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# Nucleic acid detection using BRET-beacons based on bioluminescent protein-DNA hybrids

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**Table S1: Oligonucleotide sequences**

| **Name** (domains)[a] | **Sequence**[b] |
|---|---|
| **Handle** (1) | 5'-$H_2N$-GTGATGTAGGTGGTAGAGGAA-3' |
| **Anti-handle** (1*) | 5'-TTCCTCTACCACCTACATCAC-cy3-3' |
| **Stem-Loop4** (1*-2-3-2*) | 5'-TTCCTCTACCACCTACATCACCAGCTGGAGACGTAGGGTATTGAATGTGCTG-cy3-3' |
| **Stem-Loop5** (1*-2-3-2*) | 5'-TTCCTCTACCACCTACATCACACAGCTGGAGACGTAGGGTATTGAATGTGCTGT-cy3-3' |
| **Stem-Loop6** (1*-2-3-2*) | 5'-TTCCTCTACCACCTACATCACGACAGCTGGAGACGTAGGGTATTGAATGTGCTGTC-cy3-3' |
| | |
| **Full target** (2-3*) | 5'-CAGCACATTCAATACCCTACGTCTCCA-3' |
| **Target 10** | 5'-CAGCACATTC-3' |
| **Target 11** | 5'-CAGCACATTCA-3' |
| **Target 12** | 5'-CAGCACATTCAA-3' |
| **Target 13** | 5'-CAGCACATTCAAT-3' |
| **Target 14** | 5'-CAGCACATTCAATA-3' |
| | |
| **Target A** | 5'-CATTCAATAC-3' |
| **Target B** | 5'-CTACGTCTCC-3' |
| | |
| **Stem-Loop miRNA21** | 5'-TTCCTCTACCACCTACATCACCCAGCATCAACATCAGTCTGATAAGCTAGCTGG-3' |
| **Target miRNA21** | 5'-TAGCTTATCAGACTGATGTTGA-3' |

[a]The numbers represent the individual oligonucleotides with * indicating complementary sequences.
[b]Individual colors represent individual oligonucleotide domains.

**Table S2: Mutagenesis primers**

| | |
|---|---|
| **C164S** | 5'-GGGTTACGGGTTGGCGACTGAGCGAAAGAATATTAGCTGCGGCCGC-3' |
| **G180C** | 5'-CGAGCTGCCAGAAACCGGTTGTCACCACCACCACCACCACTG-3' |

**Mutagenesis, expression and purification of NanoLuc luciferase**

The gene coding for kanamycin resistance and NanoLuc luciferase was available in a pET28a expression vector (Genscript) with an N-terminal strep-tag and a C-terminal sortase-tag and His-tag. The native cysteine was mutated to a serine (C164S) and the glycine before the N-terminal His-tag was mutated to a cysteine (G180C) using QuickChange multisite-directed mutagenesis (Agilent Technologies) according to manufacturer's protocol and the primers stated in table S2. The plasmid encoding for the mutated NanoLuc was subsequently transformed in *E. coli* BL21(DE3) (Novagen) and cultured in LB-medium supplemented with 30 mg/L kanamycin in a total volume of 500 mL. At $OD_{600}$ = 0.6 protein expression was induced by the addition of 100 µM isopropyl *β*-D-1-thioglactopyranoside (IPTG) overnight at 18 °C. Subsequently, cells were harvested by centrifugation for 10 minutes at 10,000g and lysed by resuspending the pelleted cells in BugBuster protein extraction reagent (Novagen) and Benzonase endonuclease (Novagen). The soluble fraction was obtained by centrifugation for 40 minutes at 40,000g. Finally, NanoLuc luciferase was purified from the soluble fraction by $Ni^{2+}$-affinity chromatography (Fig S1a) and the buffer was exchanged to storage buffer (20 mM Tris-HCl, 150 mM NaCl, 2 mM TCEP, pH 7.9). The correct mass was confirmed by mass spectrometry (Fig S1b-d).

