inhibitory activity. In addition, the A1-GPIb bond is much stronger when the A1 domain is anchored via the C-terminus rather than the N-terminus. This suggests that shear stress activates A1-GPIb binding in two ways. First, it applies tensile force across multimeric VWF, activating the A1 domain by dislodging this inhibitory region. Second, it applies tensile force across the A1-GPIb bond, inducing a conformational change in the C-terminal region of VWF that allosterically activates the A1 domain, perhaps in a way analogous to that seen in integrins.

**2606-Pos Board B592**

**Quantitative Guidelines for Force Calibration Through Spectral Analysis of Magnetic Tweezers Data**

**Aartjan J.W. te Velthuis**, Jacob W.J. Kerssemakers, Jan Lipfert, Nynke H. Dekker.

Single-molecule techniques are powerful tools that can be used to study the kinetics and mechanics of a variety of enzymes and their complexes. Force spectroscopy, for example, can be used to control the force applied to a single molecule and thereby facilitate the investigation of real-time nucleic acid-protein interactions. In magnetic tweezers, which offer straightforward control and compatibility with fluorescence measurements or parallel tracking modes, force measurement typically relies on the analysis of positional fluctuations through video microscopy. Significant errors in force estimate, however, may arise from incorrect spectral analysis of the Brownian motion in the magnetic tweezers. Here we investigated physical and analytical optimization procedures that can be used to improve the range over which forces can be reliably measured. To systematically probe the limitations of magnetic tweezers spectral analysis, we have developed a magnetic tweezers simulator, whose outcome was validated with experimental data. Using this simulator, we evaluate methods to correctly perform force experiments and provide guidelines for correct force calibration under configurations that can be encountered in typical magnetic tweezers experiments.

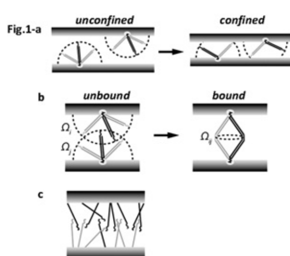
**2607-Pos Board B593**

**Lose & Gain: The Entropy Game for Surface-Tethered Binding Groups**

**Mirjam E. Leunissen**, Daan Frenkel.

The interaction of binding groups (e.g. ligand-receptor pairs) that are attached to a surface through flexible linkers does not only depend on the binding enthalpy of those groups, but also on a number of entropic contributions, namely:

- a **steric repulsion**, due to confinement of the linkers between the surfaces (Fig.1a)
- a **configurational entropy cost**, due to the restricted freedom of the linkers upon bond formation (Fig.1b)
- a **combinatorial entropy gain**, due to the multiplicity of binding configurations for the entire ensemble of binding groups (Fig.1c)




So far, these entropic effects have received little attention, despite the fact that tethered binding groups are increasingly used in, for instance, force spectroscopy investigations and self-assembling materials. Here, we investigate the entropic losses & gains in Monte Carlo simulations of surfaces functionalized with DNA 'sticky ends'. We find that each of the entropic contributions is of the same order of magnitude as the intrinsic binding strength of the sticky ends, ~5−10 kT, but that it is not *a priori* clear what their net effect is, because of their opposite sign and their dependence on e.g. the linker length and surface coverage.

**2608-Pos Board B594**

**Single Molecule Binding of CD44 to Fibrin Versus P-selectin Predicts Their Distinct Shear-dependent Interactions in Cancer**

**Phrabha S. Raman**, Christina S. Alves, Denis Wirtz, Konstantinos Konstantopoulos.

P-selectin and fibrin(ogen) play pivotal roles in the hematogenous dissemination of tumor cells. CD44 variant isoforms, CD44v, have been identified as the major functional P-selectin ligands and fibrin receptors on metastatic colon carcinoma cells. *In vivo* P-selectin mediates the initial binding/seeding and subsequent lodging of metastatic tumor cells in target organs, whereas fibrin(ogen) supports their sustained adhesion and stable implantation. *In vitro* the molecular recognition of CD44v by fibrin mediates firm adhesion at low shear, whereas CD44v-P-selectin binding supports transient rolling interactions at elevated shear stresses and low P-selectin site densities. We used single molecule force spectroscopy to provide a molecular interpretation for these two distinct adhesion events. We demonstrate that the CD44v-P-selectin relative to the CD44v-fibrin bond has a longer unstressed equilibrium lifetime, a lower susceptibility to rupture under force, and a higher tensile strength. These intrinsic differences confer the ability to the CD44v-P-selectin pair to mediate binding at higher shear stresses. Increasing the receptor-ligand contact duration (2-200 ms) did not affect the micromechanical properties of CD44v-P-selectin bond, but it increased the tensile strength of the CD44v-fibrin bond. The CD44v-fibrin bond maturation is further supported by the rapid decrease in reactive compliance and the increase in the depth of the free energy barrier of this biomolecular interaction. This bond strengthening at longer interaction times explains why CD44v binding to immobilized fibrin occurs at low shear and correspondingly high contact durations. Single molecule characterization of receptor-ligand binding can predict the shear-dependent cell-substrate adhesive interactions observed *in vitro* and *in vivo*.

