Economic Development,
Jobs, Transport
and Resources

Proteomics rocks my world!

Dr Delphine Vincent
10/11/2017

Outlines

Past

Present

- MALDI Biotyper (MBT)
- Milk proteomics (Top-Down)

Future

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Future

What I have been up to in the past…

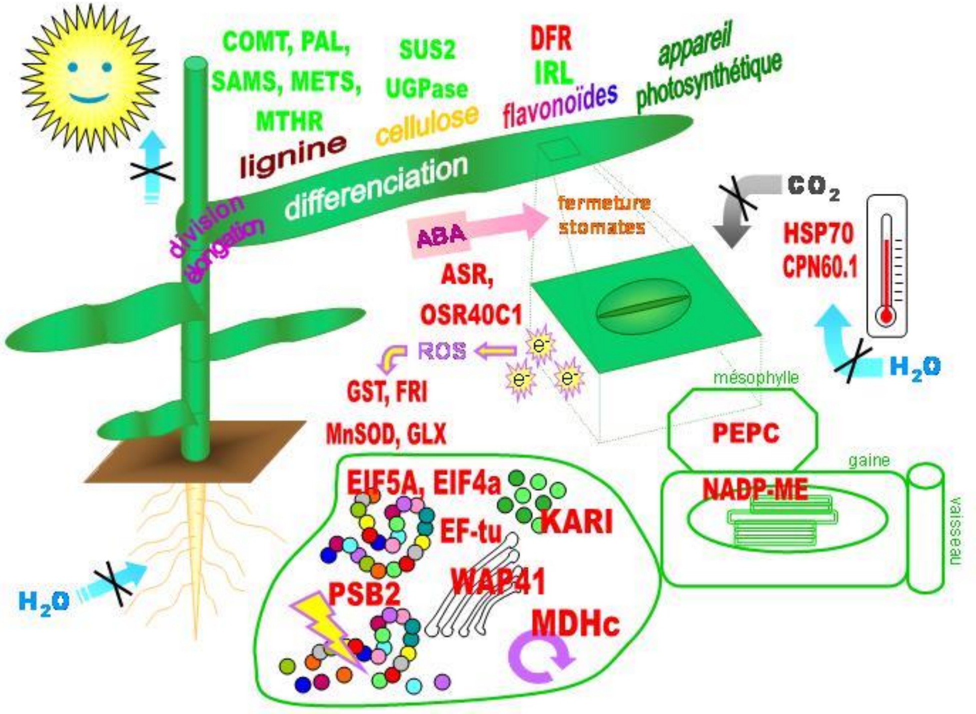
INRA
SCIENCE & IMPACT

Le Moulon
Génétique végétale UMR

1998-2003
Honours-Ph D

Corn proteins responsive to water deficit

Riccardi et al., 2004 Plant Physiol Biochem 42: 1003-1011
Ferry-Dumazet et al., 2005 Proteomics 5(8): 2069-2081
Vincent et al., 2005 Plant Physiol. 137: 949-960



UM2
UNIVERSITÉ MONTPELLIER 2
SCIENCES ET TECHNIQUES

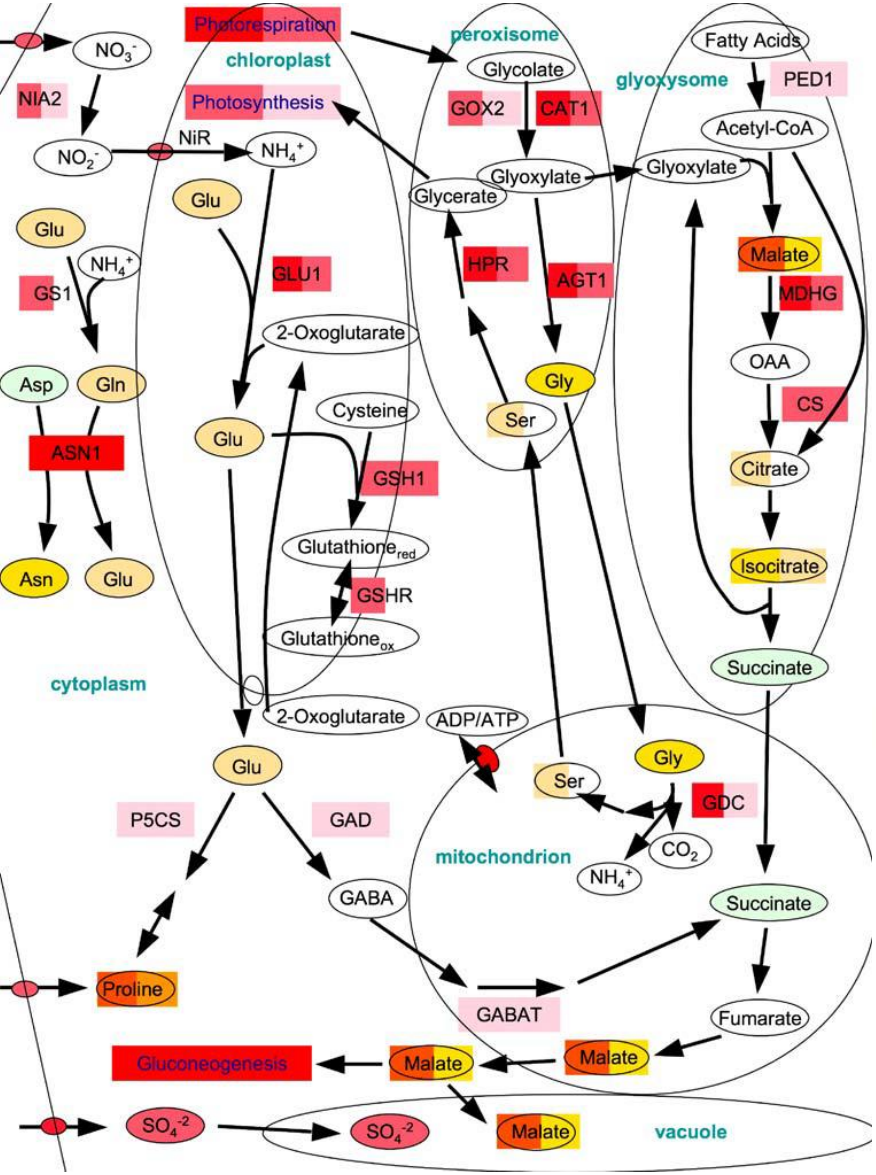
Water-stressed corn leaves slow down lignification to allow cell elongation to resume when conditions improve.

2003-2005
Post-doc 1
Stressed-grapevine proteomics

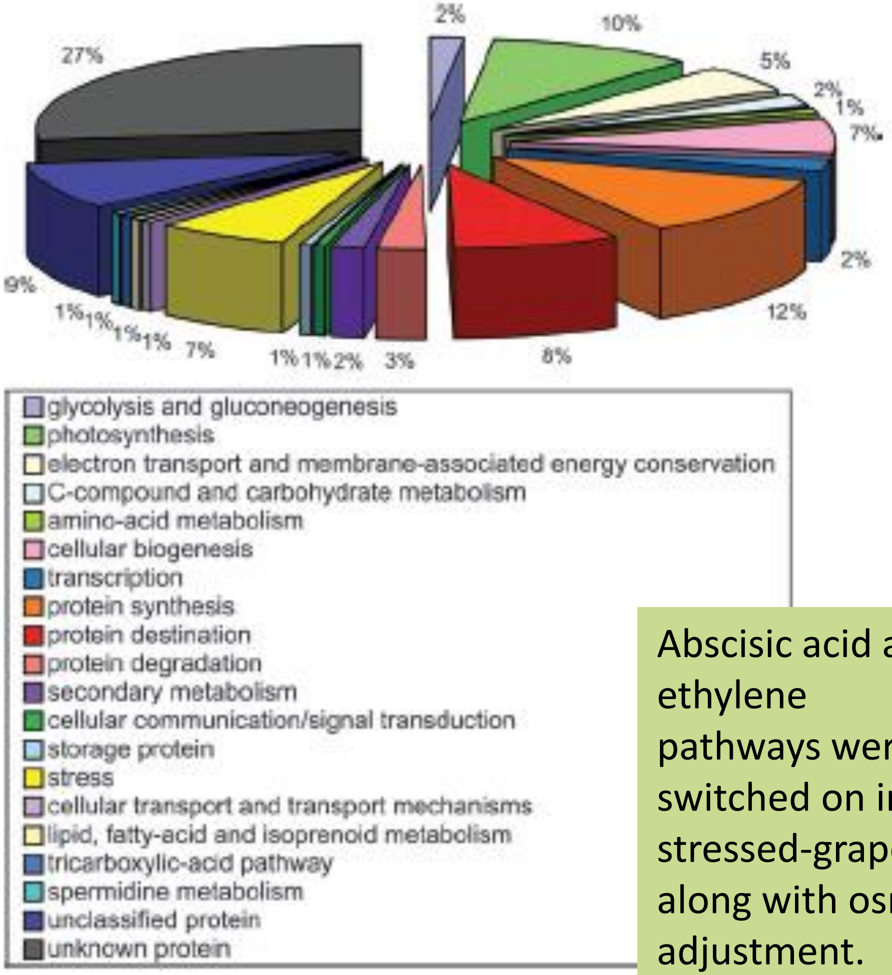
UNIVERSITY OF NEVADA
•Reno

1874

Grapevine systems biology to better understand responses to abiotic stresses



Cramer et al., 2005 Acta Hort. 689: 417-25
Vincent et al., 2006 Electrophoresis 27: 1853-65
Cramer et al., 2007 Funct. Int. Genom. 7(2):111-34
Vincent et al., 2007 J. Exp. Bot. 58 (7): 1873-92
Tattersall et al., 2007 Funct. Int. Genom. 7(4): 317-333



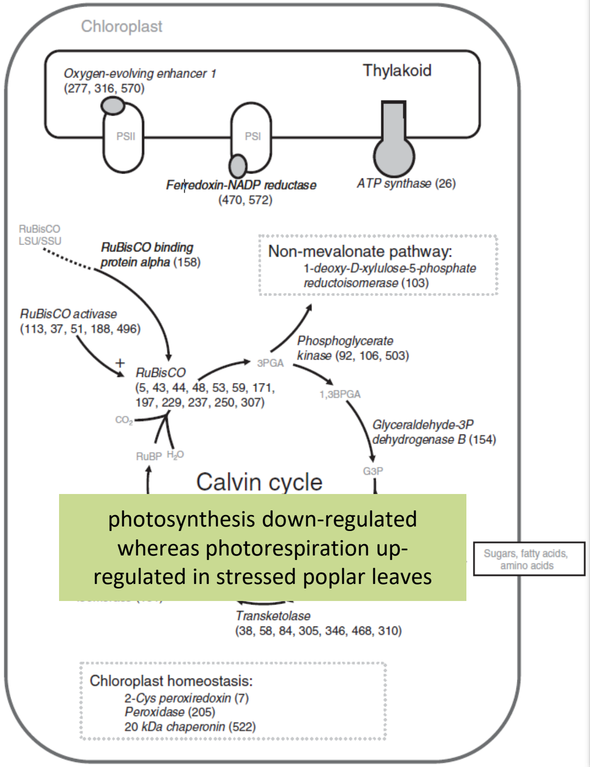
Abscisic acid and ethylene pathways were switched on in stressed-grapevines, along with osmotic adjustment.

2006
Post-doc 2
Heterosis in canola

2007-2009
Post-doc 3

1. Systems biology poplar
2. Effectors of plant-interacting fungi

Poplar response to water deficit



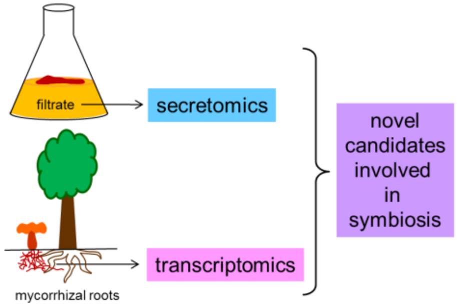
photosynthesis down-regulated whereas photorespiration up-regulated in stressed poplar leaves

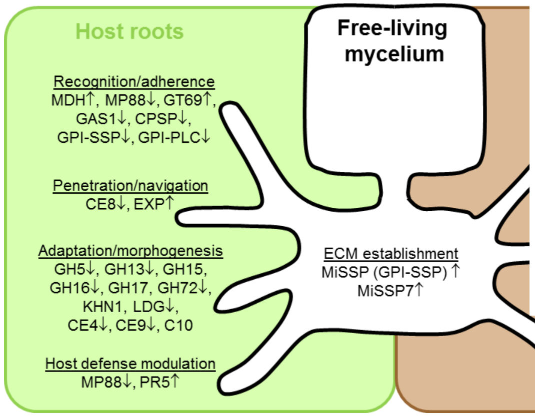
Bonhomme et al., 2009 Phytochem. 70: 988-1002
Bonhomme et al., 2009 Proteomics 9: 4121-42
Bedon et al., 2012 Plant Cell Env. 35(4):790-805

Secretomes of fungi interacting with plants

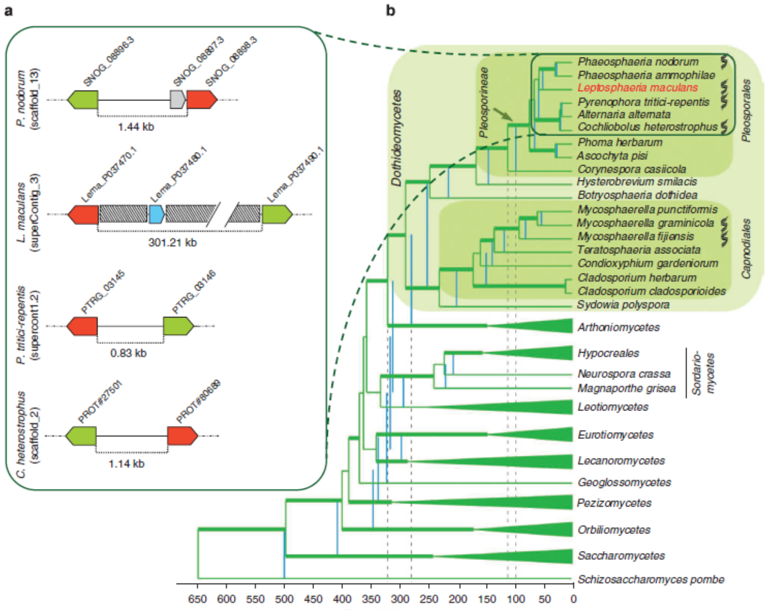
1- *Magnaporthe grisea*
rice pathogen
2- *Leptosphaeria maculans*,
canola pathogen
3- *Laccaria bicolor*
tree root symbionte

Proteogenomics: using proteomics data to validate the annotation of *Leptosphaeria maculans* sequenced genome.





Laccaria bicolor dual lifestyle:
ectomyccorhizal | free-living
host recognition | hyphal prospection



Vincent et al., 2009 Electrophoresis 30(23): 4118-36
Rouxel et al., 2011 Nat. Comm. 2(202):1-10
Vincent et al, 2012 J Prot. Res. 11(1): 157-71

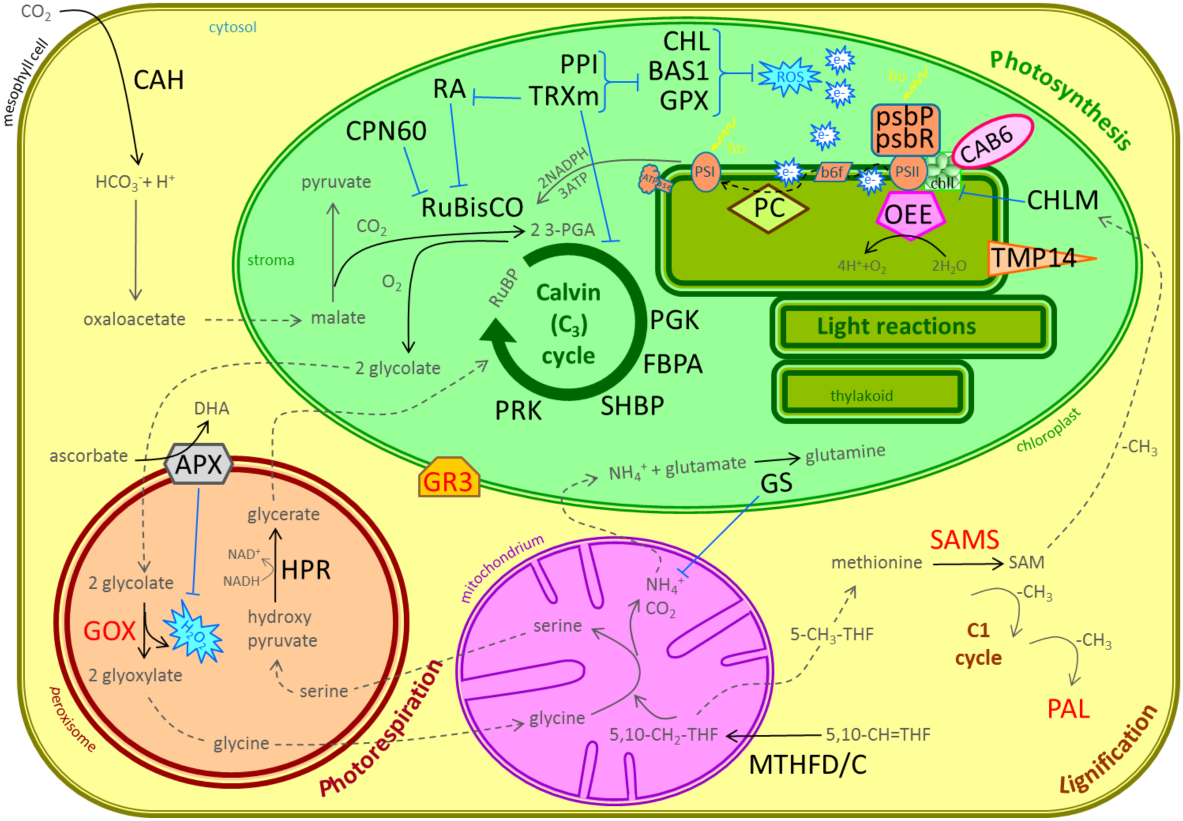
Australian
National
University

2009-2011

Post-doc 4

Staganospora nodorum Effector Toxi

Mode-of-action of SnToxA in wheat leaf



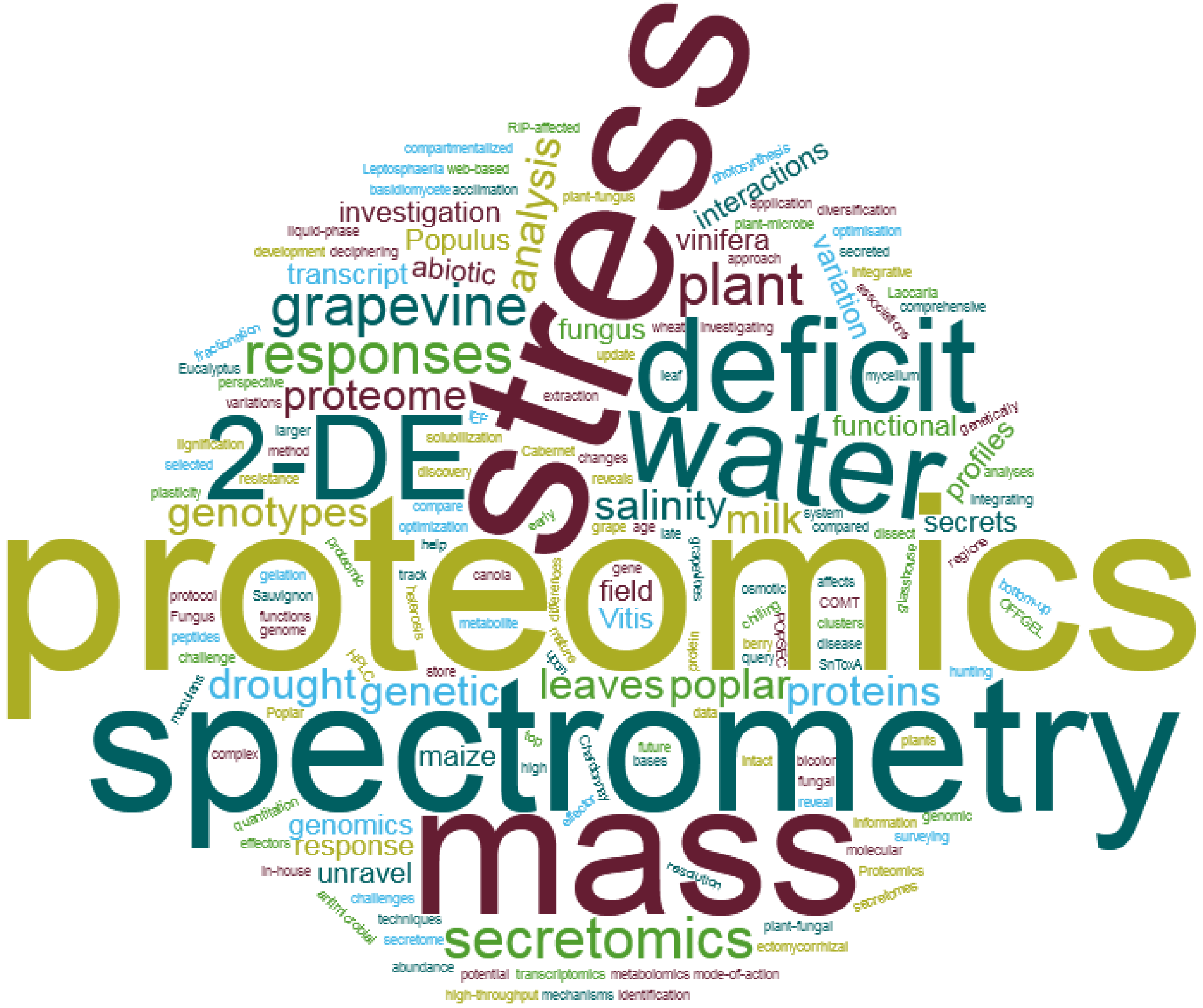
SnToxA manipulates existing host machinery (photosynthesis and TCA cycle) to cause cell death and promote infection.

Vincent & Solomon, 2011 J Int Omics

Vincent et al., 2012 Mol. Plant Pathol.

Breen et al., 2015 Front. Plant Sci.

Do I like this serial post-doctorate life?



