

表征分析案例分享

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生物药结构表征及有关物质研究



LC/LC-MS 分析:

- ✓ 消光系数: 氨基酸水解法
- ✓ 分子量: 完整/还原分子量-LC-MS
- ✓ 序列覆盖度: 肽图-LC-MS
- ✓ 二硫键: 肽图-LC-MS
- ✓ 翻译后修饰: 肽图-UPLC/UPLC-MS
- ✓ 糖型: Fast Fluor/2-AB荧光标记-UPLC-MS
- ✓ 蛋白相关杂质: UPLC-MS

聚合物分析

- ✓ AUC, DLS, DSC
- ✓ SEC-MALS etc.



其他:

- ✓ 傅里叶变换红外光谱FTIR
- ✓ 圆二色谱CD
- ✓ 荧光光谱FLR

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序列覆盖度



LC

DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP GKAPKVL IYF TSSLHSGVPS
RFSGSGSGTD FTLTISLQP EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVF IFPP
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYLSSTLT
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEK

Trypsin酶切样品检测到的肽段
Chymotrypsin酶切样品检测到的肽段

HC

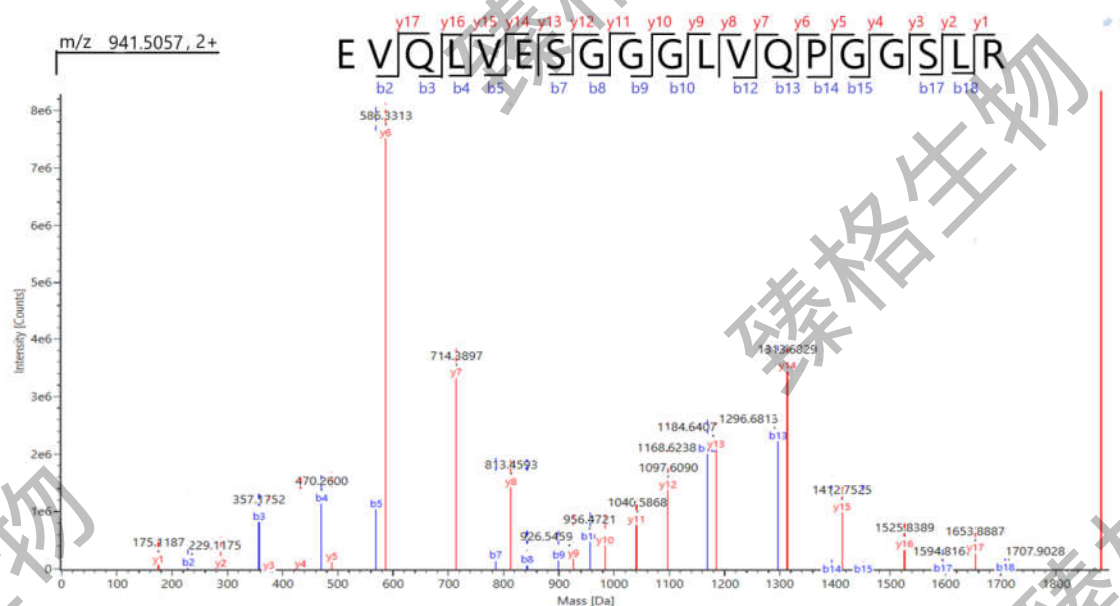
EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWRQA PGKLEWVGW INTYTGPEPT
AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP | HYYGSSHWYF DVWGQGLVT
VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL
QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKK VEPKSCDKTH TCPPCPAPEL
LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS
REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK
SRWQQGNVFS CSVMEALHN HYTQKSLSLG PGK

结论: Trypsin酶切未检测到的肽段可在 Chymotrypsin酶切的肽图中检测到, 综上 ZGQC-RS001的序列覆盖度可达100%。

序列覆盖度



Fragment label	Peptide	Expected mass (Da)	Observed mass (Da)	Mass error (ppm)
1:T1	EVQLVESGGGLVQPGGSLR	1882.0029	1882.0040	0.6

