Reconstruction de grands complexes protéiques à partir d'une surface topographique d'AFM et de leurs sous-unités constituantes

What is structural biology?

Introduction

AFM-assembly

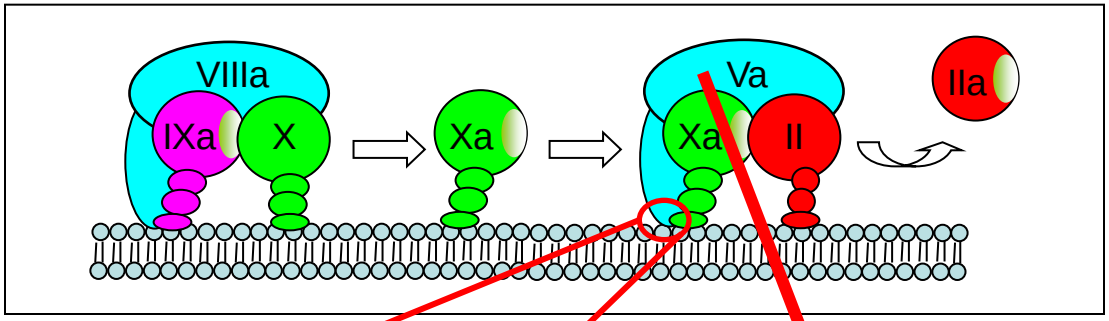
TMV reconstru.

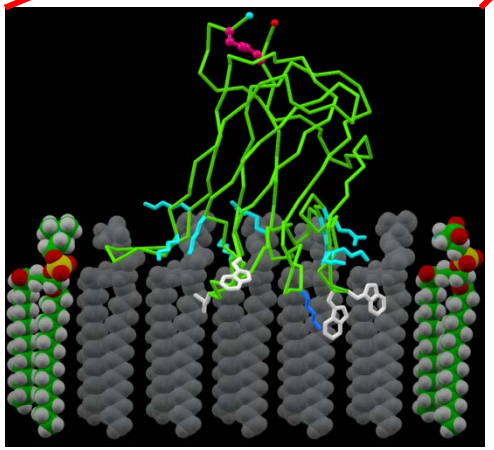
AqpZ reconstru.

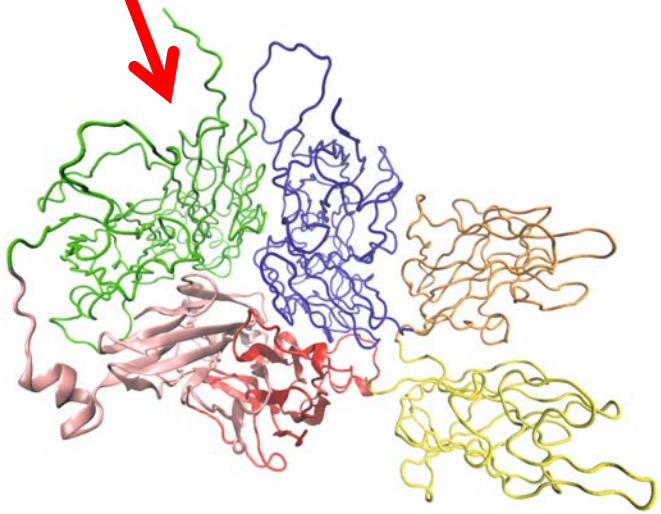
Ab reconstru.

Fva reconstru.

Summary







What is integrated structural biology

Introduction

AFM-assembly

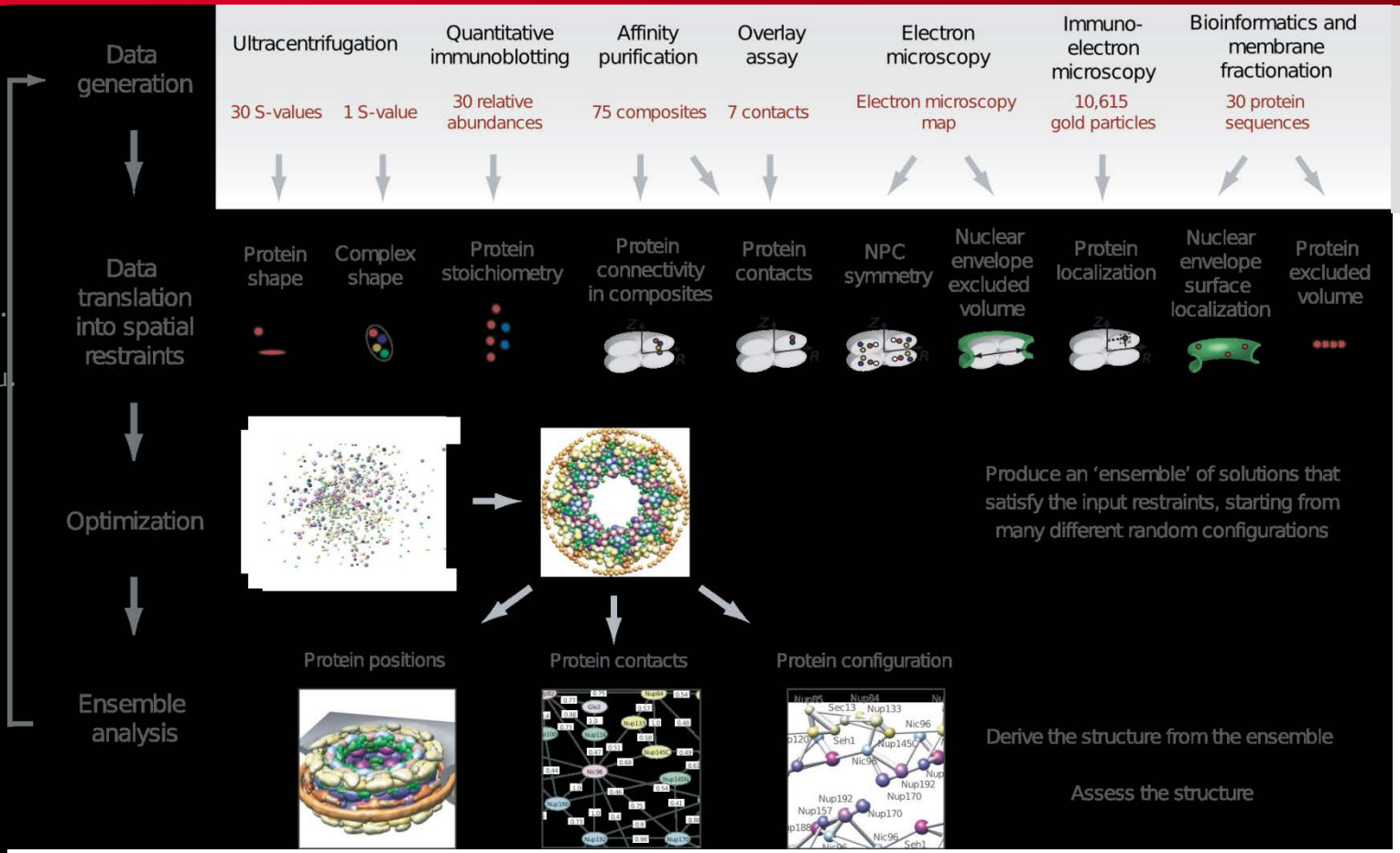
TMV reconstru.

AqpZ reconstru.

Ab reconstru.

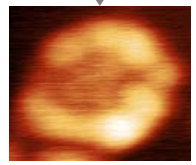
Fva reconstru.

Summary



AFM

2D surface



Alber F, Dokudovskaya S, Veenhoff LM, Zhang W, Kipper J, Devos D, Suprapto A, Karni-Schmidt O, Williams R, Chait BT, Rout MP and Sali A (2007) Determining the architectures of macromolecular assemblies. *Nature* **450**: 683-694.

+ MD-SAXS + SAXS-NMR

Determining the architectures of macromolecular assemblies

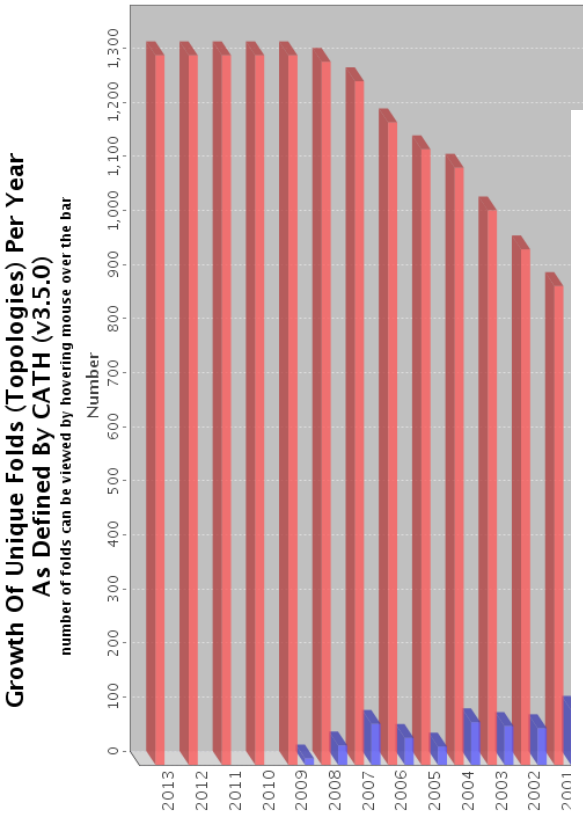
Introduction
AFM-assembly
TMV reconstru.
AqpZ reconstru.
Ab reconstru.
Fva reconstru.
Summary

