

Relating Gold Nanostar Shape to Function

A Study on the Applicability of Nanostars for
Photothermal Therapy and Cellular Uptake

William Shakespeare Morton

A thesis presented for the degree of
Doctor of Philosophy

Department of Materials
Imperial College London
London, U.K.

August 23rd 2023

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Not enough people dedicate these things to themselves,
show some self love.
Ya did good kid.

Acknowledgements

The work presented within would not have been possible without the following people:

Dr. Stefano Angioletti-Uberti, who constantly presented me with challenges and new opportunities to become a better researcher.

Dr. Fang Xie, for giving me the guidance, motivation, and resources to succeed.

Dr. Caoimhe Joyce for being the best friend and research partner anyone can ask for.

Dr. Daniel Jones for reminding me how fun it can be to discuss scientific hypotheses at rapid speed.

Dr. Alessandra Pinna, for encouraging me to pursue new opportunities in research.

Dr. Robert Vácha, Lukáš Sukeník, and the rest of Vácha lab for their friendship and guidance.

The members of the Soft Nano and Xie group for being supportive friends and researchers.

Salomé Bodet-Lefèvre, Myles Kelly, and Suzy Hill, for surviving the end of the world with me, and being my greatest friends.

To every Dungeons and Dragons group that became not only an escape, but a larger extended family.

The members of the Outdoor Club for giving my life so much more value, and making me discontent with a sedentary life indoors.

Dave, for making me laugh so hard I cried and an incredible family / research community (?).

My parents for supporting me - without question - in every possible way .

My grandparents for their constant encouragement and interest.

All of my family for their support, encouragement, and politely nodding along while I talked about research.

Everyone who loved and supported me throughout the past 4 relentless years.

And finally, to the Department of Materials, for the granting me the scholarship that made it all happen.

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Abstract

The following thesis presents a study on gold nanostars, focusing on their geometric properties, characterisation, and applications for photothermal therapy. In this application, the nanostar geometry is crucial for optimising optical properties, specifically the localised surface plasmon resonance and absorption cross-section, which can result in the particle heating when exposed to light. If the nanostar can enter a tumour, it could thermally ablate the cancerous region.

However, a fundamental challenge in nanostar characterisation is accurately estimating geometric properties such as particle volume, surface area, and the number of tips. The errors in estimation using Transmission Electron Microscopy (TEM) are first investigated through an analytical investigation, for which a library of 6000 nanostars was created. A statistical analysis suggests that current methods overestimate nanostar volumes, creating discrepancies across studies. A novel method is developed to address this shortcoming by combining Ultraviolet-visible spectroscopy (UV-Vis), TEM images, and Discrete Dipole Approximation (DDA) to characterise nanostar geometry more precisely. Once the geometry is predicted, the absorption cross-section can then be estimated using DDA.

Understanding the interaction between nanostars and cell membranes is crucial to understanding their potential use as therapeutics. A molecular dynamics method was reparametrized to better represent the binding limitations of ligands and receptors. Nanostar endocytosis was then studied, assuming cellular uptake is driven by these ligand receptor bonds. By controlling the distribution of nanostar tips the uptake speed can not only be tuned, but wholly inhibited by specific cell types, revolutionising the potential for targeted cell delivery.

In conclusion, this thesis offers a unifying method for estimating nanostar geometry, and suggests optimal geometric factors for cell targeting. While

the benefits of gold nanostars as photothermal therapeutics are highlighted, I propose that the field instead capitalises on advancements in ligand and protein design for controlling cellular processes through nanoparticle surface interactions.

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List of Acronyms

AuNS Gold nanostars

CoM Centre of mass

DDA Discrete dipole approximation

DI Deionized

EPR Enhanced permeability and retention

LSPR Localised surface plasmon resonance

MD Molecular dynamics

OLS Ordinary least squares

PTT Photothermal therapy

Rh Hydrodynamic radius

RMSE Root Mean Square Error

TEM Transmission electron microscopy

UV-Vis Ultra Violet - Visible

WCA Weeks-Chandler Anderson

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1 Introduction

1.1 Summary of Thesis Structure

The thesis is broken up into sections that build on one another to show how nanoparticle design and synthesis (Chapters 2-3) can be complimented by modelling techniques (Chapters 4-5). A brief overview of nanostar application in photothermal therapy (PTT), and a summary of the physical concepts and techniques used in this thesis can be found in the following introduction. In the next chapter a library of 6000 nanostars is created and characterised. The relationship between geometric factors, and experimental measurements is studied using statistical methods. Although this is a large library, it is by no means comprehensive, and goes to show the variety of nanoparticle shapes that fall under the nanostar nomenclature. Next, in Chapter 3, readers can find an altered version of a published article. Here, a novel methodology for characterising experimentally synthesised nanostars is introduced. The primary outcome of this chapter is the prediction of shape, which can then be used in Molecular dynamics (MD) simulations. Chapter 4 reparameterises the way cellular uptake is modelled in MD, in response to an artefact discovered throughout this work. Finally, in Chapter 5, the cellular uptake of nanostars is compared to nanospheres in MD using the parameterisation introduced in Chapter 4. Once all the chapters are presented, a final conclusion will be drawn regarding the potential for using nanostars in PTT.

1.2 Nanoparticles and Light

Gold nanospheres exhibit noteworthy opto-electronic properties when their diameter ranges between 1-100 nanometers — a domain where the surface area to volume ratio exceeds 0.01 nm^{-1} . Within this range, surface effects dominate over interactions with other substances, gravitational effects are

minimised, and electromagnetic forces are more prominent [1] .

The small size of nanoparticles means that wavelengths of light create a continuous electric and magnetic field around the nanoparticle in the visible and near infrared spectra (350-1200 nm). As the wave passes over the nanoparticle, its electron cloud aligns with the electric field. This alignment produces a dipole which oscillates with the wavelength of the electric field, as seen in Figure 1.2.1. The nanoparticle's polarizability, α , describes how easy it is to form a dipole in a given medium, whether that be air, water, or any other surrounding matter. For a spherical nanoparticle, the polarizability is isotropic, and can be simplified to the relationship seen in Equation 1.2.1 [2].

$$\alpha = 4\pi\epsilon_o r^3 \frac{\epsilon_{\text{particle}} - \epsilon_{\text{medium}}}{\epsilon_{\text{particle}} + 2\epsilon_{\text{medium}}} \quad (1.2.1)$$

In the equation above, r is the nanosphere's radius, ϵ_o is the permittivity of free space, and ϵ is the permittivity of the nanoparticle or medium as indicated by the subscripts. The permittivity describes how light moves when it interacts with a material, capable of having a real and imaginary permittivity, related to the scattering and absorption of light respectively [3].

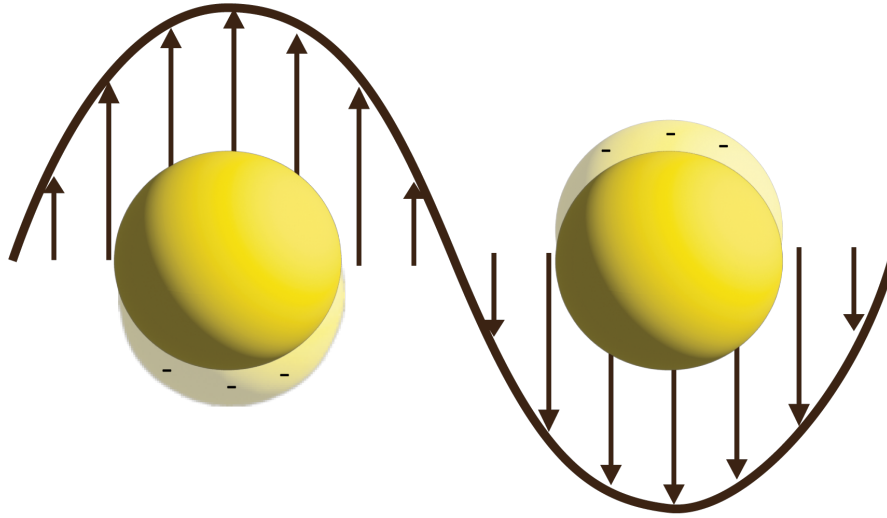


Figure 1.2.1: *The collective oscillation of valence electrons around a gold nanosphere when exposed to a wavelength of light corresponding to the Localised surface plasmon resonance (LSPR)*

Noble metal nanoparticles have a special relationship with light because,

at some wavelengths, the real part of the $\epsilon_{\text{particle}}$ is negative, meaning the denominator of Equation 1.2.1 has the potential to approach 0. The frequency of the wavelength of light that reaches this limit is known as the resonance frequency [4]. This resonance means that electrons are collectively excited, resulting in an enhanced electric field that is localised around the structure [5]. The enhanced electric field causes the nanoparticle to scatter or absorb more light than at other wavelengths. The effect is called a localised surface plasmon resonance (LSPR) and can be easily estimated for a sphere when the permittivity is well known [6]. For most bulk materials, the permittivity has been measured experimentally. However, this physical characteristic is easier to measure for bulk materials rather than nanoparticles, where the large quantity of surface atoms decreases the permittivity drastically. Therefore, several approximations have been created that can be used to apply the permittivity of bulk materials to their nano-sized counterparts [7].

A more intuitive physical analogy can be used to describe the relationships above. We have so far described how the incoming wavelength of light induces a dipole on the nanoparticle by pulling the electron cloud in alignment with the electric field. The Drude-Lorentz model relates this relationship to Hooke's law, describing the electron cloud as a mass attached to the atom's nucleus by a spring with the same resonance as the LSPR [8]. When the size of the nanoparticles increases, the restoring force is stronger, increasing the resonance and resulting in an LSPR that shifts towards higher wavelengths. A nanosphere is symmetric, meaning there is only one resonance, but changes in shape can result in multiple resonances, or modes, as the length of the spring will change based on the orientation between the particle and the wave.

While this is a rough description of the physics that makes gold nanoparticles so attractive, understanding the properties and applications in the following sections depends on the relationship between the shape of gold nanoparticles and their LSPR.

