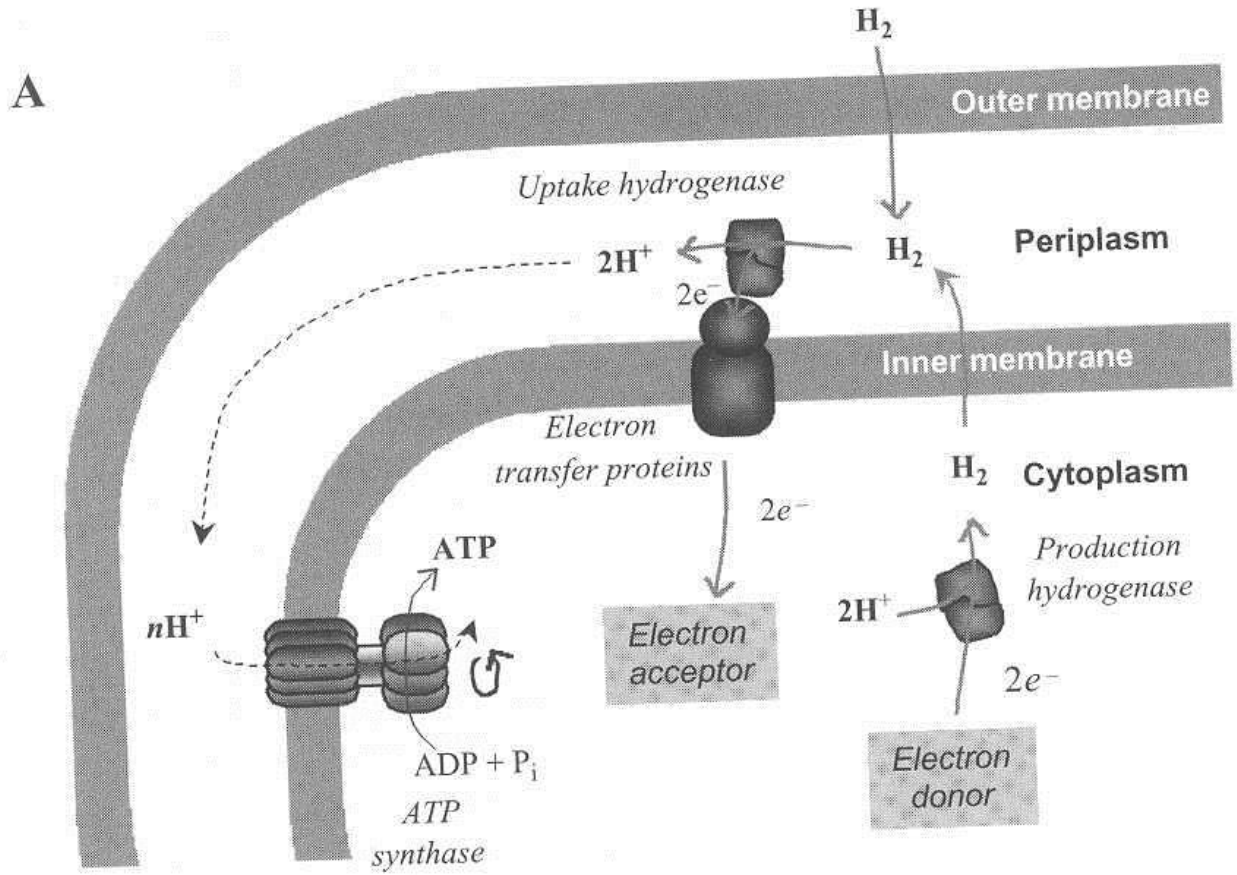
Instituto de Catálisis y Petroleoquímica

CSIC (Spanish National Council of Research)

Madrid, Spain

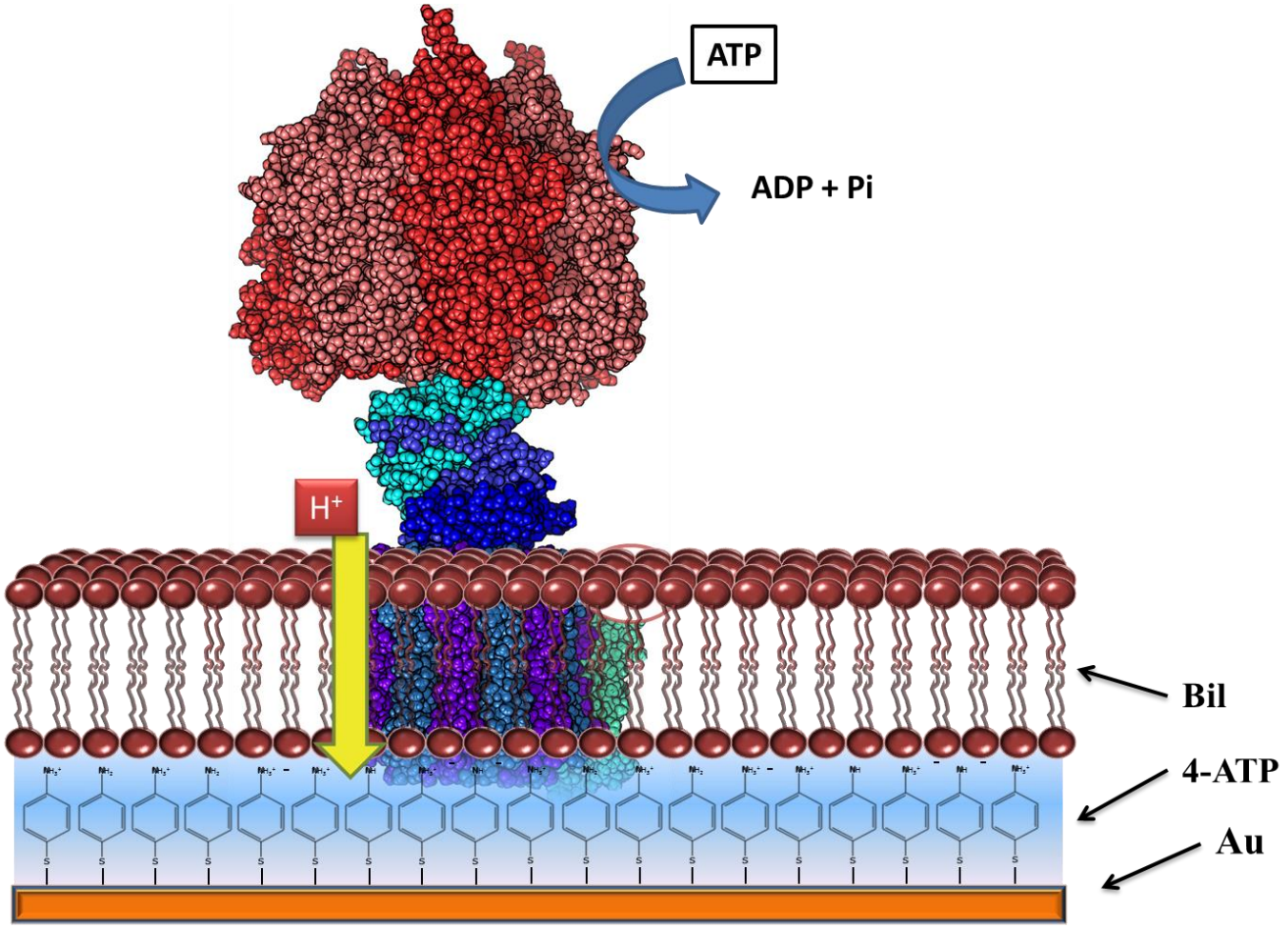
ATP synthesis coupled to the electroenzymatic activity of a hydrogenase immobilized at an electrode/biomimetic membrane interface

