AST | AMERICAN SOCIETY OF TRANSPLANTATION

Immune Approach To Primary Graft Dysfunction

Exosomes, Proteomics and Machine Learning

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CEOT CUTTING EDGE of TRANSPLANTATION

TRANSPLANT SUMMIT 2019

NO SIZE FITS ALL: *Uncovering the Potential of Personalized Transplantation*

FEBRUARY 21–23, 2019 • ARIZONA BILTMORE • PHOENIX, AZ

Disclosure

Funding: Department of Defense, the NIH and the Gerstner Foundation

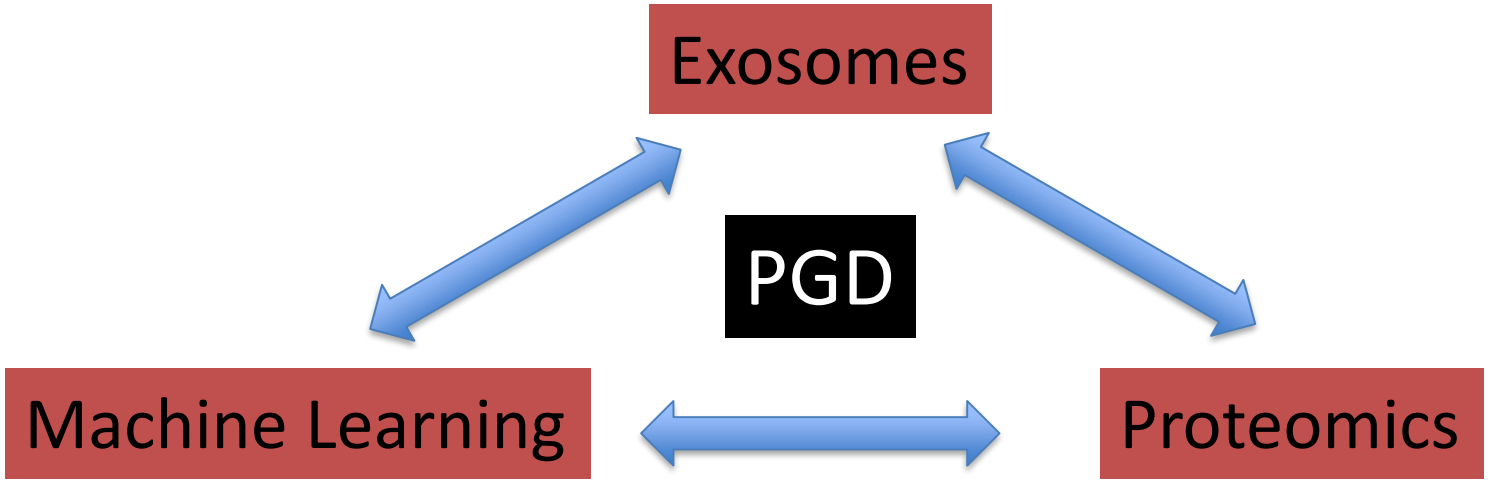
Learning Objectives

1. Delineate the role of proteomics in biomarker discovery as it relates to primary graft failure

2. Understand how we can use machine learning to analyze complex data sets

Nick Giangreco

Three Themes



Primary Graft Dysfunction

ISHLT CONSENSUS

Report from a consensus conference on primary graft dysfunction after cardiac transplantation

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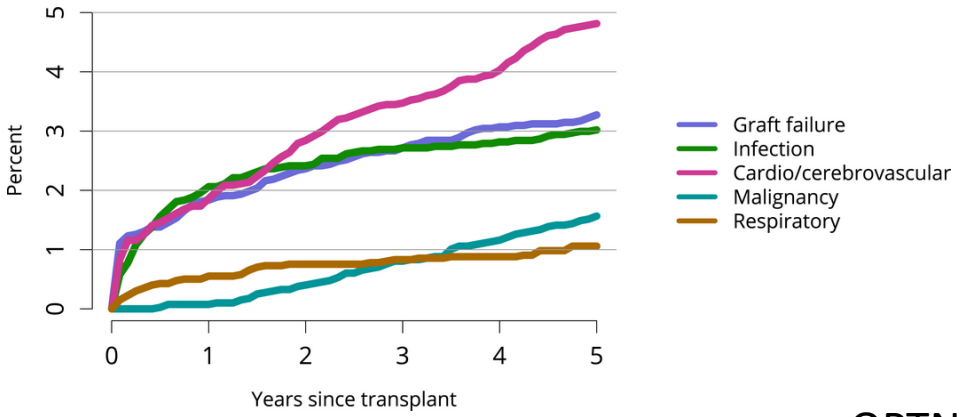
Table 6 Definition of Severity Scale for Primary Graft Dysfunction (PGD)

1. **PGD-Left ventricle (PGD-LV):**	*Mild PGD–LV*: *One* of the following criteria must be met:	LVEF ≤ 40% by echocardiography, *or* Hemodynamics with RAP > 15 mm Hg, PCWP > 20 mm Hg, CI < 2.0 L/min/m^2 (lasting more than 1 hour) requiring low-dose inotropes
	Moderate PGD-LV: Must meet one criterion from I *and* another criterion from II:	I. *One* criteria from the following: Left ventricular ejection fraction ≤ 40%, *or* Hemodynamic compromise with RAP > 15 mm Hg, PCWP > 20 mm Hg, CI < 2.0 L/min/m^2, hypotension with MAP < 70 mm Hg (lasting more than 1 hour) II. *One* criteria from the following: i. High-dose inotropes—Inotrope score > 10[a] *or* ii. Newly placed IABP (regardless of inotropes)
	Severe PGD–LV	Dependence on left or biventricular mechanical support including ECMO, LVAD, BiVAD, or percutaneous LVAD. Excludes requirement for IABP.
2. **PGD-right ventricle (PGD-RV):**	Diagnosis requires either both i and ii, or iii alone:	i. Hemodynamics with RAP > 15 mm Hg, PCWP < 15 mm Hg, CI < 2.0 L/min/m^2 ii. TPG <15 mm Hg and/or pulmonary artery systolic pressure < 50 mm Hg, *or* iii. Need for RVAD

BiVAD, biventricular assist device; CI, cardiac index; ECMO, extracorporeal membrane oxygenation; IABP, intra-aortic balloon pump; LVAD, left ventricular assist device; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure; RVAD, right ventricular assist device; TPG, transpulmonary pressure gradient.

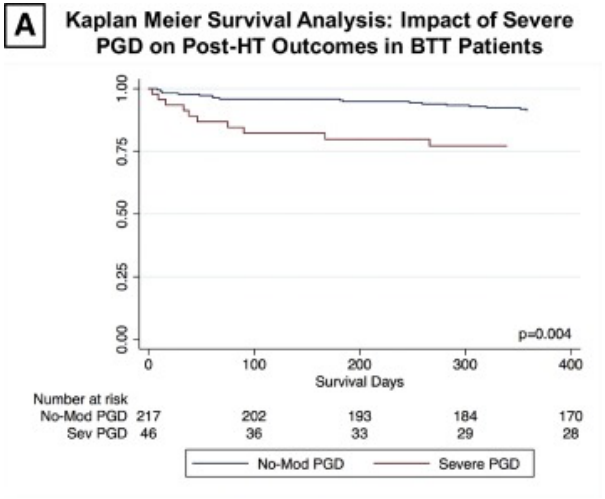
[a]Inotrope score = dopamine (×1) + dobutamine (×1) + amrinone (×1) + milrinone (×15) + epinephrine (×100) + norepinephrine (×100)[67] with each drug dosed in μg/kg/min.

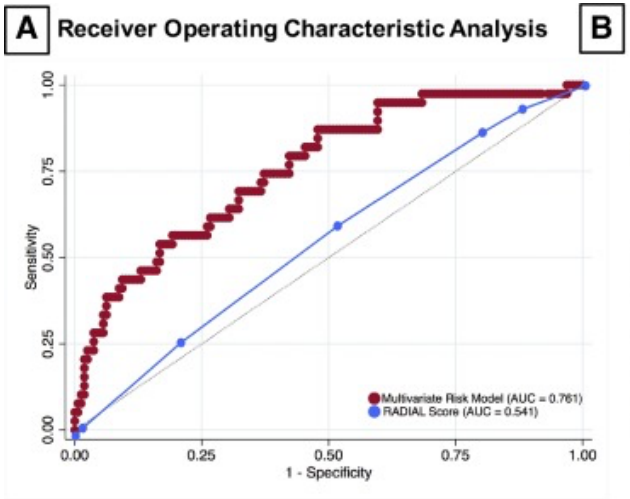
Graft dysfunction within 24 hours of transplant without a discernible cause such as rejection, pHTN, infection, etc…

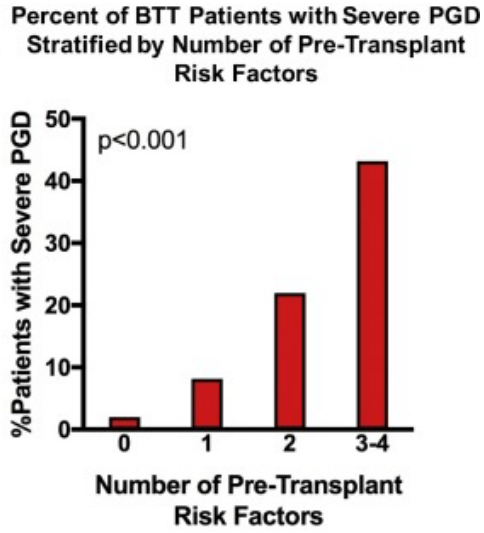


OPTN 2016

Primary Graft Dysfunction – Clinical Impact

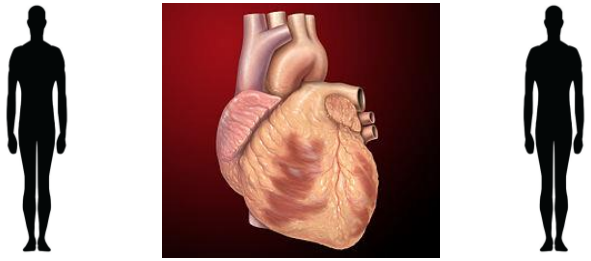






Truby et al JHLT 2018

Primary Graft Dysfunction - Etiology



Donor Graft Recepient

Acute Myocardial Dysfunction

Etiology?

