Electronic Supplementary Information (ESI)

Detailed Experimental Procedure:

For the convective assembly procedure, A 40 μL of mixture (20 μL protein solution and 20 μL AgNPs suspension) with the indicated final protein concentration was spotted at the junction of two glass slides with a micropipet. The angle between two slides was about 23^0. The velocity of the bottom stage was set to 1.0 μm/s for all measurements. The room temperature and relative humidity were 22-25 ^{0}C and 40-50% during the experiments, respectively. All SERS measurements were performed using a Renishaw InVia Raman Microscopy system equipped with a 830 nm laser. The laser power, exposure time and accumulation were 3 mW, 10s, and 1, respectively.

Table S1. Physicochemical properties of proteins used in this study.

Protein	Isoelectric point (pI)	Molecular weight (kDa)	Zeta Potential (mV)	Hyrodynamic diameter (nm)	Property
Human Serum Albumin (HSA)	4,7	66	-28,9	7,937	acidic
Immunoglobulin A (IgA)	4,63-6,85	150-350	-27,2	32,493	acidic
Hemoglobin (Hb)	6,8	64,5	-10,4	7,816	acidic
Insulin	5,3-5,35	6	-29,3	3,067	acidic
Avidin	10,5	66	+27,1	8,011	basic
Cytochrome C (Cyt c)	9,8	12	+22,5	3,718	basic

pH of Colloidal Suspensions with and without protein additions:

Table S2. pH values of only colloid and protein-colloid mixtures. All protein concentrations are 50 μg/mL.

Sample	dH_2O	1X AgNP	4X AgNP	Cyt c + 4X AgNP	HSA + 4X AgNP	IgA + 4X AgNP	Insulin + 4X AgNP
pH	6.97	8.21	8.22	7.75	7.70	7.92	7.56

As seen on Table II, the pH of only AgNP suspension is higher than AgNP-protein mixtures. Since proteins have multiple ionizable groups in their structure, they may add buffer property to the solution they are in. As they are mixed with colloidal suspension, they may not show dramatic pH change from the original suspension. Therefore, pH of the AgNP containing colloidal suspension may not change the charge status of the protein. As seen, the AgNP suspension remains basic after adding proteins with different charges.

