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MUNI

My experience with Atomic Force Microscopy

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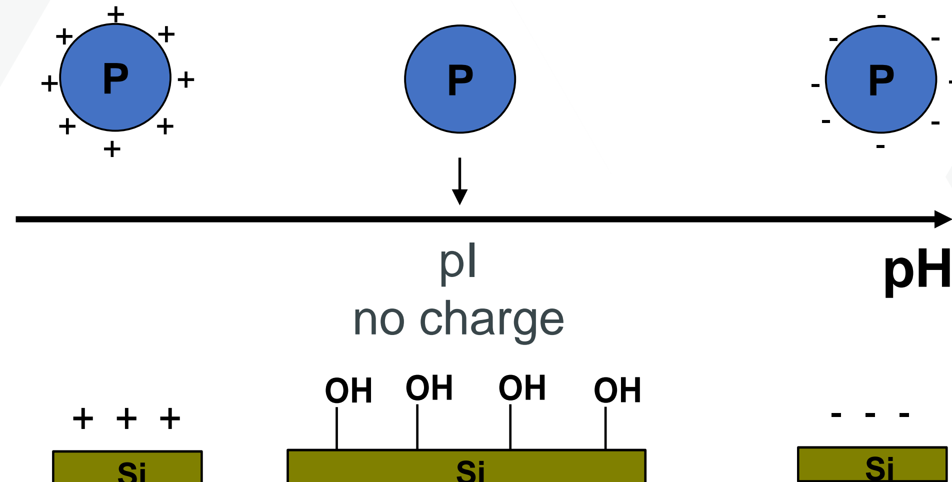
Fixation of proteins on mica

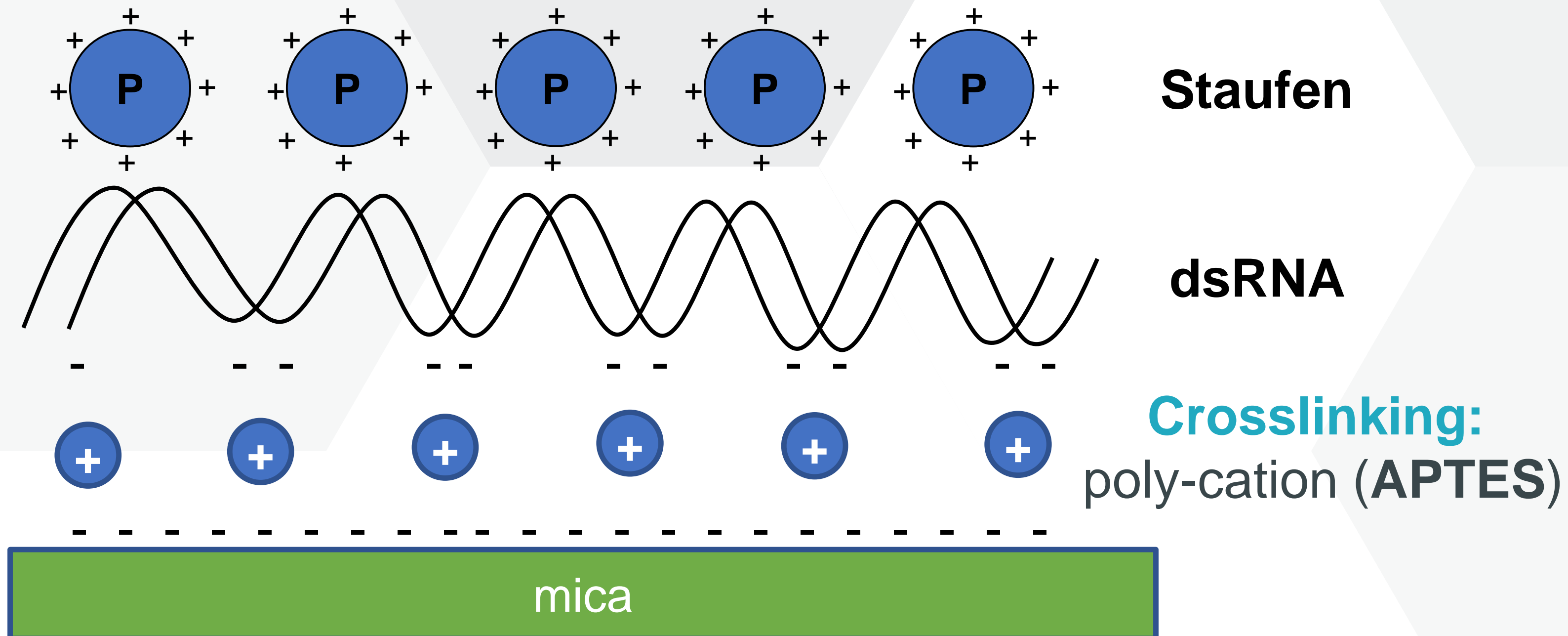
- **Protein**: charge is given by its isoelectric point (pI, pKa) + pH
- Immobilization on mica: **pKa (mica) < pH < pI** (**mica - pKa = 3.0**)
- Our proteins have pIs ranging from 8.8 till 9.5.
- This means that at pH 8.0, our proteins are positively charged and MICA is negatively charged



How do I immobilize proteins on mica surface?