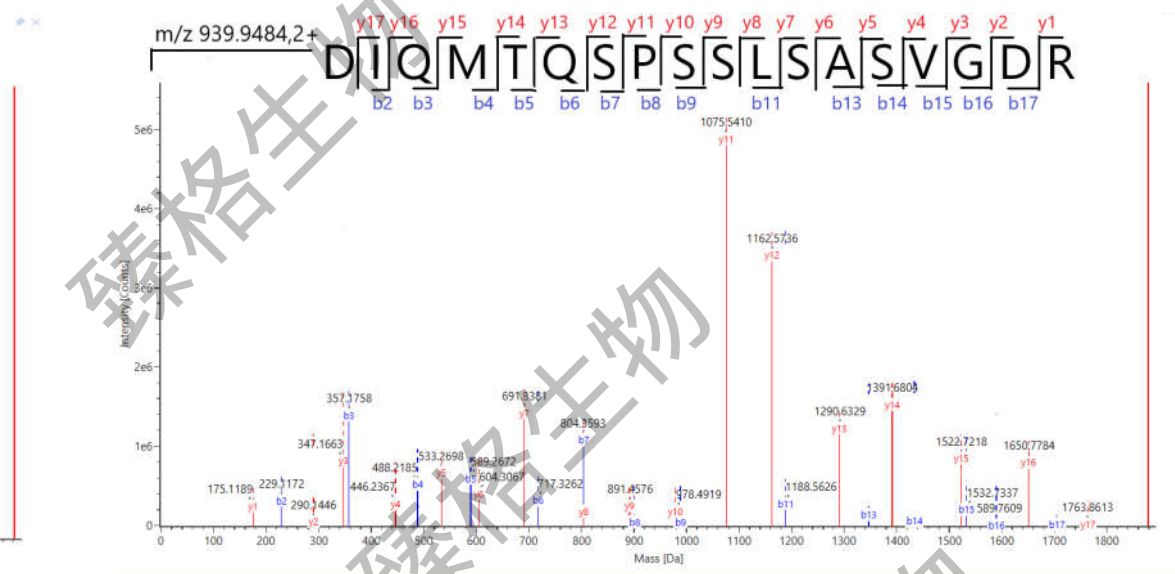
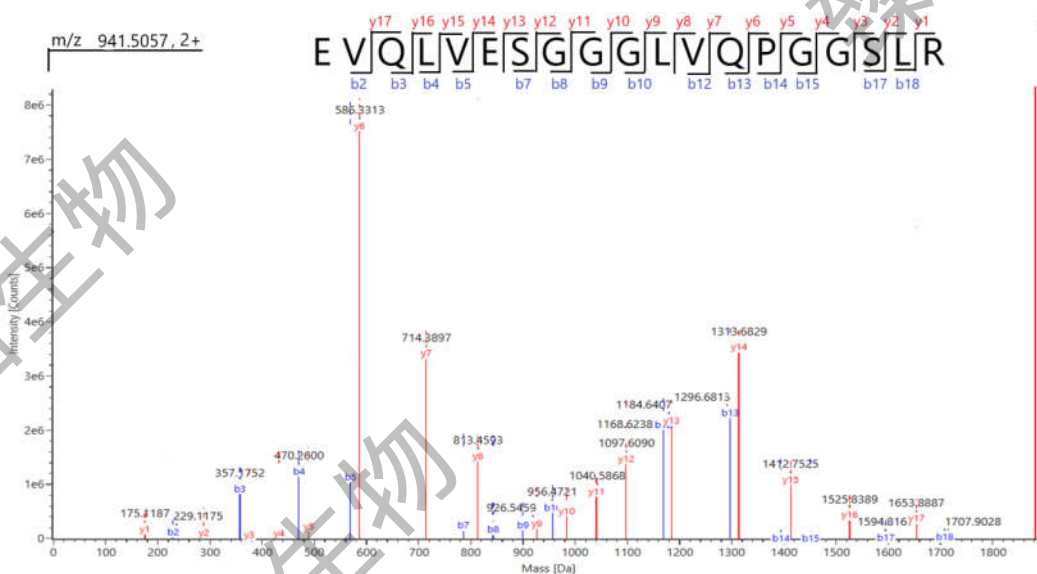


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N端序列



Fragment label	Peptide	Expected mass (Da)	Observed mass (Da)	Mass error (ppm)
1:T1	EVQLVESGGGLVQPGGSLR	1882.0029	1882.0040	0.6
2:T1	DIQMTQSPSSLSASVGDR	1878.8862	1878.8896	1.8

