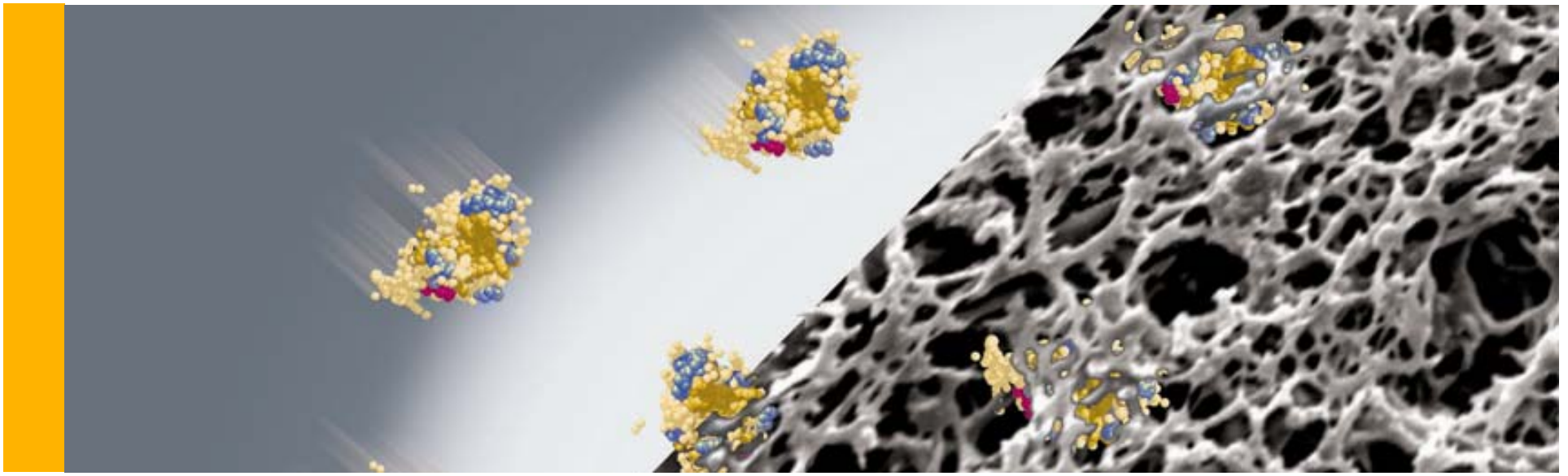


Proteomic Filter Aided Sample Preparation with the Vivacon® 500

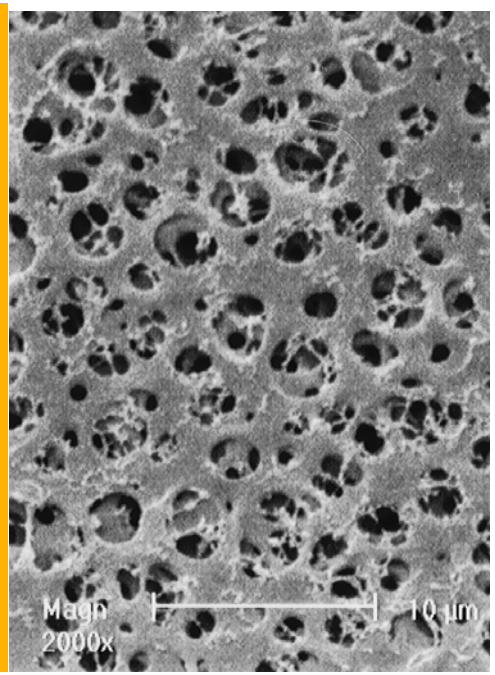
Michael Vagell, Ph.D., San Diego, January 9, 2012

Agenda

- Sartorius Laboratory Products and Services
- Executive Summary-Filter Aided Sample Preparation (FASP)
- Ultrafiltration Basics & Buffer Exchange
- FASP Method
- Comparison of the Amicon™ Microcon™ and Vivacon 500 for N-Glycoproteome Analysis
- Flu Vaccine Characterization
- Isolation of Neuropeptides
- Other Potential Applications for FASP
- Contact Information

sartorius

"For over 140 years, we have stood for trust, quality and innovation in the lab."



You may know the name Sartorius from our world famous balances, but biotechnology represents half of our business and our company has been involved with membrane filters since their early development in the 1920s.

"Sample preparation and fractionation technologies are one of the most crucial processes in proteomic analysis and biomarker discovery in solubilized samples."

J. Sep. Sci. 2009, 32, 771 – 798

Executive Summary - Filter Aided Sample Preparation (FASP)

Filter Aided Sample Preparation (FASP)[1] allows gel-free processing of biological samples solubilized with detergents for proteomic analysis by mass spectrometry. Detergents are removed by ultrafiltration using buffer exchange and after protein digestion, the peptides are separated from undigested material[2].

[1]Nature Methods 6, 359 - 362 (2009)

[2]Proteomics, 5, 1742–1745 (2005)

Buffer exchange/Diafiltration

The convective elimination of permeable solutes by the addition of fresh solvent to the retentate.

$$\frac{C}{C_o} = e^{-NS}$$

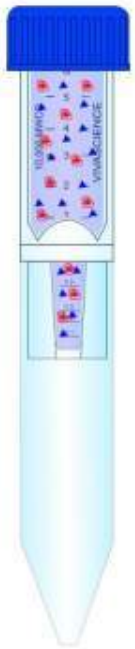
Where,

C	concentration of the solute at the given instance,
C_o	initial solute concentration
N	number of diavolumes
S	sieving/rejection coefficient

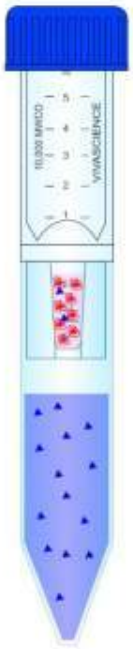
Diavolume (DV)

A volume equal to the product liquid volume to which a diafiltration buffer is added. The addition and attendant concentration theoretically dilutes contaminants by 50% for each volume added.

