Studies of Force Induced Changes in Single Molecule Systems

- **Prentiss Group**
- **Nelson Group at Harvard**
- **Whitesides Group**

Why do Single Molecule Experiments?

- **Bulk measurements give only average values**
  - **Heterogeneities are masked**
  - **Intermediate states are difficult to detect**

What sorts of Single Molecule Force Experiments are there?

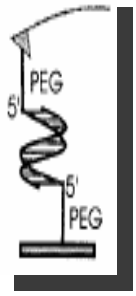
- **Adhesion Measurements**
  - **Use force to pull apart single molecule bonds**
    - » **Ligand-Receptor Studies**
    - » **DNA unzipping**
- **Elasticity Experiments**
  - **Use force to stretch single molecules**
    - » **dsDNA and ssDNA stretching provides structure and free energy information**

Receptor: A molecular structure or site on the surface or interior of a cell that binds with substances such as hormones, antigens, drugs, or neurotransmitters A molecular structure within a cell or on the surface characterised by selective binding of a specific substance and a specific physiologic effect that accompanies the binding, for example, cell surface receptors for peptide hormones, neurotransmitters, antigens, complement fragments and immunoglobulins and cytoplasmic receptors for steroid hormones.
2. A sensory nerve terminal that responds to stimuli of various kinds.

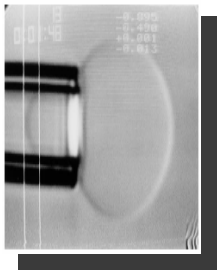
Ligand: An ion, a molecule, or a molecular group that binds to another chemical entity to form a larger complex Any molecule that binds to another, in normal usage a soluble molecule such as a hormone or neurotransmitter, that binds to a receptor. The decision as to which is the ligand and which the receptor is often a little arbitrary when the broader sense of receptor is used (where there is no implication of transduction of signal). In these cases it is probably a good rule to consider the ligand to be the smaller of the two thus in a lectin sugar interaction, the sugar would be the ligand (even though it is attached to a much larger molecule, recognition is of the saccharide).

Exerting Forces on Single Molecules
– Single Particle (Cell / Vesicle / Bead)

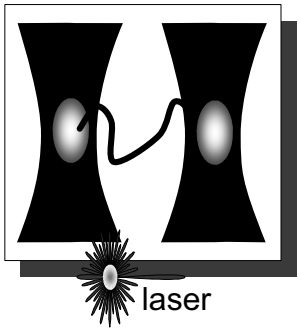
AFM



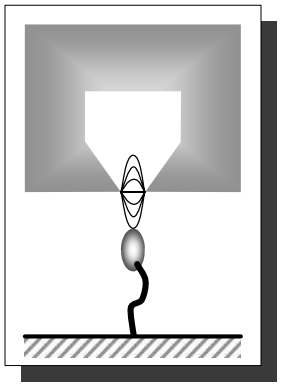
Micropipettes



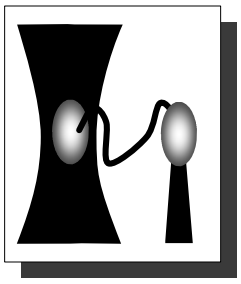
Optical Tweezers



Magnetic Tweezers



…or any combination



- *Force between single ligand receptor pairs*
- *Cell adhesion on specific surfaces*
- *Folding/Unfolding transition of polypeptides*
- *Elasticity and structure of chromosomes by aspiration*
- *…*

Meaningful data acquisition can be long and tedious since good statistics are required

Adhesion Plays an Important Role in Biology

Adhesion in Pathogenesis

Viruses

Bacteria

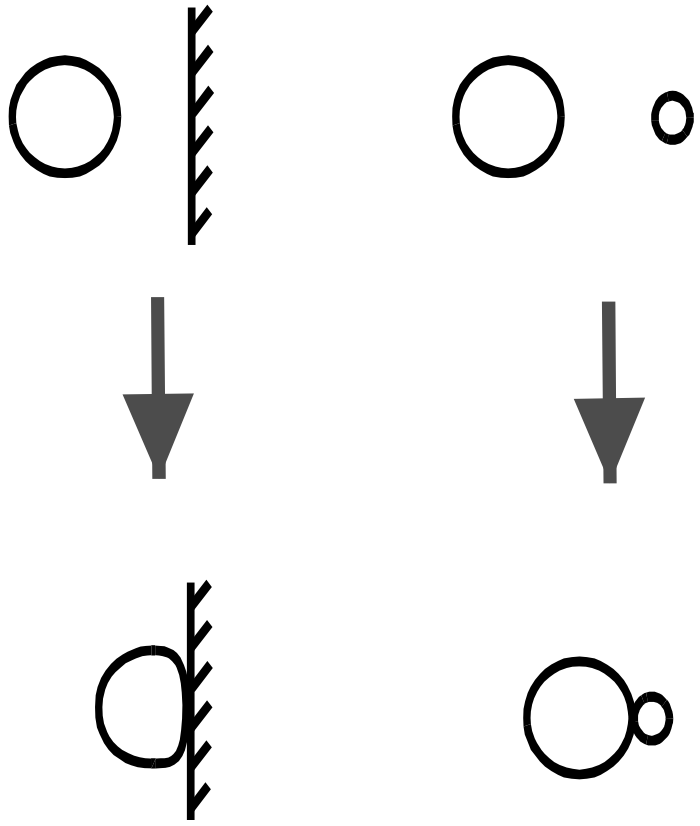
Intracellular bacteria

and rickettsia

Some toxins (ricin, cholera)

Metastasis

Inflammation



Importance of Adhesion Studies

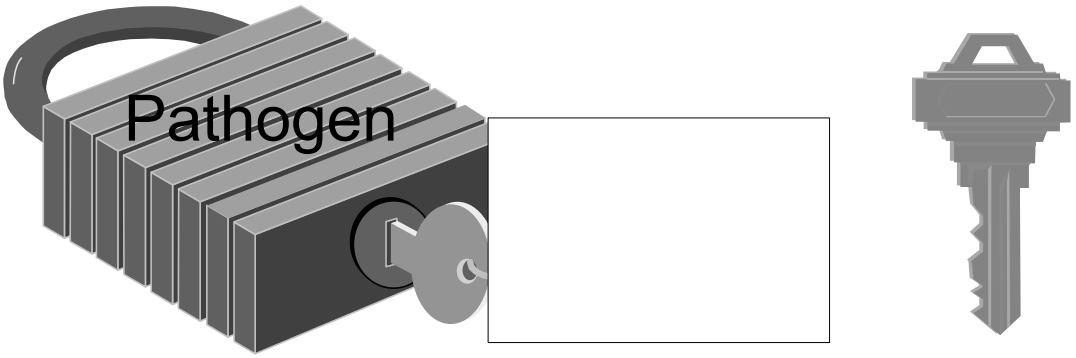
- **Basic studies of adhesion provide understanding of mechanisms governing binding**
- **Can lead to therapeutic advances by adhesion inhibition or promotion**
- **Most studies are equilibrium studies of binding to surfaces, leaving out dynamics that may be crucial**
  - **surface flow measurements are difficult because of velocity uncertainty at surfaces**

Types of Adhesion

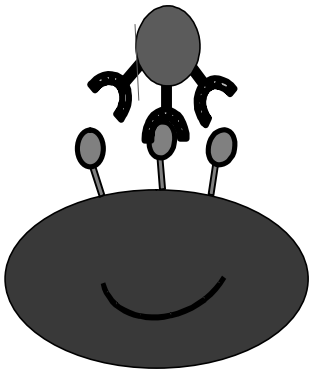
- **Non-biospecific**
  - **hydrophobic or electrostatic**
- **Biospecific and exclusively adhesive**
- **Bioespecific and functional**
  - **integrins to RGD or fibronectin**
  - **selectins binding to cadherins**

Simplest Specific Adhesion View

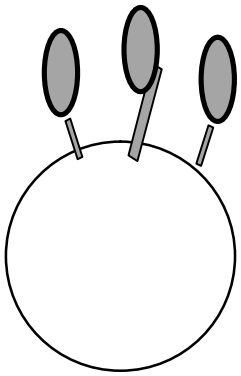
- **A pathogen (lock) selectively binds to a suitable receptor (correct key) on the surface of the cell, while not accepting incorrect binding site (wrong key)**
- **polyvalent binding is more specific**



binding sites on pathogen surface

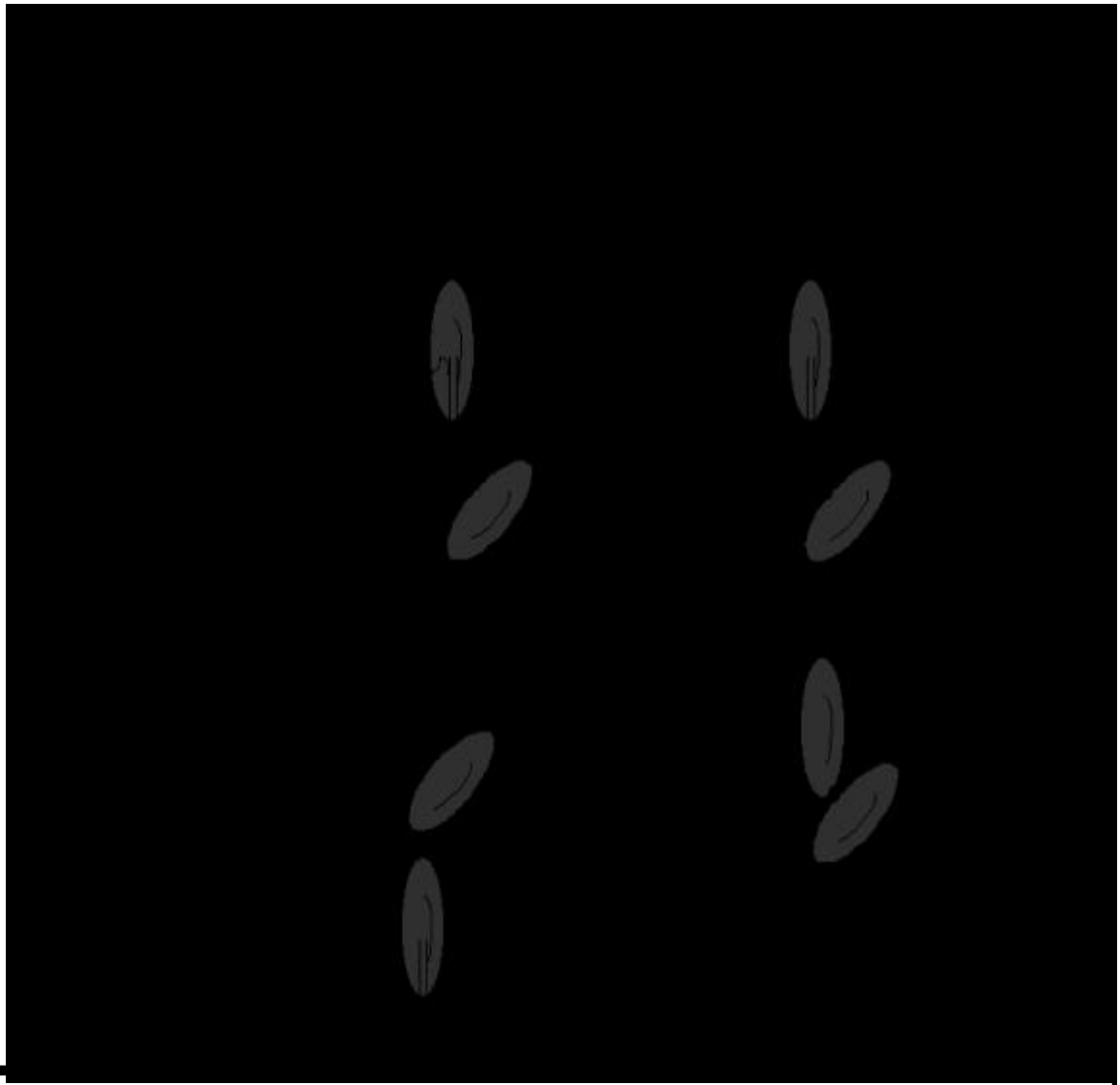


Carbohydrate on Cell Surface



Hemagglutination Assay for Virus Inhibitor

Start with cells uniformly distributed in inverted conical cuvette



After gravity

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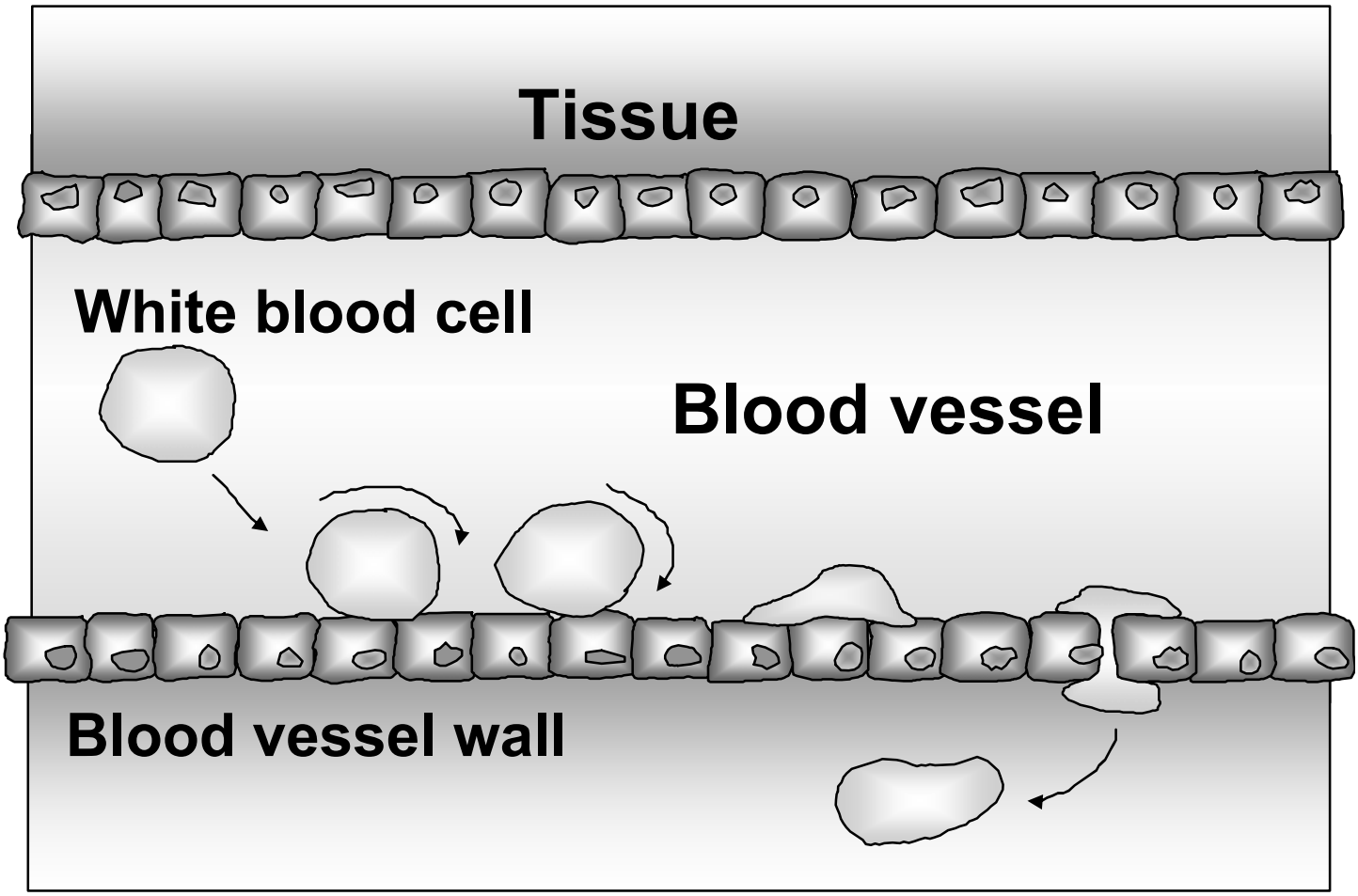
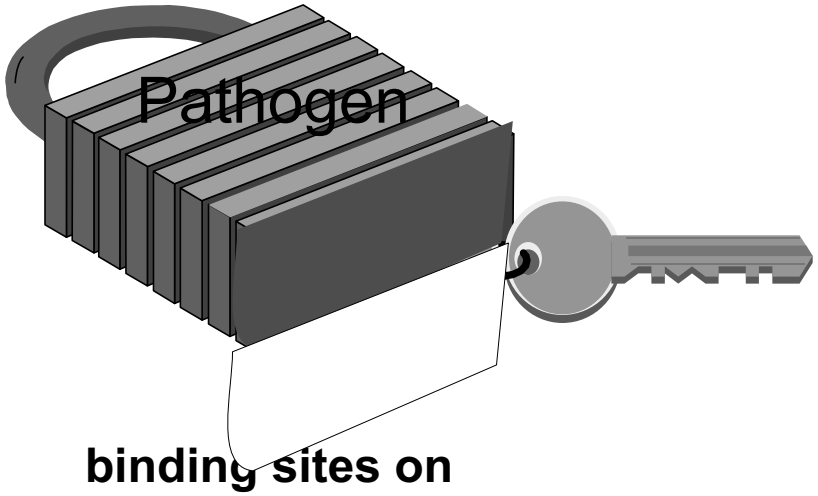
Adhesion Can be Dynamic

White blood cells adhesion to blood vessel walls at onset of inflammation

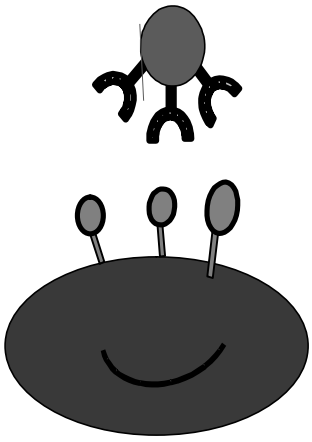


Spontaneous Unbinding of Single Bonds Occurs

- **polyvalent binding decreases unbinding probability by making deeper well**
- **if a single bond unbinds, other remaining bonds can hold pathogen to cell allowing rebind**



binding sites on pathogen surface



Carbohydrate on Cell Surface

Dynamical Control of Collisions Desirable

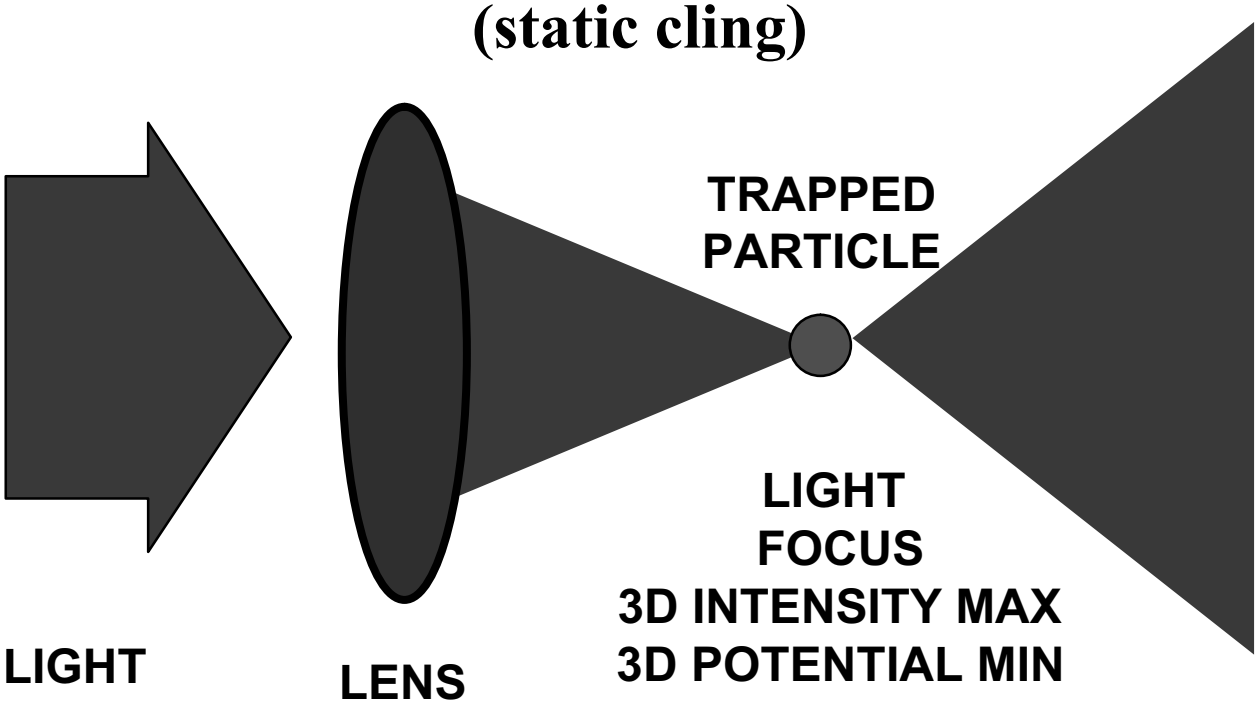
- AFM possible
- We choose optical tweezers

Optical Tweezers

Potential = - α Intensity

= - α | Electric Field |^2

(static cling)

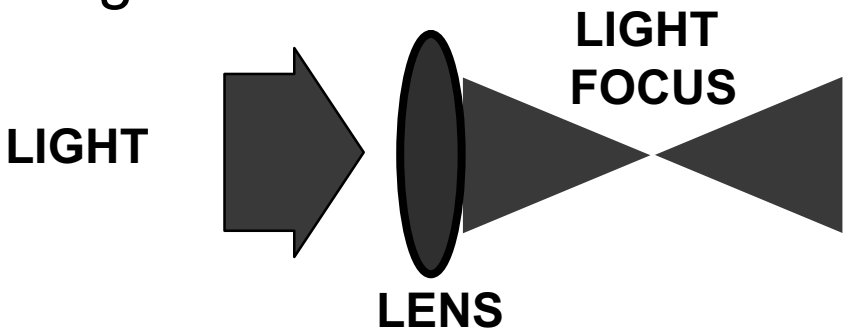


Optical Tweezers are an Excellent Tool for Research on Adhesion

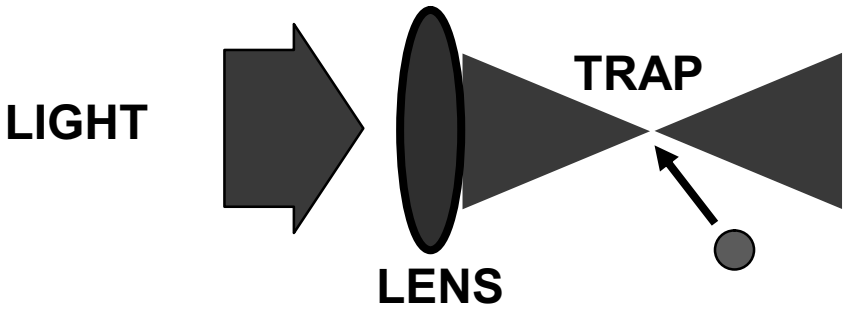
- **Tweezers based manipulation can tailor the position, orientation, velocity, duration and force of a contact between surfaces**
- **Tweezers do not require that anything be attached to the cell being manipulated**
- **Tweezers can measure the force required to separate surfaces under controlled mechanical conditions**
- **Adhesion not previously considered for tweezer measurements because binding forces >> tweezer forces**
  - **breakthrough idea: tweezers do not have to separate bound particles, but just to distinguish bound from unbound**

Optical Tweezers
Potential = - α Intensity
= - β | Electric Field |^2

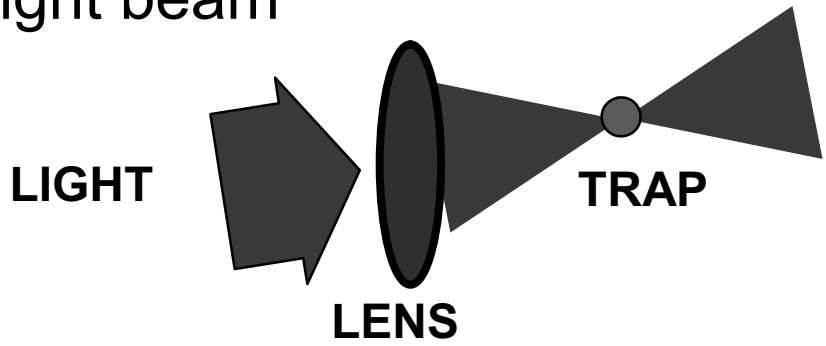
1. Establish an Electric Field Maximum by focusing a light beam



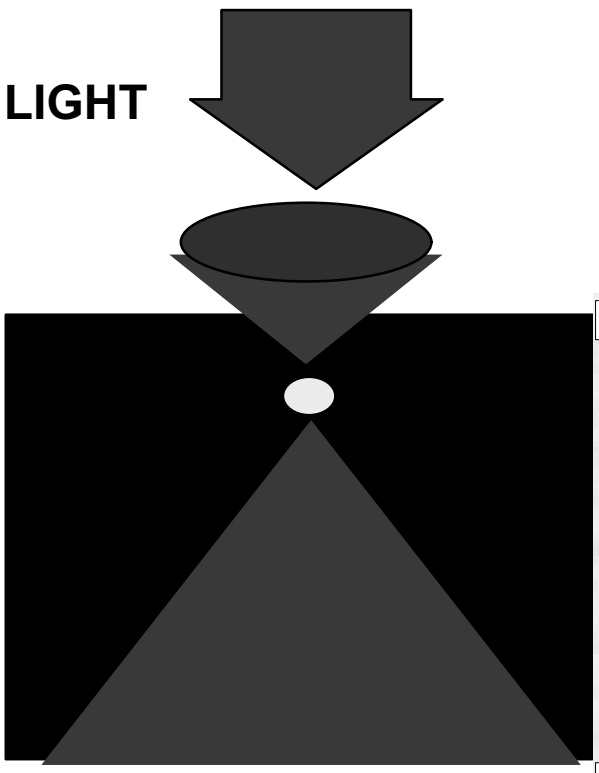
2. Particles will be attracted to the light beam focus where the Electric Field is a Maximum

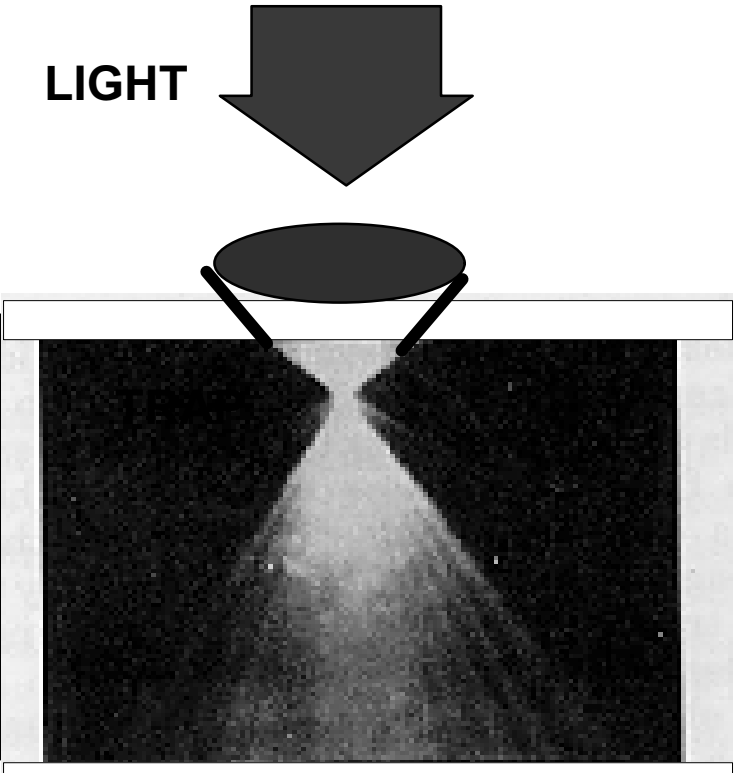


3. Particles can be manipulated by moving focus of the light beam



First Optical Tweezer





Observation of a single-beam gradient force optical trap for dielectric particles
A. Ashkin, et al , Opt L ett, **11** , 288 (1986)

Measurements of Adhesion Between a blood cell or bacteria and an artificial surface

- Used to measure pathogen bonding
- Dynamics of binding can be probed
- Used to measure inhibitors for pathogen binding

First Optical Tweezer Based Adhesion Experiments

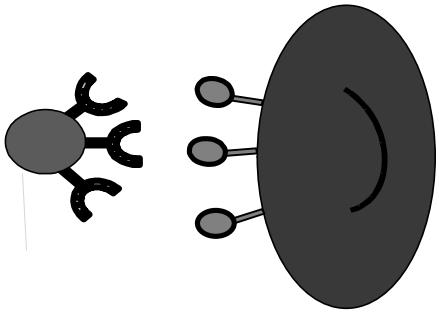
(CFLD by Phillips and Whitesides Groups)

- **Multi-beam optical tweezer controls position of two or more particles**
- **Adhesion between particles is measured by determining the tweezer force required to pull the objects apart.**
- **First experiment was on adhesion of influenza virus to red blood cell**
- **Studied effects of inhibitors**
  - **important for drug development where effective inhibition at lowest possible concentration is desired**
- **Tweezers too weak to pull apart spheres bound to cells by virus, but easily pull apart unbound cells and spheres**

