

13/11/12 **Erard et al**, Minimum set of mutations needed to optimize cyan
fluorescent proteins for live cell imaging, submitted

Supplementary Information

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Table S1: List of oligonucleotides used for mutagenesis. The mutagenic codons are bold.

T65S forward	5'-CGTGACCACCCT GAG CTGGGGCGTGCAGTGC-3'
T65S reverse	5'-GCACTGCACGCCCCAG CTC AGGGTGGTCACG-3'
S72A forward	5'-CGTGCAGTGCTT CGCC CGCTACCCGACCAC-3'
S72A reverse	5'-GTGGTCGGGGTAG CGGG CGAAGCACTGCACG-3'
Y145A forward	5'-GCTGGAGTACAAC GCC ATCAGCGACAACGTC-3'
Y145A reverse	5'-GACGTTGTCGCTGAT GGC GTTGTA CTC CAGC-3'
H148D forward	5'-CAACTACATCAG CGACA ACGTCTATATCACC-3'
H148D reverse	5'-GGTGATATAGACGTT GTC GCTGATGTAGTTG-3'
H148G forward	5'-CAACTACATCAG CGG CAACGTCTATATCACC-3'
H148G reverse	5'-GGTGATATAGACGTT GCC GCTGATGTAGTTG-3'
S175G forward	5'-CAACATCGAGGACGG CGG CGTGCAGCTCGCC-3'
S175G reverse	5'-GGCGAGCTGCAC GCC GCCGTCTCGATGTTG-3'
A206K forward	5'-CCTGAGCACCCAGTCCA AG CTGAGCAAAGACCCC-3'
A206K reverse	5'-GGGGTCTTTGCTCAG CTT GGACTGGGTGCTCAGG-3'
Y66A forward	5'-CGTGACCACCTTCGG CGCC GGCCTGATGTGC-3'
Y66A reverse	5'-GCACATCAGGCC GGC GCCGAAGGTGGTCACG-3'

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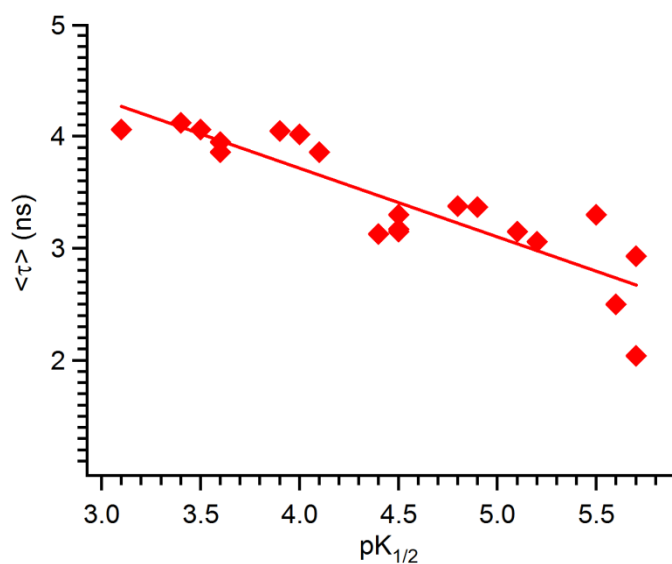


Figure S1. Correlation of the average fluorescence lifetime of CFP mutants vs their pK_{1/2}. Data from all mutants of Table 1 are plotted in the figure, and a linear fit was applied for eye guidance.

