Supporting Information to: Nano-Chemical Infrared Imaging of Membrane Proteins in Lipid Bilayers

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Experimental details

In this work several modulation schemes are combined to isolate the near-field signal. The far-field background is suppressed by demodulating the detected signal at higher harmonics of the cantilever oscillation frequency ω_t.[1] A new reference amplitude modulation scheme selectively detects only signal which is homodyne amplified by a reference arm with known phase, while rejecting the self-homodyne near-field signal that is amplified with uncontrolled phase.[2] The intensity of the reference arm is chopped at a frequency $\Omega_r \ll \omega_t$ and the resulting signal demodulated at $3\omega_t \pm \Omega_r$

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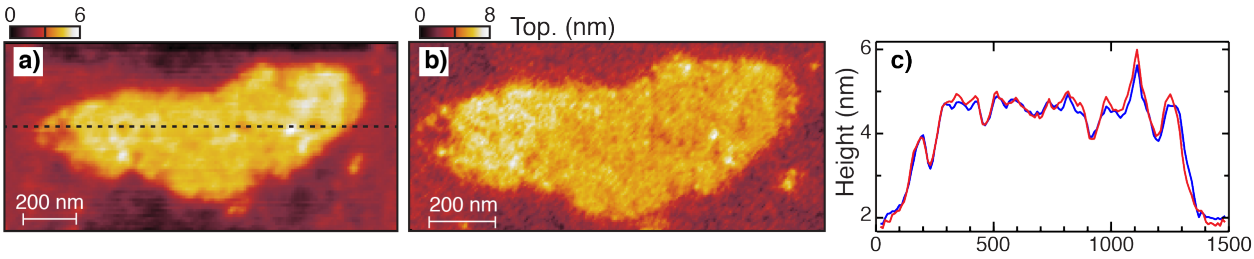

Figure S1: Tapping mode AFM topography of a membrane patch studied with a Pt-coated tip (a). Contact mode AFM topography of the same patch showing a higher spatial resolution but the same larger features in the membrane patch (b). Individual bR trimers or molecules were not observed. (c) Line cut taken along dashed line in (a) and from subsequent scan (blue and red, respectively), showing strong agreement.

using a lock-in amplifier (Zurich Instruments, HF2LI). The near-field amplitude $|A|$ and phase ϕ can then be accurately determined from the detected *s*-SNOM signal $S \propto |A|\cos(\phi - \phi_{\mathrm{ref}})$ by repeating measurements at two different discrete reference phase values.[3] We use orthogonal optical reference field phases $\phi_{\mathrm{ref},1} = \phi_{\mathrm{ref},2} + \pi/2$, which provides for simple determination of the amplitude $|A(\bar{\nu})| \propto (S^2_{\phi_{\mathrm{ref},1}} + S^2_{\phi_{\mathrm{ref},2}})^{1/2}$ and phase $\phi(\bar{\nu}) = \tan^{-1}(\mathrm{S}_{\phi_{\mathrm{ref},1}}/\mathrm{S}_{\phi_{\mathrm{ref},2}})$ of the near-field signal. This combination of two-phase homodyne amplification with amplitude modulation provides the same *s*-SNOM contrast, phase, and amplitude information as phase modulation,[4] with comparable signal-to-noise ratio (SNR), but improved stability in certain cases.

Comparison between tapping and contact mode AFM

In order to verify the disordered nature of the membrane proteins, additional characterization was performed using high resolution contact mode AFM (Asylum Instruments, Cypher) with sharp tips (Bruker SNL cantilever C, contact mode at a set point of $\sim$100 pN). The bR lattice trimers have previously been imaged on mica in an imaging buffer using this instrument and otherwise identical preparation procedures.[5] The *s*-SNOM experiments are performed using tapping mode AFM with Pt-coated tips, with apex radii $r > 10$ nm, thus precluding the resolution of single bR molecules.

To confirm the membrane disorder observed in tapping mode, membrane patch 1 (Figure 2 in the manuscript) was measured using high-resolution contact mode (Cypher) in addition to tapping

mode (Anasys), enabling direct comparison. The tapping mode and contact mode topographies of patch 1 are shown in Figure S1 (panels a and b). Identical larger topographic features are observed in both scans, with finer details resolved in contact mode, although no bR trimers are seen. The inability to resolve individual bR molecules and their trimers thus indicates a membrane lacking bR order. This disorder likely arises due to the intrinsic roughness of the template-stripped Au support substrate, its overall influence on assembly energetics, and possible effects of drying. Despite varying thickness, the membrane remains intact without tears or other holes through the patch to the Au substrate.

The tapping mode topography is highly reproducible. Morphological features in Figure S1a, including those noted as i–v in Figure 4, extend over several lines. This indicates reproducibility and precludes the effect of noise or drift. This reproducibility is further underscored by the line cut taken along the dashed line in Figure S1a (panel c, blue line, identical to Figure 4e). This is compared to the cut along the same line taken from an identical scan acquired at a later time (red line, full image scan not shown). Both lines exhibit very similar topography and the majority of features, including i–v in Figure 4e are reproducible.

Membrane patch 2

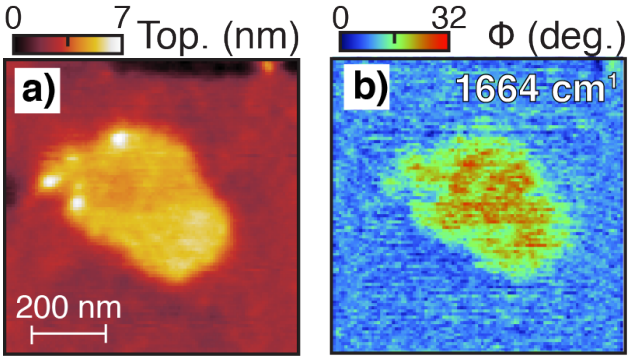

Figure S2: AFM topography with tapping mode (a) and near-field phase (b) images of purple membrane patch 2 in Figure 2.

The *s*-SNOM investigation of protein disorder was performed on multiple patches, (indicated

by 1 and 2 in Figure 2). Figure S2 shows the tapping mode topography (a) and near-field phase (b) of patch 2 at 1664 cm^{-1}. Similar bR disorder and variation in local protein concentration are observed for both patches.

References

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