Mission pour les initiatives transverses et interdisciplinaires (MITI)

Journée thématique

Fonctionnalisation de sonde et analyses de données

Toulouse, 29 novembre 2023

Interaction and regulation in redox partner proteins: electrochemical-STM and AFM-force spectroscopy

Marina I. Giannotti

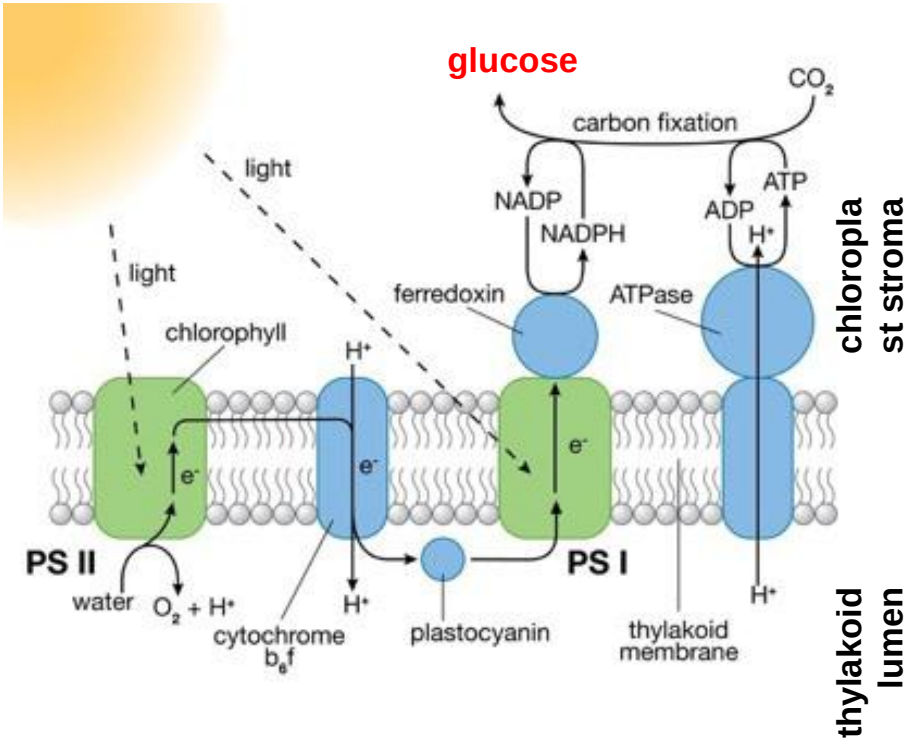
Nanoprobes & Nanoswitches

Institute for Bioengineering of Catalonia **IBEC**

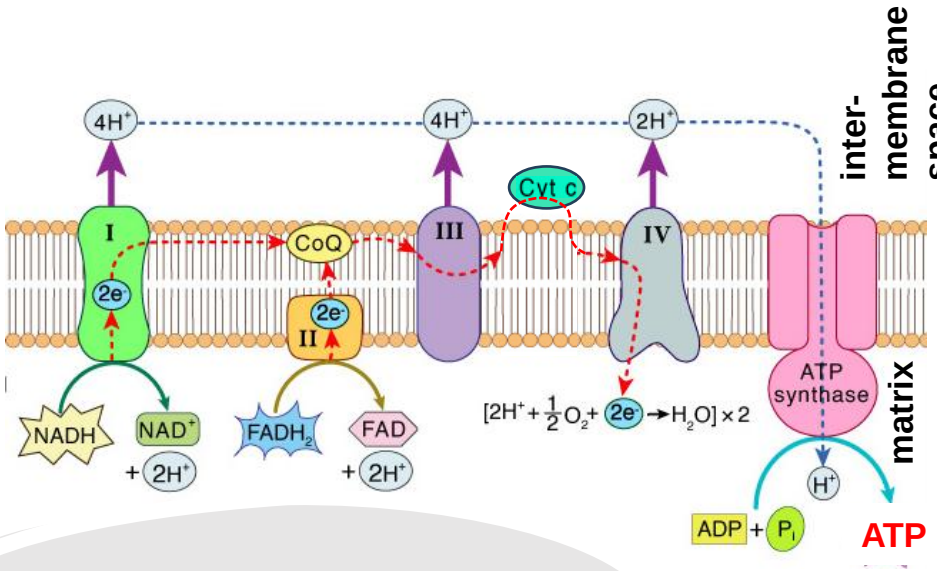
Pau Gorostiza

CHARGE TRANSPORT IN PHOTOSYNTHETIC AND RESPIRATORY COMPLEXES
protein-mediated electron transport (ET)

