Detecting environmental chemicals using immunoassay technology

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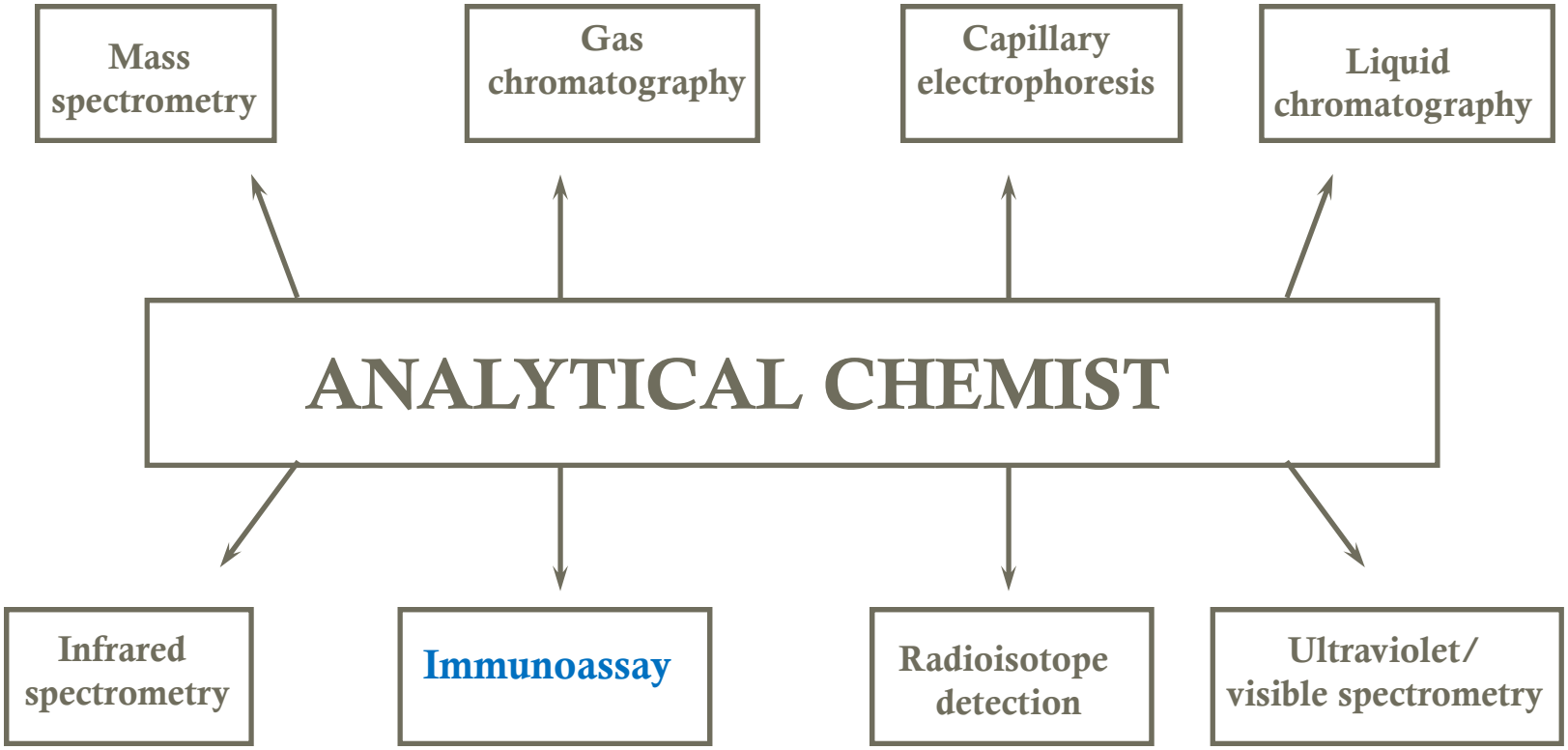
Immunoassay: a complementary technology



Immunoassay

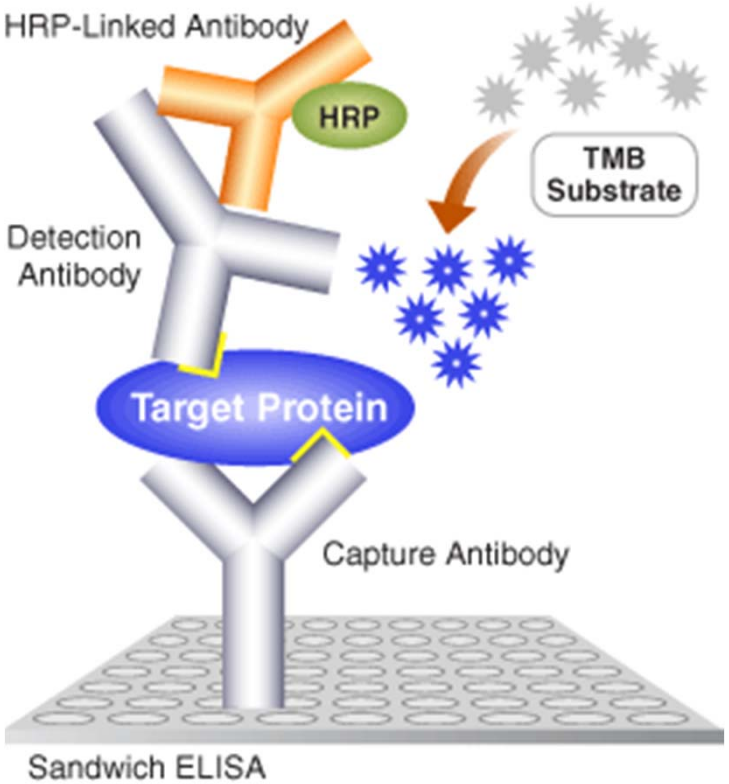
Advantages

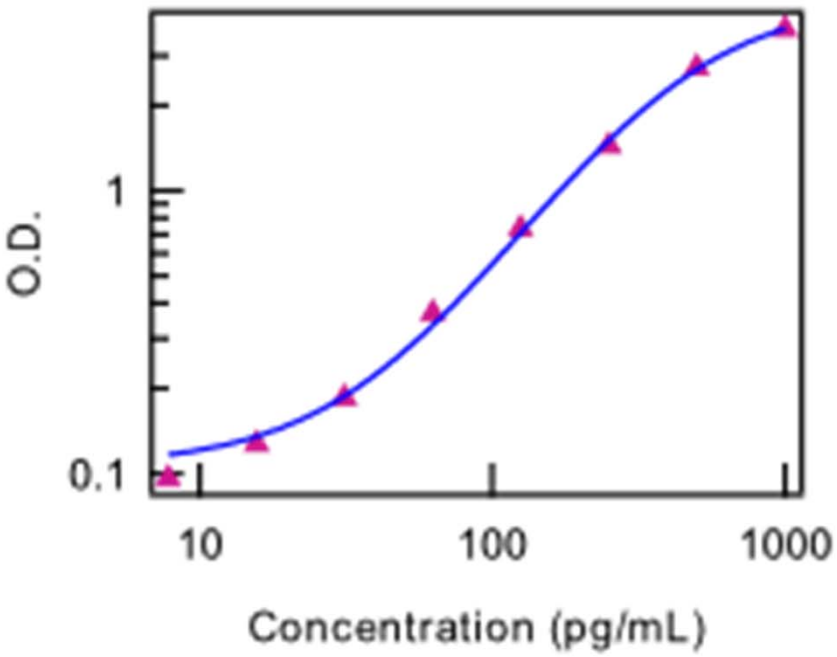
- Sensitive
- Selective
- Rapid sample preparation
- Cost-effective
- Simple to use
- Adaptable to various formats

Disadvantages

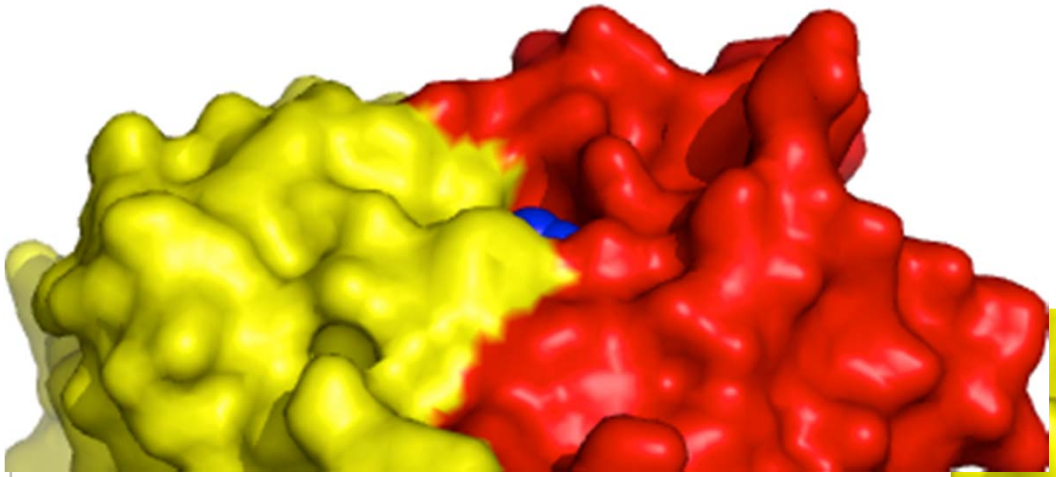
- Some targets are difficult
- Cross reactivity/Interferences
- Reagent availability
- New technology
- Difficult to apply to multianalyte analysis

