Is the most abundant protein on earth, Rubisco* slow and inefficient, optimal or both?

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*** Rubisco, *n.*** ribulose bisphosphate carboxylase oxygenase.
An enzyme present in plant chloroplasts and involved in the fixing of atmospheric carbon dioxide in photosynthesis (OED) .

Outline

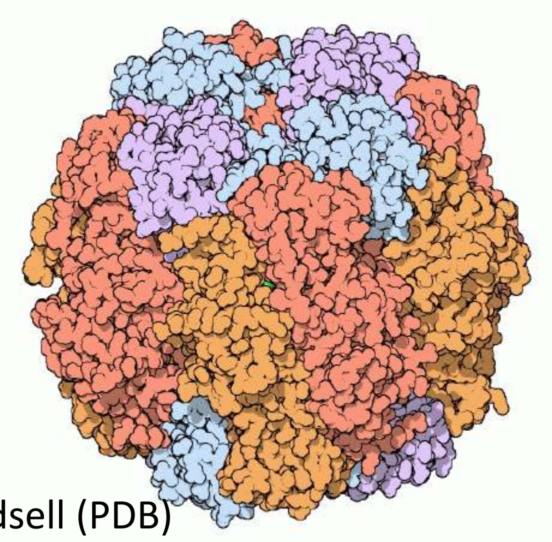
- Intro: Rubisco, an enzyme **essential** for life yet **inefficient** (?)

- Function of Rubisco in photosynthesis: **Cross-species analysis**.

- Evolution constrained to **low dimensional landscapes**:
  phenomenological power laws.

- Is Rubisco **optimal** to its habitat?

- Outlook:
  Confined plasticity - generic phenomenon?

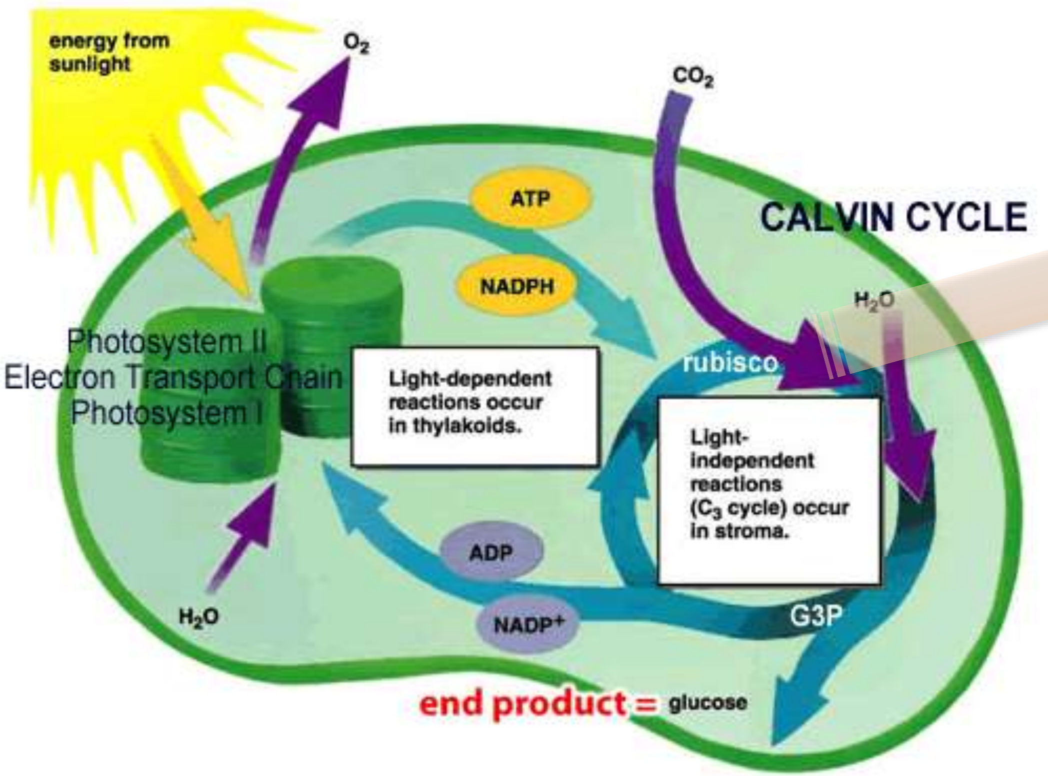


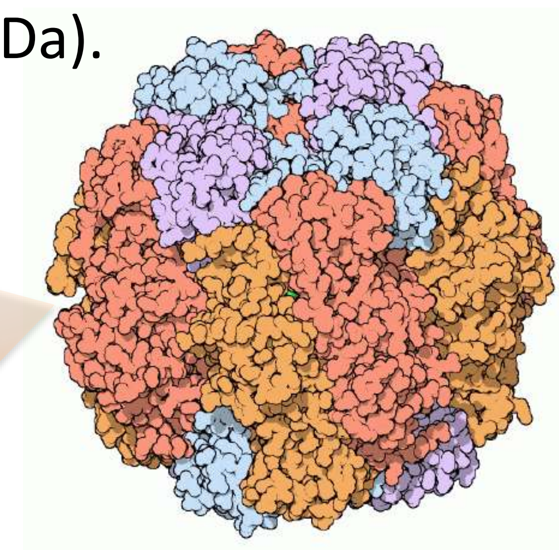
D. Goodsell (PDB)

Rubisco catalyzes carbon fixation

- Photosynthesis fixates carbon into organic forms.
- Rubisco participates in the Calvin cycle:

  captures CO_2 and releases C_3 sugars.
- Complex of 8 large + 8 small subunits (540 kDa).





D. Goodsell (PDB)

Impact of Rubisco's (in)efficiency on the biosphere

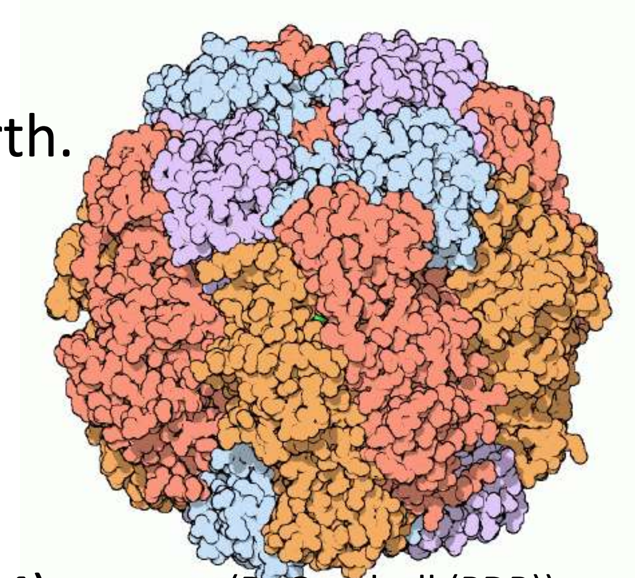
- Possibly the most abundant protein on Earth.
- Catalyzes most carbon fixation.

- **Very slow** catalysis rate (~ 3-10 CO_2 /sec).
- **Specificity:** confuses O=C=O and O=O

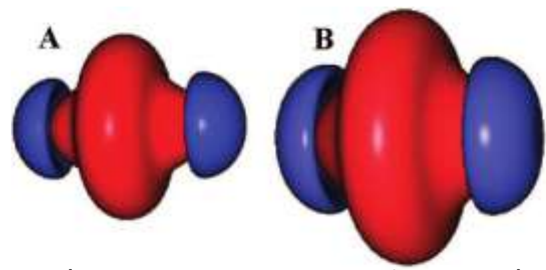
  (K_C = 10-100 and 1000 μM).

- Photorespiration instead of photosynthesis

  (wasteful release of CO_2 in daylight).

