

Distinct Degradome Signature in Triple Negative Breast Cancer Revealed by Large-Scale Comparative Peptidomic Analysis



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Breast Cancer in the U.S.

Estimated New Cases*

			Males	Females			
Prostate	233,000	27%			Breast	232,670	29%
Lung & bronchus	116,000	14%			Lung & bronchus	108,210	13%
Colorectum	71,830	8%			Colorectum	65,000	8%
Urinary bladder	56,390	7%			Uterine corpus	52,630	6%
Melanoma of the skin	43,890	5%			Thyroid	47,790	6%
Kidney & renal pelvis	39,140	5%			Non-Hodgkin lymphoma	32,530	4%
Non-Hodgkin lymphoma	38,270	4%			Melanoma of the skin	32,210	4%
Oral cavity & pharynx	30,220	4%			Kidney & renal pelvis	24,780	3%
Leukemia	30,100	4%			Pancreas	22,890	3%
Liver & intrahepatic bile duct	24,600	3%			Leukemia	22,280	3%
All Sites	855,220	100%	All Sites	810,320	100%		

Estimated Deaths



			Males	Females			
Lung & bronchus	86,930	28%			Lung & bronchus	72,330	26%
Prostate	29,480	10%			Breast	40,000	15%
Colorectum	26,270	8%			Colorectum	24,040	9%
Pancreas	20,170	7%			Pancreas	19,420	7%
Liver & intrahepatic bile duct	15,870	5%			Ovary	14,270	5%
Leukemia	14,040	5%			Leukemia	10,050	4%
Esophagus	12,450	4%			Uterine corpus	8,590	3%
Urinary bladder	11,170	4%			Non-Hodgkin lymphoma	8,520	3%
Non-Hodgkin lymphoma	10,470	3%			Liver & intrahepatic bile duct	7,130	3%
Kidney & renal pelvis	8,900	3%			Brain & other nervous system	6,230	2%
All Sites	310,010	100%	All Sites	275,710	100%		

FIGURE 1. Ten Leading Cancer Types for the Estimated New Cancer Cases and Deaths by Sex, United States, 2014.

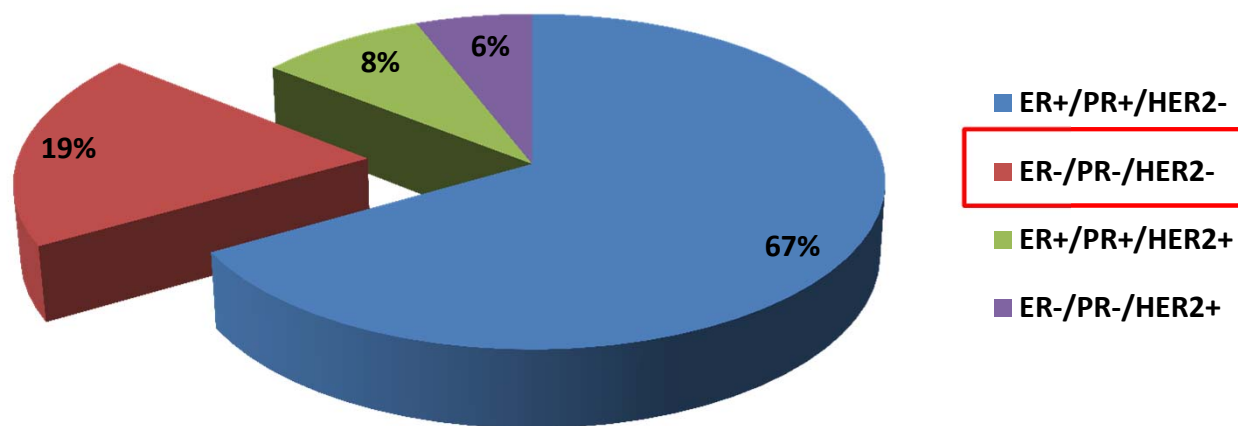
*Estimates are rounded to the nearest 10 and exclude basal cell and squamous cell skin cancers and in situ carcinoma except urinary bladder.

Triple Negative Breast Cancer

Estrogen receptor (ER)

Progesterone receptor (PR)

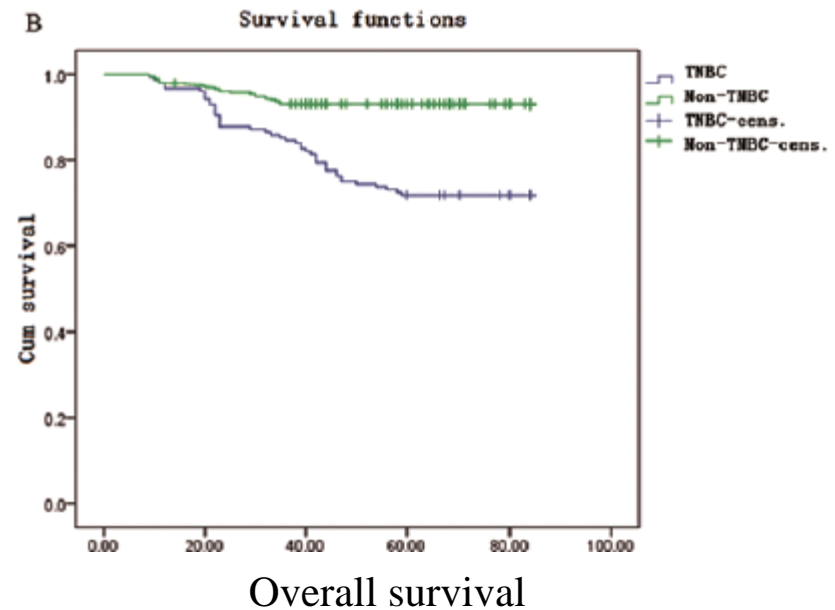
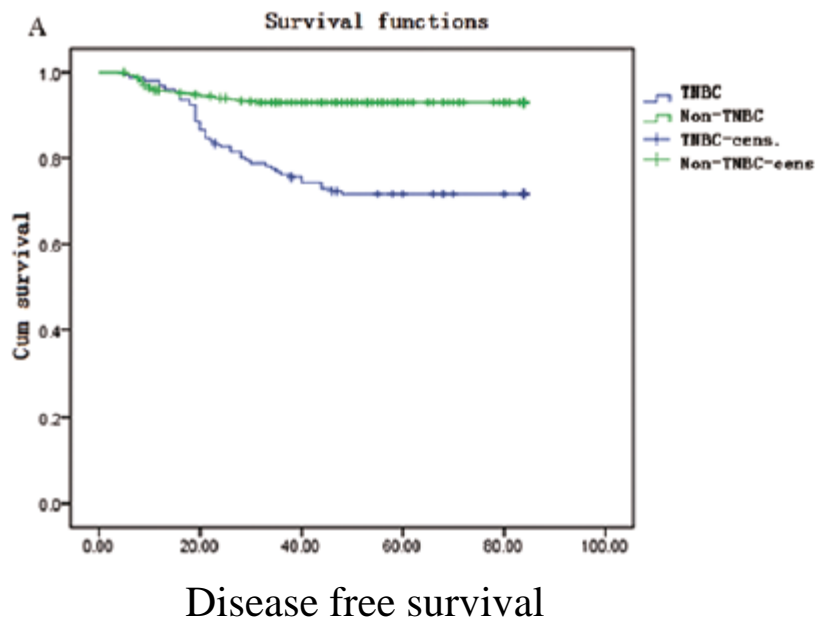
Human epidermal growth factor receptor 2 (HER2)



Triple Negative Breast Cancer

Lund et al. Cancer. 2010; 116(11):2549-2559.