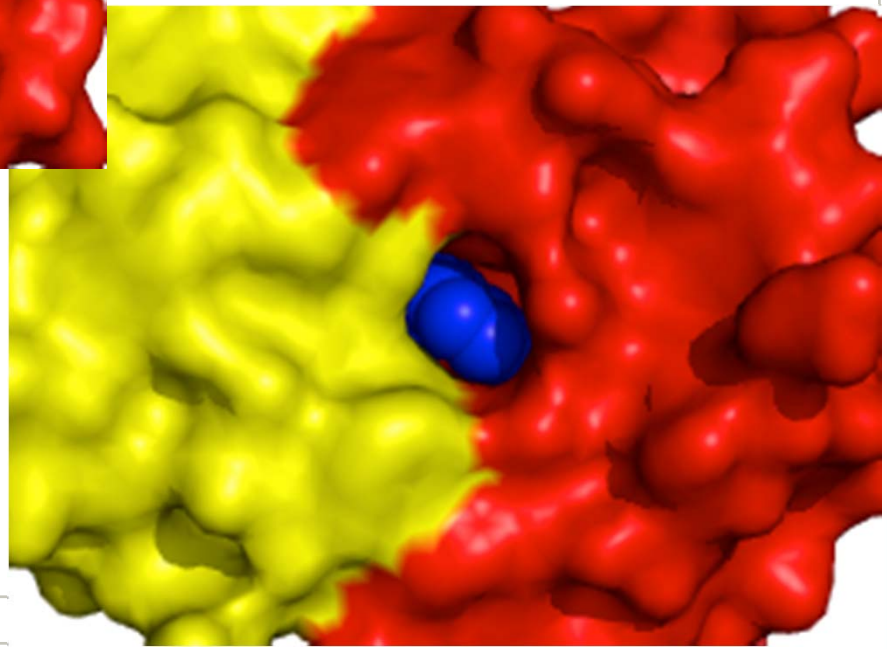
Sandwich immunoassay for protein analysis



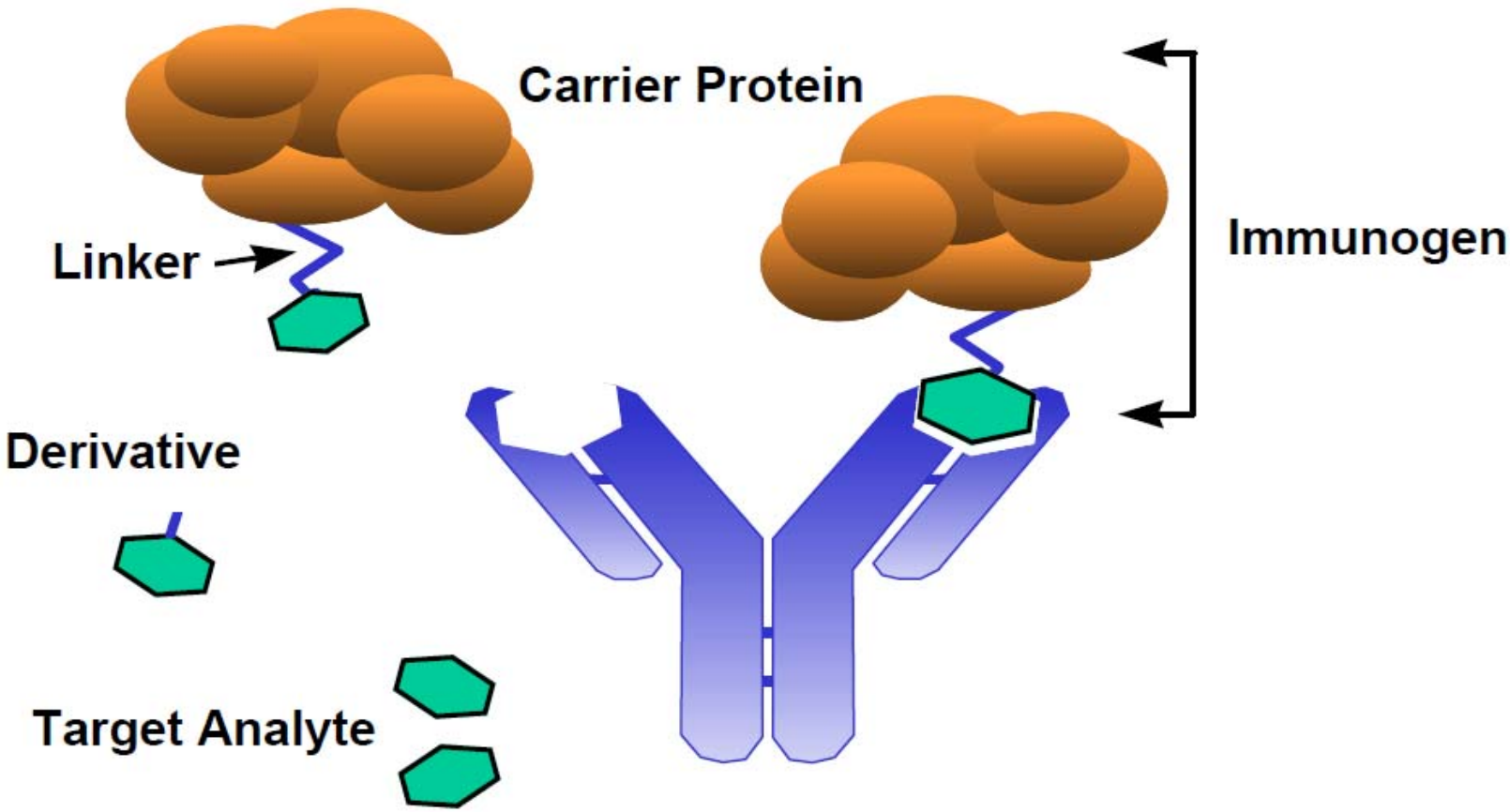


Antibody binding to a small molecule



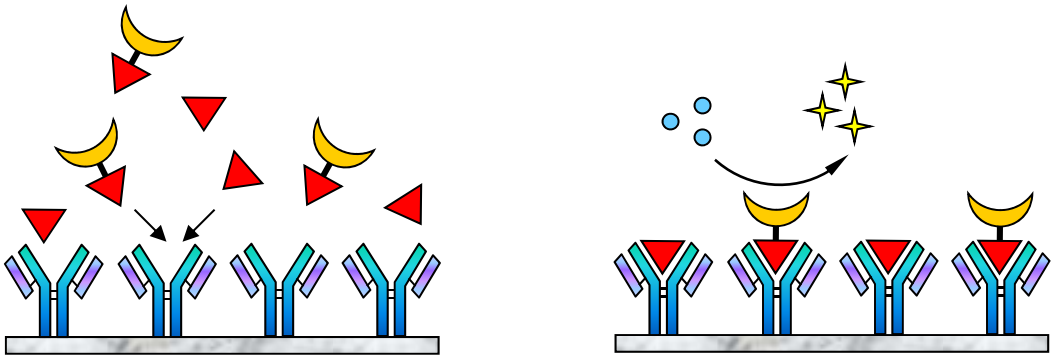


Small molecule immunoassay

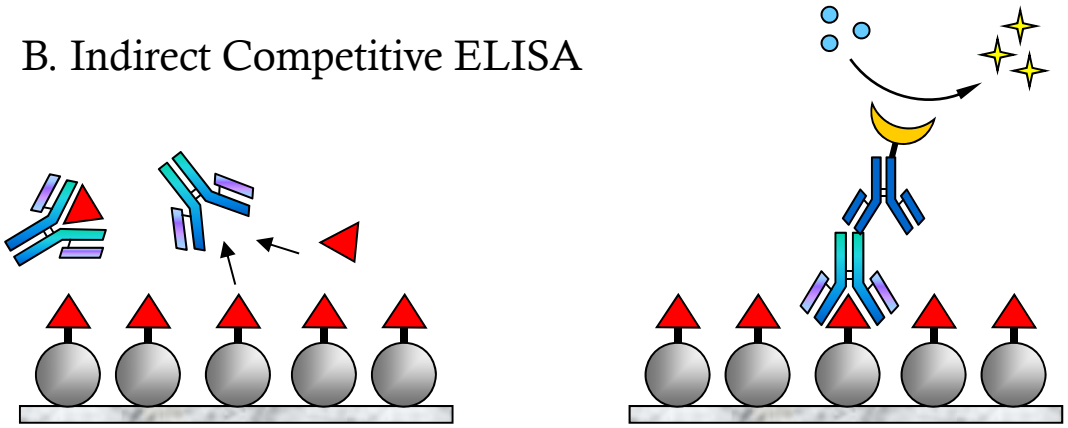


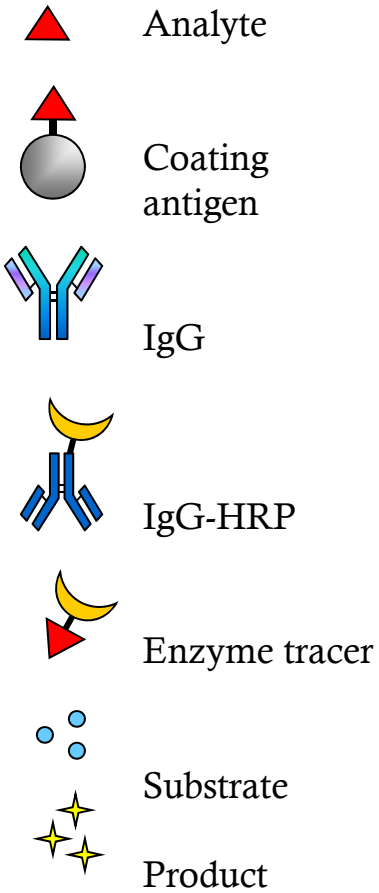
Immunoassay formats for detection of small molecules

