

# Reversible dissolution of lipid microdomains in cancer cell membranes at physiological temperature

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## Abstract

The formation of lipid microdomains (“lipid rafts”) is presumed to play an important role in various cellular functions, but their nature remains controversial. Here we report on microdomain formation in isolated, detergent-resistant membranes from MDA-MB-231 human breast cancer cells, studied by Atomic Force Microscopy (AFM). Whereas microdomains were readily observed at room temperature, they shrunk in size and mostly disappeared at higher temperatures. This shrinking in microdomain size was accompanied by a gradual reduction of the height difference between the microdomains and the surrounding membrane, consistent with the behaviour expected for lipids that are laterally segregated in liquid ordered (Lo) and liquid disordered (Ld) domains. Immunolabeling experiments demonstrated that the microdomains contained flotillin-1, a protein associated with lipid rafts. The microdomains reversibly dissolved and reappeared, respectively, on heating to and cooling below temperatures around 37 °C, which is indicative of radical changes in local membrane order close to physiological temperature.

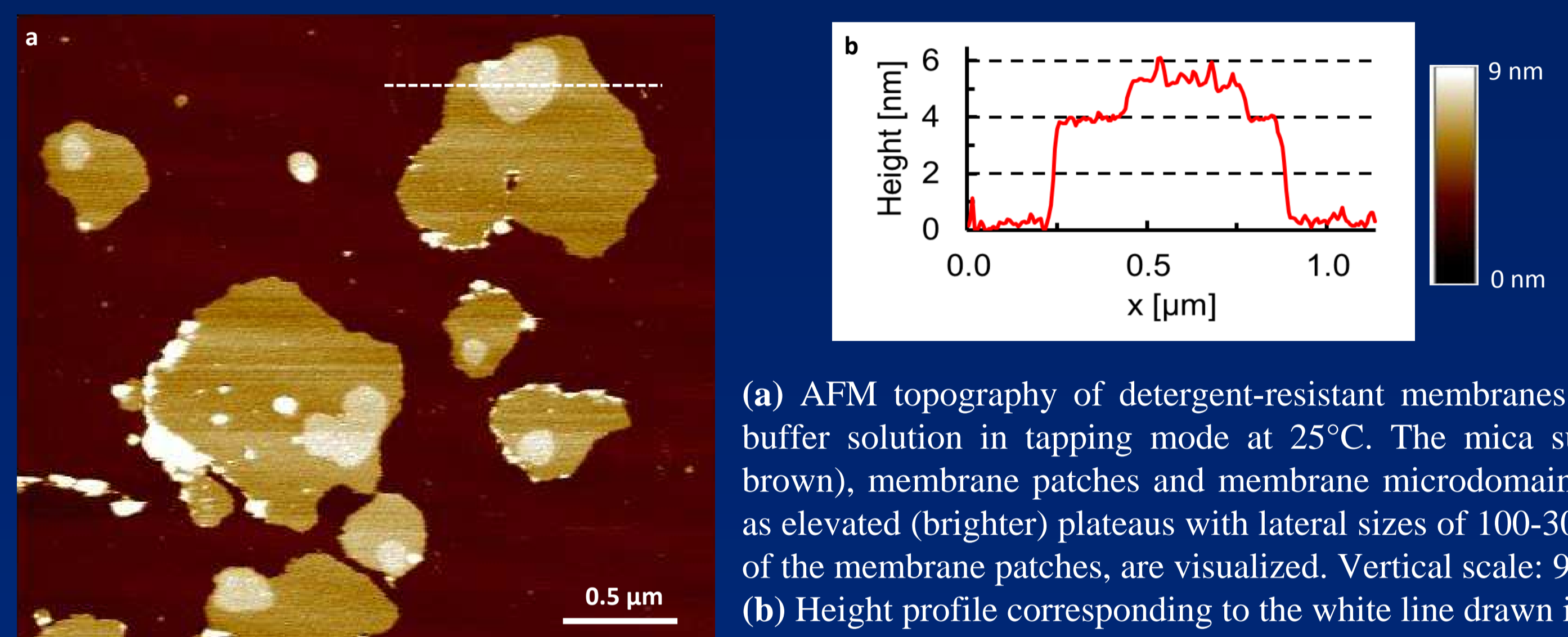
## Sample preparation

**Cell line:** Human breast cancer cell line MDA-MB-231 (ER-negative and over-expressing EGFR), derived from human mammary adenocarcinoma was obtained from Italian National Cancer Research Institute cell bank.

