Supplementary information
Protein Redox by Piezoelectric Acousto-Nanodevice

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Preparation of reduced cyt c

Cytochrome C (Sigma Aldrich) was in an oxidized state when purchased and was chemically reduced using sodium dithionite (Sigma Aldrich).[1,2] Reduced cyt c was prepared by adding cyt c to sodium dithionite in 0.1 M deoxygenated phosphate buffer solution (PBS; neutral pH) such that the final concentration of the components used throughout the experiment is 5 μM of cyt c and 5 mM of sodium dithionite. The chemical reduction of cyt c was confirmed by a UV-Vis spectrum. Oxidized cyt c solution was prepared by dissolving cyt c in 0.1 M PBS (neutral pH; deoxygenated); the final concentration used throughout the experiment is 5 μM of oxidized cyt c.

Acousto-nanodevice fabrication process

A facile three step process was adapted for preparing acousto-nanodevice[3–5] as shown in Fig. S1. Tetragonal BTO nanoparticles ($\leq$ 300 nm) were obtained from US Research Nanomaterials, Inc. A monolayer of BTO nanoparticles was deposited onto a silicon (Si) substrate by spin coating technique (step 1). Si substrate was cleaned thoroughly before spin coating. DMSO (dimethyl sulfoxide) solvent was used to disperse BTO nanoparticles uniformly and spin-coated at 3000 rpm for 60 s. Immediately after spin coating, it was baked at 180 ^{o}C for 2 min. The second step

is to deposit Au onto BTO nanoparticles (half coating) using the sputter coating method. A 10 nm thick chromium (Cr) adhesion layer was first sputtered followed by 30 nm thickness Au layer. The final step is to lift off the half-coated BTO nanoparticles (BTO-Au) from the Si substrate by sonicating the substrate in 0.1 M PBS. Scanning electron microscopy and energy-dispersive X-ray spectroscopy confirmed the formation of BTO-Au half-coated nanoparticles as shown in Fig. S1 (d, e). The liberated half-coated BTO nanoparticles were collected and further used in our experiments. We note that the yield of the fabrication was not uniform as we have a relatively large variation of the particle concentrations.

