

Investigation of Protein-Protein Interaction Using Atomic Force Microscopy

PhD Dissertation Defense

by

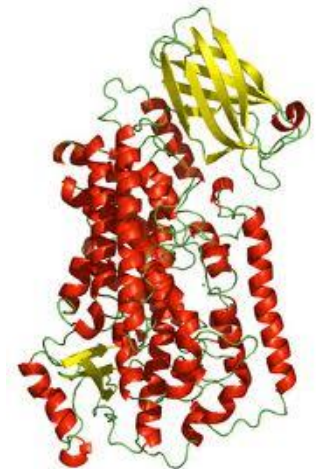
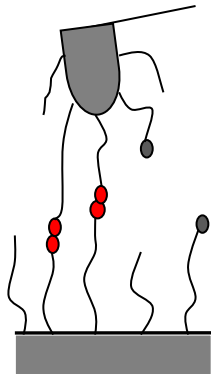
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2011

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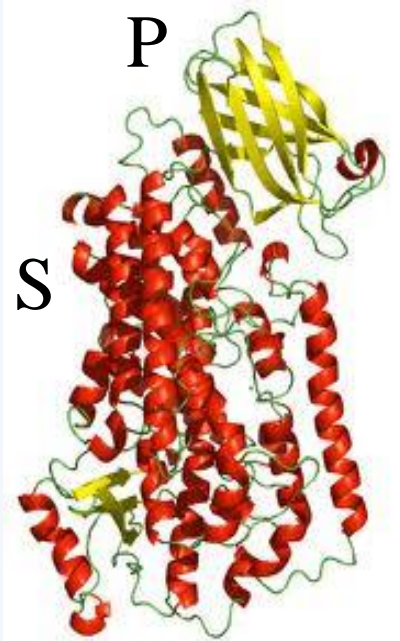


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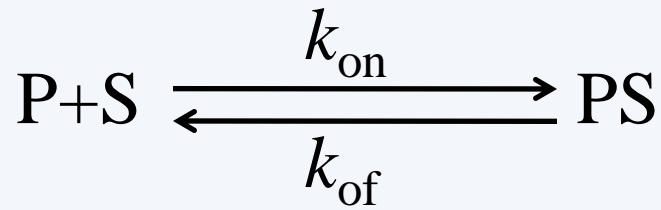
OUTLINE

- **MOTIVATION**
- **EXPERIMENTAL SETUP**
- **THEORY AND DATA ANALYSIS**
- **FORCE MEASUREMENTS**
- **DISSOCIATION RATE OF ENZYME-INHIBITOR SYSTEM**
- **RUPTURE FORCE OF COMPLEX BOND ON THE MEMBRANES OF LIVING CELLS**
- **CONCLUSIONS & FUTURE DIRECTIONS**

Can the AFM be used to investigate the proteins interaction?



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$$K_{eq} = \frac{k_{on}}{k_{off}}$$



AFM apparatus

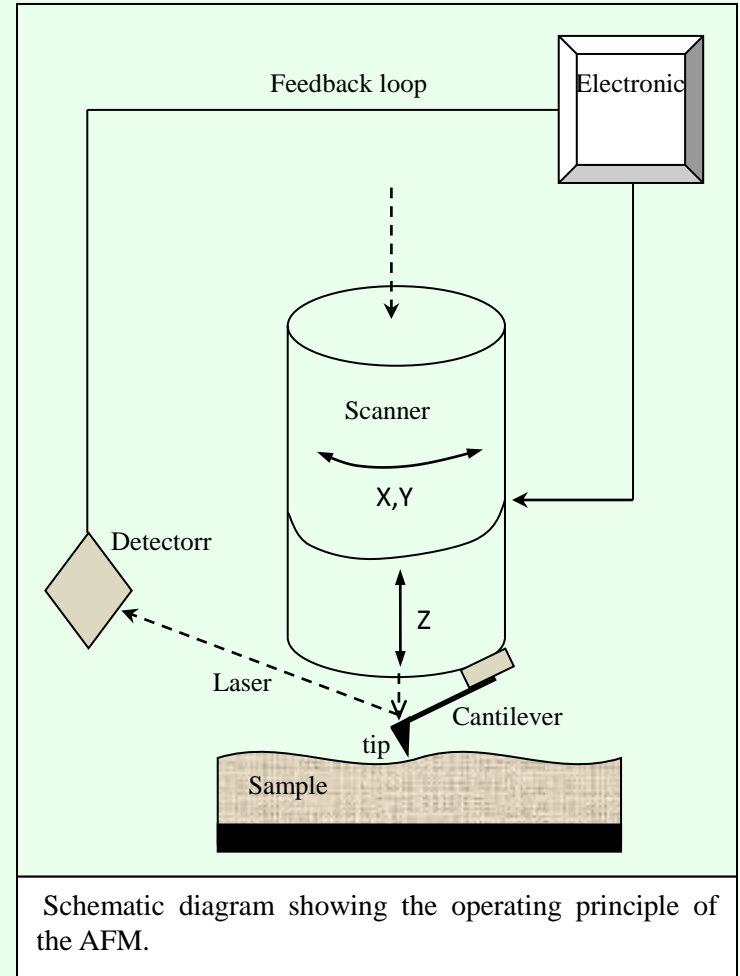
Experimental Setup



The head of the AFM

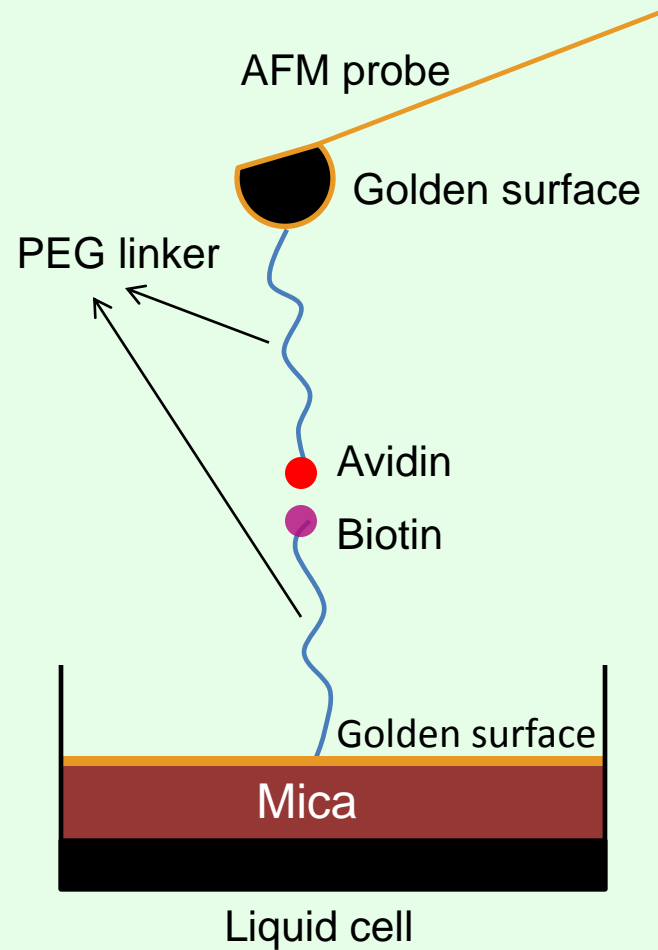


SEM images for AFM probes



Schematic diagram showing the operating principle of the AFM.