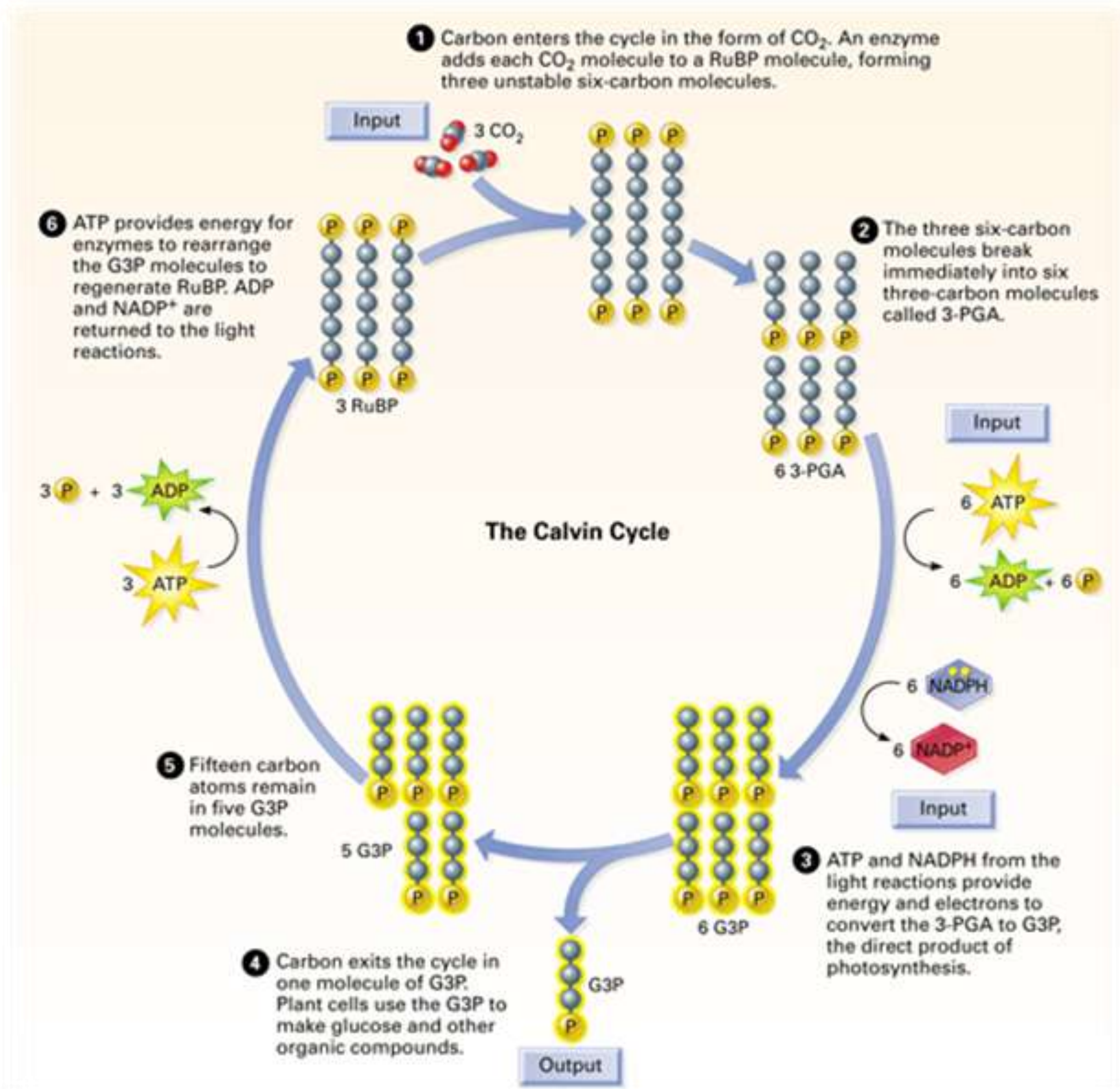


(D. Goodsell (PDB))



(Kannapan & Gready 2008)

* Calvin cycle

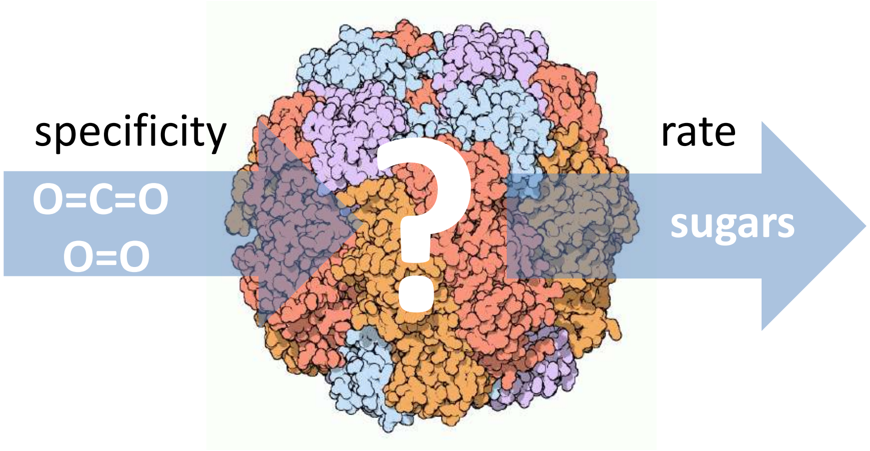


Can Rubisco be improved?

- Improve specificity and carboxylation rate?
- Ongoing effort (genetic manipulation, directed evolution)
  - little success so far.

- Already optimized by evolution? (or relic of old atmosphere?)
- Inefficiency due to biochemical constraints?
- Hint : rate-specificity tradeoff.

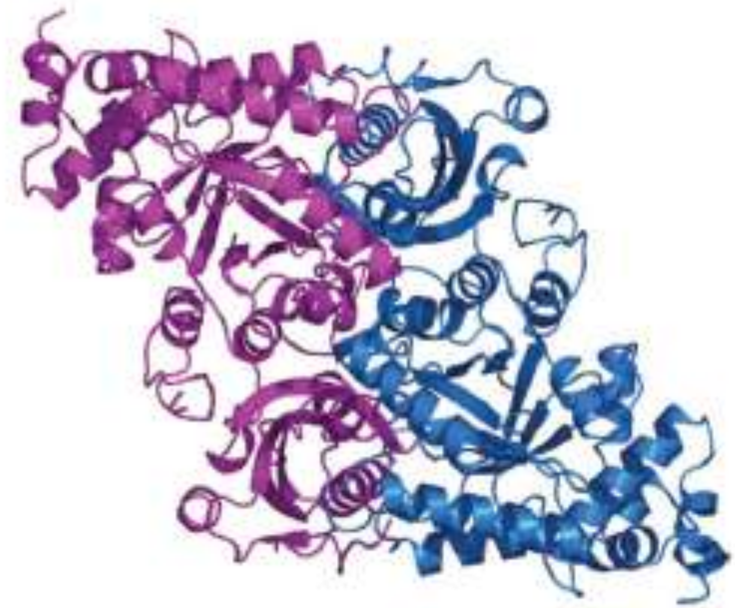
- **Is Rubisco optimal, constrained, or both?**



Rubisco has several forms

Form I

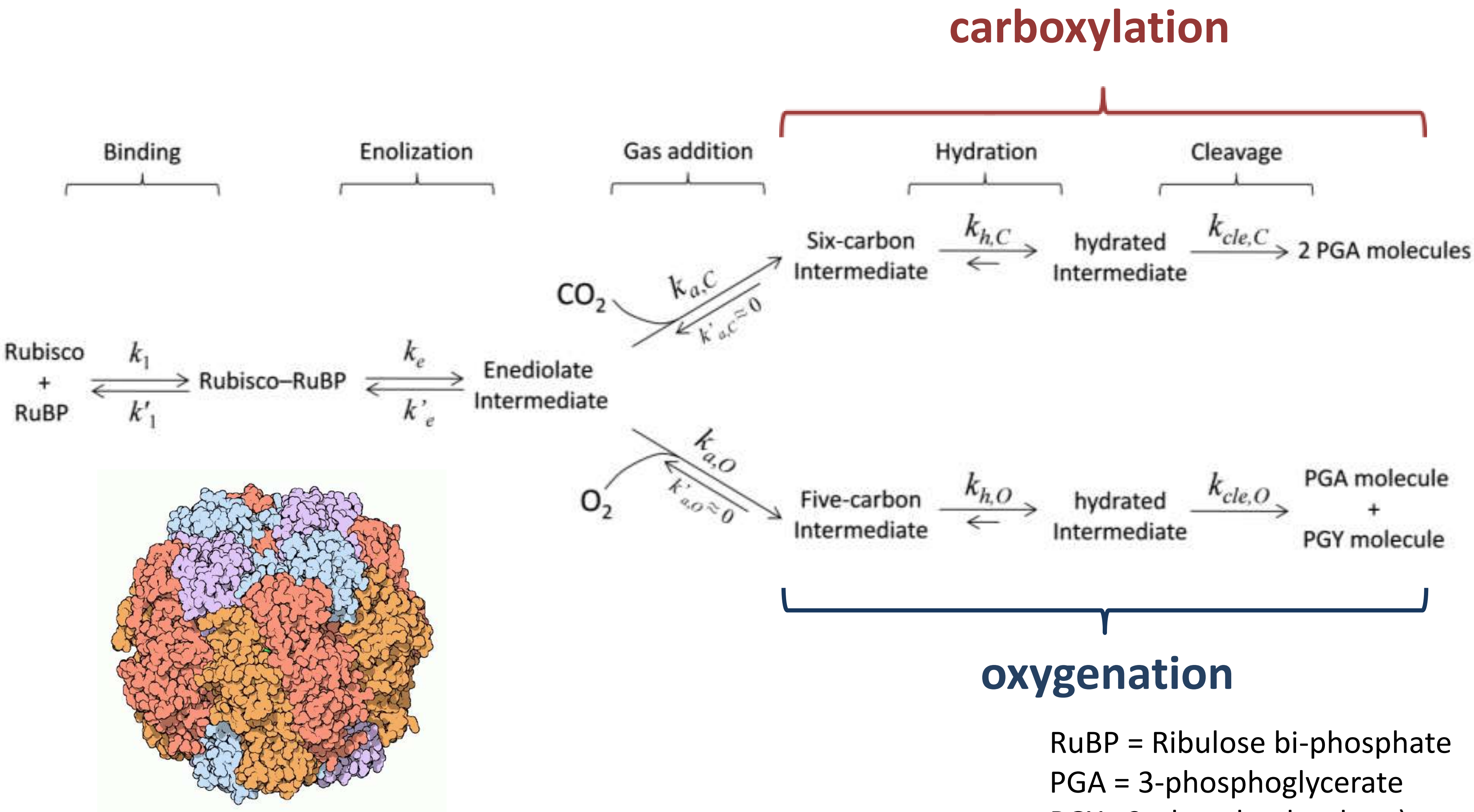
More abundant hexadecimeric form: eight large and small subunit (L_8S_8)



