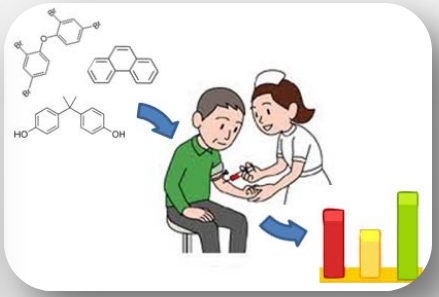
Immunoassays for small molecules that use novel single domain antibodies derived from camelids (VHH) in place of classical IgG antibodies

From mice to alpacas:
Detecting harmful chemicals using antibody technology





Candace Bever

SRP Annual Meeting || Nov 12, 2014

SUPERFUND RESEARCH PROGRAM
UCDAVIS

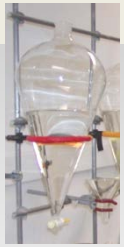
Analytical Methods

sample collected

sample returned to analytical lab

surrogate standards added

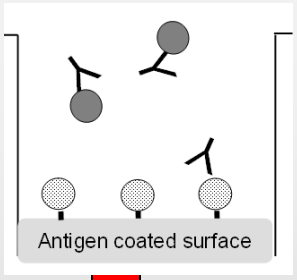
low



solvent extraction and volume reduction

high

immediately assessed by immunoassay



assessed on HPLC or GC-MS

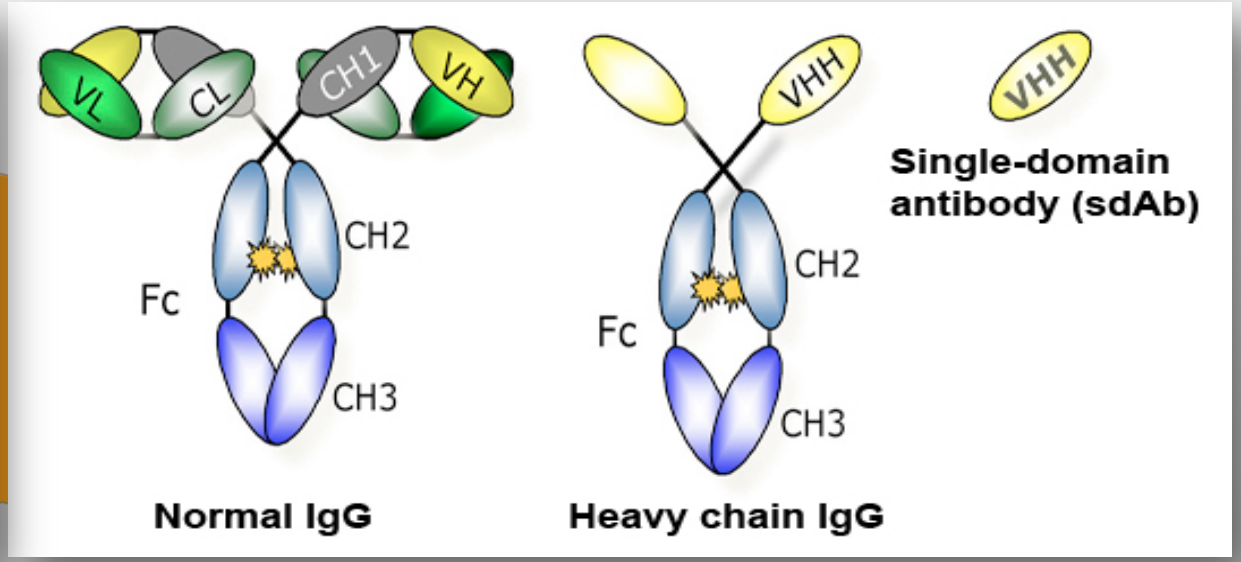
1 value corresponds to total concentration

higher resolution analysis, many compounds determined

Molecular shape **Physiochemical properties**

Why antibodies?

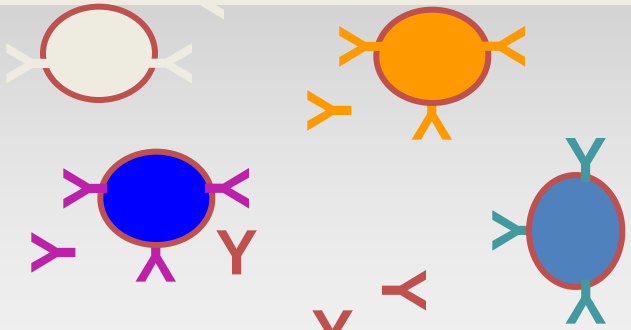
- Produced during an adaptive immune response
  - Recognize large macromolecules, viruses, bacteria, etc.
- Uniquely selective for its target
  - Similar to why you need a vaccine for a specific pathogen
- They recognize small epitopes (the size of contaminants)



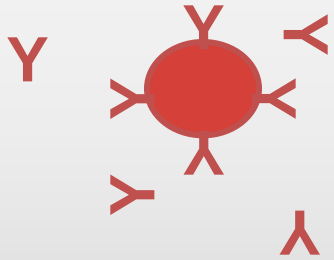
Hamers-Casterman., 1993, Nature, 363:446-448.

