

Supplementary materials

New synthetic red- and orange-emitting luciferases to upgrade in vitro and 3D cell biosensing

Maria Maddalena Calabretta^{a,b}, Denise Gregucci^{a,b} and Elisa Michelini^{a,b,c*}

^aDepartment of Chemistry “Giacomo Ciamician”, University of Bologna, Via Selmi 2, 40126, Bologna, Italy

^bCenter for Applied Biomedical Research (CRBA), Azienda Ospedaliero-Universitaria Policlinico S. Orsola-Malpighi, 40138 Bologna, Italy

^cHealth Sciences and Technologies Interdepartmental Center for Industrial Research (HSTICIR), University of Bologna, 40126, Bologna, Italy

*Correspondence:

Prof. Dr. Elisa Michelini

Department of Chemistry “Giacomo Ciamician”

University of Bologna

Via Selmi 2, 40126 Bologna, Italy

Tel.: +39 051 20 9 9533

e-mail: elisa.michelini8@unibo.it

```

1                                                                                               82
BRLuc MEDAKNIKKGPAPRYPLEDGTAGEQLHKAMKRNAQVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIVVCS
BoLuc MEDAKNIKKGPAPRYPLEDGTAGEQLHKAMKRNAQVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIVVCS
BgLuc MEDAKNIKKGPAPRYPLEDGTAGEQLHKAMKRNAQVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIVVCS
PpyLuc MEDAKNIKKGPAPRYPLEDGTAGEQLHKAMKRNAQVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIVVCS
.....

83                                                                                               164
BRLuc ENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSMGISQPTVVVFSKKGKILNVQKKLP IIQKIIIMDSKTDYQGFQSM
BoLuc ENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSMGISQPTVVVFSKKGKILNVQKKLP IIQKIIIMDSKTDYQGFQSM
BgLuc ENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSMGISQPTVVVFSKKGKILNVQKKLP IIQKIIIMDSKTDYQGFQSM
PpyLuc ENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSMNIISQPTVVVFSKKGKILNVQKKLP IIQKIIIMDSKTDYQGFQSM
.....

165                                                                                               246
BRLuc YTFVTSHLPPGFNEYDFKPEFDRDKTIALIMNSSGSTGLPKGVALPHRALCVRFSHARDPIFGNQIKPDTAILSVPVPHHG
BoLuc YTFVTSHLPPGFNEYDFKPEFDRDKTIALIMNSSGSTGLPKGVALPHRALCVRFSHARDPIFGNQIKPDTAILSVPVPHHA
BgLuc YTFVTSHLPPGFNEYDFKPEFDRDKTIALIMNSSGSTGLPKGVALPHRALCVRFSHARDPIFGNQIKPDTAILSVPVPHHA
PpyLuc YTFVTSHLPPGFNEYDFVPEFDRDKTIALIMNSSGSTGLPKGVALPHRDA CVRFSHARDPIFGNQIPDTAILSVPVPHHG
.....

247                                                                                               328
BRLuc FGMFTTLGYLICGFRVVMYRFEELFLRSLQDYKIQTALLVPTLFSFLAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVA
BoLuc FGMSTTLGYLICGFRVVMYRFEELFLRSLQDYKIQTALLVPTLFSFLAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVA
BgLuc FGMSTTLGYLICGFRVVMYRFEELFLRSLQDYKIQSALLVPTLFSFLAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVA
PpyLuc FGMFTTLGYLICGFRVVMYRFEELFLRSLQDYKIQSALLVPTLFSFVAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVA
.....

329                                                                                               410
BRLuc KGFHLPGRQGYGLTETTSAILVTPKGDDKPGAVGKVVVFFFEAKVVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNA
BoLuc KRFHLPGRQGYGLTETTSAILVTPKGDDKPGAVGKVVVFFFEAKVVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNA
BgLuc KRFHLPGRQGYGLTETTSAILVTPKGDDKPGAVGKVVVFFFEAKVVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNA
PpyLuc KRFHLPGRQGYGLTETTSAILVTPKGDDKPGAVGKVVVFFFEAKVVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNA
.....

411                                                                                               492
BRLuc LIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIRDAGVAGLPDDDAGELPAAVVLEHGKT
BoLuc LIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIRDAGVAGLPDDDAGELPAAVVLEHGKT
BgLuc LIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIRDAGVAGLPDDDAGELPAAVVLEHGKT
PpyLuc LIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIIDAGVAGLPDDDAGELPAAVVLEHGKT
.....

493                                                                                               550
BRLuc MTEKEIVDYVASQVTTAKKLRGGVVFVDEVPKGLTGKLDARKIREILIKAKKGGKII--
BoLuc MTEKEIVDYVASQVTTAKKLRGGVVFVDEVPKGLTGKLDARKIREILIKAKKGGKII--
BgLuc MTEKEIVDYVASQVTTAKKLRGGVVFVDEVPKGLTGKLDARKIREILIKAKKGGKII--
PpyLuc MTEKEIVDYVASQVTTAKKLRGGVVFVDEVPKGLTGKLDARKIREILIKAKKGGKSKI
.....