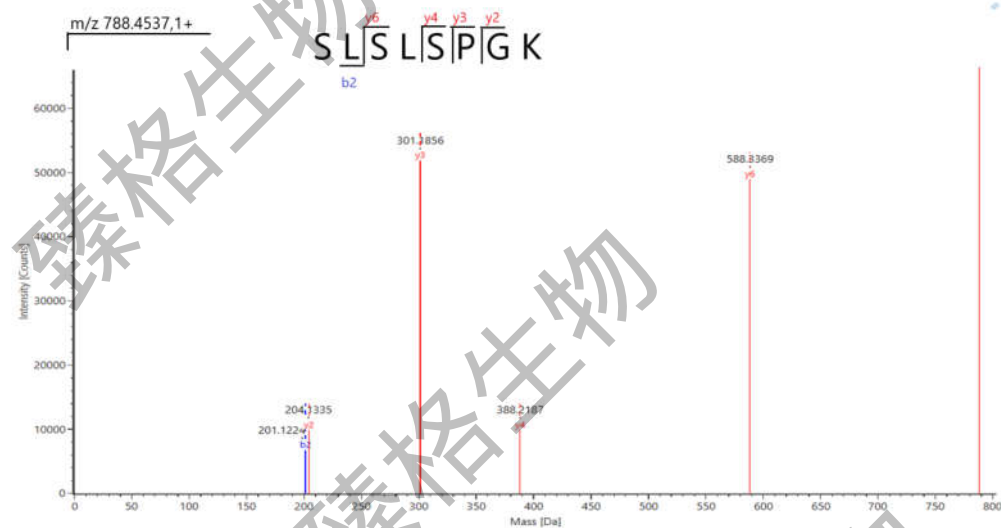
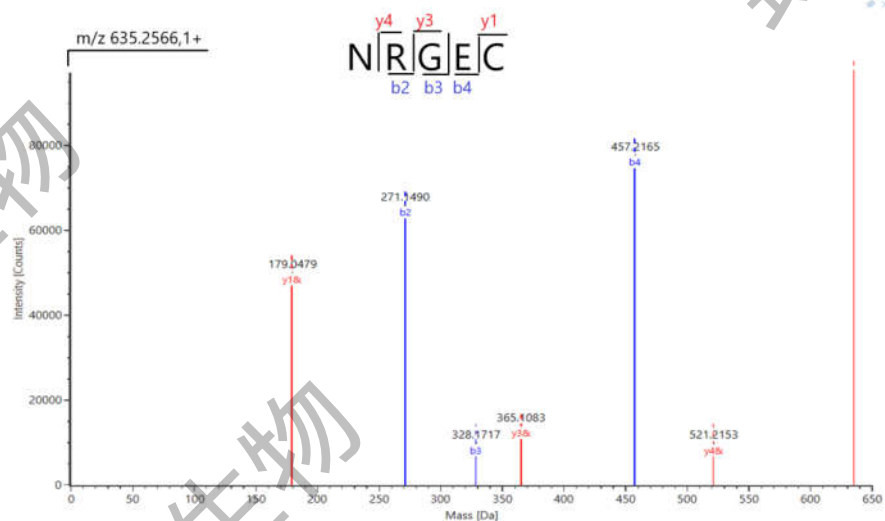


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C端序列



Fragment label	Peptide	Expected mass (Da)	Observed mass (Da)	Mass error (ppm)
1:T40	SLSLSPGK	788.4513	788.4537	3.1
2:C21	NRGEC	635.2566	635.2566	0



