Determination of *in vivo* Rubisco kinetics in *Arabidopsis*

Berkley Walker

Dr. Asaph Cousins

Maud Menten

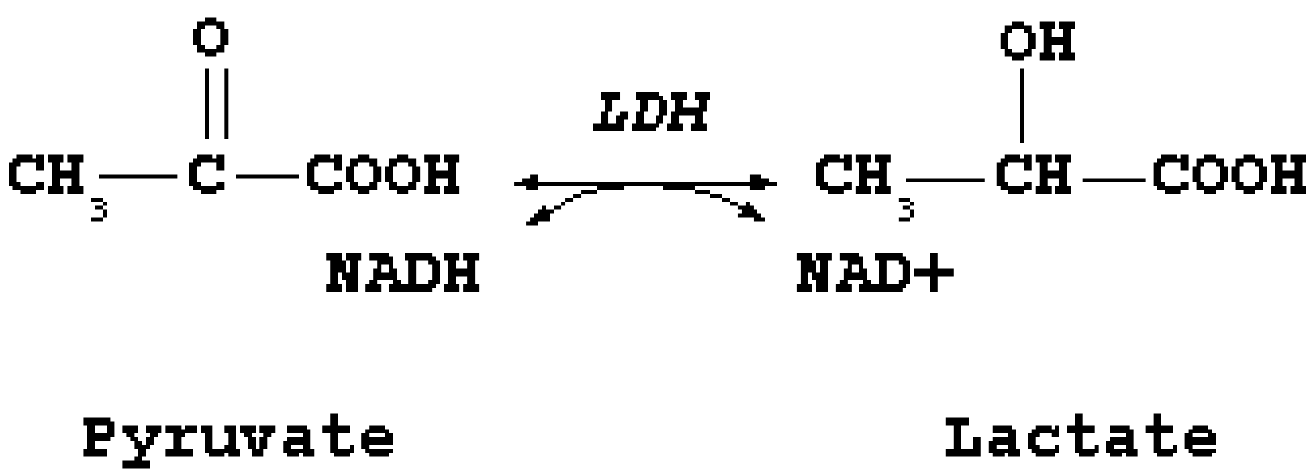
- One of the first Canadian women MD
- Studied in Germany, then the US
- Took 30 years to receive full professorship
- Studied everything from cancer to bacterial toxins
- But most importantly for today…

Michaelis-Menton Kinetics Seminar Summary

- Enzymes
- Enzyme activity can be modeled
- Competitive inhibition
- Rubisco
- *In vivo* kinetics
- Models of photosynthesis
- Uses of photosynthetic models (Who cares?)
- PLEASE ASK QUESTIONS IF I LOSE YOU!

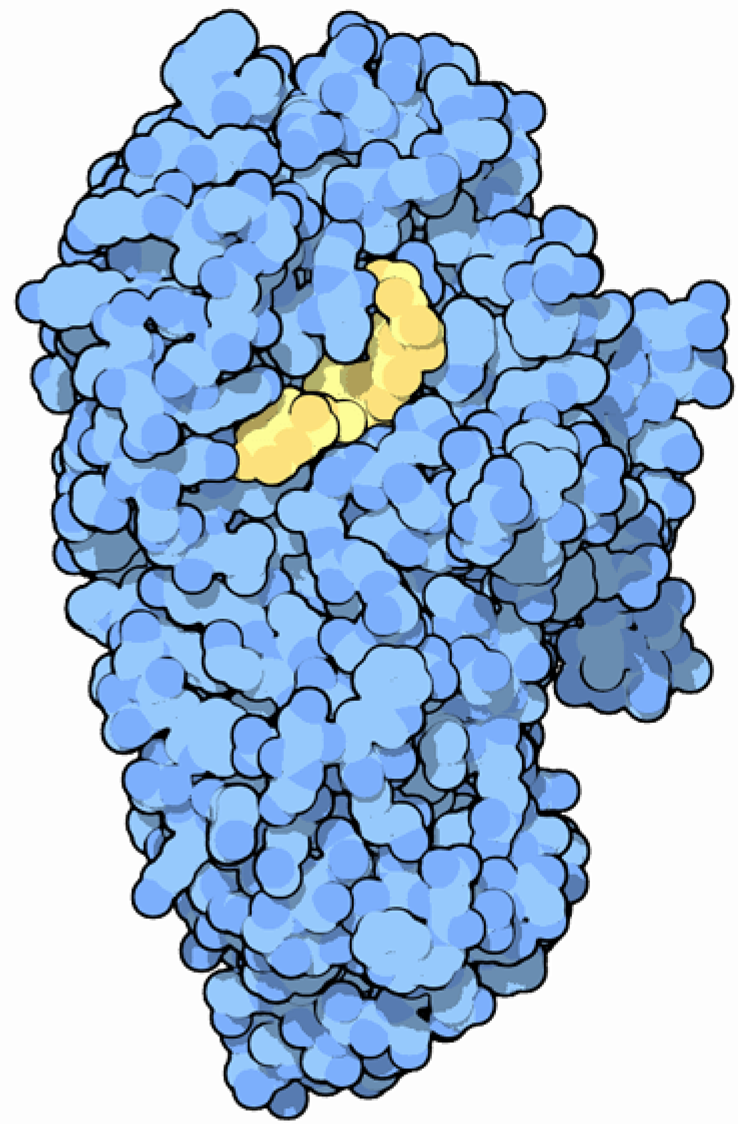
Enzyme

- Molecular machines
- Facilitate favorable reactions (Catalyze)
- Coordinate life