1.3 Nanoparticles in Medicine

1.3.1 Photothermal Therapy and Penetration Depth

The enhanced absorption of light at the LSPR is of considerable importance in photothermal therapy (PTT), where a laser irradiates tissue, heating it locally, until it dies from hyperthermia around 44°C [9]. This is a promising approach in cancer research, since a tumour can be specifically targeted, rather than exposing the entire body to treatment [10]. An added advantage is that tumours can be ablated when they are not necessarily visible or accessible, which may be limits in surgically removing them [11]. Using higher laser power can increase the temperature in the tumour, but this may be limited by the equipment, or the risk of damaging healthy tissue in the beam path. PTT dyes can be delivered to the tumour, which locally increase the efficacy of the treatment by locally absorbing more light [12]. But, the absorption decreases rapidly due to dye degradation [13].

Nanoparticles offer a potential treatment mechanism at their LSPR. Absorbed light decays through non-radiative pathways, meaning a photon of light is not released, causing the electron cloud to move rapidly and generate heat. However, nanoparticles are most useful when they can absorb incident light between 625 - 1200 nm [14]. In this regime, laser light can penetrate up to 5 mm into the body, compared to less than 1 mm outside of this range [15]. While many materials can be modified to absorb light in this window, gold is promising due to its biocompatibility and diverse synthesis methods [16]. However, the disadvantage with using gold nanoparticles, is ensuring that they are accumulated in the tumour and can be cleared from the body [17].

1.3.2 Nanoparticle Targeting

Nanoparticles are often mischaracterised as targeting tumours through the enhanced permeability and retention (EPR) effect, which describes retention of nanoparticles due to leaky cell vasculature in the tumour [18]. However, EPR is a passive effect, meaning nanoparticles still have to guide their way to the tumour. In a review of 224 manuscripts reporting nanoparticle delivery into the tumours of mice before 2016, an average of only 0.7% of the injected dose reached the tumours in each publication [19]. In 2020, the quantity of

particles retained in the tumour increased to 12% when a dose containing roughly 50 trillion nanospheres, was injected into mice, overwhelming the kidneys and liver, which generally retain the majority of a nanoparticle dose [20]. Similar methods in humans would be costly due to the number of nanoparticles needed, and potentially cause health complications from the continued administration of the therapy [21].

Active targeting, where the molecular coating on the surface of the nanoparticle is selected to bind to specific receptors on a cell surface, has shown to have more promising results [22–24]. However, even these treatments are misleading, with a more significant percentage of the injected dose reaching the tumour but an even smaller percentage, 0.0014% of injected dose, reaching the cancerous cells inside the tumour [25].

While a substantial amount has been learned from using nanotechnology to monitor and understand the functions of a tumour through imaging, as of 2021, only two nanoparticle treatments have been approved for use worldwide, and both require injection into the tumour [26–28]. Both treatments use metallic nanoparticles that interact with incoming electromagnetic waves, where one generates heat to induce hyperthermia in the tumour, and the other releases free radicals when exposed to radio waves.

While the current state of research presents significant challenges, photothermal therapy remains a glimmer of hope that may justify further research. The goal of photothermal therapy is both elegant and aggressive. Find a tumour, then heat it with a laser until the thermal damage kills cancerous cells. This technique has the same limitation as most chemotherapies: targeting tumours and limiting damage to other cells proves difficult. A critical improvement would involve selectively heating the tumour, and using low doses of laser irradiation while still generating a high temperature [29]. If nanoparticles can selectively enter tumour vasculature, these conditions could be effectively met [30]. Finally, the more heat generated from the nanoparticle, the less laser irradiation is necessary. With sufficient heat generation from low laser doses, the whole body could be irradiated without damaging healthy cells, killing smaller tumours that can be difficult to see or treat [31]. An example of the difficulty of accessing tumours can be seen in Figure 1.3.1. Here, gold nanostars could make an excellent treatment, since their LSPR can range between 600-1000 nm [32]. Early studies have shown that their structure is responsible for the increased

heating capability, raising *in vitro* cell temperatures by 13°C at a wavelength of 808 nm, compared to 6°C seen when using nanospheres [33]. However, nanostar research is concentrated on their synthesis, which has a large deviation in geometry [34]. Further research on how their geometry can influence optical properties and cellular entry are needed to justify the use of this nanoparticle in PTT.

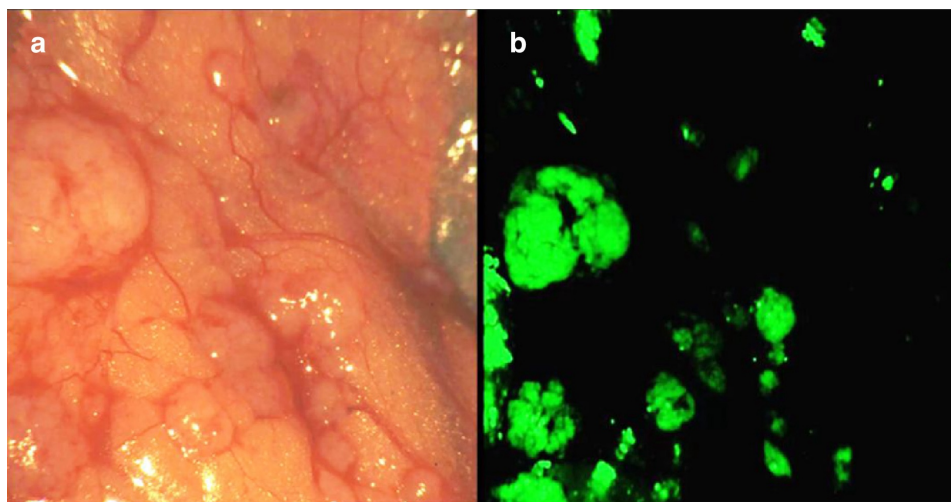


Figure 1.3.1: *White light (a) and fluorescence spectroscopy (b) image of tumours in an ovarian cancer patient. The tumour is spread throughout the imaged region, and can be difficult to access surgically. Reproduced with permission under a Creative Commons License [35].*

1.4 Material Characterisation of Nanoparticles

1.4.1 UV-Vis Absorption Spectroscopy

Ultraviolet visible (UV-Vis) Absorption Spectroscopy is often the first experimental characterisation technique used when synthesising nanoparticle solutions. Operation of such a machine may take a few minutes, and the overhead costs of running it are low. Advancements in correlating spectral measurements with particle geometry are thus highly desirable and extremely powerful [36–38]. The Beer-Lambert law describes the absorption of light by a sample in UV-Vis spectroscopy, which highlights the linear relationship between the light absorbed, A , and the product of the sample concentration,

c , the molar absorptivity, ϵ and the path length of light, l .

$$A = \epsilon cl \quad (1.4.1)$$

The amount of light absorbed is usually determined based on the fraction of light transmitted compared to a *blank* or a sample with the same materials present except for the one being analysed. If a solution of gold nanoparticles is suspended in deionised (DI) water, then the blank would be DI water, meaning that any change in the transmission is due to light absorption from the nanoparticles. This relationship can be seen in Equation 1.4.2, where I is the transmitted light intensity, and I_0 is the incident light intensity. Here, the incident light intensity is measured using the *blank*.

$$A = -\log_{10} \left(\frac{I}{I_0} \right) \quad (1.4.2)$$

The absorption cross-section of a gold nanosphere, or any other particle for that matter, is a measure of the effectiveness of the particle in absorbing light of a particular wavelength. Essentially, it indicates the likelihood that a photon, incident upon the particle, will be absorbed. It is a hypothetical area, and it gives a sense of size of the particle for the photon absorption process.

If a particle has a large absorption cross-section at a specific wavelength, it is highly likely to absorb photons of that wavelength. When gold nanospheres are excited by a wavelength of light at their resonant frequency, the LSPR dramatically enhances the absorption and scattering of light by the nanoparticles. As a result, gold nanospheres can have absorption cross-sections significantly greater than their physical cross-sectional area, resulting in a peak related to that resonance mode. Gold nanospheres only have LSPR due to their spherical symmetry, so the size of the nanosphere is easily correlated to the peak absorption using UV-Vis spectroscopy. Depending on the shape of the nanoparticle, or the distribution of shapes in a solution, the spectra may broaden due to increased collisions of conduction electrons or dispersity in particle shape, respectively [39]. An arbitrary example of a UV-Vis absorption spectra can be seen in Figure 1.4.1, where the collective oscillation of electrons at the LSPR is exemplified for a gold nanopshere.

However, it should be noted that the scattering of light into different directions may alter the amount of light absorbed by the UV-Vis detector. Therefore, it can accurately determine the wavelength of excitation, but not necessarily the absorption by a nanoparticle, as would be useful for calculating the amount a nanoparticle may heat.

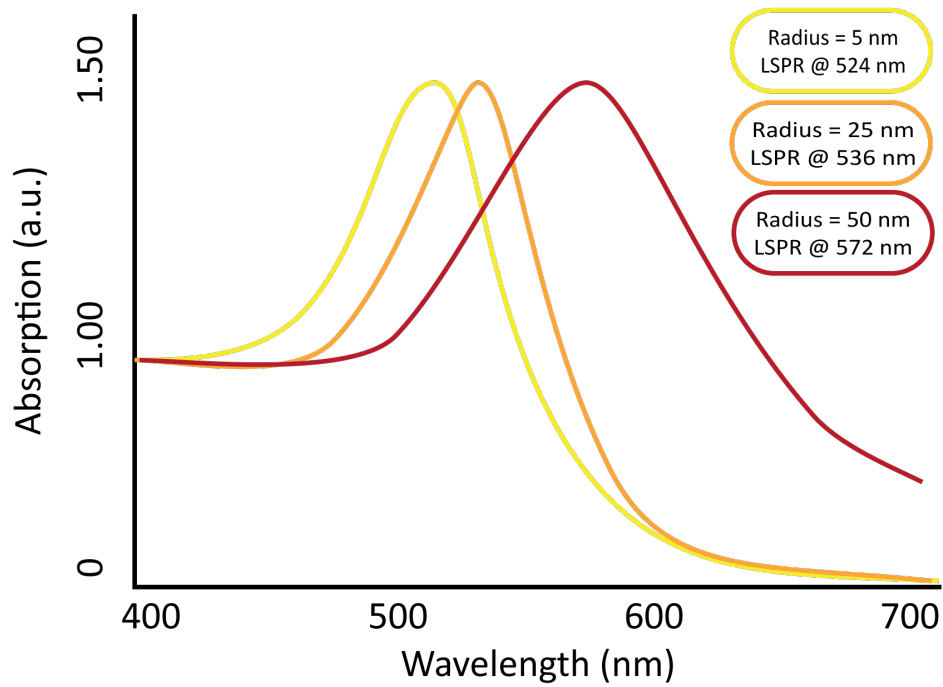


Figure 1.4.1: *An example of what could be expected from a UV-Vis absorption spectrum for gold nanospheres of increasing radius.*

1.4.2 TEM

Transmission Electron Microscopy (TEM) is a powerful technique used for characterising materials' structure, morphology, and composition at the atomic level [40, 41]. The operation of the microscope begins by generating an electron beam using a filament, often made of tungsten, which is heated to emit electrons through a process called thermionic emission. The emitted electrons are then accelerated by an electric field, typically generated by a

voltage of tens to hundreds of kilovolts, and through a series of magnetic lenses that focus the beam onto the specimen.

