

# ART: A machine learning Automated Recommendation Tool for synthetic biology

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## Abstract

Biology has changed radically in the last two decades, transitioning from a descriptive science into a design science. Synthetic biology allows us to bioengineer cells to synthesize novel valuable molecules such as renewable biofuels or anticancer drugs. However, traditional synthetic biology approaches involve ad-hoc non systematic engineering practices, which lead to long development times. Here, we present the Automated Recommendation Tool (**ART**), a tool that leverages machine learning and probabilistic modeling techniques to guide synthetic biology in a systematic fashion, without the need for a full mechanistic understanding of the biological system. Using sampling-based optimization, **ART** provides a set of recommended strains to be built in the next engineering cycle, alongside probabilistic predictions of their production levels. We demonstrate the capabilities of **ART** on simulated and real data sets and discuss possible difficulties in achieving satisfactory predictive power.

# Introduction

Metabolic engineering<sup>1</sup> enables us to bioengineer cells to synthesize novel valuable molecules such as renewable biofuels<sup>2,3</sup> or anticancer drugs.<sup>4</sup> The prospects of metabolic engineering to have a positive impact in society are on the rise, as it was considered one of the “Top Ten Emerging Technologies” by the World Economic Forum in 2016.<sup>5</sup> Furthermore, an incoming industrialized biology is expected to improve most human activities: from creating renewable bioproducts and materials, to improving crops and enabling new biomedical applications.<sup>6</sup>

However, the practice of metabolic engineering has been far from systematic, which has significantly hindered its overall impact.<sup>7</sup> Metabolic engineering has remained a collection of useful demonstrations rather than a systematic practice based on generalizable methods. This limitation has resulted in very long development times: for example, it took 150 person-years of effort to produce the antimalarial precursor artemisinin by Amyris; and 575 person-years of effort for Dupont to generate propanediol,<sup>8</sup> which is the base for their commercially available Sorona fabric.<sup>9</sup>

Synthetic biology<sup>10</sup> aims to improve genetic and metabolic engineering by applying systematic engineering principles to achieve a previously specified goal. Synthetic biology encompasses, and goes beyond, metabolic engineering: it also involves non-metabolic tasks such as, e.g., gene drives able to extinguish malaria-bearing mosquitoes<sup>11</sup> or engineering microbiomes to replace fertilizers.<sup>12</sup> This discipline is enjoying an exponential growth, as it heavily benefits from the byproducts of the genomic revolution: high-throughput multi-omics phenotyping,<sup>13,14</sup> accelerating DNA sequencing<sup>15</sup> and synthesis capabilities,<sup>16</sup> and CRISPR-enabled genetic editing.<sup>17</sup> This exponential growth is reflected in the private investment in the field, which has totalled ~\$12B in the 2009-2018 period and is rapidly accelerating (~\$2B in 2017 to ~\$4B in 2018).<sup>18</sup>

One of the synthetic biology engineering principles used to improve metabolic engineering is the Design-Build-Test-Learn (DBTL<sup>19,20</sup>) cycle: a loop used recursively to obtain a design that satisfies the desired specifications (e.g. a particular titer, rate, yield or product). The

DBTL cycle’s first step is to design (D) a biological system expected to meet the desired outcome. That design is built (B) in the next phase from DNA parts into an appropriate microbial chassis using synthetic biology tools. The next phase involves testing (T) whether the built biological system indeed works as desired in the original design, via a variety of assays: e.g. measurement of production or/and ‘omics (transcriptomics, proteomics, metabolomics) data profiling. It is extremely rare that the first design behaves as desired, and further attempts are typically needed to meet the desired specification. The Learn (L) step leverages the data previously generated to inform the next Design step so as to converge to the desired specification faster than through a random search process.

The Learn phase of the DBTL cycle has traditionally been the most weakly supported and developed,<sup>20</sup> despite its critical importance to accelerate the full cycle. The reasons are multiple, although their relative importance is not entirely clear. Arguably, the main drivers of the lack of emphasis on the L phase are: the lack of predictive power for biological systems behavior,<sup>21</sup> the reproducibility problems plaguing biological experiments,<sup>3,22–24</sup> and the traditionally limited emphasis on mathematical training for synthetic biologists.

Machine learning (ML) arises as an effective tool to predict biological system behavior and empower the Learn phase, enabled by emerging high-throughput phenotyping technologies.<sup>25</sup> Machine Learning has been used to produce driverless cars,<sup>26</sup> automate language translation,<sup>27</sup> predict sexual orientation from Facebook profiles,<sup>28</sup> predict pathway dynamics,<sup>29</sup> optimize pathways through translational control,<sup>30</sup> diagnose skin cancer,<sup>31</sup> detect tumors in breast tissues,<sup>32</sup> and predict DNA and RNA protein-binding sequences,<sup>33</sup> drug side effects<sup>34</sup> and antibiotic mechanisms of action.<sup>35</sup> However, the practice of machine learning requires statistical and mathematical expertise that is scarce, and highly competed for in other fields.<sup>36</sup>

Here, we provide a tool that leverages machine learning for synthetic biology’s purposes: the Automated Recommendation Tool (ART). ART combines the widely-used and general-purpose open source scikit-learn library<sup>37</sup> with a novel Bayesian ensemble approach, in a

manner that adapts to the particular needs of synthetic biology projects: e.g. low number of conditions, recursive DBTL cycles, and the need for uncertainty quantification. The data sets collected in the synthetic biology field are typically not large enough to allow for the use of deep learning (< 100 conditions), but our ensemble model will be able to integrate this approach when high-throughput data generation<sup>14,38</sup> and automated data collection<sup>39</sup> become widely used in the future. ART provides machine learning capabilities in an easy-to-use and intuitive manner, and is able to guide synthetic biology efforts in an effective way.

We showcase the efficacy of ART in guiding synthetic biology through four different examples: a test case with simulated data and three real cases of metabolic engineering. The test case permits us to explore how the machine learning algorithms perform when applied to systems that present different levels of difficulty when being “learnt”, as well as the effectiveness of using several DTBL cycles. The real metabolic engineering cases involve data sets from published metabolic engineering projects: renewable biofuel production, yeast bioengineering to recreate the flavor of hops in beer, and fatty alcohols synthesis. These projects illustrate what to expect under different typical metabolic engineering situations: high/low coupling of the heterologous pathway to host metabolism, complex/simple pathways, high-/low number of conditions, high/low difficulty in learning pathway behavior.

In sum, ART provides a tool specifically tailored to the synthetic biologist’s needs in order to leverage the power of machine learning to enable predictable biology. This combination of synthetic biology with machine learning and automation has the potential to revolutionize bioengineering<sup>25</sup> by enabling effective inverse design. This paper is written so as to be accessible to both the machine learning and synthetic biology readership, with the intention of providing a much needed bridge between these two very different collectives. Hence, we apologize if we put excessive emphasis on explaining basic machine learning or synthetic biology concepts: they will surely be of use to a part of the readership.

# Methods

## Key capabilities

ART leverages machine learning to improve the efficacy of bioengineering microbial strains for the production of desired bioproducts (Fig. 1). ART gets trained on available data to produce a model capable of predicting the response variable (e.g. final production of the jet fuel limonene) from the input data (e.g. proteomics data, or any other type of data that can be expressed as a vector). Furthermore, ART uses this model to recommend new inputs (e.g. proteomics profiles) that are predicted to reach our desired goal (e.g. improve production). As such, ART bridges the Learn and Design phases of a DBTL cycle.

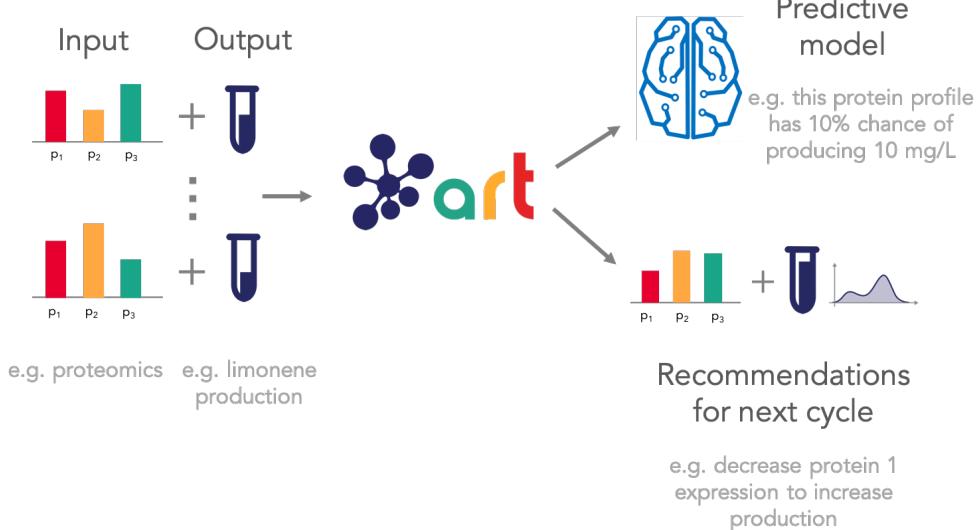


Figure 1: **ART overview.** ART uses experimental data to i) build a probabilistic predictive model that predicts output (e.g. production) from input variables (e.g. proteomics), and ii) uses this model to provide a set of recommended designs for the next experiment, along with the probabilistic predictions of the response.

ART can import data directly from Experimental Data Depot,<sup>40</sup> an online tool where experimental data and metadata are stored in a standardized manner. Alternatively, ART can import EDD-style .csv files, which use the nomenclature and structure of EDD exported files.

By training on the provided data set, ART builds a predictive model for the response as a function of the input variables. Rather than predicting point estimates of the output variable, ART provides the full probability distribution of the predictions. This rigorous quantification of uncertainty enables a principled way to test hypothetical scenarios in-silico, and to guide design of experiments in the next DBTL cycle. The Bayesian framework chosen to provide the uncertainty quantification is particularly tailored to the type of problems most often encountered in metabolic engineering: sparse data which is expensive and time consuming to generate.

With a predictive model at hand, ART can provide a set of recommendations expected to produce a desired outcome, as well as probabilistic predictions of the associated response. ART supports the following typical metabolic engineering objectives: maximization of the production of a target molecule (e.g. to increase Titer, Rate and Yield, TRY), its minimization (e.g. to decrease the toxicity), as well as specification objectives (e.g. to reach specific level of a target molecule for a desired beer taste profile). Furthermore, ART leverages the probabilistic model to estimate the probability that at least one of the provided recommendations is successful (e.g. it improves the best production obtained so far), and derives how many strain constructions would be required for a reasonable chance to achieve the desired goal.

While ART can be applied to problems with multiple output variables of interest, it currently supports only the same type of objective for all output variables. Hence, it does not support maximization of one target molecule along with minimization of another.

## Mathematical methodology

### Learning from data: a predictive model through machine learning and a novel Bayesian ensemble approach

By learning the underlying regularities in experimental data, machine learning can provide predictions without a detailed mechanistic understanding (Fig. 2). Training data is used to statistically link an input (i.e. features or independent variables) to an output (i.e. response or dependent variables) through models that are expressive enough to represent almost any relationship. After this training, the models can be used to predict the outputs for inputs that the model has never seen before.

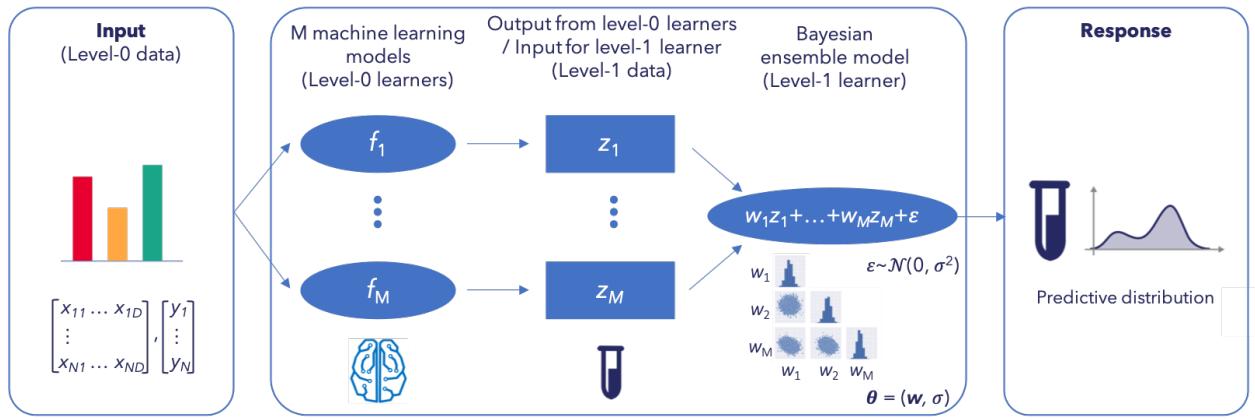


Figure 2: **ART provides a probabilistic predictive model of the response (e.g. production).** ART combines several machine learning models from the scikit-learn library with a novel Bayesian approach to predict the probability distribution of the output. The input to ART is proteomics data (or any other input data in vector format: transcriptomics, gene copy, etc.), which we call level-0 data. This level-0 data is used as input for a variety of machine learning models from the sci-kit learn library (level-0 learners) that produce a prediction of production for each model ( $z_i$ ). These predictions (level-1 data) are used as input for the Bayesian ensemble model (level-1 learner), which weights these predictions differently depending on the ability of the Bayesian ensemble model to predict the training data. The weights  $w_i$  and the variance  $\sigma$  are characterized through probability distributions, giving rise to a final prediction in the form of a full probability distribution of response levels.

Model selection is a significant challenge in machine learning, since there is a large variety of models available for learning the relationship between response and input, but none of them is optimal for all learning tasks.<sup>41</sup> Furthermore, each model features hyperparameters

(i.e. parameters that are set before the training process) that crucially affect the quality of the predictions (e.g. number of trees for random forest or degree of polynomials in polynomial regression), and finding their optimal values is not trivial.

We have sidestepped the challenge of model selection by using an ensemble model approach. This approach takes the input of various different models and has them “vote” for a particular prediction. Each of the ensemble members is trained to perform the same task and their predictions are combined to achieve an improved performance. The ensemble model can be a set of different models (heterogeneous case) or the same models with different parameters (homogeneous case). The examples of the random forest<sup>42</sup> or the super learner algorithm<sup>43</sup> have shown that simple models can be significantly improved by using a set of them (e.g. several types of decision trees in a random forest algorithm). We have chosen a heterogeneous ensemble learning approach that uses reasonable hyperparameters for each of the model types.

