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Article

Assessing CDK5 as a Nanomotor for Chemotactic Drug Delivery

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SUMMARY

Enzyme chemotaxis is a thermodynamic phenomenon in which enzymes move along a substrate concentration gradient towards regions with higher substrate concentrations. Mathematical models have been developed to understand and define this phenomenon using Michaelis-Menten kinetics and substrate binding coefficients. One key application of this phenomenon is in the field of drug delivery, where chemotaxis can steer nanovehicles towards targets along natural substrate concentrations. Such gradients exist throughout the body, including around the blood-brain barrier, and thus targeted nanovehicles can bring therapeutics through such a gradient and target themselves at a given toxic species. In patients with Alzheimer's disease, a gradient of tau protein forms in the bloodstream, and this protein is a substrate of the enzyme CDK5, a tau protein kinase that catalyzes the phosphorylation of tau protein. Through chemotaxis, CDK5 can travel along the tau protein gradient towards increasing concentrations of tau tangles and amyloid-beta proteins that are the cause of neuronal cell death in Alzheimer's patients. In order to create the momentum necessary to propel a nanovehicle along the substrate concentration gradient, an enzyme's binding affinity towards its substrate must overcome obstacles such as Brownian motion. We hypothesized that CDK5 would be able to overcome these barriers and developed a quantitative model using Michaelis-Menten kinetics to define the necessary parameters to confirm and characterize CDK5's chemotactic behavior to establish its utility in drug delivery and other applications. Our results found that CDK5's movement significantly outpaced random motion and characterized its movement for usage in drug delivery system design.

INTRODUCTION

Cyclin-dependent kinase 5 (CDK5), a tau protein kinase, is an enzyme identified in 1992 (1). It is known as a regulator in a variety of processes in the brain and nervous system. As such, the activity of CDK5 and its corresponding activators have been found to be an indicator of many neuronal diseases, including Alzheimer's disease and amyotrophic lateral sclerosis (ALS). Both of these disorders are rooted in amyloid fibril formation which introduces neurotoxicity in a manner correlated with the CDK5 activator known as p25 (2).

A hallmark of Alzheimer's disease is the formation of amyloid-beta proteins which accumulate into large structures that build up extracellularly between neurons, inhibiting neural communication and forming plaques in the brain (3). Alongside these amyloid-beta proteins are neurofibrillary tau tangles which are large aggregates of tau proteins that have misfolded and built up intracellularly in the brain, damaging core functions like memory. These proteins, specifically tau proteins, are in high concentration near the brain, but diminish in concentration in the bloodstream as the distance from the brain increases (4). This forms a concentration gradient of tau protein in the bloodstream, and most importantly, around the blood-brain-barrier or BBB.

We focused on CDK5's ability to exhibit a phenomenon known as enzyme chemotaxis, which is the movement of enzymes towards their substrates through favorable thermodynamic conditions. Enzymes exhibit the process of chemotaxis, moving along a substrate concentration gradient as they diffuse and move towards regions of high substrate concentrations (5). As such, chemotaxis is a natural phenomenon, and its effects can be amplified or neutered through the modification of the substrate gradient, but not necessarily the protein itself. Though all enzymes exhibit chemotaxis, CDKs exhibit this property with a high affinity for their substrates (6). The use of chemotaxis is especially pertinent in the field of targeted drug delivery, as the attraction between an enzyme and its substrate can be used to propel nanovehicles to a certain location in the body (7). In the case of Alzheimer's disease, many drugs are often less effective due to the fact that the liver and other parts of the body absorb much of the drug, and the low permeability of BBB leaves only a small fraction for the brain. However, using a targeted drug delivery system, a drug encapsulated in a BBB-permeable nanovehicle could use chemotaxis to move towards the therapeutic target, ensuring that a larger and more concentrated dose reaches the brain (8). Because CDK5 moves along a tau protein gradient, it could be an optimal candidate to use in a chemotactic drug delivery system, as it can move the vesicle towards higher tau concentrations, allowing Alzheimer's medication to be released and have the greatest effect. Ultimately, we aimed to characterize the chemotactic movement of CDK5 to determine its viability as a nanomotor for a potential drug delivery system.

