The Proteasix Ontology

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Overview

- Biomedical background: Peptides as Biomarkers
- Current Tools & Applications
- DEMO 1 - Proteasix Web App
- Current limitations of Proteasix Web App
- Semantic Web technologies & Proteasix Ontology (PxO)
- Concluding remarks

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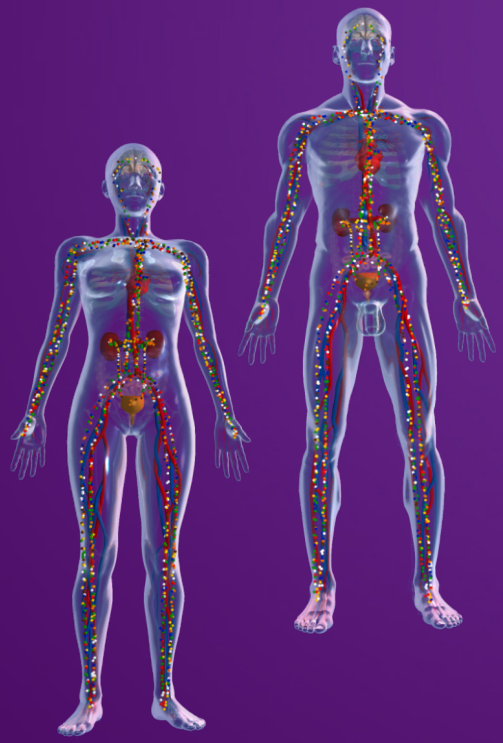
Biomedical Background

why omics?

Omics: studying “all” molecules collectively



Complex diseases cannot be adequately described by **single features:**

- Interindividual differences
- Mechanism multiplicity

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Biomedical Background

Exemplifying the challenges: CKD

	Detect	Predict evolution
Renal biopsy (kidney status)	 But too invasive	 Too late
Albuminuria (glomerular filtration)	 Not always present	 Not sensitive enough
eGFR (glomerular filtration)	 Too late	 Too late

New biomarkers: non-invasive, early changes, kidney status

(To be used in addition/combination with gold-standards)

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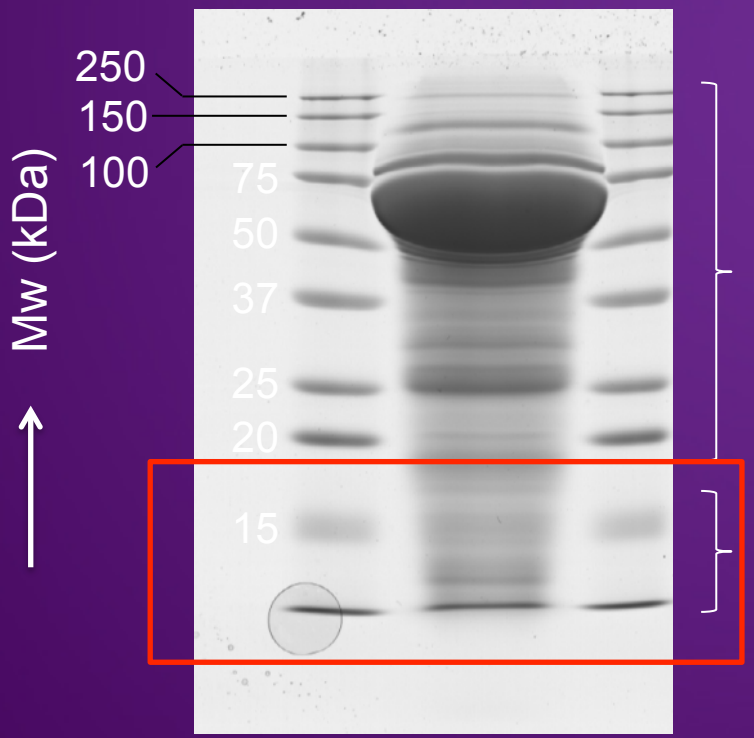
Peptides as Biomarkers

Urinary peptides

Peptide: proteolysis product (terminal post-translational modification)



Courtesy: C. Lacroix, Toulouse

- Reduced pre-analytical handling before mass spectrometry analysis
  no digestion
- Improved resistance to degradation
  already degradation products
- Peptides are filtered under physiological conditions
  detection of early events, before alteration of the filtration barrier

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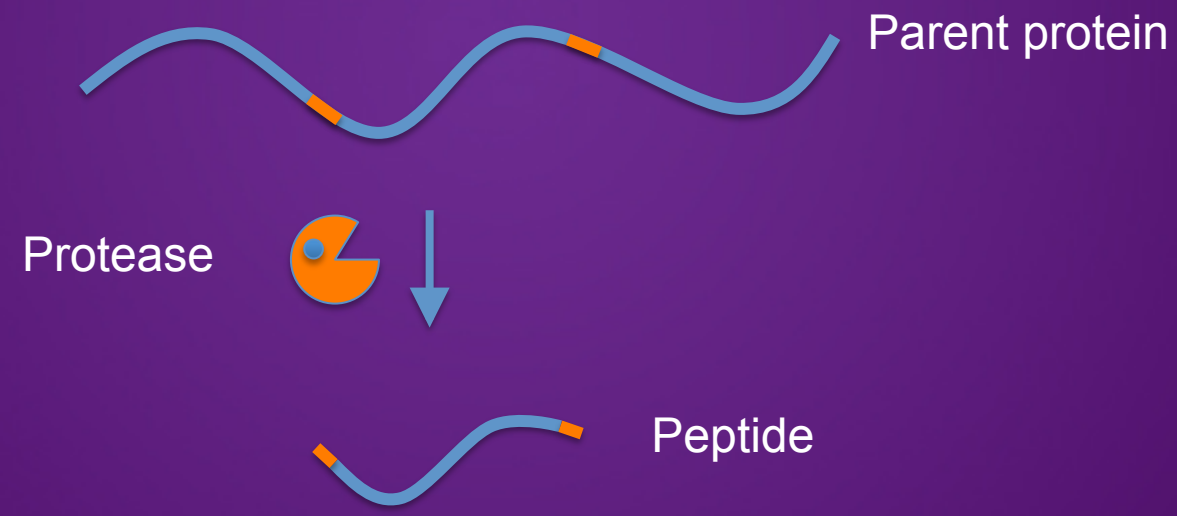
Links of urinary peptides to known processes in CKD



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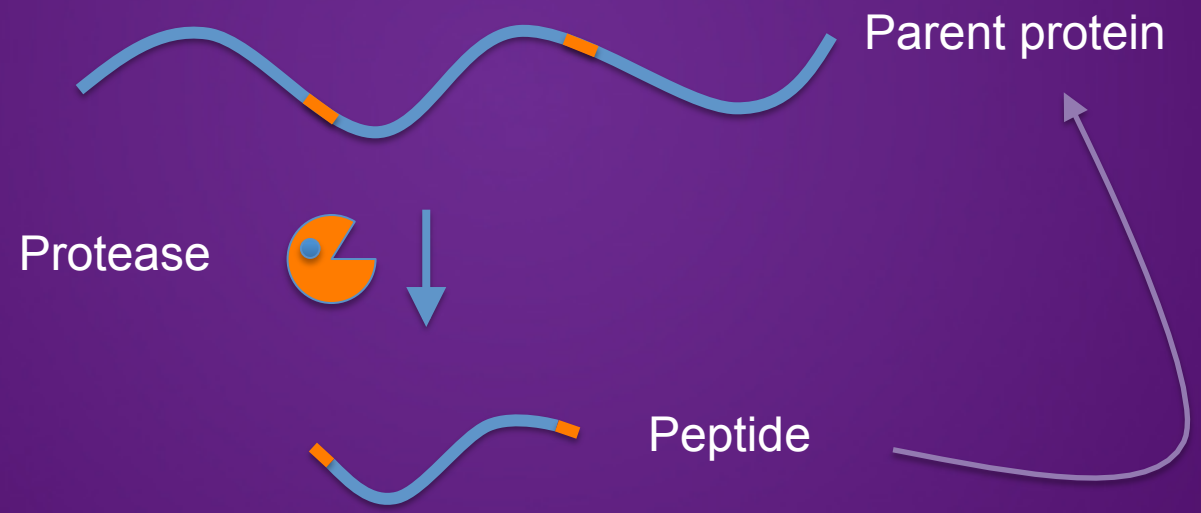
Links of urinary peptides to known processes in CKD



Parent protein is associated to pathophysiology?

