Electronic Supplementary Material (ESI) for Analyst.

SUPPORTING INFORMATION TO "A NEW LOOK AT AN OLD CLASSIC: IMPLEMENTATION OF A SERS-BASED WATER HARDNESS TITRATION"

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TABLE DES MATIERES

DETAILED CHEMICAL PROTOCOLS

CHEMICALS

Ethylenediamine tetraacetic acid, di sodium salt dihydrate (99% purity as per titration, CAS 6381-92-6r), **Eriochrome Black T** (Reagent specification as per European Pharmacopoeia, #CAS 1787-61-7), **polyethylene glycol** (MW ~20 kDa, BioUltra grade, CAS 25322-68-3), **polyethyleneimine** (branched, M_w ~25 kDa by light scattering (M_n ~10 kDa by gel permeation chromatography), 13000 – 18000 mPa·s viscosity, CAS 9002-98-6), **silver nitrate** (ACS reagent, ≥99.0%, CAS 7761-88-8), **sodium citrate tribasic dihydrate** (p.a., ≥99.0%, CAS 6132-04-3) and tetrasodium borate (99%, BioUlra, CAS1330-43-4) were purchased from Aldrich. **Glacial acetic acid** (100%, NormaPur, CAS 64-19-7) and **disodium hydrogen phosphate dodecahydrate** (102% assay, NormaPur, CAS 7558-79-4) were purchased from VWR. All pH adjustments were performed using a 1 N **sodium hydroxide** standardised solution from Aldrich. All chemicals were used as received without further purification. Evian, Volvic and Contrex water were stocked as 1.5 L bottles from a supermarket.

SYNTHESIS OF LEE-MEISEL SILVER NANOPARTICLES

Apparatus for the synthesis process. A 250 mL triple-neck round bottom flask was washed using 100 mL (1 volume) aqua regia (a mixture of 80 mL hydrochloric acid (37%wt) and 20 mL nitric acid (69%wt)) prior to synthesis, rinsed with 10 equivalent volumes of miliQ water, then with 0.5 volume of 70% ethanol afterwards and finally dried overnight in a 55°C stove. The flask was nested within a silicon oil bath on top of a magnetic heating plate. The temperature was controlled through a thermocouple inserted in the oil bath and a temperature controller. The flask was equipped with an oval stir bar and a condenser.

Synthesis. To synthesize 100 mL of silver nanoparticle solution, 20 mg silver nitrate were inserted into the flask along with 100 mL ultrapure water. The solution was brought to reflux under stirring using a 130°C set point for the oil bath. 2 mL of trisodium citrate 34.0 mM were quickly injected using a syringe into the reaction mixture through one of the side necks. The temperature was set and maintained at 100°C for 1 hour. Over time, the solution turns yellow then grey to reach a final milky green-grey colour. After cooling, the solution is stored in a closed glass bottle at 2-5°C.

Munro *et al.* have proposed on the basis of NMR data that the one-electron reduction of Ag(I) combines with a two-electron oxidation of citrate as acetonedicarboxylic acid and CO_2 (through formate).[1] The balanced chemical equation for the reduction of Ag(I) by citrate then writes as

$$2Ag^{+} + C_6H_5O_7{}^{3-} \rightarrow 2Ag + C_4H_5O_3{}^{-} + 2CO_2$$

Given this stoichiometry, silver is the limiting reagent of the reaction in our conditions. Assuming again that the reaction is quantitative, the as-synthesised solution has a 1.2 mM Ag concentration as colloidal nanoparticles and contains 88 µM residual citrate.

Removal of surface citrates. In order to achieve more reactive surfaces, the residual citrates in the previously synthesized Lee-Meisel silver NPs were displaced by supplementing the reaction mixture with sodium chloride at a final concentration of 0.2 mM, according to a protocol by Stewart *et al*. [2] The mixture was shaken via vortex mixing and incubated for 10 minutes. The solution was then aliquoted as 12.5 ml fractions into 15 mL Falcon tubes, centrifuged at 6000 rpm for 25 min on a Hettich EBA 21 centrifuge. The supernatant was discarded to leave a 600

µL NP-rich fraction which was resuspended in ultrapure water to reach back the initial volume, and homogenized using a vortex mixer and then by ultrasonication for 4 min. All aliquots were then merged into a single stock which was stored in a closed glass bottle at 2-5°C. The assumed Ag concentration as colloidal nanoparticles is 1.2 mM. At most, the residual citrate concentration is 4.2 µM and the chloride concentration is 0.01 mM. The NPs are ready to be used after one night of aging and remain functional for at least 5 days under these storing conditions.

BEAD ASSEMBLY AND CUVETTE PREPARATION FOR SERS MEASUREMENTS

STOCK SOLUTIONS

All stock solutions were prepared using volumetric glassware (glass pipettes and flask) and scales with a 1 mg or 0.1 mg precision under our experimental conditions.

Britton and Robinson buffer (BRB). An equimolar mixture of acetic, phosphoric and boric acid) 40 mM in boron at pH 8 was prepared from glacial acetic acid, disodium hydrogen phosphate and sodium tetraborate. This mother buffer was then diluted to give rise to BRB 4 mM in boron at pH 8 and BRB 4 mM in boron at pH 10.5 (pH adjustment was performed using NaOH 1N).

PEI solution. A 10 mM (in terms of monomers) mother solution was prepared by mixing 43 mg of PEI with 10 mL BRB 4 mM pH 8. The solution was diluted to a 1 mM stock solution using BRB 4 mM pH 8 as the solvent.

PEG solution. A 17%wt PEG stock was prepared by dissolving 80 g of PEG in 400 g of BRB 4 mM pH 10.5.

EBT solution. 23.3 mg of EBT were dissolved in 10 mL ultrapure water to give a 5.05 mM stock.

EDTA solutions. 379 mg EDTA disodium salt dihydrate were introduced in a 250 mL volumetric flask and dissolved in approximately 100 mL BRB 4 mM pH 10.5. The pH was monitored and adjusted to 10.5 through addition of approximately 1.7 mL NaOH 1 N. The flask was topped up to the mark using BRB 4 mM pH 10.5. The pH can drift by

∼2% overnight. On the day of SERS acquisition, the pH was adjusted to 10.5 using NaOH 1N (about 150 µL NaOH 1 N to adjust 100 mL of EDTA 4 mM). The final EDTA titer was 4.06 mM. Following the same protocol, 1861 mg of EDTA disodium salt dihydrate were introduced in a 250 mL volumetric flask along with 100 mL BRB 4 mM pH 10.5 and approximately 8 mL of a NaOH 1N. The pH was measured and adjusted to 10.5 before topping up the flask to the mark with BRB 4 mM pH 10.5. Overnight, the pH had dropped to 10.35 and was adjusted back to 10.5 to give a 19.97 mM stock. The 4.06 mM solution was diluted 5 times with BRB 4 mM pH 10.5 to give a 0.81 mM solution unsing a 10 mL volumetric pipette and a 50 mL volumetric flask.

PREPARATION OF PLAIN BEADS FOR ABSORPTION AND SERS MEASUREMENTS AND OPTICAL IMAGING

In a disposable hemolysis tube, 1.5 mL of chloride-derived Ag NP solution were inserted along 1.5 mL of PEG stock. The mixture was homogenized by vortex mixing. Next, 26 µL of PEI stock were added and the mixture was again homogenized by vortexing. The solution was transferred to a disposable PMMA spectrophotometric cuvette and left to mature 1 hour before acquiring its UV-vis absorption spectrum.

PREPARATION OF SAMPLES FOR SERS-BASED TITRATIONS

Samples for SERS-based titrations (of mineral water or of blank) were prepared according to the protocol depicted in **Fig. S5** using the stock solutions described above. The procedure involves adding into hemolysis tubes a constant volume of sample supplemented with EBT, a variable volume of appropriate buffer, a variable volume of appropriate EDTA stock such that the total of buffer and EDTA fractions remains at 600 µL, a constant volume of chloride derived Ag NP solution, a constant volume of PEG stock and a constant volume of PEI to initiate bead formation, in that order. The content of the tubes is homogenized using a vortex mixer after addition of the EDTA, NP, PEG and PEI fractions. The full mineral water titrations series in triplicate plus the blank titration series have been prepared at once, adding each reagents into the 4x18 tubes and then moving to the next one. The water samples supplemented with EBT were obtained by mixing 1 volume of EBT 5.05 mM stock (500 µL using a 1 mL graduated glass pipette) with 100 volumes of mineral water or ultrapure water (two samplings using a 25 ml volumetric pipette).

The mixtures were aged for 1 hour before launching the spectral acquisitions. Two operators were required to run the optical measurements so that acquisitions under the two excitation wavelengths were run at similar aging time. Operator A measured two titrations series under 638 nm excitation while operator B measured the two remaining series under 785 nm. They then swapped sample trays.

PREPARATION OF PLAIN BEADS FOR SERS CONTROL MEASUREMENTS

Plain beads having the same Ag concentration as those assembled in the SERS-titration sample were prepared by substituting ultrapure water for the fraction of sample water supplemented with EBT, all other components being the same.

PREPARATION OF CUVETTE FOR NORMAL RAMAN MEASUREMENT OF EBT

1,826 µL of ultrapure water were mixed with 2,100 µL BRB 4 mM pH 10.5 and 3 µL of 4.8 mM EBT solution to give rise to a 3.7 µM EBT solution at pH 9.90.

INSTRUMENTAL SET-UPS

SERS MEASUREMENTS: 638 NM & 785 NM SET-UP

Spectra under 638 nm excitation were acquired using an optical fiber-based set-up from Ocean Optics comprising a QE Pro spectrometer, a 638 nm laser module (I0638SL0050MA) and a coaxial Raman probe for measurement in backscattering configuration. The laser power was within 50-100 mW. PMMA spectrophotometric cuvettes were nested into a 4-window sample holder which had 3 windows obliterated. The integration time was 15 s and the spectra were recorded without averaging.

Spectra under 785 nm excitation were acquired on an integrated BWTEK *iRaman Plus* portable spectrometer equipped with a 785 nm laser having a maximal power of 420 mW and a laser spot size at the sample surface of 85 µm. Excitation and collection were performed in backscattering configuration using a coaxial optical fibre Raman probe. The spectrometer resolution is ~4.5 cm^{-1}. The spectral acquisition range was set to 300–2000 cm^{-1}. The acquisition time was set to 2 s and the laser power to 20%, or 15% in the few instances where the signal was saturated. Each spectrum was averaged over 3 spectral recordings.

UV-VIS MEASUREMENTS

UV-vis absorption spectra were acquired on a Jasco V-630 UV-vis spectrophotometer in PMMA disposable cuvettes.

OPTICAL IMAGING OF SERS ACTIVE AGGREGATES

Optical imaging of SERS aggregates was performed on an Olympus inverted microscope mounted with a 100 X objective. A Gene Frame (65 µL [1.5 x 1.6 cm]) was placed onto a microscopy slide and 50 µL of active aggregate solution (non-diluted) was inserted within the frame. The filled Gene Frame was closed using a glass cover slip. Care was taken to avoid any air bubbles within the reservoir.

TEM IMAGING

TEM imaging of chloride derived nanoparticles was performed on a Tecnai12 transmission electron microscope (ThermoFischer Scientific) equipped with a 4K×4K Oneview camera (Gatan), under 120kV voltage. The Cl-NP mother solution (assumed Ag concentration of 1.2 mM) was diluted 2.6 fold to limit aggregation onto the grid. Carbon coated copper grids (400 mesh) were used. Parafilm was sticked to the laboratory desk and a drop of 10 µL nanoparticle solution was placed on top pf the parafilm surface. The carbon coated copper grid was placed upside down onto the drop (shiny side of the grid in touch with the solution). The carbon coated copper grid was incubated for 5 minutes. Afterwards, a Kimwipe filter paper was used to remove residues of the drop on the edge of the carbon coated copper grid. The carbon coated copper grid is placed within a half open petri dish on a filter paper to dry. After 10 minutes the dry carbon coated copper grid can be inserted within the TEM sample holder.

SPECIATION ANALYSIS

Speciation analysis was conducted using Visual MINTEQ software with the speciation constants provided by Cheng, Ueno and Imamura.[3]

SERS AND RAMAN SPECTRAL PROCESSING

Laser 638 nm. The raw data contains 1044 data points with Raman shift ranging from 99.943 to 3309.417 cm^{-1}. Then they are truncated to keep the Raman shift range from 164.32 to 3267.65 cm-1 corresponding to 1010 data points.

Laser 785 nm. The raw data contains 1803 data points with Raman shift ranging from 296.86 to 3294.32 cm^{-1}. Then they are truncated to keep Raman shift range from 331.77 to 3200.76 cm^{-1} corresponding to 1746 data points.

