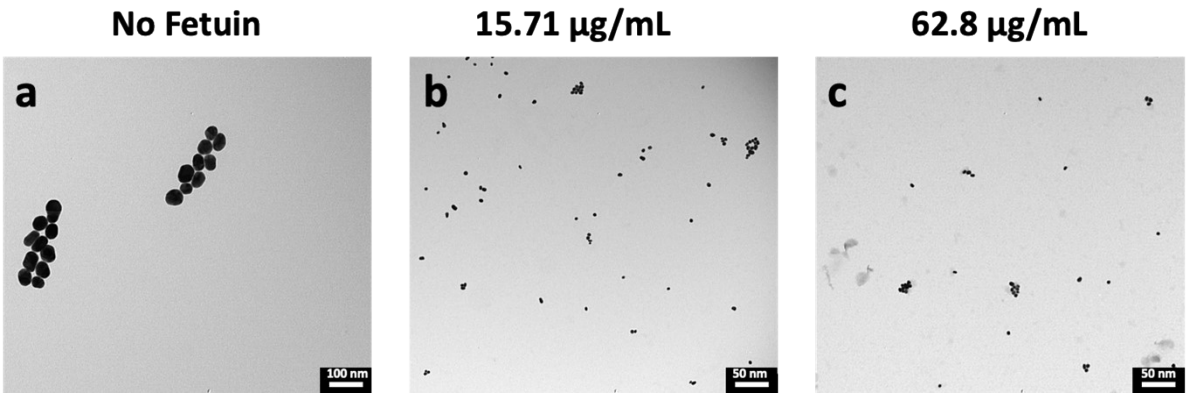

Fig. S12. TEM image of AuNP@TFMBA-WGA nanoparticles after incubation with different concentrations of Fetuin: a) 0 µg/mL, b) 15.71 µg/mL, and c) 62.8 µg/mL.

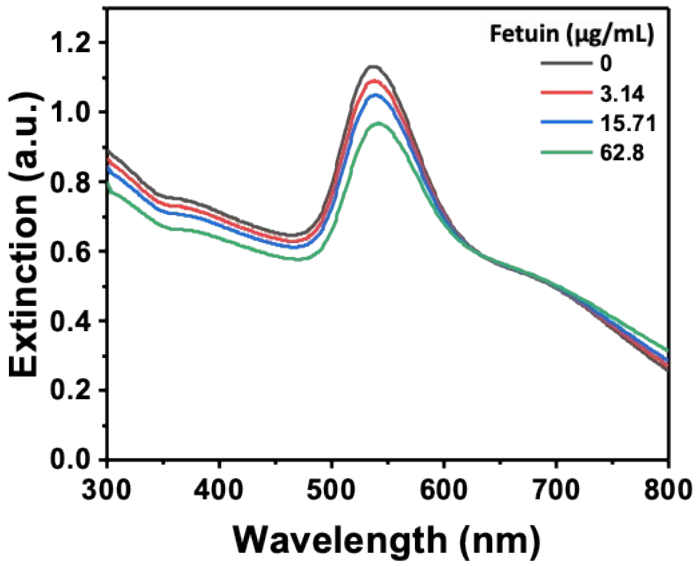

Fig. S13. Effect of concentration of Fetuin on the extinction spectra of AuNP@TFMBA-CTB SERS nanotags.