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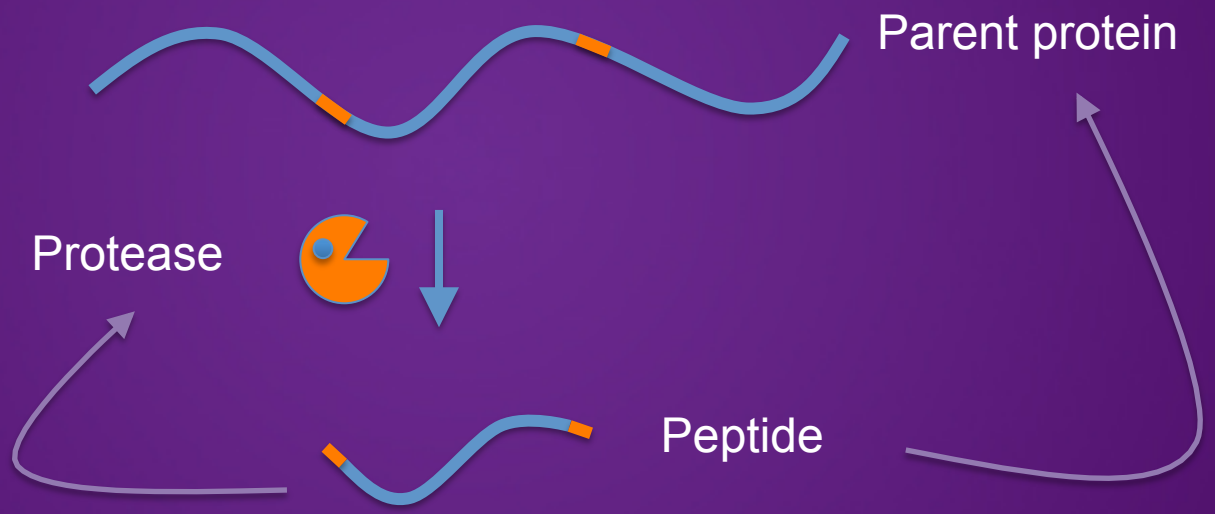
Links of urinary peptides to known processes in CKD



Parent protein is associated to pathophysiology?

And/or

Protease activity is associated to pathophysiology?

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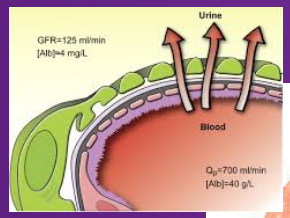
Links of urinary peptides to known processes in CKD

Parental proteins

Filtration plasma proteins

Serum albumin
Apo-A1
Beta-2-microglobulin

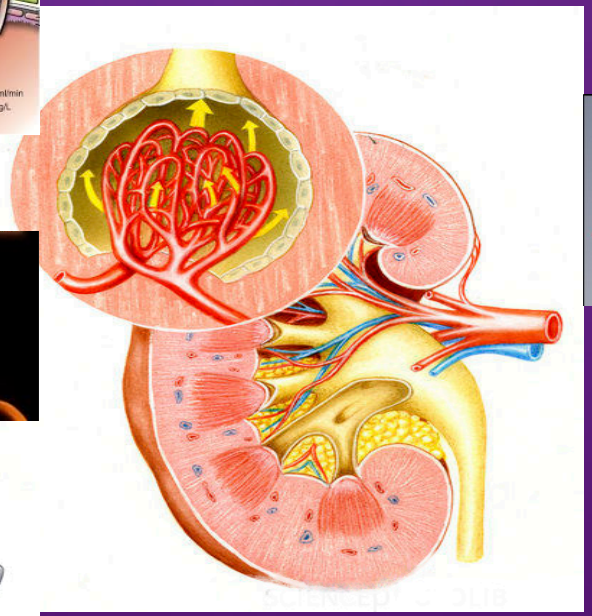


Inflammation

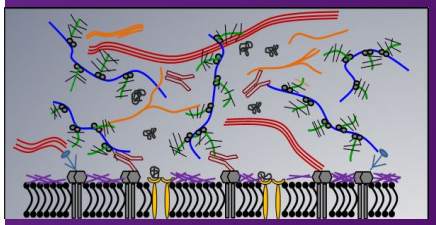
Osteopontin
Protein S100 A9
CD99

Tissue repair

Clusterin
Annexin A1



Aberrant expression of ECM (Fibrosis)



Collagen alpha I
Collagen alpha II
Collagen alpha III

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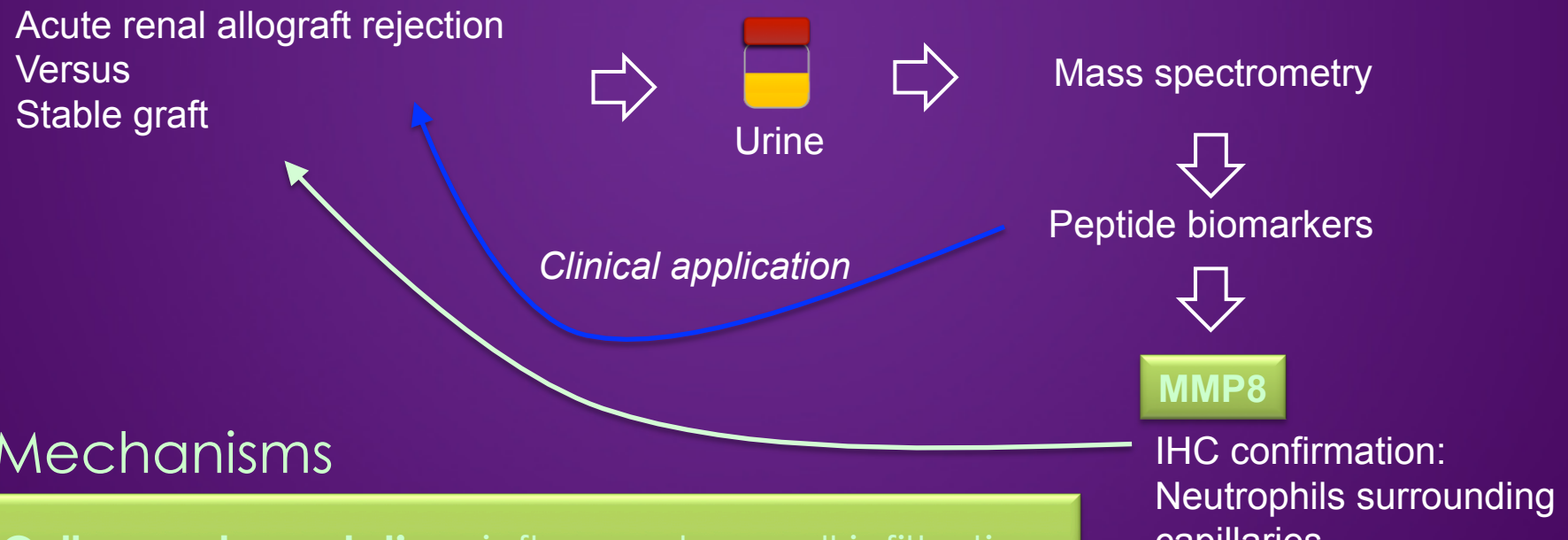
Links of urinary peptides to known processes in CKD

Proteases

Protease identification: better understanding of disease mechanisms



Mechanisms

Collagen degradation: inflammatory cell infiltration

AngII generation: macrophage recruitment

Metzger, et al. Proteomics Clin Appl 2011.

Sequence information of identified altered collagen α(I) and α (III) chain fragments in rejection samples suggested an involvement of matrix metalloproteinase-8 (MMP-8).

