Enzyme electrodes

2. Indirect electrochemical methods (mediated enzyme electrodes)

- the redox-active component of most redox enzymes is encapsulated deep inside the enzyme structure
- effective kinetic barrier for direct electron transfer

\[ \text{electron transfer rate} \equiv k_{ET} \propto e^{\beta(d-d_0)} \cdot e^{-\frac{(\Delta G^0 + \lambda)^2}{4kT\lambda}} \]

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(d)</td>
<td>distance separating acceptor and donor</td>
</tr>
<tr>
<td>(d_0)</td>
<td>Van der Waals distance</td>
</tr>
<tr>
<td>(\Delta G^0)</td>
<td>free energy</td>
</tr>
<tr>
<td>(\lambda)</td>
<td>reorganization energy</td>
</tr>
</tbody>
</table>

The electrochemical insulation of the enzyme active site by its protein shell usually precludes the possibility of any direct ET.
Enzyme electrodes

Some enzymes exhibit direct electron transfer with electrode supports

Incomplete encapsulation of the active center due to:

- presence of channels
- influence of protein orientation
- nano scale morphology of the electrode (modified metal electrodes)
Electron Transfer Mediators

Electrical contacting of redox enzymes can be established by using synthetic or biologically active charge-carriers as intermediates (which are called electron-transfer mediators).

- potential gradient between enzyme and electrode
- mediator cycled between its reduced and oxidized state
- no side reactions with the enzyme
- fast reaction with the redox center of the enzyme
- reversible electrochemistry (fast rate constant) at the electrode

a) diffusional mediators
b) mediator-functionalized electrodes
c) mediator-modified enzymes
d) enzyme bound to polymer matrix
e) interprotein electron transfer
Electron transfer mediators

a) Diffusional mediators

- diffusional penetration of the mediator into the protein
  - short distance for electron transfer

Diffusion controlled by
- hydrophobic/hydrophilic properties of enzyme and mediator
- size and shape of mediator
- electrostatic interaction

→ Ferrocene derivatives as electron acceptors for soluble oxidases (GOx)

Fe \((C_5H_5)_2\)

- redox potential \((E_M^0)\) between +0.1V to +0.4V (SCE)
- rate constant for the reaction of the reduced active center of GOx and an oxidized ferrocene \(~ 10^5 \text{ M}^{-1}\text{s}^{-1}\)
b) Mediator-functionalized electrodes

Interesting for studying affinity interactions

Electrocatalytic current developed by the C$_{60}$-modified electrode in the presence of GOx and glucose
c) Mediator-modified enzymes

Electrical “wiring” of enzymes
Chemical modification of redox enzymes with electron mediators to enable nondiffusional ET

Covalent attachment of electron mediators units at the protein periphery and inner sites yields short ET distance

- e⁻ hopping or tunneling between periphery and active sites

Glucose Oxidase covalently modified with ferrocene electron-relay groups

- increasing loading of mediators enhances electrical contacting, but decreases of enzyme activity. Optimal loading (Fc-GOx) ~ 12-13 units
→ multilayer-enzyme assemblies functionalized with ET mediators

The electrode oxidizes the ferrocene group and then the ferrocene oxidizes the Gox redox site
d) Enzyme bound to a polymer matrix

- Organic polymers provide stable biocatalyst interfaces to enzymes
- Electrical contacting of immobilized enzymes:
  - conducting polymer
  - incorporation of mediators in the polymer film

Graphite electrode modified with polyallylamine matrix containing GOx and Fc
Electron transfer mediators

→ enzymes in mediator-functionalized sol-gel matrices

ferrocene mediators

Electrical characteristic improved by the incorporation of electroconductive materials (carbon powder or metal particles)

· the presence of mediators enhances electron hopping between the enzyme and the nearest graphite particle.
Electron transfer mediators

e) Interprotein electron transfer

- Small molecular electron mediators offer little selectivity in their electronic reactions, as they exchange e\textsuperscript{-} with any acceptor or donor close to them.

