Generative Approaches to Kinetic Parameter Inference in Metabolic Networks via Latent Space Exploration

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SUMMARY

Generative machine learning methods that use neural networks to parameterize large-scale and near genome-scale kinetic models have delivered significant efficiency gains in model construction, paving the way toward high-throughput dynamic metabolism studies for biomedical and biotechnological applications. Nevertheless, challenges remain in interpreting the outputs of generative neural networks and developing strategies to quickly adapt these networks to different organisms and physiological contexts without restarting the modeling process from scratch. Here, we present a systematic framework for repurposing generative neural networks trained on one physiological context to build large-scale kinetic models tailored for another context, thereby offering a new avenue for efficiently constructing models with targeted desired properties suitable for various physiological scenarios. We showcase the effectiveness of this method through three case studies: (i) adjusting the response speed of cellular metabolism in aerobic *E. coli* cultures, (ii) improving interpretability by identifying key enzymatic steps that limit the speed of metabolic responses, and (iii) adapting our neural network to capture the distinct dynamic behavior of anaerobic *E. coli*. Given the growing adoption of generative neural networks in biological systems modeling, our approach has the potential to advance personalized medicine and accelerate the high-throughput design of cell factories by streamlining model construction across diverse living organisms.

Keywords: Systems biology, Computational biology, Metabolic networks, Dynamic (kinetic) models of metabolism, Machine Learning, Generative Neural Networks, Latent Space, Escherichia coli.

INTRODUCTION

Metabolism is an inherently adaptive dynamic process that sustains cellular functions and growth despite ongoing changes in the extracellular environment or the cell's internal state. Accurately describing the systems-wide behavior of metabolic processes is essential for advancing biomedical and biotechnological applications, such as the development of personalized therapies[1,2], understanding metabolic shifts between healthy and diseased states[3], engineering cellular factories for biochemical and pharmaceutical production[4,5], and uncovering and harnessing metabolic interactions within microbial communities[6–8]. Driven by recent advancements in omics technologies, modeling techniques, and computational tools, mathematical models play an increasingly important role in this task.[9]

The adaptability of metabolic processes to external and internal changes can be captured by dynamic (kinetic) models, which can depict the evolution of metabolite concentrations, metabolic fluxes, and enzyme concentrations over time. By incorporating information about enzyme kinetics and regulatory interactions, these models provide a quantitative framework to investigate metabolic responses to genetic and environmental perturbations, regulatory dynamics in metabolism, and its intricate interplay with other cellular processes. Dynamic models have demonstrated significant potential in diverse areas, including targeted drug development[10,11], bioproduction[12–14], environmental bioremediation[15], and the study of multi-species microbial ecologies[16]. They are also well-suited for integrating and reconciling diverse biological datasets, including multi-omics data.[14,17,18]

However, our incomplete knowledge of key biochemical and biophysical properties, such as enzyme kinetics, thermodynamics, and regulatory interactions, makes it challenging to determine the parameters behind fundamental dynamics and intrinsic complexities of metabolic processes.[19] Because of the scarcity of available experimentally measured parameters[20,21], considerable efforts have been invested in efficiently parameterizing these models using traditional approaches such as Monte Carlo sampling[22–24], and hybrid or ensemble modeling[25–28]. More recently, it was understood that integrating dynamic models with Machine Learning (ML) approaches can enhance their accuracy, scalability, and robustness.[29–34,17]

Notably, generative ML-based parameterization methods, like REKINDLE[33] and RENAISSANCE[17], have demonstrated efficiency gains of several orders of magnitude over traditional kinetic modeling frameworks. Such generative methods employ neural networks, so-called generators, to parameterize large-scale and near-genome-scale dynamic models with desirable dynamic metabolic properties. Once trained, these generators can be fine-tuned with a low amount of data via transfer learning[35,36] for studying similar physiologies, cell lines, or patients. However, to bring high-throughput dynamic studies into a practical reality, we need to address the following question: how can we repurpose and improve models built for one physiology or satisfy a specific set of model properties for use in studies with entirely different requirements? For example, can we repurpose a generator created to produce models of a wild-type strain to obtain models that capture both wild-type and recombinant physiologies? Or, how can we use a generator initially trained to produce models for a slow-growing strain to

generate models for fast-growing strains? Additionally, while improving model construction efficiency and accuracy is vital, we must go beyond and address the challenge of thoroughly interpreting generator outputs to extract biologically meaningful insights. For example, can these methods provide information useful for designing experimental strategies for biotechnological applications without requiring downstream studies[14]?

A venue to address these challenges is to explore the generator's latent space — an abstract, lower-dimensional representation of the kinetic parameter space created during the training of a generator for the studied physiology (Figure 1a). Latent space encodings of complex datasets are routinely used in many different classes of machine learning algorithms[37,38]. These encodings map multidimensional, often rugged parametric spaces onto simpler, lower-dimensional latent spaces. Machine learning algorithms can produce these nonlinear mappings between the two spaces by training on large datasets from the system of interest. These mappings can then be used to sample the latent space and generate new instances from the dataset's parametric space. Recent efforts[39–41] have focused on shedding light on the 'black box' nature of these advanced ML algorithms, with significant attention given to understanding how the features (attributes) of high-dimensional data are distributed within their latent spaces. Some studies have shown that features of complex datasets can be linearly decoupled in latent space[42,43]. This decoupling has been used to guide and control the generation process through regression-based[44] or semantic techniques[40], allowing the generation of new data points with specific or conditional features[33,45], or a weighted sum of features not present in the original dataset[43]. This means that the latent space of machine learning algorithms allows for the model reduction of complex systems and provides more flexible control of the system's properties.

In this study, we present a framework that leverages the reduced size and structured landscape of the generator's latent spaces to produce dynamic models tailored to the studied physiology (Figure 1). The framework begins by employing state-of-the-art generative ML methods, such as REKINDLE or RENAISSANCE, to create a generator that maps a simple Gaussian latent space to the relevant subspace of kinetic parameters constrained by integrated reference data (Figure 1b). Latent space perturbations are then nonlinearly transformed into the parametric space through the trained generator, producing novel kinetic parameter sets with distinct dynamic responses and properties (Figure 1c, Supplementary Note 1). This framework offers a systematic and efficient approach to exploring the dynamic properties of metabolic systems. We demonstrate the framework's versatility through three applications: (I) Fine-tuning dynamic response times in aerobically grown *E. coli*; (II) Identifying dynamic bottlenecks at the enzyme level in aerobically grown *E. coli*; (III) Repurposing trained neural network generators to create dynamic models meeting the distinct dynamic requirements of anaerobically grown *E. coli* (Figure 1d).

