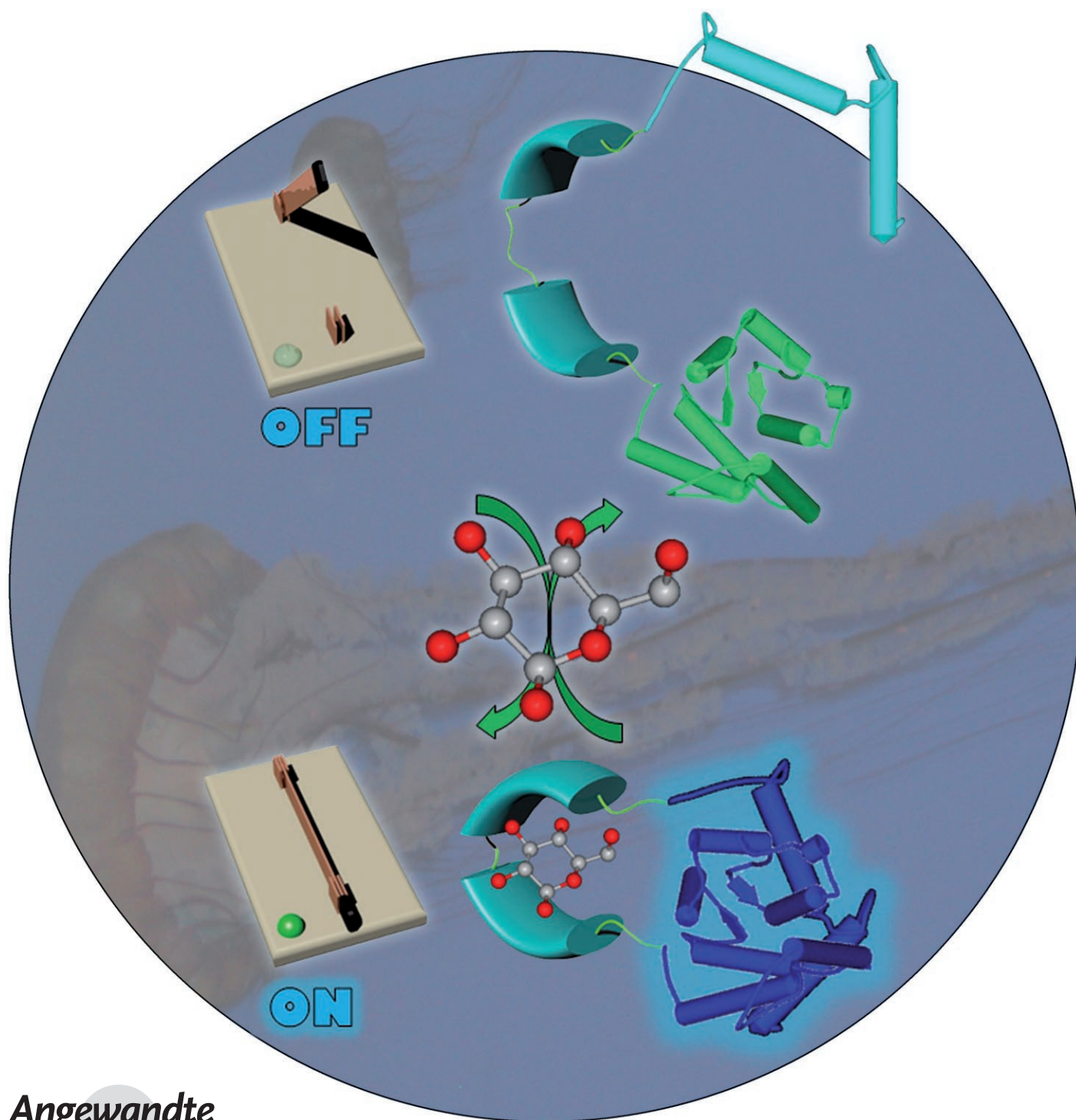


A Bioluminescent Molecular Switch For Glucose**

*Krystal Teasley Hamorsky, C. Mark Ensor, Yinan Wei, and Sylvia Daunert**



Continuous discoveries in bioengineering and more efficient molecular biology methods have allowed scientists to create new designer biomolecules with unique and distinct properties. Protein switches with optical properties are an example of such designer biomolecules that, in the presence of an environmental stimulus, demonstrate an altered response manifested by an “on/off” signal. Such molecules can be employed in the development of nanosensors, creating allosteric enzymes, and as building blocks for the fabrication of functional nanobiomaterials with unique properties. Herein, we report a bioluminescent molecular switch created by insertion of glucose binding protein (GBP) into the structure of the photoprotein aequorin (AEQ). In the presence of glucose, GBP undergoes a conformational change, bringing the two segments of AEQ together, “turning on” bioluminescence and detecting glucose. This strategy provides a general approach to molecular switches given that proteins with substrate-binding properties can be inserted into aequorin to create bioluminescent “on/off” nanosensors with potential for *in vitro*- and *in vivo*-sensitive detection, and/or imaging applications.

Bionanotechnology has led to designer biomolecules tailored to perform nanoscale engineering functions.^[1,2] Such designer biomolecules include protein switches prepared by insertion of a nucleotide sequence coding for a desired function within the gene of a protein, creating a chimeric protein with properties determined by both partners. Thus, two unrelated proteins can be fused to yield a protein switch with enhanced performance of one or both of the individual proteins.^[2] Optical molecular switches using fluorescent proteins have been developed;^[2–6] however, no reports exist on the use of their bioluminescent counterparts, despite their superior detection capabilities. In that regard, we created a bioluminescence molecular switch by inserting the binding protein GBP into the bioluminescent protein AEQ in such a way that glucose binding to GBP allosterically transduces AEQ, turning the switch “on” and generating bioluminescence emission (Figure 1).

A number of naturally occurring binding proteins are known with exquisite selectivity and sensitivity toward their substrate or substrates. Among these, the binding protein GBP^[7] was our choice as the model recognition protein for the proof-of-principle in the creation of bioluminescent molecular switches. GBP is a periplasmic binding protein

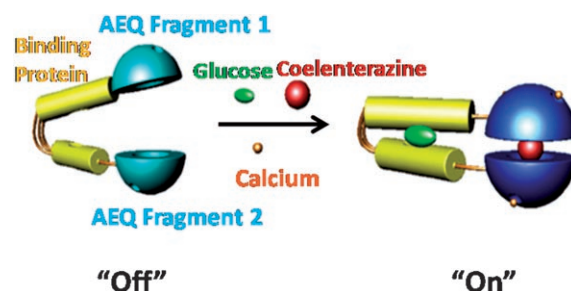