ART uses a novel probabilistic ensemble approach where the weight of each ensemble model is considered a random variable, with a probability distribution constrained by the available data. Unlike other approaches,<sup>44–47</sup> this method does not require the ensemble models to be probabilistic in nature, hence allowing us to fully exploit the popular scikit-learn library to increase accuracy by leveraging a diverse set of models (see "Related work and novelty of our ensemble approach" in supp. material). This weighted ensemble model approach produces a simple, yet powerful, way to quantify uncertainty, a critical capability when dealing with small data sets and a crucial component of AI in biological research.<sup>48</sup> Here we describe our approach for the single response variable problems, whereas the multiple variables case can be found in the "Multiple response variables" section in the supplementary material. Using a common notation in ensemble modeling we define the following levels of data and learners (see Fig. 2):

- *Level-0 data* ( $\mathcal{D}$ ) represent the historical data consisting of  $N$  known inputs and responses, i.e.  $\mathcal{D} = \{(\mathbf{x}_n, y_n), n = 1, \dots, N\}$ , where  $\mathbf{x} \in \mathcal{X} \subseteq \mathbb{R}^D$  are the features

(input) and  $y \in \mathbb{R}$  is the associated response variable. For the sake of cross-validation, the *level-0 data* are further divided into validation ( $\mathcal{D}^{(k)}$ ) and training sets ( $\mathcal{D}^{(-k)}$ ).  $\mathcal{D}^{(k)} \subset \mathcal{D}$  is the  $k$ th fold of a  $K$ -fold cross-validation obtained by randomly splitting the set  $\mathcal{D}$  into  $K$  almost equal parts; and  $\mathcal{D}^{(-k)} = \mathcal{D} \setminus \mathcal{D}^{(k)}$  is the set  $\mathcal{D}$  without the  $k$ th fold  $\mathcal{D}^{(k)}$ . Note that these sets do not overlap and cover the full available data: i.e.  $\mathcal{D}^{(k_i)} \cap \mathcal{D}^{(k_j)} = \emptyset, i \neq j$  and  $\cup_i \mathcal{D}^{(k_i)} = \mathcal{D}$ .

- *Level-0 learners* ( $f_m$ ) consist of  $M$  base learning algorithms  $f_m, m = 1, \dots, M$  used to learn from level-0 training data  $\mathcal{D}^{(-k)}$ . For ART, we have chosen the following eight algorithms from the scikit-learn library: Random Forest, Neural Network, Support Vector Regressor, Kernel Ridge Regressor, K-NN Regressor, Gaussian Process Regressor, Gradient Boosting Regressor, as well as TPOT (tree-based pipeline optimization tool<sup>49</sup>). TPOT uses genetic algorithms to find the combination of the 11 different regressors and 18 different preprocessing algorithms from scikit-learn that, properly tuned, provides the best the cross-validated performance on the training set.
- *Level-1 data* ( $\mathfrak{D}_{CV}$ ) are data derived from  $\mathcal{D}$  by leveraging cross-validated predictions of the level-0 learners. More specifically, level-1 data are given by the set  $\mathfrak{D}_{CV} = \{(\mathbf{z}_n, y_n), n = 1, \dots, N\}$ , where  $\mathbf{z}_n = (z_{1n}, \dots, z_{Mn})$  are predictions for level-0 data ( $\mathbf{x}_n \in \mathcal{D}^{(k)}$ ) of level-0 learners ( $f_m^{(-k)}$ ) trained on observations which are not in fold  $k$  ( $\mathcal{D}^{(-k)}$ ):  $z_{mn} = f_m^{(-k)}(\mathbf{x}_n), m = 1, \dots, M$ .
- The *level-1 learner* ( $F$ ), or metalearner, is a linear weighted combination of level-0 learners, with weights  $w_m, m = 1, \dots, M$  being random variables that are non-negative and normalized to one. Each  $w_m$  can be interpreted as the relative confidence in model  $m$ . Hence, given an input  $\mathbf{x}$  the response variable  $y$  is modeled as:

$$F : \quad y = \mathbf{w}^T \mathbf{f}(\mathbf{x}) + \varepsilon, \quad \varepsilon \sim \mathcal{N}(0, \sigma^2), \quad (1)$$

where  $\mathbf{w} = [w_1 \dots w_M]^T$  is the vector of weights such that  $\sum w_m = 1, w_m \geq 0$ ,  $\mathbf{f}(\mathbf{x}) = [f_1(\mathbf{x}) \dots f_M(\mathbf{x})]^T$  is the vector of level-0 learners, and  $\varepsilon$  is a normally distributed error variable with a zero mean and standard deviation  $\sigma$ . The constraint  $\sum w_m = 1$  (i.e. that the ensemble is a convex combination of the base learners) is empirically motivated but also supported by theoretical considerations.<sup>50</sup> We denote the unknown ensemble model parameters as  $\boldsymbol{\theta} \equiv (\mathbf{w}, \sigma)$ , constituted of the vector of weights and the Gaussian error standard deviation. These parameters  $\boldsymbol{\theta}$  are obtained by training  $F$  on the level-1 data  $\mathfrak{D}_{CV}$  *only*. However, the final model  $F$  to be used for generating predictions for new inputs uses  $\boldsymbol{\theta}$  inferred from level-1 data  $\mathfrak{D}_{CV}$ , and the base learners  $f_m, m = 1, \dots, M$  trained on the full original data set  $\mathcal{D}$ , rather than only on the level-0 data partitions  $\mathcal{D}^{(-k)}$ . This follows the usual procedure in developing ensemble learners<sup>51,52</sup> in the context of stacking.<sup>50</sup>

Rather than providing a single estimate of ensemble model parameters  $\boldsymbol{\theta}$  that best fit the training data, a Bayesian model provides a joint probability distribution  $p(\boldsymbol{\theta}|\mathcal{D})$  which quantifies the probability that a given set of parameters explains the training data. This Bayesian approach makes it possible to (i) make inferences about new observations, and (ii) examine the uncertainty in the model. Model parameters  $\boldsymbol{\theta}$  are characterized by full posterior distribution  $p(\boldsymbol{\theta}|\mathcal{D})$  that is inferred from level-1 data. Since this distribution is analytically intractable, we sample from it using the Markov Chain Monte Carlo (MCMC) technique,<sup>53</sup> which samples the parameter space with a frequency proportional to the desired posterior  $p(\boldsymbol{\theta}|\mathcal{D})$  (See "Markov Chain Monte Carlo sampling" section in Supp. Material).

As a result, instead of obtaining a single value as the prediction for the response variable, the ensemble model produces a full distribution that takes into account the uncertainty in model parameters. More precisely, for a new input  $\mathbf{x}^*$  (not present in  $\mathcal{D}$ ), the ensemble model  $F$  provides the probability that the response is  $y$ , when trained with data  $\mathcal{D}$  (i.e. the

full predictive posterior distribution):

$$p(y|\mathbf{x}^*, \mathcal{D}) = \int p(y|\mathbf{x}^*, \boldsymbol{\theta})p(\boldsymbol{\theta}|\mathcal{D})d\boldsymbol{\theta} = \int \mathcal{N}(y; \mathbf{w}^T \mathbf{f}, \sigma^2)p(\boldsymbol{\theta}|\mathcal{D})d\boldsymbol{\theta}. \quad (2)$$

where  $p(y|\mathbf{x}^*, \boldsymbol{\theta})$  is the predictive distribution of  $y$  given input  $\mathbf{x}^*$  and model parameters  $\boldsymbol{\theta}$ ,  $p(\boldsymbol{\theta}|\mathcal{D})$  is the posterior distribution of model parameters given data  $\mathcal{D}$ , and  $\mathbf{f} \equiv \mathbf{f}(\mathbf{x}^*)$  for the sake of clarity. Please note that although we have modeled  $p(y|\mathbf{x}^*, \boldsymbol{\theta})$  to be Gaussian (equation 1),  $p(y|\mathbf{x}^*, \mathcal{D})$  is not Gaussian due to the complexity of  $p(\boldsymbol{\theta}|\mathcal{D})$  arising from the data and other constraints.

### Optimization: suggesting next steps

The optimization phase leverages the predictive model described in the previous section to find inputs that are predicted to bring us closer to our objective (i.e. maximize or minimize response, or achieve a desired response level). In mathematical terms, we are looking for a set of  $S$  suggested inputs  $\mathbf{x}_s \in \mathcal{X}; s = 1, \dots, S$ , that optimize the response with respect to the desired objective. Specifically, we want a process that:

- i) optimizes the predicted levels of the response variable;
- ii) can explore the regions of input phase space associated with high uncertainty in predicting response, if desired;
- iii) provides a set of different recommendations, rather than only one.

These three requirements are met by solving the following optimization problem:

$$\begin{aligned} & \arg \max_{\mathbf{x}} G(\mathbf{x}) \\ \text{s.t. } & \mathbf{x} \in \mathcal{B} \end{aligned}$$

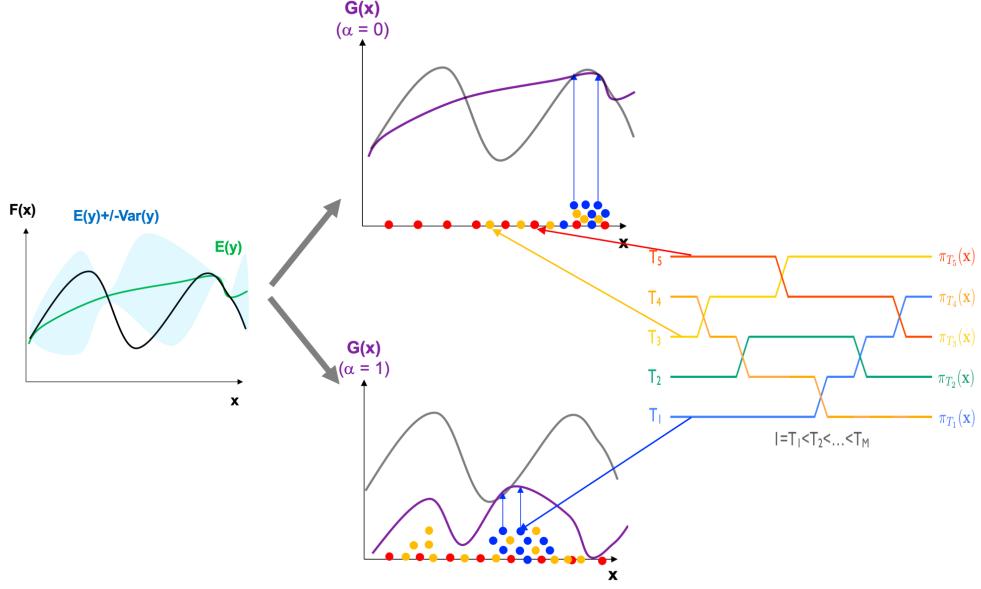
where the surrogate function  $G(\mathbf{x})$  is defined as:

$$G(\mathbf{x}) = \begin{cases} (1 - \alpha)\mathbb{E}(y) + \alpha\text{Var}(y)^{1/2} & (\text{maximization case}) \\ -(1 - \alpha)\mathbb{E}(y) + \alpha\text{Var}(y)^{1/2} & (\text{minimization case}) \\ -(1 - \alpha)||\mathbb{E}(y) - y^*||_2^2 + \alpha\text{Var}(y)^{1/2} & (\text{specification case}) \end{cases} \quad (3)$$

depending on which mode ART is operating (see Key capabilities section). Here,  $y^*$  is the target value for the response variable,  $y = y(\mathbf{x})$ ,  $\mathbb{E}(y)$  and  $\text{Var}(y)$  denote the expected value and variance respectively (see "Expected value and variance for ensemble model" in supp. material),  $||\mathbf{x}||_2^2 = \sum_i x_i^2$  denotes Euclidean distance, and the parameter  $\alpha \in [0, 1]$  represents the exploitation-exploration trade-off (see below). The constraint  $\mathbf{x} \in \mathcal{B}$  characterizes the lower and upper bounds for each input feature (e.g. protein levels cannot increase beyond a given, physical, limit). These bounds can be provided by the user (see details in the "Implementation" section in the supp. material); otherwise default values are computed from the input data as described in the "Input space set  $\mathcal{B}$ " section in the supplementary material.

Requirements i) and ii) are both addressed by borrowing an idea from Bayesian optimization: optimization of a parametrized surrogate function which accounts for both exploitation and exploration. Namely, our objective function  $G(\mathbf{x})$  takes the form of the upper confidence bound<sup>54</sup> given in terms of a weighted sum of the expected value and the variance of the response (parametrized by  $\alpha$ , Eq. (3)). This scheme accounts for both exploitation and exploration: for the maximization case, for example, for  $\alpha = 1$  we get  $G(\mathbf{x}) = \text{Var}(y)^{1/2}$ , so the algorithm suggests next steps that maximize the response variance, thus *exploring* parts of the phase space where our model shows high predictive uncertainty. For  $\alpha = 0$ , we get  $G(\mathbf{x}) = E(y)$ , and the algorithm suggests next steps that maximize the expected response, thus *exploiting* our model to obtain the best response. Intermediate values of  $\alpha$  produce a mix of both behaviors. We recommend setting  $\alpha$  to values slightly smaller than one for early-stage DBTL cycles, thus allowing for more systematic exploration of the space so as

to build a more accurate predictive model in the subsequent DBTL cycles. If the objective is purely to optimize the response, we recommend setting  $\alpha = 0$ .



**Figure 3: ART chooses recommendations for next steps by sampling the modes of a surrogate function.** The leftmost figure shows the true response  $F(\mathbf{x})$  (e.g. biofuel production to be optimized) as a function of the input features  $\mathbf{x}$  (e.g. proteomics data), as well as the predicted response after several DBTL cycles (green) and its variance (blue). Depending on whether we prefer to *explore* the phase space where the model is least accurate or *exploit* the predictive model to focus on the highest predicted responses, we will seek to optimize a surrogate function  $G(\mathbf{x})$  (equation 3) where the exploitation-exploration parameter  $\alpha = 0$  (exploitation),  $\alpha = 1$  (exploration) or anything in between. Monte Carlo sampling in combination with Parallel Tempering (right hand side of figure) produces sets of vectors  $\mathbf{x}$  that start sampling the full space (low temperatures, blue) and slowly progress towards concentrating on the nodes (high temperatures, red). Final recommendations (arrows) to improve response are provided from these low temperature samples, and chosen such that they are not too close to each other (at least 20% difference).

