Modelling Nanoparticle-Cell Interactions: A Kinetic Approach

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Abstract: This project addresses the effect of nanoparticle functionalisation on binding affinity to endothelial brain cells. This kind of characterization is fundamental to the optimization of drug delivery to the brain, allowing for treatment of neurodegenerative diseases. For this, we perform a proof of concept binding experiment on bEnd.3 cells with ApoE-functionalized and pristine PEG-PLA polymersomes, and propose a deterministic kinetic model to describe these systems.

Keywords: Biophysics, Binding kinetics.
SDGs: Health and well-being

I. INTRODUCTION

Nanoparticle-cell interactions are fundamental for living organisms. When a cell encounters a foreign object, a multitude of complex factors determine whether or not this object should be bound to the cell-membrane and/or internalised.

Understanding the factors which influence binding or internalization affinity is of great interest for the development of nanomedicines, specially when attempting to treat neurodegenerative diseases, given the blood-brain barrier's (BBB) specialised endothelial cells.

Polymersomes are vesicle-like structures composed of amphiphilic block copolymers, that is, chain molecules made up of hydrophilic and hydrophobic blocks that typically self-assemble into a spherical bi-layer membrane in aqueous media[1]. Since they can encapsulate substances in their aqueous core or within their membrane, polymersomes are used in drug-delivery to get targeted cells to inadvertently internalise the active agent inside the "inert" shell. Given their potential medical applications, polymersomes are the nanoparticles (NPs) of focus in this work.

Instead of going into the minutiae of each NP-cell interaction, it is possible to study the broad kinetics of NP binding/absorption in controlled cell populations to determine how different NP parameters affect the yield of bonded/absorbed particles. This still requires a certain understanding of the cellular mechanisms for binding and absorption, but exempts us from having to measure them directly.

Our work focuses on how a NP's binding/absorption affinity is affected by its functionalization: a functionalized polymersome has attached molecules or chemical groups in its surface, while a pristine one does not. Functionalizing NPs allows for great control of their properties, such as size, stability and solvent compatibility among others, and a great effort is being currently made to engineer more sophisticated NP surfaces destined for nanomedicine. [2][3]

Theoretically, adding an active component (ligand) to the NP should increase binding/uptake, but that is highly dependent on the precise molar ratio of ligands, and it is best to study a range of formulations in parallel. Since our experiments are limited to the use of a single functionalised formulation, they should be understood as a proof of concept for the methodology, rather than as a basis for drawing firm conclusions on NP behaviour.

Our model will describe receptor-mediated interactions, which should only take place when the particles have ligands capable of binding to the receptors on the cell surface. In practice, both pristine and functionalised NPs are bound to the cell also via non-specific binding, but our model should be able to identify the contribution of receptor binding, if there is any.

II. METHODS

A. Experimental overview

Our experiment aims to incubate cells with both pristine and functionalized fluorescent NP solutions and to gather the supernatant liquid at different time points. This supernatant is the extracellular medium, which excludes the cells and any nanoparticles that have been adsorbed onto or internalized by them. Therefore, if any particles bind to or enter the cells, the solution which we take out should have a decreased NP concentration.

We infer the NP concentration in each sample by measuring its fluorescence intensity and referencing a standard curve generated prior to each binding experiment.

B. Cell culturing

For the experiment, we culture immortalized mouse brain endothelial cells from the bEnd.3 cell line. These

