

Frame Shifts Affect the Stability of Collagen Triple Helices

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ABSTRACT: Collagen model peptides (CMPs), composed of proline–(2*S*,4*R*)-hydroxyproline–glycine (POG) repeat units, have been extensively used to study the structure and stability of triple-helical collagen – the dominant structural protein in mammals – at the molecular level. Despite the more than 50-year history of CMPs and numerous studies on the relationship between the composition of single-stranded CMPs and the thermal stability of the assembled triple helices, little attention has been paid to the effects arising from their terminal residues. Here, we show that frame-shifted CMPs, which share POG repeat units but terminate with P, O, or G, form triple helices with vastly different thermal stabilities. A melting temperature difference as high as 16 °C was found for triple helices from 20-mers Ac-OG[POG]₆-NH₂ and Ac-[POG]₆PO-NH₂ and triple helices of the constitutional isomers Ac-[POG]₇-NH₂ and Ac-[GPO]₇-NH₂ melt 10 °C apart. A combination of thermal denaturation, CD and NMR spectroscopic studies, and molecular dynamics simulations revealed that the stability differences originate from the propensity of the peptide termini to preorganize into a polyproline-II helical structure. Our results advise that care must be taken when designing peptide mimics of structural proteins as subtle changes in the terminal residues can significantly affect their properties. Our findings also provide a general and straightforward tool for tuning the stability of CMPs for applications as synthetic materials and biological probes.

INTRODUCTION

Collagen, the most abundant structural protein in mammals, possesses an intriguing hierarchical structure.^{1,2} At its lowest level, three peptide chains, each of which adopts a left-handed polyproline type II (PPII) helix with all-*trans* amide bonds, intertwine with a one residue stagger into a right-handed triple-helical assembly.¹ The tight packing of the triple helix requires every third amino acid to be glycine (Gly, G). The single strands consist, therefore, of Xaa-Yaa-Gly repeat units.¹ Proline (Pro, P) is most common in the Xaa position, (4*R*)-hydroxyproline (Hyp, O) in the Yaa position.¹⁻³ These cyclic amino acids preorganize the individual strands into the PPII conformation, which is stabilized by $n \rightarrow \pi^*$ interactions between neighboring amide bonds (Figure 1a).^{4,5} The triple helix is further stabilized by hydrogen bonds between Gly N-H groups and Xaa C=O groups of the neighboring strand (Figure 1a).¹

Synthetic collagen model peptides (CMPs) composed of 20–40 amino acids have provided deep insights into the structure-property relationship of collagen triple helices at the molecular level.¹ These studies revealed that the stability of collagen triple helices depends on steric and stereoelectronic factors.¹ Changes in the CMP structure by alterations of the residues, the attachment of functional moieties, or both, allow for modulating triple helix stability. Examples include non-canonical amino acids such as proline derivatives⁶⁻¹⁶ or peptoid residues,^{17,18} glycine surrogates,^{19,20} charged groups,^{13,21-24} or lipidation.²⁵⁻²⁷ Interchain²⁸⁻³⁶ and intrachain³⁷ tethering have also been employed to study and control CMP structure and triple helix stability. These insights were also valuable for developing synthetic materials³⁸⁻⁴⁶ and biological probes⁴⁷⁻⁵⁰ based on CMPs.

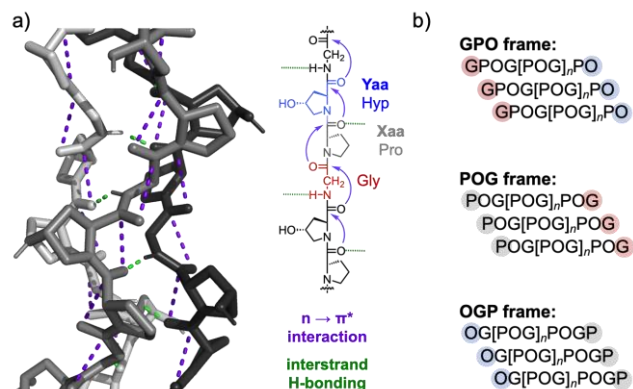


Figure 1. (a) General structure of collagen. (b) Frame-shifted constitutional CMP isomers with POG core repeats but different terminal amino acids.

