



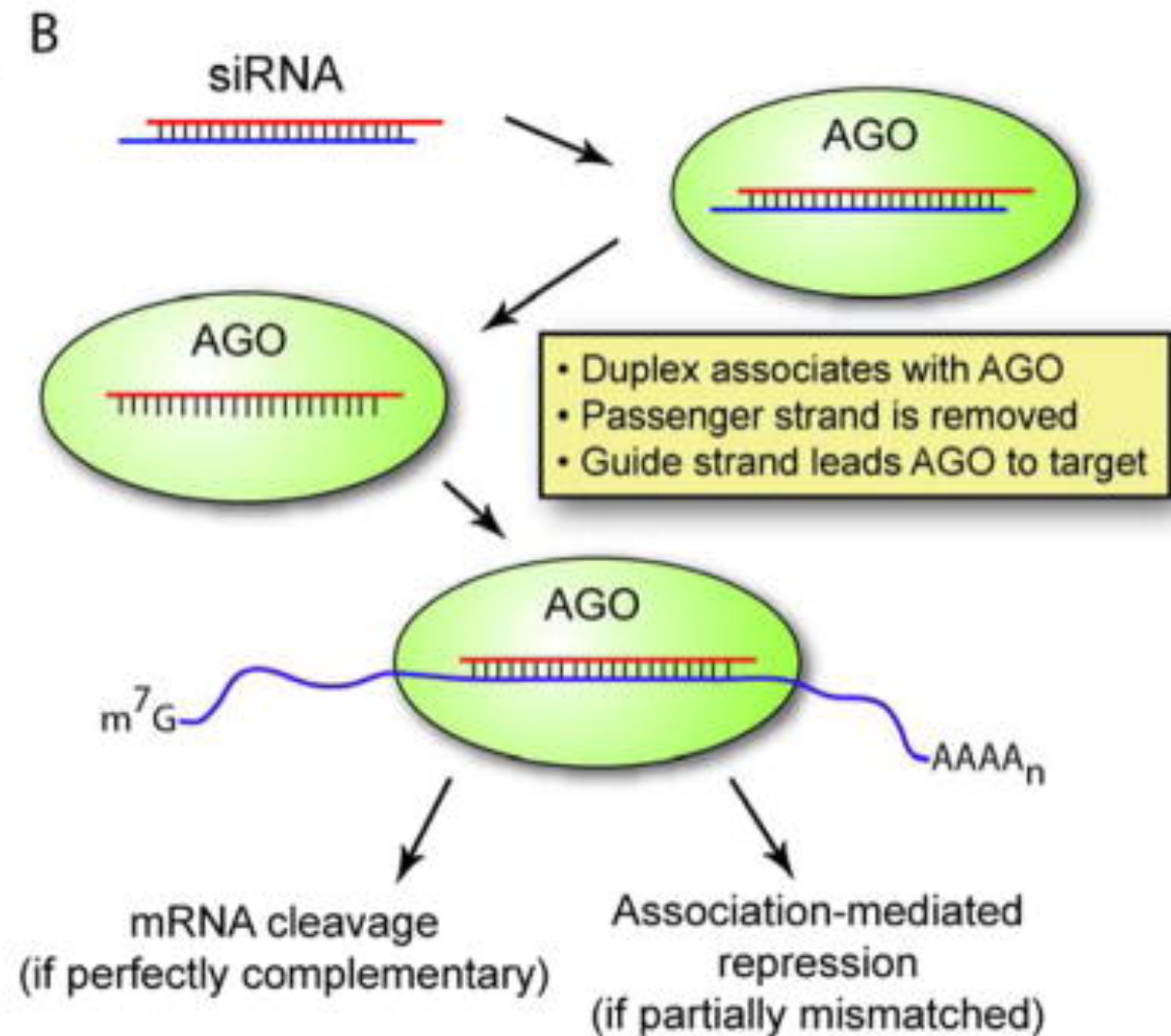
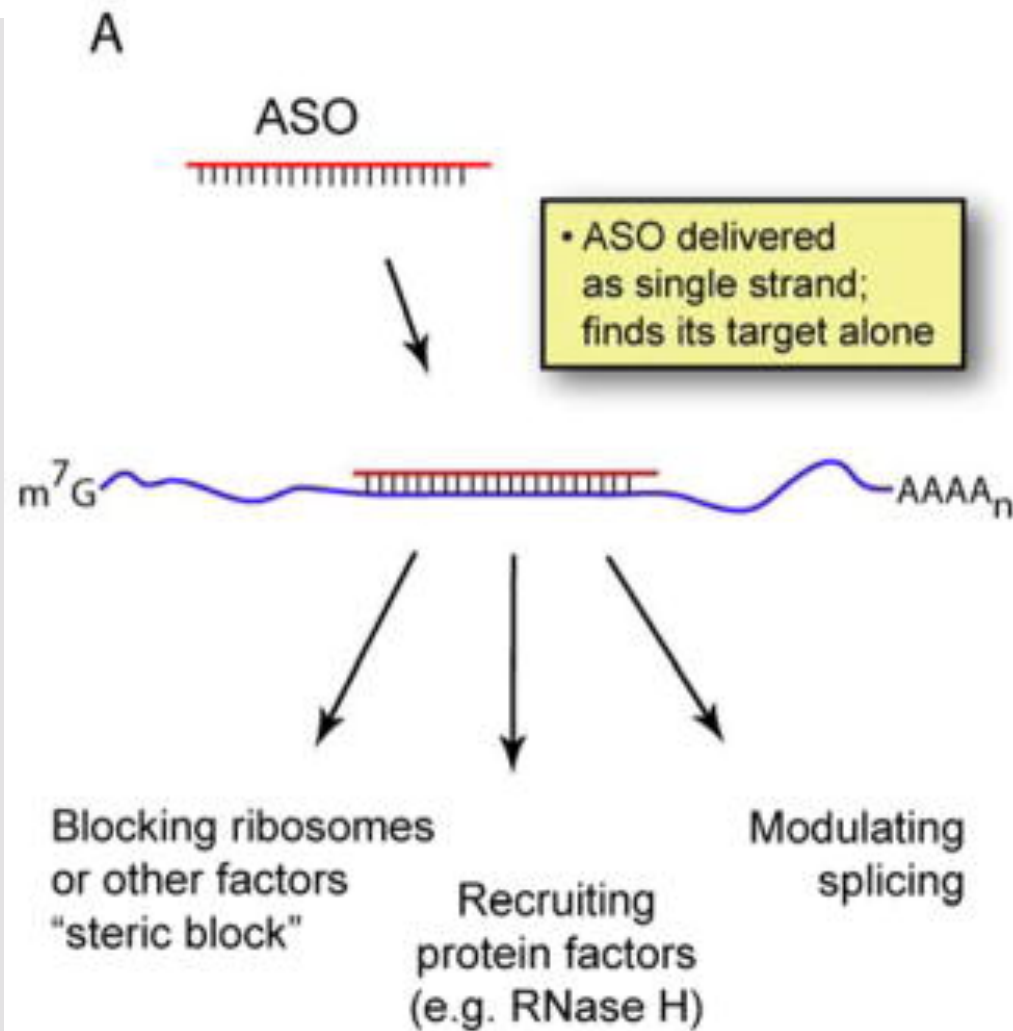
**CHARACTERIZATION OF OLIGONUCLEOTIDE
BIOTRANSFORMATION USING ION PARING
REVERSED-PHASE LIQUID CHROMATOGRAPHY
COUPLED WITH HIGH RESOLUTION MASS
SPECTROMETRY**