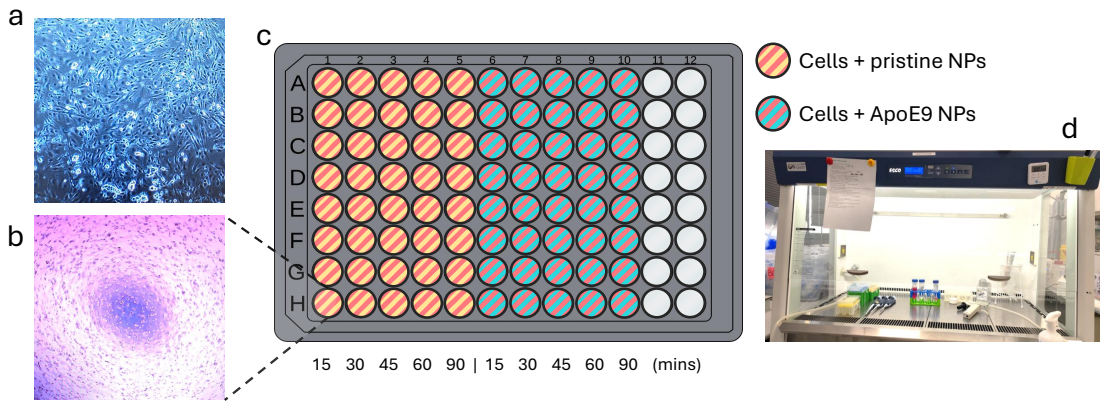

FIG. 1: **(a)** Confluent bEnd.3 cells. **(b)** bEnd.3 cells immediately after seeding in coated well. **(c)** Diagram of the cell and NP solution distribution in the 96-well plate. **(d)** Laminar flow hood used for the experiments.

cells originate from the brain tissue of a mouse with endothelioma and are further immortalised through viral treatment. Research has shown that bEnd.3 cells display the same comparative barrier characteristics as their primary (non-immortalised) counterparts, and they are commonly used in neurovascular research[4].

The cells are cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% pen-strep, kept in a humidified incubator at 37 $°C$ and 5% CO_2. The medium is tinted with Phenol-red and is changed every three days, when its red colour fades to indicate nutrient depletion.

BEnd.3 cells are adherent and form a monolayer on the surface of the culture flasks. It generally takes a few days for them to achieve 100% confluence, meaning total coverage of the flask surface. When fully confluent, the cells appear as in Figure 1a and need to be sub-cultured (split) to prevent overgrowth. We can only open the culture flask inside a laminar flow hood that filters the air to prevent contamination, Figure 1d.

Cell-splitting: the splitting of a cell culture is the process by which we dilute the cells into fresh medium. When splitting adherent cells, it is necessary to first detach the cells from their container using a dissociation agent such as Trypsin.

Our protocol is to take out and discard the old medium, rinse the cells with a saline solution (PBS), add the Trypsin and then leave the culture in the incubator for around 5 minutes. After incubation we check in the microscope if the cells have become suspended in the fluid, appearing globular. If they are suspended, we transfer the solution into a conical tube and rinse again the flask with PBS; if not, either gentle slapping of the flask or more time at the incubator might be needed.

While it facilitates detachment, Trypsin may also damage the cellular surface, compromising its receptors and adhesion molecules. This is why we dilute the Trypsin in fresh medium immediately after achieving suspension. The FBS in the medium contains agents which neutralise the Trypsin, minimising the harm.

The tube containing the suspended cells is centrifuged to sediment the cells. When sedimented, the cells form a pellet at the bottom of the tube, and the medium containing Trypsin can be discarded by decantation. We then add new medium, and use a 1 ml pipette to gently break apart the pellet and re-suspend the cells. We should aim for the suspension to be homogenous.

From this suspension we can take 20 μl for mixture with Trypan blue dye, which will allow us to perform a manual cell count in a Neubauer chamber. From the cell density thus estimated, we then take as many cells as we need from the suspended solution and place them in the culture flask, providing as much medium as is necessary for full coverage of the surface. The remaining suspended cells can be discarded, cultured in a new flask or seeded in a well-plate, Figure 1b.

Adherent cells such as bEnd.3 experience an inherent stress when suspended, and so the entire splitting process needs to be as fast as possible while preventing any contamination.

Cell-seeding: The binding experiment takes place in a 96-well plate, Figure 1c, with 0.33 cm^2 of surface per well. Before seeding we coat the wells with a solution of Collagen IV AT 0.1 mg/ml in PBS to facilitate attachment. When seeding, we want to place around 16500 cells per well, so we take the corresponding volume from the solution of suspended cells. The cells are then left to grow in the incubator for 3 days.

C. Binding procedure

The NPs used in the experiment are polyethyleneglycol–polylactic acid (PEG-PLA) polyersomes marked

with Sulfo-Cyanine7 (Cy7) for fluorescence. We have both pristine and ApoE9-functionalized NP solutions; ApoE being a peptide sequence derived from Apolipoprotein E, a protein known to facilitate access across the BBB[5], that has been coated on the NPs at 9% molar fraction.

To get the standard curve we measure the fluorescence of a range of NP solutions with known concentrations on an opaque 96-well plate. The solutions start at twice the working concentration and are diluted 1:2 in each column, the last one having just solvent instead.

For the experiment itself, we first have to aspirate the medium from the wells were the cells are. We then administer 100 μl of working-concentration solution per well, using an eight-channel multipipette. Cells in columns 1–5 receive the pristine NP solution, while those in columns 6–10 get the ApoE9-functionalized NPs (Figure 1c).