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Links of urinary peptides to known processes in CKD

Proteases

Nomenclature:

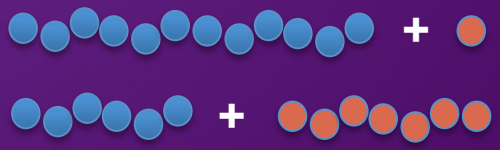
- Cleavage site: 8 amino acid sequence (P4-P3-P2-P1 **|** P1'-P2'-P3'-P4')
- Scissile bond: peptide bond between P1 and P1'

Some proteases have very **broad specificity** (e.g. Trypsin X-X-X-**R/K|** X-X-X-X)

Some proteases have very **strong specificity** (e.g. Masp2 S-L-G-R**|** K-I-Q-I)

Some proteases have **exopeptidase activity**

Some proteases have **endopeptidase activity**



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From peptides to enzymes? Cathepsin in Diabetic nephropathy

Smith et al. Hypertension 2012

- In the large conduit arteries, elastin is important in maintaining vascular compliance.
- In predialysis chronic kidney disease (CKD), **elastin degradation** is an important determinant of arterial stiffness and is associated with all-cause mortality.
- **Elastin degradation** is mediated by several **proteases**, including matrix metalloproteinase 2 and cathepsin S.
- Higher **matrix metalloproteinase 2** and **elastin-derived peptide levels** were also independently associated with preexisting cardiovascular disease.

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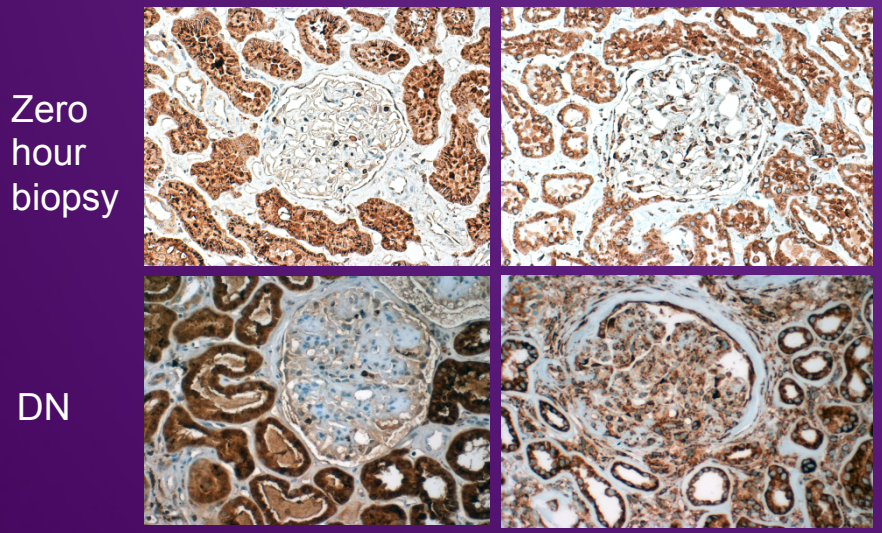
From peptides to enzymes? Cathepsin in Diabetic nephropathy

Urinary peptides in DN

→ Proteasix → Some math (Holger Husi Glasgow, UK) → **Top Predicted enzymes**

↑Cathepsin S
↑ Cathepsin V (L2)



Unpublished (Gert Mayer, Innsbruck, Austria)

Cathepsins

- Known roles in extracellular matrix (ECM) degradation.
- Cathepsin S increased in serum of CKD patients.

  Smith et al. Hypertension 2012
- Cathepsin V (L2) …??

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Tools & Applications

Peptides represent a new source of very useful biomarkers in kidney and other disease.

Peptides can help better understanding the **pathophysiological mechanisms**:

- Parental proteins
- Proteases

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Tools & Applications

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Peptides can help better understanding the **pathophysiological mechanisms**:

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Initially:

Very tedious work!

HELP ?

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Tools & Applications

Peptides can help better understanding the **pathophysiological mechanisms**

Initially:

Very tedious work!

Let's get some HELP

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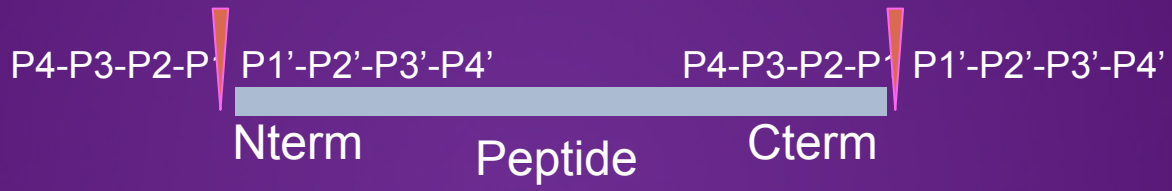
Tools & Applications

What do we need?



- 2 cleavage sites/peptides (N and C Term)
- Cleavage sites are incomplete → reconstruct full cleavage sites
- Identify proteases that can cleave these cleavage sites

We need an **automatic tool** to reconstruct the cleavage sites

We need to collect information about **cleavage site/protease**

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Tools & Applications

Exisiting tools for linking proteases, substrates, cleavage sites:

- CutDB (Proteolysis Map Project; PMAP)
- MEROPS
- TopFIND2
- Proteasix

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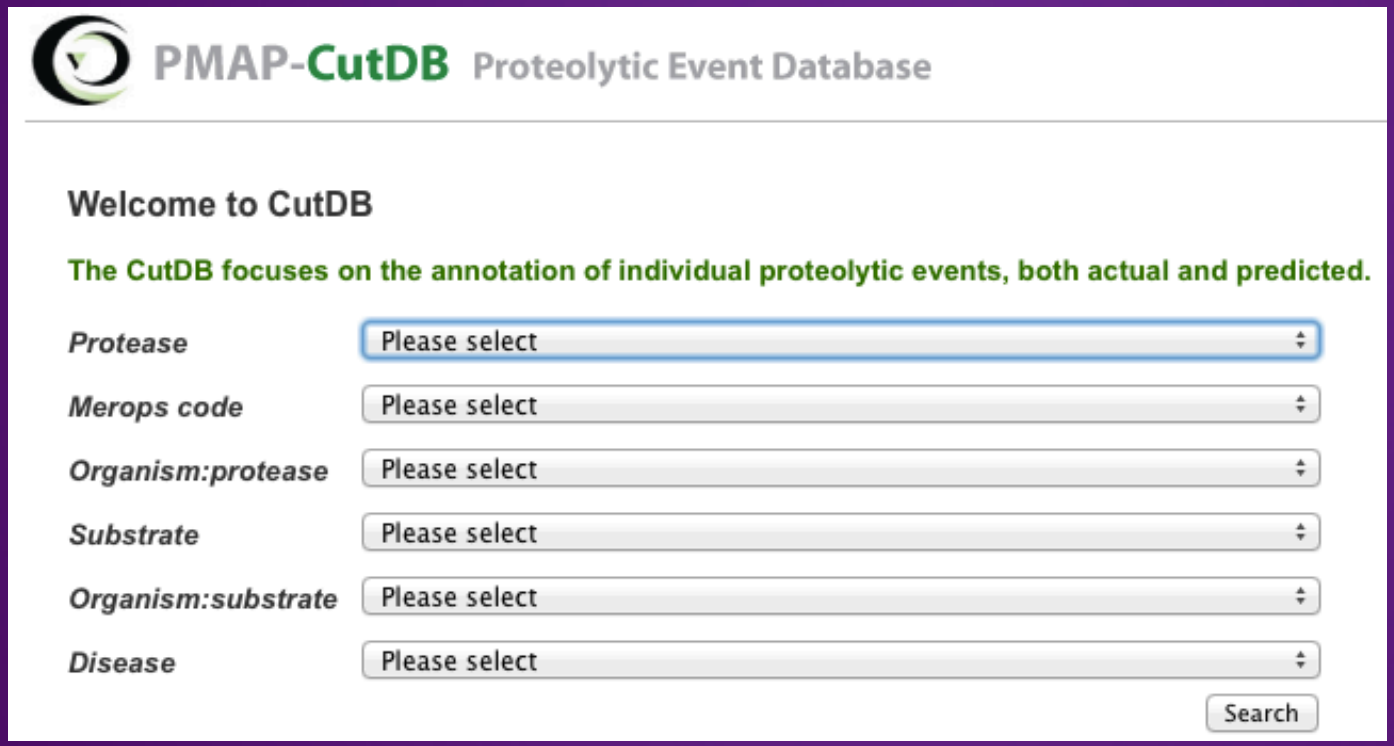
CutDB

