Supplementary Material (ESI) for Molecular BioSystems
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SUPPLEMENTARY INFORMATION

De novo helical peptides as target sequences for a specific, fluorogenic protein labelling strategy

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Contents: CD spectra of unlabelled and labelled dC10, tables of primers used for cloning and mutagenesis, fluorescence spectra of fluorogens and dithiolated adducts, and images of negative control cell labelling experiments.

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Figure S1: Normalised CD spectrum (5 mM sodium phosphate, pH 7.0, 23 °C.) of dC10 after thrombin-mediated cleavage from MBP-dC10

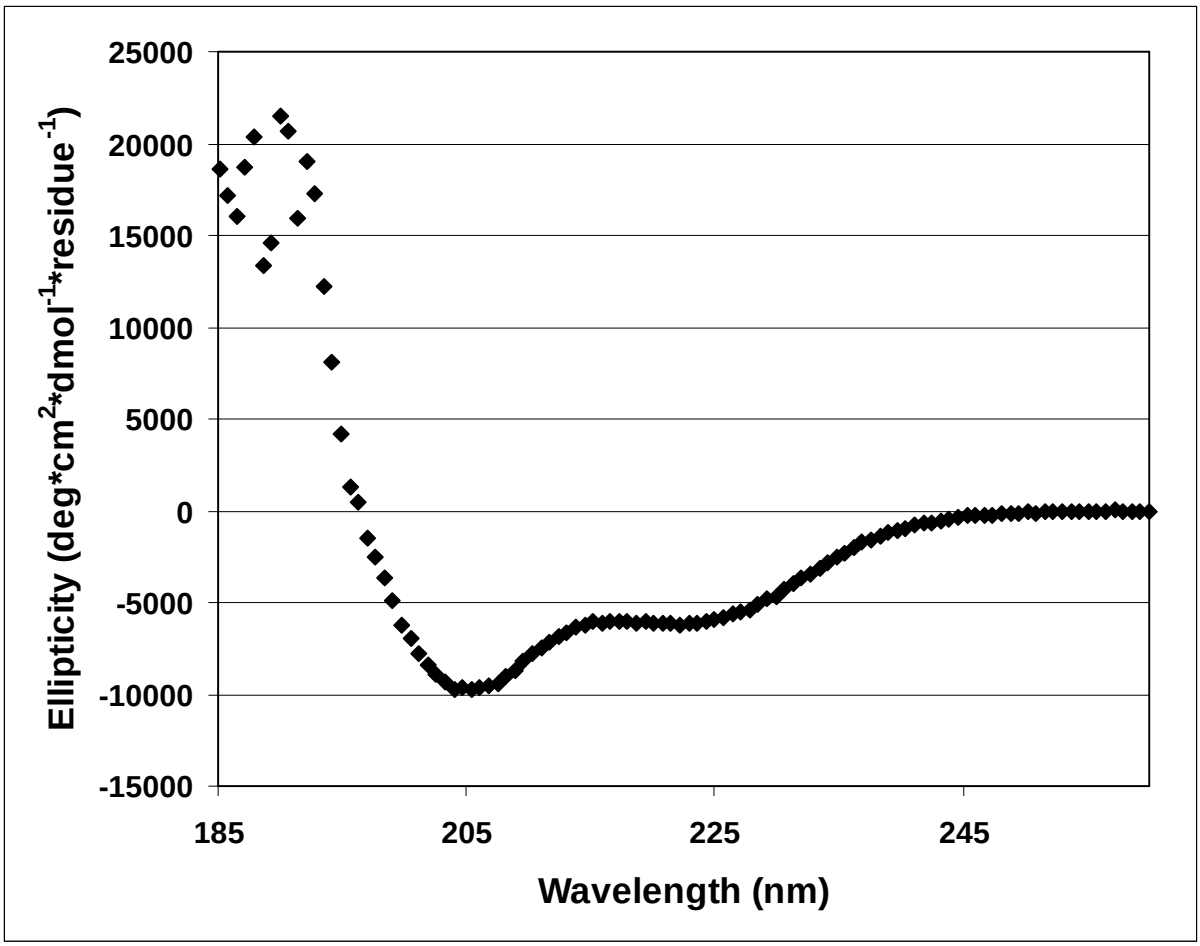

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Figure S2: Normalised CD spectrum (5 mM sodium phosphate, pH 7.0, 23 °C) of dC10 after labelling with $dM10^{M}$-FITC (**2**) and subsequent thrombin-mediated cleavage from MBP-dC10 (*cf* Figure S1)

