Databasing Drug Metabolites to Influence Design-Make-Test Drug Discovery Cycles

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CPSA USA
Clinical & Pharmaceutical Solutions through Analysis
September 26 - 29, 2016
Sheraton Bucks County Hotel, Langhorne, PA

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What is Design - Make - Test?

Drug Metabolism Scientist

Chemist

DESIGN

MAKE

TEST

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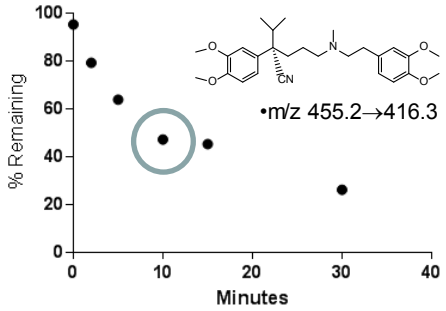
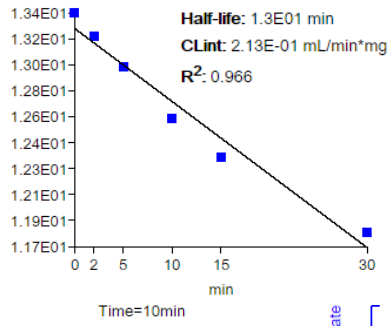
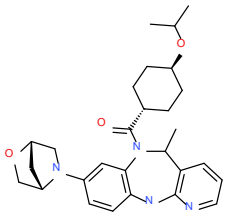
Drug Metabolism Involvement Traditionally in Test Phase

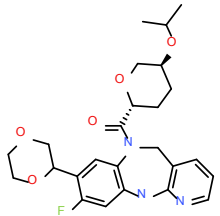
- Improve Pharmacokinetics (PK)
  - Metabolic Stability and Intrinsic Clearance (MSIC; CL_{int})
  - Metabolite Identification to aid MSIC
- Improve Safety
  - Reactive Metabolite Trapping & ID

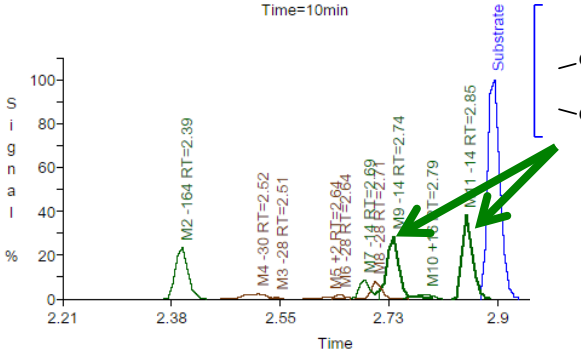


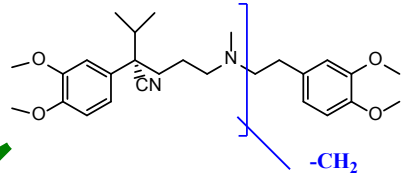




GSH
CN
Trapping







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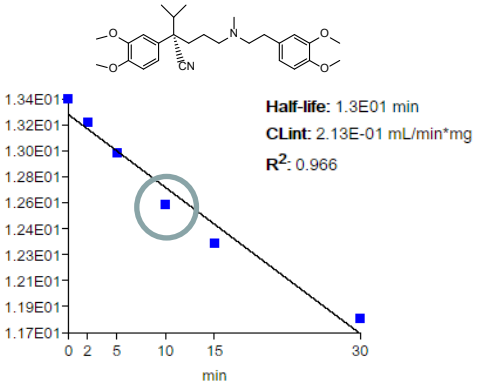
Early read on PK (or CL) via MSIC

DESIGN

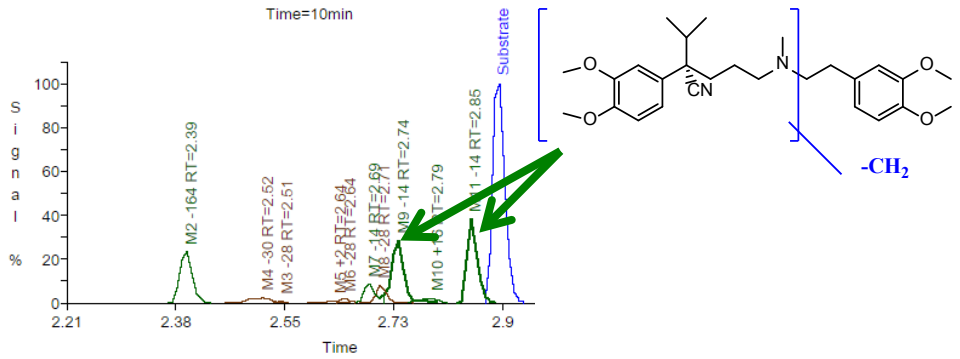
MAKE

TEST



1-2 Weeks; ~300 compounds / week

reTEST



Add another 1-2 Weeks; ~10 compounds / week

How to Better Inform?

- Structure Proposals
- Reduce Cycle Time

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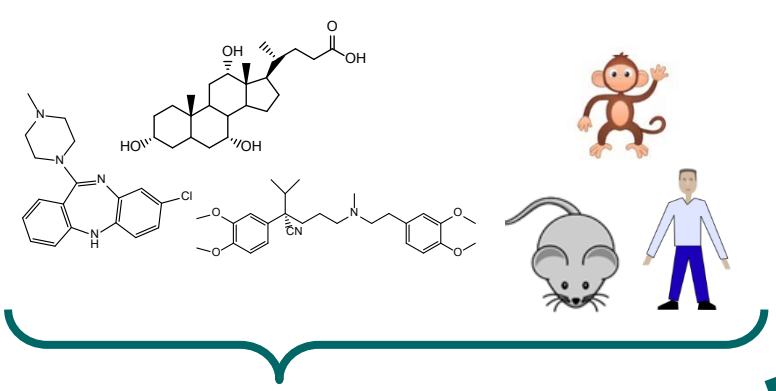
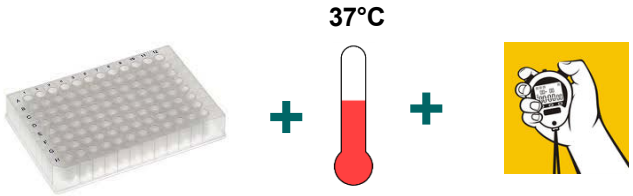
The Process of Metabolite Structure Proposal





MOLECULAR DISCOVERY WebMetabase
release-3.1.7

- Interpret
- Report / Communicate
- Store / Search
- Design

- Mass Spec Independent
- Acquisition Independent
  - DDA - Data Dependent Acquisition
  - DIA - Data Independent Acquisition
    - MS^E (Waters)
    - SWATH (Sciex)

    Sequential Windowed Acquisition of All Theoretical Fragment Ions
- In Silico Augmented
  - Site of metabolism prediction

