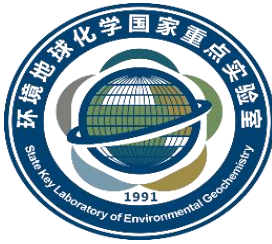




中国科学院
CHINESE ACADEMY OF SCIENCES



Photosynthetic bicarbonate photolysis masked by the rapid oxygen isotopic exchange between water and bicarbonate

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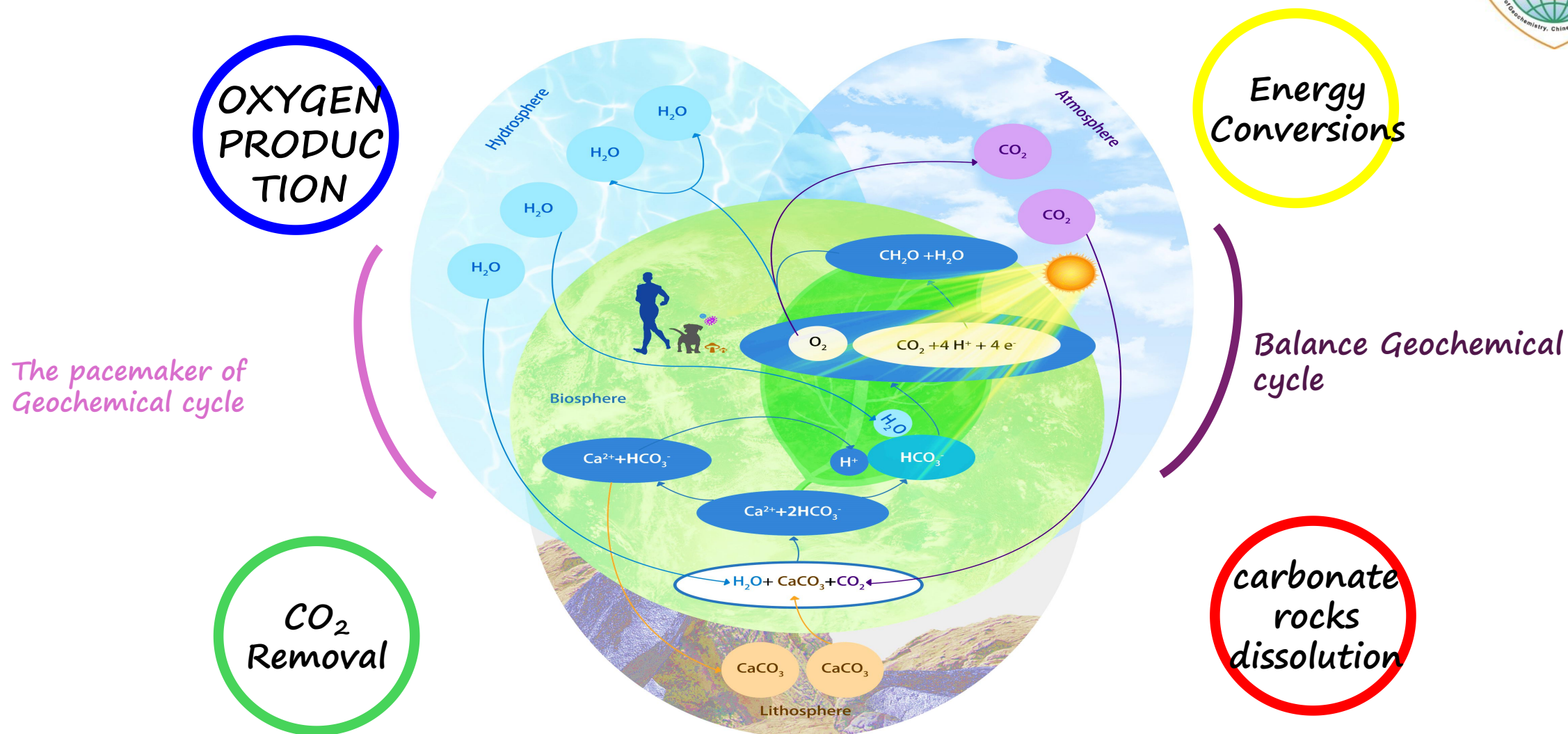


September 17, 2024, Rome, Italy





Photosynthesis is the heartbeat of life





1. What has the classic O^{18} labeling experiment proven?

1.1 Ruben, S., Randall, M., Kamen, M., & Hyde, J. L. (1941). Heavy oxygen (O^{18}) as a tracer in the study of photosynthesis. *Journal of the American Chemical Society*, 63(3), 877-879.

TABLE I

ISOTOPIC RATIO IN OXYGEN EVOLVED IN PHOTOSYNTHESIS
BY *Chlorella*^a

Expt.	Sub- strate	Time between dissolving KHCO ₃ + K ₂ CO ₃ and start of O ₂ collection, minutes		Percent. O ¹⁸ in		
		minutes	Time at end of O ₂ collec- tion, minutes	H ₂ O	HCO ₃ ⁻ + CO ₃ ²⁻	O ₂
1	0.09 M	0		0.85	0.20	..
	KHCO ₃	45	110	.85	.41 ^b	0.84
	+0.09 M	110	225	.85	.55 ^b	.85
	K ₂ CO ₃	225	350	.85	.61	.86
2	0.14 M	0		.20
	KHCO ₃	40	110	.20	.50	.20
	+0.08 M	110	185	.20	.40	.20
	K ₂ CO ₃					
3	0.06 M	0		.20	.68	..
	KHCO ₃	10	50	.20	..	.21
	+0.14 M	50	165	.20	.57	.20
	K ₂ CO ₃					

The
signal
in
oxygen
is
same
as that
in H₂O

TABLE II

ISOTOPIC RATIO IN OXYGEN EVOLVED IN PHOTOSYNTHESIS
BY *Chlorella* IN PRESENCE OF OXYGEN

O ₂ present in gas space at beginning, ml.	O ₂ produced in photosynthesis by 200 mm. ³ algae, ml.	Per cent. O ¹⁸ at end of experiment	
		Obsd.	Calcd. for no exchange
2.29 (O ¹⁸ = 0.20%)	1.55 (O ¹⁸ = 0.85%)	0.43	0.46
3.64 (O ¹⁸ = .20%)	1.18 (O ¹⁸ = .85%)	.34	.36
1.44 (O ¹⁸ = .85%)	0.73 (O ¹⁸ = .20%)	.59	.62
4.81 (O ¹⁸ = .85%)	1.22 (O ¹⁸ = .20%)	.69	.71

The
calculated
value is
always
greater
than the
observed
value
(Implying
that not
all oxygen
is from
water)

Using O^{18} as a tracer, it was found that the oxygen released during photosynthesis comes from water, not carbon dioxide.



1.1 Ruben, S., Randall, M., Kamen, M., & Hyde, J. L. (1941). Heavy oxygen (O^{18}) as a tracer in the study of photosynthesis. *Journal of the American Chemical Society*, 63(3), 877-879.

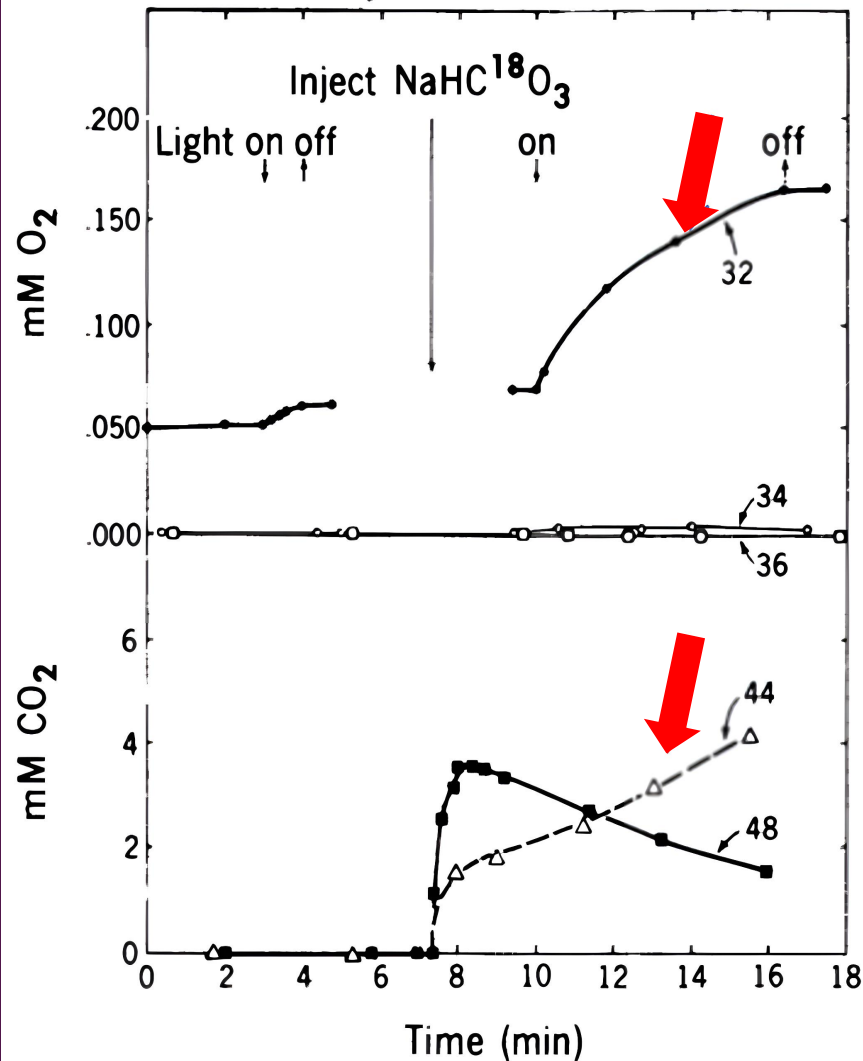
"It is apparent that the ratio of the $^{18}O/^{16}O$ evolved oxygen is identical with that of the water. Since the oxygen in OH, COOH, O—O, C=O, etc., groups exchanges but very slowly with water at room temperature and moderate pH, it seems reasonable to conclude that the oxygen originates solely from the water."