BABAK BASIRI

2019 STANFORD UNIVERSITY MS RESEARCH APPLICATIONS SYMPOSIUM
OCTOBER 10, 2019

AMGEN[®]

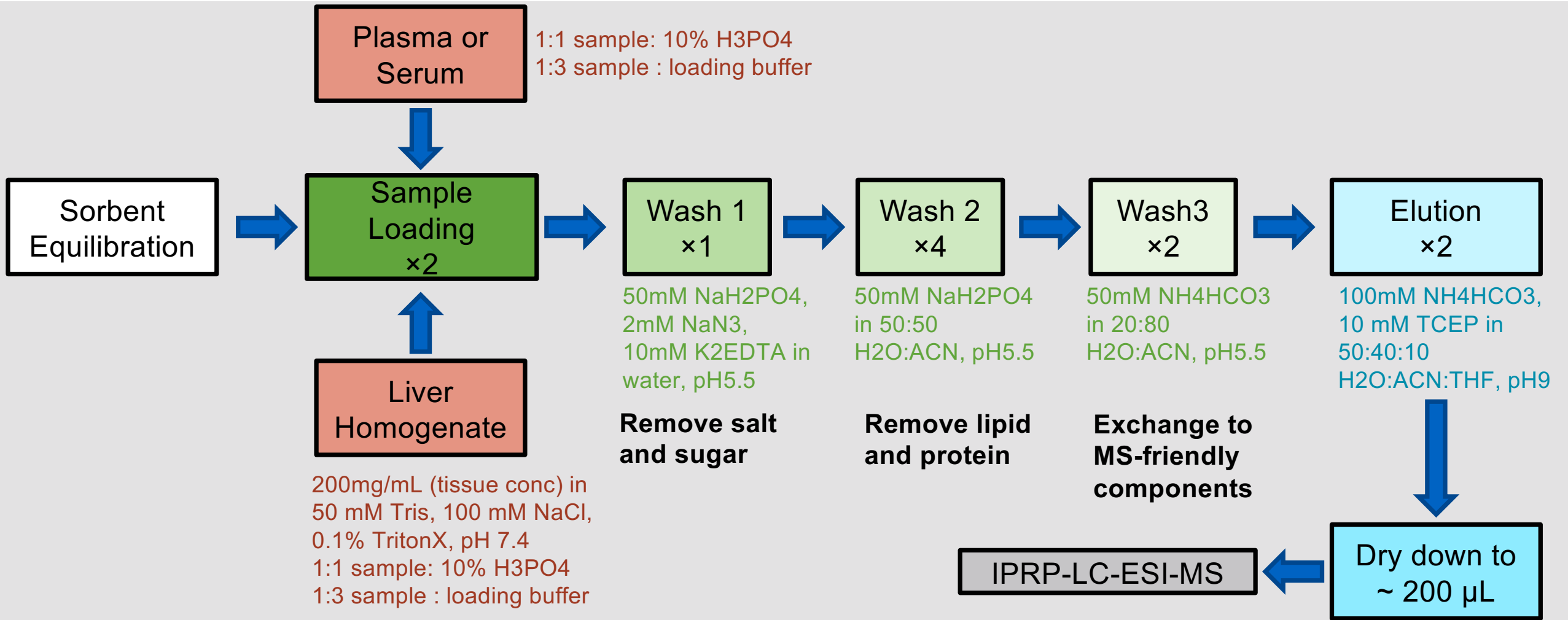
OLIGONUCLEOTIDE THERAPEUTICS HAVE TWO MAJOR MECHANISMS OF ACTION:



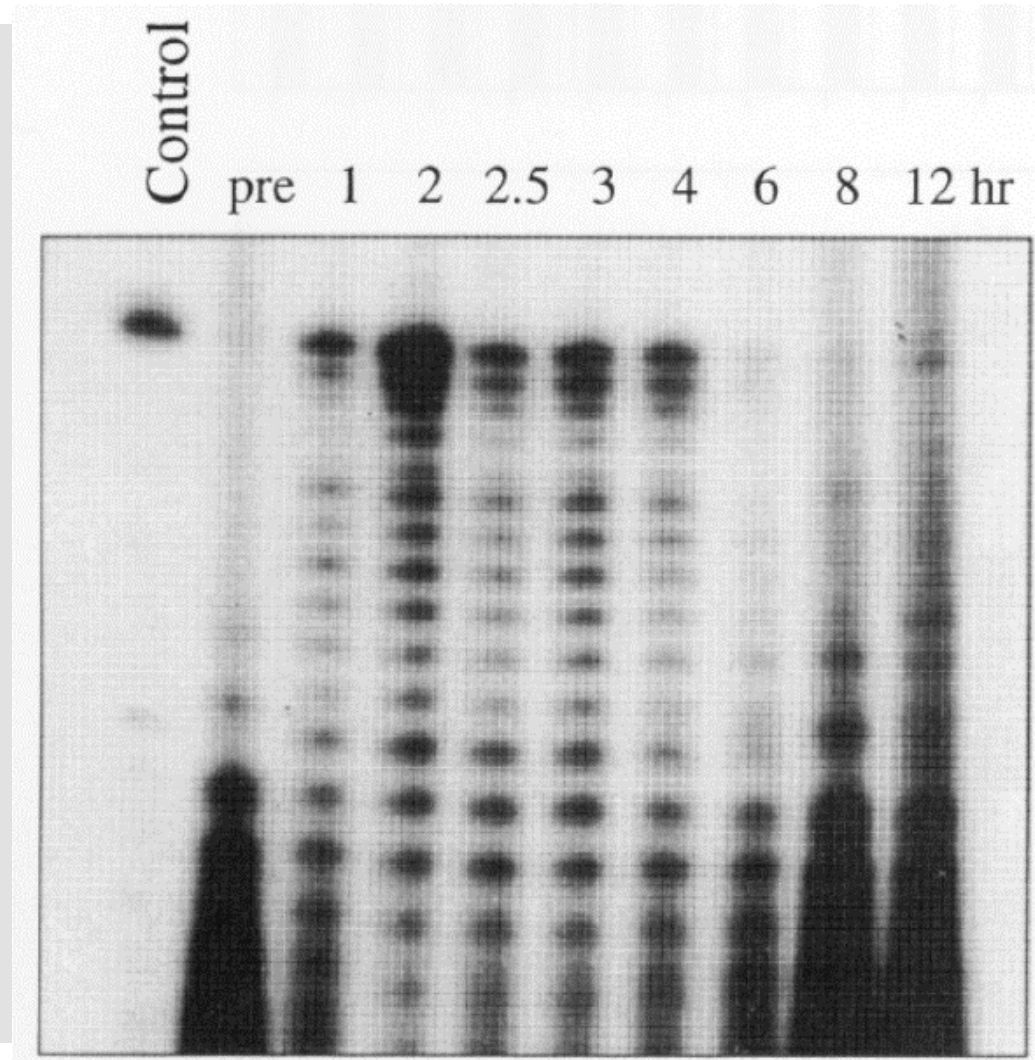
WORKFLOW OF LC-MS BASED siRNA MET ID

- **Incubation of siRNA in liver homogenate**
 - siRNAs were incubated in liver homogenate (200 mg/mL tissue in 100 mM Tris and 1 mM magnesium chloride in water, pH=6.0) for up to 48 hours.
- **Extraction of siRNA from biological matrices**
 - Phenomenex Clarity OTX SPE plates
 - Optimized SPE protocol
- **Ion-pairing RP LC**
 - Waters Oligonucleotide BEH C₁₈ (130 Å, 1.7 µm, 2.1x50mm), 80°C
 - 15 mM TEA, 400 mM HFIP in H₂O (Solvent A) or MeOH (Solvent B)
- **MS**
 - Negative mode (-3000V), full scan MS, 60K resolution

OPTIMIZED SPE PROTOCOL



THE BIOTRANSFORMATION OF OLIGONUCLEOTIDE DRUGS IS PRIMARILY MEDIATED BY NUCLEASES:



OMA AND OPA SOFTWARE PACKAGE

Oligonucleotide Mass Assembler

Sequence(s) 5'→ SBL→ 3'

OH mC~mA-mG-mC-mC-mC-mU-fU-fA-fU- OH Insert

OH OH Insert

Output file

C:\Users\bbasiri\Desktop\Test Choose

Adducts

1-2 Na⁺ 1-2 K⁺

Pt(NH₃)₀₋₂²⁺

Library loaded with 23 items!