The repetitive nature of the collagen sequence allows for the synthesis of CMPs in three different frames (Figure 1b). These frames are constitutional isomers that share the same core sequence but differ in the terminal amino acids. The first CMPs were synthesized in the 1960s by polymerization of tripeptide building blocks.⁵¹⁻⁵⁵ These early works used all three frames, Xaa-Yaa-Gly (“POG”),⁵¹ Gly-Xaa-Yaa (“GPO”),⁵²⁻⁵⁴ and Yaa-Gly-Xaa (“OGP”),^{51,55} and resulted in polymers with varying lengths. Since CMPs of defined length became available by solid-phase peptide synthesis (SPPS),⁵⁶ CMPs with the POG-frame have been the most common.^{1,57,58} The GPO frame has also been used,^{1,57,58} but examples of CMPs with the OGP frame are rare.⁵⁹ The preponderance of the POG frame likely arose from synthetic considerations. Fmoc-Pro-Hyp-Gly-OH is a standard building block for segment condensations since couplings with an activated Gly residue proceed smoothly, and the achiral Gly cannot undergo epimerization.^{34,60,61} In addition,

resins pre-loaded with Gly are readily available for the SPPS of CMPs.^{62,63} However, neither Pro nor Hyp are particularly prone to epimerization, and other sequence frames are therefore also readily available, in particular with modern coupling reagents.⁶⁴ Despite the extensive efforts to understand how structural modifications influence the stability of the collagen triple helix, little attention has been paid to stability differences arising from frame-shifted CMPs.

Herein, we show that frame-shifted CMPs assemble into collagen triple helices with significantly different thermal stabilities. The work revealed that the shortening of a CMP can increase the stability of collagen triple helices. A combination of thermal denaturation, circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopic studies, and molecular dynamics (MD) simulations elucidated that the propensity of the CMP termini for PPII-helicity is a key factor for the stability of the resulting triple helix.

RESULTS AND DISCUSSION

Thermal stability of frame-shifted collagen triple helices.

Our interest in functional collagen led us to question whether and how different frames of repeat units affect the stability of collagen triple helices. So far scientists have not paid attention to the role of the frame. For example, a recent publication reported a triple helix with GPO repeats with a thermal stability of 55 °C.¹⁸ We noticed that this melting temperature (T_m) is more than 10 °C higher than that of a triple helix with POG repeats ($T_m = 40\text{--}43$ °C), a CMP that has been studied extensively as a reference in our and other laboratories.^{11-13,20,22,29} This discrepancy led us to directly compare CMP Ac-[GPO]₇-NH₂ (**G21O**) with Ac-[POG]₇-NH₂ (**P21G**). Thermal denaturation with CD spectroscopic monitoring revealed a stark difference in thermal stability between the two triple helices of $T_m = 43$ °C (**P21G**) versus $T_m = 53$ °C (**G21O**; Figure 2a). This difference of $\Delta T_m = 10$ °C between constitutional isomers prompted us to systematically study the influence of frameshifts on the stability of collagen triple helices. First, we prepared CMPs consisting of 20, 21, and 22 amino acids in all three frames and determined the relative thermal stabilities of their triple helices (Figure 2 and Table 1). This set of CMPs covers each possible frame and combination of N-terminal and C-terminal amino acids of the Pro, Hyp, Gly triad. We used CMPs with capped termini (N-acetyl, C-amide) to avoid charge-charge interactions at the termini.²²

The thermal denaturation experiments revealed big stability differences of the triple helices, spanning a range of more than 20 °C (from $T_m = 34.0$ to $T_m = 54.5$ °C; Figure 2b). These differences occurred between frame-shifted triple helices of the same length and triple helices formed by CMPs that differ by only one amino acid more or less (Figure 3).

