Label-free Detection of Cardiac Troponin-I using Carbon Nanofiber based Nanoelectrode Arrays

Adaikkappan Periyakaruppan, Ram P. Gandhiraman, M. Meyyappan, Jessica E. Koehne

[1]Center for Nanotechnology, NASA Ames Research Center, Moffett Field, CA

A sensor platform based on vertically aligned carbon nanofibers (CNFs) has been developed. Their inherent nanometer scale, high conductivity, wide potential window, good biocompatibility and well-defined surface chemistry make them ideal candidates as biosensor electrodes. A carbon nanofiber (CNF) multiplexed array has been fabricated with 9 sensing pads, each containing 40,000 carbon nanofibers as nanoelectrodes. Here, we report the use of vertically aligned CNF nanoelectrodes for the detection of cardiac Troponin-I for the early diagnosis of myocardial infarction. Antibody, anti-troponin, probe immobilization and subsequent binding to human cardiac troponin-I were characterized using electrochemical impedance spectroscopy and cyclic voltammetry techniques. Each step of the modification process resulted in changes in electrical capacitance or resistance to charge transfer due to the changes at the electrode surface upon antibody immobilization and binding to the specific antigen. This sensor demonstrates high sensitivity, down to 0.2 ng/mL, and good selectivity making this platform a good candidate for early stage diagnosis of myocardial infarction.

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Jessica E. Koehne
NASA Ames Research Center
Moffett Field, CA

Biosensor Motivation

NASA Applications

- Astronaut health monitoring
  - Lab-on-a-chip (DNA, rRNA, ricin, cholesterol, dopamine, serotonin, pH)
- Water Quality monitoring
  - Pathogen detection on ISS and long duration missions
- Planetary exploration
  - Life on other planets

Outside Applications and Customers

- Medical Diagnostics
  - NIH, DARPA
- Environmental Monitoring
  - EPA, NIH
- Biowarfare agent detection
  - DHS, DARPA
- Food Safety
  - FDA

Astronaut Heart Health Monitoring

Microgravity and Cardiovascular Health

- Fluid Shifts
- Changes in total blood volume
- Changes in heart beat
- Diminished aerobic activity

↓

Need for on-flight diagnostics

↓

Troponin-I

- biomarker: acute myocardial infarction
- normal levels: 0.4 ng/mL and lower
- risk of heart attack: 2.0 ng/mL and above

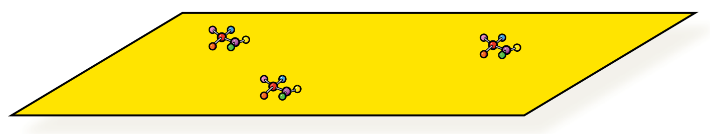
http://www.nasa.gov/exploration/humanresearch/areas_study/physiology/physiology_cardio.html

Nanoelectrodes for Sensors

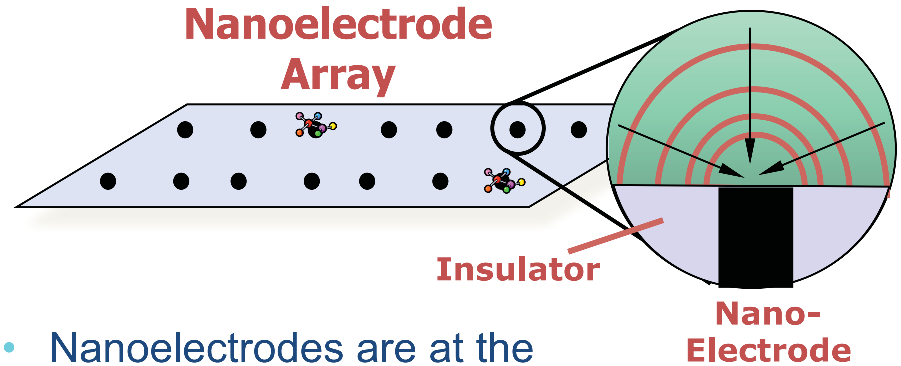
Nanoscale electrodes create a dramatic improvement in signal detection over traditional electrodes for small analyte concentrations

Background: $i_n \propto C_d^0 A$

Traditional Macroelectrode



Nanoelectrode Array

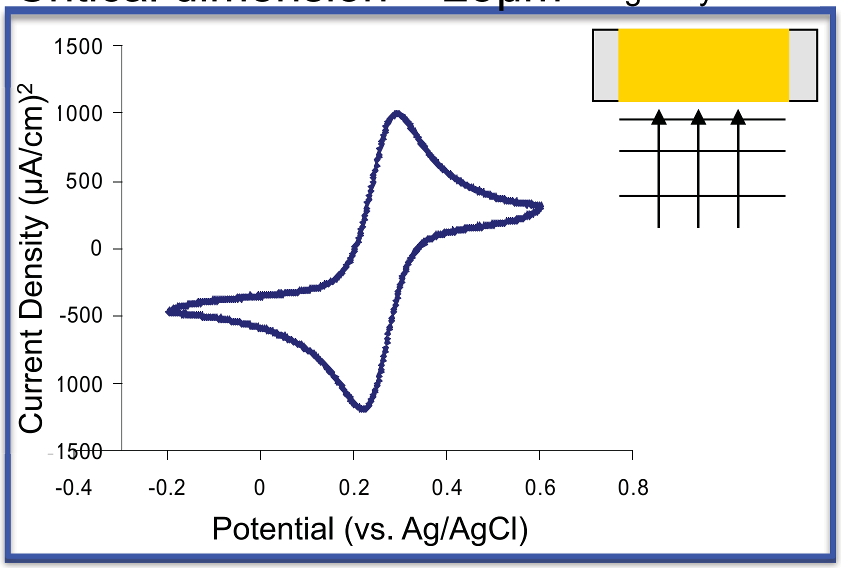


- **Scale difference** between macroelectrode and molecules is tremendous
- **Background noise** on electrode surface is therefore significant
- **Significant amount** of target molecules required

- Nanoelectrodes are at the **scale close to** molecules
- with dramatically **reduced background noise**
- Multiple electrodes results in **magnified signal** and **desired redundance** for statistical reliability.

