

Determining the Number of NH3 Ligands Required on a PAMAM Dendrimer for Lysosome Escape via the Proton Sponge Effect

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Abstract

Drug delivery systems are a necessity for certain life-saving drugs that must be delivered safely and reliably to their target cells. Dendrimers, a large branching polymer that allow for the functionalization of end groups to perform the functions necessary for drug delivery, are one of the main drug delivery systems used today, however it may take researchers a long time to develop new dendrimers due to their highly customizable nature. Our study focuses on streamlining the dendrimer design process and adding to the scientific discussion regarding the true cause of lysosome lysis through the proton sponge effect.

Drug delivery systems must overcome several obstacles to accomplish their task, one of these is the lysosome degradation pathway. The lysosomal escape mechanism that we selected to study was the proton sponge effect, a process that causes lysis through osmotic pressure.

We calculated the number of NH3 ligands (the end groups that cause the proton sponge effect), needed on a single dendrimer. We determined the number of ligands required on each dendrimer to cause lysis. Additionally, our results incline us to theorize that lysis through the proton sponge effect is caused primarily by osmotic pressure. These results will help inform researchers who develop new dendrimers.

Introduction

Drug delivery systems are crucial to some drugs' function in medicine, without a method of transport they would never reach their target [1]. Dendrimers, shown in Figure 1, are one of the main drug delivery systems used today. They are highly customizable, which is both a benefit and a flaw, as the number of parameters a researcher needs to account for is very high, leading to long development periods for new dendrimers. Specifically, dendrimers' terminal ends can be fitted with ligands that each accomplish specific tasks for the system.

We fill the gap in knowledge in the design of dendrimers and determine the number of NH3 ligands that are required to lyse an average sized lysosome in an average cell. This will help researchers quickly determine a viable ratio of ligands to put on their dendrimer system. In addition to that, our experiments will add to the scientific discussion regarding the true cause of the proton sponge effect, whether it be membrane destabilization caused by the dendrimer, or the osmotic pressure caused by the protonation of NH3 ligands and subsequent chloride influx.

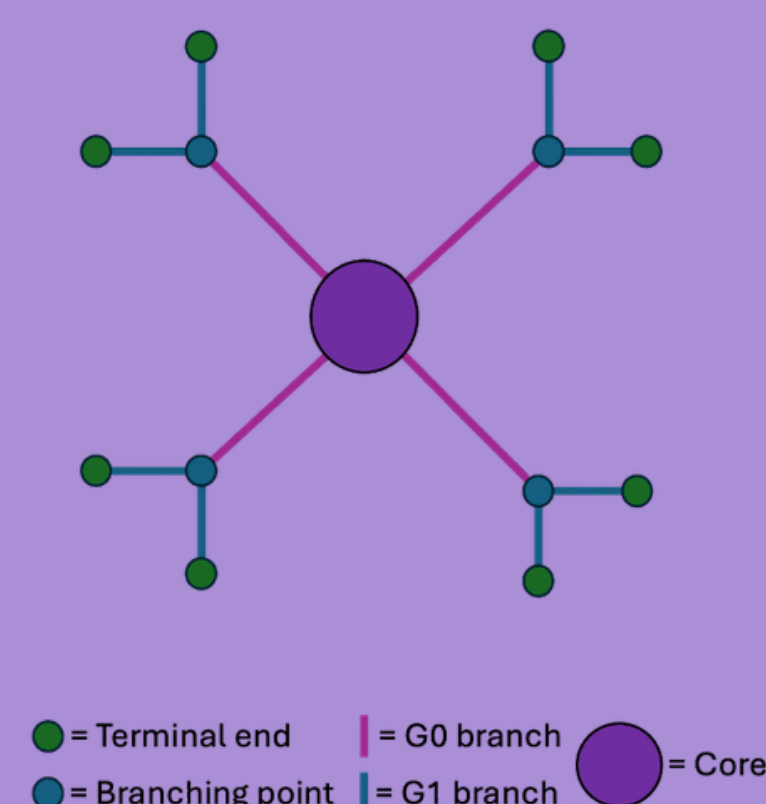


Figure 1 - Diagram of a G1 dendrimer

Methods

The experiments we performed were entirely *in silico*. We used the Python packages Numpy, Pandas, and Matplotlib to run the calculations and create graphical readouts. We also used the following equations in our simulations: the osmotic pressure equation to compare chloride ions entering/number of NH3 groups with osmotic pressure, and this equation derived from the molarity equation:

$$N = \frac{M \times v \times (6.022 \times 10^{23})}{50}$$

Equation 1

Where N is the number of dendrimers, 50 is the number of lysosomes in a cell [2], M is molarity of dendrimers outside the cell, and v is cell volume. We divided by 50 because the numerator of the fraction is the number of dendrimers per cell. This was used to estimate the number of dendrimers that would enter a single lysosome.

