Detection of post-translational modifications of peptides

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Proteomics workshop
September 12, 2006

Synopsis

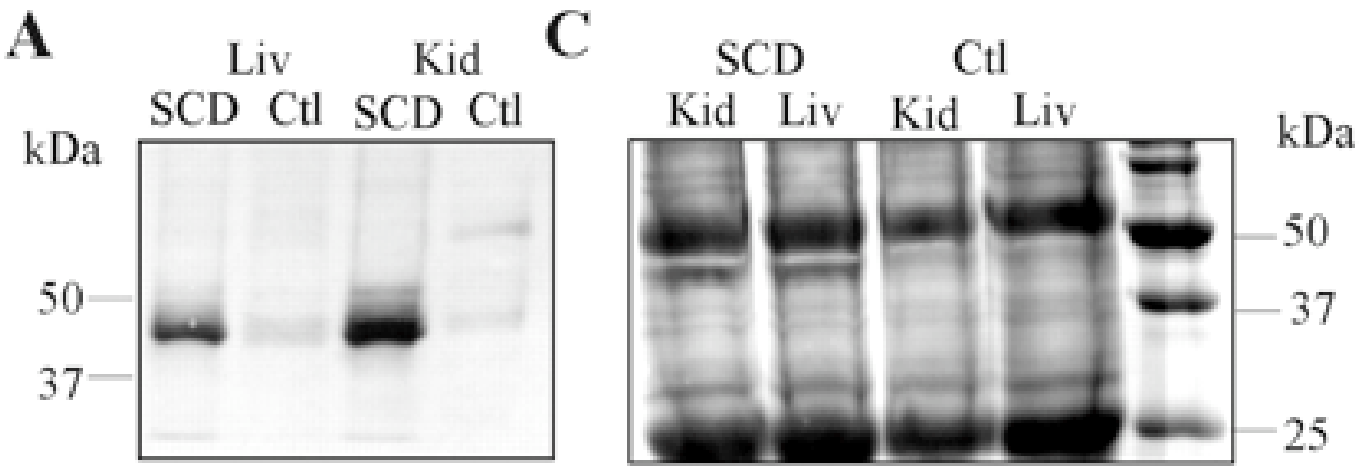
- **Detection of protein nitration - limitations (SB)**
- **Detecting O-glycosylated peptides - a job for ECD (MR)**
- **Inactivation of creatine kinase by the reactive aldehyde 4-hydroxynonenal (4HNE) (SE)**
- **hBAT, intermediates and inactivation (ES)**

Nitration of proteins

- **Peroxynitrite is a highly oxidizing and nitrating species produced by the reaction of the two radicals, nitric oxide and superoxide $NO^{\cdot} + O_2^{-\cdot} = ONO_2^-$**

- **UAB has an important place in the identification of nitrated proteins**
  - **1996 Greis et al., Arch Biochem Biophys 335: 396 (Surfactant protein A)**
  - **1997 Crow et al., J Neurochem 69: 1945 (neurofilament-L)**
  - **2000 Cassina et al., J. Biol Chem 275: 21409 (cytochrome C)**
  - **2003 Aslan et al., J Biol Chem 278: 4194 (actin)**

Nitration in sickle cell anemia

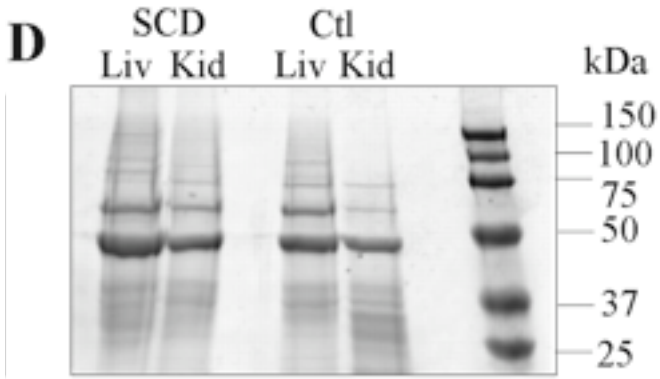


A = anti-nitrotyrosine and liver and kidney homogenates

C = immunoprecitated NO_2Tyr proteins run on SDS-PAGE and stained by Coomassie Blue

