ELECTROCHEMICAL METHODS FOR THE CONTROL AND MEASUREMENT OF BIOLOGICAL ACTIVITY

Technical Journal Club I March 14, 2017

Andreia D. Magalhães

MD-PhD Program



Zentrum für Neurowissenschaften Zürich Neuroscience Center Zurich



Overview

- Introduction to Electrochemical biosensors
- Electrochemical Measurement of the β -Galactosidase Reporter from Live Cells: A Comparison to the Miller Assay
- Electronic control of gene expression and cell behaviour in Escherichia coli through redox signalling

SyntheticBiology Research Article	nature
Electrochemical Measurement of the β-Galactosidase Reporter from Live Cells: A Comparison to the Miller Assay Tanya Tschirhart, ^{‡,§,†} Xinyi Y. Zhou, ^{‡,†} Hana Ueda, ^{§,⊥} Chen-Yu Tsao, ^{‡,§} Eunkyoung Kim, [§] Gregory F. Payne, ^{‡,§} and William E. Bentley ^{*,‡,§} [‡] Fischell Department of Bioengineering, [§] Institute for Bioscience and Biotechnology Research, [⊥] Department of Mathematics, University of Maryland, College Park, Maryland 20742, United States	ARTICLE Received 31 Mar 2016 Accepted 21 Nov 2016 Published 17 Jan 2017 DOL: 10.1038/ncomms14030 OPEN Electronic control of gene expression and cell behaviour in Escherichia coli through redox signalling Tanya Tschirhart ¹ , Eunkyoung Kim ¹ , Ryan McKay ^{1,2} , Hana Ueda ^{1,3} , Hsuan-Chen Wu ¹ , Alex Eli Pottash ^{1,2} , Amin Zargar ² , Alejandro Negrete ⁴ , Joseph Shiloach ⁴ , Gregory F. Payne ^{1,2} & William E. Bentley ^{1,2}

Electrochemical biosensors

• Biosensor: device that is able to convert a biological event into a quantifiable signal thus allowing the analysis and processing of a biological sample as an electronic signal



Grieshaber D, et al. Sensors, 2008

Electrochemical biosensors

- Electrochemical biosensors contain a biological event recognition element (enzyme, antibodies, etc.) that reacts with a high sensitivity and specificity with a target analyte and produces an electrical signal
- First biosensor: glucose oxidase (GOx) biosensor in 1962, developed by Clark
- After that and over the past decades several devices have been developed
- Biosensors can be widely applied to medical diagnostics, food quality, environmental monitoring, etc.
- Global market of about \$7 billion, with predominance of at-home health monitoring devices

Ronkainen N, et al. *Chem. Soc. Rev.*, 2010 Lee TM, et al. Sensors, 2008 Grieshaber D, et al. Sensors, 2008



pubs.acs.org/synthbio

Electrochemical Measurement of the β -Galactosidase Reporter from Live Cells: A Comparison to the Miller Assay

Tanya Tschirhart,^{‡,§,†} Xinyi Y. Zhou,^{‡,†} Hana Ueda,^{§,⊥} Chen-Yu Tsao,^{‡,§} Eunkyoung Kim,[§] Gregory F. Payne,^{‡,§} and William E. Bentley^{*,‡,§}

[‡]Fischell Department of Bioengineering, [§]Institute for Bioscience and Biotechnology Research, [⊥]Department of Mathematics, University of Maryland, College Park, Maryland 20742, United States

- The β-Galactosidase assay provides a simple and economical quantitative estimate of lacZ expression by measuring enzyme activity directly
- Ortho-nitrophenyl-β-D-galactopyranoside (ONPG) is the artificial chromogenic substrate used for this assay
 - It is hydrolysed to ONP (that is quantified by measuring absorbance) and galactose
- The extent of the enzymatic reaction can be used to compare gene expression across samples
- But this assay requires cell lysis that thus prevents continuous measurements
- The redox-active molecule p-aminophenyl β-D-galactopyranoside (PAPG) can also be used as a substrate for electrochemically measuring β-gal activity allowing for on-line monitoring of gene expression





Tschirhart T, et al. ACS Synth Biol, 2016







Tschirhart T, et al. ACS Synth Biol, 2016



Tschirhart T, et al. ACS Synth Biol, 2016



- First comparison of the electrochemical method for measuring β-gal activity in both lysed and intact cells to the gold-standard Miller assay
- The electrochemical method is a feasible alternative that enables added versatility for analysis of synthetic biology constructs, especially for real-time measurement
- This work connects, for the first time, the work of the biosensor community in enzymatic reporter detection, with the standard optical measurement techniques biologists use for the same purpose



Bacterial redox sensors

- Bacteria have evolved sensitive and specific sensors to monitor different redox signals
- These sensors convert the redox signals into regulatory outputs, which allow them to adapt to the altered redox environment
- The sensing mechanisms are many and varied
 - Thiol-based
 - Fe–S cluster-based
 - **soxR** <u>superox</u>ide response <u>r</u>egulator
 - Haem-based
 - Flavin cofactor-based
 - Pyridine nucleotides
 - Etc.

Tschirhart T, et al. Nat Commun, 2017

Green J, et al. Nature, 2004

Bacterial redox sensors

- SoxR is a dimeric transcription factor, with each monomer containing a [2Fe–2S] cluster that is maintained in a reduced form by NADPH-dependent enzymes
- When oxidized (for example, by redox-cycling drugs), SoxR activates the SoxS promoter which, in turn, regulates other genes, mainly with the aim of detoxifying the cell



Bacterial redox sensors

- Pyocyanin (Pyo) is used as an inducer of the E. coli SoxRS regulon
- Ferricyanide (Fcn) is used as an electronically regulated electron acceptor allowing the amplification of the intracellular Pyo redox cycling that leads to SoxR-mediated transcription
- Ferricyanide is approximately five orders of magnitude more soluble than oxygen in water and can be used as an alternative terminal electron acceptor in the respiratory pathway
- The reduced form, ferrocyanide, can be quantified by electrochemical methods and thus be quantitatively related to cell growth and/or cell activity



















- Use of biologically relevant redox molecules in translating electronic signals to changes in engineered bacterial gene expression
- Characterization of a functioning reversible and dose-dependent bacterial electrogenetic device coupling Pyodriven SoxR activation with electronic control of Fcn(O/R) redox form
 - Minimal rewire of cells to take advantage of native redox interactions
- This system can be tailored to produce a variety of responses and guide various behaviours
- New way of using redox molecules and electron flow for guiding biological function in bacteria

Electrochemical methods for the control and measurement of biological activity

Questions?

Thank you for your attention

ELECTROCHEMICAL METHODS FOR THE CONTROL AND MEASUREMENT OF BIOLOGICAL ACTIVITY

Technical Journal Club I March 14, 2017

Andreia D. Magalhães

MD-PhD Program



Zentrum für Neurowissenschaften Zürich Neuroscience Center Zurich