Energy generation in anaerobic bacteria by H_2 oxidation

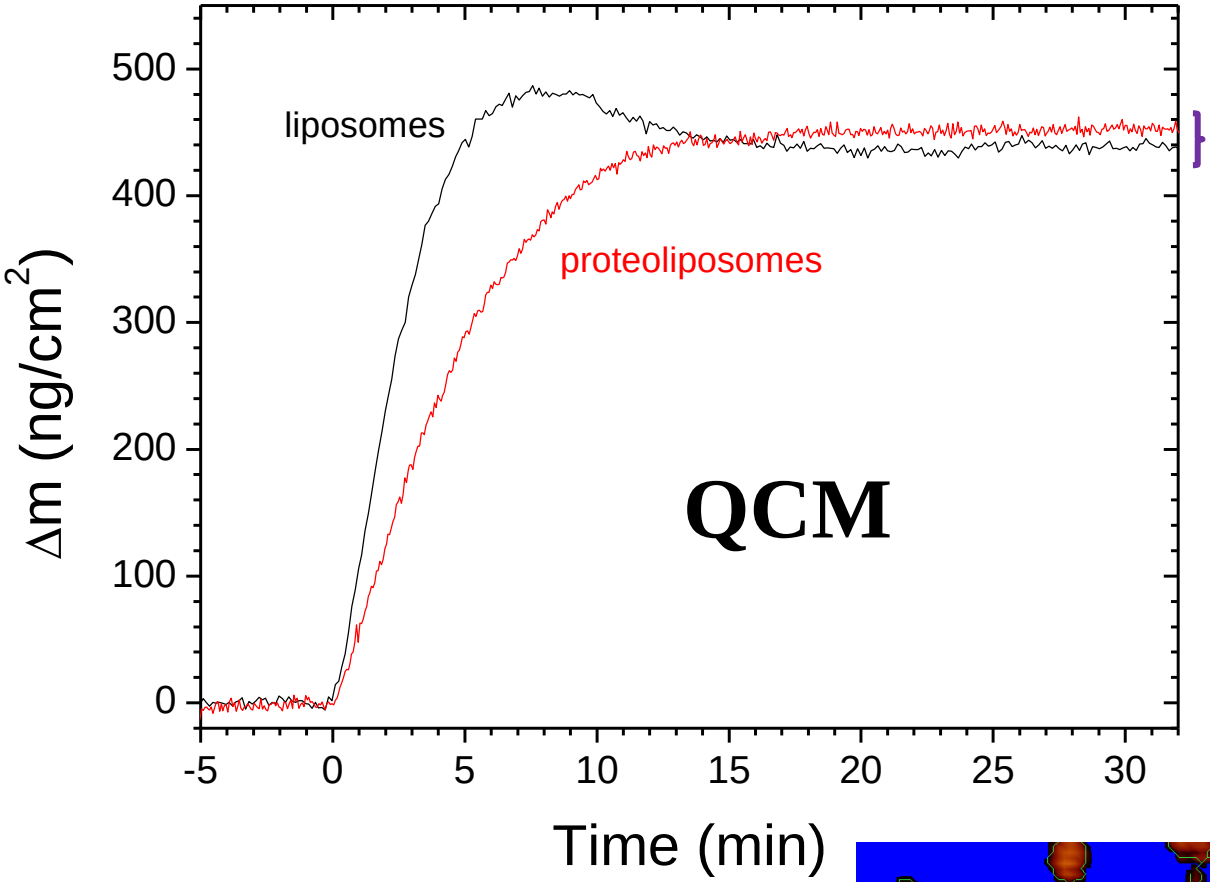


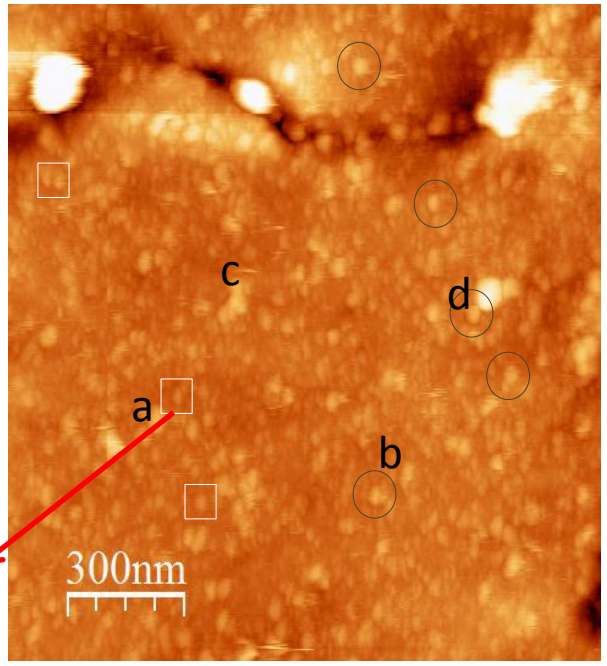
R. Cammack. Hydrogen as a Fuel, Learning from Nature. Taylor & Francis, London 2001

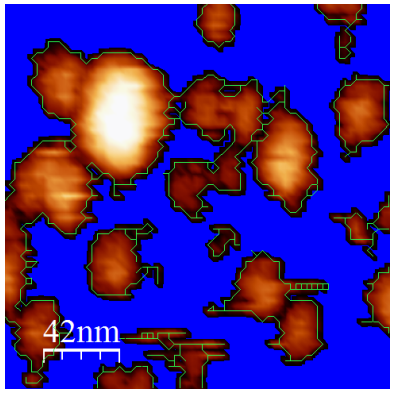
Functional reconstitution of *E. coli* ATP-synthase on a modified gold electrode



QCM and AFM study of ATP-synthase reconstitution on a supported phospholipid bilayer



