b. Dr. Counter also decides to tabulate the fate of each molecule that reaches the middle of the pore. For each molecule that comes to the point halfway across the nuclear envelope, Dr. Counter follows it to see whether it exits the pore by going into the cytoplasm or by going into the nucleus. What is the predicted difference between the number per minute of these molecules exiting into the nucleus and into the cytoplasm? Explain your reasoning.

The difference must be \textbf{zero}, as there is no net force to bias diffusion* through the pore. (If this were not true, the pore would do net transport when \([\text{GFP}]_{\text{nucl}} = [\text{GFP}]_{\text{cyto}}\), without energy input, which would violate the 2nd law of Th.)*

*or the random walk, if you prefer.

5. Isocratic anion exchange chromatography is a column chromatography technique in which negatively charged solutes bind to a positively stationary phase so that the most negatively charged solutes elute last from the column. The mobile phase is a salt solution kept at a constant concentration throughout the experiment. An experimenter finds that chromatographic conditions optimized for separation of two net negatively charged small molecules give only poor separation of proteins with approximately the same net charge. What is the most likely reason? What should be done to improve the separation of the proteins?

This is yet another form of partition chromatography, in which the amount of separation between the adjacent tails of two peaks is proportional to \(\sqrt{n}\), where \(n\) is the mean number of molecular transits between mobile and stationary phase. \(n\) is smaller for the proteins because they have lower diffusion coefficients. To increase \(n\), reduce the flow rate or lengthen the column.
6. A postdoc whose friends call him "Preps" is using gel filtration chromatography as the final step of a complex enzyme preparation. He applies a 50 μl sample to a 1.12 (diameter) \times 10 \text{ cm} (length) Sephadex column and elutes at 0.2 ml/min. Preps is happy that his protein elutes from the column in 25 min in a nicely Gaussian-shaped peak, but is annoyed that his sample is so diluted by the column that he recovers only 67% of the protein in a 1 ml fraction centered on the peak.

a. If the protein has a diffusion coefficient of $1 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, estimate the fraction of the dilution can be attributed to diffusion of the protein along the axis of the column during chromatography.

- Column cross-sectional area $A = \pi r^2 = \pi (0.56)^2 = 1 \text{ cm}^2$
- Effective cross-sectional area is less because of space occupied by beads. We'll assume this is 50%, so $A_{\text{eff}} = 0.5 \text{ cm}^2$
- Initial height of sample on column is $\frac{0.05 \text{ cm}^2}{0.5 \text{ cm}^2} = 0.1 \text{ cm}$
- Let's approximate this as a Gaussian with $\sigma = 0.05 \text{ cm}$
- For diffusion starting with an infinitely thin band, $\sigma(t) = \sqrt{2D\Delta t}$

$\text{so } t_{\text{start}} = \frac{\sigma^2}{2D} = \frac{(0.05 \text{ cm})^2}{1 \times 10^{-6} \text{ cm}^2 / \text{s}} = 2500 \text{ s}$

is the time corresponding to the loading time in the chromatography expl.

$\Rightarrow t_{\text{end}} = t_{\text{start}} + (25 \text{ min}) (60 \text{ s/min}) = 4000$

$\Rightarrow \sigma(t_{\text{end}}) = 0.09 \text{ cm}$ is the predicted width due to diffusion

- Actual width of eluted peak is $\frac{0.5 \text{ cm}^3}{0.5 \text{ cm}^2} = 1 \text{ cm}$

so diffusion accounts for $0.09/1 = 9\%$ of width
b. What are likely to be the two predominant causes of the dilution?

1. All flow paths through the column not being the same length (due to non-uniform column packing, etc.)

2. There is a statistical broadening due to transiting between the stationary and mobile phases.
   \[ \sigma_{stat} = \sqrt{n \cdot p \cdot (1-p)} \] where \( n \) = number of transits and \( p \) = probability of being in the stationary phase.