Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary information

Sub-nanometre mapping of the aquaporin-water interface with multifrequency atomic force microscopy

Maria Ricci, Roy A Quinlan, and Kislon Voïtchovsky†

†kislon.voitchovsky@durham.ac.uk

Content:

InvOLS calibration for the different harmonics

In all experiments the cantilever motion is detected by a laser which effectively measures the slope of the cantilever's deflection and not an absolute displacement. It is therefore necessary to take into account the difference in nature cantilever motion between eigenmodes when calibrating the Inverse Optical Lever Sensitivity (InvOLS). The static deflection InvOLS was obtained from a force curve acquired on a mica surface. The mica surface was assumed infinitely stiff so that the slope of deflection *vs* z-piezo displacement in the contact region was assigned to 1. Imposing a unity slope provides the value of the deflection InvOLS. We used a rectangular cantilever for all measurements and assumed the cantilever to be uniform. Under this assumption, the InvOLS of the first flexural mode (first eigenmode) is obtained by multiplying the deflection InvOLS by 1.09. The InvOLS of the scond flexural mode (second eigenmode) requires multiplying the deflection InvOLS by 0.314.[1,2] These corrections have been applied to the data presented in this paper. For the measurements conducted at (sub-) harmonics of the driving frequency, the correction was calculated according to the closest eigenmode.

