The molecular specifications of a mineral modulation sequence derived from the aragonite-promoting protein n16. Supporting Information.

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In vitro Kevlar crystal growth assays. **To determine if n16N exhibited the same mineralization behavior in the reduced versus non-reduced state, w**e employed a polyimide (Kevlar) assay for the nucleation of calcium carbonate crystals in the presence of n16N with and without reducing conditions. The protocol for the Kevlar assay has been described in detail in earlier reports.[1-3] These assays were divided into two runs, one which was conducted identically to our original non-reducing protocol, and, a parallel run wherein we utilized N_2 / H_2 degassed $CaCl_2$ stock solutions containing 50 micromolar DTT in deionized distilled water. Final n16N assay concentrations of 1×10^{-5}, 5×10^{-5}, and 1×10^{-4} M were utilzed in both runs, and the negative control conditions consisted of no added peptide. Assay conditions and sample workup for SEM imaging were conducted as described in our previous polypeptide studies.[1-3] SEM imaging was conducted using a Hitachi S-3500N SEM microscope at 5 kV after thin Au coating of samples. The SEM images presented in this report are representative of 10-20 different crystals in each assay sample. Cropping of SEM images and adjustment of brightness/darkness and contrast levels were performed using Adobe Photoshop.

Given that reducing conditions influence n16N conformational stability, we subsequently examined the effect of reduction on the mineralization capabilities of the n16N polypeptide (Supplementary Figure 1). Rhombodehdral calcite crystals grown under negative control conditions (+/- DTT) appear to be identical in terms of dimension and surface features, with only minor irregularities exhibited on the surface of calcite crystals grown in the presence of DTT. Hence, DTT itself does not significantly influence calcite crystal growth or morphology in our assay systems. The same holds true for n16N under reducing and non-reducing conditions (Supplementary Figure 1). Here, we note that each n16N assay condition generates crystals that exhibit "staircase structures" with small terrace sizes as originally reported for n16N under non-reducing conditions.[3] Thus, the

mineralization behavior of n16N appears to be the same under both reducing and non-reducing conditions.

Determination and evaluation of proton conformational shifts and coupling constants. Qualitative NMR parameters, such as proton conformational shifts ($\delta\Delta H_\alpha$)[4] and ΔJ values,[5] can be utilized to estimate the presence or absence of random coil conformation (Supplementary Figure 2). In general, ΔJ values > 1 Hz indicate significant deviation from random coil structure; (+) ΔJ values are typically indicative of β-strand conformation, (-) ΔJ values are representative of α-helix conformation, and ΔJ values for β-turn can be either (+) or (-).[5] For apo-n16N, residues A1, G7, Y9, I14, E19, R20, D21, Y23 possess $\delta\Delta H_\alpha$ > 0.1 ppm (Supplementary Figure 2), indicating that these residues exhibit significant deviation from random coil database values.[4] The remaining residues possess $\delta\Delta H_\alpha$ < 0.1 ppm, suggesting the presence of random coil or unstructured states.[4] For those residues where J-couplings could be unambiguously determined, we observe (+) ΔJ values > 1 Hz for residues Y2, H3, W13, Y16, I18, R20, K29, and the sequence block C6 – Y11, indicating that these residues are beta-strand in nature (Supplementary Figure 2). Collectively, these results qualitatively confirm that apo-n16N at neutral pH exists in a unfolded state that consists of random coil conformation and some partial beta strand structure.

Global conformation of n16N in the presence of Ca (II). CD experiments were conducted using the DTT - n16N stock solution diluted to 12 micromolar concentration in 100 micromolar Tris-HCl, pH 7.5, to which stoichiometric Ca (II) titrations were performed using microliter additions of $CaCl_2$ stock solution (99.9% pure, Sigma-Aldrich, deionized distilled water) over a range of 2:1, 1:1, 1:2, 1:4, 1:10, 1:20 n16N: metal ion ratios. CD spectra were obtained as described in the Materials and Methods section of the paper.

