affipro

Protease column portfolio 2020

AffiPro
Perfecting your proteomics

AffiPro

The aim of the company is to offer qualitatively better supporting technologies for life science mass spectrometry using novel affinity approaches and smarter fine-tuned surface chemistry

The current product portfolio includes protease columns for LC-MS and protein chips for *in-situ* sample preparation in MALDI-MS

Based in Mratin near Prague, Czech Republic
www.affipro.cz

Hydrogen Deuterium Exchange

- Hydrogen deuterium exchange (HDX) mass spectrometry is a powerful tool for studying the dynamics of higher order protein structure

- The rate of hydrogen deuterium exchange on the amide hydrogen of the protein backbone provides information about solvent accessibility

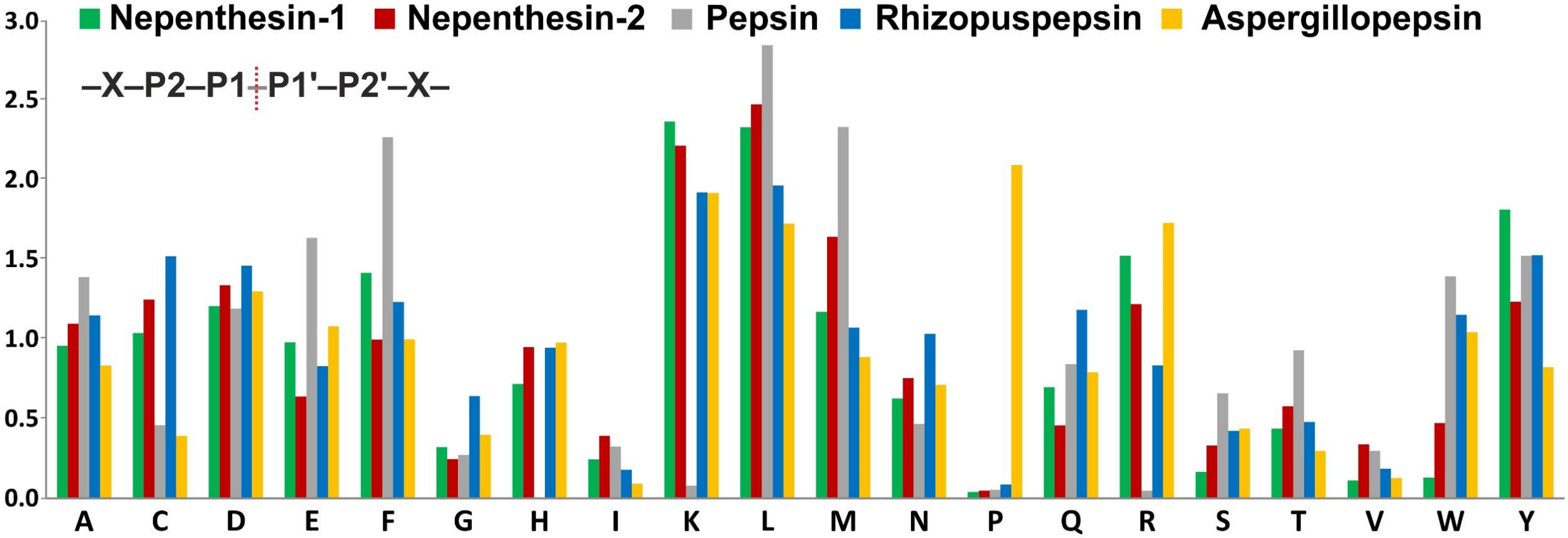
- This information can be used to deduct details about protein-ligand information, protein-protein interaction, protein folding and conformational changes

Protease digestion in HDX

- Optimization of protein digestion is a key step in the HDX experiment
- Spatial resolution and complete protein sequence coverage are the crucial factors
- Proper selection of high functioning protease column boosts digestion performance and allows to achieve full coverage of the protein sequence with many overlapping peptides
- AffiPro offers a set of five acidic protease columns with different cleavage preferences

