

Electronic supplementary information

Preparation of the apoenzyme

The enzyme Carbonic anhydrase from bovine erythrocytes (E.C. 4.2.1.1, Fluka *BioChemika*) was dialyzed (Spectra/Por[®]7 MWCO 3500 from Spectrum Laboratories) against 0.075 M Pyridine-2,6-dicarboxylic acid in 0.2 M phosphate buffer (pH 7.4) over-night in order to remove the Zn²⁺ from the enzyme. After dialysis the apoenzyme was purified by size exclusion chromatography using Biogel-P6 (Bio-Rad Laboratories). Protein concentration was determined by measuring absorbance at 280 nm ($\epsilon_{280} = 57000 \text{ M}^{-1}\text{cm}^{-1}$).

Precautions against metal contamination

The usage of glassware was avoided. All solutions (except ZnSO₄ and DNA) were treated with Chelex 100 (Sigma) before use. 0.2 M Phosphate buffer solution and 1 M Trizma[®] hydrochloride buffer solution were extracted with 0.01% Dithizone in CHCl₃. The water used for the reactions was of *TraceSelect*Ultra Quality (Fluka).

Color assay for detection of CO₂ hydration

All solutions used in this experiment were stored in an ice/water bath, in order to maintain a constant temperature of 0°C.

The reaction tube used here had a total volume of 6.5 ml and was equipped with a small magnetic stir bar. Solutions were CO₂-saturated by continuously blowing gaseous CO₂ into the tube with stirring. Reaction solutions (400 µl) contain the reagents as indicated in the legend of fig. 2.

In the absence of buffer, CO₂ is in equilibrium with H₂CO₃ and conversion of only trace amounts of CO₂ is expected. Reactions were initiated by addition of HEPES buffer (pH 9.0).