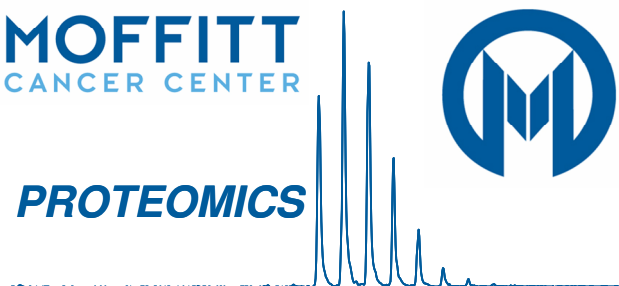


Peptide Synthesis Report

MOFFITT
CANCER CENTER

PROTEOMICS



Project Title: Internal standard for Insulin-like growth factor-binding protein 7 precursor (IBP7_HUMAN)

Submitter: C. Eric Thomas

PI: John Koomen

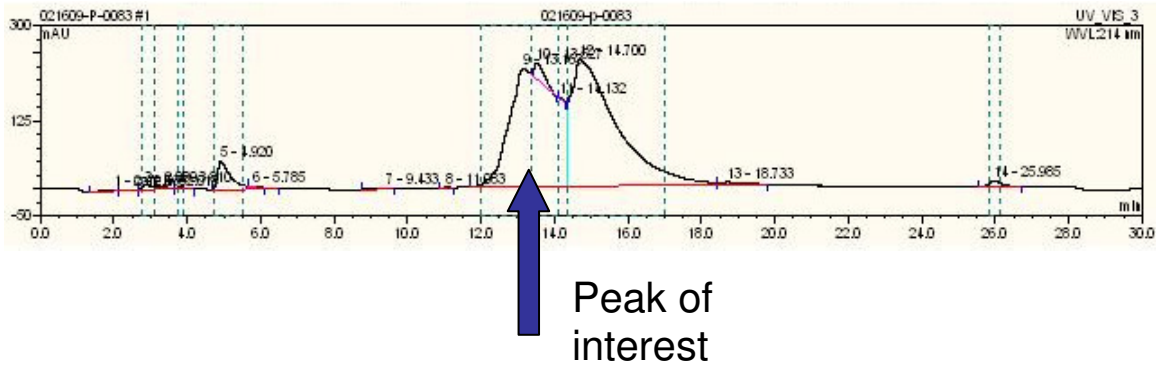
Design and Synthesis:

MPS#	Peptide Sequence	Residues	Modification	MWT Monoisotopic
p-0083	ITVVDALHEIPVK	263-275	Stable Isotope P U-13C5, 15N	1438.829

Solid state peptide synthesis (Symphony, Protein Technologies, Tucson, AZ) is used to make standards at the 25 micromole scale using standard Fmoc chemistry. Briefly, Wang resin (Novabiochem, Germany) serves as the support to initiate peptide formation. Activator solvents for the amino acid coupling consist of 150 mM N-Methylmorpholine in N-methylpyrrolidone (NMP) with 100 mM of O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU). Deprotection between couplings is achieved using 20% piperidine in NMP with 2% 1,8-diazabicyclo [5,4,0] undec-7-ene. Upon sequence completion, cleavage from the resin is performed using 95% TFA, 2.5% water, and 2.5% triisopropylsilane. Peptides are then precipitated in ice cold ethyl ether, washed twice, and resuspended in water prior to lyophilization.

Semi-preparative HPLC:

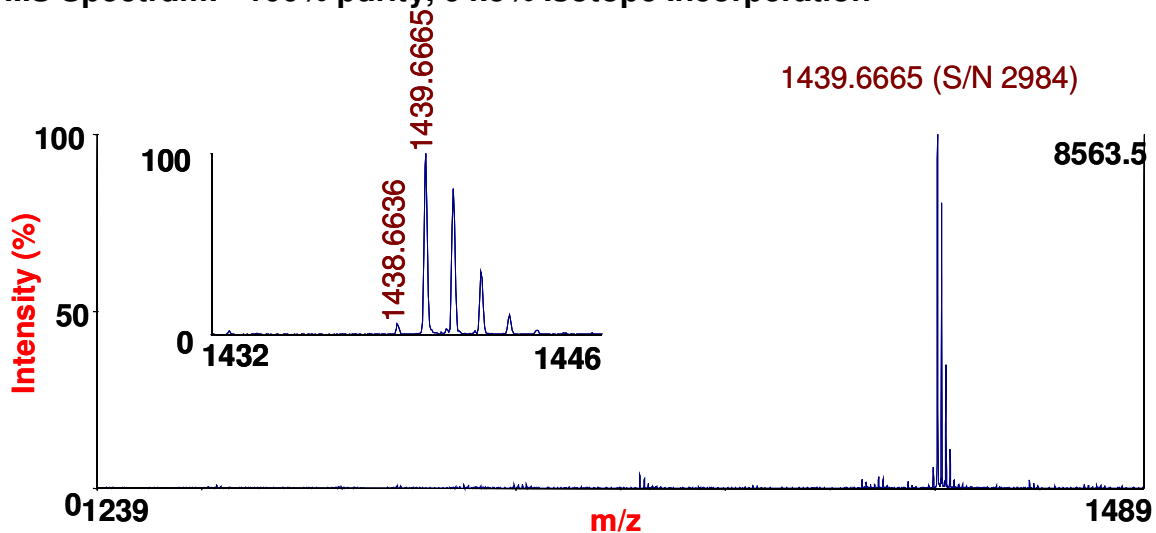
HPLC purification is performed on a semi-preparative system (U3000, Dionex, Sunnyvale, CA) using a C18 reverse phase column (TP 238, 250 x 10mm, 10-15 μ m particles, Grace Vydac, Deerfield, IL). Twenty minute gradients are run from 5% to 60% B solvent (A: 2% acetonitrile/0.1% formic acid; B: 100% acetonitrile/0.1% formic acid). Eluted peptides are measured at 214 nanometer wavelength and collected with automatic triggering (Foxy Jr, ISCO).



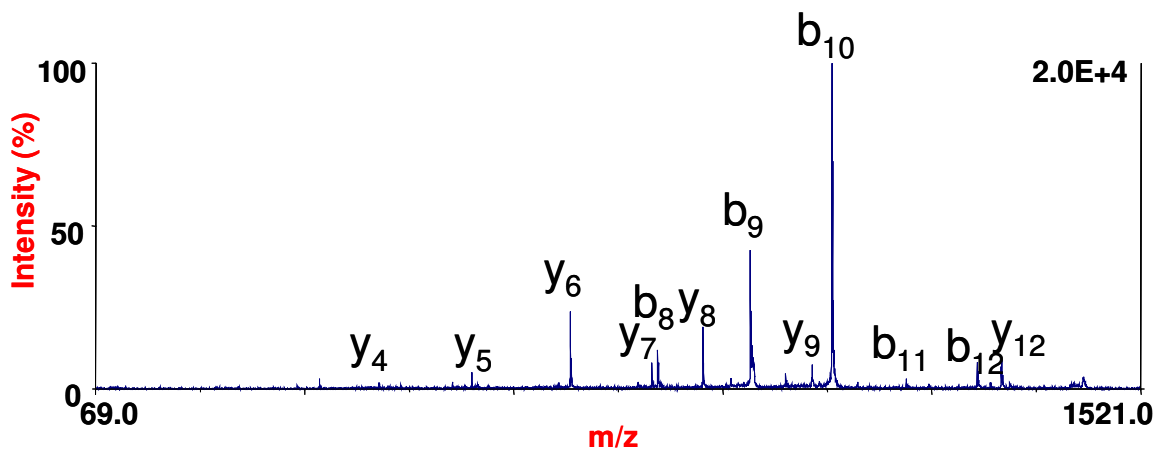
MALDI:

Purified peptides are analyzed with MALDI MS to verify purity and sequenced with MS/MS (4700, Applied Biosystems, Framingham, MA). Peptides in 2% ACN with 0.1% formic acid are mixed 1:1 with α -cyano-4-hydroxycinnamic acid (10 mg/ml) in 50% ACN and deposited in 1 microliter aliquots on the MALDI target.

MS Spectrum: ~100% purity, 94.5% Isotope Incorporation



MS/MS spectrum:



Fragment Ion table with Identified peaks:

SEQ	#	B	Y	#
I	1	114.0919	1439.837	13
T	2	215.1396	1326.753	12
V	3	314.208	1225.705	11
V	4	413.2764	1126.637	10
D	5	528.3034	1027.568	9
A	6	599.3405	912.5413	8
L	7	712.4246	841.5042	7
H	8	849.4835	728.4201	6
E	9	978.5261	591.3612	5
I	10	1091.61	462.3186	4
P* Stable Isotope	11	1194.663	349.2346	3
V	12	1293.731	246.1818	2
K	13	1421.826	147.1134	1