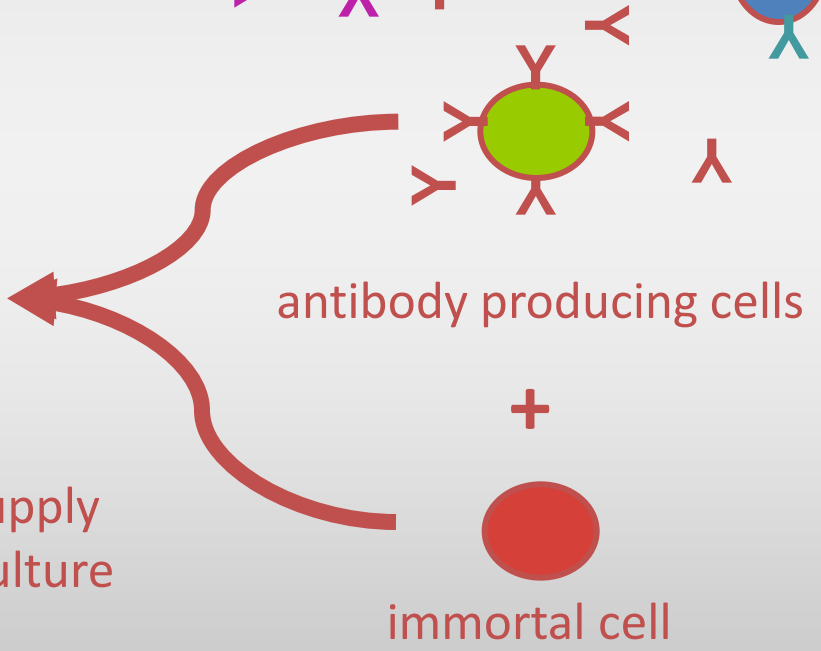
How to make antibodies to chemicals?

Polyclonal antibodies



Monoclonal antibodies





antibody producing cells

+

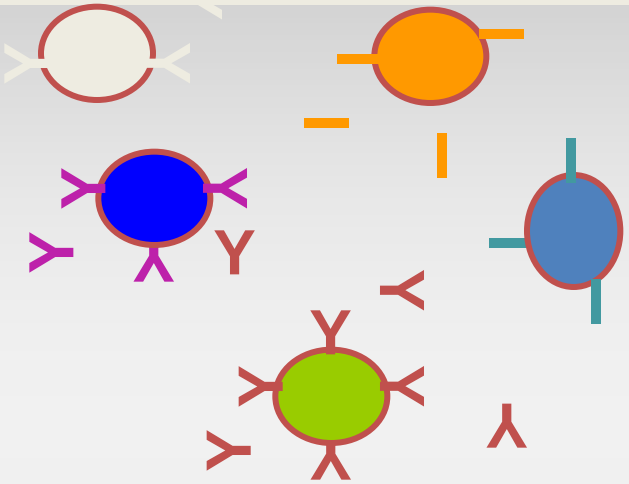
immortal cell

Provide an endless supply of antibodies in cell culture

Consistent binding site

How to make antibodies to chemicals?

Polyclonal antibodies

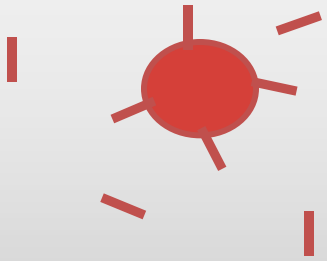


antibody producing cells

isolate antibody gene

insert gene in plasmid/virus, grow in bacteria

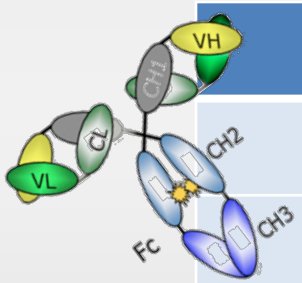
Monoclonal antibodies
"nanobodies"
"sdAb"



Provide an endless supply of antibodies in bacterial culture

Consistent binding site

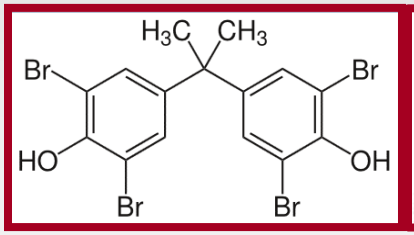
Antibody differences



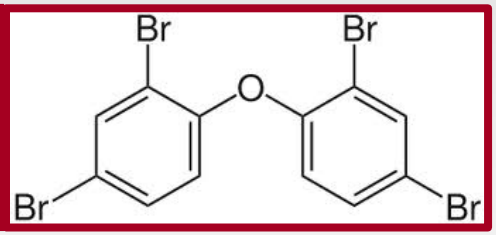
Mouse	Characteristics	Camelids
140-150 kDa	size	15-20 kDa
tetramer	structure	monomer
mammalian – doubles every day	culture	bacterial – doubles every 20 mins
mgs in weeks	yield	mgs in a day
chemically	labeling	chemically or genetically