Dual Optical Tweezers (DOT) For Biochem

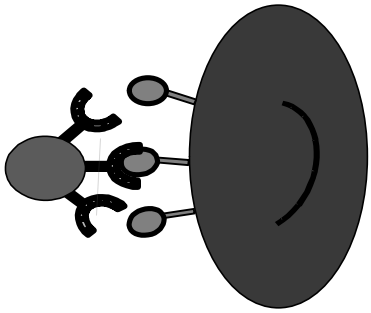
Start with virus coated sphere and cell in separate traps Time=0

coated microsphere

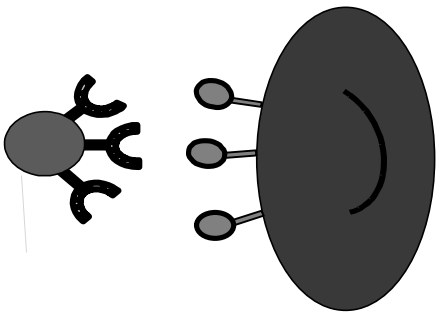


erythrocyte

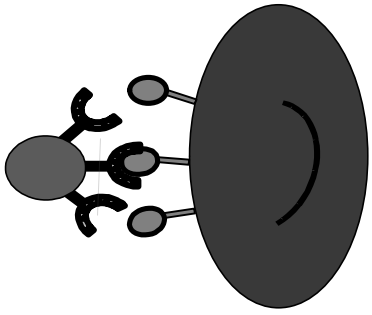
Move 2 traps together so sphere and cell collide



Move 2 traps apart, if they remain together they have bound



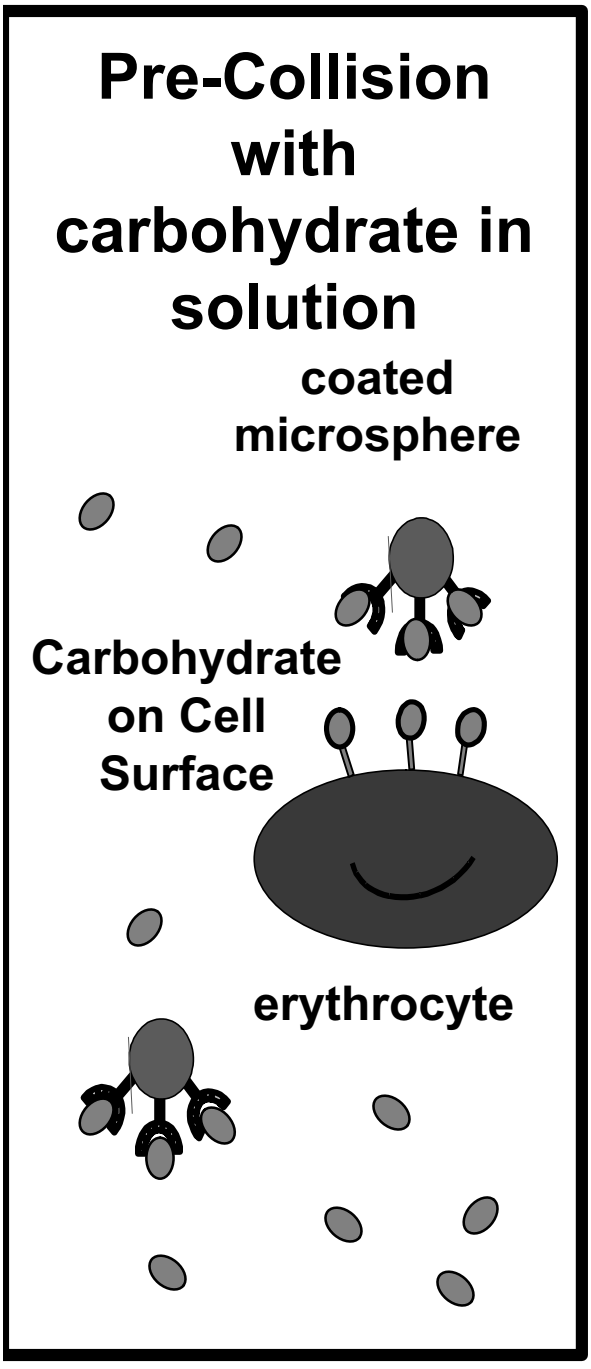
or

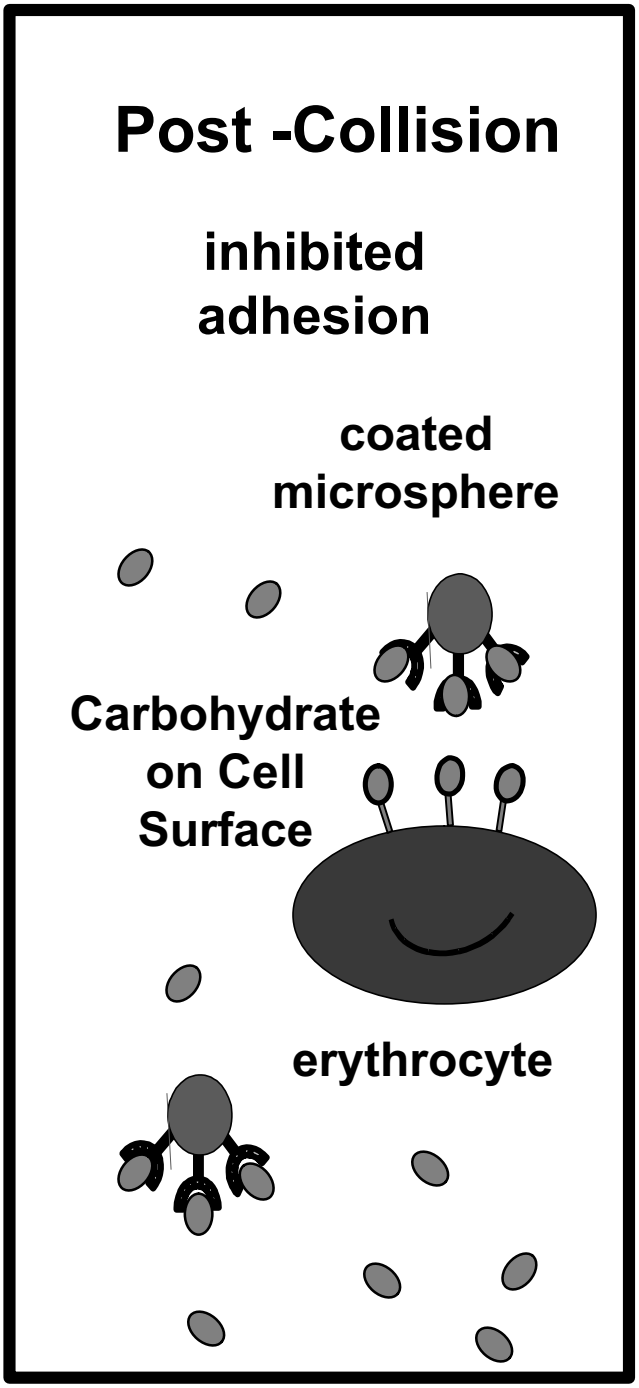


Adhesion

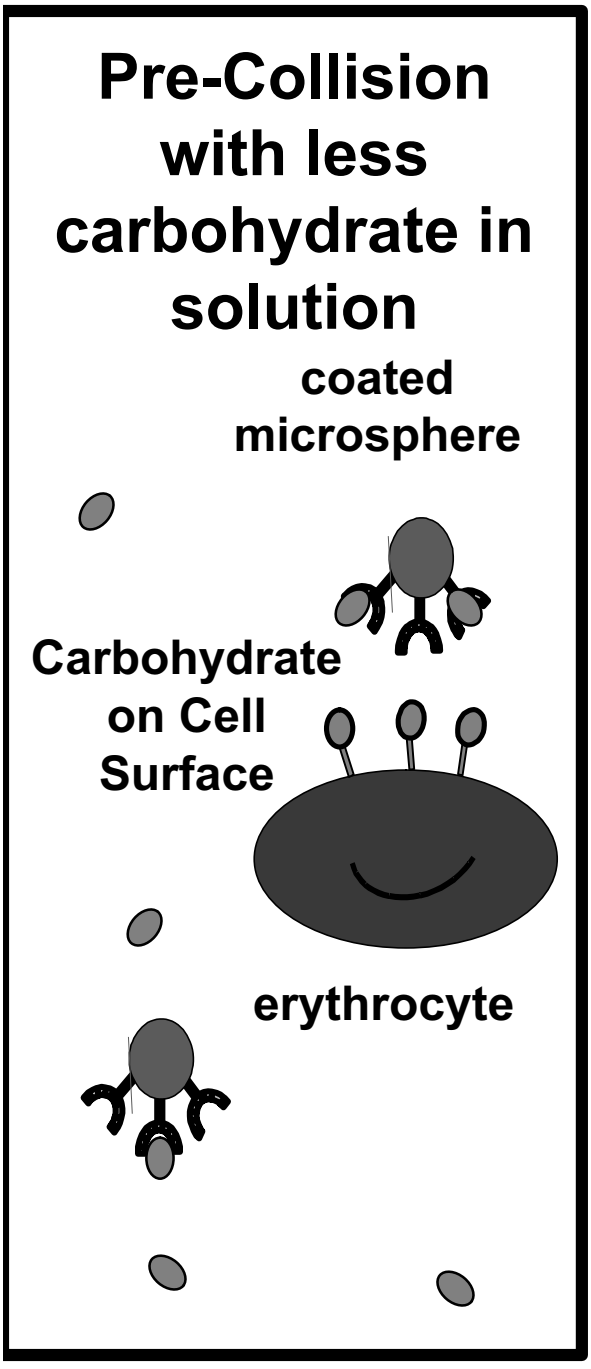
No Adhesion

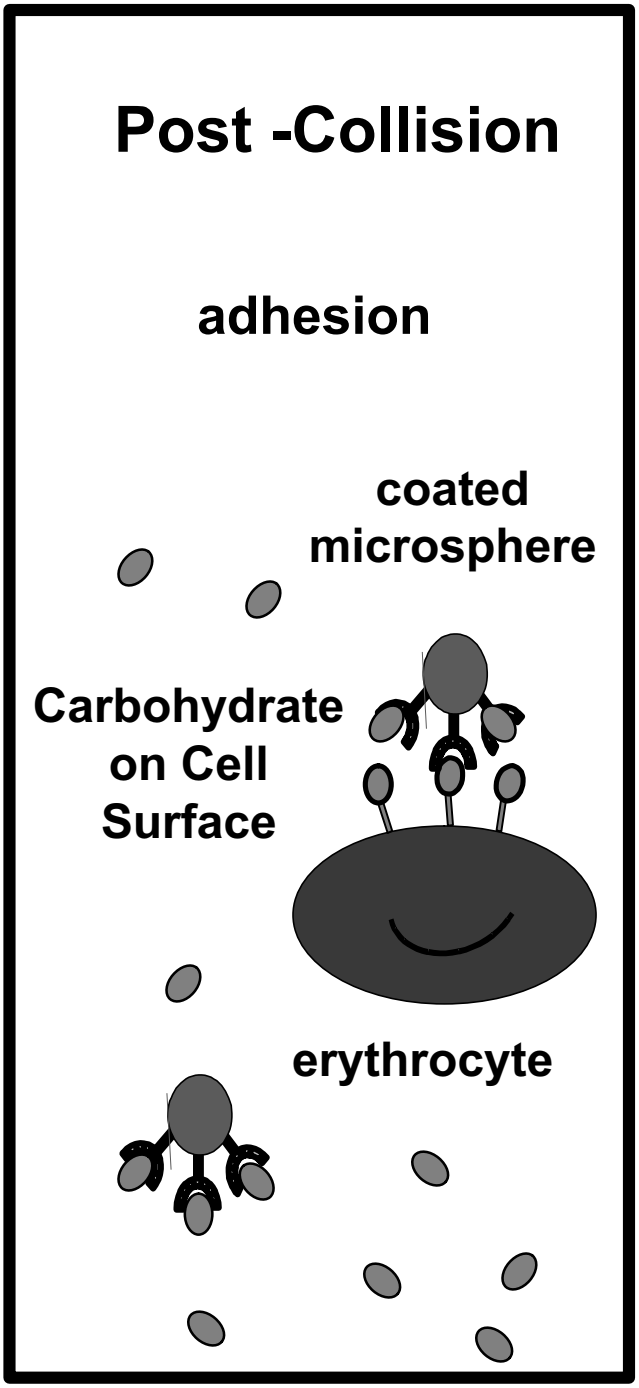
OPTCOL to Measure Inhibition of Adhesion by Soluble Carbohydrates





OPTCOL to Measure Inhibition of Adhesion by Soluble Carbohydrates





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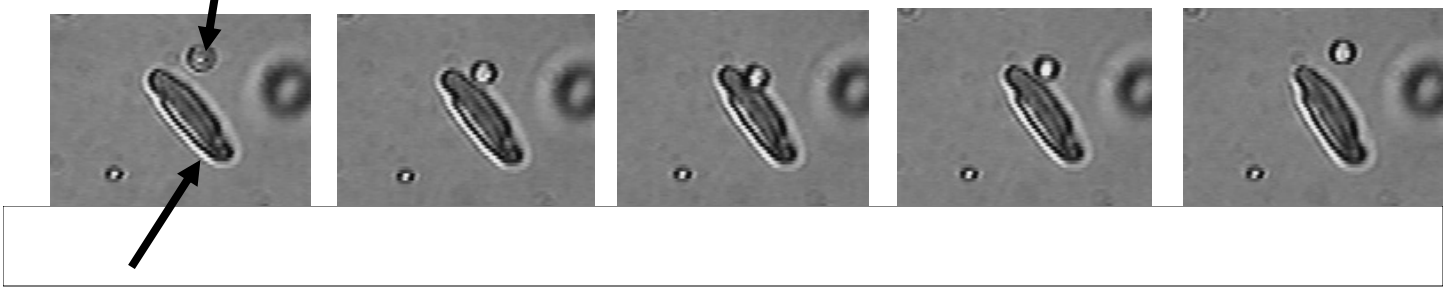
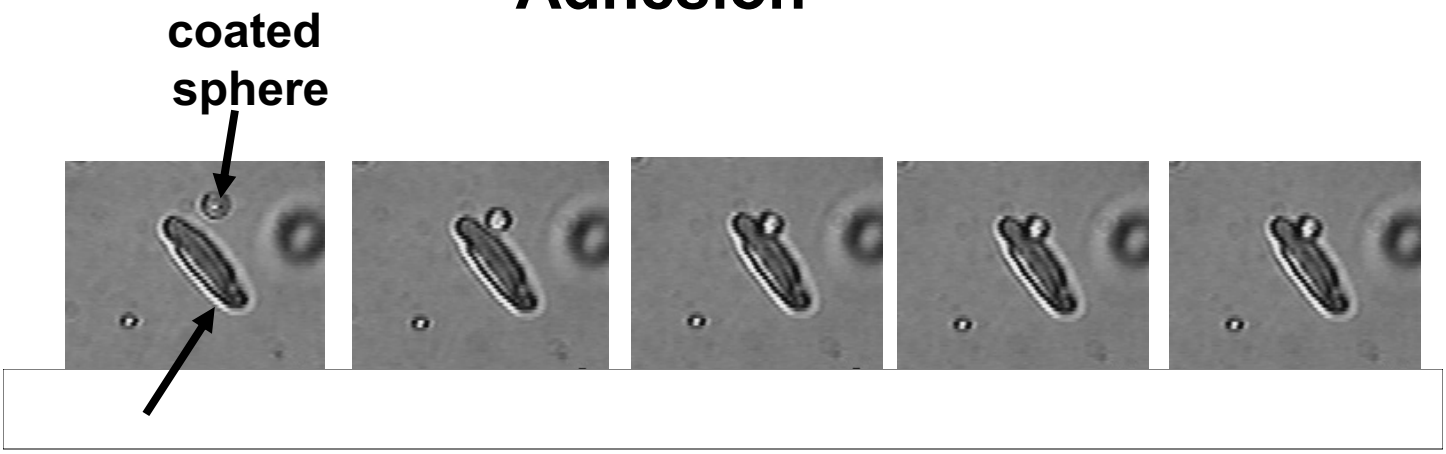
Dual Tweezer Controlled Collision

Inhibited Adhesion



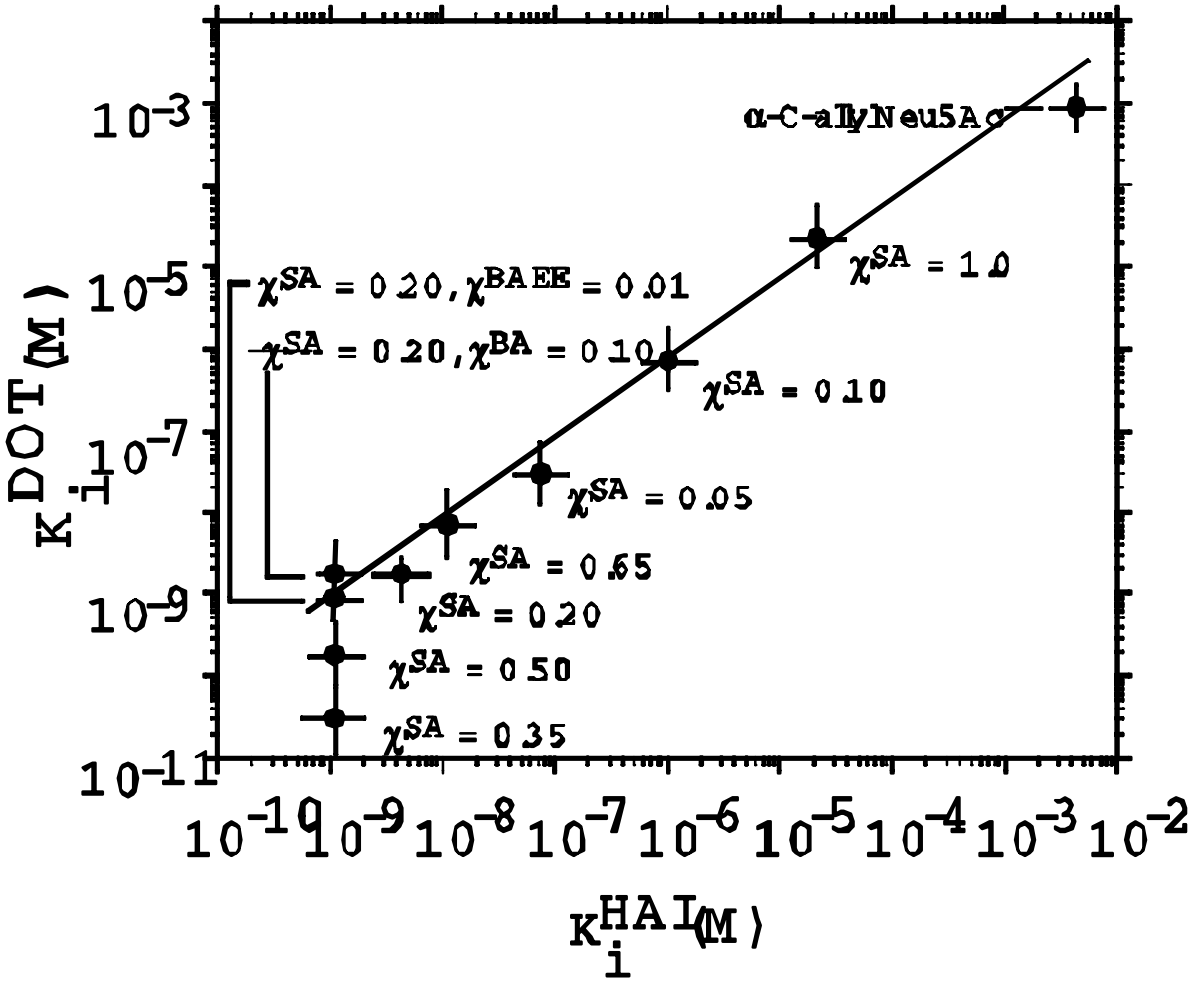
Adhesion



Ten trials. Adhesion in <3 is counted as binding, no adhesion > 10 no binding. 3-10 rare.

Results of First Tweezers Adhesion Experiment

- **Dot used to measure effectiveness of viral anti-binding agents**
  - **Passivation of bead surface vastly increases specificity of test**
  - **50% adhesion point matches standard assay in range where standard is valid (>10^{-9} molar)**
  - **Extends dynamic range 2 orders of magnitude beyond standard test (10^{-11} to 10^{-3} molar)**
  - **Agents indistinguishable in standard assay shown to have orders of magnitude different effectiveness**



Measurements of Cell Binding to Pathogen Surrogates

- **Use a molecule with known structure to probe binding**
- **Provide detailed comparison of theory and experiment**

Second Model System WGA and Erythrocyte

- **Wheat Germ Agglutinin**
  - **well characterized dimeric lectin that binds to GlcNAc and NeuAc with four binding sites**

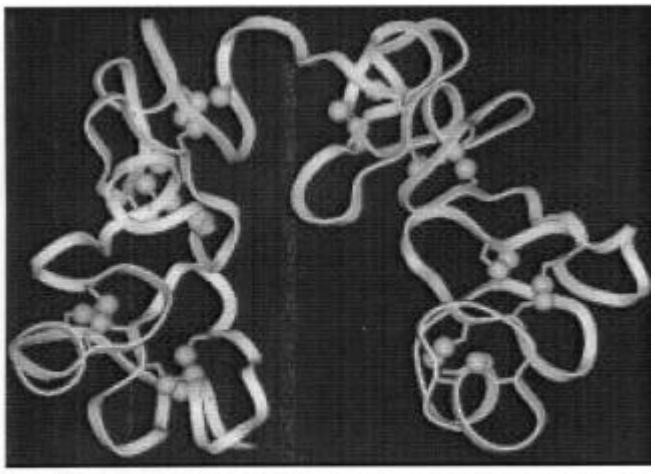


Figure 11. Ribbon representation of wheat germ agglutinin (PDB entry WGC). The sulfur atoms of the disulfide bridges are shown as small balls.

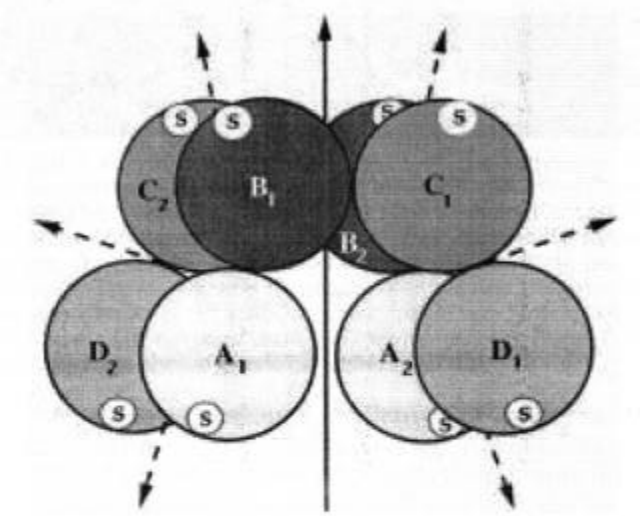
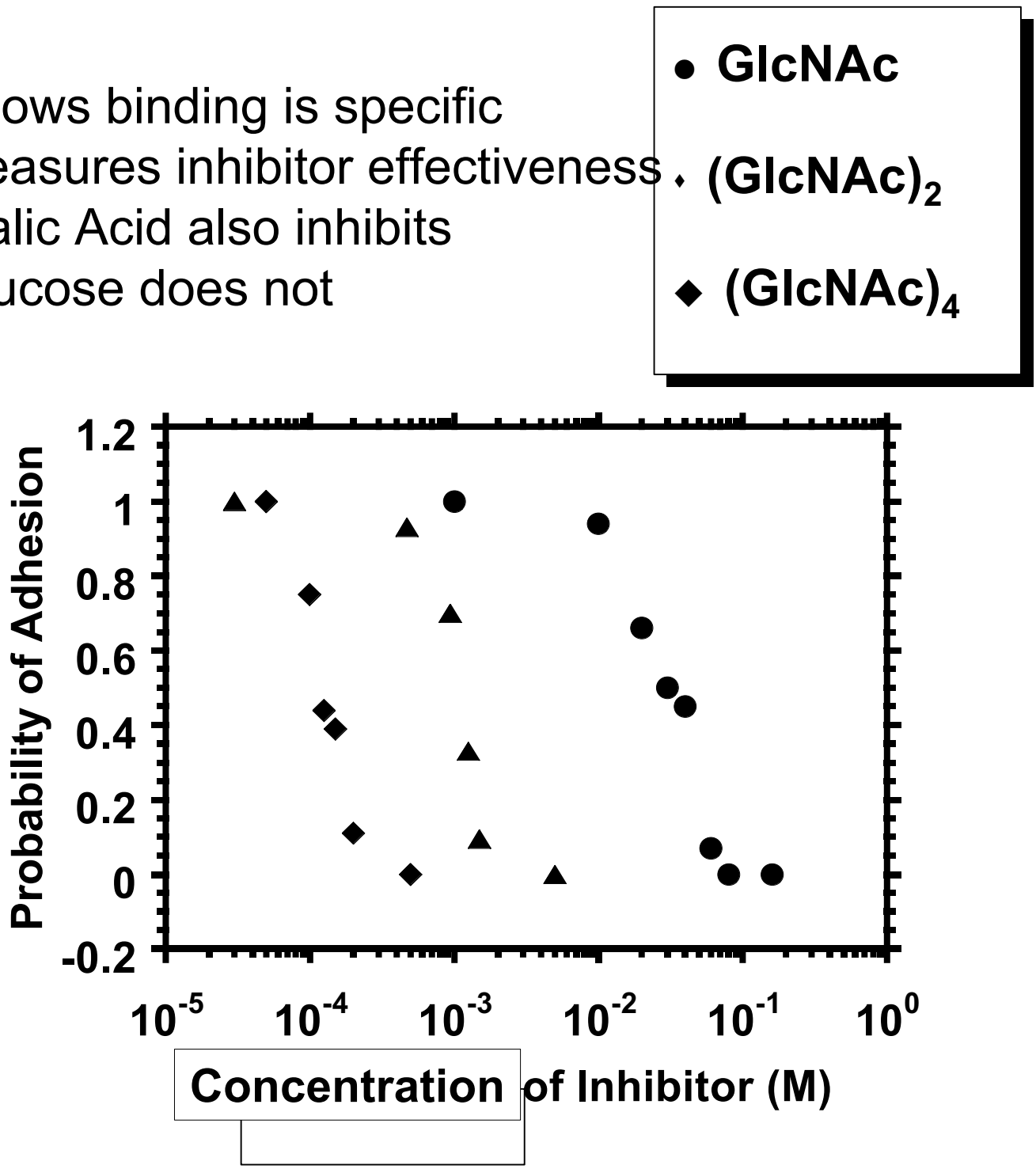


Figure 12. Schematic representation of the wheat germ agglutinin dimers. Domains are shown as large shadowed circles and labeled A1, B1, C1, D1, etc. The position of the molecular 2-fold axis is indicated by an arrow. Broken arrows represent the two types of pseudo-2-fold axes generated in the dimer interface between domains of different dimers. "S" refers to the aromatic carbohydrate-binding pocket. (Reprinted by permission from ref 51

Inhibition of WGA binding by Soluble Sugar

Shows binding is specific
Measures inhibitor effectiveness
Sialic Acid also inhibits
Glucose does not



WGA binding results

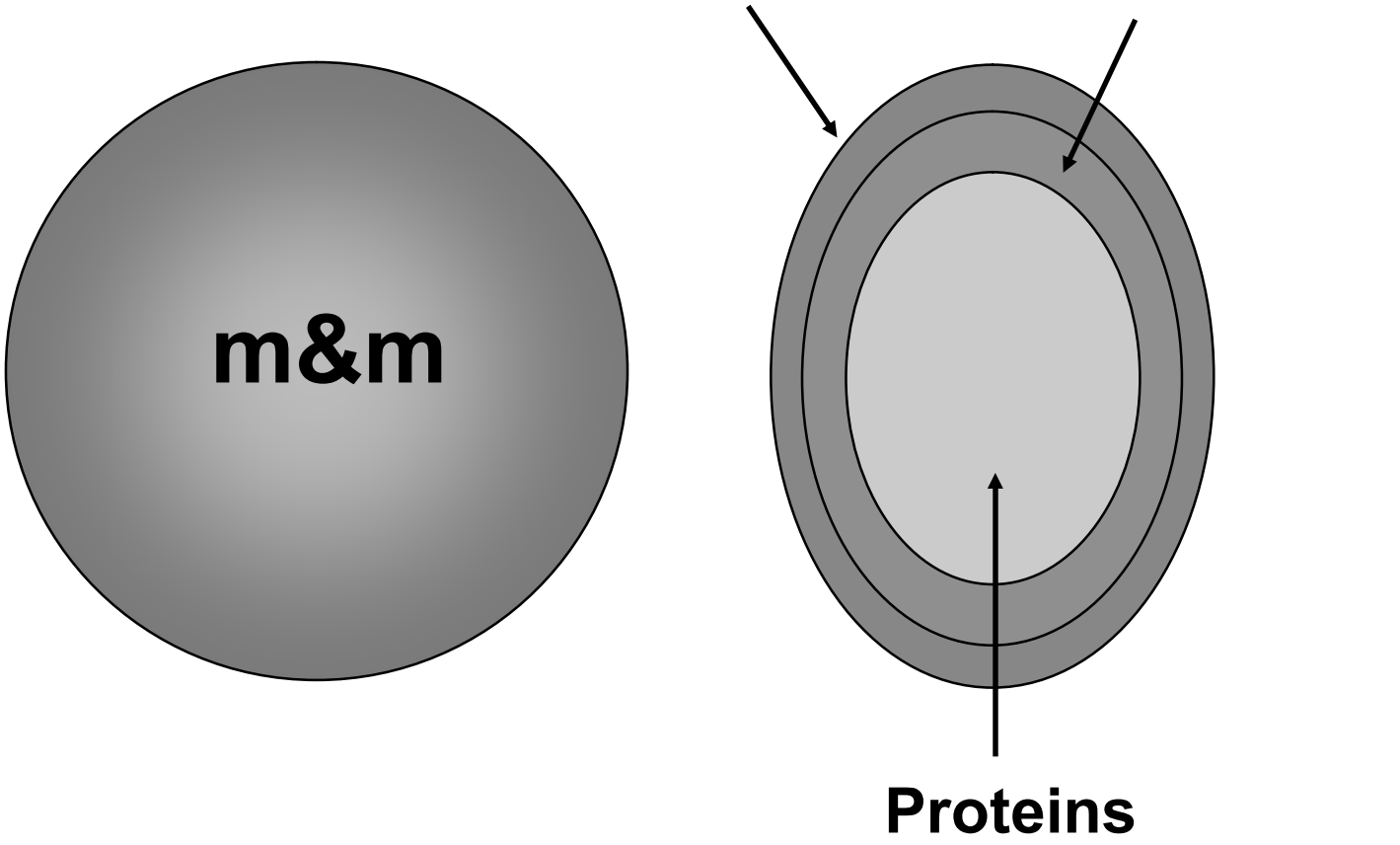
- **Uncoated spheres stick to each other and to erythrocytes**
  - **BSA blocks uncoated adhesion**
  - **Not biospecific**
    - » **electrostatic or hydrophobic**
- **EG coated spheres do not stick to each other or to cells**
- **In the absence of inhibitor, WGA coated spheres always stick to cells and could not be removed with tweezers**
- **GlcNac and sialic acid in solution inhibit the binding**
  - **inhibition concentrations for 50 % sticking are similar to concentrations required to inhibit hemagluttination**
- **Other soluble carbohydrates do not block binding**
  - **glucose had no effect**
- **No Spontaneous unbonding observed**

Bacteria Binding to an Artificial Surface

- Can precisely control surface chemistry using self-assembled monolayers
- Time dependent responses dominated by bacterial changes rather than surface changes
  - over long times remodeling of surface is still an issue
- Model system: E. coli specifically binding to mannose
  - clinical sample

Why Study adhesion to Sugars?