When the electron beam interacts with the specimen, it can be transmitted, absorbed, scattered, or cause the specimen to emit secondary electrons or X-rays. The transmitted and scattered electrons can form an image or a diffraction pattern, while the secondary electrons and X-rays can be used for compositional analysis. Images from TEM only show the projection area of the sample studied due to electrons penetrating through the material, limiting the capability of analysing more complex structures. Therefore, the sample must be thin enough (usually less than 100 nm) for the electrons to pass through.

A three-dimensional model of a nanoparticle can be obtained by tilting the sample at different angles and reconstructing the images. The methodology is both time and computationally expensive while still amounting to what may look like a set of rotating particle projections. Such complicated methods are typically unnecessary for a spherical nanoparticle as they will generate no further structural analysis.

The diameter of the spheres can be measured by determining the number of pixels in the scale bar, for which the length in nm is known, to convert the diameter of the spheres in pixels to nanometers [42]. Programs such as ImageJ can help with this conversion, and the development of computer vision programs has progressed to a point where the outlines of particles can easily be approximated, making characterisation by shape easier than ever [43–45].

1.5 Modelling of Nanostars

1.5.1 Discrete Dipole Approximation

Once the nanoparticle's shape is established, computational modelling of the electromagnetic properties can be performed using well-established methods [46–58]. Here, the focus will be on a point-based method called the discrete dipole approximation (DDA) as, further on in this work, the focus will shift to particles with more complex surfaces [59].

In DDA, points are treated as dipoles, and the polarisation, P of each dipole at point j , is found with respect to every dipole within the system. The

calculations performed by DDA involve solving a system of linear equations, with the number of equations increasing with the number of dipoles used to represent the volume of the nanoparticle. Once every polarisation has been calculated, the extinction, absorption, and scattering efficiencies (Q_{ext} , Q_{abs} , Q_{sca}) can be found using Equations 1.5.1-1.5.4 [60]. These values are essential descriptors for metallic nanoparticles since they determine if more or less light is being absorbed or scattered than would be observed for a non-plasmonically active nanoparticle with the same cross-section. In other words, these factors allow for a comparison between particles, determining which are better at scattering or absorption, which is necessary for photothermal therapy.

$$C_{\text{ext}} = \frac{4\pi k}{|E_{\text{inc}}|^2} \sum_{j=1}^N [\text{Im}(E_{\text{inc},j}^* \cdot P_j)] \quad (1.5.1)$$

$$C_{\text{abs}} = \frac{4\pi k}{|E_{\text{inc}}|^2} \sum_{j=1}^N [\text{Im}(P_j \cdot (\alpha_j^{-1} \cdot P_j^*)) - \frac{2}{3}k^3 P_j \cdot P_j^*] \quad (1.5.2)$$

$$C_{\text{sca}} = C_{\text{ext}} - C_{\text{abs}} \quad (1.5.3)$$

$$Q_x = C_x / \pi a_{\text{eff}}^2 \quad (1.5.4)$$

In the equations above, α is the polarisability of the material, k is the wavenumber of interest, $k = 2\pi/\lambda$ where λ is the wavelength of light *in vacuo*, E_{inc} is the incident electric field, $E_{\text{inc},j}^*$ is the complex conjugate of the incident electric field at point j , and a_{eff} is the effective radius of the shape. The effective radius will come up quite often throughout this thesis, and it is therefore quite important to clarify what it means now. DDA is a method used for arbitrarily shaped particles, and the number of dipoles used to represent a particle can vary drastically to obtain low or high resolution results. So, the a_{eff} is used to describe the volume of the particle if it was a perfect sphere. For example, a cube with side length of 10 nm would have a volume of 1000 nm³. A sphere with the equivalent volume would have a radius of 6.2 nm, making the $a_{\text{eff}} = 6.2$.

In Equation 1.5.4, the subscript x is a placeholder for ext, sca, or abs, as all three properties are found by dividing the cross-section, C_x , by the

effective area of a circle with radius $= a_{\text{eff}}$, to get the efficiency Q_x . Q_{ext} , which is the summation of Q_{sca} and Q_{abs} , will reach a maximum when the surface plasmons of the nanoparticle are excited, allowing computational results to be compared easily to UV-Vis absorption spectra of experimentally synthesised nanoparticles.

1.5.2 Coarse Grained Molecular Dynamics

Computer simulations can allow us to investigate biological phenomena on a length and timescale that may not be achievable experimentally. Specifically, it is used in this thesis to analyse the cellular uptake of nanostars. Here, the uptake can be compared to that of a nanosphere, which is more thoroughly studied experimentally. The goal in this comparison is to determine how the geometric differences between nanostars and nanospheres impact their interaction with biological membranes.

In all-atom molecular dynamics (MD) simulations, individual atoms are represented as beads, or points, which can be bonded together to form larger structures such as lipids and nanoparticles. Approximations of quantum mechanical interactions between atoms are used to calculate the potential energy between them. The set of functions that describe this potential energy are typically called a force field. The Newtonian equations of motion are then used to calculate atoms' future positions and velocities, given their initial position/velocity and the forces acting upon them. As the simulation progresses, the position of each atom changes in response to the forces acting upon it, as Newton's second law in Equation 1.5.5 describes.

$$\vec{F}_i = m_i \frac{\partial^2 \vec{r}_i}{\partial t^2} = -\nabla U_i \quad (1.5.5)$$

The force, $\vec{F}$, acting on a atom i , which has mass m_i and position $\vec{r}_i$, is calculated at each time step as the negative gradient of the potential energy, U_i , which is described by the force-field. This force is then used to update the position and velocity of the atom as the simulation advances.

While tracking the position of each atom in a system can be advantageous, these models can limit the timescales which can be reached [61]. To reduce the computational cost of simulations, simplifications can be made which leave only the essential physics present. There are several coarse grained parameterizations which can be used, examples of which can be seen in

Figure 1.5.1. To capture the membrane fluctuation dynamics, which are essential for nanoparticle uptake, the Cooke-Deserno parameterization is a reliable and well tested model [62]. The model parameters are inferred by finding a set of potentials that accurately reproduce experimental properties of a lipid bilayer[63]. Here, I will briefly explain how the model reduces computational costs.

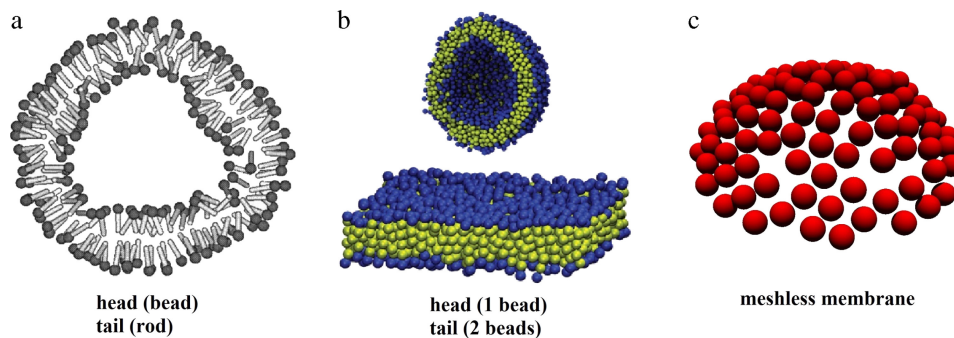


Figure 1.5.1: *Three different approximations for a coarse grained lipid bilayer membrane, with differing levels of biological relevancy. By using a rod instead of two beads to represent the tail of the lipid, there is decreased flexibility in the membrane, but the system with two beads would take longer to simulate. Reproduced with permission under a Creative Commons License [64]*

One of the largest contributors towards computational cost is the solvation of the membrane using water molecules. The position and velocity must be calculated for every atom in the system [65]. These variables are influenced by surrounding atoms, possibly through Van der Waals or coulombic interactions. In that case, this calculation must be done for every atom with respect to the atoms in the surrounding area. By using a parameterization with an implicit solvent, meaning the effects of a solvent are integrated into the force field potentials, water molecules can be removed from the system. Additionally, each lipid may normally contain on the order of 100 atoms, which are instead represented by three beads. By removing water molecules and using beads to represent a cluster of atoms, the computational costs are significantly reduced by starting the simulation with fewer atoms to begin with.

If the positions and velocities of every bead are measured at every time step, it would be convenient to use the largest time step possible. Setting a timestep dictates when the next set of calculations will take place, controlling the speed at which the system evolves. It also implies that the forces will

remain constant on each atom between the two timesteps. So, the time-step must be smaller than the fastest movement, or vibrational period, in the simulation [66]. If not, beads may overlap if the chosen time step is too large when integrating into the next position. Most potentials that are used to introduce inter-atomic forces prevent overlap by approaching infinite potential energy as the separation distance between beads is reduced below the equilibrium value. The increase in energy translates to two atoms repelling each other. Since coarse-grained simulations do not typically involve using bonds with high frequency, the time step is usually - but not always - larger than in all-atom simulations. To give a sense of scale, the timesteps in all atom simulations are dictated by the frequency of hydrogen bonds which is typically on the order of femtoseconds (10^{-15} sec) where coarse grained simulations can reach up to nanoseconds (10^{-9} sec), but the latter has more variation and is highly dependent on the system [67].

Finally, the size of the membrane can be drastically reduced. Most cells in the human body are typically micrometres in diameter, meaning a nanoparticle may interact with 0.0025% of the surface area[68]. It would be prudent to focus only on the part where the nanoparticle will interact with the membrane. From the perspective of the nanoparticle the cellular membrane is flat, similar to how we see a flat plane on a spherical Earth. So instead of modelling the entire cell membrane, periodic boundary conditions can be used to remove edge effects and represent a continuous plane. An example of a periodic boundary condition can be seen in Figure 1.5.2 using beads and a coarse-grained lipid bilayer membrane. When periodic boundary conditions are not enforced, lipid heads will move to the edge of the bilayer, in order to reduce the interactions between the hydrophobic tails and the aqueous environment. Therefore, the resulting simulation would not represent the desired system.