Pepsin
Rhisopuspepsin
Nepenthesin-1
Nepenthesin-2
Aspergillopepsin

Cleavage preferences of acidic proteases



Yang M. et al., Anal Chem. 2015, Rey M. et al., Rapid Commun Mass Spectrom. 2009

Portfolio overview

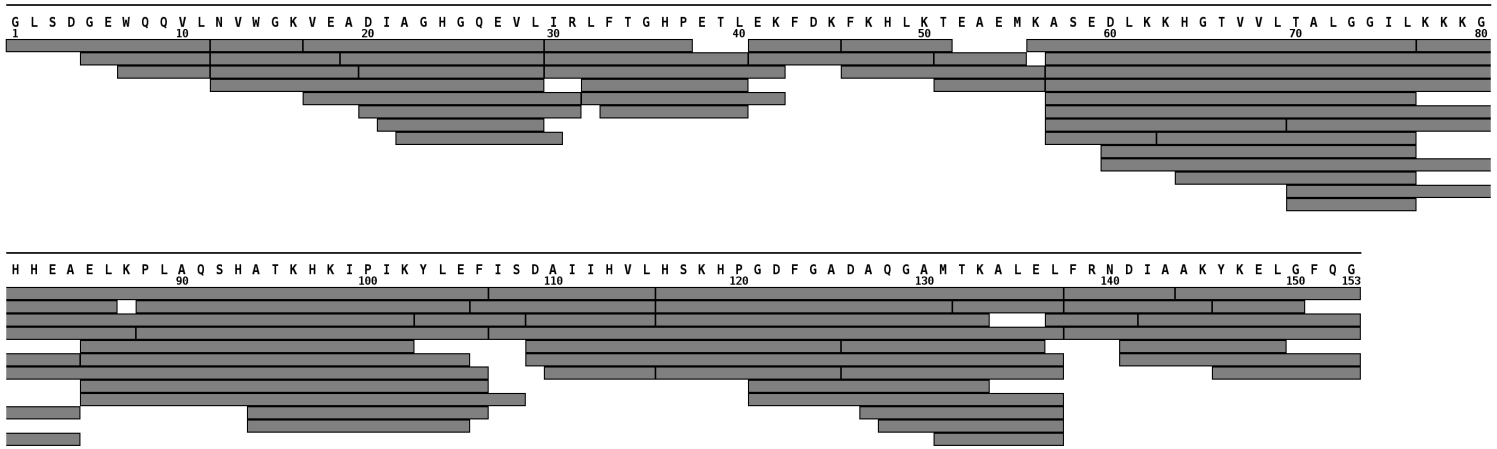
PN	Protease	i.d. [mm]	Length [mm]	Volume [uL]
AP-PC-001	**Pepsin**	2.1	20	69.3
AP-PC-001s	**Pepsin**	1	20	16.2
AP-PC-002	**Rhizopuspepsin**	2.1	20	69.3
AP-PC-002s	**Rhizopuspepsin**	1	20	16.2
AP-PC-003	**Nepenthesin-1**	2.1	20	69.3
AP-PC-003s	**Nepenthesin-1**	1	20	16.2
AP-PC-004	**Nepenthesin-2**	2.1	20	69.3
AP-PC-004s	**Nepenthesin-2**	1	20	16.2
AP-PC-005	**Aspergillopepsin**	2.1	20	69.3
AP-PC-005s	**Aspergillopepsin**	1	20	16.2

All fittings are 10-32 for 1/16” OD tubing

Nepenthesin-1

Digestion of Myoglobin, Nepenthesin-1 (AP-PC-003)

