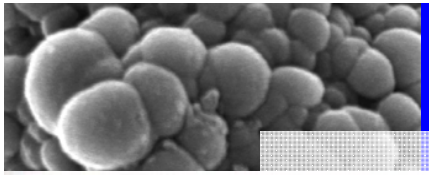

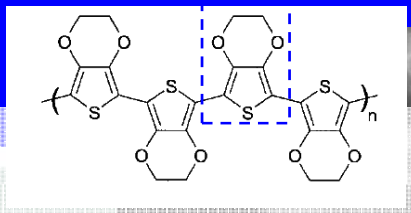

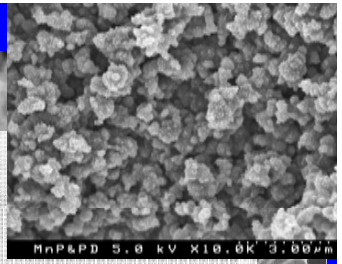

Micro- and Nano-Biosensors Applied for Tracking of the Brain Chemical Messages

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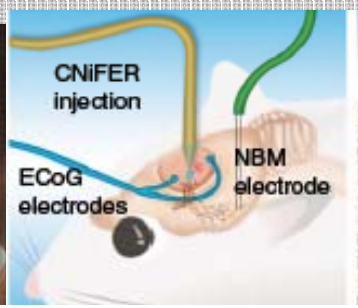
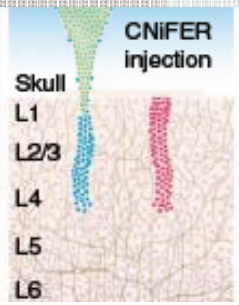
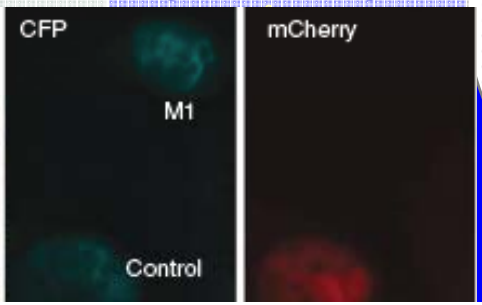
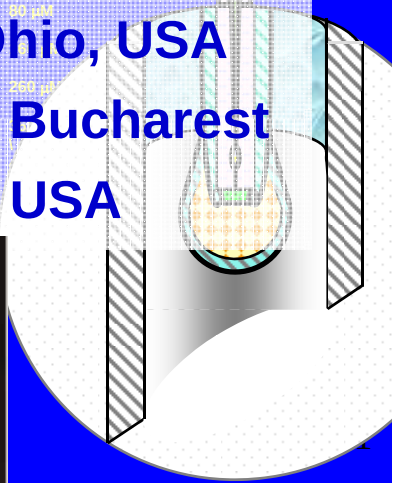








Abstract:

Every task of the nervous system, from those unconscious (i.e., heart control), to the higher cognitive functions (i. e., emotions, learning and memory, is triggered by chemical communication. This talk will outline some of the biosensor methods that could be applied to monitor this chemical signaling in the brain, in real time, with an accent on the electrochemical techniques.

A biosensor can be defined as any device that (i) uses a biological or a synthetic recognition element, in order to (ii) bind specifically to an analyte or molecule of interest and furthermore (iii) provides a physical signal (e.g. fluorescence, current, impedance) that is proportional to the amount of analyte, all these elements being intimately integrated with each other.

The micro-biosensors having a minuscule sensing tip (µEBS) offer several advantages, including: stirring insensitivity, ability to measure analyte concentrations with high spatial and temporal resolution in unstirred liquids and soft-solid specimens, with fast response times and low levels of background electrical noise.

Glucose, galactose, choline, hydrogen peroxide, nitric oxide and/or peroxynitrite are all molecules involved, one way or another, in neural controlled processes. At the same time, these are some of the analytes that were detected with our µBS (be it Clark-type or carbon-fiber type designs).

The µBS fabrication and their performance (response times, detection limit, sensitivity, etc.) will be further discussed in our talk. Consequently, these µBS could become an enabling technology for real time monitoring *in situ* of the neuro-chemical communication. Some other alternative techniques to monitor these neuro-transmitters and neuro-modulators will be also outlined.

The neuro-transmitters molecules can be grouped in three different categories vis-a-vis the electrochemical methods (ECM) of detection:

(i) Molecules readily detectable by ECM: dopamine, norepinephrine, epinephrine, serotonin, melatonin, histamine, adenosine or their metabolites, but also nitric oxide, molecular oxygen, or hydrogen peroxide

(ii) Molecules not readily electro-active *per se*. These, however, can be oxidized by an enzyme, that can be monitored by coupling this enzyme reaction with electrochemical detection. Some of the analytes herein are glutamate, acetylcholine, lactate, etc., but also choline and glucose.