In order to address (iii), as well as to avoid entrapment in local optima and search the phase space more effectively, we choose to solve the optimization problem through sampling. More specifically, we draw samples from a target distribution defined as

$$\pi(\mathbf{x}) \propto \exp(G(\mathbf{x}))p(\mathbf{x}), \quad (4)$$

where  $p(\mathbf{x}) = \mathcal{U}(\mathcal{B})$  can be interpreted as the uniform ‘prior’ on the set  $\mathcal{B}$ , and  $\exp(G(\mathbf{x}))$  as the ‘likelihood’ term of the target distribution. Sampling from  $\pi$  implies optimization of the function  $G$  (but not reversely), since the modes of the distribution  $\pi$  correspond to the optima of  $G$ . As we did in the previous section, we resort to Markov chain Monte Carlo for sampling. The target distribution is not necessarily differentiable and may well be complex. For example, if it displays more than one mode, as is often the case in practice, there is a risk that a Markov chain gets trapped in one of them. In order to make the chain explore all areas of high probability one can “flatten/melt down” the roughness of the distribution by tempering. For this purpose, we use the Parallel Tempering algorithm<sup>55</sup> for optimization of the objective function through sampling, in which multiple chains at different temperatures are used for exploration of the target distribution.

#### *Choosing recommendations for the next cycle*

After drawing a certain number of samples from  $\pi(\mathbf{x})$  we need to chose recommendations for the next cycle, making sure that they are sufficiently different from each other as well as from the input data. To do so, first we find a sample with optimal  $G(\mathbf{x})$  (note that  $G(\mathbf{x})$  values are already calculated and stored). We only accept this sample as a recommendation if there is *at least one* feature whose value is different by at least a factor  $\gamma$  (e.g. 20% difference,  $\gamma = 0.2$ ) from the values of that feature in *all* data points  $\tilde{\mathbf{x}} \in \mathcal{D}$ . Otherwise, we find the next optimal sample and check the same condition. This procedure is repeated until the desired number of recommendations are collected, and the condition involving  $\gamma$  is satisfied for all previously collected recommendations and all data points. In case all draws are exhausted without collecting the sufficient number of recommendations, we decrease the factor  $\gamma$  and repeat the procedure from the beginning. Pseudo code for this algorithm can be found in the "Pseudo algorithm for recommendations" section in the supplementary material. The probability of success for these recommendations is computed as indicated in the "Success probability calculation" section in the supplementary material.

## Implementation

ART is implemented Python 3.6 and should be used under this version. The source code can be downloaded from <https://github.com/JBEI/AutomatedRecommendationTool> and installed by the command `python setup.py install`. Figure S1 represents the main code structure and its dependencies to external packages. In the "Implementation" section of the supp. material, we provide explanations of the main modules and functions.

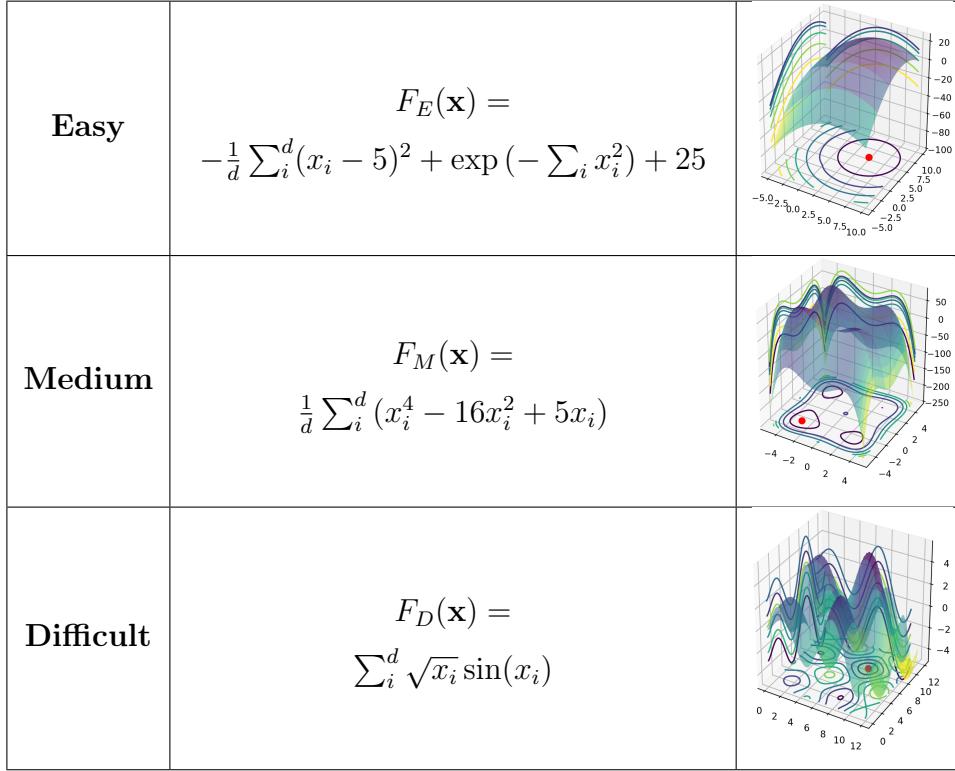
## Results and discussion

### Using simulated data to test ART

Synthetic data sets allow us to test how ART performs when confronted by problems of different difficulty and dimensionality, as well as gauge the effect of the availability of more training data. In this case, we tested the performance of ART for 1–10 DBTL cycles, three problems of increasing difficulty ( $F_E$ ,  $F_M$  and  $F_D$ , see Table 1) and three different dimensions of input space ( $D = 2, 10$  and  $50$ ), as shown in Fig. 4. We simulated the DBTL processes by starting with a training set given by 16 strains and measurements in triplicates (mimicking the 48 wells of throughput of a typical automated fermentation platform<sup>56</sup>). We limited ourselves to the maximization case, and at each DBTL cycle generated 16 recommendations that maximize the objective function given by Eq. (3). We employ a tempering strategy for the exploitation-exploration parameter, i.e. assign  $\alpha = 1$  at start for a purely exploratory optimization, and gradually decrease the value to  $\alpha = 0$  in the final DBTL cycle for the exploitative maximization of the production levels.

ART performance improves significantly as more DTBL cycles are added. Whereas the prediction error, given in terms of Mean Average Error (MAE) remains constantly low for the training set (i.e. ART is always able to reliably predict data it has already seen), the MAE for the test data (data ART has not seen) in general decreases markedly only with the addition

Table 1: Functions presenting different levels of difficulty to being learnt, used to produce synthetic data and test ART’s performance.



of more DBTL cycles. The exception are the most complicated problems: those exhibiting highest dimensionality ( $D = 50$ ), where MAE stays approximately constant, and the difficult function  $F_D$ , which exhibits a slower decrease. Furthermore, the best production obtained in the simulated process, given in terms of the highest mean predicted production, increases monotonically with more DBTL cycles: faster for easier problems and lower dimensions and more slowly for harder problems and higher dimensions. Finally, the uncertainty in those predictions decreases as more DBTL cycles proceed. Hence, more data (DBTL cycles) almost always translates into better predictions and production. However, we see that these benefits are rarely reaped with only the 2 DBTL cycles customarily used in metabolic engineering (see examples in the next sections): ART (and ML in general) becomes only truly efficient when using 5–10 DBTL cycles.

Different problems present different difficulties to being learnt (i.e. being predicted accurately), and this can only be assessed empirically. Low dimensional problems can be easily

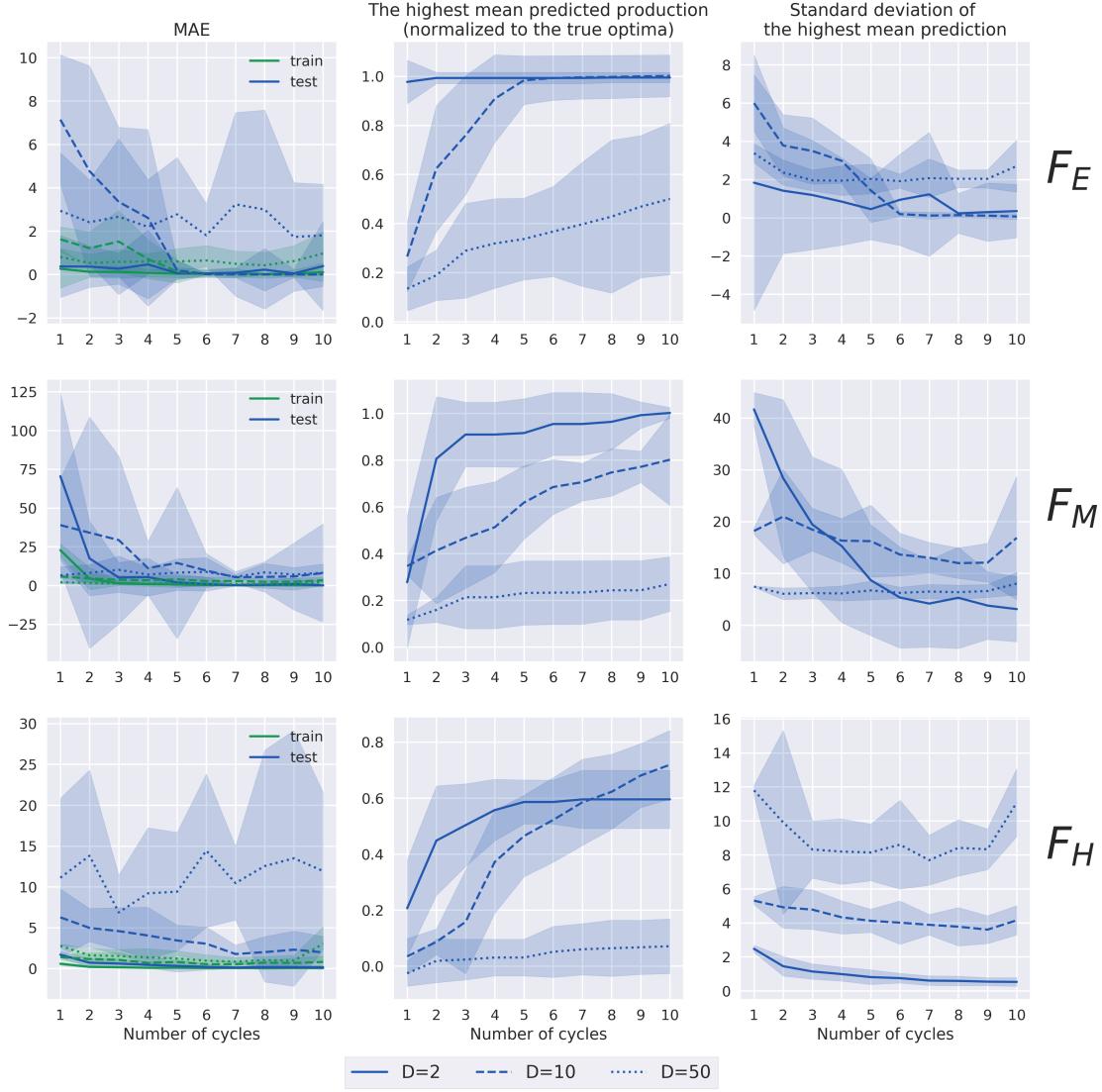


Figure 4: **ART performance improves significantly by proceeding beyond the usual two Design-Build-Test-Learn cycles.** Here we show the results of testing ART’s performance with synthetic data obtained from functions of different levels of complexity (see Table 1), different phase space dimensions (2, 10 and 50), and different amounts of training data (DBTL cycles). The leftmost column presents the Medium Average Error (MAE) in production predictions, which decreases drastically as more data (DBTL cycles) are added, with the exception of the high dimension case. The center column presents the results of the simulated metabolic engineering in terms of highest production achieved so far. The production increases monotonically with a rate that decreases as the problem is harder to learn and the dimensionality increases. The right column shows the uncertainty in ART’s production prediction, which decreases markedly with the number of DBTL cycles except for the highest number of dimensions.

learnt, whereas exploring and learning a 50 dimensions landscape is very slow (Fig. 4). Difficult problems (i.e. less monotonic landscapes) take more data to learn and traverse than easier ones. We will see this effect in terms of real experimental data when comparing the biofuel project (easy) versus the dodecanol project (hard) below. However, it is not possible to decide a priori whether a given real data project or problem will be easy or hard to learn: the only way to determine this is by checking the change in prediction accuracy as more data is added.

## Improving the production of renewable biofuel

The optimization of the production of the renewable biofuel limonene through synthetic biology will be our first demonstration of ART using real-life experimental data. Renewable biofuels are almost carbon neutral because they only release into the atmosphere the carbon dioxide that was taken up in growing the plant biomass they are produced from. Biofuels from renewable biomass have been estimated to be able to displace ~30% of petroleum consumption<sup>57</sup> and are seen as the most viable option for decarbonizing sectors that are challenging to electrify, such as heavy-duty freight and aviation.<sup>58</sup>