Figure 1. The protein switch, showing the components and triggers resulting in switching from the “off” to “on” mode.

capable of binding glucose with high selectivity.^[8] The protein consists of two globular domains linked by a flexible hinge region. Glucose binds to the hinge region, causing a conformational change, which brings the two domains together to engulf the substrate.^[9] This conformational change has been previously employed in the development of sensitive, selective, and reversible GBP-based fluorescence biosensors for glucose.^[7,10,11] It is well established that bioluminescence detection offers significant advantages over fluorescence.^[12] Thus, employing bioluminescence in the creation of a molecular switch should result in highly sensitive systems with low detection limits. To that end, we selected the photoprotein aequorin as the bioluminescent generating partner for our glucose molecular switch.

Aequorin consists of an apoprotein and a chromophore that resides in a hydrophobic binding pocket. In the presence of molecular oxygen and calcium ions, AEQ undergoes a conformational change that leads to the oxidation of the chromophore, coelenterazine, to coelenteramide with release of CO₂ and a concomitant emission of light at 469 nm.^[13] AEQ has three calcium-binding EF hands^[14] (EF hand I, III, and IV), and three triads each consisting of tryptophan, tyrosine, and histidine, which are involved in holding coelenterazine in the active site (Figure 2).^[15] Thus, these EF hands and triads are essential for the maximum activity of the protein, and their disruption could lead to loss of protein stability and bioluminescence. The creation of our molecular switch involved a rational strategy where the structure of AEQ was split into two fragments, and GBP was inserted between those fragments. In the resulting hybrid protein, the split AEQ fragments are too far apart to reassemble, and thus the molecular switch is “off”. However, the presence of glucose induces a conformational change in GBP that allows for the AEQ fragments to come together and freely re-form into one bioluminescent active entity, turning the switch “on”. A comparison of the CD spectra of GBP-AEQ in the presence or absence of glucose has shown that the protein tertiary structure rearranged upon binding glucose. (see the Supporting Information). The three-dimensional structure of AEQ demonstrates an exterior flexible loop between EF hand I and EF hand II into which we hypothesized that a large sequence might be inserted without destroying the activity of AEQ (Figure 2). Thus, GBP was inserted between amino acids 47 and 48 (Figure 3). Indeed, neither the calcium-binding sites nor the amino acids that interact with coelenterazine were disrupted. To the best of our knowledge, this is the

