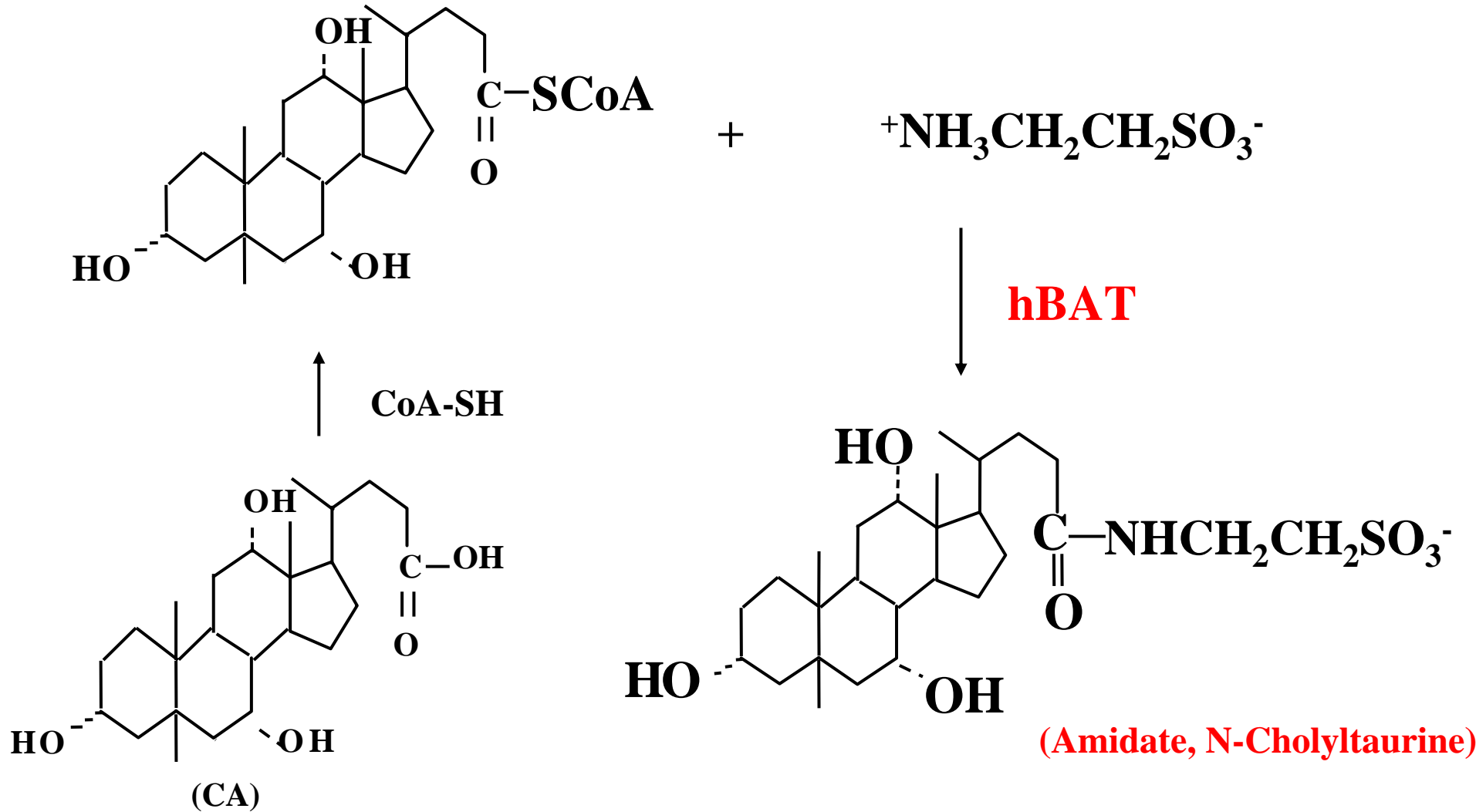


**Post-translational  
Modifications to Human Bile  
Acid CoA:Amino Acid N-  
acyltransferase**

Erin Shonsey

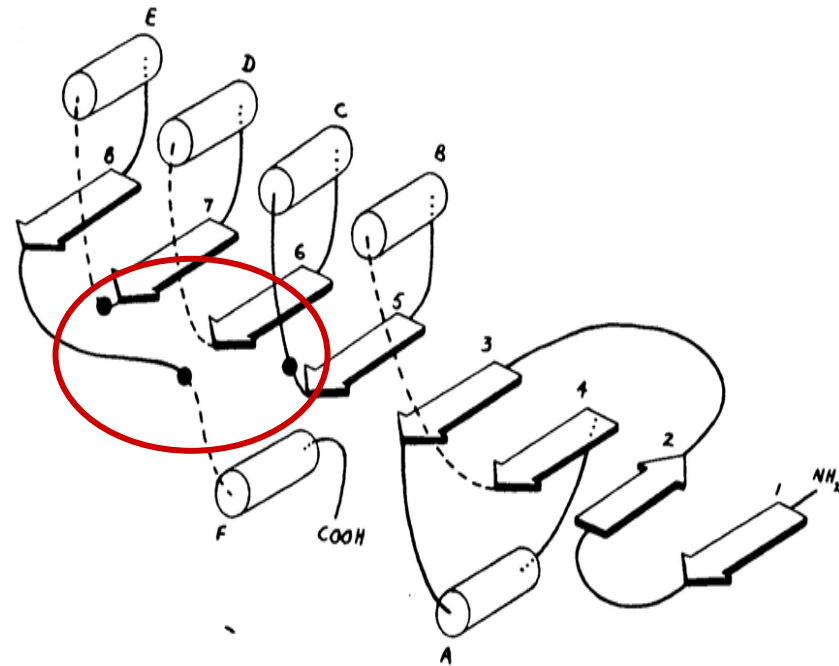