Enzymes

- Long chains of amino acids
- Chains fold to create shape
- Shape gives catalytic ability by stabilizing intermediates



Enzyme definitions

Enzyme

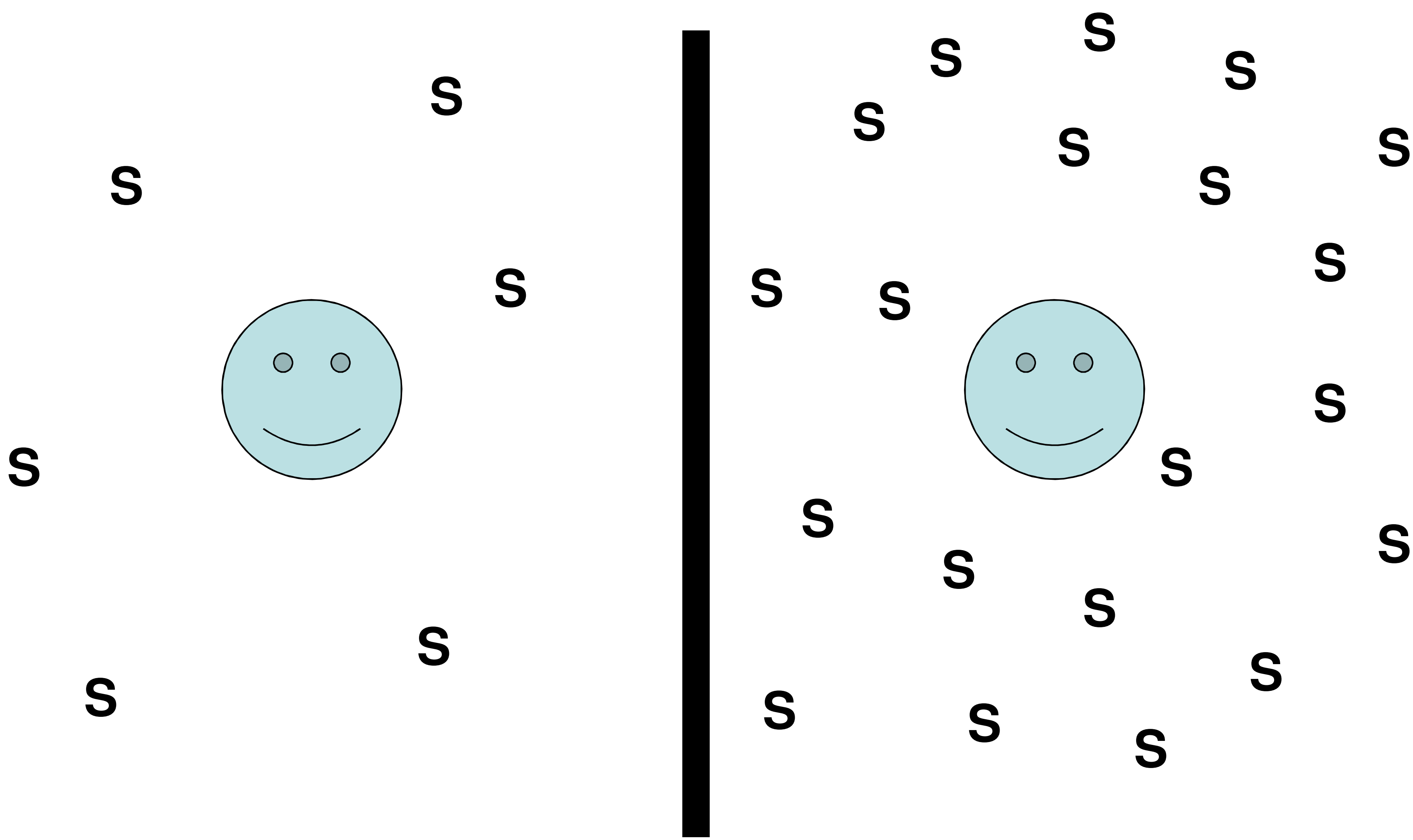
$$H_2O + CO_2 \xleftrightarrow{CA} H_2CO_3 \leftrightarrow HCO_3^- + H^+$$