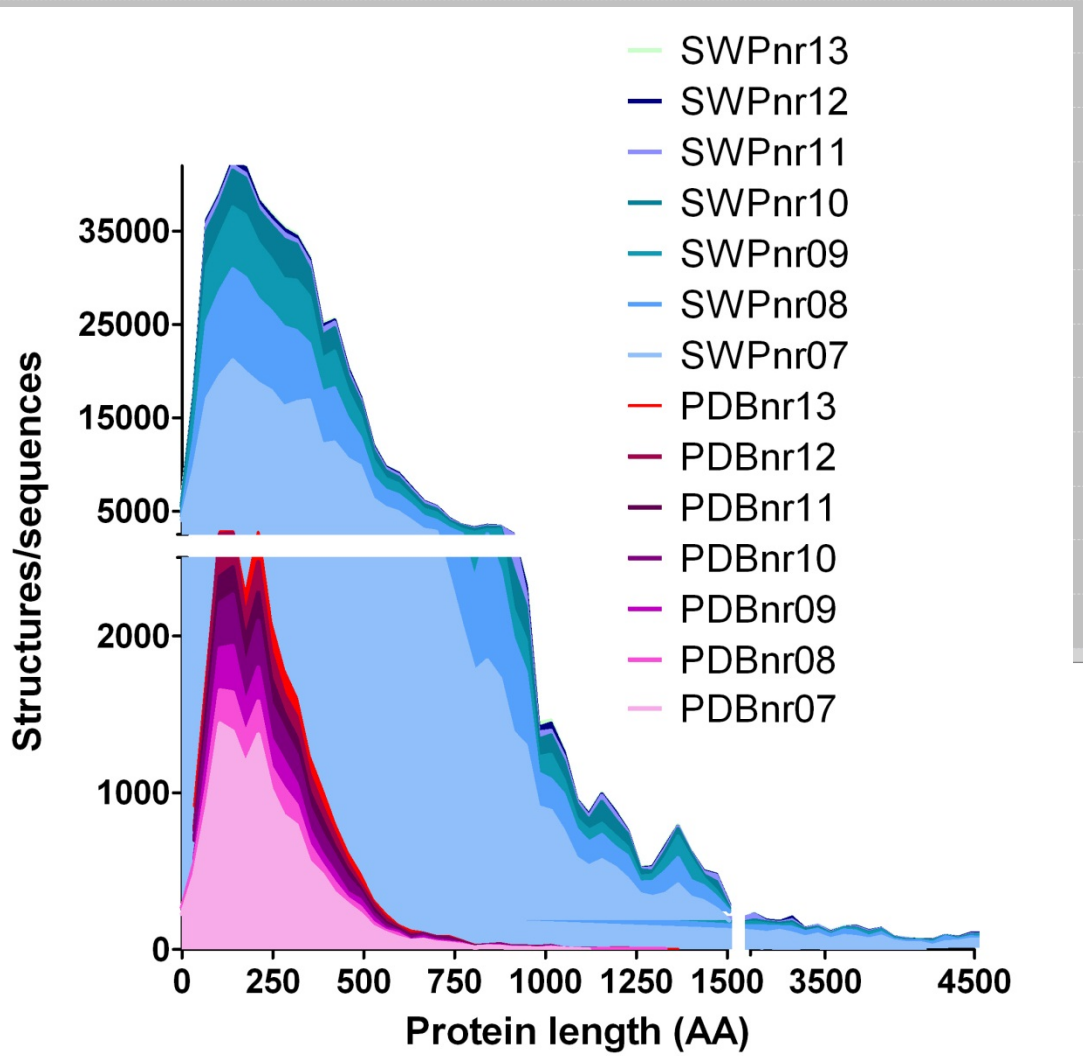
Technique	Sample	Advantages	Limitations
X-ray crystallography	•Crystal	•Very high resolution	•Requires crystals •Requires large quantity of material •Dependent of crystal contacts
NMR	•Solution	•Good "resolution" •Dynamics information	•Difficult for large systems (>50 kDa)
Cryo-EM	•Dilute frozen solution	•Low material requirement •Ideal for highly symmetric systems	•Med-low resolution •Requires large systems (>200 kDa)
SAXS/SANS	•Solution	•Large range of sample size •Kinetics and interactions	•Low resolution •Large quantity of materials (1-100 mg/ml) •Ambiguous reconstructed models
AFM	•Solution/Air •Adsorbed on surface	•Very low material requirement •Very high SNR	•Low resolution •Ambiguous reconstructed models

Adapted from:
Svergun D. I. et Koch M. H. J. (2003). Small-angle scattering studies of biological macromolecules in solution. Rep. Prog. Phys. 66, 1735–1782.

Recent evolution in protein fold discovery 2013

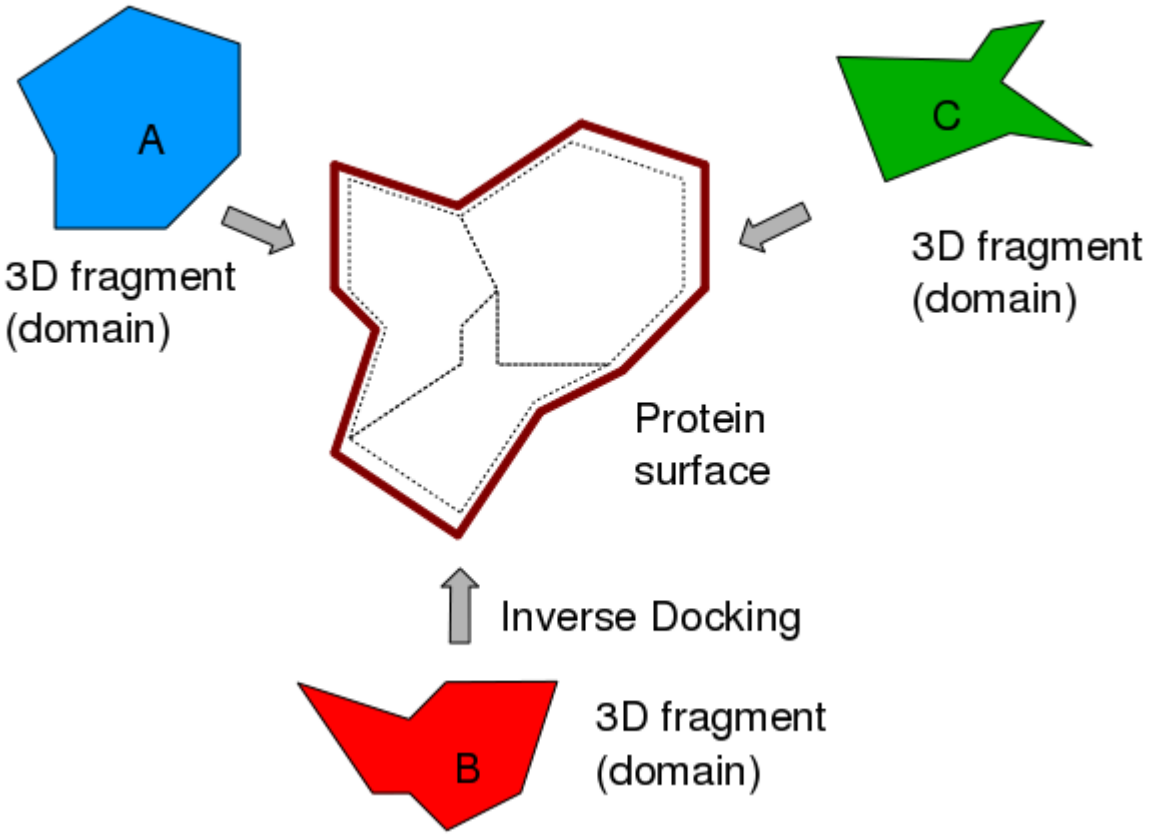
Introduction
AFM-assembly
TMV reconstru.
AqpZ reconstru.
Ab reconstru.
Fva reconstru.
Summary