- Heating 1 nM proteins at 94 °C for 3 minutes
- Cooling in ice
- Loading the samples on mica coated grids
- Washing the grids with buffer in order to remove unproperly fixed particles

RNA-protein complexes on APTES grid



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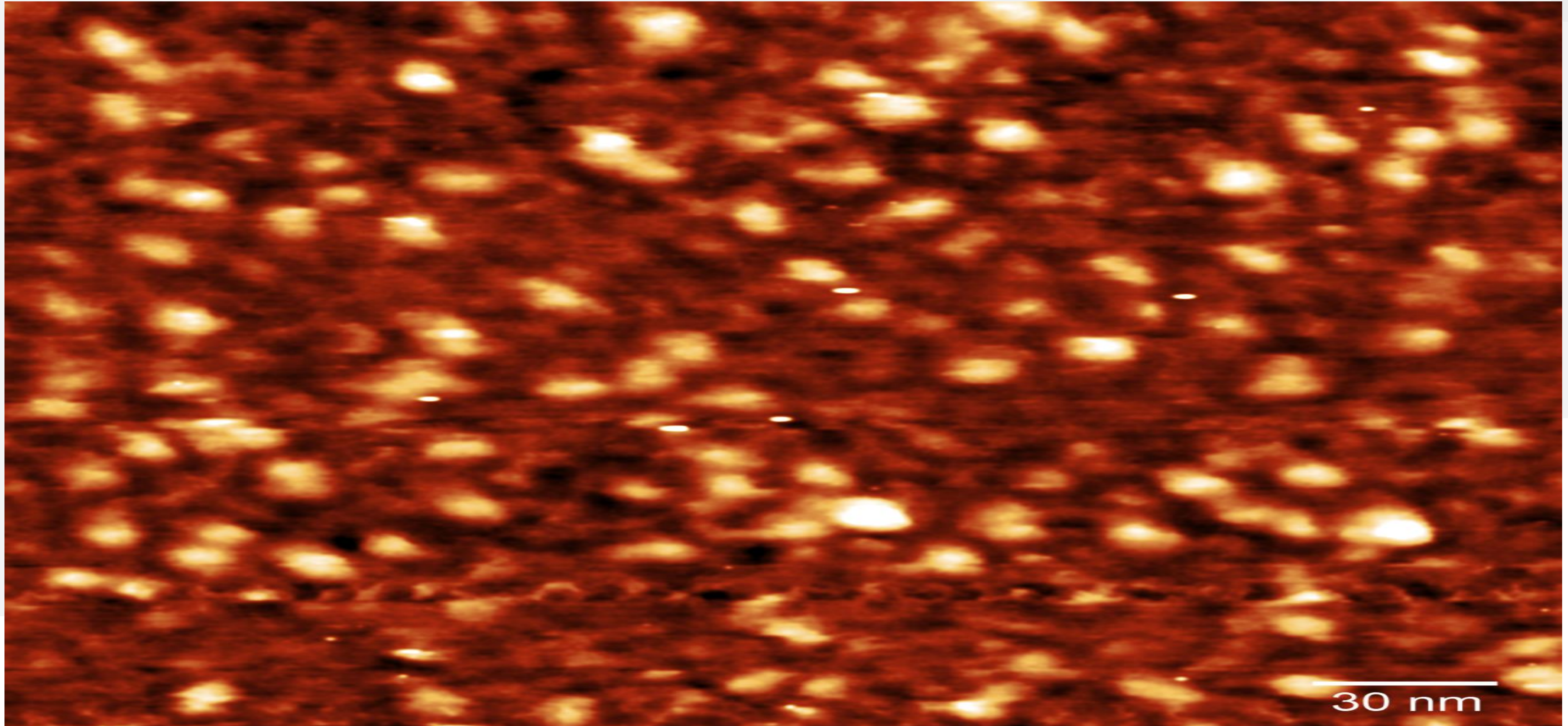
How do we immobilize proteins on aptes surface?

