# Reference Solution - Problem Set #5: Diffusion and Target Search

## Question 1: GFP Diffusion

The inert green fluorescent protein (GFP) has a free diffusion coefficient of *D* = 30 µm2 s-1 in the cell.

a) How long will it take a single GFP molecule with this value of *D* to diffuse in three dimensions the distance of

* the width of an E. coli cell (~1 µm)?
* the width of a "typical" nucleus of a human cell (~15 µm)?
* the length of an internodal cell in the alga Nitella (~1 cm)?
* the length of a spinal motor neuron that innervates a foot muscle in an adult human (~1 m)?

b) In which of the above cells is free diffusion not an efficient mechanism for the transport of macromolecules and what could be alternative mechanisms in these cases?

c) Predict how a plot of the mean squared displacement (MSD) of GFP versus time for 0-10 seconds would look like in in a human cell nucleus and compare it to a plot of MSD versus time for free diffusion of GFP.

### a) Diffusion times in different cellular contexts

To calculate the time it takes for a GFP molecule to diffuse a certain distance in three dimensions, we use the mean squared displacement (MSD) formula:

⟨r²⟩ = 6Dt

where ⟨r²⟩ is the mean squared displacement, D is the diffusion coefficient, and t is time.

Rearranging to solve for time:

t = ⟨r²⟩/(6D)

For each cellular context:

**Width of an E. coli cell (~1 µm):**
t = (1 µm)²/(6 × 30 µm² s⁻¹) = 0.0056 s ≈ **5.6 milliseconds**

**Width of a "typical" nucleus of a human cell (~15 µm):**
t = (15 µm)²/(6 × 30 µm² s⁻¹) = 1.25 s ≈ **1.25 seconds**

**Length of an internodal cell in the alga Nitella (~1 cm = 10,000 µm):**
t = (10,000 µm)²/(6 × 30 µm² s⁻¹) = 555,556 s ≈ **6.4 days**

**Length of a spinal motor neuron that innervates a foot muscle (~1 m = 10⁶ µm):**
t = (10⁶ µm)²/(6 × 30 µm² s⁻¹) = 5.56 × 10⁹ s ≈ **176 years**

### b) Efficiency of diffusion and alternative transport mechanisms

Free diffusion is not an efficient mechanism for transport in:

**Internodal cells of the alga Nitella**:
With diffusion times of several days, alternative mechanisms are necessary:

* Cytoplasmic streaming: Flow of cytoplasm along actin filaments driven by myosin motors
* Active transport via motor proteins
* Directed vesicular transport

**Spinal motor neurons**:
With diffusion times of decades to centuries, active transport is essential:

* Axonal transport system using motor proteins:
	+ Kinesin for anterograde transport (cell body → axon terminal)
	+ Dynein for retrograde transport (axon terminal → cell body)
* Fast axonal transport (100-400 mm/day) for vesicles and organelles
* Slow axonal transport (1-10 mm/day) for cytoskeletal proteins

In contrast, free diffusion works efficiently for smaller cells like E. coli (milliseconds) and within compartments like the nucleus (seconds).

### c) Mean squared displacement (MSD) of GFP in nucleus vs. free diffusion

**Free diffusion of GFP:**

* For free diffusion, MSD increases linearly with time: MSD = 6Dt
* The plot of MSD versus time would be a straight line with slope 6D = 180 µm²/s

**Diffusion of GFP in the nucleus:**

* In the nucleus, GFP experiences obstacles like chromatin, nuclear bodies, and macromolecular crowding
* This results in anomalous diffusion described by: MSD = 6Dtᵅ, where α < 1
* For short time scales (< 100 ms), the curve might appear nearly linear
* For longer time scales (1-10 s), the curve increasingly deviates from linearity, with a decreasing slope
* Eventually, the MSD may plateau as confinement by the nuclear envelope limits long-distance diffusion

The comparison would look similar to this:

* Free diffusion: Linear increase, MSD = 180t µm²
* Nuclear diffusion: Sublinear increase, MSD = 180tᵅ µm², where α ≈ 0.6-0.8

## Question 2: Target Search by p53

The transcription factor p53 diffuses in vitro in three dimensions with *D*3 = 50 µm2 s-1 through a spherical volume of 15 µm diameter filled with water and a 24 base pairs long DNA fragment with its binding site.

a) Estimate the "search time" for p53 to bind the DNA under the above conditions according to the considerations given in the review by Berg & von Hippel (DOI: 10.1146/annurev.bb.14.060185.001023) on diffusion-controlled reactions.

b) Compare the “search time” you have calculated in a) to the time it takes p53 to translocate from one end of the sphere to the other end.

c) Name three different mechanisms by which the search time of p53 to find its target site in the cell could be reduced.

### a) Estimation of search time

According to Berg & von Hippel (1985), the mean diffusion time to reach a small target of radius r in the middle of a cell of radius R (where R >> r) is given by:

τ₃ = (R²/3D₃) × (R/r)

Where:

* R = 7.5 µm (radius of the 15 µm diameter sphere)
* D₃ = 50 µm² s⁻¹ (diffusion coefficient of p53)
* r ≈ 4.1 nm (radius of the 24 bp DNA fragment, calculated as half the length of the DNA fragment: 24 bp × 0.34 nm/bp = 8.16 nm)

Substituting these values:

τ₃ = [(7.5 µm)²/(3 × 50 µm² s⁻¹)] × (7.5 µm/0.0041 µm)
τ₃ = (0.375 s) × 1829
τ₃ ≈ 686 s ≈ **11.4 minutes**

This represents the average time required for p53 to find the DNA fragment by random diffusion.

