

1 Supporting Information for

2 **BRET Nano Q-body: A Nanobody-Based Ratiometric Bioluminescent**
3 **Immunosensor for Point-of-Care Testing**

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25 **Table S1.** Primers used in this study

Primer	Sequence (5' - 3')
Inf_Nluc_EagI_back	GATCGACTATTTTCGGACG
Overlap_G4S_NlucMTX_for	AGAGCCGCCGCCGCCGCCAGAATGCGTTTCG
Overlap_G4S_NlucMTX_back	GGCGGGCGGGCTCTCATATGGCTCAAATCGAAG
Inf_Nluc_XhoI_for	GGTGGTGGTGCTCGAG
Overlap_EAAAK_NlucMTX_for	ATGTTTAGCTGCCGCTTCCGCCAGAATGCGTTC
Overlap_EAAAK_NlucMTX_back	GGAAGCGGCAGCTAAACATATGGCTCAAATCGAAG
Overlap_G4S_2_Nluc_for	TGAACCTCCCCCTCCACTACCGCCTCCACCCGCCA G
Overlap_G4S_2_MTX_back	AGTGGAGGGGGAGGTTACATATGGCTCAAATCGA AG
Overlap_G4S_3_MTX_back	AGTGGAGGGGGAGGTTACAGGCGGTGGGGGTTCTC ATATGGCTCAAATC

a



b

5'-

ATG GTC TTC ACA CTC GAA GAT TTC GTT GGG GAC TGG CGA CAG ACA GCC GGC TAC AAC CTG GAC CAA GTC
 CTT GAA CAG GGA GGT GTG TCC AGT TTG TTT CAG AAT CTC GGG GTG TCC GTA ACT CCG ATC CAA AGG ATT
 GTC CTG AGC GGT GAA AAT GGG CTG AAG ATC GAC ATC CAT GTC ATC ATC CCG TAT GAA GGT CTG AGC GGC
 GAC CAA ATG GGC CAG ATC GAA AAA ATT TTT AAG GTG GTG TAC CCT GTG GAT GAT CAT CAC TTT AAG GTG
 ATC CTG CAC TAT GGC ACA CTG GTA ATC GAC GGG GTT ACG CCG AAC ATG ATC GAC TAT TTC GGA CGG CCG
 TAT GAA GGC ATC GCC GTG TTC GAC GGC AAA AAG ATC ACT GTA ACA GGG ACC CTG TGG AAC GGC AAC AAA
 ATT ATC GAC GAG CGC CTG ATC AAC CCC GAC GGC TCC CTG CTG TTC CGA GTA ACC ATC AAC GGA GTG ACC
 GGC TGG CGG CTG TGC GAA CGC ATT CTG GCG ~~~Linker~~~ CAT ATG GCT CAA ATC GAA GTA AAC TGC TCT AAT
GAG ACC GGT CAG GTT CAG CTC GTA GAA TCG GGA GGA GGT CTG GTT CAG GCG GGT GGC AGT CTG CGC TTG
 TCA TGC GCA GCT TCT CGG CGT TCG AGT CGT AGC TGG GCG ATG GCG TGG TTT CGC CAA GCA CCA GGC AAA
 GAA CGC GAG TTT GTG GCC AAG ATC AGC GGT GAT GGG CGC TTA ACC ACG TAT GGC GAT TCG GTC AAA GGG
 CGT TTC ACC ATT AGC CGC GAT AAT GCG GAG TAT CTG GTG TAC CTT CAG ATG GAT TCC CTG AAA CCG GAA
 GAT ACA GCC GTG TAC TAC TGT GCA GCC GAC GAC AAC TAT GTG ACG GCT TCA TGG CGT TCT GGT CCG GAC
 TAT TGG GGC CAA GGC ACT CAG GTC ACC GTA TCC AGC CTC GAG CAC CAC CAC CAC CAC GGA TCC GAC
 TAC AAG GAC GAC GAT GAC AAA TAA

