

NOPP PROPOSAL PANEL SUMMARY

Project No: Naar

Title: Toxin sensor-based approach for simultaneous, in situ and autonomous monitoring of multiple HAB-toxins in three regions of the coastal US.

Lead PI/Institution: Jerome Naar/University of North Carolina at Wilmington

Collaborating institutions: Luminex Corp., NOAA, Woods Hole Oceanographic Institute, Florida Fish and Wildlife Conservation Commission, FDA

Panel scores				Avg.
1	1.5	1.5		1.3

Proposal Narrative (Brief):

Reviewer Comments and Criteria Score:

1. Relevance of the proposed research to NOPP objectives

Responds to Topic 3.B.2 - technology development for in situ HAB toxin sensors

2. Overall scientific and technical merits of the proposal

Solid proposal from technology development and application, reviewers agrees that its potential for achieving goal to developing autonomous immunoassay arrays for use with a modified Luminex-based detection system called BeadPix. BeadPix has not been developed and the reviewers were cautious that the Sept 08 proposed target date may not be met. The reviewers were impressed with the strong team on this proposal, which would ensure that adequate groundtruthing of the method and dissemination of this tool to the management community would take place. To that end, the descriptions of regional deployments, their rationale, and management application/involvement were exceptional. There was some discussion on the claims of sensitivity and rapidity, and the expectation that this technique would be semi-quantitative. But despite these potential limitations, the reviewers agreed that the outcome of this study would be a detection system superior to current technology for sensing the suite of targeted toxins. Two other significant pluses were the proof of concept of the Lead PI in the successful and rapid adaptation of the brevetoxin ELISA method to a Luminex-based microassay system. This gave confidence that the timing of developing the other assays was reasonable, especially given the availability
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of the antibodies from the co-PIs. Another positive aspect was that Year 3 was devoted to validation in the 3 regional hot spots for the target HABs. The feeling was that the third year would also serve as a buffer period if delays occurred in accomplishing fully the objectives in Years 1 and 2.

3. **Level of support of critical research objectives or operational goals such as data accessibility, education and communication**

All aspects of this proposed study are covered by experienced specialists, and adequate time is dedicated from personnel involved in heavy lifting of development and testing.

4. **Quality of proposed partnerships including the degree of broad participation within the oceanographic community and demonstration of significant partnering among at least two of the following parties: (i) academia, (ii) industry (or not-for-profit organization), and (iii) government (federal, state, local) and extent resources are shared among partners**

Strong partnership by all indicated groups – academia (UNCW), business (Luminex), non-profit (WHOI), state (FWRI), and federal (NOAA, FDA).

5. **The offeror's capabilities, related experience, and facilities or unique combinations of these that are critical to the proposal objectives**

The Lead PI, Luminex, and the partners all bring exceptional facilities and experience to accomplish the objectives.

6. **The partnership members' long-term commitment to the proposed objectives**

Many of the PIs are juggling many projects but the payoff in needed technology would be great for their existing and planned projects. They all have a reputation for producing on grants. Naar will earn his 50% salary and others at UNCW are heavily involved.

7. **The qualifications and experience of the proposed principal investigator and key personnel**

The team is greatly qualified and experienced – they are the ones you want in each region and the Lead PI has pushed the envelope in this field.

8. **Reasonableness of cost**

The budget is probably reasonable – bulk to UNCW and Luminex (perhaps a bit high for Luminex – may be a place to trim). NOAA, WHOI, FWRI, and FDA are cheap-to-free (much cost-sharing).

Overall Score:

Additional Comments (please explain in some detail):

NOPP PROPOSAL REVIEW FORM

Project No:

Title: Toxin sensor-based approach for simultaneous, in situ and autonomous monitoring of multiple HAB-toxins in three regions of the coastal US.

Lead PI/Institution: Jerome Naar/University of North Carolina at Wilmington

Collaborating institutions: Luminex Corp., NOAA, Woods Hole Oceanographic Institute, Florida Fish and Wildlife Conservation Commission, FDA

Proposal Narrative (Brief): Development of autonomous in situ toxin immunoassays using a BeadPix (Luminex) microbead array system. Proposed system would be designed for brevetoxin, PSP, and domoic acid detection, and has potential for high sensitivity, rapid, and low cost.

