# USING BLOOD HEMOGLOBIN FOR BLOOD ANALYSIS.

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# 1.-pH effect on autoxidation kinetic

The **figure ESI-1** shows the effect of the pH on the HbII.$O_2$ autoxidation (♦)and HbII.$O_2$/$H_2O_2$ reaction (■). As can be seen for low pH values, the autoxidation dominates. For pH=4 or higher the HbII.$O_2$/$H_2O_2$ dominates; for pH=5 or higher the autoxidation is negligible.

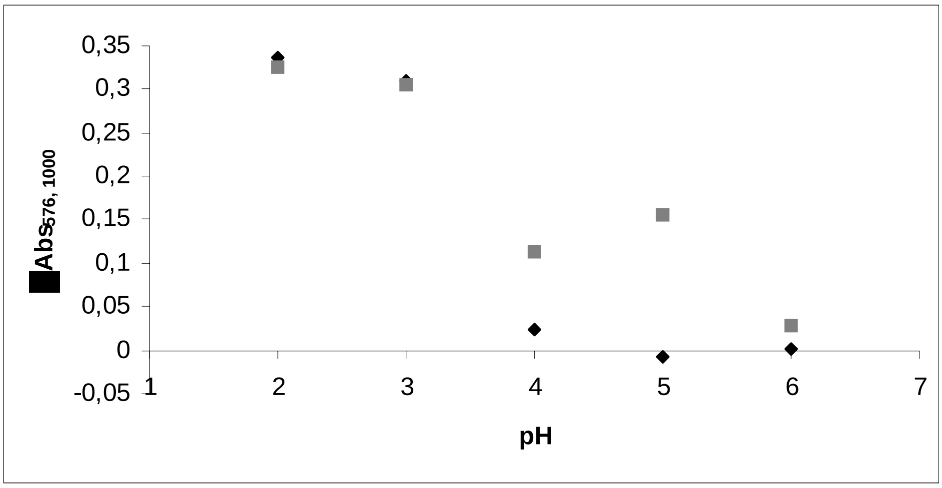


**Figure ESI-1.** Conditions: blood dilution 1/250 in citrate buffer at different pH and $2.2*10^{-5}$ M $H_2O_2$ (no $H_2O_2$ addition in the autoxidation assays)

# 2.-$H_2O_2$ calibration lines

As has been indicated the equation (9) in the main text admits several simplifications depending on the measurement times considered. Table **ESI-1** shows the most important simplifications for the purposes of this paper and figures **ESI-2** and **ESI-3** demonstrate how these simplified equations are fulfilled for the time interval given.

| t. interval | Simplification applied | Mathematical model | $K^{Hb}$, $M^{-1}s^{-1}$ |
|---|---|---|---|
| Full record | None | $\Delta Abs_{576,t} = \frac{2\Delta\varepsilon_{576}[Hb]_0[H_2O_2]_0(1-e^{([Hb]_0-2[H_2O_2]_0)k_{Hb}t})}{2[H_2O_2]_0-[Hb]_0 e^{([Hb]_0-2[H_2O_2]_0)k_{Hb}t}}$ (ESI–1) | $14 \pm 1$ |
| > 2000 s | $[Hb]_o > 2[H_2O_2]_o$ | $\Delta Abs_{576} = 2\Delta\varepsilon_{576}[H_2O_2]_o$ (ESI – 2) | |
| | $[Hb]_o < 2[H_2O_2]_o$ | $\Delta Abs_{576} = \Delta\varepsilon_{576}[Hb]_o$ (ESI – 3) | |
| < 500 s | Taylor polynomial approach ($e^{-x} \approx 1-x$) (11) | $\Delta Abs_{576,t} = \Delta\varepsilon_{576}\frac{2[Hb]_o k_{Hb} t}{1+[Hb]_0 k_{Hb} t}[H_2O_2]_0$ (ESI – 4) | $15 \pm 1$ |
| < 150 s | * Taylor polynomial approach (11)<br>* $1+[Hb]_0 k_{Hb} t \approx 1$ | $\Delta Abs_{576,t} = 2\Delta\varepsilon_{576}[Hb]_o k_{Hb}[H_2O_2]_0 t$ (ESI - 5) | $14 \pm 2$ |

