

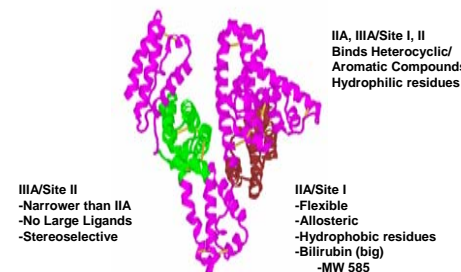
# Mining the Serum Fragmentome: Fractionation, Enrichment & Analysis of Carrier Protein-Bound Fragments

Scott A. Kuzdzal<sup>1</sup>; Mary F. Lopez<sup>1</sup>; David Sarracino<sup>2</sup>; Alvydas Mikulskis<sup>1</sup>

<sup>1</sup>PerkinElmer Life & Analytical Sciences, Boston, MA; <sup>2</sup>Harvard-Partners Center for Genetics and Genomics, Cambridge, MA

## INTRODUCTION

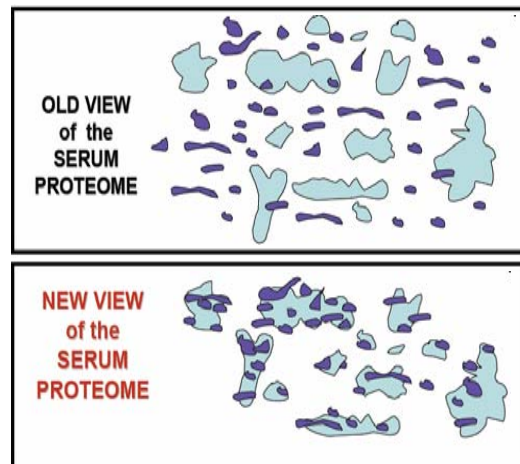
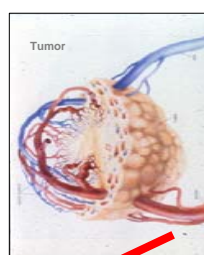
Sample complexity reduction is an essential first step for biomarker discovery. Many depletion strategies have been developed to remove the more common, abundant proteins in sera or plasma. Many of these abundant proteins, however, are 'carrier' proteins and contain a vast assortment of interesting protein fragments [Mehta et al., *Dis. Markers*. 2003-2004, V19, N1, p. 1-10]. In fact, with an albumin blood concentration exceeding  $6 \times 10^{-4}$  M, the probability that even molecules with relatively low binding affinities will be complexed with albumin is greater than 98%. Thus, many potentially interesting biomarkers may be inadvertently lost during protein depletion strategies. This presentation will focus on application of a novel Cibacron blue based membrane absorber technology (Sartorius) for the mining of the human serum fragmentome. Multiple fragments representing over 160 unique proteins were fractionated/enriched from ovarian cancer sera using this strategy and subsequently identified by FT-ICR MS.



## METHODS

Peptides carry valuable diagnostic information and most are bound to carrier proteins

- Tissues are continuously perfused by the serum
- Physiologic state reflected in serum proteomic patterns
- Small proteins and cleaved peptides are produced by proteolytic cascades
- Due to the equilibrium ratio, low mw fragments and peptides are likely to exist almost entirely in complexed association with a carrier protein (albumin), even when the innate binding affinity,  $K_f$ , is low.



Cibacron Blue captures albumin facilitates the enrichment of putative peptide and protein fragment biomarker candidates

Cibacron Blue Captures Carrier Proteins + Bound Proteins/Peptides

Elution buffers elute Proteins/Peptides

MS Serum Profile

Analysis of Albumin-Associated Peptides and Proteins from Ovarian Cancer Patients

MARK S. LOWENTHAL<sup>1</sup>, ARPITA L. MEHTA<sup>1</sup>, KRISTINA FROGALE<sup>1</sup>, RUSSELL W. BANDLE<sup>1</sup>, ROBYN P. ARAUJO<sup>1</sup>, BRIAN L. HOOD<sup>2</sup>, TIMOTHY D. VEENSTRA<sup>2</sup>, THOMAS P. CONRADS<sup>2</sup>, PAUL GOLDSMITH<sup>1</sup>, DAVID FISHMAN<sup>2</sup>, EMANUEL F. PETRICION III<sup>2</sup>, and LANCE A. LIOTTA<sup>2\*</sup>

Figure 1. Serum samples (obtained from the National Ovarian Cancer Early Detection Program (NOCEDP) and gynecologic oncology clinic at Northwestern University, Chicago, IL) were processed using prototype ProXPRESSION™ biomarker enrichment kits (PerkinElmer, Boston, MA). This Cibacron blue (CB) dye affinity chromatography-based technology is designed to capture high-abundance carrier proteins in blood (such as albumin) and dramatically enriches for the peptide and protein fragments bound to the carrier proteins. ZipPlates™ and vacuum manifold were purchased from Millipore (Bedford, MA).