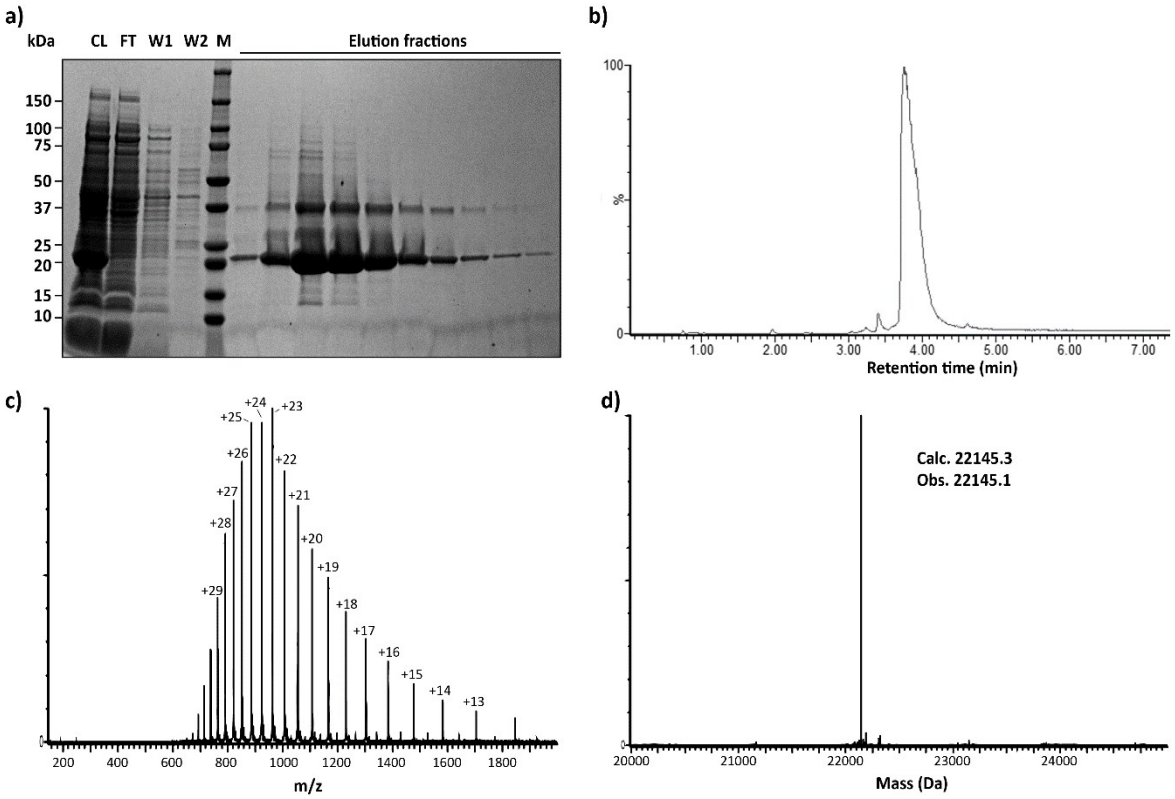


**Figure S1.** Expression, purification and characterization of NanoLuc with a C164S and G180C point mutation. **a)** Semi-native 4-20% SDS-PAGE analysis of the expression and purification of NanoLuc with a C164S and G180C point mutation ($MW_{calc}$ = 22145.3 Da). CL: Cell lysate, FT: $Ni^{2+}$-affinity column flow through, W1: $Ni^{2+}$-affinity column wash with buffer containing 20 mM imidazole, W2: $Ni^{2+}$-affinity column wash with buffer containing 60 mM imidazole, Elution fractions: elution fractions with buffer containing 500 mM imidazole. The additional band at ~37 kDa indicates the formation of disulfide-linked dimers. These were reduced by subsequently exchanging the buffer to storage buffer containing 2 mM TCEP. **b)** Liquid-Chromatography trace of NanoLuc showing a single peak with corresponding m/z spectrum **(c)** and deconvoluted mass spectrum **(d)** obtained by injecting 0.1 µL NanoLuc diluted to 0.5 mg/mL in $H_2O$ + 0.1% formic acid on an Agilent Polaris C18A RP column with a flow of 0.3 mL/min and a 15-60% acetonitrile gradient containing 0.1% formic acid and measuring mass spectra on a Xevo G2 QTof mass spectrometer in positive mode. Deconvoluting the m/z spectra was performed with MaxEnt Deconvolution software.

