PKS

Will plastic anti-bodies revolutionize the bioanalysis?

Olivier Heudi, PhD
12th EBF Open Symposium
November 22, 2019

NOVARTIS

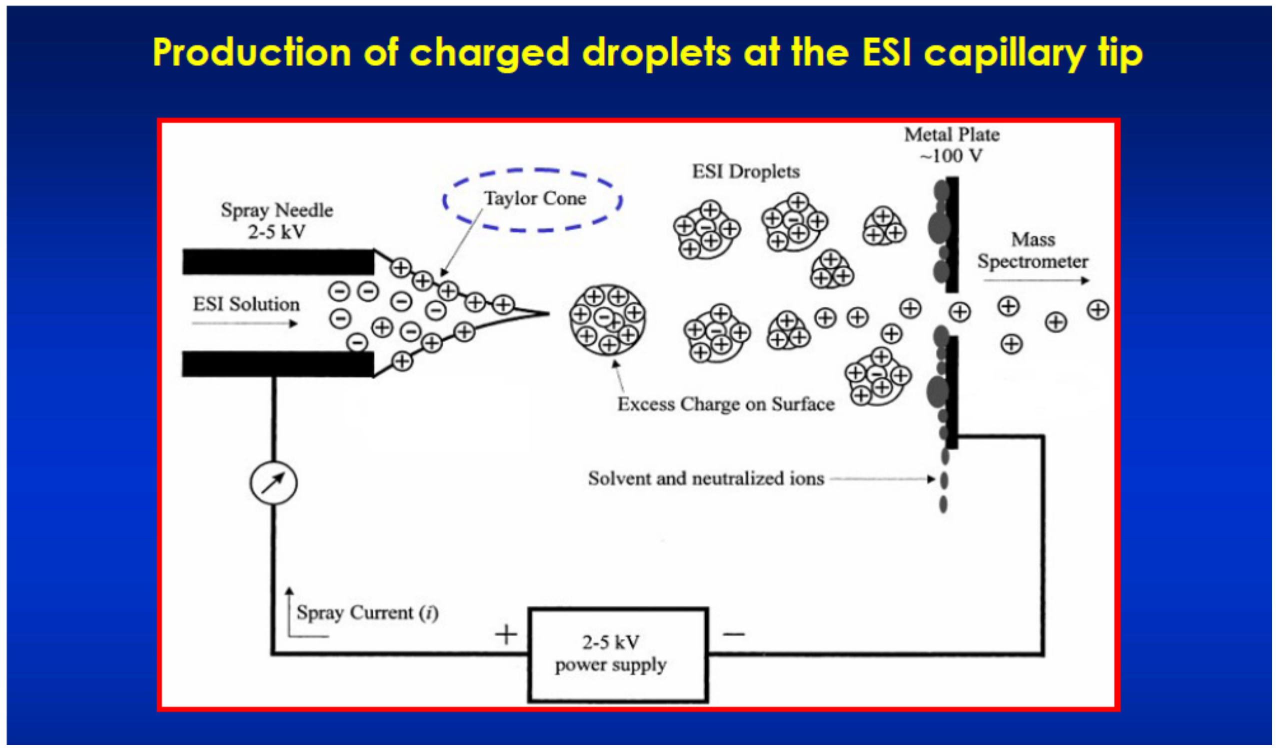
Agenda

1. Introduction
2. Sample enrichment current approach
3. MIP concept and applications
4. Conclusions

NOVARTIS

Why do we struggle when developing a bioanalytical method?

How to gain in sensitivity (*API 3200 vs API 5500*)