Spectral data were processed independently by the lead and corresponding authors using the open-source software R or Python.[4,5] Baseline corrections were performed using either asymmetric least squares smoothing in Python or polynomial fitting (*modpolyfit()* function of the "baseline" package in R).[6,7] Both spectral processing gave identical results. The data presented in the figures were treated in R.

PHYSICO-CHEMICAL CHARACTERISATIONS

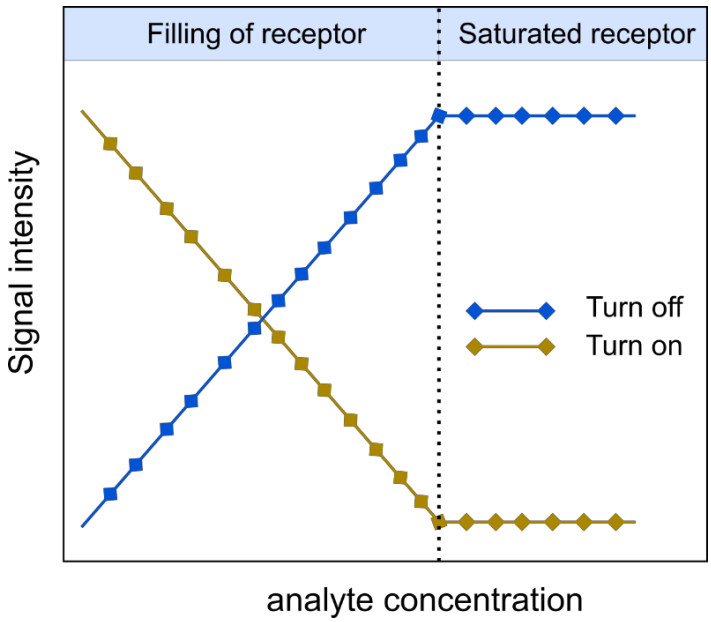
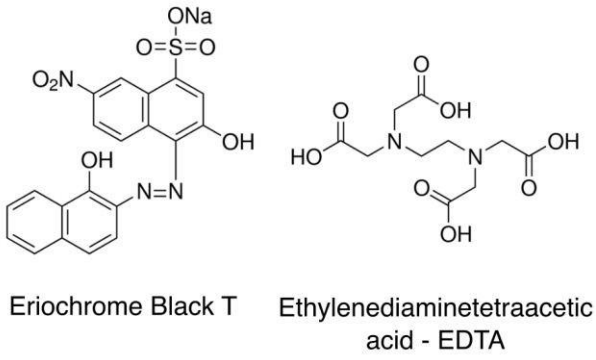


Figure S0: Schematic depiction of calibration models when detecting an analyte in spectrophotometric mode through its interaction with a receptor.



Eriochrome Black T

Ethylenediaminetetraacetic acid - EDTA

Figure S1: structures of the indicator (EBT) and chelator (EDTA) used in the water hardness titration.

(a) **(b)**

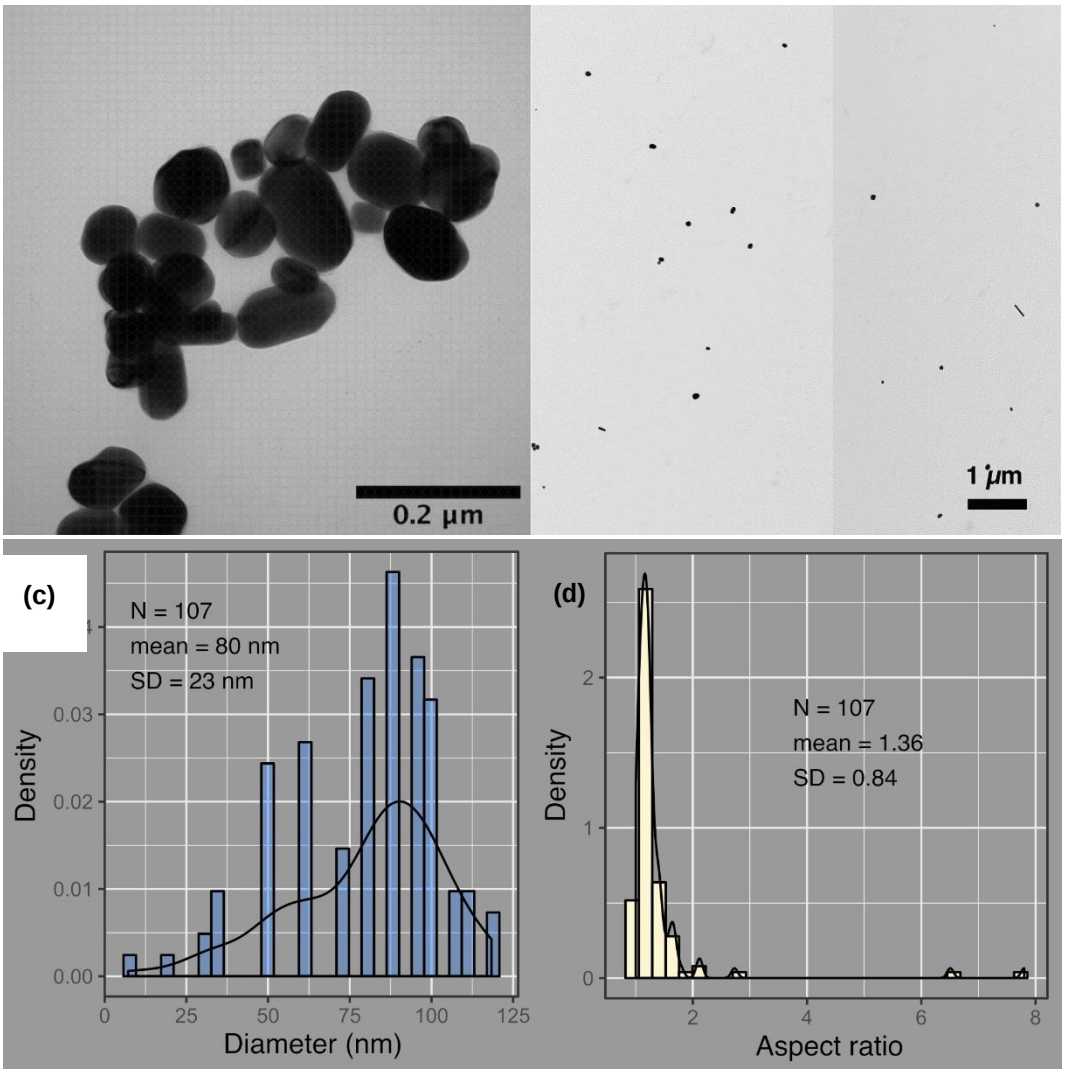


Figure S2: TEM micrograph of chloride derived NPs at high **(a)** and low magnification **(b)**. The diameter distribution, **(c)**, was determined from manually measuring the area of NPs on micrographs having the same magnification as **(b)** and assuming that the particles were spherical. The NPs have in fact an aspect ratio of 1.36 ± 0.84 **(d)**.

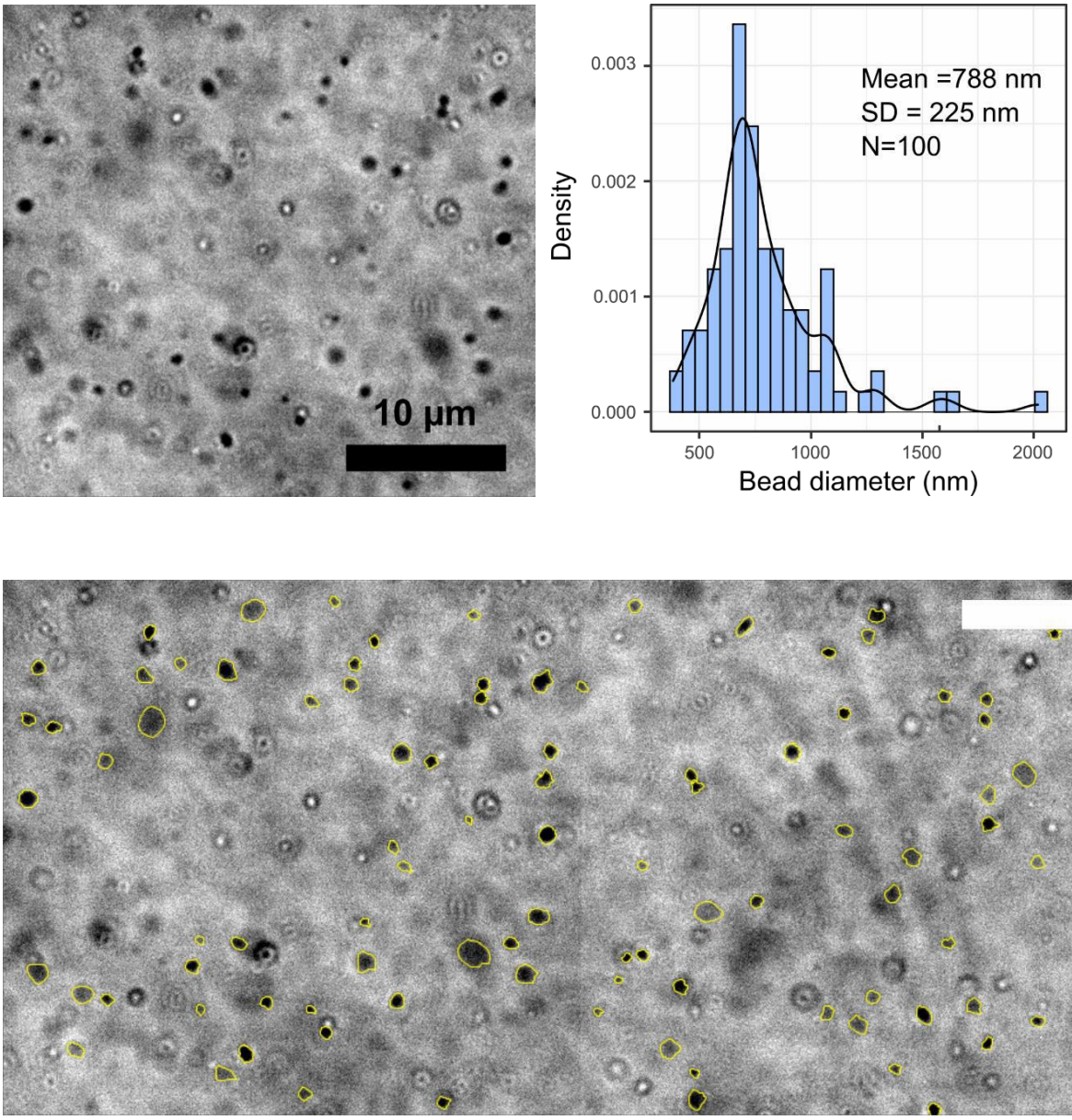


Figure S3: optical imaging of SERS aggregates (100X objective) and corresponding size distribution (up); outlines of particles detected using Image J which were used to estimate the size distribution (down). The NPs were manually outlined using the polygo selection tool.

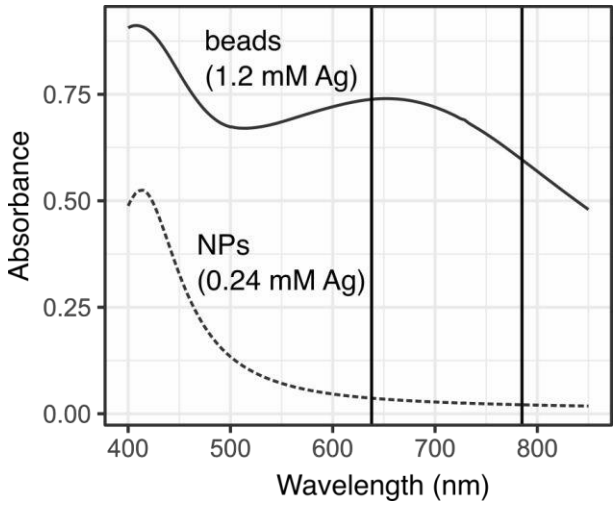


Figure S4: Extinction spectra of Cl-exchanged NPs and plain beads (synthesised without incorporated EBT). The vertical lines indicate the excitation wavelengths of the Raman spectrometers used for SERS acquisition (638 and 785 nm).