So according to Ruben The premise for concluding that photosynthetic oxygen produced from water is that the exchange rate of oxygen in groups such as OH, COOH, O-O, C=O with water at room temperature and moderate pH is very slow!



1.2 Stemler A, Radmer R (1975) Source of photosynthetic oxygen in bicarbonate-stimulated Hill reaction. Science 190(4213):457-458



"Fig. 1. Oxygen-18 content of dissolved carbon dioxide and photosynthetically evolved oxygen with time. The reaction mixture contained 0.175M NaCl, 0.1M sodium format, 0.05M N-2-hydroxyethylpiperazine - N'-2-ethanesulfonic acid (HEPES) buffer, pH 7.5, 2 mM K₃Fe(CN)₆, and chlorophyll 100 µg/ml. Light from a projection lamp was filtered through 12 inches (30.5 cm) of water and a 3-110 filter (Corning) and focused onto the reaction vessel. The intensity was about one-half of saturation. Mass numbers represented by each curve are indicated: 32 (¹⁶O₂), 34 (¹⁶-¹⁸O₂), 36 (¹⁸O₂), 44 (C¹⁶O₂), and 48 (C¹⁸O₂)."

"Bicarbonate can stimulate chloroplasts to carry out the Hill reaction (oxidizing water to molecular oxygen and reducing artificial oxidants) and release five times more oxygen. However, when bicarbonate containing 18O is applied, the released oxygen only contains 16O. Therefore, they concluded that bicarbonate cannot be the direct source of oxygen produced by photosynthesis."



1.2 Stenler A, Radmer R (1975) Source of photosynthetic oxygen in bicarbonate-stimulated Hill reaction. Science 190(4213):457-458



"Again, resorted to tracer methods. The material used was isolated broken chloroplast fragments instead of whole cells. This minimized the problems of low intracellular pH and removed nearly all carbonic anhydrase, thus effectively controlling the isotopic exchange reaction between H_2O and CO_2 . In addition, by depleting chloroplasts of HCO_3^- , oxygen evolution was made dependent on an exogenous source of this ion. When labeled bicarbonate,"

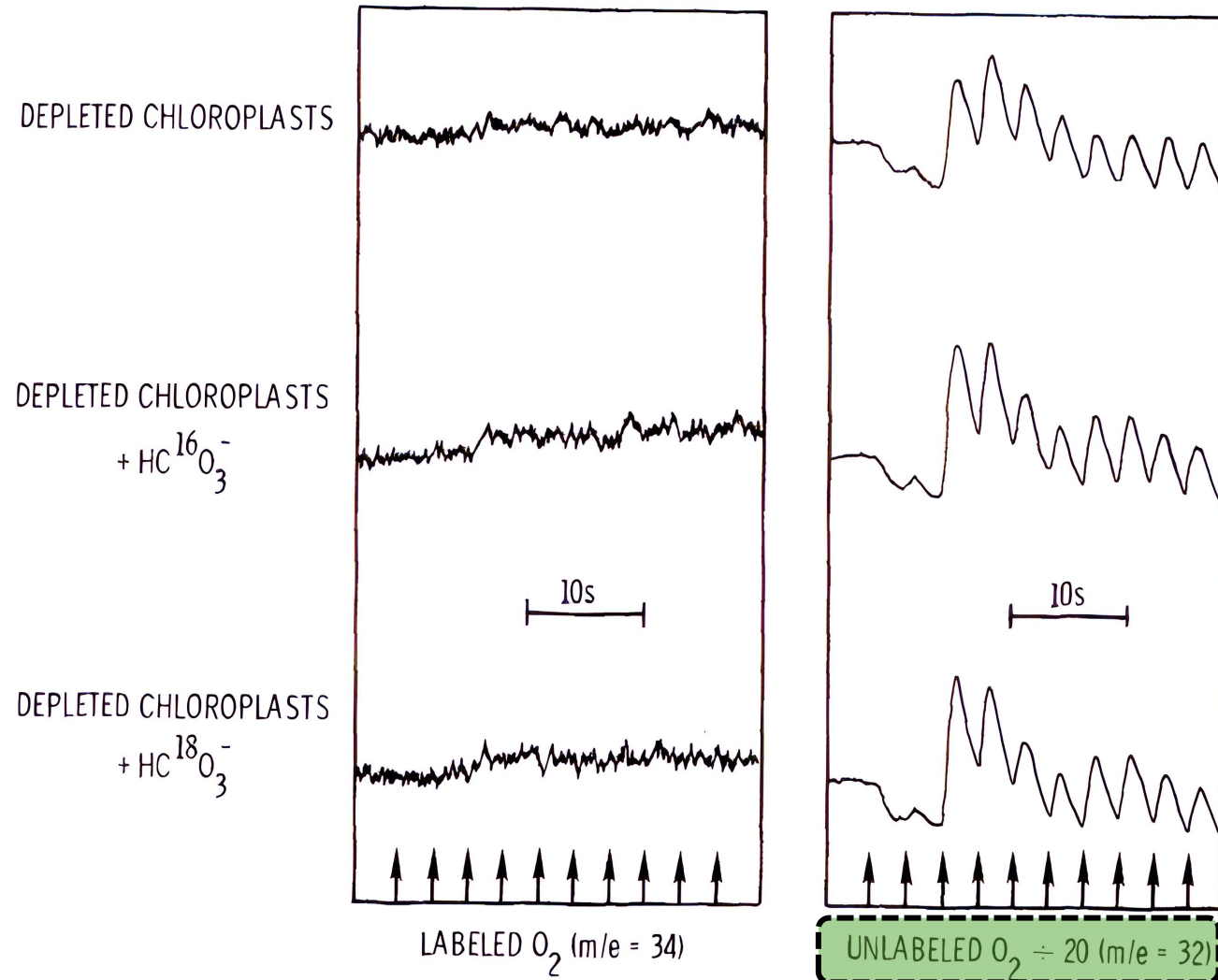


- I. Can they remove all the carbonic anhydrases?
- II. What if all the carbonic anhydrases are removed? (Where PSII is present)
(PSII has the characteristics of carbonic anhydrase) Can effectively control the isotopic exchange between H_2O and CO_2 ?



