Electronic supplementary materials *Mendeleev Commun.*, 2025, **35**, 292–294

Organization of cellulose fibrils in the capsular matrix of *Acidisarcina polymorpha* SBC82^{T} identified by synchrotron radiation

Vladislav V. Kovalenko, Svetlana E. Belova, Ksenia B. Tereshkina, Eduard V. Tereshkin, Yurii F. Krupyanskii and Nataliya G. Loiko

1. Cell sample study at the synchrotron station ID23-1 ESRF

Delivery of the sample under the X-ray beam was performed by applying a thin layer of biomass on a special sample holder mesh grid used for the study of single crystals of micron size and smaller. A view of the sample holder is shown in Figure S1. Then the whole area of the sample holder was scanned by X-ray radiation with a half of the step equal to the diameter of the X-ray beam (2 μm). Among all captured images, the image that contained a symmetric diffraction pattern from cellulose crystalline formations was selected for processing; it is presented in Figure 3 in the article.

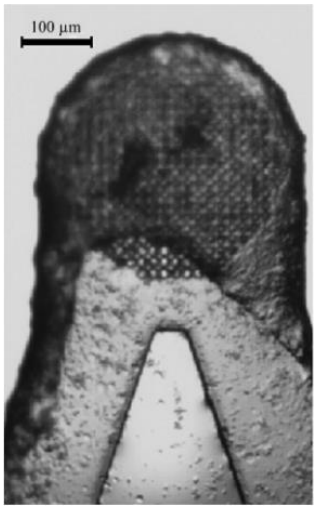


Figure S1 Illustration of sample holder with applied cell biomass.

2. Calculation of the size of cellulose-containing fibrils oriented along the main direction in the biopolymeric capsular matrix of acidobacteria from the analysis of the diffraction pattern at small angles

The size of the scattering object could be estimated from intensity variations along radial strips. Let calculate the intensity of X-ray radiation from a one-homogeneous cylinder with radius R.

According to scattering theory, the dependence of the scattering amplitude F(q) on the scattering vector **q** from an object with electron density ρ(**r**) is proportional to its Fourier transform:

$$F(\boldsymbol{q}) = \iiint \rho(\boldsymbol{r})\, \mathrm{e}^{-i(\boldsymbol{qr})}\, d\boldsymbol{r} = \iiint \rho(\boldsymbol{r})\, \mathrm{e}^{-i\left(q_x x + q_y y + q_z z\right)}\, d\boldsymbol{r} \qquad \text{(S1)}$$

For cylindrical coordinate system in real and reciprocal spaces: $x = r\cos\psi;\ y = r\sin\psi; z = z;\ q_x = q\cos\Psi;\ q_y = q\sin\Psi;\ q_z = Z$. Since in the diffraction pattern the scattering intensity is nonzero only at the equatorial line, there is no periodic structure in the scattering object along its central axis, so we can consider that the electron density of the scattering sample does not depend on the z coordinate. Then integration in formula (S1) over the variable z will give only a constant value. The symmetry of the

diffraction strips indicates the electron density independence at the angle ψ. Then the scattering amplitude can be written in the following form:

$$F(q,\Psi,Z)\sim \int_0^R \rho(r)rdr \int_0^{2\pi} exp[i(q\ r\ cos(\psi-\Psi))]d\psi = 2\pi \int_0^R \rho(r)\,J_0(qr)rdr \quad \text{(S2)}$$

By integrating over the angle ψ we obtain the zero-order Bessel function, further, considering the electron density of the scattering cylinder to be uniform, integrating over r from 0 to the cylinder radius R we finally obtain:

$$F(q)\sim 2\pi\rho \int_0^R J_0(qr)\,rdr = 2\pi\rho R\,\frac{J_1(qR)}{q} \quad \text{(S3)}$$

The scattering amplitude for an ensemble of such cylinders can be written as follows (the vector $\mathbf{v_j}$ denotes the radius-vector of the center of the j-th cylinder):[S1]

$$\Phi(\boldsymbol{q}) = \sum_{j=1}^{N} F_j(\boldsymbol{q})\exp[-i\boldsymbol{q}\boldsymbol{v_j}] \quad \text{(S4)}$$