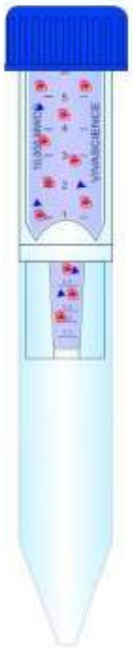
Buffer Exchange with Centrifugal Concentrators



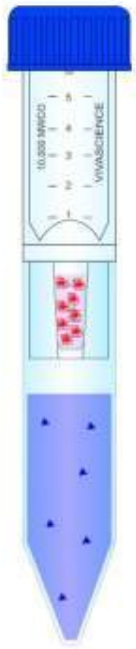
Select cut off 1/3 protein size



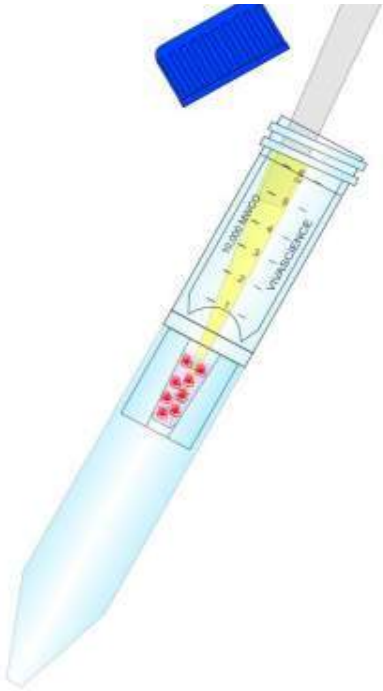
Spin; salt pass through membrane



Refill with buffer of choice



Spin to concentrate target



Recover concentrated and de-salted protein sample

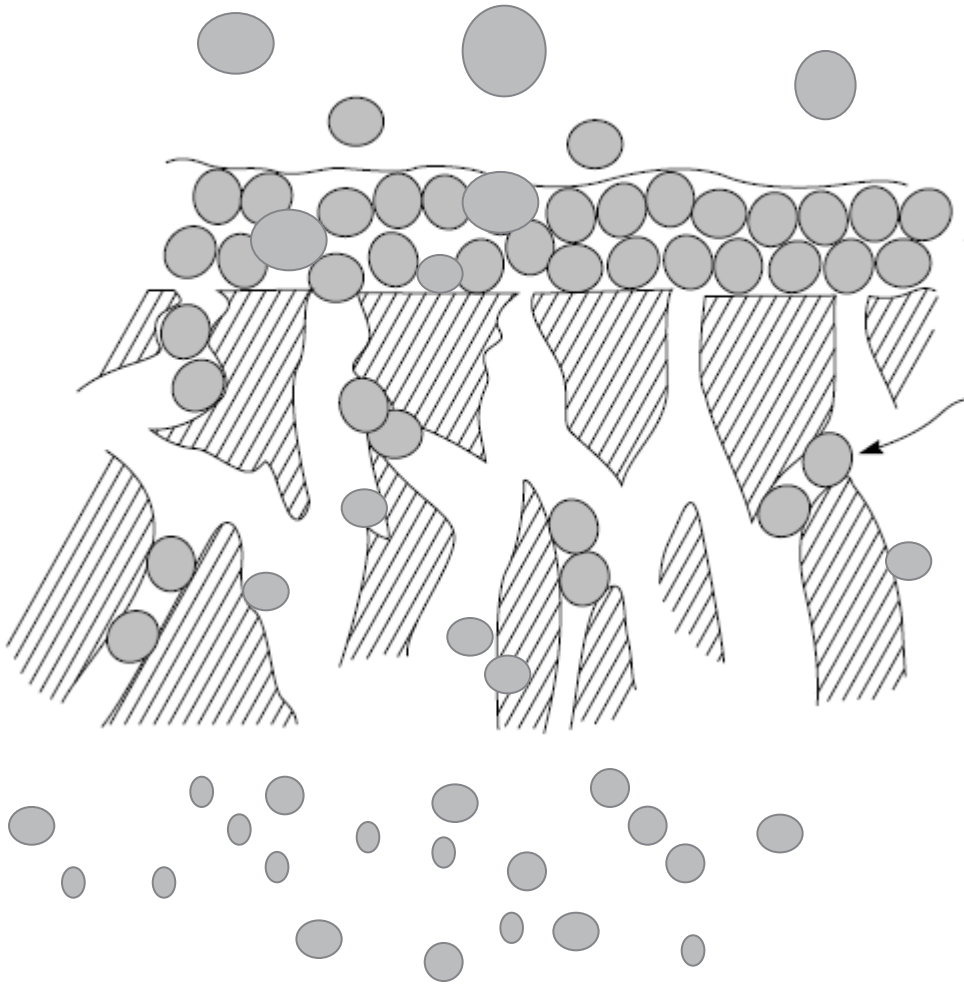
What determines whether a molecule will pass through or be retained by an ultrafiltration membrane?

- membrane factors
  - molecular weight cutoff (MWCO) ~ pore size
  - membrane composition
- particle size distribution
- concentration of macromolecules (contributing to the thickness of gel layer)
- amount of force (such as centrifugal force or transmembrane pressure)
- the hydrodynamic radius of the molecule

Factors affecting the hydrodynamic radius of a molecule:

- charge
  (determined by solution pH and amino acid composition)
- hydrophobicity
- shape (globular versus linear)
- deformation characteristics (unwinding)

Where did my protein go?