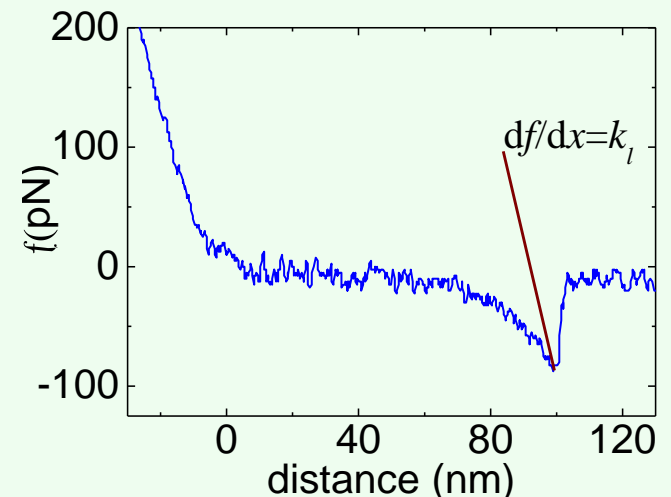
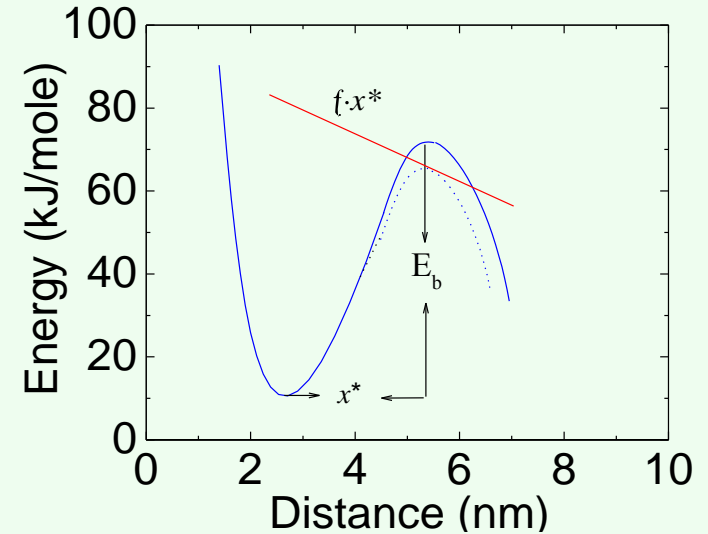
Experimental Setup



Theory

Standard Theory

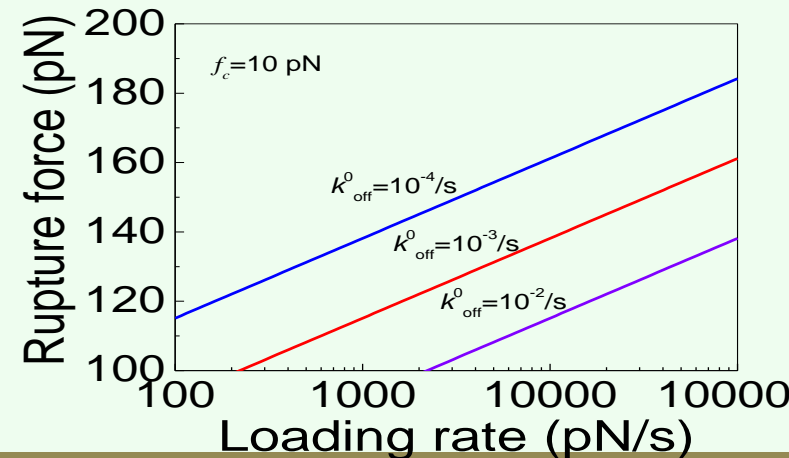
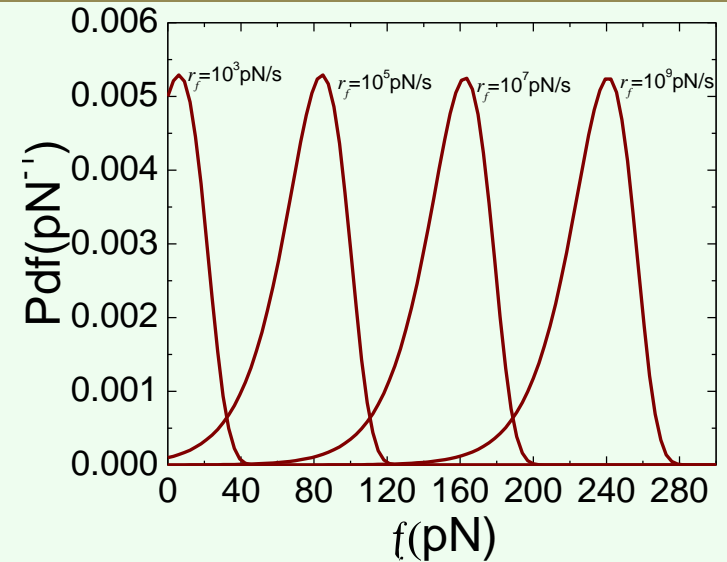
- $\frac{dS(t)}{dt} = -k_{off}(f)S(t)$
- $k_{off}(f) = k_{off}^0 \exp(fx^* / k_B T)$
- $r_f = \frac{df}{dt} = k_l v = \text{const} \tan t$