PRACTICAL RUNNING OF TITRATION

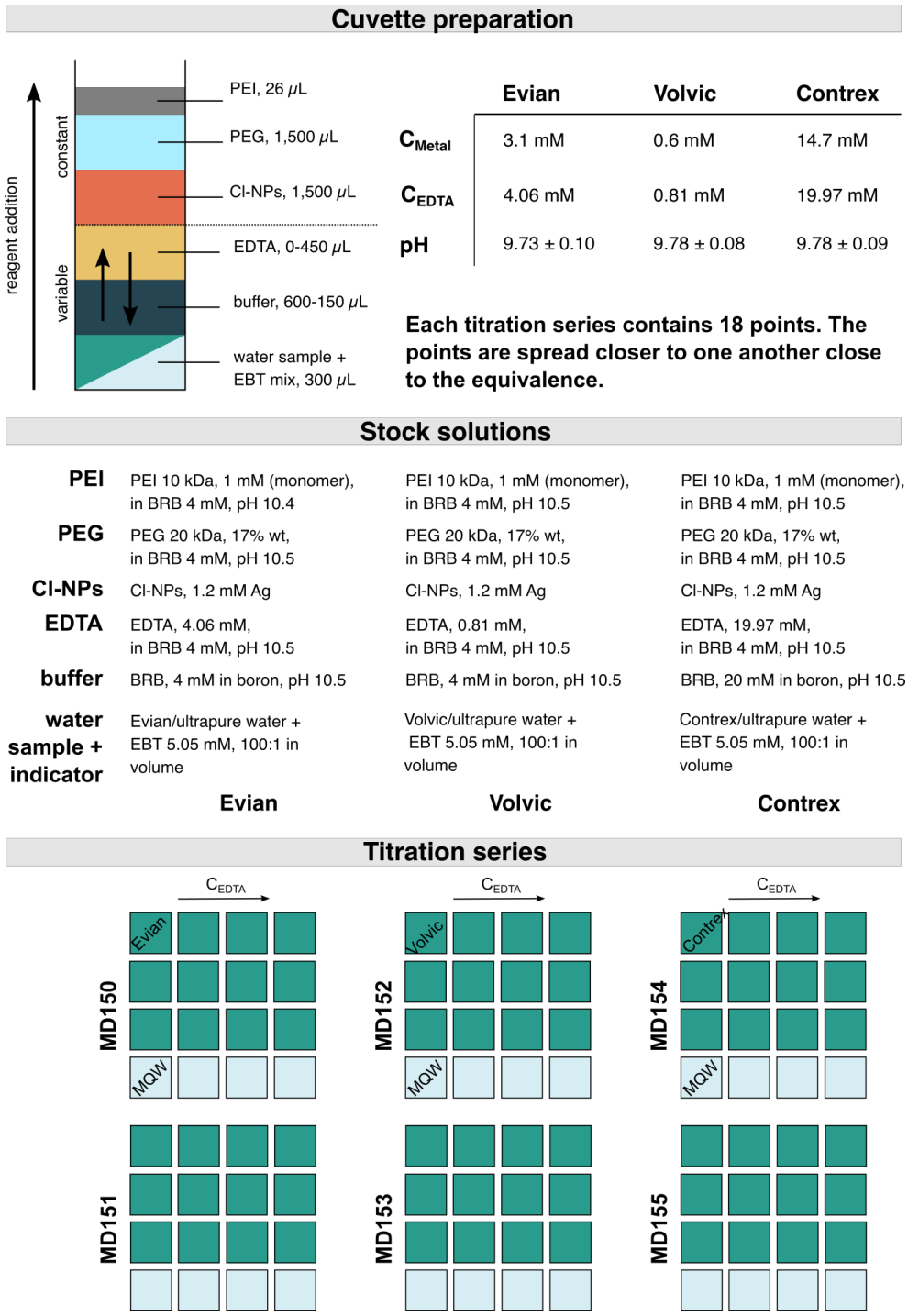


Figure S5: Graphical depiction of reagent addition to prepare the cuvettes for SERS acquisition. The reagents were added stepwise into a hemolysis tube and then transferred to a spectrophotometric cuvette. The EDTA, PEG and PEI solutions were buffered using Britton & Robinson buffer 4 mM in boron. The final pH in the cuvettes were comprised between 9.74 and 9.90. For titration samples, 18 different combinations of EDTA and water volumes were explored, the total volume of the sample being kept constant at 3,926 µL.The codes of titration series can be used to retrieve the specific data in the provided datasets.

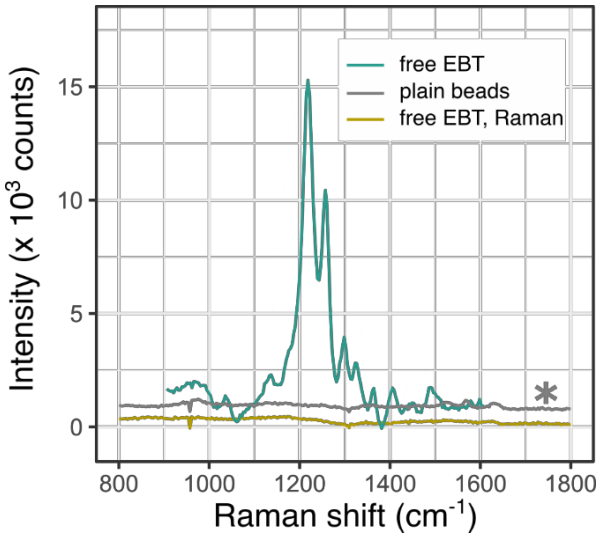


Figure S6: SERS spectra of free EBT-labelled beads and plain beads, along with normal Raman spectrum of free EBT. The pH is 10 for all samples. Where applicable, EBT concentration is 3.7 µM and Ag concentration is 0.46 mM. Note that the SERS spectrum of plain beads (*) has been offset for clarity.

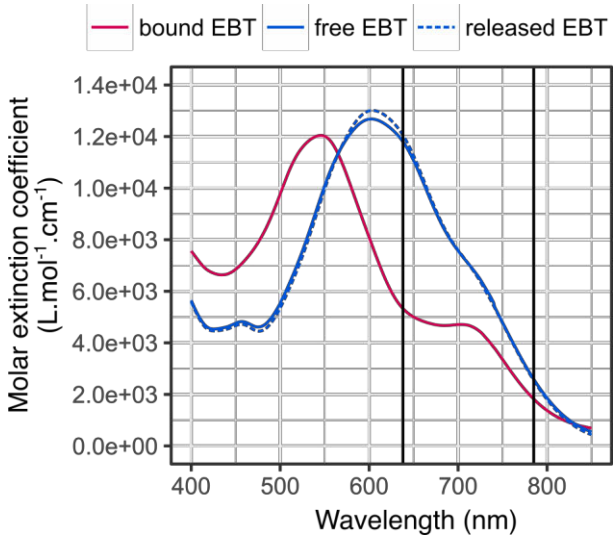


Figure S7: Visible absorption spectra at pH 10 of EBT (33 µM) in the as a free species, as a bound species (in the presence of 0.18 mM Mg^{2+} and 0.33 mM Ca^{2+}) and again as a free species upon release of Mg^{2+} through addition of 6 metal equivalents of EDTA. The vertical lines indicate the excitation wavelengths of the Raman spectrometers used for SERS acquisition (638 and 785 nm).

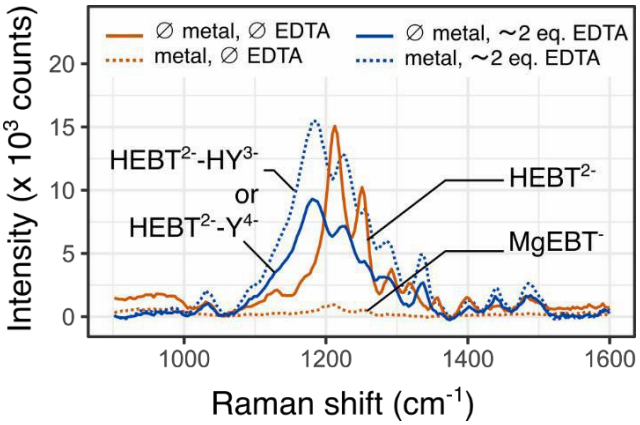


Figure S8: Tentative assignment of spectral contribution to species adsorbed on the surface of the SERS-active aggregates. According to speciation analysis at pH 10, over the course of the titration, EBT can exist as $MgEBT^-$, $CaEBT^-$ and $HEBT^{2-}$ and EDTA exists as CaY^{2-}, MgY^{2-}, HY^{3-} and Y^{4-}. In the absence of metal, EBT likely adsorbs on the surface of NPs as $HEBT^{2-}$. Upon addition of EDTA, still in the absence of metal, we hypothesize that EDTA adsorbs onto the NPs as HY^{3-} but also as Y^{4-} since it accounts for 10% of dissolved EDTA at this pH. These deprotonated forms of EDTA could engage into hydrogen bonding with $HEBT^{2-}$ on the surface of the NPs, driving the partial deprotonation of the last exchangeable proton of EBT. In the presence of a saturating amount of divalent cations, EBT adsorbs as complexes, mostly as $MgEBT^-$ since it accounts for 87.5% of all dissolved EBT. After addition of 2 eq. of EDTA, the spectrum of the hypothesized hydrogen-bonded complexes of $HEBT^{2-}$-Y^{4-} or $HEBT^{2-}$-HY^{3-} is restored. More detailed investigations would be needed to support such tentative species assignment, in particular through acquisition of spectra at well-resolved pH steps, but were beyond the scope of the present study.

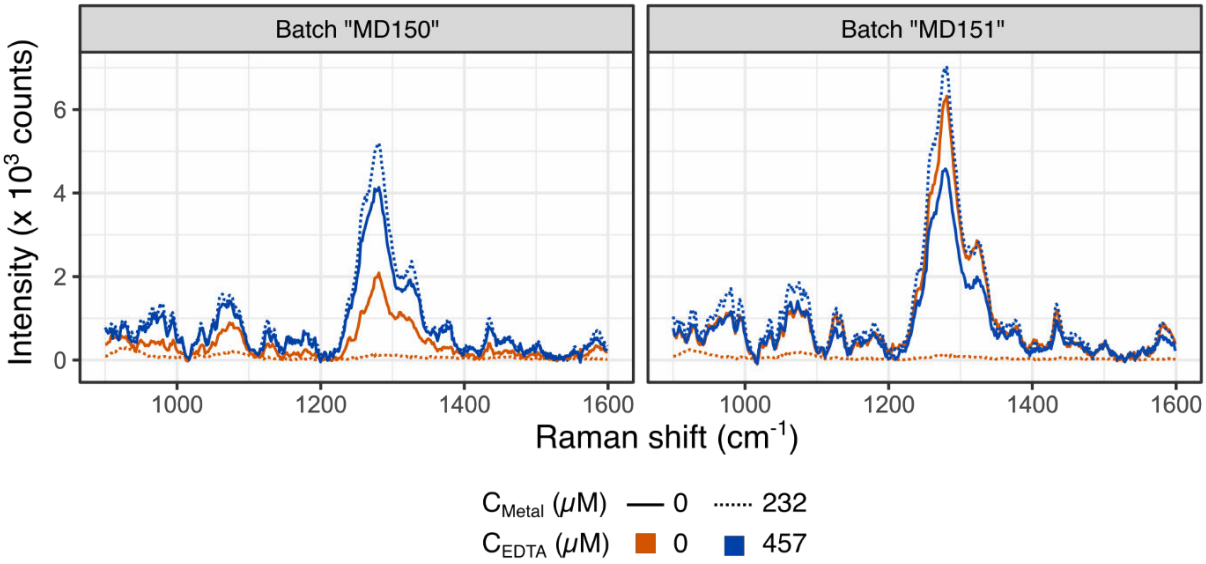


Figure S9: SERS spectra of free and bound EBT, in the presence or absence of an excess of EDTA, acquired under 785 nm irradiation. The analytical concentration of EBT is 3.7 µM in each condition. Complexation of the indicator occurs in the presence of 82 µM Mg^{2+} (and 150 µM of Ca^{2+}). The spectra have been acquired from two distinct batches of nanoparticles.

OVERVIEW OF SERS TITRATION DATA

The SERS titration data have been acquired in order to investigate the reproducibility (within a batch of nanoparticles), repeatability (between batches of particles) and transferability or robustness across water samples or across equipment (and in particular, across excitation wavelength) of the SERS-based titration of water hardness.

For each of the 3 water samples, 18 points-titration were repeated 3 times along with one additional blank titration, for two batches of particles, and were monitored at two common excitation wavelengths, resulting in a total of $18 \times (3 + 1) \times 2 \times 2 = 864$ SERS spectra. To facilitate an overview and comparison of the spectra, and an appreciation of their reproducibility, they were regrouped into lattices of 108 spectra for each water sample and irradiation wavelength. To each lattice of spectra corresponds 3 lattices of titration curves plotted from 3 spectral proxies.

The data can be downloaded from a scientific data repository (DOI: 10.5281/zenodo.6980619).[8]

In those files, the spectra were ordered as row vectors, the 17 first columns of which give the chemical composition and settings of optical measurements. The remaining columns are the baseline-corrected Raman intensities at the Raman shift given as headers.

