



# Deep learning and generative artificial intelligence methods in enzyme and cell engineering

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Efficient enzymes and microbial factories are essential to promote the transition toward a sustainable bioeconomy. This review focuses on the progress of artificial intelligence (AI) methods in accelerating the development of optimized biocatalysts and genetic networks in cells. Recent advances in AI in the field of enzyme discovery, engineering, and *de novo* design are discussed. Additionally, we highlight examples of successful applications of AI in optimizing different components in cells, from gene expression regulation to metabolic pathway optimization and design. Finally, this review emphasizes the challenges limiting the reliability and generalizability of current AI methods.

## Addresses

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## Introduction

The development of circular, bio-based processes that yield fine chemicals, enzyme biocatalysts, and engineered organisms, or remove environmental waste, is crucial for the global endeavor to move toward a sustainable bioeconomy [1]. Artificial intelligence (AI) methods can accelerate this transition by reducing the experimental effort for the development of optimized strains and biocatalysts. Here, we highlight recent advances in the applications of AI methods in designing and optimizing enzymes as biocatalysts and in cell engineering.

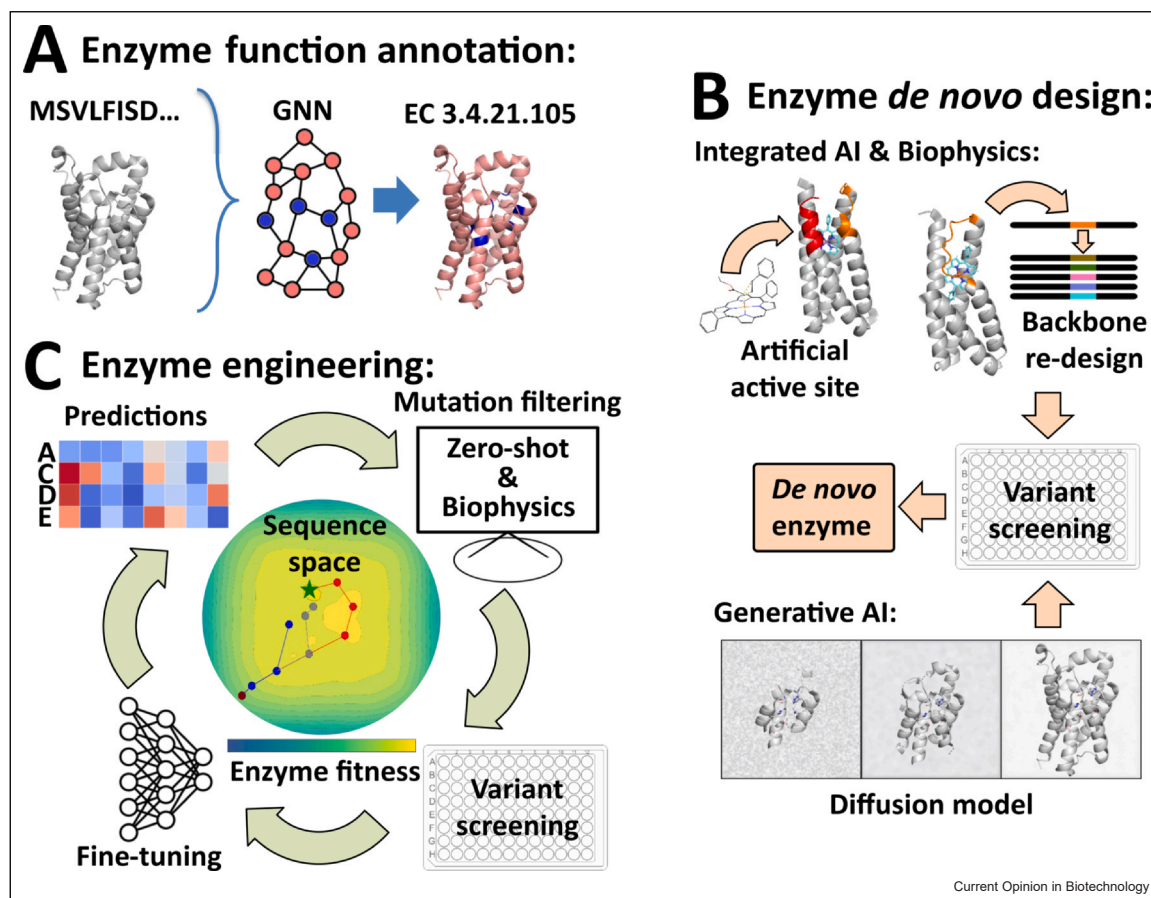
## Artificial intelligence methods in enzyme discovery and engineering