Cells are like peanut m&m's



Adhesion to cells is frequently really adhesion to sugars on the outside of the cells. Sugars can encode far more info than amino acids, so cells are often identified by the sugars on their surfaces.

Microbial Infection begins with adhesion to a sugar

Influenza virus - “Flu”
HIV - AIDS
Helicobacter pylori - Ulcers
Escherichia coli - Meningitis
Pseudomonas aeruginosa - Pneumonia
Trypanosomes - African sleeping sickness
Plasmodium falciparum - Malaria

Chronic inflammatory disease begins with adhesion to a sugar

Diabetes
Multiple sclerosis
Rheumatoid arthritis
Inflammatory bowel disease
Psoriasis
Transplant rejection
Asthma

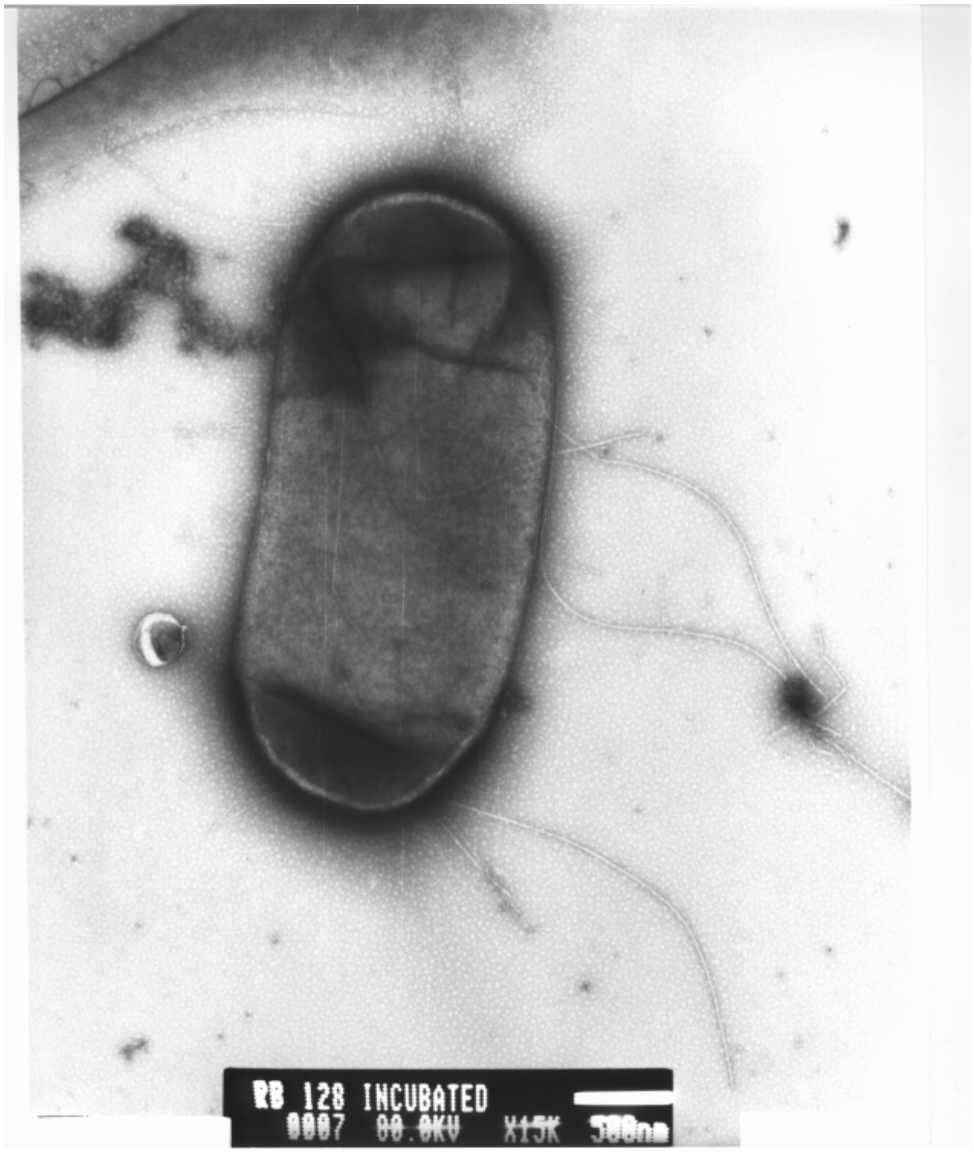
Inhibition of Adhesion with minimal dose can be therapeutically important

- Adhesion minimization can prevent infection and inflammation
- Smallest possible doses reduce side effects
- Lectins are proteins that bind mono- and oligosaccharides reversibly and with high specificity
  - not catalysts
  - not products of immune response
- Lectins frequently cause specific adhesion
- Search for inhibitors that bind to the lectins preventing pathogens from binding to cells
- Need quantitative method of evaluating inhibitors
  - measure equilibrium binding in solution
  - no dynamical information

Tweezer Based Measurements of E. Coli Binding

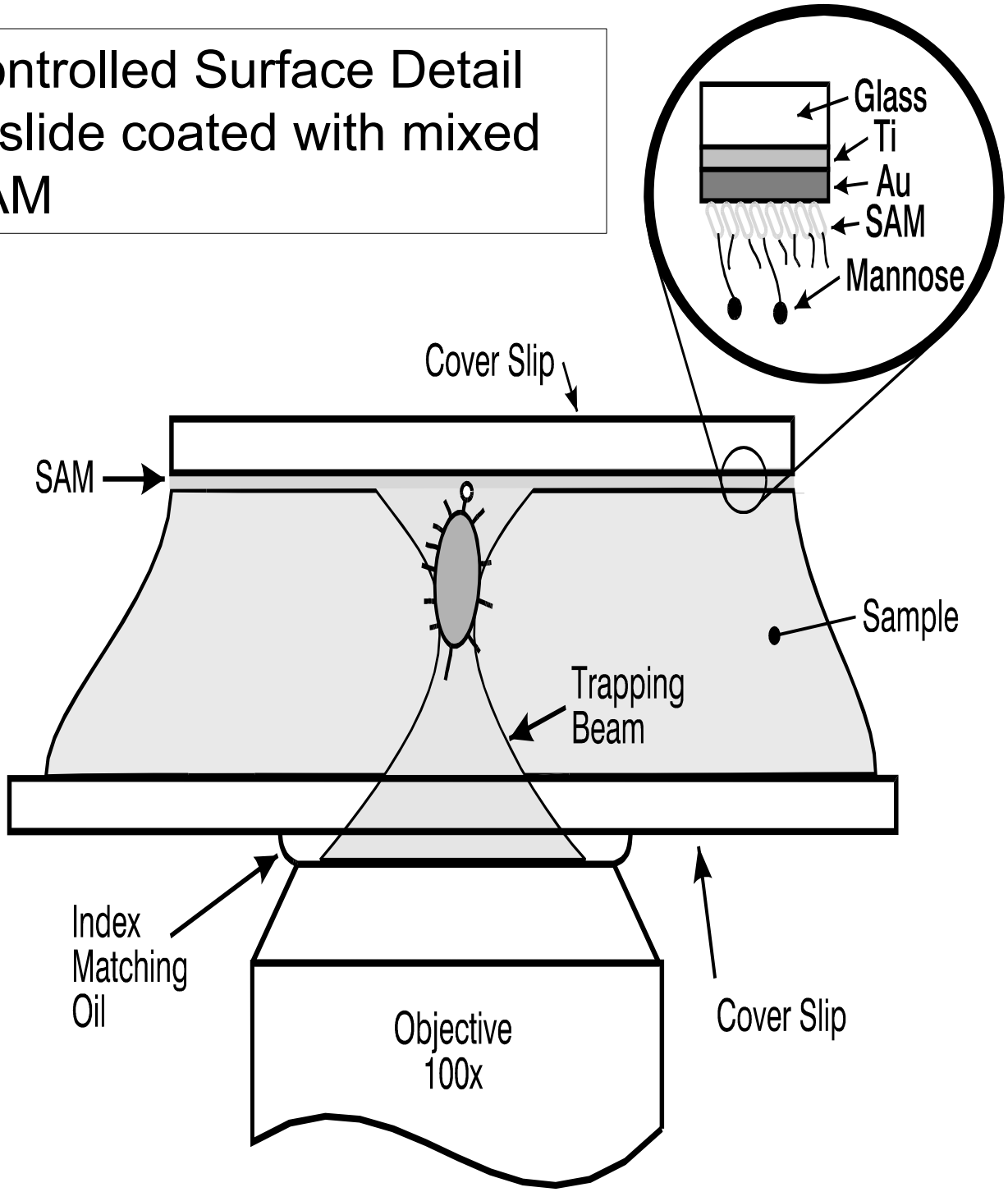
(Michael Liang and Stephen Smith)

- **Goal: Measuring the specific binding of E.coli to mannose**
- **Strategy: Control the density of mannose on a surface using self-assembled monolayers (SAMs)**

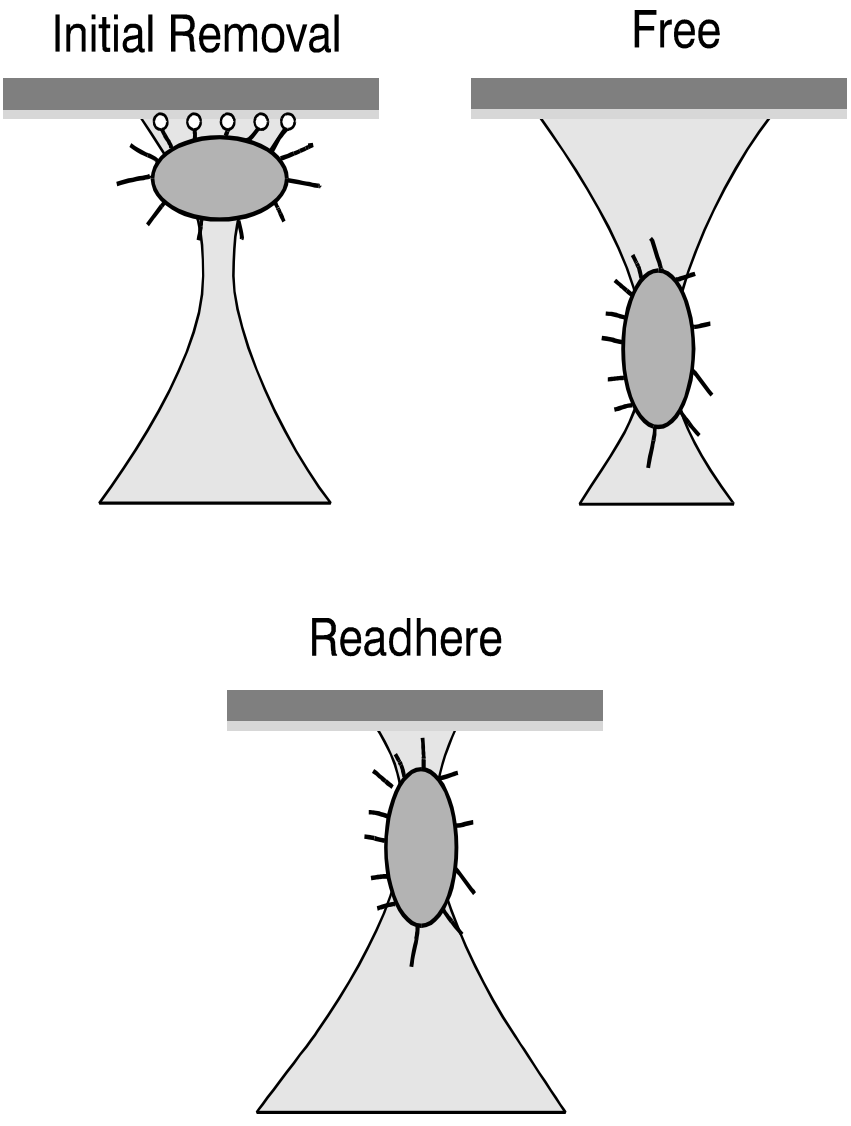


Apparatus Detail

Controlled Surface Detail of slide coated with mixed SAM

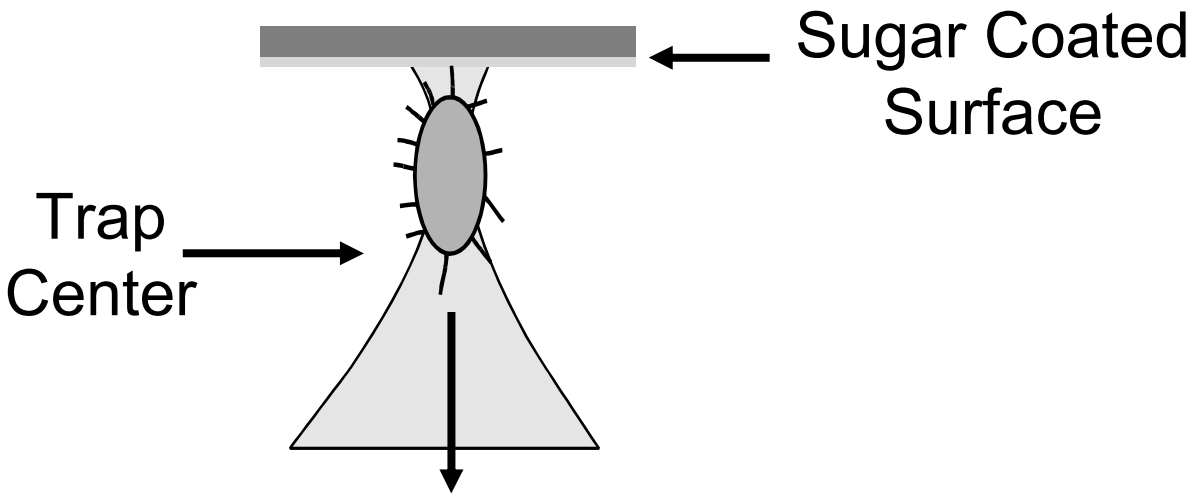


Initial Steps in Adhesion Measurement

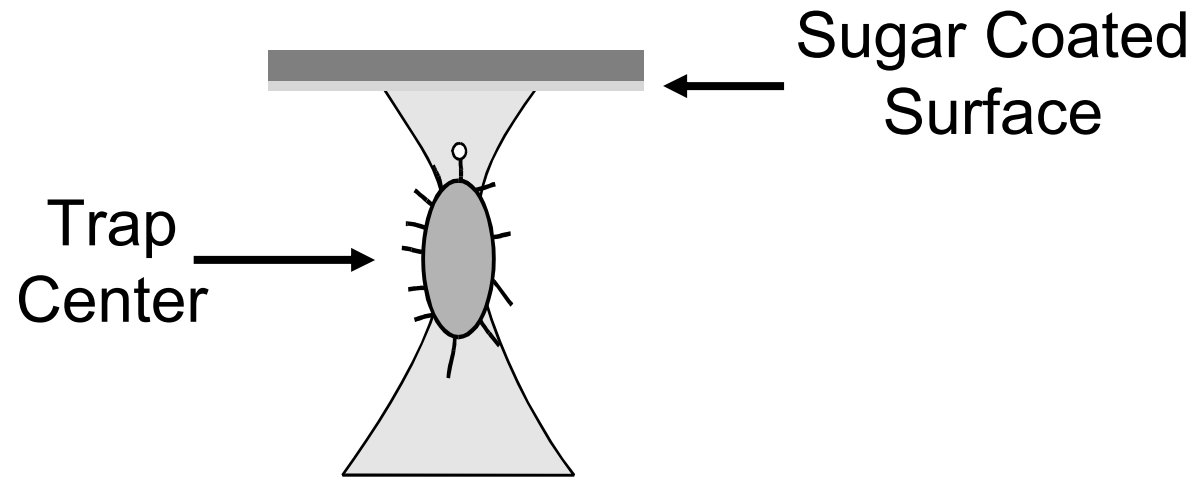


Steps in Adhesion Measurement

Place Low Power Trap Below Adhering Bacteria



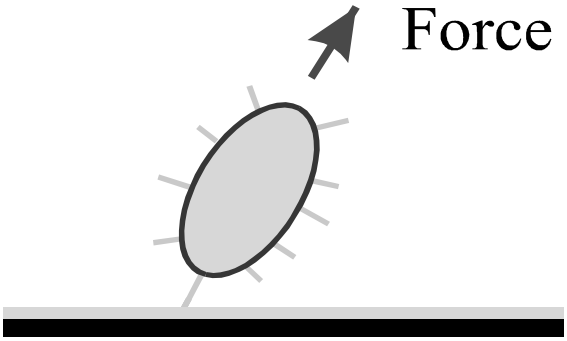
Increase Trap Strength Until Bacteria Unbinds because Tweezer Force = Bond Rupture Force



No Net Average Tweezers Force

Effect of Multiple Binding Sites on Binding Force

Single site

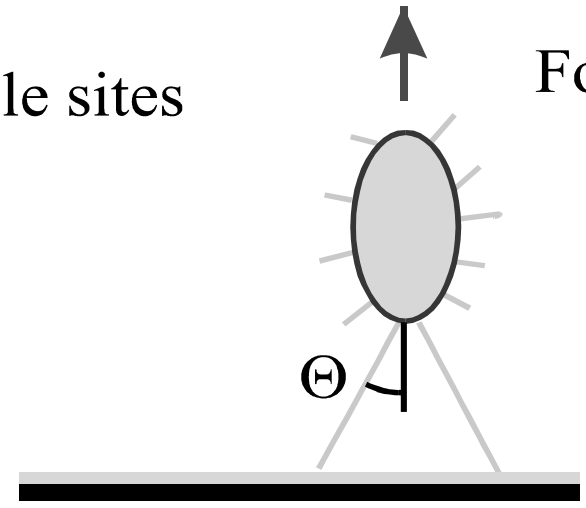


Minimum Power to remove

Binding force = N single bond for N on single site

Binding force < N single bond for multiple site

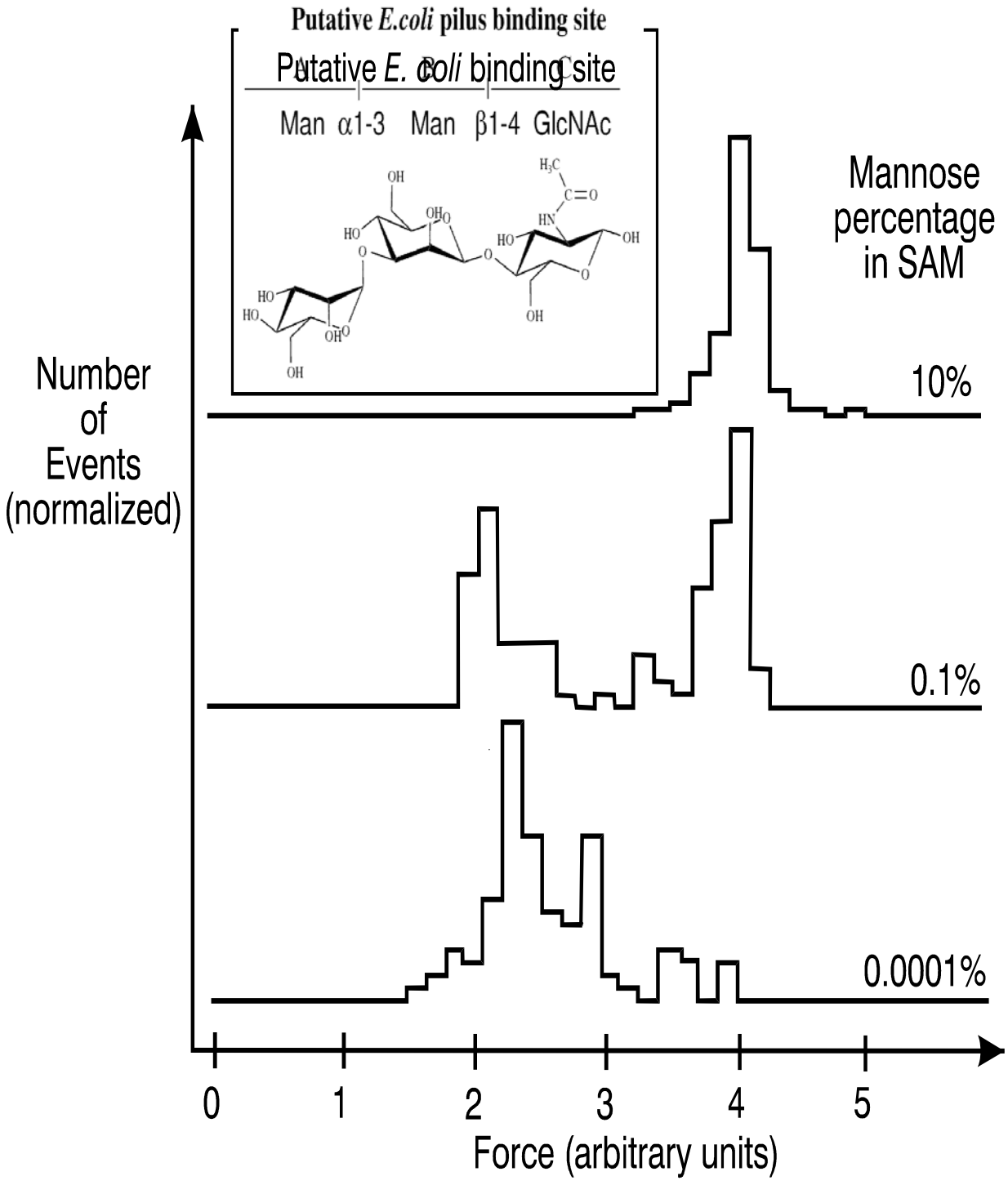
Multiple sites



Continuum of powers above minimum

Experimental Results Show 2 Bonds on 1 Site

E. coli Bond Rupture Force



Tweezer Based Measurements of E. Coli Binding

- First observation of unbinding with optical tweezers
- Demonstrated quantized specific binding of E. Coli to surfaces
- Measured effects of inhibitors on binding force
  - some inhibitors INCREASED strength of individual bonds
- Found that each pili had at most two binding sites
  - consistent with literature of dimer at end of pili with only 1 dimer/pili available
- Found negligible probability of spontaneous release of doubly bound E.coli
- Observed spontaneous release of singly bound E. coli
  - can measure time between unbinding events
- Mannose in solutions will cause bound E.coli to unbind even without tweezers

Single Molecule Binding of Ligand/Receptor Pairs isolated on artificial surfaces

- **Determine the energy landscape of the binding potential**
- **Measure the dynamics of binding and unbinding**
- **Measure the effects of inhibitors**
- **Measure steric effects**
- **Measure effects due to the presence of macroscopic surfaces**

Receptor: A molecular structure or site on the surface or interior of a cell that binds with substances such as hormones, antigens, drugs, or neurotransmitters A molecular structure within a cell or on the surface characterised by selective binding of a specific substance and a specific physiologic effect that accompanies the binding, for example, cell surface receptors for peptide hormones, neurotransmitters, antigens, complement fragments and immunoglobulins and cytoplasmic receptors for steroid hormones.
2. A sensory nerve terminal that responds to stimuli of various kinds.

Ligand: An ion, a molecule, or a molecular group that binds to another chemical entity to form a larger complex Any molecule that binds to another, in normal usage a soluble molecule such as a hormone or neurotransmitter, that binds to a receptor. The decision as to which is the ligand and which the receptor is often a little arbitrary when the broader sense of receptor is used (where there is no implication of transduction of signal). In these cases it is probably a good rule to consider the ligand to be the smaller of the two thus in a lectin sugar interaction, the sugar would be the ligand (even though it is attached to a much larger molecule, recognition is of the saccharide).