Reviewer Comments and Criteria Score:

1. Relevance of the proposed research to NOPP objectives

Innovative toxin detection method that would advance HAB detection technology significantly – very relevant. Score = 1

2. Overall scientific and technical merits of the proposal

This proposal has great promise, and covers scientific and technical aspects strongly. The team of Anderson, Heil, and Trainer ensures adequate groundtruthing and application to management. The ability to detect toxins in situ is hugely needed and I see no major flaws in these plans. The Luminex microbead technology appears to be an ideal way to apply the immunoassay arrays in the high throughput, highly sensitive way needed for early warning of HABs. In theory, the Luminex approach is quantitative (based on the intensity of a fluorochrome specific to a report molecule reaction), but I would have liked more information on the quantitative capabilities in previous applications of Luminex. This proposed method has the potential to lead to an autonomous system that detects and quantifies numerous toxins and cells, as well as deployment on AUV's. The objectives (from immunoassay development to microarray adaptation to developing an autonomous sampling unit to development of a FTI and DAI to real-time deployment and groundtruthing) is ambitious but each step is thoroughly described and solid. Naar's rapid success in adapting the brevetoxin assay lends confidence. The fact that Year 3 is dedicated to deployment

and testing gives a buffer in the case of snags in the plan (I suspect PSP adaptation and ASPU will take longer than proposed). Finally, the descriptions of regional deployments, their rationale, and management application/involvement are exceptional. Score = 1

3. Level of support of critical research objectives or operational goals such as data accessibility, education and communication

All aspects of this proposed study are covered by experienced specialists, and adequate time is dedicated from personnel involved in heavy lifting of development and testing. Score = 1

4. Quality of proposed partnerships including the degree of broad participation within the oceanographic community and demonstration of significant partnering among at least two of the following parties: (i) academia, (ii) industry (or not-for-profit organization), and (iii) government (federal, state, local) and extent resources are shared among partners

All three user groups are heavily involved. Score = 1

5. The offeror's capabilities, related experience, and facilities or unique combinations of these that are critical to the proposal objectives

Facilities etc. are excellent. Score = 1

6. The partnership members' long-term commitment to the proposed objectives

Many of the PIs are juggling many projects but the payoff in needed technology would be great for their existing and planned projects. They all have a reputation for producing on grants. Naar will earn his 50% salary and others at UNCW are heavily involved. Score = 1

7. The qualifications and experience of the proposed principal investigator and key personnel

This is a strong team. Score = 1

8. Reasonableness of cost

The budget is reasonable – bulk to UNCW and Luminex. NOAA, WHOI, FWRI, and FDA are cheap-to-free (much cost-sharing). Score = 1

Overall Score: 1 = Excellent

Additional Comments (please explain in some detail):



NOPP PROPOSAL REVIEW FORM

Project No:

Title: Toxin sensor based approach for simultaneous

Lead PI/Institution: Naar, UNC Wilmington Center for Marine Science

Collaborating institutions: Luminex Corp., Woods Hole Oceanographic Institute, Florida Fish and Wildlife Conservation Commission, NOAA, FDA

Proposal Narrative (Brief): The PIs plan to develop new analytical methods for the detection of HAB toxins; brevetoxin, domoic acid and saxitoxins and neosaxitoxins using a variation of the Luminex xMAP technology. Luminex uses microbeads which incorporate two fluorescent dyes with different emission maxima. The ratio of the two dyes varies and 100 distinct sets of microspheres with varying dye ratios are available. Beads are tagged with an analyte and a competitive displacement assay (linked vs. free toxin) is performed with antibody. A secondary antibody labeled with a fluorescent dye is used for detection. Current Luminex technology uses a flow cytometer for detection. The ratio of the first two dyes identifies the bead type (ie. The toxin tag), the degree of fluorescence of the third dye is for quantitation. Multiple bead sets for multiple toxins can be analyzed simultaneously. The proposed sensor would be similar to the Luminex microspheres but detection would be performed using magnetic beads and a digital CCD camera incorporated into a new instrument (BeadPix) currently under development at Luminex and anticipated to be available in September of '08. Antibodies for brevetoxin are currently available to the PI and preliminary studies have demonstrated the adaptation of the brevetoxin ELISA to the Luminex format with a dynamic range of 4 orders of magnitude and a detection limit of 0.01 ng/mL using toxin standards. Antibodies to domoic acid and saxitoxins and neosaxitoxins are currently not available, but are to be developed in the laboratories of collaborators Vera Trainor (NOAA) and Sherwood Hall (FDA). Luminex will be responsible for the "ruggedization" of the BeadPix instrument. Other tasks include development of an automated assay preparation module and automated sample processing module power supply and telecommunications. In year three the PIs plan to deploy the module in three locations: the Pacific northwest for domoic acid; New England for PSP toxins; Gulf of Mexico for brevetoxins. Instrument deployments will be overseen by government collaborators, NOAA and Florida