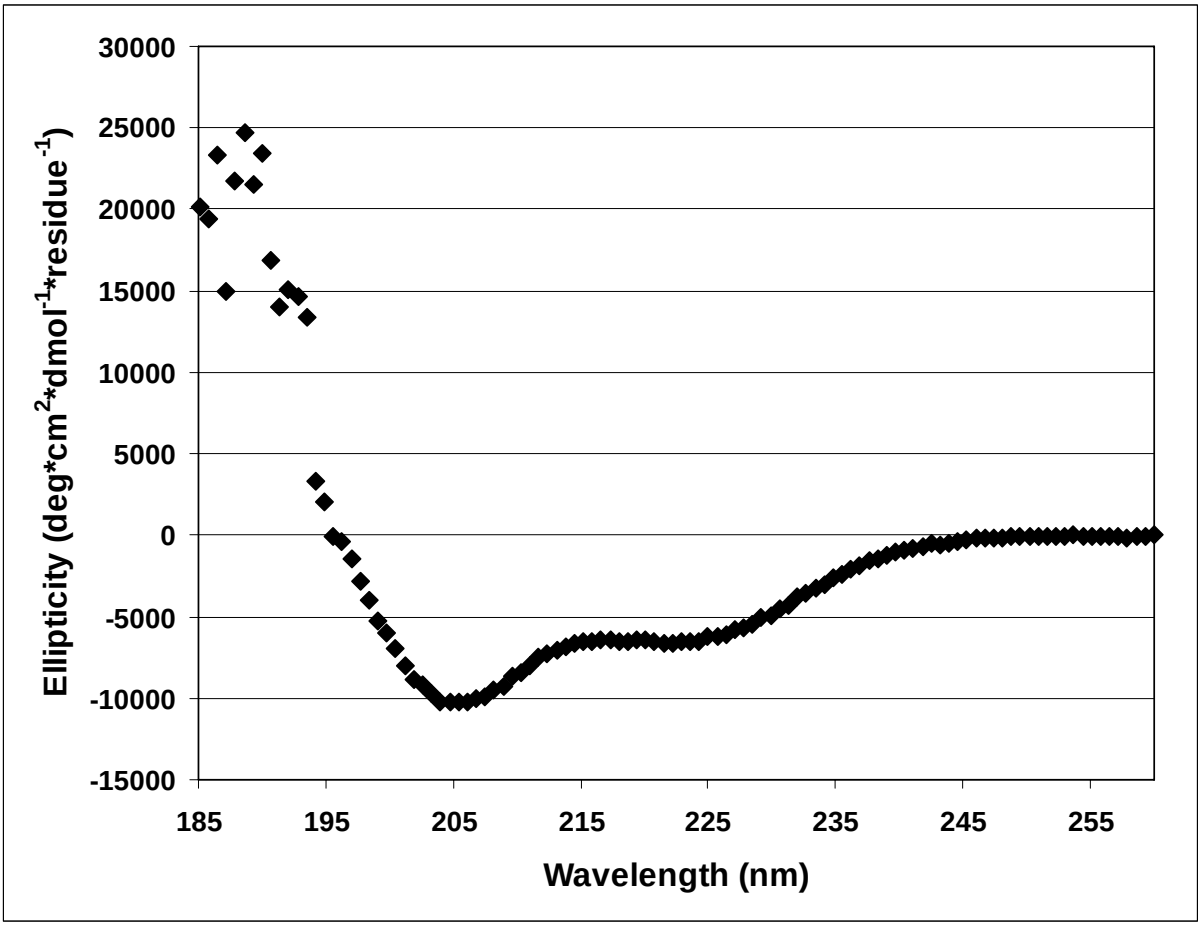

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Table S1: Experimental and predicted helicity values for thrombin-cleaved peptides dC10 and dC10 labelled with $dM10^{M}$-FITC (**2**), determined by CD in 5 mM sodium phosphate, pH 7.0, 23 °C.

Peptide	**Experimental mean residue ellipticity** [a]	**Experimental mean ellipticity** [a]	**Predicted helicity value (AGADIR)** [b]
dC10	17.74	-62.94	27.73 % [c]
dC10-dM10-FITC	18.81	-54.88	

[a] Measured at 222 nm

[b] See references 1 and 2.