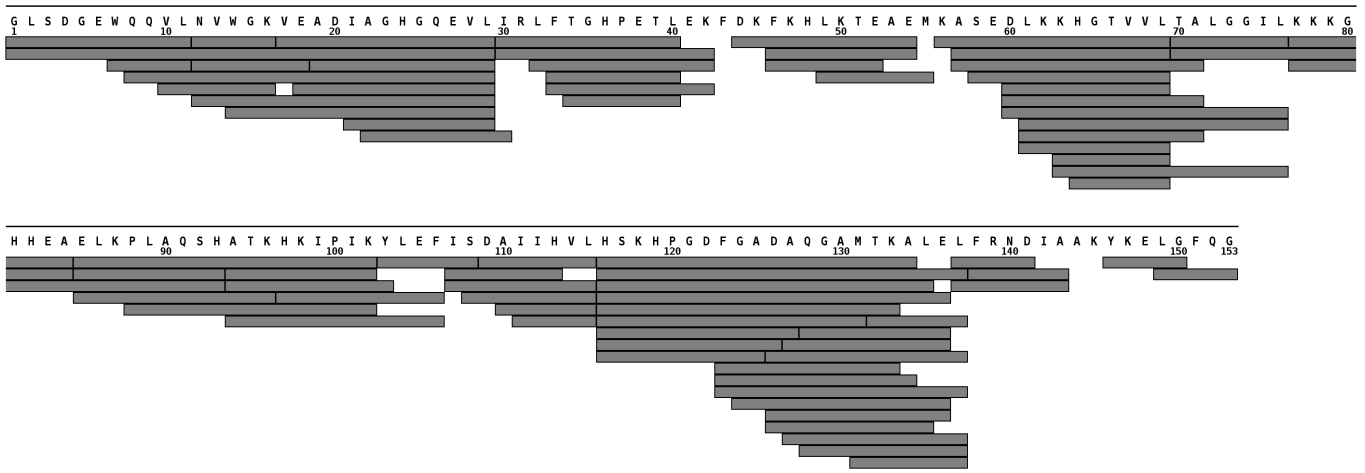
- Flow rate: **0.1mL/min**
- Temperature: 0-4°C



Nepenthesin-2

Digestion of Myoglobin, Nepenthesin-2 (AP-PC-004)

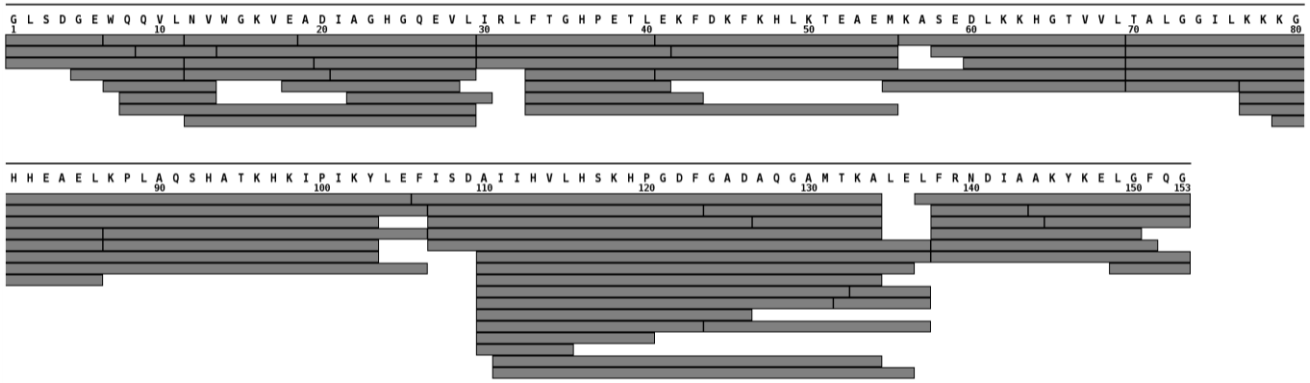
- Flow rate: **0.4mL/min**
- Temperature: 0-4°C