The intensity is proportional to the quad-ratio of the modulus of Φ(q):

$$I(q)\sim \sum_{j=1}^{N} F_j(\boldsymbol{q})F_j{}^*(\boldsymbol{q}) + \sum\sum_{j\neq k} F_j(\boldsymbol{q})F_k{}^*(\boldsymbol{q})\exp[-i\boldsymbol{q}(\boldsymbol{v_j}-\boldsymbol{v_k})] \quad \text{(S5)}$$

The first term in the right part of the equality is the sum of squares of moduli of form factors of individual cylinders. The second term for non-periodic substance oscillates near zero due to the uniform probability of distribution of the vector direction ($\mathbf{v}_j$ -$\mathbf{v}_k$) and, therefore can be neglected.[S2] For dense fibril packages with periodic structure second term in (S5) will be non-zero at wide angle region as crystal peaks, and on small angle regions only on q values around 2π/l (where l ~ 50 nm is a liner size of this fibril package). So, in the region near beamstop (q ∈ [0.5nm^{-1},2.5 nm^{-1}]) the intensity of the ensemble of identical cylindric fibrils can be written as:

$$I(q) = I_0\left(\frac{J_1(qR)}{q}\right)^2 + Bgr, \quad \text{(S6)}$$

i.e., I_0 is the dimensional constant, *Bgr* is the background scattering intensity.

To calculate the fibril radius, an approximation of the experimental scattering data at small angles of the curve (S6) was performed at Wolfram Mathematica. The best value of R was determined by RMSD procedure. The results of approximation of the experimental data by the intensity proportional to (S6) are shown in Figure S2 (A).

The matches of the minimum of the experimental points and the theoretical curve with R = 17.0 ± 0.5 Å indicates the correctness of the chosen theoretical model.

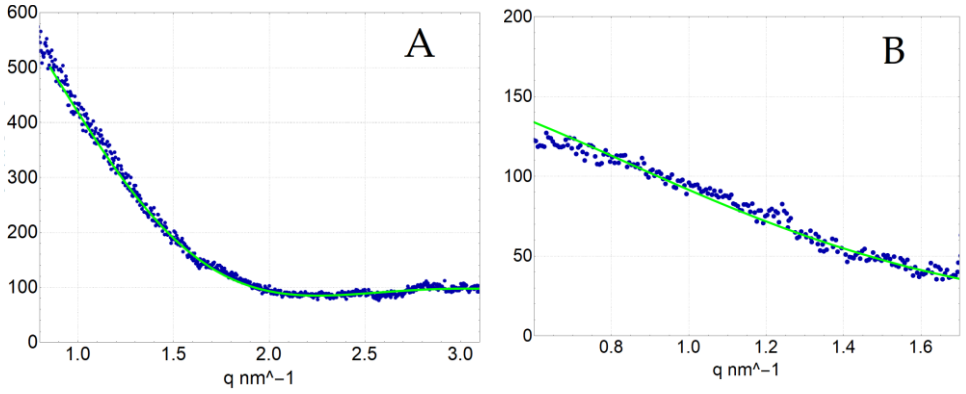


Figure S2 Approximation of the experimental data of the scattered intensity along the radial strips at small angles. (A) Intensity along the main direction, approximated by formula (S6). (B) Intensity along the complementary direction, approximated by formula (S7). Blue dots indicate experimental values. The green solid line is the theoretical curve.

3. Calculation of the size of cellulose-containing fibrils oriented along the complementary direction in the biopolymeric capsular matrix of acidobacteria from analysis of the diffraction pattern at small angles

The radial intensity distribution along the second direction located on the two-dimensional diffraction pattern was approximately perpendicular to the main one (χ_1=83°), did not contain any features at both small and wide scattering angles. The intensity along the strips at small scattering angles monotonically decayed. Therefore, such an experimental intensity cannot be described using formula (S6), because of the oscillatory behaviour of the Bessel functions.

