3-2-2007

SGER-Exploring the use of Quantum Dots to Detect the Physiology of Intact Phytoplankton Cells by Flow Cytometry

Karen Orcutt
Principal Investigator; University of Maine, Orono

Final Report for Period: 06/2006 - 11/2006 **Submitted on:** 03/02/2007
Principal Investigator: Orcutt, Karen . **Award ID:** 0530568
Organization: University of Maine
Title:
SGER-Exploring the use of Quantum Dots to Detect the Physiology of Intact Phytoplankton Cells by Flow Cytometry

Project Participants

Senior Personnel

Name: Orcutt, Karen
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Sieracki, Michael
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Wells, Mark
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Gundersen, Kjell
Worked for more than 160 Hours: Yes
Contribution to Project:

Post-doc

Name: Poulton, Nicole
Worked for more than 160 Hours: Yes
Contribution to Project:
Dr. Poulton assisted with the flow cytometric detection of Qdot labeled cells. Her time was supported by this grant under a subcontract with Bigelow Laboratories for Ocean Sciences.

Graduate Student

Undergraduate Student

Technician, Programmer

Other Participant

Research Experience for Undergraduates

Organizational Partners

Moss Landing Marine Laboratories

Other Collaborators or Contacts

Dr. Jason Smith

Activities and Findings

Research and Education Activities: (See PDF version submitted by PI at the end of the report)
Please also see Findings

Findings: (See PDF version submitted by PI at the end of the report)
Please also see activities

Training and Development:
This research project provided the PI's with experience and skill using quantum dot bioconjugates in a biological oceanographic application.

Outreach Activities:
The results of this project were presented at the Amercan Society for Limnology and Oceanography meeting in Santa Fe, NM 2007. The results of this work were also presented in three invited talks to Coastal Carolina University, University of Southern Mississippi, Department of Marine Science, Stennis Space Center, Mississippi, Louisiana Universities Marine Consortium (LUMCON) in 2006.

Journal Publications

Books or Other One-time Publications

Web/Internet Site

Other Specific Products

Contributions

Contributions within Discipline:
This work applied a new tool in immuno-detection of phytoplankton cells.

Contributions to Other Disciplines:

Contributions to Human Resource Development:

Contributions to Resources for Research and Education:

Contributions Beyond Science and Engineering:

Categories for which nothing is reported:

Any Journal
Any Book
Any Web/Internet Site
Any Product
Contributions: To Any Other Disciplines
Contributions: To Any Human Resource Development