Checking ss1: GENERATING ESI

Total mass: avg=6854.4874 mono=6851.0864

GENERATING CID

a-d,w-z,internal,

DONE! 0.374s - 119132 peaks written

Help Clear Log Run

oma - Notepad

File Edit Format View Help

#oma.lib - Defines items available for reference assembly

#Please be careful when modifying this file. Too much [tab]s can make it unusable!

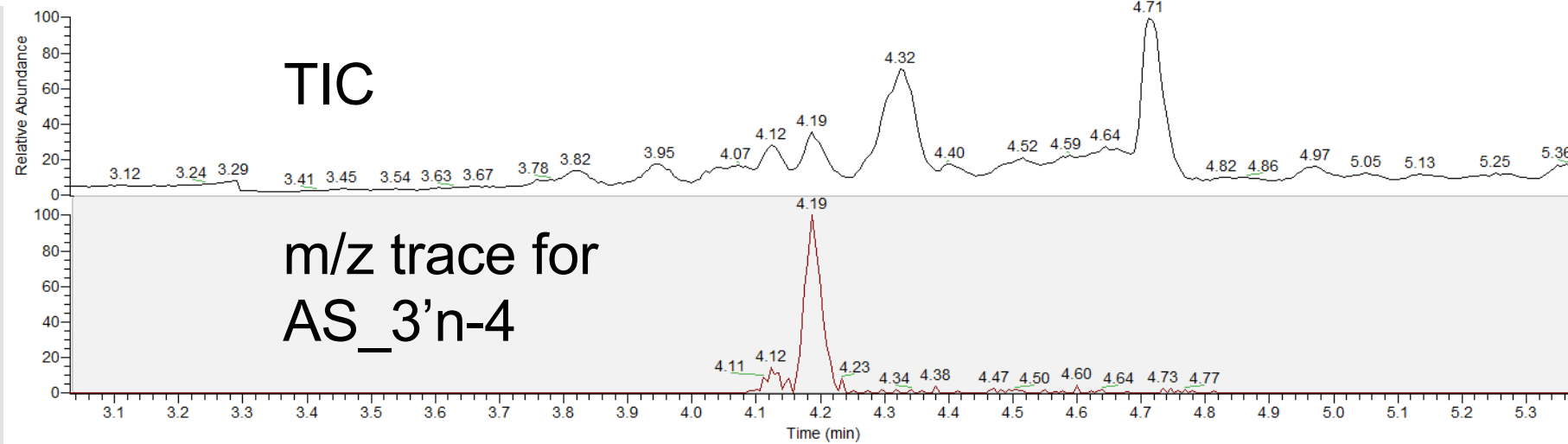
#Available chemical elements: H B C N O F Si P S Cl As Se Br I Na K Mg Ca Fe Co Cu Zn Pt

#Format:

#	Type	Abbrev	Name	Formula	Linker_only:d/w c/x	b/y	a/z
L	-		Phosphate	H;1;0;4;P;1	H;2;0;4;P;1	O;3;P;1	H;1;0;1 H;-1
L	~		Thioate	H;1;0;3;S;1;P;1	H;2;0;3;S;1;P;1	S;1;0;2;P;1	H;1;0;1 H;-1
L	-		Me-phosphonate	C;1;H;3;0;3;P;1	C;1;H;4;0;3;P;1	C;1;H;3;0;2;P;1	H;1;0;1 H;-1
L	=		Arsenic	H;1;0;4;As;1	H;2;0;4;As;1	O;3;As;1	H;1;0;1 H;-1
B	U		Uracil	H;3;C;4;N;2;O;2			
B	T		Thymine	H;5;C;5;N;2;O;2			
B	C		Cytosine	H;4;C;4;N;3;O;1			
B	G		Guanine	H;4;C;5;N;5;O;1			
B	A		Adenine	H;4;C;5;N;5			
B	X		Hypoxanthine	H;4;C;5;N;4;O;1			
B	H		Hydrogen	H;1			
B	R		Pseudouridine	H;3;C;4;N;2;O;2			
B	Z		Dummy base	H;0;C;0;N;0;O;0			
S	d		Desoxyribose	H;7;C;5;O;1			
S	r		Ribose	H;7;C;5;O;2			
S	m		MeO-ribose	H;9;C;6;O;2			
S	h		Homo-hexose	H;9;C;6;O;1			
S	f		2'F	H;6;C;5;O;1;F;1			
S	n		NAG25	H;107;C;61;N;8;O;31			
S	e		-3 GalNAC	H;68;C;37;N;5;O;16			
S	w		-2 GalNac	H;81;C;45;N;6;O;21			
S	o		-1 GalNac	H;94;C;53;N;7;O;26			
S	p		PEG linker	H;29;C;15;O;8			

