DAMPING OF PROTEINS

(continuing about the viscosity of proteins)
Q: What happens when a protein is struck by a force?

Q: Does it ring like a tuning fork (underdamped) or does it creep monotonically into new shape (overdamped)?

To answer these we need to understand the motion of proteins for which important is to study the material properties of proteins such as rigidity and the frictional forces that damp their motion.
• **Observed**: Proteins have similar densities and rigidities to hard plastics

• But due to its small size the viscous force from the surrounding fluid are large compared to the inertial forces which results in the global motions of proteins being overdamped, meaning that proteins relax monotonically into new conformations

• Thus *protein*, as a mechanical device, is like a *plastic toy*
THE MOLECULAR BASIS OF VISCOSITY

Well understood for gases but not for liquids

- **Ideal Gas**: The force needed to shear 2 adjacent planes arises from the transfer of momentum due to diffusion of the gas molecules and as rate of diffusion increases with temperature the viscosity also increases with temperature.

- **Liquids**: Viscosity of liquids is due to intermolecular bonds that break more rapidly at higher temperatures so viscosity decreases with increase in temperature.
Simple Theory of Viscosity

- This theory is based on the molecular friction between two surfaces.
- The molecules on one surface make transitory crosslinks with the molecules of adjacent surfaces.
While the cross link is attached the surfaces move through a distance that is small compared to the molecular size.

If the time for which the molecules are attached is $t_{\text{on}}$, then the rate of detachment is $1/t_{\text{on}}$.

If the speed of movement is $v$, then each molecule will be stretched by an average amount $t_{\text{on}}v$ during each attachment.

If the stiffness of each molecular bond is $k$, then the average force opposing the shear is $-kt_{\text{on}}v$. 
From that the Drag force is given by:

\[-p \kappa t_{on} v\]

where each molecule spend a fraction \(p\) of its time attached as cross link.

And the associated drag coefficient is:

\[\gamma = p \kappa t_{on}\]
INTERPRETATIONS ABOUT ‘VISCOUS DAMPING’ FROM THE FORMULA: $\gamma = PKT_{on}$

- **EXPECTED**: Damping increases with the number of molecular bonds between the surfaces and as temperature decreases $\Rightarrow$ attached time decreases $\Rightarrow$ viscosity decreases.

- **FROM FORMULA**: 
  - $t_{on}$ increases $\Rightarrow$ drag increases, because the cross links get more attached and produce greater force.
  - $k$ increases $\Rightarrow$ drag increases, because greater opposing force is generated.
Furthermore:
large ‘v’ => large shear rates => less attached time (since the shear disrupts the bonds)

Model Predicts:
as velocity gradient increases => viscosity decreases

This approach can be extended further to find expression for viscosity of liquids as follows:
- If number of molecules per unit area is \(1/\delta^2\) where \(\delta\) is the dimension for the molecule and separation of the layers is also \(\sim \delta\) then we have

\[
F/A = \text{pkt}_{\text{on}} v / \delta^2 = (p\delta k^2)(\delta^{-3})(t_{\text{on}})(v / \delta)
\]

\[
\therefore F/A \approx \omega c t_{\text{on}} dv / dx
\]

where \(\omega\) = intermolecular bond energy; \(c = \text{concentration}; t_{\text{on}} = \text{constant}\)

- Thus force is proportional to velocity gradient \((dv/dx)\) and the coefficient of viscosity is given by \(\eta = c \omega t_{\text{on}}\)
• Now in case of proteins:
  1) As the sliding of protein domains past each other also entails the breaking and un-breaking of numerous weak bonds, we expect protein movements will be slowed down due to internal viscosity.
  2) Protein friction is expected to be at least as important as solvent friction.
  3) If more long-lived crosslinks are broken, then protein friction can be much larger.
There are few works done in this regard:
1) Brenner et al. (1982) studied ‘Internal viscosity or protein friction in relaxed muscle fibers where the myosin heads make transitory crossbridges to the actin filaments
2) McCammon et al. (1977) studied molecular dynamics modeling and suggested that interior of proteins is liquid like and thus should possess viscosity
3) Ansari et al. (1992) measured the protein friction in myoglobin and found that it contributes a damping force that is 4 times greater than that from solvent
Use of scaling argument:

As the dimension of an object gets smaller, the viscous force increases relative to the inertial forces, as a result, the global motions of small and comparatively soft objects (such as proteins in aqueous solutions) are expected to be overdamped.
Consider globular protein to undergo structural change and transform to homogeneous and isotropic cube of side ‘L’, density \( \rho \), Young’s modulus \( E \), and damped by a fluid of viscosity \( \eta \).

- For mass \( m = \rho L^3 \);
  stiffness \( k = EL \);

- The drag force associated with a global conformational change that alters the shape of a protein should be given by the Stoke’s law, for which the drag coefficient \( \gamma \) responsible for structural change is given as ‘\( 3\pi \eta l \)’.
As stated in chapter 2 the condition for motion being overdamped is: \( \gamma^2 > 4mk \)

i.e. \( 4m k / \gamma^2 = (4\rho L^3 EL) / (3\pi \eta L)^2 = (2 / 3\pi^2)(\rho E / \eta^2) L^2 > 1 \)

Smaller the object, the smaller the ratio, and the less is the tendency for oscillations.

Because L decreases => \( \eta \) and \( k \) decreases in proportion to the length (resulting less oscillations)

But L decreases => \( m \) decreases thrice (i.e. much faster)

**Inertial Force Decreases More Quickly Compared to the Viscous Force**
Q: How small must a protein be to ensure that the motion is overdamped and that it does not oscillate when subject to external force?