WATER HARDNESS TITRATION OF EVIAN

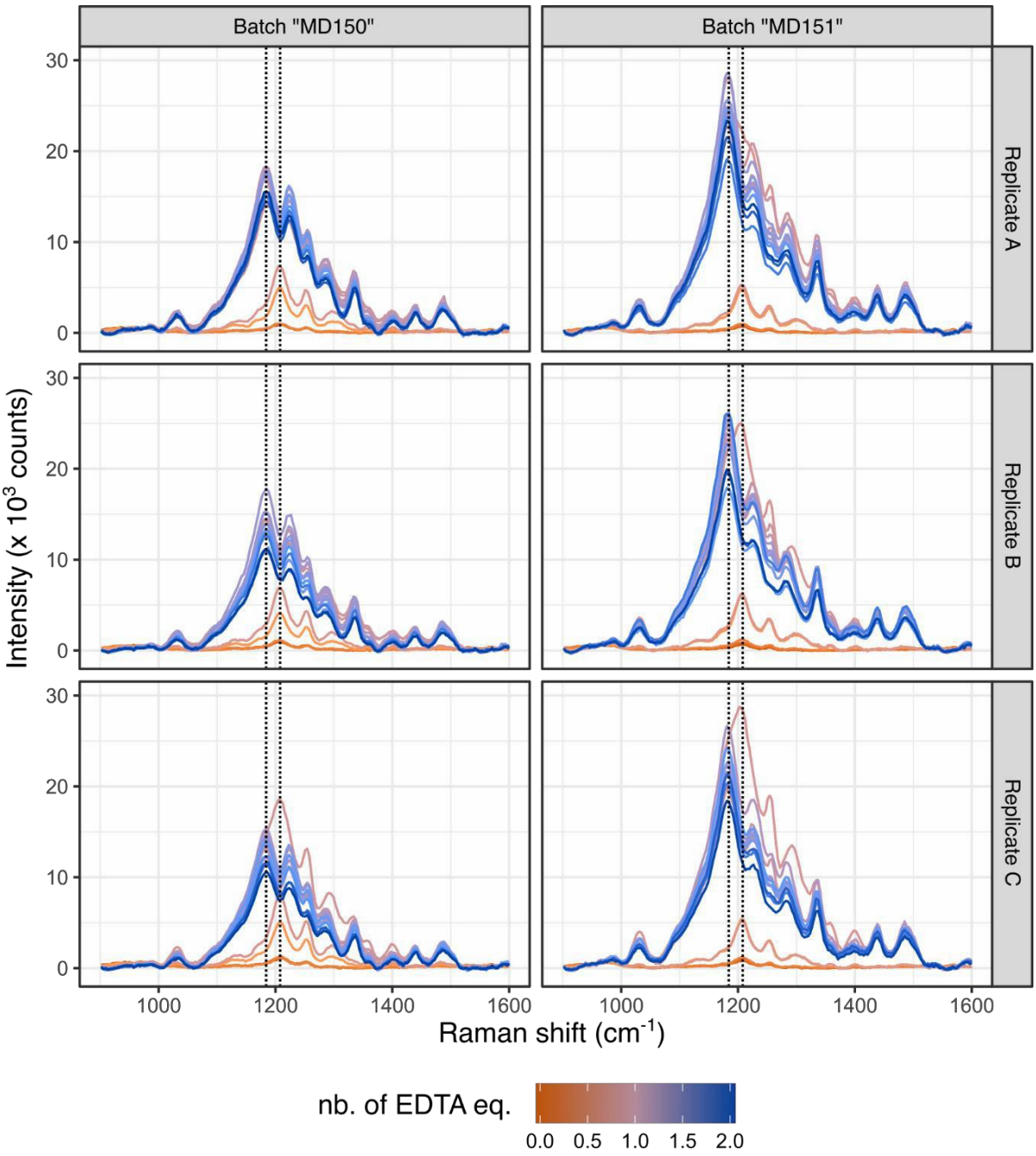


Figure S10: Replicates of Evian hardness titration monitored in SERS under 638 nm excitation as described in Figure S5. Three titration series (A, B, C) of 18 EDTA concentrations were performed using two distinct NP batches.

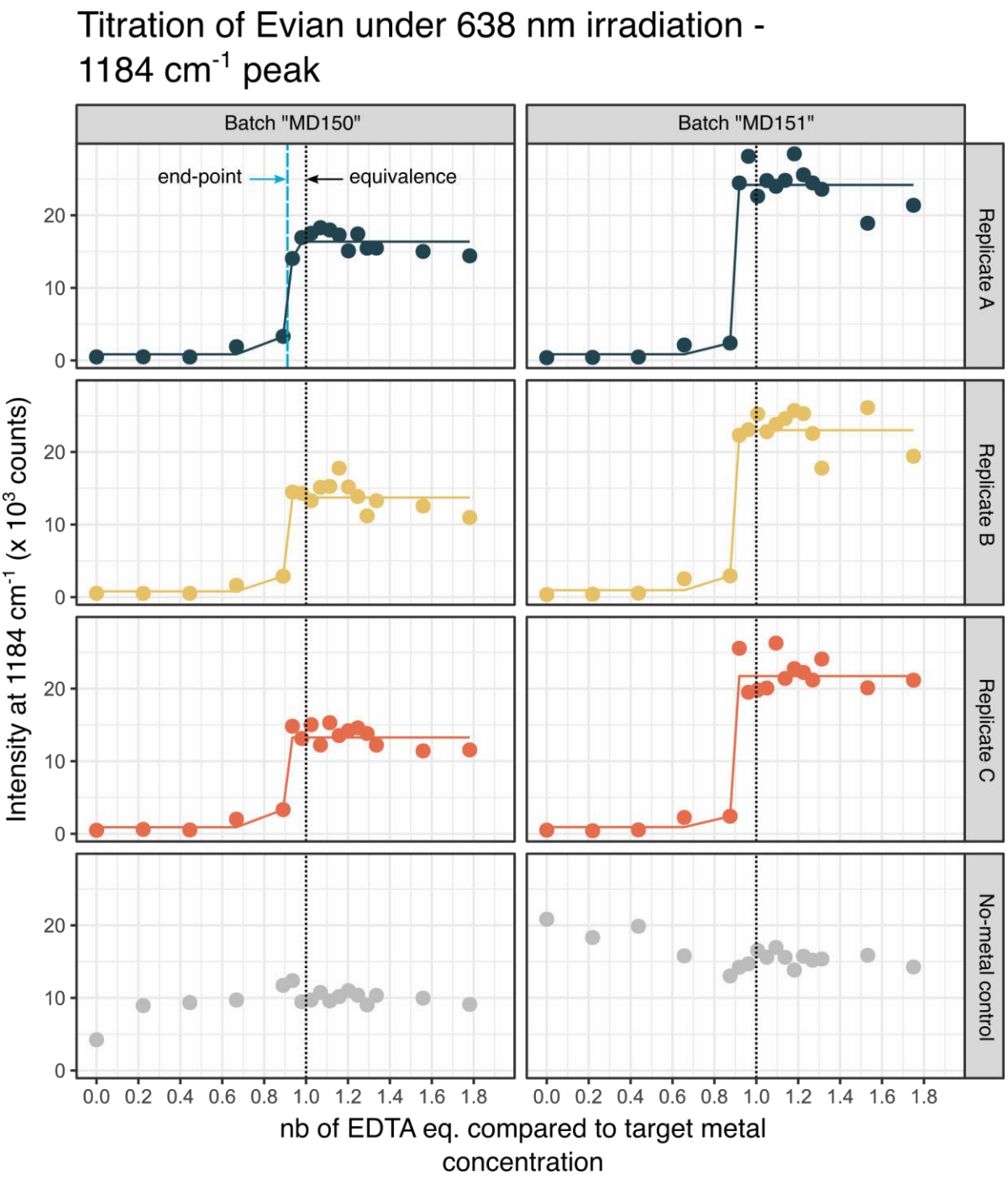


Figure S11: Evian hardness titration curves obtained from the tracking of the 1184 cm^{-1} peak intensity under 638 nm excitation. A blank titration was performed as a control by substituting ultrapure water for Evian.

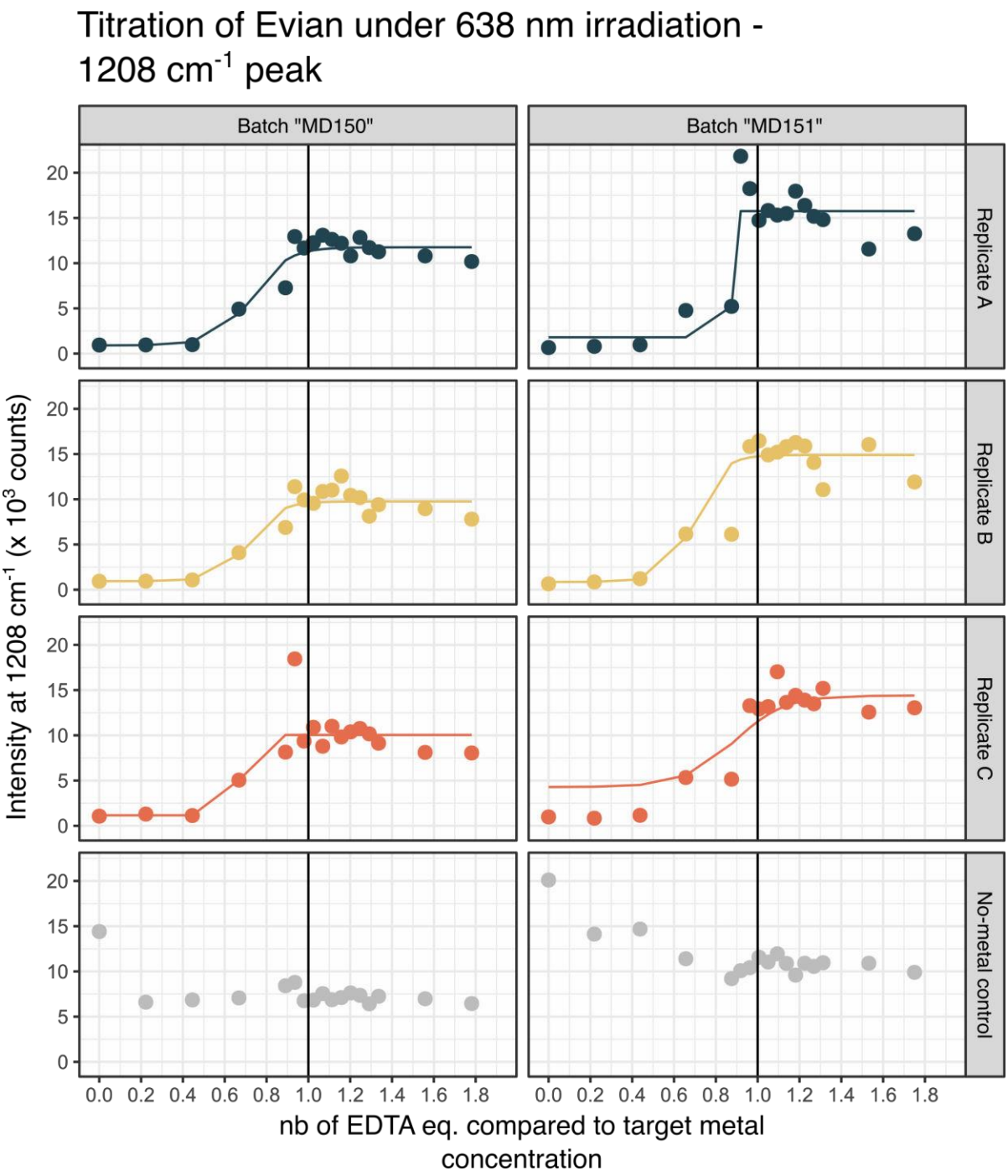


Figure S12: Evian hardness titration curves obtained from the tracking of the 1208 cm^{-1} peak intensity under 638 nm excitation.

