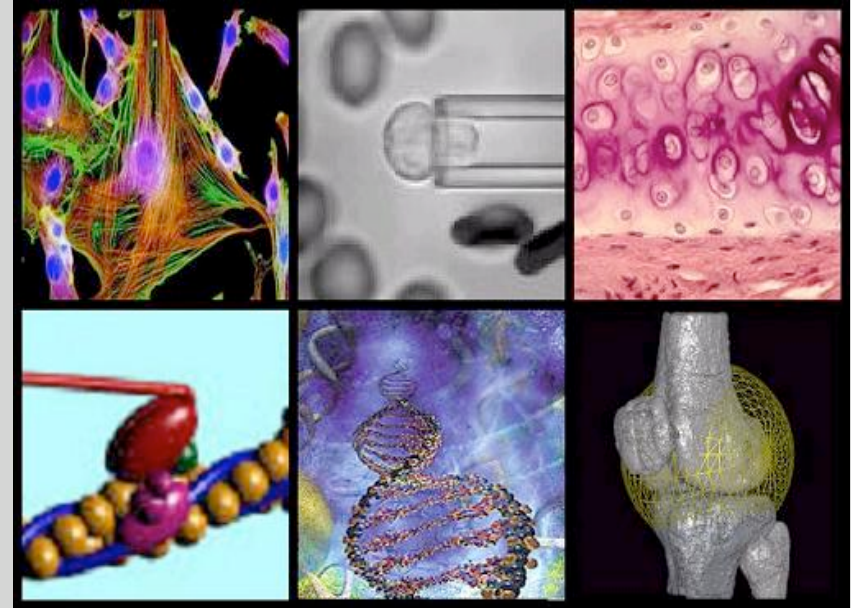


# Experiences in Teaching Undergraduate Molecular, Cell and Tissue Biomechanics



Roger Kamm  
Mechanical Engineering  
Biological Engineering  
MIT



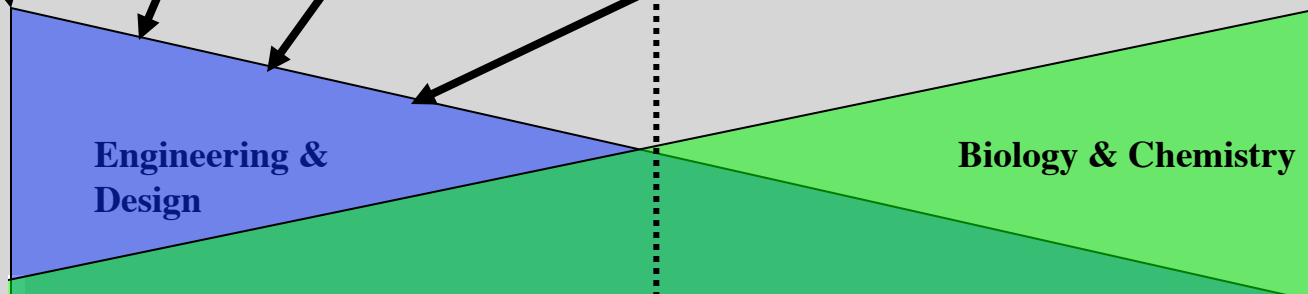
# The Bioengineering Landscape

ME major with subjects that draw upon a background in modern biology

ME major with a minor in medical or biological engineering

ME core, but with a primary focus in biological engineering

BE major for a biologically-based engineering education



# Molecular, Cellular and Tissue Biomechanics

Instructors: Typically two; one from “traditional” mechanics, and one with a molecular perspective

Pre-req: Differential equations, Molecular and Cell Biology, Statistical Thermodynamics (in major)

Instrumentation lab follows this (AFM, optical traps, microrheology)

Text: Various chapters from textbooks, readings from the literature, chapters of the text-in-progress: Molecular, Cellular and Tissue Biomechanics (Grodzinsky, Hwang, Barocas, Kamm, in progress).



