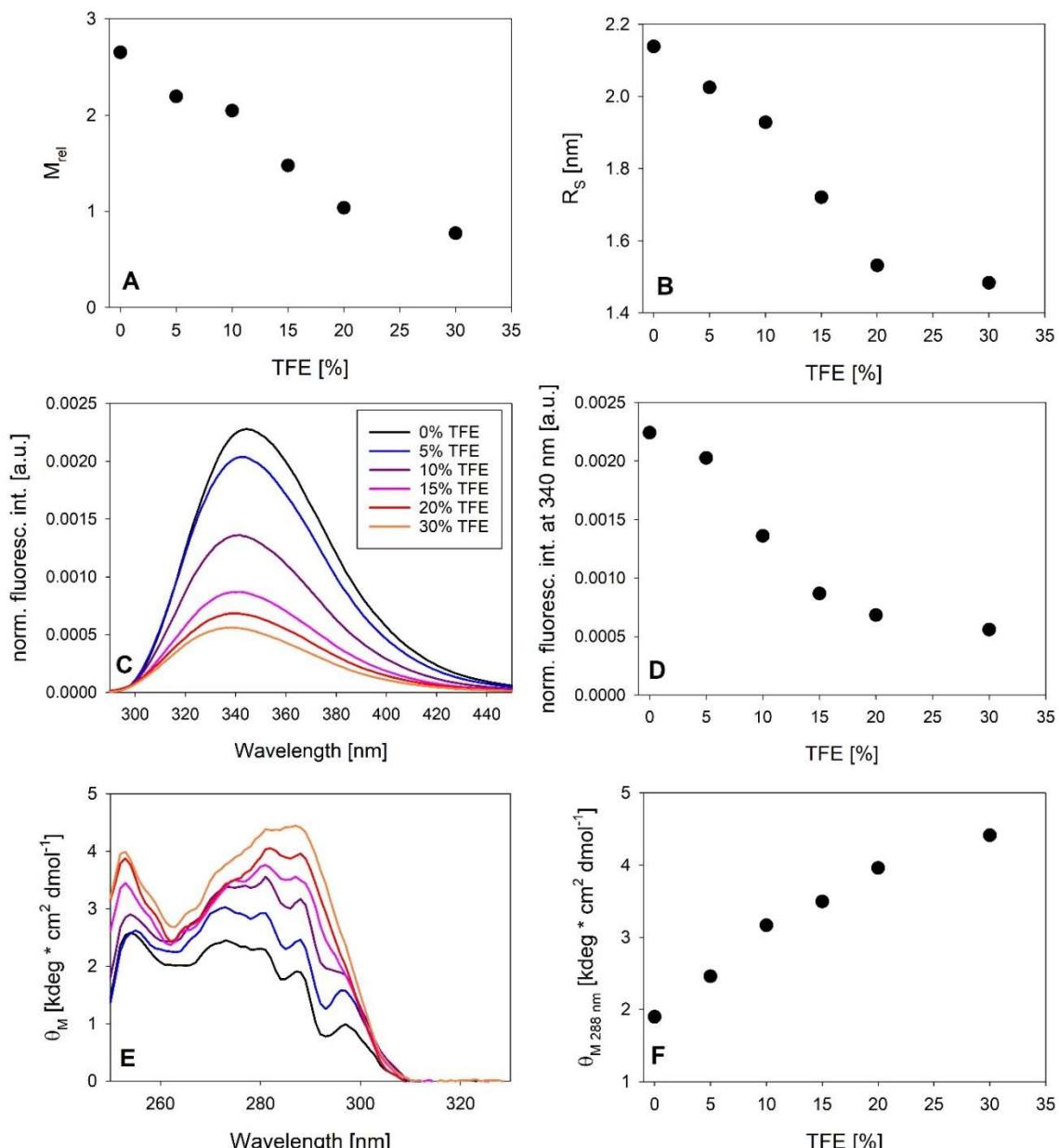
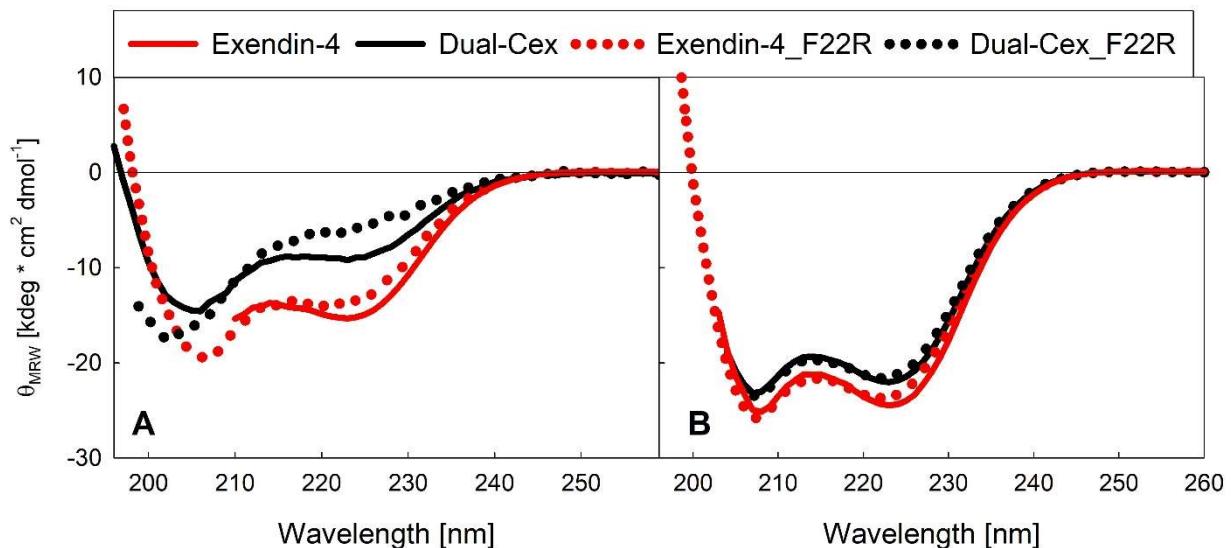