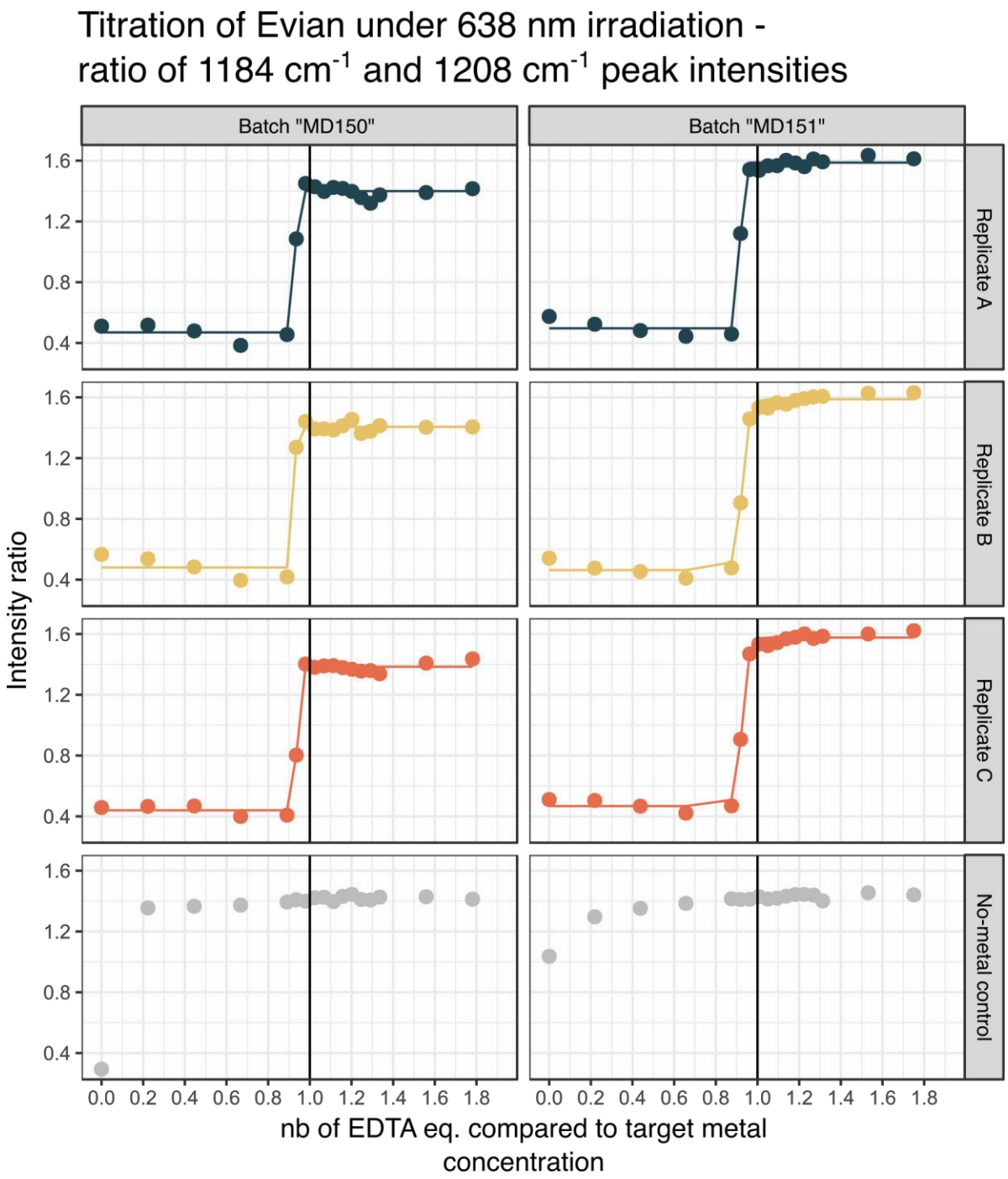


Figure S13: Evian hardness titration curves obtained from the ratio of the intensities of the 1184 and 1208 cm-1 peaks, under 638 nm excitation.

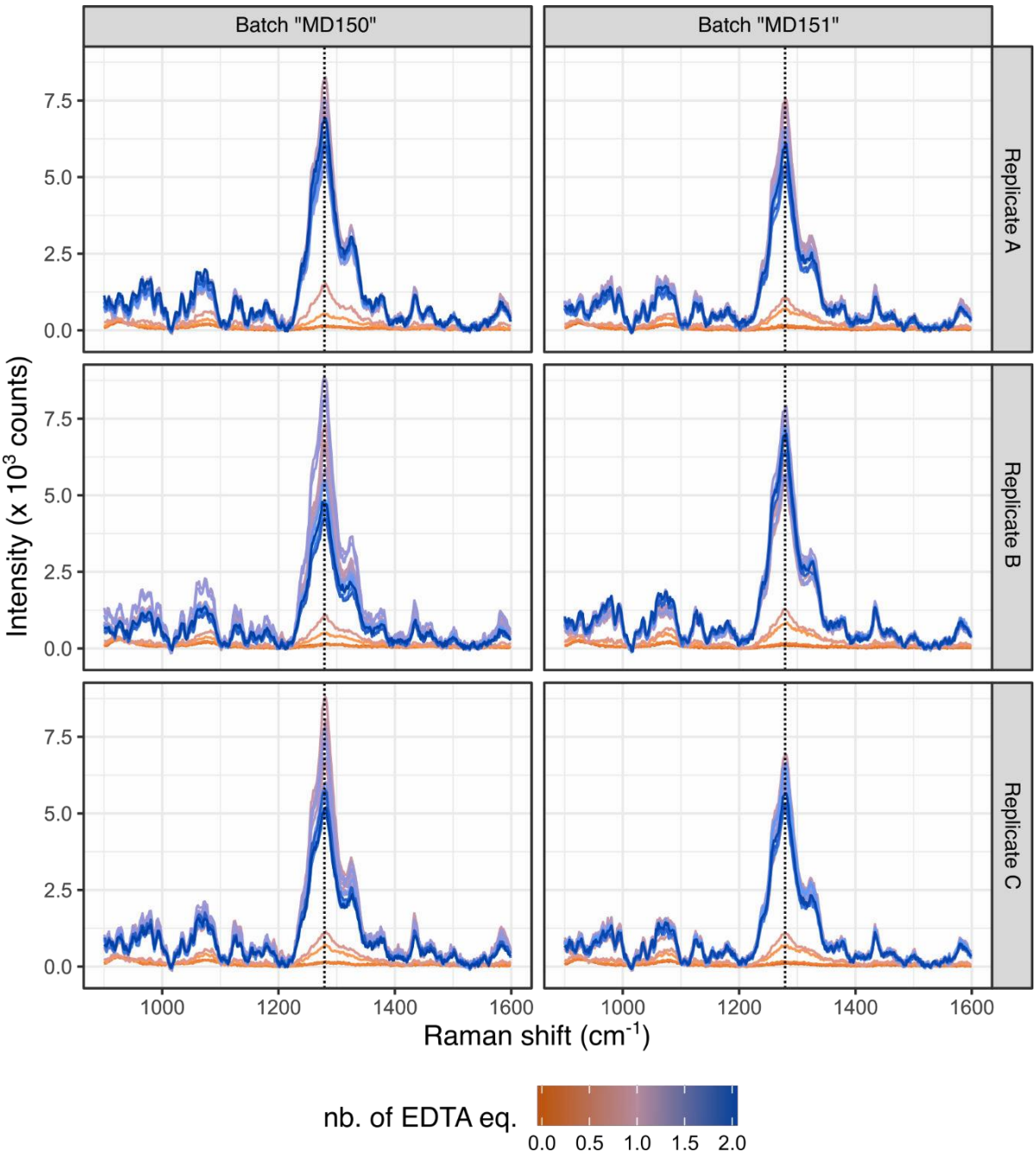


Figure S14: Replicates of Evian hardness titration monitored in SERS under 785 nm excitation as described in Figure S5. Three titration series (A, B, C) of 18 EDTA concentrations were performed using two distinct NP batches.

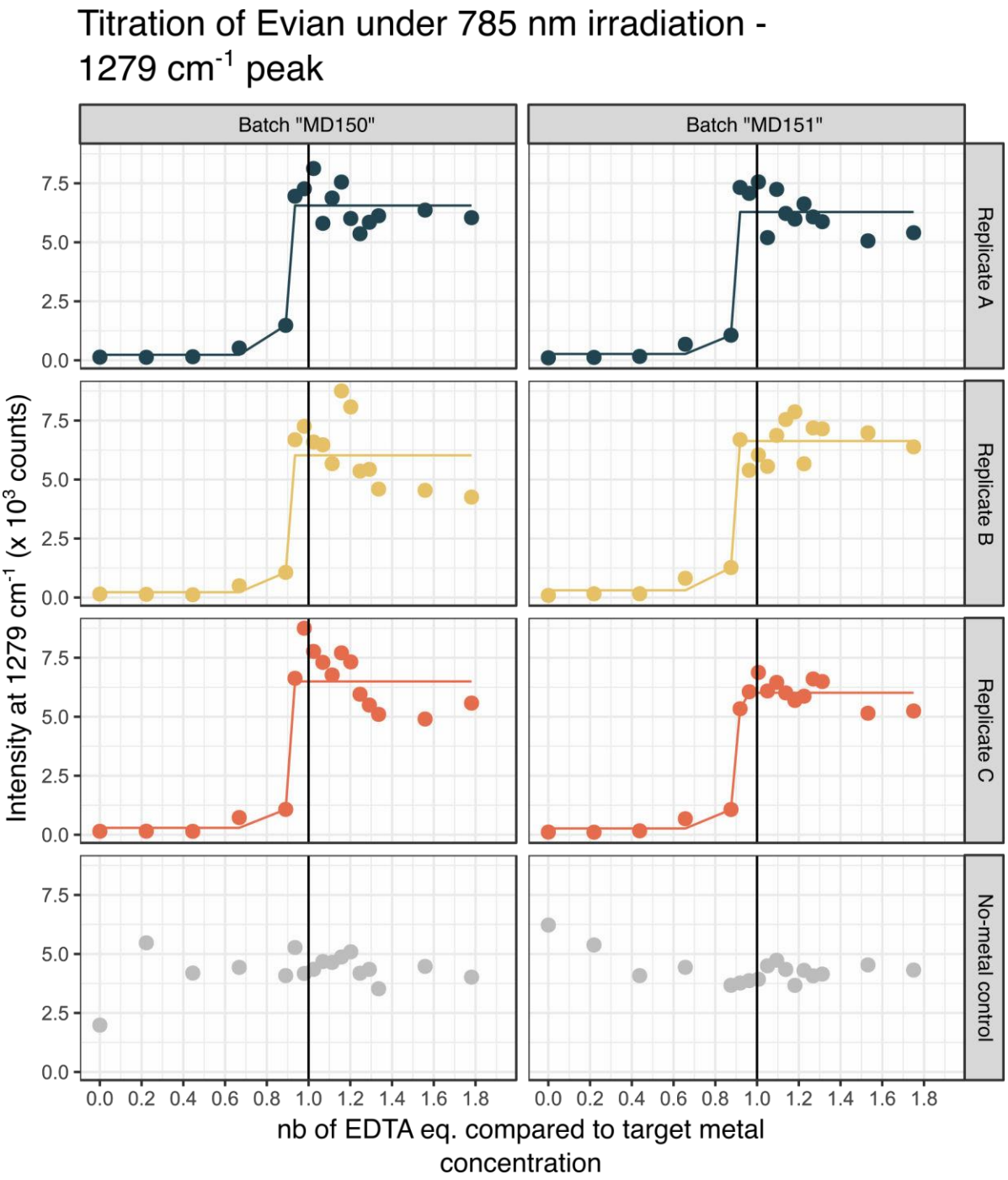


Figure S15: Evian hardness titration curves obtained from the tracking of the 1279 cm^{-1} peak intensity under 785 nm excitation.

INFLUENCE OF THE RATIO OF INDICATOR TO ANALYTE

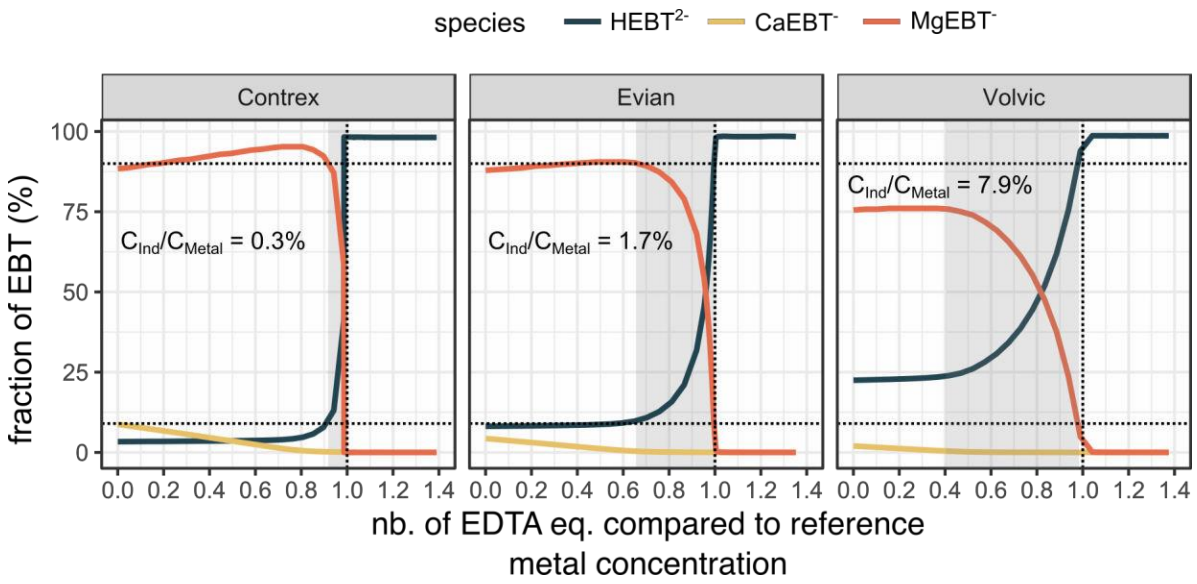


Figure S16: Speciation analysis of EBT over the course of the titrations of Contrex, Evian and Volvic. C_{Ind}/C_{Metal} refers to the ratio of indicator to metal concentrations within the cuvettes.

