Boosting Carbon Capture: Progress in Enhancing RuBisCO's Carboxylase Activity

Jeremy Zheng[1,2], Alirio Melendez Romero[1]*

[1]Department of Research Project Qualifications, Shenzhen College of International Education, 518040, Shenzhen, China

[2]University of Cambridge

***Corresponding author**

DOI: https://dx.doi.org/10.51244/IJRSI.2025.1210000343

Received: 02 November 2025; Accepted: 10 November 2025; Published: 22 November 2025

ABSTRACT

The Calvin-Benson Cycle serves as the primary carbon-fixation pathway in nearly all photosynthetic organisms. The enzyme, Ribulose Bisphosphate Carboxylase/Oxygenase (RuBisCO), is responsible for 'fixing' atmospheric CO_2 into Ribulose-Bisphosphate (RuBP), the most vital and rate-limiting factor in this cycle. Enhancing this enzyme carbon fixing ability is crucial for advancing global food security and addressing climate change. RuBisCO catalyses nearly all carbon fixation on Earth; however, RuBisCO is not a highly effective enzyme. The main contributing factor to its inefficiency is its oxygenase activity, which results in photorespiration instead of carbon fixation. Although many efforts have been made to enhance RuBisCO's carbon fixing efficiency, significant advancements are still needed. In this study we examine the current understanding of RuBisCO's catalytic mechanism, its complex oxygenase activity, evolutionary trade-offs, and new developments in RuBisCO engineering.

INTRODUCTION

The Calvin-Benson Cycle is the most dominant carbon-fixation mechanism in almost all photosynthetic organisms form prokaryotes, to protists such as algae, to green plants. As the enzyme that catalyses the immobilization/fixation of atmospheric CO_2 to Ribulose-Bisphosphate (RuBP) The enzyme Ribulose Bisphosphate Carboxylase/Oxygenase (RuBisCO) is the most crucial and the rate-limiting component of the entire cycle [1]. Improving the enzyme ability to fix carbon has great significance in promoting global food security and combating with the climate change, since almost all of carbon-fixation taken place on the earth is catalysed by RuBisCO. The primary reason for RuBisCO's inefficiency to fix atmospheric carbon is its additional oxygenase activity, which leads to photorespiration rather than carbon fixation. Numerous attempts have been made to improve its efficiency, but major breakthroughs are yet to be achieved [2]. The focus of this study is to review and share insights into the current understanding of RuBisCO's catalytic mechanism, its elusive oxygenase activity, evolutionary trade-offs, and the to highlight the progress in RuBisCO engineering.

As the primary enzyme for carbon fixation across all domains of life, the core tertiary structure of RuBisCO is highly conserved between vastly different phylogenetic clades. The key catalytic component of RuBisCO is its large core subunit, also denoted as RbcL [3]. A single *RbcL* monomer consists of a N-terminal domain, which promotes dimerization, and a TIM Barrel fold situated at the C terminal (Figure.1). This classic TIM Barrel structure consists of an inner β-sheet-based cylindrical structure with a surrounding ring of alpha-helices. The key catalytic residues are situated inside the β-barrel, with a divalent magnesium ion coordinated at the centre, forming the active site of RuBisCO. A lid-loop domain flanking the β-Barrel (also called loop-6) is an integral component of the active site and gates the entrance of the substrate RuBP [4]. When loop 6 adopts a closed conformation, the complete RuBisCO active site is formed. Of those highly conserved catalytic residues, a single strategic lysine residue is chemically modified via the carbamylation reaction, in which a single non-substrate CO_2 molecule is attacked by the nucleophilic nitrogen in the amine group of the lysine side chain, forming a

modified side chain called carbamoylysine [1]. The moiety functions as a general base during the catalysis carried out by RuBisCO, which will be described later in the article.

Figure 1

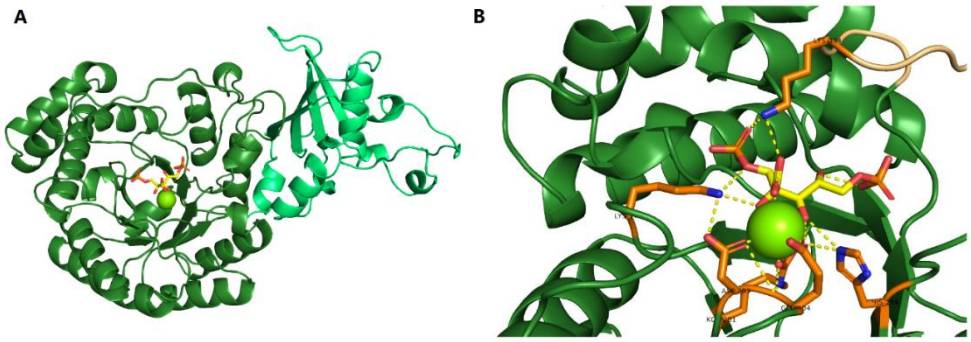

Figure.1 (A) Ribbon-band crystallographic structure of spinach RuBisCO. The catalytic TIM Barrel is coloured in olive green, whilst the N-terminal domain is in light green. A Mg2+ ion (light green) and 6C-intermediate mimic molecule (CABP) bind to the centre of the TIM barrel. **(B)** A closer look at the active site of RbcL. Note that the strategic carbamoylysine (KCX 201) is situated right below the Mg2+ ion. Crucial residues are coloured orange, whilst the lid-loop region (loop 6) is coloured light orange. The crystallographic structures were originally downloaded from the PDB RCSB database (PDB ID: 8RUC) and then processed and coloured by PyMOL.

The essential catalytic RbcL subunit forms homodimers (RbcL2) in all RuBisCO-containing organisms, and this dimer serves as the basic functional unit from prokaryotes to green plants. Apart from the major RbcL subunit, a group of RuBisCO small subunits (SSUs), also called RbcS, is present in many organisms, such as modern green plants [5]. RbcL2 and RbcS can form hetero oligomers (Figure.2), representing the quaternary structure of RuBisCO in many species. For form I RuBisCO, the major RuBisCO isoform presenting in green algae and modern plants, 8 RbcS oligomerizes with 4 pairs of RbcL2 dimers, forming a hexadecamer denotated as (RbcL)8(RbcS)8. Other forms of RuBisCO, including forms II and III, form RbcL2 homo-oligomers with different numbers of RbcL2 involved, but lacking the RbcS subunit [6]. The actual role of RuBisCO small subunit is still under investigation; some evidence suggest that the RbcS is responsible for the correct folding and assembly of RbcL, whilst others delineate a regulatory role of RbcS in RuBisCO's catalysis [7].

Figure 2

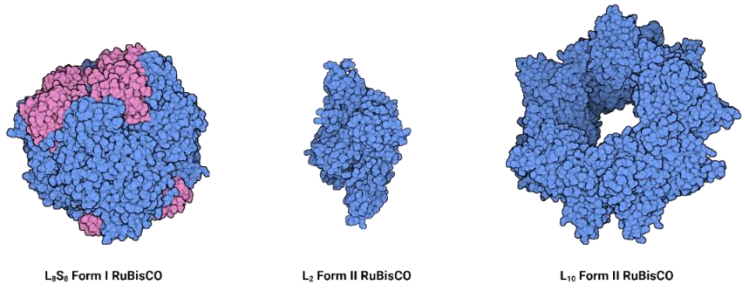

Figure.2 Different forms of functional RuBisCO units in different organisms. In modern green plants and green algae, Form I RuBisCO (Left) is dominant, and it exists as a hetero octamer formed by RbcL and RbcS subunits. In many prokaryotes, only a dimer of RbcL is present (middle). Other forms of oligomeric RuBisCO include the L_{10} decameric RuBisCO, which exists in bacterial and archaeal species (right). (PDB ID: 1RCX, 9RUB, 5MAC, converted into space-filling model and coloured by Biorender.)

The normal functioning of RuBisCO does not solely rely on the RbcL and RbcS subunits. A group of auxiliary proteins serves as RuBisCO chaperones are responsible for its correct folding, assembly, and activation [8]. For eukaryotic photosynthetic species, many of them are chloroplast specific, existing only in the stroma of the

chloroplasts. The expression of RuBisCO chaperones is crucial for the heterologous production of RuBisCO in model organisms such as *Escherichia coli,* which facilitates the further analysis of its functionality owing to the short replication cycle of prokaryotes [9], compared to the green plants. Apart from the chaperones that assist the folding process of RuBisCO, a special RuBisCO chaperone called RuBisCO Activase (RCA) is responsible for removing an inhibitory RuBP molecule bound to the inactive RuBisCO [10], and the chemical modification of the lysine residue into carbamoylysine. These actions facilitate the formation of the RuBisCO-Mg2+ complex, making the RuBisCO enzyme ready for catalysis.