## RESULTS

A rich trove of protein fragments, many from low abundance proteins or proteins not previously seen in serum, were recovered from the ovarian cancer sera. In this study, we identified a number of proteins associated with cellular proliferation, cancer and cancer signaling pathways in the ovarian cancer samples. Although we could not be certain that these peptides/proteins were exclusive to the cancer sera, many of the peptides were not found in the pooled healthy serum samples. Samples eluted from the Cibacron Blue plates were ported directly into the LC/MS/MS. This process resulted in accurate sequence identification for numerous peptides and proteins (Table 2). Multiple protein fragments (with very high identification probabilities) were obtained for most serum proteins (Table 3).

Protein	Observed Fragment	MW (Da)	P (Pep)	Protein	Observed Fragment	MW (Da)	P (Pep)
Transferrin	R.RYTAALLSPYSSTAVV TNPKE	2616.34	2.88E-04	Thyrosinase-like 3	E.KNPLPKETIEQKAGE	2113.08	3.95E-04
Glycophorin H	Q.EKSRKRCGGLVRFKDFV TAHCOGSSIN.V	3173.68	2.47E-03	Neurogranin	R.KGPGPGGGAGVAPG GAGCGG.S	1687.86	2.25E-12
Viperin-like 4	M.PSCDQPGPAFLP	1260.51	8.73E-04	Zyxin	S.LANTQPRKTPPAAS.K PKFSPVTKPTPVAS.K	3215.72	5.72E-08
Olfactory Receptor family 6, subfamily C, member 3	F.FAPGVTEFVLTAMSDR VYV.A	2435.17	3.85E-04	Alpha-synuclein isoform NAC0212	G.KTKEGLVLYGDKTKGV VWG	2059.15	3.83E-05
Lysosomal associated multipass transmembrane protein 5	D.MPHNQFRMFMFSJ	1768.86	6.40E-03	Phosphatase-like 9	A.EYPAKFKVYKSSVGA SDFPTNAL	2788.45	2.10E-04
F-box and leucine-rich repeat protein 3	L.PRAALARLRDAE.G	1451.84	1.68E-03	Catecholase 1 isoform 4	K.SVQSSGSKTKHAANS KDSRLR.Q	2655.44	2.02E-04
Acetyl-Coenzyme A acetyltransferase 2	D.KVELVIVSTRKGLI	1538.96	2.27E-04	Myosin-associated differentiation marker	M.PVTVTRITTTT.T	1391.77	4.03E-03
ELL associated factor 2	A.EASLMDM.S.S	1027.41	7.86E-03	Splicing factor, arginine-serine-rich 8 isoform 1	K.SGAKEEAGPGGAGGG SINELLVYFACKLFRIDE RALA	3839.91	4.42E-05
Coronin-2	R.GILNPGQGGSSSSQST F	1674.79	4.80E-06	Drebrin-like isoform 8	E.SAVHPREIFKOKERA G	1724.96	3.67E-05
Differentially expressed in FOC 9 isoform 1	Q.YVCSICHWNLAVLP	1723.74	3.72E-04	Dryasider-binding protein 9 isoform e	D.FDKLTDADVQLQIEQL KLF.D	2754.50	1.55E-03
Apoptoprotein L1 isoform b precursor	C.SDMEGALLR.V.S	1161.59	3.22E-03	4-7 protein isoform a	S.KPRRFGAGGY.R	1049.55	1.46E-05
Nuclear prelamins A recognition factor isoform b precursor	R.TLPMALTAICPGW.V	1276.61	2.75E-03	Developmentally regulated GTP binding protein 2	H.KPNVFKPKGGGFS TV.T	2182.20	9.22E-03
Transforming growth factor, beta-induced	R.SVRLAPVYKQLRMR.K	1819.03	1.95E-03	Discoidin, CUB and LCCL domain containing 1	G.PWAGGSSGNKPRR.E	1639.76	1.22E-03
PREDICTED: proline-rich synapse-associated protein 2	P.ESADSGVEEADTRSS DPLAETTSSTVSMSTLS SE.S	3988.76	3.52E-03	Page/Bac transposable element derived 4	E.SDQKSSSDSDGSMK W	1616.72	2.59E-03
271-activated kinase 7	K.KKIEISGSPNFEH.R	1485.77	2.04E-05	Naosh4 preproprotein	G.TCQNSGAFHC.V	1154.44	3.99E-03
Vacuolar protein sorting 16 isoform 3	G.GVSRLAHWACY.K	1375.69	6.55E-03	Calcium channel, voltage-dependent, alpha 1E subunit	T.EGKCHLRHGNS.A	1477.88	1.79E-03
Coagulation factor XII precursor	K.REQPPLSTR.N	1083.59	1.25E-04	Wheat lysine kinase	D.PERRPSMALLVYHNS ASRKS.A	2520.42	3.72E-03
Desmoglein 1 preproprotein	V.VVTGMVGSNDKGVDF.V	1555.71	1.26E-09	Epithelial protein lost in neoplasm beta	D.LKKLRSSSLKRSRFF T	2088.25	4.57E-03
Hormotin	Y.GSGSGVSSSRGVPY.E	1284.56	3.28E-04	Solute carrier family 12 (potassium/chloride cotransporter), member 7	L.NKSDQDLVLMNPG.P	1627.85	6.15E-03
Brain-specific angiogenesis inhibitor 3	I.GLVFNKLVSRDGL.D	1643.98	8.21E-03	PREDICTED: ubiquitin specific protease 2A 1	N.YELVGVVHSGQ.A	1300.69	3.46E-03
Connector enhancer of Kinas suppressor of Ras 2	A.WLDRSPP.A	920.46	4.67E-03	MAX dimerization protein 3	A.RQLKERLRSKQSLQR.D	2054.21	1.80E-03
Zonadherin isoform 1	N.SPSCSSLSGSMGSRP.C	1673.68	2.28E-03	Paraneoplastic proliferator-activated receptor (PPAR)	T.AVIDIKLKHVYSGPGE DPLDGMGVSTNSSHPM SSKHNH.S	4289.04	9.99E-04
Neck4 binding protein 2	K.RDRQKEVMCT.Q	1269.54	1.81E-03	Ca2+-dependent secretion activator isoform 2	H.MMYTYSKNCDDQDKL AVRMVDPQNMHLS	3428.76	2.43E-03
Sodium channel, voltage-gated, type K, alpha	S.EEKTKL.G	860.51	9.20E-04	ATP-binding cassette, sub-family C, member 12 isoform D	Y.SERSPPMAGATG.P	1157.59	5.49E-03
Kinogen 1	K.RPPGSPF.R	904.47	2.45E-03	SET binding protein 1	N.KDLLGGVAPP.S	1166.68	2.46E-03

