CNT-Oxidoreductase Enzyme Conjugates for Biosensors and Biofuel Cells

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1. Introduction and Background

Trauma is the national leading cause of death for people between the ages of 1 and 44. More work years are lost due to trauma than cancer and heart disease combined (according to the North Central Regional Trauma Advisory Council). In the last 50 years, not much has been done to improve trauma management on the site of injury. There is the need to develop implantable biosensors with high stability and performance for use in trauma management. Implantable biofuel cells offer an attractive alternative for powering fully implantable biomedical devices. Molecular dynamics (MD) simulation is being employed to gain a deeper understanding of the conformation and orientation of the enzyme around the nanotube as well as provide valuable insight into the electron transfer interaction between enzyme cofactor and CNT.

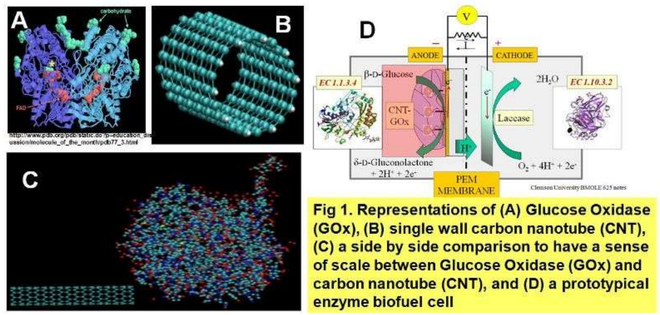


Fig 1. Representations of (A) Glucose Oxidase (GOx), (B) single wall carbon nanotube (CNT), (C) a side by side comparison to have a sense of scale between Glucose Oxidase (GOx) and carbon nanotube (CNT), and (D) a prototypical enzyme biofuel cell

2. Materials and Methods

Our initial model system includes a nine amino acid peptide (TGTGVGTGT) interacting with a CH_3 terminated self-assembled monolayer (SAM) surface and an -OH terminated SAM surface. Solvation effects will be simulated implicitly using the Generalized Born with a simple Switching (GBSW) method. The CHARMM suite of simulation tools is being used as a simulation engine conducted with the CHARMM force field.

Cleaned Single Walled Nanotubes, Glucose Oxidase (GOx), DI Water generated by a Milli-Q Ultrapure Water Purification System, materials prescribed by Sigma Aldrich for enzymatic assay , Synergy Mx Monochromator-Based Multi-Mode Microplate Reader, Soniprep 150 equipped with MSE exponential probe, Sorvall Evolution RC centrifuge (Thermo Scientific) with SLA1500 rotor, Princeton Applied Research Potentiostat/Galvanostat Model 283, Jasco J-810

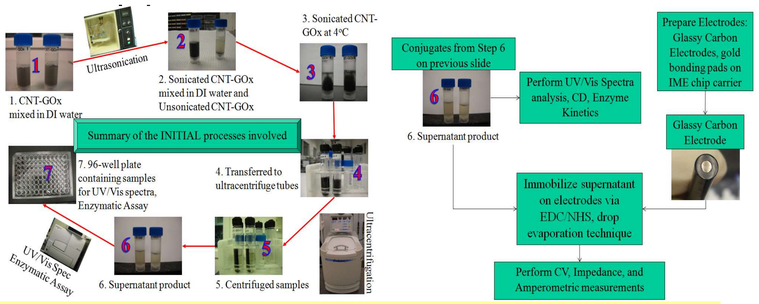
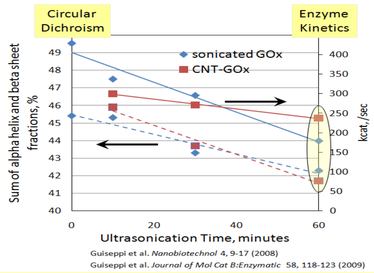