Target analytes

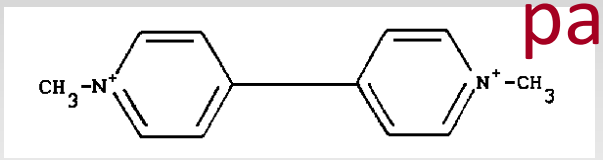
TBBPA



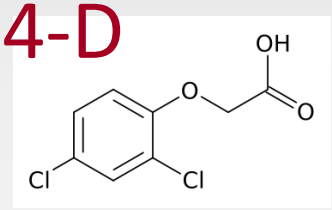
BDE-47

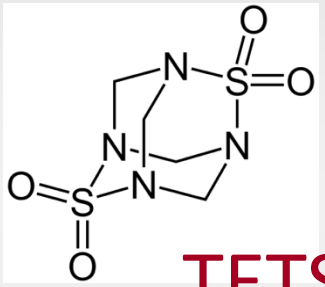


paraquat



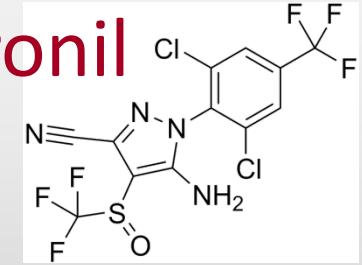
2, 4-D

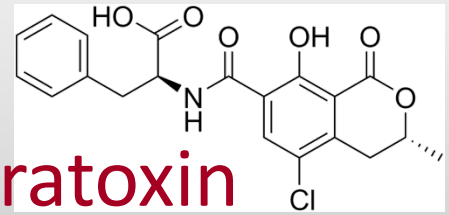




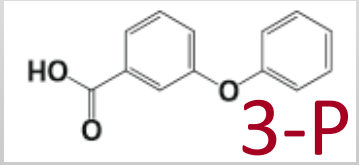
TETS

fipronil





