Early diagnosis is central to curing colorectal cancer.
Cell-based biosensors

Traditional biosensors:
- Single input
- Single output

Cell-based biosensors:
- Self-manufacturing
- Easy-to-use on-site
- Multiple outputs
- Feedback loops
Cell-based biosensors within iGEM

- iGEM Peking 2013 – Aromatic Scouts
  - Benzoic acid
- iGEM Groningen 2012 – Food Warden
- iGEM Wageningen 2014 – The BananaGuard
  - Fusaric acid
Similar course of action

Extracellular environment

Diffusion

Signaling proteins

Translation

Transcription

Promotor

Transcription
COMBs
Clickable Outer Membrane Biosensors
An aptamer-based approach to a modular and universal sensor system
Scaffold – Outer Membrane Proteins

OmpX

p-Azido-L-phenylalanine (pAzF)
Orthogonal SPAAC Click reaction

Recognition element
Orthogonal SPAAC Click reaction

Recognition Element
Recognition element - Aptamers

Source: Flandersfood
Signal domain

BRET

Split luciferase
BRET – NanoLuc & mNeonGreen

Efficient energy transfer is not possible

Resonance Energy Transfer

Intensity

Intensity
Split luciferase - NanoBit

A)

B)

Substrate → Product + Light
Plasmid Design

- pETDuet-1
- pEVOL-pAzF
Plasmid Design

- Transcription
- Translation
Steps towards a working sensor

1. Localization in the outer membrane & the click reaction
2. Testing whether the signaling components work
3. Testing whether DBCO-functionalized DNA can be clicked and hybridized
4. Testing whether proximity invokes a response with a complementary DNA test
Membrane localization & click

Localization & Click

Signaling components

DNA-Click & Hybridization

Complementary DNA test

Incubation

Washing
Membrane localization & click

- Localization & Click
- Signaling components
- DNA-Click & Hybridization
- Complementary DNA test
Functioning of the signal domain

- Localization & Click
- Signaling components
- DNA-Click & Hybridization
- Complementary DNA test

OmpX-NanoLuc & OmpX-mNeongreen

Bioluminescence (a.u.)

Wavelength (nm)
Functioning of the signal domain

- Localization & Click
- Signaling components
- DNA-Click & Hybridization
- Complementary DNA test

OmpX-NanoBit

![Graph showing bioluminescence vs. wavelength with OmpX-NanoBit, negative controls, and NanoBit emission marked.]

- Bioluminescence (a.u.)
- Wavelength (nm)
Clicking and hybridizing DNA

- Localization & Click
- Signaling components
- DNA-Click & Hybridization
- Complementary DNA test

Incubation → Washing → Cy5

& → TAMRA
Clicking and hybridizing DNA

- Localization & Click
- Signaling components
- DNA-Click & Hybridization
- Complementary DNA test

DNA Hybridization Test

Cy5

TAMRA
Complementary DNA test

- Localization & Click
- Signaling components
- DNA-Click & Hybridization
- Complementary DNA test

Spacer

1* → 2*
Complementary DNA test

- Localization & Click
- Signaling components
- DNA-Click & Hybridization
- Complementary DNA test

Complementary DNA assay with mNeongreen and Nanoluc

Fluorescence (a.u.)

Wavelength (nm)

- Clicked DNA with complementary DNA
- Clicked DNA without complementary DNA
- mNeongreen emission
- NanoLuc Emission
Complementary DNA test

- Localization & Click
- Signaling components
- DNA-Click & Hybridization
- Complementary DNA test

Graph: Complementary DNA assay with NanoBit

- Clicked DNA with complementary DNA
- Clicked DNA without complementary DNA
- NanoBit Emission
Complementary DNA test

- Localization & Click
- Signaling components
- DNA-Click & Hybridization
- Complementary DNA test

**DNA Triggered Signaling - NanoBiT**

- **Bioluminescence (a.u.)**
- **Concentration oligo (nM)**

**DNA Triggered Signaling - Negative Control**

- **Bioluminescence (a.u.)**
- **Complementary DNA (nM)**
In silico troubleshooting

• Model COMBs in *E. coli* to increase the signal
• Considered expression levels
In silico result

BRET signal increase on ligand binding

Signal increase (%) vs. Ligand concentration (nM)

- 10,000 COMBs
- 50,000 COMBs
Future

• Decrease protein expression levels
• Test with aptamers
• Coupling COMBs to signaling pathways
• Three application scenarios: 
  Q fever 
  pesticide overuse and 
  detection of intestinal disease

• Involving stakeholders

• Discussed synthetic biology with 
  students and lay people

• Co-organized a symposium
Achievements

• Expressed and characterized individual COMBs
• Modeled COMBs in *E. coli*
• Registered 6 new parts, including NanoBiT and mNeonGreen
• First step towards a universal and modular cell-based biosensor system
The Cloning guide

The first step towards a successful iGEM Project

Integrated DNA Tech
@idtdna

#iGEM collaboration at its finest: The Cloning Guide
idtb.io/SFQzD @Eindhoven_iGEM #iGEM2015
pic.twitter.com/e4arVHUOZ4

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2 likes
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COMBs
Clickable Outer Membrane Biosensors
Generation of Aptamers

Combinatorial Nucleic Acid Synthesis

Selection by incubation & washing

Amplification and mutation (e.g. via a dirty PCR)

Typically 8-15 times

Recovery of selected oligonucleotides

High-affinity oligo’s
Bio-orthogonal tRNA & tRNA synthetase

Stop Codon

Non-natural Release Factor

Peptide chain with non-natural amino acid

Peptide chain
Protein Expression

$pEVOL-pAzF$  $pETDuet-1$

Transcription

Translation
Two components:
- Stochastic diffusion model for ligand binding
- Monte-Carlo Tethered Particle Simulation to simulate linker
Dimerization of DNA-fluorophores to clicked DNA

[A] OmpX-NanoLuc & OmpX-mNeonGreen
With DBCO-DNA and complementary DNA-fluorophores

[B] OmpX-NanoLuc & OmpX-mNeonGreen
Without DBCO-DNA, with complementary DNA-fluorophores