Identification of Novel Proteolytic Pathways by Proteomics

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BURNHAM INSTITUTE
for MEDICAL RESEARCH
From Research, the Power to Cure

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Proteolytic Signatures

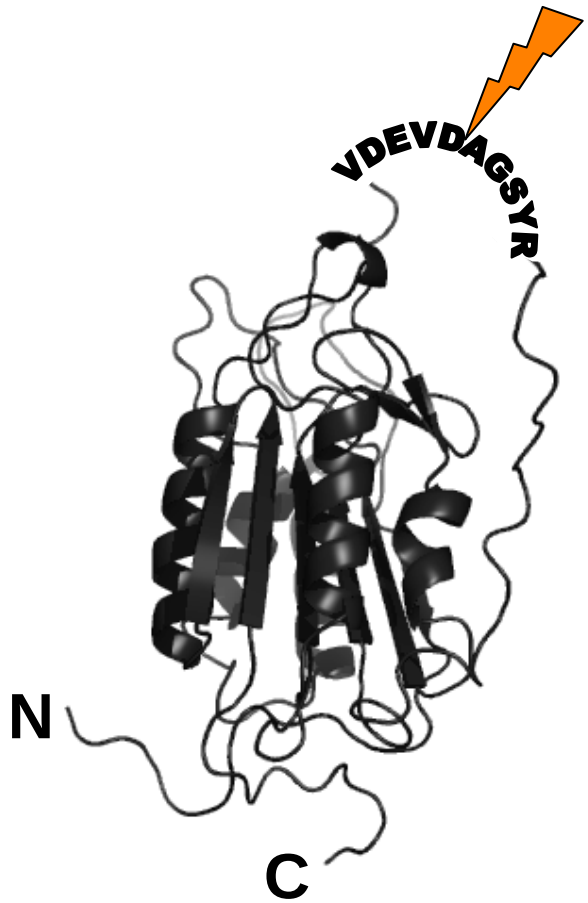
- The Chemistry
- What we are interested in
- Samples of progress
- Outreach collaborations