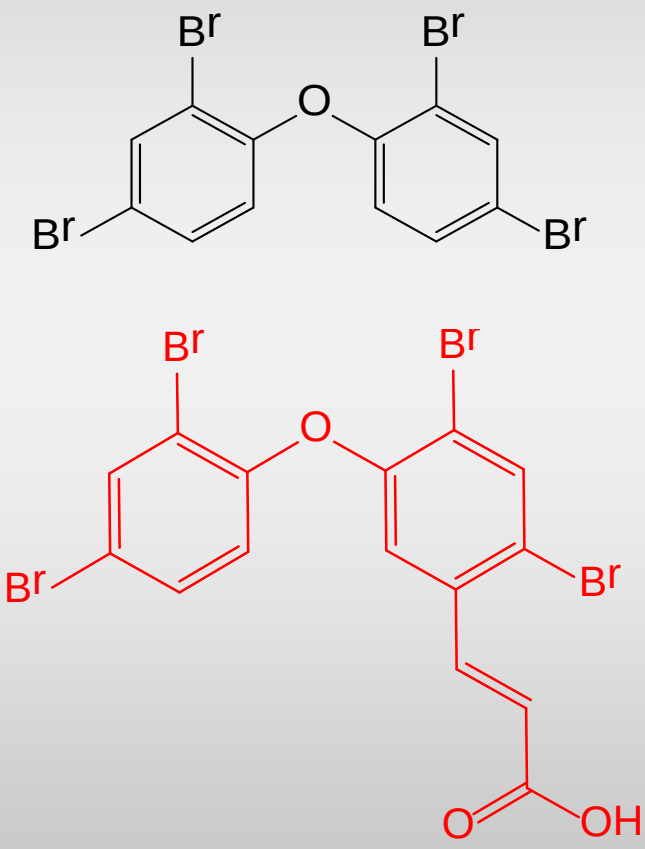
ochratoxin



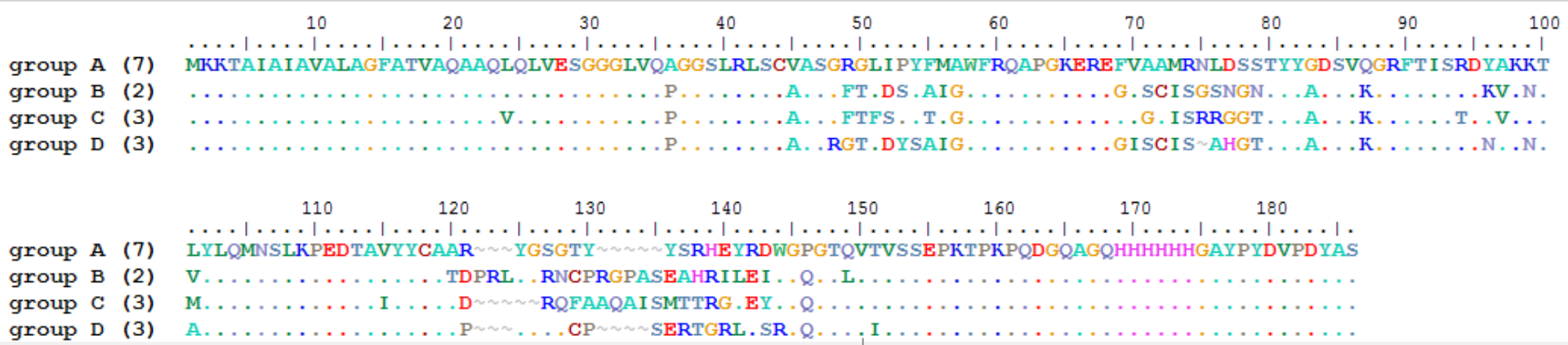
3-PBA

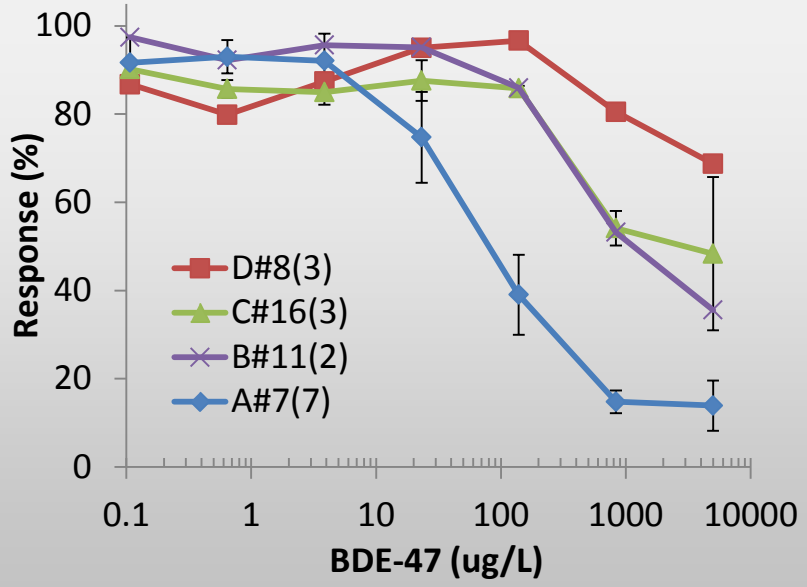
*see posters

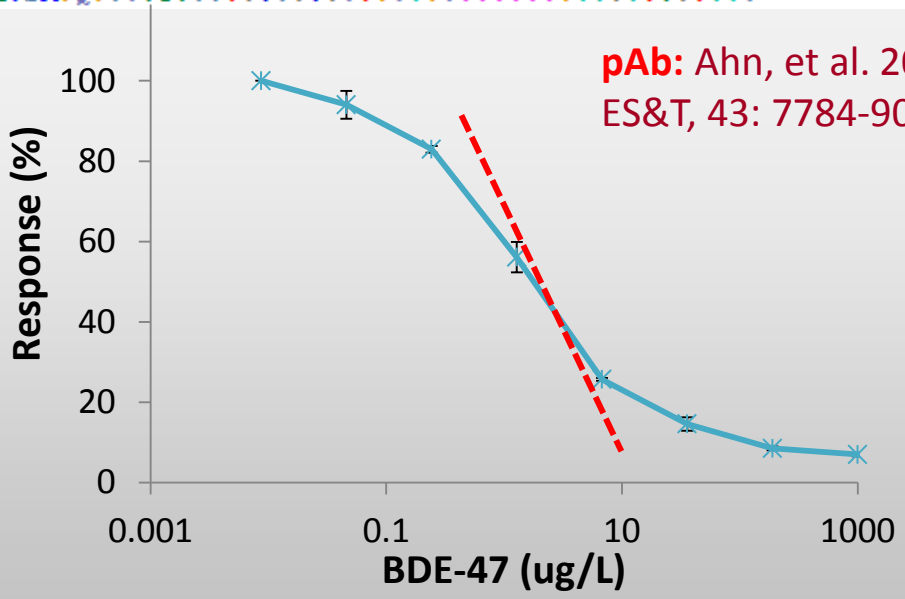
VHH Antibody production to BDE-47



VHH Antibody production



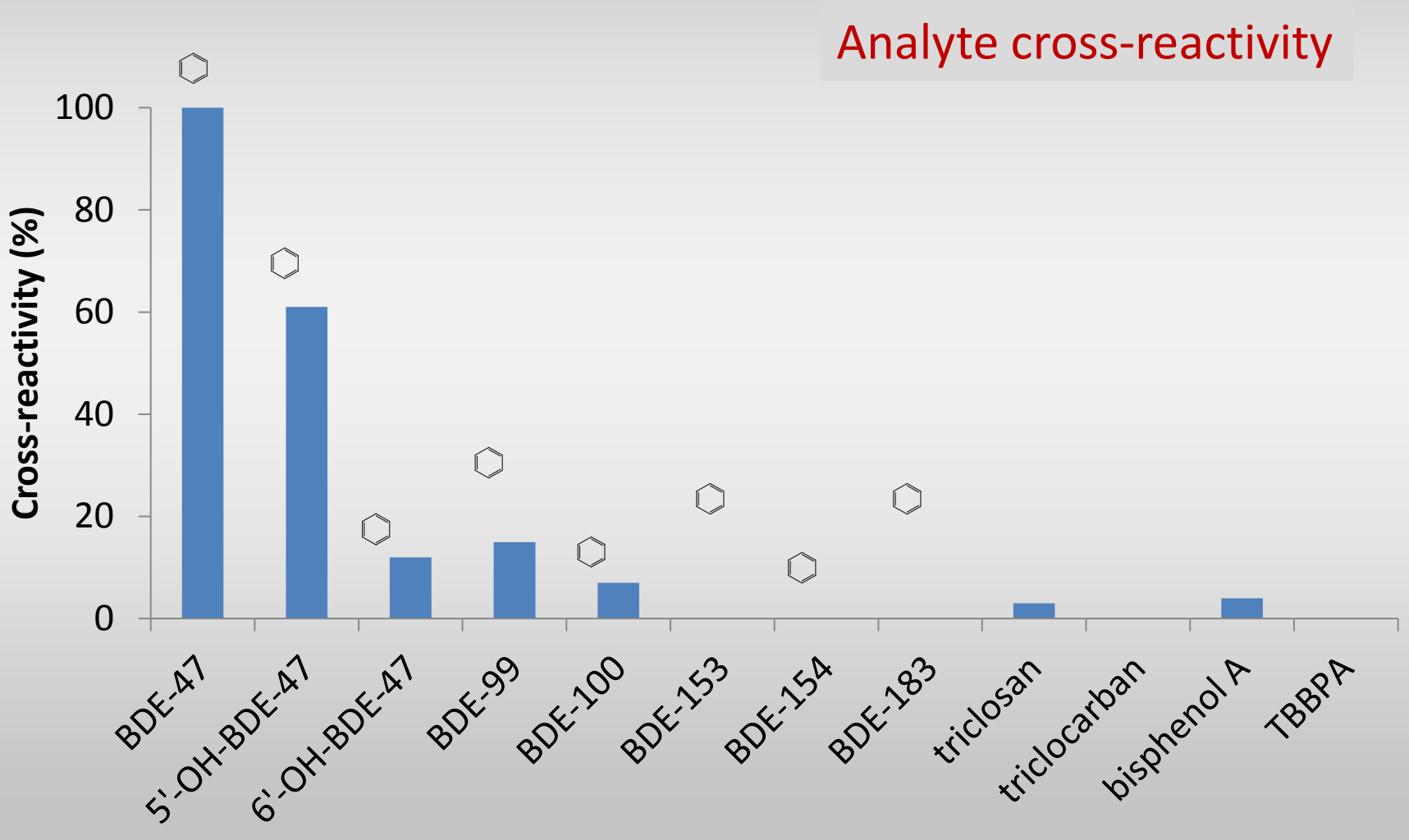




pAb: Ahn, et al. 2009. ES&T, 43: 7784-90.

