Sample Preparation for Proteomics and Mass Spectrometry Analysis

BMG/PHR 744

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01/21/2011

Why is sample preparation so important?

- key step in analysis that affects

• reproducibility, accuracy of results

• durability of instrumentation, columns

• speed of analysis (increased efficiency)

• cost of analysis

Pre-analytical factors:

Sample collection:

- Collection tubes: e.g. serum vs plasma
- Additives: eg. anticoagulants for plasma, antibacterial for urine?
- Centrifugation/filtration

Sample storage:

- Do you need to aliquot?
- Storage temperature?
- Freeze thaw cycles? Temperatures?

What are we interested in determining?

- molecular weight determination
- identifying proteins
- biomarker discovery
- characterizing post-translational modifications
  (e.g. glycosylation, phosphorylation)
- protein-protein interaction
  (glycosylation, phosphorylation)

Sample preparation generally involves:

- Extraction or isolation of specific analytes
- Cleaning up – remove interfering substances, contaminants
- Concentration of analytes
- Derivatization

Choice of techniques used in sample preparation depends on:

- starting material
  - simple vs complex matrix
  - volume
- subsequent analysis required
- through-put
  - how many samples need to be analyzed?

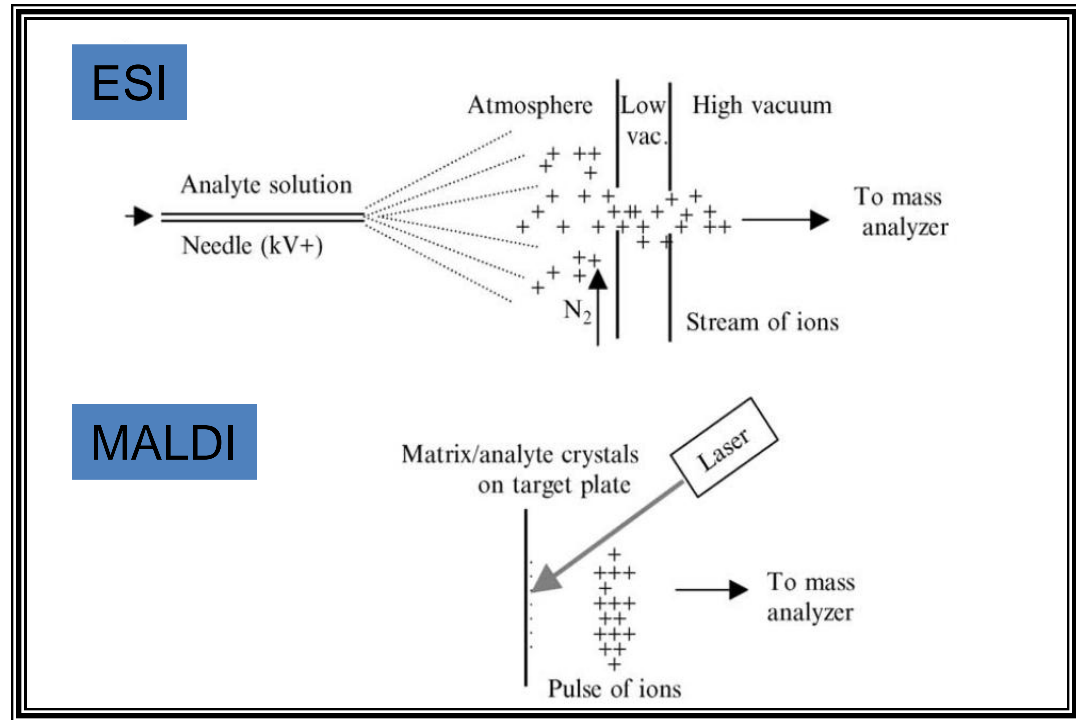
Biofluids: Serum, plasma, urine, Saliva, Broncheoalveolar lavage (BALF), Nipple Aspirate

Eknoyan, *American Journal of Kidney Diseases*, **49**, 2007, 865-872

Subsequent analysis?

Urine color wheel

Mass Spectrometer



Baldwin M.A., Mass spectrometers for the analysis of biomolecules, Methods in Enzymology, Vol 402

Starting material: simple or complex

Proteomics. 2005 Aug;5(13):3226-45.
Omenn GS et. al.,

Overview of the HUPO Plasma Proteome Project: results from the pilot phase with 35 collaborating laboratories and multiple analytical groups, generating a core dataset of 3020 proteins and a publicly-available database.

Genome Biol. 2006; 7(9): R80.
Adachi J., et al.,

The human urinary proteome contains more than 1500 proteins, including a large proportion of membrane proteins

General Sample Preparation Methods

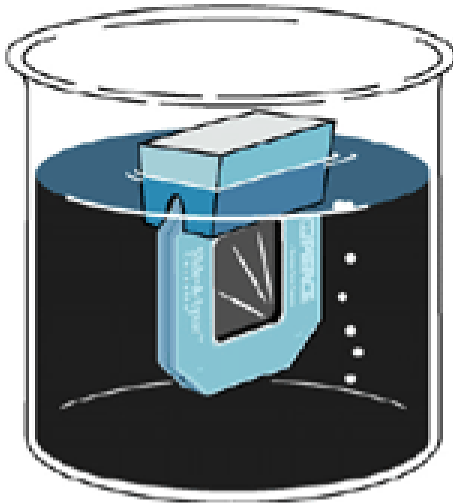
- ❖Membrane based
  - •Dialysis
  - •Ultrafiltration

- ❖Protein precipitation
  (Tricarboxylic acid, NH_4SO_4, MeOH)