The parameterisation used within takes advantage of these techniques to create a model membrane which has the necessary physical properties to simulate cellular uptake of nanoparticles.

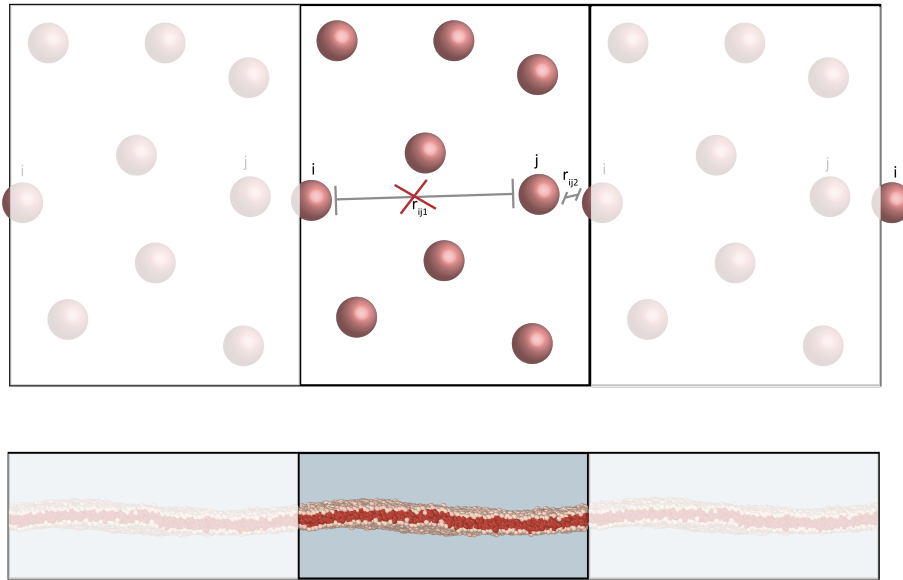


Figure 1.5.2: *Top: Beads floating within a simulation box where periodic boundary conditions are used. When calculating the distance between bead i and j , the shortest distance will be used. Bottom: The cross section of a simulation of a coarse grained lipid bilayer membrane, where the tan particles are hydrophilic lipid heads, and the red particles are hydrophobic lipid tails. If the periodic boundary conditions were removed, the lipid heads would move towards the edges, completely enveloping the hydrophobic tails and minimising their interaction with the surrounding aqueous environment.*

2 Analytical Models of Nanostars

2.1 Background

Understanding the correlation between the structure and function of nanostructures has necessitated the optimisation and reproducibility of their synthesis through material selection and wet-chemistry techniques. One such nanostructure, the nanostar, has presented challenges in characterisation due to its complex morphology [69]. Gold nanostars are known by various names, including nano urchins, nanoflowers, bumpy, rough, and virus-like nanoparticles [70–73]. Although some of these names suggest subtle differences in the particle’s morphology, such as low spike length, large tip curvature, and high density of tips, there is currently no specific criterion for distinguishing between them. For simplicity, we will refer to these particles as nanostars throughout this discussion.

A nanostar without any tips is just a nanosphere, where the localised surface plasmon resonance (LSPR) sits in the visible range between 500-650 nm, depending on the size. The tips are responsible for increasing the wavelength of the nanostar LSPR to 700-1200 nm, a desirable quality for photothermal therapy since lasers of higher wavelength can penetrate deeper into the body [34, 74]. While tips create a larger adsorption area, they also make the surface potentially less accessible to the membrane, an aspect that is often overlooked [75]. Moreover, the same features that might increase nanostar’s utility can also make these particles toxic towards cells, potentially harming the patients they are created to help [76]. Since nanostars with high aspect-ratio tips can perforate the cell membrane, causing leakage it is crucial to fully characterise the nanostar shape and its impact on cell viability [77, 78]. Unfortunately, the task is not straightforward.

Unlike nanospheres, to which nanostars are often compared, the geometrical features cannot be determined by looking at the Ultraviolet-visible (UV-Vis) absorption spectra of the nanostars in solution.

The nanostar shape can only be unequivocally determined based on visual inspection using transmission electron microscopy (TEM) or scanning electron microscopy, the former of which will be explored further in the following chapter [79]. The current commercial standard for selling a monodisperse solution of nanostars is to report the LSPR, the core diameter, and the tip length [80]. Even in this case, the description fails to capture the tip radius, the number of tips, and the deviation in tip length and radius across the nanostars. The best categorisation to date was done by Boselli et al., which utilised TEM images, computer vision, and a principal component analysis based on the Fourier transform of the nanostar contours [81]. While this powerful technique can sort nanostars into various groups by similarity, such as having many short tips, it fails to account for the large variability within each shape category, or determine the remaining shape factors mentioned.

Since the best characterisations of nanostars so far have been derived from TEM images, this chapter aims to determine the information that can be obtained from these 2D projections, when the 3D geometry is known. Therefore, this section will ignore the opto-electronic properties of nanostars, and will rely on a large dataset to analyse trends.

2.2 Methods

I coded all of the methods mentioned in this chapter unless stated otherwise. The Python code developed is a part of the published GitHub repository that accompanies the publication in the following chapter (shakespearemorton/starscat).

2.2.1 3D Nanostars

Two different shape generators were developed to create nanostars, based on a collection of points, for the two computational methods used in this thesis. The volumetric method, used for Discrete Dipole Approximation (DDA), involves the placement of points on an integer lattice, a requirement for the DDA. These were created by determining if a point lies outside of the sphere or right circular cone, representing a nanostar core and tip respectively. The collection of points makes up a set of dipoles representing

the volume of the nanostar. The surface-based method, used for Molecular Dynamics (MD), has finer control over the structure and reduces the total number of points in the system. The surface-based method places equally spaced points across the nanostar's surface, where the separation distance between points is kept constant. The even spacing is achieved by using a Fibonacci lattice, the pseudocode for which can be seen in Algorithm 1 [82]. The Fibonacci lattice is a convenient solution, because the surface area of the sphere is known. Additionally the same code can be used to pick equally spaced points around the sphere, to place the tips.

Algorithm 1 Generate Fibonacci Lattice on a Spherical Surface

```

1: function GENERATEFIBONACCI LATTICE(numberOfPoints, radius)
2:   goldenAngle  $\leftarrow \pi(3 - \sqrt{5})$  ▷ Golden angle in degrees
3:   points  $\leftarrow []$  ▷ List to store points
4:   for i  $\leftarrow 1$  to numberOfPoints do
5:     latitude  $\leftarrow \arcsin(-1 + 2 \times i / \text{numberOfPoints})$ 
6:     longitude  $\leftarrow (\text{goldenAngle} \times i) \bmod 360$ 
7:     x  $\leftarrow \text{radius} \times \cos(\text{latitude}) \times \cos(\text{longitude})$ 
8:     y  $\leftarrow \text{radius} \times \cos(\text{latitude}) \times \sin(\text{longitude})$ 
9:     z  $\leftarrow \text{radius} \times \sin(\text{latitude})$ 
10:    points.append((x, y, z))
11:  end for
12:  return points
13: end function

```

Experimentally, the radius of a nanostar tip is measured tangent to the nanostar core, and the tip height is taken as a measure from the end of the tip to the same tangent line. Therefore, the height and radius of the cone are extended to intersect the spherical core of the particle. To achieve this, the height of the cones are increased based on the radius of the core. The tip radius is also adjustable, and the angle between the base and the start of the rounded tip is modified accordingly. The pseudocode to achieve this is seen in Algorithm 2.

A schematic showing different nanostar production stages can be seen in Figure 2.2.1.

Algorithm 2 Generate Intersecting Right Round Cone

```

1: function ROUND Cone( $h, rBase, rTip, rSphere$ )
2:   A flat top right round cone is generated
3:    $angCone \leftarrow$   $\triangleright$  cone angle measured using  $h$  and  $rBase$ 
4:    $inc \leftarrow -\sqrt{rSphere^2 - rBase^2} + rSphere \triangleright$  Increase in Cone Height
5:    $rAdj \leftarrow (h + inc) / \tan(angCone)$ 
6:    $hCap \leftarrow$   $\triangleright$  determine the height where tip radius begins
7:    $hTotal \leftarrow h + inc - hCap$ 
8:    $fitX \leftarrow [rAdj, rTip]$ 
9:    $fitY \leftarrow [0, hTotal]$ 
10:   $slope, intercept \leftarrow \text{LinFit}(fitX, fitY) \triangleright$  Line from  $rBase$  to  $rTip$ 
11:   $heights \leftarrow \text{range}(0, hTotal)$ 
12:   $radii \leftarrow [] \triangleright$  List to store radius(height)
13:  for  $h$  in  $heights$  do
14:    Append  $(h - intercept) / slope$  to  $radii$ 
15:  end for
16:   $conePts \leftarrow [] \triangleright$  List to store points
17:  for  $index$  in  $\text{range}(\text{Length of } heights)$  do
18:    if  $radii[index] \geq hCap$  then
19:       $angles \leftarrow \text{range}(0, 2\pi)$ 
20:      for  $angle$  in  $angles$  do
21:         $x \leftarrow \cos(angle) \times radii[index]$ 
22:         $y \leftarrow \sin(angle) \times radii[index]$ 
23:         $z \leftarrow heights[index]$ 
24:        Append  $[x, y, z]$  to  $conePts$ 
25:      end for
26:    end if
27:  end for
28:  The rounded tip is generated
29:   $roundedTip \leftarrow \text{GenerateFibonacciLattice}(hCap)$ 
30:  for  $pt$  in  $roundedTip$  do
31:    if  $pt[z] \geq 0$  then  $\triangleright$  Only use top hemisphere ( $z$ -coord)
32:       $pt[z] \leftarrow pt[z] + hTotal \triangleright$  Move to top of cone
33:      Append  $pt$  to  $conePts$ 
34:    end if
35:  end for
36:  return  $conePts$ 
37: end function