Contributions: To Any Resources for Research and Education

Contributions: To Any Beyond Science and Engineering

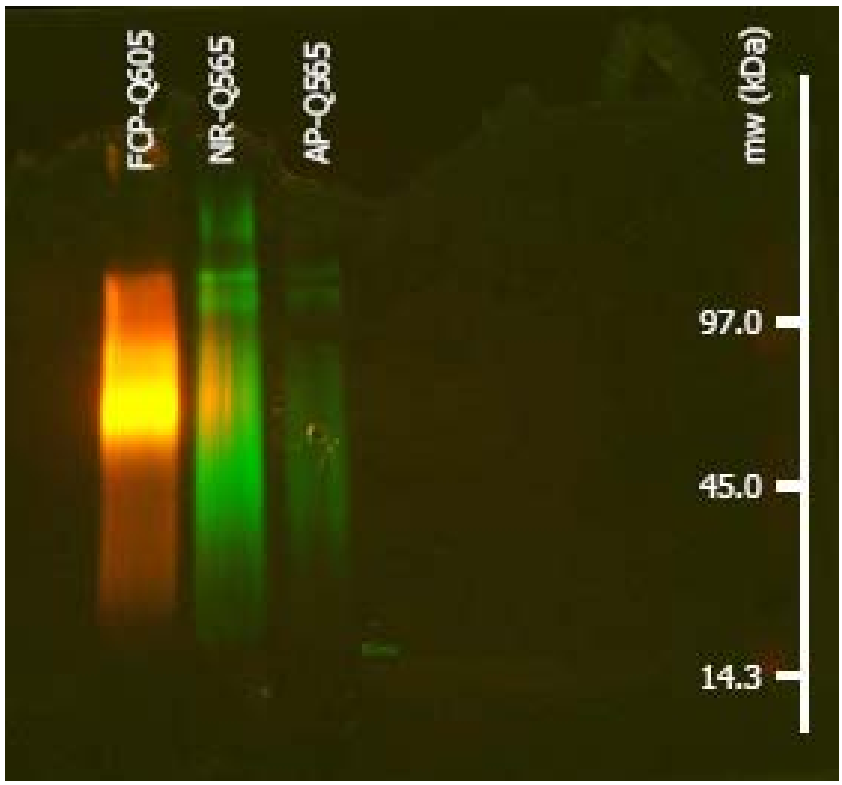

Figure 1
Direct visualization of Qdot bioconjugagates on SDS-PAGE. The Qdot conjugates are FCP-Q605, NR-Q565 and AP-Q565 (a P-stress protein).

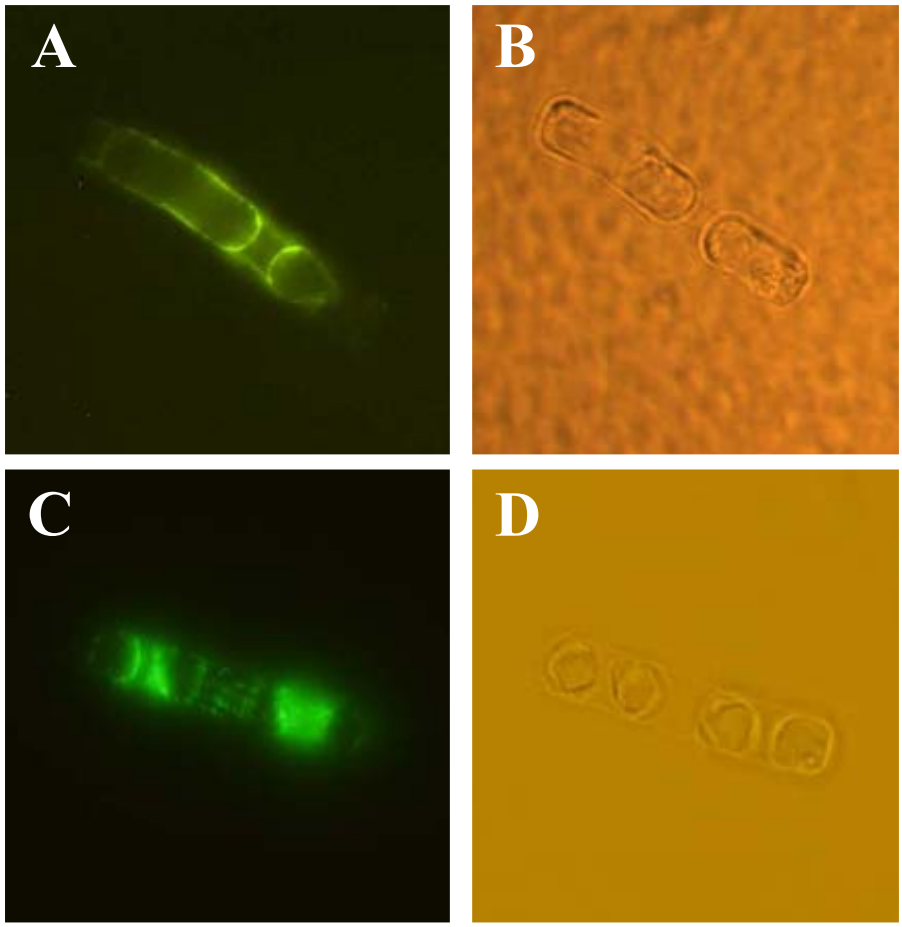

Figure 2
EFM image (A) and LM image (B) of *Skeletonema costatum* stained with NR-Q565 conjugate.
EFM image (C) and LM image (D) of *S. costatum* stained with FCP-Q565 conjugate.