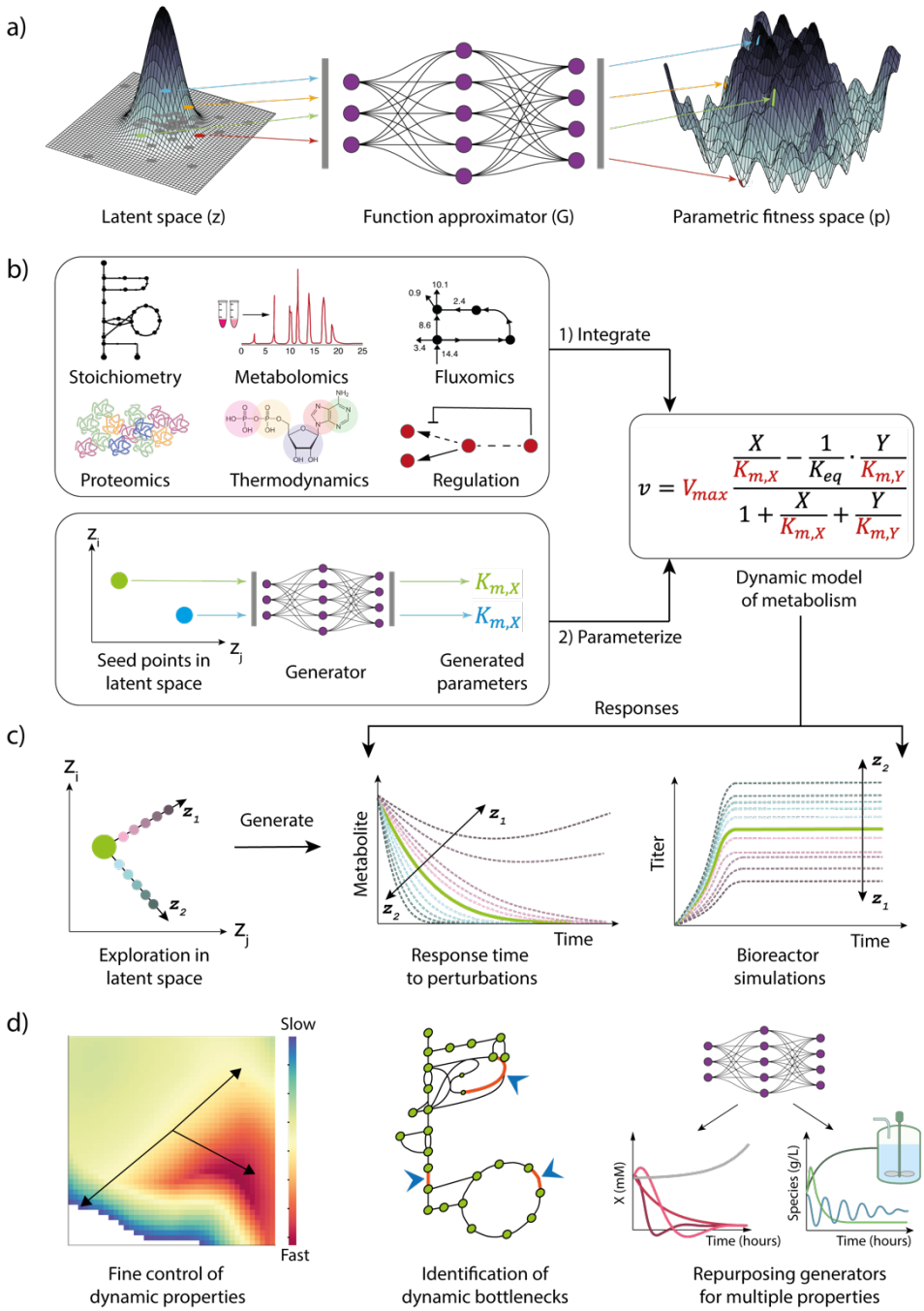

Figure 1. The framework for exploring the dynamic landscapes of metabolism through latent space ***(a)*** *Nonlinear function approximators, e.g., generative neural networks (G), map a complex parametric space (p) to a simpler latent space (z).* ***(b)*** *Generative learning-based methods produce kinetic parameters that satisfy desired dynamic properties, after relevant quantitative and structural information has been integrated.* ***(c)*** *Exploring around a latent seed point of a kinetic parameter set allows for generating novel parameter sets giving rise to dynamic models with distinct dynamic properties* ***(d)*** *A few applications of the methodology presented in this paper.*

RESULTS

Navigating dynamic properties of metabolic networks

Metabolic networks are characterized by their complex topology and intricate dynamics, with metabolic reactions from different pathways operating across a range of time scales.[46,47] Reactions in glycolysis, the pentose phosphate pathway, the citric acid cycle, and the electron transport chain operate with short time constants. Conversely, protein synthesis, DNA replication, and RNA transcription exhibit longer time scales, reflecting the time-intensive process of their molecular assembly. A fundamental requirement of dynamic models based on mechanistic rate laws is to capture such dynamic properties of metabolism while aligning with experimental observations.[14,17] To meet this requirement, we explored the latent space of generator neural networks and investigated how to systematically create biologically relevant dynamic models — models that capture the time scales and behaviors of metabolic processes (Methods). We quantified the dynamics of the parameterized kinetic model using the dominant time constant, τ_{max}. The dominant time constant corresponds to the slowest decaying mode in a system's response, meaning this time constant has the greatest impact on the long-term evolution of the system dynamics (Methods).

To this end, we used the REKINDLE framework to train generators that produce biologically relevant dynamic models of *E. coli* central carbon metabolism, parameterized by 259 independent kinetic parameters (Methods, Supplementary Figures 3, 4). After training, we selected a generator with a high incidence of biologically relevant models to generate 100 kinetic parameter sets, 80 of which were biologically relevant, using a 127-dimensional Gaussian vector as the latent input. We randomly chose one of the 80 models and explored how modifying its corresponding latent input affects the dynamic properties of the resulting parameterized metabolic network (Figure 1c). As a first step, we increased each latent feature by 50% individually and assessed its impact on the resulting generated kinetic parameter set. We found that even a single latent feature change altered multiple kinetic parameters due to the nonlinear behavior of the neural network (Figure 2a, Methods). Some latent features had a more pronounced effect on the model than others, whereas some did not impact the kinetic parameters at all. This suggests that certain latent features may encode nonlinearities or key structural properties of the metabolic network, with only a subset playing a significant role in shaping kinetic parameters.

Next, we examined how the dominant time constants, τ_{max}, of the parameterized system change as we alter each latent feature. We found that altering each latent feature affected τ_{max} (Figure 2b). Some changes made the model faster (τ_{max} decreased), while others made it slower (τ_{max} increased). We also tested different levels of latent feature modification (10%, 25%, and 50%) and observed the change in τ_{max} with the modification magnitude (Supplementary Fig 5). Interestingly, the effect on τ_{max} reversed when latent features were decreased by 50% compared to when they increased by 50% (Supplementary Fig 6). For example, increasing latent feature 22 by 50% decreased τ_{max} by 14%, while decreasing it by 50% increased τ_{max} by 13%.

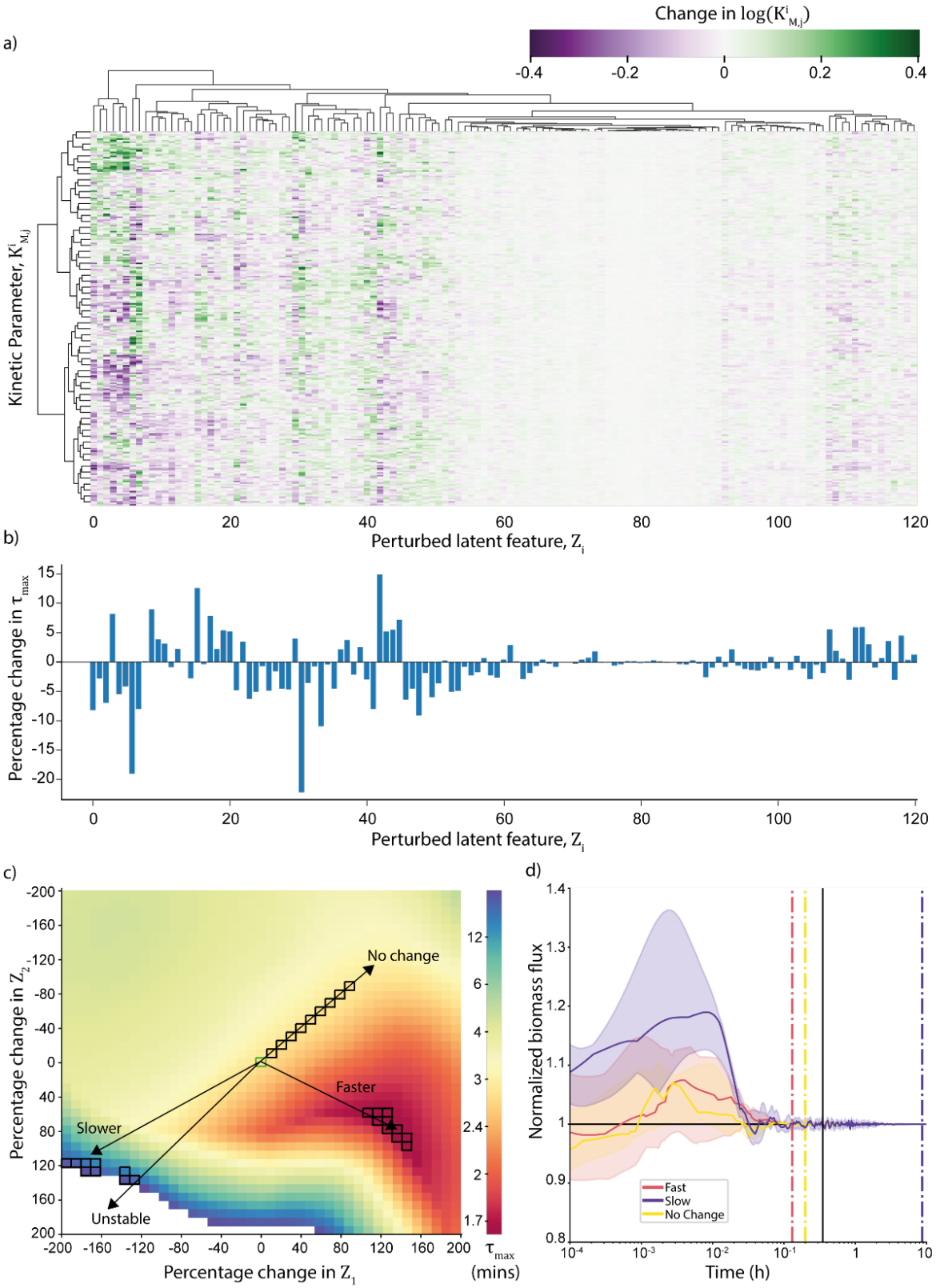
a)
Change in log(K^i_M,j)
-0.4
-0.2
0
0.2
0.4
Kinetic Parameter, K^i_M,j
0
20
40
60
80
100
120
Perturbed latent feature, Z_i
b)
Percentage change in τ_max
15
10
5
0
-5
-10
-15
-20
0
20
40
60
80
100
120
Perturbed latent feature, Z_i
c)
Percentage change in Z_2
-200
-160
-120
-80
-40
0
40
80
120
160
200
No change
Faster
Slower
Unstable
12
6
4
3
2.4
2
1.7
τ_max
(mins)
-200 -160 -120 -80 -40 0 40 80 120 160 200
Percentage change in Z_1
d)
Normalized biomass flux
1.4
1.3
1.2
1.1
1
0.9
0.8
Fast
Slow
No Change
10^-4
10^-3
10^-2
10^-1
1
10
Time (h)