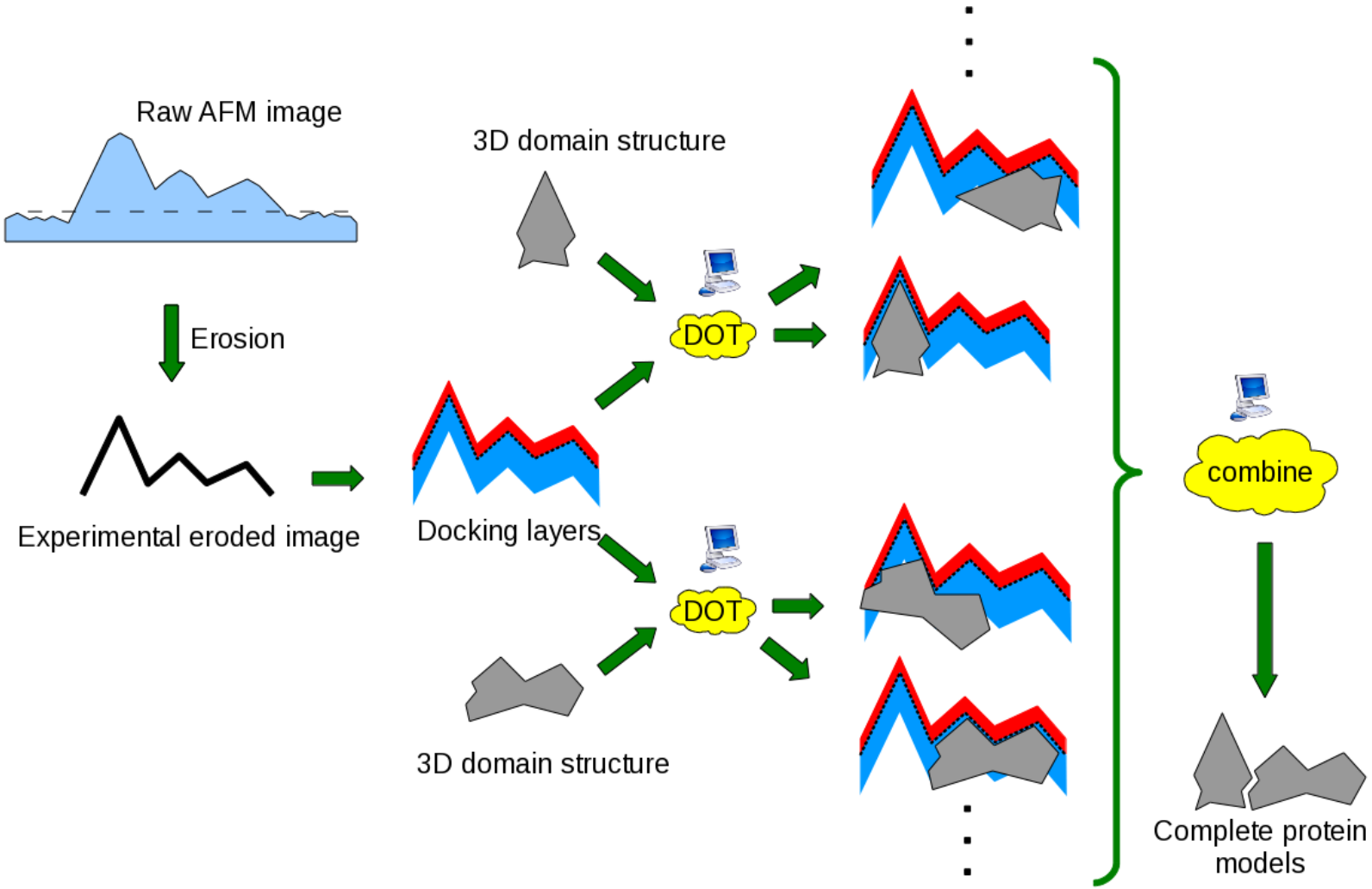
The AFM-ASSEMBLY concept

Introduction
AFM-assembly
TMV reconstru.
AqpZ reconstru.
Ab reconstru.
Fva reconstru.
Summary



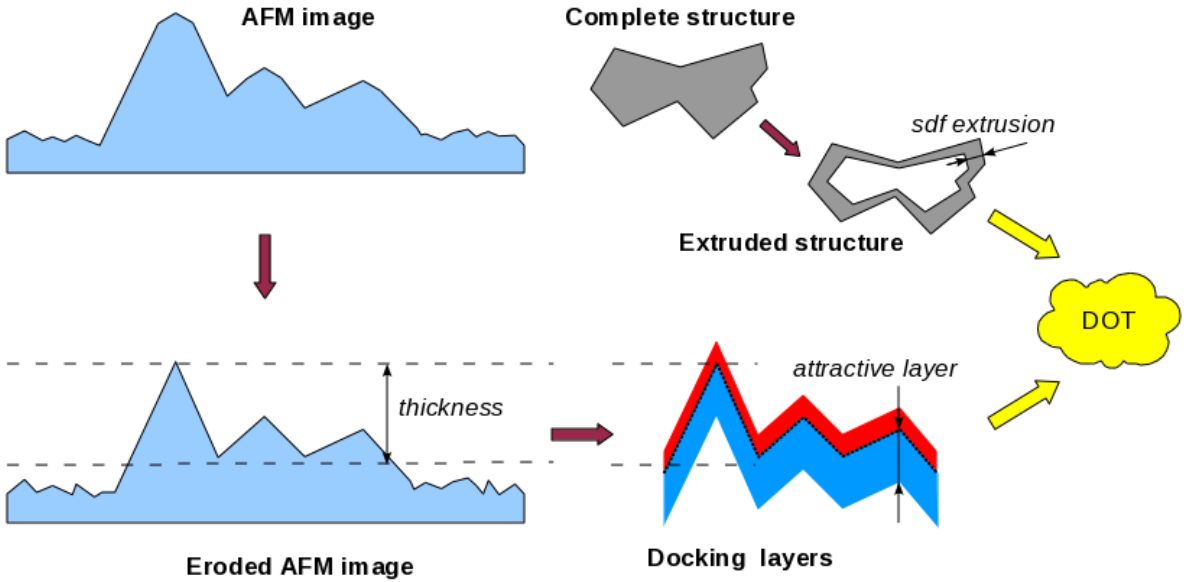
The AFM-ASSEMBLY protocol

Introduction
AFM-assembly
TMV reconstru.
AqpZ reconstru.
Ab reconstru.
Fva reconstru.
Summary

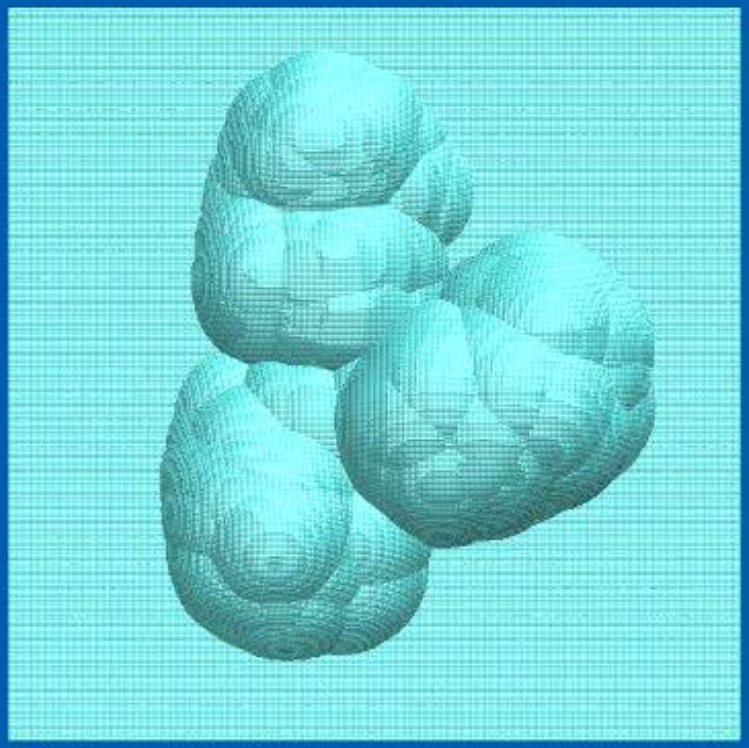


The AFM-ASSEMBLY protocol with extrusion

Introduction
AFM-assembly
TMV reconstru.
AqpZ reconstru.
Ab reconstru.
Fva reconstru.
Summary



http://biodev.cea.fr/adepth



DockAFM: docking atomic coordinates in AFM surfaces

Introduction

AFM-assembly

TMV reconstru.

AqpZ reconstru.

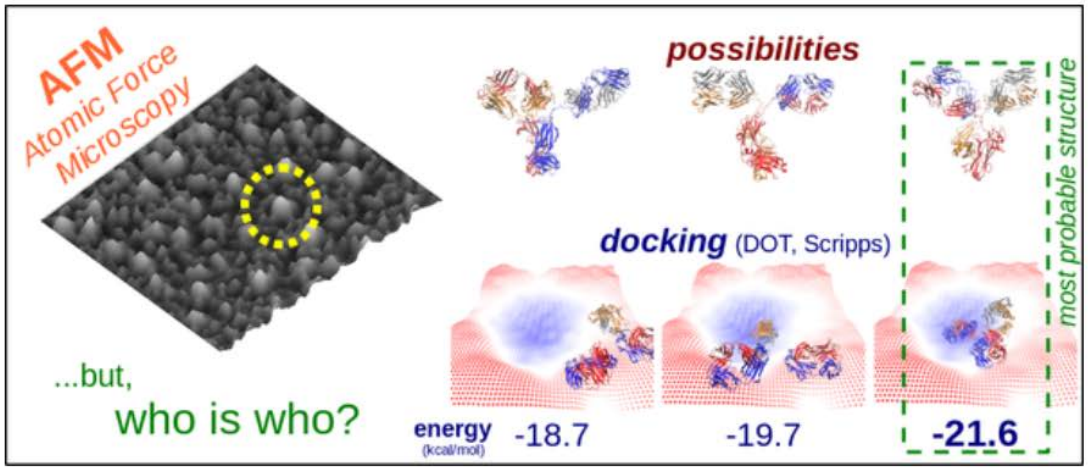
Ab reconstru.

Fva reconstru.

Summary

DockAFM

docking structures under an AFM surface



Download:

user manual

example

tools

RC Chaves JL Pellequer (2013)

Comment and request:
Coordinator

1. Input

structure (.pdb) : Parcourir...

AFM image (.txt) : Parcourir...

Image z threshold in Å : 0.0

Erosion tip (h, r, a) : 50 Å 10.0 Å 15 °

http://biodev.cea.fr/dockafm

Finally, the interesting part…

Introduction
AFM-assembly
TMV reconstru.
AqpZ reconstru.
Ab reconstru.
Fva reconstru.
Summary

Proofs of concept:

- TMV virus
- Aquaporin Z

Applications:

- Conformational variation in antibodies
- Alternative conformation of FVa

Proof of concept: reconstruction of TMV

Introduction

AFM-assembly

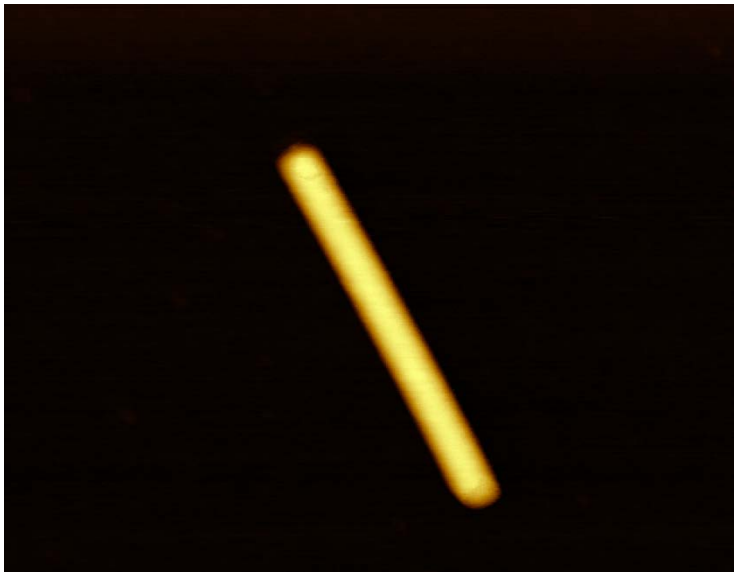
TMV reconstru.