# Evolution of MCT Biomechanics

- First taught in 1995 as an advanced UG course
- By 1999, enrollment had grown from 12 to 50+ and the course was divided into separate graduate and undergraduate versions
- Graduate version was specified as a required core subject for Biological Engineering students (Bioengineering track)
- UG version required in the UG major started in 2005
- UG version now offered each Spring with an enrollment of ~70 students; grad version, ~35 students
- Students drawn from BE, ME, DMSE, Biol, Chem, Phy, Chem E, CEE



## MOLECULAR MECHANICS

Biomolecules and intermolecular forces  
Single molecule biopolymer mechanics  
Formation and dissolution of bonds under force  
Motion at the molecular/macromolecular level

## TISSUE MECHANICS

Molecular structure --> physical properties  
Continuum, elastic models (stress, strain, constitutive laws)  
Viscoelasticity  
Poroelasticity  
Electrochemical effects on tissue properties

## CELLULAR MECHANICS

Structure/function/properties of the cell  
Biomembranes  
The cytoskeleton  
Cell adhesion and aggregation  
Cell migration  
Mechanotransduction

Full syllabus and course materials can be found on OpenCourseWare:

# Guiding Principles

- Build from the fundamentals in molecular and cellular biology
- Selection of topics driven by the important biological issues
- Develop an understanding that bridges from molecules, to cells to tissues
- Allow the course to evolve with the field



# Some Learning Objectives

- To understand the fundamental concepts of mechanics and be able to apply them to simple problems in the deformation of continuous media
- To understand the underlying basis for the mechanical properties of molecules, cells and tissues
- To be able to model biological materials using methods appropriate over diverse length scales
- To be familiar with the wide spectrum of measurement techniques that are currently used to determine mechanical properties of biological materials
- To appreciate the close interconnections between mechanics and biology/chemistry of living systems



# Some of the Challenges

- Diverse backgrounds (students from many different majors, including non-engineering)
- No single textbook that adequately covers molecular, cell and tissue mechanics
- Keeping the material up-to-date (rapidly evolving field)
- Mathematics is always a problem
- Difficult concepts (stat mech, scaling analysis, tensorial quantities, poroelasticity)
- The need for compromises (depth vs. breadth, keeping in the biology)



# Spec sheets term projects: Collagen

## SP\_COL1 – Collagen Type I

An Engineer's Handbook

Authors:

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Jewel Sharpe [jsharp@mit.edu]

### Description

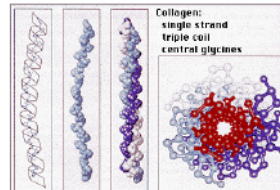
SP\_COL1 assemblies consist of Collagen Type I, a structural protein. Collagen is the most abundant protein in mammals, and Collagen Type I is the most common form. Its assemblies provide tensile strength and elasticity to bone, skin, arteries, muscle, tendon, and a majority of other connective tissue environments.

Subparts: Collagen microfibrils  
→ fibrils → sheets and ropes

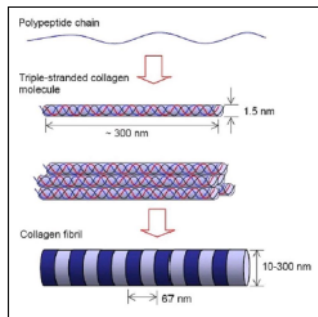
### Molecular Structure

#### From polypeptides...

At its most basic level, SP\_COL1 consists of helical polypeptides consisting mainly of Gly-X-Y repeats. These polypeptides come in two forms:  $\alpha 1$ , which has short nonhelical ends, and  $\alpha 2$ , which does not. The collagen microfibril forms when two  $\alpha 1$  chains and one  $\alpha 2$  chain assemble into a heterotrimer. This microfibril is the smallest unit of SP\_COL1 found in its native, final environment, and its structure is the primary source of SP\_COL1's mechanical tensile strength. The three polypeptide chains are held together by hydrogen bonds, which originate from the hydrogen side chains of all the glycine residues in each polypeptide's helical region.



**Collagen microfibril:** Diagram showing the side-views of the structure of 1, then 3, collagen polypeptides, as well as a top-down view of the heterotrimer. Highlighted in red are the glycine residues, which contribute the hydrogen bonds that hold the microfibril together. The role of the glycines explains their regular appearance in the chains, and their strong conservation across species.



From microfibril to fibril: diagram showing relative sizes.