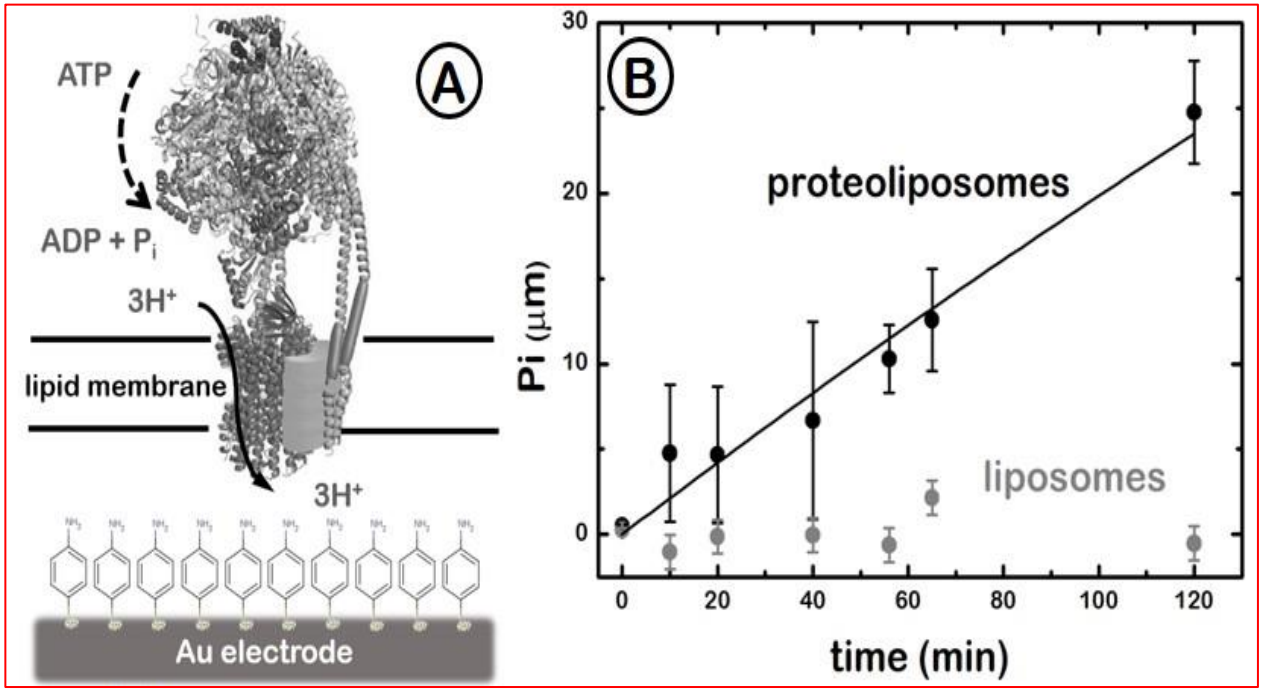




AFM

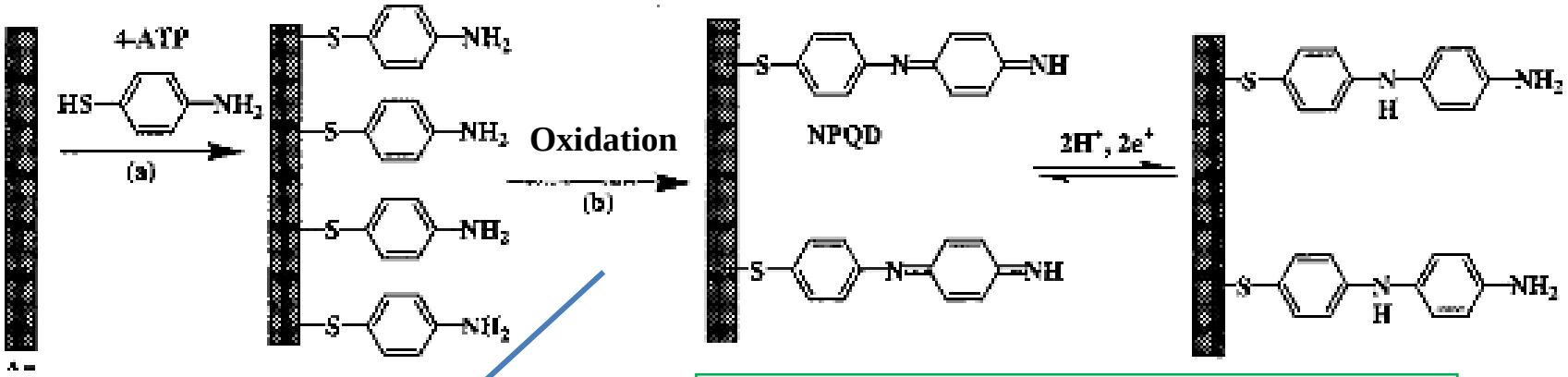
O. Gutiérrez-Sanz, P. Natale, I. Márquez, M. C. Marques, S. Zacarias, M. Pita, I. A. C. Pereira, I. López-Montero, A. L. De Lacey, M. Vélez. Angew. Chem. Int. Ed. 55 (2016) 6216-6220.

ATP hydrolysis activity of ATPase reconstituted on a gold electrode

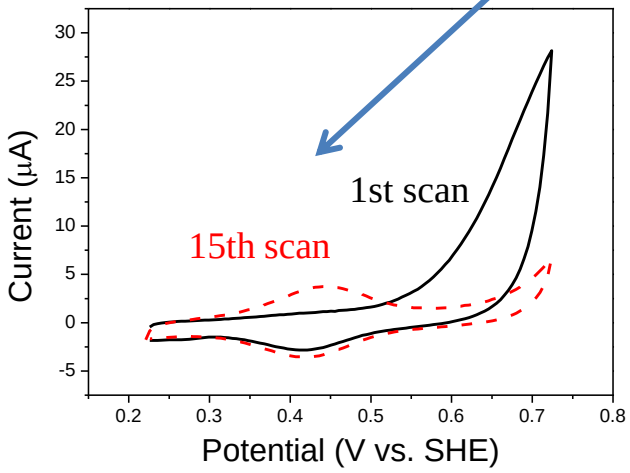


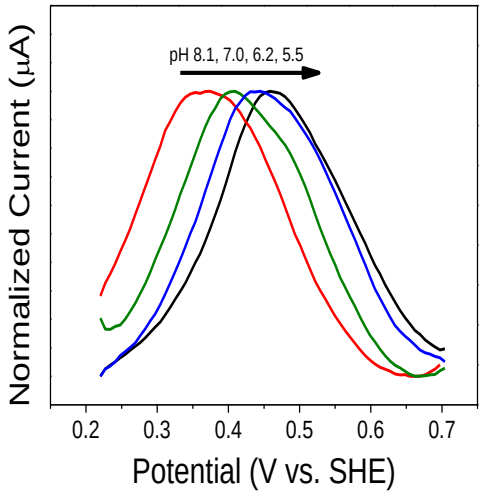
Spectrophotometric detection of phosphate production with green malachite

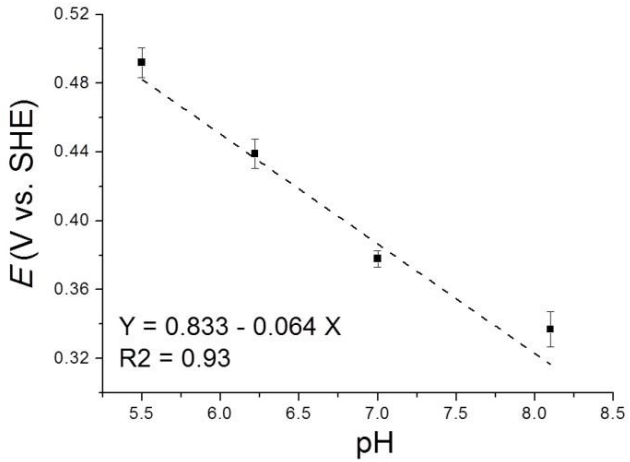
Redox probe for measuring proton translocation across the supported bilayer