Figure 2. Latent space augmentation enables control of metabolic network dynamics ***(a)*** *Change in all log-transformed K_M values from the original model when all 127 latent features are increased by 50% (Methods).* ***(b)*** *Corresponding percentage change in τ_{max} from original model when all 127 latent features are increased by 50% one by one.* ***(c)*** *τ_{max} values of the resulting models when the 2 most effective latent features which decreased τ_{max} are modified between ±200% from the original model simultaneously.* ***(d)*** *Dynamic responses of normalized Biomass flux of chosen models from Figure 2c (black squares). The black line indicates the doubling time of wild-type E. coli. Color-dashed lines represent time points when the median response returns within 5% of the reference steady-state value.*

This reversal was consistent across all latent features, suggesting that modifying latent features in a neural network-generated kinetic model enables easy control of dynamic responses.

To further investigate this observation, we selected the two latent features with the highest impact on τ_{max} (Figure 2b), which make the model dynamics faster. We perturbed these features simultaneously, varying them between ±200% in 5% increments, and analyzed τ_{max} of the resulting 1681 models (Figure 2c). The results indicate that the dynamics of the models change based on different combinations of perturbations — some becoming slower, others faster, some exhibiting local instability, while some remaining unchanged — depending on the direction within the 2-dimensional latent space (Figure 2c). To validate this, we selected the 20 fastest, 20 slowest, and 20 models with no dynamic changes from the 1681 models (Figure 2c, squares). We perturbed the steady metabolite concentrations by up to ±50% and analyzed the response times of the selected models (Methods). The non-linear responses confirmed the conclusions obtained by analyzing the dominant times. The fastest models, achieved through latent space manipulation, settled back to the reference steady state of biomass flux much quicker (7.8 mins, Figure 2d, red dashed line) compared to the models with no dynamic changes (11.9 mins, yellow dashed line) and the slowest models (522.5 mins, purple dashed line). Similar trends were observed for ADP, ATP, and NADH concentrations (Supplementary Figure 7). This demonstrates that simple and systematic transformations in the latent space of a trained generator can reliably generate models with diverse dynamic properties and bias these properties towards specific requirements.

Discovering dynamic bottlenecks in metabolic networks

Altering individual latent features provides systematic and precise means to control the dynamic properties of a metabolic model. Yet, for biological discovery, it is essential to identify which specific kinetic parameters dictate the dynamic behavior of the metabolic network, particularly τ_{max}, for a given parameterized model. To identify these kinetic parameters, we first examined how individual kinetic parameters responded to changes in the two most important latent features (Figure 2c) and found that all parameters varied smoothly with these modifications (Supplementary Figure 8). Next, we computed correlations between τ_{max} and all 259 individual kinetic parameters, $K_{M,i}^{j}$, across the 1681 models from Figure 2c (Methods). Many kinetic parameters showed strong correlation or anti-correlation with τ_{max} (Figure 3a, left), whereas such strong correlations were absent for models

generated through random sampling in the latent space (Figure 3a, right). This difference arises because modifying key latent features with the highest impact on τ_{max} alters the kinetic parameters that govern τ_{max} in a systematic manner, whereas random sampling does not ensure proximity in parameter space, leading to arbitrary variations in τ_{max} values.

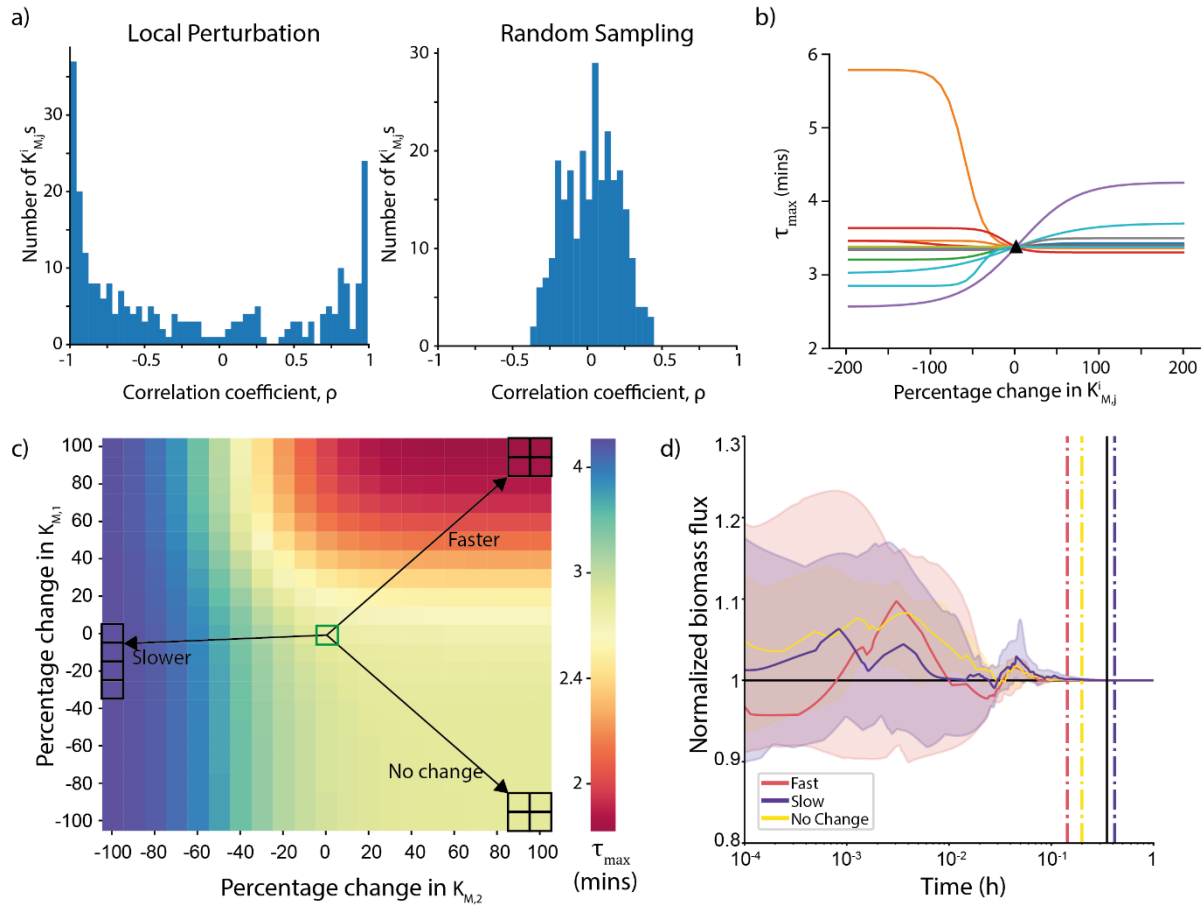

Figure 3. Enhancing interpretability at the parametric level with latent space augmented datasets ***(a)*** *Spearman Correlation coefficients between τ_{max} of the parameterized system and all 259 kinetic parameters (left) of all 1681 models obtained in Figure 2c (right) when 100 models are randomly sampled from the generator* ***(b)*** *τ_{max} values when 10 highest correlated/anti-correlated) parameters from 3a are discretely perturbed from the values of the original model. Black triangle represents τ_{max} the original model.* ***(c)*** *τ_{max} of resulting models when the top 2 parameters with highest impact on τ_{max} in 3b are perturbed simultaneously from the original model.* ***(d)*** *Dynamic responses of normalized biomass flux of chosen models from Figure 2c (black squares). The black line indicates the doubling time of wild-type E. coli. Color-dashed lines represent time points when the median response returns within 5% of the reference steady-state value.*

