Netherlands Bioinformatics Centre

Sequence-based prediction of protein secretion success in *Aspergillus niger*

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DSM

Introduction

- *Aspergillus niger* is widely used for industrial enzyme production
  - It has a high secretion capacity
  - Its fermentation is “generally recognized as safe” (GRAS)

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fermentor

pectinases

wine clarification

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fermentor

pectinases

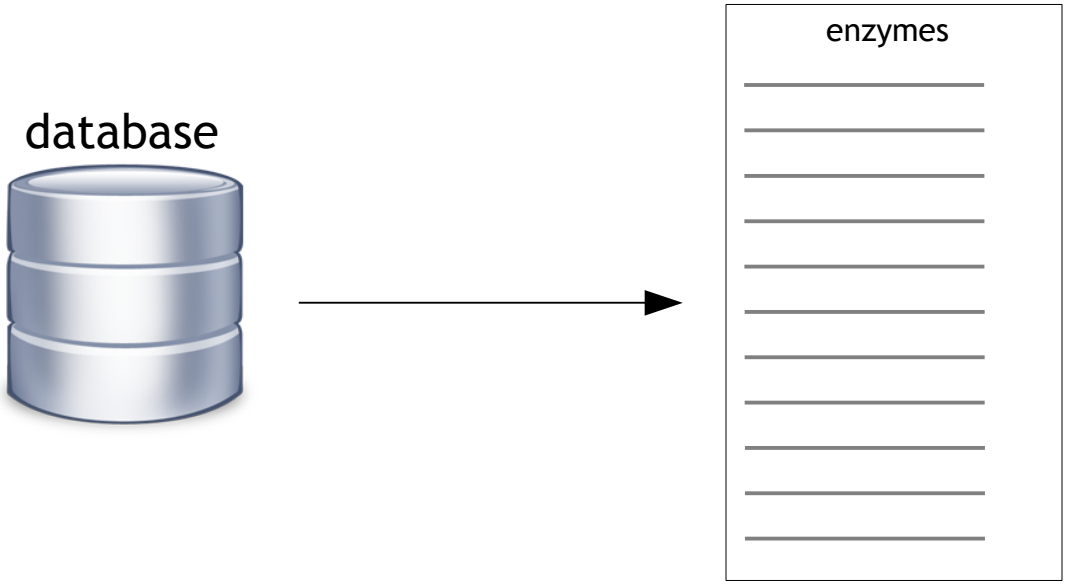
glucoamylase

wine clarification

high fructose corn syrup

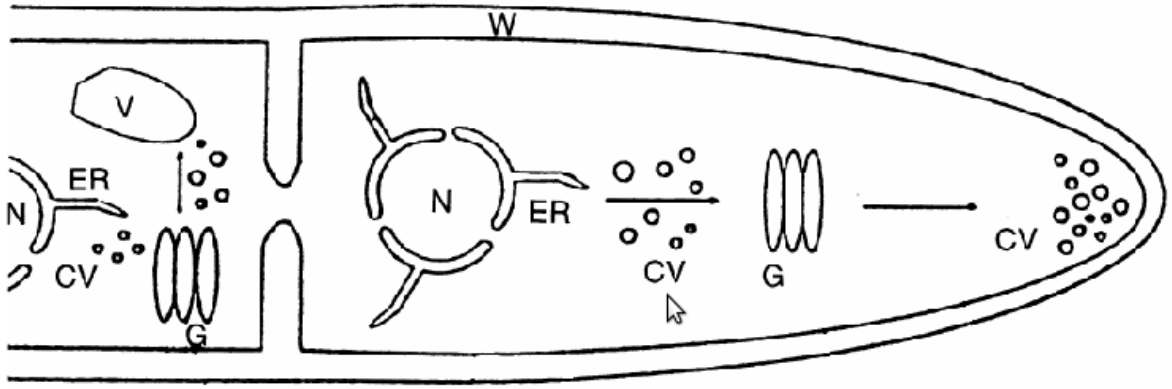
Introduction

- *Enzyme production:* gene over-expression using a strong constitutive promoter
- *Enzyme selection:* proteins from genome databases with predicted signal peptide



Introduction

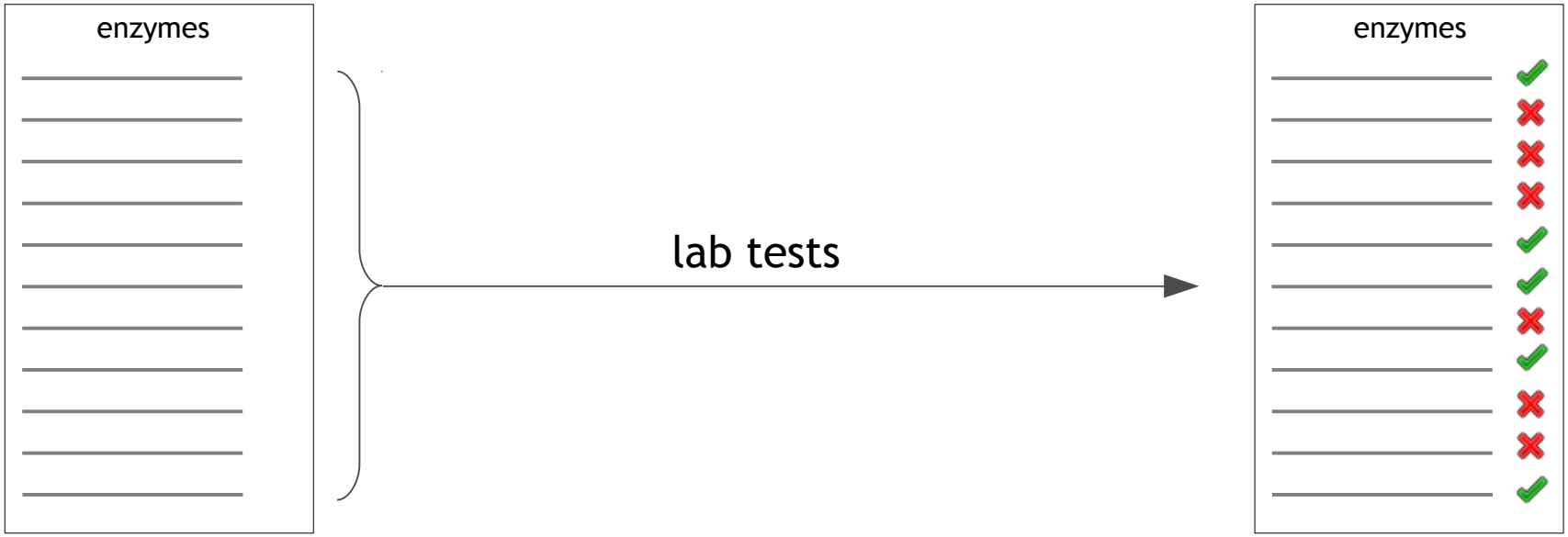
- A protein with an n-terminal signal peptide does not guarantee successful production and secretion when over-expressed
  - Protein can be translocated to different location
  - Over-expression can cause protein misfolding