Substrates/
Reactants

Products

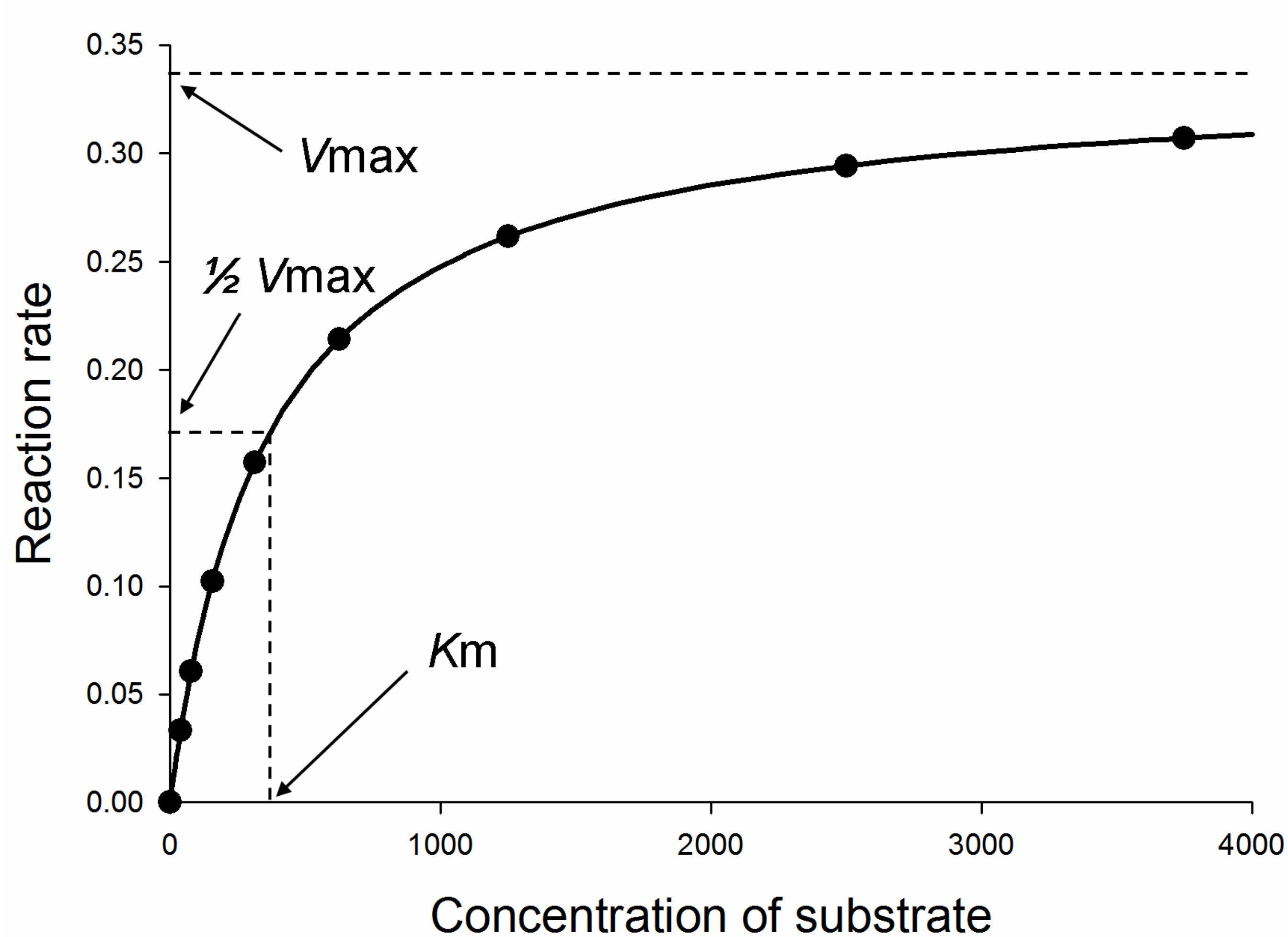
Modeling of enzymes

Which enzyme is faster? (higher activity)



Modeling enzymes

- Enzyme activity will increase with substrate concentration to a certain point
- Enzyme activity will saturate
- Single-substrate enzymes can be modeled by…

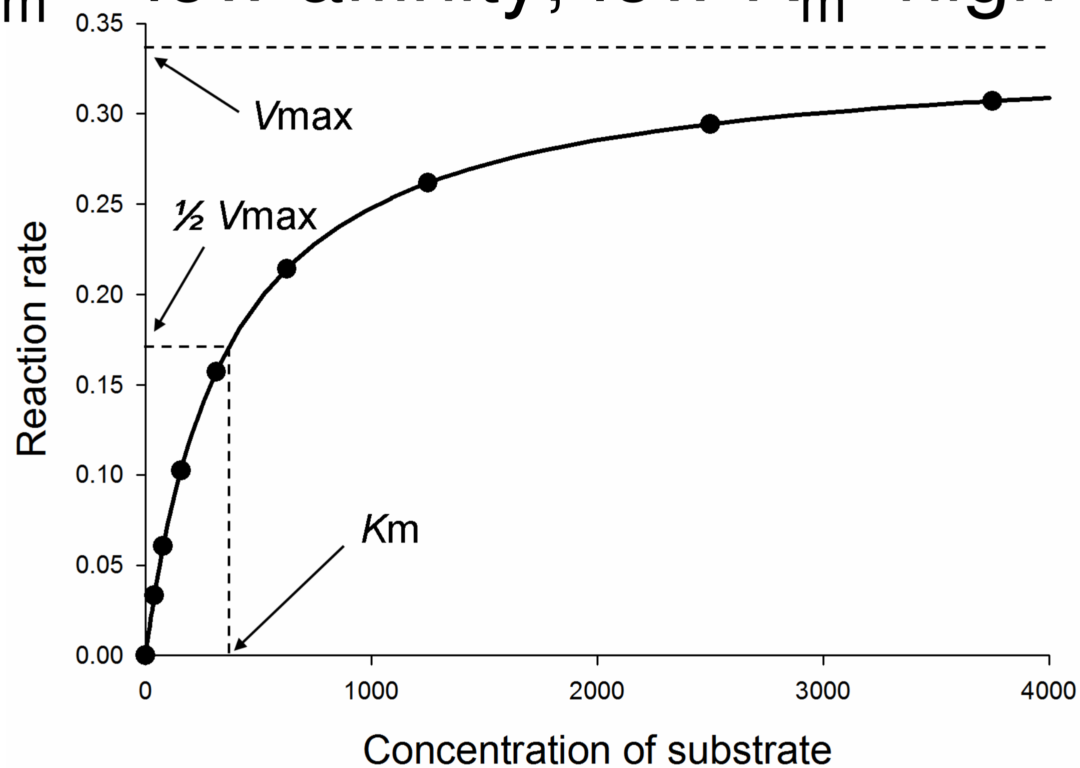


$$V_o = \frac{v_{\max}[S]}{K_m + [S]}$$

V_o = reaction rate

Modeling enzymes

- K_m and v_{max} describe the affinity and maximum velocity
- High K_m = low affinity, low K_m=high affinity