PLANT PHOTOSYNTHETIC CHAIN



MITOCHONDRIAL RESPIRATORY CHAIN

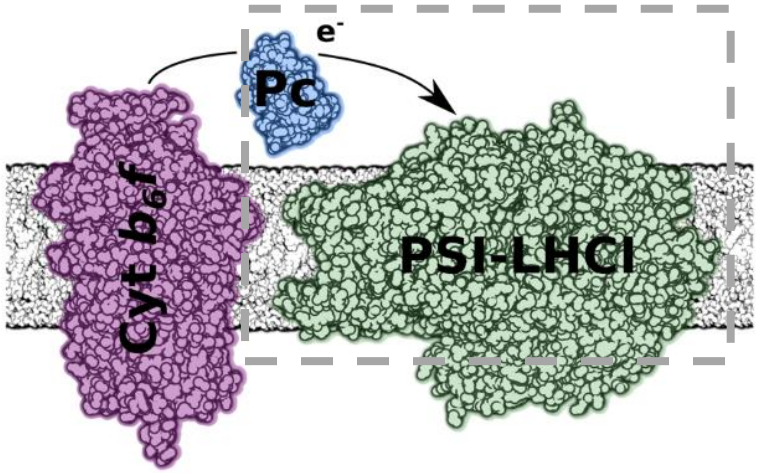


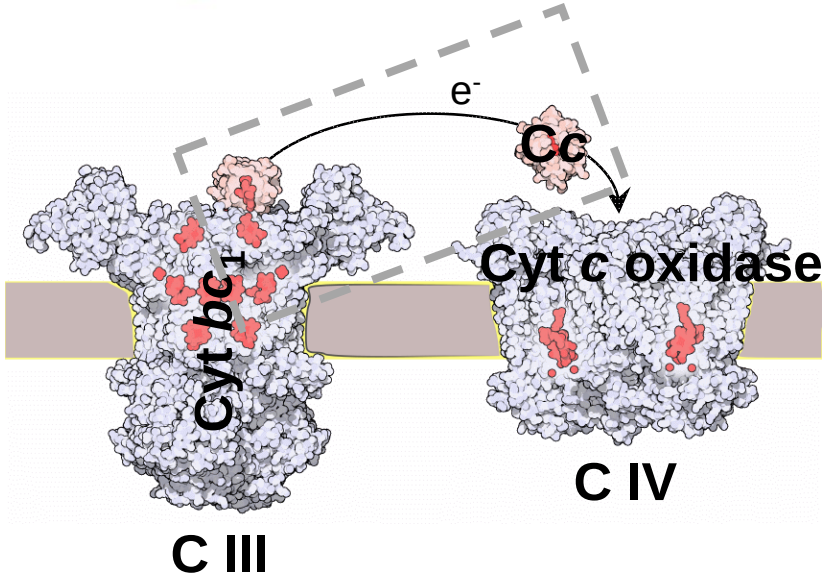
sustained and efficient electron flow

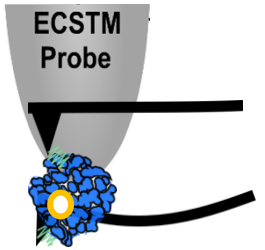
reorganization energy ↓

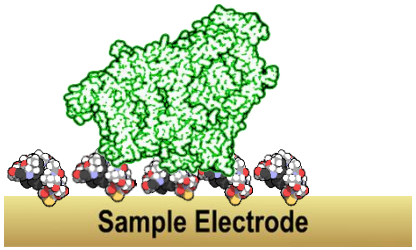
turn-over ↑

inter-biomolecular interactions at the nanoscale

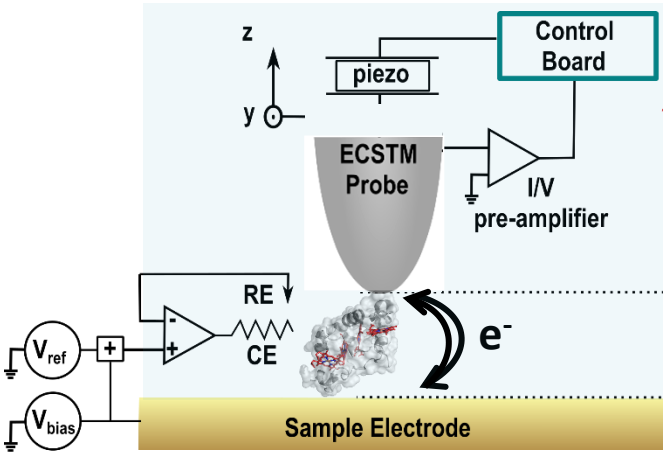








