Deep learning-based $k_{\text{cat}}$ prediction enables improved enzyme-constrained model reconstruction

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Enzyme turnover numbers ($k_{\text{cat}}$) are key to understanding cellular metabolism, proteome allocation and physiological diversity, but experimentally measured $k_{\text{cat}}$ data are sparse and noisy. Here we provide a deep learning approach (DLKcat) for high-throughput $k_{\text{cat}}$ prediction for metabolic enzymes from any organism merely from substrate structures and protein sequences. DLKcat can capture $k_{\text{cat}}$ changes for mutated enzymes and identify amino acid residues with a strong impact on $k_{\text{cat}}$ values. We applied this approach to predict genome-scale $k_{\text{cat}}$ values for more than 300 yeast species. Additionally, we designed a Bayesian pipeline to parameterize enzyme-constrained genome-scale metabolic models from predicted $k_{\text{cat}}$ values. The resulting models outperformed the corresponding original enzyme-constrained genome-scale metabolic models from previous pipelines in predicting phenotypes and proteomes, and enabled us to explain phenotypic differences. DLKcat and the enzyme-constrained genome-scale metabolic model construction pipeline are valuable tools to uncover global trends of enzyme kinetics and physiological diversity, and to further elucidate cellular metabolism on a large scale.

The enzyme turnover number ($k_{\text{cat}}$), which defines the maximum chemical conversion rate of a reaction, is a critical parameter for understanding the metabolism, proteome allocation, growth and physiology of a certain organism. There are large collections of $k_{\text{cat}}$ values available in the enzyme databases BRENDA$^1$ and SABIO-RK$^2$, which are, however, still sparse compared to the variety of existing organisms and metabolic enzymes, largely due to the lack of high-throughput methods for $k_{\text{cat}}$ measurement. Additionally, experimentally measured $k_{\text{cat}}$ values have considerable variability due to varying assay conditions such as pH, cofactor availability and experimental methods$^6$. Altogether, the sparse collection and considerable noise limit the use of $k_{\text{cat}}$ data for global analysis and may mask enzyme evolution trends.

In particular, enzyme-constrained genome-scale metabolic models (ecGEMs), where the whole-cell metabolic network is constrained by enzyme catalytic capacities and thereby able to accurately simulate the maximum growth abilities, metabolic shifts and proteome allocations, rely heavily on genome-scale $k_{\text{cat}}$ values$^7$. Over the past decade, ecGEMs (or models following the concept of enzyme constraints) have been separately developed for several well-studied organisms$^8$ including Escherichia coli$^9$, Saccharomyces cerevisiae$^{10,11}$, Chinese hamster ovary cells$^{12}$ and Homo sapiens$^{13}$. Due to the limitations of $k_{\text{cat}}$ measurements$^13$ and the reliance on enzyme commission (EC) number annotations to search for $k_{\text{cat}}$ values in those developed pipelines$^{14,15}$, the reconstruction of ecGEMs for lesser-studied organisms or large-scale reconstruction for multiple organisms has remained a challenge$^{7,14}$. Moreover, even for those well-studied organisms, the $k_{\text{cat}}$ coverage is far from complete$^{16,17,18}$. In a S. cerevisiae ecGEM, only 5% of all enzymatic reactions have fully matched $k_{\text{cat}}$ values in BRENDA$^1$. When data are missing, previous ecGEM reconstruction pipelines typically assume $k_{\text{cat}}$ values from similar substrates, reactions or other organisms, which can result in model predictions deviating from experimental observations$^1$. There is a clear requirement for obtaining large-scale $k_{\text{cat}}$ values to improve model accuracy and yield more reliable phenotype simulations$^{15}$.

Deep learning has been applied and shown great performance in modelling chemical spaces$^{16}$, gene expression$^9$, enzyme-related parameters such as enzyme affinity$^17$ and EC numbers$^18$. Previously, Heckmann and colleagues employed machine learning approaches to predict E. coli $k_{\text{cat}}$ values based on features such as average metabolic fluxes and catalytic sites obtained from protein structures$^{19}$. However, such features are typically hard to obtain, which allows the application of this approach only to the most well-studied organisms such as E. coli.

To this end, we developed a deep learning approach (DLKcat) that uses substrate structures and protein sequences as inputs, and demonstrated its capability for the large-scale prediction of $k_{\text{cat}}$ values for various organisms, as well as for identifying key amino acid residues that affect these predictions. We showcased the predictive power of the deep learning model by predicting genome-scale $k_{\text{cat}}$ profiles for 343 yeast/fungi species, accounting for more than 300,000 enzymes and 3,000 substrates. The predicted $k_{\text{cat}}$ profiles enabled reconstruction of 343 ecGEMs for the yeast/fungi species through an automatic Bayesian-based pipeline, which can accurately simulate growth phenotypes among yeast species and identify the phenotype-related key enzymes.

Results
Construction of a deep learning approach for $k_{\text{cat}}$ prediction. The deep learning approach DLKcat was developed by combining a graph neural network (GNN) for substrates and a convolutional neural network (CNN) for proteins (Fig. 1). Substrates were represented as molecular graphs converted from the simplified...
molecular-input line-entry system (SMILES), and protein sequences were split into overlapping n-gram amino acids (the string of contiguous sequences consisting of n items). We generated a comprehensive dataset from the BRENDA and SABIO-RK databases to train the neural network. Incomplete database entries with missing information and redundant entries were filtered out to ensure a dataset of unique entries with substrate name, substrate SMILES information, EC number, protein sequence, organism name and \( k_{cat} \) value. The final dataset contained 16,838 unique entries catalogued by 7,822 unique protein sequences from 851 organisms and converting 2,672 unique substrates (Supplementary Figs. 1 and 2). This dataset was randomly split into training, validation and test datasets by 80%, 10% and 10%, respectively, while five times of random splitting indicated the robustness of the deep learning model.