Pepsin

Digestion of Myoglobin, Pepsin (AP-PC-001)

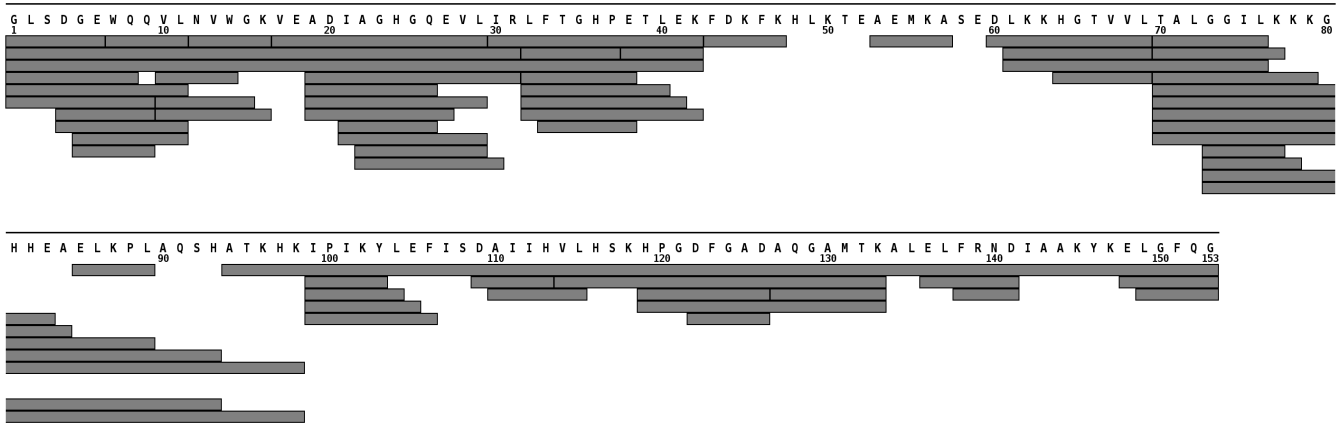
- 1pFlow rate: **0.1mL/min**
- Temperature: 0-4°C



Rhizopuspepsin

Digestion of Myoglobin, Rhizopuspepsin (AP-PC-002)

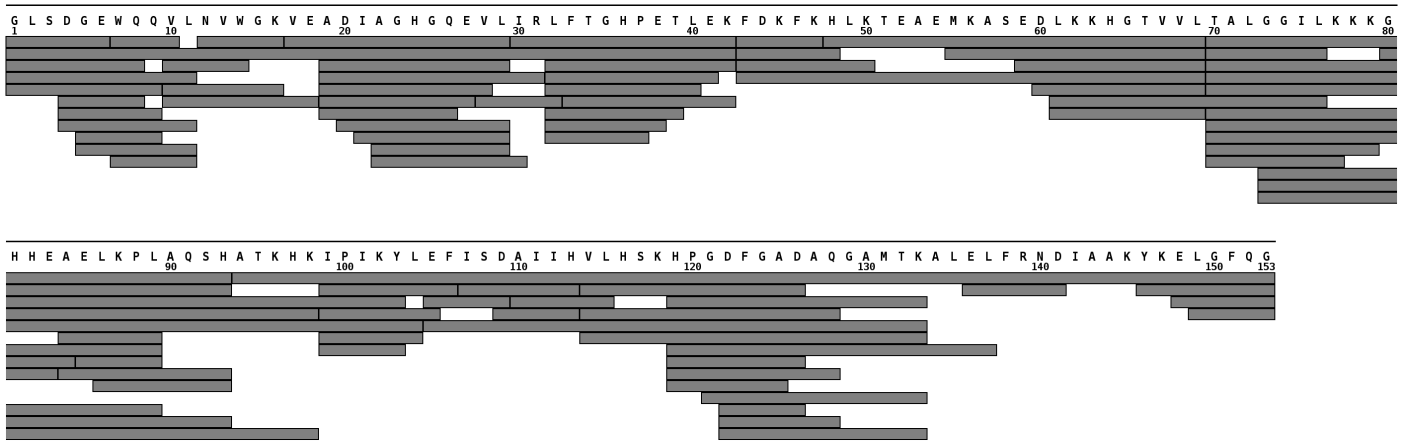
- 1pFlow rate: **0.2mL/min**
- Temperature: 0-4°C