Frame-shifted triple helices of the same length. Within the 20-mer series, Ac-[POG]₆PO-NH₂ (**P20O**) forms the most stable ($T_m = 50.0$ °C), Ac-[OGP]₆OG-NH₂ (**O20G**) the least stable ($T_m = 34.0$ °C) triple helix. Thus, their thermal stability difference (ΔT_m) is 16.0 °C (Figure 2b). High ΔT_m values of ~10 °C were also observed in the 21- and 22-mer series. These thermal stability differences of the frame-shifted collagen triple helices of the same length are striking since they are similar to those of triple helices that differ in length by an entire tripeptide repeat unit. For example, the 24-mer Ac-[POG]₈-NH₂ (**P24G**) forms a triple helix that is 11.7 °C more stable ($T_m = 54.3$ °C) than the shorter 21-mer homolog **P21G** ($T_m = 42.6$ °C; Table 1,

Figure S1). The isomeric 21- and 24-mer pair Ac-[GPO]₈-NH₂ (**G24O**, $T_m = 63.3$ °C) and **G21O** ($T_m = 52.8$ °C) exhibits a similar difference of $\Delta T_m = 10.5$ °C (Table 1, Figure S1). Thus, frame-shifting is an alternative to the elongation of CMPs to access collagen triple helices with desired stability.

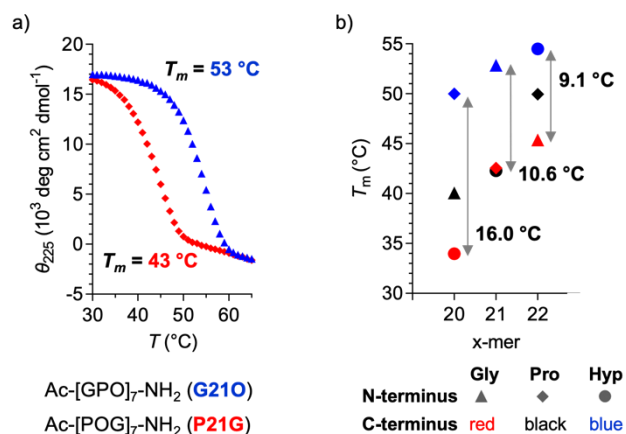


Figure 2. (a) Thermal denaturation and T_m values of Ac-[GPO]₇-NH₂ (**G21O**) and Ac-[POG]₇-NH₂ (**P21G**). (b) Graphic representation of the T_m and ΔT_m values of collagen triple helices formed by frame-shifted CMP 20-, 21- and 22-mers.

Table 1. Thermal stability of collagen triple helices formed by frame-shifted 20–22-mer CMPs, and 24-mers P24G and G24O.

CMP	Symbol ^a	Code ^b	T_m (°C) ^c
Ac-[GPO] ₆ GP-NH ₂	▲	G20P	40.0
Ac-[POG] ₆ PO-NH ₂	◆	P20O	50.0
Ac-[OGP] ₆ OG-NH ₂	●	O20G	34.0
Ac-[GPO] ₇ -NH ₂	▲	G21O	52.8
Ac-[POG] ₇ -NH ₂	◆	P21G	42.6
Ac-[OGP] ₇ -NH ₂	●	O21P	42.3
Ac-[GPO] ₇ G-NH ₂	▲	G22G	45.4
Ac-[POG] ₇ P-NH ₂	◆	P22P	50.0
Ac-[OGP] ₇ O-NH ₂	●	O22O	54.5
Ac-[GPO] ₈ -NH ₂	–	G24O	63.3
Ac-[POG] ₈ -NH ₂	–	P24G	54.3

^aSymbol as used in Figures 2, 3 and 5. ^bCode for CMPs: N-terminal amino acid, total length, C-terminal amino acid. ^cDetermined by thermal denaturation (heating rate 1 °C/114 s; 200 μ M) monitored by CD spectroscopy, $N = 2\text{--}4$, standard deviation (SD) ≤ 0.4 °C.

Triple helices differing by one amino acid at the N- or C-terminus. The ΔT_m values of ~11 °C of 21- versus 24-mers (**P21G** versus **P24G** and **G21O** versus **G24O**) suggest that one additional amino acid increases the thermal stability of collagen triple helices on average by 3–4 °C. This expectation is in stark contrast to the observed stability differences of triple helices that differ by one amino acid. In fact, an additional amino acid does not necessarily increase triple helix stability.

