BRET-based detection of succinate

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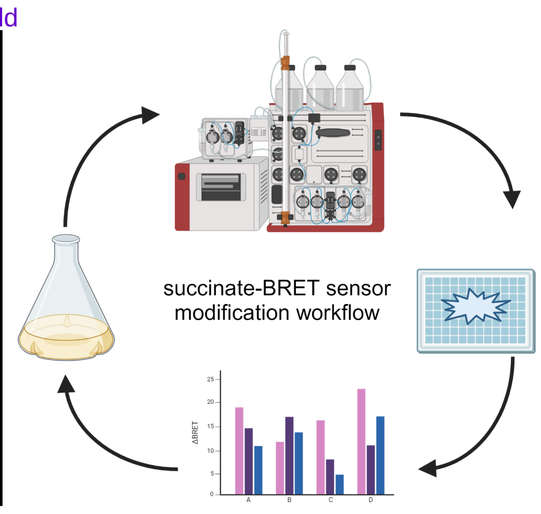
Structural basis and initial design of a BRET sensor for succinate

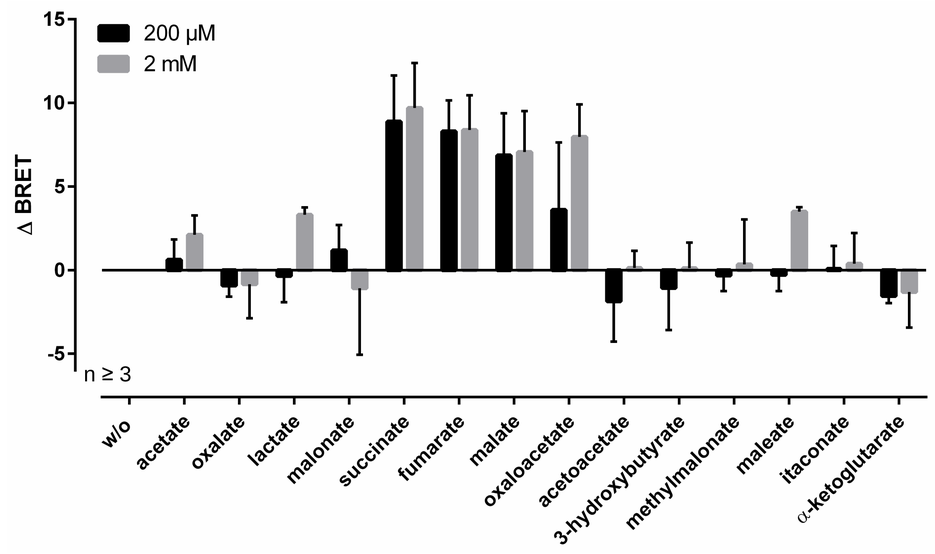
Succinate is a key intermediate of the tricarboxylic acid cycle, a fundamental pathway governing aerobic energy metabolism. Novel physiological roles for succinate have emerged including its involvement in muscle fiber remodeling and immunity. Accumulation of succinate has been observed in various pathological states including chronic inflammation, ischemia and cancer. Despite succinate‘s increasing significance, current methodologies for its detection *in vivo* or in live cells remain inadequate. This project aims to fill this gap with a succinate-specific BRET-Sensor that allows label-free detection and dynamic measurements of succinate concentrations under different metabolic conditions.

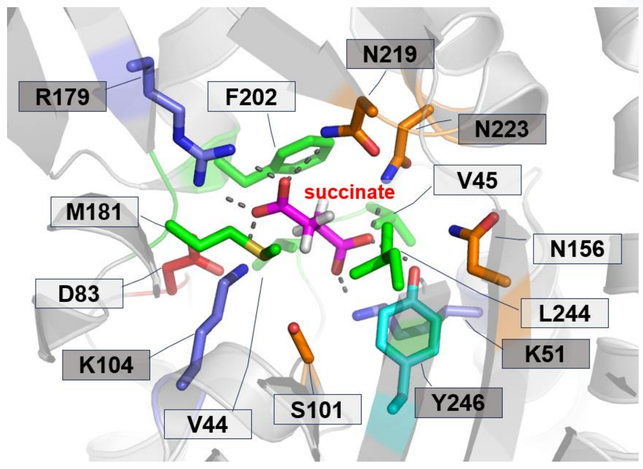
A potential succinate-BRET sensor was generated based on the periplasmic bacterial protein dctp1 from *Shewanella oneidensis* (PDB 4MX6) (with A01) The protein encoding the sensor has been successfully expressed in *E.coli* BL-21 (DE3). Protein purification is carried out via a 10xHis-tag and Ni-immobilized metal affinity chromatography. The purified protein was tested and evaluated.