### Molecular Structure

#### ...to Fibrils

Individual microfibrils average around 300nm long and 1.5nm in diameter. In the formation of SP\_COL1 assemblies, hundreds or thousands of these microfibrils aggregate to form the next level of collagen organization: the fibril. Individual fibrils can reach many microns in length, and their diameters range from 10 to 300nm. Collagen Type I microfibrils form 67nm-periodic cross-striated fibrils in most extracellular spaces, possibly as a way to provide both elastic and viscoelastic properties. At a molecular level, it is these striated fibrils that provide tensile strength and elasticity by allowing cell attachment and macromolecule anchoring.

## SP\_COL1 – Collagen Type I

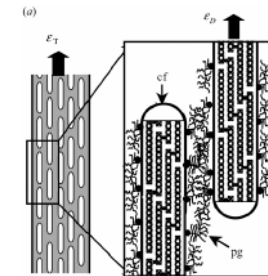
An Engineer's Handbook

Authors:

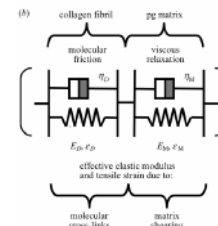
Sophia Mian [smian@mit.edu]  
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### Viscoelastic Modeling

SP\_COL1 assemblies can be accurately characterized through a viscoelastic model. Experiments show that under an applied strain, SP\_COL1 assemblies exhibit a time-dependent strain response. However, the final strain value is time-independent, characteristic of Voigt model-like behavior. In its native environment, SP\_COL1 fibers and sheets are covered with proteoglycan matrices, which exhibit properties similar to the SP\_COL1 fibers themselves. The physical arrangement is schematized in figure part a).



Collagen-Proteoglycan Arrangement.



Series-Voigt model of SP\_COL1 in its native environment

### Viscoelastic Behavior of SP\_COL1 and Proteoglycan Matrix Series-Voigt model of SP\_COL1 in its native environment

Given the placement of the proteoglycan matrix relative to the SP\_COL1 fibers, the most logical (and empirically accurate) model places the collagen fibril and proteoglycan matrix in series, with each represented by a Voigt model arrangement, as in figure part b). Empirically, the fibril "dashpot" is much less viscous than the proteoglycan one: at higher strain rates, the ratio of fibril to total extension increases. Fibril alone also exhibits greater tensile strength with increasing strain rate, consistent with the Voigt model.

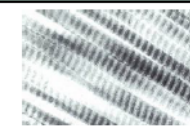
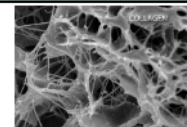
### Representative Measurements

Direct measurements of the mechanical properties of pure SP\_COL1 assemblies yielded the following results:

**Collagen Membrane**  
max stress:  $58 \pm 5$  MPa  
strain:  $7 \pm 0.5\%$   
 $E \sim 800$  MPa

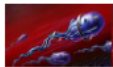
**Collagen Fiber**  
max stress: 120 MPa  
strain: 9.5%  
 $E \sim 1200$  MPa

For comparison  
High-Density Polyethylene  
 $E \sim 1400$  MPa.



# Bacterial Flagellum

BFM\_001



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<http://mechanome.openwetware.org/registry/index.php/Part:flagellum>

## Background

### Description of Bacterial Flagellum

Bacterial flagellum is an organelle made up of a bacterial reversible rotary motor and a thin helical filament 15  $\mu\text{m}$  long and 20 nm wide, responsible for locomotion. The bacterial motor has a molecular mass of 11 Mda with 13 component proteins. The 45 nm in diameter rotary motor is attached to the cell envelope and rotates the helical filament at a rate of about 20,000 rpm or several hundred Hz, with an energy consumption of about  $10^{-16}$  W and an almost 100% energy conversion efficiency. The bacterial motor is powered by an electrochemical gradient of  $\text{H}^+$  and  $\text{Na}^+$  ions. The motor can switch direction depending on which way the helical filament is rotated.

Sowa, Yoshiyuki, and Richard M. Berry. "Bacterial Flagellar Motor." *Quarterly Reviews of Biophysics* 41 (2008): 103-32.



Figure 1: 3D image of bacterial flagellum swimming straight

"Revealing the mystery of the bacterial flagellum - A self-assembling nanomachine with fine switching capability - - MEXT Nanotechnology Network Center of Japan." Revealing the mystery of the bacterial flagellum. 5 Feb. 2004. NANONET. 05 May 2009 <<http://www.nanonet.go.jp/english/maimag/2004/011a.htm>>.