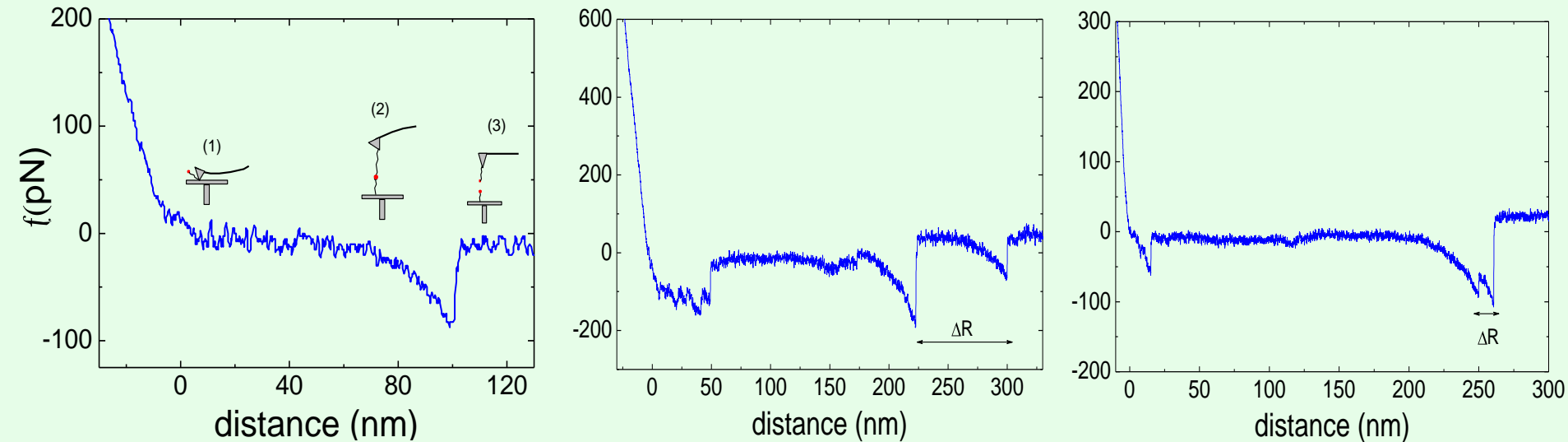
Theory

- $$Pdf(f) = \frac{k_{off}^0}{r_f} \exp\left(\frac{f}{f_c}\right) \exp\left[\frac{k_{off}^0 f_c}{r_f} \left(1 - \exp\left(\frac{f}{f_c}\right)\right)\right]$$

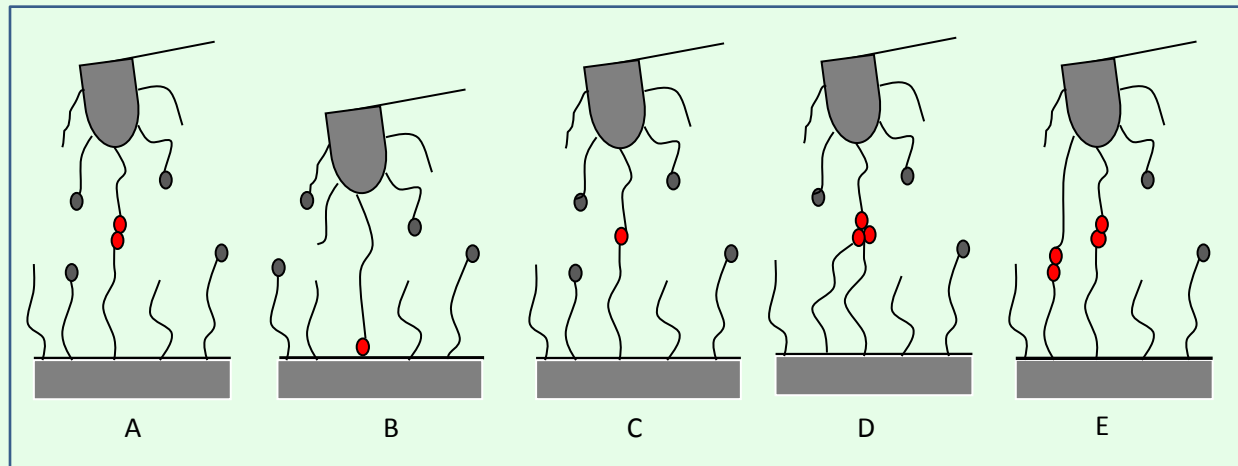
- $$f_p = f_c \ln\left(\frac{r_f}{k_{off}^0 f_c}\right)$$



Force Measurements (Biotin-Avidin)



- Three examples of typical force curves, which read rupture forces and predict different scenarios of molecules interaction.