4mx6 – succinate / SiaP, similar fold



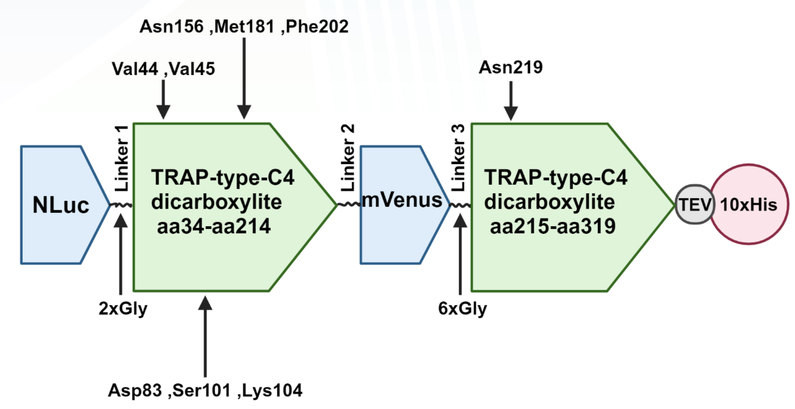
Carboxylic acids were categorized by chain length. The sensor displays specificity for C4-dicarboxylic acids. Given the coexistence of fumarate, malate, and oxaloacetate with succinate in biological fluids , there is a need to enhance the sensor's specificity.





-------H-bonds (dark background label)

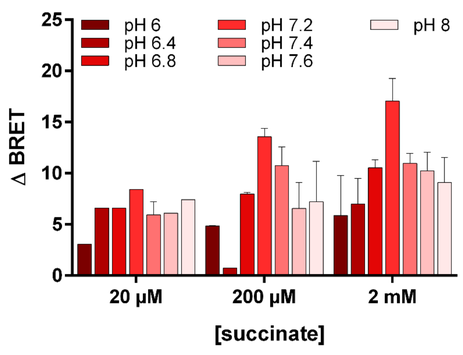
Red – negatively charged
Green – hydrophobic
Blue – positively charged
Orange – uncharged, hydrophilic
Cyan – tyrosine (aromatic, OH group)



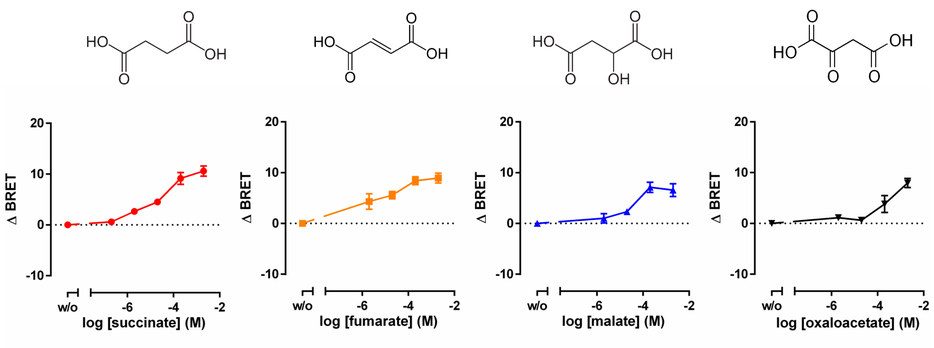
- 41 modifications planned
- 30 modifications expressed in BL-21 (DE3)
- 20 modifications tested via BRET-assay

At least 6 side chains are in direct interaction with succinate via H-bonds. The binding pocket is subdivided into microregions with specific biophysical properties. We want to modify these by site-directed mutagenesis to increase the specificity and sensitivity of the sensor. This could be achieved by narrowing the binding site to exclude larger substrates like oxaloacetate, which carries a keto-group at C2 that is absent in succinate, or by modifying the glycin-linker’s lengths and flexibility.

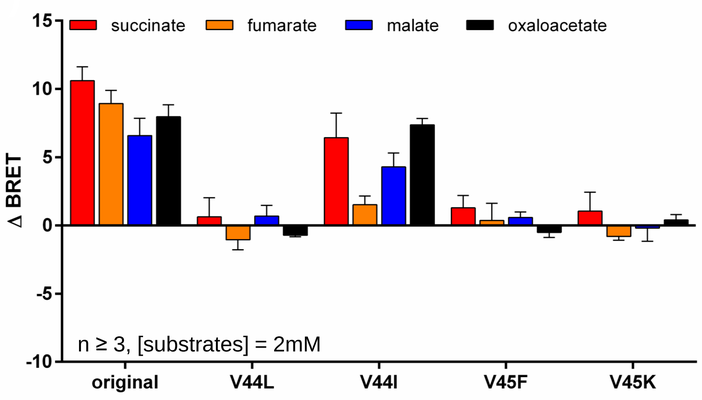
The sensor exhibits peak sensitivity at pH 7.2

