Supporting Information for:

Temperature-controlled Electrospray Ionization Mass Spectrometry as a Tool to Study Collagen Homo- and Heterotrimers

Martin Köhler, Adrien Marchand, Nina B. Hentzen, Jasmine Egli, Alina I. Begley, Helma Wennemers* and Renato Zenobi*

Department of Chemistry and Applied Biosciences, ETH Zurich, 8093, Zurich, Switzerland

*Correspondence and requests for materials should be addressed to
R.Z. (email: zenobi@org.chem.ethz.ch, Tel.: +41 44 632 43 76)
and H.W. (email: helma.wennemers@org.chem.ethz.ch, Tel.: +41 44 633 37 77)

Contents of supporting information:

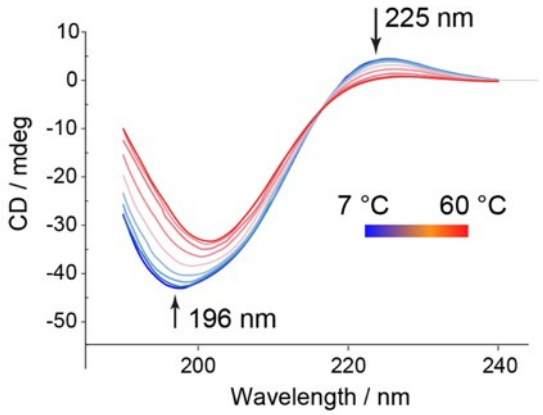

Figure S1. CD spectroscopy of CMP **A** (50 µM in 10 mM aq. NH_4Ac at pH 7) at different temperatures ranging from 7 °C to 60 °C. Two CD bands were detected at 225 nm and 196 nm, which are indicative of triple helix formation.

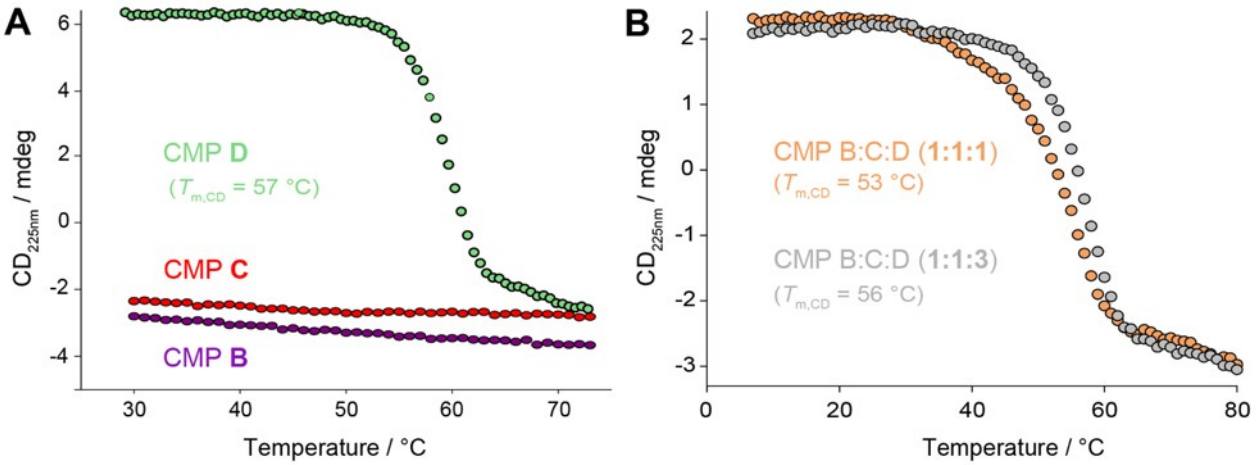

Figure S2. (**A**) CD spectroscopic thermal denaturation studies of CMP **B** (purple), **C** (red) and **D** (green). 50 µM solutions in 10 mM aq. NH_4Ac at pH 7 were used. Only CMP **D** showed a characteristic, sigmoidal melting curve with a $T_{M,CD}$ = 57 °C (heating rate of 1 °C/min). (**B**) CD spectroscopic thermal denaturation studies of mixtures of CMPs **B, C, and D** in molar ratios of 1:1:1 (orange) and 1:1:3 (gray). 100 µM solutions in 10 mM aq. NH_4Ac at pH 7 were used. The following melting temperatures were obtained: **B**:**C**:**D** (1:1:1): $T_{M,CD}$ = 53 °C and **B**:**C**:**D** (1:1:3): $T_{M,CD}$ = 56 °C (heating rate of 1 °C/min).