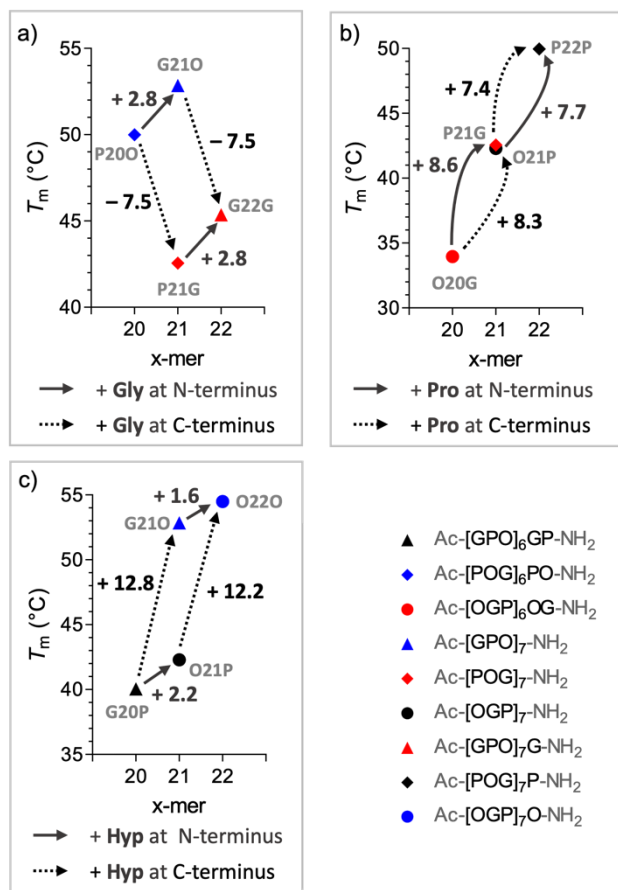


Figure 3. Parallelograms illustrate the effect of the addition of (a) Gly, (b) Pro, or (c) Hyp to the N-terminus (solid-line arrows) or C-terminus (dashed-line arrows) on the thermal stability of collagen triple helices. Numbers next to the arrows denote the ΔT_m in $^{\circ}\text{C}$. Note, the scales of the y-axes are not identical in the three panels.

The addition of a Gly residue to the N-terminus of Ac-[POG]₇-NH₂ (**P21G**, $T_m = 42.6^{\circ}\text{C}$) increased the T_m by 2.8°C (**G22G**, $T_m = 45.4^{\circ}\text{C}$). The omission of the C-terminal Gly boosted, however, triple helix stability by 7.5°C (Ac-[POG]₆-PO-NH₂ **P20O**, $T_m = 50.0^{\circ}\text{C}$; Figure 3a) – despite the shorter length! This is a striking result, which illustrates that the shortening of a CMP can result in a more stable triple helix. The observed ΔT_m values for an additional N- or C-terminal Gly residue were identical throughout the series (N-terminal Gly: $\Delta T_m = +2.8^{\circ}\text{C}$; C-terminal Gly: $\Delta T_m = -7.5^{\circ}\text{C}$) and allowed for the construction of a parallelogram between triple helices that differ by a terminal Gly residue (Figure 3a). The addition of a Pro residue had a similar effect at both termini and resulted in an increase of T_m of about 8°C , an increase that is more than double the expected value (Figure 3b). An additional N-terminal Hyp had a minor effect of $\Delta T_m \approx +2^{\circ}\text{C}$, while an additional Hyp at the C-terminus had the highest stabilizing effect with a ΔT_m of almost 13°C (Figure 3c).

These results show that the length, but even more importantly, the nature of the terminal amino acids determines the stability of collagen triple helices. The data illustrate how frame-shifting and the choice of the terminal residues of CMPs provide simple tools to tune the stability of collagen triple helices. The findings provide a guide for the design of the most stable – or, if desired, least stable – triple helix at a given length:

1. At the C-terminus, a Hyp residue has the biggest stabilizing effect ($\Delta T_m \approx +13^{\circ}\text{C}$).
2. At the N-terminus, a Pro residue provides the largest stabilization ($\Delta T_m \approx +8^{\circ}\text{C}$).
3. At the C-terminus, a Gly residue destabilizes the triple helix significantly ($\Delta T_m = -7.5^{\circ}\text{C}$).
4. The C-terminal residue impacts triple helix stability more than the N-terminal residue.

Taken together, CMPs with a Pro-Hyp diad at both the C- and N-termini form, at a given length, the most stable triple helices. This design requires a total number of $3n-1$ amino acids where n denotes the number of POG repeat units.