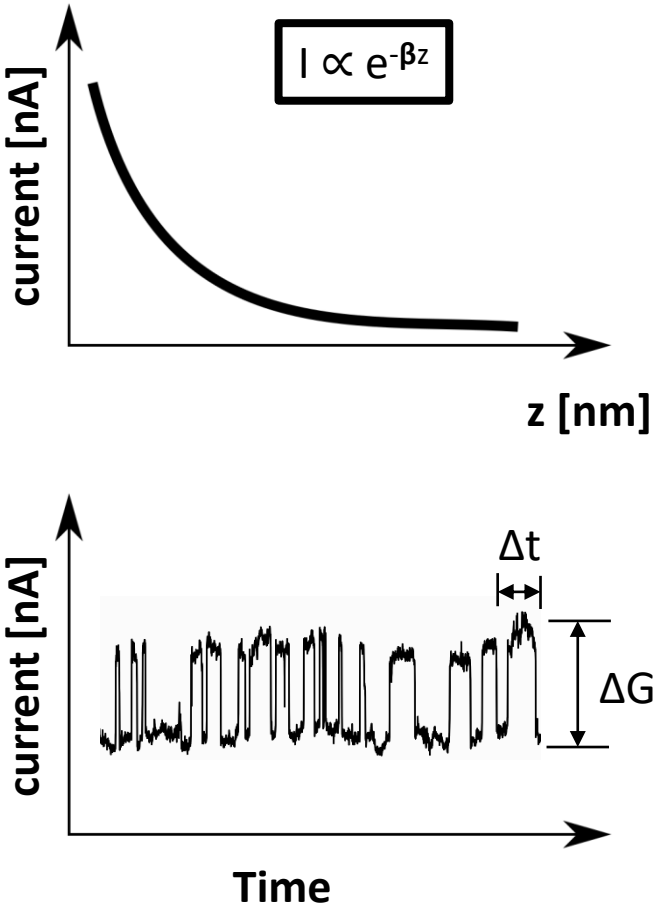
Electrochemical STM



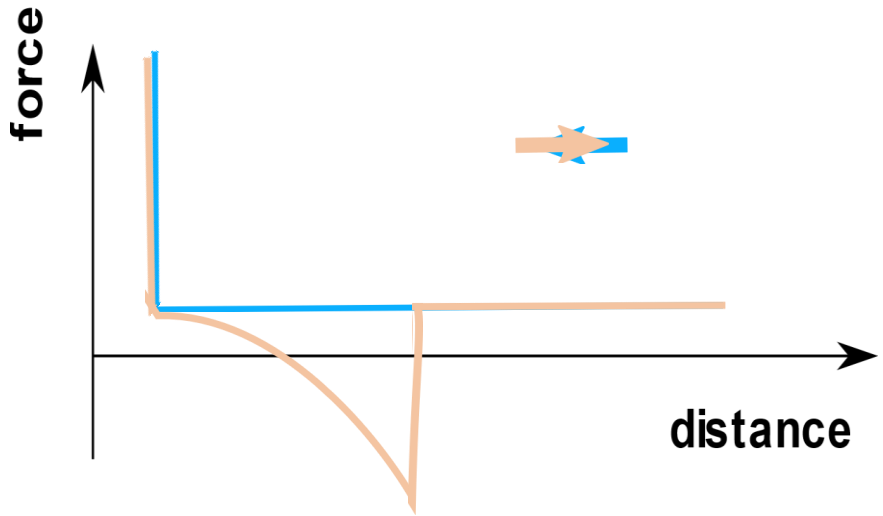
$U_{bias}=U_{probe}-U_{sample}$

$U_{bias} < 0 \quad I > 0$

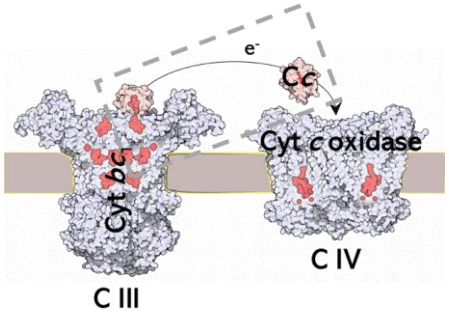
$U_{bias} > 0 \quad I < 0$

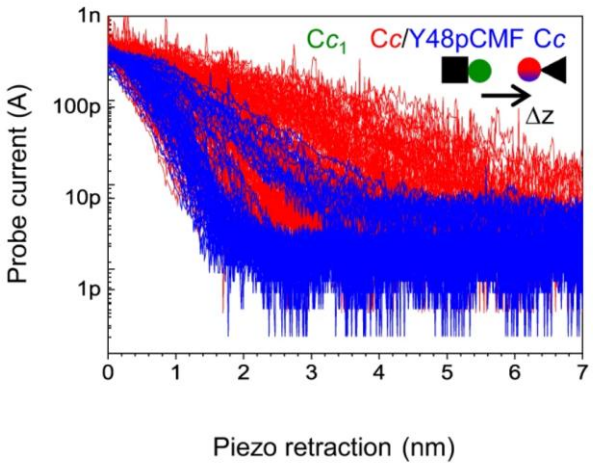


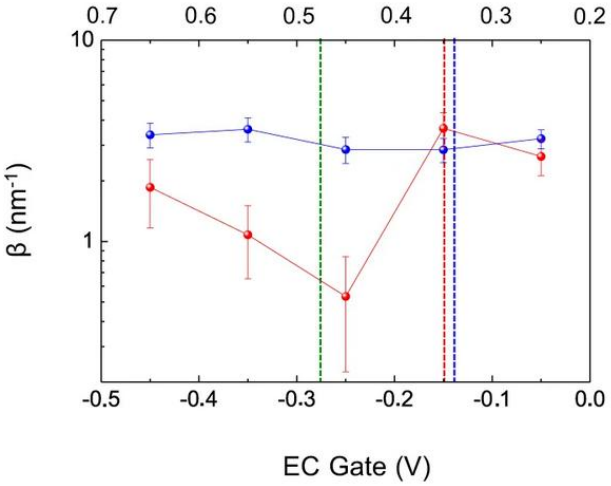
AFM force spectroscopy

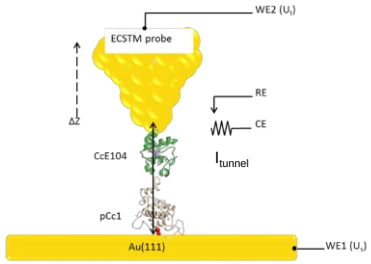


Cytochrome *c* (C*c*)– cytochrome c_1 (Cc_1)