(iii) Neuro-peptides and some aminoacid neuro-transmitters (i e Glycine; neuroactive peptides) not detectable with electrochemical methods at this time, in situ

Fabrication methods for microbiosensors
Immobilization the recognition interface (enzymes, synthetic markers, etc)

- Electroactive polymers (EAPs) Pyrrole, Aniline, Thiophene
- Redox gels
- Electrodeposited films
- Sol-gel immobilization

Limitations of microbiosensors

- Interferents
  - Use mediators to lower operational potential
  - Permselective membranes w. size or charge exclusion

- Oxygen, pH, temp changes
  - Cause occasional erroneous signals
  - Can be compensated by hardware

- Sensitivity loss in vivo from
  - Fouling or capsule effect, when implanted
  - Enzyme activity due to actions of proteases

Advantages of microbiosensors

- Extremely useful analytical tools
- Direct measurement of brain neurochemical
- Coupled with other physiological recordings or complementary techniques

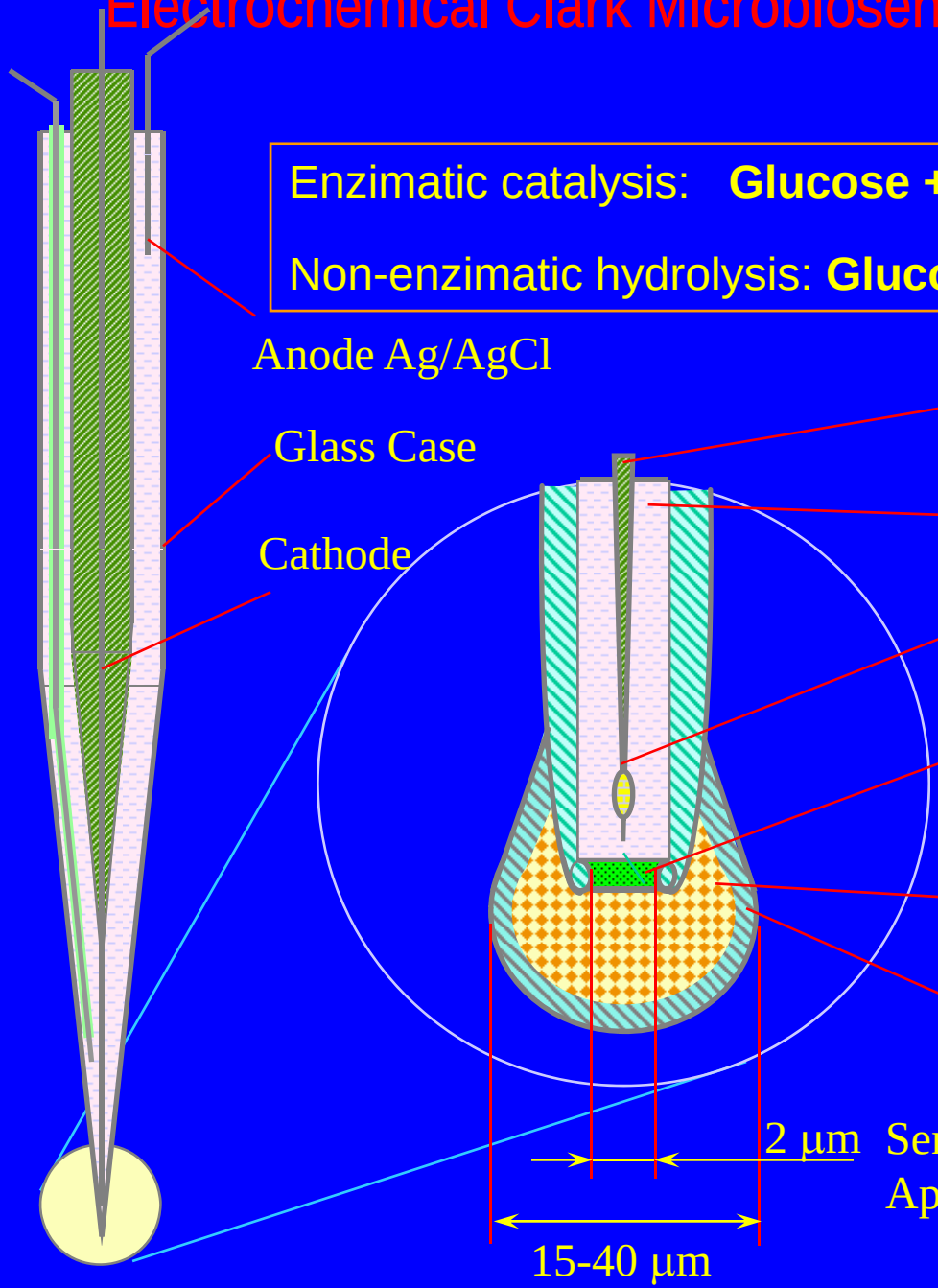
Analyte	Application	Refs
Glutamate	Release from photoreceptors during light stimulation (in vitro) Electrode arrays: KCl-induced release, glutamate clearance (in vivo)	Burmeister et al, 2001;2002; 2003
	KCl-induced release from cultured neurons, hippocampal slices	Poitry et al, 1997 Nakajima et al, 2003
	Multisite recording from hippocampal slices	Kasai et al, 2001
Adenosine	Release from brainstem during cardiorespiratory reflexes (in vivo)	Dale et al, 2002 Gourine et al, 2002
	Release from spinal cord during locomotion (in vivo)	Llaudet et al, 2003
	Release from hippocampal brain slices during hypoxia	Frengueli, 2003
Acetyl-choline /Choline	Detection of exogenous choline, measurement of endogenous cholinesterase activity (in vivo)	Burmeister et al, 2003; Garguilo & Michael, 1995; 1996
	Electrode arrays: KCl-induced release of choline; activity of endogenous cholinesterase (in vivo)	Burmeister et al, 2003; Cui et al, 2001
	Selectivity of detection in vivo	Parikh, 2004
	Detection of Ach and choline in vivo: KCl-induced release	Mitchell, 2004
	Choline microbiosensor	Peteu et al, 1996