Quantitative Information K_{on} K_{off}

Law of Mass action

According to the law of mass action, the reversible reaction A + B ? AB can be described as two reactions that occur at characteristic rates that depend on the energy landscape of the bond, reactant concentration, and temperature only

$d[AB]/dt = k_{on} [A] [B]$

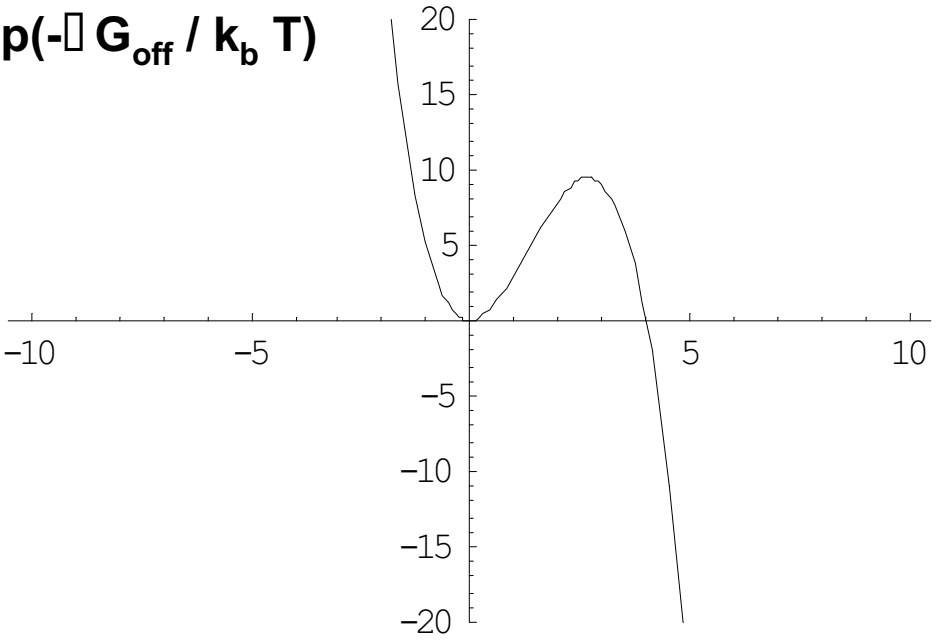
$d[A]/dt = d[B]/dt = k_{off} [AB]$

where k_{on} and k_{off} are constant functions of temperature

Kon and Koff in terms of Free Energy Difference ΔG_{on} ΔG_{off}

$k_{on} = v_{on} \exp(-\Delta G_{on} / k_b T)$

$k_{off} = v_{off} \exp(-\Delta G_{off} / k_b T)$



Effect of Applied Force

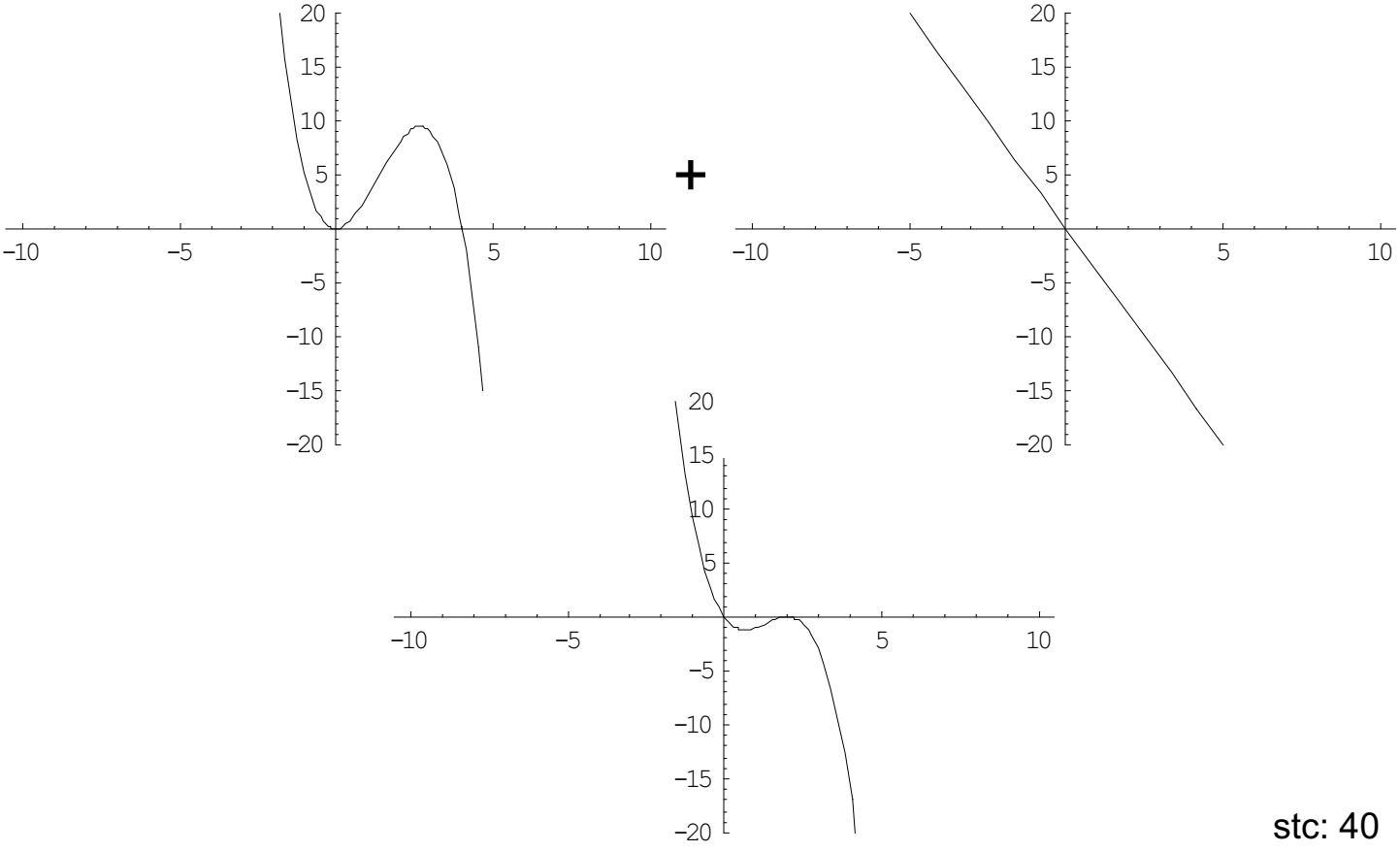
According to the Bell model,

$W = -F_{magnetic}\, d$

$\Delta G(F) = \Delta G(0) - F x$

where x is the reaction coordinate, which, in a simple model, is roughly the distance that A and B must be separated to break the bond.

$k_{off}(F) = k_{off}(0) \exp(F x / k_b T)$



Measuring K_{off} (0) and x using Applied Force

- **The off rate, k_{off} (F) is measured by examining the number of unbroken bonds as a function of time:**

- **$N(t) = N(0) \exp(-t\, k_{off}(F))$**

  - **By measuring the k_{off} (F) rate at various forces, it is possible to extrapolate the k_{off} rate at zero force (k_{off} (0) or k_{off} in solution) as well as to calculate the unbinding reaction distance (x) and the transition state barrier (DG). The graph of F vs k_{off} (F) can be fitted to the decay equation:**

- **$k_{off}(F) = k_{off}(0) \exp(F\, x/k_b\, T)$**

- **If more than one type of bond is present, or there is more than one minima in the potential then multiple decay times will be present, each of which can be treated as described above.**

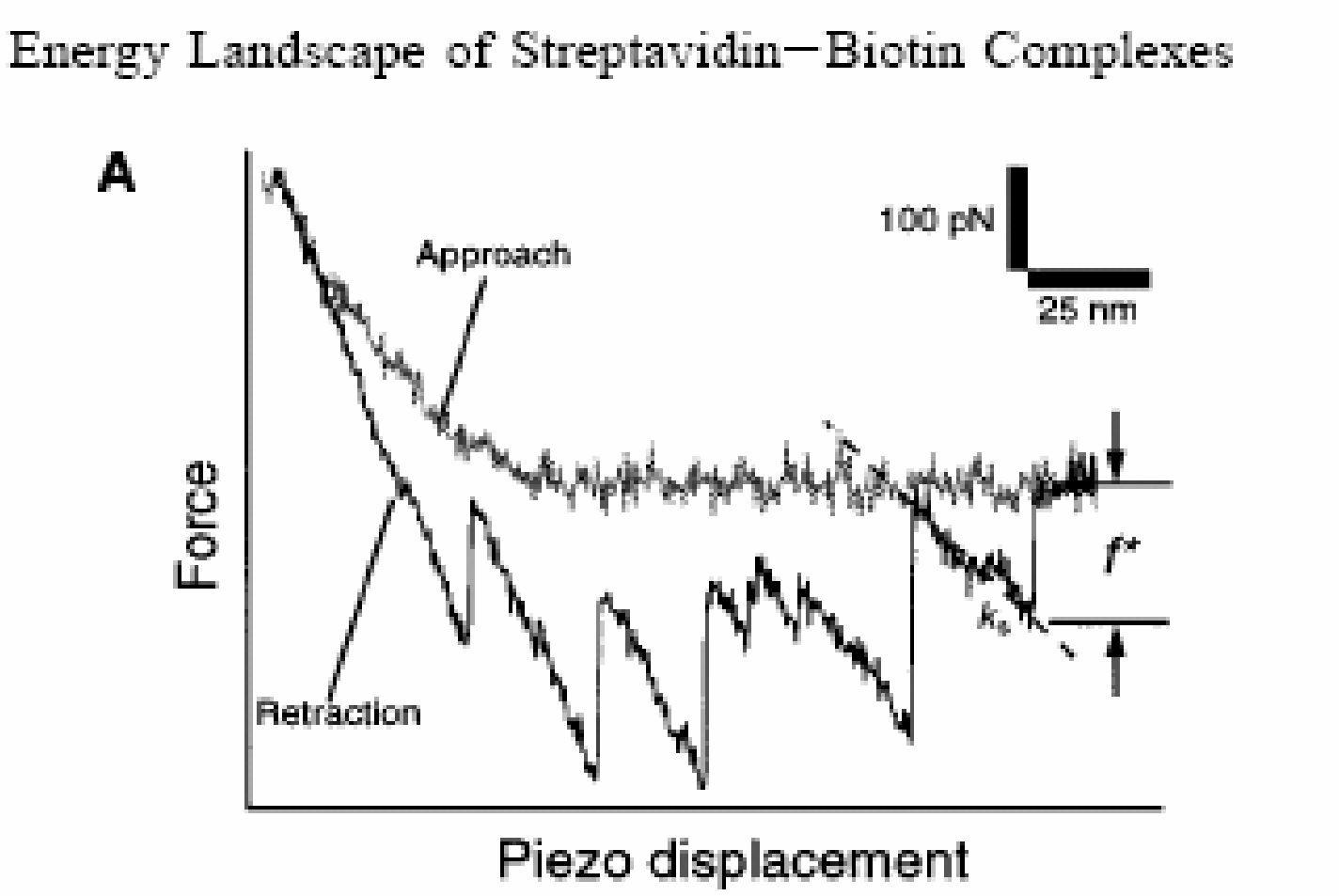
AFM Results

(force depends on time)

$$k_{off} = v_{off} \exp(-\Delta G_{off} / k_b T)$$

$$k_{off}(F) = k_{off}(0) \exp(F x / k_b T)$$

Measure rupture force and distance

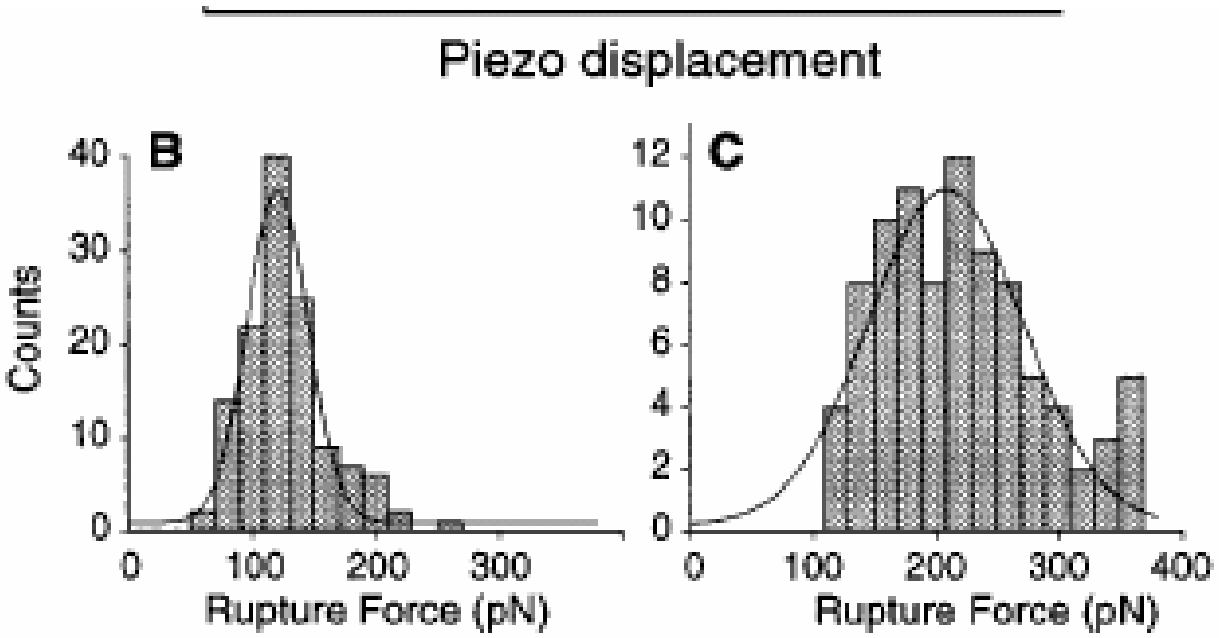


AFM Results

distribution of rupture forces as a function of loading rate

198 pN/sec

2300 pN/sec



AFM Results

evidence for an intermediate state

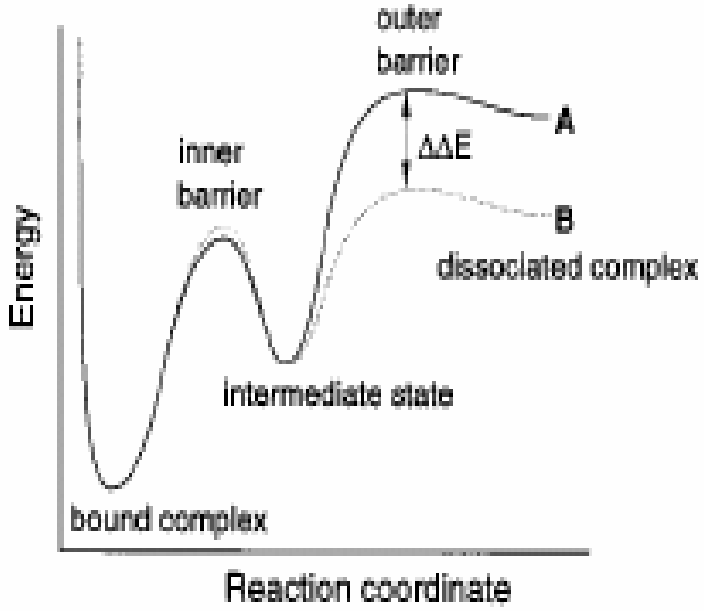


FIGURE 4: Conceptual energy landscapes of the (A) streptavidin−biotin interaction and the (B) W120F−biotin interaction.

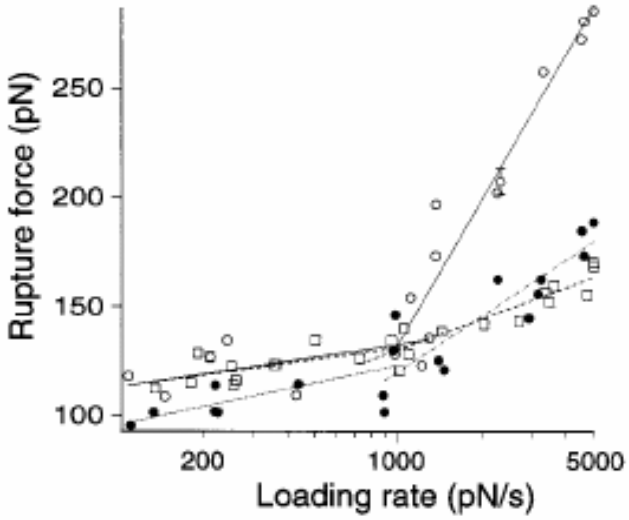
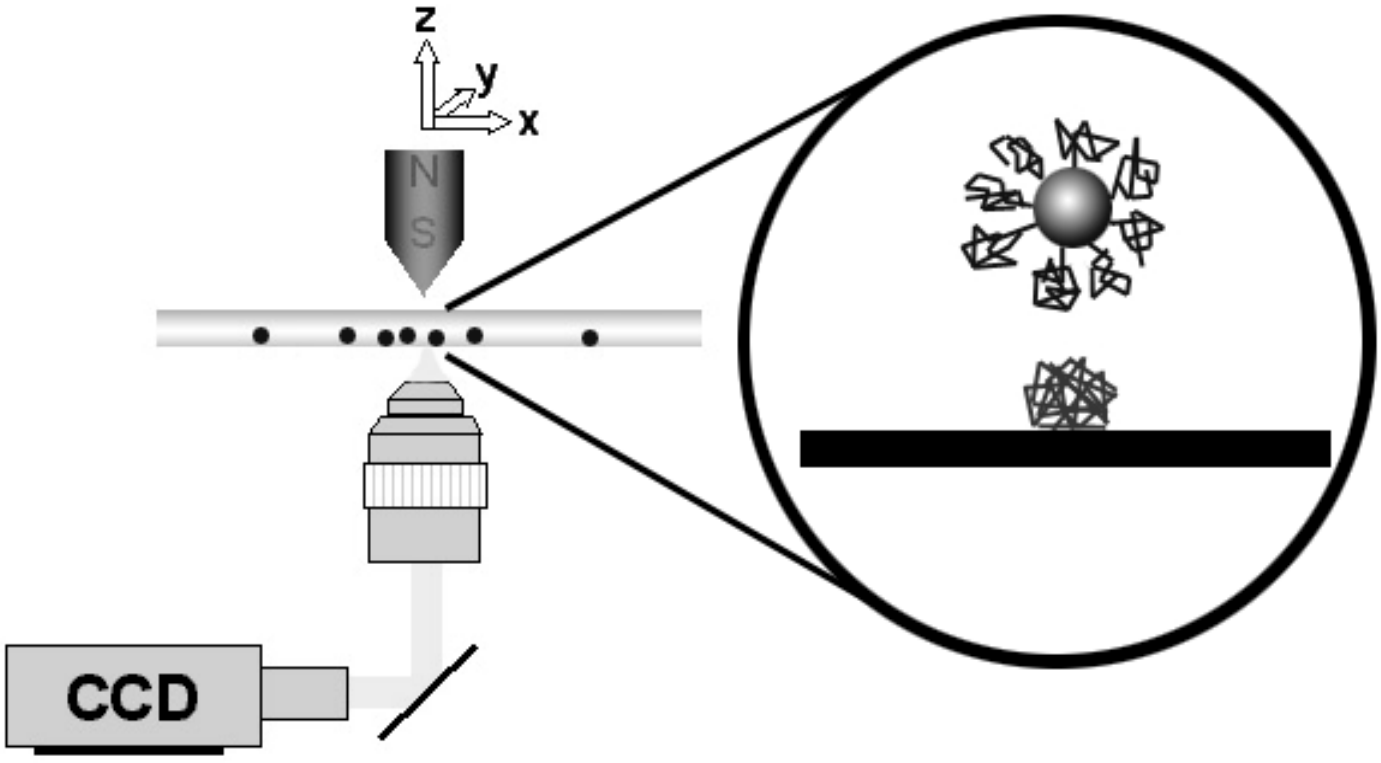


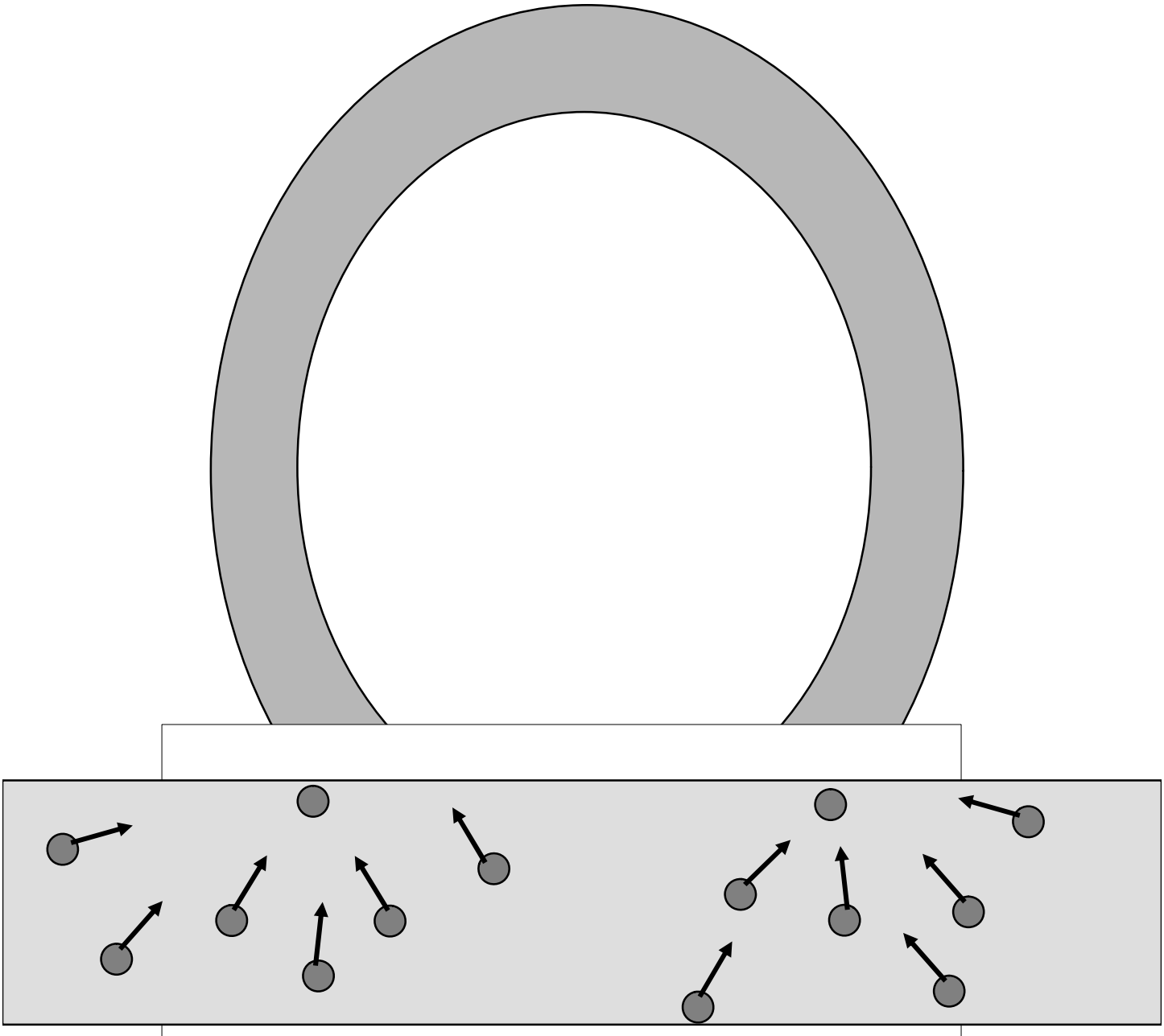
FIGURE 3: Loading rate dependence of the rupture force in the unbinding of the streptavidin−biotin (○), avidin−biotin (□), and W120F−biotin (●). Both regimes in force spectra were fitted to the Bell model. Standard errors of all data points were less than 5% of the mean value. Representative error bars were placed on selected data points.

Ligand-Receptor Binding using Magnetic Tweezers at constant force



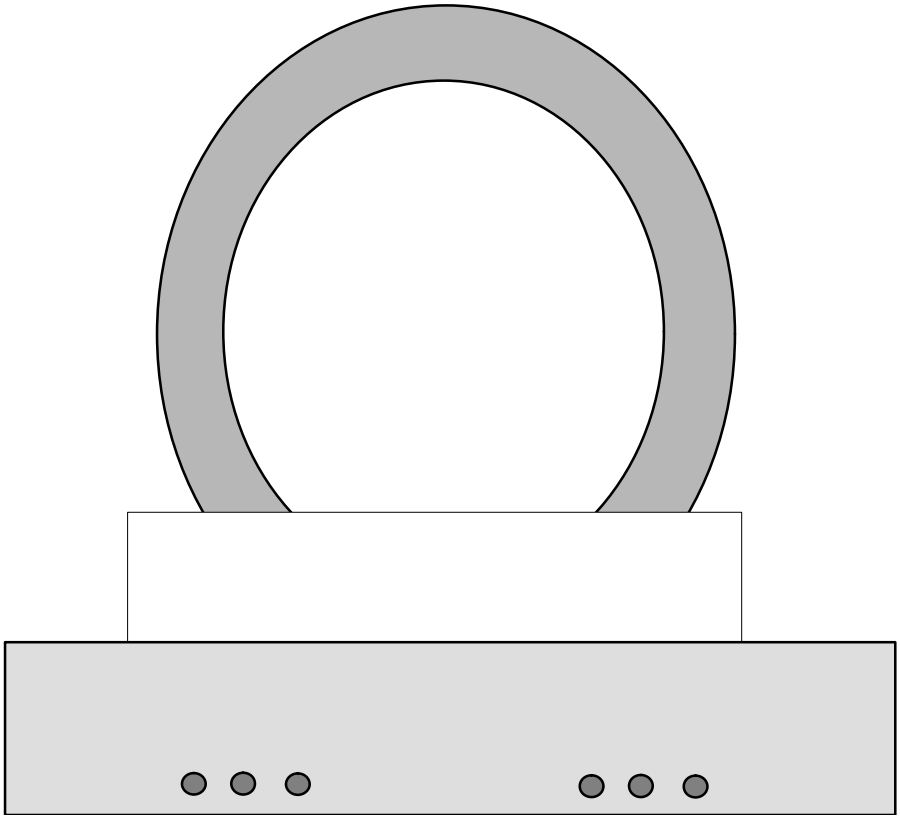
- Single molecule measurements of ligand- receptor interactions.
- Compare with other techniques (relate single molecule results to bulk properties).
- Probe energy landscape.
- Surface confined molecules.
- Good statistics possible with parallel measurements.

Magnetic Crystals of Paramagnetic Beads Energy= |m||B|



Beads are attracted to B field MAXIMUM

Beads Attached to a surface are pulled away, exerting a force on the molecule attaching the bead to the surface



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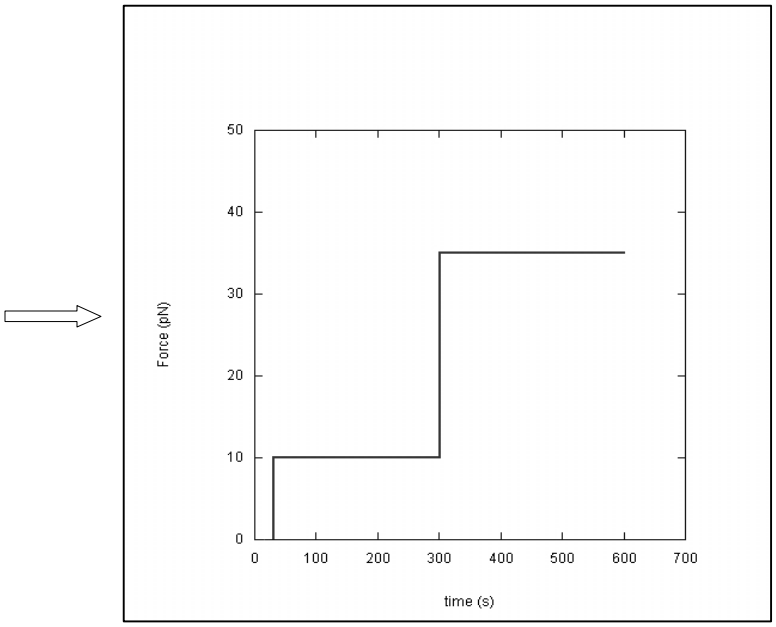
Magnetic Tweezer Based Adhesion Measurements

•**Experiment:**

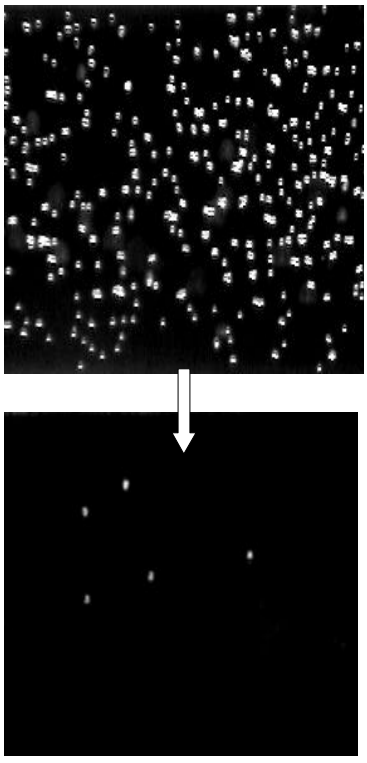
Allow the beads to contact the surface for 30'' .

Apply a low force ~ 5-10 pN

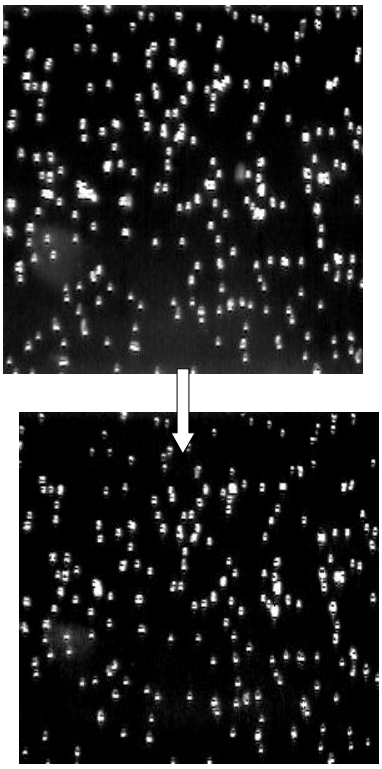
Apply force required to unbind ligand- receptor pairs.