Gouka et al, 1997

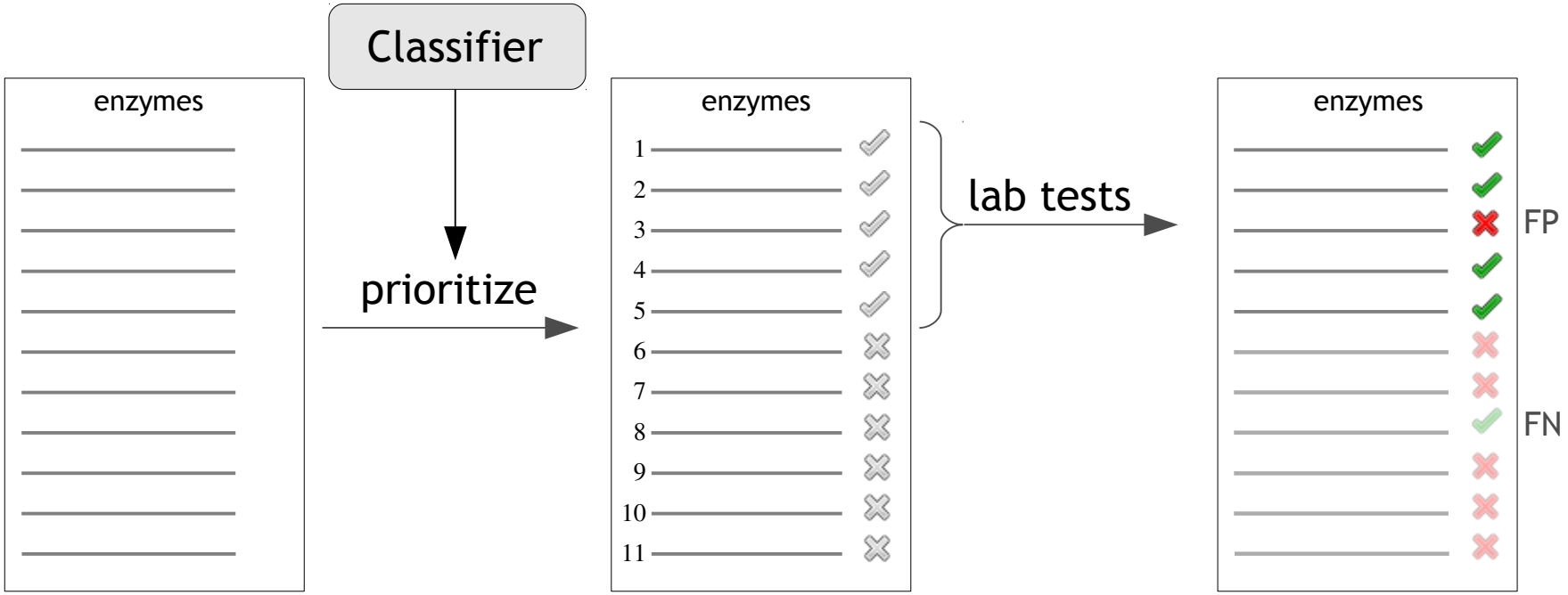
Introduction

- **Costly** lab work needed to test for successful production of over-expressed enzymes
  - secretion
  - high production rate



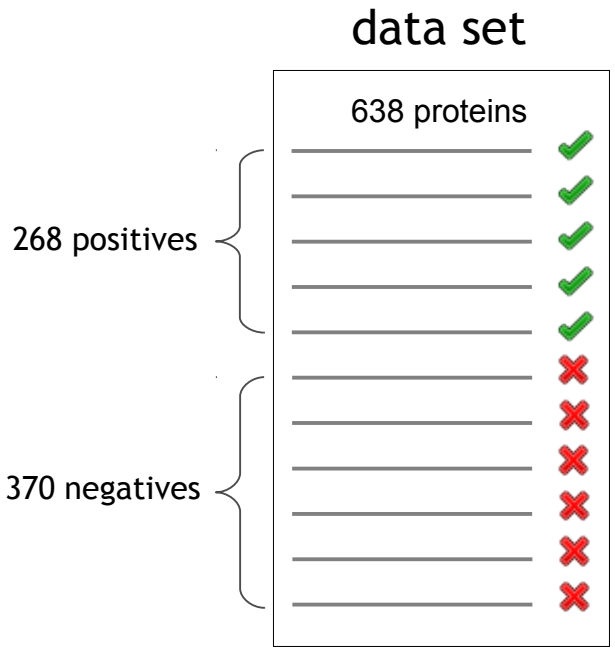
Introduction

- A classifier can greatly reduce the amount of lab work, at the cost of missing some successful enzymes



Data set

- Data set with success scores of 638 over-expressed *A. niger* proteins is used to train and test a classifier