Database of protease/cleavage site

Not peptide-centric; No batch searches



ID	Protease	Organism [protease]	Substrate	Organism [substrate]	Cut-site	Structure	Update	Detail
18127	matrix metallopeptidase-2 M10.003	Gallus gallus	collagen, type I, alpha 1	Bos taurus	GPQG-IAGQ 952-953		May 25 2006 YI	Detail

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MEROPS

Database of protease specificity

Not peptide-centric; No batch searches

Specificity matrix								
Amino acid	**P4**	**P3**	**P2**	**P1**	**P1'**	**P2'**	**P3'**	**P4'**
Gly	252	121	430	482	60	125	590	290
Pro	196	769	70	210	8	15	29	236
Ala	253	368	642	506	110	235	538	332
Val	199	447	129	45	280	444	274	272
Leu	259	364	178	81	1389	390	112	297
Ile	159	288	70	33	557	351	81	168
Met	82	100	44	67	150	97	47	59
Phe	128	81	48	58	177	143	100	126
Tyr	97	51	48	54	131	91	61	53
Trp	11	16	9	39	44	42	25	23
Ser	178	116	483	333	79	229	486	235
Thr	145	93	86	112	61	252	221	162
Cys	37	42	51	39	70	24	11	40
Asn	88	67	130	376	35	120	167	139
Gln	166	79	226	152	129	220	128	149
Asp	148	12	87	206	9	25	161	278
Glu	169	42	161	266	33	123	147	241
Lys	169	54	180	133	66	309	171	190
Arg	138	26	192	105	13	99	26	38
His	92	80	52	120	14	81	42	76

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TopFIND2

WELCOME

TopFIND is the first public knowledgebase and analysis resource for protein termini and protease processing

More than 290,000 N- and C-termini and more than 33,000 cleavages listed

Covers H. sapiens, M. musculus, S. cerevisiae, A. thaliana and E. coli

Database

Tool

WHAT TopFIND PROVIDES

Integration of protein termini & function with proteolytic processing, alternative transcription & translation

Displays proteases and substrates within their protease web including detailed evidence information

TopFINDer automates analysis and functional annotation of proteomics-derived termini sets

PathFINDer identifies indirect protease-substrate connections for the evaluation of complex processes

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Proteasix Web-based version

Content:

Human proteases (230), mouse proteases (160), rat proteases (105)

>20000 protease/cleavage site association (CutDB, MEROPS)

318 protease specifity matrices for prediction (MEROPS)

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Content:

Human proteases (230), mouse proteases (160), rat proteases (105)

>20000 protease/cleavage site association (CutDB, MEROPS)

318 protease specifity matrices for prediction (MEROPS)

Functionality:

Visualisation of protease specificity

Cleavage site reconstruction (based on UniprotKB)

Prediction of proteases

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Proteasix Web-based version

http://sysvasc.cs.man.ac.uk

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Current Limitations of Proteasix Web App

- To refine the predictions, we need to know:
  - ❑ the species in which our proteins exist
  - ❑ the function of the protease
  - ❑ where the proteins live, i.e. which cellular component

- At the moment there is NO distinction between
  - ❑ **Exopeptidase activity**
  - ❑ **Endopeptidase activity**

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- At the moment there
  - ❑ **Exopeptidas**
  - ❑ **Endopeptidas**

tween

We need to incorporate more knowledge

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Ontology

Challenge:
how to go from information to **Knowledge**

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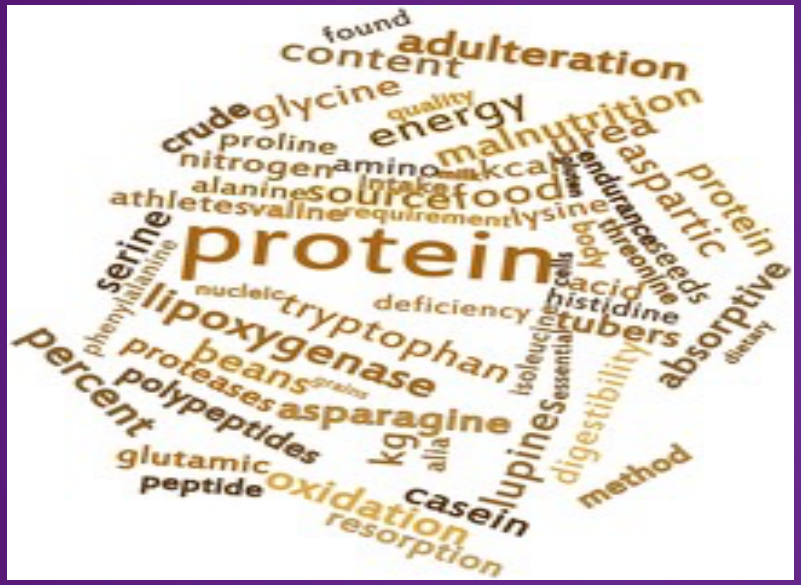
Ontology



In informatics and computer science, an **ontology** is a representation of the **shared background knowledge** for a community.

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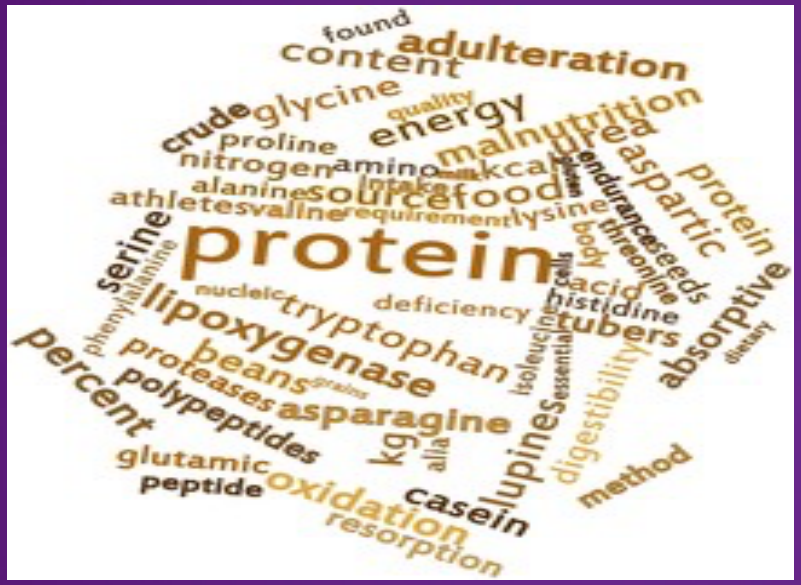
Ontology



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UniProtKB - P02768 (ALBU_HUMAN)

Protein	**Serum albumin**
Gene	**ALB**
Organism	*Homo sapiens (Human)*

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The goal with a computer science ontology is to make knowledge of a domain *computationally useful*

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An **ontology** is a description of the **concepts** and **relationships** that can exist

Categories or classes:

- Protein
- Gene
- Organism

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The goal with a computer science ontology is to make knowledge of a domain *computationally useful*.