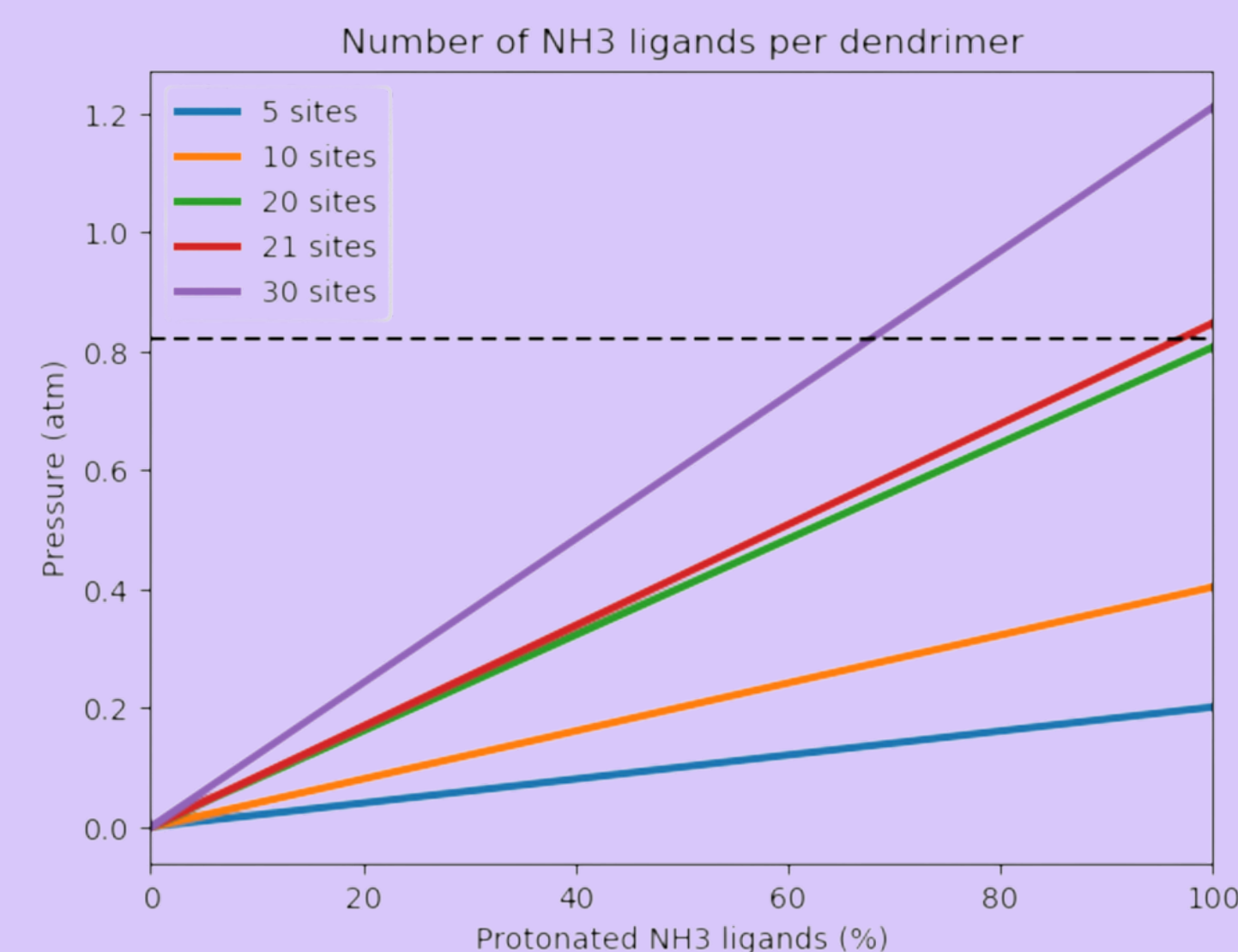


Figure 2 - Number of NH3 ligands required on a dendrimer (note that site is used as a synonym of ligand in the legend)

Results

As can be seen in figure 2, we determined that for a lysosome with 250nm radius [3](average sized), 62'500 dendrimers (the number obtained from Equation 1 using 350 nM dendrimer concentration, a non-cytotoxic concentration [4], and the volume of a cardiomyocyte) would need 21 NH3 ligands, or sites, each. Our graph also used the value of 0.82 atm of pressure [5] as a threshold to cause lysosome lysis.

Discussion

This value of ligands per dendrimer is useful to anyone attempting to build a dendrimer drug delivery system, as it will give them a baseline for the requirements a dendrimer must meet to induce the proton sponge effect and lyse the lysosome.

Additionally, our experiments demonstrate that it is mathematically possible to cause lysosome lysis solely with osmotic pressure, without including membrane destabilization. We therefore theorize that osmotic pressure may be the primary cause of lysis through the proton sponge effect.

Conclusion

We found that it takes 21 NH3 ligands per dendrimer to rupture an average sized lysosome. We recommend that anyone who wishes to continue this study delve deeper into the kinetics of this reaction to determine exact number of ligands required to lyse varying sizes of lysosomes. As stated earlier, we only used average values, therefore creating a table of lysosome size to number of ligands required would be beneficial.

References

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