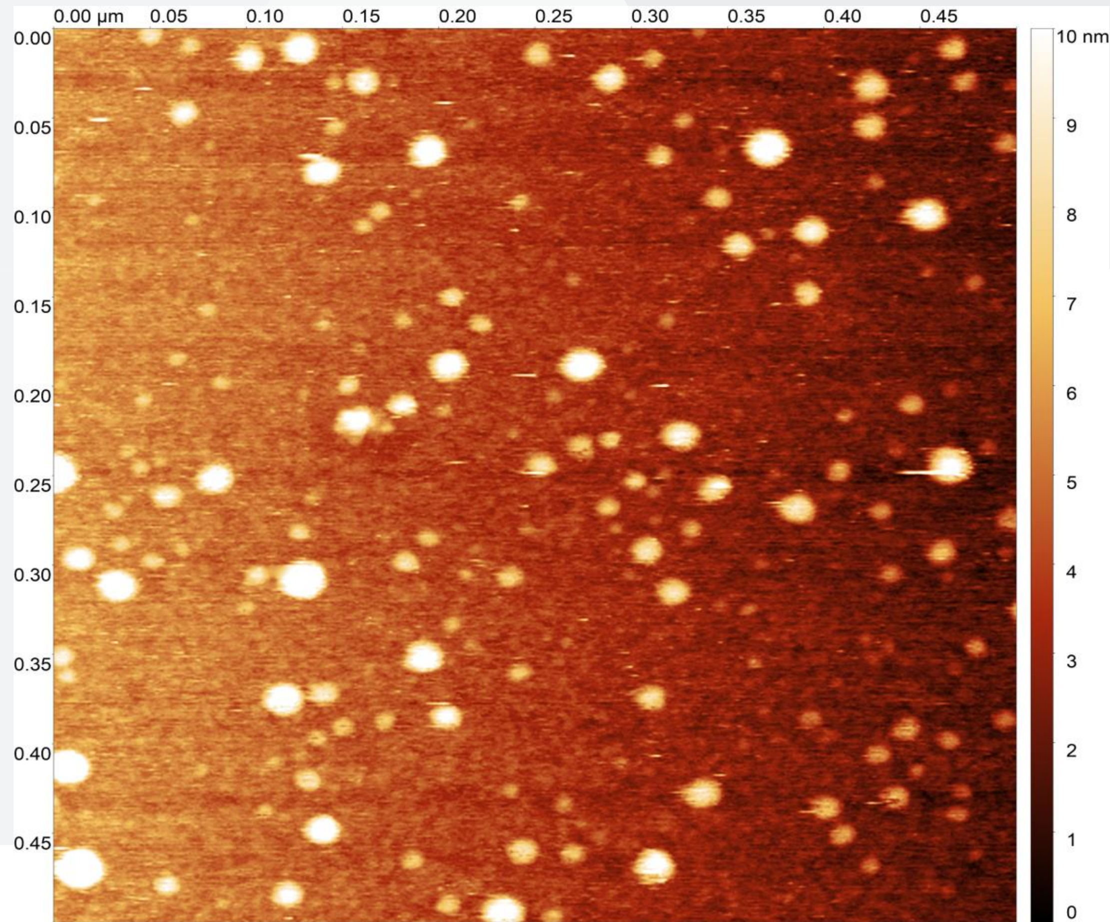
- Heating 1 nM proteins at 94 °C and 2 nM RNAs at 72 °C for 3 minutes
- Cooling in ice
- Loading RNA samples on APTES coated grids for 2 minutes
- Washing the grid with the buffer
- Loading protein samples on the grid
- Washing the grid with the buffer
- Observing the samples at AFM