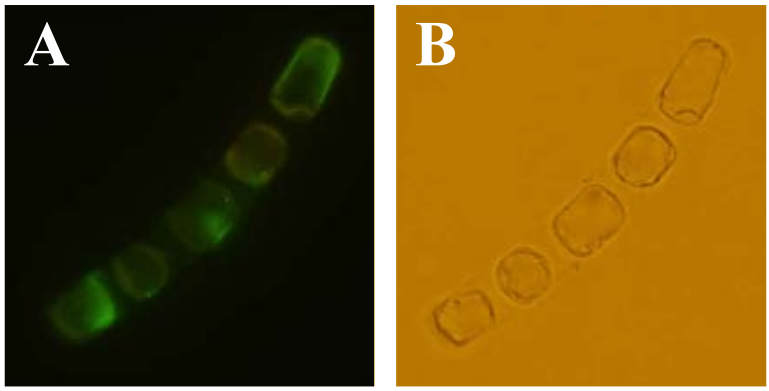

Figure 3
EFM image (A) and LM image (B) of *S. costatum* stained with FCP primary antibody and FITC labeled secondary antibody.

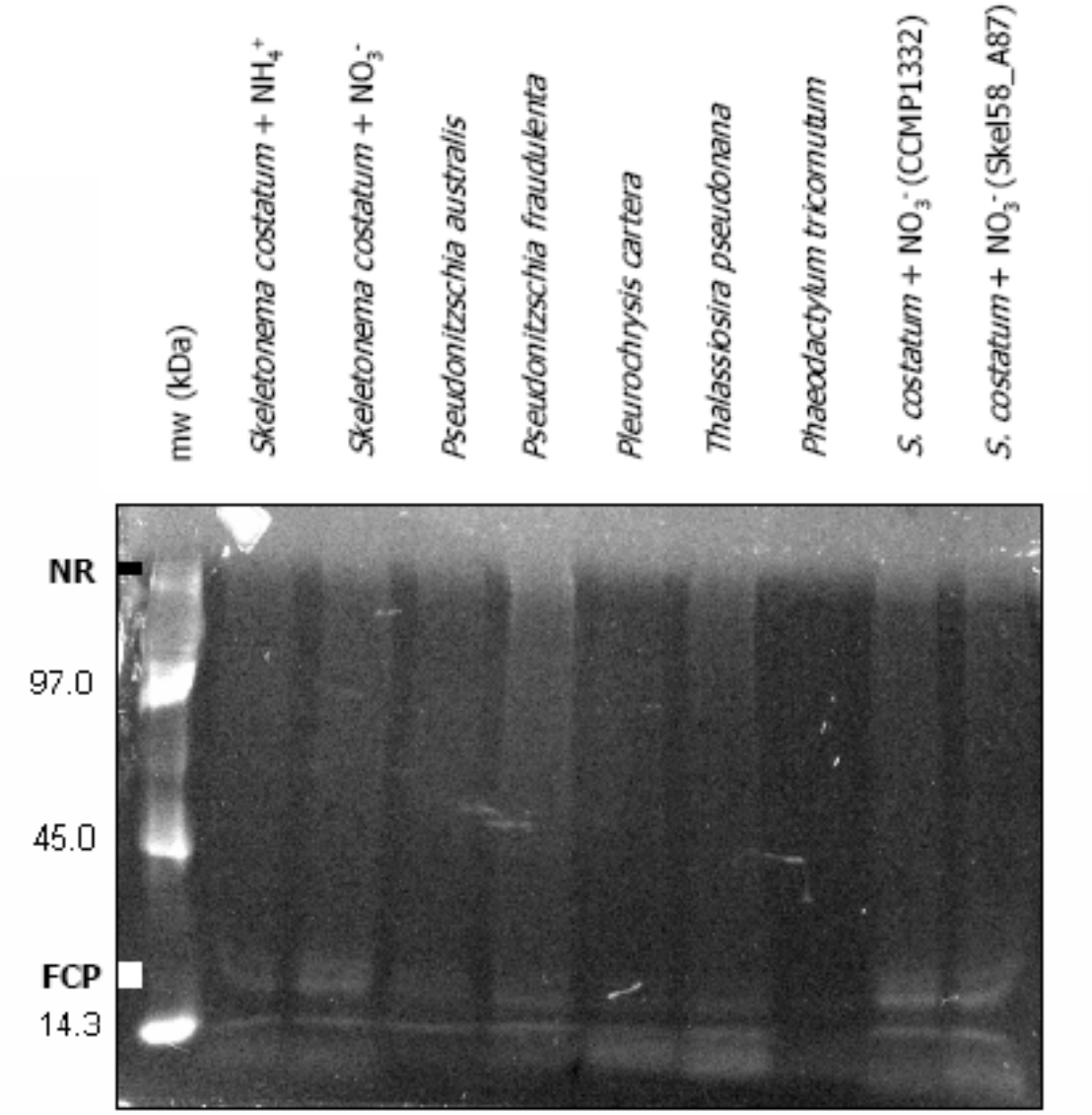

Figure 4
In-gel direct immunodetection of NR and FCP in SDS-PAGE using Qdot bioconjugates for a selection of phytoplankton cultures.