METHODOLOGY

This study is intended to comprehensively review the current scientific perceptions and developments regarding the carboxylase activity of Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) for greater carbon capture. A systematic literature search was used for the study, aimed at the identification, analysis, and synthesis of information from peer-reviewed, scientific literature.

Literature Search Strategy

A comprehensive and wide search of literature was performed on major academic databases such as, but not limited to, PubMed, Scopus, Web of Science, and Google Scholar. The search strategy made use of keyword combinations and phrases associated with RuBisCO and carbon fixation such as: "RuBisCO," "carbon capture," "carboxylase activity," "oxygenase activity," "photorespiration," "RuBisCO engineering," "site-directed mutagenesis," "directed evolution," "rational design," "molecular grafting," "ancient RuBisCO," "carbon dioxide concentrating mechanisms (CCMs)," "carboxysomes," "pyrenoids," "RuBisCO activase (RCA)," "glycolate pathway," and "synthetic carbon fixation pathways." Identified publications including original articles, review articles and relevant publications from government or international organisations, were reviewed to capture the most relevant studies.

Inclusion and Exclusion Criteria

Studies were included in this review if they:

- Provided information on the structure, catalytic mechanism, and evolutionary biology of RuBisCO.
- Addressed the inherent inefficiencies of RuBisCO, particularly with respect to its oxygenase activity and photorespiration.
- Described experimental and computational attempts to improve RuBisCO carboxylation activity or specificity.
- Investigated alternative strategies of enhancing carbon fixation, e.g., carbon dioxide concentrating mechanisms or synthetic carbon fixation pathways.
- Discussed the role of partner proteins like RuBisCO Activase (RCA) in modulating RuBisCO function.
- Presented critical findings, models or theoretical frameworks related to enhancing carbon capture efficiency in plants or other photosynthetic organisms.

Studies were excluded if they: focused solely on the non-photosynthetic carbon metabolism or had no close relation whatsoever with any improvement to RuBisCO efficiency or carbon-fixing pathways.

Data Extraction and Synthesis

Relevant information concerning the objectives of the review was extracted with diligence from the selected literature. Such information included:

- The molecular structure and conformational dynamics of RuBisCO subunits and complexes.
- Detailed mechanisms of carboxylation and oxygenation reactions.
- Kinetic parameters (kcat-CO2, Km-CO2, Sc/o) and evolutionary trade-offs.
- Specific techniques employed for RuBisCO modifications (e.g., mutations, gene transfer, AI-based design).

- Architectural and functional details of the CCMs (carboxysomes, pyrenoids) and their constituents.
- Mechanisms of RuBisCO activase, as well as strategies for its optimization.
- Design and performance of modified glycolate pathways and synthetic carbon fixation cycles.
- Reported improvements of carbon fixation rates, biomass accumulation, or related physiological parameters in engineered systems.

The extracted information was then critically analysed, categorised, and synthesised to extract key points, common challenges, promising strategies and emerging trends in RuBisCO engineering and carbon capture enhancement. Major emphasis was made on the feasibility, possible impacts and limitations of each of the discussed approaches.

Study Structure

The study is organised to first establish the fundamental importance and inherent limitations of RuBisCO, followed by an exploration of its catalytic mechanisms and evolutionary context. Subsequent sections delve into various strategies developed to overcome these limitations, categorised by their focus on modifying the enzyme itself or its surrounding biochemical environment. The discussion progresses from classical protein engineering techniques to advanced computational design, molecular grafting, and the implementation of sophisticated carbon concentrating mechanisms and synthetic pathways. The review concludes by proposing a combinatorial strategy to integrate these diverse approaches for synergistic effects, aiming to provide a roadmap for future research in boosting carbon capture.

The Catalytic Activity of RuBisCO

The catalytic mechanism of RuBisCO demonstrates the multi-step nature of the entire process (Figure.3). The mechanism can be divided into four major stages [11]: First, the activation of RuBisCO, which is catalysed by the chaperone RCA, as we have mentioned earlier. Second, the generation of the 6C enediolate from RuBP, which is essential for both the carboxylation and the oxygenation reaction. This procedure is initiated by the negatively charged, basic carbamoylysine residue, which deprotonates the C3-carbon of RuBP, causing the formation of a carbon double bond and the further deprotonation of the C3-hydroxy group, resulting in the generation of two negative charges on the intermediate. The central Mg^{2+} stabilizes the enediolate intermediate via the formation of coordinate bonds with the two negatively charged oxygen atoms [11].

The third stage of the carboxylase mechanism is believed to occur in a concerted manner [12]: it is initiated by the nucleophilic attack of a H_2O on C3-carbon, followed by the C2-C3 π bond breakage and the nucleophilic attack of C2-carbon on the substrate CO_2, forming a 6-carbon unstable intermediate. In the last stage, the complete breaking of the C2-C3 linkage is eventually completed via the deprotonation of the C3-hydroxy group by the carbamoylysine residue [13], causing the release of two 3-phosphoglycerate (3-PGA) products from the active site.

Figure 3.

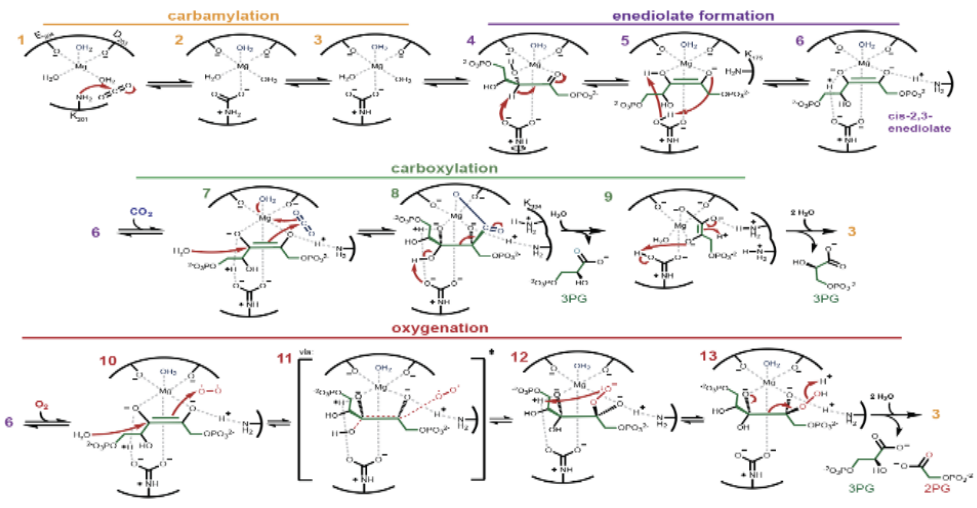

Figure.3 The detailed catalytic mechanism of RuBisCO. An Mg^{2+} bound enediolate intermediate is required by both the carboxylation and the oxygenation reaction. Note that the exact detail of the oxygenation reaction remains enigmatic (see text). (Source: Prywes *et al.*, 2023) [2].

The oxygenase activity of RuBisCO shares the common stage 1 and 2 with its carboxylase activity, since the enediolate intermediate is required in both reactions. The exact details of the oxygenation are long-debated, and no definitive solution has yet been proposed. The primary reason for its enigmatic property is primarily due to the spin-chemistry [14], the enediolate intermediate, which does not contain any unpaired electrons, adopting the singlet electron configuration, is prohibited from reacting with the biradical, triplet oxygen molecule in the absence of any catalytic transition metal cofactors.

Potential mechanisms have been proposed to explain how RuBisCO overcomes this restriction. The first one, called Single-electron-transfer (SET), states that the enediolate first transfers one electron to the O_2, forming a positive radical enediolate (Ene.+) and a superoxide radical (O^{2-}) [14]. The second theory called intersystem crossing (ISC), proposes that one of the molecules (either the enediolate or O_2) alters its electronic state to achieve a match. It could be the transition of the singlet enediolate to triplet enediolate, caused by the bond distortion between C2 and C3, or potentially the excitation of triplet O_2 to singlet O_2 [14]. Further characterizations and understandings of RuBisCO's oxygenase activity are essential for the further enhancement of its specificity.