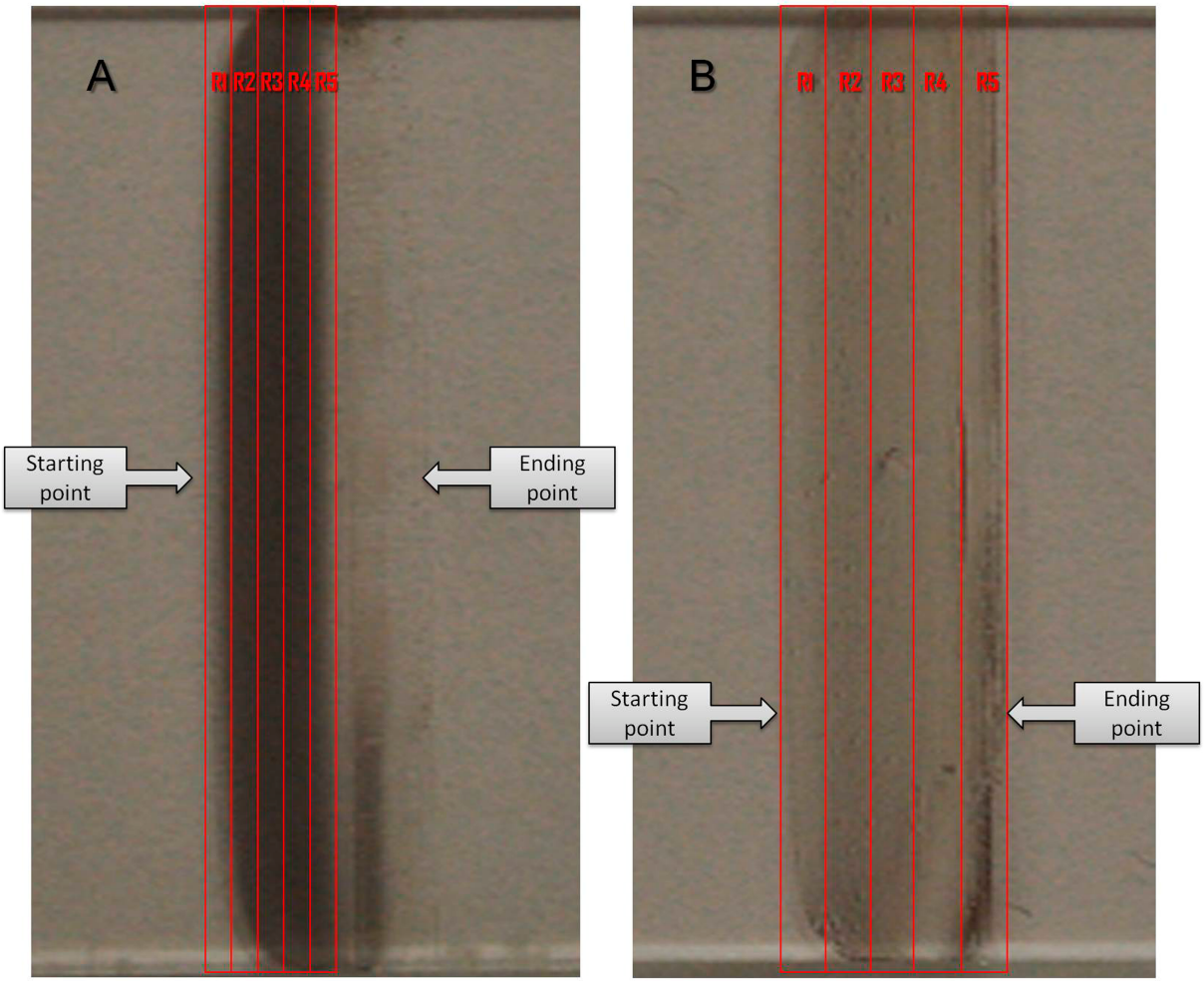

Fig. S1 Divisions of the assembly area for the positively-charged proteins Avidin-Cyt c (A) and the negatively-charged proteins IgA-Hb (B).

As seen, the assembly region of the negatively charged proteins is larger than the assembly region of the positively charged proteins. Negatively charged AgNPs interact with positively charged proteins and, due to the effective packaging, they sediment in a smaller area. The distance of each region on convectively assembled area of Avidin-Cyt c-AgNP is 500 μm and IgA-Hb-AgNP is 900 μm.