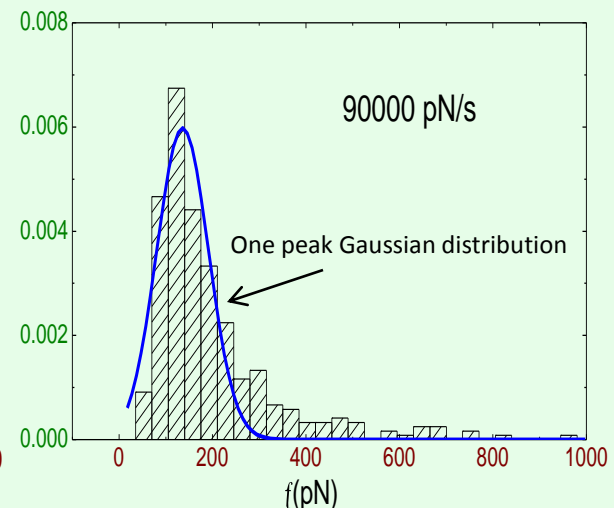
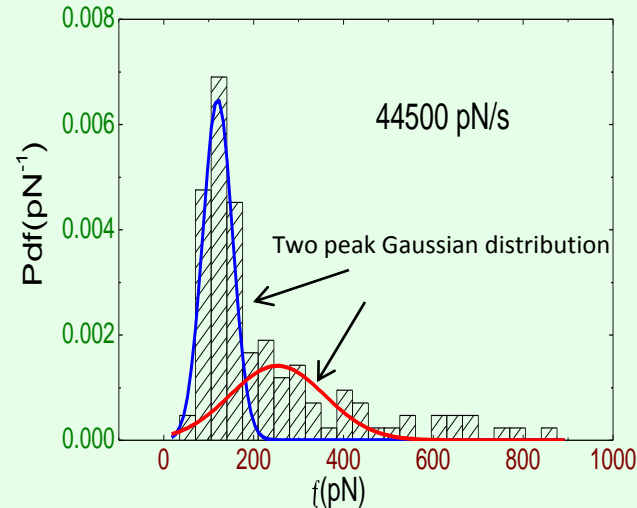
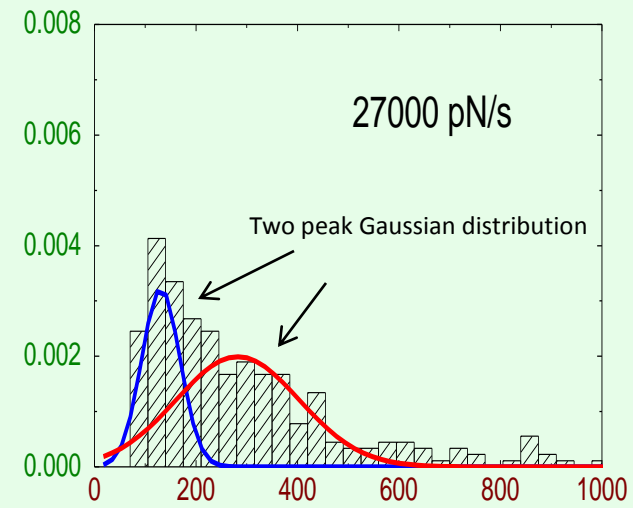
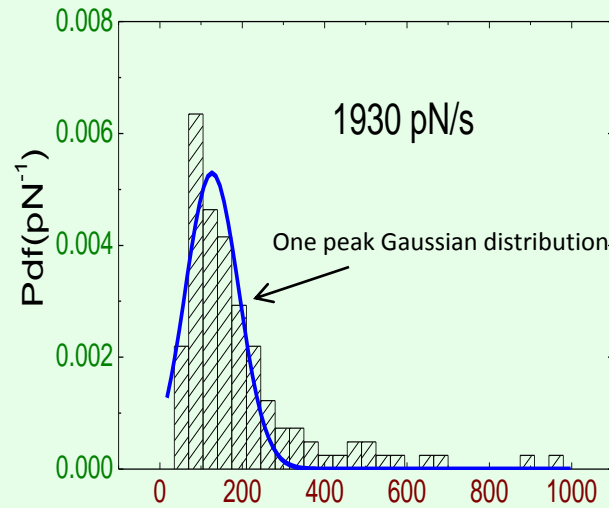


Force Measurements (Biotin-Avidin)

- Rupture force histograms, and Gaussian fitting.

- Pdf: probability distribution function.

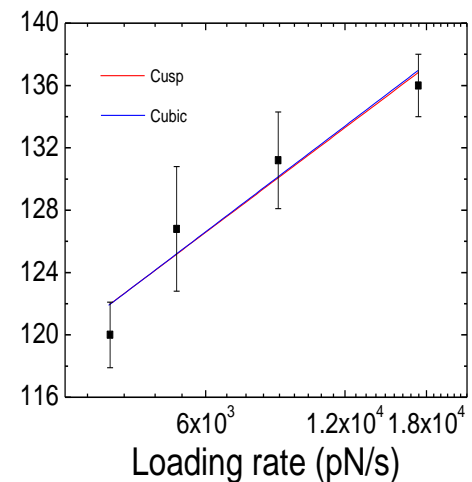
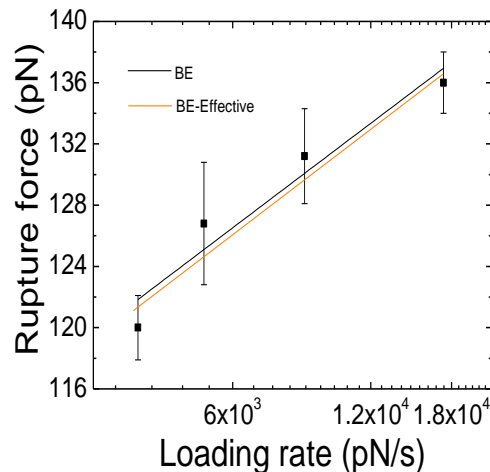
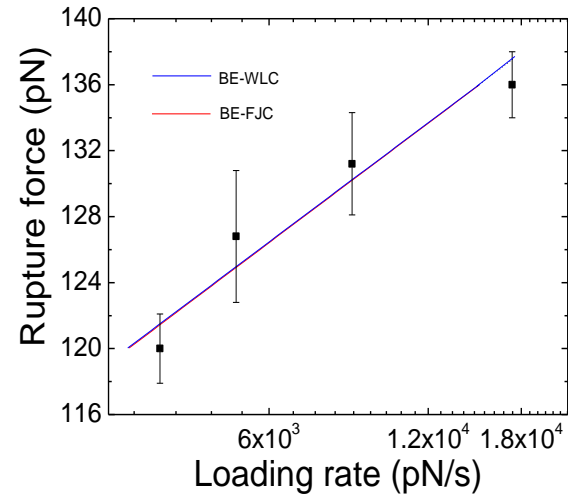
$$\text{Probability} = \int_{f_1}^{f_2} \text{pdf}(f) df$$



Results (Biotin-Avidin)

- Extracted values of the bond length (x^*), kinetic off rate (k_{off}^0) and activation barrier (E_b^0) for the complex bond Biotin-Avidin using the six different models discussed in the text.

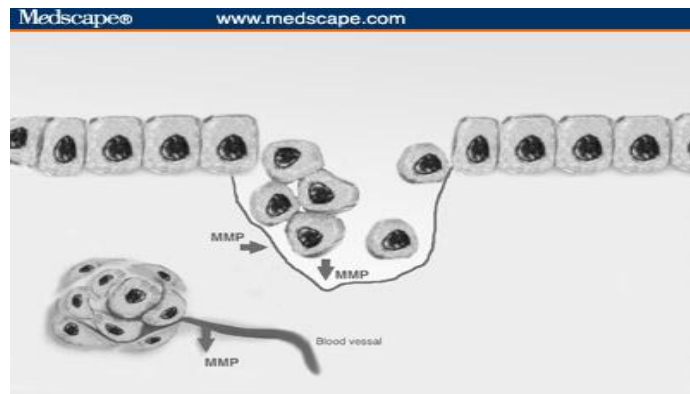
Model	$x^*(\text{nm})$	$k_{off}^0 (\text{s}^{-1}) \times 10^{-3}$	E_b^0 (kJ/mole)
Standard theory	0.42±0.07	8.32±2.6	
BE-Effective	0.42±0.07	1.6±0.5	72.70±0.77
Cusp	0.43±0.06	1.2±0.3	73.41±0.62
Cubic	0.43±0.06	1.4±0.4	73.03±0.70
BE-FJC	0.40±0.70	2.4±1.2	71.70±1.23
BE-WLC	0.41±0.07	1.1±0.7	73.63±1.50