Potential Evolutionary and Biochemical Trade-offs of RuBisCO

Given that this carbon-fixation enzyme has evolved for billions of years, A tricky question emerged: why modern RuBisCO enzymes are so catalytically inefficient. This leads to various different hypotheses: Some early postulations have a pessimistic view, predicting that RuBisCO enzymes have already evolved to their optimal state, and the oxygenation reaction is inevitable due to the extreme disparity between the atmospheric CO_2 and O_2 concentration [15. Some other researchers, however, predict that there is a trade-off among some of the kinetic parameters of the RuBisCO enzyme. A negative correlation between the catalytic rate (kcat-co2) and specificity (Sc/o) was observed in many kinetic studies [16], which is also supported by the fact that the RuBisCO of many organisms that deploys a carbon dioxide concentrating mechanism (CCM) have high Kcat-CO_2 and low Sc/o.

Other studies, however, concluded that the negative correlation between these two parameters becomes unsolid as the sample size increases [17]. Instead, a strong positive correlation between Kcat-CO_2/Km-CO_2 and Kcat-O_2 /Km-O_2 was observed, suggesting that the Sc/o value is highly constrained by evolution. This finding can also be explained by the catalytic mechanism: if the energy level of the enediolate intermediate increases, both the carboxylation and the oxygenation reaction will happen more readily, without discrimination (Figure.4). Apart from the hypotheses on kinetic trade-offs, some phylogenetic studies suggest that the inefficiency of RuBisCO was largely due to its evolutionary history. Events such as the great oxidation event (GOE) may have a long-lasting negative impact on RuBisCO, which is further exacerbated by its slow evolution speed [18]. A persuasive answer has not been proposed, some important yet unknown evolutionary constraints may exist.

Figure 4.

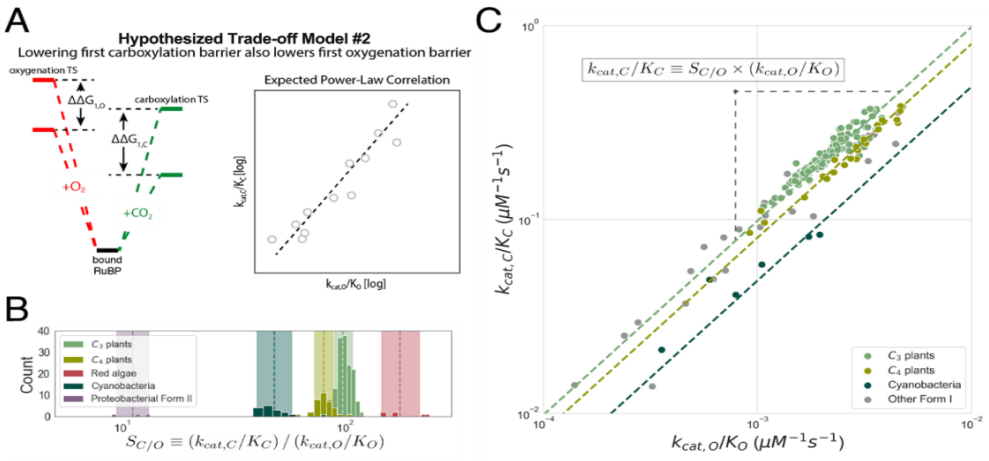

Figure.4 The strong positive correlation between Kcat-CO_2/Km-CO_2 and Kcat-O_2/Km-O_2 can be explained by RuBisCO's catalytic mechanism. This indicates a relatively constant Sc/o. Some clades (such as Red algae) may have slightly greater Sc/o compared to others. (Source: Flamholz *et al.*, 2019) [17].

Current Developments in RuBisCO Enhancing Strategies

The extensive research on the functionality of RuBisCO is accompanied with numerous attempts to improve its parameters in the past few decades (Figure.5). The major objectives of RuBisCO engineering are to either increase its specificity (carboxylase to oxygenase ratio, S(c/o)), or improving the rate constant (Kcat) of the carboxylase reaction, or both. Those improvement strategies focusing on the RuBisCO includes the site-directed mutagenesis and directed-evolution [19], which are largely ineffective owing to the historical evolutionary constraint imposed to the enzyme. An alternative approach is to computationally re-engineer RuBisCO via rational design, which holds its potential in the era of generative artificial intelligence. Alternatively, grafting high-efficiency RuBisCO from foreign species such as red algae into green plants also shows promise [20], while predicting the sequence of ancient RuBisCO from geological periods of decreasing CO_2 concentration using phylogenetic study, serves as a novel approach to discover other high-efficiency variants of RuBisCO [21].

Alternative to those strategies focusing on the improvement of RuBisCO itself, other approaches aim to re-engineer the systems surround this enzyme. The carbon dioxide concentrating mechanism (CCM) that exists in bacteria and algae could be harnessed to increase the S(c/o) ratio, via the compartmentalization of RuBisCO in a CO_2-rich chamber. These CCMs include the proteinaceous particles called carboxysomes in photosynthetic bacteria, and membranous organelles named as pyrenoids in eukaryotic algae species [22]. Furthermore, the enzyme RCA is also a target of improvement, its re-engineering is also a potential strategy to boost the activity of the RuBisCO enzyme [10].

Figure 5.

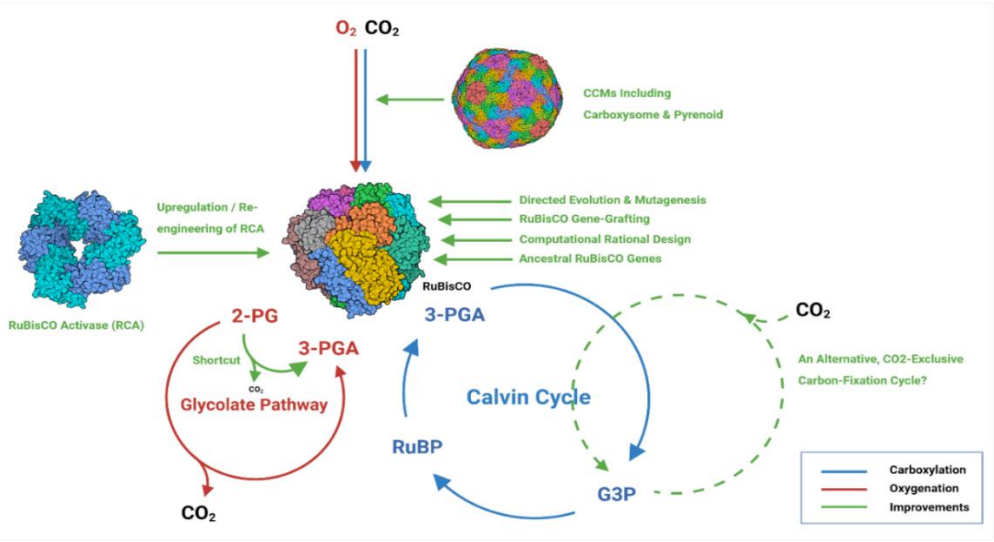

Figure.5 A summary of current strategies to enhance the functionality of the RuBisCO enzyme. Many of them focuses on the RuBisCO enzyme itself, aiming to achieve maximum specificity and catalytic rate via changing the amino acid sequence of RuBisCO. These methods include: directed evolution and mutagenesis, RuBisCO gene transferring, rational design via computations, and deduction of ancestral RuBisCO sequences. Other methods focus on altering the surrounding environment of the RuBisCO enzyme, which includes the CCMs and engineering RCA. Other proposed innovative strategies include the optimization of the glycolate pathway, or the *de novo* construction of an alternative cycle using synthetic biology. (The diagram was illustrated using Biorender.)