Therefore, we sought to test the hypothesis that CDK5 is

a thermodynamically viable mechanism for chemotactic drug delivery in the presence of tau protein buildups in patients with neuronal disease. In creating an in silico environment to test this idea, we also aimed to discover the relevant mathematical parameters involved in CDK5's chemotaxis so that the enzyme could be utilized by future researchers to design drug delivery mechanisms.

The use of nanomotors in drug delivery is often powered by enzyme chemotaxis, and the strength of the nanomotor is often determined by the kinetics of the reaction between the enzyme and its substrate. Fast reactions tend to have stronger affinities between the enzyme and its substrate, which results in a stronger nanomotor. As such, analyzing the kinetics of enzymes as a whole is critical to understanding how they behave as nanomotors to power the movement of chemotactic vesicles.

The current Michaelis-Menten model of enzyme kinetics describes the chemotaxis of enzymes by using their substrate-binding affinity, catalytic turnover, and level of diffusion enhancement in order to quantify the movement of a given enzyme along a gradient based on certain constants (9). We designed computational simulations using equations from the Michaelis-Menten kinetics model. In order to determine CDK5's viability as a nanomotor, we used a derivation of the Michaelis-Menten equation that uses several parameters to calculate the net chemotactic velocity. We then compared this velocity to another simulation that quantified the displacement of CDK5 particles from Brownian motion. Brownian motion is the random movement of particles in a system, and it is the main force that acts against enzyme chemotaxis (8). As such, we wanted to test whether CDK5 diffused at a high enough velocity that it was able to outpace Brownian motion and travel down its enzyme substrate concentration gradient. To do this we created a simulation based on the standard equation of Brownian motion and then characterized the displacement of CDK5 by modeling Brownian motion as a Weiner process, which is a stochastic mathematical process that is continuous and uses Gaussian increments that are normally distributed, based on its diffusion coefficient in the presence of a tau protein gradient.

Overall, our models holistically characterized the chemotactic movement of the enzyme CDK5 and the counteracting forces against its chemotaxis along a tau protein gradient. We established in silico that CDK5 has the necessary thermodynamic characteristics and enzyme-substrate binding affinity to overcome alternative forces like Brownian motion, the random motion of particles in a fluid medium, and thus could be used for chemotactic drug delivery. Moreover, our model computed the characteristics of CDK5 that could be used in future drug delivery research, including its net chemotactic velocity and reaction rate so that future researchers can simulate and create systems that affix it to nanovehicles like liposomes and are able to accurately predict the movement of those nanovehicles along a CDK5 substrate concentration gradient.

RESULTS

In order to complete our in silico model of CDK5's kinetics, we first performed a literature review to analyze the various parameters of CDK5. For example, we determined the diffusivity of the enzyme in the absence of substrate, the fraction of free enzymes in the system, and the total chemotactic velocity of all enzymes if the system were to chemotax simultaneously. Utilizing these parameters, with the amount of substrate acting as the independent variable, we were able to calculate and visualize the constants of CDK5 transportation in Figures 1-6.

In **Figure 1**, through the use of the Michaelis-Menten equations, we graphed the rate of the phosphorylation of tau protein by CDK5 as the tau protein concentration (in micromolar) increased, using the average concentration ranges used in in vitro studies (6). This was 80 to 160 μM of tau protein. The rate of phosphorylation graph illustrated that the reaction velocity increased proportionally with the concentration of tau protein. Next, we determined the fraction of free enzymes using the concentration of tau protein as the independent variable again, as demonstrated in (**Figure 2**). With this graph, we observed an inverse relationship between the two variables, showing lower amounts of unused or free enzymes as the tau concentration increased. As a result of the decrease in unphosphorylated tau, as well as fewer unbound enzymes in the system, chemotaxis slows down gradually. By utilizing these parameters, we were able to plot the net chemotactic velocity of the enzyme using substrate concentration as an independent variable. We discovered that as substrate concentration increases, the net chemotactic velocity decreases, approaching 3.287E5 nm/s as the tau concentration reaches the average maximum of 160 μM and a velocity of 4.247E5 nm/s for free enzymes at the same concentration, illustrated in **Figure 4**. Using all

