Supplemental information

Lucina pectinata Oxyhemoglobin (II-III) heterodimer pH susceptibility

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Method

Kinetics and optical absorption

Dissociation of O_2 from HbII-HbIII was determined at pH 5 and 7 by monitoring the decay of the (HbII-HbIII)-O_2 absorption signal at 414 nm, using a SHIMADZU UV-2600 spectrophotometer. Briefly, small volumes (140 µL) of 98 µM (HbII-HbIII)-O_2 solution were mixed rapidly with a large volume of deoxygenated buffer (2 mL) at pH 7 and 5. The kinetic traces, as well as spectra associated with the kinetics, were acquired for a period of 3 hr.

Results

Figure S1 shows that at pH 5, O_2 dissociation from (HbII-HbIII)-O_2 is near 1.6 times faster by than at pH 7, with k_{off} values of 0.0051 s^{-1} and 0.0026 s^{-1}, respectively. The k_{off} results are approximately within the range of the reported values for HbII (0.056 s^{-1}) and recombinant HbII (0.053 s^{-1}) by Ramos, *et al.*, and HbII (0.011 s^{-1}) and HbIII (0.008 s^{-1}) by Kraus *et. al*.

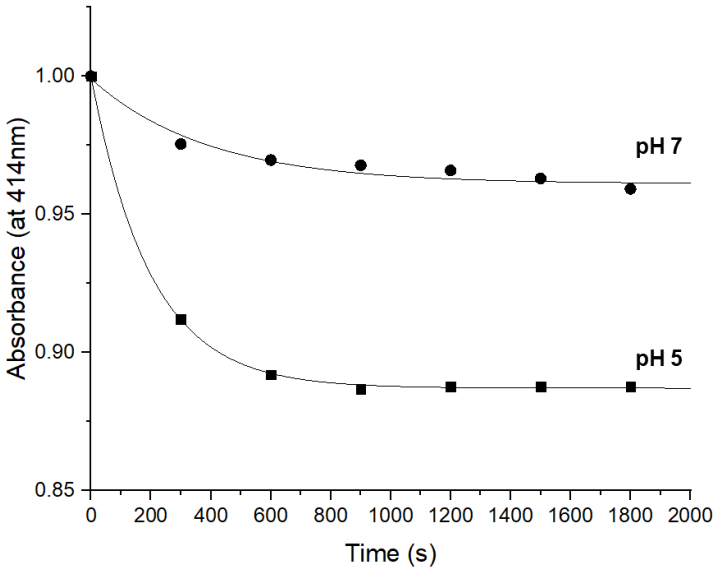

Figure S1: Kinetic traces of O_2 dissociation at pH 5 (squares) and 7 (circles).

References

C. Ramos, R. Pietri, W. Lorenzo, E. Román, L. B. Granell, C. L. Cadilla, J. López-Garriga, Protein J. 29 (2010) 143-151.

D. Kraus, J.B. Wittenberg, J. Biol. Chem. 265 (1990) 16043–16053.