Site-Directed Mutagenesis & Directed-Evolution

As one of the earliest strategies to improve the functionality of RuBisCO, many attempts in the 80s and 90s of the last centuries aim to enhance the specificity (Sc/o) and the catalytic rate (Kcat-CO_2) of the enzyme; Sc/o can

also be represented mathematically by the partition coefficient, or τ , which is the gradient of carboxylase activity against the oxygenase activity of the RuBisCO enzyme (Gutteridge *et al.*, 1984):

$$\tau = S\ {}^{c}/_{o} = \frac{kcat[CO2]}{Km[CO2]} \Big/ \frac{kcat[O2]}{Km[O2]}$$

Owing to the lack of accurate X-ray crystallographic model and overall characterization of the structural domains of RuBisCO, these earliest attempts of site-directed mutation didn't make substantial progressions in the improvement of the partition coefficient of RuBisCO, and usually resulted in a decline in the enzymatic activity, such as the one carried out by Gutteridge *et al.* [23], on the RuBisCO of the photoautotrophic bacterium *Rhodospirillum rubrum.* Series of mutagenesis were conducted by various different research groups after the first high-resolution crystallographic structure of RuBisCO was solved by Andersson [24] in the mid-90s. Some mutations target the amino acid residues at or adjacent to the active site, whilst many others concentrate on a dynamic loops structure termed as loop-6 (See Figure. 1). Though the rationale of many of these mutations were inferred from the characterized catalytic mechanism of RuBisCO, many ended up compromising the enzymatic activity. Some site-directions, though, resulted in a modest improvement in either the catalytic rate (Kcat-CO_2) or specificity (Sc/o) [19].

Whilst these rational, site-directed mutations could only explore a limited portion of the sequence space (all the possible permutations and combinations of amino acid residues in a polypeptide of give length) near the wild-type, the "irrational" approach of directed-evolution (DE) allows for a wider discovery of the potentially optimal coordinates in the sequence space (Figure.7). The process of directed-evolution involves rounds of selection on microorganisms that carries the expression vector containing the coding-sequence (CDS) of the gene to be optimized. Variations in the sequence of CDS is introduced via different methods, including chemical mutagenesis and error-prone PCR. A strong selective pressure is then imposed on the bacterial population, followed by the amplification of the potentially fitter genotype in the population, which can then be sequenced.

Escherichia coli strains expressing different types of RuBisCO chaperones was commonly used as the chassis organism for directed evolution. The selective pressure can be readily introduced via an enzyme named as phosphoribulokinase (prk) (Figure.6). The enzyme phosphorylates a common metabolite – Ribulose-5-phosphate (Ru5P) in the pentose phosphate pathway (PPP) of the bacteria, generating Ribulose-bisphosphate (RuBP), which cannot be catabolized by other enzymes in *E. coli* thereby creating a metabolic dead-end. The uncontrolled accumulation of RuBP imposes a toxic effect on the bacteria, which can only be alleviated by the newly introduced enzyme RuBisCO, which uses RuBP as the substrate. It was believed that, after rounds of this selection, E. coli lineages with enhanced RuBisCO functionality will emerge [19]. Variant strategies that involve the perturbation of the glycolytic enzymes were also designed, which makes RuBP the only viable source of energy [2].

Figure 6

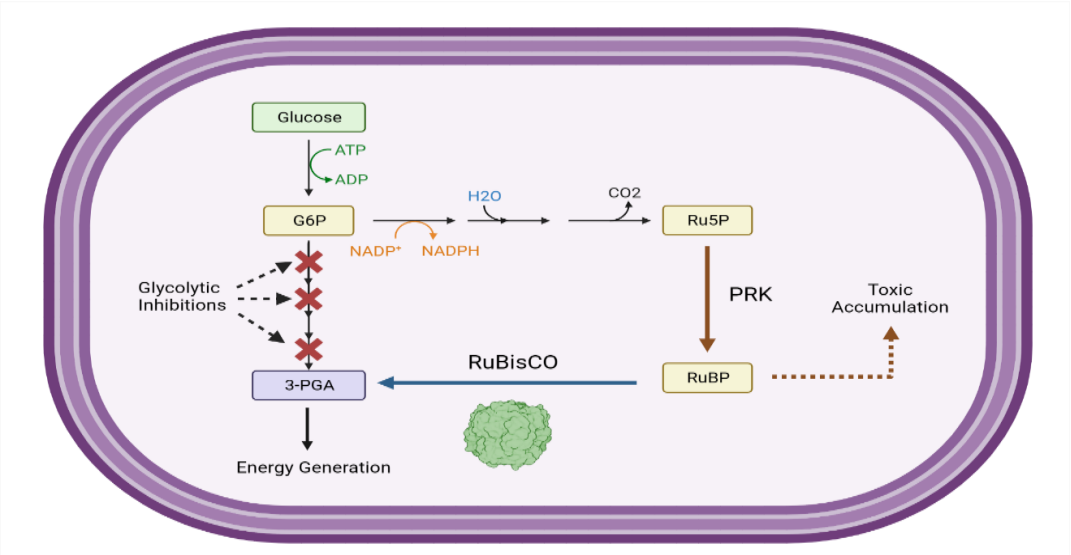

Figure.6 Escherichia coli as the chassis for directed evolution. A toxic pathway (brown) containing the enzyme PRK can be introduced into the metabolic network. If RuBP cannot be efficiently removed by RuBisCO (blue arrow), its toxic accumulation will hamper the growth of the bacteria, thus exerting a selective pressure. Knocking out the glycolytic enzymes (red crosses) can further enhance this selective pressure by making PRK-RuBisCO the only energy acquisition pathway. (The idea was derived from Cai *et al.*, 2014, the diagram was illustrated by Biorender.)

A relatively successful DE carried out by Cai *et al.* had allegedly achieved 85% increase in specific carboxylation activity (U/mg) and a 45% increase in the catalytic efficiency (Kcat [CO_2]/Km[CO_2]). It was also mentioned though, that their selection system based on the bacterial colony size may include false-positive results, and the Km [CO_2] value was higher in the mutant variants, suggesting a decrease in the affinity to CO_2. The RuBisCO template utilized by different research groups to carry out directed evolution vary, which hinders the direct comparison between them. Moreover, most of them could only achieve an improvement in one of the parameters (Catalytic efficiency or Substrate specificity) whilst compromising the other [2].

Computation-Aided Rational Design

The recent developments in artificial intelligence (AI) have been catalysing new waves of breakthrough in bioinformatics and structural biology. Algorithms including Alphafold2 have shown their promising capability in the sequence - based protein 3D structure prediction [25], whilst others like Protein MPNN opens a new gate in protein structural design, by generating the amino acid sequences for a desired structure [26]. The recent emergence of generative AI, including the algorithm RF diffusion, sheds light on the rapid development of novel artificial enzymes with novel functions [27].

The AI-guided enzyme design and optimization usually involves two essential processes: *In silico* modelling, which involves the construction of the protein scaffolds and the use of deep-learning. Rounds of computational optimizations then select a group of candidates for the second procedure – *In vitro* verification, which involves the measurement of kinetic parameters such as Kcat and Km of the enzymes [28]. The two main processes are cooperative and complementary, the result from *In vitro* can in turn be used for the next round of *in silico* design. Recently, the use of a deep learning based algorithm named as “hallucination” has achieved a major milestone in the *de novo* design of the enzyme luciferase [28], with kinetic parameters comparable to that of the wild type, and a significant improvement in substrate selectivity is also observed. If the same principle could be applied to the enzyme RuBisCO in near future, we could expect new RuBisCO variants with improved Kcat-CO_2 or Sc/o been engineered. A more recent, open-source generative AI model developed by the same research group named as RF diffusion [27] further illuminates the potential of *de novo* protein design and active-site modelling.

Dynamic loop structures in many enzymes had become a major target for the functional improvement and substrate specificity-switching [29]. These loops control the gating of the active-site, adopting open or closed conformations to regulate enzyme activity, and forms a portion of the active-site, thereby contributing to the specificity of enzyme catalysis. This is exemplified by many classic examples such as the beta-lactamase and protein tyrosine-phosphatases [29]. The activity of RuBisCO is also determined by the dynamic loop termed as loop-6 (see previous sections), which controls the entry of substrates and forms a portion of the active-site. If generative AIs such as RF diffusion can be deployed to re-engineer the loop-6, an enhancement in RuBisCO’s specificity towards CO_2 could be envisioned.

A major obstacle of computationally aided RuBisCO re-engineering is imposed by the structural complexity of this enzyme. The harsh requirement of multiple chloroplast chaperones during the folding process and the hexadecameric nature of the enzyme itself exerts a greater level of difficulty in modelling its structure and mode of action. This is largely different from the monomeric luciferase enzymes as is previously described. Though the rational, AI-aided optimization of RuBisCO faces many challenges, it holds the greatest potential in discovering the superlative RuBisCO variants, due to its much wider exploration of the sequence space compared to site-directed mutations and directed-evolutions (Figure.7).

Figure 7.