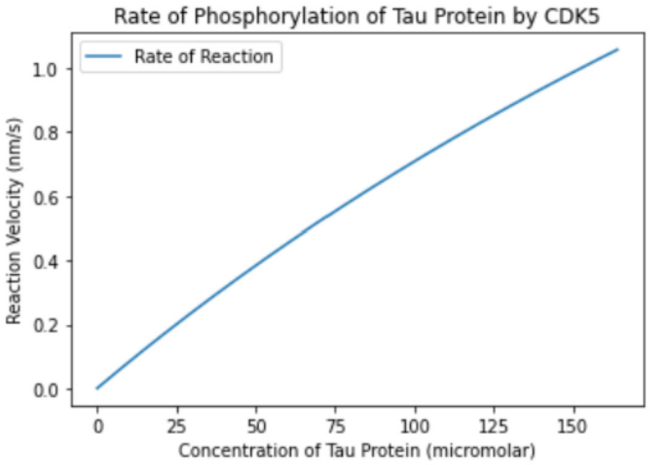

Figure 1: Reaction rate of CDK5 to phosphorylate tau protein. As the reaction progresses, the concentration of unphosphorylated tau decreases because CDK5 is phosphorylating available tau protein. The rate of reaction decreases, which slows chemotaxis. Plotted the concentration of tau protein in micromolar against reaction velocity in nm/s.

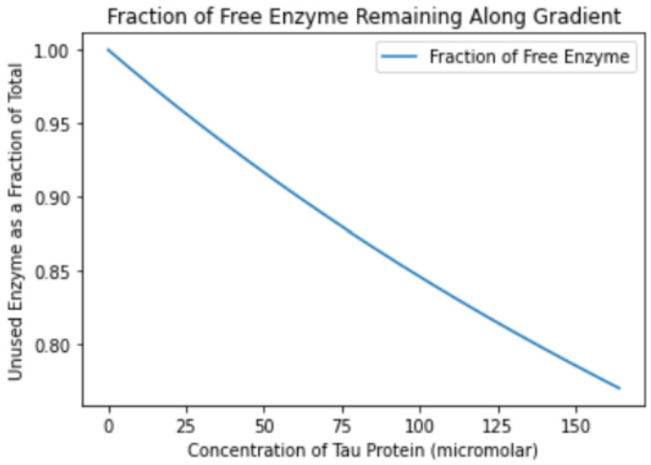

Figure 2: Fraction of free enzyme remaining as substrate increases. Used derived Michaelis-Menten equations to plot the concentration of tau protein in micromolar against the fraction of enzyme available.

of this, we developed a table (**Table 1**) to portray our data. Additionally, using the data displayed in Table 1, we created a particle diffusion model comparing mean Brownian displacement based on random motion with the chemotactic velocities of CDK5 to see if the force or Brownian motion could be overcome (**Figure 5**). We simulated 100 CDK5 particles in a particle diffusion model using the parameters we had generated from Michaelis-Menten kinetics. We ran this model over 1000 seconds and created a visualization of the trajectory of each simulated particle as well as the mean squared displacement of the particles in the system. Because the diffusion coefficient is not perfectly uniform, we accounted for error and fitted a diffusion coefficient of 1.203E7, as calculated by the Stokes-Einstein Equation, into our model. Finally, we calculated the mean squared displacement based

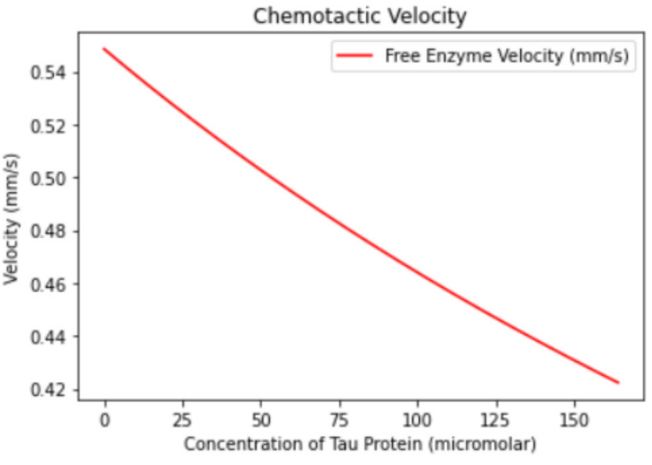

Figure 3: Chemotactic velocity of free enzymes. The chemotactic velocity of free enzymes assumes all enzymes in a system chemotax which is then multiplied with the fraction of free enzymes to calculate the net chemotactic velocity. The graph shows a decrease in chemotactic velocity of free enzymes as tau concentrations (μM) increase.

