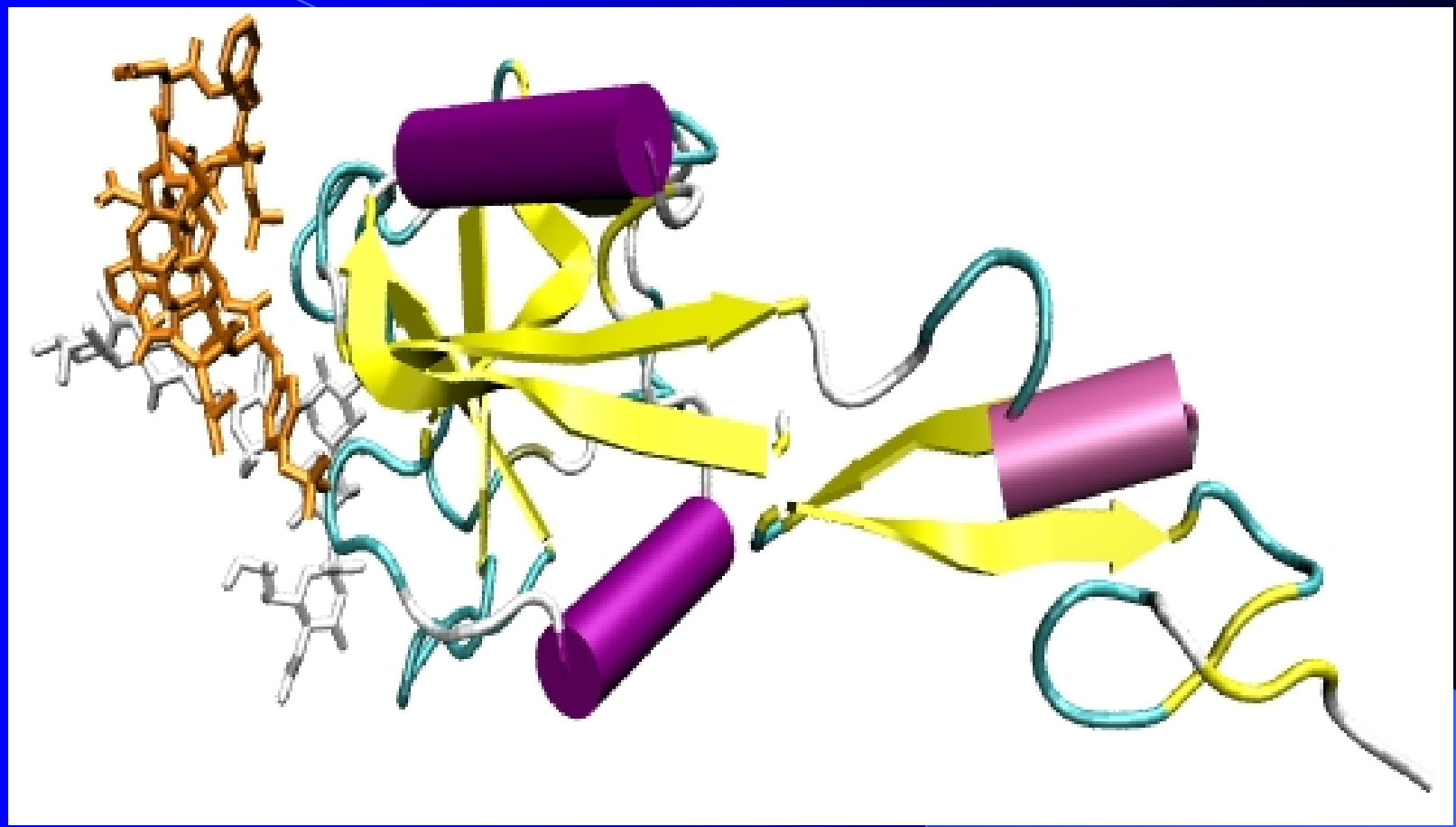
Single molecule studies of cell adhesion complexes: transition from *"catch"* to *"slip"* bonds



Outline:

1. Single molecule spectroscopy of protein-protein interaction:

- pulling force measurements (AFM, observables)
- recent applications

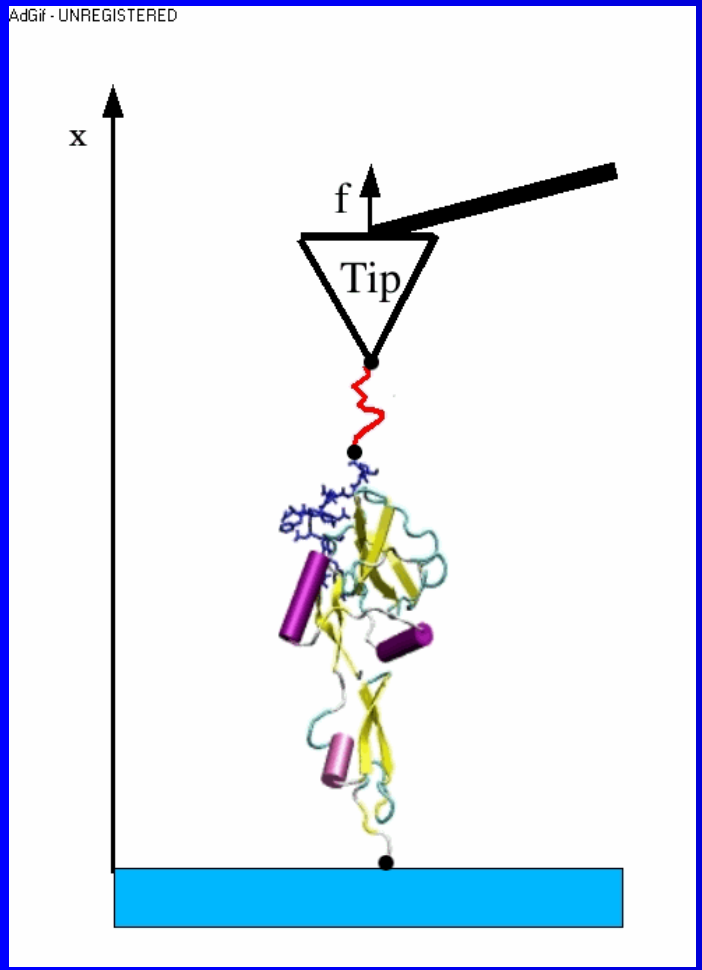
2. Dynamics of cell-adhesion complexes involving selectins:

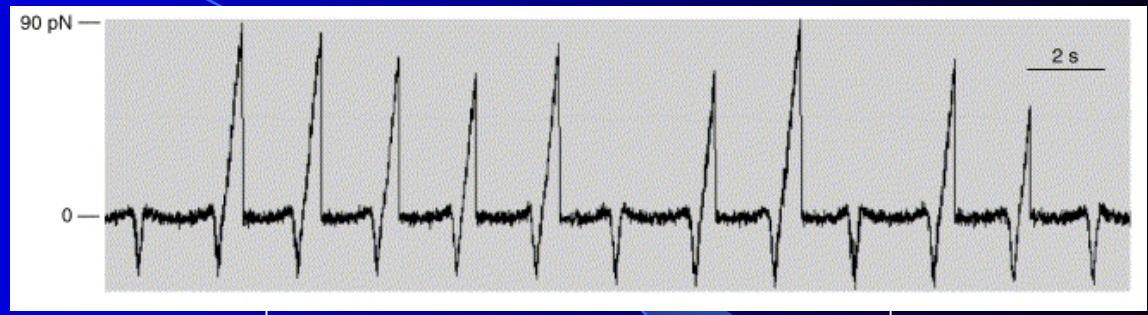
- biological function (rolling of leukocytes)
- AFM assays of forced unbinding (*"catch-slip"*)
- kinetic model

3. Simulations of P-selectin forced unbinding trajectories:

- coarse-grained models of protein complexes
- unbinding dynamics of P-selectin-PSGL-1 complex

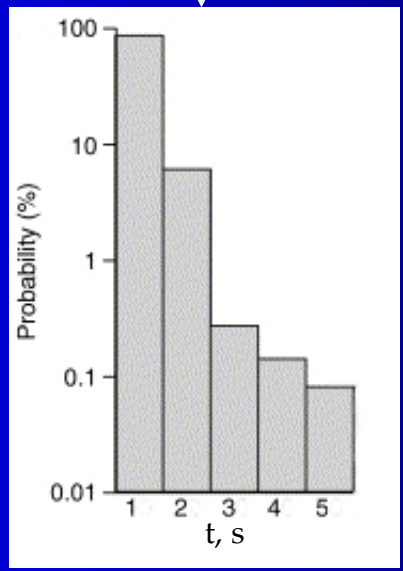
1. Single molecule spectroscopy of protein-protein interactions:
Protein unbinding assays employing pulling force