September 12, 2006

# Conjugation of Bile Acids

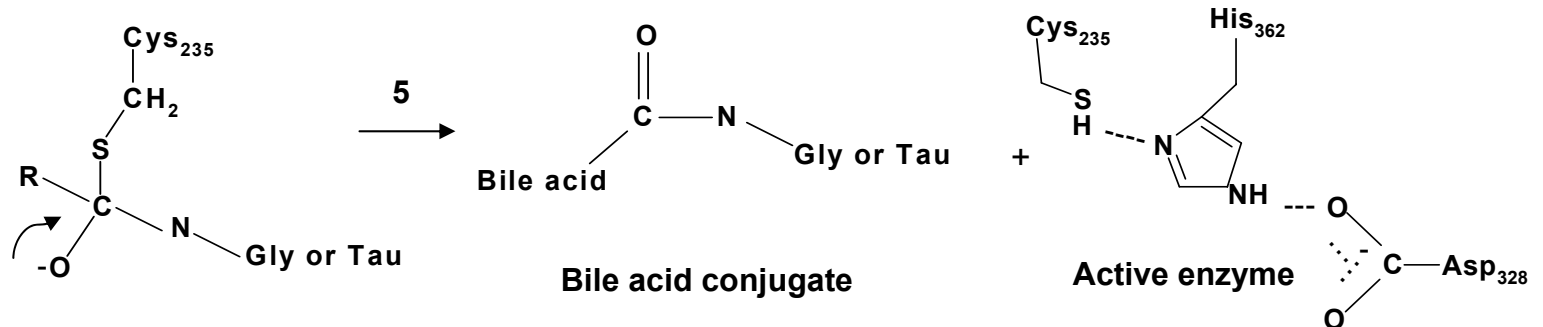
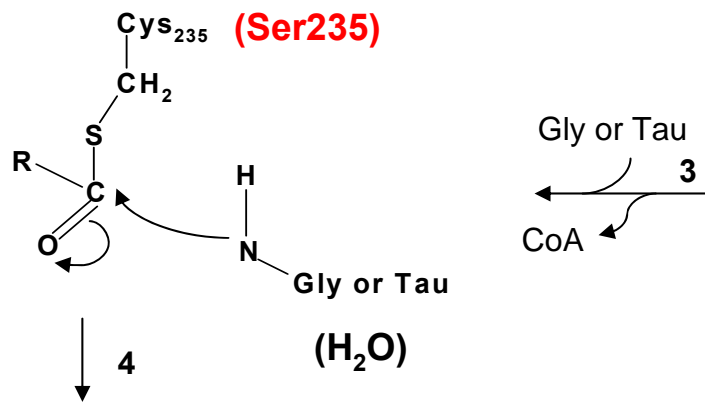
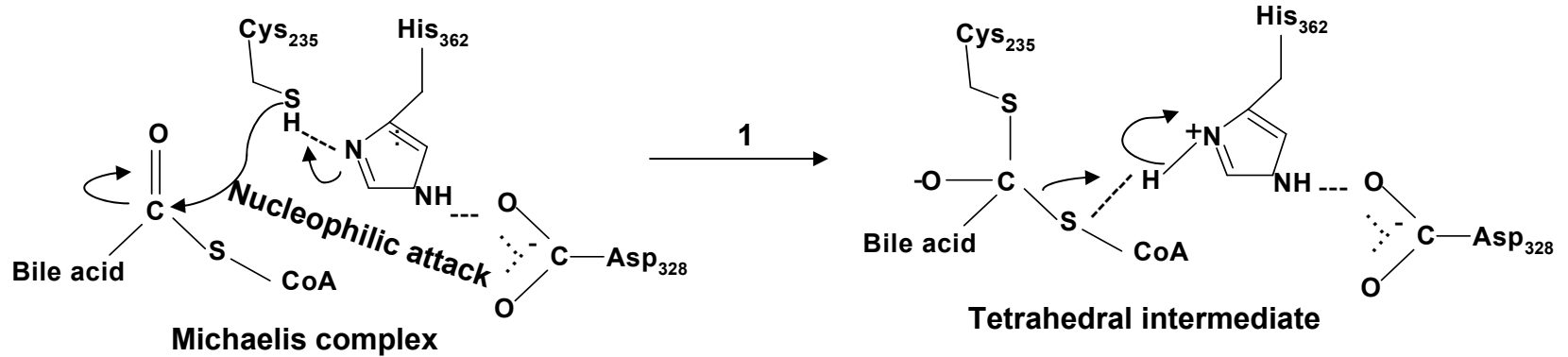