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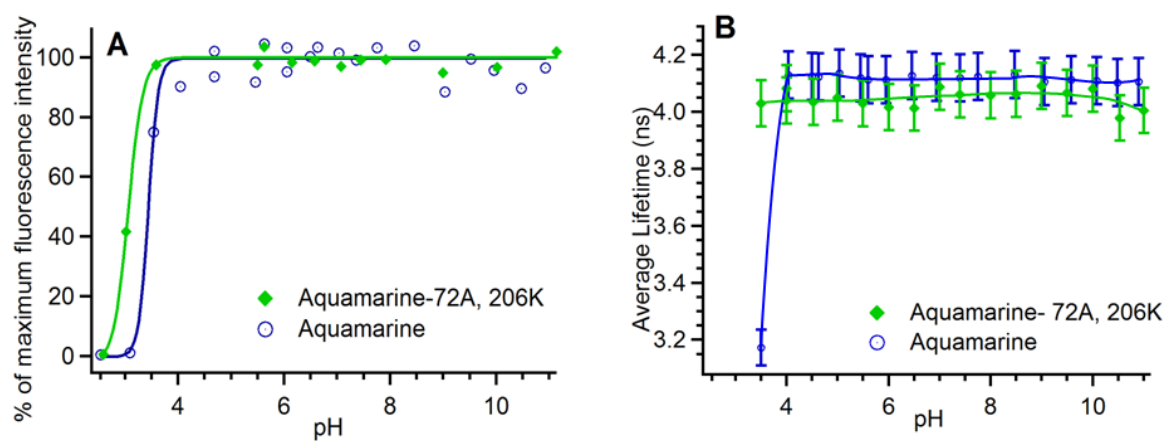


Figure S2. Extended pH stability of Aquamarine-72A-206K vs Aquamarine. (A) fluorescence intensity and (B) average lifetime.

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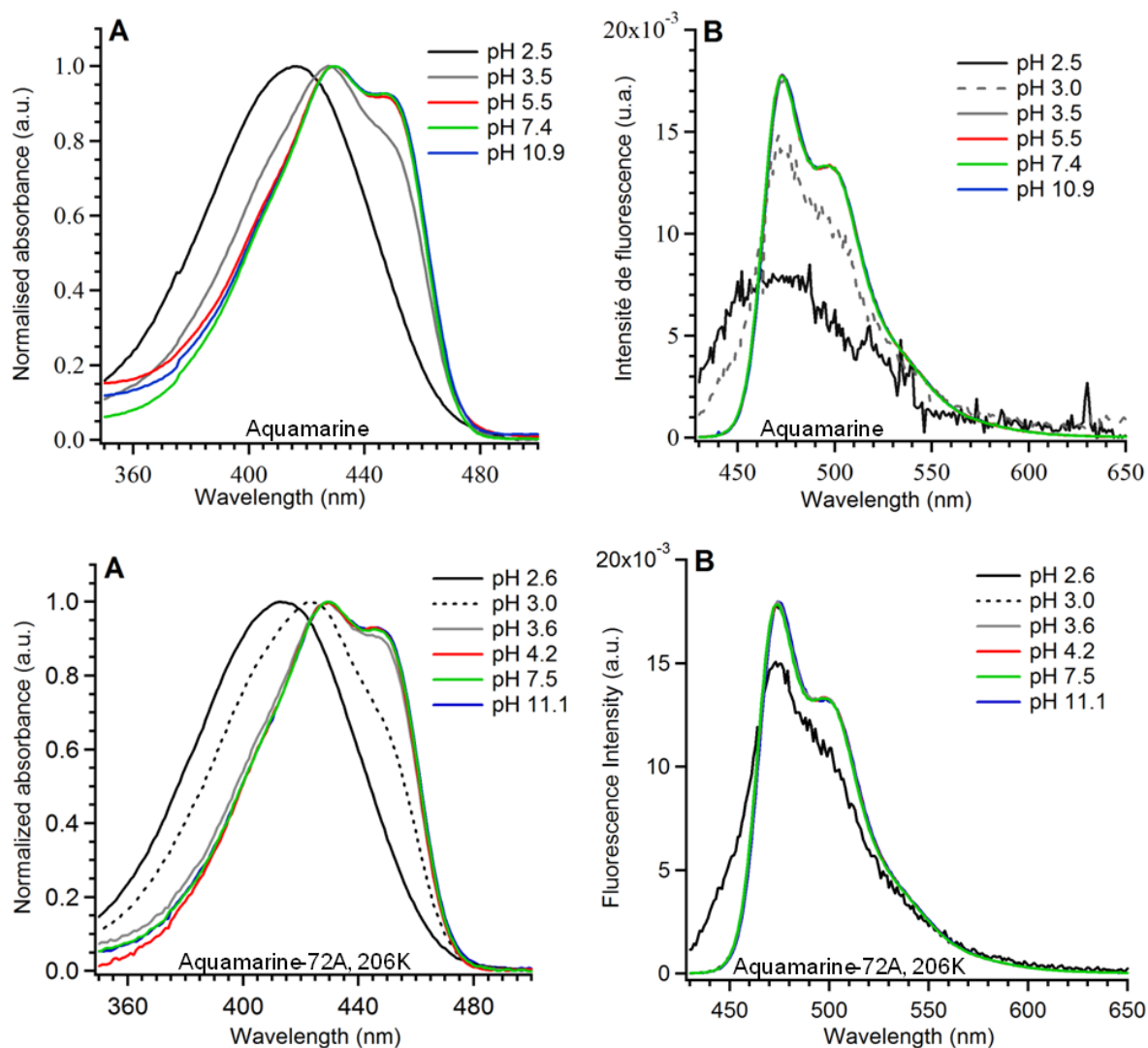