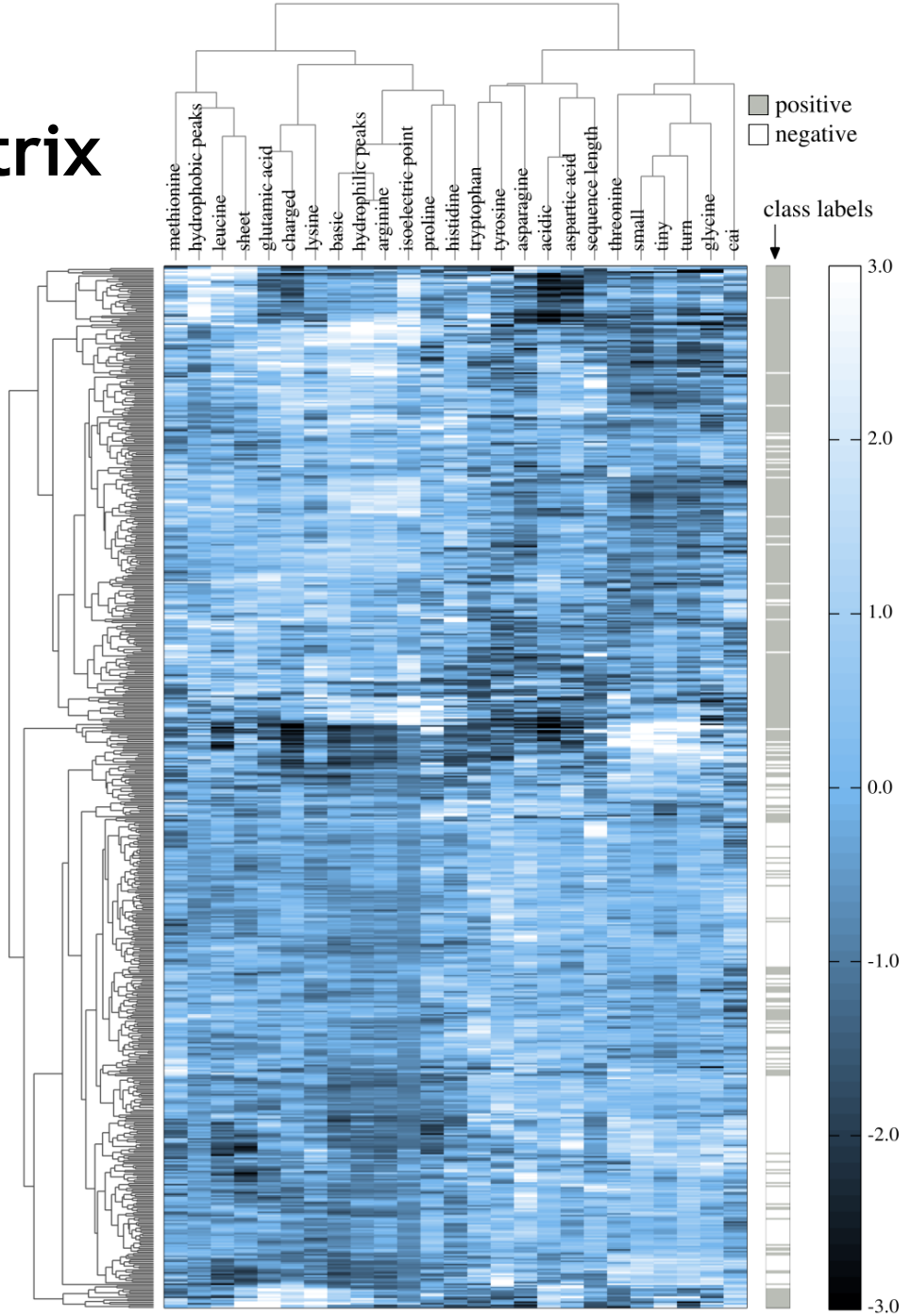
Features

- 39 sequence based features calculated
- 25 features with p-value < 0.001 (two-sample *t*-test) selected for classification

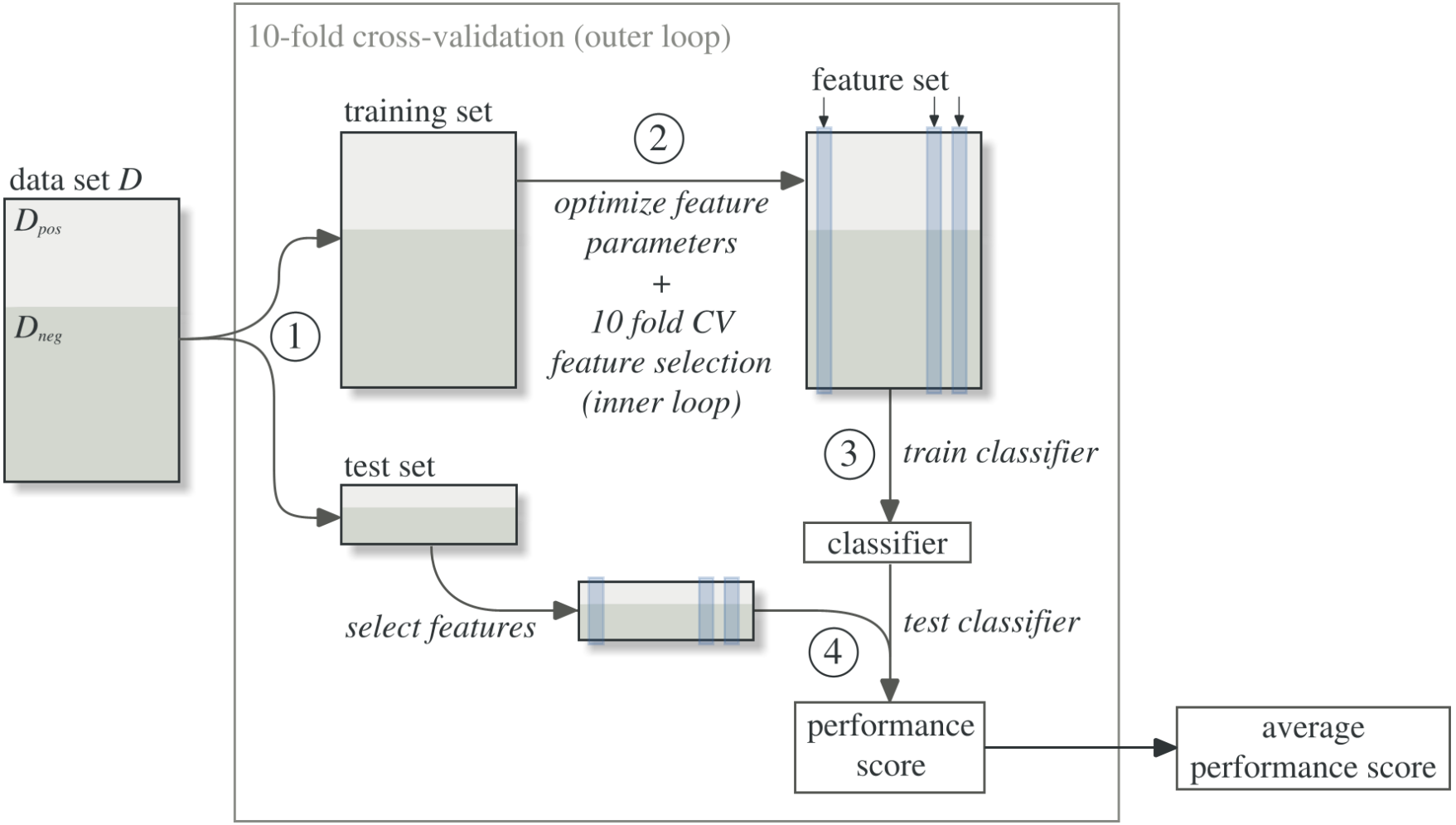
Nucleotide composition	4
GC-content	1
CAI index	1
Amino acid composition	20
Amino acid set composition	8
Hydrophobic/phylic peaks	2
GRAVY-score	1
Isoelectric point (pI)	1
Sequence length	1