Form II

Found in bacteria: only large subunit dimmer (L_2)

Rubisco fixates carbon in a multistage process

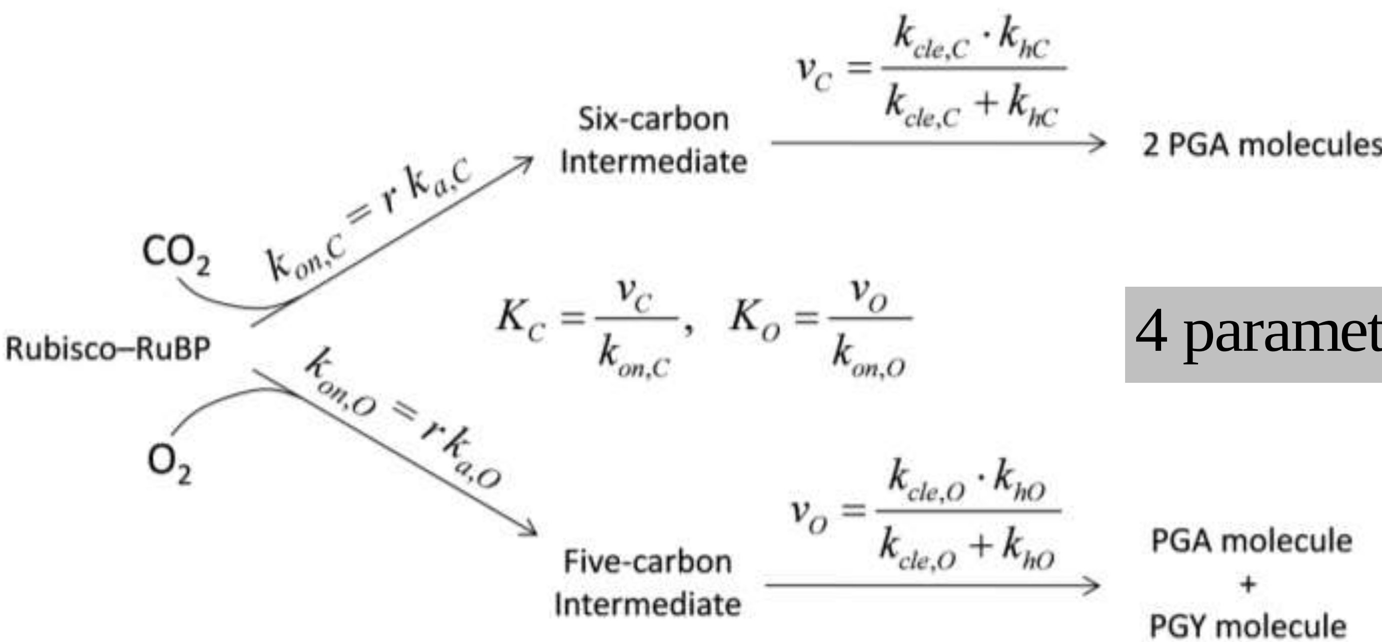


RuBP = Ribulose bi-phosphate
PGA = 3-phosphoglycerate
PGY =2-phosphoglycolate)

Rubisco kinetics is effectively Michaelis-Menten

$$\begin{pmatrix}\text{carboxylation}\\ \text{per Rubisco}\end{pmatrix} = \frac{v_C}{1+\frac{K_C}{[CO_2]}\left(1+\frac{[O_2]}{K_O}\right)}$$

carboxylation



4 parameters: K_C v_C K_O v_C

oxygenation

$$\begin{pmatrix}\text{oxygenation}\\ \text{per Rubisco}\end{pmatrix} = \frac{v_O}{1+\frac{K_O}{[O_2]}\left(1+\frac{[CO_2]}{K_C}\right)}$$

Energy landscape from MM Kinetics

Gas addition

$$k_{on,C} = v_C / K_C = \exp(-\Delta G_{1,C})$$

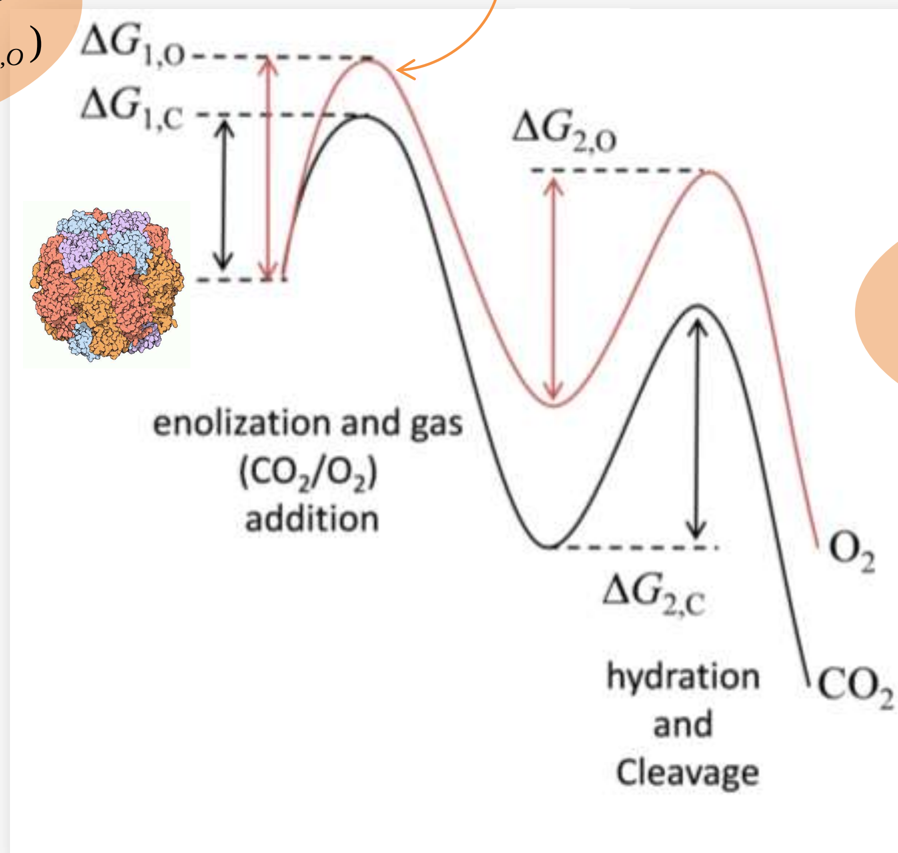
$$k_{on,O} = v_O / K_O = \exp(-\Delta G_{1,O})$$

Specificity $S = \frac{k_{on,C}}{k_{on,O}} = \exp\left(\Delta G_{1,O} - \Delta G_{1,C}\right)$