Next, we selected the 10 kinetic parameters with the strongest correlation or anti-correlation with τ_{max} and individually varied them between ±100% in 10% increments to observe their impact on the τ_{max} values. Notably, not all strongly correlated parameters had the same impact on τ_{max} (Figure 3b). This is because some parameters, despite their correlation, do not directly affect τ_{max} and are inherently

‘sloppy’ with respect to the system’s dynamics. Their correlation arises from the non-linear mapping of the generator neural network between the latent space and the parametric space, which enforces proportional changes in these kinetic parameters when modifying the selected latent features.

Based on this analysis, we identified the two parameters with the highest impact on τ_{max} by measuring the total deviation from the original model’s τ_{max} (Figure 3b, black triangle), filtering out sloppy parameters that vary widely without affecting τ_{max}. We then perturbed these two parameters, $K_{M,nadph}^{ME2}$ and K_{M,mal_L}^{MDH}, simultaneously between ±100% in 10% increments and examined the resulting τ_{max} values (Figure 3c). Similar to the results in Figure 2c, we found that modifying only these 2 out of 259 parameters significantly changed τ_{max}, thereby affecting the overall dynamic response time of the entire metabolic network.

To validate this finding, we again performed nonlinear integration of the 20 fastest, 20 slowest, and 20 models with no dynamic changes by perturbing the reference metabolite concentrations by up to ±50% and analyzing their response times (Figure 3c, squares). The fastest models returned to the reference steady state of biomass flux much sooner (8.6 mins, Figure 3d, red dashed) compared to the models whose dynamics did not change (11.9 mins, yellow dashed), and the slowest models (24.9 mins, purple dashed). Similar trends were observed for ADP, ATP, and NADH concentrations (Supplementary Figure 9), demonstrating that latent space manipulation enables direct identification of the key parameters governing a model’s dynamic properties. In this case, $K_{M,nadph}^{ME2}$ and K_{M,mal_L}^{MDH} primarily control τ_{max} of the parameterized dynamic model.

Since kinetic parameters are inferred to match experimentally measured steady-state metabolite concentrations and fluxes, one can yield different sets of kinetic parameters for different reference steady states. Consequently, $K_{M,nadph}^{ME2}$ and K_{M,mal_L}^{MDH} do not necessarily control τ_{max} in every model, as the dynamic properties of metabolic networks arise from complex interdependencies among kinetic parameters. Thus, for each set of kinetic parameters and the corresponding reference state of concentrations and fluxes, different parameters may emerge as key controllers of τ_{max}. To address this, we next investigated whether certain parameters or enzymes consistently act as dynamic bottlenecks in *E. coli* central carbon metabolism both within models satisfying a given steady state and across models from multiple steady states.

To this end, we sampled four different thermodynamically feasible reference profiles of fluxes and metabolite concentrations using Thermodynamic Flux Balance Analysis[48] (Methods). For each reference state, we employed REKINDLE to obtain generators providing biologically relevant dynamics. We then generated 100 models per state and selected relevant models with τ_{max} ≤ 6.67 mins. Finally, for each relevant model, we repeated the analysis from Figures 2 and 3, where we: (i) perturbed latent space features individually, (ii) identified those with the highest impact on τ_{max}, and (iii) determined the top 3 kinetic parameters using correlation and filtering from the resulting set of models. We focused on the top 3 parameters, as those beyond them had a negligible impact on τ_{max} in most cases (Figure 3b). We then identified enzymes whose associated kinetic parameters

consistently affected τ_{max} across all steady states, with key reactions in the TCA cycle and glycolysis playing a dominant role (Supplementary Figure 10). Indeed, kinetic parameters associated with Pyruvate Dehydrogenase (PDH), Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), and Citrate Synthase (CS) primarily determined τ_{max} across the network (Figure 4a).

To substantiate this result, we modified the kinetic parameters of these 3 enzymatic reactions one at a time and evaluated the τ_{max} of the resulting models. We found that changing specific parameters, such as $K_{m,g3p}^{GAPD}$, $K_{m,cit}^{CS}$, and $K_{m,nad}^{PDH}$, consistently affected the τ_{max} of all models across all steady states. In contrast, alterations to other parameters within the same metabolic reactions had little to no effect (Supplementary Figure 11). This suggests that $K_{m,g3p}^{GAPD}$, $K_{m,cit}^{CS}$, and $K_{m,nad}^{PDH}$, act as global dynamic bottlenecks, regulating τ_{max} of the studied *E. coli* physiology. We also examined if such important enzymes were co-occurring for any particular steady state profile, but we did not find strong evidence of it (Supplementary Note 2).

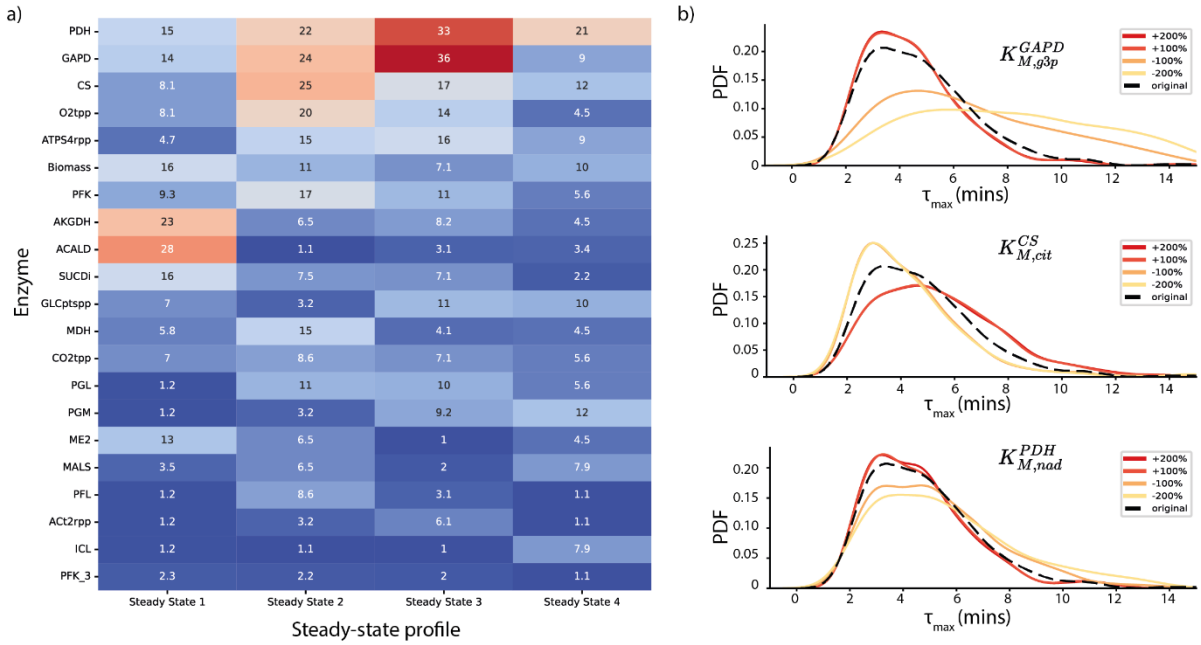

Figure 4. Discovering general dynamic bottlenecks in the central carbon metabolism of *E. coli* (a) *Occurrence of enzymes (in %) whose associated kinetic parameters significantly affect τ_{max} of the E. coli central carbon metabolism across 4 different steady-state profiles. Red color denotes high occurrence, while blue indicates low occurrence.* ***(b)*** *Distribution of τ_{max} across models when $K_{m,g3p}^{GAPD}$ (top), $K_{m,cit}^{CS}$ (middle) , and $K_{m,nad}^{PDH}$ (bottom) are perturbed from their original values by different magnitudes (colored lines). The black dashed line represents the τ_{max} distribution of the original, unperturbed models.*