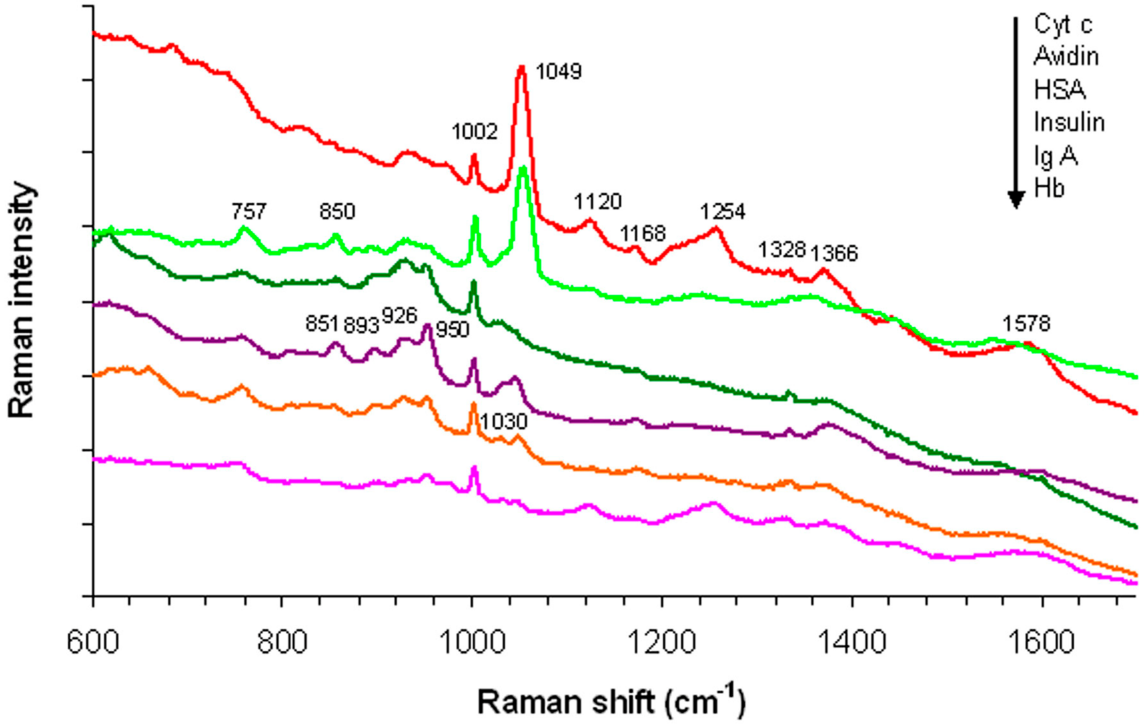

Fig. S2 The SERS spectra of each protein used in the study.

Reproducibility of SERS Spectra Obtained from the Assembled Area:

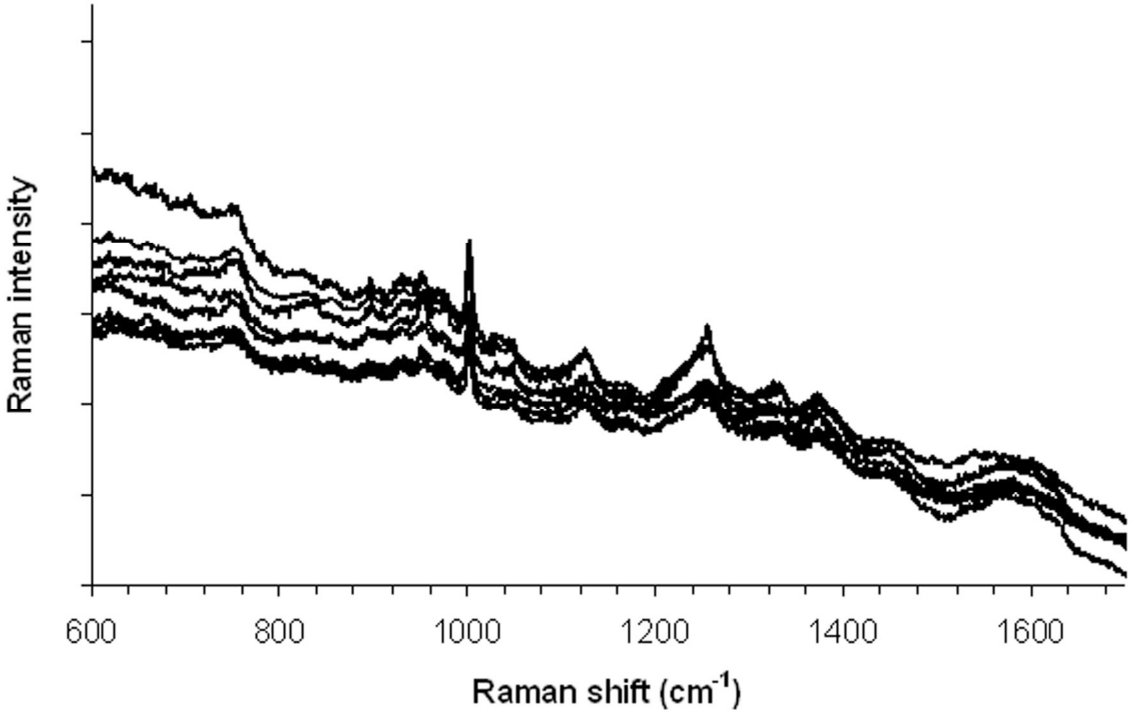

Fig. S3 The reproducibility of SERS spectra of Hemoglobin on the assembled area but different spots

