REAGENTLESS HAND-HELD REAL-TIME EVANESCENT OPTICAL CHEM-BIO DETECTION USING BIOMIMETIC RECEPTOR AND LIGAND NANOSURFACES

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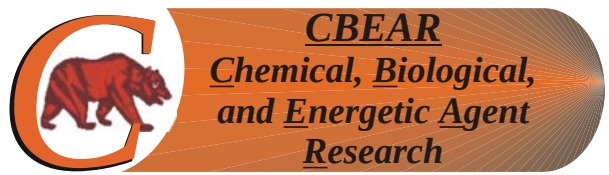
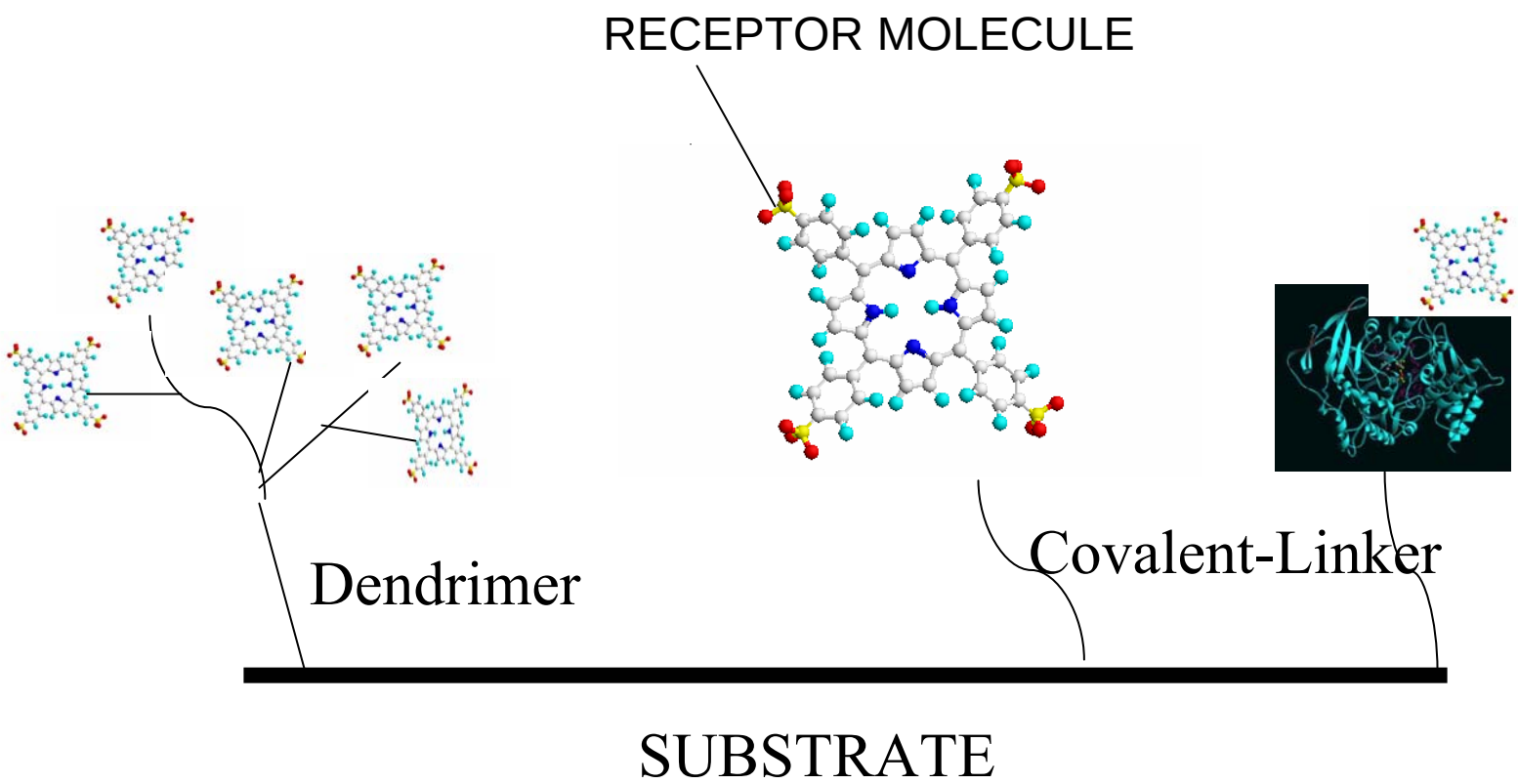
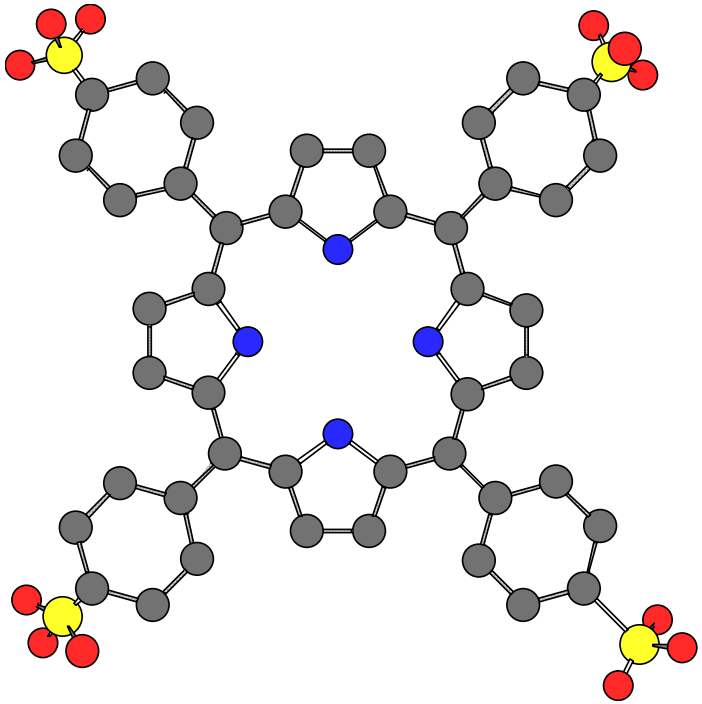