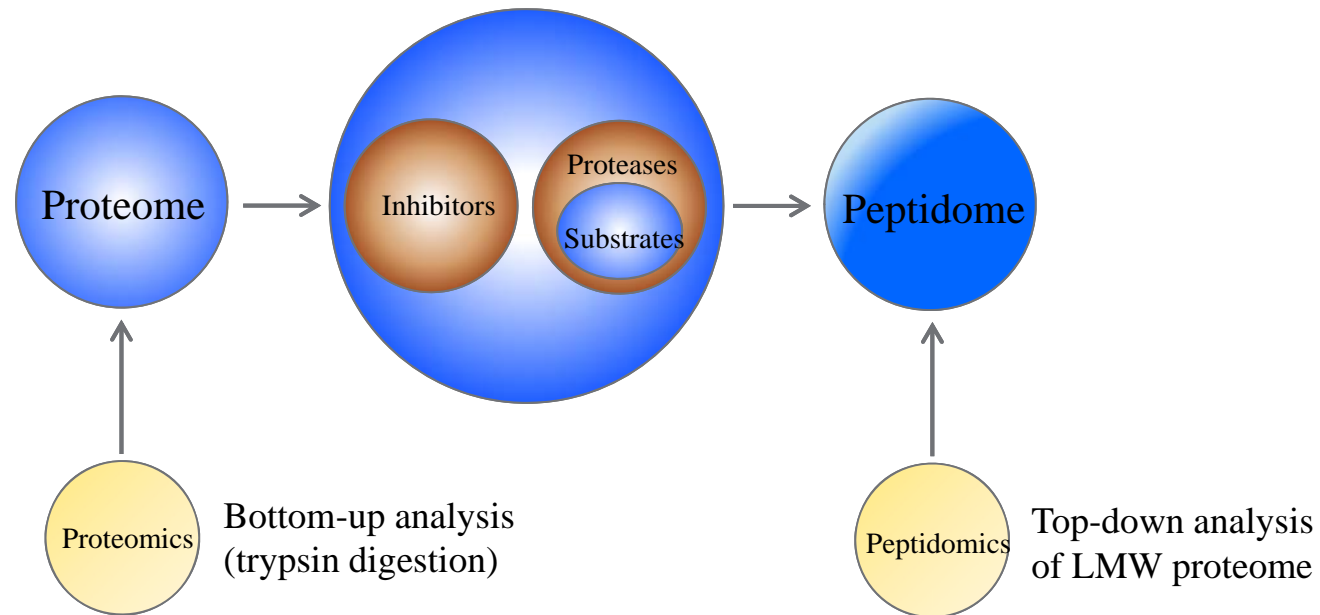
TNBC is associated with poor survival



Yuan et al. MOLECULAR AND CLINICAL ONCOLOGY 2: 245-251, 2014

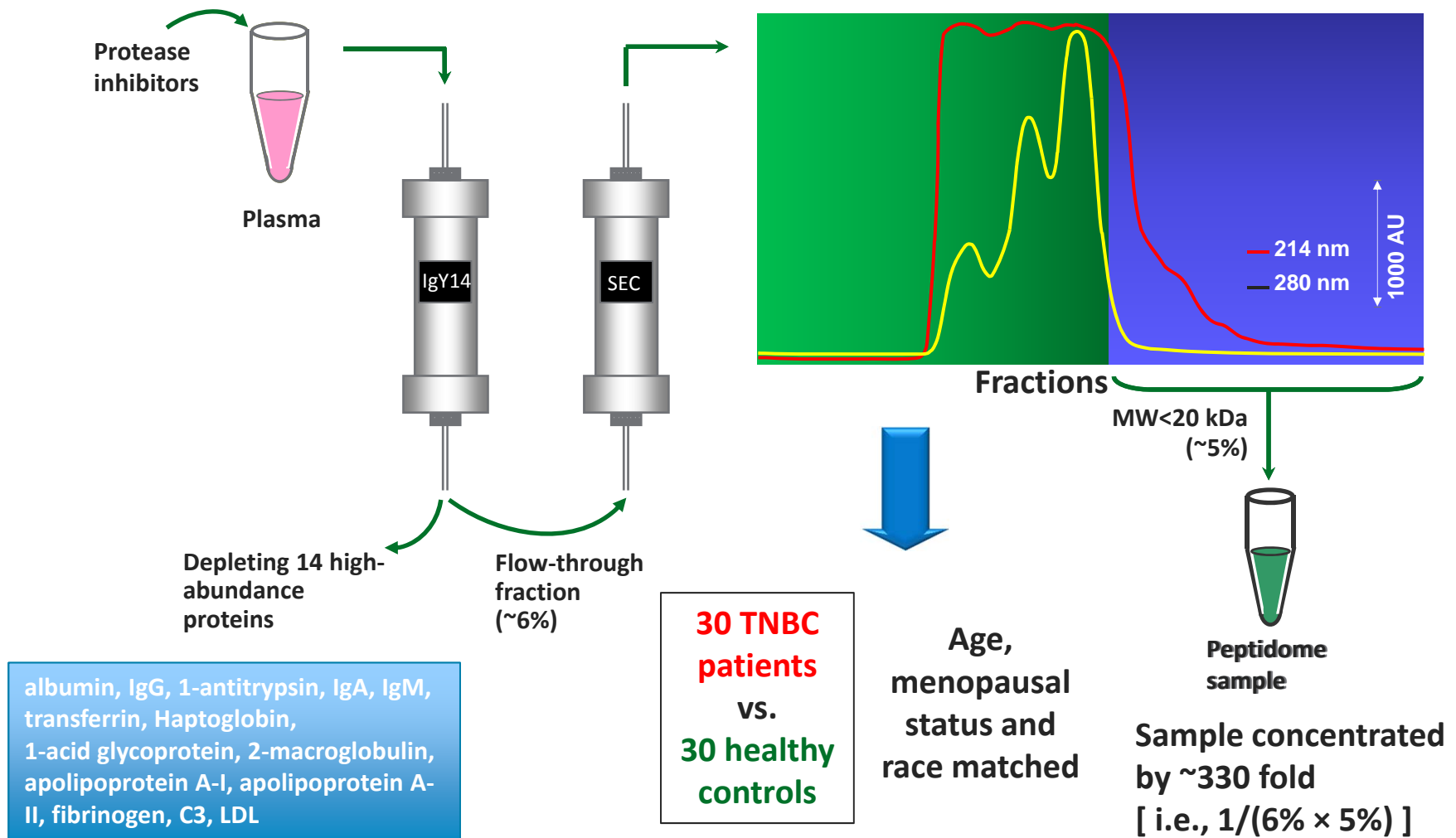
Need effective biomarkers for TNBC!

