Novel immunoanalytical methods for monitoring levels of environmental pollutants

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Exposure quantification is a critical component of risk assessment, but can be time-consuming and cost-prohibitive. Immunoanalytical methods allow for fast, high-throughput assessment of xenobiotic levels in the environment, as well internal dose measurement. Our group has developed immunoreagents and sensitive assays to target several endocrine disruptors, including polybrominated flame retardants and antimicrobial chemicals triclocarban (TCC) and triclosan (TCS). Short random peptides expressed on a surface of bacteriophage can be selected based on their high-affinity binding to antibody-analyte complex and used for non-competitive phage anti-immunocomplex assay (PHAIA). Bound phage DNA was then quantified by a real-time PCR (PHAIA-PCR), to provide a high resolution assay for 3-phenoxybenzoic acid (3-PBA) a key urinary biomarker of exposure to pyrethroid pesticides. Next, novel single domain antibodies (sdAbs) were developed to target 3-PBA and the herbicide paraquat. Recombinant antibodies are desirable due to low cost and ease of production, but the complexity of mammalian immunoglobulins prevents their expression in bacteria. Camelid heavy chain antibodies naturally lack light chains, but maintain high affinity binding. The variable domains from heavy chain antibodies can be selected using phage libraries and expressed in E. coli. Llama-derived sdAbs against TCC were coupled to 20-50 nm $NaYF_4$:Yb:Tm lanthanide nanoparticles encapsulated in a gold nanoshell to develop a lateral flow portable biosensor. In addition to their robust fluorescent properties, these up-converting nanoparticles are uniquely suitable for direct analyte detection in complex environmental matrices, such as wastewaters, because their excitation at near-infrared wavelengths limits autofluorescence from biological samples. In conclusion, the methods presented here will facilitate identification of hazardous chemicals in situ at Superfund sites and evaluation of human exposure.

RESULTS

Heterologous polyclonal immunoassay for antimicrobial triclocarban (TCC)

In polyclonal antibody-based immunoassays for small molecules, the relative binding affinity of the target analyte can be increased by utilizing relatively low affinity haptens (heterologous haptens) in the competitive assay format. Coating antigen with heterologous hapten was employed to measure TCC levels.

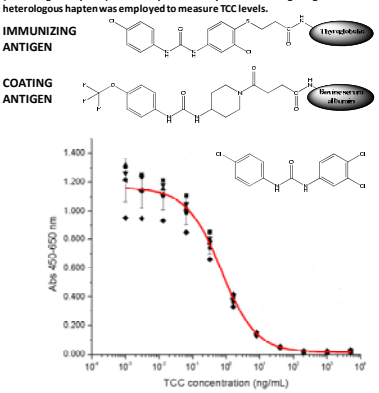

Figure 1. Competitive indirect enzyme-linked immunosorbent assay (ELISA) was performed with a rabbit polyclonal anti-TCC antibody; and with horseradish peroxidase (HRP) and 3,3',5,5'-tetramethylbenzidine (TMB) detection (four parameter curve: IC50=0.69 µg/L).

Non-competitive phage anti-immunocomplex real-time polymerase chain reaction (PHAIA-PCR) for detection of 3-phenoxybenzoic acid (3-PBA)

A library of bacteriophage-displayed random short peptides provides immense binding diversity. The library can be utilized for selection of anti-immunocomplex peptide recognizing antibody with a bound analyte. Phage-displayed peptides can be therefore used for the development of a non-competitive immunoassay for small molecule targets. Bound phage can be quantified by the use of real-time PCR (RT-PCR) with fluorophore-labeled primers corresponding to arabinose promoter or the peptide encoding sequence on the phagemid.

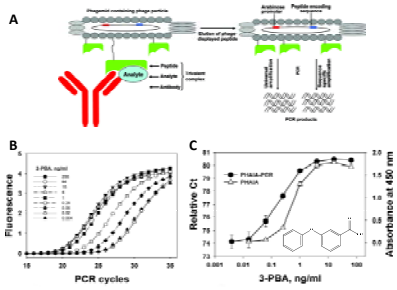

Figure 2. A. Schematic diagram of the PHAIA-PCR. **B.** Phage particles eluted after incubation with various concentrations of analyte were used for RT-PCR with 5'-FAM labeled primers. **C.** Comparison of performance for standard PHAIA-based immunoassay (HRP and TMB detection) and PHAIA-PCR (Ct threshold values) in detection of 3-PBA.

