

BIOMARKERS OF EXPOSURE TO HAZARDOUS SUBSTANCES

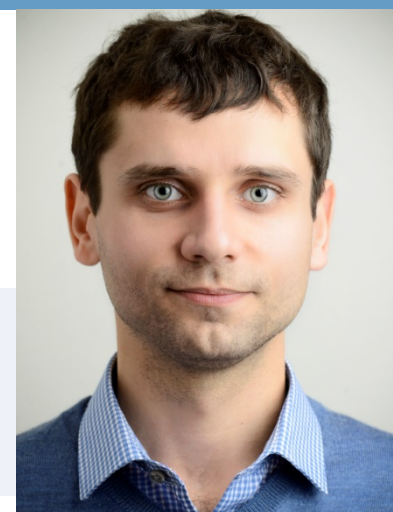
PROJECT 3: Immunoassays for human and environmental monitoring

UC DAVIS SUPERFUND RESEARCH PROGRAM
FUNDED SINCE 1987



NATALIA VASYLIEVA, *PhD*
Bioanalytical Chemistry

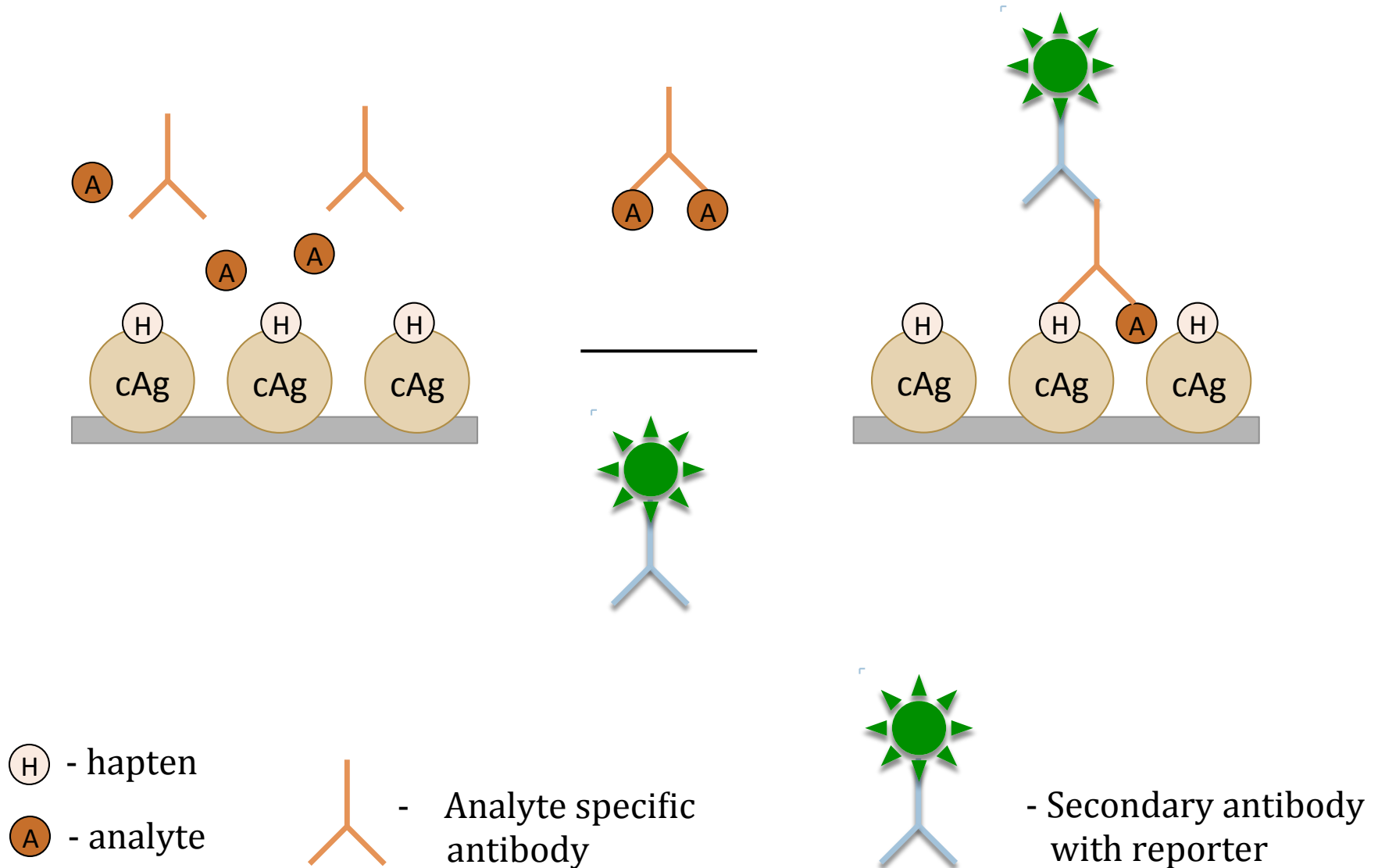