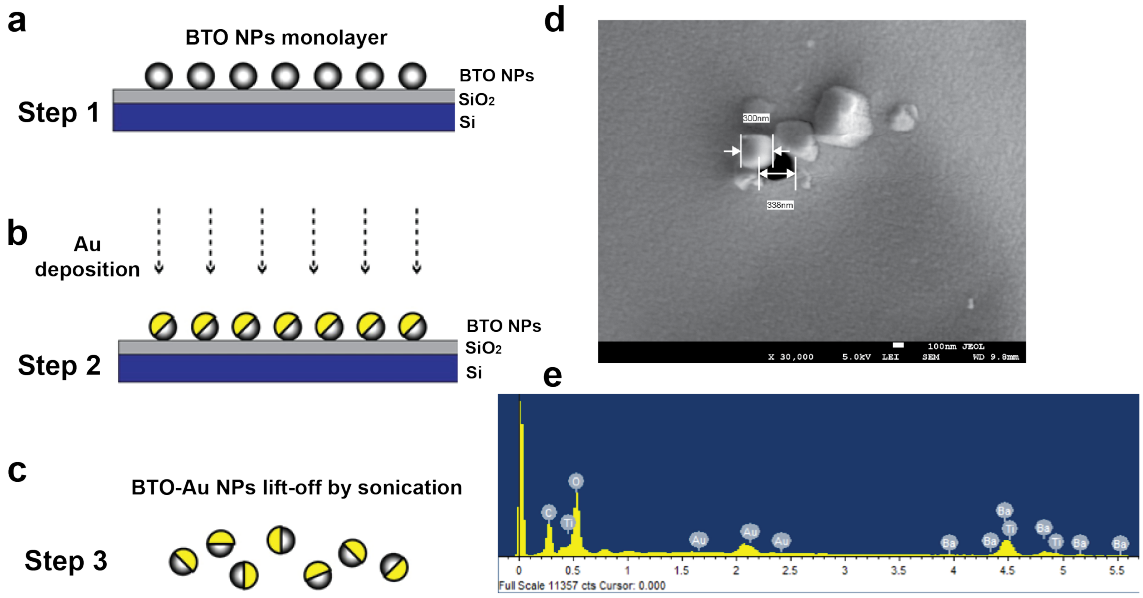

Figure S1: Acousto-nanodevice fabrication process and characterization (a) Monolayer deposition of BTO nanoparticles onto Si substrate by spin coating technique (b) metal deposition (Au) onto BTO nanoparticles monolayer by sputtering technique (c) lift off of BTO-Au Janus nanoparticles by sonication process (d) Scanning Electron Micrograph of half coated BTO nanoparticles (BTO-Au) peeling off the Au layer due to sonication process (e) EDS (Energy-dispersive X-ray spectroscopy) spectrum of BTO-Au nanoparticles confirming the Au coating on BTO nanoparticles.

Sample preparation for UV-Vis spectroscopy

UV-vis spectroscopy samples were prepared by mixing acousto-nanodevice or the control devices (BTO nanoparticles or BTO nanoparticles with Cr coating) and cyt c (oxidized or reduced) in

0.1 M PBS (neutral pH; deoxygenated). BTO nanoparticles concentration of 0.1 μM and cyt c concentration (both oxidized cyt c and reduced cyt c) of 5 μM was maintained in all the conjugates, which were freshly prepared just before recording the UV-Vis spectrum.

UV-Vis absorption spectroscopy

UV-Vis absorption spectrometer (Jasco V-670) was used to confirm the redox state of the cyt c. The parameters used for recording the absorption spectrum are as follows:
wavelength range - 300 nm to 800 nm, data interval - 0.2 nm, scan speed - 200 nm/min, response - medium, UV/Vis bandwidth - 2 nm, NIR bandwidth - 8 nm. Baseline correction was obtained before measurements using 0.1M PBS solution.

Scanning electron microscopy

The surface morphology of acousto-nanodevice were characterized using Field Emission Scanning Electron Microscope (JEOL, JSM-7600F). Elemental analysis of acousto-nanodevice were performed using Energy-dispersive X-ray spectroscopy (JEOL, JSM-7600F).

Ultrasonic irradiation and intensity measurement

The acoustic intensity of the applied ultrasound was measured using a fiber-optics hydrophone (Precision Acoustics, UK). The system output was monitored using an oscilloscope (Tektronix MDO3054). The measured acoustic intensity was calculated based on the measured peak pressure using the following formula. (I: acoustic intensity, p: peak pressure [Pa], ρ: density of water [kg/m^3], c: speed of sound in water [m/s])

$$I = \frac{p^2}{2\rho \cdot c} \; [W/m^2]$$

Data fitting

An in house-written python code was used for peak-fitting; the fitting was carried by finding the least-squared error. We found that Lorenztian peak fitting to be more suitable than the Gaussian fitting. To estimate the degree of oxidation, we fixed the peak positions and the relative ratios of the different peaks for oxidized and reduced cyt c. The peak positions and the relative ratios were determined by fitting reference oxidized cyt c (as purchased) and chemically reduced cyt c, which showed great agreements with the published literature. The absorption peaks (soret region) assignable to oxidized cyt c and reduced cyt c were fixed at 409 and 415 nm respectively.[6] The ratio of individual absorption peaks (near the Q band) of oxidized cyt c and reduced cyt c were fixed with respect to their corresponding soret peak.[2,6]

Peak decomposition of Reduced cyt c

The reduced cyt c (reference spectrum) was fitted with Lorenztian function as given in Fig. S2. The peak decomposition shows that the spectra is mostly made out of the reduced cyt c-related peaks shown as green shade area with the slight contribution of oxidation shown as blue shaded area.