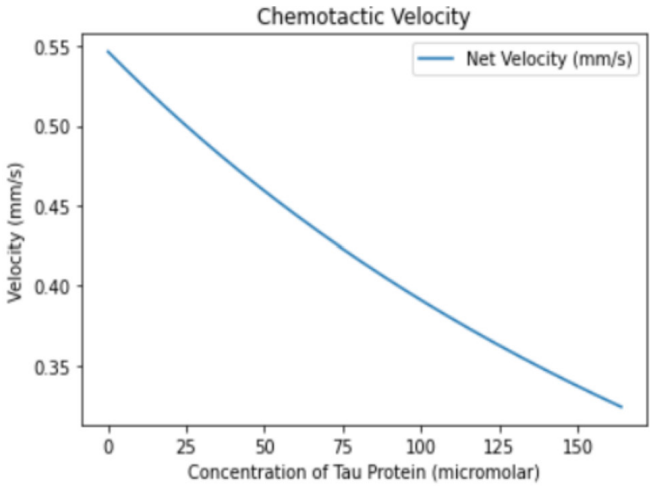

Figure 4: Net chemotactic velocity. The net velocity multiplies the free enzyme velocity by the fraction of free enzymes available in the system. Thus, as the concentration of tau in μM increases, the net velocity in mm/s decreases.

on the diffusion of CDK5, and was determined to be 8.501E3 nm. When comparing the mean displacement with the theoretical diffusion coefficient of CDK5, the diffusion is 1416 times as strong as the Brownian displacement; thus CDK5 particles in a chemotactic system would predictably be able to diffuse along the concentration gradient of tau protein.

DISCUSSION

Based on our enzyme models, we were able to characterize the enzyme kinetics of CDK5, as well as explore its viability as a potential nanomotor for targeted drug delivery. The results of our analysis conclude that the chemotactic properties of the enzyme CDK5 allow its velocity to outpace Brownian diffusion by a factor of 1416. Moreover, the characterization of CDK5 provides the basis for further research on this enzyme to explore its interactions with its substrate. Based on our

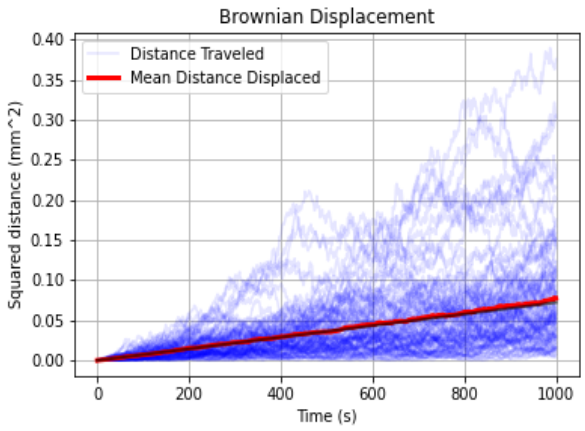

Figure 5: Distance traveled by particles and average distance displaced. The Wiener process and Stokes-Einstein equation were used in tandem to simulate particle movement depicted in **Figure 3,** and it shows that CDK5 can theoretically overcome Brownian Displacement.

Substrate Concentration (μM))	Net Velocity (mm/s)	Reaction Rate (nm/s)	Fraction of Free Enzyme	Free Enzyme Velocity (mm/s)
80	0.416	0.585	0.873	0.479
85	0.41	0.617	0.866	0.475
90	0.403	0.648	0.859	0.471
95	0.397	0.679	0.852	0.468
100	0.391	0.709	0.846	0.464
105	0.385	0.739	0.839	0.46
110	0.379	0.768	0.833	0.457
115	0.374	0.797	0.827	0.453
120	0.368	0.825	0.821	0.45
125	0.363	0.853	0.815	0.447
130	0.357	0.881	0.809	0.443
135	0.352	0.908	0.803	0.44
140	0.347	0.935	0.797	0.437
145	0.342	0.961	0.791	0.434
150	0.337	0.987	0.785	0.431
155	0.332	1.013	0.78	0.428
160	0.328	1.038	0.774	0.425

Table 1: Simulated data on chemotactic movement in relation to substrate concentrations of tau protein kinase. Used fundamental chemotaxis formulas to calculate changes in movement as substrate concentration increased. Includes net and free enzyme velocity in nm/s, reaction rate, and the fraction of free enzymes as graphed in the other figures.

results, we can confirm that CDK5 is a viable chemotactic nanomotor that can outpace Brownian diffusion and could be incorporated into a targeted drug delivery system, based on its interactions with its substrate the tau protein.