Analyte	Application	Refs
ATP	ATP Release of ATP from spinal cord during locomotion (in vivo)	Llaudet et al, 2005
	Release of ATP from ventral medulla during hypoxia (in vivo)	Gourine et al, 2005
	Release of ATP in the embryonic retina and its role in the control of development (in vitro)	Pearson et al, 2005
Glucose	Glucose levels in striatum of free moving rats	Lowry et al, 1998
	Anaesthesia and glucose levels (in vivo)	Lowry & Fillenz, 1998
	Glucose levels in cortex during the sleep–wake cycle (in vivo)	Netchiporouk , 2001
	O2 dependence of measurements in vivo	Dixon et al, 2002
	Glucose microbiosensors	Peteu et al, 1996
	Mapping cellular motility and chemotaxis (glucose, galactose)	Peteu et al, 1998
Lactate	Lactate Changes in lactate coupled to neural activation	Hu & Wilson, 1997
	Lactate levels during sleep wake cycle (in vivo)	Shram et al, 2002
	Lactate release and metabolism in retinal glial cells (in vitro)	Poitry et al, 2000
	Lactate release from cultured astroglia	Poitry-Yamate et al, 2002
	Electrode array: KCl-induced changes in lactate release (in vivo)	Burmeister , 2005
H2O2	H2O2 Release in striatum in response to electrical stimulation (in vivo)	Kulagina & Michael, 2003
	H2O2 microbiosensor	Peteu et al, 1996

Electrochemical Clark Microbiosensors for Glucose, Choline, H2O2

Enzimatic catalysis: **Glucose + O_2 → Gluconolactone + H_2O_2**

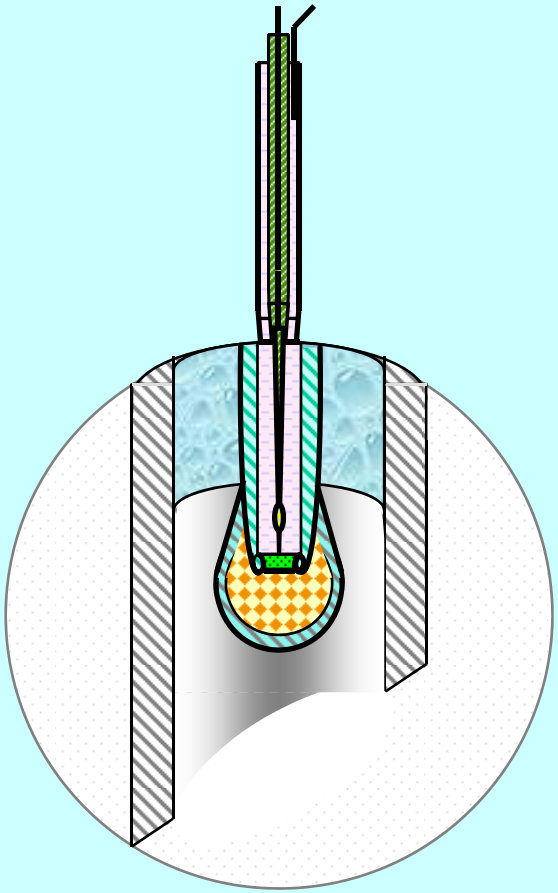
Non-enzimatic hydrolysis: **Gluconolactone + H_2O → Gluconic acid**



[Peteu , Worden et al, 1996; 1998]

Effects of long-term *in situ* use

Needle-type design



[Peteu , Worden et al, 1996; 1998]

PON detection current status

- **Chemiluminescence, fluorescence**
- **LC-MS: peroxynitrite-derived 3-nitrotyrosine in rat μvessels**
- **Electrochemical: modified C μfiber amperometry**

  (Xue et al, *An. Chem* 2000)
- **Electrochemical: unmediated signature on platinized C μfiber**

  (Amatore et al, *Chem Eur J* 2001)