An **ontology** is a description of the **concepts** and **relationships** that can exist

Categories or classes:

- Protein
- Gene
- Organism

Relationships:

P02768 is-a protein

ALB is-a gene

P02768 only_in_taxon Human

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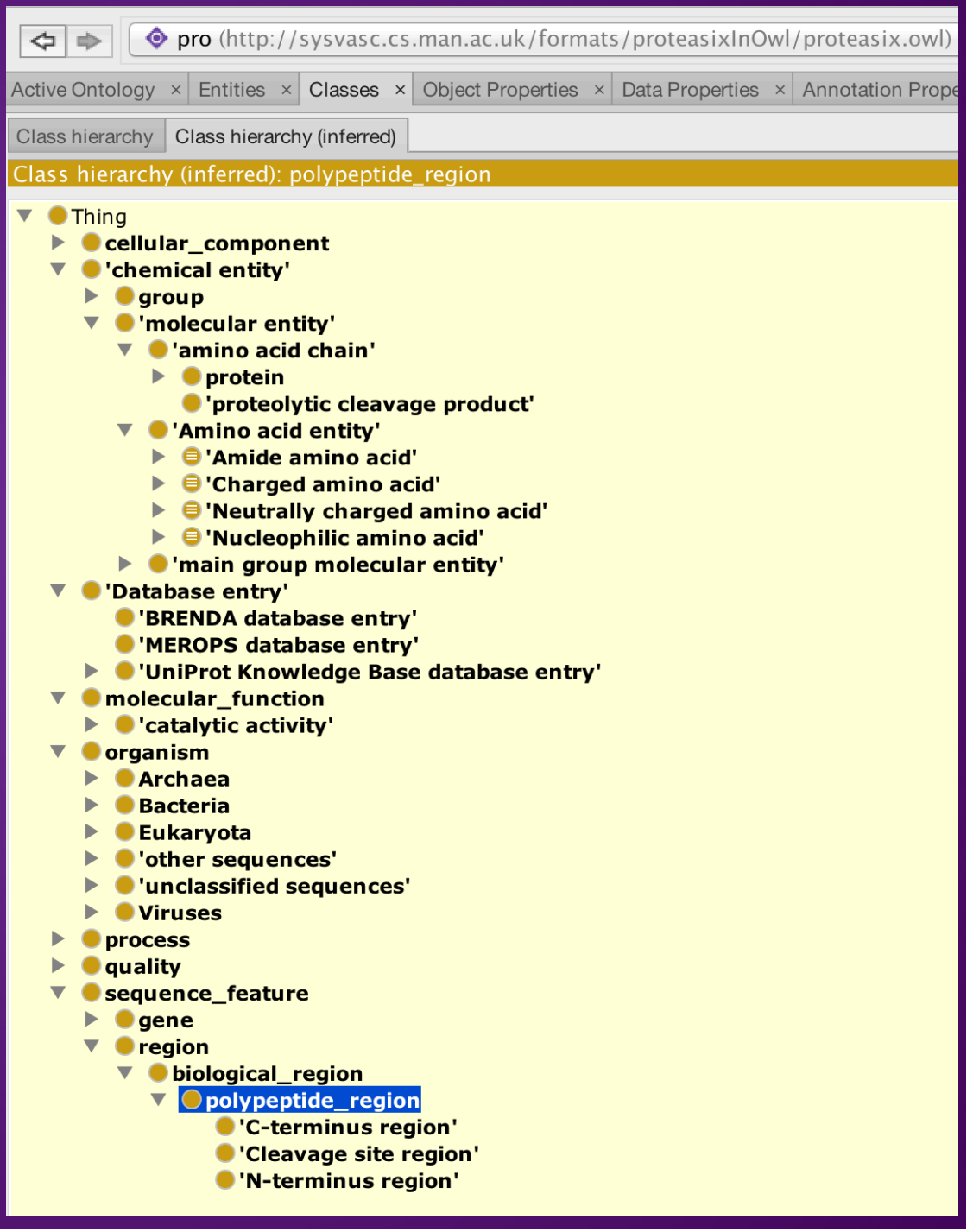
Current Work:
Proteasix Ontology (PxO)

Concepts



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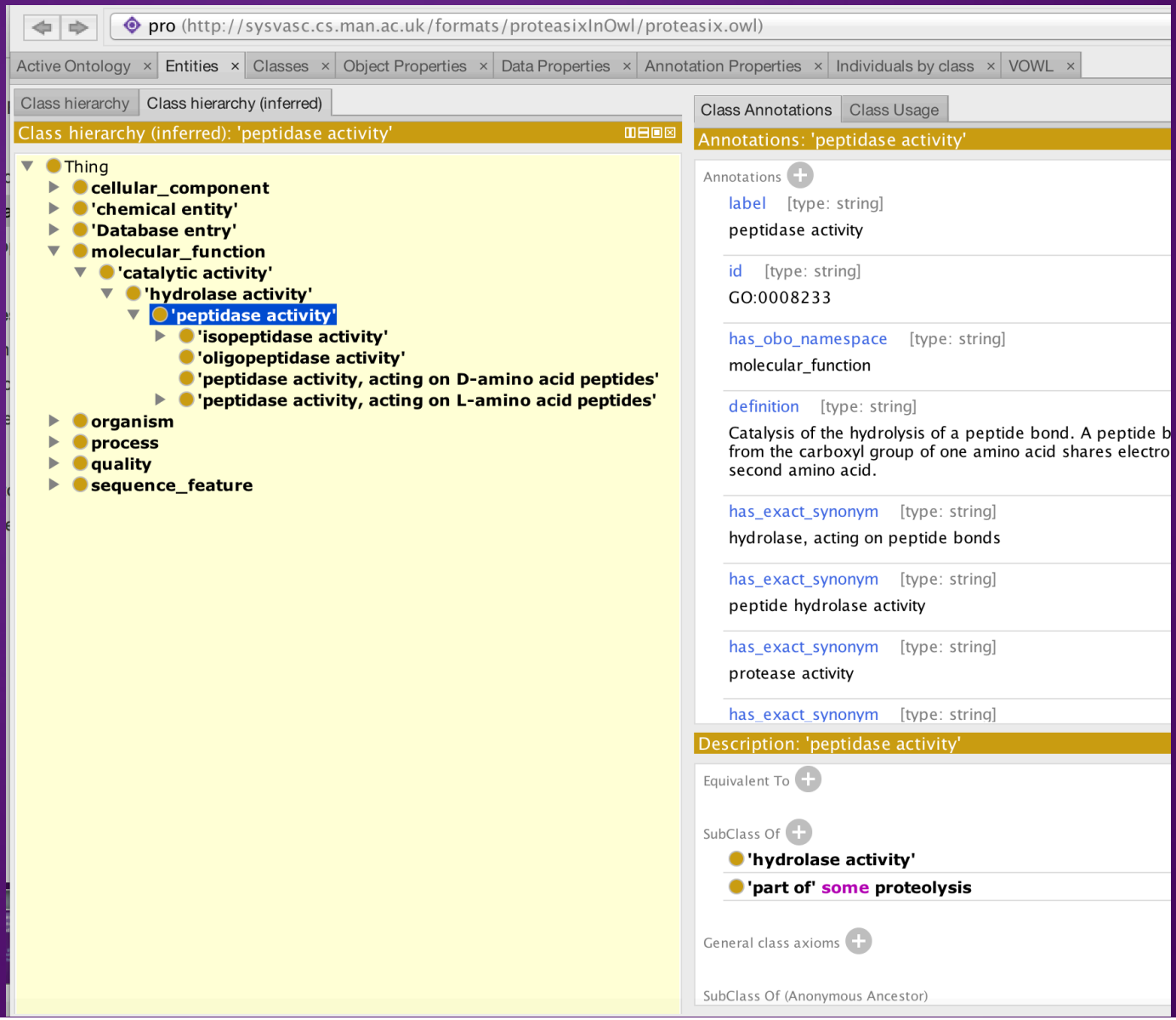
Current Work: *Proteasix Ontology (PxO)*