What are the molecular reasons for the effect of the terminal residues on the stability of collagen triple helices?

Collagen triple helices rely on interstrand H-bonds and PPII-helicity of the individual strands (Figure 1a).¹ Both factors contribute hand-in-hand to triple helix stability as efficient interstrand H-bonding is only possible with PPII-helical strands. PPII helices are stabilized by $n \rightarrow \pi^*$ interactions between neighboring amide bonds and involve delocalization of the non-bonding electrons of O_{i-1} into the π^* orbital of the $\text{C}_i=\text{O}_i$ bond.^{4,5,65-67} φ and ψ angles of -75° and $+145^{\circ}$, respectively, are ideal for effective $n \rightarrow \pi^*$ donation and PPII helicity.^{66,68} The cyclic structure of Pro and Hyp favors these angles and preorganizes single-stranded CMPs into PPII helices. Our data imply that effective preorganization requires a Pro-Hyp diad (C-terminal Hyp, $\Delta T_m \approx +13^{\circ}\text{C}$; N-terminal Pro, $\Delta T_m \approx +8^{\circ}\text{C}$). A single cyclic amino acid at either terminus – Pro at the C-terminus ($\Delta T_m \approx +8^{\circ}\text{C}$) and Hyp at the N-terminus ($\Delta T_m \approx +2^{\circ}\text{C}$) – provides less stabilization. In contrast, the Gly residue is not predisposed to accommodate the φ and ψ angles of the PPII helix, consistent with the observed weak stabilizing effect at the N-terminus ($\Delta T_m = +2.8^{\circ}\text{C}$) and strong destabilizing effect at the C-terminus ($\Delta T_m = -7.5^{\circ}\text{C}$).

At a given length of a CMP, the resulting triple helices have the same number of theoretically possible H-bonds. Our data, therefore, imply that PPII-helical preorganization of the termini, especially the C-terminus, defines the stability of frame-shifted collagen triple helices (Figure 4). The observed destabilizing effect by a Gly at the C-terminus suggests that this residue causes repulsion, possibly by adopting dihedral angles that do not match with a PPII helix. Consequently, $n \rightarrow \pi^*$ interactions and H-bonding would be favored by the C-terminal Pro-Hyp diad but not a Gly residue.

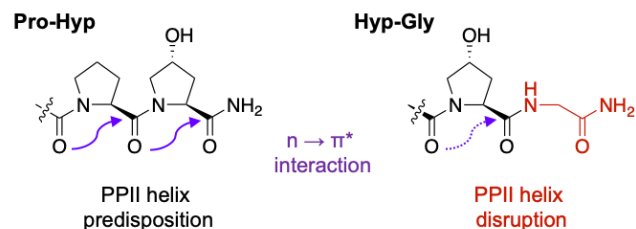


Figure 4. C-terminal Pro-Hyp diad (left) and Hyp-Gly (right).

To probe the role of H-bonding and the effect of terminal Gly residues in more detail we carried out molecular dynamics (MD) simulations and NMR spectroscopic experiments.

Molecular Dynamics Simulations – The persistence of H-bonding depends on the CMP frame. We performed MD simulations with 21-mer CMPs **G21O**, **P21G**, and **O21P** as representative examples of triple helices with different termini. For each CMP, we performed 100 ns long MD simulations with water as an explicit solvent. The simulations were carried out at 300 K and 400 K in 5 and 10 replicas, respectively⁶⁹ using the GROMOS software package⁷⁰ and the GROMOS 54A7 force field.⁷¹ The starting structures were based on a crystal structure of collagen (PDB ID: 3B0S).⁷²

At 300 K, the triple helices largely retained their structure but unfolded during the course of the simulation at 400 K (Figure S2). In agreement with previous findings,^{73–75} the unfolding of all three frame-shifted triple helices started at the N-terminus, and the C-terminus retained its helical structure for a longer period of time (Figure S3 and S4).⁷⁶ While the N-terminal unfolding of the three isomeric triple helices took place at a similar rate, the unfolding rate at their C-termini differed. The triple helix of **G21O** unfolded the slowest (Figure S3), in line with the thermal stability of the frame-shifted collagen triple helices.