Deep learning model performance for \( k_{cat} \) prediction. The effects of hyperparameters on deep learning performance were evaluated by learning curves (Supplementary Fig. 4). With the selected optimal parameters \((r\)-radius substrate subgraphs, in which \( r \) is the number of hops from a vertex of substrate structure, 2; \( n \)-gram amino acids, 3; vector dimensionality, 20; time steps in GNN, 3; number of layers in CNN, 3\), the deep learning model was trained. The root mean square error (r.m.s.e.) of \( k_{cat} \) predictions gradually decreased with increasing epoch (Fig. 2a), where one epoch is one iteration of the dataset passing through the neural network. A final deep learning model trained and stored for further use had a r.m.s.e. of 1.06 for the test dataset, signifying that predicted and measured \( k_{cat} \) values were overall within one order of magnitude (Fig. 2a). A high predictive accuracy could be observed on both the whole dataset (training, validation and test datasets) (Fig. 2b; Pearson’s \( r = 0.88 \)) and the test dataset (Supplementary Fig. 5a; Pearson’s \( r = 0.71 \); Supplementary Fig. 5b for test dataset where at least either the substrate or enzyme was not present in the training dataset; Pearson’s \( r = 0.70 \)). The predicted \( k_{cat} \) values were categorized according to the metabolic context of the enzymes (Supplementary Table 1), and enzymes involved in primary central and energy metabolism yielded significantly higher \( k_{cat} \) values than enzymes involved in intermediary and secondary metabolism (Supplementary Fig. 5c), in agreement with previous observations.

The deep learning model was able to show enzyme promiscuity. Understanding enzyme promiscuity and the related underground metabolism is a key topic in evolutionary biology. DLKcat-predicted \( k_{cat} \) values (Fig. 2c) were higher for preferred substrates (median \( k_{cat} = 11.07 \text{s}^{-1} \)) compared to alternative substrates (median \( k_{cat} = 6.01 \text{s}^{-1} \); \( P = 1.3 \times 10^{-12} \)) and random substrates (median \( k_{cat} = 3.51 \text{s}^{-1} \); \( P = 9.3 \times 10^{-4} \)) for promiscuous enzymes in the whole dataset, while the same trend was identified in the test dataset (Supplementary Fig. 5d; \( P < 0.05 \)). The concept of native and underground metabolism could be enriched with the rich experimental \( k_{cat} \) data that are available for human aldo–keto reductase and 61 substrates, where DLKcat could differentiate (Fig. 2d; \( P = 0.0039 \)) between native (top 10% experimental \( k_{cat} \) values, median \( = 2.22 \text{s}^{-1} \)) and underground (last 10%, median \( = 0.04 \text{s}^{-1} \)) substrates.

**Prediction and interpretation of \( k_{cat} \) of mutated enzymes.** Beyond good overall performance (Fig. 2b), DLKcat was able to capture the effects of amino acid substitutions on the \( k_{cat} \) values of individual enzymes. The annotated dataset was divided into wild-type enzymes and mutated enzymes with amino acid substitutions. As the median \( k_{cat} \) of mutant enzymes was lower than that of wild-type enzymes (Supplementary Fig. 6a), the deep learning model was a good \( k_{cat} \) predictor for both wild-type enzymes (Fig. 3a for the whole dataset; Pearson’s \( r = 0.87 \); Supplementary Fig. 6b for the test dataset; Pearson’s \( r = 0.65 \) and mutated enzymes (Fig. 3b for the whole dataset; Pearson’s \( r = 0.90 \); Supplementary Fig. 6c for the test dataset; Pearson’s \( r = 0.78 \)). Several well-studied enzyme–substrate pairs were collected from the literature, where each pair had \( k_{cat} \) values reported for at least 25 unique single or multiple amino acid substitutions (Supplementary Table 2). The predicted and experimentally measured \( k_{cat} \) values correlated very well (Pearson’s \( r = 0.94 \); Fig. 3c). The experimentally measured \( k_{cat} \) values were further grouped as within a 0.5-fold to 2.0-fold change of wild-type \( k_{cat} \) (‘wild-type-like \( k_{cat} \)’ or less than a 0.5-fold change of wild-type \( k_{cat} \) (‘decreased \( k_{cat} \)’). The scarcity of mutated enzymes with \( k_{cat} \) values over twofold of the wild-type \( k_{cat} \) values precluded defining the ‘increased \( k_{cat} \)’ group. DLKcat was able to capture the effects of small changes in protein sequences on the activities of individual enzymes, as the decreased \( k_{cat} \) group contained significantly lower predicted \( k_{cat} \) values compared to the wild-type-like \( k_{cat} \) group, for all enzyme–substrate pairs (Fig. 3d).

To investigate which amino acid residues dominate enzyme activity, we applied a neural attention mechanism to back-trace important signals from the neural network output towards its input. This approach assigns attention weights to each amino acid residue, quantitatively describing its importance for the predicted enzyme activity. Attention weights were calculated for the wild-type \( H. sapiens \) purine nucleoside phosphorylase (PNP) with inosine as substrate, as rich mutation data are available for this enzyme–substrate...
The $k_{cat}$ prediction for 343 yeast/fungi species. We previously reconstructed GEMs for 332 yeast species plus 11 out-group fungi, but only expanded 14 of them to ecGEMs using the original pipeline\textsuperscript{18} due to the limited available $k_{cat}$ data\textsuperscript{44}. As DLKcat allows prediction of almost all $k_{cat}$ values for metabolic enzymes against any substrates for any species, this enabled the generation of ecGEMs for all 343 yeast/fungi species, predicting $k_{cat}$ values for around three million enzyme–substrate pairs (Supplementary Fig. 7). Yeast and fungal specialist enzymes (with narrow substrate specificity) had higher $k_{cat}$ values compared with generalist (that is, promiscuous) enzymes that catalyse more than one reaction in the same species under evolutionary pressure are adapted to have higher $k_{cat}$ values, which is undesirable as in vitro $k_{cat}$ values can be considerably different from in vivo\textsuperscript{45}. To resolve these uncertainties, we adopted a Bayesian genome-scale modelling approach\textsuperscript{32}. Here, we used predicted $k_{cat}$ values as mean values for prior distributions and experimentally measured phenotypes to update these to obtain posterior $k_{cat}$ distributions. For this, experimental growth data on yeast/fungi species were collected, collating 371 entries for 53 species with 16 carbon sources (Supplementary Table 5 and Supplementary Fig. 9). A sequential Monte-Carlo-based approximate Bayesian computation (SMC-ABC) approach\textsuperscript{32} was implemented to sample the $k_{cat}$ values, after validating its generality with the ecGEM of S. cerevisiae, which had the most abundant experimental data (Supplementary Fig. 10). The ecGEMs parameterized with the mean values of sampled posterior $k_{cat}$ values are hereafter represented as posterior-mean-DL-ecGEMs.