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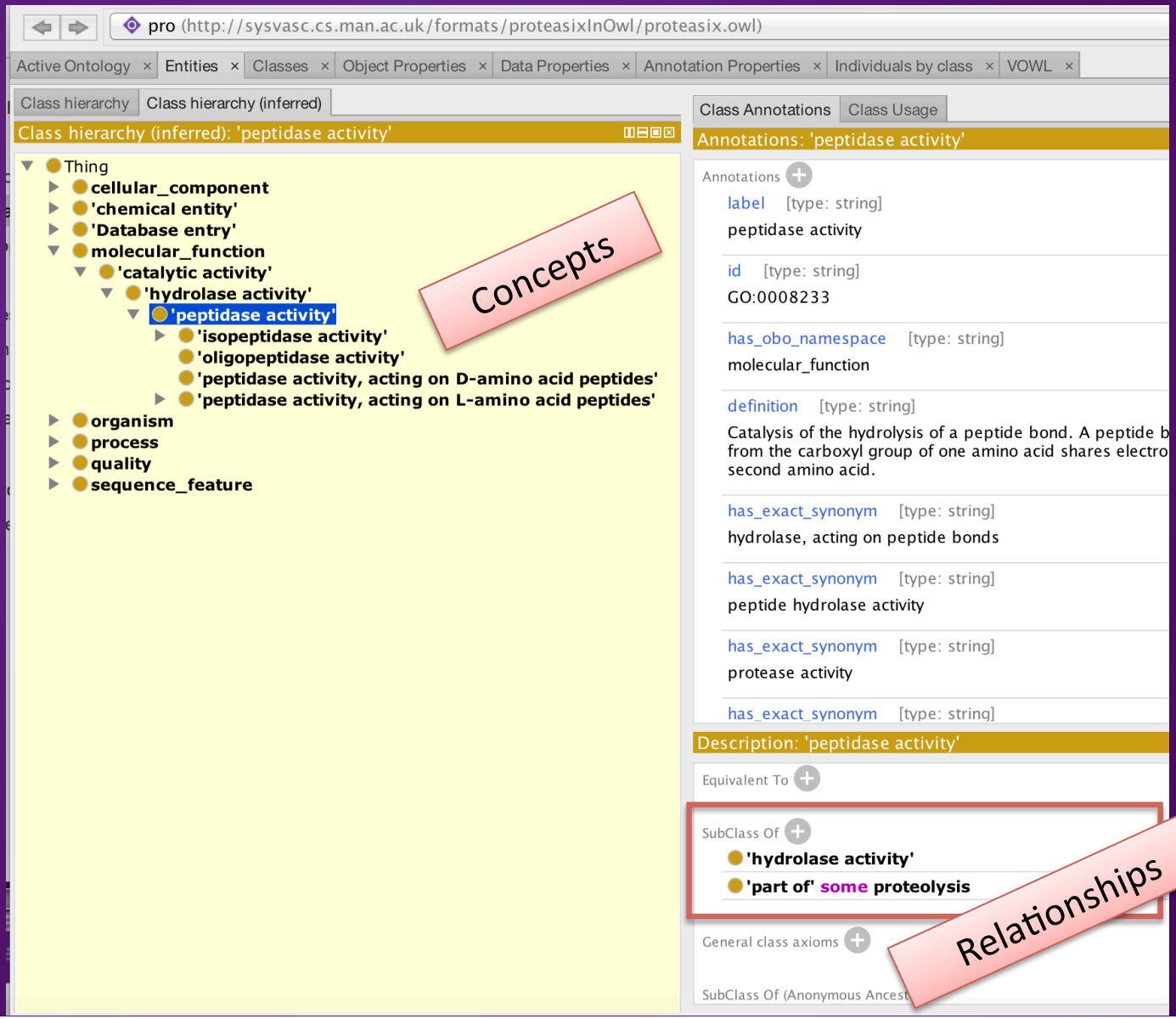
Current Work: *Proteasix Ontology (PxO)*



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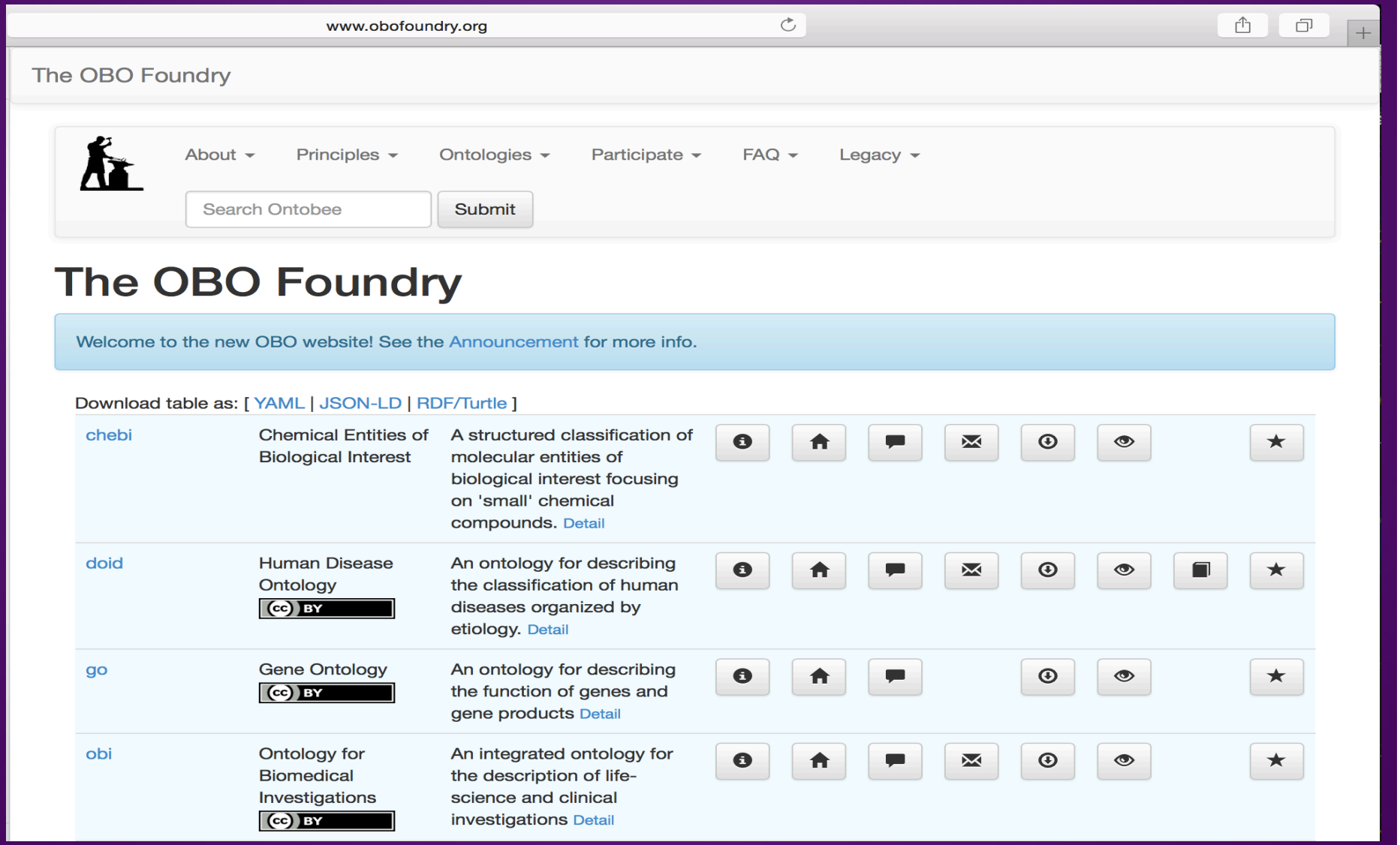
Proteasix Ontology (PxO) reuse ontologies