Clustered feature matrix

- Hierarchical clustering of the proteins and features already shows class separability

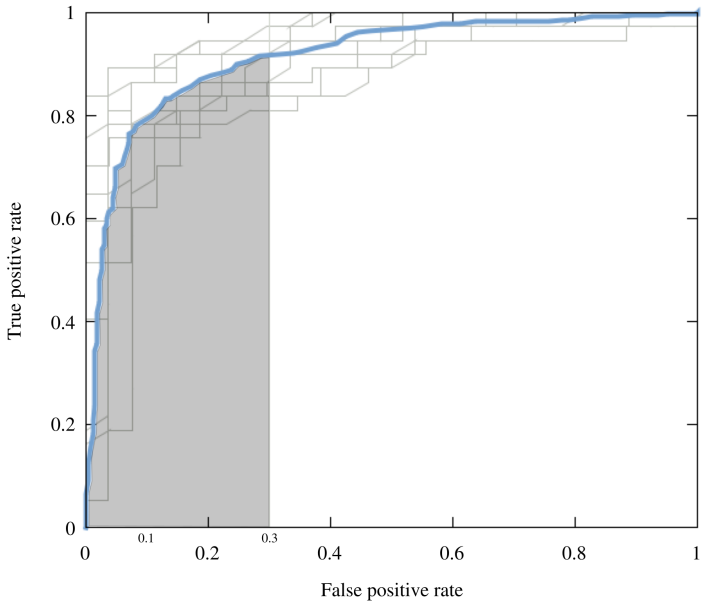


Training and validation protocol

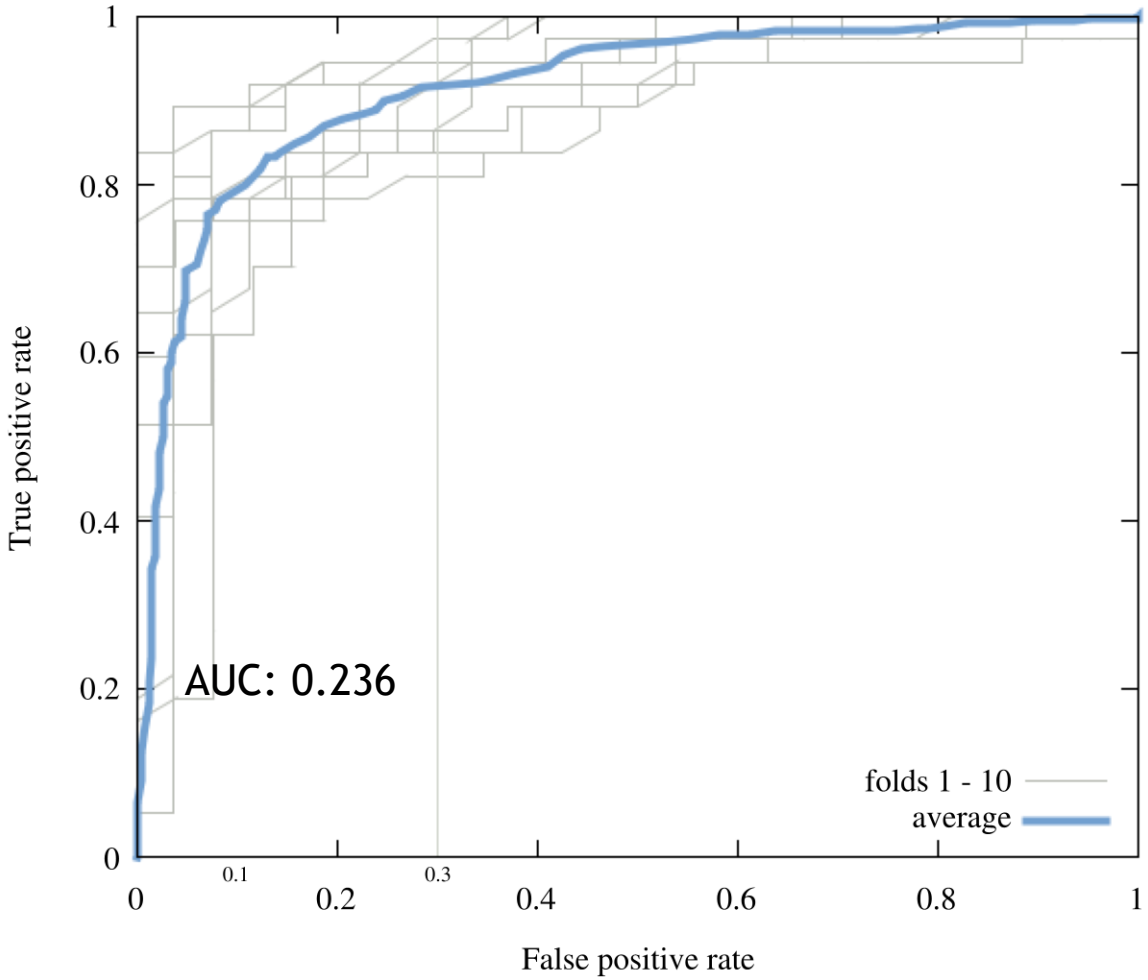


Performance evaluation

- The area under the curve (AUC) of the range 0% to 30% false positive rate of the ROC curve is used as performance measure, because we are mostly interested in low false positive rates.