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翻译后修饰

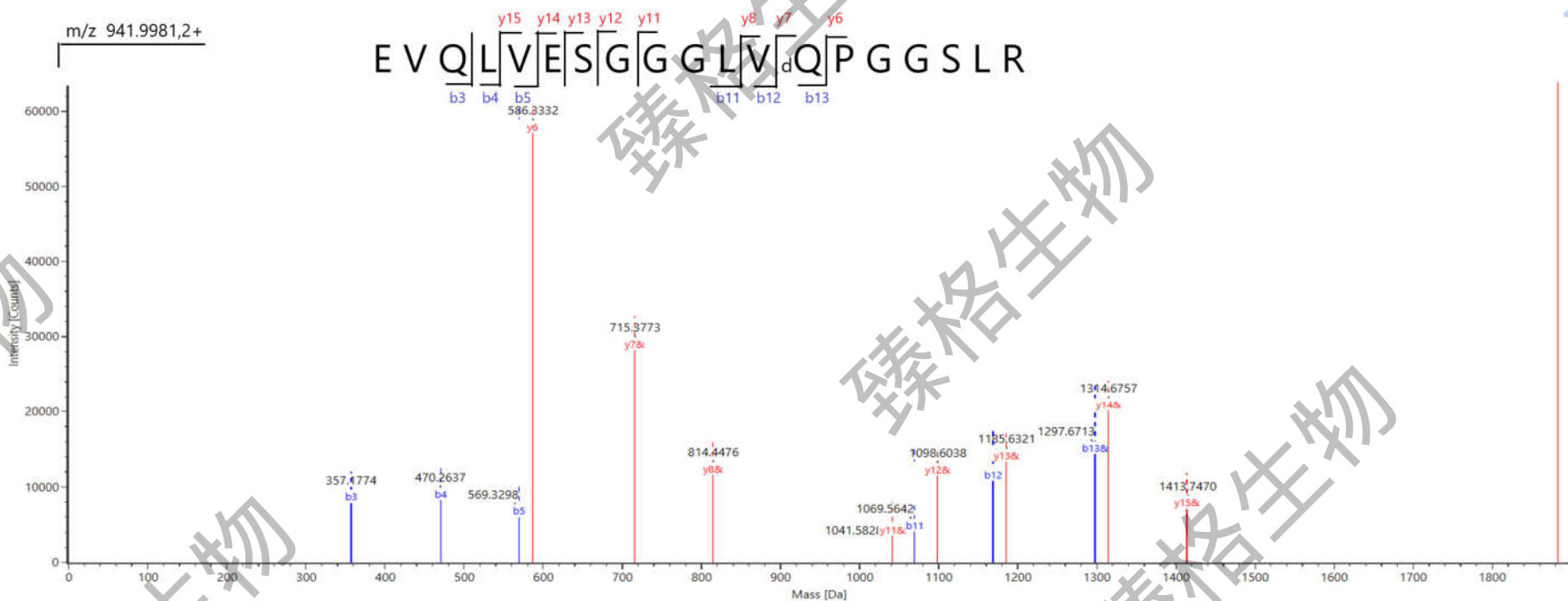


Fragment label	Peptide	Modifiers	Position	(%)
HC: T1	EVQLVESGGGLVQPGGSLR	Deamidation Q	Q13	1.30
		Deamidation Q	Q3	1.22
HC: T8	STAYLQMNSLR	Deamidation Q	Q82	0.98
HC: T19	DTLMISR	Oxidation M	M258	2.34
HC: T24	VVSVLTVLHQDWLNGK	Deamidation N	N321	1.74
HC: T34	NQVSLTCLVK	Carbamidomethyl C, Deamidation N	C373,N367	0.91
HC: T35	GFYPSDIAVEWESNGQPENNYK	Deamidation N	N390	1.13
HC: T39	WQQGNVFSCSVMHEALHNHYTQK	Carbamidomethyl C, Deamidation Q	C431,Q424	1.08
		Carbamidomethyl C, Deamidation Q	C431,Q425	1.06
HC: T40	SLSLSPGK	-Lysine C-TERM	K453	99.09
LC: T1	DIQMTQSPSSLSASVGDR	Deamidation Q	Q6	1.70
		Deamidation Q	Q3	1.65
		Oxidation M	M4	1.20
LC: T8	TVAAPSVFIFPPSDEQLK	Deamidation Q	Q124	0.98
LC: T16	VYACEVTHQGLSSPVTK	Carbamidomethyl C, Deamidation Q	C194,Q199	0.96

翻译后修饰



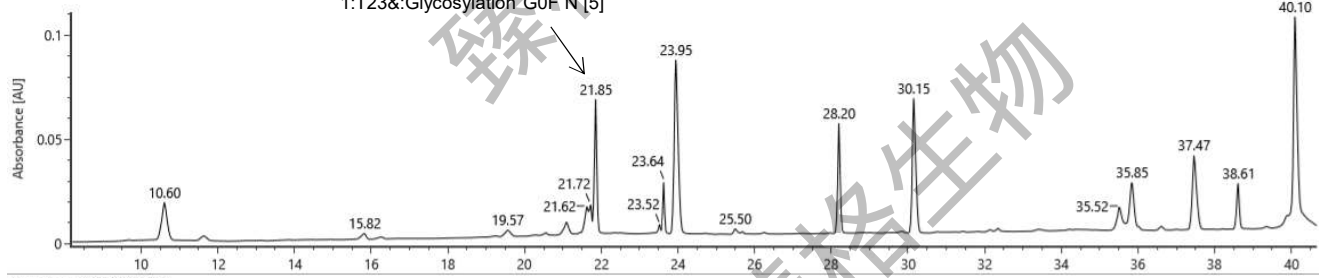
Fragment label	Peptide	Modifiers	Position	Expected mass (Da)	Observed mass (Da)	Mass error (ppm)
1:T1	EVQLVESGGGLVQPG GSLR	Deamidation Q	Q13	1882.9869	1882.9889	1.1



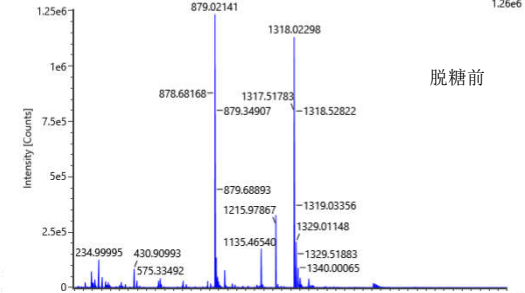
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糖基化位点