NOVARTIS

How to gain in sensitivity : sample preparation cleanness matter

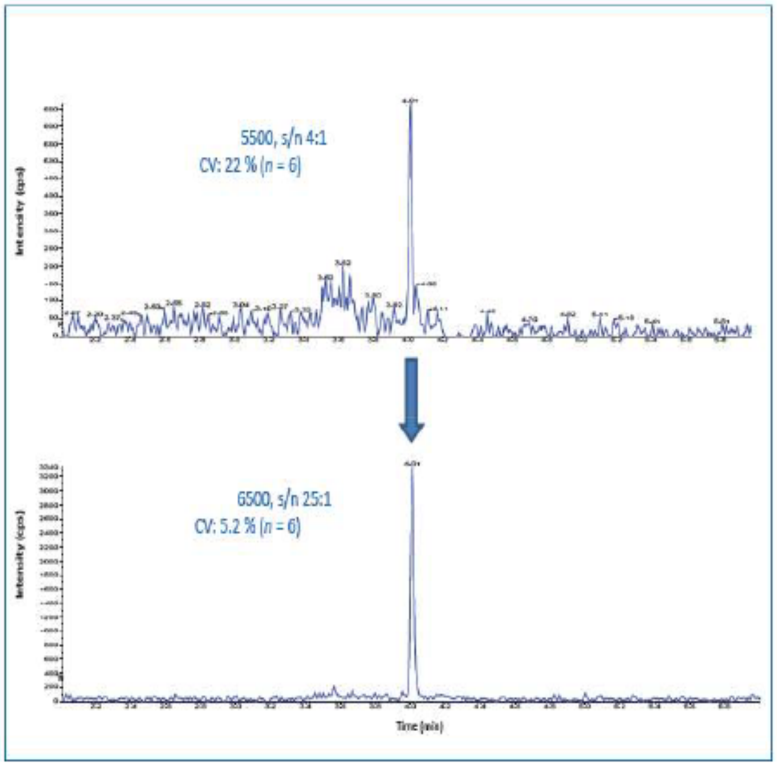
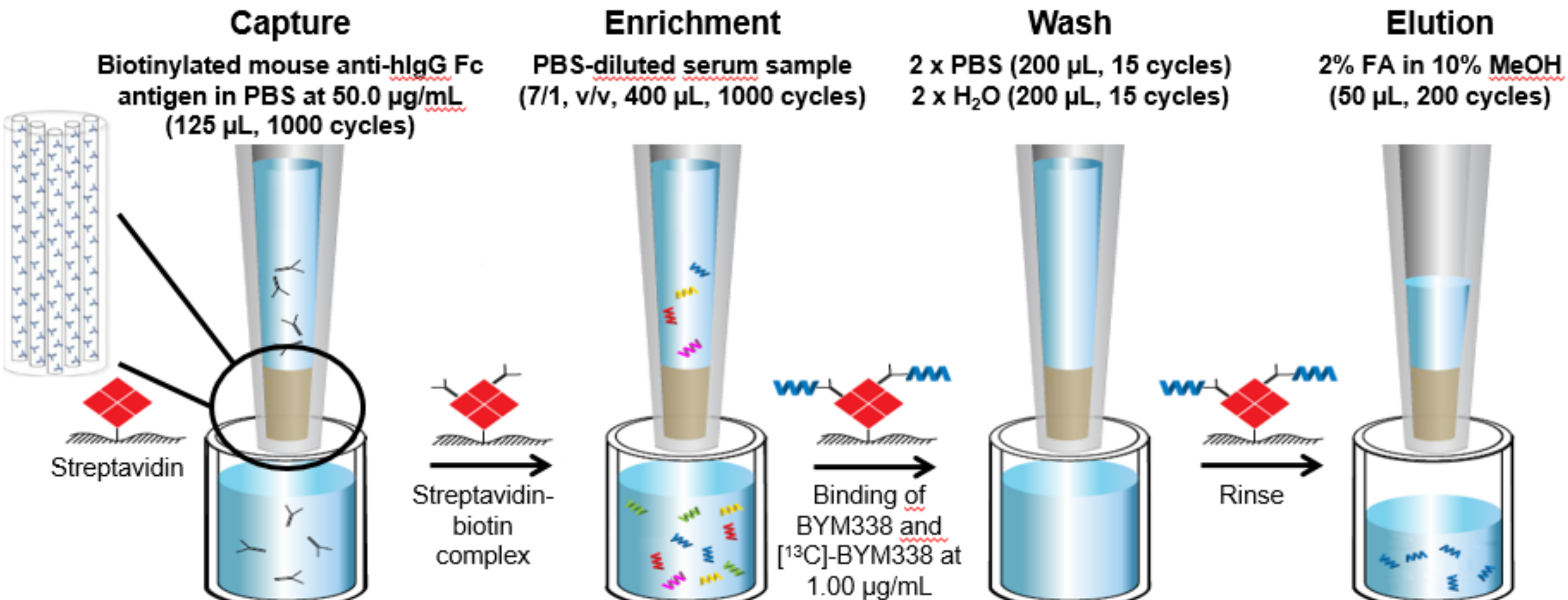


Figure 1. 5500 and 6500 response comparison demonstrating improved LLOQ precision with increased response factor. Extracts were chromatographed on a Waters Acquity HSS C18 column (2.1 x 100 mm; 1.7µm) using a Shimadzu Nexera LC-30AD UHPLC system.

