Conformationally Adaptive Biosensors

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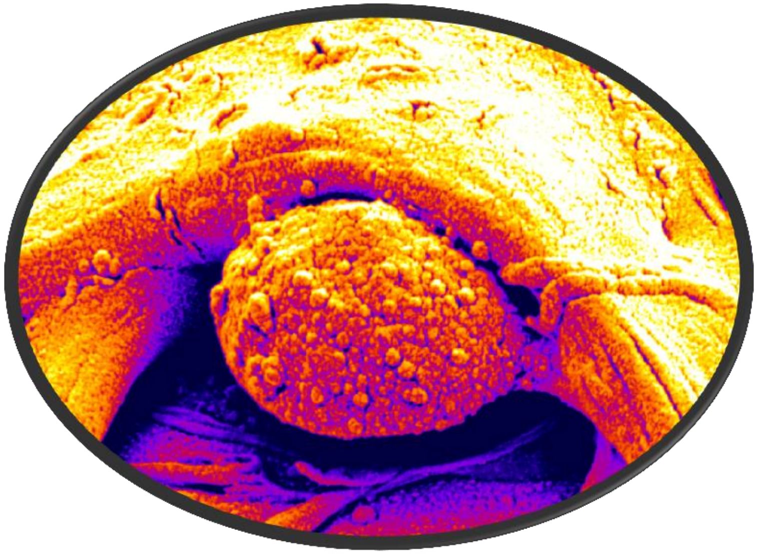
Croatia - February 22, **2018.**

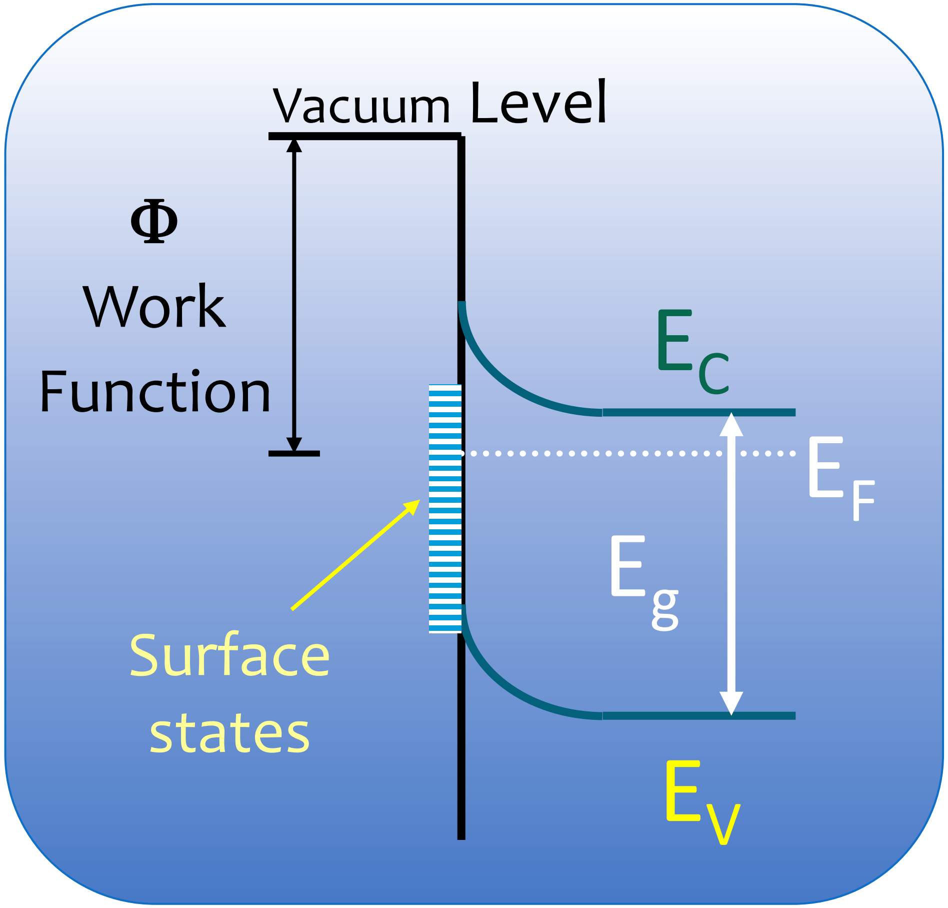
Molecular Conformations Role in Electrical Biosensing

- ✓ **Molecule - Semiconductor**
- ✓ **Small Molecules**
- ✓ **Enzymes**
- ✓ **Ions**



One Electron Energy Level Diagram

n-Type Semiconductor



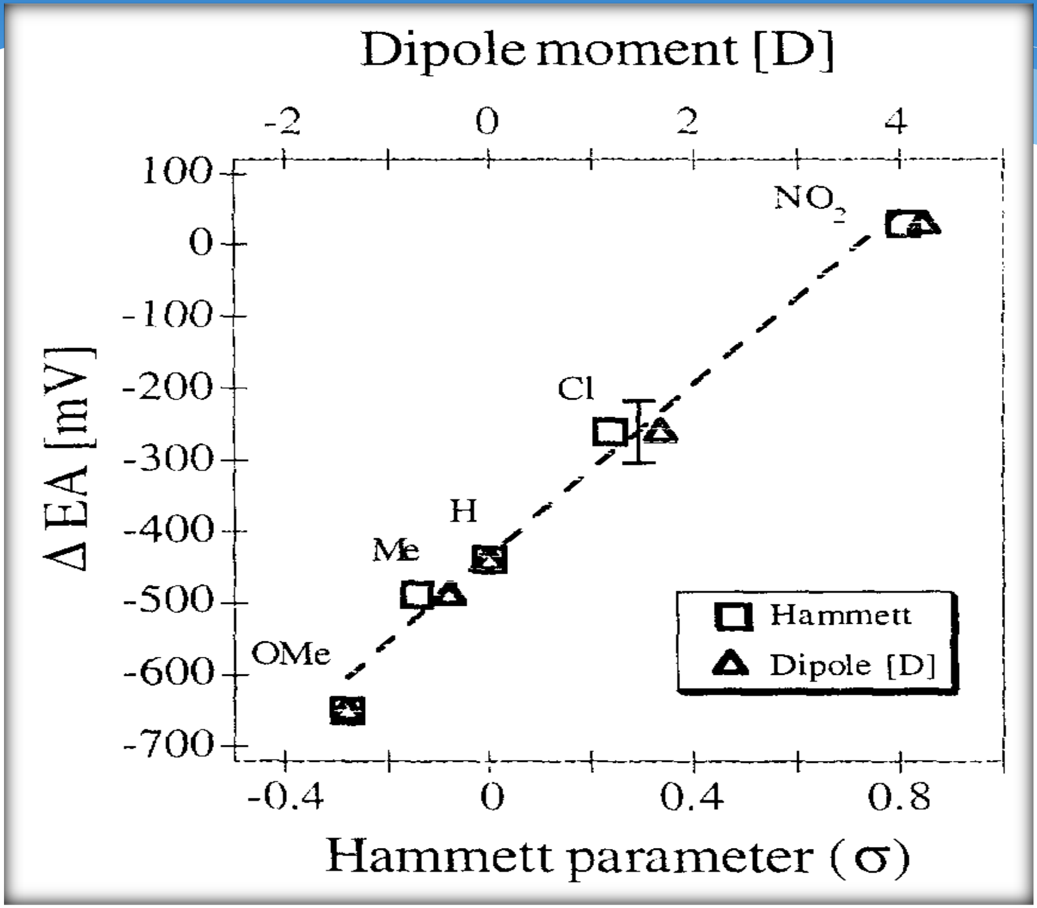
EA
Electron Affinity

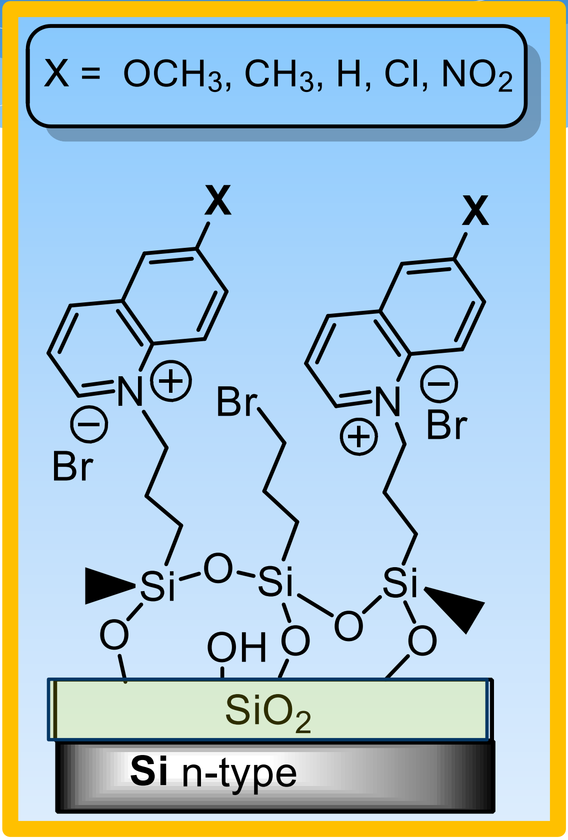
BB
Band Bending

$$\Phi = EA + BB + (E_C - E_F)$$

$$\Delta\Phi = \Delta EA + \Delta BB$$

Silicon – molecule electronic interface

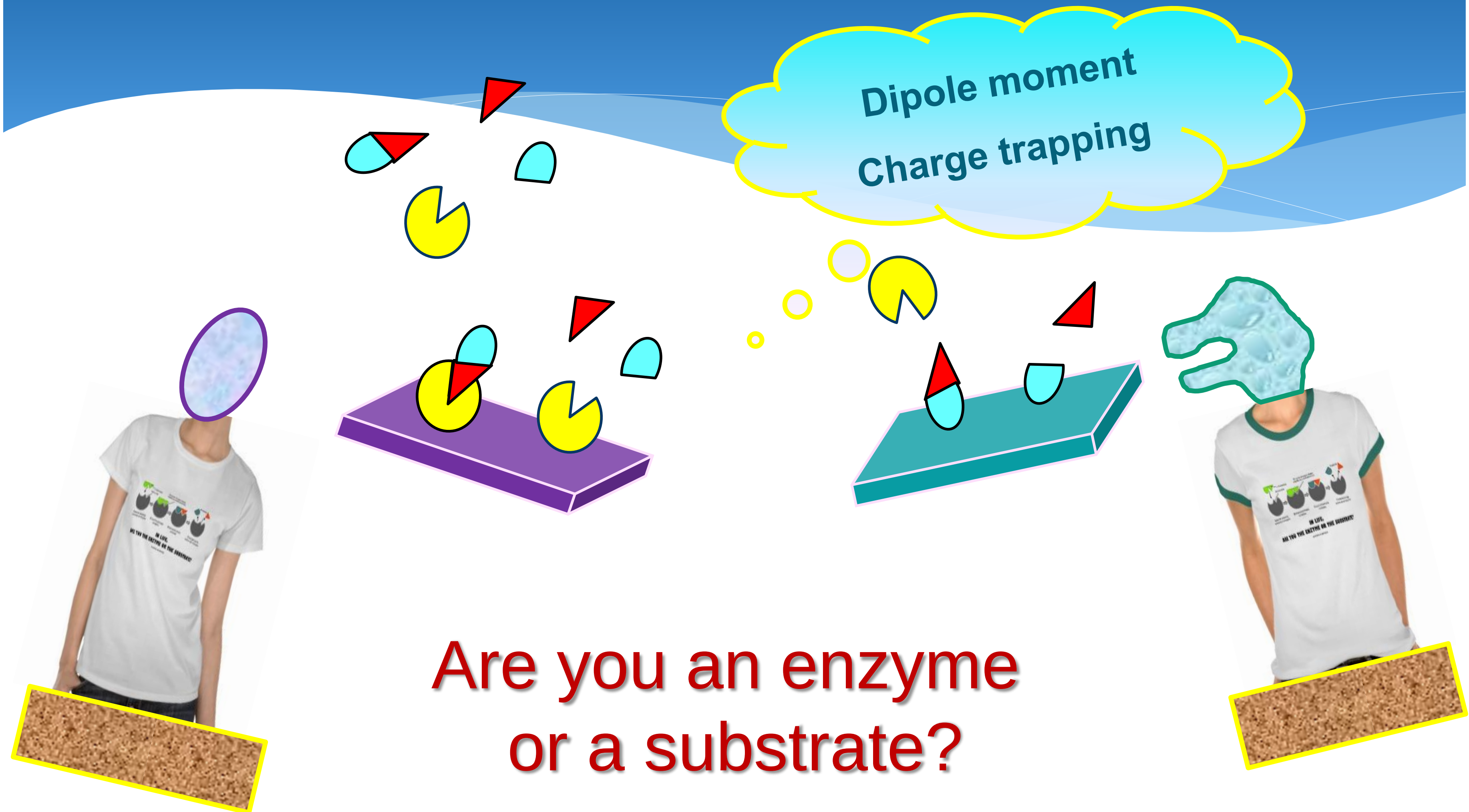




Chem. Phys. Lett. ***1997***
J. Phys. Chem. C **2010**

J. Am. Chem. Soc. **2008**

Molecular Transducers Effect

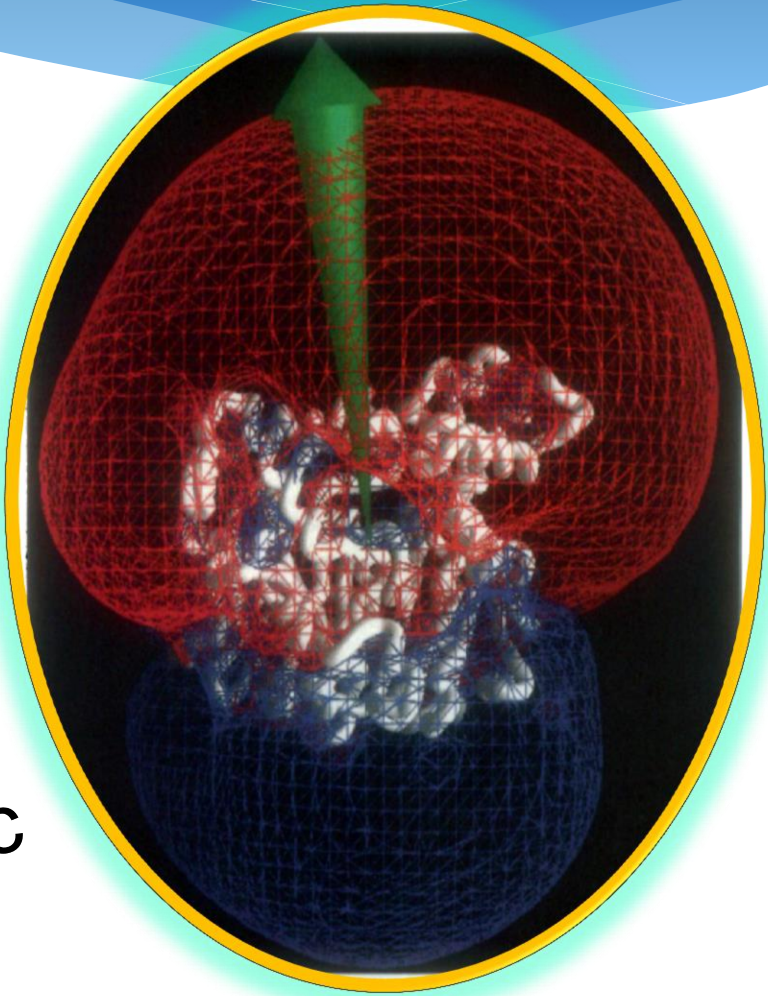


Are you an enzyme or a substrate?

Biosens. Bioelectron. **2009**, *24*, 2384
IEEE Sens. J., **2011**, 11, 2007-2015

Sensing strategy for biorecognition

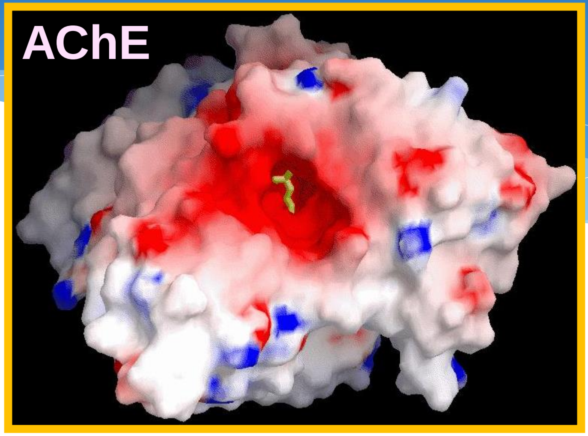
- Dipole moment
- Depolarization
- Shielding