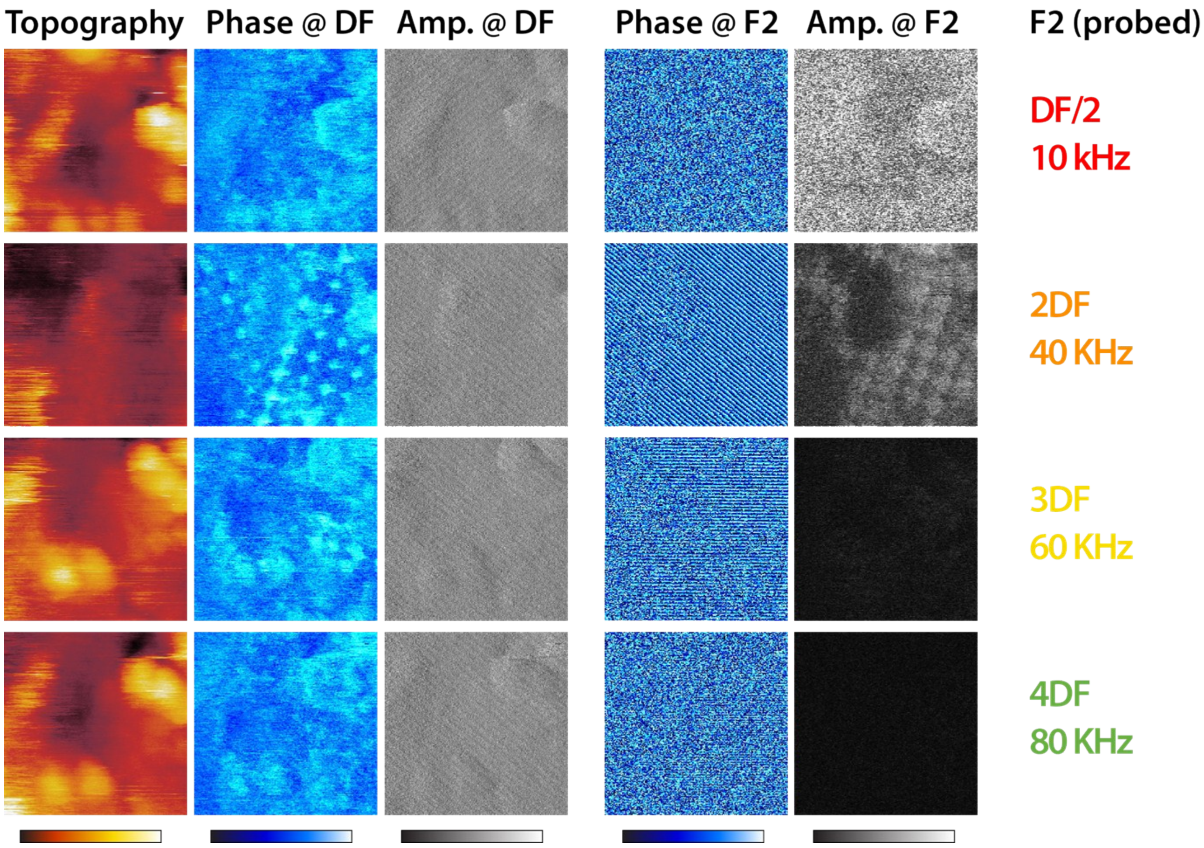

Fig S1 Full set of data used for Fig. 3. Each line of images present data acquired simultaneously. The first three images show the topography, phase and amplitude at the driving frequency (DF) where the feedback operates (operation in AM1-AFM). Here DF = 20.15 kHz. During the imaging, the oscillation amplitude and phase are simultaneously recorded at a different frequency F2, indicated in colour on the right. The spatial variations of amplitude and phase at F2 are shown in the 2 last columns. F2 is always a harmonic or sub-harmonic of DF and is hence also expressed as a function of DF. A new image is acquired for each line. The quality of the image slowly deteriorates with the experiment due sweeping of material by the tip, but this does not affect findings regarding inter-harmonics energy transfer. The phase does not show any meaningful information for any of the harmonics. Spatial variation of the amplitude at (DF/2) only captures hints of large topographic features. Amplitude at the second harmonic (2DF) is clearly correlated with surface features, while little or nothing is visible for higher harmonics.

All images are 50 nm xy, captured over a same location. The colour scale bars at DF represent 6 nm in topography, 12° in phase and 120 pm in amplitude. The colour scale bars at F2 represents 360° in phase (consistent with undefined phase) and 120 pm in amplitude. The free amplitude at DF is 1.2 nm (free) with an imaging setpoint to free amplitude ratio of 0.9