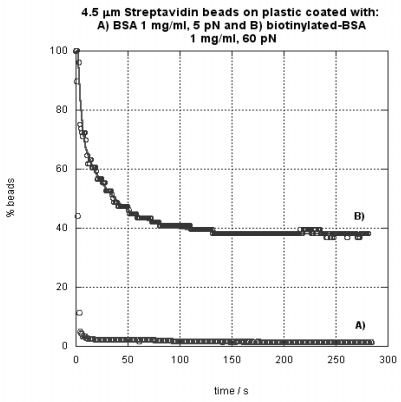


Negative control



Positive control after low force





Non-Specific Binding

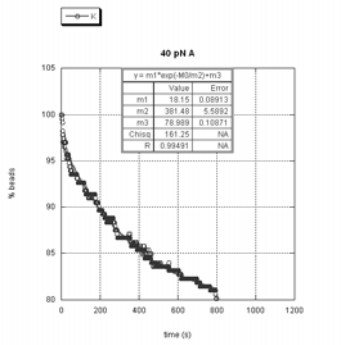
- A big issue in early experiments
- A big problem for bead based assays
- Can combat with appropriate surface coatings
- Inert to protein binding not the same as inert to bead binding

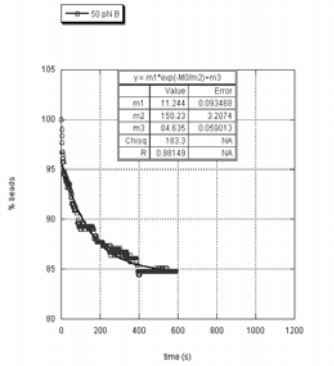
Different “Inert” Surfaces

SURFACE	BEADS	SOLUTION	LOW FORCE	HIGH FORCE	CONTROL
BSA on PVC	**Streptavidin**	**Buffer PBS**	**40-60 % beads remaining**		**(-)**
BSA on PVC or PVC	**Streptavidin**	**Buffer PBS / Tween 20, 0.05%**	**< 5 % beads remaining**		**(-)**
Biotinylated-BSA on PVC	**Streptavidin**	**Buffer PBS / Tween 20, 0.05 %**	**90 % beads**	**40 – 80 % remain depending on force**	**(+)**
Biotinylated-BSA on PVC	**Streptavidin and incubated with biotin 10 mM – 1 μM**	**Buffer PBS / Tween 20, 0.05 %**	**< 5 % beads remaining**		**(-)**
Biotinylated-BSA on PVC and incubated with streptavidin	**Streptavidin**	**Buffer PBS / Tween 20, 0.05 %**	**< 5 % beads remaining**		**(-)**
BSA : Biotinylated-BSA: 500 :1, on PVC	**Streptavidin**	**Buffer PBS / Tween 0.05 %**	**< 15 % beads remaining**		**(-)**
Streptavidin on PVC or PVC	**Biotin PEO- or biotinPEO + streptavidin**	**Buffer PBS / Tween 0.05% or + biotin 10 mM**	**< 5 % beads remaining**		**(-)**

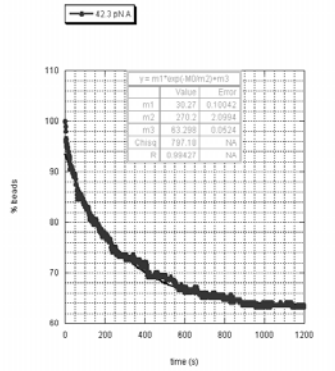
Results

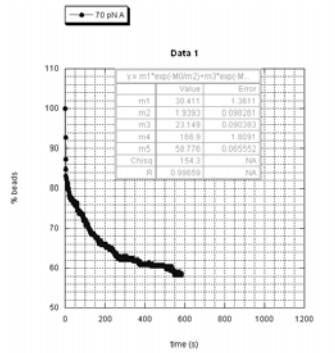
Avidin

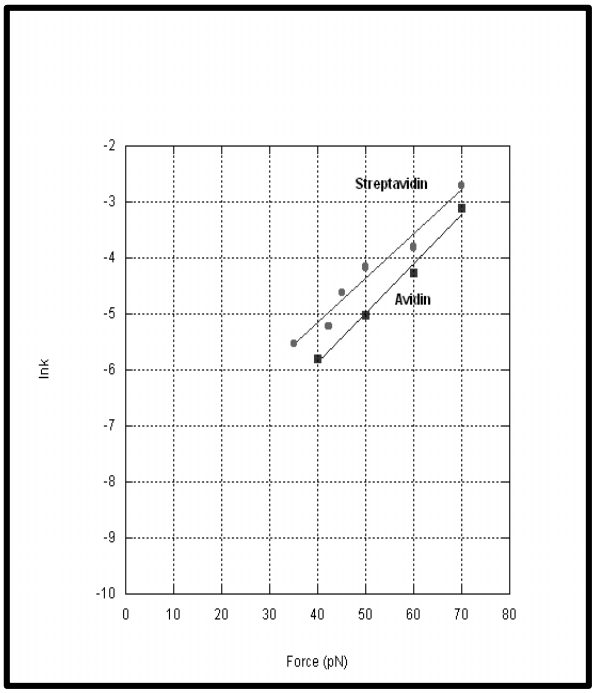




Streptavidin







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Comparison with Other Techniques

Table 1. Apparent Kinetic Constants of Dissociation, k_1^{app}, for Streptavidin Mutants Bound to Biotin-Terminated SAMs[a]

		$k_1^{app}\,(s)^{-1}$		
	$k_{off}\,(s^{-1})$		SAMs formed from **2** and **3**	
streptavidin type	solution[24,27]	SAMs formed from **1**	$\chi_2 = 0.15$	$\chi_2 = 0.007$
wild-type	$(3.3 \pm 0.1) \times 10^{-6}$		$(1.37 \pm 0.08) \times 10^{-4}$	$(3.31 \pm 0.16) \times 10^{-4}$
Y43A	$(5.7 \pm 0.1) \times 10^{-4}$	$(4.16 \pm 0.17) \times 10^{-4}$	$(2.05 \pm 0.04) \times 10^{-3}$	$(7.25 \pm 0.40) \times 10^{-2}$
W120A	*b*	$(5.40 \pm 0.08) \times 10^{-2}$	0.336 ± 0.019	2.44 ± 0.24

[a] The rate constants of dissociation of streptavidin−biotin in solution are smaller than on the surface and were obtained from refs 24 and 27. [b] Dissociation of biotin from W120A is too fast (<60 s) for an accurate determination of k_{off}.

AFM: Moy

Table 1: Bell Model Parameters from f^* vs log (r_f) Relation

ligand−receptor pair	loading rate range (pN/s)	x_β (nm)	k^o (s^{-1})	$\Delta\Delta E^a$ (k_BT)
streptavidin−biotin	100−1000	0.49	1.67×10^{-5}	
	1000−5000	0.05	2.09	
avidin−biotin	100−1000	0.53	6.45×10^{-6}	0.95
	1000−5000	0.20	0.08	3.26
W120F−biotin	100−1000	0.31	6.70×10^{-3}	−5.99
	1000−5000	0.11	1.05	0.69

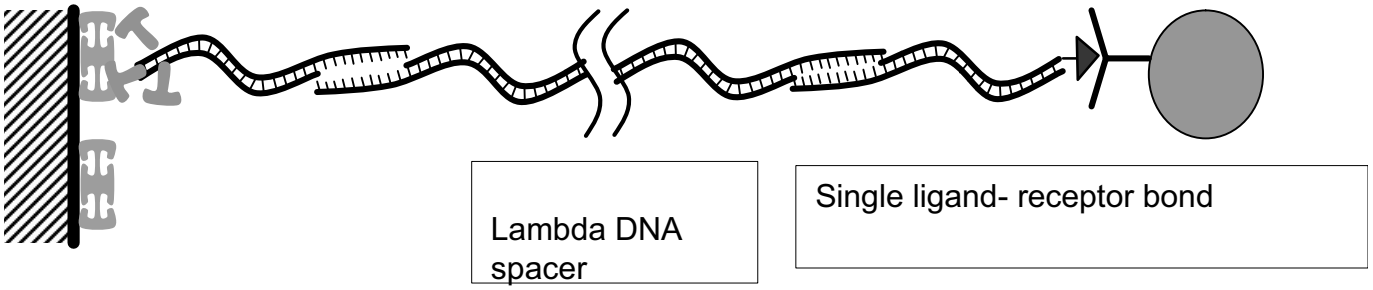
[a] $\Delta\Delta E$ is relative to streptavidin-biotin binding energy.

These results: avidin $k_{off} = 9.10^{-5}$, x= 0.36 nm; streptavidin $k_{off} = 1.4.10^{-4}$, x= 0.38 nm.

BFP (Evans, 1999): avidin- biotin 38 to 85 pN regime: x=0.3 nm.

Results for CA on SAMs also showed higher k_{off} values for surface attached ligand-receptor complexes (Whitesides JACS 117, 12009).

Guaranteed Single Molecule



Use a long spacer with only one single binding site

Long linkers on both sides allow better comparison with solution values

Summary of Ligand-Receptor Binding using Magnetic Tweezers

Accomplishments

✓ **Surface: designed to reduce non-specific interactions significantly (<1%).**

✓ **Interaction studied: biotin- avidin (streptavidin).**

✓ **Calculation of parameters at constant force: reaction off rate, unbinding reaction distance.**

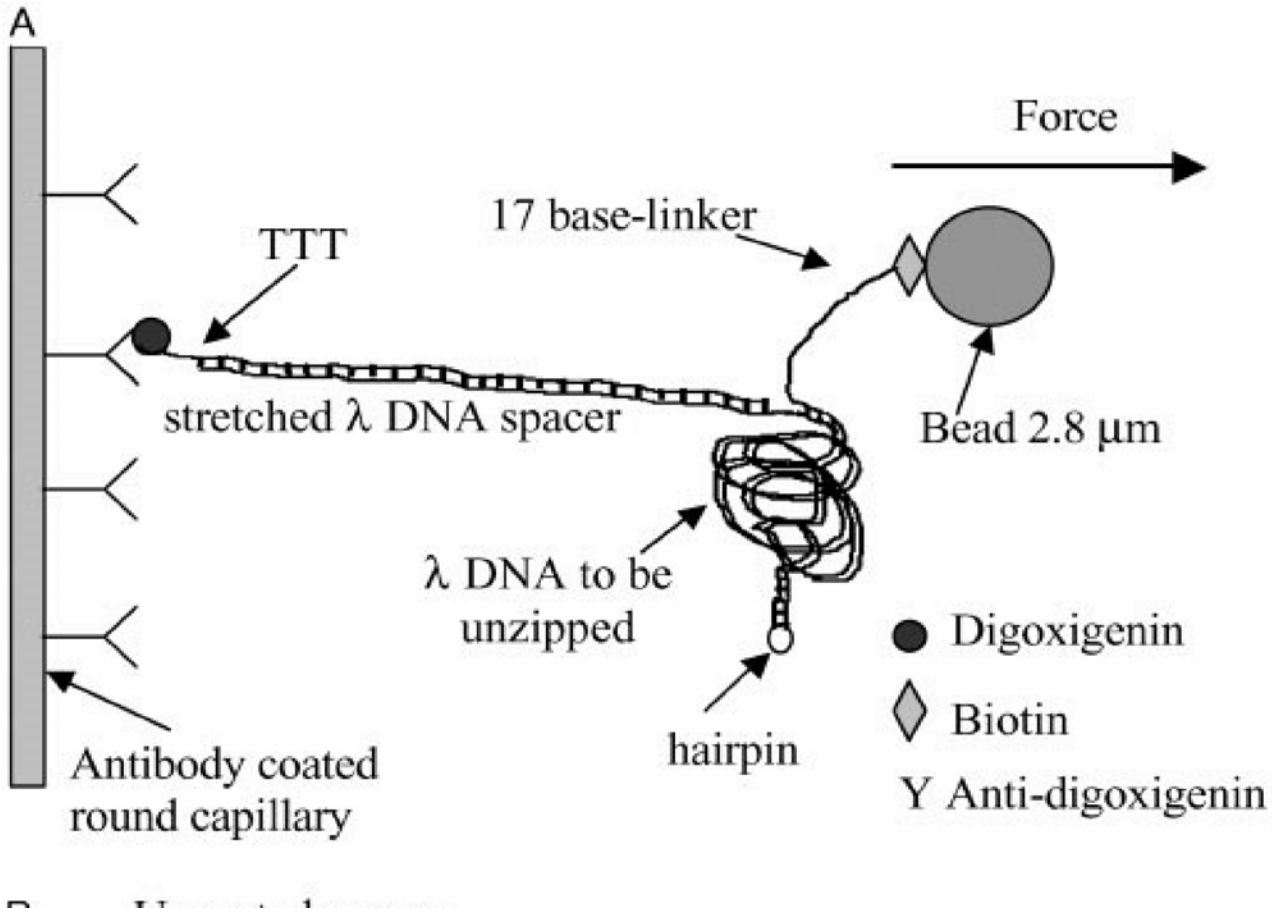
Future Work

- **Include tethers of different lengths to approach solution conditions.**

- **Other surfaces: gold.**

- **Study other ligand- receptor couples such as sulfonamides-carbonic anhidrase or ligand (receptors) on cell surfaces.**

Unzipping Lambda phage dsDNA using magnetic tweezers



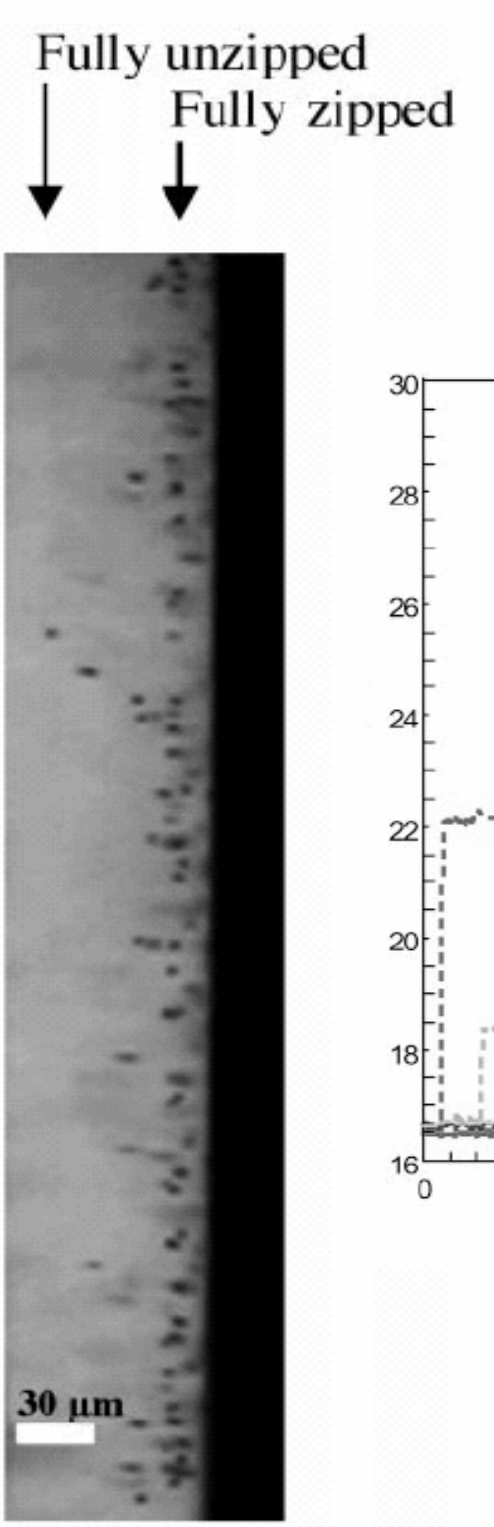
What is the physics?

Unzipping occurs when the free energy of the single stranded is lower than the free energy of the double stranded

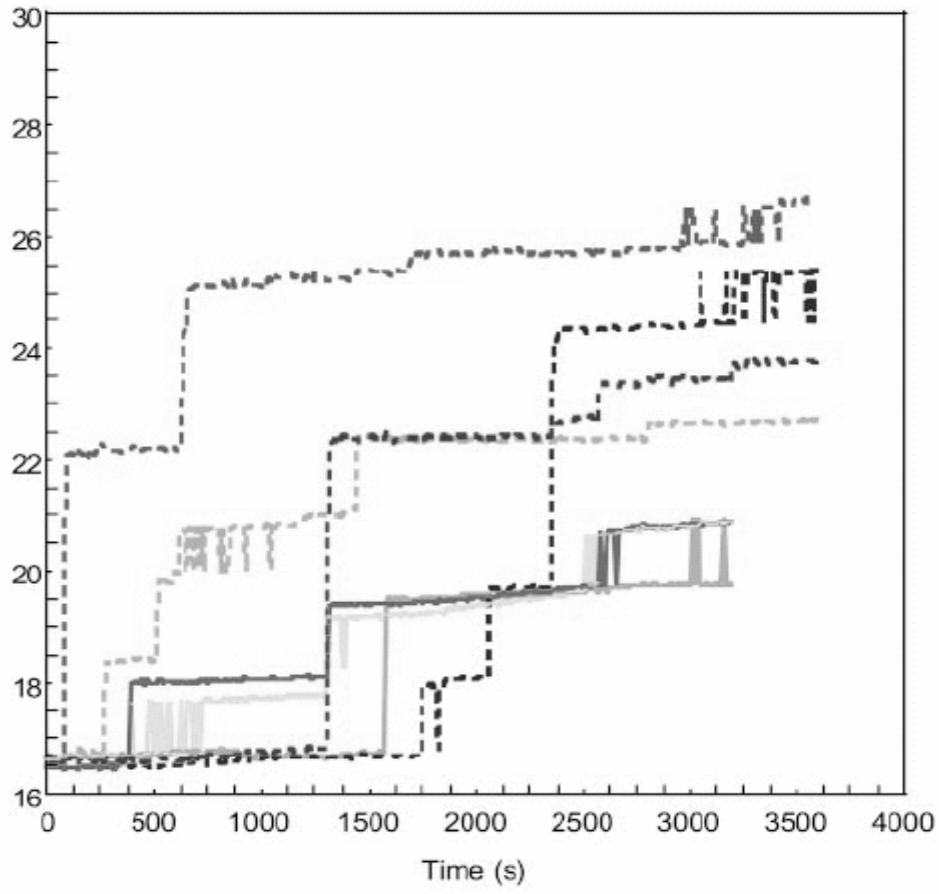
- **Contribution due to entropy difference favors single stranded**
- **Contribution do to enthalpy difference favors double stranded**
- **Applied force aids separation**

  $\Delta G = g_b - 2\, g_u\, (F)$

Observations of the Sequence Dependence of dsDNA unzipping

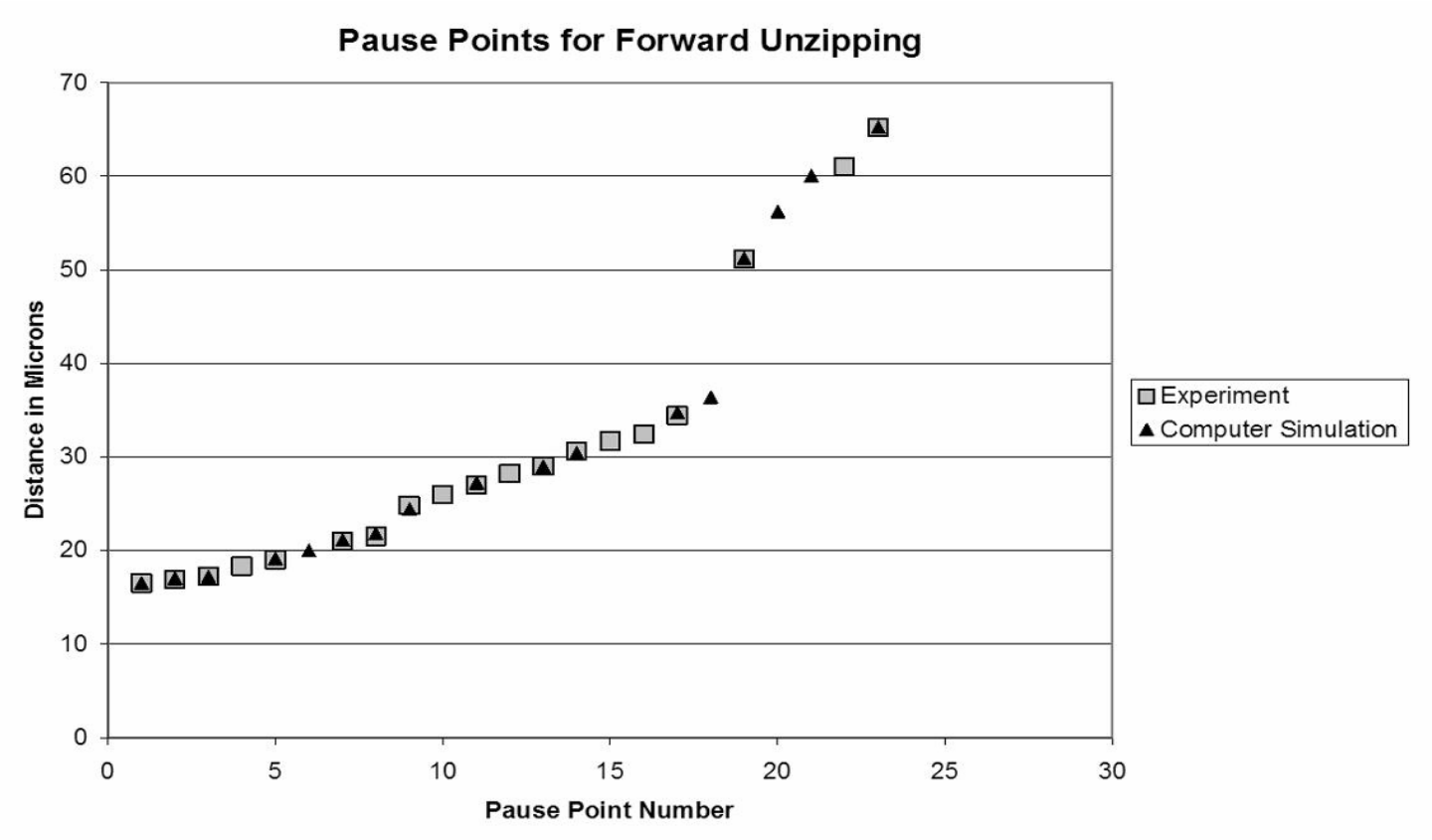


Bead Positions vs Time



Comparison with Theory

Averaged Potential Theory did not work well
Monte-Carlo simulation is a good match



Accomplishments

✓ **Pauses in force-induced unzipping of double stranded were successfully predicted by Monte Carlo, but not by coarse graining**

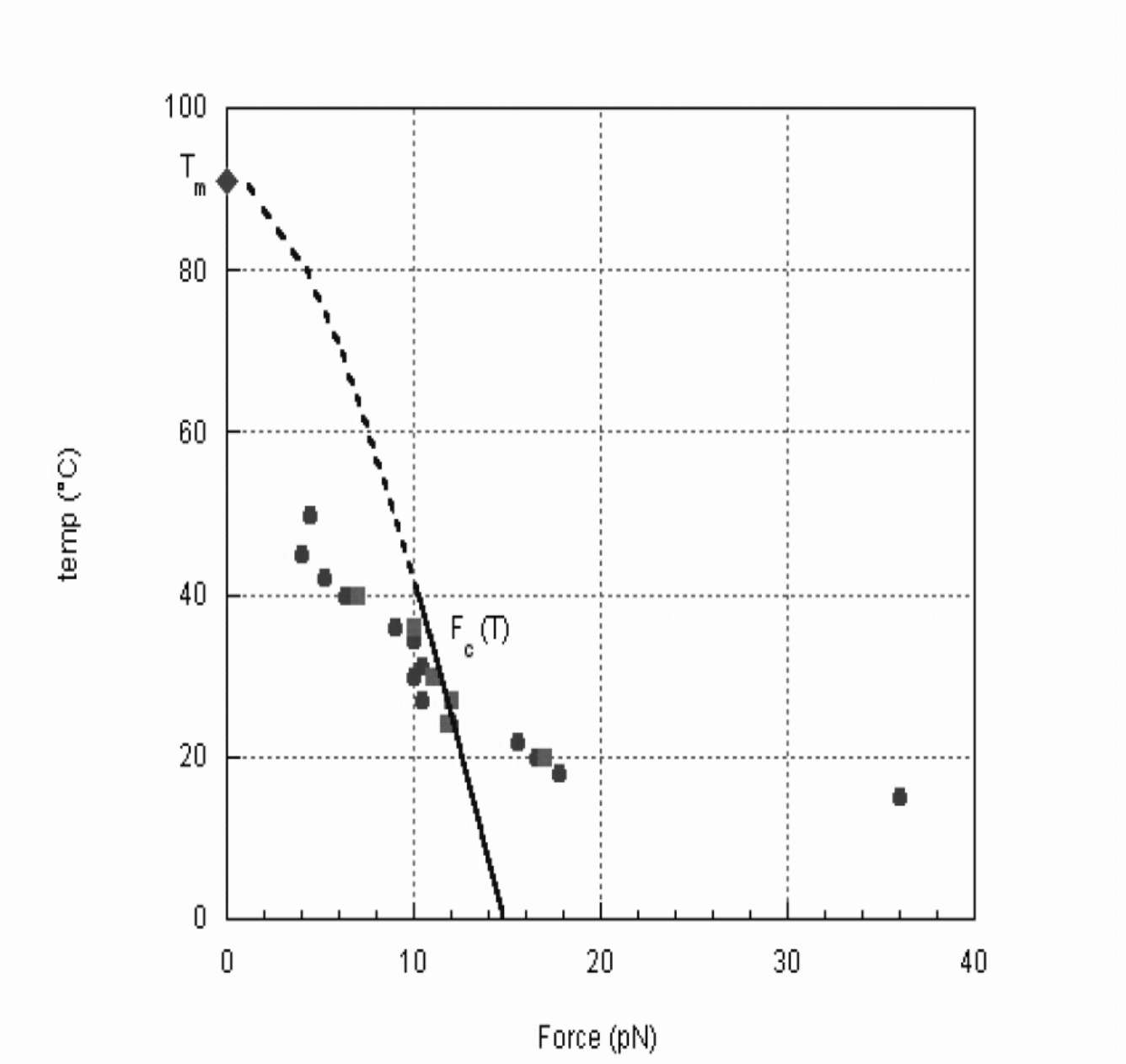
Future Work

- **Investigate interactions beyond nearest neighbors**
- **Explore the kinetics of unzipping through simulations**

Temperature Dependence of Unzipping

- $\Delta G = \Delta H - T\Delta S$
  - unzipping should get easier at higher temperatures
  - DH and DS assumed independent of temperature
- Present results as a phase diagram in the force temperature plane
  - at zero force the transition temperature should be the melting temperature
- Allows comparison of theory with experiment
  - temperature dependence important for DNA chips, PCR, and projection of melting data to in vivo conditions

Phase Diagram of DNA Unzipping



•Surprises at high and low temperature, dependence on thermal history

New Biophysics?