```

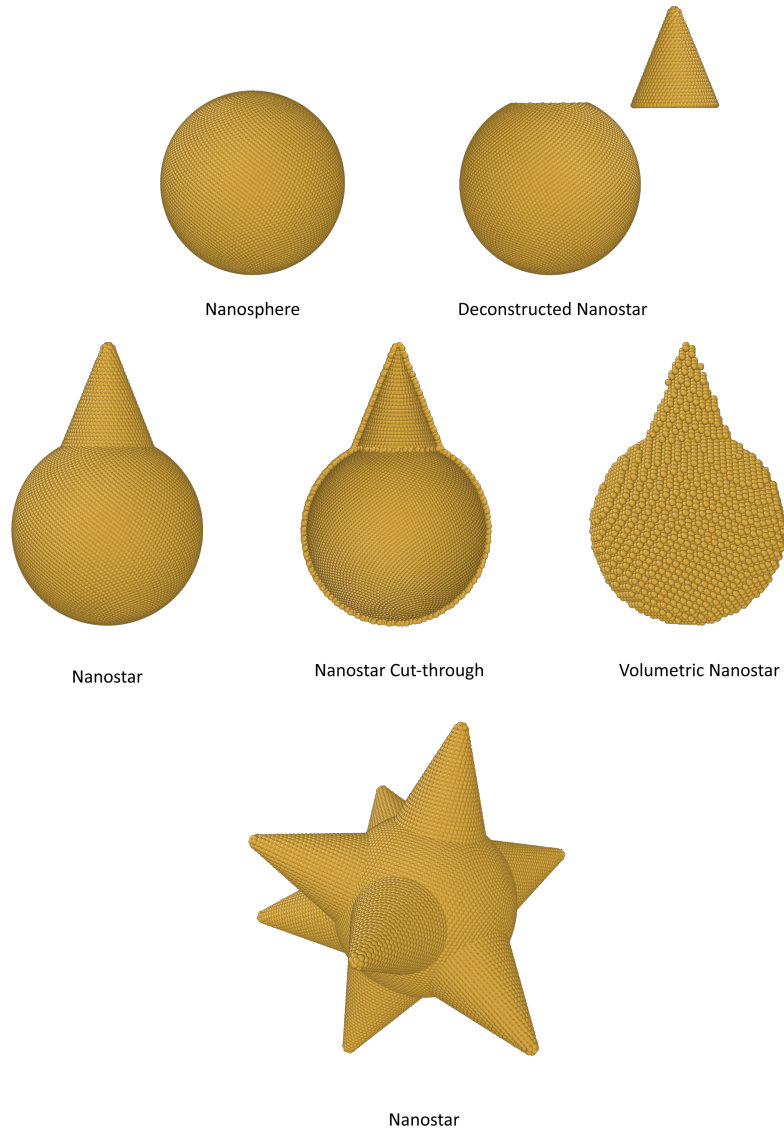


Figure 2.2.1: To develop the surface-based model of a nanostar, the spherical core is first created. Then, a spherical cap is removed to accommodate the addition of a tip. Since the tip length is the measure from the end of the tip to the point of intersection tangent to the core, the base of the tip is extended to intersect with the surface of the sphere. The volumetric model requires placing a point on an integer lattice, meaning the resolution is poorer than the surface model.

2.2.2 Projection Area

The projection area of each nanostar is measured similarly to how it would be experimentally. Each star being a collection of points with coordinates in the X, Y, and Z direction, is projected onto the XY plane. The projection area is calculated by taking a sphere of radius α , sometimes referred to as an alpha sphere for this method, and rotating it along the outside of the collection of points. A line is drawn between every set of points with which the sphere comes into contact. If the alpha sphere is too large, a low-resolution shape is obtained, whereas an alpha sphere that is too small will create a surface inside the volume of the points. Since points are placed every 1 nm along the surface of the nanostar, a sphere of radius $\alpha = 1$ nm is sufficient to capture the shape of the nanostar. This assumption was confirmed by spot-checking the outlines developed through graphs like those in Figure 2.2.2. In order to measure variation, the star is then rotated using a rotation matrix that aligns one point on the nanostar to 200 equally spaced points on a unit sphere. The projection area is then averaged over all rotations. The convex hull, which is the area of a shape that uses the minimum amount of lines to completely enclose the set of points, was found using an in-built SciPy package [83]. Finally, the solidity, which is the particle's projection area divided by its convex hull, can be calculated, acting as a good indicator for roughness [84].

Often, geometric properties of nanostars, such as volume, are estimated using the measured projection area from TEM images [85]. The effective radius of the nanostar, a_{eff} , or the radius of a circle with an area similar to the projection area. This radius can then be taken and used to find the volume of a sphere with the same radius. When reporting the results from the nanostars below, the percent error between the experimental a_{eff} and the a_{eff} of a sphere with the same volume of the nanostar, is reported to demonstrate the predictability of nanostar shapes from TEM images.

2.2.3 Contact Area

The maximum contact area (CA) and the percent of the surface in contact ($\hat{C} = 100 * \frac{CA}{S}$), where S is the surface area, between a nanoparticle and a lipid bilayer membrane is crucial for initiating endocytosis and is hypothesised to influence the delivery of nanoparticles into cells [86]. Here the lipid bilayer

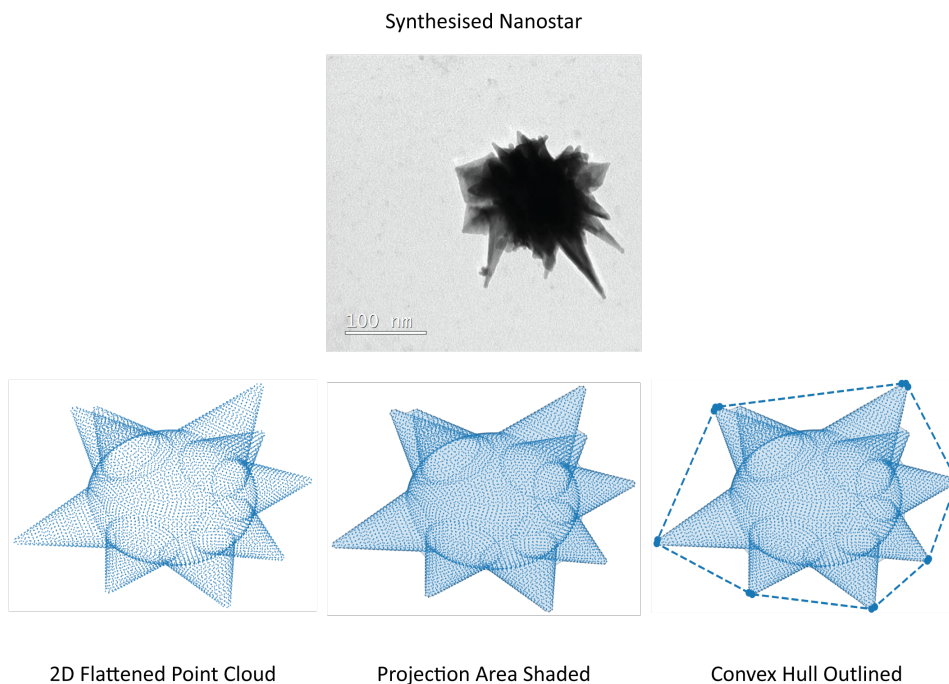


Figure 2.2.2: Measuring the projection area of the synthesised nanostar, a_{eff} would be the radius of a circle with a similar area, which assumes spherical uniformity of the nanostar. Measuring the same property from the modelled nanostars involves flattening the point cloud representing the surface so that all points lie on the plane $Z = 0$. The projection area is used to determine the experimental a_{eff} . The actual a_{eff} is found by determining the radius of a sphere with the same volume of the nanostar since this is known for the modelled particles. Finally, the projection area can be divided by the convex hull to find the solidity of the nanostar - a good metric of roughness.

is treated as a flat surface, and the lowest 5 nm are considered to be within interaction distance with the bilayer. I will elaborate more on these effects later in Chapter 5, where nanoparticles' endocytosis is discussed in full detail. To measure the amount of contact area, the point on the nanostar surface with the lowest Z position was moved to the XY plane at $Z = 0$. Once there, the number of beads within 5 nm of the plane at $Z = 0$ was summed, and the area was converted from beads to nm^2 by assuming $1 \text{ bead} = 1 \text{ nm}^2$. A distance of 5 nm was chosen to represent an average lipid bilayer fluctuation, meaning that these areas of the nanostar are accessible to the membrane. The maximum contact area over all 200 rotations was then reported for each

nanostar. Figure 2.2.3 shows the surface beads within 5 nm of a flat surface. The orientation shown has the most contact points, making it the preferred surface for membrane interactions.

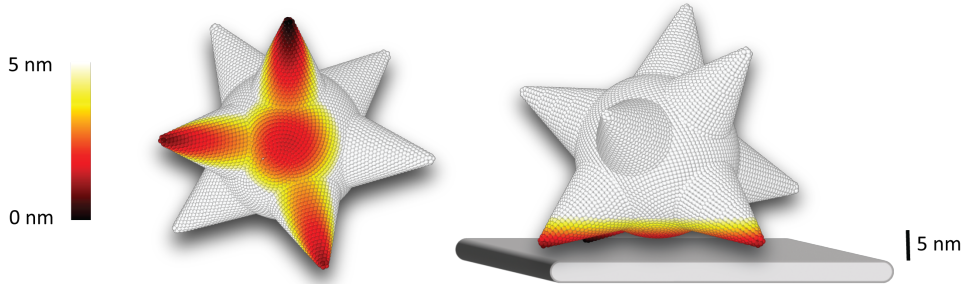


Figure 2.2.3: *Orientation of the given nanostar resulting in the highest $\hat{C}$ between the nanostar and a flat surface. Colouring is applied to the particles to show the distance from the surface, with white beads sitting greater than 5 nm above the surface.*

2.2.4 Correlation Analysis

One of the main outcomes from this chapter is to understand the geometric features of a nanostar that can have its geometry predicted using TEM imaging. By replicating the imaging procedure, while knowing the exact volume, surface area, and geometry of the nanostar, variables that are strong predictors of the possible error in approximations from TEM imaging can be found. Unfortunately, the relationships between variables in this study are inherently convoluted, complicating the disentangling of each variable's effects on the measured quantities. For example, if solidity increases with tip length, there might be a stronger correlation to the volume, which can go unnoticed. One must carefully note that while these relationships, identified as correlations, indicate a statistical relationship between variables, they do not imply causation. To investigate the nature of these relationships, a random forest algorithm was employed from the Sci-Kit Learn package [87]. This algorithm is apt for handling high-dimensional data and ascertaining the strength of relationships between input variables and outputs. While many other methods exist, the implementation of a random forest is straightforward, and the data does not require any pre-processing. The dataset was split into a training set, comprising a randomly selected 80% of the nanostars,

and a testing set with the remaining 20%. This ratio was chosen to balance the need for sufficient training data against the risks of overfitting and memorisation [88]. The algorithm's performance was gauged by measuring the Root Mean Squared Error (RMSE) between predictions for the training set and the actual values in the test set. The RMSE is effective for this purpose due to its penalty for larger errors and its interpretability in the same units as the dependent variable, as seen in Equation 2.2.1, where n is the number of samples, y_i is the actual value, and $\hat{y}_i$ is the predicted value. Model parameters were adjusted to minimise RMSE from test data set in a one-shot run, rather than using a validation set of data. A maximum depth of 10, minimum samples per leaf of 1, minimum samples to split an internal node of 2, and 200 trees were used as the parameters for the random forest algorithm.