**2609-Pos Board B595**

**Single-Molecule Studies of the Parallel Unfolding Pathways of Maltose Binding Protein (MBP)**

Vasudha Aggarwal, Rajendra Kulothungan S., Saranya Rajaram, Balamurali M.M., Raghavan Varadarajan, **Sri Rama Koti Ainavarapu**.

Maltose binding protein (MBP) is a 370aa protein found in the periplasmic space of gram-negative bacteria and serves as a receptor for osmotic shock sensitivity and chemotaxis in response to the maltose and other maltodextrins from the environment. X-ray crystallographic experiments have shown a ligand induced conformational change in the binding protein structure, resulting in the two N- and C- terminal clefts coming closer to each other and thus enclosing the ligand molecule in the spatially buried binding groove. Here, we are interested in capturing the effect of the ligands on the protein conformation, one molecule at a time, by using atomic force microscope. Through single- molecule experiments, we are trying to understand the effect of maltose and maltotriose on the mechanical stability and the unfolding pathways of MBP. Our results have shown that MBP unfolds via at least two different pathways: one being a two-state unfolding pathway occurring in 38% cases whereas the other contains a mechanically stable intermediate occurring in the remaining 62% cases. The main unfolding peak is observed at ~75 pN, unraveling a contour length of 100 $\pm$ 8 nm whereas the intermediate state occurs at almost half the length of the main state, i.e. at 50 $\pm$ 8 nm and at force of ~45 pN. Although the presence of its ligand, maltose, shows no change in the unfolding force or the contour length of both the states during unfolding, but an increase has been observed in the frequency of occurrence of the intermediate (79%). This increase is more pronounced in case of maltotriose bound protein (83%). This indicates that the ligand bound MBP prefers to unfold via an intermediate.

**2610-Pos Board B596**

**Investigating the Role of the Alpha-C domain in Fibrin Fiber Mechanics**

**Nathan Hudson**, Lifang Ping, Olamide Olusesi, E. Timothy O'Brien, Richard Superfine, Susan Lord, Michael Falvo.

Fibrin fibers, which form the mesh network structure of blood clots, display a hierarchical design. The fibrin molecule is a rotationally symmetric dimer consisting of two identical subunits, each composed of three nonidentical polypeptide chains: $\alpha$, $\beta$, and $\gamma$. The central region (E), where the N-termini of all 6 chains are connected via disulfide bridges, is connected to two outer, beta-sheet rich regions (labeled the $\beta$C, and $\gamma$C and collectively called the D region) by a triple-helix coiled coil. The C-terminal region of the alpha chain contains two distinct parts: the connector region (amino acids 221-391 in human) and the $\alpha$C domain (a392-610 in human). Within the connector region are a series of tandem amino acid repeats, leading to a largely disordered structure, while the terminal region is thought to contain beta sheet structure. The fibrin monomer polymerizes into a half-staggered protofibril via specific interactions; these protofibrils then laterally aggregate to form a fiber.

We recently published a report showing that the extensibility of fibrin fibers was directly related to the length of the alpha-C connector region across various species. Chicken fibers, which have no connector region, fail at strains of $47 \pm 23\%$, while human fibers fail at strains of >200%. To investigate this effect further, we have begun mechanical tests of two recombinant fibrin variants, A$\alpha$251, in which the $\alpha$C connector region of the human fibrin molecule has been truncated at amino acid 251, and a human/chicken hybrid fibrin molecule consisting of the amino acids 1- 197 of the human A$\alpha$ chain linked to residues 199-487 of chicken A$\alpha$ chain. Preliminary results indicate that A$\alpha$251 has breaking strains similar to normal human fibers ($185 \pm 46\%$) while the human/chicken hybrid has breaking strains similar to the chicken fibers ($70 \pm 35\%$).