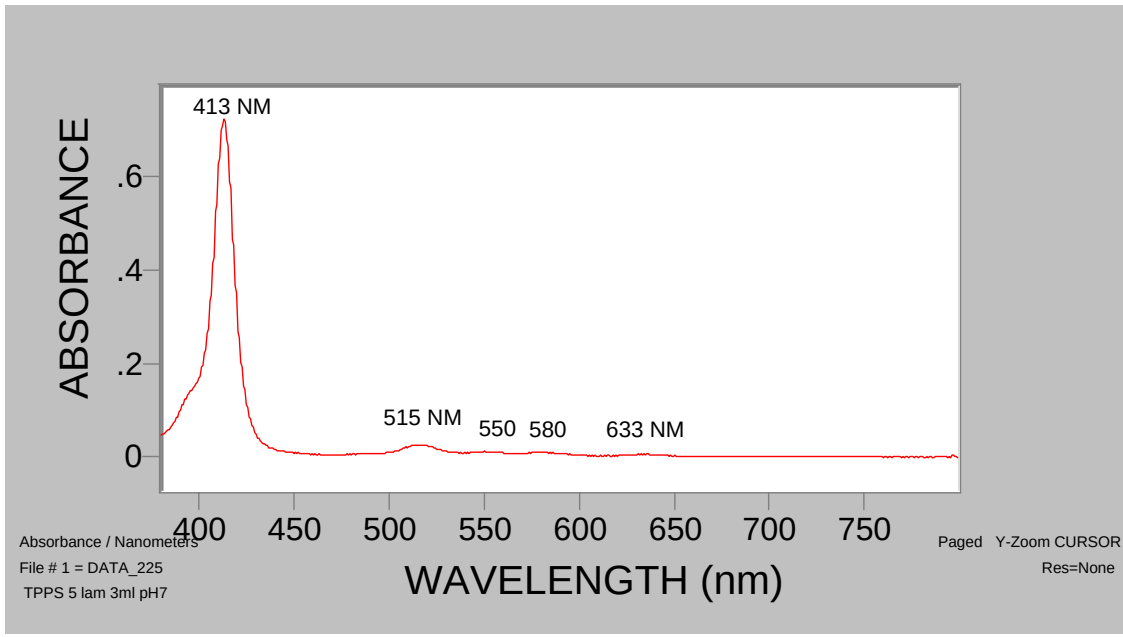
"Some days you eat the bear; some days the bear eats you!"

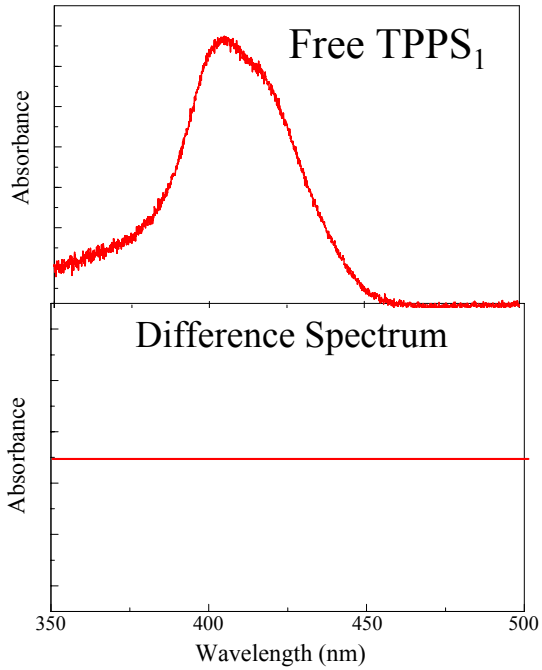
Chemical Structure of Surface Monolayer

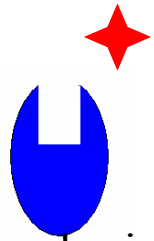




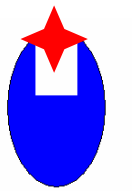
Porphyrin? What's that?