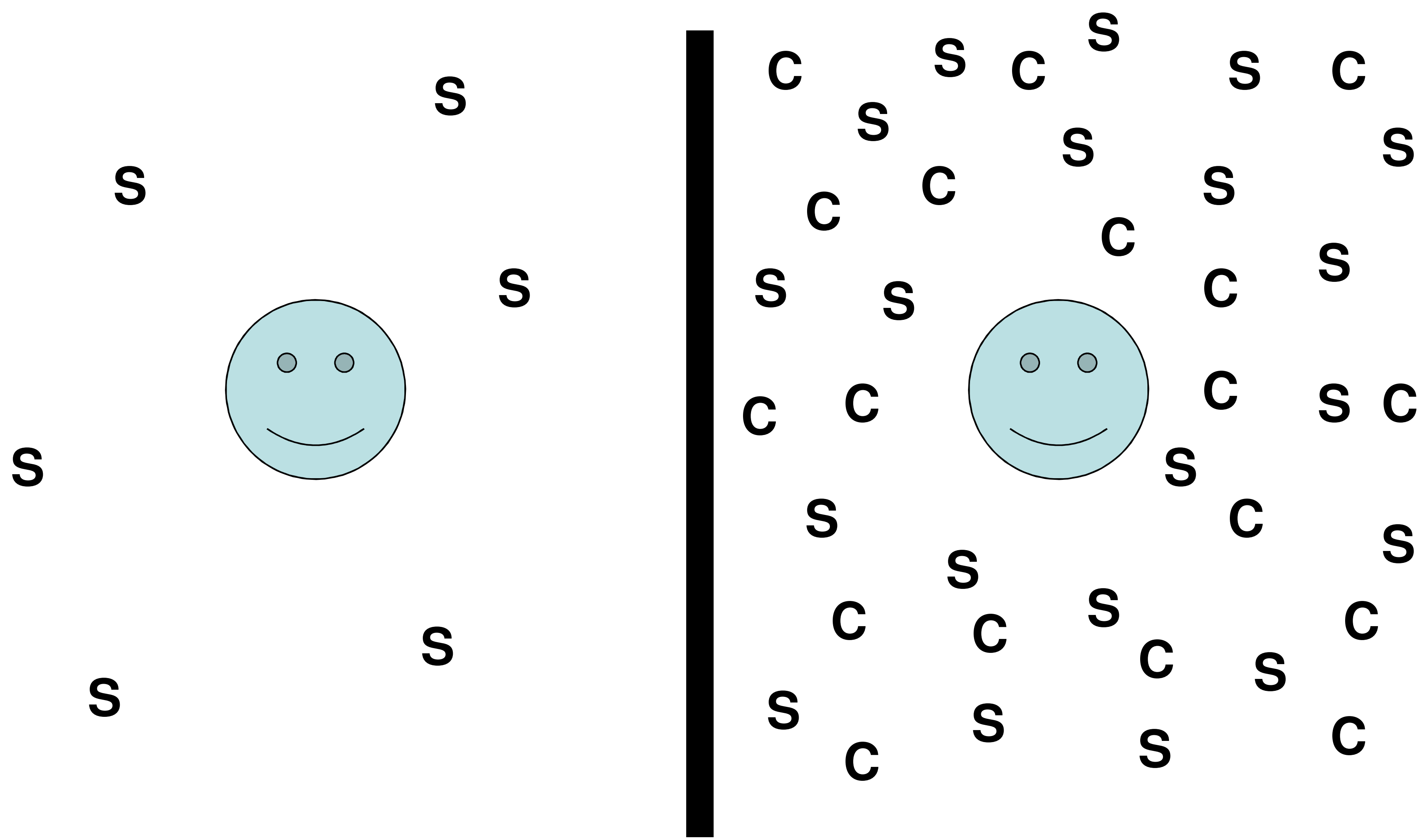


Competitive Inhibition

- Previous equation only works with a single substrate
- What if there is a second molecule the enzyme reacts with?

Modeling of enzymes

Which enzyme is faster?