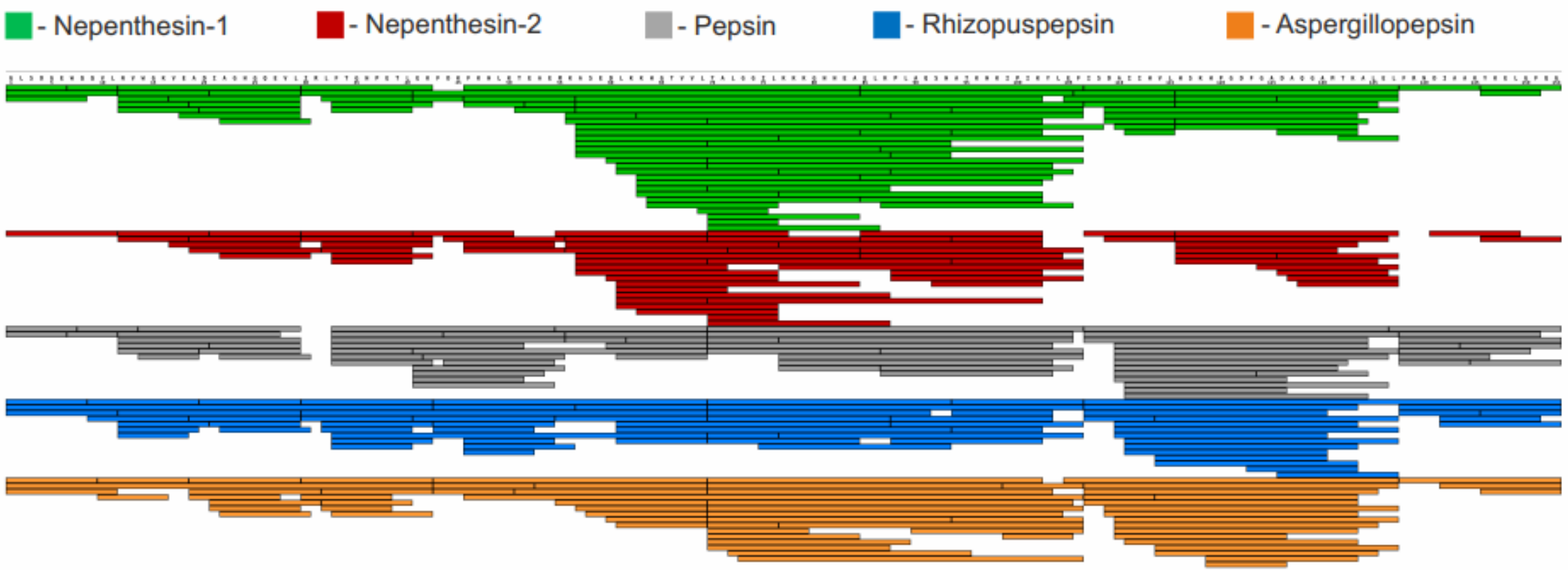
Aspergillopepsin

Digestion of Myoglobin, Aspergillopepsin (AP-PC-005)

- Flow rate: **0.4mL/min**
- Temperature: 0-4°C



Comparison of the proteolytic activity (Myoglobin)