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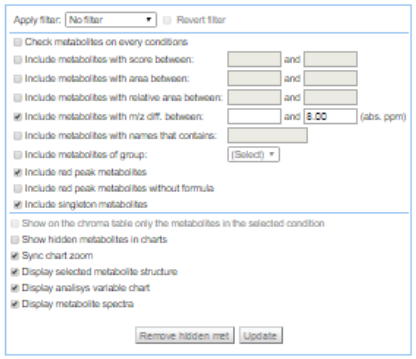
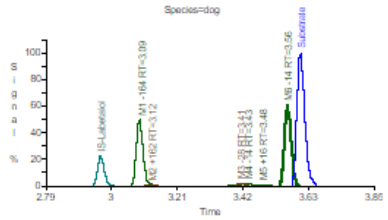
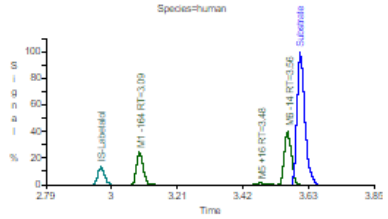
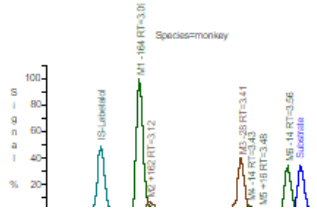
Interactive Data Views in WebMetabase



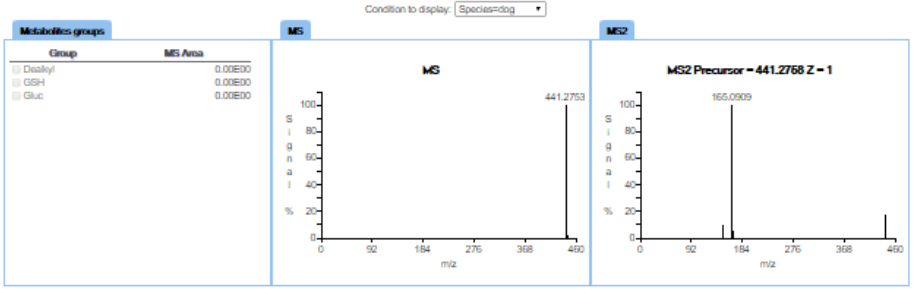


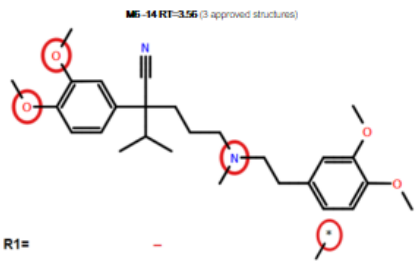




Name	RT	m/z	Mass shift	m/z diff (ppm)	Z	Area ABS	Area %	ion formula	Max score
IS-Labetalol	2.96 -2.97	329.1860 -329.1865		1.68 -2.98		1.23E05 -1.39E05			
M1 -164 RT=3.09	3.09	291.2065 -291.2082	-164.0837	-5.28 -0.66	1	1.56E05 -2.54E05	11.60 -45.49	$[C_{17}H_{26}N_2O_2 + H]^+$	408.9
M2 +162 RT=3.12	3.12	617.3057 -617.3087	+162.0164	-2.97 -1.89	1	4.23E03 -1.83E04	0.31 -3.34	$[C_{32}H_{44}N_2O_{10} + H]^+$	540.8
M3 -28 RT=3.41	3.41	427.2584 -427.2595	-28.0313	-0.82 -1.82	1	8.66E03 -1.05E05	0.65 -19.19	$[C_{25}H_{34}N_2O_4 + H]^+$	400.0
M4 -14 RT=3.43	3.43 -3.45	441.2747 -441.2756	-14.0157	-1.94 -0.10	1	9.37E03 -3.27E04	1.10 -2.43	$[C_{26}H_{36}N_2O_4 + H]^+$	400.0
M5 +16 RT=3.48	3.48 -3.49	471.2855 -471.2873	+15.9949	-4.17 --0.22	1	1.43E03 -2.08E04	0.26 -1.55	$[C_{27}H_{38}N_2O_5 + H]^+$	493.3
M6 -14 RT=3.56	3.56 -3.57	441.2753 -441.2759	-14.0157	-2.59 --1.13	1	8.17E04 -4.04E05	14.90 -30.13	$[C_{26}H_{36}N_2O_4 + H]^+$	482.4
Substrate	3.61	455.2911 -455.2918		-3.02 --1.48	1	1.08E06 -8.28E04	63.26 -15.11	$[C_{27}H_{38}N_2O_4 + H]^+$	

Condition to display: Species=dog





MD MOLECULAR DISCOVERY **WebMetabase**

release-3.1.7

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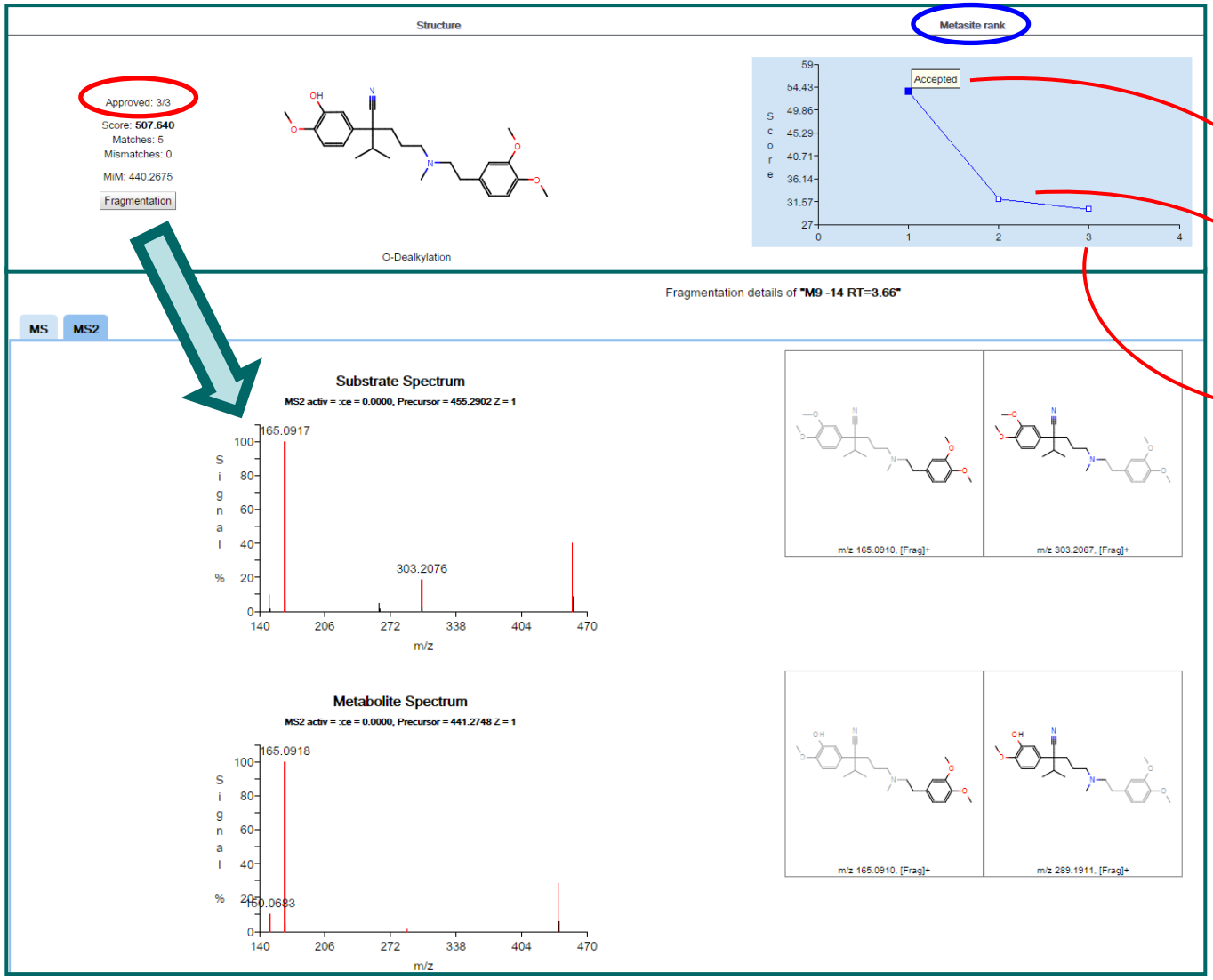
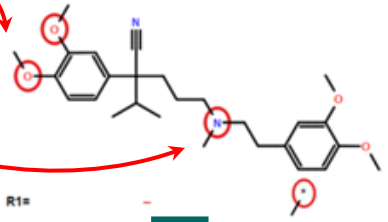
Data Interpretation & More Informed Proposals

