Title:
Validation of biological recognition elements for signal transduction as first step in the development of whole cell biosensors

Authors & affiliations:
Luis F. Spitta, Christa Baumstark-Khan, Sebastian Diegeler, Sebastian Feles, Bernd Henschenmacher, Bikash Konda, and Christine E. Hellweg
DLR-Institute of Aerospace Medicine, Linder Höhe, 51147 Köln, Germany

Abstract: (Your abstract must use Normal style and must fit in this box. Your abstract should be no longer than 300 words. The box will 'expand' over 2 pages as you add text/diagrams into it.)
Preparation of Your Abstract
1. The title should be as brief as possible but long enough to indicate clearly the nature of the study. Capitalise the first letter of the first word ONLY (place names excluded). No full stop at the end.
2. Abstracts should state briefly and clearly the purpose, methods, results and conclusions of the work.
Introduction: Clearly state the purpose of the abstract
Methods: Describe your selection of observations or experimental subjects clearly
Results: Present your results in a logical sequence in text, tables and illustrations
Discussion: Emphasize new and important aspects of the study and conclusions that are drawn from them

Choosing the proper combination of receptor element, cell type and measurable signal requires major consideration for developing cell-based biosensors. In order to use physiologically relevant cellular responses towards (geno)toxic conditions, information on the mechanism of action and of the expected outcome of exposure needs to be considered.
Two mammalian cells’ signaling pathways acting in response to genotoxic stress were investigated with respect to contribute to possible biosensor development: Nuclear factor-xB (NF-xB) and Nuclear factor erythroid 2-related factor (NRF2) signaling. NF-xB functions as transcription factor that controls a broad range of biological processes connected to anti-apoptotic and inflammatory cellular responses. In the non-induced state, NF-xB is retained in the cytosol by binding to IxB proteins; upon activation by genotoxins IxB is phosphorylated by an ATM-dependent IKK complex and targeted for proteasomal degradation thereby liberating NF-xB for translocation to the nucleus where it induces target gene expression. Nrf2 plays an important role in the antioxidant response pathway. In non-induced state NRF2 is cytoplasmatically inhibited by the cytoskeletal Keap1 protein. Oxidative stress conditions lead to Nrf2 liberation and its nuclear translocation. Nrf2 binding to the ARE (antioxidant response element) region of their promoters induces gene expression of target genes coding for antioxidant enzymes protecting the cell from oxidative damage.

Figure 1: The genotoxic stress-induced NF-xB subpathway and the oxidative stress-induced Nfr2 pathway:
in the non-induced state the transcription factors NF-xB and Nfr2 are hold in the cytoplasm by their inhibitors IxB and Keap1; upon activation the transcription factors are set free from their inhibitory proteins and translate to the nucleus where they enhance transcription of their target genes involved in inflammation and cellular defense.
For monitoring DNA damage dependent signaling in human embryonic kidney cells (HEK/293), the Dual-Luciferase Reporter (DLR) Assay was used with the reporter plasmids pGL4.32 or pGL4.37 permitting NF-κB or Nrf2 controlled induced expression of firefly (*Photinus pyralis*) luciferase and with the control reporter plasmid pRL-CMV providing constitutive expression of sea pansy (*Renilla reniformis*) luciferase. Time and concentration dependent analysis of luminescence induction by the classical NF-κB and ARE inducers tumor necrosis factor α (TNF-α) and tert-butylhydroquinone (tBHQ) show induction kinetics to be maximal at 5 to 8.5 h of incubation and at 18 h, respectively. For both reporter pathways a concentration dependency in luminescence induction by the inducers under investigation was demonstrated over a wide concentration range (from 0.3 to 20 ng ml⁻¹ for TNF-α and from 12.5 to 200 µmol l⁻¹ for tBHQ).

![Figure 2](image1.png)  
**Figure 2:** Dual luciferase assay for tBHQ induced Nrf2 dependent signaling in transiently transfected HEK/293 cells after 18 h of incubation with the inducer

![Figure 3](image2.png)  
**Figure 3:** Dual luciferase assay for TNF-α induced NF-κB dependent signaling in transiently transfected HEK/293 cells after 5 h incubation with the inducer. Maximal induction is about 15-fold, half maximal induction is achieved with 3.8 ng ml⁻¹ TNF-α
**Title:** Validation of biological recognition elements for signal transduction as first step in the development of whole cell biosensors

**Authors:** L.F. Spitta, C. Baumstark-Khan, S. Diegeler, S. Feles, B. Henschenmacher, B. Konda, C.E. Hellweg

**Presenting Author:** L.F. Spitta

---

**ABSTRACT REF NO: BIOS2016_1392**

Biosensors 2016

25–27 May 2016

Gothenburg, Sweden


referiert

Poster

Nicht eingeladen