Opportunity?

Primary Graft Dysfunction – Precision Medicine

Donor

"an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person"

-NIH

Primary Graft Dysfunction – Precision Medicine

Donor

"an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person"

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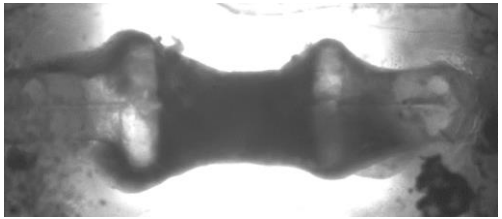
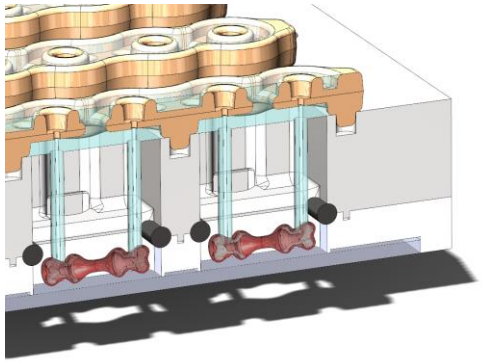
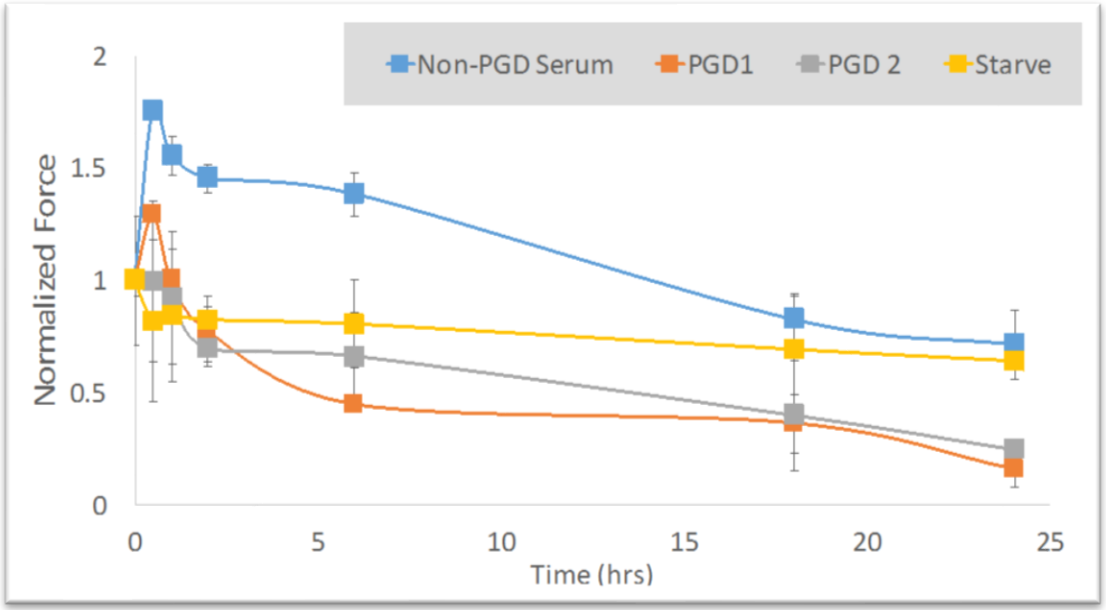
Hostile Donor?

Circulating factors in the blood of transplant recipients impede cardiomyocyte function at the cellular level, leading to overall organ dysfunction.

Primary Graft Dysfunction – Hostile Donor



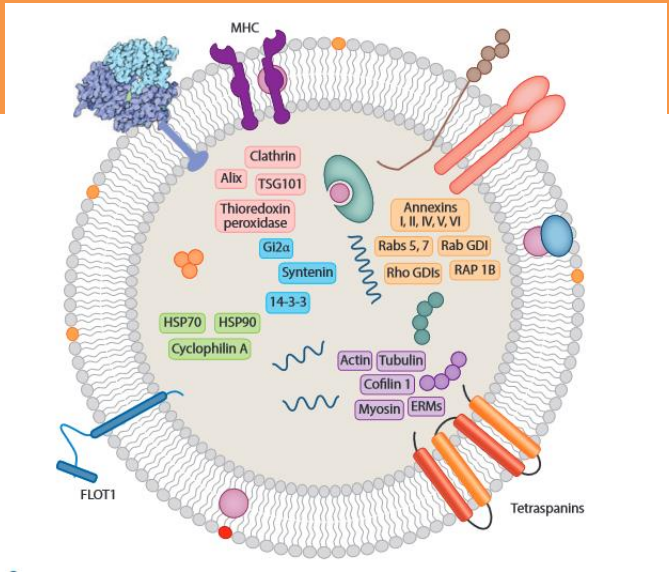




Exosomes

Characterize the “Hostile Milieu” Hypothesis

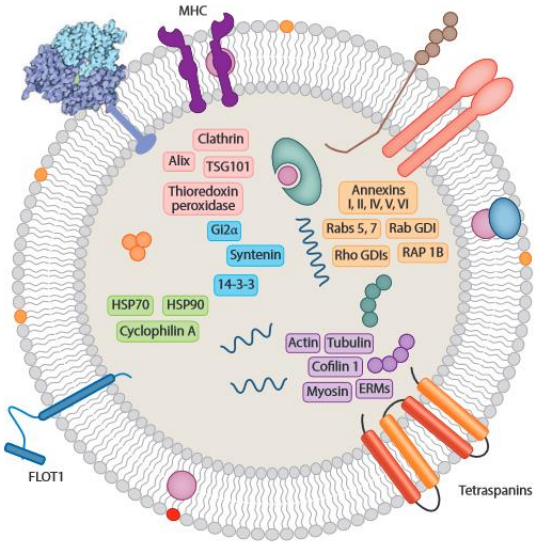
- Small 30-120nm cell derived vesicles that are likely present in all body fluids
- Released by cells as MVB merge with the cytoplasmic membrane
- Complex cargo including miRNA, mRNA, proteins and specialized lipids
- Delivered to recipient cells through endocytosis and have
- Burgeoning understanding of the role of exosomes in disease



Origin	Physiological Role
DC and B cells	Metastasis,
Tumors	MS and TIA
Vasculature	Prions
Pathogens	
Adaptive Immunity	