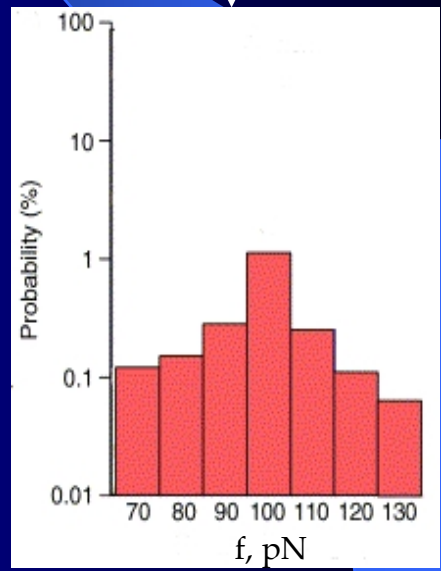




f-constant

$f(t)=r_f t$





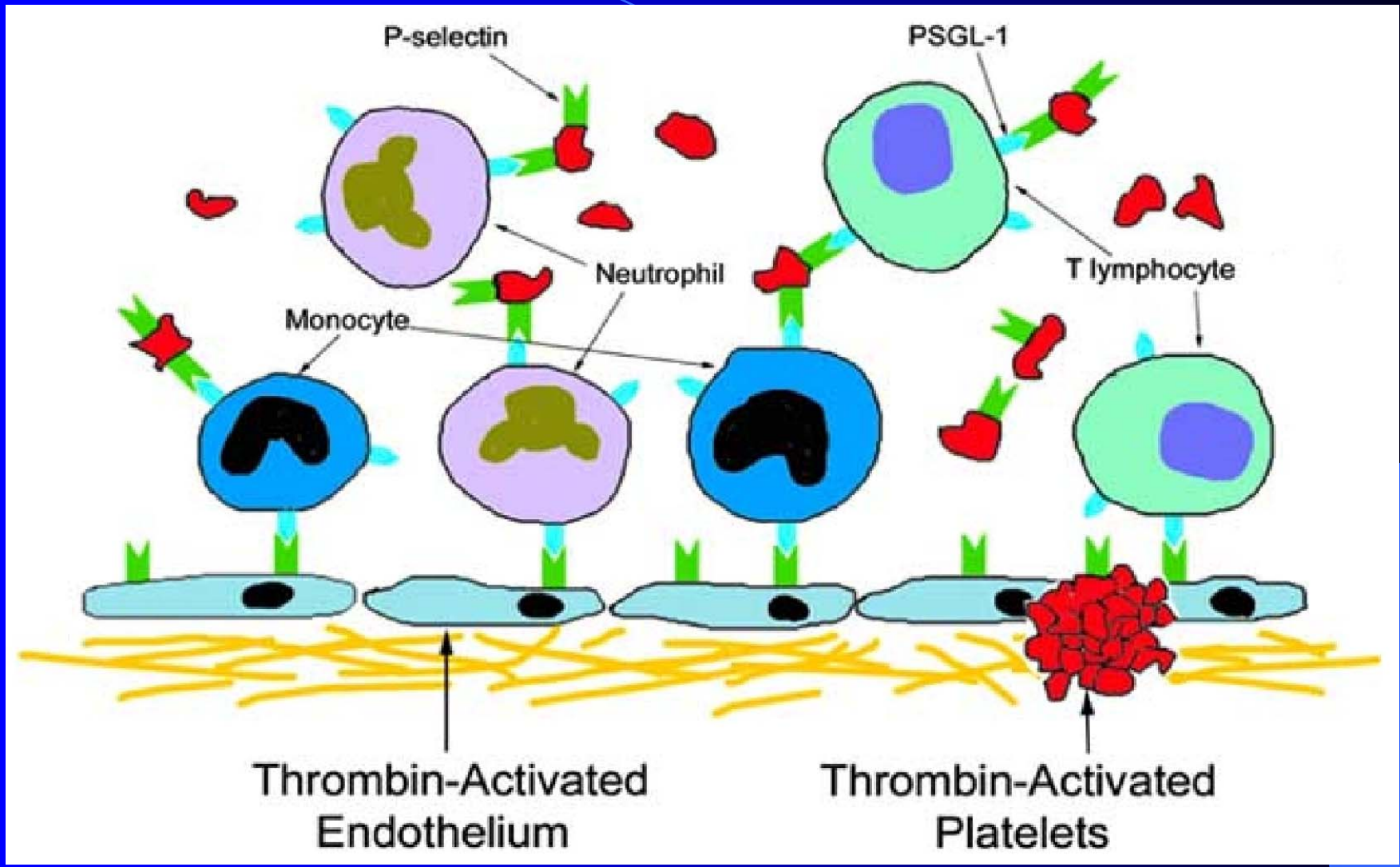
J. Weisel, H. Shuman, R. Litvinov, Curr Opin Struct Biol, ***13****, 227 (2003); M. Schlierf, H. Li, J. Fernandez, PNAS,* ***101****, 7299 (2004); J. Liphardt, D. Smith, C. Bustamante, Curr Opin Struct Biol,* ***19****, 279 (2000); J.-F. Allemand, D. Bensimon, V. Croquette, ibid,* ***13****, 266 (2003); S. Weiss, Science,* ***283****, 1676 (1999); E. Evans, PNAS,* ***98****, 3784 (2001)*

Recent studies of forced single molecule protein-protein unbinding

Receptor* and receptor-bearing surface	Ligand* and ligand-bearing surface	Measuring technique	Reported loading or shear rate/stress	Rupture forces (pN)
Adhesion molecules				
GPIIb-IIIa ($\alpha_{IIb}\beta_3$)	Fibrinogen	Hydrodynamic flow	0.6–2.9 N/m^2 shear stress	70–150 (15.6%)
Covalently bound to latex beads	Free in solution			150–230 (16%)
				230–310 (17%)
$\alpha_{IIb}\beta_3$	GSSSGRGDSPA	AFM	12 nN/s	~93
On native adherent platelets	Covalently bound to tips via glutaraldehyde			
$\alpha_{IIb}\beta_3$	Fibrinogen	LT	20 nN/s	60–150,
On resting or activated native adherent platelets	Covalently bound to latex beads via carbodiimide			
Covalently bound to modified silica beads via glutaraldehyde				
$\alpha_V\beta_3$	GRGDSP	AFM	30 nN/s†	42 ± 4
$\alpha_5\beta_1$	GRGDSP			32 ± 2
$\alpha_V\beta_3$	Osteopontin			50 ± 2
$\alpha_V\beta_3$	Echistatin			97 ± 15
All on adherent osteoclasts partly fixed with paraformaldehyde	All adsorbed on tips via noncovalently bound PEG			
GP Ib-IX ($\alpha\beta$IX)	von Willebrand factor (vWF)	LT	Not reported	6.5 ± 0.8
				8.8 ± 0.3
$\alpha\beta$IX	Ultralarge vWF			11.4 ± 2.1
$\alpha\beta$IX	A1 domain of vWF			11.5
On native transfected CHO cells	Adsorbed on latex beads			
P-selectin	PSGL-1	AFM	168 nN/s†	159 ± 30
Bound to silanized glass cover slips via biotin–avidin	Bound to silanized tips via biotin–avidin			
P-selectin	PSGL-1	AFM	250 nN/s†	175
Bound to cantilever as Fc-chimera via anti-Fc-Ab	On intact neutrophils			
PSGL-1 and other selectins' ligands	P-, E-, L-selectins or peripheral node addressin	Hydrodynamic flow	0.5–5.0 dyn/cm^2 shear stress	37–250

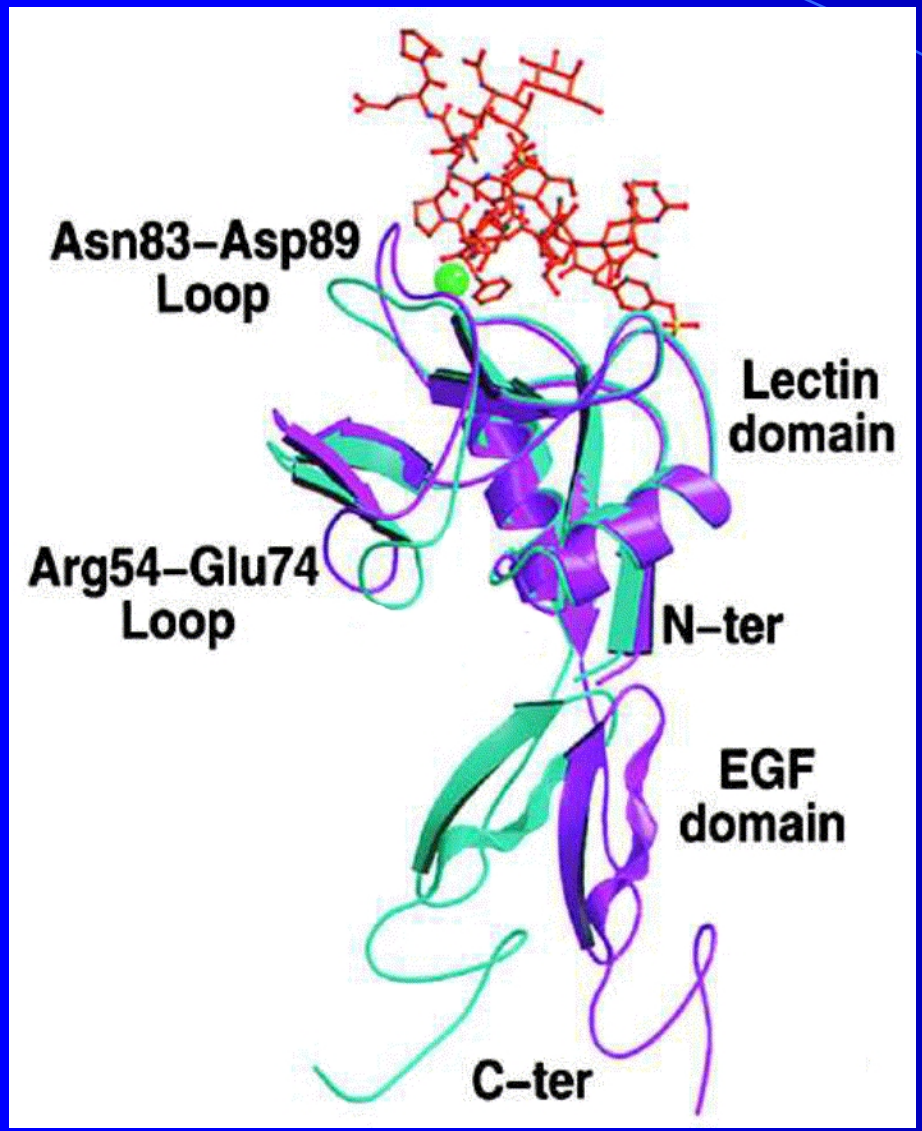
J. Weisel, H. Shuman, R. Litvinov, Curr Opin Struct Biol, ***13****, 227 (2003)*

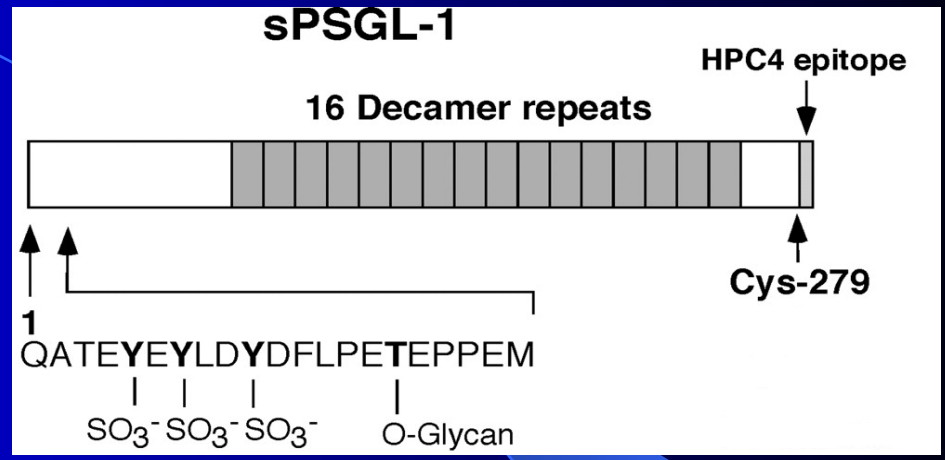
2. Biological functions of selectin cell-adhesion complexes: rolling of leukocytes

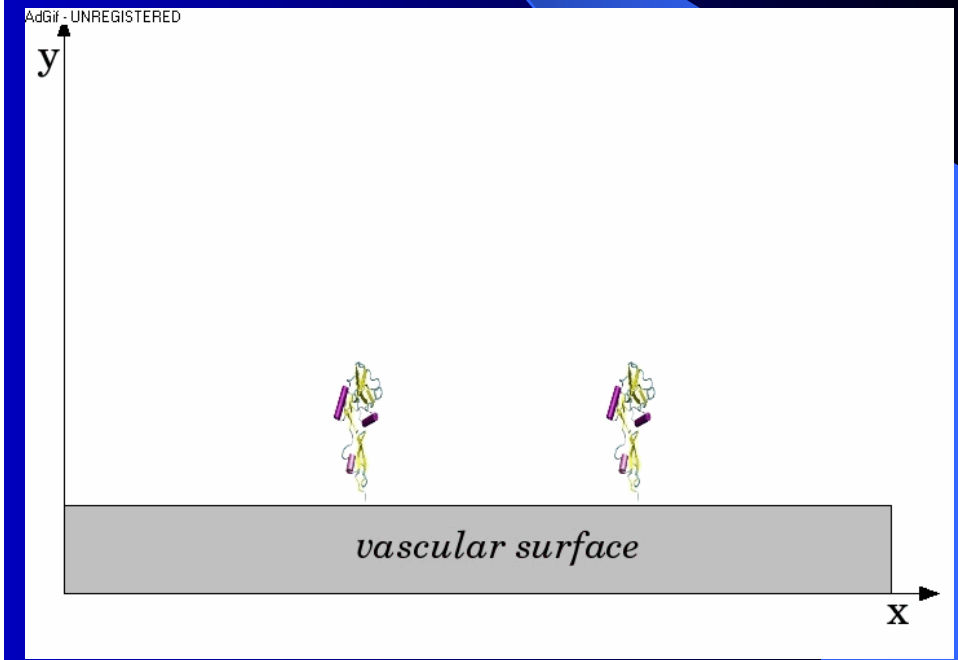


J.-G. Geng, M. Chen, K.-C. Chou, Curr Med Chem, ***11****, 2153 (2004); L. M. Coussens, Z. Werb, Nature,* ***420,*** *860 (2002); Y. J. Kim, L. Borgis, N. M. Varki, A. Varki, PNAS, 95, 9325 (1998)*