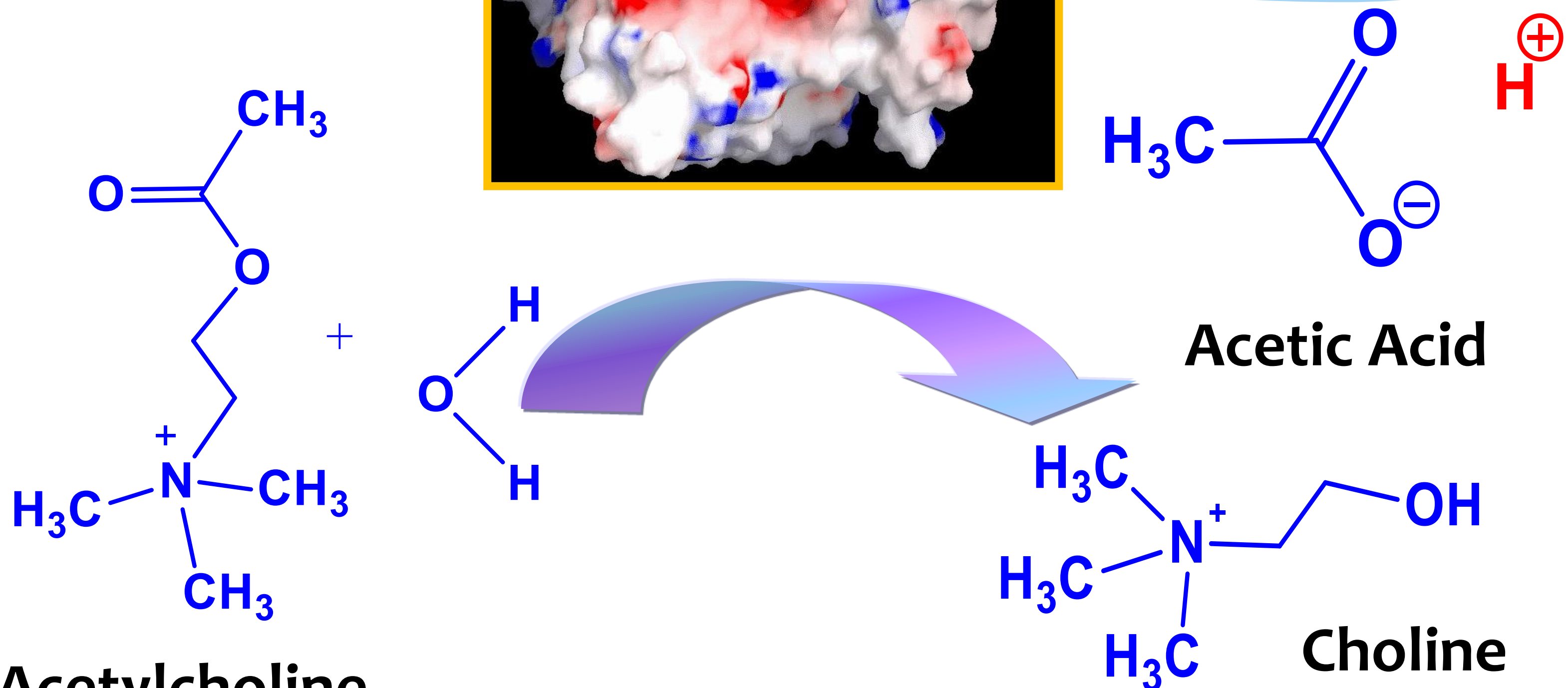


AChE ➔ [ES] ➔ Ch + Ac

$\mu \approx 1200$ D

AChE biocatalytic activity



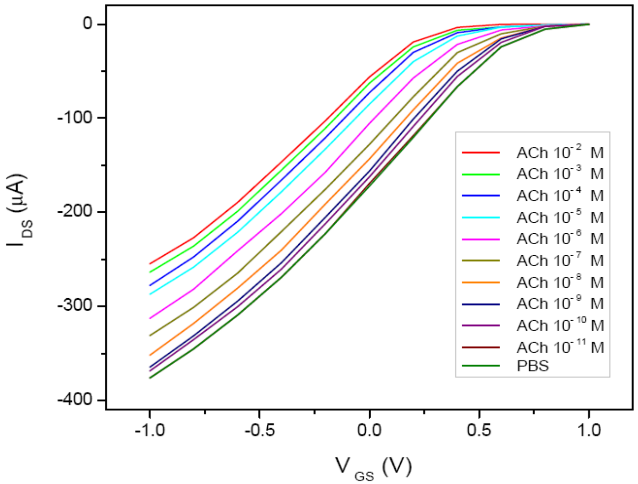


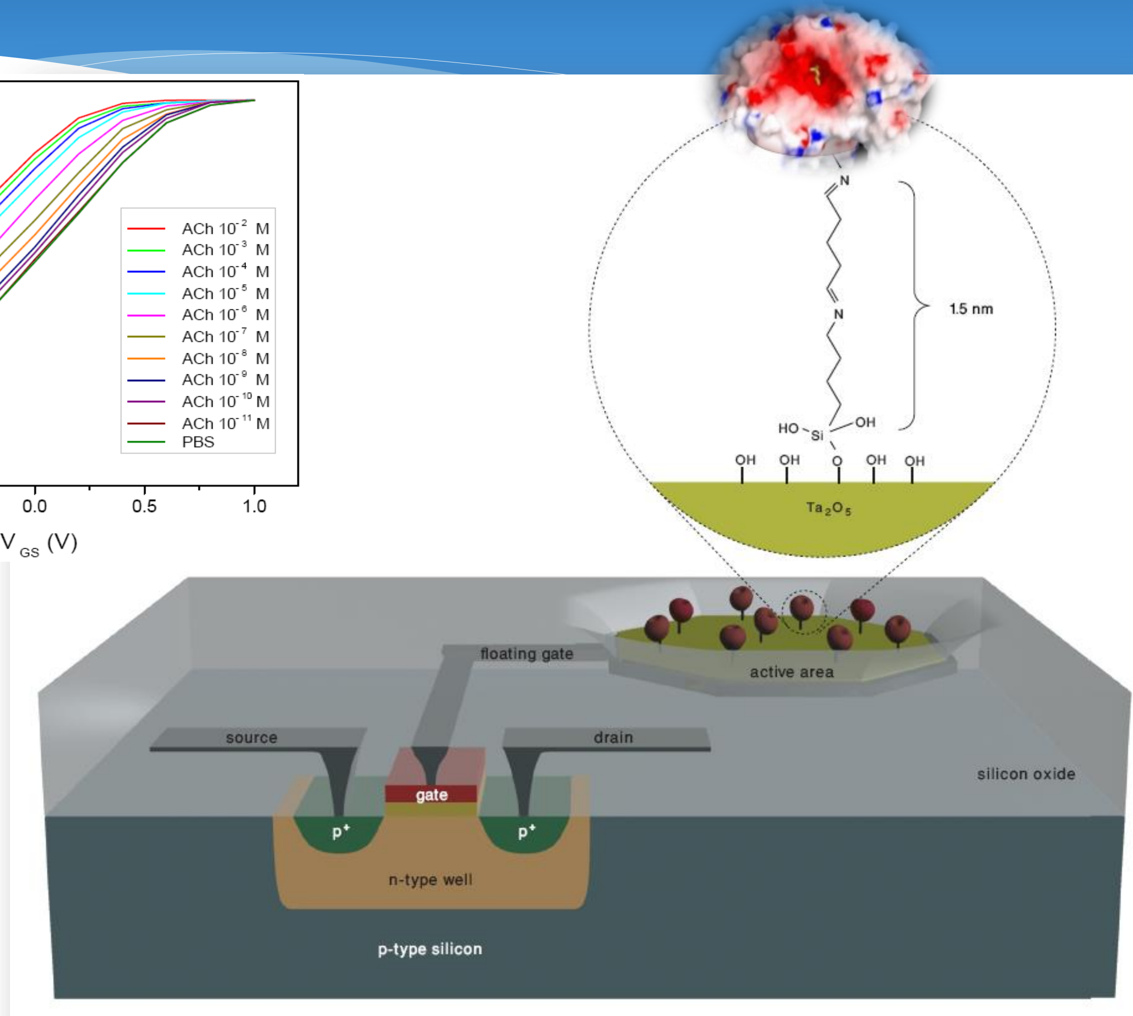
Acetylcholine

Acetic Acid

Choline

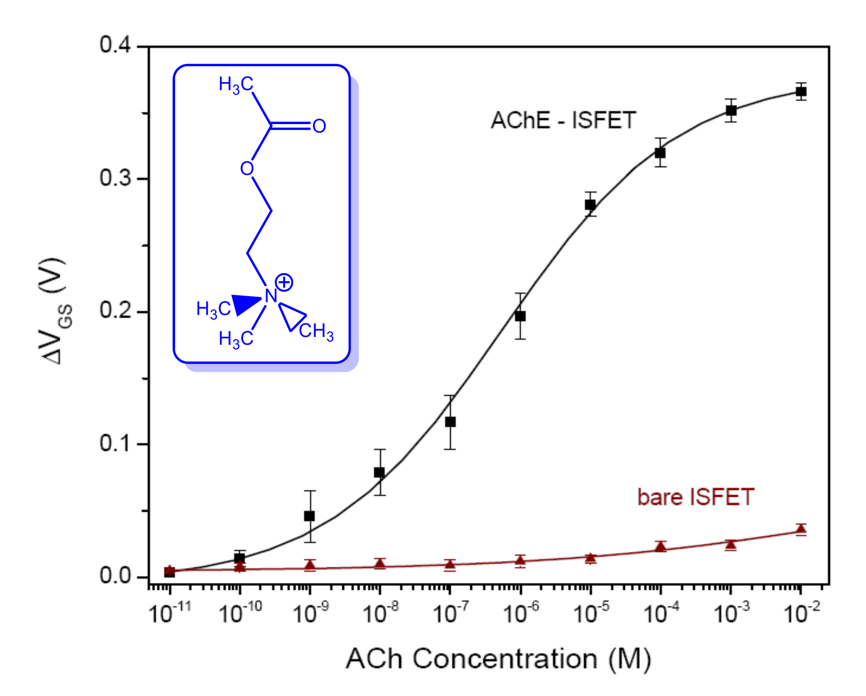
AChE - floating-gate EnFET

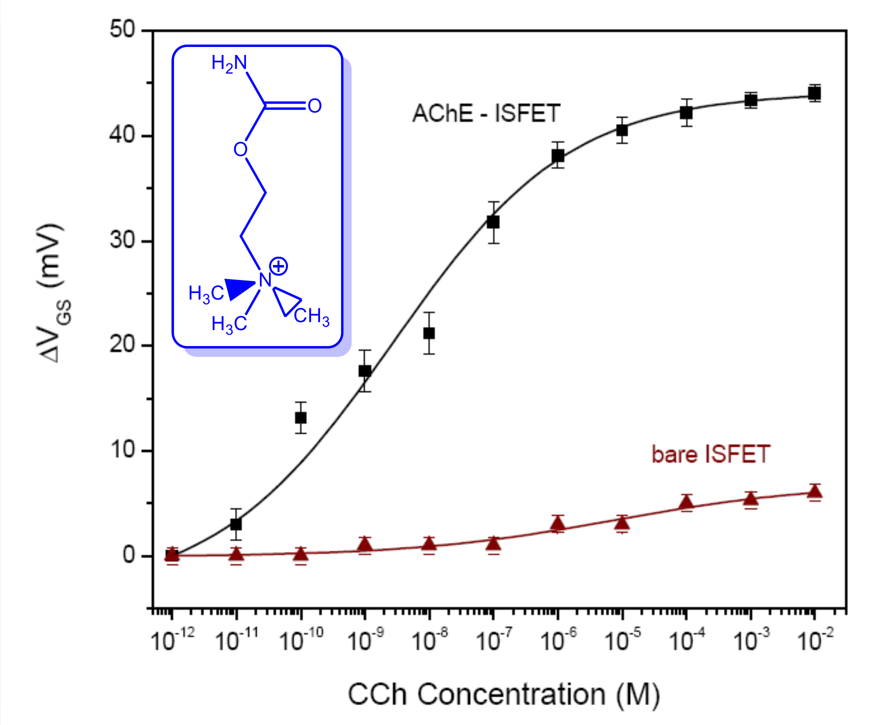




EnFET dose response to ACh

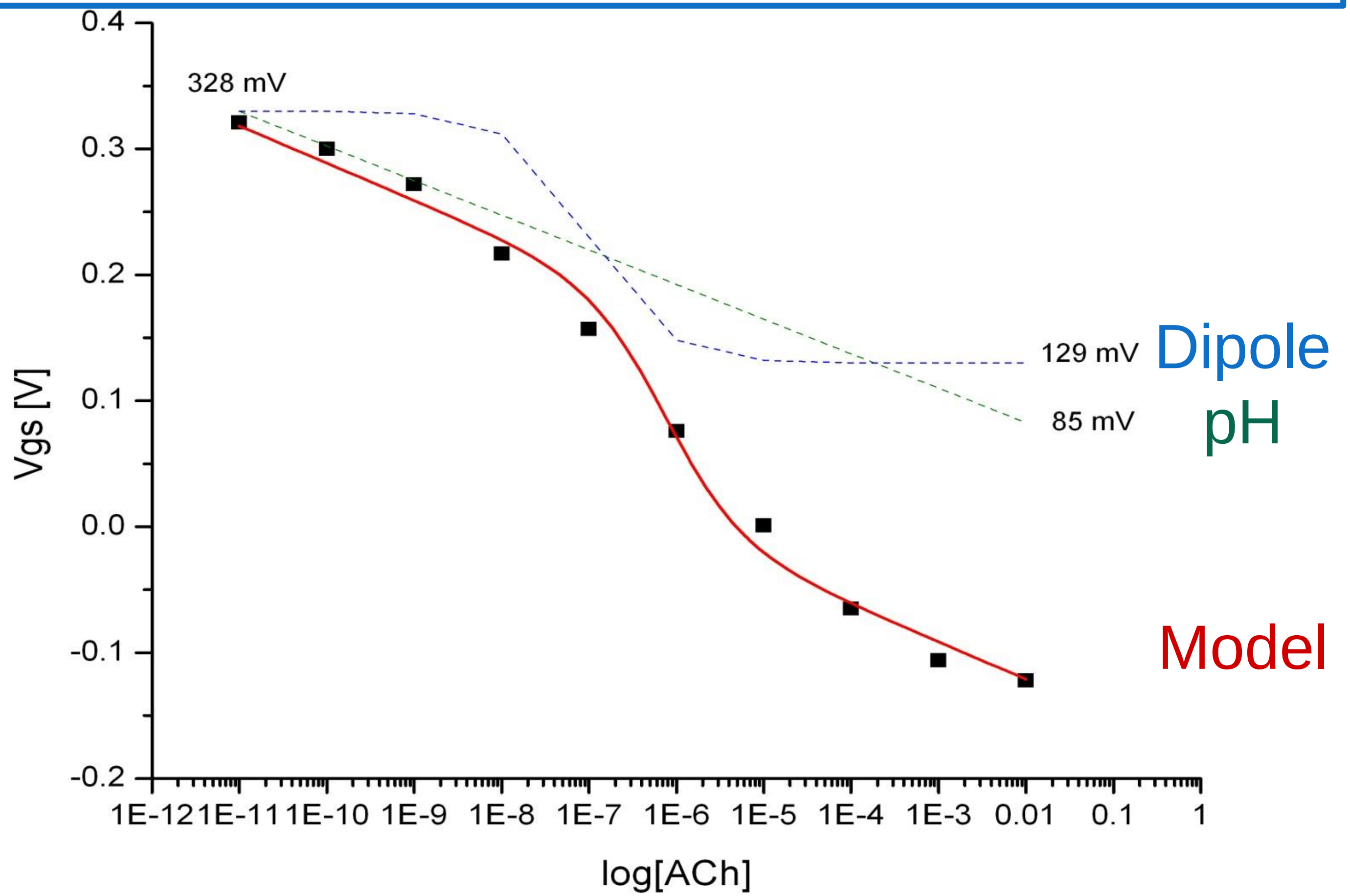
and the competitive inhibitor CCh





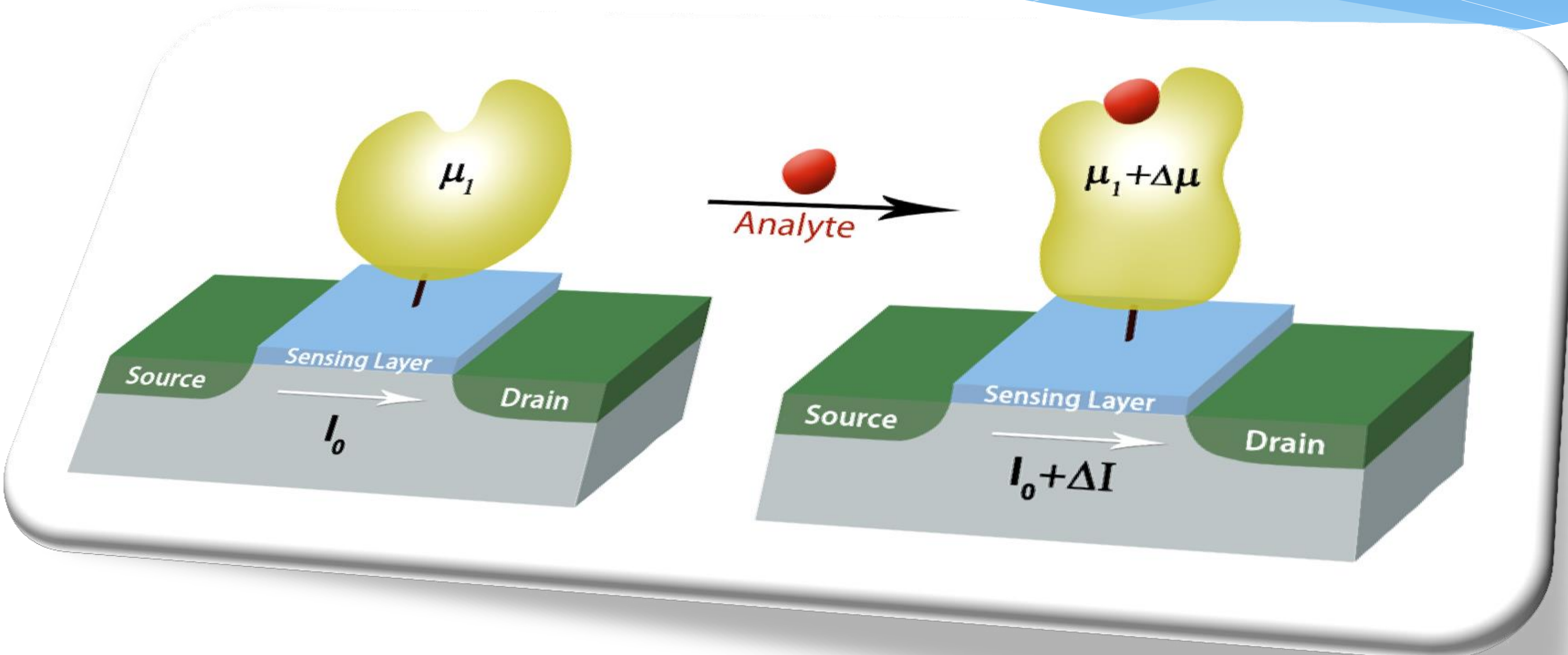
Model Fitting

$$V_{GS}(S) = V_0 - 2.303\frac{k_B T}{q}\frac{\beta}{\beta+1}\left(pH_{pzc} - pH(S)\right) - \frac{N_S(S)\cdot\Delta\mu\cdot\cos\theta}{\varepsilon\varepsilon_0}$$



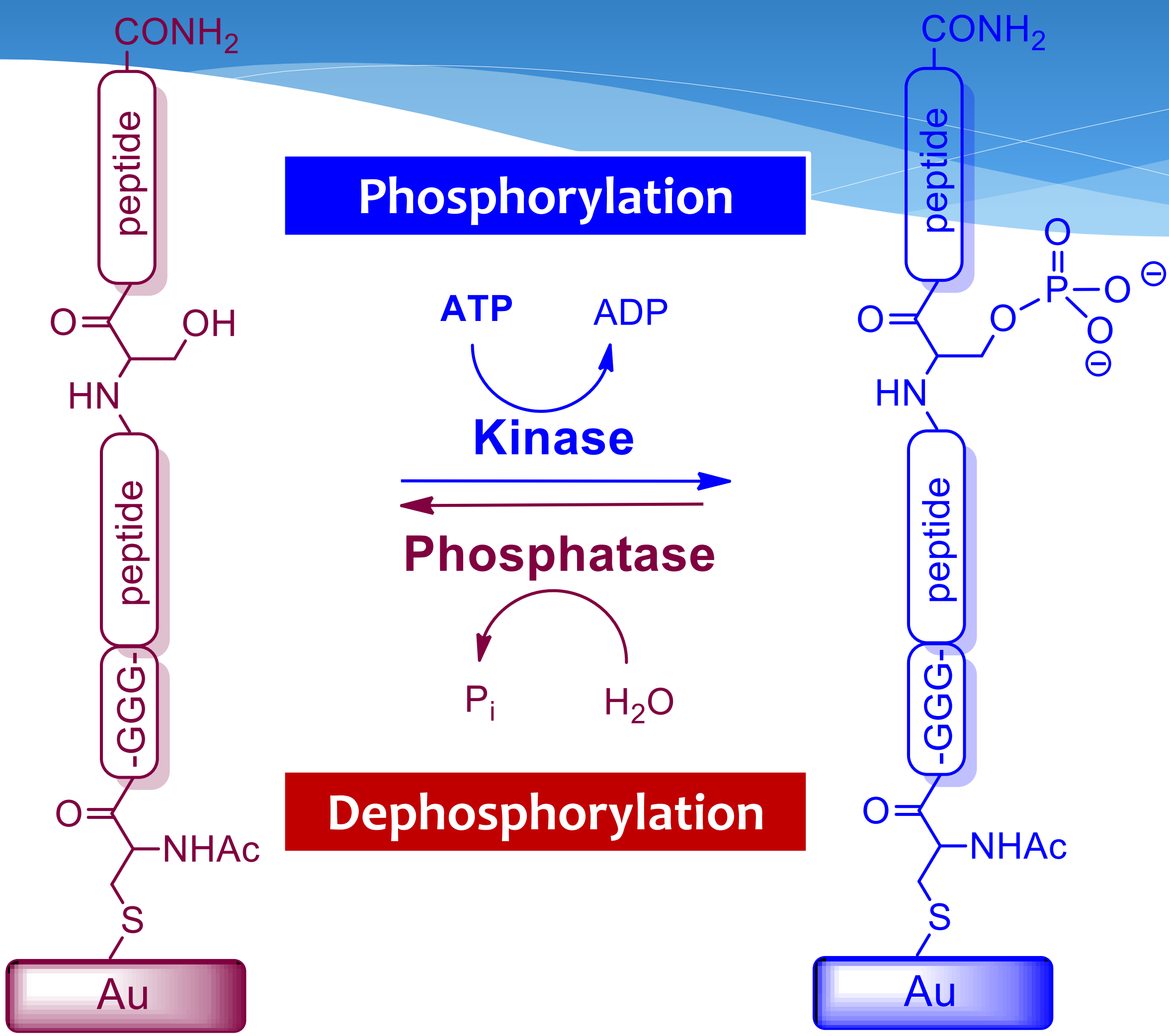
The device's response is affected by both protons and dipoles

Direct detection of biorecognition via dipole sensing mechanism



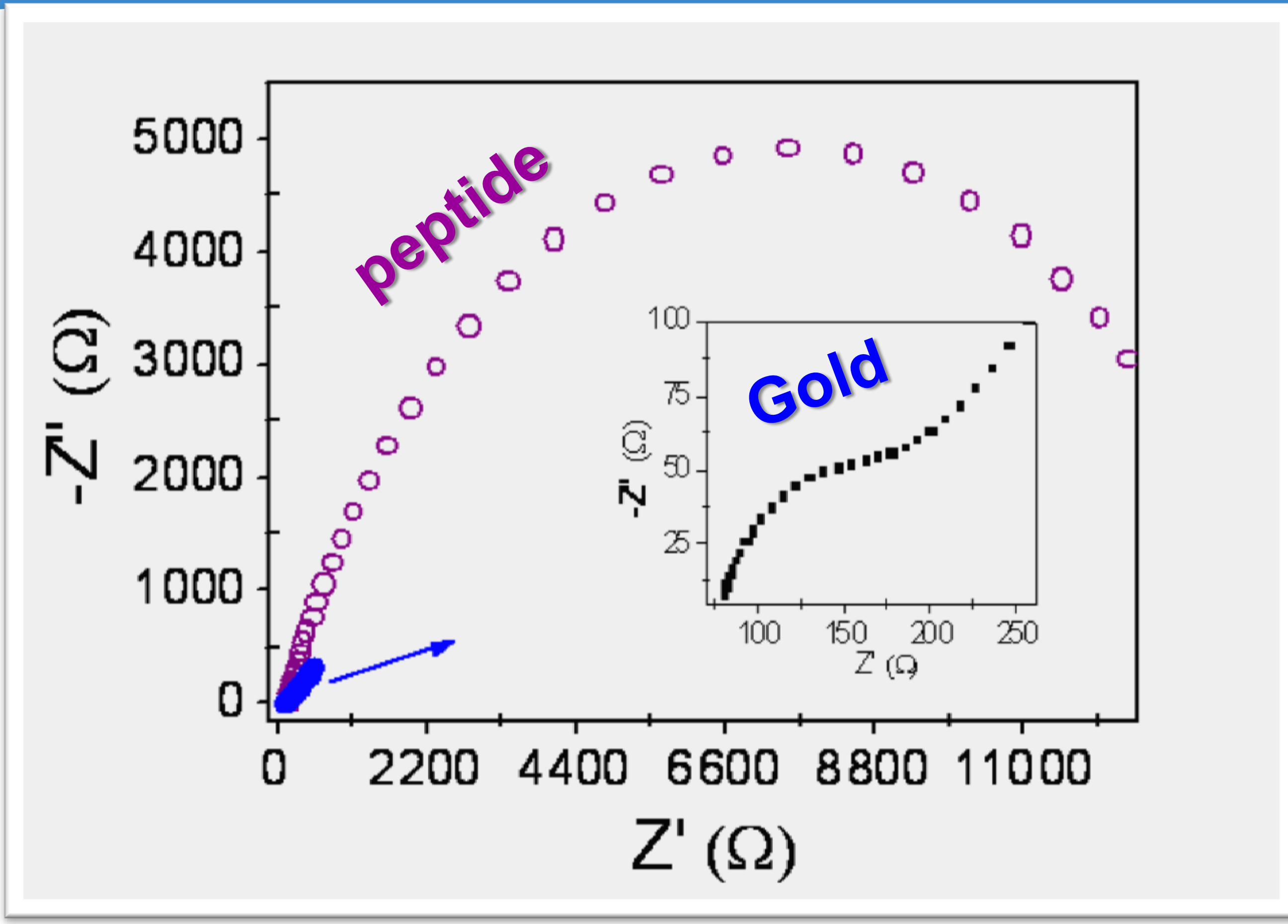
J Am Chem Soc **2009**, *131*, 4788
Biosens. Bioelectron. **2009**, *24*, 2384
IEEE Sens. J. **2011**, 11, 2007
Inter. J. Uncon. Comp. **2012**, 8, 325