The Bayesian learning processes for S. cerevisiae and non-conventional yeast Yarrowia lipolytica are shown as examples (Fig. 4 and Supplementary Fig. 11). We calculated r.m.s.e. values between measurements and predictions for batch and chemostat growth of S. cerevisiae and Y. lipolytica under different carbon sources. After several generations, the ecGEMs parameterized with sampled posterior $k_{cat}$ values achieved a r.m.s.e. lower than one (Fig. 4a and Supplementary Fig. 11a), which showed they could accurately describe the experimental observations. For instance, the S. cerevisiae ecGEM captured the metabolic shift at increasing growth rate (Fig. 4b)—known as the Crabtree effect\textsuperscript{33)—while Y. lipolytica respired at its maximum growth rate (Supplementary Fig. 11b). Principal component analysis for all generated $k_{cat}$ sets (9,800 sets for S. cerevisiae and 4,900 sets for Y. lipolytica) showed a gradual move from the prior distribution to the distinct posterior distribution (Fig. 4c and Supplementary Fig. 11c). The Bayesian learning process affected more variance than mean predicted $k_{cat}$ values (Fig. 4d,e). For S. cerevisiae, 1,057 enzyme–substrate pairs reduced their $k_{cat}$ variance (Šidák-adjusted one-tailed F-test, $P<0.01$), while only 532 pairs changed their mean predicted $k_{cat}$ (Šidák-adjusted Welch’s t-test, $P<0.01$), which were randomly distributed across metabolic subsystems (Supplementary Table 6; two-sided Fisher’s exact test, $P>0.25$). For Y. lipolytica, the values were 1,224 and 646

**Fig. 2 | Deep learning model performance for $k_{cat}$ prediction.** a, The r.m.s.e. of $k_{cat}$ prediction during the training process. b, Performance of the final deep learning model. The correlation between predicted $k_{cat}$ values and those present in the whole dataset (training, validation and test datasets) was evaluated. The brightness of colour represents the density of data points. Student’s $t$-test was used to calculate the $P$ value for Pearson’s correlation. c, Enzyme promiscuity analysis on the whole dataset. For enzymes with multiple substrates, we divided the substrates into preferred and alternative by their experimentally measured $k_{cat}$ value, and then used the predicted $k_{cat}$ values for this box plot. Random substrates were randomly chosen from the compound dataset in our training data, except for the documented substrates and products for the tested enzyme. We evaluated 945 promiscuous enzymes in the whole dataset ($n=945$ for preferred substrates, $n=4,238$ for alternative substrates, $n=945$ for random substrates). d, Comparison of the predicted $k_{cat}$ values for the native substrates and the underground substrates with the human aldo–keto reductase enzyme as a case study. Here, we defined those substrates with the top 10% catalytic ability (experimental $k_{cat}$ value) as the native substrates ($n=6$), while those with the last 10% catalytic ability (experimental $k_{cat}$ value) were considered as the underground substrates as defined in the reference ($n=6$)\textsuperscript{31}. In each box plot (c and d), the central band represents the median value, the box represents the upper and lower quartiles and the whiskers extend up to 1.5 times the interquartile range beyond the box range. A two-sided Wilcoxon rank sum test was used to calculate the $P$ values in c and d.

**Bayesian approach for 343 ecGEM reconstructions.** Using the predicted $k_{cat}$ values for 343 yeast/fungi species, we generated 343 ‘DL-ecGEMs’ (ecGEMs parameterized with $k_{cat}$ values from DLKcat). The training data for the deep learning model were primarily measured in vitro, which implies that DLKcat also predicts in vitro $k_{cat}$ values, which is undesired as in vitro $k_{cat}$ values can be considerably different from in vivo\textsuperscript{45}. To resolve these uncertainties, we adopted a Bayesian genome-scale modelling approach\textsuperscript{32}. Here, we used predicted $k_{cat}$ values as mean values for prior distributions and experimentally measured phenotypes to update these to obtain posterior $k_{cat}$ distributions. For this, experimental growth data on yeast/fungi species were collected, collating 371 entries for 53 species with 16 carbon sources (Supplementary Table 5 and Supplementary Fig. 9). A sequential Monte-Carlo-based approximate Bayesian computation (SMC-ABC) approach\textsuperscript{32} was implemented to sample the $k_{cat}$ values, after validating its generality with the ecGEM of S. cerevisiae, which had the most abundant experimental data (Supplementary Fig. 10). The ecGEMs parameterized with the mean values of sampled posterior $k_{cat}$ values are hereafter represented as posterior-mean-DL-ecGEMs.

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Deep learning and Bayesian approaches improve ecGEM quality. We subsequently generated posterior-mean-ecGEMs from corresponding DL-ecGEMs for all the 343 yeast/fungi species. For comparison, we also built ‘original-ecGEMs’ for the same species with a $k_{\text{cat}}$ parameterization strategy that assigns measured $k_{\text{cat}}$ values from BRENDA$^\dagger$ and SABIO-RK$^\ddagger$ to enzyme/reaction pairs as was done in previous pipelines$^{24}$. We were able to reconstruct original-ecGEMs for all 343 yeast/fungi species only after assuming that orthologs across yeast species had the same EC number annotation as in *S. cerevisiae*. In case of missing data, certain flexibility was introduced by matching the $k_{\text{cat}}$ value to other substrates or organisms, or even introducing wild cards in the EC number. The original-ecGEMs yielded $k_{\text{cat}}$ values for ~40% of enzymes and generated enzymatic constraints for ~60% of enzyme-annotated reactions, while DL-ecGEMs and their derived posterior-mean-ecGEMs covered $k_{\text{cat}}$ values for ~80% of enzymes and defined enzymatic constraints for ~90% of enzymatic reactions (Fig. 5a,b for 343 yeast/fungi species; Supplementary Fig. 12a,b for *S. cerevisiae*). While original-ecGEMs had fewer assigned $k_{\text{cat}}$ values, their reconstruction pipeline also relied heavily on correct enzyme EC number annotations and available measured $k_{\text{cat}}$ values in the databases, contrasting with the DL-ecGEM reconstruction, which relied only on protein sequences and substrate SMILES information while resulting in a higher coverage. In DL-ecGEMs and posterior-mean-ecGEMs the only missing $k_{\text{cat}}$ values were for generic substrates without defined SMILES information (such as generic compounds phosphatidate and thioredoxin).