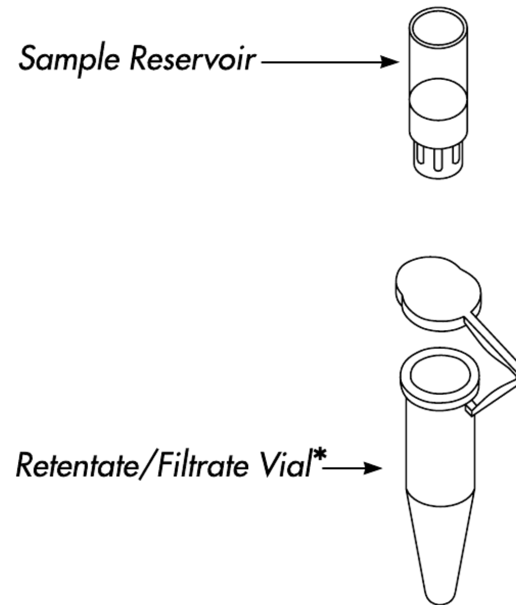
- ❖Liquid-liquid extraction

- ❖Solid phase extraction

Desalting, concentration, buffer exchange



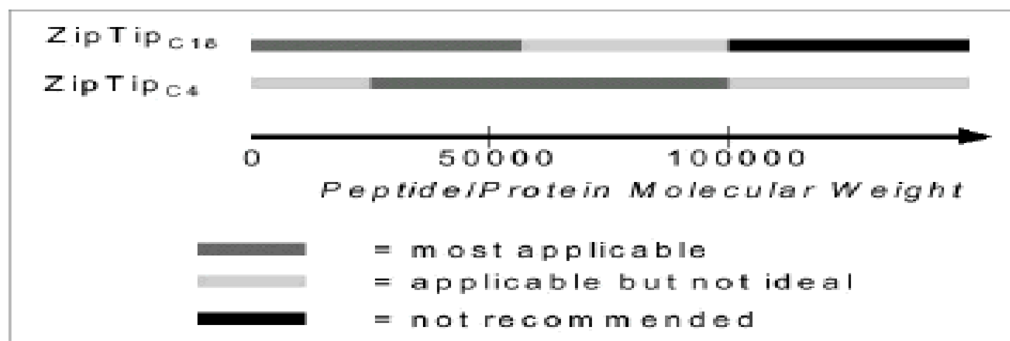
Slide-A-Lyzer™ Dialysis (Pierce)



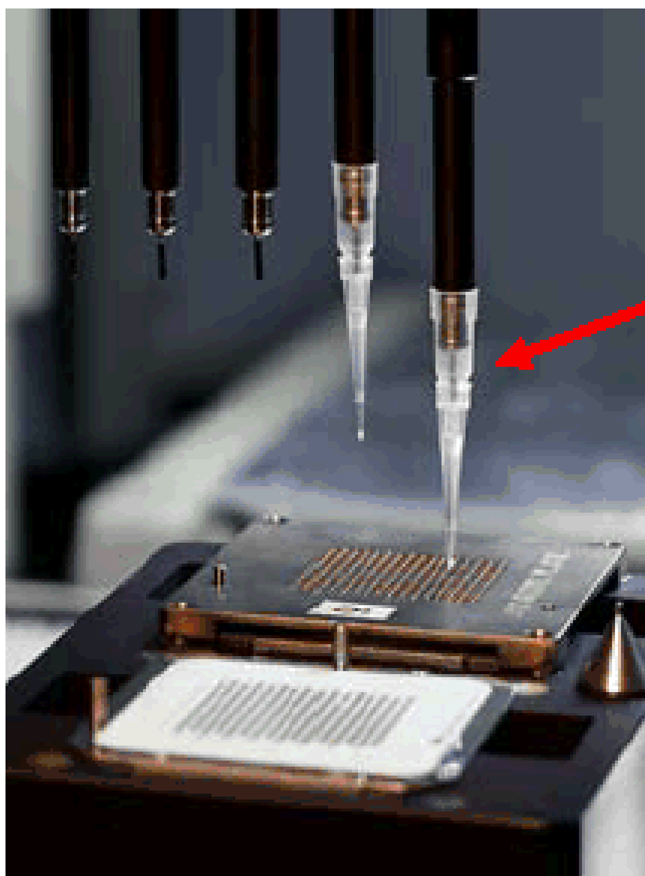
Microcon centrifugal device (Millipore)

http://biotech.matcmadison.edu/resources/proteins/labManual/chapter_4/section4_3.htm

http://www.millipore .com



http://www.millipore.com/techpublications/tech1/ps2342enus



Micromass Mass*PREP*™ and PE MultiPROBE® compatibility. Integrated device containing an adapter collar press fit into a ZipTip$_{\mu\text{-}C18}$ containing a 200 nl bed of C18 allowing elution in minimal volumes.