# Catalytic Triad of BAT

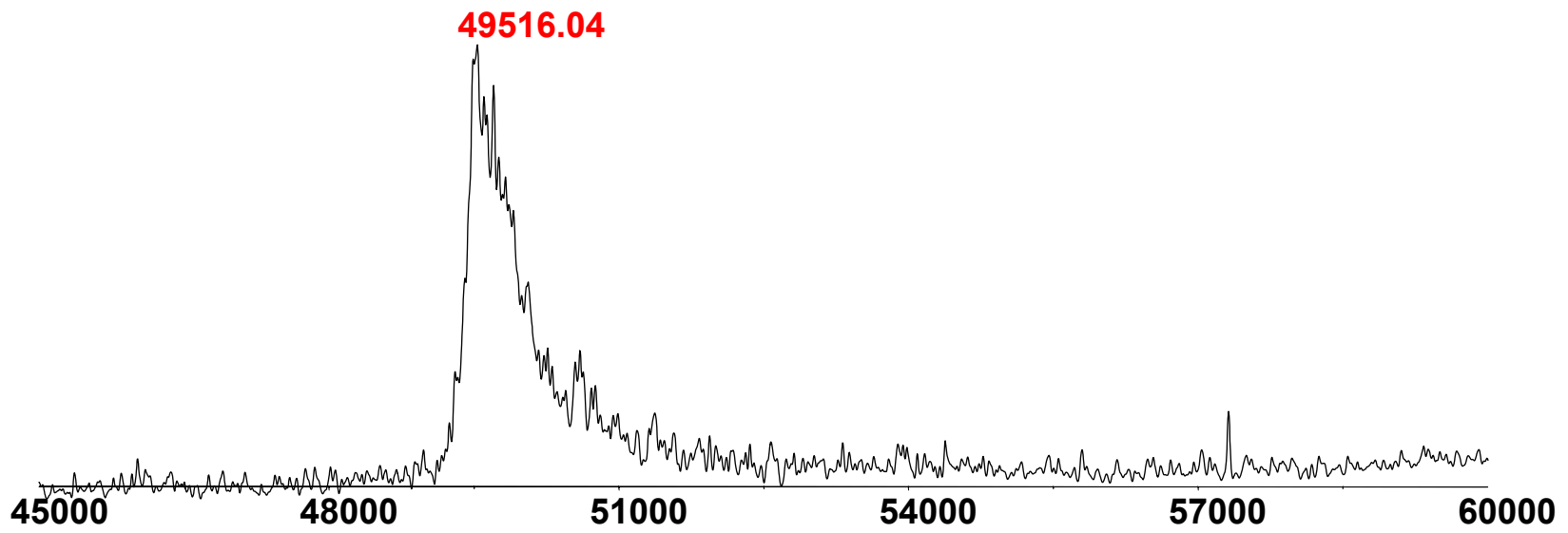
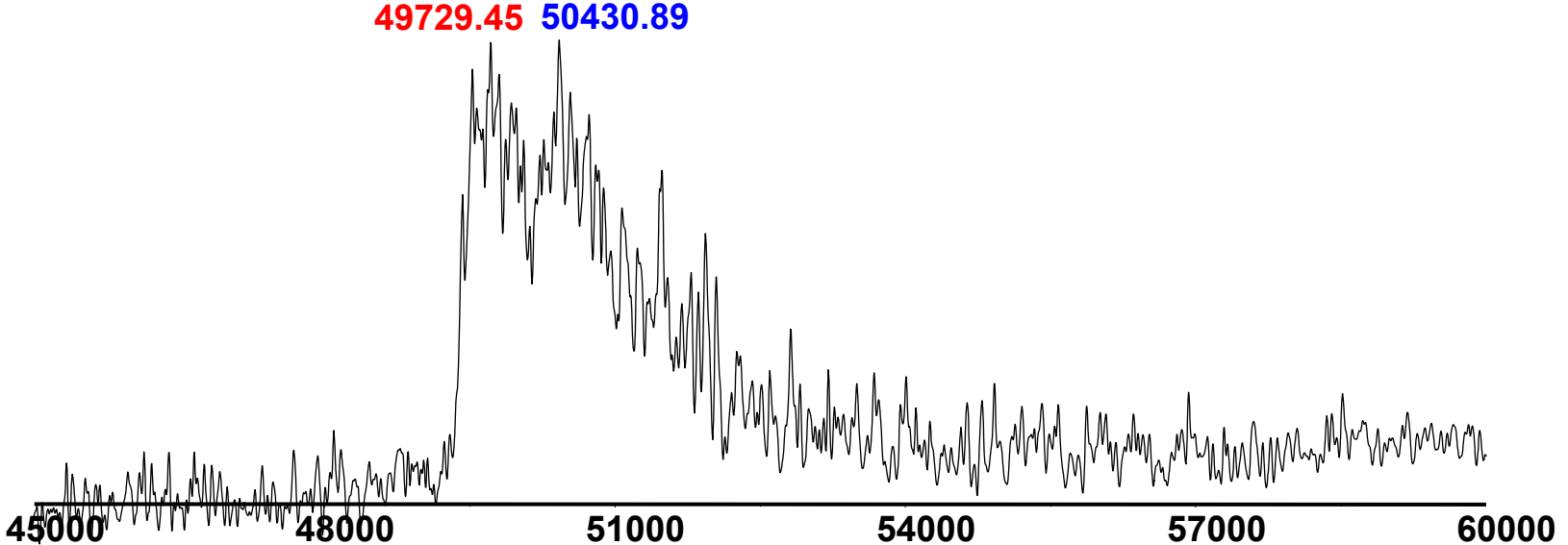
- Various mutation and other studies performed previously led to the hypothesis that BAT works through a catalytic triad
- The members of this catalytic triad are believed to be Cys235, His362, and Asp328