BOGDAN BARNYCH, *PhD*
Organic Chemistry



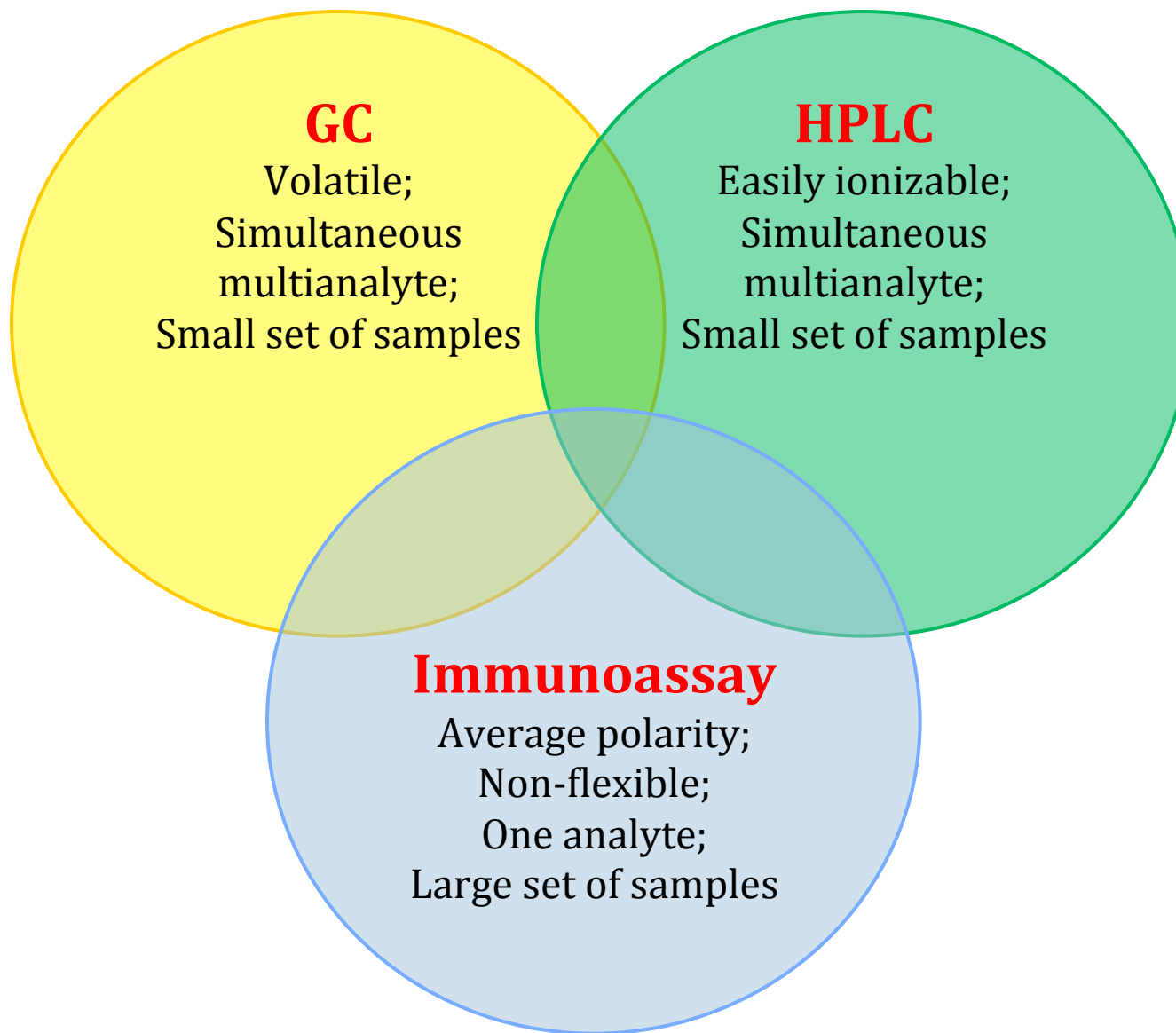
Outline

- Principle of immunoassay
- Position of immunoassay among other analytical tools
- Criteria for choosing target analytes
- Nanobodies[®] – modern reagents for immunoassay development
- Examples