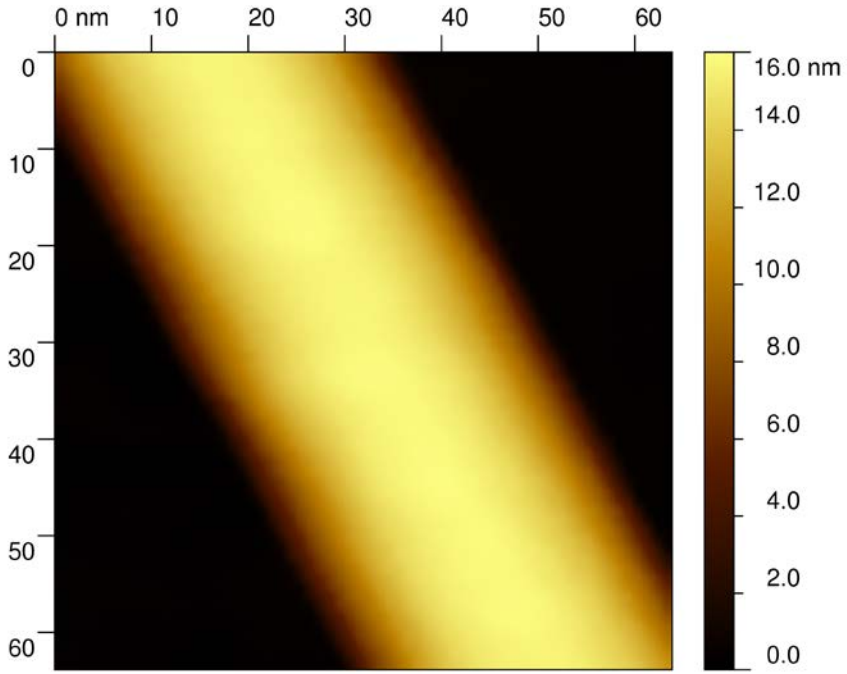
AqpZ reconstru.

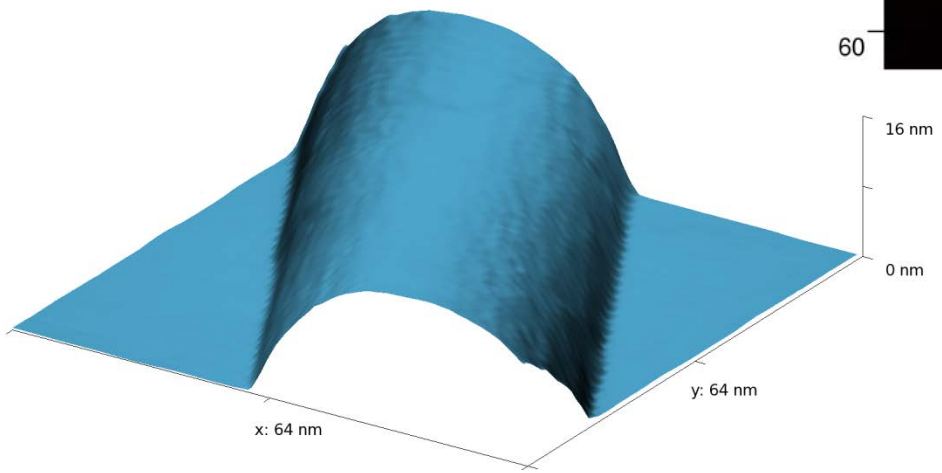
Ab reconstru.

Fva reconstru.

Summary

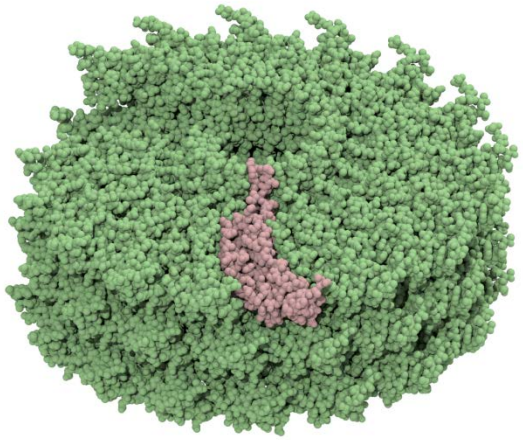


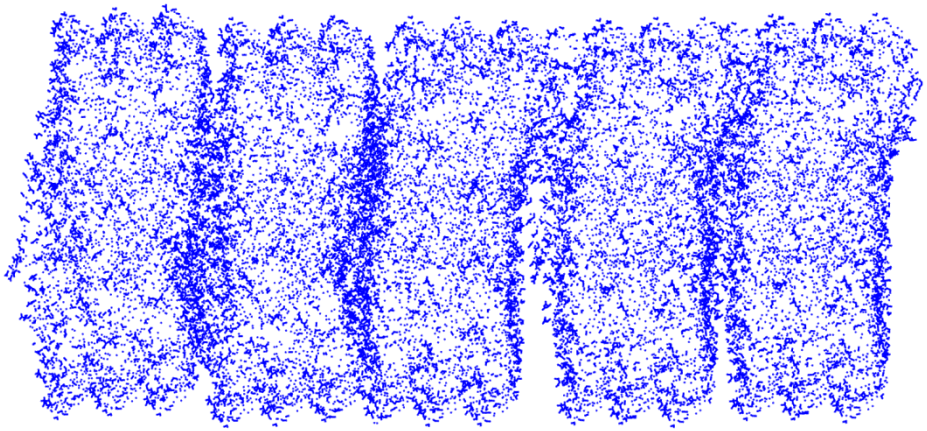


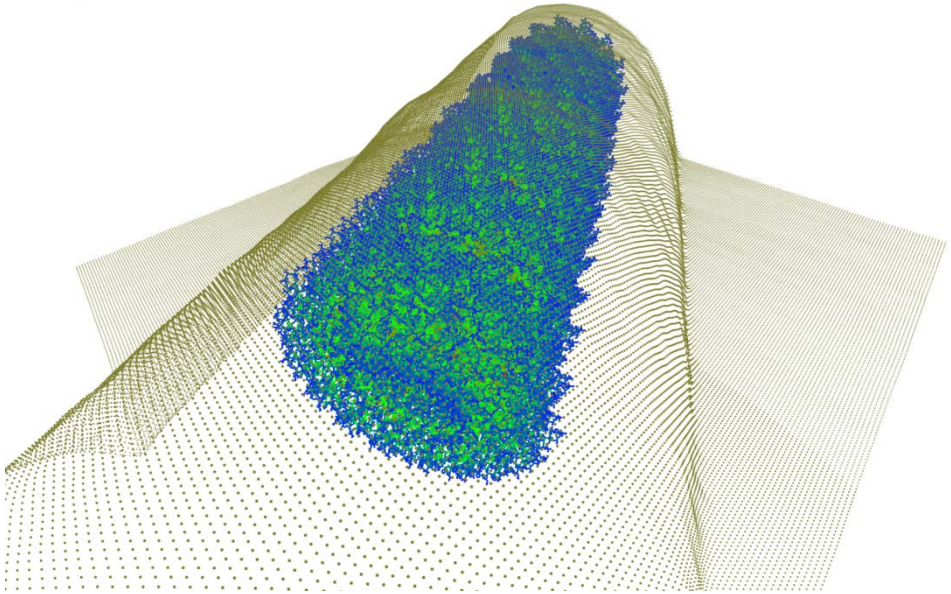


Proof of concept: reconstruction of TMV

Introduction
AFM-assembly
TMV reconstru.
AqpZ reconstru.
Ab reconstru.
Fva reconstru.
Summary