Aims and Significance of Detecting Peroxynitrite (PON)

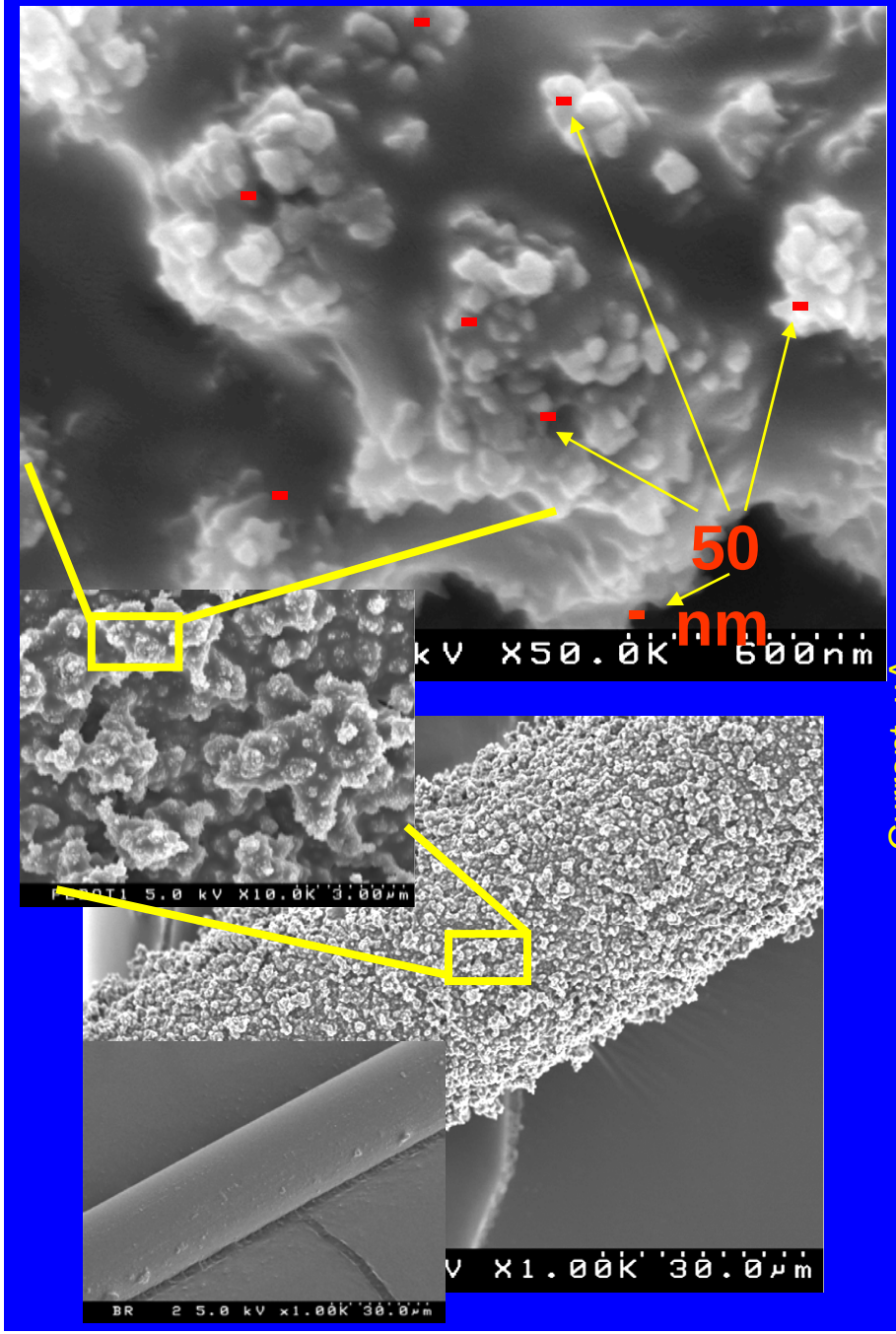
Peroxynitrite Anion in Animal Cells $O{=}N{-}O{-}O^-$

- **Generated by Nitric Oxide reaction w. Superoxide anion in organelles, cells**
- **Powerful oxidizing and nitrating agent**
- **Implicated in pathogenesis of Alzheimer's, Parkinson's, cancer, AIDS**

Pathogenetic role of peroxynitrite in Traumatic Brain Injury (TBI) is demonstrating by the beneficial effects of NOS inhibitor and peroxynitrite scavengers in reducing neuronal injury and improving neurological recovery following injury (Pacher, Beckam, Liaudet, 2007).