We performed a Ca (II) titiration of n16N and monitored the conformation of this sequence using CD spectrometry (Supplementary Figure 3). Here, under reducing conditions, we confirm that the apo form of n16N adopts a random coil conformation, as evidenced by the presence of a major negative ellipticity band ($\pi - \pi^*$) near 195 – 198 nm.[1-3] With the introduction of Ca (II), we note that the adsorption wavelength of the $\pi - \pi^*$ ellipticity band does not shift. This indicates that reduced n16N in the presence of Ca (II), maintains a global, unfolded conformation that is random-coil in nature.[1-3]

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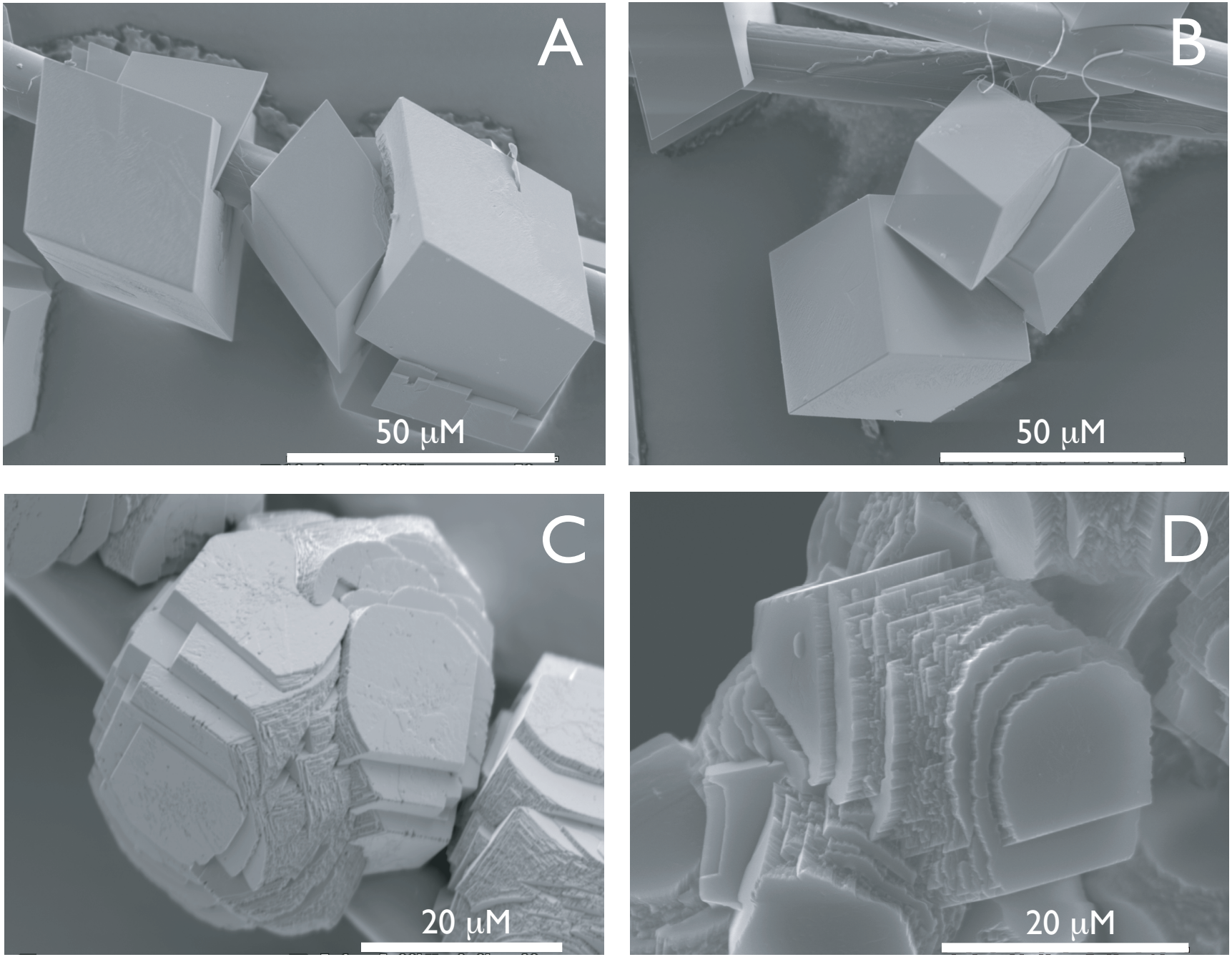
Supplementary Figure Legends for Collino and Evans, 2008

Supplementary Figure 1: *Scanning electron microscopy images of in vitro Kevlar calcium carbonate assay systems*. (**A**) negative control assay, which features typical rhombohedral calcite crystals; (**B**) negative control assay, reducing conditions; (**C**) n16N, 100 µM, no reducing conditions; (**D**) n16N, 100 µM, reducing conditions. Scale bar dimensions are provided in each figure. Note that in these assays the exposed Miller planes of the rhombohedral calcite crystals in the negative control and in the experimental samples are the {104}.