ANALYTE	Precursor m/z	Product m/z	Signal Gain	S/N Gain
methamphetamine	150.1	91.0	5.6	3.9
clomipramine	315.2	242.1	6.8	2.3
ketoconazole	531.2	489.0	5.2	3.8
ginsenoside Rb2	1101.6	789.5	9.5	2.1
verapamil	455.3	165.1	4.2	2.4
erythromycin	734.5	558.4	12.6	2.8
nigericin	742.5	657.4	10.1	5.6
tylosin	916.5	174.1	4.0	2.2
cyclosporin	1202.8	1184.8	6.8	4.7
lidocaine	235.2	86.1	3.5	1.7

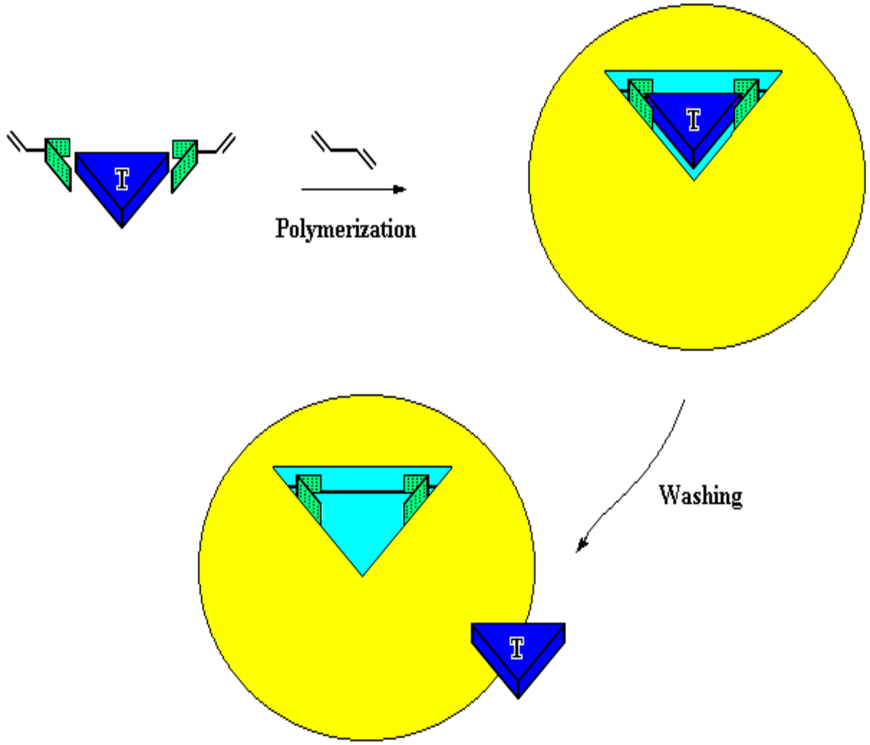
Summary of observed signal and S/N gains 5500 Vs 6500

Sample enrichment current approach : immunocapture



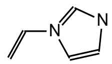
Molecular Imprinting Overview

- Pre-selected binding monomers self-assemble around the target (T) and are then polymerized to form MIPs
- MIPs are very stable & robust
- Polymers are inexpensive
- MIPs work in organic solvents and in water
- MIPs are easily functionalised
- In theory, MIPs can be prepared for any target
- MIPs have similar affinity and specificity to antibodies

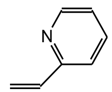


NOVARTIS

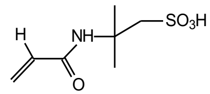
Diverse library of monomers



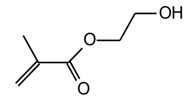
1-VINYLIMIDAZOLE



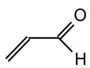
2-VINYLPYRIDINE



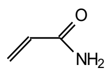
2-ACRYLAMIDO-2-METHYL-1-PROPANESULFONIC ACID (AMPSA)



