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Measurements drive progress in directed evolution for precise engineering of biological systems

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Abstract

Precise engineering of biological systems requires quantitative, high-throughput measurements, exemplified by progress in directed evolution. New approaches allow high-throughput measurements of phenotypes and their corresponding genotypes. When integrated into directed evolution, these quantitative approaches enable the precise engineering of biological function. At the same time, the increasingly routine availability of large, high-quality data sets supports the integration of machine learning with directed evolution. Together, these advances herald striking capabilities for engineering biology.

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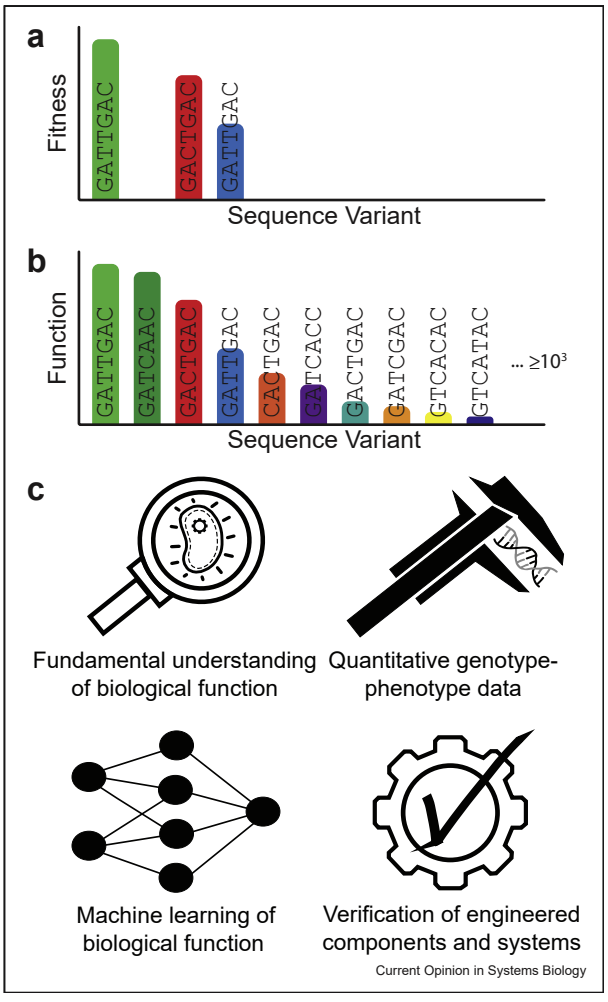
Introduction

Measurements are foundational to engineering biology. The design–build–test–learn workflow at the heart of engineering biology relies on meaningful measurements to quantify, design, predict, and verify the performance of biological components and systems [1,2]. Measurements validate the success of an engineered function, find the limits of performance, and provide a basis for reproducibility and interoperability [3–5]. Many current and proposed applications of engineering biology, such as living therapeutics [6,7], bioremediation [8], and materials manufacturing [9], depend on functions with precisely defined performance [10]. Engineering such specified functions requires measurements to quantitatively characterize the performance of biological parts and systems.

Directed evolution is often the most effective approach to engineering biology (Figure 1) [11,12]. Today, directed evolution uses artificial selections to optimize biological functions, for example, increasing enzymatic activity, binding affinity, or fluorescence signal of an existing biological construct. Directed evolution begins by building libraries of sequence variants, frequently through mutagenesis of a natural gene or organism. Artificial selections then enrich sequence variants with the desired function, often by connecting the target function to the growth rate of a cell, although many other methods are routinely used, such as fluorescence-activated cell sorting [13]. After selection, tens — or, more ambitiously, hundreds — of surviving sequence variants are quantitatively characterized and evaluated against the engineering goal. While directed evolution indirectly optimizes a biological function by connecting the desired function to organismal fitness, engineering biological functions with precise specifications will require high-throughput measurements (HTMs) that provide quantitative information on the performance of many individual sequence variants.