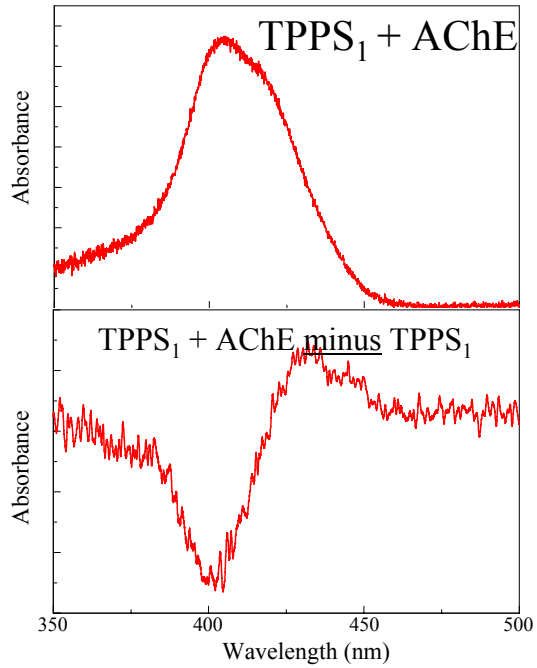


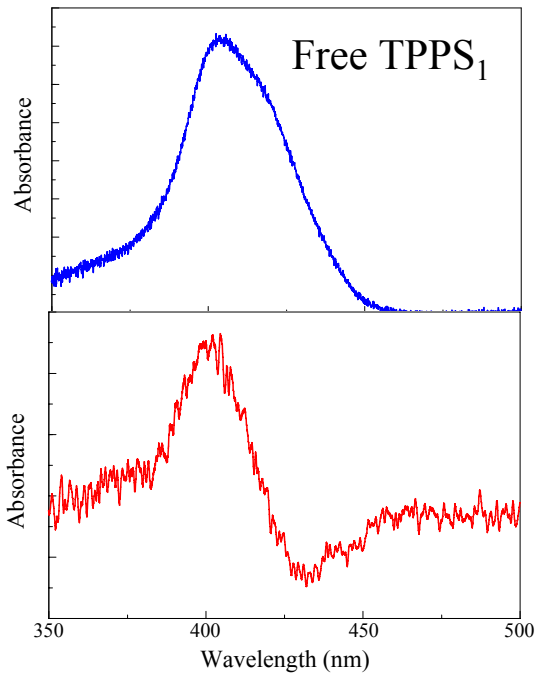


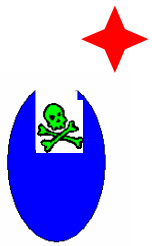
1) Porphyrin in solution



2) Formation of $TPPS_1$-AChE complex



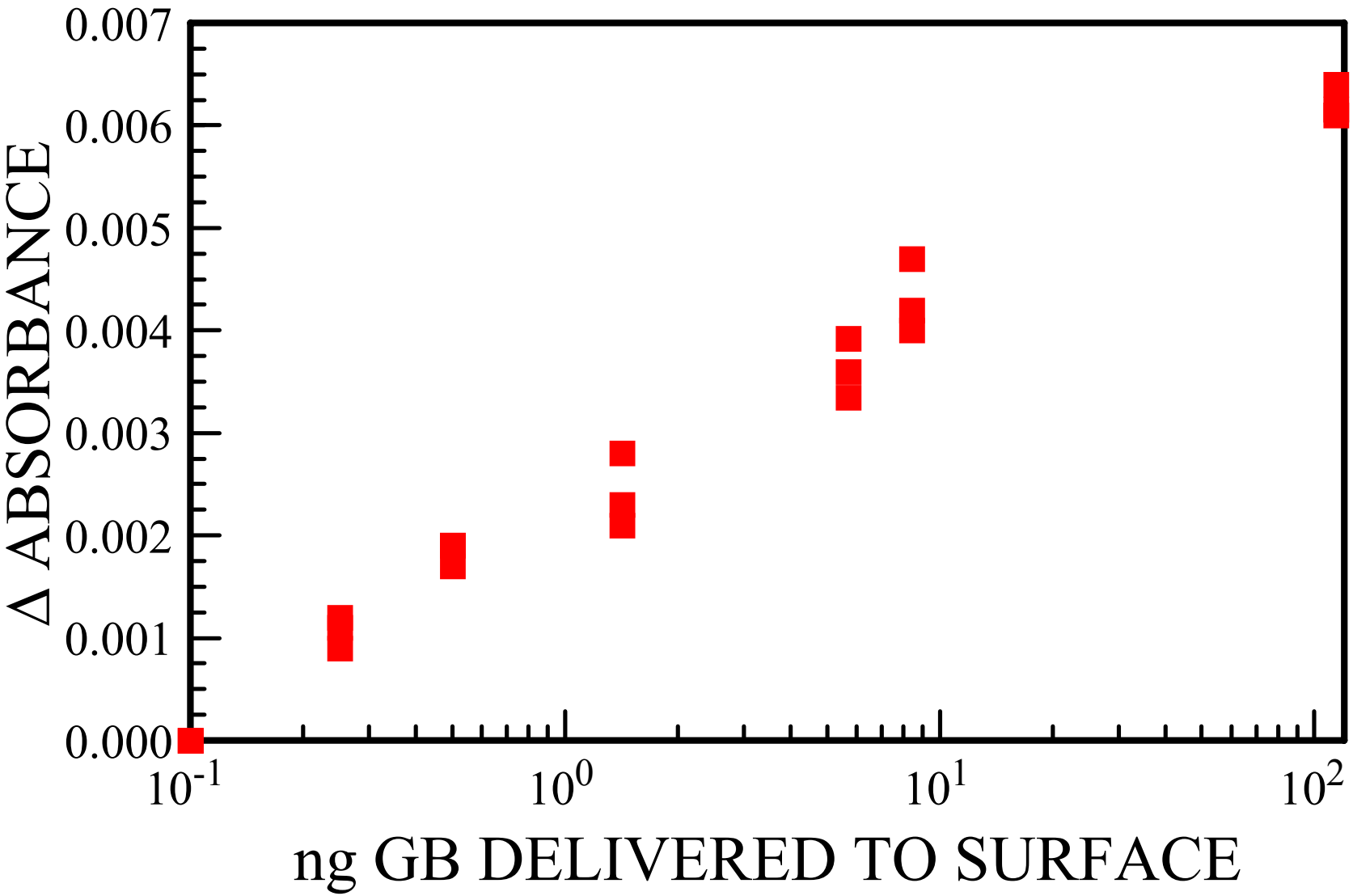




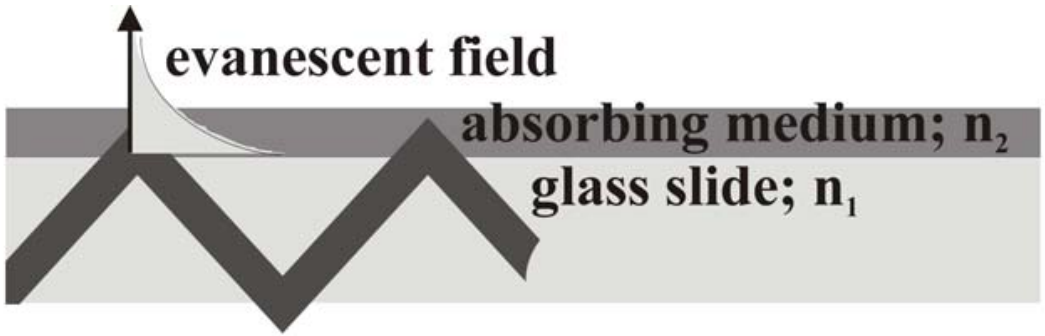
3) Dissociation of $TPPS_1$AChE Complex

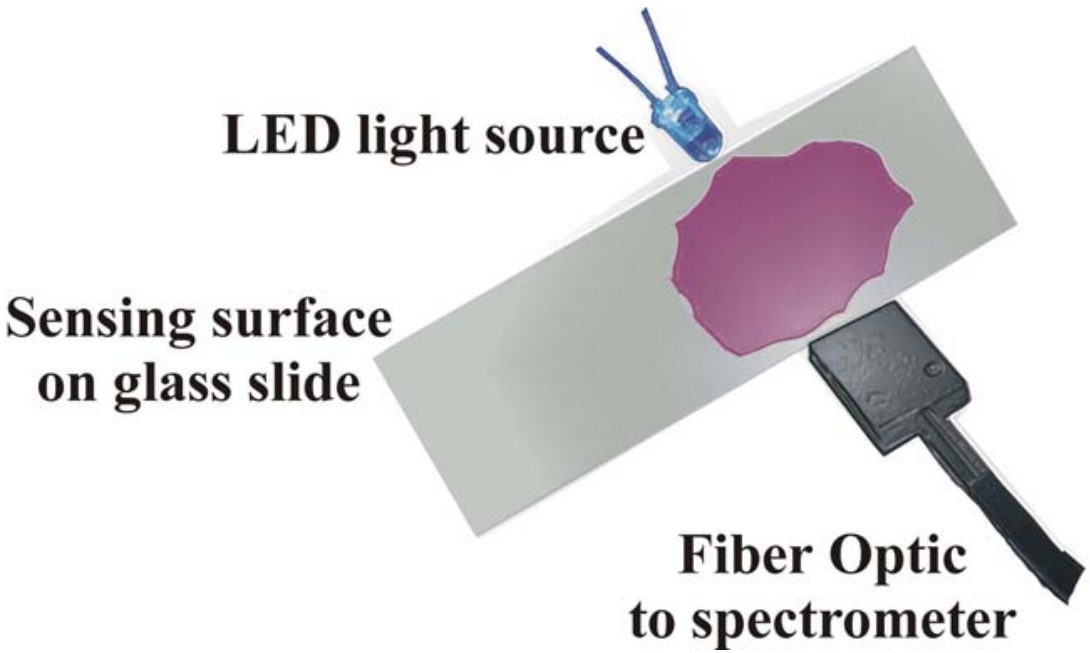
$TPPS_1$-AChE + Inhibitor <u>minus</u> $TPPS_1$-AChE

RESPONSE OF AChE SURFACE TO AEROSOL GB



EVANESCENT ABSORBANCE MEASUREMENT





WHAT CAN WE DETECT?

- Cyanide (1 ppb gas or 1 μg/l; 10 ppm upper limit)
- Sarin (0.1 ng/liter liquid, > 10 ng/liter upper limit; 25 ng gas, >10 μg upper limit)
- Dipicolinic acid found in anthrax exospore (1.5 ppb solution, 250 ppb upper limit)
- Pentachlorophenol (1 ppb in solution; 1 ng/liter)
- Paraoxon (0.007 ppb in solution, 10 ppb upper limit)
- Diazinon (0.01 ppb in solution, >10 ppb upper limit)
- HD vapor (0.1 ppb; >10 ppb upper)
- Benzene, naphthalene, hydrazine, formaldehyde and other TICs.
- Ozone
- CO_2 (0.1% or 1000 ppm LOD gas)

WE DON'T USE

- Antibodies
- PCR, DNA, or RNA
- Primers, aptamers
- Buffers, substrate solutions
- Secondary enzymatic reactions
- Preconcentrator (It is one itself)

HOW FAST CAN WE DETECT?

In many cases, less than

1 second.

HOW SENSITIVE IS IT?

Most published sensitive measurement so far is

7 ppt (0.007 ppb)

We can do 30-fold better.

LIFETIME?

