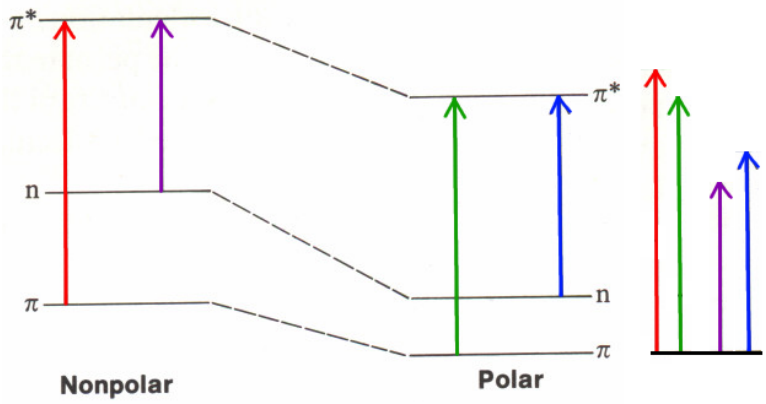
A macromolecule is moved from a non-polar solvent to a polar solvent, which causes a change in energy states as shown below. When moving to the polar solvent, do you expect a red or blue shift in the absorption profile, or none at all for (a) the n to π* transition, and (b) the π to π* transition? Explain why. Arrows on the right are drawn to scale for comparison.



In this case, the energy for each transition changes as the molecules is moved from a non-polar to a polar environment. First, the energy of each electronic level is lower, but that is not what is observed in the absorbance spectrum. Here, only the sizes of the transitions matter. This is illustrated simply by comparing the height of the transitions. **a)** For the *n* to *π** transition, the energy increases as the molecules is moved to a polar environment, because the blue line is higher than the purple line. This means that the wavelength would have to be lower. A lower wavelength corresponds to a higher energy. So for the *n* to *π** transition, we see a blue shift. **b)** For the *π* to *π** transition, the energy decreases, so there a red shift is observed.

3a. (12 points)
A linear dsDNA molecule of 200 bp is dissolved in pure water, 15 mM NaCl, and 500 mM NaCl and measured in the analytical ultracentrifuge in these three salt concentrations. The following $s_{20,w}$ and $D_{20,w}$ were obtained:

In water: $s_{20,w}$: 5.039x10^{-13}s, $D_{20,w}$: 2.215x10^{-7} cm^2/sec
In 15 mM NaCl: $s_{20,w}$: 5.123x10^{-13}s, $D_{20,w}$: 2.073x10^{-7} cm^2/sec
In 500 mM NaCl: $s_{20,w}$: 5.179x10^{-13}s, $D_{20,w}$: 1.952x10^{-7} cm^2/sec

Assuming each basepair contributes 660 g/mol, calculate the following values for each observation above:

Partial specific volume ($\bar{\nu}$):
Frictional coefficient:
volume of one molecule:
Frictional ratio:

Note: The molar mass of the DNA molecule does not change when salt is added

Useful constants: the viscosity (η) of water at 20°C is 0.0100194 poise, and the density (ρ) of water at 20°C is 0.998234g/ml. The gas constant (R) is 8.314621e7 erg/(K*mol) and Avogadro's number (N) is 6.022141e23

Show all work & equations used.

Diffusion coefficient: $D = \frac{RT}{Nf}$ Sedimentation coefficient: $s = \frac{M(1-\bar{\nu}\rho)}{Nf}$

Stokes-Einstein Relationship: $f_0 = 6\pi\eta r$ Frictional coefficient: $\phi = \frac{f}{f_0}$

To calculate the vbar, we combine equations for *s* and *D* and solve for vbar:

$$\frac{s}{D} = \frac{M(1-\bar{\nu}\rho)}{RT} \qquad \bar{\nu} = \left(1 - \frac{sRT}{DM}\right)\frac{1}{\rho}$$

To calculate the frictional coefficient, we can use either the sedimentation or diffusion coefficient equation: $f = \frac{M(1-\bar{\nu}\rho)}{Ns}$ $f = \frac{RT}{ND}$