COMPARISON OF SERS-BASED AND SPECTROPHOMETRIC WATER HARDNESS TITRATIONS

In the conditions in which the Evian SERS titrations were run, the EBT concentration was 3.8 µM or 1.7% of the reference metal concentration in the cuvette mix. Under those conditions, not only would the colorimetric transition range have extended over ~0.35 eq. of EDTA *vs.* metal, making for an unprecise equivalence determination, EBT would have contributed only ~0.05 to the absorption of the cuvette, altogether precluding naked eye inspection of the colour switch. Spectrophotometric monitoring in these conditions would have been needed.

On the basis of the molar extinction coefficients determined in **Fig. S7**, we simulated the titration curve that would be obtained when tracking the absorption bands having maxima at 550 and 600 nm respectively or their ratio (**Fig. S17**). They display slopes and inflexion points at values comparable to those observed in the 1184 cm^{-1} peak or 1184/1208 ratio-based SERS titration curves (97% trueness, 90 % switch achieved over 0.23 eq. of titrant in the case of absorption *vs.* 94 % trueness and 90% switch achieved over just under 0.1 eq. of titrant in the case of the ratiometric SERS monitoring). This evidences that EBT is about as good an indicator in SERS as it is in absorption spectrometry and proves the feasibility of using colorimetric indicators to implement SERS-monitored complexometric titrations.

Moreover, the projected absorbance range spanned over the titration deserves a comment. The simulated titration curves (**Fig. S17**) imply that absorbances on the order of 0.04 and varying by just 0.007 absorbance units can be instrumentally resolved. At those absorbance levels, a signal-to-noise ratio of 100, which is commonly provided by entry-level absorption spectrometers, is enough to distinguish between the bound and free states of the indicator. However, disposable cuvettes, which were used in our protocol and are an absolute necessity of field measurements, have intrinsic absorbances that typically vary by 0.005 absorbance units. Given this cuvette-to-cuvette variability, a

sharp titration curve could not have been obtained from absorption readings with our sample preparation protocol. Yet it was when monitoring the titration in SERS.

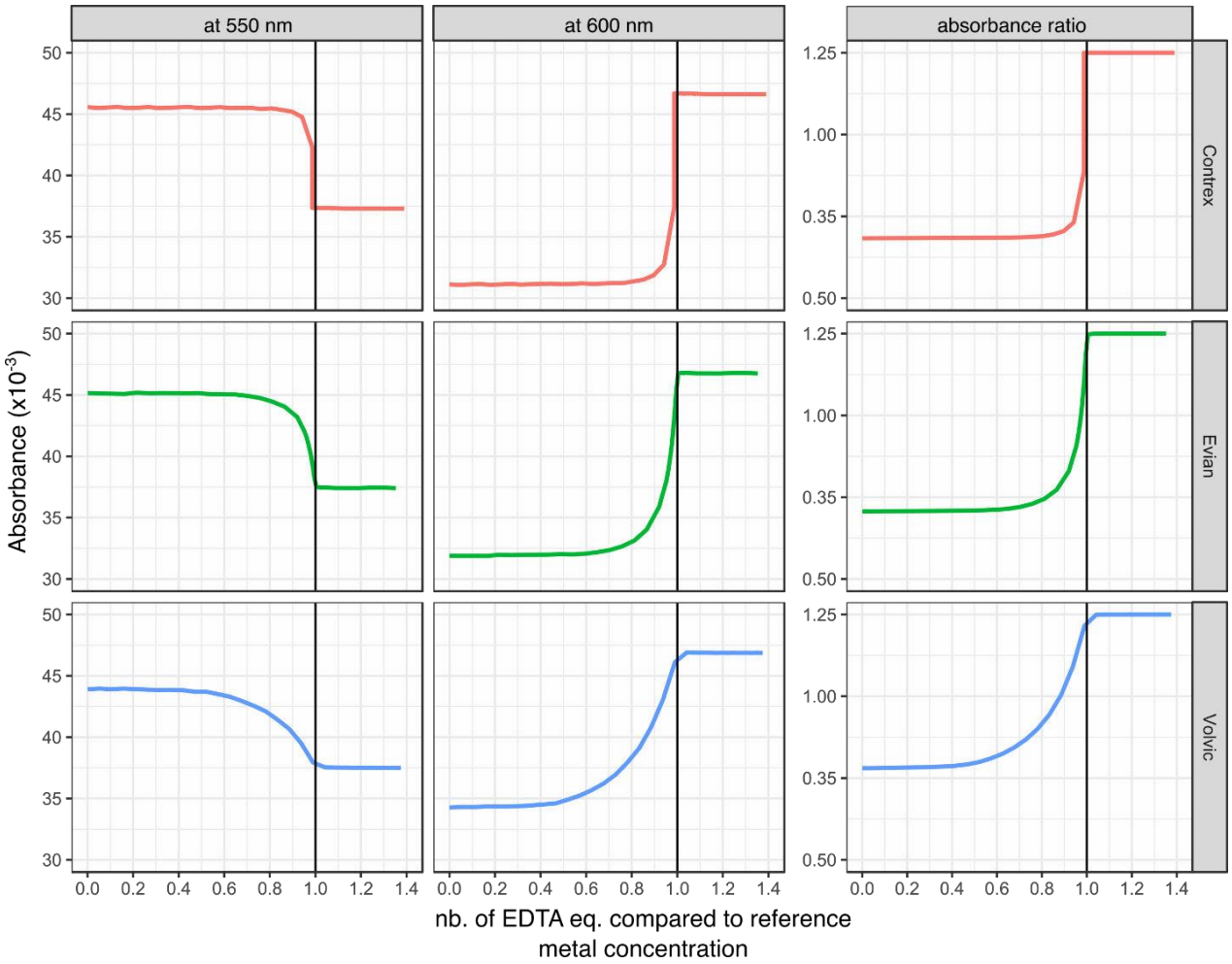


Figure S17: Simulation of absorption-based titration curves of Evian, Volvic and Contrex water in the concentration conditions used for the SERS titrations (*i.e.* 3.7 µM EBT). The simulations are based on the molar extinction coefficients determined in Fig. S6.

WATER HARDNESS TITRATION OF VOLVIC

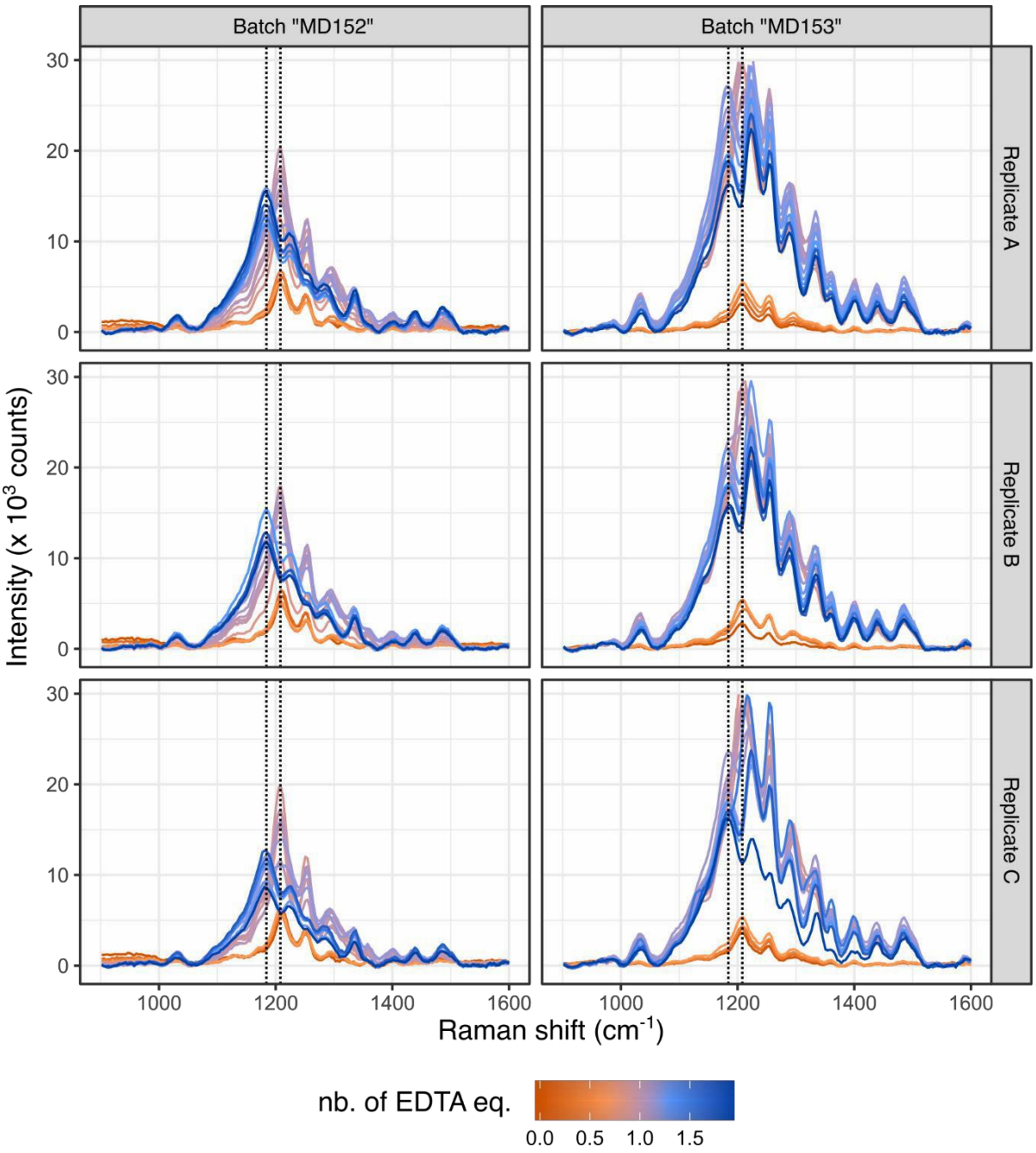


Figure S18: Replicates of Volvic hardness titration monitored in SERS under 638 nm excitation as described in Figure S5. Three titration series (A, B, C) of 18 EDTA concentrations were performed using two distinct NP batches.

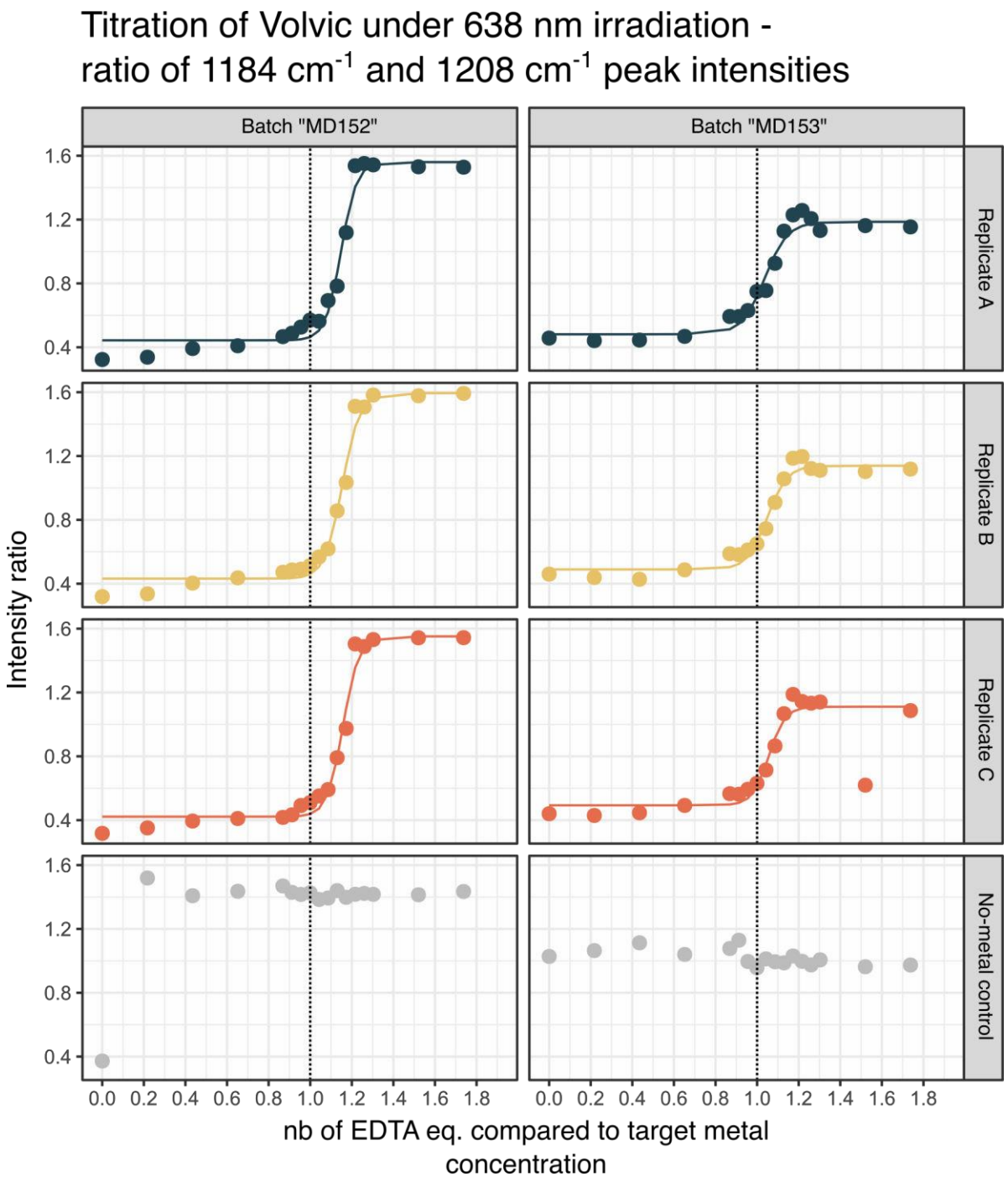


Figure S19: Volvic hardness titration curves obtained from the ratio of the intensities of the 1184 and 1208 cm-1 peaks, under 638 nm excitation.