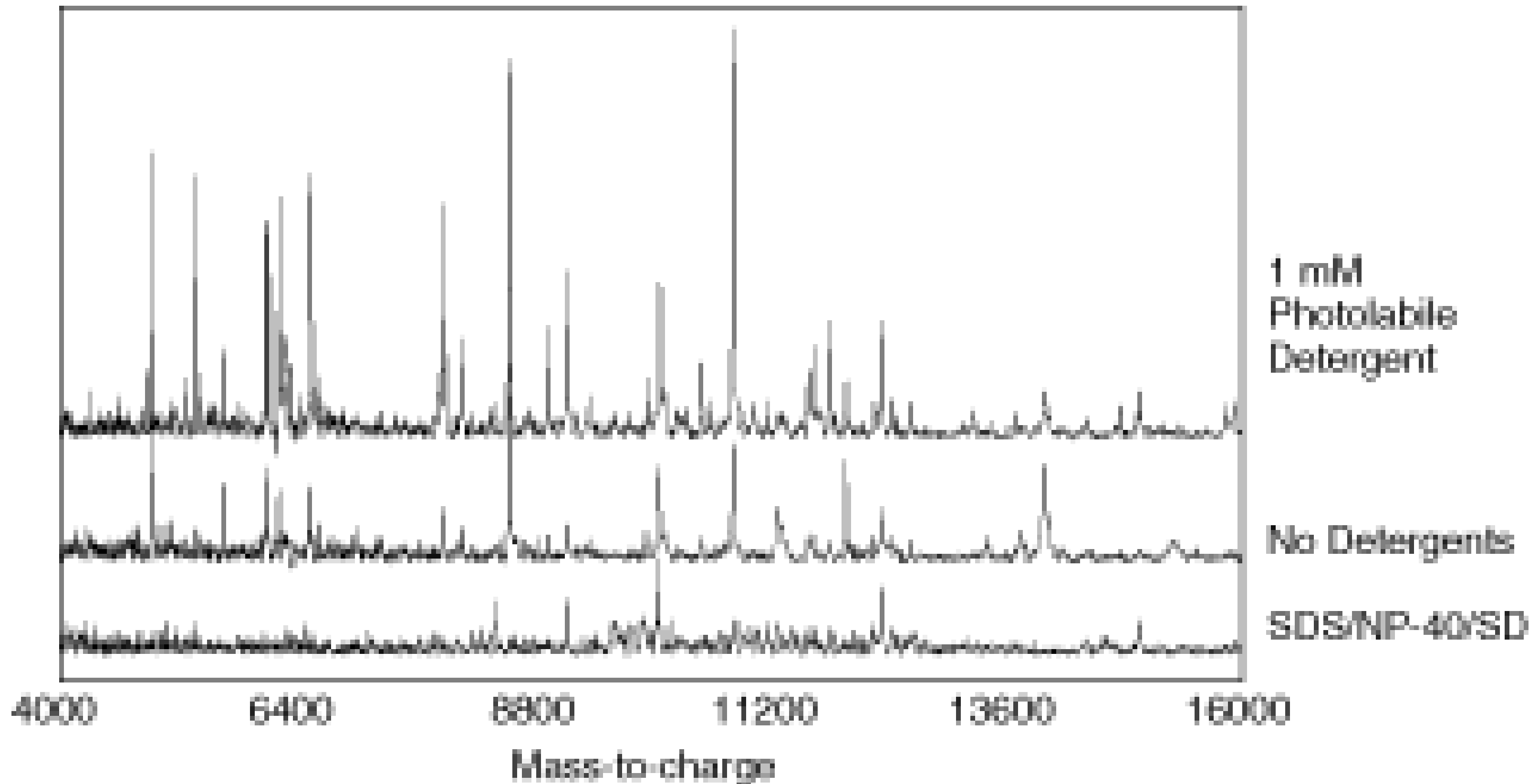
Additional Automation Protocols:

- ABI Symbiot™
- Bruker MAP™ II and MAP II/8
- Genomic Solution ProMS™
- Tecan Genesis

Automation reduces the labor involved with sample processing and target spotting.

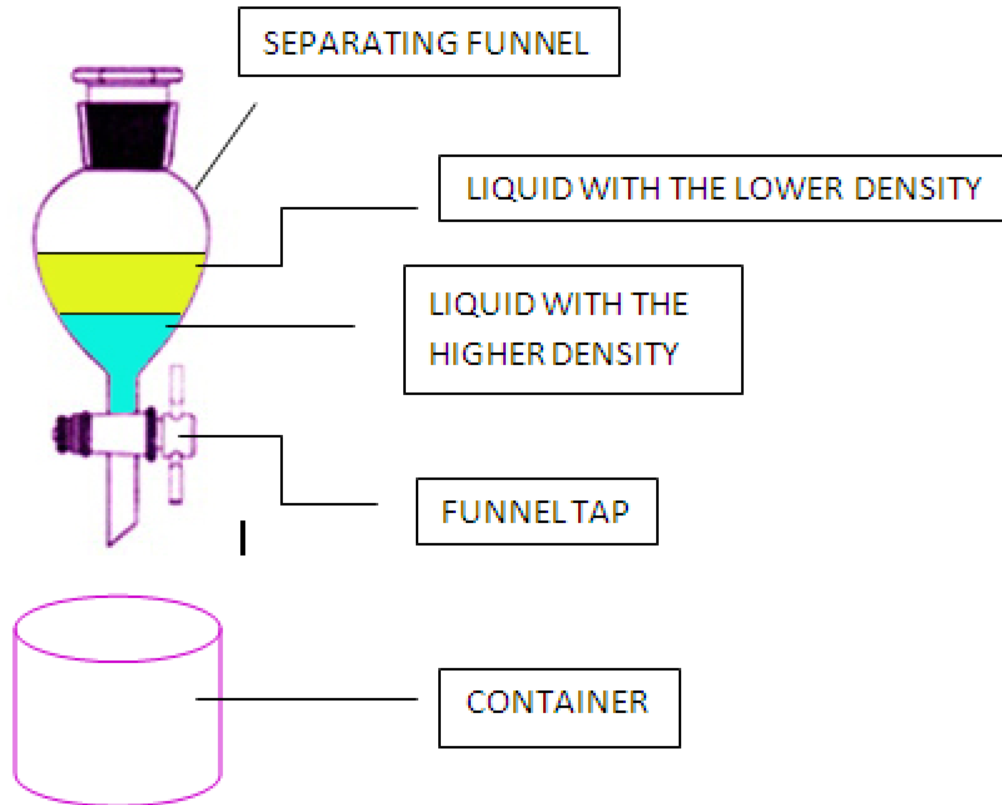
http://www.millipore.com/techpublications/tech1/ps2342enus

Effect of detergents – example of use of nonacid cleavable detergents designed for MS



J. Mass. Spectrom. 2005:40, 1319-1326

Liquid-Liquid Extraction



http://www.tutorbene.com/index.aspx?PageID=92

Liquid-Liquid Extraction

Basic Principle: Differential partitioning between two liquid phases

Disadvantages:

- Time consuming
- Low recovery and reproducibility of results
- Large quantities of solvent required
- Not as easy to adapt to high throughput protocols

Basic Principle of SPE

Extraction of target analyte(s) from a liquid sample matrix to a solid sorbent using interactions between the analyte(s) and the sorbent.