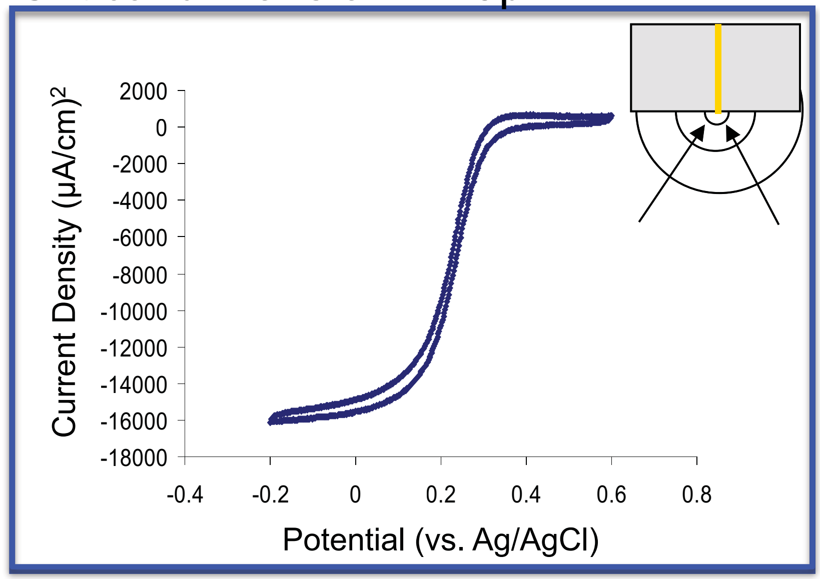
Macroelectrode vs. Nanoelectrode

Critical dimension > 25μm glassy carbon



Semi-infinite planar linear diffusion

Critical dimension < 25μm carbon nanofiber



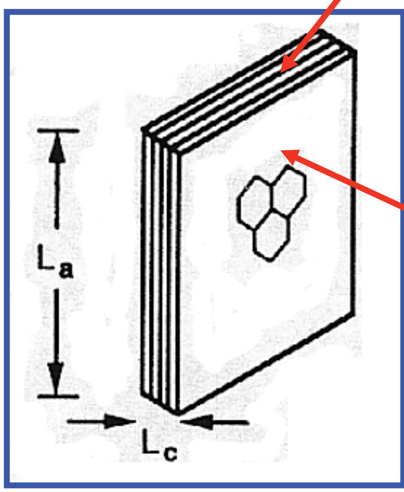
Semi-infinite hemispherical diffusion:
Current exhibits a steady state
Diffusion layer is approximately 6r

Nanostructured Ensemble or Array Electrode

- Spatial Resolution: defined by **r**
- Sensitivity: signal to noise
    - $i_s/i_n \approx nFC_0D_0/\mathbf{r}$

Carbon Nanofibers (CNFs)

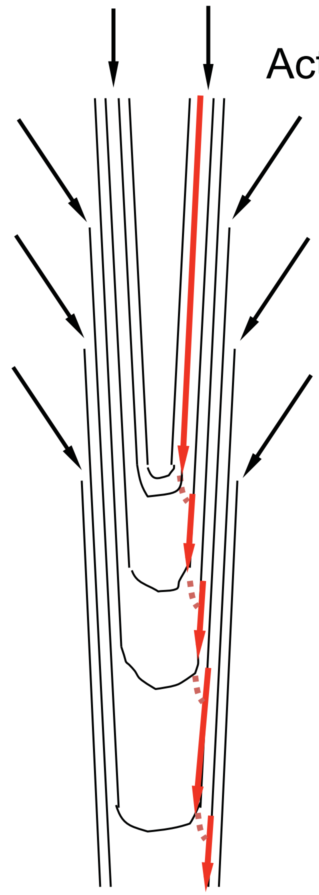
HOPG



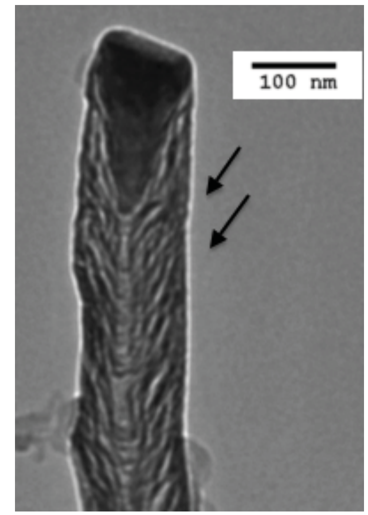
Edge Plane:
(1) High electron transfer rate (~ 0.1 cm/s)
(2) Very high specific capacitance (>60 μF/cm^2)

Basal Plane:
(1) Low electron transfer rate (< 10^{-7} cm/s)
(2) Anomalously low capacitance (~1.9 μF/cm^2)