Theory of competitive ELISA



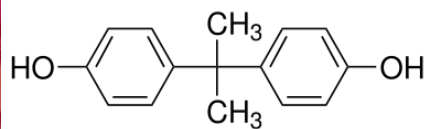
Position of immunoassay



Target analytes



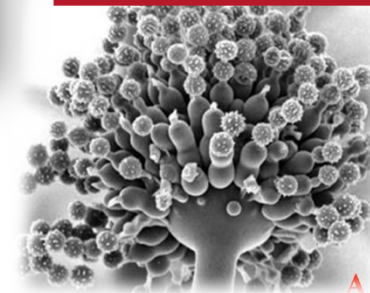
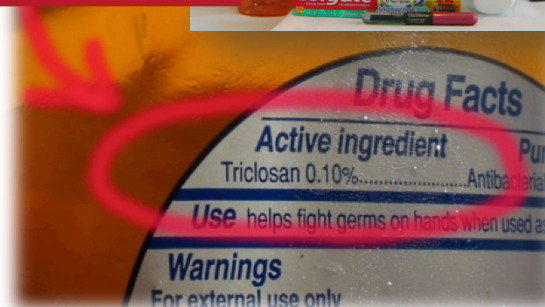
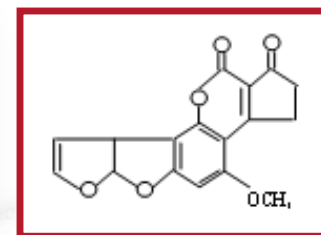
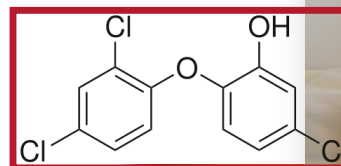
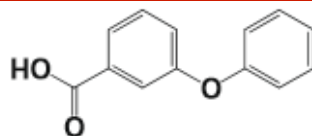
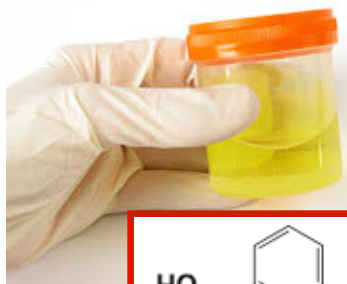
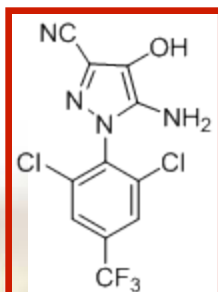
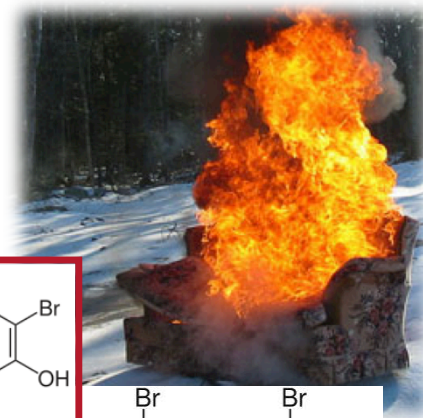
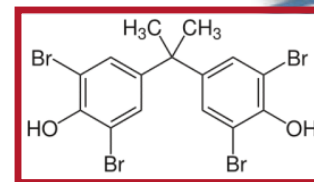
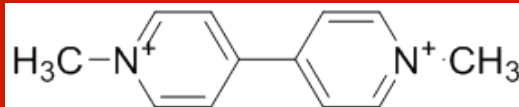
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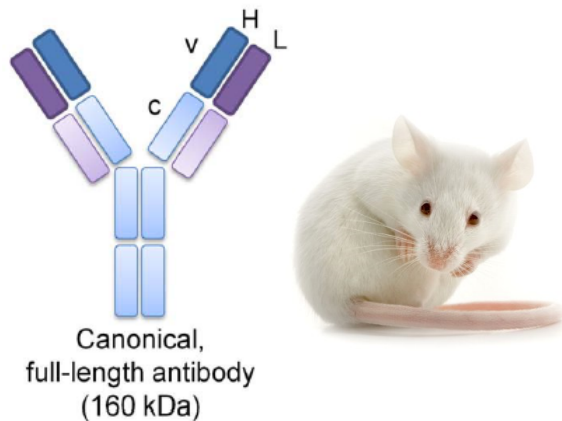
WARNING:
Pesticides are
Dangerous to
Your Health!



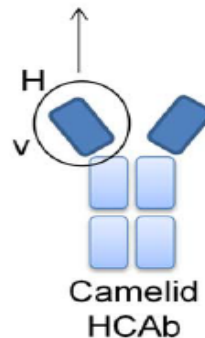
Stop Endocrine Disrupting Chemicals!