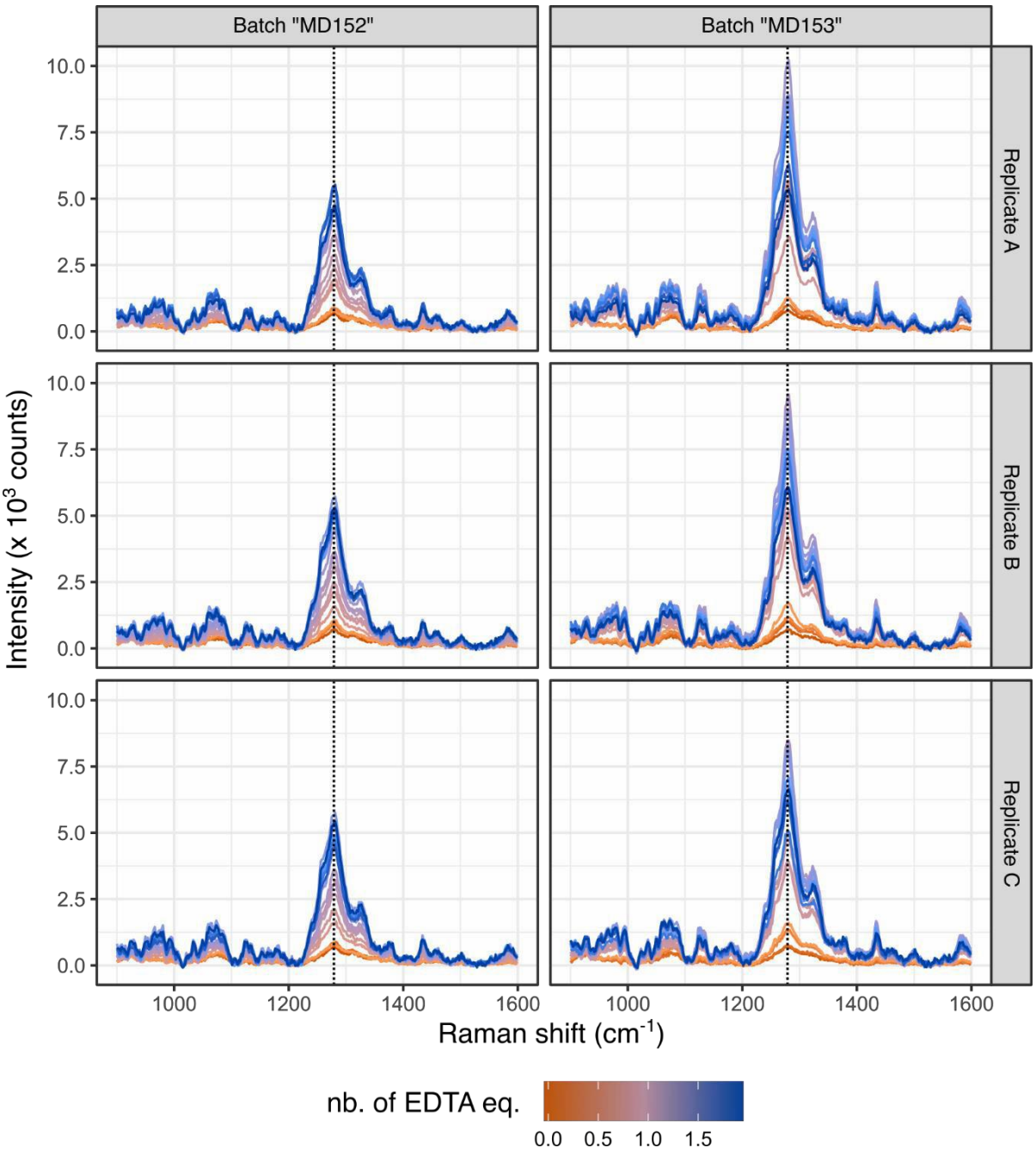


Figure S20: Replicates of Volvic hardness titration monitored in SERS under 785 nm excitation as described in Figure S5. Three titration series (A, B, C) of 18 EDTA concentrations were performed using two distinct NP batches.

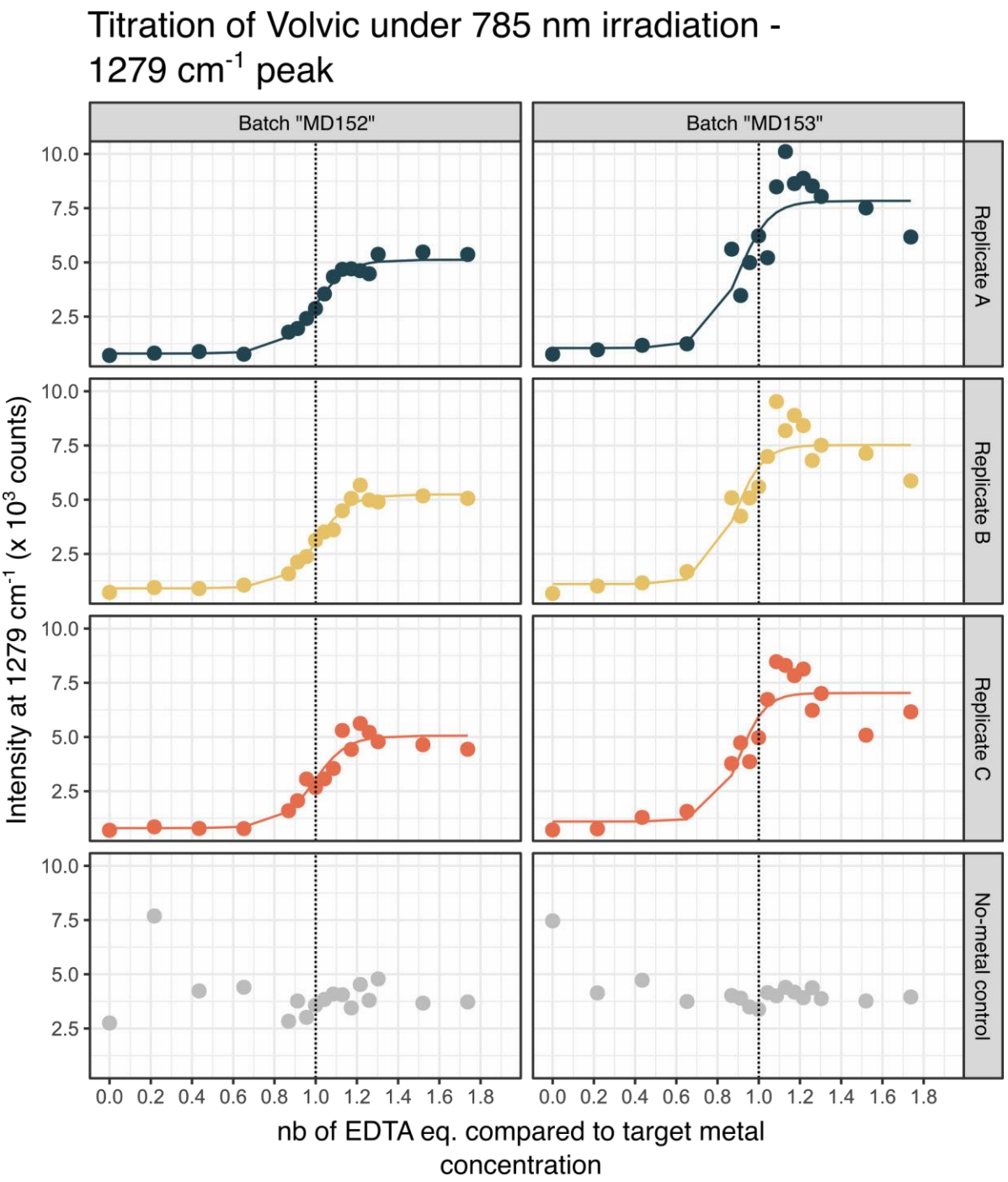


Figure S21: Volvic hardness titration curves obtained from the tracking of the 1279 cm^{-1} peak intensity under 785 nm excitation.

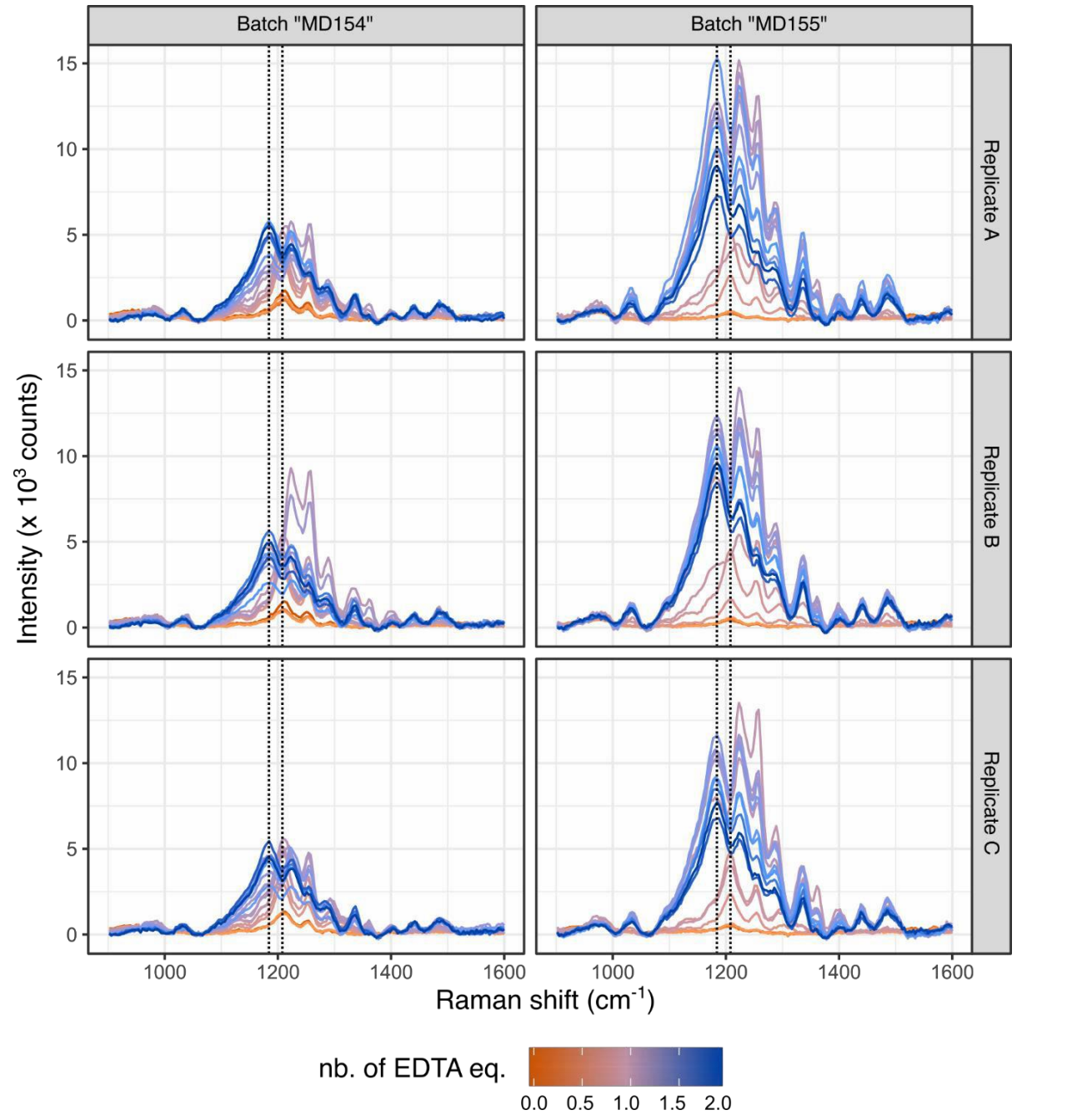


Figure S22: Replicates of Contrex hardness titration monitored in SERS under 638 nm excitation as described in Figure S5. Three titration series (A, B, C) of 18 EDTA concentrations were performed using two distinct NP batches.