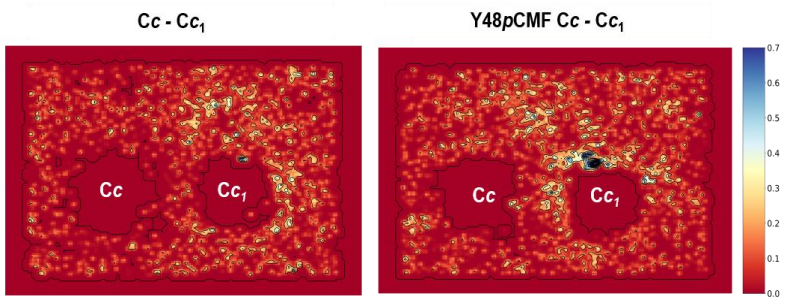


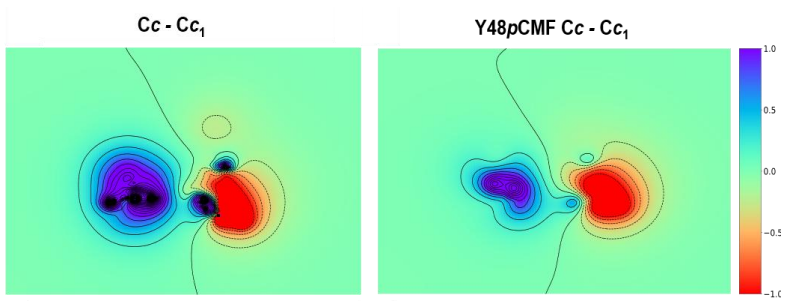




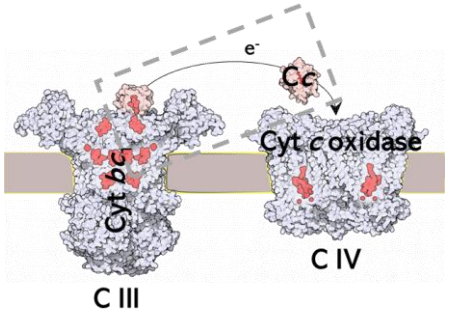
Lagunas et al. Nat. Commun. 2018

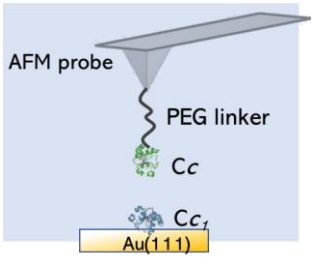
Gomila et al. Nat. Commun. 2022

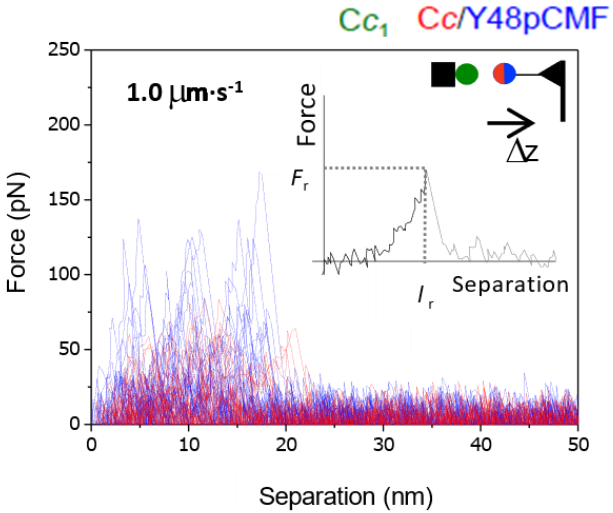


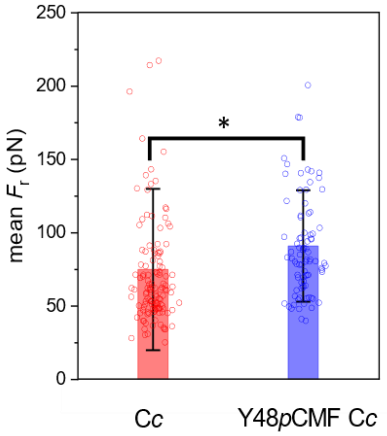


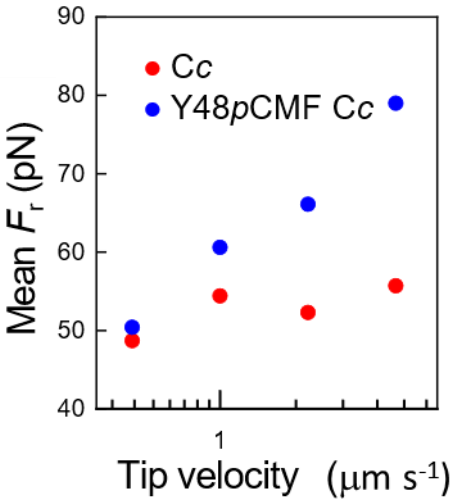
Cytochrome *c* (C*c*)– cytochrome c_1 (Cc_1)

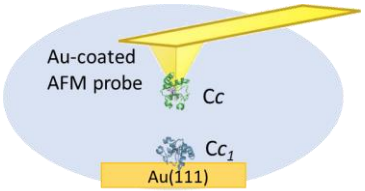








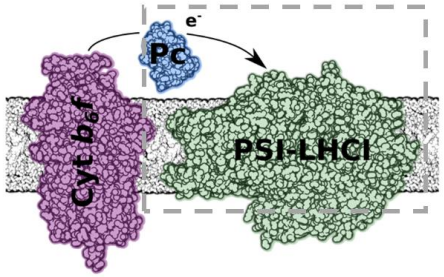


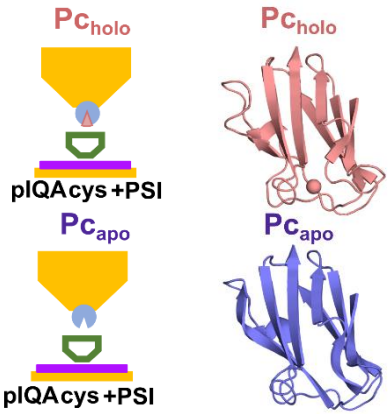


$$F' = \frac{k_B T}{x_\beta} \cdot \left[(\ln r) + \ln \frac{x_\beta}{k_{off} \cdot k_B T} \right]$$

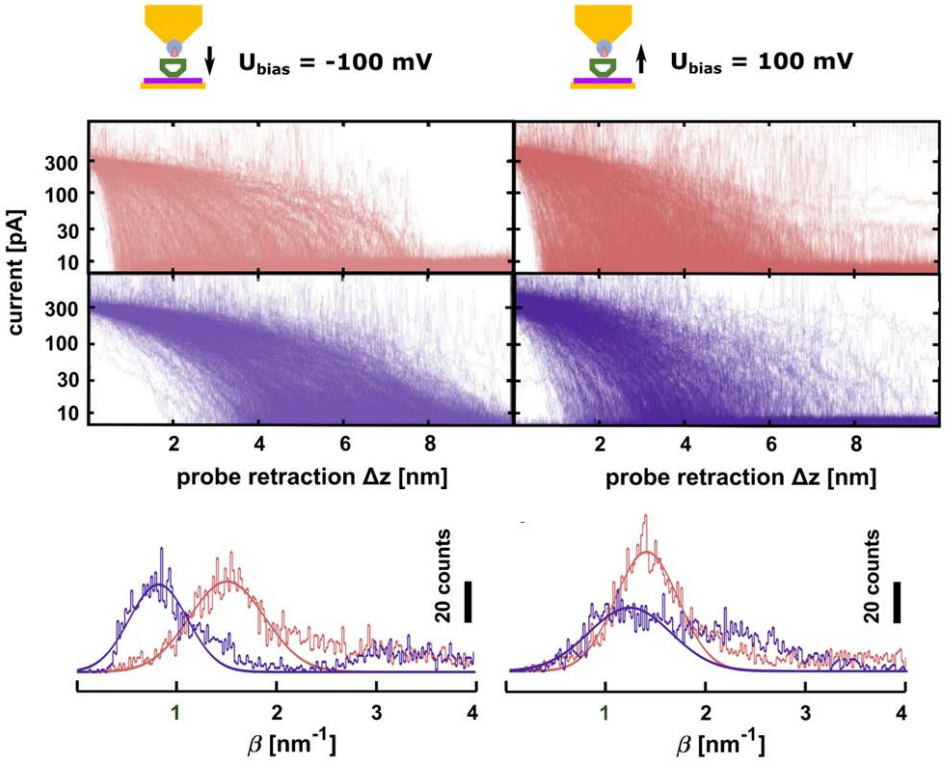
Gomila et al. Nat. Commun. 2022

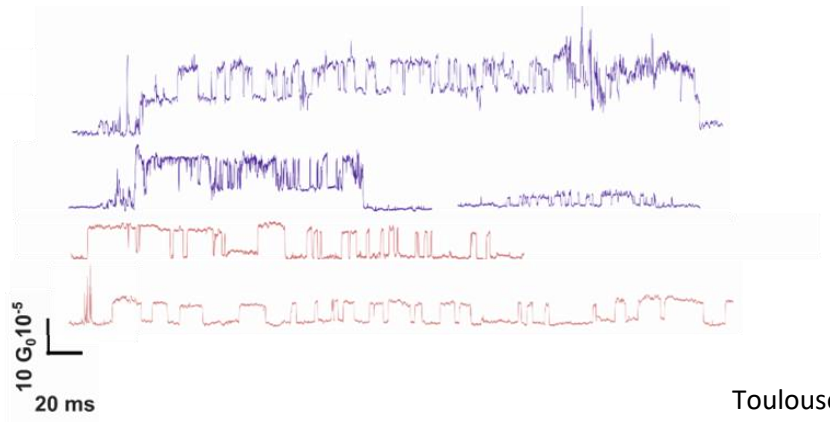
PSI – Plastocyanin (Pc)



