Magnetic nano-sensors for sensitive protein detection

Adam Seger, Ph.D.
Electrical Engineer
Nanoengineered Biomedical Devices

• What does it mean?

• What do we need?
  – A biologically relevant problem
  – A technology that can address that problem

• How do we get there?
  – Get engineers together with scientists
ELISA

• Enzyme Linked Immunosorbent Assay
• Method established 1971
  – Replaced radioimmunoassay
• Chromogenic, fluorogenic
• Detect proteins, antibodies, peptides...
ELISA

http://www.epitomics.com/images/products/sandwich.jpg
Giant Magnetoresistance (GMR)

- Discovered in 1988
  - Albert Fert and Peter Gruenberg
- Nobel Prize in 2007
Giant Magnetoresistance

http://www.nims.go.jp/apfim/GMR.html
What Happens When We Combine These Technologies?
We Get MagArray!

**Simple, ultra-sensitive, multiplex immunoassay system**
MagArray System

What do we mean by:

• **Simple** - Small, portable system about the size of a tissue box...No moving parts...No microfluidics... Basic, easy step process.

• **Ultra-sensitive** - Have been able to distinguish concentrations as low as 50 attomolar. Depending on the target protein, typical detection limits are in the femtomolar / picomolar range.

• **Multiplex** - 80 individual sensors per chip. One chip per sample. Factoring in controls and replicates, researchers typically analyze 10-20 proteins per chip.
MagArray History

- Founded in 2005 by team of Stanford professors who adapted a magnetic nanoparticle technology from the computer disk drive industry for use in medical diagnostics
- Focus on market need to better detect proteins and develop biomarker panels
- Basic technology development from 2007-2011 funded primarily by federal grants and contracts
- Partnership with Aisin Seiki in 2011
- Currently located in life sciences incubator in Sunnyvale
- Working with alpha customers on application development
How the MagArray System Works

Sample added to chip with pre-functionalized capture antibodies

Target antigen binds to capture antibody

Detection antibody binds to target antigen

Magnetic nanoparticle binds to detection antibody

Magnetic nanoparticle generates magnetic field that induces electric current in GMR sensor
Real Time, Multiplexed Results
Superior Sensitivity and Dynamic Range

Compared to ELISA, the MagArray system was able to detect CEA at a much lower concentration and across a wider dynamic range.
Flexible Assay Protocols

【Standard Assay】
• About 1 hour
• Excellent sensitivity

【Express Assay】
• < 10 minutes
• Homogeneous Assay
• Can be run with whole blood

【Enhanced Sensitivity】
• Best sensitivity
• Same basic process
• Ideal for low abundance proteins
Standard vs Express Assay Performance

**Standard Curve for cTnI detection:**
**Serum (30min) vs. Whole Blood (9min)**

- **MagArray Signal (ppm)** vs. **Concentration (pg/ml)**
- **blood (10min)**
- **50% serum (30min)**
Minimal Sample Volume

5 µl samples

Ran EGFR/HE4 in Duplicate

Ran Autoantibodies in Duplicate

Extra Vial for Repeats

Diluted 1:100
Wash-Free Assay for Analyzing Cross Reactivity

Add magnetic nanoparticles, then detection antibodies sequentially: anti-EGFR, anti-CEA, and anti-EpCAM
Sequential Addition of Antibodies

Antibodies can be added in a stepwise process to identify and isolate cross reactivity issues.
Advantages

In addition to superior performance in sensitivity, dynamic range, multiplexibility, size, cost, etc., the following unique characteristics are worth highlighting:

• **Real Time Read Out** – Sensors are scanned every 4 seconds, and results are displayed as they are generated, thus allowing researchers to monitor binding as it takes place. Extremely useful for “go/no go” decisions in a fast paced environment.

• **Whole Blood** – Optical interference caused by red blood cells is not a factor for MagArray’s magnetic technology. While sensitivity is slightly less than serum, whole blood can be used as a suitable sample type.

• **Sequential Addition of Antibodies** – MagArray’s open well system allows for detection antibodies to be sequentially added at any stage during the process. Researchers can identify and isolate cross reactivity issues in multiplex assays, as well as design assay procedures to circumvent certain cross reactivity problems.

• **No Wash** - MagArray sensors only detect magnetic particles that have bound to the analytes of interest. There is no need to wash after the addition of label. This is of particular advantage when analyzing low affinity proteins that tend to get washed away during typical assay procedures.

• **Small Sample Volume** - Assays can be run using as little as 10-20ul. MagArray’s system allows researchers to run multiplex assays on minute samples from small animals such as mice or rats. It is also extremely valuable for limited human samples that cannot be readily re-obtained.
Notable Papers

Matrix-insensitive protein assays push the limits of biosensors in medicine
Richard S. Gaster1,2,3,11, Drew A. Hall3,11, Carsten H. Nielsen4,6, Sebastian J. Osterfeld7,8, Heng Yu5, Kathleen E. Murphy9, Robert J. Wilson10, Boris Mermann3, Joseph C. Liao3,10, Sanjiv S. Gambhir4,3,10 & Shan X. Wang5,7,10

Quantification of protein interactions and solution transport using high-density GMR sensor arrays
Richard S. Gaster12, Liang Xu12, Shu-Jen Han12, Robert J. Wilson2, Sebastian J. Osterfeld8, Heng Yu12 and Shan X. Wang12

Multiplex protein assays based on real-time magnetic nanotag sensing
Sebastian J. Osterfeld1,2, Heng Yu2,1, Richard S. Gaster2, Stefano Caramuta2, Liang Xu2, Shu-Jen Han2, Drew A. Hall2, Robert J. Wilson2, Shouheng Sun2, Robert L. White2,4, Ronald W. Davis2,4, Nader Pourmand2,4,6,2 and Shan X. Wang2,4,6,2

Autoassembly Protein Arrays for Analyzing Antibody Cross-Reactivity
Richard S. Gaster,1,† Drew A. Hall,3 and Shan X. Wang*1

Sensitive giant magnetoresistive-based immunoassay for multiplex mycotoxin detection
Andy C. Mak2,8, Sebastian J. Osterfeld5, Heng Yu4, Shan X. Wang5, Ronald W. Davis2, Olufisayo A. Jejelowo6, Nader Pourmand2,8