Catalysis

$$v_C = \exp(-\Delta G_{2,C})$$

$$v_O = \exp(-\Delta G_{2,O})$$



* Rubisco fixates carbon in a multistage process

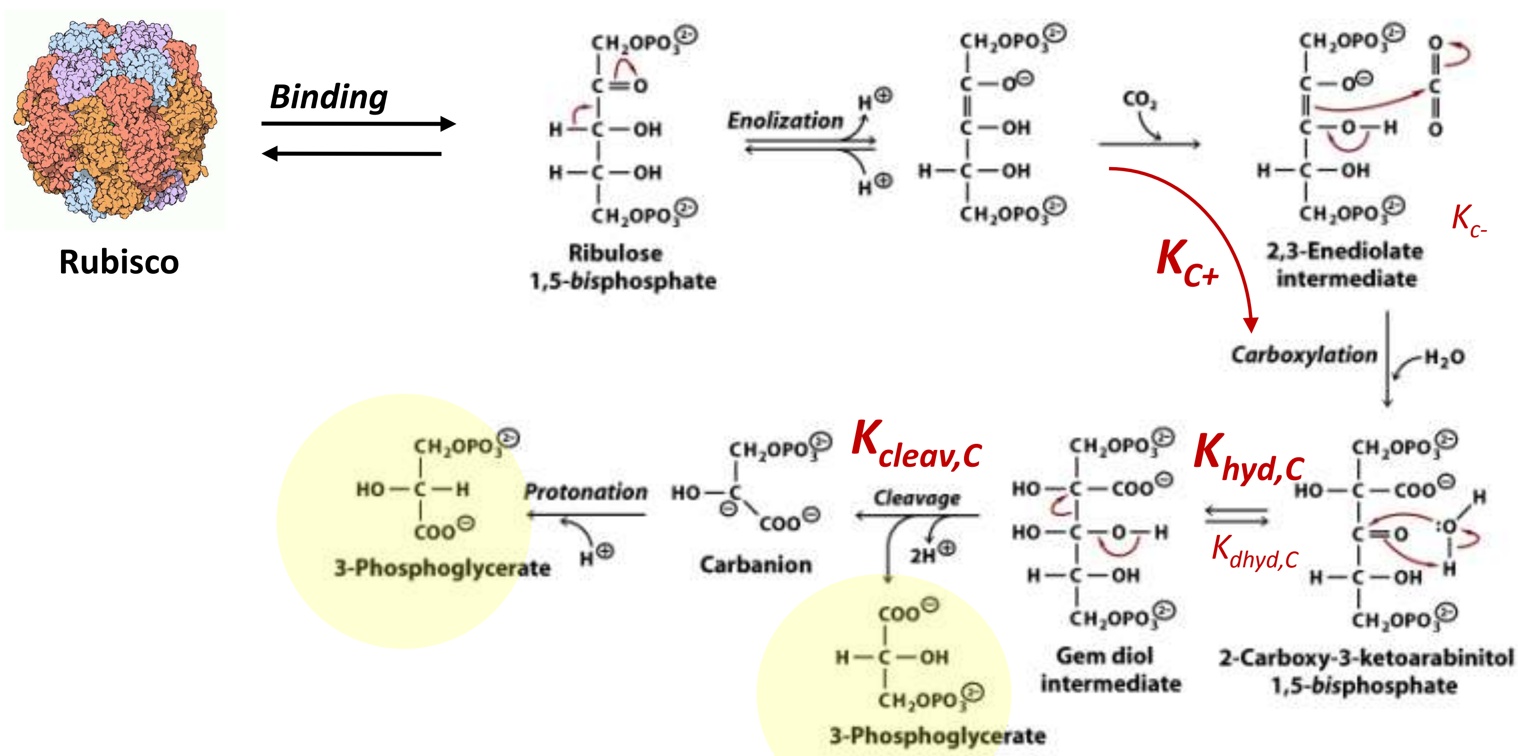


Figure 15-20 Principles of Biochemistry, 4/e

Specificity of Rubisco is rather low

- **Specificity** = ratio of gas addition rates

$$S=\frac{\left(\text{carboxylation}/[CO_2]\right)}{\left(\text{oxygenation}/[O_2]\right)}=\frac{\left(v_C/K_C\right)}{\left(v_O/K_O\right)}=\frac{k_{on,C}}{k_{on,O}}.$$

Range: 40-160

- Why oxygenation? **Biochemical constraints** due to O_2-CO_2 similarity?

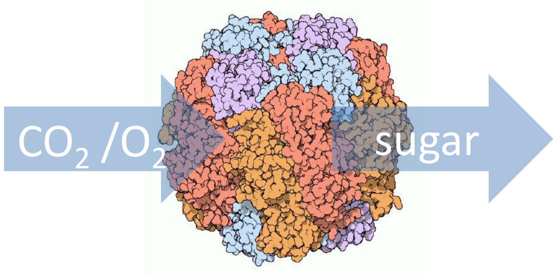
- Still, variation in specificity – **selection pressures**?

- Negative S - v_C correlation **(interplay between selection and constraints ?)**
  low $[CO_2]/[O_2]$ → high S, low v_C and vice versa.

- $[CO_2]/[O_2]$ determined by environment + concentration mechanism (CCM).

Kinetic parameters of Rubisco are correlated

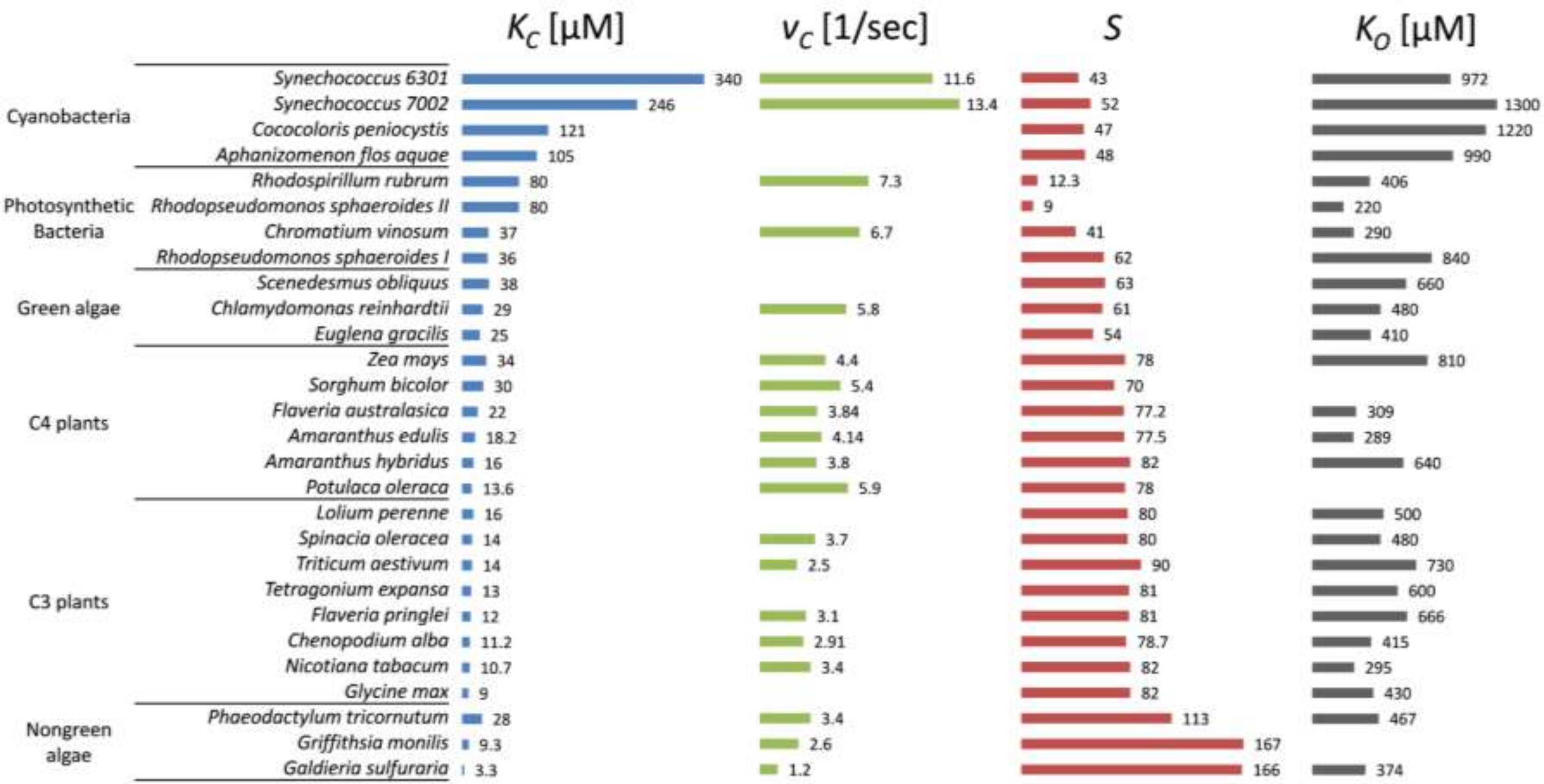
- Analysis of data from 28 eukaryotes and prokaryotes: Bacteria, cyanobacteria, green/non-green algae, C_3/C_4 plants.
- 4 kinetic parameters (S, v_C, K_C, K_O).



$$\text{carboxylation} = \frac{v_C}{1+\frac{K_C}{[CO_2]}\left(1+\frac{[O_2]}{K_O}\right)},$$

$$\text{oxygenation} = \frac{v_O}{1+\frac{K_O}{[O_2]}\left(1+\frac{[CO_2]}{K_C}\right)}$$