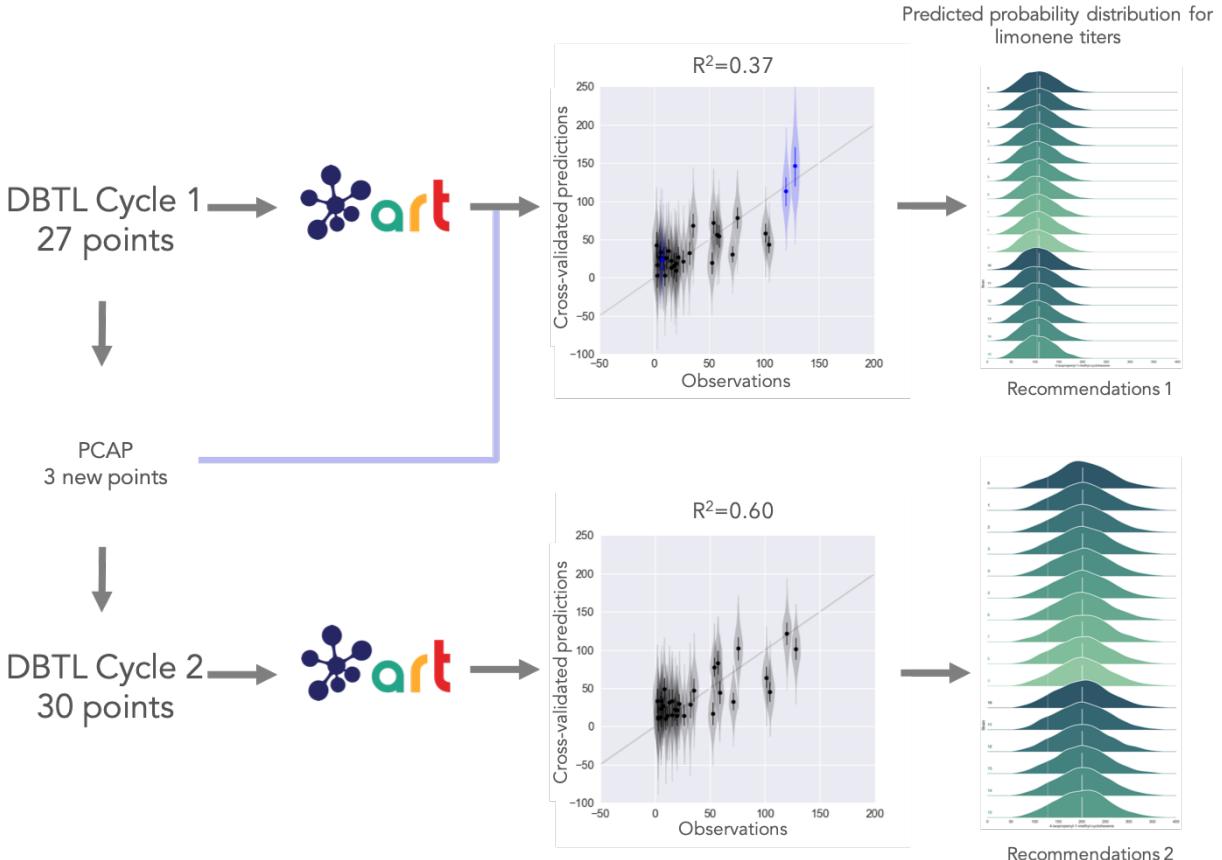
Limonene is a molecule that can be chemically converted to several pharmaceutical and commodity chemicals.<sup>59</sup> If hydrogenated, for example, it has low freezing point and is immiscible with water, characteristics which are ideal for next generation jet-biofuels and fuel additives that enhance cold weather performance.<sup>60,61</sup> Limonene has been traditionally obtained from plant biomass, as a byproduct of orange juice production, but fluctuations in availability, scale and cost limit its use as biofuel.<sup>62</sup> The insertion of the plant genes responsible for the synthesis of limonene in a host organism (e.g. a bacteria), however, offers a scalable and cheaper alternative through synthetic biology. Limonene has been produced in *E. coli* through an expansion of the celebrated mevalonate pathway,<sup>63</sup> used to produce the antimalarial precursor artemisinin<sup>64</sup> and the biofuel farnesene,<sup>65</sup> and which forms the technological base on which the company Amyris was founded (valued ~\$300M ca. 2019). This

version of the mevalonate pathway is composed of seven genes obtained from such different organisms as *S. cerevesiae*, *S. aureus*, and *E. coli*, to which two genes have been added: a geranyl-diphosphate synthase and a limonene synthase obtained from the plants *A. grandis* and *M. spicata*.

For this demonstration, we use historical data from Alonso-Gutierrez et al.<sup>66</sup>, where 27 different variants of the pathway (using different promoters, induction times and induction strengths) were built. Data collected for each variant involved limonene production and protein expression for each of the nine proteins involved in the synthetic pathway. These data were used to feed Principal Component Analysis of Proteomics (PCAP),<sup>66</sup> an algorithm using principal component analysis to suggest new pathway designs. The PCAP recommendations, used to engineer new strains, resulted in a 40% increase in production for limonene, and 200% for bisabolene (a molecule obtained from the same base pathway). This small amount of conditions (i.e. data rows) available to train the algorithms is typical of synthetic biology/metabolic engineering projects. Although we expect automation to change the picture in the future,<sup>25</sup> the lack of large amounts of data has determined the machine learning approach in ART (i.e. no deep neural networks).

ART is able to not only recapitulate the successful predictions obtained by PCAP improving limonene production, but also provides a systematic way to obtain them as well as the corresponding uncertainty quantification. In this case, the inputs for training ART are the concentrations for each of the nine proteins in the heterologous pathway (features), and the production of limonene (response). The objective is to maximize limonene production. We have data for two DBTL cycles, and we use ART to explore what would have happened if we have used ART instead of PCAP for this project.

We used the data from DBLT cycle 1 to test the predictions provided for ART and suggest new strain designs (i.e. proteomics profiles for the pathway genes). The model trained with the initial 27 conditions provided reasonable cross-validated predictions for production ( $R^2 = 0.37$ ) of this set, as well as the three strains which were created for



**Figure 5: Improving renewable biofuel (limonene) production through ART.** We used the first DBTL cycle data (27 strains) to train ART and recommend new protein targets. The ART recommendations were very similar to the protein concentrations that eventually led to a 40% increase in production (Fig. 6), and the model predicts production levels which are very close to the measured ones in the second DBTL cycle (three blue points in the upper graph). Adding those three points from DBTL cycle 2 provides a total of 30 strains for training that lead to recommendations predicted to exhibit even higher production (bottom part of the graph).

DBTL cycle 2 at the behest of PCAP (Fig. 5). This suggests that ART would have easily recapitulated the PCAP results. Indeed, the ART recommendations are very close to the PCAP recommendations, as shown in Fig. 6. Moreover, the ART recommendations are predicted to exhibit higher production than the PCAP recommendations. While we cannot experimentally test this, ART's capability to predict the result of the PCAP results suggest ART's results would have improved upon those of PCAP. Interestingly, we see that while the quantitative predictions of each of the individual models were not very accurate, they

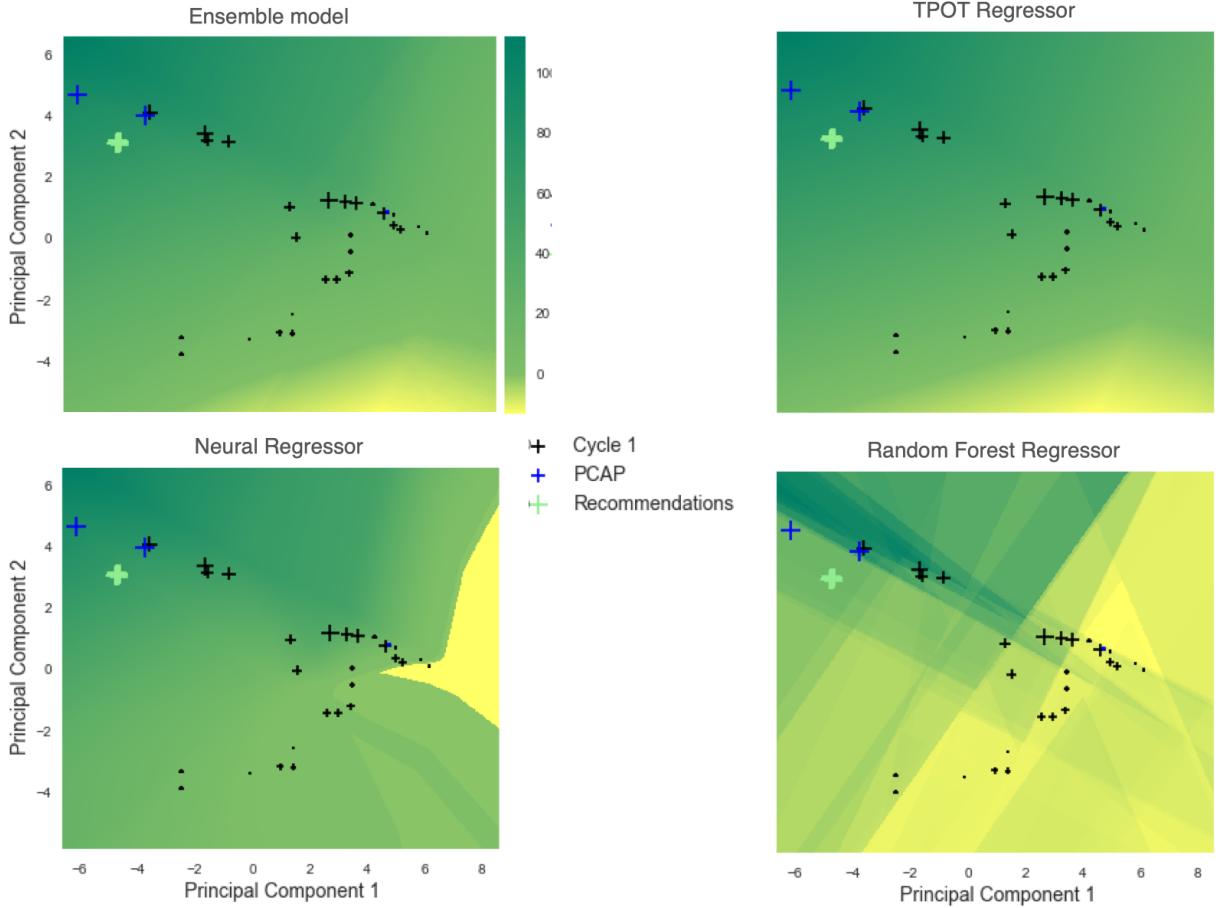
all signaled towards the same direction in order to improve production, hence showing the importance of the ensemble approach (Fig. 6).

Training ART with experimental results from DBTL cycles 1 and 2 results in even better predictions ( $R^2 = 0.60$ ), highlighting the importance of the availability of large amounts of data to train ML models. This new model suggests new sets of strains predicted to produce even higher amounts of limonene.

## Brewing hop flavoured beer without hops by engineering specific levels of production of linalool and geraniol

Our second example involves using the metabolic engineering of yeast (*S. cerevisiae*) to produce hoppy beer without the need for hops. To this end, the ethanol-producing yeast used to brew the beer was modified to also synthesize the metabolites (linalool, L and geraniol, G) that impart hoppy flavor.<sup>67</sup> Synthesizing linalool and geraniol through synthetic biology is economically advantageous because growing hops is water and energetically intensive, and their taste is very variable from crop to crop. Indeed, a startup (Berkeley Brewing Science<sup>68</sup>) was generated from this technology.

ART is able to reproduce the same type of predictions that required correlation analyses and three different types of mathematical models in the original publication, providing a systematic approach to beer flavor design. The challenge is different in this case as compared to the previous case (limonene): instead of trying to maximize production, the goal is to reach a particular level of linalool and geraniol so as to match a known beer tasting profile (e.g. Hop Hunter, Pale Ale, Torpedo or Tropical IPA, Fig. 7). ART can provide this type of recommendations, as well. For this case, the inputs are the levels for the four different proteins involved in the pathway, and the output are the concentrations of the two target molecules (L and G), for which we have desired targets. We have data for two DBTL cycles involving 50 different strains (19 strains for the first DBTL cycle and 31 for the second one, Fig. 7). As in the previous case, we use this data to simulate the outcomes we would have

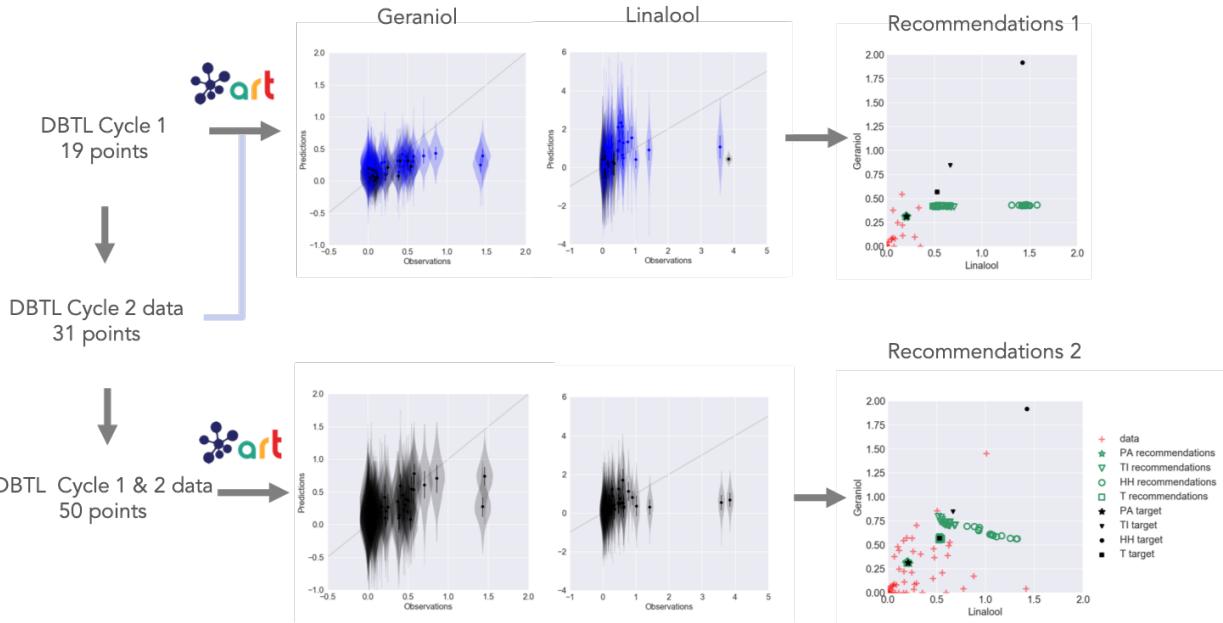


**Figure 6: All machine learning algorithms point in the same direction to improve limonene production, in spite of quantitative differences in prediction.** The color heatmap indicates the limonene production predicted for each point in the proteomics phase space for a Random Forest, a Neural Regressor, TPOT and the final ensemble model that leverages all the models and conforms the base algorithm used by ART. Although the models differ significantly in the actual quantitative predictions of production, the same qualitative trends can be seen in all models (i.e. explore upper left quadrant for higher production), justifying the ensemble approach followed by ART. The ART recommendations (light green) are very close to the PCAP recommendations (blue) that were experimentally tested to improve production by 40%.

obtained in case ART had been available for this project.

The first DBTL cycle provides a limited number of 19 strains to train the model, which performs very well on this training set but poorly on the test set provided by the 31 strains from DBTL cycle 2 (Fig. 7). Despite this small amount of training data and the poor performance on test data, the model trained in DBTL cycle 1 is able to recommend new

protein profiles that are predicted to reach the Pale Ale target (Fig. 7), which are very close to the protein profiles that were eventually used to meet the target. Similarly, this DBTL cycle 1 model was almost able to reach (in predictions) the L & G levels for the Torpedo beer, which will be finally achieved in DBTL cycle 2, once more training data is available. For the Tropical IPA or Hop Hunter, recommendations from this model were not close to the target.



**Figure 7: Using ART to engineer yeast to produce hoppy beer without the need for hops.** The 19 strains in the first DBTL cycles were used to train ART, but it did not show an impressive predictive power. In spite of it, ART is still able to recommend protein profiles predicted to reach the Pale Ale target flavor profile, and others which were close to the Torpedo profile. Adding the 31 strains for the second DBTL cycle allows us to recommend profiles which are predicted to reach targets for all beers except Hop Hunter, which displays a very different metabolite profile from all others.