### History of Bacterial Flagellum Research

1676: Antonie van Leeuwenhoek observes motility of bacteria under microscope  
1880: Wilhelm Pfeffer discovers bacteria do chemotaxis (1969: Julius Adler adds to this work, attributes sensing to proteins, named chemoreceptors)

Early 1900s: Bacteria are found to have flagella, motility in bacteria attributed to flagella, flagella appears helical

1970s: Berg & Anderson (1973) and Silverman & Simon (1974) discover that bacterial flagellum move by true rotation, not by propagation of helical waves, as other researchers thought. Silverman & Simon discovered this by tethering bacterial cells by mutated filaments and observing cell body rotation with a light microscope. The rotations of tethered or swimming bacteria have also been studied using dark-field (DF), laser DF, differential interference contrast (DIC) and fluorescence microscopy.

Recently, the rotation of flagellum is studied by attaching submicron polystyrene beads to truncated flagellar filaments of immobilized cells and recording rotation with back focal plane interferometry or high-speed fluorescence microscopy. The drag coefficient by the bead is the approximately the same as the drag caused by the part of the filament that has been truncated.

Berg, H.C. & Anderson, R.A. (1973) Bacteria swim by rotating their flagellar filaments. *Nature*.

Silverman, M. & Simon, M. (1974). Flagellar rotation and the mechanism of bacterial motility. *Nature*.

Biological Motors

BFM\_001



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<http://mechanome.openwetware.org/registry/index.php/Part:flagellum>

## Structural Components

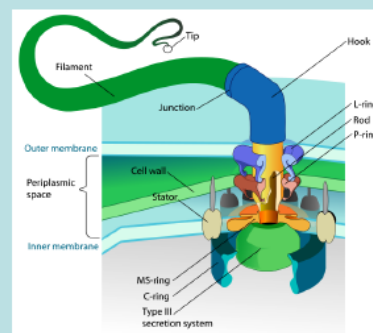


Figure 2: Flagellum of gram-negative bacteria

"File:Flagellum base diagram en.svg -" Wikipedia, the free encyclopedia. 2008. 07 May 2009 <[http://en.wikipedia.org/wiki/File:Flagellum\\_base\\_diagram.svg](http://en.wikipedia.org/wiki/File:Flagellum_base_diagram.svg)>.

### Hook and Filament

The hook and the filament are thin tubular polymers made up of tubulin. They are made up of either short or long 11 helical protofilaments. The alternating and mixing of these different sized protofilaments create a helical shape. The motors can switch rotation between counterclockwise (looking in direction from filament to motor) and clockwise directions. When the motor is rotating counterclockwise, the cell propels smoothly because the filaments are in a bundle while for clockwise rotation, a filament is forced out of the bundle of filaments making the cell change direction, called "tumble". Even one motor switch can cause reorientation. The motor switching rate is controlled by a sensory/signaling protein network.

Sowa, Y. (2008).

### Motor Structure

#### Rotor and Stator

The structure of the bacterial flagellar motor has been primarily studied by electron microscopy (EM), as well as biochemical and genetic studies. It is one of the largest molecular machines in bacteria. Just like other rotary motors, the bacterial flagellar motor has a rotor and stator. The rotor rotates with respect to the cell and is attached to the filament by a joint called the hook. The stator, the part that remains stationary to the cell, is attached to the bacterial cell wall. The stators are composed of two proteins MotA and MotB for  $\text{H}^+$  driven motors (found in *E. coli* or *salmonella*) or PomA and PomB in  $\text{Na}^+$  driven motors.

#### Basal Body

The basal body is the core of the motor and consists of up to 45 nm rings: L ring, P ring, MS ring, and the C ring. They span across the inner cytoplasmic membrane, peptidoglycan cell wall, and outer membrane of the bacteria. The L ring and P ring act as bushings between the rotor and cellular envelope and are embedded in the outer membrane and peptidoglycan layer, respectively. The rod connects the hook to the MS ring in the cytoplasmic membrane. The MS ring is the first part of the motor to assemble, and so is believed to be the base upon which the rest of the motor is built. The cytoplasmic side of the MS ring is attached to the C ring, where it is believed torque generation occurs.

Sowa, Y. (2008).

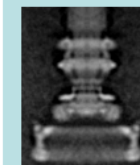


Figure 3: Composite electron micrograph of basal body and hook of bacteria

Francis, N. R., et al., "Isolation, Characterization and Structure of Bacterial Flagellar Motors Containing the Switch Complex." *Journal of Molecular Biology* 235 (1994): 1261-270.