**Figure S1.** Multiple sequence alignment (Clustal O 1.3.4) of proglucagon-derived peptides. Conserved sequence clusters are highlighted. Red colors depict a cluster of conserved hydrophobic amino acids

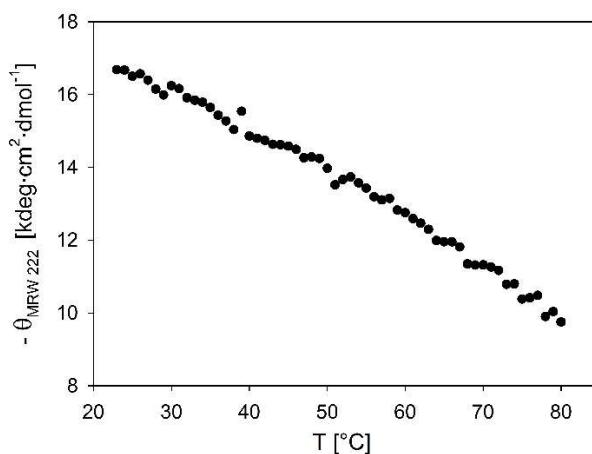


**Figure S2.** Titration of exendin-4 in PBS, pH 7.4 with TFE. (A) and (B) show relative molecular masses  $M_{\text{rel}}$  (A) and Stokes radii  $R_s$  (B) of 0.3 mM exendin-4 with increasing concentrations of TFE measured at 23 °C. Trp fluorescence (C) and near-UV CD (E) spectra of 0.1 mM exendin-4 were measured with increasing concentrations of TFE. Fluorescence intensity at 340 nm and molar ellipticity at 288 nm were plotted as a function of

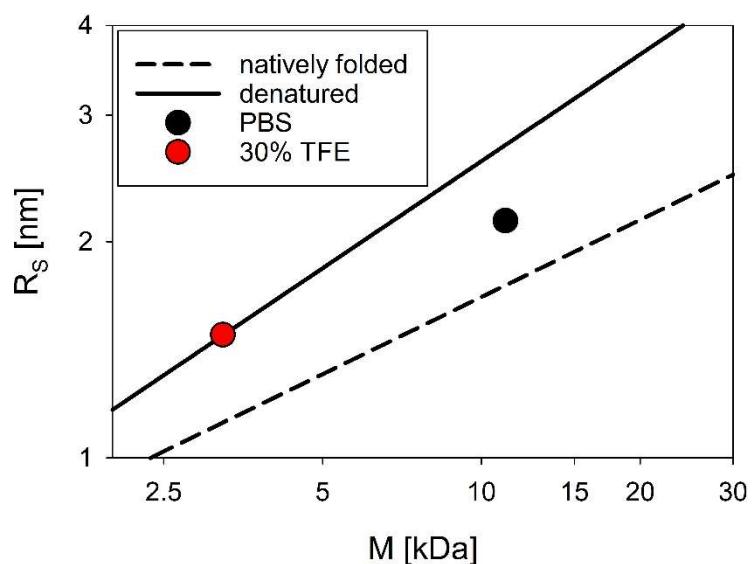
TFE concentration in (D) and (F), respectively. Fluorescence intensity is shown normalized to peptide concentration and solvent contribution.



