## Methods

### Dataset Compilation

The dataset was compiled by manually collecting information about the primary sequence and substrate specificity of TEs from scientific literature. The dataset included primary sequence and accompanying in vivo *E. coli* product distributions for 116 acyl-ACP plant TEs previously reported in scientific and patent literature [1]–[4]. *E. coli* was chosen because it remains the most common and facile method for characterization of heterologous TEs. The product distribution data was subsequently used to classify each TE into discrete categories to be used in a classification framework. Based on their product distributions, the TEs were divided into three categories, 1) the “medium-chain” category contained TE which resulted in distributions of at least 50% C8 to C12 free fatty acids, 2) the “long-chain” category contained TE which produced 50% C14 to C18 free fatty acids and less than 10% C8 to C12 free fatty acids and 3) the “mixed distribution” category contained TE which yielded distributions between 10% and 50% C8 to C12 free fatty acids. For the regression framework, each TE was assigned a number which represented the fraction of the total free fatty acid distribution constituted of C8 to C12 free fatty acids.

### Feature Extraction

In this work, the following three feature representation methods were employed to encode primary sequence information of the enzymes into fixed length vectors.

#### k-spectrum kernel

The k-spectrum kernel proposed by Leslie et. al. [5] is the set of all k-length contiguous subsequences present in a given input sequence. If we assume to be the set of all characters of a sequence, to be a k-mer and to be an indicator function that is 1 if occurs in a protein sequence and 0 otherwise, then the feature map is as follows:

The k-spectrum kernel, is:

#### Grouped amino acid encoded k-spectrum kernel

The grouped amino acid encoded k-spectrum kernel performs an additional pre-processing step on the primary sequences of the enzymes before encoding them into a feature vector representation similar to the k-spectrum kernel. In the preprocessing step, 20 amino acid types which can occur at a particular position in the sequence of an enzyme were categorized into five classes according to their physicochemical properties, hydrophobicity, charge and molecular size. The five classes were the aliphatic group (: GAVLMI), aromatic group (: FYW), positive charge group (: KRH), negative charged group (: DE) and uncharged group (: STCPNQ). Finally, each amino acid in the sequence was replaced by its respective class to create an encoded sequence with only five different types of values per position. For example, a primary sequence ‘MLTPWS’ is represented as . Using the encoded representation, the k-spectrum kernel was used to represent the protein sequence. It should be noted that the preprocessing step significantly reduces the set of characters of the sequence and can help prevent overfitting since the kernel feature map size will be much lower than the original feature map size obtained by the k-spectrum kernel. The reduced feature map size will require much lower number of parameters to train a classifier and thus may prevent overfitting.

#### Automatic position detection method for feature extraction

The automated position selection method selects the most important amino acid residue positions based on their statistical correlation with the labels (substrate specificity category of the enzymes). In this method, multiple sequence alignment of the enzymes was performed using the mafft tool [6]. The aligned sequences were transformed into a one-hot encoded representation of each amino acid position. Theoretically, each position could be encoded by a 21 dimensional feature vector (20 types of amino acids and a gap) but in most cases a position was encoded by a vector of length 5 or even less since the types of amino acids in a specific position were usually conserved. The encoded feature space was used to select the best features which contributed the most towards the target variable’s or label’s variability. The feature selection technique was conducted using the SelectKBest module in scikit-learn [7]. Anova F-test, Chi-2 test or Mutual information can be used as the correlation metric between the features and labels based on which the positions were selected. The best ranked features were mapped back from the expanded one hot encoded feature space back to the original amino acid positions. These positions were recorded as the most important determinant of enzyme substrate specificity. The method had a parameter, n, that denotes the number of positions among all the aligned amino acid sequence positions that it must extract using the feature selection algorithm before stopping. After selecting the n most important positions, amino acids in those positions were represented as a one hot encoded feature vector. Each position may also contain a gap apart from the 20 types of amino acids (result of a multiple sequence alignment). Thus a 21-dimensional one hot encoded feature vector was used to represent each position. The length of the feature space obtained as a result of this encoding was 21 \* n. The correlation metric and the number of positions to be selected can be determined through hyperparameter optimization.