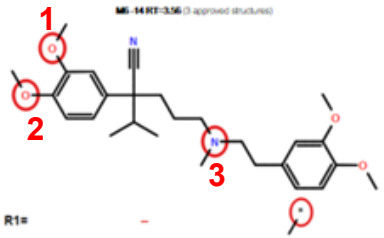


Zamora et al J. Med-.Chem. 2003
Cruciani et al., *J.Med.Chem.* 2005

$$P_{SoM,i} = (1 + w_e)E_i(1 + w_r)R_i$$

enzyme cavity flexibility accessibility	cofactor reactivity substrate reactivity mechanism of reaction
Recognition process	Reaction process
Binding affinity	Kinetic process





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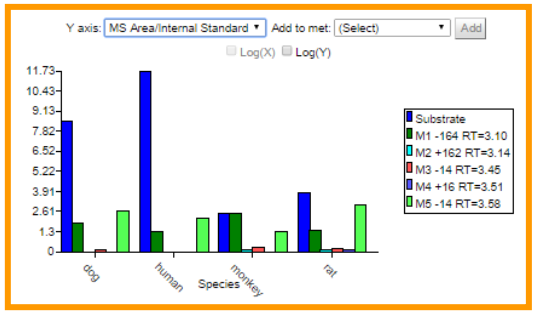
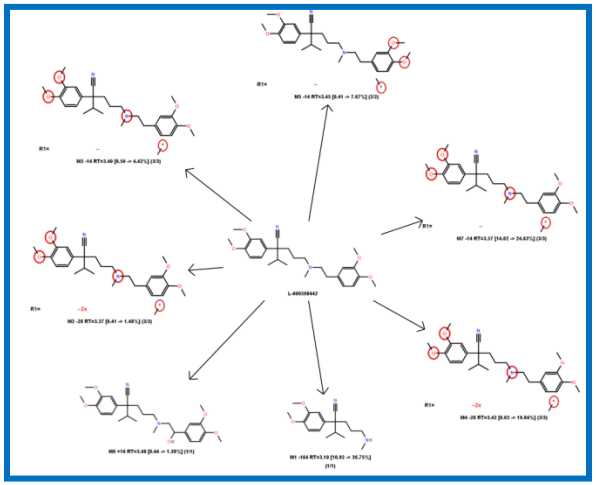
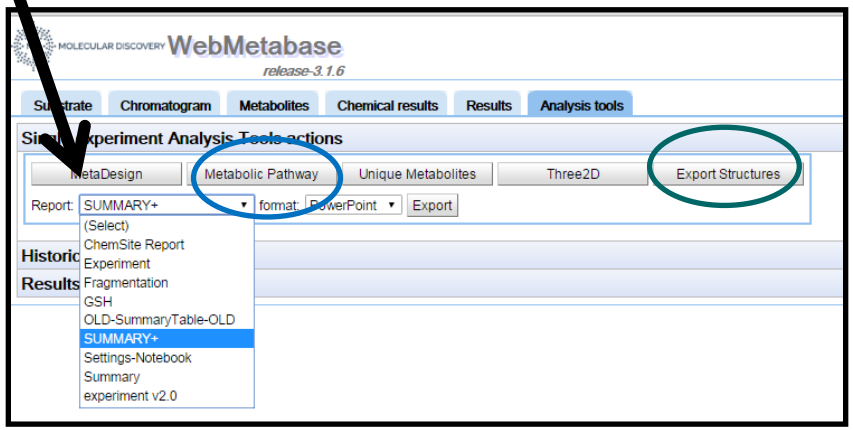
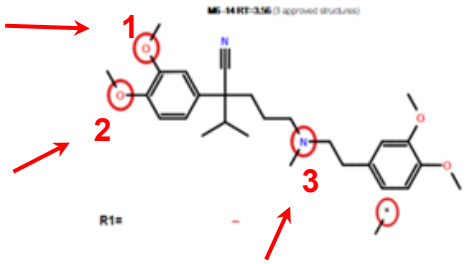
Communicating More Informed Structure Proposals via WebMetabase

- Metabolite chart
- Metabolic pathway
- Export structures in .sdf
  - in silico modeling
- Summary in PowerPoint
  - Metadata, chromatograms, metabolic pathway, table of metabolites, metabolite chart
  - make modifications before sending
    - add “appropriate” Metasite prediction
- Multi experiment reporting via Search









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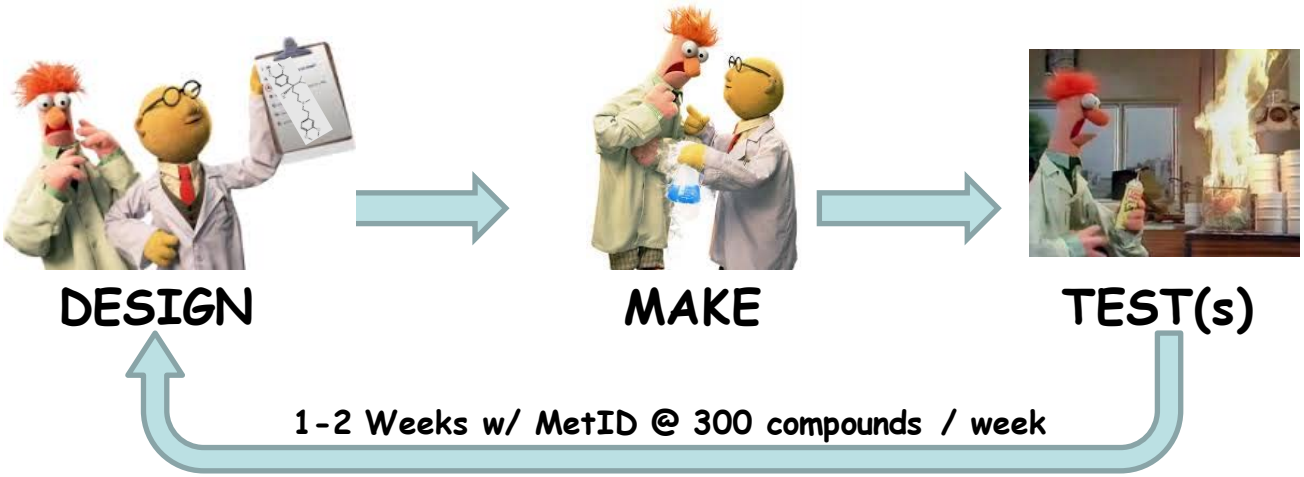
Routine WebMetabase Use Began in 2015