Aslan et al., JBC 278:4194

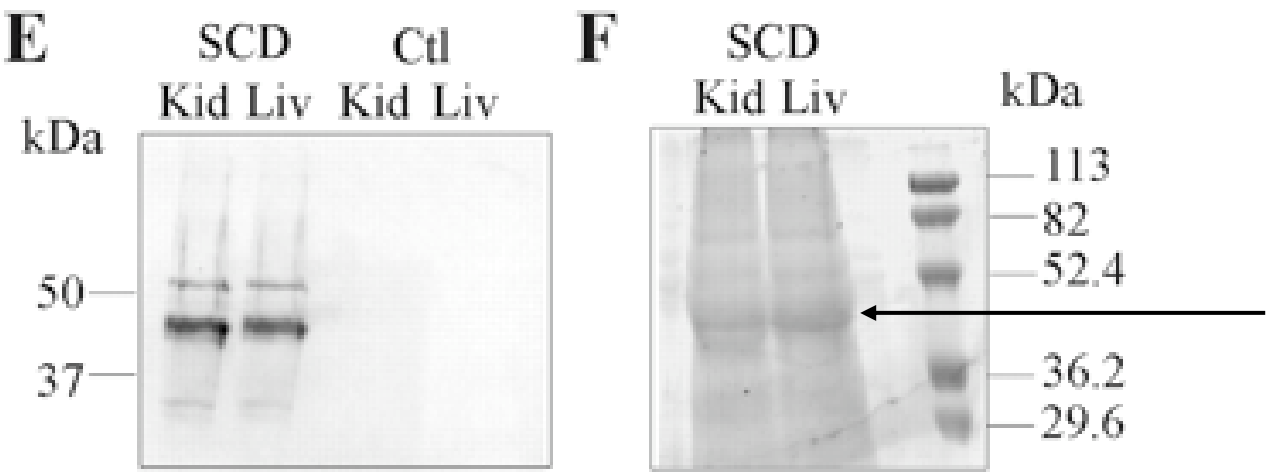
Multiple purification steps needed for nitrated actin



D = actin-enriched proteins run on SDS-PAGE and stained with Coomassie Blue

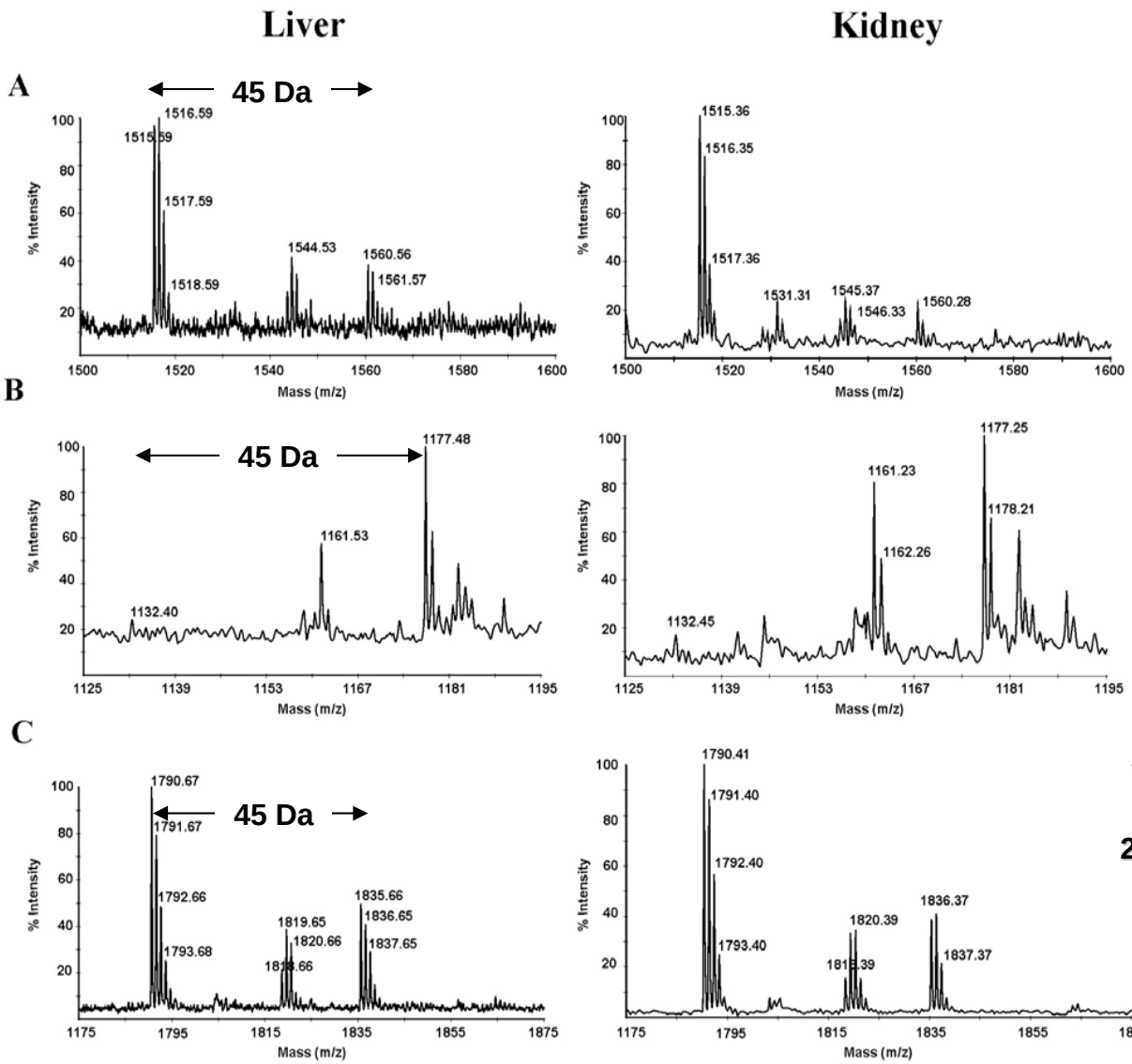
E = Western blot with anti-nitrotyrosine of actin-enriched proteins