Proteins can be used as EM showing high selectivity

→ Soluble cytochromes as ET mediators

Bioelectrocatalyzed reduction of O\textsubscript{2} by COx mediated by cyt c and cyt c\textsubscript{551} in a multistep ET
Amperometric DNA sensors

**General DNA sensor design**

<table>
<thead>
<tr>
<th>Type of sensor</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct DNA electrochemistry</td>
<td>Highly sensitive (femtomoles of target); requires no labeling step; amenable to a range of electrodes</td>
<td>High background signals; cannot be multiplexed; destroys the sample</td>
</tr>
<tr>
<td>Indirect DNA electrochemistry</td>
<td>Highly sensitive (attomoles of target); usually requires no labeling step; multiple-target detection at same electrode</td>
<td>Probe substrate can be difficult to prepare; destroys the sample</td>
</tr>
<tr>
<td>DNA-specific redox indicator detection</td>
<td>Moderate to high sensitivity (femtomoles of target); well suited to multiple-target detection; samples remain unaltered</td>
<td>Chemical labeling step required unless ‘sandwich’ method used; sequence variations can be problematic</td>
</tr>
<tr>
<td>Nanoparticle-based electrochemistry amplification</td>
<td>Extremely sensitive (femtomole to zeptomole range, $10^{-15}$ to $10^{-21}$ moles); well suited to multiple-target detection with different nanoparticles</td>
<td>Many development steps in assay; reliability and robustness of surface structures problematic; sample usually destroyed</td>
</tr>
<tr>
<td>DNA-mediated charge transport</td>
<td>Highly sensitive (femtomole range) and simple assay; requires no labeling; uniquely well suited for mismatch detection; sequence independent; amenable to multiplexing; applicable to DNA-protein sensing step</td>
<td>Biochemical preparation of target sample required</td>
</tr>
</tbody>
</table>

Direct electrochemistry with DNA

Electrochemical activity of nucleic acids bases

- Thymine
- Cytosine
- Adenine
- Guanine

: reduction at mercury electrodes
: oxidation at carbon electrodes
: chemically reversible red/ox of G at mercury electrodes

- irreversible cathodic reduction of CA observed in ssDNA even at neutral pH, when suitable salt (0.3M) screens the negative charged phosphates of the NAs
- large potentials are needed → high background current
- destructive process
Indirect electrochemistry with DNA

Use of electrochemical mediators

→ Complexes of Ruthenium and Osmium to mediate the electrochemical oxidation of guanine

· The electrode oxidizes the reduced metal complexes which then come into contact with DNA

· Guanine residues in DNA can reduce the metal complex, regenerating the reduced mediator

How to detect the hybridization process?

dsDNA is more negative than ssDNA

⇒ after hybridization the oxidized metal complex (positively charged) is attracted more efficiently to the dsDNA
Specific redox indicator detection

Interaction between small redox molecules and NAs

A

Electrostatic

Binding

Groove

Intercalation

Fe(CN₆)⁴⁻

Ir_complex

DM

Ru⁺

MB

Groove-binder
electrostatic

Fe(CN₆)⁴⁻

Ru(NH₃)₅Cl²⁺

B

Ir(bpy)(phen)(phi)³⁺

DM (daunomycin)

MB (methylene blue)
Specific redox indicator detection

DNA modified with osmium tetroxide bipyridine (Os,bipy)

III: reduction of A and C

1: Os-modified DNA
2: unmodified ssDNA
Electrochemical detection of single-base mismatches

This method takes advantage of the electronic structure of double-helical DNA, using intercalated redox probe molecules, to report perturbation in base stacking.

- Current flows through the well stacked DNA and the redox intercalator DM can be either reduce or oxidize.
- Stacking mismatches reduce (or even suppress) the current flowing along the DNA.
DNA-mediated charge transfer

Electrocatalysis enhanced detection

Current flows through the well stacked DNA to reduce MB$^+$ to LB$. LB goes on to reduce ferricyanide in solution, regenerating MB$^+$ catalytically.

DNA sequence: SH-5'-AGTACAGTCATCGCG

CV on dsDNA-modified Au electrodes

fully base-paired

single mismatch
In-vivo amperometric measurement of neurotransmitters

applications

- Need for understanding the neurochemical activity of the living mammalian brain
- To know about: concentration, lifetime and regulation of electroactive substances in the extracellular space of the living brain.