A. Direct Competitive ELISA

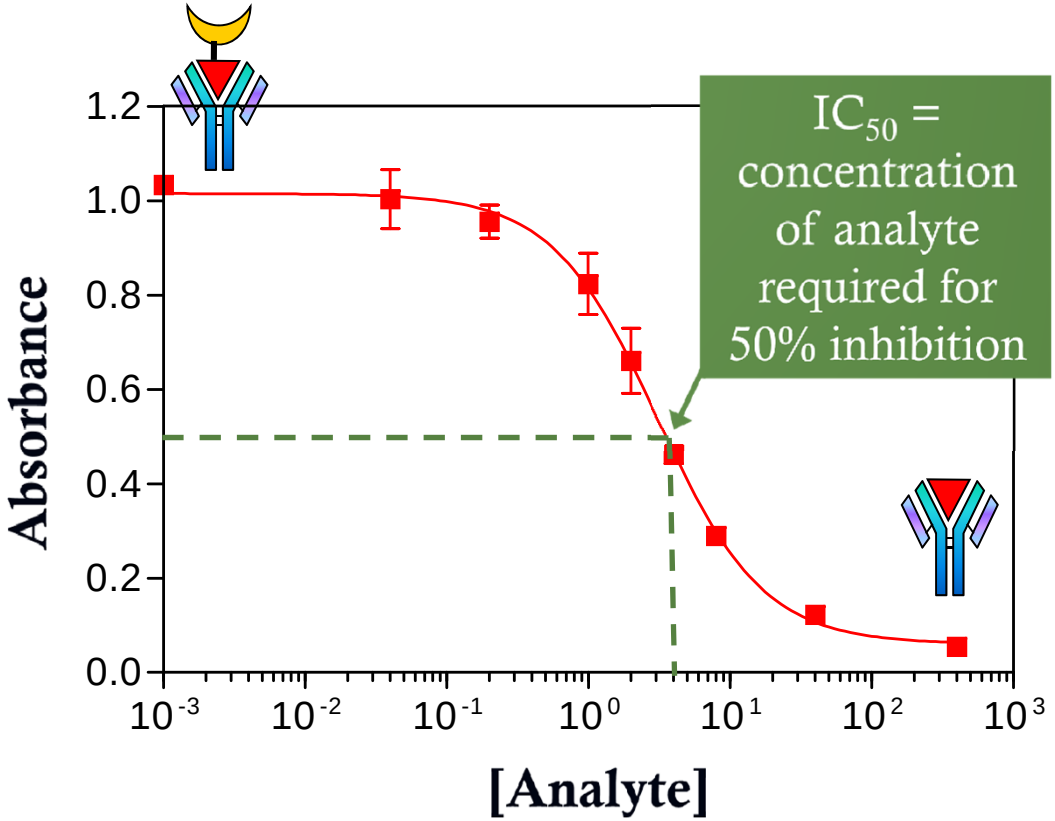


B. Indirect Competitive ELISA



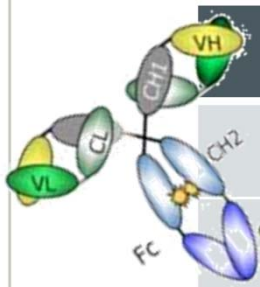


Calibration curve for competitive immunoassay



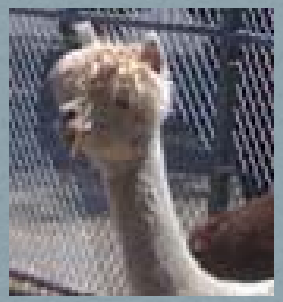
- Reasonable **signal:noise** ratio
  - High maximum absorbance
  - Low background absorbance
- High **sensitivity**
  - low limit of detection
  - steep slope (-0.75 to -1)
- Useful range of quantitation
  - number of points – well distributed

Antibody differences

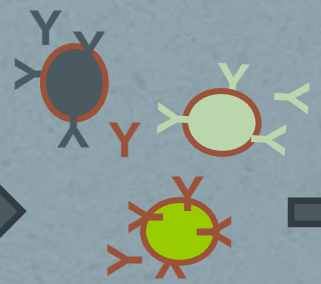


Murine	Characteristics	Camelids
140-150 kDa	size	15-20 kDa
tetramer	structure	monomer
mammalian – doubles every day	culture	bacterial – doubles every 20 mins
mgs in weeks	yield	mgs in a day
chemically	labeling	chemically or genetically

