Quality criteria for biomolecule imaging

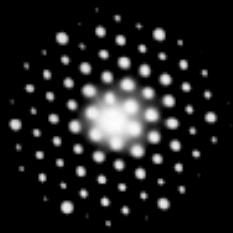
Ignacio Casuso (Simon Scheuring's team)

AFM is not alone in biomolecule studies. Standardization crosstalk

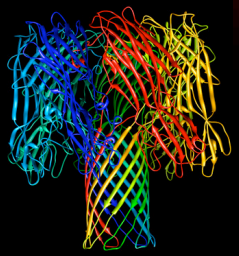
Crystallography

Purification
Crystallization
Freezing

X-Ray Diffraction



3D Structure Reconstitution

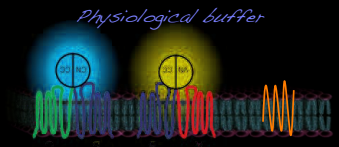


FACTS

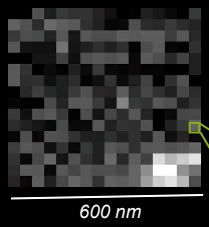
+ Angstrom Resolution
- - - - - - -
- Far from native enviroment
- Frozen

Fluorescence Microscopy

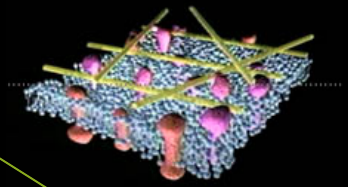
Molecule Labeling



Motion Tracking



Models & Cartoons

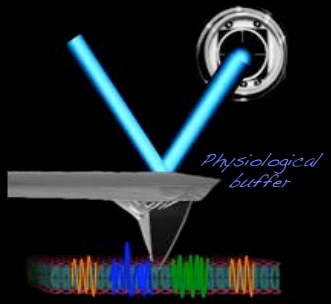


FACTS

+ High specificity of labels
+ Miillisecond time resolution
- - - - - - -
- Labeling distorts molecules
- Molecular environment not seen

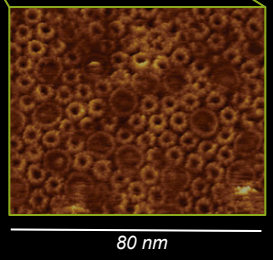
Atomic Force Microscopy

Tip-Sample Contact



Topographic Profile

Topographic Map of Molecular Environment

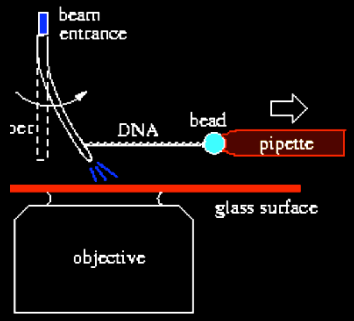


FACTS

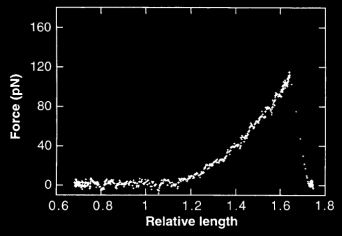
+ Surrounding observed
+ No labeling
- - - - - - -
- Slow (minutes/frame)
- Low molecular recognition

