Analysis of Future Biodetection Systems

Biodetection Sampling Systems 2
Spectroscopy Systems 4
Systems Integration 7
DNA Based Detection Technologies 11
Ligand Based Technologies 14
Transduction Systems 18

This report is for the Future of Biodetection Workshop, held in Santa Fe, NM, on September 26 & 27, 2006. The report is based on the facilitators' notes and those of note takers who were present during breakout sessions. We have made an attempt to capture all of the points made by the group.

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Analysis of Future Biodetection Systems

Biodetection Sampling Systems
Session Lead: Greg Kaduchak
Presenter: Gary Long

Summary
The session was a follow up to a sobering talk given by Dr. Gary W. Long, Tetracore, Inc.. The talk addressed a brief review of the state of technology in sampling for biodetection with a vision of where the technology can be in 5-10 years. Dr. Long then led this discussion session dedicated to stimulate conversation concerning sampling issues.

The session began with concerns about the breadth of the sample collection problem:

- Sample to warn or diagnose
- Sample recovery vs. detection technique
- Sample preservation
- Purifying sample of inhibitors / interferrents
- Sample matrix

The group then focused on the question of standards. There are standards for detectors, but a general lack of standards for sampling. Labs have different protocols and get different results. A biodetection roadmap would identify a gap in the area of sampling standards for better quantification of detection thresholds. But, standards are customer driven and application specific.

The conversation then shifted to autonomous sensing. Topics of discussion included:

- How much sample is needed and how secretly do you need to do it?
- How can you separate the target material from its surroundings (soil, fecal matter, etc.)
- Ideal sampler would need to be small and be able to separate material, lyse and release DNA from a droplet, etc.
- How could we get to that point?
- How could we make it affordable?
- Different media

The next key thoughts included sample preparation with relation to enhanced detection. Better sample purification steps can produce samples that do not require culturing for detection.

Next, the group discussed combined sampling/detection (surveillance) through living organisms. What about looking at an organism's response (host-pathogen relationship) as a way to a detection mechanism? What you're looking for is a canary – a detector of anything unusual. In the absence of sophisticated detection technology, is surveillance of infected organisms a more efficient and cost effective means of detection.

Back to the standards issue, the group addressed the question of 'what is dangerous?':

Analysis of Future Biodetection Systems

- What concentration of e. coli, etc.
- What about who is eating it.
- How infectious is each organism (different)….
- How do we assess the risk?

Reality is that we don't exactly know how to deal with and detect the threats we already know about and understand, let alone new engineered threats.

In the end it was found difficult to separate discussion of the complexities of detection and sample collection due to the diversity of the problem set.

Spectroscopy Systems

Session Lead: José Olivares

Presenter: Luis Garcia-Rubio

Summary

Dr. Luis Garcia-Rubio, U. South Florida, made a presentation on aspects that need to be considered and can be extracted from spectroscopy based sensors. These include detection of chemical and physical markers of organisms. The presentation then focused primarily on the use of spectrophotometric techniques for identification and classification of organisms, although the conclusion was that no one technology will be able to provide all of the properties that would be desirable. Dr. Garcia-Rubio's vision was that through miniaturization of spectroscopy based systems, multiple systems can be brought to bear on a particular problem with orthogonal capabilities making the identification and classification much more reliable.

During the breakout session, the group primarily focused on discussing the vision and gaps to spectroscopy based systems by answering five primary questions.

1. ***Are there areas of technology that need to be added to the vision of the speaker?***
    - Miniaturization – was identified as the main vision that the speaker brought forth and the power which miniaturization brings by combining multiple analytical systems in one platform.
    - Trigger based detectors – the primary contribution that spectroscopy based sensors will have in biodetection will be as triggers in a system.
    - Identification systems – the power of physicochemical properties in the identification of organisms was pointed out.
    - The value will come from the use of multiple spectroscopy techniques which in conjunction are able to provide a more definitive and reliable identification than any one single technology. These could include such technologies as mentioned in the presentation (mass spectrometry, infrared spectroscopy, raman spectroscopy, multidimensional fluorescence, and UV-Vis spectroscopy) and others identified by the workgroup, e.g., photoacoustics, multiphoton spectroscopy, NMR, ESR and other technologies to enhance or provide multiple spectral recognition systems.
    - Adding other hardware, e.g., tunable lasers, along with the spectrophotometric technologies described by the speaker would add more versatility and power to the technology.
    - It was agreed that the power of multidimensional spectroscopy, through multispectral spectroscopic analysis, remained to be properly exploited for biodetection systems.
    - The addition of surface based nanotechnologies to enhance signals and add specificity to the analysis would add multidimensional capabilities to these types of sensors.
    - These systems could be used to look at the systemic response of the human bioresponse; as a canary that can be monitored.