The number of H-bonds remained similar for the three triple helices during the simulation at 300 K (Figure 5a). At 400 K, the **G21O** triple helix with the C-terminal Pro-Hyp diad retained the H-bonds for a longer period of time compared to the other, less stable isomeric triple helices (Figure 5b, S5, and S6). The higher retention of H-bonds was particularly significant in the C-terminal region of the triple helix. The MD simulations thus corroborate an interplay between PPII helical preorganization at the termini and H-bonding as a key factor that determines the stability of frame-shifted collagen triple helices.

These conclusions were further supported by the solvent-accessible surface area (SASA) of the N- and C-terminal amino acid triads of the frame-shifted triple helices in the simulations at 300 K. A lower SASA correlates with a more tightly packed triple helix. At the N-terminus, the SASA followed the order POG < OGP < GPO (**P21G** < **O21P** < **G21O**), while the order was reversed at the C-terminus, GPO < OGP < POG (**G21O** < **O21P** < **P21G**) (Figure S7). Thus, the triple helices with a terminal Pro-Hyp diad exhibit the lowest SASA, and the triple helices with a terminal Gly the highest. These findings are consistent with tightly packed triple-helical assemblies with extensive interstrand contacts in the case of the terminal Pro-Hyp diads. This result further supports the importance of preorganization by two consecutive cyclic amino acids for the packing and hence stability of CMP triple helices.

NMR spectroscopy – Probing the conformational flexibility of Gly residues at the N- and C-termini. The observed strong destabilization of triple-helical collagen by a C-terminal Gly suggests that unfavorable dihedral angles disturb the triple-helical assembly. The MD simulations indeed predicted a significant population of ϕ dihedral angles around 180° and +75° for both the N- and the C-terminal Gly residues at 300 K (Figure S8–10), angles that are not favorable for PPII helicity. We reasoned that the N-terminal Gly could avoid repulsion through, *e.g.*, *trans/cis* isomerization of the tertiary Gly-Pro amide bond but not the secondary C-terminal Hyp-Gly bond. An N-terminal Gly should therefore exhibit greater conformational flexibility than a C-terminal Gly.

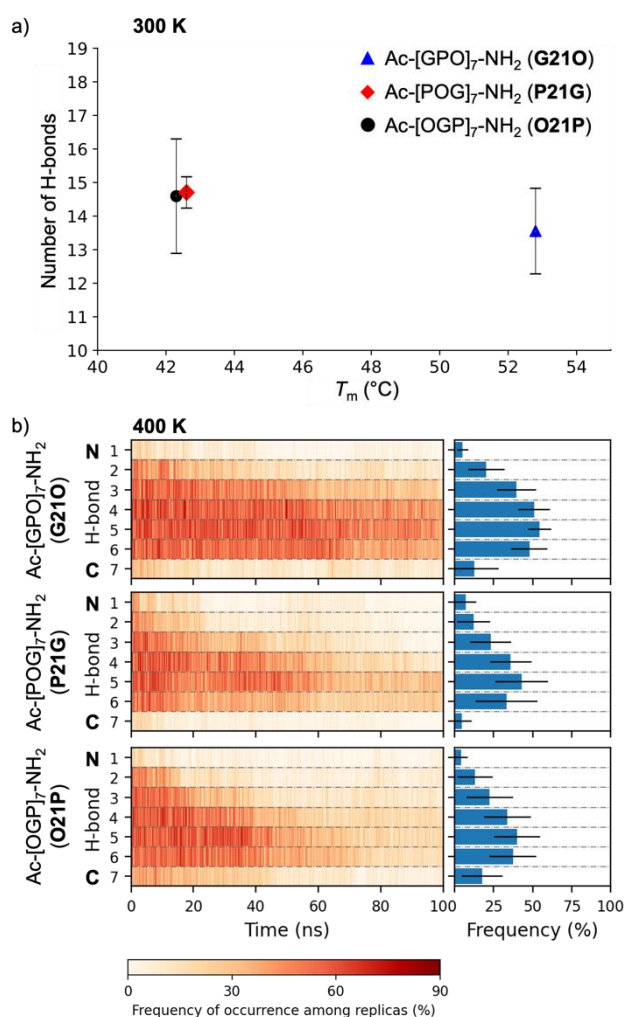


Figure 5. (a) Plot of the total number of H-bonds (mean \pm SD) in triple helices formed from frame-shifted constitutional isomers of 21-mer CMPs as obtained from MD simulations at 300 K versus their melting temperature determined by CD. (b) Occurrence of H-bonds in frame-shifted collagen triple helices over the time course of the MD simulations at 400 K. Each line represents an average of three equivalent H-bonds between the leading, middle, and lagging strands. *Left*: heatmap of the occurrence frequency among replicas over time. *Right*: mean \pm SD of the occurrence.

