Notes B

Slide 2. The left-handed wrapping of DNA in the nucleosome core particle obviously changes the writhe of DNA. We have seen that it also alters the helical repeat and thus changes the DNA twist. We therefore expect that nucleosome formation in the presence of topoisomerase on a circular DNA molecule, changes the linking number of the circle. What is the linking change per nucleosome? Walter Keller addressed this question experimentally in a beautiful series of experiments. He isolated SV40 DNA, the circular genome of the Simian Virus 40, linearized the circle and ligated it in the presence of different concentrations of ethidium bromide (samples 1-10). This yields a spectrum of topoisomer distributions with different means. (The more ethidium bromide was added, the smaller the mean linking number of the distribution).

Sample 1 was ligated in the absence of ethidium bromide and sample 11 was (untreated) SV40 DNA I. Electrophoresis was performed at room temperature (gels a, c-f) or at 4 degrees Celsius (gel b), in the absence (gels a and b) or the presence of different EthdBr concentrations (gels c-f). The overlapping distributions, which can be resolved under different electrophoretic conditions, allow for measuring the linking deficit of native SV40 DNA to relaxed SV40 DNA. The mean linking difference between two overlapping distributions is determined by measuring the linking difference between the distribution centers. (See Depew and Wang, 1975).

Slide 3. A plot of ethidium bromide concentration versus the mean linking deficit is shown on the left. Also indicated is the mean linking deficit of native SV40 DNA as determined with the help of adjacent overlapping distributions. The number found was -24. We now can isolate SV40 chromosomes and look at them under the electron microscope to count the number of nucleosomes, as was done by Sargostio et al. We see that the linking deficit (the number of negative supercoils) agrees very well with the number of nucleosome on the circle.

Slide 4. The Whitehead link is an example for two closed space curves (link) that are topologically linked but have linking number 0. The reason is that one of the two components of the link is knotted. (See Colin Adams, The Knot Book, Owl Books 2002).
Slide 6. The twist measures the number of revolutions of \( v \) about the ribbon axis gamma. As long as gamma lies within a plane, this notion is sufficient to define twist. We intuitively use the plane as a reference frame to determine the number of revolutions of \( v \) about gamma, as indicated in the slide (upper left corner). Things are getting more complicated when gamma is not confined to a plane, as shown below. In this case, the question arises which reference frame to choose? The problem is solved by determining the rate of revolution in a “moving reference frame” as we move along gamma. The moving reference frame is defined at each point of gamma by the tangential unit vector to gamma, \( T \), the unit vector \( v \), orthogonal to \( T \), and \( N \), the vector product of \( T \) and \( v \). The three vectors \( T \), \( v \), and \( N \), thus define, in this order, a right-handed orthonormal basis at each point of gamma. We now consider the orthonormal basis \( T \), \( v \), \( N \) at time \( t \) and describe \( v \) at time \( t + \Delta t \) within this reference frame. In general, \( v(t + \Delta t) \) is not orthogonal to \( N(t) \) or \( T(t) \) anymore. We are interested in the angle \( \Delta \theta \) by which \( v(t + \Delta t) \) has rotated toward \( N(t) \), and thus about \( T \), within the time interval \( \Delta t \). Since \( v \) has unit length, this angle is equal to the arc length over \( \Delta \theta \). For small \( \Delta t \) and thus small \( \Delta \theta \) the arc length equals the leg opposite to \( \Delta \theta \), which is equal to the projection of \( \Delta v \) into \( N \), i.e. the dot product of \( N \) and \( \Delta v \).

Slide 7. The angular rate of rotation is the limiting value of \( \Delta \theta \) divided by \( \Delta t \) as \( \Delta t \) tends toward zero. Note that the definition implies that the twist is positive if \( v \) rotates about \( T \) clockwise, and negative if it rotates counterclockwise.

Slide 8. We define the twist of the ribbon as the integral of the angular rate of rotation over the curve gamma divided by \( 2\pi \). Since we are interested in the number of revolutions rather than the arc length, we divide the integral by \( 2\pi \). (Remember that \( 2\pi \) is the arc length of the unit circle).

Slide 9. Imagine a surface \( M \) with a space curve gamma lying in \( M \). Let gamma be the axis of a ribbon, with \( v \) standing orthogonally of \( M \), or more precisely, the tangential plane of \( M \) at each point of gamma. This defines a ribbon that stands orthogonally on \( M \) and follows \( \gamma \). We now define the surface twist, \( STw \), of \( M \) along \( \gamma \) as the twist of the ribbon \((\gamma, v)\). The twist of \((\gamma, v)\) is determined the surface geometry.
**Slide 10.** Let $(\gamma, v)$ be the reference ribbon orthogonal to $M$ along $\gamma$. A reference ribbon that lies in rather than stands on the surface, $(\gamma, w)$ with $\langle v | w \rangle = 0$, has the same twist as $(\gamma, v)$. This is intuitively clear. A proof can be found in the accompanying Math Notes.

**Slide 11.** Shown here is a ribbon lying in the surface of a cylinder. Calculating its twist gives us the surface twist of the cylinder surface along the helical path of the ribbon axis. Applying the definition of twist, we find that the surface twist equals $n \times \sin \alpha$, where $n$ is the number of turns about the cylinder axis and $\alpha$ is the helical pitch angle. A proof of this equation can be found in the mathematical notes. Note that the surface twist increases as $\alpha$ increases. It reaches its maximum at a pitch angle of $90^\circ$.

**Slide 13.** Now imagine a DNA molecule lying in a surface $M$ with its axis following the indicated curve $\gamma$. We describe the DNA molecule by the ribbon $(\gamma, w)$. The winding number measures how many times and in which direction $w$ rotates past $v$, the orthonormal vector field on $M$ along gamma.

**Slide 14.** We can experimentally measure the winding number of DNA by exposing DNA lying a surface to small concentrations of DNase I. The periodicity with which the DNA becomes accessible to DNase, which occurs every other turn of the helix, allow us to determine the winding number of DNA with respect to the surface. The length of the DNA molecule measured in base pairs divided by the winding number is called the helical repeat, which is what we measure in this experiment.

**Slide 16.** The twist decomposition theorem allows us to calculate the twist of a DNA molecule, whose axis $\gamma$ lies in a surface $M$, from surface geometry and an experimentally accessible quantity, the winding number. A proof of this theorem can be found in the Math Notes.

**Slide 17.** The naïve concept of twist coincides with the concept of winding number in the case of a flat surface.

**Slide 18.** We can use the linking theorem to prove that the writhe of a space curve in the surface of a sphere is zero. We may use any ribbon whose axis is congruent with
the space curve to determine the curve’s writhe. Let gamma be a unit speed curve in the surface of a sphere and v the normal vector to the surface of the sphere. The linking number of the ribbon (γ, v) - which is the normal reference ribbon - is obviously zero. Its twist is zero as well, since T = γ’ = (1/c) v’. The latter equality follows from v = cγ.