Peptidomics: promising, but challenging



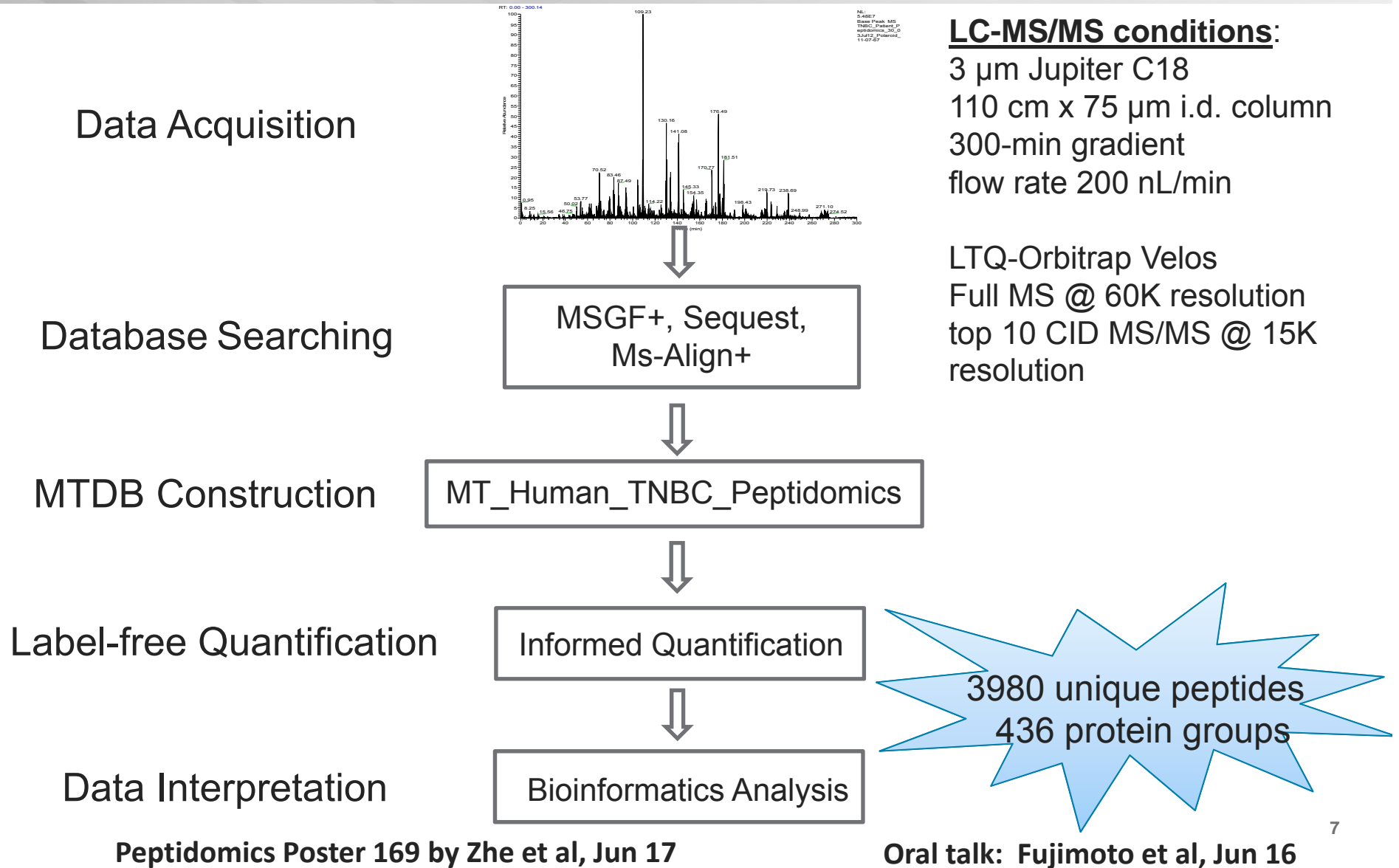
- ✓ Proteolysis affects protein abundances, function and activities
- ✓ Elevated expression/activities in cancer (e.g., OE of MMPs in BrCa: tumor growth)
- ✓ Provides a complementary (or otherwise unseen) view of the proteome
- Challenges in sample preparation and informatics analysis

Peptidome isolation from blood plasma



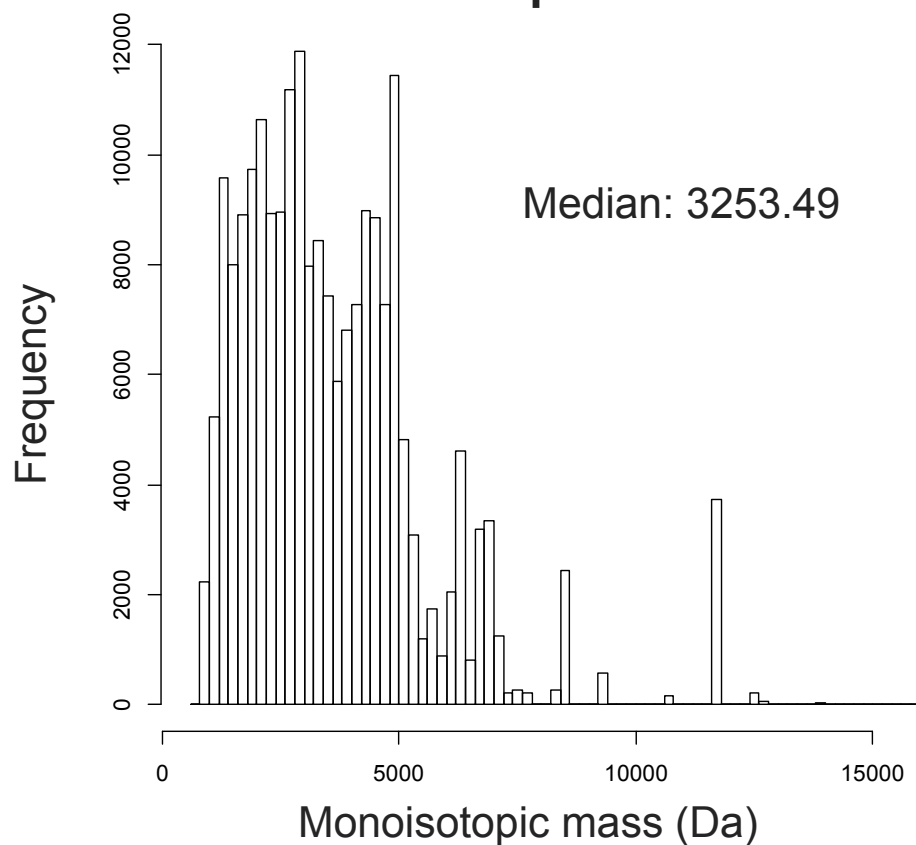
Shen et al., J Proteome Res. 2010, 9(5):2339-46
 Shen et al., PLoS One. 2010, 5(10):e13133