2012
Baby break!

2013-now
Senior research Scientist
DPI/DEPI/DEDJTR/AVR Agribio

Mass Spec paradise

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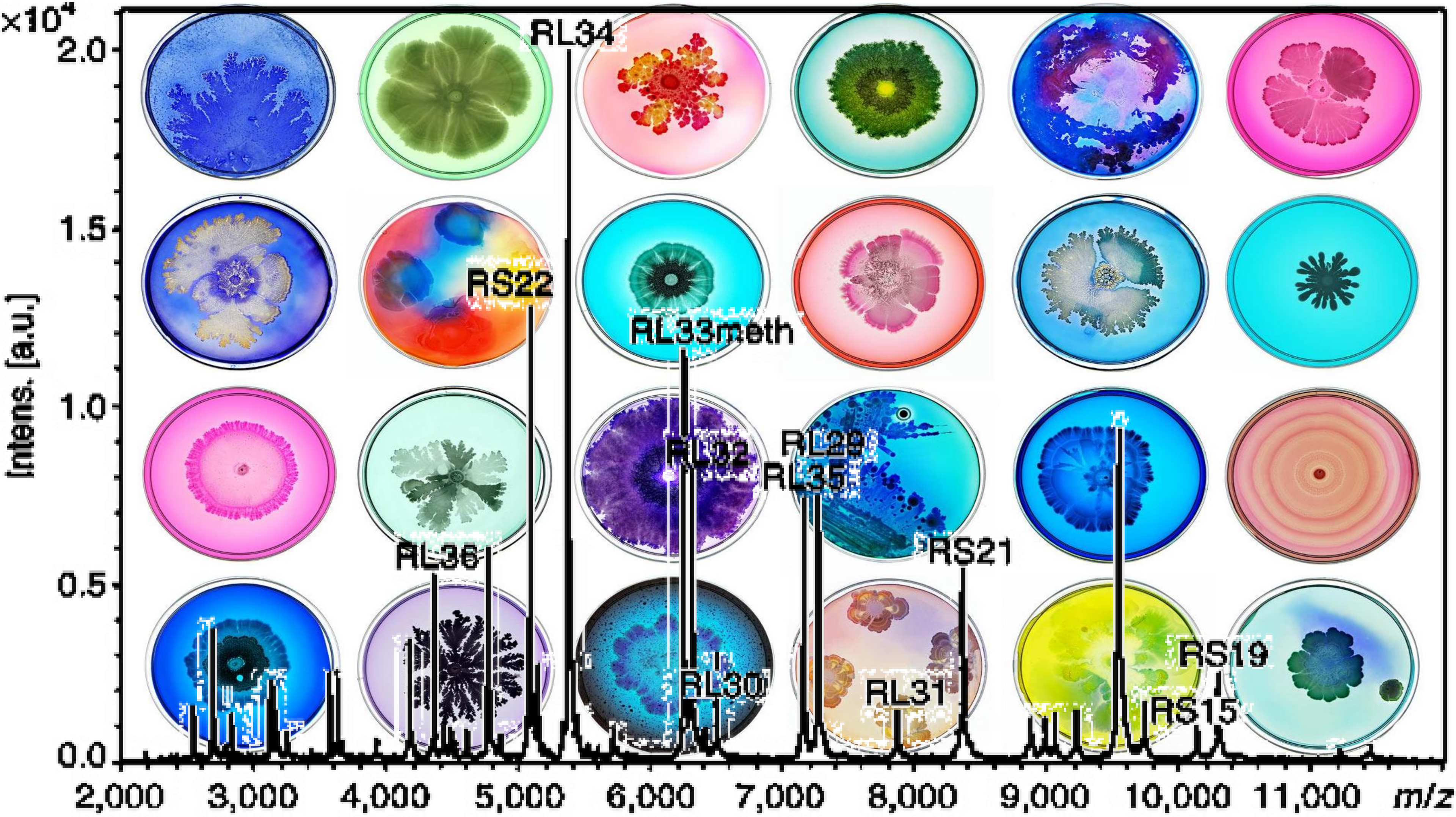
Microbial identification by MALDI-TOF MS



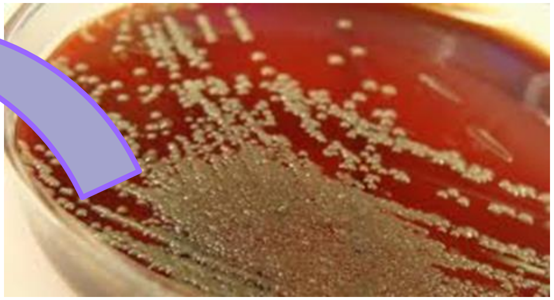
MALDI Biotyper (MBT)

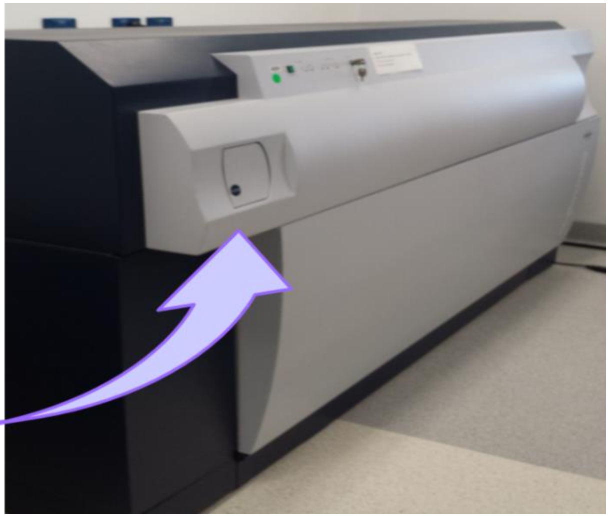
Traditional methods of identifying microorganisms

- Gram stain
- Culture on selective media
- Biochemical tests
- 16S sequencing

Some limitations of traditional methods

- Time consuming
- Labor-intensive
- Expensive





MALDI-Biotyper

+ Robust and Easy
+ Quick and cheap
+ Accurate
-Dependent upon database (mostly animal bacteria)
6000 entries of bacteria and yeasts

MBT principles

The most abundant i

Sinorhizobium melilot

Bacillus subtili

Borrelia burgdorfe

Treponema pallidur

Helicobacter pylo

Escherichia co

Haemophilus influenza

Rickettsia prowazek

Mycoplasma sp

Aquifex aeolicu

Thermatoga maritim

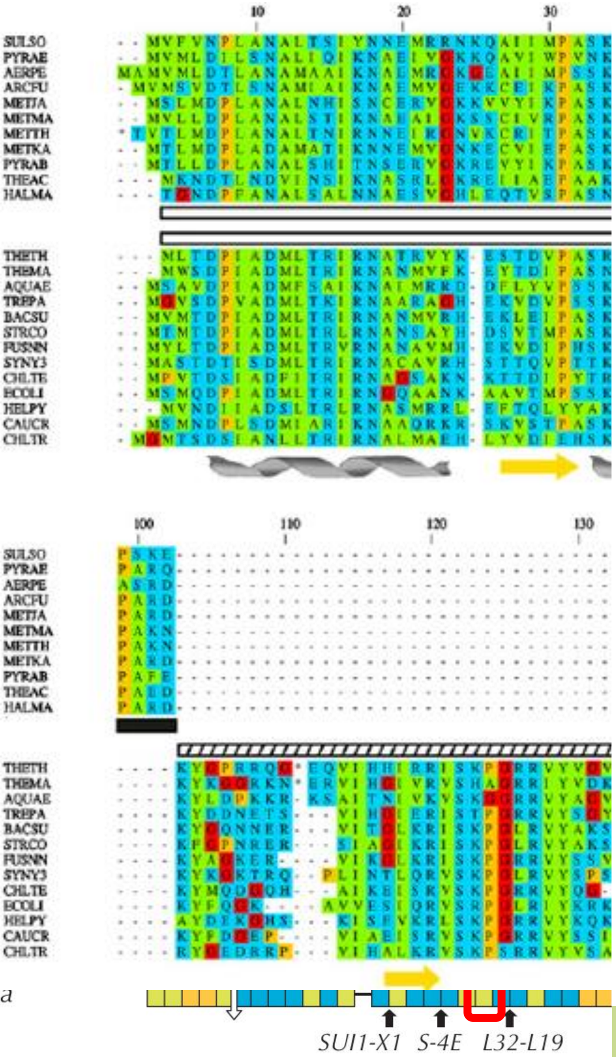
Deinococcus radioduran

Mycobacterium tuberculosi

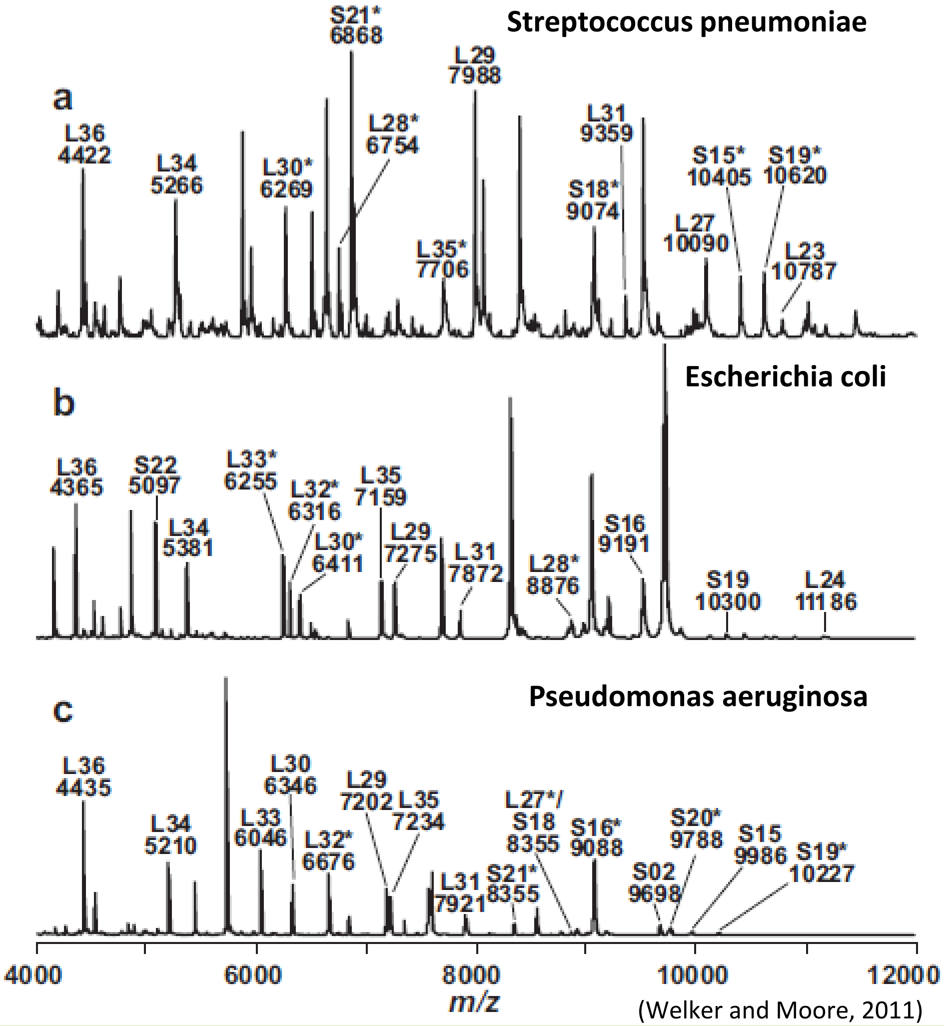
Chlamydia sp

Synechocysti

Archaea



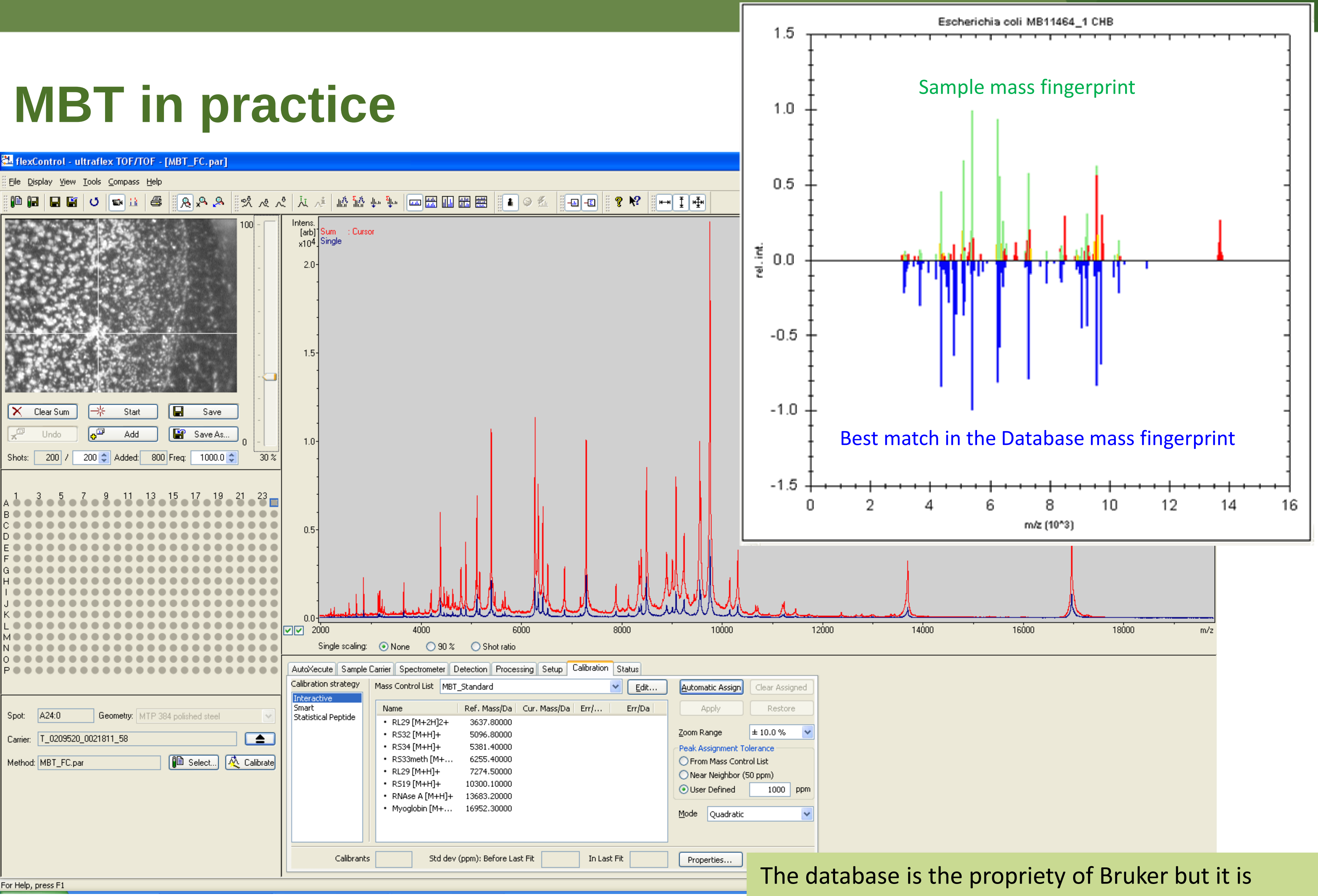
(Barloy-Hubler et al. 2001)



(Welker and Moore, 2011)

Conjointly, these slight AA variations across dozens of major proteins will produce a **unique pattern** (mass fingerprint) for each species.

MBT in practice



Shots: 200 / 200 Added: 800 Freq: 1000.0 30 %

Single scaling: ● None ○ 90 % ○ Shot ratio

Spot: A24:0 Geometry: MTP 384 polished steel

Carrier: T_0209520_0021811_58

Method: MBT_FC.par

Calibration strategy: Interactive, Smart, Statistical Peptide

Mass Control List: MBT_Standard

Name	Ref. Mass/Da	Cur. Mass/Da	Err/...	Err/Da
RL29 [M+2H]2+	3637.80000			
RS32 [M+H]+	5096.80000			
RS34 [M+H]+	5381.40000			
RS33meth [M+...	6255.40000			
RL29 [M+H]+	7274.50000			
RS19 [M+H]+	10300.10000			
RNAse A [M+H]+	13683.20000			
Myoglobin [M+...	16952.30000			

Zoom Range: ± 10.0 %

Peak Assignment Tolerance
○ From Mass Control List
○ Near Neighbor (50 ppm)
● User Defined 1000 ppm

Mode: Quadratic

Calibrants Std dev (ppm): Before Last Fit In Last Fit

For Help, press F1

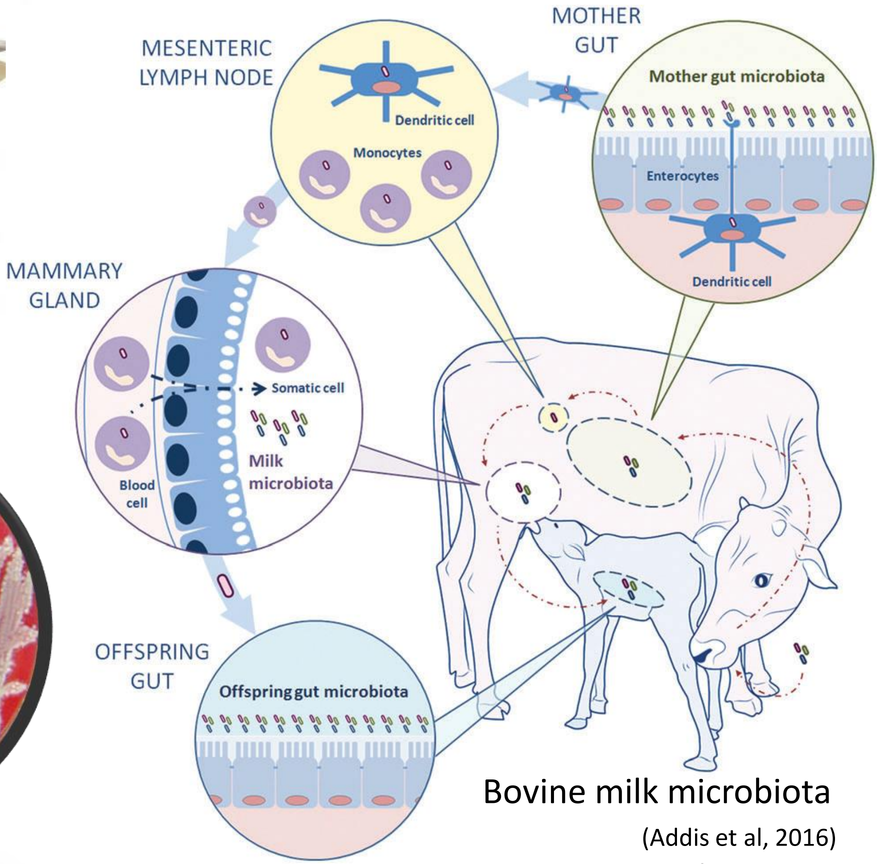
The database is the propriety of Bruker but it is possible to add new entries (plant and soil microbes are under-represented).

Biotyping (proteotyping) of milk microbes

Such technology helps validating metagenomics results but is only applicable to lab-friendly microbes as the 1st step is to grow them.

List of some of the species identified in cow’s milk:

Organism	MBT score
Bacillus licheniformis	2.257
Candida kefyr	2.045
Enterococcus faecium	2.353
Pseudomonas koreensis	2.165
Pseudomonas lundensis	2.086
Pseudomonas rhodesiae	2.053
Pseudomonas tolaasii	2.180
Raoultella terrigena	2.589
Staphylococcus aureus	2.357
Staphylococcus auricularis	2.023
Staphylococcus chromogenes	2.193
Staphylococcus epidermidis	2.246
Staphylococcus haemolyticus	2.111
Staphylococcus hyicus	2.102
Staphylococcus intermedius	2.134
Staphylococcus vitulinus	2.213
Staphylococcus warneri	2.023
Streptococcus agalactiae	2.456
Streptococcus dysgalactiae	2.124
Streptococcus uberis	2.184



Bovine milk microbiota

(Addis et al, 2016)

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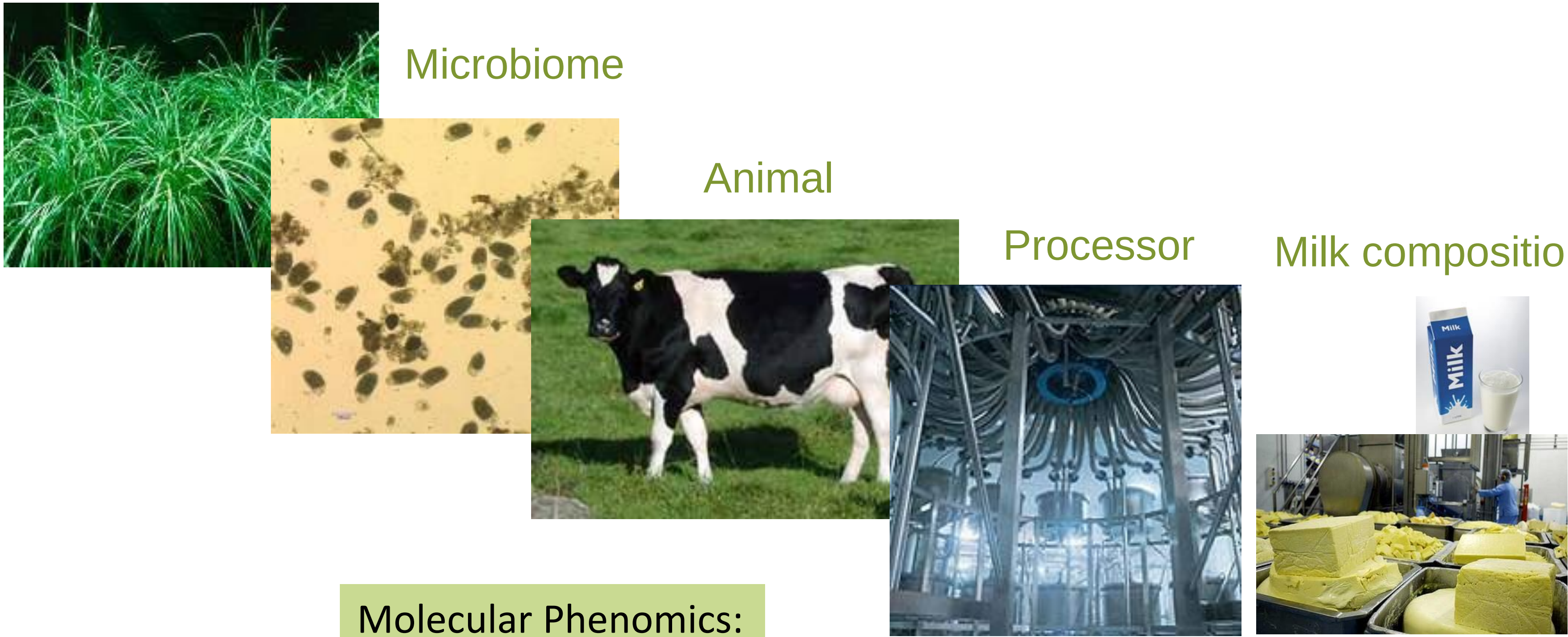
Milk Systems Biology



Feeding diet

Microbiome

Animal

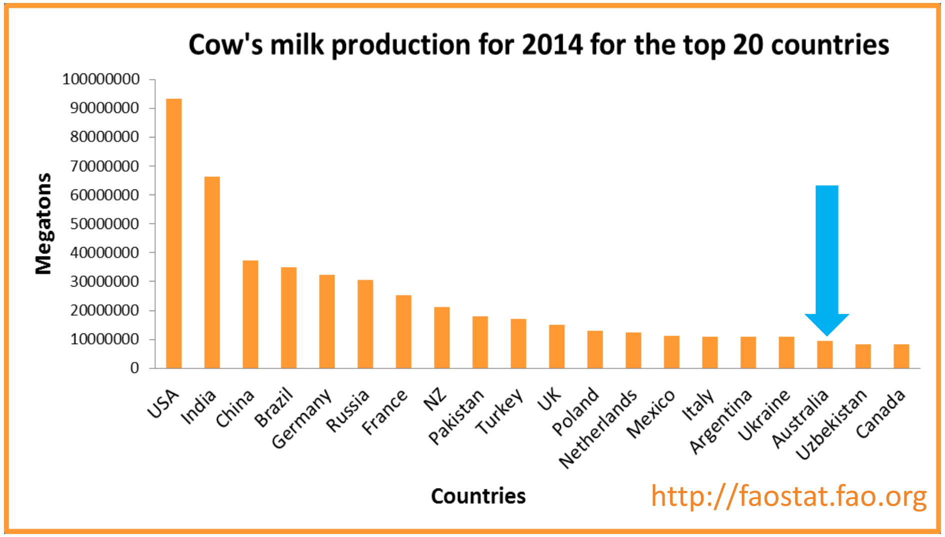
Processor

Milk composition

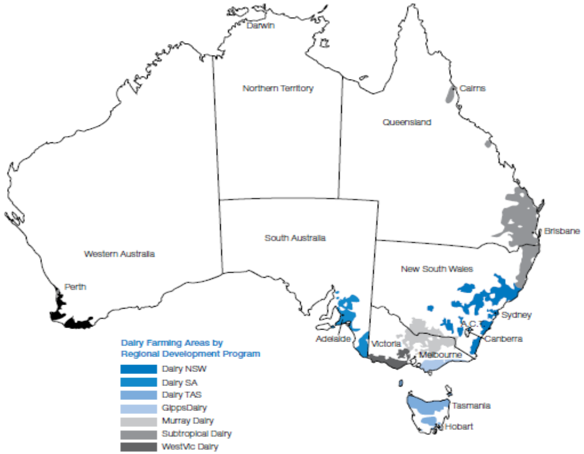
<u>Molecular Phenomics:</u>
Microbes
Proteins
Oligosaccharides
Lipids
Metabolites

Cow’s milk production

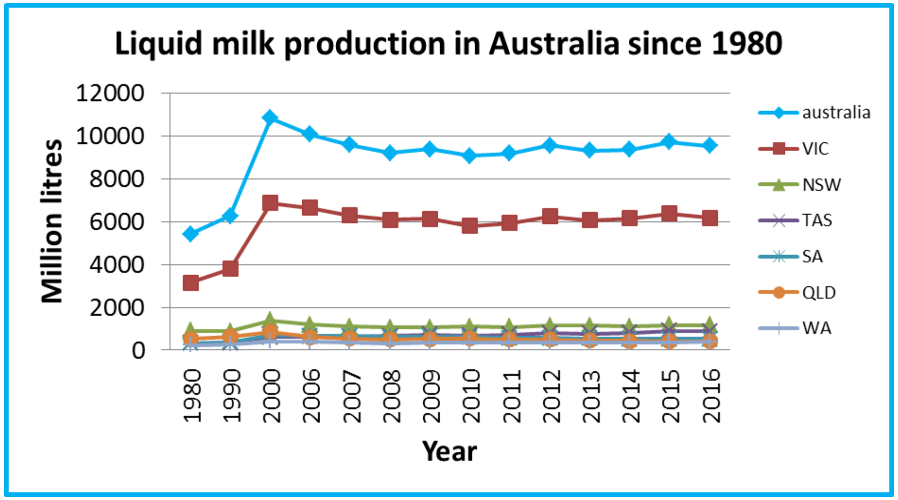
Milk production is concentrated in the temperate zone of Australia. It is strongly seasonal in the key south-eastern dairying regions, reflecting the predominantly pasture-based nature of the industry.