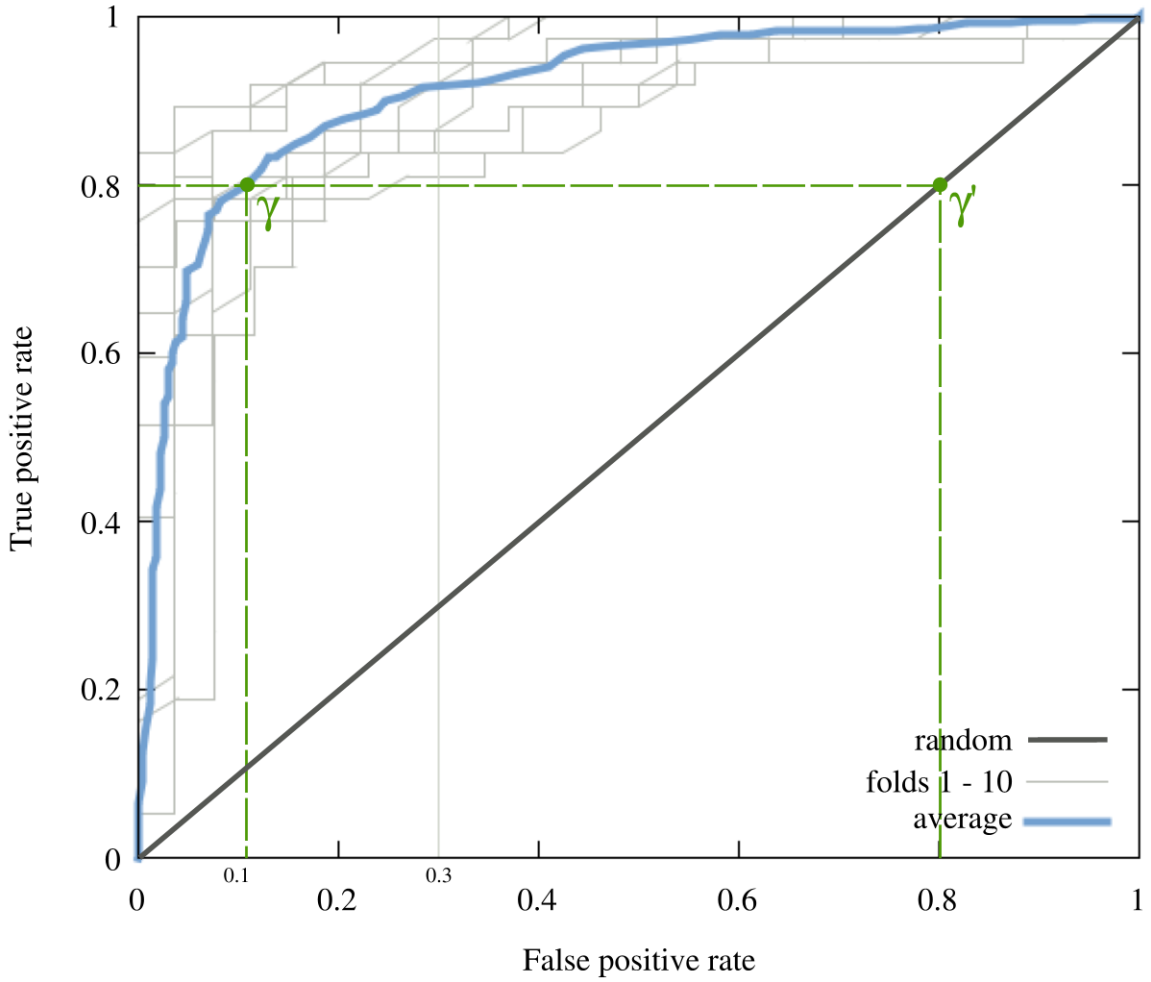
Results: linear discriminant classifier



Results: example

Example situation:
A list of 100 enzymes:
42 positives and
58 negatives

		γ
Classifier	TP-rate	*0.80*
	TP	*34*
		γ
	FP-rate	*0.10*
	FP	*6*
	lab tests	*40*
		γ'
Random selection	FP-rate	*0.80*
	FP	*46*
	lab tests	*80*
$\text{lab tests}_{\text{random}} / \text{lab tests}_{\text{classifier}}$		*2.00*



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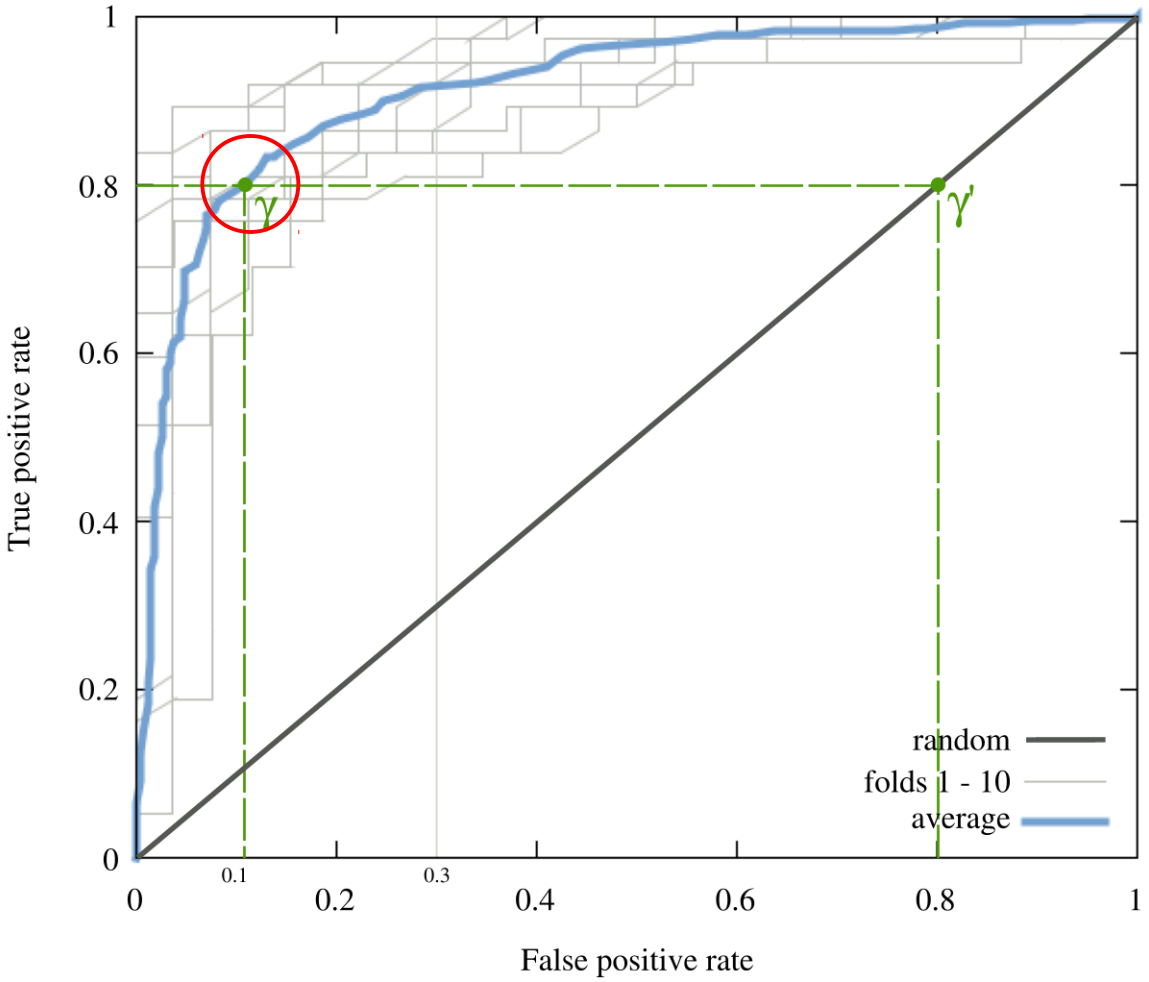