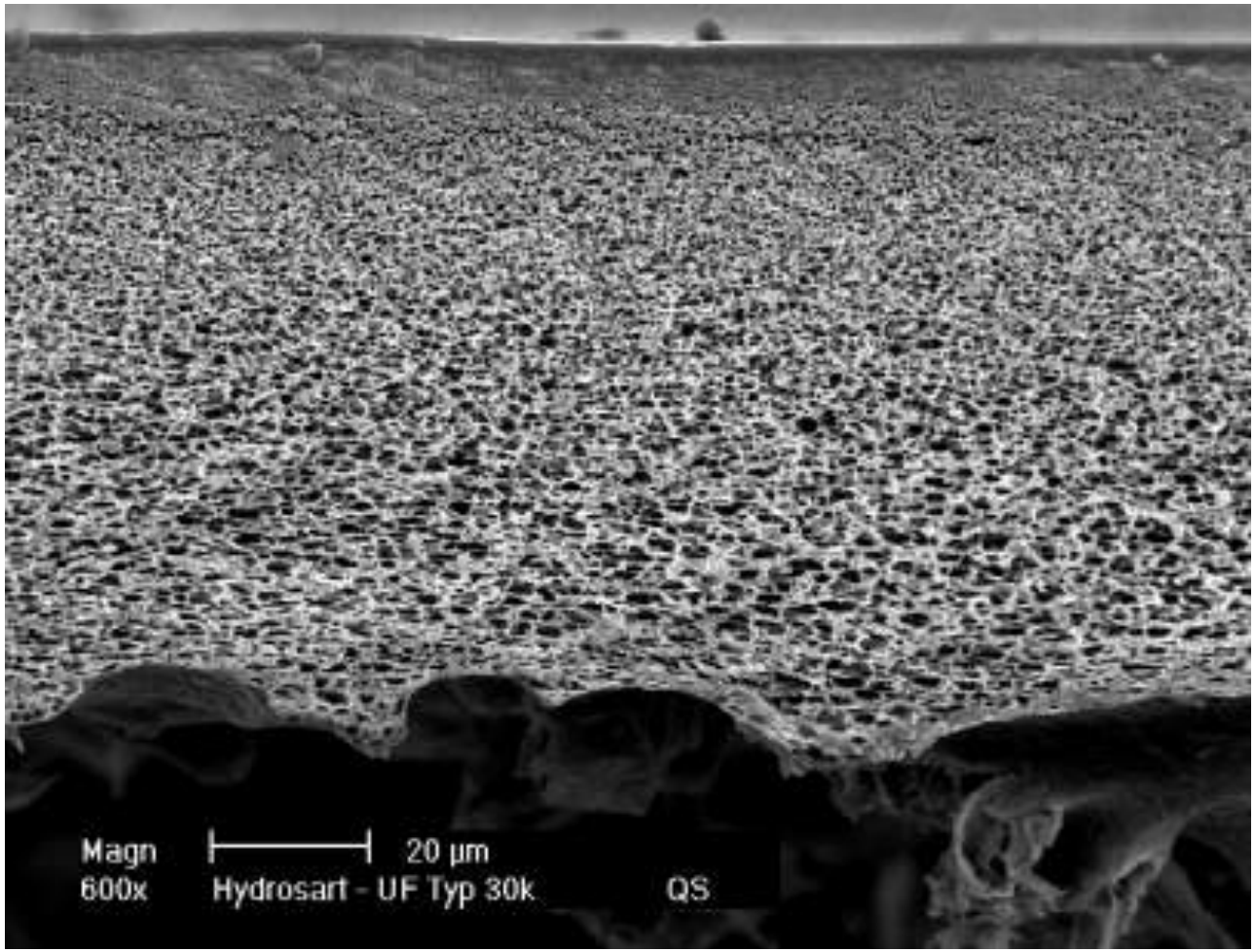


surface fouling at gel layer

internal fouling

- nonspecific adsorption
- size exclusion

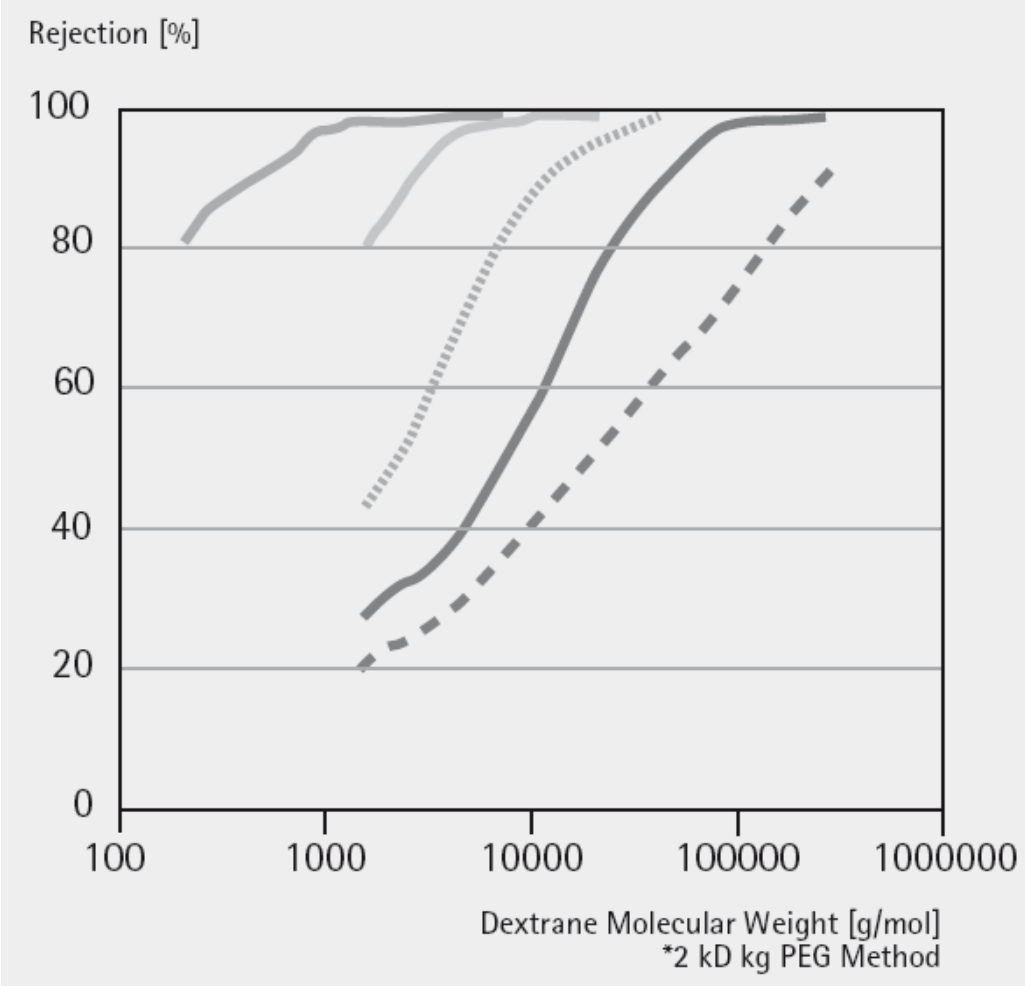
Ultrafiltration Membrane Cross Section



Skin Layer

Drainage & Support Layer

Rejection Profiles of Different Membrane Cutoffs



FASP method

1. Cell or tissue lysates can be prepared in the presence of high concentrations of detergent. Disulfide bridges are reduced with dithiothreitol (DTT). Detergent micelles and protein detergent complexes are dissociated in the presence of 8 M urea. The detergent, DTT and other low-molecular-weight components are removed by utrafiltration (buffer exchange).
