Protein Extracting Electrodes Based on Hydrophobic Graphite Cloth

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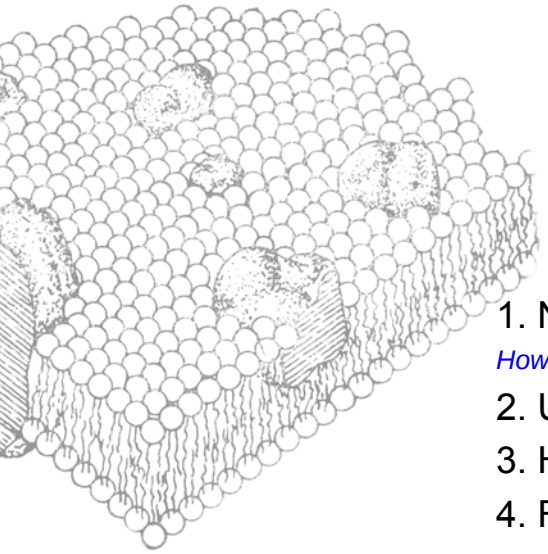
- Disadvantages of Classical Approach
- Thin Liquid Films
- Protein Extracting Electrodes

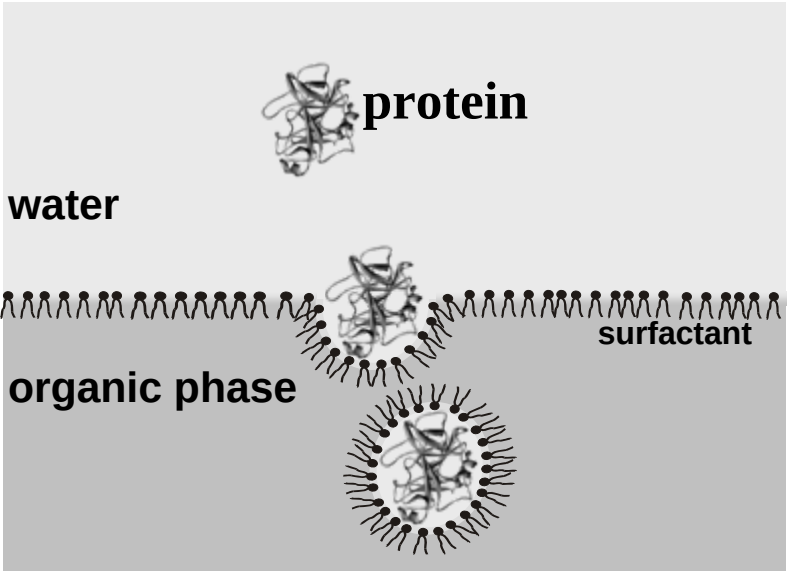
I. Disadvantages of Classical Approach

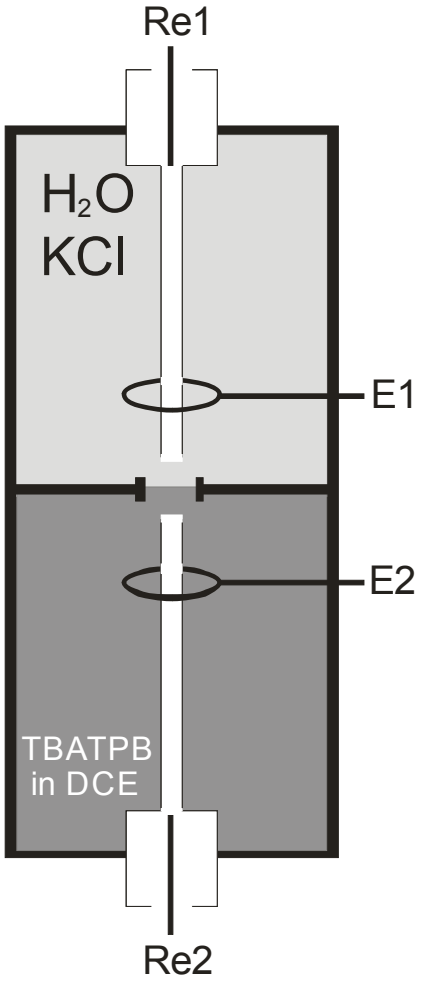


1. Necessity to use polar organic solvents

However the higher dielectric permittivity, the lower average diameter of micelles

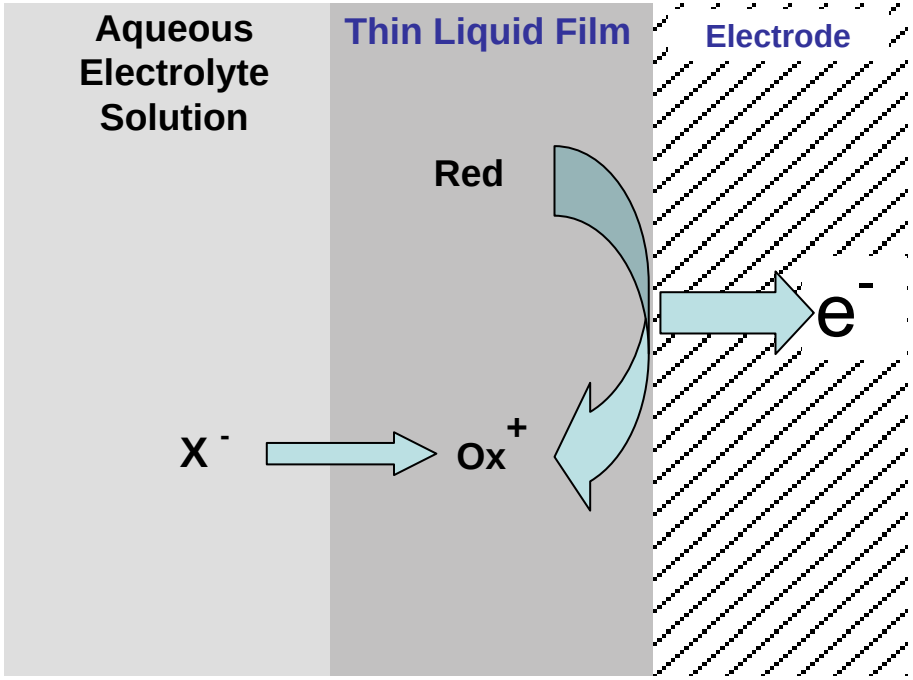
2. Unstable interface
3. High surfactant concentrations lead to interface shatter
4. Four electrode potentiostats





Cell with Interface Between Two Immiscible Electrolyte Solutions

II. Thin Liquid Films



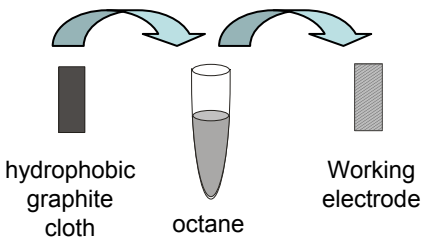
C. Shi, F.C. Anson. Anal.Chem., 70, (1998), 3114.