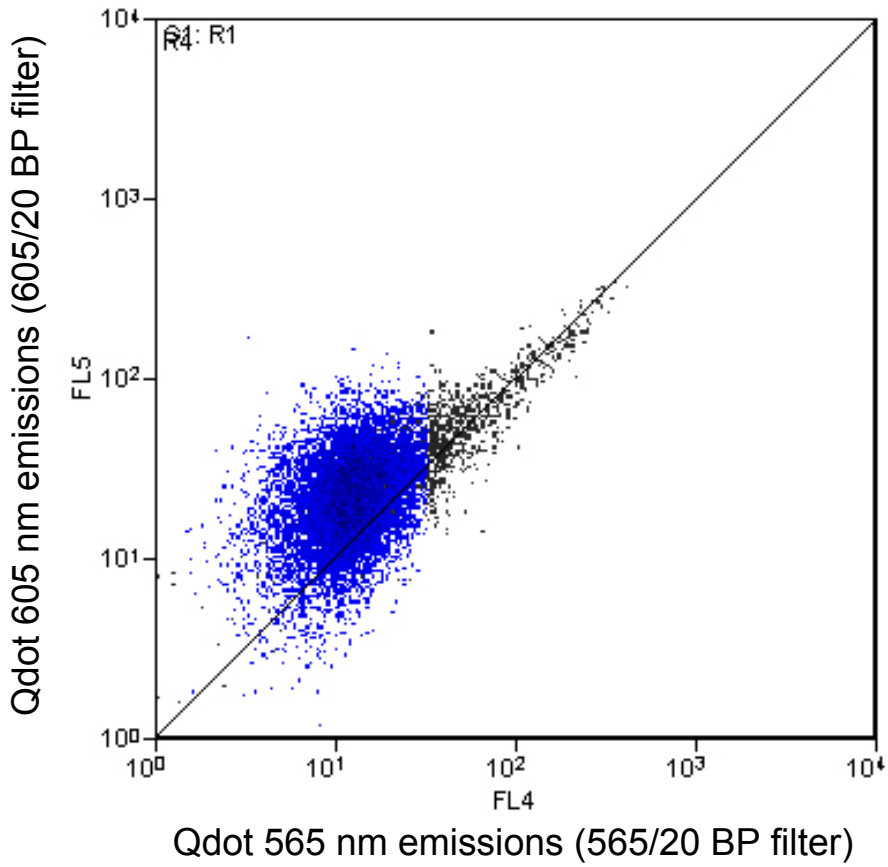

Figure 5
Culture of *S. costatum* stained with FCP-Q605 conjugate and detected on a Dako Cytomation MoFlo cytometer equipped with a 100-mW 488-nm air cooled argon laser and a 90-mW 365-nm water cooled laser. Q605-FCP fluorescence can be seen as a deviation from the one to one ratio.

