

# Machine learning network-constrained regression of epigenetic data

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# Declaration

I Sivo V. Daskalov of Corpus Christi College, being a candidate for the M.Phil in Advanced Computer Science, hereby declare that this report and the work described in it are my own work, unaided except as may be specified below, and that the report does not contain material that has already been used to any substantial extent for a comparable purpose.

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# Abstract

Computational biology often involves working with high-dimensional data. Penalized regression methods are often used on such data, as they can effectively perform feature selection. Several approaches for network-constrained regression have been suggested in literature over the recent years. They use prior knowledge in the form of a network to exploit known relationships between predictors. Synthetic datasets have been generated to do parameter tuning for the various implemented methods.

We suggest an approach for cooperative parameter tuning in the context of multiple alternative methods that share common input and goals. The aim is to tune the different regression methods iteratively, in a way that increases agreement between their coefficients. Neighboring values on the tuning parameter grid are considered for each method and iteration, selecting the set of values that achieves largest correlation with the averaged coefficients of all other methods for the previous iteration. Given enough iterations and granularity of the tuning grids, this process converges.

We also implement a simple approach to aggregate the coefficients produced by the various regression methods. Each predictor is considered relevant if it corresponds to a non-zero coefficient in a certain fraction of the underlying methods. Once a consensus has been reached through this form of voting, ordinary least squares estimation is used to fit only the relevant predictors to the data.

The common way of parameter tuning by minimization of the prediction mean squared error is implemented alongside our suggested approach. The comparison is discussed and a set of tuning parameters is assembled for use on real data. Gene methylation and expression data has been processed with the implemented algorithms. A map is created that shows methylation of which genes affects the expression levels of each gene.



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# Chapter 1

## Introduction

Epigenetics [1] studies the heritable traits that cannot be explained by changes in the DNA sequence. Examples of epigenetic mechanisms include DNA methylation and histone modification. These mechanisms adjust the expression level of genes [2], which allows organisms to dynamically adapt to changes in the environment.

Disruption of gene expression levels is related to the development of various diseases [3]. For example, the epigenetic deactivation of certain tumor suppressor genes commonly leads to the development of cancer [4]. The expression levels of certain genes can therefore be used as additional tools in early diagnostics of cancer, as prognosis factors and as predictors of response to treatment.

Good understanding of the relationship between DNA methylation and gene expression is important for both cancer prevention and epigenetic disease treatment. We have used the gene methylation and expression level data discussed in [5] to explore this relationship. One of the goals in this project is to produce a map that shows the methylation of which genes affects the expression levels of each gene.

Several methods [6, 7, 8, 9, 10, 11, 12] have been implemented and considered for use with real data. The hyperparameters for each method have been

tuned with the use of synthetic datasets as suggested in [8]. This is done because ground truth remains unknown for the relationship between gene methylation and expression.

A novel method of hyperparameter tuning is developed as an alternative to the widely used method of minimizing the cross-validated mean squared test error. In our context we have a bundle of regression methods that operate on the same training data and share a common goal - to correctly identify the relationship between predictor and target variables. Instead of tuning the various regression methods independently, our approach performs cooperative hyperparameter tuning on all methods simultaneously. It uses an iterative algorithm to increase the similarity of estimated coefficients for the various methods by tuning their hyperparameters. For each method and iteration, the method's parameter grid neighborhood is searched for a set of parameters that maximizes the correlation between its estimated coefficients and the averaged estimates of all other methods for the previous iteration. When this process converges a set of parameters is defined for each method that maximizes the overall agreement across the whole set of methods.

The various regression methods discussed in this project minimize different cost functions. As a result, each method exhibits specific strengths and weaknesses. The relative performance of the methods depends on the dataset used. For this reason it is impossible to predict their effectiveness on an arbitrary real data set with no access to ground truth. We have developed a simple way to merge the estimation results of the method bundle. It is designed to balance the behavior of any individual method. Each of the predictor variables is considered important if it has non-zero coefficients in a fraction of the methods above a given threshold. This approach for variable selection in practice implements a voting system where each predictor must achieve a certain electoral threshold to be selected. Ordinary least squares estimation is then performed only using the set of selected variables.