The volume of a molecule can be obtained from the molar mass and the partial specific volume:

$$V = \frac{M\bar{\nu}}{N}$$

The calculation of the frictional coefficient requires calculation of f_0 which is the frictional coefficient of a molecule if it were squeezed into a perfect sphere. The Stokes-Einstein relationship gives this value if we know the volume of this molecule (see calculation above). Using the equation for the volume of a sphere, we can calculate:

$\frac{4}{3}\pi r_0^3 = \frac{M\bar{v}}{N}$ solving for r_0: $r_0 = \frac{3M\bar{v}}{4\pi N}$ then $f_0 = 6\pi\eta r_0$ and using f from above solve for φ.

System:	vbar:	f:	volume:	f/f0:
in Water:	0.580949 ml/g,	1.82729e-07,	1.27339e-19 cm^3,	3.10009
in 15 mM NaCl:	0.544627 ml/g,	1.95246e-07,	1.19377e-19 cm^3,	3.3845
in 500 mM NaCl:	0.510983 ml/g,	2.07349e-07,	1.12003e-19 cm^3,	3.67151

3b. (4 points)
Identify the direction in which each variable is changing when [salt] is increased. Explain why this trend is observed for each of these variables. What is happing to the DNA molecule as [salt] is changing?

The vbar is decreasing, as salt increases. When the vbar is decreasing, the molecule's density is increasing, in this case the density is increasing because less water is bound. When there are no sodium ions around, the strongly negative backbone charge makes the surrounding water reorient its dipole moments to align with the backbone charge, and making it appear as if the water is bound and cosedimenting.

The frictional coefficient is actually increasing when less water is bound, because the hydration shell has a more globular shape than the naked DNA molecule with the extra water molecules bound. So the less water is bound, the less globular the overall sedimenting particle will be, even though the charges on the backbone of the DNA will be neutralized by sodium ions, and the molecule will be more flexible.

The volume of a particle increases when less salt is present, because more water is bound, increasing the volume of a molecule.

The frictional ratio has the same trend as the frictional coefficient. Less water bound means less globular. This is also the reason the diffusion decreases with increasing salt. The s-value increases with increasing salt due to a combination of effects. Even though the friction goes up with increasing salt concentration, which should decrease sedimentation speed, the change in density of the particle outweighs this effect. So the more globular particle is actually much less dense and therefore does not sediment as fast.

4. (4 points)
A researcher measures the binding coefficient of a fluorescently labeled RNA molecule to an unlabeled protein target by mixing 200 nM of the labeled RNA molecule with 800 nM of unlabeled protein target and performing a fluorescence sedimentation velocity experiments of the mixture and the labeled RNA molecule by itself, generating a single 2.3S peak for the labeled RNA molecule by itself. The mixture produces two peaks, one at 2.3S (30% of the fluorescent signal) and another at 6.1 S (70% of the fluorescent signal). The unlabeled target is measured separately in a UV detection experiment and produces a single peak at 4.8 S. What is the Kd for this interaction in molar concentration. Show formulas used.

The 2.3 S peak is from the labeled molecule in its free form. The 6.1S sediments faster than the 4.8 S control peak from the target by itself, hence, it represents the complex of target and labeled molecule, likely a 1:1 complex. When the RNA binds the protein, the formed complex binds a portion of the labeled RNA molecules. This portion is proportional to the RNA binding, so we have 30% free RNA and 70% bound RNA, even if only a portion of the RNA is labeled (assuming labeled and unlabeled RNA bind equally strong). Since we know the starting concentrations (200 nM for the RNA and 800 nM for the protein), we can write a Kd equation:

A = RNA, B = protein

$$Kd = \frac{[A][B]}{[AB]}$$

Here, [A] is the 30% of the starting concentration of [A], i.e. 0.3*200 nM = 60 nM, [AB] equals 70% of the RNA's starting concentration, i.e., 0.7 * 200 nM = 140 nM. Since 140 nM of the starting concentration of [B] are consumed by the formed complex, 800 nM – 140 nM = 660 nM remain unbound [B]. Hence:

$$Kd = \frac{[60][660]}{[140]} = 282.86\,nM$$