Repurposing generators for multiple dynamic properties

Dynamic models of metabolism often need to satisfy multiple dynamic requirements to facilitate applications in systems and synthetic biology design and testing. One common application of dynamic

models is in predicting cellular responses in bioreactors[50], where cells are used as 'biofactories' to produce a non-native compound or overproduce a native compound. Kinetic models need to accurately capture experimental fermentation curves in terms of growth, yield, and titers before being used for optimizing strain design. A recent study[14] showed that only a small fraction of models obtained by traditional sampling-based kinetic modeling frameworks (212 out of 91,852 models, i.e., 0.23%) successfully capture dynamic properties of metabolism and experimental fermentation data. Moreover, this fraction decreased even further when sampled models were tested on different recombinant strains in addition to the wild-type physiology. Therefore, deriving models that simultaneously satisfy multiple dynamic properties or multiple physiologies requires considerable computational resources and systematic downstream processing. Thus, we aimed to leverage latent space manipulation to repurpose trained generators – originally designed for a single dynamic property – to construct models that satisfy multiple dynamic requirements.

Building on this approach, we demonstrate how generators trained to produce models with valid τ_{max} can be repurposed to generate models that satisfy specific fermentation data for anaerobic *E. coli* using latent space manipulation. We used RENAISSANCE[17] to train generator neural networks to generate models with valid linearized dynamics for the anaerobic *E. coli* model (Methods, Supplementary Figure 12). Selecting the generator with the highest incidence of valid models (30%), we generated 1000 models. After integrating initial experimental conditions, we performed bioreactor simulations on the 300 valid models (Methods). While we observed a range of responses, none of the 300 models perfectly fit the experimental data (Figure 5a). This was expected, as the generator was not explicitly trained to produce models satisfying this specific fermentation dataset.

To obtain models that more closely fit the experimental data, we selected one that approximated the observed trends but exhibited lower final biomass titers and a faster glucose uptake rate (red dashed line, Figure 5a). Using its corresponding latent space input, we investigated whether latent space manipulation could generate models with improved agreement. To quantify the goodness of fit, we calculated the average deviation (E) between simulated responses and experimental data, following Varma et. al[49] (Methods). The average deviation of the chosen model (E^*) was $E^*_{biomass}$= 0.011 g/l and E^*_{glc}= 0.753 mM). As a first step in exploring latent space, we modified each of the 99 latent features of the chosen model one at a time. Each feature was perturbed between ±100% in 1% increments, resulting in 101 modified models per feature. We then simulated the bioreactor responses for all modified models and analyzed the final biomass titers. Consistent with previous findings, perturbing certain latent features significantly impacted the final biomass titers, while most had little to no impact (Figure 5b, Supplementary Figure 13). However, unlike in previous studies, we observed that some models became locally unstable upon latent feature perturbation.

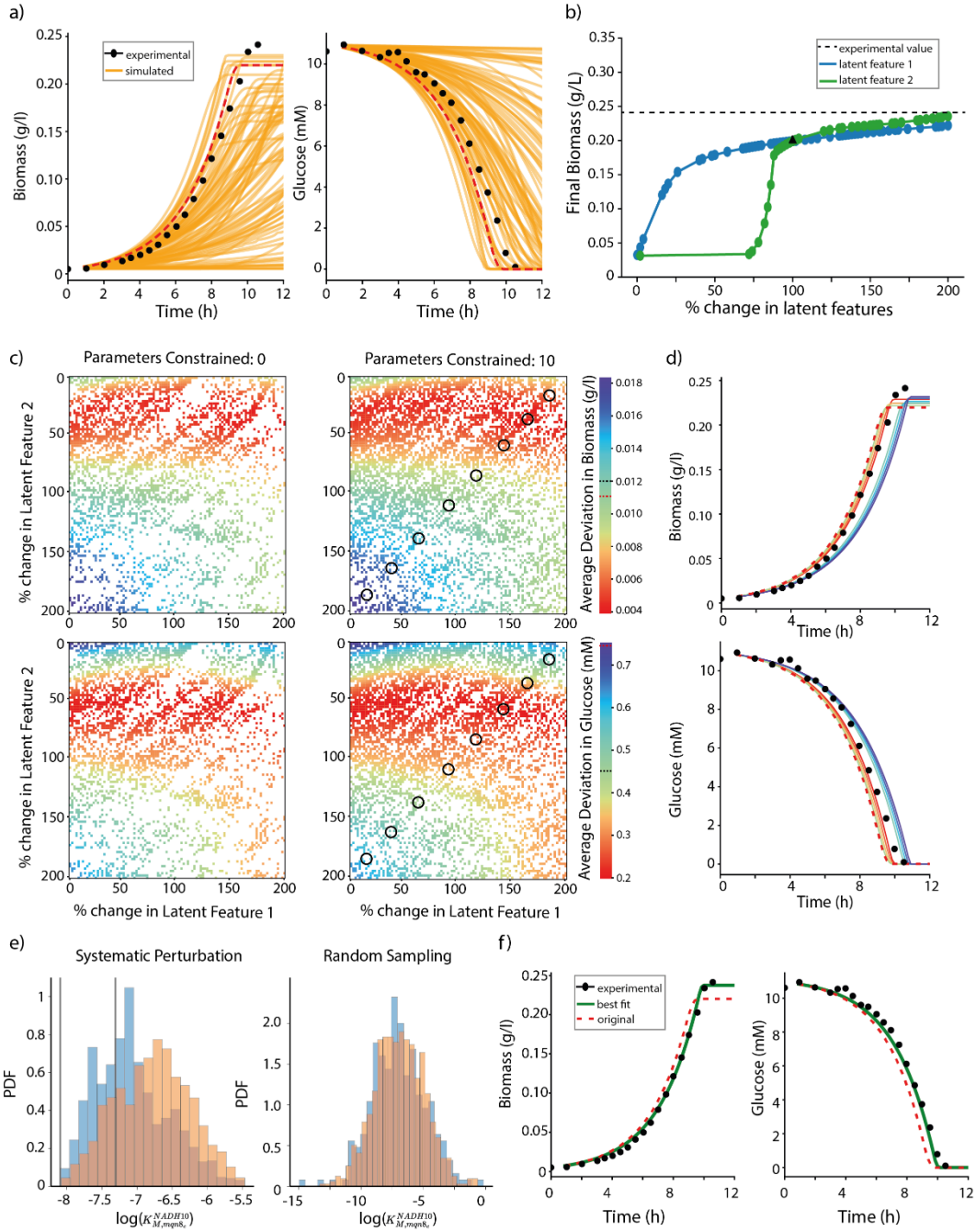

Figure 5: Repurposing generators to enforce multiple dynamic properties of metabolic models ***(a)*** *Bioreactor responses of 300 randomly sampled models generated by a neural network trained for relevant linearized dynamics of anaerobic E. coli metabolism for Growth or Biomass (left) and Glucose (right). The response marked in dashed red is chosen for further studies* ***(b)*** *Final biomass titers of model responses when two most important latent features are perturbed from their original values. The black triangle represents the final biomass titer of the original model (red line from Figure 5a)* ***(c)*** *(right) Average deviations of simulated responses of biomass (top) and glucose (bottom) from experimental bioreactor datapoints when the two latent features in Figure 5b are perturbed simultaneously. (left) Average deviations of simulated responses of biomass (top) and glucose (bottom) from experimental bioreactor data points when the two latent features in Figure 5b are*

perturbed simultaneously, and 10 parameters are constrained, as explained in Figure 5d. The dashed black line on the color bar represents average deviations of predicted responses for the same system as obtained by Varma et al[49]*.* ***(d)*** *Distributions of the parameter* $K_{M,mqn8}^{NADH10}$ *in generated models when models are obtained through: systematic perturbation of the latent point of the model in Figure 5a (top) and random sampling of the latent space of the trained generator (bottom). Gray vertical lines in the top panel represent the 30th percentile values for this parameter that favors stability. NADH10: NADH dehydrogenase (Menaquinone-8, 0 protons), mqn8_c: Menaquinone-8.* ***(e)*** *Bioreactor responses of 8 models sampled from Figure 5c, Right panel, black dots. The responses are colored according to the corresponding sampling space as mapped in the color bar in Figure 5c. The red dashed line represents the original model response.* ***(f)*** *The bioreactor response of the best model (green) obtained through latent space exploration, and the original model response (red dashed).*

Given that fitting the biomass titers was more difficult than the glucose uptake rate, we selected the two latent features with the highest impact on the biomass titers (Figure 5b). Since increasing these two features also increased final biomass titers, we perturbed them between 0 and 200% of their original values in 1% increments (Figure 5b). Among the generated 10201 new models, 29% had valid τ_{max} (Methods). We then performed bioreactor simulations for the valid models and evaluated their average deviations from experimental values (Figure 5c, left). Even though 71% of the models were unstable, we observed distinct trends in goodness-of-fit depending on perturbation levels in the selected latent features.