- **In the high temp low force regime effects of bubbles and hairpins have been ignored**
  - **detailed simulation by R. Bonshudt suggests hairpins do not play a role**
  - **Libchaber data supports bubbles, but this is not yet widely held**
- **In low temp regime a structural change appears to take place**
  - **supported by CD data**
  - **supported by force vs extension data**
  - **supported by history dependence**
  - **consistent with previous short sequence data on B' to B transition**

More new biophysics

- **Unzipping force is buffer insensitive from 20-40 C**
  - **stretching curves change drastically**
  - **melting temperature changes of 30 C**
- **Biological robustness makes sense, detailed mechanism still not understood**

Summary Phase Diagram of DNA Unzipping

Accomplishments

✓ **Between 24-35ºC good projections from bulk thermodynamic using nearest neighbor, insensitive to buffer**
✓**Above 35ºC unzipping force depends on buffer and bubbles in the dsDNA may be important**
✓**Below 24ºC dsDNA conformational changes may play a role**
✓**Demonstrate that dsDNA parameters depend on previous history of the sample**

Future Work

•**Evaluate effect of sequence dependent conformation (e.g. B' or A tract)**

•**Improve predictive models for PCR primers**

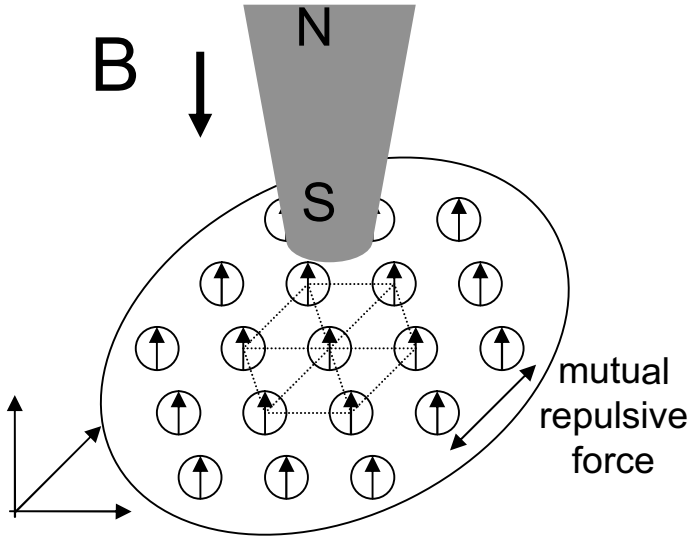
•**Develop rapid lower temperature PCR**

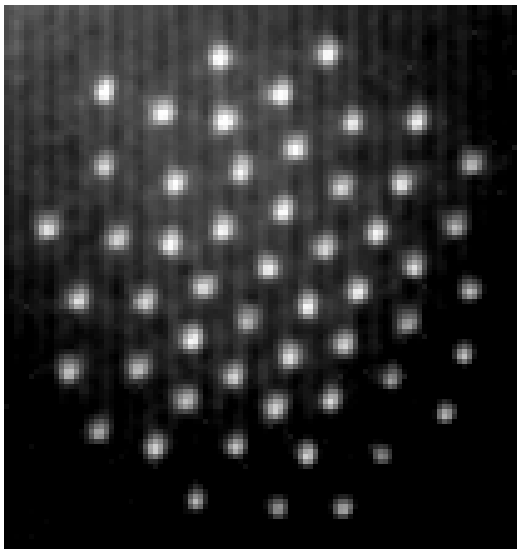
•**Understand history dependence of dsDNA parameters**

Magnetic Field Induced Lattices

Goal: Assemble small particles into an ordered hexagonal lattice

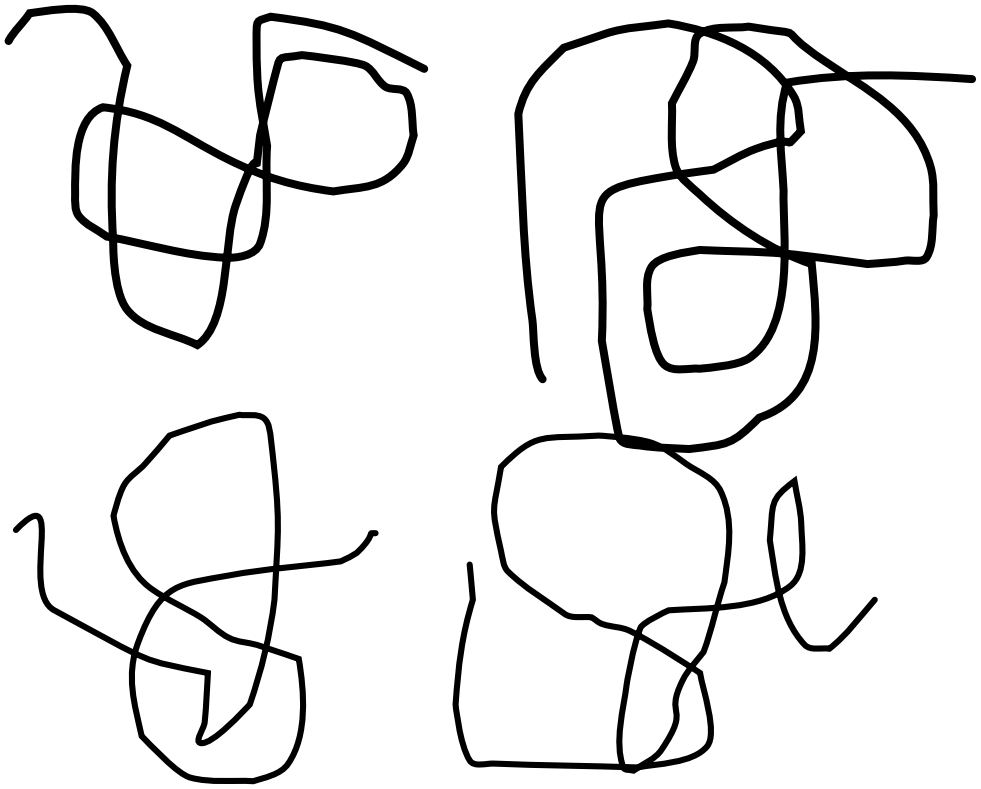
Strategy: Use a magnetic field gradient to attract paramagnetic particles to a center , and dipole/dipole interaction to keep them slightly separated





The beads form an ordered crystal with a spacing increasing as |B|/(grad|B|)

It is often Desirable to Join Long DNA Strands

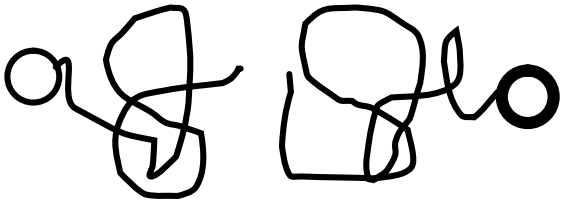


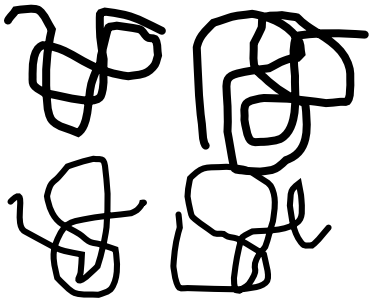
Random walk is not sufficient to bring ends together increasing concentration makes a gel the rate of end attachment DECREASES

Lattice Based Assembly

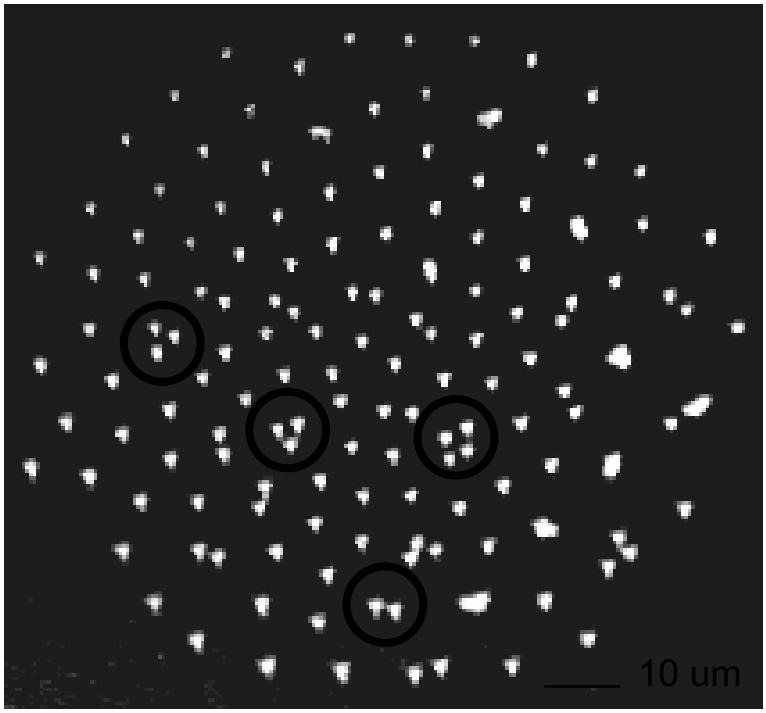
Goal: Attach two separate double strands of DNA to form one longer double strand, and detect successful assembly

Strategy: Attach the DNA to magnetic beads, form beads in crystal, detect DNA attachment as crystal defects



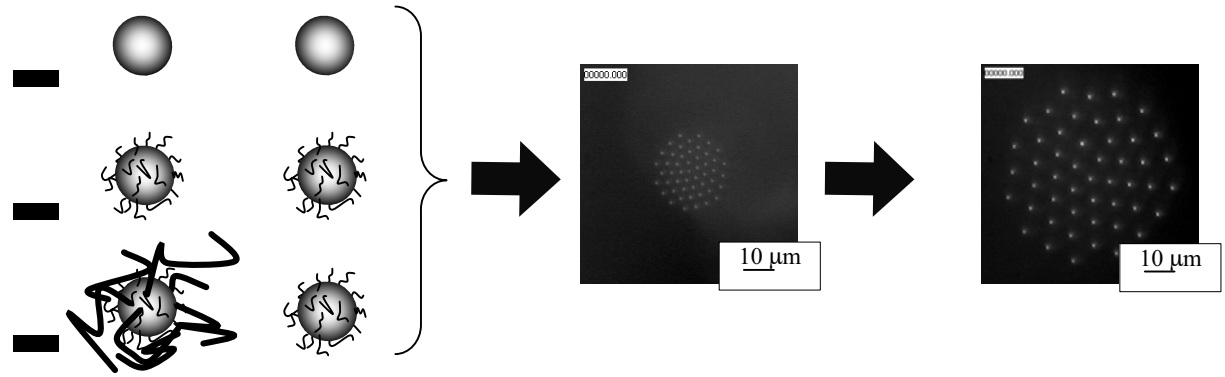


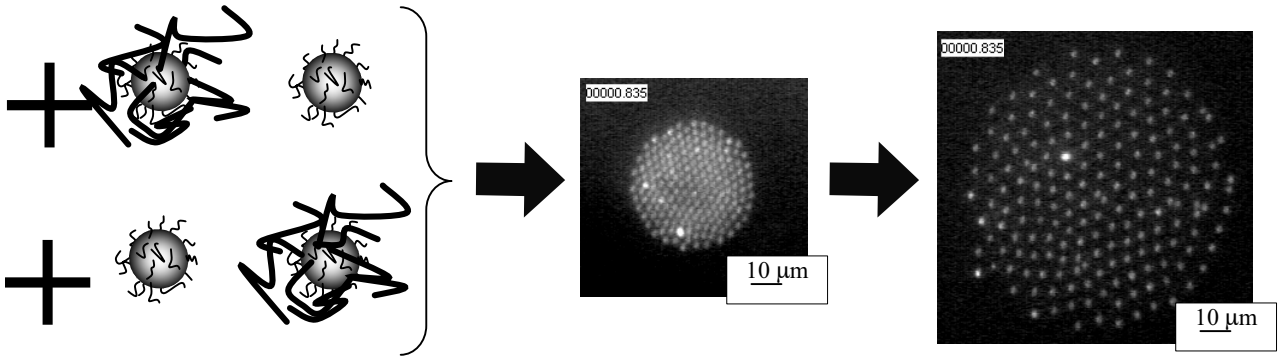
Random walk is not sufficient to bring ends together increasing concentration makes a gel the rate of end attachment DECREASES



DNA concentration is low, but probability of joining ends is high.

Examples



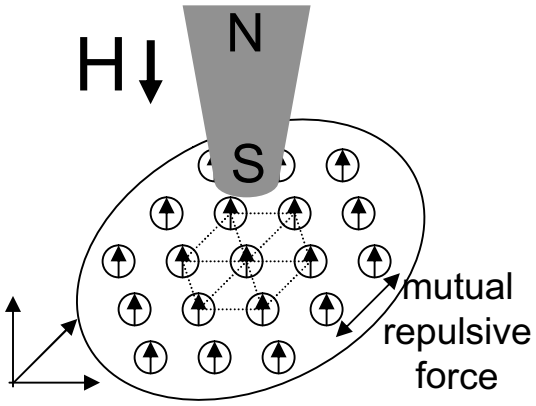


Join DNA Ends by Bringing Beads Together

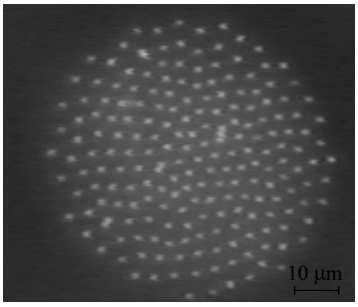
DNA concentration is low, but probability of joining ends is high.

Magnetic tweezers

Self-Assembled Crystal

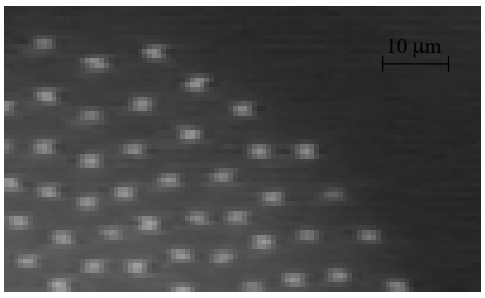


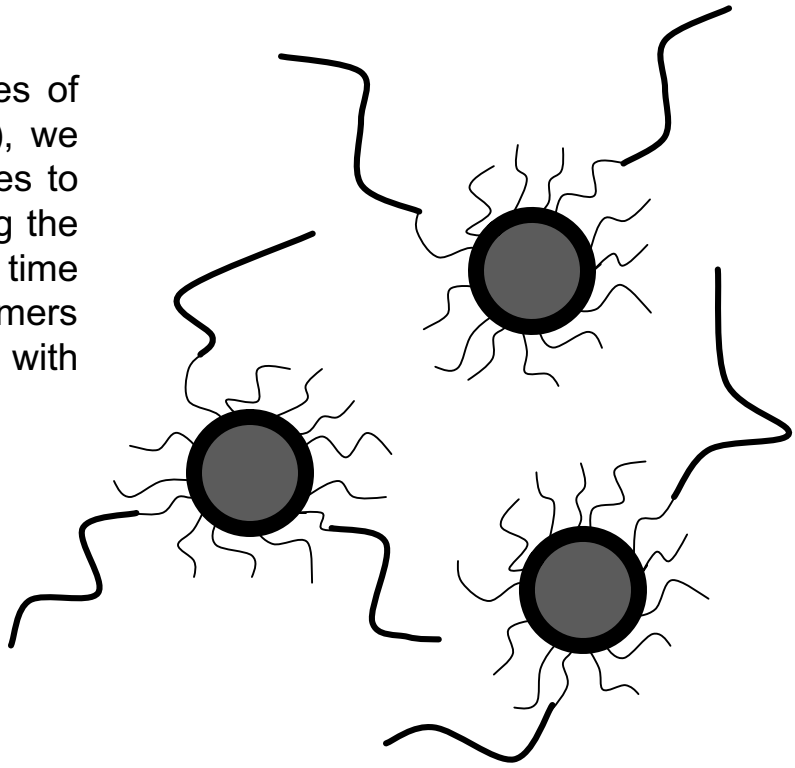
In the presence of a vertically oriented magnetic field, micron-sized super-paramagnetic beads form a 2-Dimensional crystal at the surface of a droplet of water. The spacing between beads can be adjusted by varying the magnetic field strength. We image the crystal using video microscopy.



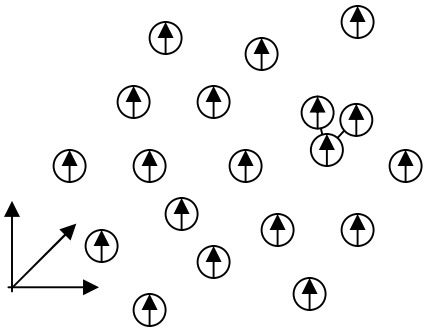
Binding Properties of Large Molecules

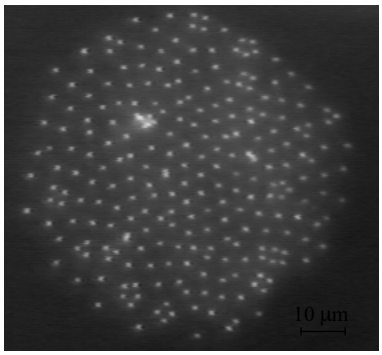
In order to study the properties of long polymers (such as DNA), we chemically attach the molecules to the beads. Then, by controlling the crystal spacing, we control the time and distance at which the polymers interact with each other and with other beads..





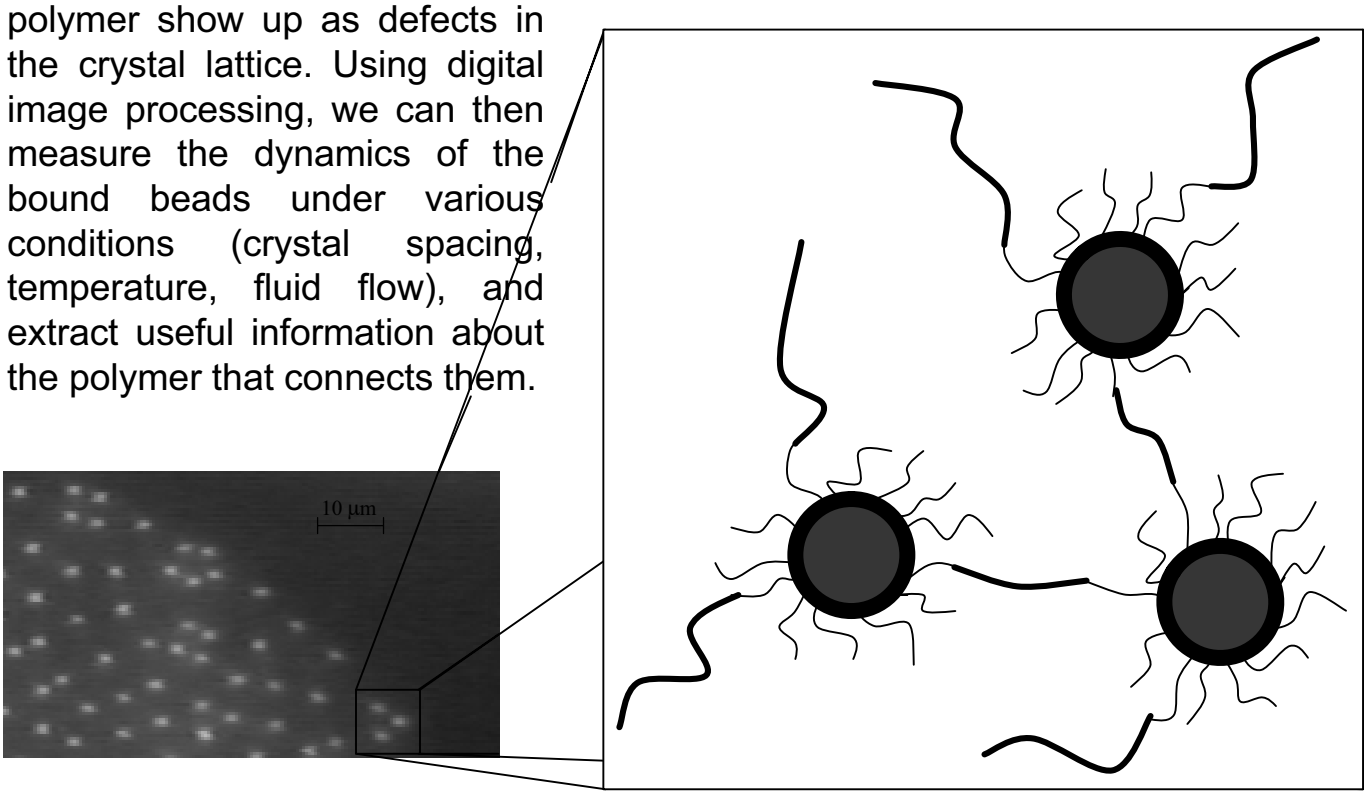
Crystal Defects





Bound Beads

Beads bound to each other by a polymer show up as defects in the crystal lattice. Using digital image processing, we can then measure the dynamics of the bound beads under various conditions (crystal spacing, temperature, fluid flow), and extract useful information about the polymer that connects them.



Results of Lattice Based Measurements

- **Most probable binding difference = radius of gyration**
- **Highly peaked distribution**

Adhesion For Microfabrication

- **Biospecific interactions can be used to build structures from combinations of cells and non-living material**
- **Useful for creating and studying bio-materials interfaces**
- **Methods for creating microstructures that can be disassembled at will**

Macrofabrication: Strategies for Microfab

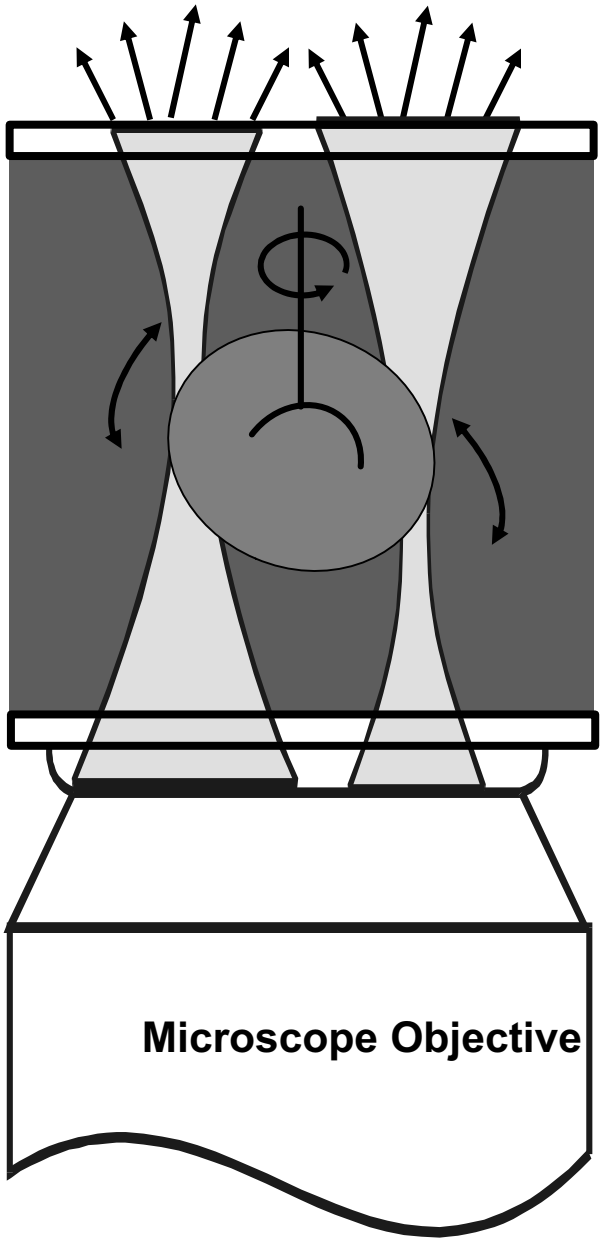
Essential Research Areas for Developing Functional Devices with Biological Components

Materials for Controlling the Interactions of Cells and Proteins with Synthetic Surfaces

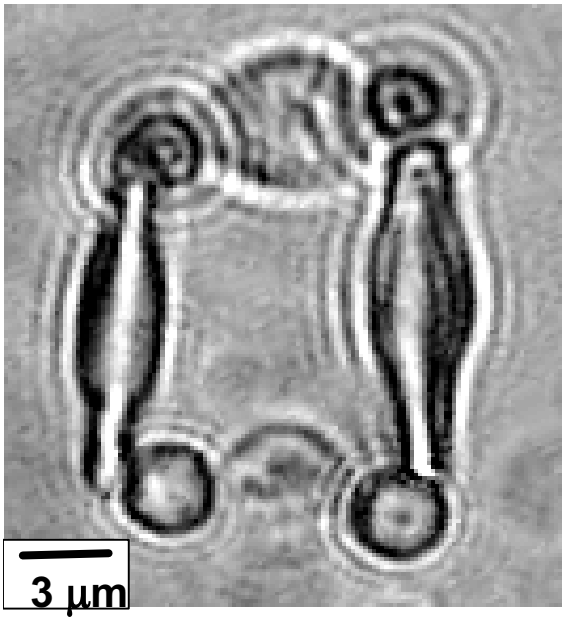
Strategies to Control the Environment of Cells in 2D and 3D Arrays and Assemblies

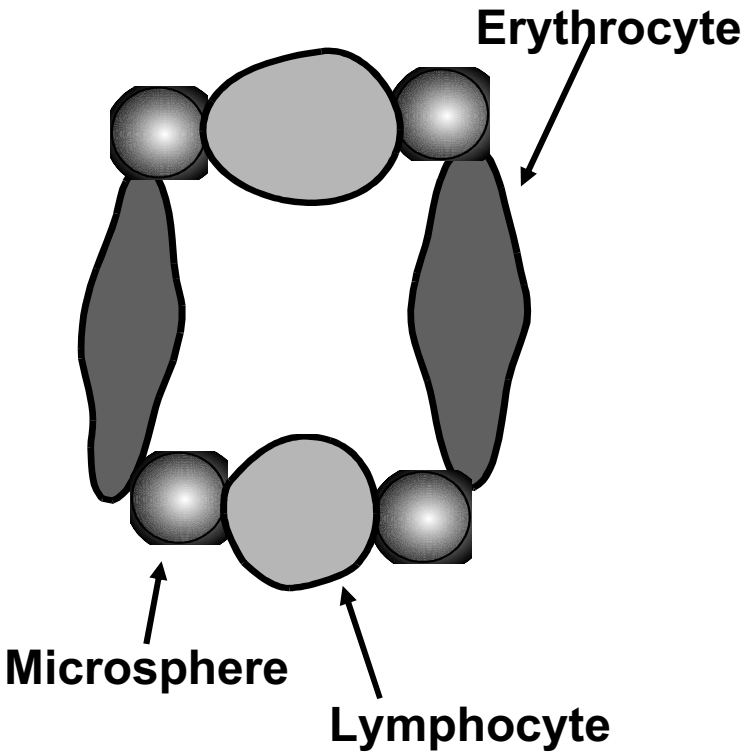
Strategies to Detect and Transduce Biological Signals and Responses

Multiple-Beam Optical Tweezer to Orient Ery

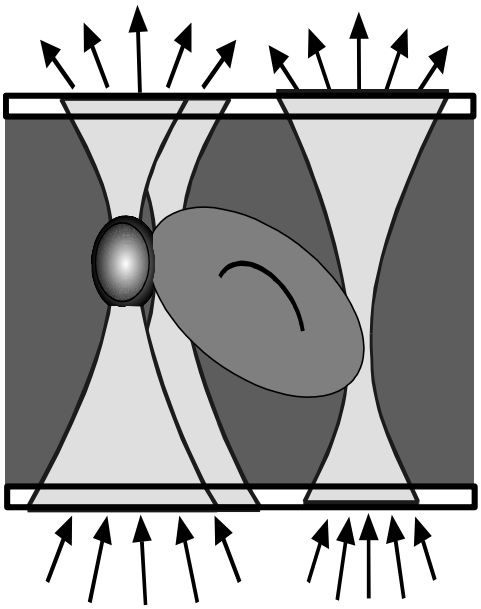


Erythrocytes and Lymphocytes in One Assembly





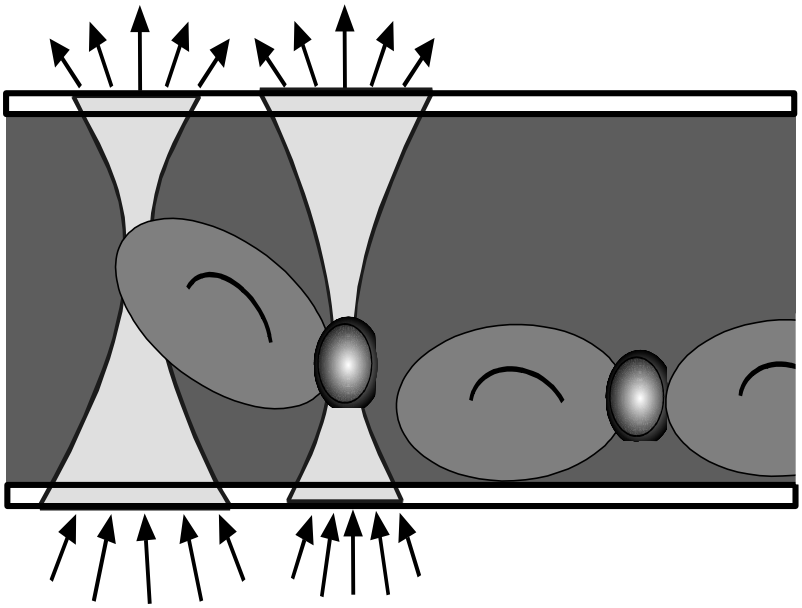
Light-Driven Microfabrication using WGA coated Spheres



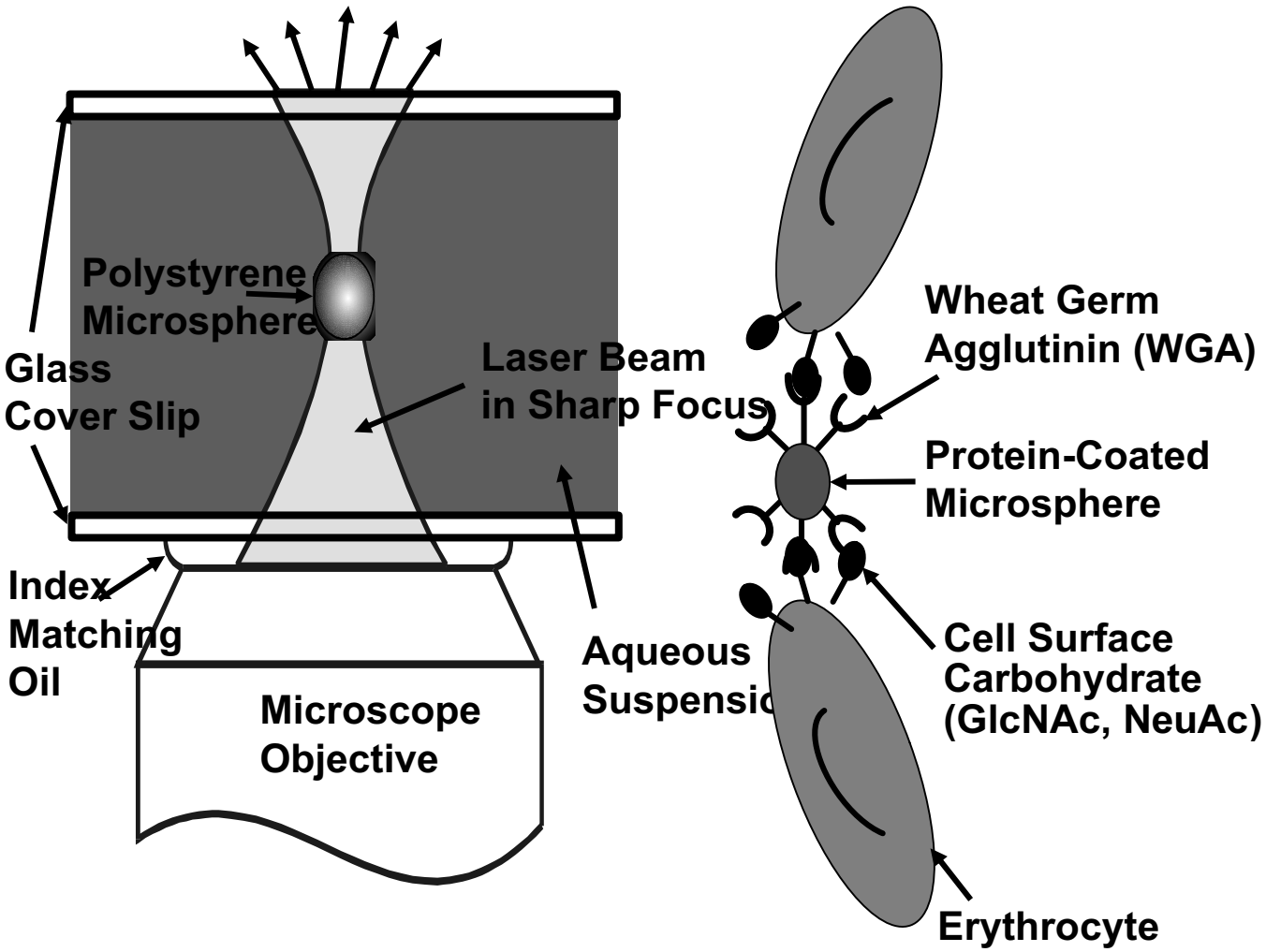
A) Attach sphere to erythrocyte

B) Attach erythrocyte and sphere to growing assembly that rests on the surface of the cover slip