2. Thiols are carboxyamidomethylated with iodoacetamide (IAA) and excess reagent is removed by ultrafiltration.
3. Repeated washes with 8 M urea remove any remaining detergent.
4. The protein suspension is digested with endoprotease, and the resulting peptides are collected in the filtrate. High-molecular-weight molecules including the endoprotease are retained on the filter.

http://www.biochem.mpg.de/mann/fasp/index.html

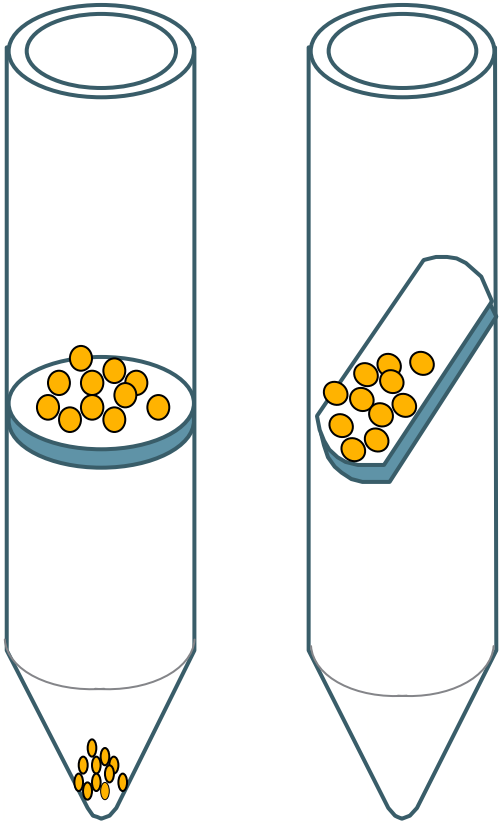
Nature Methods 6, 359 - 362 (2009)

The Amicon Microcon and the Vivacon are similar in terms of size and membrane orientation.

