

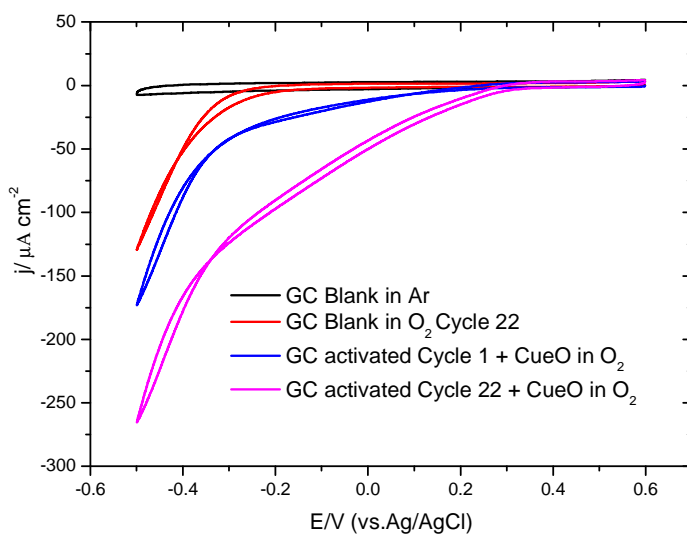
SUPPORTING INFORMATION FOR:

Comprehensive study of the enzymatic catalysis of the Electrochemical Oxygen Reduction Reaction (ORR) by immobilized Copper efflux oxidase (CueO) from *E.Coli*

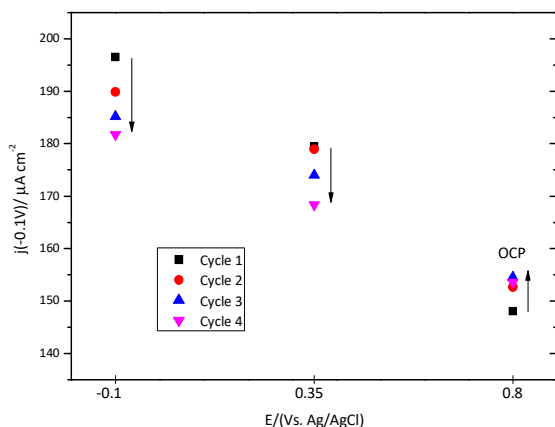
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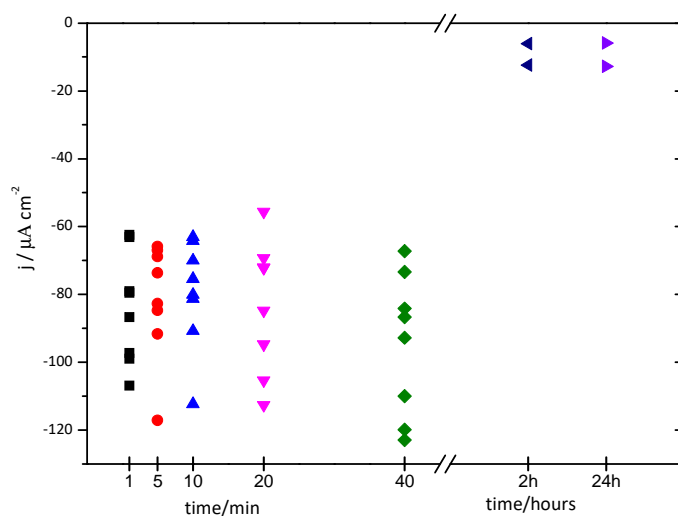
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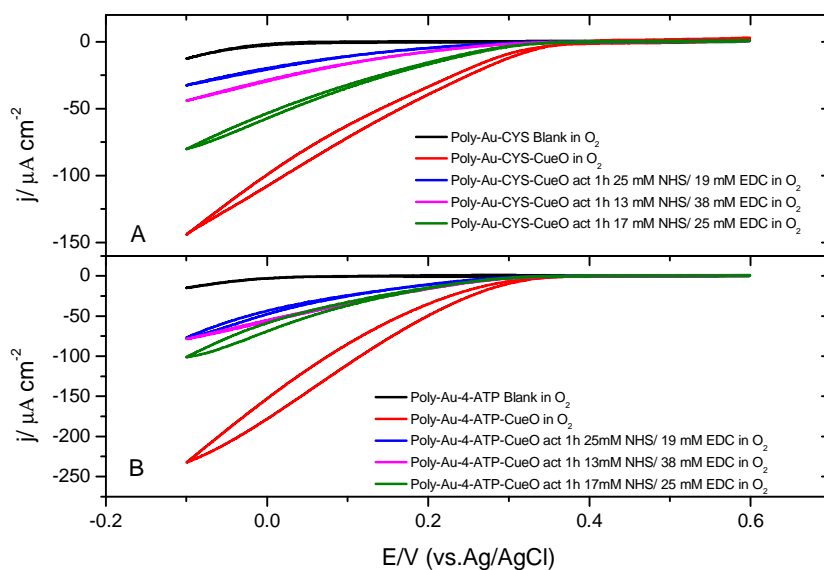
**Figure S1.** Cyclic voltammogram for CueO immobilized on an activated (during 22 cycles) bare GC in an O<sub>2</sub> saturated PBS, pH 6.5. Blue line: 1<sup>st</sup> cycle. Pink line: 22<sup>nd</sup> cycle. GC blank in Ar atmosphere (black line). GC blank in O<sub>2</sub> atmosphere (cycle 22) (Red line). Scan rate: 20 mVs<sup>-1</sup>.