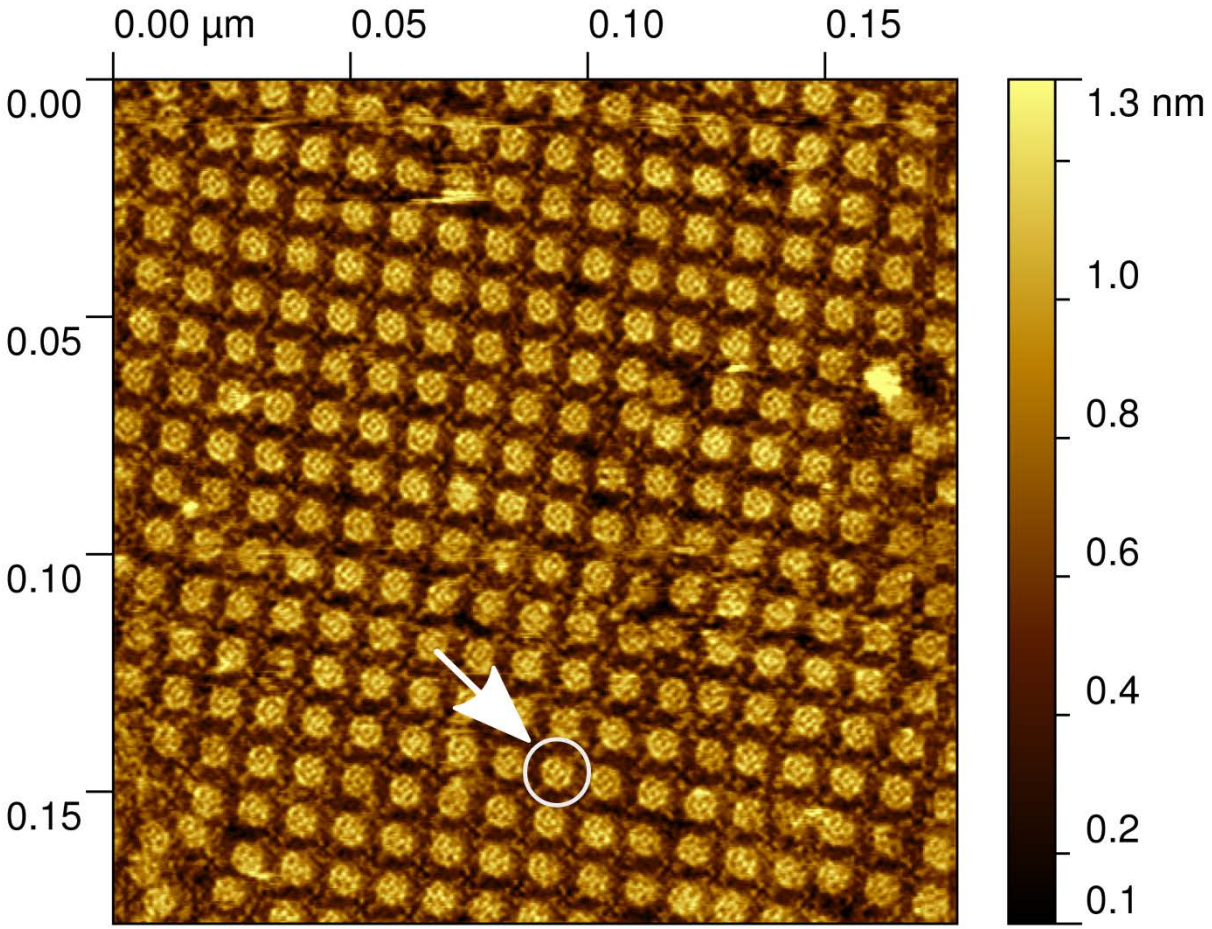




Trinh, M.-H., Odorico, M., Pique, M.E., Teulon, J.-M., Roberts, V.A., Ten Eyck, L.F., Getzoff, E.D., Parot, P., Chen, S.-w.W., and Pellequer, J.-L. (2012). Computational reconstruction of multidomain proteins using atomic force microscopy data. Structure **20:** *113-120*.

Reconstruction of a tetrameric membrane protein

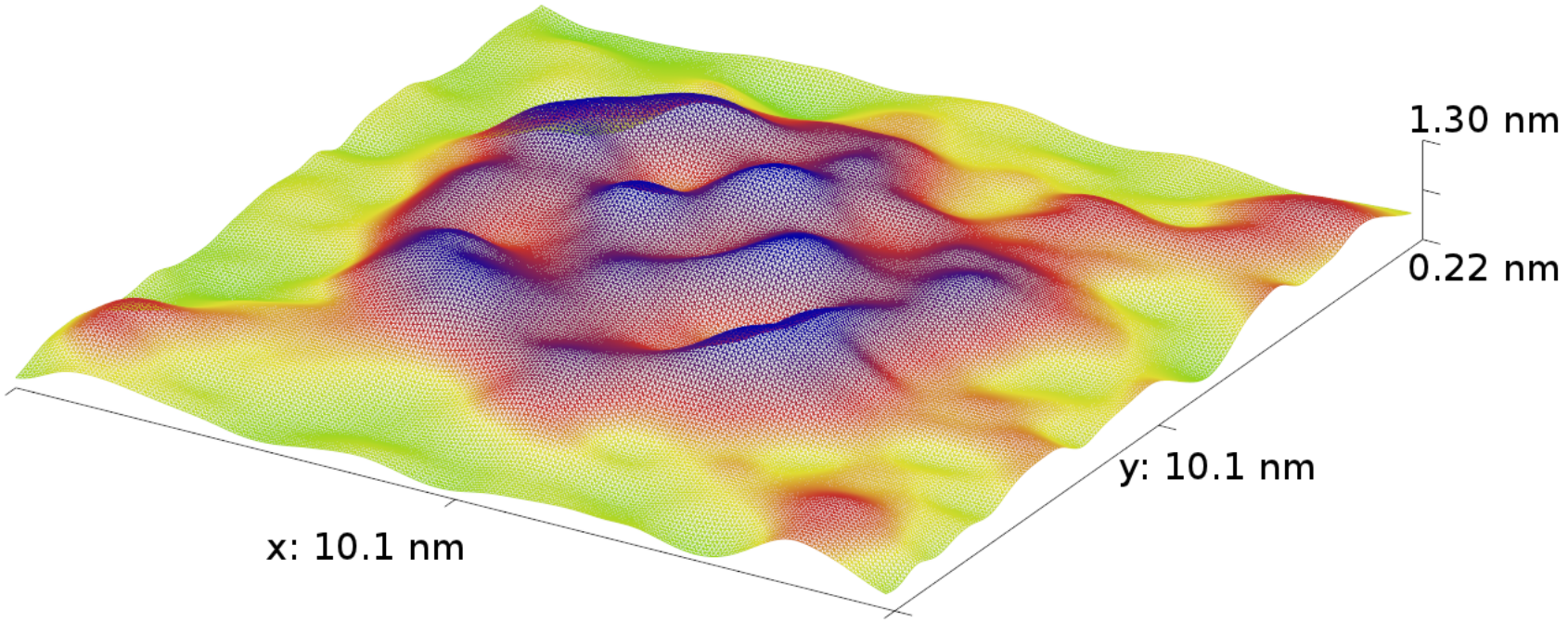
Introduction
AFM-assembly
TMV reconstru.
AqpZ reconstru
Ab reconstru.
Fva reconstru.
Summary



Scheuring, S., Ringler, P., Borgnia, M., Stahlberg, H., Damiel, M., Agre, P., and Engel, J. (1999). High resolution AFM topographs of the Escherichia coli water channel aquaporin Z. EMBO J. *18*, 4981-4987.

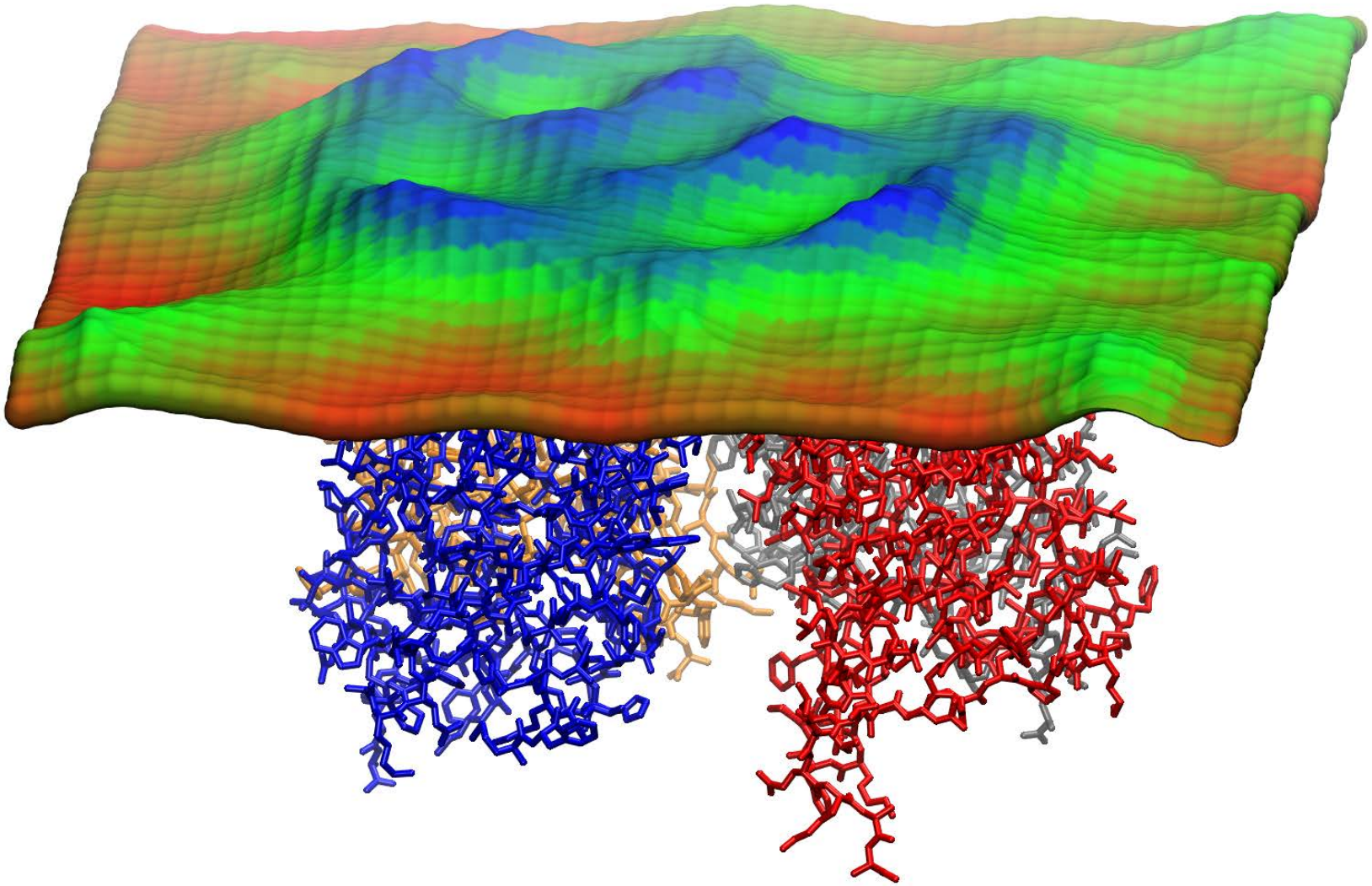
Reconstruction of a tetrameric membrane protein

