BRET-BASED AFFINITY ASSAY FOR CREATININE IN BLOOD PLASMA WITH SMARTPHONE READOUT

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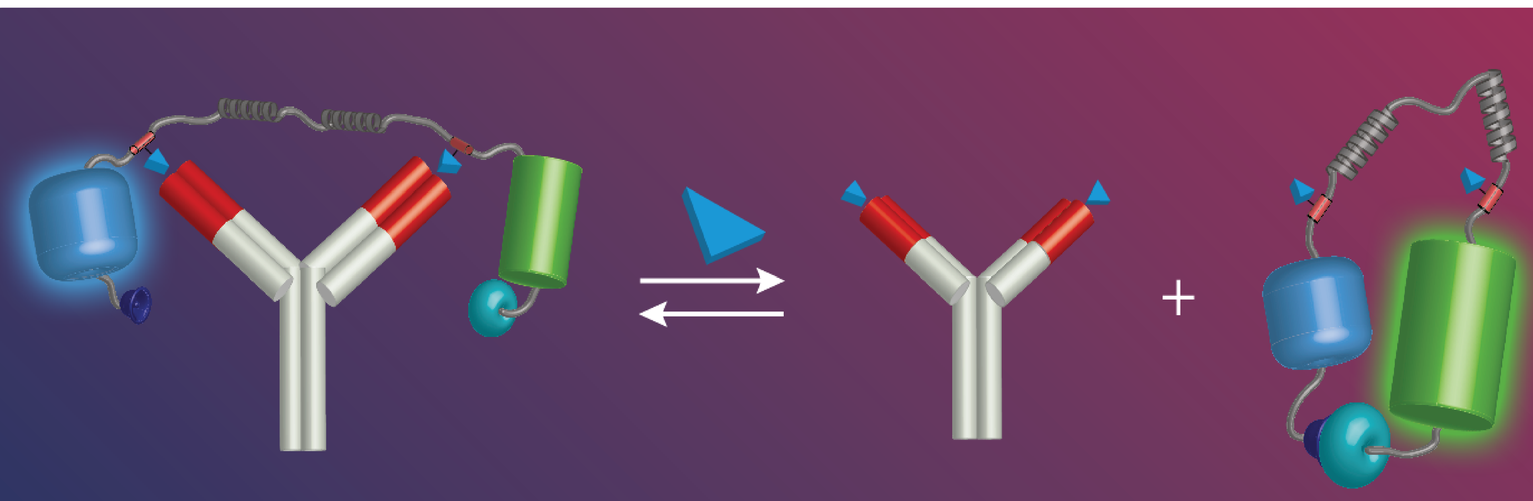
INTRODUCTION

TU Eindhoven SensUs Team 2016 has developed a novel, modular creatinine biosensor based on a bioluminescence resonance energy transfer (BRET) affinity assay for determination of creatinine concentration in blood plasma. The readout is performed by ratiometric color analysis using a smartphone. Therefore, this biosensor is suitable for point-of-care applications.



The single-protein sensor consists of a blue-light emitting luciferase NanoLuc, connected via a semi-flexible linker to the green fluorescent protein mNeonGreen. In the linker, two anti-creatinine antibody epitopes are introduced. These will compete with creatinine for anti-creatinine antibodies. Binding of creatinine to the antibodies results into release and recombination of the protein complex by helper domains, shifting the emission from blue to green due to BRET. [1]

METHODS

PROTEIN COMPLEX FUNCTIONALIZATION

Introduction of the unnatural amino acid para-azidophenylalanine (pAzF) in the protein sequence [2] allows incorporation of creatinine epitopes via dibenzocylcooctyl (DBCO) strain promoted click chemistry (Figure 1).

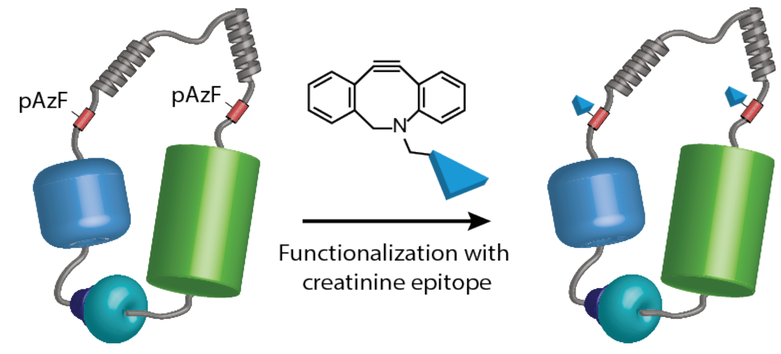


Figure 1. A pET28a plasmid encoding the BRET-sensor was transformed in *E. coli* BL21 cells. pAzF was introduced via a pEVOL vector encoding a tRNA/tRNA synthetase pair. [3] Subsequently, DBCO-creatinine was conjugated to the BRET-sensor using click chemistry.