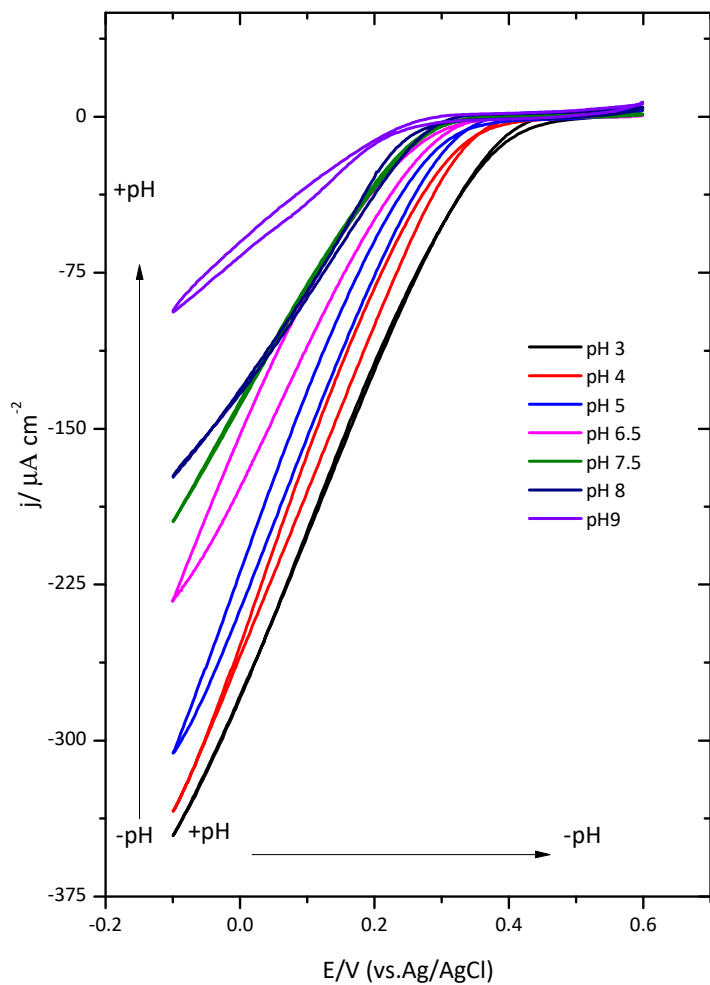
**Figure S2.** Data for the O<sub>2</sub> reduction catalytic density (for the first four cycles, cycle 1 (black square), cycle 2 (red circle), cycle 3 (blue up-triangle) and cycle 4 (pink down-triangle) after protein submission to OCP, 0.6, 0.35 and -0.1 V potential values.