Fig 2. Representation of the methods applied to sample preparation and analysis

3. Results and Discussion

The conjugate system exhibits preservation of both enzymatic activity and enzymatic structure after undergoing sonication for varying time periods. It is worth noting that over a relatively long period of time (60 min), the enzymatic activity of the CNT-GOx conjugate system is 30% higher than sonicated GOx while the secondary structure for the conjugate system displayed a higher rate of conformational change. It is argued that one possible reason for a higher activity in the conjugate system over the sonicated GOx is due to the absorbance of some of the ultrasonic energies by the presence of dispersed CNT. The UV/Vis spectra, displays an observable peak shift (~280nm) between the CNT-GOx conjugate system, sonicated GOx, and pristine GOx demonstrating the effect of CNT on GOx. A closer look at the spectra over the 330nm to 500nm range gives precursory confirmation that the CNTs affect the conformational structure of GOx.



- Sum of alpha helix and beta sheet fractions (%):
  - CNT-GOx B (sonicated for 300 seconds): 38.0%
  - pGOx (unsonicated / 0 seconds): 38.5%
- Modeled: Helix (34%), β-sheet (23%)*
- Reported: Helix (34%), β-sheet (14%)

*http://www.pdb.org/pdb/explore/remediatedSequence.do?structureId=1CF3

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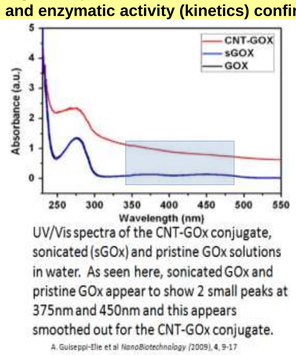
Fig 3. Enzymatic structural confirmation obtained by circular dichroism spectroscopy and enzymatic activity (kinetics) confirmation.



UV/Vis spectra of the CNT-GOx conjugate, sonicated (sGOx) and pristine GOx solutions in water. As seen here, sonicated GOx and pristine GOx appear to show 2 small peaks at 375nm and 450nm and this appears smoothed out for the CNT-GOx conjugate.

A. Guiseppi-Elie et al *NanoBiotechnology* (2005), 4, 9-17

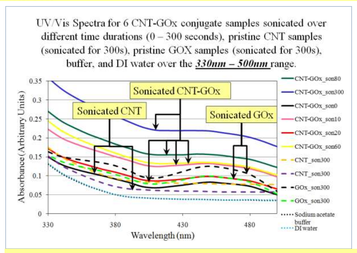


Fig 4. UV/Vis spectra taken using the Synergy Mx Monochromator-Based Multi-Mode Microplate Reader with samples placed in a 96-well plate.

Performing multiple scan rate cyclic voltammetry (MSRCV), affords us the opportunity to extract the diffusion coefficients of electroactive species before the immobilization of the conjugate system on the electrode

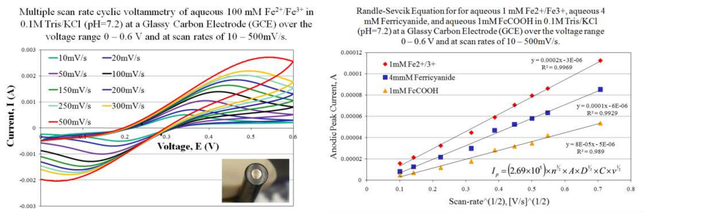


Fig 5. (A) Multiple scan rate cyclic voltametry (MSRCV) of aqueous 100mM Fe^{2+}/Fe^{3+} in 0.1M Tris KCl (pH=7.2) at a Glassy Carbon Electrode (GCE) over the voltage range 0 – 0.6 V and at scan rates of 10 – 500 mV/s. (B) Application of the Randle-Sevcik equation to extract the apparent diffusion coefficient for different electroactive solutions.

It is demonstrated that the cysteamine modified MDEA electrode gives rise to a 200% increase in the effective area versus an unmodified gold electrode.