Competitive inhibition

- Need to account for the specificity (K_i) and concentration ([I]) of the inhibitor
- What happens as [I] increases? Decreases?

$$V_o = \frac{v_{\max}[S]}{K_m + [S]}$$

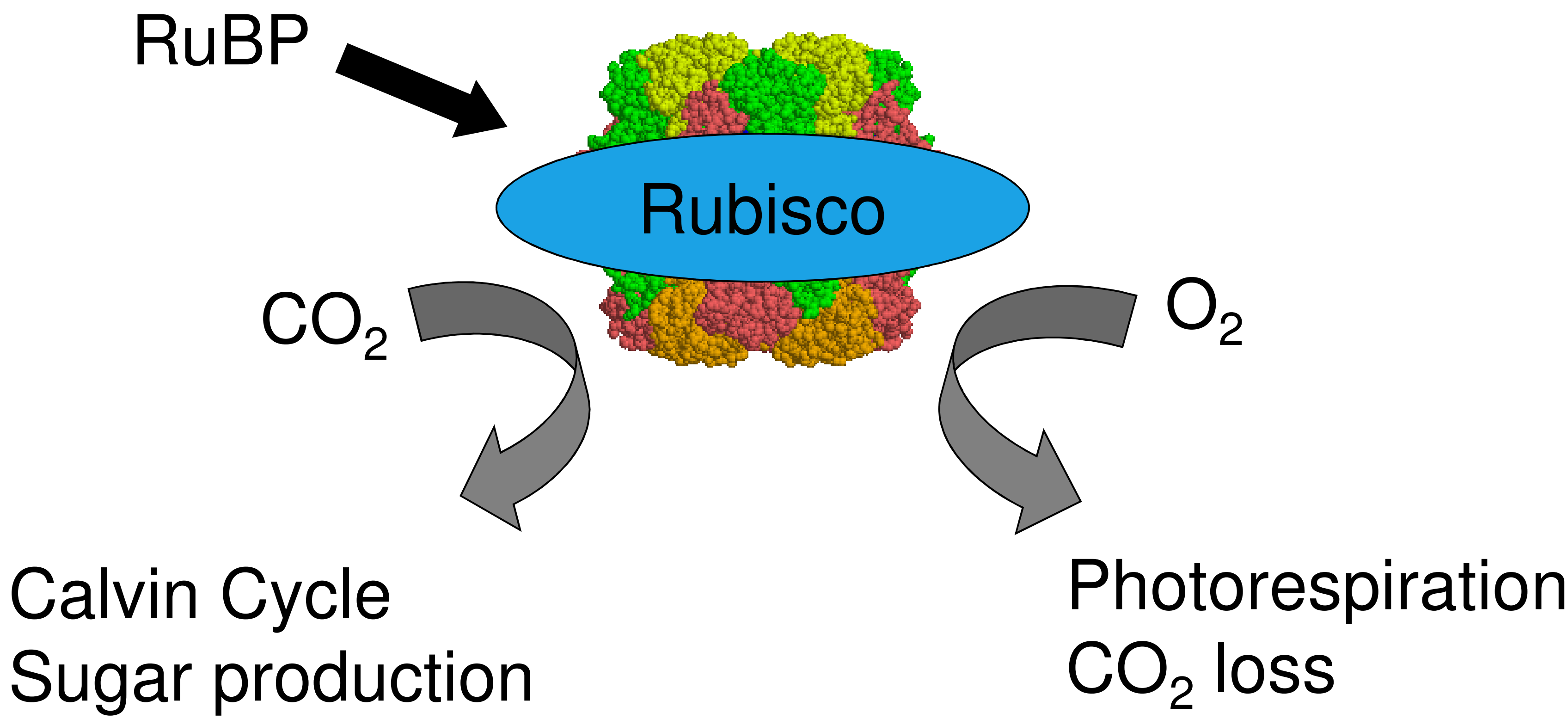
One substrate

$$V_o = \frac{v_{\max}[S]}{K_m\left(1 + \frac{[I]}{k_i}\right) + [S]}$$

Competitive inhibition

Rubisco

- Most abundant form of organic N on the planet
- Captures CO_2 in the first step of sugar production
- Often the limiting step to photosynthesis
- Ribulose 1-5 bisphosphate Carboxylase Oxygenase



