Supporting Text

Spectral Subtractions. In the text we referred to the absorptive difference spectrum: $(G_{79}^{*}+G_{79}^{**})-0.25G_{79}^{**}$. The pump–probe spectra were independently measured for each sample and used to obtain the complex absorptive spectrum of $G_{79}^{*}+G_{79}^{**}$ and of the homodimer G_{79}^{**}. The real parts of these spectra are plotted in Figs. 4d and 4c. Because the isotopomer pump-probe spectra were so weak there was an uncertainty of $\approx 0.06\pi$ in the phase of these absorptive spectra. The helix concentrations of the various samples were not precisely equal ($\pm 20\%$) and normalization was uncertain. The spectrum of G_{79}^{**} was subtracted from that of $G_{79}^{*}+G_{79}^{**}$ until the ^{13}C=^{18}O diagonal absorption region intensity was reduced by 50%, which required between 0.22 and 0.28 of the G_{79}^{**} spectrum for a series of samples, rather than the ideal fraction of 0.25 for perfectly normalized signals. The G_{79}^{**} spectrum used in the indicated subtraction was obtained for a range of small (few percent) phase corrections to ensure that the imbalance between positive and negative contributions in the difference spectrum was minimized when averaged across the complete spectral window (1,500–1,700 cm^{-1}). This overall procedure for all phase choices and for all samples gave very similar basic spectral characteristics in the isotopomer region, including the cross-peak, as by simply subtracting 25% of the real part of the G_{79}^{**} spectrum. There are more significant variations in the main helix band region of 1,620–1,670 cm^{-1}. The resulting difference spectrum is pure heterodimer in the ^{13}C= ^{18}O region but not at ^{13}C=^{16}O, which maintains 25% of the G_{79}^{*} intensity. However, the procedure we used does subtract ≈25% of the ^{13}C=^{16}O natural abundance peak. The additional G_{79}^{*} contribution was not subtracted.

Polarization Measurement. In the polarization measurements, we obtained the $\langle xxxx\rangle$ and $\langle xyyx\rangle$ echo signals of both the homodimer sample G_{79}^{**} and the mixture sample $G_{79}^{*}+G_{79}^{**}$. The difference absorptive spectrum of $(G_{79}^{*}+G_{79}^{**})-0.25G_{79}^{**}$ was calculated for each of the two polarizations according to the above-mentioned procedure. For each 2D absorptive spectrum $\underset{\sim}{S}(\omega_\tau, \omega_t)$ a spectral slice was chosen as follows:

$\int_{-\infty}^{\infty} \mathrm{Re}[\underset{\sim}{S}(\omega_\tau, \omega_t)]G(\omega_\tau)d\omega_\tau$ where $G(\omega_\tau)$ is a Gaussian profile centered at $\omega_\tau = \omega_{^{13}C=^{18}O}$ having full width at half maximum of 12 cm^{-1}. This slice was subsequently least-squares fitted to a series of overlapping spectral bands along the ω_t axis in the region of 1,574–1,655 cm^{-1} (Fig. 5*a* gives an example) to obtain the ^{13}C=^{18}O diagonal peak contribution and the cross peak between the two isotopomer levels solely from the pure heterodimers G_{79}^{**}/G_{79}^{*} and G_{79}^{*}/G_{79}^{**}.

The Dipole–Dipole Angular Factor. The factor $\kappa(\Omega)$ in the text is defined by:

$$\kappa(\theta_1, \theta_2, \phi_1, \phi_2) = \hat{\mu}_1 \cdot \hat{\mu}_2 - 3\hat{\mu}_1 \cdot \hat{y}\hat{\mu}_2 \cdot \hat{y} \qquad \textbf{[1]}$$

where θ_1, ϕ_1 and θ_2, ϕ_2 are the polar angles of the two dipole vectors and $\hat{y}$ is the unit line joining them, then:

$$\hat{\mu}_i = \cos\theta_i \hat{z}_i + \sin\theta_i(\cos\phi_i \hat{x}_i + \sin\phi_i \hat{y}_i) \qquad \textbf{[2]}$$

