Poster

THEORY OF TOPOGRAPHY AND RECOGNITION IMAGING BY DYNAMIC FORCE MICROSCOPY

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Since the beginnings of Atomic Force Microscopy (AFM), measurement techniques have steadily evolved leading to a growing field of successful applications in biology. Although force spectroscopy (consisting in recording force vs. distance curves) represents an excellent method for studying one key issue in biological processes, molecular recognition, until recently it was impossible to record recognition maps at the same resolution and imaging speed as the conventional maps of topography, lateral force, phase, etc. The introduction of simultaneous Topography and RECognition (TREC) imaging [1,2] provided a simple and fast Dynamic Force Microscopy mode capable of simultaneously recording images of topography and specific recognition between the molecules at scanning tip and sample. This is achieved by evaluating the maxima and minima of the cantilever oscillation separately in intermittent contact mode. In this poster, we aim to theoretically confirm the working principle of TREC and identify the experimental conditions needed in order to obtain optimal sensitivity.

To that end, the dynamic response of the cantilever-tip system in water was simulated as a damped, driven harmonic oscillator with additional terms describing the interaction with the sample. The non-specific tip-sample interactions considered were van-der-Waals forces and Derjaguin-Muller-Toporov contact repulsion [3]. Specific recognition forces (F_{rec}) between the ligand tethered to the tip and its epitopes on the sample surface was modelled in different ways. On one hand, interactions depending on the molecular linker were simulated. Therefor, a harmonic potential with

$$F_{\mathrm{rec}}(d) = -k_{\mathrm{l}}(d - L_{\mathrm{opt}})$$

centred at the linker's "optimum extension" L_{opt} (d: instantaneous tip-sample distance, k_{l}: linker's force constant) or the worm-like chain model

$$F_{\mathrm{rec}}(d) = -\frac{k_B T}{L_p}\left[\frac{1}{4\left(1 - d/L_0\right)^2} - \frac{1}{4} + \frac{d}{L_0}\right]$$

(k_B: Boltzmann's constant, T: temperature, L_0: linker's contour length, L_p: linker's persistence length) were used. In both cases, the oscillation amplitude was chosen to be slightly smaller than the tether length [1]. On the other hand, the effect of additional van-der-Waals attraction (intruduced by means of a locally higher Hamaker constant without considering any ligand) was studied. The resulting equations of motion were integrated using a fifth-order Runge-Kutta algorithm.

Our results show that when $Q \approx 1$, indeed variations in sample topography and the presence of binding sites affect different parts of the cantilever's oscillation. The recognition events modelled by a harmonic potential or a worm-like chain lead to qualitatively equal results and give very similar numerical results when reasonable values for a typical PEG linker are chosen for L_{opt}, k_{l}, L_0, and L_p. Whereas recognition sites of all three types cause the upper half of the deflection period to diminish (oscillation maxima decrease), topographic changes

mainly influence the lower half of the cantilever oscillation (deflection minima adapt to topography).

In order to efficiently distinguish contributions from topography and recognition events (and to keep the scanning tip at a constant distance from the sample), a feedback loop based on the deflection minima has to be employed. Only then, the signal provided by the deflection maxima contains the true recognition information and the vertical piezo movement compensating the minima signal accurately represents topography. An example of this situation is given in fig. 1, where the scanning of a sample with mixed topographic and recognition features (modelled as harmonic potential) is simulated. As can be seen, the recognition signal (built from the deflection maxima) is not affected by the substrate topography and vice versa. This insight is not readily available in the experiment since the presence of molecular recognition sites on a sample is always linked to the topographic detection of the respective epitopes.

In view of these findings, from a theoretical point of view we show that Dynamic Force Microscopy employing a low quality factor ($Q \approx 1$) and an oscillation amplitude adequate for the chosen cross-linker can be used in TREC mode to simultaneously map topographic and recognition information. Simulations point out that a feedback loop based on the deflection minima signal succeeds in the optimal separation of both signals. Our results indicate that molecular recognition in TREC can be usefully modelled by both a harmonic potential or a worm-like chain model, and that even other attractive interactions like locally enhanced van-der-Waals forces could be detected by evaluating the cantilever's deflection maxima.

References:

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Figures:

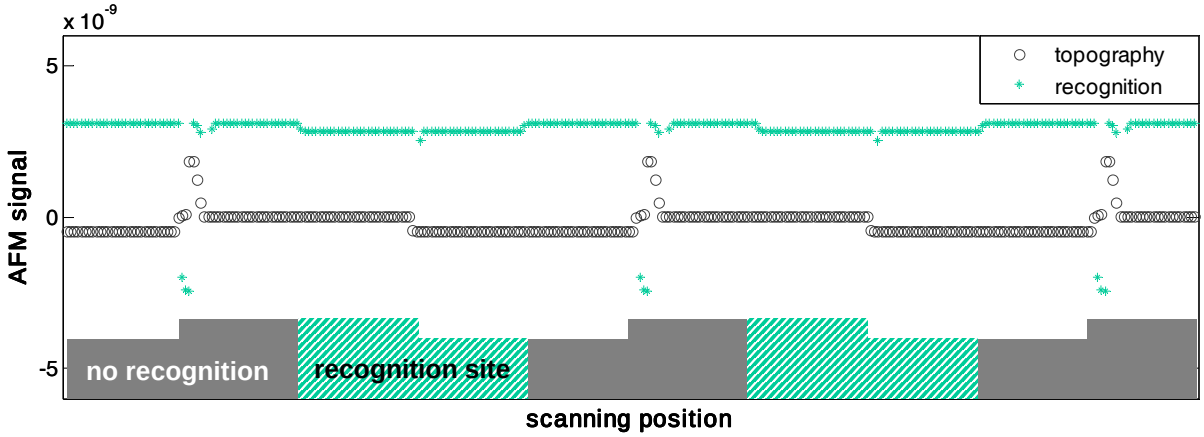

Figure 1. Simulated scan line of an area containing both topographic and recognition features. The sketch at the bottom represents the sample scanned (striped portions are recognition sites). It can be seen that the topography signal represents an accurate measure of the sample's height, whereas the recognition signal identifies the epitopes on the sample.