Biological Motors

# Kinesin

## Kin\_Mot001

Kin\_ATP → MT Transporter

<http://mechanome.openwetware.org/registry/index.php/Part:kinesin>



Author updates:  
Matt Lang [mjl@mit.edu]  
Ricardo Gonzalez [rgr@mit.edu]

Last Update: 8 April 2009

### Description of Motor Protein

A Biological motor (Kin\_Mot) that is responsible for cargo transport along track=microtubules. Sub-parts: Catalytic domain, cargo domain, coiled coil stalk. Motor Specs: Unloaded velocity: 800nm/s; Stall force: 6pN; Track: microtubule; + end directed; Step distance: 8.2nm. Processivity: ~1um. Two headed and coordinated. Fuel source: ATP, 1ATP/step; ATP/micron, 125. Efficiency 80%.

### Sub-component of Device: Kinesin Motility Assay

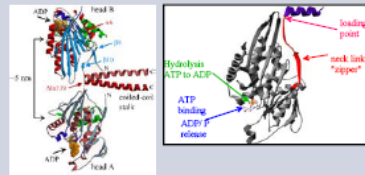
Kin\_mot001, MT001,  
ATP001, Tax001

### Kinesin-1

The most widely studied form of kinesin is Kinesin-1, also referred to in the literature as conventional kinesin. Conventional kinesin was the first kinesin motor to be identified and purified from cell extracts (Lawrence et al., 2004). Structurally, conventional kinesin is made up of two monomers and each, in turn, is made up of an N-terminal motor head, a neck linker, a long coiled-coil dimerization region and a globular tail domain. In addition, the active form of conventional kinesin is a dimer with the coiled-coil regions of two monomers wound together to form a 70 nm stalk.

Lawrence, C. J., R. K. Dawe, et al. (2004). "A standardized kinesin nomenclature." *J Cell Biol* 167(1): 19-22.

### Structure/Subcomponents



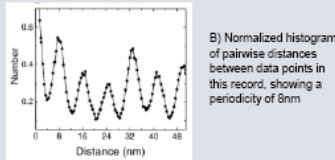
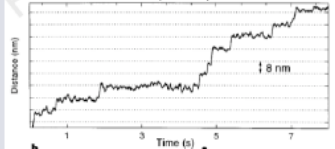
Crystal structure of dimeric kinesin

### Part Compatibility SwissProt ID

Structures: 2KIN (rat), 1MKJ (human),

### Motility

A) Sample record of movement at 2uM ATP, showing the elementary steps (solid line).



B) Normalized histogram of pairwise distances between data points in this record, showing a periodicity of 8nm

$$V_{max}: 800 \text{ nm s}^{-1} \quad V_{act}(F, [ATP]) = \frac{d \cdot k_{cat}(F)[ATP]^n}{k_{cat}(F) + k_b(F) + [ATP]^n}$$

kcat: 2E-09 M  
kb: 1.2

### 8nm per ATP

Work by Schnitzer et al. was fundamental at defining experimentally based constraints on theories of kinesin movement or walking. In their work, Schnitzer et al. set out to determine the coupling ration, defined as the number of ATP molecules hydrolyzed per mechanical advance, for kinesin. Using the technique of optical-trapping interferometry, Schnitzer et al. were able to measure, at subnanometer resolution, the average rate of movement of a kinesin molecule that had been tagged to silica beads and deposited onto immobilized microtubules. This work led to the conclusion that at near-zero load, kinesin hydrolyses a single ATP molecule per 8-nm advance.

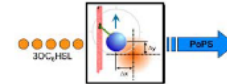
Schnitzer, M. J. and S. M. Block (1997). "Kinesin hydrolyses one ATP per 8-nm step." *Nature* 388(6640): 386-90.