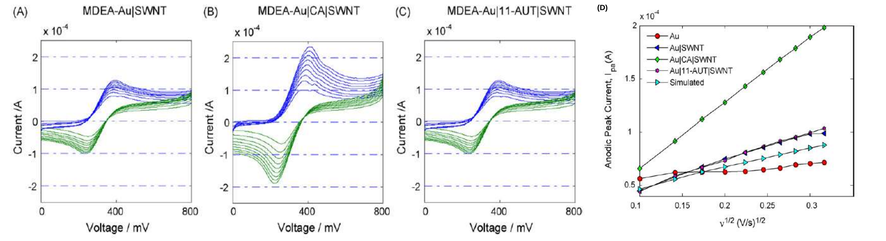


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Fig 6. (A) Multiple scan rate cyclic voltammetry (MSRCV) of (A) Unmodified MDEA-Au 100mm electrode with adsorbed acid-chopped SWNT (B) Cysteamine modified MDEA-Au 100mm electrode conjugated to acid chopped SWNT and (C) 11-AUT modified MDEA-Au 100mm electrode conjugated to acid chopped SWNT. This was done in phosphate buffered 0.1 M KCl solution (pH=7.2) over the voltage range 0 to 0.8 V and at scan rates of 10 – 100 mV/s at RT. (D) Application of the Randle-Sevcik equation to extract the apparent diffusion coefficient for each system. This method makes it possible to determine the area of detectors available per electrode surface area.

Results from peptides interacting with SAM surfaces is shown comparing implicit and explicit solvent methods. While the generalized Born model slightly overestimates the desolvation energy-penalty for the adsorption of polar peptides compared to the explicit model, it exhibits a good balance between speed and accuracy.

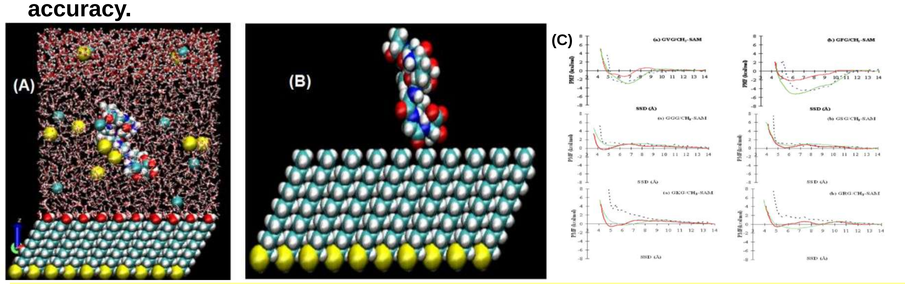

Fig. 7 (A) Snapshot of a model system containing a TGTG-V-GTGT peptide solvated in physiological saline with explicit TIP3P water over an –OH terminated SAM surface *(Vellore et al., Langmuir 2010).*
(B) Snapshot of the same model system with implicit solvent over a CH_3 terminated SAM surface. (C) Potential of Mean Force (PMF) profiles at 310K between the CH_3-terminated SAM surface and (a, b) hydrophobic peptides [GVG, GFG], (c, d) neutral hydrophilic peptides [GGG, GSG], (e, f) charged peptides [GKG, GRG] obtained using the C22/TIP3P explicit water model (thick red line), the C22/GBMV implicit solvent model (thin green line), and the C19/ACE implicit solvent model (dotted black line) *[Sun et al., J. Comput. Chem., 2007].*

4. Conclusions

- ❑ Sonication is shown to not have adverse effects on the CNT-GOx conjugate system although prolonged sonication does modify the enzyme's secondary structure while improving activity by 30%
- ❑ Cystamine aids the spontaneous immobilization of CNTs on electrode surfaces as well as increase the effective area available by 200% compared to an unmodified gold electrode
- ❑ The *Generalized Born with a simple switching* implicit solvent method displayed improbable adsorption behavior relative to substantial experimental evidence. This calls for fine tuning of the model parameters to address the overestimation of hydrophobic and hydrophilic interactions.

5. Key References

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