**Table ESI-1.-** Different forms of equation (9) depending of the time interval considered

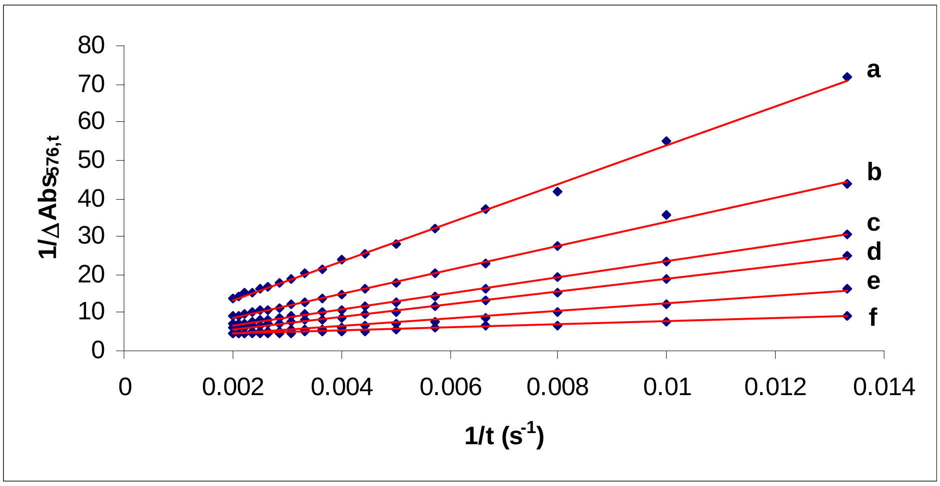


**Figure ESI-2.** Inverse of $\Delta Abs_{576,t}$ change as a function of the inverse of time fitting according to equation (ESI-4) where there is a linear relationship.

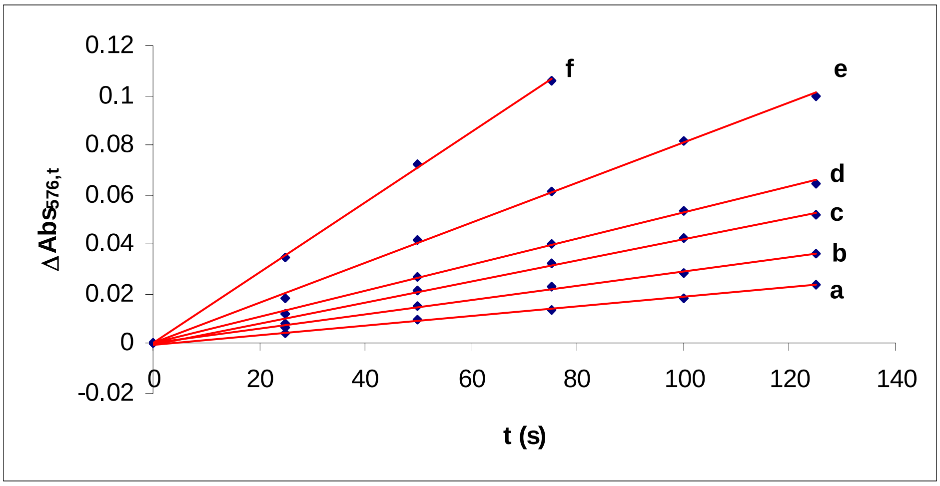


**Figure ESI-3.** $\Delta Abs_{576,t}$ change as a function of the inverse of time fitting according to equation (ESI-5).

# 3.- Study of the Hb/GOx electron transfer

**1) The conclusion given in (Page 6, Main text)**

**a)** *The only Hb species reacting with GOx is HbIII (the same oxidation state as in HRP) yielding $HbIV^+$ by intramolecular reduction,*

can be supported by results given in figure **ESI-4**. This figure shows the $\Delta Abs_{576}$ variation versus time for different GOx concentrations. As can be seen, when the GOx concentration increases the absorbance variation decreases owing to the fact that the HbIV concentration increases as a result of the reaction between HbIII and GOx (see figure 2 of the Main test: the difference between molar absorptivities is lower for $HbIIO_2$/HbIV than for $HbIIO_2$/HbIII so that the absorbance variation is lower in the first case).