If we use the idea that also along this direction the main scattering element are cylindrical fibrils, we can try to search for an approximation function of intensity from the scattering vector in the form of the average value of the scattered intensity from cylinders with a known distribution function by the value of their radius D(R):

$$I(q) \sim \int_{R_{min}}^{R_{max}} \left(\frac{J_1(qR)}{q}\right)^2 D(R)dR + Bgr \qquad \text{(S7)}$$

The Schults-Zimm distribution was chosen as the distribution function, which describes well the mass and size distribution of polymers[S3,S4] and can describe the asymmetry with respect to the mean value.

$$D(d) = \left(\frac{R}{R_{mean}}\right)^z \frac{(1+z)^{(1+z)}}{\Gamma(1+z)} e^{-\frac{(1+z)R}{R_{mean}}}, \qquad \text{(S8)}$$

i.e., R_{mean} denotes the average value of the cylinder radius, $\sigma = \frac{R_{mean}^2}{\sqrt{1+z}}$ – standard deviation, a Γ(1+z) – Eulerian gamma function.

The RMSD approximation procedure of the experimental data was performed in Wolfram Mathematica. The matching results of the approximation of the experimental data with the theoretical curve (S7) are shown in Figure S2 (B). The average value of the radius was R_{mean}= 10 Å. At the same time, the standard deviation was quite large σ=5.9 Å.

It is possible that the fibrils oriented along the complementary direction act as binding material for the cellulose fibrils located in the main direction. Although it is not possible to determine the certain ratio of fibrils along the main and complementary directions because the entire backspace region is not available to us. Nevertheless, if we take into account the intensities at all available scattering angles and assume that the ratio of integral values of intensities along the principal and complementary directions is equal to the square of the ratio of masses of the scattering objects, the mass ratio can be estimated as follows ~ 9:1.

4. Calculation of the relative orientation of crystalline domains of cellulose-containing fibrils oriented along the main direction in the biopolymer capsule matrix of acidobacteria from the analysis of the diffraction pattern at large angles

The diffraction peaks in the previous section has a form of arcs. This effect occurs due to the non-zero value of tilt angle for periodic fibrils bundles relative to the main axis. The intensity distribution over the azimuthal angle (χ) on these arcs can be used to estimate the distribution of the tilt angle of periodic packages relative to the main direction of the fibril axis.

The value of the intensity distribution H(χ) as a function of the azimuthal angle x is related to the probability distribution for a periodic packages to have a tilt angle μ from the main axis f(μ) by the following relation[S5]

$$H(\chi) = 4\int_{\chi}^{\pi/2} \frac{f(\mu)sin(\mu)}{\sqrt{cos^2\chi - cos^2\mu}}d\mu \qquad \text{(S9)}$$

To obtain the orientation distribution of the periodic packeges of fibrils, we need to solve the integral equation (S9). To write equation (S9) more conveniently, we replace the variables x=cos(χ); y=cos(μ), then we obtain the following expression

$$H(x) = 4\int_{0}^{x} \frac{f(y)}{\sqrt{x^2 - y^2}} \qquad \text{(S10)}$$

Multiply the left and right parts of the equation by $\frac{x}{\sqrt{u^2-x^2}}$ and integrate by the variable *x* from 0 to *u*

$$\int_0^u \frac{x\,H(x)dx}{\sqrt{u^2 - x^2}} = 4\int_0^u \frac{x}{\sqrt{u^2 - x^2}}\left[\int_0^x \frac{f(y)}{\sqrt{x^2 - y^2}}dy\right] \qquad \text{(S11)}$$

Then replace the limits of integration by the Dirichlet rule:

$$\int_0^u \frac{x\,H(x)dx}{\sqrt{u^2 - x^2}} = 2\int_0^u f(y)\left[\int_y^u \frac{2x}{\sqrt{x^2 - y^2}\sqrt{u^2 - x^2}}dx\right]dy \qquad \text{(S12)}$$