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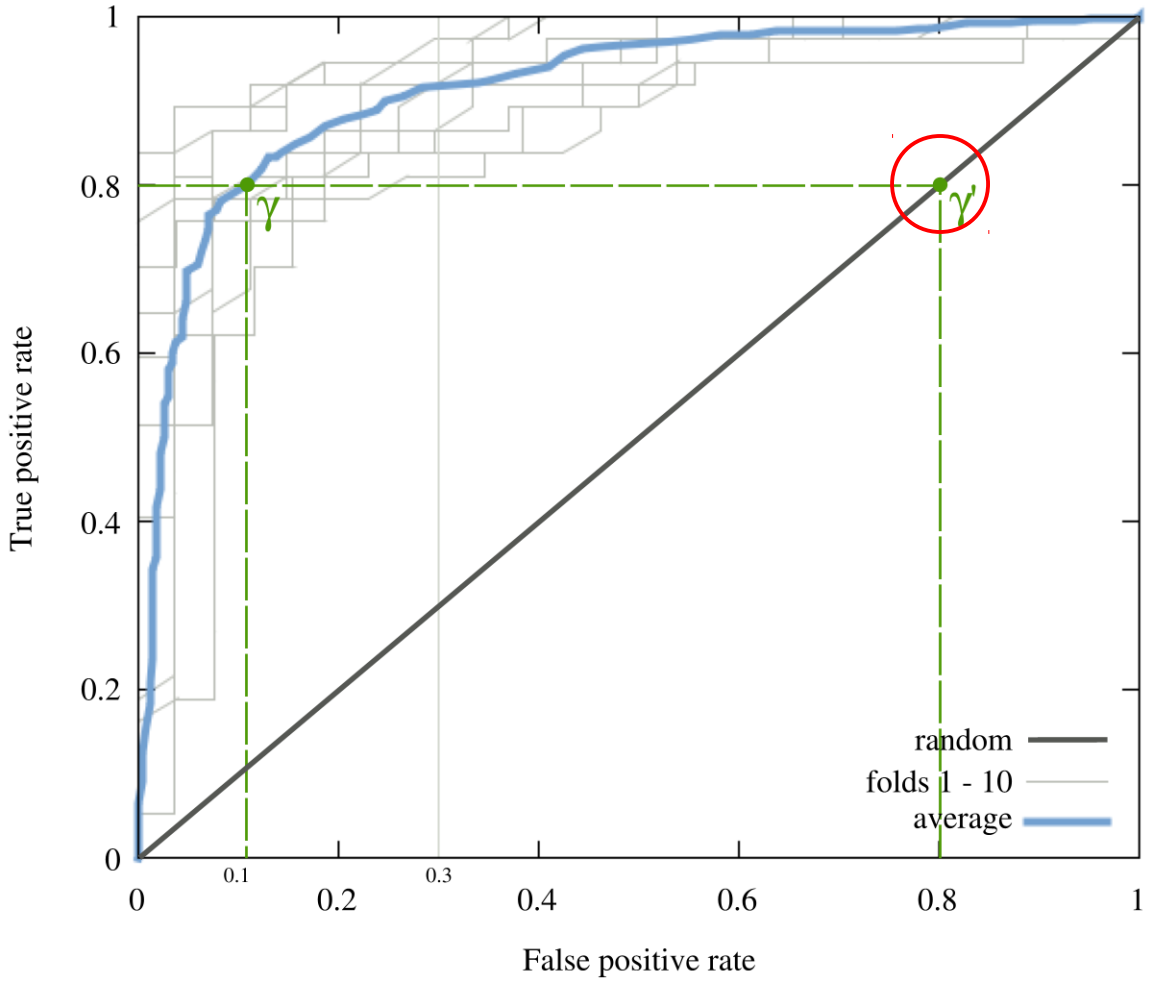

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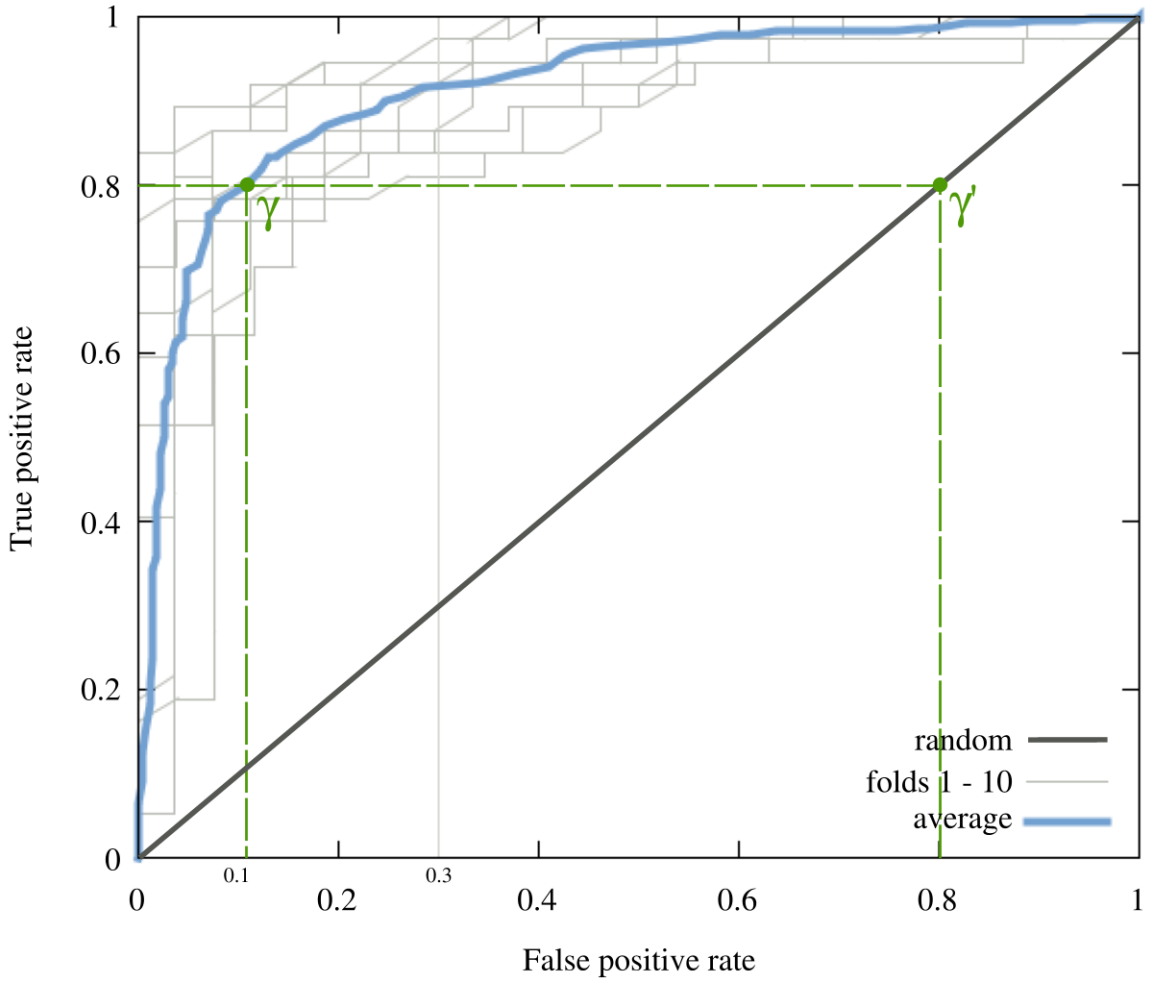

