

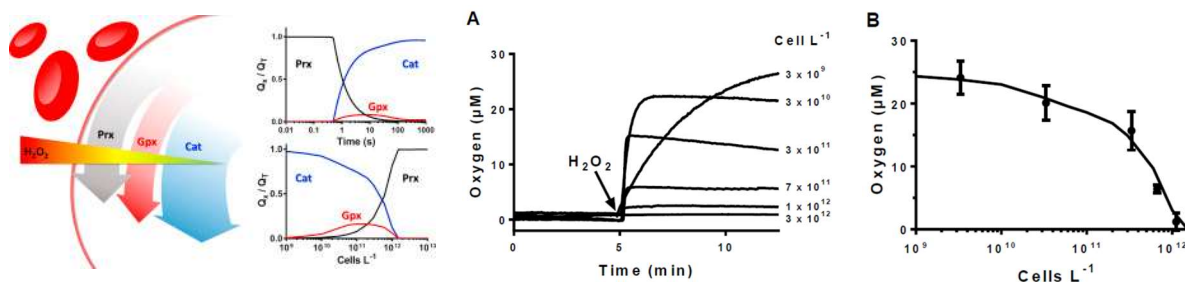
High-Resolution Fluorespirometry and kinetics

Kinetic and stoichiometric constraints determine the pathway of H₂O₂ consumption by red blood cells

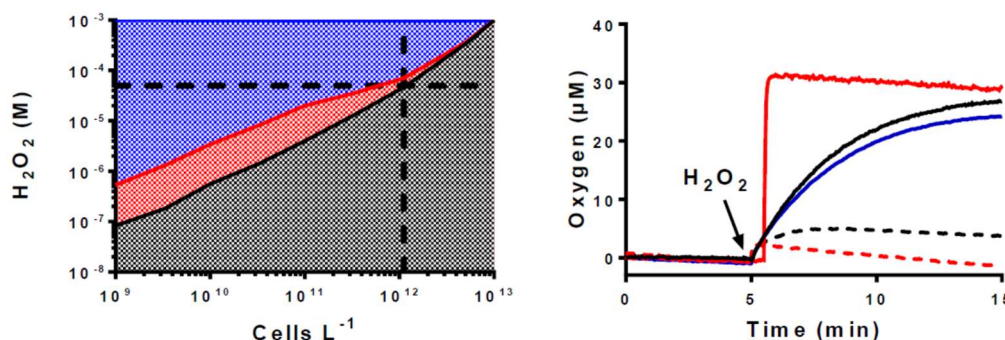


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The antioxidant systems in red blood cells are evaluated measuring the oxygen released by the intracellular catalase with H₂O₂



Production of oxygen from H₂O₂ by RBC. Graphical abstract. A. Oxygen concentration was measured in the presence of 3 × 10⁹, 3 × 10¹⁰, 3 × 10¹¹, 7 × 10¹¹, 1 × 10¹², and 3 × 10¹² cell L⁻¹ before and after the addition of 50 µM H₂O₂ (arrow). B. Correlation between oxygen production and cell density. The dots represent maximal oxygen production at each cell density while the line was obtained by simulations *in silico*.



Effect of enzyme inhibitors. (Left) Maximum H₂O₂ concentration consumed by Prx (black line), Gpx (red line) and catalase (blue line) was simulated at different cell densities. Black dashed area indicates the experimental space where Prx in RBC can be studied; the red dashed area indicates where GPx will be most active and the blue dashed area indicates the conditions under which catalase will be the main enzyme consuming H₂O₂. The dashed lines highlight 50 µM H₂O₂ (horizontal line) and 1.1 × 10¹² cell L⁻¹ (vertical line). (Right) Representative oxygen production profile obtained after the addition of 50 µM H₂O₂ (arrow) to 3 × 10⁹ cell L⁻¹ (black lines) or to an equivalent amount of catalase (2 µM, red lines). Both, cells and catalase were pre-incubated for 30 min at 37 °C in the absence (continuous lines) or presence (dashed lines) of 2 mM sodium azide. Oxygen evolution from cells pre-treated in the same condition with 50 mM NEM (continuous blue line) is also shown.

Reference: Orrico F, Möller MN, Cassina A, Denicola A, Thomson L (2018) Kinetic and stoichiometric constraints determine the pathway of Catalase; Glutathione peroxidase; Hydrogen peroxide; Peroxiredoxin; Reaction kinetic; Reaction rate; Red blood cells consumption by red blood cells. Free Radic Biol Med 121:231-9.