Besides the improved $k_{\text{cat}}$ coverage, the posterior-mean-ecGEMs and DL-ecGEMs also outperformed original-ecGEMs in the prediction of exchange rates (Fig. 5c for 53 species with reported phenotype; Supplementary Fig. 12c for *S. cerevisiae*) and maximum growth rates under various carbon sources and oxygen availabilities (Fig. 5d and Supplementary Fig. 13 for 53 species with reported growth phenotype; Supplementary Fig. 12d for *S. cerevisiae*). Moreover, we used these three types of ecGEMs to predict required protein abundances and compared this with published quantitative proteomics data from four species with different carbon sources, culture modes and medium set-ups (Supplementary Table 7). Proteome predictions from DL-ecGEMs and posterior-mean-ecGEMs had the lowest r.m.s.e. values, while DL-ecGEMs had already reduced the r.m.s.e. by 30% when compared to original-ecGEMs (Fig. 5c for four species with absolute proteome data). Combined, the current pipeline not only increases $k_{\text{cat}}$ coverage but also contributes to ecGEMs better representing the 343 fungi/yeast species.

The $k_{\text{cat}}$ comparison identifies phenotype-related enzymes. The predicted $k_{\text{cat}}$ values were furthermore able to distinguish between Crabtree positive and negative yeast species. There is much interest in understanding the presence of the Crabtree phenotype among yeast species$^{45,50}$, and a model of *S. cerevisiae* energy metabolism has previously been used to interpret this phenotype by comparing protein efficiency (that is, ATP produced per protein mass per time) in its two energy-producing pathways$^5$. It was postulated that the Crabtree effect is related to the high-yield (HY) pathway (containing the Embden–Meyerhof–Parnas pathway, the tricarboxylic acid (TCA) cycle and the electron transport chain), having a lower protein efficiency than the low-yield (LY) pathway (containing Embden–Meyerhof–Parnas plus ethanol formation; Fig. 6a$^\dagger$). We here used the posterior-mean-ecGEMs of 102 yeast species with experimental reported Crabtree phenotype (25 positive; 77 negative) to similarly calculate the protein efficiencies of the HY and LY pathways.
Of the 102 species, 89% followed the trend that Crabtree positive species have a higher LY efficiency, suggesting that Crabtree positive yeasts’ LY pathways are more protein efficient than their HY pathways for producing the same amount of ATP (Supplementary Table 8). For five commonly studied species, the results are shown in Fig. 6b, and even though ATP yields in their HY pathways may vary across species, primarily due to the presence of respiratory complex I, they still followed the same trend (Supplementary Table 8). Inconsistencies in strains where the HY/LY protein efficiency ratio did not trend with the Crabtree effect might be due to additional regulation not considered in ecGEMs.

With the predicted $k_{cat}$ profiles for yeast species, we could investigate whether key enzymes show different $k_{cat}$ values among 25 Crabtree positive and 77 negative species. Of the enzymes in the energy-producing pathways, only pyruvate kinase, citrate synthase, fumarase and phosphoglucone isomerase had significantly different $k_{cat}$ values (Fig. 6c). Since fumarase and phosphoglucone isomerase can operate in reversible directions, it is unclear how the $k_{cat}$ difference relates to the Crabtree effect. The $k_{cat}$ values of pyruvate kinase were higher in Crabtree positive species ($P = 0.0066$; Fig. 6c). This aligns with the fact that increasing pyruvate kinase activity in the Crabtree positive Schizosaccharomyces pombe increases its fermentation ratio, decreases the growth dependence on respiration and provides resistance to growth-inhibiting effects of antimycin A, which inhibits respiratory complex III (ref. 37). Citrate synthase catalyses the first and rate-limiting step of the TCA cycle, condensing acetyl-coenzyme A and oxaloacetate to citrate. The $k_{cat}$ values of citrate synthase of Crabtree negative species are higher ($P = 0.008$), which would benefit metabolic flux from entering the TCA cycle (Fig. 6a,c). This is consistent with $^{13}$C-metabolic flux analysis that showed that Crabtree negative species have higher TCA flux.

**Discussion**

The diversity of biochemical reactions and organisms makes it difficult to generate genome-scale $k_{cat}$ profiles. Here we presented the deep learning approach DLKcat to predict $k_{cat}$ values of all metabolic enzymes against their substrates, requiring only the substrate SMILES information and protein sequences of the enzymes as input, yielding a versatile $k_{cat}$ prediction tool for any species.

DLKcat can capture $k_{cat}$ changes towards precise single amino acid substitutions, enabling attention weight calculations that identify the amino acid residues majorly impacting enzyme activity. Amino acid substitution is a powerful technique in the enzyme evolution field and routinely used to probe enzyme catalytic mechanisms. Particularly, most substitution experiments perform mutagenesis in the substrate binding site region, since it is hypothesized that the binding region would have a high impact towards catalytic activity. However, it has been reported that remote regions can have a profound impact on catalytic activity. Here, we identified not only high attention weights for amino acid residues in the inosine binding region of human PNP enzyme, but also various non-binding residue sites with high attention weights, suggesting that those residues may also majorly impact catalytic activity and deserve further validation. DLKcat can thereby serve as a valuable part of the protein engineering toolbox.

Predicted genome-scale $k_{cat}$ profiles can facilitate the reconstruction of enzyme-constrained models of metabolism, from both curated and automatically generated basic (non-ec) GEMs. The
Fig. 5 | Evaluation of three ecGEM modelling pipelines including original-ecGEM, DL-ecGEM and posterior-mean-ecGEM reconstruction. 

**a, b.** Enzymatic constraint coverage comparison for enzymes (a) and enzymatic reactions (b) of 343 yeast/fungi species. The r.m.s.e. for the phenotype prediction for 53 species with phenotype data. 

**c, d.** Growth prediction of posterior-mean-ecGEMs for 53 species with phenotype data. 

**e.** Original-ecGEMs were constructed following the pipeline to extract $k_{\text{cat}}$ values from 100 posterior datasets after the Bayesian training process. Culture conditions for the labels on the $x$ axis of those proteome datasets can be found in Supplementary Table 7, and the collected proteome datasets are available in the GitHub repository: sce, S. cerevisiae; kla, Kluyveromyces lactis; kmx, K. marxianus; yli, Y. lipolytica. DL, deep learning-predicted. In the violin plot (a, b and e), white shaded box limits stands for the upper and lower quartiles; the central line limits stands for the 1.5x interquartile range.

Deep learning-predicted $k_{\text{cat}}$ process proved to be a more comprehensive but still practical alternative to matching in vitro $k_{\text{cat}}$ values from the BRENDA and SABIO-RK databases, as is common in original-ecGEM reconstruction pipelines such as the GECKO and MOMENT. By not depending on EC number annotation, DLKcat is not the only kinetic parameter.