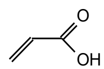
2-HYDROXYETHYL METHACRYLATE



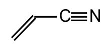
ACROLEIN



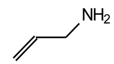
ACRYLAMIDE



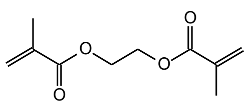
ACRYLIC ACID



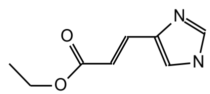
ACRYLONITRILE



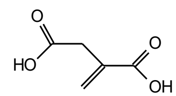
ALLYLAMINE



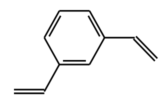
ETHYLENE GLYCOL DIMETHACRYLATE (EGDMA)



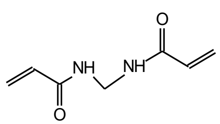
UROCANIC ACID ETHYL ESTER (UAEE)



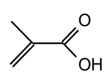
ITACONIC ACID (IA)



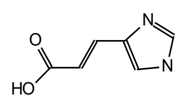
m-DIVINYLBENZENE



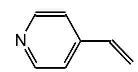
N,N-METHYLENEBISACRYLAMIDE



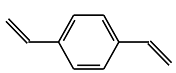
METHACRYLIC ACID (MAA)



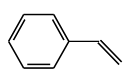
UROCANIC ACID (UA)



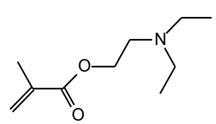
4-VINYLPYRIDINE



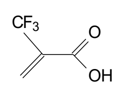
p-DIVINYLBENZENE



STYRENE



2-(DIETHYLAMINO)ETHYL METHACRYLATE (DEAEM)



TRIFLUOROMETHACRYLIC ACID (TFMAA)

~5000 in Literature

MIP applications

- Imaging
- Protein-protein interaction
- Diagnostics
- Viral detection
- Bioanalysis : ELISA or LC-MS/MS
- Dot blot detection
- Western blot
- Sensing
- Biomarker detection

Antibodies *vs* NanoMIPs

Feature	Antibody	nanoMIPs
Affinities • **Typical/High** • **Lower affinities?**	Good • 10-100nM • By selection: difficult	Excellent • ~10nM • Yes: by design
Surface area of interaction	Fixed	Variable by design No library synthesis required
Diversity: number of monomer units	20	5000
Speed of development	3 months Polyclonal 7-10 months Monoclonal	2-6 weeks
Speed of manufacture (per batch; no QC)	c. 4 weeks	Hours/Days
Batch to Batch Variability	Very poor (Polyclonals) Excellent (Monoclonals)	Excellent
Robustness • **Thermal** • **Enzyme** • **Chemical**	 • Moderate • Poor • Poor	 • Excellent (> 121°C) • Excellent (not biodegradable) • Excellent
Ease of integration	Moderate (known limits)	Excellent
Typical Shelf life	6-24 months	c. 5 years or more
Protection against duplication	Easy to clone/duplicate from sequence Scaffold not protected	Proprietary technology required Formulation patents possible (cf. composition of matter)