Peptide Monolayer as Enzyme Substrate



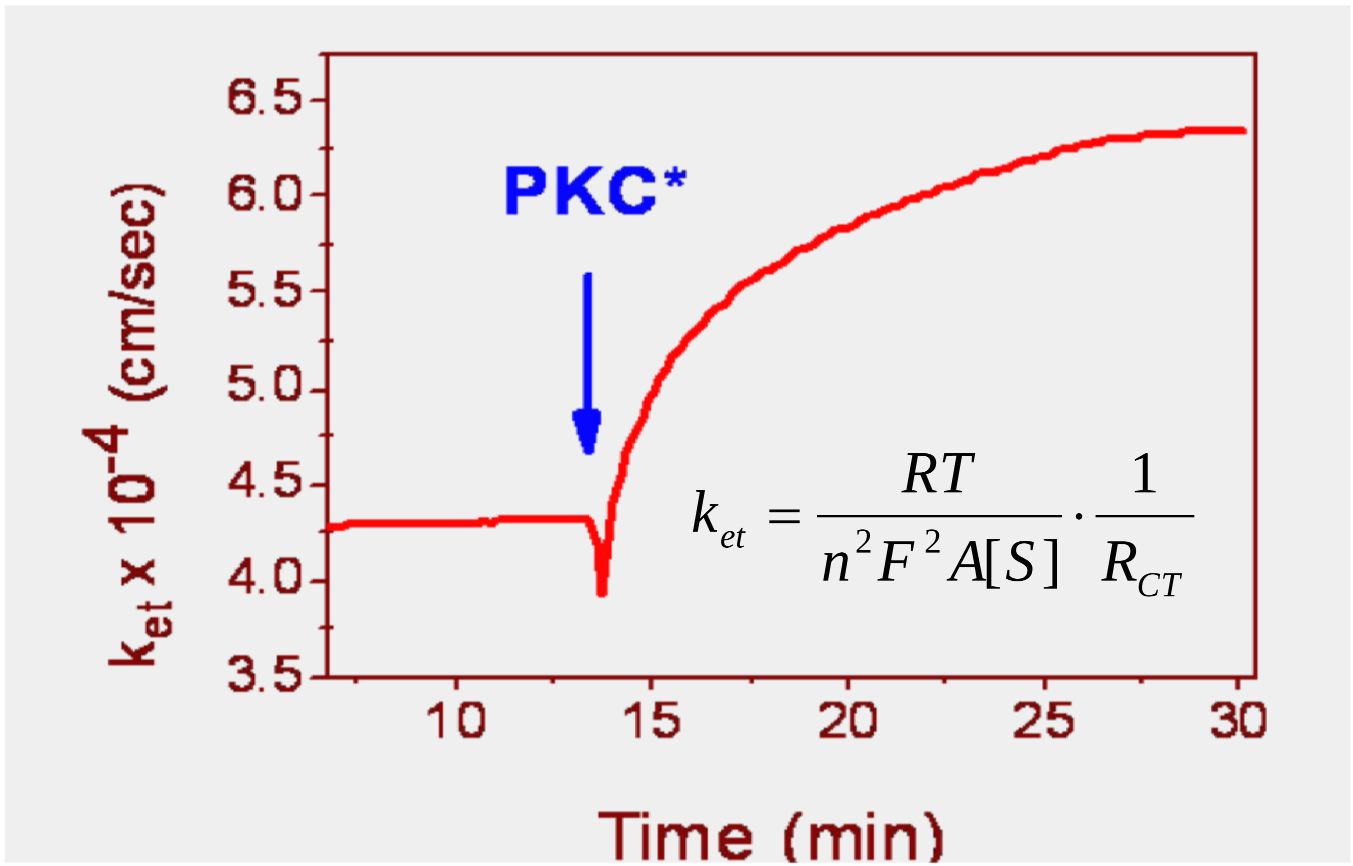
Electrochemical Impedance Spectroscopy

Ferricyanide $[Fe^{3+}(CN)_6]^{3-} + e^- \rightarrow [Fe^{2+}(CN)_6]^{4-}$ Ferrocyanide



Formation of densely packed peptide-monolayer on Gold electrode

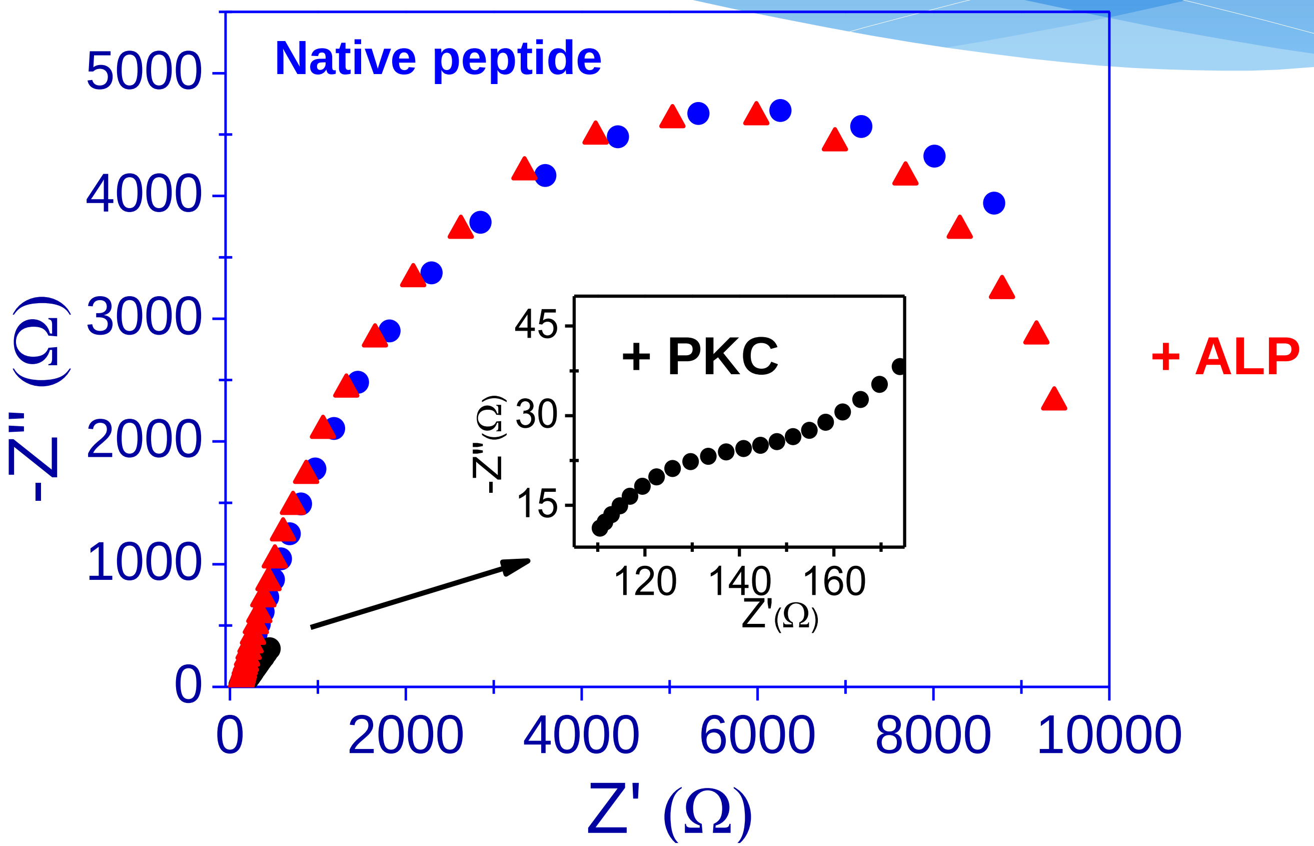
Phosphorylation kinetics



Peptide monolayer on Au electrode:
Redox couple free AC-impedance study

Phosphorylation/dephosphorylation of peptidic monolayer on Au electrode

Au-CGGGPPRRSSIRNAH

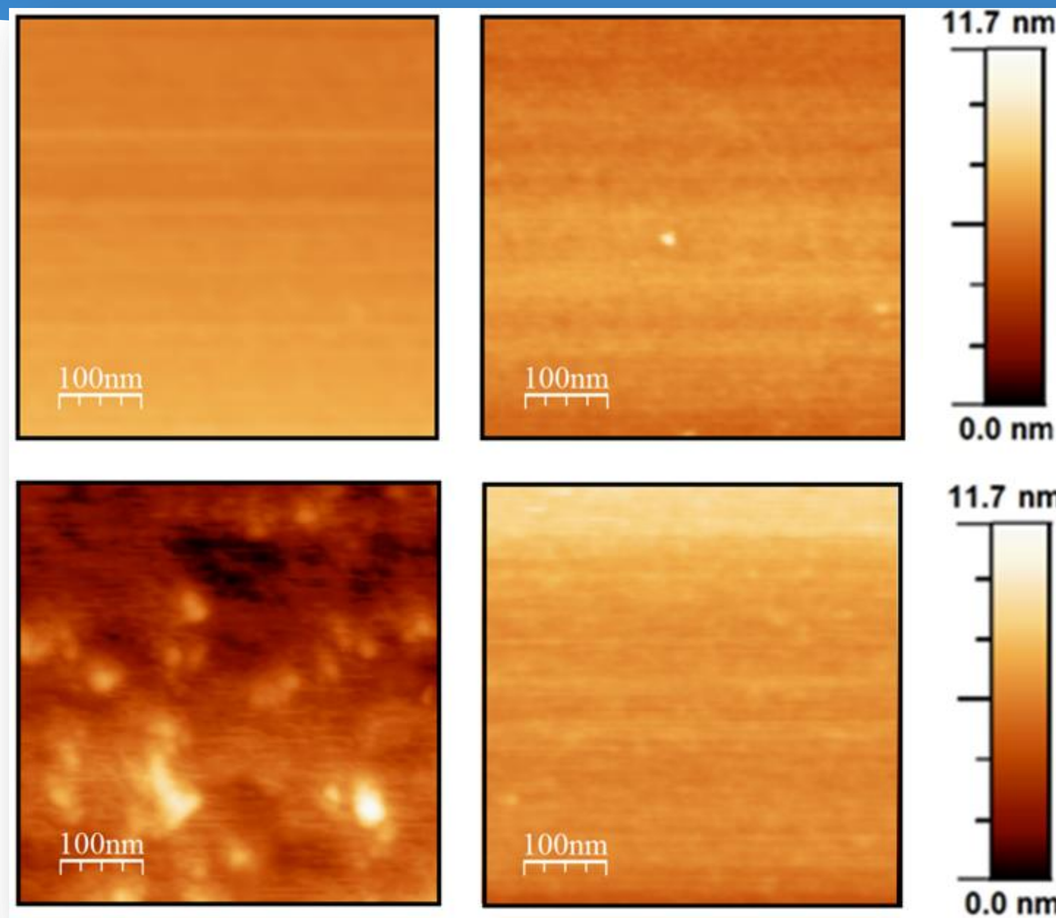


Langmuir **2011**, *27*, 11212

AFM topography changes of peptide monolayer
following kinase & phosphatase enzymatic activity

Silicon
$\rho = 1.3$ Å

PKC
$\rho = 9.6$ Å

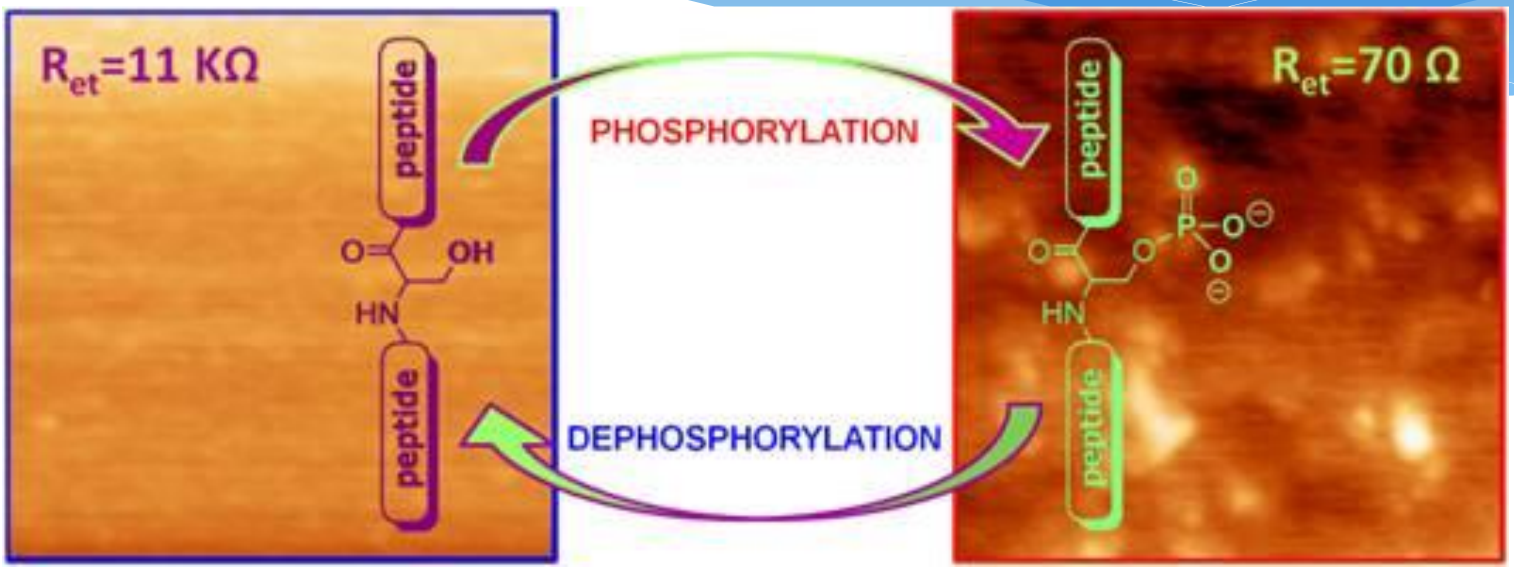


C1-peptide
$\rho = 2.2$ Å

ALP
$\rho = 2.2$ Å

ρ = RMS roughness

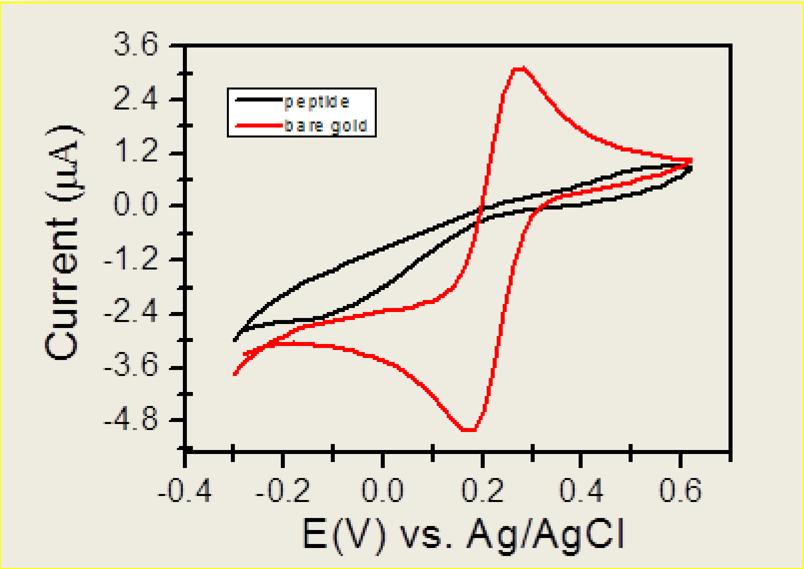
Kinase-Phosphatase Biosensor



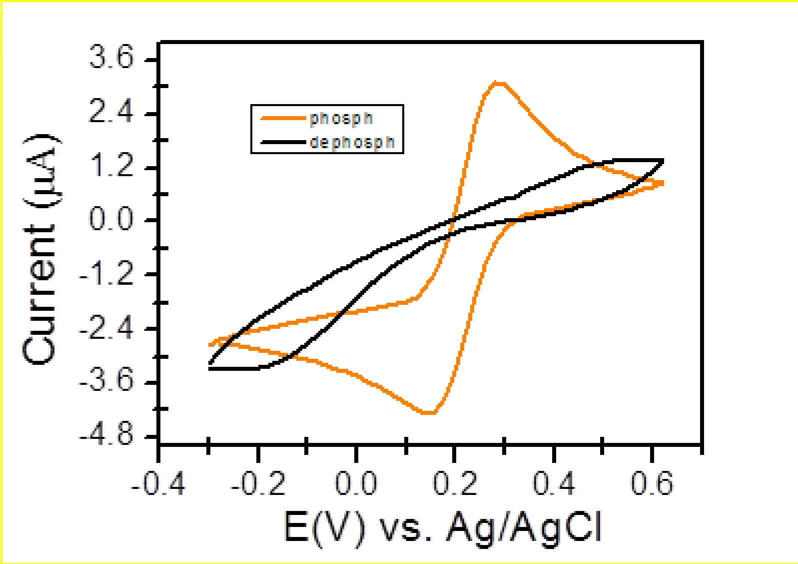
Langmuir **2011**, *27*, 11212-11221
Biopolymers **2015**, *104*, 515-520
Chem. Sci. **2015**, *6*, 4756 – 4766
Sci. Reports **2016**, *6*, 36793