2. Dynamics of forced unbinding of P-selectins from PSGL-1



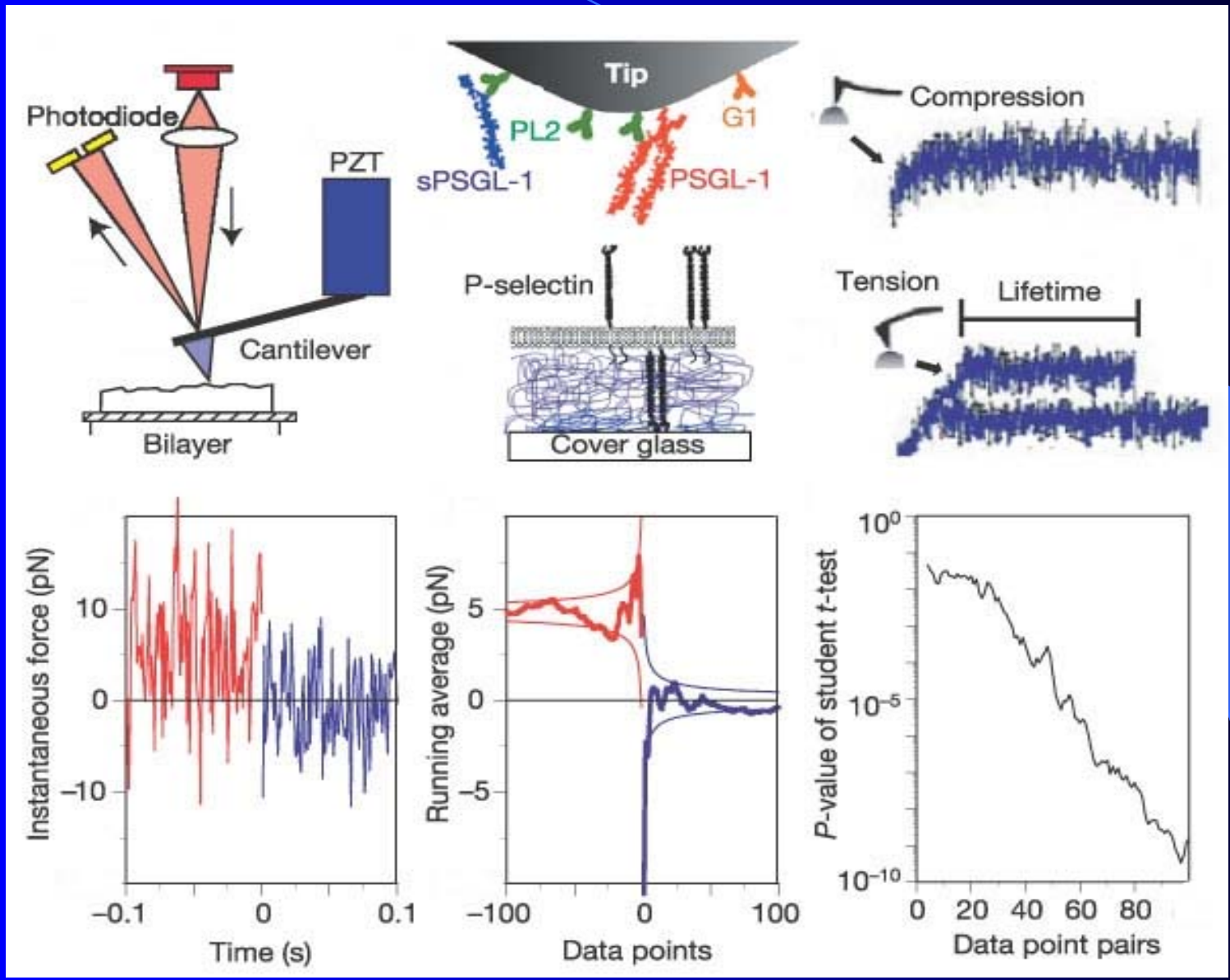




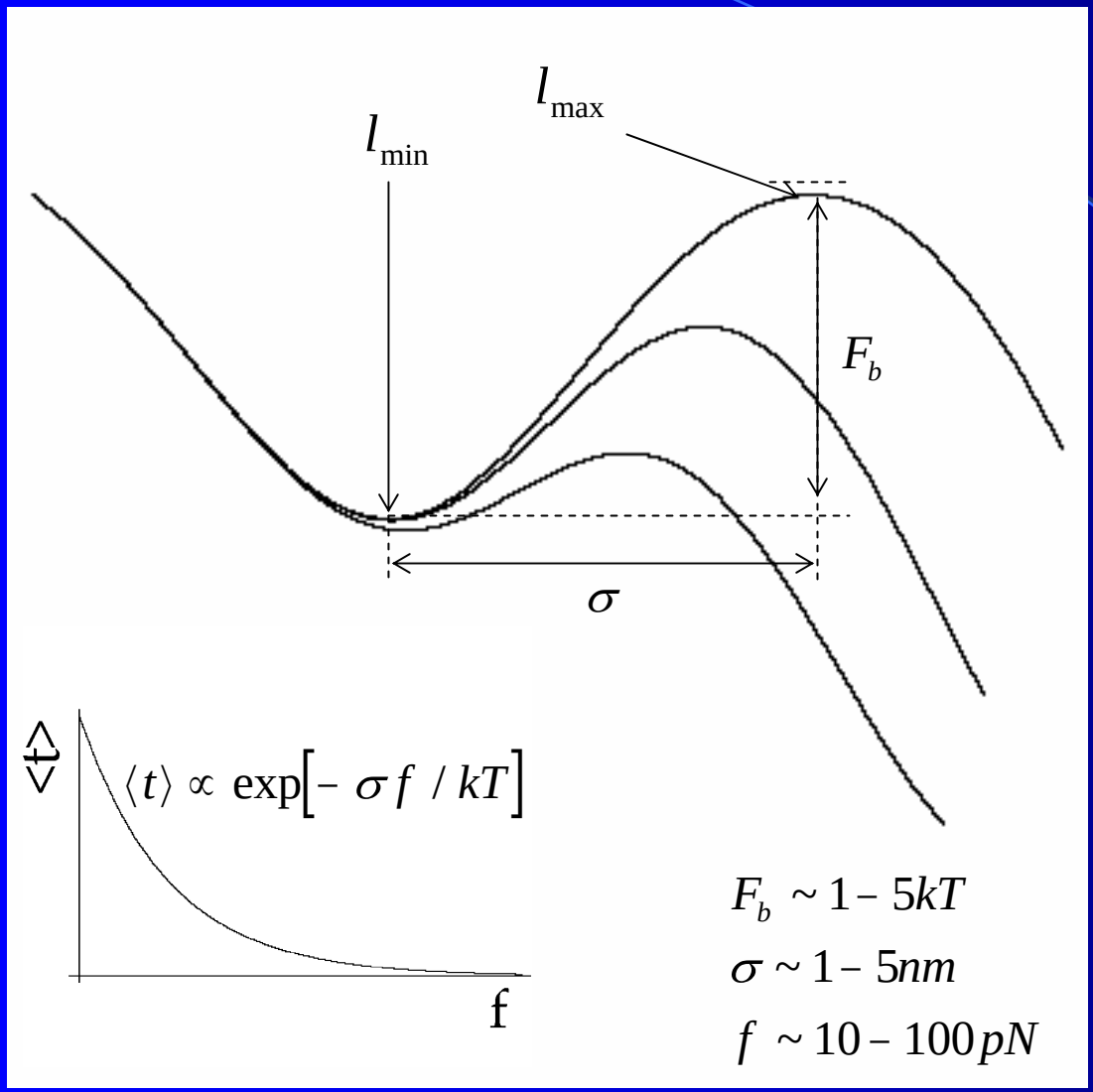
*T. Springer et al, J Cell Biol, **138**, 1169 (1997); R. McEver, R. Cummings, J Clin Invest, **100**, 485 (1997); D. Vestweber, J. Blanks, Physiol Rev, **79**, 181 (1999)*

AFM measurements of forced unbinding of P-selectins

*(B. T. Marshall et al, Nature, **423**, 190, 2003)*



Rate of unbinding under force: Kramers-Smoluchowski theory



f=0-case:

$$k_0 = \frac{D}{l_{min} l_{max}} \exp\left[-F_b / kT\right]$$

$$l_{min,max} = \sqrt{2\pi kT / \kappa_{min,max}}$$

f>0-case:

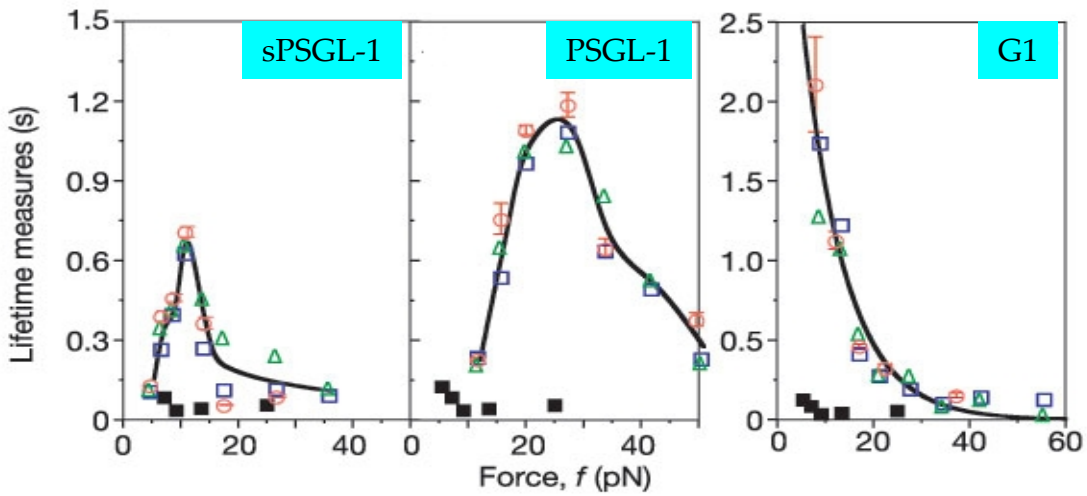
$$F_b \xrightarrow{f} F_b - \sigma \cdot f$$

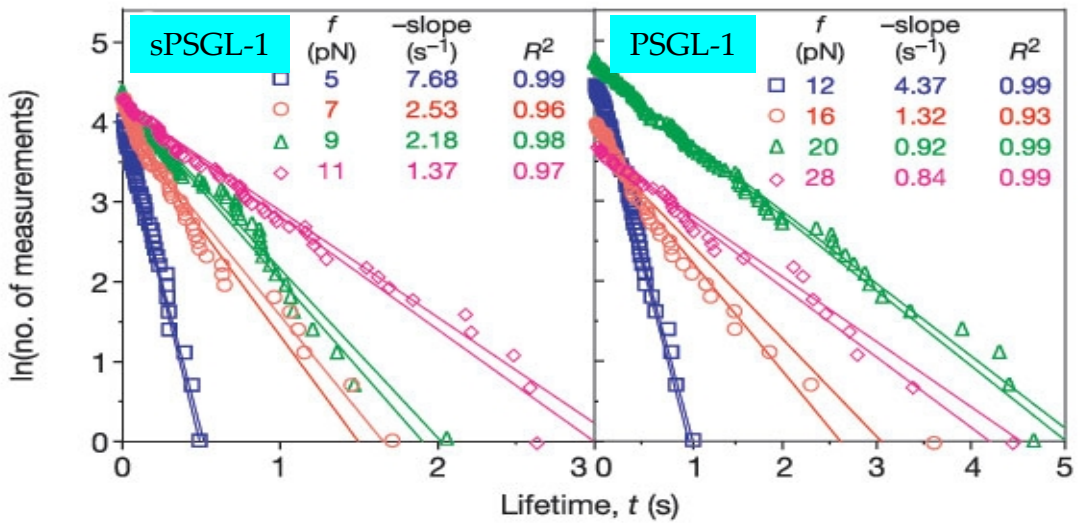
$$k_0 \xrightarrow{f} k = k_0 \exp\left[\sigma f / kT\right]$$