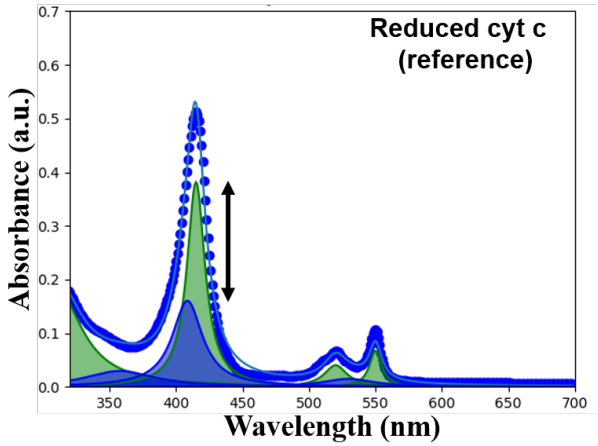

Figure S2: Lorentzian fit for UV-Vis spectrum of reduced cyt c (reference)

Acoustically triggered protein oxidation

To confirm the role of ultrasound and surface metal coating, 3 controls groups namely BTO-reduced cyt c (no sonication), BTO-reduced cyt c (sonication), BTO-Au-reduced cyt c (no sonication) and the test group BTO-Au-reduced cyt c (sonication) were used for the study. It is clear from the graph (Fig. S3) that only the combination of acousto-nanodevice and ultrasonic irradiation [BTO-Au-reduced cyt c (sonication)] induces measurable change in the spectra. The role of ultrasound, and hence the alternating polarization of the acousto-nanodevice, is verified to be significant in cyt c oxidation; without ultrasound irradiation, there is no change in the absorption spectra [BTO-Au-reduced cyt c (no sonication). From [BTO-reduced cyt c (sonication)] spectrum, the effect of gold half-coating can be seen; no oxidation effect was observed under 30 minutes of ultrasonic irradiation in the absence of the surface metal coating, indicating that the nanoparticles themselves are ineffective in controlling the redox state of cyt c. Only when both the acousto-nanodevice and ultrasound is combined, there was measureable oxidation effect on reduced cyt c; blue shift of $\sim$ 5 nm around the soret region (415 nm to 410 nm) as given in Fig S3 (inset).

Stability of reduced cyt c

The stability of chemically reduced cyt c over a time period of 120 min (without sonication) and 30 min (under sonication) were examined. Reduced cyt c remained stable without reverting back to oxidized state in both the absence as well as the presence of ultrasound as shown in Fig. S4. This again confirms the role of BTO-Au and ultrasound in the oxidation of reduced cyt c; the proposed mechanism.

Interaction of reduced cyt c with BTO-Cr Acousto-nanodevice

BTO-Cr acousto-nanodevice fabrication process is similar to that of BTO-Au fabrication process. Instead of Au, a 30 nm thick Cr metal layer was deposited on to BTO nanoparticles.

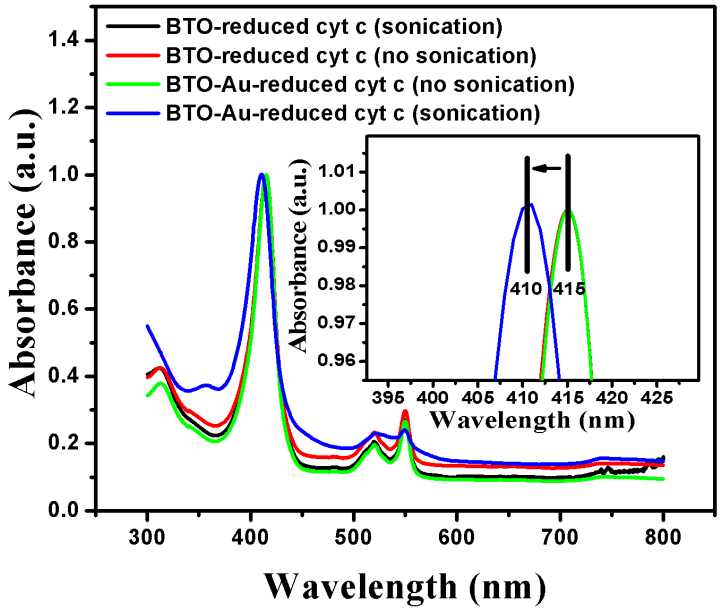

Figure S3: The UV-Vis spectrum of BTO-reduced cyt c (no sonication), BTO-reduced cyt c (sonication), BTO-Au-reduced cyt c (no sonication) and BTO-Au-reduced cyt c (sonication). Except for BTO-Au-reduced cyt c (sonication), there is no shift in wavelength (blue shift) for other conjugates.