Antibody differences



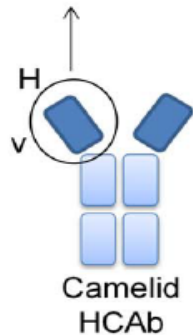
Single-domain antibody/Nanobody®
(Nb or VHH or V_{NAR}) (15 kDa)



Mouse	Characteristics	Camelids
150 kDa	size	15 kDa
unstable	stability	very stable
mammalian – doubles every day	culture	bacterial – doubles every 20 mins
mgs in weeks	yield	mgs in a day
chemically	engineering	chemically or genetically

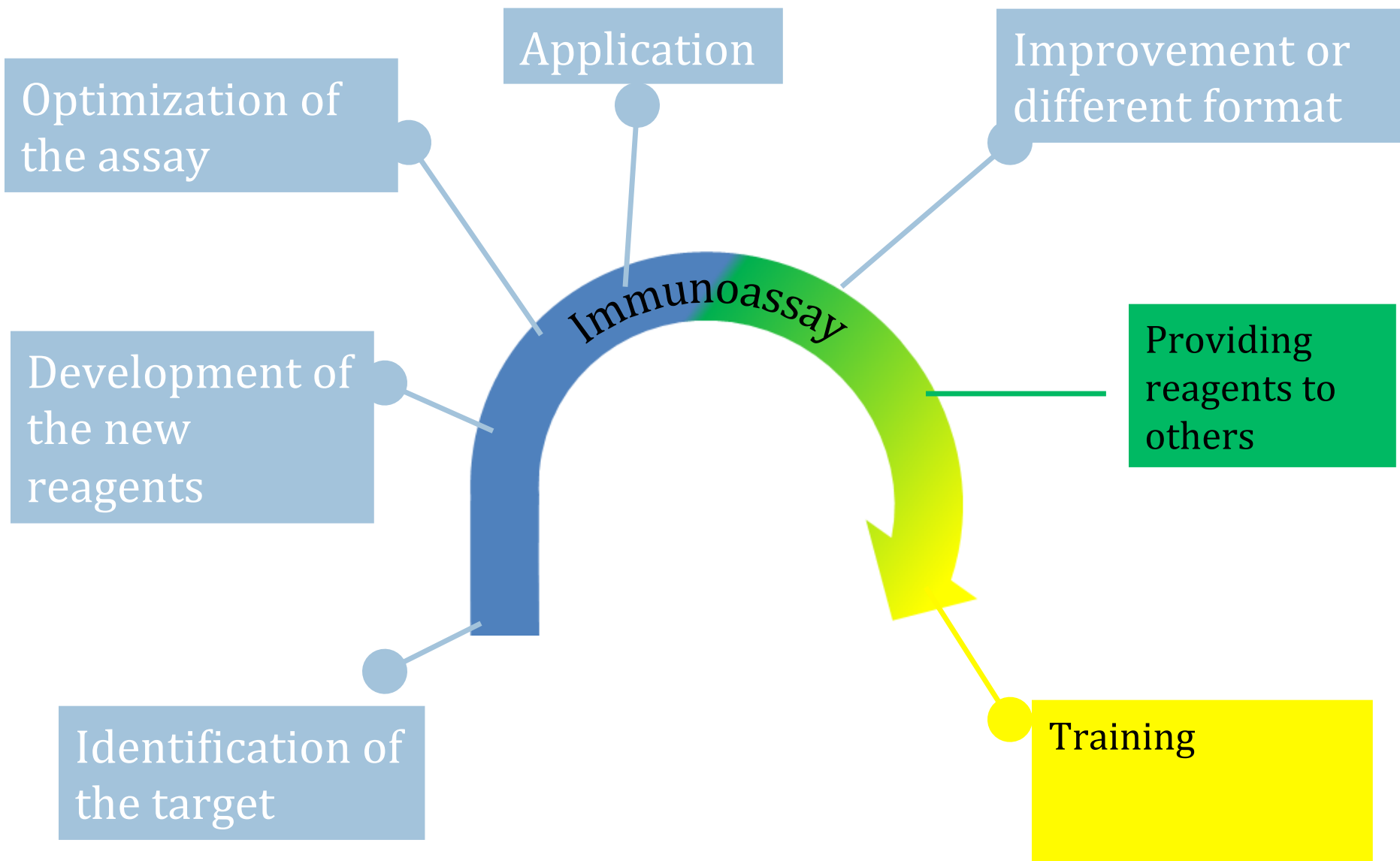
Nanobody advantages

Single-domain antibody/Nanobody®
(Nb or VHH or V_{NAR}) (15 kDa)

