CHEMBIOCHEM

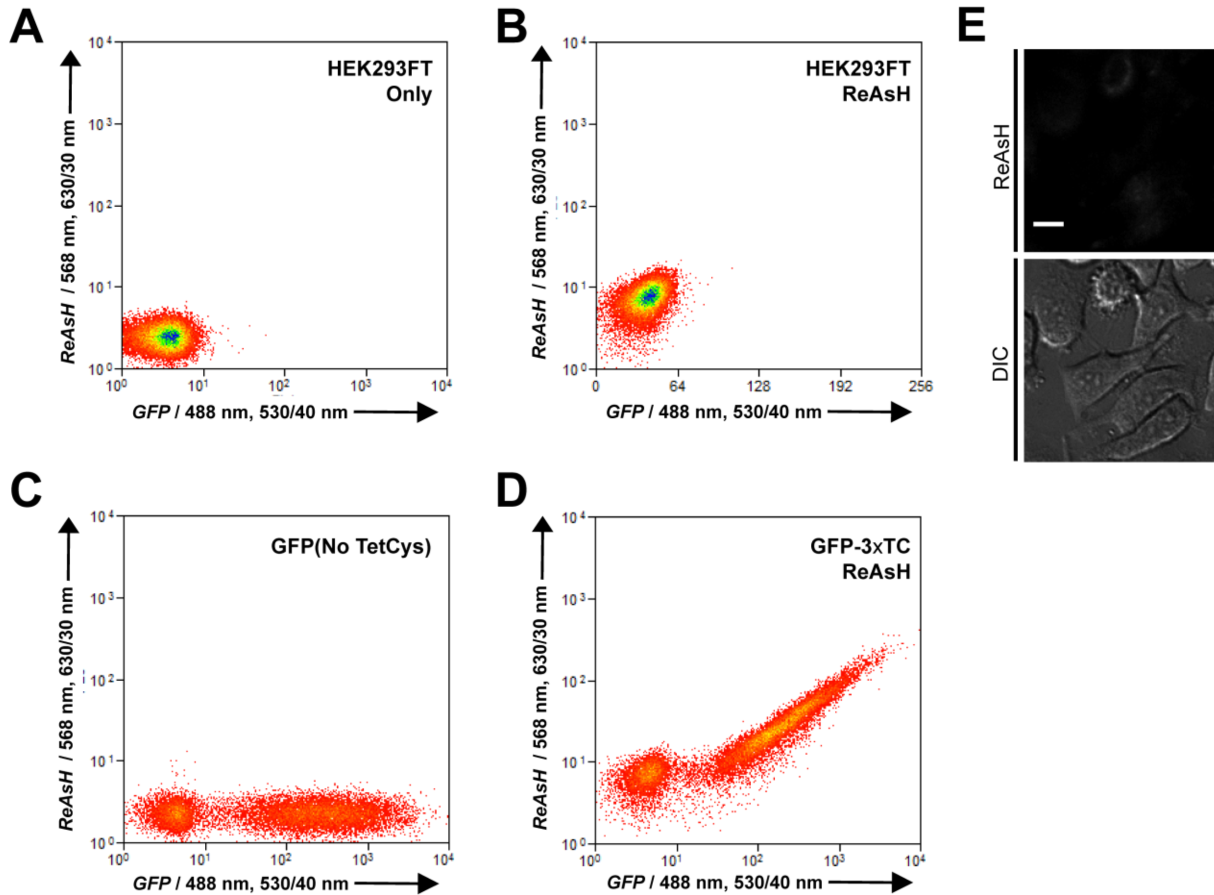
Supporting Information

© Copyright Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, 2010

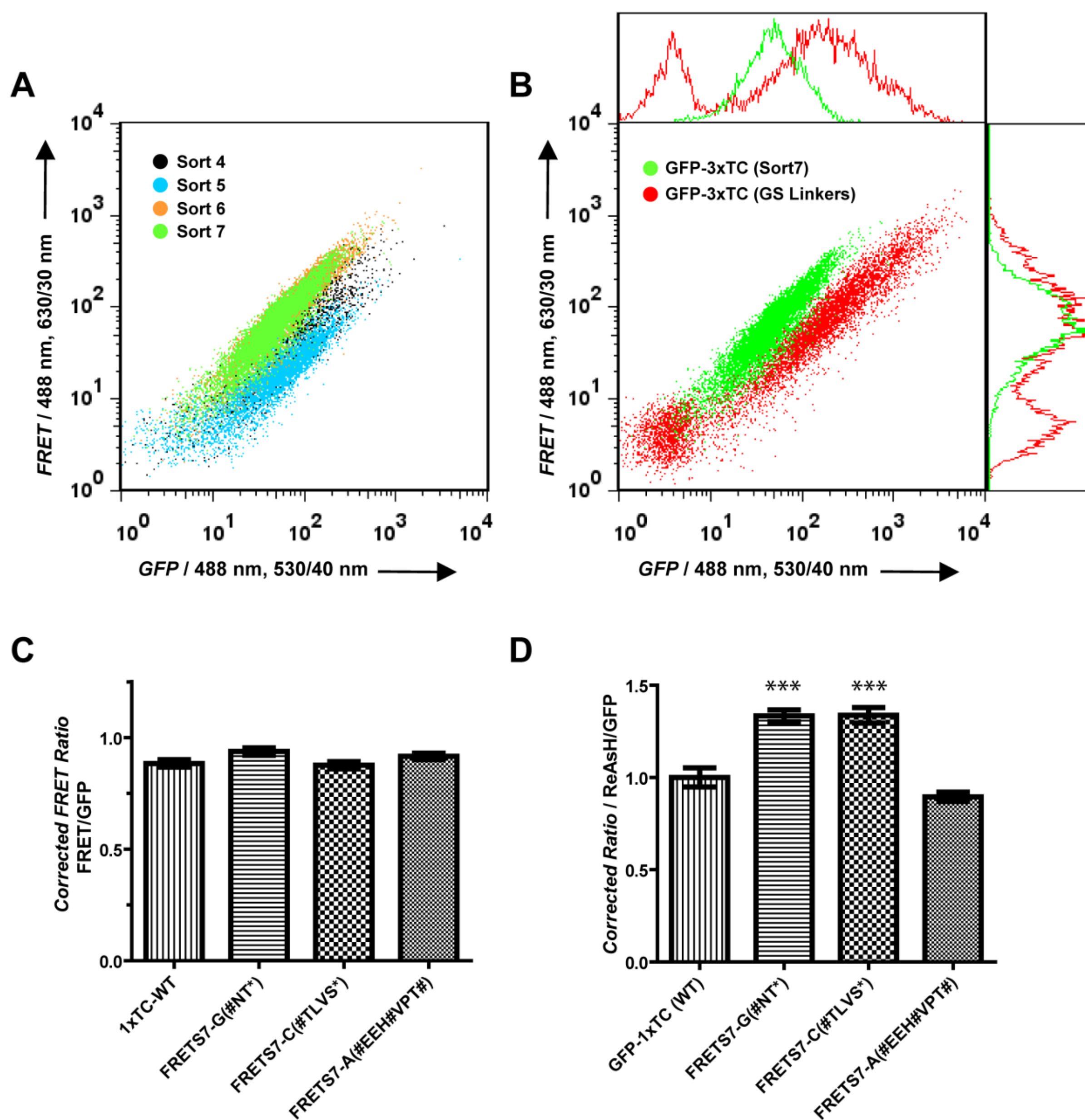
FACS-Based Selection of Tandem Tetracysteine Peptides with Improved ReAsH Brightness in Live Cells

Schuyler B. Van Engelenburg, Theresa Nahreini, and Amy E. Palmer*[a]