Cow's milk production for 2014 for the top 20 countries

http://faostat.fao.org

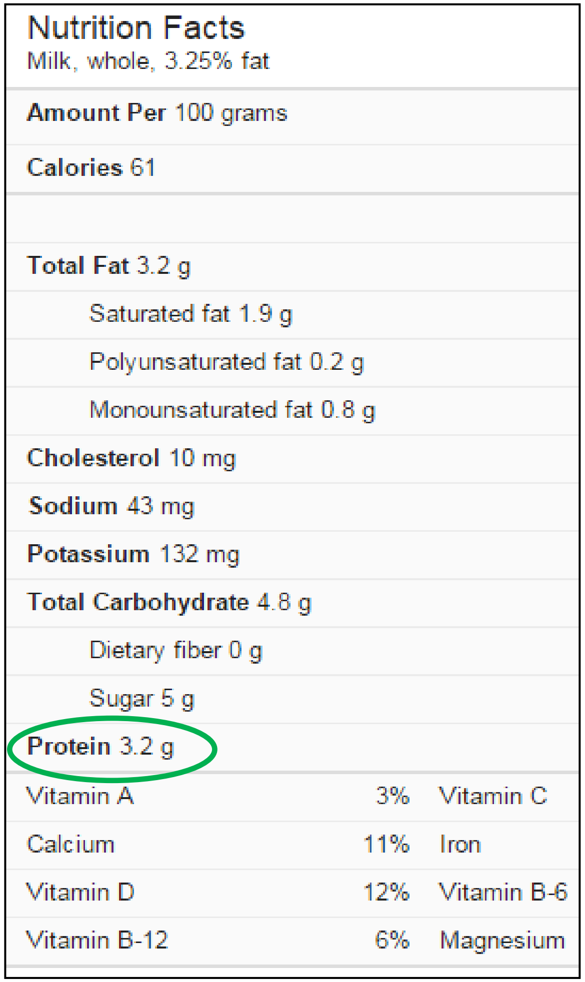


Dairy Australia, 2016


Liquid milk production in Australia since 1980

Milk components

Milk: emulsion of fat globules, a suspension of casein micelles (casein, calcium, phosphorous), suspended in an aqueous phase, with solubilized lactose, whey proteins, and some minerals.

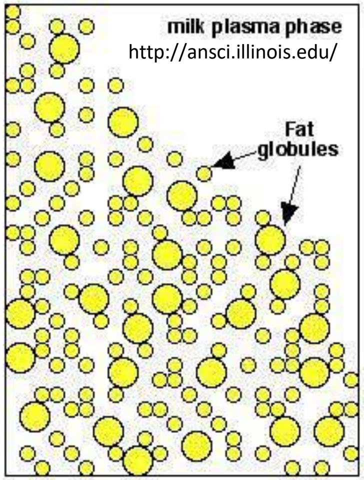


Nutrition Facts
Milk, whole, 3.25% fat

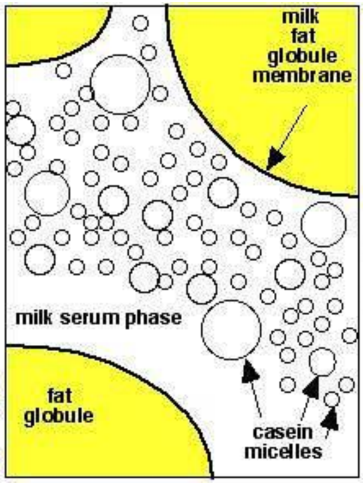
Amount Per 100 grams

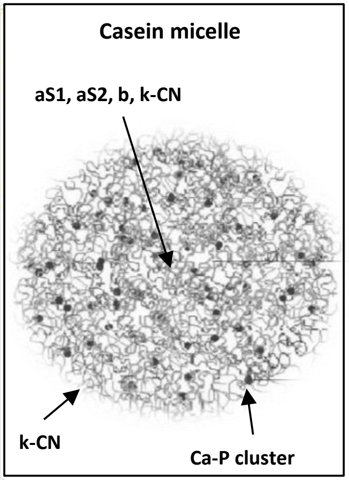
Calories 61

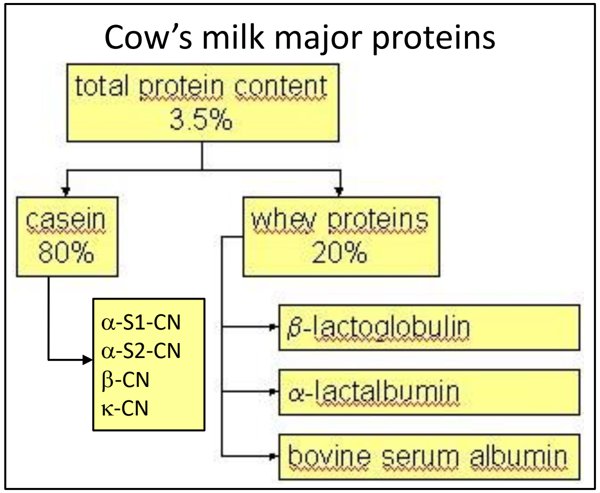
Total Fat 3.2 g			
Saturated fat 1.9 g			
Polyunsaturated fat 0.2 g			
Monounsaturated fat 0.8 g			
Cholesterol 10 mg			
Sodium 43 mg			
Potassium 132 mg			
Total Carbohydrate 4.8 g			
Dietary fiber 0 g			
Sugar 5 g			
Protein 3.2 g			
Vitamin A	3%	Vitamin C	
Calcium	11%	Iron	
Vitamin D	12%	Vitamin B-6	
Vitamin B-12	6%	Magnesium	



http://ansci.illinois.edu/





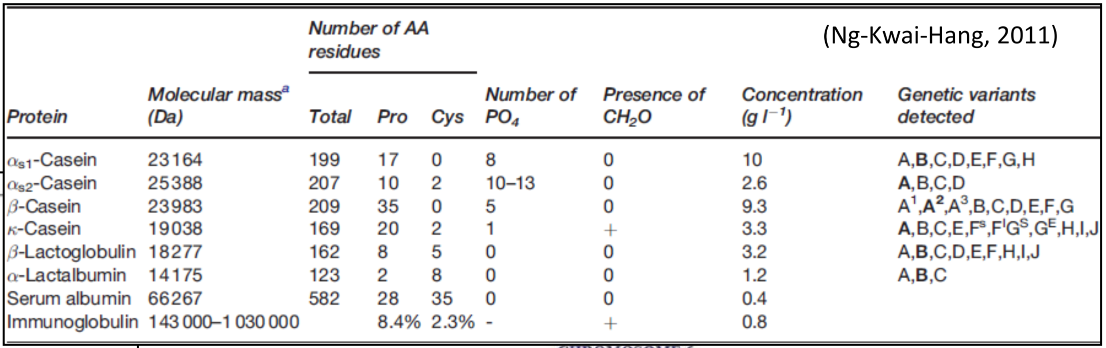


Cow's milk major proteins

Protein	Concentration (g/L)
aS1-CN	10.0
b-CN	9.3
k-CN	3.3
b-LG	3.2
aS2-CN	2.6
a-LA	1.2
g-CN	0.8
Ig	0.8
PP/8F/8S	0.5
BSA	0.4
MFGM	0.4
PP3	0.3
Lactoferrin	0.1
Transferrin	0.1
Total	33.0

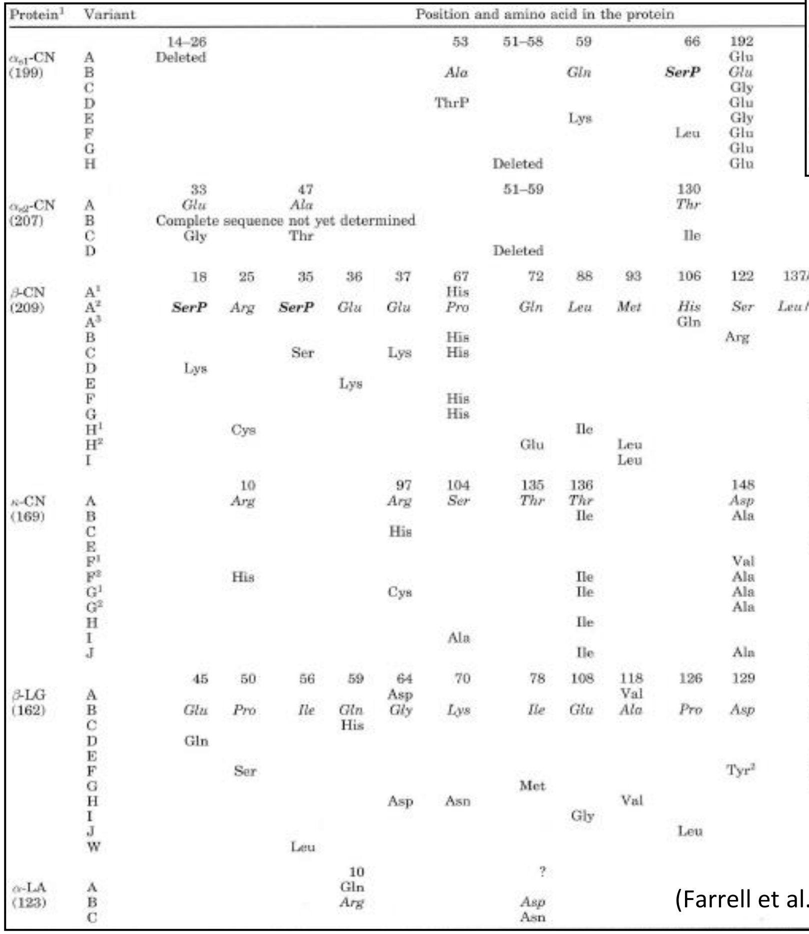
Milk allelic protein variants

Major milk proteins in cattle are encoded by highly polymorphic genes, with up to 47 protein allelic variants identified.



(Ng-Kwai-Hang, 2011)

Protein	Molecular mass[a] (Da)	Number of AA residues: Total	Pro	Cys	Number of PO_4	Presence of CH_2O	Concentration $(g\ l^{-1})$	Genetic variants detected
α_{s1}-Casein	23 164	199	17	0	8	0	10	A,**B**,C,D,E,F,G,H
α_{s2}-Casein	25 388	207	10	2	10–13	0	2.6	**A**,B,C,D
β-Casein	23 983	209	35	0	5	0	9.3	A^1,$\mathbf{A^2}$,A^3,B,C,D,E,F,G
κ-Casein	19 038	169	20	2	1	+	3.3	**A**,B,C,E,F^s,F^IG^S,G^E,H,I,J
β-Lactoglobulin	18 277	162	8	5	0	0	3.2	A,**B**,C,D,E,F,H,I,J
α-Lactalbumin	14 175	123	2	8	0	0	1.2	A,**B**,C
Serum albumin	66 267	582	28	35	0	0	0.4	
Immunoglobulin	143 000–1 030 000		8.4%	2.3%	-	+	0.8	



Position and amino acid in the protein

Protein[1]	Variant	14–26	53	51–58	59	66	192
α_{s1}-CN (199)	A	Deleted					Glu
	B		*Ala*		*Gln*	***SerP***	*Glu*
	C						Gly
	D		ThrP				Glu
	E				Lys		Gly
	F					Leu	Glu
	G						Glu
	H			Deleted			Glu

Protein[1]	Variant	33	47	51–59	130
α_{s2}-CN (207)	A	*Glu*	*Ala*		*Thr*
	B	Complete sequence not yet determined			
	C	Gly	Thr		Ile
	D			Deleted	

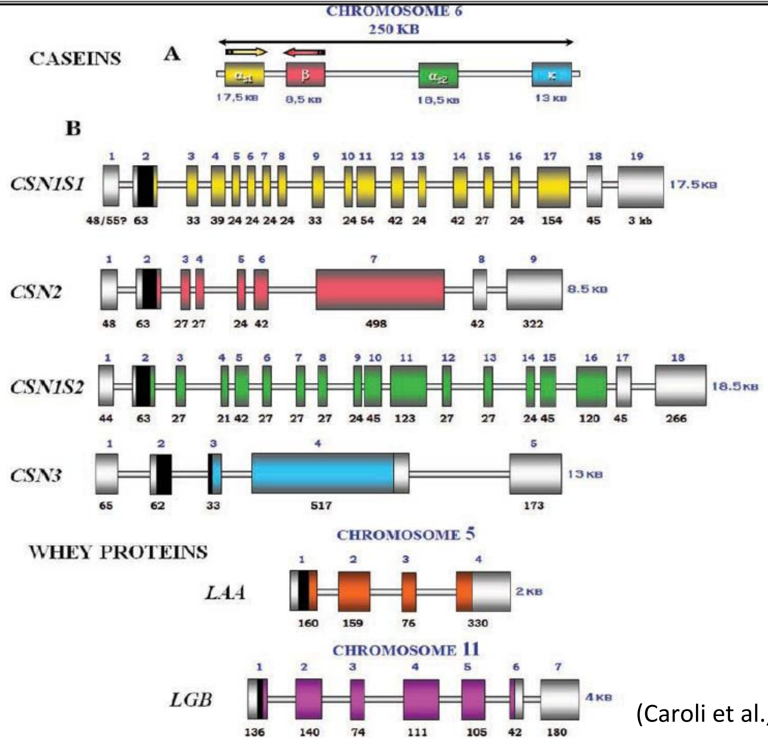
Protein[1]	Variant	18	25	35	36	37	67	72	88	93	106	122	137/138	152	?
β-CN (209)	A^1						His								
	A^2	***SerP***	*Arg*	***SerP***	*Glu*	*Glu*	*Pro*	*Gln*	*Leu*	*Met*	*His*	*Ser*	*Leu/Pro*	*Pro*	*Gln*
	A^3										Gln				
	B						His					Arg			
	C			Ser		Lys	His								
	D	Lys													
	E				Lys										
	F						His							Leu	
	G						His						Leu		
	H^1		Cys						Ile						
	H^2							Glu		Leu					Glu
	I									Leu					

Protein[1]	Variant	10	97	104	135	136	148	155
κ-CN (169)	A	*Arg*	*Arg*	*Ser*	*Thr*	*Thr*	*Asp*	*Ser*
	B					Ile	Ala	
	C		His					
	E							Gly
	F^1						Val	
	F^2	His				Ile	Ala	
	G^1		Cys			Ile	Ala	
	G^2						Ala	
	H					Ile		
	I			Ala				
	J					Ile	Ala	Arg

Protein[1]	Variant	45	50	56	59	64	70	78	108	118	126	129	158
β-LG (162)	A					Asp				Val			
	B	*Glu*	*Pro*	*Ile*	*Gln*	*Gly*	*Lys*	*Ile*	*Glu*	*Ala*	*Pro*	*Asp*	*Glu*
	C				His								
	D	Gln											
	E												Gly
	F		Ser									Tyr^2	Gly
	G							Met					Gly
	H					Asp	Asn			Val			
	I								Gly				
	J										Leu		
	W			Leu									

Protein[1]	Variant	10	?
α-LA (123)	A	Gln	
	B	*Arg*	*Asp*
	C		Asn

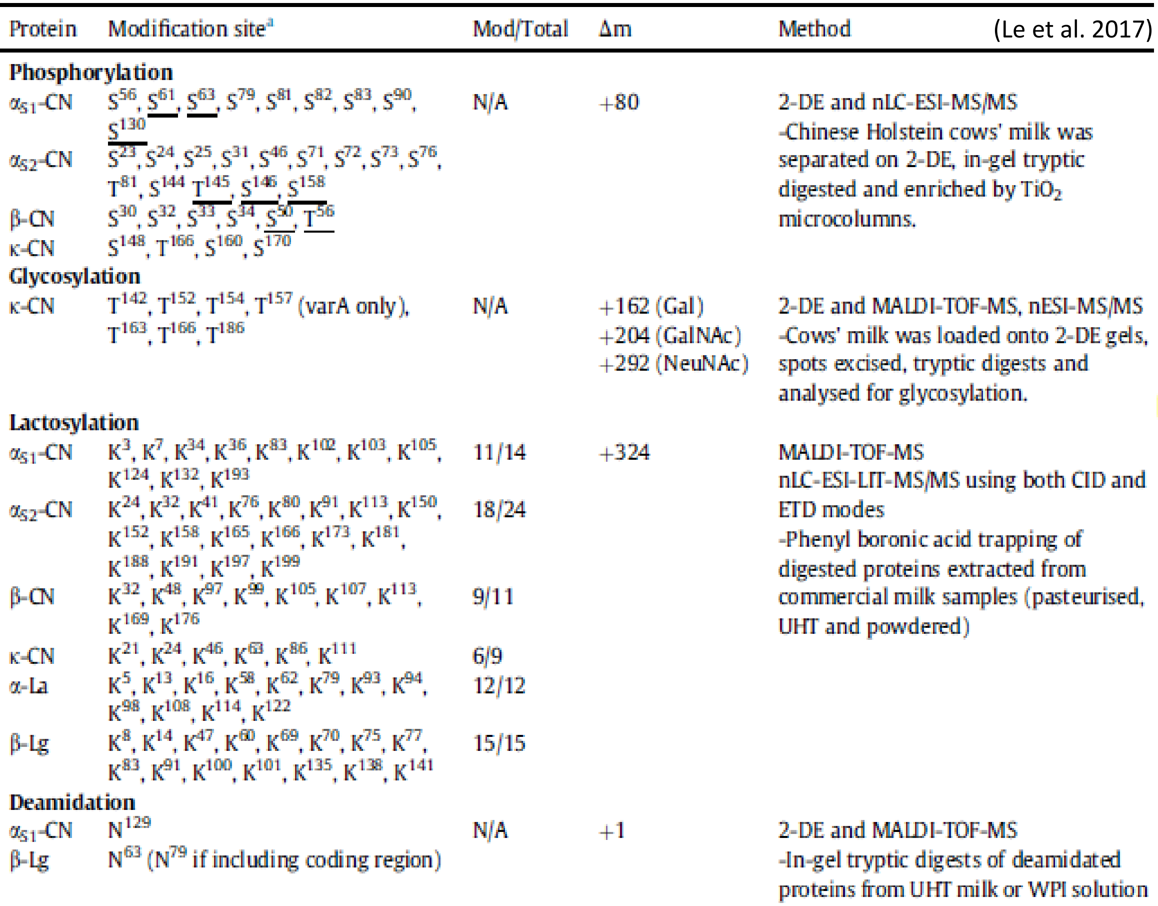
(Farrell et al., 2004)



(Caroli et al., 2011)

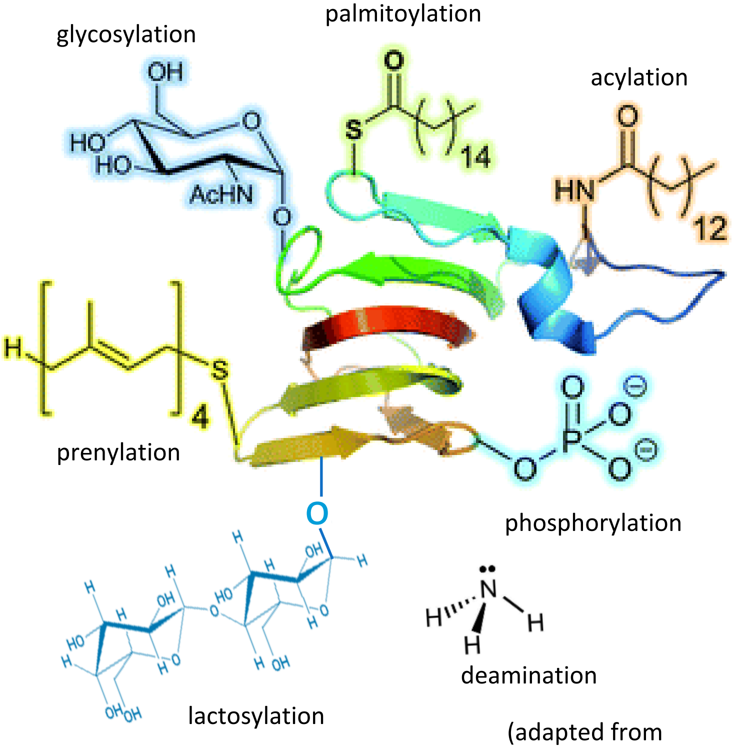
Milk protein post-translational modifications (PTMs)

Milk also contains many proteoforms. PTM is the second pathway after genetic polymorphism by which the milk proteome is expanded. PTMs are critical to the functions of milk proteins (e.g., calcium binding and micelle stability, charge, solubility, susceptibility to proteases, hydrophobicity/hydrophilicity). PTMs are identified by MS. Caseins are phosphorylated and/or glycosylated.