- Recognition and differentiation of virulent strains based on host responses could be measured using spectroscopy based systems.

2. ***Is/are the vision(s) paradigm shifts, disruptive technologies, or incremental improvements to current technology?***
    - The group felt that the hardware is not a paradigm shift or disruptive technology for UV/Vis and other traditional spectroscopy systems.
    - But the algorithms used to extract information for classification from the spectra can be new disruptive technology.
    - Certainly, hardware miniaturization can be disruptive and leads to low cost instruments that can be used in sensor networks.
    - Integration of these spectroscopy based technologies with nanotechnology and chemistry in order to provide high specificity and sensitivity could be disruptive.
    - Miniaturization and cost reduction for other technologies such as mass spectrometry could be equally as powerful.
    - Similarly addition of other key hardware components, e.g., optical fiber technologies and fixed filter systems in front of the spectrometer, can add new capabilities to these technologies.
    - Dual applications of the technology beyond defense applications, to point of care and clinical applications (as examples) are key markets that these technologies will need to move into for success.

3. ***What gaps exist in order to fulfill the vision(s)?***
    - The physico-chemical properties and characteristics required for identification and classification of an organism need to be further understood. This understanding will lead to spectral properties that can be associated with phenotypic characteristics for classification and acceptance by the community.
    - It was not known as to how far these technologies can be used in order to address other questions of interest to bioforensics, e.g., where was the organism made, how was it made, by whom, etc.
    - Addition of orthogonal spectroscopy based technologies and specific ligands remained as untapped areas that could enhance these types of sensors.
    - Tailoring of these technologies towards applications in health care vs. defense presents different types of scenarios and fielding (conops) questions which will need to be addressed. Yet, there may be overlap in these that can be leveraged. As examples of paradigms in applications where the technology can be applied but all of the implications and overlaps not well understood are in home health care and air sampling of "sick buildings".
    - As with all such systems, determination of what is a feasible cost that any particular market sector will bear will remain a major issue that needs to be addressed, is a reasonable cost to the consumer a $10-20 system or should it be $100-1000?

4. ***What major breakthroughs in science and basic research need to be made?***
    - Multiple areas for future research and development where identified by the group. These include:
        - Systematic study of spectral signatures of organisms and backgrounds
        - Multidimensional / multispectral studies
        - Spatial resolution within the internal structure of the organisms
        - Time domain – response to the environment of organisms which the technology will need to be sensitive to
        - What matrix characteristics need to be understood, exploited, or removed
        - Coupling of spectroscopy systems with specific ligands, functionalized materials, e.g, toxin detection
    - Other areas that will need to be addressed are better associated with the implementation of such technologies, such as:
        - The sensitivity of the detection system. Which is dependent on question being asked, background, building, host response
        - The design features that need to be added to the sensor dependent on the application, e.g., sampling capabilities, and requirements for calibration and standardization in the field
    - The common problems where most or all elements of a system have dual use applications that can drive economy of scale and efficiency.
        - Detection to treat (public health)
        - Detection to warn (public security)
        - Detection for prosecution (law enforcement)
        - Detection for intent (intelligence)
    - The features and technological achievements that will "bring the technology to the individual level"

5. ***What resources/investments are needed to fulfill these gaps?***
    - The development of interdisciplinary teams of technologists, and teaming between users, technology developers and policy makers will be key to making any of these technologies successful in their intended applications.
    - Investments in dual use systems will make the technology pull much faster and successful in acceptance. This will require education in areas of overlap for technology development and application.
    - Finally, changes in regulatory approval system will need to be implemented for non-traditional systems to make it into the public sector market. In the mean time the use the veterinary market as a back door to achieve development is seen as an alternative. This will need to be matched with education or understanding of quality requirements through out the development cycle of any of these technologies.