AffiPro acidic protease columns protocol for online digestion

- The columns can be connected to any HPLC or LC/mass spectrometry system
- The degree of digestion can be controlled by changing flow rate, pressure or temperature.
- The columns are compatible with common buffers including **3 M urea, 1 M thiourea or 3 M guanidine hydrochloride**.
- The pressure limit of the columns is **2000 psi**.
- **The columns must not be exposed to solutions with pH greater than 4.5 or organic solvents**.
- The columns can be tested for enzyme activity using myoglobin.
- The columns should be stored with endcaps at 4°C in digestion buffer or in solution with pH lower than 4.5 without organic solvents.
- Before first use or after a longer storage, unplug the trap column and flush the protease column for 5min at flow rate 0.1 mL/min with digestion solvent.
- Autodigest peptides could be observed in the first run or after longer storage. Inject standard protein for several times to settle the column.

What is Nepenthesin?

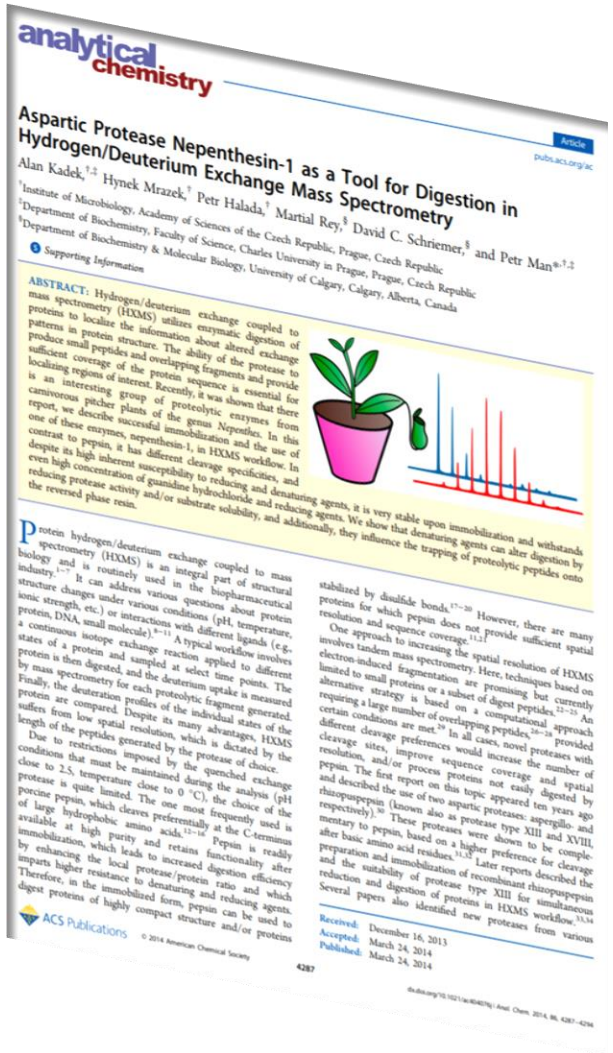
New group of interesting proteolytic enzymes from carnivorous pitcher plants of the genus *Nepenthes*

Their proteolytic activity is very useful for hydrogen/deuterium exchange mass spectrometry (HX-MS)

In contrast to pepsin, it has different cleavage specificities, and despite its high inherent susceptibility to reducing and denaturing agents, it is very stable upon immobilization and withstands even high concentration of guanidine hydrochloride and reducing agents

Nepenthesin II shares many properties with Nepenthesin I, such as fast digestion at reduced temperature and pH, and broad cleavage specificity, but in addition, it cleaves C terminal to tryptophan