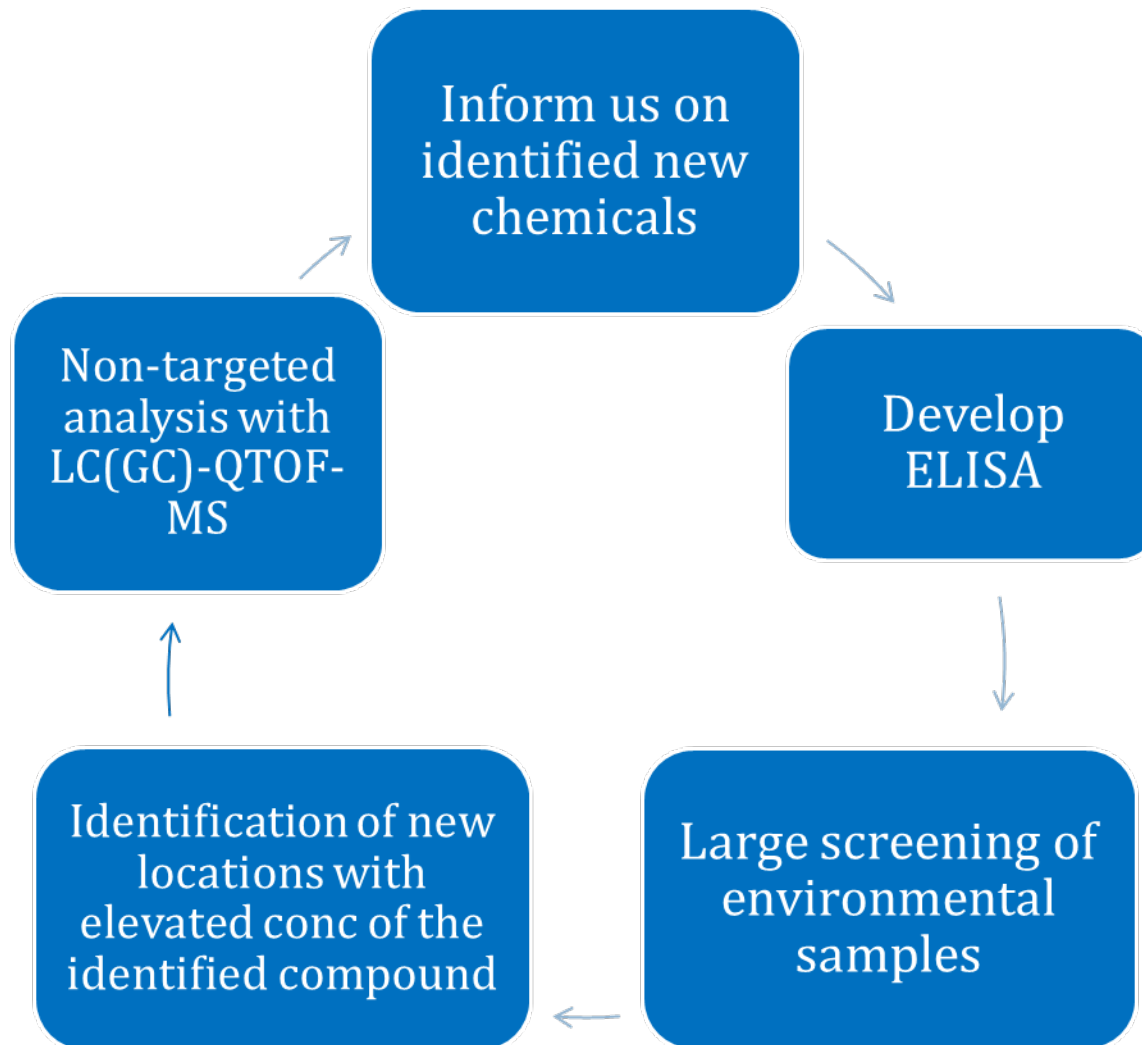


- inexpensive mass production – *E. coli*
- 15K or 90% size reduction over IgG ->high density for sensitive biosensors
- thermally stable for field assays
- ease of expression and genetic manipulation
- recreation of nanobody from published sequence

Interaction with SRP and other collaborators



Identification of new targets in collaboration with Thomas Young's team UC Davis SRP

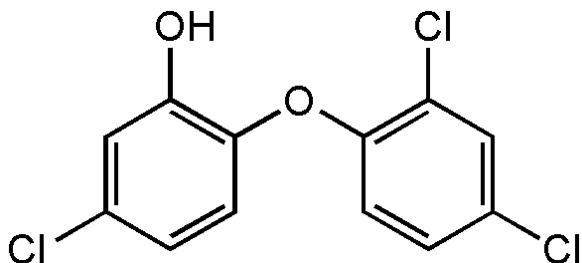



Examples:

- pyrethroids
- fipronil and its metabolites
- triclosan



Triclosan



- Antimicrobial additive
- NHANES study: (n=2517, 75% detection rate, LOD 2 μ g/L)
 - Wolff et al., 2007, EHP; Calafat et al., 2008, EHP
-  Found in >50% of rivers surveyed
 - max concentration: 2.3 μ g/L (med. 0.14 μ g/L)
 - Kolpin, et al. ES&T, 2002

*G. Cherednichenko, ... B. D. Hammock, ...I. N. Pessah, *P Natl Acad Sci U S A* **2012**, 109, 14158-14163.

M. F. Yueh, K. Taniguchi, S. J. Chen, R. M. Evans, B. D. Hammock, M. Karin, R. H. Tukey, *P Natl Acad Sci USA* **2014, 111, 17200-17205.