Data analysis workflow

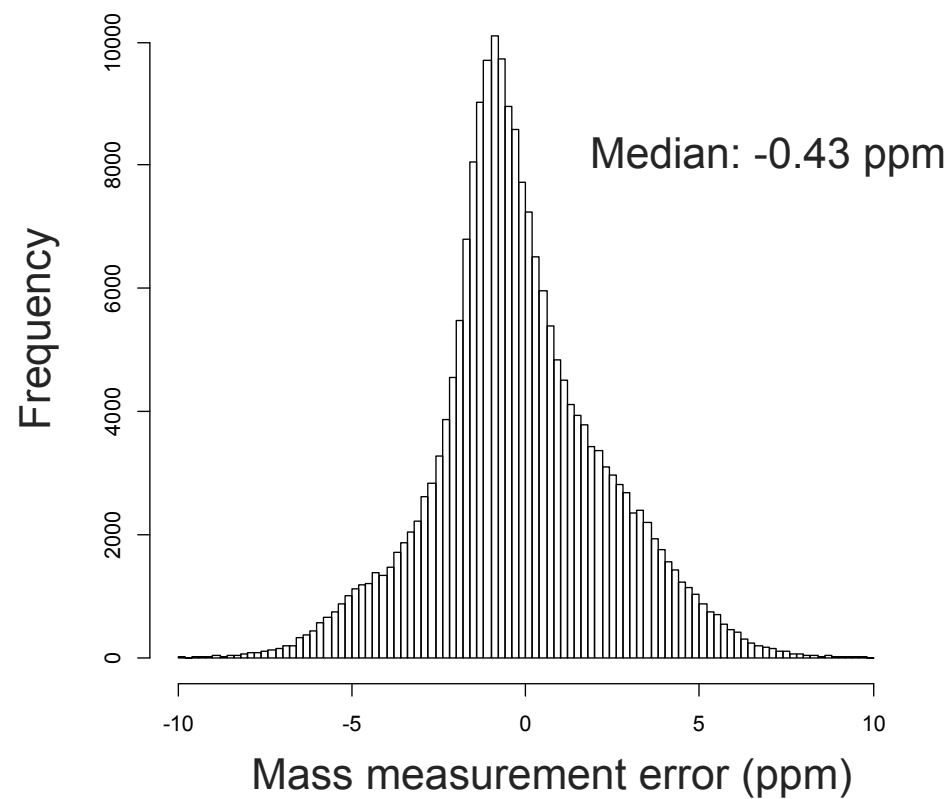


Distribution of precursor mass and mass measurement error

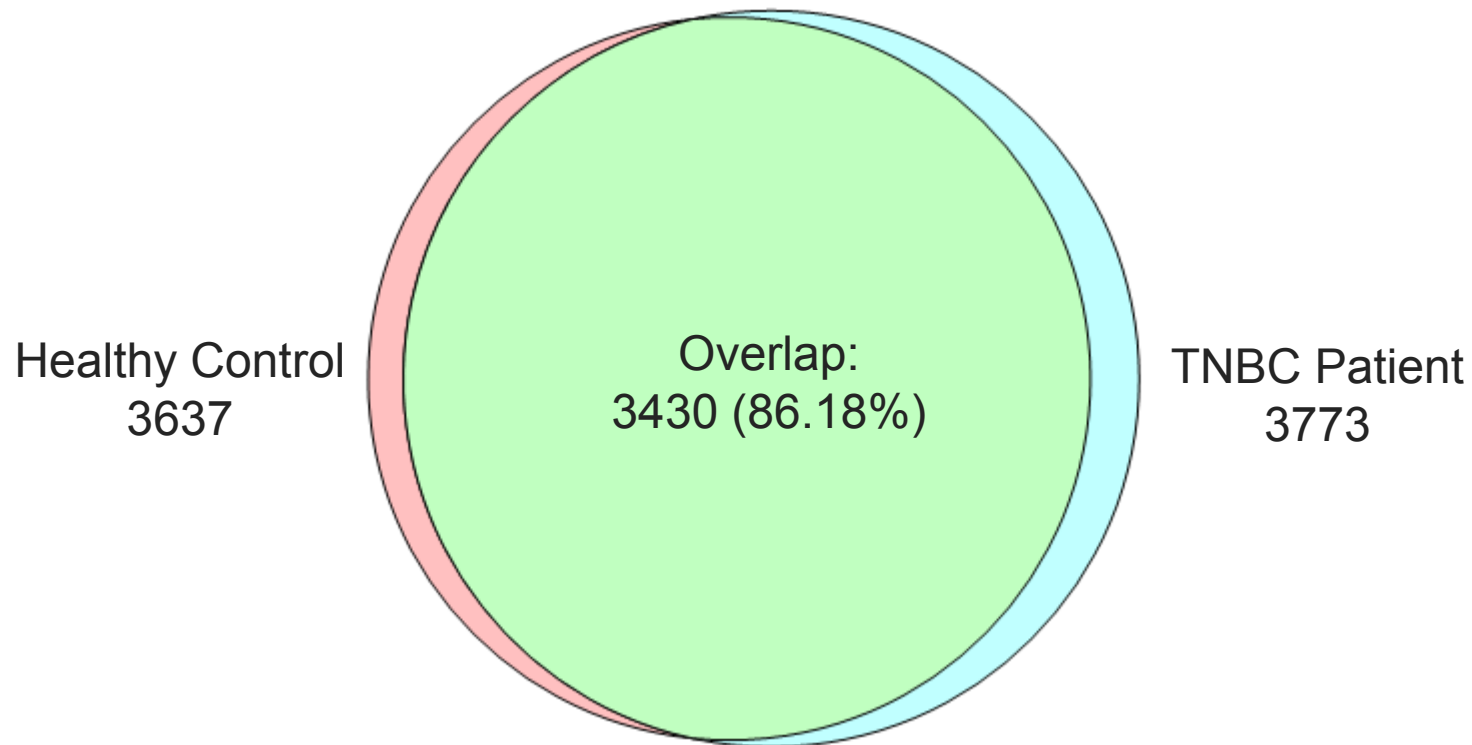
Distribution of precursor mass



Distribution of mass measurement error