Nanobody library construction

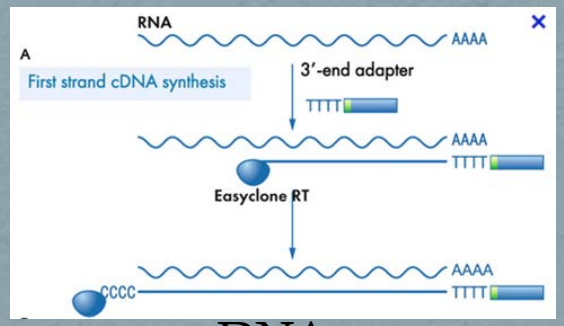


immunization

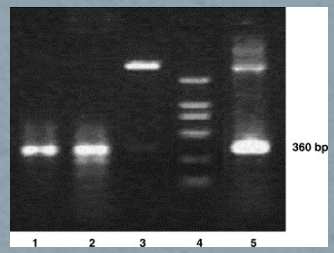


Collect B-cells

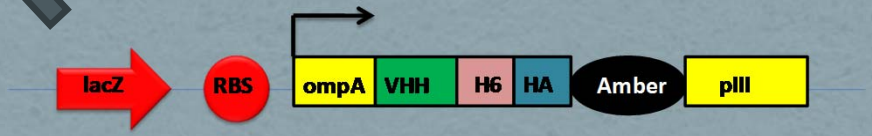
Isolate mRNA



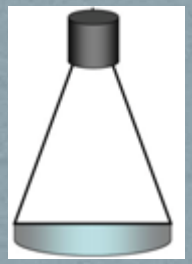
cDNA library



Digest to produce VHH insert

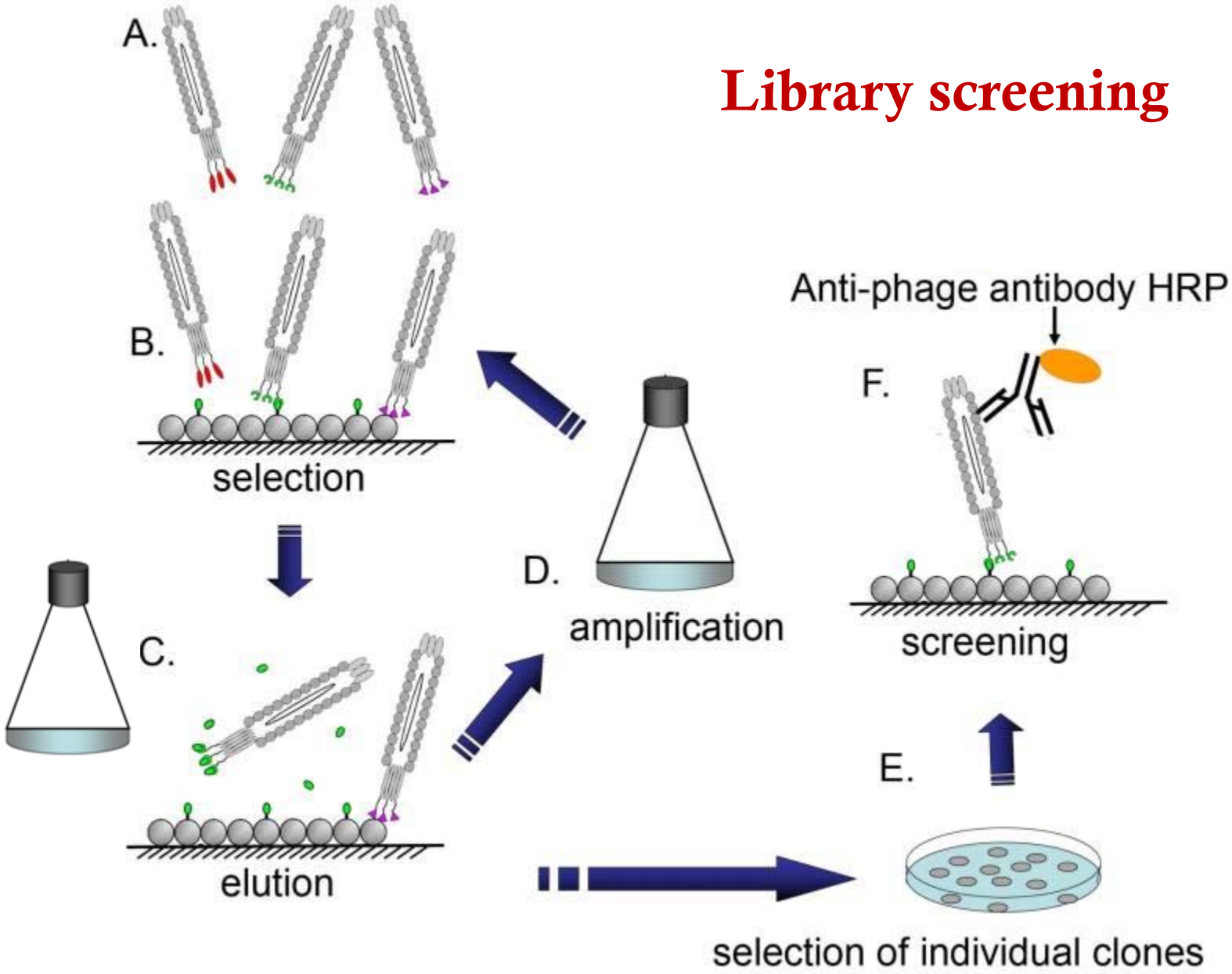


Ligation of VHH into pComb3X



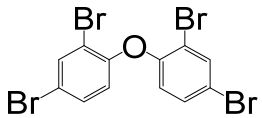
amplification

Library screening

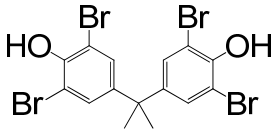


Nanobodies developed or under development to date in the Hammock Laboratory

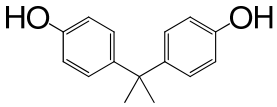
- BDE-47



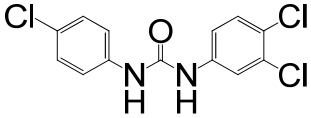
- Tetrabromobisphenol A



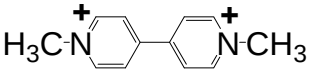
- Bisphenol A



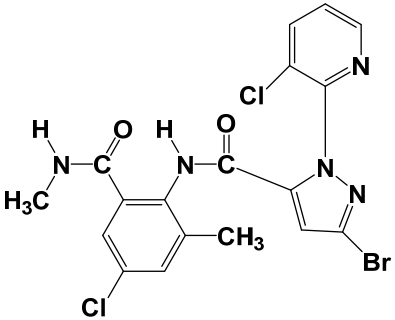
- Triclocarban



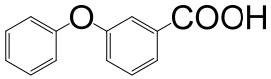
- Paraquat



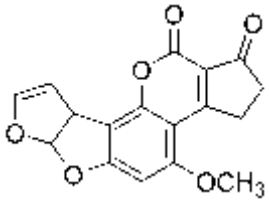
- Chlorantraniliprole



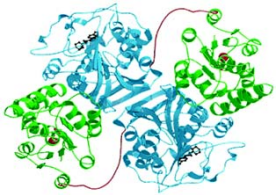
- 3-Phenoxybenzoic acid



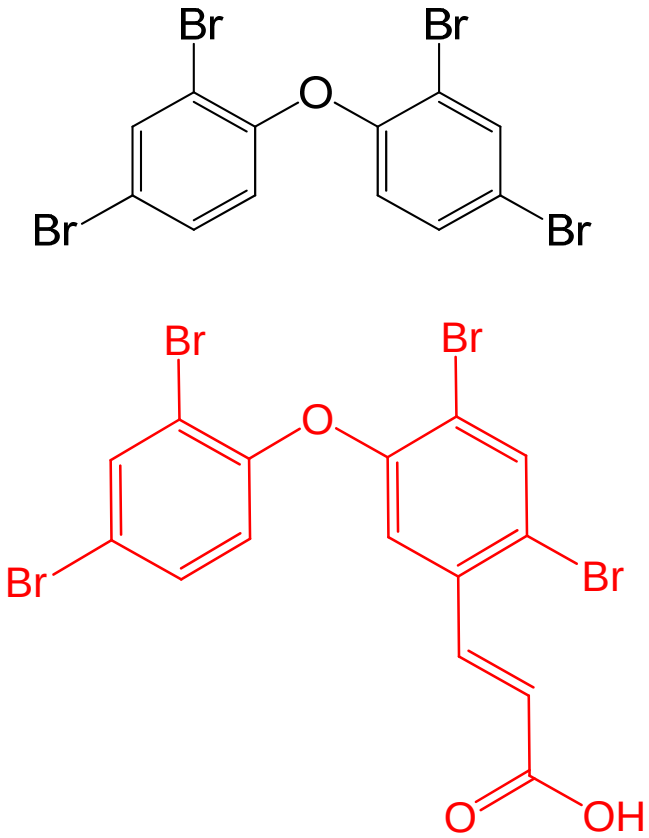
- Aflatoxins



- Microsomal and soluble epoxide hydrolase



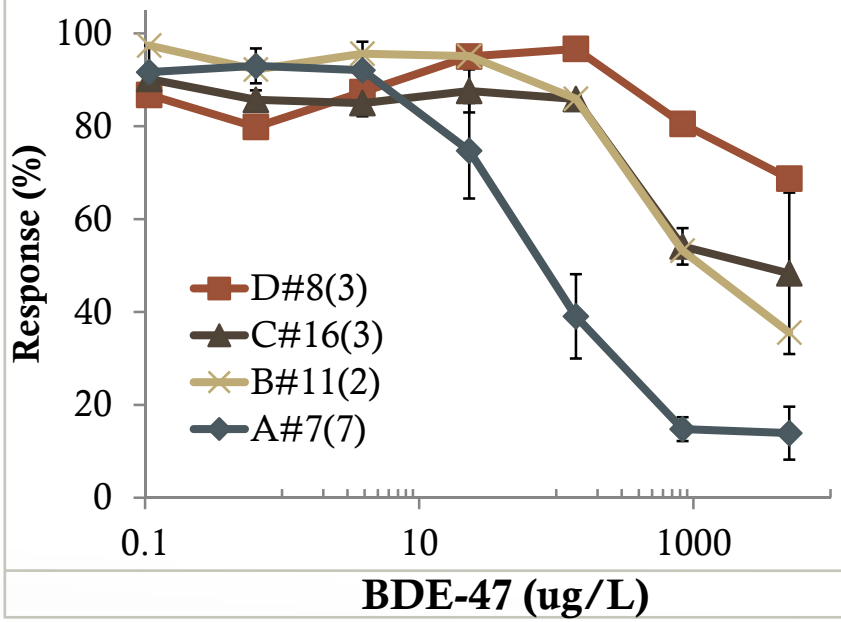
Nanobody production to a target analyte

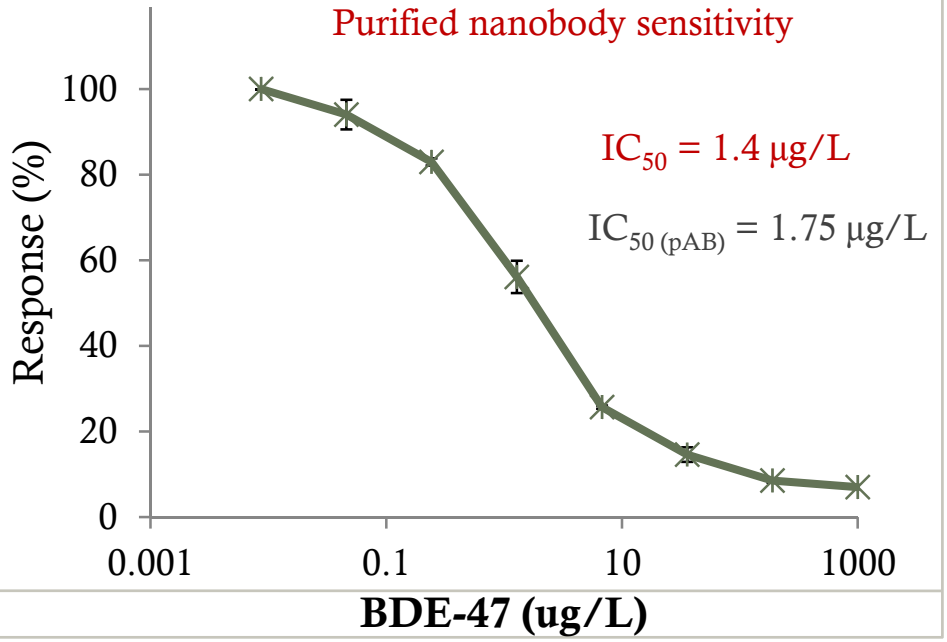


Nanobody production to BDE-47