Cyclic voltammograms of peptide phosphorylation and dephosphorylation

Electrode - Peptide



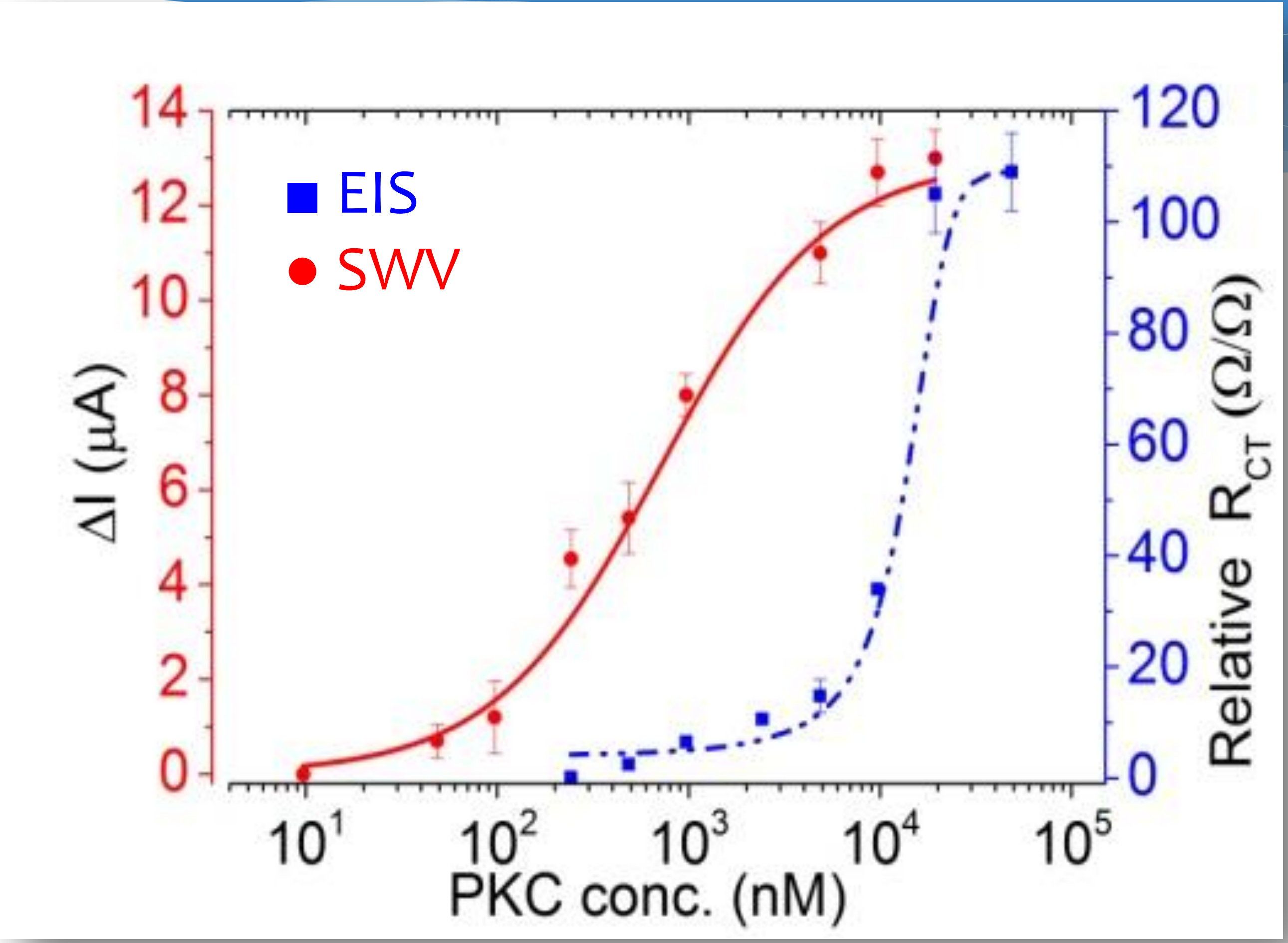
PKC - ALP



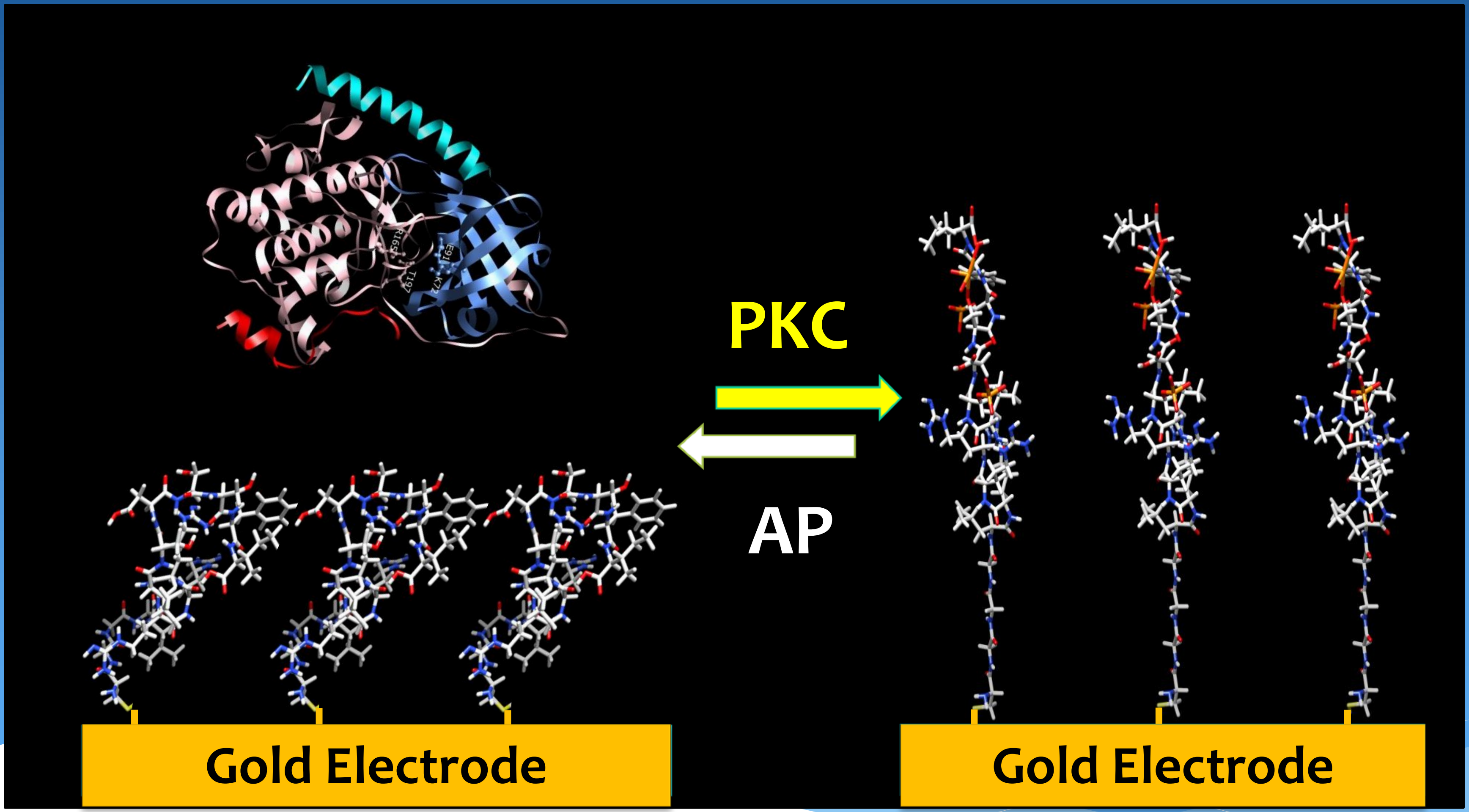
$$[Fe^{3+}(CN)_6]^{3-} + e^- \rightarrow [Fe^{2+}(CN)_6]^{4-}$$

PKC Dose response

Impedimetric vs. Amperometric Sensing



Structural Model



Biosensor for early detection of lung cancer

* Lung cancer is one of the most lethal kinds of cancer
* Due to late discovery of the disease, only 10-15% of the patients recover
* only 10% of the patients are non-smokers

Biomarker discovery via Phosphoproteomics

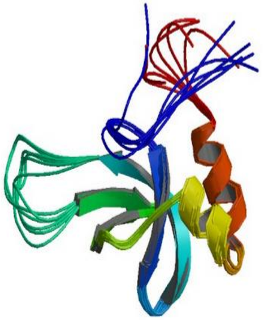
Lung cancer tissue

1,451 phosphoproteins (candidate biomarkers)

T / N > 5

Related to Lung cancer

HDGF (Ser165)

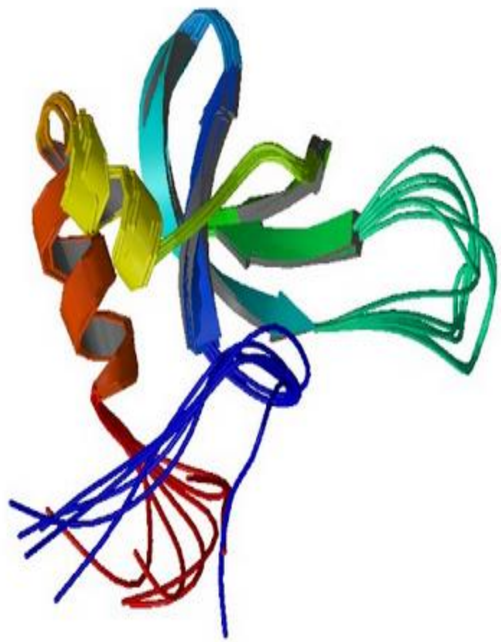


Amit E. et al., *Chem. Sci*. 2015

HDGF

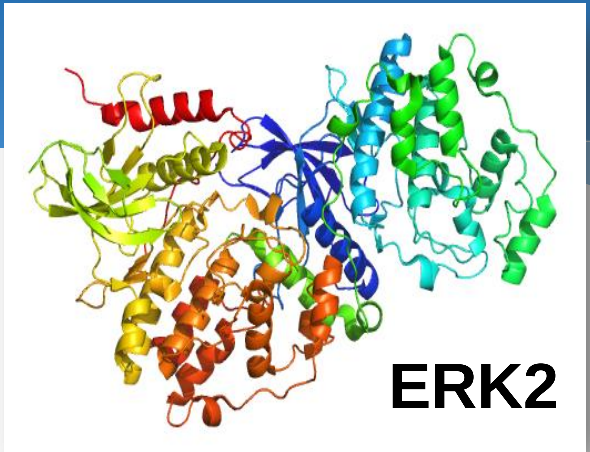
Hepatoma Derived Growth Factor

- **A mitotic factor**
- **Highly expressed in embryonic development**
- **Related to many kinds of cancer, including lung cancer**
- ***In vitro* experiments showed that ERK2 phosphorylates Ser165**

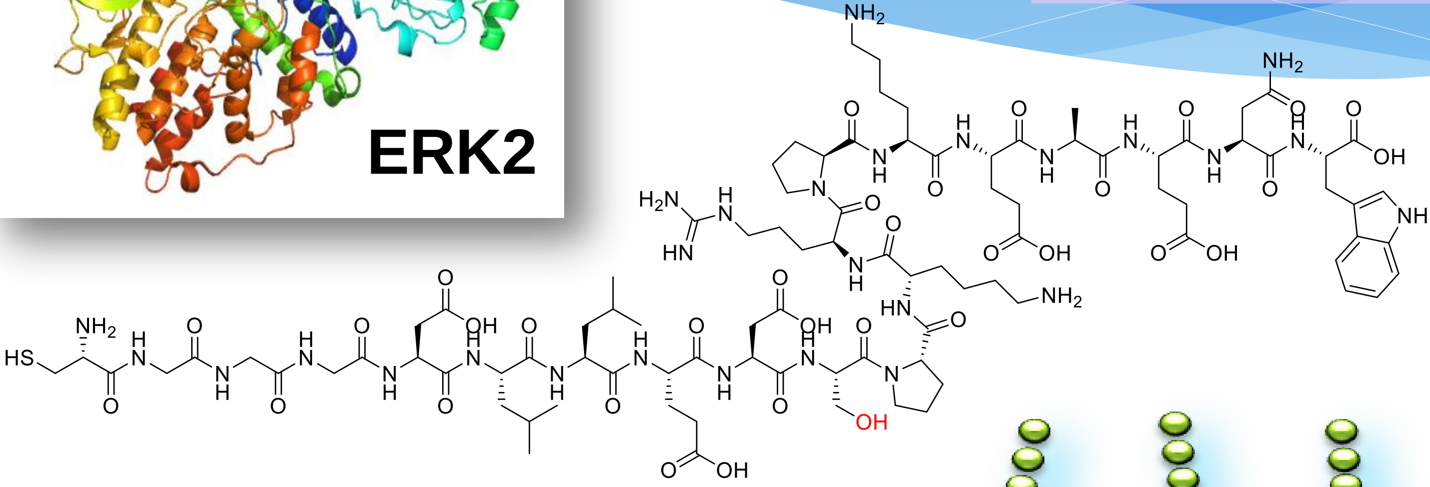


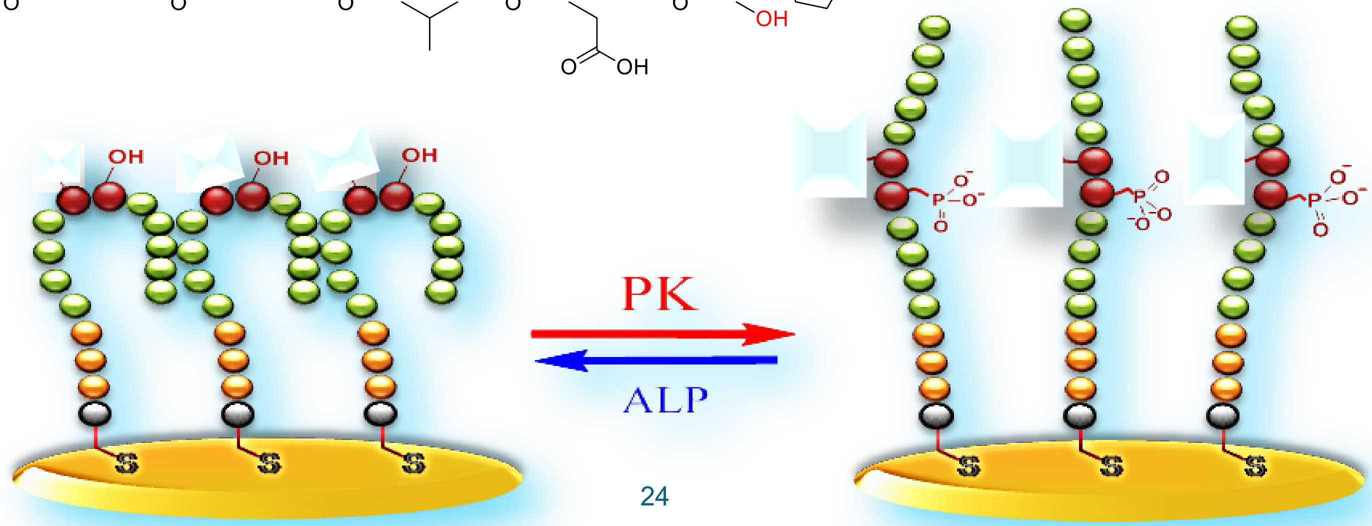
Mao, J. et al., *Cancer Sci*. 2008
Everett, A. D. et al., *BMC Cell Biol.* 2011