Item name: QC200190501
Channel name: TUV 214

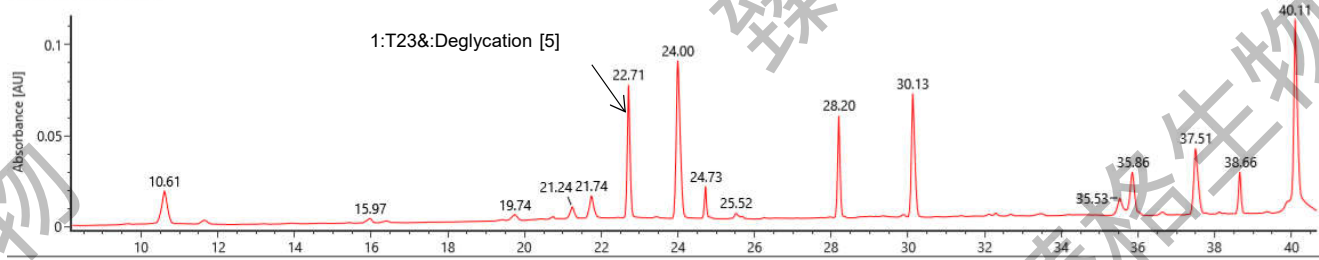


Item name: QC200190501 Channel name: 1: Average Time 21.9196 min : TOF MS¹ (100-2500) GeV E...
Item description:

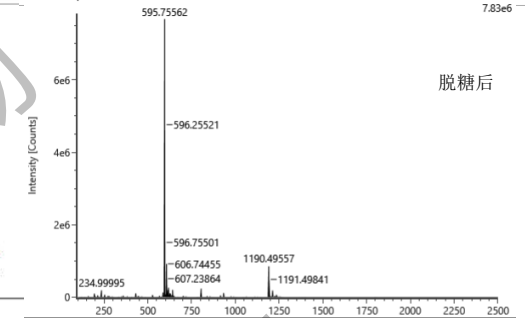


脱糖前

Item name: QC200190502
Channel name: TUV 214



Item name: QC200190502 Channel name: 1: Average Time 22.7998 min : TOF MS¹ (100-2500) GeV E...
Item description:



脱糖后

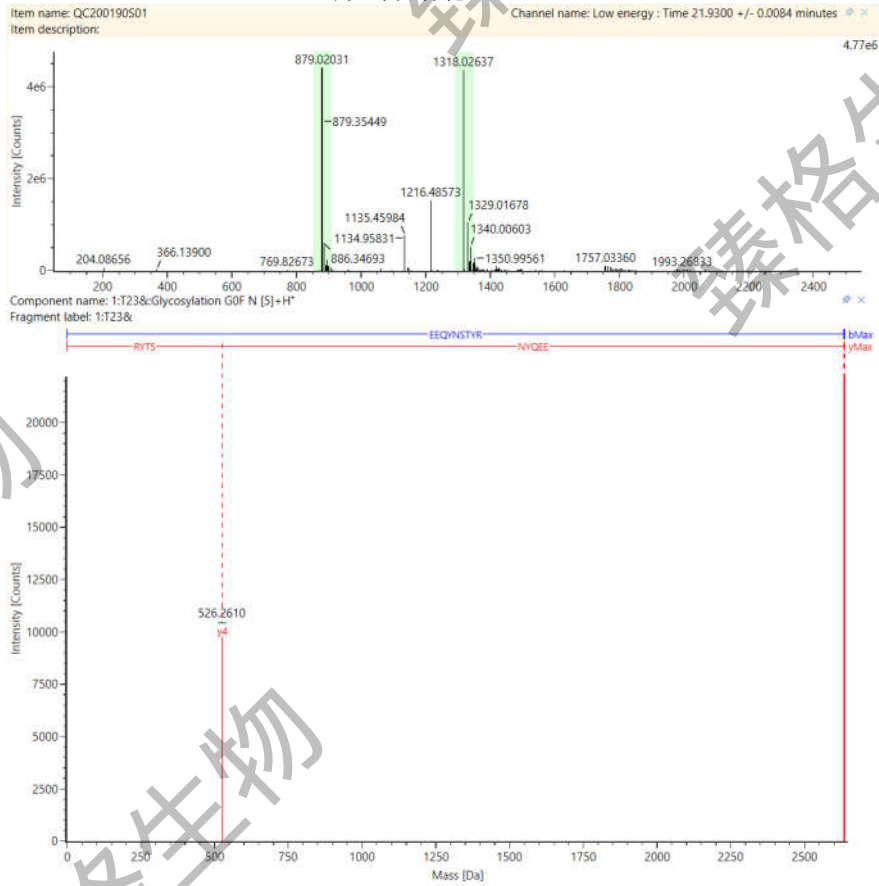
Sample	Fragment label	Peptide	Modifiers	Modifier site	Sequence start	Sequence end	Expected mass (Da)	Observed mass (Da)	Mass error (ppm)	Observed RT (min)	Expected m/z	Observed m/z	Charge
脱糖前	1:T23&	EEQYNSTYR	Glycosylation GOF N [5]	N303	299	307	2634.04588	2634.0421	-1.4	21.93	1317.5266	1317.5247	2
脱糖后			Deglycation [5]				1190.49603	1190.4972	1	22.79	595.7517	595.7523	2