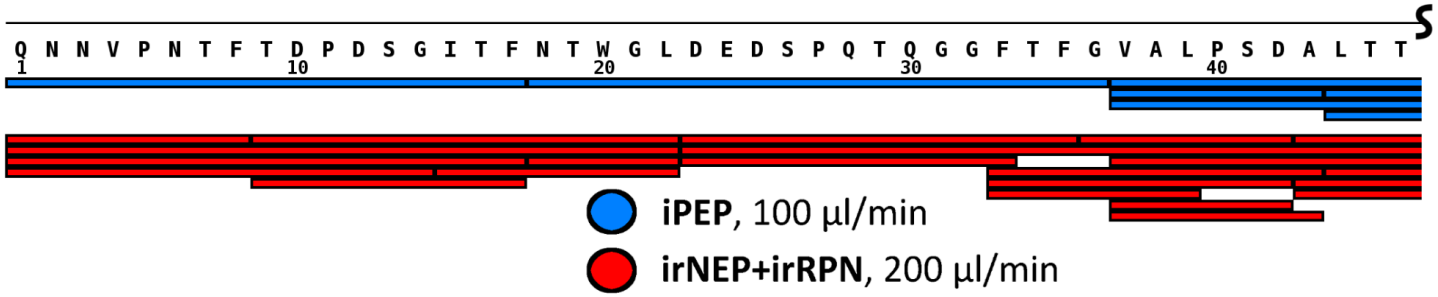
DOI: 10.1021/acs.analchem.5b00831 | Anal. Chem. 2015, 87, 6681−6687