Solid Phase extraction – selectivity and efficiency

- widely used

- many advantages
  - versatile: different modes of retention, RP, ion exchange, affinity binding; several combinations of sorbents and solvents, variable sample volumes

  - simple, scalable, easier to automate for increased reproducibility and throughput

http://www.chemistry.adelaide.edu.au/external/soc-rel/content/spe.htm

Reservoir

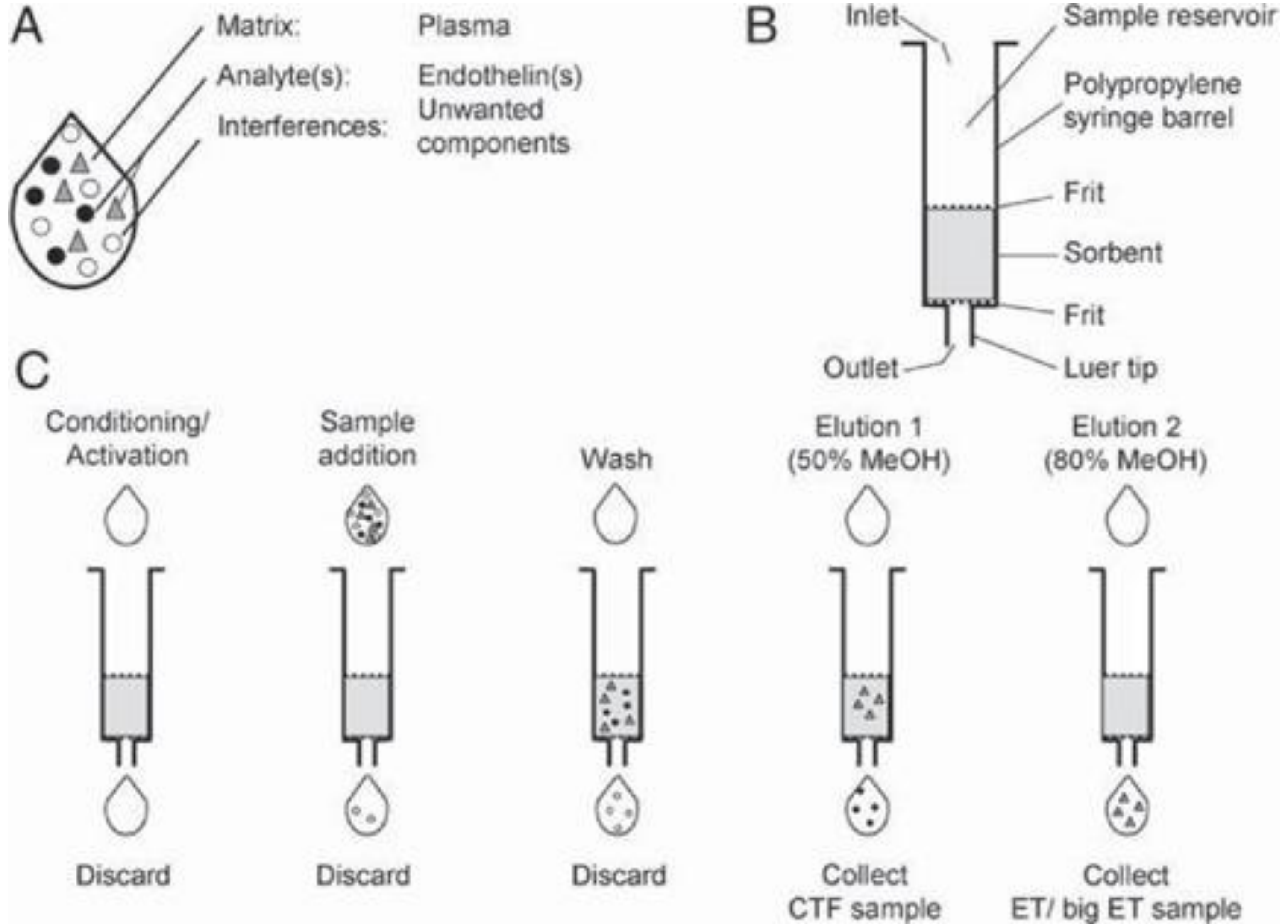
Sorbent Bed

Frits
(polyethylene, stainless steel or teflon)

Luer Tip

http://www.biotage.com/DynPage.aspx?id=35833

SPE - Schematic



http://www.springerimages.com/Images/RSS/1-10.1385_1-59259-289-9_021-0

Different Sorbents based on various functional groups:

- **Ion exchange** : extracting ionic analytes
  anionic – aminopropyl, quaternary amine
  cationic – carboxylic acid, benzene sulfonic acid

- **Polar** : “Normal Phase”: extracting polar analyte(s) from non-polar solutions
  sorbent: silica, alumina, cyano …

- **Non-polar** : “Reverse Phase”: extracting non-polar or hydrophobic analyte(s) from polar solutions
  sorbent: C18, C8, C4, phenyl …

- **Mixed mode**

Choice of solvent in different steps:

- Conditioning – prepare sorbent for effective interaction with target analyte(s)
- Sample loading – retain samples
- Wash – remove weakly bound interfering substances
- Elution – desorb target analyte(s)

Use:

- "Weak" solvent for retention and wash steps
- "Strong" solvent for elution step