**Figure S3.** Far-UV CD spectra of exendin-4 and Dual-Cex and the respective F22R mutants of both peptides were measured in PBS (A) and in 30% TFE (B) at pH 7.4 at concentrations < 0.1 mM which grant a monomeric state of all peptides



**Figure S4.** Temperature dependence of  $\theta_{222}$  from far UV CD spectra for exendin-4 at 0.4 mM at pH 7.4



**Figure S5.** Compactness of exendin-4 in PBS, pH 7.4 (black) and in PBS + 30% TFE, pH 7.4 (red). Compactness of ~0.4 mM exendin-4 and at 23 °C, visualized using scaling laws of the type  $R_s = a \cdot M^b$ . Reference data sets reporting on the average compactness of natively folded (dashed line) and denatured (solid line) proteins are shown for comparison.

**Table S1.**  $^1\text{H}$ -chemical shifts of Dual-Cex in 70%  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (9:1), 100 mM acetate buffer, pH 4.6 and 30% TFE at 310 K<sup>1</sup>.

No.	Residue	NH	H $\alpha$	H $\beta$	Others
1	His	-			$\delta$ : 7.28, $\epsilon$ : 8.27
2	Ser	Broad	4.58	3.96/3.92	
3	Gln	8.70	4.45	2.21/2.08	$\gamma$ : 2.44, NH2: 7.43/6.72
4	Gly	8.40	4.03		
5	Thr	7.90	4.37	4.22	$\gamma$ : 1.17
6	Phe	8.18	4.70	3.22/3.15	$\delta$ : 7.30, $\epsilon$ : 7.36, $\zeta$ : 7.30
7	Thr	7.87	4.28	4.28	$\gamma$ : 1.23
8	Ser	8.10	4.42	4.01/3.92	
9	Asp	8.29	4.63	2.78/2.73	
10	Leu	8.12	4.20	1.67	$\gamma$ : 1.67, $\delta$ : 0.94, $\delta'$ : 0.89
11	Ser	8.14	4.22	4.03/3.99	
12	Lys	7.78	4.29	1.98/1.95	$\gamma$ : 1.60/1.50, $\delta$ : 1.75, $\epsilon$ : 3.03
13	Gln	8.01	4.24	2.22/2.18	$\gamma$ : 2.45, NH2: 7.27/6.63
14	Met	8.25	4.36	2.15	$\gamma$ : 2.68/2.59, $\epsilon$ : 2.05
15	Asp	8.22	4.59	2.86/2.78	
16	Ser	8.18	4.32	4.08/4.02	
17	Arg	8.08	4.24	2.06/2.00	$\gamma$ : 1.79, $\delta$ : 3.27, $\epsilon$ : 7.51

<sup>1</sup> Proton chemical shifts are referenced to sodium-3-(Trimethylsilyl)propionate-2,2,3,3-d<sub>4</sub>.

18	Arg	8.12	4.25	1.99	$\gamma$ : 1.86/1.72, $\delta$ : 3.26, $\varepsilon$ : 7.26
19	Ala	8.05	4.28	1.58	
20	Gln	8.145	4.08	2.27	$\gamma$ : 2.58/2.49, NH2: 7.39/6.61
21	Asp	8.32	4.54	2.96/2.68	
22	Phe	8.26	4.34	3.39/3.30	$\delta$ : 7.22, $\varepsilon$ : 7.34, $\zeta$ : 7.38
23	Ile	8.51	3.76	2.14	$\beta$ -Me: 1.02, $\gamma$ : 1.92/1.43, $\delta$ : 0.96
24	Glu	8.32	4.03	2.29/2.22	$\gamma$ : 2.51
25	Trp	8.10	4.30	3.76/3.26	H1: 9.76, H2: 7.18, H4: 7.28, H5: 7.06, H6: 7.13, H7: 7.27
26	Leu	8.55	3.40	1.95/1.42	$\gamma$ : 1.64, $\delta$ : 0.85, $\delta'$ : 0.80
27	Lys	8.84	3.93	1.97/1.92	$\gamma$ : 1.68/1.47, $\delta$ : 1.68, $\varepsilon$ : 2.96
28	Asn	7.58	4.76	3.00/2.78	NH2: 7.62/6.88
29	Gly	7.66	4.19/3.55		
30	Gly	8.24	2.77/1.42		
31	Pro	-	4.53	2.46/2.03	$\gamma$ : 2.08/2.03, $\delta$ : 3.65/3.05
32	Ser	7.67	4.43	3.97/3.92	
33	Ser	8.04	4.33	3.99/3.77	
34	Gly	7.84	4.29/3.81		
35	Ala	8.06	4.89	1.49	
36	Pro	-	4.63	2.21/1.87	$\gamma$ : 2.02, $\delta$ : 3.85/3.69
37	Pro	-	3.13	1.47/1.05	$\gamma$ : 1.80/1.62, $\delta$ : 3.45

38	Pro	-	4.36	2.24/1.96	$\gamma$ : 1.89, $\delta$ : 3.16/3.02
39	Ser	7.93	4.34	3.88/3.80	NH2: 7.41/6.93

**Table S2.**  $^1\text{H}$ -chemical shifts of Dual-Cex in  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (9:1), 100 mM acetate buffer, pH 4.6 at 310 K<sup>2</sup>.

