LOSCHMIDT LABORATORIES

IRrIS: the first BRET-based bioluminescent system engineered in nature's image

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The principle and benefits of BRET

BRET = bioluminescence resonance energy transfer

- **Highly efficient** mechanism of **radiationless** transfer of energy from a donor bioluminescent enzyme (luciferase) to an acceptor fluorescent protein through a connection faciliated by **couloumbic interactions**
- The energy transfer occurs **over distances up to 100 Å** and requires proper orientation and energetical compatibility of donor and acceptor
- The result is **bioluminescence-powered fluorescence**, where the luciferase acts as a biocatalytic **engine powering a lightbulb** (the fluorescent protein), which may be exchanged or altered to fit the needs of various applications
- BRET-based bioimaging probes are **powerful, sensitive and highly customizable** alternatives to established fluorescent or bioluminescent probes, combining the benefits and mitigating the disadvantages of both approaches (**Fig. 1**)

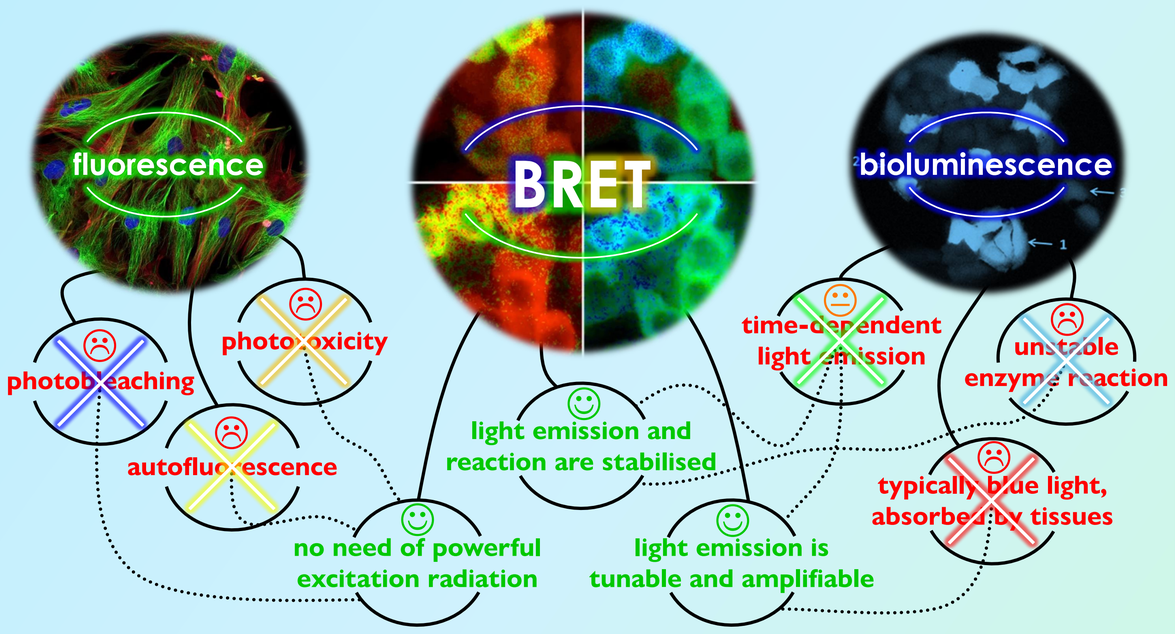
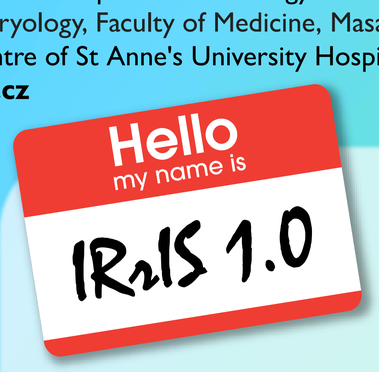


Fig. 1: The most significant disadvantages of utilisation of fluorescence and luminescence as probes for bioimaging and their comparison with bioimaging probes utilising BRET-based bioluminescence; dotted lines connect the corresponding properties relevant for *in vivo* bioimaging probes.