What I saw at the beginning…

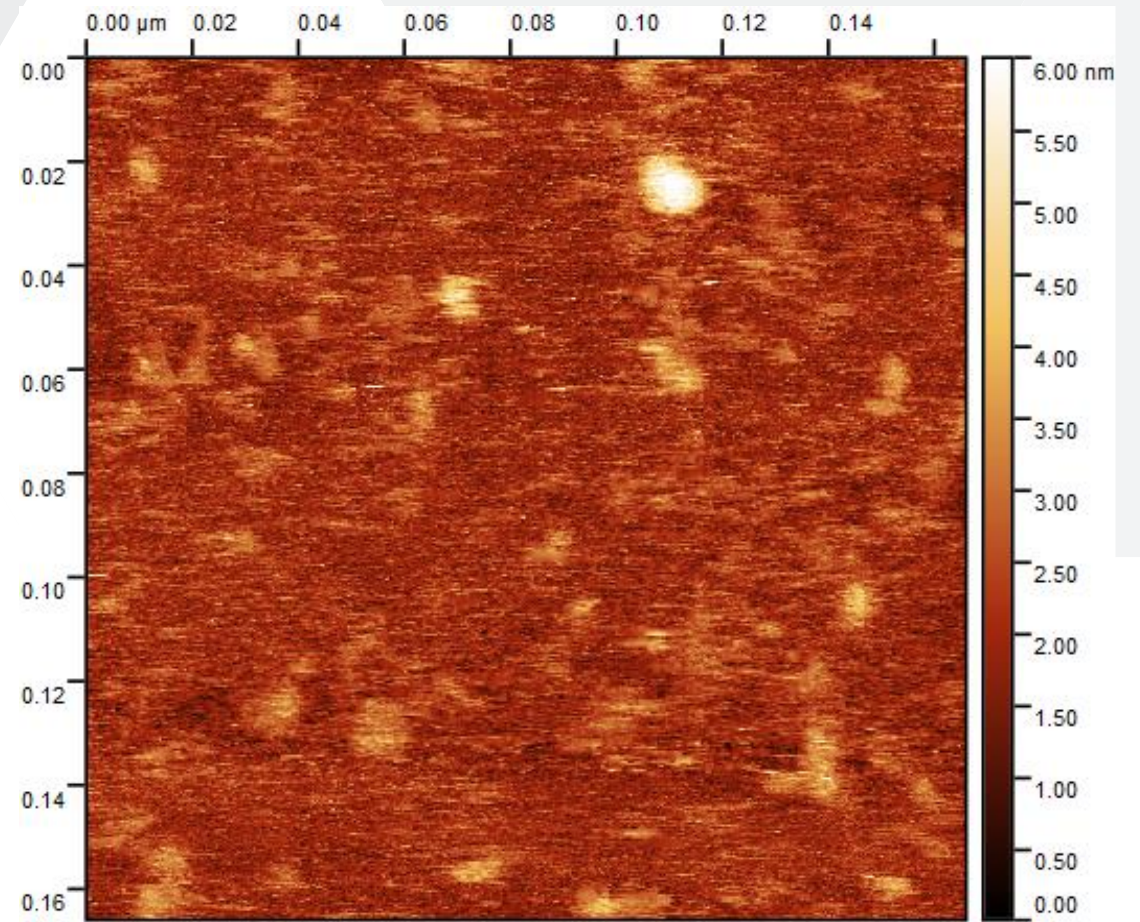


RBD3-End 80 µM

How did I improve?