CREATININE EPITOPE SYNTHESIS

DBCO-creatinine is synthesized (Figure 2A) and subsequently clicked to obtain fully functional anti-creatinine antibody recognition sites on the BRET-sensor. NMR and LCMS confirms the purity of DBCO-creatinine.

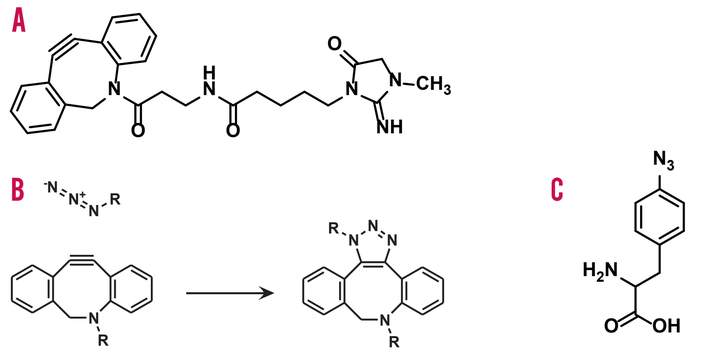


Figure 2. A: DBCO-creatinine is synthesized in a three step synthesis with an overall yield of 28%. [4] B: DBCO click reaction to functionalize BRET-sensor. C: para-azidophenylalanine which is incorporated in the BRET-sensor to facilitate the DBCO click reaction.

CLICK REACTION CONFIRMATION

Anti-creatinine antibody addition causes a decrease in green emission intensity proving successful incorporation of DBCO-creatinine (Figure 3).

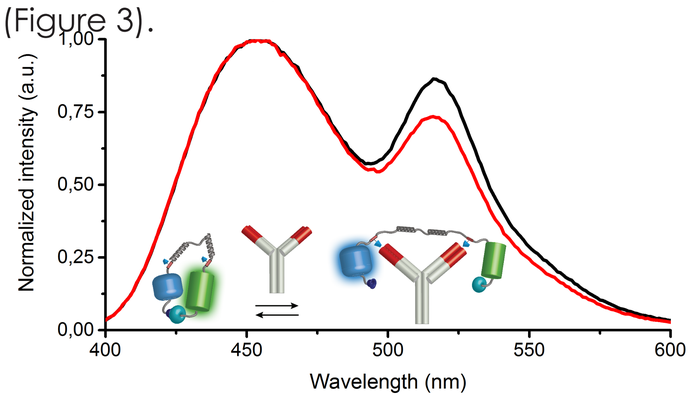
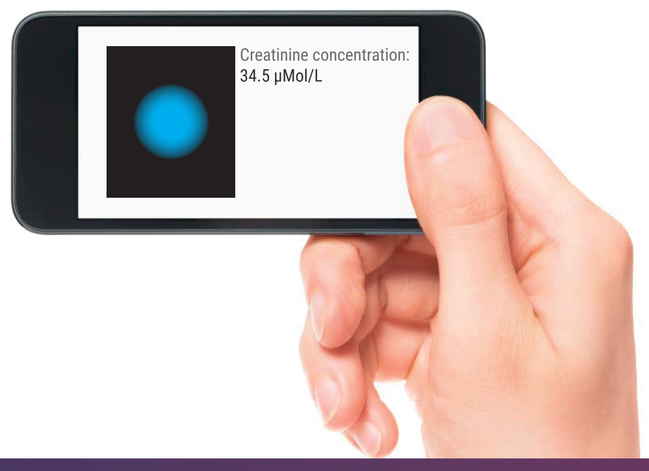


Figure 3. Emission spectrum of BRET-sensor (5 nM) with (red) and without (black) anti-creatinine antibodies. After antibody addition (1 µM) the peak of mNeonGreen at 520 nm is decreased in intensity, indicating a functional BRET sensor. The insert shows the reaction of the antibody with the BRET-sensor.

SMARTPHONE READOUT

- Optical readout using camera
- 3D printed readout adapter for Samsung Galaxy S7 and Nokia Lumia 920
- Cartridge-based readout system



RESULTS

- Responsive to creatinine in buffer and 100% bovine blood plasma
- Detection range from 100 µM – 10 mM in buffer