### b) Comparison with translocation time

The time for p53 to diffuse from one end of the sphere to the other (a distance of 15 µm) can be calculated using the MSD formula:

t = (15 µm)²/(6 × 50 µm² s⁻¹) = 0.75 s

The search time (11.4 minutes) is approximately 915 times longer than the time required for p53 to translocate across the entire sphere (0.75 s). This dramatic difference illustrates the challenge of finding a specific small target in three-dimensional space, even when the diffusion process itself is relatively fast.

### c) Mechanisms to reduce search time

Three mechanisms that could reduce the search time for p53 to find its target site:

**1. Facilitated diffusion (sliding)**

* p53 can bind non-specifically to DNA and slide along the DNA backbone through one-dimensional diffusion
* This reduces the search dimensionality from 3D to 1D
* p53 can scan approximately 100-200 base pairs during each DNA binding event
* Sliding is particularly effective for locating binding sites once the protein has encountered the DNA

**2. Intersegmental transfer**

* p53 can transfer directly between two DNA segments that are close in 3D space but distant in sequence
* This allows the protein to "jump" across different regions of DNA without fully dissociating
* This mechanism is effective in the crowded nuclear environment where different DNA segments come into close proximity
* p53's tetrameric structure facilitates simultaneous binding to multiple DNA segments

**3. Reduced volume through nuclear compartmentalization**

* The nucleus is compartmentalized, with specific proteins concentrated in certain regions
* Transcription factors like p53 are often enriched near transcriptionally active regions
* This effectively reduces the search volume, lowering the time required to find target sites
* Electrostatic interactions between positively charged p53 domains and negatively charged DNA enhance association rates

These mechanisms work synergistically in vivo to reduce the search time from minutes to seconds, enabling more efficient gene regulation.

## Question 3: One-Dimensional Diffusion Along DNA

TFX can slide along long DNAs by one-dimensional diffusion with a diffusion constant *D*1 = 2·10-4 µm2 s-1. For unspecific binding to the DNA it dissociates with a kinetic rate constant of *k*off = 0.1 s-1 from the DNA.

a) On an average, the protein moves the length *l*slide along the DNA before it dissociates.

The value of *l*slide is given by  Show how this expression for *l*slide can be derived.

b) Calculate *l*slide for the values of *D*1 and *k*off given above as well as the time it takes TFX to translocate a distance of *l*slide by diffusion in three dimensions with *D*3 = 50 µm2 s-1.

c) Discuss if one-dimensional diffusion along the DNA will speed up the search time of a transcription factor for finding its target DNA sequence with the parameters given above..

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### a) Derivation of sliding length

On average, the protein moves the length l\_slide along the DNA before it dissociates, given by:

l\_slide = √(2D₁/k\_off)

Derivation:

1. The mean squared displacement in one dimension is given by:
⟨x²⟩ = 2D₁t
2. The average time the protein remains bound to DNA before dissociating is:
t = 1/k\_off
3. Substituting this time into the MSD equation:
⟨x²⟩ = 2D₁ × (1/k\_off) = 2D₁/k\_off
4. Taking the square root to find the average distance:
l\_slide = √⟨x²⟩ = √(2D₁/k\_off)

This represents the average distance the protein slides along DNA before dissociating.

### b) Calculation of l\_slide and 3D diffusion time

**Calculation of l\_slide:**

l\_slide = √(2D₁/k\_off) = √(2 × 2·10⁻⁴ µm² s⁻¹/0.1 s⁻¹) = √(4·10⁻³ µm²) ≈ 0.063 µm = 63 nm

Since DNA has a rise of 0.34 nm per base pair, this corresponds to approximately 186 base pairs.

**Time to translocate the same distance in 3D:**

Using D₃ = 50 µm² s⁻¹ and the MSD formula for 3D diffusion:

t = (0.063 µm)²/(6 × 50 µm² s⁻¹) ≈ 1.3 × 10⁻⁵ s = 13 microseconds

This is approximately 750,000 times faster than the time spent sliding the same distance in 1D (10 seconds).

### c) Efficiency of one-dimensional diffusion for target search

Despite the significantly slower 1D diffusion compared to 3D diffusion, one-dimensional sliding along DNA can still substantially accelerate the overall search process through several mechanisms:

**Reduction of dimensionality:**

* 1D sliding reduces the search space from three dimensions to one
* While 1D diffusion is slower, it allows the protein to scan DNA bases sequentially
* The protein can examine approximately 186 bp during each binding event without missing any potential binding sites

**Antenna effect:**

* The ability to bind DNA at any position and then slide to the target site effectively increases the "target size"
* Instead of having to directly contact a specific sequence, the protein can land within the sliding length and still locate the target
* This increases the effective target size from a few base pairs to ~186 bp

**Optimal search strategy:**

* The most efficient search strategy combines periods of 3D diffusion with periods of 1D sliding
* Theory suggests an optimal search involves roughly equal time spent in each mode
* For the given parameters (D₁ = 2·10⁻⁴ µm² s⁻¹, k\_off = 0.1 s⁻¹), the protein spends 10 seconds in the 1D mode per binding event
* This creates a balance between broad exploration (3D) and detailed scanning (1D)

For TFX with the given parameters, one-dimensional diffusion would enhance the search process despite being slower than 3D diffusion. The optimal ratio of 3D to 1D search depends on factors like DNA concentration, the spatial organization of the genome, and specific properties of the transcription factor.

## References

Berg, O.G., and von Hippel, P.H. (1985). Diffusion-controlled macromolecular interactions. Annu Rev Biophys Biophys Chem 14, 131-160.