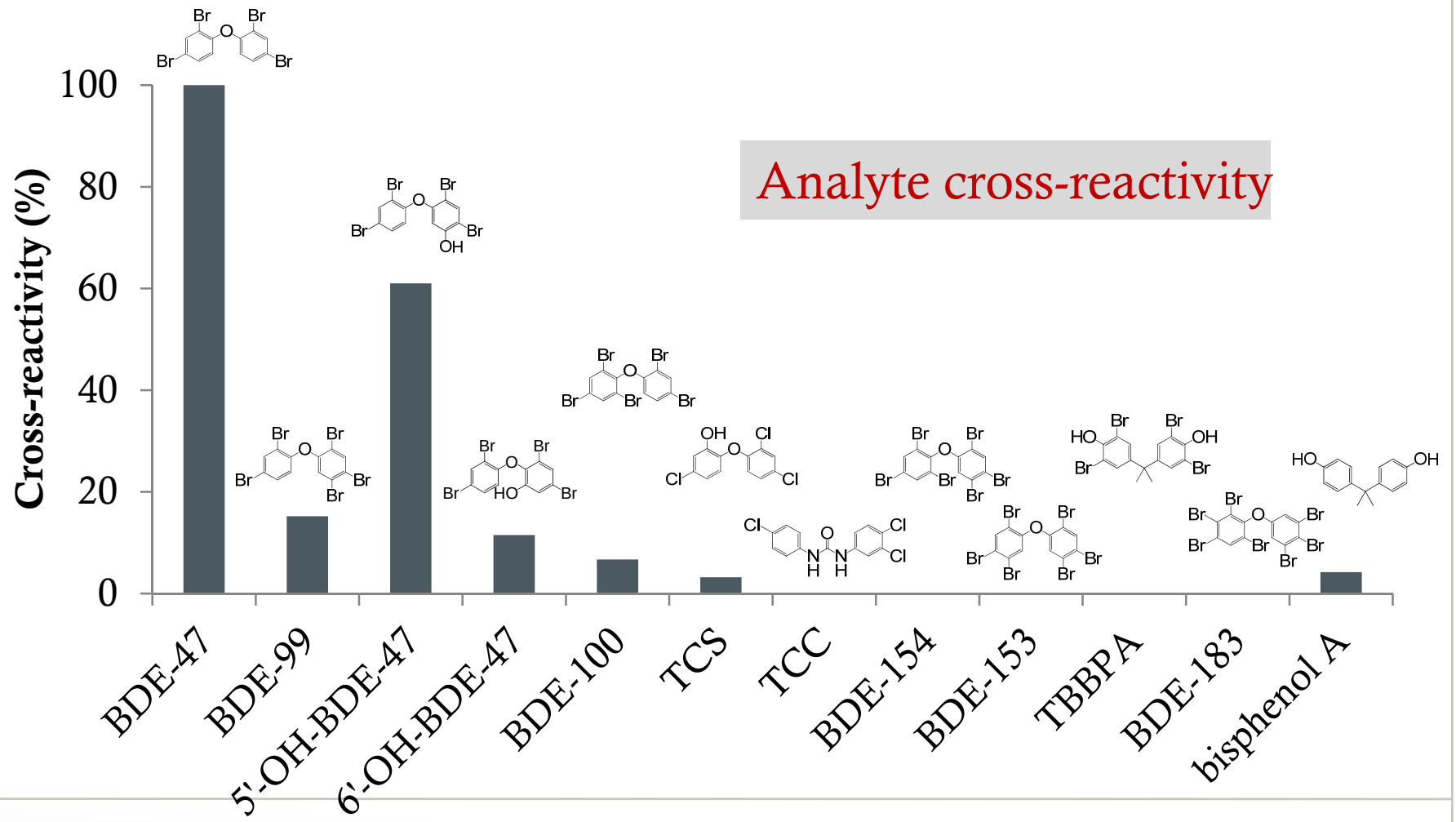
```
                    10        20        30        40        50        60        70        80        90        100
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
group A (7)  MKKTAIAIAVALAGFATVAQAAQLQLVESGGGLVQAGGSLRLSCVASGRGLIPYFMAWFRQAPGKEREFVAAMRNLDSSTYYGDSVQGRFTISRDYAKKT
group B (2)  ..................................P........A...FT.DS.AIG...........G.SCISGSNGN...A...K........KV.N.
group C (3)  ......................V...........P........A...FTFS..T.G.............G.ISRRGGT...A...K......T..V...
group D (3)  ..................................P........A..RGT.DYSAIG...........GISCIS~AHGT...A...K........N..N.

                    110       120       130       140       150       160       170       180
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|.
group A (7)  LYLQMNSLKPEDTAVYYCAAR~~~YGSGTY~~~~~YSRHEYRDWGPGTQVTVSSEPKTPKPQDGQAGQHHHHHHGAYPYDVPDYAS
group B (2)  V.................TDPRL..RNCPRGPASEAHRILEI..Q..L.....................................
group C (3)  M............I.....D~~~~~RQFAAQAISMTTRG.EY..Q.....................................
group D (3)  A..................P~~~....CP~~~~SERTGRL.SR.Q....I.....................................
```