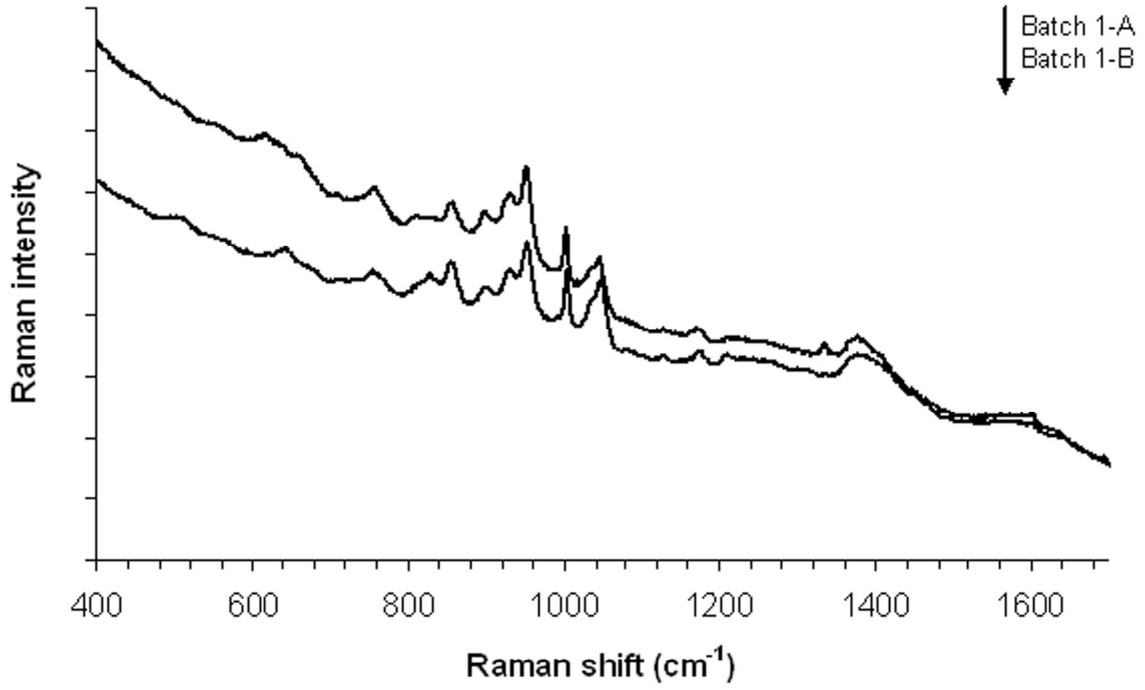

Fig. S4 The SERS spectra of Insulin with same batch of colloid in different times.