Active sites



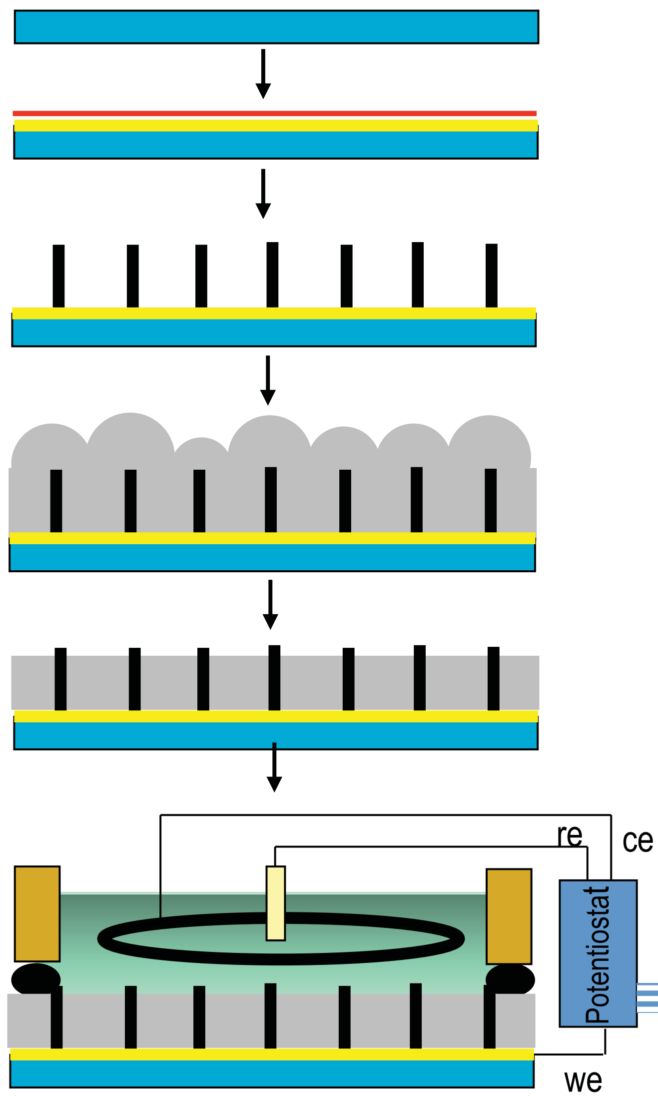
Bamboo-like CNFs



TEM of CNF

R. L. McCreery, A. J. Bard, in *Electroanalytical Chemistry*, Ed., 1991, 17, 221.

CNF Array Preparation



(1) Coat silicon wafer with underlying Cr metal & Ni catalyst metal

(2) Growth of Vertically Aligned CNF Array by Plasma Enhanced Chemical Vapor Deposition (PECVD)

(2) Dielectric Encapsulation of silicon dioxide by TEOS Chemical Vapor Deposition (TEOS CVD)

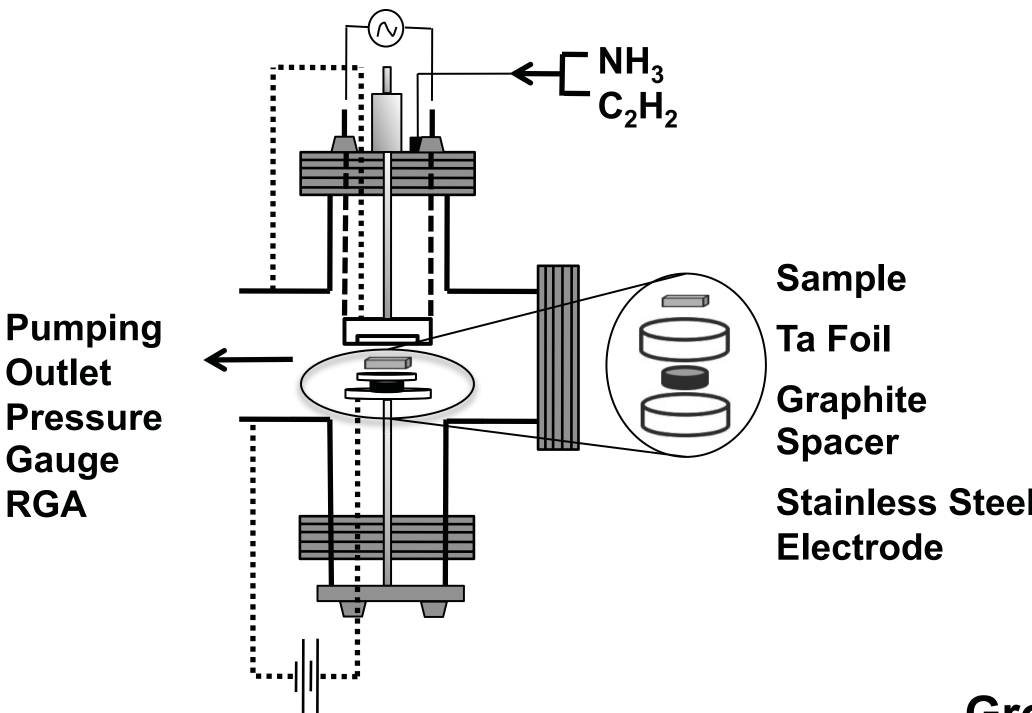
(3) Planarization by Chemical Mechanical Polishing (CMP)

(5) Electrochemical Characterization

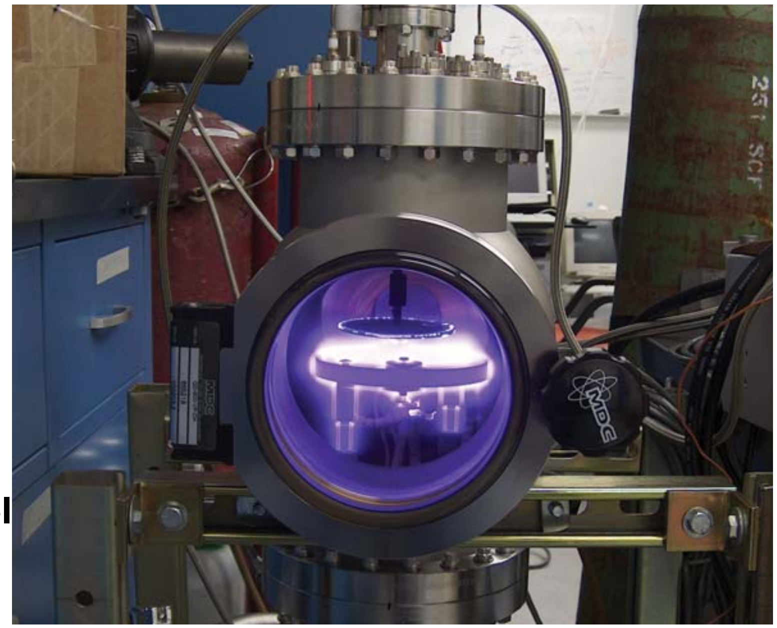
Cruden, B., et al., *Appl. Phys. Lett.* **2003**, 94, 4070-4078.