[*] K. Teasley Hamorsky, Dr. C. M. Ensor, Dr. Y. Wei, Prof. S. Daunert
Department of Chemistry
University of Kentucky
Lexington, KY 40506-0055 (USA)
Fax: (+1) 859-323-1069
E-mail: daunert@uky.edu

[**] This work was supported in part by grants from the National Institutes of Health and the National Aeronautics and Space Administration. We thank the Office of the Vice President of Research at the University of Kentucky for a University Research Professorship to S.D. K.T. acknowledges support from a Gill Fellowship, and S.D. from a Gill Eminent Professorship.

Supporting information for this article, including details of the experimental procedures, is available on the WWW under <http://www.angewandte.org> or from the author.

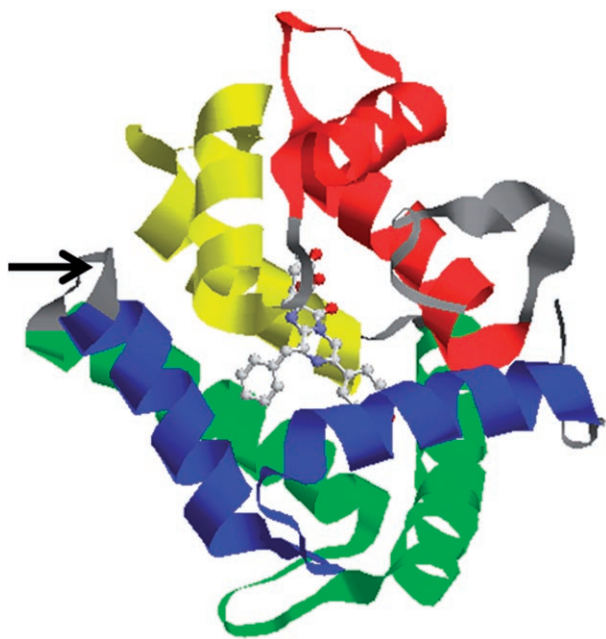


Figure 2. Three-dimensional structure of aequorin (AEQ) with coelenterazine (1J3; molecule visible in the center, with C gray, N blue, O red). EF Hand I shown in blue, EF hand III yellow, EF hand IV red, non-calcium-binding hand II green. The arrow indicates the insertion site.



Figure 3. The three-dimensional structure of the GBP-AEQ molecular switch in solution. The glucose-binding protein (GBP, right) is inserted into the split aequorin (left, between hands I (blue) and II (green)).

first time that AEQ has been split into fragments, and that the AEQ fragments have demonstrated “on” bioluminescence upon a molecular recognition event.

In the presence of glucose, the molecular switch showed an increase in bioluminescence intensity proportional to the levels of glucose, with glucose detection observed down to 1.0×10^{-7} M (Figure 4). The switch is “on” and “off” between 1.0×10^{-7} and 1.0×10^{-2} M concentrations of glucose, which encompasses the normal, hypoglycemic, and hyperglycemic

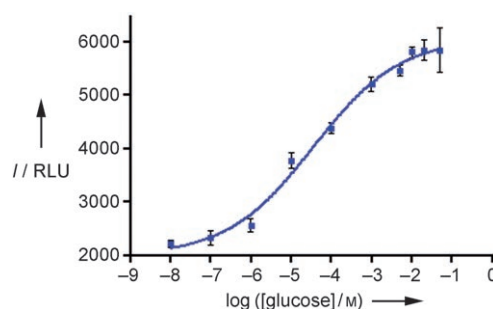


Figure 4. Glucose response with intensity I in relative light units (RLU). The performance of the molecular switch to sensing glucose was evaluated by employing a concentration of 5.0×10^{-8} M of hybrid protein. The data points are an average of three measurements ± 1 standard deviation.

glucose levels in blood, from 2 to 20 mM glucose. Furthermore, the sugars known to not bind to GBP had no effect on the molecular switch, showing that GBP confers its high selectivity pattern for glucose to the GBP-AEQ switch (Figure 5). Thus, the molecular switch could be useful in the clinical detection of glucose.