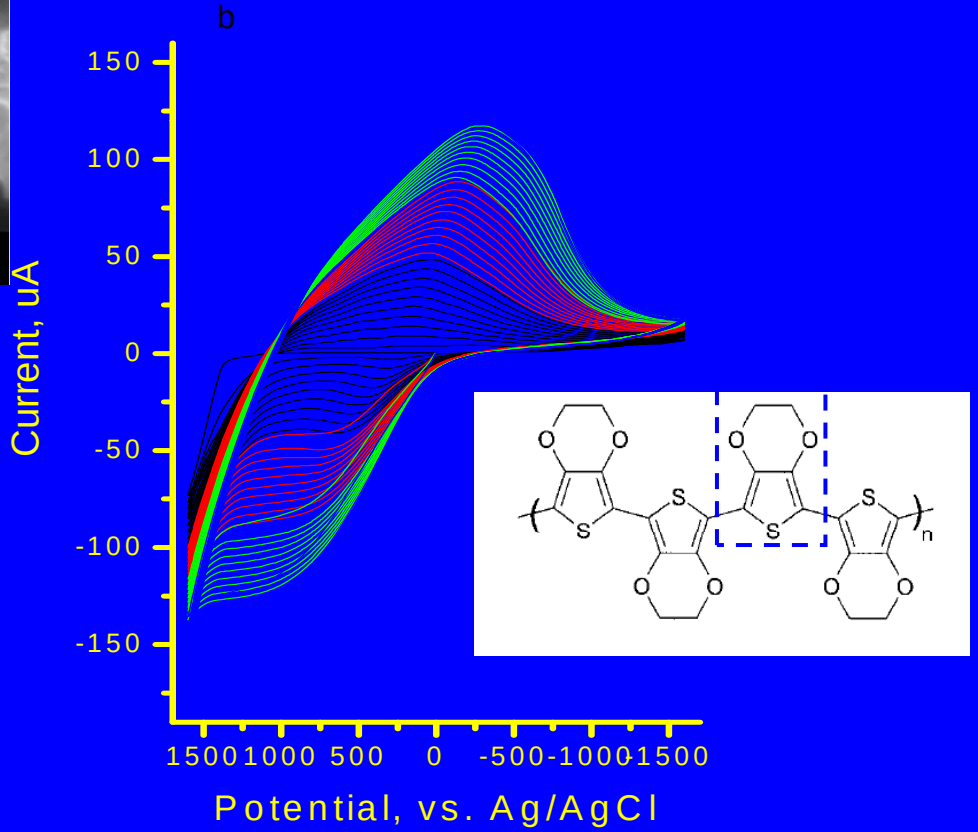
Peroxynitrite formation and/or protein nitration have an important role in neurodegenerative disorders and suggest that the neutralization of this reactive species may offer significant therapeutic benefits in patients suffering from these devastating diseases (Pacher, Beckam, Liaudet).