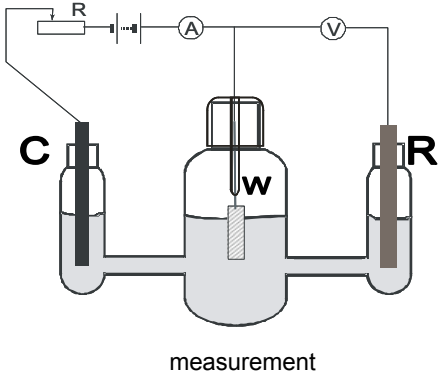
Advantages of electrode shielded with thin liquid layer:

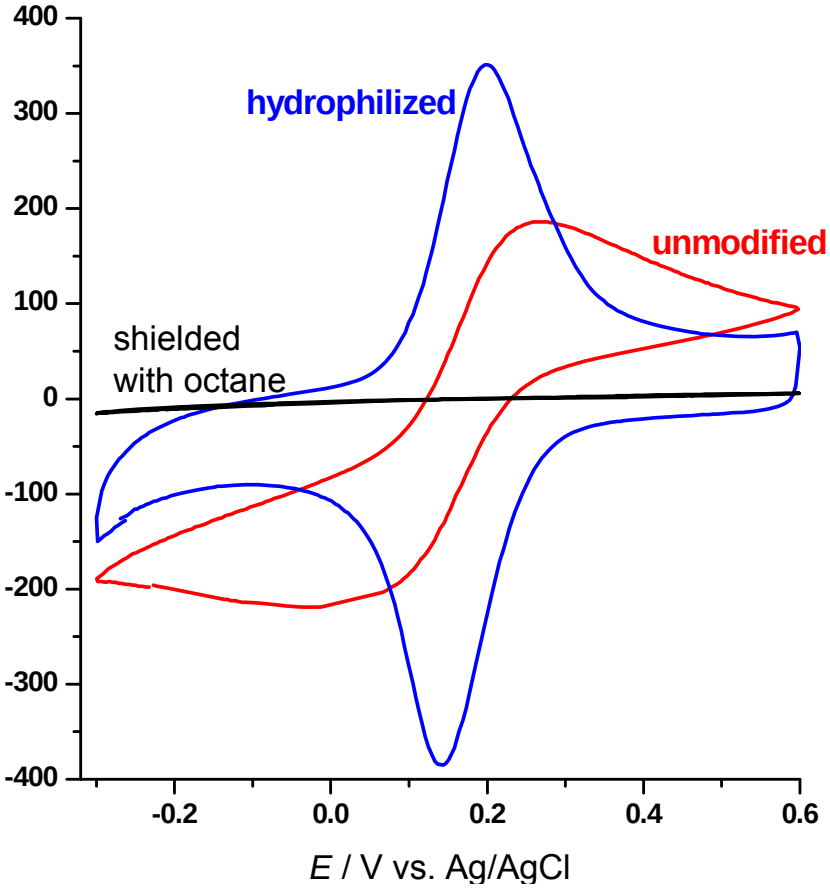
- Common three-electrode cell
- Liquid|Liquid interface is reproducible and easy to be formed
- Non-polar water-immiscible liquids can be used!!!

Surface Shielding

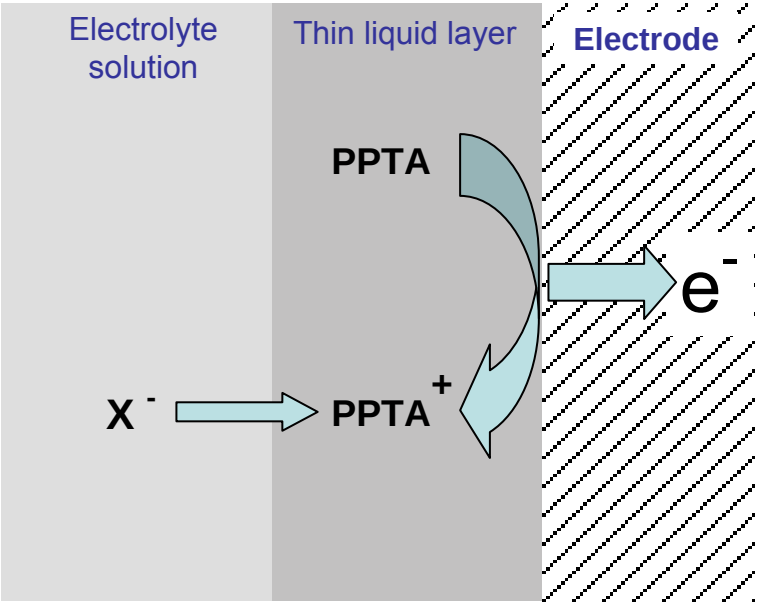
hydrophobic graphite electrode	thin liquid film	water + $Fe(CN)_6^{3+/4+}$

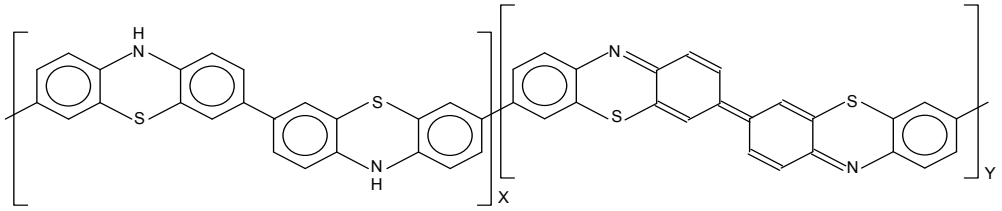




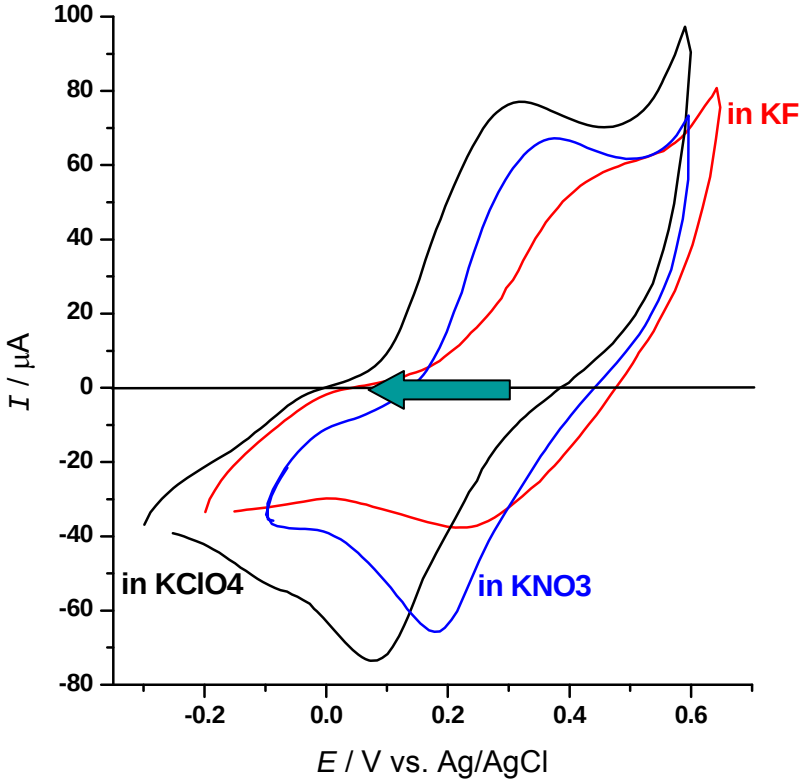


Thermodynamics of Interfacial Ion Transfer





Water-Insoluble Redox Polymer Polyphenotiazine (PPTA)