Analysis of Future Biodetection Systems

Systems Integration

Session Lead: Kristin Omberg

Presenter: David Cullin

Summary

The Systems Integration session was focused on the development of end-to-end systems for the detection of biological agents. While advances in detection technologies are necessary to improve current biodetection systems, they are not sufficient in and of themselves; these technologies must be incorporated into a coherent system, including software and hardware components and concepts of operation, to fully meet end-user requirements.

Plenary Session

Dr. David Cullin gave the plenary talk. He began by presenting the context for deriving biodetection system requirements: namely, the threat they are designed to mitigate. Over the past two decades, this threat has changed substantially. Twenty years ago, the United States was primarily concerned about the Soviet Union. The threat was reasonably well defined in terms of agents of concern, potential delivery systems, and instances for use. The current threat, however, is poorly defined. The list of potential agents is evolving rapidly; a wide range of possible delivery systems can be used; and use of biological agents can no longer be restricted to specific scenarios. The design of a coherent system to address the current threat is significantly more challenging than in the past.

Many biodetection systems in use today were designed under the old paradigm. While they may be adapted for use under current threat conditions, they are not ideal. Dr. Cullin presented a diagram showing data sources and flows in the current DoD and DHS biodetection systems. Included in the diagram were potential sources of error. DoD and DHS require high levels of confidence in their biodetection systems. To mitigate the effects of sources of error, current concepts of operation involve bundling detector data together with additional data streams, such as medical surveillance. A final decision on a biodetection event therefore typically involves multiple data sources, only one of which is the detector itself. Dr. Cullin noted that this produces two potential paths for improving the performance of biodetection systems: the detector technology can be improved to reduce potential error in the detector data; or data fusion technology can be improved, to reduce potential error in the overall system.

Dr. Cullin then summarized the current DoD Guardian and DHS BioWatch systems, including their general systems architectures and operations. He also addressed future technology roll-outs, such as the DHS BAND program. He stressed the importance economics have had in the deployment of these systems, and the pressure that will be imposed on future systems to minimize operational costs.

Dr. Cullin also discussed the particular application of facility monitoring for biological agents, why it is important, how it may be leveraged in overall urban systems, and the key issues involved in monitoring high profile buildings. He presented a summary of the

National Research Council's report on facility monitoring, and introduced a diagram for how their recommendations could be implemented in a cost-effective fashion.

He concluded by discussing potential areas for improvement in biodetection systems technologies. Detection, identification, and post-event sampling are all critical areas where improvement is needed, as is data fusion. As a final point, he reminded the audience that unless a biodetection system is "rugged and reliable," it is unlikely to be successful in a field deployment.

Breakout Session

The breakout session began with a discussion of the technologies deployed in current systems. In both the current DoD and DHS systems, collection, detection, and identification are three distinct steps. Collectors are employed to concentrate aerosol samples onto media which are taken to a laboratory for preliminary and confirmatory analyses using polymerase chain reaction (PCR) and immunoassays.

Coherent design of a biodetection system is contingent upon well defined requirements for the system, or alternatively, development of a process by which to define requirements. The breakout session discussed, in general terms, what a biodetection system should do. There was consensus that system requirements are not widely known. DHS and DoD have elucidated requirements for their existing and next-generation systems, but the audience was not familiar with them. After the breakout session, several participants suggested LANL hold a series of internal meetings after the completion of the workshop to familiarize laboratory staff with programs that are defined and ongoing in this area. There was, however, disagreement as to whether requirements for new biodetection systems should be contingent upon existing programs, or formulated *a priori* with involvement from stakeholders, including potential sponsors and the public health community (the intended end-user).

The session participant concurred that system requirements should be based on risk. Agents detected, dissemination methods, and media monitored should be determined based on a risk assessment, such as those that are currently underway at DHS and have been completed by DoD. There was not widespread familiarity with these risk assessments in the breakout; this is another potential area for internal education.