### Ensemble Method for Classification

Our ensemble framework involved three base learners which provided an output to a meta learner that predicted the enzyme specificity class. Although all of our base learners were trained using the same principle (either SVM or NN), the heterogeneity among them was governed by the three different feature representation techniques described in the Feature Extraction section which were used to encode the set of enzyme sequences. The outputs of the base learners were passed on to the meta learner that used a majority voting scheme to predict the enzyme specificity category; if the predictions of the three base learners were respectively, then the prediction of the meta learner was . The workflow of our ensemble model is presented in Figure Y.

#### The Base Learner

##### Support Vector Machine

The Support Vector Machine based learner of enzyme specificity prediction included Principal Component Analysis (PCA) for dimensionality reduction of the feature space followed by a Support Vector Classifier [8] to predict enzyme specificity class. The PCA based dimensionality reduction step was carried out to decrease the number of parameters required to train an SVM model and make the model more generalizable. The one versus one strategy was used for multi-class classification. The number of PCA components, SVM model kernel, regularization parameter C and kernel coefficient gamma were selected by optimizing these hyperparameters using a 3-fold cross validation scheme described in the Model Training subsection.

##### Neural Network

The NN based learner of enzyme specificity prediction included Principal Component Analysis for dimensionality reduction of the feature space followed by an Artificial Neural Network based classifier to predict enzyme specificity class. The PCA based dimensionality reduction step was carried out to decrease the number of parameters required to train an NN model and make the model more generalizable. The number of PCA components, hidden layer size of NN, initial learning rate and L2 regularization parameter alpha were selected by optimizing these hyperparameters using a 3-fold cross validation scheme described in the Model Training subsection.

#### Model Training

The model was trained using python’s numpy and scikit-learn modules [7], [9]. At first, a random seed was specified using numpy to reproduce results. The dataset of 116 TE enzyme sequences labeled according to their corresponding substrate specificity category was divided into a training set and a validation set by a 75-25 percentage split. The training set of sequences was encoded by the three different feature representation techniques, described in the [Feature Extraction](#_Feature_Extraction) section, into three distinct feature vector representation of the sequences. The distinct feature vectors of the training set of sequences were used to train three separate base learners operating on the same principle (either PCA+SVM or PCA+NN). The hyperparameters of the base learners (number of components of PCA, kernel type, regularization parameter C, kernel coefficient in case of the SVM based learner or hidden layer size initial learning rate and regularization parameter in case of the NN based learner) were optimized using the GridSearchCV module of scikit-learn and setting the cross validation split to 3. The three base models with optimized set of hyperparameters were used to independently predict the substrate specificity category of enzymes in both training and validation sets. The output predictions of these base learners were passed on to a meta learner that used a hard-voting based majority vote classifier to output the final prediction of the enzyme substrate specificity class. In case there is a three-way tie between outputs of the base learners, the prediction of the positional feature builder based learners (which performed the best among all the base learners) was selected as the ensemble output. The training and validation accuracies of the three base learners and the ensemble model were recorded. This whole procedure was repeated 10,000 times by varying the random seed specified initially, which resulted in different training and validation set, thus affecting the model performance and yielding a distribution of training and validation set accuracies for the three base learners and the ensemble model. The objective of training our model multiple times was to check its robustness to the training set. In addition to the above mentioned hyperparameters, the k-mer motif and GAA encoded motif builder had an additional hyperparameter k that denotes the length of the motif to be considered. It was set to be 7 for both the models based on a separate validation study mentioned in detail in Appendix.

### Support Vector Machine for Regression

The SVM based regression model involved Principal Component Analysis for dimensionality reduction of the feature space followed by a Support Vector Regressor to predict enzyme substrate specificity. The model was trained similar to the classification framework. However, instead of using an ensemble of SVMs, an individual SVM was used to predict the substrate specificity. The grouped amino acid encoded spectrum kernel was the only feature extraction technique used in this case. The number of PCA components, SVM model kernel, regularization parameter C and kernel coefficient gamma were selected by optimizing these hyperparameters using a 3-fold cross validation scheme.