Even though the DLKcat-based pipeline yields ecGEMs with superior performance over original-ecGEMs, various challenges remain. For example, while our deep learning model can distinguish alternative from randomly chosen substrates for promiscuous enzymes (Fig. 2c), it still predicts a level of kinetic activity towards random substrates that is likely too high. This behaviour can be explained by the limited availability of negative data: cases where an enzyme–substrate pair did not result in catalysis. Increased reporting of negative datasets, where non-detected activity for enzyme–substrate pairs are reported and collected by enzyme databases, could enhance future deep learning models in terms of defining true negatives. In addition, DLKcat did not consider the effect of environmental factors such as pH and temperature, but combining DLKcat with other emerging machine learning tools, such as for enzyme optimal temperature prediction, would enable future investigation on the impact of environmental parameters on enzyme activities.

Another challenge relates to reactions involving multiple substrates and those catalysed by heteromeric enzyme complexes. The multiple substrate SMILES and protein sequences that can be defined for such reactions can all function with DLKcat, thereby yielding multiple predicted $k_{\text{cat}}$ values for one reaction. We currently select the maximum $k_{\text{cat}}$ values in those cases, but it would be favourable to devise an approach that can predict one $k_{\text{cat}}$ value for each multi-substrate and/or heteromeric enzyme.

In addition, DLKcat-derived DL-ecGEMs and posterior-mean-ecGEMs inherit limitations from basic (non-ec) GEMs, where the steady-state assumption that is central to constraint-based modelling allows one to determine metabolic fluxes but does not readily consider regulatory behaviours. While ecGEMs drastically reduce the solution space of constraint-based models to cellular feasible capacities, $k_{\text{cat}}$ is not the only kinetic parameter that determines reaction rate, as for example, affinity constants play influential roles. However, as constraint-based models cannot predict internal metabolite concentrations, it is currently not feasible to readily consider the influence of those parameters. Nonetheless, $k_{\text{cat}}$ values are also important parameters in other resource allocation models such as proteome-constrained GEMs and metabolism/macromolecular-expression models. Despite improved predictions and more applications, how to define $k_{\text{cat}}$ values has also remained a challenge in the reconstruction of those models. Such resource allocation models and ecGEMs share the assertion that cells need to allocate their limited proteome to different pathways to achieve faster growth or better fitness, while the proteome cost for each reaction is similarly defined by the flux and the kinetic rate of the enzyme. Deep learning-predicted $k_{\text{cat}}$ values for the metabolic parts of those models can therefore improve their quality and performance, although other challenging kinetic parameters, for example, ribosomal catalytic rates, to be determined in those model formulations cannot be obtained from DLKcat. In addition, model formulations that particularly focus on describing enzyme kinetics could benefit from deep learning-predicted $k_{\text{cat}}$ values, so that our DLKcat approach can find a broad application in the modelling field.

In conclusion, we showed that DLKcat yields realistic $k_{\text{cat}}$ values that can be used to direct future genetic engineering, understand
enzyme evolution and reconstruct ecGEMs to predict metabolic fluxes and phenotypes. Besides that, we envision many other possible uses of this deep learning-based $k_{\text{cat}}$ prediction tool, such as a tool in genome mining and Genome-Wide Association Studies analysis. The developed automatic Bayesian ecGEM reconstruction pipeline will be instrumental for further use in ecGEM reconstruction, for omics data incorporation and analysis.

Methods

Dataset preparation for deep learning model development. The dataset used for deep learning model construction was extracted from the BRENDAs and SABIO-RK databases on 10 July 2020 by customized scripts via application programming interface. We generated a comprehensive dataset including the substrate name, organism information, EC number, protein identifier (UniProt ID), enzyme type and $k_{\text{cat}}$ values. As the overall majority of $k_{\text{cat}}$ values reported in BRENDAs and SABIO-RK do not specify their assay conditions, such as pH and temperature, we decided not to include the features in order to maintain the training dataset size and variety. In addition, substrate SMILES, a string notation to represent the substrate structure, was extracted using substrate name to query the PubChem compound database, which is the largest database of chemical compound information and is easy to access.

As different substrates usually have various synonyms in different databases and GEMs, we used a customized Python-based script to ensure that the same canonical SMILES information could be output for the same substrates with various synonyms, which is essential to help filter redundant entries obtained from different databases. Several rounds of data cleaning were performed to ensure quality (Supplementary Fig. 2). Protein sequences were queried with two methods: for entries with UniProt ID, the amino acid sequences could be obtained via the application programming interface of the UniProt with the help of Biopython v.1.78 (https://biopython.org/); and for entries without UniProt ID, the amino acid sequences were acquired from the UniProt$^{48}$ and the BRENDAs$^{57}$ databases based on their EC number and organism information. After that, the sequences of those entries with wild-type enzymes were mapped directly, and the sequences of those entries with mutated enzymes were changed according to the mutated sites. Finally, the remaining entries formed the high-quality dataset for deep learning model construction. Detailed numbers for the data cleaning can be found in Supplementary Fig. 2.

Construction of the deep learning pipeline. In this work, we developed an end-to-end learning approach for in vitro $k_{\text{cat}}$ value prediction by combining a GNN for substrates and a CNN for proteins. The integration of GNN and CNN can naturally be used to handle pairs of data with different structures, that is, molecular graphs and protein sequences. In this approach, substrates are represented as molecular graphs where the vertices are atoms and the edges are chemical bonds, while proteins are represented as sequences in which the characters are amino acids.

For substrates, there are just a few types of chemical atoms (for example, carbon and hydrogen) and chemical bonds (for example, single bond and double bond). To obtain more learning parameters, we employed $r$-radius subgraphs to get the vector representations, which are induced by the neighbouring vertices and edges within radius $r$ from a vertex$^{60}$. First, substrate SMILES information was converted to a molecular graph using RDKIT v.2020.09.1 (https://rdkit.org). Given a substrate graph, the GNN can update each atom vector and its neighbouring atom vectors transformed by the neural network via a nonlinear function, for example, ReLU (ref. 60). In addition, two transitions were developed in the GNN, including vertex transitions and edge transitions. The aim of transitions is to ensure that the local information of vertices and edges is propagated in the graph by iterating the process and summing neighbouring embeddings. The final output of the GNN is a set of real-valued molecular vector representations for substrates.