O. R. Raj *et al. Langmuir*, 17, 7378-7386 (2001)

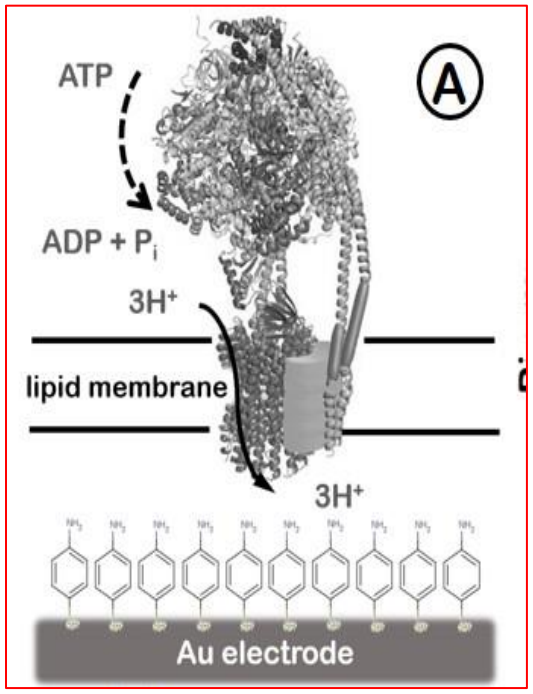


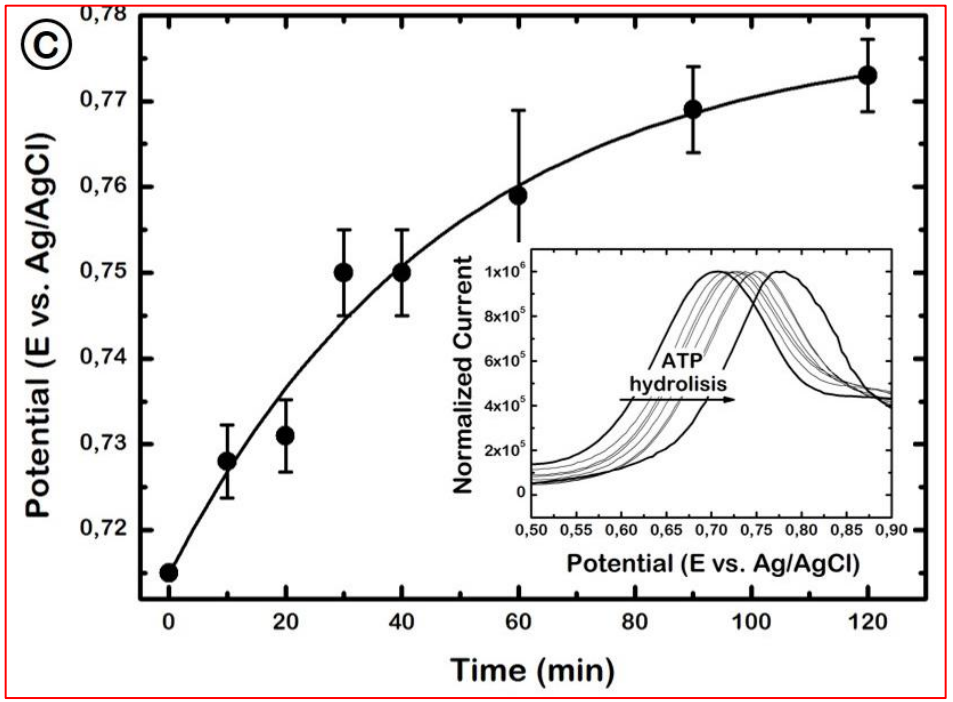




Differential pulse voltammetry measurements

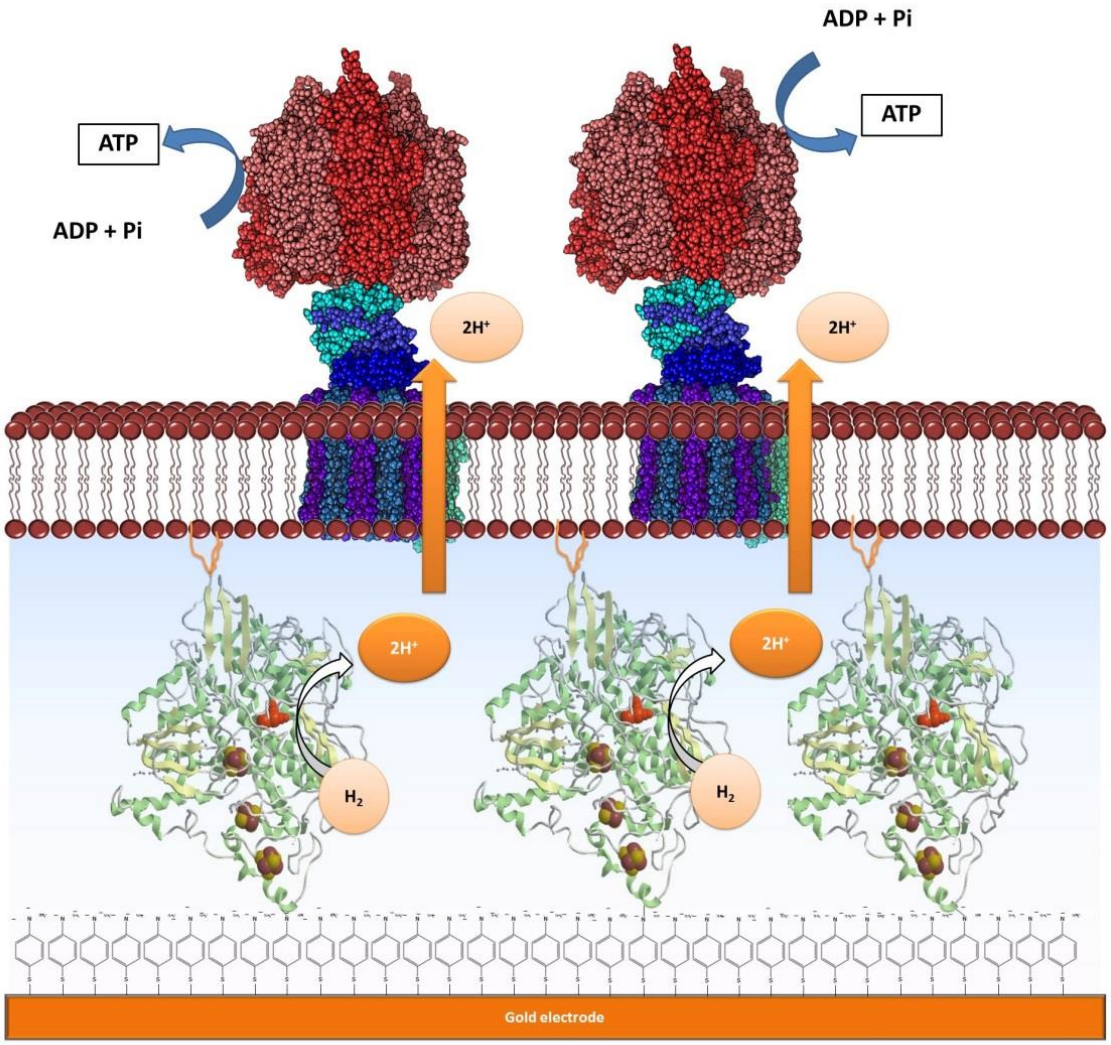
Proton pumping by ATPase while hydrolyzing ATP