**DNA and protein sequence of NanoLuc (C164S), (G180C)**

```
                                    1
atgtggtctcatcctcaatttgaaaaaatggtatttactcttgaagattttgtcggtgat
 M  W  S  H  P  Q  F  E  K  M  V  F  T  L  E  D  F  V  G  D

 10                            20
tggcgccagaccgccggctataacctggaccaagtgcttgaacagggcggggttagcagc
 W  R  Q  T  A  G  Y  N  L  D  Q  V  L  E  Q  G  G  V  S  S

 30                            40
ctgtttcaaaacctgggggtgagtgtcacgccaattcagcgcatcgttctgtcgggagag
 L  F  Q  N  L  G  V  S  V  T  P  I  Q  R  I  V  L  S  G  E

 50                            60
aatggtctgaaaatcgatatccacgtcattatcccgtacgaaggtctttctggtgatcag
 N  G  L  K  I  D  I  H  V  I  I  P  Y  E  G  L  S  G  D  Q

 70                            80
atggggcagatagaaaaaatattcaaagtggtgtacccagtagacgatcatcacttcaag
 M  G  Q  I  E  K  I  F  K  V  V  Y  P  V  D  D  H  H  F  K

 90                            100
gttatactgcactatggcaccctcgttatcgatggcgttactccgaatatgatcgattac
 V  I  L  H  Y  G  T  L  V  I  D  G  V  T  P  N  M  I  D  Y

 110                           120
tttgggcgtccttatgaaggtattgcggtgttcgacggtaaaaaaattacggttaccggg
 F  G  R  P  Y  E  G  I  A  V  F  D  G  K  K  I  T  V  T  G

 130                           140
acgctctggaatggtaataaaatcattgatgagcgcttgataaacccagatggcagcctt
 T  L  W  N  G  N  K  I  I  D  E  R  L  I  N  P  D  G  S  L

 150                           160
ctgttcagagttacgataaacggggttacgggttggcgactgagcgaaagaatattagct
 L  F  R  V  T  I  N  G  V  T  G  W  R  L  S  E  R  I  L  A

 170                           180
gcggccgcactcgagctgccagaaaccggttgtcaccaccaccaccaccac
 A  A  A  L  E  L  P  E  T  G  C  H  H  H  H  H  H
```

**Conjugation of handle oligonucleotide to NanoLuc**

The handle oligonucleotide functionalized with a 5' primary amine was obtained HPLC purified from Integrated DNA Technologies and dissolved in PBS (100 mM NaPi, 150 mM NaCl, pH7.2) to a final concentration of 1 mM. Subsequently, 20 equivalents of Sulfo-SMCC (Thermo Scientific) dissolved in DMSO to a final concentration of 20 mM were added and incubated for 2 hours at room temperature with continuous shaking at 850 rpm. The maleimide-functionalized handle oligonucleotide was purified by 3 consecutive ethanol precipitations. In short, 10 % (v/v) 5 M NaCl and 300 % (v/v) ice cold ethanol was added and the solution was stored at -30 °C for 1 hour. After centrifugation at 14,000 rpm for 15 minutes at 4 °C the supernatant was removed and the pellet was dried under vacuum.

Before conjugating the handle oligonucleotide to NanoLuc, the protein was first buffer exchanged to 100 mM sodium phosphate at pH 7.0 using a PD-10 desalting column to remove TCEP. Subsequently, the handle oligonucleotide was added in a 3-fold molar excess to ~100 µM NanoLuc and allowed to react for 2 hours at room temperature with continuous shaking at 850 rpm. Finally, the ODN-NanoLuc conjugate was purified by $Ni^{2+}$-affinity chromatography and anion exchange chromatography to remove excess handle oligonucleotide and unreacted NanoLuc, respectively. In short, the reaction mixture was loaded on a prepacked His-bind resin column and the excess of handle oligonucleotides was removed by washing the column with wash buffer (20 mM Tris-HCl, 250 mM NaCl, 60 mM imidazole, pH 7.9). By subsequently washing the column with elution buffer (20 mM Tris-HCl, 250 mM NaCl, 500 mM imidazole, pH 7.9) the unreacted NanoLuc and ODN-NanoLuc were eluted. The elution fractions were pooled and the buffer was exchanged to a low ionic strength buffer (20 mM Tris-HCl, pH 7.0) using a PD-10 desalting column and directly loaded on a Strong Anion-exchange Spin Column (Thermo Scientific). Unreacted NanoLuc was eluted by washing the anion exchange spin column with a low ionic strength buffer (20 mM Tris-HCl, 0-300 mM NaCl, pH 7.0). Finally, pure ODN-NanoLuc was eluted with a high ionic strength buffer (20 mM Tris-HCl, 1 M NaCl, pH 7.0). The concentration of ODN-NanoLuc was determined by measuring the absorbance at 260 nm and the theoretical extinction coefficient of the handle oligonucleotide.