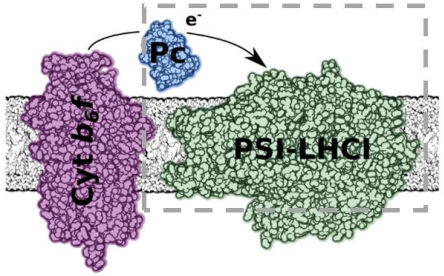


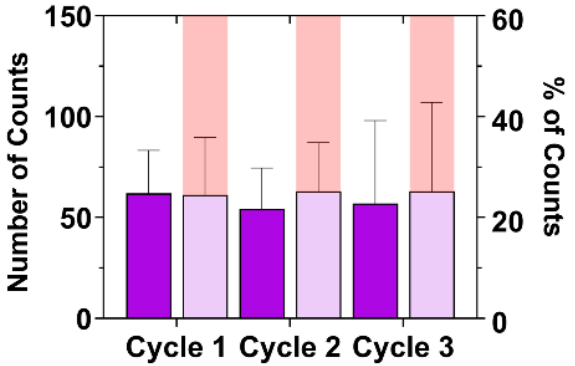
López-Ortíz et al. ACS Nano 2023

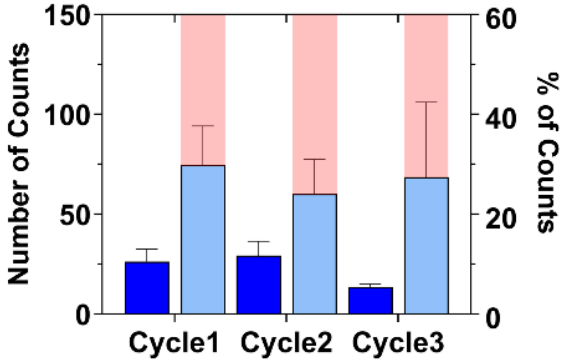


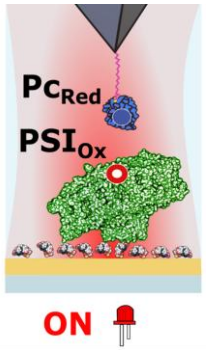


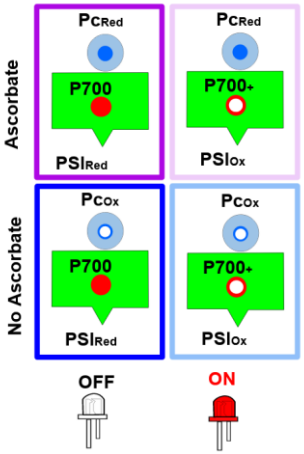
PSI – Plastocyanin (Pc)

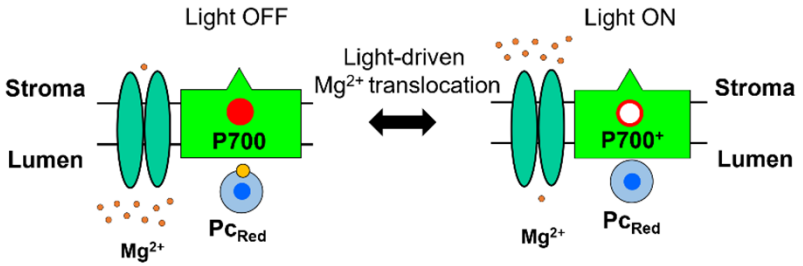


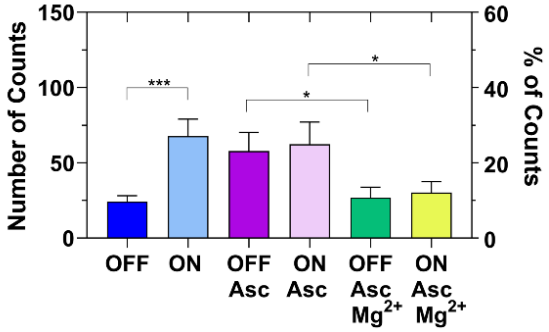


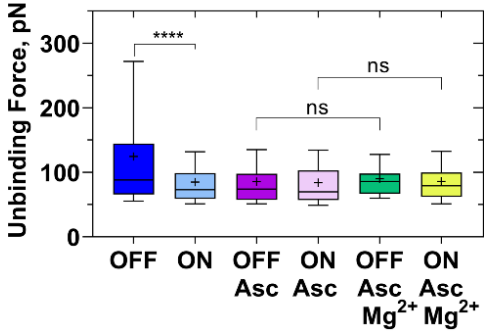












Zamora et al. ACS Nano 2022

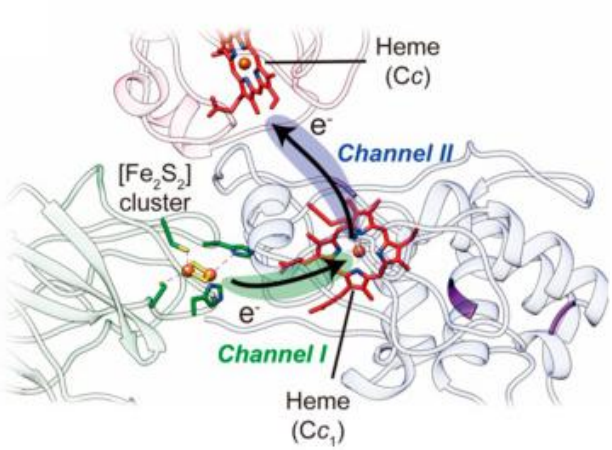
Main observations

- ✓ **long-range ET** in PSI-Pc and C*c*-Cc_1 in aqueous solution, ion depletion conduit (ECSTM on oriented protein cognates)
  - ✓ charge exchange distance and conductance is modulated by the cooper ion in Pc (PSI-Pc)
  - ✓ Phosphorylation impairs ET (C*c*-Cc_1)