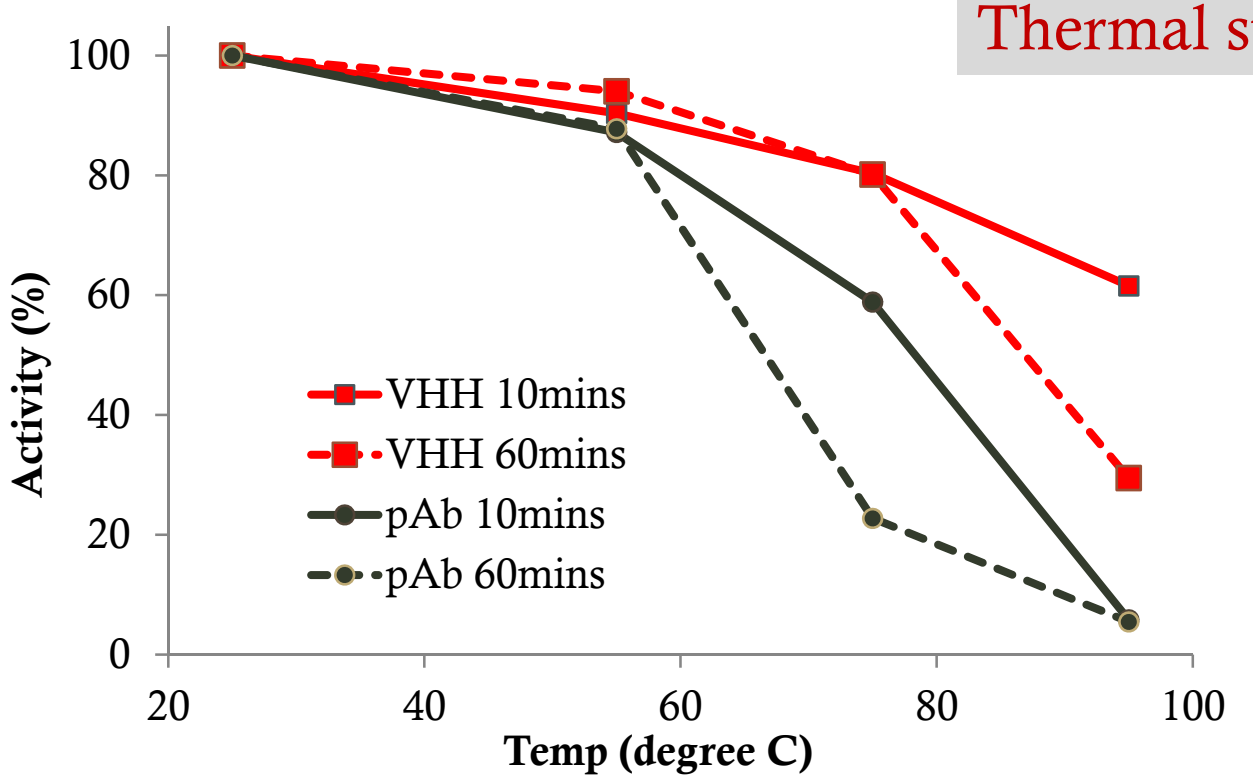


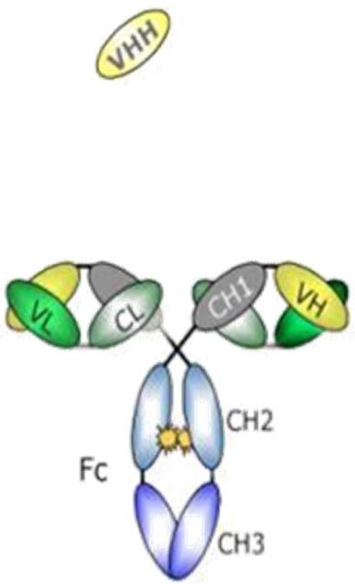
Nanobody characterization



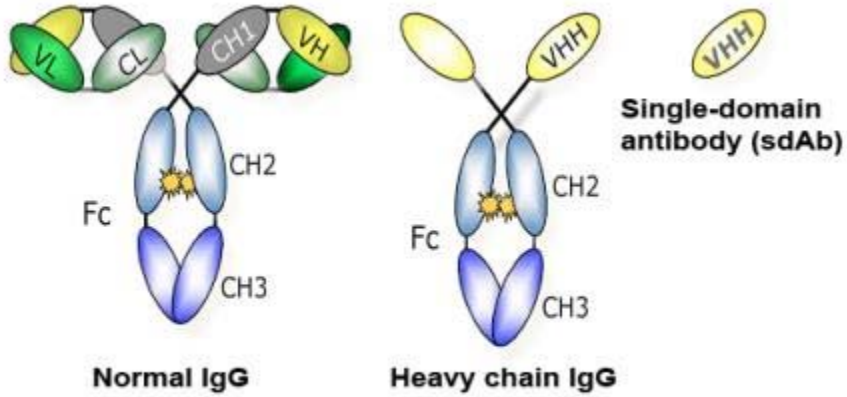
Antibody characterization

Thermal stability





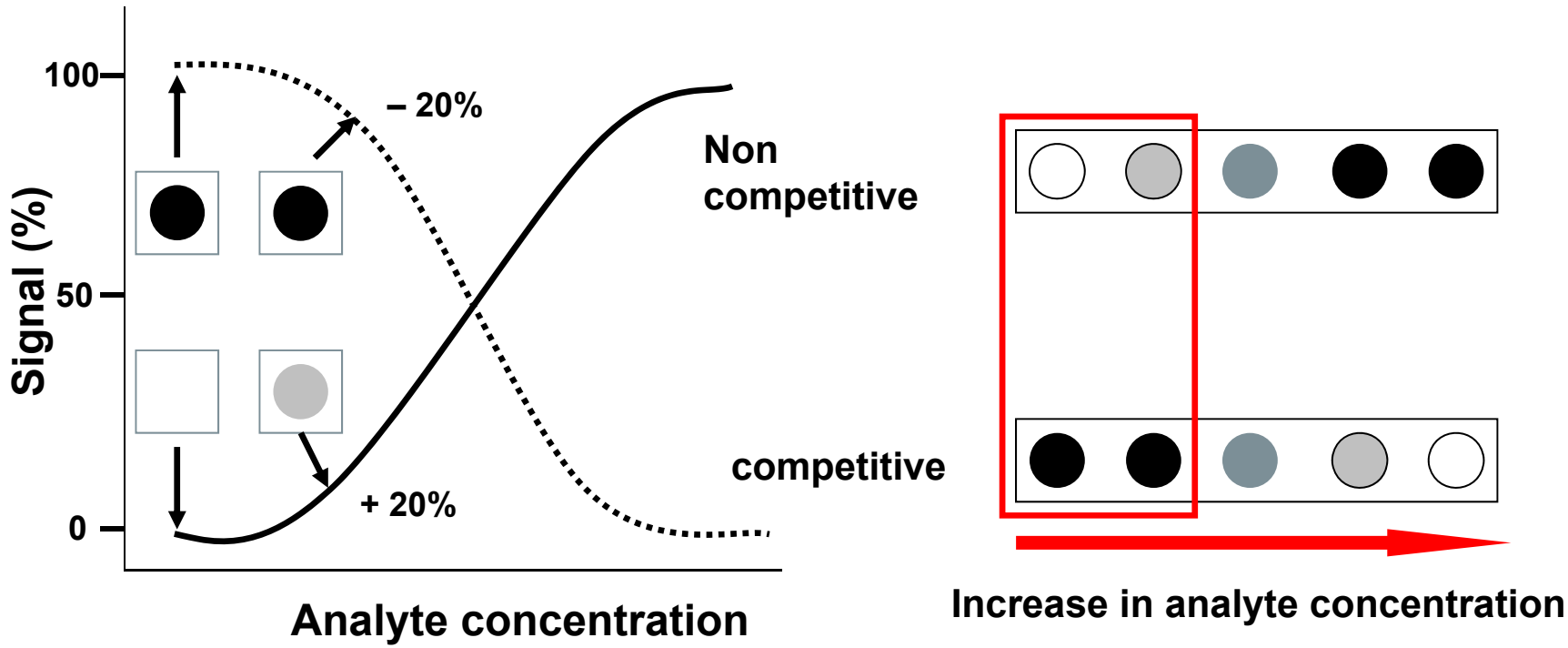
Nanobody® (sdAb) summary



- Ease of genetic manipulation
  - Labeled constructs
- Well-expressed products
  - Easy to scale up/improved costs
- High chemical/temperature stability and solubility
  - Decreased matrix effects
  - Ease of storage, efficient refolding

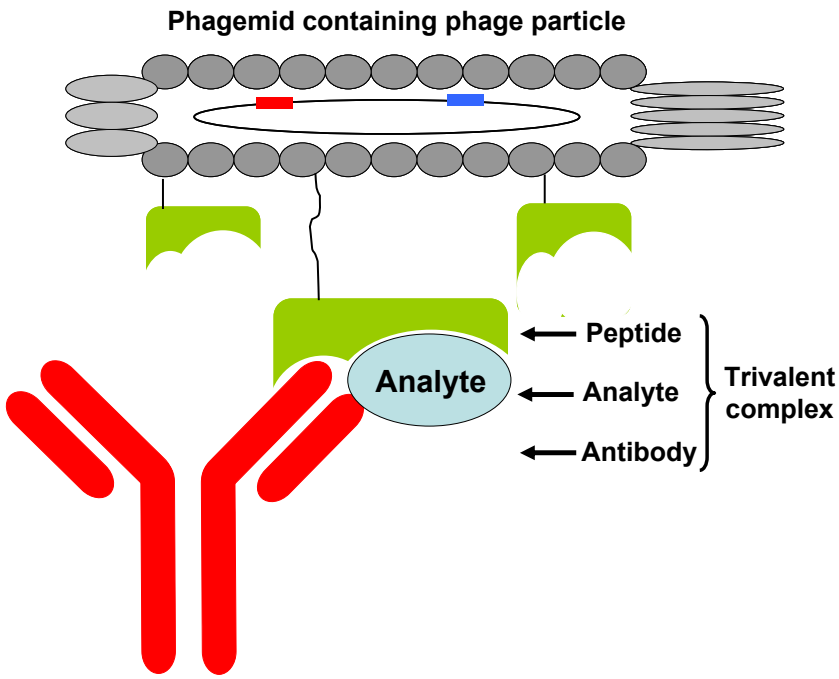
Employs phage-display technology…

Competitive vs noncompetitive assays



- Non competitive assays provide clear difference in signals at low concentrations of analyte from signals at zero concentrations

Phage displayed peptide selection

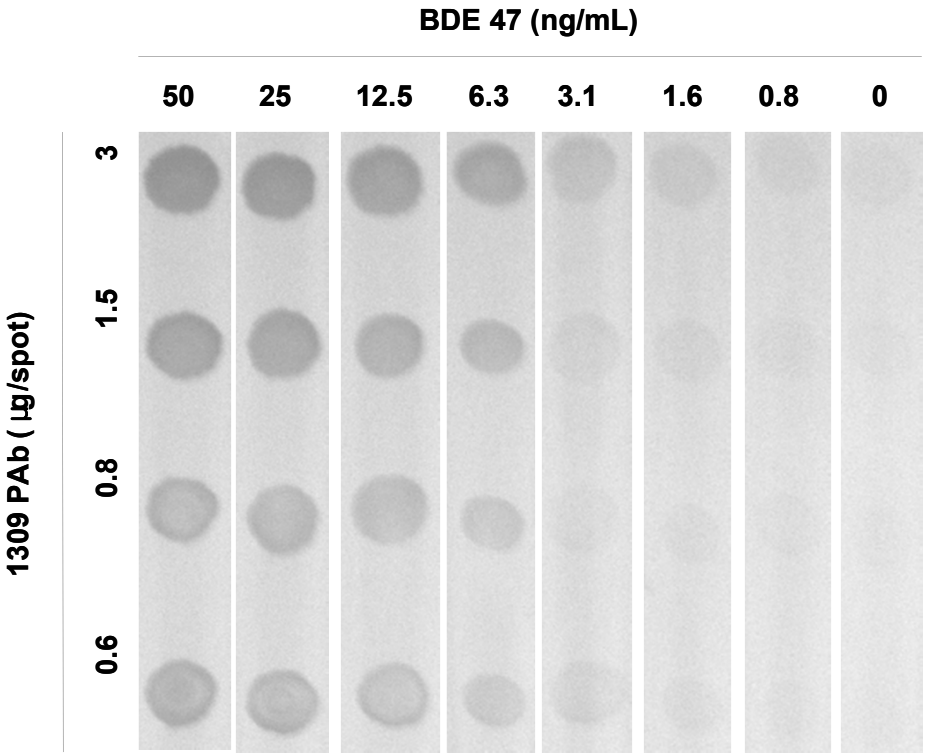


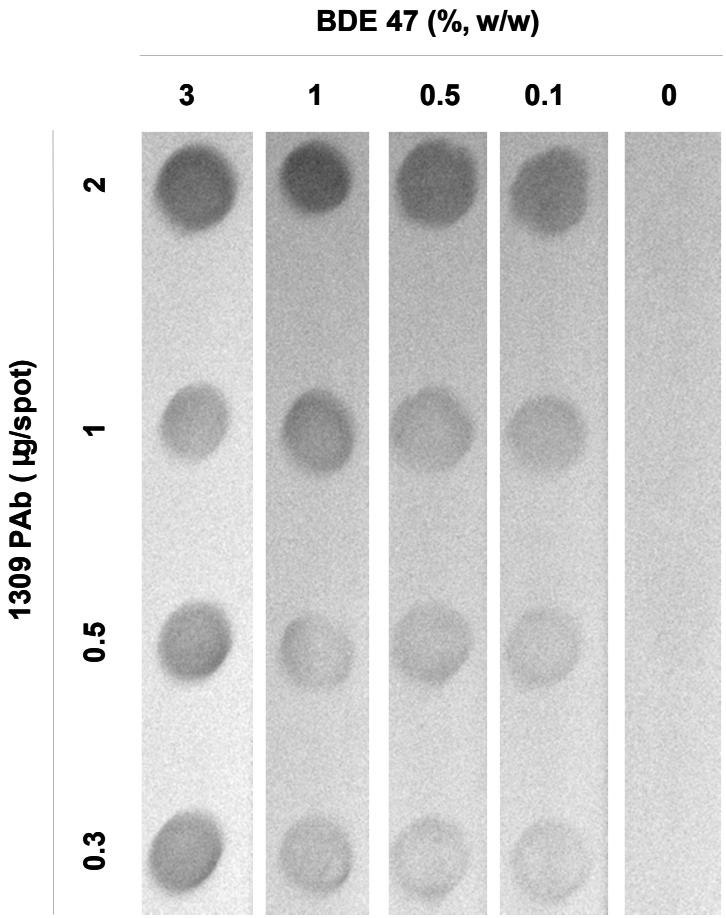
Phage displayed peptides effectively result in sandwich type assays for small molecules.

PHAIAs improve assay sensitivity

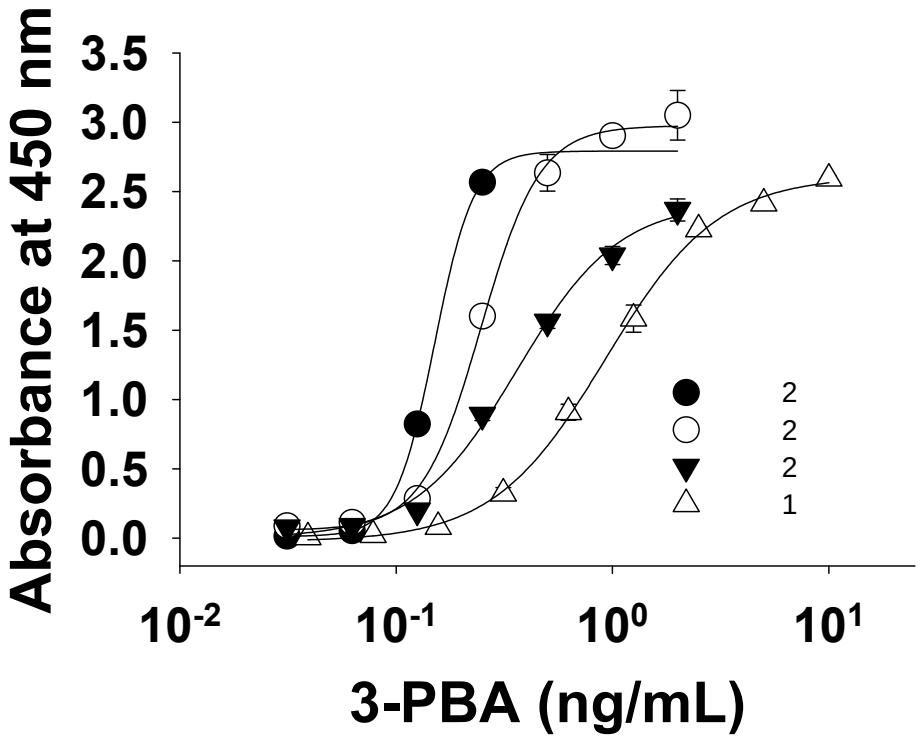
Analyte	Competitive ELISA IC_{50} (ng/ml)	Noncompetitive PHAIA SC_{50} (ng/ml)	Sensitivity Enhancement
Molinate	69.2	5.0	14x
Atrazine	0.64	0.051	12x
Digoxin	2.1	0.22	10x
3-Phenoxybenzoic acid	1.65	0.31	5x
BDE-47	1.7	0.7	2x