Similarly, by using the CNN to scan protein sequences, we can obtain low-dimensional vector representations for protein sequences transformed by the neural network via a nonlinear function, for example, ReLU. To apply the CNN to proteins, we defined ‘words’ in protein sequence and split a protein sequence into an overlapping $n$-gram (n = 1, 2, 3) of amino acids$^{61}$. In this work, to avoid low-frequency words in the learning representations, a relatively smaller $n$-gram number of 1, 2 or 3 was set. Then, we translated protein sequences into various word embeddings. Following this, the CNN used a filter function, shown in equation (1), to compute the hidden vectors from the input word embeddings and weight matrix. After that, we obtained a set of hidden vectors for these split subsequences based on $n$-gram amino acid splitting.

$$c_i^{(l)} = f(W_{\text{conv}}c_i^{(l-1)} + b_{\text{conv}})$$

where $f$ is a nonlinear activation function (for example, ReLU); $W_{\text{conv}}$ is the weight matrix and $b_{\text{conv}}$ is the bias vector; and $l$ are the serial numbers of a set of hidden vectors; and $c_i^{(l-1)}$ and $c_i^{(l)}$ are the hidden vectors for the protein sequence.

Other, also important parameters of the neural networks (CNN and GNN) were set as follows: number of convolutional layers in CNN, 2, 3 or 4; number of time steps in GNN, 2, 3 or 4; window size, 11 (fixed); $r$-radius, 0, 1 or 2; and vector dimensionality, 5, 10 or 20. These different settings were explored based on the coefficient of determination ($R^2$) in equation (2) during the hyperparameter tuning to find which hyperparameter is better for improving the deep learning performance. The $R^2$ was calculated by scikit-learn v.0.23.2 (https://scikit-learn.org/stable/). And finally, we used the optimal hyperparameters to train our deep learning model.

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2}$$

where $y_i$ is the predicted $k_{\text{cat}}$ value, $\hat{y}_i$ is the experimental $k_{\text{cat}}$ value, $\bar{y}$ is the average of the experimental $k_{\text{cat}}$ values and $n$ is the total number of items in the dataset (validation dataset or test dataset).

After the acquisition of the substrate molecular vector representations and the protein sequence vector representations, we concatenated them together along
with an output vector \( \mathbf{k}_{\text{cat}} \) to train the deep learning model using the neural attention mechanism. During the training process, all the datasets were shuffled at the first step, and then were randomly split into a training dataset, validation dataset and test dataset at the ratio of 80%:10%:10%. Given a set of substrate–protein pairs and the \( \mathbf{k}_{\text{cat}} \) values in the training dataset, the aim of the training process is to minimize its loss function. The best model was chosen according to the minimal \( \text{r.m.s.e.} \), shown in equation (3), on the validation dataset with the least spread between the training dataset and validation dataset. For building and training models, the PyTorch v.1.4.0 software package was used and accessed using the Python package v.3.7.6 interface under CUDA/10.1.243. In addition, data processing was mainly implemented by NumPy v.1.20.2, SciPy v.1.5.2 and pandas v.1.1.3. Data visualization was implemented by Matplotlib v.3.3.2 and seaborn v.0.11.0.

\[
\text{r.m.s.e.} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (y_i - \hat{y}_i)^2}
\]

where \( y_i \) is the predicted \( \mathbf{k}_{\text{cat}} \) value, \( \hat{y}_i \) is the experimental \( \mathbf{k}_{\text{cat}} \) value and \( n \) is the total number of items in the dataset (validation dataset or test dataset).

**Enzyme promiscuity analysis based on deep learning model.** For enzyme promiscuity, we explored whether the deep learning model can identify substrate preference for promiscuous enzymes. For each promiscuous enzyme, we defined promiscuity, we explored whether the deep learning model can identify substrate preferences in the corresponding enzyme. Using the trained deep learning model, we predicted and compared the \( \mathbf{k}_{\text{cat}} \) values for the preferred, alternative and random substrates on various promiscuous enzymes. In order to identify high-quality promiscuous enzymes, entries with an experimentally measured \( \mathbf{k}_{\text{cat}} \) value less than \( \sim 2 \times 10^{-3} \) in a log, scale were excluded in this analysis.

**Validation of deep learning-based \( \mathbf{k}_{\text{cat}} \) values.** According to the classification of metabolic pathways, metabolic contexts were mainly divided into four different subsystems: (1) primary metabolism (carbohydrate and energy), involving the main carbon and energy metabolism, for example, glycolysis/gluconeogenesis, TCA cycle, pentose phosphate pathway, and so on; (2) primary metabolism (amino acids, fatty acids and nucleotides); (3) intermediate metabolism, related to the biosynthesis and degradation of cellular components, such as coenzymes and cofactors; and (4) secondary metabolism. To explore the metabolic subsystems for all of the wild-type enzymes in the experimental dataset, the module in the KEGG database was used to assign metabolic pathways for enzyme–substrate pairs by linking the detailed metabolic pathway in the KEGG application programming interface with the EC number annotated in each enzyme–substrate pair. Detailed classification can be found in Supplementary Table 1. Using the trained deep learning model, the predicted \( \mathbf{k}_{\text{cat}} \) values were generated for all the enzyme–substrate pairs.

**Interpretation of the reasoning of deep learning.** To interpret which subsequences or residue sites are more important for the substrate, the neural attention mechanism was employed by assigning attention weights to the substrates or residue sites. The neural attention mechanism was employed by assigning attention weights to the subsequences or residue sites that are more important for the substrate, the neural network, as shown in equations (3) and (6). By considering the embeddings of \( \mathbf{y}_{\text{substrate}} \), the attention weight vector for each sequence was accessible in equation (7), which represents the importance signals of the protein sequence towards the enzyme activity for a certain substrate.

**Prediction of \( \mathbf{k}_{\text{cat}} \) values for 343 yeast/fungi species.** The GEMs of 343 yeast/fungi species were automatically reconstructed in our previous paper from a yeast/ fungus pan-GEM, which was derived from the well-curated Yeast8 of *S. cerevisiae* combined with the pan-GEMs. For each model, all reversible enzymatic reactions were split into forward and backward reactions. Reactions catalysed by isoenzymes were also split into multiple reactions with one enzyme complex for each reaction. Substrates were extracted from the model and mapped to the MetaNetX database to get SMILES information using annotated MetaNet identifiers (IDs) for metabolites. Protein IDs for the enzymes were from the model genomes. Protein sequences were queried by the protein ID in the protein FASTA file for each species. Reaction IDs, substrate names, substrate SMILES information and protein IDs were combined as the input file for the deep learning to predict model.