Supplementary Figure 2: (**A**) $\delta\Delta H\alpha$ *($\Delta H\alpha$, observed - $\Delta H\alpha$, random coil) and* (**B**) ΔJ *($^3J_{observed} - {}^3J_{random\ coil}$) values obtained for apo-n16N at pH 7.5, 293 K*. For (A), (-) values represent upfield shifts, (+) values represent downfield shifts. No corrections have been made for terminal residue effects; however, corrected chemical shift values were utilized for Pro, X-Pro nearest neighbor effects.[4] Proton chemical shifts were referenced from internal d_4-TSP. The dashed lines represent the random coil threshold value (i.e., $\delta\Delta H\alpha \pm 0.1$ ppm; $\Delta J \pm 1$ Hz). "X" = not determined.

Supplementary Figure 3: *CD spectra of 12 micromolar n16N (reduced) in 100 micromolar Tris-HCl, pH 7.5, in the apo form and in the presence of stoichiometric amounts of $CaCl_2$.*

Supplementary Figure 4: *Ramachandran φ, ψ dihedral angle distribution plot for apo-n16N conformer libraries*. Scatterplots represent dihedral distributions for XPLOR SA/MD - generated conformer ensemble (n = 10 for each sequence).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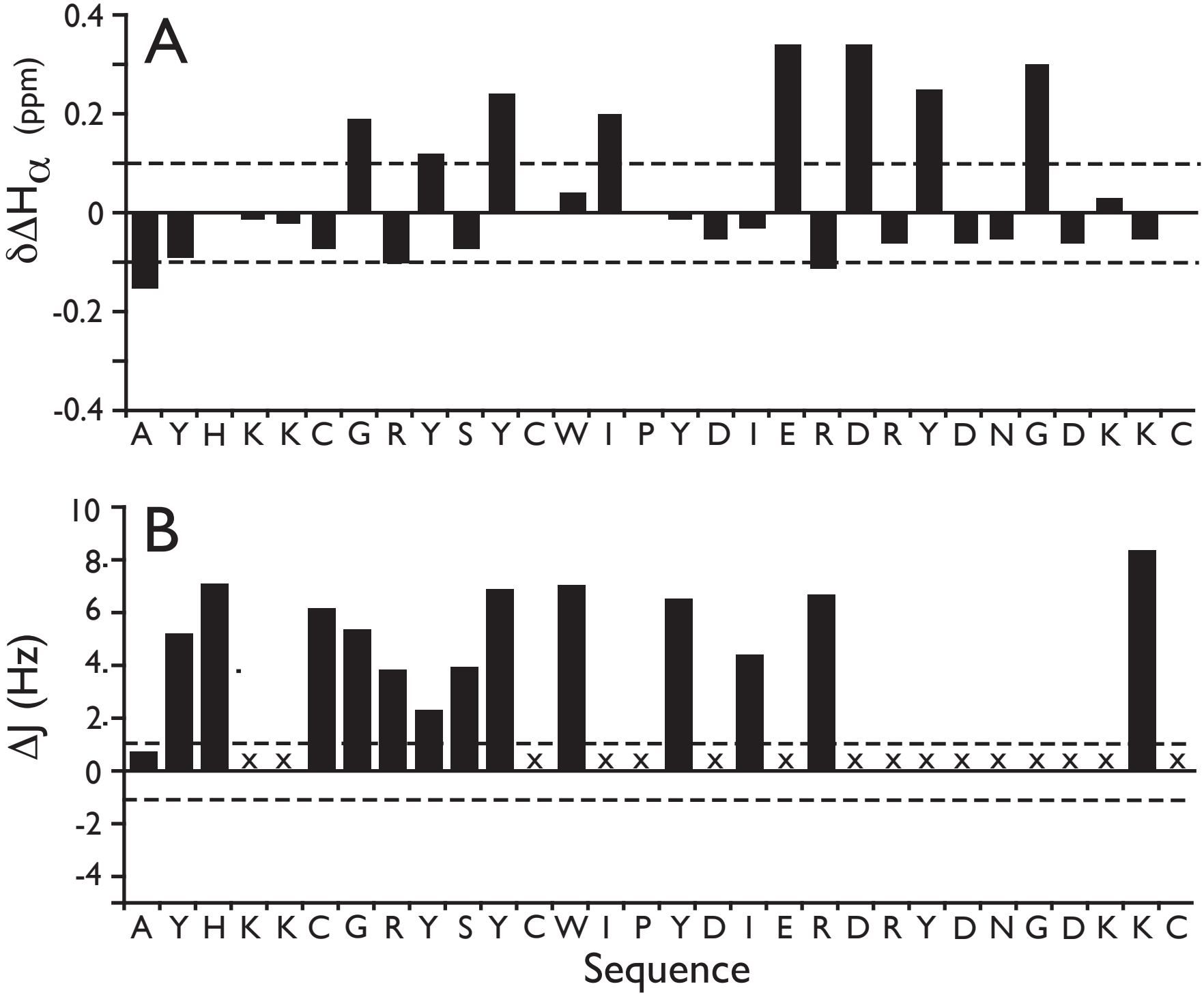
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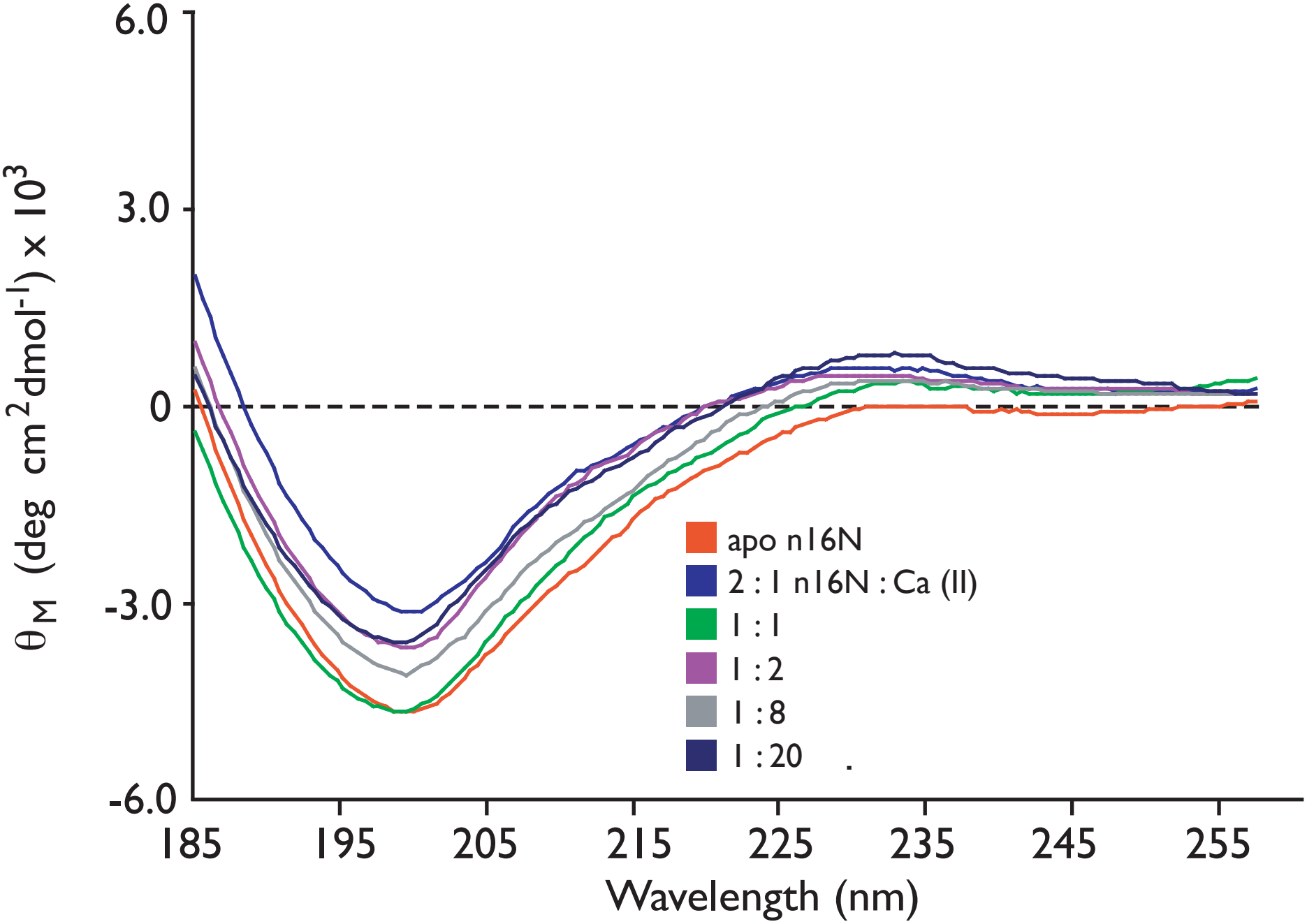
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Wavelength (nm)
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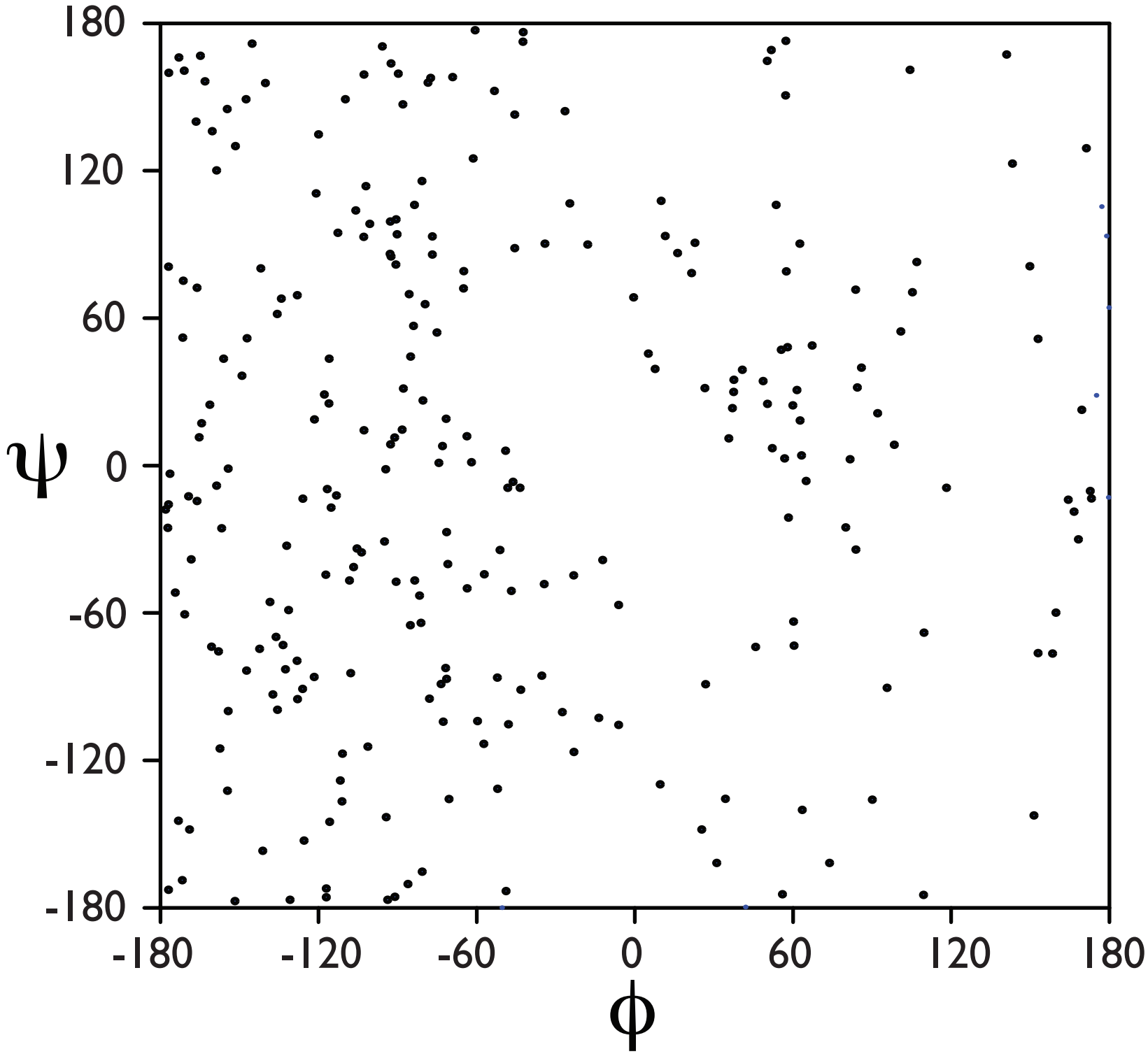
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