As part of the development of system requirements, the breakout discussed three potential timescales of action for a biodetection system. "Detect to treat" systems, like those currently in use by DHS and DoD, provide information on a biological event in time for prophylactic treatment of the exposed. "Detect to warn" systems, which are not currently mature enough for widespread deployment, provide information on a biological event in time to warn those in the area that an event has occurred, and allow them to take measures to prevent or minimize exposure. "Detect to prevent" systems, which are largely theoretical, would detect prior to the dissemination of agent, and could be used to prevent the dissemination event entirely.

Analysis of Future Biodetection Systems

If the goal of a biodetection system is to inform public health, and provide sufficient information for them to take public health actions (e.g. deployment and administration of countermeasures, quarantine, etc), there was general consensus that the system should help the user figure out what's really happening. This is a gap in current systems, which often provide incomplete information and are unable to differentiate between a naturally occurring event and a deliberate release. The system should also help with attribution. There was general consensus that the system should be an integrated, end-to-end system that provides the user with a means to do detection, response, and attribution, analogous to Ebay, which allows users to find an item, chat with the seller, and purchase it in a simple and transparent fashion.

The concept of dual-use biodetection systems was discussed several times. Dual-use systems could be used to detect a terrorist attack as well as a naturally occurring outbreak. Dual-use is an attractive concept, as it provides users with a routine use for the system and can be used to leverage some of the costs. However, there has been very little research done to determine whether dual-use is a realistic concept. While it certainly should be strongly considered, dual-use is only practical if it can be accomplished without adversely affecting the performance of the system.

A final discussion in the breakout session touched on the issue of standards. There are currently no standards in place for biodetection systems. The breakout session was divided on whether there should be one set of standards for all systems, or whether this might be counterproductive. It would be useful for all customers to be able to share information across disparate biodetection systems; however, this must be accomplished without degrading the performance of the system relative to the users' specific needs.

Systems Integration Synopsis

- Gap: Definition of what a biodetection system should do (requirements)
    - Can/should this be defined by current federal programs?
    - Can/should this be defined in consultation with public health?
        - Have to include the end-user

- Goal of a biodetection system: "Public Health Actionable Information"
    - Detect to treat: state of the art
    - Detect to warn/protect: future goal
    - Detect to prevent (pre-attack)

- Current state of the art: evidence marshalling (combining environmental, medical, etc.)
    - Gap: Current systems are unable to differentiate between naturally occurring and deliberate releases
    - Gap: Dose-response tie to system requirements
    - Gap: Viability

Analysis of Future Biodetection Systems

- What should a biodetection system for public health include?
    - Risk-based system design
        - Gap: Risk communication
    - Dual use
        - Gap: Can you combine the two in an effective manner?
            - Can we think of creative ways to combine them?
        - Benefit: Gets public health people used to the technologies
        - Also addresses economic issue
    - Should be able to help you figure out what's really going on
        - Should be able to differentiate between terrorist attack and natural outbreak
        - Attribution
        - Gap: Data fusion and analysis
            - Gap: Understanding what information is relevant and figuring out how to make information better
    - One system that does it all, end to end—detection to response to attribution
        - Ebay
    - Gap: Standards
        - Can you standardize across chem/bio/rad? Should you?
        - Information sharing architectures

DNA Based Detection Technologies

Session Lead: Hong Cai

Presenter: Stephen Apatow

Summary

The DNA Based Detection Technologies discussion panel comprised of ~ 20 experts from a well diversified academic institutions, industrial entities and state/government agencies. The majority of them are experts in human and veterinary clinical diagnosis. The rest of panel is experts and specialists in biotechnology development, domestic and international law and policy development. The key consensus points reached in our discussion panel are:

(1) Biodefense must encompass the public heath surveillance component – a global biodefense surveillance and reporting network has to be formed to be meaningful to the biodefense mission.