Next, we investigated whether the instability of perturbed models could be reduced by comparing the distributions of kinetic parameters from two sources: (i) random sampling of the generator's latent space (1,000 models, Figure 5a), and (ii) systematic perturbation of latent space inputs (10,201 models, Figure 5c). We found that parameters derived from systematic perturbation provided better delineation between stable and unstable models than those obtained through random sampling (Supplementary Figure 14). An example of this delineation for $K_{M,mqn8}^{NADH10}$ is shown in Figure 5d. To quantify these differences, we ranked all 478 kinetic parameters by measuring the divergence between stable and unstable model distributions using Jensen-Shannon (JS) entropy (Methods). The 10 parameters with the highest distribution differences were constrained within ranges that favor stability in the 10201 perturbed models (Figure 5d, vertical black lines). Constraining these 10 parameters increased the local stability incidence from 29% to 47% (Figure 5c, left) and improved the resolution of the original perturbed set of models (Figure 5c, right).

We then repeated this procedure by constraining the top 20, 50, 100, 150, and 200 parameters, and observed that local stability increased up to 60% (Supplementary Figure 15). With improved stability through latent space analysis, we next examined the bioreactor responses of the constrained models (Figure 5c, right; Supplementary Figure 16, 17). Many constrained models exhibited a better fit than the original unperturbed model (Supplementary Figure 16, 17). Indeed, constraining just 10 parameters not only yielded better fitting models but also introduced a range of responses with different dynamics and final biomass titers, enabling finer tuning (Figure 5e). Increasing the number of constrained parameters further improved model performance, with the best-fitting model obtained by

constraining 200 parameters, achieving an average deviation E^{best}, where $E^{best}_{biomass} = 0.004$ g/l and $E^{best}_{glc} = 0.330$ mM, representing a 63% and 56% improvement in goodness-of-fit for biomass and glucose predictions, respectively, compared to the original model. These findings demonstrate that the latent space of neural networks can be leveraged to repurpose generators trained for a specific dynamic property, enabling them to satisfy additional constraints without requiring explicit retraining.

DISCUSSION

Kinetic models of metabolism allow us to integrate and consolidate data from cellular processes and predict transient and steady-state metabolic responses in a quantitative and deterministic manner. This capability makes them invaluable tools for numerous applications in biomedical and biotechnological sciences. Recently developed data-driven generative methods, such as REKINDLE{Citation} and RENAISSANCE, have significantly improved the efficiency of kinetic model parameterization, enabling dynamic studies across multiple physiologies and large patient cohorts. As the applications of these methods will inevitably extend to other domains of biotechnology and health, research efforts in this field would greatly benefit from the ability to repurpose generative neural networks designed for a specific physiology and/or organism to other studies. This would allow researchers to study new physiologies and organisms without undergoing the lengthy modeling process again. The methodology proposed in this paper aims to address this challenge by using the latent space of neural networks employed in these advanced methods to repurpose generators and allow them to encompass diverse dynamic properties while enhancing interpretability.

As computational and modeling techniques continue to advance, in silico models will increasingly align with real biological systems, ultimately evolving into digital replicas of cellular processes. These models will provide fundamental insights while reducing reliance on extensive experimental studies. Generative neural networks inherently encode higher-level or emergent system properties in their latent space during training. By manipulating these latent embeddings, we can systematically access and control these properties. Moreover, latent space encodings facilitate model reduction by decreasing the dimensionality of the parametric space, enabling fine-tuned control over emergent dynamic properties using simple linear transformations while maintaining reference steady-state conditions. These transformations generate structured datasets that are well-suited for statistical analysis, allowing researchers to identify key system parameters or parameter combinations that govern network-level properties—an approach not feasible with conventional random parameter sampling in kinetic model parameterization.

We demonstrate how latent space exploration of generative neural networks provides system-level insights that can be used by both modelers and experimentalists alike. For example, simple latent space transformations can identify general dynamic bottlenecks in metabolic networks (Figure 4), guiding targeted experimental interventions without requiring complex downstream analyses. Additionally, repurposed generators that produce models satisfying multiple dynamic criteria can serve as

robust starting points for hypothesis testing—both experimentally and in silico—offering advantages beyond the standard "off-the-shelf" application of these methods.

We further demonstrate how latent space exploration can be used to repurpose generators trained for a single property to produce models satisfying multiple dynamic constraints. In principle, this repurposing can be extended to incorporate increasingly complex properties, significantly reducing computational costs associated with pruning models for specific traits. Such an approach could streamline *in silico* strain design pipelines like NOMAD[13,14]. Additionally, since this methodology is model-agnostic, it can be applied to parameterize any dynamical system and fulfill diverse dynamic requirements, as long as trained generators are available. As generative machine learning methods like REKINDLE and RENAISSANCE become more widely used across metabolic studies and organism-specific designs, the availability of trained generators will increase, further enhancing the utility and accessibility of our proposed methodology.

METHODS

Dynamic models of *E. coli* metabolism

E. coli central carbon metabolism

The kinetic nonlinear models employed in this study represent the central carbon metabolism of *Escherichia coli* in its wild-type form. These models are based on the models by Varma and Palsson[51] and have been extensively analyzed using the SKimPy[52] toolbox and the REKINDLE[33] method. The model encompasses 64 metabolites, distributed between the cytosol and the extracellular space, and includes 65 reactions, of which 16 are transport reactions. Among the 64 metabolites, 15 are designated as boundary metabolites, as they are localized in the extracellular space. The remaining 49 metabolites are confined to the cytosol, where their dynamics are governed by a system of ordinary differential equations (ODEs) describing mass balances. Each reaction was assigned a kinetic mechanism based on its stoichiometry, and the system was subsequently parameterized using 411 kinetic parameters. Out of these 259 were concentration associated Michaelis Menten constants, K_Ms, and the remaining 152 parameters are maximal reaction velocity rates, V_{max}s.

Anaerobic metabolism of *E. coli*

The kinetic model was constructed using a reduced stoichiometric network of the central carbon metabolism of *E. coli* grown under anaerobic conditions. The reduced stoichiometry was built using redGEM[53] and lumpGEM[54] and includes core pathways like glycolysis, PPP, TCA, pyruvate metabolism and a lumped reaction for growth. When building the stoichiometry, we blocked the flux through oxygen uptake and any electron transport chain reaction that used oxygen as an electron acceptor to ensure the model accurately represented anaerobic conditions. The resulting steady-state model had 180 reactions, including 40 boundary reactions and 34 transport reactions. It included 141 metabolites in total, of which 104 were intracellular and 37 were extracellular, corresponding to 119 unique metabolites.

The model was tailored to a specific context by imposing physiological and experimental data from the study conducted by Varma and Palsson.[49] The growth rate was set to 0.4322 h^{-1} and the glucose uptake rate to 18.50 mmol gDW^{-1} h^{-1} (gram per weight per hour). Extracellular metabolites not

reported as secreted or found in the media were constrained to their minimum feasible secretion/uptake rate, and a maximum possible concentration of 10^{-4} M, an order of magnitude lower than the reported concentrations of fermentation products. Known extracellular concentrations for media components were imposed with a +-20% error. For the primary fermentation products (ethanol, formate, acetate) the steady-state concentrations were also constrained to 10^{-4} M. Next, constraints were imposed on thermodynamic variables, calculated using the group contribution method[55,56] to ensure that the sampled flux directionalities and metabolite concentrations were consistent with the second law of thermodynamics.