The model for the second DBTL cycle leverages the full 50 strains from cycles 1 and 2 for training and, although its accuracy is lower, it is enough to provide recommendations that are predicted to attain three out of four targets. The Pale Ale target was already matched in the first cycle and the new recommendations maintain the same profile. The Torpedo target was almost achieved in the first cycle and is predicted to be reached in the second cycle.

The Tropical IPA cycle 1 recommendations were off target, but the cycle 2 recommendations are predicted to reach the desired target. Finally, Hop Hunter L&G levels are very different from all other beers and cycle 1 results, so neither cycle 1 or 2 recommendations can predict protein profiles achieving this profile. ART has not seen any scenarios with such high levels of L&G, and cannot extrapolate well into that part of the metabolic phase space.

## Improving dodecanol production

The final example is one of a failure (or at least a mitigated success), from which as much can be learnt as from the previous successes. Opgenorth et al.<sup>69</sup> used machine learning to drive two DBTL cycles to improve production of 1-dodecanol, a medium-chain fatty acid used in detergents, emulsifiers, lubricants and cosmetics. Although a 20% increase in production was achieved, several shortcomings in this general approach (mapping proteomics data to production) to leverage machine learning to guide metabolic engineering were evidenced: the machine learning algorithms were not able to produce accurate predictions with the low amount of data available for training, and the tools available to reach the desired target protein levels were not accurate enough.

This project consisted of two DBTL cycles comprising 36 and 24 strains, respectively, for three different pathways. The goal was to modulate the protein expression by choosing Ribosome Binding Sites (RBSs, the mRNA sites to which ribosomes bind in order to translate proteins) of different strengths, and test two alternative routes to the final product. The idea was for the machine learning to operate on a small number of variables ( $\sim 3$  RBSs) that, at the same time, provided significant control over the pathway. The input for ART consisted of the concentrations for each of three proteins (different for each of the three pathways), and the goal was to maximize 1-dodecanol production.

The first challenge involved the limited predictive power of the machine learning model in this project. As shown in Fig. 8 for one of the pathways, ART prediction accuracy is completely compromised in this example. The causes seem to be double: a small training

set and a strong connection of the pathway to the rest of host metabolism. The initial 36 strains were divided into three different designs (pathways), decimating the predictive power of ART. This claim is supported by the S5 figure in Opgenorth et al.<sup>69</sup>, which shows the predictive error decreasing with the amount of strains/designs. Now, it is complicated to estimate the number of strains needed for accurate predictions because that depends on the complexity of the problem to be learnt (see synthetic data set section). In this case, the problem is harder to learn than the previous two examples: the mevalonate pathway used in these examples is fully exogenous (i.e. built from external genetic parts) to the final yeast host and hence, free of the metabolic regulation that is certainly present for the dodecanol producing pathway. The dodecanol pathway depends on fatty acid biosynthesis which is vital for cell survival (it produces the cell membrane), and has to be therefore tightly regulated. This characteristic makes it more difficult to learn its behavior by ART using only pathway protein levels (instead of adding also proteins from other parts of host metabolism).

A second challenge compounding the first one involves being unable to reach the target protein levels proposed by ART to increase production. This difficulty precludes not only bioengineering, but also testing the validity of the ART model. For this case, both the mechanistic (RBS calculator<sup>70,71</sup>) and machine learning-based (EMOPEC<sup>72</sup>) tools proved to be very inaccurate for bioengineering purposes: e.g. a prescribed 6-fold increase in protein expression could only be matched with a 2-fold increase. Moreover, non-target effects (i.e. changing the RBS for a gene significantly affects protein expression for other genes in the pathway) were abundant, further adding to the difficulty.

A third, unexpected, challenge was the inability of constructing several strains in the Build phase due to toxic effects engendered by the proposed protein profiles. This phenomenon materialized through mutations in the final plasmid in the production strain or no colonies after the transformation. The prediction of these effects in the Build phase represents an important target for future ML efforts, with the capability to not only enhance bioengineering but reveal fundamental biological knowledge.

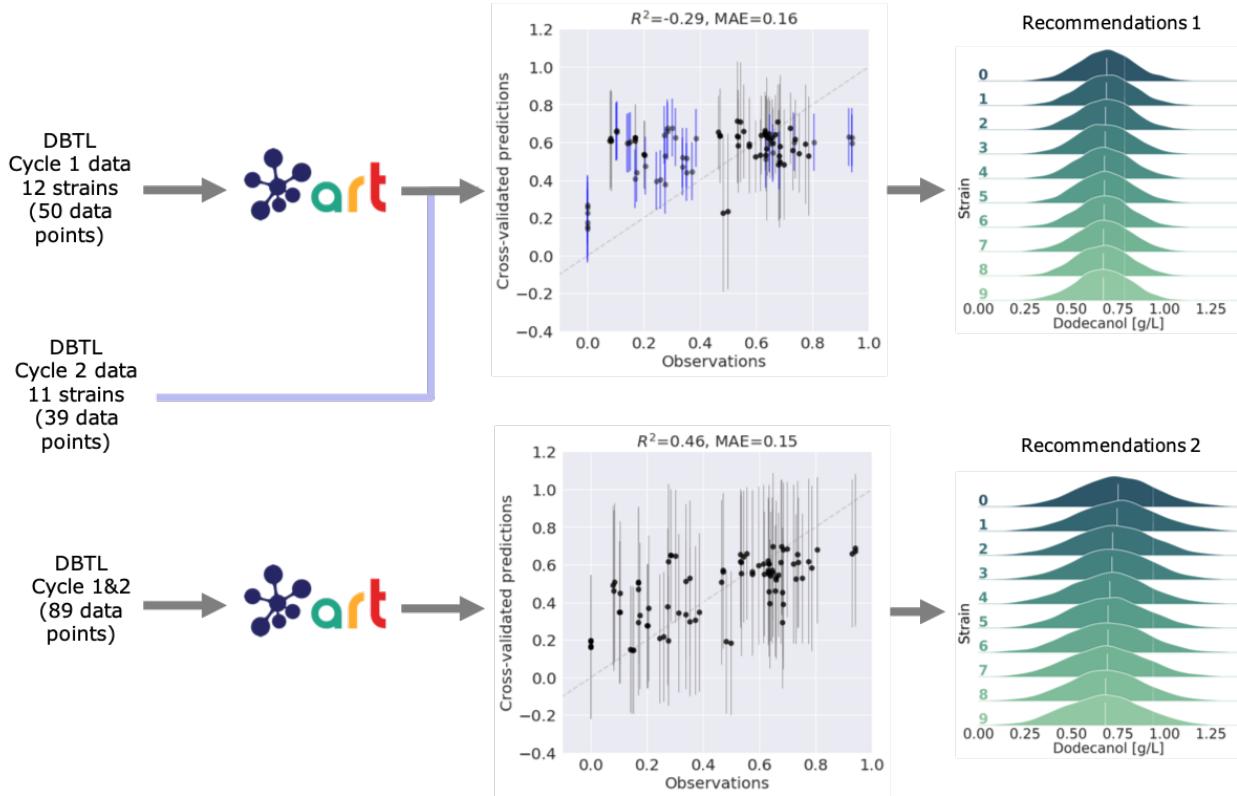


Figure 8: **Leveraging ART to improve dodecanol production.** The scarce amount of initial data (12 strains) plus the strong tie of the pathway to metabolism (fatty acids) produce a model that is scarcely predictive (top of the graph). Adding data from both cycles (1 and 2) improves predictions notably (bottom graph).

In spite of all these challenges, the ML-based approach was able to improve production with respect to the first cycle by  $\sim 20\%$ ,<sup>69</sup> 6-fold higher than the best published titer for the same fermentation conditions.<sup>73</sup> While this  $\sim 20\%$  was not transformational, one can imagine systematically applying this approach in several DBTL cycles and, if comparable increases are obtained, obtaining gains of  $\sim 250\%$  in 5 DBTL cycles or  $\sim 620\%$  in 10 cycles.

## Conclusion

ART is a tool that provides synthetic biologists easy access to machine learning techniques. ART takes as input a set of vectors of measurements (e.g. a set of proteomics measurements for several proteins, or transcripts for several genes) along with their corresponding systems responses (e.g. associated biofuel production) and provides a predictive model, as well as recommendations for the next round (e.g. new proteomics targets predicted to improve production in the next round).

ART combines the scikit-learn library with Bayesian inference and MCMC sampling, and is optimized for the conditions encountered in metabolic engineering: small sample sizes, recursive DBTL cycles and the need for uncertainty quantification. ART uses a novel ensemble approach where the weight of each ensemble model is considered a random variable with a probability distribution constrained by the available data. Unlike other approaches, this method does not require the ensemble models to be probabilistic in nature, hence allowing us to fully exploit the popular scikit-learn library to increase accuracy by leveraging a diverse set of models. This weighted ensemble model produces a simple, yet powerful, approach to quantify uncertainty, a critical capability when dealing with small data sets and a crucial component of AI in biological research.<sup>48</sup> While ART is adapted to synthetic biology's special needs and characteristics, its implementation is general enough that it is easily applicable to other problems of similar characteristics. ART is perfectly integrated with the Experiment Data Depot<sup>40</sup> and the Inventory of Composable Elements,<sup>74</sup> forming part of a growing family of tools that standardize and facilitate synthetic biology.

We have showcased the use of ART on a case with synthetic data sets and three real metabolic engineering cases from the published literature. The synthetic data case involves data generated for several production landscapes of increasing complexity and dimensionality. This case allowed us to test ART for different difficulties of the production landscape to be learnt by the algorithms, as well as different numbers of DBTL cycles. We have seen that while easy landscapes provide production increases readily after the first cycle, the most complicated ones require  $> 5$  cycles to start producing satisfactory results. In all cases, results improved with the number of DBTL cycles, underlying the importance of designing experiments that continue for  $\sim 10$  cycles rather than halting the project if results do not improve in the first few cycles.

The demonstration cases using real data involve engineering *E. coli* and *S. cerevisiae* to produce the renewable biofuel limonene, synthesize metabolites that produce hoppy flavor in beer, and generate dodecanol from fatty acid biosynthesis. Although we were able to produce useful recommendations with as low as 11 (hopless beer) or 27 (limonene) conditions, we also found situations in which such low amounts of data was insufficient for meaningful predictions (dodecanol). It is impossible to determine a priori how much data will be necessary for accurate predictions, since this depends on the difficulty of the relationships to be learnt (e.g. the amount of coupling between the studied pathway and host metabolism). However, one thing is clear: two DBTL cycles (which was as much as was available for all these examples) are rarely sufficient for guaranteed convergence of the learning process. We do find, though, that accurate quantitative predictions are not required to effectively guide bioengineering: our ensemble approach can successfully leverage qualitative agreement between the models in the ensemble to compensate for the lack of accuracy. Among the possible pitfalls in the current approach is the possibility that recommended target protein profiles cannot be accurately reached, since the tools to produce specified protein levels are still imperfect. This area needs further investment in order to accelerate bioengineering and make it more reliable, hence enabling design to a required specification.

While ART is a useful tool in guiding bioengineering, it represents just an initial step in applying machine learning to synthetic biology. Future improvements under consideration include adding a pathway cost (\$) function, enabling categorical/discrete input variables, the inclusion of classification problems, adding new optimization methods, incorporating covariance of level-0 models into the ensemble model, and incorporating input space errors into learners. These may not be the preferred list of improvements for every user, so ART’s dual license allows for modification by third parties for research purposes, as long as the modifications are offered to the original repository. Hence, users are encouraged to enhance it in ways that satisfy their needs. Commercial users must license the software (see <https://github.com/JBEI/AutomatedRecommendationTool> for details).

ART provides effective decision-making in the context of synthetic biology, and facilitates the combination of machine learning and automation that can disrupt synthetic biology.<sup>25</sup> Combining ML with recent developments in macroscale lab automation,<sup>56,75</sup> microfluidics<sup>21,39,76–78</sup> and cloud labs<sup>79</sup> may enable self-driving laboratories,<sup>80</sup> which augment automated experimentation platforms with artificial intelligence to facilitate autonomous experimentation. We believe that fully leveraging AI and automation can catalyze a similar step forward in synthetic biology as CRISPR-enabled genetic editing, high-throughput multi-omics phenotyping, and exponentially growing DNA synthesis capabilities have produced in the recent past.

## Competing interests

The authors declare that they have no competing interests.

## Author’s contributions

Z.C., T.R. and H.G.M. conceived the original idea. T.R. and Z.C. developed methodology, designed the software, wrote the code and performed computer experiments. T.R. designed

simulated benchmarks and performed numerical experiments. T.R. analyzed all results. H.G.M., T.R. and Z.C. wrote the paper.

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## Supplementary material

### Markov Chain Monte Carlo sampling

The posterior distribution  $p(\boldsymbol{\theta}|\mathcal{D})$  (probability that the parameters  $\theta$  fit the data  $\mathcal{D}$ , used in equation 2) is obtained by applying Bayes' formula. The posterior is defined through a prior  $p(\boldsymbol{\theta})$  and a likelihood function  $p(\mathcal{D}|\boldsymbol{\theta})$ , i.e.

$$p(\boldsymbol{\theta}|\mathcal{D}) \propto p(\mathcal{D}|\boldsymbol{\theta})p(\boldsymbol{\theta}).$$

We define the prior to be  $p(\boldsymbol{\theta}) = p(\mathbf{w})p(\sigma)$ , where  $p(\mathbf{w})$  is a Dirichlet distribution with uniform parameters, which ensures the constraint on weights is satisfied, and  $p(\sigma)$  is a half normal distribution with mean = 0 and standard deviation = 10. The likelihood function follows directly from Eq. (1).

### Expected value and variance for ensemble model

From equation (1), we can easily compute the expected value

$$\mathbb{E}(y) = \mathbb{E}(\mathbf{w}^T \mathbf{f} + \varepsilon) = \mathbb{E}(\mathbf{w})^T \mathbf{f} \quad (5)$$

and variance

$$\text{Var}(y) = \mathbf{f}^T \text{Var}(\mathbf{w}) \mathbf{f} + \text{Var}(\varepsilon) \quad (6)$$

of the response, which will be needed for the optimization phase in order to create the surrogate function  $G(\mathbf{x})$  (Eq. 3).