**Lipid raft isolation:** Membrane fractions were isolated by ultracentrifugation on 5-30% linear sucrose gradient in presence of Triton X-100; every fraction was characterized for its content in lipids and proteins.

**AFM imaging:** Purified membranes were diluted 1:30 in distilled water. 50  $\mu$ L of diluted samples were floated on a freshly cleaved mica leaf, previously activated with 100  $\mu$ L of adsorption buffer (150 mM KCl, 25 mM MgCl<sub>2</sub>, 10 mM Tris/HCl pH 7.5). After 10 min, the sample was gently rinsed with recording buffer (150 mM KCl, 10 mM Hepes/Tris, pH 7.5). Samples were imaged in recording buffer in tapping mode by a Nanoscope Multimode IIIa AFM.

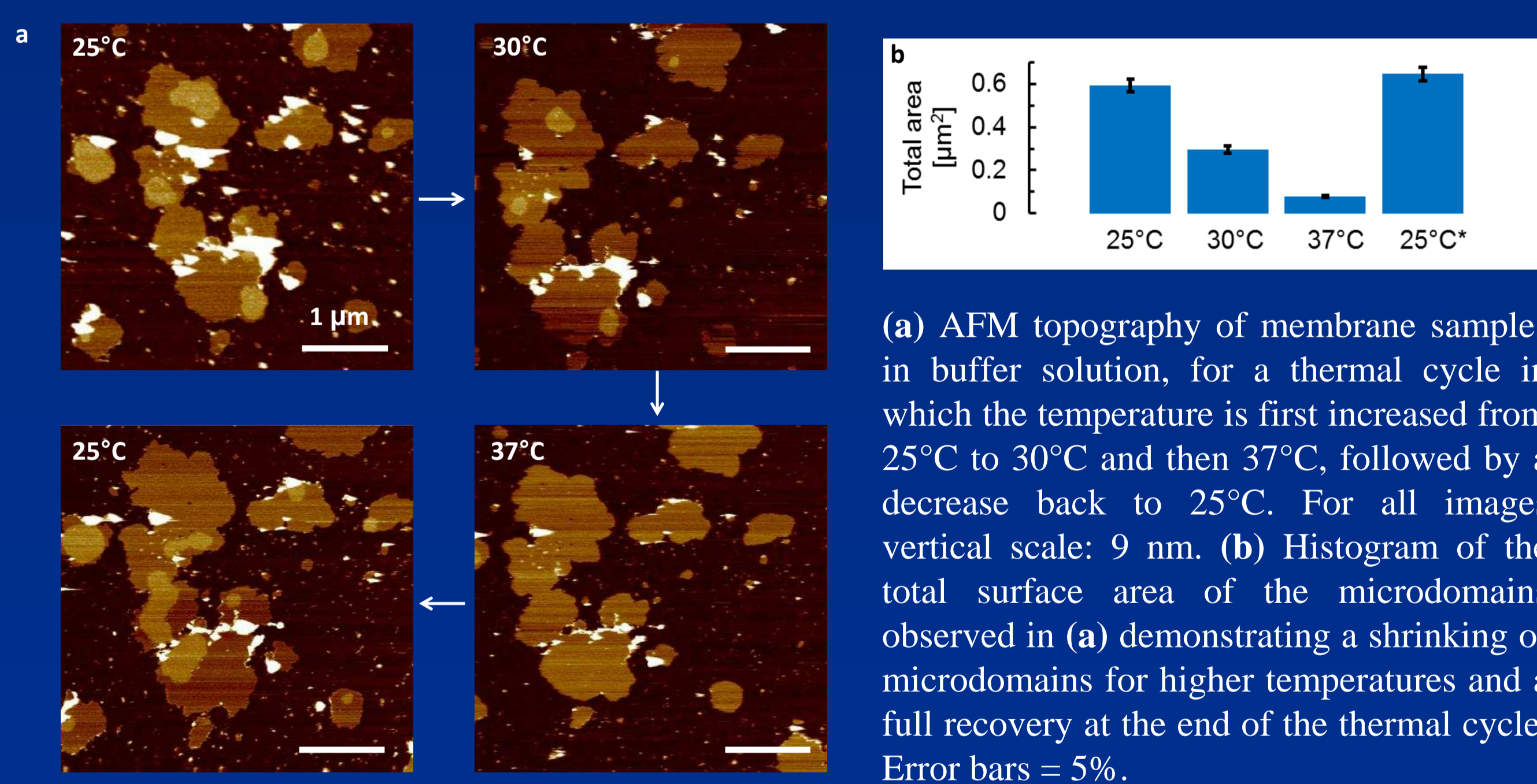
## Appearance of microdomains in plasma membranes for human breast cancer cells