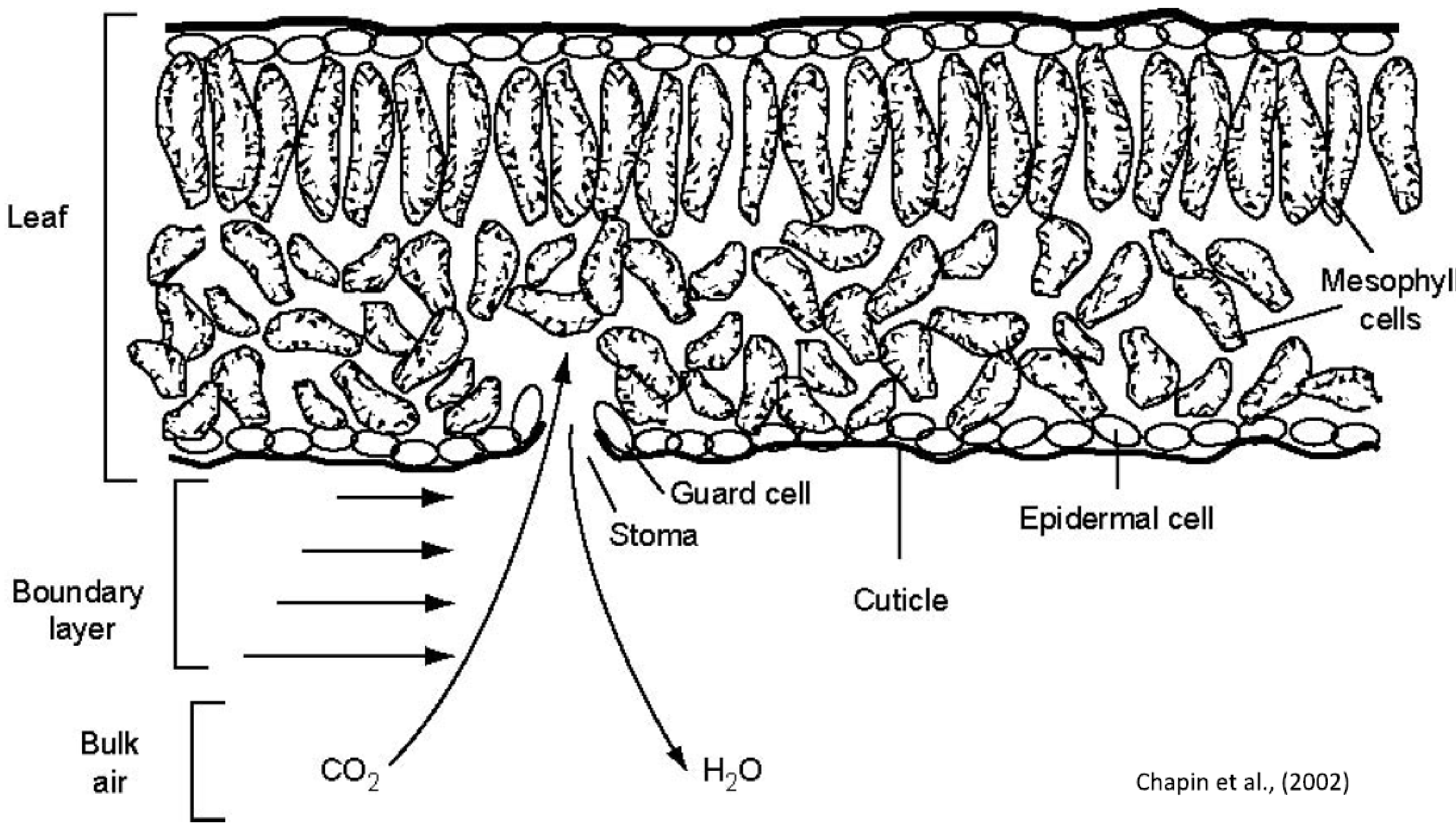
- The schizophrenic enzyme essential for life on earth
- Modeled with competitive inhibition

In vivo kinetics

- Data from earlier from *in vitro*
- Enzymes are in different conditions in a living plant
- How can we determine the *in vivo* kinetic descriptions of Rubisco?

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

How gas exchange works



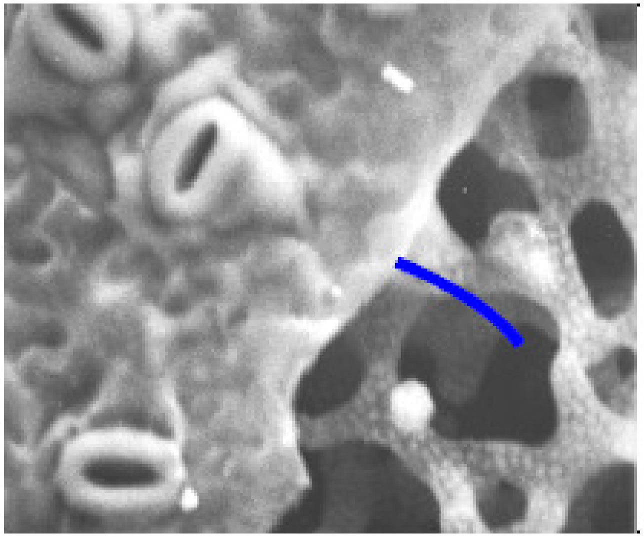
Chapin et al., (2002)