1 nM Staufen1 without pre-heating and observed at 22 °C



Preheated 1 nM Staufen1 and observed at 37 °C

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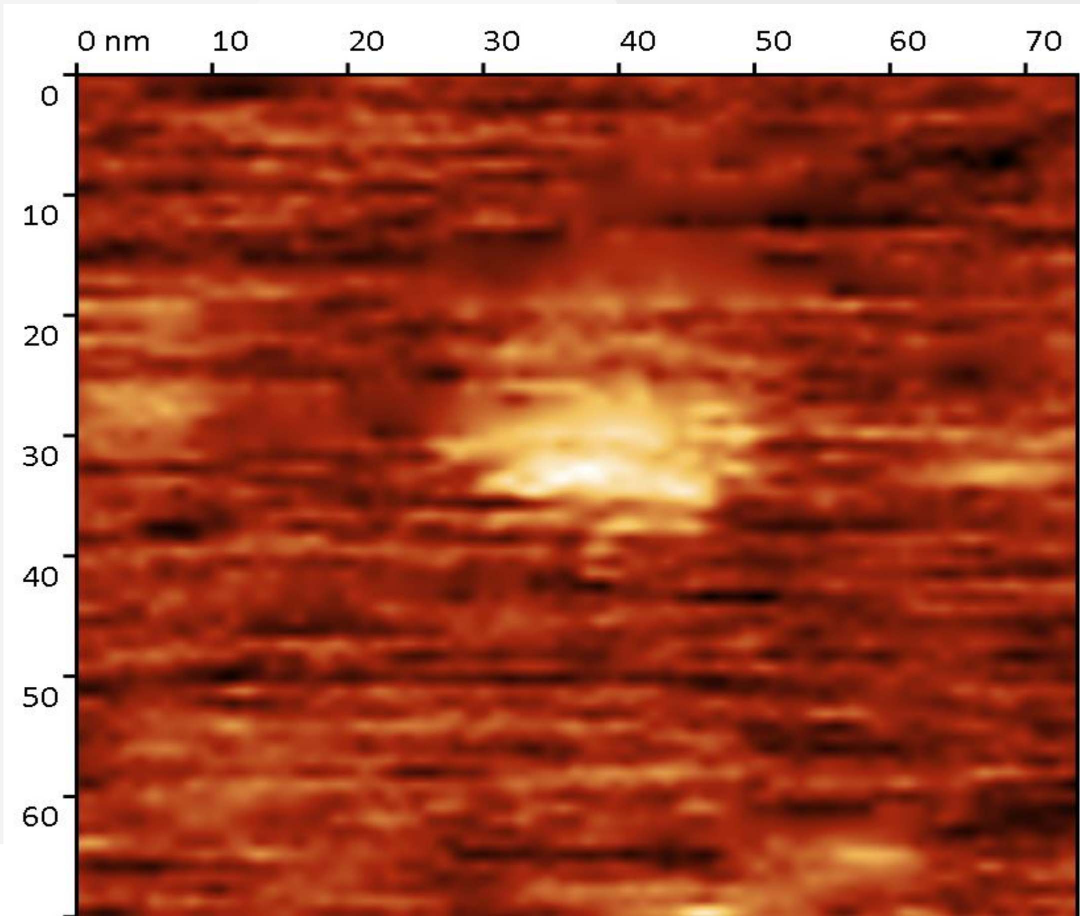
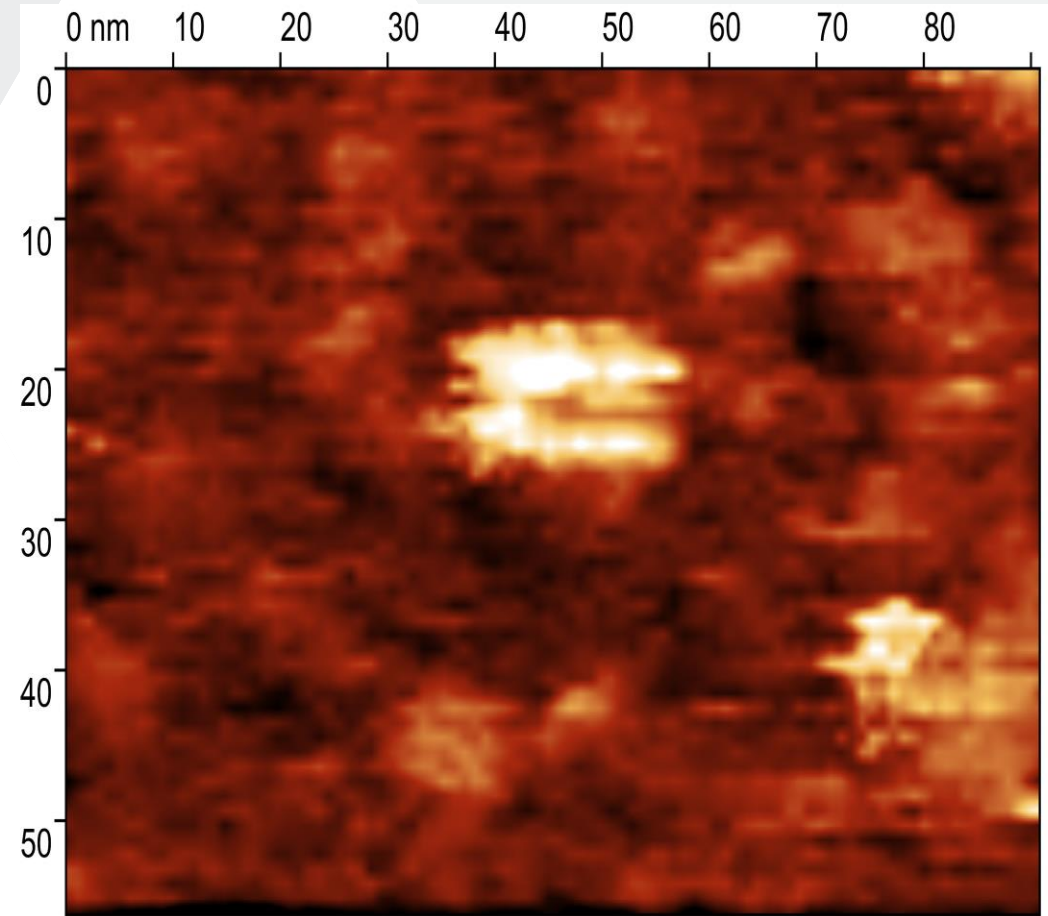
Examples of pictures we made by AFM

Peak force amplitude	6 nm
Peak force frequency	2 kHz
Peak force	30 pN
Lift heigth	12 nm
Tip radius	5 nm
Spring constant	0.084 N/m