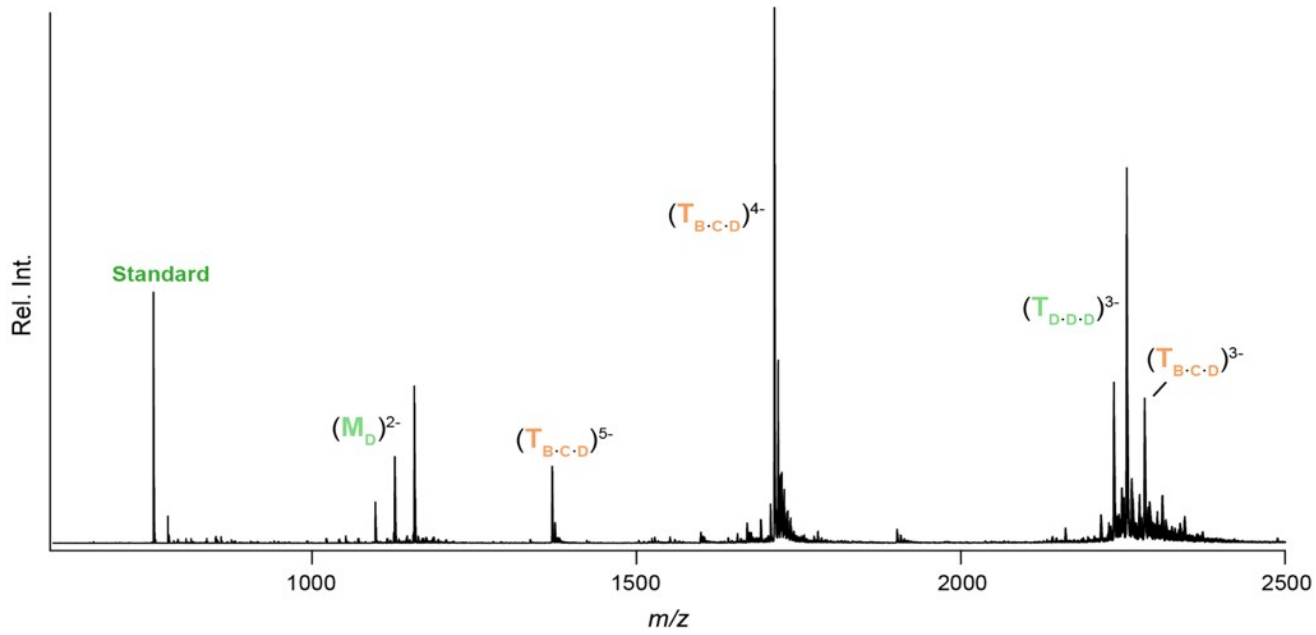

Figure S3. Native MS spectrum of an annealed mixture of CMPs **B**:**C**:**D** (1:1:3). 100 µM of total peptide concentration in 10 mM aq. NH_4Ac at pH 7 was used. *m/z* Signals corresponding to the specific **B**·**C**·**D** heterotrimer and the **D**·**D**·**D** homotrimer are present.