<table>
<thead>
<tr>
<th>substrates</th>
<th>concentrations</th>
</tr>
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<tbody>
<tr>
<td>Dopamine</td>
<td>Lactate</td>
</tr>
<tr>
<td>Glutamate</td>
<td>( \text{H}_2\text{O}_2 )</td>
</tr>
<tr>
<td>Nitric acid</td>
<td>( \text{O}_2 )</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
</tr>
</tbody>
</table>

- Understand pathological conditions affecting the human brain:
  - Parkinson’s disease, Alzheimer’s disease, traumatic brain injuries, schizophrenia, etc.

some demanding conditions

- measurements with micrometer spatial resolution
- little disruption of the delicate brain tissue

----- carbon fiber ----- microelectrodes ( \( \Phi \approx 10\mu\text{m} \) )

neuronal cell in the mammalian brain \( \approx 10\text{-}20\mu\text{m} \) diameter

blood capillaries separated by distances of 20-100 \( \mu\text{m} \)
After an action potential arrives at a nerve terminal, a finite number of synaptic vesicles release their neurotransmitters into the extracellular space.

- **diffusion process**
- **clearance process**
  - i) transport back to nerve terminals
  - ii) metabolized into inactive products

\[
\frac{\partial C(x,t)}{\partial t} \equiv D \frac{\partial^2 C(x,t)}{\partial x^2} - kC(x,t)
\]

- Concentration time dependence
- Diffusion term
- Clearance term
Release of neurotransmitters

\[ \frac{\partial C(x,t)}{\partial t} = D \frac{\partial^2 C(x,t)}{\partial x^2} - kC(x,t) \]

\[ C(x,t) = \frac{C_0}{2} \left[ \text{erf} \left( \frac{h-x}{2\sqrt{Dt}} \right) + \text{erf} \left( \frac{h+x}{2\sqrt{Dt}} \right) \right] \exp(-kt) \]

large diffusion effect: after 50ms the maximum concentration is less than 2% of the original value

the concentration profile does not extended more than a few micrometers from the source

h=100nm
D=2.4\times10^{-6} \text{ cm}^2/\text{s}

In-vivo amperometric measurement of neurotransmitters

\( h=100\text{nm} \)
Monitoring electrically evoked dopamine release

Electrical stimulation of dopamine axons that passes through a brain region called the medial forebrain bundle (MFB).

Small current pulses stimulate action potential which travel along the axon and evoke dopamine release.

Before (solid line) and after (dotted line) administration of nomifensine, which inhibits dopamine transporter.

Tissue damage when big electrodes are used.
Detection of other substances

Make use of the selectivity which offers the incorporation of enzymes

Most of the enzyme sensors are based on oxidase enzymes that generate H$_2$O$_2$, a substance that can be oxidized at the electrode surface, but at high potentials

- large background currents (low sensitivity) and oxidation of other compounds (low specificity)

Glutamate carbon fiber microelectrode

Carbon fiber microelectrode functionalized with horseradish peroxidase (HRP), glutamate oxidase (GOx), and a polymer with Os$^{2+}$/Os$^{3+}$ redox couple

- Glutamate is oxidized by GOx producing H$_2$O$_2$, which is reduced by HRP.

- HRP is reduced by the Os$^{2+}$/Os$^{3+}$ redox couple, which is subsequently reduced at the electrode by applying a very low potential (-100mV vs Ag/AgCl)
Detection of glutamate

TTX (tetrodotoxin) inhibits the propagation of action potentials and the subsequent release of neurotransmitters.

Detection of \( \text{H}_2\text{O}_2 \)

Many neurodegenerative diseases, for instance Parkinson’s disease, are thought to result from oxidative stress, a condition characterized by an excess of reactive oxygen species, such as \( \text{H}_2\text{O}_2 \).

An increase of dopamine may induce an overproduction of peroxide.

Electrically evoked dopamine release results in a biphasic \( \text{H}_2\text{O}_2 \) increase.