# Chapter 2

## Background and Related Work

This chapter briefly reviews the main concepts of linear regression. We describe in detail the various methods for penalized regression found in literature and used in this project.

### 2.1 Linear Regression

Let us consider an entity with a number of scalar measurable (observable) properties, e.g. temperature, weight, dimensions. We can define a matrix  $X$  of  $n$  rows and  $p$  columns, such that each column contains the observed values of a particular property and each row represents an independent observation of values for all properties. Let us also define a vector  $y$  of length  $n$  containing the corresponding observed values of an arbitrary property of interest.

Linear regression is a method for modeling the relationships between a scalar dependent (target) variable  $y$  and a number of explanatory variables (predictors)  $X_1, \dots, X_p$ . It assumes that this relationship is linear and assigns a regression coefficient  $\beta_i$  to each predictor  $X_i$ , as well as a constant (offset) term  $\beta_0$ . The linear regression model takes the form shown in Equation 2.1

$$y_i = \beta_0 1 + \beta_1 X_{i1} + \beta_2 X_{i2} + \dots + \beta_p X_{ip} + \epsilon_i, \quad \text{for } i = 1, 2, \dots, n \quad (2.1)$$

where  $\epsilon_i$  represents noise, capturing all external factors influencing the target values, such as inaccuracy of measurement. The error  $\epsilon_i$  introduces cannot be predicted or reduced.

## 2.2 Ordinary Least Squares Estimation

Ordinary least squares (OLS) is a method of estimating the unknown parameters  $\beta$  in a linear regression model. It aims to minimize the sum of squared deviations of the observed values from the model prediction (2.2), also called residual sum of squares (RSS).

$$L(\beta) = \sum_{i=1}^N (y_i - x_i^T \beta)^2 \quad (2.2)$$

The parameter estimate  $\hat{\beta}$  for the linear regression model is obtained as shown in equation 2.3 through the minimization of the objective function  $S(\beta)$ .

$$\hat{\beta} = \operatorname{argmin}_{\beta \in R} S(\beta) = L(\beta) \quad (2.3)$$

## 2.3 Penalized Regression

Penalized regression methods introduce a penalty  $P(\beta)$  to the objective function  $S(\beta)$  in addition to the loss function  $L(\beta)$ .  $P$  penalizes values of the unknown parameters that are considered unrealistic in the current context, which is done to obtain a more meaningful estimation. One or more regularization parameters  $\lambda_i$  can be used to balance the effect of any introduced penalties by scaling them. The general form of penalized regression is shown in Equation 2.4.

$$S(\beta) = L(\beta) + P(\beta) \quad (2.4)$$

### 2.3.1 Ridge regression

Ridge regression [13], also called Tikhonov or L2 regularization, is used to penalize large values in the  $\beta$  estimate. The penalty, shown in Equation 2.5, causes the parameter estimates of the less important predictors to be shrunk, but remain non-zero. As a result, L2 regularization does not perform feature selection.

$$P(\beta) = \lambda \sqrt{\sum_{i=1}^p \beta_i^2} \quad (2.5)$$

### 2.3.2 Lasso

The least absolute shrinkage and selection operator (LASSO) was introduced by Tibshirani in [6]. It produces a sparse coefficient vector, whose remaining non-zero elements define a subset of the most relevant predictors. Model sparsity is especially important in high-dimensional problems, such as those arising when processing epigenetic data. The L1 penalty, shown in Equation 2.6, performs both variable selection and regularization.

$$P(\beta) = \lambda \sum_{i=1}^p |\beta_i| \quad (2.6)$$

### 2.3.3 Elastic Net

The Elastic Net [7], suggested by Zou and Hastie, linearly combines the L1 (2.6) and L2 (2.5) penalties. This approach overcomes the individual limitations of the Lasso and Ridge methods. The elastic net penalty, shown in Equation 2.7, is adjusted by two hyperparameters  $\lambda_1$  and  $\lambda_2$ , one for each of the two penalty terms.