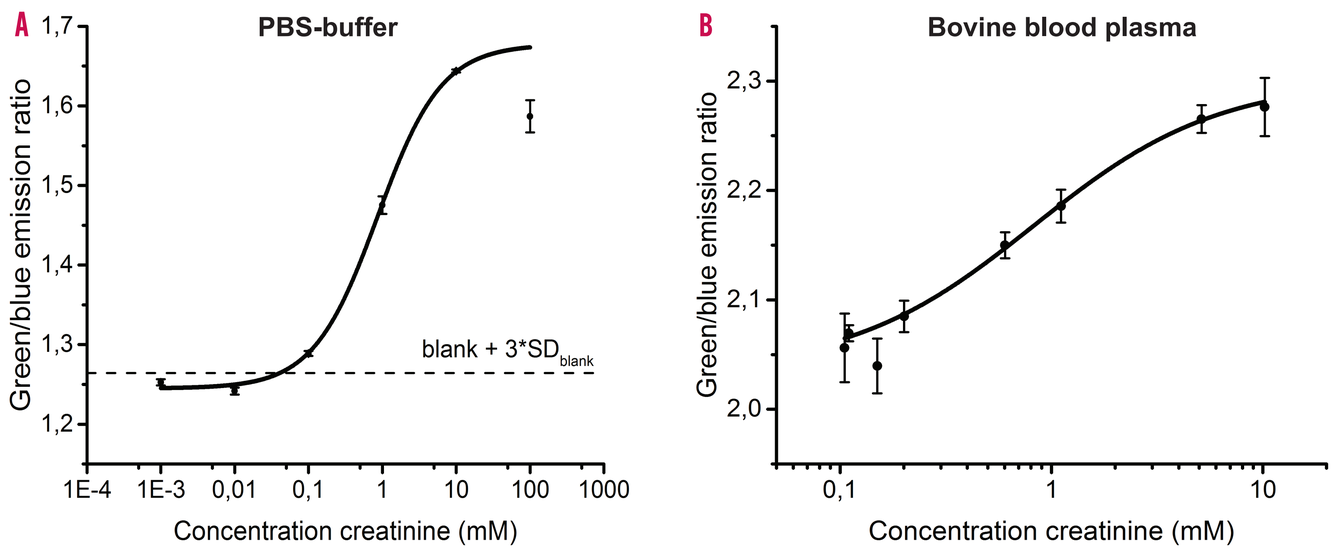


Figure 4. Creatinine titration experiments of BRET-sensor (5 nM) (A) in buffer solution and (B) in 100% plasma. A magenta filter is used to measure the intensity of 400-450 nm and a green filter to measure the intensity of 500-550 nm. Experiments are performed in a 384 well plate in a Tecan Infinite plate reader.

[1] R. Arts, I. den Hartog, S.E. Zijlema, V. Thijssen, S.H. van der Beelen and M. Merkx (2016). Detection of Antibodies in Blood Plasma Using Bioluminescent Sensor Proteins and a Smartphone. *Analytical Chemistry*, 88(8), pp. 4525-4532.
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SensUs T.E.S.T. RANDOX Jena Bioscience www.jenabioscience.com TU/e

THE FUTURE OF T.E.S.T.

INTRODUCTION

In the Netherlands, 1.7 million people suffer from chronic kidney damage and early detection is necessary to prevent kidney failure. Especially patients with diabetes form a high-risk group [1]. Therefore, it is important to screen and monitor the kidney function over time. Creatinine is an indicator that can be used to identify kidney damage. Elevated creatinine levels correspond to a decreased kidney function. For this reason, student team T.E.S.T. has developed a new biosensor which can measure the creatinine concentration in blood plasma.

	Competing Systems	Performance of T.E.S.T.
Precision	0.1 mg/dl	t.b.d.
Range	0.2-20 mg/dl	1.1-110 mg/dl
Sample Volume	1.2 µL	20 µL
Speed	30 sec	5 min
Cost per Instrument	€ 400,-	€ 20,-
Cost per Cartridge	€ 7,-	€ 0,67

SCIENCE

The used assay technology is suitable for other biomarkers, as previous research at the TU/e proved this modular design [2, 3]. The cartridge will be developed to contain one spot for each biomarker to be measured. Further development of the app allows a simultaneous measurement of these biomarkers, increasing clinical relevance.

APPLICATIONS

In the future a quick check at the general practitioner is possible with instant results. Follow up and treatment monitoring will be the future in home care settings.

BUSINESS

There are 5000 general practitioners in The Netherlands that form the initial consuming market for our biosensor [4]. When further developed, this biosensor could be used by patients themselves increasing the consuming market even more.

TECHNOLOGY

The app will be developed in such a way that it can run on different operating systems. Also a vibration function could be added so shaking isn't nescessary anymore. Also the readout will be optimized.

INDUSTRY

The cartridge and the device can be easily manufactured by applying readily available mass production methods such as injection molding. Protein engineering and chemical syntheses can be performed via medium laboratory scale set-ups.

[1] National Kidney Foundation. (2015). Diabetes - A Major Risk Factor for Kidney Disease.
[2] Arts, R., Den Hartog, I., Zijlema, S. E., Vito Thijssen, Van der Beelen, S. H. E., and Merkx, M. (2016). Detection of Antibodies in Blood Plasma Using Bioluminescent Sensor Proteins and a Smartphone.
[3] Griss, R., Schena, A., Reymond, L., Patiny, L., Werner, D., Tinberg, C. E., … Johnsson, K. (2014). Bioluminescent sensor proteins for point-of-care therapeutic drug monitoring.
[4] Landelijke Huisartsen Vereniging. (n.d.). Feiten en Cijfers Huisartsenzorg.

T.E.S.T.