Application of the ELISAs: Triclosan study with Dr. Pezzoli and Dr. Gabriel Anaya SRP UCSD



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Los Laureles Canyon Dust Pilot Study

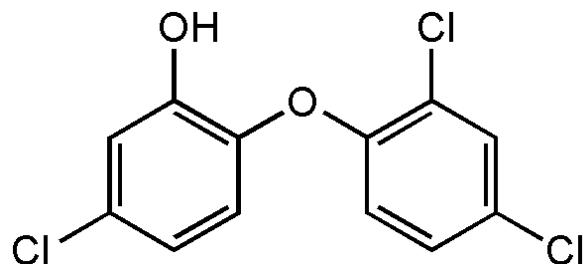
aimed in identifying environmental public health concern at the US-Mexico border, in the San Diego-Tijuana city region



- Household dust, urine, and demographic data was collected from 46 residents
- 388 household environmental public health survey conducted
- Household dust was analyzed for:
 - heavy metals
 - bisphenol A
 - Phthalates
 - Triclosan -> **found triclosan**

• Urine samples were analyzed for triclosan by UC Davis SRP partners

Triclosan



□ Antimicrobial additive



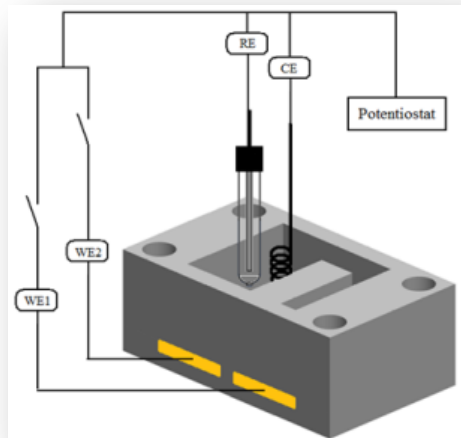
□ **Our data showed that the US/Mexican population at the border was at significant health risk since the mean concentration of triclosan in the urine samples we found was high**