# Charge Relay Mechanism shared by hBAT, thioesterases, and a large group of hydrolases

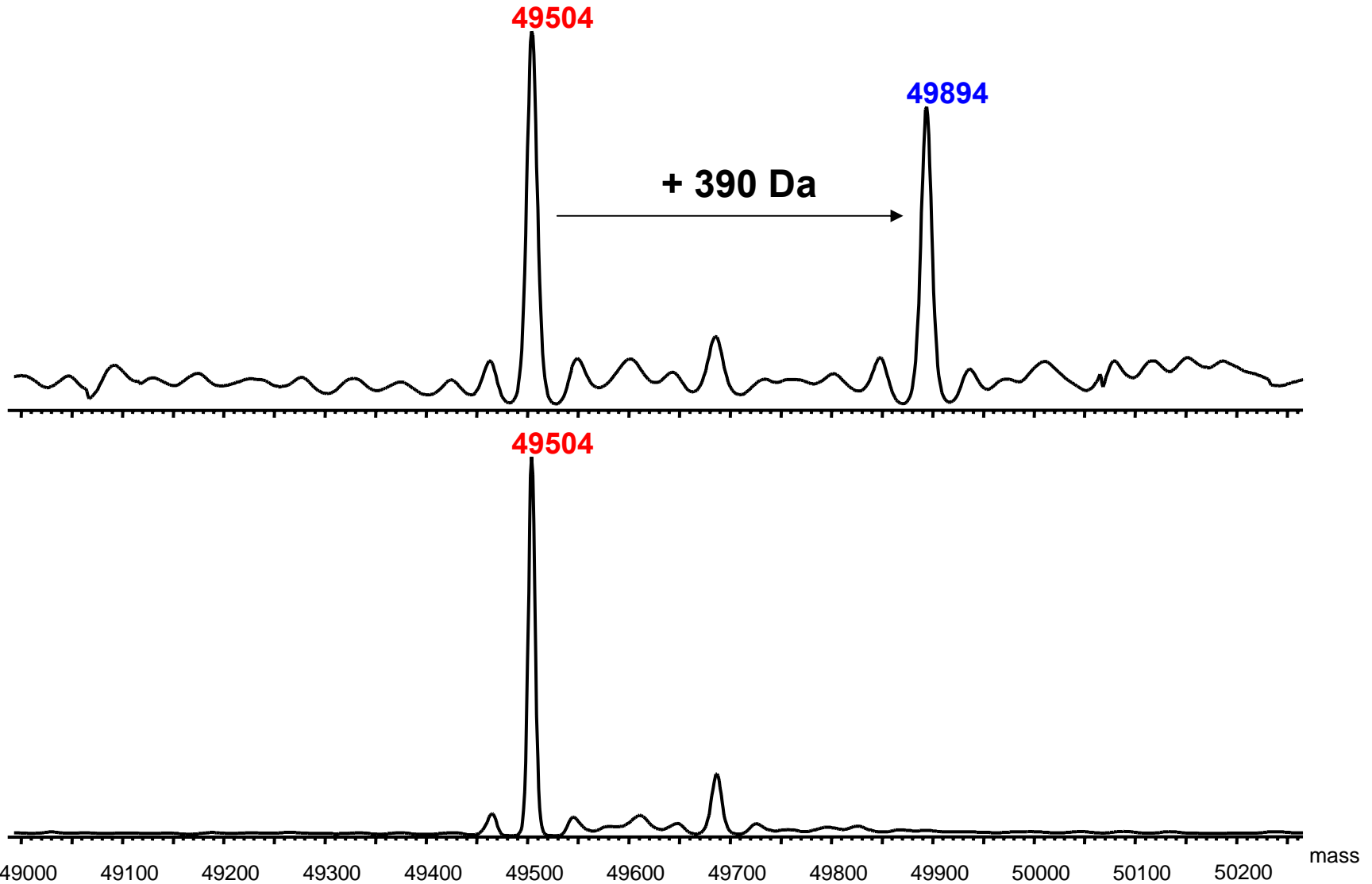


# MALDI-TOF MS Analysis of hBAT-Avi/cholic acid intermediate

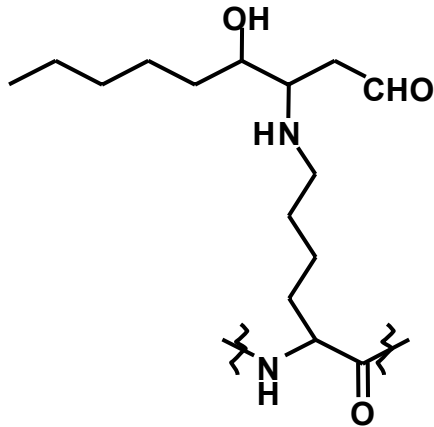


Mass (m/z)

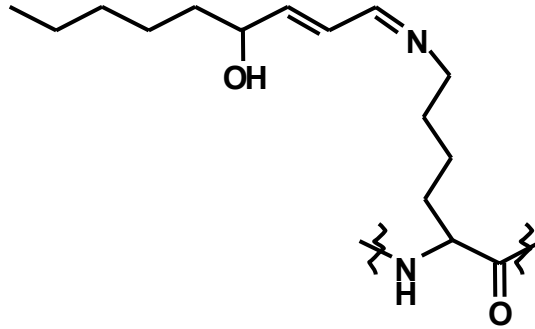
# Deconvoluted Q-TOF MS Analysis of hBAT-Avi/cholic acid intermediate



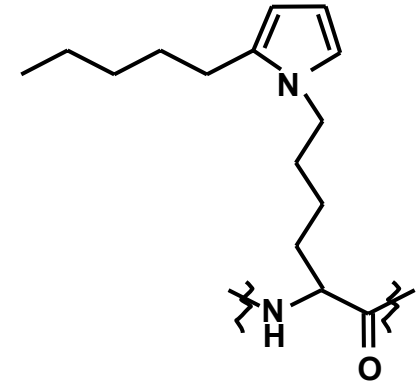
# Amino Acids Modified by 4HNE



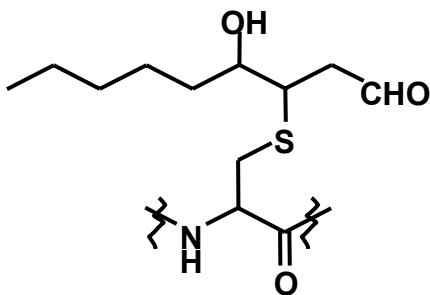
4HNE-Modified Lysine  
Michael Addition



4HNE-Modified Lysine  
Schiff Base Adduct



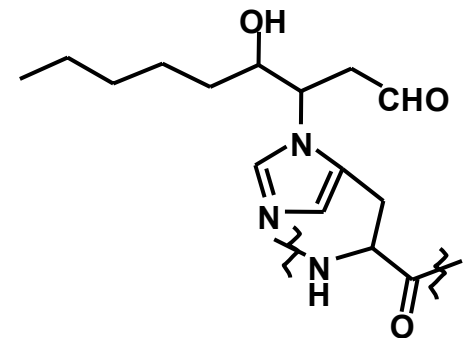
4HNE-Modified Lysine  
2-Pentylpyrrole Adduct



4HNE-Modified Cysteine  
Michael Addition

Adduct	Mol wt
Michael	156
Schiff base	138
Pentylpyrrole	120

J Am Soc Mass Spectrom. 2004  
Aug;15(8):1136-47.



4HNE-Modified Histidine  
Michael Addition

# Potential 4HNE targets in hBAT

MIQLTATPVSALVDEPVHIRATGLIPFQMVSFQASLEDENGDMF  
YSQAHYRANEFGEVDLNHASSLGGDYMGVHPMGLFWSLKPEKLL  
TRLLKRDVMNRPFQVQVKLYDLELIVNNKVASAPKASLTLEWY  
VAPGVTRIKVREGRLRGALFLPPGEGLEFPVIDLFGGLGGLLEF  
RASLLASRGFASLALAYHNYEDLPKPEVTDLEYFEEAANFLLR  
HPKVFGSGVGVVSVCGQVQIGLSMAIYLYKQVTATVLLINGTNFPF  
GIPQVYHGQIHQPLPHSAQLISTNALGLLELYRTFETTQVGASQ  
YLFPIEEAQGQFLFIVGEGDKTINSKAHAEQAIGQLKRHGKNNW  
TLLSYPGAGHLIEPPYSPLCCASTTHDLRLHWGGEVIPHAAAQE  
HAWKEIQRFRLRKHLLIPDVTSQL