Profiling Proteolytic Signatures

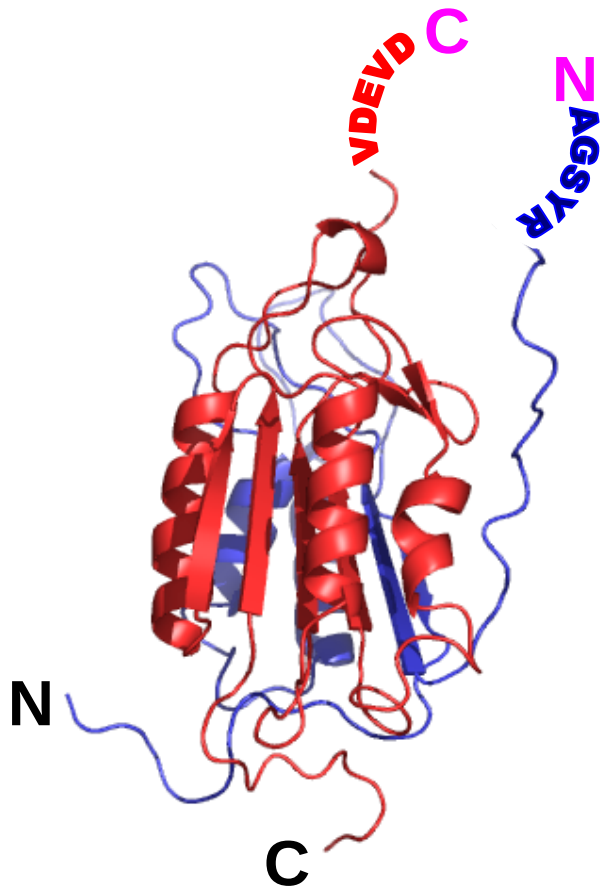
Mari Enockson

John Timmer

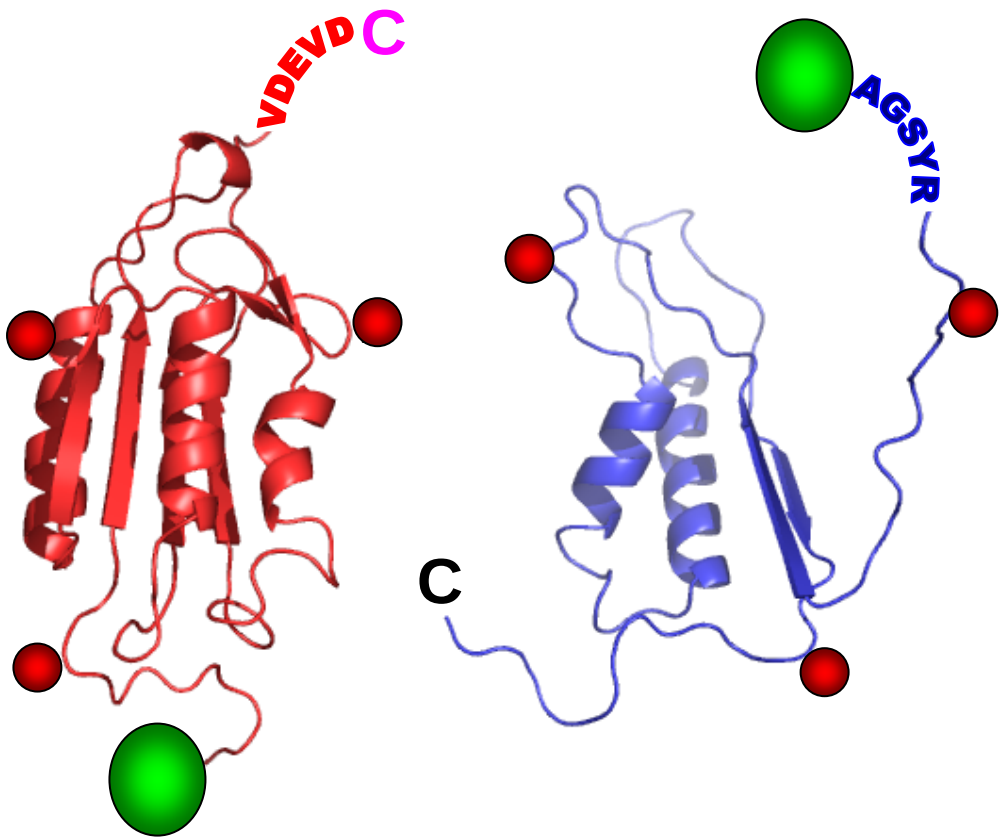
Specific limited proteolysis



Specific limited proteolysis

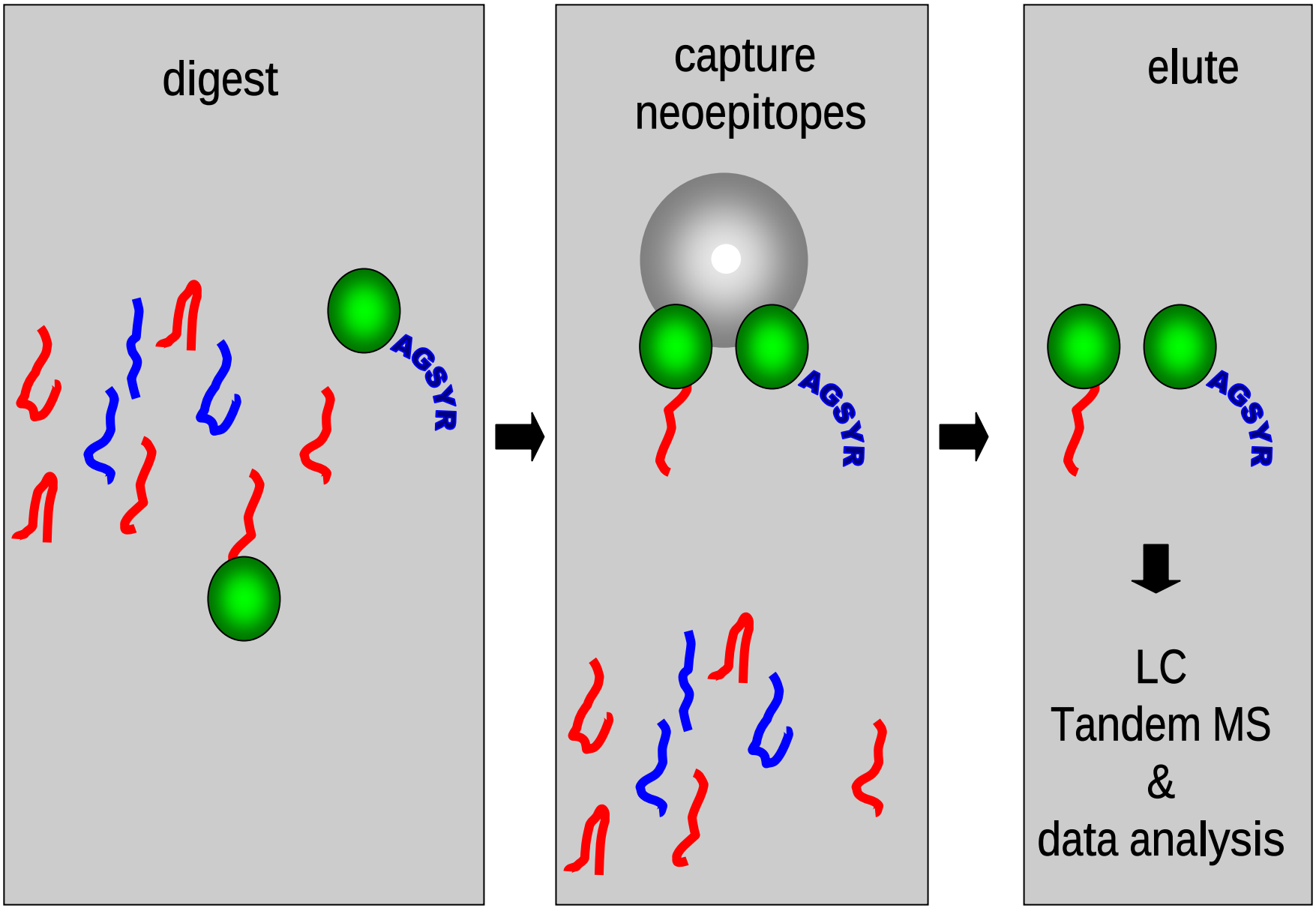


Specific limited chemistry



Block Lys side-chains

Affinity label N-terminus


digest
AGSYR
capture
neoepitopes
AGSYR
elute
AGSYR
LC
Tandem MS
&
data analysis

Proteolytic Signatures

- Define the proteolytic “fingerprint” of a biological sample
  - Trace back to culpable proteases
  - Chemical genomics & activity-based probes
- Protease substrate validation and discovery
- Proteases in Disease
  - Parallel samples
  - System purtubation
- Biomarker & therapeutic target discovery
  - Proteases can be imaged
  - Proteolytic products are stable

