

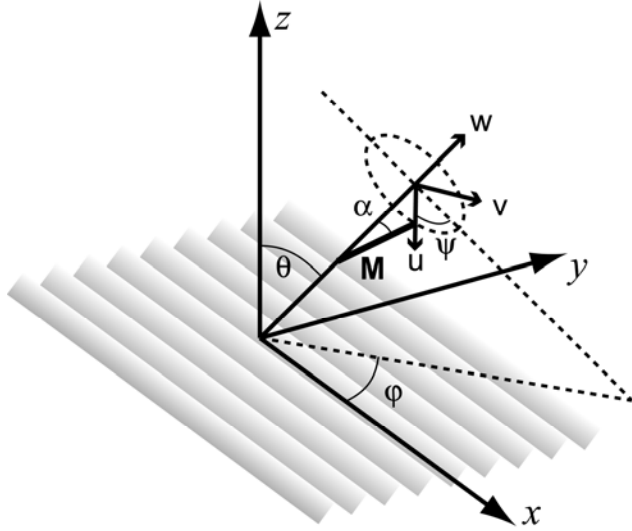
Supporting Information

Samples for PM-IRRAS measurements were required to be on conductive metal substrates. For this purpose, a thin metal film was evaporated on glass slides using a BOC Edwards Auto 306 electron-beam evaporator. An adhesion layer of 5 nm of titanium was evaporated first, followed by 30 nm of gold capped with a 1 nm layer of titanium on top of the gold to produce a hydrophilic oxide surface. Prior to use, evaporated metal substrates were cleaned in oxygen plasma using a PlasmaLab 80 reactive ion etcher (Oxford Instruments).

Applied Nanostructures ACT silicon cantilevers (300 kHz resonant frequency, 40 N/m spring constant) were used for AFM imaging.

The orientation of a TDM (M) of a single molecule may be described with respect to a fixed space coordinate system (x, y, z) by using the Euler angles as shown in Schematic S1. The molecular coordinate system is described by the u, v , and w axes, where w denotes the main axis of the molecule or supramolecular structure. The z axis is chosen to be normal to the substrate while the x and y axes are chosen to lie in the substrate plane. In the case of the PA nanofibers, w denotes the long axis of the nanofiber structure, and the x axis is chosen to be parallel to the direction of the patterned channels. The tilt (θ) and azimuthal (φ) angles describe the orientation of w with respect to the space coordinate system. The orientation of the TDM with respect to the nanofiber coordinate system is described by a tilt (α) and a twist (ψ) angle.

Schematic S1. Fixed space coordinate system (x, y, z), nanofiber coordinate system (u, v, w), and the angles ($\theta, \varphi, \psi, \alpha$) used to describe the orientation of the nanofiber and TDMs (M).



The direction cosines K_x , K_y , and K_z are the square of the components of the unit vector of the TDM along each axis of the spatial coordinate system and are related to their respective extinction coefficients by a constant of proportionality.¹ Given the absorption intensities E_x , E_y , and E_z for a given vibration obtained experimentally from spectra polarized along each fixed space axis, the direction cosines can be calculated from $K_i = E_i/\Sigma E$, where $\Sigma E = E_x + E_y + E_z$. The function of K_i with respect to α and the three Euler angles θ , φ , and ψ is determined by straightforward geometric arguments as shown by Zbinden.² The components of the TDM along each axis of the space coordinate system (M_x , M_y , and M_z) are related to the components of the TDM along each axis of the molecular coordinate system (M_u , M_v , and M_w) as follows:

$$\begin{pmatrix} M_x \\ M_y \\ M_z \end{pmatrix} = \begin{pmatrix} \cos \psi \cos \theta \cos \varphi + \sin \psi \sin \varphi & \sin \psi \cos \theta \cos \varphi - \cos \psi \sin \varphi & \cos \varphi \sin \theta \\ \cos \psi \cos \theta \sin \varphi - \sin \psi \cos \varphi & \sin \psi \cos \theta \sin \varphi + \cos \psi \cos \varphi & \sin \varphi \sin \theta \\ -\sin \theta \cos \psi & -\sin \theta \sin \psi & \cos \theta \end{pmatrix} \begin{pmatrix} M_u \\ M_v \\ M_w \end{pmatrix} \quad (\text{S.1})$$