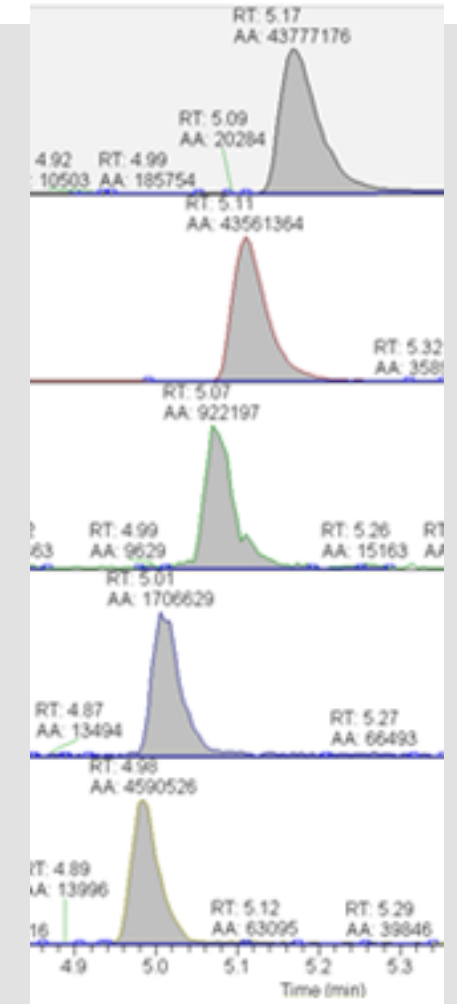
METABOLITES CAN BE CONFIDENTLY ANNOTATED FROM THE FULL-SCAN MS

RT: 3.03 - 5.38

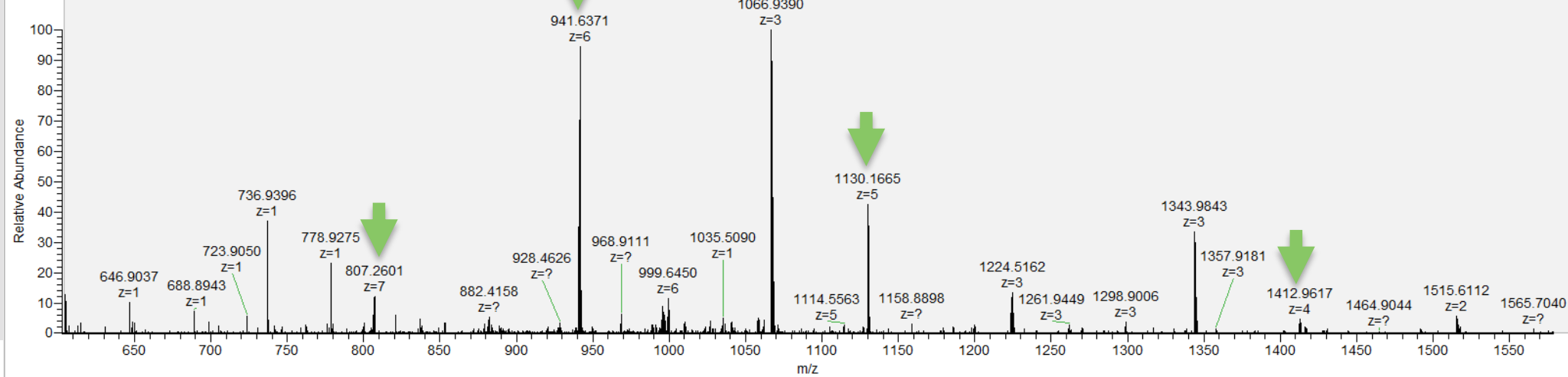


NL: 2.71E7
m/z= 600.00-2400.00 F: FTMS - p ESI sid=60.00 Full ms [600.0000-2400.0000] MS spe_3851_lh1_mase

NL: 6.86E4
Base Peak m/z= 941.25-941.35 F: FTMS - p ESI sid=60.00 Full ms [600.0000-2400.0000] MS spe_3851_lh1_mase