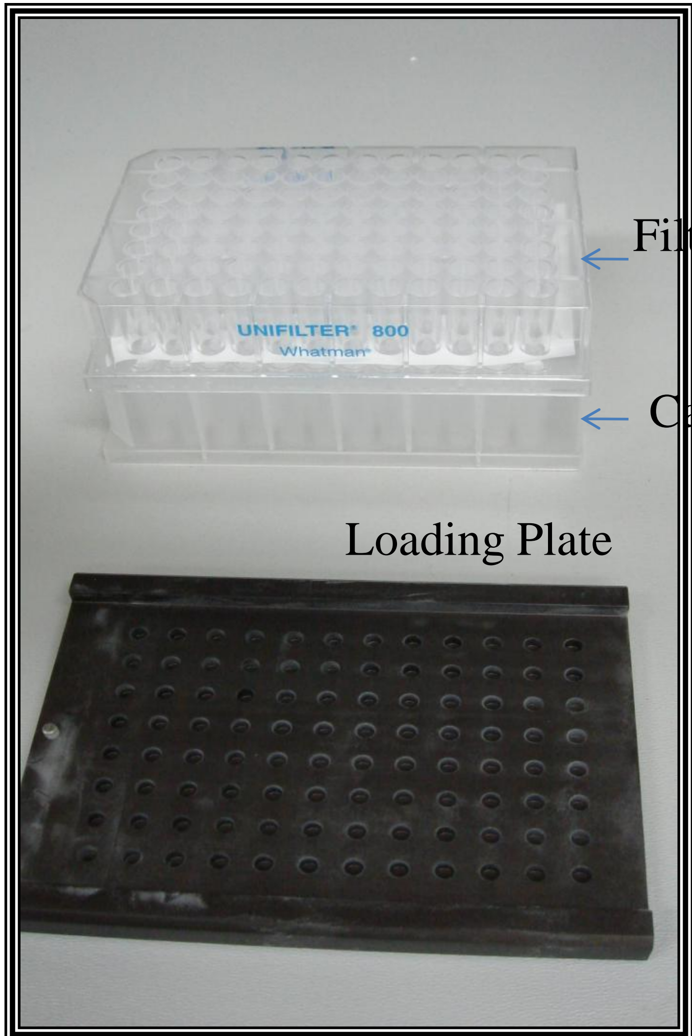
Fixed-well Plate

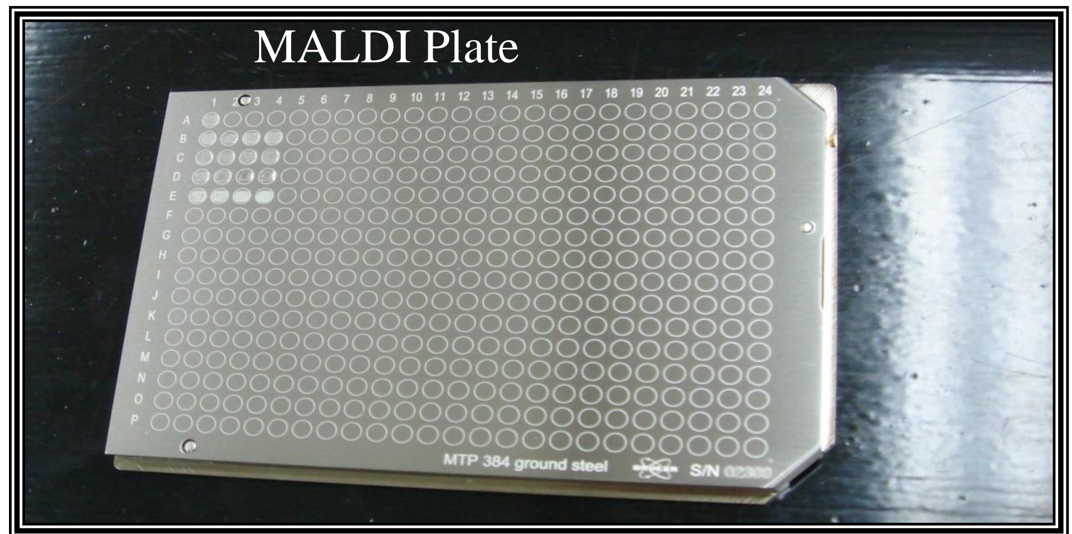
Modular array
Plate

http://www.biotage.com/DynPage.aspx?id=36890

C18 Packed Filter Plate

1) *Add C18 and Organic to activate*
2) *Remove Organic*
3) *Add Sample*
4) *Adsorb by Shaking*
5) *Centrifuge off Non-Binding Proteins*
6) *Elute With Organic*
7) *Spot to MALDI target Plate*