F = Actin-enriched proteins bound to anti-nitrotyrosine affinity phase, eluted and run on SDS-PAGE and stained with Coomassie Blue



Aslan et al., JBC 278:4194

MALDI-TOF identification of NO_2Tyr peptides in actin - note the degradation pattern



^{85}IWHHTFYNELR^{95}

^{197}GYSFTTTAER^{206}

^{239}SYELPDGQVITIGNER^{254}

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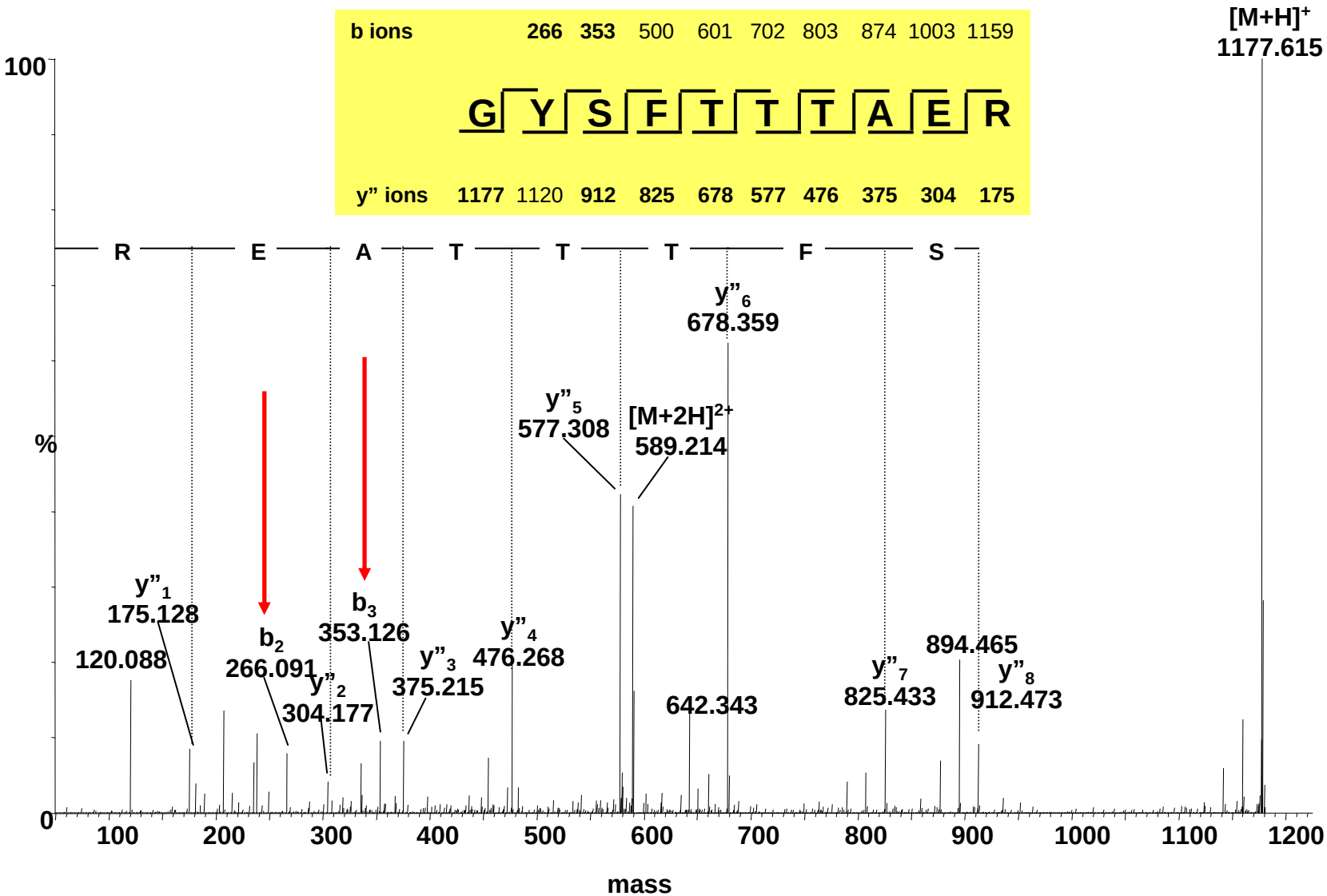
September 12, 2006