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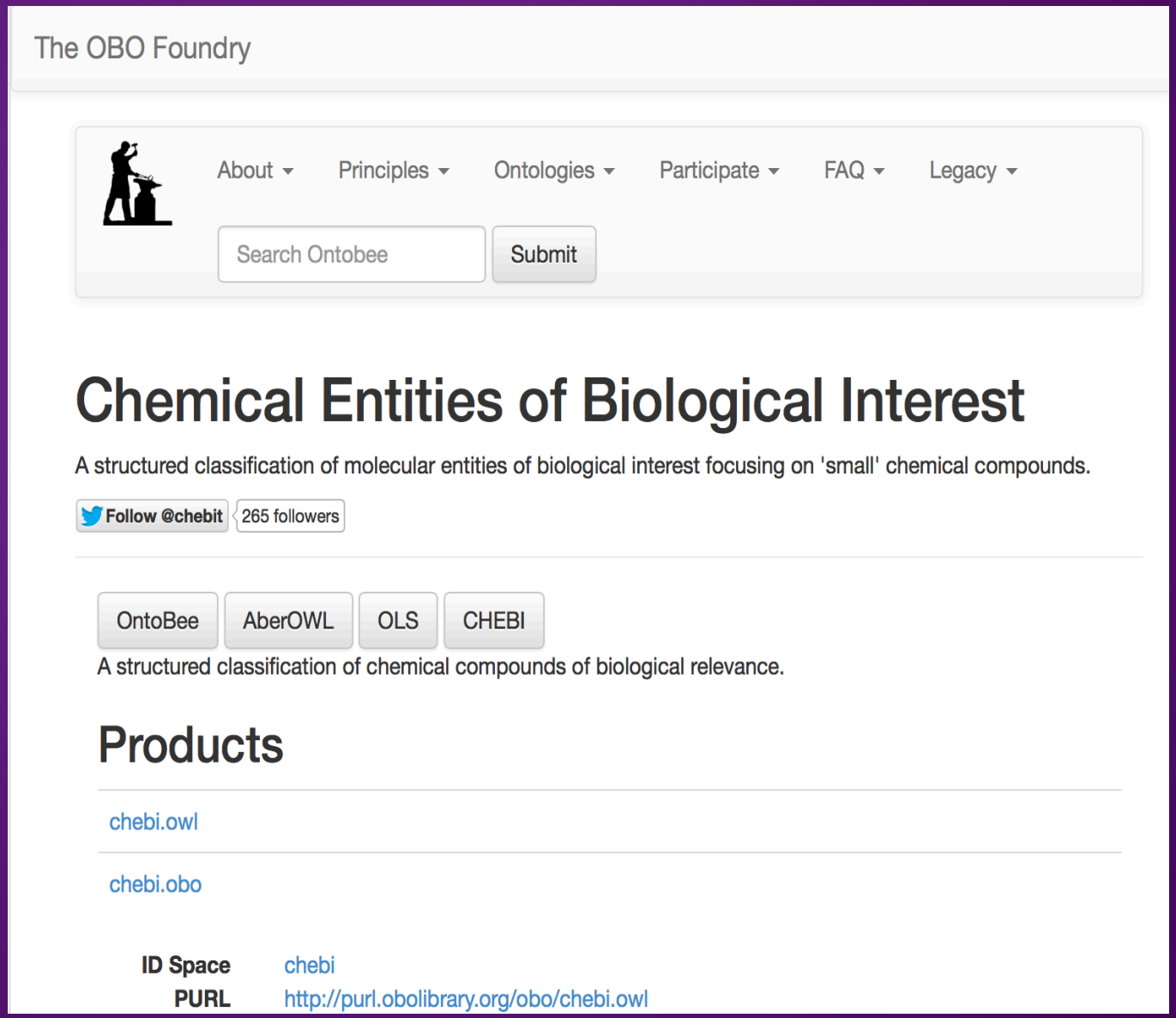
Proteasix Ontology (PxO) reuse ontologies

chemical entity

molecular entity

Alanine



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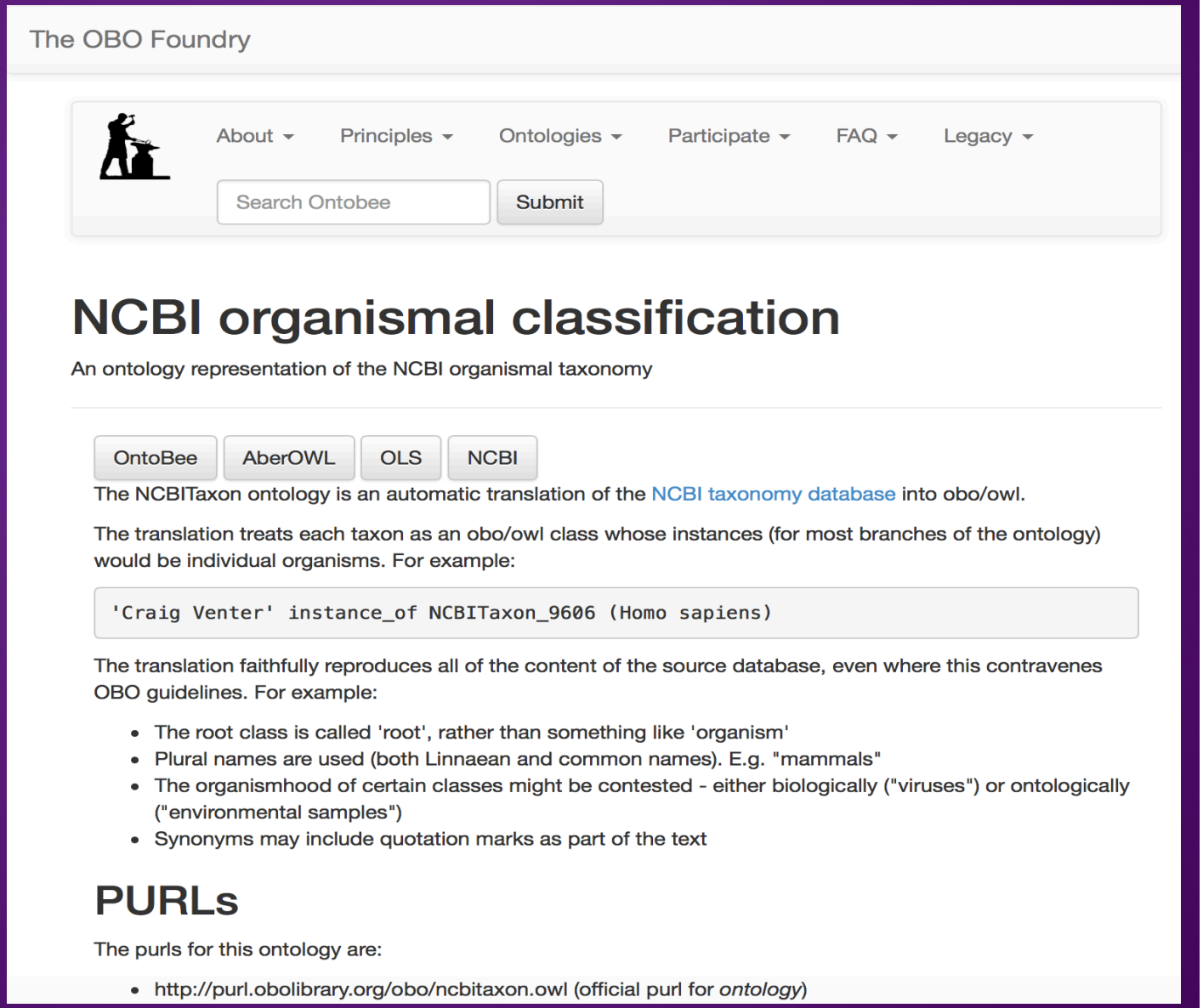
Proteasix Ontology (PxO) reuse ontologies