Protein	Modification site[a]	Mod/Total	Δm	Method
Phosphorylation				
α_{S1}-CN	S^{56}, S^{61}, S^{63}, S^{79}, S^{81}, S^{82}, S^{83}, S^{90}, S^{130}	N/A	+80	2-DE and nLC-ESI-MS/MS -Chinese Holstein cows' milk was separated on 2-DE, in-gel tryptic digested and enriched by TiO_2 microcolumns.
α_{S2}-CN	S^{23}, S^{24}, S^{25}, S^{31}, S^{46}, S^{71}, S^{72}, S^{73}, S^{76}, T^{81}, S^{144} T^{145}, S^{146}, S^{158}			
β-CN	S^{30}, S^{32}, S^{33}, S^{34}, S^{50}, T^{56}			
κ-CN	S^{148}, T^{166}, S^{160}, S^{170}			
Glycosylation				
κ-CN	T^{142}, T^{152}, T^{154}, T^{157} (varA only), T^{163}, T^{166}, T^{186}	N/A	+162 (Gal) +204 (GalNAc) +292 (NeuNAc)	2-DE and MALDI-TOF-MS, nESI-MS/MS -Cows' milk was loaded onto 2-DE gels, spots excised, tryptic digests and analysed for glycosylation.
Lactosylation				
α_{S1}-CN	K^{3}, K^{7}, K^{34}, K^{36}, K^{83}, K^{102}, K^{103}, K^{105}, K^{124}, K^{132}, K^{193}	11/14	+324	MALDI-TOF-MS nLC-ESI-LIT-MS/MS using both CID and ETD modes -Phenyl boronic acid trapping of digested proteins extracted from commercial milk samples (pasteurised, UHT and powdered)
α_{S2}-CN	K^{24}, K^{32}, K^{41}, K^{76}, K^{80}, K^{91}, K^{113}, K^{150}, K^{152}, K^{158}, K^{165}, K^{166}, K^{173}, K^{181}, K^{188}, K^{191}, K^{197}, K^{199}	18/24		
β-CN	K^{32}, K^{48}, K^{97}, K^{99}, K^{105}, K^{107}, K^{113}, K^{169}, K^{176}	9/11		
κ-CN	K^{21}, K^{24}, K^{46}, K^{63}, K^{86}, K^{111}	6/9		
α-La	K^{5}, K^{13}, K^{16}, K^{58}, K^{62}, K^{79}, K^{93}, K^{94}, K^{98}, K^{108}, K^{114}, K^{122}	12/12		
β-Lg	K^{8}, K^{14}, K^{47}, K^{60}, K^{69}, K^{70}, K^{75}, K^{77}, K^{83}, K^{91}, K^{100}, K^{101}, K^{135}, K^{138}, K^{141}	15/15		
Deamidation				
α_{S1}-CN	N^{129}	N/A	+1	2-DE and MALDI-TOF-MS -In-gel tryptic digests of deamidated proteins from UHT milk or WPI solution
β-Lg	N^{63} (N^{79} if including coding region)			

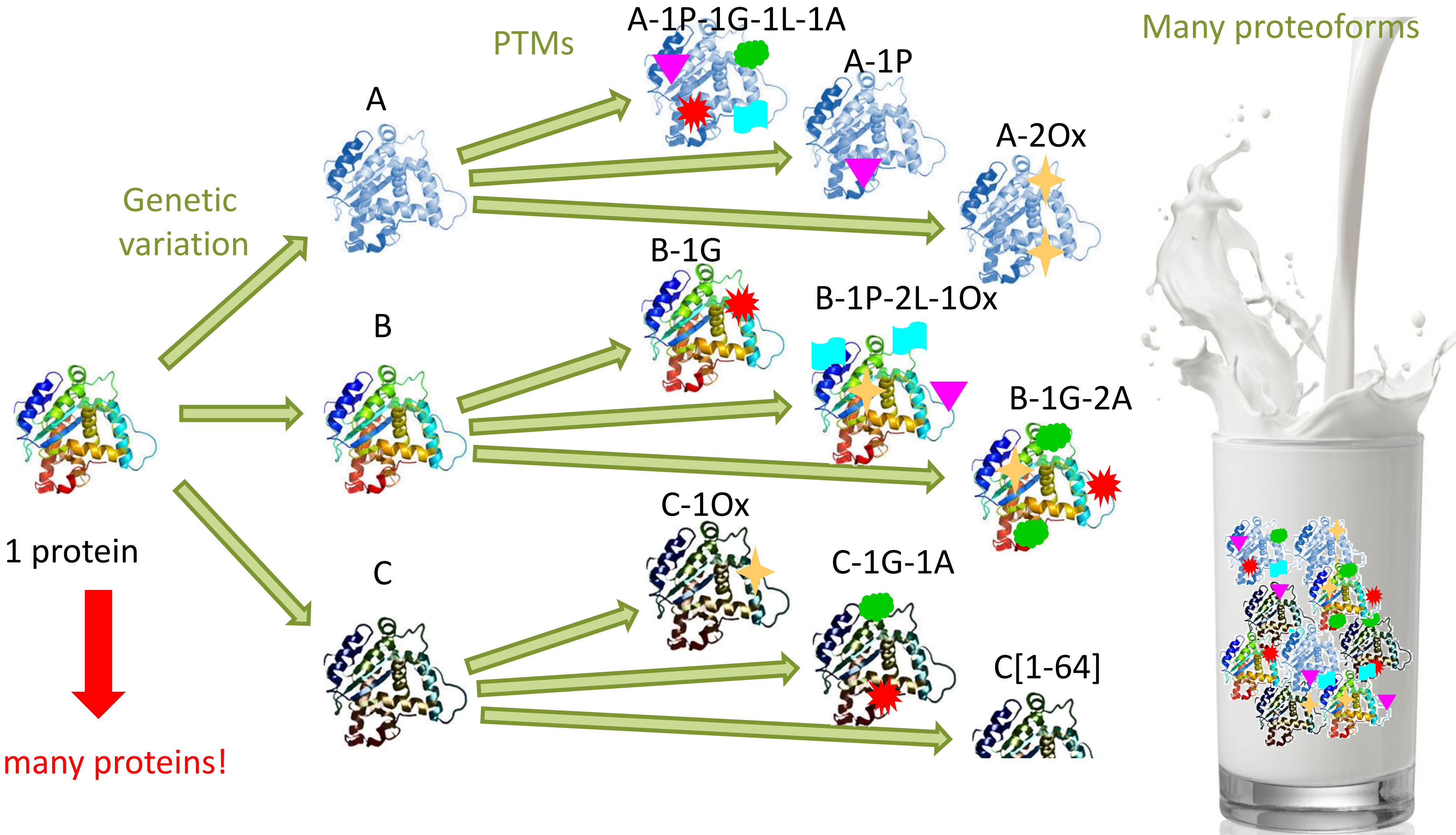
(Le et al. 2017)



(adapted from Heal and Tate, 2010)

Complex milk proteome

An image is worth a thousand words!



1 protein

many proteins!

Genetic variation

A

B

C

PTMs

A-1P-1G-1L-1A

A-1P

A-2Ox

B-1G

B-1P-2L-1Ox

B-1G-2A

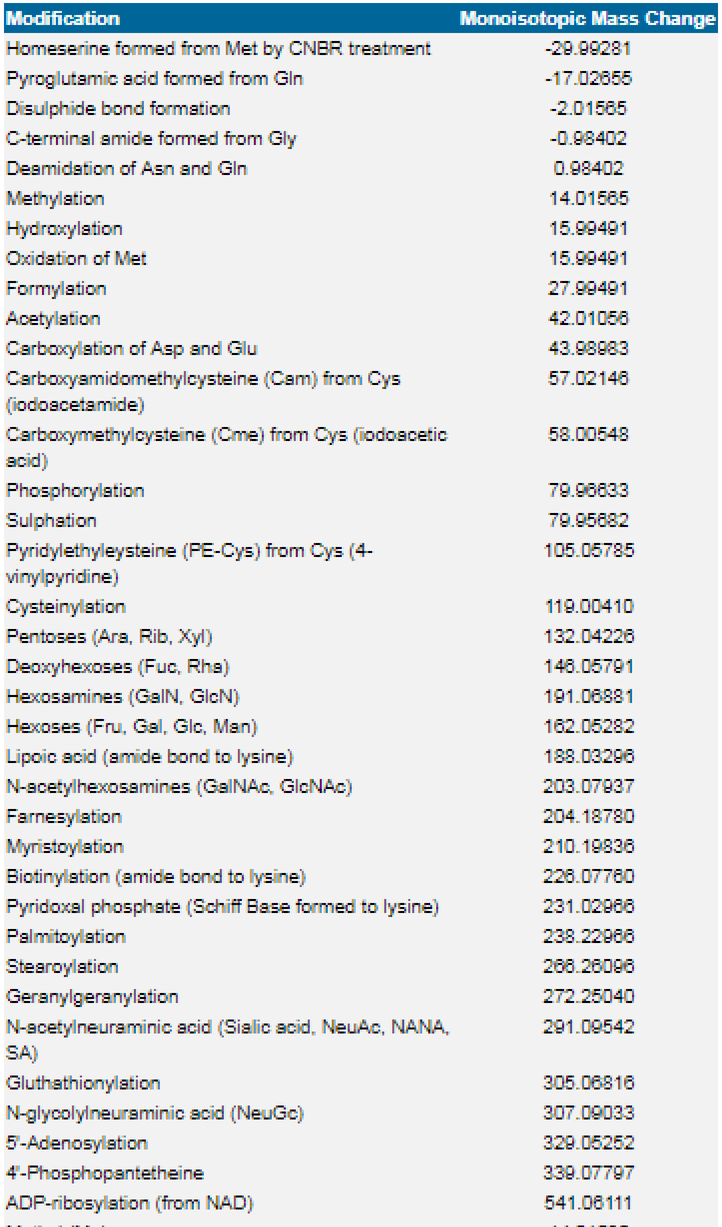
C-1Ox

C-1G-1A

C[1-64]

Many proteoforms

Complex milk proteome

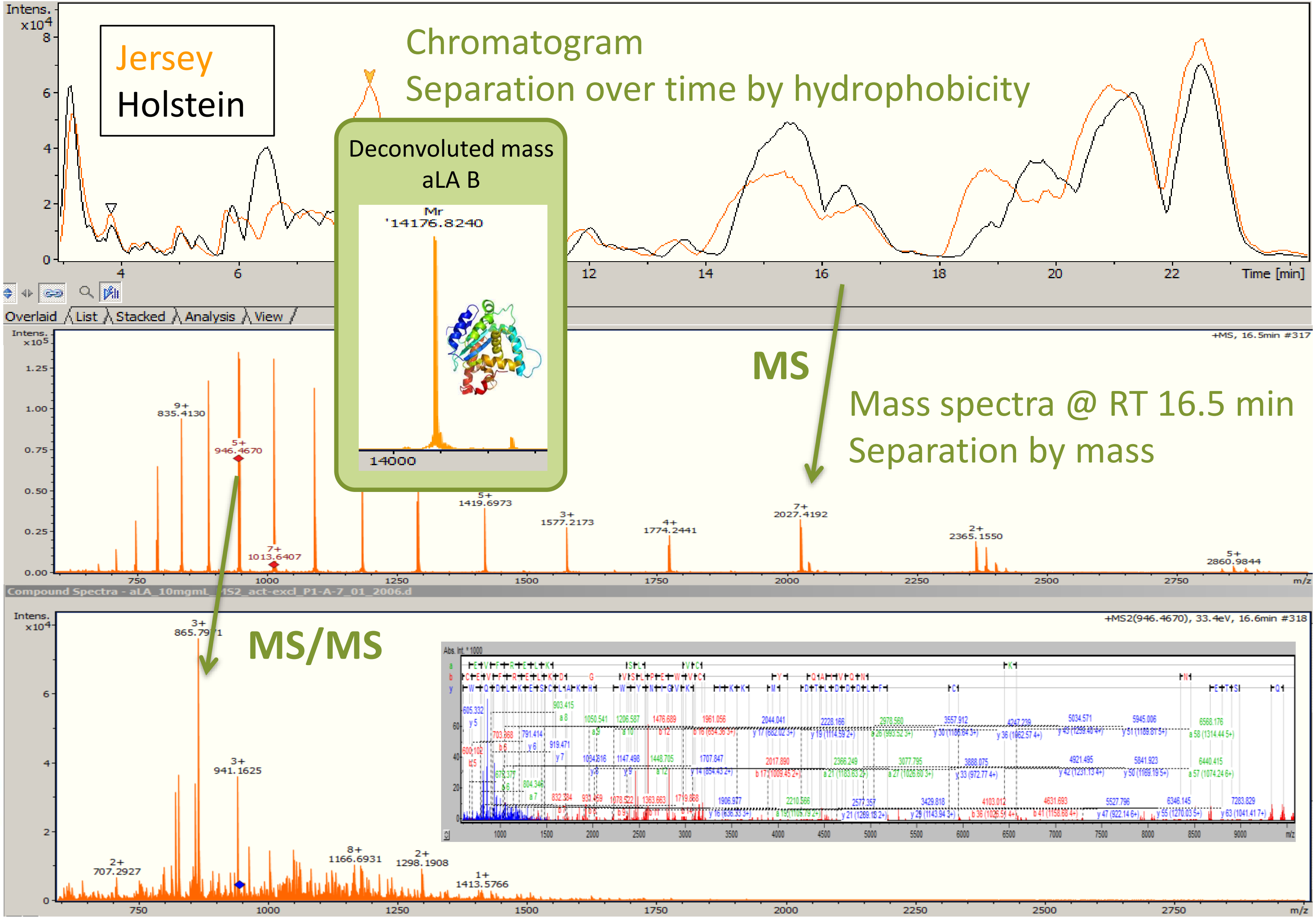


Modification	Monoisotopic Mass Change
Homeserine formed from Met by CNBR treatment	-29.99281
Pyroglutamic acid formed from Gln	-17.02655
Disulphide bond formation	-2.01565
C-terminal amide formed from Gly	-0.98402
Deamidation of Asn and Gln	0.98402
Methylation	14.01565
Hydroxylation	15.99491
Oxidation of Met	15.99491
Formylation	27.99491
Acetylation	42.01056
Carboxylation of Asp and Glu	43.98983
Carboxyamidomethylcysteine (Cam) from Cys (iodoacetamide)	57.02146
Carboxymethylcysteine (Cme) from Cys (iodoacetic acid)	58.00548
Phosphorylation	79.96633
Sulphation	79.95682
Pyridylethyleysteine (PE-Cys) from Cys (4-vinylpyridine)	105.05785
Cysteinylation	119.00410
Pentoses (Ara, Rib, Xyl)	132.04226
Deoxyhexoses (Fuc, Rha)	146.05791
Hexosamines (GalN, GlcN)	191.06881
Hexoses (Fru, Gal, Glc, Man)	162.05282
Lipoic acid (amide bond to lysine)	188.03296
N-acetylhexosamines (GalNAc, GlcNAc)	203.07937
Farnesylation	204.18780
Myristoylation	210.19836
Biotinylation (amide bond to lysine)	226.07760
Pyridoxal phosphate (Schiff Base formed to lysine)	231.02966
Palmitoylation	238.22966
Stearoylation	266.26096
Geranylgeranylation	272.25040
N-acetylneuraminic acid (Sialic acid, NeuAc, NANA, SA)	291.09542
Gluthathionylation	305.06816
N-glycolylneuraminic acid (NeuGc)	307.09033
5'-Adenosylation	329.05252
4'-Phosphopantetheine	339.07797
ADP-ribosylation (from NAD)	541.06111

Protein name	Mo mass	PTM type	Mass shift	Mo mass w/ PTM
aS1CN A	21415.636	8P	639.731	22055.367
aS1CN B	22960.472	8P	639.731	23600.203
aS1CN C	22888.450	8P	639.731	23528.181
aS1CN D	22990.482	8P	639.731	23630.213
aS1CN E	22888.487	8P	639.731	23528.218
aS1CN F	22986.524	8P	639.731	23626.255
aS1CN G	22960.472	8P	639.731	23600.203
aS1CN H	22030.060	8P	639.731	22669.791
aS2CN A	24333.343	10P	799.663	25133.006
aS2CN B	24333.343	10P	799.663	25133.006
aS2CN C	24303.369	10P	799.663	25103.032
aS2CN D	23393.961	10P	799.663	24193.624
bCN A1	23608.317	5P	399.832	24008.149
bCN A2	23568.311	5P	399.832	23968.143
bCN A3	23559.311	5P	399.832	23959.143
bCN B	23677.386	5P	399.832	24077.218
bCN C	23607.370	5P	399.832	24007.202
bCN D	23609.374	5P	399.832	24009.206
bCN E	23567.364	5P	399.832	23967.196
bCN F	23624.349	5P	399.832	24024.181
bCN G	23624.349	5P	399.832	24024.181
bCN H1	23515.219	5P	399.832	23915.051
bCN H2	23552.323	5P	399.832	23952.155
bCN I	23550.355	5P	399.832	23950.187
kCN A	18945.557	pyroGlu+1P	79.966	19025.524
kCN B	18913.604	pyroGlu+1P	79.966	18993.571
kCN C	18926.515	pyroGlu+1P	79.966	19006.482
kCN E	18915.547	pyroGlu+1P	79.966	18995.514
kCN F1	18929.599	pyroGlu+1P	79.966	19009.566
kCN F2	18894.561	pyroGlu+1P	79.966	18974.528
kCN G1	18860.512	pyroGlu+1P	79.966	18940.479
kCN G2	18901.567	pyroGlu+1P	79.966	18981.534
kCN H	18957.593	pyroGlu+1P	79.966	19037.560
kCN I	18929.562	pyroGlu+1P	79.966	19009.529
kCN J	18982.673	pyroGlu+1P	79.966	19062.640
bLG A	18355.446	none	0.000	18355.446
bLG B	18269.409	none	0.000	18269.409
bLG C	18278.409	none	0.000	18278.409
bLG D	18268.425	none	0.000	18268.425
bLG E	18245.424	none	0.000	18245.424
bLG F	18187.367	none	0.000	18187.367
bLG G	18215.344	none	0.000	18215.344
bLG H	18341.393	none	0.000	18341.393
bLG I	18197.388	none	0.000	18197.388
bLG J	18285.440	none	0.000	18285.440
bLG W	18269.409	none	0.000	18269.409
aLA A	14148.756	none	0.000	14148.756
aLA B	14176.798	none	0.000	14176.798
aLA C	14175.814	none	0.000	14175.814

Potentially, thousands of proteoforms

LC-MS and LC-MS/MS analyses of intact milk proteins



Jersey
Holstein

Chromatogram
Separation over time by hydrophobicity

Deconvoluted mass
aLA B

MS

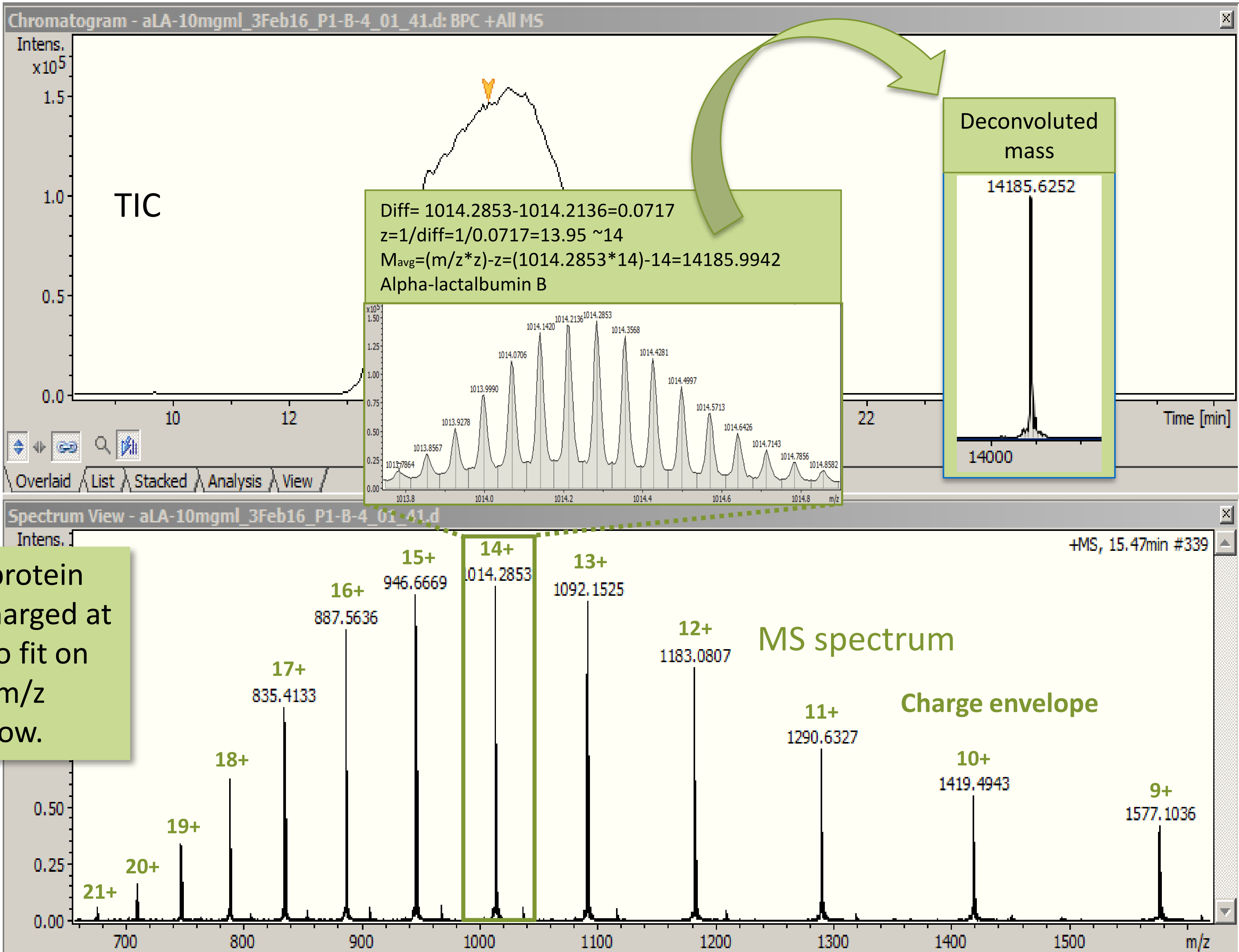
Mass spectra @ RT 16.5 min
Separation by mass

MS/MS

aLA B [1-123] top-down sequencing

EQLTKCEVFRELKDLKGYGGVSLPEWVCTTFHTSGYDTQAIVQNNDSTEYGLFQINNKIWCKDDQNPHSSNICNISCDKFLDDDLTDDIMCVKKILDKVGINYWLAHKALCSEKLDQWLCEKL

Charge state of an intact protein and deconvolution



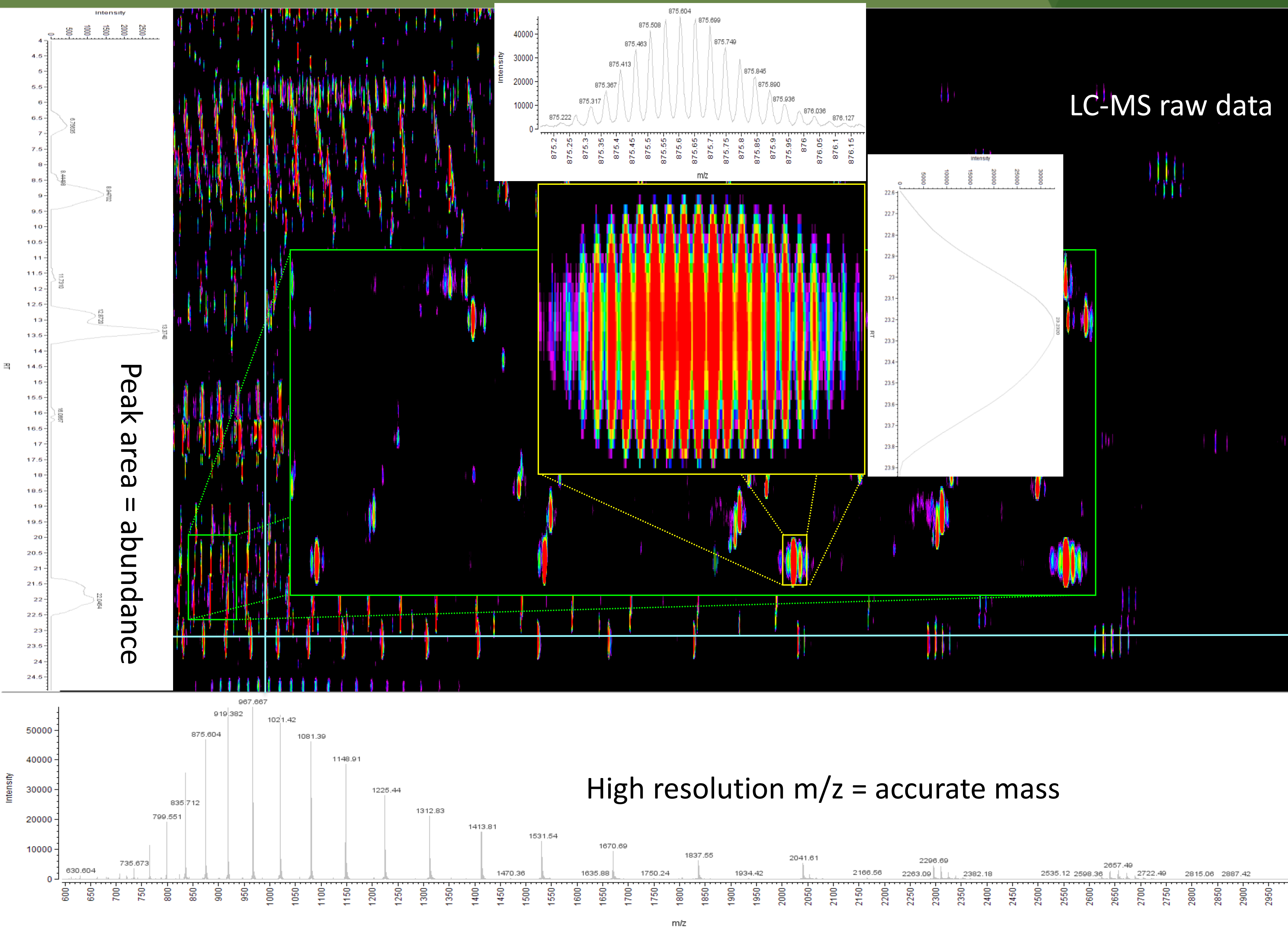
Diff= 1014.2853-1014.2136=0.0717

z=1/diff=1/0.0717=13.95 ~14

M_{avg}=(m/z*z)-z=(1014.2853*14)-14=14185.9942

Alpha-lactalbumin B

This 14185 D protein needs to be charged at least 8 times to fit on the 300-2000 m/z scanning window.