[c] Value determined for cleaved peptide at pH 7.0, 23 °C and ionic strength of 0.1. Similar values of 28.90 % and 28.53 % were determined at ionic strength of 0.025, at pH 7.4 and pH 7.0, respectively.

[1] Muñoz , V.; Serrano, L. : Development of the Multiple Sequence Approximation Within the AGADIR Model of α-Helix Formation: Comparison with Zimm–Bragg and Lifson–Roig Formalisms. *Biopolymers* **1997**, *41*, 495-509.

[2] The AGADIR algorithm is available on-line at http://www.embl-heidelberg.de/Services/serrano/agadir/agadir-start.html.

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Table S2: Nucleotide sequences of dC10 helices studied herein. Mutations relative to parent dC10 are underlined. See Table 6 for corresponding amino acid sequences.

Helix Name	Nucleotide sequence
dC10	5'-CTGAGCGCTGCTGAGTGCGCTGCTAGAGAAGCTGCATGCAGAGAA GCTGCAGCTAGAGCTGGAGGAAAGTAG-3'
dC10-H2	5'-CTGCACGCTGCTGAGTGCGCTGCTAGAGAAGCTGCATGCAGAGAA GCTGCAGCTAGAGCTGGAGGAAAGTAG-3'
dC10-H7	5'-CTGAGCGCTGCTGAGTGCCACGCTAGAGAAGCTGCATGCAGAGAA GCTGCAGCTAGAGCTGGAGGAAAGTAG-3'
dC10-H9	5'-CTGAGCGCTGCTGAGTGCGCTGCTCACGAAGCTGCATGCAGAGAA GCTGCAGCTAGAGCTGGAGGAAAGTAG-3'
dC10-H2H17	5'-CTGCACGCTGCTGAGTGCGCTGCTAGAGAAGCTGCATGCAGAGAA GCTCACGCTAGAGCTGGAGGAAAGTAG-3'

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Table S3: Primers used for site-directed mutagenesis.

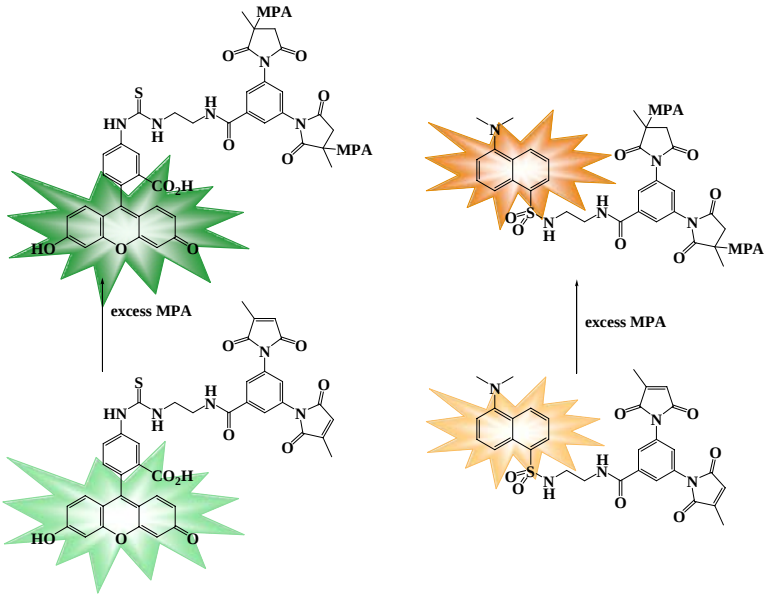
Mutant dC10 helix	Primer sequence
dC10-H2	5'-GGCTCTCTCGAGCTGCACGCTGCTGAGTGCGCT-3' 5'-AGCGCACTCAGCAGCGTGCAGCTCGAGAGAGCC-3'
dC10-H7	5'-GCGCTGCTGAGTGCCACGCTAGAGAAGCTGC-3' 5'-GCAGCTTCTCTAGCGTGGCACTCAGCAGCGC-3'
dC10-H9	5'-GCTGAGTGCGCTGCTCACGAAGCTGCATGCAGAG-3' 5'-CTCTGCATGCAGCTTCGTGAGCAGCGCACTCAGC-3'
dC10-H2H17	5'-GCATGCAGAGAAGCTCACGCTAGAGCTGGAGG-3' 5'-CCTCCAGCTCTAGCGTGAGCTTCTCTGCATGC-3' 5'-GGCTCTCTCGAGCTGCACGCTGCTGAGTGCGCT-3' 5'-AGCGCACTCAGCAGCGTGCAGCTCGAGAGAGCC-3'