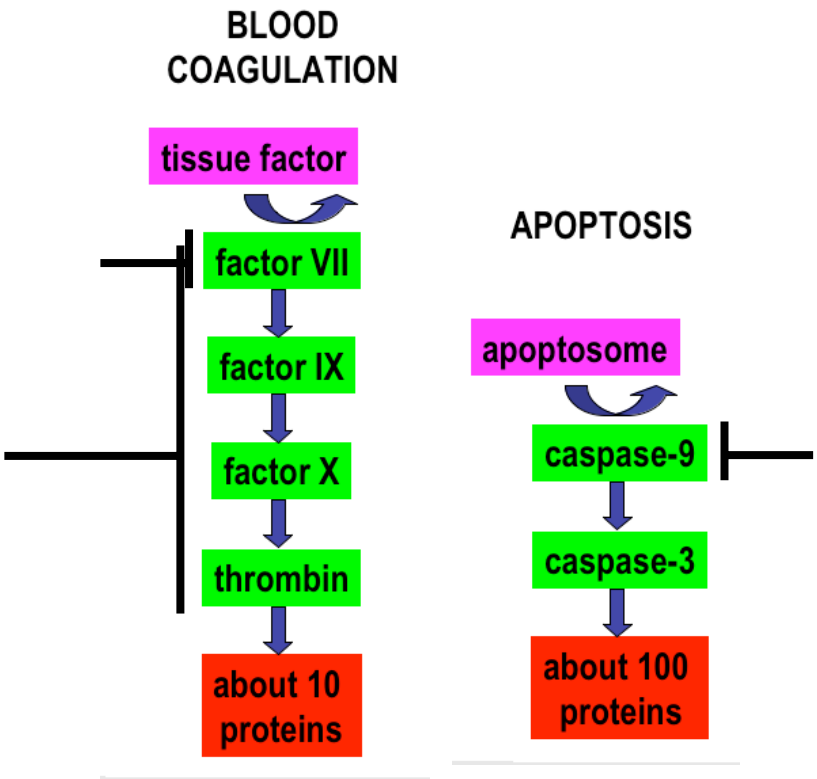
Proteolytic Signatures

Proteome simplification

One peptide only for most proteins

2-3 peptides for internally processed proteins

Tracking the origin of a proteolytic signature



proteolytic products

Validation

- *E. coli* soluble proteins
  - Met N-termini
  - Met-removed N-termini
  - Signal peptide processing
- Yeast proteins
  - ….
- Mouse proteins
  - ….
  - Mitochondrial import
- Human plasma and HEK293 cell proteins
  - ….
  - Mitochondrial import
  - Secreted proteins

NATIVE N-TERMINI (UNPROCESSED)

E. Coli Soluble Proteins (Met intact)

-.MFTGIVQGTAK.L

-.MFTGSIVAIVTPMDEKGNVCR.A

-.MHENQQPQTEAFELSAAER.E

-.MHITYDLPVAIDDIIEAK.Q

-.MHITYDLPVAIDDIIEAKQR.L

-.MIDKSAFVHPTAIVEE.G

-.MIDTTLPLTDIHR.H

-.MIGLVGK.K

NATIVE N-TERMINI (Initiator *Met* Removed)