17 His

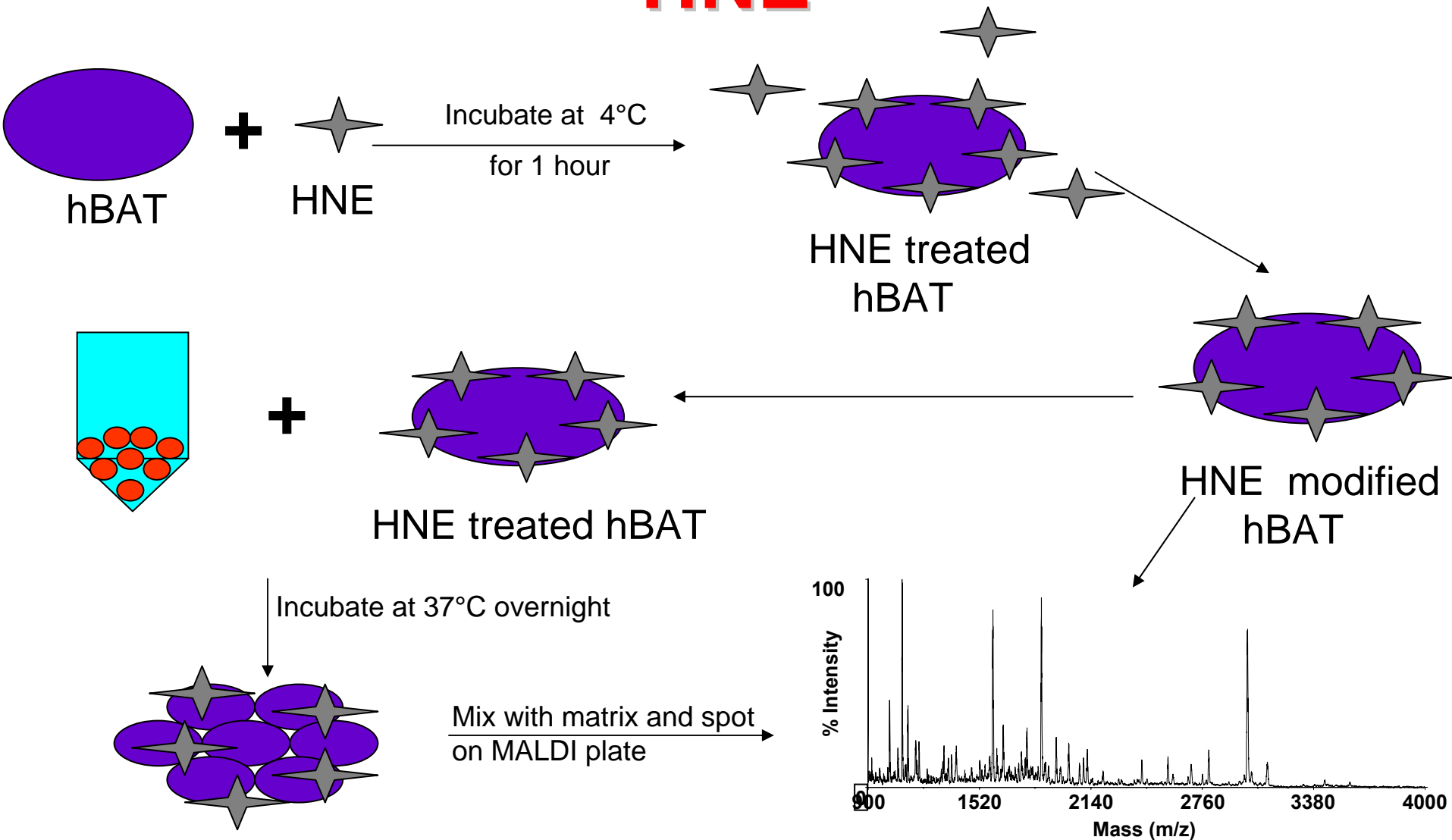
16 Lys

19 Arg

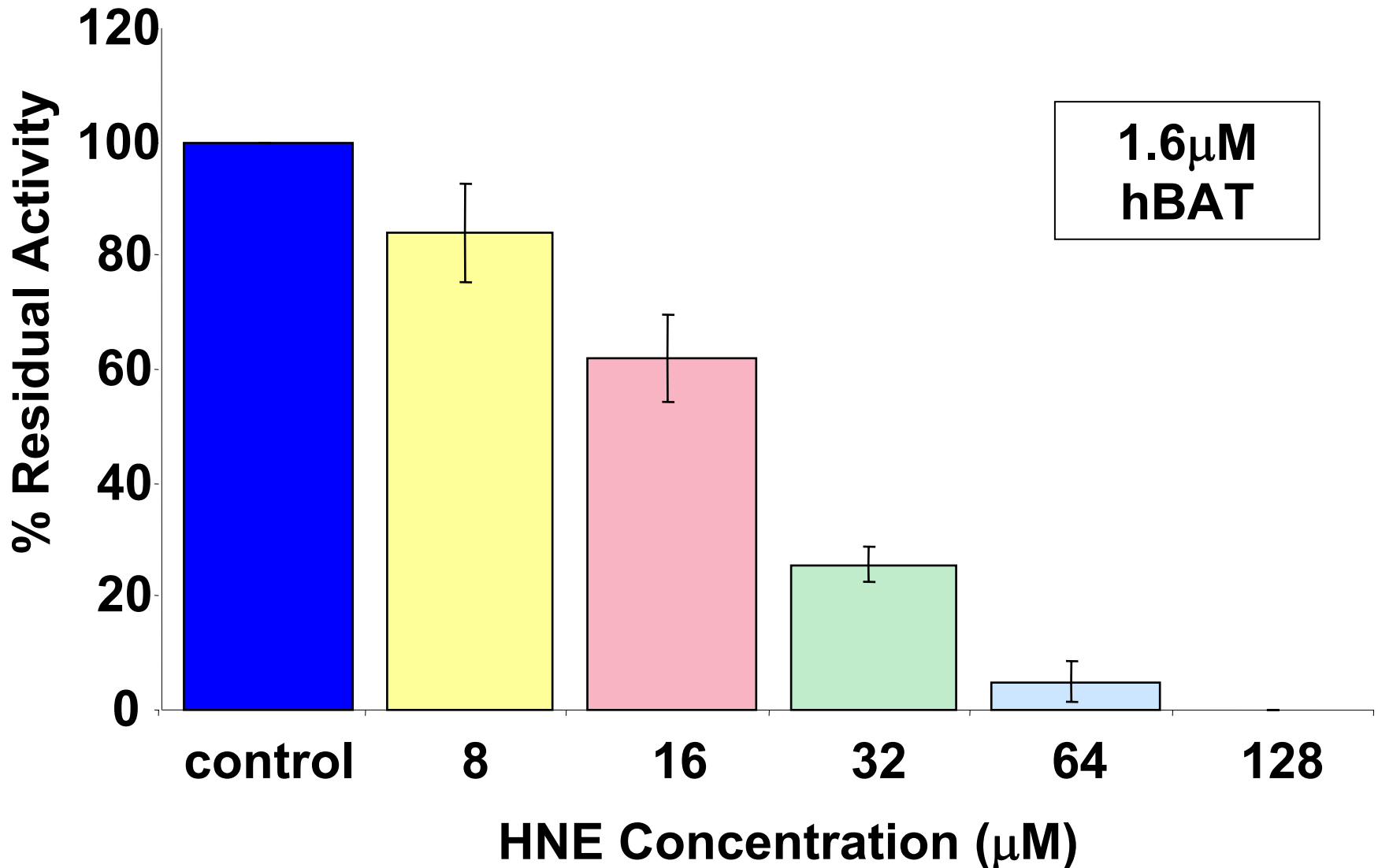
3 Cys