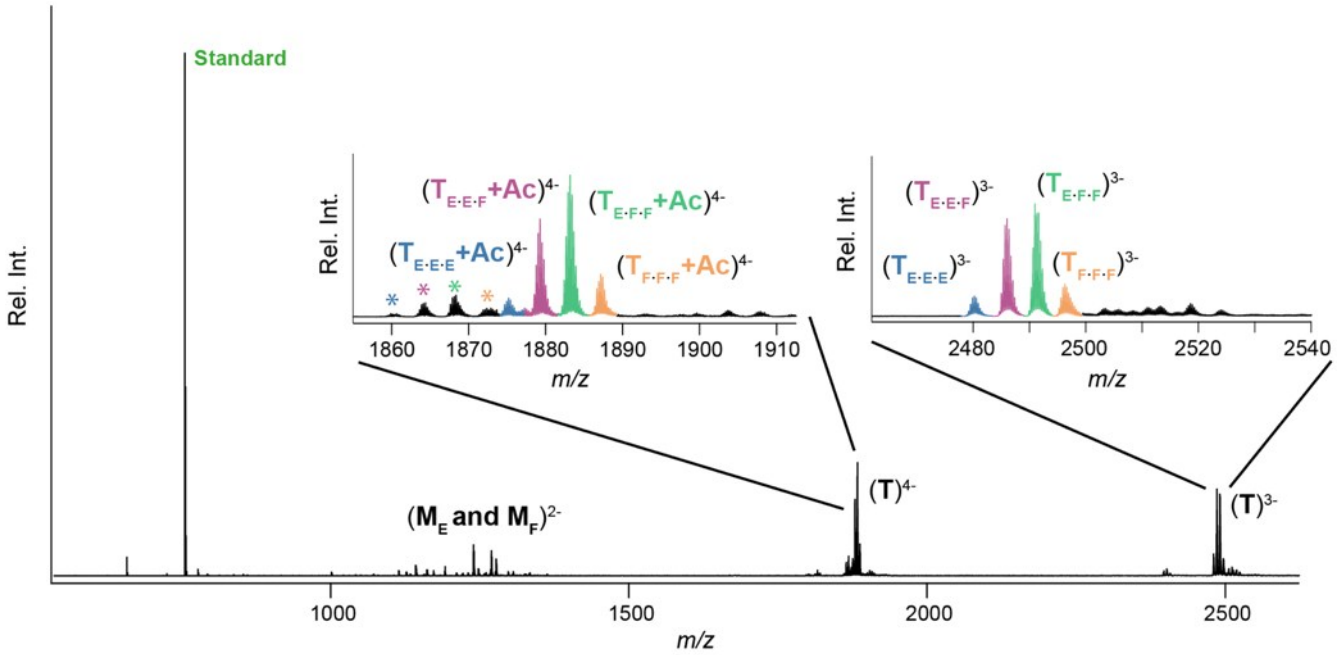

Figure S4. Native MS spectrum of an annealed mixture of CMPs **E** and **F** (1:1). 50 µM solutions in 10 mM aq. NH_4Ac at pH 7 were used. *m/z* Signals corresponding to the **E**·**E**·**E** (blue) and **F**·**F**·**F** (orange) homotrimers and **E**·**F**·**F** (green) and **E**·**E**·**F** (pruple) heterotrimers were detected. Charge states 3- and 4- were observed and enlarged spectra of the trimeric species are shown. Beside the acetate adducts of the fourfold negatively charged trimers, we also detected the deprotonated species (labeled with *).

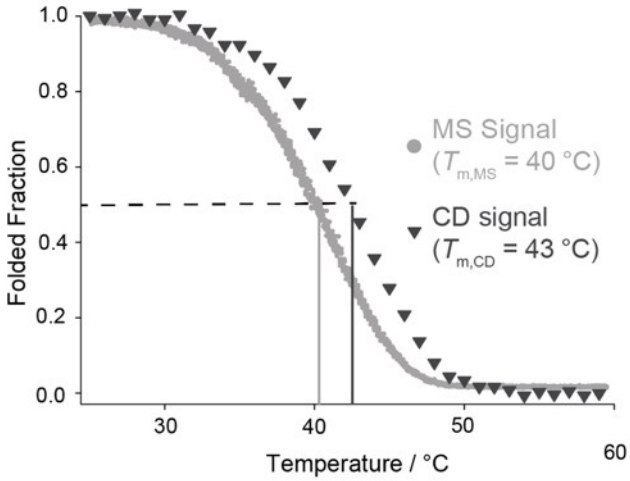

Figure S5. Folded fractions of the triple helices formed in a mixture of CMPs **E** and **F** (1:1) as a function of temperature, as monitored by CD spectroscopy (triangles) and MS (dots). 50 µM solutions in 10 mM aq. NH_4Ac at pH 7 were used. For comparison with melting temperatures obtained by CD spectroscopy, the signals of all trimeric species detected by temperature-controlled MS were summed up and normalized. The obtained melting temperatures of $T_{m,CD}$ = 43 °C and $T_{m,MS}$ = 40 °C are in good agreement (heating rate of 1°C/min).

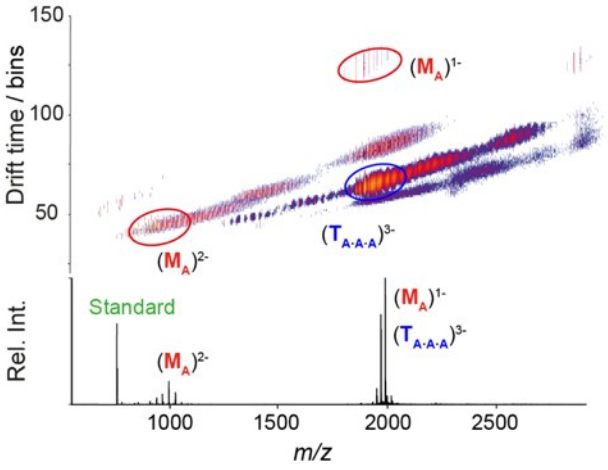

Figure S6. Native ESI MS spectrum of CMP **A** with the corresponding ion mobility. The signal of the triply negatively charged **A**·**A**·**A** homotrimer ($T_{A\cdot A\cdot A}$) overlaps with the signal of the singly charged CMP **A** monomer (M_A).

Table S1. Comparison of T_m values as determined by thermal denaturation studies[a] using CD spectroscopy or temperature-controlled nESI MS as monitoring tools. All values determined at a heating rate of 1 °C/min for 50 µM (or 100 µM for B·C·D) solutions in 10 mM aq. NH_4Ac at pH 7.

Entry	Composition of triple helix	$T_{m,CD}$ / °C	$T_{m,MS}$ / °C
1	A·A·A	46	45
2	B·C·D	53	53
3	D·D·D	57	55
4	E·E·E	38	35
5	E·E·F	n.d.	38
6	E·F·F	n.d.	42
7	F·F·F	46	43