Aslan et al., JBC 278:4194

MSMS of actin tryptic peptide 197-206



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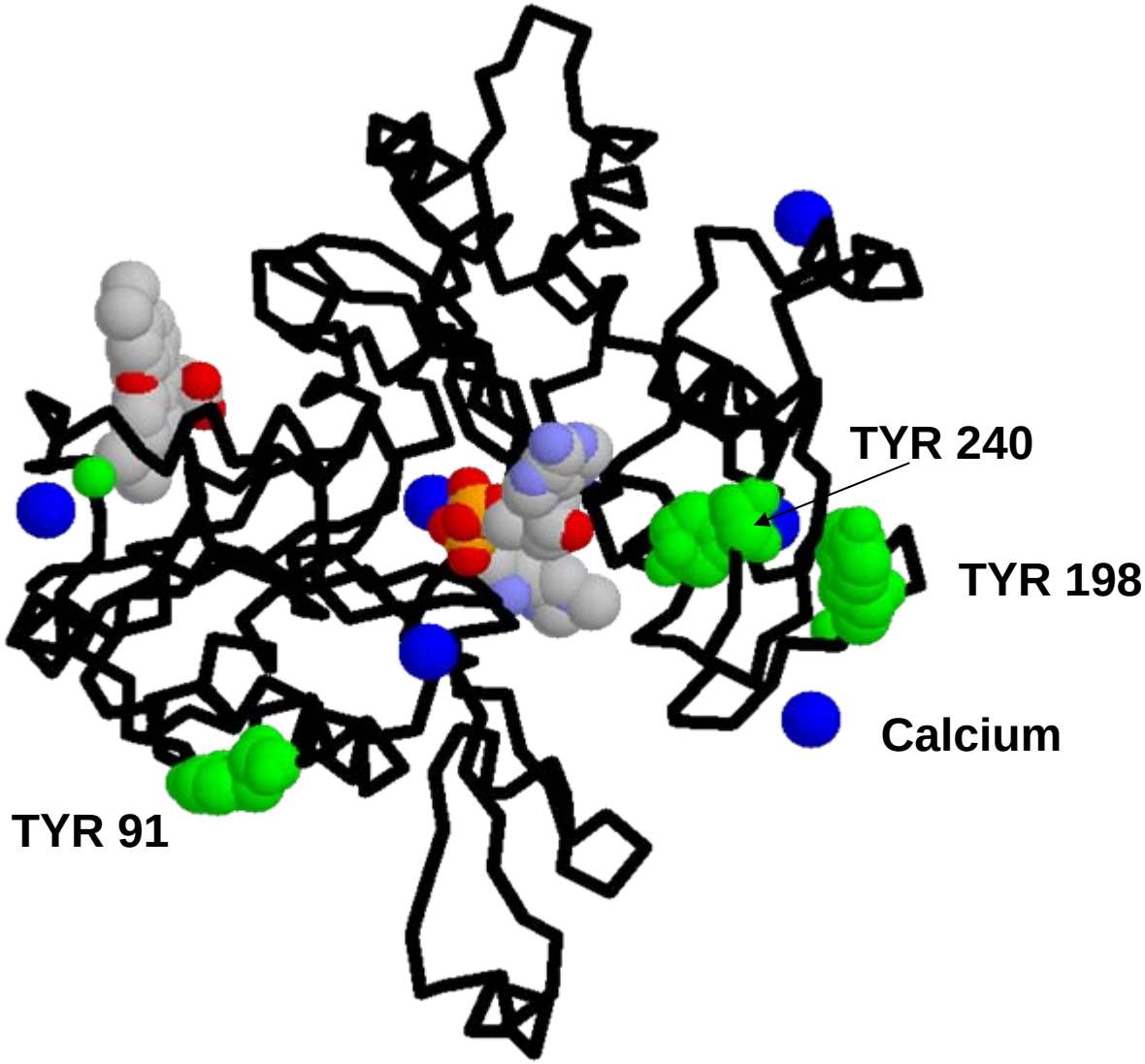
In Vivo Nitrated Actin