### Related work and novelty of our ensemble approach.

Our ensemble approach is based on stacking,<sup>50</sup> where different ensemble members are trained on the same training set and whose outputs are then combined, as opposed to techniques

that manipulate the training set (e.g. bagging<sup>81</sup>) or those that sequentially add new models into the ensemble (e.g. boosting<sup>82</sup>). Different approaches for constructing ensemble of models using the Bayesian framework have been already considered. For example, Bayesian Model Averaging (BMA)<sup>44</sup> builds an ensemble model as a linear combination of the individual members in which the weights are given by the posterior probabilities of models. The weights therefore crucially depend on marginal likelihood under each model, which is challenging to compute. BMA accounts for uncertainty about which model is correct but assumes that only one of them is, and as a consequence, it has the tendency of selecting the one model that is closest to the generating distribution. Agnostic Bayesian learning of ensembles<sup>83</sup> differs from BMA in the way the weights are calculated. Instead of finding the best predictor from the model class (assuming that the observed data is generated by one of them), this method aims to find the best predictor in terms of the lowest expected loss. The weights are calculated as posterior probability that each model is the one with the lowest loss. Bayesian model combination (BMC)<sup>45</sup> seeks the combination of models that is closest to the generating distribution by heavily weighting the most probable combination of models, instead of doing so for the most probable one. BMC samples from the space of possible ensembles by randomly drawing weights from a Dirichlet distribution with uniform parameters. The Bayesian Additive Regression Trees (BART)<sup>47</sup> method is one of the homogeneous ensemble approaches. It models the ensemble as a (nonweighted) sum of regression trees whose parameters, and ensemble error standard deviation, are defined thought their posterior distributions given data and sampled using MCMC. Yao et al.<sup>46</sup> suggest a predictive model in terms of a weighted combination of predictive distributions for each probabilistic model in the ensemble. This approach can be seen as a generalization of stacking for point estimation to predictive distributions.

All of these models, except of BMC and our model, have weights being point estimates, obtained usually by minimizing some error function. In contrast, we define them as random variables, and in contrast to BMC, our weights are defined through full joint posterior distri-

bution given data. BMC is the closest in design to our approach, but it was formulated only in the context of classifiers. Only BART does include a random error term in the ensemble, apart from our model. Unlike BMA, BMC or models of Yao et al.<sup>46</sup>, Chipman et al.<sup>47</sup>, our approach does not require that the predictors are themselves probabilistic, and therefore can readily leverage various scikit-learn models. The main differences are summarized in Table S1.

Table S1: Feature differences between Bayesian based ensemble modeling approaches.

Method	Weighted average	Probabilistic base models	Probabilistic weights	Regression	Classification	Ensemble error term
BMA <sup>44</sup>	✓	✓	✗	✓	✓	✗
BMC <sup>45</sup>	✓	✓	✓✗	✗	✓	✗
BART <sup>47</sup>	✗	✓	✗	✓	✗	✓
Stacking predictive distributions <sup>46</sup>	✓	✓	✗	✓	✓	✗
Agnostic Bayes <sup>83</sup>	✓	✓✗	✗	✓	✓	✗
This work	✓	✗	✓	✓	✗	✓

Although our model has some features that were previously considered in the literature, the approach presented here however, to the best of our knowledge, is novel in the fact that the metalearner is modeled as a Bayesian linear regression model, whose parameters are inferred from data combined with a prior that satisfies the constraints on the ‘voting’ nature of ensemble learners.

## Input space set $\mathcal{B}$

The bounds for the input space  $\mathcal{B}$  for  $G(\mathbf{x})$  (Eq. 3) can be provided by the user (see details in the Implementation subsection). Otherwise, default values are computed from the input

data defining the feasible space as:

$$\begin{aligned}\mathcal{B} &= \{\tilde{\mathbf{x}} \in \mathbb{R}^D \mid L_d - \Delta_d \leq \tilde{x}^d \leq U_d + \Delta_d, d = 1, \dots, D\} \\ \Delta_d &= (U_d - L_d)\epsilon ; \quad U_d = \max_{1 \leq n \leq N}(x_n^d) ; \quad L_d = \min_{1 \leq n \leq N}(x_n^d) \\ (\mathbf{x}_n, y_n) &\in \mathcal{D}, \quad n = 1, \dots, N\end{aligned}\tag{7}$$

## Success probability calculation

Our probabilistic model enables us to quantify uncertainty by systematically estimating the probability of success for the provided recommendations. Of practical interest are the probability that a single recommendation is successful, and the probability that at least one recommendation of several provided is successful.

We define success for response  $y$  (in general) through set  $\mathcal{S} = \{y \mid y \sim p_{\mathcal{S}}(y)\}$ , where probability distribution for success is

$$p_{\mathcal{S}}(y) = \begin{cases} \mathcal{U}(y^*, U) & \text{(maximization case)} \\ \mathcal{U}(L, y^*) & \text{(minimization case)} \\ \mathcal{N}(y^*, \sigma_{y^*}^2) & \text{(specification case)}, \end{cases}\tag{8}$$

with  $y^*$  being a success value defined by the user (e.g. the best production so far improved by a factor of 20% for the maximization case);  $L, U$  the lower and upper bounds, respectively, for the uniform distribution; and  $\sigma_{y^*}^2$  being the variance of the normal distribution around the target value  $y^*$  for the specification case. The success probability of interest, i.e. the posterior probability distribution of success given recommendation  $\mathbf{x}^r$  is then

$$p(\mathcal{S} \mid \mathbf{x}^r) = \int p_{\mathcal{S}}(y) p(y \mid \mathbf{x}^r, \mathcal{D}) dy$$

and is approximated using draws from the posterior predictive distribution (2) as

$$p(\mathcal{S}|\mathbf{x}^r) \approx \begin{cases} \frac{1}{N_s} \sum_{i=1}^{N_s} \mathbb{I}_{\mathcal{S}}(y_i) & \text{(maximization/minimization case)} \\ \frac{1}{N_s} \sum_{i=1}^{N_s} \mathcal{N}(y_i; y^*, \sigma_{y^*}^2) & \text{(specification case)} \end{cases} \quad (9)$$

where  $y_i \sim p(y|\mathbf{x}^r, \mathcal{D})$ ,  $i = 1, \dots, N_s$ , and  $\mathbb{I}_{\mathcal{A}}(y) = 1$  if  $y \in \mathcal{A}$ , 0 if  $y \notin \mathcal{A}$ .

In case of multiple recommendations  $\{\mathbf{x}^r\}$ , we provide the probability of success of least one of the recommendations only for maximization and minimization types of objectives. This probability is calculated as one minus the probability  $p(\mathcal{F}|\{\mathbf{x}^r\})$  that all recommendations fail, where

$$p(\mathcal{F}|\{\mathbf{x}^r\}) \approx \frac{1}{N_s} \sum_{i=1}^{N_s} \mathbb{I}_{\mathcal{F}}(\{y_i^r\}), \quad \{y_i^r\} \sim p(y|\{\mathbf{x}^r\}, \mathcal{D}), i = 1, \dots, N_s, r = 1, \dots, N_r,$$

and the *failure* set  $\mathcal{F} = \{\{y^r\} | y^r \notin \mathcal{S}, \forall r = 1, \dots, N_r\}$  consists of outcomes that are not successes for all of the recommendations. Since the chosen recommendations are not necessarily independent, we sample  $\{y_i^r\}$  jointly for all  $\{\mathbf{x}^r\}$ , i.e.  $i$ -th sample has the same model parameters  $(w_i, \sigma_i, \varepsilon_{ij} \sim \mathcal{N}(0, \sigma_i^2)$  from Eq. 1) for all recommendations.

In case of multiple recommendations  $\{\mathbf{x}^r\}$ , we calculate the probability of success of at least one of the recommendations as one minus the probability that all recommendations fail, i.e.  $1 - p(\mathcal{F}|\{\mathbf{x}^r\})$ , where

$$p(\mathcal{F}|\{\mathbf{x}^r\}) \approx \frac{1}{N_s} \sum_{i=1}^{N_s} \mathbb{I}_{\mathcal{F}}(\{y_i^r\}), \quad \{y_i^r\} \sim p(y|\{\mathbf{x}^r\}, \mathcal{D}), i = 1, \dots, N_s, r = 1, \dots, N_r,$$

and the *failure* set  $\mathcal{F} = \{\{y^r\} | y^r \notin \mathcal{S}, \forall r = 1, \dots, N_r\}$  consists of outcomes that are not successes for all of the recommendations. Since the chosen recommendations are not necessarily independent, we sample  $\{y_i^r\}$  jointly for all  $\{\mathbf{x}^r\}$ , i.e.  $i$ -th sample has the same model parameters  $(w_i, \sigma_i, \varepsilon_{ij} \sim \mathcal{N}(0, \sigma_i^2)$  from Eq. 1) for all recommendations.

## Multiple response variables

For multiple response variable problems (e.g. trying to hit a predetermined value of metabolite  $a$  and metabolite  $b$  simultaneously, as in the case of the hopless beer), we assume that the response variables are conditionally independent given input vector  $\mathbf{x}$ , and build a separate predictive model  $p_j(y_j|\mathbf{x}, \mathcal{D})$  for each variable  $y_j, j = 1, \dots, J$ . We then define the objective function for the optimization phase as

$$G(\mathbf{x}) = (1 - \alpha) \sum_{j=1}^J \mathbb{E}(y_j) + \alpha \sum_{j=1}^J \text{Var}(y_j)^{1/2}$$

in case of maximization, and analogously adding the summation of expectation and variance terms in the corresponding functions for minimization and specification objectives (Eq. 3). The probability of success for multiple variables is then defined as

$$p(\mathcal{S}_1, \dots, \mathcal{S}_J | \mathbf{x}) = \prod_{j=1}^J p(\mathcal{S}_j | \mathbf{x}^r)$$

Future work will address the limitation of the independence assumption and take into account possible correlations among multiple response variables.

## Pseudo algorithm for recommendations

### Implementation

#### Modules

`art.py` is the core module that defines the class `RecommendationEngine` with functions for loading data (into the format required for machine learning models), building predictive models and optimization.

Module `constants.py` contains assignments to all constants appearing throughout other modules. Those include default values for some of the optional user input parameters (Ta-

---

**Algorithm 1**

---

```
1: Input:  $N_r$ : number of recommendations  
    $\{\mathbf{x}_n\}_{n=1}^{N_s}$ : samples from  $\pi(\mathbf{x})$   
    $E_a$ : engineering accuracy  
    $\mathcal{D}_{\mathbf{x}}$ : input variable experimental data  
   type of the distance between recommendations  
2: Output: XXXXX  
3:  $draws \leftarrow \{\mathbf{x}_n\}_{n=1}^{N_s}$  {remaining draws}  
4:  $rec = \emptyset$   
5: while  $i = 1, \dots, N_r$  do  
6:    $r \leftarrow$  a sample from  $draws$  with maximal  $F(\mathbf{x})$  { $F(\mathbf{x})$  is already calculated}  
7:   if check the condition***** then  
8:      $rec = \{rec, r\}$   
9:   end if  
10:   $draws \leftarrow draws \setminus$  set of all draws with the same  $F(\mathbf{x})$   
11: end while  
12: return  $rec$ 
```

---

ble S3), hyperparameters for `scikit-learn` models and simulation setups for PyMC3 and `PTMCMCSampler` functions.

Module `utilities.py` is a suite of functions that facilitate ART’s computations but can be used independently. It includes functions for loading studies (with `edd-utils` or directly from files), metrics for evaluation of predictive models, identifying and filtering noisy data, etc.

Module `plot.py` contains a set of functions for visualization of different quantities obtained during an ART run, including functions of relevance to final users (e.g. true vs. predicted values) as well as those providing insights into intermediate steps (e.g. predictive models surfaces, space exploration from optimization, recommendations distributions).

All modules can be easily further extended by future contributors to ART.

## Importing a study

Studies can be loaded directly from EDD by calling a function from the `utility.py` module that relies on `edd-utils` package:

```
dataframe = load_study(edd_study_slug=edd_study_slug, edd_server=edd_server)
```

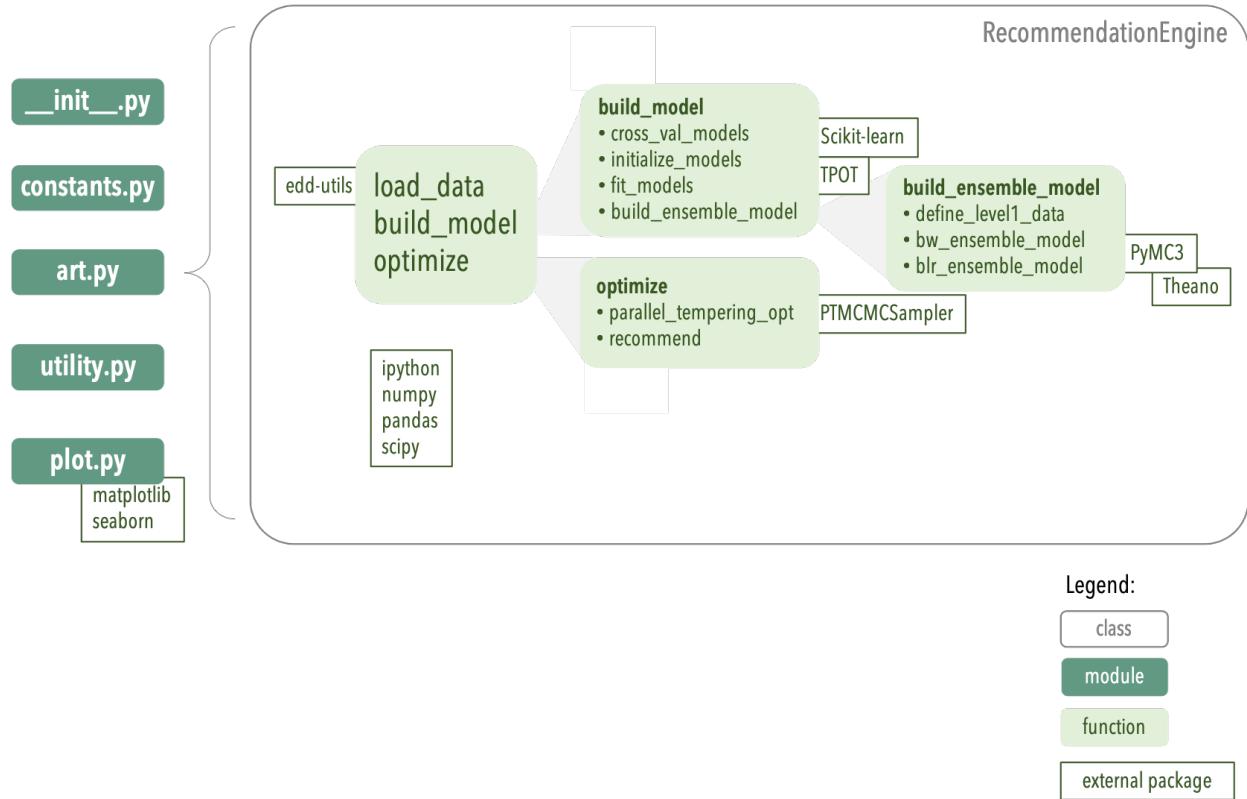


Figure S1: The main ART source code structure and dependencies.