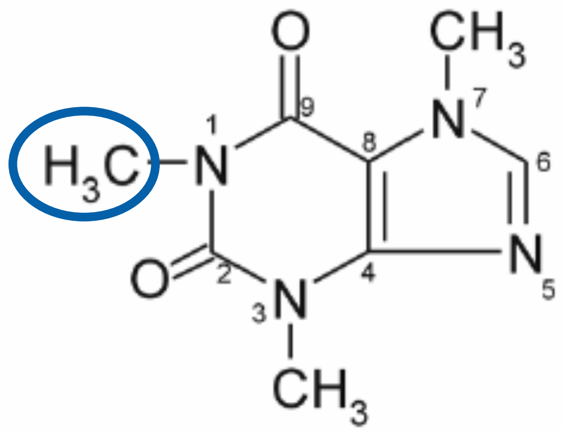
NOVARTIS

Comparison of MIPs and antibodies in ELISA

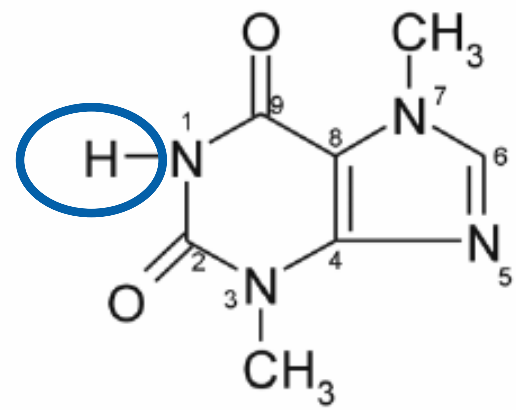
Template	MIP size, nm	Detection limit for assay with MIP, nM	Detection limit in assay with antibodies, nM
Biotin	104±6	$1,20 \times 10^{-3}$	$2,54 \times 10^{-3}$
L-Thyroxine	164±11	$8,07 \times 10^{-3}$	17,5
Glucosamine	138±16	$4,01 \times 10^{-4}$	$3,38 \times 10^{-4}$
Fumonisine B2	94±4	$6,12 \times 10^{-3}$	$2,5 \times 10^{-2}$
Haemoglobine	149±15	$8,7 \times 10^{-2}$	$1,54 \times 10^{-4}$
Glycated haemoglobin (“polyclonal”)	103±14	$2,46 \times 10^{-3}$	-
Glycated haemoglobin (“monoclonal”)*	103±14	$9,49 \times 10^{-3}$	$2,38 \times 10^{-4}$

MIP diameter and detection limits vs detection limits for antibodies against a range of small molecule targets. In contrast to antibodies MIPs had no cross-reactivity for non-glycated haemoglobin

MIP: does that really work?



Caffeine



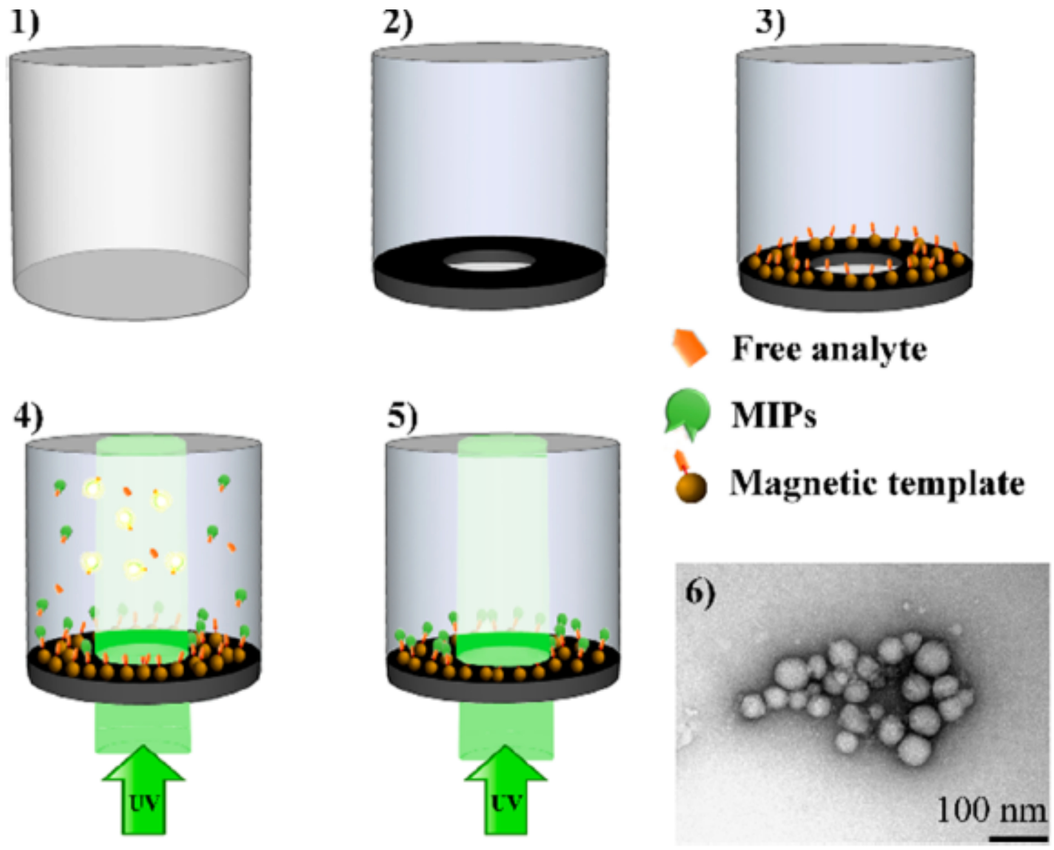
Theophylline

Korean J. Chem. Eng., **21**(4), 853-857 (2004)
SHORT COMMUNICATION

Solid Extraction of Caffeine and Theophylline from Green Tea by Molecular Imprinted Polymers