We probed the flexibility of the terminal Gly residues by ^1H - ^{15}N NMR relaxation and heteronuclear nuclear Overhauser effect (NOE) experiments.^{77,78} For these experiments, we prepared analogs of peptides **P21G** and **G22G** with ^{15}N -labeled terminal Gly residues and peptide **P200** containing an ^{15}N labeled central Gly residue (Figure 6a, left). ^1H - ^{15}N HSQC spectra of peptides **P21G** and **G22G** with labeled terminal Gly residues showed three distinct signals corresponding to the leading, middle, and lagging strand of the triple-helical (TH) assembly as well as a signal corresponding to the residual, non-assembled single strand (SS) peptide (Figure 6a; orange and purple).⁷⁹ The spectrum of peptide **P200** with the labeled central Gly residue showed only two signals corresponding to the triple-helical and single-stranded species; the leading, middle, and lagging strands of the triple helix being indistinguishable (Figure 6b; grey).

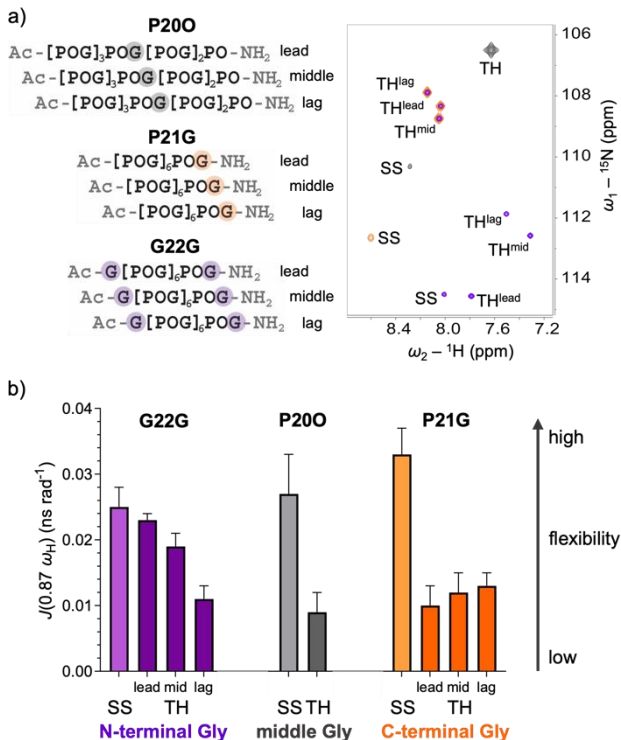


Figure 6. (a) ¹⁵N-labeled CMPs **P200**, **P21G**, and **G22G** and their ¹H-¹⁵N HSQC spectra (3 mM in 50 mM phosphate buffer/D₂O 95:5, pH 7.4). ¹⁵N-labeled Gly residues are highlighted, for the strand assignment see Figure S12. SS, single strand; TH, triple helix. (b) Plot of the spectral density function, J , evaluated for N-terminal, central and C-terminal Gly-¹⁵N residues of CMP single strands (SS) and triple helices (TH) at frequency $0.87 \times \omega_H$. Error bars represent \pm SD.