Table 1. Partial listing of some of the protein fragments identified using the Cibacron Blue ProXPRESSION Kit. Thousands of fragments representing over 160 human serum proteins have been identified.

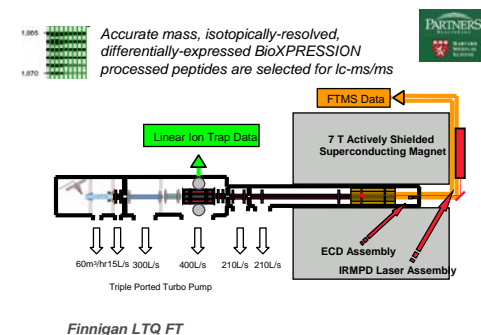


Figure 2. Ultra High Resolution Tandem Mass Spectrometry *de novo* sequencing was performed on a Finnigan LTQ FT.



Figure 3. Processing of 96 samples using ProXPRESSION Biomarker HT Kits. These kits fractionate/enrich carrier-protein bound protein fragments (processing may be automated on liquid handlers).

## CONCLUSIONS

In summary, we identified ca. 162 proteins from peptides and protein fragments bound to carrier proteins in a period of 2-3 weeks from ovarian cancer patient serum samples. These fragment analytes represent a remarkable collection of peptides derived from proteins involved in cellular inflammation, differentiation, signaling, apoptosis, transcriptional regulation and other regulatory mechanisms. It is remarkable that this rich variety of low abundance species are so well represented in the fraction bound to serum albumin.