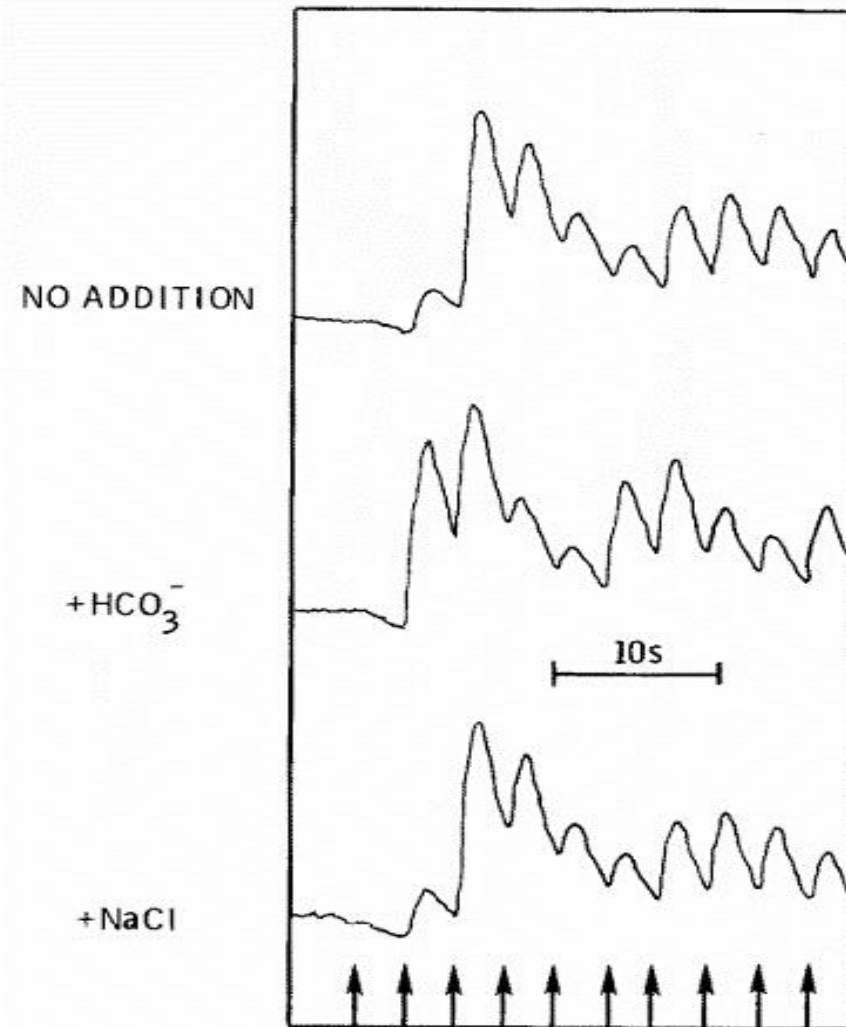
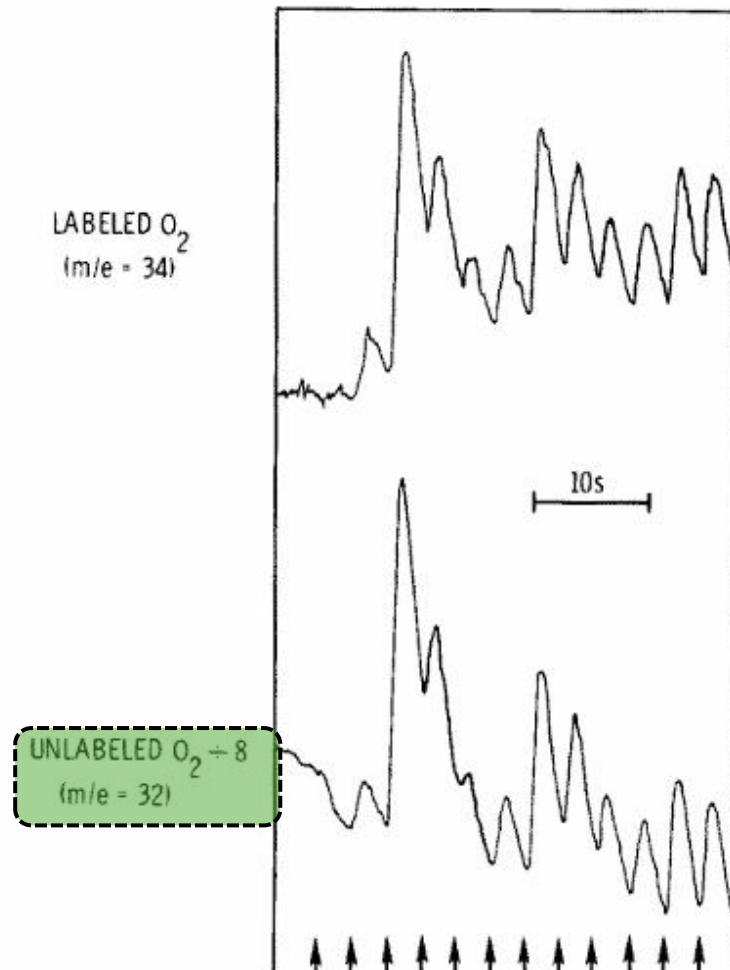


1.3 Radmer R, Ollinger O (1980) Isotopic composition of photosynthetic O₂ flash yields in the presence of H₂¹⁸O and HC¹⁸O₃⁻. FEBS Lett 110(1):57-61.



"Fig. 1. Evolution of ¹⁸O₂ and unlabeled O₂ elicited by short saturating light flashes in HCO₃⁻ depleted chloroplasts, depleted chloroplasts reactivated with unlabeled HCO₃⁻, and depleted chloroplasts reactivated with HC¹⁸O₃⁻. The unlabeled O₂ signal (m/e = 32) was attenuated 20-fold compared to ¹⁸O₂ (m/e = 34) signal. The solid arrows in this and the following two figures mark the firing of the xenon flash (3 s spacing). For the HC¹⁸O₃⁻ experiment, the isotopic composition of the CO₂ at the beginning of the flash sequence was C¹⁸O₂ (m/e = 48), 0.031; C¹⁶¹⁸O₂ (m/e = 46), 0.320; CO₂ (m/e = 44), 0.649."

1.3 Radmer R, Ollinger O (1980) Isotopic composition of photosynthetic O₂ flash yields in the presence of H₂¹⁸O and HC¹⁸O₃⁻. FEBS Lett 110(1):57-61.



"Fig. 3. Effect of bicarbonate on the second flash yield in the presence of ferricyanide. The buffer was reaction medium (see section 2) amended with 1 mM K₃ Fe(CN)₆. Top trace, depleted chloroplasts; middle trace, depleted chloroplasts plus 10 mM NaHCO₃; bottom trace, depleted chloroplasts plus 10 mM NaCl."

"Fig. 2. O₂ flash yields observed when H₂ ¹⁸O (top) or unlabeled (bottom) H₂ O was added in total darkness to dark-adapted chloroplasts. Buffer: 0.9 ml Tricine (50 mM, pH 7.4) plus 0.1 ml H₂ ¹⁸O (71 atom%) or H₂ ¹⁶O, H₂ O (3 μl) was also added to 25 μl chloroplast suspension. After mixing, 10 μl of these chloroplasts were layered on the mass spectrometer inlet. The unlabeled O₂ signal was attenuated 8-fold compared to the labeled (m/e = 34) signal."



1.3 Radmer R, Ollinger O (1980) Isotopic composition of photosynthetic O₂ flash yields in the presence of H₂¹⁸O and HC¹⁸O₃⁻. FEBS Lett 110(1):57-61.



" isotope previously added. This discrepancy did not compromise the validity of the conclusions but was somewhat disturbing. We therefore monitored the H₂¹⁸O/H₂¹⁶O ratio in situ by determining the C¹⁸O₂ : C¹⁶O₂ ratios before illumination in preparations containing 10 mM HCO₃⁻ and 100 units of carbonic anhydrase. The isotope ratio of the evolved O₂ in these experiments was found to agree with the measured H₂¹⁸O/H₂¹⁶O ratio. "

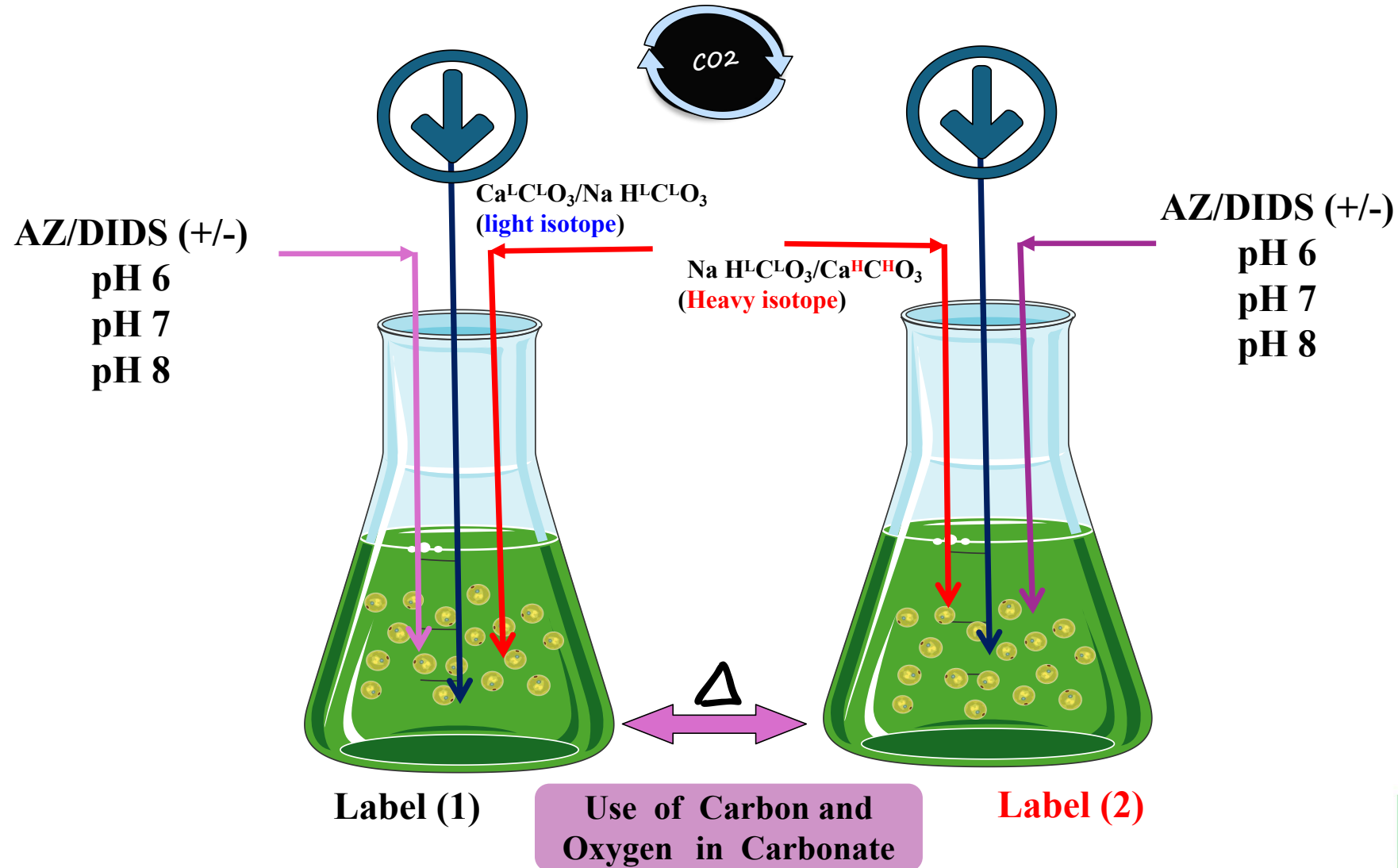
"labeled O₂, a point we verified using H₂¹⁸O, we concluded that CO₂ or HCO₃⁻ contributed ≤1% to the O₂ evolved by single turnovers of the O₂ evolving system. Therefore, these data, along with earlier find-"