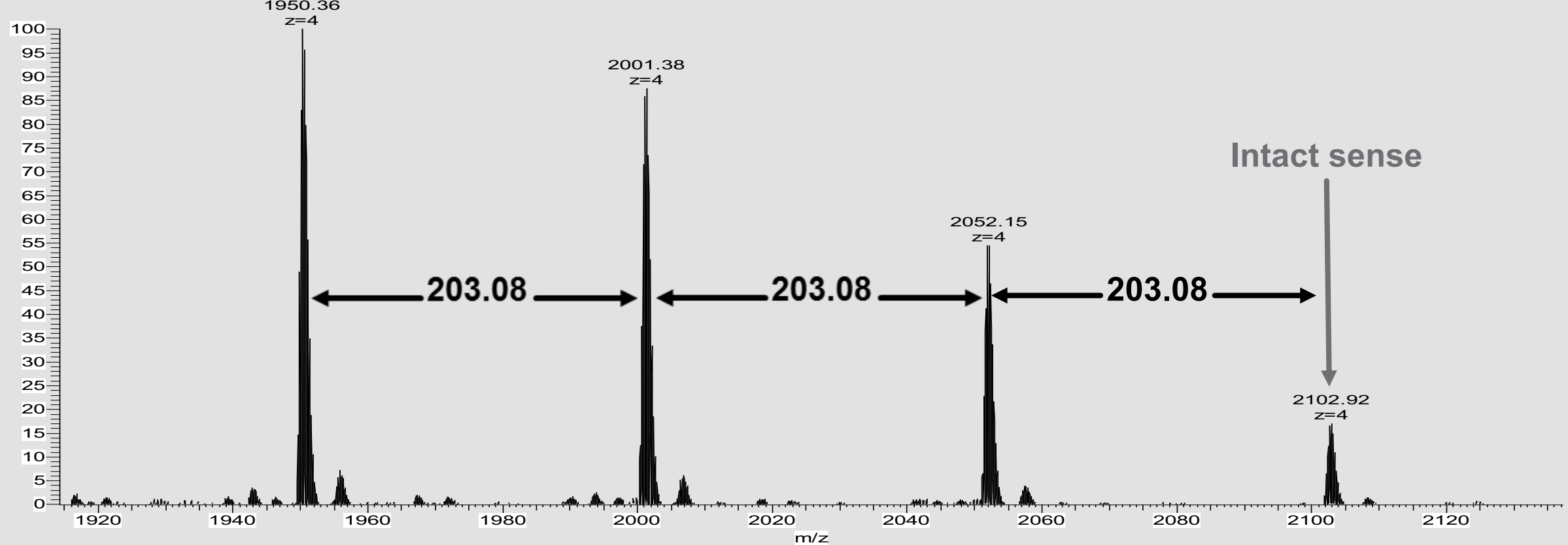


spe_3851_lh1_mase #741 RT: 4.19 AV: 1 NL: 2.13E5
F: FTMS - p ESI sid=60.00 Full ms [600.0000-2400.0000]