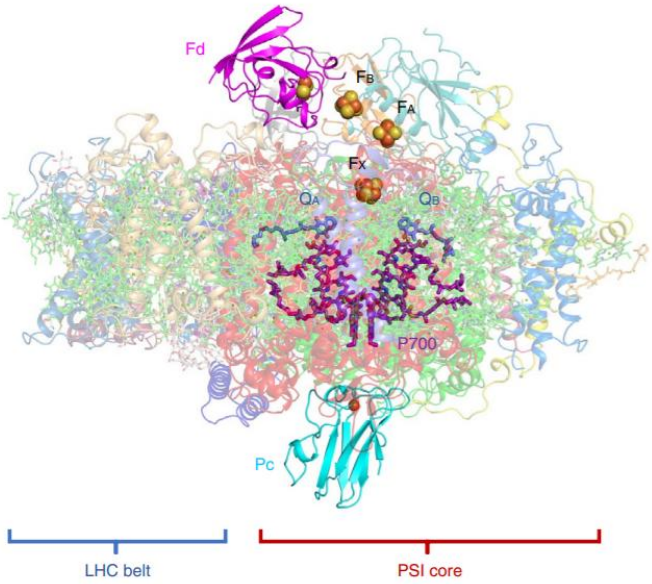
binding as a regulatory mechanism for ET rates
rather than a necessary condition for ET

- ✓ **specific** but **weak binding**, necessary for high efficiency and turn-over rate (AFM-FS on oriented protein cognates)
  - ✓ regulated by redox state, Mg^{2+} ions in solution (PSI-Pc),
  - ✓ regulated by chemical modifications (PTM) like phosphorylation (C*c*-Cc_1)

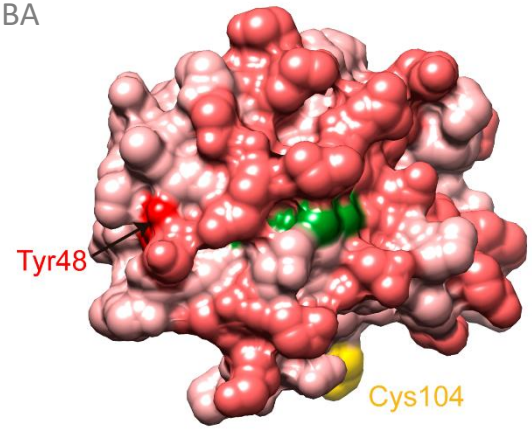
Protein immobilized with right orientation



Pérez-Mejías et al. BBA Bioenergetics 2020



Caspy et al. Nature Plants 2020



C*c*-SH
E104C mutant

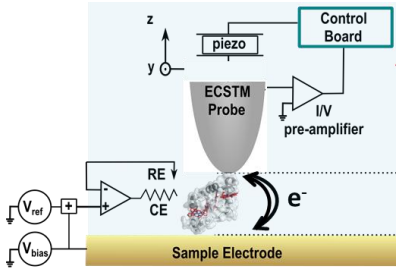
Cytochrome *c*

Pc-SH
mutant with three extra residues at the C-terminal:
Thr-Cys-Gly

Plastocyanin

Fonctionnalisation de sonde: *Interaction and regulation in redox partner proteins: ECSTM and AFM-FS*

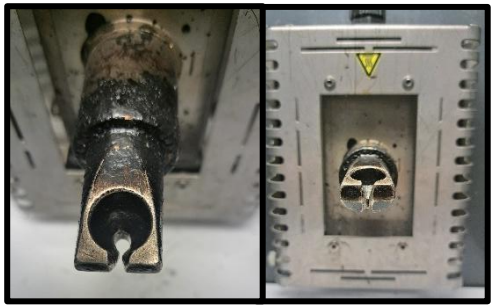
GT Fonctionnalisation



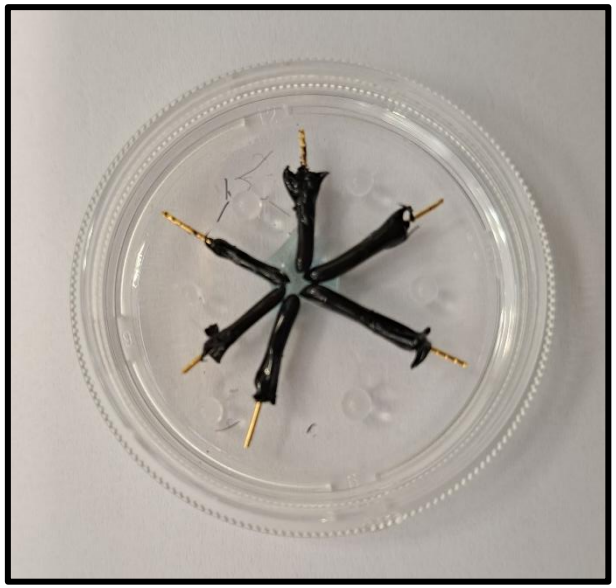
Electrochemical STM

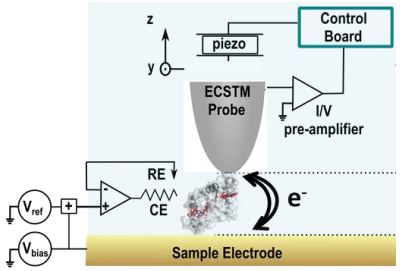
Au ECSTM Probes. Functionalization with $Pc_{apo/holo}$

Mechanically sharpen the Au wire → Coat with Apiezon wax → Au probes placed in a PDMS-coated Petri dish → 1 hour incubation at 4ºC of purified Pc_{apo}