Immunochemical formats

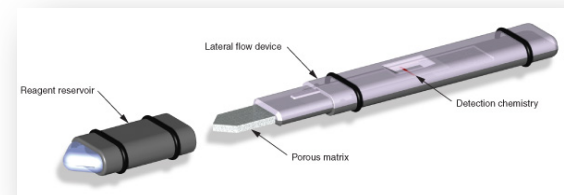
ELISA



Electrochemical Impedance Spectroscopy



Lateral Flow Strip



Microfluidic device



Optical biosensor

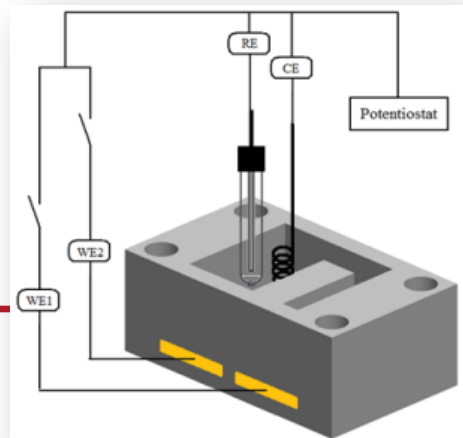


Provide reagents for the development of new Immunochemical formats - biosensors

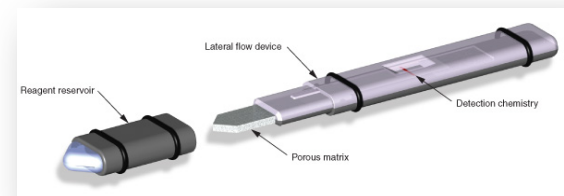
ELISA



Electrochemical
Impedance
Spectroscopy



Lateral Flow Strip



Microfluidic device

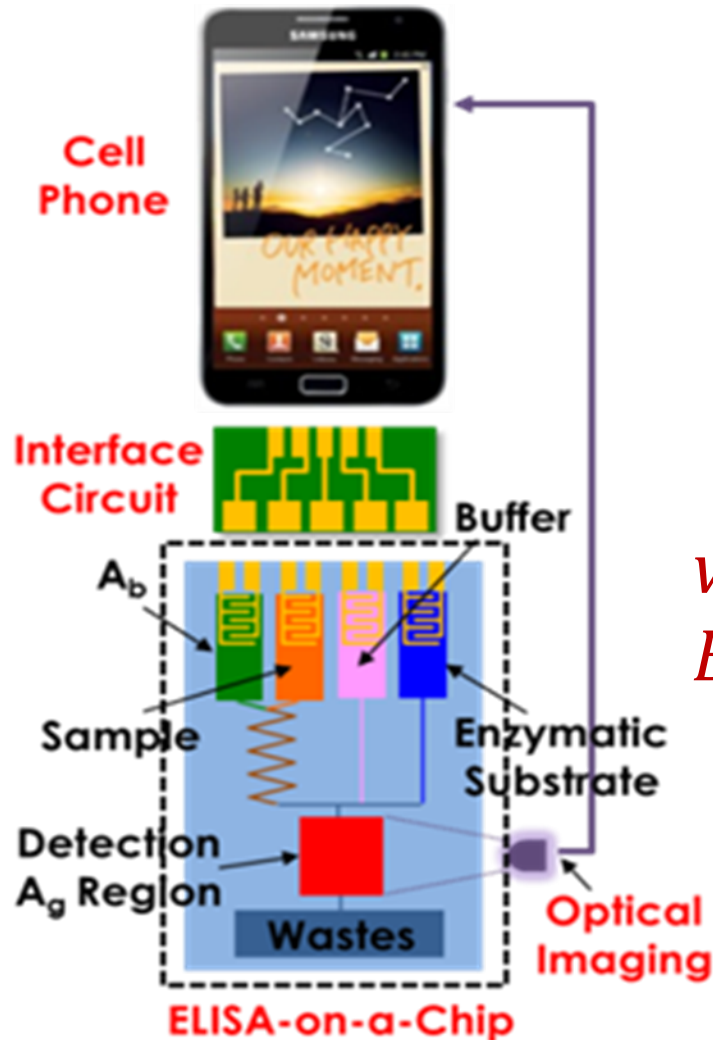


**Collaboration with
SRP partners**
Dr. Tingrui Pan and
Dr. Ian Kennedy

Optical biosensor



Nanobody utilization in a sensor format



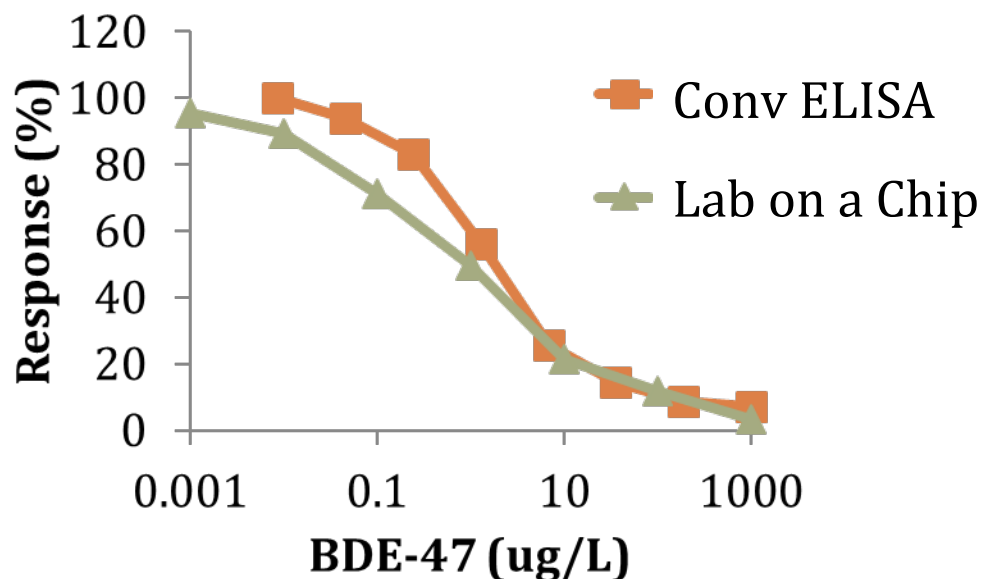
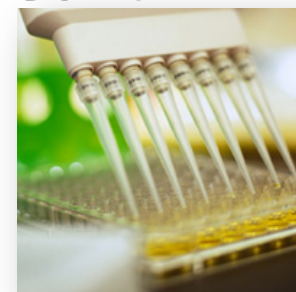
*with Dr. Tingrui Pan UCD SRP
Biomedical Engineering*