Introduction
AFM-assembly
TMV reconstru.
AqpZ reconstru
Ab reconstru.
Fva reconstru.
Summary



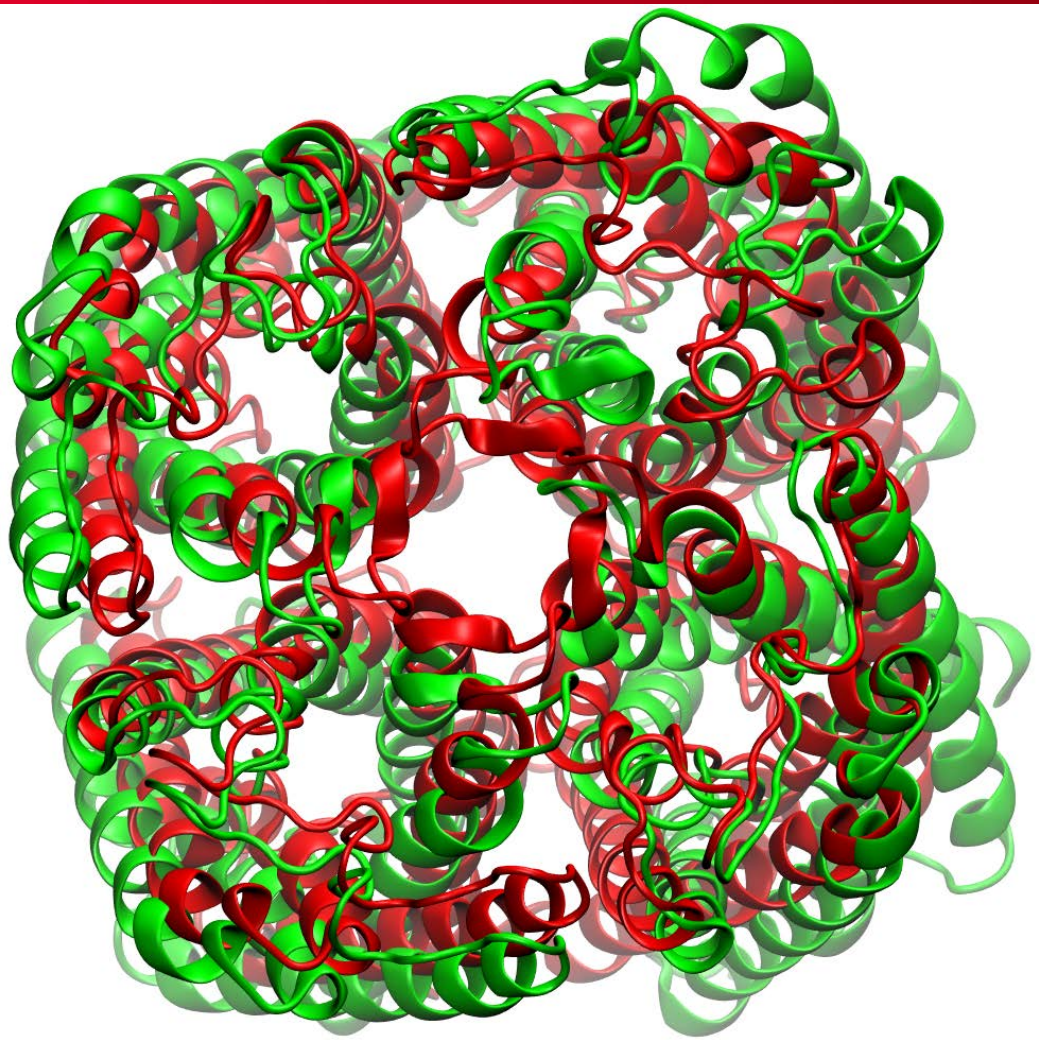
Reconstruction of a tetrameric membrane protein

Introduction
AFM-assembly
TMV reconstru.
AqpZ reconstru
Ab reconstru.
Fva reconstru.
Summary



Reconstruction of a tetrameric membrane protein

Introduction
AFM-assembly
TMV reconstru.
AqpZ reconstru
Ab reconstru.
Fva reconstru.
Summary



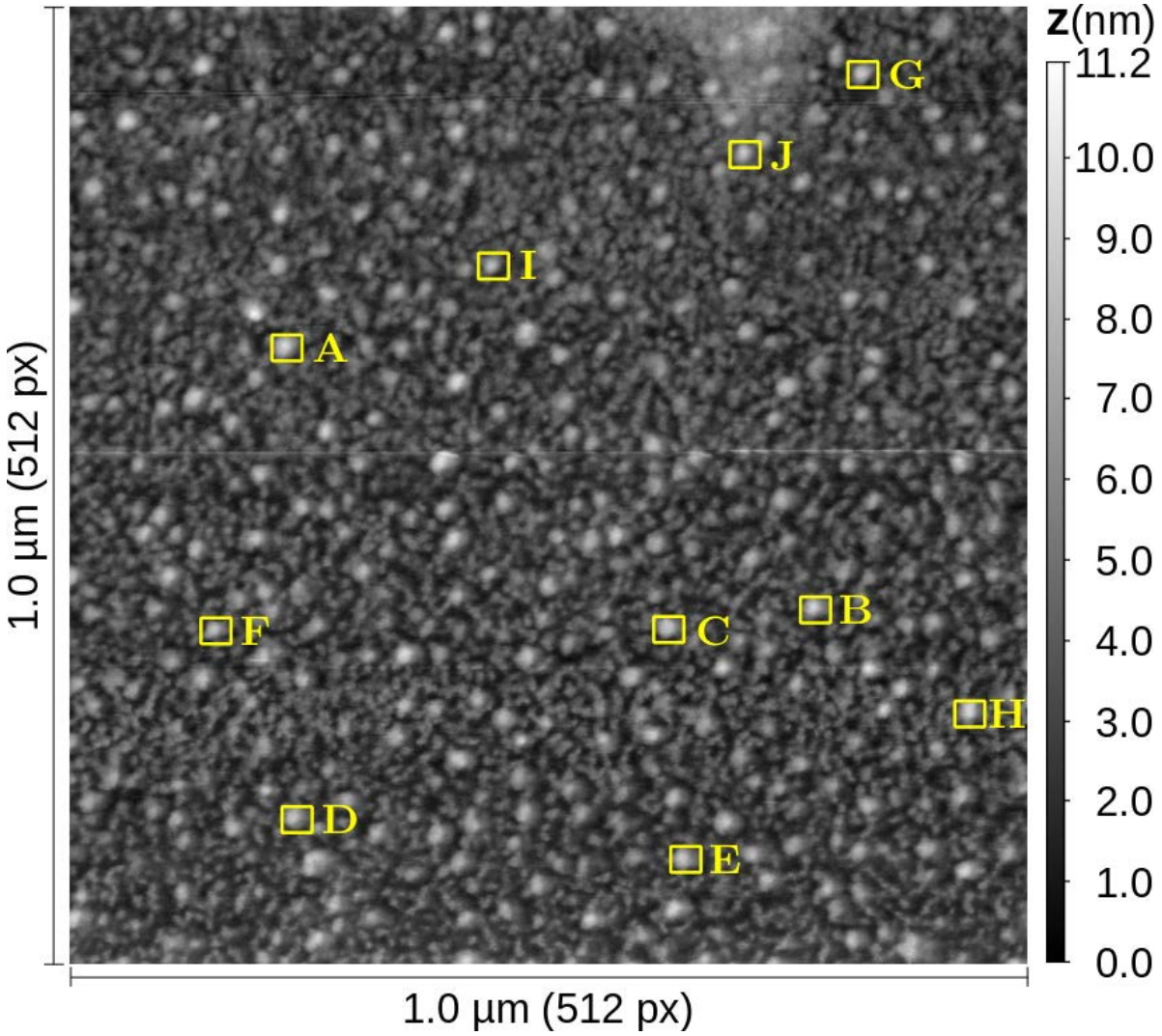
RMSD= 4.5 Å

$RMSD_{X\text{-}PLOR}$= 3.5 Å

Trinh, M.-H., Odorico, M., Pique, M.E., Teulon, J.-M., Roberts, V.A., Ten Eyck, L.F., Getzoff, E.D., Parot, P., Chen, S.-w.W., and Pellequer, J.-L. (2012). Computational reconstruction of multidomain proteins using atomic force microscopy data. Structure **20:** *113-120*.