CNF Growth by Plasma Enhanced Chemical Vapor Deposition (PECVD)

PECVD Reactor Schematic



Custom Built PECVD Reactor



Growth Process

- Heated to 650 C
- Plasma discharge 500 W, 530 V, 0.97 A
- 150 sccm NH_3/50 sccm C_2H_2, 5-6 torr
- Growth rate- 1000 nm/min
- Quality is good, alignment is good

Cruden, B., et al., *Appl. Phys. Lett.* **2003**, 94, 4070-4078.

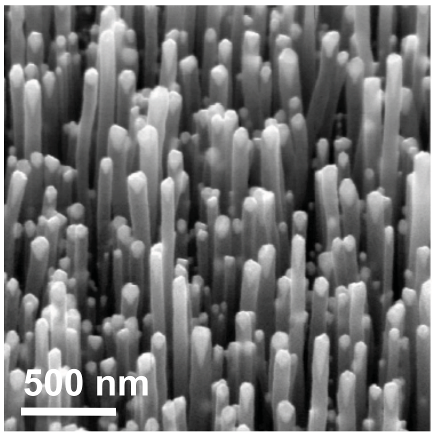
Define CNF Placement by Catalyst Placement

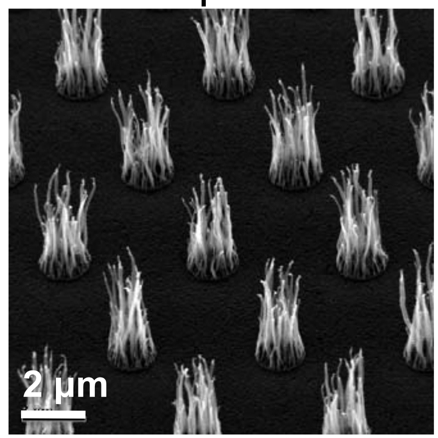
Continuous Layer of Catalyst

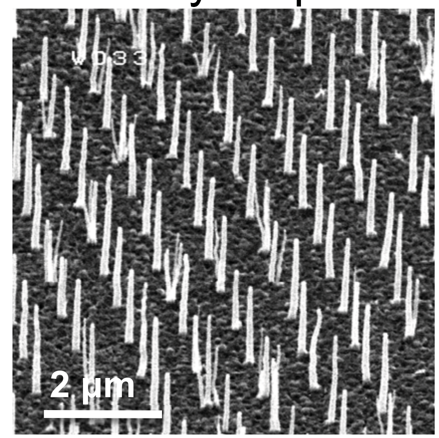