The user should provide the study slug (last part of the study web address) and, if the study is not located on the default (public) EDD server, the url to the EDD server. Alternatively, a study can be loaded from an EDD-style csv file, by providing a path to the file and calling the same function

```
dataframe = load_study(data_file=data_file)
```

Either approach will return a pandas dataframe containing all information in the study, which can be pre-processed before running ART, if needed.

## Running ART

ART can be run by instantiating an object from the `RecommendationEngine` class by:

```
art = RecommendationEngine(dataframe, **art_params)
```

The first argument is the dataframe created in the previous step (from an EDD study

or data file import). If there is no data preprocessing, the dataframe is ready to be passed as an argument. Otherwise, the user should make sure that the dataframe contains at least the required columns: `Line Name`, `Measurement Type` and `Value`. Furthermore, line names should always contain a hyphen (“-”) denoting replicates (see Table S2), and this character should be exclusively used for this purpose (this is critical for creating partitions for cross-validation).

Table S2: Valid and non valid examples of entries of the `Line Name` column in the dataframe passed to start an ART run.

<span style="color: green;">✓</span> Valid	<span style="color: red;">✗</span> Non valid
<code>LineNameX-1</code>	<code>LineNameX1</code>
<code>LineNameX-2</code>	<code>LineNameX2</code>
<code>LineNameX-r1</code>	<code>Line-NameX1</code>
<code>LineNameX-r2</code>	<code>Line-NameX2</code>
<code>LineNameX-R1</code>	<code>Line-Name-X1</code>
<code>LineNameX-R2</code>	<code>Line-Name-X2</code>
...	...

The second argument is a dictionary of key-value pairs defining several required and optional keyword arguments (summarized in Table S3) for generation of the `art` object.

### *Building the model*

The level-0 models are first initialized and then fitted through the `_initialize_models` and `_fit_models` functions respectively, which rely on the `scikit-learn` and `tpot` packages. To build the final predictive model, first the level-1 data is created by storing cross-validated predictions of level-0 models into a `theano` variable that is shared across the functions from the PyMC3 package. Finally, the parameters of the ensemble model are sampled within the function `_ensemble_model`, which stores the inferred model and traces that are later used for predictive posterior probability calculation, as well as first and second moments from the traces, used for estimation of the first two moments of the predictive posterior distribution using Eq. (5)–(6).

By default, ART builds the models using all available data and evaluates the final, ensemble model, as well as all level-0 models, on the same data. Optionally, if specified by the user

Table S3: ART input parameters. Required parameters are marked with an asterisk.

Name	Meaning
<code>input_var</code>	List of input variables*
<code>bounds_file</code>	Path to the file with upper and lower bounds for each input variable (default <code>None</code> )
<code>response_var</code>	List of response variables*
<code>build_model</code>	Flag for building a predictive model (default <code>True</code> )
<code>cross_val</code>	Flag for performing cross-validation (default <code>False</code> )
<code>ensemble_model</code>	Type of the ensemble model (default ‘BW’)
<code>num_models</code>	Number of level-0 models (default 8)
<code>recommend</code>	Flag for performing optimization and providing recommendations (default <code>True</code> )
<code>objective</code>	Type of the objective (default ‘maximize’)
<code>threshold</code>	Relative threshold for defining success (default 0)
<code>target_value</code>	Target value for the specification objective (default <code>None</code> )
<code>num_recommendations</code>	Number of recommendations for the next cycle (default 16)
<code>rel_eng_accuracy</code>	Relative engineering accuracy or required relative distance between recommendations (default 0.2)
<code>niter</code>	Number of iterations to use for $T = 1$ chain in parallel tempering (default 100000)
<code>alpha</code>	Parameter determining the level of exploration during the optimization (value between 0 and 1, default <code>None</code> )
<code>output_directory</code>	Path of the output directory (default <code>./results/response_var_time_suffix</code> )
<code>verbose</code>	Amount of information displayed (default 0)
<code>seed</code>	Random seed for reproducible runs (default <code>None</code> )

through the input flag `cross_val`, ART will evaluate the models on 10-fold cross-validated predictions, through the function `_cross_val_models`. This computation lasts roughly 10 times longer. Evaluating models on new data, unseen by the models, can also be done by calling:

```
art.evaluate_models(X=X_new, y=y_new)
```

### *Optimization*

ART performs optimization by first creating a set of draws from

```
draws = art.parallel_tempering_opt()
```

which relies on the `PTMCMCSampler` package. Here, an object from the class `TargetModel` is being created. This class provides a template for and can be replaced by other types of ob-

jective functions (or target distributions) for parallel tempering type of optimization, as long as it contains functions defining loglikelihood and logprior calculation (see Eq. 4). Also, the whole optimization procedure may well be replaced by an alternative routine. For example, if the dimension of the input space is relatively small, a grid search could be performed, or even evaluation of the objective at each point for discrete variables. Lastly, out of all draws collected by optimizing the specified objective, ART finds a set of recommendations by `art.recommend(draws, rel_eng_accuracy=rel_eng_accuracy, distance_type='at_least_one')` which ensures that each recommendation is different from all others and all input data by a factor of `rel_eng_accuracy` ( $\gamma$ ) in at least one of the components.

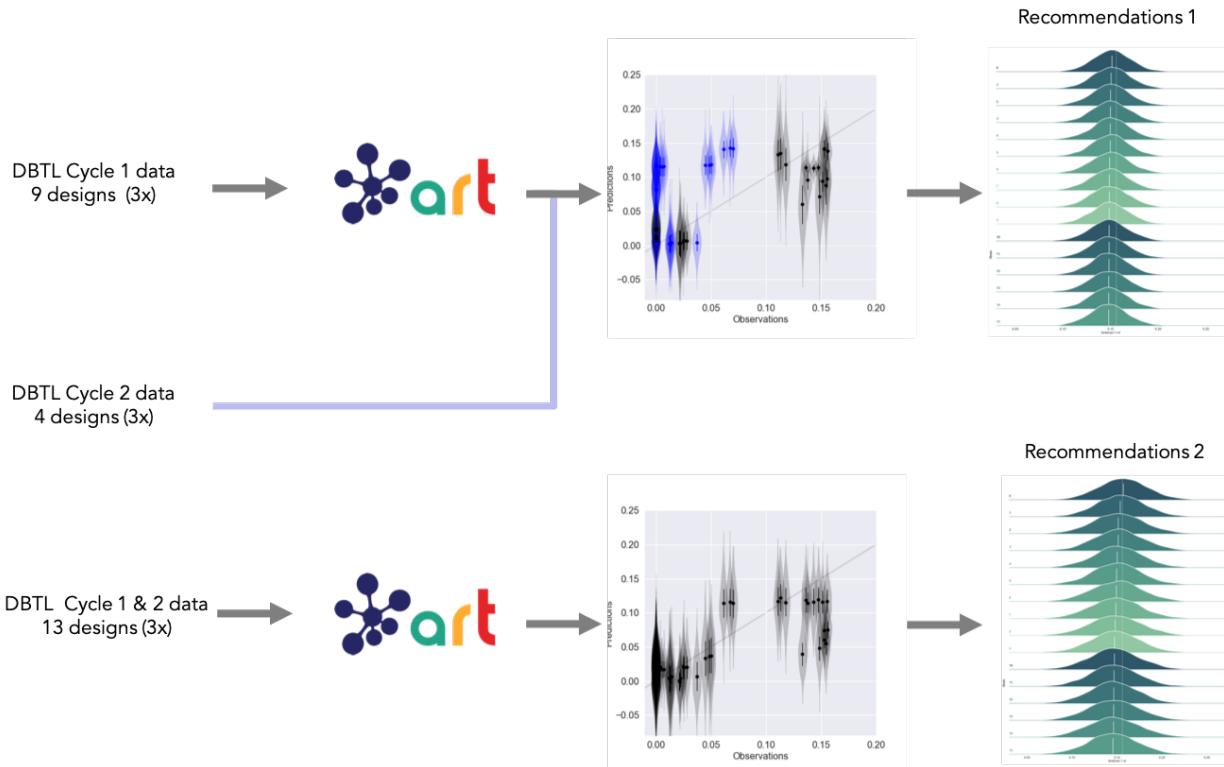


Figure S2: Dodecanol Pathway 2

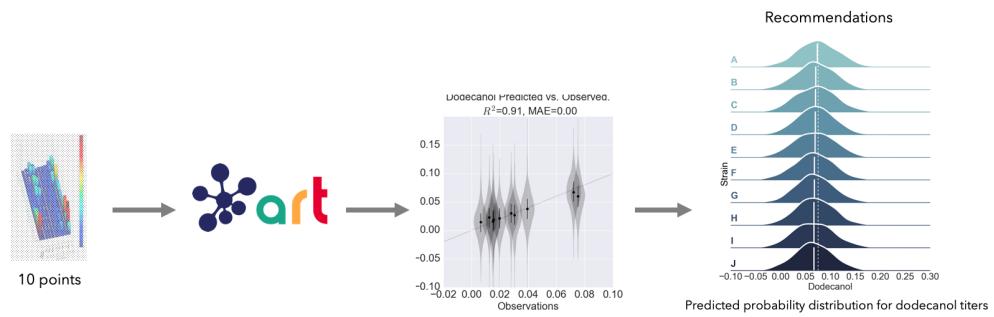


Figure S3: Dodecanol Pathway 3

## References

- (1) Stephanopoulos, G. Metabolic fluxes and metabolic engineering. *Metabolic engineering* **1999**, *1*, 1–11.
- (2) Beller, H. R.; Lee, T. S.; Katz, L. Natural products as biofuels and bio-based chemicals: fatty acids and isoprenoids. *Natural product reports* **2015**, *32*, 1508–1526.
- (3) Chubukov, V.; Mukhopadhyay, A.; Petzold, C. J.; Keasling, J. D.; Martín, H. G. Synthetic and systems biology for microbial production of commodity chemicals. *npj Systems Biology and Applications* **2016**, *2*, 16009.
- (4) Ajikumar, P. K.; Xiao, W.-H.; Tyo, K. E.; Wang, Y.; Simeon, F.; Leonard, E.; Mucha, O.; Phon, T. H.; Pfeifer, B.; Stephanopoulos, G. Isoprenoid pathway optimization for Taxol precursor overproduction in *Escherichia coli*. *Science* **2010**, *330*, 70–74.
- (5) Cann, O. These are the top 10 emerging technologies of 2016. World Economic Forum website <https://www.weforum.org/agenda/2016/06/top-10-emergingtechnologies-2016>. 2016.
- (6) Council, N. R.; ADDAUTHORS, *Industrialization of Biology: A Roadmap to Accelerate the Advanced Manufacturing of Chemicals*; National Academies Press, 2015.
- (7) Yadav, V. G.; De Mey, M.; Lim, C. G.; Ajikumar, P. K.; Stephanopoulos, G. The future of metabolic engineering and synthetic biology: towards a systematic practice. *Metabolic engineering* **2012**, *14*, 233–241.
- (8) Hodgman, C. E.; Jewett, M. C. Cell-free synthetic biology: thinking outside the cell. *Metabolic engineering* **2012**, *14*, 261–269.
- (9) Kurian, J. V. A new polymer platform for the future—Sorona® from corn derived 1, 3-propanediol. *Journal of Polymers and the Environment* **2005**, *13*, 159–167.

- (10) Cameron, D. E.; Bashor, C. J.; Collins, J. J. A brief history of synthetic biology. *Nature Reviews Microbiology* **2014**, *12*, 381.
- (11) Kyrou, K.; Hammond, A. M.; Galizi, R.; Kranjc, N.; Burt, A.; Beaghton, A. K.; Nolan, T.; Crisanti, A. A CRISPR–Cas9 gene drive targeting doublesex causes complete population suppression in caged *Anopheles gambiae* mosquitoes. *Nature biotechnology* **2018**, *36*, 1062.
- (12) Temme, K.; Tamsir, A.; Bloch, S.; Clark, R.; Emily, T.; Hammill, K.; Higgins, D.; Davis-Richardson, A. Methods and compositions for improving plant traits. 2019; US Patent App. 16/192,738.
- (13) Chen, Y.; Guenther, J. M.; Gin, J. W.; Chan, L. J. G.; Costello, Z.; Ogorzalek, T. L.; Tran, H. M.; Blake-Hedges, J. M.; Keasling, J. D.; Adams, P. D.; Garcia Martin, H.; Hillson, N. J.; Petzold, C. J. Automated “Cells-To-Peptides” Sample Preparation Workflow for High-Throughput, Quantitative Proteomic Assays of Microbes. *Journal of proteome research* **2019**, *18*, 3752–3761.
- (14) Fuhrer, T.; Zamboni, N. High-throughput discovery metabolomics. *Current opinion in biotechnology* **2015**, *31*, 73–78.
- (15) Stephens, Z. D.; Lee, S. Y.; Faghri, F.; Campbell, R. H.; Zhai, C.; Efron, M. J.; Iyer, R.; Schatz, M. C.; Sinha, S.; Robinson, G. E. Big data: astronomical or genonomical? *PLoS biology* **2015**, *13*, e1002195.
- (16) Ma, S.; Tang, N.; Tian, J. DNA synthesis, assembly and applications in synthetic biology. *Current opinion in chemical biology* **2012**, *16*, 260–267.
- (17) Doudna, J. A.; Charpentier, E. The new frontier of genome engineering with CRISPR-Cas9. *Science* **2014**, *346*, 1258096.

