

## Etude dynamique et structurale de biomolécules par microscopie HS-AFM

### Partners

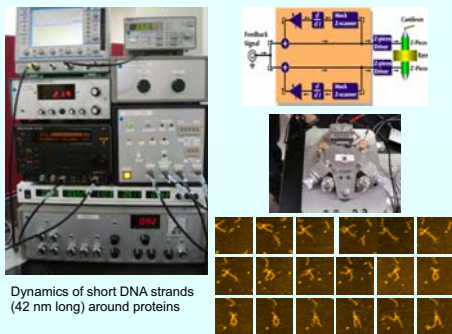
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The objective of the HS-NANO-BIO-IMAGING project is the implementation of high-speed AFM (HS-AFM) developed by the Kanazawa's university in France and to promote the technology by the production of biologically important results worldwide. We expect to be able, using the HS-AFM, to contribute novel knowledge about dynamic processes and structure-function relationships of individual molecules. Although all consortium members are experienced bio-AFMists, we expect to face various technical obstacles.

In this context, one clearly sees the growing expectation of:

- Characterization of supramolecular organization in model or native membranes;
- Advance in active devices for biosensing;
- Improvement of high-speed AFM in term of dynamics and resolution.

### HighSpeed AFM prototype



In its actual configuration the HS-AFM, developed by T. Ando in Kanazawa University, is a very powerful equipment which however presents the disadvantage of being difficult to drive.

The work program has been divided into three phases:

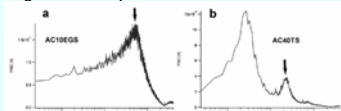
- Structural and dynamic characterization of the supramolecular assemblies extended to cell membrane dynamics,
- Development of specific substrate and more particularly characterization of substrate-supramolecular coupling for biosensor development.
- Implementation of the high-speed AFM prototype in term of scan area size, high speed and high resolution imaging, and minimization of tip-sample interaction force.

In collaboration with the consortium groups of E. Lesniewska in Dijon, and S. Scheuring, in Paris, and C. Le Grimmellec in Montpellier, P. Hinterdorfer in Linz, and Veeco Instruments Co. (Santa Barbara, CA), the Toshio Ando's group is organizing the first Single Molecule Biology Symposium in Japan:

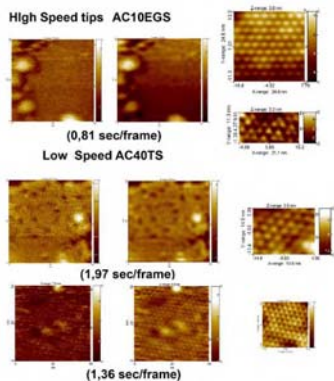
Next event  
**Symposium WBMA'09**  
Watching Biomolecules in Action  
Osaka, Japan  
December 15-17, 2009  
<http://www.s.kanazawa-u.ac.jp/phys/biophys/WBMA09/index.htm>

Development of the High-speed Atomic Force microscope (HS-AFM) working in liquid and equipped of an efficient feedback giving access to high resolution imaging of soft biological samples is expected to have a decisive impact on the understanding of structure-function relationships in supramolecular assemblies and complex systems (review in ref.). A second problem is the cost of commercial high-speed cantilevers (~400 € per unit), which become prohibitive considering that, like in common AFMs, several tips are generally necessary for one experiment. The Montpellier HS-AFM group (P. Dosset, J. Kokavecz, C. Le Grimmellec, P-E. Milhiet, J. Soullier) is actually progressing on HS-AFM image treatment for films, electronic components, cantilevers and the study of test (bacteriorhodopsin) and complex (Influenza A virus) systems. Two examples of progress made are illustrated below:

1) Use of low speed cantilevers in HS-AFM: We performed a systematic study on the use of soft, low speed, cantilevers for intermediate speed imaging. We found that Olympus BLAC40TS can be used to image soft samples down to 2 images per sec, choosing the second harmonic peak for feedback (Thermal spectrum, Fig. b) compare to Olympus -AC10EGS HS cantilevers (Thermal spectrum, Fig. a) which is essential for a larger spreading of the technique.