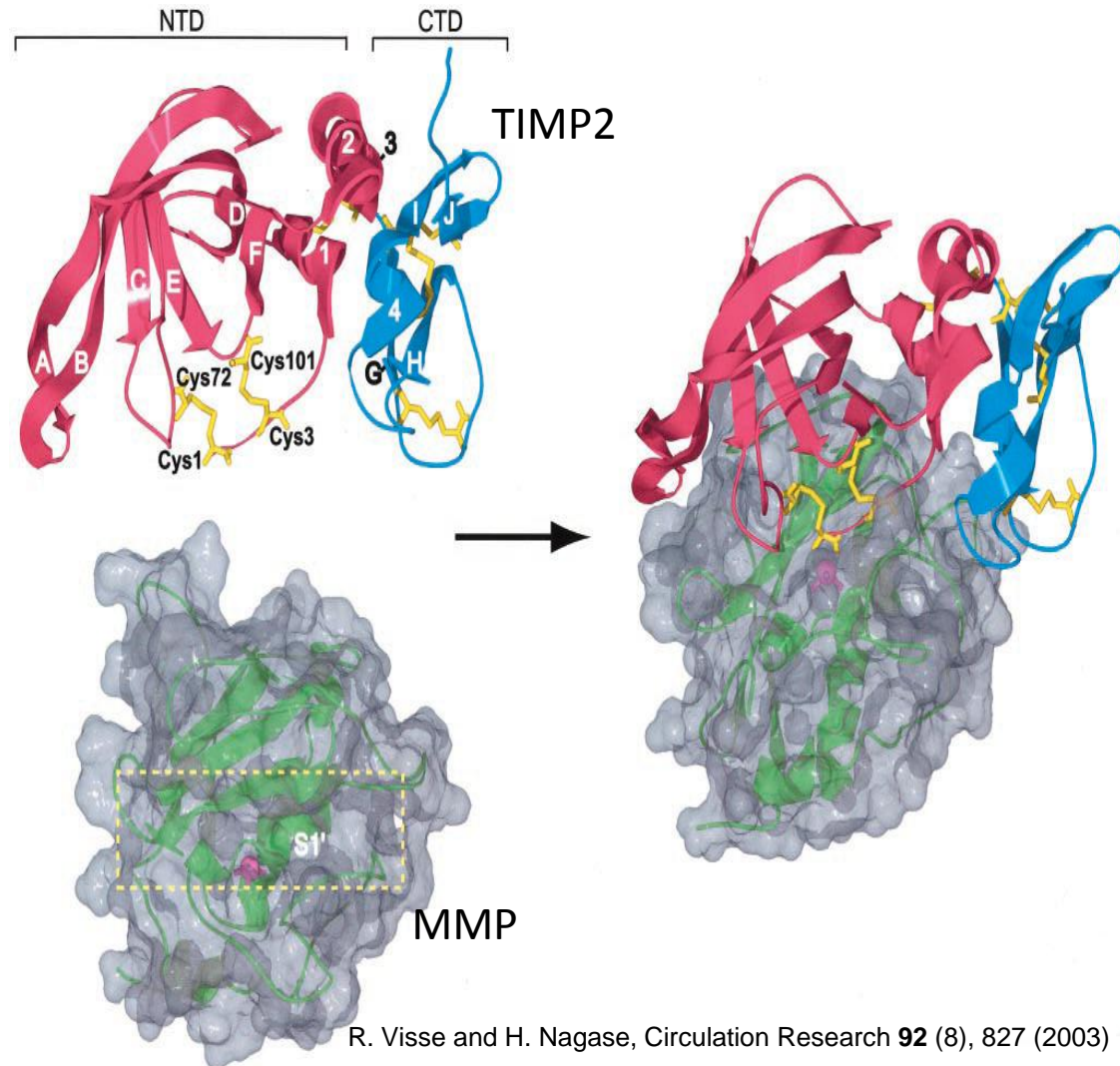
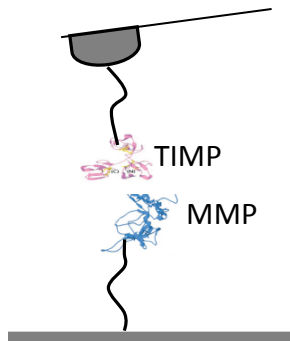
Force Measurements (TIMP-MMP)

MMP: Matrix metalloproteinases

TIMP: Tissue inhibitor matrix metalloproteinases



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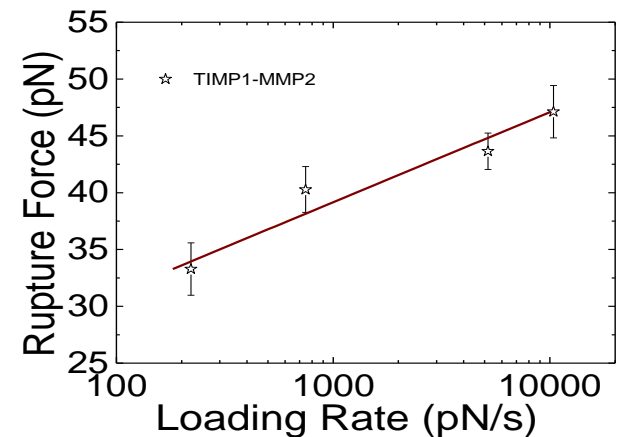
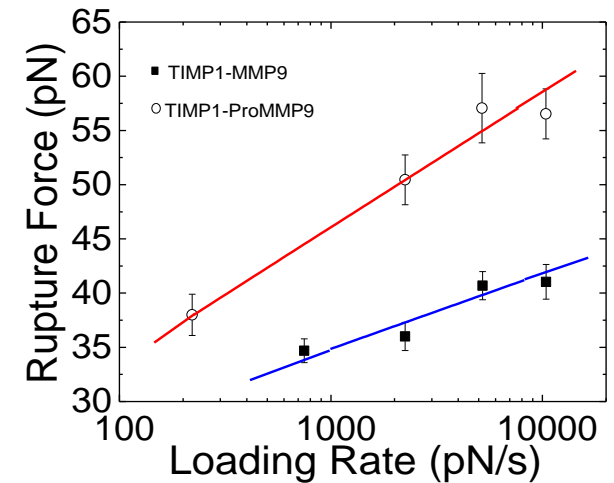


R. Visse and H. Nagase, *Circulation Research* **92** (8), 827 (2003)

Results (TIMP1-MMP)

- Kinetic Off Rates, bond lengths and activation energies of TIMP1 and MMP as given by BE-WLC.