~120ºC





Electrochemical STM

Au ECSTM Probes. Functionalization with $Pc_{apo/holo}$

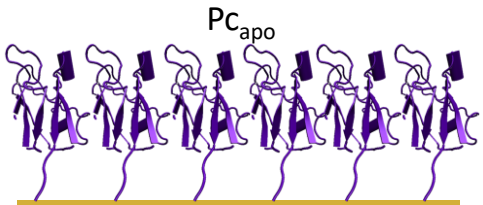
Rinse with MES the unbound Pc_{apo}

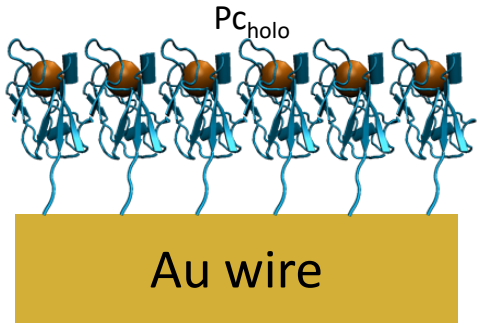
1 hour incubation at 4°C of $CuSO_4$ – MES solution

Rinse with MES the unbound Cu

Pc_{holo} is ready for experiments

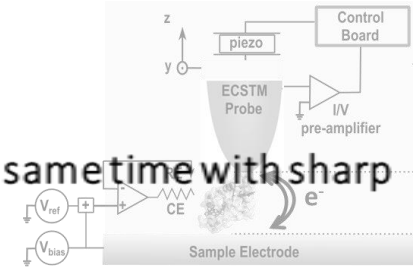
Pc_{apo} is ready for experiments





Fonctionnalisation de sonde: *Interaction and regulation in redox partner proteins: ECSTM and AFM-FS*

GT Fonctionnalisation



Au ECSTM PROBES FUNCTIONALIZATION:

1. Mechanically sharpen the gold wire: cut the Au wire and pull and cut at the same time with sharp hand-held steel wire cutter to obtain the tip
2. Coat twice the Au wire with Apiezon wax heated at ~120ºC with a home-built heat-coating applicator
3. Place 6 probes looking at each other in a polydimethylsiloxane (PDMS)-coated Petri dish
4. Place 100 µL drop of purified protein (Pc_{apo}) solution soaking all probe tips and incubate for 1 hour at 4ºC
5. Put drops of water around the probes to maintain humidity
6. Extract the protein (Pc_{apo}) drop and rinse with protein buffer to eliminate the unbound protein
7. For Pc_{holo} incubate 1 hour more at 4ºC with a solution of MES 5 mM pH = 5.5 with 100 mM of $CuSO_4$ and rinse again with MES after to eliminate the unbound Cu^{2+}

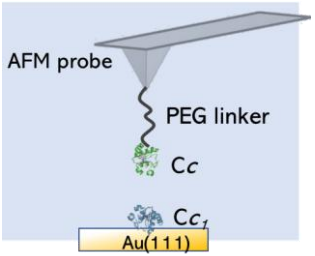
Lagunas et al. Nat. Commun. 2018

Gomila et al. Nat. Commun. 2022

López-Ortíz et al. ACS Nano 2023

Fonctionnalisation de sonde: *Interaction and regulation in redox partner proteins: ECSTM and AFM-FS*

GT Fonctionnalisation

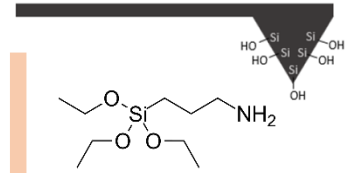


AFM force spectroscopy

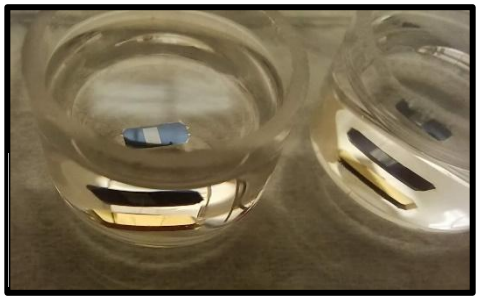
Si/Si_3N_4 AFM probes. Functionalization with PEG-protein

Surface activation O_2/UV

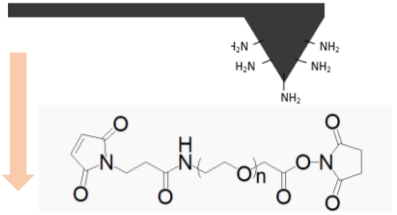
APTES functionalization (vapor phase) ON

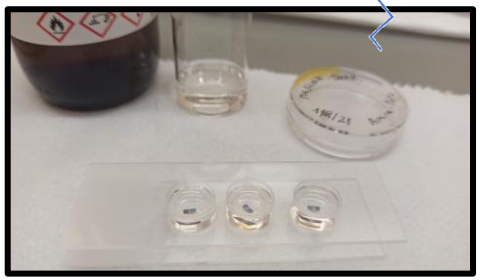


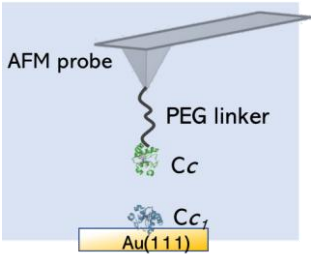
Rinse chloroform and ethanol, dry N_2 (can be stored)



React with heterobifunctional PEG (+ TEA) /chloroform 1,5 h







AFM force spectroscopy

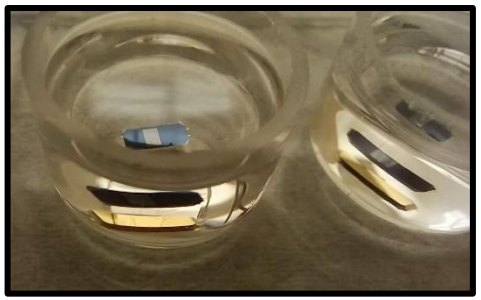
Si/Si_3N_4 AFM probes. Functionalization with PEG-protein

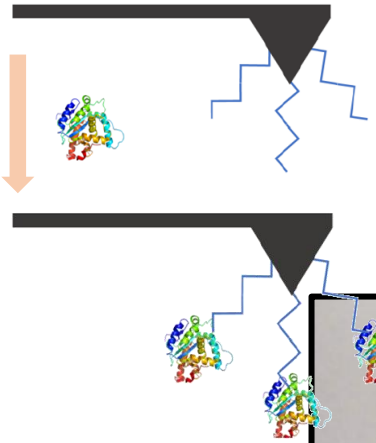
Rinse chloroform and ethanol and MilliQ Water, dry N_2