Design a kinase specific biosensor for early detection of lung cancer

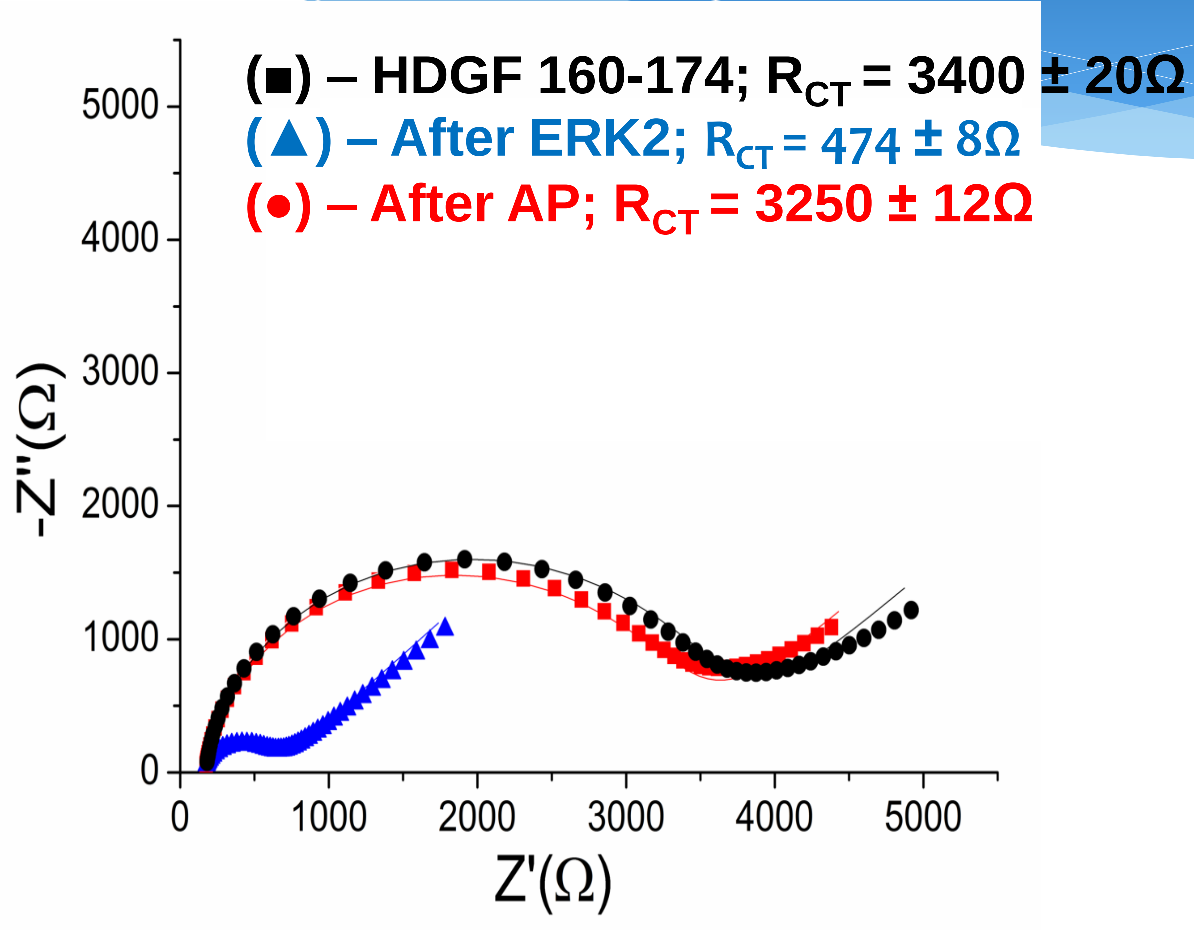


HDGF 160-174





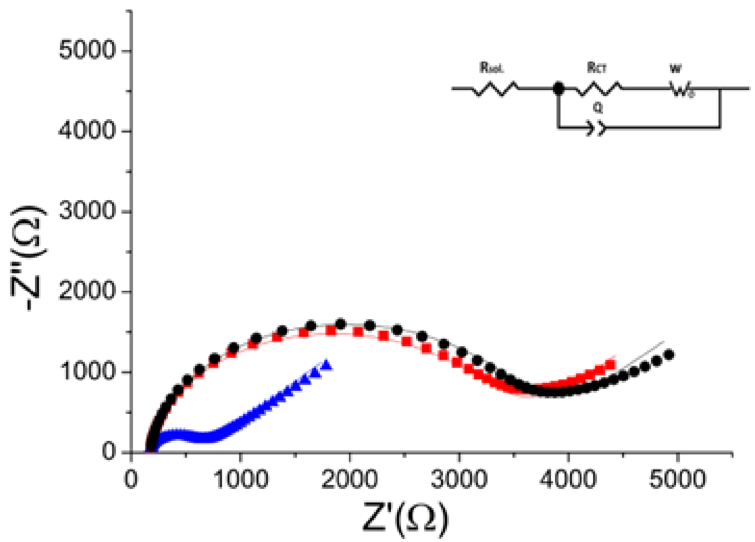
ERK2 phosphorylation is reversible



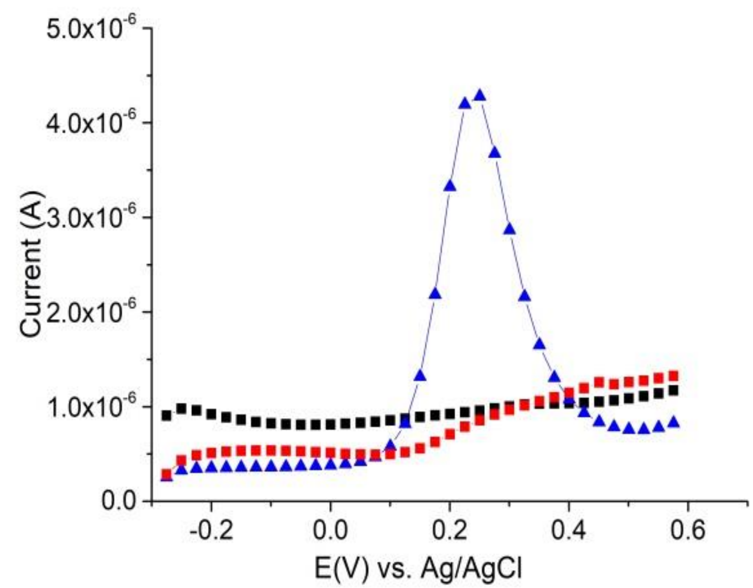
Amit E. et al., *Chem. Sci*. 2015

Sensitivity and reversibility of the HDGF biomarker

EIS

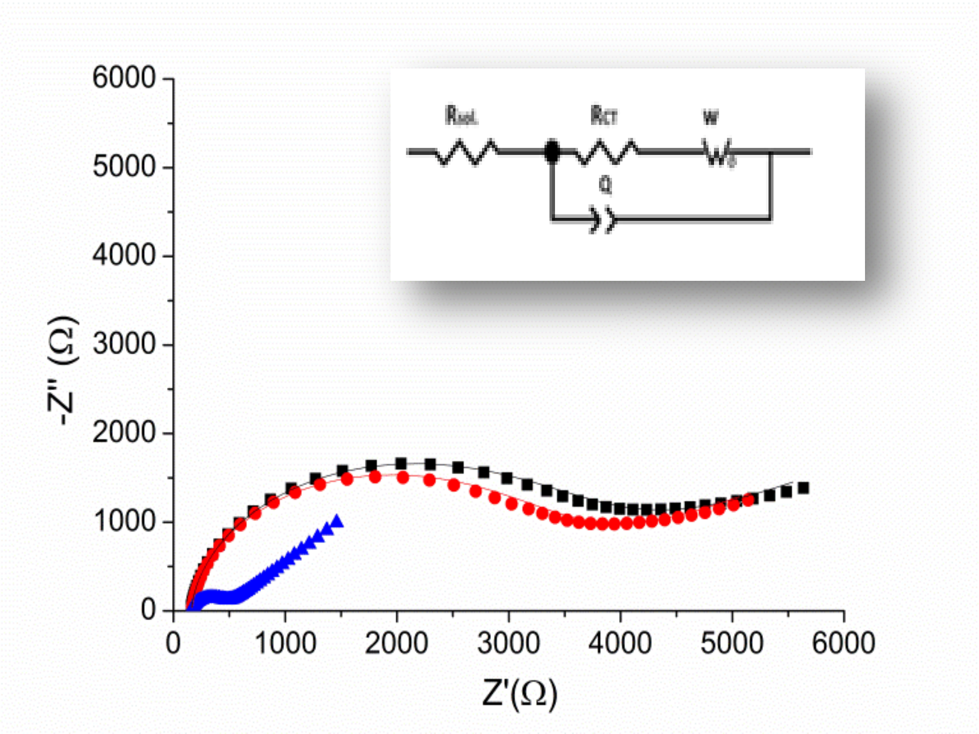


SWV



(■) – peptide (●) – ERK2 (▲) – AP

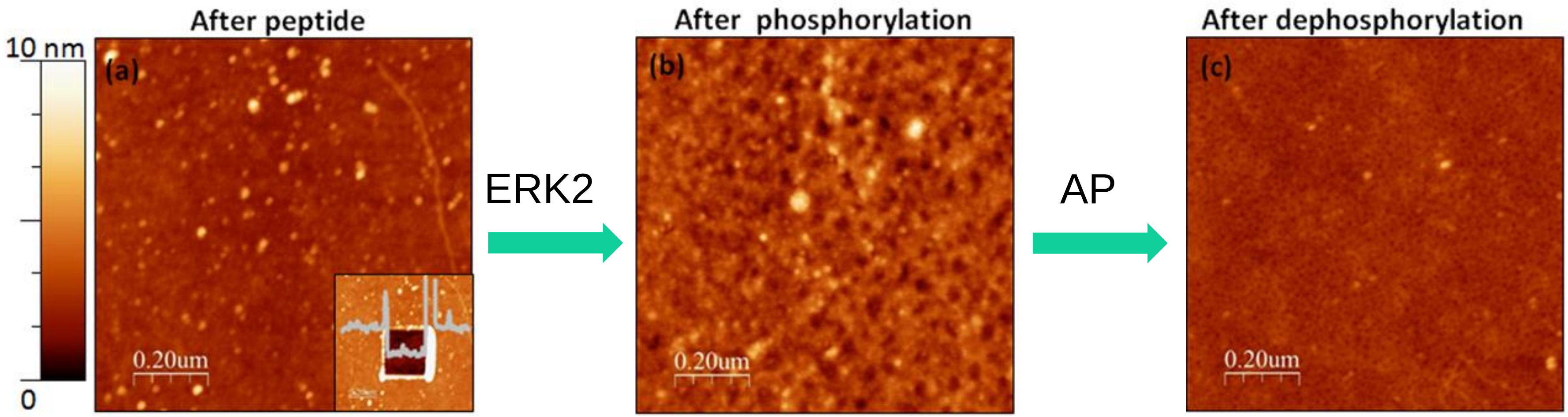
Selectivity of the HDGF biomarker



(■) – peptide
(●) – CaMK2
(▲) – ERK2

AFM measurements

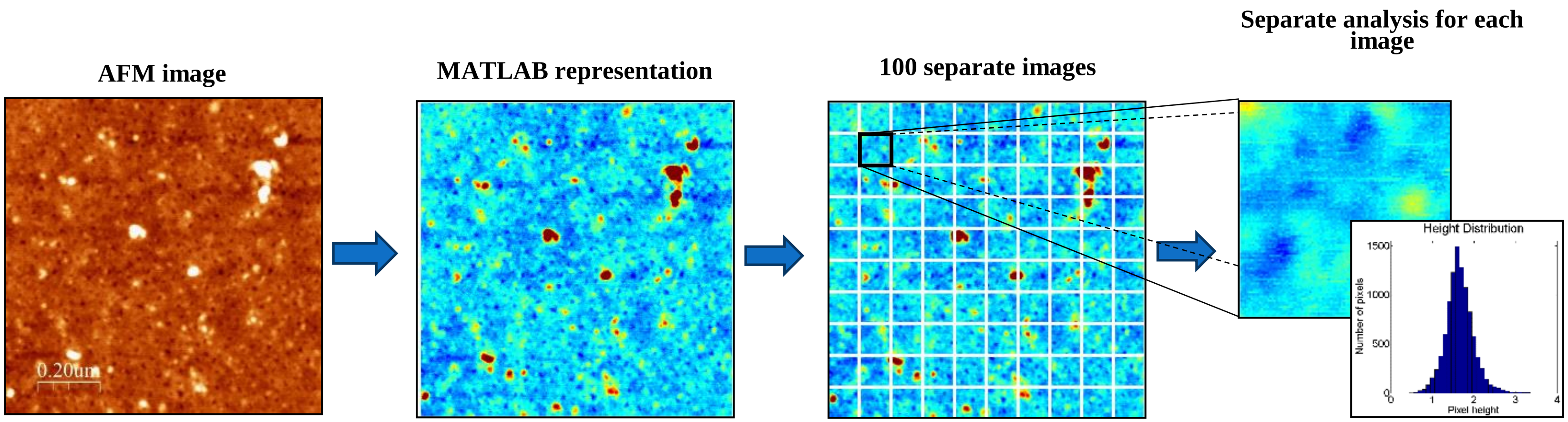
the surface is rougher after phosphorylation



Zhuravel R. et al., *Sci. Reports* **2016**

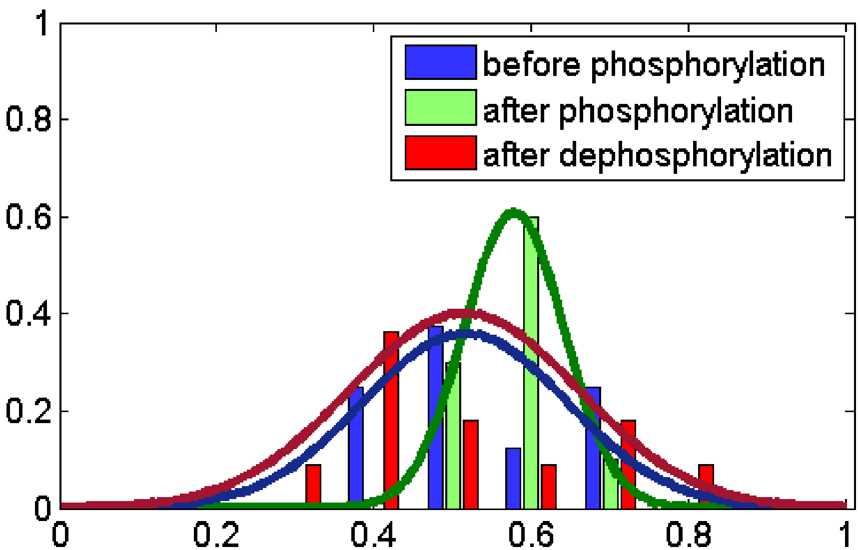
High resolution AFM

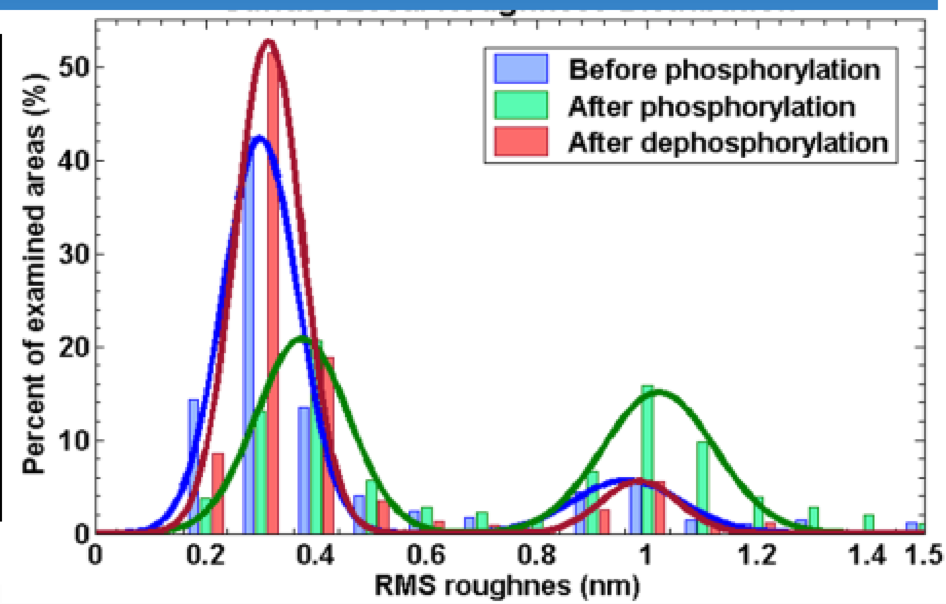
Characterization and analysis



$$R_{RMS} = \sqrt{\frac{1}{n}\sum(Z - Z_{mean}\ )^2}$$

High resolution roughness analysis





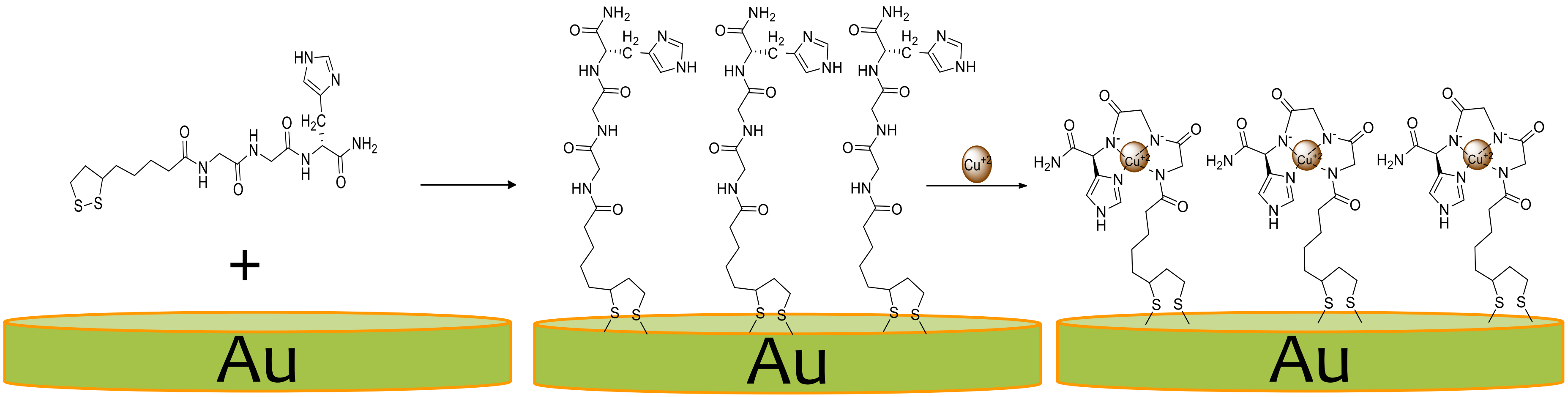
- Global roughness increases upon phosphorylation and recovers upon dephosphorylation

- Local roughness analysis reveals a bi-modal distribution with smooth-rough transition and up-shifting

Ions Biosensing

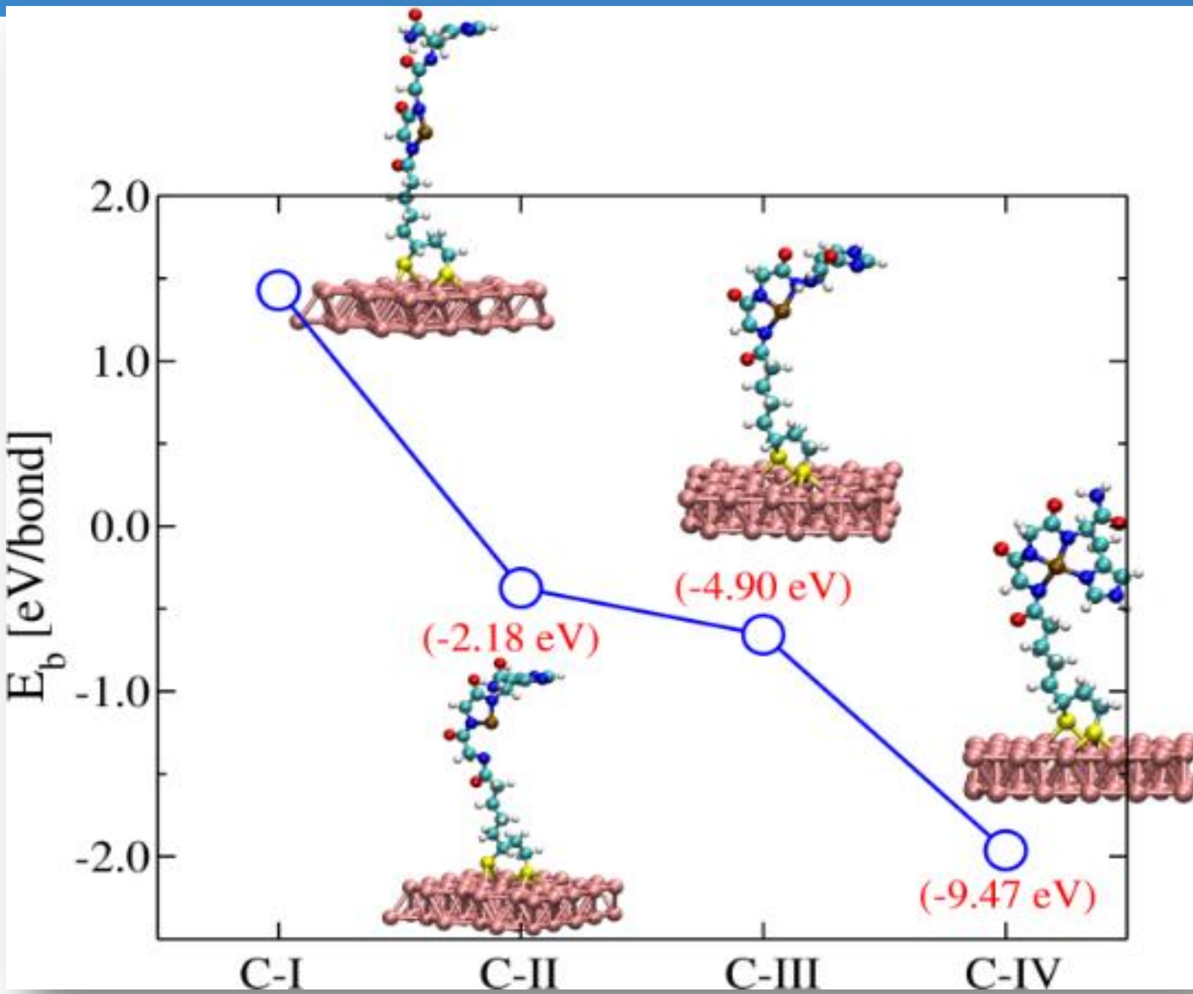
Impairment of serum Zn^{2+} to Cu^{2+} ratio was found to correlate with many disease states, including immunological and inflammatory disorders, autism, Alzheimer's disease, skin diseases and cancer.

Peptide Biosensor for Cu(II) Ions

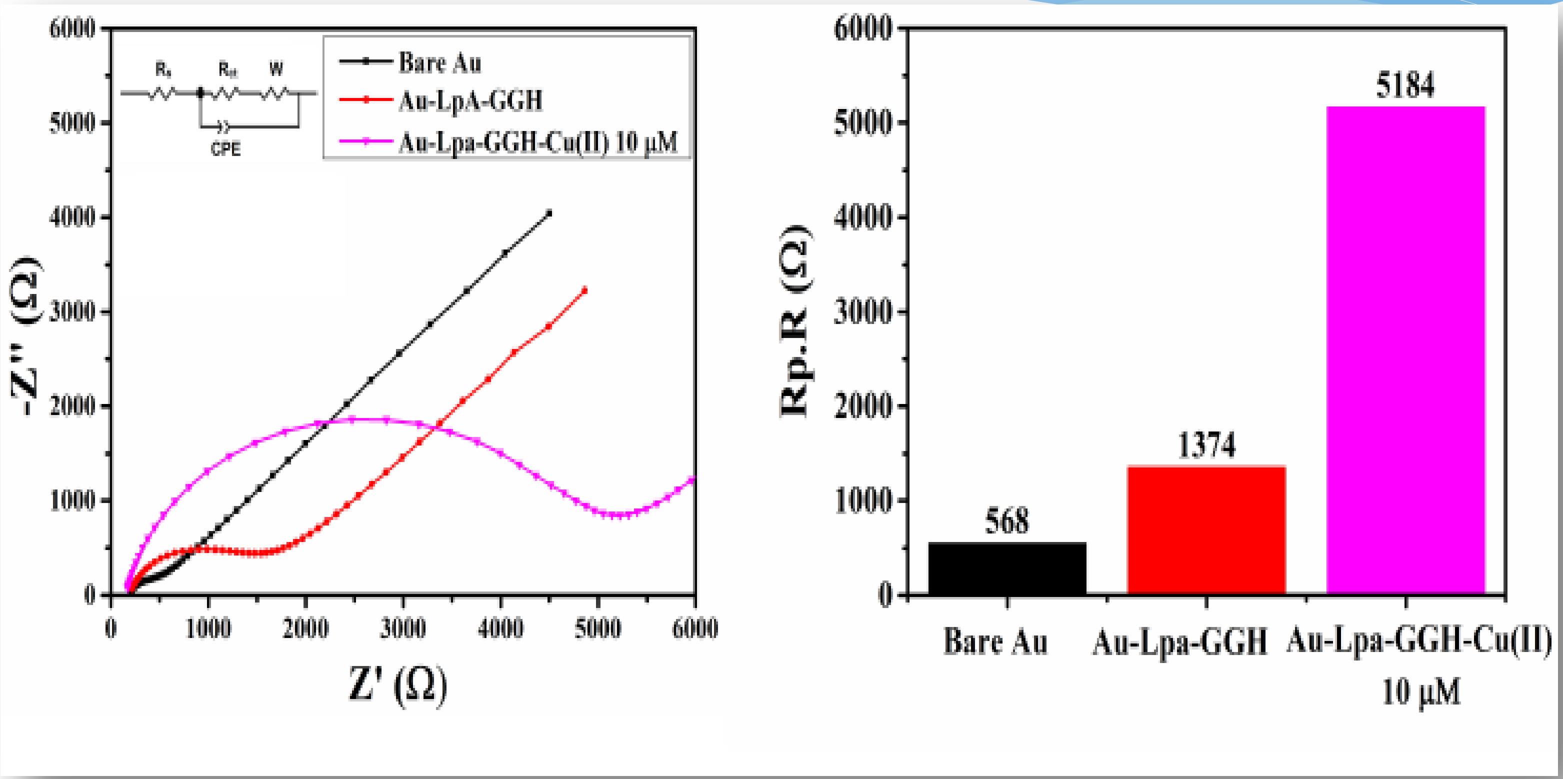


GGH sensing platform

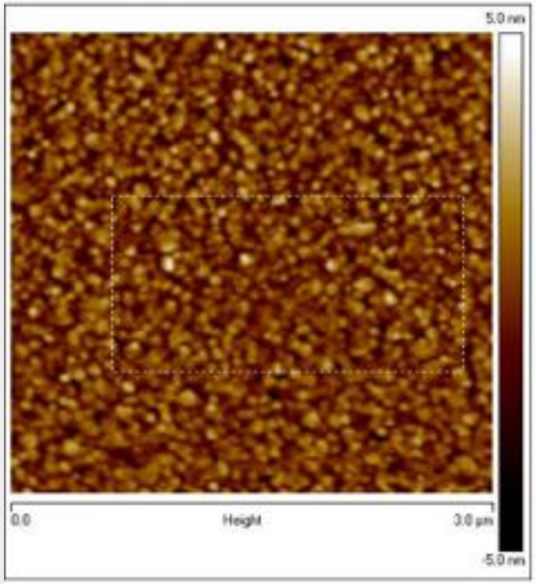
DFT calculation of the ion binding energy per N-Cu bond



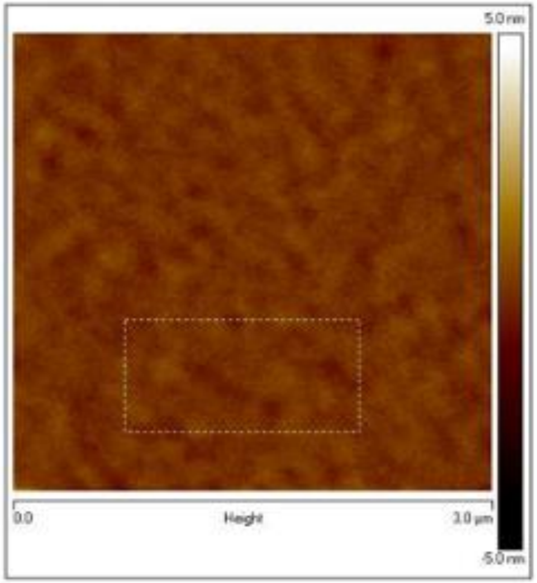
Impedimetric sensing of GGH-Cu(II)