(2) A meaningful global biodefense and surveillance network comprises of two complementary portions, human and veterinary surveillance, and environmental surveillance. The current health care management system must undergo a fundamental and conceptual paradigm change to accommodate the point-of-care human and veterinary DNA-based molecular diagnosis. The federal government has to be willing to complement private effort and stand on top of the environmental surveillance effort.

(3) The technologies for an affordable, rapid, multiplexed and field nucleic acid-based test must be developed for the global biodefense mission. More specifically, the technical components, including rapid isothermal amplification technologies (or alternative signal amplification strategy), rapid and multiplexed detection platforms, rapid and efficient nucleic acid extraction methods, have to be developed for implementation in the global surveillance and biodefense network.

(4) The pathogen molecular signatures as well as their natural background have to be understood for data interpretation and subsequent management algorisms have to be developed and assessed for their efficacy and impact on public health.

(5) The development of affordable dual use DNA-based detection technology is the only financial feasible mechanism to keep the surveillance working alive and meaningful for long term.

(6) The standardization of detection protocols, licensing policies, reporting system across different agencies and nations must be achieved for a global surveillance network.

(7) All the technology development must have a viable commercialization path for final implementation. Too many technologies have been fallen into the "valley of death" due to the lack of the clear vision and realistic market assessment prior to development.

(8) Active communication among policy makers, technology developers and clinical specialist is critical for future biodefense network establishment and implementation.

(9) The threat and impact of the engineered biothreat has to be understood for any pandemic and biothreat defense.

Technology Gaps

- Rapid, affordable and highly sensitive home-usage vs reference lab-use nucleic acid tests must be developed.
- Faster, cheaper, better, more for less (multiplexed detection), field-use, easy-to-use nucleic acid-based technology has to be developed. The majority of current molecular testing is done in reference or research laboratories due to the need for sophisticated instrumentation and highly trained personnel.
- An efficient, instrumentation-free nucleic acid extraction and clean up method has to be developed to provide highly pure nucleic acid for amplification and detection.
- A rapid, specific and highly efficient amplification strategy has to be developed to replace PCR and eliminate thermal cyclers. All current isothermal amplification methods, such as SDA, TMA, are slow and inefficient.
- An affordable, sensitive, instrumentation-free and multiplexed detection array method must be developed for nucleic acid detection.
- An integrated, inexpensive and stand-alone device has to be developed for global surveillance, preferably for home-use, to track human and animal diseases.
- A comprehensive, rapid, inexpensive and high-resolution nucleic acid array with molecular signature extraction algorism should be developed for reference laboratories and WHO for rapid and accurate assessment of disease (engineered and naturally occurring).
- A bioinformatic signal extraction algorithm has to be developed for timely assessment of disease progression globally.
- Alternative amplification-free technologies should be developed for nucleic acid-based detection. Examples includes, single molecular detection, aptamer development, rapid signal reporting systems, etc.

Knowledge and Policy Gaps

- Technology transfer from research labs to point-of-care
- Lack of funding
- Lack of universal standard for validation, FDA-, USDA-, OIE-, WHO-approved, approval for use in different countries
- Lack of understanding of engineered biothreats
- Lack of communication between end users and R&D people (resulting in not the right product)
- Lack of understanding the concerns and needs from public health and biodefense communities (e.g. dual use technologies that do not meet the needs of either party)

- Lack of assessment of pros and cons of point-of-care vs. home-based diagnosis (liabilities, benefit)
- Lack of sense of impact of global technology
- Lack of understanding of how to use the data (e.g. expression microarray data, signature extraction, relevance to the disease prevalence)
- Global reporting capability during outbreak (US vs world, individual vs reference labs)
- Point-of-care assays and immediate responses (treatment)
- Paradigm change in the health care system (cost and benefit justification)

What major breakthroughs in science and basic research need to be made?
- Understanding of background (animal and human, in different countries)
- International collaboration policies
- Basic concept and paradigm change of health care system (overall outcome management, cost management model), change of paradigm of human and animal care systems
- A good animal and environmental sample preparation method (purity, recovery)