Photolithography Defined Catalyst Spots

Electron Beam Lithography Defined Catalyst Spots

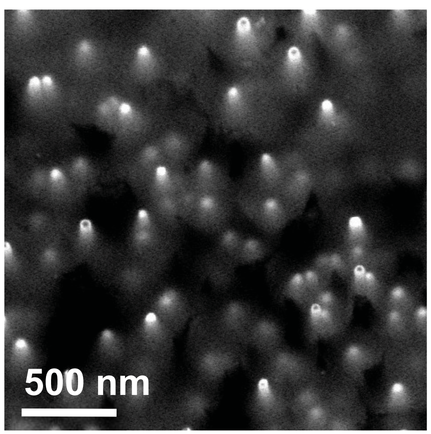
As Grown CNFs

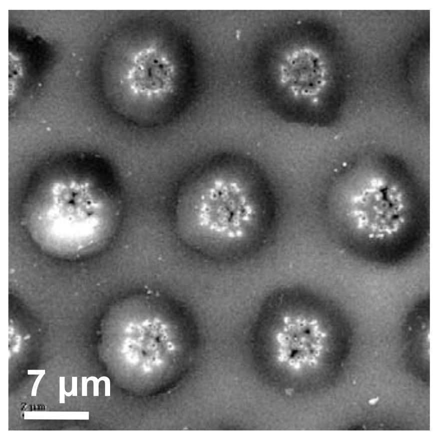


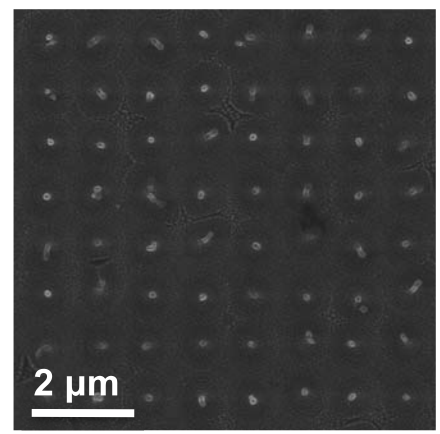




SiO_2 Encapsulated CNFs

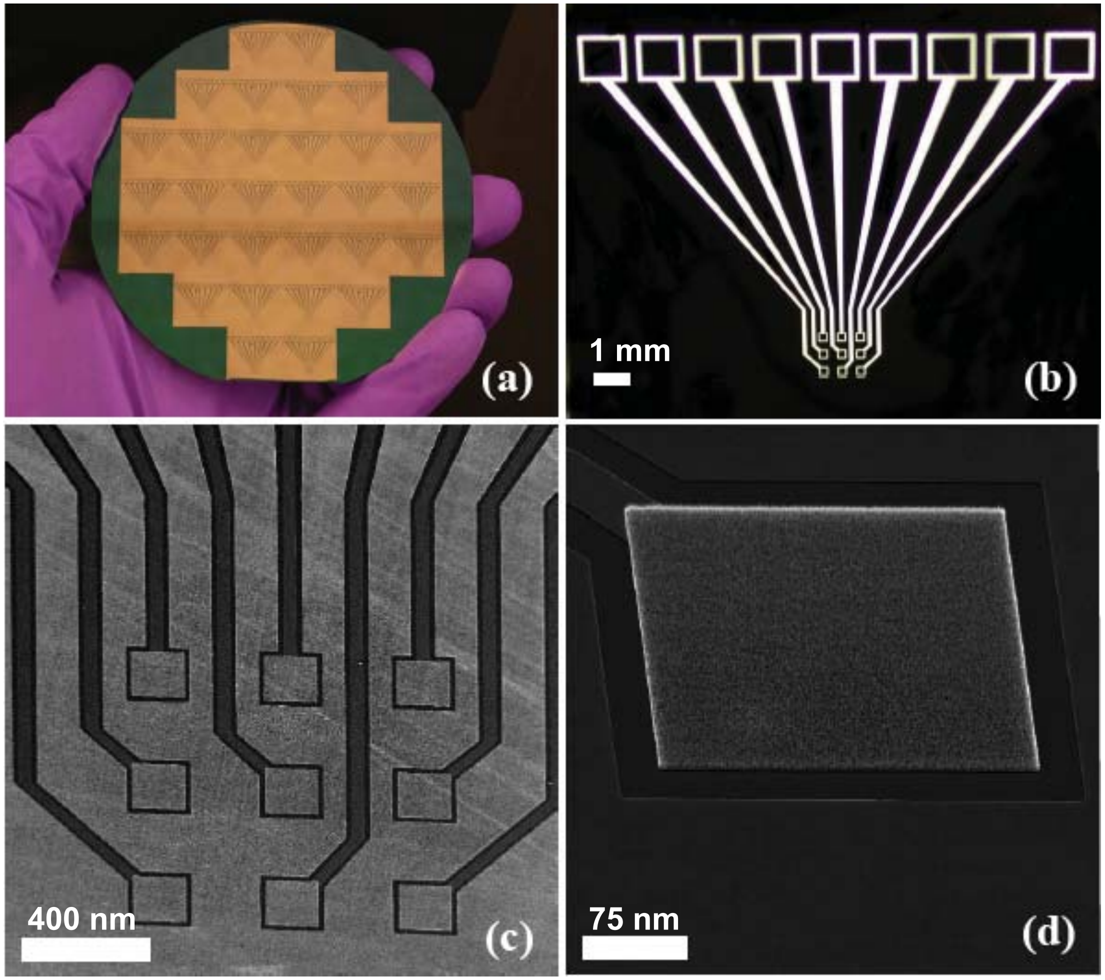






Fabrication of 3x3 Array

30 devices on a 4” Si wafer



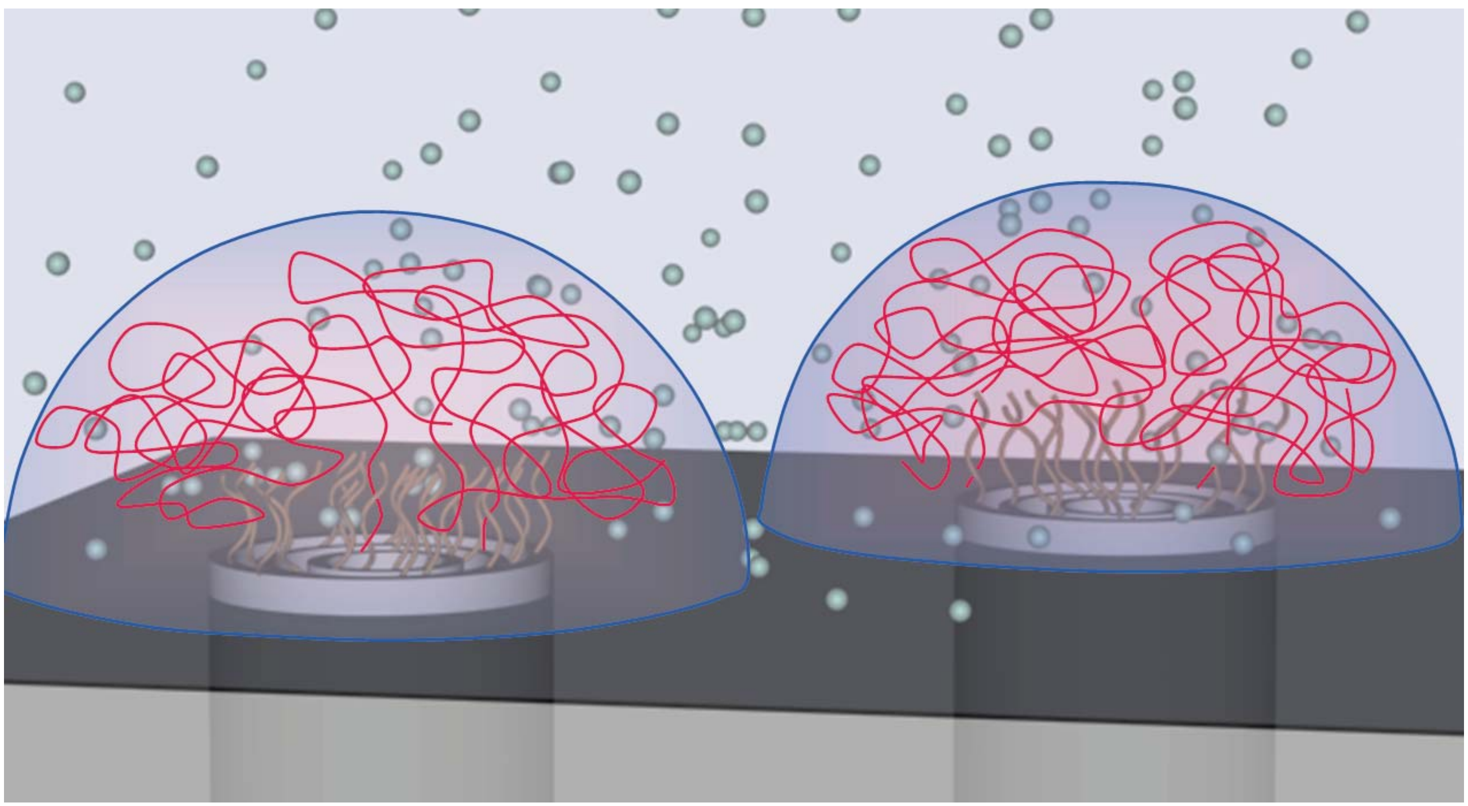
- 200 μm by 200 μm electrode dimensions
- 9 individually addressed electrodes
- potentially 9 different target molecules

