



U.S. Department of Health and Human Services
National Institutes of Health



**National Heart
Lung and Blood Institute**

NHLBI Proteomic Centers

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Proteomics Working Group

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Moving from Genomics to Proteomics

- Where are the gaps
- What are the major challenges
- What can the NHLBI do now

Main Recommendation

- Technology Development – and its application



Goal of the NHLBI Proteomics Initiative

- **Highly Interactive, Multi-Disciplinary Centers**
- **Couple Technology Development with Biology in Order to Facilitate Application**
- **Foster Blue Sky approaches in Proteomic Technology Development and it's application**



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Lung and Blood Institute

NHLBI Proteomics Initiative



<http://www.nhlbi-proteomics.org/>



Biological Applications

Oxidative Stress in Cardiovascular Disease
Cardiac Hypertrophy
Vascular Biology
Blood Pressure
Cystic Fibrosis
Auto-Immune Diseases
Ischemic/Hypoxic Disorders

Cardiovascular Development
Insulin Resistance
Hematopoiesis
Sleep
Macrophage Biology
Immune Signaling
Inflammation of the Airways

Technology Development

Protein Abundance
Protein Profiling
3-D Proteomics
Free Flow Electrophoresis
Mass Spectrometry Analysis of Complex
Mixtures
Flow Cytometry
Microcapillary-based Secreted Protein
Detection
Array-based Antigen Profiling

Protein-Protein Interactions
Modification Detection
2D Separation Technology
Microfluidic Devices
Mass Spectrometry of Intact Proteins
Modeling of Proteomics Systems
Protein Detecting Microarrays
Synthetic Capture Agents



Interactions, Communication, Dissemination

- **NHLBI Proteomic Web Site**
- **NHLBI Proteomic Investigator Meetings**
 - Twice a year: April and September
- **NHLBI Proteomic NewsSpots**
 - Highlights of activities in the Centers
- **Resources, Tools, and Training**
- **Meetings**



<http://www.nhlbi-proteomics.org/>

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PROTEOMICS

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NewsSpots
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Department of Health and Human Services

Welcome

The National Institute of Heart Lung and Blood has established a consortium of 10 highly interactive, multi-disciplinary Proteomic Centers to enhance and develop innovative proteomic technologies and apply them to relevant biological questions that will advance our knowledge of heart, lung, blood, and sleep health and disease.

This NHLBI Proteomics Initiative is intended to complement and enhance the NHLBI's ongoing research programs, which include a substantial investment in clinical research, genomic research, basic biology, technologies, and training and education programs.

 **"Visit the ProteomicsPortal!"**

Read about Yale University's molecular tools and protocols for RNA and DNA aptamers in the [Winter/December NewsSpots](#)



Test Tube Evolution Cycle



<http://www.nhlbi-proteomics.org/newspots/>

NewsSpots

Volume 3, Issue 3, Fall 2006



UTMB CHIP CAN IDENTIFY LOW ABUNDANCE TRANSCRIPTION FACTOR PROTEINS

Thioaptamers are nucleic acids containing randomly selected thiophosphate backbone substitutions that show enhanced affinity, selectivity and nuclease resistance relative to unmodified aptamers [1]. In the past several years, scientists at the University of Texas Medical Branch in Galveston (UTMB) have identified a number of thioaptamers against a broad range of proteins, including transcription factor proteins such as NF- κ B and AP-1, the RNase H domain of HIV reverse transcriptase and viral envelope proteins.

In collaboration with CIPHERGEN Biosystems, UTMB scientists have anchored one of the AP-1 thioaptamers, XBY-S2, on ProteinChip Array surfaces to screen and identify bound proteins by mass spectrometry. The team has successfully directly identified specific "on-chip" captured proteins from pre-fractionated crude nuclear extracts by direct coupling a thioaptamer XBY-S2 to the pre-activated ProteinChip array surfaces. Five hnRNP proteins have been identified [2].

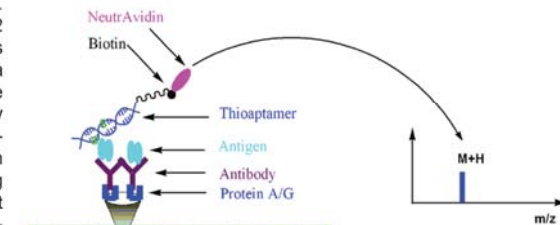
While the XBY-S2 thioaptamer was developed as a decoy to a subset of AP-1 proteins, the team did not observe any AP-1 proteins in the "on-chip" capture and digestion protocol described by Wang et al. [2]. They believe that the higher cellular concentrations of the hnRNP proteins relative to the AP-1

transcription factors limited the identification of any AP-1 proteins. In order to detect AP-1 proteins that bind to the XBY-S2 thioaptamer, they developed an alternative "on-chip" sandwich assay (see figure). They have shown that XBY-S2 binds to AP-1 proteins CREM-1 and Fra-1 while control oligonucleotides with scrambled sequence and poly(IC)7 with biotin tags do not.

This demonstration of highly specific "on-chip" protein identification by a novel antibody/thioaptamer sandwich assay shows great promise in further developing specific proteomics arrays capable of identifying (and ultimately quantifying) low-abundance proteins such as AP-1 transcription factors [2].

References

1. Yang, X, Gorenstein DG. Progress in thioaptamer development. *Curr Drug Targets* 5: 705-15, 2004.
2. Wang H, Yang X, Bowick GC, Herzog NK, Luxon BA, Lomas LO, Gorenstein DG. Identification of proteins bound to a thioaptamer probe on a proteomics array. *Biochem Biophys Res Commun* 347: 586-93, 2006.



Schematic view of biotinylated thioaptamer and immuno-captured protein interaction detected by NeutrAvidin protein signal.