If the two dipoles are interchangeable by a twofold axis parallel to $\hat{z}$ it is found that: $\cos\theta_1 = \cos\theta_2$; $\sin\theta_1 = \sin\theta_2$; $\cos\phi_1 = -\cos\phi_2$; and $\sin\phi_1 = -\sin\phi_2$. The two-fold axis implies that $\phi_2 = \phi_1 + \pi$ so that by defining $\hat{\mu}_1 \cdot \hat{\mu}_2 = \cos\theta_{12}$ and replacing ϕ_i by ϕ, after some manipulation and defining $2P_2(\cos\phi) = (3\cos^2\phi - 1)$ yields the equation quoted in the text:

$$\kappa(\theta_{12}, \phi) = 1 + (\cos\theta_{12} - 1)P_2(\cos\phi) \qquad \textbf{[3]}$$

The Relationship Between θ_{12} and the Crossing Angle. The dipole 1 makes an angle β with the A helix axis $\hat{z}_A$ thus:

$$\hat{\mu}_1 = \cos\beta \hat{z}_A + \sin\beta(\cos\phi_A \hat{x}_A + \sin\phi_A \hat{y}_A) \qquad \textbf{[4]}$$

where $\hat{y}_A$ and $\hat{y}_B$ (for helix B) form a plane with the twofold axis, which interchanges them, whereas $\hat{x}_A$ and $\hat{x}_B$ are perpendicular to this plane. In this picture each dipole is located on the surface of a cone whose axis is the relevant helix axis (i.e., 1 on A and 2 on B). These definitions lead to: $\hat{y}_A \cdot \hat{y}_B = -\cos\theta_{AB}$; $x_A \cdot x_B = -1$; $\hat{z}_A \cdot \hat{z}_B = \cos\theta_{AB}$; $\hat{z}_A \cdot \hat{y}_B = \hat{z}_B \cdot \hat{y}_A = \sin\theta_{AB}$; and $\phi_A = \phi_B = \phi_H$, where θ_{AB} is the dihedral or crossing angle between the two helices considered to be perfect α-helices for the five residues at the crossing region. The angles θ_{12} and β are then related to the helix crossing angle and the azimuth of a dipole ϕ_H around its helix axis as follows:

$$\cos\theta_{12} = \cos\theta_{AB}(\cos^2\beta - \sin^2\beta \sin^2\phi_H) + \sin\theta_{AB}\sin 2\beta \sin\phi_H - \sin^2\beta\cos^2\phi_H \qquad \textbf{[5]}$$

This is the relationship used in the discussion of the crossing angle.

Determination of the Azimuthal Range of Angles ϕ_H from NMR Data. A helix axis segment is generated for the four consecutive amino acids [78–81] that include residue Gly-79. The cross product of the unit vectors created by the bisectors of the angles formed by the first three α-carbons (Cα1, Cα2, Cα3) and the second three α-carbons (Cα2, Cα3, Cα4) gives the direction cosines of the local helix axis ($\hat{z}_A$ or $\hat{z}_B$). From the NMR atomic coordinates of the GpA TM helix dimer (1), the two amide-I transition dipole vectors ($\hat{\mu}_1$ and $\hat{\mu}_2$) at Gly-79 were obtained by assuming the dipole tilts 20° from the carbonyl bond toward the C–N bond in the plane of the peptide group. The dyad axis unit vector is $\hat{C}_2 = (\hat{\mu}_1 + \hat{\mu}_2)/|\hat{\mu}_1 + \hat{\mu}_2|$, which locates the $\hat{y}_A$ axis. The angle between the dipole on helix A and $\hat{z}_A$ is $\beta_A = \arccos(\hat{\mu}_1 \cdot \hat{z}_A)$, and the azimuthal angle $\phi_A = \arcsin\left[(\hat{\mu}_1 \cdot \hat{y}_A)/\sin\beta_A\right]$. The same analysis can be applied to helix B for the 20 model structures reported for 1AFO (1). We find $\beta_A = \beta_B = \beta = 30 \pm 1^\circ$. In the estimate of the crossing angle, we used $\beta = 32^\circ$ and $\theta_{12} = 108^\circ$ as indicated in the text. The azimuthal angles are $\phi_A = \phi_B = \phi_H = -73 \pm 7^\circ$.

1. MacKenzie KR, Prestegard JH, Engelman DM (1997) *Science* 276:131–133.