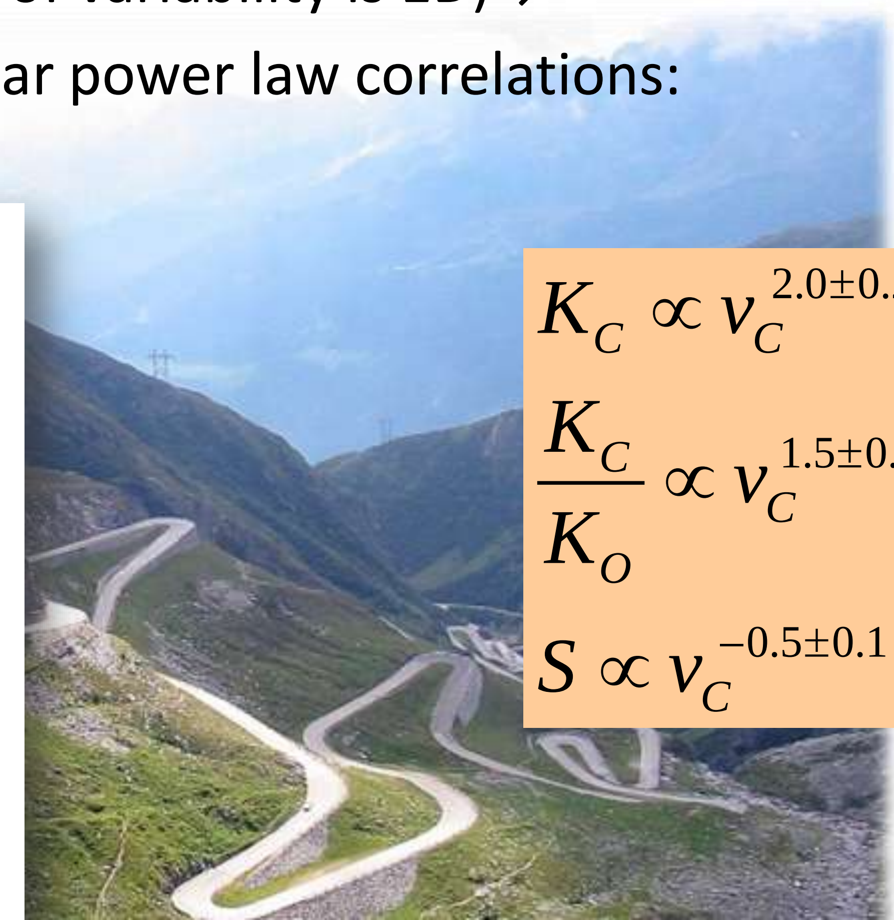
Cross-species analysis exhibits strong correlation

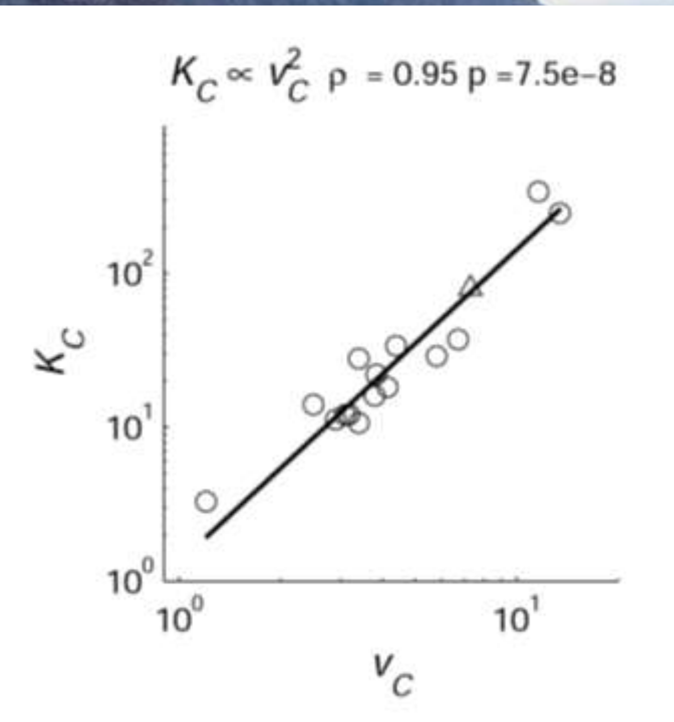


Parameter space is approximately 1D

- Data resides in 4D space (S, v_C , K_C , K_O).
- But constrained to **1D power law** (linear in log scale).
- PCA analysis (>90% of variability is 1D)→

clear power law correlations:





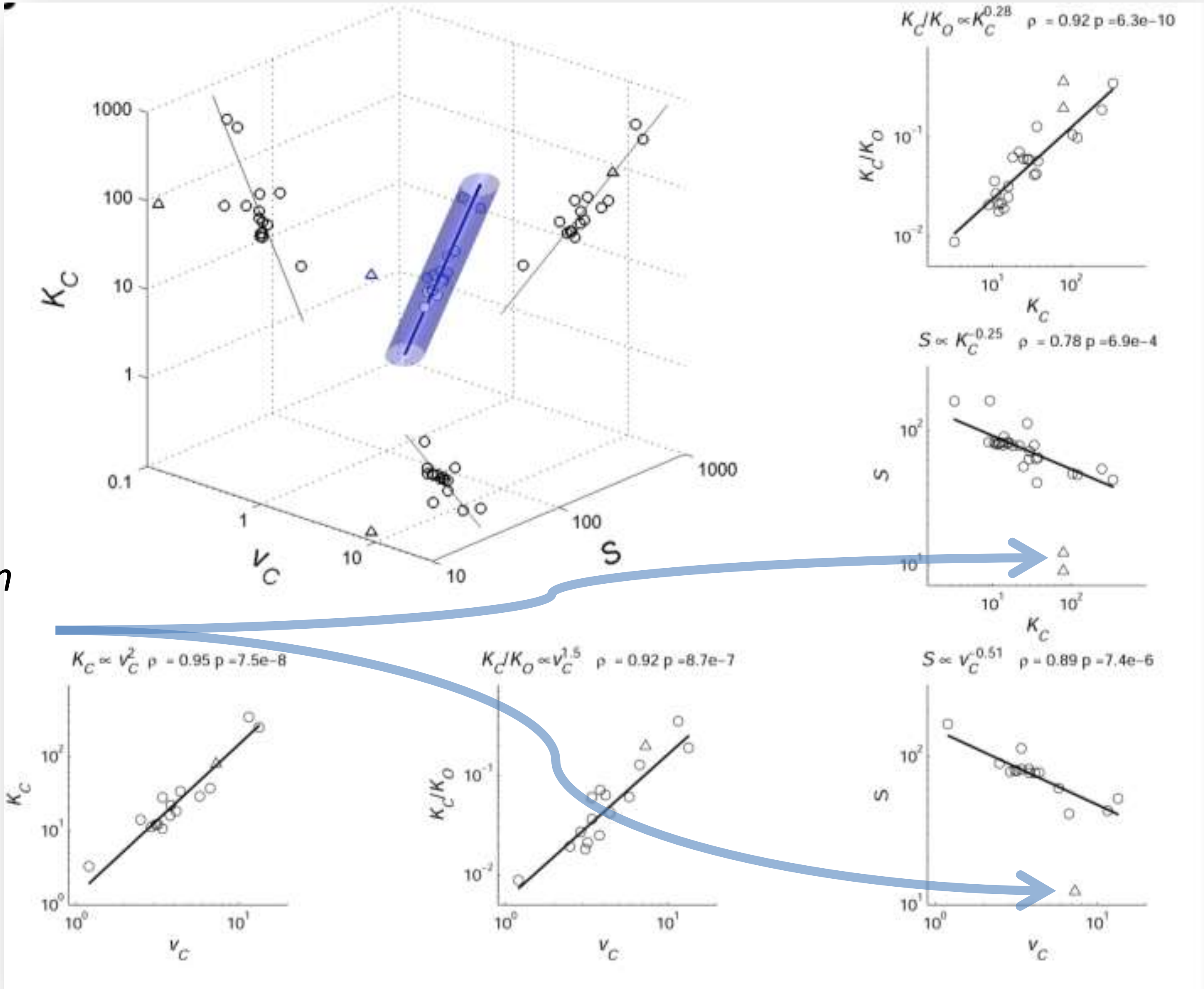
$$K_C \propto v_C^{2.0\pm0.2} \qquad \mu\text{M}$$

$$\frac{K_C}{K_O} \propto v_C^{1.5\pm0.2}$$

$$S \propto v_C^{-0.5\pm0.1} \qquad \mu\text{M}$$

Effective 1D landscape for Rubisco evolution

- All organisms scattered around a straight line in 4D parameter space.