Reconstruction of dynamical structures of antibodies

Introduction
AFM-assembly
TMV reconstru.
AqpZ reconstru.
Ab reconstru.
Fva reconstru.
Summary



Reconstruction of dynamical structures of antibodies

Introduction

AFM-assembly

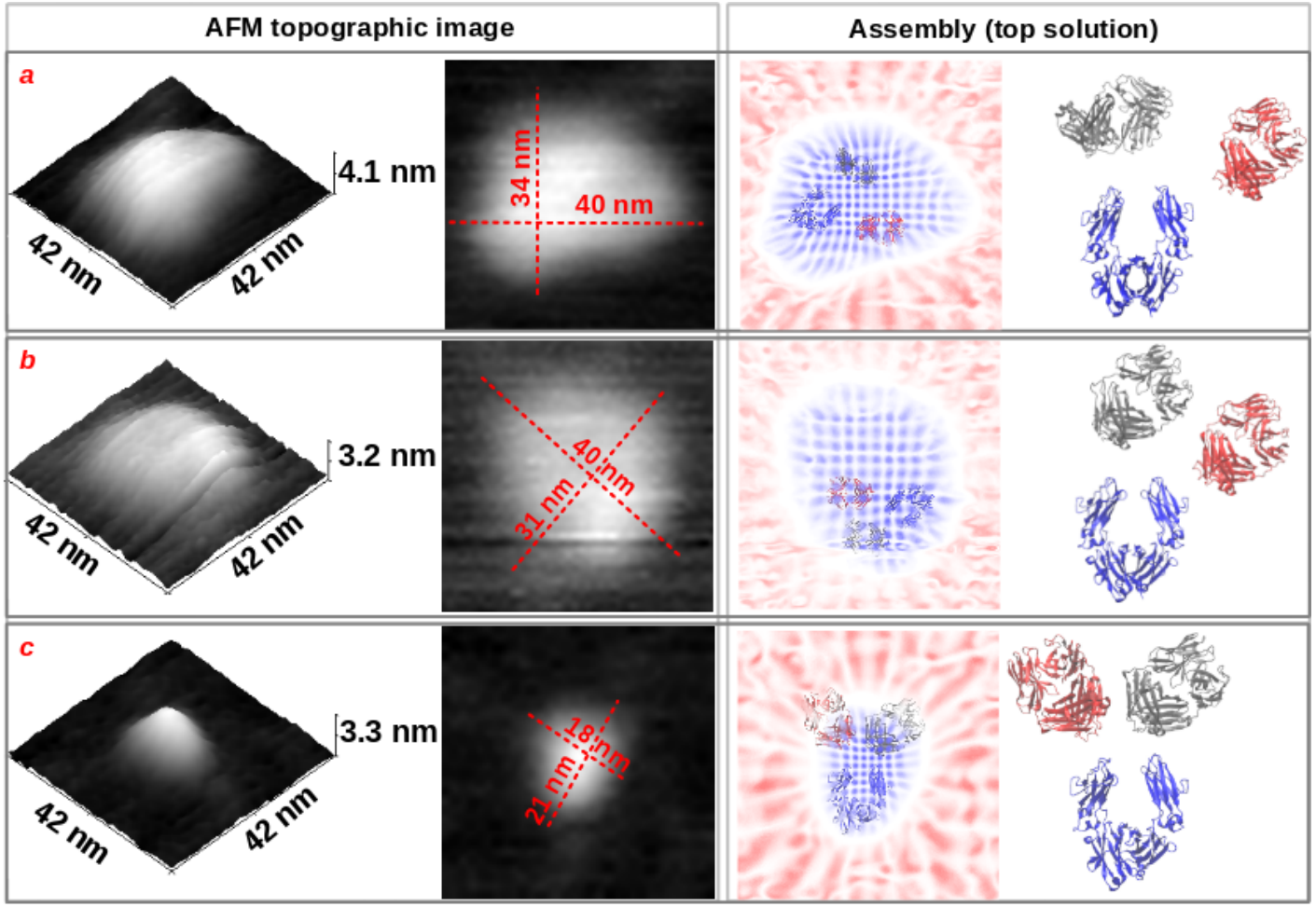
TMV reconstru.

AqpZ reconstru.

Ab reconstru.

Fva reconstru.

Summary



The images were interpolated linearly with scale = 4, 4 and 8 resulting in spacial resolution of 2.4, 2.4 and 1.7 Å/px, respectively.
The top assembly solution resulted from the combine of the first 2000 docking positions (6 deg resolution) and with distance constrain for the Fc-Fab linking distance range of d = 35-52 nm.
The combine score is -1.29E+6, -1.38E+6 and -9.48E+5 respectively.

Reconstruction of dynamical structures of antibodies

Introduction
AFM-assembly
TMV reconstru.
AqpZ reconstru.
Ab reconstru.
Fva reconstru.
Summary

	Linker					*Domains*					
	separation (Å)		axis (°)			axis (°)			planes (°)		
Obj	|L1|	|L2|	L1^L2	L1^Fc	L2^Fc	Fab1^Fab2	Fab1^Fc	Fab2^Fc	Fab1^Fab2	Fab1^Fc	Fab2^Fc
A	58.5	49.1	63.1	118.7	144.1	23.8	29.5	50.7	31.5	55.1	46.9
B	60.5	51.4	87.3	104.7	144.5	31.6	28.1	37.5	43.3	83.3	41.7
C	59.7	51.0	74.8	121.1	140.1	22.9	40.9	31.0	58.0	83.0	28.5
D	56.4	50.9	103.2	101.1	138.8	49.8	34.2	32.9	32.1	68.1	37.1
E	54.8	58.6	68.8	145.9	130.4	25.7	53.7	44.4	32.8	46.5	13.8
F	59.4	59.3	20.7	145.4	150.8	97.2	45.2	90.0	60.1	40.3	85.2
G	58.4	47.4	44.1	158.9	157.0	11.5	43.7	53.3	54.6	47.8	86.6
H	56.6	61.6	77.5	97.8	128.2	31.1	70.0	57.3	64.0	45.6	24.9
I	49.3	45.9	71.5	113.9	154.1	90.2	37.8	97.3	67.9	35.0	61.9
J	54.5	38.4	35.7	117.4	121.5	40.6	51.7	48.3	43.3	48.5	54.3
mean	**57**	**51**	**65**	**122**	**141**	**42**	**43**	**54**	**49**	**55**	**48**
range	49:60	38:62	21:103	98:159	121:157	12:97	28:70	31:97	31:68	35:83	14:87
1HZH[a,b]	34.8	45.7	41.8	135.4	107.0	143.4	70.2	73.3	57.7	79.6	89.7
1IGT[a,b]	51.6	35.5	50.6	113.4	146.0	169.5	112.6	64.9	35.0	73.1	88.1
1IGY[a,b]	36.6	34.0	74.6	72.5	116.7	112.8	99.7	56.6	39.2	56.4	85.5

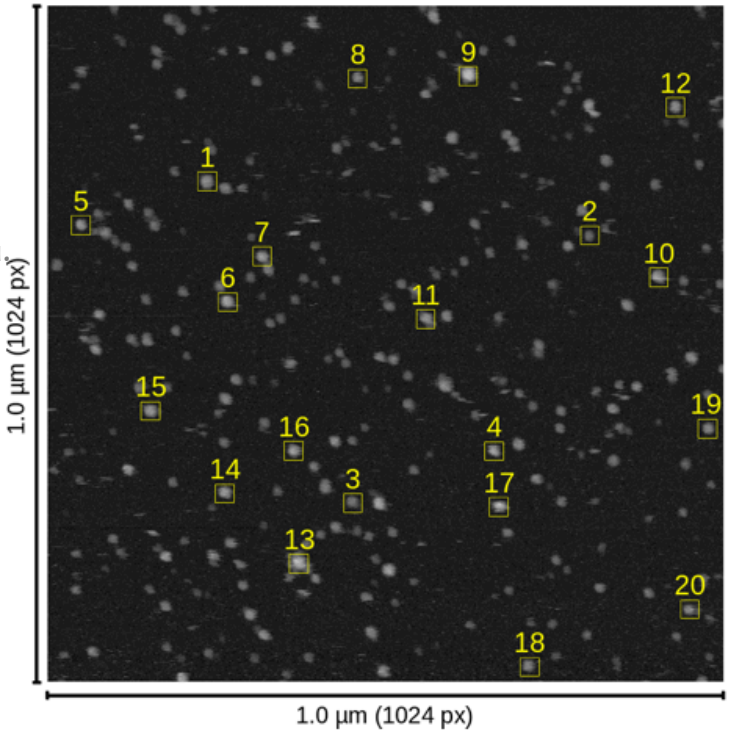
Chaves RC, Teulon J-M, Odorico M, Parot P, Chen S-wW and Pellequer J-L (2013) Conformational dynamics of individual antibodies using computational docking and AFM. *J. Mol. Recognit.* ***26: 596-604.***

Reconstruction of coagulation FVa

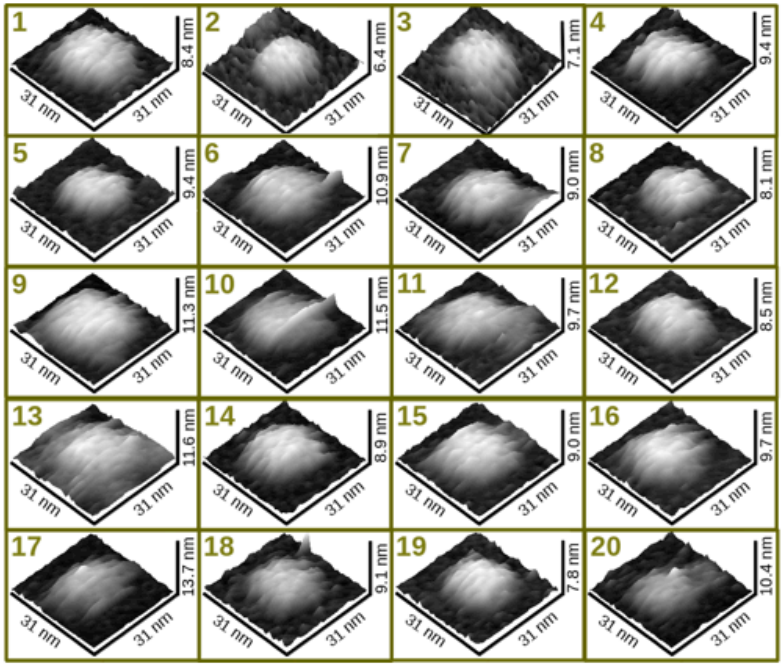
Introduction
AFM-assembly
TMV reconstru.
AqpZ reconstru.
Ab reconstru.
Fva reconstru.
Summary

activated Factor V (**FVa**) imaged by AFM

Resolution: 9.75 Å/px (raw)



average height = 9.5 (6.4 : 13.7) nm



Multimode 8, Nanoscope V (Bruker)
ScanAsyst/**PeakForce**, 1 Hz, force amplitude 25 nm
Tip OTR8 (r=2nm, K= 0.57 N/m)
liquid
mica