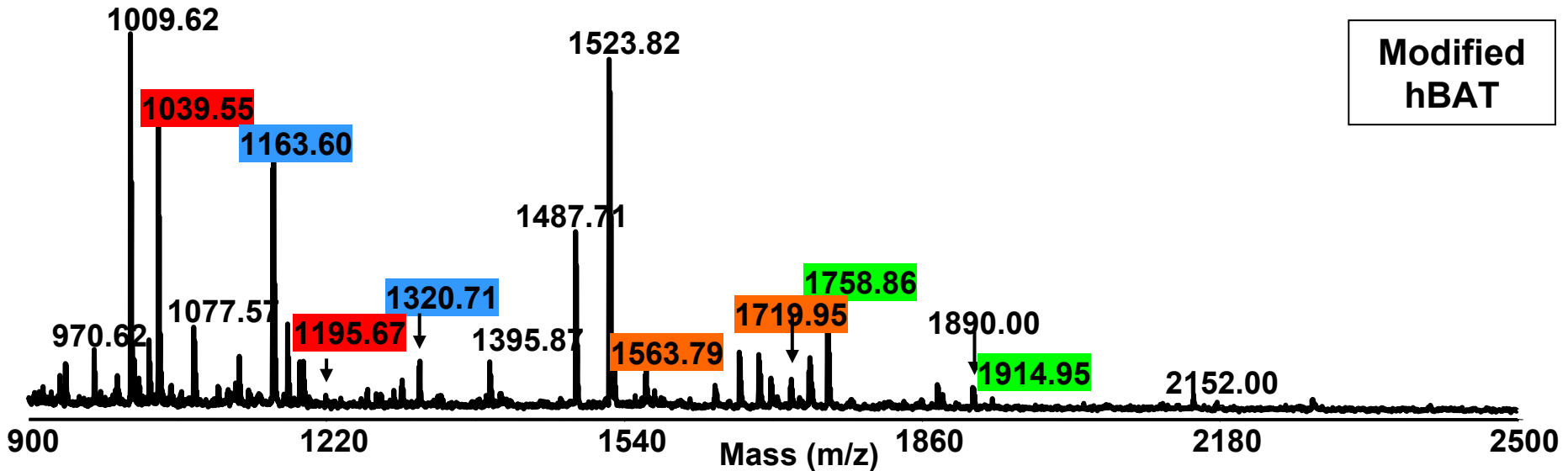
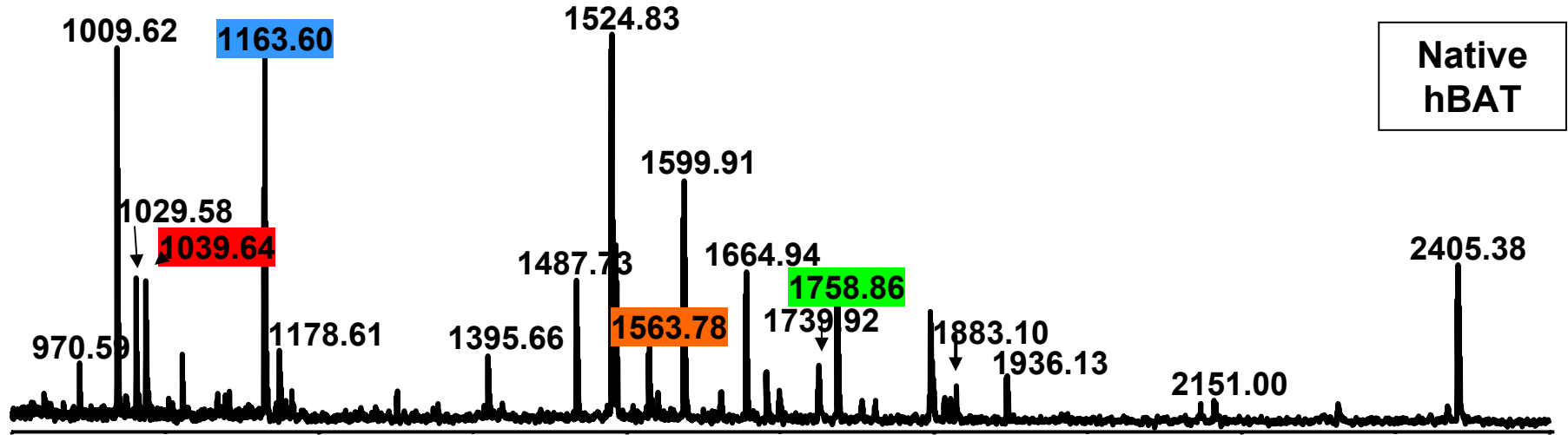
# *In vitro* Modification of hBAT with HNE



