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 scans of CMP A at different temperatures
Figure S2. Thermal denaturation profiles via CD spectroscopy of CMPs B, C, D and B:C:D mixtures
Figure S3. Native MS spectrum of an annealed mixture of CMP B:C:D (1:1:3)
Figure S4. Native MS spectrum of an annealed mixture of CMP E:F (1:1)
Figure S5. Comparison of melting curves of CMP E:F (1:1) obtained via CD spectroscopy and MS
Figure S6. Native MS spectrum of CMP A with corresponding ion mobility data
Table S1. Overview of $T_m$ values obtained via CD spectroscopy and MS
Figure S1. CD spectroscopy of CMP A (50 µM in 10 mM aq. NH₄Ac 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. NH₄Ac at pH 7 were used. Only CMP D showed a characteristic, sigmoidal melting curve with a $T_{M,CD} = 57 \, ^\circ$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. NH₄Ac at pH 7 were used. The following melting temperatures were obtained: B:C:D (1:1:1): $T_{M,CD} = 53 \, ^\circ$C and B:C:D (1:1:3): $T_{M,CD} = 56 \, ^\circ$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. NH₄Ac 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. NH₄Ac 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 (purple) 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. NH₄Ac 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 $T_{m,CD} = 43 \, ^\circ C$ and $T_{m,MS} = 40 \, ^\circ C$ 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 $A\cdot A\cdot A$ 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\textsuperscript{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\textsubscript{4}Ac at pH 7.

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