SUPPORTING INFORMATION

Understanding Cholesterol-Mycolic Acid-Phosphatidylcholine Interactions: Advancing Electrochemical Detection of Tuberculosis

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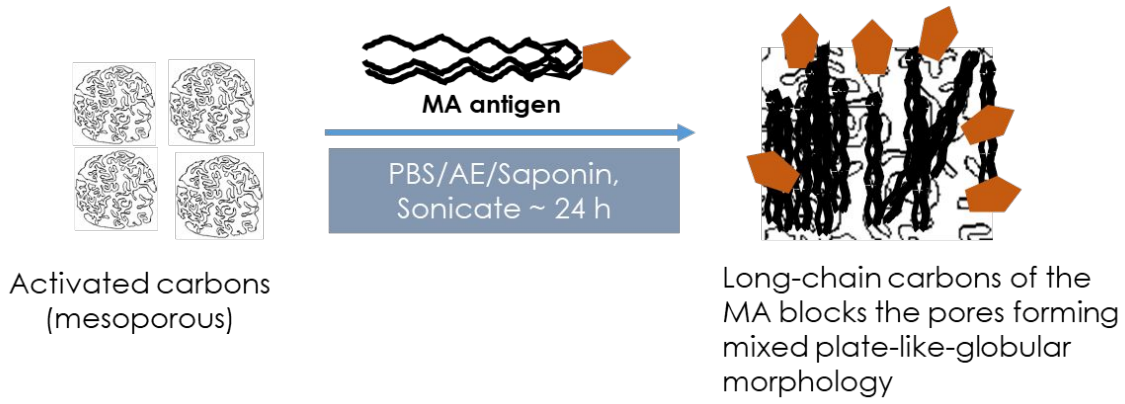

Figure S1: Cartoon representation of the synthesis of activated carbon-encapsulated mycolic acid antigens.

Physico-Chemical Characterization

The morphology of activated carbon and activated carbon-encapsulated mycolic acid and / or phosphatidylcholine were determined using a Zeiss Ultra Plus 55 field emission scanning electron microscope (**SEM**) operated at 2 kV in secondary electron detection mode. The samples were prepared by placing the powder on the carbon stickers attached to an aluminum holder. Nitrogen gas adsorption-desorption experiments, Brunauer–Emmett–Teller (**BET**) analysis, was performed using the Micromeritics TriStar II 3000 area and porosity analyzer instrument to probe the specific surface areas, porosity, and pore size of the electrocatalysts. Raman spectra for the activated carbon, activated carbon encapsulated mycolic acid and/or phosphatidylcholine were recorded with a Renishaw inVita Raman microscope using a Nb-YAG laser with an excitation wavelength of 532 nm and a granting with 1800 lines mm-1 yielding a spectral resolution of ca 1.2 cm^{-1}. The spot size of the sample was in the focal plane ca 2 μm using an output power of 0.5mW. Spectra were recorded for 30 seconds and accumulated 50 times to eliminate cosmic rays and to obtain a high signal to noise and signal to background ratio. Peak fitting is achieved by employing

Lorentzian peaks assuming four components for the carbon spectrum between 1000 and 1800 cm^{-1}. X-ray diffraction (XRD) patterns of the sample were collected using a X'Pert-Pro MDP diffractometer with theta/theta geometry (step width: 0.0263 $^{o}s^{-1}$), operating a copper tube at 40 kV and 40 mA. The instrumental resolution function is characterized with the NIST SRM 660a (LaB_6) standard. The XRD patterns were carried out in the 2Θ range between 10 and 100^{0}. Qualitative phase analysis of the samples was conducted with Bruker EVA using PDF database. The X-ray photoelectron spectra (XPS) were obtained at activated carbon, activated carbon-encapsulated mycolic acid and / or Phosphatidylcholine using a Physical Electronics model 5400 spectrometer system with monochromatic Mg K α radiation at 1253.6 eV at take-off angles of 15° and 45°.