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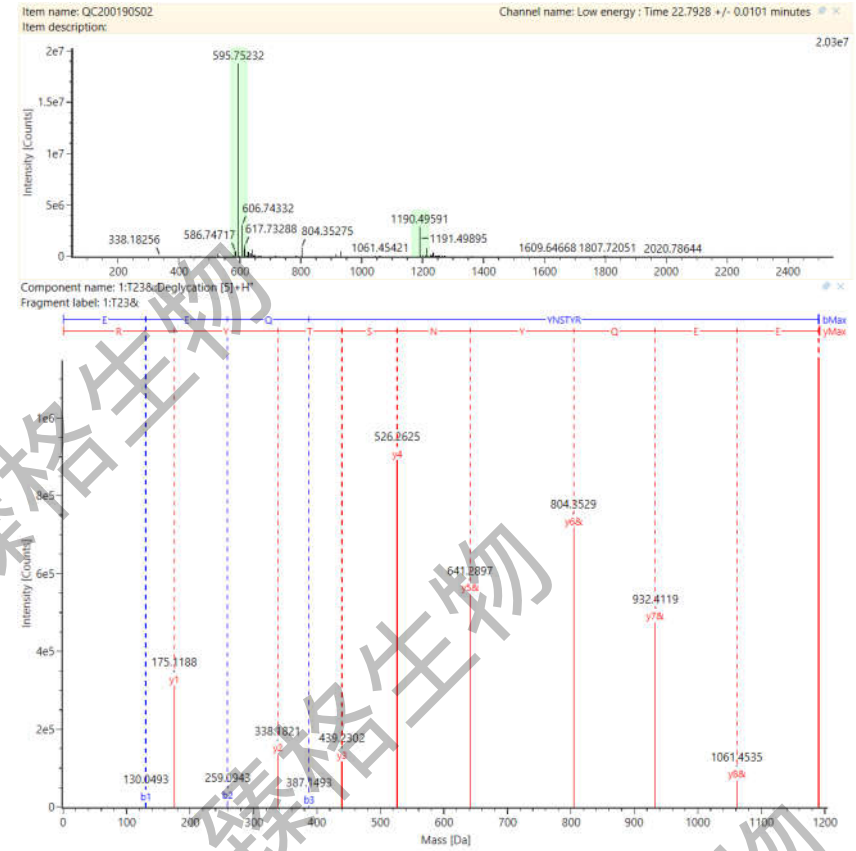
糖基化位点



脱糖前



脱糖后



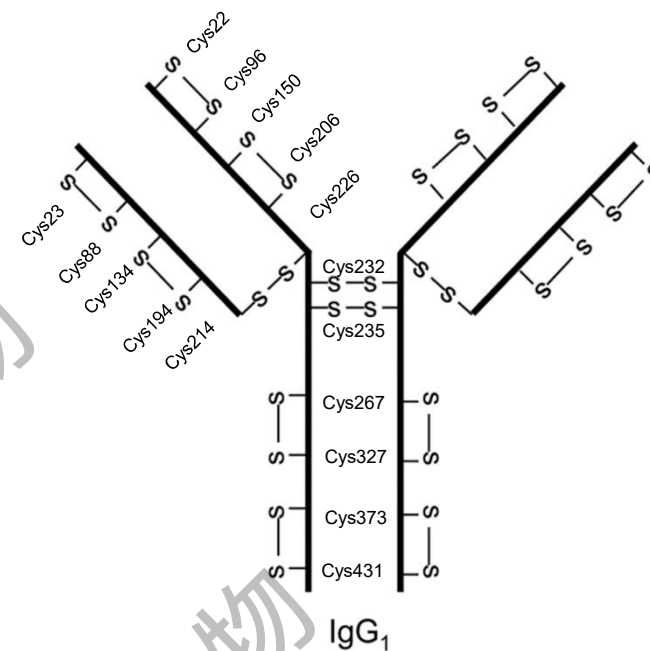
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10