At each time point we need to take the plate out of the incubator and transfer the solution from the corresponding columns into a new opaque 96-well plate (e.g. at 45 minutes we get the solution from columns 3 and 8). After removing the liquid from a well, we provide the cells with PBS to prevent them from dying before the counting.

Once we have transferred the solution from each column, we perform a fluorescence reading on the opaque plate containing the solutions and proceed with the cell count. For this, we prepare a Hoechst 33342 1 to 1000 solution in PBS and incubate the cells in each well with 30 μl of this solution for 20 minutes. Hoechst 33342 is a fluorescent dye that stains DNA, and allows us to estimate cell count from a florescence reading.

We perform all the fluorescence readings on a TECAN Spark microplate reader using methods specifically configured for the measuring of Cy7 and Hoechst 33342 fluorescence. The method for Cy7 provides the fluorescence intensity in arbitrary units, while the Hoechst 33342 method is calibrated to directly provide the cell count.

III. RESULTS

The binding experiment was performed twice, using solutions of $5 \cdot 10^{10}$ and $7 \cdot 10^{10}$ NPs/ml. We want to work not with concentrations, but with concentrations per cell, to account for each cell's contribution to the binding. We therefore divide the NP concentration (estimated from fluorescence) in each well by its corresponding cell count, obtaining the free particle concentrations per cell, ρ_{P}.

Now, we should be able to calculate the initial particle concentration per cell, ρ_{P0}, in each condition (Pristine/ApoE9) using the known concentration of the respective initial solution (either $5 \cdot 10^{10}$ or $7 \cdot 10^{10}$ NPs/ml) and the average cell count for all those wells. But as we can see in Table I, the standard deviation in cell population is very high, leading to large uncertainties in our ρ_{P0} values. This probably means that the cell solution with which we seeded the wells was not properly homogenised.

Even if we disregarded this, some of the experimental ρ_{P} values for time t>0 are significantly larger than the previously calculated ρ_{P0}. This is why in Figure 2 a and b we see normalised concentrations greater than one. This obviously has no physical meaning, since it would imply that the cells are somehow generating more particles. The cause for this could be inhomogeneous distribution of the dissolved NPs, or improper calibration from the standard curves, Figure 2 c and d. We also suggest that leftover medium containing Phenol red (which was aspirated, maybe improperly, before introducing the NP solutions) could have inflated the fluorescence readings, simulating higher NP concentrations.

TABLE I: Average cell count (N_c) and nanoparticle concentration per cell (ρ_{P0}) for experiments using i) $5 \cdot 10^{10}$ and ii) $7 \cdot 10^{10}$ NP/ml solutions. Uncertainties represent standard deviation for N_c and propagated error for ρ_{P0}.

	N_c (10^4 cells)	ρ_{P0} (10^6 NP/cell·ml)
i) Pristine	3.8 ± 0.6	1.3 ± 0.2
ApoE9	3.9 ± 0.1	1.3 ± 0.5
ii) Pristine	3.1 ± 0.7	2.3 ± 0.5
ApoE9	3.2 ± 0.1	2.2 ± 0.8

We also have to discuss the size of the error bars of the data in Figure 2 a and b. These uncertainties only account for the standard deviation among wells, because the μl imprecision introduced by the pipettes is negligible before the human error. Since we are dividing each well's concentration by its own cell count, irregular cell-seeding does not come into play here. Inhomogeneity of the NP solution is again a likely culprit, and the improper aspiration of the old medium which we just proposed could also be causing these highly deviated readings independent of the actual NP concentration.

Another source of error is improper pipetting, such as overly tilting the pipette or aspirating air. These mistakes can cause irregular volume uptake/release and lead to the formation of bubbles that disturb fluorescence readings. Additionally, the splittings performed on the culture may have been too slow, leading to Trypsin over-exposure and damage to the cells' receptors.

With all, the experiments have failed at showing greater binding of the functionalised particles via receptor-mediated pathways. We could attribute this to the molar ratio with which the NPs were functionalised, but it is clear the data is unreliable and further experimentation would be necessary to make any such statements.

