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Supporting information for article:

Custom AFM for X-ray beamlines: *in situ* biological investigations under physiological conditions

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S1- Filter setting used during the acquisition of the Reflectivity data.

Figure S1 shows the transmitted beam after the filter as a function of $Q_z$ employed for the acquisition of a Reflectivity curve. The flux at the sample position without filters was $2 \times 10^{13}$ photons/s.

Figure S1: Transmitted beam used for the acquisition of the Reflectivity curves as a function of $Q_z$. 
S2- Beam-Tip alignment:
The AFM cantilever and the beam have been aligned either by measuring the current flowing in the cantilever (see Figure 4d in the main manuscript) or by Scanning X-Ray Transmission Microscopy. Figure S2 shows the transmitted beam scans measured at different heights with the ID03 detector. The inset presents schematically the scan geometry. Black curve: the beam crossing the optical fiber and subsequently the AFM chip. Red curve: beam crossing solely the optical fiber. Blue curve: beam crossing the AFM cantilever.

![Image](image_url)

Figure S2: transmitted beam scans measured along the X-Axes at different heights corresponding to different colours. Inset: pictorial scheme of the measurement set up.

S3- Reflectivity for DPPC: Best fit parameters for data shown in Figure 4c of the main manuscript:

Derived values for thicknesses (d), electron density (ρ) and the roughness parameter (σ) of the first head-groups H₁ (the closest to the substrate), the two hydrocarbon chains C₁ and C₂, the CH₃ group and the second head-groups H₂ from the best fit of the data presented in Figure 4c. σ₁₂₀, σᵦₒ₂ and σᵦᵣ are the roughness parameters for the water layer below the head-groups H₁, the SiO₂ and the Silicon Substrate.

| Parameter | DPPC |
|-----------|------|
| dₜ₁ (nm)  | 0.76 |
| dₙ₁ (nm)  | 1.55 |
| dₜ₂ (nm)  | 0.49 |
| dₙ₂ (nm)  | 1.60 |
| dₜ₃ (nm)  | 1.09 |
| ρₜ₁ (e/A³) | 0.58 |
| ρₙ₁ (e/A³) | 0.31 |
| ρₜ₃ (e/A³) | 0.23 |
S4 Reflectivity for DOPC: Best fit parameters for data shown in Figure 5c of the main manuscript:

Derived values for thicknesses (d), electron density (ρ) and the roughness parameter (σ) of the first head-groups H₁ (the closest to the substrate), the two hydrocarbon chains C₁ and C₂, the CH₃ group and the second head-groups H₂ from the best fit of the data presented in Figure 5c. σ₁₂₀, σ₁₂₃ and σ₁₄ are the roughness parameters for the water layer below the head-groups H₁, the SiO₂ and the Silicon Substrate.

| Parameter  | DOPC (blue data) | DOPC (red data) |
|------------|------------------|-----------------|
| ρ₈ (e/A³)  | 0.48             | 0.43            |
| ρ₂₁ (e/A³) | 0.26             | 0.30            |
| ρ₂₃ (e/A³) | 0.21             | 0.27            |
| ρ₂₄ (e/A³) | 0.28             | 0.35            |
| ρ₂₅ (e/A³) | 0.40             | 0.38            |
| σ₈ (at/A)  | 1.24             | 1.01            |
| σ₁ (at/A)  | 2.17             | 2.05            |
| σ₂₁ (at/A) | 2.19             | 2.05            |
| σ₂₂ (at/A) | 2.05             | 2.05            |
| σ₂₃ (at/A) | 2.27             | 1.94            |
| σ₂₄ (at/A) | 1.01             | 1.01            |
| σ₂₅ (at/A) | 2.84             | 2.84            |
| σ₂₆ (at/A) | 9.36             | 9.36            |