$$P(\beta) = \lambda_1 \sum_{i=1}^p |\beta_i| + \lambda_2 \sqrt{\sum_{i=1}^p \beta_i^2} \quad (2.7)$$

## 2.4 Network-constrained regularization

Various approaches for network-constrained regularization have been developed in recent years. They enable the use of prior knowledge in the form of a network in the parameter estimation process. This allows methods to consider known relationships between predictors. In the context of epigenetic research, prior knowledge could be provided as a gene network representing known interactions between genes. Biological knowledge about the predictors should lead to a better understanding of the data and improved (biological) meaningfulness of the results.

For all network-constrained regularization approaches presented in this section, we define the following notation:

Let us consider a network that is represented by a weighted graph  $G = (V, E, W)$ , where  $V$  is the set of vertices corresponding to the  $p$  predictors,  $E$  is the set of edges and  $W$  contains their corresponding weights. An edge between the vertices  $u$  and  $v$  is represented as  $u \sim v$  and its edge weight is  $w(u, v)$ . Let us define the degree  $d_v$  of a vertex  $v$  as  $d_v = \sum_{u \sim v} w(u, v)$ .

### 2.4.1 Grace

The first approach for network-constrained regularization was suggested by Li and Li [8]. The alias "Grace" is derived from the method's full name "GRAPh Constrained Estimation". The penalty function, shown in Equation 2.8, contains two terms - an  $L1$  penalty for variable selection and a second term that performs the network penalization.

$$P(\beta) = \lambda_1 \sum_{i=1}^p |\beta_i| + \lambda_2 \sum_{u \sim v} \left( \frac{\beta_u}{\sqrt{d_u}} - \frac{\beta_v}{\sqrt{d_v}} \right)^2 w(u, v) \quad (2.8)$$

The penalty is designed to smooth the parameters  $\beta$  over the gene network. This is achieved by penalizing the scaled difference of the coefficients between neighboring vertices in the network. The penalty encourages genes with a higher degree in the network (e.g. hub genes) to have larger coefficients.

### 2.4.2 aGrace

One drawback of the original Grace approach is that it performs poorly when the coefficients of two linked predictors have different signs. This scenario is feasible because one of the two genes could be negatively correlated with the target, in which case the coefficients of both genes will be penalized.

Li and Li proposed a modification [9] that performs adaptive graph-constrained regularization (aGrace) to solve this issue. It uses an initial coefficient estimate  $\tilde{\beta}_v$  obtained through OLSE (2.2) if  $p < n$  or Elastic Net (2.3.3) otherwise. The adaptive Grace penalty function is shown in Equation 2.9.

$$P(\beta) = \lambda_1 \sum_{i=1}^p |\beta_i| + \lambda_2 \sum_{u \sim v} \left( \frac{\text{sign}(\tilde{\beta}_u)\beta_u}{\sqrt{d_u}} - \frac{\text{sign}(\tilde{\beta}_v)\beta_v}{\sqrt{d_v}} \right)^2 w(u, v), \quad (2.9)$$

where the multiplier  $\text{sign}(\tilde{\beta}_u) = \begin{cases} -1 & \text{if } \tilde{\beta}_u < 0 \\ 1 & \text{otherwise} \end{cases}$  adjusts the sign of each fraction as suggested by the initial estimate  $\tilde{\beta}$ .