- For a rigid (i.e. molecular weight less than 100 kDa) protein in water: $\eta^2/\varrho E \approx 1_{\text{nm}^2}$. So according to above equation motion of protein with diameter less than characteristic length $L = (3\pi/2)\sqrt{(\eta^2/\varrho E)} \approx 5_{\text{nm}}$ will be overdamped.

- This is true for ~1000 amino acids.
Proteins in fluids are considered as mass and spring with damping model.

Now motion of sphere (diameter $L$) held by spring (stiffness $k$) in viscous fluid (viscosity $\eta$) can be solved using NAVIER-STROKE`S EQUATION.

The motion is overdamped or underdamped is decided based on REYNOLDS NUMBER ($R_{AC}$) being greater than or less than unity.
If $R_{AC} << 1$ STROKE`S LAW holds and $R_{AC} = (kL\eta) / (12\pi\eta^2)$. This implies $L \sim 6\text{nm}$(for $k=\text{EL}$) which agrees with the previous values for the diameter of sphere.

Now if such a body oscillates with the frequency $\omega$ then the inertial forces can be neglected provided $\omega << (4\eta) / (\rho L^2)$.

Stroke`s law doesnot hold for small motions that have high enough frequency but if time constant of the motion is greater than 6ps then inertial forces can be neglected.
The ratio of the inertial to viscous force is:
\[ \frac{3x(1+2x/9)}{2(1+x)} \] where \( x^2 = \frac{L^2 \rho \omega}{8 \eta} \)

Because the resonance frequency is
\[ \sim (k/m)^{0.5} \equiv \left( \frac{E}{\rho} \right)^{0.5}/L, \]
it follows that the characteristic length below which the viscous forces are larger than the inertial forces is \( \sim 8 \text{nm} \) which is in agreement with previous calculations.
Rigidity of allosteric, energy transducing proteins such as motor proteins and the ribosomes is much less than rigid proteins like cytoskeleton.

Consider a structural change of around 1nm associated with a work of $10^{-21}$ J. This gives the stiffness value as $0.2 \text{n/m}$ which is really less. This low value of stiffness corresponds to much greater length $\sim 50 \text{nm}$.

This implies that the motion of large protein molecules such as ribosomes ($L \sim 30 \text{nm}$) would also be overdamped.
Consider the ribosome, a globular protein of diameter \( \sim 30\text{nm} \). If they were not rigid then it would have an overdamped motion. But if they were very rigid like 1GPa and the only damping came from the surrounding fluid, then it would oscillate at a frequency of 5GHz corresponding to a period of nearly 200ps.

i.e. the oscillations would die out after only a few cycles.
The magnitude of oscillations would depend on the size of the force. If work equal to $10^{-19} \text{ J}$ is done on the protein and if we consider the chemical energy being converted to mechanical energy within protein then amplitude of deformation will be of the order of $\sim 0.8 \text{ Å}$.

The oscillations, if they occurred, would be very small indeed. So even if they occurred they would not play important roles in the chemistry of protein synthesis.
The protein friction due to fluid like nature of the interiors of proteins would further dampen the tendency to oscillate. As ansari et al. (1992) suggested that the internal damping is 4 times more than external (i.e. due to fluid).

Elongated proteins are more damped than globular proteins of the same molecular weight. As the aspect ratio \( \frac{L}{\sqrt{A}} \) increases, the damping increases while stiffness decreases. It is observed that if aspect ratio is 10 then the characteristics length for stretching motion is \( \sim 136 \text{nm} \) while that of bending motions is \( \sim 4200 \text{nm} \). Thus motion of large axial ratio will always be overdamped.

The longer the filament the more highly damped. This is a scaling behavior opposite to that of globular proteins. This leads to an important conclusion that the motion of cytoskeleton is overdamped.
Quality of motion of proteins is very important for understanding how it works.

The understanding that proteins are overdamped suggests they can be viewed as mechanical devices that move monotonically into new structural states in response to applied forces.

Also, it rules out the ideas of high frequency resonances and long-distance information transfer and processing in proteins.
Motion of cytoskeleton and cells are also overdamped

- **Expected**: Based on scaling argument motion of linear molecules with larger dimensions to be underdamped
- **Observed**: The larger the molecule more is its motion damped as the intracellular viscosity is very high
- It appears that the cytoskeletal proteins can readily diffuse through the pores, but the motion of larger particles, such as ribosomes and organelles, is severely restricted
Even in where the cytoskeletal filaments are highly aligned and tightly crosslinked for maximum rigidity, the viscous force dominates inertial ones.

Thus even though it is conceivable that the rigidity of the whole cell could be large enough to result in underdamped motion, the cytoskeletal filaments are too sparsely crosslinked to make the network sufficiently rigid.
The rigidity of the cytoskeletal proteins such as actin, tubulin, and keratin, which serve structural roles in cell, is similar to that of hard plastics but substantially less than metal, glass or wood.

This is because proteins are held by relatively weak van der waals force.

The rigidity of protein machines is expected to be substantially less than the structural proteins.
- As proteins move and change shape, they experience damping force from surrounding fluid as well as from internal friction

- These viscous force arise from rapidly making and breaking of bonds

- Due to small size of proteins the viscous force is generally much greater than the inertial force.
Consequently the global motions of proteins, especially less rigid ones, are highly overdamped.

They creep rather than oscillate when subject to applied forces.

The motion of long, thin cytoskeletal filaments are also overdamped, due to their large aspect ratios. This, in turn, causes the motion of cells to be overdamped.
THANK YOU!