二硫键检测



Fragment label	Linkage	Expected mass (Da)	Observed mass (Da)	Mass error (ppm)	Frangementation confirm
1:T2-1:T5	Cys 23=Cys 88	7344.40414	7344.3887	-2.1	✓
1:T9-1:T16	Cys 134=Cys 194	3556.7563	3556.7554	-0.2	✓
1:T17-18-2:T17	Cys 214=Cys 226	1261.4936	1261.4927	-0.7	✓
2:T2-2:T9	Cys 22=Cys 96	3371.4806	3371.4799	-0.2	✓
2:T12-2:T13	Cys 150=Cys 206	7917.92663	7917.9179	-1.1	✓
2:T18-3:T18	Cys 232=Cys 232, Cys 235=Cys 235	5455.79065	5455.776	-2.7	✓
2:T20-2:T26-27	Cys 267=Cys 327	2757.34336	2757.3435	0.1	✓
2:T34-2:T39	Cys 373=Cys 431	3845.83088	3845.8282	-0.7	✓



手动核查的重要性-----序列覆盖度



Filters

Match all of these expressions

- Fragment label does not contain
- Fragment label does not contain
- Fragment label does not contain

Match all of these expressions

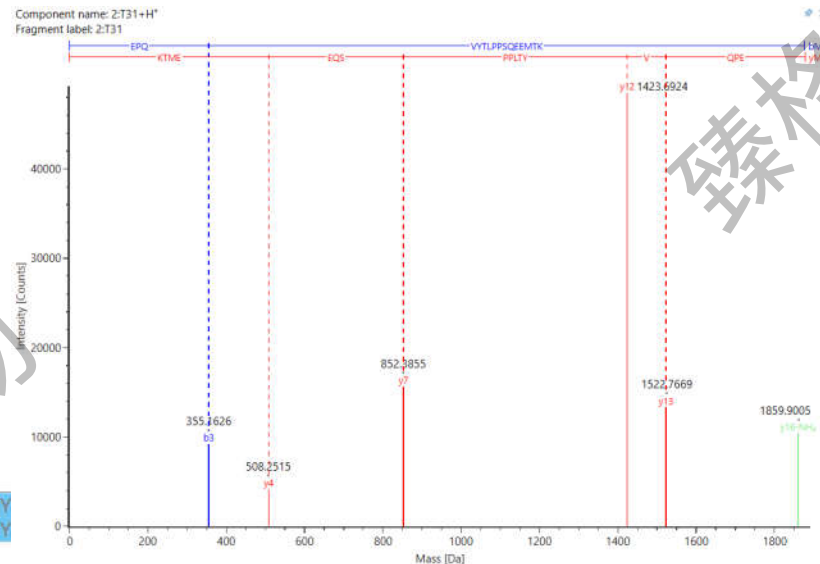
- % Matched 1st Gen Primary Ions (%) is greater than or equal %
- Matched 1st Gen Primary Ions is greater than or equal

Match all of these expressions

- Primary Ions Intensity Flag is

Identified: 93%

1: 1 to 100	DIQLTQSPAS	LAVSLGQRAT	ISCKASQSV	YDGD SYLNWY	QQIPGQPPKL	LIYDASNLYS	GIPPRFSGSG	SGTDFEWH	PVEKVDAATY	
1: 101 to 200	TFGGGKLEI	KRTVAAPSVF	IFPPSDEQLK	SGTASVCLL	NNFYPRKAVK	QWKVDNALQS	GNSQESVTEQ	DAKDSYSL	STLTLKADY	
1: 201 to 218	THQGLSSPVT	KSFNR GEC								
2: 1 to 100	QVQLQQSGAE	LVRPGSSVRI	SCKASGYAFS	SYMMWVKQR	PGQGLEWIGQ	IWPGDGDTHY	NGHFKGRATL	TADPSSSTAY	MQLSSLASED	SAVYFCARRE
2: 101 to 200	TTTVGRYYA	MDYWGQGITV	TVSSASTKGP	SVEPLAPCSR	STSESTAALG	CLVKDYFPEP	VTVSMNSGAK	ISGVHTFPAV	LQSSGLYSLS	SVTVTPSSSL
2: 201 to 300	GTKTYTCND	HKPSNTKVDK	RVESKYGPPC	PPCPAPEFLG	GPSVFLFPPK	PKDTLMISRT	PEVTCVVDV	SQEDPEVQFN	WYVDGVEVHN	AKTKPREEQF
2: 301 to 400	NSTYRVVSVL	TVLHQDWLNG	KEYKCKVSNK	GLPSSIEKTI	SKAMGQPRFP	QVYTLPPSQE	EMTKIQVSLT	CLVKGFYPSD	IAVEVESNGQ	PENNYKTTPE
2: 401 to 500	VLDSDGSFFL	YSRLTVDKSR	WQEGNVFSCS	VMHEALHMHY	TQKLSLSLGL	KGGGGSGGGG	SDIQMTQSPS	SLSASVGRDV	TIICRASQDI	RNYLMWYQDI
2: 501 to 600	PGKAPKLLIY	YTSRLESQVP	SRFSGSGSGT	DYTLTISLSQ	PEDFATYYCQ	QGNTLPWTFG	CGTKVEIKGG	GGSGGGSGG	GGSEVQLVES	GGGLVQPPGG
2: 601 to 700	LRLSQAASGY	SFTGQTNWV	RQAPGKCLEW	VALINPYKGV	TTYADSVKGR	FTISVDKSKN	TAYLQMNLSR	AEDTAVYYCA	RSYGYGDSOW	YFDVWQGTI
2: 701 to 705	VTVSS									