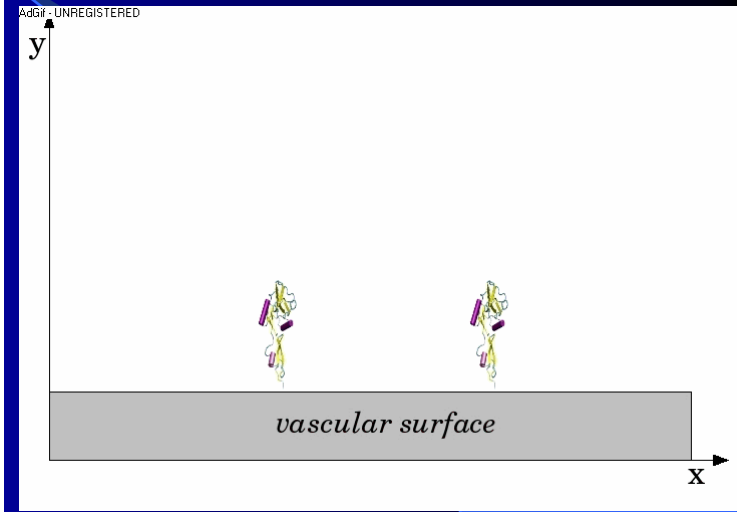
H. Kramers, Physica, ***7****,* ***284*** *(1940); E. Evans & K. Ritchie, Biophys. J.,* ***76****, 2439 (1999); G. Bell, Science,* ***200****,* ***618*** *(1978)*

The average lifetimes measured by AFM:

*B. T. Marshall et al, Nature, **423**, 190, (2003)*



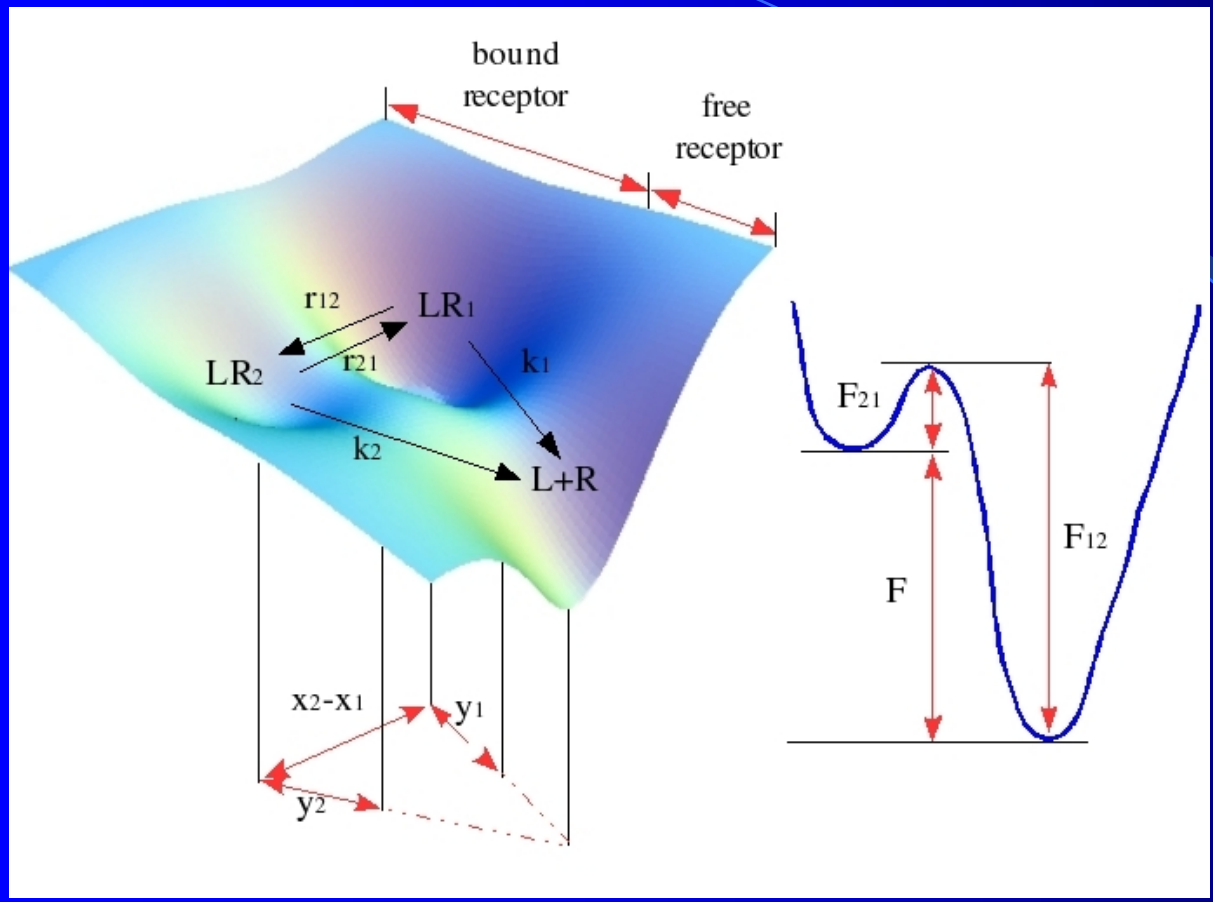




Catch-slip transition couples translational (blood flow) & rotational (rolling) degrees of freedom

*K. Sarangapani et al, J Biol Chem, **279**, 2291 (2004); R. Alon, D. Hammer, T. Springer, Nature, **374**, 539 (1995)*

Two-state kinetics of unbinding of P-selectins: energy landscape



$$r_{12} = r_{10} \exp[-F_{12} / kT]$$
$$r_{21} = r_{20} \exp[-F_{21} / kT]$$

$$k_1 = k_{10} \exp[y_1 f / kT]$$
$$k_2 = k_{20} \exp[y_2 f / kT]$$

$$\frac{r_{10}}{r_{20}} \exp[-F / kT] = K_{eq}$$
$$[LR]_{10} = 1/(K_{eq} + 1),\ [LR]_{20} = K_{eq} / (K_{eq} + 1)$$

f

$$K^*_{eq} = K_{eq} \exp[(x_2 - x_1) f / kT]$$
$$[LR]^*_{10} = 1/(K^*_{eq} + 1),\ [LR]^*_{20} = K^*_{eq} / (K^*_{eq} + 1)$$

V. Barsegov, D. Thirumalai, PNAS, ***102, 1835*** *(2005)*

The average lifetime of P-selectin complex with sPSGL-1 and G1 (constant force experiment)

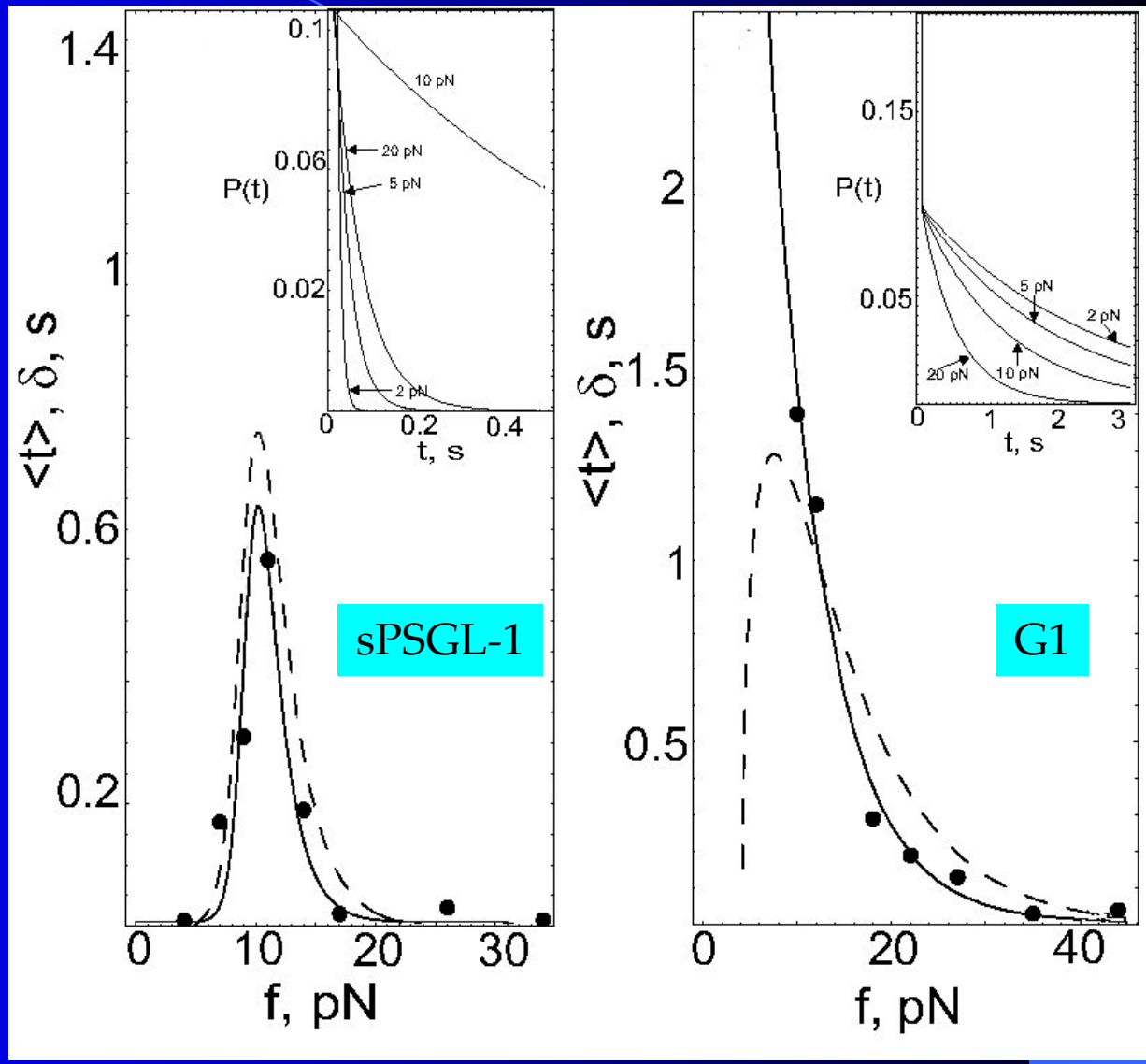
$$\frac{d[LR]_1}{dt} = -(r_{12} + k_1)[LR]_1 + r_{21}[LR]_2$$