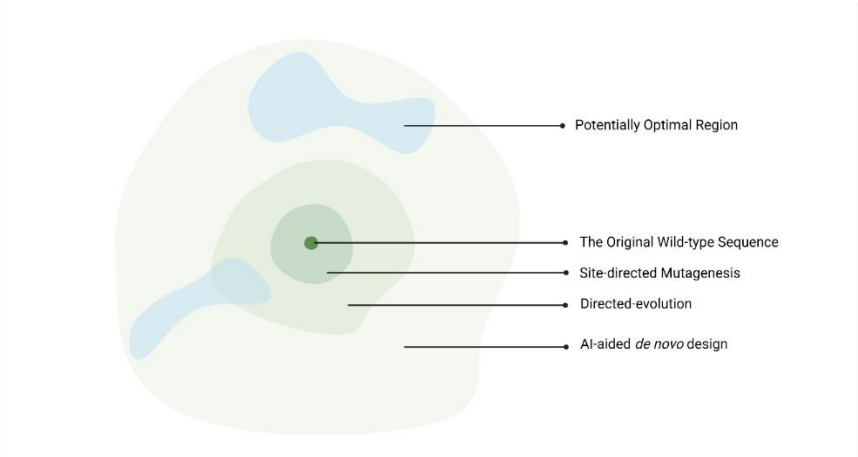

Figure.7 Visualizing the concept of sequence space. The central dark green point is the original sequence. Different improvement strategies have different extent of coverage to the entire sequence space. AI-aided design holds the greatest development potential owing to its wide sequence space coverage. (The diagram was illustrated via Biorender.)

RuBisCO Molecular-Grafting

Although the enigmatic catalytic mechanisms and molecular complexities of RuBisCO enzymes may hinder the optimization of our existing RuBisCO enzymes in plants, there could be a more straight-forward solution. Variants of Form-I RuBisCO in photosynthetic eukaryotes often shows greater catalytic efficiency and sometimes, greater specificity towards CO_2.

The molecular grafting and heterologous expression of algal RuBisCO in plants may appears as a tantalizing alternative solution, since it appears not requiring any of the complex and time-consuming molecular engineering in the previous methods. However, many of the initial attempts in RuBisCO molecular grafting ended up with fiasco, owing to the misfolding of newly introduced red algal RuBisCO in plant's chloroplasts [30]. This is mainly due to the incompatibility of the chaperone system between red algae and plants. It was highly pitiful that, in a study carried out in 2018 [31], the RuBisCO from red algae *Griffithsia monilis* (GmRuBisCO) failed to function normally when heterologous expressed in tobacco plants. Owing to its spectacular catalytic efficiency, the successful grafting of GmRuBisCO would have great significance to the lant growth. This failure presents as a setback for this methodology.

It was later discovered that the RuBisCO variant from purple bacterium *Rhodobacter sphaeroides* is less susceptible to the incompatibility of chaperone system [32]. This variant (RsRuBisCO) could achieve a stable expression and assembly in tobacco chloroplasts, and the further introduction of one of its endogenous chaperones RsRCA greatly enhanced the activity of RsRuBisCO. This achievement presents as a substantial progress in the heterologous expression of algal RuBisCO in plants and shed light on other RuBisCO grafting. In 2023, a research group successfully utilized the RsRuBisCO as a platform for the introduction of molecular characteristics from GmRuBisCO into tobacco plants [20]. By designing and characterizing a series of different RsRuBisCO-GmRuBisCO chimeras, it was found that, by introducing two characteristic amino acid residues (329A and 332V) on the loop-6 of GmRuBisCO to the one of RsRuBisCO, the chimera still exhibits normal expression and folding, whilst the kcat-co2 parameter increased by 60%, efficiency of CO_2-fixation increased by 22% and a 7% increase in Sc/o was observed. This presents as a major progression in RuBisCO engineering, since the tobacco plants transformed with the engineered chimeric RsRuBisCO exhibits a twofold growth rate compared to the plant that expresses wild-type RsRuBisCO [20].

Reviving Ancient RuBisCO Variants

Apart from the utilization of existing RuBisCO variants, another very innovative approach emerged recently is to use phylogenetic study to elucidate the amino acid sequence of an ancient specie's RuBisCO, which functioned

when the atmospheric O_2 and CO_2 concentration was vastly different from our current atmosphere. In the study carried out by Lin *et al.*, 2022 [7], a phylogenetic tree was constructed for both Large and Small subunits of *Solanaceae* RuBisCO using Bayesian inference and Maximum likelihood.

The amino acid sequence at each different nodes (common ancestors) was predicted, representing the ancestral RuBisCO variants. Lin *et al.* [7] reasoned that the periodic decrease in atmospheric CO2 in the past millions of years leaded to the emergence of C4 plant and carbon dioxide concentrating mechanisms (CCMs), and these C4 RuBisCO exhibit greater kcat-co2, which could be very adaptive to the current rapidly increasing CO_2 level.

After the kinetics assay on these predicted RuBisCO were performed, it was observed that many of them exhibits superior kcat-co2 whilst their Sc/o remains largely the same as modern C3 *Solanaceae* plants. This study sheds light on the development of a RuBisCO variant which is optimized to the rapidly increasing atmospheric CO_2 concentration during the climate change in the Anthropocene.

Carbon Dioxide Concentrating Mechanisms (CCMs)

Apart from the intricated structural optimization of the RuBisCO itself, many alternative approaches focus on the improvement of the peripheral environment of existing RuBisCO. One category of strategies, called carbon dioxide concentration mechanisms (CCMs) was inspired by the spatial separation of carbon fixation and Calvin cycle in C4 plants, which creates a high local concentration of CO_2 in bundle-sheath cells [1]. The two prevailing CCM machineries are carboxysomes and pyrenoids, one derived from eubacteria, whilst the other one is from algae. Both are described below.

Carboxysomes

As the bacterial CCM machinery, carboxysomes are proteinaceous, multi-protein complexes that generally adopts an icosahedral shape [33]. The carboxysomes complex contains hundreds of RuBisCO and carbonic anhydrase enzymes in their inner cavity. The shell of carboxysomes is formed by a diverse collection of structural proteins, inter-linked with various scaffolding proteins that connects the outer shell with the RuBisCO enzymes inside (Figure.8). The proteinaceous shell itself exhibits selective permeability, while it is poorly permeable to gases including O_2 and CO_2, it is readily permeable to bicarbonate ions. This design allows the bicarbonate ions (HCO3-) that rapidly diffused into the central compartment to be trapped via the enzyme carbonic anhydrase, which converts it into CO_2, thereby creating a high local CO_2 concentration that favour the catalysis by RuBisCO [33].

Figure 8.

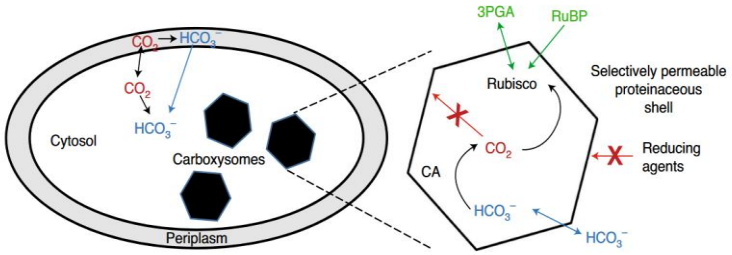

Figure.8 The molecular details of carboxysomes. Note that the proteinaceous shell is neither permeable to gases such as O2 and CO2 or reducing agents. Bicarbonate ions (HCO3-) can readily diffuses into the shell and converted into CO2 by the enzyme carbonic anhydrase (CA). (Source: Zang *et al.*, 2021) [34].

Different isoforms of carboxysome exists in the diverse eubacterial domain. The alpha-carboxysome primarily exists in alpha-proteobacterium and other bacteria that possess Form-IA RuBisCO (See sections on the evolution and phylogeny of RuBisCO), CsoS2 is the main scaffolding protein that is responsible for the assembly of RuBisCO in this type [35]. Another form of carboxysome is the beta-carboxysome, which exists in cyanobacteria species and contains Form-IB RuBisCO. The scaffold protein CcmM is mainly responsible for the assembly of RuBisCO in this type [34]. The spatial arrangement of RuBisCO indifferent carboxysomes vary. For example,

the alpha-carboxysome from *Cyanobium* sp. PCC 7001 exhibits concentric RuBisCO arrangement of RuBisCO, while the alpha carboxysome in *Halothiobacillus neapolitanus* exhibits spiral arrangement [36].