E. Coli Soluble Proteins (Met removed)

M.GTTTMGVKLDDATR.E

M.GTTTMGVKLDDATRE.R

M.PANARSHAVLTTE.S

M.PEATPFQVMIVDDHPLMR.R

M.PPLNGAVM[147]HPVAHTGVRKM[147]VDK.I

M.PQQNYLDELTPAFTSLLAIK.E

M.PTSHENALQQR.C

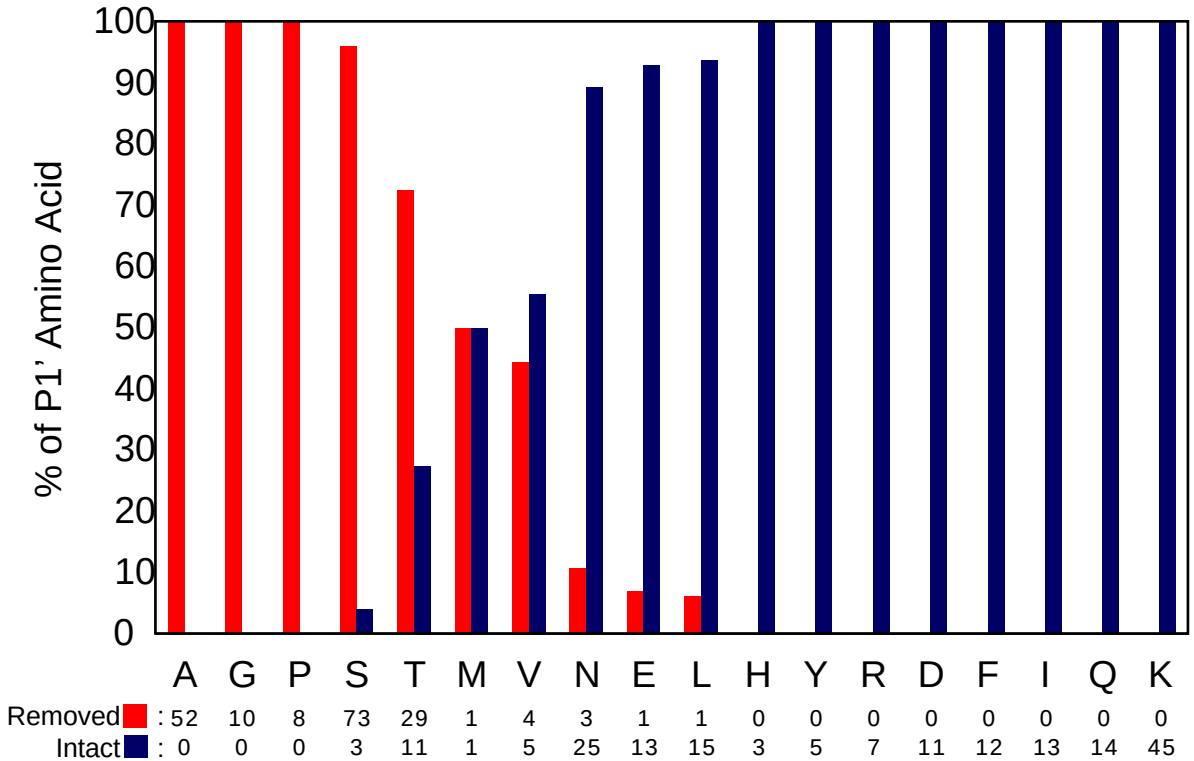
M.PVITLPDGSQR.H

M.PVITLPDGSQRHYD.H

M.SCEELEIVWNNIKAEAR.T

M.SCPVIELTQQLIR.R

Confirming the *in vivo* activity of eMetAP



- **Assemble a database of observed eMetAP substrates**
- **Define the specificity of eMetAP *in vivo***
- **"Real life is mostly binary"**