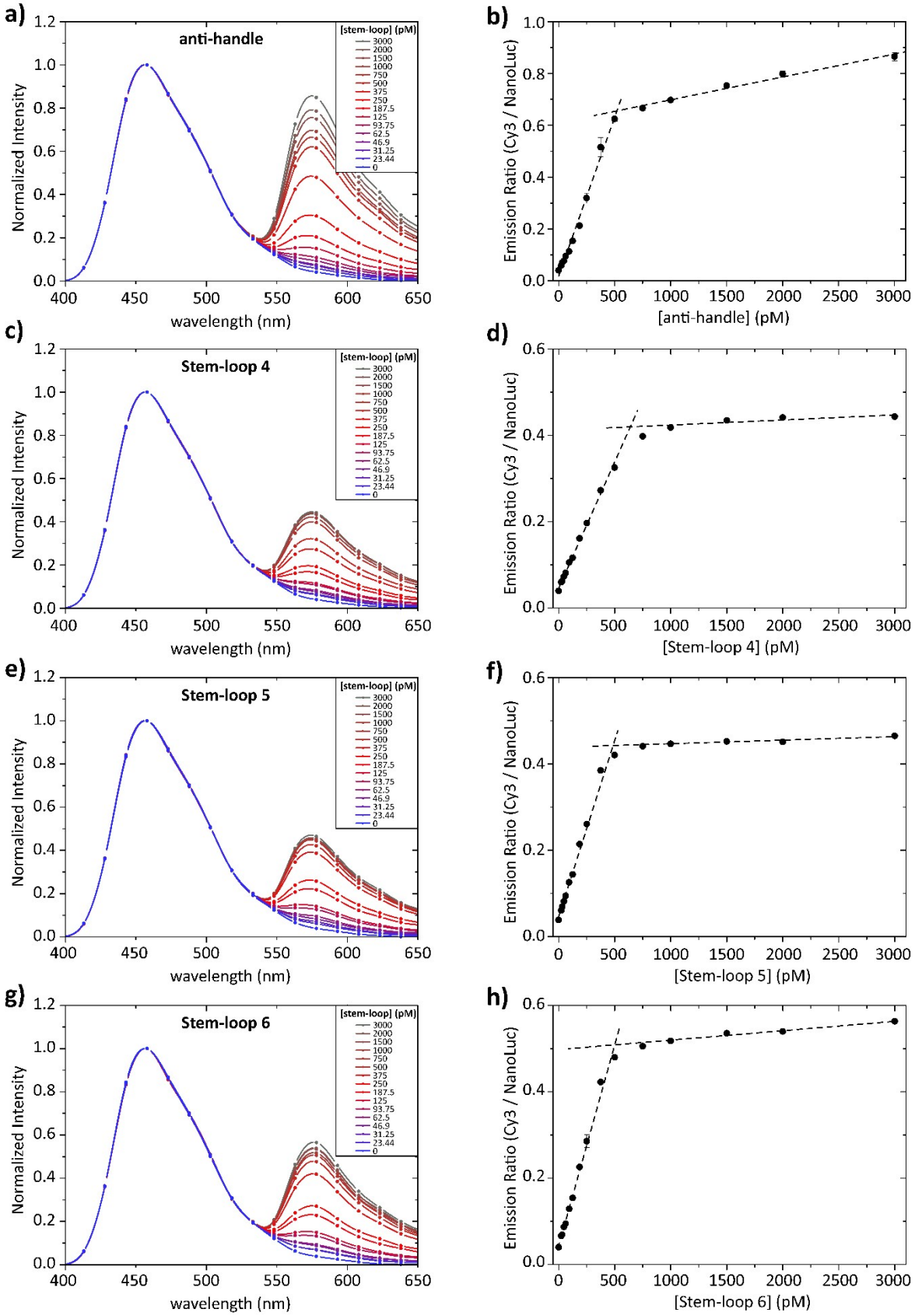


**Figure S2.** Titration of various concentrations of stem-loop with different stem lengths to 500 pM ODN-NanoLuc. Normalized bioluminescence intensities and corresponding Cy3/NanoLuc emission ratios as a function of stem-loop concentration for **a,b)** anti-handle, **c,d)** Stem-loop 4, **e,f)** Stem-loop 5 and **g,h)** Stem-loop 6. Triplicate measurements were performed in TE/Mg/NaCl buffer supplemented with 1 mg/mL BSA (10mM Tris-HCl, 1 mM EDTA, 12.5 mM $MgCl_2$, 150 mM NaCl, 1 mg/mL BSA, pH 8.0, 1:5000 substrate). Error bars represent the standard deviation of triplicate measurements.