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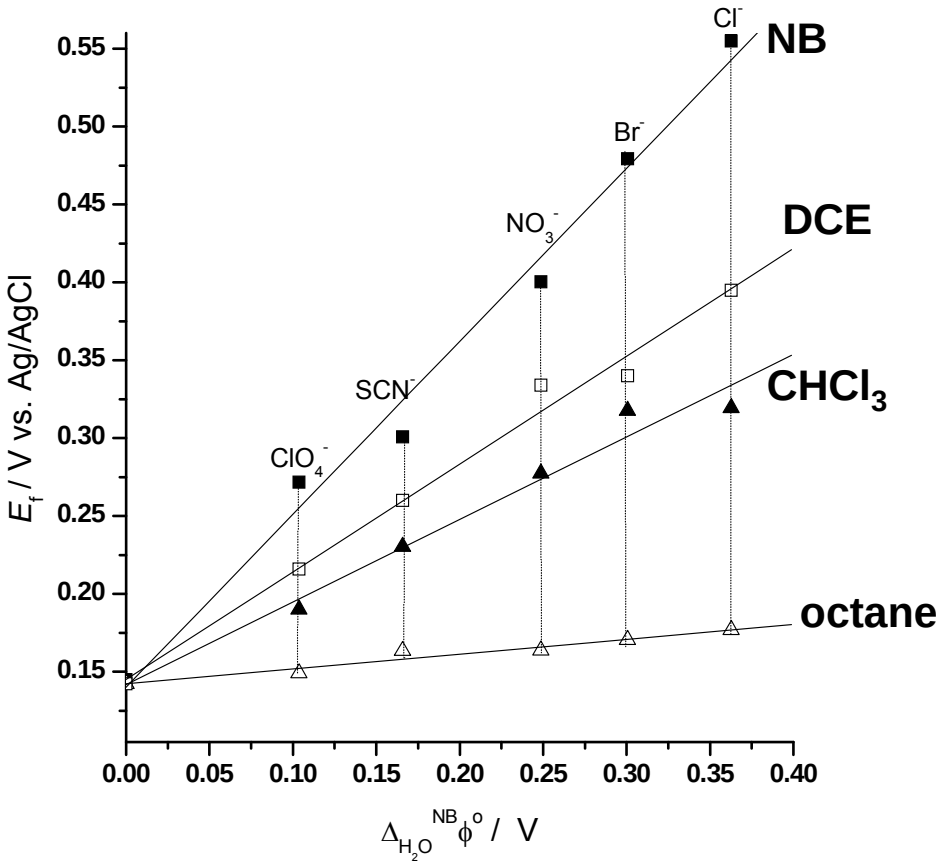
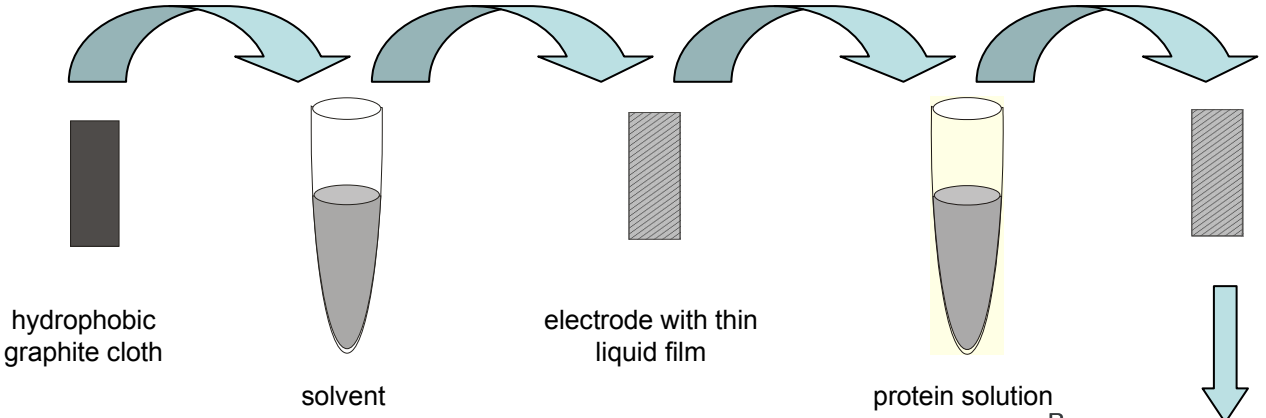


Gibbs free energy of interfacial ion transfer is determined by the entropy in low-polar organic solvents.

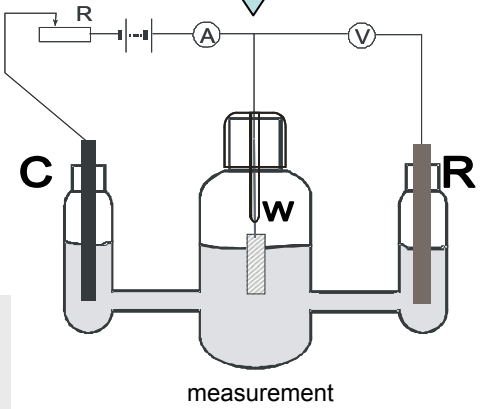
A.A. Karyakin, M.Yu. Vagin et al. J.Phys.Chem. B, 108, (2004), 11591