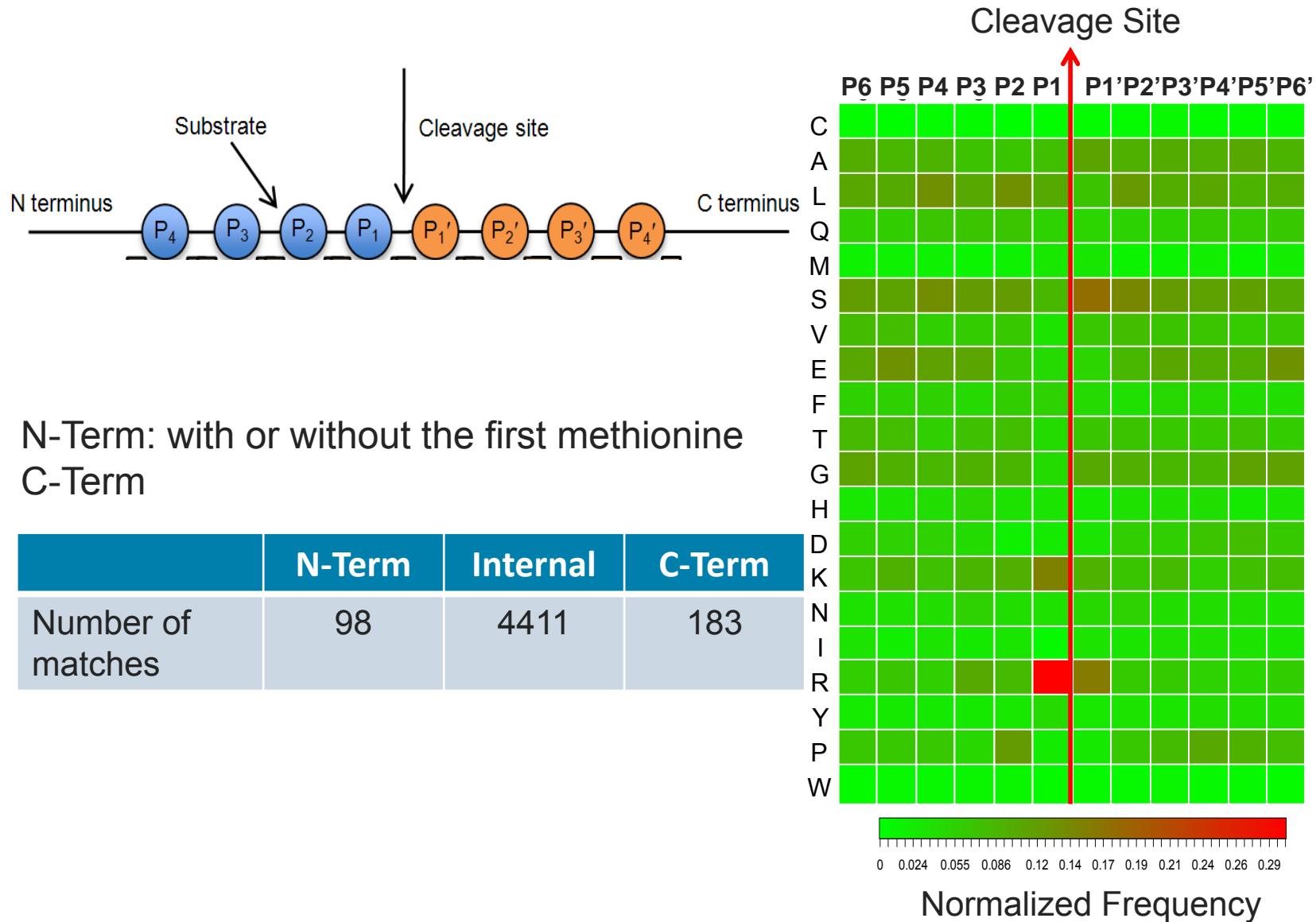


Comparison of peptide IDs in the two groups



Total: 3980 unique peptide IDs

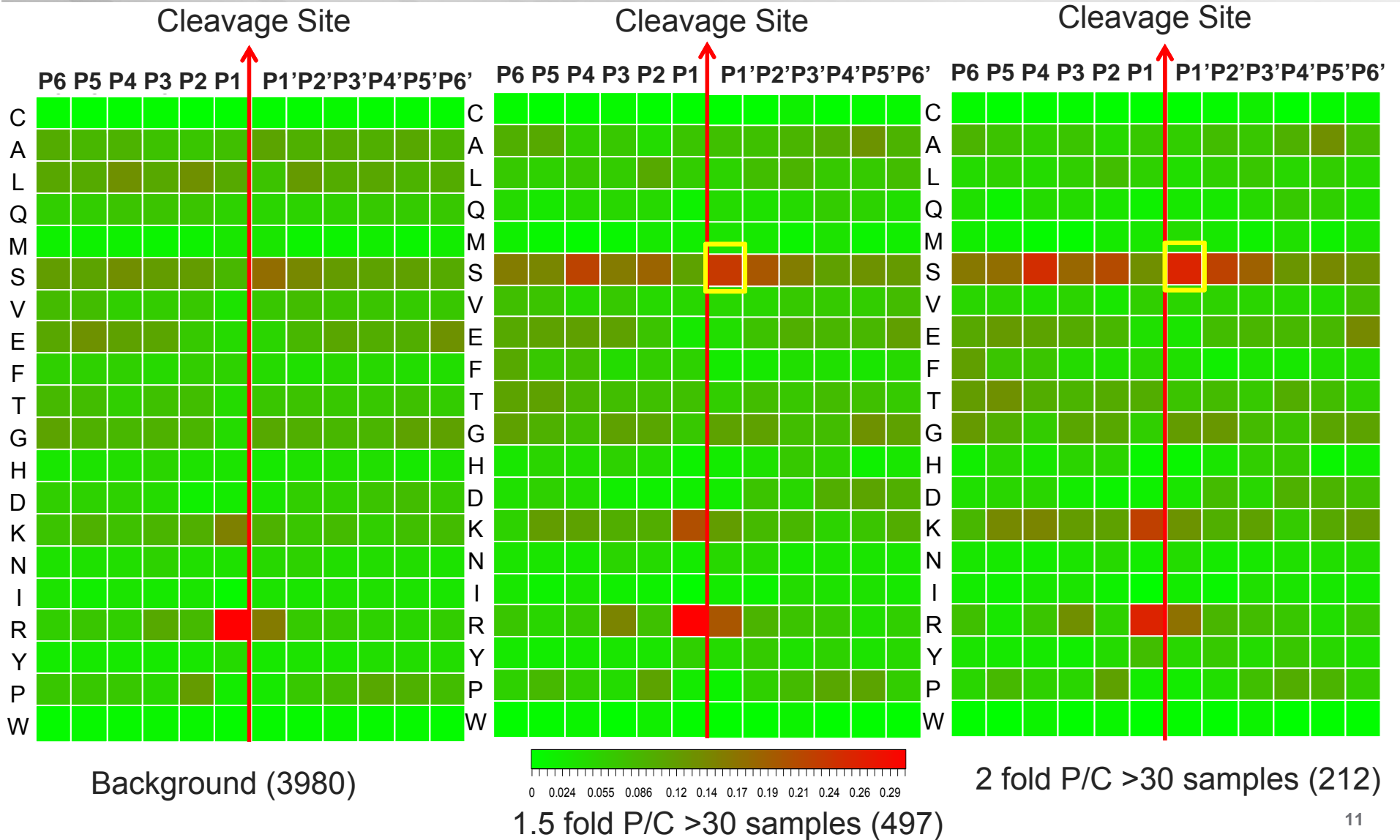
Trypsin like protease cleavage specificity in all identified peptides