Bever, et al. 2014. Anal Chem, 86(15): 7875-82

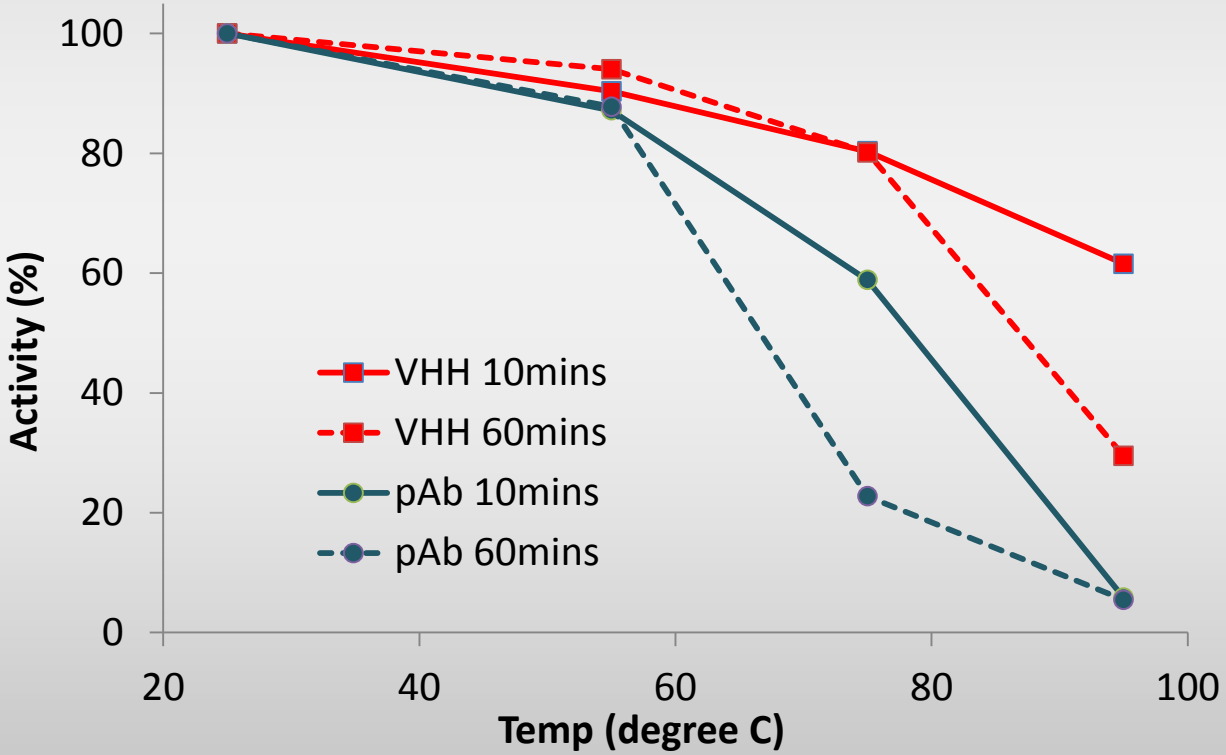
VHH Antibody characterization

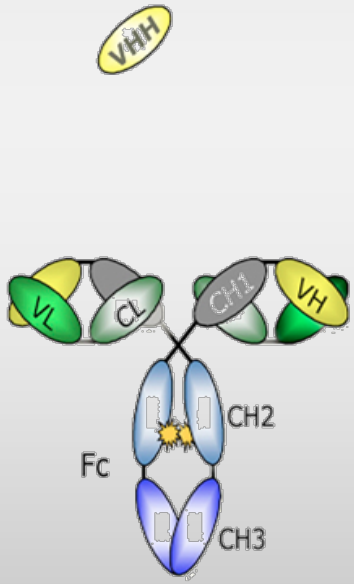


Bever, et al. 2014. Anal Chem, 86(15): 7875-82

Antibody characterization

Thermal stability

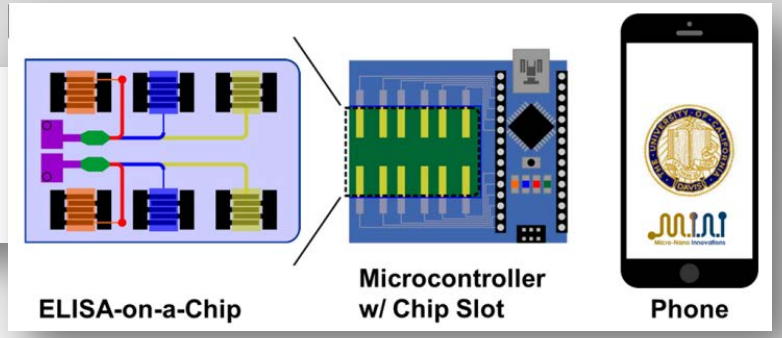