Using different types of carboxysomes may have different advantages. For example, the compatibility of beta-carboxysome with plant's form-IB RuBisCO is greater, whilst the alpha-carboxysome can harbour the very catalytically efficient Form-IA RuBisCO. Although the successful assembly of bacterial Carboxysomes in plant chloroplasts have not been achieved, extensive characterizations on their structure have been conducted in recent years, and it was predicted that, the incorporation of carboxysome CCM in plant chloroplast can significantly boost the efficiency of carbon fixation.

Pyrenoids

The eukaryotic CCM machinery, also known as the pyrenoids, is a complex subcellular structure formed by portions of the thylakoid membrane and a starch sheath [37]. This CCM machinery primarily presents in red and green algal species. During the carbon assimilation process, CO_2 first diffuses across the chloroplast membranes and enter the stroma, then trapped by the enzyme carbonic anhydrase and converted into HCO^{3-}. This bicarbonate ion can then be transported via protein channels and enter the thylakoid lumen, which has lower pH (6) compared to the stroma (8). Therefore, HCO^{3-} can be converted back to CO_2 by a thylakoid carbonic anhydrase, and diffuses into the matrix of the pyrenoid complex, where RuBisCO enzymes are situated (Figure.9). The starch shell peripheral to the matrix impedes the diffusion of gases, leading to the accumulation of CO_2 and the exclusion of O_2 in the matrix. Furthermore, in the extra low atmospheric CO_2 concentration, bicarbonate ions could be actively transported across the chloroplast membranes [38].

Figure 9.

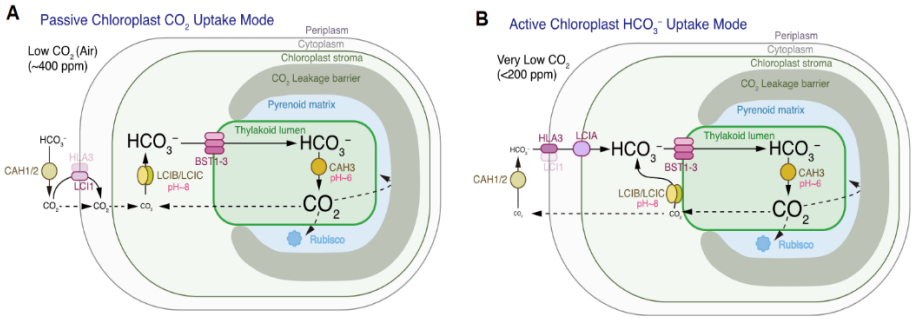

Figure.9 The molecular mechanism of the pyrenoid complex. CO_2 can either be actively pumped into the stroma or passively diffuses into the stroma, depending on the atmospheric CO_2 concentration. Stromal carbonic anhydrases (LCIB/LCIC) and thylakoid carbonic anhydrase (CAH3) work cooperatively to convert the carbon into the suitable form (HCO^{3-}/CO_2). (Source: He, Crans and Jonikas, 2023) [37].

In the study carried out by Fei *et al.*, 2022 [38], the authors proposed a strategy that could be applied to implement the pyrenoid complex in modern plants (Figure.10).

The initial step is the localization of RuBisCO enzymes in a closely packed matrix that is adjacent to the thylakoid membrane. The stromal carbonic anhydrase will also be excluded in this process.

The second stage involves the fixation of carbonic anhydrases in the thylakoid lumen, ideally bordering the RuBisCO matrix complex. These two initial steps do not significantly increase either the CO_2 flux or the cost of carbon fixation. The objective of the third stage is to introduce bicarbonate ion transporters to the thylakoid membrane, allowing the passage of HCO^{3-} from stroma into thylakoid lumen.

Finally, a starch sheath is constructed in the 4th stage that excludes O_2 and allows CO_2 to accumulate in the matrix. The third stage drastically increases the net flux of CO_2 but also increases the number of ATP consumed per CO_2 fixed. The fourth stage finally reduces this number to just 1.3 ATP, completing the pyrenoid engineering [38].

Figure 10.

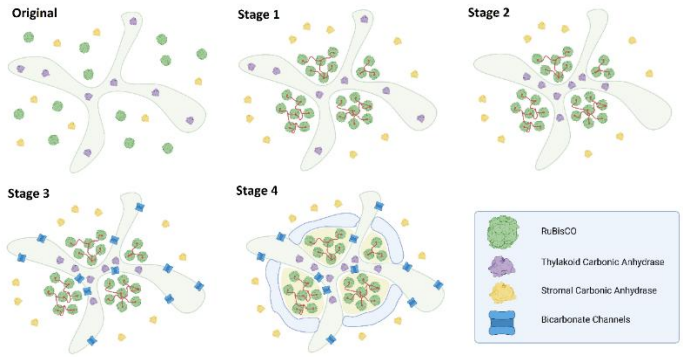

Figure.10 A stepwise pyrenoid construction strategy in modern plants. Originally, stromal and thylakoid carbonic anhydrases, along with RuBisCO enzymes, are diffusely dispersed. The initial step is the fixation of RuBisCO via scaffolding proteins near the thylakoid junctions. This is followed by the immobilization of carbonic anhydrase in thylakoid lumen and the introduction of HCO^{3-} channels to the thylakoid membrane. Finally, a starch sheath is introduced to isolate the matrix from O_2 and allow CO_2 to build up. (The idea was from Fei *et al.*, 2022, the diagram is constructed using Biorender.)

Overexpression of RuBisCO-Activase (RCA)

Event after the successful folding and assembly of RuBisCO large and small subunits in the stroma, the activity of RuBisCO is still dependent on multiple factors, such as the stromal pH, Mg^{2+} concentration, and, perhaps most importantly, the presence of RuBisCO Activase (RCA), an ATP-dependent chaperone protein [39] The RCA enzyme is a homohexamer consisting of 6 identical RCA monomers, it is an AAA+ ATPase family protein that utilizes ATP hydrolysis to facilitate its own conformational change [40]. The RCA in modern plants also has a C-terminal domain with a disulfide bridge, indicating that the enzymatic activity can be indirectly regulated by light via the changes in the redox environment. This structure is absent in cyanobacterial RCA.

The recruitment and binding of RCA onto the RuBisCO L_8S_8 complex can efficiently induce its conformational change, causing the release of an inhibitory RuBP molecule, allowing the active site lysine residue (K201) to be modified, becoming a carbamoylysine (KCX201) residue. This residue is crucial for the coordination of the Mg^{2+} ion required to stabilize the enediolate intermediate during the catalysis (See the section on catalytic mechanisms) [39]. Due to its indispensable role in the enhancement of RuBisCO activity, many research groups, such as the one that expresses RsRuBisCO in tobacco plants (previously described) [32], utilize it as an alternative approach to boost the functionality of RuBisCO.

The overexpression of RCA was indeed proved to be successful in enhancing the growth rate plants. In the study carried out by Bi *et al.*, 2017 [41], overexpression of RCA in *Cucumis sativus* (Cucumber) indeed resulted in the enhance plant growth, whilst the study carried out by Qu *et al.*, 2021 [42] in *Oryza sativa* (Rice) shows that the co-overexpression of RuBisCO and RCA achieved a greater growth rate and dry mass at 40 Celsius compared to the wild type. This finding implies that the overexpression of RCA could be utilized strategy to increase the heat resistance of modern crops, securing the food supply during the imminent climate change. Furthermore, it was observed that RCA is complexed with some alpha-carboxysomes [43], showing that RCA can be implemented together with the CCMs to achieve a synergistic effect in enhancing the carbon-fixation rate.

Glycolate Pathway Shortcuts

Nearly all the strategies previously described in this review aim to enhance the carboxylase activity of RuBisCO and maximally suppress photorespiration. The glycolate pathway is a complex and energy-consuming cycle that involves multiple organelles including peroxisome and mitochondria [44]. However, an alternative improvement strategy is to engineer a shortcut for photorespiration.

In the study carried out by South *et al.*, 2019 [45], three alternative glycolate pathways were designed using synthetic biology approaches (Figure.11), then separately implemented in different tobacco plants. These

alternative pathways are peroxisome and mitochondria-independent, only involves enzymatic catalysis inside stroma, thus are predicted to have greater efficiency. After the *in vivo* experiment in tobacco plants, a 13% increase in the dry mass was observed in the plant adopting alternative pathway-1 compared to the wild-type, whilst a 18% increase was observed in the third alternative pathway. The transcriptional suppression of a glycolate efflux transporter called PLGG1 using RNAi (RNA interference) further improved the alternative pathway-3, with a 24% increase in dry mass compared to the wild-type [45].