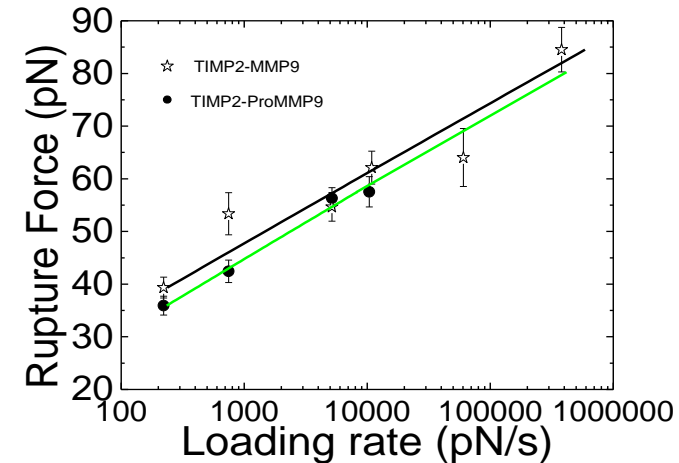
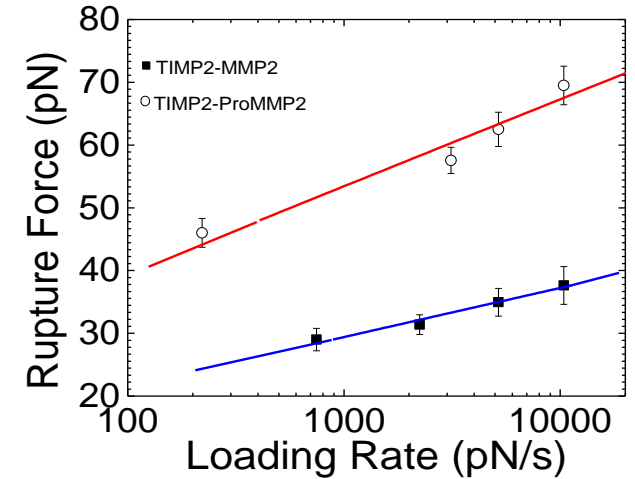
Complex bond	k_{off}^0 (s ⁻¹) × 10 ⁻³	x^* (nm)	E_b^0 (kJ/mole)
TIMP1-MMP2	1.6±0.1	1.2±0.20	72.7±0.5
TIMP1-ProMMP2	No binding	No binding	No binding
TIMP1-MMP9	1.4±0.1	1.4±0.30	73.0±0.5
TIMP1-ProMMP9	14.0±1.7	0.83±0.10	67.4±0.9



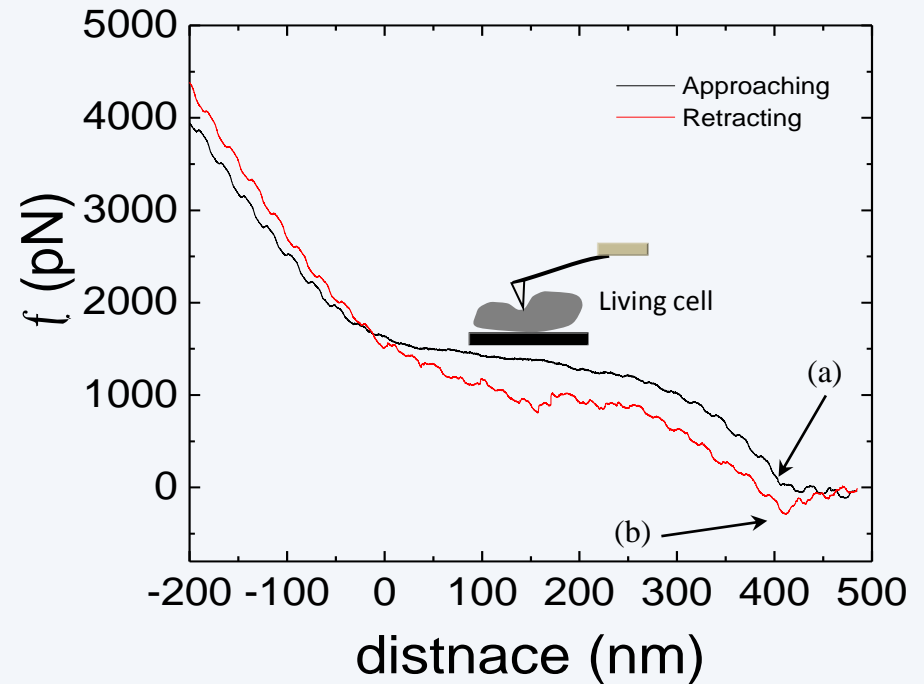
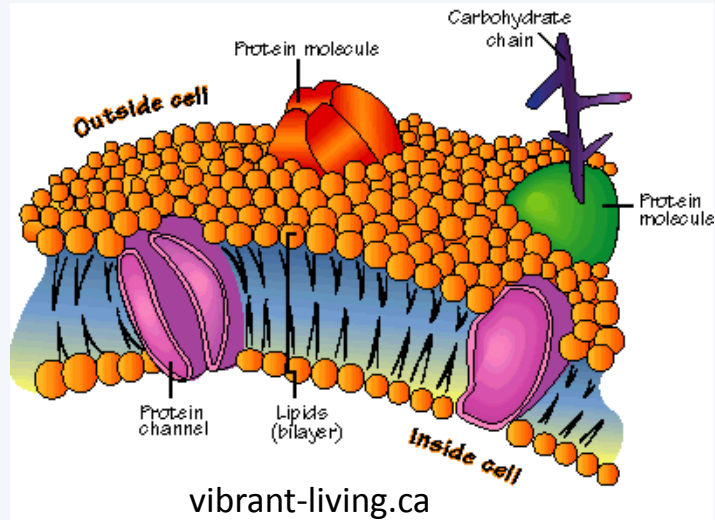
Results (TIMP2-MMP)

- Kinetic Off Rates, bond lengths and activation energies of TIMP2 and MMP as given by BE-WLC.