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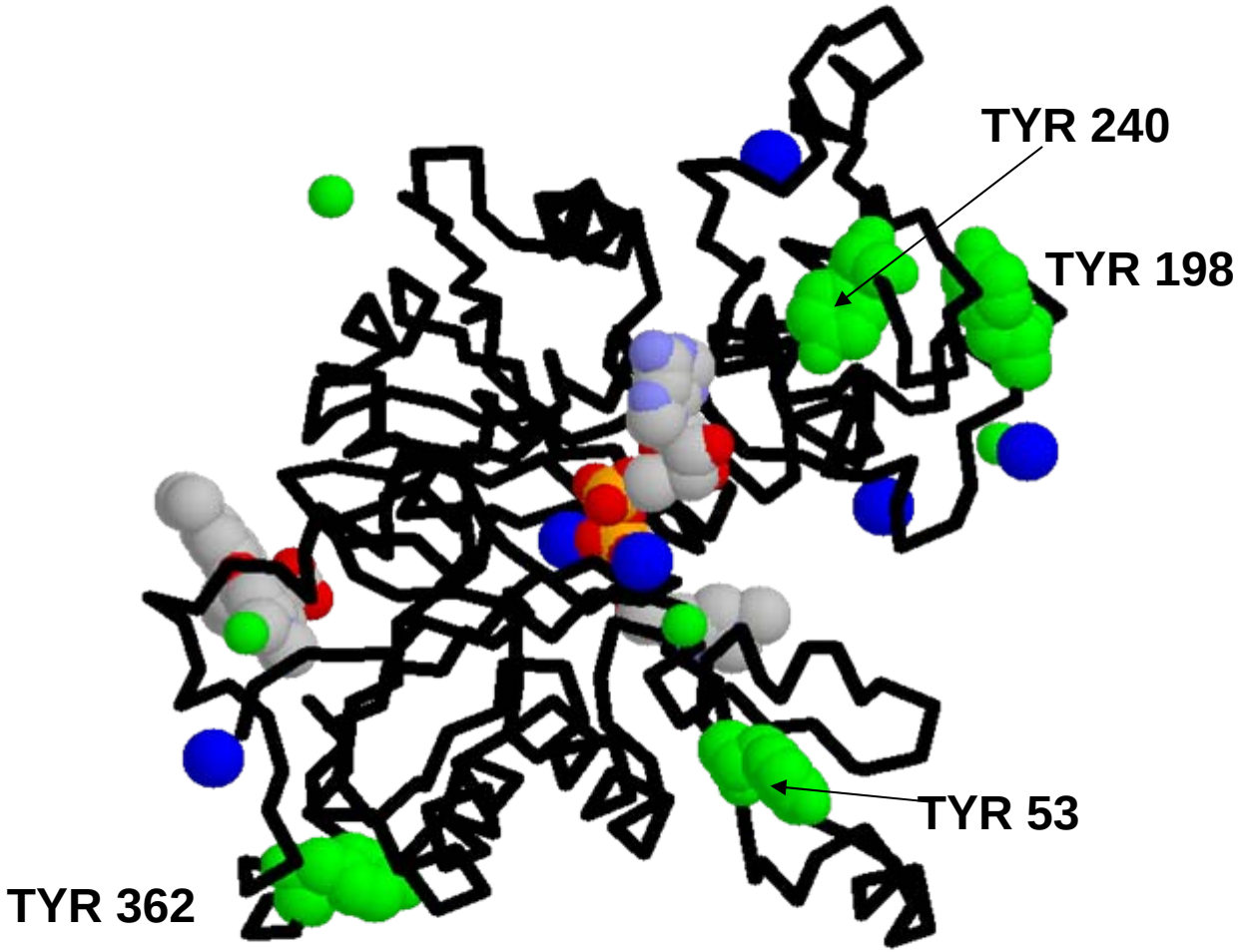
Thanks to
Amanda Isom d.