- Porphyrin surfaces are useable for

OVER 4 years

without special storage (room, unsealed)

- Enzymatic surfaces are useable for

>480 days sealed

(and counting)

Current Functional Modular Prototype

NEW GENERATION BIOMIMETIC BIOSENSOR

- Instead of binding a specific enzyme, we bind a specific protein or molecule that will bind the cell, virus, toxin, or whatever.
- After that, everything is the same.
- Same hardware
- Same slides, different coating material!
- Different proteins and/or porphyrins (we buy)

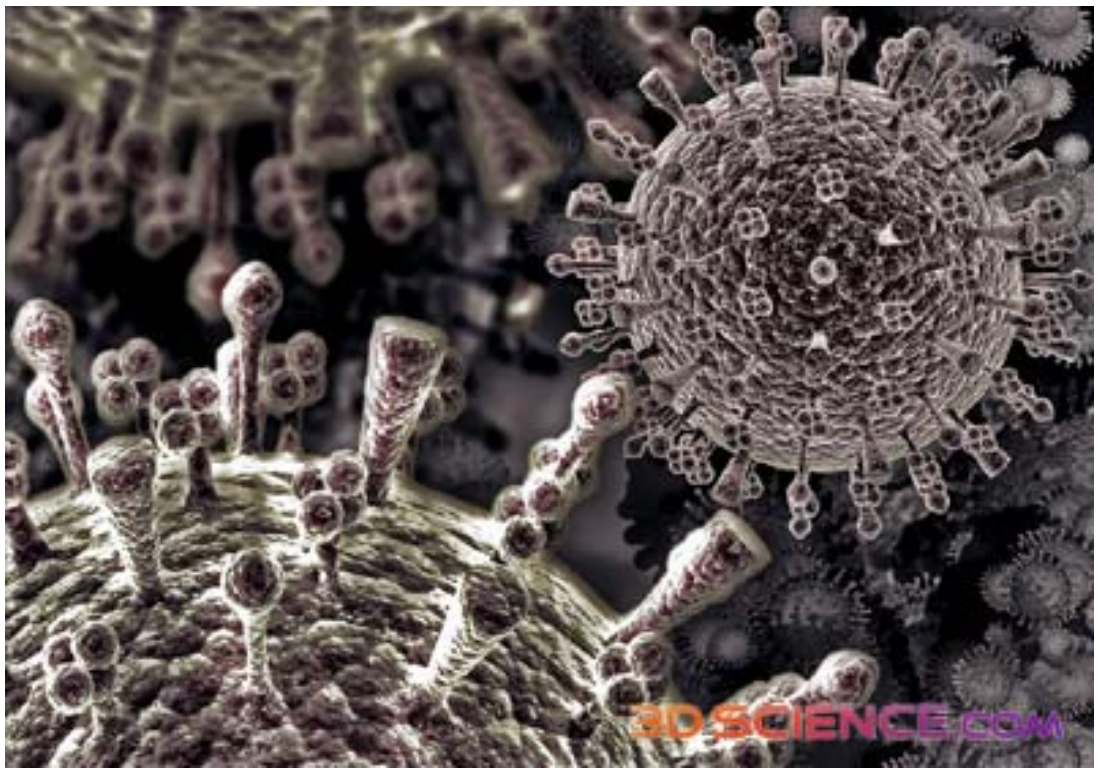
NATURE HAS ALREADY SOLVED THE PROBLEM!

WHAT CAN BE DETECTED WITH THIS BIOSENSOR?

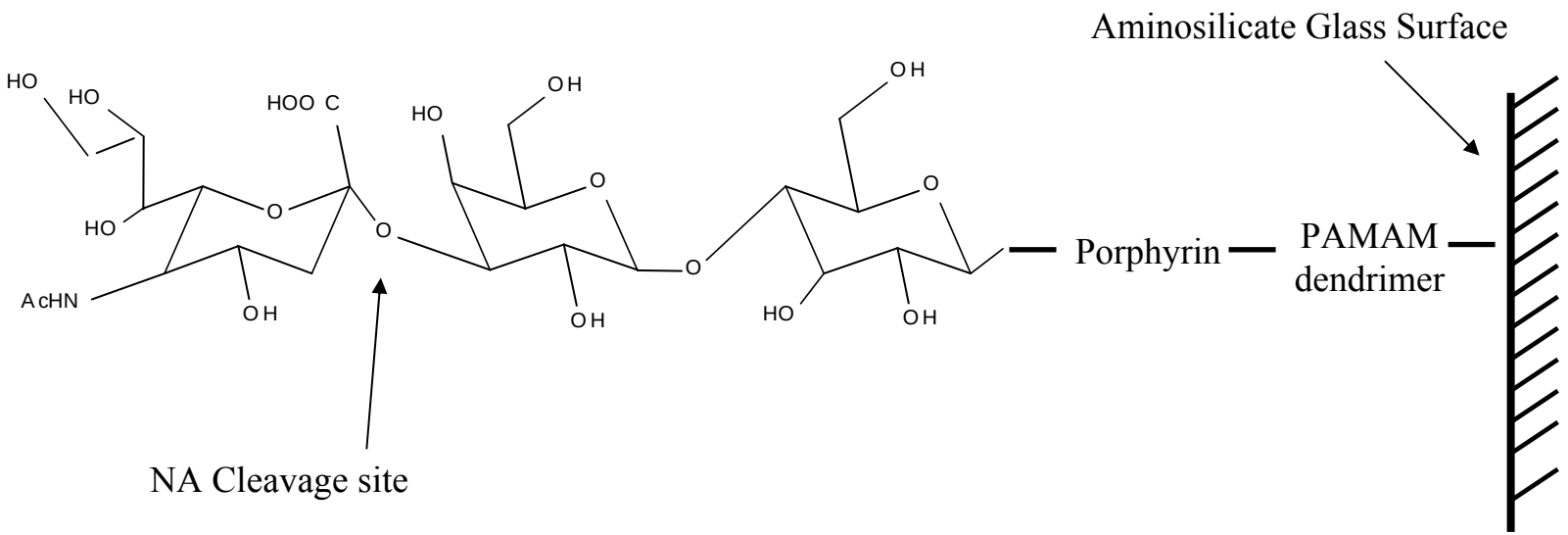
- PSA (prostate specific antigen)
- Several lymphomas secrete a carbohydrate called “T-antigen” into the blood stream which can be used as an indicator of some cancers.
- Ricin can be bound by ConA and other lectins.
- Cholera toxin; other toxins as well.
- Influenza virus; other viruses.
- Bacteria of all sorts.

