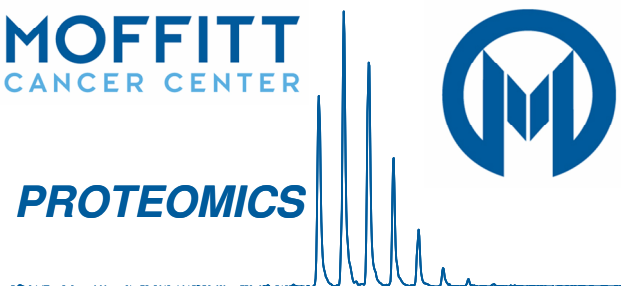


Peptide Synthesis Report

MOFFITT
CANCER CENTER

PROTEOMICS



Project Title: GAPDH internal standard

Submitter: Yun Xiang

PI: John Koomen

Design and Synthesis:

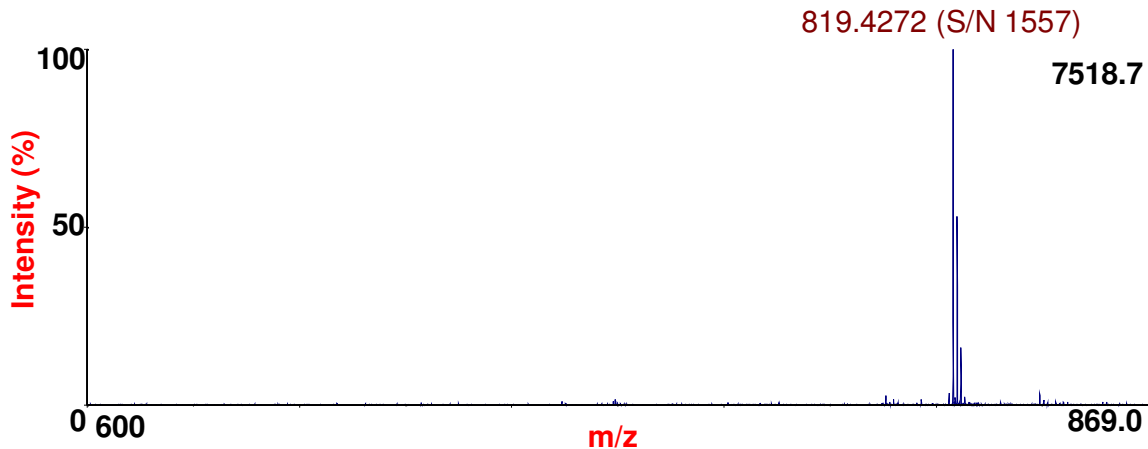
MPS#	Peptide Sequence	Residues	Modification	MWT Monoisotopic
p-0324	VGVNCFAR		G7 to A	818.439

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.

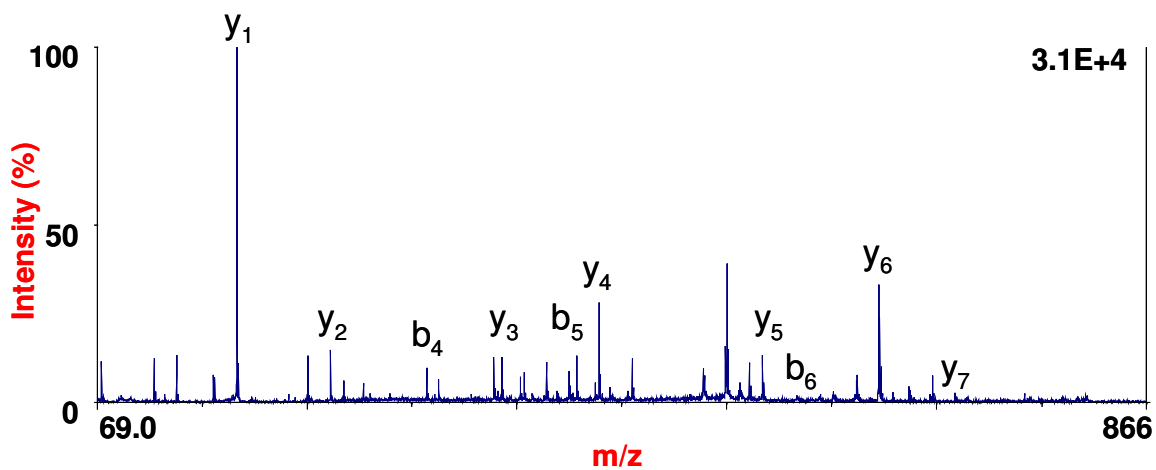
MALDI:

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% pure



MS/MS spectrum:



Fragment Ion table with Identified peaks:

Seq	#	B	Y	# (+1)
V	1	100.07628	819.44775	8
G	2	157.09775	720.37934	7
V	3	256.16616	663.35788	6
N	4	370.20909	564.28946	5
G	5	427.23055	450.24654	4
F	6	574.29897	393.22507	3
A	7	645.33608	246.15666	2
R	8	801.43719	175.11955	1