In Vitro Nitrated Actin



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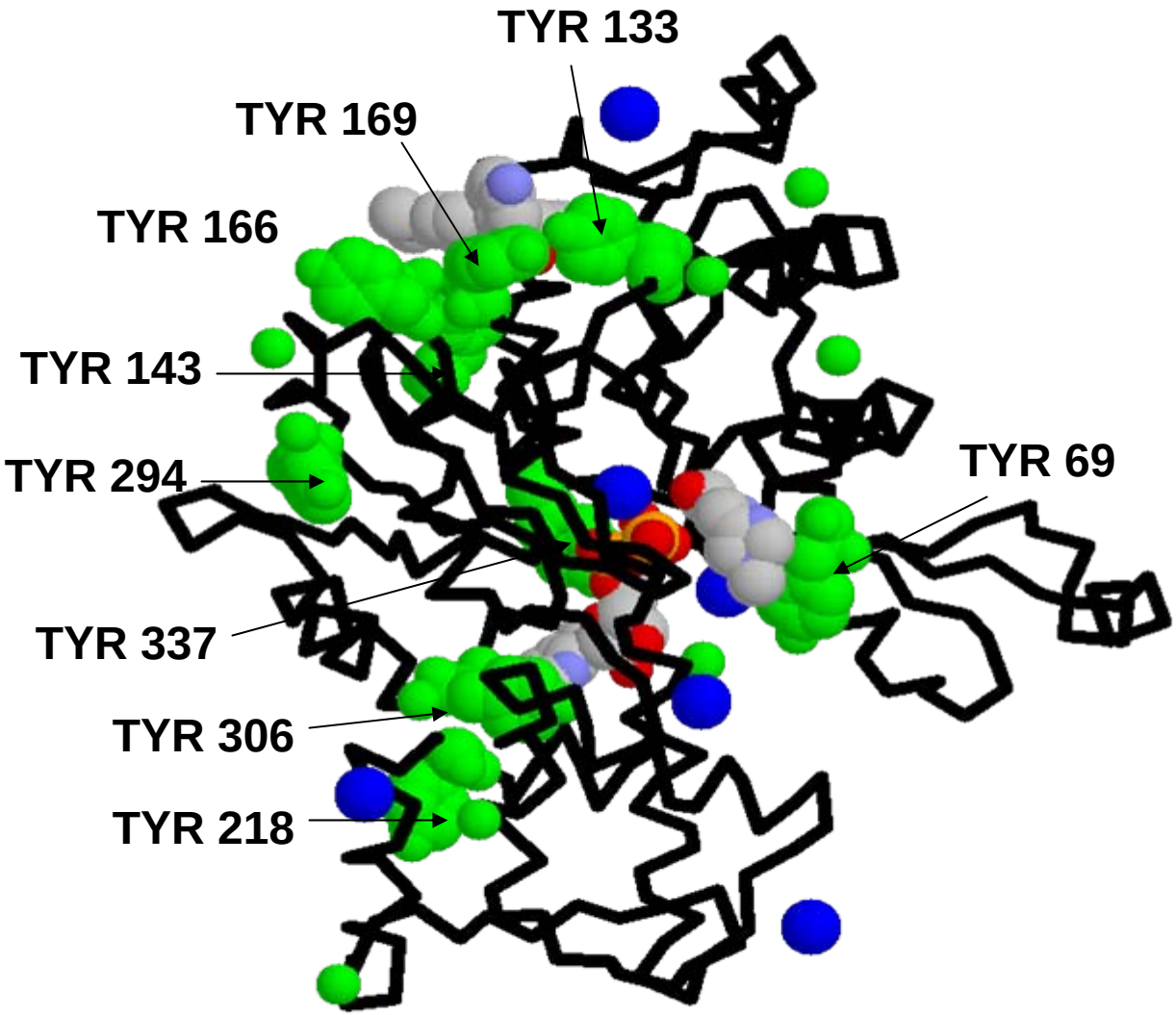
Undetected actin peptides with tyrosine nitration

```
  1 MDDDIAALVV DNGSGMCKAG FAGDDAPRAV FPSIVGRPRH QGVMVGMGQK
 51 DSYVGDEAQS KRGILTLKYP IEHGIVTNWD DMEKIWHHTF YNELRVAPEE
101 HPVLLTEAPL NPKANREKMT QIMFETFNTP AMYVAIQAVL SLYASGRTTG
151 IVMDSGDGVT HTVPIYEGYA LPHAILRLDL AGRDLTDYLM KILTERGYSF
201 TTTAEREIVR DIKEKLCYVA LDFEQEMATA ASSSSLEKSY ELPDGQVITI
251 GNERFRCPEA LFQPSFLGME SCGIHETTFN SIMKCDVDIR KDLYANTVLS
301 GGTTMYPGIA DRMQKEITAL APSTMKIKII APPERKYSVW IGGSILASLS
351 TFQQMWISKQ EYDESGPSIV HRKCF
```