AFM is not alone in biomolecule studies. Standardization crosstalk

Micropipette

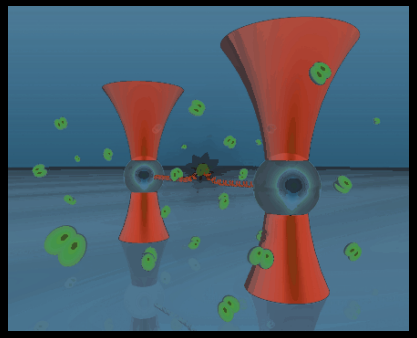


DNA elasticity

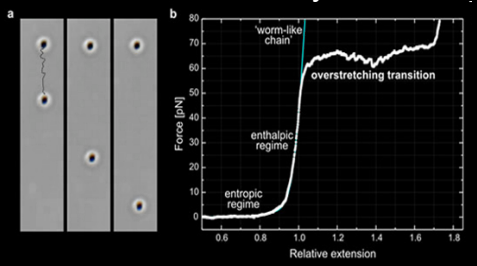


Force sensitivity ~ 1 pN

Optical Tweezers



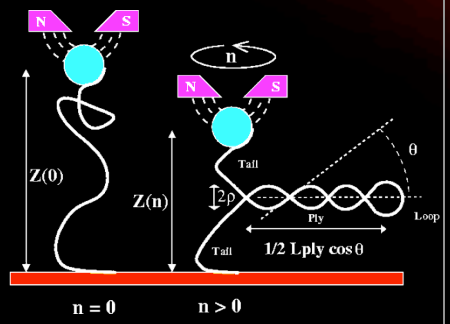
DNA elasticity



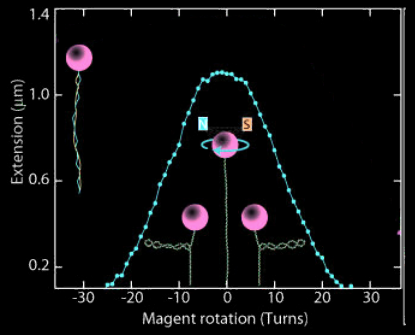
Force sensitivity ~ 0.1 pN

(AFM ~ 10 pN)

Magnetic Tweezers



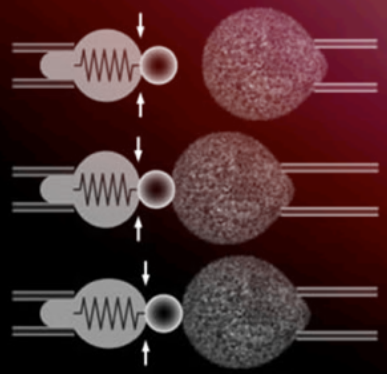
Controlled number of torsion turns on DNA



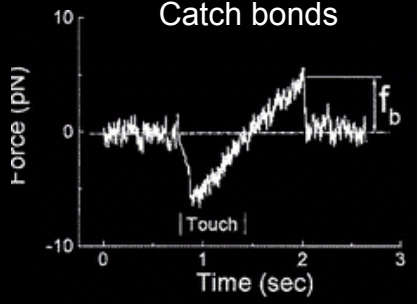
Extension Force sensitivity ~ 0.1 pN

(AFM no turns)