Bever, et al. 2014. Anal Chem, 86(15): 7875-82

VHH utilization in sensor formats

UCDAVIS
BIOMEDICAL ENGINEERING



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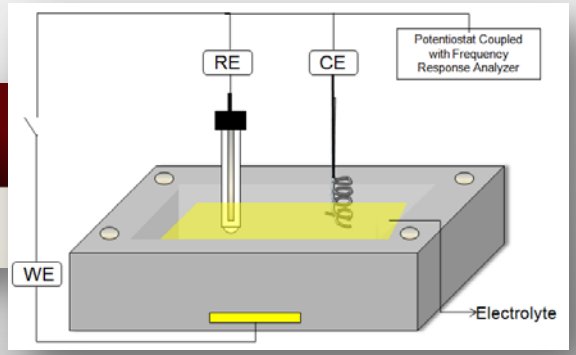
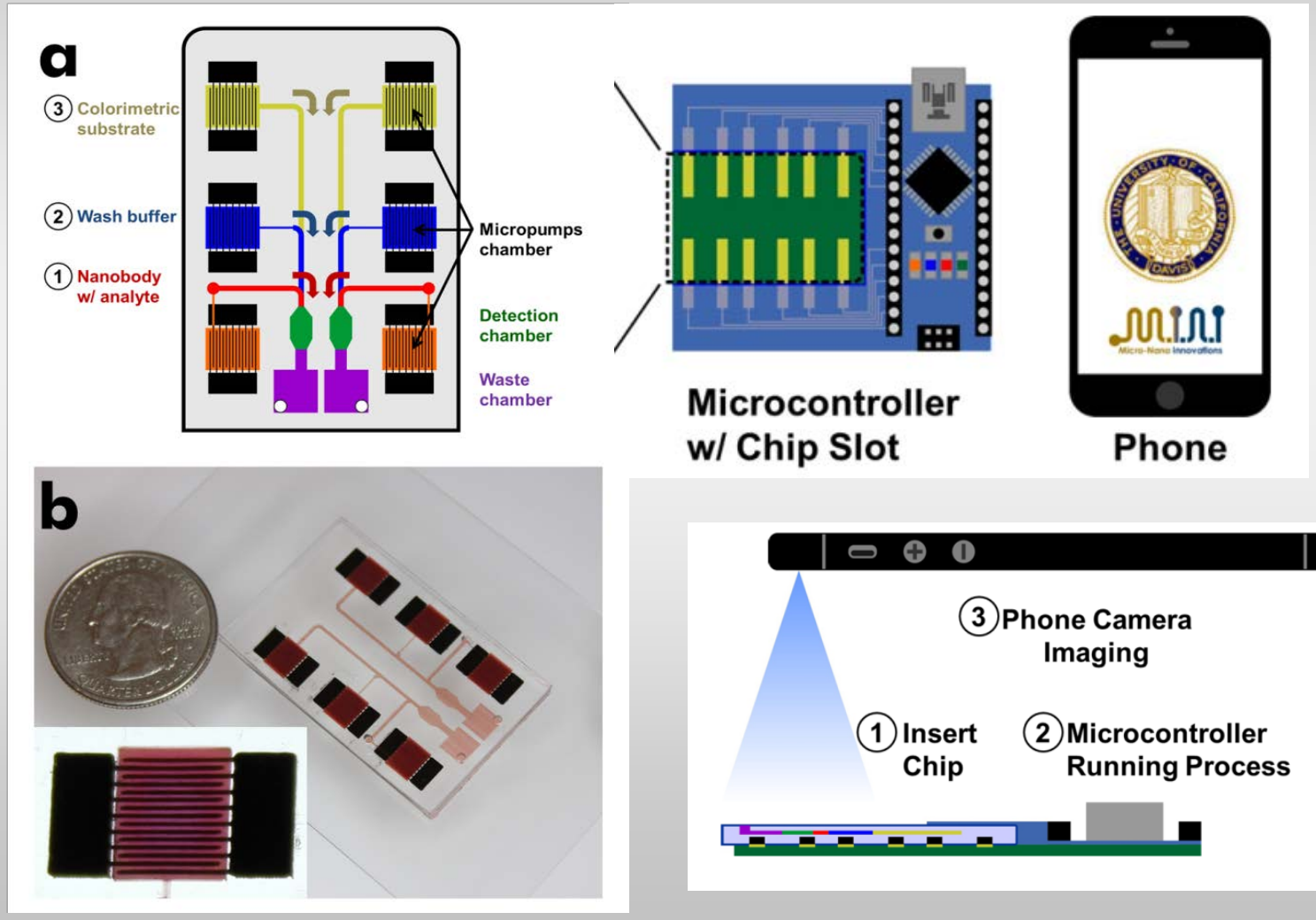
MATERIALS TECHNOLOGY CENTER