PEDOT-Hemin Modified Microbiosensors for Peroxynitrite



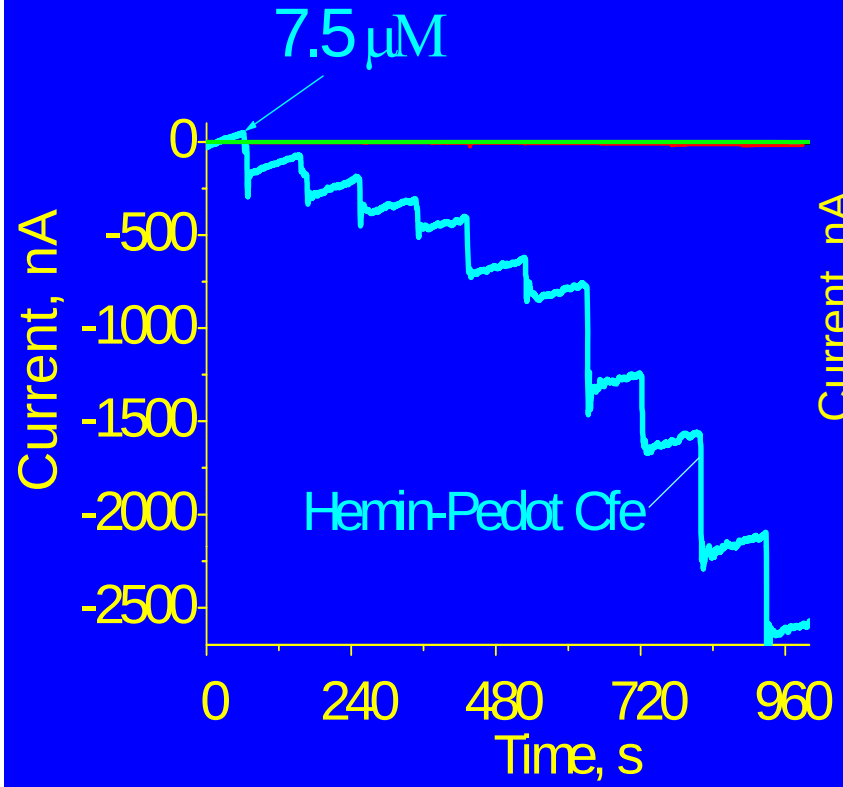
PEDOT *vs* Polypyrrole

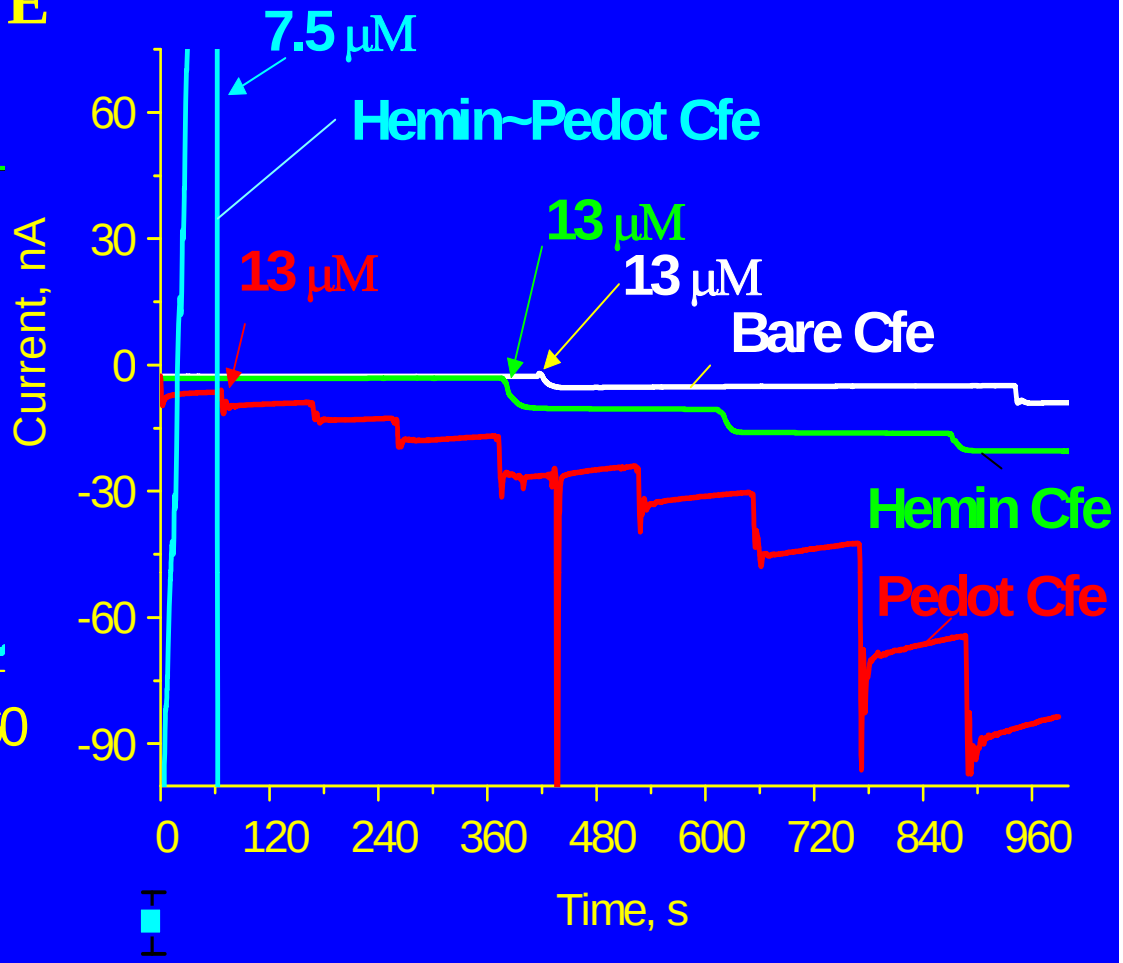
- Higher electrochemical stability
- Better conservation of conductivity, charge
- Higher ionization potential (5 eV *vs* 4 eV)
- Better protection => O_2 oxidative damage