### Accelerating enzyme discovery

While millions of protein sequences are known, only a fraction have experimental function annotations such as Enzyme Commission (EC) numbers. In 2023, CLEAN (contrastive learning-enabled enzyme annotation) [2], a model trained on enzyme sequences, outperformed the then state-of-the-art, sequence homology-based BLASTp [3] method on the Price-149 benchmark [4] of 149 challenging enzyme sequence-function pairs, achieving an F1 score of 0.495 versus 0.385. This demonstrated that machine learning can surpass traditional similarity searches in EC prediction. In 2024, CLEAN was upgraded to CLEAN-contact [5], which uses structure-based residue contact maps interpreted via the ResNet-50 computer vision model [6] and the protein language model (PLM) ESM-1b [7], improving prediction performance (F1 score of 0.525 on Price-149).

Graph neural networks (GNNs) further integrate structural data into AI training, as exemplified by GraphEC [8] and TopEC [9] (Figure 1). Both models follow similar approaches of using protein structures predicted from sequence to build graph representations. Initially, GraphEC predicts the enzyme's active site within its model framework, while TopEC employs P2Rank [10]. Both then predict EC numbers based on the feature importance assigned to the active site residues. GraphEC further aims to predict the optimum pH value of the identified enzymatic reaction; however, due to low data availability, this was only evaluated on roughly 4000 enzymes, with 52% of optimum pH values at 7–8. GraphEC outperforms CLEAN on the Price-149 benchmark (F1 score of 0.613), while TopEC performs on par with CLEAN on the custom, larger benchmark TopEnzyme [11] developed by the TopEC authors. This is notable, as TopEC was evaluated with both a 30% sequence similarity cutoff and a structure-based data split using FoldSeek clusters [12] to prevent data leakage between training and test sets. GraphEC and CLEAN use more permissive cutoffs at 40% and 50% sequence identity, respectively. This increases the likelihood of homologous proteins occurring in the training and test sets, artificially boosting the models' prediction performance [13,14]. Besides the increasing availability of training data, progress in EC number predictions can also be expected from the refinement of protein embeddings used for model training. A recent preprint shows that simple multilayer-perceptron models trained

Figure 1



Workflows using AI methods in enzyme discovery and engineering. **(a)** Enzymes suitable for specific biocatalytic applications can be identified via AI-based function predictions for unannotated sequences. **(b)** They can also be designed *de novo* by inserting artificial active sites into protein scaffolds or by designing novel backbones around defined active site residues and ligands. **(c)** For the optimization of enzyme properties for biocatalysis, AI can be integrated into directed evolution workflows to learn from experimental screening results and predict new mutations, or be applied for computational filtering of predicted mutations to minimize experimental efforts.

on embeddings from the PLM ESM2 [7] or the multi-modal models ProTrek [15] and OneProt can outperform CLEAN and TopEC in enzyme classification [16].

### **De novo design of novel enzymes**

*De novo* designed enzymes are artificial proteins, tailored to catalyze a specific target reaction. Recent progress in the development of AI methods and protein design workflows has enabled the creation of ‘new-to-nature’ enzymes able to efficiently produce high-value compounds, inspiring several recent review articles [17–21].

Recent successful enzyme designs follow one of two main strategies. The first starts from a computational active site model (theozyme) and uses 3D structure diffusion followed by inverse folding to generate stable and soluble protein backbones around it [21,22]. The second strategy designs a minimal active site into known enzyme scaffolds. Although both methods can yield soluble and stable

enzymes, the enzymes generally show low initial activities [21]. Hou et al. recently demonstrated that highly stable and active designs can be achieved [18]. Building on a four-helix bundle scaffold designed in 2013 and refined for more than a decade [23], they combined predictions from biophysical modeling and the message-passing neural network LigandMPNN (Ligand Message-Passing Neural Network) [24] with X-ray crystallography and minimal experimental screening to design variants of a small (< 13 kDa) porphyrin binding protein able to catalyze the cyclopropanation of styrene. The top variant achieves a 99% yield, with high substrate turnover and a diastereomeric ratio of 98.5:1.5 for the trans-(*S,S*) product. It retains its activity in up to 70% ethanol and exhibits a melting temperature of > 90°C, highlighting the biocatalytic potential of expert-designed enzymes.