Yeast MAP1 and MAP2 Specificity

Yeast MAP1 ko: very slow growth
Yeast MAP2 ko: mild phenotype

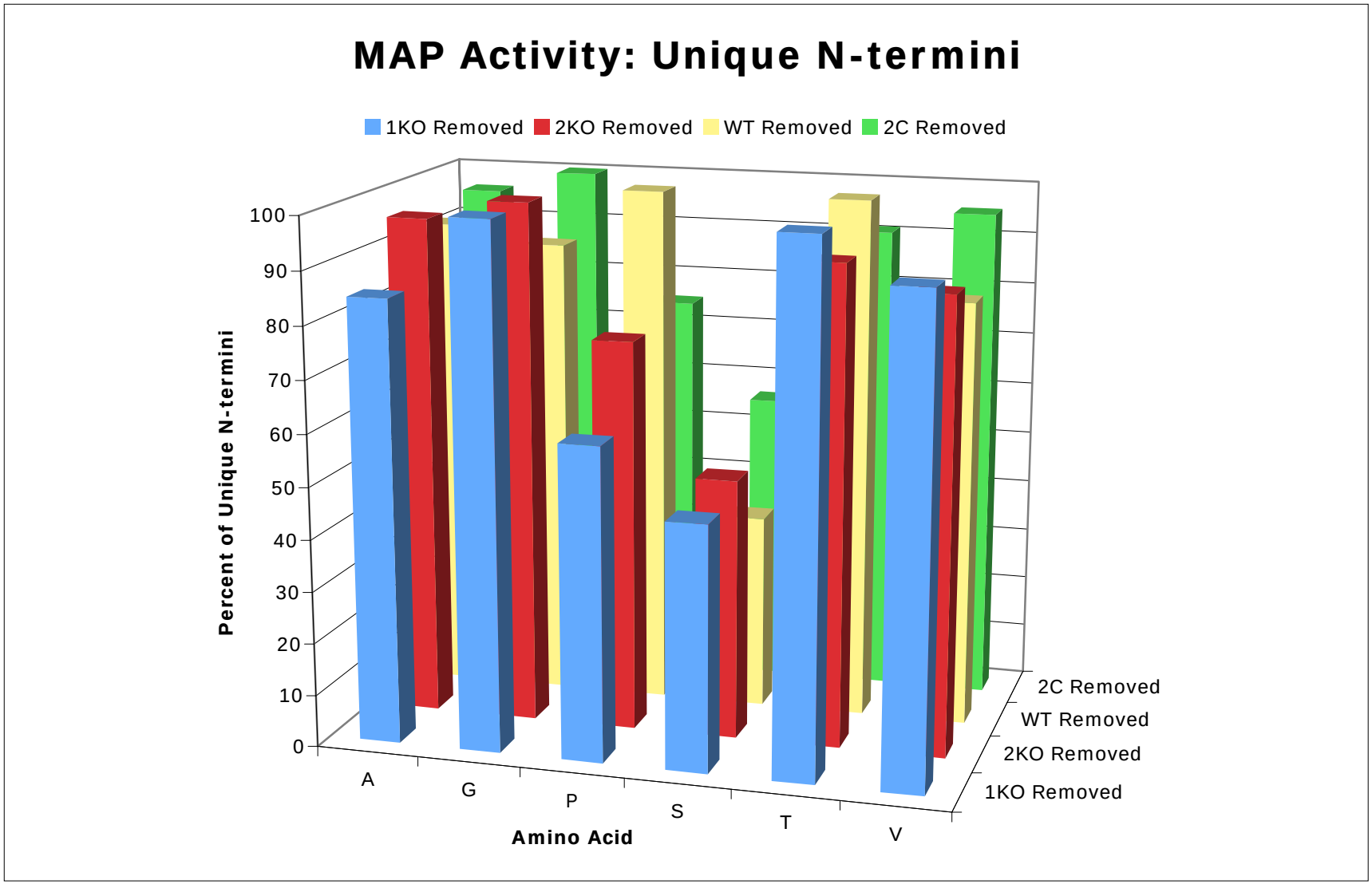
Complement MAP1 ko with MAP2: restores growth

Question: is there a general failure of Met removal in the MAP1 ko?

N-terminome of:

- **MAP 1 ko**
- **MAP 2 ko**
- **WT**
- **MAP1 ko complemented with MAP2**

Yeast MAP1 and MAP2 Specificity



Detection of Aminopeptidase Activity in Human Serum

Alpha-1-antichymotrypsin

N-terminus HPNSPLDEENLTQENQDRGTHVDLGLASANVDFAFSL 60

trimmed
- NSPLDEENLTQENQDRGTHVDLGLASANVDFAFSL 60
- SPLDEENLTQENQDRGTHVDLGLASANVDFAFSL 60

Hemopexin

N-terminus TPLPPTSAHGNVAEGETKPDPDVTERCSDGWSFDATT 60

trimmed
- LPPTSAHGNVAEGETKPDPDVTERCSDGWSFDATT 60
- PPTSAHGNVAEGETKPDPDVTERCSDGWSFDATT 60
- PTSAHGNVAEGETKPDPDVTERCSDGWSFDATT 60
- TSAHGNVAEGETKPDPDVTERCSDGWSFDATT 60
- SAHGNVAEGETKPDPDVTERCSDGWSFDATT 60
- HGNVAEGETKPDPDVTERCSDGWSFDATT 60