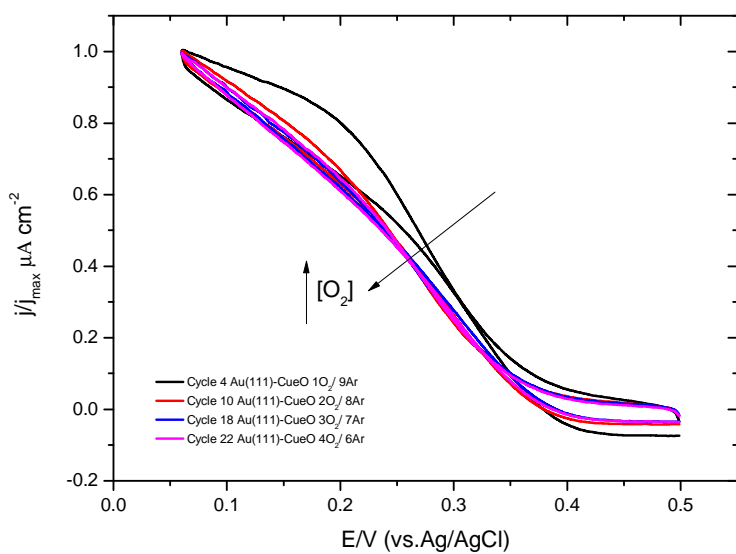
**Figure S3.** CueO current density dependence with immobilization time on Au(111). O<sub>2</sub> saturated sodium PBS, pH=6.5. Data were collected in the steady curve for a potential value of 0.2 V.



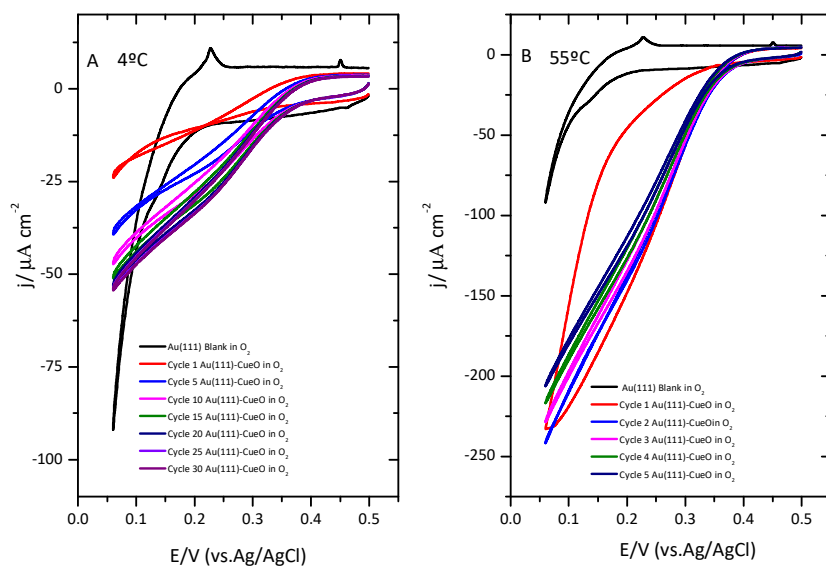
**Figure S4.** Cyclic voltammograms for CueO activated in presence of different NHS/EDC mixture solutions: blue line (25 mM NHS/ 19 mM de EDC), pink line (13 mM NHS/ 38 mM de EDC), green line (17 mM NHS/ 25 mM de EDC). Black line: Poly-Au-CYS blank (A) and (B) Poly-Au-4-ATP blank (B). Red line: Poly-Au-CYS-CueO (A) Poly-Au-4-ATP-CueO (B). O<sub>2</sub> saturated sodium PBS. Scan rate: 20 mVs<sup>-1</sup>.