cbic_200900689_sm_miscellaneous_information.pdf

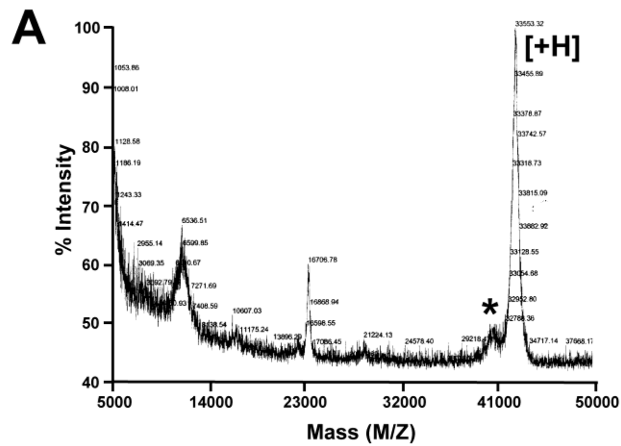


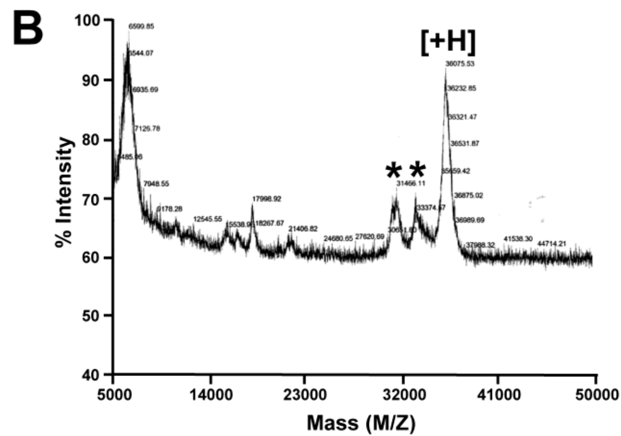
Supplementary Figure 1. Control experiments demonstrate specificity for ReAsH binding to the GFP-3×TC. FACS quantification of fluorescence levels for: (**a**) HEK293FT cells only, (**b**) ReAsH stained and BAL destained HEK293FT cells not expressing GFP-3×TC, (**c**) HEK293FT cells expressing wild-type eGFP only, and (**d**) ReAsH stained and BAL destained HEK293FT cells expressing the rationally designed GFP-3×TC protein. Note the increased level of fluorescence (>10^1) for specifically stained HEK293FT cells. (**e**) Microscopic analysis of ReAsH treated and BAL destained HEK293FT cells negative for GFP-3×TC. ReAsH Image is thresholded to same level as images from Figure 2. Typically, ≤ 30% of the fluorescence signal is attributed to background ReAsH staining which is corrected by subtraction in all experiments described. Scale bar represents 10 μm.

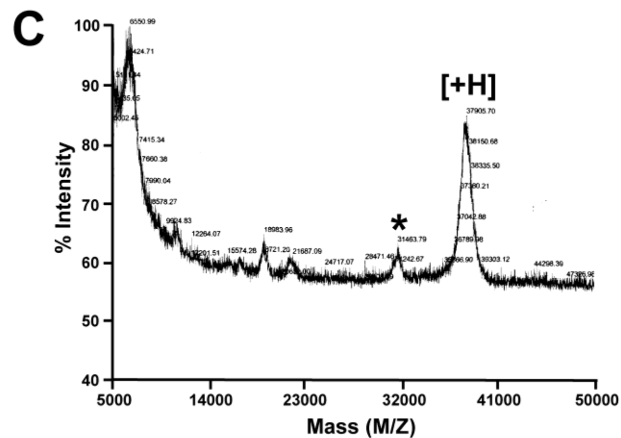


Supplementary Figure 2. FRET-based FACS and fluorescence microscopy analysis of the GFP-3×TC library reveals the core 1×TC motif is sufficient for maximal FRET/GFP ratio. (A) Enrichment of GFP-TC clones with improved FRET properties. Sorts 4-7 were normalized to an isogenic HEK293FT cell line stably expressing a rationally designed GFP-3×TC (GS spacers) protein. Histograms represent n = 5.0×10^3 cell events. (B) FRET-based FACS analysis reveals GFP-TC variants with improved properties relative to the GFP-3×TC (GS spacer) peptide. However, only one GFP-3×TC clone (GFP-#EEH#VPT#) was isolated from the final sort. The remainder of the isolated clones contained stop codon mutations on or within the first randomized liker of the 3×TC motif yielding only one biarsenical binding site with short C-terminal amino acid extensions. (C) Single cell fluorescence microscopy shows no significant improvement in the FRET ratio (FRET/GFP) when compared to the single core TC motif alone

(n $\geq$ 60 cells for each of three independent experiments). (D) Modest improvements ($\leq$ 1.3-fold) in the ReAsH brightness (ReAsH intensity normalized to the GFP intensity) are observed for two truncated GFP-1×TC variants compared with the core GFP-1×TC peptide (n $\geq$ 60 cells for three independent experiments; *** $P < 0.001$). The only GFP-3×TC tag selected by this sorting strategy displayed no improvement in ReAsH fluorescence relative to GFP. Error bars represent SEM.







Supplementary Figure 3. MALDI-TOF mass spectra of ReAsH saturated GFP-Tetracysteine variants. Metal affinity chromatography purified GFP-TetCys constructs were subjected to 5-fold molar excess ReAsH (relative to the predicted number of binding sites) and desalted for mass spectrometry analysis. (**a**) GFP-1×TC (expected MW: 33.689 KDa; predicted MW: 33.553 KDa), (**b**) GFP-2×TC (expected MW: 37.489 KDa; predicted MW: 36.075 KDa), and (**c**) GFP-3×TC20 (expected MW: 38.160 KDa; predicted MW: 37.905 KDa) show appropriate +1

MALDI-TOF mass spectra and minimal proteolysis and/or cysteine oxidation. Asterisk (*) denotes minimal potential proteolysis products and or oxidized GFP-TetCys protein unable to bind ReAsH.