One missing characteristic in this model is the lack of pharmacokinetic parameters. There was little literature on CDK5 and its properties, which limited the models that could be used to characterize it, as many of them required parameters and constants that had not been experimentally verified. In addition, there were not many existing models that helped characterize enzyme chemotaxis, as the phenomenon has been relatively unexplored and has only recently been considered as a potential nanomotor. As such, it was difficult to provide data on all of the chemotactic aspects of CDK5. Another factor that could create disparities between the model and in vivo experiments with CDK5 is that it requires activator proteins to bind to allosteric sites on the enzyme for the substrate to bind (2). If the activator proteins are not bound to the enzyme, the substrate will not bind to the active site, and chemotaxis will not occur. As a result, different environments may expose the enzyme to different proteins which can change its binding affinity with the substrate. While in silico and in vitro environments can control for this, it will vary when this system is tested in the human body. One way that a drug delivery system could resolve this issue is via the integration of cyclin-CDK5 complexes into the drug delivery vesicles.

Since we determined that CDK5 was able to overcome Brownian diffusion, a chemotactic drug delivery system would be able to travel towards the therapeutic target. Once past the blood-brain barrier, the vesicle could release therapeutic treatments to mitigate or possibly cure the effects of diseases like Alzheimer's and Parkinson's. Not only does this research allow for the characterization of CDK5, but it also allows the experiment to be applied to other concepts, furthering the field of controlled drug delivery systems.

Our team didn't have access to resources this year and couldn't experiment in vitro, so we decided to model and design this project in silico. As such, future considerations for this project include in vitro, and eventually in vivo testing of our chemotaxis-based controlled drug delivery system. These experiments could help us verify the results of our simulations by allowing us to control for more factors and test our system under various conditions. For instance, we could verify the chemotactic properties of this enzyme and its interactions with tau proteins by experimenting with CDK5 attached to a liposome. This would allow for more specific drug design by creating data on what enzyme levels are needed to consistently overcome Brownian motion. In terms of therapeutic development, this approach could allow for the creation of a nanoparticle-based system that uses PEGylated and glucose-coated liposomes to cross the blood-brain barrier while carrying a drug that can eliminate a therapeutic target (ex. amyloid-beta oligomers). Such a system could function by using active CDK5 to phosphorylate tau proteins as CDK5 traveled across the concentration gradient. As CDK5 is able to phosphorylate tau proteins regardless of their structure, the vesicle would be able to use the uniquely high concentration of substrates created by Alzheimer's disease in addition to the healthy baseline. Further in vivo research would need to be performed to understand the nature of liposomal delivery based on the CDK5-tau protein catalysis-based positive chemotaxis and the phospholipid-based interactions occurring in the negative direction. While some health concerns with a CDK5-driven system exist, exogenous CDK5 should not significantly affect Alzheimer's disease patients in the same way that endogenous activated CDK5 enzymes do.

MATERIALS AND METHODS

Michaelis-Menten

In order to assess the viability of CDK5 as a nanomotor for drug delivery, we used a modified version of the Michaelis Menten equation to plot the net chemotactic velocity over an interval of tau concentration. We used this equation (10):

$$U_{chem}^{net} = f_E u_{chem} = D_E^0 \frac{K_m}{K_m + [S]} \frac{\partial_x [S]}{K_d + [S]}$$

In order to derive this equation, we started with an existing equation in the literature (10), shown here:

$$U^{E}_{chem} = D^{0}_{E} \frac{\partial_x [S]}{K_d + [S]}$$

This equation quantifies the chemotactic velocity if all free enzymes in the system were to chemotax. We multiplied this value by the rate of reaction, which quantifies the fraction of free enzymes that remain as substrate concentration increases. Ultimately, this resulted in the final equation for the net chemotactic velocity of the enzyme (U_chem^net). In this equation, D_E^0 represents the diffusivity of the enzyme in the absence of substrate. The variable K_m represents the Michaelis constant, which is the concentration of the substrate for a specific enzyme when the reaction rate for that enzyme is half of its maximum reaction rate. The term [S] acts as the independent variable in this model, and it represents substrate concentration. The term ∂_x [S] represents the concentration gradient of the substrate molecules, which can be calculated by dividing substrate concentration by the average capillary length in the body. Finally, the term K_d is the dissociation constant of the enzyme-substrate complex. By determining the values of these parameters for our specific system, we were able to plot the net chemotactic velocity at different substrate concentrations.