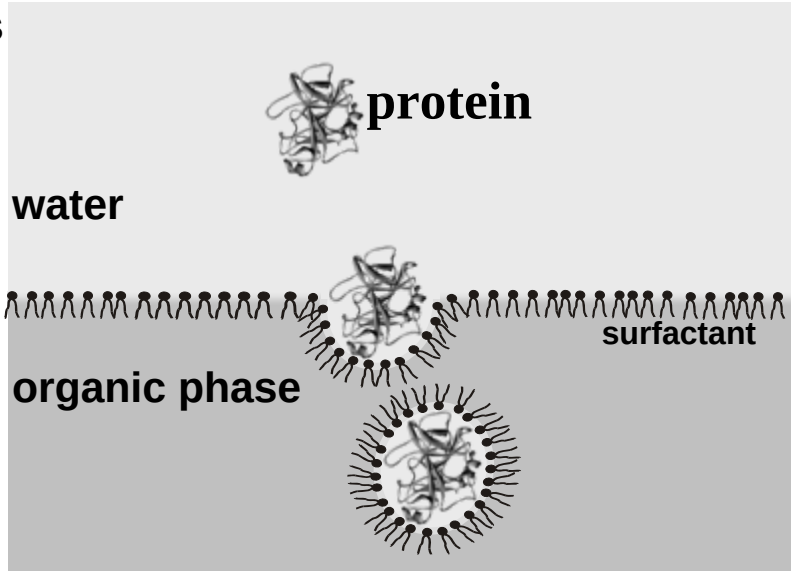
III. Protein Extracting Electrodes

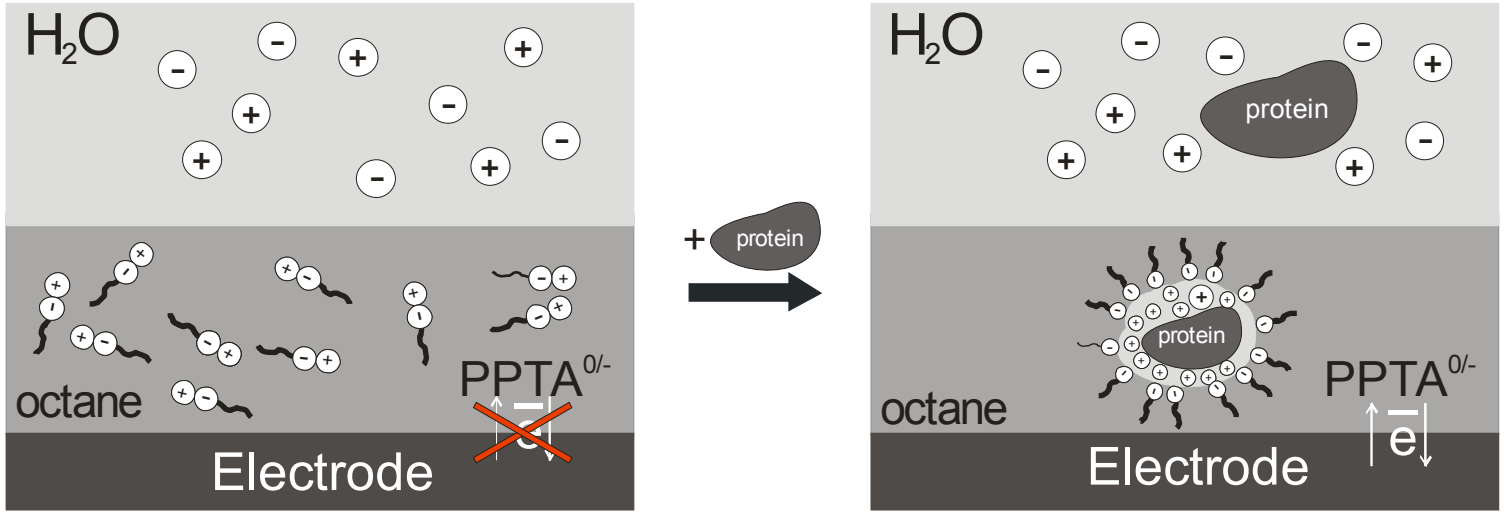


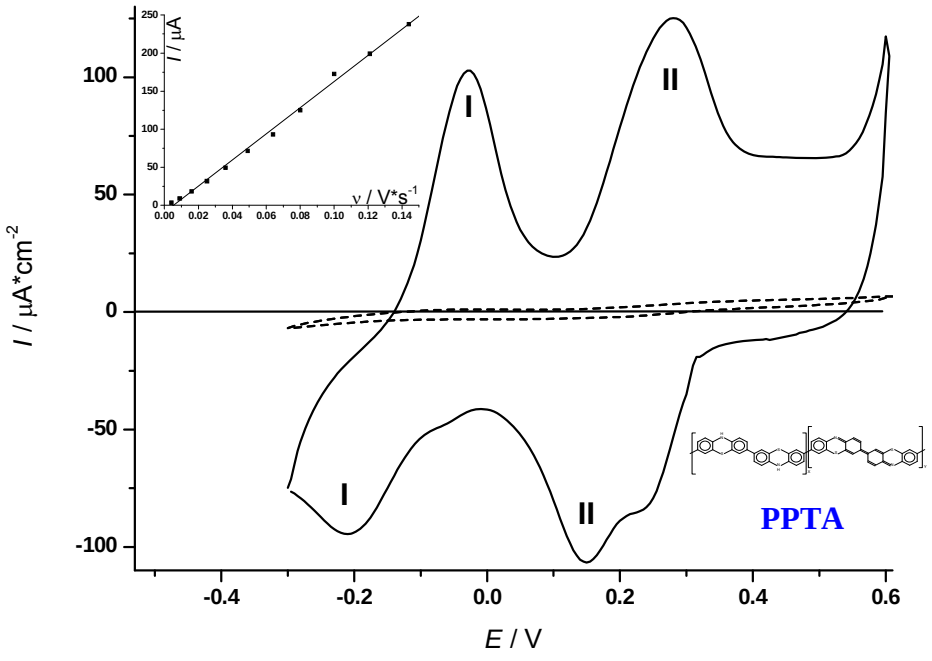
Small redox-inactive proteins are an important analytes:

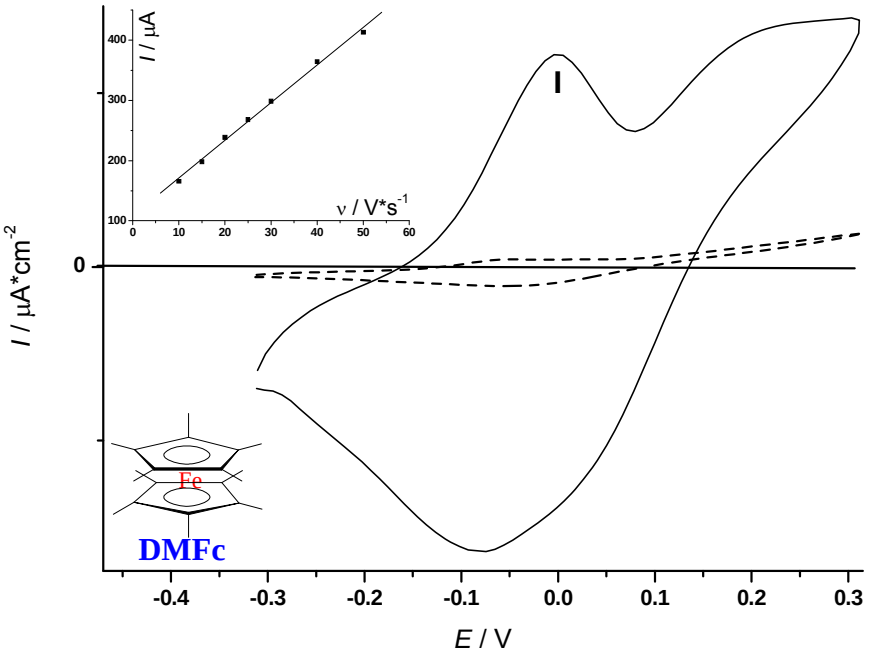
- precursors of certain pathologies
- regulators of physiological activity
- toxins for human or animals
- markers of dangerous viruses