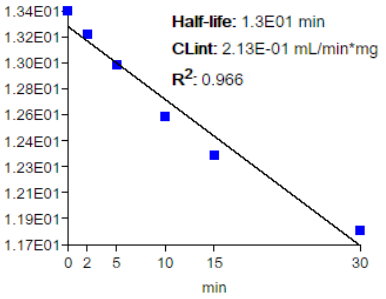
- The “Users” - today consist of:
  - mainly the MetID Scientists (~15)
  - a few ADME Principle Investigators
  - a few Medicinal Chemists

- The Data - standard non-labeled in vitro MetID assay
  - microsomes or hepatocytes
  - generally 1-time point (15, 30, 60, or 120 min)
  - species comparison (typically 2-4 species)
  - ~10 compounds per week

- ~600 Merck compounds across ~40 programs in WebMetabase

To Influence Tomorrow's Design... Need More Data and More Timely





- 2016 effort to...
  - acquire MSIC data via high resolution mass spectrometry (HRMS)
    - identify mass spectrometry platforms; identify CRO partners
  - automate software identification via MassMetasite / WebMetabase
  - visualize MetID data in tools like Spotfire® TIBCO Software (IT & Structural Chemistry)

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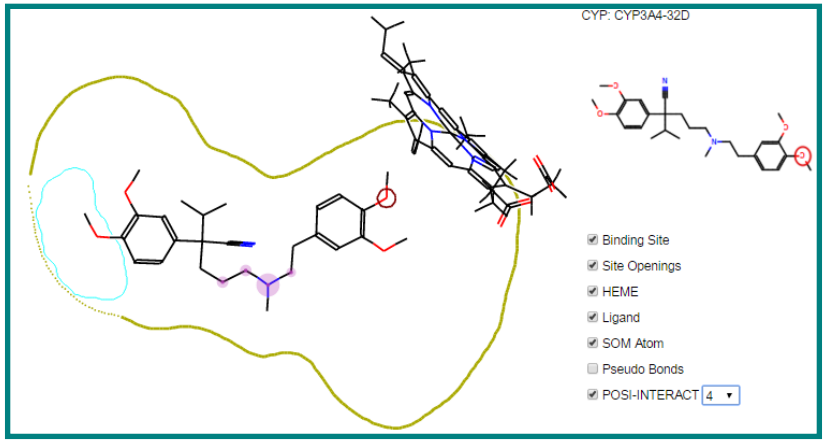
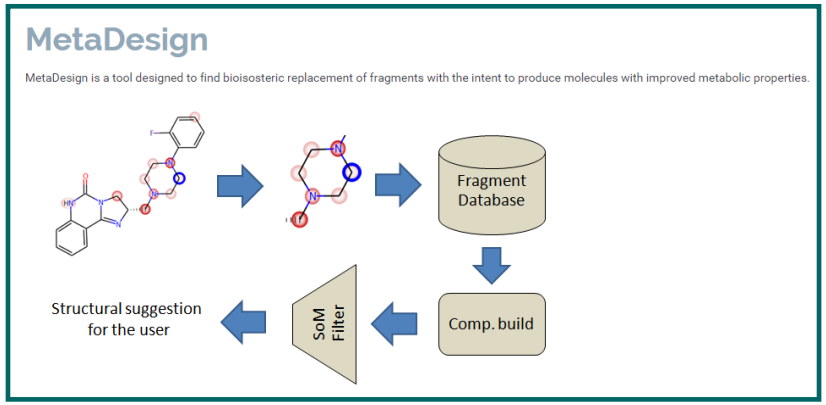
Influence Tomorrow's Design... with Ideating Tools in WebMetabase

- Three2D - CYP interaction modeling when positioning the site of metabolism next to the heme.



- MetaDesign - replace metabolic soft spots with bioisosteres



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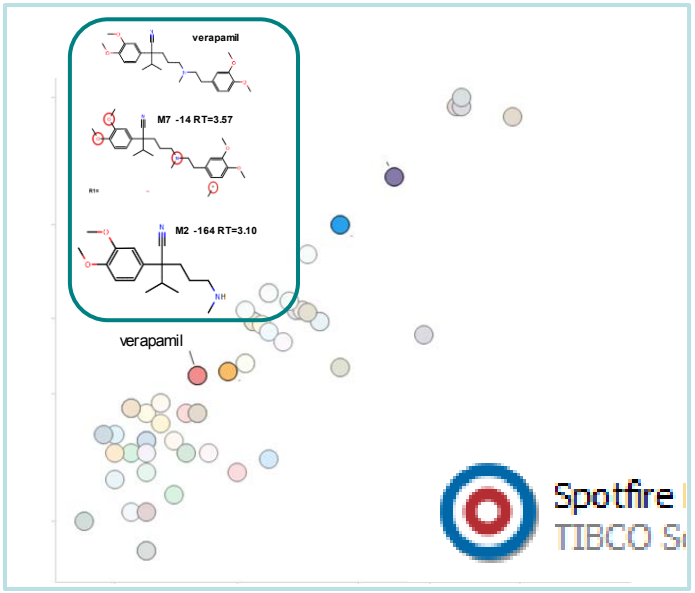
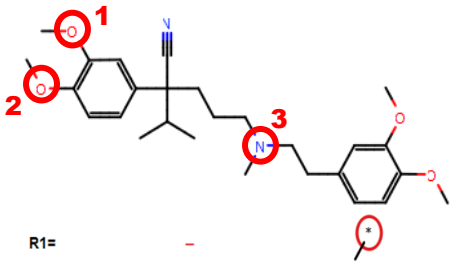
Tomorrow's Drug Metabolism in Design - Make - Test... More Timely and More Info

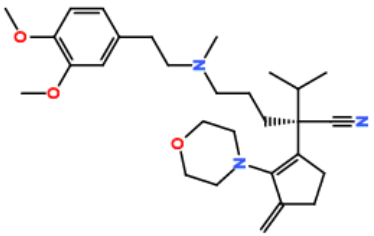
- Auto-generated info...
  - Metasite augmented Markush
  - Three2D
  - MetaDesign
- Get info out of WebMetabase and...
  - into other predictive tools (e.g. ADMET workbench)
  - use for model dev (e.g. phase II conjugation prediction)
  - visualize with other data in Spotfire



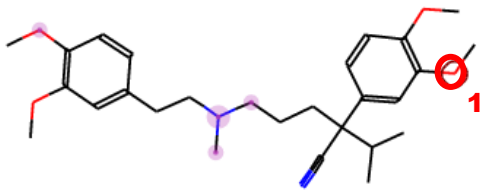
In silico augmented proposal



Design proposal



Docking for CYP active site interaction(s)