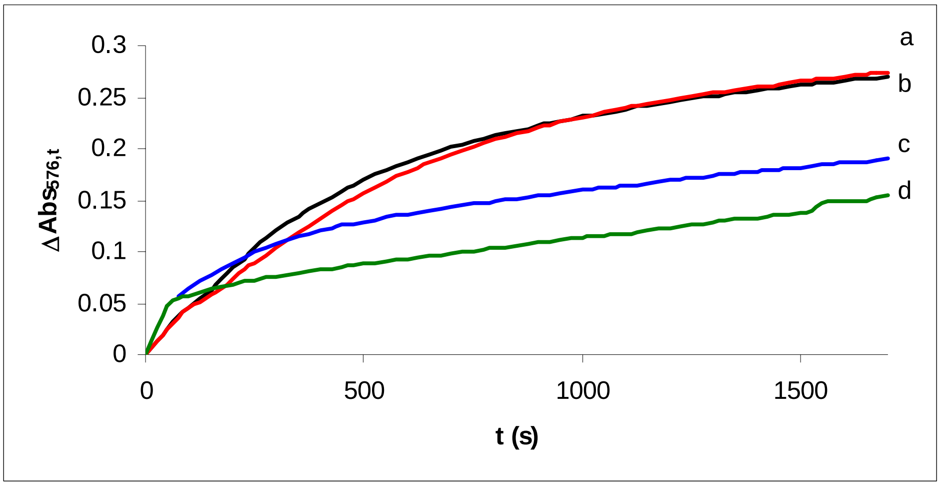


**Figure ESI-4.** $\Delta Abs_{576,t}$ change as a function of time for different GOx concentrations in absence of azide. Experimental conditions: $4.3*10^{-5}$ M Hb, $5.6*10^{-5}$ M glucose concentrations and different GOX concentrations: **a)** $6.2*10^{-7}$ M (red); **b)** $1.2*10^{-6}$ M (black); **c)** $1.0*10^{-5}$ M (blue); **d)** $5.0*10^{-5}$ M (green).

**2) The conclusion given in (Page 6, Main text)**

**b)** *Azide partially inhibits the electron transfer between HbIII and GOx*

is supported by data in figure **ESI-5**.

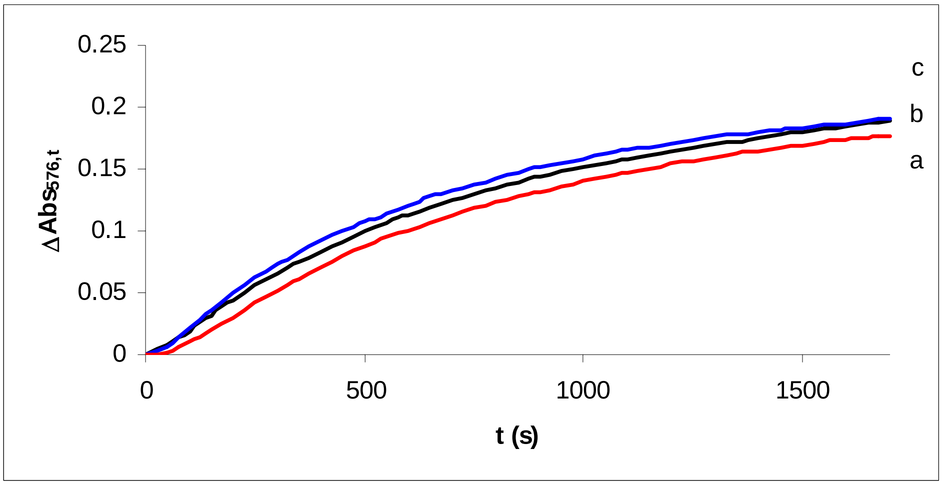


**Figure ESI-5.** $\Delta Abs_{576,t}$ change as a function of time for different GOx concentrations in presence of azide. Experimental conditions: $4.3*10^{-5}$ M Hemoglobin , $5.6*10^{-5}$ M glucose concentrations and different GOx concentrations: **a)** $9.1*10^{-7}$ M (red); **b)** $5.1*10^{-6}$ M (black); **c)**$1.0*10^{-5}$ M (blue).