Biomembrane Force Probe



Catch bonds

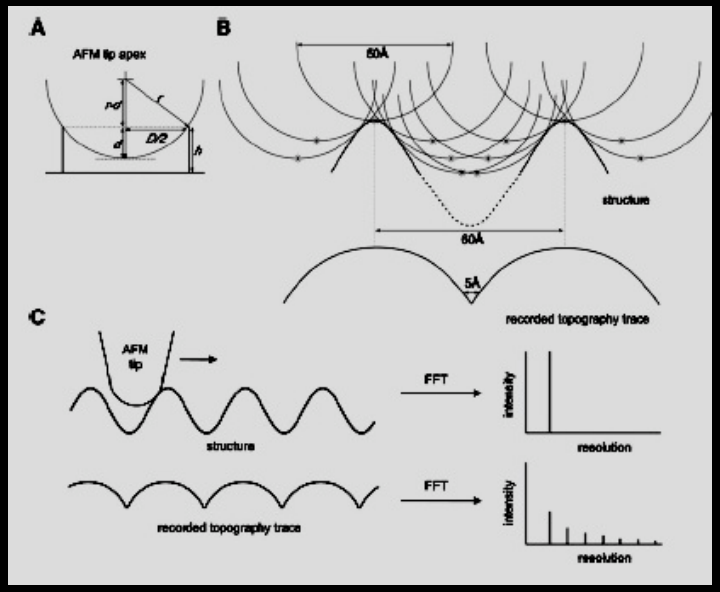


Tunable wide range of force sensitivity 1000-0.001 pN

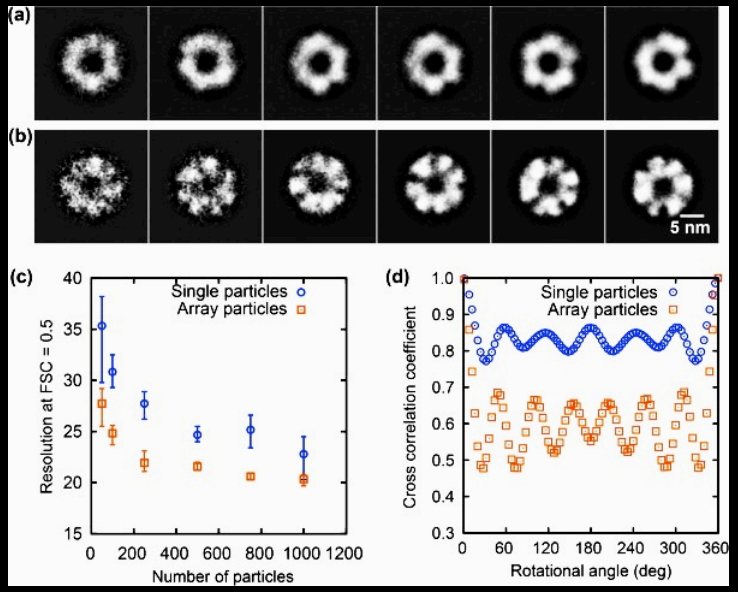
The resolution in AFM and Electron Microscopy