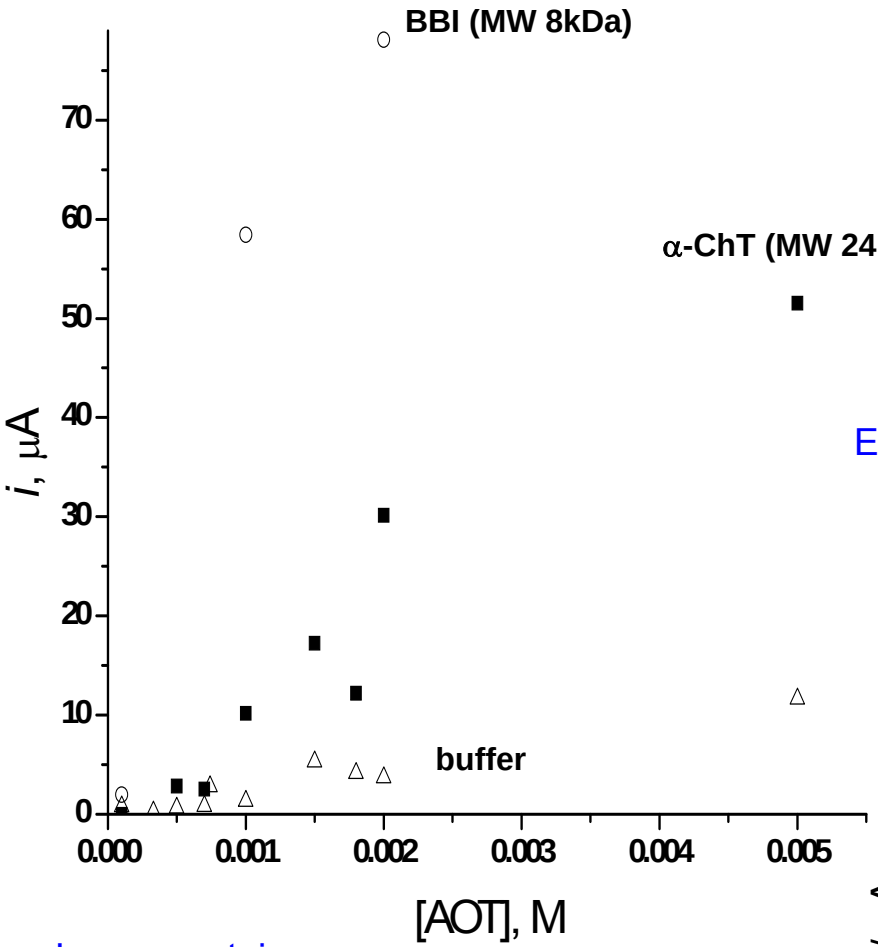






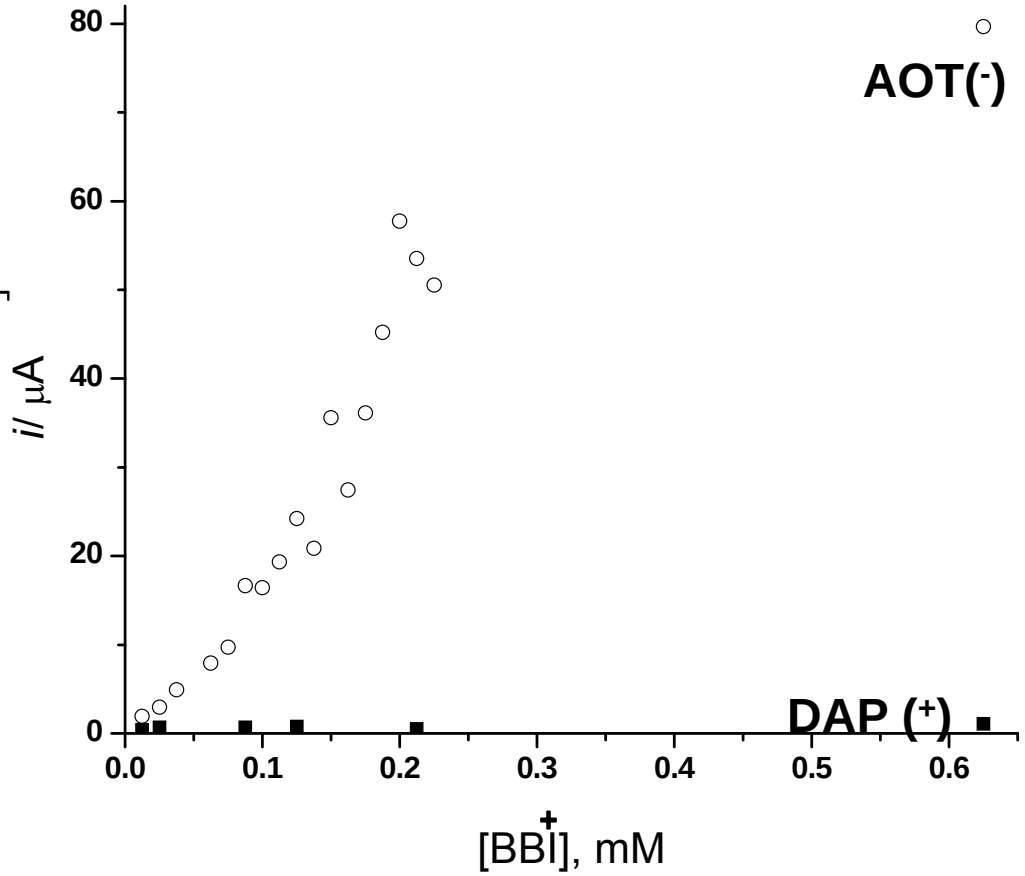


M.Yu. Vagin et al. J.Electroanal.Chem., 584, (2005), 110

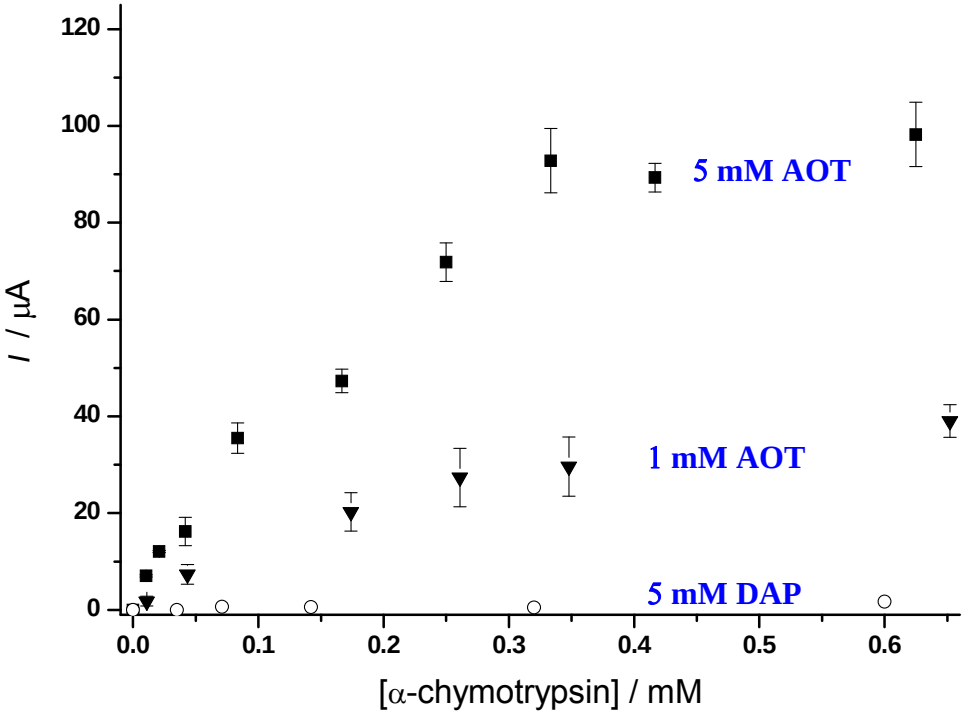


Larger proteins
requires larger amounts of surfactant

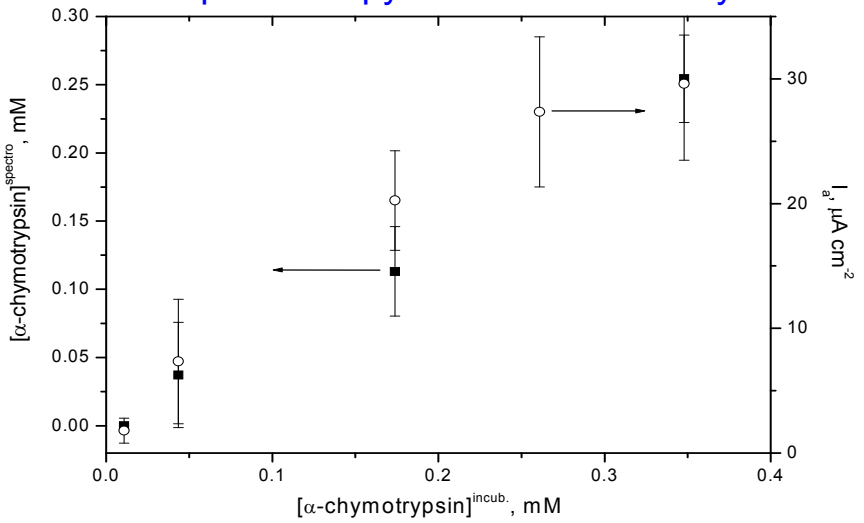