# Inactivation of hBAT by HNE



# MALDI-TOF MS of Chymotrypsin Digest of HNE modified hBAT

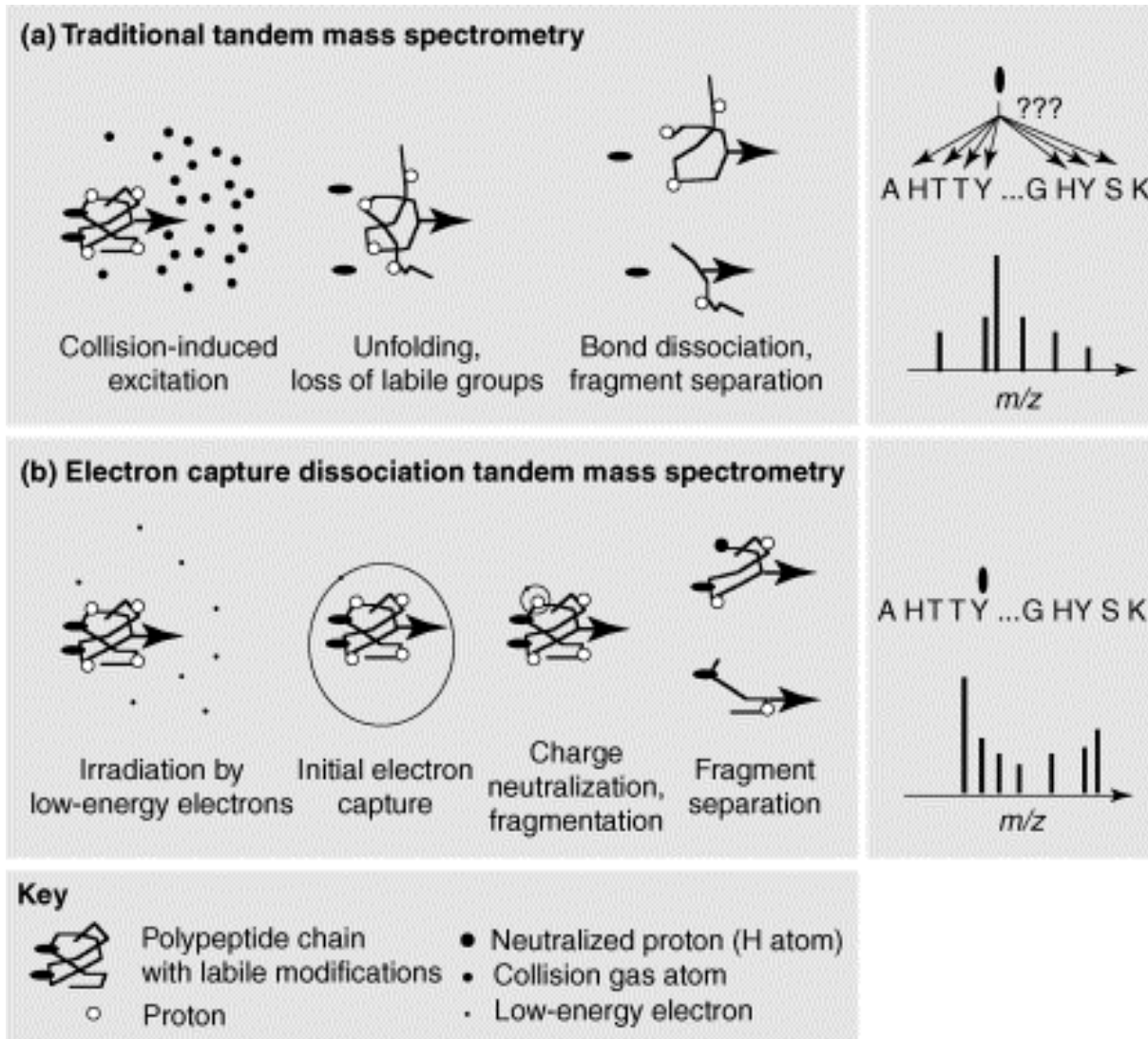


# Histidine Residues Modified by 1 mM HNE Found by QTOF

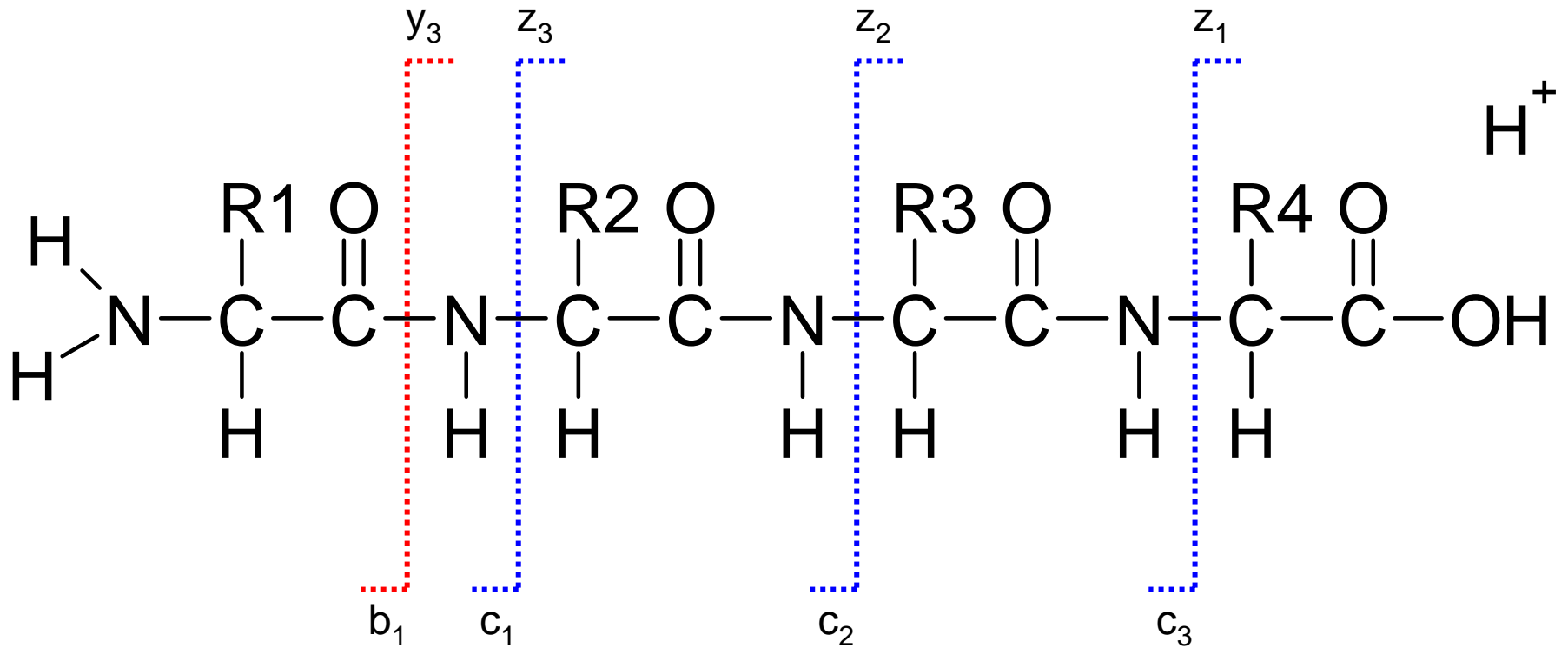
Residue Numbers	Sequence	Unmodified Mass	Modified Mass	Mass Shift
185-201	GFASLALAY <b>H</b> NYEDLP	1937	2093	156
271-283	<b>H</b> GQIQPLPHSAQ	1563	1719	156
271-283	HGQI <b>H</b> QPLP <b>H</b> SAQ	1563	1875	312
336-345	<b>A</b> HAEQAIGQLK	1164	1320	156
382-400	LHWGGEVIPHAAAQE <b>H</b> AWK	2136	2292	156