Figure 11.

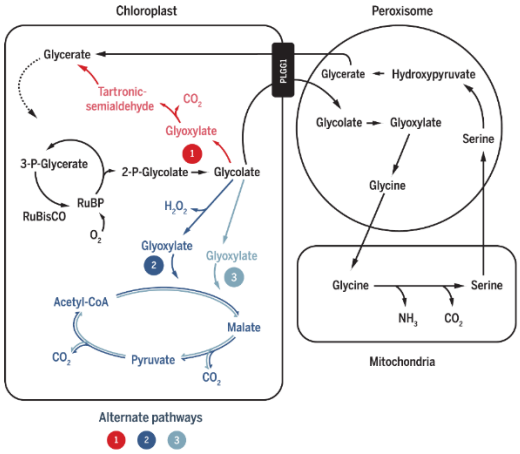

Figure.11 Three alternative synthetic glycolate pathways. The first alternative pathway (AP-1) implements a three-enzyme system that directly converts glycolate to glycerate, losing one CO_2. The second and third alternative pathways (AP-2 AND AP-3) redirects glycolate to the glyoxylate cycle, where it is used to synthesize malate. The two pathways are different at the electron receptor they use. AP-2 uses O_2, while AP-3 uses NAD+. The H_2O_2 generated by AP-2 requires an extra enzyme (catalase) to decompose it. (Source: South *et al.*, 2019) [45].

Synthetic Carbon Fixation Pathways

The disappointing inefficiency of RuBisCO in the Calvin cycle leads to the idea of whether this troublesome enzyme could be simply bypassed. However, this leads to a tricky question: which carbon-fixating enzyme will be the best alternative?

Many enzymes involved in carbon-fixation only functions in the anaerobic conditions, such as the 2-ketoglutarate ferredoxin oxidoreductase (KGOR) involved in the reductive TCA (rTCA) cycle, and the formate dehydrogenase involved in the Wood-Ljungdahl pathway [46]. Both were originated from anaerobic autotrophs, not suitable for plant engineering. Other types of carboxylases, including the reducing carboxylases (for example, crotonyl-CoA carboxylase) and the ATP-dependent carboxylases (for example, phosphoenolpyruvate carboxylase (PEP carboxylase)), are not integrated with the pentose phosphate pathway (PPP), the pathway relied by the Calvin cycle to regenerate RuBP.

To better utilize these O_2-tolerant exotic carboxylases, heavy synthetic biology approaches should be applied to devise artificial catalytic cycles that can be integrated into plant metabolism.

Significant progresses have been recently achieved in the design of artificial carbon-fixation pathways. In research carried out in 2022 by Luo *et al.* [47], a chimeric, synthetic carbon-fixation pathway, named as rGPS (reductive glyoxylate-pyruvate synthesis) pathway was proposed (Figure.12).

The rGPS cycle itself was constructed from three complementary synthetic pathways: rPS (reductive Pyruvate Synthesis), rGS (reductive Glyoxylate Synthesis) and an MCG (Malyl-CoA-Glycerate) shuttle. The rGS pathway was primarily constructed based on the C4/CAM carbon-fixation pathway in plants, utilizing the high-efficiency, CO_2 - exclusive PEP carboxylase that is ATP-dependent.

The conversion of 3C Pyruvate to 4C oxaloacetate is then followed by its transformation into 4C malyl-CoA, which is then cleaved into 2C glyoxylate and 2C acetyl-CoA.

The glyoxylate molecules are salvaged by the MCG pathway, forming glycerate. The extra acetyl-CoA then goes through the rPS pathway, which is a chimera of the natural ethylmalonyl-CoA pathway from *Rhodobacter Sphaeroides* [48] and the mesaconate pathway. The 3C pyruvate molecules were regenerated at the end, making it a self-replenishing Cycle.

Figure 12.

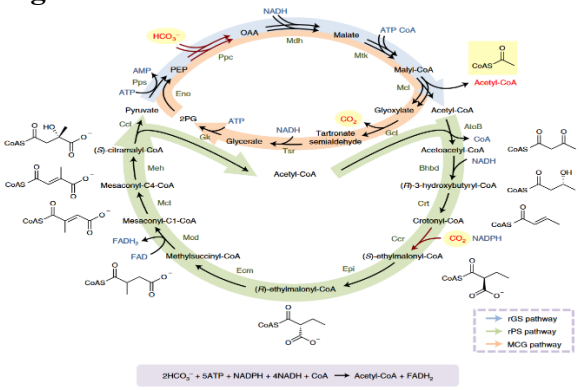

Figure.12 The synthetic, *in vitro* rGPS cycle was constructed using various enzymes from different natural metabolic pathways. The key carbon-fixating enzymes (PEP carboxylase and crotonyl-CoA reductase are O_2 insensitive, presented as a major advantage.) Note that the output molecule shown is acetyl-CoA, but could also be the 3C pyruvate, source: Luo *et al.*, 2022 [47]

The key advantage of this design is the leverage of O_2-insensitive PEP carboxylase and crotonyl-CoA reductase. This synthetic rGPS cycle was proven to be successful in *in vitro* experiments, in which cofactors and reducing equivalent are constantly provided [47] However, there is still a long way to go for a truly self-sustaining, light-driven carbon-fixation cycle to finally emerge. The metabolic integration with modern plant chloroplasts is also a challenge for this chimeric pathway.

DISCUSSIONS & CONCLUSIONS

The various strategies discussed in this review, to boost carbon-fixation rate, have their own advantages and drawbacks, and most studies are usually just focused on a single optimisation strategy. It is worth notice that none of the current strategies have led to the emergence of a superlative RuBisCO or a novel carbon-fixation pathway. It will be a more sensible approach to leverage these current strategies simultaneously and letting them to achieve a synergistic effect. In the end, I propose a combinatorial strategy that integrates multiple improvements in one design:

1. RuBisCO enzymes with improved Kcat- CO_2 or Sc/o by molecular-grafting, AI-aided design and/or phylogenetic revival.
2. Optimized RuBisCO complexed with CCM machineries.
3. Upregulated expression of RCA, designed to form complex with the CCM.
4. Knockout of glycolate efflux protein, photorespiratory shortcut in chloroplast.

As the enzyme responsible for greater than 99.5% of annual CO_2 absorption globally, RuBisCO is such an enzyme with huge significance to the welfare of humankind and the biosphere. The optimization of RuBisCO will be a valuable strategy to combat the ever-rising CO_2 concentration and the imminent climate change, via the sequestration of CO_2 into the form of biomass. Even if the trend of climate change cannot be impeded, RuBisCO engineering, along with other strategies in plant synthetic biology, will increase the resistance and adaptability of our crops to the drastic changes in the environmental conditions, guaranteeing the food security for our species in the next century.

REFERENCES

1. Nelson, D.L. and Cox, M.M. (2021) Lehninger principles of biochemistry. Eighth edition. New York, NY: Macmillan International, Eighth Edition.