HTMs have proven their utility over the past twenty years, for example, with the widespread and routine implementation of flow cytometry to quantify the phenotypes of cells. More recent innovations in sequencing render HTMs of phenotypes and their associated genotypes accessible. These innovative approaches can advance precise engineering of biological function. In particular, deep sequencing has been adapted to directly quantify the performance of 10^6 protein variants [14–16]. This allows thorough characterization of evolutionary paths, more predictive design of biomolecules, and general consideration of a more complete suite of engineering options from a given biological system than previously accessible. Arriving at an optimal design then becomes a much more tractable task or achievable at all. Common examples of HTMs for this purpose include

Figure 1



High-throughput measurements (HTMs) integrate into engineering biology workflows to enable precise engineering of biological function: **(a)** Directed evolution selects a limited subset of sequence variants with desirable properties from a library of diverse sequence variants. After an artificial selection, only a few sequence variants are evaluated, leaving most sequence variants uncharacterized. **(b)** HTMs enhance directed evolution by measuring each member of a library for its ability to meet the engineering goal. **(c)** The comprehensive information resulting from HTMs offers many benefits toward precise engineering of biological function.

deep sequencing of sorted populations following fluorescence-activated cell sorting (sort-seq [17,18]), measuring changes to the relative abundance of DNA sequences during growth (bar-seq [19–22], and deep mutational scanning [23–25]). Multiple HTMs may also be combined to relate phenotypic performance to the genotypic context of the cellular host. For example, RNA-seq combined with ribo-seq quantifies both transcriptional and translational kinetics, which uncovered previously unknown mechanisms of cellular burden [26]. Given the challenges imposed by biological complexity, HTMs are uniquely suited to capturing the heterogeneity of a biomolecular or cellular population. The resulting data relating genotypes to phenotypes can inform workflows to achieve increasingly sophisticated engineered function [15,27].

HTMs enhance directed evolution for precise engineering of biological systems

Integrating HTMs into directed evolution gives unprecedented insights into the process for engineering biological systems, as well as actionable information to optimize both the workflow and engineered product. HTMs can characterize the genotype and phenotype of every sequence variant within a library to overcome common limitations, such as the exponential distribution in sequence diversity that typically occurs during library construction [28], and enable new capabilities, such as identifying the genotypes of poorly performing sequence variants. Typically, the diversity in a library is assayed only minimally, for example, by sequencing a few sequence variants at random before a selection. These minimal measurements fail to illuminate the diversity within the library and only guarantee that the most grievous potential biases, such as significant imbalances of nucleotides, were avoided during library construction. Instead, measuring the size and diversity of the entire library before selection can identify biases that occur during library construction, and these biases can be accounted for downstream in the directed evolution workflow. In this way, HTMs ensure that the required genetic and phenotypic diversity exists within a library at the outset of a directed evolution experiment. Monitoring changes in the diversity throughout experimental evolution yields the relative enrichment, and thus the functional success, of each sequence variant in a population, including exceptionally rare genotypes often lost during bottlenecking [15,29]. The wealth of information collected from HTMs of an entire library can inform more optimal conditions for further selections, shorten the path to reach the engineering target, and increase the likelihood of success. More broadly, directed evolution with HTMs will increase our fundamental understanding of biology and expand the scope and applicability of future efforts in engineering biology.

HTMs enable the precise engineering of biological systems by quantitatively characterizing the function of biomolecules *in vitro* and decoupled from cellular fitness. An effective engineering biology workflow alters the biophysical properties of a biomolecule, for example, by delivering a specified reaction rate, binding affinity, or metabolic flux. However, conventional methods to assay these properties are slow and laborious [30,31], often requiring the purification of individual biomolecules, and allow for the processing of only a few samples. HTMs speed the engineering workflow considerably by directly and quantitatively assaying

sample function *in vitro*. Early practical examples include repurposed flow cells from deep sequencing platforms to evaluate nucleic acid binding [32], while more recent demonstrations applied a similar approach to quantify kinetic properties [33,34]. By directly characterizing the kinetics of many functional biomolecules, rather than their associated selection fitness, HTMs circumvent the need to tie the function of a biomolecule to the fitness of an organism or some other reproductive advantage [32,35—37]. In this way, HTMs enable new capabilities in the engineering workflow, as well as the ability to effectively engineer a broader range of targets and associated functions.