Influenza Virus

- Contains two glycoproteins
  - Hemagglutinin (HA) binds sialic acid
  - Neuraminidase (NA) cleaves sialic acid

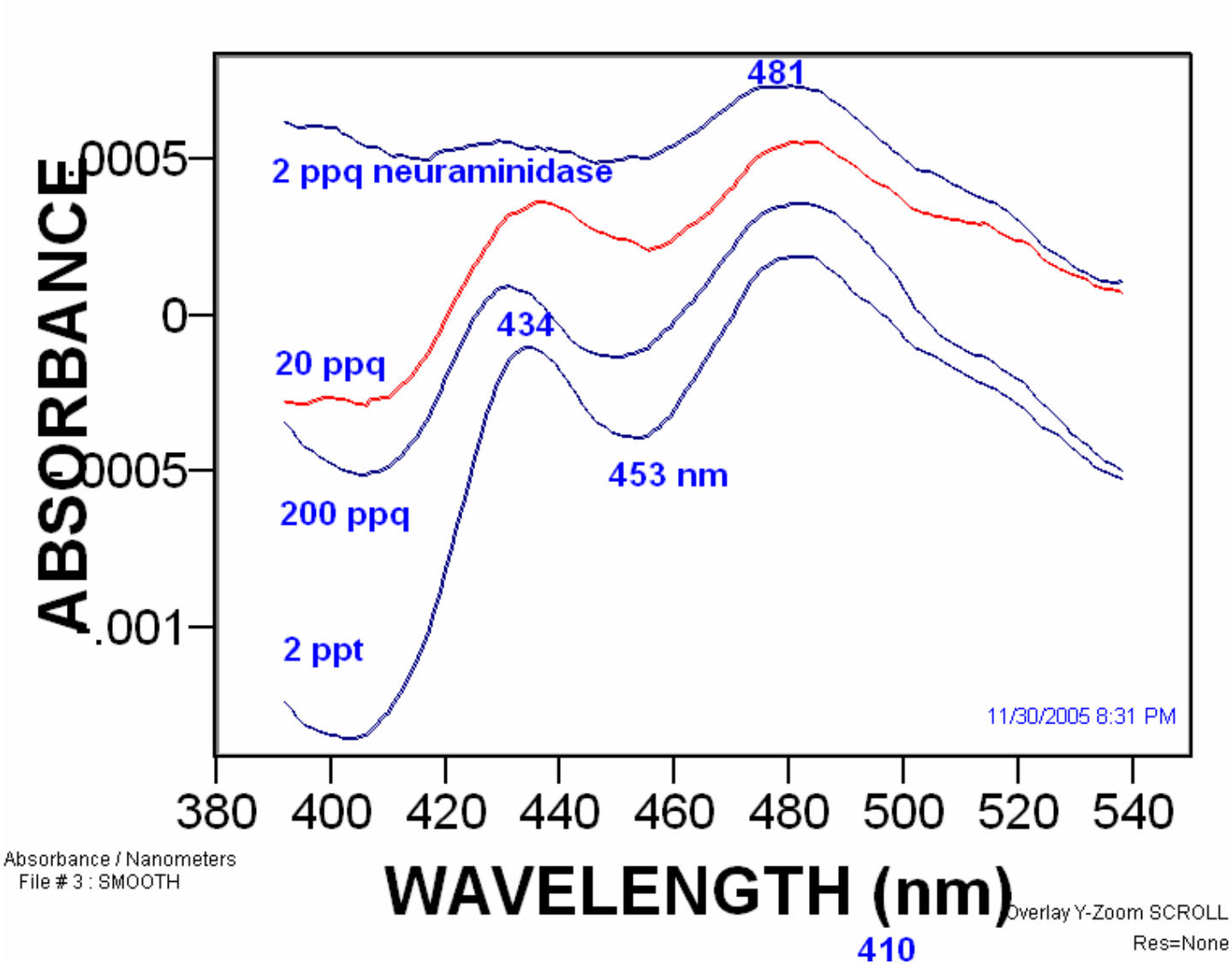


For the detection of influenza a sialic acid-porphyrin derivative is covalently immobilized.