What resources/investments are needed to fill these gaps?
- Development of affordable, easy-to-use, rapid nucleic acid assays for home use and reference lab use
- Technology development in nucleic acid extraction (clean up enzyme inhibitors), isothermal amplification, multiplexed, dual use detection platforms
- Molecular signature development (background signature development included)
- Bioinformatic algorithm for signature extraction
- Provide technology maturation funds to decrease the cycle time of product development (tech transfer funds)
- Provide assistant for clinical validation and FDA approval
- Perform a comprehensive assessment of the infrastructure and healthcare concepts needed to make a sensible impact in biodefense
- Perform an assessment of what is the information needed to meet the geo/eco/political needs
- Perform a need-based assessment, e.g. dual use vs. specific applications
- Perform a pilot implementation case study to assess the function of NA technology (with focused target pathogens)

Additional Comments
- Too complex, too disruptive, should be careful about the use of information.
- US vs third world country response? What is the new international law? Universal standard?
- There are liability issues with home-based tests. Not feasible? Do we want to put out all of the different tests on markets?
- The infectious disease outbreak may not the best model for biodefense.

Analysis of Future Biodetection Systems

Ligand Based Technologies

Session Lead: Jennifer Martinez

Presenter: Brian Kay

Summary

Brian Kay started the discussion on ligands by presenting a nice overview lecture on ligands and ligand generation. His lecture included peptides, scFv, antibodies, and alternative protein scaffolds. Without the time to review all ligands in one presentation, the ligand break-out group added ligands that were not thoroughly discussed within the lecture. We broadly defined ligands as an affinity reagent that utilizes non-hybridization based specificity. As a group we then attempted to review and compare the ligand types, as a tool for those outside the ligand community. [It should be noted that this is a starting point comparison and is in no way complete.] The breakout group then considered ligands for deployable (possibly autonomous) sensors. We discussed attributes of ideal ligands and what technological gaps exist for realizing some of the ideal attributes.

Ligand Comparison:

- *Peptides*
  - μM affinity, specific (less than antibodies?)
  - Easily made synthetically and can be made thermal/protease stable
  - Easily disseminated (once sequence is published anyone can synthesize the ligand)
  - Selection and synthesis can be automated
  - Selection time ~ 3 days, not as good a ligand for small molecule antigens
  - Uses: autonomous sensing, laboratory tests, therapeutics.
- *Aptamers*
  - μM (sub-micromolar affinity possible) affinity
  - Can be specific
  - Chemically synthesized
  - Can be made stable with substitutions, possibly more thermal stable than other ligands (would need to see a back-to-back test)
  - Easily disseminated (once sequence is published anyone can synthesize the ligand)
  - Selection and synthesis can be automated
  - Selection is ~2 weeks; has the advantage that synthetic derivatives can be added to the starting materials to make a larger diversity in the libraries
  - May be better for basic proteins/DNA binding proteins than other proteins
  - Can be made into amplification/detection scaffolds such as aptazymes
  - Can be made to recognize small molecules
  - Uses: autonomous sensing, laboratory tests, therapeutics (if enhanced affinity/half-life)
- *scFv*
  - nM affinity (sub-nanomolar affinity possible)
  - Specific
  - Higher affinity and higher specificity(?) than peptides and aptamers

- Lower stability than full length antibodies, but can be fused with enzymes and stabilizing proteins
- Made through recombinant technology
- Not easily disseminated
- Selection and recombinate production can be automated
- Selection >3 days
- Can be made to recognize small molecules
- Can be made against targets that are toxic in animals (i.e. toxins and pathogens)
- Uses: research reagents, laboratory tests, already used as therapeutics (i.e. if made into humanized antibodies).