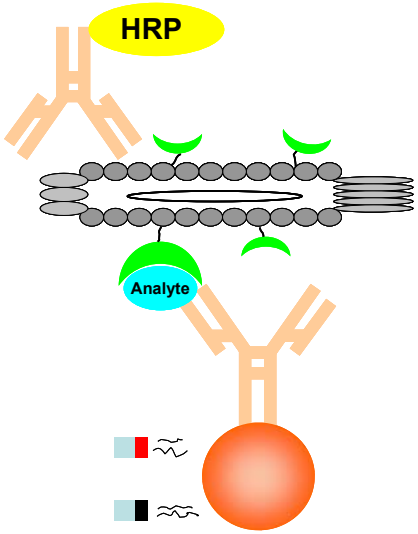
Phage peptide-based dipstick assay



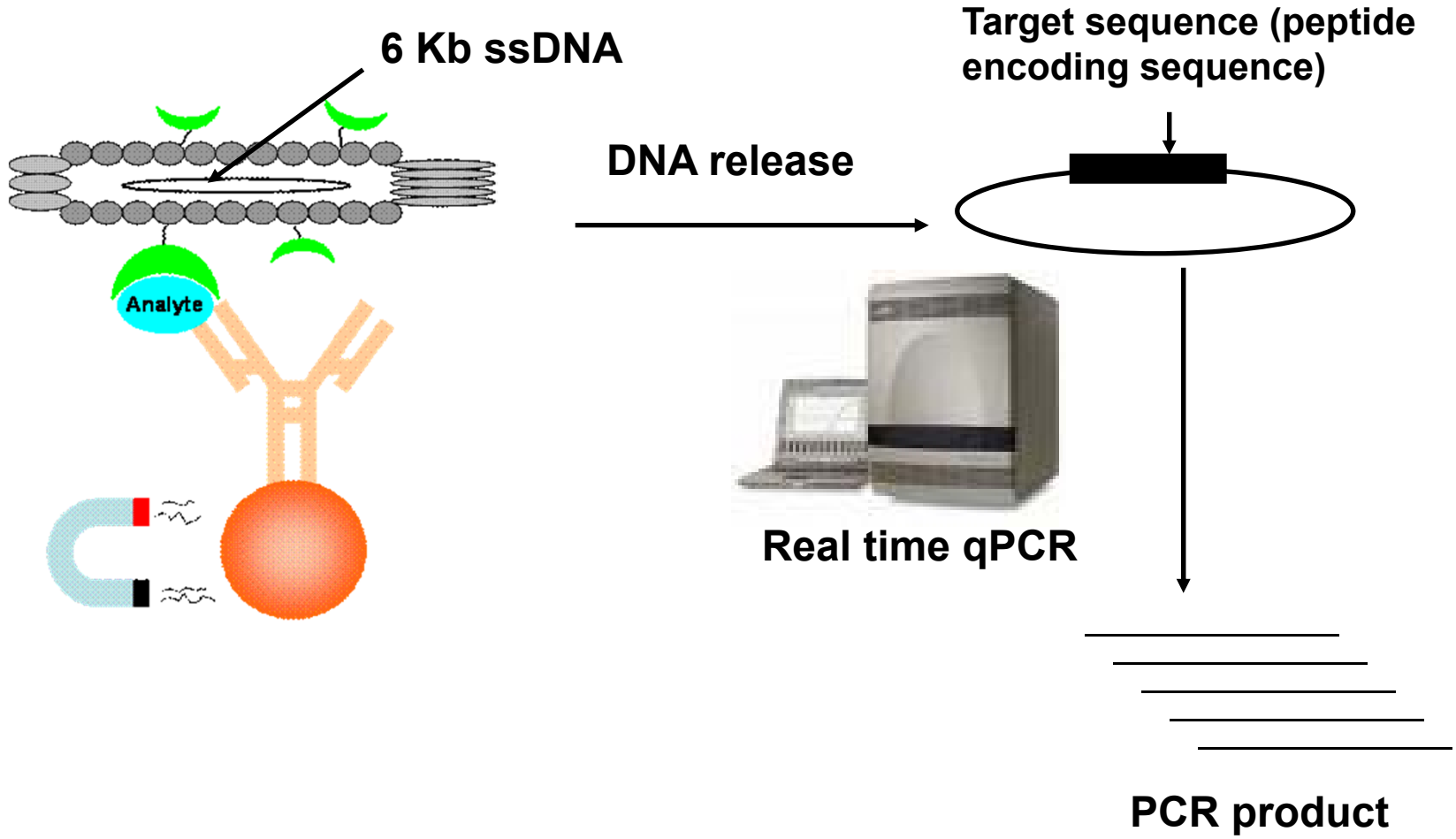


Bead-based PHAIA

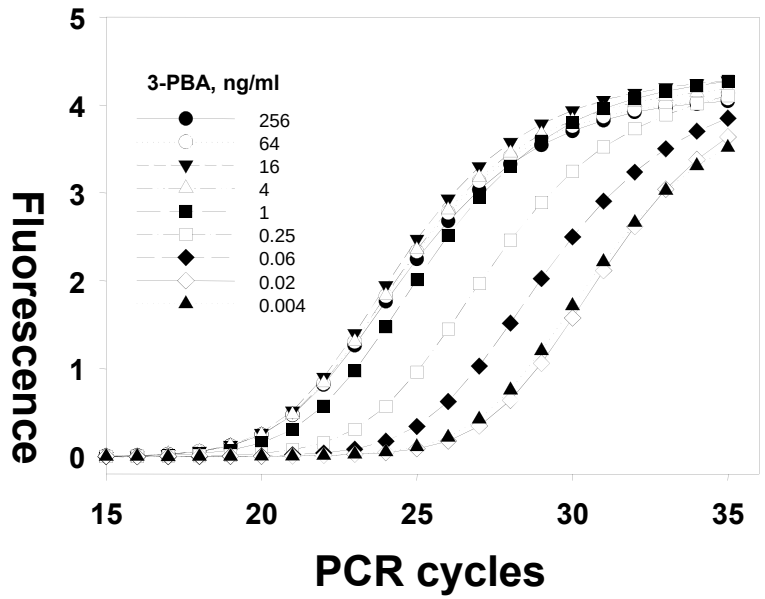


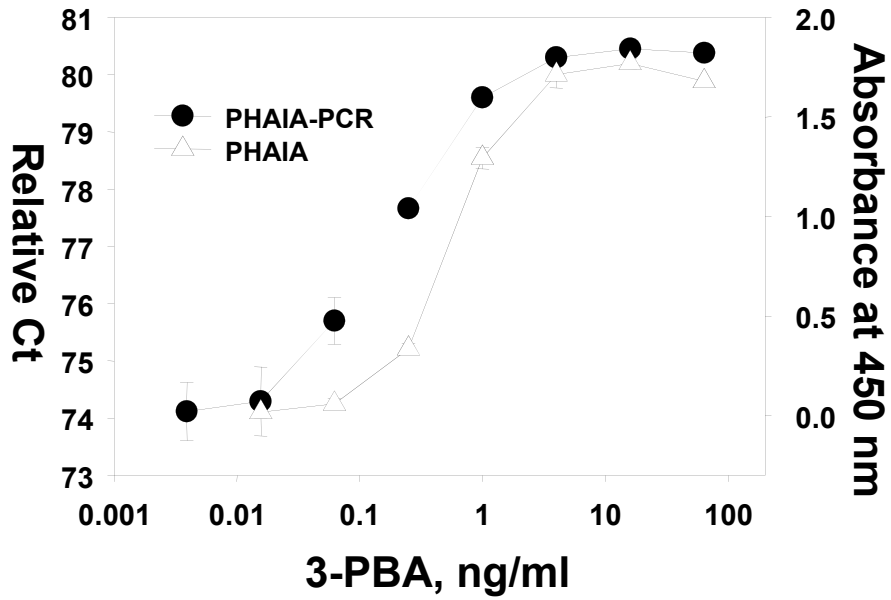


Phage peptide mediated real time quantitative PCR

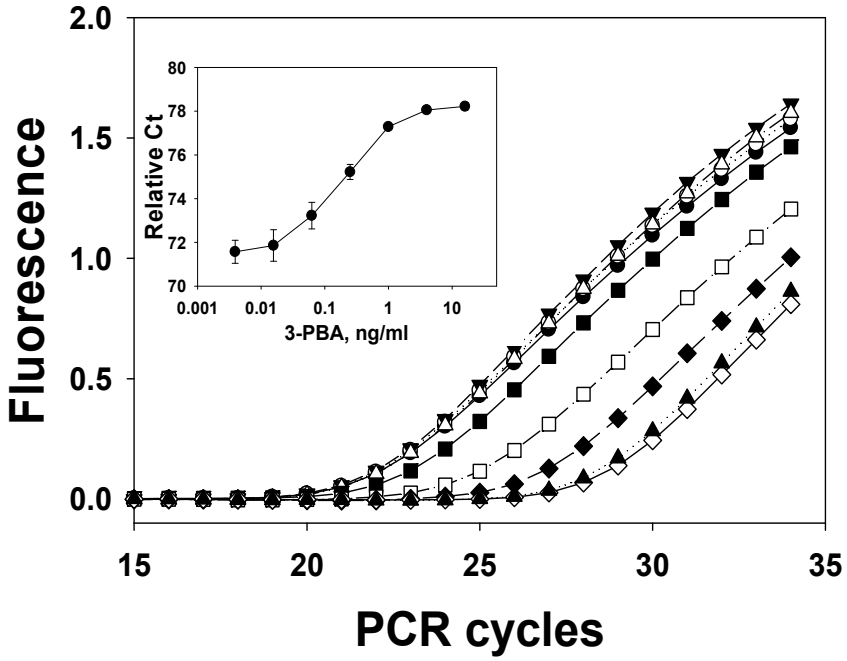


PHAIA-qPCR is more sensitive than PHAIA

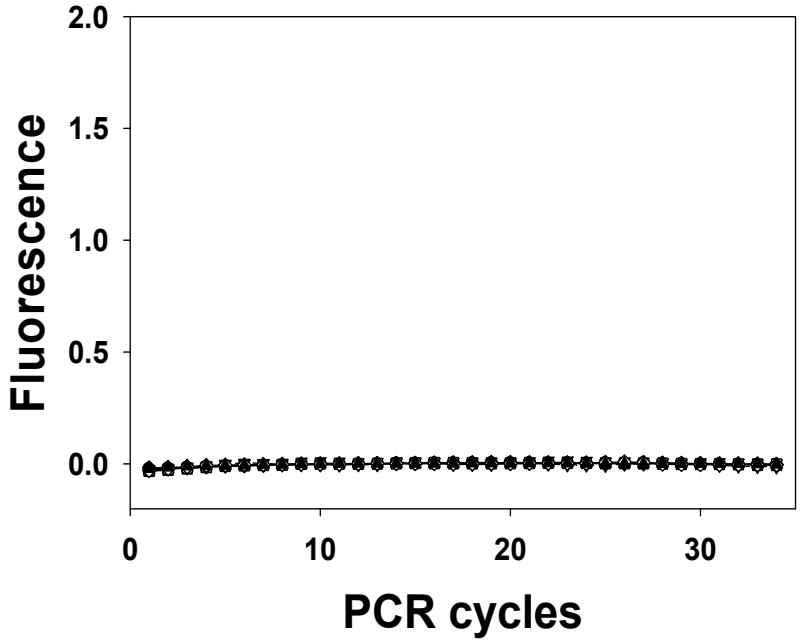




PHAIA-qPCR can be selective



3-PBA



molinate

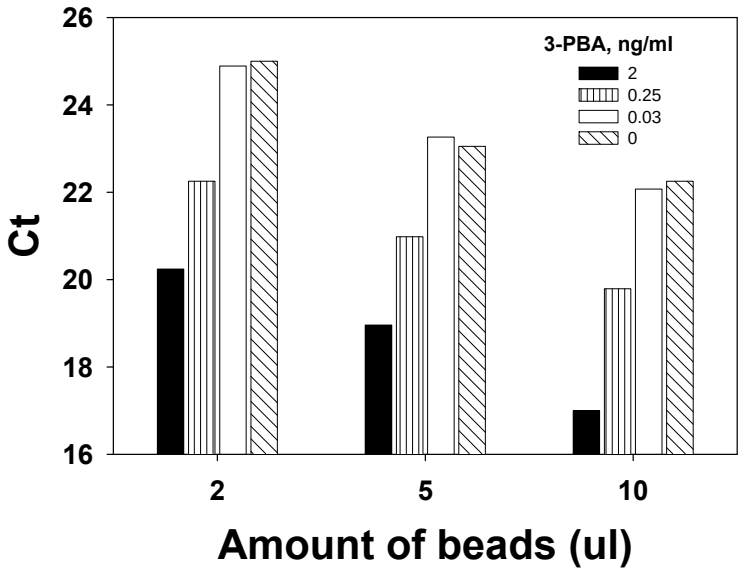
Assay validation

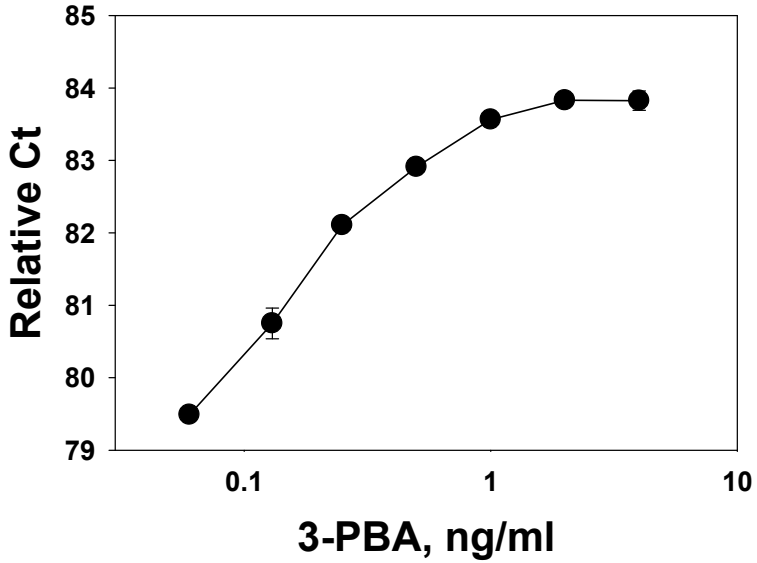
Spiking (ng/ml)	[a]Expected detection after dilution	Run 1		Run 2		CV (%)
		Detection	Recovery (%)	Detection	Recovery (%)	
Recovery of 3-PBA						
50	5	5.0 ± 0.2	100	5.1 ± 0.1	102	3.1
20	2	2.2 ± 0.1	110	2.3 ± 0.06	115	4.3
5	0.5	0.4 ± 0.05	80	0.5 ± 0.04	100	10
2	0.2	0.18 ± 0.02	90	0.2 ± 0.01	100	10

[a]detection level by the PHAIA-qPCR from diluted samples