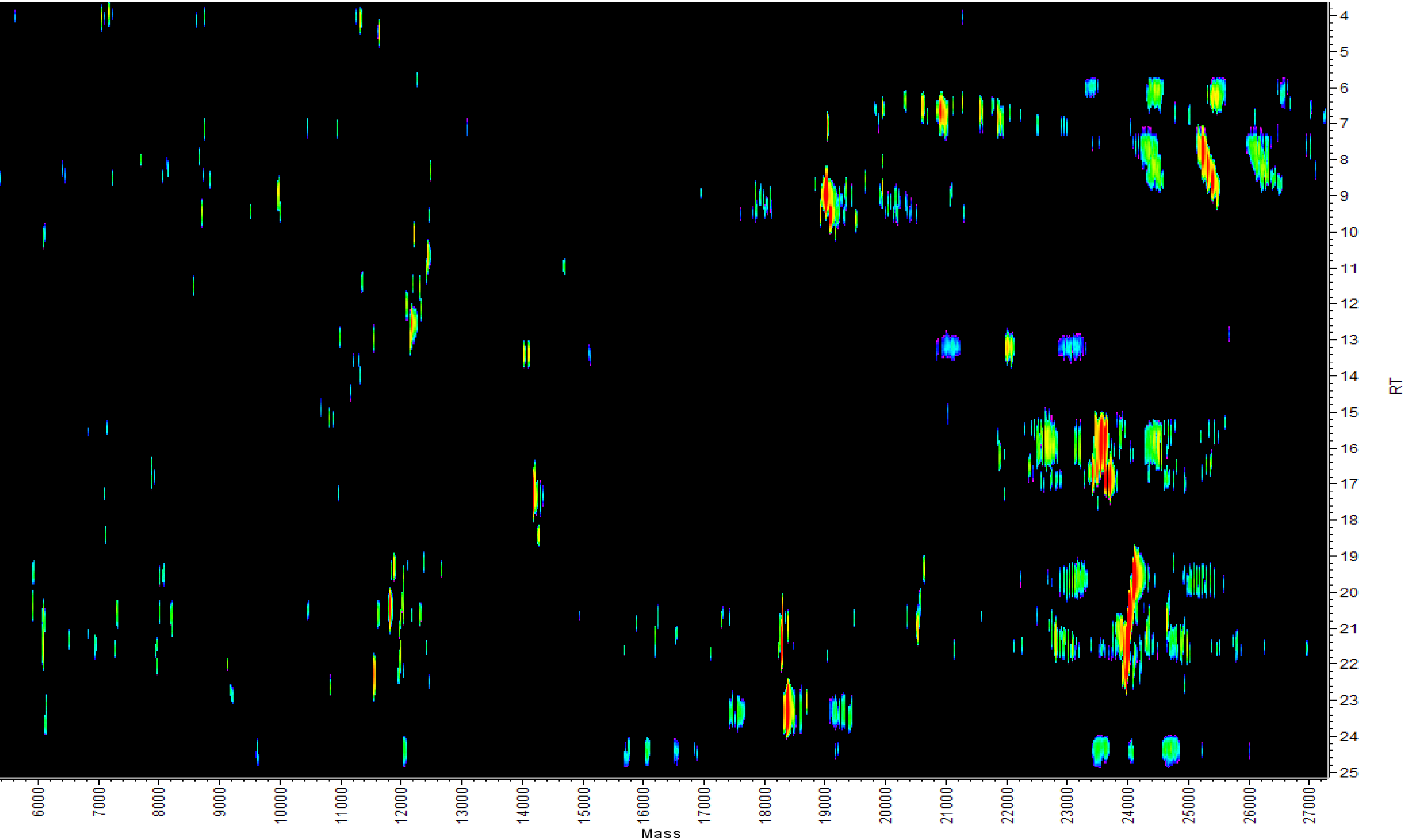
LC-MS raw data

Peak area = abundance

High resolution m/z = accurate mass

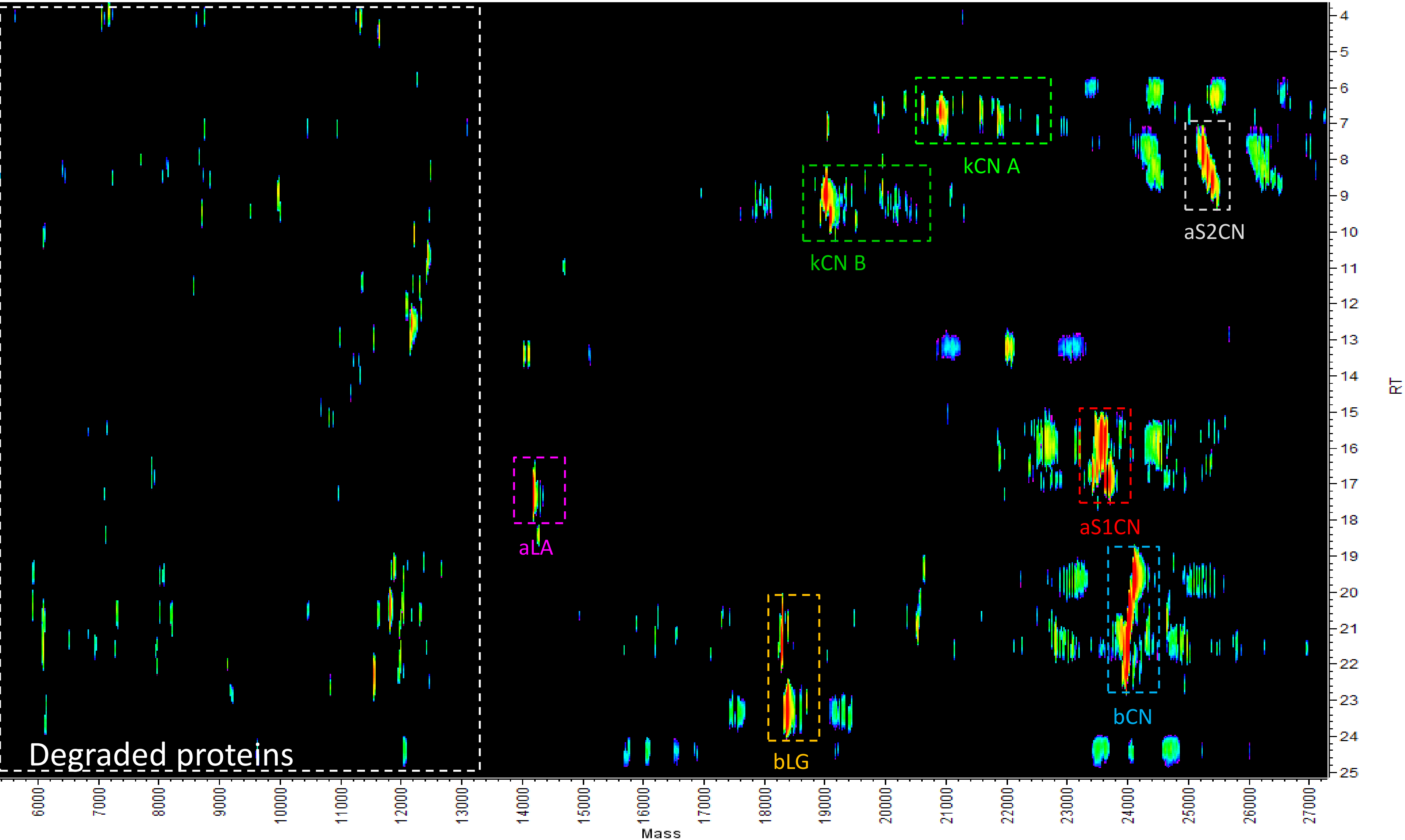
Map of deconvoluted proteins

Known proteoforms of the most abundant milk proteins (caseins and major whey proteins) only account for some of the peaks. MS/MS analyses are needed to identify the other peaks.



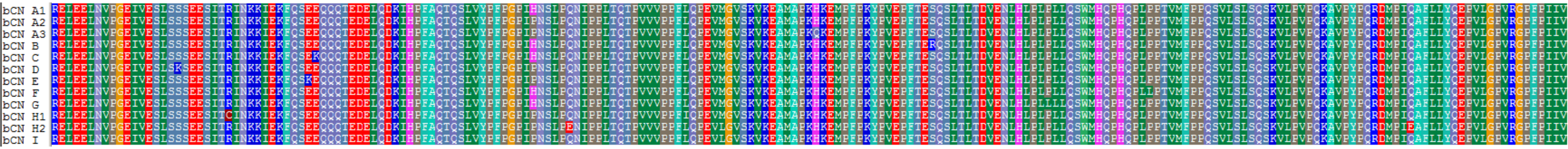
Map of deconvoluted proteins

Known proteoforms of the most abundant milk proteins (caseins and major whey proteins) only account for some of the peaks. MS/MS analyses are needed to identify the other peaks.



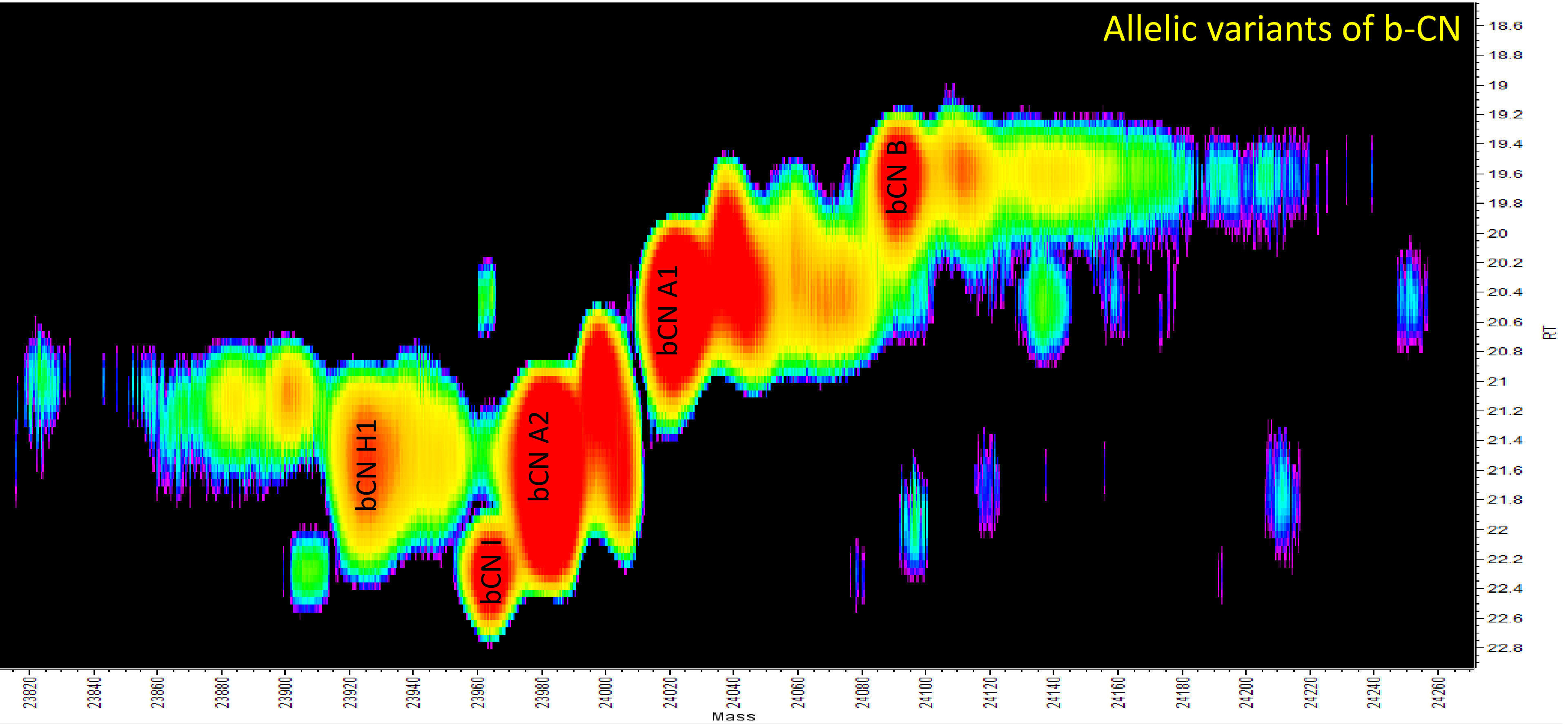
Zoom in on beta-caseins: alleles

There are 12 known allelic variants of bovine beta-caseins, differing by 1 or 2 AA at a time (out of 209).



```
bCN A1 RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIHNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN A2 RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIPNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN A3 RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIPNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKQKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN B  RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIHNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTERQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN C  RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEKQQQTEDELQDKIHPFAQTQSLVYPFPGPIHNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN D  RELEELNVPGEIVESLSKSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIPNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN E  RELEELNVPGEIVESLSSSEESITRINKKIEKFQSKEQQQTEDELQDKIHPFAQTQSLVYPFPGPIPNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN F  RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIHNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLLPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN G  RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIHNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLLLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN H1 RELEELNVPGEIVESLSSSEESITCINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIPNSLPQNIPPLTQTPVVVPPFIQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN H2 RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIPNSLPENIPPLTQTPVVVPPFLQPEVLGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIEAFLLYQEPVLGPVRGPFPIIV
bCN I  RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIPNSLPQNIPPLTQTPVVVPPFLQPEVLGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
```

In this example, 5 allelic variants of beta-caseins could be identified: A1, A2, B, H1 and I.

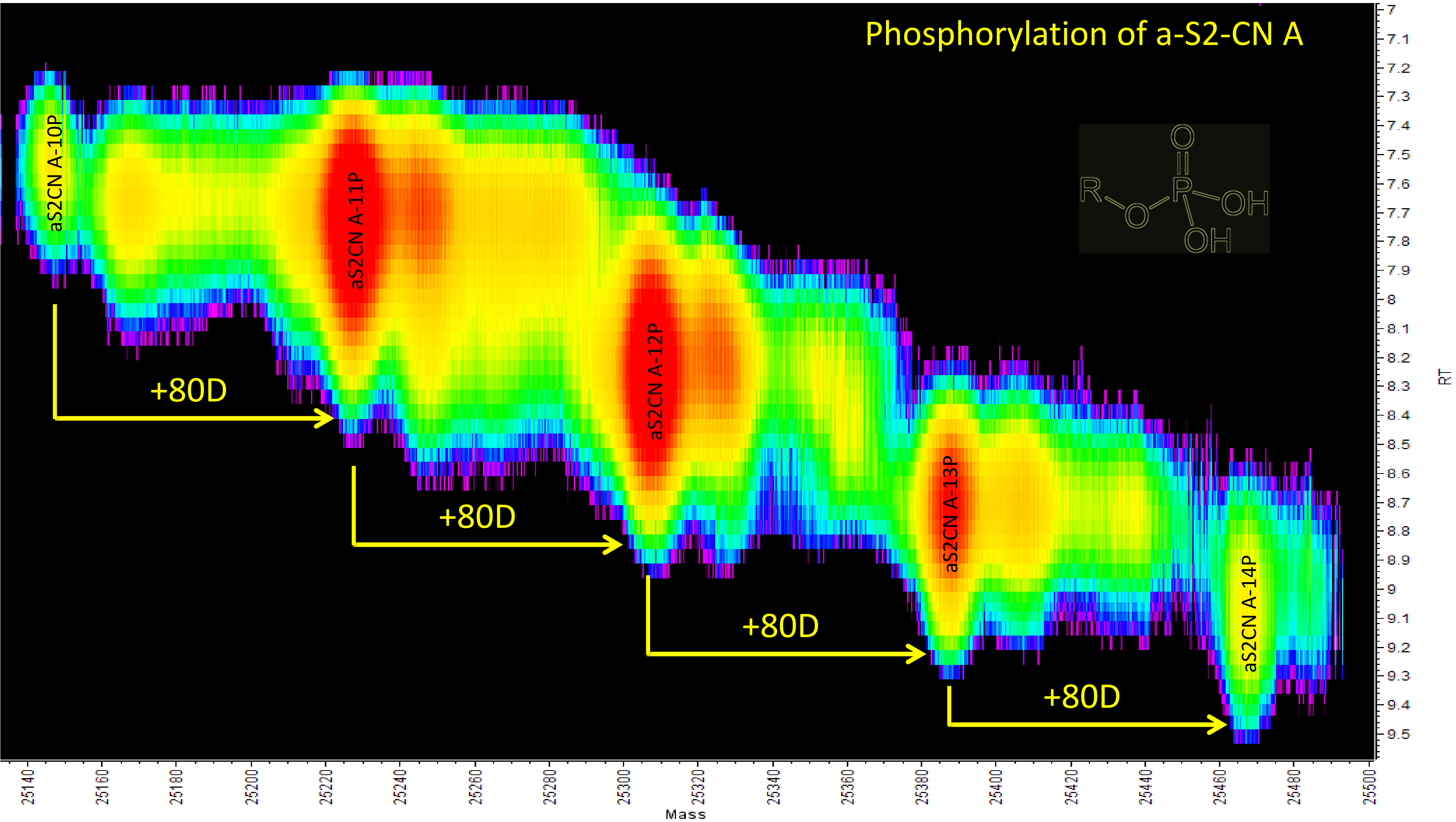


Zoom in on alpha-S2-caseins: PTMs

Alpha-S2-caseins contain up to 14 phosphate groups attached to Serine residues (S), with the prominent proteoforms containing 11 or 12 phosphate groups.

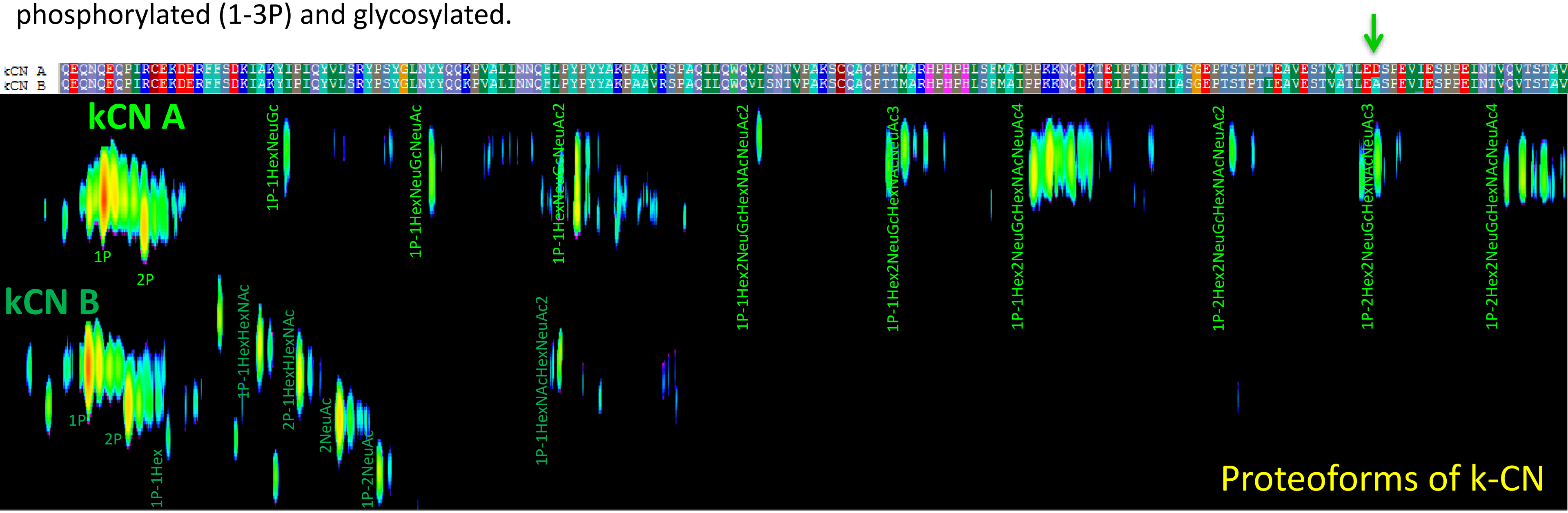
8-10 13 31 55-57 61 143

aS2CN A-11P [1-207] KNTMEHVSSSEESIISQETYKQEKNMAINPSKENLCSTFCKEVVRNANEEEYSIGSSSEESAEVATEEVKITVDDKHYQKALNEINQFYQKFPQYLQYLYQGPIVLNPWDQVKRNAVPITPTLNREQLSTSEENSKKTVDMESTEVFTKKTKLTEEEKNRLNFLKKISQRYQKFALPQYLKTVYQHQKAMKPWIQPKTKVIPYVRYL



Zoom in on kappa-caseins: alleles and PTMs

There are 12 known allelic variants of kappa-caseins, the most prominent ones being variants A and B. Kappa-proteins are phosphorylated (1-3P) and glycosylated.