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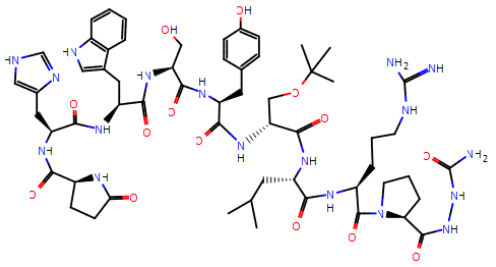
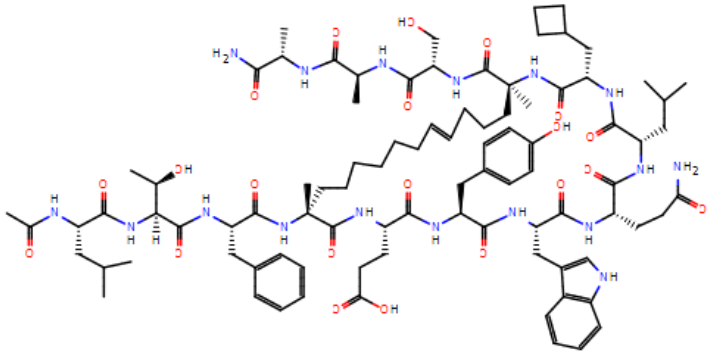
Metabolite Databasing and Predictive Tools for Peptides are Trailing those for Small Molecules

- Design - Make - Test Challenge: oral bioavailable therapeutic peptides
- Potential Key - understand the metabolic stability of these peptides within the body and in vitro systems that mimic bodily conditions
- Build Tools - to aid therapeutic peptide Design-Make-Test cycles
  - process high resolution mass spec data of peptides
    - multi-charged, non-natural amino acid containing, stapled, etc.
    - identify metabolites - possibilities large
  - visualize, communicate, database, and even interrogate large sets of peptide metabolic stability data





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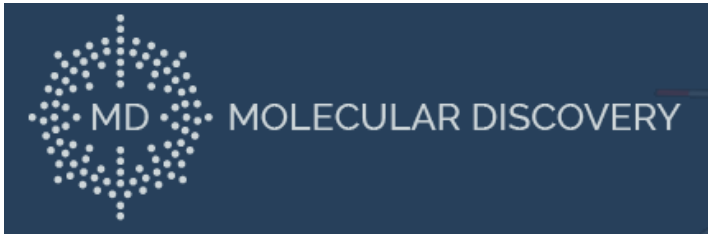
Collaborative Project to Interrogate Metabolic Stability Data of Peptides

- with Lead Molecular Design (LMD) and Molecular Discovery (MD)



- Merck internship to generate / provide metabolic stability data (parent loss and metabolite formation over time) for 30-40 peptides in variety of in vitro systems
  - No sharable data sets currently exist at Merck ("firewalled" or limited variables)

- PhD project of LMD to develop new peptide version of WebMetabase for viewing, searching, and data interrogation capabilities.

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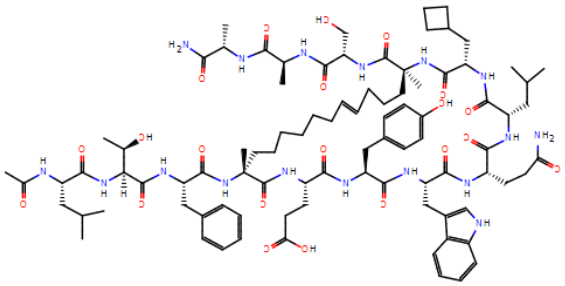
What Peptides to Study?

- Chang et al (2013) PNAS 110:E3445-3454:
  - 20+ analogs of stapled peptide ATSP-7041
  - Acteyl-LTF-X-EYWQL-Cba-Y-SAA-amide

    X = R-stereochemistry for alkenyl side chain (CH2-CH2-CH2-CH2-CH2-CH2-CH=CH2) and α-methyl substitution

    Y = S-stereochemistry for alkenyl side chain (CH2-CH2-CH2-CH=CH2) and α-methyl substitution

    Cba = cyclcobutyl-Ala



Agency for Science, Technology and Research
SINGAPORE

- Wang et al. (2015) Mol. Pharmaceutics 12, 966−973:

molecular pharmaceutics

Article

pubs.acs.org/molecularpharmaceutics

Toward Oral Delivery of Biopharmaceuticals: An Assessment of the Gastrointestinal Stability of 17 Peptide Drugs

Jie Wang, Vipul Yadav, Alice L. Smart, Shinichiro Tajiri,[†] and Abdul W. Basit*

Department of Pharmaceutics, UCL School of Pharmacy, University College London, WC1N 1AX London, U.K.

Peptides

Buserelin
Calcitonin (salmon)
Deslorelin
Glucagon
Gonadorelin
Goserelin
Histrelin
Leuprolide
Octreotide
Oxytocin
Secretin Human
Terlipressin
Lys-Vasopressin

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What Matrices or Systems to Study?

- MD wish-list has many human proteases

- In-house cookbook:
  - porcine pepsin and elastase
  - bovine trypsin and α-chymotrypsin
  - Simulated intestinal fluid (SIF) - pancreatin
    - pancreatin has trypsin, amylase, and lipase
  - with and without protease (buffer controls)

- Matrices of interest
  - blood, plasma, and serum
  - tritosomes (lysosomal lysate)
  - intestinal - contents (e.g. feces), microsomes, cytosol, S9
  - liver - microsomes, cytosol, S9, lysosomes, homogenate

- Does species / gender of matrix matter?

Tier-1

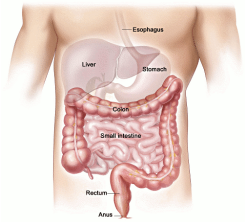
caspase-1
caspase-3
caspase-6
caspase-7
cathepsin B
cathepsin D
cathepsin G
cathepsin K
cathepsin L
elastase-2
granzyme A
granzyme B
matrix metallopeptidase-2 (MMP2)
MMP7
MMP9
thrombin
trypsin-1

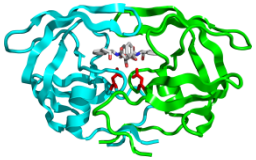
Tier-2

HIV-1 retro pepsin
Calpain-1
Caspase-8
Matrix metallopeptidase-3
Chymotrypsin A (bovine)
Elastase-2
Plasmin
Glutamyl peptidase I
Furin
Signal peptidase I
Thylakoidal processing peptidase
Signalase

The Process of Metabolic Stability

Acteyl-LTF-X-EYWQL-Cba-Y-SAA-amide





Proteases

\+ 37°C +

Use of HP D300e ability to dispense nL of DMSO stock

Mass Spectrometer

- Thermo Ion-Trap
- Thermo Orbitrap
- Agilent Q-TOF
- Waters Q-TOF
- ABSciex Triple-TOF
- Bruker Q-TOF