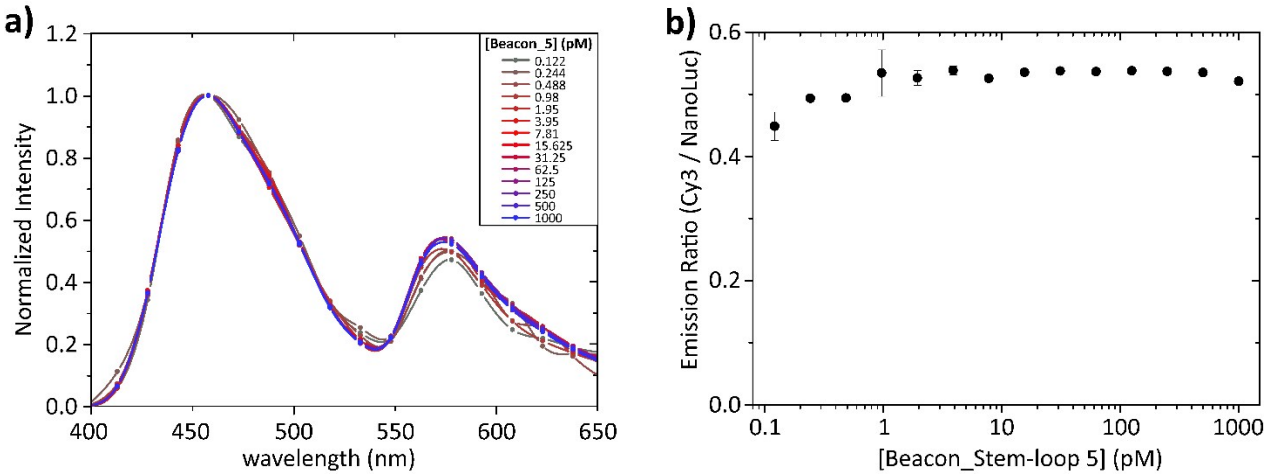


**Figure S3.** Stability of the assembled intramolecular BRET-beacon at different concentrations. 5 nM ODN-NanoLuc was prehybridized with 10 nM Stem-loop 5 and subsequently the assembled BRET-beacon was diluted to concentrations between 0 – 1000 pM of the stem-loop and incubated for 1 hour at room temperature. **a)** Normalized bioluminescence emission spectra at various BRET-beacon concentrations and **b)** corresponding Cy3/NanoLuc emission ratios. Triplicate measurements were performed in TE/Mg/NaCl buffer supplemented with 1 mg/mL BSA (10mM Tris-HCl, 1 mM EDTA, 12.5 mM $MgCl_2$,150 mM NaCl, 1 mg/mL BSA, pH 8.0, 1:5000 substrate). Error bars represent the standard deviation of triplicate measurements.

$$R = A + (B - A) * \frac{([Beacon] + K_{d,app} + [Target]) - \sqrt{([Beacon] + K_{d,app} + [Target])^2 - 4[Beacon][Target]}}{2[Beacon]},$$

(Eq. S1)

In equation S1, R is the measured emission ratio, A the emission ratio in absence of target, B the emission ratio at saturating target concentrations, [Beacon] the concentration of the BRET-beacon (i.e. the concentration of the stem-loop) and [Target] the concentration of target oligonucleotide.

**Table S3. Apparent affinities derived from fitting equation S1 to the data in main text Figure 4**

| | Stem-loop 4 | Stem-loop 5 | Stem-loop 6 |
|---|---|---|---|
| Target 14 | 0.22 ± 0.02 nM | 0.25 ± 0.02 nM | 14.46 ± 0.61 nM |
| Target 13 | 0.20 ± 0.02 nM | 1.60 ± 0.10 nM | 77.1 ± 3.3 nM |
| Target 12 | 0.82 ± 0.07 nM | 12.2 ± 0.8 nM | 402 ± 16.9 nM |
| Target 11 | 10.01 ± 0.83 nM | 104.4 ± 6.8 nM | 3215 ± 144 nM |
| Target 10 | 112.2 ± 9.3 nM | 1009 ± 66 nM | 22320 ± 2284 nM |