Fish and Wildlife Research Institute (FWRI). The results from the BeadPix instrument will be compared to those obtained by standard analytical methods.

Reviewer Comments and Criteria Score:

1. Relevance of the proposed research to NOPP objectives

This proposal responds to Topic 3. B.2 of the BAA. Sensors for Measurement of Biological, Bio-Optical or Chemical Properties of the Ocean; Development of the next generation of biological, chemical, optical and; biooptical field sensors; harmful algal bloom (HAB) HAB toxins in ocean, coastal, and Great Lakes waters.

2. Overall scientific and technical merits of the proposal

Overall this is an excellent proposal with a high probability of success. The proposal is well written, the plans and division of tasks are well laid out. The assembled team represents the leaders in the field of HAB toxins. All of the necessary materials and expertise are available in this proposal. The downside; The chemistry for the coupling to the beads will apparently be different than that used for the Luminex beads. It is poorly described in the proposal. [Is the “capture moiety” antibody or toxin?] In the past, Luminex has been used almost exclusively for the detection of large biomolecules (protein, DNA, RNA, protein toxins). Few examples of its application to small molecule detection have been reported. This reviewer found only a single example (TNT). The standard chemistry was not successful and required some modification. The assay will not be rapid. The assay is based on an ELISA and will require all of the incubation and washing steps of any ELISA assay. It is not clear that, under real life conditions, this assay will be as sensitive as other assays that utilize some sort of signal amplification. Nonetheless, this technology has not been applied to HAB toxins and only once (that this reviewer can identify) to small molecules, so we just don’t know the answers and this team can provide them.

3. Level of support of critical research objectives or operational goals such as data accessibility, education and communication

The investigators in this proposals are leaders in the field of HAB toxins and have the necessary expertise to carry out the project. Facilities are excellent. The necessary materials, cultures, antibodies and toxins are available or can be obtained by the investigators. The project is highly leveraged with other funded projects such as ECOHOB (to FWRI) an NIEHS sponsored program project (UNCW)

4. Quality of proposed partnerships including the degree of broad participation within the oceanographic community and demonstration of significant partnering among at least two of the following parties: (i) academia, (ii) industry (or not-for-profit organization), and (iii) government (federal, state, local) and extent resources are shared among partners

The proposal represents a partnership between academia (UNC Wilmington),

business (Luminex corporation), Non-profit (Woods Hole), State agency (FWRI), and federal agencies (NOAA and FDA). The bulk of the budget goes to UNCW. A significant portion of the budget goes to Luminex (35%) which seems high to develop an instrument they are already developing. FWRI and FDA are not requesting funds. The project is highly leveraged through programs such as ECOHAB, an NIEHS sponsored program project (UNCW), which is viewed as a strength.

5. The offeror's capabilities, related experience, and facilities or unique combinations of these that are critical to the proposal objectives

outstanding

6. The partnership members' long-term commitment to the proposed objectives

The PIs, Jerome Naar, Vera Trainor, Sherwood Hall, Don Anderson, Cindy Heil and others all have extensive backgrounds in HAB toxins or oceanography. Successful implementation of the proposed technology would advance research in the field of HAB toxins. It is not clear that this technology would be adapted to monitoring programs as it may be costly.

7. The qualifications and experience of the proposed principal investigator and key personnel

Outstanding

8. Reasonableness of cost

Considering the number of partners, the leveraging of other projects. The lack of budget to two government agencies the costs seem reasonable. The Luminex budget seems a bit high.

Overall Score (1 = Excellent, 2 = Very Good, 3 = Good, 4 = Fair, 5 = Poor): 1.5

Additional Comments (please explain in some detail):