-3'

DNA Sequences of linker

No linker: 5'- no DNA sequence -3'

Flexible linker: 5'- GGT GGA GGC GGT TCT -3'5'- GGT GGA GGC GGT AGT GGA GGG GGA GGT TCA -3'5'- GGT GGA GGC GGT AGT GGA GGG GGA GGT TCA GGC GGT GGG GGT TCT -3'Rigid linker: 5'- GAA GCG GCA GCT AAA -3'

c

MVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYEGLSGDMQGIKIFKVVYP
 VDDHHFKVILHYGTLVIDGVTNMDYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLFRVTVINGVTGWRLCERIL
 A~Linker~HMAQIEVNGSNETGQVQLVESGGGLVQAGGSLRLSCAASRRSSRSWAMAWFRQAPGKEREFVAKISGDGRLTT
 YGDSVKGRFTISRDNAEYLVYLLQMDSLKPEDTAVYYCAADDNYVTASWRSGPDYWGQGTQVTVSSLEHHHHHHGSDYKDD
 DDK*

Amino acid sequences of linker

No linker: no amino acid sequence

Flexible linker: GGGGSGGGSGGGGSGGGSGGGSGGGGSRigid linker: EAAAAK

Figure S1. DNA and amino acid sequences of BRET nano Q-bodies. (a) The schematic structure of BRET nano Q-bodies. (b) The DNA sequences of BRET nano Q-bodies. The letters colored dark blue represent NanoLuc; The letters highlighted with dark grey represents linkers. The letters colored light yellow represent cys-tag and cystine was bold and underlined. The grey represents anti-MTX VHH. 6 × His-tag and FLAG-tag were highlighted with light grey and ice blue, respectively. (c) The amino acid sequences of BRET nano Q-bodies.

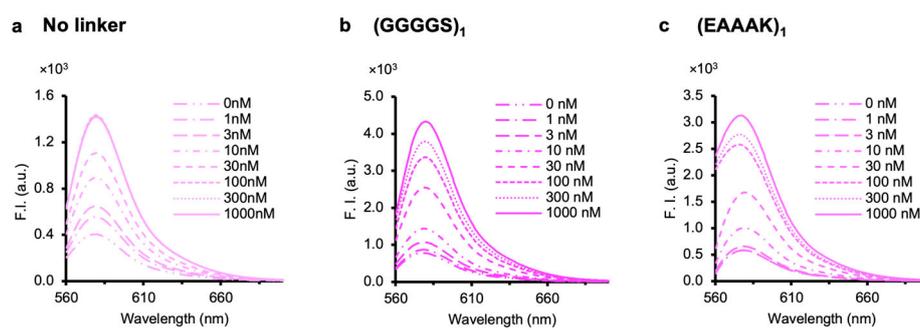


Figure S2. The fluorescence spectra of BRET nano Q-bodies with various concentrations of MTX during optimizing the linker flexibility. F.I.; fluorescence intensity. (a) NanoLuc-TAMRA-MTXVHH. (b) NanoLuc-GGGGS₁-TAMRA-MTXVHH. (c) NanoLuc-EAAAK₁-TAMRA-MTXVHH.

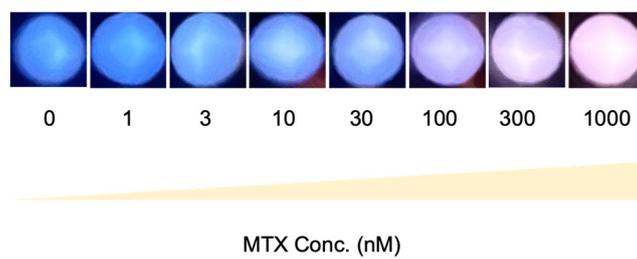


Figure S3. The bioluminescence photos of BRET nano Q-body with various concentrations of MTX taken by smartphone.