$$\frac{d[LR]_2}{dt} = r_{12}[LR]_1 - (r_{21} + k_2)[LR]_2$$

$$[LR] = [LR]_1 + [LR]_2$$

$$\langle t^n \rangle = \int_0^\infty dt\, [LR](t)\, t^n$$

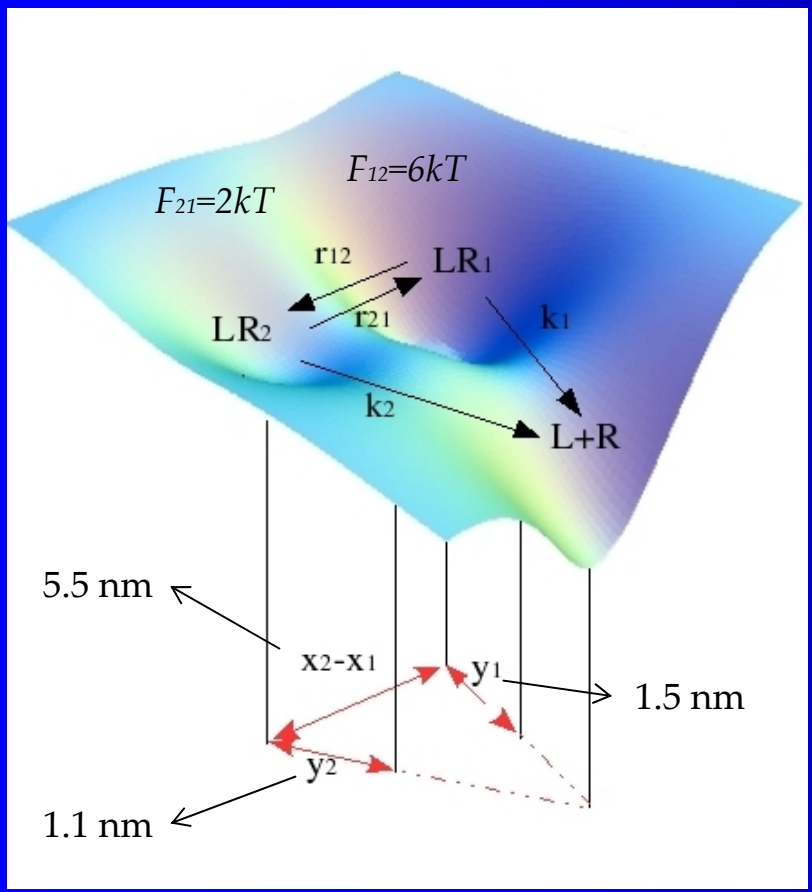
$$\delta(t) = \sqrt{\langle t^2 \rangle - \langle t \rangle^2}$$



Model parameters for P-selectin complex with ligands

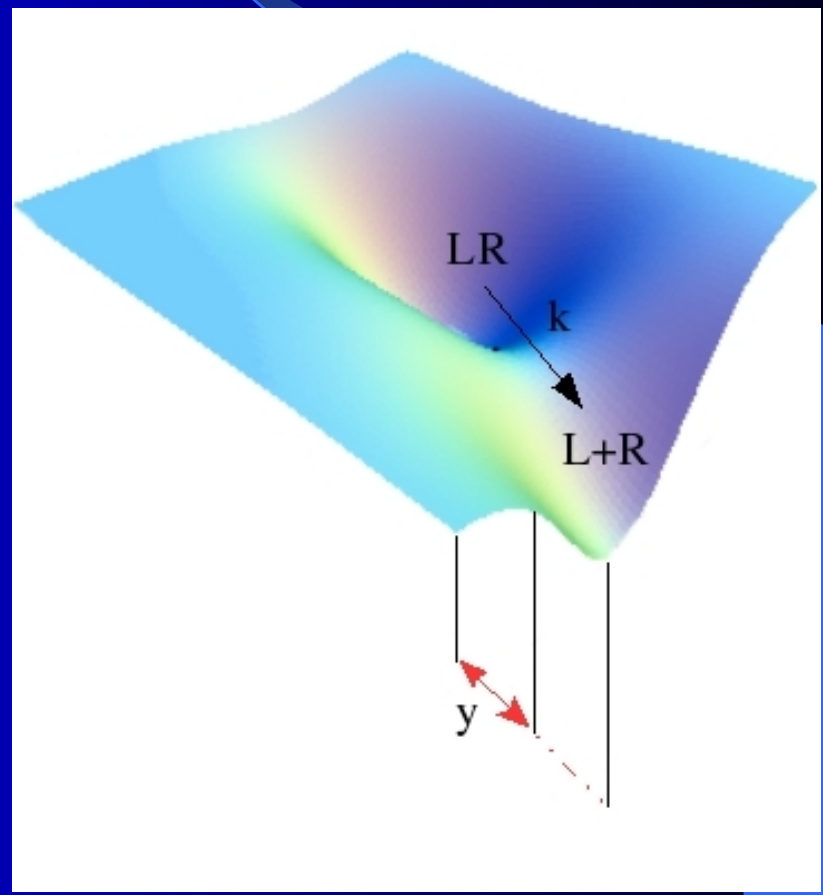
sPSGL-1

$$r_{20} = 40\,s^{-1} >> r_{10} = 5\,s^{-1}$$
$$k_{10} = 100\,s^{-1} >> k_{20} = 0.05\,s^{-1}$$



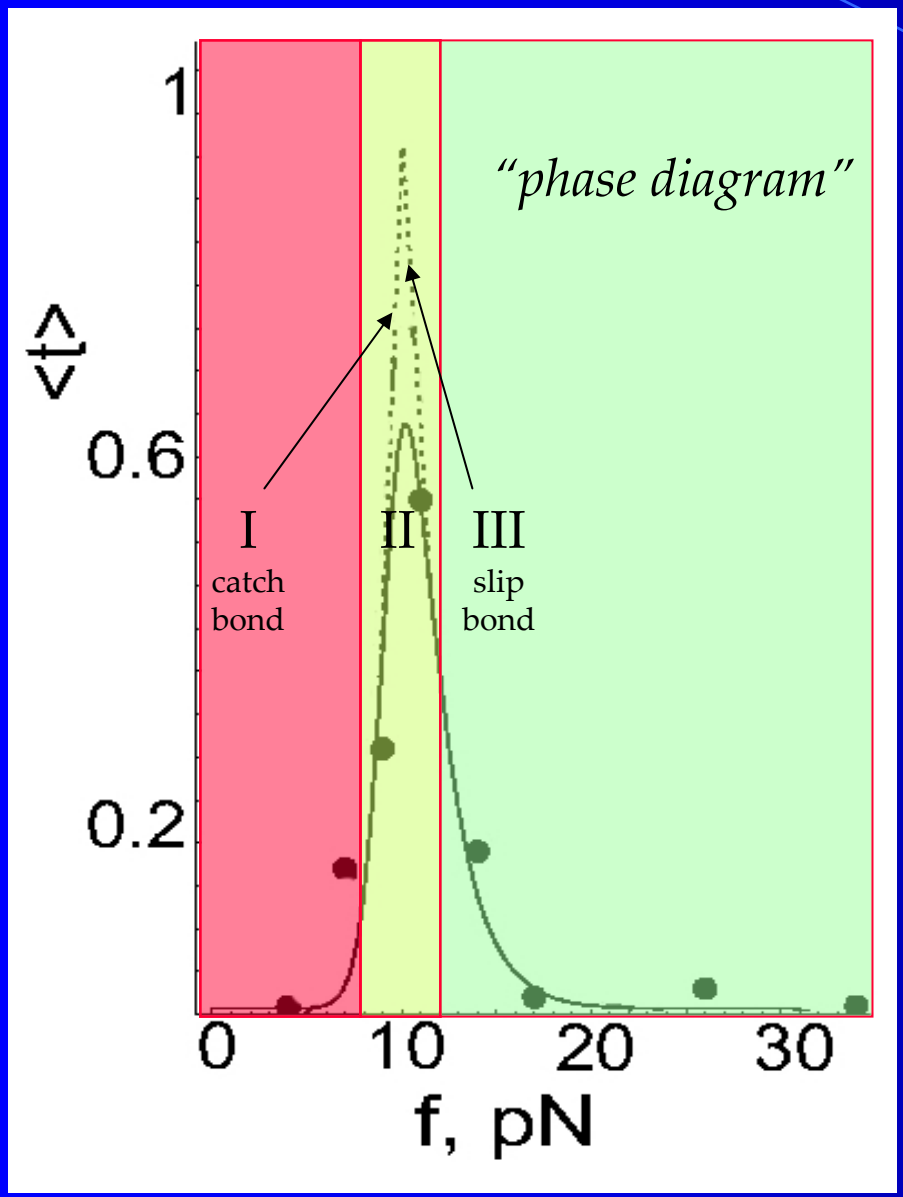
G1

$$r_{20} = r_{10} = 10\,s^{-1}; k_{10} = k_{20} = 0.35\,s^{-1}$$
$$y_1 = y_2 = 0.32\,nm$$



