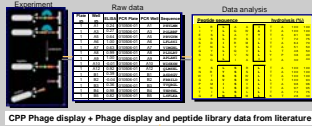


ProfileDB

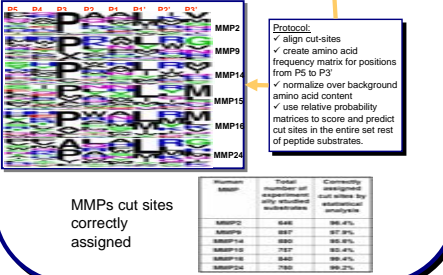
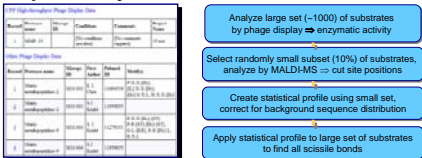
Database of substrate recognition profiles



Specificity Models

Sequence Context

Specificity model for human MMPs



MMPs cut sites correctly assigned

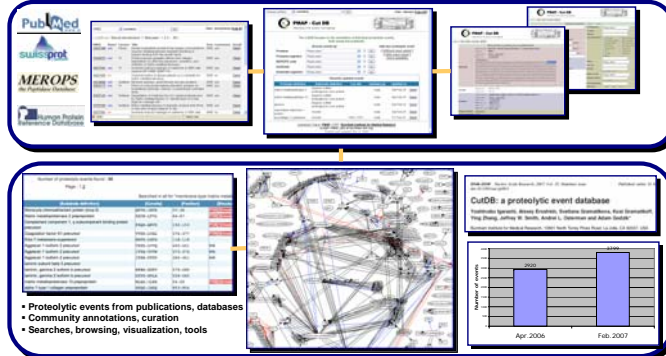
Example: protease specificity controls superantigen-induced response in streptococcal infection



Experimental Evidence

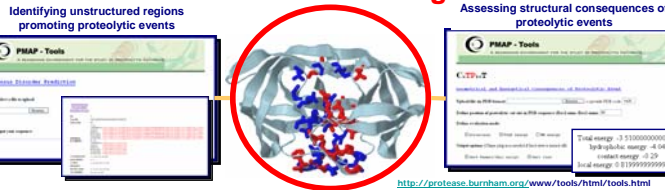
Streptococcal Mitogenic Exotoxin, SmeZ, is the Most Susceptible MTH Streptococcal Superantigen to Degradation by the Streptococcal Cysteine Protease, SpeBSM

CutDB, Database of Proteolytic Events



- Proteolytic events from publications, databases
- Community annotations, curation
- Searches, browsing, visualization, tools

Substrates and Cleavage Sites

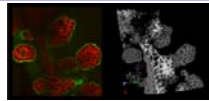


Cellular Context

Localization

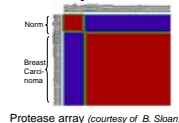
RefSeq ID	Cell Location 1	Score	Cell Location 1	Score	Name
NP_059581.1	cytoplasmic	0.31	cytoplasmic	0.30	cytoskeletal protein
NP_055092.2	cytoplasmic	0.69	cytoplasmic	0.52	cytoskeletal protein
NP_001025111.1	cytoplasmic	0.65	ER	0.11	cytoskeletal protein
NP_058997.1	ER	0.59	cytoplasmic membrane	0.16	cytoskeletal protein
NP_054142.2	cytoplasmic	0.58	ER	0.03	cytoskeletal protein
NP_017899.2	cytoplasmic	0.69	cytoplasmic	0.68	cytoskeletal protein
NP_056099.2	cytoplasmic	0.54	cytoplasmic	0.53	cytoskeletal protein
NP_004792.2	cytoplasmic	0.72	cytoplasmic	0.11	cytoskeletal protein
NP_011070181.1	cytoplasmic	0.70	cytoplasmic	0.07	cytoskeletal protein
NP_004448.2	ER	0.42	cytoplasmic	0.17	cytoskeletal protein

Predictions for all possible substrates (MultiLoc algorithm)



Protease imaging. Coculture of mcf10a with N1me myofibroblasts. Laminin 5 - green, actin - red. (Courtesy of B. Sitan and N. Polyak)

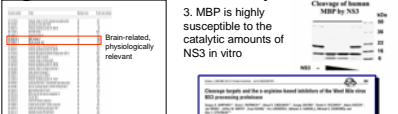
Expression



Protease array (courtesy of B. Sitan)

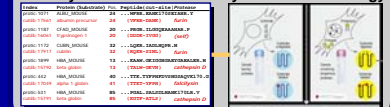
Myelin Basic Protein – Predicted Target in West Nile Virus encephalitis

- West Nile virus is an emerging global threat.
- NS3 protease, which is essential for the proteolytic processing of the viral polyprotein precursor, could identify in virus pathogenicity
- Can we find its target in human?



PROTIC-DB

Proteolytic events identified by PROTIC technology



Analysis and tentative assignment of observed events by CutDB comparison

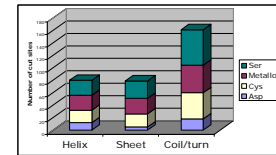
Structural Context

What are the topological preferences and constraints of proteases?

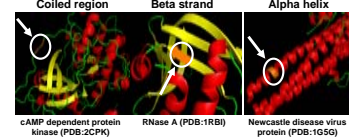


1,364 protein 3D structures with cut-sites

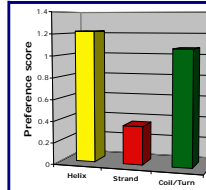
The analysis was performed for > 1,300 PDB structures of proteins with proteolytic events recorded



Cut-sites in 3D. Examples



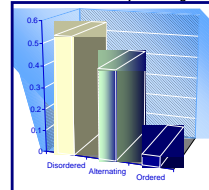
Secondary structure preferences



Preference score: N (cut-sites) / N (all residues) in a given secondary structure

- Strong discriminations against beta-strands
- First noted by S.J. Hubbard (1999) BSA: 1382-1391
- Surprisingly high preference for alpha-helices

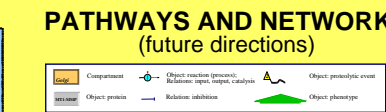
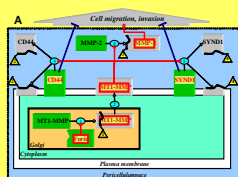
Disorder and 'shape-shifting'



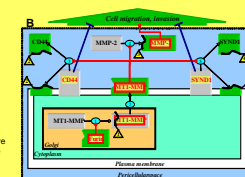
Regions of alternating order/disorder ('shape shifters') are flexible regions that are disordered in some but ordered in other environmental conditions (as reflected in respective PDB structures)

- The probability of a proteolytic event in alternating or disordered regions is ~10-fold higher than in ordered regions

PATHWAYS AND NETWORKS (future directions)



A fragment of MT-MMP network illustrating the concept of process diagrams and their application for modeling states of the network ('progressive pairing'). Transition between non-invasive (A) and invasive states (B) is promoted by a series of reactions triggered by activation and secretion of MT1-MMP. Most of the reactions shown in the diagram lead to proteolytic events (numbered triangles linked to CutDB) changing the pattern of presence (green objects) and absence (gray objects) of protein species whose functional activity promotes (as for MMP-2) or inhibits (as for CD44 and Syndecan-1) the phenotype.



TRP-1

TRP-3