Recent research in the field now focuses on integrating additional sources of valuable information into the

design process. For example, generative graph models such as the Distributional Graphormer framework [25] and BioEmu [26] predict structural ensembles of enzymes without relying on computationally expensive molecular dynamics simulations.

### Optimizing enzyme properties

Enzyme engineering typically relies on directed evolution (DE), which consists of iterative cycles of variant generation, experimental screening, and recombination of beneficial mutations [27]. Along with efficient experimental screening methods and computational modeling, AI methods have long been applied to learn how mutations affect enzyme properties [28].

AI methods in enzyme engineering follow two principal frameworks. Zero-shot predictions commonly rely on unsupervised training of models on large datasets of protein sequences, potentially enhanced with structural data [29]. A standout model is the PLM ESM3 [30], trained to generate numerical embeddings from amino acid patterns and epistatic relationships, containing information about a protein's 'fitness'. Fitness scores of many recent zero-shot predictors, while not directly predicting measurable enzyme parameters, can be correlated with experimental properties, including stability and activity, as highlighted in the continuously updated ProteinGym benchmark dataset (proteingym.org, last accessed 06.10.2025) [31]. Importantly, these zero-shot predictions vary widely in performance across enzyme datasets, limiting their reliability in iterative enzyme engineering workflows. Zero-shot methods trained to predict specific enzyme properties, such as  $K_m$  or  $k_{cat}$  values, exemplify this limitation. Here, predictions on substrates outside the training data remain challenging, as highlighted for the model ESP: "Hence, we conclude that ESP only achieves high accuracies for new enzyme-small molecule pairs if the small molecule was present among the ~1400 substrates of our training set" [32]. Additionally, models trained for application on broad enzyme and substrate classes often disregard important factors such as temperature, solvent composition, and pH, which affect  $K_m$  and  $k_{cat}$  values and vary widely across biocatalytic processes and enzyme engineering campaigns [29]. However, recent models like MPEK [33] are starting to address this challenge.

When high-quality training data is available for a target enzyme, supervised AI models present a viable alternative to zero-shot predictions [34]. Traditional regression models continue to be employed due to their ability to encode smaller but complex datasets and their high interpretability. Landwehr et al. successfully applied an augmented ridge regression model in combination with zero-shot selection methods and a cell-free, high-throughput screening (HTS) platform to rapidly evolve a promiscuous amide synthetase into nine variants,

optimized to specifically produce different amide products with improved activity [35]. Similarly, large PLMs can also be integrated into enzyme engineering workflows by using fine-tuning to increase their accuracy in engineering iterations. Notably, a recent evaluation of eight PLMs fine-tuned for different protein engineering tasks revealed that, while fine-tuning significantly improved stability predictions regarding resilience to protease degradation, only five out of the eight models also showed improved predictive ability in replicating experimentally assessed mutational landscapes of three nonenzyme proteins [36]. Despite rapid progress, accurate AI predictions of mutation effects remain challenging. The most reliable predictions can be expected from workflows that integrate multidimensional experimental data to train AI models for enzyme-specific predictions, followed by rigorous variant filtering with biophysics-based modeling and AI-based zero-shot predictions, to reduce experimental screening efforts, while broadening sequence-space exploration.

### Artificial intelligence methods in cell engineering

Cell engineering involves targeted genetic modifications to engineer specific aspects of cellular function via the control of gene expression or the design of novel genetic circuits (Figure 2). AI-powered algorithms can aid in this process and guide cell engineering for industrial purposes (Table 1).