### 2.4.3 GBLasso

One concern regarding the adaptive grace (2.4.2) method is the difficulty to estimate the sign adjustment of all  $\beta_i$ , for which  $\tilde{\beta}_i = 0$ . To discard the need for this estimation, Pan et al. proposed an alternative approach [10]. The authors suggested the penalty function shown in Equation 2.10.

$$P(\beta) = \lambda 2^{1/\gamma'} \sum_{u \sim v} \left( \frac{|b_u|^\gamma}{w_u} + \frac{|b_v|^\gamma}{w_v} \right)^{1/\gamma}, \quad (2.10)$$

where  $\gamma > 1$  and  $\lambda > 0$  are hyperparameters and  $\gamma'$  satisfies  $\frac{1}{\gamma'} + \frac{1}{\gamma} = 1$ . The denominator  $w_i$  is a weight function attributed to each node. Three types of weight functions, dependent on the node's degree  $d_i$  and/or  $\gamma$ , were initially considered by the authors:  $w_i = d_i^{(\gamma+1)/2}$ ,  $w_i = d_i$  and  $w_i = d_i^\gamma$ .

A simplification of the penalty function is presented in [11]. The authors have selected a node weight function of  $w_i = d_i^{\gamma/2}$  and the penalty sum multiplier  $\lambda 2^{1/\gamma'}$  has been modified to depend exclusively on  $\lambda$ . The simplified penalty function is shown in Equation 2.11 and referred to with the alias GBLasso in this paper.

$$P(\beta) = \lambda \sum_{u \sim v} \left[ \left( \frac{|b_u|}{\sqrt{d_u}} \right)^\gamma + \left( \frac{|b_v|}{\sqrt{d_v}} \right)^\gamma \right]^{1/\gamma} \quad (2.11)$$

#### 2.4.4 Linf and aLinf

##### Linf

Luo et al. [11] continued researching the GBLasso method (2.4.3). They noted that as  $\gamma \rightarrow \infty$  the GBLasso penalty (2.11) is transformed into Equation 2.12. This penalty is linear and we denote the method as  $L_\infty$  (Linf).

$$P(\beta) = \lambda \sum_{u \sim v} \max \left( \frac{|\beta_u|}{\sqrt{d_u}}, \frac{|\beta_v|}{\sqrt{d_v}} \right) \quad (2.12)$$

The authors also suggest an equivalent formulation of the GBLasso-based regression as the following constrained minimization problem:

$$\begin{aligned} S(\beta) &= \sum_{i=1}^n (y_i - x_i^T \beta)^2 \\ \text{subject to } &\sum_{u \sim v} \left[ \left( \frac{|b_u|}{\sqrt{d_u}} \right)^\gamma + \left( \frac{|b_v|}{\sqrt{d_v}} \right)^\gamma \right]^{1/\gamma} \leq C \end{aligned} \quad (2.13)$$

Similarly, regression with the  $L_\infty$  penalty can be equivalently defined as:

$$\begin{aligned} S(\beta) &= \sum_{i=1}^n (y_i - x_i^T \beta)^2 \\ \text{subject to } &\sum_{u \sim v} \max \left( \frac{|\beta_u|}{\sqrt{d_u}}, \frac{|\beta_v|}{\sqrt{d_v}} \right) \leq C \end{aligned} \quad (2.14)$$



## aLinf

The authors suggest an additional modification to reduce bias in the parameter estimates of the standard Linf method. Similarly to [9], they propose a two-step approach using an initial parameter estimate  $\tilde{\beta}$ , obtained with the  $L_\infty$  method. The adaptive penalty, denoted as  $aL_\infty$  (aLinf), is shown in Equation 2.15.

$$P(\beta) = \lambda \sum_{u \sim v} \left| \frac{\text{sign}(\tilde{\beta}_u)\beta_u}{\sqrt{d_u}} - \frac{\text{sign}(\tilde{\beta}_v)\beta_v}{\sqrt{d_v}} \right| \quad (2.15)$$

The following constrained minimization problem can be defined to implement the  $aL_\infty$  approach:

$$\begin{aligned} S(\beta) &= \sum_{i=1}^n (y_i - x_i^T \beta)^2 \\ \text{subject to } \sum_{u \sim v} \left| \frac{\text{sign}(\tilde{\beta}_u)\beta_u}{\sqrt{d_u}} - \frac{\text{sign}(\tilde{\beta}_v)\beta_v}{\sqrt{d_v}} \right| &\leq E \end{aligned} \quad (2.16)$$

### 2.4.5 TTLP and LTLP



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