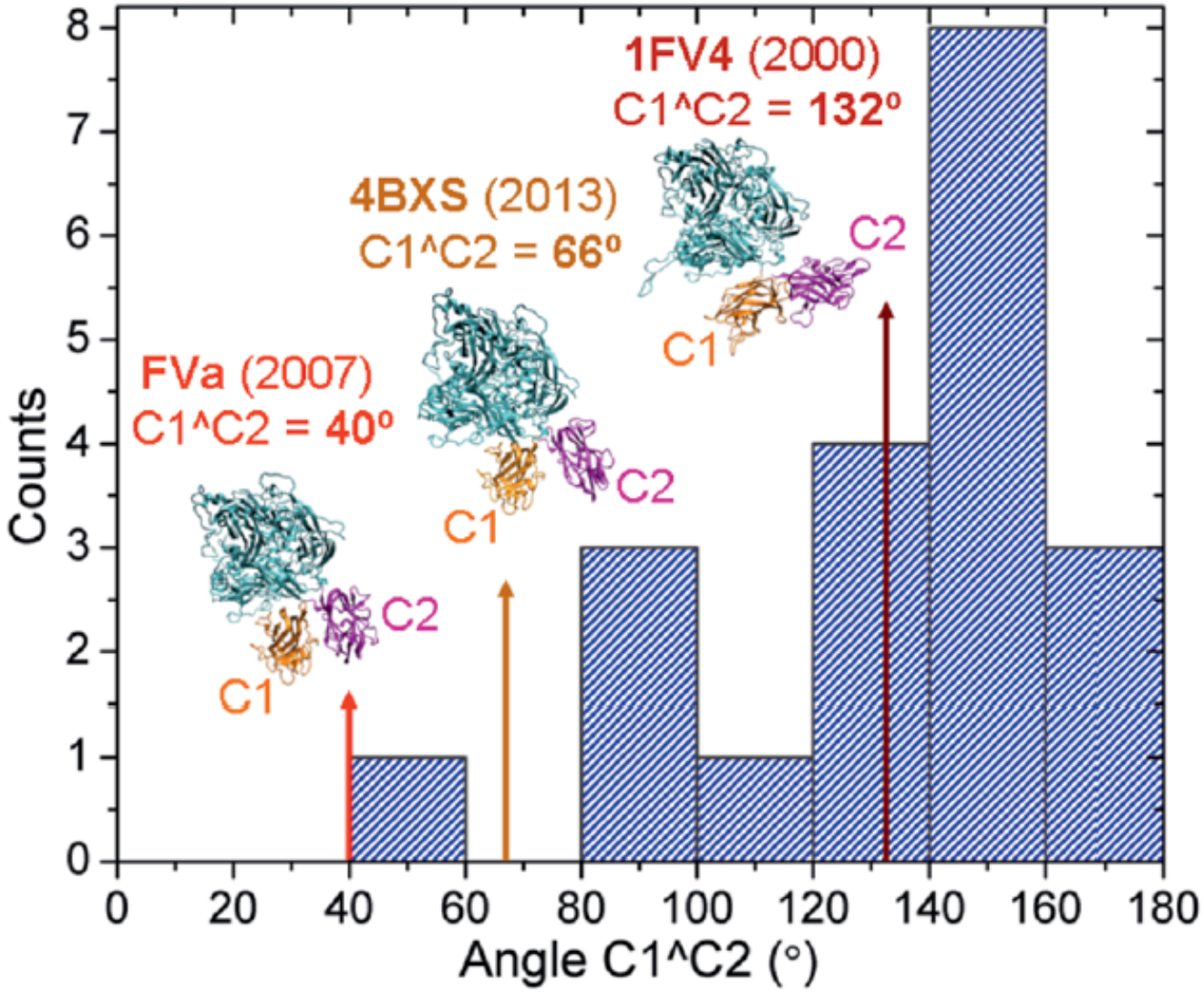
11s4_fva_e6_1,5nM_mgcl2_liquid_otr8_z1_1um.003

mica treated with $MgCl^{2+}$ (20 mM), 1h at RT.
Factor Va 60 µl (9 nM)
Buffer HEPES (20mM, 150 mM NaCl, pH 7,1)
imaged in liquid (50 µl)

molecules not completely fixed, still some movement

Reconstruction of coagulation FVa

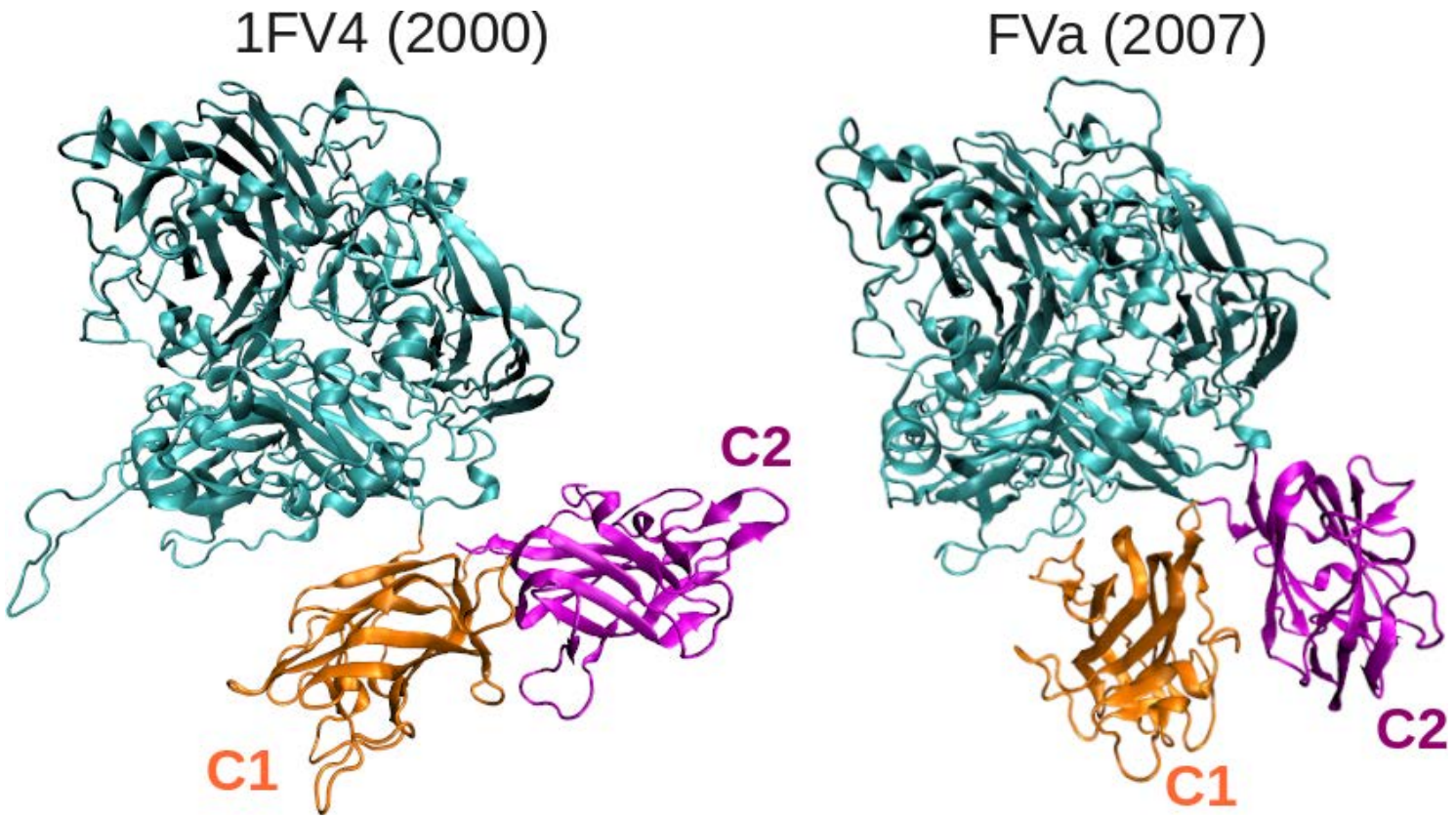
Introduction
AFM-assembly
TMV reconstru.
AqpZ reconstru.
Ab reconstru.
Fva reconstru.
Summary



Chaves RC, Dahmane S, Odorico M, Nicolaes GAF and Pellequer J-L (2014) Factor Va 3D structure reconstruction using Atomic Force Microscopy. *Thromb. Haemost.* **112**: 1167-1173

Reconstruction of coagulation FVa

Introduction
AFM-assembly
TMV reconstru.
AqpZ reconstru.
Ab reconstru.
Fva reconstru.
Summary



Chaves RC, Dahmane S, Odorico M, Nicolaes GAF and Pellequer J-L (2014) Factor Va 3D structure reconstruction using Atomic Force Microscopy. *Thromb. Haemost.* **112**: 1167-1173

AFM-assembly summary

Introduction
AFM-assembly
TMV reconstru.
AqpZ reconstru.
Ab reconstru.
Fva reconstru.
Summary

- Reconstruction protocol for assembling large molecular structure
- Dedicated image processing developments
- Promising results concerning membrane proteins

- Need for automatization of the protocol
- Need for application to other real HR AFM topographic images
- Possibility to incorporate additional data (EM, SAXS)

Acknowledgments

Grenoble 2015

Paramè Modifier

Next AFMBioMed Summer School will be organized in Grenoble end of August 2015.

Additional information will be posted here ASAP. Meanwhile, you can contact the organizer:

Jean-Luc Pellequer

jean-luc.pellequer at ibs.fr

Instructors (to be completed)

Sébastien Janel (IPL, Lille)

Simon Scheuring (INSERM, Marseille)

Hermann Schillers (Univ. Muenster)

Rui Chaves
Yannick Delcuze
Michael Odorico
Jean-Marie Teulon
Shu-wen W. Chen
Christian Godon
Pierre Parot
Minh-Hieu Trinh

Merci pour votre attention