```

Figure S1: Amino acid alignment of the BrLuc, BoLuc, BgLuc and PpyLuc luciferases. The red highlighted residues indicate the mutated amino acids.

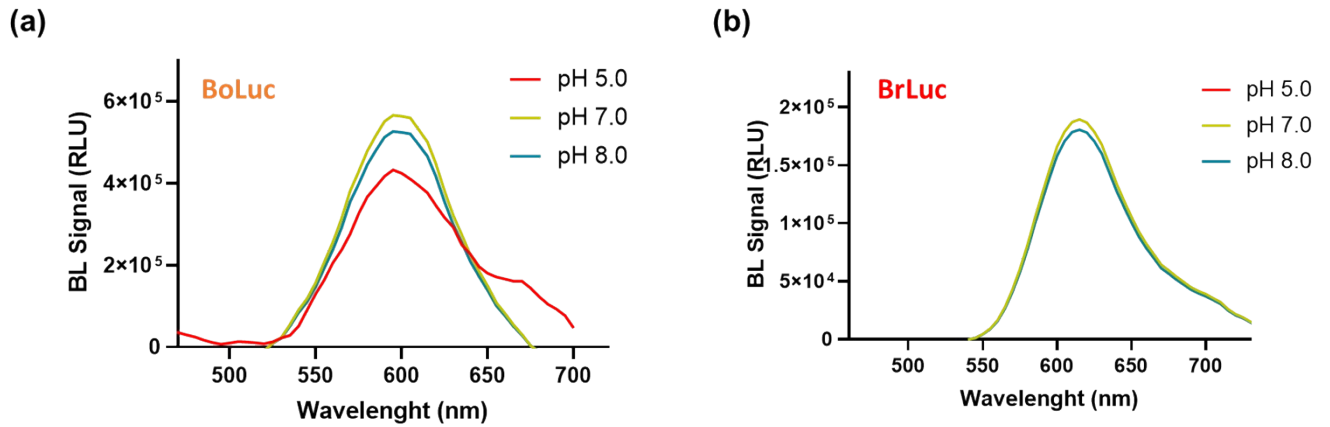


Figure S2: (a) BoLuc mutant and (b) BrLuc mutant emission spectra obtained at different pH (5.0, 7.0 and 8.0) with D-Luciferin substrate. BrLuc emission at pH 5.0 was not detectable.

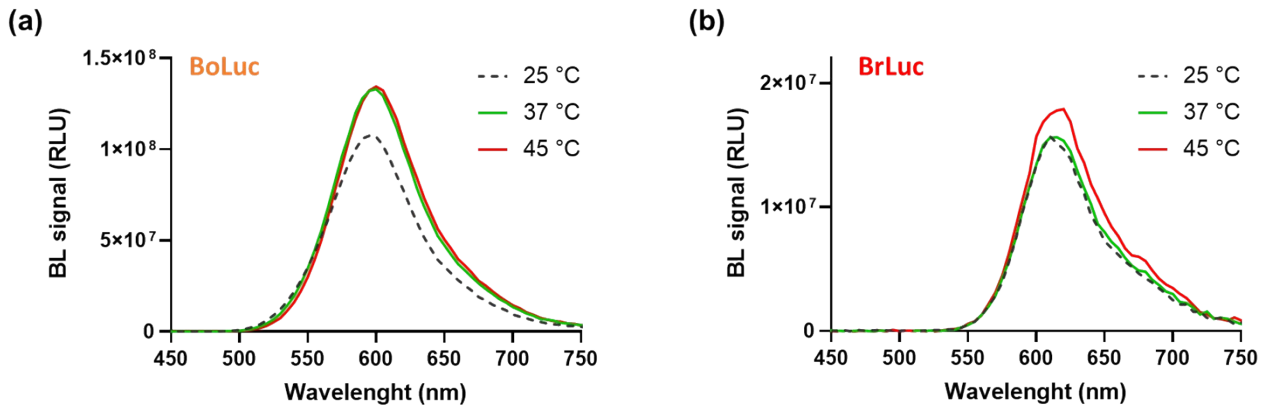


Figure S3: (a) BoLuc mutant and (b) BrLuc mutant emission spectra obtained at different temperature (25°, 37° and 45°C) with the D-Luciferin substrate.

Table S1. Kinetic Parameters of BgLuc, BoLuc and BrLuc mutants

Luc enzyme	Km (μM)	Kcat (cps/M)*	Kcat/ Km
BgLuc	20.9 ± 0.5	4.91×10^8	2.93×10^{10}
BoLuc	8.2 ± 0.2	4.97×10^8	6.06×10^7
BrLuc	196 ± 11	1.50×10^8	7.92×10^5

*Apparent kcat values were calculated by dividing the apparent Vmax (cps) by the luciferase concentration (6.6×10^{-6} M)