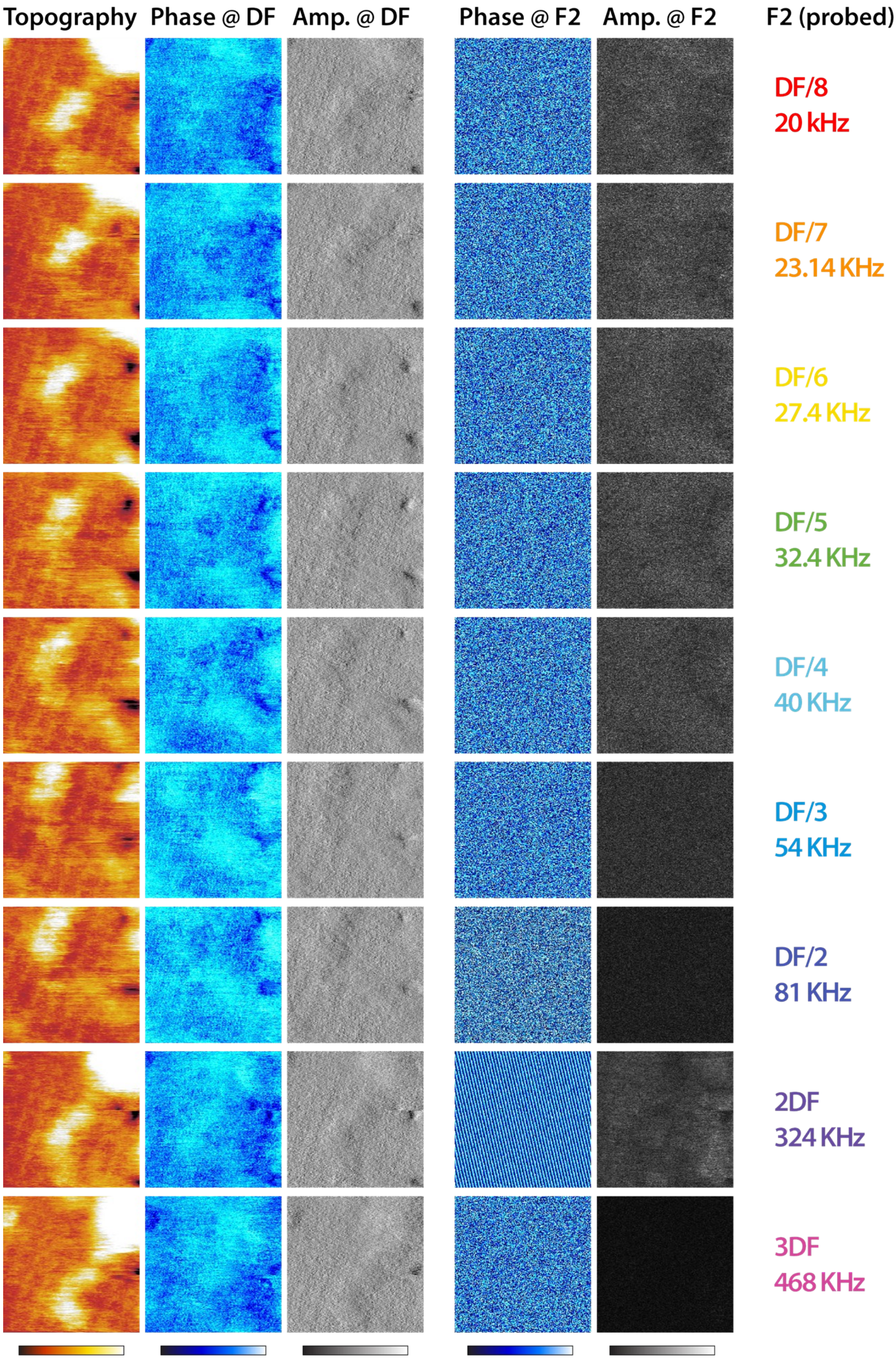

Fig S2 Full set of data used for Fig. 4. Each line of images present data acquired simultaneously. The first three images show the topography, phase and amplitude at the driving frequency (DF) where

the feedback operates (operation in AM2-AFM). Here DF = 162 kHz. During the imaging, the oscillation amplitude and phase are simultaneously recorded at a different frequency F2, indicated in colour on the right. The spatial variations of F2 are shown in the 2 last images in each line. F2 is always a harmonic or sub-harmonic of DF and is also expressed as a function of DF.
A new image is acquired for each line. The phase does not show any meaningful information for any of the harmonics. Spatial variations in amplitude show some correlation with topographic features only for 2D, while little or nothing is visible for other (sub-) harmonics.
All images are 50 nm xy, captured over a same location. The colour scale bars represent 4 nm in topography (a), 10° in phase (b) and 30 pm in amplitude (c). The grey scale in (e) represents 30 pm for all images, which show a fraction of same area of the membrane as (a-c). The free amplitude is 260 pm with an imaging setpoint to free amplitude ratio close to 0.9.