Figure S3. pH dependence of the spectral properties of Aquamarine (top) and Aquamarine-72A-206K (bottom). (A) Absorption and (B) emission spectra normalized to maximum of the chromophore band.

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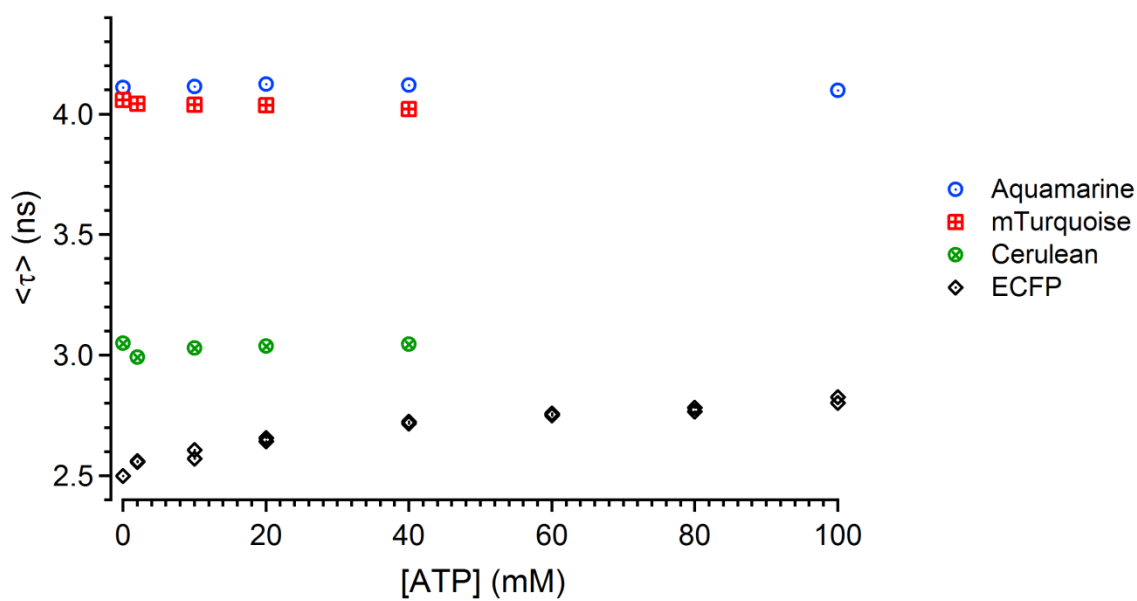


Figure S4. Variations of the average fluorescence lifetime of purified CFP variants in the presence of ATP.