Magnetic bead-based PHAIA-qPCR

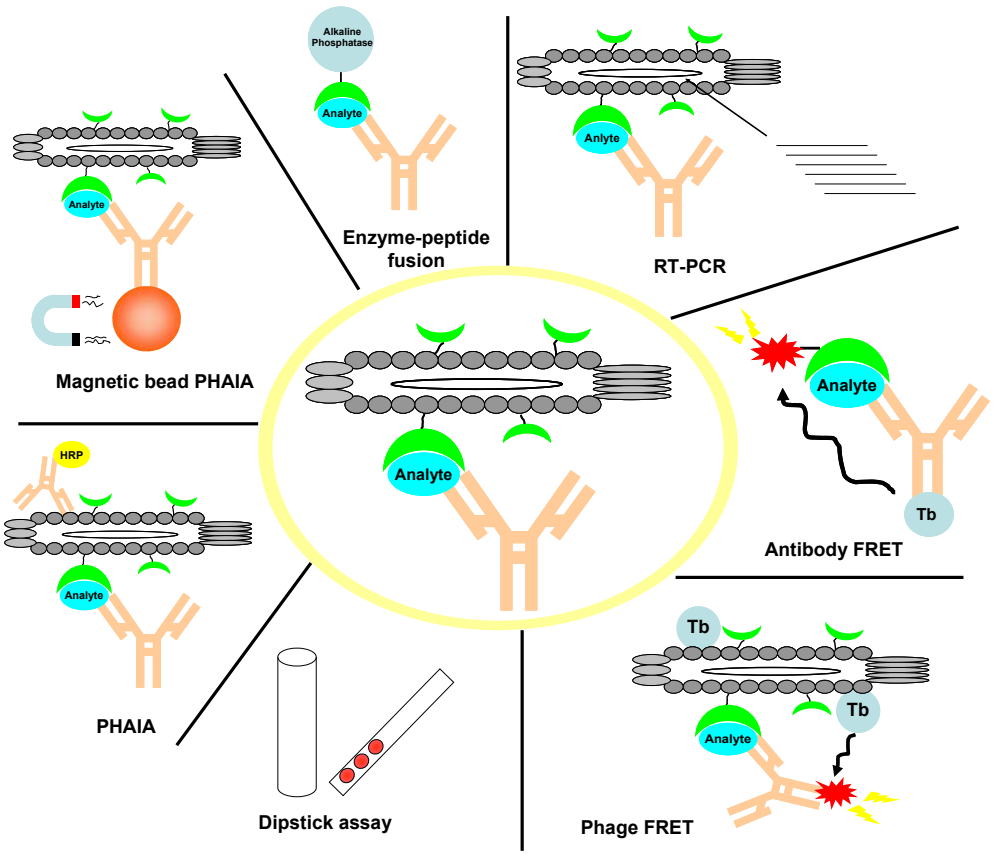
	Bead-based qPCR	PHAIA
Ab coating	Not necessary	1 h at 37 ^{o}C
Blocking	Not necessary when beads are stored in PBS containing 1% BSA	1 h at 37 ^{o}C
Interaction	1 h at RT	1 h at RT
Secondary Ab	Not necessary	1 h at RT
Detection	30 min	15 min
Assay time	1h and 30 min	4 h and 15 min





Phage displayed peptide assays

- All the advantages of ‘sandwich-type’ assays
- Often improves sensitivity compared to competitive assay
- Easy to produce, but requires an antibody
- Multiple assay formats possible



Acknowledgements

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NIH Fogarty International Center TW05718

Resources

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- Additional publications at www.biopestlab.ucdavis.edu/immunoassay/

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