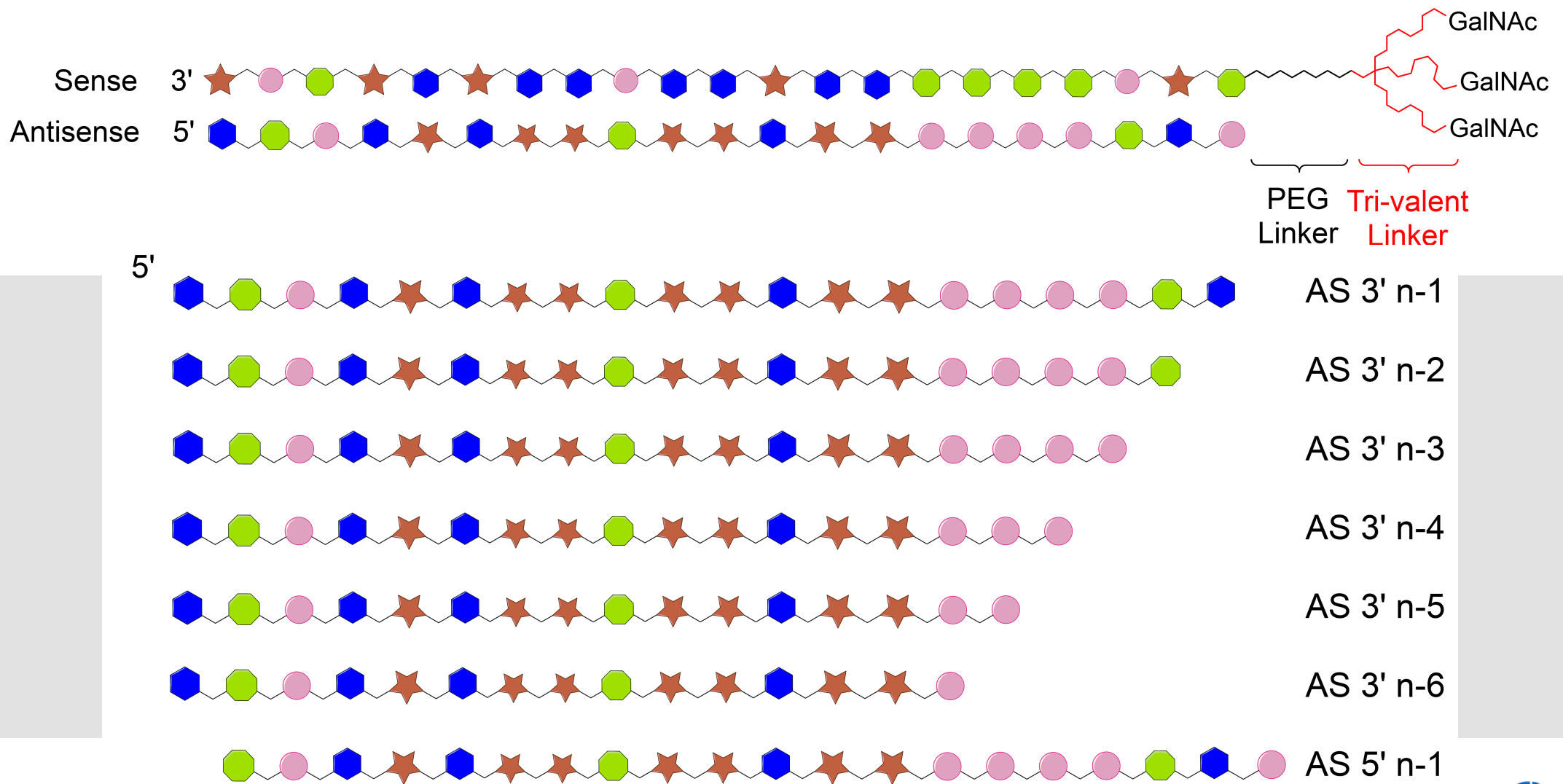


SENSE STRAND IS MAINLY METABOLIZED VIA THE SUCCESSIVE REMOVAL OF ITS *GalNAc* RESIDUES *in vitro*

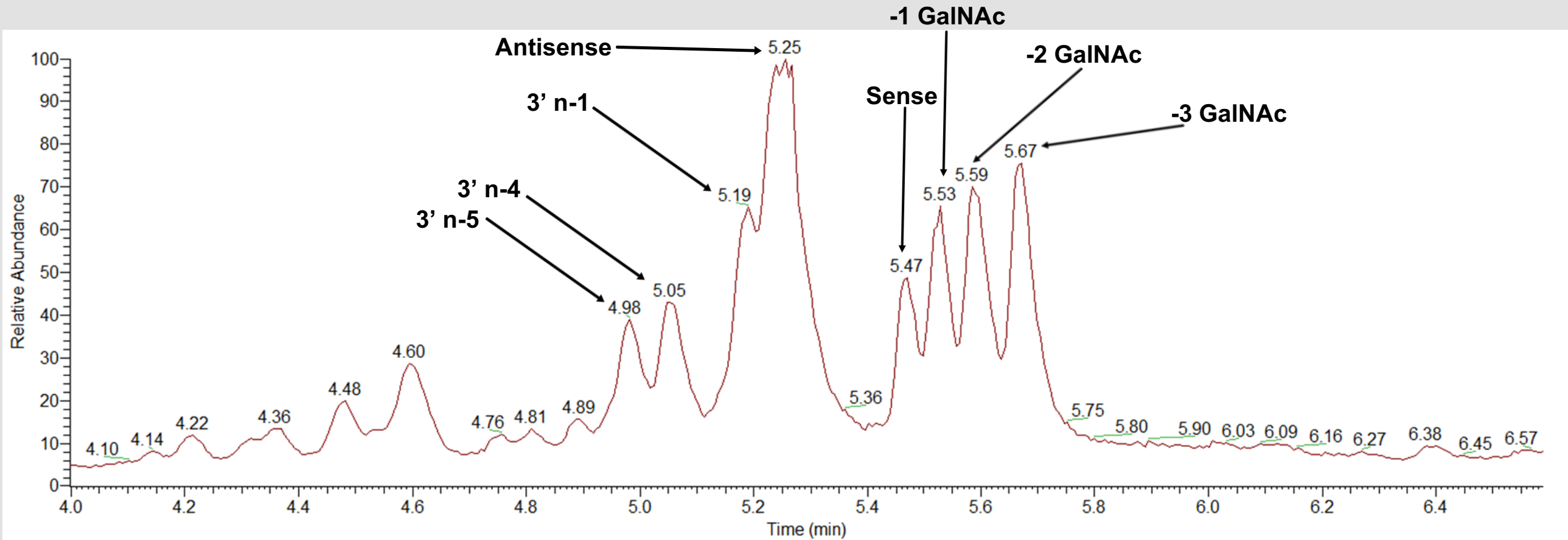
48h_pH_5 #1565-1715 RT: 6.23-6.64 AV: 151 NL: 1.53L
T: FTMS - p ESI sid=60.00 Full ms [800.0000-2400.0000]