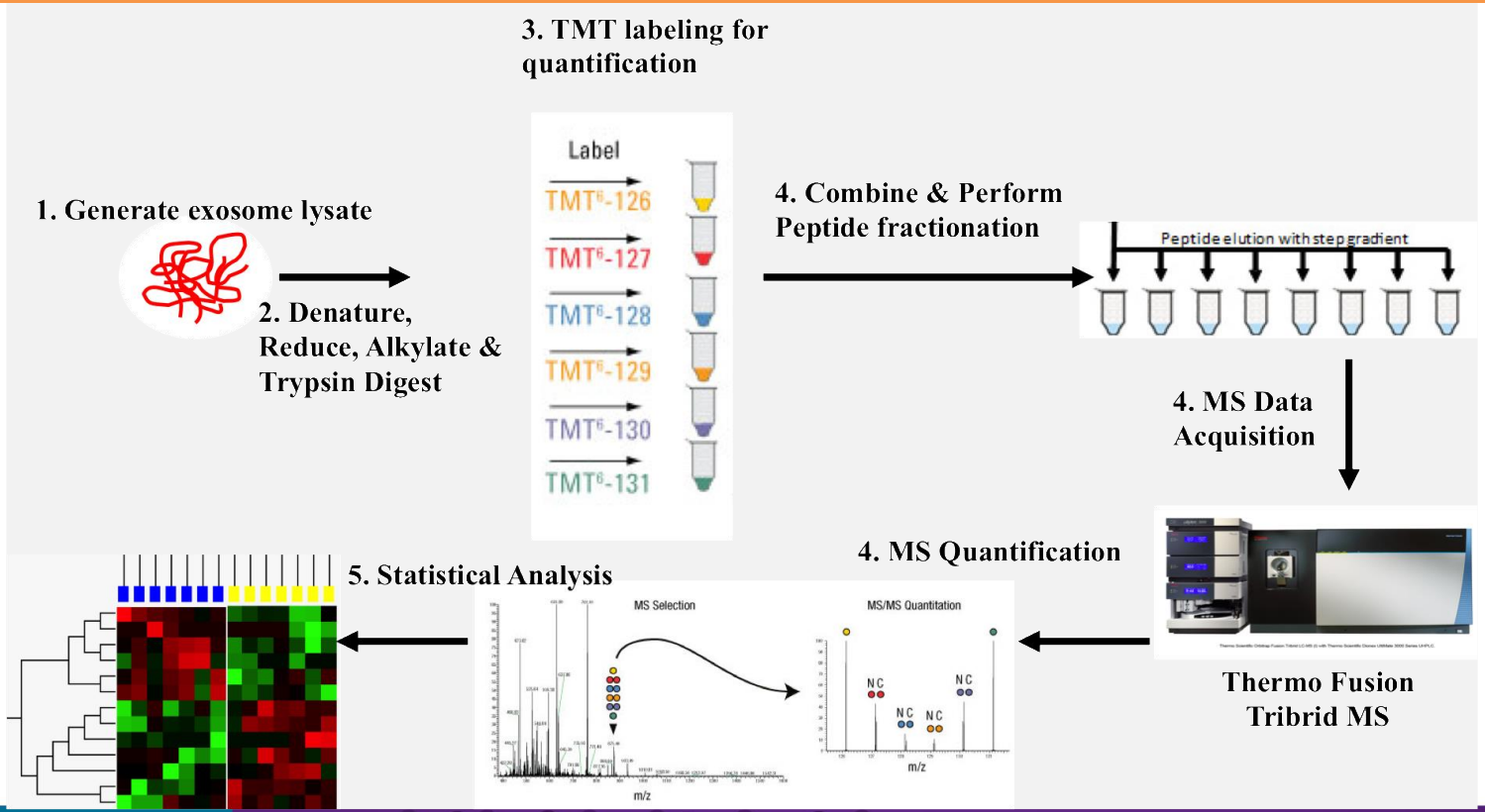
Exosomes

- Can be rapidly isolated using immuno-affinity methods or ultrafiltration from accessible fluids (blood/urine)
- Secreted in both normal and pathological states
- Exosomal miRNA have been shown to be clinically relevant biomarkers for multiple cancers
- Exosomal proteins have been found to be potentially useful biomarkers in cancer, neurological disease and kidney disease
- Importantly, cargo is very **stable** compared to proteins and RNA in serum

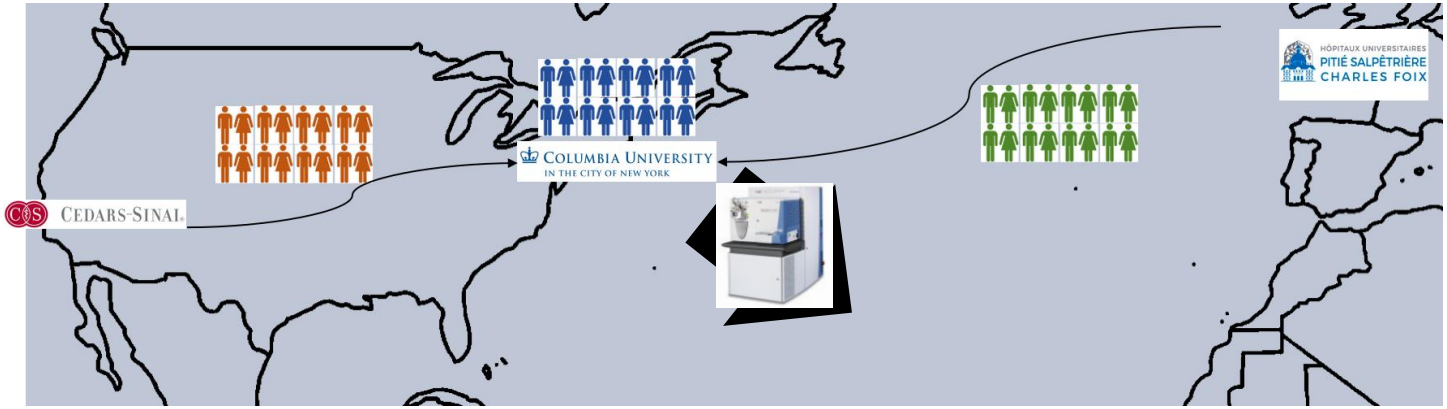


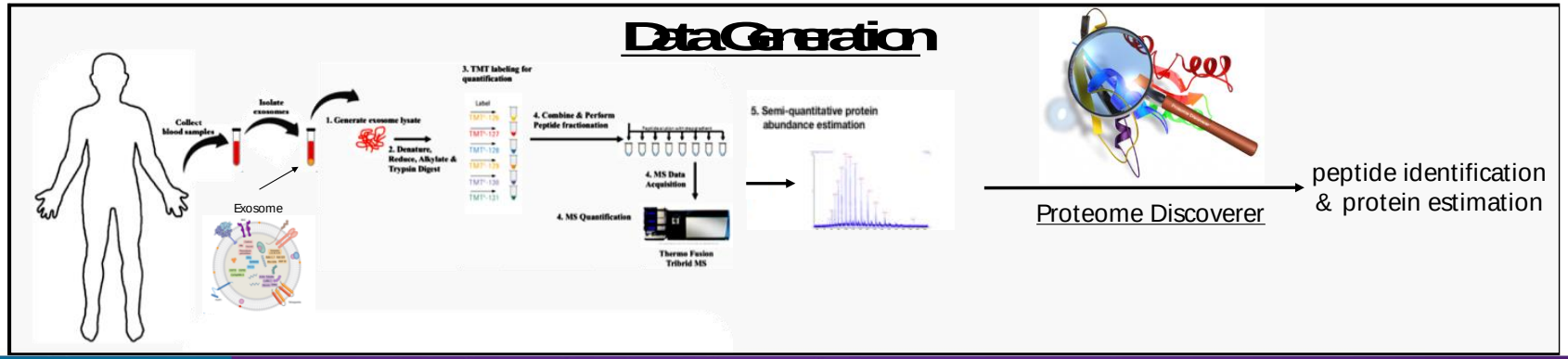
Protein	Disease Biomarker
Fetulin-A	AKI
EGFRvIII	Glioblastoma
Phospho-Tau	Alzheimers
α-synuclein	parkinsons

Proteomics on Pre-Transplant Serum Exosomes



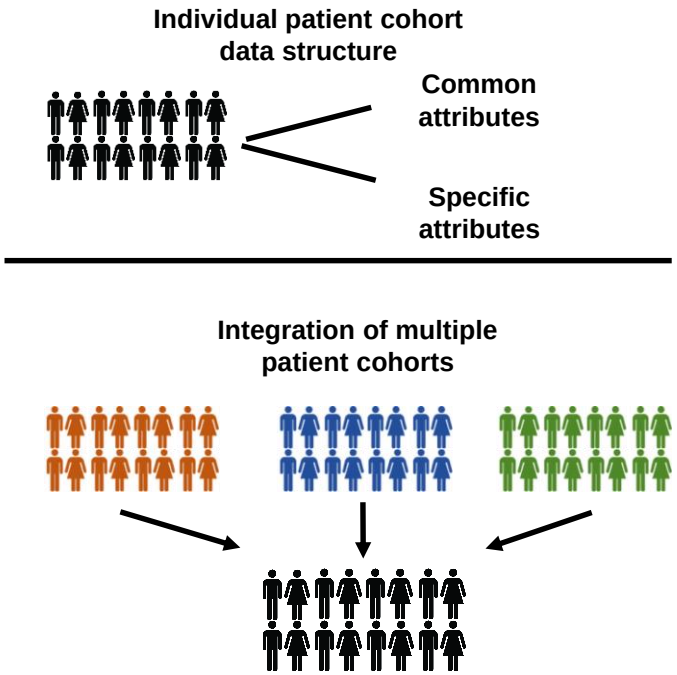
Workflow


CEDARS-SINAI
COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK
HÔPITAUX UNIVERSITAIRES
PITIÉ SALPÊTRIÈRE
CHARLES FOIX


Data Generation
Collect blood samples
Isolate exosomes
Exosome
1. Generate exosome lysate
2. Denature, Reduce, Alkylate & Trypsin Digest
3. TMT labeling for quantification
4. Combine & Perform Peptide fractionation
4. MS Data Acquisition
4. MS Quantification
Thermo Fusion Tribrid MS
5. Semi-quantitative protein abundance estimation
Proteome Discoverer
peptide identification & protein estimation

cohorts

https://github.com/ngiangre/cohorts



`cohorts` is:

- Modular
  - Each cohort is its own data structure
- Customizable
  - Each cohort has attributes for individual and integrative cohort analyses
- Flexible
  - Can use built in functions and use custom code leveraging the cohort attributes

Proteomics

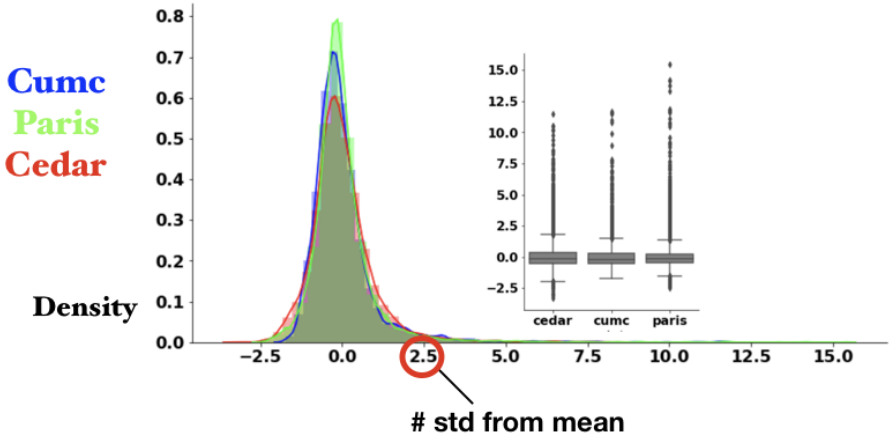
There are two questions we can try to answer with proteomics

1. Mechanistic: Do the proteins tell us something about PGD. Is there a pathway discernible in differential protein analysis?