bR best AFM topo, Res reported 0.5nm - three external membrane loops reported

bR EM projection maps Res reported 0.7 nm – seven external membrane features



Tip artefacts
FTT -> Not real resolution

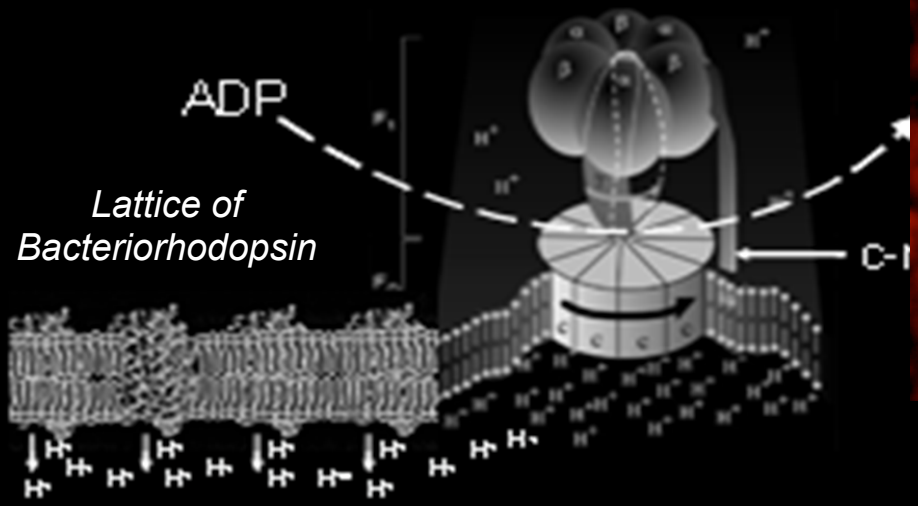


White noise on top of signal
FTT -> Real resolution

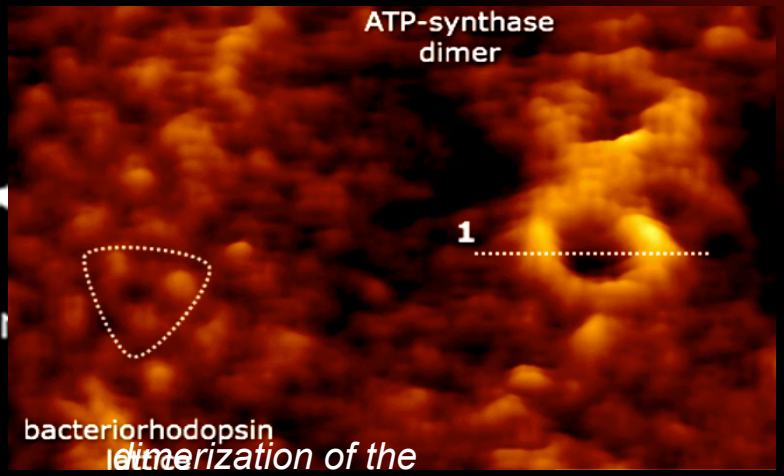
The ATP-synthase C-ring // the overall image must be shown

Which is the location of the c-rings in the membrane ?

Expected to be close to the H^+ producing bR lattice



H^+ production and gradient



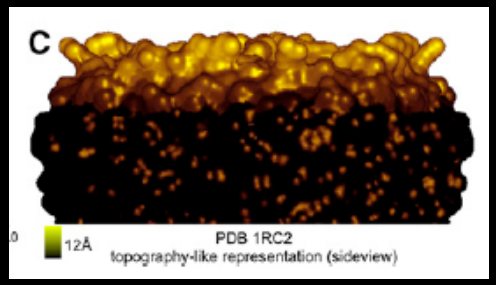
dimerization of the c-ring is observed

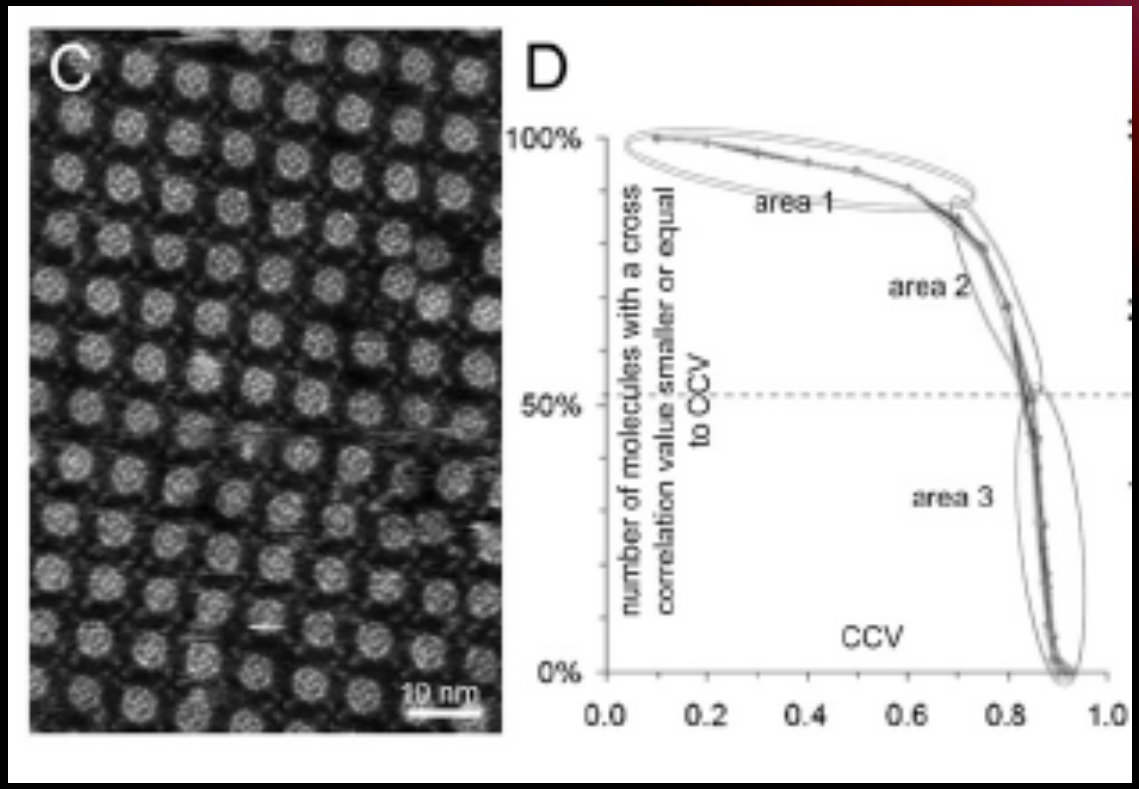
It is not enough with a highly zoomed and post treated image