The first term in this equation represents the diffusivity of the enzyme in the absence of substrate. In order to calculate the diffusivity of the enzyme, we used the Stokes-Einstein equation, shown here (11):

$$D = \frac{k_b T}{6\pi\eta r}$$

In this equation, which solves for the enzyme's diffusivity (D), k_b represents the Boltzmann constant, a widely known physical constant that equals 1.380649 x 10-23 joule per kelvin. *T* represents the temperature of the system, in kelvin. In addition, *η* is the viscosity of the medium, and *r* is the radius of the particle itself (in our case, the radius of CDK5). To evaluate this equation, we consulted with a professor who had published several papers about chemotaxis regarding which parameters to use for *in-silico* simulation. Ultimately, we used a temperature of 296 kelvin, a radius of 4.5 nm, and a viscosity of .004, which gave us a diffusivity of 1.20448474 x 10^{-11} meters squared per second. When converted to nanometers squared per second, the diffusivity of CDK5 was calculated as 12,044,847. For the following terms in the equation, we needed the value of the Michaelis constant of CDK5, which we found in the existing literature to be 549 micromolar (12). Along with the Michaelis constant, we consulted past experimental literature and found the dissociation constant, which happened to be the same as the Michaelis constant in the case of CDK5, 549 micromolar. We used substrate concentration as our independent variable, and for the concentration gradient, we divided the average tau concentration in the brain of 874.5 ng/mg by the average capillary length in the human body to get 50 micromolar per nanometer (13). While this only makes chemotaxis viable when close to the BBB, the flow of blood will bring our drug in close proximity to the BBB, where chemotaxis can help it move along the gradient. This allowed us to fill in all the necessary constants for our equation and create a graph that plots the relationship between substrate concentration and net chemotactic velocity for CDK5.

Brownian Motion:

Brownian motion is the randomized movement of particles in a system and is one of the key factors in determining whether an enzyme can chemotax down a gradient or not, as it can create fluctuations that are stronger than the diffusion of a particle down its concentration gradient, preventing that particle from moving quickly in the correct direction (14). The mean squared displacement of particles can be calculated with the equation $< |\vec{r}(t) - \vec{r}(t+\tau)|^2 >= 2DN\tau >$ where $\vec{r}(t)$ is the position of the particle at the time defined by *t*, *N* is the number of dimensions being modeled, *D* is the diffusion coefficient of the particle, described by Michaelis-Menten kinetics as diffusivity, and τ is the interval of time being modeled (15). This phenomenon is oftentimes modeled by using a Wiener process. This process can be described with the equation W(*t*+*τ*)-W(*t*) ~ *N*(*0*,*τ*) where *τ* is the instant of time, *t* is the time delay, and *N* is a random, normally distributed variable with a variance equivalent to *t* and mean equal to zero (15). Ultimately, by substituting *τ* for *2DNτ* in *W*(*t*+*τ*)-*W*(*t*) ~ *N*(*0*,*2DNτ*) . Brownian motion can be described by a Wiener process where the displacement of particles at a given instant will be normally distributed. By utilizing this process alongside the coefficient D that we had already calculated in our original characterization of CDK5, we were able to design a simulation of CDK5 particles to find out the mean squared displacement.

The simulation was written in Python with the physics library PyBroMo. Using CDK5's parameters, 100 independent trajectories were introduced into the simulation and randomly sampled over 1000 time steps that each represented one second. By comparing the expected distance from the origin for each point with the actual distance from each point, the mean displacement per particle per second was found and then averaged across the simulation to the extent of the displacement induced by Brownian motion. Using the mean squared displacement equation, the theoretical displacement of each particle was also calculated and plotted.

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REFERENCES

1. Shupp, Alison, et al. "Biological Functions of CDK5 and Potential CDK5 Targeted Clinical Treatments." *Oncotarget*, Impact Journals LLC, 7 Mar. 2017, www.ncbi.nlm.nih.gov/pmc/articles/PMC5370047/.
2. Dhavan, Rani, and Li-Huei Tsai. "A Decade of CDK5."