V. Barsegov, D. Thirumalai, PNAS, ***102, 1835*** *(2005)*

Transition from *"catch"* to *"slip"* bonds for P-selectin with sPSGL-1



$$3 \le f \le 10-15\,pN$$

$$r_{12} >> r_{21}, k_1; x_2 - x_1 >> y_1$$

$$k_{eff} = k_1 / K_{eq}^{*} + k_2$$

$f < 3pN$

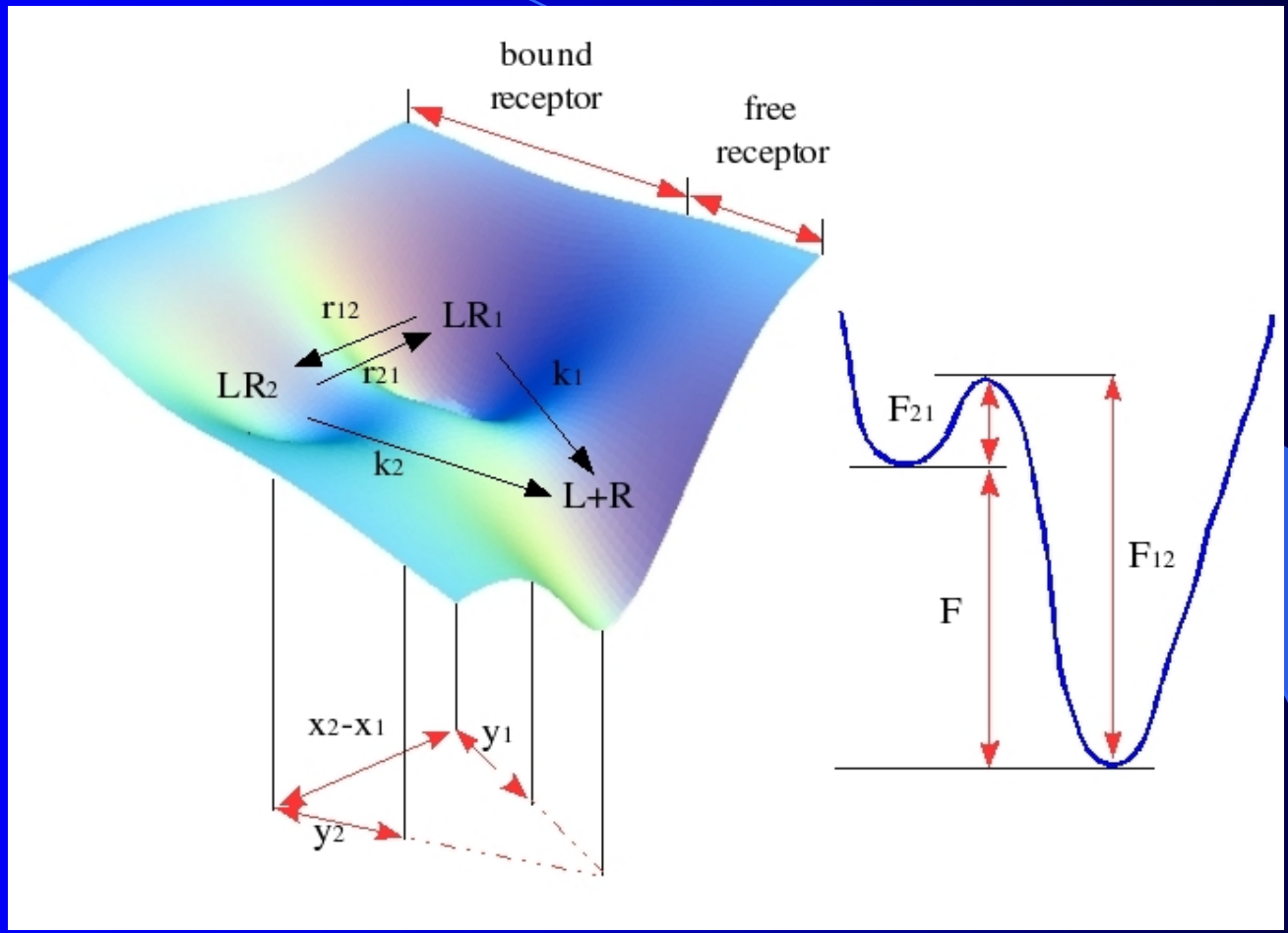
$f > 15pN$

$$k_{catch} \approx k_1 / K_{eq}^{*}$$

$$k_{slip} \approx k_2$$

V. Barsegov, D. Thirumalai, PNAS, ***102, 1835*** *(2005)*

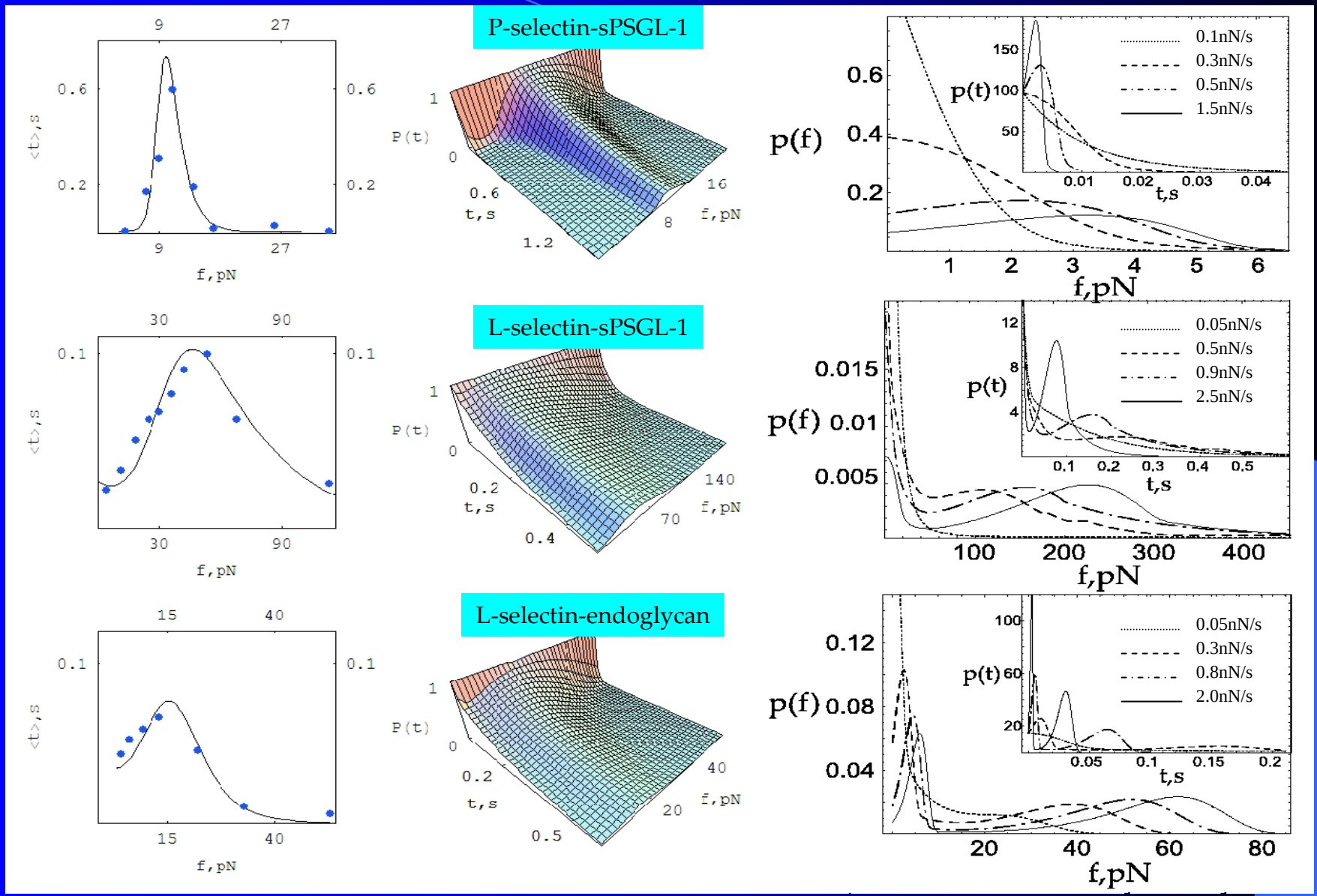
Free-energy landscape of cell adhesion complexes with selectins



1. How general is the two-state model?

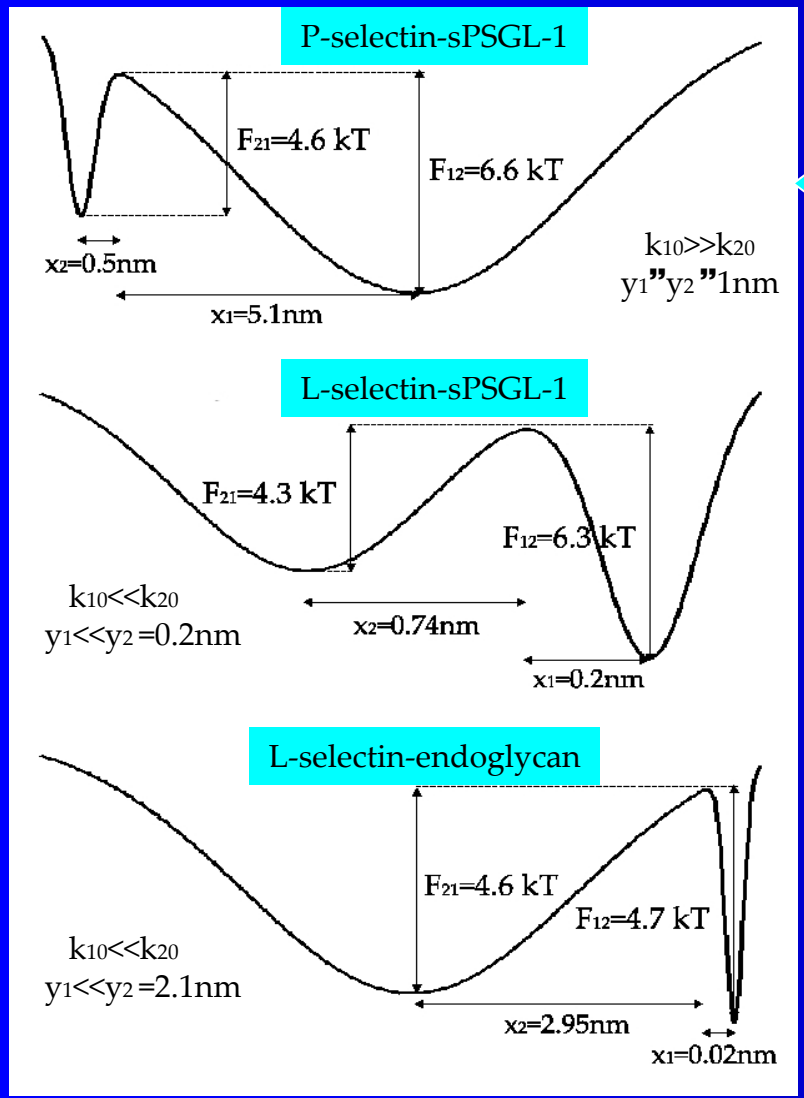
2. What is the molecular origin of catch-slip transition?

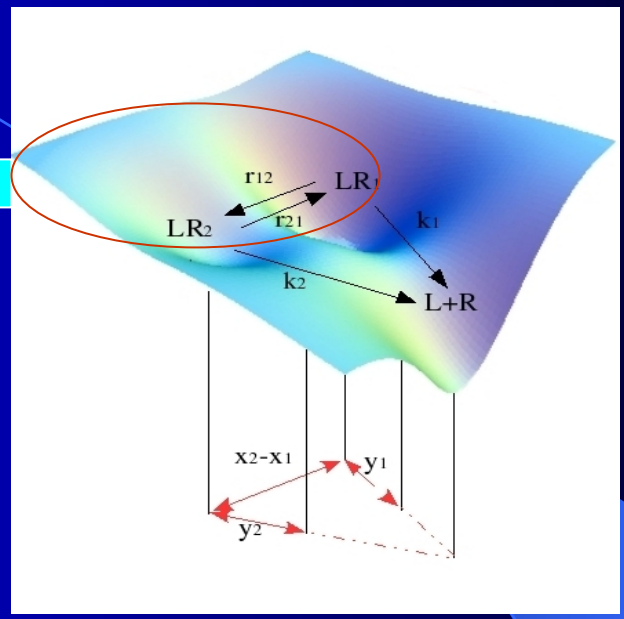
Dynamics of forced unbinding of P- and L-selectins