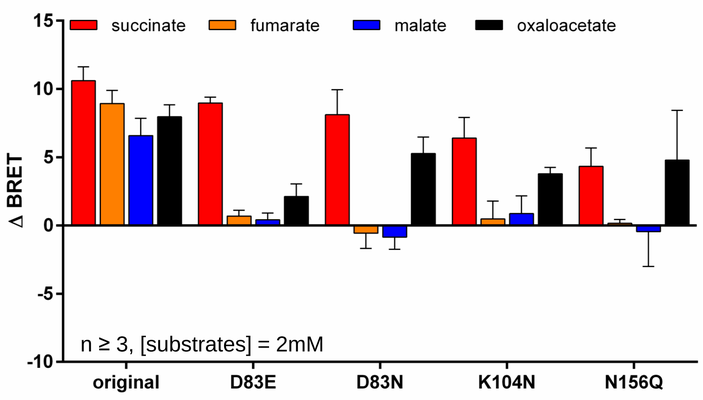


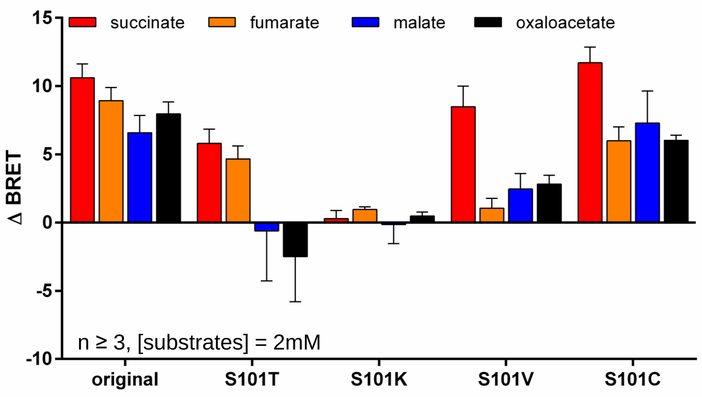
BRET response to increasing concentrations of C4-dicarboxylic acids

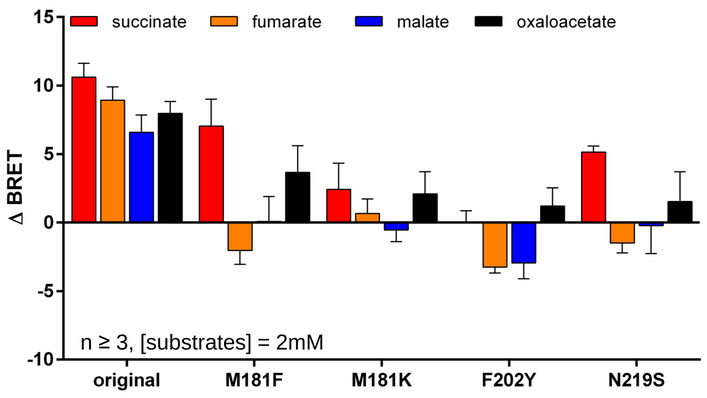


Mutational data

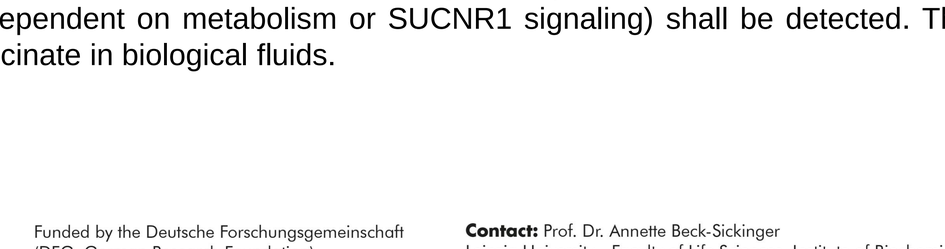








S101T exhibits specificity towards succinate and fumarate



2xGly in the first linker enhances sensitivity

The mutation S101T resulted in a substantial decrease in the signals for malate and oxaloacetate, likely due to a narrowing of the binding pocket. The signal for succinate and fumarate exhibited only a minor reduction. 2xGly in the first linker instead of 4xGly resulted in an amplification of the succinate signal compared to the original sensor. With the mutation D83E, a significant signal was only detectable upon stimulation with 2 mM succinate. It is conceivable that combining D83E with a 2xGly first linker could enhance the specificity and sensitivity of the sensor.

Outlook: Once the desired specificity and sensitivity are achieved for the succinate-BRET sensor protein, we will clone it into an eukaryotic expression vector, that allows for heterologous overexpression in eukaryotic cells. With that, intracellular succinate concentrations (e.g., dependent on metabolism or SUCNR1 signaling) shall be detected. The BRET sensor could also be utilized in a broad range of applications, such as a diagnostic tool to detect succinate in biological fluids.

Deutsche Forschungsgemeinschaft
German Research Foundation
UNIVERSITÄT LEIPZIG
CHARITÉ UNIVERSITÄTSMEDIZIN BERLIN
Heinrich Heine Universität Düsseldorf
MARTIN-LUTHER-UNIVERSITÄT HALLE-WITTENBERG

Funded by the Deutsche Forschungsgemeinschaft
(DFG, German Research Foundation)
SFB1423 - Project ID 421152132

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