MD MOLECULAR DISCOVERY **WebMetabase**

release-3.1.7

- Interpret
- Report / Communicate
- Store / Search
- Interrogate
- Design

Mass-MetaSite3 © Molecular Discovery

- Mass Spec Independent
- Data Acquisition Independent

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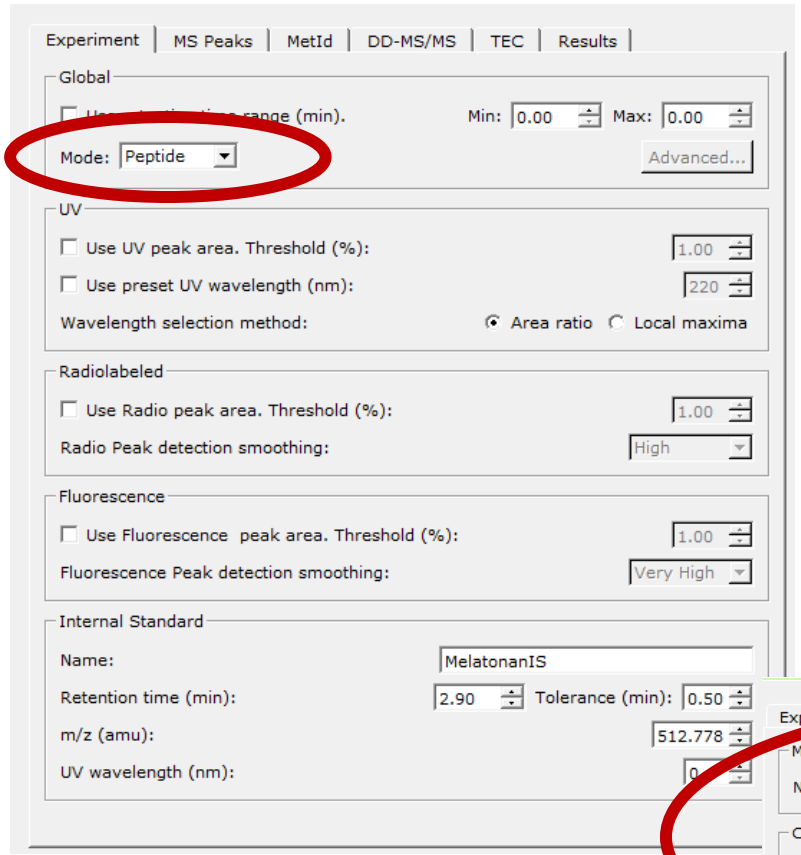
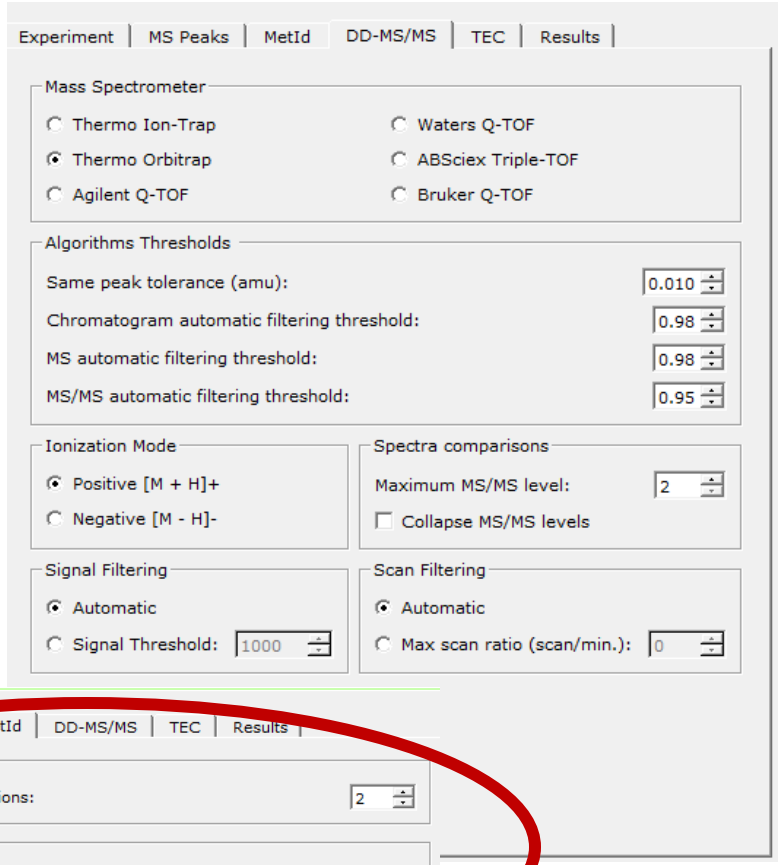
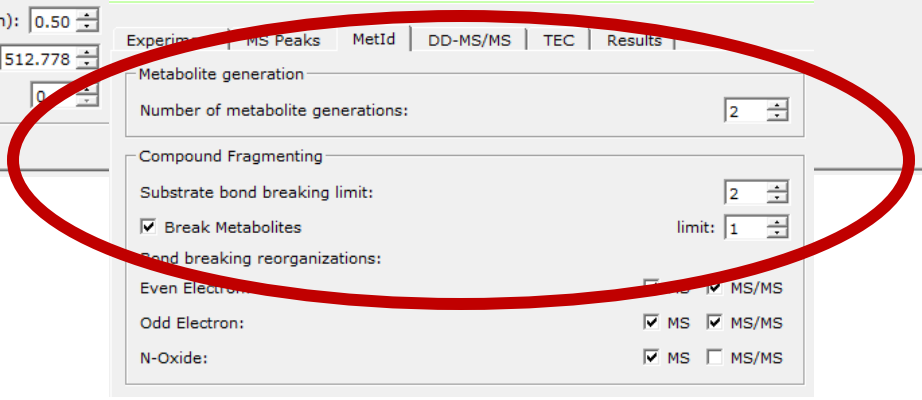
Important MassMetasite Settings







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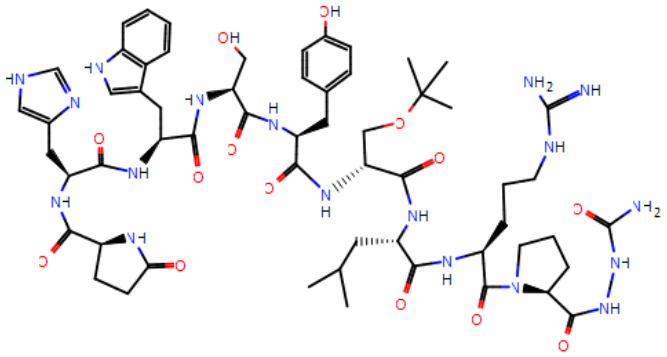
Peptides in Wang et al. (2015) Mol. Pharmaceutics 12, 966−973

Peptides
Buserelin
Calcitonin (salmon)
Deslorelin
Glucagon
Gonadorelin
Goserelin
Histrelin
Leuprolide
Octreotide
Oxytocin
Secretin Human
Terlipressin
Lys-Vasopressin



molecular pharmaceutics

Article

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Toward Oral Delivery of Biopharmaceuticals: An Assessment of the Gastrointestinal Stability of 17 Peptide Drugs

Jie Wang, Vipul Yadav, Alice L. Smart, Shinichiro Tajiri,† and Abdul W. Basit*

Department of Pharmaceutics, UCL School of Pharmacy, University College London, WC1N 1AX London, U.K.