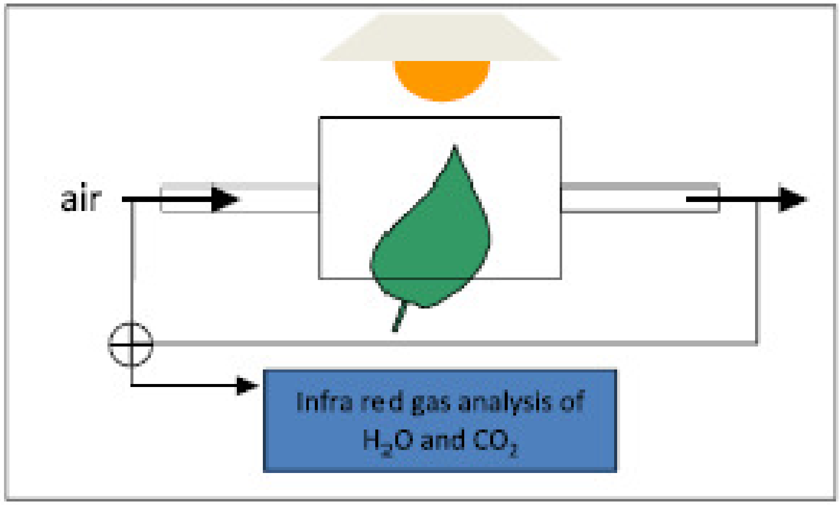
How gas exchange works

How gas exchange works

CO_2

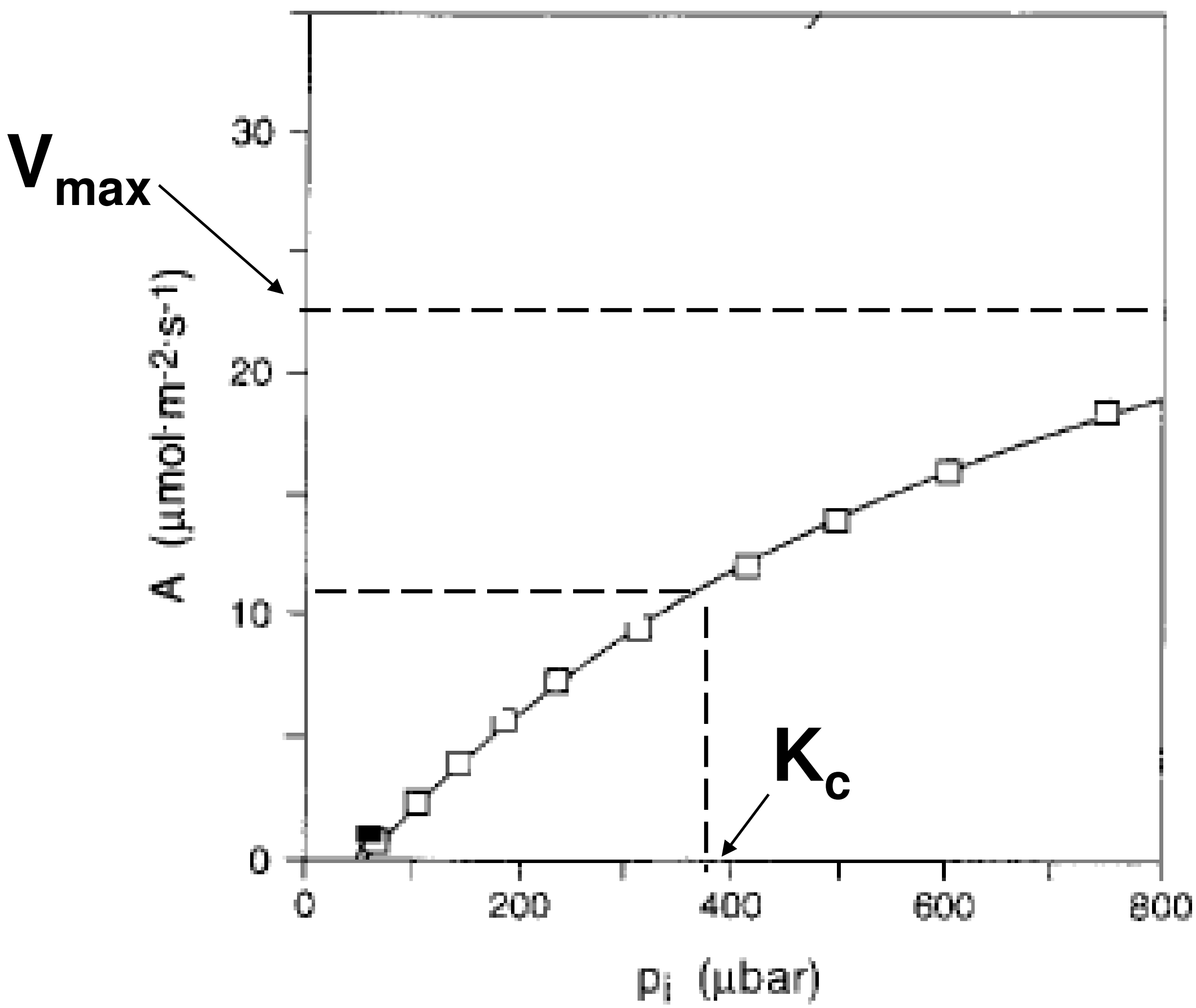
H_2O



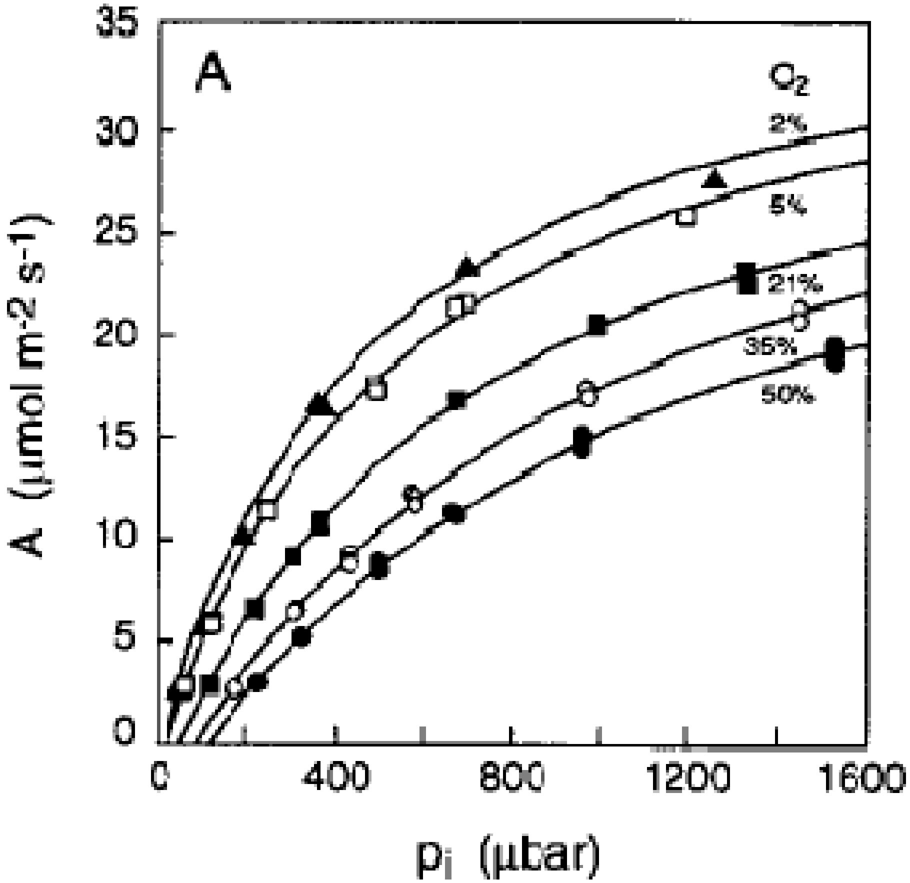


Micrograph by J Evans and S von Caemmerer

In vivo Kinetics



von Caemmerer S, Evans J, Hudson G, Andrews T (1994) The kinetics of ribulose-1, 5-bisphosphate carboxylase/oxygenase in vivo inferred from measurements of photosynthesis in leaves of transgenic tobacco. Planta **195:** 88-97



$$V_c = \frac{v_{\max}[CO_2]}{K_m\left(1 + \frac{[O_2]}{k_o}\right) + [CO_2]}$$

Leaf Models of photosynthesis

$$Assimilation = V_c - 0.5V_o - R_d$$

