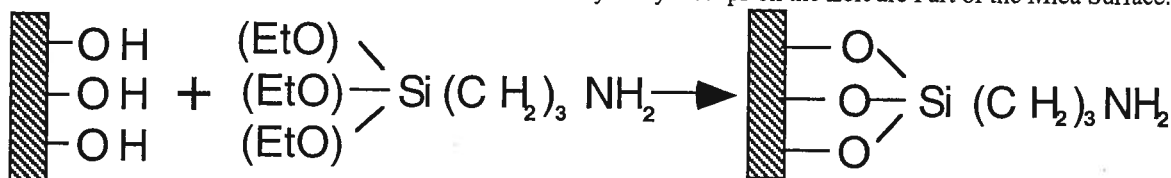


331.7 Silanized Mica (AP-mica): DNA, RNA and Nucleoprotein Complexes Immobilized on AP-mica

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We describe here a sample preparation procedure for AFM with the use of functionalized mica substrates. This technique allows routine visualization of DNA, RNA and nucleoprotein complexes with AFM [1-5]. The method is based on covalent bonding of 3-aminopropyltriethoxy silane (APTES) molecules to mica (i.e., creating positive charges at the mica surface) [1,2]. The reaction is shown schematically in Figure 7a.

Figure 331.7a Mica Modification with APTES. The Hydroxy Groups on the Left are Part of the Mica Surface.



The amino groups of APTES are covalently bound to the freshly cleaved mica surface, giving it properties similar to anion exchange resins used in affinity chromatography. The amino groups, after exposure to a water solution, become positively charged in a rather broad range of pH; aliphatic amino-groups have a pK of around 10.5. Therefore, DNA, which is a negatively charged polymer, should adhere strongly to this surface. We checked the DNA binding to AP-mica directly by use of radiolabeled DNA [1]. AFM images of mica substrates modified with APTES in toluene solutions showed that the surfaces are very rough if concentration of APTES is 1 μ M or more. The surfaces are smooth if APTES concentration in solution is 100nM [1]. The concentration of APTES in solution needs to be so low that mica modification can be performed in APTES vapors at ambient conditions.

331.7.1 Materials

- Commercially available 3-aminopropyltriethoxy silane (e.g. Fluka and Chemika-BioChemika in Switzerland, Aldrich in the USA)

Note: Ask for a freshly prepared chemical, redistill it and store the chemical under argon to prevent polymerization.

- Mica substrate: any type of commercially available mica sheets (e.g., green or ruby mica).
- Water: double glass distilled or de-ionized water filtered through a 0.5 μ m filter.

331.7.2 Substrate Preparation

Place two plastic caps—cut them from regular eppendorf tubes—on the bottom of a 2l desiccator and evacuate it with a regular vacuum pump (to -28 inches Hg is sufficient) and fill with Ar. Cleave mica sheets to make them as thin as 0.05-0.1mm. Put 30 μ l of APTES into one plastic cap and 10 μ l of N,N-diisopropylethylamine (Aldrich) into another cap. Mount mica strips at the top of the desiccator and leave the reaction to proceed for 1-2 hours. After that, remove the cap with APTES and purge argon for 2 minutes. Leave the sheets to cure for 1-2 days; then AP-mica substrates are ready for the sample deposition.

Note: A dry argon atmosphere is crucial for obtaining substrates for AFM studies and for substrate storage. Allow the gas to flow while you open the desiccator. Under these conditions the AP-mica substrates retain their activity for at least a month.

331.7.3 Sample Preparation

1. **The Droplet Procedure.** Place 50 μ l of DNA (RNA, protein-DNA complex) solution (0.1 μ g/ml) in the middle of an AP-mica substrate (usually 1x1cm², or the size of the sample puck) for 5 minutes. Then rinse the surface thoroughly to remove all buffer components and dry (i.e., blow with dry gas or dry in vacuum). The sample is ready for imaging.
2. **The Immersion Procedure.** Immerse pieces of AP-mica into 0.1 μ g/ml DNA solutions (RNA, nucleoprotein complexes) and allow the sample to adsorb for 30-60 minutes. Take the strips out of the solution, rinse with water and dry (see above). The sample is ready for imaging.