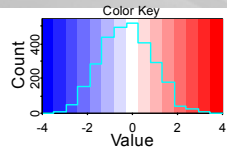
N-Term: with or without the first methionine
C-Term

	N-Term	Internal	C-Term
Number of matches	98	4411	183

Serine related protease cleavage specificity in TNBC group

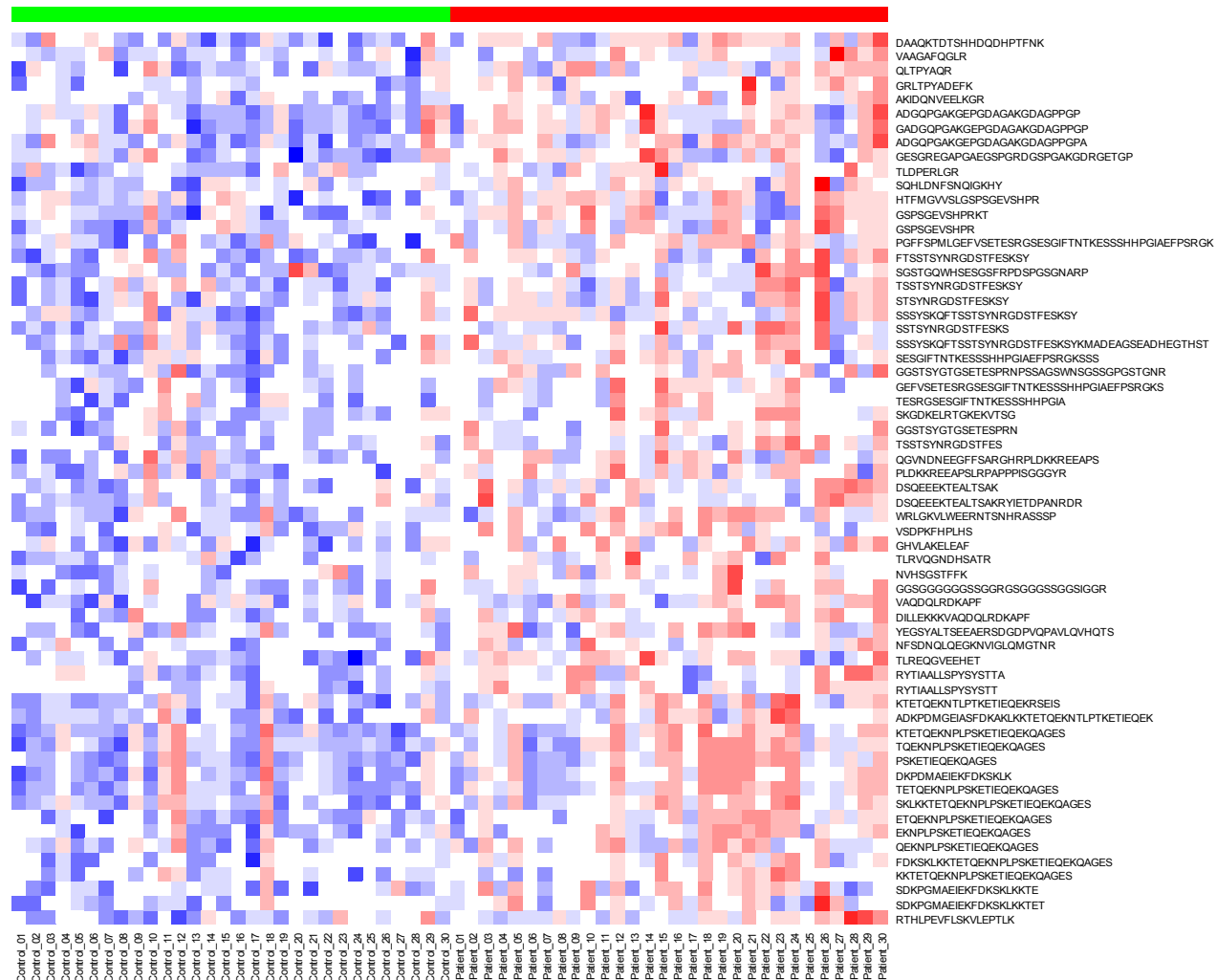


Significantly increased peptidome level in TNBC samples



Heatmap

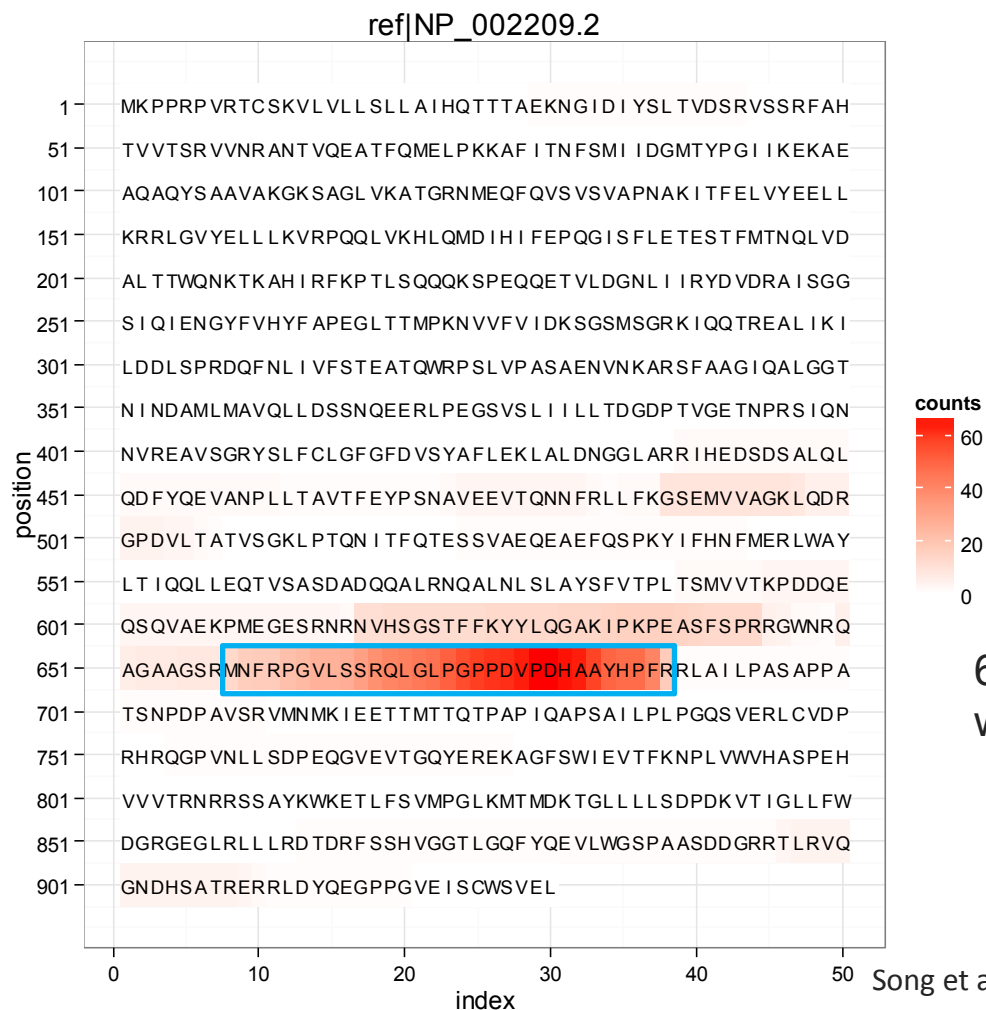
Healthy Control TNBC Patients



Protein name	Protein description	Peptide
A2GL_HUMAN	Leucine-rich alpha-2-glycoprotein	1
APOA4_HUMAN	Apolipoprotein A-IV;	3
CO1A1_HUMAN	Collagen alpha-1(I)	4
CO3_HUMAN	Complement C3	1
FA5_HUMAN	Coagulation factor V	1
FIBA_HUMAN	Fibrinogen alpha chain	15
FIBB_HUMAN	Fibrinogen beta chain	2
GELS_HUMAN	Gelsolin	2
GLR_HUMAN	Glucagon receptor	1
IBP3_HUMAN	Insulin-like growth factor-binding protein 3	1
IGF2_HUMAN	Insulin-like growth factor II	1
ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4	2
FETUA_HUMAN	Alpha-2-HS-glycoprotein	3
A1AT_HUMAN	Alpha-1-antitrypsin	1
K2C1_HUMAN	Keratin, type II cytoskeletal 1	1
MA1A1_HUMAN	Mannosyl-oligosaccharide 1,2-alpha-mannosidase IA;	2