BTO-Cr did not cause oxidation of reduced cyt c even at different time intervals (18, 30 min) both in the absence and presence of sonication. No blue shift was observed as depicted in the UV-Vis spectrum of BTO-Cr-reduced cyt c (Fig.S5 a-b). Thus,the surface plasmonic effect at the metallic surface as a dominant factor for oxidation effect is ruled out. Moreover, the fact that there is no modulation of the oxidation state of proteins under BTO-Cr is indicative of importance of the metal work function in determining the effect of the acousto-nanodevice; whether it will induce oxidation or reduction.

Statistical analysis

Statistical analysis of all the independent experimental data was performed using the Student's t-test with JMP® Pro 14.0. All the experiments were repeated at least quadruple. Data were

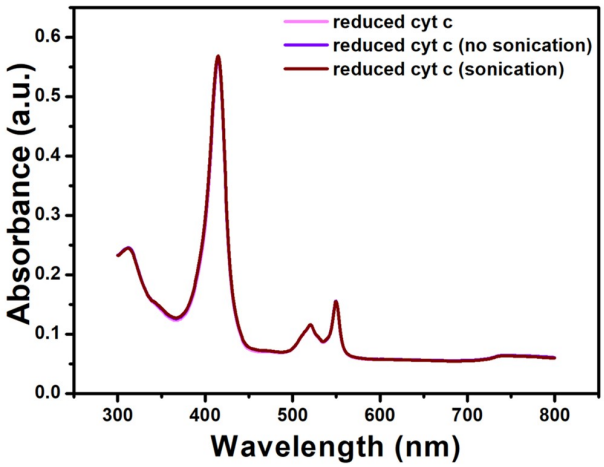

Figure S4: Stability of reduced cyt c both in the presence of sonication (30 min) and absence of sonication (120 min).

considered statistically significant when p-value is less than 0.05.

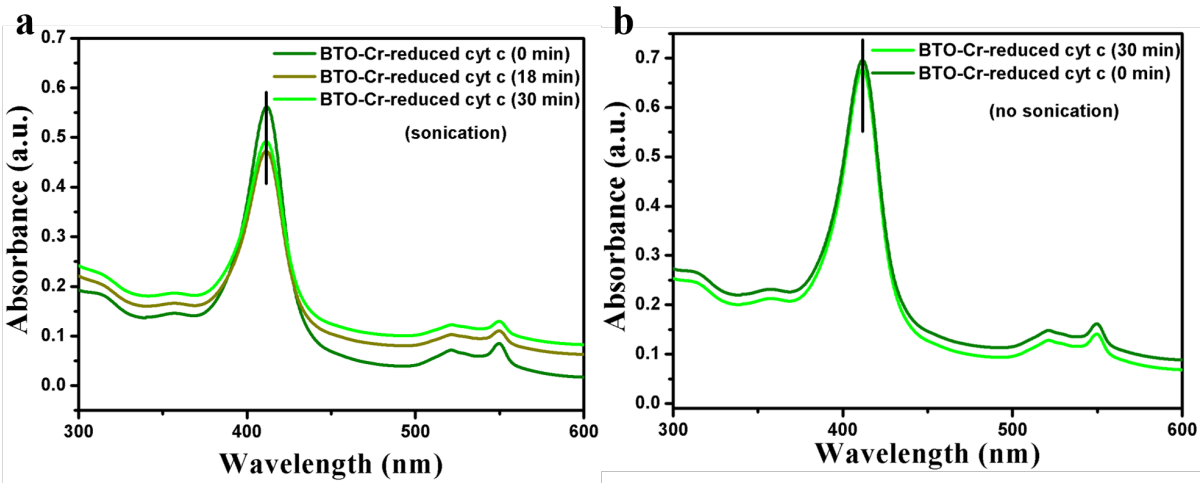

Figure S5: Interaction of reduced cyt c with BTO-Cr nanoparticles. UV-Vis spectrum of BTO-Cr-reduced cyt c at 0, 18 and 30 min time intervals showing no oxidation effect (a) in the presence of ultrasound as well as (b) in the absence of ultrasound.

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