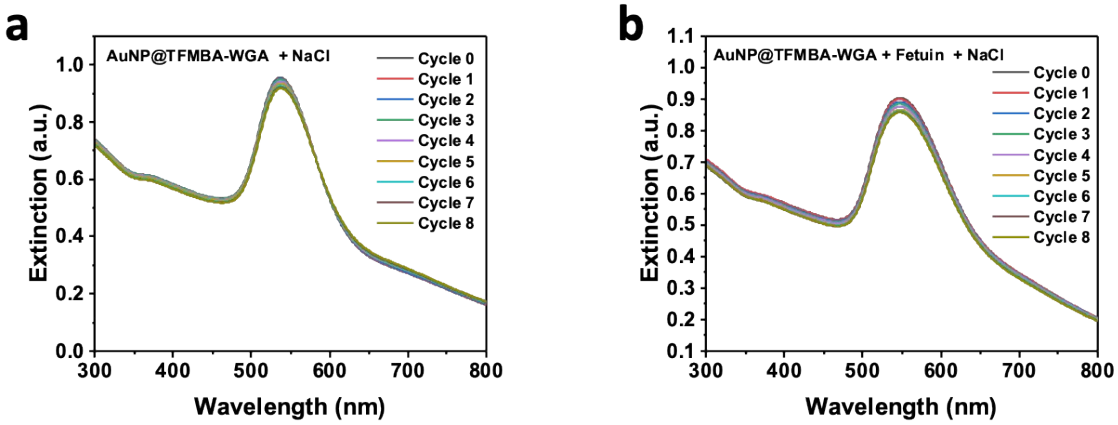

Fig. S14. Salt stability study for (a) AuNP@TFMBA-WGA and (b) AuNP@TFMBA-WGA incubated with Fetuin (62.8 µg/mL) samples. In each cycle 1.5 µL of NaCl (0.5 M) was added to 1 mL of samples.