No.	residue	NH	H $\alpha$	H $\beta$	Others
1	His		4.33	3.36	$\delta$ : 7.34, $\epsilon$ : 8.47
2	Ser		4.54	3.88	
3	Gln	8.69	4.42	2.16/2.03	$\gamma$ : 2.41, NH2: 7.48/6.83
4	Gly	8.41	3.98		
5	Thr	7.95	4.31	4.12	$\gamma$ : 1.12
6	Phe	8.31	4.73	3.17/3.03	$\delta$ : 7.25, $\epsilon$ : 7.33, $\zeta$ : 7.28
7	Thr	8.00	4.33	4.20	$\gamma$ : 1.17
8	Ser	8.21	4.42	3.91/3.85	
9	Asp	8.32	4.62	2.76/2.66	
10	Leu	8.19	4.26	1.65	$\gamma$ : 1.65, $\delta$ : 0.92, $\delta'$ : 0.85
11	Ser	8.21	4.31	3.95/3.90	
12	Lys	7.92	4.30	1.89/1.78	$\gamma$ : 1.47/1.41, $\delta$ : 1.68, $\epsilon$ : 2.99
13	Gln	8.06	4.29	2.13/2.01	$\gamma$ : 2.37, NH2: 7.44/6.78
14	Met	8.21	4.41	2.07/2.02	$\gamma$ : 2.58/2.52, $\epsilon$ : 2.03
15	Asp	8.22	4.61	2.75	
16	Ser	8.21	4.33	3.96/3.91	
17	Arg	8.19	4.26	1.91/1.85	$\gamma$ : 1.67/1.62, $\delta$ : 3.19, $\epsilon$ : 7.37

<sup>2</sup> Proton chemical shifts are referenced to sodium-3-(Trimethylsilyl)propionate-2,2,3,3-d<sub>4</sub>.

18	Arg	8.09	4.28	1.89/1.83	$\gamma$ : 1.70/1.64, $\delta$ : 3.18, $\varepsilon$ : 7.26
19	Ala	8.18	4.26	1.48	
20	Gln	8.25	4.18	2.12	$\gamma$ : 2.43, NH2: 7.52/6.79
21	Asp	8.24	4.52	2.77/2.67	
22	Phe	8.14	4.33	3.19/3.14	$\delta$ : 7.17, $\varepsilon$ : 7.32, $\zeta$ : 7.34
23	Ile	7.97	3.79	1.98	$\beta$ -Me: 0.88, $\gamma$ : 1.65/1.30, $\delta$ : 0.89
24	Glu	8.12	4.07	2.09	$\gamma$ : 2.39
25	Trp	7.97	4.38	3.45/3.24	H1: 9.88, H2: 7.18, H4: 7.32, H5: 7.07, H6: 7.17, H7: 7.36
26	Leu	8.16	3.68	1.70/1.46	$\gamma$ : 1.53, $\delta$ : 0.84, $\delta'$ : 0.80
27	Lys	8.11	4.05	1.85	$\gamma$ : 1.52/1.40, $\delta$ : 1.65, $\varepsilon$ : 2.94
28	Asn	7.85	4.74	2.91/2.75	NH2: 7.54/6.88
29	Gly	7.84	4.03/3.71		
30	Gly	7.98	3.33/2.75		
31	Pro	-	4.46	2.34/1.98	$\gamma$ : 2.00, $\delta$ : 3.58/3.22
32	Ser	8.06	4.45	3.92/3.89	
33	Ser	8.12	4.41	3.94/3.80	
34	Gly	8.11	4.09/3.90		
35	Ala	7.96	4.67	1.38	
36	Pro	-	4.61	2.22/1.83	$\gamma$ : 2.00, $\delta$ : 3.80/3.62

37	Pro	-	3.93	1.69	$\gamma$ : 1.90/1.86, $\delta$ : 3.65/3.51
38	Pro	-	4.38	2.26/1.90	$\gamma$ : 1.90, $\delta$ : 3.45/3.29
39	Ser	8.16	4.35	3.87/3.82	NH2: 7.50/7.08