Density Functional Theory (DFT) Simulation / Calculation Details

Density Functional Theory (DFT) studies were carried out with the super-computational facilities at the Centre for High Performance Computing (CHPC, Cape Town, South Africa) utilizing the BIOVIA Material Studio and employing adsorption locator tool module.[1] Cholesterol, phosphatidylcholine (PC) and all Mycolic acids (MA) strains: (Alpha; Methoxy-cis, Methoxy-trans; Keto-cis and Keto-trans) were modelled using the suites' building tools. The cleaning was done on each molecule using the material studio cleaning tool. Each of the above structures were subjected to relaxation calculations, and for convergences to be achieved, threshold energies were set at 10^{-6} eV. PC was later adsorbed onto MA and the new system relaxed to form PC/MA. Adsorption of cholesterol was then carried out (using BIOVIA Material Studio adsorption suite / adsorption locator) on PC and all strains of MA, and PC/MA structures. The minimum adsorption distance was set at 5 Å. The adsorption locator module was used to predict the preferred adsorption mechanistic models, in which the first model was found to be more stable. In order to verify the

occurrence of certain interactions in the adsorption mechanism, the adsorption locator module could only predict one preferred adsorption mechanistic model as the most stable. DMoI3, another module of the BIOVIA Materials Studio, was used to calculate electronic properties. The same threshold energy as that used for adsorption was set for these calculations of electronic properties and energies. To guarantee reliable theoretical results, Condensed-phase Optimization Molecular Potential for Atomistic Simulation Studies (COMPASS) forcefield was used.[2]

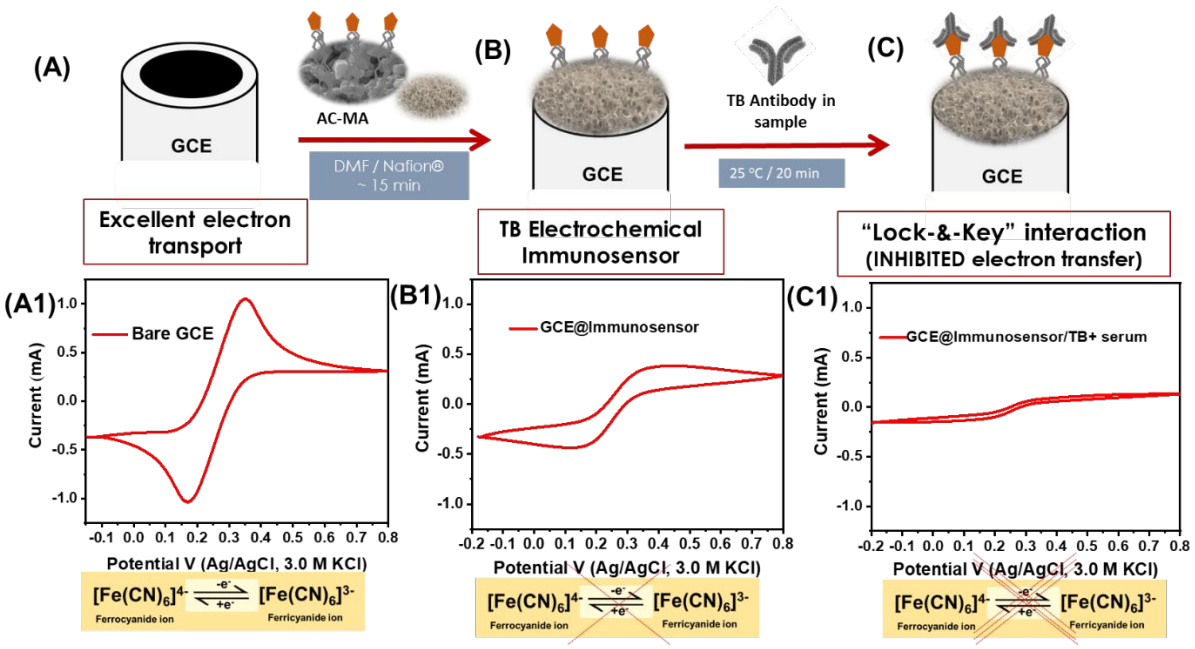

Figure S2: Fabrication of TB immunosensor as described in this work starting from the (A) bare GCE, (B) immobilization of the AC-MA with saponin to form the TB immunosensor, and (C) the immobilization of TB antibody to form the lock-and-key with the surface AMA antigen. The sensing mechanism and the resultant voltammograms for the carbon-encapsulated mycolic acid antigen as electrochemical immunosensor for tuberculosis: the resultant CVs of (A1) bare GCE, (B1) TB Immunosensor, and (C1) Immunosensor surface interacting with the antibody to obtain the lock-and-key structure.