The components of the TDM with respect to the molecular coordinate system are related to the absolute magnitude of the TDM M and the angle α by simple relations:

$$\begin{pmatrix} M_u \\ M_v \\ M_w \end{pmatrix} = \begin{pmatrix} M \sin \alpha \\ 0 \\ M \cos \alpha \end{pmatrix} \quad (\text{S.2})$$

Inserting equations S.2 into S.1 yields expressions for M_x , M_y , and M_z in terms of α , θ , φ , and ψ . Knowing that the direction cosines are proportional to the square of the components of the TDM as $K_i = (M_i/M)^2$, we obtain:

$$K_x = (\cos \psi \cos \theta \cos \varphi \sin \alpha + \sin \psi \sin \varphi \sin \alpha + \cos \varphi \sin \theta \cos \alpha)^2 \quad (\text{S.3})$$

$$K_y = (\cos \psi \cos \theta \sin \varphi \sin \alpha - \sin \psi \cos \varphi \sin \alpha + \sin \varphi \sin \theta \cos \alpha)^2 \quad (\text{S.4})$$

$$K_z = (-\sin \theta \cos \psi \sin \alpha + \cos \theta \cos \alpha)^2 \quad (\text{S.5})$$

The nanofibers are assumed to have cylindrical symmetry about their long axis, so the distribution of ψ is taken to be uniform. Cylindrical symmetry allows us to integrate the expressions for the direction cosines over the angle ψ to yield:

$$K_x = \frac{1}{2} \cos^2 \theta \cos^2 \varphi \sin^2 \alpha + \frac{1}{2} \sin^2 \varphi \sin^2 \alpha + \cos^2 \varphi \sin^2 \theta \cos^2 \alpha \quad (\text{S.6})$$

$$K_y = \frac{1}{2} \cos^2 \theta \sin^2 \varphi \sin^2 \alpha + \frac{1}{2} \cos^2 \varphi \sin^2 \alpha + \sin^2 \varphi \sin^2 \theta \cos^2 \alpha \quad (\text{S.7})$$

$$K_z = \frac{1}{2} \sin^2 \theta \sin^2 \alpha + \cos^2 \theta \cos^2 \alpha \quad (\text{S.8})$$

The patterned nanofibers can be thought of as a biaxially oriented film where the nanofibers preferentially align perpendicular to the z axis and parallel to the x axis within the xy

plane. Stein^{3, 4} introduced two orientation parameters, f_θ and f_φ , to describe alignment in biaxially oriented films:

$$f_\theta = \frac{3\langle \cos^2 \theta \rangle - 1}{2} \quad (\text{S.9})$$

$$f_\varphi = 2\langle \cos^2 \varphi \rangle - 1 \quad (\text{S.10})$$

where $\langle \cos^2 \theta \rangle$ and $\langle \cos^2 \varphi \rangle$ are the mean values of $\cos^2 \theta$ and $\cos^2 \varphi$ for a distribution of TDMS in the film. f_θ is equal to the space-averaged second order Legendre polynomial P_2 or Saupe order parameter S_{zz} describing alignment along the z axis out of the substrate plane.^{5, 6} f_φ can be thought of as a two-dimensional order parameter quantifying the in-plane alignment of directors along the x axis. Pelletier and coworkers⁷ rewrote the expressions for K_i given by Zbinden in terms of these two parameters assuming cylindrical symmetry about the w axis, resulting in the following:

$$K_x = \left[\left(\frac{1}{2} \sin^2 \alpha - \frac{1}{3} \right) (f_\theta + f_\theta f_\varphi - f_\varphi) + \frac{1}{3} \right] \quad (\text{S.11})$$

$$K_y = \left[\left(\frac{1}{2} \sin^2 \alpha - \frac{1}{3} \right) (f_\theta - f_\theta f_\varphi + f_\varphi) + \frac{1}{3} \right] \quad (\text{S.12})$$

$$K_z = \left[\left(\cos^2 \alpha - \frac{1}{3} \right) f_\theta + \frac{1}{3} \right] \quad (\text{S.13})$$