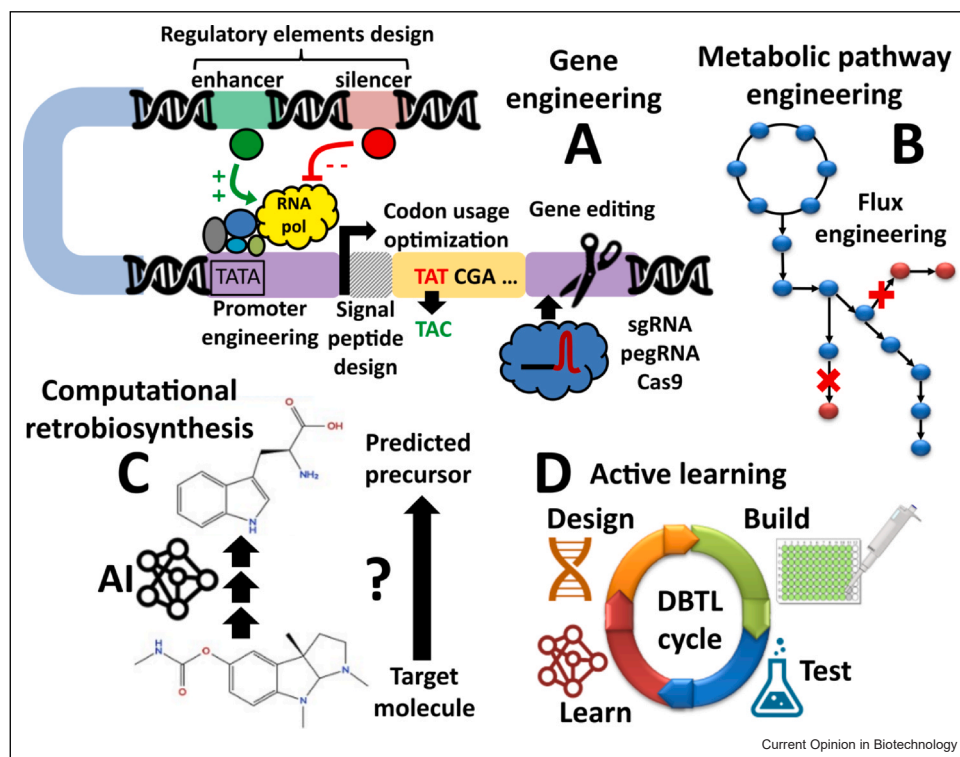
#### Promoter engineering

Promoters are short DNA sequences that recruit RNA polymerases to initiate gene transcription. Engineering them can boost protein expression. Several deep learning methods have been developed for this purpose. DeepSEED [40] combines a convolutional neural network (CNN) model with a generative adversarial network (GAN) to design synthetic promoters in *Escherichia coli* and mammalian cells, 83% of which showed up to 1.6-fold higher activities. However, GANs instability and convergence issues [45] have prompted the need for alternative models. Variational autoencoders and diffusion models paired with CNNs or transformers have been applied for designing efficient promoters in cyanobacteria [41] and *E. coli* [45]. Notably, Du et al.'s diffusion model [45] outperformed GANs in creating functional synthetic promoters in *E. coli*.

#### Optimizing DNA/RNA sequences

Codon optimization boosts protein expression but must find a balance between host codon bias, GC content, and mRNA folding. CodonTransformer [48], a generative transformer trained on >1 M DNA-protein pairs, optimizes sequences by reducing the number of negative regulatory elements. While CodonTransformer remains untested, other deep learning models have been

Figure 2



Engineering targets and approaches used for cell optimization. (a) Cells can be engineered by optimizing DNA sequences involved in the expression or the regulation of specific genes. (b) By engineering BGCs via sequence optimization or gene knock-in/knock-out, metabolic fluxes can be modified to optimize biosynthetic yields or cell growth. (c) *De novo* metabolic pathway design via RBS can be used to introduce new metabolic pathways in cells. (d) Active learning uses DBTL cycles that integrate predictions from theoretical models with iterative HTS to guide strain optimization.

experimentally validated. As an example, Ravi et al.'s recurrent neural network (RNN) model [43] predicts sequences with reduced rare codon usage, raising mRNA expression by 30–40-fold in HeLa cells. CUSTOM [49], a random forest model, predicts optimal codon usage to optimize tissue-specific protein production, which was experimentally validated in two human cell lines. DeepCodon [50], a transformer model, focuses on preserving important rare codons in *E. coli* genes to boost gene transcription. Experimental validation showed that DeepCodon preserved ~90% of functional rare codons and increased the expression levels of two enzymes in *E. coli*. GEMORNA, a large language model (LLM) trained on over 1 million natural protein sequences from 115 mammalian species, optimizes protein expression by 15–41-fold [44] in human cells. Another RNA language model predicts mutations that increase rRNA thermostability, generating ribosomal units that stayed active *in vitro* after a pre-incubation at 65°C [51].

### Engineering protein secretion and translocation

Signal peptides (SPs) are short N-terminal sequences that direct proteins to the secretory pathway or across the

plasma membrane. Engineering SPs can boost recombinant protein secretion and improve protein translocation. Deep learning models [52,53], such as TSignal and USPNet, are currently the most accurate methods for predicting SPs across all domains of life. AlphaFold's ability to model SPs in specific conformations can also be leveraged to filter out false positives predicted by the former methods [54]. Predictions from generative models were also experimentally validated in several studies. For example, optimized SPs generated by the SPgo generative model [55] increased the secretion of recombinant peptides by ~150-fold, while Wu et al. [56] used gradient boosting to design novel SPs with up to 2.9 times enhanced secretion efficiency in *Yarrowia lipolytica*. Additionally, ProtGPS [57] can generate protein sequences with high subcellular localization specificity, as confirmed by cell imaging experiments.