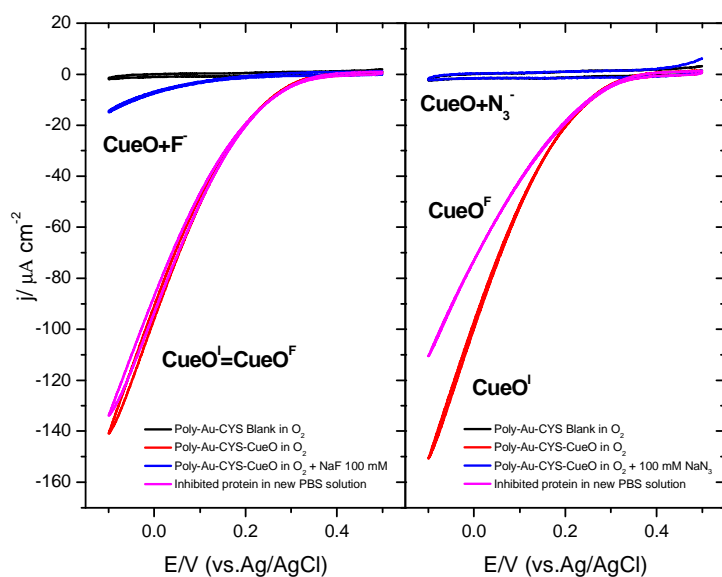
**Figure S5.** CueO behavior over Au(111) modified with 4-ATP in an  $\text{O}_2$  solution at different pHs values between 3 and 9. Scan rate:  $20 \text{ mVs}^{-1}$ .



**Figure S6.** Cyclic voltammogram for the O<sub>2</sub> reduction normalized current densities.

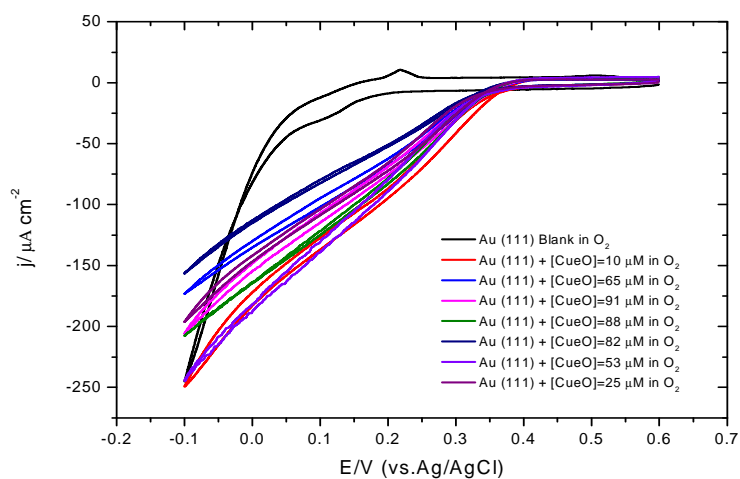


**Figure S7.** Monitorization of the CueO cyclic voltammograms measured at two different temperatures: (A) 4°C and (B) 55°C. Scan rate: 20 mVs<sup>-1</sup>.



**Figure S8.** Reversibility of the CueO inhibition process. A)  $F^-$  inhibition reversibility process. Cell 1) Poly-Au-CYS blank (black line) and maximum ORR current density for a poly-Au-CYS-CueO modified electrode in absence of inhibitors (red line). Cell 2) ORR current density for a poly-Au-CYS-CueO modified electrode in presence of 100 mM NaF or  $NaN_3$  concentration (blue line). Transference of the inhibited protein modified electrode to the Cell 1) maximum ORR current density for the inhibited poly-Au-CYS-CueO modified electrode in new PBS solution without containing inhibitors (pink line). Scan rate: 2 mVs<sup>-1</sup>

1.



**Figure S9.** Dependence of the CueO cyclic voltammetry ORR catalysis with the protein concentration. Scan rate: 20 mVs<sup>-1</sup>.