Nature Reviews Molecular Cell Biology, Nature Publishing Group, 1 Oct. 2001, www.nature.com/articles/35096019.
3. Murphy, M Paul, and Harry LeVine. "Alzheimer's Disease and the Amyloid-Beta Peptide." *Journal of Alzheimer's Disease : JAD*, U.S. National Library of Medicine, 2010, www.ncbi.nlm.nih.gov/pmc/articles/PMC2813509/.
4. Mandell, James W., and Gary A. Banker. "A Spatial Gradient of Tau Protein Phosphorylation in Nascent Axons." *Journal of Neuroscience*, Society for Neuroscience, 15 Sept. 1996, www.jneurosci.org/content/16/18/5727.short.
5. Agudo-Canalejo, Jaime, et al. "Phoresis and Enhanced Diffusion Compete in Enzyme Chemotaxis." *ACS Publications*, ACS Publications, 19 Mar. 2018, pubs.acs.org/doi/10.1021/acs.nanolett.8b00717.
6. Hashiguchi, Mitsuko, et al. "Truncation of CDK5 Activator P35 Induces Intensive Phosphorylation of SER202/Thr205 of Human Tau." *Journal of Biological Chemistry*, Elsevier, 10 Sept. 2002, www.sciencedirect.com/science/article/pii/S0021925819718080.
7. Somasundar, Ambika, et al. "Positive and Negative Chemotaxis of Enzyme-Coated Liposome Motors." *Nature Nanotechnology*, Nature Publishing Group, 18 Nov. 2019, www.nature.com/articles/s41565-019-0578-8.
8. Newby, Jay M., et al. "Technological Strategies to Estimate and Control Diffusive Passage Times through the Mucus Barrier in Mucosal Drug Delivery." *Advanced Drug Delivery Reviews*, Elsevier, 12 Dec. 2017, www.sciencedirect.com/science/article/pii/S0169409X17303058.
9. Ross, Callum, et al. "Liposome Delivery Systems for the Treatment of Alzheimer's Disease." *International Journal of Nanomedicine*, Dove Medical Press, 12 Dec. 2018, www.ncbi.nlm.nih.gov/pmc/articles/PMC6296687/#:~:text=Targeted%20nanoliposomes%20represent%20a%20viable,BBB)%20and%20into%20brain%20cells.
10. Mohajerani, Farzad, et al. "A Theory of Enzyme Chemotaxis: From Experiments to Modeling." *Penn State*, American Chemical Society, 30 Oct. 2018, pennstate.pure.elsevier.com/en/publications/a-theory-of-enzyme-chemotaxis-from-experiments-to-modeling.
11. Mortensen, Kim I., et al. "Confined Brownian Motion Tracked with Motion Blur: Estimating Diffusion Coefficient and Size of Confining Space." *Frontiers*, Frontiers, 13 Jan. 2021, www.frontiersin.org/articles/10.3389/fphy.2020.583202/full.
12. Peterson, Dylan W, et al. "No Difference in Kinetics of Tau or Histone Phosphorylation by CDK5/P25 versus Cdk5/p35 in Vitro." *Proceedings of the National Academy of Sciences of the United States of America*, National Academy of Sciences, 16 Feb. 2010, www.ncbi.nlm.nih.gov/pmc/articles/PMC2840295/.
13. Han, Pengcheng, et al. "A Quantitative Analysis of Brain Soluble Tau and the Tau Secretion Factor." *Journal of Neuropathology and Experimental Neurology*, Oxford University Press, 1 Jan. 2017, www.ncbi.nlm.nih.gov/pmc/articles/PMC5901078/.
14. Tweedy, Luke, et al. "Self-Generated Chemoattractant Gradients: Attractant Depletion Extends the Range and Robustness of Chemotaxis." *PLOS Biology*, Public Library of Science, 16 Mar. 2016, journals.plos.org/plosbiology/article?id=10.1371%2Fjournal.pbio.1002404.
15. Ingargiola, Antonino. "Tritemio/Pybromo: Version 0.7." *Zenodo*, 1 Nov. 2017, zenodo.org/record/1039986#.YNFNUqhKhPY.