A total of 10,000 steady-state profiles, consistent with the integrated data, were sampled from this context-specific model using thermodynamics-based flux balance analysis implemented in the pyTFA tool.[57] Each steady-state profile includes metabolite concentrations, metabolic fluxes, and thermodynamic variables. Once these profiles were obtained, we generated kinetic models around each steady state. We used the profile with index 3709 as a reference for the study titled "Repurposing generators for multiple dynamic properties" and RENAISSANCE for the parameterization.

The kinetic model was built with SkIMpy[52] and comprises 63 mass balances and 138 rate laws with various mechanisms related to the associated reaction. Specifically, there are 21 Convenience mechanisms[58], 55 Generalized Reversible Hill[59], 2 Bi-Uni Reversible Hill[59], 41 Reversible Michaelis-Menten, 18 Uni-Bi Reversible Hill[59], and one multi-substrate Monod (Irreversible Michaelis-Menten) for the growth rate. These mechanisms are parametrized by 463 Michaelis constants, K_Ms, and 138 maximal velocities, V_{max}s, totaling 601 kinetic parameters.

Valid dynamic responses of *E. coli* metabolism

Once the structure of the ODEs has been defined and steady state data has been integrated as outlined in the sections above, we need to parameterize the model to fully define it. Once parameterized, the model exhibits different dynamic behaviours depending on the exact values of the kinetic parameters, not all of which are biologically valid or physiologically desirable.

In this study, we define kinetic models as stable if they exhibit local stability in the reference steady state profile of fluxes and metabolite concentrations and if all characteristic times of the aperiodic model response remain within physiologically and biologically plausible limits. To assess local stability and time constants, we compute the Jacobian matrix of the dynamic system[22]. The stability of a given model is determined by the eigenvalues of the Jacobian: a model is considered locally stable if all eigenvalues have negative real parts, whereas the presence of any positive real part indicates instability.

For models classified as locally stable, we define the characteristic time constant, τ_{max}, of the linearized system as the inverse of the real part of the largest eigenvalue of the Jacobian, λ_{max}. These time constants provide insights into the model's dynamic behavior. Fast metabolic processes, such as glycolysis and the electron transport chain, are associated with small time constants, whereas biosynthetic processes operate on longer timescales. The characteristic timescales must remain within biologically and physically relevant bounds.

To ensure physiological validity, we impose the constraint that the aperiodic model response should not exceed the timescale of cell division. Specifically, we enforce that all characteristic response times be at least three times faster than the cell's doubling time, ensuring that metabolic perturbations settle within 5% of the steady-state level before the next division. Additional constraints on response

dynamics are also considered, such as ensuring that biochemical responses occur on a timescale slower than proton diffusion within the cell. Models satisfying these criteria are expected to accurately capture the metabolic dynamics observed in an organism.

In this study, we classify models as valid or invalid based on these physiological constraints. For aerobic *E. coli*, the dynamic responses of our models should be at least three times faster than the wild-type doubling time (~21 min)[60]; that is, the dominant time constant of the model's responses should be smaller than ~7 minutes. Similarly, anaerobically grown *E. coli* has a doubling time of ~120 minutes[49]; thus the dominant time constant of the model's responses should be under ~40 minutes to be deemed valid.

Training generators to produce dynamically valid models

In this study, we use deep generative learning frameworks like REKINDLE[33] and RENAISSANCE[17] to parameterize the ODEs describing the dynamic model. These methods require a fully defined mathematical structure of the metabolic system and the integration of context-specific multi-omics reference data prior to parameterization (Figure 1b). Once these structural and data constraints are established, the frameworks employ distinct optimization strategies: REKINDLE utilizes adversarial training[38], while RENAISSANCE relies on Natural Evolution Strategies (NES)[61,62]. These methods train neural networks to generate kinetic parameter sets that ensure the parameterized model exhibits desirable dynamic properties while satisfying the reference data, as described in the section above.

REKINDLE was used to train generators for obtaining valid models of *E. coli* central carbon metabolism for all the studies showcased in Figure 2,3 and 4.

Training data for REKINDLE

REKINDLE requires labelled kinetic parameter sets from traditional sampling-based frameworks as training data. For this purpose, 5000 parameter sets were sampled for each of the 4 steady state profiles using the ORACLE framework[23,63,64] . The training set had an incidence of 48%, 15%, 28% and 22% valid models for the 4 steady states, respectively. This training data was log-transformed and labelled as valid or invalid as described in the previous section before training.

REKINDLE training

REKINDLE was employed on the training data to generate parameter sets with valid dynamic responses separately for each steady state. 3 statistical repeats were done for each steady state with randomly initialized generator and discriminator. For every repeat, training was deemed complete and successful when the generator and discriminator losses had stabilized and the discriminator accuracy reached ~50% (Supplementary Figure 1), indicating the discriminator was not able to distinguish between samples from the training data and the generator's samples. The incidence of valid models throughout the training process was recorded (Supplementary Figure 2) and the generators with highest incidences across the repeats were chosen for conducting further studies for each steady state.

RENAISSANCE was used to train generators for obtaining valid models of anaerobically grown *E. coli* metabolism for the studies showcased in Figure 5.

RENAISSANCE training

RENAISSANCE was employed with following hyperparameters: generator population, $n = 20$, search radius, $\sigma = 10^{-2}$, learning rate, $\alpha = 10^{-3}$, and learning rate decay, $d = 5\%$. The optimization was

done with 10 statistical repeats with a randomly initialized population of generators, and the incidence of valid models was stored (Supplementary Figure 12). The generator with the highest incidence of valid models across all replicates was chosen for further studies.

Neural network architecture

In REKINDLE, the GANs were implemented as conditional GANs[45]. The discriminator network was composed of three layers that have a total of 18,319 parameters: layer 1, Dense with 32 units, Dropout (0.5); layer 2, Dense with 64 units, Dropout (0.5); layer 3, Dense with 128 units, Dropout (0.5). The generator network was composed of three layers that have a total of 315,779 parameters: layer 1, Dense with 128 units, BatchNormalization, Dropout (0.5); layer 2, Dense with 256 units, BatchNormalization, Dropout (0.5); layer 3, Dense with 512 units, BatchNormalization, Dropout (0.5). We used the binary cross-entropy loss and the Adam optimizer with a learning rate of 0.0002 for training both the networks.

In RENAISSANCE, the generator neural networks were composed of three layers with 1,076,352 parameters: layer 1, dense, with 256 units, dropout (0.5); layer 2, dense, with 512 units, dropout (0.5); and layer 3, dense, with 1,024 units, dropout (0.5).

All software programs were implemented in Python (v3.6).

Bioreactor simulations of anaerobically grown *E. coli*

The developed and parameterized kinetic models of anaerobic *E. coli* can be used to simulate a batch fermentation process. The bioreactor is modeled by adding two additional types of ordinary differential equations (ODEs)[52]: one for monitoring the number of cells growing in the bioreactor according to the growth rate and another that accounts for the uptake and secretion of metabolites shared between the medium and intracellular metabolism (e.g., glucose uptake and acetate secretion). We initialize the bioreactor model with steady-state concentrations and fluxes used in the kinetic model parameterization process, as well as the experimental-derived inoculum of the cells and the concentrations of the medium derived from the Varma and Palsson study.[49] By integrating the model over 12 hours, we can compare its results with experimental fermentation data.[49]

Non-linear integration of parameterized models

To observe the dynamic behavior of parameterized model, we randomly perturb the reference steady state metabolite concentration (X_{ref}) and flux profile of the model up to ±50%. We next integrate the ODEs using this perturbed state X' as the initial condition $X(t=0) = X_0$. To check if a model came back to the reference steady state, we monitor the L_2 norm of the metabolite concentrations at a given point of time $X(t)$ and the reference concentration X_0. If the metabolite concentration at 21 mins (doubling time of *E. coli*) is less than 5% of the reference steady state we classify the model as having returned back to the steady state, i.e., we test,

$$\left\|X(t)_{t=21mins} - X_{ref}\right\| < 0.05\left\|X_{ref}\right\|$$

Spearman Correlation Coefficient

For two random variables X and Y, we compute the Spearman correlation coefficient as,

$$r_s = \frac{cov(R(X), R(Y))}{\sigma_{R(X)}\sigma_{R(Y)}}$$

where $R(X)$ and $R(Y)$ are the ranks of X and Y, respectively, $cov(R(X), R(Y))$ the covariance of the rank variables, and $\sigma_{R(X)}$ and $\sigma_{R(Y)}$ are the standard deviations of the rank variables.