Biosensing Using CNF Array

Objectives:

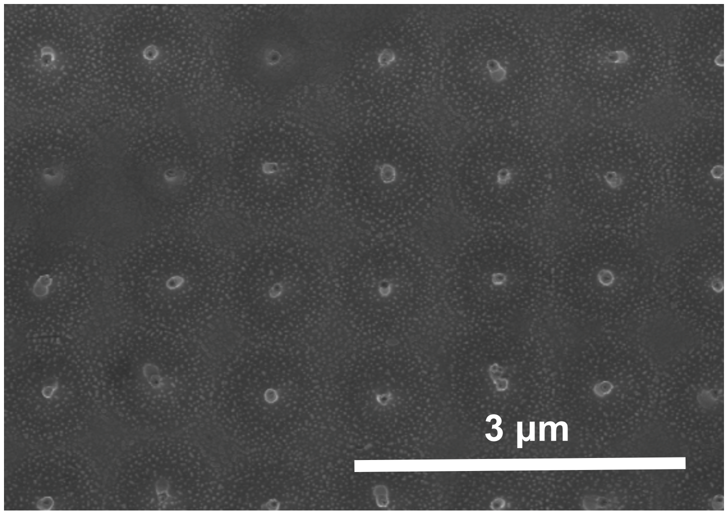
The objective is to test an ultrasmall biosensor for:

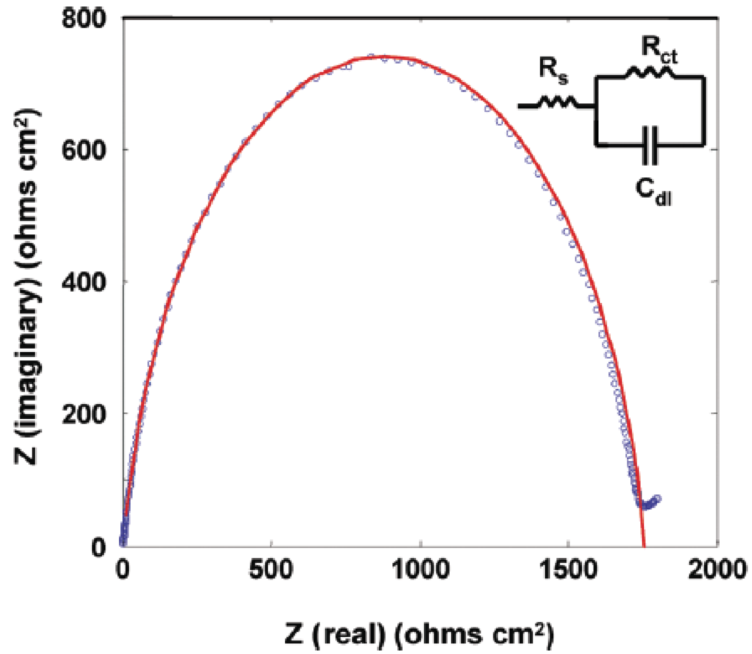
1) point of care diagnostics for astronaut health monitoring