(a) AFM topography of detergent-resistant membranes recorded in buffer solution in tapping mode at 25°C. The mica support (dark brown), membrane patches and membrane microdomains, appearing as elevated (brighter) plateaus with lateral sizes of 100-300 nm on top of the membrane patches, are visualized. Vertical scale: 9 nm. (b) Height profile corresponding to the white line drawn in (a).

The microdomains protruded 1-2 nm from the surrounding membrane, suggesting a locally enhanced order, and with larger roughness indicating significant protein content

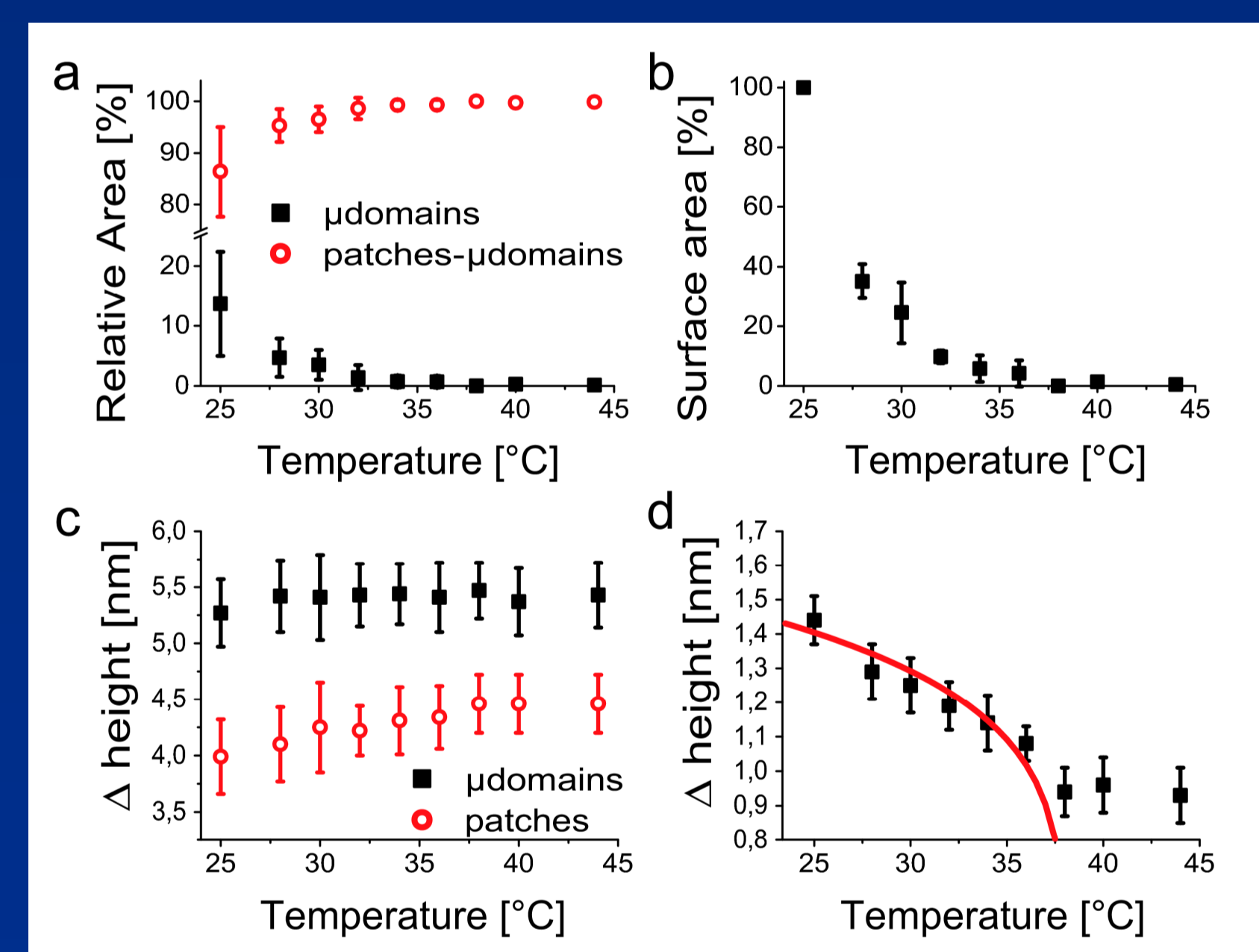
## Reversible dissolution of microdomains at physiological temperature