Average deviation of simulated bioreactor responses from experimental data

The average deviation, E, between experimental data, $y_{exp}(t_i)$, and simulated data, $y_{exp}(t_i)$, at time points at t_i for $i = 1, \ldots, N$, is calculated as,

$$E = \frac{1}{N}\sum_{i=1}^{N} |y_{exp}(t_i) - y_{sim}(t_i)|$$

This metric was used for comparison with Varma et. al[49], where it was first introduced.

Jensen-Shannon (JS) Distance

For two separate probability distributions P(x) and Q(x) over the same random variable x, we can measure how different or similar these distributions are using the Jensen-Shannon divergence or distance as,

$$D_{JS}(P||Q) = \frac{1}{2}D_{KL}(P||M) + \frac{1}{2}D_{KL}(M||Q)$$

where

$$M(x) = \frac{1}{2}(P(x) + Q(x))$$

and $D_{KL}(P||Q)$ is the Kullback-Leibler divergence from Q(x) to P(x) formulated as,

$$D_{KL}(P||Q) = \begin{cases} \Sigma P(x)\, log\left(\frac{P(x)}{Q(x)}\right) & \text{if } P(x) \neq Q(x) \\ 0 & \text{if } P(x) = Q(x) \end{cases}$$

The JS divergence is symmetric and is 0 for identical distributions, with a higher divergence score representing higher dissimilarity.

Clustering of heatmaps

The clustering of all heatmap plots in this study (Figure 2a, Supplementary Figure 1) was done using the $\mathrm{clustermap}$ function of the Seaborn library[65] in Python.

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CONTRIBUTIONS

S.C. and L.M. conceptualized the overall methodology, V.H. and L.M. supervised the research and acquired funding, S.C and I.T. curated the data, models, and computational resources, S.C., O.G., and I.T. designed the code, S.C, O.G, and I.T analyzed the data and validated results, S.C and L.M visualized the results, S.C prepared the original draft of the manuscript, S.C, I.T., and L.M. reviewed and edited the draft. All authors read and commented on the manuscript.

CONFLICT OF INTEREST

The authors declare no financial or commercial conflict of interest.

ABBREVIATIONS

ML, Machine Learning, RENAISSANCE, REconstruction of dyNAmIc models through Stratified Sampling using Artificial Neural networks and Concept of Evolution strategies,
REKINDLE, REconstruction of KINetic models of metabolism using Deep LEarning,
TFA, Thermodynamics-based Flux Balance Analysis, ODE, Ordinary Differential Equations.

SUPPORTING INFORMATION

Supplementary Note 1: Augmenting latent space of neural networks for generating kinetic parameter sets.

Supplementary Note 2: Co-occurrence of enzyme parameters in determining dynamics of metabolic networks.

Supplementary Figure 1: The co-occurences of enzymes whose associated kinetic parameters emerge as the primary bottlenecks (top 3) for determining τ_{max} of the network for 4 different steady state profiles represented in (a) (b) (c) and (d) as explained in Fig 4. The color bar represents the number of co-occurences with darker shades of blue representing higher number of co-occurences.

Supplementary Figure 2: Enzymes which whose associated kinetic parameters emerge as the top 3 parameters affecting τ_{max} of the *E. coli* central carbon metabolism network for 4 different steady state profiles. The models are sorted from slowest (dark blue) to fastest (yellow) depending on their dominant time constant $\tau_{max.}$

Supplementary Figure 3: (Upper panel) Discriminator and generator loss (Lower Panel) Discriminator accuracy during REKINDLE training for (a) steady state 1 (b) steady state 2 (c) steady state 3 (d) steady state 4 as described in the Methods section for *E. coli* central carbon metabolism.

Supplementary Figure 4: Incidence of valid models for (a) steady state 1 (b) steady state 2 (c) steady state 3 (d) steady state 4, with 3 statistical repeats in each case as described in the Methods section for *E. coli* central carbon metabolism.

Supplementary Figure 5: Percentage change in dominant time constant (τ_{max}) of the model analyzed in Figure 2 when each latent feature is increased by 10% (green), 25% (orange) and 50% (blue) respectively.

Supplementary Figure 6: Percentage change in dominant time constant (τ_{max}) of the model analyzed in Figure 2 when each latent feature is increased by 50% (orange) and decreased by 50% (blue) respectively.

Supplementary Figure 7: Normalized dynamic responses of (left) ATP (middle) ADP and (right) NADP concentrations of chosen models from Figure 2c (black squares). Black line indicates doubling time of WT *E. coli*. Color dashed lines represent time points when median response comes back to within 5% of the reference steady state value. Note that for ATP and NADH the slow models (purple) do not come back to steady state in 1 hour.

Supplementary Figure 8: Non exhaustive examples of change in individual kinetic parameters values for 1681 models obtained in Figure 2c for (a)$K_{M,xu5p}^{RPE}$(b) $K_{M,dhap}^{FBA}$ (c) $K_{M,nadph}^{ICDHyr}$ (d) $K_{M,co2}^{PDH}$ (e) $K_{M,r5p}^{TKT1}$ (f) $K_{M,coa}^{SUCOAS}$. For exhaustive list check supplementary data and instructions for generating figures.

Supplementary Figure 9: Dynamic responses of normalized (left) ATP (middle) ADP and (right) NADP concentrations of chosen models from Figure 3c (black squares). Black line indicates doubling time of WT E. coli. Color dashed lines represent time points when median response comes back to within 1% of the reference steady state value. Note that for ADP the slow models (purple) do not come back to steady state in 1 hour.

Supplementary Figure 10: Percentage occurrence of enzymes affecting τ_{max} of the parameterized model across 4 different steady states for *E. coli* central carbon metabolism. An enzyme is counted as important if an associated kinetic parameter significantly affects the τ_{max} of the entire model.

Supplementary Figure 11: Distribution of τ_{max} of models when (top) $K_{m,nadh}^{GAPD}$ (middle) $K_{m,oaa}^{CS}$ (bottom) $K_{m,co2}^{PDH}$ are modified from their original values by different magnitudes (colored lines). The black dashed line represents the distribution of τ_{max} of the original unperturbed models

Supplementary Figure 12: Incidence of relevant models (Methods) for anaerobic *E. coli* metabolism obtained using RENAISSANCE for 50 generations for 10 statistical repeats (different colored lines). The repeat with the highest incidence (gray line) was selected for further studies.

Supplementary Figure 13: Non exhaustive visualization of final biomass titers of model responses when individual latent features are perturbed from their original values (as explained in Figure 5). For exhaustive list check supplementary data and instructions for generating figures.

Supplementary Figure 14: Distributions of the parameter (a) $K_{M,lac_D}^{D-LACt2pp}$ (b) $K_{M,ru5p_D}^{RPI}$ (c) $K_{M,nadh}^{GAPD}$ (d) $K_{M,nadph}^{GND}$ (e) $K_{M,atp}^{GLNS}$ (b) $K_{M,pyr}^{PPS}$ (c) $K_{M,nadh}^{NADH10}$ (d) $K_{M,h}^{Biomass}$ in generated models when (left) models are obtained through systematic perturbation of the latent point of the model in Fig 5a (right) when models are obtained through random sampling of the latent space of the trained generator. Gray vertical lines in top panel represent the 30th percentile values for this parameter that favors stability.

Supplementary Figure 15: Percentage of stable models of anaerobic *E. coli* metabolism achieved when different number of individual parameters are constrained as explained in Figure 5e and Supplementary Figure 12.

Supplementary Figure 16: Simulated bioreactor responses of locally stable models of anerobic *E. coli* metabolism obtained by perturbing latent features of the original model when (a) No parameters (b) 10 parameters (c) 100 parameters and (d) 200 parameters are constrained to ranges that favor stability as explained in Figure 5, Supplementary Figure 12 and Supplementary Figure 13. Red line represents the original unperturbed model and green line represents the best model obtained with

lowest average deviation (Methods) from experimental values. Black dots represent experimental values.

Supplementary Figure 17: Average deviations of simulated bioreactor responses of anaerobic *E. coli* metabolism for (left) biomass and (bottom) glucose from experimental bioreactor datapoints when the two latent features in figure 5b are perturbed simultaneously and (a) no parameters (b) 10 parameters (c) 100 parameters and (d) 200 parameters are constrained to ranges that favor stability as explained in Figure 5, Supplementary Figure 12 and Supplementary Figure 13.

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