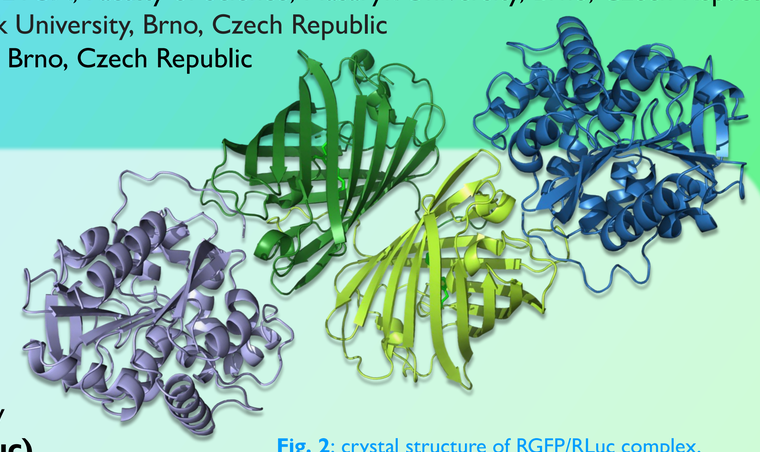


Fig. 2: crystal structure of RGFP/RLuc complex. RGFP molecules are in green, RLuc molecules are in blue.

- Genetic fusion of biologically compatible **luciferase (RLuc)** and **green fluorescent protein (RGFP)**, comprising the key actors in the bioluminescent system of *Renilla reniformis*
- Prototype **BRET-based bioluminescent system** engineered to mimic the natural structure and function of RGFP/RLuc complex (**Fig. 2**) showing technologically beneficial properties, but very poor stability and fuctional dependency on the reaction conditions
- Fusion in IRrIS stabilises the protein interaction **without interfering** with the natural interaction interface within RGFP/RLuc, preserving its character and beneficial traits
- IRrIS is a **competitive**, **stable** and **robust** bioluminescent system (**Fig. 3**), showing:
  - **superior light-emitting properties** including intensive, long-lasting emission with maximum at 509 nm, not easily absorbed by living tissues
  - **excellent stability and performance** in physiological conditions, enabling its use as an *in vivo* bioimaging probe
  - **~100% efficiency of energy transfer** from RLuc to RGFP via BRET

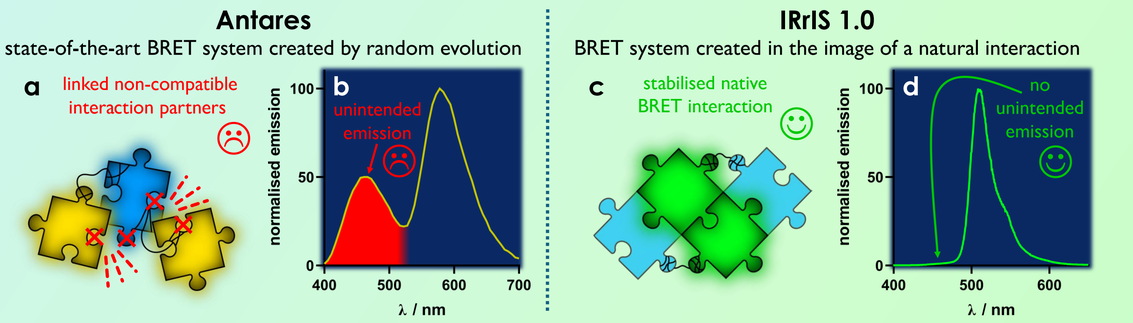


Fig. 3: Schematic model (**a**) and emission spectra (**b**) of a state-of-the-art BRET system Antares created by N- and C-terminal fusion of two molecules of *Entacmaea*-derived fluorescent protein with *Ophlophorus*-derived luciferase and its comparison with schematic model (**c**) and emission spectra (**b**) of IRrIS 1.0, created by an optimised fusion of all-*Renilla*-derived components.