SDPR_HUMAN	Serum deprivation-response protein	1
TAGL2_HUMAN	Transgelin-2	1
TLN1_HUMAN	Talin-1	1
TTHY_HUMAN	Transthyretin	2
TYB10_HUMAN	Thymosin beta-10	2
TYB4_HUMAN	Thymosin beta-4	11
TYB4Y_HUMAN	Thymosin beta-4, Y-chromosomal	2
VTDB_HUMAN	Vitamin D-binding protein	1

Highly proteolytically active region in ITIH4

119 unique peptides identified for ITIH4



66 Unique peptides within the blue box

Song et al. Clinical Chemistry 52, No. 6, 2006

Region

658 – 688

31

Proline-rich (PRR) potential bioactive peptide

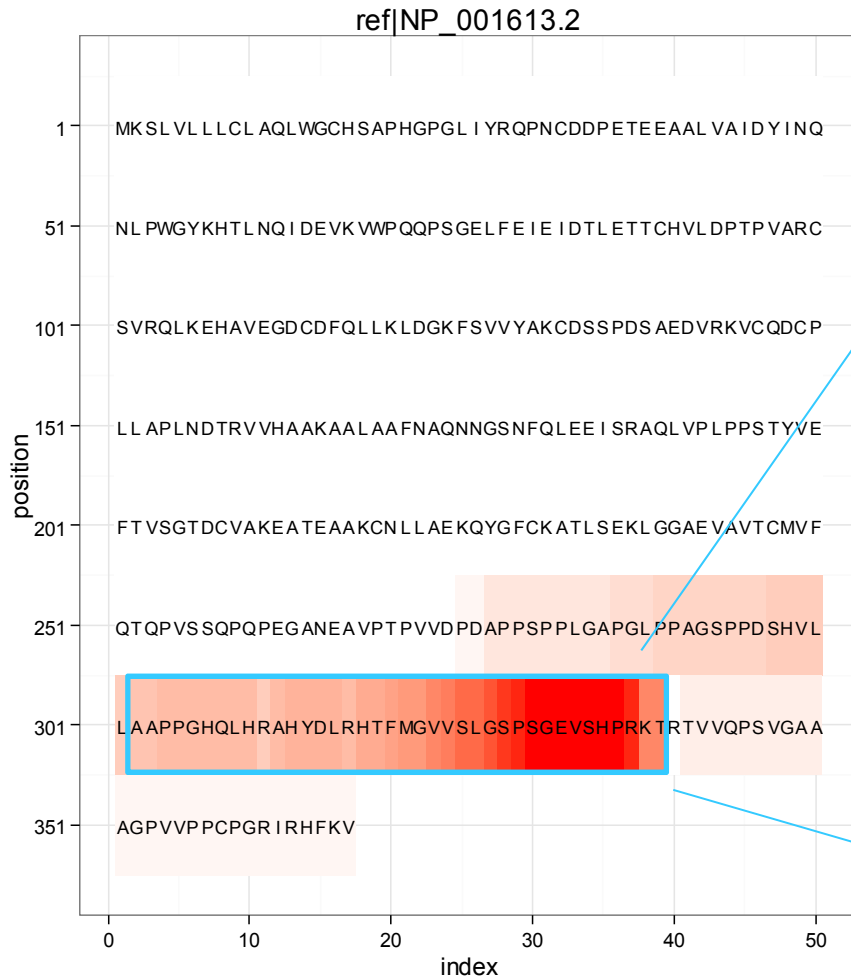
14

Highly proteolytically active region in AHSG

30 unique peptides identified for AHSG

Patient/Control Ratio

Red: >1
Green: <1



AAPPGHQLHRAHYDL

PPGHQLHR

AHYDLRHTFMG

AHYDLRHTFMGVVSLGSPSGEVSHPR

AHYDLRHTFMGVVSLGSPSGEVSHPRKT

HYDLRHTFMGVVSLGSPSGEVSHPRKT

HTFMGVVSLGSPSGEVSHPR

HTFMGVVSLGSPSGEVSHPRKT

FMGVVSLGSPSGEVSHPRKT

MGVVSLGSPSGEVSHPRKT

VVSLGSPSGEVSHPR

VVSLGSPSGEVSHPR

VVSLGSPSGEVSHPRKT

VSLGSPSGEVSHPR

SLGSPSGEVSHPR

SLGSPSGEVSHPRKT

GSPSGEVSHPR

GSPSGEVSHPRKT

SPSGEVSHPR

SPSGEVSHPRKT

PSGEVSHPRKT

SGEVSHPR

C-term:

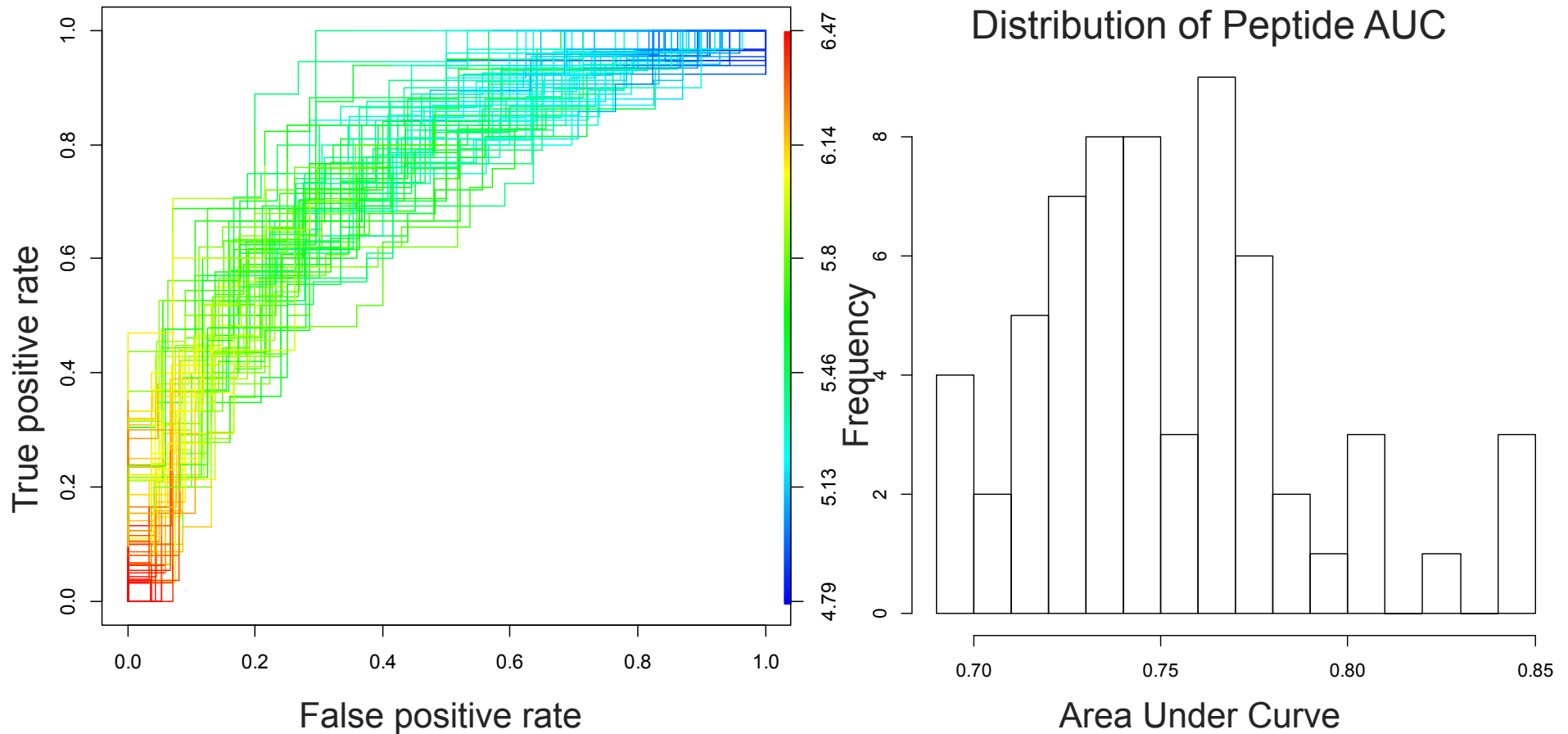
R	9
T	10
other	3

Ratio:

2.89

22 peptides within
blue box

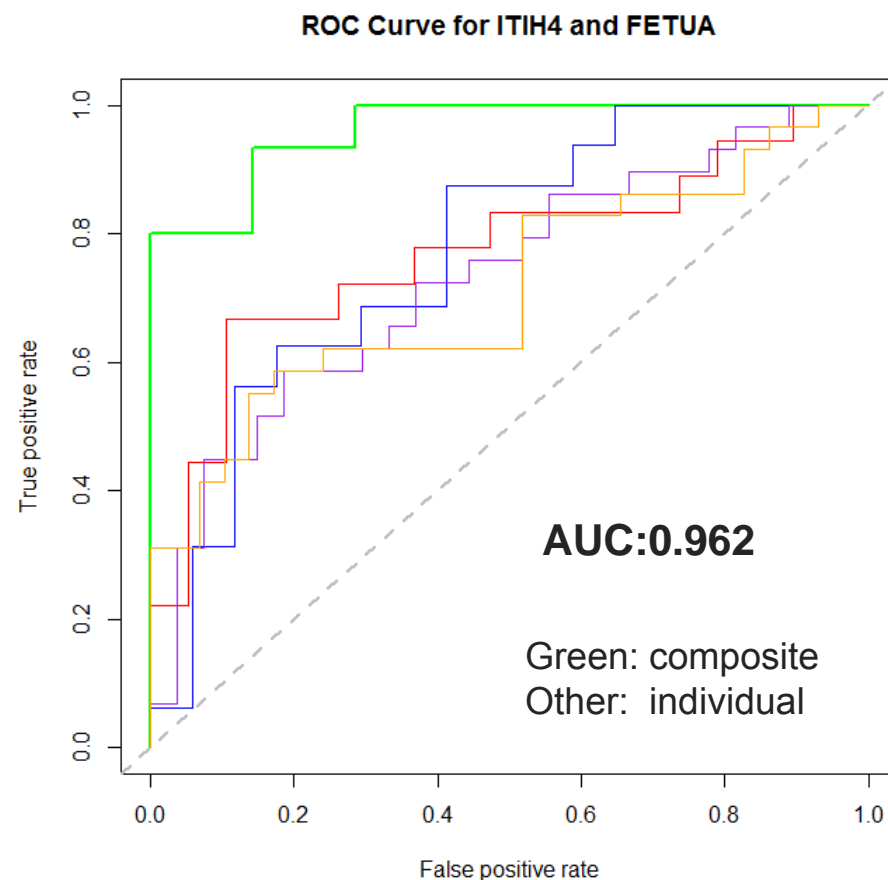
ROC curve for all 62 significant peptides



Most peptides showed moderate AUC value.

ROC curve consisting of ITIH4 and AHSG

	AHSG_1	AHSG_2	ITIH4_1	ITIH4_2
AUC	0.769	0.772	0.731	0.712



29 samples
(14 Control, 15 TNBC)

Peptidome signature peptide of ITIH4 and AHSG pair can be a potential cooperative biomarkers for TNBC

Summary

- Enhanced peptidome/degradome activity observed in the cancer group
- 62 significantly-changing peptides separate TNBC group and control group
- Enhanced P1' serine related protease activity in TNBC group
- Peptidome signature of peptide pairs from ITIH4-AHSG are a potential indicator for TNBC

Perspectives

- Peptidomic biomarker discovery in plasma is interesting but still challenging
- Future work may focus on tumor peptidomics to identify cancer specific biomarker and follow up in plasma and serum

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