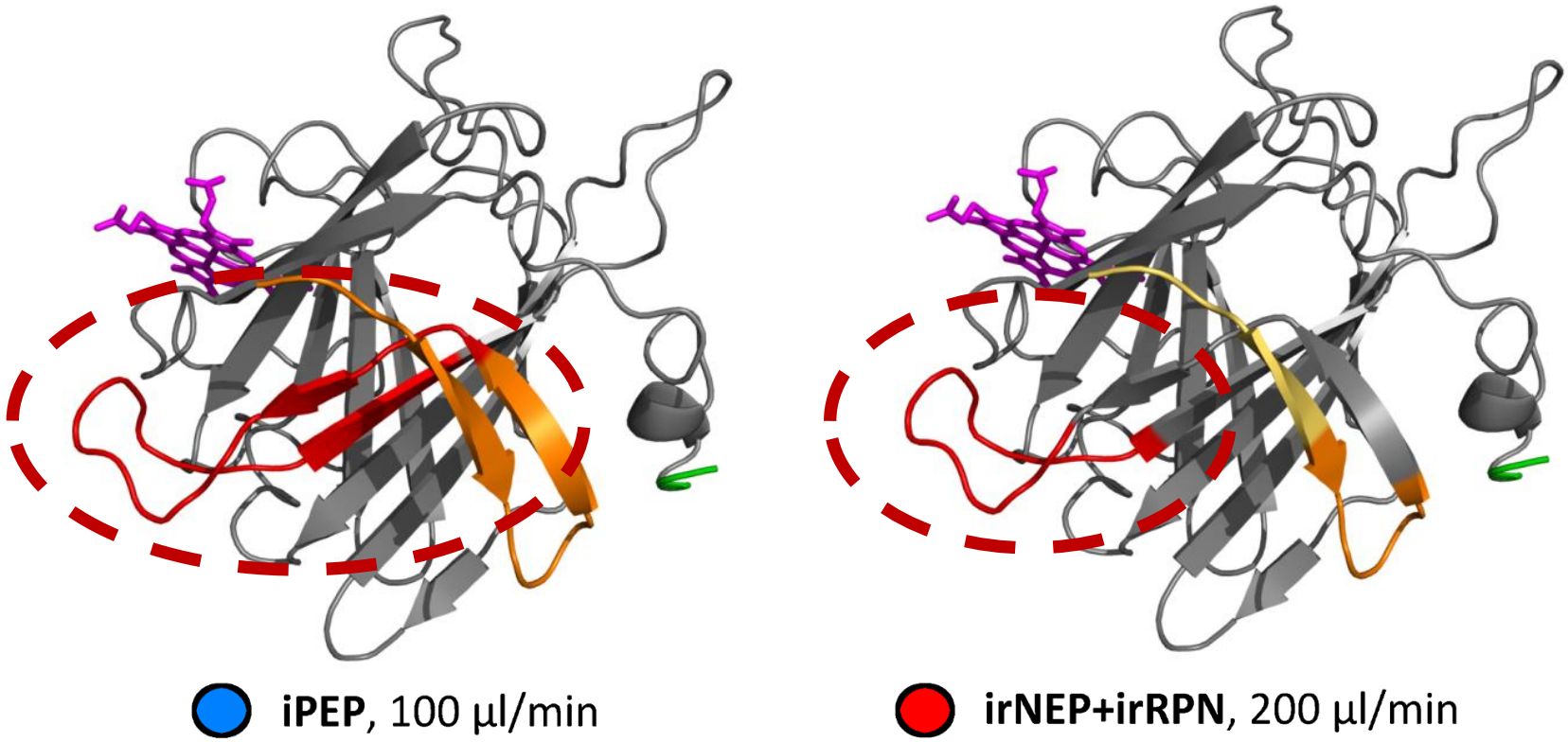


dx.doi.org/10.1021/ac404076j | Anal. Chem. 2014, 86, 4287−4294

Proteases Combination – Improved Spatial Resolution

Combining the proteases provides more information, which makes a strong scientific argument for more diverse protease column portfolio





https://doi.org/10.1016/j.bbagen.2016.11.016 | Kadek et al., Biochim Biophys Acta (2017)

Examples of reported customer use

University of Leeds

Article | Open Access | Published: 01 May 2020

Inter-domain dynamics in the chaperone SurA and multi-site binding to its outer membrane protein clients

Antonio N. Calabrese, Bob Schiffrin, Matthew Watson, Theodoros K. Karamanos, Martin Walko, Julia R. Humes, Jim E. Horne, Paul White, Andrew J. Wilson, Antreas C. Kalli, Roman Tuma, Alison E. Ashcroft, David J. Brockwell & Sheena E. Radford

Nature Communications **11**, Article number: 2155 (2020) | Cite this article

2656 Accesses | **2** Citations | **93** Altmetric | Metrics

Abstract

The periplasmic chaperone SurA plays a key role in outer membrane protein (OMP) biogenesis. *E. coli* SurA comprises a core domain and two peptidylprolyl isomerase domains (P1 and P2), but its mechanisms of client binding and chaperone function have remained unclear. Here, we use chemical cross-linking, hydrogen-deuterium exchange mass spectrometry, single-molecule FRET and molecular dynamics simulations to map the client binding site(s) on SurA and interrogate the role of conformational dynamics in OMP recognition. We demonstrate that SurA samples an array of conformations in solution in which P2 primarily lies closer to the core/P1 domains than suggested in the SurA crystal structure.

https://www.nature.com/articles/s41467-020-15702-1

The Hebrew University of Jerusalem

Biochemistry

Defining Hsp33's Redox-regulated Chaperone Activity and Mapping Conformational Changes on Hsp33 Using Hydrogen-deuterium Exchange Mass Spectrometry

doi: 10.3791/57806 Published: June 7, 2018

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Summary

One of the most challenging stress conditions that organisms encounter during their lifetime involves the accumulation of oxidants. During oxidative stress, cells heavily rely on molecular chaperones. Here, we present methods used to investigate the redox-regulated anti-aggregation activity, as well as to monitor structural changes governing the chaperone function using HDX-MS.

https://www.jove.com/t/57806/defining-hsp33-s-redox-regulated-chaperone-activity-mapping

Customer testimonials in the USA

Large portion of our customers in North America are in biopharma and biotech companies and do not publish nor discuss their protocols and molecules publicly. However, we can provide testimony of some leading academic KOLs in the HDX field who are using AffiPro columns.

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John Engen Laboratory

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The Engen Laboratory website:
http://www.hxms.neu.edu/index.htm

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