For the patterned nanofibers, θ is close to 90° and f_θ approaches -0.5. We choose to concentrate our quantitative evaluations on the amide I and amide A vibrations known to be aligned mostly parallel to the nanofiber long axis (α approaching 0). While the orientation

parameters cannot be determined exactly from the three polarized spectra alone, upper and lower bound values of f_ϕ can be calculated assuming specific values of α and θ . The lower bound is obtained assuming that the TDM is oriented parallel to the nanofiber long axis ($\alpha = 0$) and that the non-zero value of K_z is due to tilt of the nanofiber out of the xy plane ($f_\theta > -0.5$). Solving for f_ϕ from equations S.11 – S.13 gives

$$f_{\phi,\min} = \frac{K_x - K_y}{K_x + K_y} \quad (\text{S.14})$$

Considering a different idealized case in which all of the nanofibers are lying perfectly parallel to the substrate ($f_\theta = -0.5$), any observed non-zero value of K_z is attributable to an average tilt of the TDM off the axis of the nanofiber ($\alpha > 0$). This scenario yields an upper bound value for f_ϕ given by

$$f_{\phi,\max} = \frac{K_x - K_y}{K_x + K_y - 2K_z} \quad (\text{S.15})$$

Assuming cylindrical symmetry, it is impossible to distinguish whether this value of α is due to inherent tilt of the TDM out of the plane of the parallel β -sheet, twist of the β -sheet about the nanofiber axis, or disorder in the self-assembled structure. We can at least obtain a maximum value of α by selecting the smallest value of K_z for each molecule and each vibration and solving for $f_\theta = -0.5$:

$$\alpha_{\max} = \arcsin\left(\sqrt{2K_z}\right) \quad (\text{S.16})$$

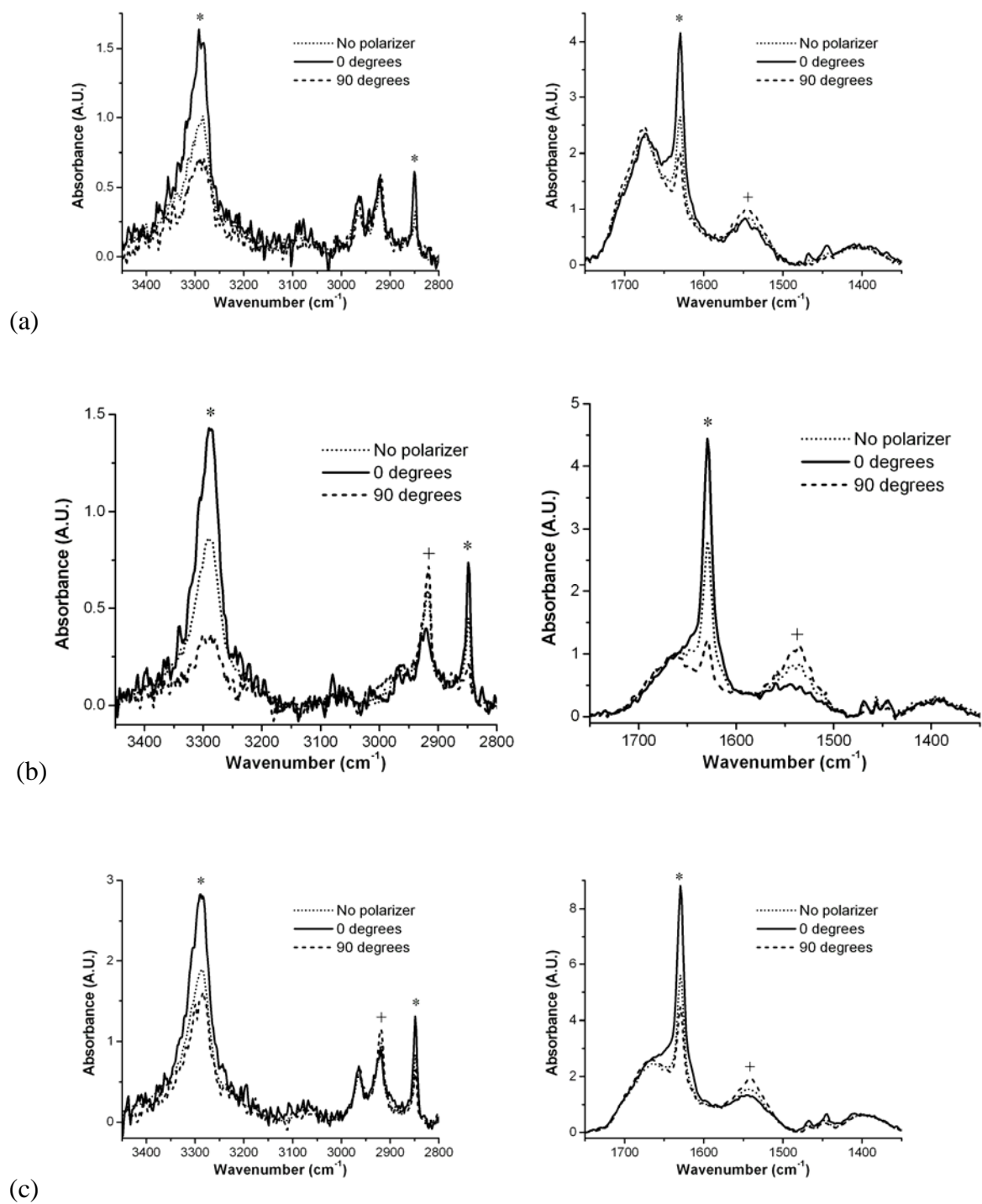


Figure S1. Polarized transmission FTIR spectra of nanofibers of **1** aligned in gratings of (a) 833 nm and (b) 278 nm period by sonication assisted solution embossing. (c) Spectra of **1** embossed in 416 nm period gratings without the aid of sonication also show some nanofiber alignment.

Spectra are normalized to the intensity of the residual silane Si-CH₃ peak at 1261 cm⁻¹. Transition dipole moments oriented parallel (*) and perpendicular (+) to the fiber structure are identified as the incident polarization is rotated from 0° to 90° relative to the alignment direction.