### Engineering gene transcription regulation

Cis-regulatory elements (CREs) regulate gene transcription and can be optimized via deep learning. The CREATor transformer model [39] captures distal CRE patterns up to 2 Mb from target genes across cell types, outperforming prior methods. In addition to improved



**Table 1****Deep learning architectures used for enzyme and cell engineering.**

Architecture	Example of application	Advantages	Drawbacks
Artificial neural networks (ANN)	Metabolic flux modeling [38]	Straightforward training, lower complexity, less risk of overfitting	Poor at modeling positional/long-range correlations in sequences
CNN	Enhancer detection [39]	Good at detecting spatial hierarchies and patterns between features	Computationally expensive, limited modeling of long-range correlations
GAN	Promoter engineering [40]	Can be combined with a CNN for pretraining, generative application	Training instability of GANs, convergence issues, biases in generated data
Variational autoencoders	Promoter engineering [41]	Dimensionality reduction, generative application, robust to model perturbation	Biases in generated data due to compressed latent space representation
Reinforcement learning/active learning	Metabolic pathway optimization [42]	Efficient exploration of design space with experimental feedback	Experiment costs, convergence risk to local optimum, definition of a reward criterion
RNN	Codon optimization [43]	Modeling of sequential and time-dependent information in data	Similar issues as for ANN, gradient vanishing problem
Transformer models/LLM	<i>De novo</i> RNA design [44]	Good at modeling long-range correlations, strong generalization, generative tasks	Computationally expensive, risk of overfitting, requires diverse and large datasets
Diffusion models	Promoter design [45]	Enhanced generative sampling and increased stability compared to GANs	Computationally expensive, can generate biologically unrealistic sequences
GNN	Function prediction [9] Metabolic flux optimization [46]	Good at modeling spatial features and relational information between multimodal data, high interpretability	Memory-intensive training, risk of overfitting, over-squashing problem [47], scalability issues

Source: Adapted from ref. [37].

detection, recent generative models have demonstrated excellent performance in producing functional CREs with *in vivo* activity. DREAM [58], combining a CNN model trained on millions of *Drosophila* enhancers, generated enhancers 3.9 times more active than the strongest natural *Drosophila* enhancer. Another approach [59] used transfer learning to design synthetic enhancers in *Drosophila*, 78% of which showed *in vivo* tissue-specific activity, while CODA [60], a CNN model paired with a simulated annealing algorithm, was developed to design tissue-specific CREs active in mice and zebrafish. Finally, iterative retraining of generative CNNs was successfully used to create ultra-short human enhancers showing improved cell-specificity when tested in different human cell lines [61].

### Engineering gene editing systems

Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9 (CRISPR/Cas9) enables precise genome editing through single guide RNA (sgRNA)-guided Cas9 nucleases. Data-driven models can predict sgRNA binding specificity and design sgRNA with superior activity. DeepCRISTL [62] uses transfer learning to predict on-target activity across cellular contexts and showed consistently higher Spearman correlation coefficients than other competing methods across 13 cellular contexts. For predicting off-target sites, CRISPR-DIPOFF [63] (a RNN model) achieves comparable prediction accuracy to CRISPR-IP [64] (a CNN model), with the latter offering a superior precision-recall balance. For generating

novel sgRNAs, sgRNA Gen [65], a transformer model, learns from AlphaFold3 ipTM scores to create synthetic sgRNAs that showed *in vivo* genome editing activity in *Bacillus subtilis*. Besides this, LLMs [66,67] have also been used to design Cas9 nucleases with improved binding properties. ProGen2 [66], a language model retrained on 1 M CRISPR operon sequences, generated an active Cas9 variant with improved on-target specificity, with a 95% off-target activity reduction when tested in a human cell line.