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (y_i - \hat{y}_i)^2} \quad (2.2.1)$$

The algorithm will select samples from the training dataset and determine the importance of each input variable on the desired observable. Significant variables will be heavily relied upon to determine an accurate output given specific inputs. However, while random forests excel in prediction, they do not explicitly characterize the nature of relationships between influential predictors and the output variable. The algorithm may predict that there is a strong connection between the velocity of a thrown ball and its kinetic energy. However, it will not know that the relationship depends on the velocity squared.

2.2.5 Library of Nanostars

An assortment of 6000 nanostars was generated using the methods presented here and analysed to give the reader a scale of the input parameters used to generate nanostars, and the outputs that were measured. The following tables and trends highlight the geometric relationships expected to drive nanostar performance.

2.3 Results and Discussion

2.3.1 Value Ranges

Before embarking on an analysis to characterise geometric relationships, it is imperative to have a scale to work with. In Table 2.1, the minimum and maximum of each dependent and independent variable are reported. However, not every combination of dependent variables was tested due to geometric constraints. For example, a nanostar with a core radius of 8 nm could not support 30 tips with a radius of 6 nm.

Table 2.1: Range of independent and dependent variables from 6,000 nanostars.

The table shows the extent of nanostar shape factors: core radius ($Core_{rad}$), tip radius (Tip_{rad}), tip length (Tip_{len}), and number of tips (N_{tips}). These geometric properties are related to: the surface area to volume ratio (S/V), the error in predicting volume from projection area ($\% Error_{a_{eff}}$), the contact area with a flat plane (CA), and the percent of the surface area in contact with a plane ($\hat{C}$).

	$Core_{rad}$ (nm)	Tip_{rad} (nm)	Tip_{len} (nm)	N_{tips}	S/V (nm^{-1})	$Error_{a_{eff}}$ (%)	CA (nm^2)	$\hat{C}$ (%)
Min	8	3	3	1	.20	0	141	2
Max	15	6	14	30	.87	39.8	1459	43

2.3.2 Volume and Surface Area

As mentioned in the previous chapter, many of the electromagnetic benefits of nanoparticles come from their high surface area-to-volume ratio, S/V . The spherical core has a S/V that is proportional to $\frac{1}{r}$ suggesting that nanostars with larger cores will result in smaller S/V , which is what Table 2.2 demonstrates. Adding more tips would increase the ratio, so the ten nanostars with the lowest ratio all have one tip, allowing them to lie flat on a 2D surface, resulting in a high $\hat{C}$. If we consider the tip's surface area to volume ratio, keeping volume fixed, longer tips will have a greater surface area than those with a wider radius. This dependence is reflected in Table 2.3, where nanostars with smaller cores are adorned with many long tips that maximise their surface area to volume ratio.

Table 2.2: The nanostars with the ten lowest values of S/V . These nanostars maximise the core radius while minimising the singular tip that it is forced to have on the surface.

Particle (ID)	Core _{rad} (nm)	Tip _{rad} (nm)	Tip _{len} (nm)	N _{tips}	S/V (nm ⁻¹)	Error _{a_{eff}} (%)	CA (nm ²)	$\hat{C}$ (%)
5453	15	3	3	1	0.201	0.1	488	17
5602	15	3	4	1	0.201	0.2	496	17
5723	15	3	5	1	0.202	0.3	504	18
5456	15	3	3	2	0.202	0.4	501	18
5498	15	4	3	1	0.202	0.2	501	18
5636	15	4	4	1	0.202	0.3	505	18
5853	15	3	6	1	0.202	0.5	513	18
5761	15	4	5	1	0.202	0.4	514	18
5603	15	3	4	2	0.202	0.6	496	17
5872	15	4	6	1	0.202	0.5	523	18

Table 2.3: The nanostars with the ten highest values of S/V . These nanostars exhibit a small spherical core, with a high density of long, thin, tips to increase surface area.

Particle (ID)	Core _{rad} (nm)	Tip _{rad} (nm)	Tip _{len} (nm)	N _{tips}	S/V (nm ⁻¹)	Error _{a_{eff}} (%)	CA (nm ²)	$\hat{C}$ (%)
672	8	3	13	29	0.872	39.0	196	4
759	8	3	14	29	0.870	39.8	175	4
645	8	3	13	28	0.859	38.7	190	4
707	8	3	14	28	0.853	39.4	170	4
610	8	3	12	29	0.850	37.0	191	4
644	8	3	13	26	0.837	38.3	205	5
648	8	3	13	27	0.832	38.1	212	5
555	8	3	14	26	0.830	38.9	189	4
541	8	3	14	24	0.828	39.5	158	4
426	8	3	10	29	0.827	34.0	203	6

2.3.3 Contact Area

Many of the nanostars in Table 2.2 had high $\hat{C}$ due to the ease of lying flat against a plane with a single tip. Keeping the spherical core and the edges of the tips in contact with a single plane will increase $\hat{C}$, but the geometric constraints are highly dependent on the distribution of tips around the nanostar. In Table 2.4, many tips keep the nanostar away from the surface, and increasing the length of the tips increases the surface area, resulting in an even lower percentage of the surface being in contact. As the length of tips increases, the distance between them will also increase proportionally

to the arc length of a circle. The angle between tips remains the same, but the total radius of the nanostar increases, resulting in less contact with a flat plane. A large tip radius is also used to increase the separation distance between tips while simultaneously increasing surface area. The results in Table 2.5 are less straightforward. Achieving a large $\hat{C}$ necessitates having small core radius, 8 nm, because a larger core radius would result in more of the surface that is no longer in contact with the plane. A few tips, 2-5, with a high surface area are also able to lie on the same plane. The tips are long but with a small radius, since a larger radius would result in more surface area not in contact with the plane. However, the tips are not at their maximum length, suggesting that if they were longer, they might push the nanostar’s core further from the surface. These relationships are highly dependent on how tips are placed on the surface. Since the model distributes them evenly across the surface, there may be a bias in the results shown here.

Table 2.4: *The nanostars with the ten lowest values of $\hat{C}$. These nanostars exhibit a high density of long and wide tips that keep the nanoparticle away from the flat surface.*

Particle (ID)	Core _{rad} (nm)	Tip _{rad} (nm)	Tip _{len} (nm)	N _{tips}	S/V (nm ⁻¹)	Error _{a_{eff}} (%)	CA (nm ²)	$\hat{C}$ (%)
5947	14	6	14	29	0.478	25.9	235	2
5678	14	4	14	29	0.405	27.0	167	2
5921	14	6	14	28	0.485	26.9	232	2
5816	14	6	14	24	0.440	26.1	201	2
5661	14	4	14	28	0.401	26.8	166	2
5609	14	4	14	24	0.397	26.8	154	2
4358	13	4	14	29	0.448	28.5	166	2
5782	14	5	14	29	0.416	26.1	202	2
5762	14	5	14	28	0.411	26.0	197	2
5837	14	6	14	25	0.438	25.3	220	2

2.3.4 Predictability

In Tables 2.2-2.5, the percent error in a_{eff} is shown as a metric of predictability, or how well the volume of the particle can be predicted based on the information from a 2D projection obtained from a TEM image. The lower the error, the better the nanostar can be characterised by TEM alone. In total, the error ranged from 0-40%, and in almost all cases, the

Table 2.5: The nanostars with the ten highest values of $\hat{C}$. These nanostars use between 2-5 tips with a small radius, which can be kept in a single plane to maximise contact.

Particle (ID)	Core _{rad} (nm)	Tip _{rad} (nm)	Tip _{len} (nm)	N _{tips}	S/V (nm ⁻¹)	Error _{a_{eff}} (%)	CA (nm ²)	$\hat{C}$ (%)
86	8	3	9	5	0.493	9.8	536	43
75	8	3	9	4	0.475	7.5	497	43
73	8	3	8	5	0.479	8.6	506	43
175	8	3	13	2	0.445	10.8	452	43
151	8	3	12	2	0.440	10.0	438	42
29	8	3	8	3	0.449	5.1	436	42
83	8	3	10	4	0.486	8.5	500	42
54	8	3	8	4	0.465	6.6	461	42
92	8	3	10	5	0.504	11.0	537	42
194	8	3	14	2	0.451	11.6	450	41

a_{eff} estimated from the projection area is larger than that calculated from the known volume of the nanostar. Tips drive the overestimation because they are not spherically symmetric and lie out of plane, making it difficult to determine their length and radius. The tables above seem to contain the outer limits of the range of errors, suggesting a high correlation between the two factors studied; S/V and $\hat{C}$. The random tree algorithm was applied to the data, using the geometric inputs (core radius, tip radius, etc.) along with surface area, volume, S/V, number of contacts, $\hat{C}$, and solidity as inputs, to predict % Error_{a_{eff}}.

The algorithm results showed that 72% of its ability to predict test data came from $\hat{C}$, and another 19% came from S/V, with a RMSE of 0.41%. Additionally, the total number of contacts, C , only contributed 3% of the model's predictability, while the surface area and volume alone contributed less than 1%. Now the disadvantages of the random tree algorithm become apparent. Does predictability increase or decrease with S/V and $\hat{C}$? A logarithmic relationship is seen between S/V and % Error_{a_{eff}}, while $\hat{C}$ exhibited a negative linear relationship when graphed.