- With Kinetic parameters for Rubisco one can then model CO_2 assimilation rates under a given condition

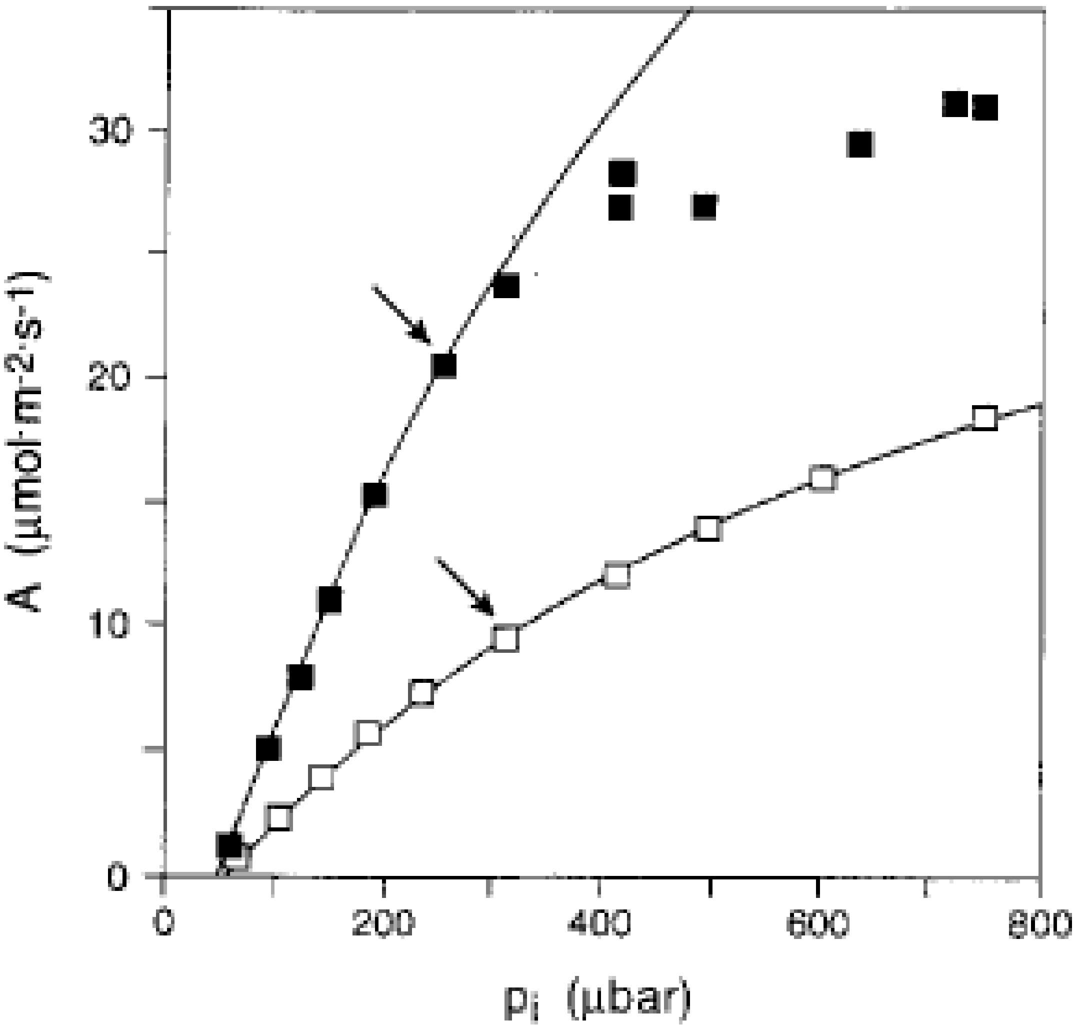
Models of Photosynthesis
Why should I care?

- How fast will a plant fix carbon in a given CO_2 concentration?
- How does photosynthesis respond to changing temperature?
- What are inefficiencies in photosynthesis?
- Understand plant energy use

What this project will involve:

- Measuring leaf gas exchange of CO_2 under different conditions (Temp and atm)
- Fitting these measurements to models of Rubisco kinetics to determine constants
- Using these constants to predict photosynthesis in model plants

In vivo Kinetics



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