Truth in Advertising

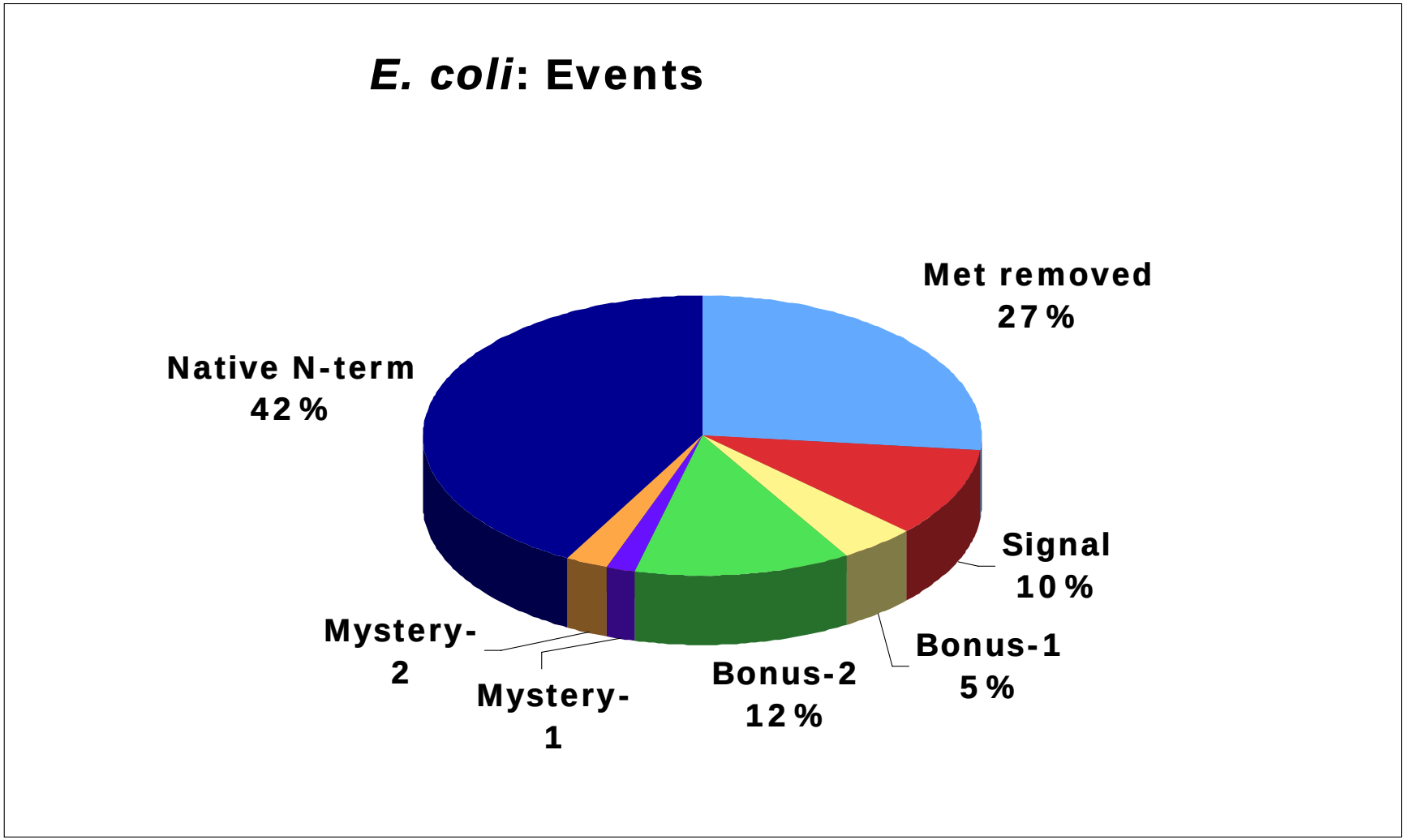
“Bonus” and “Mystery” peptides

Peptide annotation :

☑ N-terminal	-.MKFNPFVTSDR.S
☑ N-terminal Met removed	M.PGHLQEGFGCVVTNR.F
☑ Signal peptide removed	A.DAPEEEDHVLVLR.K
☑ Mito. transit peptide removed	F.ASGANFEYIIAEKR.G
☑Annotated	-

☐ Bonus-1 (unannotated)	A.PEVLPKPR.M
☐ Bonus-2 “tryptic” N and C-term.	R.AAVPSGASTGIYEALELR.D
☐ Mystery-1 “tryptic” N-term, ? C-term	R.RGEVGIYQVQLRALEH.V
☐Mystery-2 no “tryptic” either side	G.SDAKRGGPGGFQR.P