To better describe the relationship, a linear regression model was created using ordinary least squares (OLS) regression to determine the linear combination of $\log_{10}(\text{S/V})$ and $\hat{C}$ that could accurately predict % Error_{a_{eff}}. The RMSE for this model was 2.02%, suggesting that there is a strong relationship between predicted and observed values using the following

equation:

$$\%Error_{a_{eff}} = 18.02 * \log_{10}(S/V) - 0.053 * \hat{C} + 41.11 \quad (2.3.1)$$

The accuracy of the predictions can also be seen in Figure 2.3.1 and Figure 2.3.2. The overestimation of error by the model may be attributed to the exclusion of other predictors, or the nanostars used in the study.

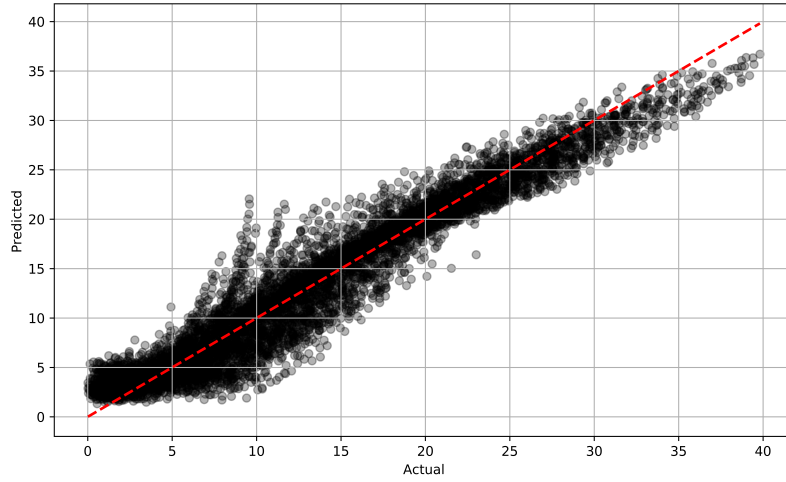


Figure 2.3.1: Predicted values of $\% Error_{a_{eff}}$ by a OLS regression model between $\log_{10}(S/V)$ and $\hat{C}$. A red line is overlayed where the predicted and actual values are identical.

Since nanostar tips tend to overestimate a_{eff} , the fewer tips that can lie in a single plane, the less impact they will have on increasing the projection area. The overestimation is supported by the negative slope attributed to $\hat{C}$, which shows that the less surface area that can lie on a single plane, the lower the error. Despite the predicted error having little correlation to the volume and surface area individually, their ratio is a predictive factor, which can be exemplified in Particle 5947 found in Table 2.4. As the number of tips on the surface of the nanostar increases, it eventually approaches the volume of a sphere, even though the surface area is quite large. If the nanostar core radius increases, it can support tips with smaller radii, which means they could be closer together, increasing the packing on the nanostar surface.

This geometric relationship can also be seen as solidity, which had 1.6%

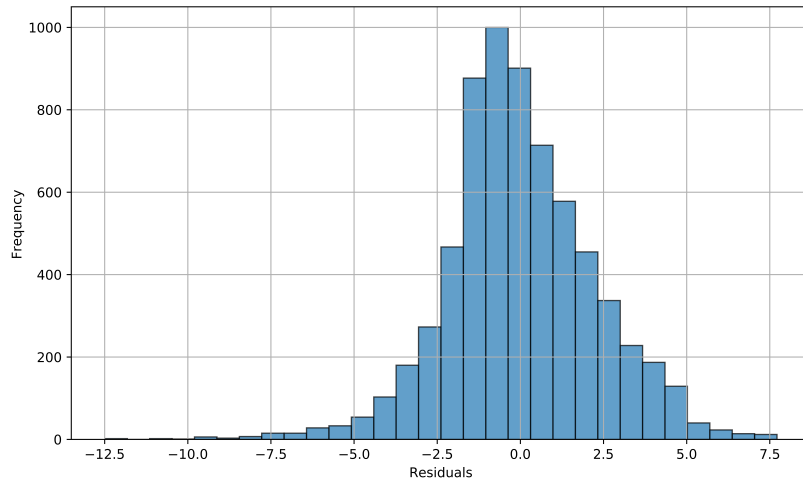


Figure 2.3.2: A histogram of the residuals from Figure 2.3.1. The slight skew towards negative values implies that Equation 2.3.1 tends to overestimate the $\%Error_{a_{eff}}$.

importance in determining the outputs of the random tree algorithm. While the importance was low, this is likely due to solidity being correlated, but not the causation of the effect seen in predictability.

It is crucial to clarify that the discussion so far has demonstrated the ability to accurately predict a_{eff} from TEM images, which can only be directly attributed to the volume of the nanostars. The surface area must be substantially large to predict the volume in the first place. A similar OLS regression model between the predicted a_{eff} and $\log_{10}(SA)$ revealed a positive, highly linear correlation with an RMSE of 0.6. The values seem promising, but this relationship is believed to be an artefact of the experimental conditions. Since all nanostar tips were the same size, it would be easy to attribute the surface area to a_{eff} using the analysis above. However, in an actual synthesis of nanostars, there is non-uniformity in tip radius and length, which would likely reduce the strength of the relationship seen here.

2.4 Conclusions

The difficulty in characterising the geometry of nanostars experimentally means assumptions are made from the projection area of TEM images. The

library of modelled nanostars generated here can be helpful in quantifying the confidence of these assumptions. After measuring core radius and tip length experimentally, the range of surface areas and volumes for that specific synthesis can be determined. The reference library can be instrumental on its own, but in the future should also be expanded to include variation in nanostar tip length and radius. The accuracy of characterising a 3D nanoparticle using a 2D projection area has been characterised. Nanostars with many long thin tips are expected to have a large S/V ratio, limiting their ability to be characterised through TEM. However, it is expected that the volume will often be overestimated. Since the addition of tips to a nanostar increases S/V , a better method for determining nanostar geometry is necessary.

3 Modelling Nanostar Geometry

Notes

The following chapter was adapted from the peer reviewed journal article: Morton, W., Joyce, C., Taylor, J., Ryan, M., Angioletti-Uberti, S., & Xie, F. (2022). Modeling Au Nanostar Geometry in Bulk Solutions. *Journal of Physical Chemistry C*. <https://doi.org/10.1021/acs.jpcc.2c07520>

The method for synthesising gold nanostars was taught to me by Caoimhe Joyce.

The operation of the Thermo Scientific iCAP 6000 Series ICP Spectrometer for Inductively Coupled Plasma Optical Emission Spectroscopy was performed by Johnny Taylor.

Mary Ryan, Stefano angioletti-Uberti, and Fang Xie acted as advisors throughout the research project.

All other work, analysis, and writing was done by myself.

3.1 Background

Bulk properties of plasmonic nanoparticles with one or two geometric factors, like nanospheres or nanorods, are easily measured. A straightforward technique would involve measuring the average geometry of the structures using a few transmission electron microscopy (TEM) images. The radius of a sphere and the aspect ratio of a rod are easily determined by 2D projections of the nanoparticles. Dynamic light scattering and ultraviolet-visible (UV-Vis) spectroscopy are two additional methods that could be employed, and all three methods have the ability to estimate the polydispersity of the bulk solutions. The simplicity with which these nanoparticles can be described is directly attributed to both the simplicity of the structures themselves, and the large quantity of research that has been done on them. Despite the publications involving nanostars, there have been few attempts to define

their average properties. Figure 3.1.1 exemplifies the difficulty by showing the reader a monodisperse solution of nanostars, and challenging them to describe the average particle.

Electron microscopy and UV-Vis spectroscopy have been crucial for developing correlations between the optical and geometric properties of other nanostructures [90, 91] [92]. Shape factors have been exploited to relate the aspect ratios of nanorods to localised surface plasma resonances (LSPRs), determine the transmissivity of nanohole arrays, and relate the aspect ratio of gold nanostar tips to LSPRs [78, 93–95]. When used in unison, microscopy, spectroscopy, and electromagnetic modelling can predict the optical properties of synthesised nanostructures, or guide synthesis towards designer structures made for specific applications. The goal of this chapter is to produce a similar methodology for nanostars, using these techniques to show the limits of structural prediction and to successfully describe an average solution of nanostars.

Angle-resolved TEM images have given the most accurate depictions of individual gold nanostars (AuNS), at the cost of a significant amount of experimental time [96–99]. Theoretical studies have suggested that the resonance modes of a complex structure are based on hybridisation between the resonance mode(s), or eigenstates, of the elementary parts that make up a hybrid structure, and the volumetric ratio between them [100]. In the case of a AuNS, these elementary parts are the core (sphere) and tips (cones) [101]. Unfortunately, these measurements would require a researcher to find the average tip shape on many individual AuNS in order to find a bulk average. Due to the complexity of this process, AuNS are commonly described by their resonance wavelength. While the structures of individual nanostars can be explored and modelled, the results are not necessarily representative of the bulk solution [102].

AuNS are predominantly used as photothermal particles, transferring light into heat at higher wavelengths, or for localising electric fields at their tips. The former can be ideal for inducing hyperthermia in cells, PTT, while the latter is helpful for applications such as surface-enhanced Raman scattering and fluorescence-based sensing, which can be improved by using AuNS with many tips [34, 102]. Therefore, it is desirable for the method developed here to approximate the number of tips on each nanostar.

Quantifying the approximate number of tips can give insight into the

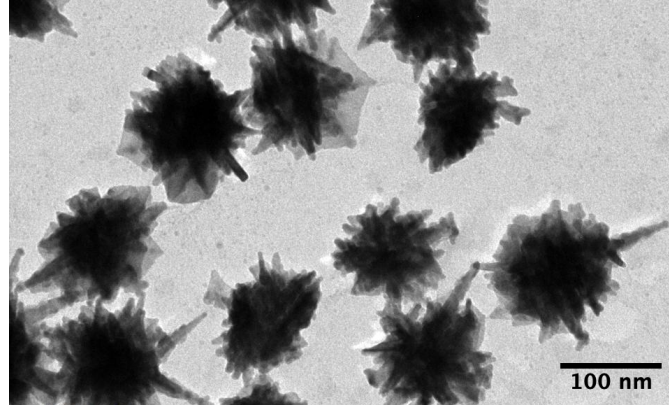


Figure 3.1.1: TEM image of a solution of AuNS with a polydispersity of 0.036, as measured by dynamic light scattering. Despite the monodispersity of the solution, a range of core radii and tip lengths can be identified. The solution was synthesised using the method described in this chapter. Reproduced with permission from Morton, W. et al [89]

particle's surface area, a crucial step for understanding their utility for photothermal therapy (PTT) [103, 104]. However, depending on the tip's geometry, there can be an increase in uptake into cells, degradation of cell membranes, or higher adsorption to the outside of the membrane [103]. It is therefore increasingly necessary to correlate the geometric characteristics of these nanostructures to their optical properties and their cellular uptake, immune system evasion and cargo delivery [81, 104].

