A surface functionalized nanoporous titania integrated microfluidic biochip

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Supplementary Information. Details of Experimental Section

Reagents. All chemicals including titanium (IV) butoxide, chitosan, cholesterol, cholesterol oxidase and cholesterol esterase are of analytical grade and have been purchased from Sigma Aldrich. Sylgard 184 is from Dow Corning (Midland, MI, USA). SU-8-100 negative photoresist, positive photoresist (Shipley-1818) and SU-8 developer from Microchem (Newton, MA, USA). ITO coated glass slides (film thickness ~150-300 Å) with a resistance of 70-100 Ω/square were obtained from Vin Karola Instrument.

Solution Preparation. The stock solutions of ChEt (1mg dl^{-1}) and ChOx (1mg dl^{-1}) are freshly prepared in phosphate buffer (50mM) at pH 7.0. Cholesterol oleate solution (500mg/dl) is first dissolved in 1% polidocanol (Brij) as a surfactant by heating/stirring resulting in clear and colourless suspension and final volume is made by addition of 0.9% NaCl solution. Further, different concentrations of cholesterol oleate have been diluted to 2-500 mg/dl using 0.9% NaCl solution.

Instrumentation

The XPS measurements have been conducted in binding energy the range, 0-1100 eV to confirm ChEt-ChOx functionalization of nanoporous antTiO_2-CH matrix. The surface morphological studies have been investigated using scanning electron microscope (SEM, LEO-440). AFM image of antTiO_2 nanoparticles have been recorded using model Multimode-V, Vicco Instrument (tapping mode). Surface profiling and the thicknesses of the films structure are estimated using an optical profiling system (NanoMap-D, AepTechnlogy, U.S.A.). The cyclic voltammetry, electrochemical impedance spectroscopy (EIS) and chronoamperometric measurements have been made on an Autolab Potentiostat/Galvanostat (Eco Chemie, The Netherlands).The pore size distribution and total surface area of the TiO_2 and antTiO_2-CH are calculated by means of the Brunauer-Emmett-Teller (BET) and BJH method and Autosorb1 software (Quantachrome Instruments, USA). The TiO_2 and ant TiO_2-CH samples are degassed at 150^{o}C and 50^{o}C for 5 h respectively. The specific surface areas were estimated by the use of BET method, and the pore size distributions are concluded with the Barrett-Joyner-Halenda (BJH) method by using the nitrogen desorption branches of the isotherms. Raman spectral analysis was carried out using WiTec, Germany which has a laser light source of 532 nm wavelength. Electrochemical investigations for this nanopore microfluidic chip have been carried out using an Autolab Potentiostat/Galvanostat (FRA analyzer) in phosphate buffer saline [PBS, (50 mM, pH 7.0, 0.9% NaCl)] containing $[Fe(CN)_6]^{3-/4-}$ (5mM). The flow of buffer solution and analyte are optimized using electrochemical technique through this chip and 1μL/min is found to be optimum flow rate.

Figure S1: Ti2p XPS spectra of the antTiO$_2$-CH/ITO film (i) and ChEt-ChOx/antTiO$_2$-CH/ITO (ii) film after deconvolution.

