ATP synthesis coupled to the electroenzymatic activity of a hydrogenase immobilized at an electrode/biomimetic membrane interface
Energy generation in anaerobic bacteria by H₂ oxidation

Functional reconstitution of *E. coli* ATP-synthase on a modified gold electrode
QCM and AFM study of ATP-synthase reconstitution on a supported phospholipid bilayer

\[ \Delta m \text{ (ng/cm}^2\text{)} \]

\[ \text{Time (min)} \]

\[ \sim 18 \text{ ng/cm}^2 \text{ ATP-ase} \]

ATP hydrolysis activity of ATPase reconstituted on a gold electrode

Spectrophotometric detection of phosphate production with green malachite
Redox probe for measuring proton translocation across the supported bilayer


**Figure:**
- **Left panel:** Diagram of a redox probe showing 4-ATP and the supported bilayer with chemical structures.
- **Middle panel:** Comparison of current (µA) with potential (V vs. SHE) for 1st and 15th scans.
- **Right panel:** Normalized current (µA) at pH 8.1, 7.0, 6.2, 5.5, showing a decreasing trend with respect to pH. The linear regression equation is given as $Y = 0.833 - 0.064X$, with an R² of 0.93.

**Differential pulse voltammetry measurements**
Proton pumping by ATPase while hydrolyzing ATP

The reconstituted ATPase on the electrode is functional and is mostly oriented in the correct way
Co-immobilization of NiFeSe Hase and ATPase on a gold electrode with a supported phospholipid bilayer
Phospholipid bilayer formation on top of a hydrogenase monolayer covalently bound to 4-aminthiophenol-Au

Before treatment with Triton X100

After treatment with Triton X100

AFM characterization

Generation a proton gradient across the supported phospholipid bilayer

The electrocatalytic oxidation of H₂ by the immobilized hydrogenase induces a pH gradient across the biomimetic membrane

Co-immobilization of NiFeSe Hase and ATPase on a gold electrode with a supported phospholipid bilayer
ATP production coupled to electroenzymatic H₂ oxidation

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The membrane-bound Ni-Fe-Se hydrogenase from *Desulfovibrio vulgaris* Hildenborough

**Catalytic advantages:**
- Activation is fast upon reduction
- High H$_2$-production activity
- Oxygen tolerant during H$_2$-production activity

Phospholipid bilayer formation on top of a hydrogenase monolayer covalently bound to 4-aminothiophenol-Au

Cyclic voltammetry of electrocatalytic oxidation of H₂ by DET

Generation a proton gradient across the supported phospholipid bilayer

Control experiments

![Graph showing normalized current vs. potential (V vs. SHE) for different gases.](image-url)
- Further characterization and optimization of the ATP regeneration system.
- Improve the operational stability of the process
- Is it the hydrogenase, the ATPase, the Au-SAM or the PhBL integrity that limits the stability?
- To study oxygen sensitivity of the process.
- To couple a biochemical reaction to the ATP regeneration system.
QCM characterization of phospholipid bilayer formation on top of a hydrogenase monolayer

Hydrogenase immobilization: $\Delta (f_{N}/N)_{7} = -42$ Hz, $\Delta \text{Diss}_{7} (10^{-6}) = 4.4$, coverage = 8 pmol/cm$^2$

Phospholipid bilayer formation: $\Delta (f_{N}/N)_{7} = -21$ Hz, $\Delta \text{Diss}_{7} (10^{-6}) = 2$