- *Alternative Protein Scaffolds*
  - *See Brian Kays lecture for examples*
  - Growing area that will possibly fill in some problems with scFv, peptides, and other ligands
  - May have higher *in vivo* efficacy
  - Some scaffolds have affinities and specificity close to scFv. These scaffolds are newer and are still largely research projects
  - Applications are further downstream
  - The amount of development money and time will largely depend on final use (i.e. what "drives" their production)
- *Full Antibodies*
  - Gold standard and highly usable/high comfort level
  - nM-pM affinity
  - Can be highly specific (however some research suggests that 90% of all monoclonals can recognize other antigens when screened across many antigens, this could be found true for most ligands)
  - Antibodies are not as stable as peptides (antibodies are proteins that can denature), however they are more stable than many proteins and can be made more stable with additives
  - Not easily disseminated
  - Not easily created: immunization is required and they are often not fully characterized (i.e. it takes a lot of money to get sequences and make identical ligands, hence more frequent immunizations are required once the antibody stock is depleted; creating a new uncharacterized ligands)
  - Requires humanization for therapeutics
  - There are new technologies that may make creation of antibodies more reproducible and quicker (i.e. immunization of chickens with DNA (utilize the high-throughput of the food industry), there is concern that the affinity of resultant antibodies is lower than those derived from mice/bunnies, overall this technique is still not fully characterized)
  - Can be made to recognize small molecules
  - Large number of companies with technology to produce antibodies
  - Uses: therapeutics, laboratory tests, research reagents, and *possibly* autonomous sensing (with modifications, testing etc).

Analysis of Future Biodetection Systems

- *Carbohydrates*
  - µM-mM affinity (possibly increase with avidity, i.e. GM1/cholera)
  - Can be specific
  - Research and development is in infancy
  - Not as good for small molecules
  - Disseminated from published molecular structure
  - Selection: carbohydrates are typically rational designed at this stage (with a few exceptions there are few combinatorial scaffolds linking selection and amplification of carbohydrates)
  - Synthesis is difficult (not automated and currently requires excellent synthetic skills)
  - Possibly some conjugation issues (use will require a facile conjugation method)
- *Polymers (MIP alternative?)/Nontraditional (i.e. dendrimers*
  - Less proven
  - Avidity effect for affinity/specificity
  - Possible chemical sensing cross-over
  - *Possibly* better at small molecule sensing
  - *S*till unproven in large arenas
  - May be much more stable (i.e. polymer)
  - [Note: this discussion group had few experts in nontraditional ligands]
- *Chimeras of Ligands*
  - Pairing ligands (homo or hetero types) can increase specificity

What ligands did we miss in the discussion?

- Modified aptamers (i.e. thiol-aptamers)
- Carbohydrates
- Lipids
- Biomimetics or polymers
- Mirror image molecules (stabilized to natural degradation pathways)
- Functionalized dendrimers and functionalized nanoparticles (these are more display and detection modalities than ligands)

Deployable Sensors: What are the ideal attributes of a molecular recognition ligand for deployable sensing (Holy Grail)?

- Matrix independent (tolerant of differences in salt, pH, viscosity, high protein load, etc)
- Recyclable or cheap (easily and reproducibly manufactured)
- Robust: temperature (0-120 F), shock, and matrix stable
- Easy to use
- Compatible with all ligand scaffolds for multiplexing
- Redundant ligand sets for one pathogen/sensor platforms
- High affinity: pM sensitivity or lower affinity with redundancy (i.e. mimic of natural processes)
- Specific: strain specific or redundancy in low specificity

Analysis of Future Biodetection Systems

- Ligands that can be virtually designed
- Binding of antigen triggers signal event or amplification (i.e. turn on enzyme cascade or fluorescence etc)
- Forensics: enable determination of where or how made, enable sample archive
- Differentiates viable vs. nonviable organisms
- Learning system that can make new ligands within a sensor (i.e. click based?). On-the-fly detection of evolving threats
- Self-repairing/self-detoxifying
- Metabolite sensing
- Enables
    - Continuous monitoring (don't have to wait for cycling)
    - Concentration determination
    - High signal-to-noise (in part specificity)
    - Reagentless (i.e. one ligand and not a sandwich)
    - Remote detection