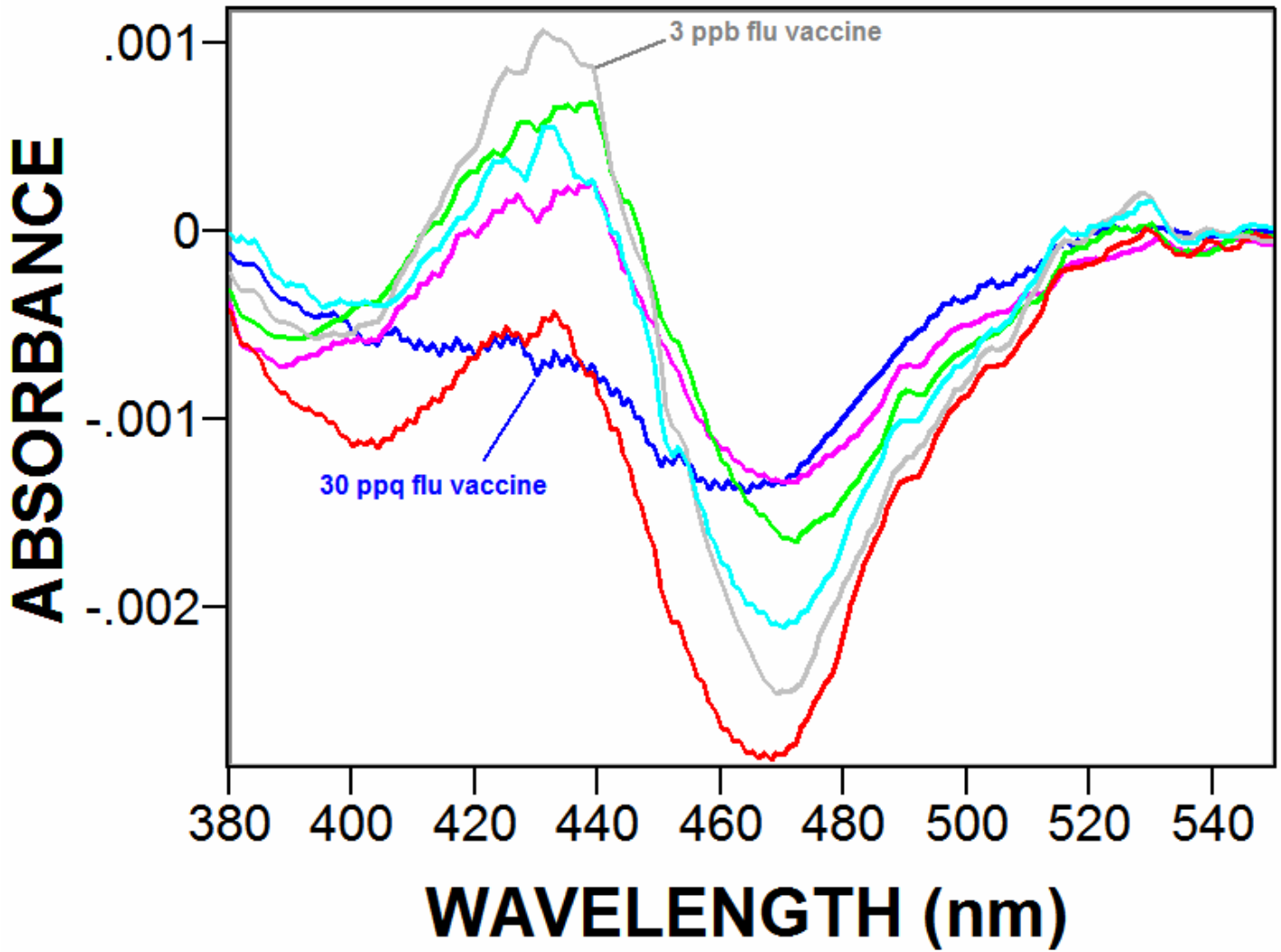


Cleavage of the sialic acid will alter the electron density distribution and thus the spectrum of the porphyrin.

Difference spectra of porphyrin slides exposed to NA



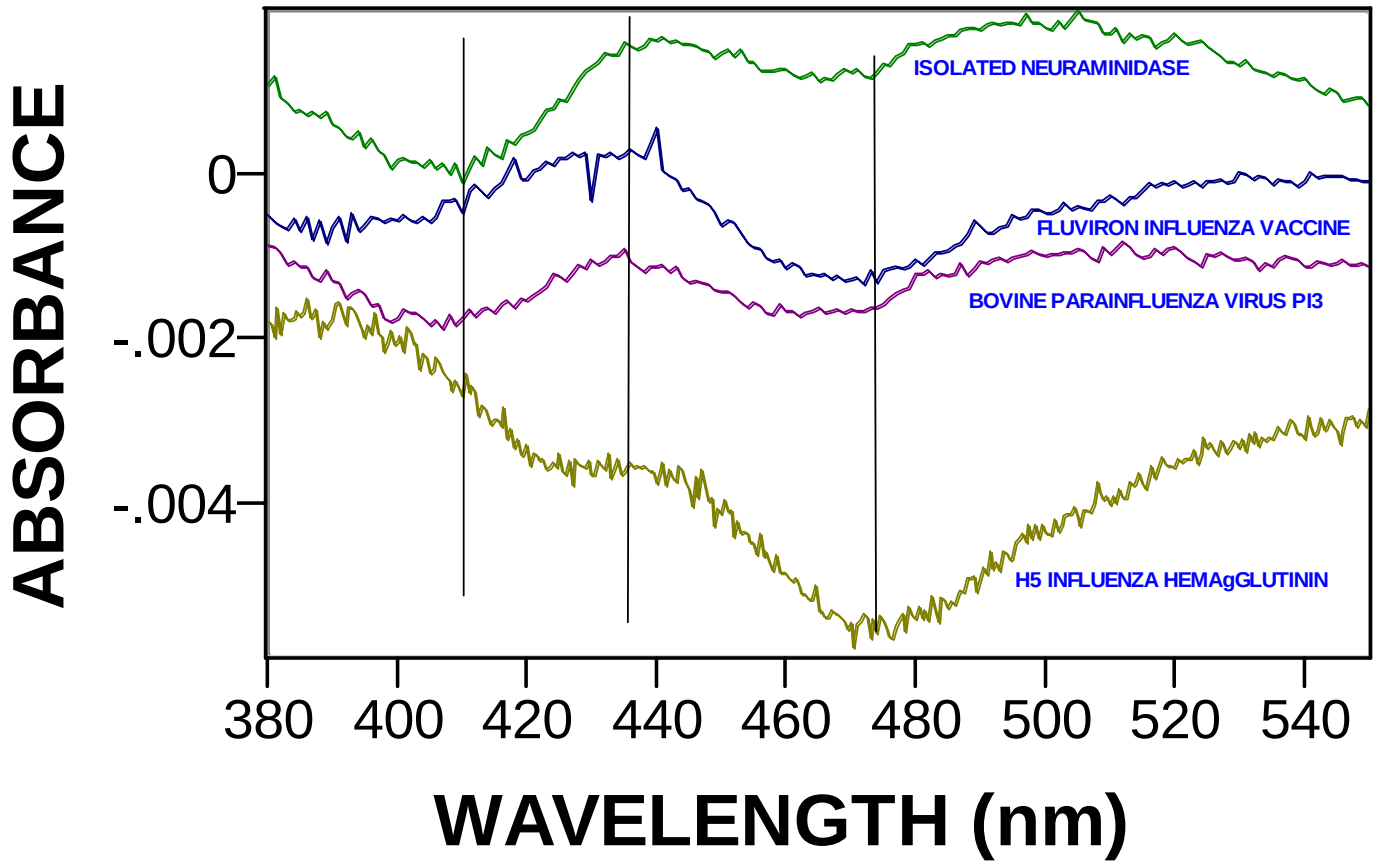
Difference spectrum of porphyrin slides challenged with human influenza virus



