

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 20 May 97	3. REPORT TYPE AND DATES COVERED Final Technical Report 1 Sep 93-30 Sep95	
4. TITLE AND SUBTITLE Luminescence-Based Biosensor Systems		5. FUNDING NUMBERS G N00014-93-1-0991	
6. AUTHOR(S) Thomas O. Baldwin and Miriam M. Ziegler		7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Texas Agricultural Experiment Station Administration Bldg., Room 6 Contracts & Grants Office College Station TX 77843-2147	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of Naval Research ATTN: Code 341 800 North Quincy Street Arlington VA 22217-5660		8. PERFORMING ORGANIZATION REPORT NUMBER 500777	
11. SUPPLEMENTARY NOTES			
12a. DISTRIBUTION/AVAILABILITY STATEMENT APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED.		12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) Progress has been made in two related areas during the period of this grant. In the area of luminescence-based biosensor systems, we have developed sensitive methods to detect antibiotics in biological materials and foods using bioluminescence. We believe that our methods can be automated and applied to the rapid detection of antibiotics in biological fluids, food sources, and other materials. In related studies, the crystal structure of bacterial luciferase has been determined at 2.4 Å resolution. This accomplishment represents a major step forward in our effort to understand this enzyme and how it works, including the details of its folding. Knowledge of the structure is crucial also to efforts to derivatize the enzyme for development of new generation biosensor systems.			
19970530 164			
DTIC QUALITY INSPECTED 4			
14. SUBJECT TERMS bioluminescence, biosensors, bacterial luciferase, antibiotics		15. NUMBER OF PAGES 5	
		16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL

FINAL TECHNICAL REPORT

OFFICE OF NAVAL RESEARCH

ONR Grant #

N00014-93-1-0991

Grant Title

**Luminescence-Based Biosensor
Systems**

Grant Period

September 1, 1993 - September 30, 1995

Principal Investigators

Thomas O. Baldwin and Miriam M. Ziegler

**Performing Organization:
The Texas Agricultural Experiment Station
Contracts & Grants
Administration Building Room 6
College Station, TX 77843-2147**

TAES ACCOUNT NUMBER: 500777

Accomplishments: Progress has been made in two related areas during the period of this grant. One area is in the development of luminescence-based biosensor systems, and the other is in the advancement of basic knowledge that will be crucial to long-term efforts to develop additional strategies for biosensor technologies.

We have developed sensitive methods to detect antibiotics in biological materials and foods using bioluminescence. The method takes advantage of the cell-density dependent (quorum sensing) system that controls the expression of bioluminescence. Exposure of cells preinduction to even low levels of antibiotics causes a slight attenuation of the induction process that is amplified as the culture grows, and is manifested by large differences in the intensity of light emission postinduction. We believe that our methods can be automated and applied to the rapid detection of antibiotics in biological fluids, food sources, and other materials.

In related studies, the high resolution crystal structure of bacterial luciferase has been determined (see refereed publication 11, below, and Figure 1). This accomplishment represents a major step forward in our effort to understand this enzyme and how it works, including the details of its folding. Knowledge of the structure is crucial also to efforts to derivatize the enzyme for development of new generation biosensor systems. While knowledge of the structure is an important milestone, utilization of the information will require much additional effort. We are making excellent progress in related studies under ONR grant #N0001496-1-0087, and we hope that future studies on this system will continue to be supported by the Office of Naval Research.



Figure 1. Stereo image of an α -carbon trace of the α and β subunits of bacterial luciferase from *Vibrio harveyi* (from the 2.4 Å structure, refereed publication #11 below).

Publications (1993-1995):

Refereed Publications

1. Devine, J. H., Kutuzova, G., Green, V. W., Ugarova, N. and Baldwin, T. O. (1993) Luciferase from the European firefly *Luciola mingrelica*: Cloning and nucleotide sequence of the cDNA, overexpression in *E. coli* and purification of the enzyme. *Biochim. Biophys. Acta* **1173**, 121-132.
2. Ziegler, M. M., Goldberg, M. E., Chaffotte, A. F. and Baldwin, T. O. (1993) Refolding of luciferase subunits from urea and assembly of the active heterodimer. Evidence for folding intermediates that precede and follow the dimerization step on the pathway to the active form of the enzyme. *J. Biol. Chem.* **268**, 10760-10765.
3. Baldwin, T. O., Ziegler, M. M., Chaffotte, A. F. and Goldberg, M. E. (1993) Contribution of folding steps involving the individual subunits of bacterial luciferase to the assembly of the active heterodimeric enzyme. *J. Biol. Chem.* **268**, 10766-10772.
4. Clark, A. C., Sinclair, J. F. and Baldwin, T. O. (1993) Folding of bacterial luciferase involves a non-native heterodimeric intermediate in equilibrium with the native enzyme and the unfolded subunits. *J. Biol. Chem.* **268**, 10773-10779.
5. Sinclair, J. F., Waddle, J. J., Waddill, E. F. and Baldwin, T. O. (1993) Purified native subunits of bacterial luciferase are active in the bioluminescence reaction but fail to assemble into the $\alpha\beta$ structure. *Biochemistry* **32**, 5036-5044.
6. Abu-Soud, H., Clark, A. C., Francisco, W. A., Baldwin, T. O. and Raushel, F. M. (1993) Kinetic destabilization of the hydroxyperoxyflavin intermediate by site-directed modification of the reactive thiol in bacterial luciferase. *J. Biol. Chem.* **268**, 7699-7706.
7. Francisco, W. A., Abu-Soud, H. M., Baldwin, T. O. and Raushel, F. M. (1993) Interaction of bacterial luciferase with the aldehyde substrates and inhibitors. *J. Biol. Chem.* **268**, 24734-24741.
8. Smith, D. J., Zhu, H., Kolatkar, P. R., Tam, L.-T., Baldwin, T. O., Roe, B. A., Broyles, R. H., and Riggs, A. F. (1993) The hemoglobins of the bullfrog, *Rana catesbeiana*. The cDNA-derived amino acid sequences of the α chains of adult hemoglobins B and C: Their role in deoxygenation-induced aggregation. *J. Biol. Chem.* **268**, 26961-26971.
9. Sinclair, J. F., Ziegler, M. M. and Baldwin, T. O. (1994) Kinetic partitioning during protein folding yields multiple native states. *Nature Struct. Biol.* **1**, 320-326.