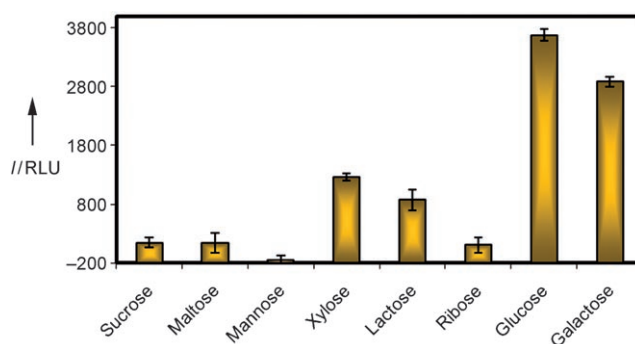


Figure 5. Light intensity I in the presence of 10 mM of various sugars. For each sugar, I is given as the difference between the value with and that without sugar present. The data points are an average of three measurements ± 1 standard deviation.

In summary, we have demonstrated that genetic manipulation to create a hybrid protein of two unrelated proteins, namely AEQ and GBP, can result in a functional bioluminescent molecular switch in which the on/off mode is tuned by the recognition of GBP toward glucose. This is the first time that aequorin has been rationally split into two fragments that, upon a molecular recognition event, come together and emit bioluminescence forming the basis for the creation of the first bioluminescent molecular switch. More significantly, this work demonstrates the generation of a whole new family of genetically encoded bioluminescent molecular switches by introduction of a binding protein into the structure of the spliced AEQ. A variety of hinge-motion binding proteins, such as periplasmic binding proteins, enzymes, transcriptional regulators, or messenger proteins could be employed in such molecular switches. It is envisioned that these newly created protein chimeras with distinct properties and functions could

be employed in a variety of applications, such as in highly sensitive and selective biosensing nanosystems, or as functional building blocks for the fabrication of bottom-up smart bionanomaterials with unique characteristics for the incorporation into nanodevices. Further, this work provides useful insight into the areas of genetically engineered chimeric proteins, allowing for the design and production of a series of other unique designer biomolecules.

Received: September 26, 2007

Revised: December 12, 2007

Published online: March 27, 2008

Keywords: binding proteins · luminescence · molecular switches · photoproteins · protein engineering

-
- [1] P. Fortina, L. J. Kricka, S. Surrey, P. Grodzinski, *Trends Biotechnol.* **2005**, 23, 168.
- [2] M. Ostermeier, *Protein Eng. Des. Sel.* **2005**, 18, 359.
- [3] G. S. Baird, D. A. Zacharias, R. Y. Tsien, *Proc. Natl. Acad. Sci. USA* **1999**, 96, 11241.
- [4] N. Doi, H. Yanagawa, *FEBS Lett.* **1999**, 453, 305.
- [5] M. S. Siegel, E. Y. Isacoff, *Neuron* **1997**, 19, 735.
- [6] M. Ferraz Rosa, A. Vera, A. Aris, A. Villaverde, *Microb. Cell Fact.* **2006**, 5, 15.
- [7] E. A. Moschou, L. G. Bachas, S. K. Deo, *Anal. Chem.* **2006**, 78, 6692.
- [8] L. L. E. Salins, R. A. Ware, C. M. Ensor, S. Daunert, *Anal. Biochem.* **2001**, 294, 19.
- [9] L. A. Luck, J. J. Falke, *Biochemistry* **1991**, 30, 6484.
- [10] M. Fehr, S. Lalonde, I. Lager, M. W. Wolff, W. B. Frommer, *J. Biol. Chem.* **2003**, 278, 19127.
- [11] K. Deuschle, S. Okumoto, M. Fehr, L. L. Looger, L. Kozhukh, W. B. Frommer, *Protein Sci.* **2005**, 14, 2304.
- [12] M. Mirasoli, S. K. Deo, J. C. Lewis, A. Roda, S. Daunert, *Anal. Biochem.* **2002**, 306, 204.
- [13] O. Shimomura, F. H. Johnson, *Tetrahedron Lett.* **1973**, 14, 2963.
- [14] F. I. Tsuji, S. Inouye, T. Goto, Y. Sakaki, *Proc. Natl. Acad. Sci. USA* **1986**, 83, 8107.
- [15] J. F. Head, S. Inouye, K. Teranishi, O. Shimomura, *Nature* **2000**, 405, 372.
-