They were uneasy and wondering whether there was a rapid exchange of oxygen isotopes of water and bicarbonate caused by carbonic anhydrase.



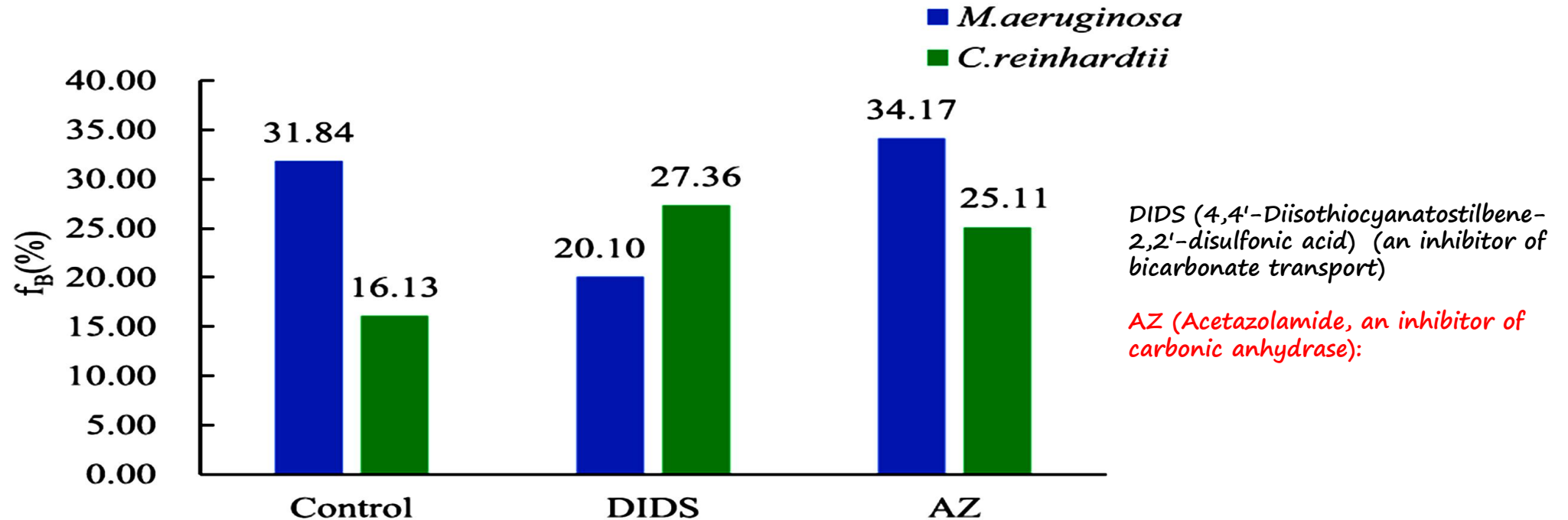
2. Uncover the veil of oxygen isotopic exchange between HCO_3^- and H_2O during photosynthesis

2.1 The bi-element (carbon and oxygen) bidirectional isotopic tracer culture technology





2.2 The use of carbon and oxygen from added bicarbonate by microalga in the full nutrition medium

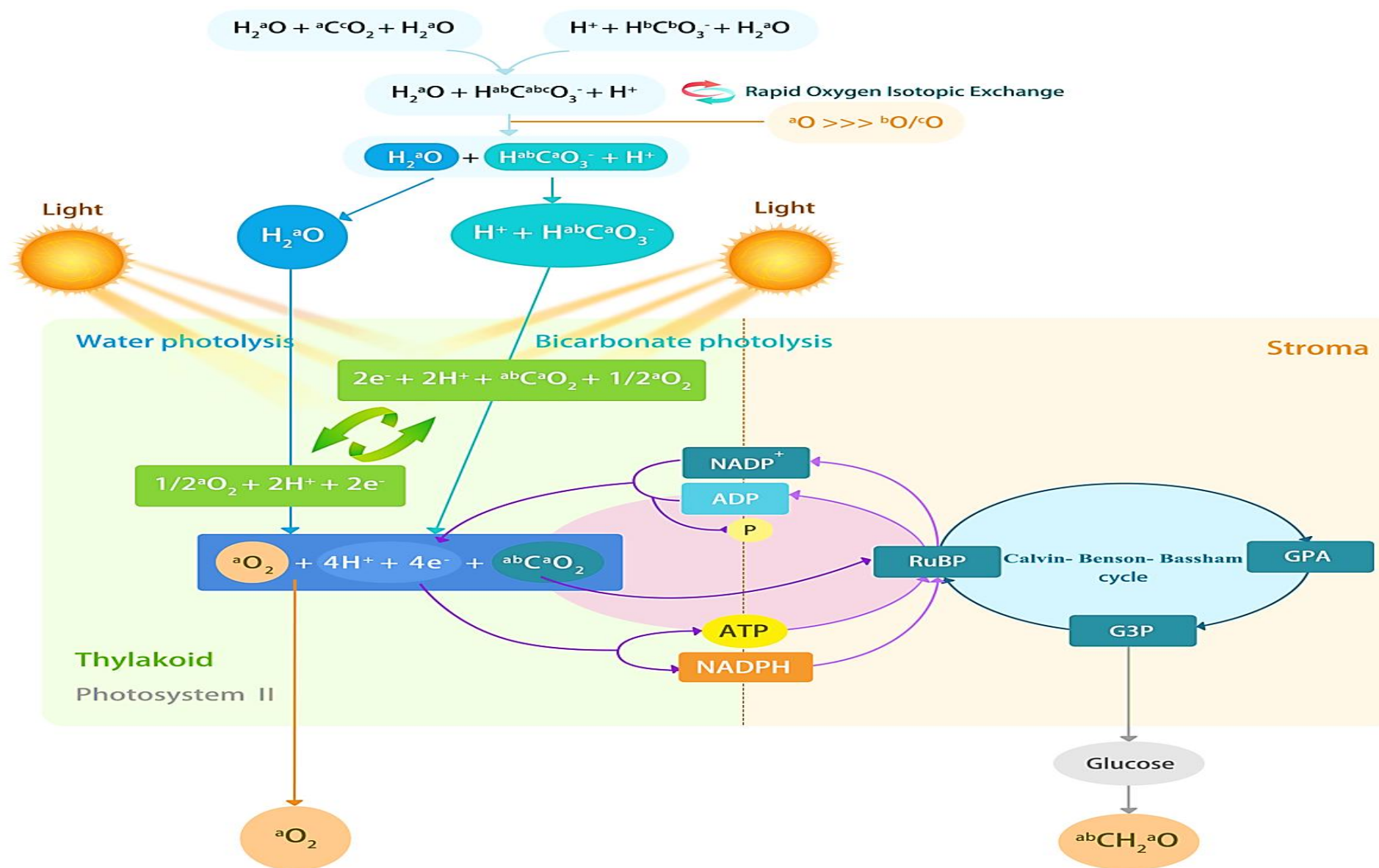


Proportion $f_B(\%)$ of carbon utilization in HCO_3^- of *Microcystis aeruginosa* and *Chlamydomonas reinhardtii* in control group, DIDS treatment group and AZ treatment group, respectively

The oxygen signal of bicarbonate did not appear in the algae's organic matter.