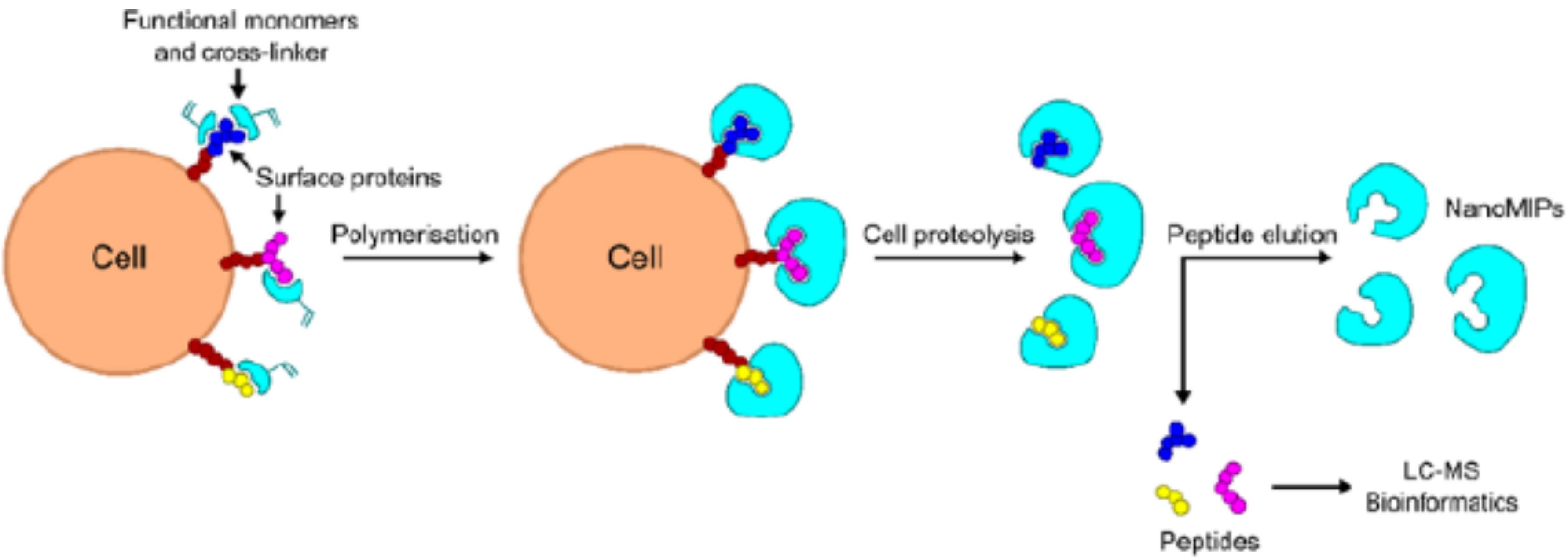
Dexian Wang, Seung Pyo Hong and Kyung Ho Row†

MINA test for Methyl parathion i plasma



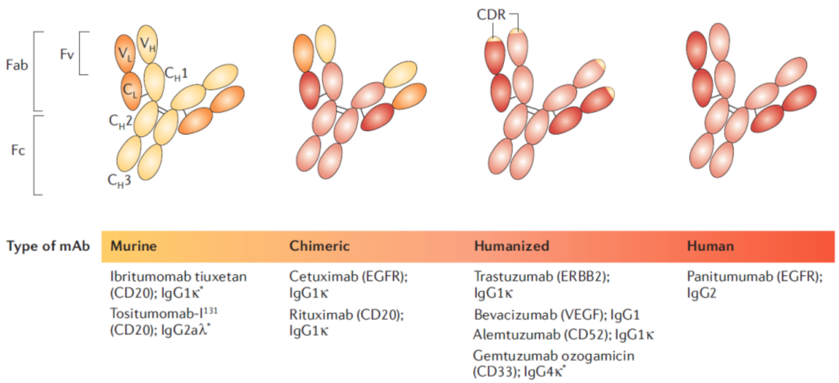
Cem Esen et al. *Analytical Chemistry* 2019 *91* (1), 958-964

