Supporting Information for

Quantitative Understanding of Energy Transfer between Fluorescent Proteins Connected via Flexible Peptide Linkers

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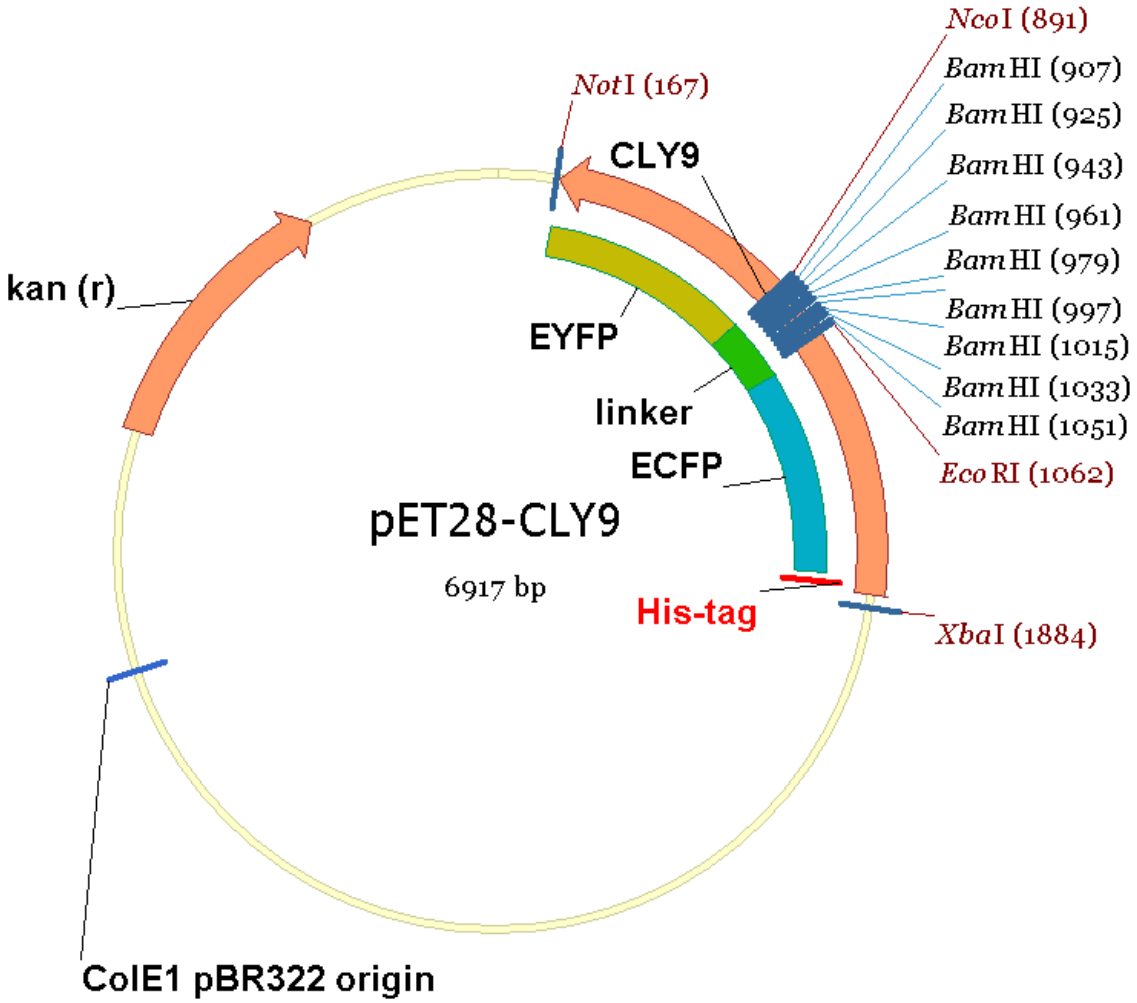

Figure S1. Vector map of pET28-CLY9, indicating the open reading frame of CLY9 and various other features.

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----------------------------------------MetGlySerSerHisHisHisHisHisHi
TCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACTATGGGCAGCAGCCATCATCATCATCATCA
Xba I                                     start


sSerSerGlyLeuValProArgGlySerHisMetValSerLysGlyGluGluLeuPheThrGlyValVal
CAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTG

ProIleLeuValGluLeuAspGlyAspValAsnGlyHisLysPheSerValSerGlyGluGlyGluGlyA
CCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCG

spAlaThrTyrGlyLysLeuThrLeuLysPheIleCysThrThrGlyLysLeuProValProTrpProTh
ATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCAC

rLeuValThrThrLeuThrTrpGlyValGlnCysPheSerArgTyrProAspHisMetLysGlnHisAsp
CCTCGTGACCACCCTGACCTGGGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGAC

PhePheLysSerAlaMetProGluGlyTyrValGlnGluArgThrIlePhePheLysAspAspGlyAsnT
TTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACT

yrLysThrArgAlaGluValLysPheGluGlyAspThrLeuValAsnArgIleGluLeuLysGlyIleAs
ACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGA

pPheLysGluAspGlyAsnIleLeuGlyHisLysLeuGluTyrAsnTyrIleSerHisAsnValTyrIle
CTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACATCAGCCACAACGTCTATATC

ThrAlaAspLysGlnLysAsnGlyIleLysAlaAsnPheLysIleArgHisAsnIleGluAspGlySerV
ACCGCCGACAAGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCG

alGlnLeuAlaAspHisTyrGlnGlnAsnThrProIleGlyAspGlyProValLeuLeuProAspAsnHi
TGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCA

sTyrLeuSerThrGlnSerAlaLeuSerLysAspProAsnGluLysArgAspHisMetValLeuLeuGlu
CTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAG

PheValThrAlaAlaGlyIleThrLeuGlyMetAspGluLeuTyrLysSerGlyIleArgGlyGlySerG
TTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTCCGGAATTCGTGGTGGATCCG
                                                      EcoR I      BamH I
lyGlySerGlyGlySerGlyGlySerGlyGlySerGlyGlySerGlyGlySerGlyGlySerGlyGlySe
GTGGATCAGGTGGATCCGGTGGTAGTGGTGGATCCGGAGGTTCTGGTGGATCCGGTGGTTCAGGTGGATC
            BamH I              BamH I              BamH I              BamH

rGlyGlySerGlyGlySerGlyGlySerGlyGlySerGlyGlySerGlyGlySerGlyGlySerGlyGly
CGGTGGATCTGGTGGATCCGGTGGTAGTGGTGGATCCGGTGGATCTGGTGGATCCGGTGGTAGTGGTGGA
I             BamH I              BamH I              BamH I              Bam

SerGlyGlySerThrMetValSerLysGlyGluGluLeuPheThrGlyValValProIleLeuValGluL
TCCGGAGGTAGCACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGC
H I           Nco I
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euAspGlyAspValAsnGlyHisLysPheSerValSerGlyGluGlyGluGlyAspAlaThrTyrGlyLy
TGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAA

sLeuThrLeuLysPheIleCysThrThrGlyLysLeuProValProTrpProThrLeuValThrThrPhe
GCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTC

GlyTyrGlyLeuGlnCysPheAlaArgTyrProAspHisMetLysGlnHisAspPhePheLysSerAlaM
GGCTACGGCCTGCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCA

etProGluGlyTyrValGlnGluArgThrIlePhePheLysAspAspGlyAsnTyrLysThrArgAlaGl
TGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGA

uValLysPheGluGlyAspThrLeuValAsnArgIleGluLeuLysGlyIleAspPheLysGluAspGly
GGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGC

AsnIleLeuGlyHisLysLeuGluTyrAsnTyrAsnSerHisAsnValTyrIleMetAlaAspLysGlnL
AACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGA

ysAsnGlyIleLysValAsnPheLysIleArgHisAsnIleGluAspGlySerValGlnLeuAlaAspHi
AGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCA

sTyrGlnGlnAsnThrProIleGlyAspGlyProValLeuLeuProAspAsnHisTyrLeuSerTyrGln
CTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAG

SerAlaLeuSerLysAspProAsnGluLysArgAspHisMetValLeuLeuGluPheValThrAlaAlaG
TCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCG

lyIleThrLeuGlyMetAspGluLeuTyrLys***---------
GGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCCGC
                                 stop Not I
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Figure S2. The DNA sequence between the *Xba* I and *Not* I sites that differs from pET-28a(+) and the amino acid translation of the open reading frame of CLY9 are depicted below. Important features are underlined (His-tag, red; ECFP, blue; linker, green; EYFP, yellow).

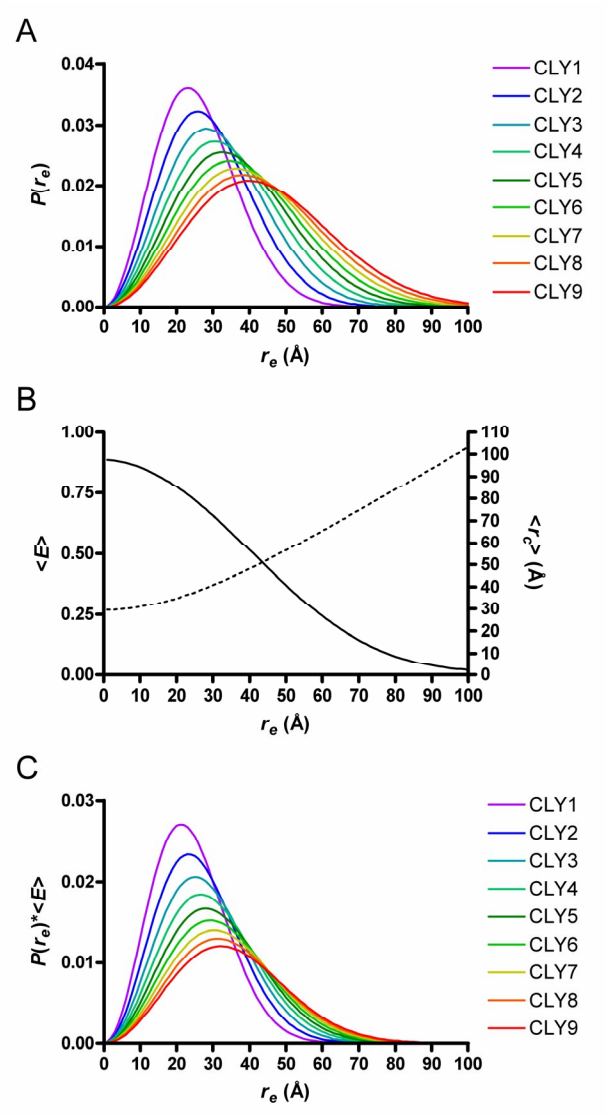

Figure S3: Modeling the energy transfer efficiency for CLYx using the Gaussian chain model to describe the peptide linker. (A) Distribution functions showing the probability $P(r_e)$ for each end-to-end distance of the peptide linker for CLY1-9 calculated using the Gaussian chain model assuming a characteristic ratio of 2.3. (B) Plot showing the average energy transfer ($\langle E \rangle$, solid line) and the average interchromophore distance ($\langle r_c \rangle$, dashed line) as a function of r_e. (C) The contribution of each r_e to the overall energy transfer $\langle E \rangle_{ensemble}$, calculated by multiplying the probability of each r_e with the corresponding $\langle E \rangle$.