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LC Method Comparison

	Wang et al.
Column	Luna C18 150 x 4.6 mm, 5 um
Temperature	33°C
Injection Volume	50µL
Flow rate	1 mL/min
UV absorbance	214 nm
Mobile Phase	Water (0.1%TFA)/acetonitrile (78:22)
Stop Solution	3 volumes methanol
HPLC System	Agilent Technologies, 1260 Infinity
UV detector	Diode array model G1329B
Pepsin Concentration	3.2mg/mL pH(25°C) 1.2
Initial Peptide Concentration	0.67µM
Run Time	≥18 min

	In house
Column	Aquity BEH C18, 100 x 2.1 mm, 1.7um,
Temperature	60°C
Injection Volume	3µL
Flow rate	0.5 mL/min
UV absorbance	N/A
Mobile Phase	Water (0.1%FA) /acetonitrile (0.1%FA)
Stop Solution	2 volumes 80:20 Acetonitrile:FA
HPLC System	Waters Aquity
Instrument	Thermo Scientific LTQ Orbitrap
Pepsin Concentration	0.1mg/mL pH(25°C) 1.0-1.4
Initial Peptide Concentration	10µM

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Time (min)	Flow (mL/min)	%A	%B
Initial	0.50	90	10
0.50	0.50	90	10
3.50	0.50	60	40
4.00	0.50	10	90
4.20	0.50	90	10
5.25	0.50	10	90
6.00	0.50	90	10

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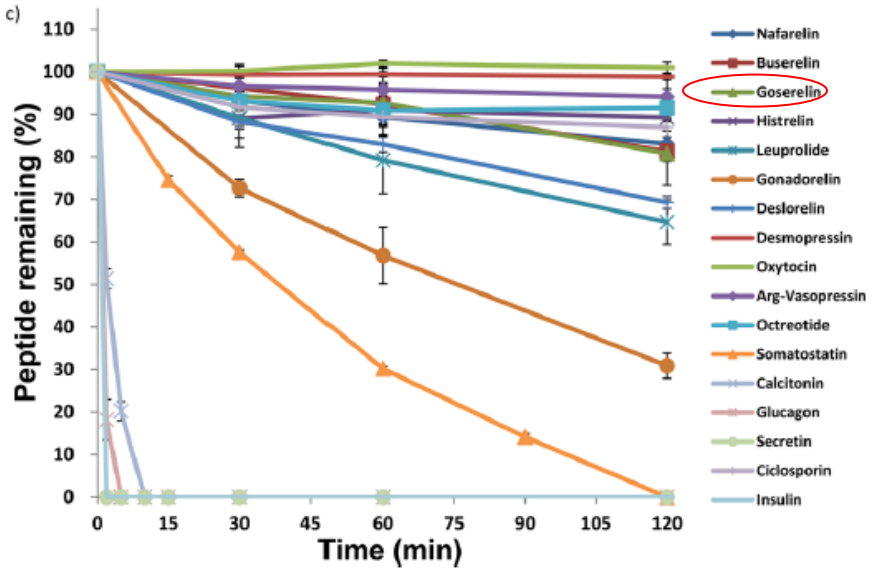
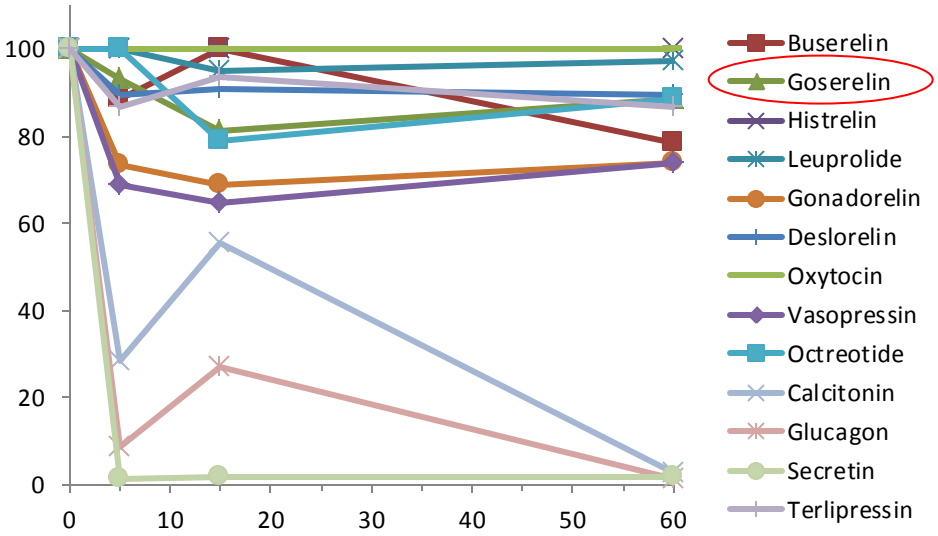
Parent Loss for Goserelin in Pepsin in Gastric Simulated Fluid

Wang et al.	
Pepsin Concentration	3.2mg/mL pH(25°C) 1.2
Initial Peptide Concentration	0.67μM
Parent Loss	~10%

In House (intern Alison Bateman)	
Pepsin Concentration	0.1mg/mL pH(25°C) 1.0-1.4
Initial Peptide Concentration	10μM
Parent Loss	~10%





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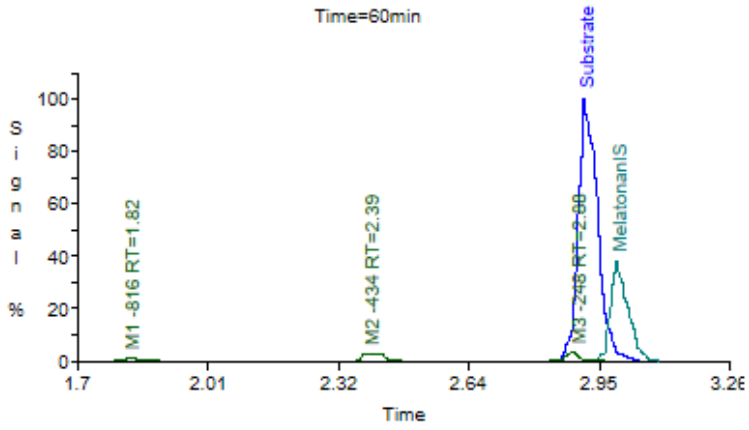
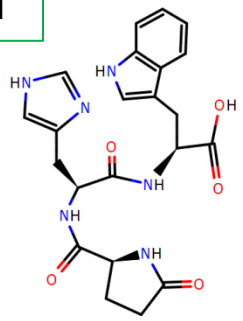
Visualize Parent Loss (Goserelin) and Metabolite Formation in WebMetabase



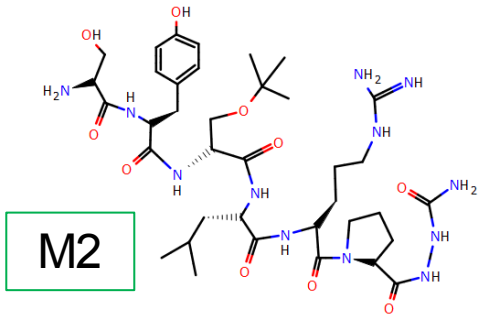
Matrix:
Pepsin in Simulated Gastric Fluid