2. Classification: Can we use a protein or a set of proteins to help us predict PGD. Are there "features" can correctly classify transplant candidates at high risk versus those at low risk of PGD

Proteomics

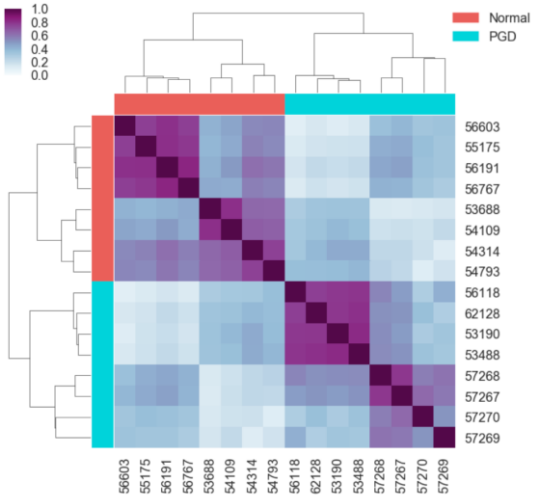
Data distribution

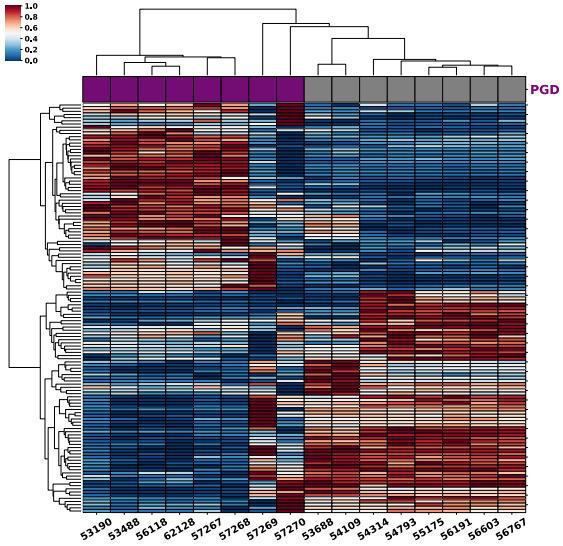


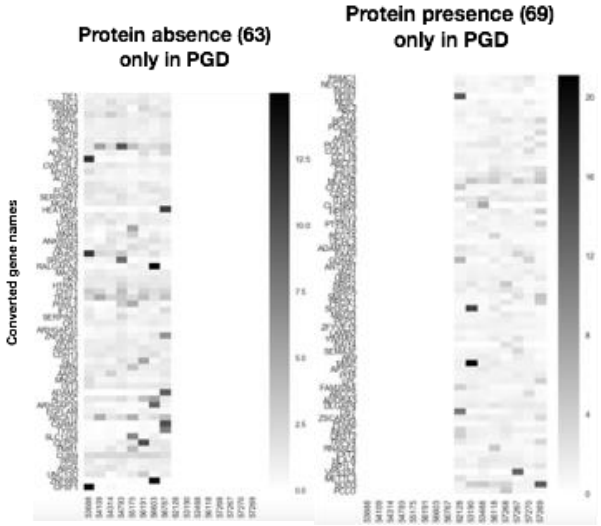
	# Data Files	# Proteins	# Replicates	# Samples	# Groups
Columbia	2	1204	64	16	5
Cedar-Sinai	2	729	150	44	7
Paris	2	934	108	29	8

Prospective Cohort - Columbia

PGD Patients Have Different Exosome Proteomics Prior to Transplant







Prospective Cohort - Columbia

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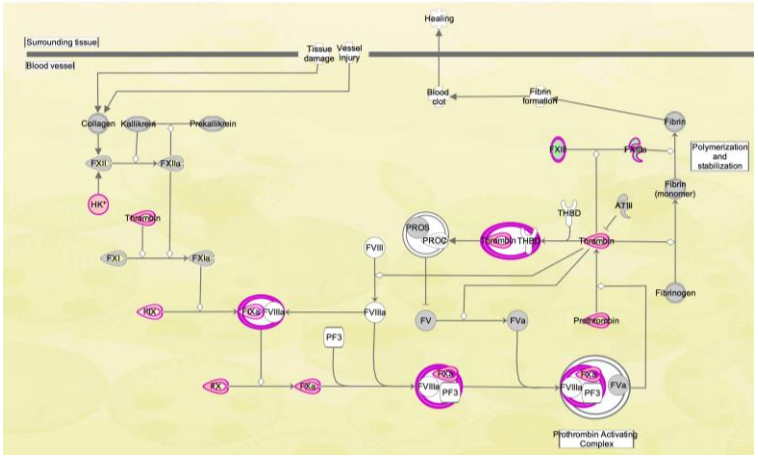
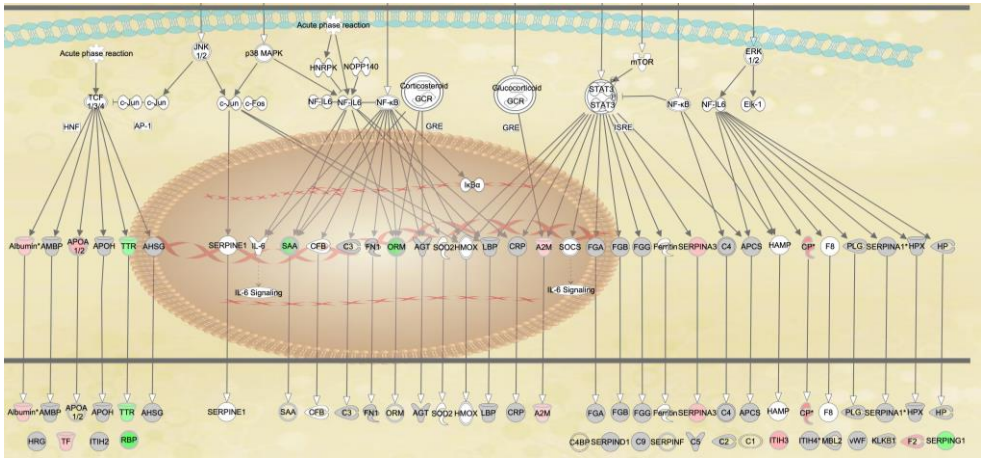
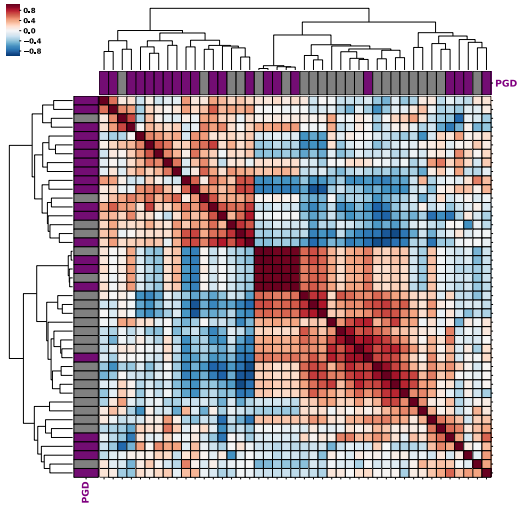
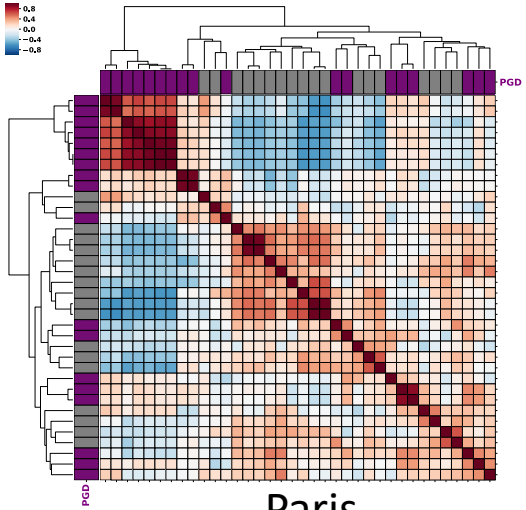