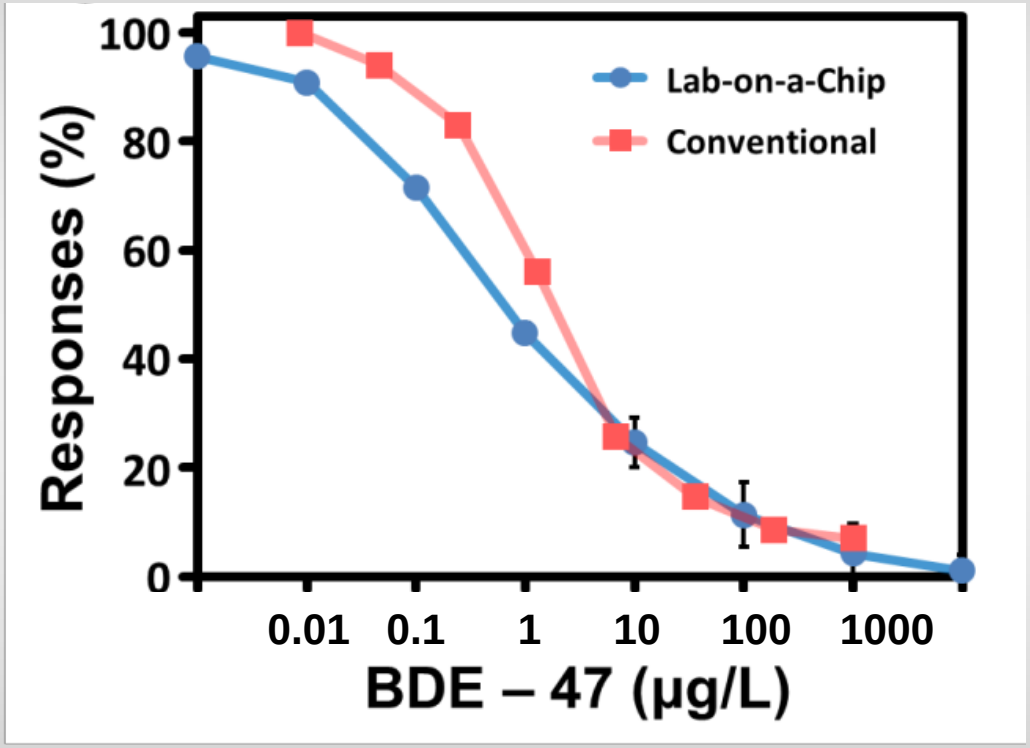
*see poster about the sensor technologies