# Electron Capture Dissociation

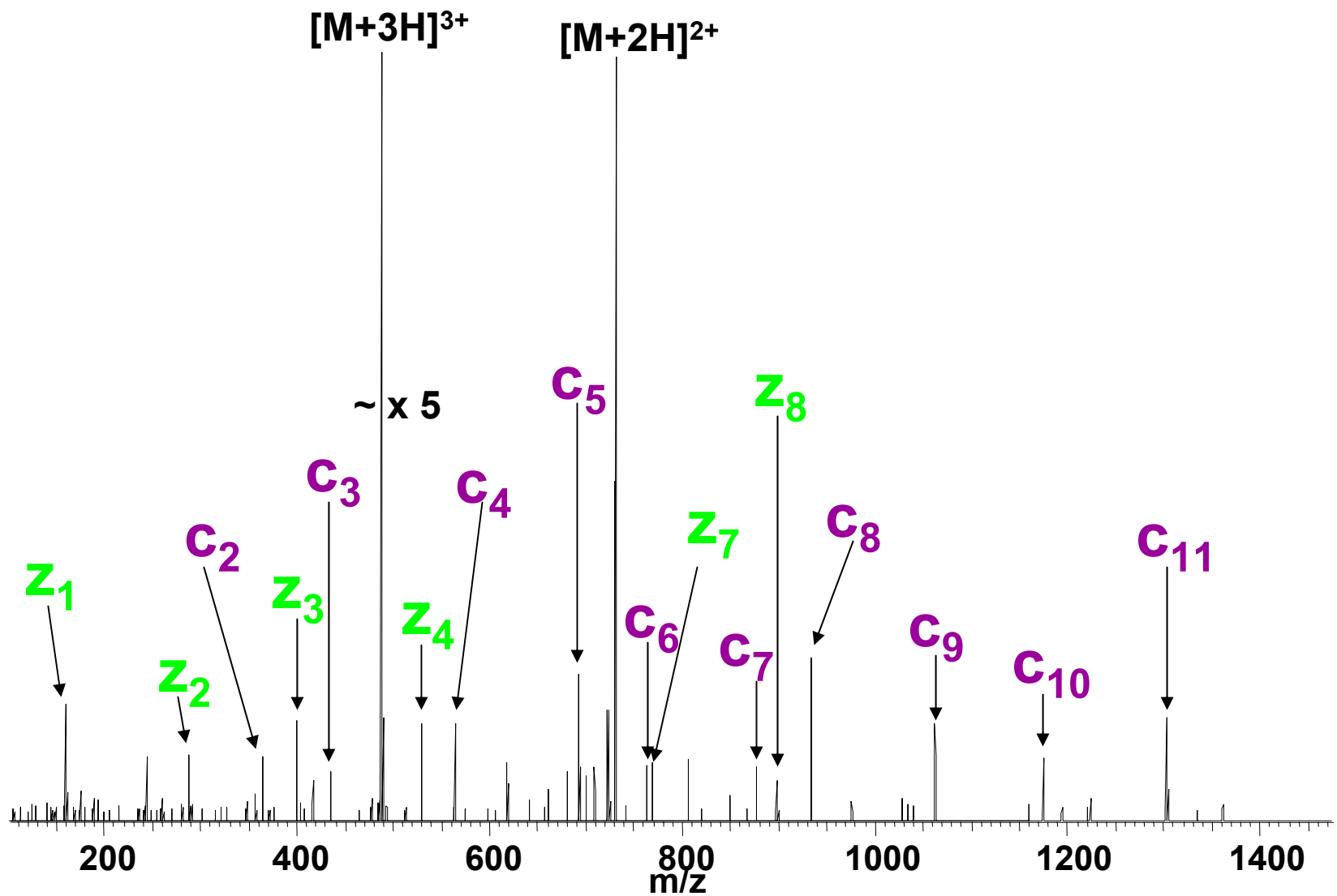


# ECD Fragmentation



# MS/MS Spectra Using ECD of [MH]<sup>+</sup> 1460.8408

A H A E Q A I G Q L K R





# Sequence Coverage Using FT-ICR/MS

MIQLTATPVSA LVDEPVHIRATGLIPFQMVSFQASL  
EDENGDMFY SQAHYRANEFGEVDLNHASSLGGDYMG  
VHPMGLFWSLKPEKLLTRLLKRDVMNRPFQVQVKLY  
DLELIVNNKVASAPKASLTLERWYVAPGVTRIKVRE  
GRLRGALFLPPGEGLEFPGVIDLFGGLGGLLEFRASL  
LASRGFASLALAYHNYEDLPRKPEVTDLEYFEEAAN  
FLLRHPKVFGSGVGVVSV CQGVQIGLSMAIYLKQVT  
ATV LINGTNFPFGIPQVYHGQIHQPLPHSAQLISTN  
ALGLLELYRTFETTQVGASQYLFPIEEAQQGQFLFIV  
GEGDKTINSKAHAEQAIGQLKRHGKNNWTLLSYPGA  
GHLIEPPYSPLCCASTTHDLRLHWGGEVIPHAAAQE  
HAWKEIQRFLRKHLIPDVTSQL

**70.09% Sequence Coverage**

# Amino Acids Modified by HNE Using FT-ICR/MS

Peptide	Modified Amino Acid				
	128 $\mu$ M HNE	64 $\mu$ M HNE	32 $\mu$ M HNE	16 $\mu$ M HNE	8 $\mu$ M HNE
A <b>H</b> AEQAIGQLKR	H336	H336	H336		H336
RLHWGGEVIPHAAAQE <b>H</b> AWK	H397	H397	H397	H397	
AQGQFLFIVGEGD <b>K</b> TINS <b>K</b>	K329, K334	K329, K334	K329, K334	K329, K334	K329, K334
MIQLTATPVSALVDEPV <b>H</b> IR	H18	H18			
RANEFGEVDLNHASSLGGDYMGVHPMGLFWSLKPEK	1*156				
NNWTLLSYPGAG <b>H</b> LIEPPYSPL <b>C</b> CAST <b>H</b> DLR	H362, C372, C373, H378				

# Summary

- **A covalent enzyme intermediate was identified between hBAT and cholic acid**
- **LC-ESI-MS/MS on the Q-tof identified 6 modifications at 1 mM HNE**
- **LC-ESI-FT-ICR/MS identified up to 10 modifications at 128  $\mu$ M, down to 3 modifications at 8  $\mu$ M**
- **4HNE may first alter sites in the region of the catalytic triad**

# Acknowledgements

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Shannon Eliuk

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## Mass Spec Shared

## Facility:

Marion Kirk

Landon Wilson