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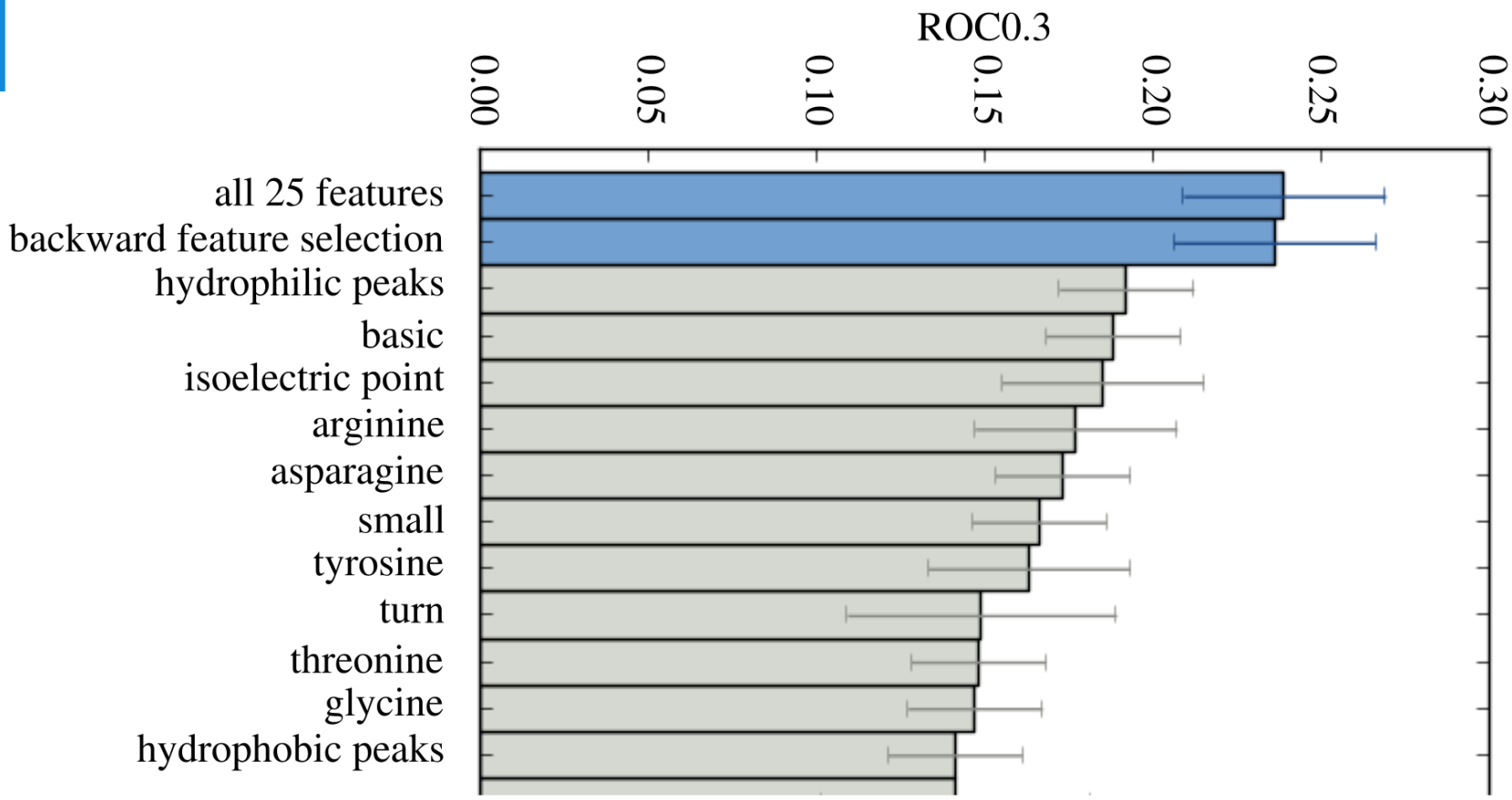
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all 25 features
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basic
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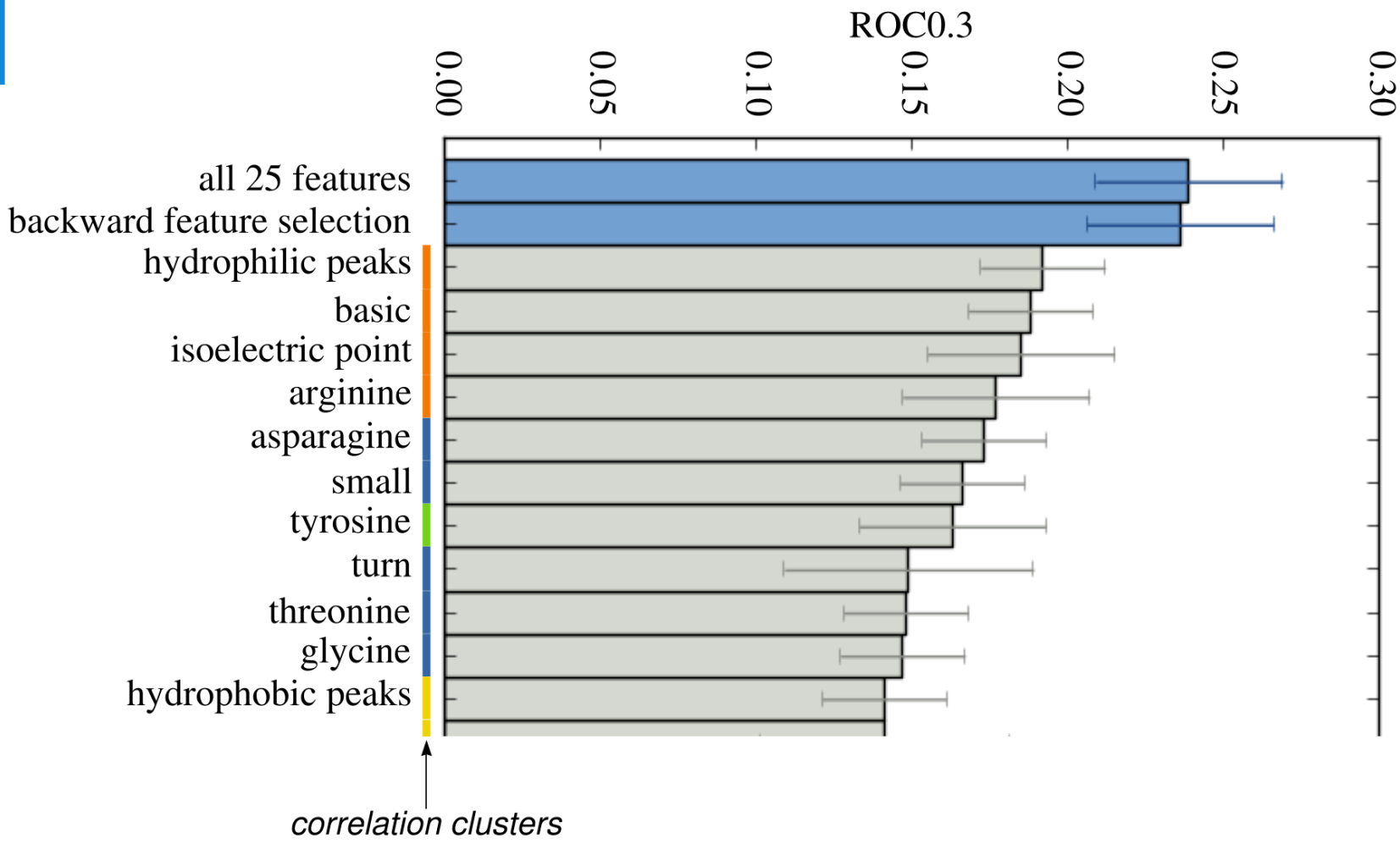
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correlation clusters