Human

Rat

Mouse



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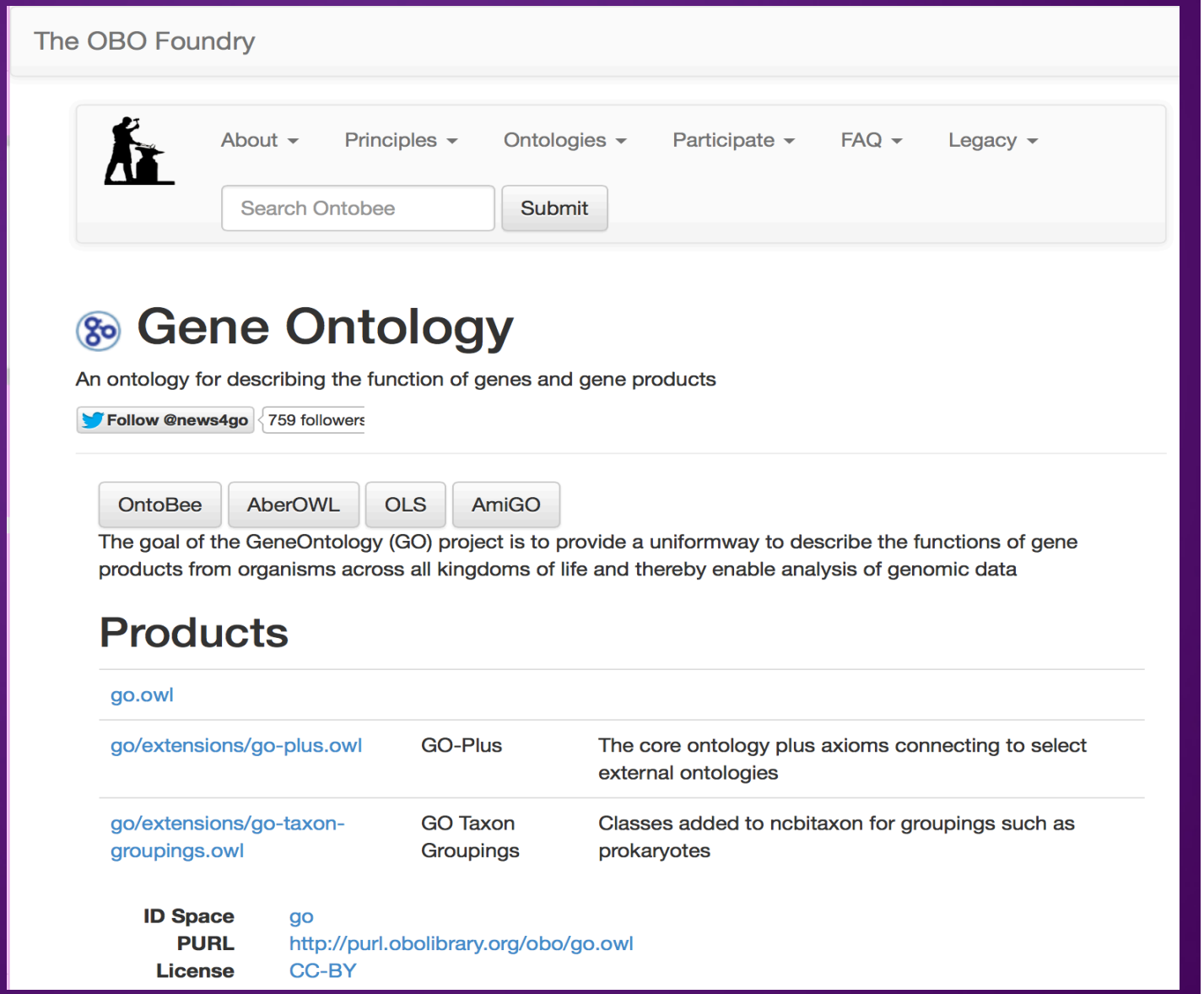
Proteasix Ontology (PxO) reuse ontologies

molecular_function

cellular_component

biological_process



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Proteasix Ontology (PxO) reuse ontologies

Protein

amino acid chain

proteolytic cleavage product

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The term "**Semantic Web**" refers to W3C's vision of the *Web of linked data*

Ontologies are considered one of the pillars of the **Semantic Web**

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Semantic Web technologies has reached to a degree of maturity

Semantic Web technologies

NEW Proteasix

SWRL (rules)

OWL-S (services)

SPARQL (queries)

OWL (ontologies)

JSON-LD

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Current Work: *Proteasix Ontology (PxO)*

- UniProt KB proteins (Swiss-prot and TrEMBL)
  - ❑ Organised by Taxons following PRO ontology
  - ❑ Annotated with GO biological_process; GO cellular_component; and GO molecular_function
- Model cleavage sites patterns for
  - ❑ **Exopeptidase activity**
  - ❑ **Endopeptidase activity**

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For using Peptides as Biomarkers, we need more data.. and data linkage..

Semantic Web technologies

OWL (ontologies)

Let's glue the relevant knowledge together

Class: polypeptide_region

SubClassOf:

biological_region
part_of some protein,
associated_with some 'proteolytic cleavage product',
only_in_taxon some organism

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For using Peptides as Biomarkers, we need more data.. and data linkage..

In Swiss-Prot there are proteins annotated with GO:0004252, which is serine-type endopeptidase activity

Semantic Web technologies

SPARQL
(queries)

Let's get them!

```
SELECT ?x  FROM <file:./ontofiles/Swiss-Prot.owl>
{
      ?x rdf:type owl:Class;
            rdfs:subClassOf  [ a    owl:Restriction ;
            owl:onProperty      pr:has_function ;
            owl:someValuesFrom  obo:GO_0004252  ] .
 }
```

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For using Peptides as Biomarkers, we need more data.. and data linkage..

Can we retrieve from Swiss-Prot ALL the proteins annotated with any DESCENDANT of Peptidase activity GO_0008233 ?

Semantic Web technologies

SPARQL
(queries)

Let's get Pepetidase Activities from GO,
e.g. serine-type endopeptidase activity GO:0004252

```
GRAPH <file:./ontofiles/
GO_TAXONOMY_molecular_function.owl>
{?C rdfs:subClassOf+ obo:GO_0008233 . } .
```

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For using Peptides as Biomarkers, we need more data.. and data linkage..

Can we retrieve from Swiss-Prot ALL the proteins annotated with any DESCENDANT of Peptidase activity GO_0008233 ?

Semantic Web technologies

SPARQL
(queries)

YES! let's get proteins annotated with Pepetidase Activities from GO

```
SELECT ?x  FROM <file:./ontofiles/Swiss-Prot.owl)>
FROM NAMED <file:./ontofiles/GO_TAXONOMY_molecular_function.owl>
 {
GRAPH <file:./ontofiles/GO_TAXONOMY_molecular_function.owl>
            {?C rdfs:subClassOf+ obo:GO_0008233 . } .
{       ?x rdf:type owl:Class;
            rdfs:subClassOf  [ a  owl:Restriction ;
            owl:onProperty      pr:has_function ;
            owl:someValuesFrom   ?C ] .
  }  }
```

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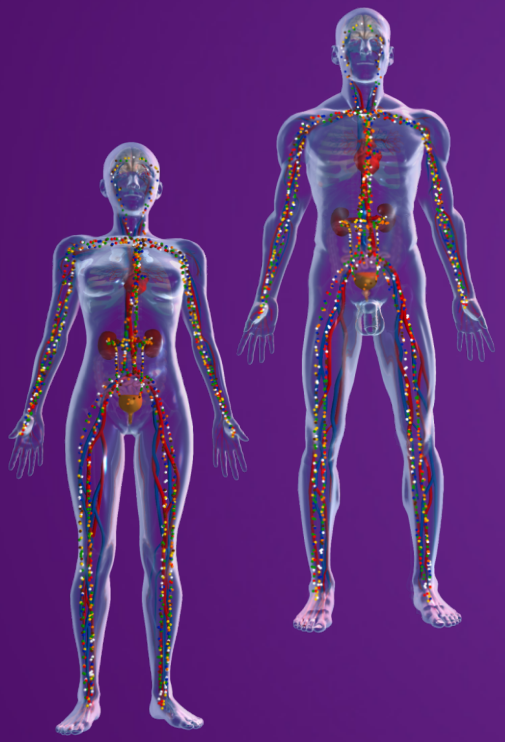
Concluding remarks



TopFIND2 and Proteasix can help to automatically predict modification of protease activity

Current work: exploring the benefits of using Proteasix ontology (PxO) in the Semantic-Web version of Proteasix

Hard Limits:

- Prediction always need validation
- Data available (over-representation of some proteases, other are missing)

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ACKNOWLEDGMENT: to Julie Klein for providing

- Biomedical background
- Comparison of Current Tools & Applications

RF lab
renalfibrosis.fr

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SysVASC