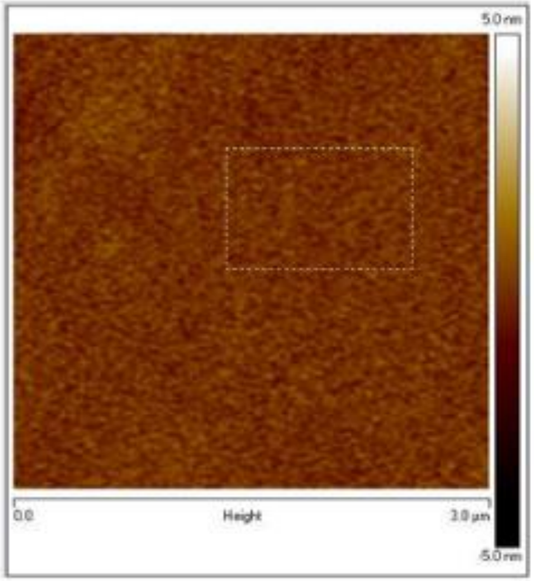
AFM Topography



Bare Si/Au
Ra= 0.893 nm

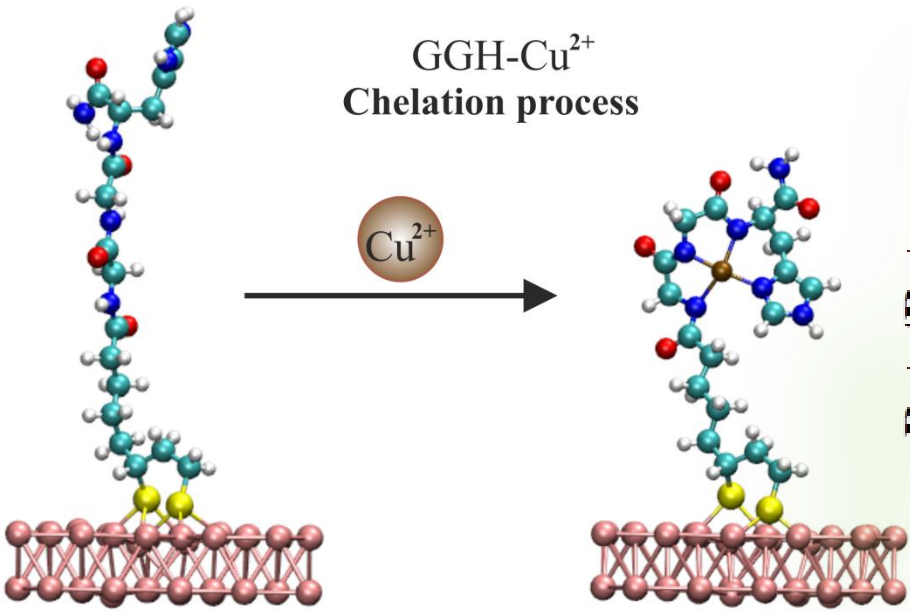


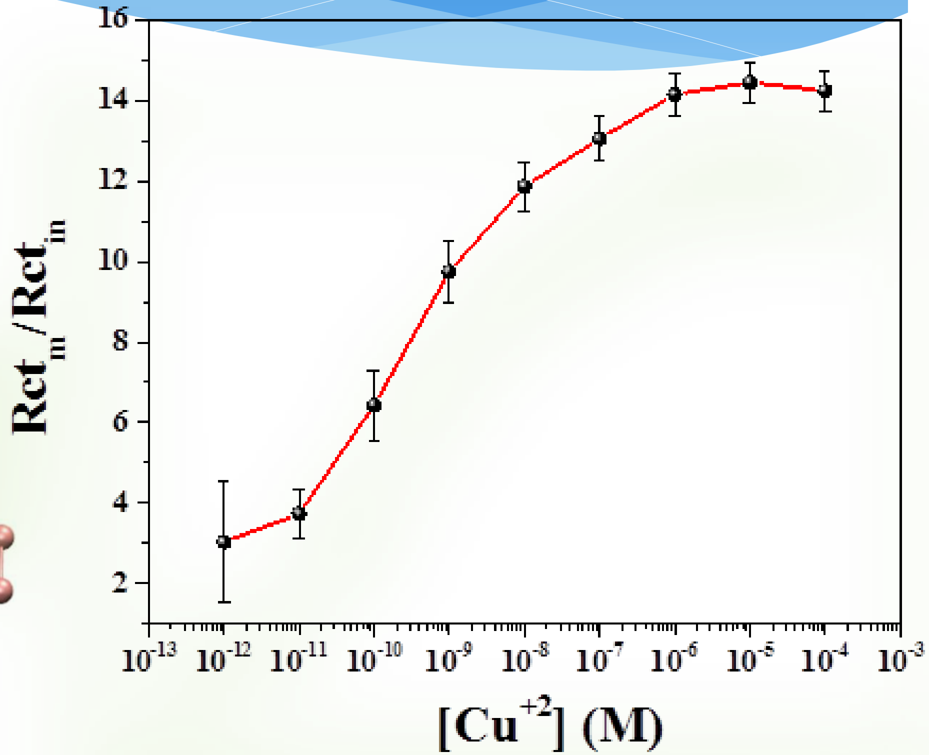
Si/Au-LpA-GGH
Ra= 0.177 nm



Si/Au-LpA-GGH-Cu
Ra= 0.284 nm

Cu(II) Ions Biosensor





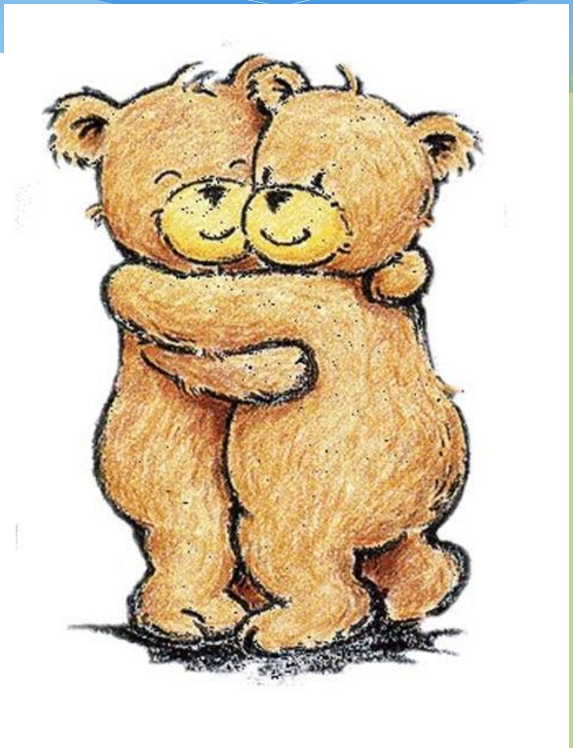
μ = 6.5 D

μ = 10.2 D

Summary and outlook

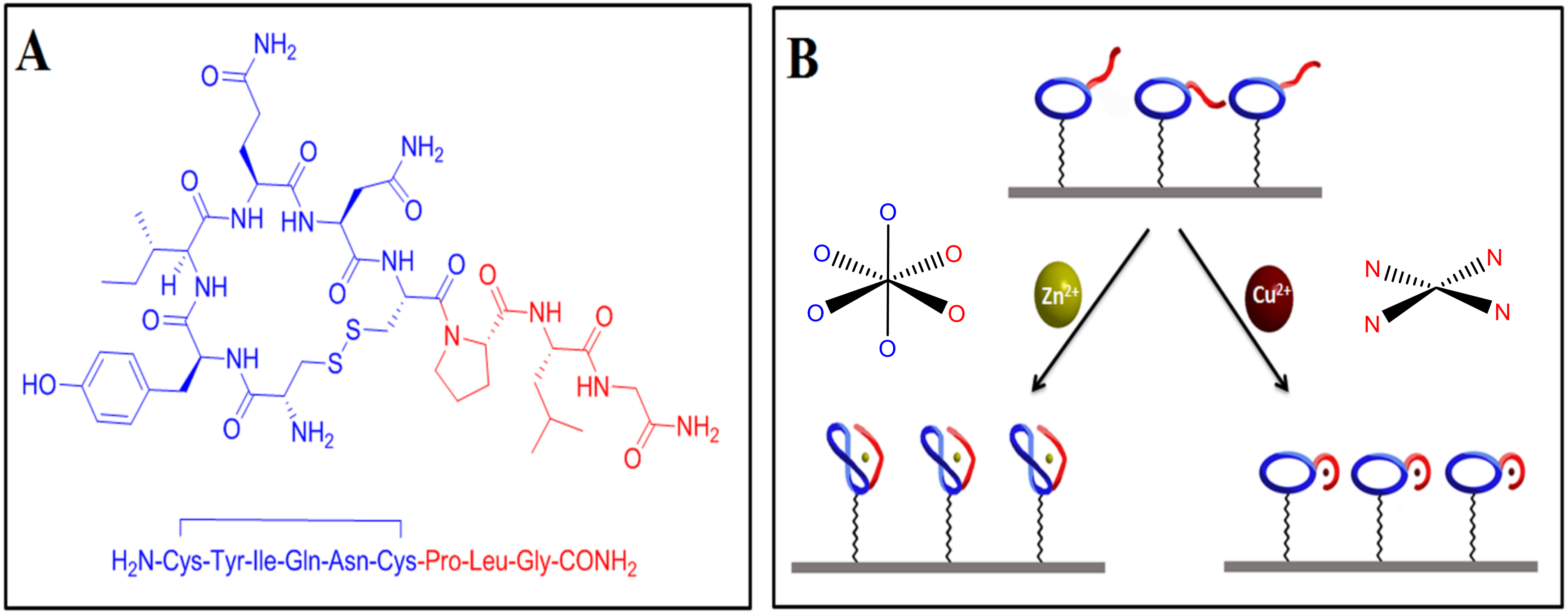
- ✓ **Sensitive biosensor for neurotransmitters:**
  nerve-gas detectors & artificial synapse
- ✓ **Highly selective biosensor for kinases:**
  cancer diagnostics and drug screening
- ✓ **Peptide based metal-ions sensor:**
  novel environmental & medical applications

Oxytocin, The Love Hormone



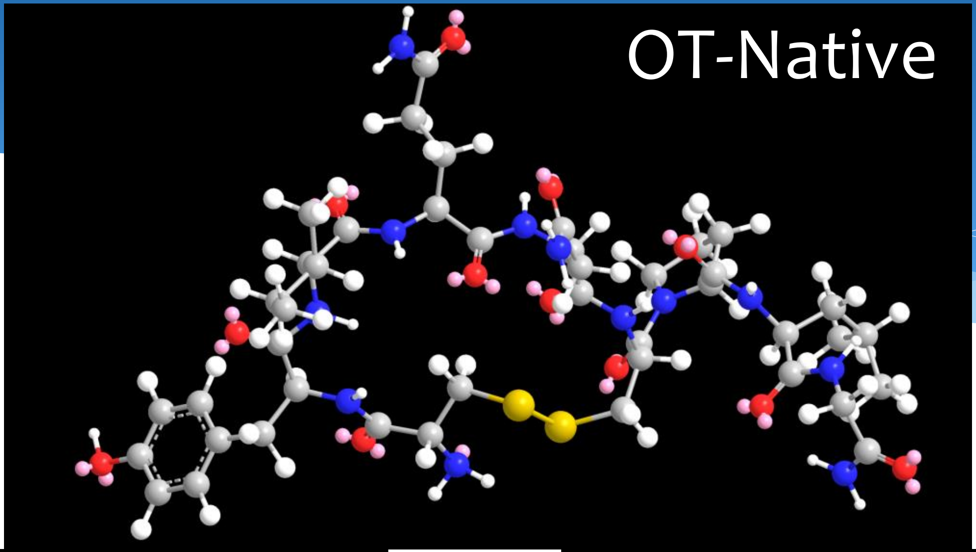
Oxytocin complexation with metal-ions

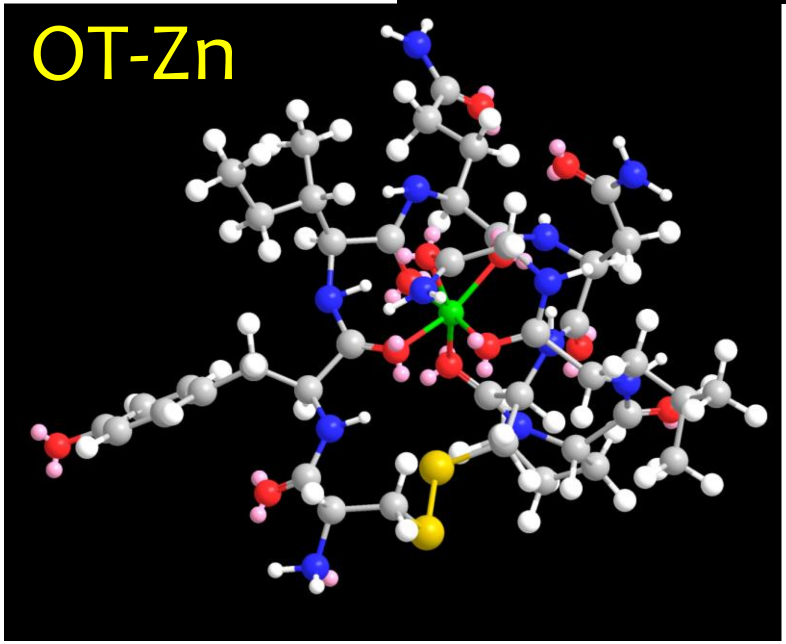
Ring and **Tail**

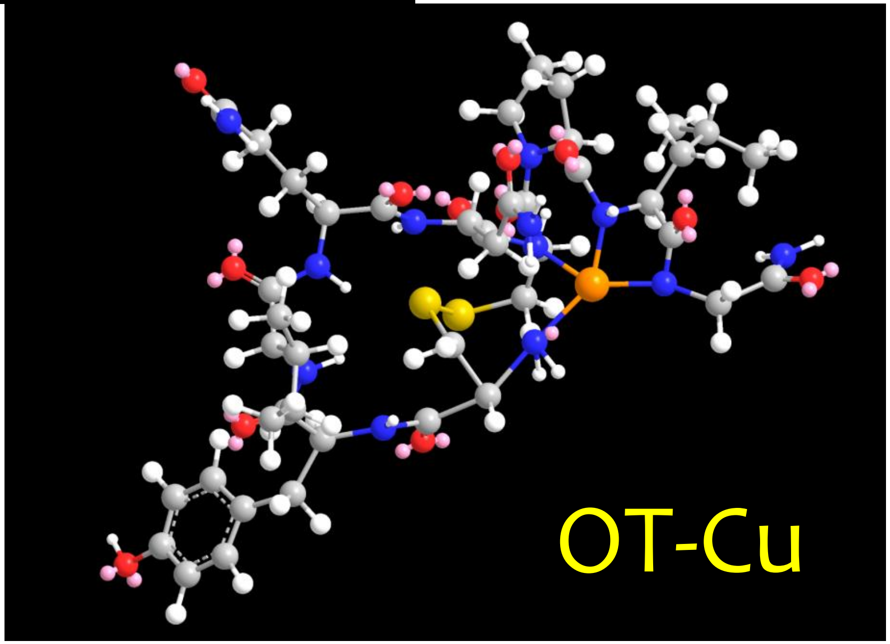


ACS Omega **2017**, *2*, 8770−8778.