Truth in Advertising

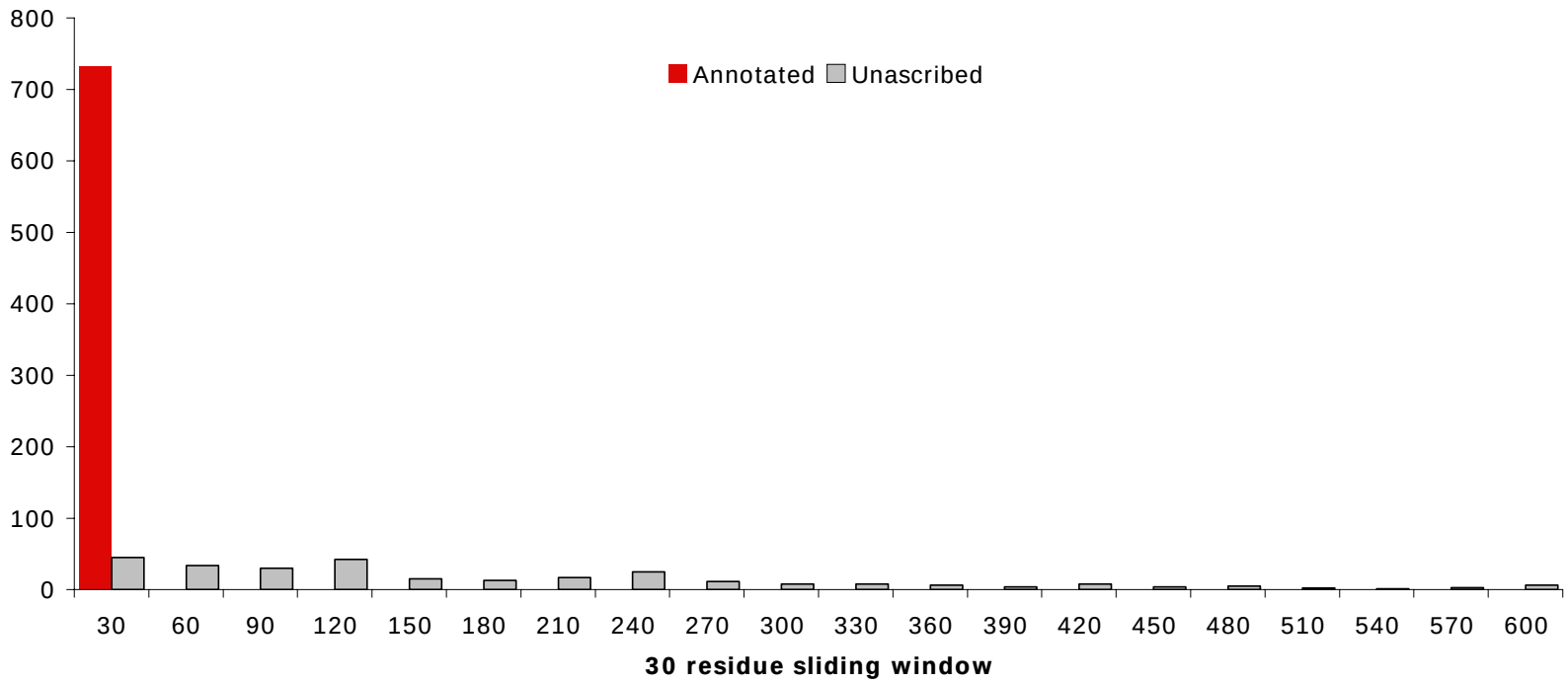


Validation by Annotation

The distribution of spectra that have previously been annotated, or which can be clearly annotated by homology, clusters heavily in the first 30 residues. This is because most E. coli proteins isolated contain a free amino group at the N-terminus (plus or minus Met, or following signal peptide removal).

Therefore it logically holds that the unascribed spectra represent yet to be annotated proteolytic cleavage events.

This is an example of what we mean by "biological validation"