Development of single domain heavy chain antibody fragments (VHHs)

Recombinant antibodies provide distinct advantages, such as ease of production and genetic modification. Camelids, including alpacas and llamas, produce naturally immunoglobulins that consist only of heavy chains, but retain binding affinities of conventional antibodies. Variable domains of these heavy chain antibodies (VHHs) can be expressed on a surface of filamentous bacteriophage and selected for desired binding affinities in the process of panning.

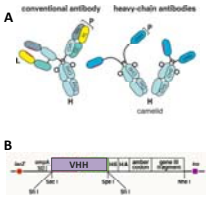

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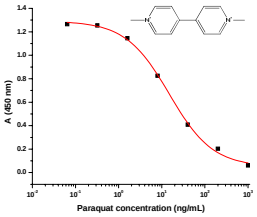
Figure 3. A. The distinction between conventional antibodies and heavy chain antibodies. **B.** A scheme of phagemid pComb3X used for VHH cloning. **C.** The alpaca herd used for immunizations and blood collection.

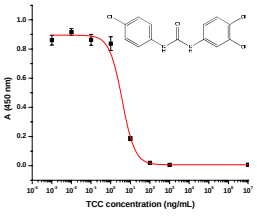
Phage-displayed VHH ELISA for detection of herbicide paraquat

Figure 4. Library of phage-displayed VHHs from B-lymphocytes of immunized alpaca was panned for anti-paraquat VHHs. After 4 rounds of panning, individual clones were screened. Positive clone 2P4-11 was displayed on a phage surface and tested in a competitive indirect ELISA with HRP and TMB detection (four parameter curve: IC_{50}=14.879 µg/L).



Soluble VHH ELISA for detection of TCC

Figure 5. Library of phage-displayed VHHs from B-lymphocytes of immunized llama was panned for anti-TCC VHHs. After 4 rounds of panning, individual clones were screened. Positive clone T7 was expressed as a soluble protein in TOP10F´ *E.coli* and purified. Competitive indirect ELISA with HRP and TMB detection was performed (four parameter curve: IC_{50}=3.798 µg/L).



Gold-shell coated up-converting nanoparticles (UCNPs) for analytical use in complex biological matrices

Nanophosphors containing rare earth metals erbium (Er) and thulium (Tm) absorb in the near-infrared region (975-980 nm) and emit in the UV-visible range, a phenomenon well-known as up-conversion. As a result these robust fluorophores can avoid autofluorescence common after UV excitation in biological materials. Encapsulation of 20-40 nm UCNPs in gold nanoshells amplifies the luminescence ~9.1-fold for the green emission and ~ 6.7-fold for the red emission by surface plasmon resonance.

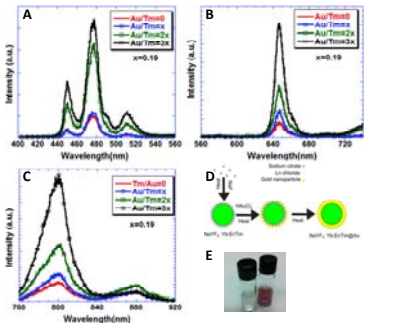


Figure 6. A, B, and C. Enhanced visible and NIR emissions from particles with different levels of gold coating on cubic-$NaYF_4$:20%Yb:1%Tm nanoparticles. All solutions were excited at 975 nm. **D.** A one-pot method to synthesize core-shell photonic material. Citrate was chosen to perform the dual role of stabilizing the phosphor surface and reduce the gold chloride on top of the phosphor. **E.** Colloidal suspensions of nanophosphors.

CONCLUSIONS

- **Sensitive polyclonal immunoassay for antimicrobial triclocarban was developed by utilizing competition with heterologous hapten**
- **Non-competitive immunoassay with phage-displayed anti-immunocomplex peptides and PCR-based detection was optimized for 3-phenoxybenzoic acid , a biomarker of exposure to pyrethroid pesticides, and improved sensitivity was achieved**
- **Recombinant antibody fragments were selected from immunized camelid VHH libraries and developed into assays for triclocarban and herbicide paraquat**
- **Gold-coated up-converting lanthanide nanoparticles were synthesized that provide robust fluorescence with low background in biological samples**

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