Gaps

- Ligand development technology that is easily transferable: full package of development and use (controls/protocals etc) or commercial companies that make ligand development fast and cheap.
- Faster characterization: more high-throughput methods for determining sensitivity/specificity/eptitopes in many matrices; stability determination/modifications/new platforms for easy manufacturing
- Better/faster synthesis
- BSL3-4: high-throughput ligand screening, high-throughput cell-based screening
- Increased sensitivity/affinity of alternative scaffolds relative antibodies: overcome some of the stability problems and provide ligands with signal transduction capabilities, as two examples.
- High-throughput design and selection of competitors for quencher release/configurational changes (e.g. for small molecule and some FRET based competition assays)
- Standards for testing: back-to-back testing of ligands. Set up a ligand comparison: given these 10 antigens, what ligands can you generate, demonstrating their properties (ease in conjugation, stability, signal amplification, specificity, affinity, time for production etc)
- Ligands for metabolites (these might enable tests for: viability, forensics, engineered threats, concentration determination)
- High-throughput selection, amplification, and synthesis for other biopolymer ligands (i.e. carbohydrates)
- Abilities to express the entire proteome of an organism quickly, so as to generate ligands in high-throughput manner against many in a fast manner
- Gap in prioritizing funding/development

Analysis of Future Biodetection Systems

Transduction Systems

Session Lead: Steve Graves

Presenter: Larry Sklar

Summary

The session began with a stimulating talk by Dr. Larry Sklar on the mechanisms that cells use to sense their environment with exquisite sensitivity. The goal of the talk was to provide examples in nature that could provide lessons in sensor development, which is justified as nature has had billions of years to develop systems to detect molecules of biological interest in the environment. Specific examples within the talk included the detection of photons by rhodopsin and G-protein signaling cascades in neutrophils. Several key elements of biological sensor systems were brought out, including non-linear response (signal cascades), sensitive switch events that can respond to the flux of events to limit over response to spurious background signals, and signal termination and regeneration to allow for sensor control. The use of these approaches in both the optical detection systems of the eye and neutrophil detection were considered. The talk led to a very stimulating breakout session on signal transduction.

Key Functions of a Sensor

- Recognition of environmental signals
- Signal amplification
- Controlled termination of sensor response
- Modulation of gain of signal strength
- Self determination of sensor viability (not organism viability)
- Rapid response time
- Response discrimination *i.e.* specificity
- Reagentless
- Reversibility

Topics of Specific Interest

- The sensor density required to detect environmental signals – with the specific example of rhodopsin where every photon that strikes the eye is detected.
- Detector efficiency – revolved around discussions as to how best ensure that every potential event resulted in a signal – It was asserted that the following are critical for signal transduction efficiency:
    - Receptor/detector geometry
    - Signal feedback
    - Capture efficiency
    - Serial vs. parallel detection and receptor system
    - Pattern recognition
    - Recognition driving action

Analysis of Future Biodetection Systems

Key Thoughts on Topics of Specific Interest

- Knowledge of the natural modulation of signals can greatly assist signal transduction by allowing frequency filters to remove background.
- There should be a second step such as a cascade. You want to link the recognition to a switch.
- Receptors are easy. Molecular switch. You need to go from recognition to switch. Something that turns on immediately.

Gaps

- What should be the density of the detection system in order to detect one binding event?
- Development of the ideal switch.
    - What is the perfect switch? What are the options? What is the biology of switching?
    - Where does the switch live? Cellular, artificial, membrane, nanostructures
- Technology to match detector size to sample size
- Amplification of target or signal
- Phase sensitive detection
- Learning from the environment
    - Background characterization
    - Understanding the flux needed for detection and response
- Assay limitations that may impose challenges to the transducer
- Can a 100 percent detection efficiency be achieved
- Reversibility

Key Thoughts on Gaps

The primary issue identified was background characterization in that almost any signal transduction system will be able to detect and provide a response in a background free environment. The challenge is to detect above background, particularly environmental background. If that is not characterized in terms of natural fauna expected over the entire time frame then resolving real events vs. normal background will be very difficult.

Opportunities Identified

- Mimicking inflammatory mediators
- Understanding catalytic systems in cell that can be used for detection
- Modeling of background associated with the signal generation
- Orthogonal detection/transduction detection system
- Confirmatory detection/transduction systems
- Use biology to detect biology and even chemistry
- Nanotechnology modeled around living systems
- New flourescent molecules
- Understanding sensor network response
- Making transgenic detectors from living systems

Key Thoughts on Opportunities Identified

The real opportunity identified was felt to be using living systems as model signal transduction systems. Lessons from such systems will be very valuable.