Note: This procedure is convenient if deposition needs to be done in strict temperature-controlled conditions. In this case, incubate the solution at a pre-set temperature for 10-15 minutes.

Note: DNA concentration may be increased if molecules as small as several hundred base pairs (bp) are deposited. Otherwise, we recommend to decrease the DNA concentration if the length is more than 20kbp. For example, concentration of lambda DNA (~48kb) should be about 0.01 μ g/ml [1,4].

331.7.4 Imaging Conditions

The prepared samples can be imaged in air (dry atmosphere is recommended), in propanol or under water (cf. [1,3,4]). There is no special requirement for imaging procedures.

331.7.5 General Comments

A large range of concentrations can be used with AP-mica [1,2,5-9]. Deposition can also be performed in a wide range of temperatures (0 - 60°C, [2,5]) and pH. These are unique features in comparison with other sample preparation methods for AFM [5-9] and are very important for sample depositions, which are stable at specific environmental conditions. AP-mica is very stable; this allows, for example, preparation of the substrate well in advance of deposition. Also, once deposited onto AP-mica the samples are stable for several months if they are stored at ambient conditions.

331.7.6 References

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331.7.7 Comments Regarding Yuri Lyubchenko's Recipe

It is very important to use fresh APTES. With older bottles, samples fail to stick to it and the surface is unusually rough. When not in use, store the APTES bottle in a desiccator in the refrigerator. Flood the bottle with dry argon before replacing the cap. Also, be sure that you use dry argon when preparing the AP-mica and also when storing it. Use chromatographic grade argon and pass it through a molecular sieve drying column. Use a real vacuum pump. Lab vacuum ports are typically too weak (~15 inches Hg rather than the needed ~28 inches Hg). A small 4 inch glass desiccator (Sigma #D1920) can be used instead of a 2l desiccator. Premount the mica on a metal sample puck. Immediately before processing, cleave the mica and place it on a magnet mounted to the top of the desiccator.

It is also possible to lay the mica face down right on top of an eppendorf cap containing the APTES rather than mounting it up toward the top of the desiccator. In this case the processing time can be reduced to about 15 minutes. Using the N,N-diisopropylethylamine is also optional. According to Yuri Lyubchenko it renders the atmosphere as neutral as possible since the APTES is hydrolyzed at acidic pH, but it seems not to be essential. You may also try processing the mica for longer than the suggested 1-2 hours. This may make it more rough, however. Optimize the time for your own system since such factors as desiccator size and vacuum strength affect the product.

331.8 Sources of Mica

Spruce Pine Mica
Telephone: 828-765-4241
Fax 828-765-7192

Ted Pella, Inc.
P.O. Box 492477, Redding, CA 96049-2477
Telephone: 530-243-2200; 800-237-3526

We recommend that you do not cut mica with scissors but punch it out into discs using a punch set (e.g., available through McMaster-Carr). This prevents the deterioration of the crystal. We use 5 minute epoxy in our lab to glue punched mica discs to steel sample pucks.

331.9 Poly-L-Lysine Coating

To prepare poly-L-lysine adhesive for binding cells to a petri dish:

1. Use a 0.01% Poly-L-Lysine solution (e.g., Sigma #P-4832) in water. Polylysine has a molecular weight range of 150k-300k and should be stored in a refrigerator.
2. Incubate 50 μ L of the solution, or enough to cover the desired area, on a clean 12mm round coverglass.
3. Incubate for 15 minutes and then rinse with water or PBS.

Note: It is also possible to use lower weight poly-l-lysine (~30k) and incubate for 30 minutes.