- Outliers: *R. rubrum* & *R. sphaeroides* (form II of Rubisco).

Correlations indicate related energy barriers

easier CO_2 addition

$\Updownarrow$

harder hydration & cleavage

$$\Delta G_{1,C} + \Delta G_{2,C} \approx \text{constant}$$

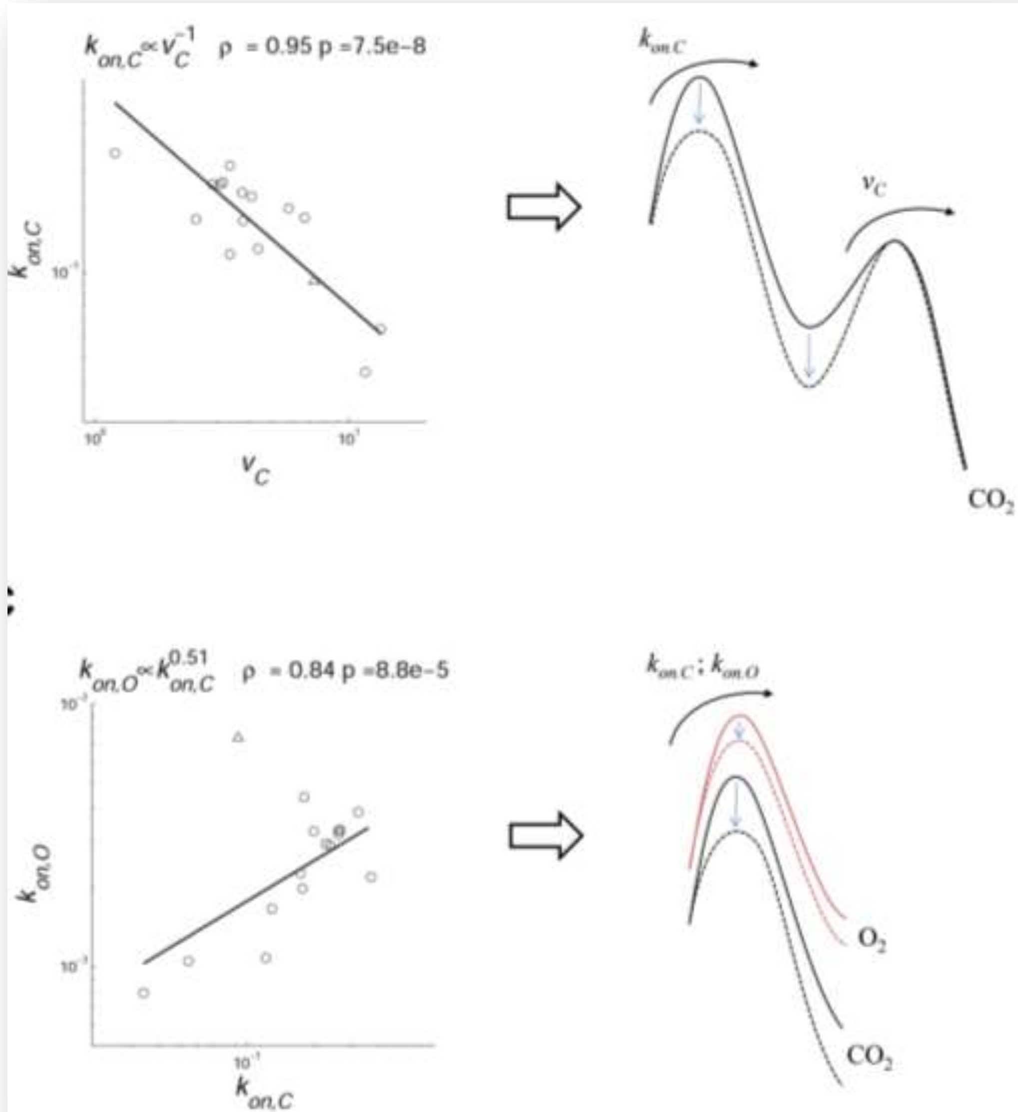
easier O_2 addition

$\Updownarrow$

much easier CO_2 addition

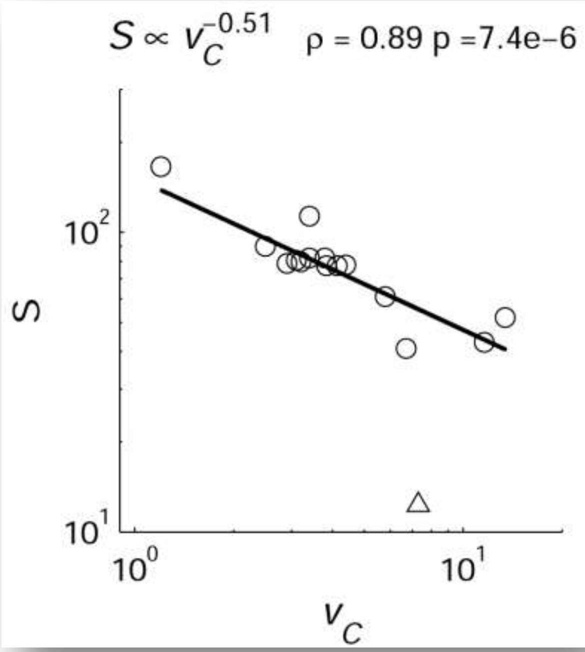
$$\Delta\Delta G_{1,C} \approx 2 \cdot \Delta\Delta G_{1,O}$$

“Conformational proofreading” (Savir & TT)



Tradeoff between specificity and carboxylation rate

- Specificity $S = k_{on,C}/k_{on,O}$.
- $S \leftrightarrow v_C$ tradeoff results from these **two basic** tradeoffs.



- Hints for possible optimality of Rubsico.
- Evolution in constrained ~1D landscape.

- Possible mechanistic picture: partition of deformation energy between two stages of carboxylation, binding and catalysis.

$$\Delta G_{1,C} + \Delta G_{2,C} \approx \text{constant}$$

Is Rubisco optimal? (under design constraints)

- Performance measure

net photosynthesis rate:

CO_2fixed – CO_2lost by photoresp:

$$f = \text{carboxylation} - \tfrac{1}{2} \cdot \text{oxygenation}$$

- "Design constraints":

$$K_C \propto v_C^{2.0 \pm 0.2}$$

$$\frac{K_C}{K_O} \propto v_C^{1.5 \pm 0.2}$$

$$S \propto v_C^{-0.51 \pm 0.1}$$

$$f = \frac{v_C - 3 \cdot 10^{-3} \cdot \left([O_2]/[CO_2]\right) \cdot v_C^{3/2}}{1 + 1.3 \cdot v_C^2 / [CO_2] + 5 \cdot 10^{-3} \cdot \left([O_2]/[CO_2]\right) \cdot v_C^{3/2}},$$

Look for optimality in 1D landscape (coordinate v_C) in given environment ($[CO_2]$, $[O_2]$).

Environments - CO_2:O_2 content

- **Atmosphere:** 21% O_2, 0.04% CO_2 (volume).

- **C_3 Plants: No CCM.** Aqueous solution in equilibrium with atmosphere. Gradient due to CO_2 consumption. [CO_2] ~7 µM, [O_2]~ 250µM.

- **C_4 Plants: 10 fold CCM.** Rubisco resides in bundle sheath cells where [CO_2] is raised by pumping mechanism (CCM): [CO_2] ~80 µM [O_2] ≤ equilibrium.

- **Aquatic species (Algae, Cyanobacteria): 100 fold CCM.** [C_i] is ~ 1-100 fold relative to passive concentration in algae and may reach a 1000 fold for HCO_3^- in cyanobacteria. Thus, [CO_2]~ 1-100 mM. "typical" [CO_2]~ 250µM.