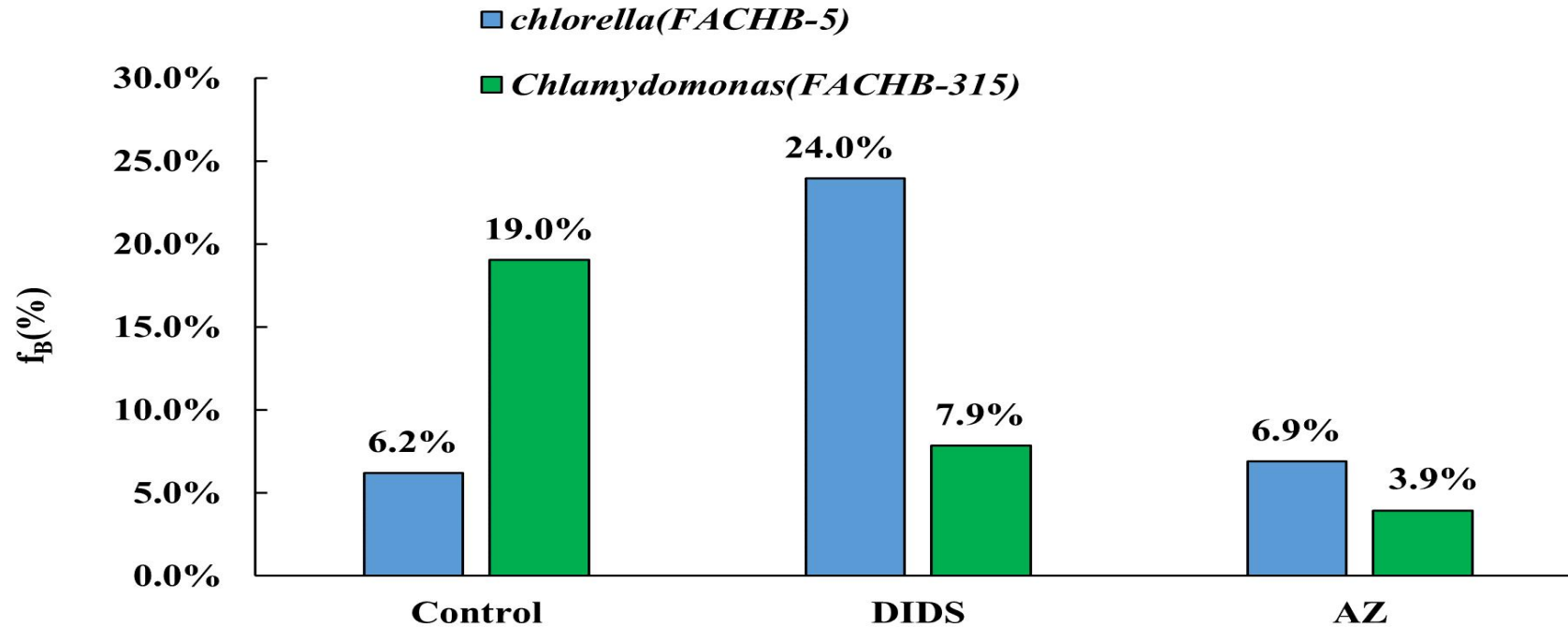
This indicates that there is a rapid exchange of oxygen isotopes between bicarbonates and water during the photosynthesis of microalgae, and photosynthetic oxygen release does not exclude the direct contribution of bicarbonates.

2.2 The use of carbon and oxygen from added bicarbonate by microalga in the full nutrition medium





2.3 The use of carbon and oxygen from added calcium carbonate by microalga in the full nutrition medium



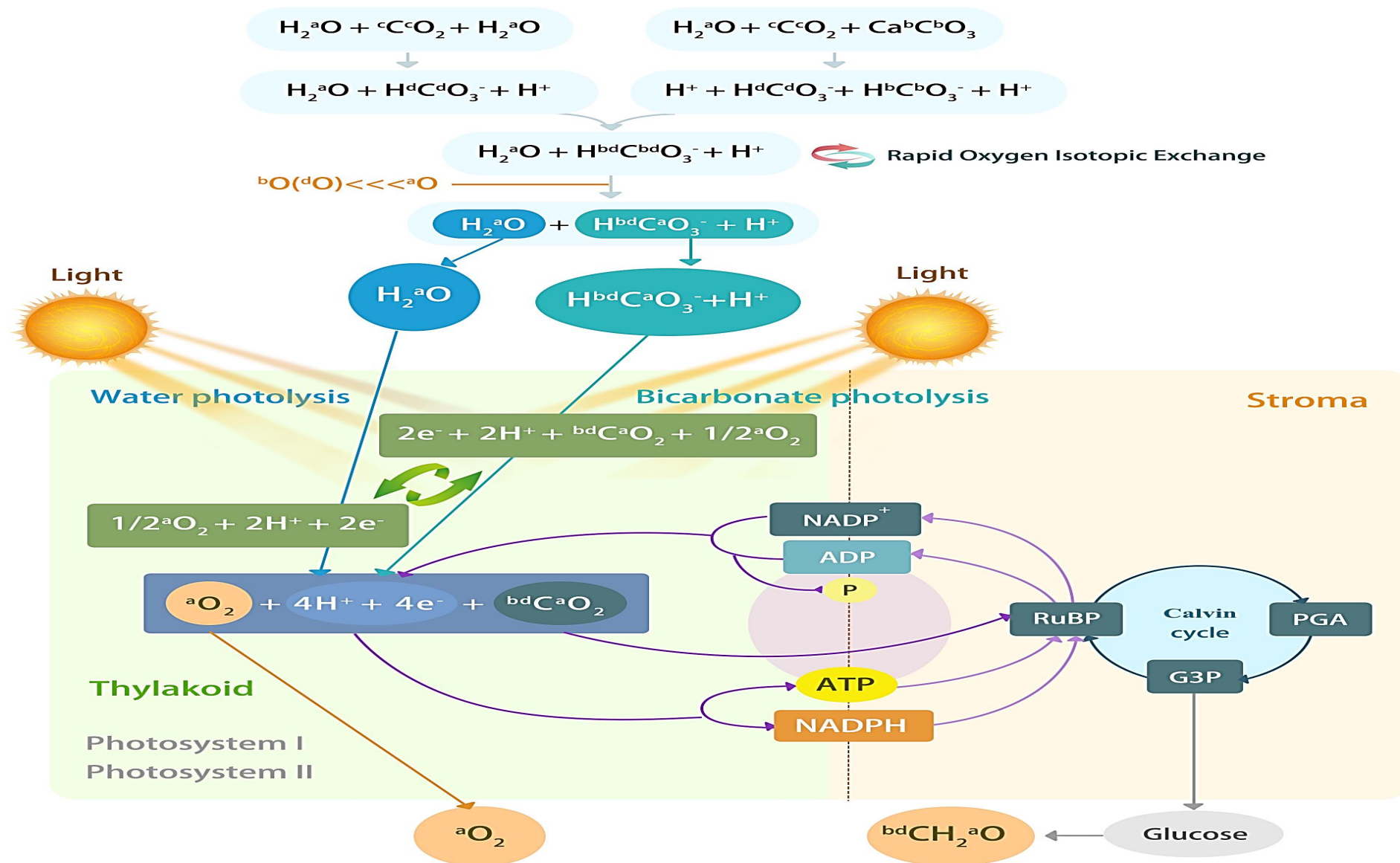
Proportion $f_B(\%)$ of carbon utilization in calcium carbonate of *Microcystis aeruginosa* and *Chlamydomonas reinhardtii* in control group, DIDS treatment group and AZ treatment group, respectively

The oxygen signal of calcium carbonate did not appear in the algae's organic matter.

This indicates that there is a rapid exchange of oxygen isotopes between bicarbonates and water during the photosynthesis of microalgae, and photosynthetic oxygen release does not exclude the direct contribution of bicarbonates.



2.3 The use of carbon and oxygen from added calcium carbonate by microalga in the full nutrition medium





2.4 The use of carbon and oxygen from added calcium carbonate by microalga in the calcium-magnesium-depleted medium



The utilization of carbon and oxygen from added calcite by microalgae in the calcium-magnesium-depleted medium

Species	Treatments	C(%)	O (%)	C:O
<i>Chlorella vulgaris</i>	pH 6	12.63	10.48	1.21:1.00
<i>Chlorella vulgaris</i>	pH 7	12.26	8.97	1.37:1.00
<i>Chlorella vulgaris</i>	pH 8	8.10	7.17	1.13:1.00
<i>Chlamydomonas reinhardtii</i>	pH 6	16.89	17.01	0.99:1.00
<i>Chlamydomonas reinhardtii</i>	pH 7	16.60	13.07	1.27:1.00
<i>Chlamydomonas reinhardtii</i>	pH 8	10.05	7.48	1.34:1.00
<i>Microcystis aeruginosa</i>	pH 6	17.64	14.74	1.20:1.00
<i>Microcystis aeruginosa</i>	pH 7	16.93	13.55	1.25:1.00
<i>Microcystis aeruginosa</i>	pH 8	7.65	7.00	1.09:1.00