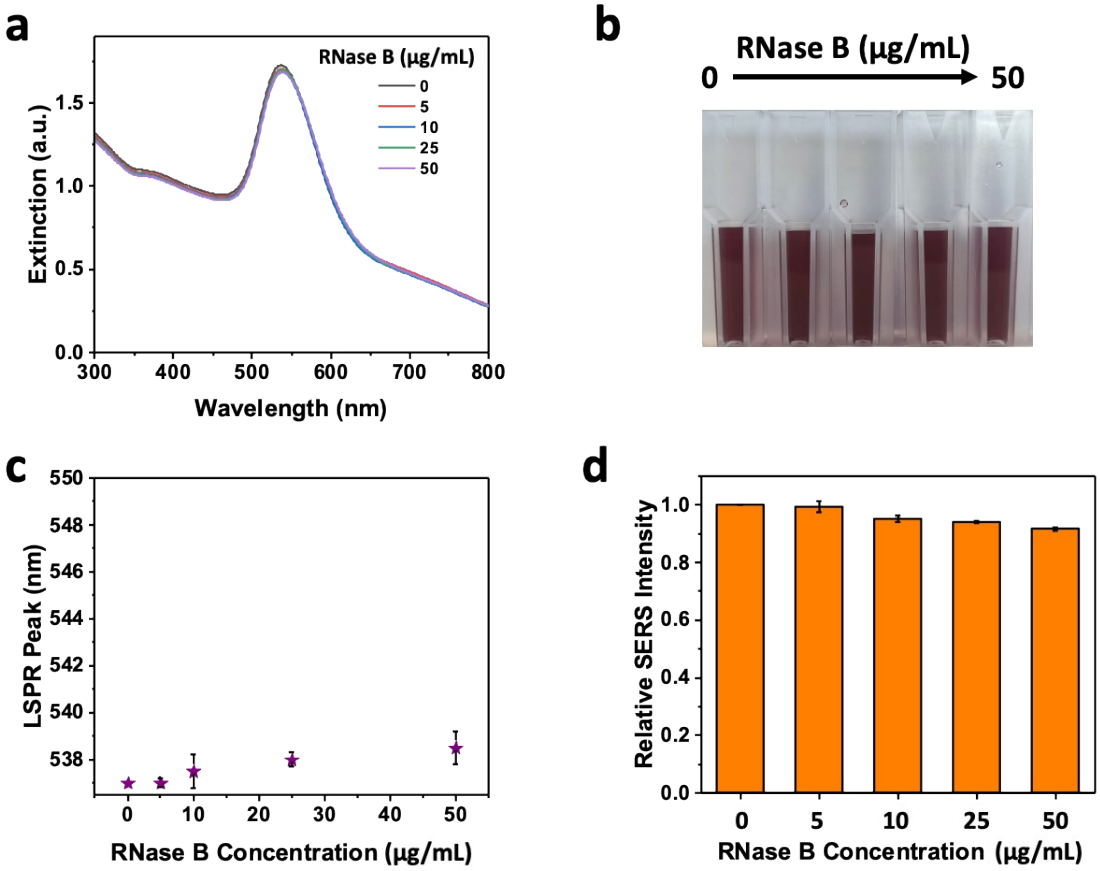

Fig. S15. a) UV-Vis spectra, b) images of solution, c) LSPR peak, and d) Relative SERS intensity of AuNP@TFMBA-WGA nanoparticles incubated with different concentrations of RNase B compared to a sample with no RNase B. ANOVA statistical analysis did not show any significant differece.

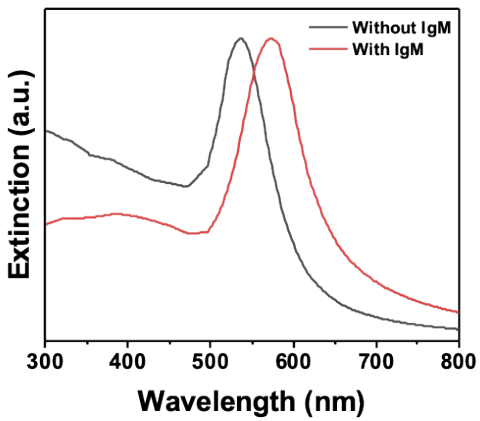

Fig. S16. Simulated extinction spectra for a cluster of seven AuNPs (50 nm) with interparticle distances of 35 nm in water, with and without IgM.

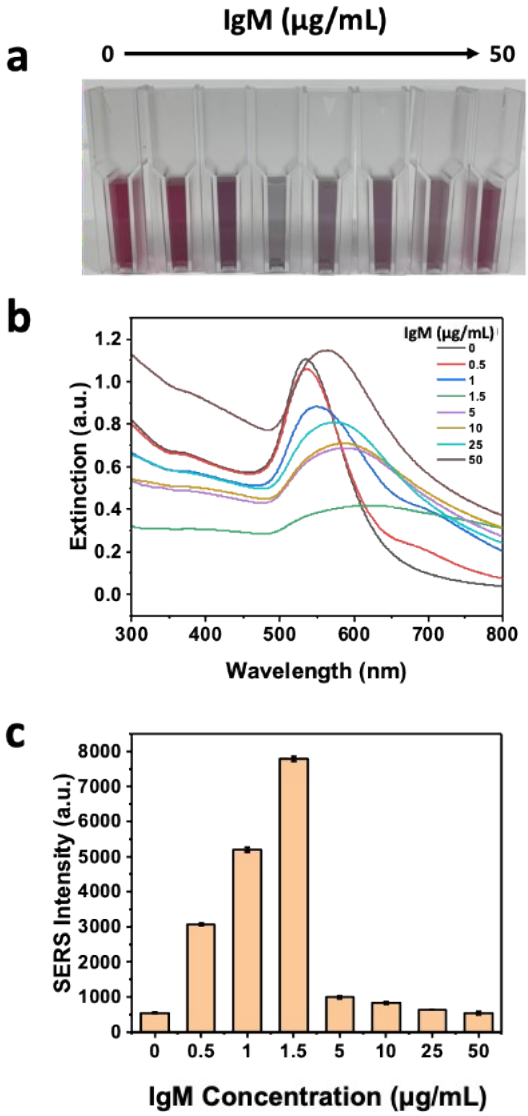

Fig. S17. a) Solution colour change, b) UV-Visible spectra and c) SERS intensity of AuNP@TFMBA after incubation with different concentrations of IgM.

References

1. P. B. Johnson and R. W. Christy, *Physical Review B*, 1972, **6**, 4370-4379.
2. J. Vörös, *Biophys J*, 2004, **87**, 553-561.