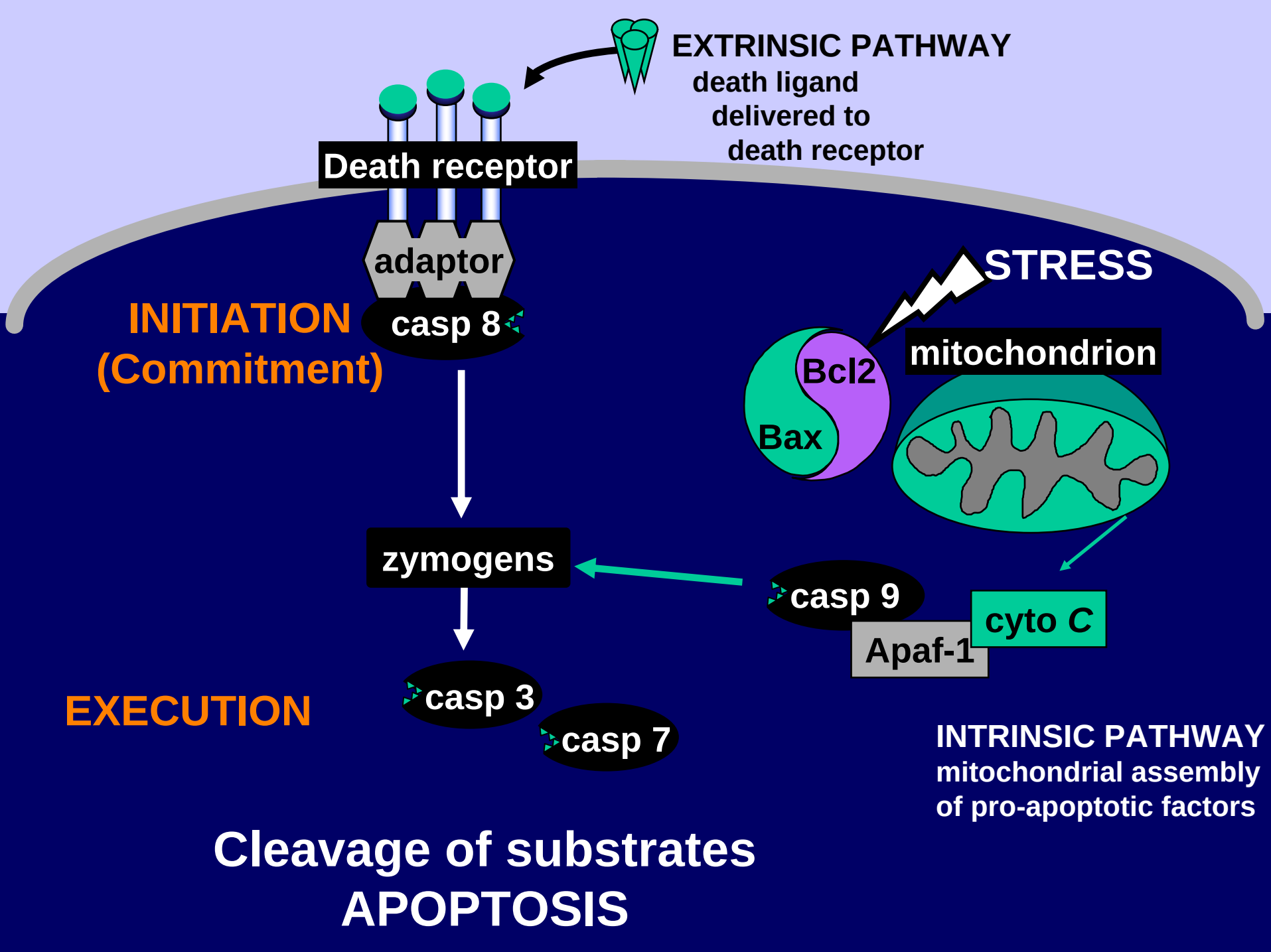
Ongoing Biological Outreach Projects

• Yie Hwa Chang - Saint Louis University
(Yeast and human MetAPs)

• Craig Walsh - UC Irvine
Andreas Strasser - WEHI, Melbourne
(T cell activation)

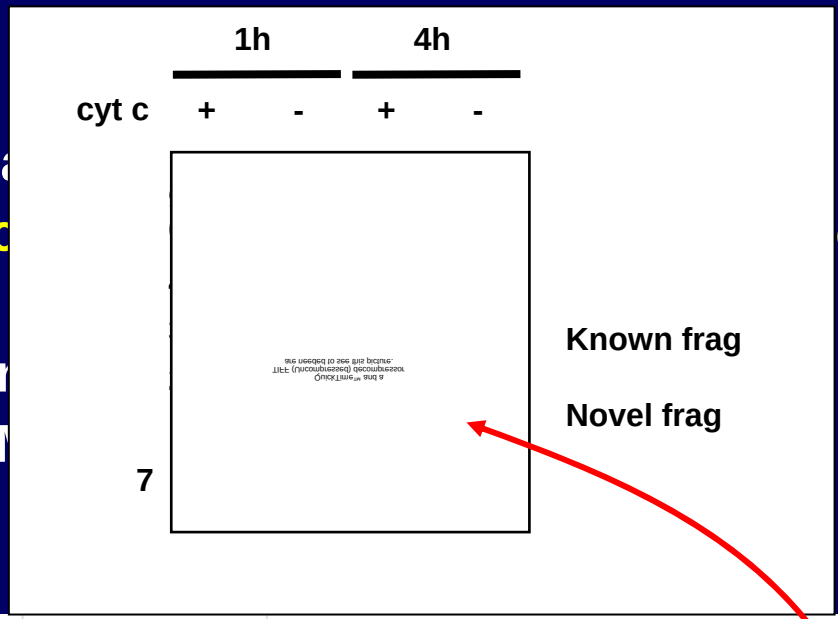
• Bruce Hay - California Institute of Technology
(DRONC apoptosis/growth compensation)

• Sharon Reed - UCSD
(*Toxoplasma gondii* cysteine proteases)


EXTRINSIC PATHWAY
death ligand
delivered to
death receptor
Death receptor
adaptor
casp 8
INITIATION
(Commitment)
STRESS
mitochondrion
Bcl2
Bax
zymogens
casp 9
cyto C
Apaf-1
EXECUTION
casp 3
casp 7
INTRINSIC PATHWAY
mitochondrial assembly
of pro-apoptotic factors
Cleavage of substrates
APOPTOSIS

Signatures of proteolysis

- Initiate a … “neoepitopes”
- Specific … c products
- Tryptic …
- Enrich …
- LC/MS/…



peptide	SwissProt	Name	Cleavage site	P1	Fischer 2003
MK*VTLK*ELCWLLR	Q5T2J2	Novel protein.	KTLAD-MKVTL	745	No
PPKLNAFIMDK	NMD3B_HUMAN	Glutamate [NMDA] receptor subunit 3B	MLTSD-PPKLN	735	No
VDYK*SGTPM*QSAAKAPYLAKFK	Q8WUK7	Hypothetical protein.	AIVLD-VDYKS	291	No
GLGVARPHYGSVLD	IQGA1_HUMAN	Ras GTPase-activating-like protein IQGAP1	ADEVD-GLGVA	8	No
CGAGK*DSLEK*QEESITVQTMMNTLR	SCRN1_HUMAN	Secernin-1.	EDHLD-CGAGK	234	No
GGK*LDVGNAEVK*LEEENR	BAP31_HUMAN	B-cell receptor-associated protein 31	GAAVD-GGKLD	163	Known
GLAVTPTPVPVVGSQMTR	U2AF2_HUMAN	Splicing factor U2AF 65 kDa subunit	TMTPD-GLAVT	128	No
GVTHTVPIYEGYALPHAILR	ACTB_HUMAN	Actin, cytoplasmic 1	MDSGD-GVTHT	157	DIFF
MGEIASFDK*AK*	TYB10_HUMAN	Thymosin beta-10.	ADKPD-MGEIA	5	No
PTGTYHGDSDLQLER	TBB2C_HUMAN,	Tubulin beta-2C chain	EHGID-PTGTY	31	No

Thanks for the help

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Andrei Osterman
Alexey Eroshkin

Andy Tao - Purdue

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