Microcon

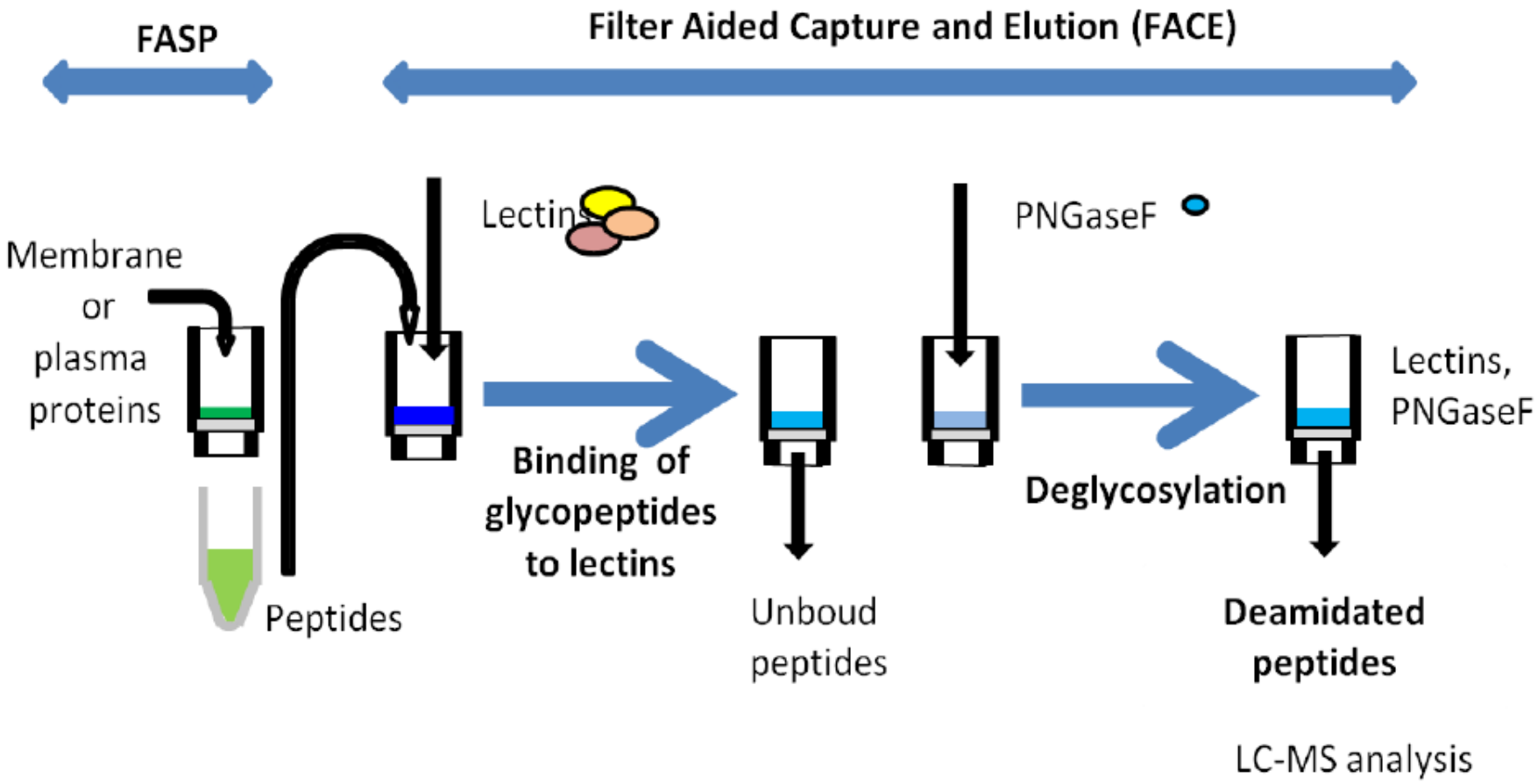
Vivacon 500

Horizontal versus Vertical Membrane Configuration



For most applications, vertical membrane configurations are preferred.
However, in FASP, the vertical membrane configuration, with its increased surface area, can result in fewer peptides passing into the filtrate.