Retrospective Cohort – Cedars and Pitie Salpetriere

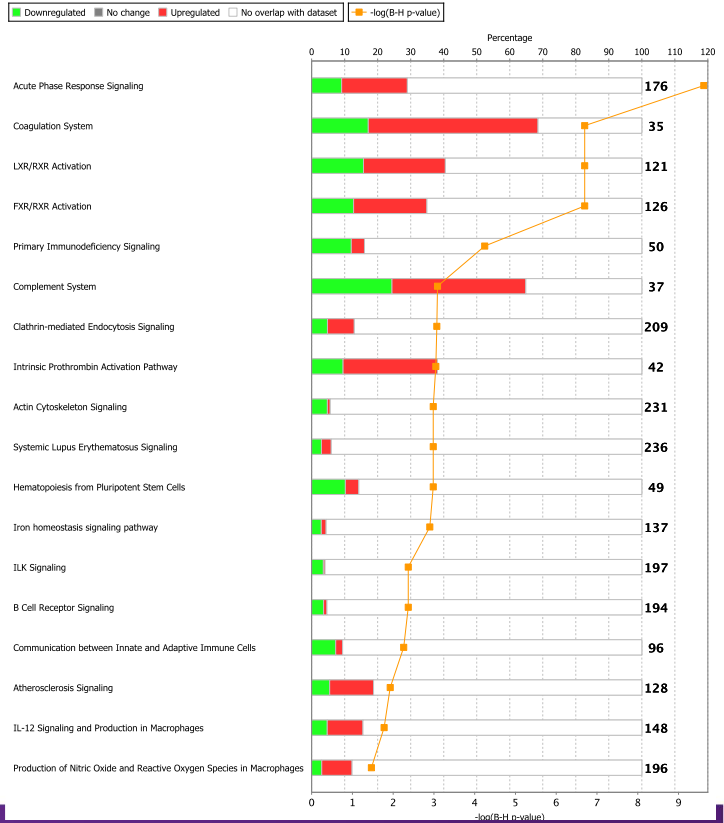


Cedars



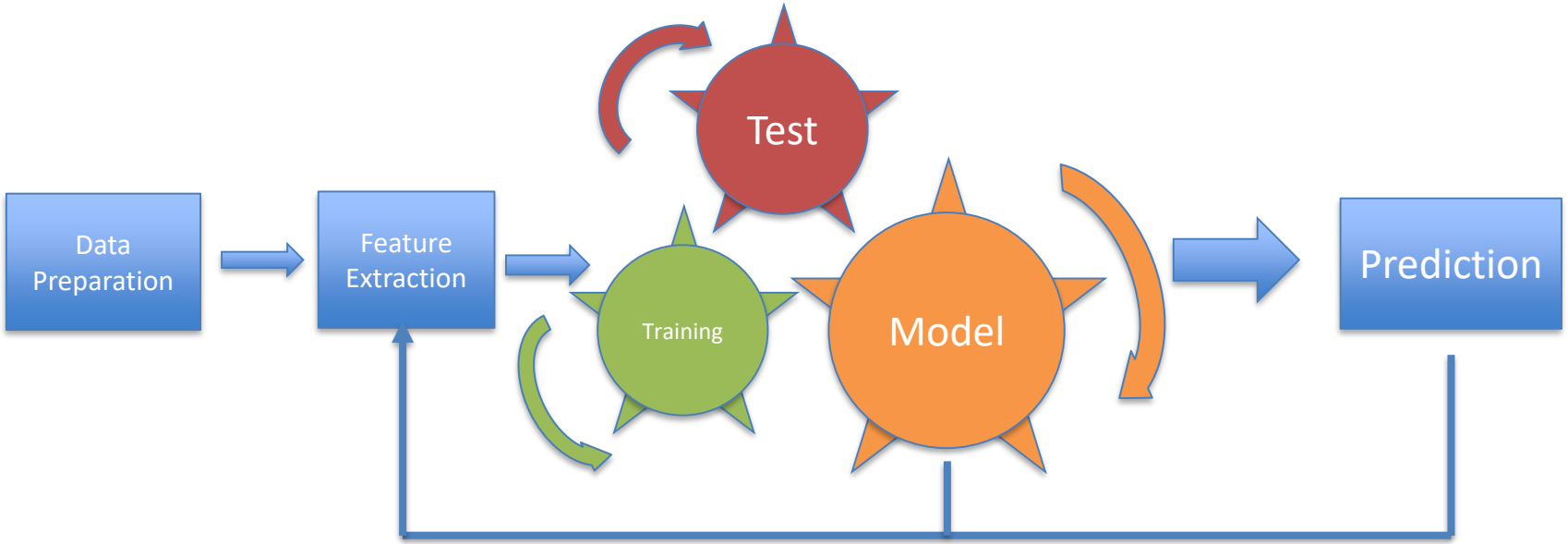
Paris

Low Correlation
Small number of differential proteins
PGD did not separate out



Classification – Machine Learning

Machine Learning: Building mathematical models using training data in order to make predictions or decisions on test data without explicitly programmed to perform the task. Important to this is progressive improvement in that task with more data and training.

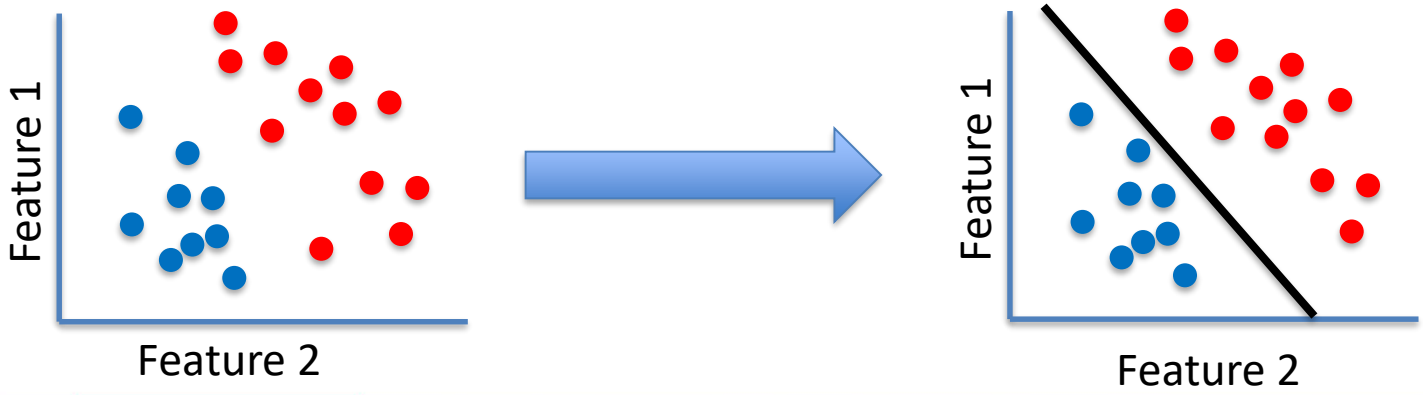


Classification Machine Learning

Different Algorithms

Multidimensional Feature Space

Allows identification of boundaries between two states



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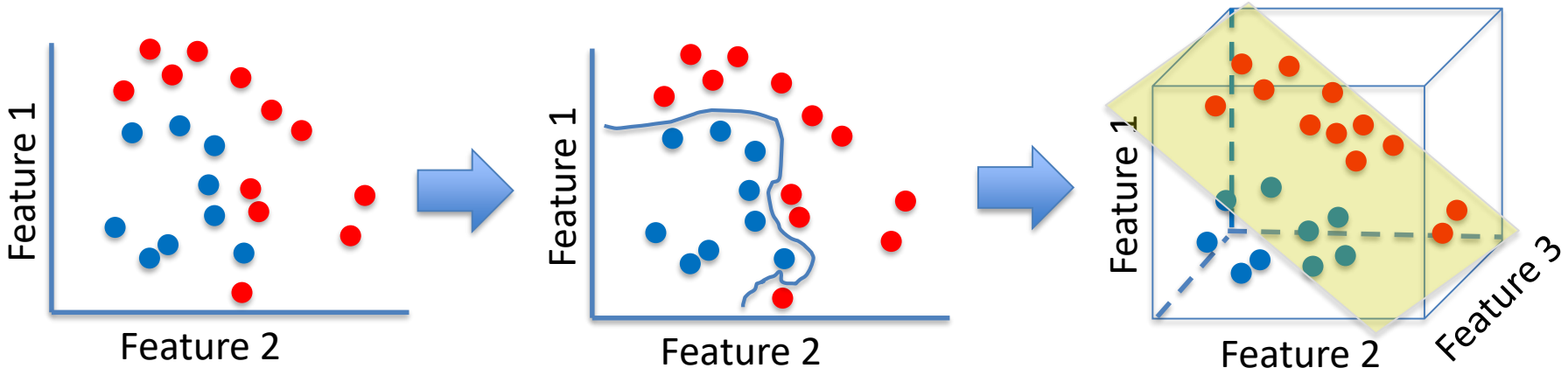