### Conditions (abridged)

Chemical: =1 ATP/step  
Mechanical: >2 substeps/cycle  
Output: Velocity measured nm/s  
[ATP]: 1mM  
Stall Force: 6pN  
Chassis: 2 headed

Biological Motors

## Kin\_Mot001



<http://mechanome.openwetware.org/registry/index.php/Part:kinesin>

Authors:  
Matt Lang [mjl@mit.edu]  
Ricardo Gonzalez [rgr@mit.edu]

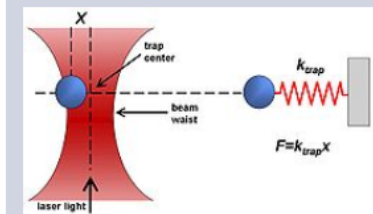
Last Update: 19 October 2007

### Tools to study kinesin

Experimental observations of kinesin motility were elusive for many years given the challenge of measuring nanometer length scales with precise spatial and temporal resolution. Not only are motor steps small, in the order of nanometers, but Brownian motion at these scales produces a significant amount of background noise. The magnitude of the thermal energy,  $kT$ , is  $\sim 4pN$ , and is comparable to the energy required to move a kinesin tagged to a bead, making it difficult to distinguish individual steps. Despite these challenges, key technologies such as optical trapping and fluorescence imaging with one-nanometer accuracy (FIONA) have allowed the direct measurement of kinesin movement.

### Optical Trapping

Optical trapping allows for the manipulation of dielectric beads, onto which kinesin have been attached and placed on top of microtubules (motility assay), by exerting a force through a highly focused laser beam. The trap basically behaves as a Hookean spring, keeping the motor stabilized against random motion. The applied force will eventually stall the motor, allowing, for instance, a direct measurement of how much force the motor can generate. In addition, optical trapping also allows for direct measurements of the stepwise motion of molecular motors and was used to determine that kinesin advances in 8nm steps. (Moffitt et al 2008).



Optical traps behave just like a Hookean spring. They can be used to apply variable forces or in clamp-mode, applying a constant load.

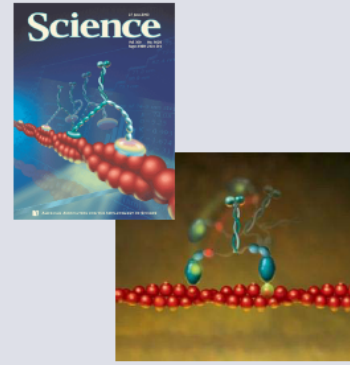
Moffitt JR, Chemla YR, Izhsy D, Bustamante C, "Differential detection of dual traps improves the spatial resolution of optical tweezers", *PNAS* (2006), 103(24): 9006-9011.

### FIONA

A novel technique recently developed to study molecular motor is that of FIONA (Kural et al., 2005). FIONA builds on the fact that in light microscopy, a point-like fluorescent object cannot be observed better than at  $\sim 250 \text{ nm}$  in the visible spectrum of light because of the diffraction limit. The objects position, however, can be localized with high precision by determining the center of the diffraction-limited spot. Using this technique, it has been established that a single organic dye can be localized within a nanometer. When applied to the study of kinesin, for instance, FIONA allows for the direct observation of the displacement undertaken by an individually tagged head.

Comert Kural, Hamza Balci, Paul R. Selvin. Molecular Motors One at a Time: FIONA to the Rescue. *Journal of Physics Condensed Matter. Special issue on Molecular Motors*, 17, S3979-S3995 (2005)

Biological Motors



Registry of Standard Biological Parts

making life better, one motor at a time

License: Public

# What works and what doesn't

- Topics capture the interest of the students, especially the molecular and cell mechanics
- Now that we require stat thermo, molecular mechanics is a breeze
- Term papers (e.g., a “spec sheets” for biology)
- Traditional concepts in mechanics too compressed



How can we offer students an opportunity to continue to explore biomechanics *after* an introductory course?



# Biomechanics Teaching Consortium

- **Motivation:**

- A need for more advanced, graduate electives
- Wide availability of distance learning facilities

- **The Plan (one scenario):**

- Four consortium members, each of whom “host” one subject
- Subject taught by “the leading expert” in the field
- A course each institution currently offers
- Each course taught every other year and broadcast to other consortium members
- Courses selected and periodically assessed by an independent body
- Students receive full credit from their own institution
- Local faculty liaison plus TA/grader at each remote site



# Biomechanics Teaching Consortium

- **The Benefit:**

- Four courses, one per term, cycled over 2 years, in exchange for one course taught yearly
- Start-up costs can be recouped in 10 years

- **Future enhancements**

- Web-based labs
- Expand consortium to include non-hosting (fee paying?) members
- Make videotapes of lectures available – next phase of OCW?