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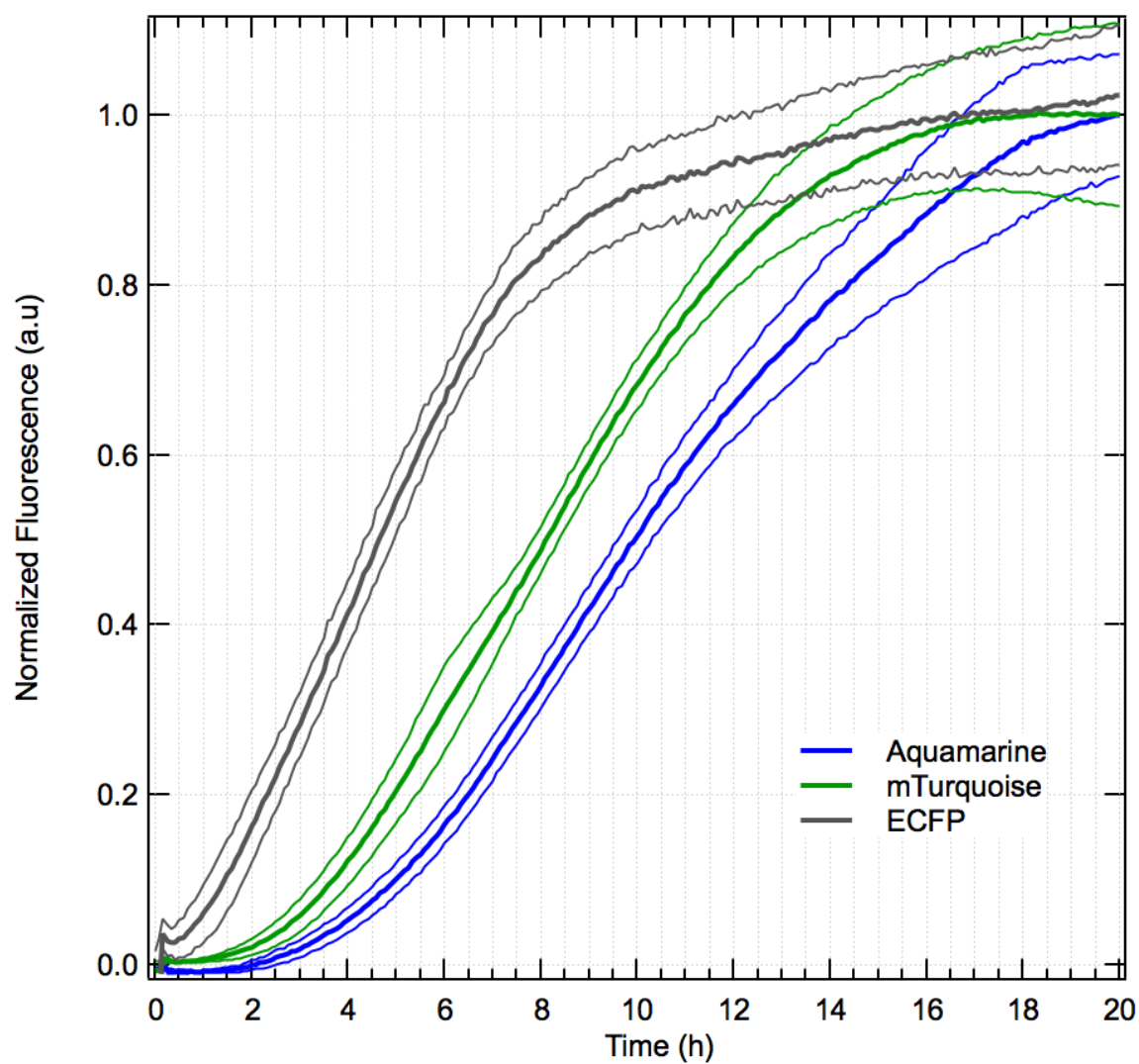


Figure S5. Kinetics of production and maturation of Aquamarine, mTurquoise and ECFP in *E. coli*. The bold line is the average of 10 to 15 wells from two independent experiments for each variant, thinner line represents SD.