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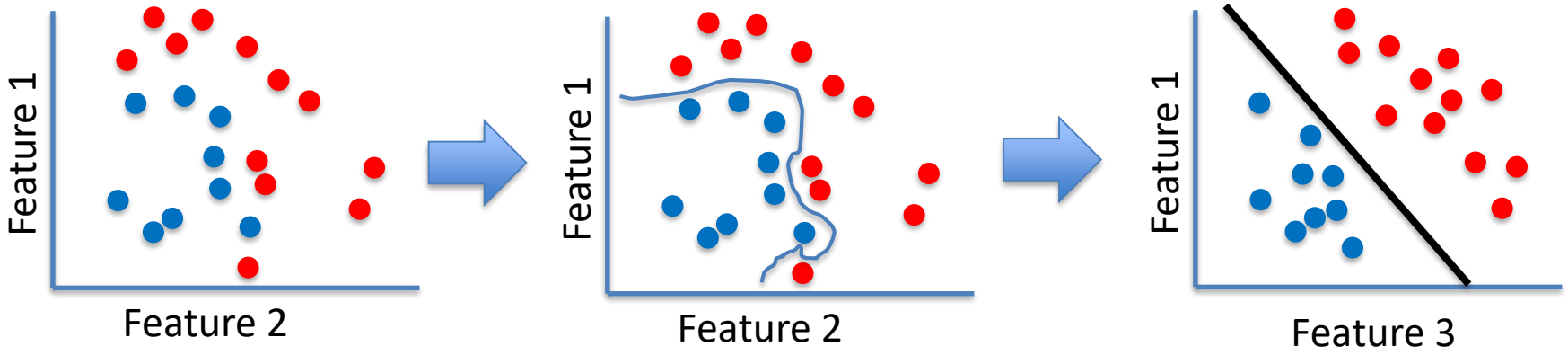

Classification Machine Learning

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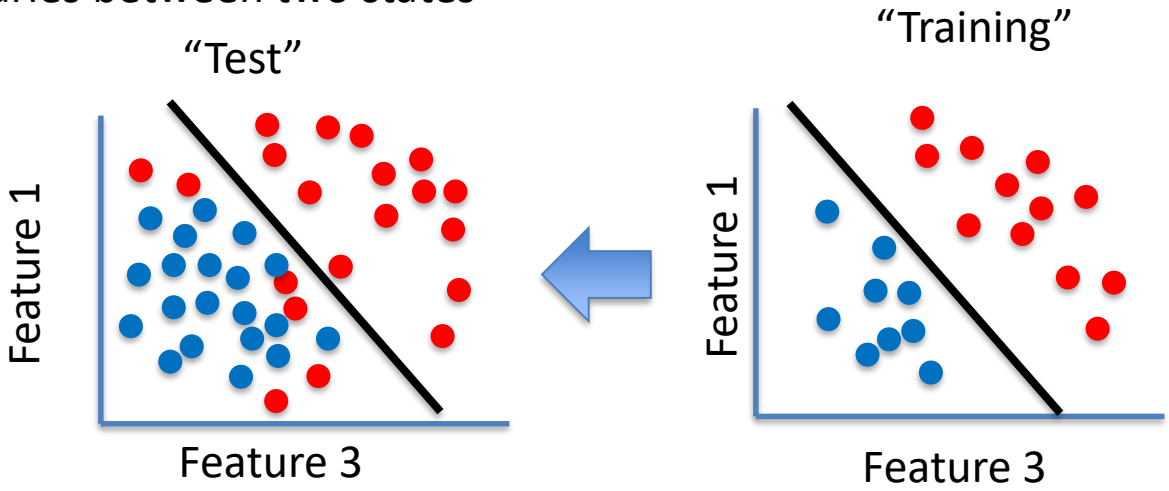
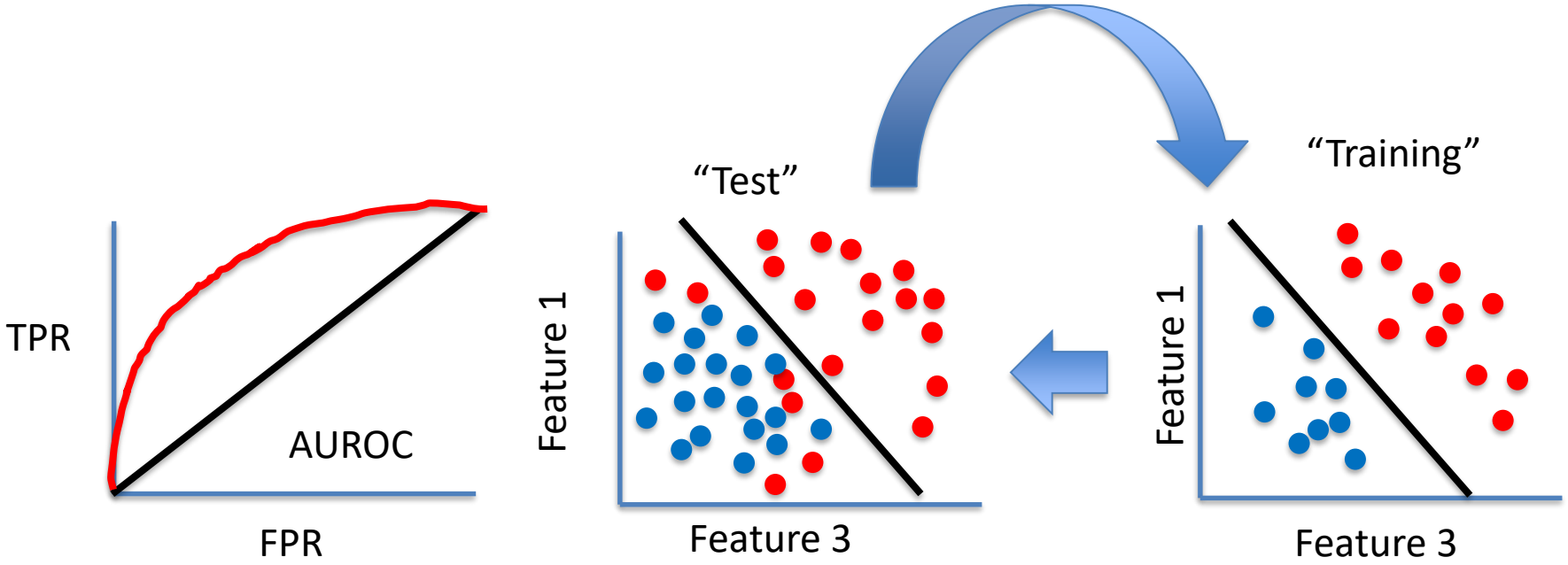
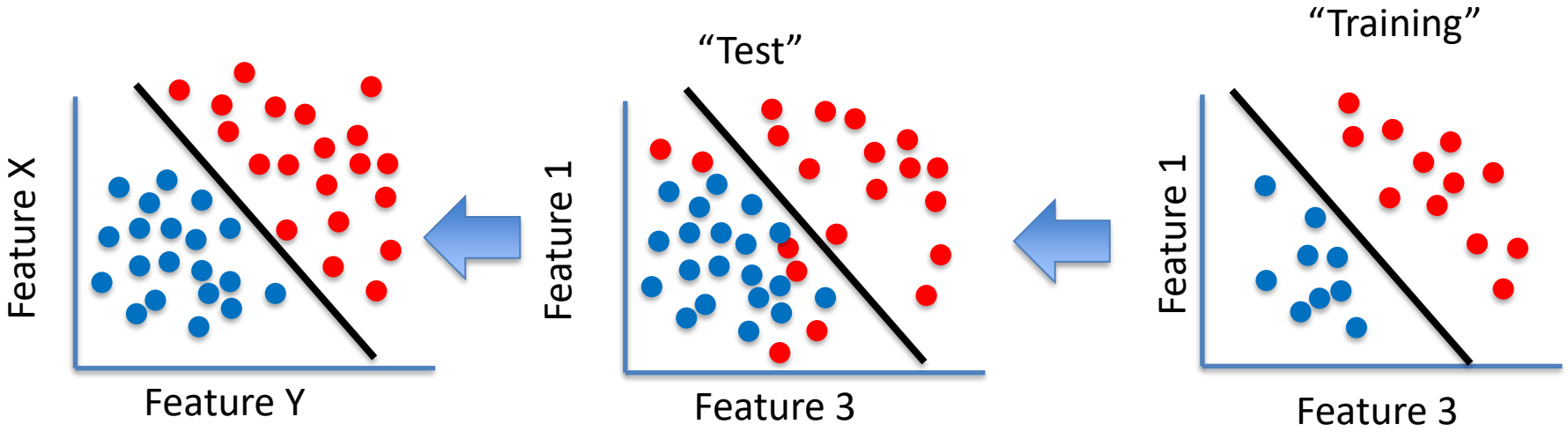