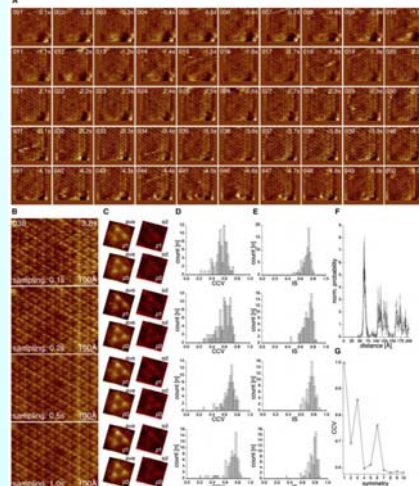


As shown below, the use of AC40TS allows to get good resolution images at ~ 1 image per sec. This renders preliminary studies on the HS-AFM imaging of various samples affordable.



Imaging of H1N1 Influenza virus (protocol under development): so far HS-AFM was applied to the imaging of structures with limited (<10 nm) differences between the minimal and maximal height. We have chosen to image unfixed Influenza virus in liquid to check the efficiency of the feedback for corrugated soft biological structures. As shown below, architecture of the virus envelope is accessible to HS AFM using HS-cantilevers and commercial cantilevers. The acquisition time for an image is < 200 times that of conventional AFMs.

High-speed atomic force microscopy becoming a reference tool for the study of dynamic biological processes. The spatial and time resolutions of HS-AFM are on the order of nanometers and milliseconds, respectively, and allow structural and functional characterization of biological processes at the single-molecule level. In this work we present contact-mode HS-AFM movies of purple membranes containing two-dimensional arrays of bacteriorhodopsin (bR). In high-resolution movies acquired at a 100 ms frame acquisition time, the substructure on individual bR trimers was visualized. In regions in between different bR arrays, dynamic topographies were observed and interpreted as motion of the bR trimers. Similarly, motion of bR monomers in the vicinity of lattice defects in the purple membrane was observed. Our findings indicate that the bR arrays are in a mobile association-dissociation equilibrium. HS-AFM on membranes provides novel perspectives for analyzing the membrane diffusion processes of non labeled molecules.



High-resolution contact-mode HS-AFM of the extracellular surface of individual bacteriorhodopsin (bR) molecules. (A) Fifty aligned images (660 Å x 660 Å, 200 pixel x 200 pixel) recorded at 100 ms time intervals, from 5 s of a longer HS-AFM movie. The image quality is consistent and depicts the trimeric assembly of individual bR molecules in the purple membrane (PM). (B) From top to bottom: one individual frame (frame sampling time: 100 ms; pixel sampling time: 2.5 ms), and merge of two, five, and 10 subsequent frames with 200 ms, 500 ms, and 1 s acquisition sampling time. (C) Averages and SD maps (n = 46) corresponding to the images shown in B; the averages have a full color range of 0.5 Å < height < 6.7 Å, and the SD maps have a full color scale of 0.5 Å < SD < 2.3 Å, 0.5 Å < SD < 2.1 Å, 0.4 Å < SD < 1.8 Å, and 0.4 Å < SD < 1.6 Å, respectively. (D) Histogram showing the number of individual molecules found in the images shown in B as function of their CCV with a noise-free ensemble average. (E) Histogram showing the number of individual molecules found in the images shown in B as function of their IS. (F) Average (black line) and SD (gray lines) of the PCF between bR trimers, peaking at 63 Å (FWHM: 5 Å) of five individual image frames, showing the high precision and stability of the scanner. (G) Symmetry analysis of the average calculated from one image frame (B and C, top) peaking for threefold symmetry at 0.85.

### Publications

Ignacio Casuso, Noriyuki Kodera, Christian Le Grimmellec, Toshio Ando & Simon Scheuring. Contact-mode high-resolution high-speed atomic force microscopy movies of the purple membrane. *Biophys J.* 97 (5): 1354-1361, 2009.

Marie-Cécile Giocondi; Daisuke Yamamoto, Eric Lesniewska, Pierre-Emmanuel Milhiet, Toshio Ando, Christian Le Grimmellec. Surface topography of membrane domains. *Biochimica et Biophysica Acta Biomembranes*, In Press, 2009.

### Communications

P.E. Milhiet. Real time imaging of the growth of amyloid-like fibrils of lithostathine using high speed AFM. P.E. Milhiet. XI International Scanning Probe Microscopy Conference 2009, Madrid June 17-19, 2009

S. Scheuring. High-resolution AFM of membrane proteins in native membranes. XI International Scanning Probe Microscopy Conference 2009, Madrid June 17-19, 2009

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