Supplemental Figure

Biophysical Characterization of the Engineered T20 Binding Protein 5H-ex.

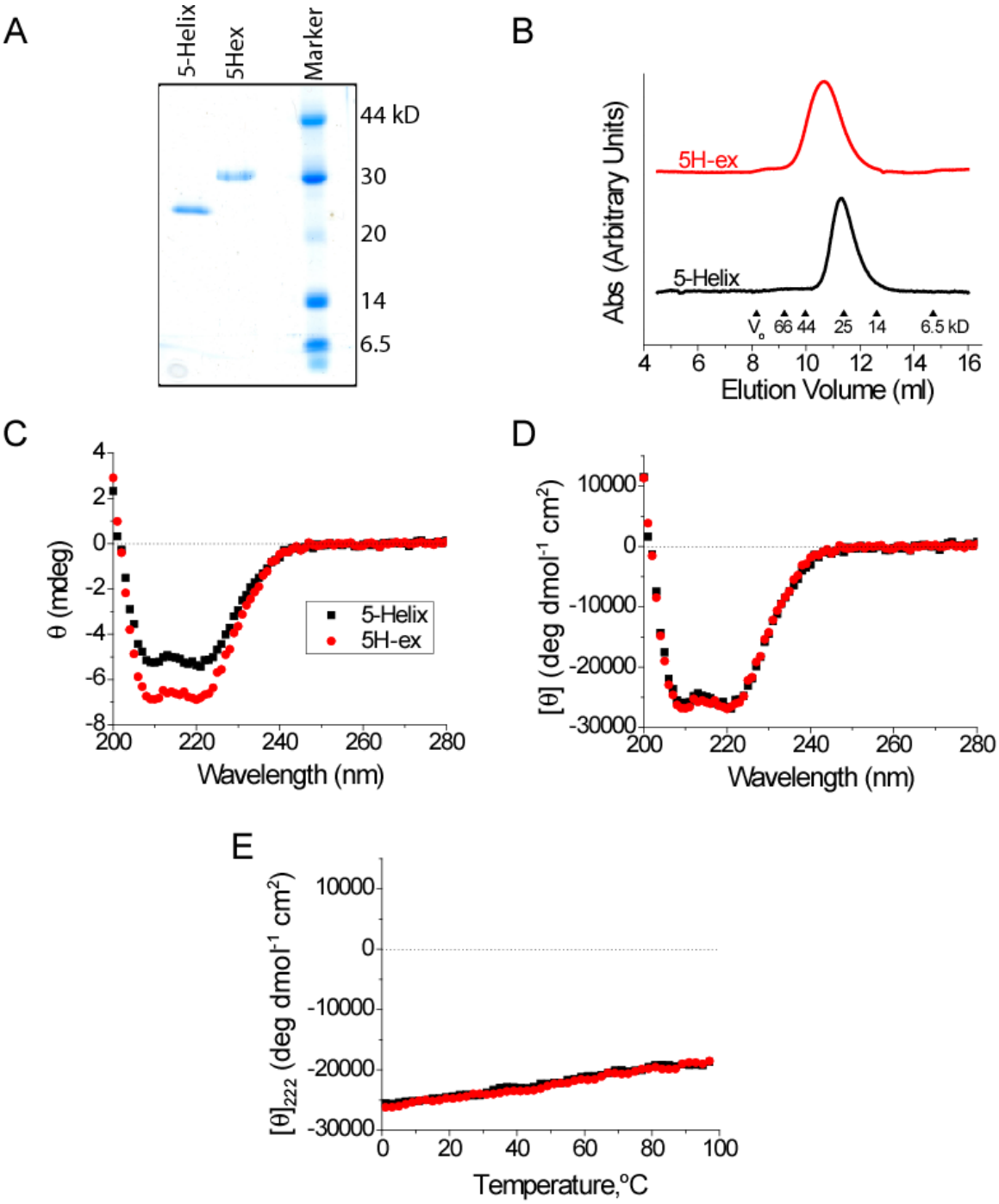
(A) SDS-PAGE analysis of purified 5-Helix (MW 25.4 kD) and 5H-ex (MW 31.6 kD). Approximately 2 μg protein was loaded per lane, and bands were visualized by staining with Gelcode Blue (Pierce). Molecular weights of the standards (GE) run in the lane labeled "Marker" are shown to the right.

(B) Analytical size exclusion chromatography of 5-Helix (black) and 5H-ex (red). Samples (100 μl of 5 μM protein) were loaded onto a Superdex 75HR 10/300 column (GE) running tris-buffered saline (TBS—50 mM tris pH 8, 100 mM NaCl) at 4^{o}C. The triangles below the chromatograms represent the elution volumes of molecular weight standards (BioRad and GE) run under the same conditions. V_o indicates the column void volume.

(C) Circular dichroism (CD) spectra of 5-Helix (black) and 5H-ex (red). Spectra were obtained from protein solutions (0.9 μM protein in TBS at 4^{o}C) in a 1 mm quartz cuvette using a Jasco J-810 spectropolarimeter. The larger absolute ellipticity (θ) at 222 and 208 nm for 5H-ex is consistent with the greater number of helical residues for this enlarged protein.

(D) The CD spectra in **C** converted to mean residue ellipticity ([θ]). The depth of the well at 222 nm indicates that the 5H-ex protein adopts ~95% of the helical content expected from the design.

(E) Thermal stability of 5-Helix (black) and 5H-ex (red) monitored by mean residue ellipticity at 222 nM. The experiments were performed using a protein concentration of 0.9 μM in a stirred 1-cm quartz cuvette. All CD experiments were performed in triplicate with quantitatively similar results.


A
5-Helix
5Hex
Marker
44 kD
30
20
14
6.5
B
Abs (Arbitrary Units)
5H-ex
5-Helix
V0
66
44
25
14
6.5 kD
4
6
8
10
12
14
16
Elution Volume (ml)
C
θ (mdeg)
4
2
0
-2
-4
-6
-8
5-Helix
5H-ex
200
220
240
260
280
Wavelength (nm)
D
[θ] (deg dmol-1 cm2)
10000
0
-10000
-20000
-30000
200
220
240
260
280
Wavelength (nm)
E
[θ]222 (deg dmol-1 cm2)
10000
0
-10000
-20000
-30000
0
20
40
60
80
100
Temperature,°C