Proteins/peptides of interest are usually of low abundance

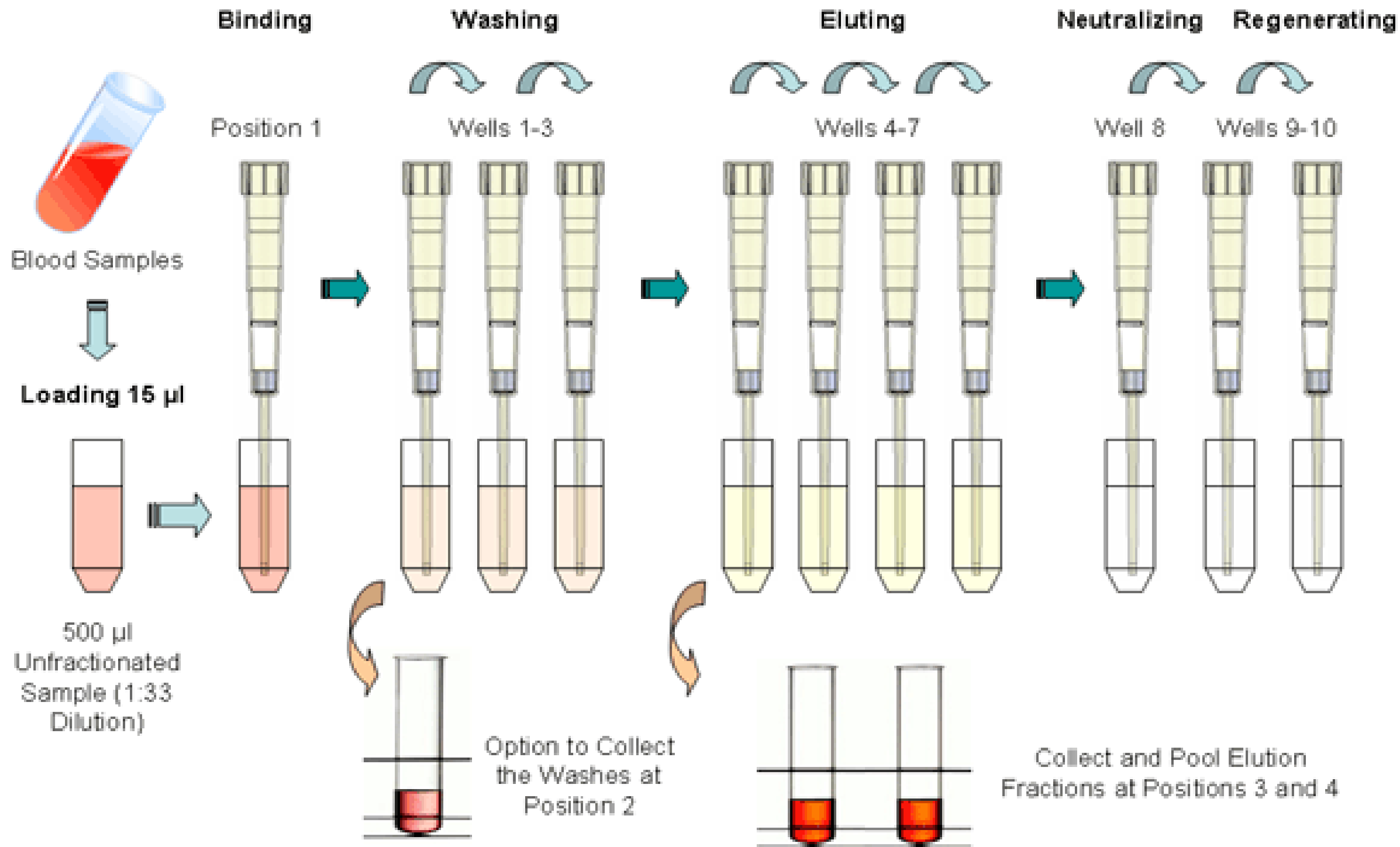
For eg. in serum/plasma:

Large dynamic range mg/ml (serum albumin) to pg/ml (interleukins)
-difficult to detect and quantify low abundance proteins/peptides

Top 12 most abundant serum proteins:

- HSA (Human Serum Albumin)
- IgG
- Fibrinogen
- Transferrin
- IgA
- IgM
- Haptoglobin
- a2-Macroglobulin
- a1-Acid Glycoprotein
- a1-Antitrypsin
- Apo A-I
- Apo A-II

Depletion techniques to remove most abundant proteins



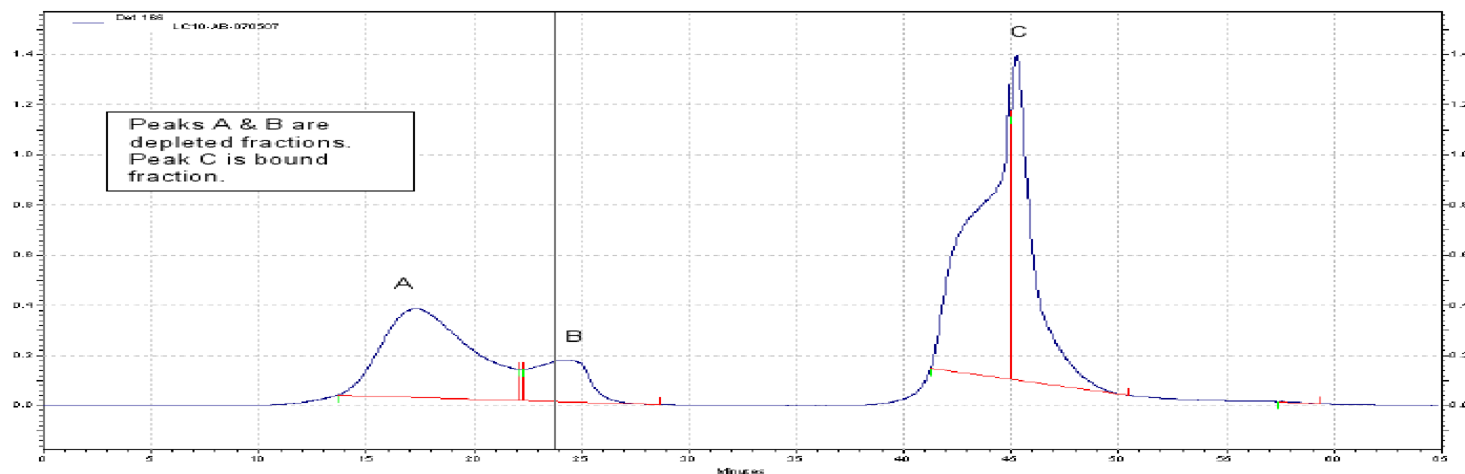
http://www.sigmaaldrich.com/life-science/proteomics/protein-sample-preparation/protein-depletion-products/seppro-depletion-resins/seppro-tips/seppro-tip-fractionation-study.html