2. Prywes, N. et al. (2023) 'Rubisco Function, Evolution, and Engineering', Annual Review of Biochemistry, 92(1), pp. 385–410.
3. Taylor, T.C. and Andersson, I. (1997) 'The structure of the complex between rubisco and its natural substrate ribulose 1,5-bisphosphate', Journal of Molecular Biology, 265(4), pp. 432–444.
4. Taylor, T.C. and Andersson, I. (1996) 'Structural transitions during activation and ligand binding in hexadecameric Rubisco inferred from the crystal structure of the activated unliganded spinach enzyme', Nature Structural Biology, 3(1), pp. 95–101.
5. Valegård, K. et al. (2018) 'Structure of Rubisco from Arabidopsis thaliana in complex with 2-carboxyarabinitol-1,5-bisphosphate', Acta Crystallographica Section D Structural Biology, 74(1), pp. 1–9.
6. Gunn, L.H., Valegård, K. and Andersson, I. (2017) 'A unique structural domain in Methanococcoides burtonii ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) acts as a small subunit mimic', Journal of Biological Chemistry, 292(16), pp. 6838–6850.
7. Lin, M.T. et al. (2020) 'Small subunits can determine enzyme kinetics of tobacco Rubisco expressed in Escherichia coli', Nature Plants, 6(10), pp. 1289–1299.
8. Li, Q. et al. (2022) 'Structural insights into cyanobacterial RuBisCO assembly coordinated by two chaperones Raf1 and RbcX', Cell Discovery, 8(1), p. 93.
9. Aigner, H. et al. (2017) 'Plant RuBisCo assembly in E. coli with five chloroplast chaperones including BSD2', Science, 358(6368), pp. 1272–1278.
10. Waheeda, K. et al. (2023) 'Molecular mechanism of Rubisco activase: Dynamic assembly and Rubisco remodeling', Frontiers in Molecular Biosciences, 10, p. 1125922.
11. Tcherkez, G. (2013) 'Modelling the reaction mechanism of ribulose-1,5-bisphosphate carboxylase/oxygenase and consequences for kinetic parameters', Plant, Cell & Environment, 36(9), pp. 1586–1596.
12. Cummins, P.L., Kannappan, B. and Gready, J.E. (2018) 'Revised mechanism of carboxylation of ribulose-1,5-biphosphate by rubisco from large scale quantum chemical calculations', Journal of Computational Chemistry, 39(21), pp. 1656–1665.
13. Cleland, W.W. et al. (1998) 'Mechanism of Rubisco: The Carbamate as General Base', Chemical Reviews, 98(2), pp. 549–562.
14. Bathellier, C. et al. (2020) 'Ribulose 1,5-bisphosphate carboxylase/oxygenase activates O 2 by electron transfer', Proceedings of the National Academy of Sciences, 117(39), pp. 24234–24242.
15. Tcherkez, G.G.B., Farquhar, G.D. and Andrews, T.J. (2006) 'Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized', Proceedings of the National Academy of Sciences, 103(19), pp. 7246–7251.
16. Shih, P.M. et al. (2016) 'Biochemical characterization of predicted Precambrian RuBisCO', Nature Communications, 7, p. 10382.
17. Flamholz, A.I. et al. (2019) 'Revisiting Trade-offs between Rubisco Kinetic Parameters', Biochemistry, 58(31), pp. 3365–3376.
18. Bouvier, J.W. et al. (2021) 'Rubisco Adaptation Is More Limited by Phylogenetic Constraint Than by Catalytic Trade-off', Molecular Biology and Evolution. Edited by J. Echave, 38(7), pp. 2880–2896.
19. Cai, Z. et al. (2014) 'Development of an activity-directed selection system enabled significant improvement of the carboxylation efficiency of Rubisco', Protein & Cell, 5(7), pp. 552–562.
20. Zhou, Y. et al. (2023) 'Grafting Rhodobacter sphaeroides with red algae Rubisco to accelerate catalysis and plant growth', Nature Plants, 9(6), pp. 978–986.
21. Lin, M.T. et al. (2022) 'Improving the efficiency of Rubisco by resurrecting its ancestors in the family Solanaceae', Science Advances, 8(15), p. eabm6871.
22. .Meyer, M.T. et al. (2020) 'Assembly of the algal CO 2 -fixing organelle, the pyrenoid, is guided by a Rubisco-binding motif', Science Advances, 6(46), p. eabd2408.
23. Gutteridge, S. et al. (1984) 'A site-specific mutation within the active site of ribulose-1,5-bisphosphate carboxylase of Rhodospirillum rubrum', The EMBO Journal, 3(12), pp. 2737–2743.
24. Andersson, I. (1996) 'Large Structures at High Resolution: The 1.6 Å Crystal Structure of Spinach Ribulose-1,5- Bisphosphate Carboxylase/Oxygenase Complexed with 2-Carboxyarabinitol Bisphosphate', Journal of Molecular Biology, 259(1), pp. 160–174.
25. Jumper, J. et al. (2021) 'Highly accurate protein structure prediction with AlphaFold', Nature, 596(7873), pp. 583–589.

26. Dauparas, J. et al. (2022) 'Robust deep learning–based protein sequence design using ProteinMPNN', Science, 378(6615), pp. 49–56.
27. Watson, J.L. et al. (2023) 'De novo design of protein structure and function with RFdiffusion', Nature, 620(7976), pp. 1089–1100.
28. Yeh, A.H.-W. et al. (2023) 'De novo design of luciferases using deep learning', Nature, 614(7949), pp. 774–780.
29. Corbella, M., Pinto, G.P. and Kamerlin, S.C.L. (2023) 'Loop dynamics and the evolution of enzyme activity', Nature Reviews Chemistry, 7(8), pp. 536–547.
30. 30. Whitney, S.M. et al. (2001) 'Form I Rubiscos from non-green algae are expressed abundantly but not assembled in tobacco chloroplasts', The Plant Journal, 26(5), pp. 535–547.
31. Lin, M.T. and Hanson, M.R. (2018) 'Red algal Rubisco fails to accumulate in transplastomic tobacco expressing Griffithsia monilis RbcL and RbcS genes', Plant Direct, 2(2), p. e00045.
32. Gunn, L.H. et al. (2020) 'The dependency of red Rubisco on its cognate activase for enhancing plant photosynthesis and growth', Proceedings of the National Academy of Sciences, 117(41), pp. 25890–25896.
33. Long, B.M. et al. (2016) 'Cyanobacterial CO2-concentrating mechanism components: function and prospects for plant metabolic engineering', Current Opinion in Plant Biology, 31, pp. 1–8.
34. Zang, K. et al. (2021) 'Scaffolding protein CcmM directs multiprotein phase separation in β-carboxysome biogenesis', Nature Structural & Molecular Biology, 28(11), pp. 909–922.
35. Oltrogge, L.M. et al. (2020) 'Multivalent interactions between CsoS2 and Rubisco mediate α-carboxysome formation', Nature Structural & Molecular Biology, 27(3), pp. 281–287.
36. Ni, T. et al. (2022) 'Structure and assembly of cargo Rubisco in two native α-carboxysomes', Nature Communications, 13(1), p. 4299.
37. He, S., Crans, V.L. and Jonikas, M.C. (2023) 'The pyrenoid: the eukaryotic CO2-concentrating organelle', The Plant Cell, 35(9), pp. 3236–3259.
38. Fei, C. et al. (2022) 'Modelling the pyrenoid-based CO2-concentrating mechanism provides insights into its operating principles and a roadmap for its engineering into crops', Nature Plants, 8(5), pp. 583–595.
39. Karthick P.V., et al. (2024) Improving Crop Yield through Increasing Carbon Gain and Reducing Carbon Loss. Plants 2024, 13(10), pp.1317-1333. https://doi.org/10.3390/plants13101317
40. Stainbrook, S.C., et al., (2024) C4 grasses employ distinct strategies to acclimate rubisco activase to heat stress. Bioscience Reports (2024) 44 (10): BSR20240353. doi: 10.1042/BSR20240353
41. Bi, H. et al. (2017) 'Overexpression of the rubisco activase gene improves growth and low temperature and weak light tolerance in Cucumis sativus', Physiologia Plantarum, 161(2), pp. 224–234.
42. Qu, Y. et al. (2021) 'Overexpression of both Rubisco and Rubisco activase rescues rice photosynthesis and biomass under heat stress', Plant, Cell & Environment, 44(7), pp. 2308–2320.
43. Tsai, Y.-C.C. et al. (2022) 'The CbbQO-type rubisco activases encoded in carboxysome gene clusters can activate carboxysomal form IA rubiscos', Journal of Biological Chemistry, 298(1), p. 101476.
44. Zhang, Z., at al., 2024. Photorespiration in plant adaptation to environmental changes. Crop and Environment. 3 (4), pp. 203-212
45. South, P.F. et al. (2019) 'Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field', Science, 363(6422), p. eaat9077.
46. Cocon, K., D., and Lui, P. (2024) The potential of RuBisCO in CO2 capture and utilization. Progress in Energy and Combustion Science. 105, 101184. https://doi.org/10.1016/j.pecs.2024.101184
47. Luo, S. et al. (2022) 'A cell-free self-replenishing CO2-fixing system', Nature Catalysis, 5(2), pp. 154–162.
48. Erb, T.J. et al. (2007) 'Synthesis of C 5 -dicarboxylic acids from C 2 -units involving crotonyl-CoA carboxylase/reductase: The ethylmalonyl-CoA pathway', Proceedings of the National Academy of Sciences, 104(25), pp. 10631–10636.