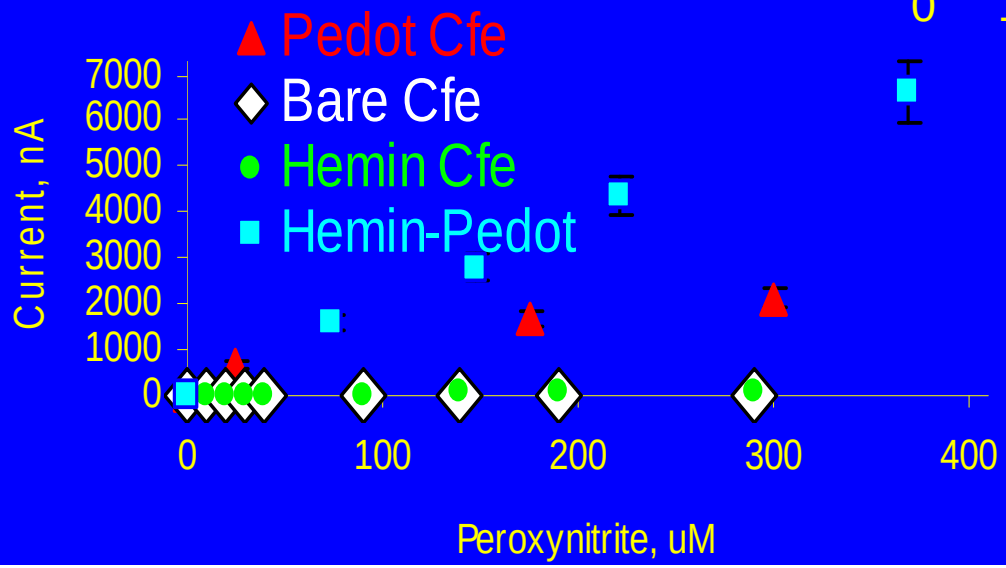


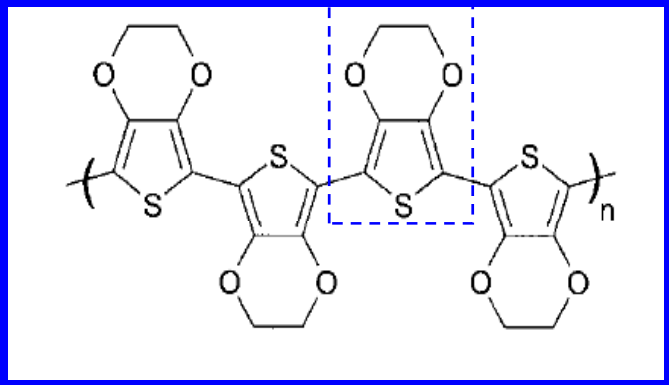
[Peteu, Bayachou,et al 2006; 2010]