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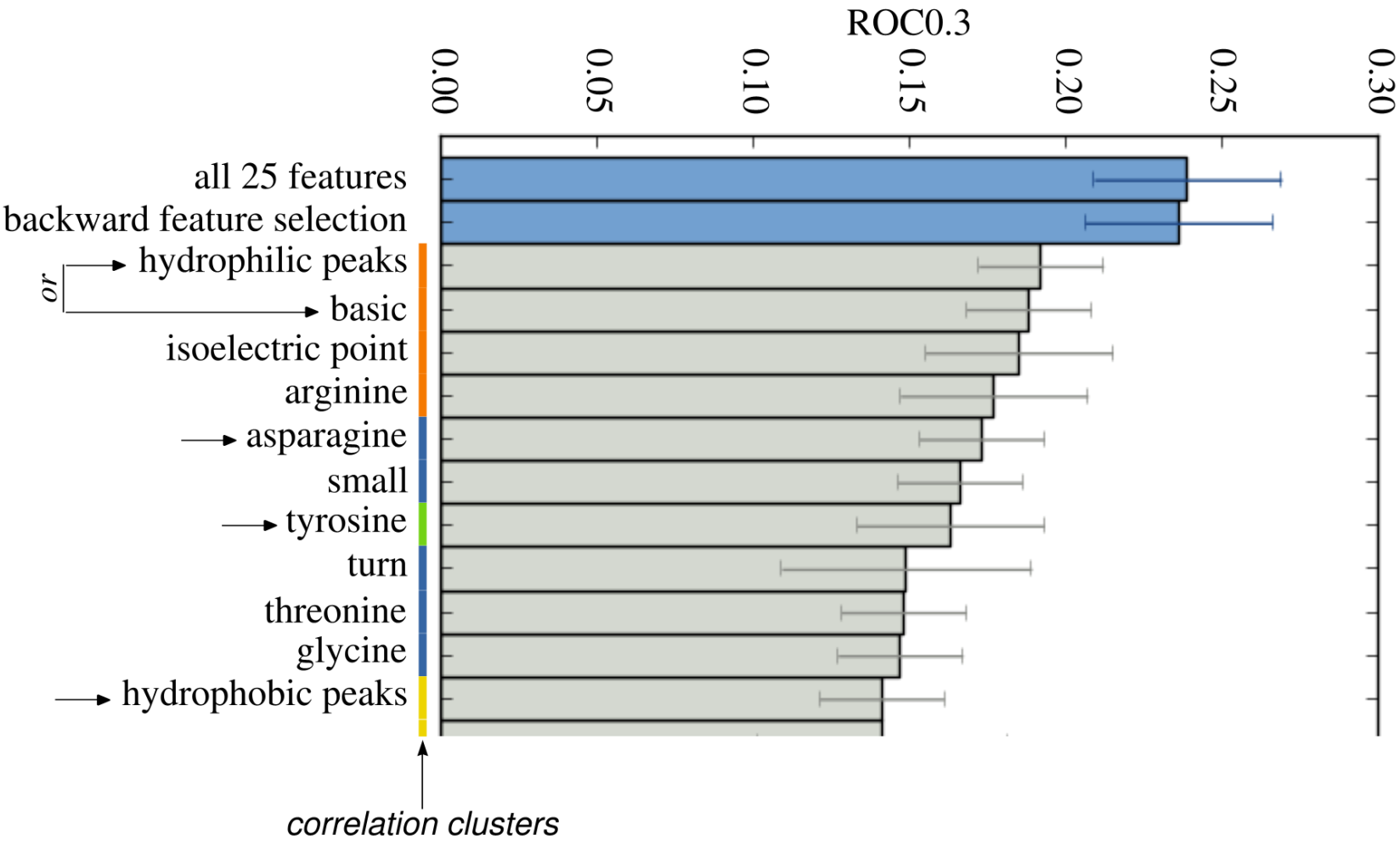
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Further research

- Separation n-terminal signal peptide and rest of the sequence
- Taking into account predicted secondary structure and solvent accessibility
- Using string kernels to include information on the location of amino acid
- Same study on a data set with heterologous proteins

Acknowledgements

nbic netherlands bioinformatics centre

DSM

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