Table 1.
Timetable for IgY 14 LC2 column

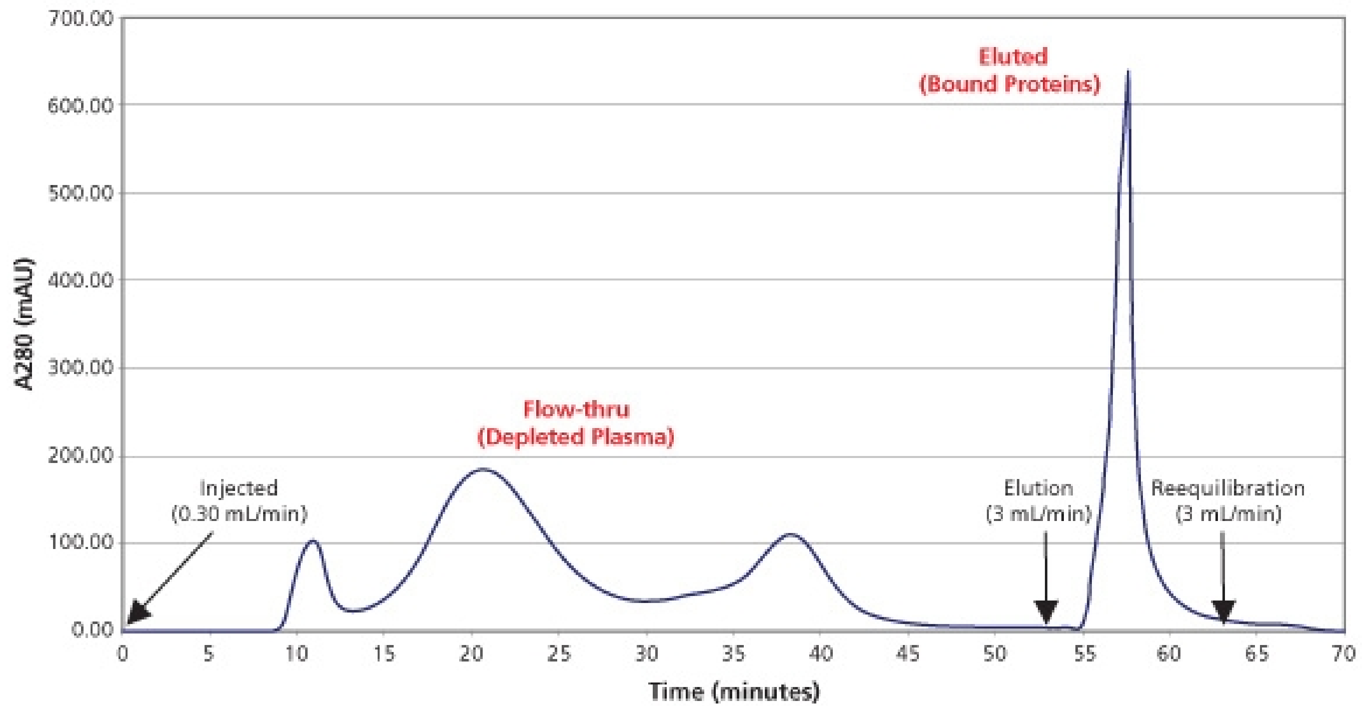
Cycle	Time (minutes)	1x Dilution Buffer (%)	1x Stripping Buffer (%)	1x Neutralization Buffer (%)	Flow Rate (ml/minute)	Maximum Pressure (psi)
Injection						
Wash	0	100	0	0	0.2	350
Wash	17.01	100	0	0	1.5	350
Elution	22.01	0	100	0	1.5	350
Neutralization	36.01	0	0	100	1.5	350
Re-equilibration	42.01	100	0	0	1.5	350
Stop	50.00					

Method for 6.4 x 63.0 mm column is optimized for Beckman System Gold HPLC, Pump Module 1 Type: 118, Detector Model: 166.

Results
Figure 1.
Typical Depletion Chromatogram



Seppro IgY 14 LC-2 column

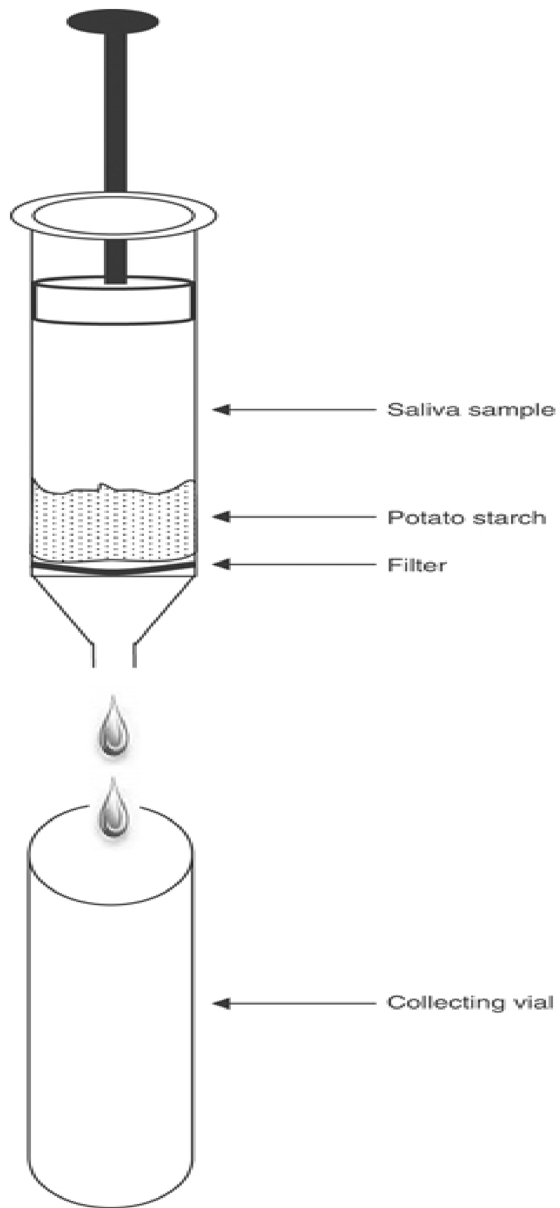


http://www.sigmaaldrich.com/life-science/proteomics/protein-sample-preparation/protein-depletion-products/seppro-depletion-resins/proteoprep-20-lc.html

The ProteoPrep 20 Technology depletes the following proteins:

Albumin
IgGs
Transferrin
Fibrinogen
IgAs
α-2-Antitrypsin
IgMs
α-1-Antitrypsin
Complement C3
Haptoglobulin

Apolipoprotein A1
Apolipoprotein A2
Apolipoprotein B
Acid-1-Glycoprotein
Ceruloplasmin
Complement C4
Complement C1q
IgDs
Prealbumin
Plasminogen