An illustrative demonstration of enzyme engineering and directed evolution with high-throughput measurements identified enzymes that were both highly active and selective toward a target substrate [38]. Typical selections for this conversion are inefficient, often selecting for highly active enzymes with poor substrate specificity. By using HTMs to evaluate every enzyme in a library for activity toward both the original substrate and the new substrate, the researchers could simply choose the optimal balance between these two design parameters [38]. The activity and specificity of thousands of different enzymes were characterized, and further evaluation of a few chosen enzymes confirmed the effectiveness of this approach. In another recent example, HTMs quantified the function of nearly 10^6 allosteric transcription factors [15]. This allowed the researchers to choose transcription factors targeting three different functional parameters with quantitative specifications, representing unprecedented control over allosteric function [15]. Importantly, HTMs enabled precise engineering of biomolecules in both studies, by achieving multiple engineering design goals simultaneously.

HTMs allow integration of machine learning into directed evolution

HTMs increase the utility and practicality of machine learning (ML) approaches to engineering biology. HTMs provide information crucial to construct predictive models of biological function. Simple, linear ML models trained on small, low-throughput data sets produce biased predictions that underestimate the effect of genetic substitutions on the phenotype, limiting the potential for the rational design of new function [39]. In contrast, ML models trained on HTMs can efficiently explore vast spaces for genetic design and model generation [40]. The large data sets resulting from HTMs are also required to train deep neural networks (DNNs) [41], which readily capture nonlinear effects of multiple genetic substitutions and enable the rational design of biological parts on a scale orders of magnitude larger than previously possible [42]. In addition, while directed evolution gains information on high-performance sequence variants, HTMs capture quantitative information on all sequence variants, even those that perform poorly. Overall, HTMs can constitute comprehensive, unbiased training sets that sample a usefully large space relating genotypes to phenotypes, and ML methods, such as DNNs, trained on these data promise to better predict engineered function.

ML techniques can also increase the value of HTMs in directed evolution. When searching a large space of sequence variants, ML approaches can learn lower dimensional representations of a library (e.g. embedding models) to achieve a practical reduction in the design space [43,44]. These approaches effectively display complex biological data to drive intuition-guided experiments and inform library design for directed evolution. These approaches also provide meaningful insights into the underlying biochemical mechanisms driving evolution by representing similarity between function through distance in the embedding space. This has particular value when there are multiple design objectives or when new or novel categories of phenotypes are desired or unexpectedly arise [15]. DNNs often provide only black-box predictions, offering no insight into the underlying biophysical processes. Understanding and interpreting these predictions mechanistically from a biomolecular perspective, however, will become increasingly valuable to engineering efforts. Demonstrated approaches for interpretable DNN models include embedding DNN models inside biophysical models [45] and using post-hoc approaches to identify relevant motifs that contribute heavily to a model's predictions [46]. These mechanistic insights then augment the ability to design new functions for engineered biological systems. The growing use of HTMs, together with the development of new ML approaches, is drastically improving our ability to engineer biology; we expect massive and synergistic gains in the rate at which new functions are engineered.

Conclusions and outlook

HTMs are well matched to our current understanding of inherently complex biological systems and so are well poised to advance engineering biology with directed evolution. Still, a need persists for measurement assurance. Standard materials and methods to evaluate measurement performance of new techniques against established methods would facilitate the development of novel tools relevant to HTMs. Standards for reporting methods and data along with published results [47], as well as the development of data repositories suited to storing and curating HTMs of genotypes and phenotypes [48], can aid comparability and interoperability. These open platforms support confidence in reported results and decrease redundant workflows by informing

other engineering goals. The widespread publication of HTMs may also reveal novel phenotypes beyond the expected capabilities of the biological parts being engineered, increasing the utility of those engineering targets [15].

HTMs are revolutionizing the utility of directed evolution, enabling a new rigor suited to the precise engineering of biology required for applications outside the laboratory. The increase in quantitation in engineering biology dovetails with industrial best practices, where traceable measurements are routinely implemented for quality and control for the burgeoning bioeconomy. The growing use of laboratory automation further complements HTMs and the precise engineering of biological systems, as evidenced by investment in biofoundries coupled to pipelines for large-scale data collection [49]. Although balancing even two design goals remains novel for engineering biology, this infrastructure will advance capabilities in engineering biology toward a better match with the routine achievements of other engineering fields. Rather than approaching an engineering task with rigid and narrow expectations, we become better equipped through measurement to learn from biology. Surveying the landscape of biological function through HTMs may then instill a renewed sense of wonder and possibility, perhaps ultimately freeing us from the limitations of building only what we can imagine.

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Conflict of interest statement

Nothing declared.

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