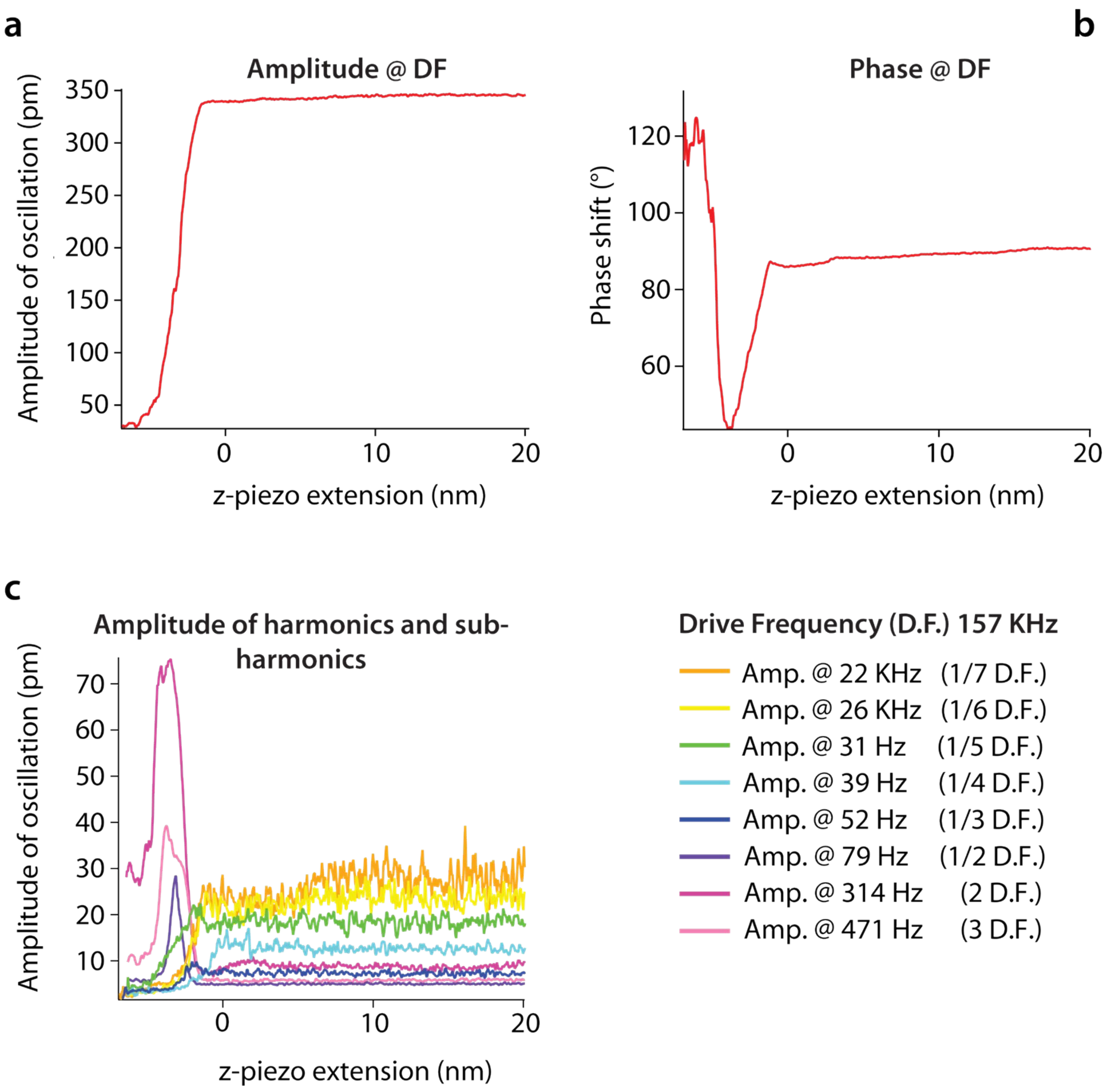

Fig S3 Spectroscopy amplitude- and phase-distance curves acquired with the cantilever driven at a frequency near its second eigenmode (DF = 157 kHz). The zero is defined arbitrarily as the z-piezo extension past which the cantilever experiences a static positive deflection. The amplitude (a) shows little variation before zero while the phase (b) exhibit a small but reproducible shift at ~3nm already. This could be explained by the first 'direct' contact between the tip and the membrane, consistent with the harsh imaging conditions observed when working in AM2-AFM. The harmonics and sub-harmonics as a function of distance show some enhancement at close distances only for 1/2 DF, 2 DF and 3DF, with the maximum enhancement for 2 DF, consistent with the images in Fig. S2. Each curve shown in (a), (b) and (c) is an average over at least 10 raw data curves.

Supplementary References

1 H.-J. Butt and M. Jaschke, *Nanotechnology*, 1995, **6**, 1–7.
2 D. Kiracofe and A. Raman, *J. Appl. Phys.*, 2010, **108**, 034320–034324.