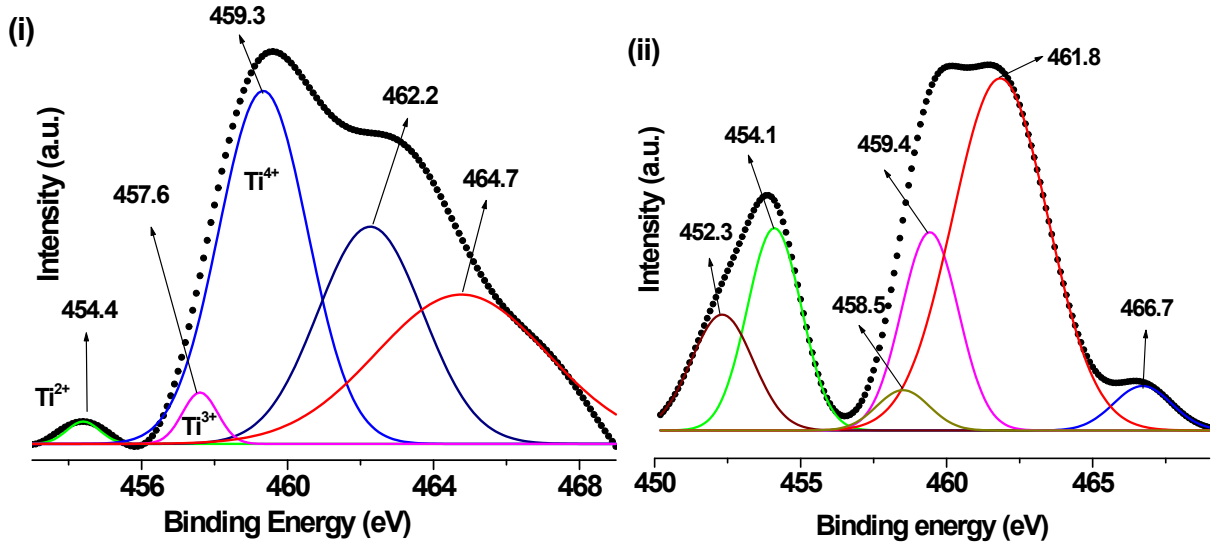


Figure S2:CV of ChEt-ChOx/antTiO$_2$-CH/ITO bioelectrode as a function of scan rate [20-160 (mV/s)] in ascending order, inset: anodic and cathodic peak current *vs* (scan rate mV/s)$^{-1/2}$.

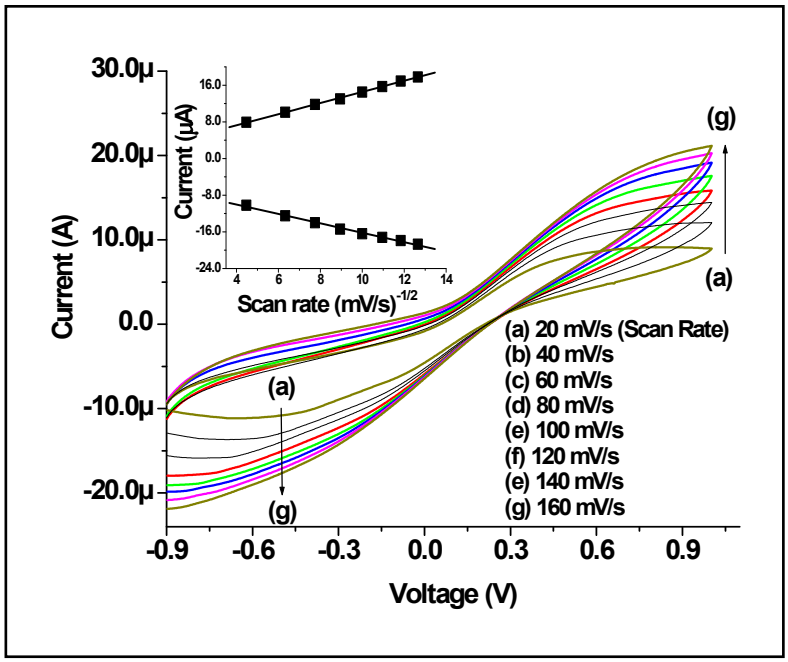


Figure S3 Chronoamperometric response curve of antTiO$_2$-CH/ITO electrode at different flow rates.

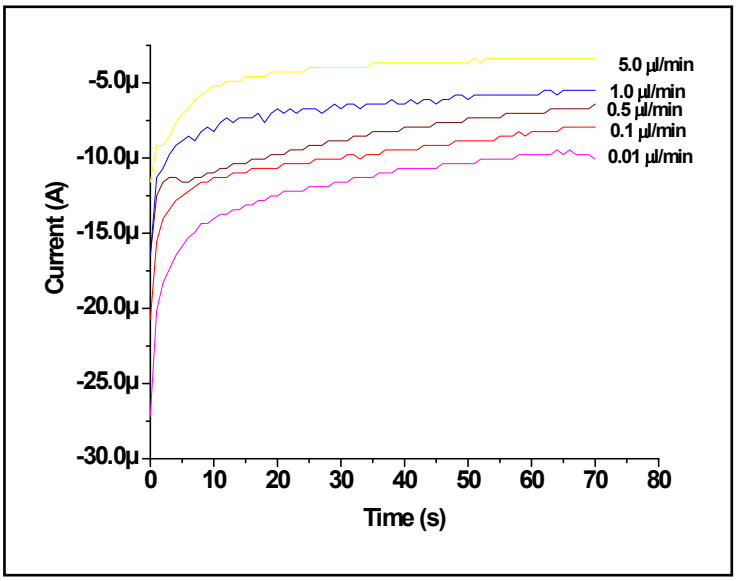


Figure S4 Chronoamperometric response curve of ChEt-ChOx/antTiO$_2$-CH/ITO bioelectrode at different flow rates.