- (18) Cumbers, J. Synthetic Biology Has Raised \$12.4 Billion. Here Are Five Sectors It Will Soon Disrupt. 2019; <https://www.forbes.com/sites/johncumbers/2019/09/04/synthetic-biology-has-raised-124-billion-here-are-five-sectors-it-will-soon-disrupt/#40b2b2cb3a14>.
- (19) Petzold, C. J.; Chan, L. J. G.; Nhan, M.; Adams, P. D. Analytics for metabolic engineering. *Frontiers in bioengineering and biotechnology* **2015**, *3*, 135.
- (20) Nielsen, J.; Keasling, J. D. Engineering cellular metabolism. *Cell* **2016**, *164*, 1185–1197.
- (21) Gardner, T. S. Synthetic biology: from hype to impact. *Trends in biotechnology* **2013**, *31*, 123–125.
- (22) Prinz, F.; Schlange, T.; Asadullah, K. Believe it or not: how much can we rely on published data on potential drug targets? *Nature reviews Drug discovery* **2011**, *10*, 712.
- (23) Baker, M. 1,500 scientists lift the lid on reproducibility. *Nature News* **2016**, *533*, 452.
- (24) Begley, C. G.; Ellis, L. M. Drug development: Raise standards for preclinical cancer research. *Nature* **2012**, *483*, 531.
- (25) Carbonell, P.; Radivojević, T.; Martin, H. G. Opportunities at the Intersection of Synthetic Biology, Machine Learning, and Automation. *ACS Synth. Biol.* **2019**, *8*, 1474–1477.
- (26) Thrun, S. Toward robotic cars. *Communications of the ACM* **2010**, *53*, 99–106.
- (27) Wu, Y.; Schuster, M.; Chen, Z.; Le, Q. V.; Norouzi, M.; Macherey, W.; Krikun, M.; Cao, Y.; Gao, Q.; Macherey, K.; ADDAUTHORS, Google’s neural machine translation system: Bridging the gap between human and machine translation. *arXiv preprint arXiv:1609.08144* **2016**,

- (28) Kosinski, M.; Stillwell, D.; Graepel, T. Private traits and attributes are predictable from digital records of human behavior. *Proceedings of the National Academy of Sciences* **2013**, *110*, 5802–5805.
- (29) Costello, Z.; Martin, H. G. A machine learning approach to predict metabolic pathway dynamics from time-series multiomics data. *NPJ systems biology and applications* **2018**, *4*, 19.
- (30) Jervis, A. J.; Carbonell, P.; Vinaixa, M.; Dunstan, M. S.; Hollywood, K. A.; Robinson, C. J.; Rattray, N. J.; Yan, C.; Swainston, N.; Currin, A.; ADDAUTHORS, Machine learning of designed translational control allows predictive pathway optimization in *Escherichia coli*. *ACS synthetic biology* **2018**, *8*, 127–136.
- (31) Esteva, A.; Kuprel, B.; Novoa, R. A.; Ko, J.; Swetter, S. M.; Blau, H. M.; Thrun, S. Dermatologist-level classification of skin cancer with deep neural networks. *Nature* **2017**, *542*, 115.
- (32) Paeng, K.; Hwang, S.; Park, S.; Kim, M. *Deep Learning in Medical Image Analysis and Multimodal Learning for Clinical Decision Support*; Springer, 2017; pp 231–239.
- (33) Alipanahi, B.; Delong, A.; Weirauch, M. T.; Frey, B. J. Predicting the sequence specificities of DNA-and RNA-binding proteins by deep learning. *Nature biotechnology* **2015**, *33*, 831.
- (34) Shaked, I.; Oberhardt, M. A.; Atias, N.; Sharan, R.; Ruppin, E. Metabolic network prediction of drug side effects. *Cell systems* **2016**, *2*, 209–213.
- (35) Yang, J. H.; Wright, S. N.; Hamblin, M.; McCloskey, D.; Alcantar, M. A.; Schröubers, L.; Lopatkin, A. J.; Satish, S.; Nili, A.; Palsson, B. O.; ADDAUTHORS, A White-Box Machine Learning Approach for Revealing Antibiotic Mechanisms of Action. *Cell* **2019**, *177*, 1649–1661.

- (36) Metz, C. AI Researchers Are Making More Than \$1 Million, Even at a Nonprofit. *The New York Times* **2018**,
- (37) Pedregosa, F.; Varoquaux, G.; Gramfort, A.; Michel, V.; Thirion, B.; Grisel, O.; Blondel, M.; Prettenhofer, P.; Weiss, R.; Dubourg, V.; ADDAUTHORS, Scikit-learn: Machine learning in Python. *Journal of machine learning research* **2011**, *12*, 2825–2830.
- (38) Batth, T. S.; Singh, P.; Ramakrishnan, V. R.; Sousa, M. M.; Chan, L. J. G.; Tran, H. M.; Luning, E. G.; Pan, E. H.; Vu, K. M.; Keasling, J. D.; ADDAUTHORS, A targeted proteomics toolkit for high-throughput absolute quantification of Escherichia coli proteins. *Metabolic engineering* **2014**, *26*, 48–56.
- (39) Heinemann, J.; Deng, K.; Shih, S. C.; Gao, J.; Adams, P. D.; Singh, A. K.; Northen, T. R. On-chip integration of droplet microfluidics and nanostructure-initiator mass spectrometry for enzyme screening. *Lab on a Chip* **2017**, *17*, 323–331.
- (40) Morrell, W. C. et al. The Experiment Data Depot: A Web-Based Software Tool for Biological Experimental Data Storage, Sharing, and Visualization. *ACS Synth. Biol.* **2017**, *6*, 2248–2259.
- (41) Wolpert, D. The Lack of A Priori Distinctions between Learning Algorithms. *Neural Computation* **1996**, *8*, 1341–1390.
- (42) Ho, T. K. Random Decision Forests. *Proceedings of 3rd International Conference on Document Analysis and Recognition* **1995**,
- (43) van der Laan, M.; Polley, E.; Hubbard, A. Super Learner. *Statistical Applications in Genetics and Molecular Biology* **2007**, *6*.
- (44) Hoeting, J. A.; Madigan, D.; Raftery, A. E.; Volinsky, C. T. Bayesian model averaging: a tutorial. *Statistical Science* **1999**, *14*, 382–417.

- (45) Monteith, K.; Carroll, J. L.; Seppi, K.; Martinez, T. Turning Bayesian model averaging into Bayesian model combination. The 2011 International Joint Conference on Neural Networks. 2011.
- (46) Yao, Y.; Vehtari, A.; Simpson, D.; Gelman, A. Using Stacking to Average Bayesian Predictive Distributions (with Discussion). *Bayesian Analysis* **2018**, *13*, 917–1003.
- (47) Chipman, H. A.; George, E. I.; McCulloch, R. E. Bayesian Ensemble Learning. Proceedings of the 19th International Conference on Neural Information Processing Systems. 2006; pp 265–272.
- (48) Begoli, E.; Bhattacharya, T.; Kusnezov, D. The need for uncertainty quantification in machine-assisted medical decision making. *Nature Machine Intelligence* **2019**, *1*, 20.
- (49) Olson, R. S.; Urbanowicz, R. J.; Andrews, P. C.; Lavender, N. A.; Kidd, L. C.; Moore, J. H. In *Applications of Evolutionary Computation: 19th European Conference, EvoApplications 2016, Porto, Portugal, March 30–April 1, 2016, Proceedings, Part I*; Squillero, G., Burelli, P., Eds.; Springer International Publishing, 2016; Chapter Automating Biomedical Data Science Through Tree-Based Pipeline Optimization, pp 123–137.
- (50) Breiman, L. Stacked regressions. *Machine Learning* **1996**, *24*, 49–64.
- (51) LeDell, E. Scalable Ensemble Learning and Computationally Efficient Variance Estimation. Ph.D. thesis, University of California, Berkeley, 2015.
- (52) Aldave, R. Systematic Ensemble Learning and Extensions for Regression. Ph.D. thesis, Université de Sherbrooke, 2015.
- (53) Brooks, S., Gelman, A., Jones, G., Meng, X.-L., Eds. *Handbook of Markov Chain Monte Carlo*; CRC press, 2011.

- (54) Snoek, J.; Larochelle, H.; Adams, R. P. Practical Bayesian Optimization of Machine Learning Algorithms. NIPS'12 Proceedings of the 25th International Conference on Neural Information Processing Systems. 2012; pp 2951–2959.
- (55) Earl, D. J.; Deem, M. W. Parallel tempering: Theory, applications, and new perspectives. *Physical Chemistry Chemical Physics* **2005**, *7*.
- (56) Unthan, S.; Radek, A.; Wiechert, W.; Oldiges, M.; Noack, S. Bioprocess automation on a Mini Pilot Plant enables fast quantitative microbial phenotyping. *Microbial cell factories* **2015**, *14*, 32.
- (57) Langholtz, M.; Stokes, B.; Eaton, L. 2016 Billion-ton report: Advancing domestic resources for a thriving bioeconomy, Volume 1: Economic availability of feedstock. *Oak Ridge National Laboratory, Oak Ridge, Tennessee, managed by UT-Battelle, LLC for the US Department of Energy* **2016**, 2016, 1–411.
- (58) Renouard-Vallet, G.; Saballus, M.; Schmithals, G.; Schirmer, J.; Kallo, J.; Friedrich, K. A. Improving the environmental impact of civil aircraft by fuel cell technology: concepts and technological progress. *Energy & Environmental Science* **2010**, *3*, 1458–1468.
- (59) Keasling, J. D. Manufacturing molecules through metabolic engineering. *Science* **2010**, *330*, 1355–1358.
- (60) Tracy, N. I.; Chen, D.; Crunkleton, D. W.; Price, G. L. Hydrogenated monoterpenes as diesel fuel additives. *Fuel* **2009**, *88*, 2238–2240.
- (61) Ryder, J. A. Jet fuel compositions. 2009; US Patent 7,589,243.
- (62) Duetz, W.; Bouwmeester, H.; Van Beilen, J.; Witholt, B. Biotransformation of limonene by bacteria, fungi, yeasts, and plants. *Applied microbiology and biotechnology* **2003**, *61*, 269–277.

- (63) Alonso-Gutierrez, J.; Chan, R.; Batt, T. S.; Adams, P. D.; Keasling, J. D.; Petzold, C. J.; Lee, T. S. Metabolic engineering of *Escherichia coli* for limonene and perillyl alcohol production. *Metabolic engineering* **2013**, *19*, 33–41.
- (64) Paddon, C. J.; Westfall, P. J.; Pitera, D. J.; Benjamin, K.; Fisher, K.; McPhee, D.; Leavell, M.; Tai, A.; Main, A.; Eng, D.; ADDAUTHORS, High-level semi-synthetic production of the potent antimalarial artemisinin. *Nature* **2013**, *496*, 528.
- (65) Meadows, A. L.; Hawkins, K. M.; Tsegaye, Y.; Antipov, E.; Kim, Y.; Raetz, L.; Dahl, R. H.; Tai, A.; Mahatdejkul-Meadows, T.; Xu, L.; ADDAUTHORS, Rewriting yeast central carbon metabolism for industrial isoprenoid production. *Nature* **2016**, *537*, 694.
- (66) Alonso-Gutierrez, J.; Kim, E.-M.; Batt, T. S.; Cho, N.; Hu, Q.; Chan, L. J. G.; Petzold, C. J.; Hillson, N. J.; D. Adams, P.; Keasling, J. D.; Martin, H. G.; SoonLee, T. Principal component analysis of proteomics (PCAP) as a tool to direct metabolic engineering. *Metabolic Engineering* **2015**, *28*, 123–133.
- (67) Denby, C. M.; Li, R. A.; Vu, V. T.; Costello, Z.; Lin, W.; Chan, L. J. G.; Williams, J.; Donaldson, B.; Bamforth, C. W.; Christopher J. Petzold, H. V. S.; Martin, H. G.; Keasling, J. D. Industrial brewing yeast engineered for the production of primary flavor determinants in hopped beer. *Nature Communications* **2018**, *9*, 965.
- (68) <https://www.crunchbase.com/organization/berkeley-brewing-science#section-overview>.
- (69) Opgenorth, P. et al. Lessons from Two Designâ€¢Buildâ€¢Testâ€¢Learn Cycles of Dodecanol Production in *Escherichia coli* Aided by Machine Learning. *ACS Synth. Biol.* **2019**, *8*, 1337–1351.
- (70) Salis, H. M.; Mirsky, E. A.; Voigt, C. A. Automated design of synthetic ribosome binding sites to control protein expression. *Nature biotechnology* **2009**, *27*, 946.

- (71) Espah Borujeni, A.; Channarasappa, A. S.; Salis, H. M. Translation rate is controlled by coupled trade-offs between site accessibility, selective RNA unfolding and sliding at upstream standby sites. *Nucleic acids research* **2013**, *42*, 2646–2659.
- (72) Bonde, M. T.; Pedersen, M.; Klausen, M. S.; Jensen, S. I.; Wulff, T.; Harrison, S.; Nielsen, A. T.; Herrgård, M. J.; Sommer, M. O. Predictable tuning of protein expression in bacteria. *Nature methods* **2016**, *13*, 233.
- (73) Liu, A.; Tan, X.; Yao, L.; Lu, X. Fatty alcohol production in engineered *E. coli* expressing *Marinobacter* fatty acyl-CoA reductases. *Applied microbiology and biotechnology* **2013**, *97*, 7061–7071.
- (74) T.S., H.; Z., D.; H., P.; J., C.; N.J., H.; J.D., K. Design, implementation and practice of JBEI-ICE: an open source biological part registry platform and tools. *Nucleic Acids Res.* **2012**, *40*.
- (75) Granda, J. M.; Donina, L.; Dragone, V.; Long, D.-L.; Cronin, L. Controlling an organic synthesis robot with machine learning to search for new reactivity. *Nature* **2018**, *559*, 377.
- (76) Le, K.; Tan, C.; Gupta, S.; Guhan, T.; Barkhordarian, H.; Lull, J.; Stevens, J.; Munro, T. A novel mammalian cell line development platform utilizing nanofluidics and optoelectro positioning technology. *Biotechnology progress* **2018**, *34*, 1438–1446.
- (77) Iwai, K.; Ando, D.; Kim, P. W.; Gach, P. C.; Raje, M.; Duncomb, T. A.; Heinemann, J. V.; Northen, T. R.; Martin, H. G.; Hillson, N. J.; ADDAUTHORS, Automated flow-based/digital microfluidic platform integrated with onsite electroporation process for multiplex genetic engineering applications. 2018 IEEE Micro Electro Mechanical Systems (MEMS). 2018; pp 1229–1232.
- (78) Gach, P. C.; Shih, S. C.; Sustarich, J.; Keasling, J. D.; Hillson, N. J.; Adams, P. D.;

- Singh, A. K. A droplet microfluidic platform for automating genetic engineering. *ACS synthetic biology* **2016**, *5*, 426–433.
- (79) Hayden, E. C. The automated lab. *Nature News* **2014**, *516*, 131.
- (80) Häse, F.; Roch, L. M.; Aspuru-Guzik, A. Next-generation experimentation with self-driving laboratories. *Trends in Chemistry* **2019**,
- (81) Breiman, L. Bagging Predictors. *Machine Learning* **1996**, *24*, 123–140.
- (82) Freund, Y.; Schapire, R. E. A Decision-Theoretic Generalization of On-Line Learning and an Application to Boosting. *Journal of Computer and System Sciences* **1997**, *55*, 119–139.
- (83) Lacoste, A.; Marchand, M.; Laviolette, F.; Larochelle, H. Agnostic Bayesian Learning of Ensembles. Proceedings of the 31st International Conference on Machine Learning. 2014; pp 611–619.