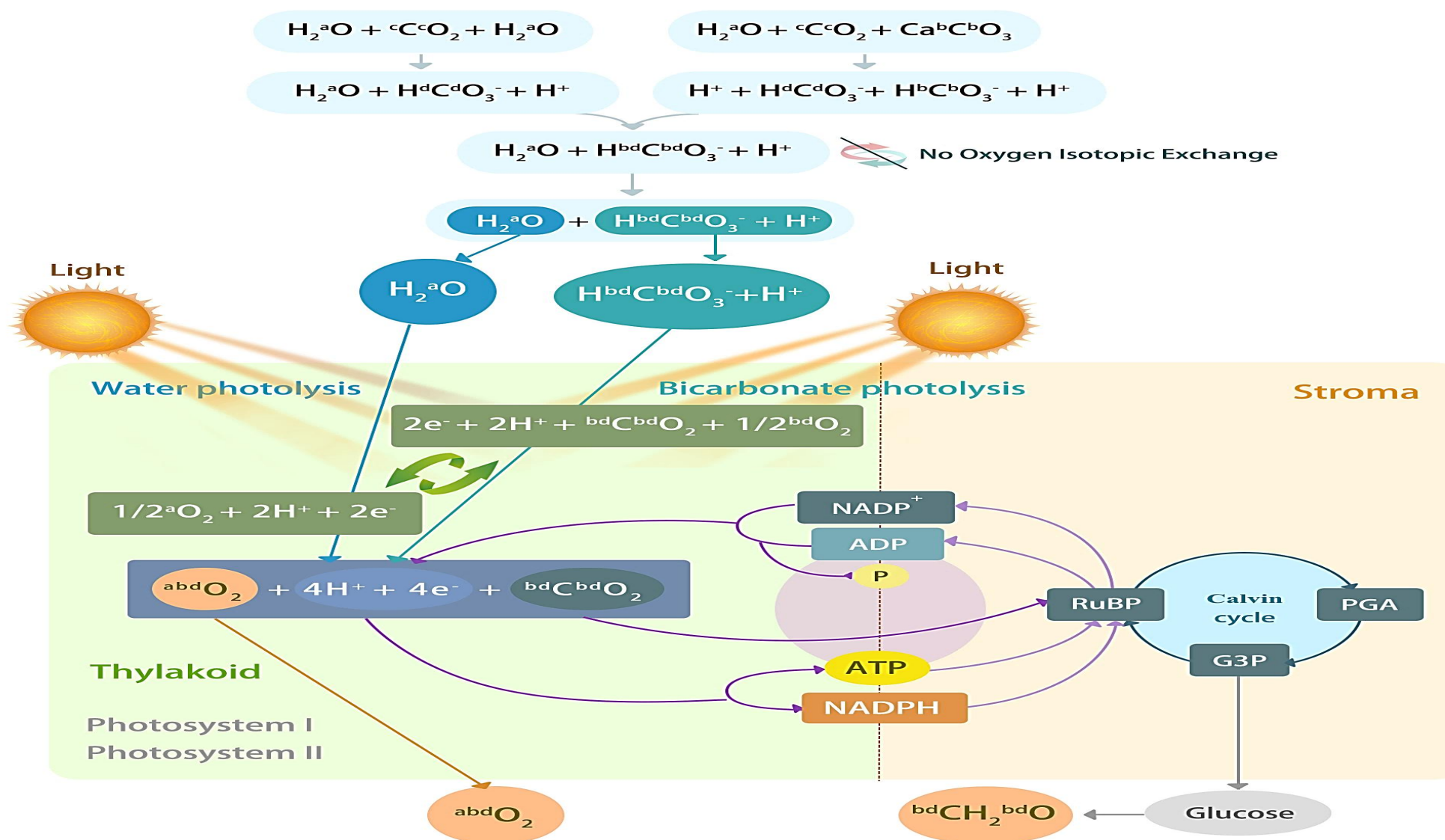
2.4 The use of carbon and oxygen from added calcium carbonate by microalga in the calcium-magnesium-depleted medium

The carbon/oxygen utilization ratios of the above microalgae were (0.99~1.37):1. This indicates that under calcium-magnesium-depleted conditions, microalgae undergo slow exchange or even no exchange of oxygen isotopes between bicarbonates and water during photosynthesis

No exchange of oxygen isotopes between bicarbonates and water during photosynthesis : the carbon/oxygen utilization ratios of microalgae was 1:1.