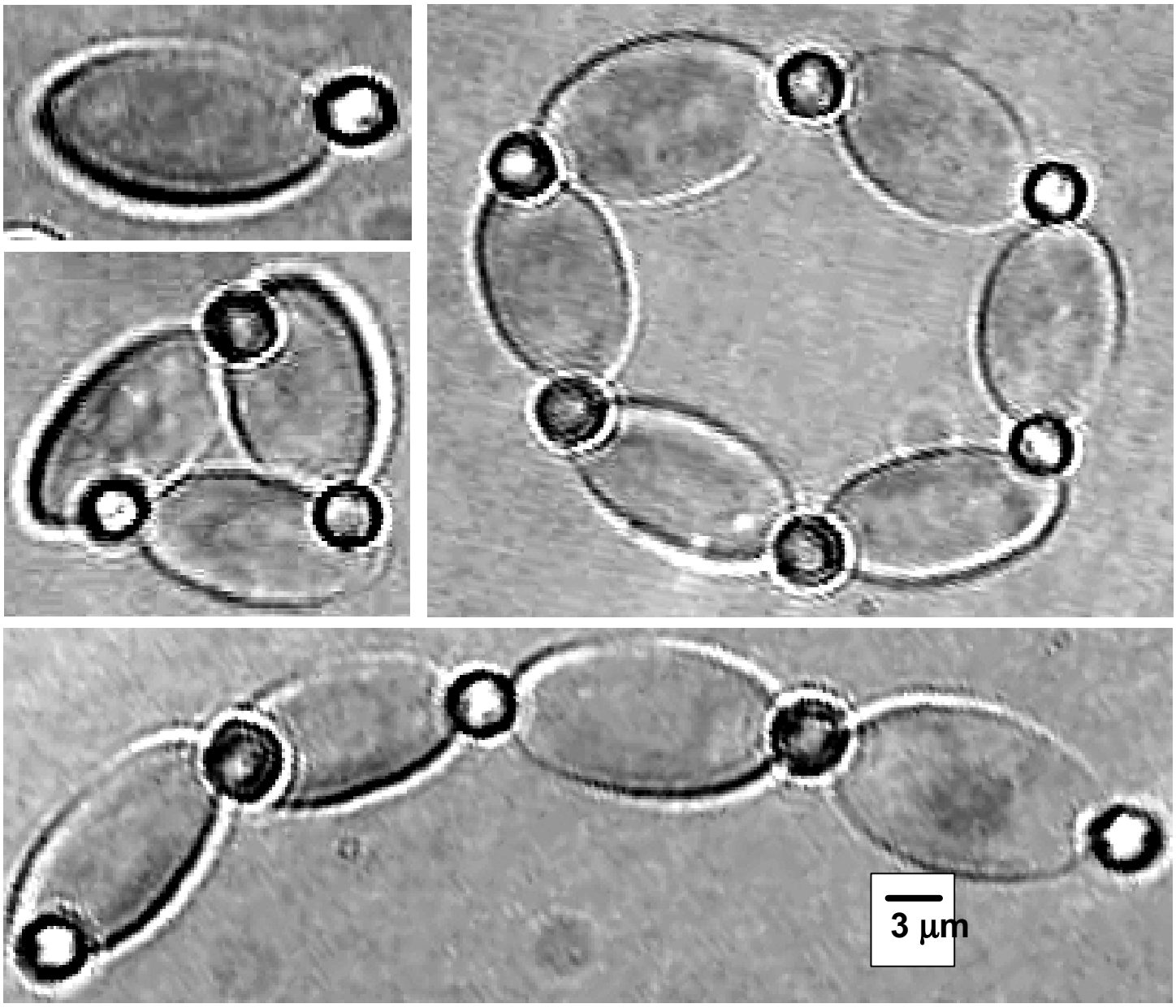
C) Iterate to generate 2D and 3D arrays



Optical Tweezers, Protein-Coated Microspheres, and Erythrocytes in Microfabrication

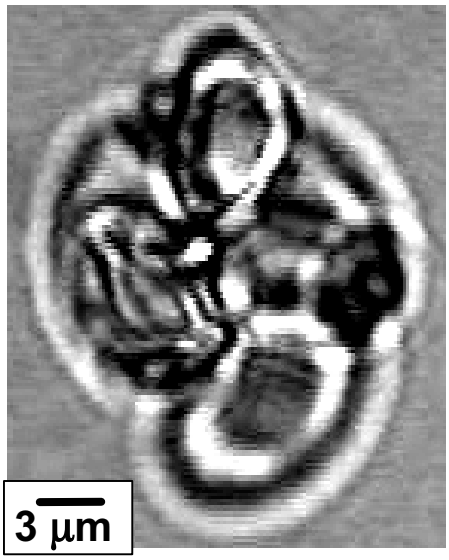


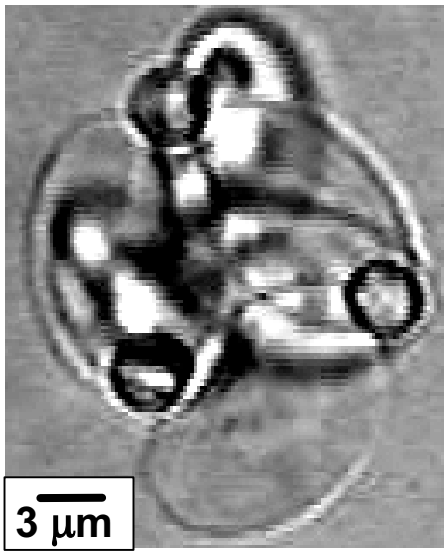
Fabrication of 2D Arrays of Erythrocytes

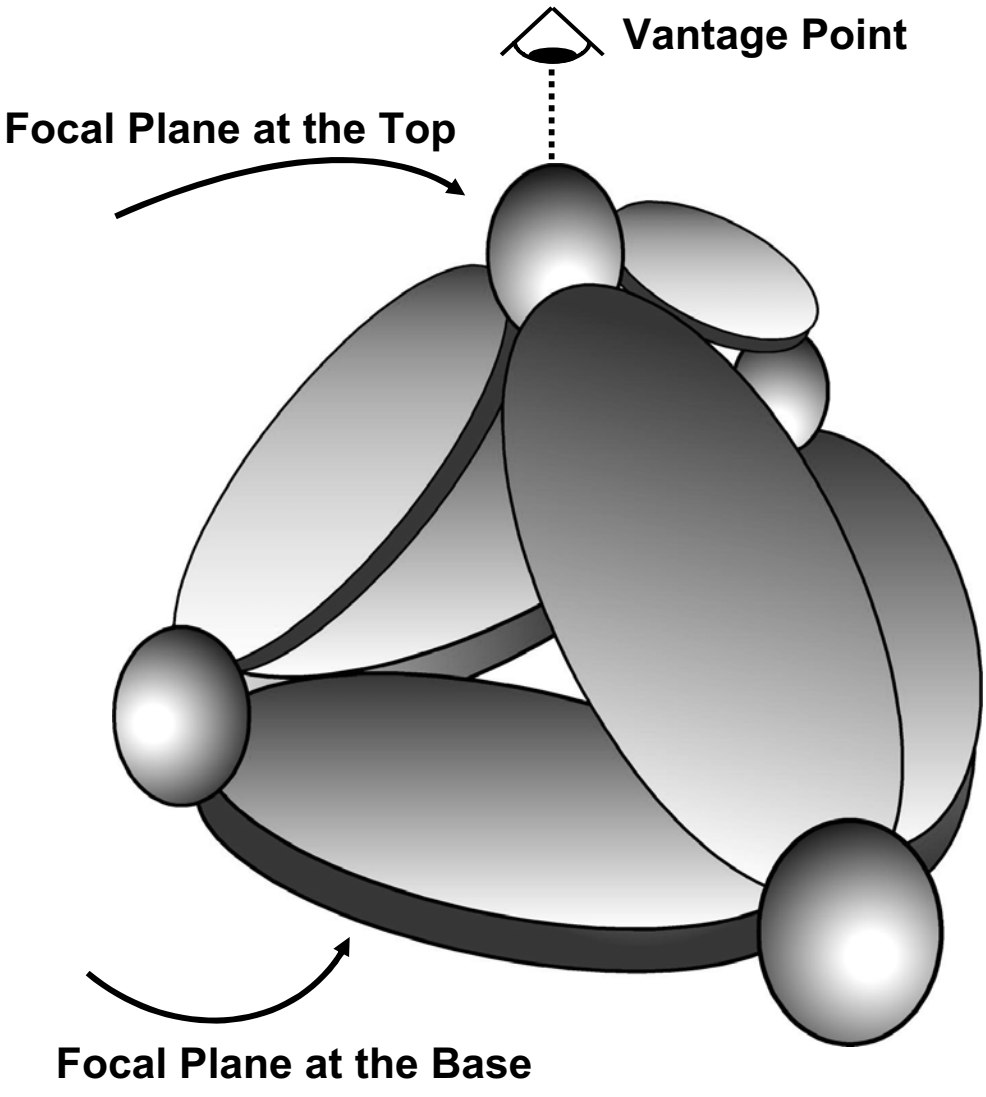


stc: 79

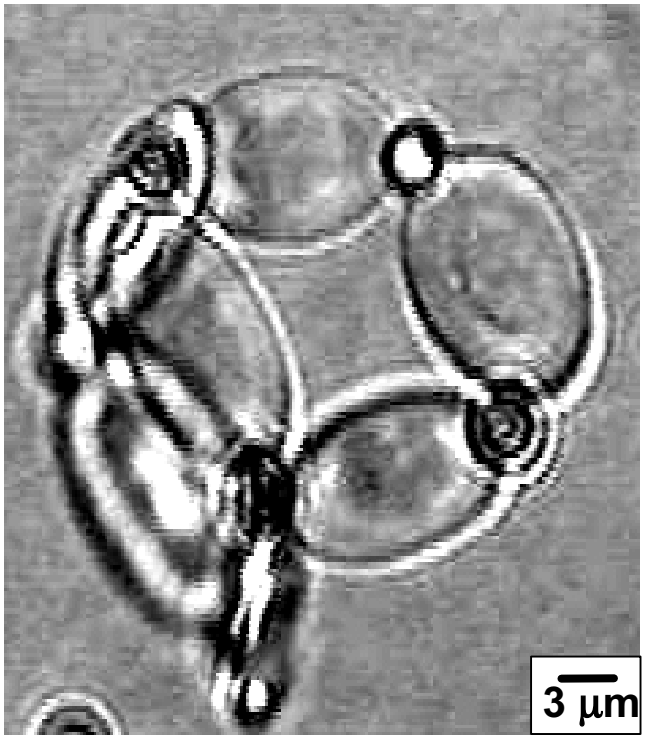
3D Fabrication: A Tetrahedral Array of Erythrocytes

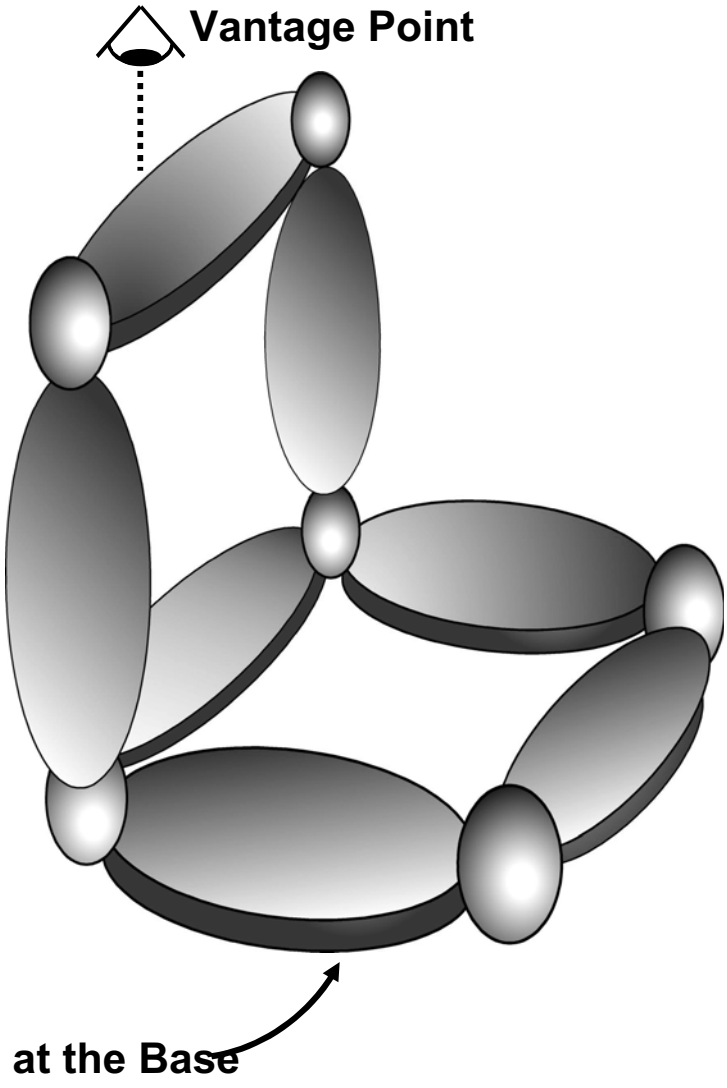






3D Fabrication: Perpendicular Planes





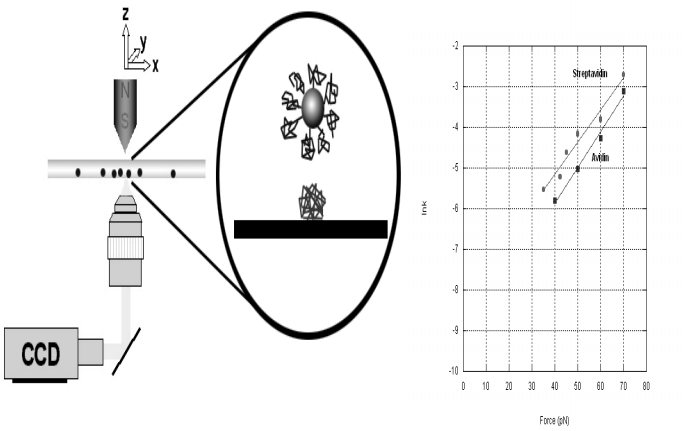
Ligand-Receptor Binding using Magnetic Tweezers

Prentiss and Whitesides Groups

Objectives

- Single molecule measurements of ligand-receptor interactions
- Relate single molecule results to bulk properties
- Evaluate effects of constraints due to surface
- Discriminate non-specific vs specific binding

Approach



Accomplishments

✓ Surface designed to reduce non-specific interactions significantly.
✓Non-specific vs specific discrimination enhanced by applied force (NRL idea also)
✓ Interaction studied: biotin-avidin (streptavidin).
✓ Calculation of parameters at constant force: reaction off rate, unbinding reaction distance.

Future Work

- Study other ligand- receptor couples such as sulfonamides-carbonic anhydrase or ligand (receptors) on cell surfaces.
- Investigate inhibitor dynamics
- Include tethers of different lengths to approach solution conditions.
- Develop efficient fieldable detectors for fast results in unfiltered samples

Magnetic Cell Organization

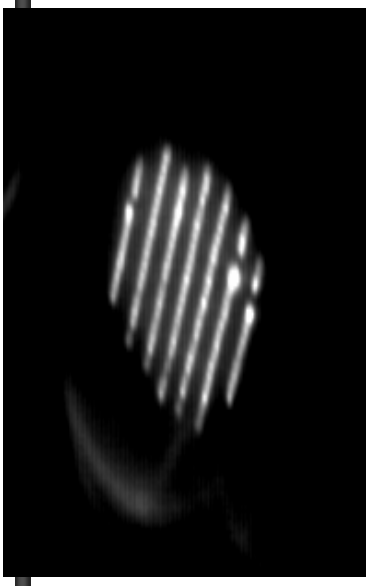
Ingber and Prentiss Groups

Objectives

•Use magnetic fields to organize and self-assemble cellular structures.

•Magnetize cells through ingestion of small (20nm) paramagnetic beads

Approach



Self-assembled pillars

Accomplishments

✓ Successful magnetization of cells

✓Assembly of planar cell configurations in solution, single cell layer possible

✓ Magnetic beads assembled in 3-D structures.

Future Work

• More complex self-assembled shapes, such as 3-D matrix of cell stacks

• Assembly in growth media solutions

• Allow cellular assemblies to grow into tissue

Phase Diagram of DNA Unzipping

Ingber, Nelson, Prentiss Groups

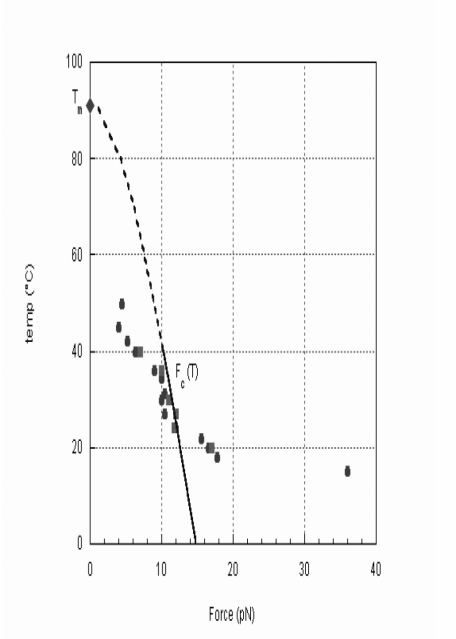
Objectives

• Separate double stranded DNA by applying a constant force at temperatures between 15°C to 50°C

•Validate existing theoretical models of dsDNA binding

• Evaluate temperature dependence of the free energy of dsDNA

Approach



Accomplishments

✓ Between 24-35ºC good projections from bulk thermodynamic using nearest neighbor, insensitive to buffer

✓Above 35ºC unzipping force depends on buffer and bubbles in the dsDNA may be important

✓Below 24ºC dsDNA conformational changes may play a role

✓Demonstrate that dsDNA parameters depend on previous history of the sample

Future Work

•Evaluate effect of sequence dependent conformation (e.g. B' or A tract)

•Improve predictive models for PCR primers

•Develop rapid lower temperature PCR

•Understand history dependence of dsDNA parameters

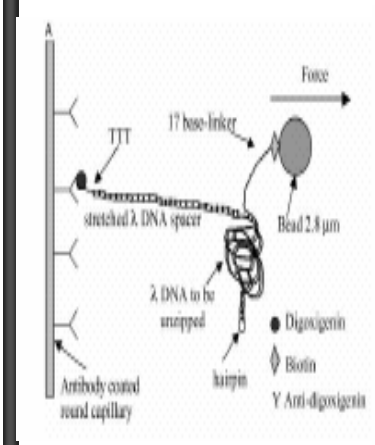
Large force over small Area

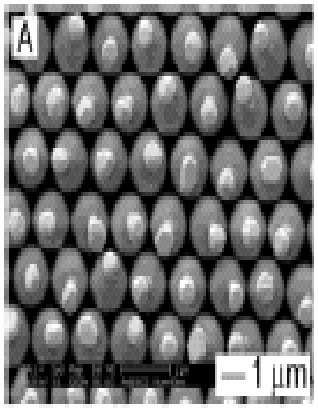
Ingber, Prentiss,Whitesides,Xia Groups

Objectives

- **Exert large force on single receptors on living cells**

Approach





Accomplishments

- ✓ **Fabricated functionalizable nanostructures on larger spheres**
- ✓**Used phage dsDNA to transfer force to a single molecule on a surface**
- ✓**Developed a flow cell to exert >10nN force on a bead attached to a surface via phage (at >nN colloid may be required)**

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- **Use on living cells**

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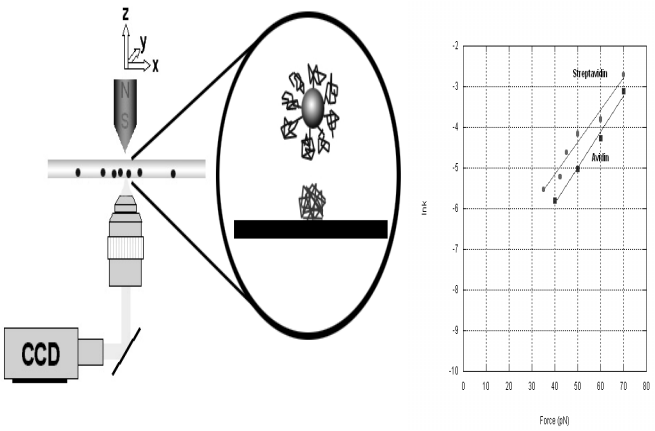

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•Use magnetic fields to organize and self-assemble cellular structures.

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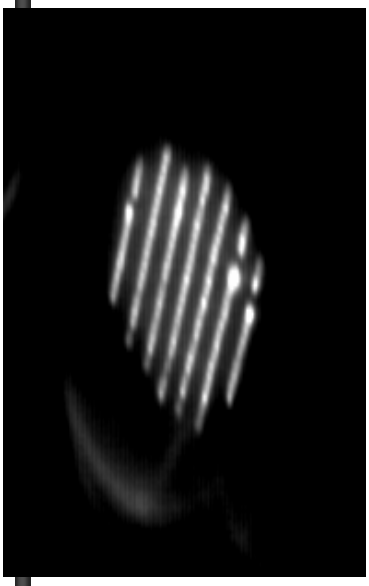

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✓ Magnetic beads assembled in 3-D structures.

Future Work

• More complex self-assembled shapes, such as 3-D matrix of cell stacks

• Assembly in growth media solutions

• Allow cellular assemblies to grow into tissue

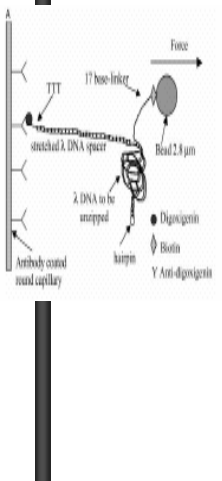
Sequence Dependence of dsDNA unzipping

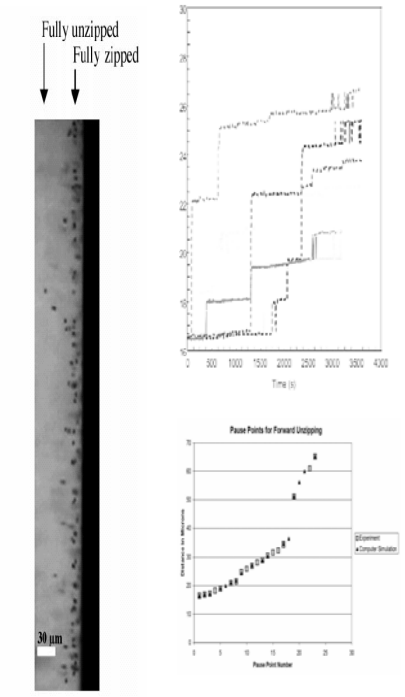
Ingber, Nelson, Prentiss Groups

Objectives

• Demonstrate that pauses in DNA unzipping are sequence dependent

Approach





Accomplishments

✓ Pauses in force-induced unzipping of double stranded were successfully predicted by Monte Carlo, but not by coarse graining

Future Work

•Investigate interactions beyond nearest neighbors

•Explore the kinetics of unzipping through simulations

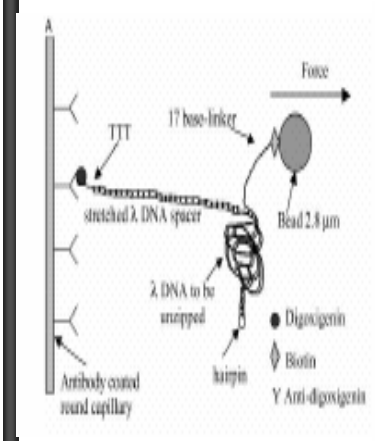
Large force over small Area

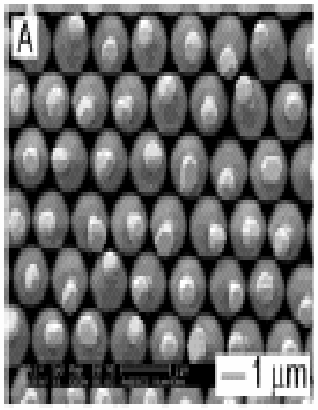
Ingber, Prentiss,Whitesides,Xia Groups

Objectives

- **Exert large force on single receptors on living cells**

Approach





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Future Work

- **Use on living cells**

Gradients and Dipoles

Opposite and Equal Pull -> No Motion

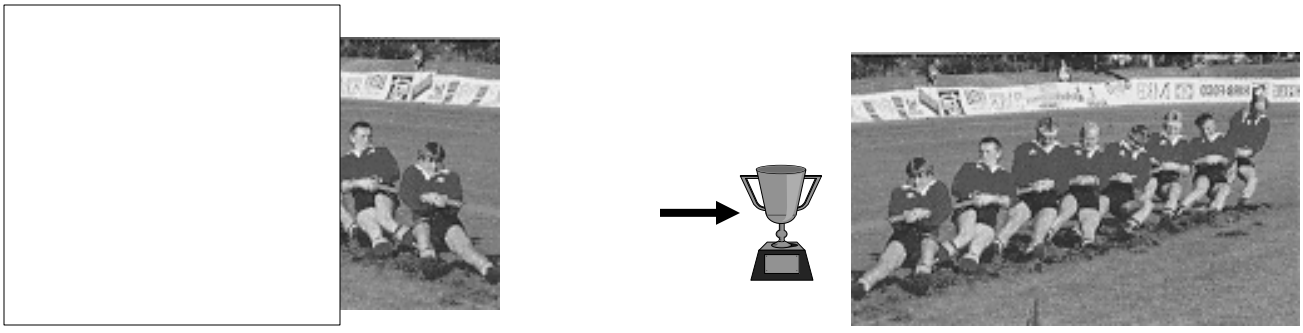
time=0

time=T

Opposite and Unequal Pull -> Motion

time=0

time=T



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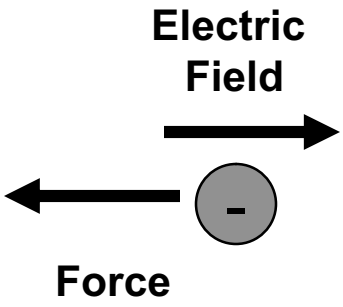
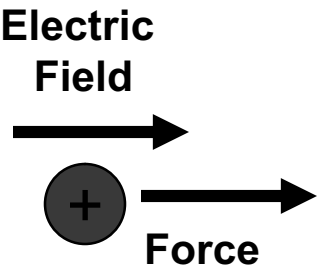
Gradients and Dipoles II

Negative q in E

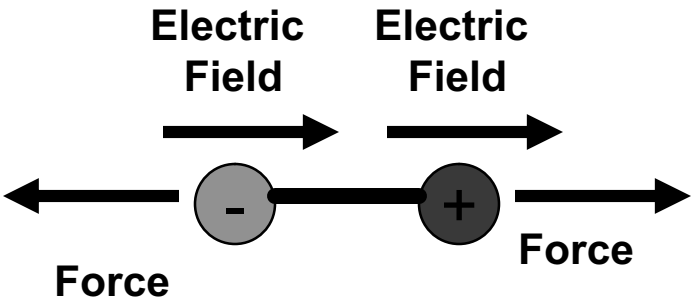


Positive q in E



Dipole

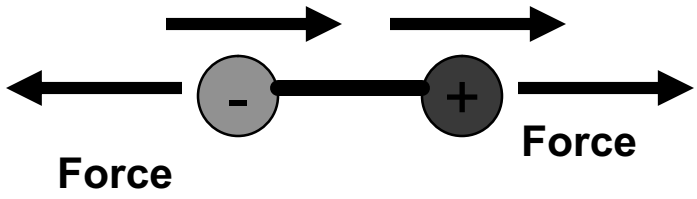
(Attached Negative and Positive q)