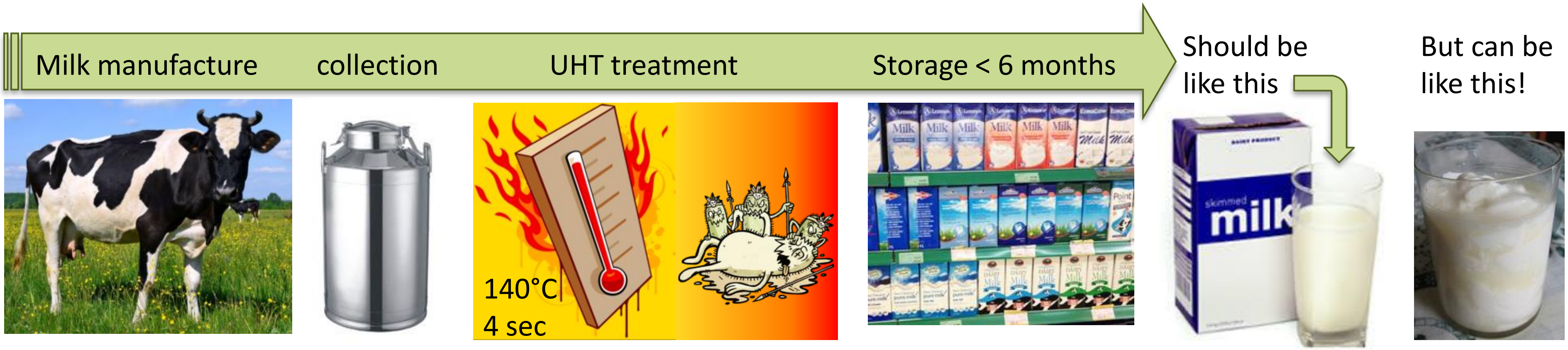


Proteoforms of k-CN

Case study: storage of UHT milk

(next slides…)

UHT principle: making milk safe to drink

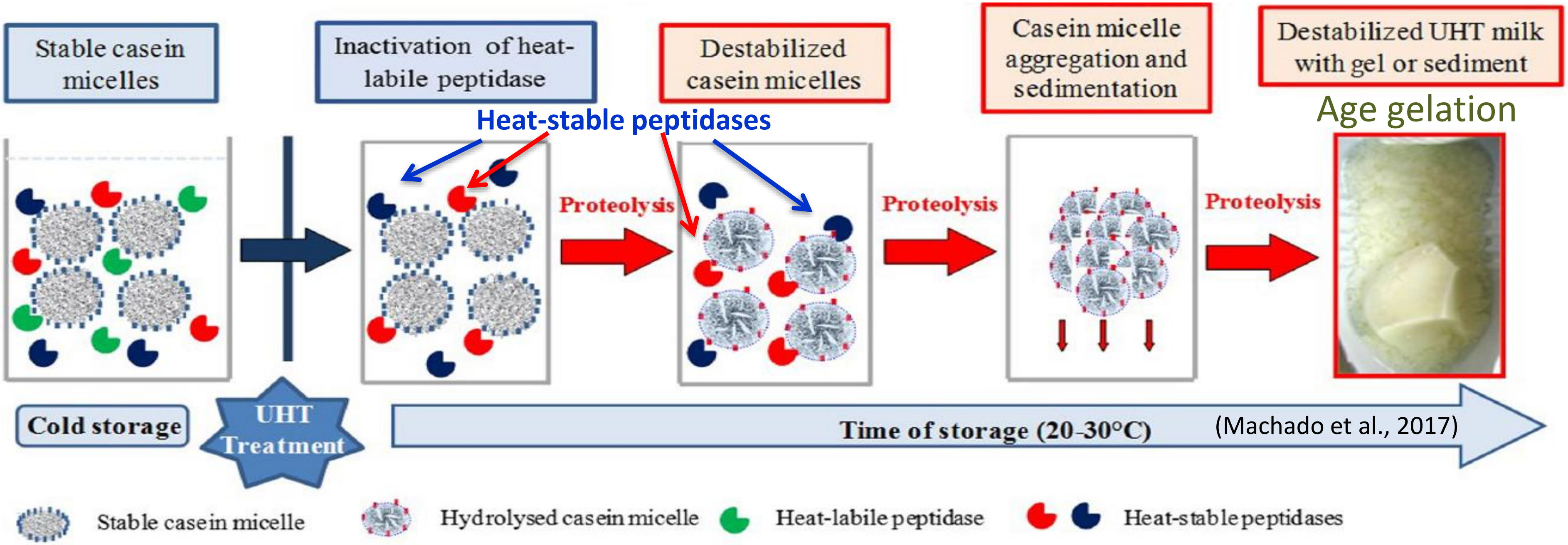


Milk manufacture → collection → UHT treatment → Storage < 6 months

Should be like this

But can be like this!

WHY?



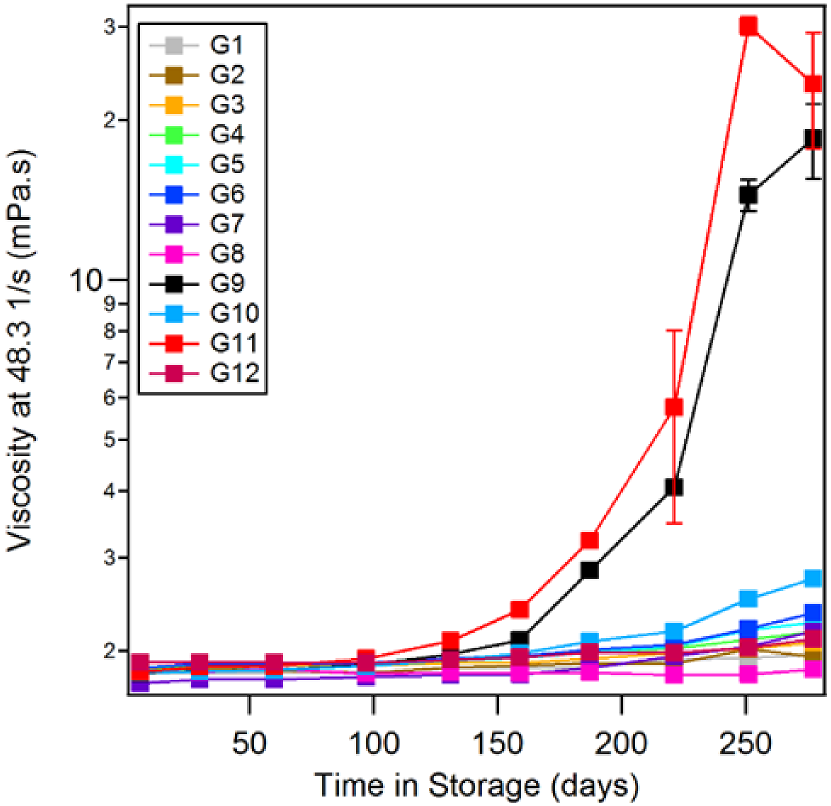
Age gelation

(Machado et al., 2017)

Study rationale: can phenotype improve UHT milk shelf-life?

Cows genotyped based on their major protein variants and grouped accordingly. The 12 groups of milk were UHT-treated and stored for 9 months at room temperature. Physical, metagenomics and top-down proteomics analyses were performed.

Group	k-CN	b-CN	b-LG
G1	**AA**	**A1A1**	**AB**
G2		**A1A1**	**BB**
G3		**A1A2**	**AB**
G4		**A1A2**	**BB**
G5		**A2A2**	**AB**
G6		**A2A2**	**BB**
G7	**AB**	**A1A1**	**AB**
G8		**A1A1**	**BB**
G9		**A1A2**	**AB**
G10		**A1A2**	**BB**
G11		**A2A2**	**AB**
G12		**A2A2**	**BB**

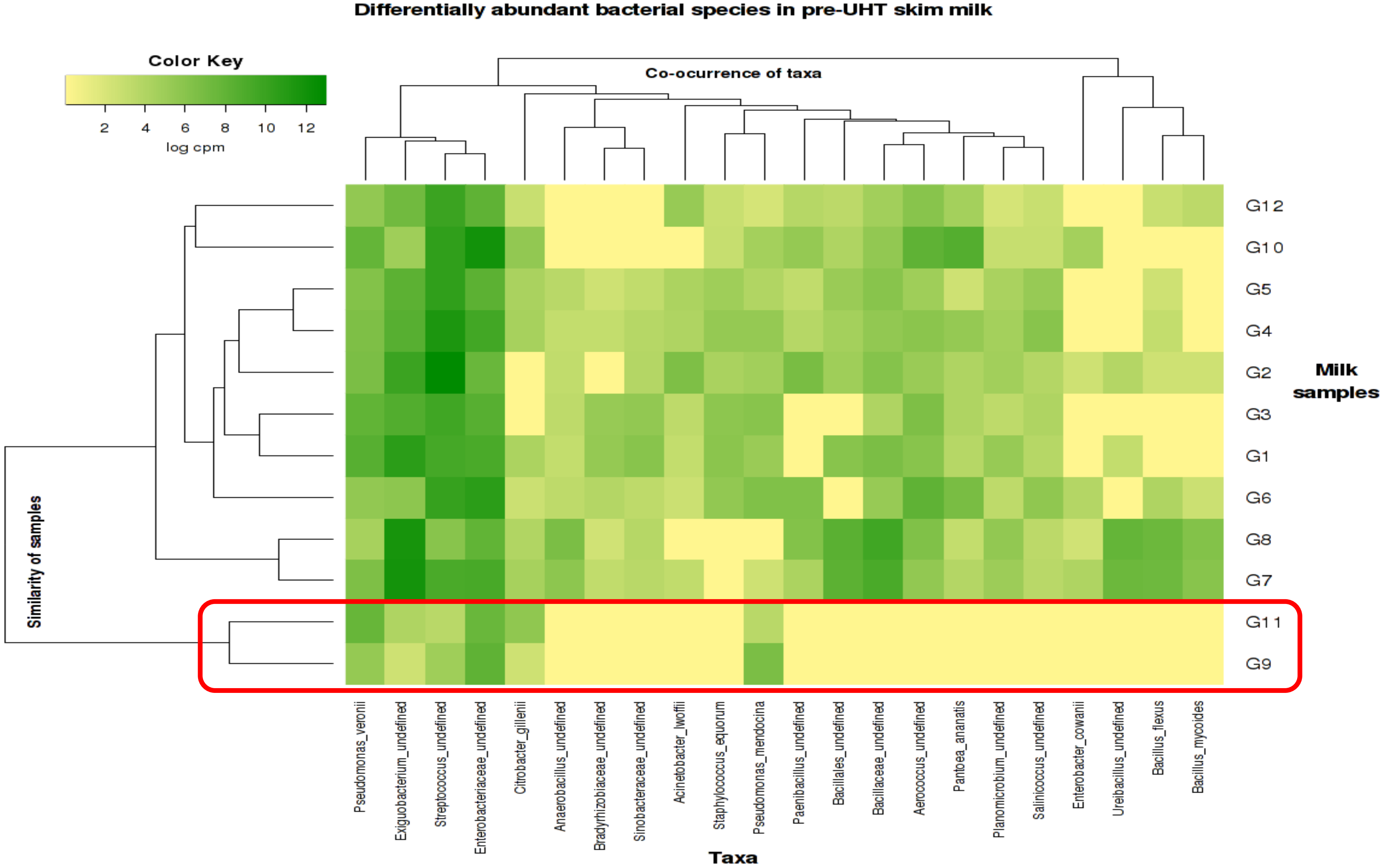


Milk viscosity in group 9 (kCN AB bCN A1A2 bLG AB) and group 11 (kCN AB bCN A2A2 bLG AB) increases from 6 months onwards.

They are the only groups displaying age gelation.

UHT Study: metagenomics

Keith Savin, heatmap, R 3.4.0, June 2017

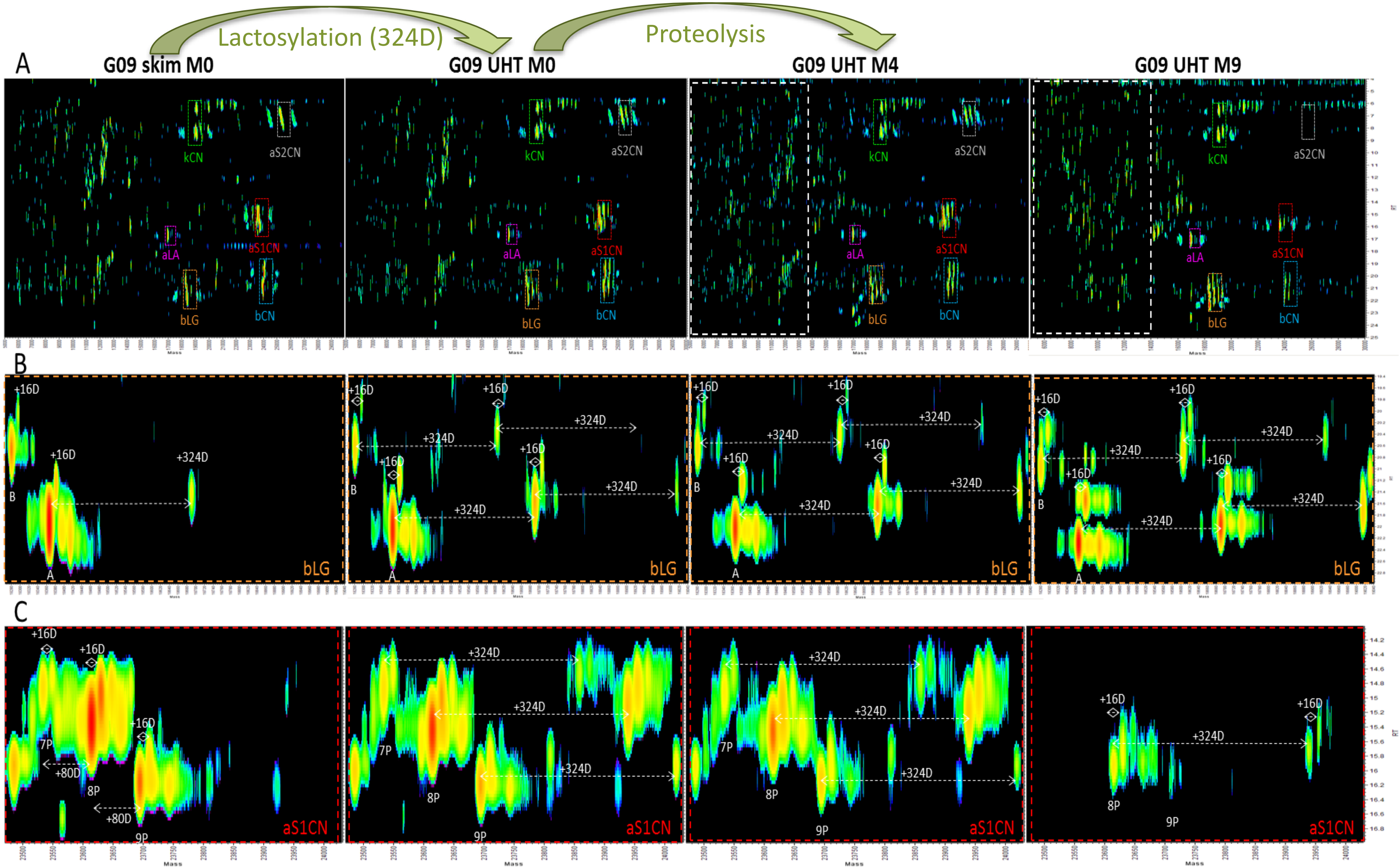


Differentially abundant bacterial species in pre-UHT skim milk

Groups 9 and 11 contain very similar types of bacteria and differ from the other 10 groups by lacking Pantoea ananatis, a Gammaproteobacterium and 4 members of the Bacillus genus (Keith Savin).

UHT Study: top-down proteomics – 2-D maps

Maps of deconvoluted proteins in skim milk and UHT milk over time.

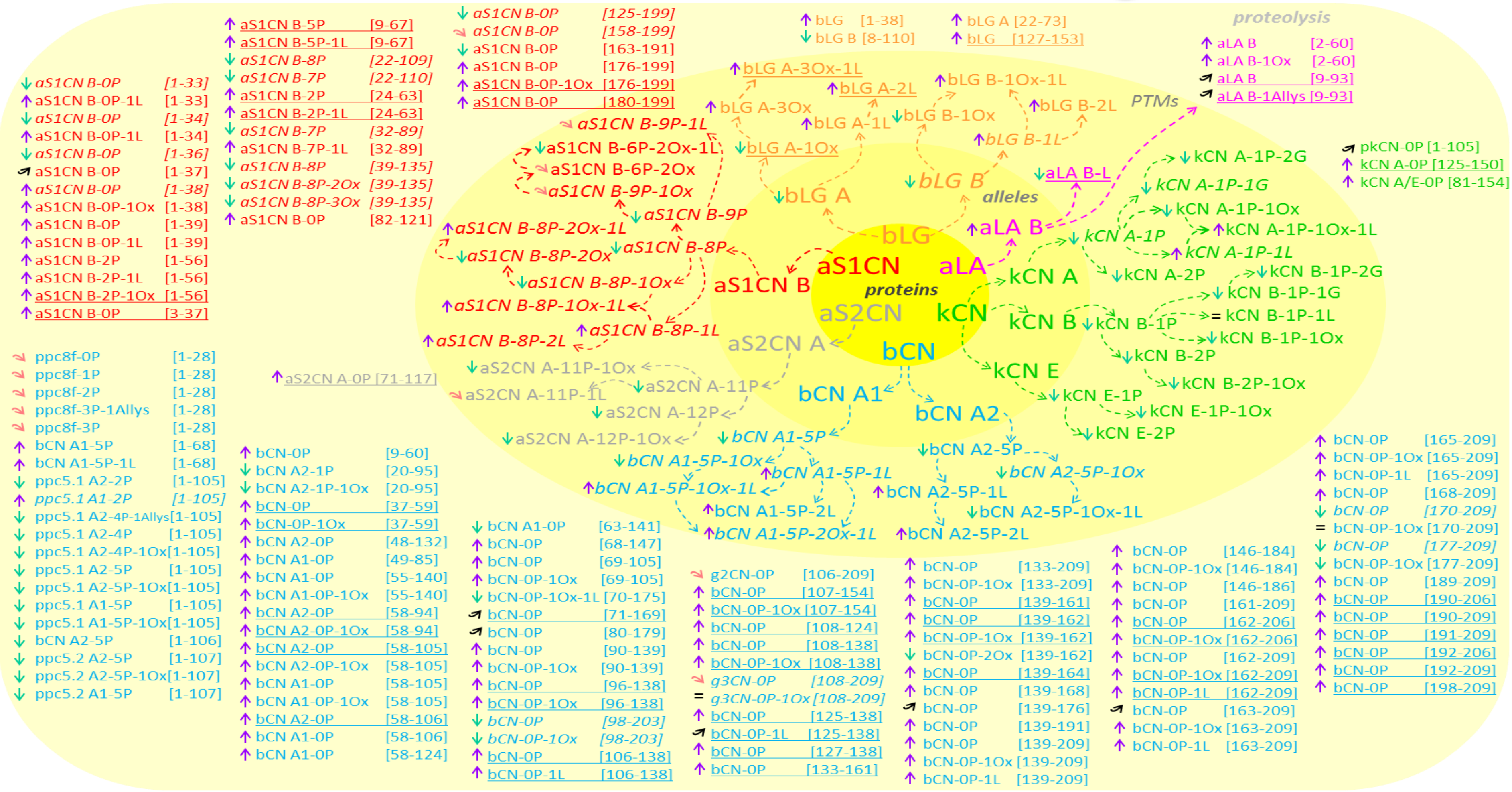


UHT Study: top-down proteomics – annotated proteoforms

Summary of all the proteoforms identified in UHT milk:

209 protein compounds =
58 intact proteoforms
+ 151 degradation products

Protein type	Main proteoform	Allysine	Glycosylation	Lactosylation	Lactosylation + Oxidation	Lysis	Oxidation	Oxidation + Lysis	Phosphorylation	TOTAL
aLA	1	1	0	1	0	2	0	1	0	6
aS1CN	1	0	0	10	3	20	4	6	1	44
aS2CN	1	0	0	1	0	1	2	0	1	5
bCN	2	2	0	11	3	67	2	30	0	117
bLG	2	0	0	4	2	4	3	0	0	15
kCN	3	0	4	2	1	3	4	0	3	17



↓ *aS1CN B-0P [1-33]*
↑ aS1CN B-0P-1L [1-33]
↓ *aS1CN B-0P [1-34]*
↑ aS1CN B-0P-1L [1-34]
↓ *aS1CN B-0P [1-36]*
↗ aS1CN B-0P [1-37]
↑ *aS1CN B-0P [1-38]*
↑ aS1CN B-0P-1Ox [1-38]
↑ aS1CN B-0P [1-39]
↑ aS1CN B-0P-1L [1-39]
↑ aS1CN B-2P [1-56]
↑ aS1CN B-2P-1L [1-56]
↑ aS1CN B-2P-1Ox [1-56]
↑ aS1CN B-0P [3-37]

↑ aS1CN B-5P [9-67]
↑ aS1CN B-5P-1L [9-67]
↓ *aS1CN B-8P [22-109]*
↓ *aS1CN B-7P [22-110]*
↑ aS1CN B-2P [24-63]
↑ aS1CN B-2P-1L [24-63]
↓ *aS1CN B-7P [32-89]*
↑ aS1CN B-7P-1L [32-89]
↓ *aS1CN B-8P [39-135]*
↓ *aS1CN B-8P-2Ox [39-135]*
↓ *aS1CN B-8P-3Ox [39-135]*
↑ aS1CN B-0P [82-121]

↓ *aS1CN B-0P [125-199]*
↘ *aS1CN B-0P [158-199]*
↓ aS1CN B-0P [163-191]
↑ aS1CN B-0P [176-199]
↑ aS1CN B-0P-1Ox [176-199]
↑ aS1CN B-0P [180-199]

↑ bLG [1-38]
↓ bLG B [8-110]

↑ bLG A [22-73]
↑ bLG [127-153]

proteolysis

↑ aLA B [2-60]
↑ aLA B-1Ox [2-60]
↗ aLA B [9-93]
↗ aLA B-1Allys [9-93]

↑ bLG A-3Ox-1L
↑ bLG A-2L
↑ bLG B-1Ox-1L
↑ bLG A-3Ox
↑ bLG A-1L
↓ bLG B-1Ox
↑ bLG B-2L
PTMs
↑ *bLG B-1L*
↓ bLG A-1Ox
↓ bLG A
↓ *bLG B*
alleles
↓ aLA B-L
↑ aLA B
bLG

↘ *aS1CN B-9P-1L*
↓ aS1CN B-6P-2Ox-1L
↘ aS1CN B-6P-2Ox
↘ *aS1CN B-9P-1Ox*
↓ *aS1CN B-9P*
↑ *aS1CN B-8P-2Ox-1L*
↓ *aS1CN B-8P-2Ox*
↓ *aS1CN B-8P*
↓ *aS1CN B-8P-1Ox*
↑ *aS1CN B-8P-1Ox-1L*
↑ *aS1CN B-8P-1L*
↑ *aS1CN B-8P-2L*
aS1CN B

aS1CN
aLA
proteins
aS2CN
kCN
bCN

aS2CN A
↑ aS2CN A-0P [71-117]
↓ aS2CN A-11P-1Ox
↘ aS2CN A-11P-1L
↓ aS2CN A-11P
↓ aS2CN A-12P
↓ aS2CN A-12P-1Ox

bCN A1
bCN A2
↓ *bCN A1-5P*
↓ *bCN A1-5P-1Ox*
↑ *bCN A1-5P-1L*
↑ *bCN A1-5P-1Ox-1L*
↑ bCN A1-5P-2L
↑ *bCN A1-5P-2Ox-1L*
↓ bCN A2-5P
↓ *bCN A2-5P-1Ox*
↑ bCN A2-5P-1L
↓ bCN A2-5P-1Ox-1L
↑ bCN A2-5P-2L

↗ pkCN-0P [1-105]
↑ kCN A-0P [125-150]
↑ kCN A/E-0P [81-154]

kCN A
↓ *kCN A-1P*
↓ kCN A-1P-2G
↓ *kCN A-1P-1G*
↓ kCN A-1P-1Ox
↑ kCN A-1P-1Ox-1L
↑ *kCN A-1P-1L*
↓ kCN A-2P
kCN B
↓ kCN B-1P
↓ kCN B-1P-2G
↓ kCN B-1P-1G
= kCN B-1P-1L
↓ kCN B-1P-1Ox
↓ kCN B-2P
↓ kCN B-2P-1Ox
kCN E
↓ kCN E-1P
↓ kCN E-1P-1Ox
↓ kCN E-2P