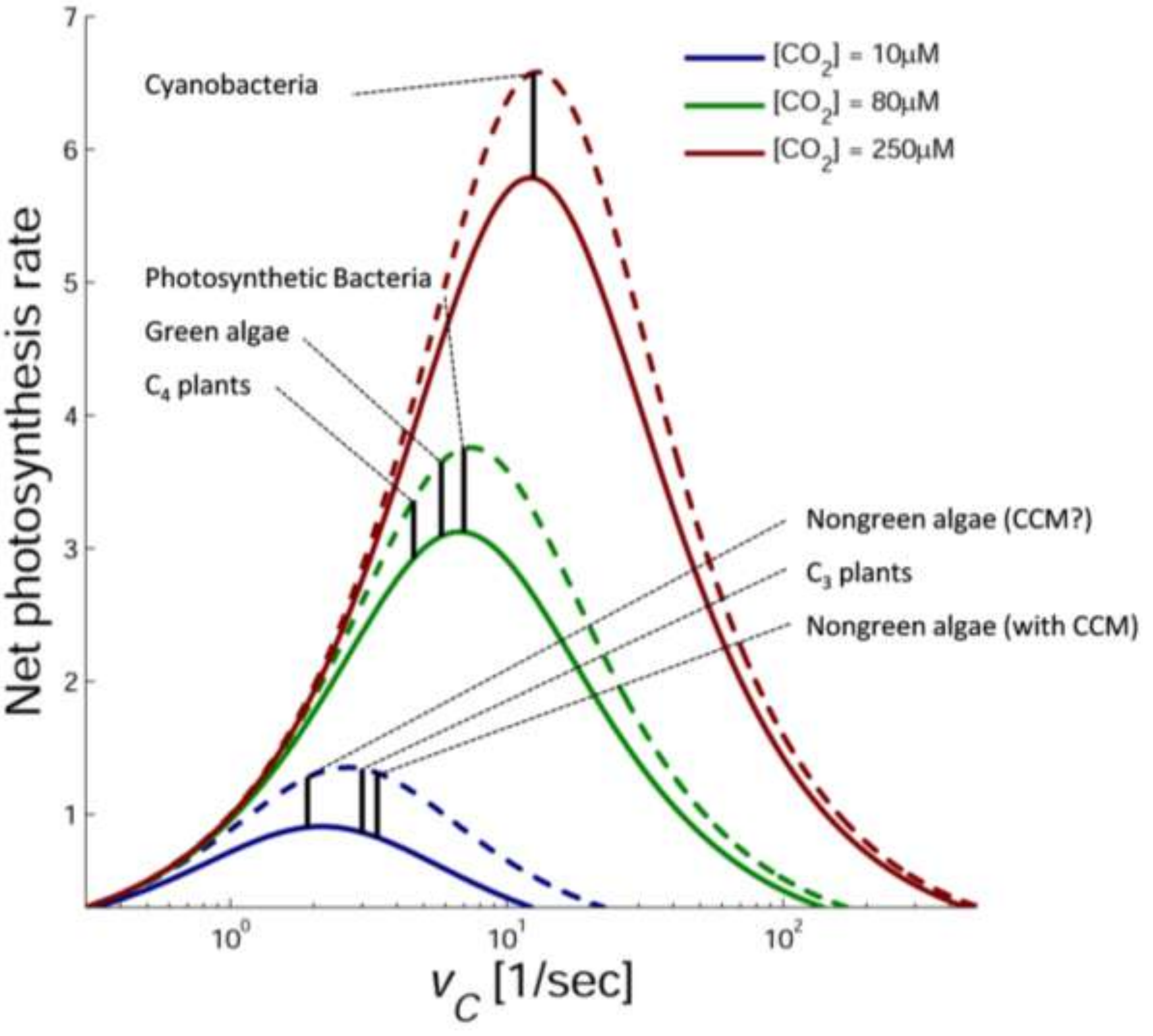
- **Photosynthetic bacteria: (uncertainty ~ CCM 10 fold).** prosper in the anaerobic parts of all kinds of aquatic environments.

Rubisco are nearly optimal to their habitats

- Max(f) as function of v_C in given habitat (O_2 and CO_2).

$$f = \frac{v_C - 0.003 \cdot \frac{[O_2]}{[CO_2]} \cdot v_C^{3/2}}{1 + 1.3 \cdot \frac{1}{[CO_2]} \cdot v_C^2 + 0.005 \cdot \frac{[O_2]}{[CO_2]} \cdot v_C^{3/2}},$$

- All organism classes are nearly optimal.



Simple "design rules" of optimal Rubisco

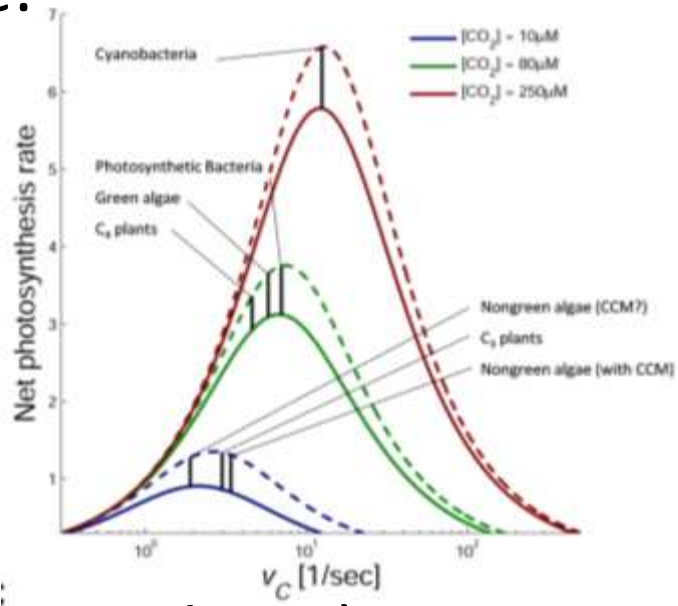
- By maximizing photosynthesis rate:

$$v_C^* = 0.86 \cdot [CO_2]^{1/2} (1 - \delta_O)$$

$$K_C^* = [CO_2](1 - 2 \cdot \delta_O)$$

$$S_C^* = 164 \cdot [CO_2]^{-1/4} (1 + 0.5 \cdot \delta_O).$$

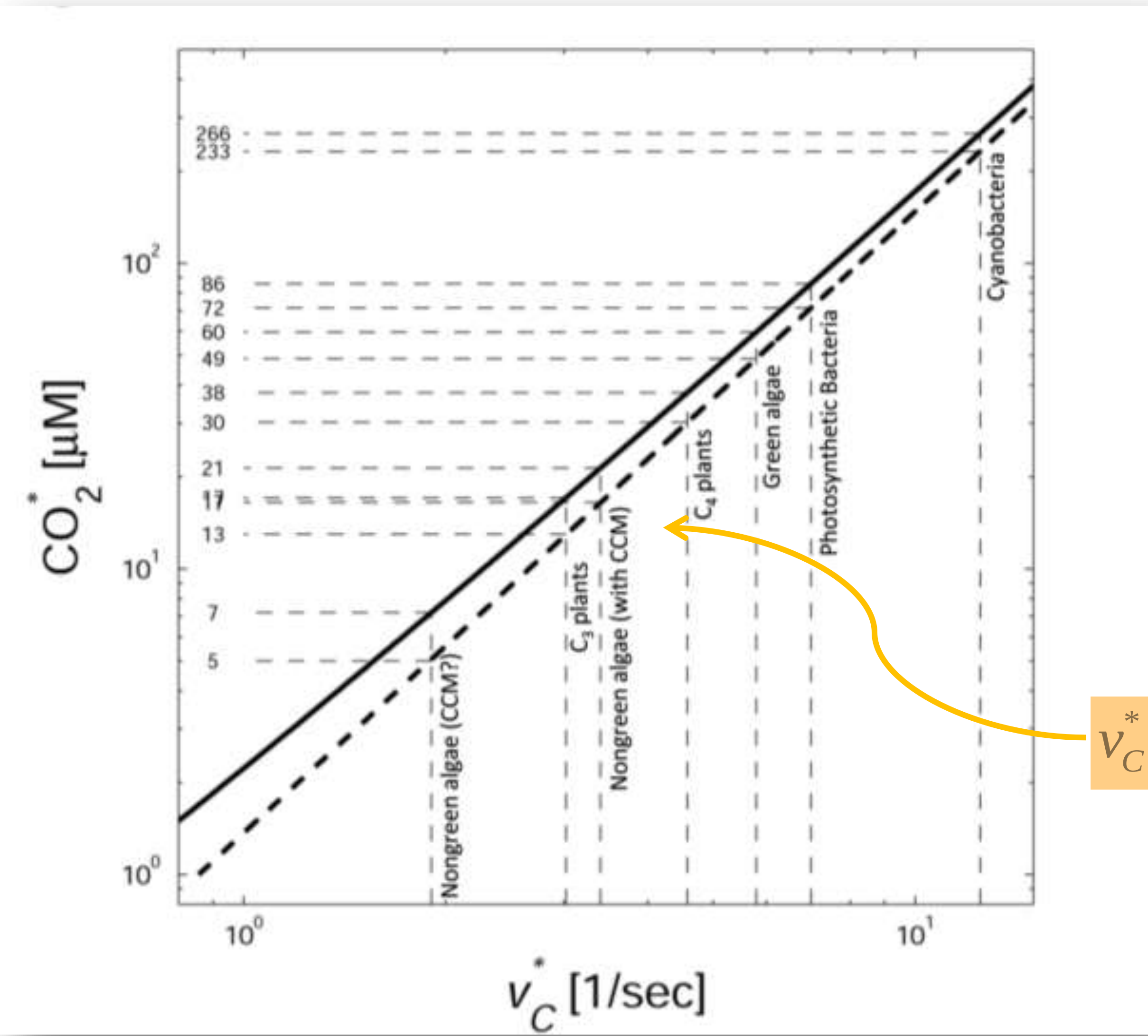
($\delta_O = 10^{-3} \cdot [O_2][CO_2]^{-1/4}$ is the oxygen effect 0-15%)