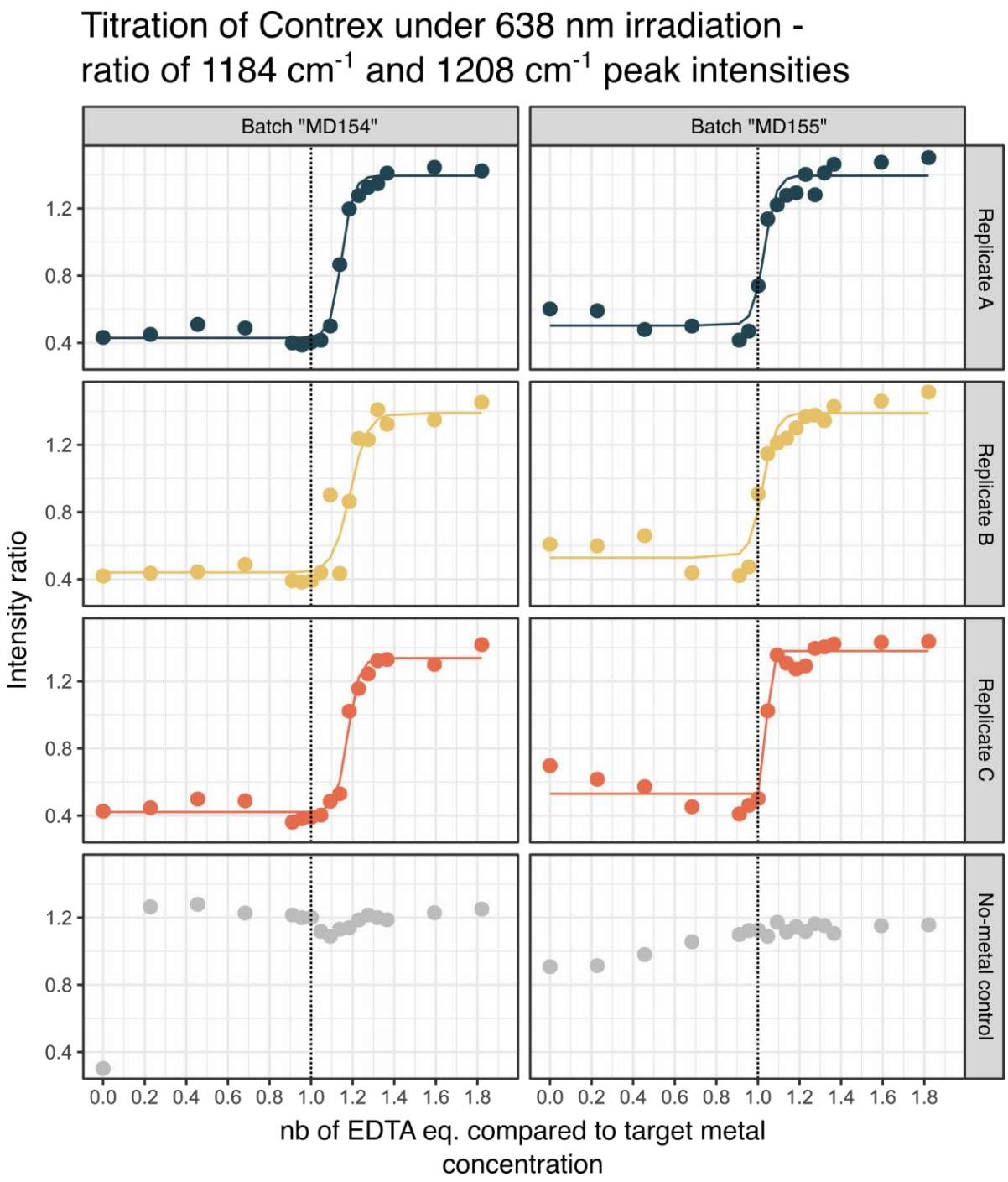


Figure S23: Contrex hardness titration curves obtained from the ratio of the intensities of the 1184 and 1208 cm-1 peaks, under 638 nm excitation.

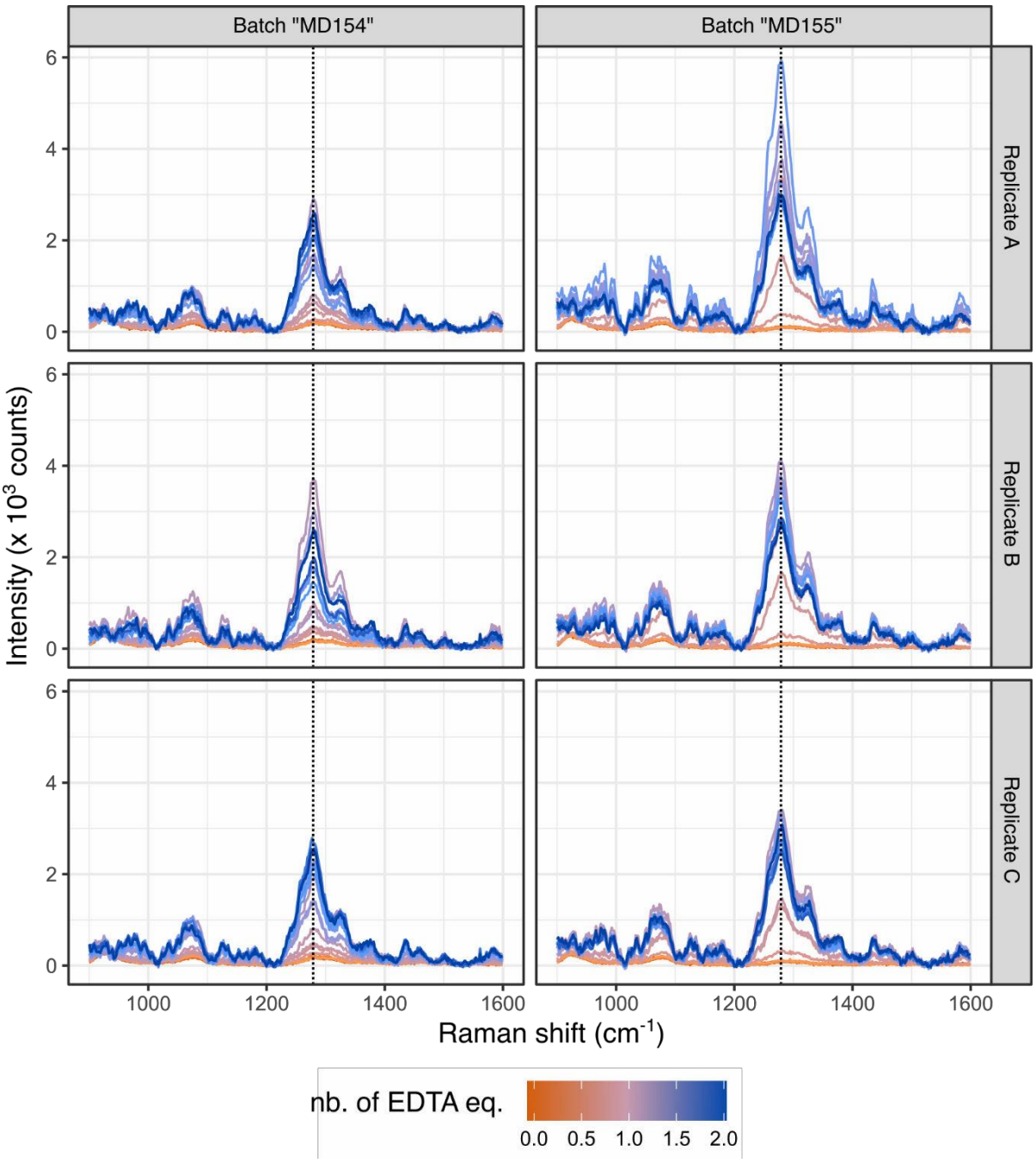


Figure S24: Replicates of Contrex hardness titration monitored in SERS under 785 nm excitation as described in Figure S5. Three titration series (A, B, C) of 18 EDTA concentrations were performed using two distinct NP batches.

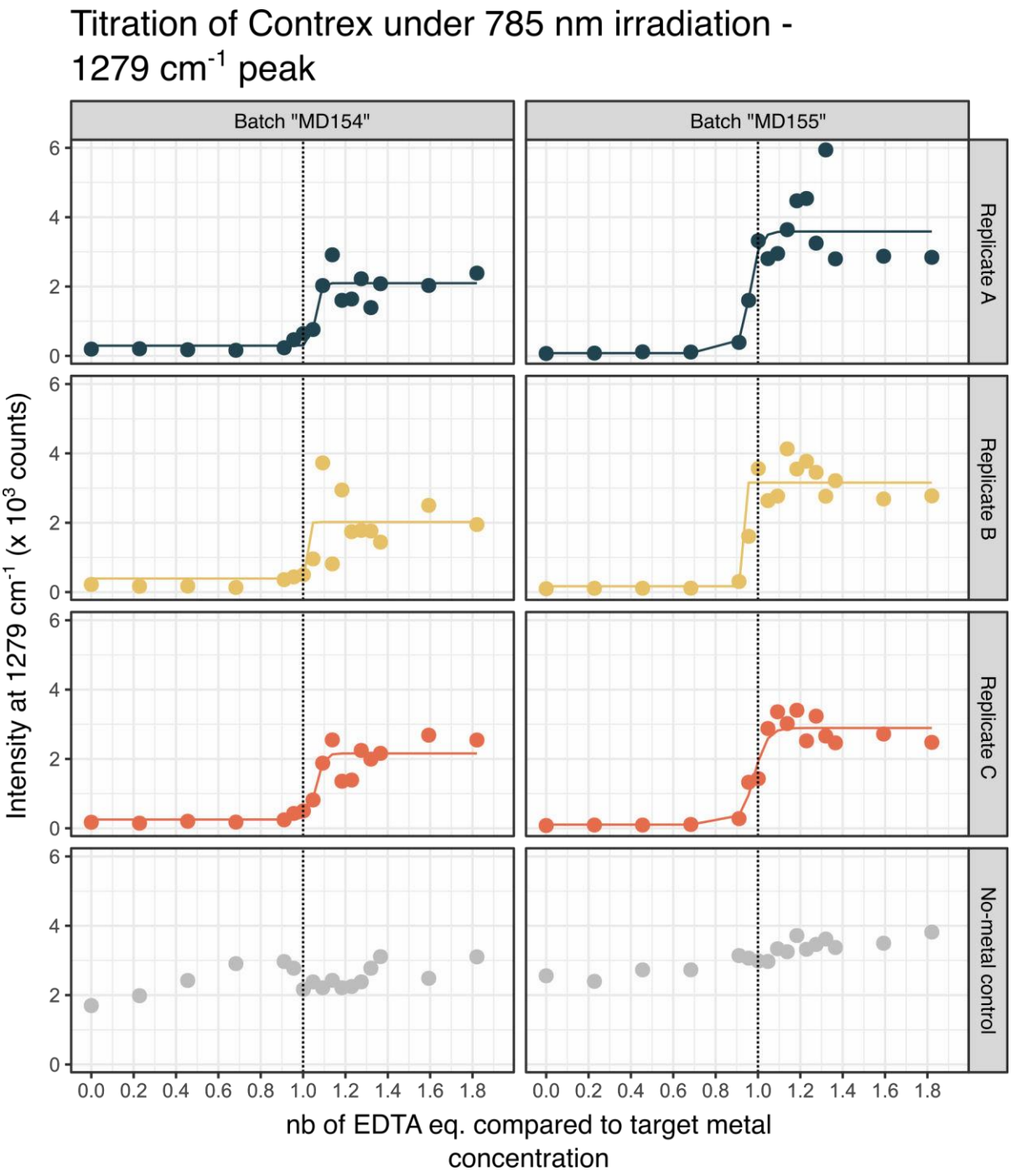


Figure S25: Contrex hardness titration curves obtained from the tracking of the 1279 cm^{-1} peak intensity under 785 nm excitation.

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