Oxytocin Conformations

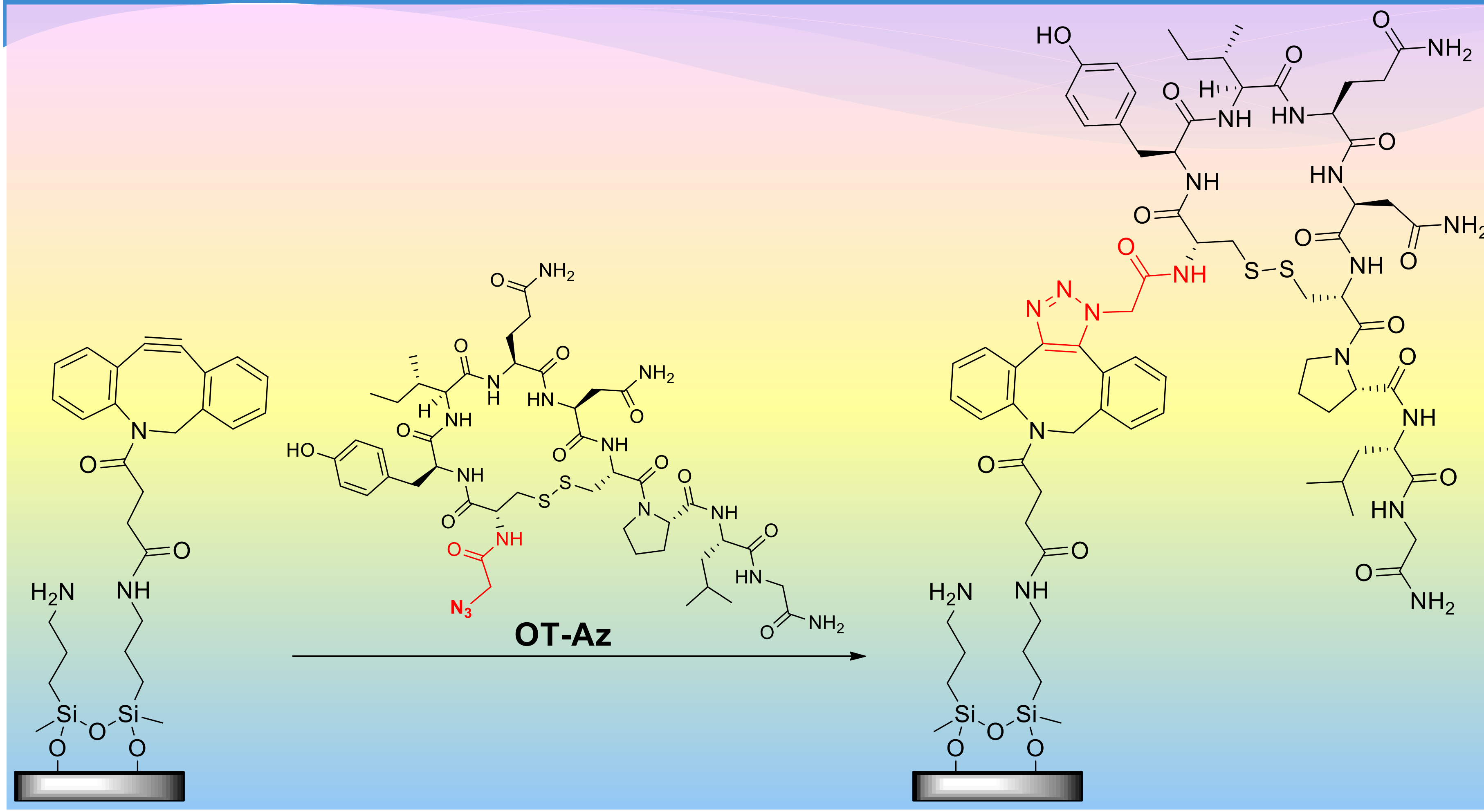






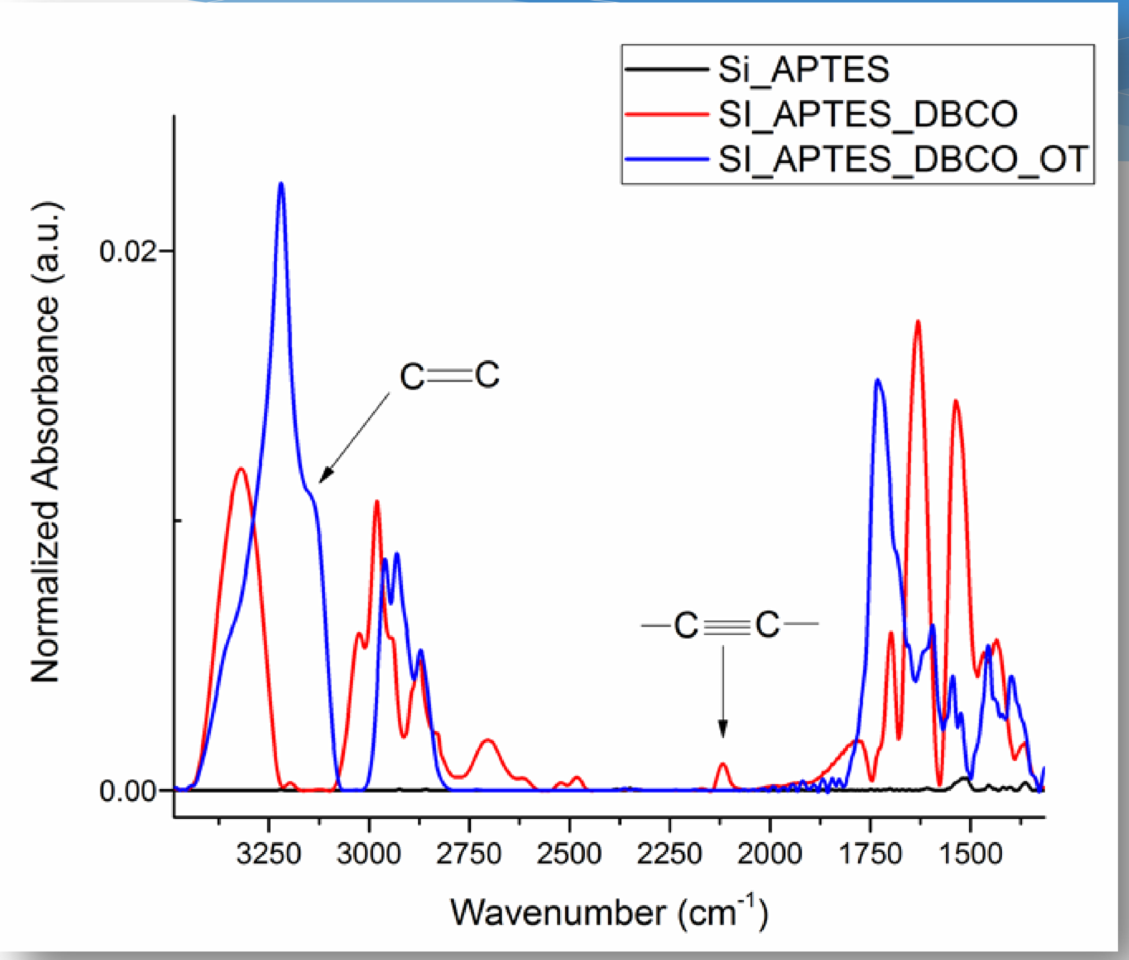
Oxytocin assembly on oxide surfaces

via Cu free click chemistry



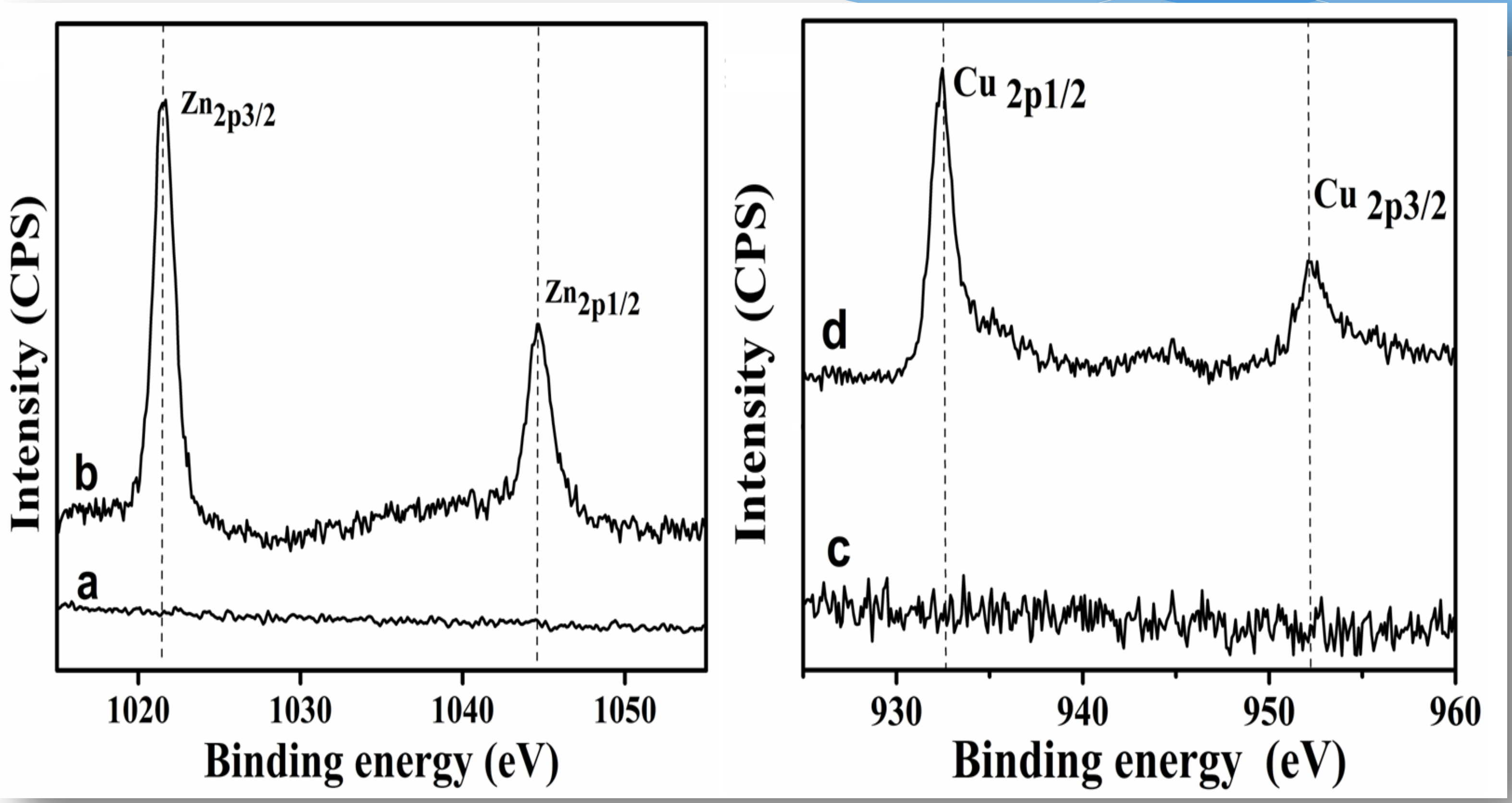
AR-FTIR spectra of oxytocin assembly

surface coupling by click chemistry

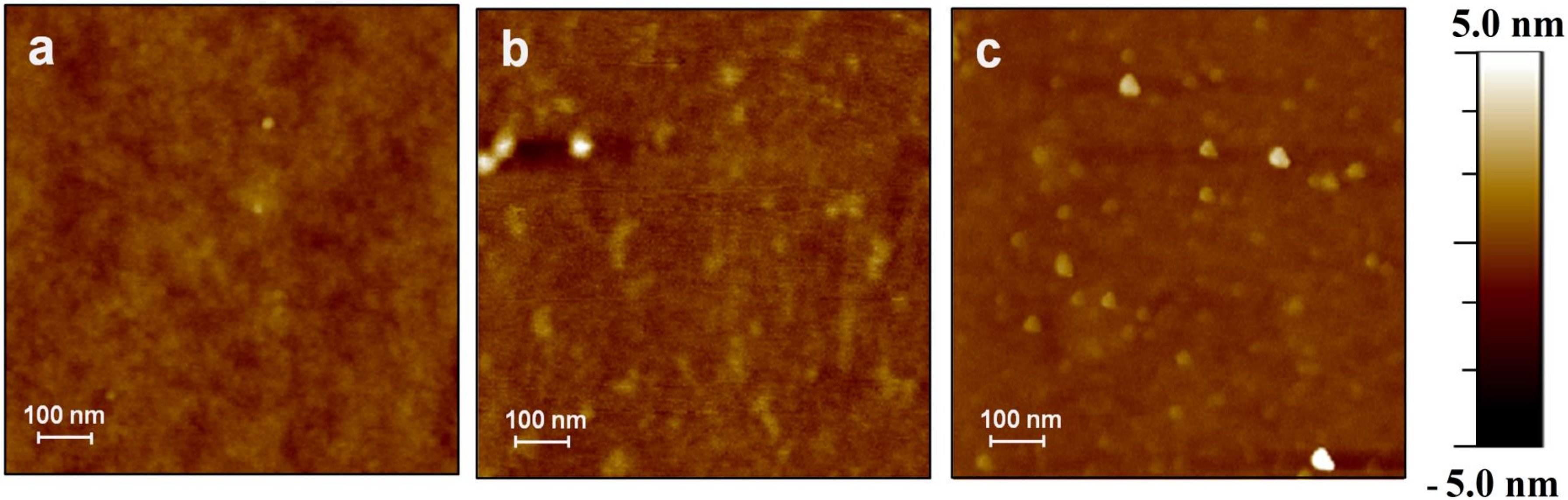


XPS spectra of oxytocin monolayer

before and after incubation in 1 μM Zn^{2+} and Cu^{2+} solutions



AFM topography of oxytocin sensor
following ion binding

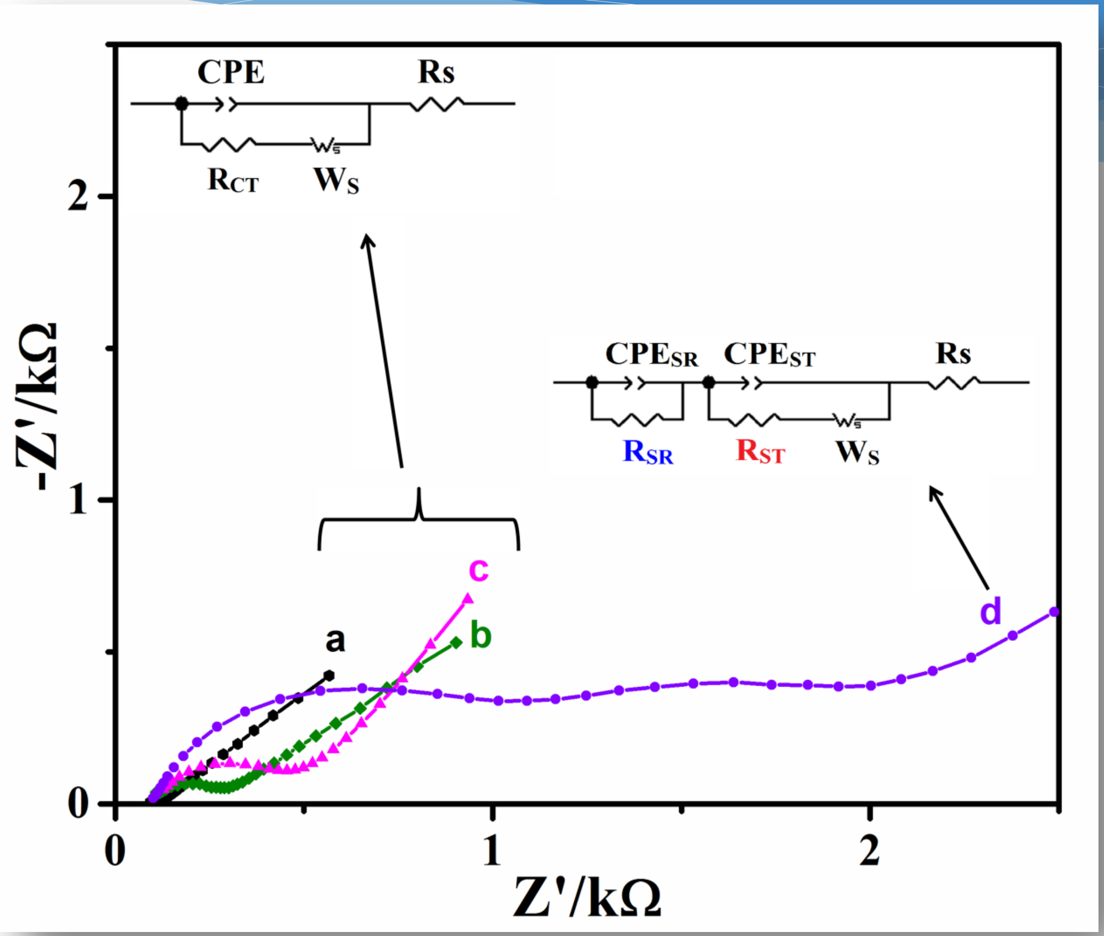


RMS roughness
ρ = 2.9 Å

1 nM Zn^{2+}
ρ = 4.8 Å

1 nM Cu^{2+}
ρ = 2.0 Å

Nyquist plots for Oxytocin assembly on GC electrodes

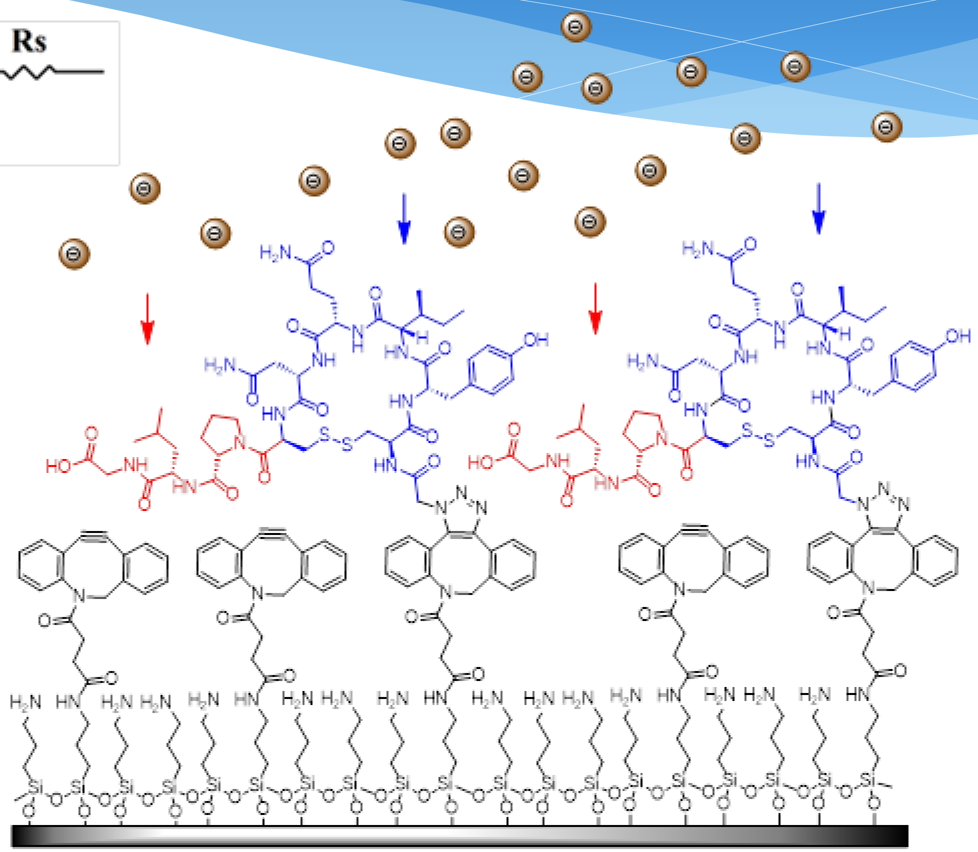


Redox couple diffusion pathway through the oxytocin layer to the electrode

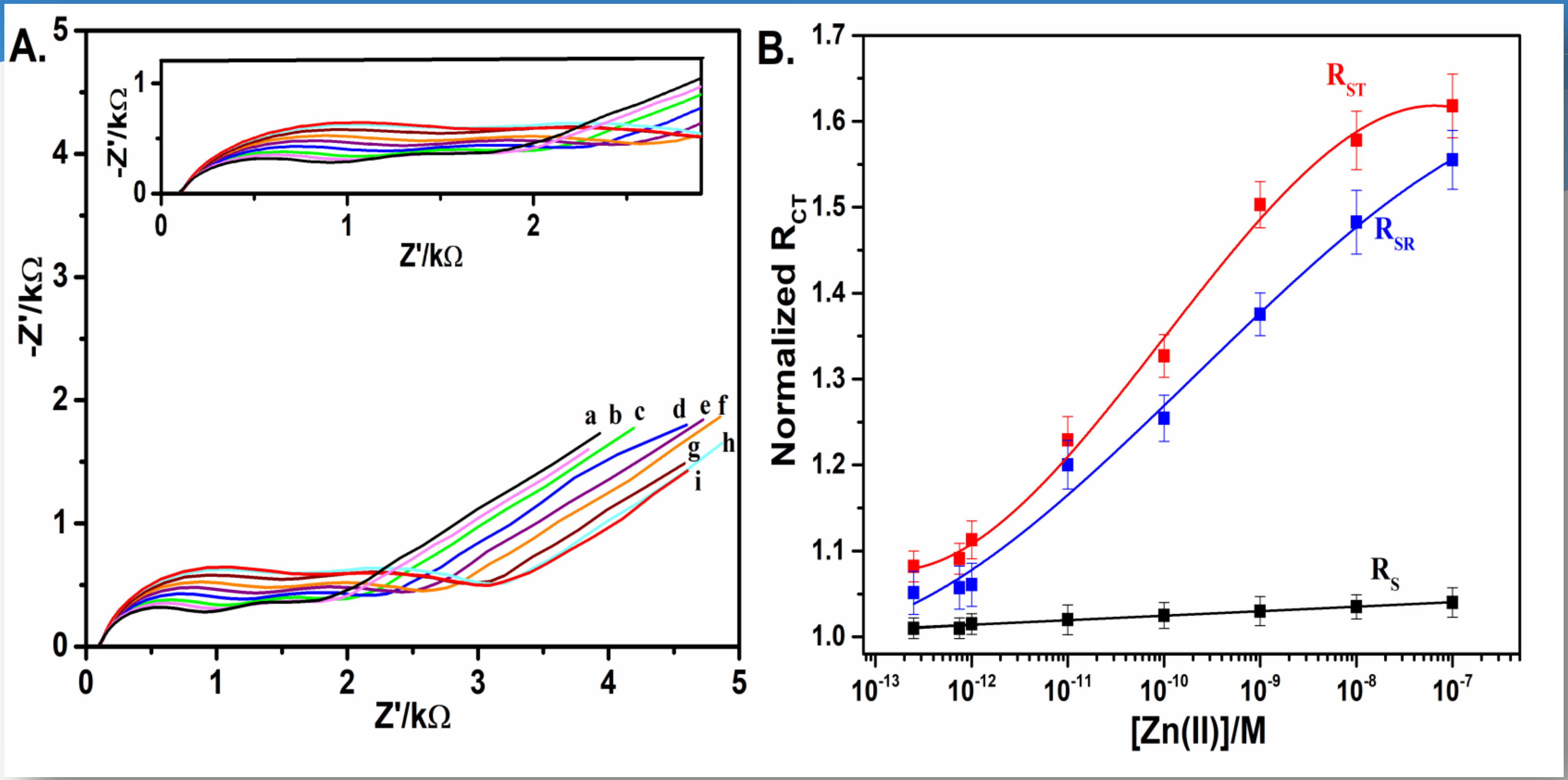
Two diffusion pathways:

OT-Ring

OT-Tail

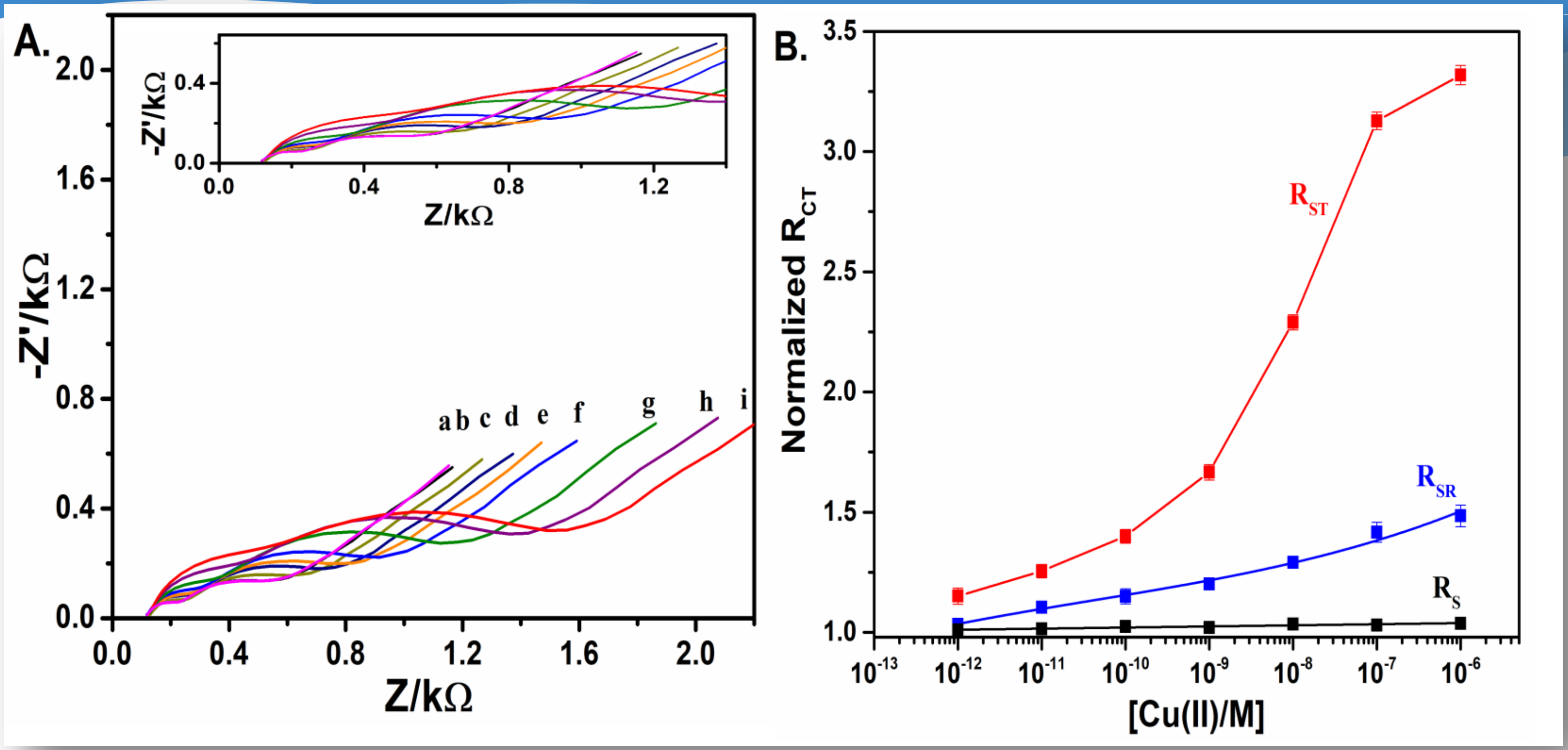


OT-Sensor dose response to Zn^{2+}



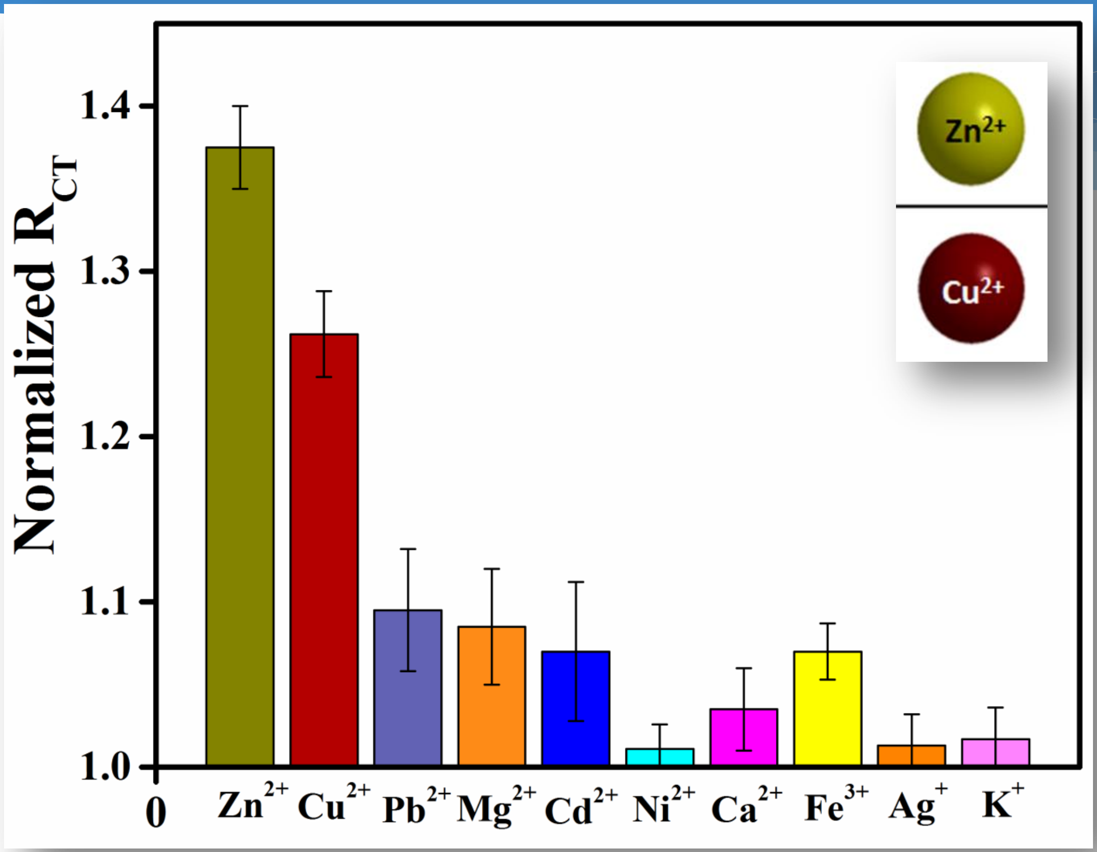
Limit of detection = 100 fM for Zn^{++}

OT-Sensor dose response to Cu^{2+}



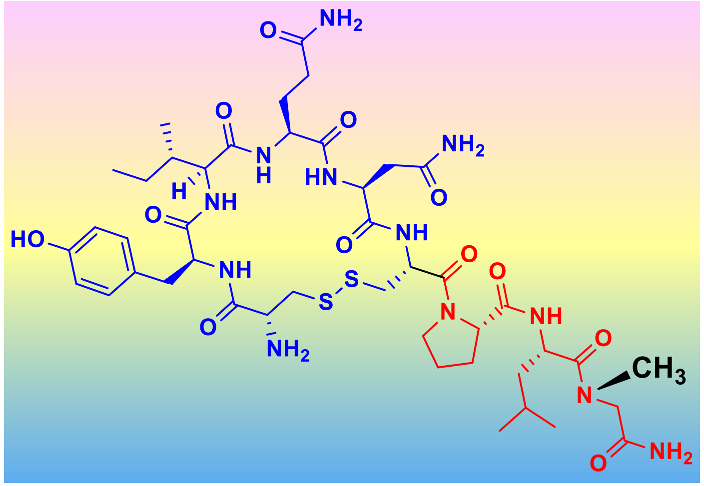
Limit of detection = 500 fM for Cu^{++}

OT-Sensor response
towards various metal ions

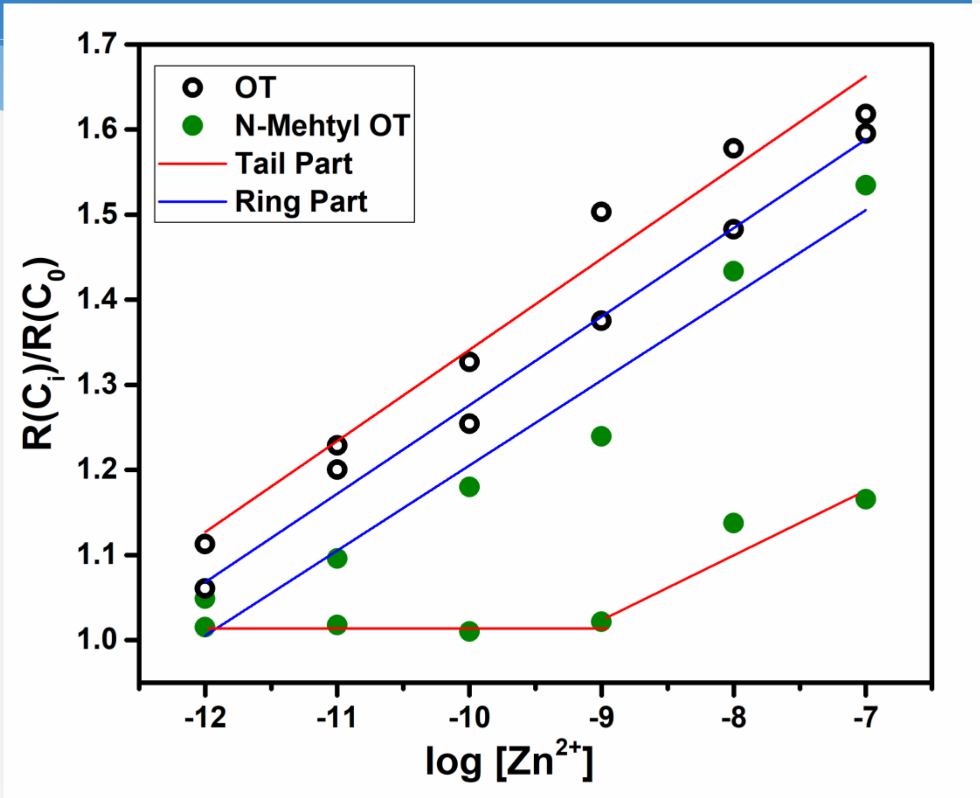


M^{2+} in 1nM concentration

Selectivity *via* OT N-methylation



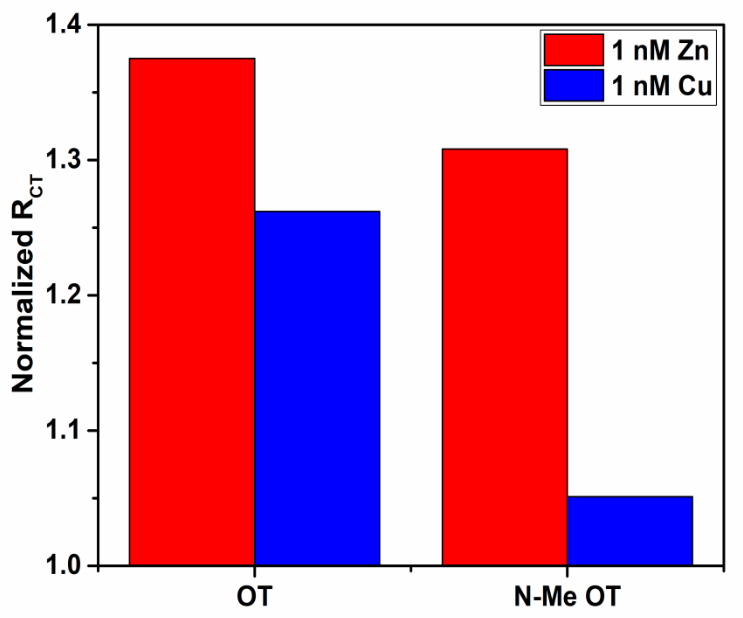
N-Methyl Oxytocin



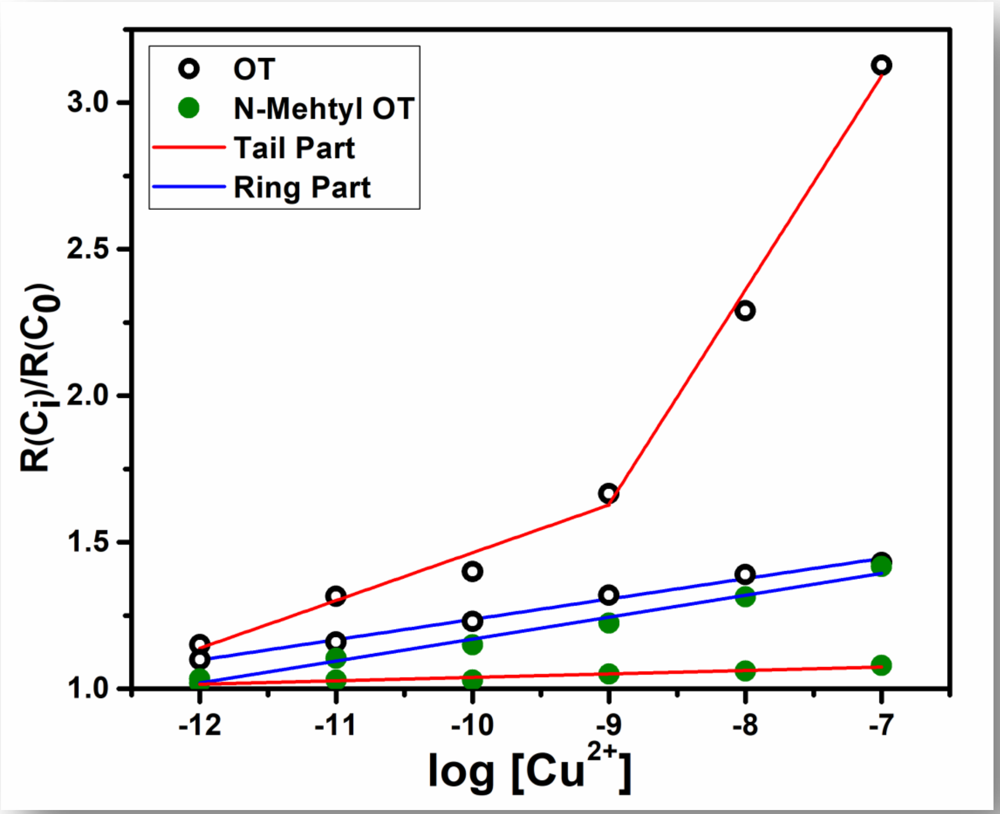
Dose response to Zn^{2+}
before and after methylation of OT

Selectivity *via* OT N-methylation

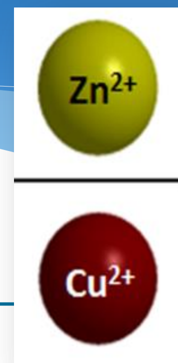
Dose response to Cu^{2+}
before and after methylation of OT

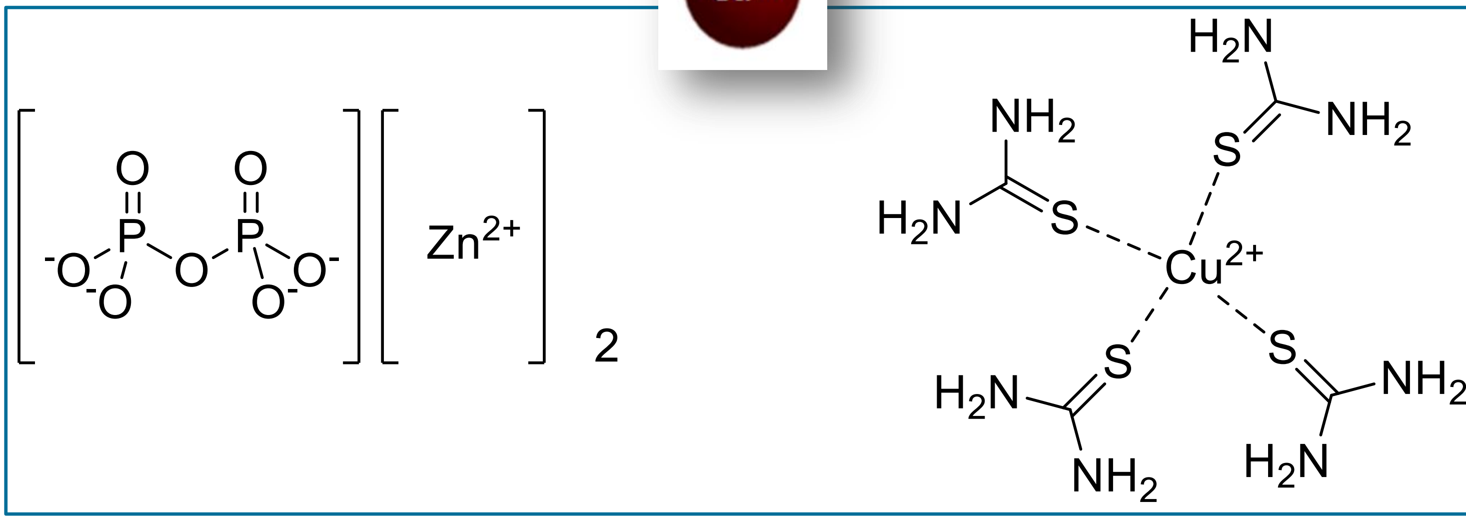


Selective detection of 1 nM Zn^{2+}
& negligible response from 1 nM Cu^{2+}



Selectivity *via* ion-masking

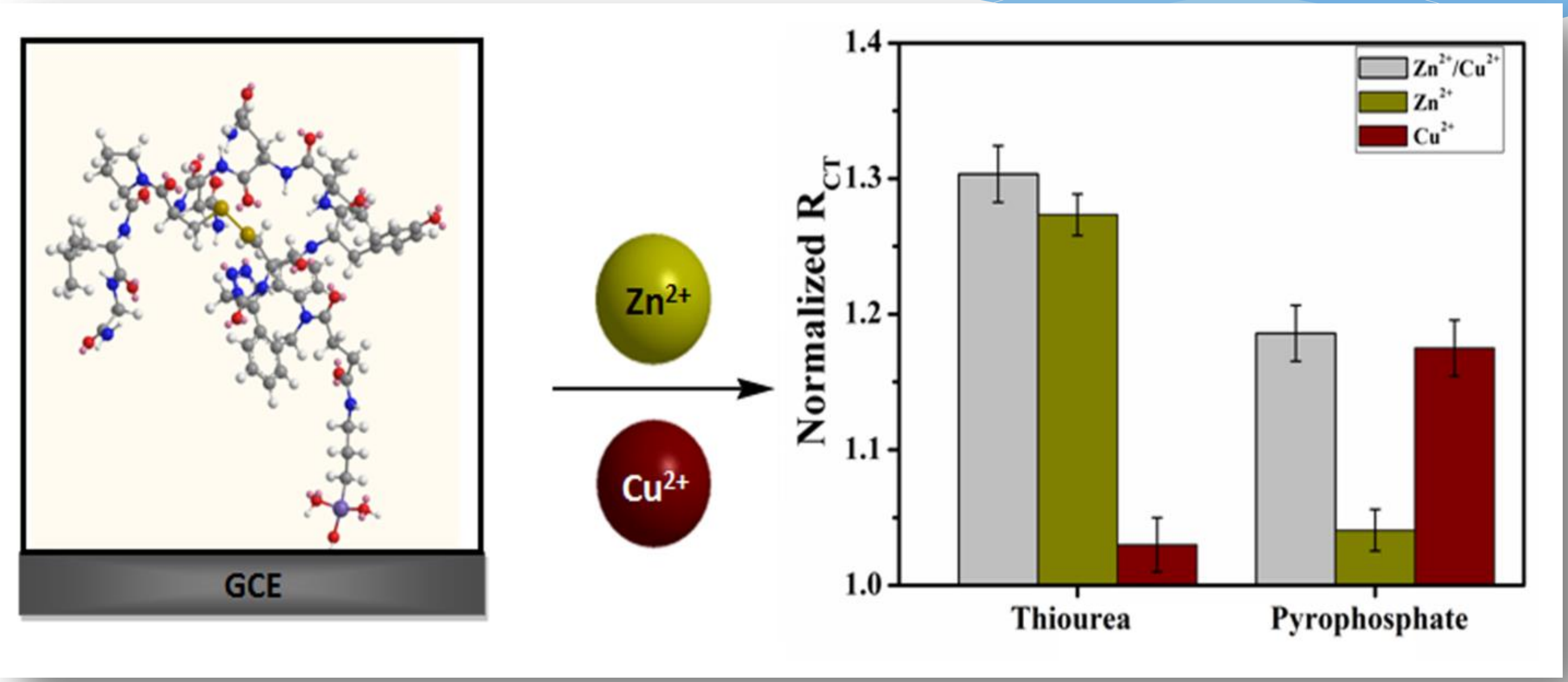




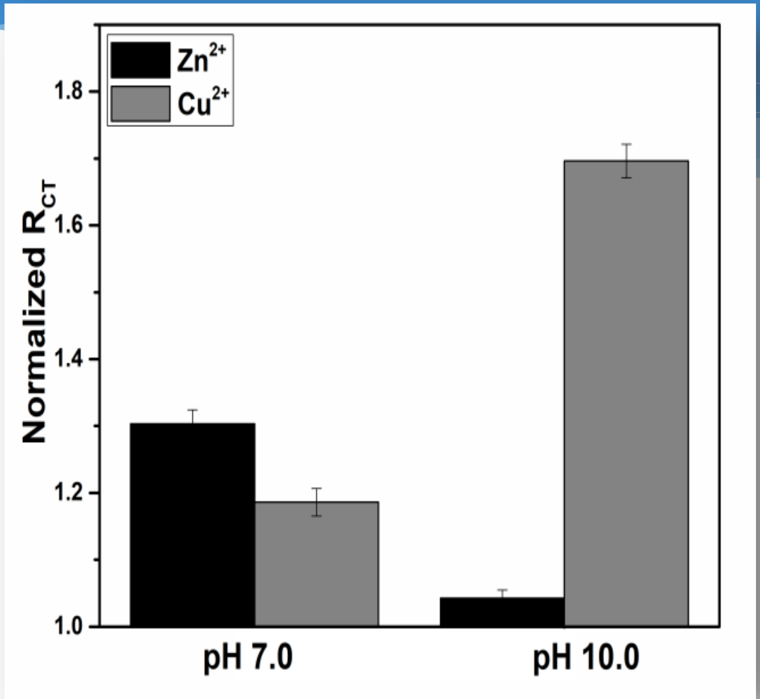
pyrophosphate (PP) and thiourea (TU) masking agents

OT-Sensor selectivity

towards zinc and copper ions

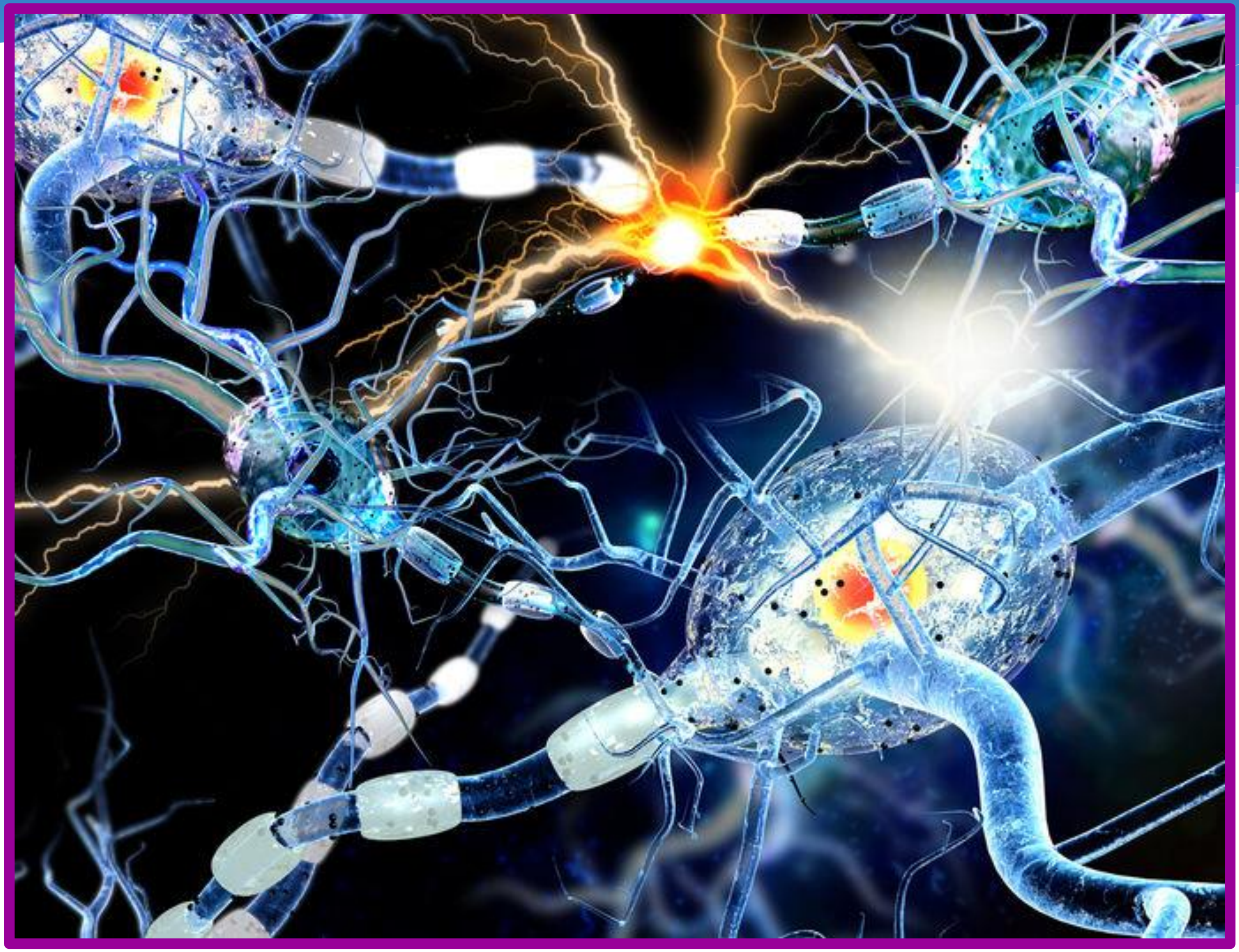


Selectivity by pH change



Selective detection of 1 nM Cu^{2+} at pH 10.0
and negligible response from 1 nM Zn^{2+}at the same pH

Multiple Sclerosis (MS)
the immune system attacks myelin sheaths



Sera samples diagnosis
healthy vs. MS patients

Sera sample	EIS of OT-Sensor		ICP-MS	
	Zn^{2+} [nM]	Cu^{2+} [nM]	Zn^{2+} [nM]	Cu^{2+} [nM]
Healthy	57.5	8.5	54.7	9.4
MS	8.6 - 11.5	5.3 - 4.0	9.6 - 10.6	4.4 - 4.5

Zn^{2+} to Cu^{2+} ratio in healthy ~ 6
and MS patients ~ 2

Summary

- Conformationally adaptive biosensors
- Micro- to Pico-molar sensitivity to Zn^{2+} or Cu^{2+}
- Selectivity for Zn^{2+} or Cu^{2+} was demonstrated:

  masking, pH and N-Me
- Diagnosis: Zn^{2+} to Cu^{2+} ratio in Multiple Sclerosis
- Point-of-care sensing devices

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