Electrostatic interactions between protein and polar groups of surfactant drastically affect the response



Concentration dependencies

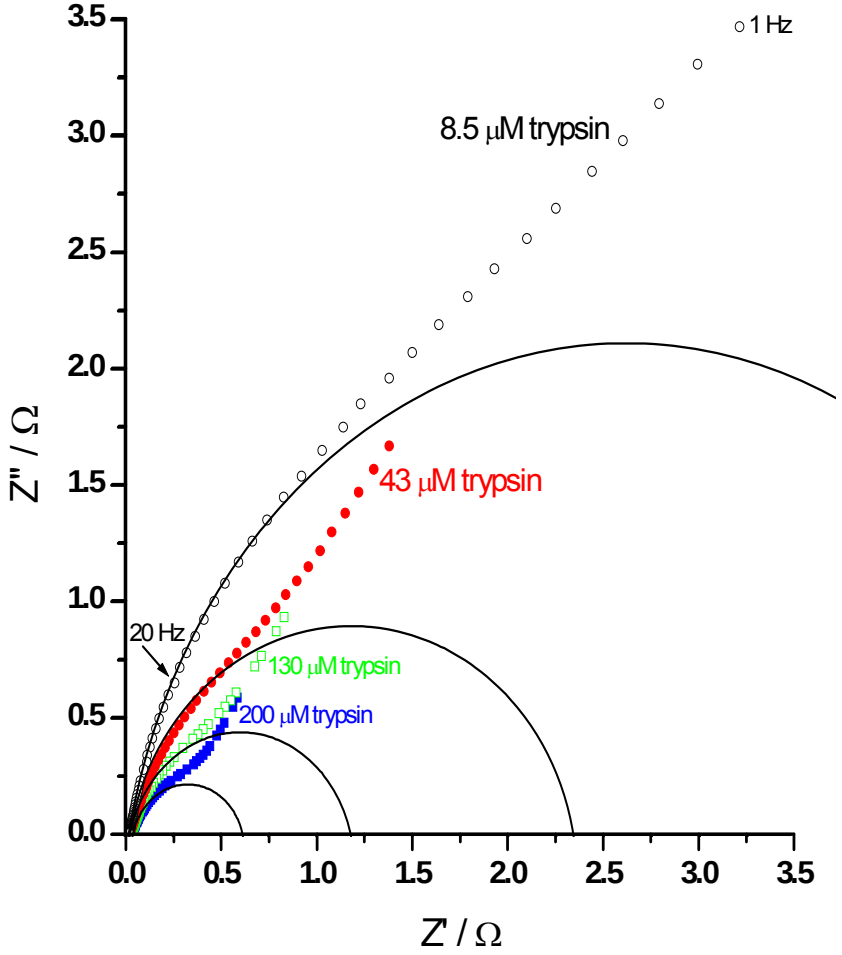


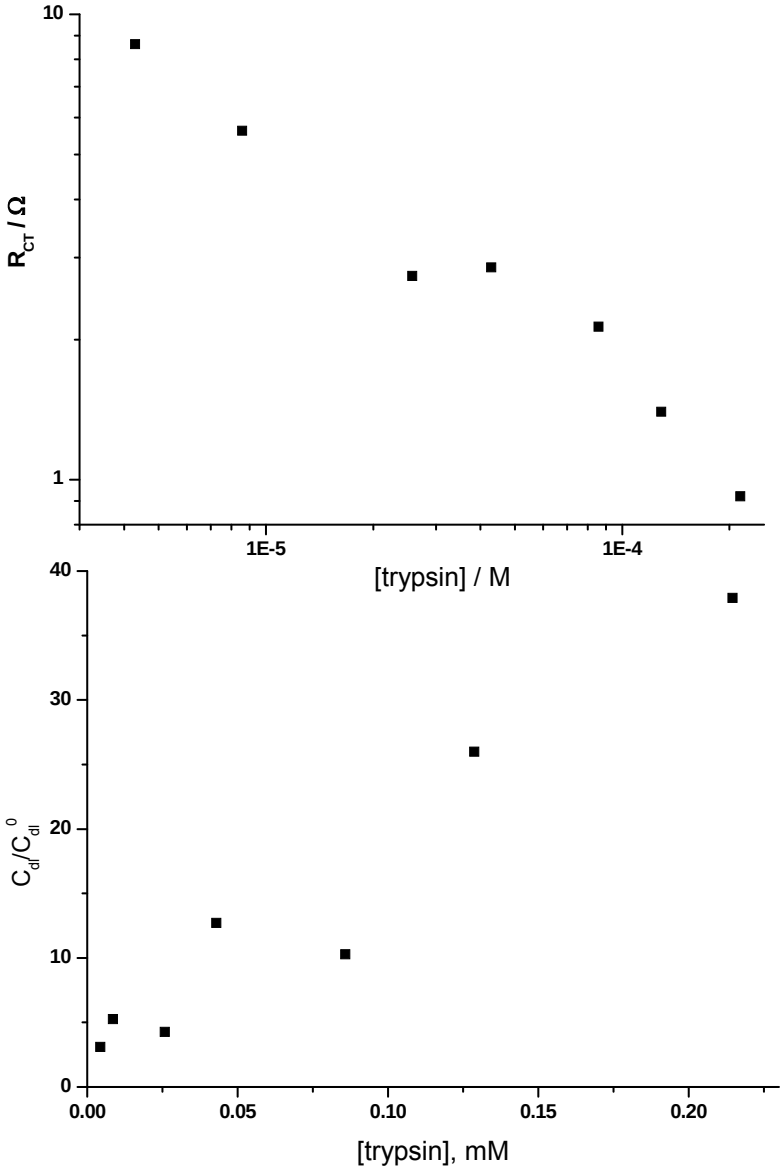
α-ChT enzyme activity measured in shielding layer
"Spectroscopy vs Electrochemistry"