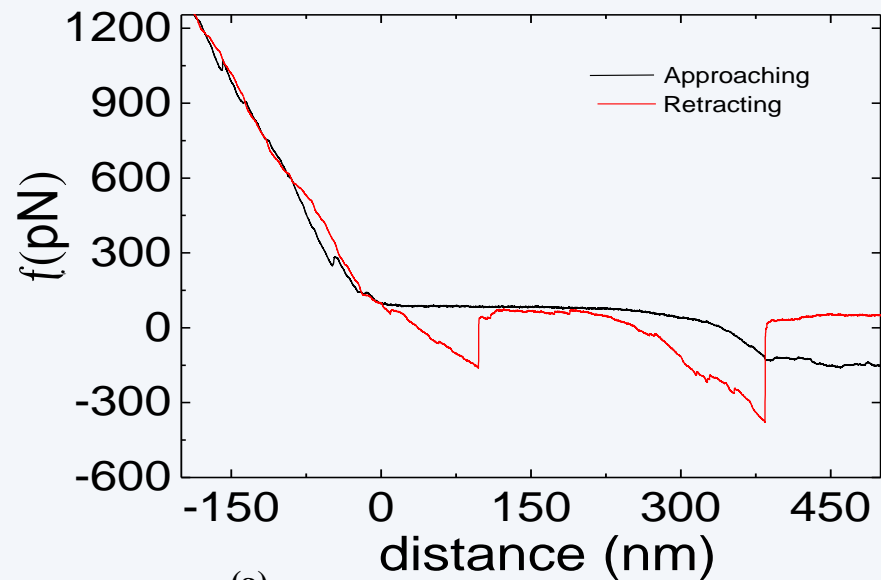
Complex bond	$k_{off}^0 (s^{-1}) \times 10^{-3}$	$x^* (nm)$	$E_b^0 (kJ/mole)$
TIMP2-MMP2	9.7±1.1	0.72±0.11	68.3±0.8
TIMP2-ProMMP2	19.4±2.5	1.25±0.13	66.5±1.0
TIMP2-MMP9	18.7±2.7	0.75±0.11	66.6±1.1
TIMP2-ProMMP9	40.0±5.4	0.72±0.06	64.8±1.0



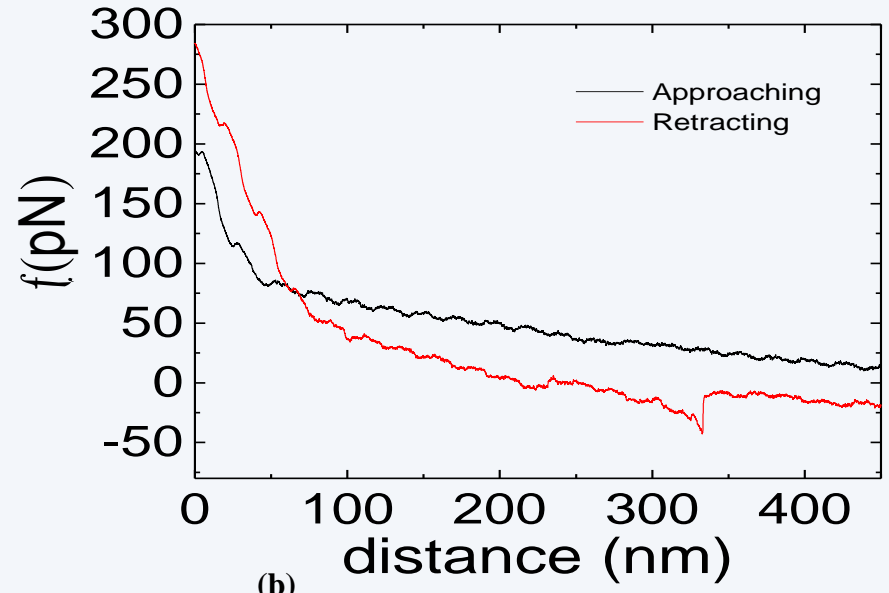
Force Measurements (TIMP-MT1-MMP) Living Cells



Force Measurements (TIMP-MT1-MMP) Living Cells



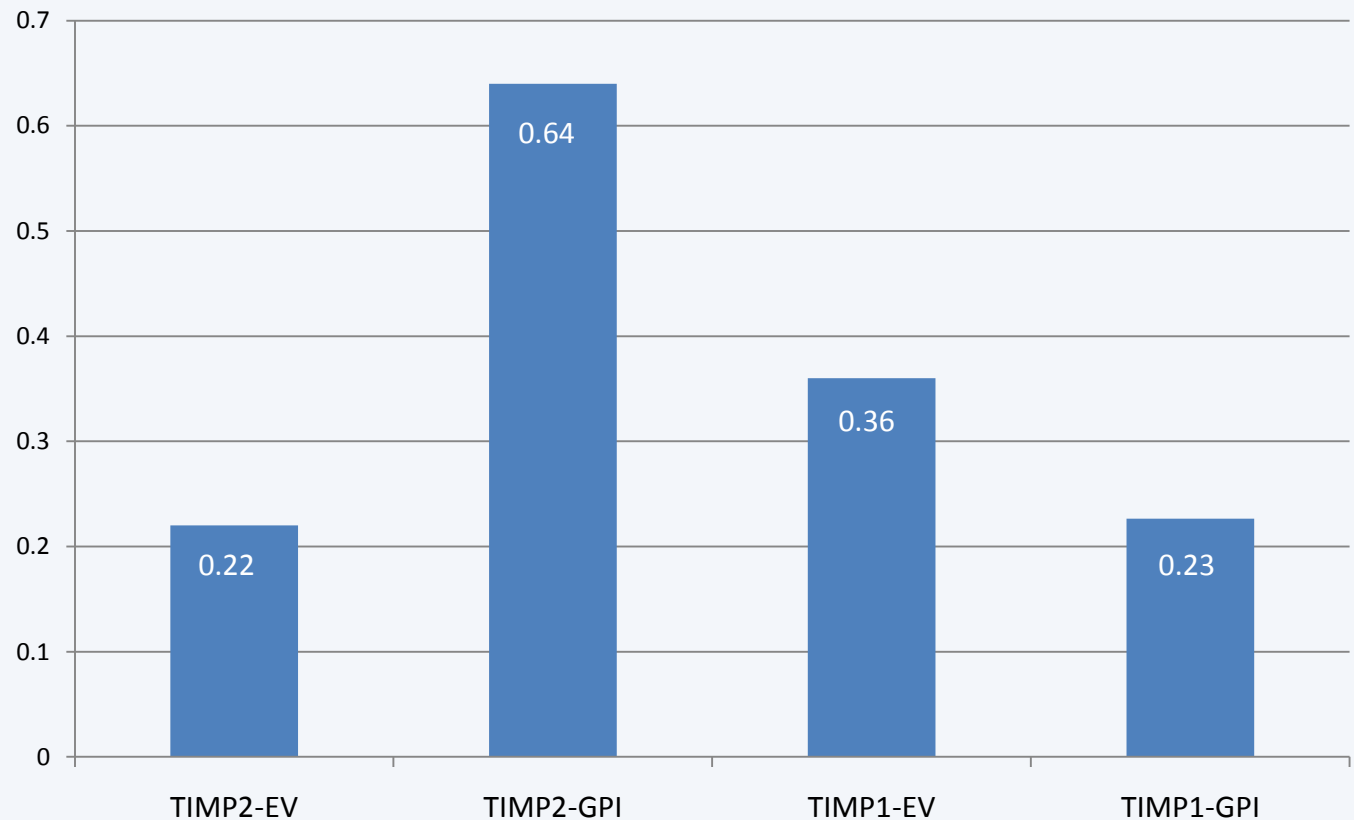
(a)