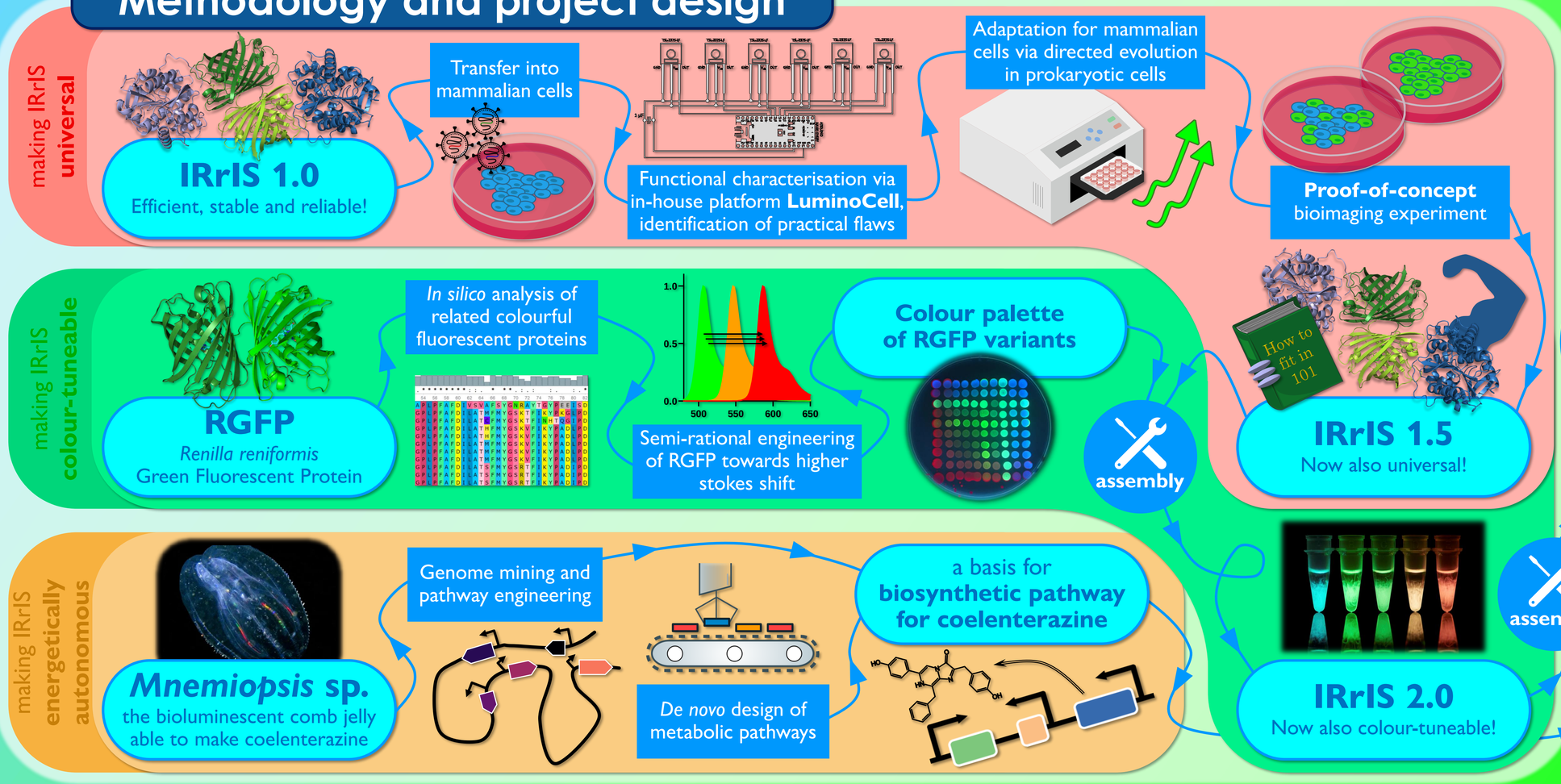
Objectives of the project

Building on the basis of the existing prototype of IRrIS ...

1) ...make IRrIS **universal** = the system should be able to work in both prokaryotic and eukaryotic cells
2) ...make IRrIS **colour-tuneable** = create a colour palette of IRrIS variants for various application purposes
3) ...lay the base for making IRrIS **energetically autonomous** = progress towards identification/construction of a biosynthetic pathway for coelenterazine (CTZ; the substrate of RLuc)

...to make IRrIS a complete and customizable system for use as a bioimaging probe!

Methodology and project design



Project prospects

IRrIS 3.0

Now better than ever before!

First BRET-based bioluminescent system **based on a natural BRET system** rather than *de novo* engineering, which is:

universal (able to work in eukaryotic and prokaryotic cells)

efficient (near 100 % BRET efficiency, not wasting any energy in the transfer)

stable (able of long-term function in a physiological environment)

reliable (with expectable output regardless of circumstances)

tuneable (with selection of output emission characteristics), and

energetically autonomous (able to supply its own energy as biosynthetically sourced luciferin),

optimised for use as **ultrasensitive probe** for real-time bioimaging!

making IRrIS **complete**

The first author is a Brno Ph.D. Talent Scholarship Holder – Funded by the Brno City Municipality

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