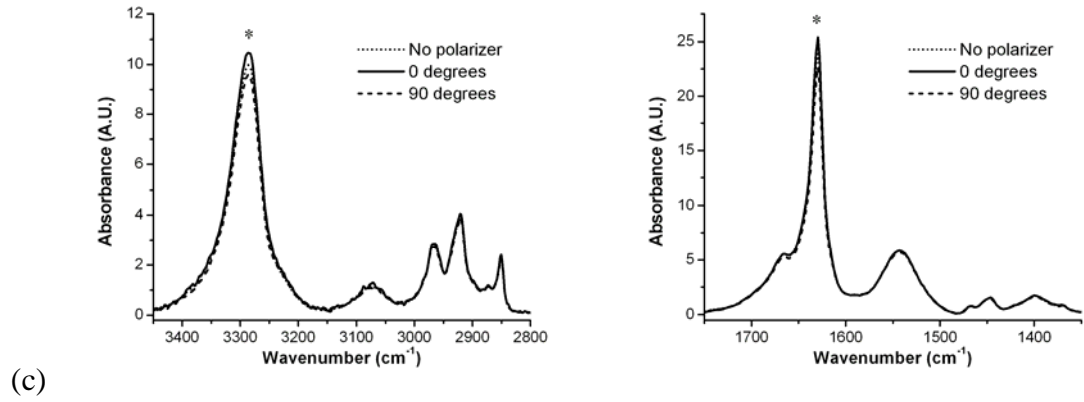
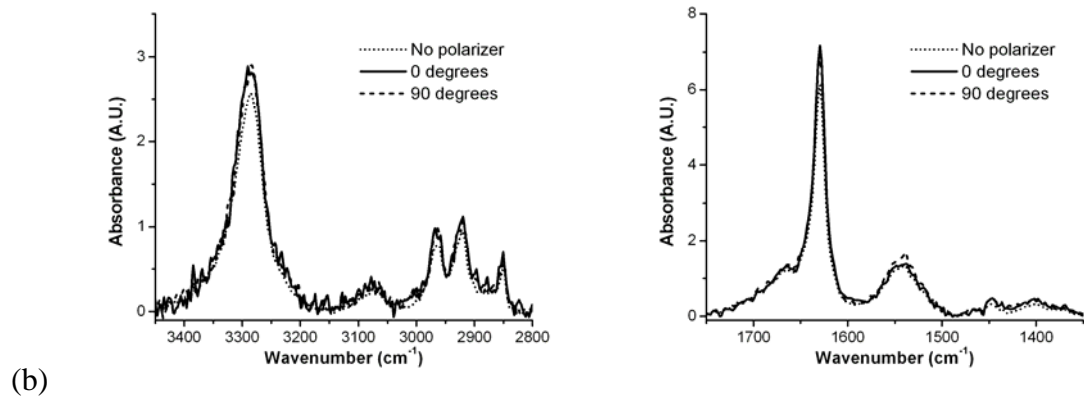
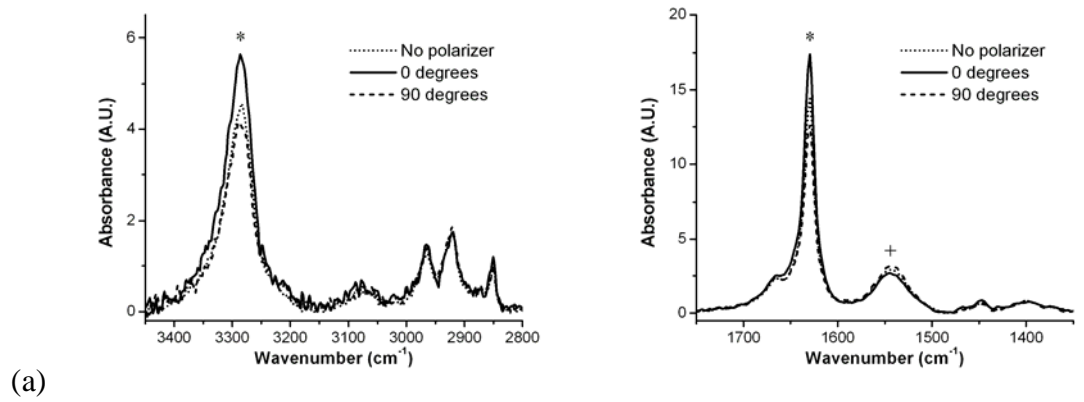


Figure S2. Polarized transmission FTIR spectra of nanofibers of **2** aligned in gratings of (a) 833 nm and (b) 278 nm period by sonication assisted solution embossing. The larger grating size shows some nanofiber alignment, but the smaller grating size shows almost no alignment. (c) Spectra of **2** embossed in 416 nm period gratings without the aid of sonication also show minimal alignment. Spectra are normalized to the intensity of the residual silane peak at 1261 cm^{-1} . Transition dipole moments oriented parallel (*) and perpendicular (+) to the fiber structure are identified as the incident polarization is rotated from 0° to 90° relative to the alignment direction.

Table S1. Absorption peaks analyzed and their assignments.

Assignment	Description	Observed Wavenumber (cm^{-1}) for 1	Observed Wavenumber (cm^{-1}) for 2
Amide A	Amide N-H stretch	3292	3289
Amide B	Fermi resonance of amide A and amide II	3071	3074
$\nu_{as}(\text{CH}_3)$	CH_3 antisymmetric stretch	2963	2964
$\nu_{as}(\text{CH}_2)$	CH_2 antisymmetric stretch	2920	2923
$\nu_s(\text{CH}_3)$	CH_3 symmetric stretch	2866	2872
$\nu_s(\text{CH}_2)$	CH_2 symmetric stretch	2849	2850
Amide I	Non- β -sheet C=O stretch	~1660	1670
	β -sheet C=O stretch	1629	1630
Amide II	Amide C-N stretch and N-H bend	1544	1543

References

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