(a) AFM topography of membrane samples in buffer solution, for a thermal cycle in which the temperature is first increased from 25°C to 30°C and then 37°C, followed by a decrease back to 25°C. For all images vertical scale: 9 nm. (b) Histogram of the total surface area of the microdomains observed in (a) demonstrating a shrinking of microdomains for higher temperatures and a full recovery at the end of the thermal cycle. Error bars = 5%.

The microdomains showed a marked temperature dependence, whereas the overall dimensions of the membrane patches appeared unchanged

## Temperature dependence of microdomain size and height

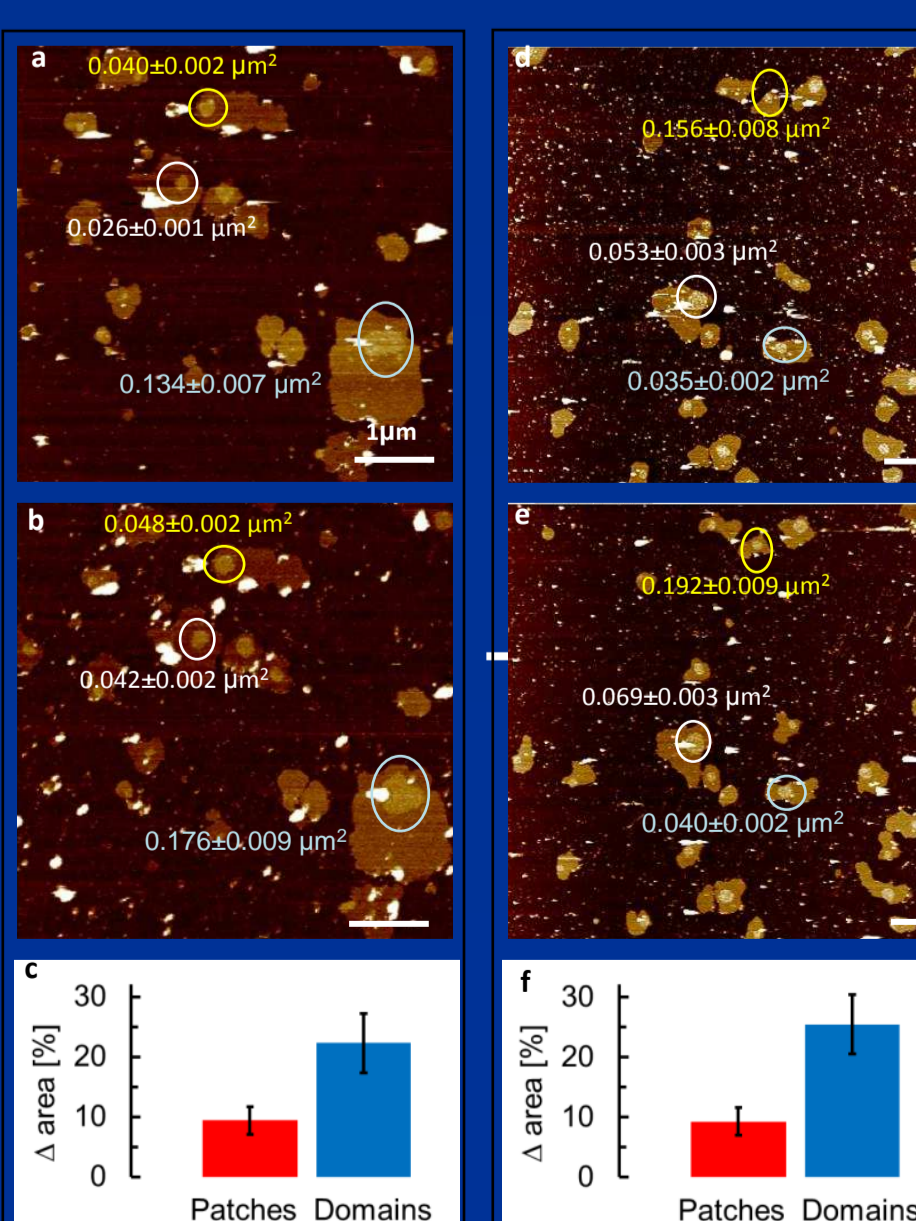


(a) Plot of the relative areas of microdomains and patches-microdomains as a function of the temperature. (b) Scattering of the surface area of microdomains at different temperatures and normalized to their surface area at 25°C. (c) Plot of the microdomain and patch heights, both with respect to the mica, as a function of the temperature. (d) Scattering of the height for microdomains at different temperatures measured with respect to the surrounding membrane patch surface. The red line is a fit to the data (below 37°C) with critical behavior as predicted by the Ising model  $A(T_c - T)^n$  with  $n=1/8$ , yielding  $T_c=37.8^\circ\text{C}$  and  $A=0.994 \text{ nm}^2/\text{C}$ . Data (mean  $\pm$  SD) obtained analyzing different thermal cycles ( $n=16$ ) for all the plots.

The temperature dependence of height difference between the microdomains and the surrounding membrane, was consistent with the behavior expected close to a critical phase transition as described by the 2D Ising model  $A(T_c - T)^n$

## AFM immunolabelling of microdomains

Before thermal cycle After thermal cycle



(a-d-e) AFM topography images of membrane samples in buffer solution at room temperature, before (left) and after (right) a thermal cycle (25°C-37°C-40°C-25°C). For all images vertical scale: 9 nm.