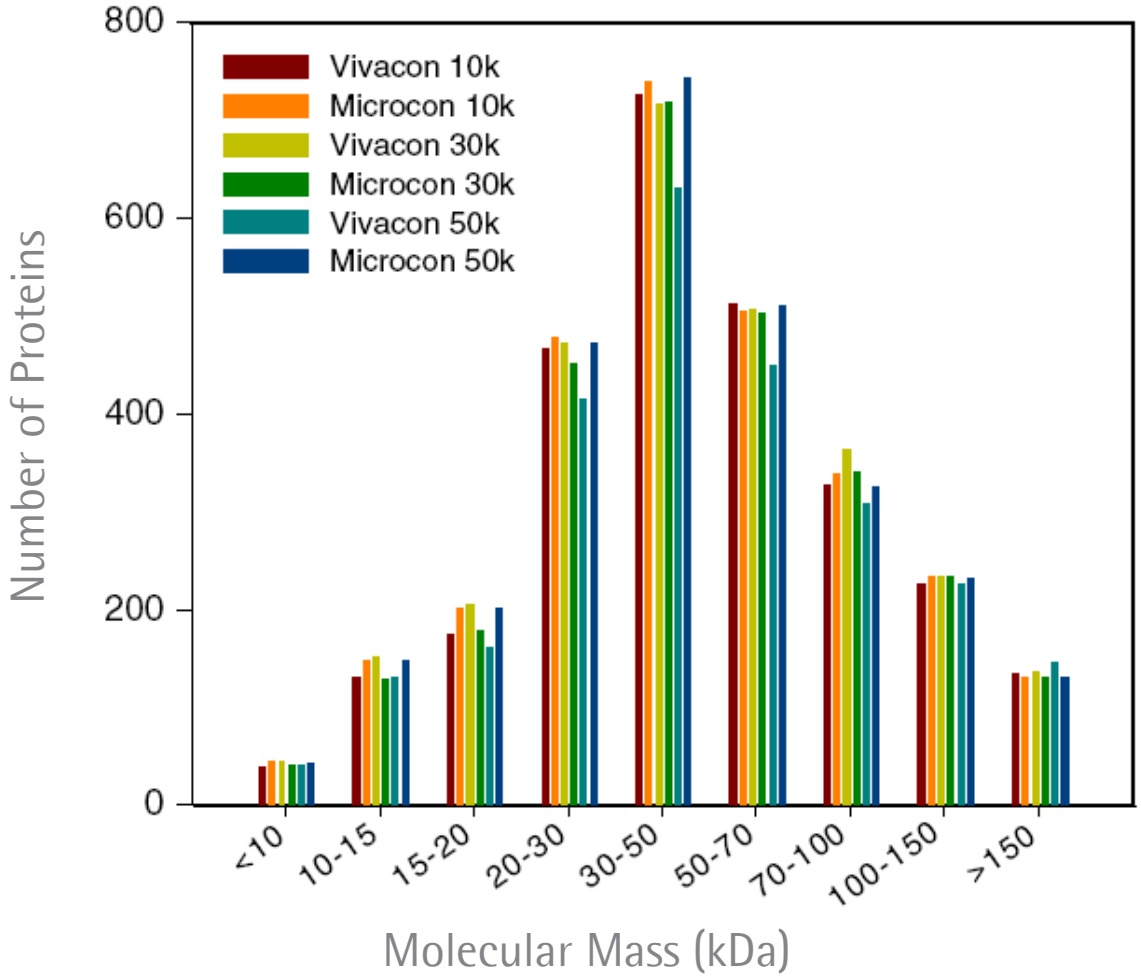
N-Glycoproteome Analysis



http://www.biochem.mpg.de/mann/fasp/n_glyco_fasp/index.html

Cell 141, 897–907, May 28, 2010

Distribution of the molecular masses of identified proteins



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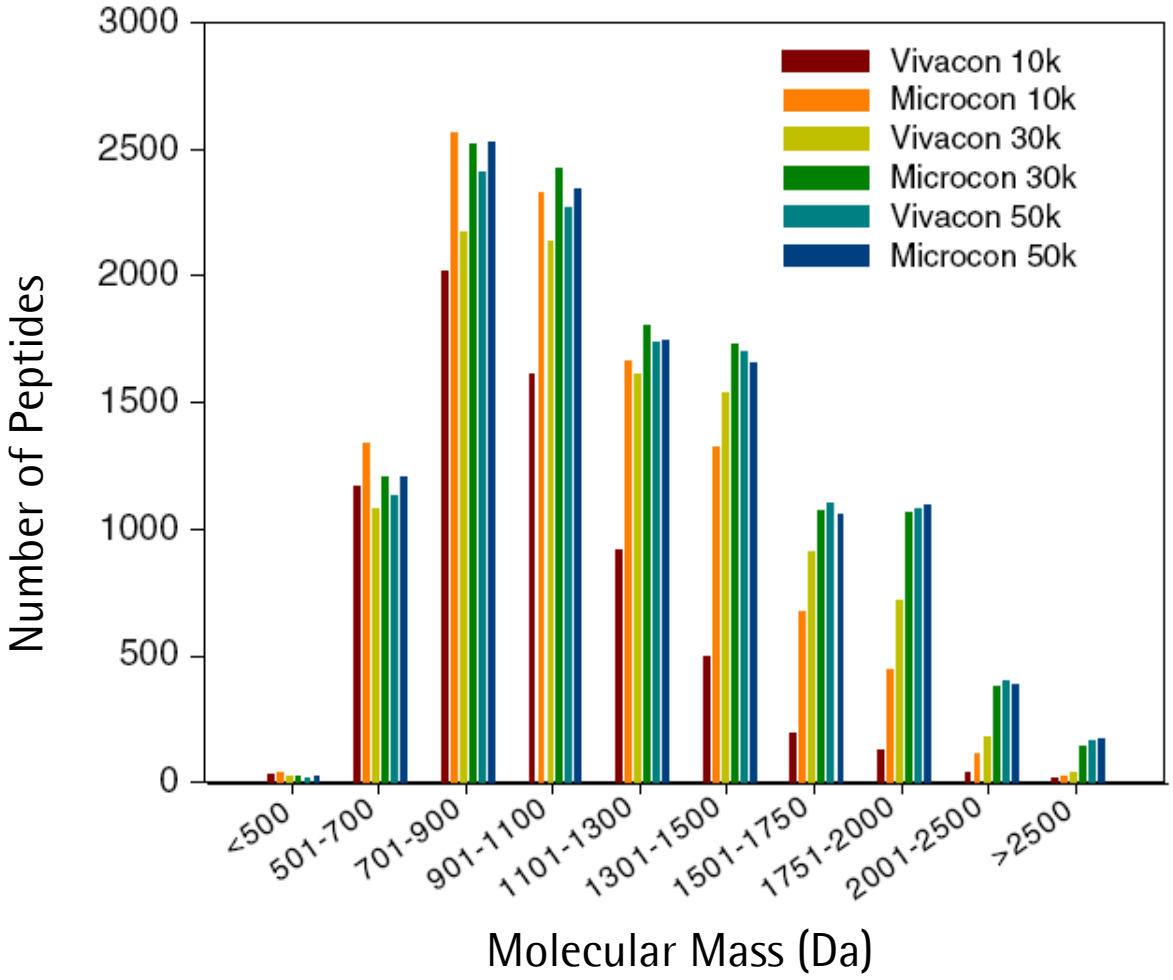
Distribution of the molecular masses of identified peptides