For assessing the relative flexibility of the labeled Gly residues, we first determined the longitudinal and transversal relaxation times (T_1 and T_2) and the heteronuclear NOEs for the amide NH-groups (Tables S1 and S2). We then used reduced spectral density mapping to determine the values of the spectral density function J at frequencies 0 , $0.87 \times \omega_H$ and ω_N , where ω_H and ω_N are Larmor frequencies of ¹H and ¹⁵N nuclei, respectively (Figure 6b and S11, Tables S3 and S4).⁸⁰⁻⁸² Internal motion on the ps–ns time scale increases $J(0.87 \times \omega_H)$ and decreases $J(0)$, while exchange processes on the μ s–ms timescale increase $J(0)$.⁸³

The results show that the flexibility of the N-terminal Gly-¹⁵N in the triple helix is higher than that of C-terminal Gly-¹⁵N (Figure 6b). The flexibility of the N-terminal Gly-¹⁵N in the leading, middle, and lagging strands differed significantly. The flexibility of the Gly-¹⁵N in the leading strand is high, similar to that in the single-stranded CMP, while the flexibility of the equivalent Gly-¹⁵N in the lagging strand is significantly lower, close to that of a Gly-¹⁵N in the middle of a triple helix (Figure 6b). These data reflect that the N-terminal Gly of the lagging strand is still part of the triple helix whereas the N-terminal Gly of the middle and leading strand reside in the overhanging ends. A similar qualitative trend was observed at the C-terminus, but the magnitude of the flexibility of the C-terminal Gly-¹⁵N residues was close to that of a Gly-¹⁵N in the middle of a triple helix for all three strands. Thus, the C-terminal Gly residues are conformationally more constrained than the N-terminal Gly residues. This finding implies that the

N-terminal Gly residues are conformationally rather flexible and can, thus, avoid unfavorable repulsion. In contrast, the NMR data is consistent with a narrow conformational preference of the C-terminal Gly residues that destabilizes the collagen triple helix.

CONCLUSION

In this account, we showed through a combined experimental and computational approach that the N- and C-terminal amino acids have a profound impact on the thermal stability of collagen triple helices. We found a melting temperature difference of up to 16 °C between triple helices derived from frame-shifted CMP 20-mers. Even CMP 21-mers, which are constitutional isomers with identical amino acid composition, exhibited a more than 10 °C T_m change upon frameshift. These magnitudes of triple helix stabilization are at the same level, or higher, as those achieved through the incorporation of stabilizing non-canonical amino acids into collagen triple helices.^{6,9,18,19,25-27}

This influence of a seemingly subtle amino acid shift on the properties of CMP-derived triple helices has not been recognized so far. For example, a recent study compared the triple helix stabilities of aza-glycine substituted CMPs with the GPO frame to a reference CMP with the POG frame.²⁰ Our findings suggest that part of the observed stability differences arose from the frameshift. Also, computational models have been put forth for predicting the stability of collagen triple helices formed by CMPs.⁸⁴ So far, these models have not taken the effect of frameshifts into account. Our work provides the basis for the refinement of these predictive tools.

The herein presented results show that careful consideration needs to be given to the frame of a collagen peptide and the nature of the terminal amino acids when designing triple helical assemblies. In particular, the C-terminal amino acid needs to be carefully chosen since it has a larger effect on triple helix stability than the N-terminal amino acid. At both termini, the greatest triple helix stabilization is achieved with a Pro-Hyp diad. This “dipeptide clamp” promotes preorganization into a PPII helical structure, the prerequisite for the formation of an ordered collagen triple helix. In contrast, a C-terminal Gly disturbs PPII helicity and destabilizes triple-helical collagen.

The results provide a straightforward general tool to tailor the stability of synthetic CMP triple helices at a given length, without the need of incorporating non-proteinogenic amino acids. We envision that frameshift considerations will be especially relevant for the design of collagen hybridizing peptide-based biological probes and therapeutics.⁴⁷⁻⁵⁰ Here, Raines and coworkers have shown that collagen hybridizing peptides need to remain single-stranded and retain a high propensity for triple-helical assembly with locally disturbed native collagen.^{85,86} Tuning the triple helix stability is thus key for such CMPs. The presented data provide a guide for choosing the appropriate frameshift and terminus, including the site for the attachment of, *e.g.*, a sensor or payload. In addition, synthetic CMPs have produced versatile materials with often intriguing structures.³⁸⁻⁴⁶ We foresee that our work will provide a useful guide to fine-tune the design and stability of such materials.

ASSOCIATED CONTENT

Supporting Information.

The Supporting information is available free of charge at <http://xxx.xxx.xxx>.

Supplementary figures referenced in the main text, synthetic protocols and analytical data of CMPs, details on the CD and NMR experiments as well as the MD simulations.

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Notes

The authors declare no competing financial interest.

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