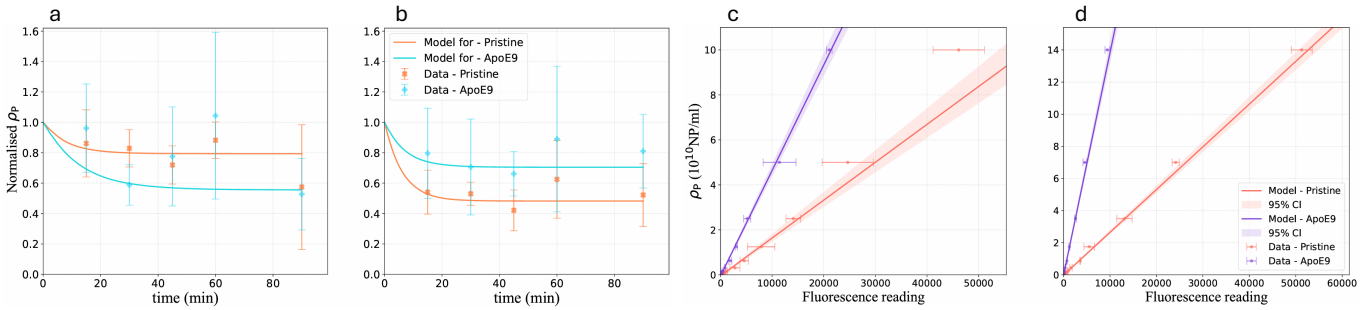

FIG. 2: Free particle concentration per cell (ρ_{P}) normalised with respect to the initial particle concentration per cell (ρ_{P0}) for the experiments on **(a)** $5 \cdot 10^{10}$ and **(b)** $7 \cdot 10^{10}$ NP/ml solutions. The plot features the average ρ_{P} at each time with its standard deviation as the error bar, and the Model from Equation 2 fitted to the data.
(c) and **(d)** are their respective standard curves, featuring the data points, the regressed curve and its 95% confidence interval (CI). The fluorescence reading is in arbitrary units.

IV. MODELLING

Our model is fully deterministic, since to consider stochastic effects would require a magnitude of data far exceeding what we have, and we want to avoid overfitting. Thus, we assume that free particles (P) and free receptors on the cell surface (R) associate to form particle-receptor complexes (PR_ν) in the way of a reversible chemical reaction:

$$\mathrm{P} \; + \; \nu R \; \underset{k_{\mathrm{off}}}{\overset{k_{\mathrm{on}}}{\rightleftharpoons}} \; PR_\nu$$

We are indicating here that particles and receptors can only be either free and available for binding or already locked in complex form, which can only disassociate. The association and dissociation rates, k_{on} and k_{off}, provide the tendency for the reaction to go in either direction.

The valence for the receptors, ν, contemplates that a single particle might need to be intercepted by multiple receptors, or that a single receptor could bind several particles, but let us emphasise that this two-state system is a very broad approximation that makes sense only when studying the tendencies of the process for an entire cell population.

Now, we can formulate the differential equations for the kinetics of the reaction:

$$\begin{aligned} \frac{\mathrm{d}\rho_{\mathrm{P}}}{\mathrm{dt}} &= -k_{\mathrm{on}} \cdot \rho_{\mathrm{P}} \rho_{\mathrm{R}}^{\nu} + k_{\mathrm{off}} \rho_{\mathrm{PR}\nu} \\ \frac{\mathrm{d}\rho_{\mathrm{R}}}{\mathrm{dt}} &= -\nu \cdot k_{\mathrm{on}} \cdot \rho_{\mathrm{P}} \rho_{\mathrm{R}}^{\nu} + \nu \cdot k_{\mathrm{off}} \rho_{\mathrm{PR}\nu} \\ \frac{\mathrm{d}\rho_{\mathrm{PR}\nu}}{\mathrm{dt}} &= +k_{\mathrm{on}} \cdot \rho_{\mathrm{P}} \rho_{\mathrm{R}}^{\nu} - k_{\mathrm{off}} \rho_{\mathrm{PR}\nu} \end{aligned} \tag{1}$$

This is a fairly simple system of non-linear, non-partial differential equations, where each ρ_{i} refers to the concentration of i. The three equations are not independent, though, and we can model the entire system with a single simplified ODE:

$$\begin{aligned} \frac{\mathrm{d}\rho_{\mathrm{P}}}{\mathrm{dt}} = &-k_{\mathrm{on}} \cdot \rho_{\mathrm{P}} (\rho_{\mathrm{R\ total}} - \nu(\rho_{Ptotal} - \rho_{\mathrm{P}}))^{\nu} \\ &+k_{\mathrm{off}} (\rho_{\mathrm{P\ total}} - \rho_{\mathrm{P}}) \end{aligned} \tag{2}$$

Where we have used that the complex concentration is equal to the concentration of non-free particles ($\rho_{Ptotal} - \rho_{\mathrm{P}}$), and that the receptor concentration is its initial concentration ($\rho_{\mathrm{R\ total}}$) minus ν times the complex concentration. Here ρ_{Ptotal} is our known ρ_{P0}, while the initial density of receptors per cell, $\rho_{\mathrm{R\ total}}$, is an unknown parameter which would need to be fit.