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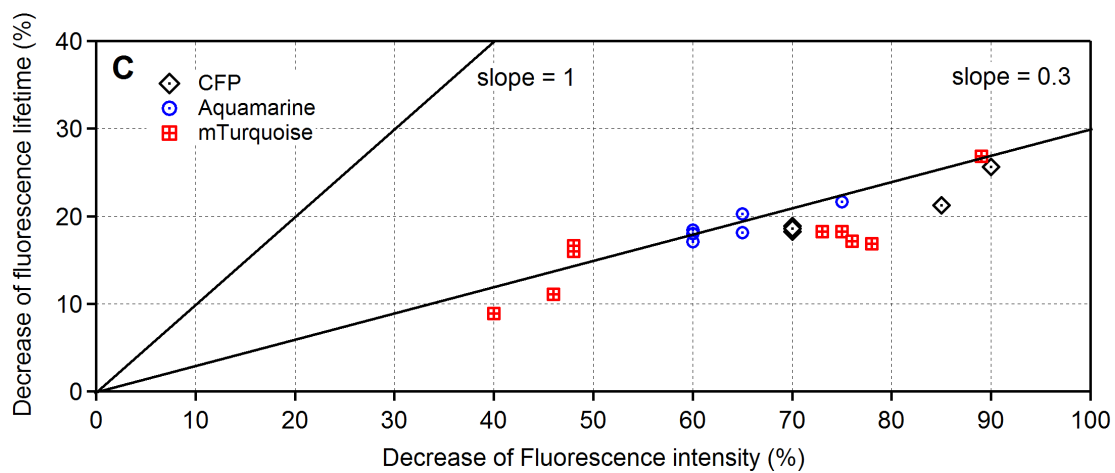


Figure S6. Fluorescence lifetime changes during irreversible photobleaching of CFPs. Black slopes were plotted for eye guidance only.

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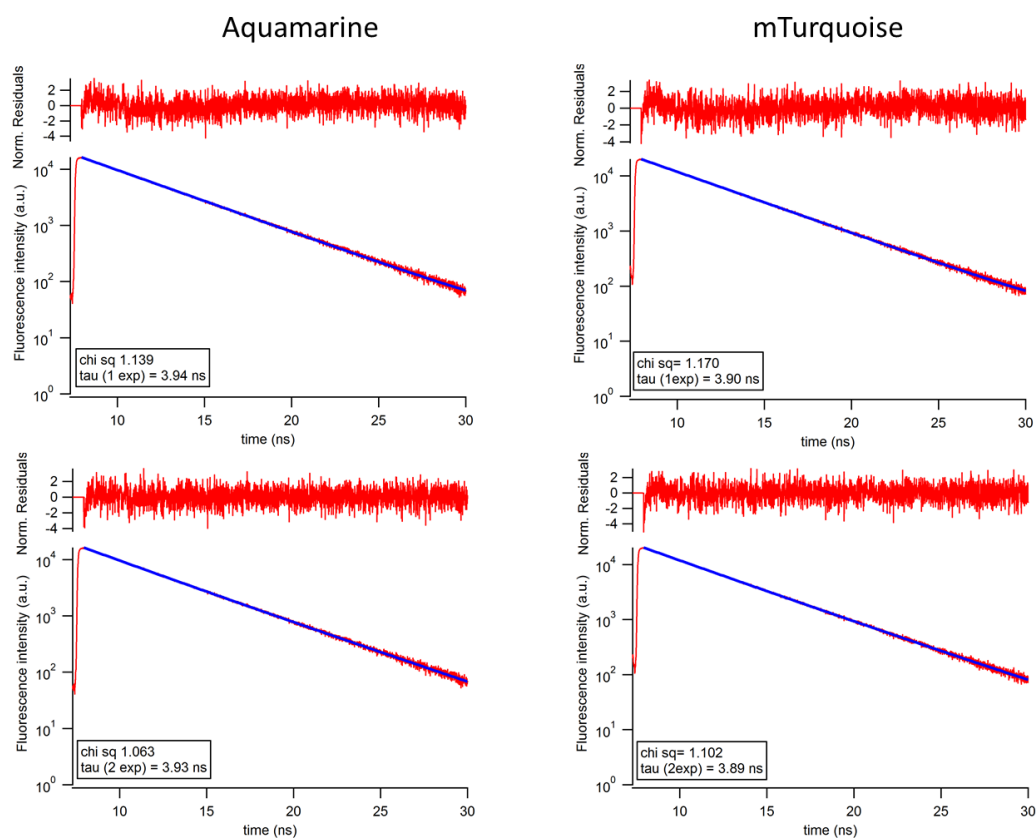


Figure S7. Examples of fits of TCSPC histograms collected from cytosolic Aquamarine and mTurquoise. Single exponential fits (top) and bi-exponential fits (bottom) of Aquamarine (left) and mTurquoise (right). The decay fit (blue), normalized residuals, chi square and average lifetime are shown.