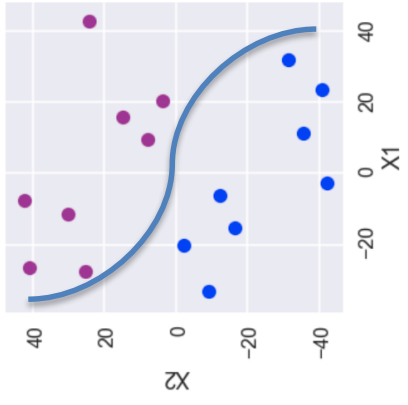
Classification Machine Learning

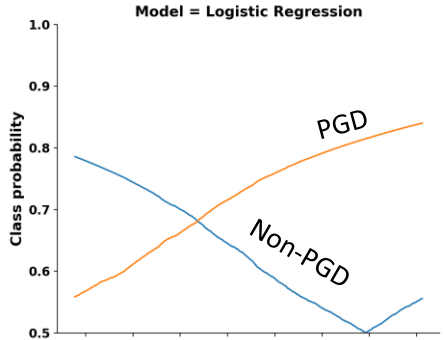


Classification Machine Learning

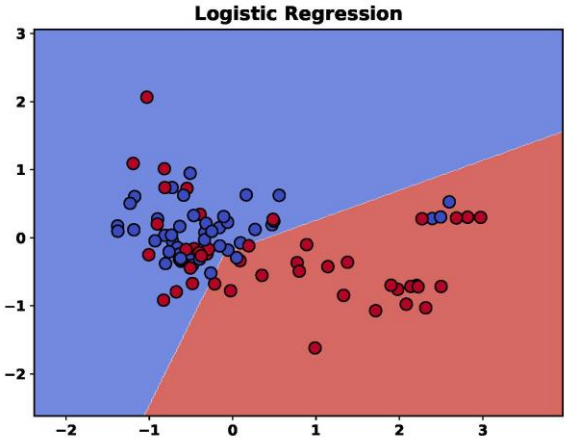


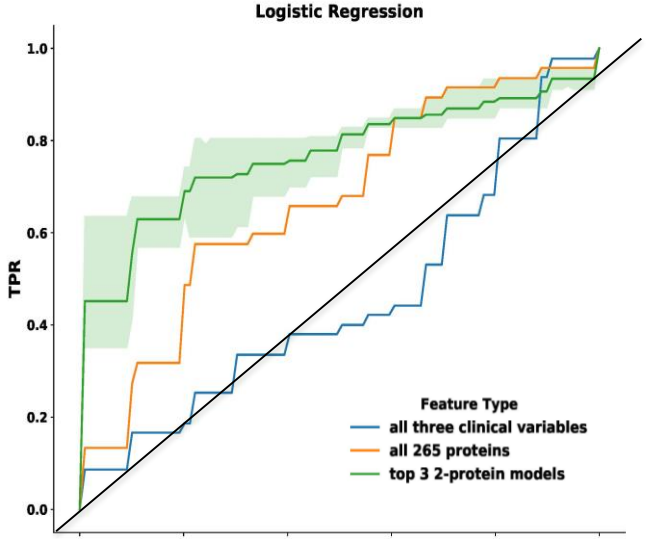
PGD– Prediction Using Logistic Regression





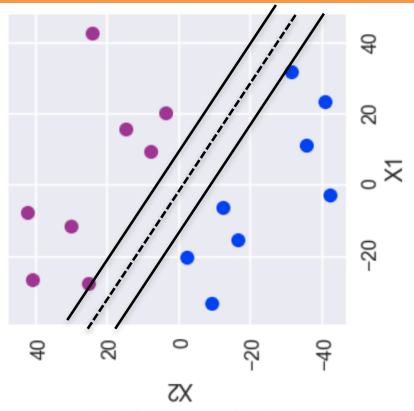
Protein Expression

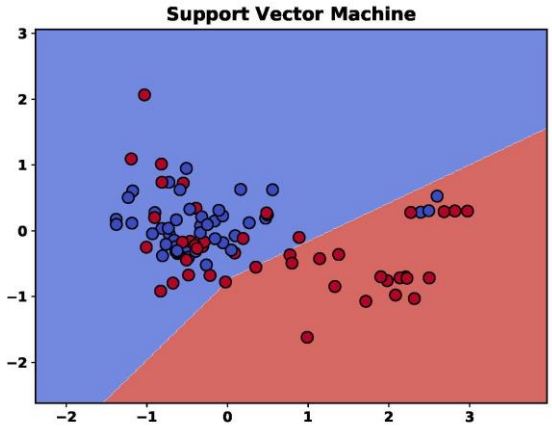


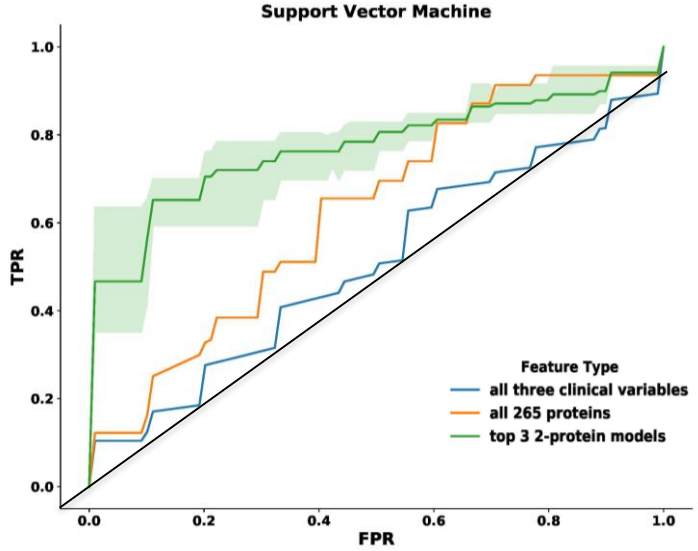


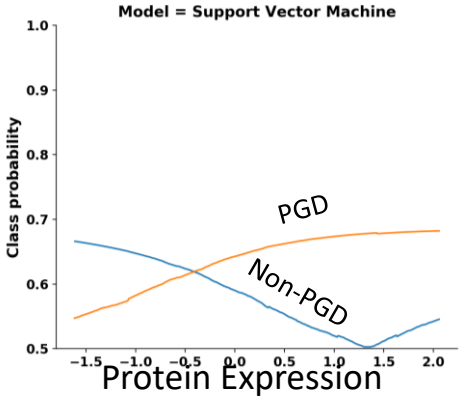
Feature	AUROC	TPR	TNR
Top Two Proteins	0.803	0.57	0.85

PGD– Prediction Using Support Vector Machines



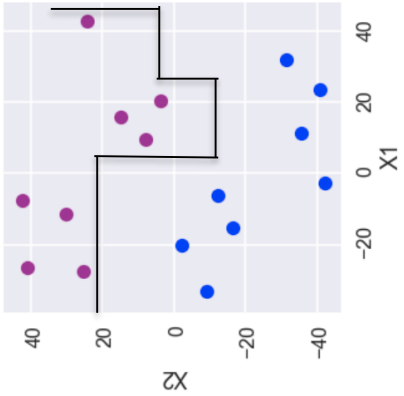


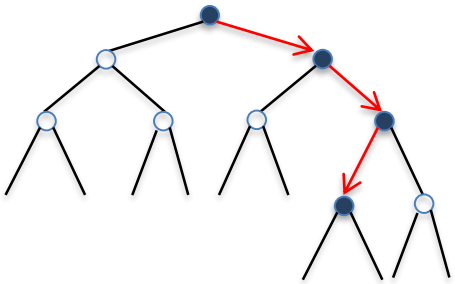


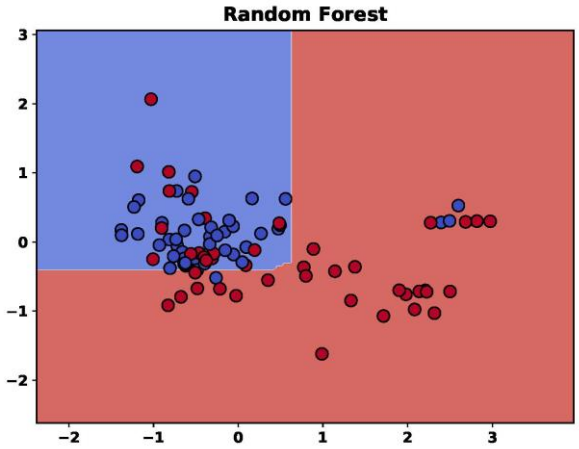


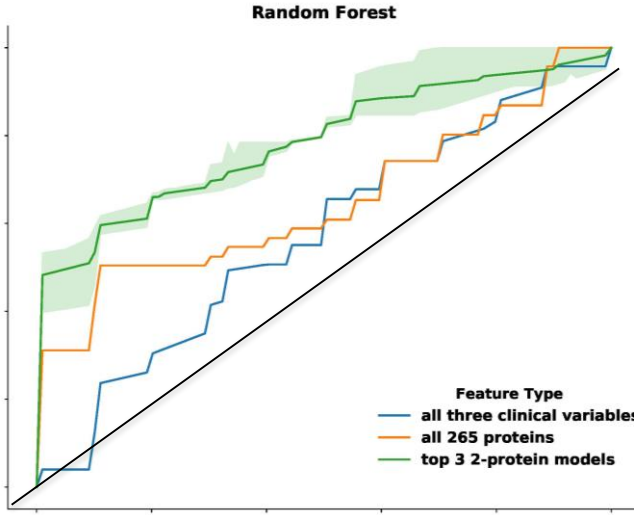
Feature	AUROC	TPR	TNR
Best 2 Protein	0.805	0.681	0.938