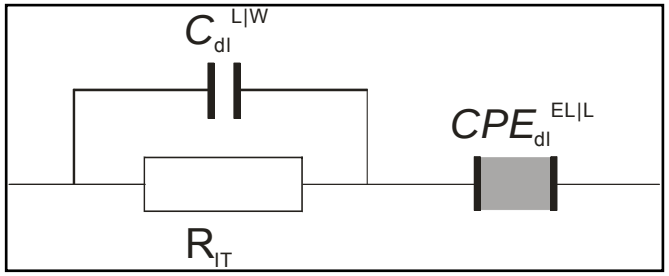
Protein	Molecular Weigh, kDa	Glycosilated	Anodic Current Increase, times	Slope of calibration curve
BBI	8	**no**	25.6	1.36
cytochrom C	12.4	**no**	65.3	1.2
α-chymotrypsin	24	**no**	7.4	0.5
recombinant HRP	34	**no**	35	not determined
native HRP	44	**yes**	4	0.3
recombinant FDH	88	**no**	8	0.45

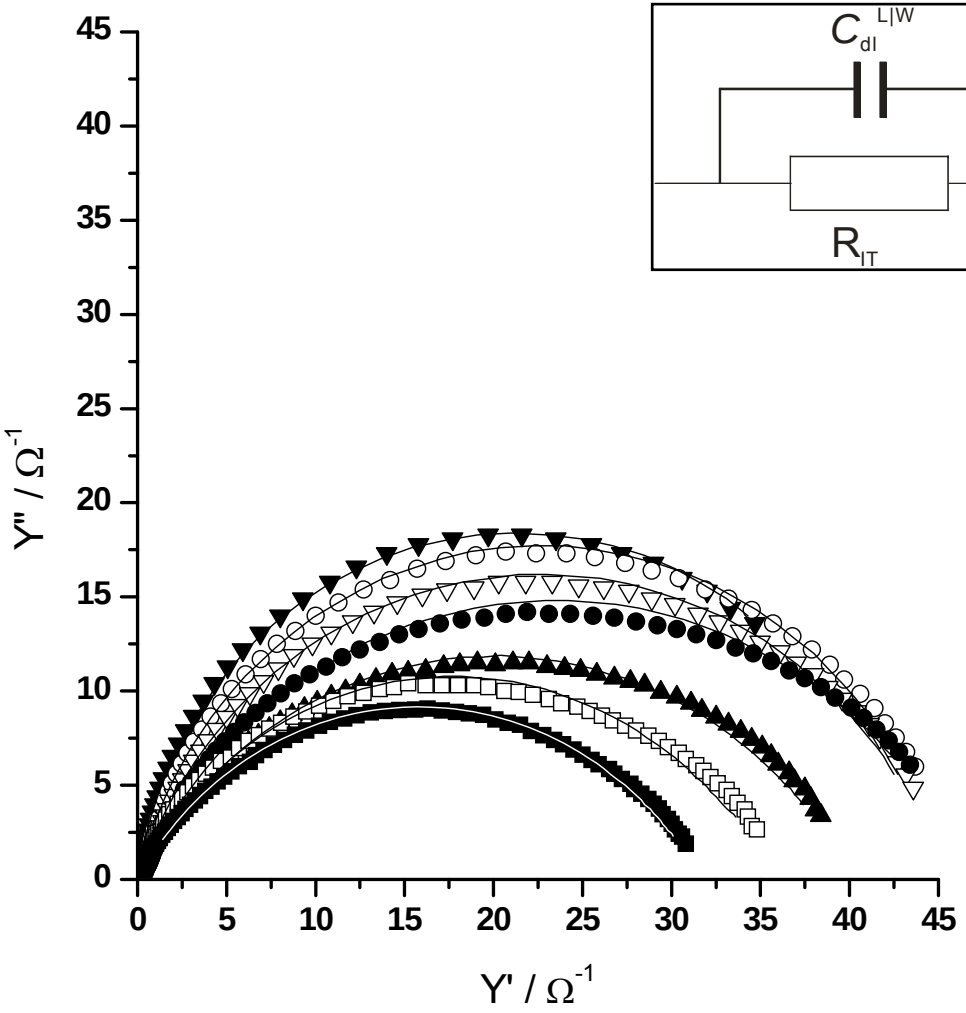
Concentration dependence of impedance

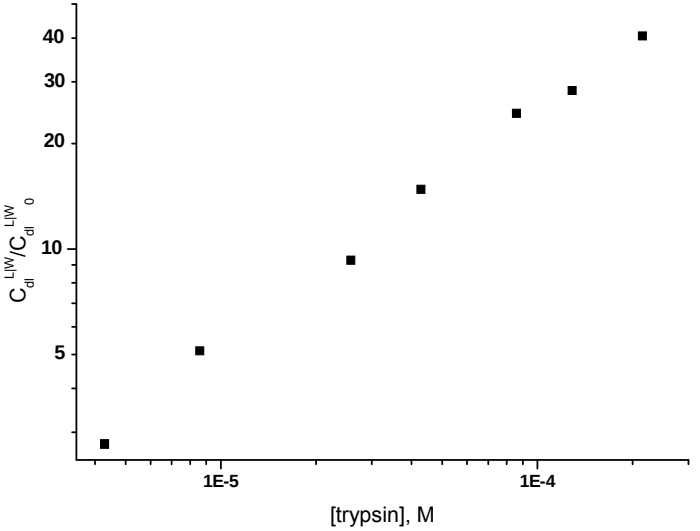




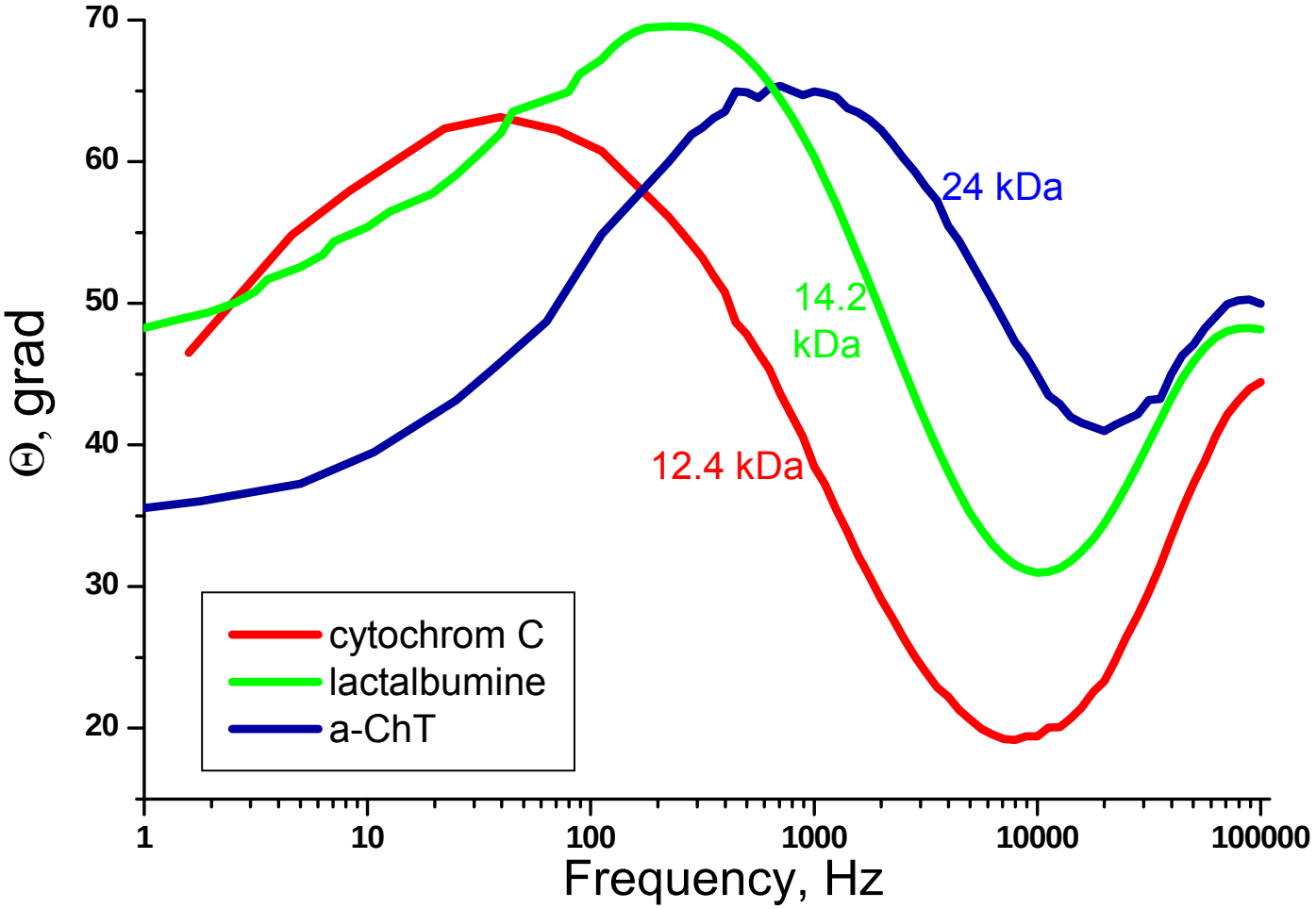
Concentration dependence of impedance







Protein Identification is Possible



Conclusions

- Thin films modified electrodes allow electrochemical studies of IT across the liquid|liquid interface
- Protein extraction can be detected with electrochemistry
- Certain selectivity can be obtained with impedance

Acknowledgments:

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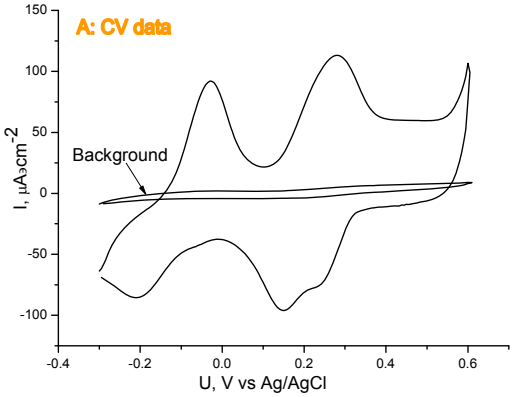
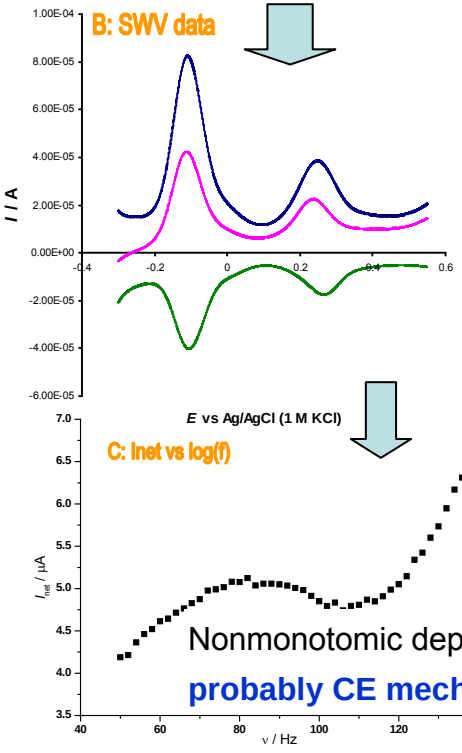
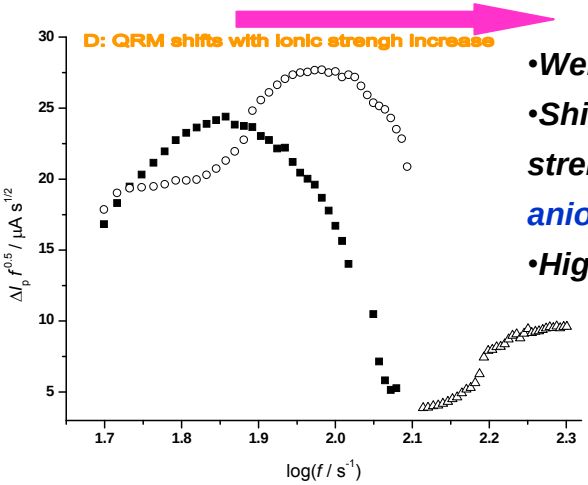
Thank You!

Towards Understanding of Mechanism

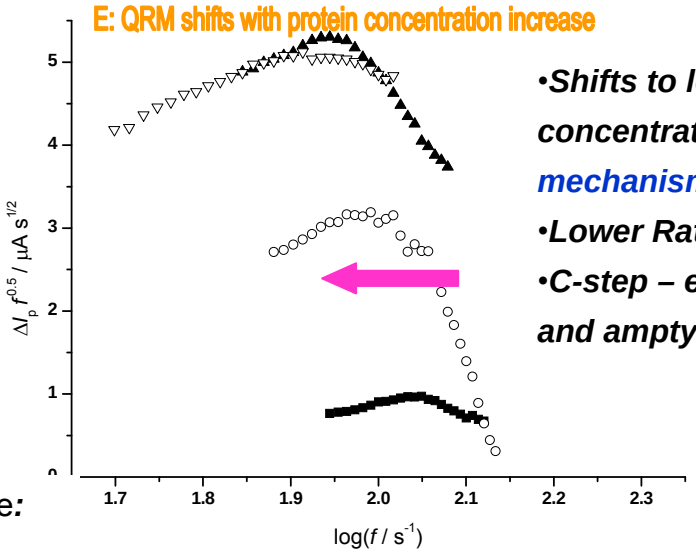




Nonmonotomic dependance*:*
probably CE mechanism



- ***Well-defined QRM***
- ***Shifts to higher rates (frequencies) with ionic strengh increase: cathions expulsion from or anions enter into reversed micelles***
- ***Higher Rate of C-step???***



- ***Shifts to lower rates (frequencies) with protein concentration increase: effect on C-step of CE mechanism***
- ***Lower Rate of C-step***
- ***C-step – exchange of proteins between loaded and ampty micelles ???***

Other Proofs:

- ***back extraction by buffer with high ionic strength***
- ***direct optical measurement of enzyme activity in reversed micelles in octane***