How much is this, REALLY?

- 30 ppq (0.03 pptrillion) is about 5×10^{-17} M neuraminidase
- This is about 30×10^{6} NA/liter
- In our sample, this is about 6000 NA molecules.
- The average virus surface has about 400 NA molecules. We can bind HA, too!
- If we could bind ALL the NA on a virus, this means we had 15 virus in our sample.

THE SPECTRAL RESPONSE IS DIFFERENT AND SPECIFIC FOR DIFFERENT VIRUS AND ANTIGENS!



WE CAN DETECT:

Our sensors can detect:

- T- antigen 100 ppt
- Cholera Toxin at 100 ppq
- Human influenza at approx. 15-50 virus/ml

AND we can distinguish between Human and Avian and bovine parainfluenza!

(Still, don't smear chickens on your face!)

WHERE DO WE GO FROM HERE?

We have the protocols to detect:

- Shigella
- Neisseria
  - Meningitis
  - Gonorrhoea
- Listeria
- Staph
- Strep
- Rabies
- Polio
- Ebola
- Dengue
- SARS
- RSV
- Norwalk Virus

And more!

What Can Be Used as Receptors?

- Carbohydrates
- Lipids
- Nucleic acids
- Proteins (Your genetically engineered protein here!)
- Phage!

ADDITIONALLY

- The surfaces can be archival
- Live vs dead microbes and intact vs fragments can be determined (that is a whole different additional presentation)
- Intact vs fragments of:
  - Microbes
  - Spores
  - Virus

FALSE POSITIVES?

Biological False Positives can be caused by:

- –Similarly reactive biological entities (similar to cross-reactivity of antibodies); choose your molecules wisely and use multiple receptors with LOGIC!
- –Fragments of the biological entity
- –Dead Cells

False positive rate is that expected using antibody-based assays.

BARRIERS TO READINESS?

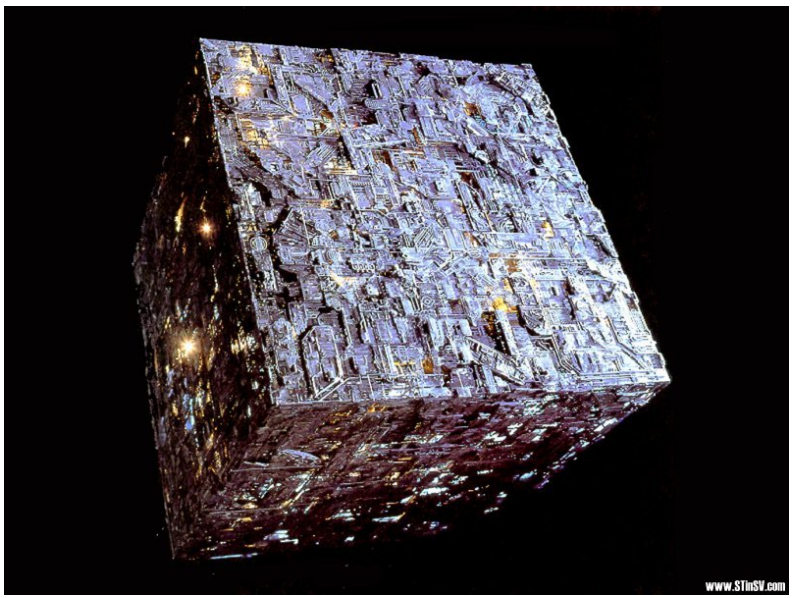
- 4 PROTOTYPES already exist and have been used since 2001.
- We do not use **Simulants** since the "normal" simulants such as MPA, DMMP, etc have ***NO*** effect on our enzyme-based sensors simply because the "simulants" are non-toxic to the enzyme and do not affect the cholinesterases. MPA, DMMP, etc are simulants on the basis of physical properties, not physiological/toxicological properties.
- This means that testing of the sensors must involve "real" toxic or somewhat toxic agents.
- And that costs $$$$$ since we must measure effects of:
  - Concentration dependence
  - Temperature
  - pH
  - Humidity

Testing must be done for each agent; the cost and time of testing presents a barrier for any new technology.

Some have told me directly "Nobody can do this". My response" "Almost nobody".

Thank You for trying to pay attention!

These technologies are patented or pending and available for licensing.



Resistance is futile. You will be assimilated.

CBEAR
Chemical, Biological, and Energetic Agent Research

"Some days you eat the bear; some days the bear eats you!"