Let's calculate the values of the integral $I = \int_y^u \frac{2x}{\sqrt{x^2-y^2}\sqrt{u^2-x^2}}dx$. To do this, let's replace the variables $t = \frac{u^2-x^2}{u^2-y^2}$, in new variables the integration is performed within the range from *t*=0 to *t*=1, and $2xdx = -(u^2 - y^2)dt$. After the described transformations the value of I will take the form:

$$I = -\int_1^0 \frac{(u^2 - y^2)dt}{\sqrt{u^2 - y^2 - t(u^2 - y^2)}\sqrt{u^2 - u^2 + t(u^2 - y^2)}} = \int_0^1 \frac{dt}{\sqrt{1-t}\sqrt{t}} = \pi \qquad \text{(S13)}$$

Thus, formula (S13) is transformed into:

$$\int_0^u \frac{x\,H(x)dx}{\sqrt{u^2-x^2}} = 2\pi \int_0^u f(y)\,dy \qquad \text{(S14)}$$

Differentiating both parts of the equation by d*u*, using Leibniz's integral rule for differentiation, we obtain:

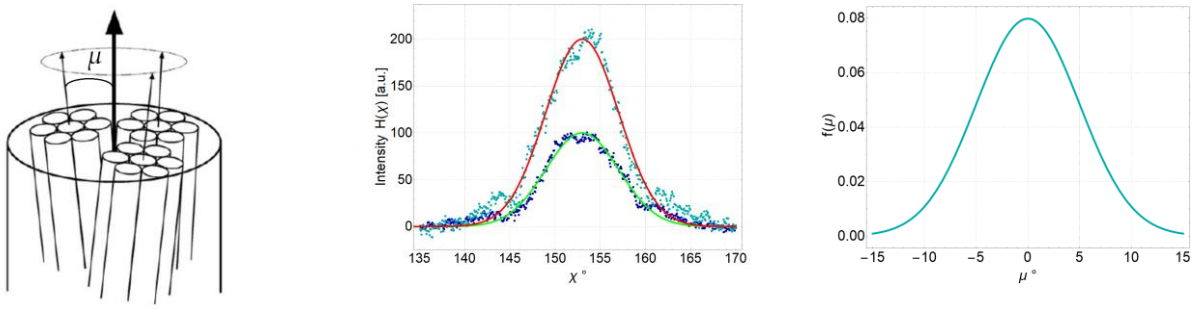
$$f(u) = \frac{1}{2\pi}\frac{d}{du}\int_0^u \frac{x\,H(x)dx}{\sqrt{u^2-x^2}} \qquad \text{(S15)}$$

Returning to the original variables μ and χ, we obtain:

$$f(\mu) = \frac{1}{2\pi}\frac{d}{dcos(\mu)}\int_0^{\mu} \frac{H(x)\cos(\chi)dcos(\chi)}{\sqrt{cos(\mu)^2-\cos(\chi)^2}} \qquad \text{(S16)}$$

Now, to calculate the distribution of fibrils inside the biopolymer matrix (Figure S3A), it is sufficient to numerically calculate the integral (S15) by approximating the angular intensity distribution H(χ) by a Gaussian normal distribution. Figure S3B shows the approximation of the angular distributions H(χ) for the four diffraction peaks and their smooth approximation. The results of calculating the tilt angle distribution of crystal domains are shown in Figure S3C. All calculations were performed in Wolfram Mathematica.

Thus, the calculations have shown that the fibrils in the periodic packeges are arranged strictly parallel, and the mean square deviation of the deviation angle is σ=5°.



A B C

Figure S3 (A) Shematic representation of hierarchy of order within biopolymer matrix. The angle of crystall domains with respect to main axis illustrates the origin of axial disorder in the X-ray diffraction pattern. Although crystall domains represents here as seven hexagonally packed fibrils, real domains size calculated from arc widths is 200 fibrils. (B) Cyan and blue dots are the H(χ) intensities of the diffraction arcs, solid red and green lines are RMSD best-fit curve proportional to gauss normal distribution function. (C) Pobability distribution function (f(μ)) of tilt angle μ calculated with (S16).

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