**Analysis of \( \mathbf{k}_{\text{cat}} \) values and \( \text{dN}/\text{dS} \) for yeast/fungi species.** In a previous study, the genomes of 343 yeast/fungi species combined with comprehensive genome annotations were publicly available. The gene-level \( \text{dN}/\text{dS} \) of gene sequences for pairs of orthologous genes from the 343 species were calculated with yn00 from PAML v.4.7 (ref. 64). For this computational framework, the input is the single-copy ortholog groups, and the output is the gene-level \( \text{dN}/\text{dS} \) values extracted from the PAML output files. By mapping the predicted \( \mathbf{k}_{\text{cat}} \) values with the gene-level \( \text{dN}/\text{dS} \) values via the bridge of protein ID, a global analysis was performed between the \( \mathbf{k}_{\text{cat}} \) values and the \( \text{dN}/\text{dS} \) values for 343 yeast/fungi species across the out-group (11 fungal species) together with 12 major clades divided by the genus-level phylogeny for 352 yeast species.

**ecGEM reconstruction.** Besides the constraints in basic (non-ec) GEM, shown in equations (8) and (9), ecGEMs are reconstructed by adding enzymatic constraints, shown in equations (10) and (11).

\[
\begin{align*}
\text{Subject to } & S \times v = 0 \\
& I_b \leq v_j \leq I_{ub} \\
& |E_j| \leq \frac{k_{\text{cat}}^j}{k_{\text{cat}}^j} \times |E_i|
\end{align*}
\]

where \( I_{lb} \) and \( I_{ub} \) are the lower bound and upper bound of the rate for the reaction \( j \).

\[
\begin{align*}
\mathbf{C} &= \{c_1^{(i)}, c_2^{(i)}, \ldots, c_N^{(i)}\} \\
\mathbf{h}_{\text{substrate}} &= f(W_{\text{substrate}} + b) \\
h_i &= f(W_{i\text{out}}c_i + b) \\
\alpha_i &= \sigma(h_{\text{substrate}}^t) \\
\end{align*}
\]
complex, we used the maximum value among all subunits to represent the $k_{\text{cat}}$ for the complex. Subunit protein stoichiometry information was multiplied before comparison. We assumed the same enzyme complex stoichiometry information for yeasts as that of S. cerevisiae, which is collected from the Protein Data Bank in Europe database (https://www.ebi.ac.uk/pdbe/) as well as the Complex Portal (www.ebi.ac.uk/complexportal).

Posterior-mean-ecGEM reconstruction was parameterized by mean $k_{\text{cat}}$ values from accepted posterior distribution. The $k_{\text{cat}}$ values in the DL-ecGEMs combined with the m.s.e. (which in the log scale is the $k_{\text{cat}}$ prediction were used as mean values and variance to make the prior distribution. Each $k_{\text{cat}}$ was described with a log normal distribution $N(k_{\text{cat}}, 1)$. This prior iteratively morphs into a posterior through multiple generations. For each generation, we sampled 126 $k_{\text{cat}}$ datasets within the distribution; 100 among those 126 datasets with a smaller distance (see next section for the SMC-ABC distance calculation) between the phenotype prediction and prediction of the model phenotype were kept to make the distribution for the next generation. Until the distance was lower than the cut-off (m.s.e. for phenotype prediction of 1), we accepted the final distribution as the posterior distribution.

SMC-ABC distance function. Experimental growth data and related exchange rates in batch and chemostat conditions were collected for the yeast/fungi species, which are available in Supplementary Table 5. The distance function was designed as the r.m.s.e. between the simulated and experimental phenotypes. To have a metric for the variance of phenotype prediction of both flux and maximum growth potential, r.m.s.e. was designed in two parts (each part may contain multiple measurement entries such as growth with a different medium). The first part addresses flux predictions in this part checks whether the model predicts similar fluxes when the carbon uptake rate is constrained, as experimentally measured. In this part, all data points for the species are used, and all measured exo-metabolite exchange fluxes are used for comparison. The second part addresses the prediction of the maximum growth rate potential. This part checks the maximum growth rate of the model prediction against the experimental measurement for one species on a certain experimentally tested medium. In this part, only the batch condition with maximum growth rate measurement was tested. No carbon uptake rate or other exchange rate was constrained in the model. Growth maximization was set as the objective function. After simulation, only the maximum growth rate and the carbon uptake rates were used for comparison with measurement.

After running the above two parts of the simulations, the r.m.s.e. for each part can be calculated. All measured and simulated rates were normalized by multiplying the carbon numbers of the corresponding metabolites before calculation of r.m.s.e. The carbon number for biomass is 41 (the mean value for the molecular weight of 1 carbon moles (Cmol) biomass of yeast is 24.42 g (ref. 3)); the biomass equals 1,000 mg). Note that if the substrate or by-product does not contain any carbon, such as $O_2$, then the normalizing number is 1. Then the average r.m.s.e. of both simulations was used to represent the distance. The SMC-ABC search stopped once the r.m.s.e. reached the accepted value or reached the maximum generation. The accepted value for the distance was set to be lower than 1, and the maximum generation was set to be 100.

Simulations with ecGEMs. We performed different kinds of simulations using the ecGEMs, including simulations of growth and protein abundance. Different media and growth conditions were set to match the experiment measurement conditions, for example, using xylose as the carbon source or anaerobic conditions. Since there are no measured total protein abundances in the biomass for all yeast/fungi species, we used the protein content mass to serve as the default total protein abundance for each species and used a factor of 0.5 to serve as the ratio of the metabolic protein to the total protein.

As for the protein abundance simulation, the medium was set to match the experimental condition as mentioned above. For the chemostat condition, the growth rate was fixed as the dilution rate, and the carbon source uptake rate was minimized, which is a normal set-up for the simulation of the chemostat condition. For the batch condition, the growth rate maximization was used as the objective. Then, the simulated protein abundances, which can be extracted from the fluxes, were compared for the difference in the mean values and the variance. Welch’s t-test was used to test the significance for the mean values, while a one-tailed P-test was used for the reduced variances. The cut-off for the significance was set to 0.01 for the adjusted $P$ value corrected by the Šidák method. $P$-VALUE ADJUST (https://github.com/nunofachada/pval_adjust) was used in the analysis.

Proteome data processing. We normalized the collected relative proteome datasets using the identical condition of the absolute proteome data from the literature following the same method as in ref. 44. The reference absolute datasets for those relative proteome datasets were documented in the collected file in the GitHub repository.