The fitted curves for our data appear in Figure 2 a and b, but we have already discussed the unreliability of the data and find little value on the values of the four parameters fitted from five dubious data points.

We can reduce the number of fitting parameters by normalising the ODE, defining a characteristic time $\tau = t \cdot k_{\mathrm{off}}$ with which we can integrate the expression and explore the behaviour of the system for different parameters. We impose $\rho_{Ptotal} = 1$ to work with the normalised particle concentration (x) and divide equation 2 by k_{off} to get a dimensionless expression:

$$\frac{\mathrm{d}x}{\mathrm{d}\tau} = -\tilde{K} \cdot x(\alpha - \nu(1-x))^{\nu} + (1-x) \tag{3}$$

This leaves us with three parameters: $\tilde{K} = k_{\mathrm{on}}/k_{\mathrm{off}}$, $\alpha = \rho_{\mathrm{R\ total}}/\rho_{\mathrm{R\ total}}$ and ν. In practice, when integrating this expression, the value of α seems to have the least effect on the overall behaviour. We thus fix its value to $\alpha = 0.25$, which appears reasonable from other NP-cell binding fittings performed in the group. These previous fittings also suggest that nu should be somewhere between one and two receptors per particle for NPs of this size, so we integrate using ν =1, 1.5, 2. We note that non-integer values for ν can either represent the statistical average over the entire population or be interpreted as reflecting partial contributions from individual receptors to the binding of a NP, but this is not something we can in any way interpret from our data.

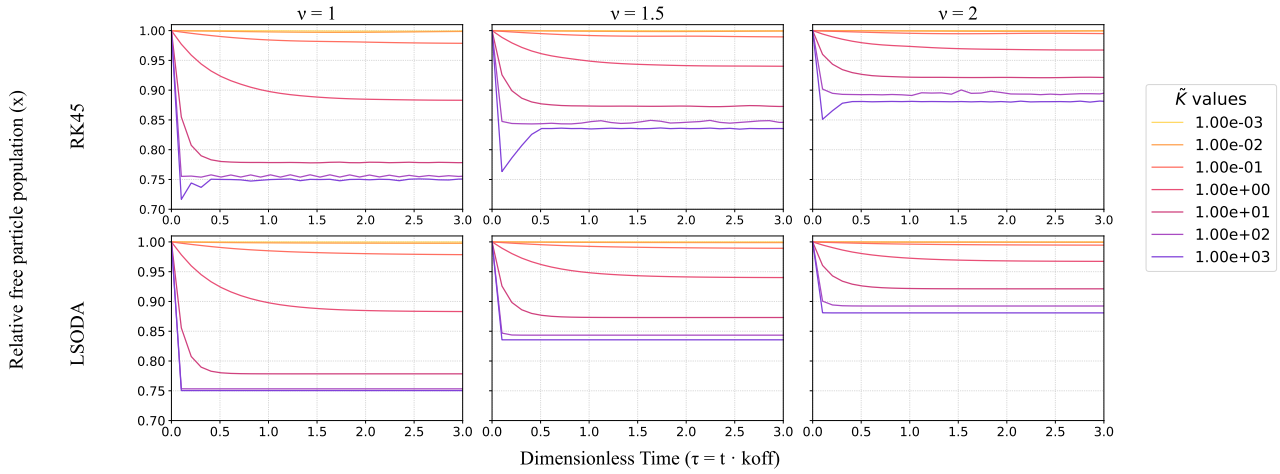

FIG. 3: Solutions for Equation 3 with fixed $\alpha = 0.25$ integrated using 4th order Runge-Kutta (RK45) and the Livermore Solver for Ordinary Differential equations (LSODA).

Lastly, for each ν, we explore a range of $\tilde{K} = k_{on}/k_{off}$ across several orders of magnitude. This is the most important parameter, as it contains the balance between the rates of association and dissociation.