69	YPIEHGIVTNWDDMEK	= 1991.89
133/143	MTQIMFETFNTPAMYVAIQAVLSLYASGR	= 3298.60, 3343.59
166/169	TTGIVMDSGDGVTHTVPIYEGYALPHAILR	= 3230.64, 3275.63
188	DLTDYLMK	= 1043.48
218	LCYVALDFEQEMATAASSSSLEK	= 2539.81
294/306	DLYANTVLSGGTTMYPGIADR	= 2260.06, 2305.05
337	YSVWIGGSILASLSTFQQMWISK	= 2647.33

Tyrosine Residues Not Nitrated



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Alternative digestion with Glu-C

```
  1 MDDDIAALVV DNGSGMCKAG FAGDDAPRAV FPSIVGRPRH QGVMVGMGQK
 51 DSYVGDEAQS KRGILTLKYP IEHGIVTNWD DMEKIWHHTF YNELRVAPEE
101 HPVLLTEAPL NPKANREKMT QIMFETFNTP AMYVAIQAVL SLYASGRTTG
151 IVMDSGDGVT HTVPIYEGYA LPHAILRLDL AGRDLTDYLM KILTERGYSF
201 TTTAEREIVR DIKEKLCYVA LDFEQEMATA ASSSSLEKSY ELPDGQVITI
251 GNERFRCPEA LFQPSFLGME SCGIHETTFN SIMKCDVDIR KDLYANTVLS
301 GGTTMYPGIA DRMQKEITAL APSTMKIKII APPERKYSVW IGGSILASLS
351 TFQQMWISKQ EYDESGPSIV HRKCF
```

53	SYVGD	= 585.22	198	RGYSFTTTAE	= 1177.52
69	AQSKRGILTLKYPIE	= 1761.99	218	KLCYVALD	= 969.48
91	KIWHHTFYNE	= 1419.65	240	KSYE	= 571.24
133/143	TFNTPAMYVAIQAVLSLYASGRTTGIVMD	= 3090.56, 3135.54	294/306	LYANTVLSGGTTMYPGIAD	= 1988.93, 2033.92
166	GVTHTVPIYE	= 1160.56	337	RKYSVWIGGSILASLSTFQQMWISKQE	= 3188.63
169	GYALPHAILRLD	= 1384.74	362	YD	= 342.10
188	YLMKILTE	= 1055.55			

Use of Glu-C would reveal whether ^{69}Y, ^{166}Y, ^{169}Y, ^{188}Y, ^{218}Y and possibly $^{294/306}$Y are nitrated

Key points to remember

- **Actin is a highly abundant protein in cells**
- **Proteins in the 40-44 kDa range are frequently heavily contaminated with actin**
- **2D-IEF/SDS-PAGE can help separate actin from other proteins**
- **Actin can be nitrated**
- **However, nitration is a low abundance event**
- **So, even detection of nitration of actin requires a preliminary immunopurification**
- **Nitration of β-actin is restricted to the protein surface; however, this may be an artifact of the distribution of trypsin cleavage sites in actin**