Calculation of protein cost and efficiency. To calculate the protein cost of the HY pathway, the glucose uptake rate was fixed at 1 mmolDW$^{-1}$ h$^{-1}$, and the non-growth associated maintenance energy (NGAM) reaction was maximized. The total protein pool reaction was then minimized by fixing the NGAM reaction at the maximized value. The minimized flux through the total protein pool reaction is the protein cost of the HY pathway for converting one glucose to ATP. As for the protein cost calculation of the IY pathway, the glucose uptake rate was fixed at 1 mmolDW$^{-1}$ h$^{-1}$, and ethanol production was maximized. Then the ethanol exchange rate was fixed at the maximized value, and NGAM was maximized. After that, NGAM was also fixed at the maximized value, and the total protein pool was minimized to calculate the protein cost for the IY pathway. We also examined the physicochemical interaction that better characterizes pathways were all inactive during this simulation. Protein efficiency is defined as the protein cost for producing one flux ATP in each pathway.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. Protein sequence FASTA files, deep learning predicted $k_{\text{cat}}$ values, GEMs, original-ecGEMs, DL-ecGEMs and posterior-mean-ecGEMs for 343 yeast/fungi species are available as a supplementary dataset on Zenodo: https://doi.org/10.5281/zenodo.6438262. Collected proteome data are available in the GitHub repository: https://github.com/SysBioChalmers/DLKcat. Databases including BRENDA (https://www.brenda-enzymes.org), SAIBO-RK (http://sabiork.h-its.org/), UniProt database (https://www.uniprot.org/) and PubChem (https://pubchem.ncbi.nlm.nih.gov) were used in the DLKcat model construction. KEGG (http://www.genome.jp/) was used in the evaluation of the DLKcat performance. Databases including the MetaNetX database (https://www.metanetx.org/), the Protein Data Bank in Europe database (https://www.ebi.ac.uk/pdbe/), and the Complex Portal (https://www.ebi.ac.uk/complexportal) were used in the ecGEM reconstruction. The authors declare that all data supporting the findings and for reproducing all figures of this study are available within the paper and its Supplementary Information. Source data are provided with this paper.

Code availability. To facilitate further usage, we provide all codes and detailed instruction in the GitHub repository: https://github.com/SysBioChalmers/DLKcat. A user-friendly example for $k_{\text{cat}}$ prediction is also included in the repository.

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Software and code

Policy information about availability of computer code

Data collection

The dataset used for deep learning model construction was extracted from the BRENDA and SABIO-RK database on 10 July 2020 by customized scripts via Application Programming Interface (API). The whole dataset is available in GitHub repository: https://github.com/SysBioChalmers/DLXcat/tree/master/DeepLearningApproach/Data/database. Literature collected experimental data used in the development of ecGEMs were stored in the supplementary file and the references were stated in the same file.

Data analysis

All code for the analysis and reproducing all figures are included in the GitHub repository: https://github.com/SysBioChalmers/DLXcat. The deep learning model processes uses Python based code, the version for the python packages:

Python v3.7.6 (Anaconda installation recommended)
pyTorch v1.4.0
scikit-learn v0.23.2
Biopython v1.78
RDKIT 2020.09.1
seaborn v0.11.0
Matplotlib v3.3.2
pandas v1.1.3
SciPy v1.5.2
NumPy v1.20.2
dN/dS analysis used the Python based code, the version for the python package:

PAML v4.7

The Bayesian learning approach uses Matlab code, the version for all Matlab packages are listed as below:

MATLAB (R2019b)
The COBRA toolbox for MATLAB [version 3.2].
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Protein sequence FASTA files, deep learning predicted kcal values, GEMs, original-ecGEMs, DL-ecGEMs and Posterior-mean-ecGEMs for 343 yeast/fungi species are available as Supplementary Dataset on the Zenodo: https://doi.org/10.5281/zenodo.5797013. Collected proteome data are available in the GitHub repository (https://github.com/SysBioChalmers/DLkcat/tree/master/BayesianApproach/Data/Proteome_ref.xlsx). All other collected datasets such as the training dataset and the deep learning model were available in the GitHub repository: https://github.com/SysBioChalmers/DLkcat. Databases including BRENDA (https://www.brenda-enzymes.org), SABIO-RK (http://sabiork.h-its.org/) UniProt database (https://www.uniprot.org/), PubChem (https://pubchem.ncbi.nlm.nih.gov) were used in the DLkcat model reconstruction. KEGG (http://www.kegg.jp/) was used in the evaluation of the DLkcat performance. Databases including MetaNetX database (https://www.metanetx.org/), PDB database (https://www.ebi.ac.uk/pdbe/) as well as the Complex Portal (https://www.ebi.ac.uk/complexportal) were used in the ecGEM reconstruction. The authors declare that all data supporting the findings and for reproducing all figures of this study are available within the paper and Supplementary files.

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Sample size

For the deep learning part, the dataset was randomly split into training, validation and test dataset by 80%, 10%, and 10%, respectively. The performance of the deep learning model was evaluated directly from the validation dataset, the testing dataset and the subset of the testing dataset without any assumption of underlying distributions. The sample size in the SMC-ABC part was chosen based on the computational power we have. We chose 126 samples per iteration for parallel purpose.

Data exclusions

In the section of data collection and preprocessing for deep learning model construction, we excluded the incomplete dataset that cannot be used in the deep learning model development. This part is described in the Methods.

Replication

For the deep learning approach, the model training process was repeated twice using the best hyperparameters after hyperparameter tuning to make sure our conclusions could be successfully reproduced. The SMC-ABC part was repeated once without specifying the random seed. All attempts at replication were successful. The conclusions in the manuscript was successfully reproduced. All scripts for reproducibility are included in the GitHub repository.

Randomization

As for the SMC-ABC approach, all values were randomly sampled from the prior distribution without any seed.

Blinding

Not relevant with this computational study, but instead publicly available enzyme kinetics data from the BRENDA and SABIO-RK database was used.

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We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.
### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ | Antibodies            |
| ☒ | Eukaryotic cell lines |
| ☒ | Palaeontology and archaeology |
| ☒ | Animals and other organisms |
| ☒ | Human research participants |
| ☒ | Clinical data         |
| ☒ | Dual use research of concern |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ | ChiP-seq              |
| ☒ | Flow cytometry        |
| ☒ | MRI-based neuroimaging |