PGD– Prediction Using Random Forest



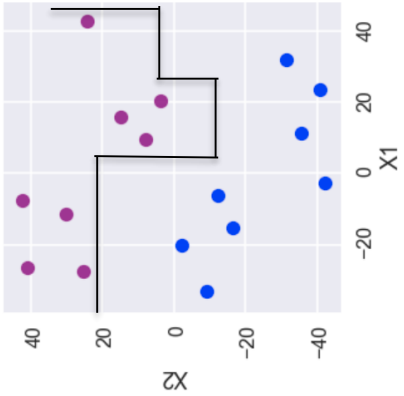


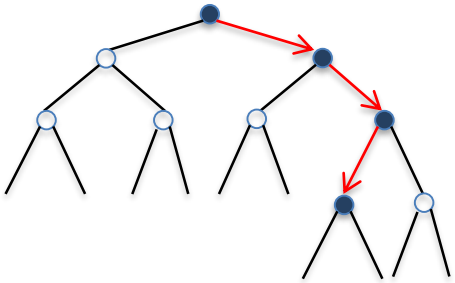


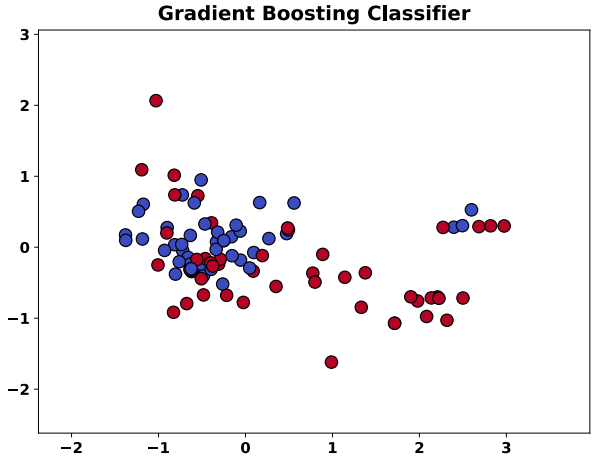


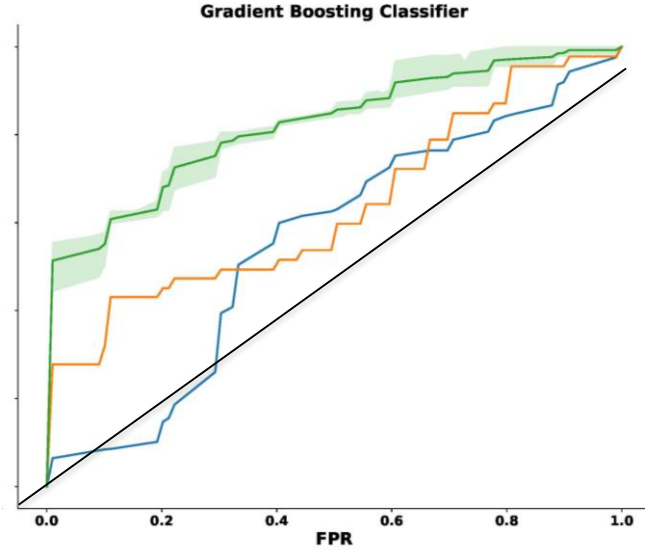
Feature	AUROC	TPR	TNR
Best 2 Protein	0.807	0.660	0.875

PGD– Gradient Boosting







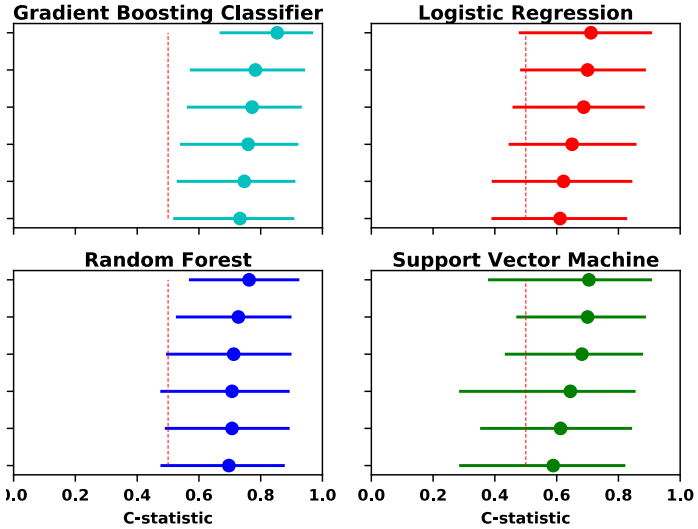


Feature	AUROC	TPR	TNR
Best 2 Protein	0.829	0.72	0.79

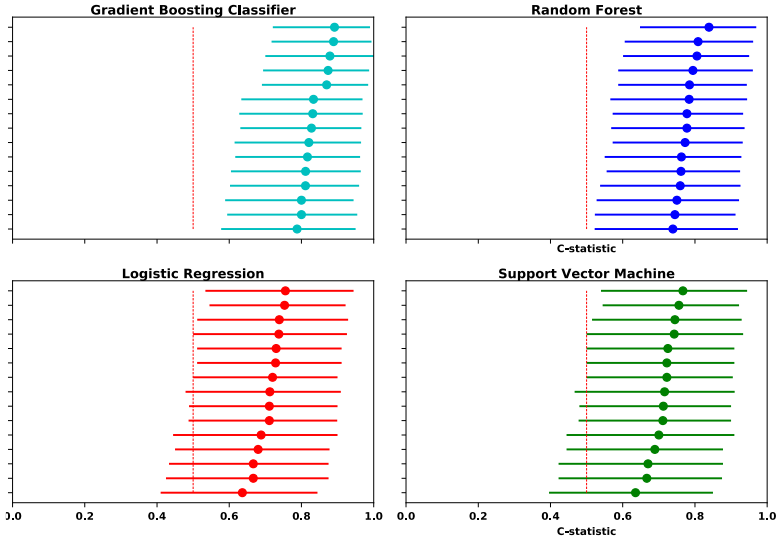
Best boundary may not be clinical

Bootstrapping with Recursive Feature Extraction

Constraint to One Protein

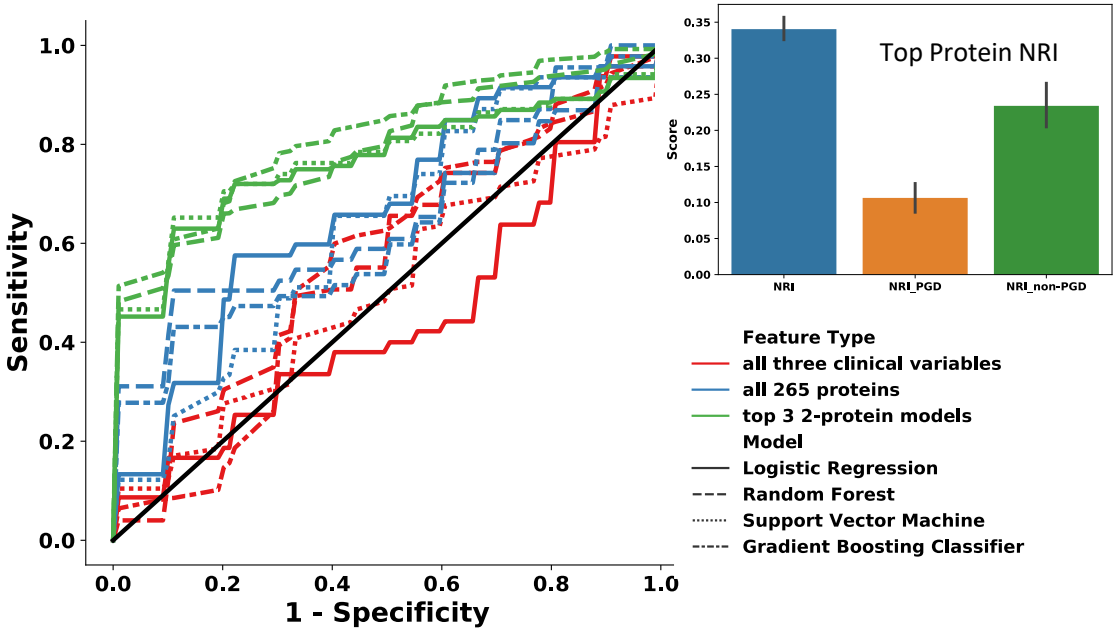


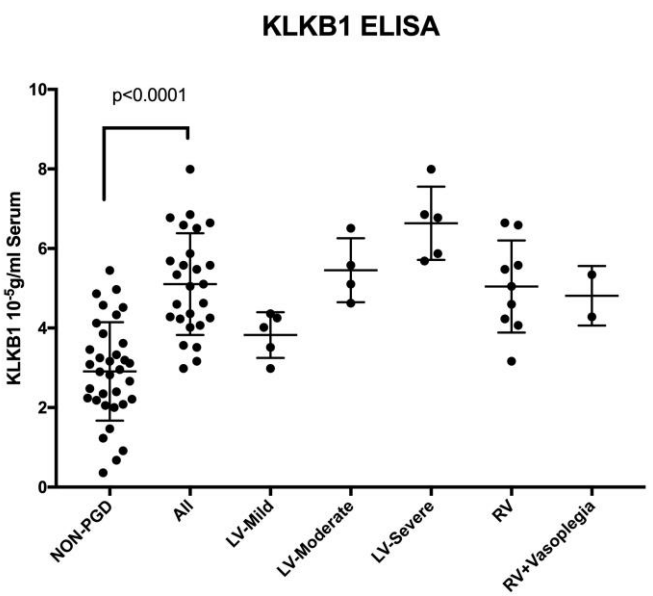
Constraint to Two Protein



More Dimensions → Better Prediction

Overall Prediction Performance





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