2.4 The use of carbon and oxygen from added calcium carbonate by microalga in the calcium-magnesium-depleted medium

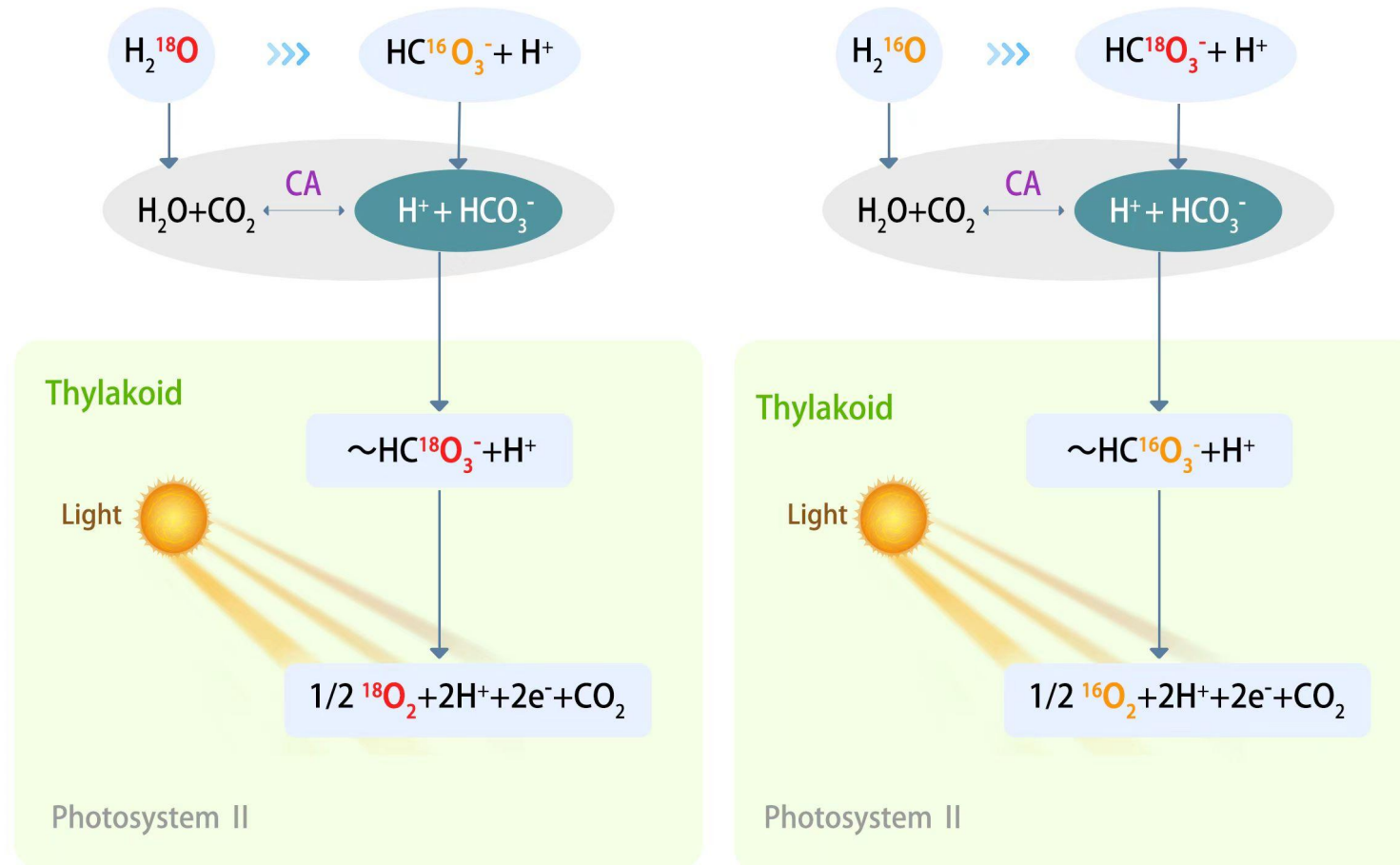




3. Complete exchange of oxygen isotopes to explain the ^{18}O labeled experiments

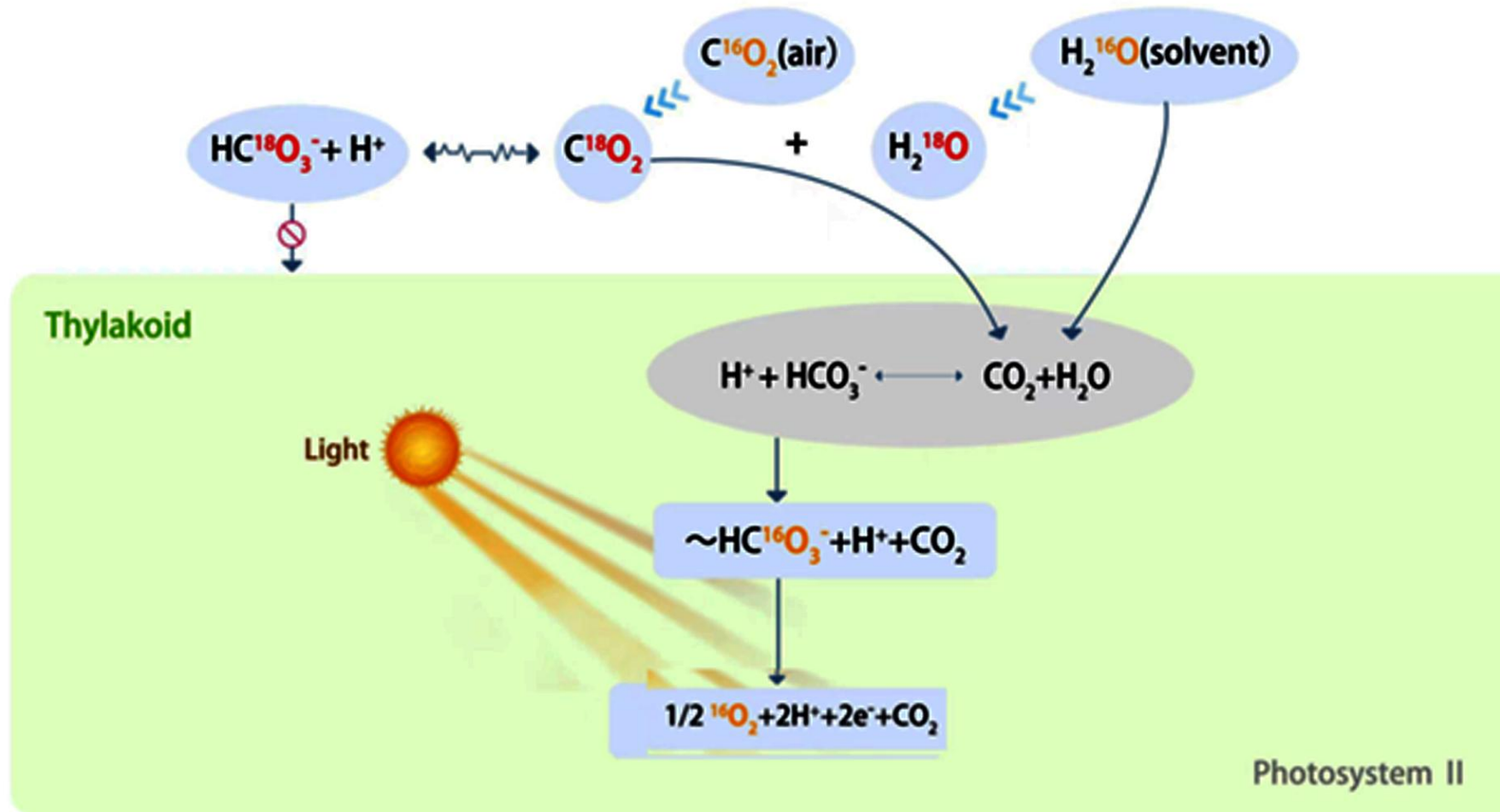


3.1 Ruben, S., Randall, M., Kamen, M., & Hyde, J. L. (1941). Heavy oxygen (O^{18}) as a tracer in the study of photosynthesis. *Journal of the American Chemical Society*, 63(3), 877-879.



Complete oxygen isotope exchange between bicarbonate and water occurs, allowing the released oxygen to retain only the oxygen signal from water and eliminating the oxygen signal from bicarbonate.

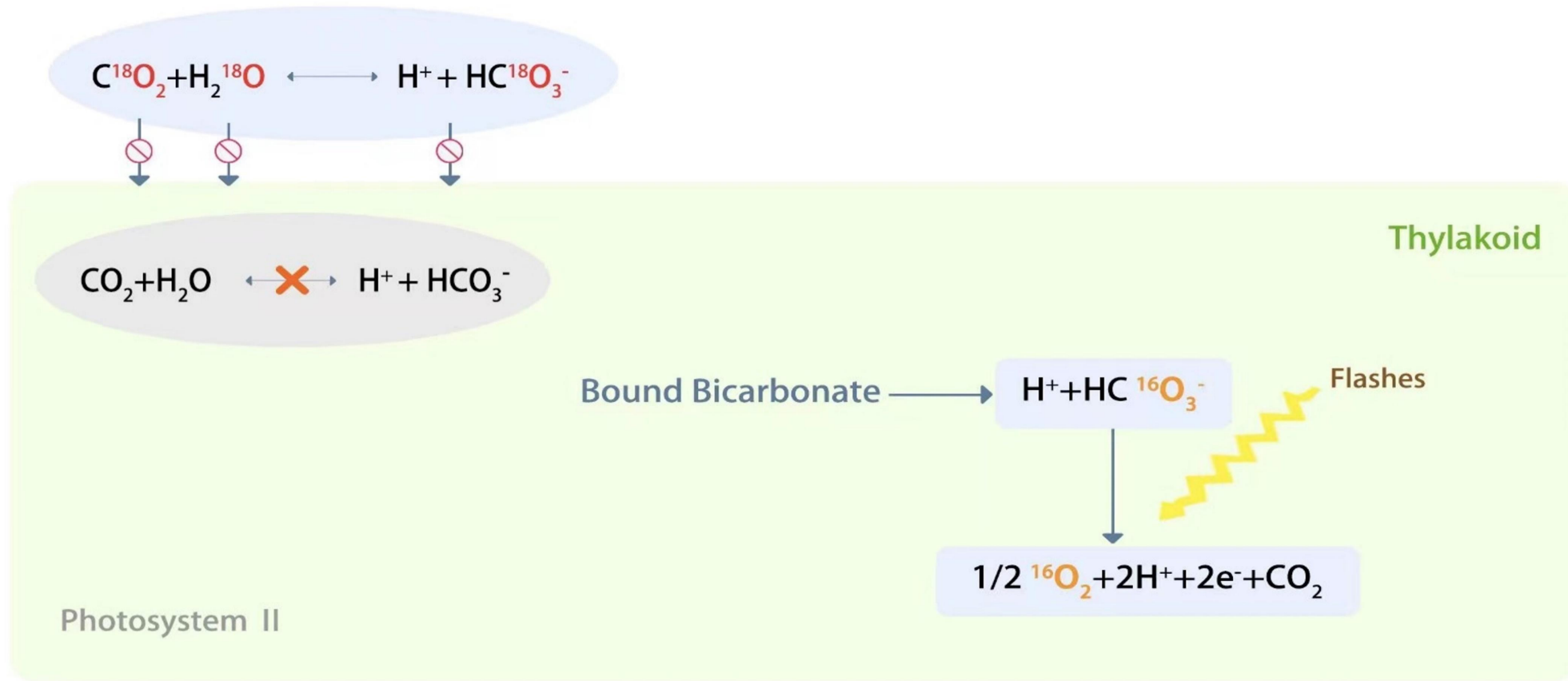
3.2 Stemler A, Radmer R (1975) Source of photosynthetic oxygen in bicarbonate-stimulated Hill reaction. Science 190(4213):457-458



Complete oxygen isotope exchange between bicarbonate and water occurs, allowing the released oxygen to retain only the oxygen signal from water and eliminating the oxygen signal from bicarbonate.