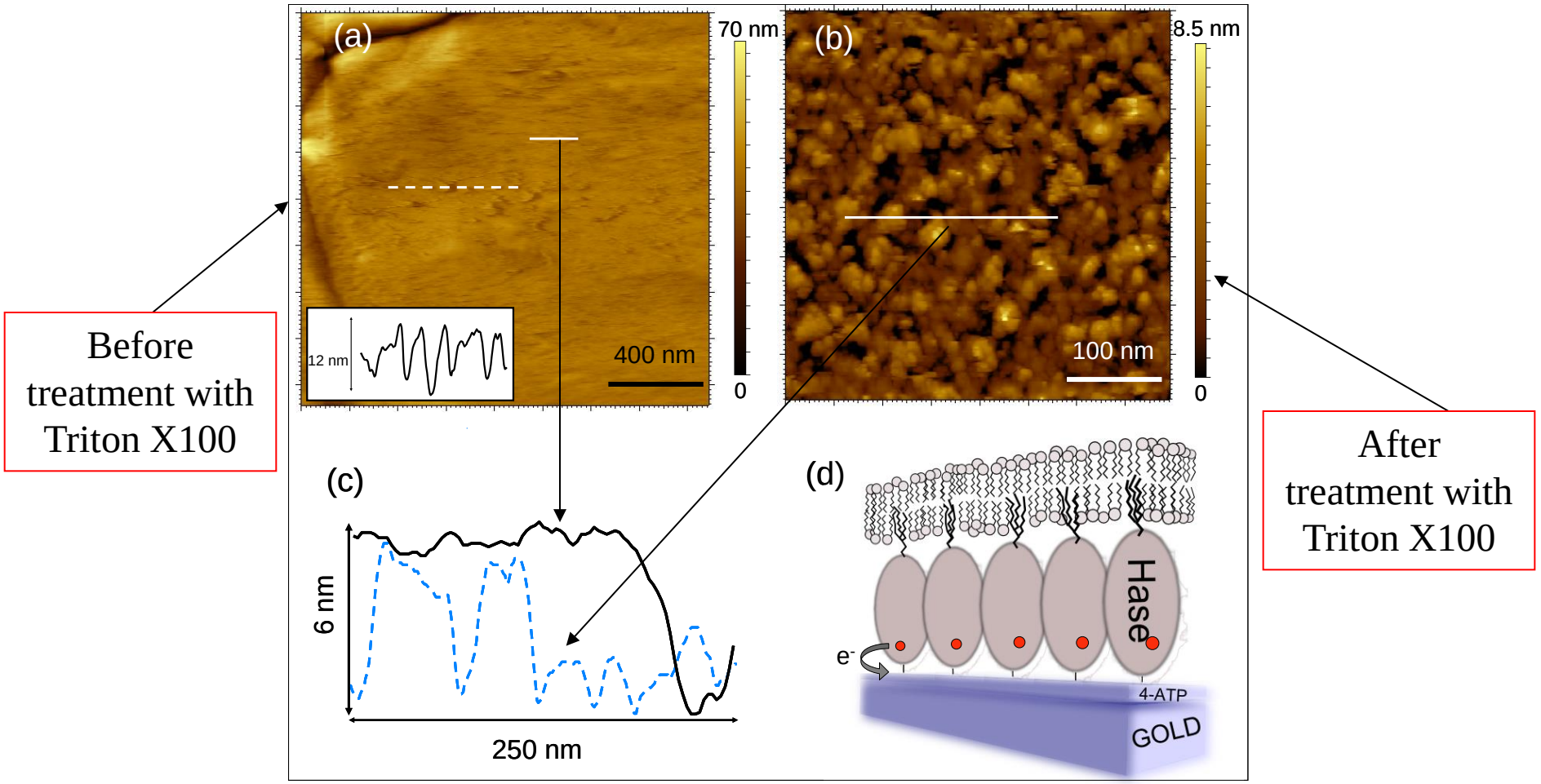


The reconstituted ATPase on the electrode is functional and is mostly oriented in the correct way

Co-immobilization of NiFeSe Hase and ATPase on a gold electrode with a supported phospholipid bilayer



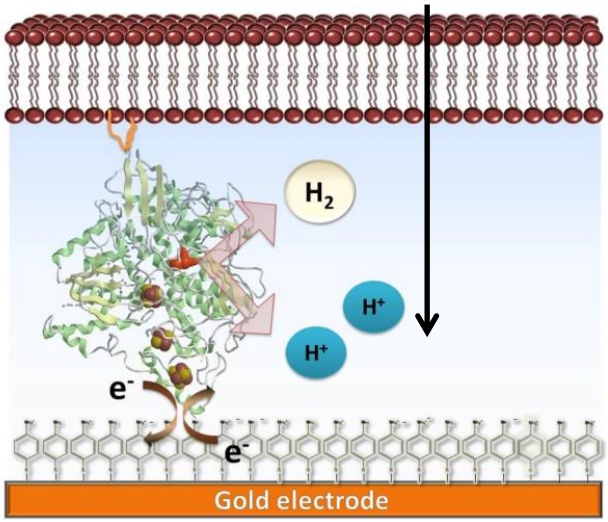
Phospholipid bilayer formation on top of a hydrogenase monolayer covalently bound to 4-aminothiophenol-Au