10. Fedorov, A. N. and Baldwin, T. O. (1995) Contribution of cotranslational folding to the rate of formation of native protein structure. *Proc. Natl. Acad. Sci.* **92**, 1227-1231.
11. Fisher, A. J., Raushel, F. M., Baldwin, T. O. and Rayment, I. (1995) The three-dimensional structure of bacterial luciferase from *Vibrio harveyi* at 2.4 Å resolution. *Biochemistry* **34**, 6581-6586.

Book chapters, symposium contributions, reviews, etc

1. Francisco, W. A., Abu-Soud, H. M., Raushel, F. M. and Baldwin, T. O. (1993) Studies on the kinetic mechanism of the bacterial luciferase-catalyzed reaction. In Bioluminescence and Chemiluminescence, eds. A. A. Szalay, L. J. Kricka, and P. Stanley (John Wiley & Sons) pp. 113-117.
2. Ziegler, M. M., Clark, A. C., Sinclair, J. F., Chaffotte, A. F., Goldberg, M. E. and Baldwin, T. O. (1993) Folding and assembly of the subunits of bacterial luciferase. In Bioluminescence and Chemiluminescence, eds. A. A. Szalay, L. J. Kricka, and P. Stanley (John Wiley & Sons) pp. 178-182.
3. Kutuzova, G. D. and Baldwin, T. O. (1993) Two cysteinyl residues conserved in all insect luciferases are not essential for the activity of *Luciola mingrelica* firefly luciferase. In Bioluminescence and Chemiluminescence, eds. A. A. Szalay, L. J. Kricka, and P. Stanley (John Wiley & Sons) pp. 89-93.
4. Gelmini, S., Pinzani, P., Orlando, C., Sestini, R., Baldwin, T. O. and Pazzagli, M. (1993) Chloramphenicol acetyl-transferase, firefly and bacterial luciferases as reporter genes in transfection of mammalian cells. In Bioluminescence and Chemiluminescence, eds. A. A. Szalay, L. J. Kricka, and P. Stanley (John Wiley & Sons) pp. 200-206.
5. Okumura, K., Chlumsky, L., Baldwin, T. O. and Kado, C. I. (1993) Stable expression of a *Vibrio* luciferase is amplified by the Ω -translation enhancer in transgenic plants. In Bioluminescence and Chemiluminescence, eds. A. A. Szalay, L. J. Kricka, and P. Stanley (John Wiley & Sons) pp. 157-162.
6. Francisco, W. A., Abu-Soud, H. M., Raushel, F. M., and Baldwin, T. O. (1994) Kinetic mechanism of the bacterial luciferase-catalyzed reaction. In Flavins and Flavoproteins 1993, ed. K. Yagi (Walter de Gruyter, Berlin) pp. 829-837.
7. Baldwin, T. O., Ziegler, M. M., Sinclair, J. F., Clark, A. C., Chaffotte, A.-F. and Goldberg, M. E. (1994) Mechanism of folding and assembly of heterodimeric bacterial luciferase. In Flavins and Flavoproteins 1993, ed. K. Yagi (Walter de Gruyter, Berlin) pp. 823-828.

8. Baldwin, T. O., Sinclair, J. F., Clark, A. C. and Ziegler, M. M. (1994) Molecular biology of the folding and assembly of the subunits of bacterial luciferase. In Bioluminescence and Chemiluminescence: Fundamentals and Applied Aspects, eds. A. K. Campbell, L. J. Kricka, and P. E. Stanley (John Wiley & Sons, Chicester and New York) pp. 501-508.
9. Christopher, J. A., Raso, S. W., Ziegler, M. M. and Baldwin, T. O. (1995) Enzymes. In Molecular Biology and Biotechnology: A Comprehensive Desk Reference, ed. R. A. Meyers (VCH Publishers, Weinheim, New York, Basel and Cambridge) pp. 295-301.
10. Sitnikov, D. M., Schineller, J. B. and Baldwin, T. O. (1995) Transcriptional regulation of bioluminescence genes from *Vibrio fischeri*. *Molecular Microbiology* **17**, 801-812.
11. Baldwin, T. O., Christopher, J. A., Raushel, F. M., Sinclair, J. F., Ziegler, M. M., Fisher, A. J., and Rayment, I. (1995) Structure of bacterial luciferase. *Curr. Opin. Struct. Biol.* **5**, 798-809.