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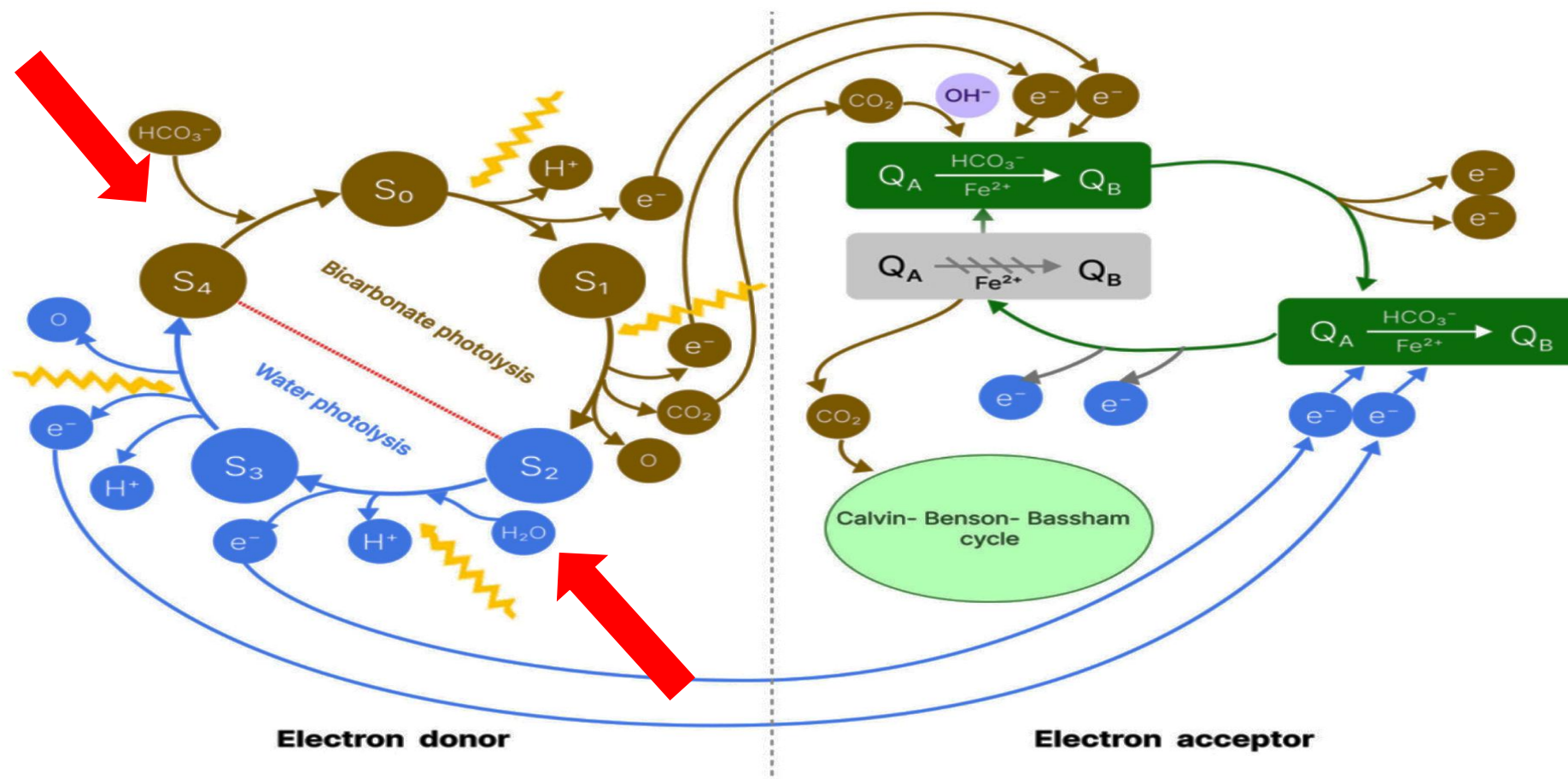
Under the action of flashes, the bound bicarbonate is not labeled, so the released oxygen is also not labeled with O¹⁸, and the amount of oxygen released is only 1/8 of that under continuous illumination.





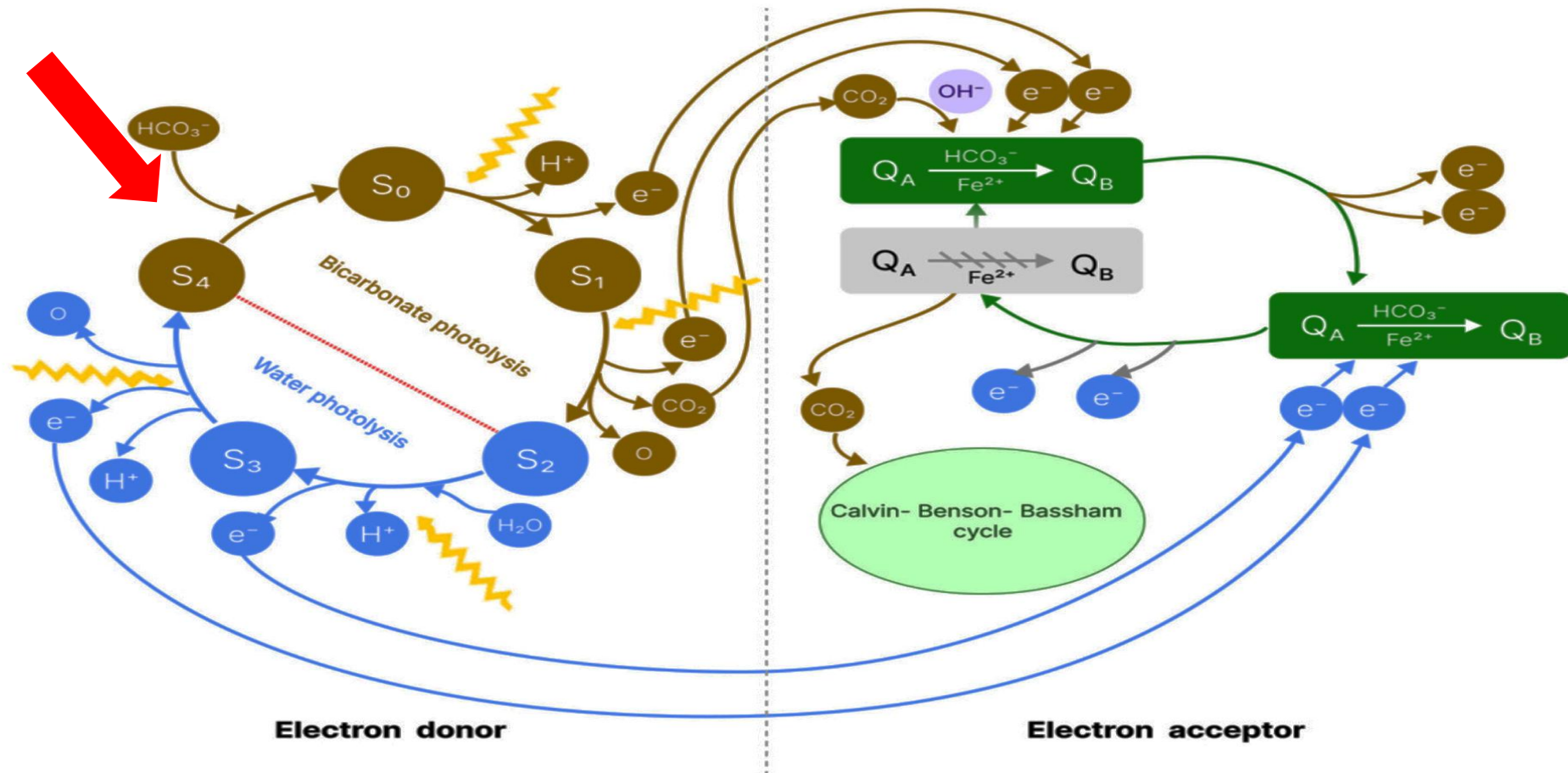
4. Modify the Kok-Joliot cycle and explain the Dole effect

4.1 Modify the Kok-Joliot cycle



Bicarbonate is incorporated into the $S_4 \rightarrow S_0$ transition state of the Kok-Joliot cycle, while water is incorporated into the $S_2 \rightarrow S_3$ transition state.(WHY)

4.2 Why is S4 transition state not observable in the Kok-Joliot cycle?



The photolysis of bicarbonate requires only two-thirds of the free energy required for the photolysis of water; the rate of bicarbonate photolysis is significantly faster than that of water photolysis. As a result, the energy barrier in the S₃→S₄→S₀ transition state is low, and the S₄ transition state is extremely short-lived, making it difficult to capture with current instrumentation.



4.3 Explain the Dole effect

$\delta^{18}\text{O}$ and ^{18}O content of different components in the atmosphere and seawater (data from [Metzner 1975](#)) (maximum oxygen exchange coefficient between carbon dioxide and bicarbonate is 1.012, data from [Reid and Urey \(1943\)](#)).

	$\delta^{18}\text{O}$	^{18}O content
Water in seawater	0‰	0.1995
Atmospheric O_2	22.06‰	0.2039
	23.56‰	0.2042
Atmospheric O_2 (average)	23.06‰ (Calculated value)	0.2041
HCO_3^- in seawater (maximum exchange)	54.14‰	0.2103
HCO_3^- in seawater (moderate exchange)	47.62‰	0.2090
HCO_3^- in seawater (no exchange)	41.60‰	0.2078



4.3 Explain the Dole effect

	$\delta^{18}\text{O}$	^{18}O content
Water in seawater	0‰	0.1995
Atmospheric O_2	23.56‰	0.2042
HCO_3^- in seawater (moderate exchange)	47.62‰	0.2090

The enrichment of ^{18}O in atmospheric O_2 is nearly 24‰ (currently 23.56‰) higher than that in seawater, and this enrichment of ^{18}O is known as the Dole effect. Since the vast majority of atmospheric O_2 originates from photosynthetic oxygen release, the Dole effect can be explained as the result of equal contributions from the photolysis of bicarbonate and water. When the degree of oxygen isotope exchange between carbon dioxide and bicarbonate is moderate, the $\delta^{18}\text{O}$ of bicarbonate in seawater is 47.62‰, and $\delta\text{O}=0.5*0+0.5*47.62\text{‰}$, resulting in $\delta\text{O} = 23.82\text{‰}$, which is very close to the measured value of 23.56‰.



5. Inspiration and Outlook



It scientifically answers the questions of the co-evolution of the elements in the Earth's spheres and the sources that maintain chemical stoichiometry in nature, providing new insights into the origin and evolution of oxygenic photosynthetic organisms, theoretical basis for managing carbon and water balances, and ultimate solutions to carbon sequestration issues.

By constructing a new type of artificial photosynthetic reactor that couples' photoreactions and dark reactions to absorb atmospheric CO₂ and produce food, energy, and necessities, it brings breakthrough solutions for the coordinated development of the food-energy-water system.

Thank you for your attention.
感谢您的关注。