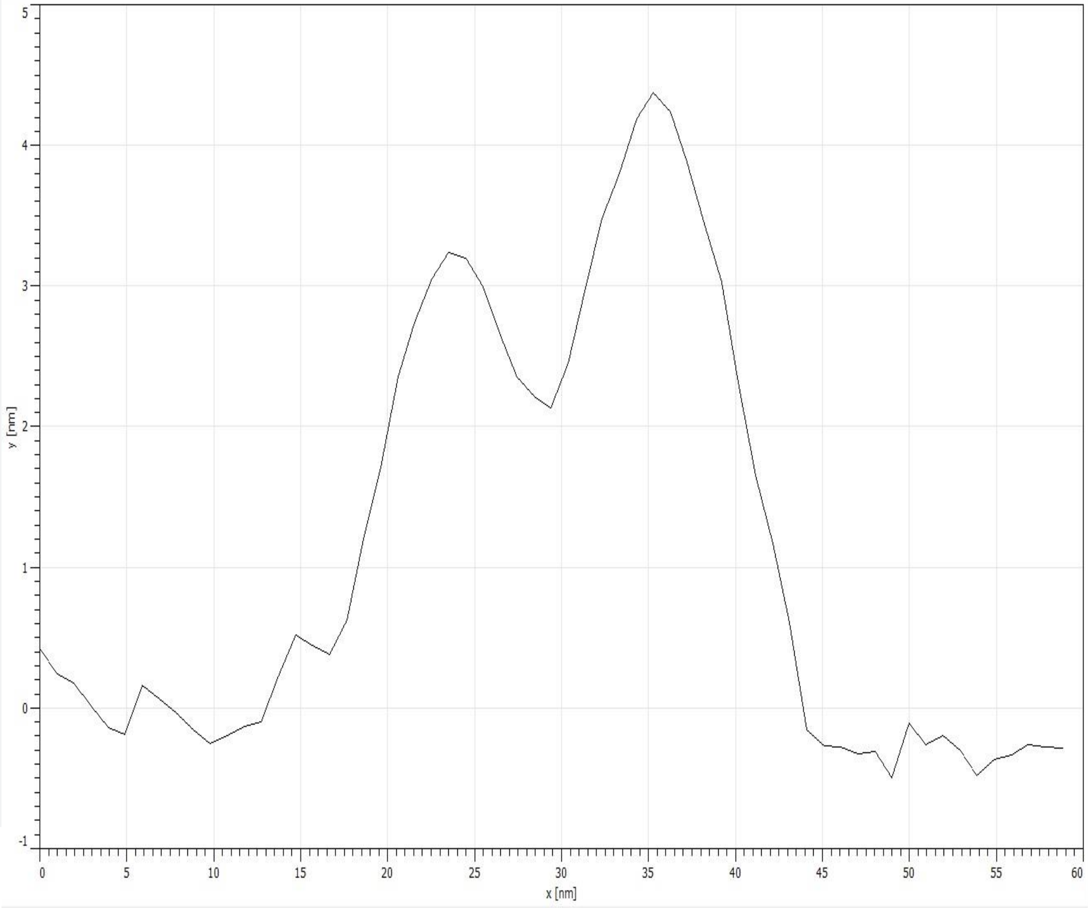


RBD3-End dimer

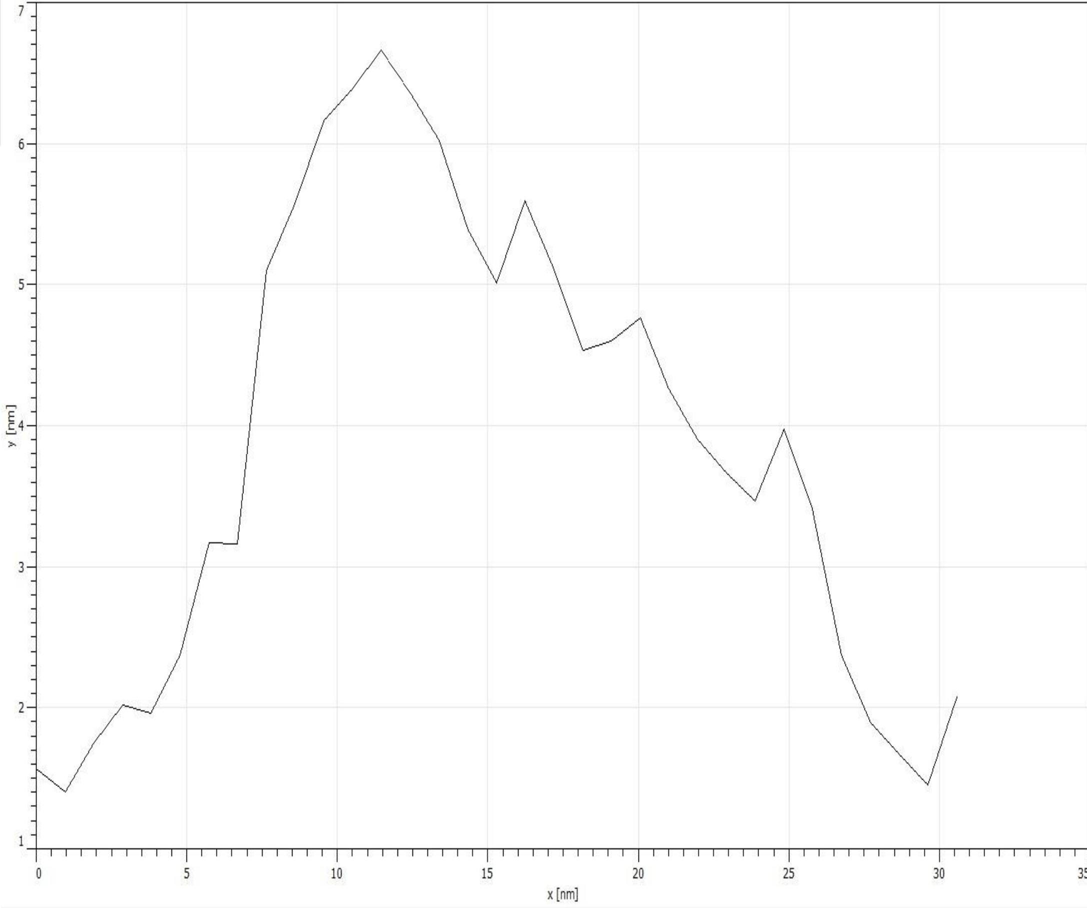


RBD3-end/19bp RNA complex. Stoichiometry 2:1

Section surface profile: a useful way to get data

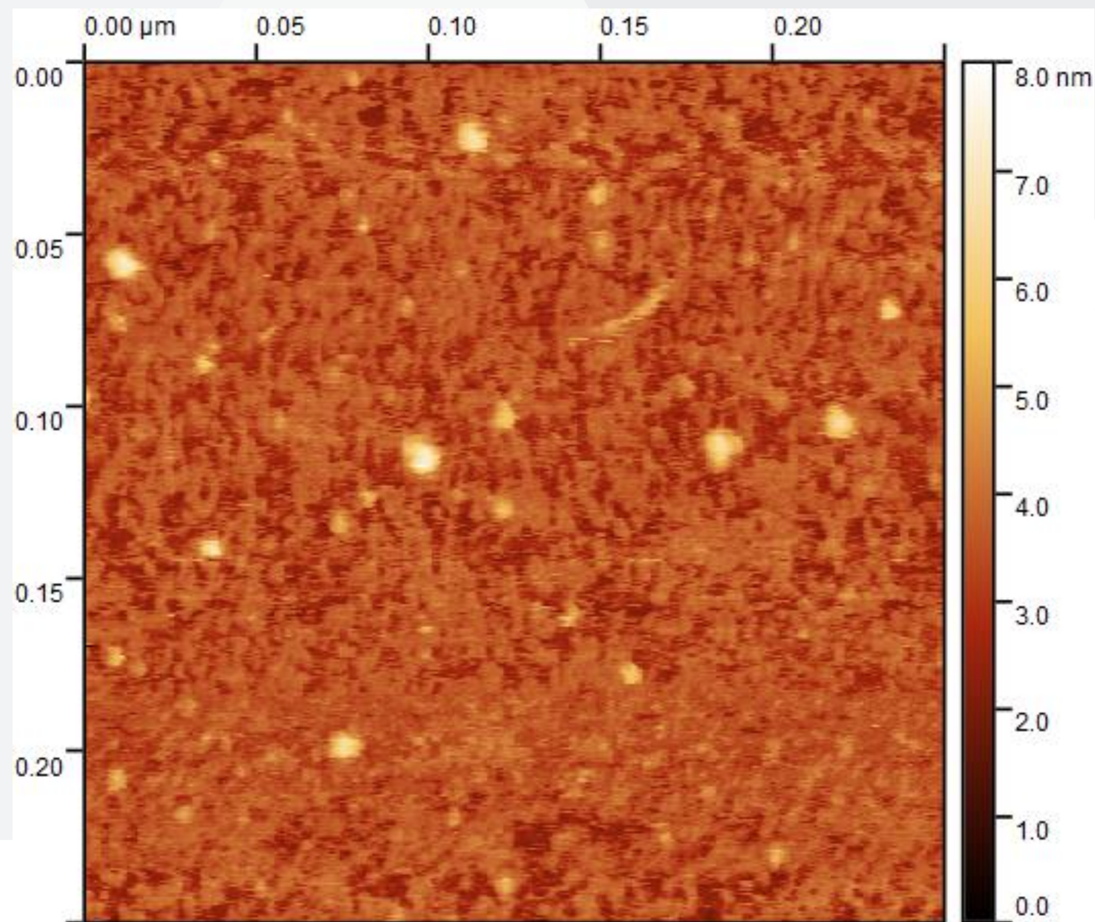


RBD3-End

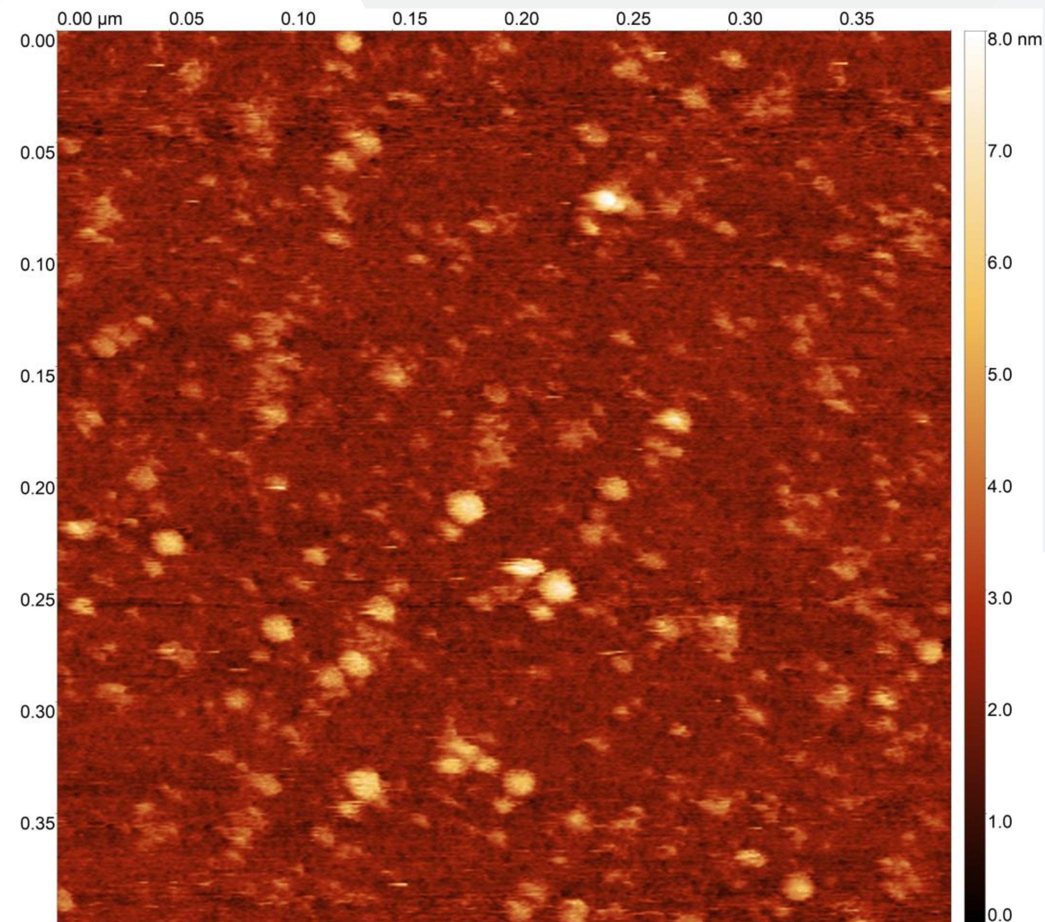


RBD3-EndΔSSM

RNA-small molecule interaction



RNA control



RNA treated with small molecule

What will we do?

- Continue examining our samples by AFM
- Find new settings of parameters and probes for better resolution
- Create model of our biomolecules
- Improve our data by processing multiple images
- Interacting with AFM experts

In conclusion

- AFM represents a fast and reliable method to analyze the samples, but it requires an optimization work that depends on many factors
- It is a technique that observe biomolecules in quasi-natural conditions
- Along with other techniques (EFM, SAXS, X-Ray crystallography, Sec-Mals, AUC, MS etc.), AFM is a valid tool to study biomolecules

Acknowledgements

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Thank You