Molecule must be identified in its envoriment

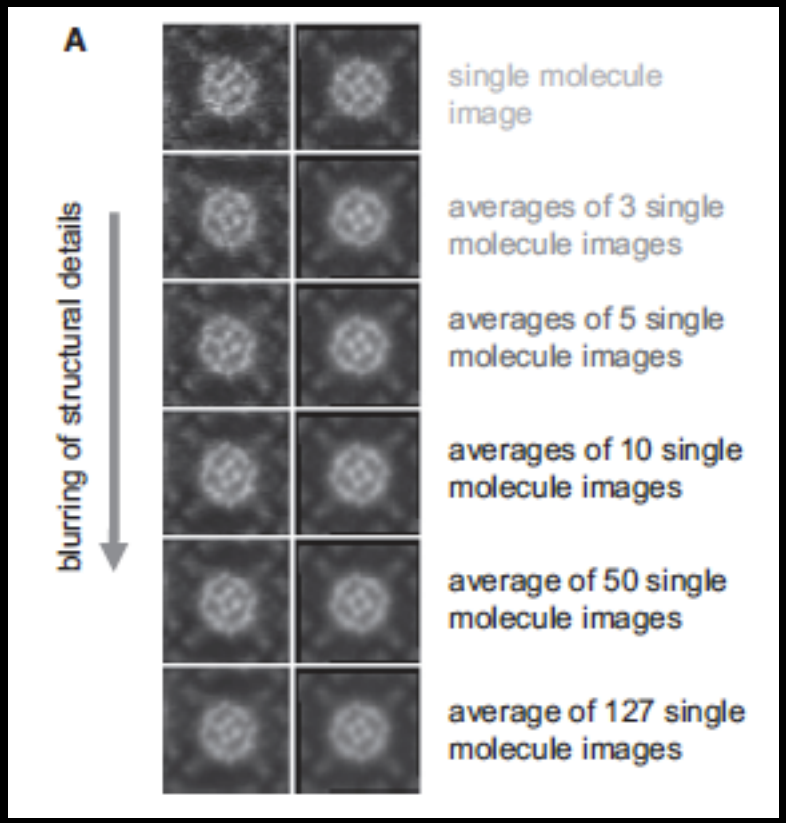
The AqpC lattice, quality of individual proteins images