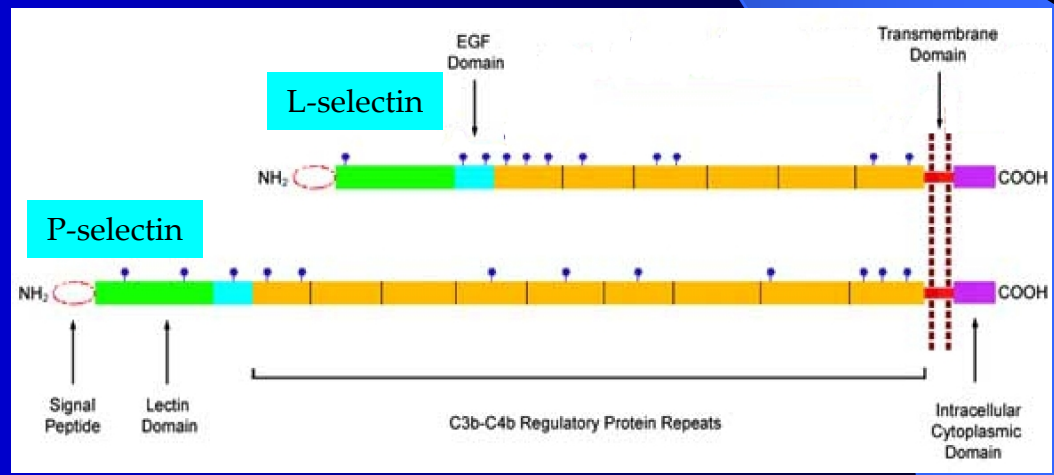


K. K. Sarangapani et al, J Biol Chem, ***279****, 2291 (2004)*

P- and L-selectin binding interface



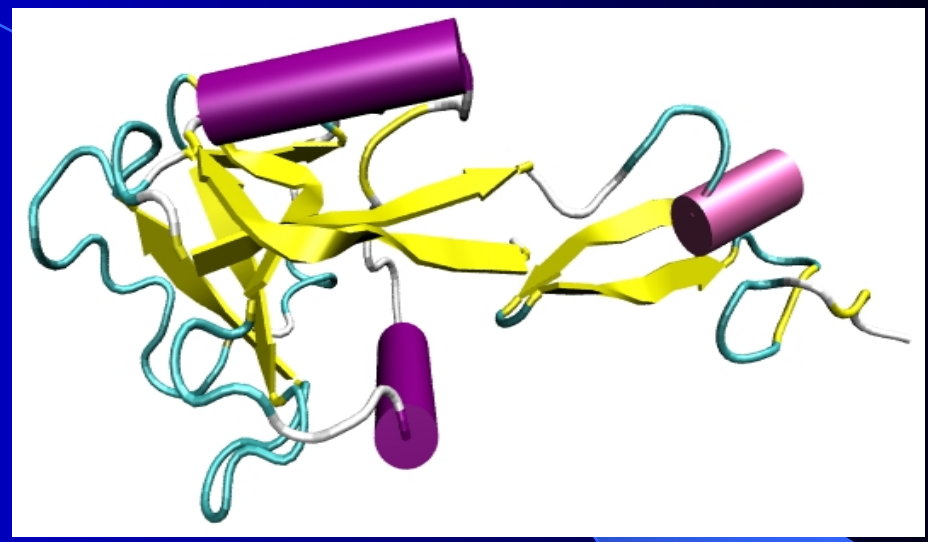




V. Barsegov, D. Thirumalai, manuscript in preparation

3. Computer simulation of P-selectin forced unbinding trajectories: coarse-grained models of protein-protein complexes

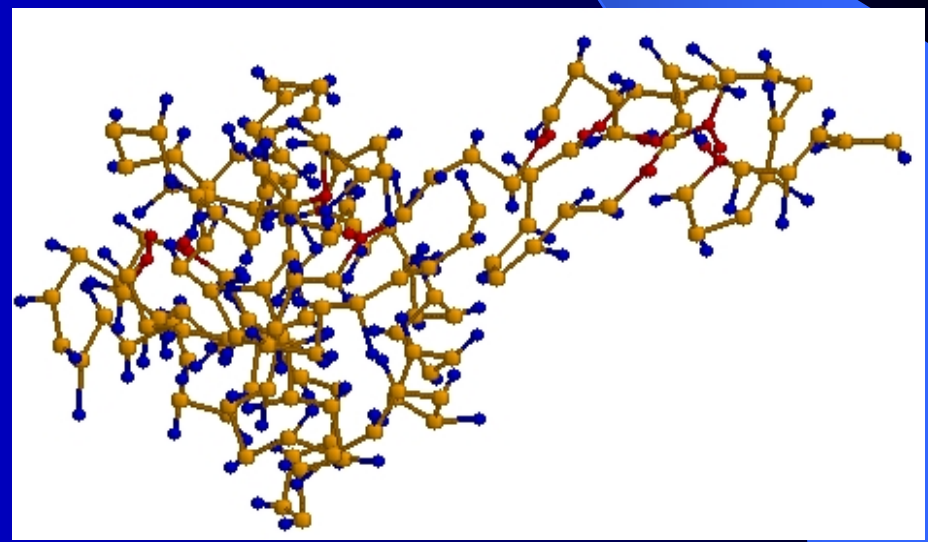
I. Coarse-grained model for P-selectin:



step 1: creating structure file of C_α & centers of mass of residues from PDB structure of P-selectin (*www.rcsb.org*)

- mimicking hydrogen bonds
- modeling S-S bonds

step 2: computing potential energy of obtained conformation of P-selectin:



$$V_R = V_{BL} + V_{SBC} + V_{BA} + V_{DIH} + V_{HB} + V_{NON} + V_{SS}$$

*K. Dill et al, Protein Sci, **4**, 561 (1995);*
*D. Thirumalai, D. Klimov, Curr Opin Struct Biol, **9**, 197 (1999); PNAS, **97**, 2544 (2000);*
*J. Bryngelson et al, Protein, **21**, 167 (1995);*
*M. Karplus, A. Sali, Curr Opin Struct Biol, **5**, 58 (1995);*
*Kolinski, J. Skolnick, Polymer, **45**, 511 (2004)*

II. Coarse-grained model for sPSGL-1

step 3: creating structure file from PDB data for sPSGL-1 for

step 4: repeating step 2 for sPSGL-1:

III. Contact map for the binding interface

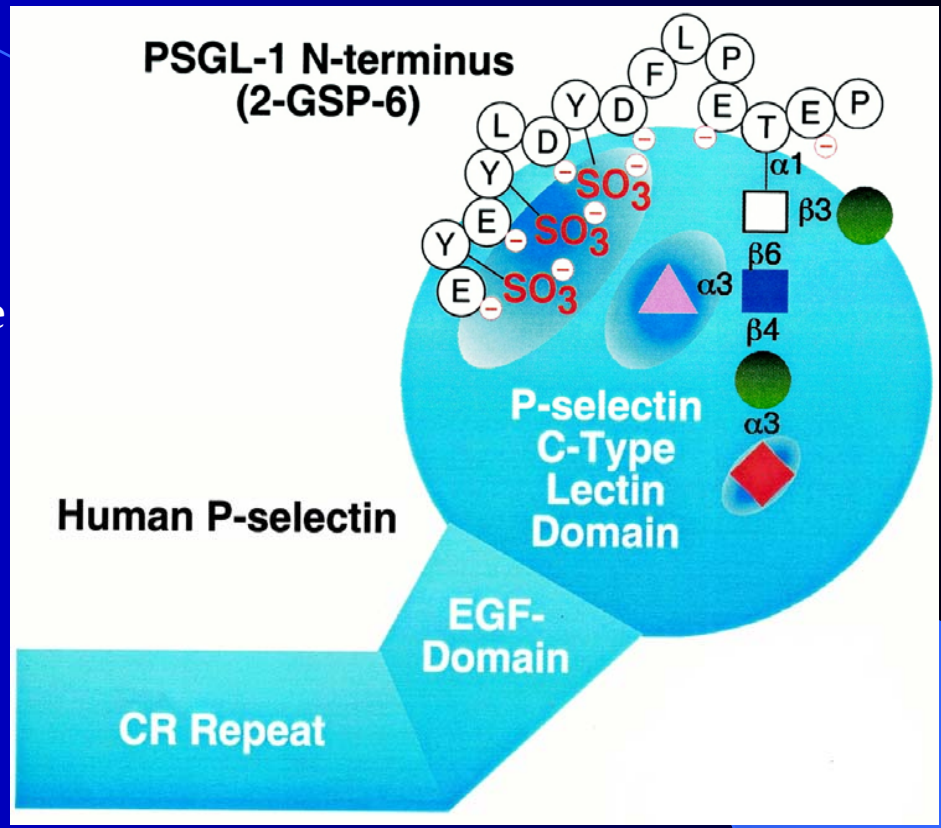
step 5: computing binding energy for the P-selectin complex with sPSGL-1

step 6: computing total energy of the protein-protein complex $V_{TOT} = V_R + V_L + V_{LR}$

step 7: pulling sPSGL-1 by following LD:

$$\eta \frac{d}{dt} X_j = -\frac{\partial V_{TOT}^f}{\partial X_j} + G_j(t)$$

$$V_{TOT}^f = V_{TOT} - f \cdot x$$

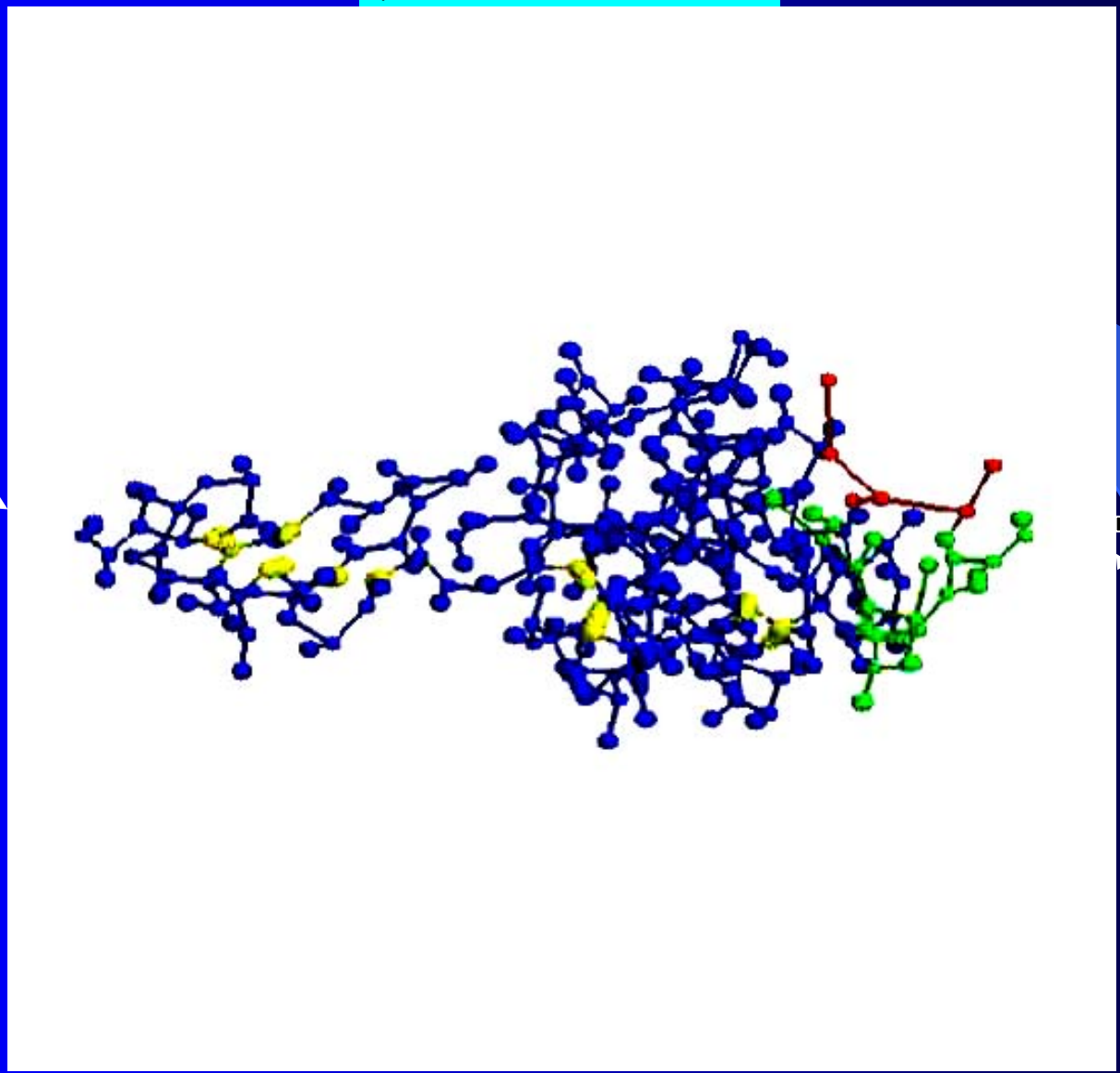


*R. Cummings et al, J Biol Chem, **274**, 24838 (1999);*
*W. Sommers et al, Cell, **103, 467, (2000)***