NanoMIPS formation using cell surface proteins as the template



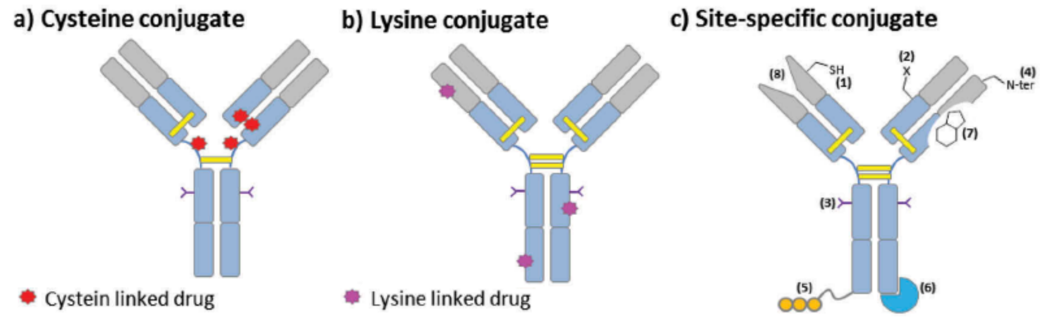
High flexibility and binding capacity of MIPs

Monoclonal antibodies (mAbs)



Imai K, Takaoka A, *Nat. Rev. Cancer*, 2006, 6, 714-27

Antibody-drug conjugates (ADCs)

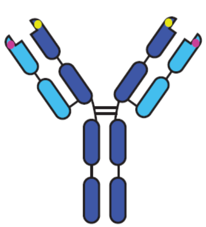


Beck A et al., *Expert Rev. Proteomics*, 2016, 13(2), 157-83

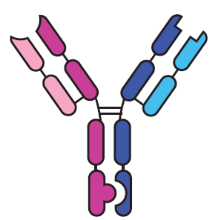
Bispecific antibodies (bsAbs)

Bispecific IgG 150 kDa

DAF
(two in one)

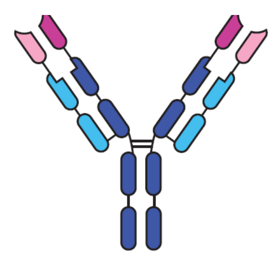


Knob-into-Hole
(KiH)