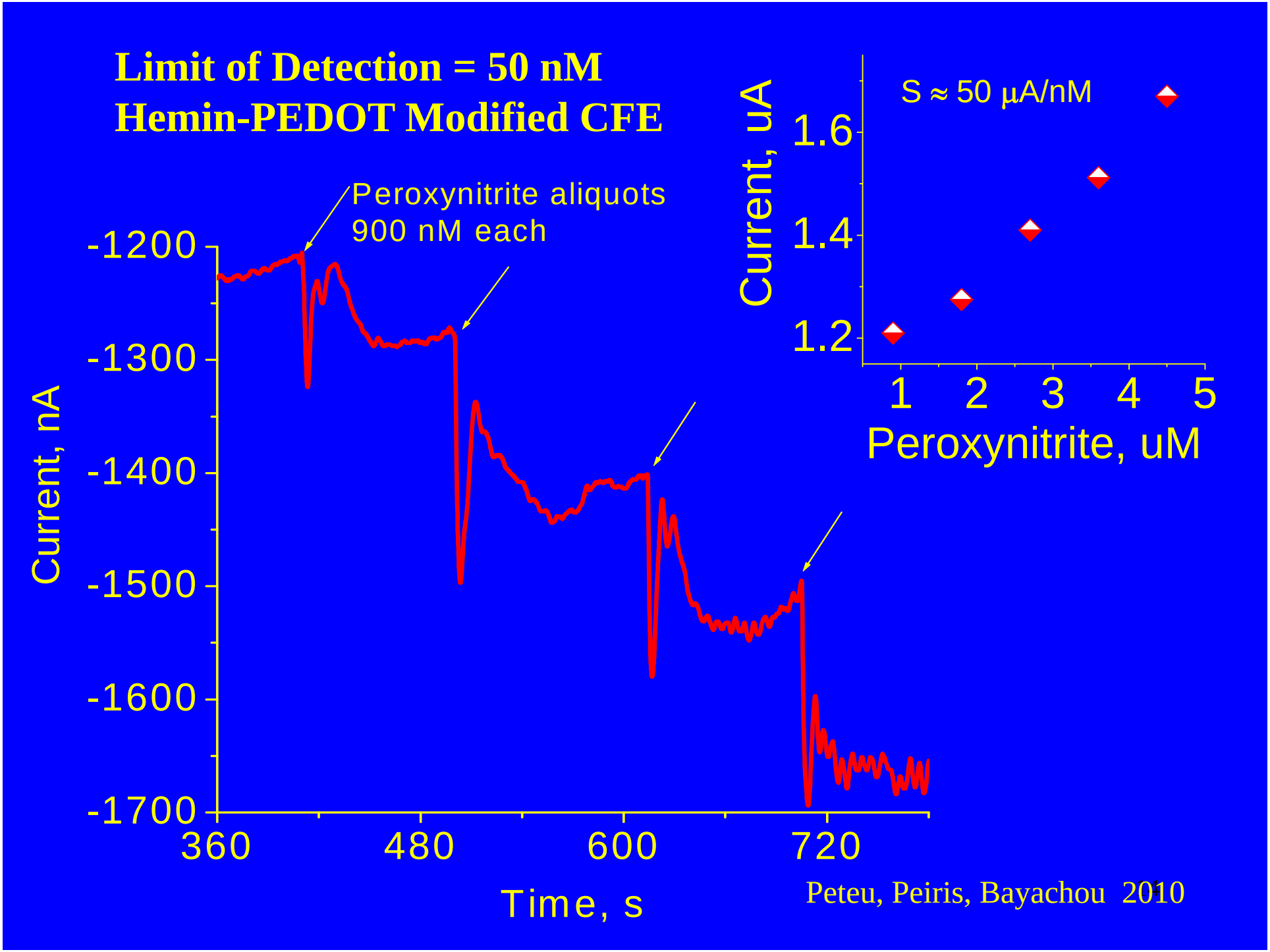
Hemin-PEDOT Modified CFE



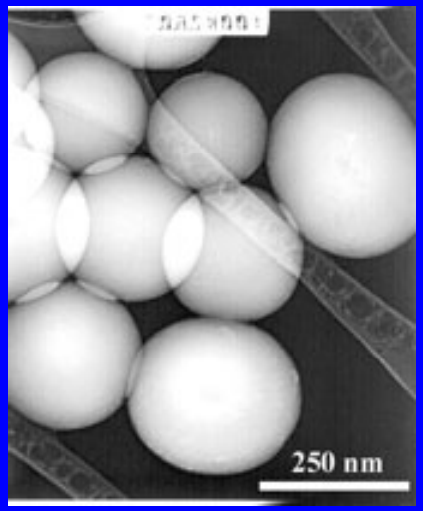


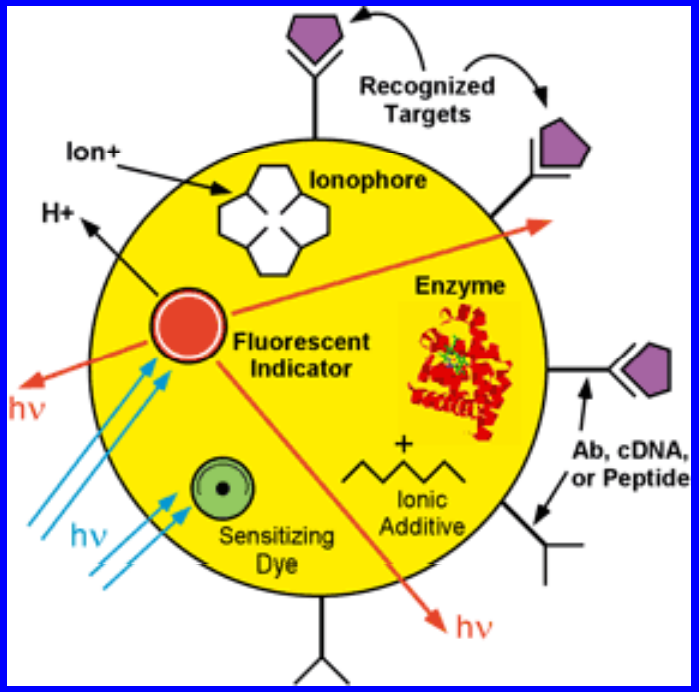


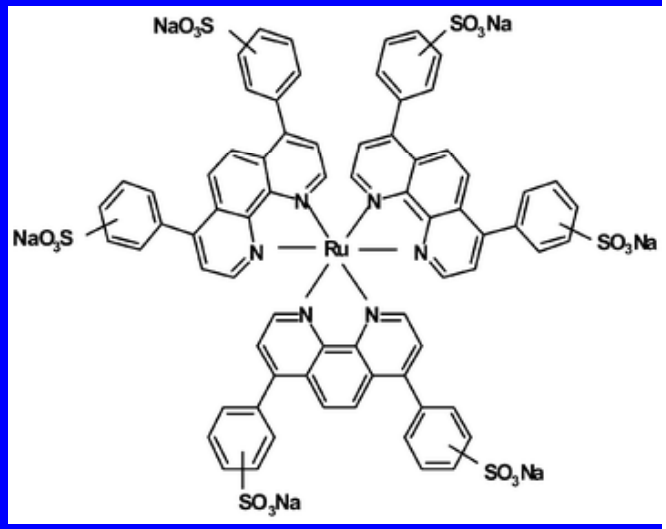



Limit of Detection = 50 nM
Hemin-PEDOT Modified CFE
Peroxynitrite aliquots
900 nM each
Current, nA
-1200
-1300
-1400
-1500
-1600
-1700
360
480
600
720
Time, s
Current, uA
1.6
1.4
1.2
S ≈ 50 µA/nM
1
2
3
4
5
Peroxynitrite, uM
Peteu, Peiris, Bayachou 2010

Optochemical Fiberless NanoBiosensors for Glucose, NO, pO_2







- **Fluorescent nanobiosensor, matrix materials and options**
- **Sizes range from 20 to 200 nm in diameter**

Kopelman Laboratory, the University of Michigan

Future outlook

- Measure release of neurochemicals from single neurons
- New smaller, smarter and faster sensors
- New multi-analyte sensor arrays
- Novel sensors for new neuro-chemicals of interest
- New clinical applications