Peptide:
Goserelin
Exact Mass:1268.6414

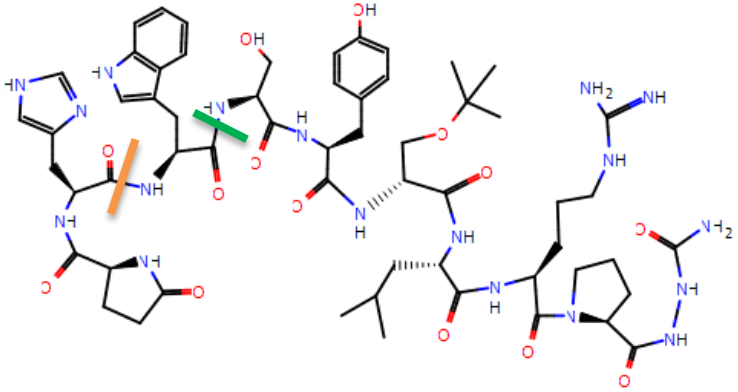
M1



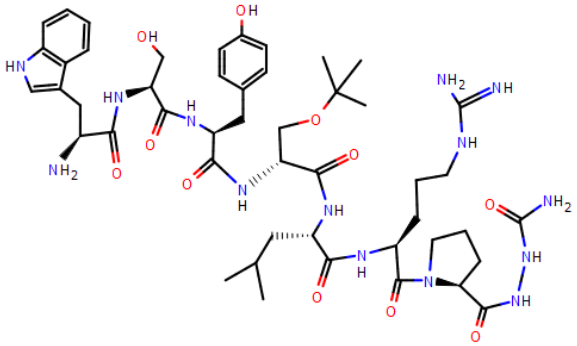
M2



Substrate



M3



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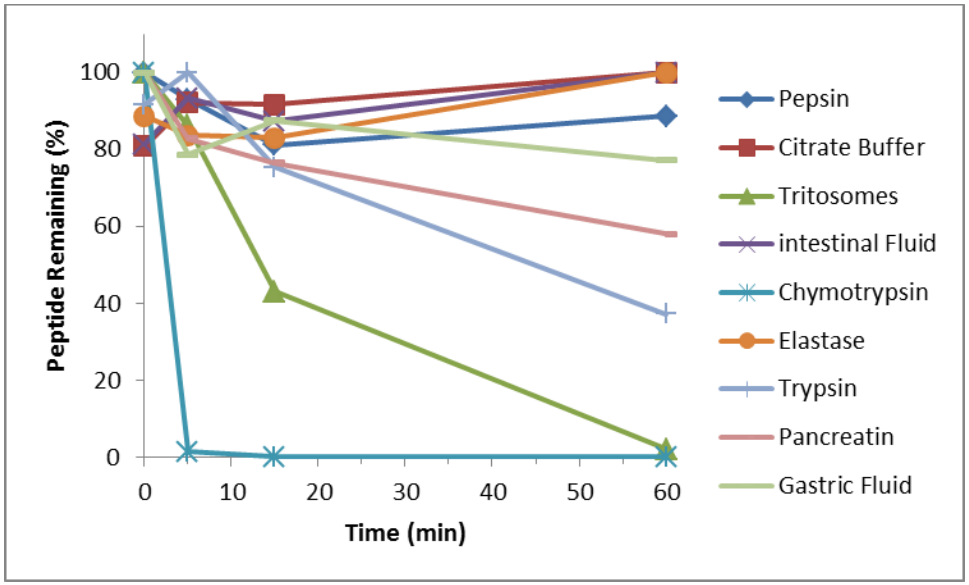
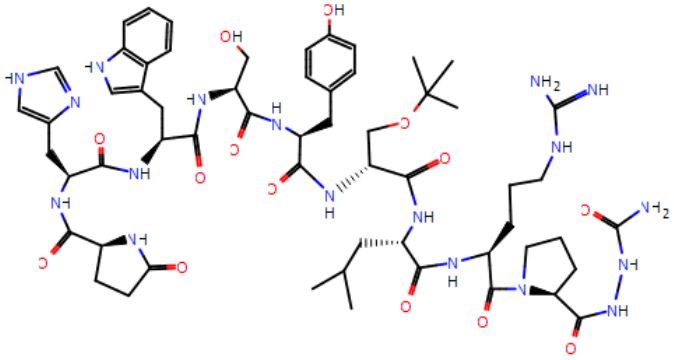
Goserelin stability in different matrices over time

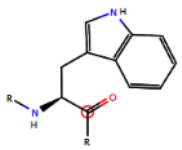
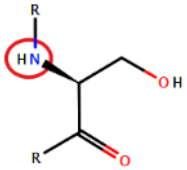
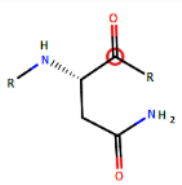
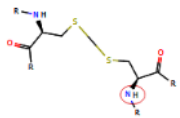
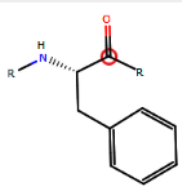
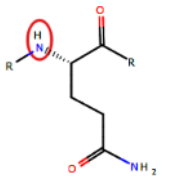
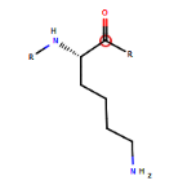
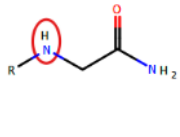




- Manually plotted in excel
- WebMetabase can do if setup proper data input (protocol)

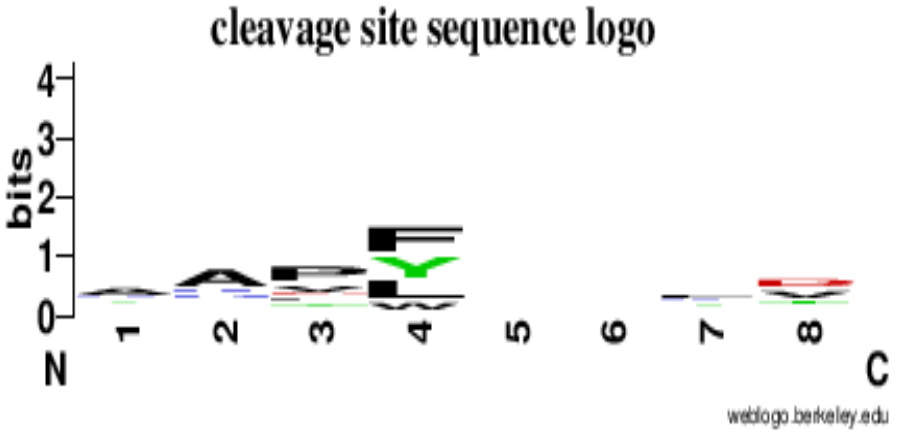
Frequency Analysis of Chymotrypsin Metabolism of Thirteen Wang et al. Peptides

Global results | Detailed results

Monomer C	Monomer N	Matrix	Bondtype	Amount of times this bond was met	Amount of times this bond was broken once
		Chymotrypsin		6	6
		Chymotrypsin		1	2
		Chymotrypsin		2	2
		Chymotrypsin		2	2

Tatiana Radchenko; Ph.D. candidate

Can One Harness a Peptide Database to Visualize Monomer Specificities with Respect to Cleavage?



http://merops.sanger.ac.uk/about/glossary.shtml#SUBSITE

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Data Challenges and Next Steps to Aid Design

- Visualization and Usage!
  - big complicated molecules - finite screen space
  - frequency analyses; structure-activity relationships (SAR)
  - data aggregation with use of software tools like Spotfire
  - linking / exporting data to other software tools

- Automation!
  - data generation - need more data
    - incubation
    - metabolite identification (software automated MetID)
  - computation
    - small molecule - CYP interactions
    - design

Acknowledgments

- Molecular Discovery and Lead Molecular Design
- Mark Cancilla and the PPDM Metabolite Identification & Tissue Distribution group
- Anthony Partridge, Tomi Sawyer, & A*STAR
- Rodger Tracy and Ken Koeplinger
- Alison Bateman and Tatiana Radchenko
- Kevin Bateman and Conrad Raab