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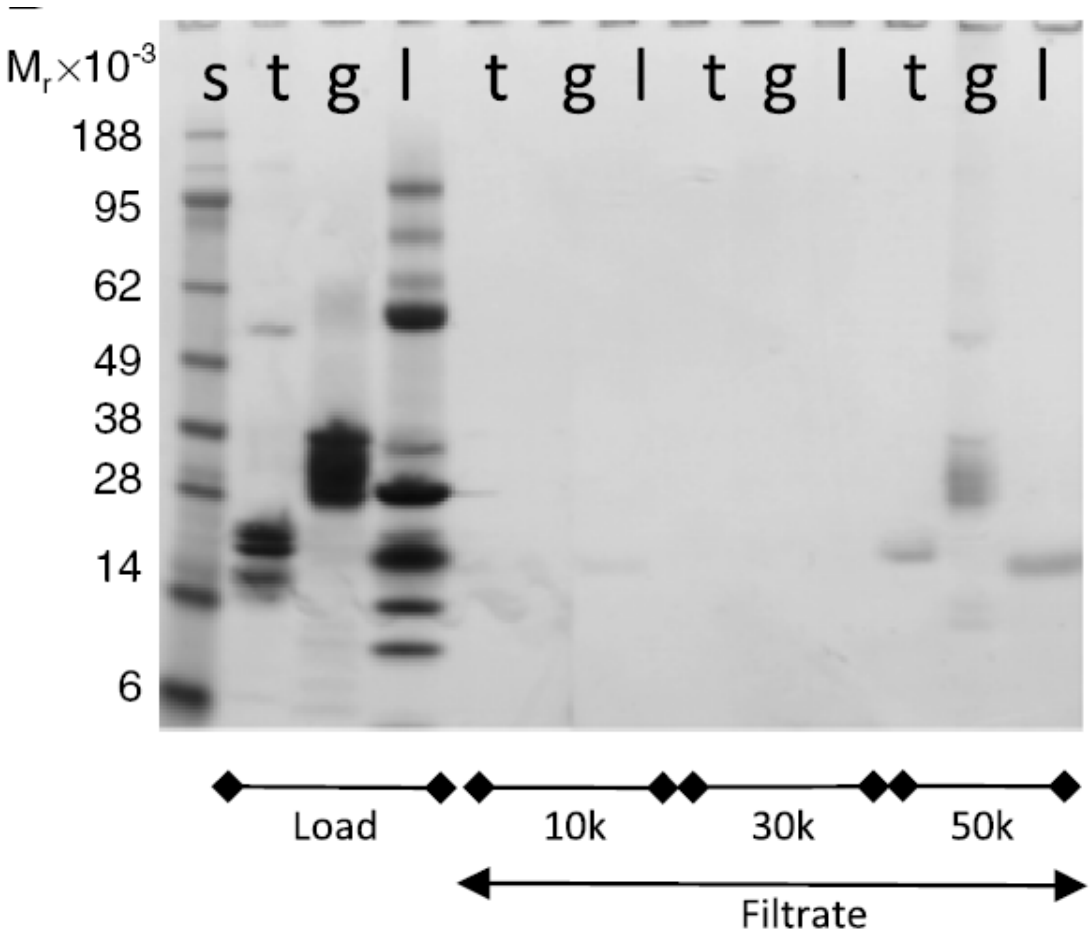
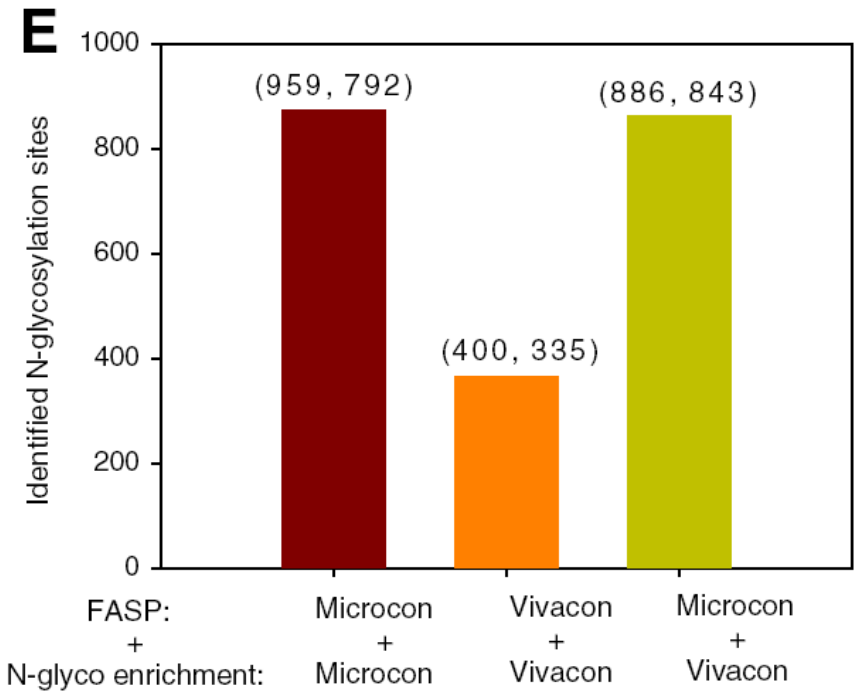


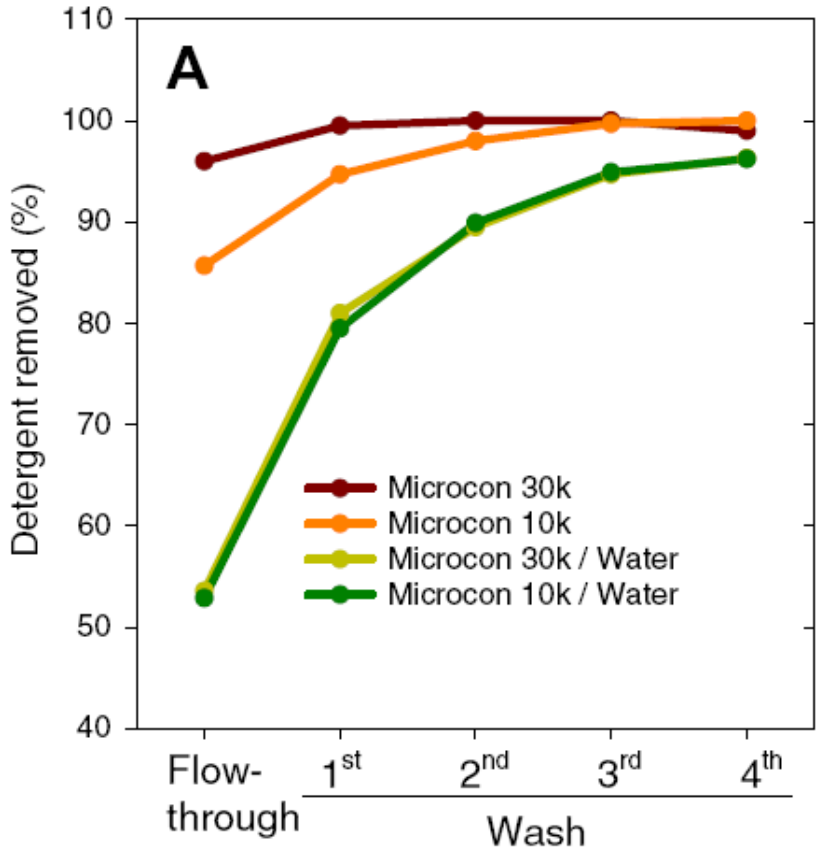
Retention of trypsin (t), GluC (g), and lectin mixture (l) containing ConA, WGA, and RC-20 on Microcon filter with nominal molecular weight cutoffs of 10, 30, and 50 k.