Quantum dots (Qdots) were successfully conjugated to primary antibodies (Qdot bioconjugate) against enzyme targets nitrate reductase (NR) and fucoxanthin complexing protein (FCP) specific for *Skeletonema costatum* (CCMP 1332). This was verified by Sodium Dodecyl Sulfate Polyacrylamide Electrophoresis (SDS-PAGE) of the Qdot bioconjugates which migrated at the correct molecular weight (Figure 1). Qdot bioconjugates were not successfully made with the primary antibody against alkaline phosphatase (AP) specific for *Prorocentrum minimum* (CCMP 1329). Cultures of *P. minimum* were grown under low P concentrations (verified by the alkaline phosphatase MUF-P assay) but the initial trials with Q565 conjugated to chicken AP antibody (IgY) failed. We then perfomed experiments using Q-dots commercially conjugated to a secondary antibody (goat anti-chicken conjugate) and with the original procedure using a secondary antibody conjugated to phycoerythrin (goat anti-chicken PE) as described by Dyhrman & Palenik (2001). Both attempts did not produce labelling of cells. The failure of the Qdot conjugation procedure may have been due to the fact that the Q-dot conjugation kit (Invitrogen) was not developed for egg yolk produced IgY antibodies; they are primarily developed for IgG antibodies. These two types of antibodies have different chemical structures. However, our experiments suggest that it was most likely not a conjugation problem as other modes of immunological detection failed using the AP primary antibody. Our results suggest that the most likely reason for the lack of conjugation was due to an extremely low titer of the AP antibody as verified by SDS-PAGE (see Figure 1).

We labeled active sites expressing NR in the cell membrane and FCP in the thykaloid membrane separately using Qdot bioconjugates (Qdot 565 and 605) in single diatom cells (Figure 2). Simultaneous detection of both probes was made difficult due to the close proximity of the two membrane–bound proteins (near the cell wall). We detected NR and FCP in intact cells of *S. costatum* using epifluorescence microscopy with a clarity and definition that was not obtainable with conventional organic fluorophores (FITC conjugated to secondary antibody; Figure 3). Specificity of the Qdot bioconjugates for target proteins was verified by SDS-PAGE as well as the cross-reactivity of the probes with other diatom cells (Figure 4).

Detection of FCP labeled cells by flow cytometry was more successful than with NR labeled cells due to higher protein abundance of FCP. This limited the experiments on sensitivity of Qdot detection that we originally planned. This work was also complicated by what appeared to be problems in preserving the stained diatom cells and initial problems associated with the staining technique. The quantum dot conjugation kit (Quantum Dot Corp.) was a new product on the market at the start of this proposal. The method description and handling of chemicals, as initially described by the manufacturer, Quantum Dot Corporation, was not optimal and lead to insufficient labeling of cells in the early phase of the project. There were also quality control issues with the quantum dot products that produced delays in receiving their product. After corporate mergers (first by Molecular Probes, which in turn was incorporated by Invitrogen) the methods were improved and optimized. Complications with background pigment inference were another issue that became clear during the initial trials on the flow cytometer. Efforts to reduce the background fluorescence included purchase of more specific filters to improve detection of Q565 and Q605. Several procedures for bleaching the remaining pigments in the diatom cells were also tested, but this only created secondary products that still interfered with the flow cytometer. The most abundant protein (FCP) could be detected by flow cytometry (Figures 5). Qdot staining of diatoms appears in very finite regions of the cell (Figures 2) and this may cause difficulty in detection with flow cytometry when protein abundance is low as in the case for NR. Another factor is the possibility that the Qdot radiative rate is relatively slow and this, coupled with low abundance, may impede detection in standard flow cytometry. However, the application of Qdot bioconjugates in flow cytometry is a promising technique when used with extracellular proteins or intracellular proteins in high abundance.

In summary, we have successfully conjugated Qdots to primary antibodies and applied these probes for cellular detection using epifluorescenet microscopy and flow cytometry. Although experiments with flow cytometric detection was not as straightforward as originally planned in the proposal, we were able to detect cells with high protein abundance. By using Qdot bioconjugates, we detected NR and FCP using epifluorescent microscopy in single cells of *S. costatum* with a clarity and definition that was not obtainable with conventional organic fluorophores. Qdot bioconjugates make

ideal probes for surface or intracellular protein immuno-localization using epifluorescent microscopy because they do not photobleach. Therefore, Qdot bioconjugate probes have great potential in the field of biological oceanography.