↘ ppc8f-0P [1-28]
↘ ppc8f-1P [1-28]
↘ ppc8f-2P [1-28]
↘ ppc8f-3P-1Allys [1-28]
↘ ppc8f-3P [1-28]
↑ bCN A1-5P [1-68]
↑ bCN A1-5P-1L [1-68]
↓ ppc5.1 A2-2P [1-105]
↑ *ppc5.1 A1-2P [1-105]*
↓ ppc5.1 A2-4P-1Allys [1-105]
↓ ppc5.1 A2-4P [1-105]
↓ ppc5.1 A2-4P-1Ox [1-105]
↓ ppc5.1 A2-5P [1-105]
↓ ppc5.1 A2-5P-1Ox [1-105]
↓ ppc5.1 A1-5P [1-105]
↓ ppc5.1 A1-5P-1Ox [1-105]
↓ bCN A2-5P [1-106]
↓ ppc5.2 A2-5P [1-107]
↓ ppc5.2 A2-5P-1Ox [1-107]
↓ ppc5.2 A1-5P [1-107]

↑ bCN-0P [9-60]
↓ bCN A2-1P [20-95]
↓ bCN A2-1P-1Ox [20-95]
↑ bCN-0P [37-59]
↑ bCN-0P-1Ox [37-59]
↑ bCN A2-0P [48-132]
↑ bCN A1-0P [49-85]
↑ bCN A1-0P [55-140]
↑ bCN A1-0P-1Ox [55-140]
↑ bCN A2-0P [58-94]
↑ bCN A2-0P-1Ox [58-94]
↑ bCN A2-0P [58-105]
↑ bCN A2-0P-1Ox [58-105]
↑ bCN A1-0P [58-105]
↑ bCN A1-0P-1Ox [58-105]
↑ bCN A2-0P [58-106]
↑ bCN A1-0P [58-106]
↑ bCN A1-0P [58-124]

↓ bCN A1-0P [63-141]
↑ bCN-0P [68-147]
↑ bCN-0P [69-105]
↑ bCN-0P-1Ox [69-105]
↓ bCN-0P-1Ox-1L [70-175]
↗ bCN-0P [71-169]
↗ bCN-0P [80-179]
↑ bCN-0P [90-139]
↑ bCN-0P-1Ox [90-139]
↑ bCN-0P [96-138]
↑ bCN-0P-1Ox [96-138]
↓ *bCN-0P [98-203]*
↓ *bCN-0P-1Ox [98-203]*
↑ bCN-0P [106-138]
↑ bCN-0P-1L [106-138]

↘ g2CN-0P [106-209]
↑ bCN-0P [107-154]
↑ bCN-0P-1Ox [107-154]
↑ bCN-0P [108-124]
↑ bCN-0P [108-138]
↑ bCN-0P-1Ox [108-138]
↘ *g3CN-0P [108-209]*
= *g3CN-0P-1Ox [108-209]*
↑ bCN-0P [125-138]
↗ bCN-0P-1L [125-138]
↑ bCN-0P [127-138]
↑ bCN-0P [133-161]

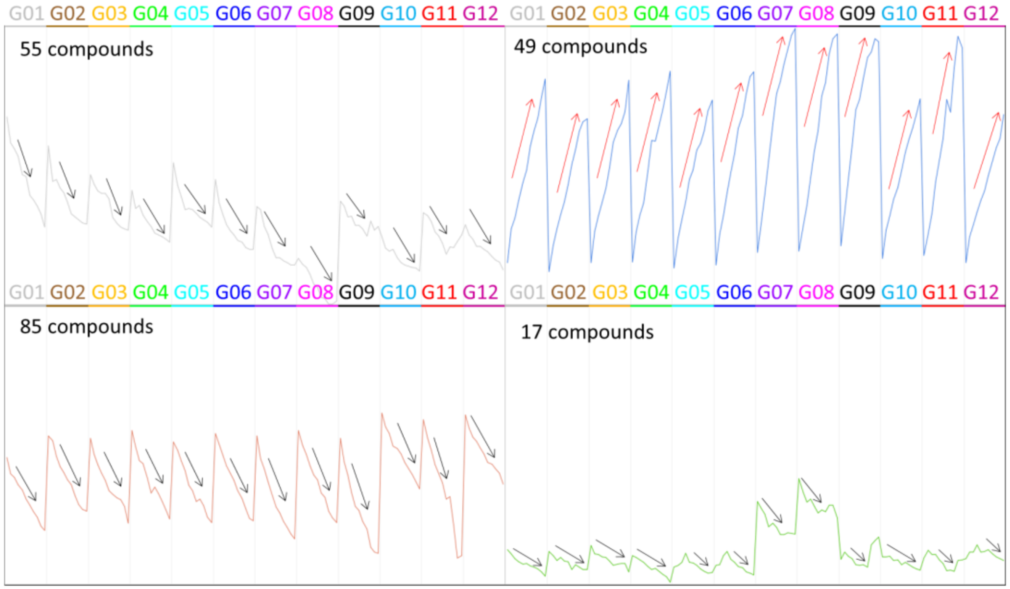
↑ bCN-0P [133-209]
↑ bCN-0P-1Ox [133-209]
↑ bCN-0P [139-161]
↑ bCN-0P [139-162]
↑ bCN-0P-1Ox [139-162]
↓ bCN-0P-2Ox [139-162]
↑ bCN-0P [139-164]
↑ bCN-0P [139-168]
↗ bCN-0P [139-176]
↑ bCN-0P [139-191]
↑ bCN-0P [139-209]
↑ bCN-0P-1Ox [139-209]
↑ bCN-0P-1L [139-209]

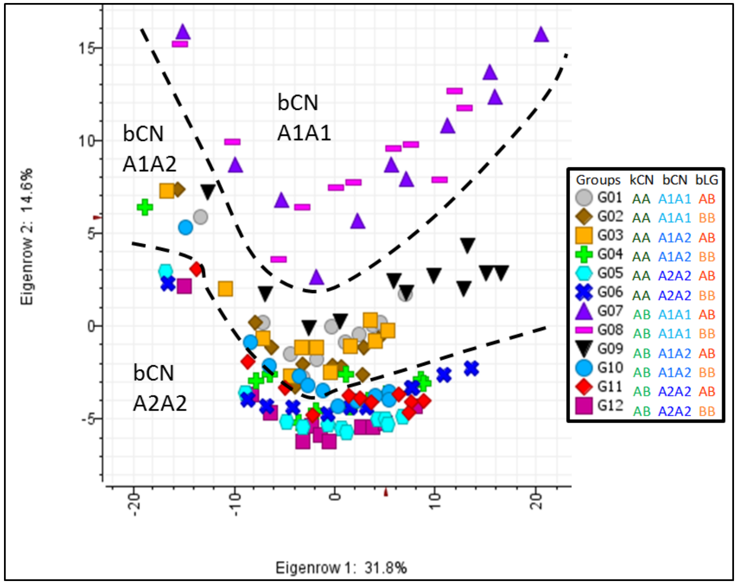
↑ bCN-0P [146-184]
↑ bCN-0P-1Ox [146-184]
↑ bCN-0P [146-186]
↑ bCN-0P [161-209]
↑ bCN-0P [162-206]
↑ bCN-0P-1Ox [162-206]
↑ bCN-0P [162-209]
↑ bCN-0P-1Ox [162-209]
↑ bCN-0P-1L [162-209]
↗ bCN-0P [163-209]
↑ bCN-0P-1Ox [163-209]
↑ bCN-0P-1L [163-209]

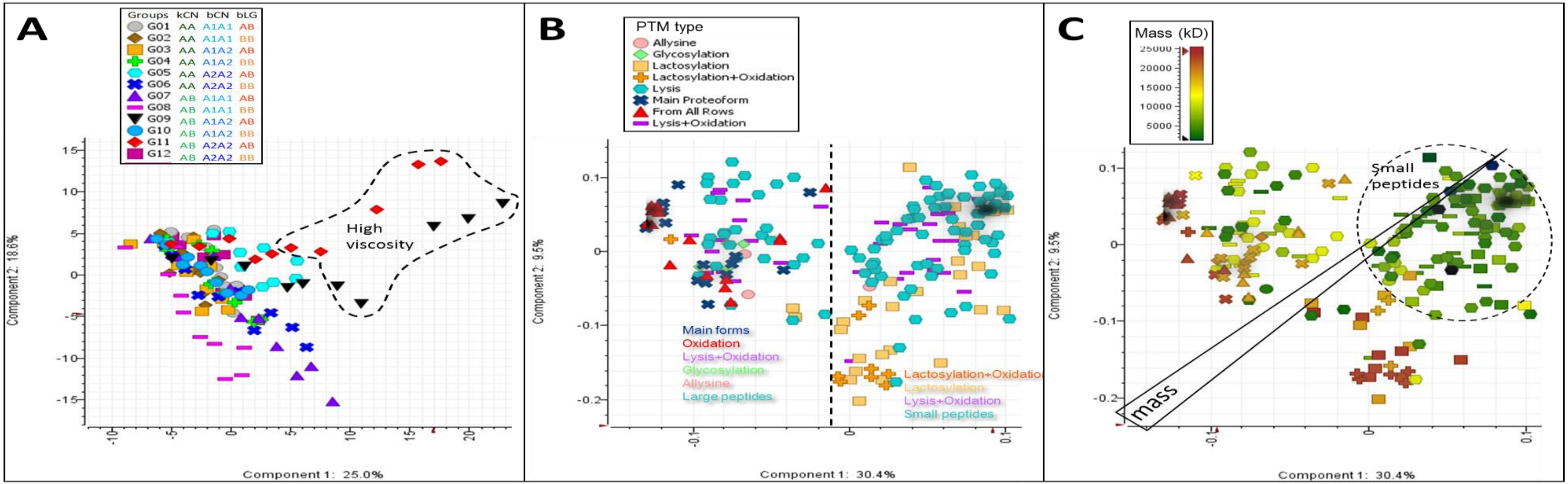
↑ bCN-0P [165-209]
↑ bCN-0P-1Ox [165-209]
↑ bCN-0P-1L [165-209]
↑ bCN-0P [168-209]
↓ *bCN-0P [170-209]*
= bCN-0P-1Ox [170-209]
↓ *bCN-0P [177-209]*
↓ bCN-0P-1Ox [177-209]
↑ bCN-0P [189-209]
↑ bCN-0P [190-206]
↑ bCN-0P [190-209]
↑ bCN-0P [191-209]
↑ bCN-0P [192-206]
↑ bCN-0P [192-209]
↑ bCN-0P [198-209]

UHT Study: top-down proteomics – statistical analyses

2 main profiles: decrease of intact proteins and increase of degraded proteins (SOM, PCA, PLS).

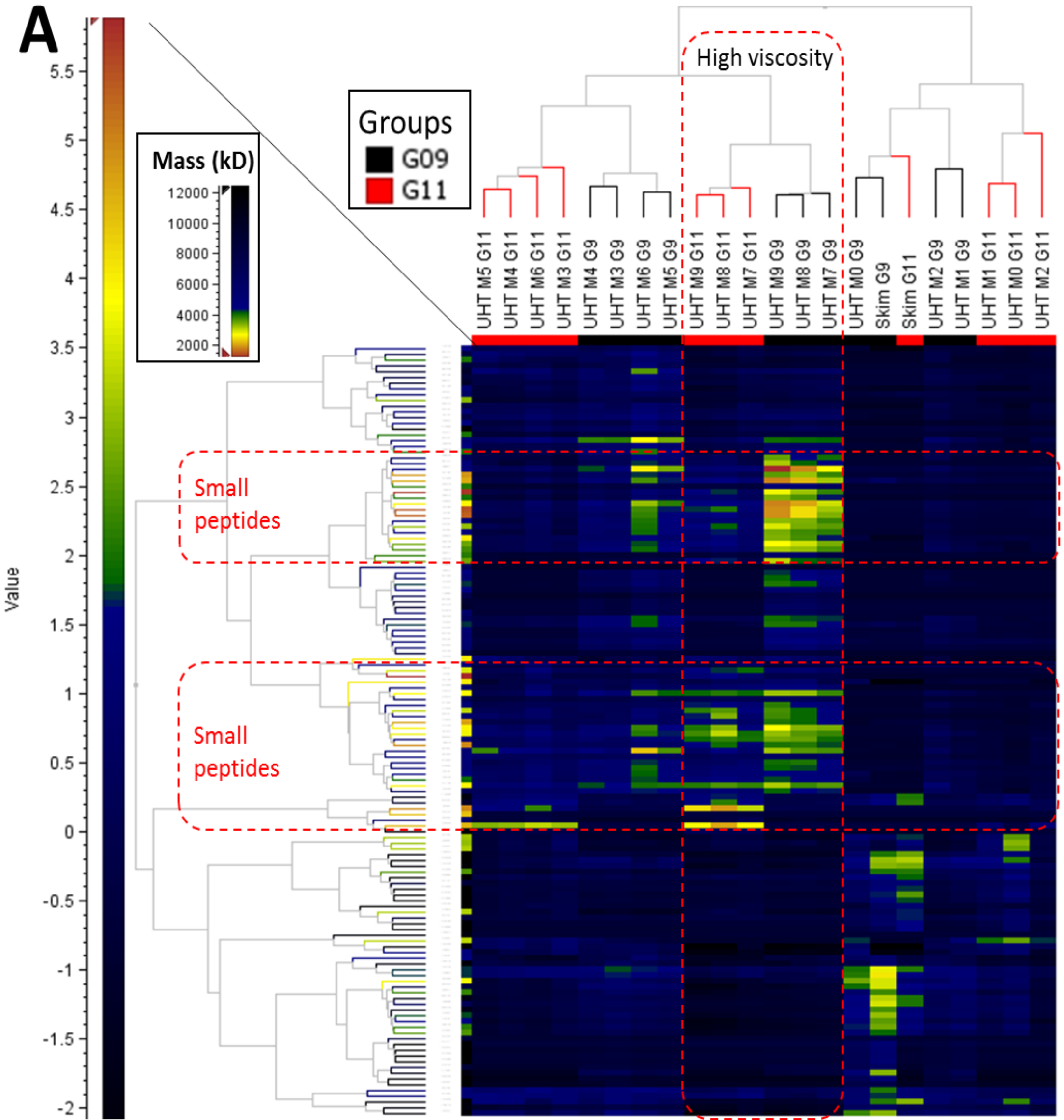




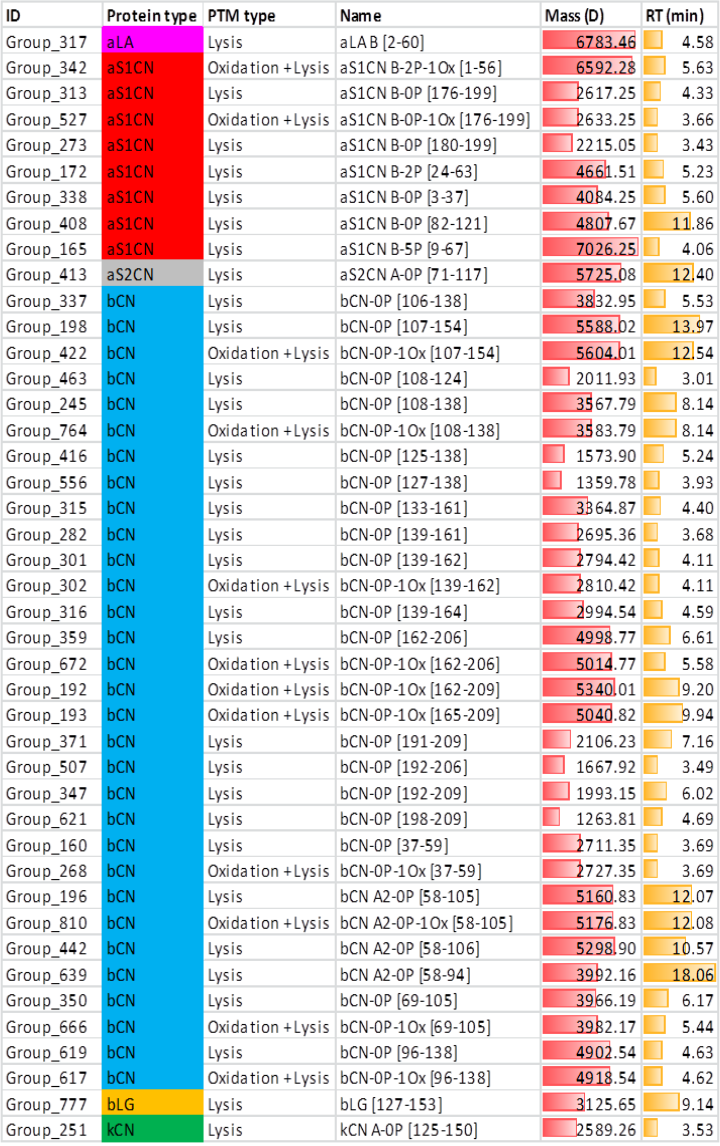


UHT Study: top-down proteomics – high viscosity markers

Confirming PLS results, HCA and correlation analyses list the biomarkers of high viscosity. They are the smallest peptides.



B



ID	Protein type	PTM type	Name	Mass (D)	RT (min)
Group_317	aLA	Lysis	aLA B [2-60]	6783.46	4.58
Group_342	aS1CN	Oxidation +Lysis	aS1CN B-2P-1Ox [1-56]	6592.28	5.63
Group_313	aS1CN	Lysis	aS1CN B-0P [176-199]	2617.25	4.33
Group_527	aS1CN	Oxidation +Lysis	aS1CN B-0P-1Ox [176-199]	2633.25	3.66
Group_273	aS1CN	Lysis	aS1CN B-0P [180-199]	2215.05	3.43
Group_172	aS1CN	Lysis	aS1CN B-2P [24-63]	4661.51	5.23
Group_338	aS1CN	Lysis	aS1CN B-0P [3-37]	4084.25	5.60
Group_408	aS1CN	Lysis	aS1CN B-0P [82-121]	4807.67	11.86
Group_165	aS1CN	Lysis	aS1CN B-5P [9-67]	7026.25	4.06
Group_413	aS2CN	Lysis	aS2CN A-0P [71-117]	5725.08	12.40
Group_337	bCN	Lysis	bCN-0P [106-138]	3832.95	5.53
Group_198	bCN	Lysis	bCN-0P [107-154]	5588.02	13.97
Group_422	bCN	Oxidation +Lysis	bCN-0P-1Ox [107-154]	5604.01	12.54
Group_463	bCN	Lysis	bCN-0P [108-124]	2011.93	3.01
Group_245	bCN	Lysis	bCN-0P [108-138]	3567.79	8.14
Group_764	bCN	Oxidation +Lysis	bCN-0P-1Ox [108-138]	3583.79	8.14
Group_416	bCN	Lysis	bCN-0P [125-138]	1573.90	5.24
Group_556	bCN	Lysis	bCN-0P [127-138]	1359.78	3.93
Group_315	bCN	Lysis	bCN-0P [133-161]	3364.87	4.40
Group_282	bCN	Lysis	bCN-0P [139-161]	2695.36	3.68
Group_301	bCN	Lysis	bCN-0P [139-162]	2794.42	4.11
Group_302	bCN	Oxidation +Lysis	bCN-0P-1Ox [139-162]	2810.42	4.11
Group_316	bCN	Lysis	bCN-0P [139-164]	2994.54	4.59
Group_359	bCN	Lysis	bCN-0P [162-206]	4998.77	6.61
Group_672	bCN	Oxidation +Lysis	bCN-0P-1Ox [162-206]	5014.77	5.58
Group_192	bCN	Oxidation +Lysis	bCN-0P-1Ox [162-209]	5340.01	9.20
Group_193	bCN	Oxidation +Lysis	bCN-0P-1Ox [165-209]	5040.82	9.94
Group_371	bCN	Lysis	bCN-0P [191-209]	2106.23	7.16
Group_507	bCN	Lysis	bCN-0P [192-206]	1667.92	3.49
Group_347	bCN	Lysis	bCN-0P [192-209]	1993.15	6.02
Group_621	bCN	Lysis	bCN-0P [198-209]	1263.81	4.69
Group_160	bCN	Lysis	bCN-0P [37-59]	2711.35	3.69
Group_268	bCN	Oxidation +Lysis	bCN-0P-1Ox [37-59]	2727.35	3.69
Group_196	bCN	Lysis	bCN A2-0P [58-105]	5160.83	12.07
Group_810	bCN	Oxidation +Lysis	bCN A2-0P-1Ox [58-105]	5176.83	12.08
Group_442	bCN	Lysis	bCN A2-0P [58-106]	5298.90	10.57
Group_639	bCN	Lysis	bCN A2-0P [58-94]	3992.16	18.06
Group_350	bCN	Lysis	bCN-0P [69-105]	3966.19	6.17
Group_666	bCN	Oxidation +Lysis	bCN-0P-1Ox [69-105]	3982.17	5.44
Group_619	bCN	Lysis	bCN-0P [96-138]	4902.54	4.63
Group_617	bCN	Oxidation +Lysis	bCN-0P-1Ox [96-138]	4918.54	4.62
Group_777	bLG	Lysis	bLG [127-153]	3125.65	9.14
Group_251	kCN	Lysis	kCN A-0P [125-150]	2589.26	3.53

UHT Study: top-down proteomics – alpha-casein

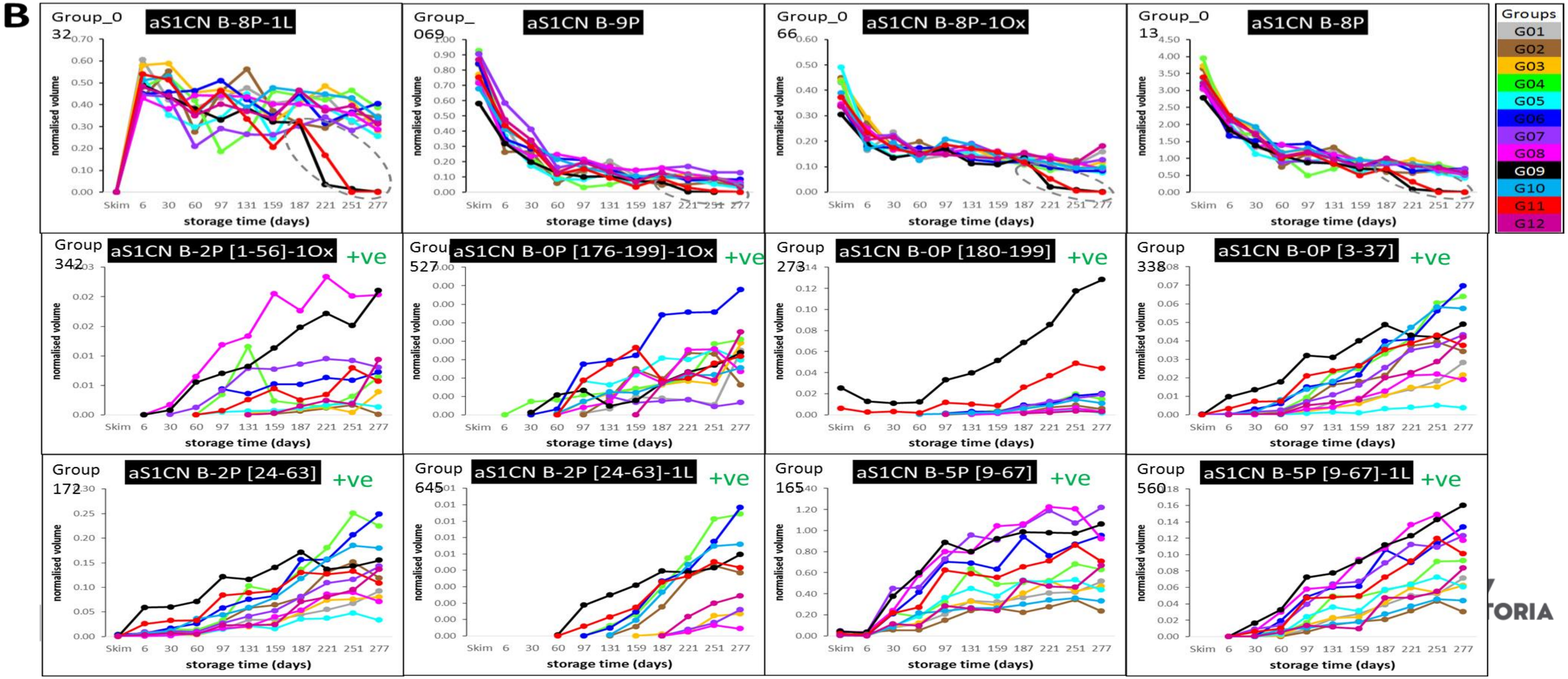
Here are examples of alpha-casein compounds associated with viscosity.