Format comparison

Lab on a Chip



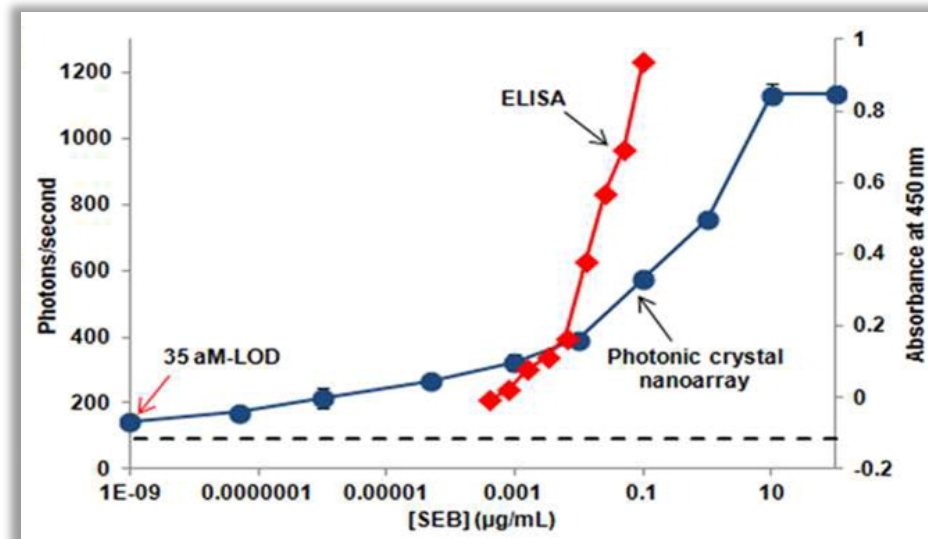
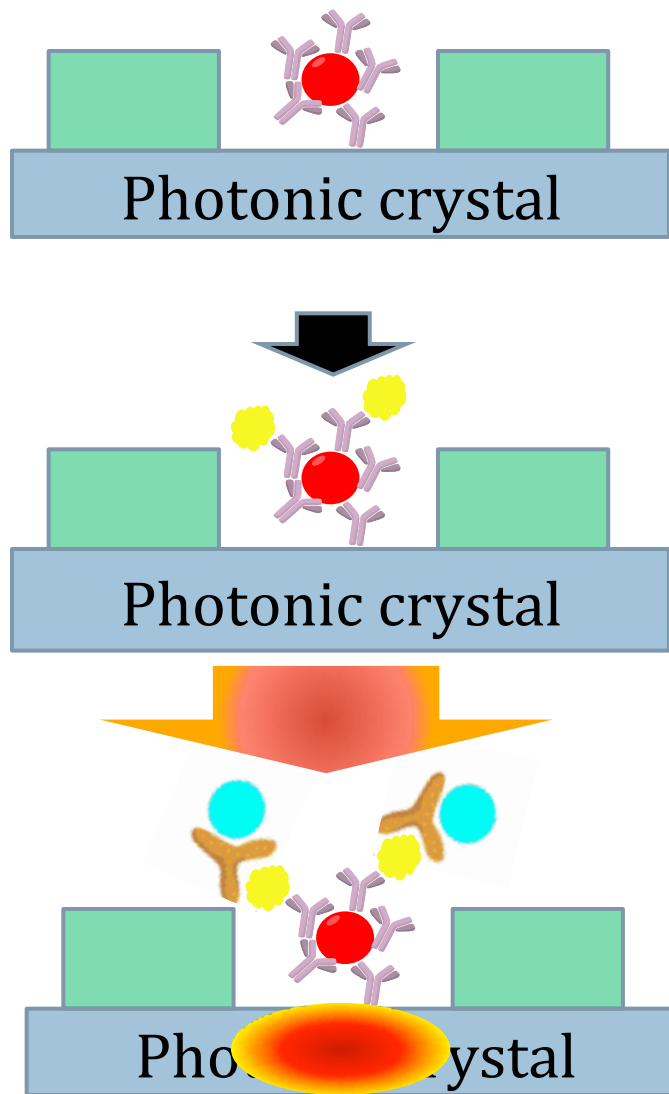
Conv. ELISA



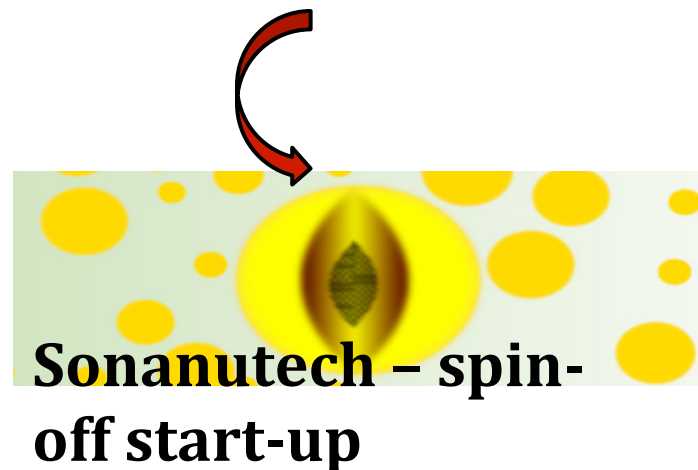
Advantages:

- ☐ Small amount of reagents needed
- ☐ Small sample volume
- ☐ Rapid detection time
- ☐ Easy visualization/analysis
- ☐ Portable

Optical biosensors in collaboration with Dr. Kennedy, UCD SRP



10^6 -fold increase in sensitivity



Collaborations and training

1. Training of international scholars about immunoassays and novel antibody reagents (nanobodies) (China, Thailand, Ireland, France, USA)
2. Taught industry experts how to produce novel antibodies (Antibodies Inc)
3. Provide reagents and research support to academic (USA, UK, USEPA) and private laboratories (Mars Inc, ManRos Therapeutics)

Acknowledgments



Hammock lab : <http://www.biopestlab.ucdavis.edu/>

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