Electrochemical Impedance Spectroscopy of CNF Electrode

ultralow density CNF

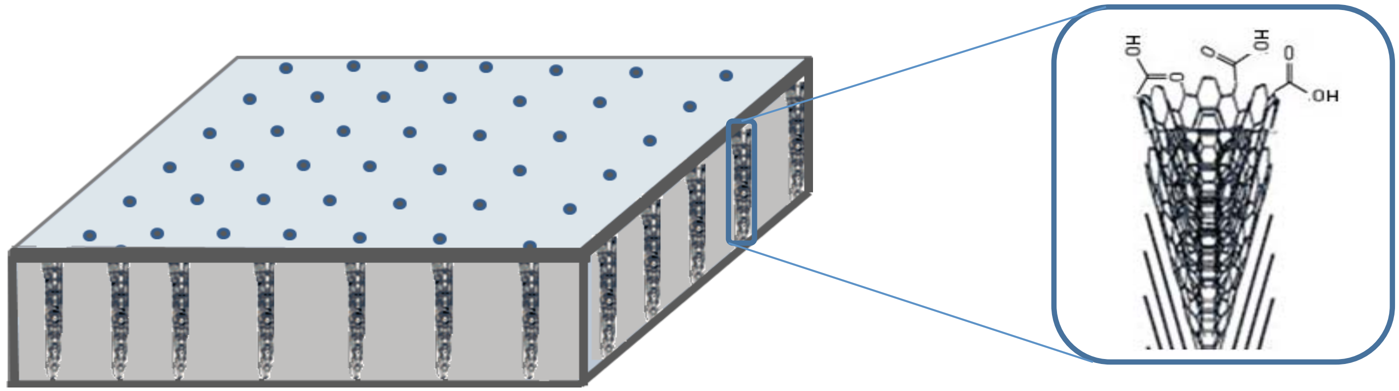


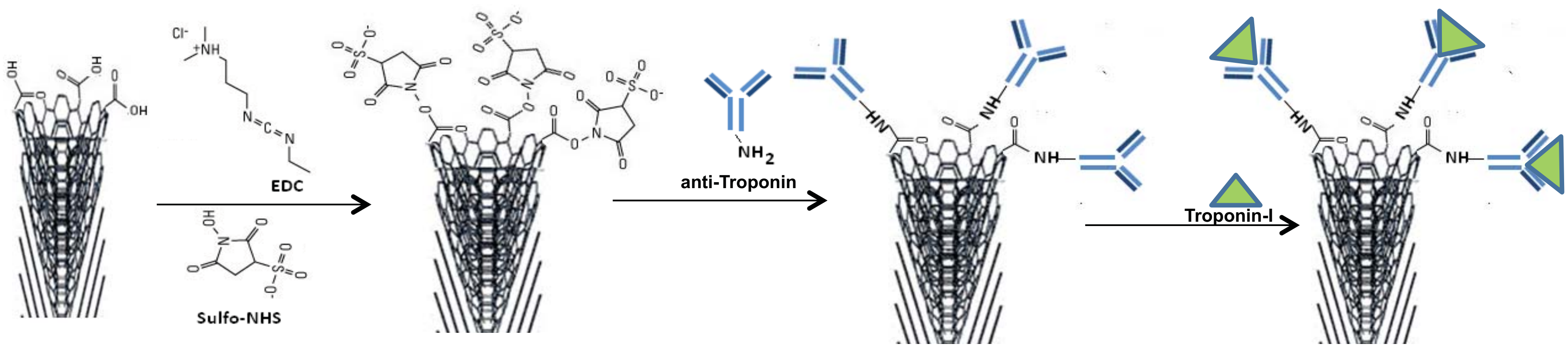


Fitting Parameters	Randomly Grown CNF	CNF (low density)	CNF (ultralow density)
I (A/mm^2)	7.1 X 10^{-6}	1.8 X 10^{-6}	2.5 X 10^{-7}
R_{ct} (KΩ)	N/A	1.8	17.3
CPE (µF)	906	3.3	2.5
n	0.79	0.89	0.91