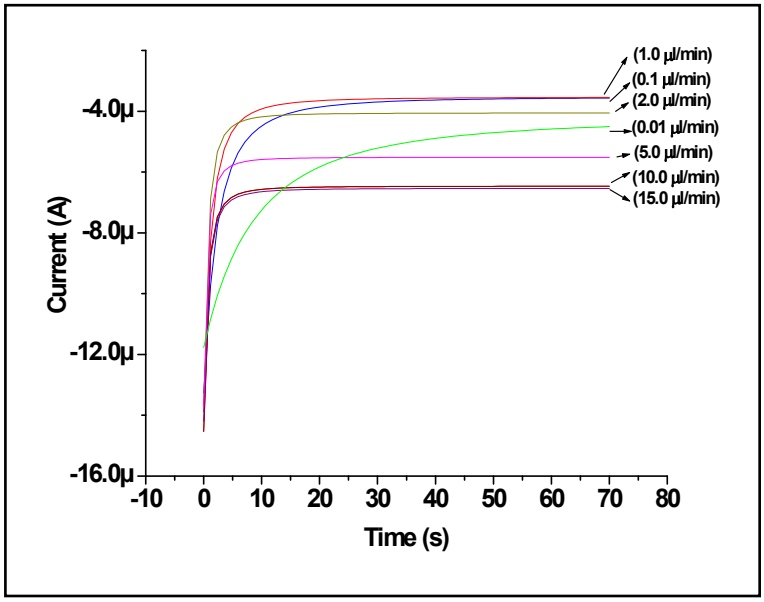


Figure S5 EIS spectra of ChEt-ChOx/antTiO$_2$-CH/ITO bioelectrode as a function of flow rate (0.01-5.0µL/min), inset: plot between charge transfer resistance (R_{ct2}) and flow rate (µL/min).

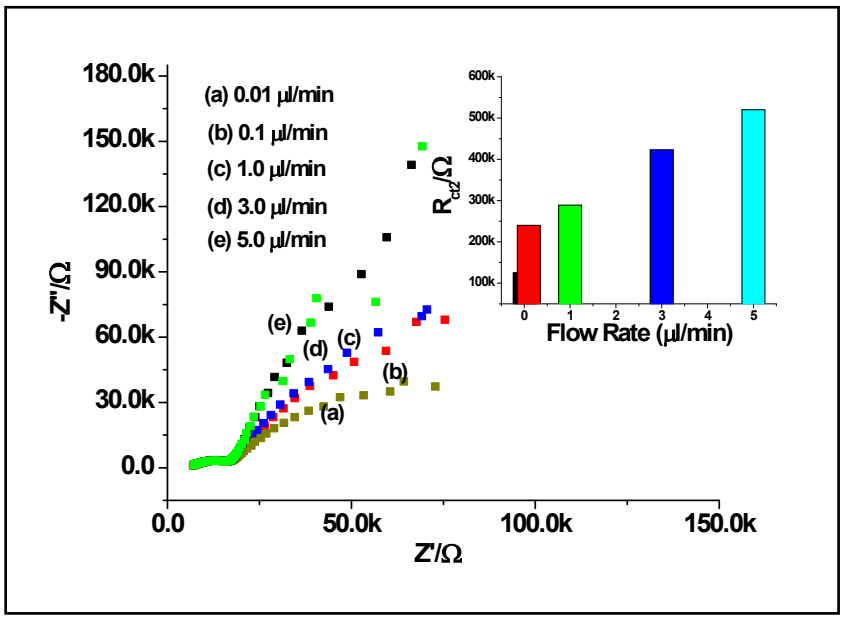


Figure S6 (i) Dispersion angles versus potentials for ChEt-ChOx/antTiO_2-CH/ITO bioelectrode and (ii)the variation of double layer capacitance as a function of potential.