A

Phosphorylation sites: 41, 46 48, 64 66-68, 75, 115

aS1CN B-8P [1-199]	RPKHPIKHQGLPQEVLNENLLRFFVAPFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVEQKHIQKEDVPSERYLGYLEQLLRLKKYKVPQLEIVPNSAEERLHSMKEGIHAQQKEPMIGVNQELAYFYPELFRQFYQLDAYPSGAWYYVPLGTQYTDAPSFSDIPNPIGSENSEKTTMPLW
aS1CN B-9P [1-199]	RPKHPIKHQGLPQEVLNENLLRFFVAPFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVEQKHIQKEDVPSERYLGYLEQLLRLKKYKVPQLEIVPNSAEERLHSMKEGIHAQQKEPMIGVNQELAYFYPELFRQFYQLDAYPSGAWYYVPLGTQYTDAPSFSDIPNPIGSENSEKTTMPLW
aS1CN B-0P [1-33]	RPKHPIKHQGLPQEVLNENLLRFFVAPFPEVFG
aS1CN B-0P [1-34]	RPKHPIKHQGLPQEVLNENLLRFFVAPFPEVFGK
aS1CN B-0P [1-36]	RPKHPIKHQGLPQEVLNENLLRFFVAPFPEVFGKEK
aS1CN B-0P [1-37]	RPKHPIKHQGLPQEVLNENLLRFFVAPFPEVFGKEKV
aS1CN B-0P [1-38]	RPKHPIKHQGLPQEVLNENLLRFFVAPFPEVFGKEKVN
aS1CN B-0P [1-39]	RPKHPIKHQGLPQEVLNENLLRFFVAPFPEVFGKEKVNE
aS1CN B-2P [1-56]	RPKHPIKHQGLPQEVLNENLLRFFVAPFPEVFGKEKVNELSKDIGSESTEDQAMED
aS1CN B-0P [3-37]	KHPIKHQGLPQEVLNENLLRFFVAPFPEVFGKEKV
aS1CN B-5P [9-67]	QGLPQEVLNENLLRFFVAPFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESISS
aS1CN B-8P [22-109]	RFFVAPFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVEQKHIQKEDVPSERYLGYLEQLLRLKKYKVPQL
aS1CN B-7P [22-110]	RFFVAPFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVEQKHIQKEDVPSERYLGYLEQLLRLKKYKVPQLE
aS1CN B-2P [24-63]	FVAPFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAE
aS1CN B-7P [32-89]	FGKEKVNELSKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVEQKHIQKEDVPSE
aS1CN B-8P [39-135]	ELSKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVEQKHIQKEDVPSERYLGYLEQLLRLKKYKVPQLEIVPNSAEERLHSMKEGIHAQQKEPM
aS1CN B-0P [82-121]	IQKEDVPSERYLGYLEQLLRLKKYKVPQLEIVPNSAEERLH
aS1CN B-0P [125-199]	EGIHAQQKEPMIGVNQELAYFYPELFRQFYQLDAYPSGAWYYVPLGTQYTDAPSFSDIPNPIGSENSEKTTMPLW
aS1CN B-0P [158-199]	LDAYPSGAWYYVPLGTQYTDAPSFSDIPNPIGSENSEKTTMPLW
aS1CN B-0P [163-191]	AWYYVPLGTQYTDAPSFSDIPNPIGSENS
aS1CN B-0P [176-199]	APSFSDIPNPIGSENSEKTTMPLW
aS1CN B-0P [180-199]	SDIPNPIGSENSEKTTMPLW

B

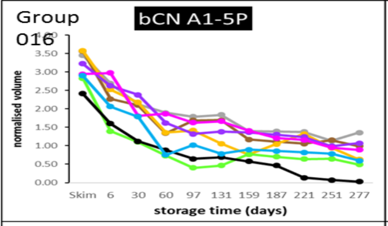


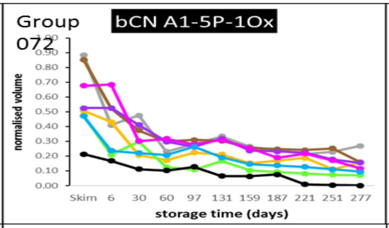
UHT Study: top-down proteomics – beta-casein

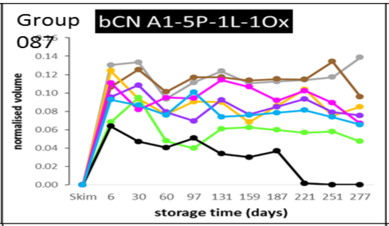
bCN A1-5P [1-209]	RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIHNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN A2-5P [1-209]	RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIPNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
ppc8f [1-28]	RELEELNVPGEIVESLSSSEESITRINK
bCN A1-5P [1-68]	RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIHN
ppc5.1 A1 [1-105]	RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIHNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPK
ppc5.1 A2 [1-105]	RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIPNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPK
bCN A2-5P [1-106]	RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIPNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKH
ppc5.2 A1 [1-107]	RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIHNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHK
ppc5.2 A2 [1-107]	RELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIPNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHK
bCN-0P [9-60]	PGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVY
bCN A2-1P [20-95]	EESITRINKKIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIHNSLPQNIPPLTQTPVVVPPFLQPEVMGV
ppc8s A2 [29-105]	KIEKFQSEEQQQTEDELQDKIHPFAQTQSLVYPFPGPIPNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPK
bCN-0P [37-59]	EQQQTEDELQDKIHPFAQTQSLV
bCN A2-0P [48-132]	KIHPFAQTQSLVYPFPGPIPNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVEN
bCN A1-0P [49-85]	IHPFAQTQSLVYPFPGPIHNSLPQNIPPLTQTPVVVP
bCN A1-0P [55-140]	TQSLVYPFPGPIHNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLL
bCN A2-0P [58-94]	LVYPFPGPIPNSLPQNIPPLTQTPVVVPPFLQPEVMG
bCN A1-0P [58-105]	LVYPFPGPIHNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPK
bCN A2-0P [58-105]	LVYPFPGPIPNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPK
bCN A1-0P [58-106]	LVYPFPGPIHNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKH
bCN A2-0P [58-106]	LVYPFPGPIPNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKH
bCN A1-0P [58-124]	LVYPFPGPIHNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQS
bCNA1-0P [63-141]	PGPIHNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQ
bCN-0P [68-147]	NSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQP
bCN-0P [69-105]	SLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPK
bCN-0P [70-175]	LPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQ
bCN-0P [70-175]	LPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQ
bCN-0P [71-169]	PQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSK
bCN-0P [80-179]	TPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVP
bCN-0P [90-139]	PEVMGVSKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPL
bCN-0P [96-138]	SKVKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLP
bCN-0P [98-203]	VKEAMAPKHKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRG
bCN-0P [106-138]	HKEMPFPKYPVEPFTESQSLTLTDVENLHLPLP
g2CN [106-209]	HKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN-0P [107-154]	KEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPT
bCN-0P [108-124]	EMPFPKYPVEPFTESQS
bCN-0P [108-138]	EMPFPKYPVEPFTESQSLTLTDVENLHLPLP
g3CN [108-209]	EMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN-0P [125-138]	LTLTDVENLHLPLP
bCN-0P [127-138]	LTDVENLHLPLP
bCN-0P [133-161]	LHLPLPLLQSWMHQPHQPLPPTVMFPPQS
bCN-0P [133-209]	LHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN-0P [139-161]	LLQSWMHQPHQPLPPTVMFPPQS
bCN-0P [139-162]	LLQSWMHQPHQPLPPTVMFPPQSV
bCN-0P [139-164]	LLQSWMHQPHQPLPPTVMFPPQSVLS
bCN-0P [139-168]	LLQSWMHQPHQPLPPTVMFPPQSVLSLSQS
bCN-0P [139-176]	LLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQK
bCN-0P [139-191]	LLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMPIQAFL
bCN-0P [146-184]	QPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRD
bCN-0P [146-186]	QPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAVPYPQRDMP
bCN-0P [161-209]	SVLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN-0P [162-206]	VLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFP
bCN-0P [162-209]	VLSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN-0P [163-209]	LSLSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN-0P [165-209]	LSQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN-0P [168-209]	QSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN-0P [170-209]	AVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
bCN-0P [177-209]	DMPIQAFLLYQEPVLGPVRGPFPIIV
bCN-0P [189-209]	AFLLYQEPVLGPVRGPFPIIV
bCN-0P [190-206]	FLLYQEPVLGPVRGPFP
bCN-0P [190-209]	FLLYQEPVLGPVRGPFPIIV
bCN-0P [191-209]	LLYQEPVLGPVRGPFPIIV
bCN-0P [192-206]	LYQEPVLGPVRGPFP
bCN-0P [192-209]	LYQEPVLGPVRGPFPIIV
bCN-0P [198-209]	LGPVRGPFPIIV

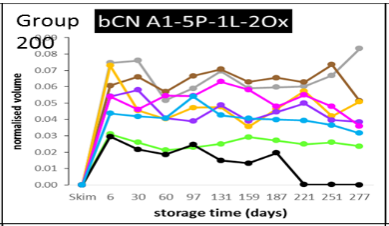
UHT Study: top-down proteomics – beta-casein

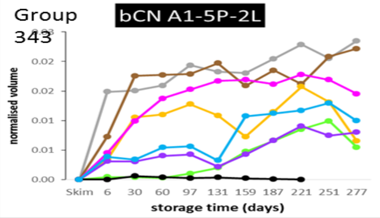
Here are examples of beta-casein compounds associated with viscosity.

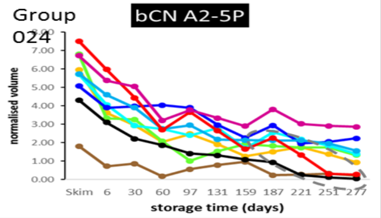


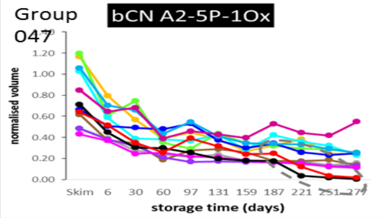


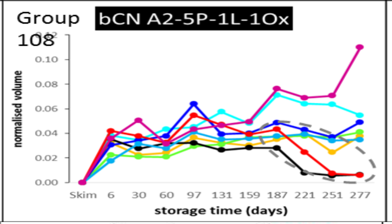


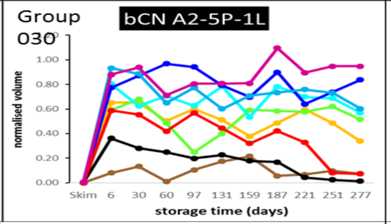


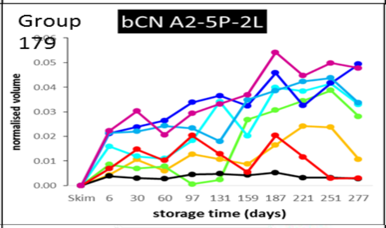


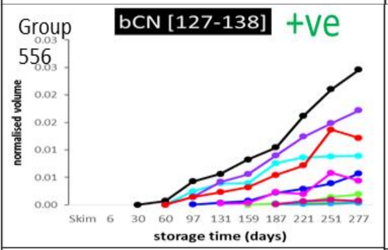


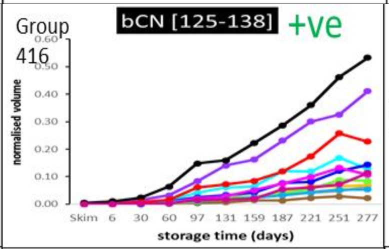


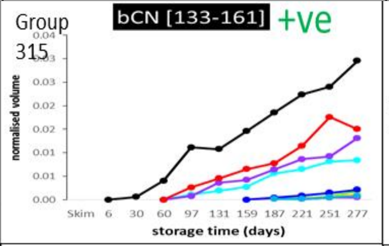


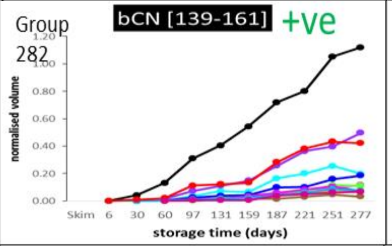


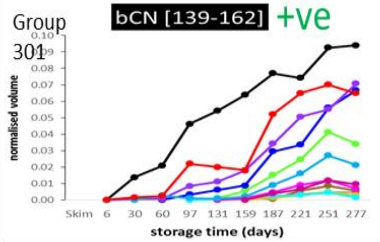


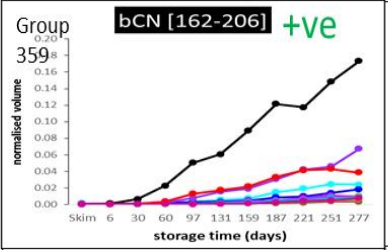


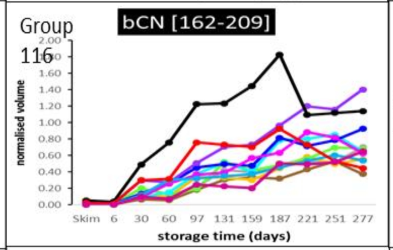


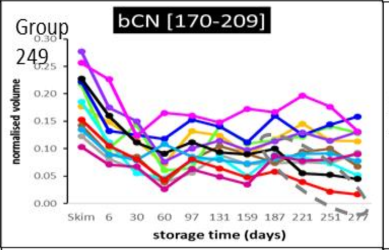


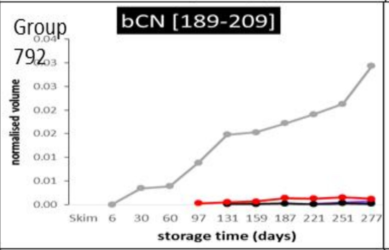


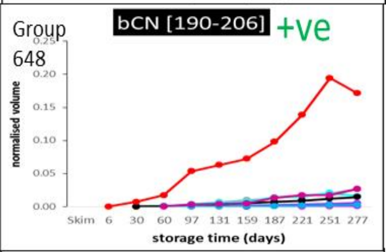


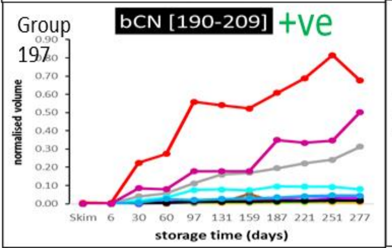


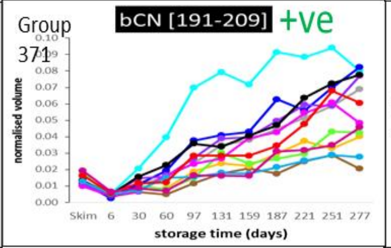


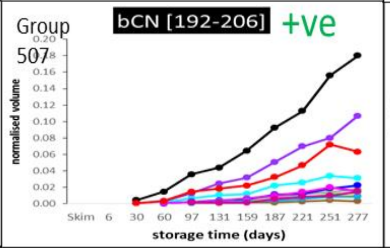


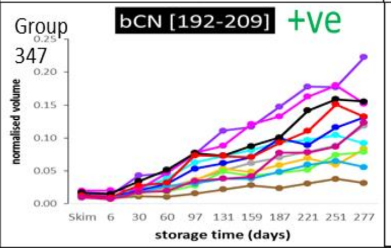


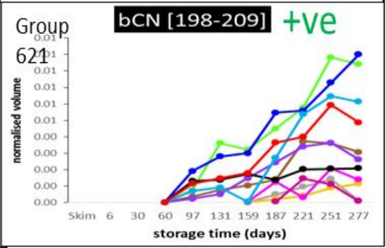












UHT Study: conclusion

Three general trends from top-down proteomics analyses:

- Decreases in abundance all of the native intact milk proteins identified over storage time of UHT milk.

- Occurrence of lactosylated proteins following UHT treatment.

- Accumulation of peptides over storage time of UHT milk.

With respect to viscosity, milks subject to age gelation (Groups 9 and 11) accumulate small peptides mostly from beta-caseins.
These peptides could arise from protease activities from thermostable bacteria.

Proteolysis would be a leading mechanism for the instability of stored milks.

Outlines

Past

Present

- MALDI Biotyper (MBT)
- Milk proteomics (Top-Down)

Future

MALDI Biotyper (MBT)

- Implement libraries with more entries of sequenced organisms (bacteria, fungi…)

Bottom-up proteomics

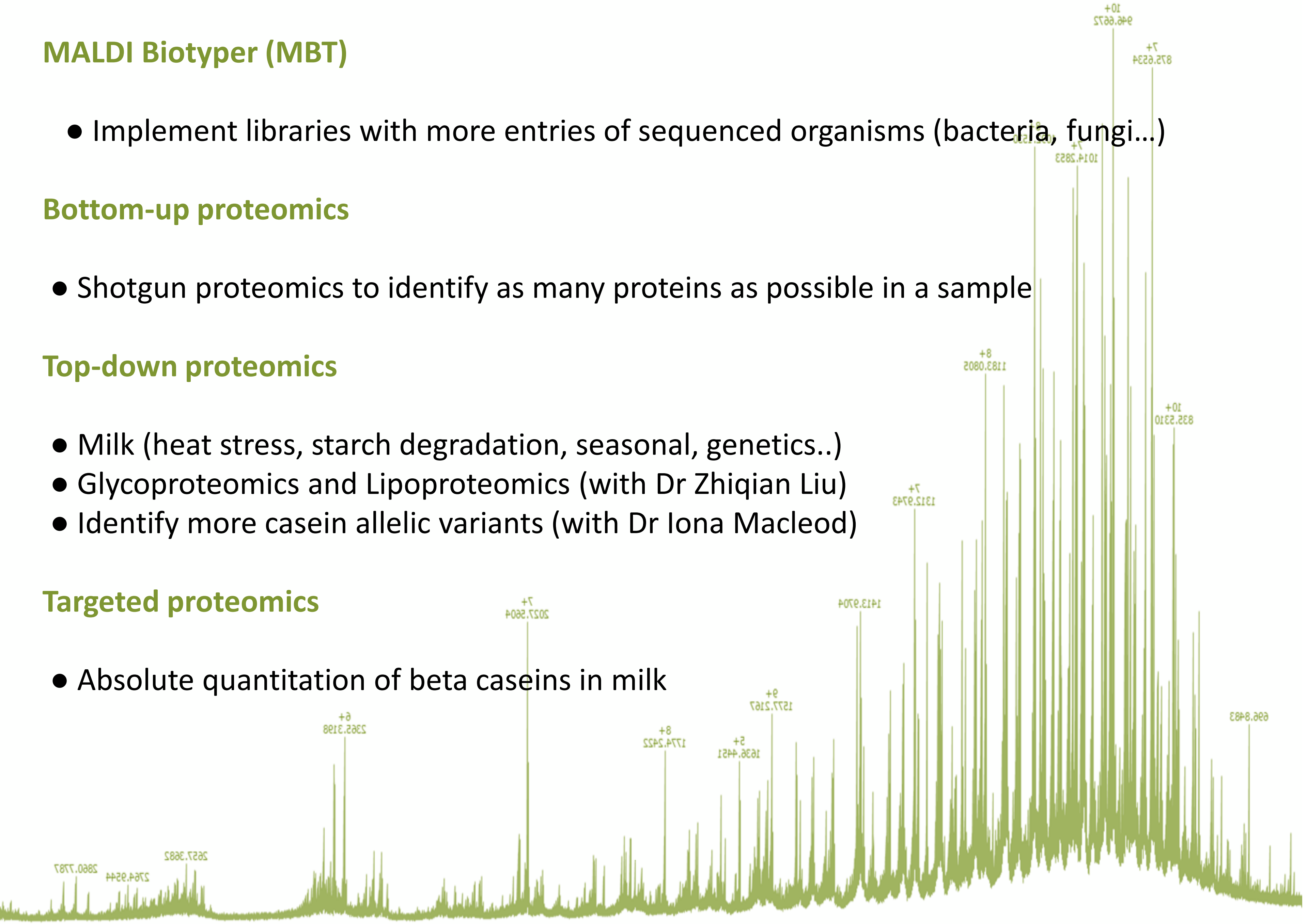
- Shotgun proteomics to identify as many proteins as possible in a sample

Top-down proteomics

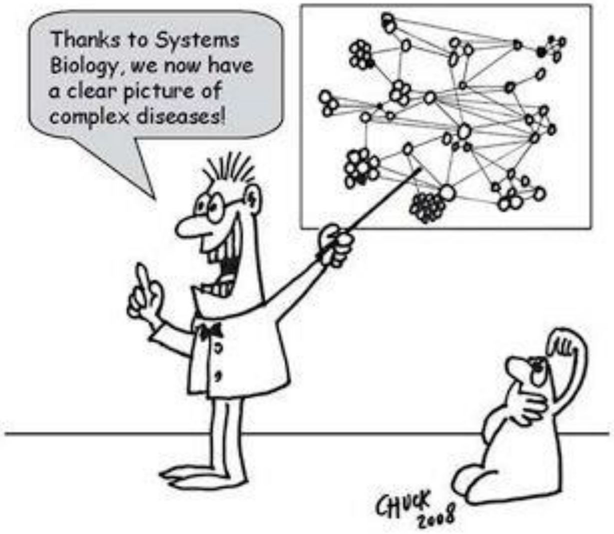
- Milk (heat stress, starch degradation, seasonal, genetics..)
- Glycoproteomics and Lipoproteomics (with Dr Zhiqian Liu)
- Identify more casein allelic variants (with Dr Iona Macleod)

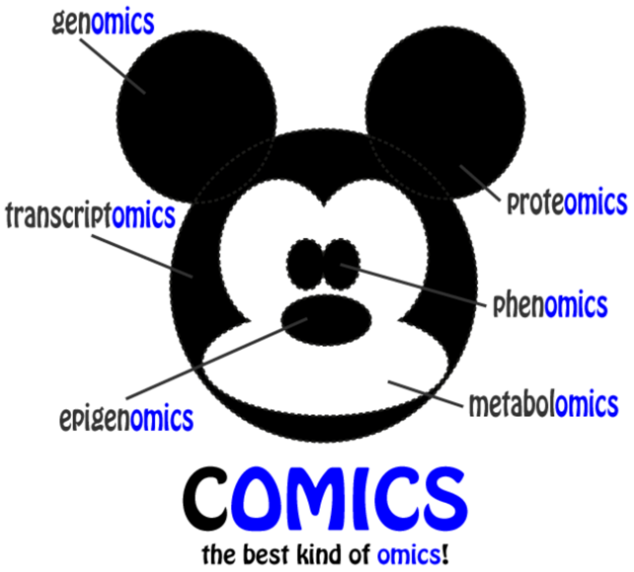
Targeted proteomics

- Absolute quantitation of beta caseins in milk



Thank you


Thanks to Systems Biology, we now have a clear picture of complex diseases!
CHUCK 2008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I'd say proteomics.
Same as always: Economics.
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