Amylase depletion in whole saliva

Electrophoresis Vol.29, 20 Pages: 4150-4157

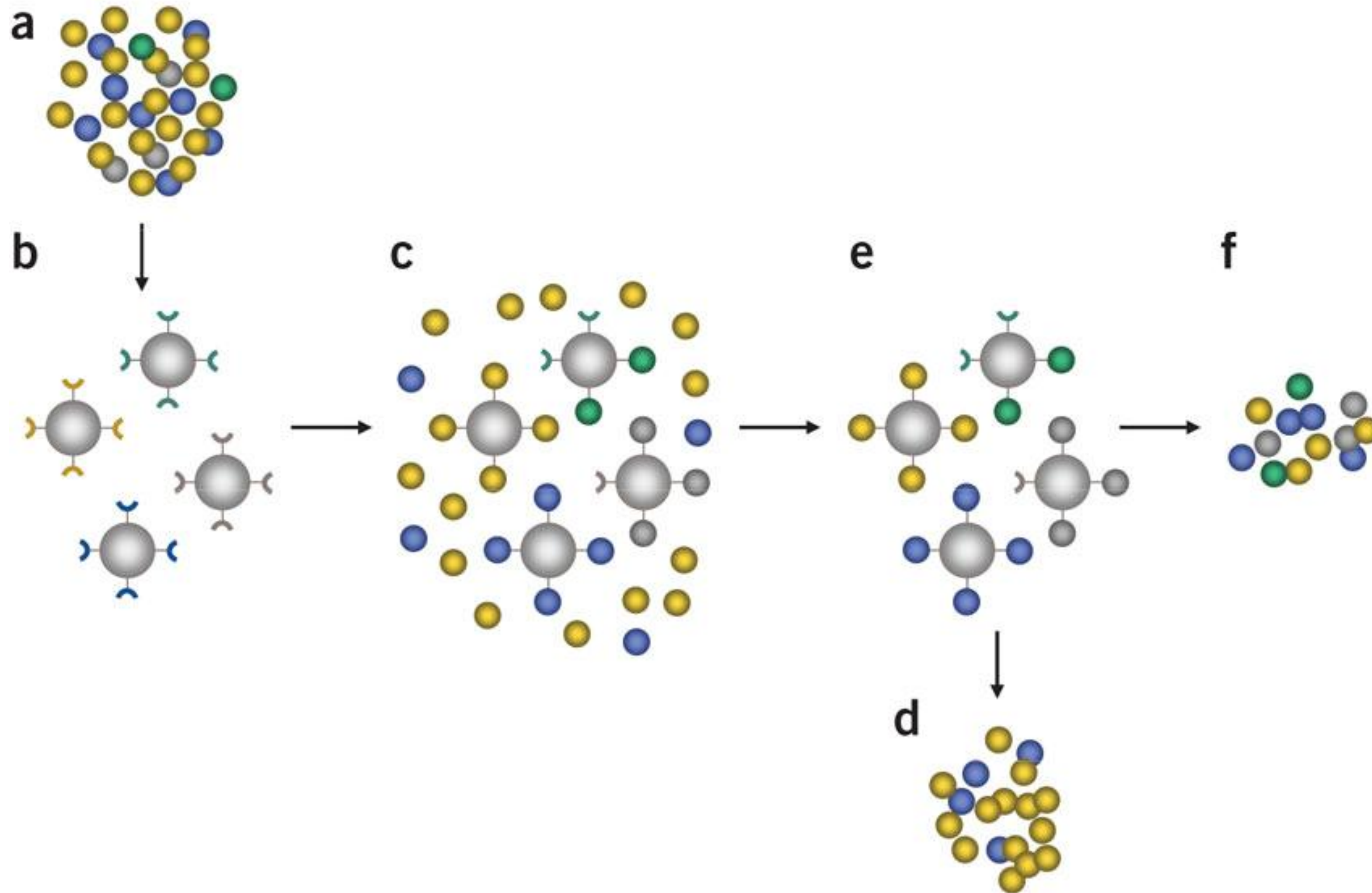
Protein Equalizer Technology : the quest for a "democratic proteome".

Proteomics. 2006 Jul;6(14):3980-92
Righetti PG, Boschetti E, Lomas L, Citterio A.
Department of Chemistry, Materials and Chemical Engineering Giulio Natta, Politecnico di Milano, Milano, Italy. piergiorgio.righetti@polimi.it

"*This comprises a diverse library of combinatorial ligands coupled to spherical porous beads. When these beads come into contact with complex proteomes (e.g. human urine and serum, egg white, and any cell lysate, for that matter) of widely differing protein composition and relative abundances, they are able to "equalize" the protein population, by sharply reducing the concentration of the most abundant components, while simultaneously enhancing the concentration of the most dilute species*."

- Non-antibody-based
- Uses combinatorial peptide libraries bound to chromatographic beads

Protein Equalizer Technology



Guerrrier et al., (2008) *Nature Protocols*, Vol 3, 883-890
http://www.nature.com/nprot/journal/v3/n5/fig_tab/nprot.2008.59_F1.html

Summary – Key points

Design your protocols carefully:
- avoid detergents, salts etc that interfere with ionization and/or detection
- avoid introducing contaminants – such as keratin from skin, hair

Use physicochemical properties of analyte(s) to design effective clean-up, fractionation techniques

Use simplest possible procedure that allows desired result (sample recovery, throughput)

References

Bodzon-Kulakowska, A., et al., (2007) *Methods for samples preparation in proteomic research*. Journal of Chromatography B, **849**, 1–31

Gilar, M., et al., (2001) *Advances in sample preparation in electromigration,chromatographic and mass spectrometric separation methods.* Journal of Chromatography A, **909**, 111–135

Guerrier, L., et al., (2008) *Reduction of dynamic protein concentration range of biological extracts for the discovery of low-abundance proteins by means of hexapeptide ligand library.* Nature Protocols, **3**(5), 883-890