S. Siddiqui, P. U. Arumugam, H. Chen, J. Li, M. Meyyappan, *ACS Nano,* **2010**, 4, 955-961.

Surface Preparation of CNF Electrode

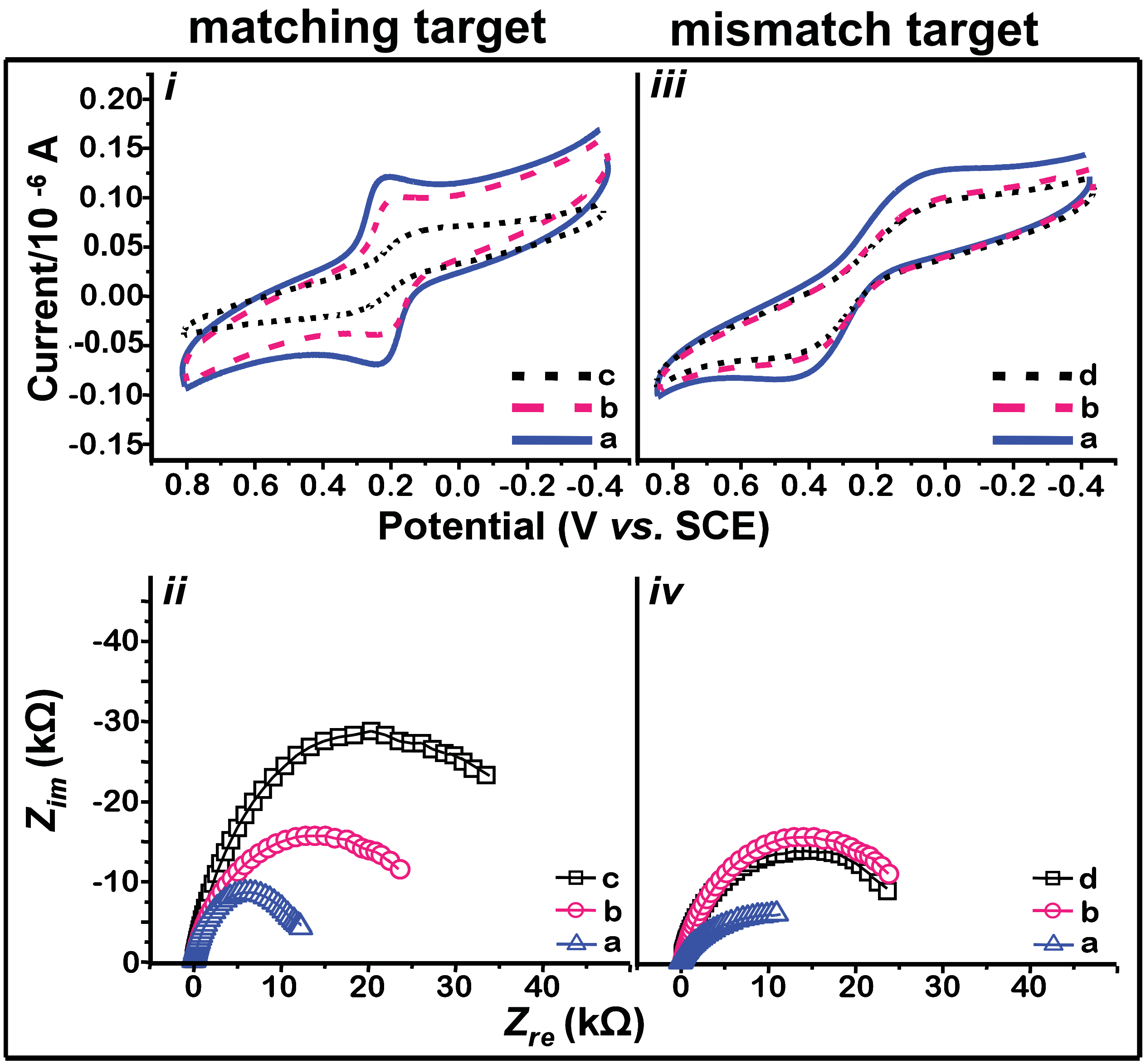




Troponin-I Detection

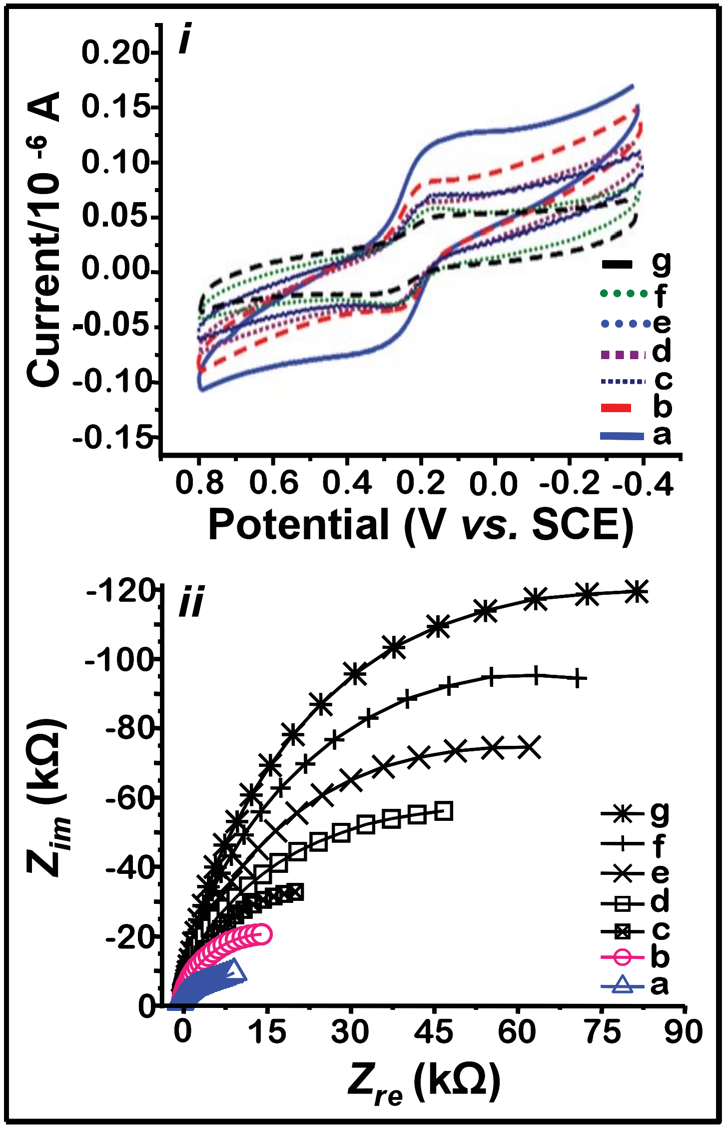
Blue: bare electrode
Pink: with anti-troponin
Black: with anti-troponin and protein

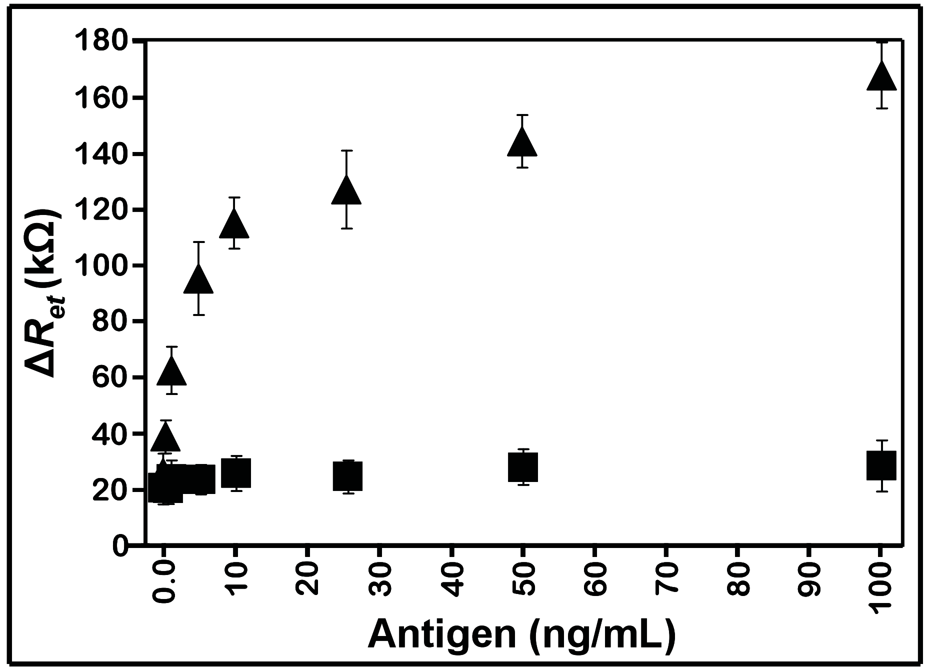
Increase in R_{ct} observed upon anti-troponin immobilization and matching protein binding



A. Periyakaruppan, R. P. Gandhiraman, M. Meyyappan, J. E. Koehne, *Anal. Chem.*, **2013**, 85, 3858-3863.

Troponin-I Concentration Study





Troponin-I concentration range: 100 ng/mL to 0.25 ng/mL
Detection down to 0.25 ng/mL

A. Periyakaruppan, R. P. Gandhiraman, M. Meyyappan, J. E. Koehne, *Anal. Chem.,* **2013**, 85, 3858-3863.

Summary

- Carbon nanofibers can be used to as nanoscale electrodes to reduce background noise while maintaining large sampling volume
- Carbon nanofiber nanoelectrode arrays are easily fabricated using standard silicon processing
  - CNF spacing defined by photolithography and e-beam lithography
- Carbon nanofibers have been used as sensitive nanoelectrodes for cyclic voltammetry and electrochemical impedance spectroscopy investigations
- Changes in R_{ct} are measured after antibody immobilization and protein binding
- Carbon nanofiber nanoelectrode arrays have been used to detect down to 0.25 ng/mL troponin-I

Ames Research Center

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