该filter条件下，序列覆盖度为93%，存在假阳性（黑色加粗），且存在漏匹配肽段（红色框）。

- 去除假阳性保证结果质量
- 每段序列附二级谱图
 - 便于数据核查（internal and external）
 - 防止后期返工查找谱图导致的更多时间成本

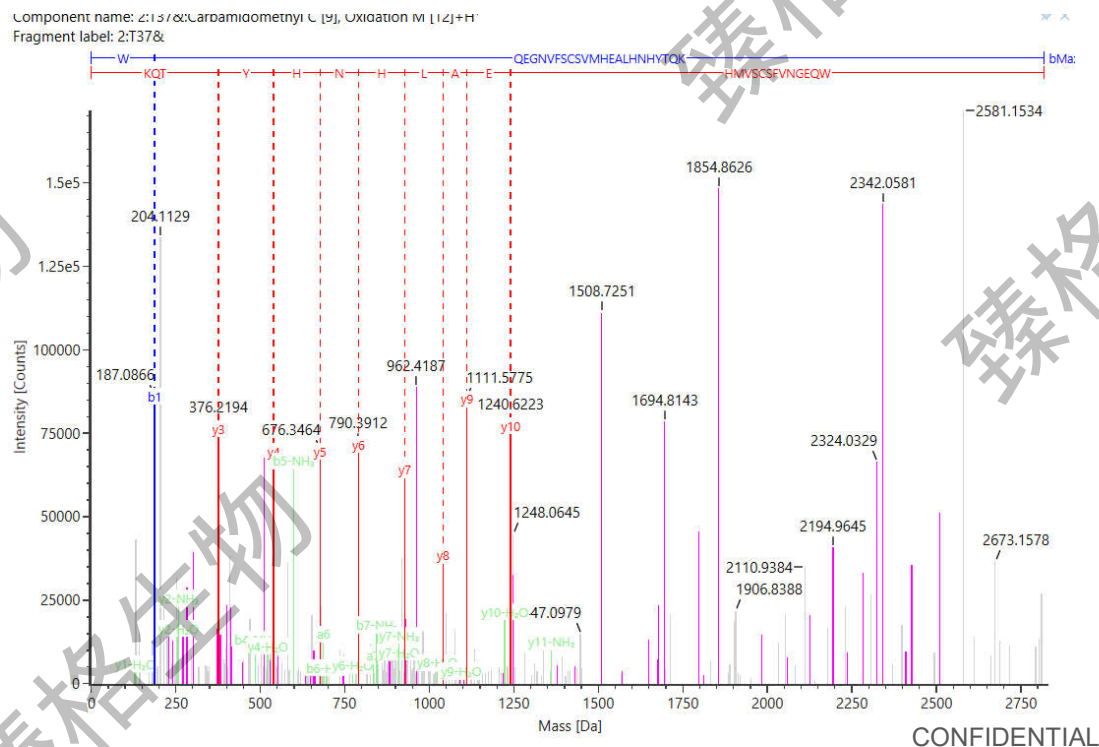
手动核查的重要性

翻译后修饰



使用Trypsin酶切数据修改process参数，增加各翻译后修饰项，重新process，调用Filter方法，初步筛选出存在的修饰信息，但存在大量假阳性结果，需人工确认。

肽段	序列	修饰	保留时间
2:T37	WQEGNVFSCSVMHEALHNHYTQK	N/A	52.64
2:T37*	WQEGNVFSCSVMHEALHNHYTQK	Oxidation (M)	52.63



该氧化修饰各参数均符合设置的filter条件，但二级图杂峰较多，且未得到修饰位点处的碎片离子。另外保留时间与未修饰肽段保留时间一致，此处为显著假阳性结果。