### Gene discovery and retrobiosynthesis for metabolic pathway design

Identifying biosynthetic gene clusters (BGCs) is crucial for metabolic engineering. Deep learning has improved the detection and classification of BGCs in metagenomes [68]. Large BGCs and enzyme reaction databases provide reaction templates and rules [69] that can be used for computational retrobiosynthesis (RBS). RBS refers to methods that predict plausible enzymatic routes from a target molecule to a precursor. However, rule-based RBS is limited by conservative predictions and convergence issues. Deep learning-based RBS, learning from reaction data without predefined rules, offers better generalization and predictive capabilities [70]. BioRetro [71], combining a generative model with AND-OR tree search, reached 81.6% top-10 accuracy on single-step retrosynthesis prediction tasks and successfully predicted 19 biosynthetic pathways reported in literature. Comparatively, READRetro [72], a dual-representation model, achieved 85.3% prediction success with 72.8%

and 26.6% hit rates for building blocks and pathways, respectively. As an example, READRetro correctly identified *L*-phenylalanine as a building block of benzylacetone. It also predicted the biosynthetic pathways of several classes of secondary metabolites and suggested a possible synthetic pathway for menisdaurilide, a metabolite without a known biosynthetic route [72]. Notably, a recent prompt-driven model [73] showed an improved performance on reaction class diversity, making it easier to explore alternative pathways beyond biases inherited from training data.

### Optimizing metabolic pathways

Metabolic modeling traditionally uses flux balance analysis (FBA) with constrained mechanistic models under pseudo-steady-state assumptions. AI methods can integrate FBA with omics datasets, improving generalization beyond mechanistic constraints. Recent machine learning models [74,75] trained on FBA data identified detrimental reactions in *E. coli* and *Synechocystis*, enabling flux redirection toward growth or CO<sub>2</sub> fixation with strong experimental validation. An enzyme-constrained FBA model [76] (ecMTM) trained on machine-learned  $k_{\text{cat}}$  values accurately predicted *Myceliophthora thermophila*'s substrate utilization hierarchy between five carbon sources, in agreement with experiments. ecMTM also identified potential enzyme targets that could be engineered to enhance the yields of key products, such as malate and ethanol. While these methods are designed for fine-tuning a predefined combination of known parameters, metabolic engineering often requires identifying new parameters to improve over iterative optimization trials. Active learning [77] can address this challenge by using iterative design-build-test-learn (DBTL) cycles that combine strain mutagenesis, HTS, and machine learning classification to identify critical features to optimize until convergence is met. A recent approach [78] successfully increased *p*-coumaric acid yields in *Saccharomyces cerevisiae* by 68%. Multi-agent reinforcement learning [42] offers an alternative for efficient design-space exploration and was used to optimize *L*-tryptophan production in two microbial strains.

### Limitations of artificial intelligence methods

AI methods face significant limitations despite their potential. Models trained on limited datasets specific to particular organisms or conditions may lack transferability. Unmodeled factors like competing pathway kinetics, regulatory constraints, and toxicity effects can reduce the scalability of metabolic models. As exemplified in one study [79], experimental validation of engineering predictions may fail due to ignored parameters in the models. Limited training data availability also affects generative models, which, unless constrained by well-trained classifiers, may generate biologically unrealistic designs or suffer from inadequate sampling

due to mode or posterior collapse [45,80]. Active learning's experimental costs limit its feasibility, favoring methods using small datasets over large neural networks for efficient convergence. Here, however, improper data splitting, such as random splits [38], and class imbalance can limit generalizability and predictive capabilities. The FlowGAT model exemplifies class imbalance issues, showing strong prediction bias toward essential genes, which compromises nonessential gene predictions [46]. These limitations underscore the importance of large, diverse, and well-balanced datasets to ensure reliable biological predictions.

### Conclusion

AI methods are rapidly advancing biotechnology, accelerating enzyme and cell engineering for various use-cases, ranging from high-value fine chemical production with optimized microbial factories to potential applications in biomedicine. Still, the successful integration of AI methods into biotechnological processes remains challenging. More standardization in handling data sparsity and imbalance is crucial to enable the fair evaluation of model reliability and generalizability. Overcoming these challenges is a prerequisite to effectively using AI for identifying or designing novel enzymes, optimizing biocatalytic processes, and building efficient cell factories. Future developments in these fields will be key to facilitating the transition from fossil fuel dependence toward a sustainable global bioeconomy.

### CRediT authorship contribution statement

**Steffen Docter:** Conceptualization, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Benoit David:** Conceptualization, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Holger Gohlke:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

### Data Availability

No data were used for the research described in the article.

### Declaration of Competing Interest

The authors declare that there is no potential conflict of interest.

### Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used the BLABLADOR large language model server provided by the Helmholtz AI Artificial Intelligence Cooperation Unit for editorial purposes. After using this

service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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