# Testing hypotheses on cellular C fluxes in Emiliania huxleyi by means of kinetic models

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### Abstract

Coccolithophores play a crucial role in the marine carbon (C) cycle and thus it is interesting to know how they will respond to climate change and ocean acidification. The interplay between intracellular metabolic processes and the marine carbonate system is still not well understood. We have tested different hypotheses concerning the uptake and the flux of inorganic C species inside the cell by means of kinetic models.

#### Pathway of C through chloroplast



## Models of cellular C fluxes

The models are constrained by experimental data of *Emiliania huxleyi*.

**Isotopic C fractionation** diffusive influx of CO<sub>2</sub>

#### Pathway of C through cell

A potential pathway of inorganic C through the chloroplast.  $CO_2$  follows its concentration gradient into the chloroplast (permeability coefficient: 0.19 m·h<sup>-1</sup>). The gradient is established via a combination of different pH values in the chloroplastintern compartments, carbonic anhydrase (CA) activity, and a reduced  $CO_2$  diffusiveness into and out of the thylakoid/pyrenoid complex (permeability coefficient: 2 mm·h<sup>-1</sup>). The model is constrained by the POC production data of Rokitta & Rost (2012) and the cytosolic  $[CO_2]$  calculated by the cell model.

**Results (fig.)**: By means of the proposed  $\begin{bmatrix} 100 \\ 80 \\ 60 \end{bmatrix}$ mechanism, a large part of CO<sub>2</sub> needed  $\begin{bmatrix} 90 \\ 80 \\ 60 \end{bmatrix}$ for POC production can be provided.  $\begin{bmatrix} 90 \\ 80 \\ 60 \\ 40 \end{bmatrix}$ Under High Light and High C (HLHC)  $\begin{bmatrix} 20 \\ 20 \end{bmatrix}$ 

In contrast to other C fractionation models,  ${}^{13}CO_2$  and <sup>12</sup>CO<sub>2</sub> diffuse independently of each other.  $^{12}CO_{2}$ The  $CO_2$  permeability coefficient of the plas-  $^{13}CO_2$  $^{13}CO_{2} \stackrel{13}{\longleftrightarrow} ^{13}CO_{2}$ ma membrane is  $0.58 \text{ m} \cdot \text{h}^{-1}$  (aquaporinbased value measured plasma membrane by Uehlein et al., 2004), the one of the chloroplast envelope is one third of this value, i.e., 0.19 m·h<sup>-1</sup>. Inside the chloroplast,  $CO_2$  is fixed into POC with  ${}^{13}R_{CO_2}({}^{13}CO_2:{}^{12}CO_2) = {}^{13}R_{POC}$  $(PO^{13}C : PO^{12}C)$ . The latter value as well as external  $[CO_2]$ 

were measured by Rost et al. (2002) for differently acclimated cells.

Results (fig.): 33 (orange squares) out of 35 data points of Rost et al. (2002) can be described by our model. Owing to RubisCO's discrimination against <sup>13</sup>CO<sub>2</sub>, the latter CO<sub>2</sub> isotope accumulates inside the chloroplast and reduces the diffusive influx of <sup>13</sup>CO<sub>2</sub> into the cell. An efflux of CO<sub>2</sub> from the cell is thus not necessary to explain the lowered <sup>13</sup>C signal of POC. A diffusive influx of CO<sub>2</sub> may actually provide a large part of inorganic C to organic C fixation. Based on the assumption that external CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> feed particulate organic carbon (POC) and particulate inorganic carbon (PIC) production, respectively (Berry et al., 2002, Kottmeier et al., in prep.), a basic cell model is established that consists of 2 compartments: the cytosol and located within the cytosol the cocco-

lith vesicle.  $CO_2$  enters the cytosol via diffusion with a permeability coefficient of 0.07 m·h<sup>-1</sup> (aquaporin-based value of Prasad et al., 1998).  $HCO_3^-$  follows its concentration gradient into the cell with a permeability coefficient of 0.35 mm·h<sup>-1</sup>. Chloroplast and mitochondria are implemented as  $CO_2$  sink and source, respectively. The carbonate system is resolved dynamically inside both compartments. The model is constrained by POC, PIC, and external carbonate system data of Rokitta and Rost (2012) who acclimated *E. huxleyi* to four different conditions (high/low light and high/low C availability). EE in the figure stands for energy equivalents.

HCO<sub>2</sub>

HCO<sub>3</sub>

CO<sub>3</sub><sup>2-</sup>

as well as under Low Light (LLLC and LLHC) more than 80%, while under High Light and Low C (HLLC) only up to ~60% can be provided. Shaded areas indicate the range of cytosolic  $[CO_2]$  that is calculated by the cell



model when assuming two different permeability coefficients for the plasma membrane:  $0.07 \text{ m}\cdot\text{h}^{-1}$  (Prasad et al., 1998) or  $0.58 \text{ m}\cdot\text{h}^{-1}$  (Uehlein et al., 2004).

# Conclusion

mitochondri

An active accumulation of inorganic C inside the cell that leads to a diffusive  $CO_2$  efflux is currently favoured in literature. Our approaches, in turn, show that a diffusive  $CO_2$  influx may contribute strongly to photosynthetic C assimilation in *E. huxleyi*.

**Results (red arrows in fig.)**:  $CO_2$  and  $HCO_3^-$  pass the cytosol without being interconverted. According to the model, diffusive  $CO_2$  influx can provide inorganic C for POC production.

#### Literature:

Berry, L., Taylor, A. R., Lucken, U., Ryan, K. P., & Brownlee, C. 2002. Calcification and inorganic carbon acquisition in coccolithophores. Functional Plant Biology, 29, 15 289–299.
 Kottmeier, D. M. Manuscript in preparation.

Prasad, G. V. R., Coury, L. A., Finn, F., & Zeidel, M. L. 1998. Reconstituted aquaporin 1 water channels transport CO<sub>2</sub> across membranes. The Journal of Biological Chemistry, 273(50), 33123-33126.
Rokitta, S. D., & Rost, B. 2012. Effects of CO<sub>2</sub> and their modulation by light in the life cycle stages of th coccolithophore *Emiliania huxleyi*. Limnology and Oceanography, 57(2), 607-618.
Rost, B., Zondervan, I., & Riebesell, U. 2002. Light-dependent carbon isotope fractionation in the coccolithophorid *Emiliania huxleyi*. Limnology and Oceanography, 47(1), 120-128.
Uehlein, N., Otto, B., Hanson, D. T., Fischer, M., McDowell, N., & Kaldenhoff, R. 2008. Function of *Nicotiana tabacum* aquaporins as chloroplast gas pores challenges the concept of membrane CO<sub>2</sub> permeability. The Plant Cell, 20, 648-657.