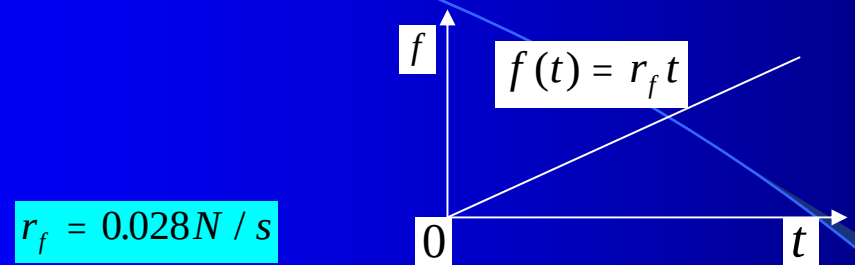
V. Barsegov, D. Klimov, D. Thirumalai (manuscript in preparation)

Simulation of P-selectin-sPSGL-1-interaction: constant loading rate

$r_f = 0.028N / s, t = 0.5\mu s$

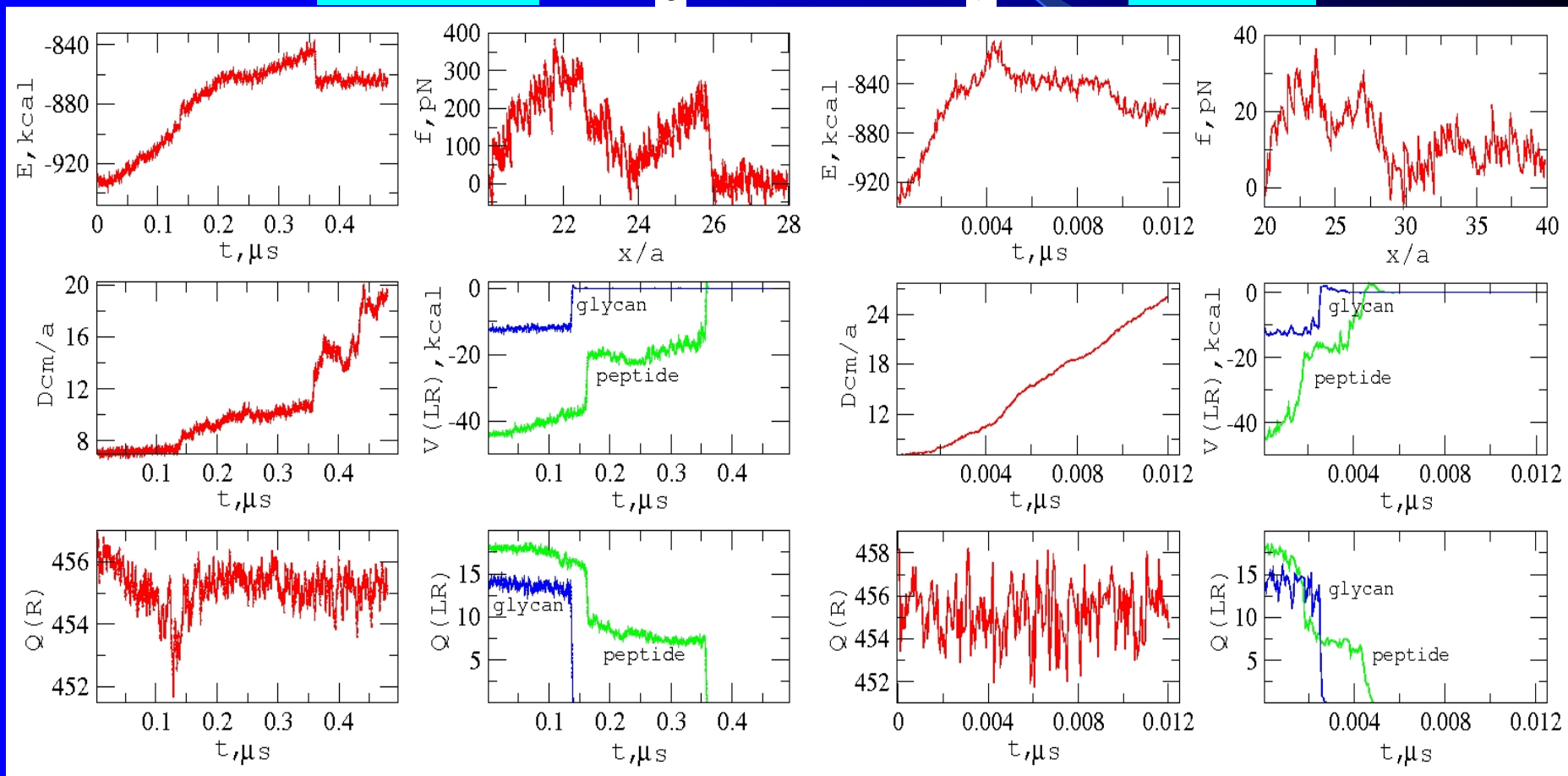


Single trajectories of the energies, forces and number of native contacts for P-selectin-sPSGL-1-interaction



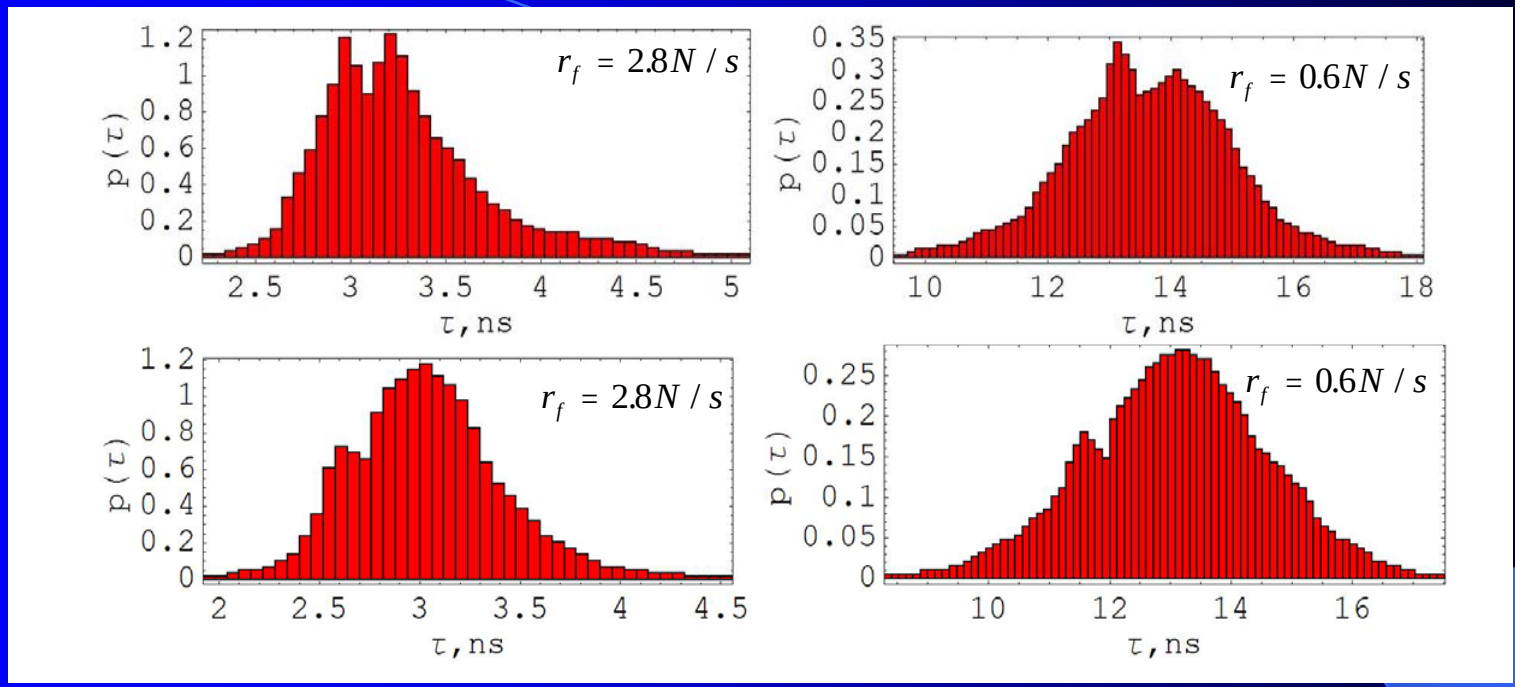
$r_f = 0.028 N/s$

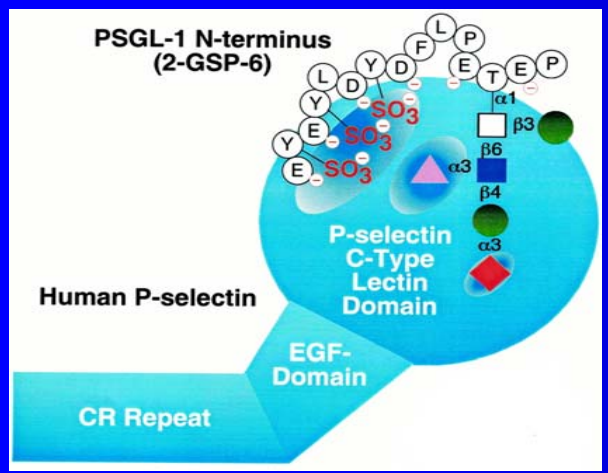
$r_f = 2.8 N/s$

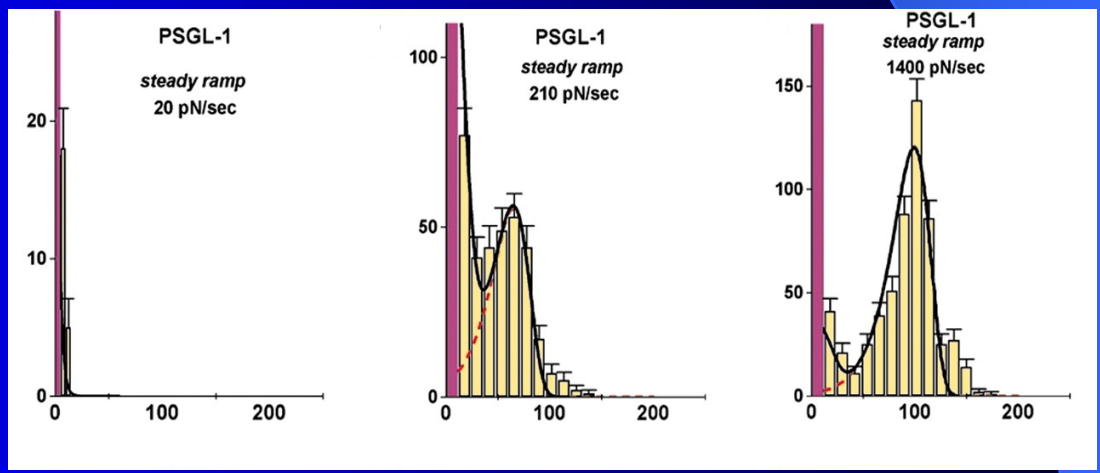


V. Barsegov, D. Klimov, D. Thirumalai (manuscript in preparation)

The evidence of the "catch-slip" dynamics from computer simulations







E. Evans, A. Leung, V. Heinrich & C. Zhu, PNAS, ***101, 11281*** *(2004)*

Main Results:

1. Theory of protein-protein interactions for cell-adhesion complexes:

- estimation of interaction parameters for both sPSGL-1 & antibody G1
- application to cell adhesion complexes involving L-selectins

2. Methodology for computer simulation of protein-protein interactions:

- coarse-grained model for simulations of protein-protein interactions
- application to P-selectin-PSGL-1 (evidence for “catch-slip” dynamics)