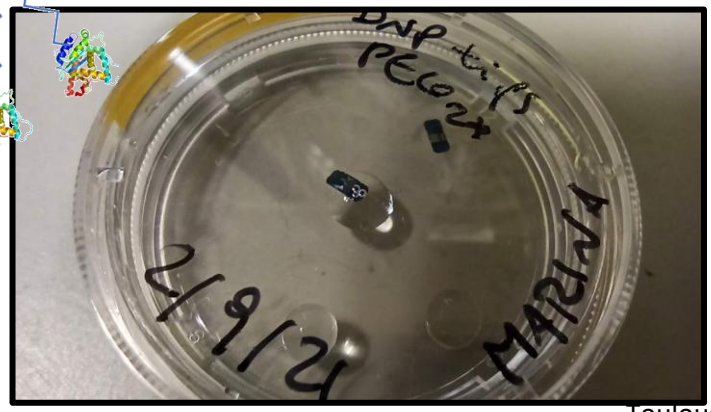
1 hour incubation at 4°C of purified Pc_{apo}

Rinse with buffer

1 hour incubation at 4°C of $CuSO_4$ – MES solution



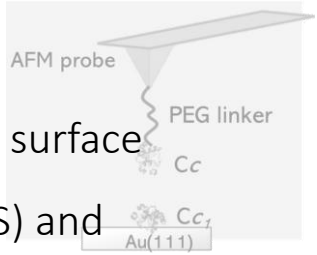




Rinse with MES the unbound Cu

Pc_{holo} is ready for experiments

Fonctionnalisation de sonde: *Interaction and regulation in redox partner proteins: ECSTM and AFM-FS*



Si/Si_2N_3 AFM PROBES FUNCTIONALIZATION:

1. Treat 15 min with a UV/Ozone ProCleaner (BioForce Nanosciences) to activate surface
2. Amino-functionalize in an atmosphere of 3-aminopropyltriethoxysilane (APTES) and triethylamine (TEA). Inside the desiccator previously flooded with Ar or N_2. Two containers with 45 µL APTES and 15 µL TEA, separately, close to the probes (on clean inert surface), vacuum on for 5 min. After 1.5 h, remove APTES and TEA containers, and vacuum is restored. Overnight, RT
3. Rinse chloroform (2x) and ethanol (2x) and dry under gentle N_2 flow. When not used immediately, they can be stored in a desiccator under Ar
4. Place in glass wells with a 0.5 mL solution 1 mg/mL heterobifunctional PEG (Mal-PEG27-NHS) and 5 µL of TEA in chloroform, 1.5 h, RT. Rinse with chloroform, ethanol and MilliQ water
5. Place 3 probes looking at each in a PDMS-coated Petri dish. Place 25 µL drop of purified protein solution (1 mg/mL) over all probe tips and incubate for 1 hour at 4ºC
6. Rinse with protein buffer to eliminate the unbound protein

Gomila et al. Nat. Commun. 2022

Zamora et al. ACS Nano 2022

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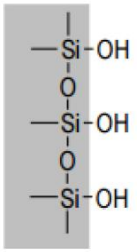
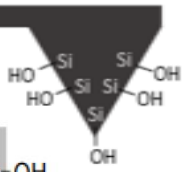
1)

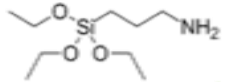
Si

ambient
atmosphere

(or piranha)
(or ozone)

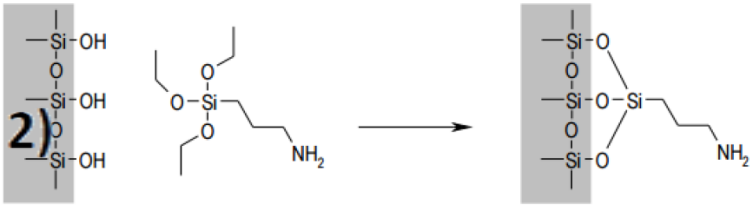


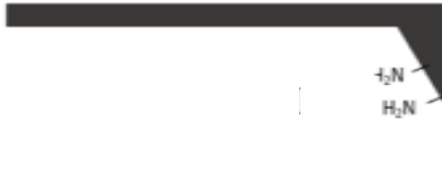


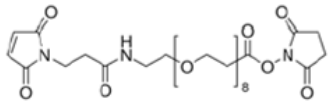


APTES

2)

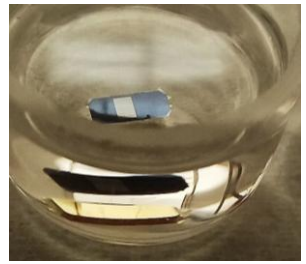


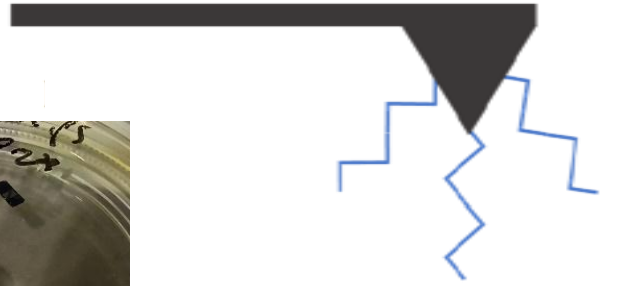




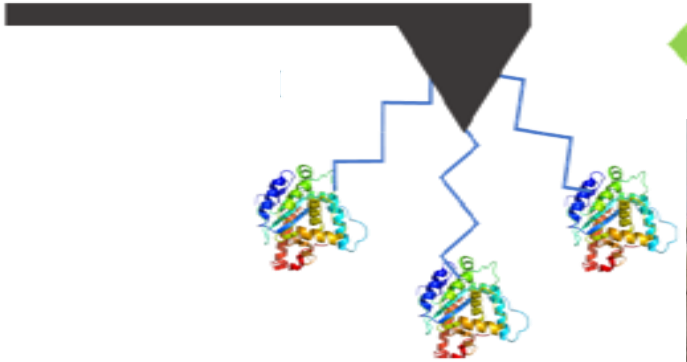
Linker

3)





Protein



4)

Zamora et al. ACS Nano 2022, 16, 15155.

Gomila et al. Nat. Commun. 2022, 13, 7100.