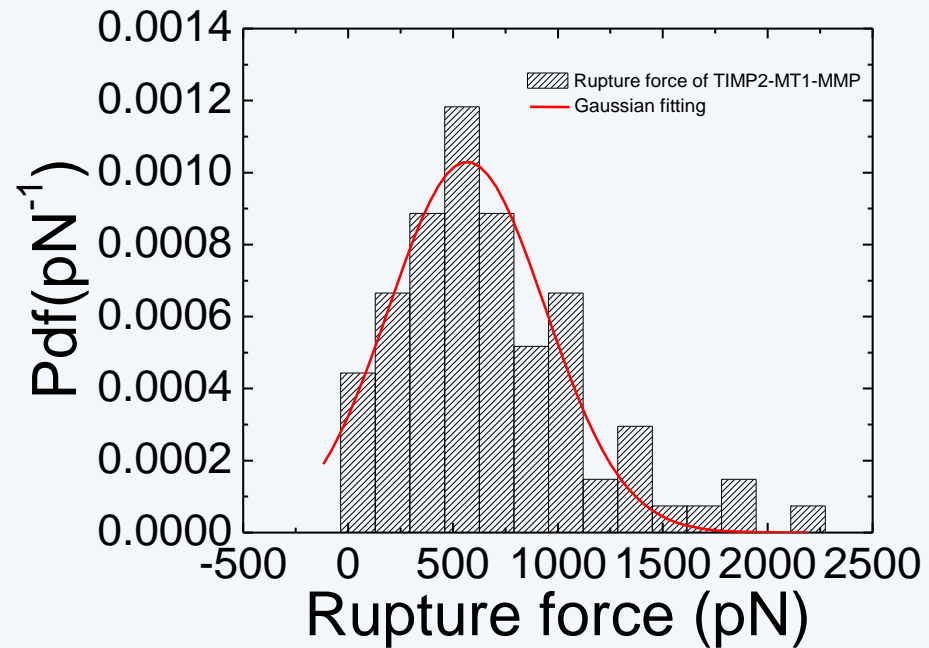
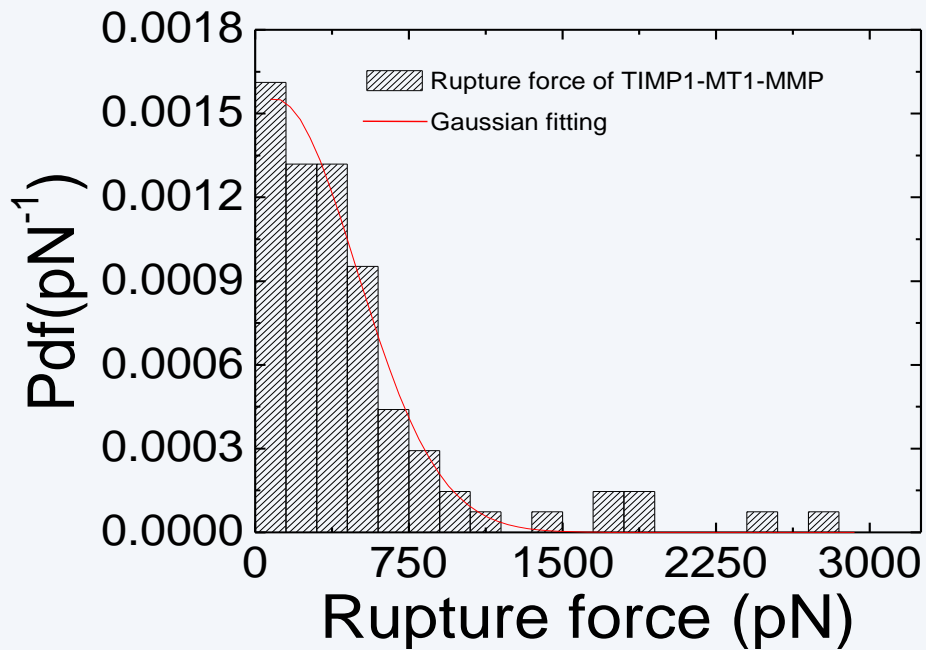
(b)

Results(TIMP-MT1-MMP) Living Cells

- Binding probability of TIMP1, TIM2 to the receptors MT1-MMP using two types of cells, GPI and EV.



Results(TIMP-MT1-MMP) Living Cells



Conclusions

- The AFM can investigate proteins interaction and provides useful parameters.
- Using PEGs as cross linkers promotes the dissociation of the proteins bond.
- The TIMP2 inhibitor binds all MMP enzymes including MMP2, ProMMP2, MMP9 and ProMMP9. The TIMP1 inhibitor binds MMP9, ProMMP9 and MMP2, but it does not bind ProMMP2. The complex bond TIMP-MMP slowly dissociates with rate 10^{-3} s^{-1} , which indicates to high affinity.
- Unlike TIMP1, TIMP2 binds the receptors MT1-MMP on the membrane of living cell. The strength of the bond TIMP2-MT1-MMP is several hundreds of pico-Newtons

*And last but not the least, THANKS TO the
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Dr. George Matei



Dr. Mircea Pantea



Venkatesh Subba Rao



Dr. Shah Khan



Jason Esmacher



Lindsay Runyan