Lab-on-a-chip



Chen, et al. 2014. Biomicrofluidics, 8: 064101

Lab-on-a-chip

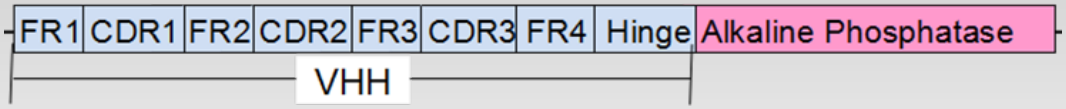


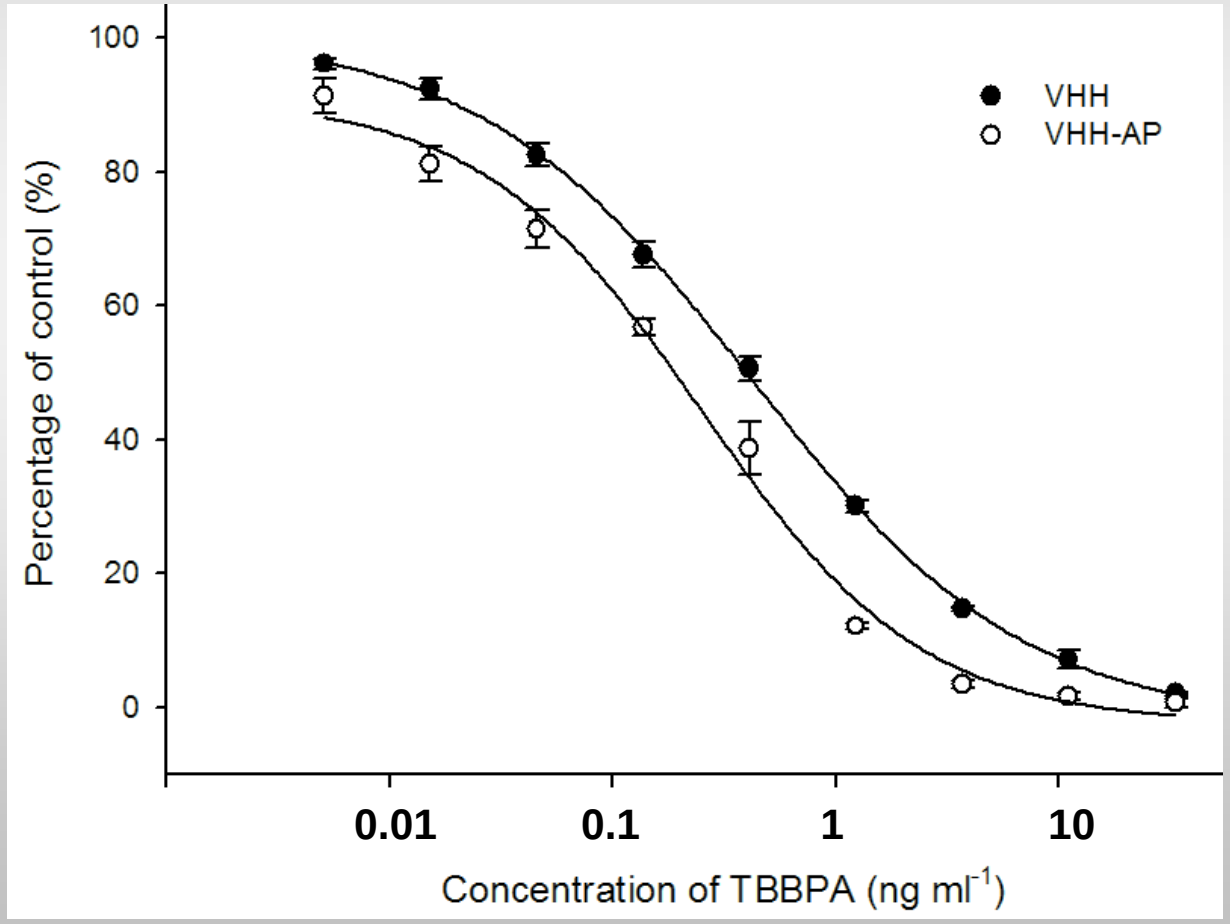
Advantages:

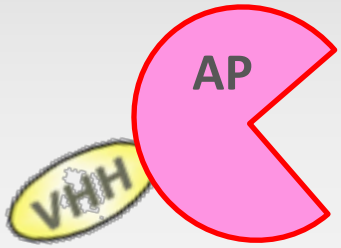
- Comparable sensitivities
- Small amount of power needed (portability)
- Small volumes needed
  - Reagents
  - Samples
- Rapid detection time
- Ease of visualization/analysis

Chen, et al. 2014. Biomicrofluidics, 8: 064101

Genetic manipulation of VHH





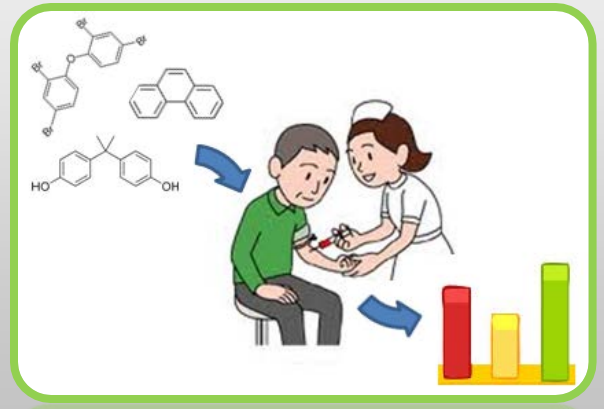


Advantages:

- Comparable sensitivities
- Less steps needed, reduces analysis time

Summary:
Developing Chemical Detection Methods Using VHH

- VHH
  - Ease of production
  - Comparable sensitivity to conventional IgG reagents
  - Selective for environmental contaminant
  - Avenues for genetic manipulations
  - Ease of incorporation into biosensor formats
- Future work:
  - Sample testing
  - On-site portable analysis



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