Untreated membrane patches (a) were incubated with anti flotillin-1 antibodies. After 60 min of treatment (b) the area of microdomains protruding from the membrane patches had increased. (c) Histogram of the surface area increase for membrane patches,  $9.3 \pm 2.3\%$  (mean  $\pm$  SD;  $n=28$ ), and microdomains,  $22.3 \pm 4.9\%$  (mean  $\pm$  SD;  $n=25$ ), after 60 min antibody incubation.

(d, e) As (a, b) but for membrane patches that have undergone a thermal cycle prior to the immunolabelling. (f) Histogram of the surface area increase for membrane patches,  $9.2 \pm 2.5\%$  (mean  $\pm$  SD;  $n=15$ ), and microdomains,  $25.3 \pm 6.3\%$  (mean  $\pm$  SD;  $n=17$ ), after 60 min antibody incubation.

The microdomain surface area increase is significantly larger than that of the overall membrane patches. Importantly, this increase was the same irrespective of whether the immunolabelling was performed before or after a thermal cycle

## Conclusions

- Static microdomains (lateral sizes  $\sim$  100-300 nm) protruding 1-2 nm from the surrounding membrane and with increased roughness were visualized in detergent resistant membranes purified from human breast cancer cells
- Microdomains reversibly dissolved and reappeared, respectively, on heating to and cooling below a critical temperature  $T_c = 37 \pm 1^\circ\text{C}$
- The observed dissolution and condensation of microdomains is consistent with a mixing-demixing transition between a Lo and Ld phase as supported by the height difference between both phases, which below  $T_c$  can be described by the 2D Ising model
- Immunolabelling demonstrated that the microdomains contained flotillin-1, a protein associated with lipid rafts. As the labelling was equally successful before and after the thermal cycle, it follows that the protein took part in the microdomain reformation process
- As the  $T_c$  corresponds to physiological temperature, these observations strongly suggest that the membrane is tuned to exploit the radical changes in its local ordering at a critical transition (e.g. to mediate cell signaling, responding to intra- or extracellular triggers)