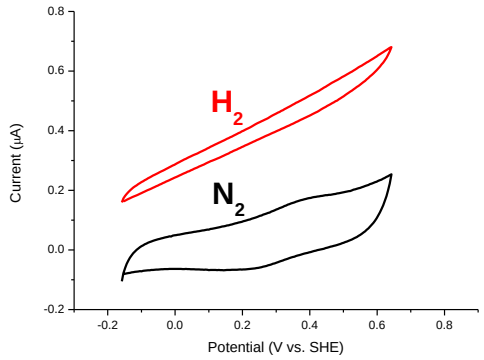


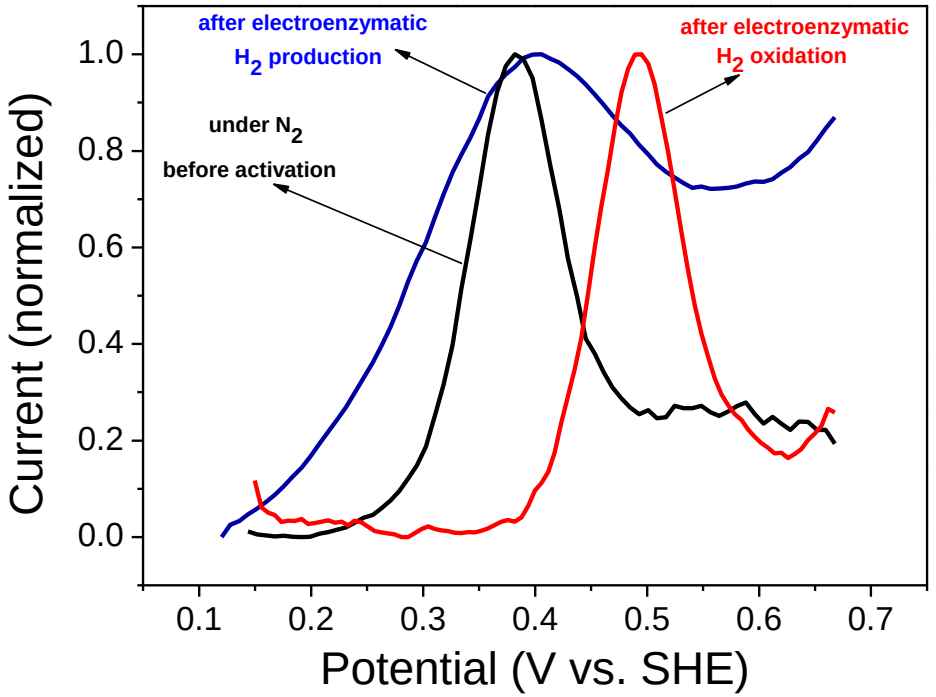
AFM characterization

Gutiérrez-Sánchez et al. Langmuir 27 (2011) 6449-6457

Generation a proton gradient across the supported phospholipid bilayer



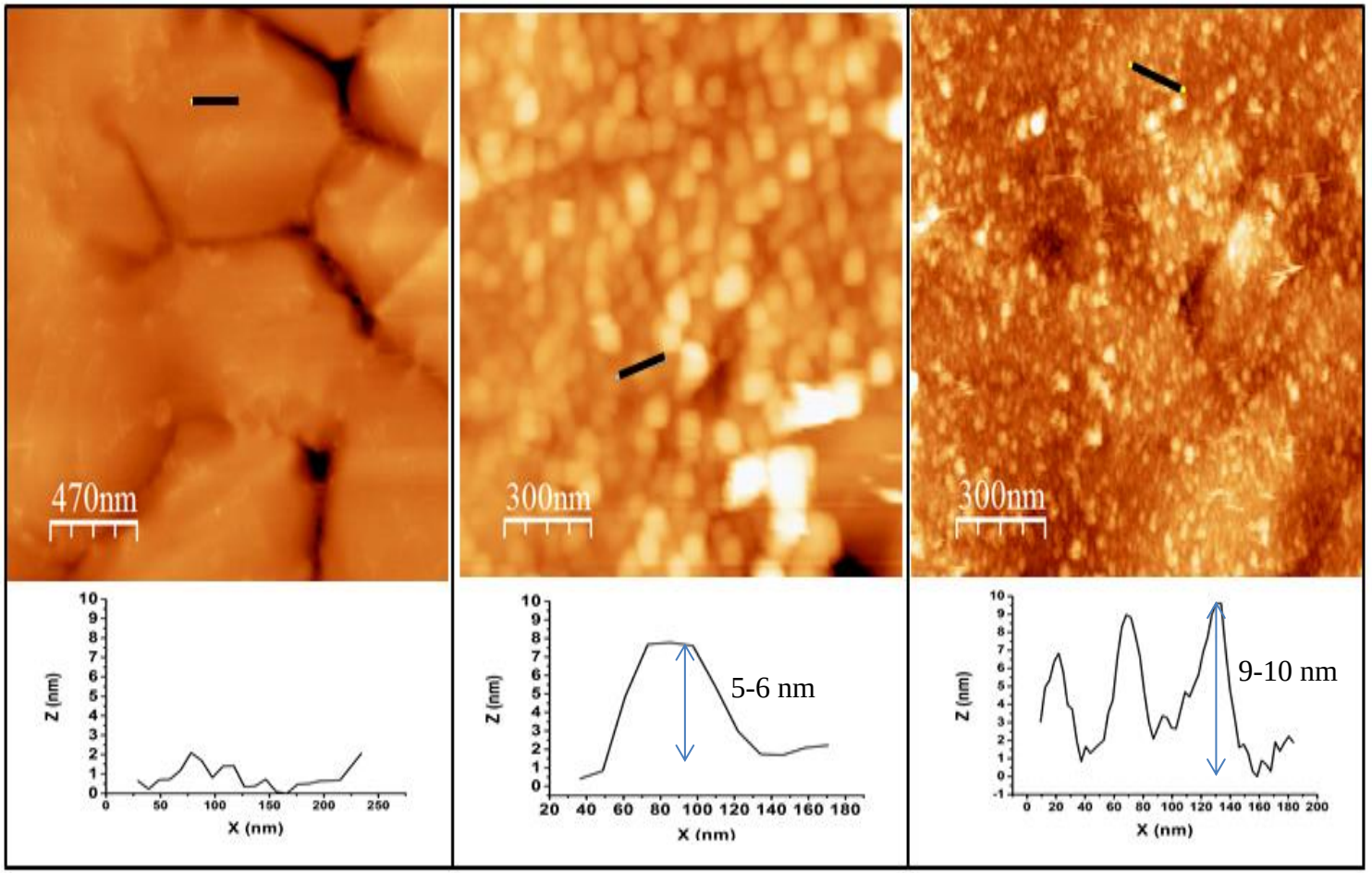




The electrocatalytic oxidation of H_2 by the immobilized hydrogenase induces a pH gradient across the biomimetic membrane

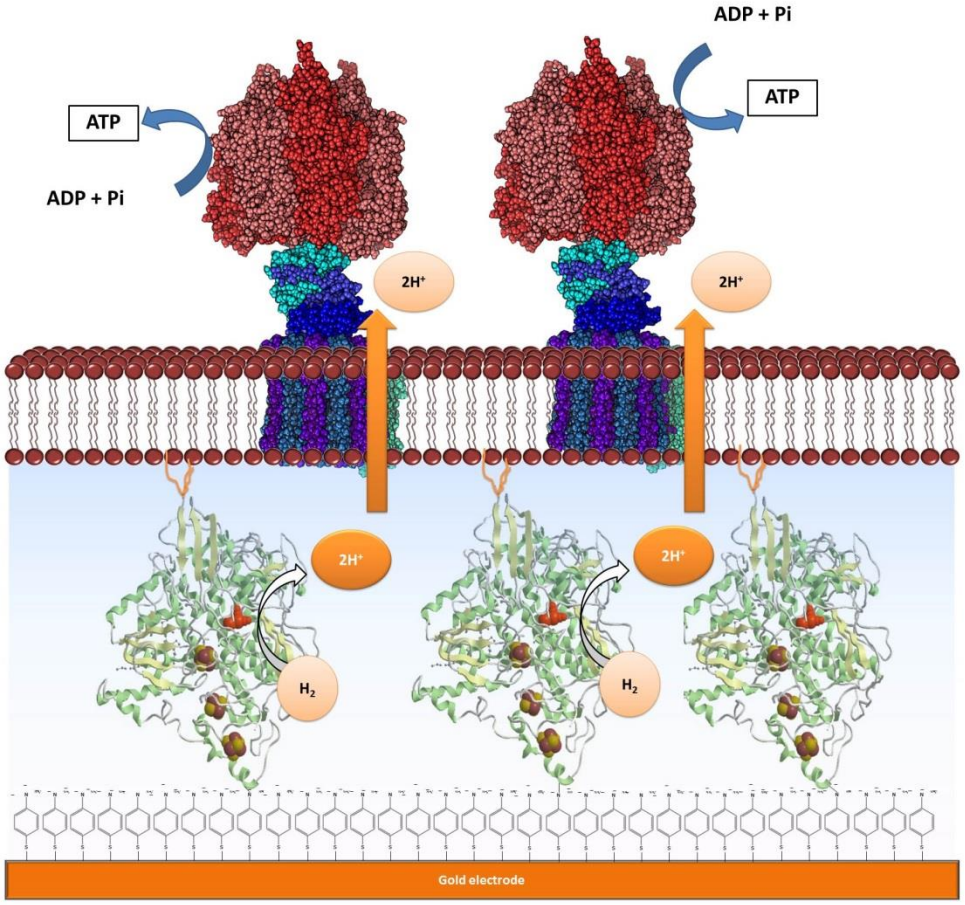
O. Gutiérrez-Sanz, C. Tapia, M. C. Marques, S. Zacarias, M. Vélez, I. A. C. Pereira, A. L. De Lacey. Angew. Chem. Int. Ed. 54 (2015) 2684-2687.