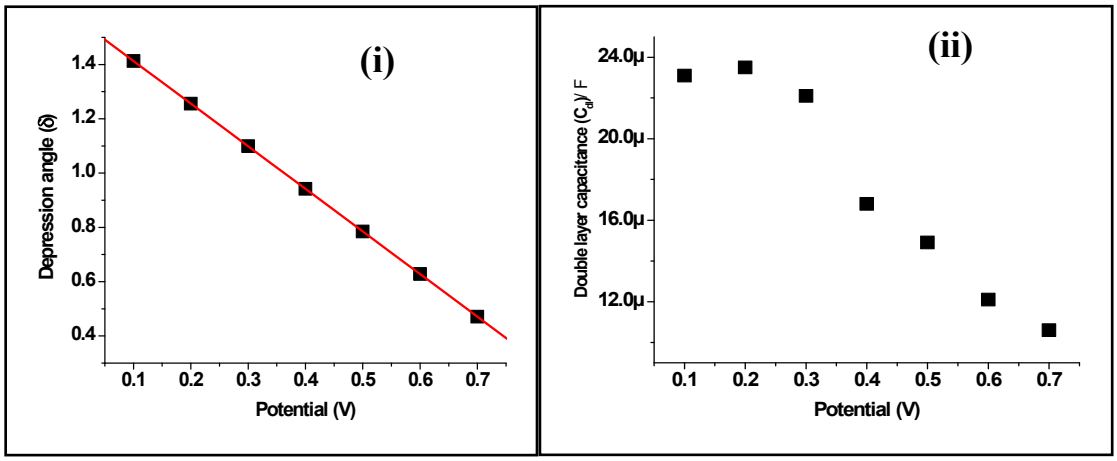


Figure S7 Plot between R_{ct2} and concentration of cholesterol oleate [2-500 mg/dl].

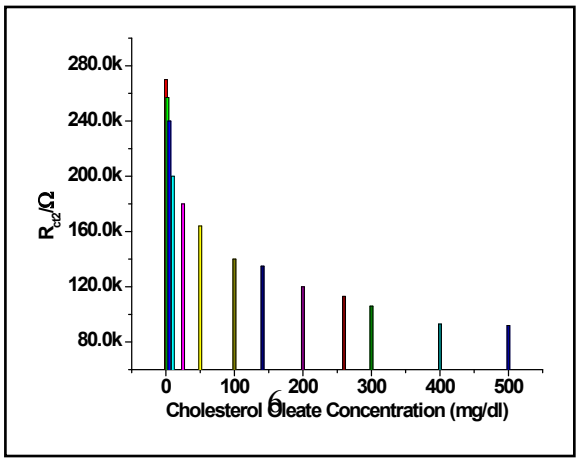
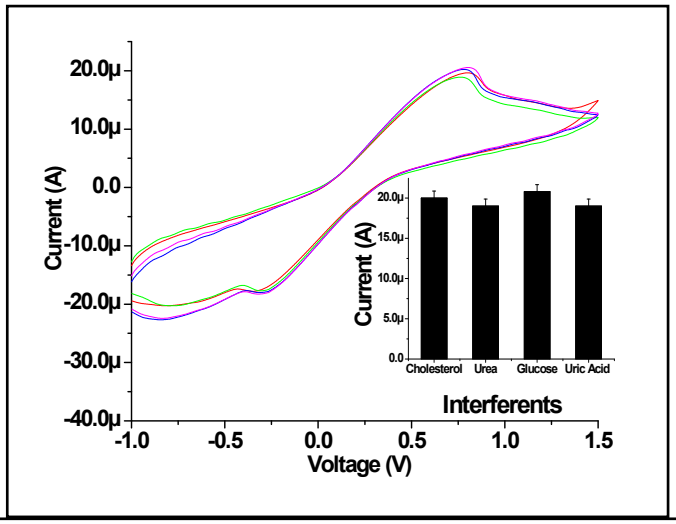


Figure S8 The selectivity studies in presence of various analytes



Elements/Atomic concentration (%)	**C (FWHM)**	**O(FWHM)**	**N(FWHM)**
antTiO$_2$–CH /ITO	43.3 (1.96)	34.8 (2.08)	1.23(3.6)
ChEt-ChOx/ antTiO$_2$–CH /ITO	61.3 (3.34)	25.7 (3.02)	2.48 (1.9)

Table S1: Atomic concentration (%) and full width half maxima (FWHM) of C, O and N elements present in antTiO_2–CH/ITO and ChEt-ChOx/antTiO_2–CH/ITO electrodes obtained from XPS analysis.