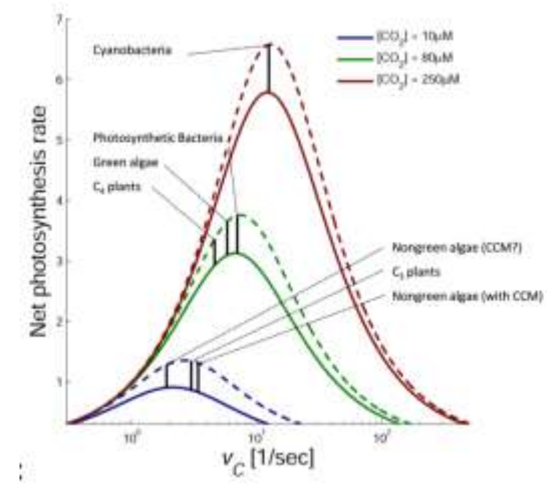


- The optimal photosynthesis rate (per Rubisco):

$$\boxed{f^* \approx 0.43 \cdot [CO_2]^{1/2} (1 - 2 \cdot \delta_O) \sim \frac{1}{2} v_C^*}$$

Simple power laws for optimality

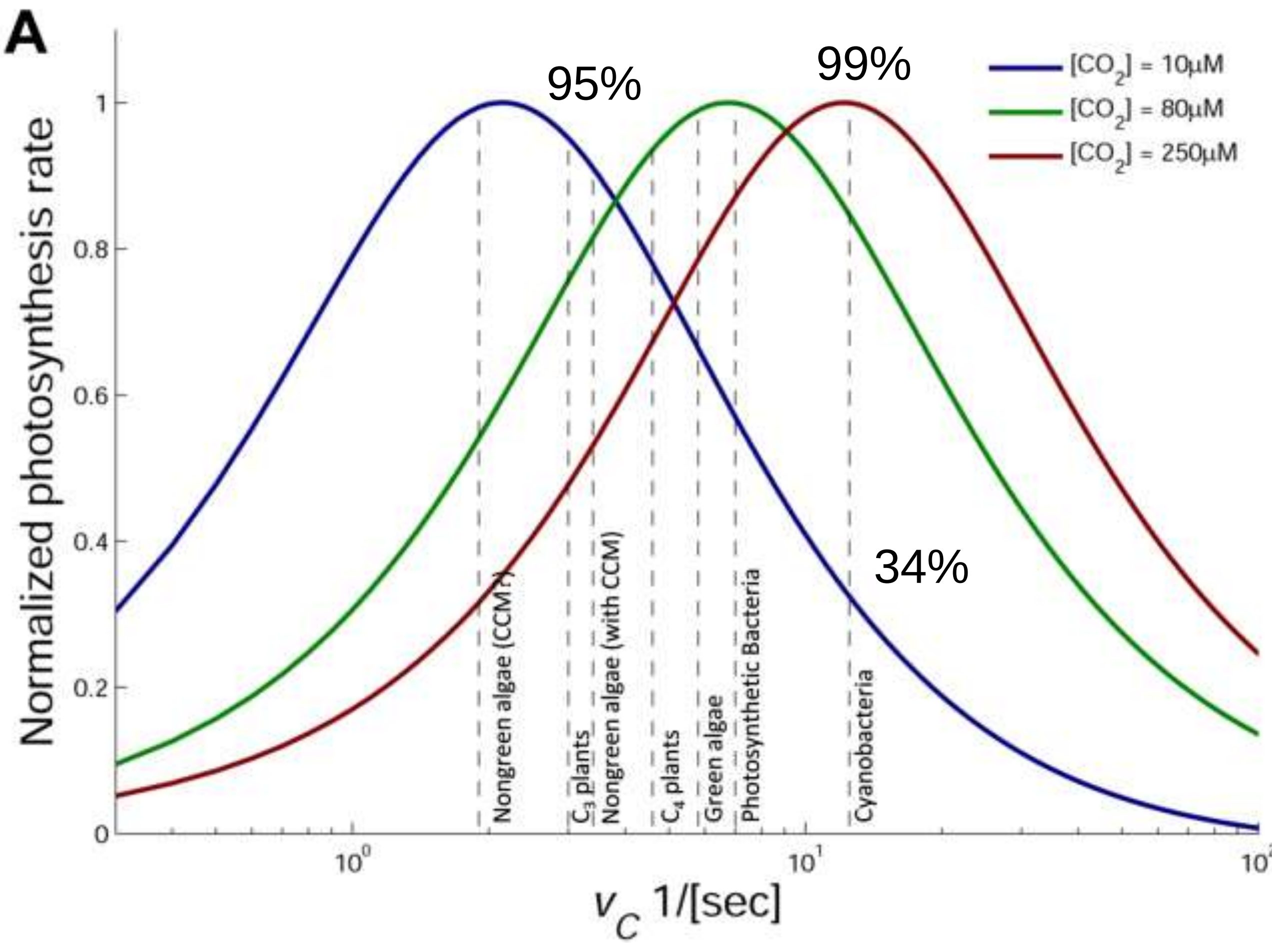




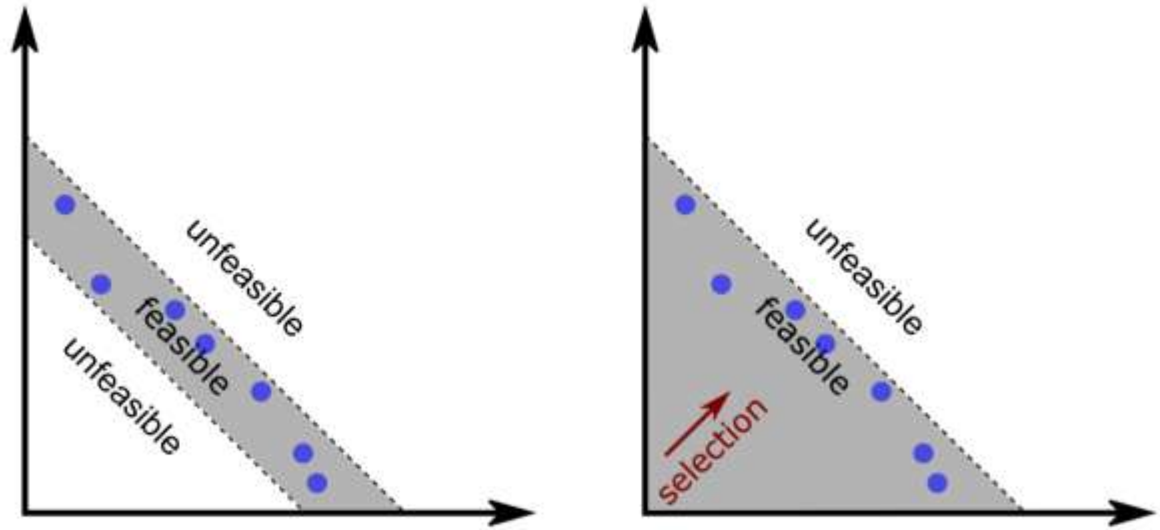
$$v_C^* = 0.86 \cdot [CO_2]^{1/2} \left(1 - \delta_O\right)$$

• Possible experimental tests: Evolution in response to changes in habitat or translocation of foreign Rubisco

Rubisco effect on fitness



Interplay between evolution and constraints



- Kinetic parameters with negative correlation such as S and vC.
- Hint: fluctuations around the line stronger for parameters that affect NPR weakly (K_O vs. K_C).
- Possible test: point mutation survey.

Conclusions

- Rubisco evolve in **1D landscape** of simple power laws.
- Rubisco is slow and inefficient

  yet **nearly optimal** in this landscape.
- **Simple optimality relations** ($f \sim v_C \sim [CO_2]^{1/2}$, $K_C \approx [CO_2]$)

- Suggests co-evolution of CCM and kinetic parameters.
- **Possible experimental tests**:

  - evolution of C3 plants in changing environment.

  - " switching" Rubisco between organisms.

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(PNAS 2010)

Questions, future directions, generalization

- **Form II outliers:** Could specificity and carboxylation activity be localized?
- Response of Rubisco to **long term climate changes**?
- Can one **improve** Rubisco? CCM?
- **Structural Mechanism?**
- **Constrained plasticity** in low dimensional landscapes:
  A generic phenomenon in proteins? (preliminary data).
- Examining other strongly selected proteins