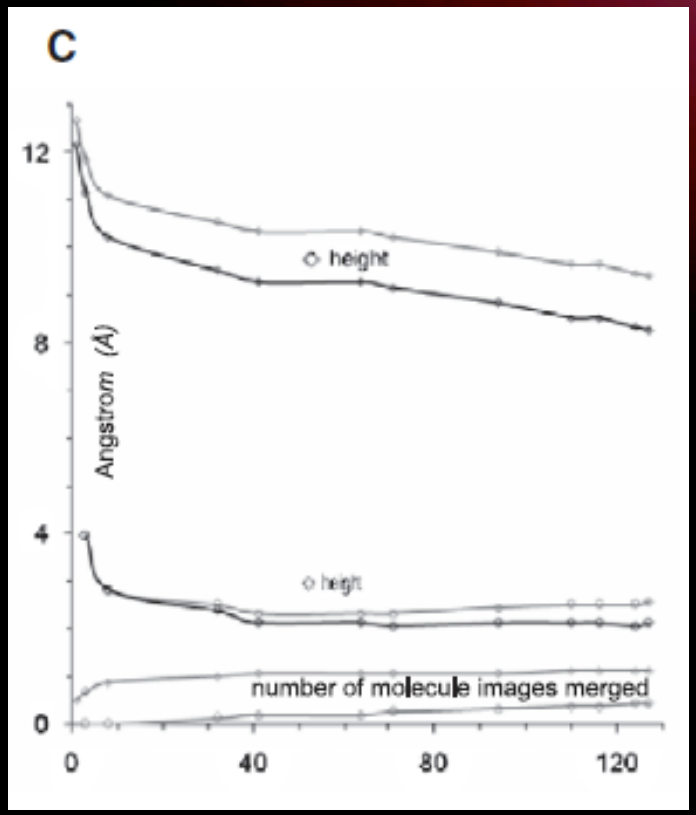
Only molecules CCV>0.75 should be acceptable





Be aware of averaging



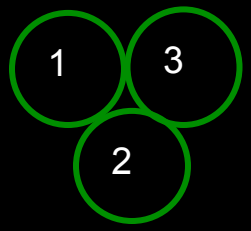


Best to pre select the best molecules before averaging

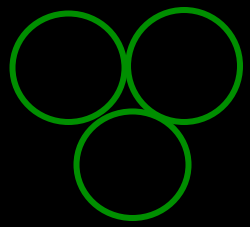
Criterion for pre-selecting the best imaged molecules

Biomolecules form agglomerates of identical subunits

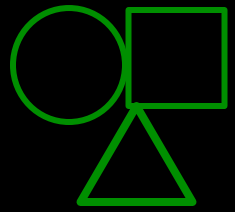
1=2=3



Cross correlation between the subunits of the molecules can be used as criteria of the quality of imaging



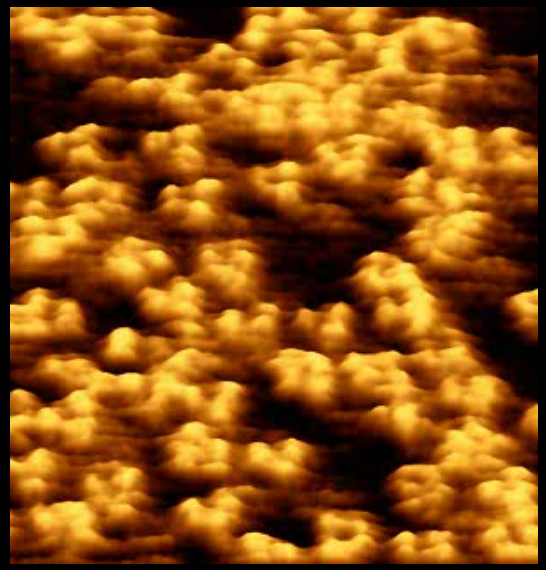
good



bad

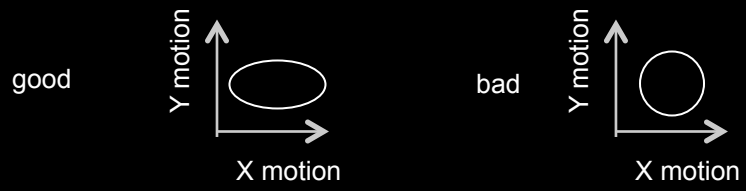
Only molecules CCV >0.75 between subunits should be acceptable

Tip motion can influence molecular motion



Criteria

1) There should be the same motion in the fast scan axe and the slow scan axe



2) Scanning at different speeds should not change the observed dynamics (ex. average speed)

TIPs, Top Importance Part of afm

Standardization

?

Cantilever (k), tip (applied force), apex (resolution)

?

EM (it can contaminate your tip, does not resolve apex)

Real imaging tests are the only way