Reprinted from Analytical Biochemistry, Vol. 410(2), Jacek R. Wiśniewski, Dorota F. Zielinska, Matthias Mann, Comparison of ultrafiltration units for proteomic and N-glycoproteomic analysis by the filter-aided sample preparation method, Pages 307-309, Copyright (2010), with permission from Elsevier.



Total lysates of mouse liver were processed according to the N-glyco FASP procedure using Microcon 30 k and Vivacon 30 k filters, and deglycosylated peptides were analyzed by LC–MS/MS.

Removal of 0.2% SDS using Microcon 10k and 30k concentrators by repeated elution with 8 M urea or water.



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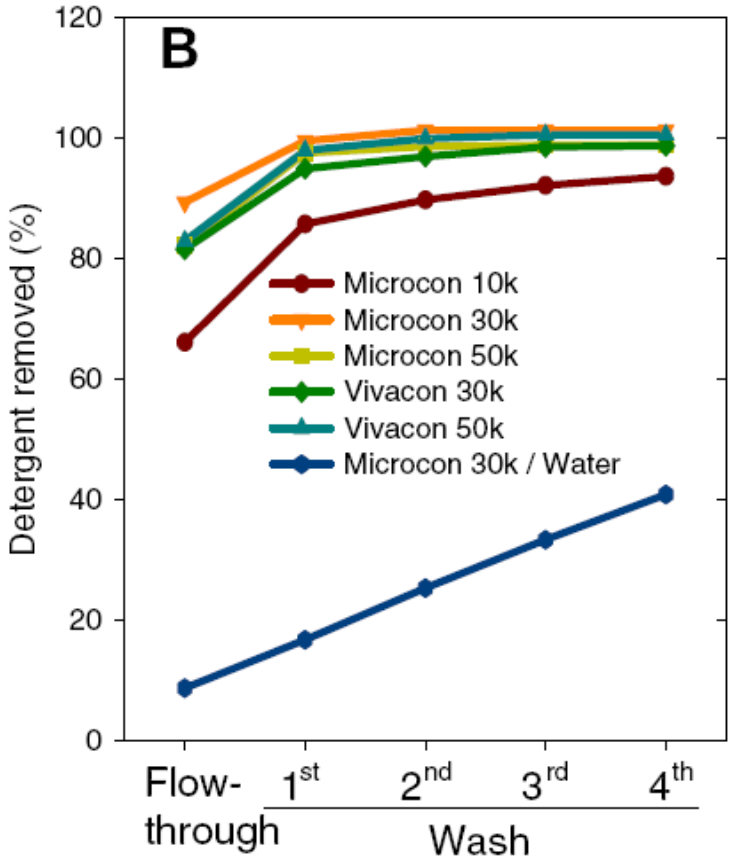
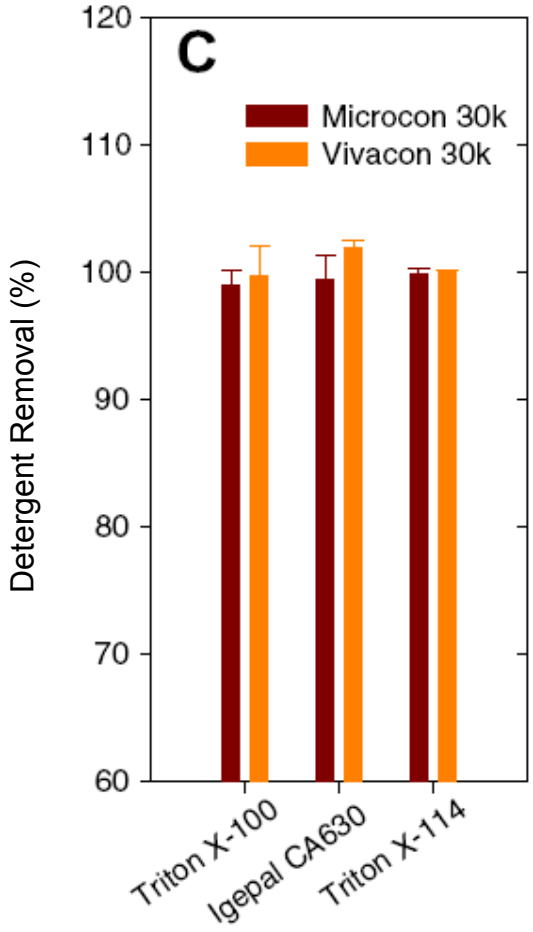


Removal of 0.2% (w/v) solutions of Triton X-100, Triton X-114, and Igepal CA630 by repeated centrifugation-mediated elutions and sample dilutions.

Extent of detergent removal after three washing steps.



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Flu Vaccine Characterization

Creskey, et al. used a variation of filter aided sample preparation where peptide-N-glycosidase F (PNGase F) was used before endopeptidase cleavage to deglycosylate the influenza virus proteins.

The use of a mass spectroscopy based method yielded not only more information, but significantly shortened the assay development time over antibody-based methods.

Anal. Biochem. 406 (2010) 193–203

Even without using FASP, the Vivacon 500 is useful for neuropeptide research

Petruzziello, et. al. homogenized brain tissue in up to 50% methanol/0.2% formic acid, and then used a 10k MWCO Vivacon 500 to separate the neuropeptides (into the filtrate) from other proteins.

J. Proteome Res., Just Accepted Manuscript
DOI: 10.1021/pr200709j • Publication Date (Web): 09 Nov 2011

Other potential applications for Filter Aided Sample Preparation (FASP)/Filter Aided Capture and Elution (FACE):

- phosphoprotein analysis[1]
- other posttranslational analysis of peptides
- analysis of peptide-protein interactions

[1]Cell 141, 897–907, May 28, 2010

What about scaling up from 500 µl to 2 ml?

The Vivacon 2 also has a horizontal membrane orientation and is available with the same Hydrosart membranes as the Vivacon 500.