Opposite and Equal Pull -> No Motion

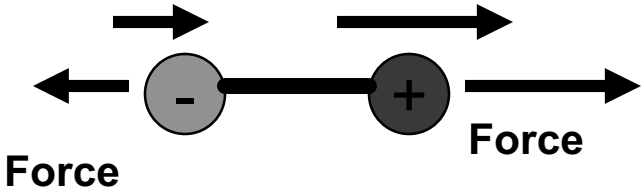
Gradients and Dipoles III

Uniform Field

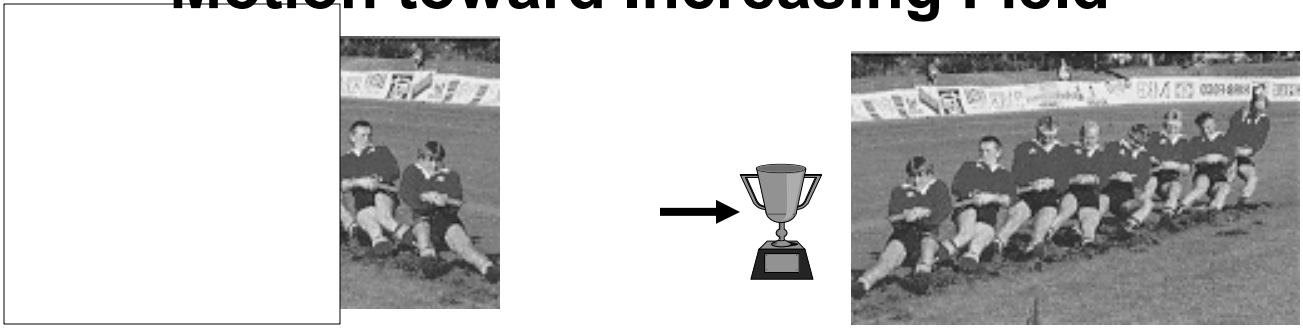


Opposite and Equal Pull -> No Motion

Non-uniform Field



Opposite and Unequal Pull -> Motion toward Increasing Field

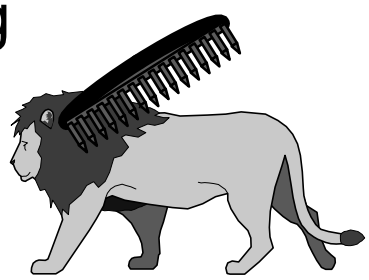


Static Cling Tweezers

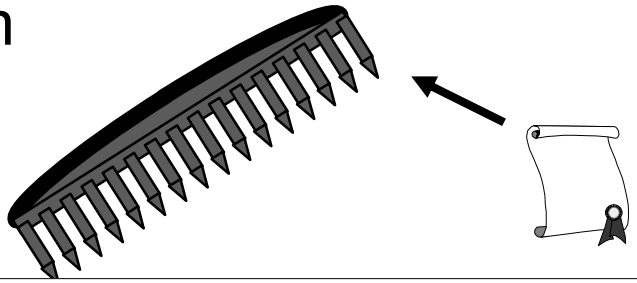
Potential= - α | Electric Field |^2

same origin as optical tweezers potential

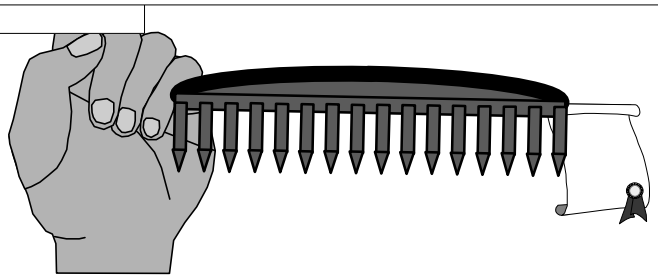
1. Establish an Electric Field Maximum at the comb by charging



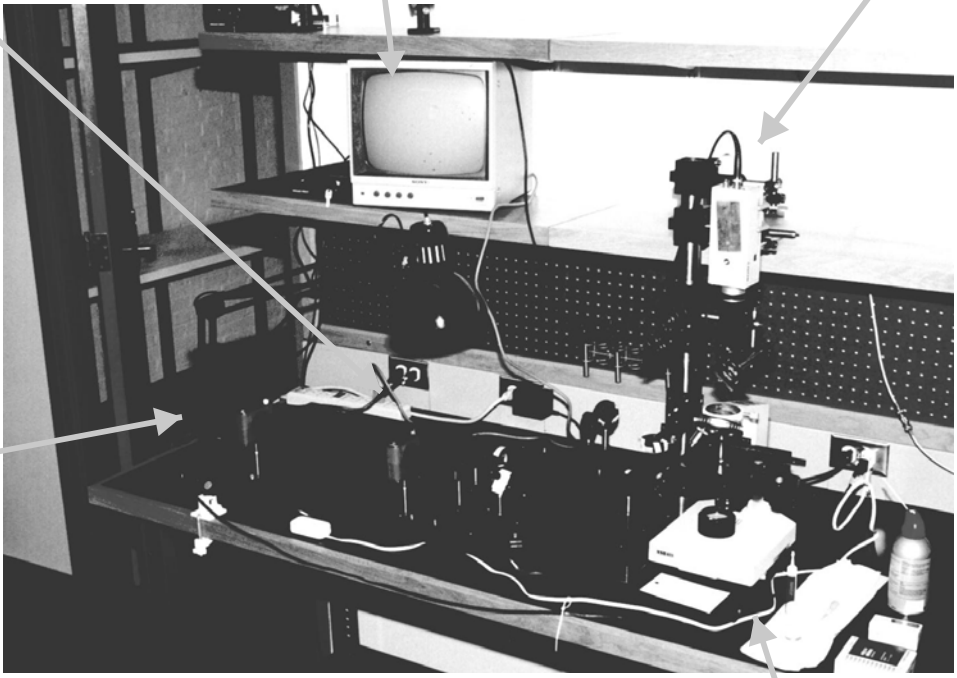
2. Particles will be attracted to the comb where the electric field is a maximum and potential is a minimum



3. Particles at the potential minimum can be manipulated by moving the comb, even if they are too small to hold in a hand



Simple Tweezers

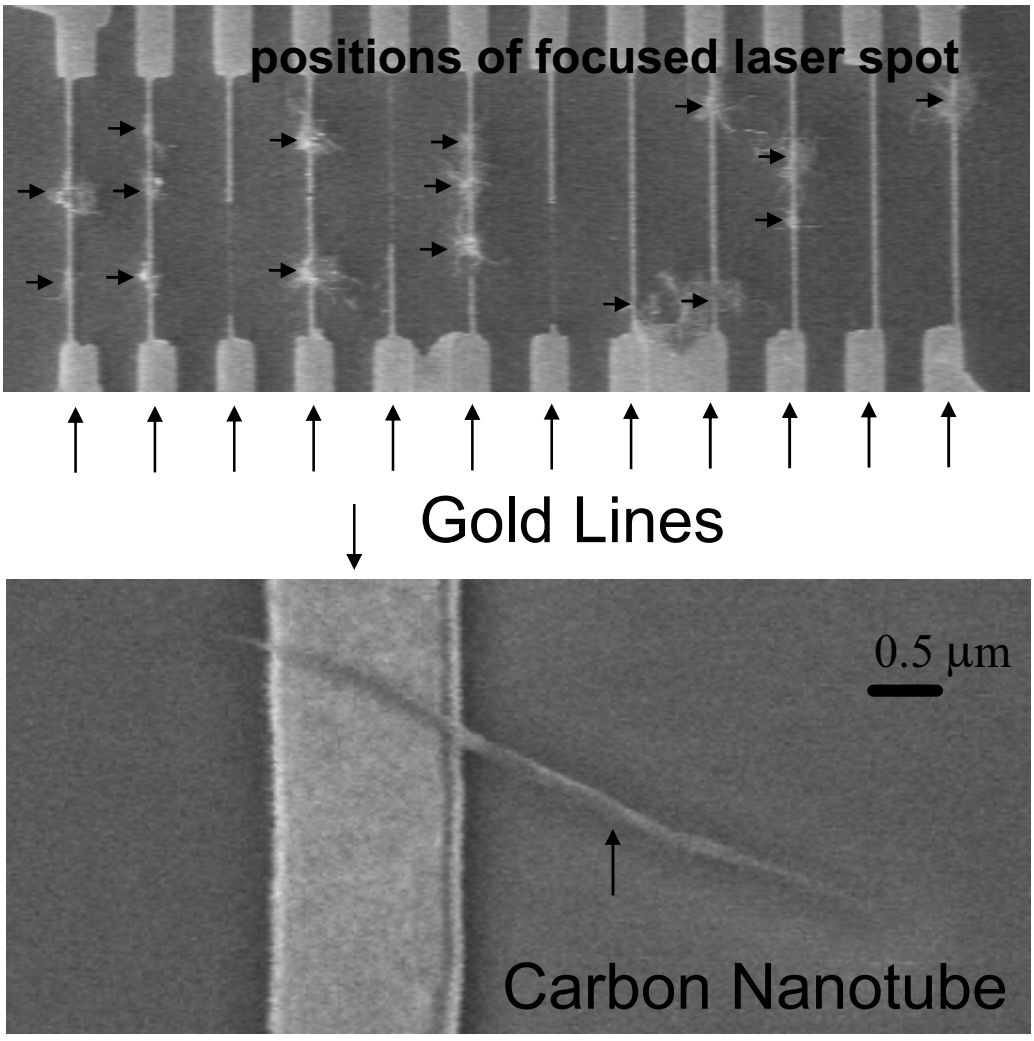


Cost ~ $5k

Light Assisted Self-Assembly

(Patrycja Paruch)

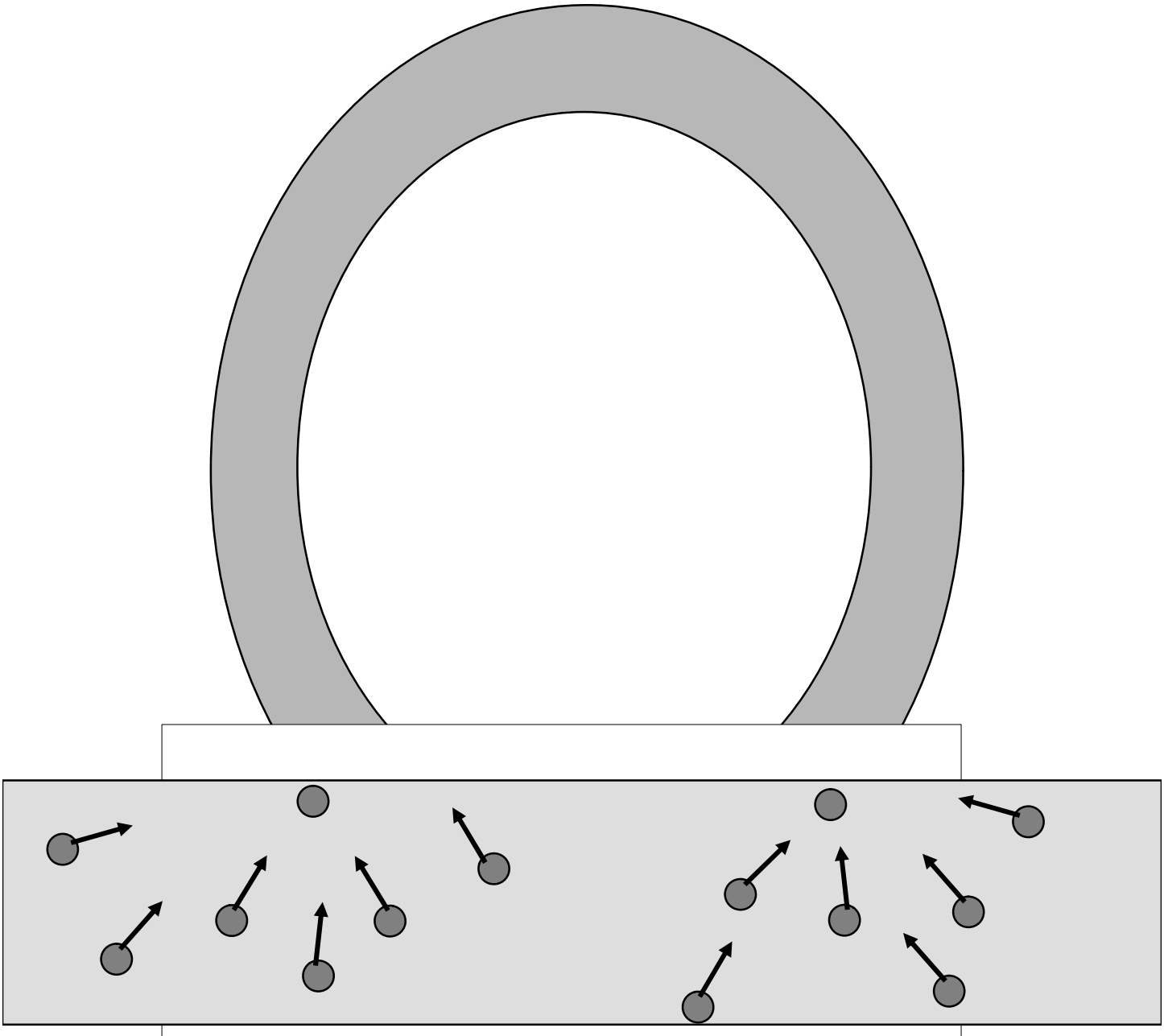
- **Goal: Deposited carbon nanotubes across a gap between two conductors for room temperature Single Electron Transistor**
- **Strategy: Use light force enhancement at conductor edge to attract nanotubes to gates**
- **Achievements: Deposited nanotubes by centering tweezer on the edge a conductor**



Scaling Laws

- **Ball bearings self assemble under gravity < 10 micron particles do not**
- **Depends on assumptions**
- **For constant magnetization**
  - **Surface B field r**
  - **Field gradient is constant at the surface**
  - **magnetic moment of bead a r^3**
  - **External B field dipole a r3**
  - **Dipole/Dipole**

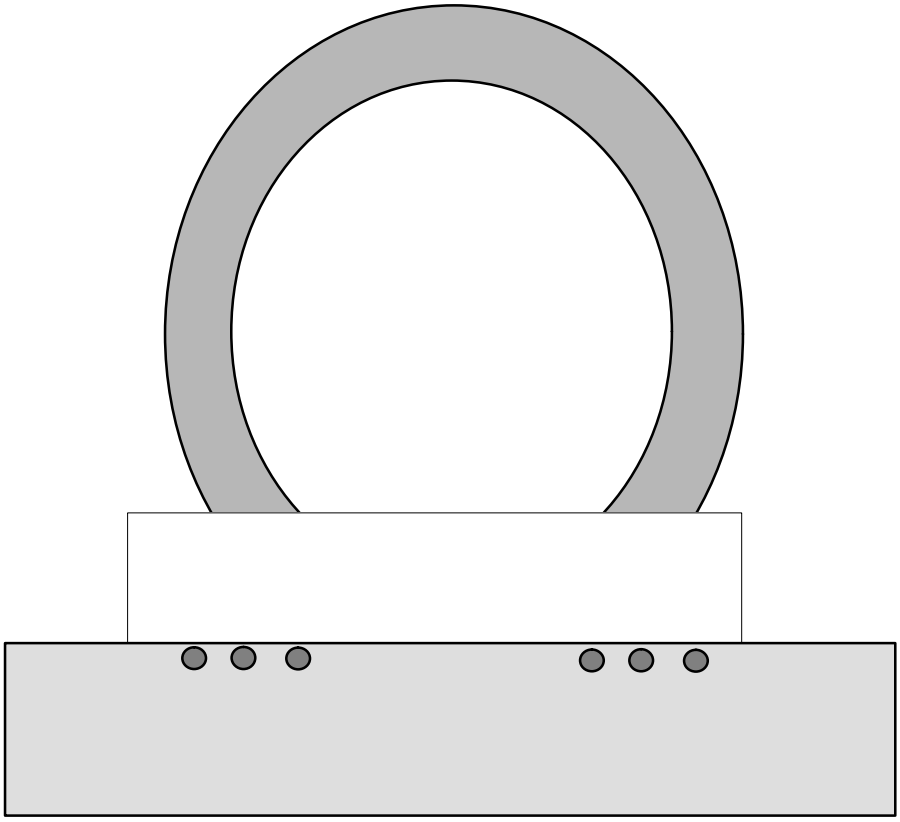
Magnetic Crystals of Paramagnetic Beads Energy= |m||B|



Beads are attracted to B field MAXIMUM

stc: 97

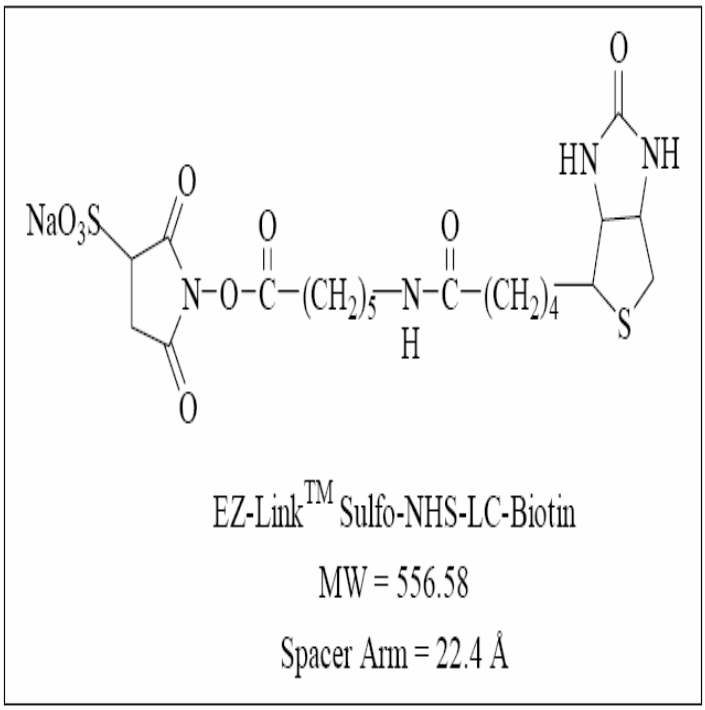
Magnetic Crystals of Paramagnetic Beads Energy= |m||B|

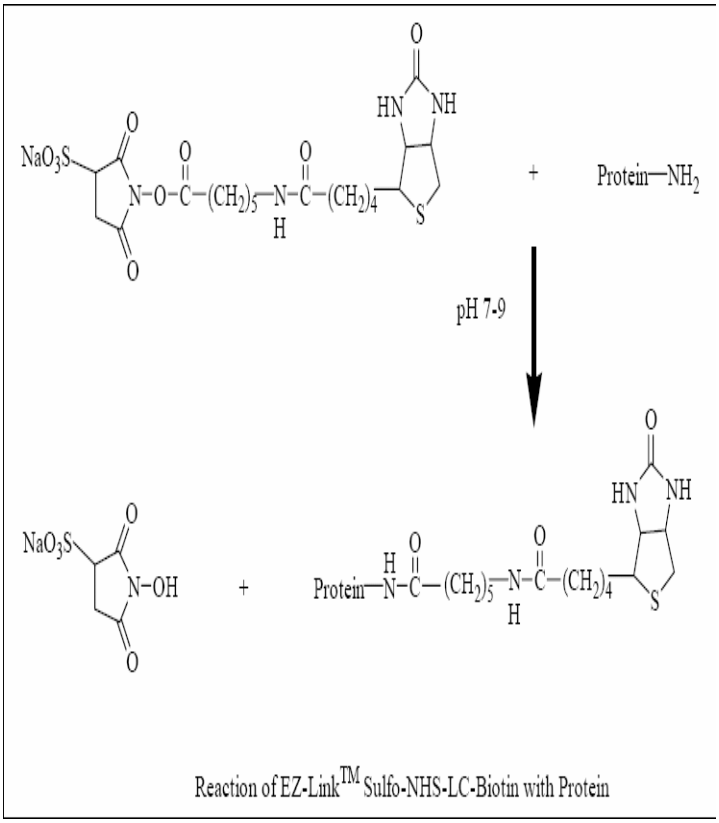


Surface Tension Keeps Beads in the water, so the beads accumulate at the surface, at the points where |B| is a maximum . If B is perpendicular to the water surface, the dipole moments are parallel, so neighboring beads repel.

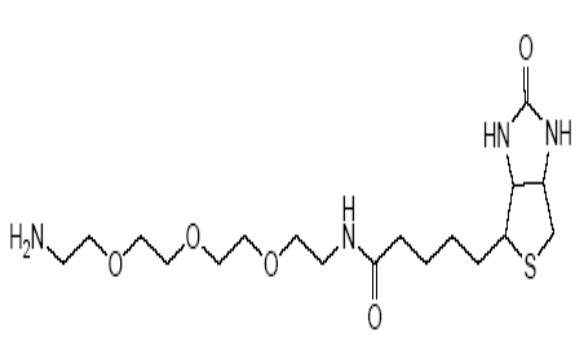
stc: 98

BSA modified with biotin on PVC (streptavidin (avidin) coated beads).

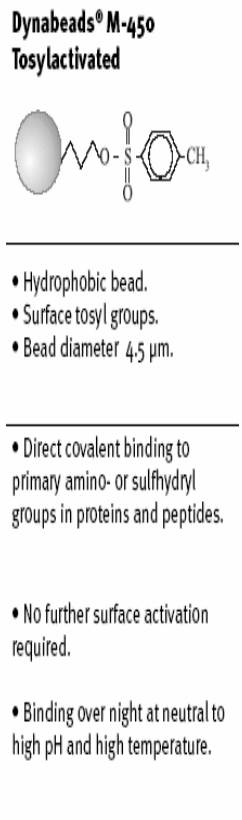


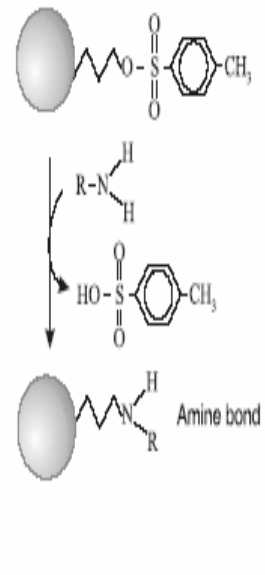


Beads modified with biotin (streptavidin (avidin) on PVC).



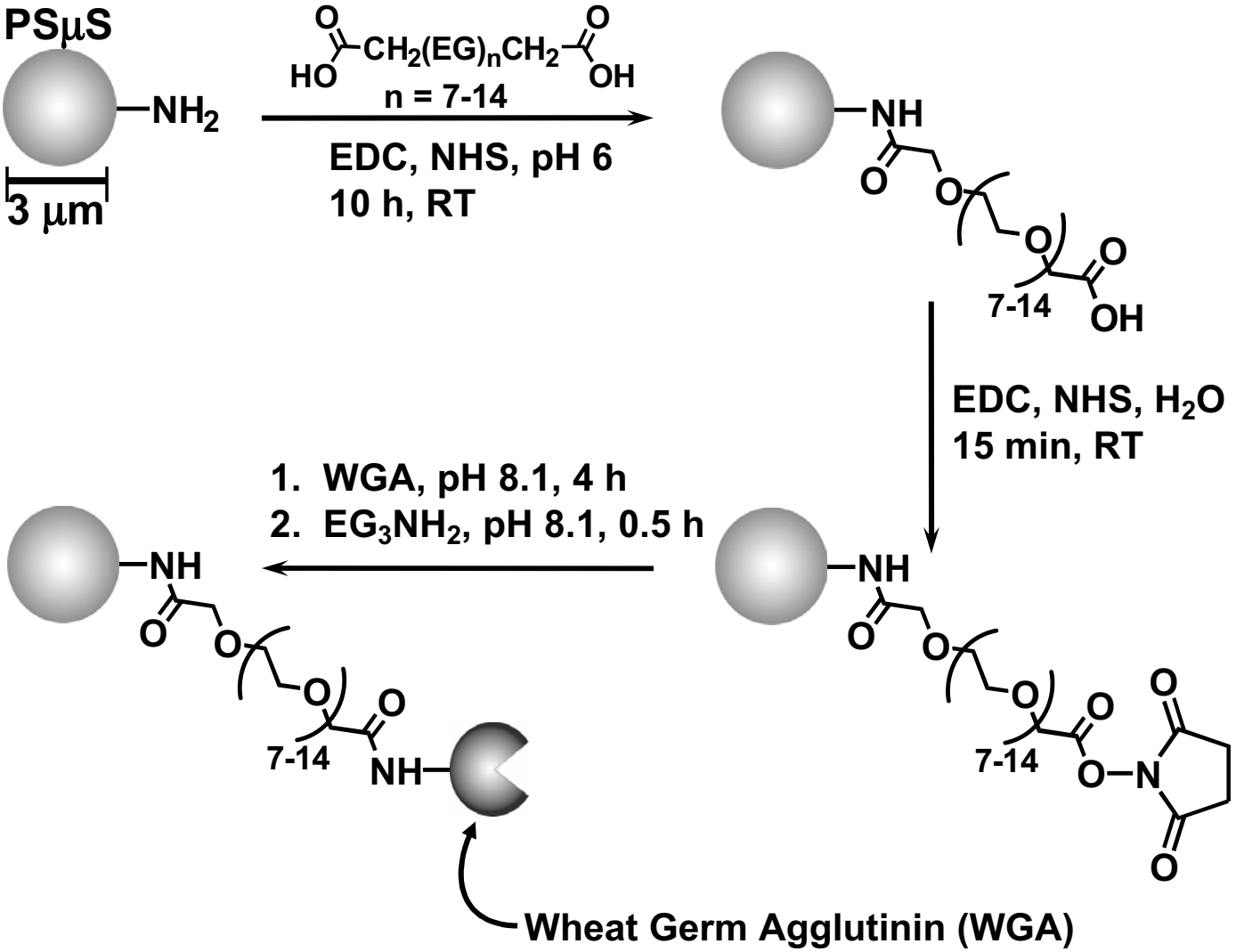
•EZ-Link® Biotin-LC-PEO-Am
Spacer arm: 22.9Å





stc: 99

Conjugation of WGA to Polystyrene Microspheres



stc: 100

Higher forces: positive assays.

- **Dissociation of specific bonds?**
- **Desorption from the surface (b-BSA) ?**

⇩

- **Avidin coated beads: no unbinding, even at highest forces probably multiple bonds but no desorption of biotinylated- BSA.**

⇩

- **Avidin (streptavidin) experiments were repeated with free biotin: 0.01-0.1 nM.**

$k_{off} = v_{off} \exp(-DG_{}$
$k_{off}(F) = k_{off}(0) \exp$

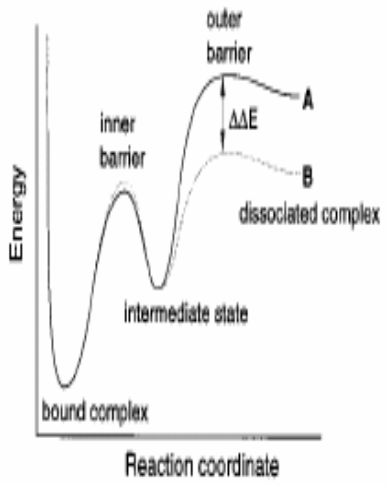


FIGURE 4: Conceptual energy landscapes of the (A) streptavidin–biotin interaction and the (B) W120F–biotin interaction.

Energy Landscape of Streptavidin–Biotin Complexes

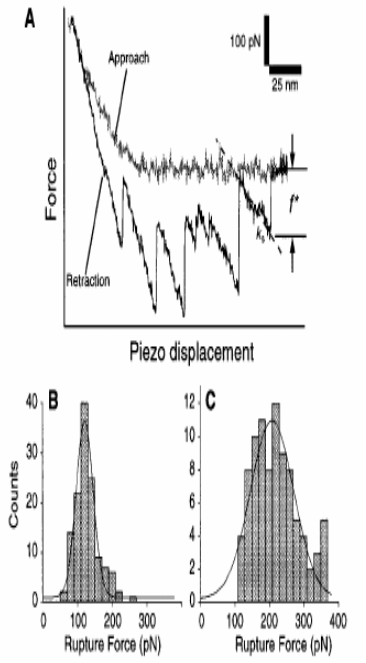


FIGURE 2: (A) Force vs displacement curves of the interaction between a streptavidin-functionalized tip and a biotinylated agarose bead. The measurement recorded the force on the AFM cantilever on approach and retraction of the cantilever from the agarose bead. f^* is the rupture force. k_S is the slope of the force vs displacement curve. The measurement was carried out with a cantilever spring constant of 30 mN/m and a scan speed, v_c, of 600 nm/s. Each cantilever was individually calibrated by thermal fluctuation analysis to determine its spring constant (*26*). Histograms of the adhesion force between a streptavidin tip and biotin bead at loading rates of (B) 198 pN/s (k_S = 2.3 mN/m and v_c = 86 nm/s) and (C) 2300 pN/s (k_S = 2.3 mN/m and v_c = 1 μm/s). Both histograms were fitted to a Gaussian function (*2*). The centers of the force distribution (B) and (C) are 126 ± 2.3 (SEM) pN (N = 256) and 207 ± 5.8 (SEM) pN (N = 100), respectively. All AFM force measurements were carried out in PBS and at 25 ± 1 °C.

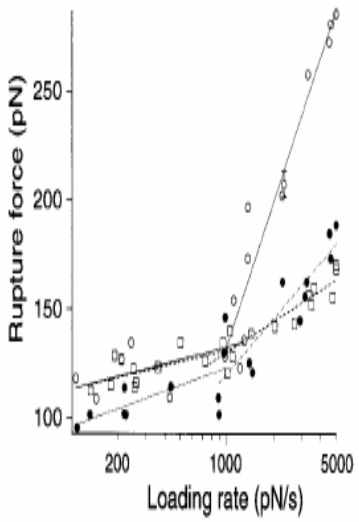


FIGURE 3: Loading rate dependence of the rupture force in the unbinding of the streptavidin–biotin (○), avidin–biotin (□), and W120F–biotin (●). Both regimes in force spectra were fitted to the Bell model. Standard errors of all data points were less than 5% of the mean value. Representative error bars were placed on selected data points.