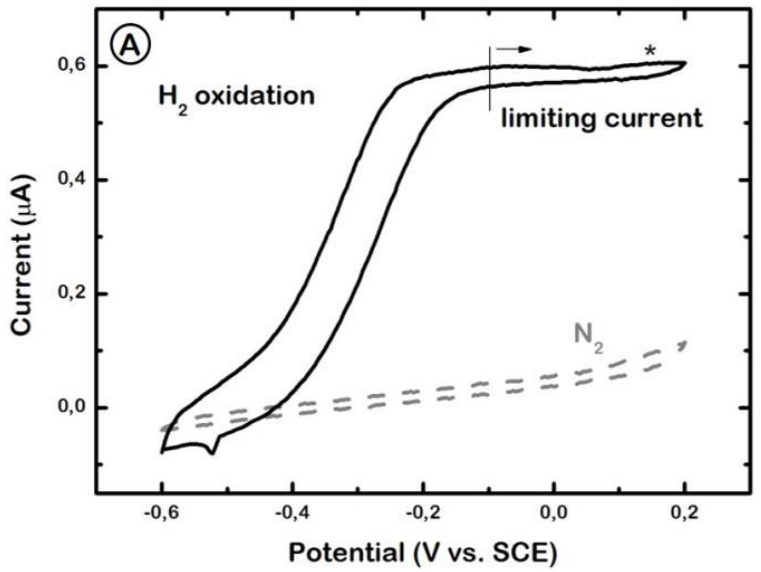
Co-immobilization of NiFeSe Hase and ATPase on a gold electrode with a supported phospholipid bilayer

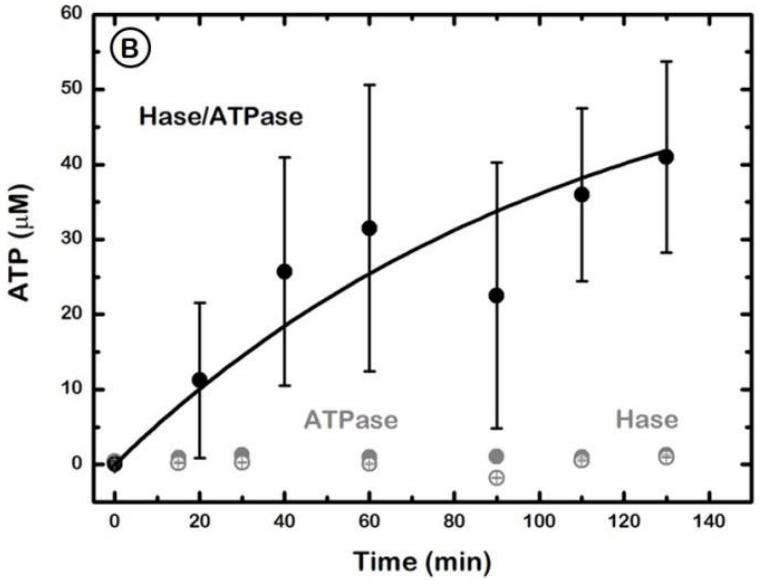


Au **Au-Hase** **Au-Hase-PhBL-ATPase**

ATP production coupled to electroenzymatic H_2 oxidation







O. Gutiérrez-Sanz, P. Natale, I. Márquez, M. C. Marques, S. Zacarias, M. Pita, I. A. C. Pereira, I. López-Montero, A. L. De Lacey, M. Vélez. Angew. Chem. Int. Ed. 55 (2016) 6216-6220.

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  - · Marcos Pita
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  - · Marisela Vélez
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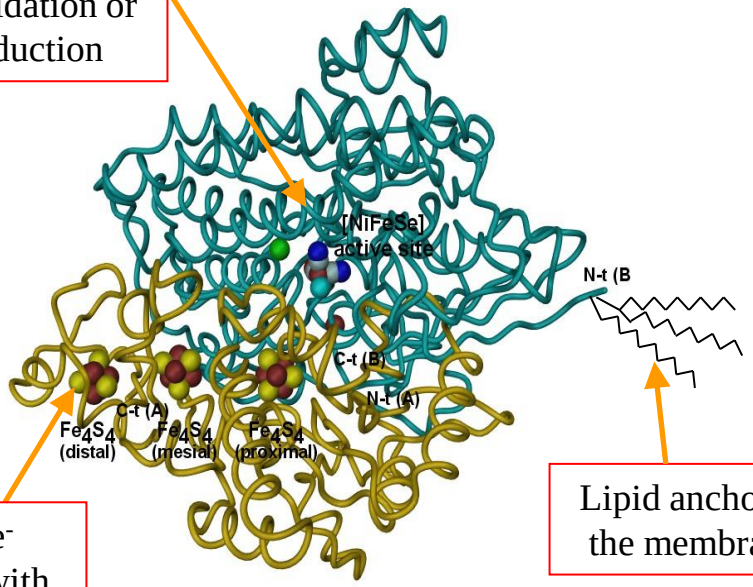
  - · Marta Marques
  - · Sonia Zacarias
- Universidad Complutense de Madrid, Spain
  - · Paolo Natale
  - · Iván López-Montero

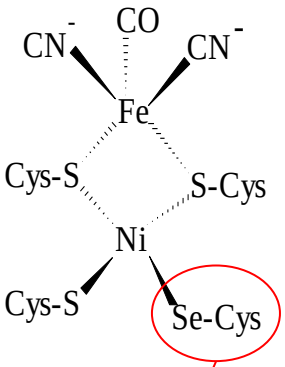
The membrane-bound Ni-Fe-Se hydrogenase from *Desulfovibrio vulgaris* Hildenborough

Active site for H_2 oxidation or production



Site for e^- exchange with redox partner

Lipid anchor to the membrane



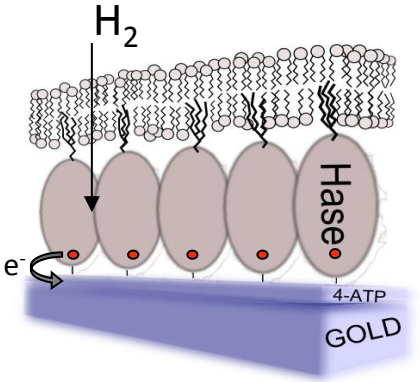
A selenocysteine replaces a cysteine ligand

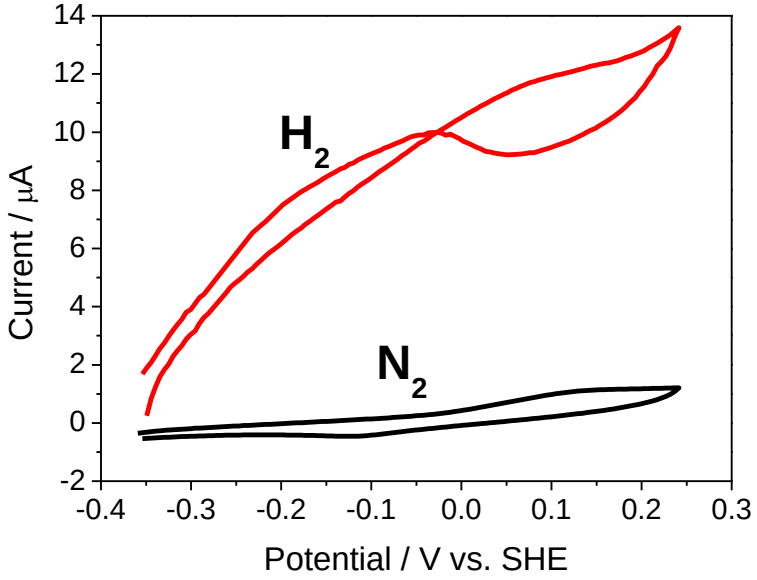
Catalytic advantages:

- Activation is fast upon reduction
- High H_2-production activity
- Oxygen tolerant during H_2-production activity

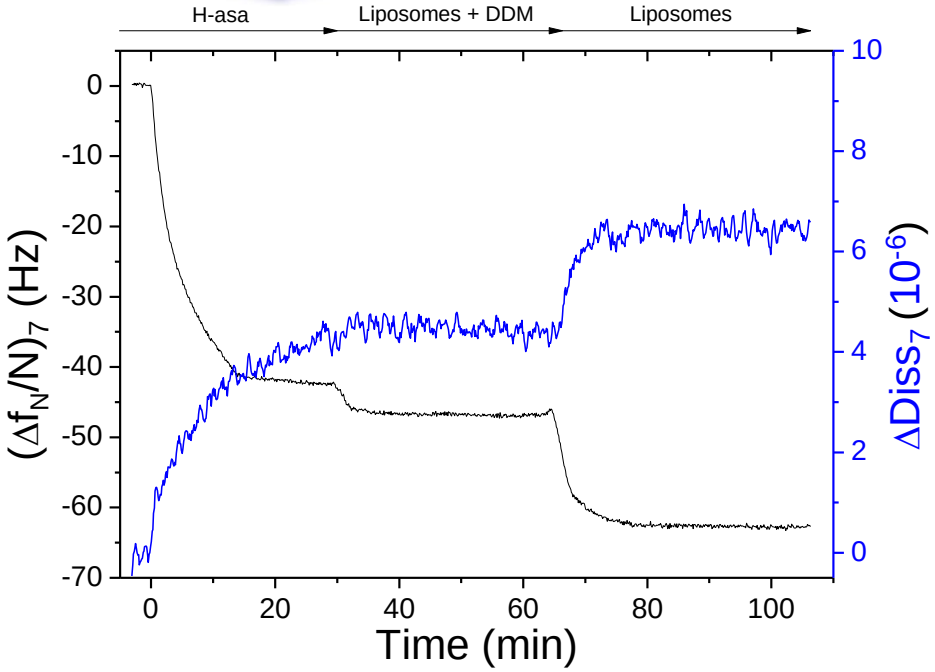
Valente et al. FEBS Lett. 581 (2007) 3341-3344; Marques et al. J. Mol. Biol. 396 (2010) 893-907.

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





Cyclic voltammetry of electrocatalytic oxidation of H_2 by DET

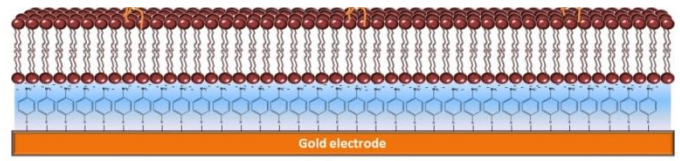


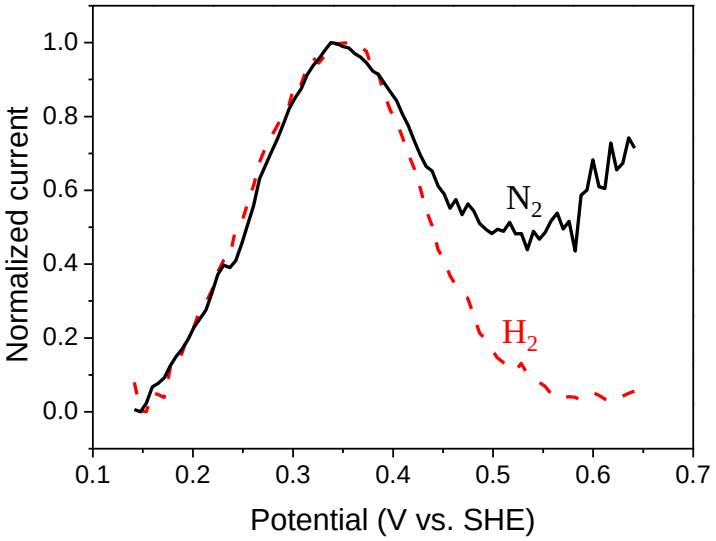
QCM characterization

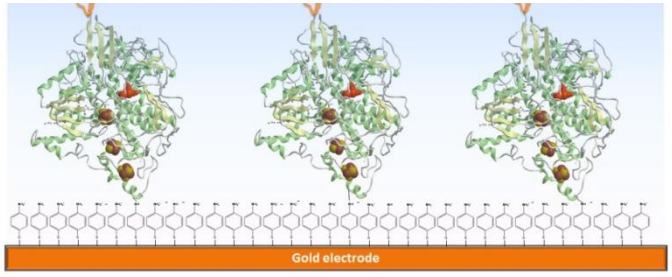
Gutiérrez-Sánchez et al. Langmuir 27 (2011) 6449-6457

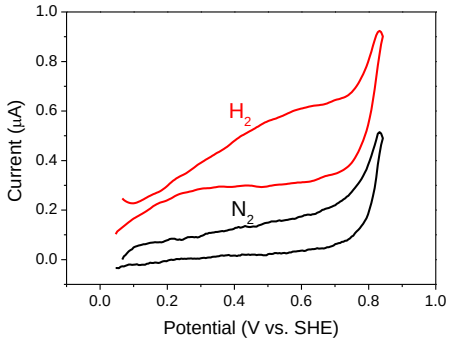
Generation a proton gradient across the supported phospholipid bilayer

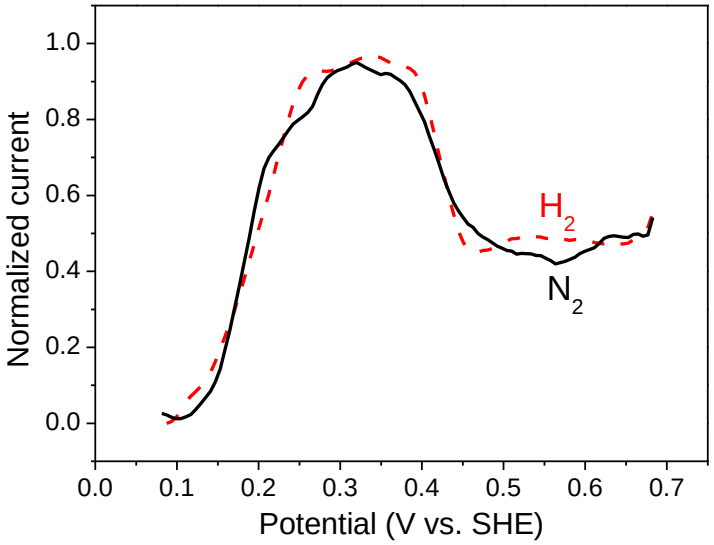
Control experiments











Future work

- Further caracterization 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, Δ $Diss_7$ (10^{-6}) = 4.4, coverage= 8 pmol/cm^2

Phospholipid bilayer formation: Δ $(f_N/N)_7$ = -21 Hz, Δ $Diss_7$ (10^{-6}) = 2