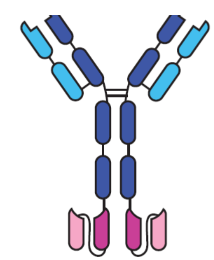


Appended IgG <150 kDa

Dual variable domain
(DVD)-IgG



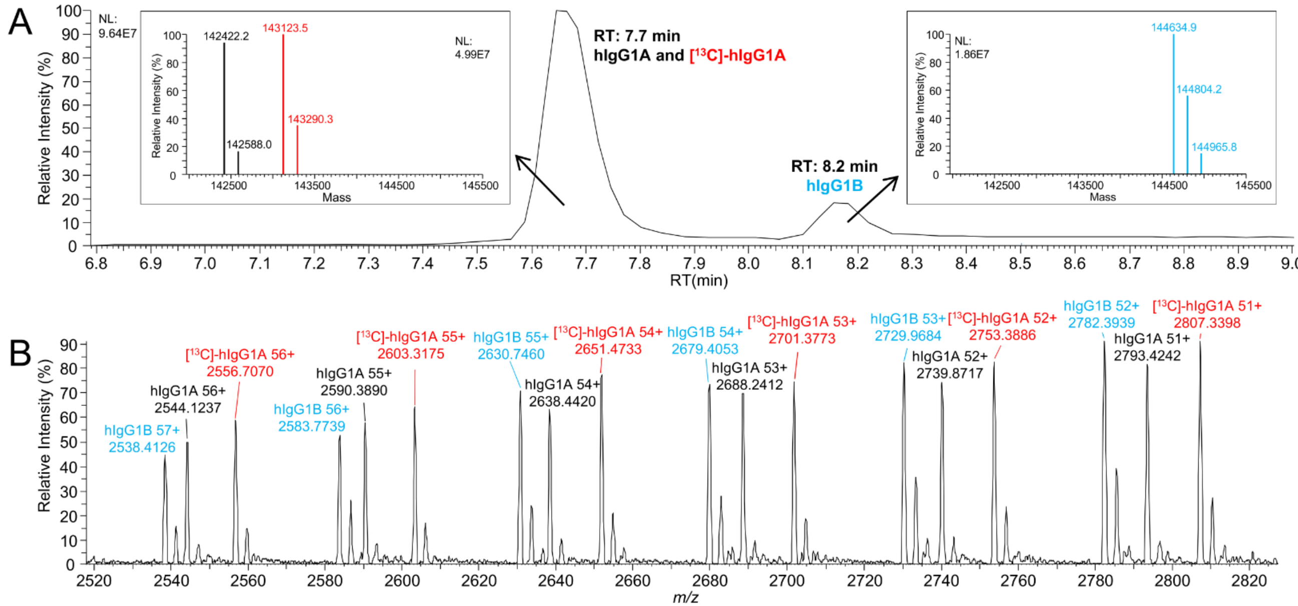
scFV-IgG



Spiess C, Zhai Q, Carter PJ, *Mol. Immunol.*, 2015, 67, 95-106

Multiplexing capabilities

Different hIgG1s spiked in rat serum



		Nominal QC concentration in rat serum (µg/mL)			
		8.00	5.00	0.250	0.100
Therapeutic protein		Inter-day accuracy and precision (n=9)			
hIgG1A, RT 7.7 min	Mean concentration (µg/mL)	**8.21**	**5.38**	**0.276**[b]	**0.110**
r^2=0.9891±0.0009	Inter-day accuracy (% bias)	2.6	7.6	10.4	10.2
	Inter-day precision (% CV)	8.8	5.2	7.5	7.3
hIgG1B[a], RT 8.2 min	Mean concentration (µg/mL)	**7.92**[b]	**5.04**	**0.254**	**-**
r^2=0.9840±0.0063	Inter-day accuracy (% bias)	-1.0	0.9	1.6	-
	Inter-day precision (% CV)	9.8	8.1	9.6	-

[a] LLOQ was set to 0.250 µg/mL. [b] n=8, one replicate did not meet accuracy acceptance criterion of ±20% and was excluded from calculations

Lanshoeft C, Cianférani S, Heudi O, *Anal. Chem.*, 2017, doi: 10.1021/acs.analchem.6b04997

Some questions about MIP 1/2

- How long does that take to design a MIP for a small molecule?

  6-8 weeks depending upon how much testing is required for the application

- Do you also design MIP for large molecules such as therapeutic antibodies?

  Yes, we can design MIPs to whole proteins, this can be done using the whole protein or by using a suitable peptide epitope. This way the whole protein is not required (especially relevant for rare/expensive proteins). We also have a proprietary approach to find exposed epitopes on molecular imprinting, but for this we do require small amounts of the whole protein.

- Is it possible to have those MIP coated in plate or ship or different type of stationary phase to increase the sample enrichment throughout?

  Yes, MIPs can be immobilized on surfaces/stationary phases for ease of handling. Also, stationary phases/resins can be made entirely out or MIPs and packed into extraction cartridges.

Some questions about MIP 2/2

- Can we multiplex the MIP in order to fish out several compounds simultaneously?

  Yes but within limits. This is more suited to MIPs in bead/bulk format, and may not be suitable for molecules which are "too different", for instance, imprinting both acidic and basic targets in the same MIP might result in poor performance of the adsorbent. Alternatively more than one MIP can be packed into one column to achieve similar results.

- What is the cost of the MIP development and can one use it twice?

  Typically the cost for the first phase development of a MIP to proof of concept is £25,000, this may vary depending upon the specific application and any particular functionalisation required. Particularly when used in packed columns for target concentration, MIPs can be re-used many times (10's to 100's). The exact number will depend on how stringent or aggressive the column regeneration or CiP conditions are.

- Would it be possible to have a sandwich assay with two MIPS – of course the second MIP could be labelled with thing like biotin?

  We have looked into this on microplates but with limited success - we believe that the MIPs are too large and that steric hindrance is the issue. However, it worked well in solution, for a medium sized molecule and protein (vancomycin and pepsin) using MIPs made against different regions of the target.

Conclusions

- Exceptionally robust, non-biodegradable affinity reagents
  - Stable at extreme pH's (e.g. pH1, HCl or H_2SO_4)
  - Resistance to organic solvents
  - Thermal stability: no cold chain required, autoclaving is possible
- Easily functionalised e.g. Fluorescence, biotin, PEG, +/-ve charges
- No Immune response required
- Selectable affinity to match requirements
- Scaffold is always complementary to target
  - Self assembly process uses target as the template
  - Wide range of targets from small molecules to whole viruses
- Rapid development & manufacture
  - First pass development in 4-6 weeks
  - Subsequent manufacturing cycles take hours

Thank you