We integrate the dimensionless equation for these parameters using 4th order Runge-Kutta method and the Livermore Solver for Ordinary Differential equations (LSODA) as implemented by scipy.integrate's solve_ivp function, Figure 3. Since the LSODA solutions are much smoother, it seems that our expression is too stiff for an explicit integration method such as Runge-Kutta

From this analysis it is clear that higher valences and lower $\tilde{K}$ (higher dissociation rates) will limit the capacity for complex formation and thus prevent the free particle population from decreasing past a certain value. Nevertheless, an initial drop of free particles is present even at instances where k_{off} surpasses k_{on} ($\tilde{K} < 1$). After the monotonous descent, the system quickly stagnates.

Comparing the curves fitted to our data in Figure 2 a and b with the solutions in Figure 3, it seems our data best corresponds to a $\tilde{K}$ somewhere around 1 and 10, with association rate is at least the same as, or an order of magnitude higher, than the dissociation rate. We can also guess that the curves that plateau around 0.8 have a $\nu \approx 1$, while the curves stagnating at 0.6 would have $\nu < 1$, which hadn't been previously contemplated.

V. CONCLUSIONS

From this study we cannot conclude that the functionalisation of the PEG-PLA polymersomes with ApoE9 at 9% molar had any distinct impact on binding affinity with bEnd.3 cells. Our experimental data was clearly compromised by human error, and an experiment featuring more formulations would be better suited to find any meaningful conclusions. In any case, we were able to develop a deterministic kinetic binding model to study the behaviour of this system for different binding parameters.

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[1] LoPresti C, Lomas H, Massignani M, Smarta T, Battaglia G. Polymersomes: nature inspired nanometer sized compartments. J Mater Chem. 2009;19:3576-3590.

[2] Wieszczycka K, Staszak K, Woźniak-Budych MJ, Litowczenko J, Maciejewska BM, Jurga S. Surface functionalization – The way for advanced applications of smart materials. Coord Chem Reviews. 2021;436:213846.

[3] Shang L, Nienhaus K, Jiang X, et al. Nanoparticle interactions with live cells: Quantitative fluorescence microscopy of nanoparticle size effects. Beilstein J Nanotechnol. 2014;5:2388-2397. Published 2014 Dec 11.

[4] He F, Yin F, Peng J, Li KZ, Wu LW, Deng XL. Zhongguo Dang Dai Er Ke Za Zhi. 2010;12(6):474-478.

[5] Wagner S, Zensi A, Wien SL, et al. Uptake mechanism of ApoE-modified nanoparticles on brain capillary endothelial cells as a blood-brain barrier model. PLoS One. 2012;7(3):e32568.

Modelant Interaccions entre Cèl·lules i Nanopartícules: Un Plantejament Cinètic

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Resum: Aquest projecte aborda l'efecte que té funcionalitzar una nanopartícula sobre la seva afinitat d'interacció amb les cèl·lules endotelials del cervell. Aquest tipus de caracterització és fonamental per optimitzar l'administració de fàrmacs al cervell, facilitant el tractament de malalties neurodegeneratives. Com a prova de concepte, es duu a terme un experiment amb cèl·lules de la línia bEnd.3 per comparar l'afinitat d'unió de polimerosomes de PEG-PLA pristins i funcionalitzats amb ApoE9. Posteriorment, es planteja un model cinètic determinista per descriure aquest tipus de sistemes.
Paraules clau: Biofísica, Cinètica d'unió.
ODSs: Salut i benestar

1. Fi de la es desigualtats		10. Reducció de les desigualtats	
2. Fam zero		11. Ciutats i comunitats sostenibles	
3. Salut i benestar	X	12. Consum i producció responsables	
4. Educació de qualitat		13. Acció climàtica	
5. Igualtat de gènere		14. Vida submarina	
6. Aigua neta i sanejament		15. Vida terrestre	
7. Energia neta i sostenible		16. Pau, justícia i institucions sòlides	
8. Treball digne i creixement econòmic		17. Aliança pels objectius	
9. Indústria, innovació, infraestructures			

El tema d'aquest treball està relacionat amb l'objectiu de desenvolupament sostenible de Salut i benestar perquè aborda la recerca necessària per dissenyar fàrmacs capaços de penetrar la vasculatura del cervell. Caracteritzant les propietats que faciliten l'adsorció i absorció de nanopartícules per cèl·lules endotelials del cervell, facilitem l'enginyeria de nanopartícules que puguin administrar agents actius pel tractament de malalties neurodegeneratives.