Table S4: Primers used for cloning of MBP-dC10-R8A9 and MBP-dC10-A9

Mutant dC10 helix	Primer sequence
MBP-dC10-R8A9	5'-TCGAGCTGAGTGCGGCGGAATGTGCGCGTGCAGAAGCAGC ATGCCGCGAAGCGGCGGCGCGCGCGGGTGGCAAATGA-3' 5'-AGCTTCATTTGCCACCCGCGCGCGCCGCCGCTTCGCGGCAT GCTGCTTCTGCACGCGCACATTCCGCCGCACTCAGC-3'
MBP-dC10-A9	5'- TCGAGCTGAGTGCGGCGGAATGTGCGGCAGCAGAAGCA GCATGCCGCGAAGCGGCGGCGCGCGCGGGTGGCAAATGA-3' 5'- AGCTTCATTTGCCACCCGCGCGCGCCGCCGCTTCGCGGC ATGCTGCTTCTGCTGCCGCACATTCCGCCGCACTCAGC-3'

Figure S3: **A**: Fluorogenic reactions of fluorogens dM10^{M}-FITC (**2**) and dM10^{M}-dansyl (**3**) with excess MPA in 50 mM HEPES (pH 7.5) with 5 % DMSO. **B**: Fluorescence spectra recorded before and after addition reactions of 2.5 µM **2** (λ_{exc} = 494 nm, λ_{fl} = 517 nm) and 75 µM **3** (λ_{exc} = 331 nm, λ_{fl} = 551 nm), showing the fluorescence enhancements reported in Table 5.

A



B

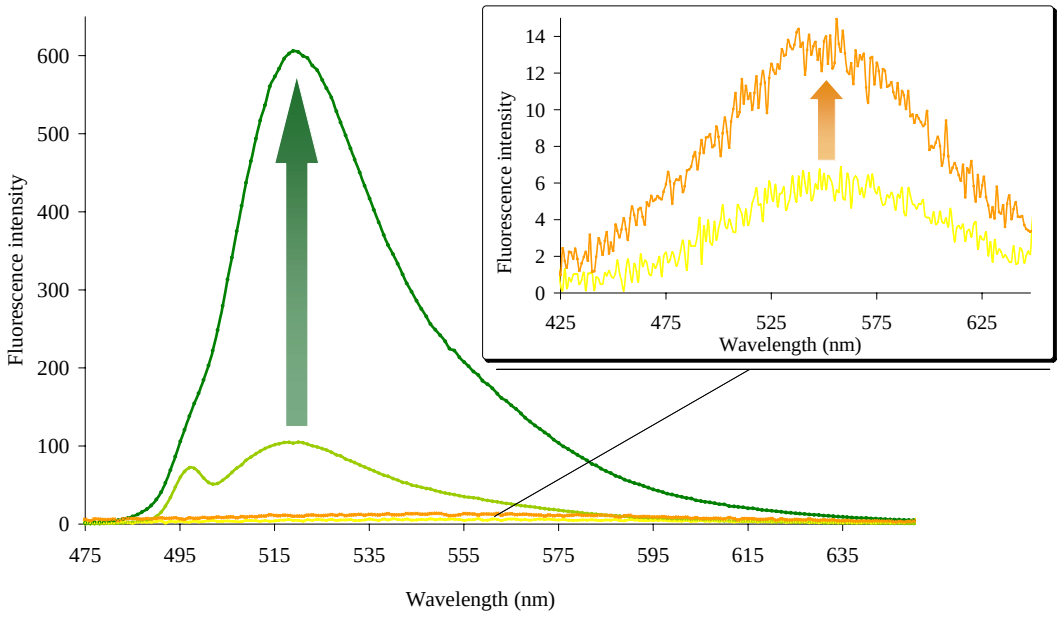


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Figure S4: Negative control experiment for labelling of EGF receptors. **A**: Phase-contrast image of HEK 293 cells expressing EGFR (*not* tagged with dC10 sequence) **B**: Confocal image after incubation with 20 μM dM10^{M}-FITC (**2**) for 20 minutes, showing *no* cell surface labelling. Non-specific green fluorescence is attributed to dead cell debris. **C**: Red fluorescent image using the same brightness and contrast settings as B, after incubation with rhodamine-labelled EGF, showing proper expression of EGFR on the cell surface.