ANTISENSE (GUIDE) STRAND METABOLITES

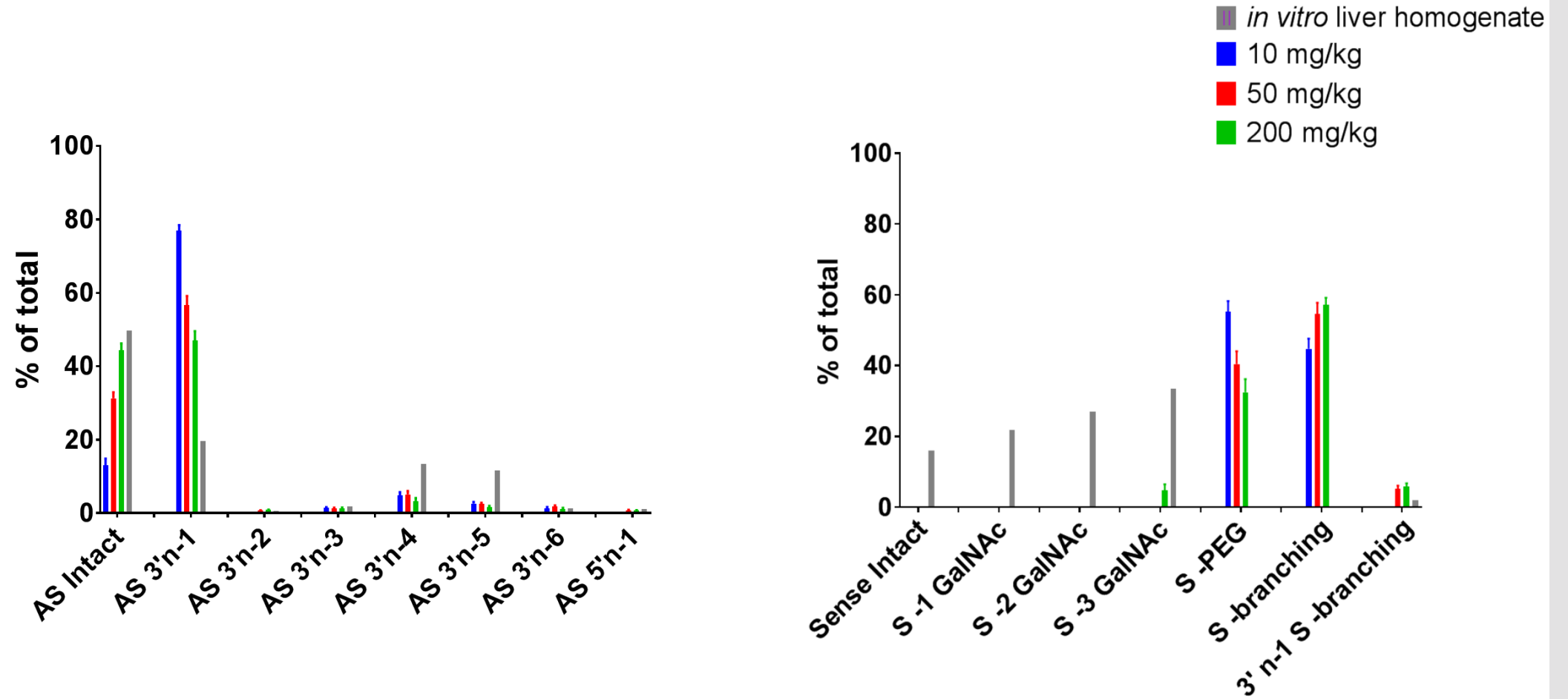


REPRESENTATIVE CHROMATOGRAM



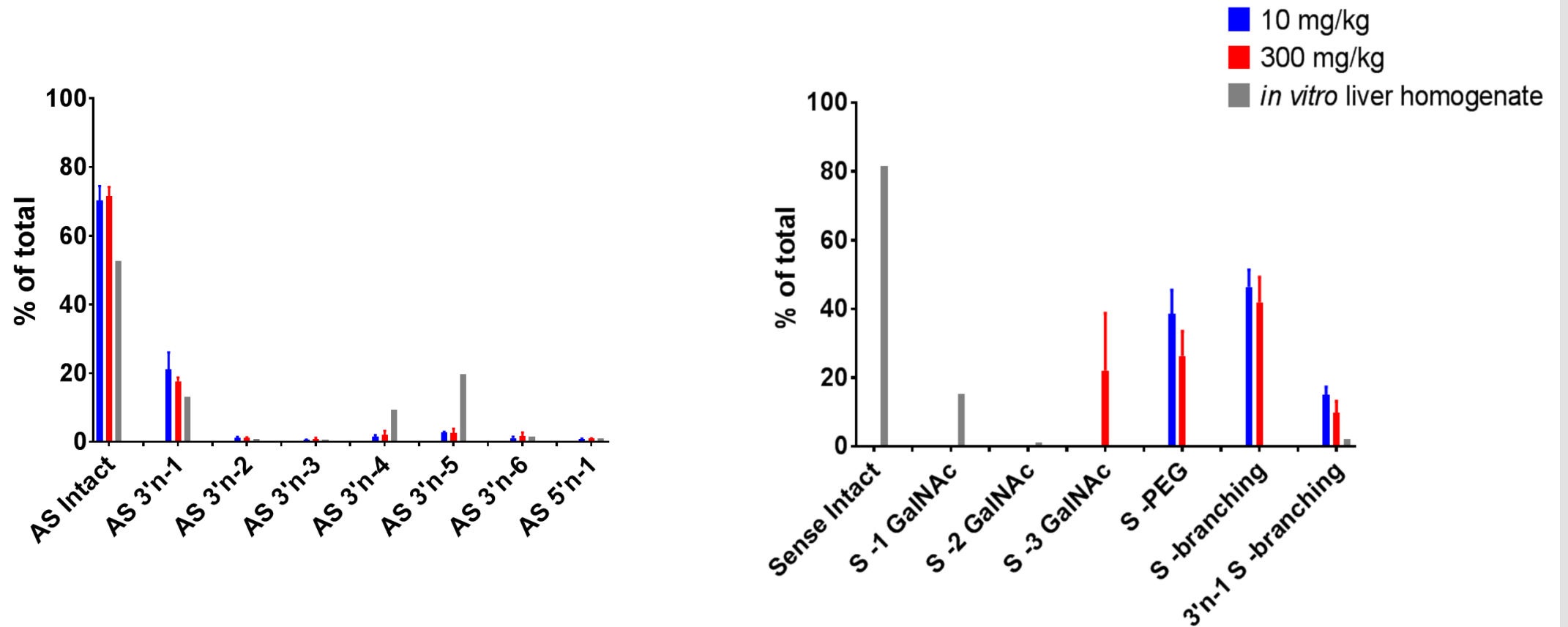
IN VITRO/IN VIVO CORRELATION

a) Rat



IN VITRO/IN VIVO CORRELATION

b) NHP



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- **Dan Rock**