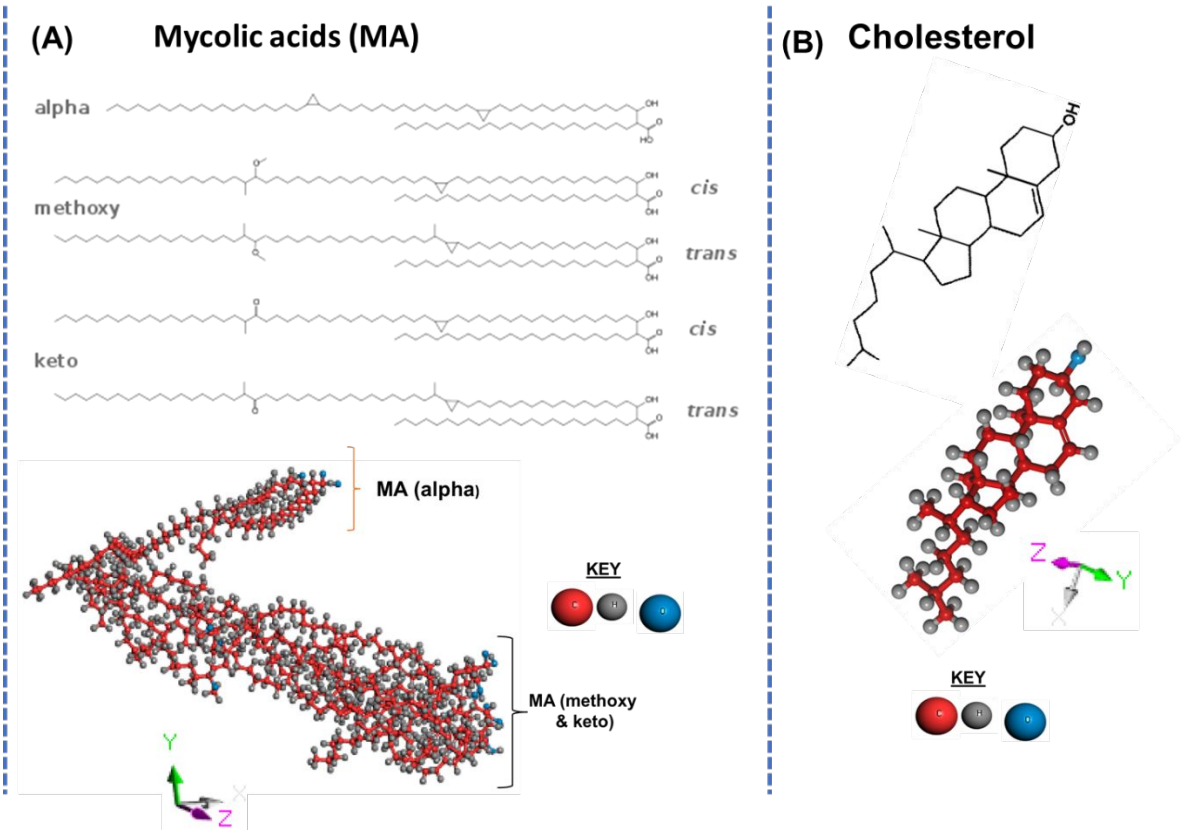

Figure S3: Molecular structures of (A) mycolic acid and (B) cholesterol

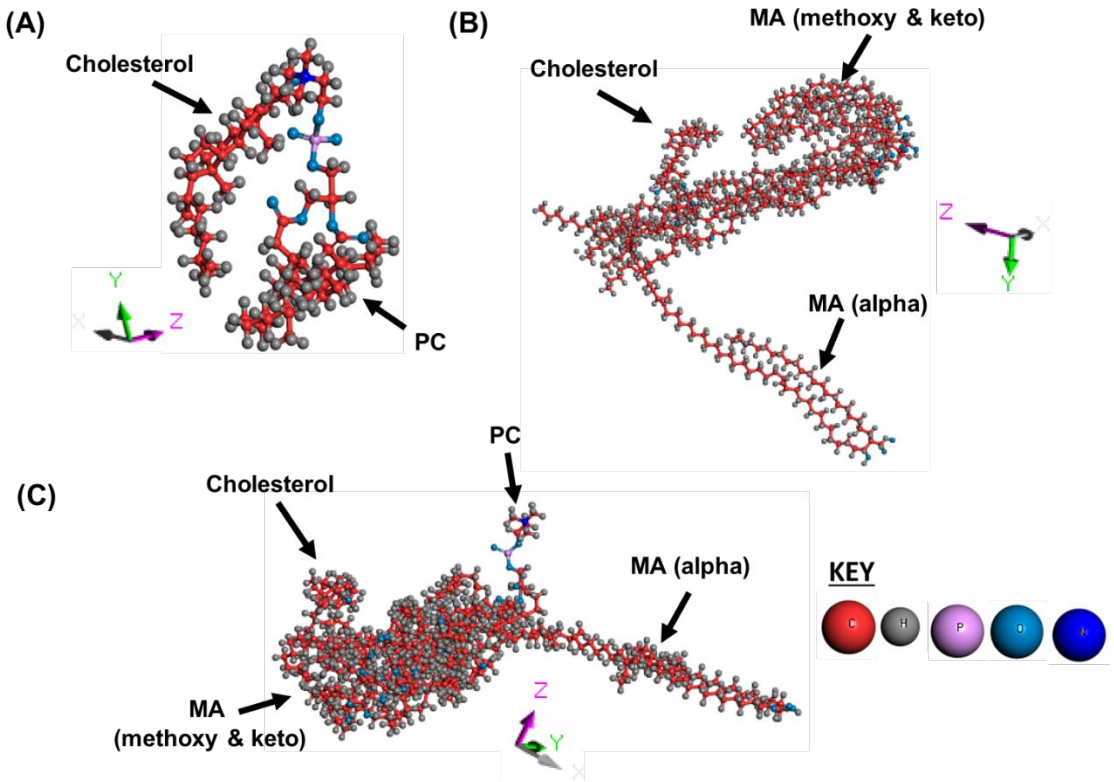

Figure S4: The interactions between (A) cholesterol (Ch) and mycolic acids (MA), (B) Ch and MA, and (C) Ch and PC/MA. All are stabilized structure as generated from the DFT simulation

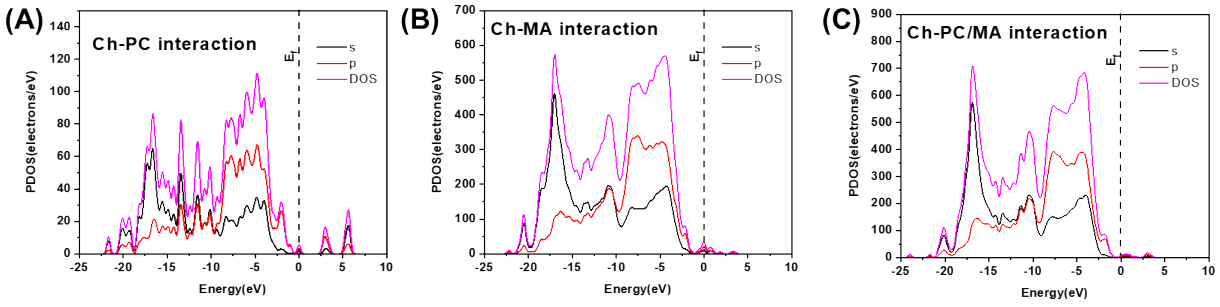

Figure S5: Typical projected partial density of states (PDOS) vs. energy of cholesterol adsorbed onto (A) PC, (B) MA, and (C) PC/MA.

DFT Co-ordinates

These can be accessed using any relevant software such as VESTA software. PLEASE NOTE that no commercial use is allowed without permission of the authors. Any academic user must observe professional ethics by fully acknowledging the authors. The co-ordinates include as follows:

1. **Mycolic acid**

Mycolic acid.xyz

2. **Cholesterol**

cholesterol.xyz

3. **Cholesterol adsorbed on PC**

Cholesteral_adsorbed_on_PC-MA.xyz

4. **Cholesterol adsorbed on MA**

Cholesteral_adsorbed_on_MA.xyz

5. **Cholesterol adsorbed on PC**

Chlostreral_adsorbed_on_PC.xyz

Table S1: Characteristics of the activated carbons used in this work.

Parameter	**Value**
BET Surface area	622.2057 m^2/g
Langmuir Surface Area	1031.9224 m^2/g
Total pore volume	0.360006 cm^3/g
Micropore volume	0.050566 cm^3/g
Adsorption average pore width (4V/A by BET)	2.31439 nm

Table S2: Summary of the DFT calculated values of the structural parameters for the adsorption of cholesterol on different molecules.

Molecule	**D.M (Debve)**	**Bond length (Å)**	**P-band (ε_p) (eV)**
PC	26.109	3.745	-12.2874
MA	521.828	2.748	-11.3922
PC/MA	146.210	2.351	-11.2695

Key: PC (phosphatidylcholine); MA (mycolic acid); D.M (Dipole moment); E_f (Fermi energy); ε_p (p-band centre)

References

1. D Systèmes, "Biovia materials studio." Dassault Systèmes: San Diego, CA, USA (2019).

2. D. Rigby, "Fluid density predictions using the COMPASS force field." Fluid phase equilibria 217.1 (2004): 77-87