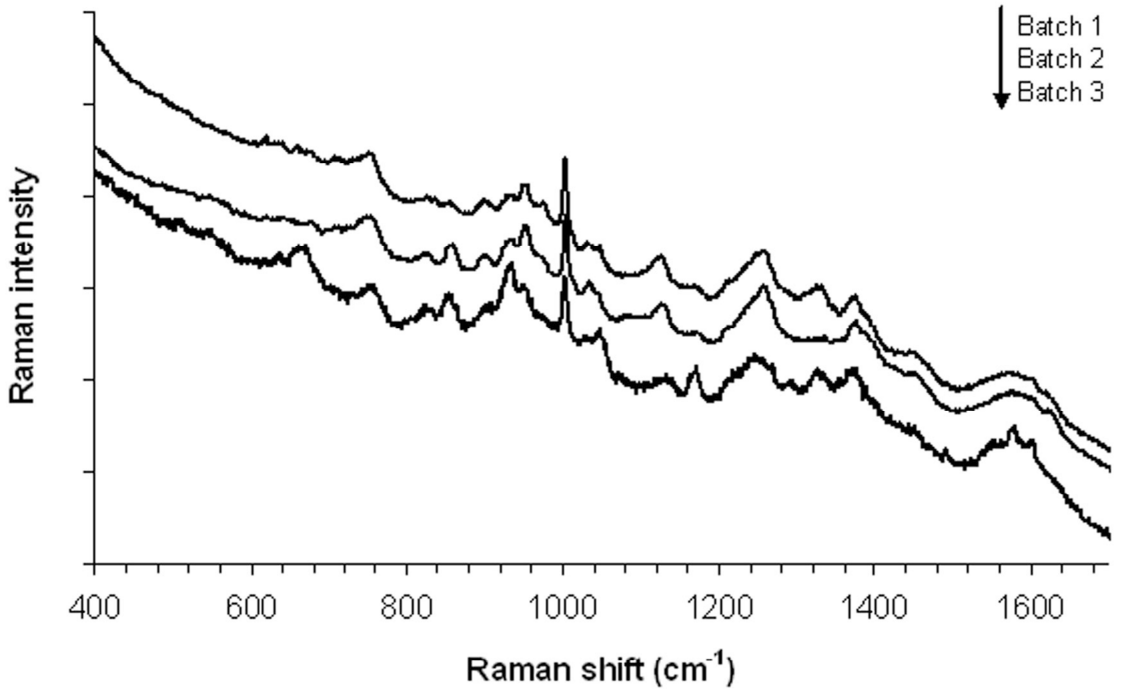

Fig. S5 The SERS spectra of Hb with different batches of colloidal suspensions

Figure 3-5 shows the spot-to-spot on the assembled area, sample-to-sample (the same batch of AgNP colloid but different times of the day or different days), and batch-to-batch reproducibility. As seen the reproducibility is satisfactory for the comparison of the spectra.

SERS Spectra of HSA and Avidin Mixture:

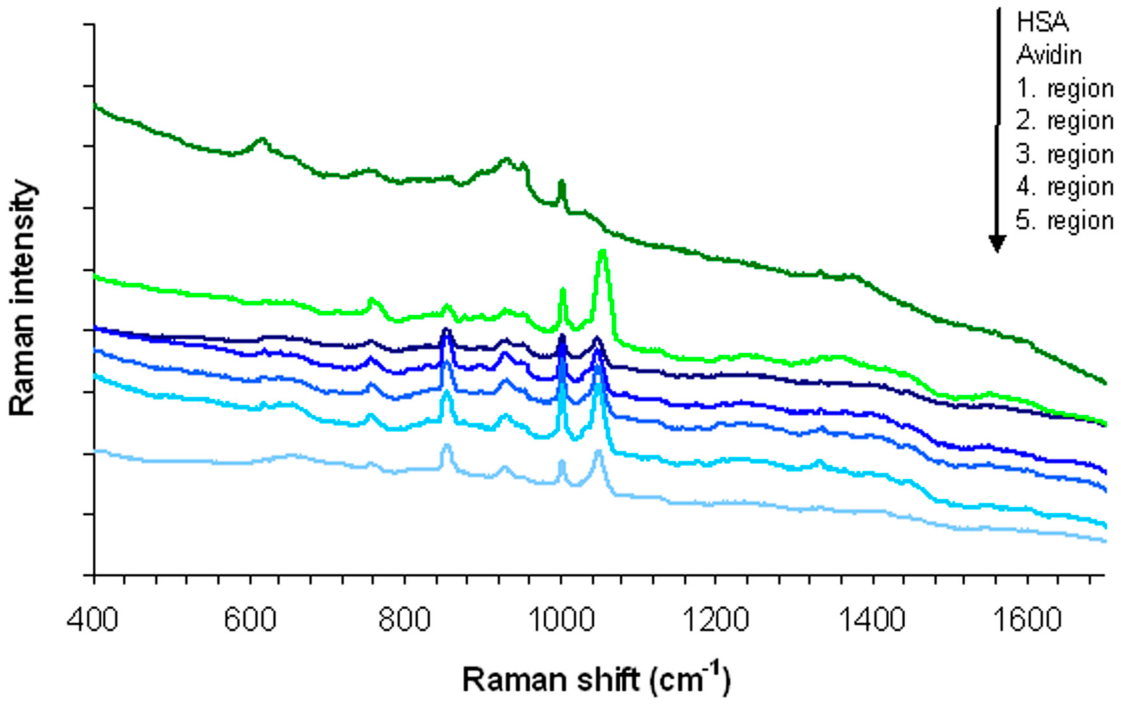

Fig. S6 The SERS spectra of HSA-avidin-colloid mixture

As seen in Figure 6, each spectrum from different regions is almost same and has a high consistency with the spectrum of avidin. This shows that proteins are not separated significantly. Proteins with different charges highly interact with each other and colloidal NPs, which prevents the differential distribution of proteins in the assembled area. However, our studies continue to explore the experimental conditions such as pH and temperature to achieve the goal.