This introduction shows that a wider adoption of AuNS depends upon their reproducibility and characterisation, but standard methods are not necessarily applicable [69]. A simple method is presented to generate average geometric and optical information about nanostars based on their UV-Vis spectra and TEM images, setting a precedent to include electromagnetic models as a necessary characterisation method, complimentary to experimental observables.

3.2 Methods

Modelled AuNS using the point-based volume method described in Chapter 1 vary in size but are always comprised of more than 10^4 dipoles to meet the inter-dipole spacing criterion, $|m|kd < 0.5$. Here, m is the complex refractive

index of gold, k is the wavenumber of light, and d is the interdipole spacing. In all calculations presented here, the largest value of $|m|kd$ used was 0.0384.

AuNS are run in DDSCAT, a free DDA simulation software, surrounded by an aqueous environment with refractive index (RI) = 1.33 to simulate water [53]. The dispersion information for Au is the commonly used Johnson and Christy dielectric constants [105]. While the Johnson and Christy constants were obtained from a film of bulk gold rather than a gold nanoparticle, the study aims to find relationships that can be used to characterise the nanoparticles. Each particle is generated 30 times and exposed to just one polarisation. Since tips are placed randomly around the particle, this ensures that an average, 'bulk' response is considered.

To experimentally create the AuNS used for the study, gold chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), silver nitrate ($AgNO_3$), sodium citrate tribasic dehydrate, L-ascorbic acid (AA) were purchased from Sigma-Aldrich, United Kingdom. Hydrochloric acid (37 %) was acquired from VWR International, United Kingdom. The heterobifunctional polymer thiol-PEG(7500)-amine was purchased from JenKem Technology, USA. For all synthesis and experiments, Milli-Q Type 1 ultrapure water was used.

AuNS were synthesised via a seed-mediated method. To make the initial seeds from which the nanostars were grown, 100 mL of a 0.25 mM aqueous $HAuCl_4 \cdot 3H_2O$ solution was heated to boiling in a 250 mL Erlenmeyer flask under magnetic stirring. Then, to make Au seeds of 41 nm, 0.25 mL of a 3.3 % (w/v) aqueous sodium citrate solution was added to the flask under vigorous stirring. After approximately 2 minutes, the solution appeared blue-purple, quickly turning a deep pink-red colour. The solution was left to stir under heat for a further 10 minutes to ensure full completion of the seed synthesis. This solution was then cooled in an ice bath, and its volume was made back up to 100 mL with Milli-Q water. The seeds were stable and could be stored long-term at 4 °C. Two sets of AuNS were grown using the seeds and growth solutions of different Au concentrations. Growth solutions were added by mixing 10 mL of a 0.2 mM ('small') or a 0.6 mM ('large') $HAuCl_4 \cdot 3H_2O$ solution containing 20 μ L of 1 M HCl and 600 μ L of seeds. While this solution was vortexed at high speed, 60 μ L of 10 mM $AgNO_3$ was followed quickly by 100 μ L of 100 mM AA . After 30 seconds of vortexing, the fully formed nanostars were then stabilised with 100 μ L of a 10 mM PEG spacer (7500 Dalton) containing a thiol group at one end and an amine

at the other.

The absorption spectra of the seeds and nanostars were recorded using a Cary5000 UV-Vis - NIR spectrophotometer in the wavelength range between 400 and 1200 nm. For approximate determination of nanostar hydrodynamic radius (R_h) and uniformity, dynamic light scattering was performed using a Malvern Zetasizer Nano ZSP. The structure and morphology of the nanostar samples were determined using a JEOL JEM2100 Plus transmission electron microscope operating at an acceleration voltage of 200 kV. Size measurements were collected using ImageJ. Gold concentrations were determined by inductively coupled plasma optical emission spectroscopy using a Thermo Scientific iCAP 6000 Series ICP Spectrometer. AuNS were centrifuged, filtered, and dissolved by adding 1 mL concentrated aqua regia (prepared freshly from HCl and HNO₃ in a 3:1 molar ratio) to 1 mL gold nanostar suspensions. After 10 minutes, 6 mL Type 1 Ultrapure water was added, making the final volume 8 mL before measurement.

3.3 Results and Discussion

3.3.1 Non-Uniform Tips

A core radius of 24 nm is chosen to represent a synthesisable structure that can be accurately simulated within a reasonable time. Tip length and radius are randomly chosen from a Gaussian distribution, centred on an average tip of length = 24 nm and radius = 12 nm, with standard deviation increasing from 1-30% of the mean.

A substantial decrease in Q_{ext} and a broadening of the full-width half maximum occurs as the variance of the Gaussian increases, and particles lose uniformity (Figure 3.3.1). Competing LSPRs from non-uniform tips are suspected to be responsible for these effects, which is valid for polydisperse particle solutions in literature [36]. The LSPR and volume change as polydispersity increases, but these shifts are small and without correlation, with the range being only 17 nm for the LSPR and $7.2 \times 10^{-3} \text{ nm}^3$ for the volume. The lack of variation suggests that there is strong correlation between the volume of the AuNS, and the optical properties, which will be further explored in Section 3.3.2.

Since non-uniformity in the width, length, or both increases the full-width

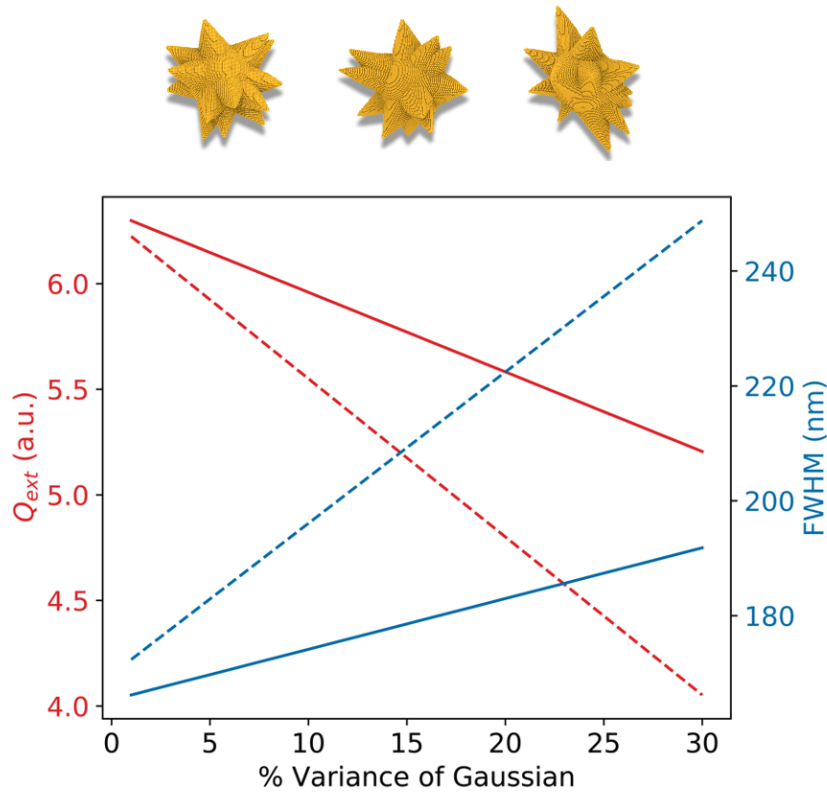


Figure 3.3.1: Change in Q_{ext} (red) and full-width half maximum (blue) with increasing non-uniformity of tip radii (dashed) & length (solid). Shown above are example DDA models of nanostars with increasing tip variability. Reproduced with permission from Morton, W. et al [89]

half maximum, it is impossible to discern from which geometric factor the experimental full-width half maximum originates. A constant core radius was used in this study, but it can be expected that including variation in core radius, which is commonly seen experimentally, will introduce another indistinguishable source of polydispersity. While this limitation is an unfortunate consequence of the complexity of the nanostructure, it also narrows the scope of study quite considerably. If there is no way to differentiate the source of polydispersity, then there is no need to include it in the model. Therefore, only the average tip dimensions will be used when comparing the modelled data to the experimental UV-Vis. This

simplification also has the added benefit of reducing the number of nanostars that need to be modelled.

3.3.2 Number of Tips

Understanding the scattering and absorption potential of AuNS is crucial for determining their optimal biomedical use, with strong absorption being ideal for photothermal therapies, and scattering for fluorescence enhancement. Therefore, a standard metric is to compare the $\frac{Q_{\text{sca}}}{Q_{\text{abs}}}$, which can help to determine their interaction with fluorophores and cells. Increasing the number of tips on a core of radius = 24 nm, from 1 to 40, causes an increase in the Q_{ext} at the LSPR, which is reported in Figure 3.3.2. The values in Figure 3.3.2 are scaled so a sphere with no tips lies at 0 to isolate the effect of tips. At lower volumes, Q_{ext} is dominated by Q_{abs} , but as the particle size increases, the scattering contribution increases, similar to gold nanospheres (Figure 3.3.2 A) [106, 107]. Regardless of the tip dimensions or aspect ratio, the same patterns are followed but with different rates of increase depending on the tip used. In comparison, the molar extinction increases with volume^{2/3}, regardless of tip dimensions, using the empirical relationship suggested experimentally by De Puig et al. and shown in Figure 3.3.2 B. [96].

3.3.3 Core Size

A robust model for a bulk solution of AuNS must consider the volumetric ratio between the core and tips. Inferring this ratio from the EM model would allow the approximate volume of the tips to be calculated since the core volume can easily be found in TEM images, unlike the number of tips. To date, the best approximation for the volume of a nanostar is obtained by using the volume of a sphere with the same projected area as the nanostar, the effective radius, a_{eff} . A comprehensive model to determine exact geometric features should be based on the observable a_{eff} and validated by modelling optical properties. In this section, by varying the core radius while adding identical tips, the relationship between the optical properties of nanostars and the volumetric ratio between elementary parts can be determined.

Increasing the core radius causes the LSPR of the structure to shift towards wavelengths of higher energy, which pushes the LSPR towards the resonance

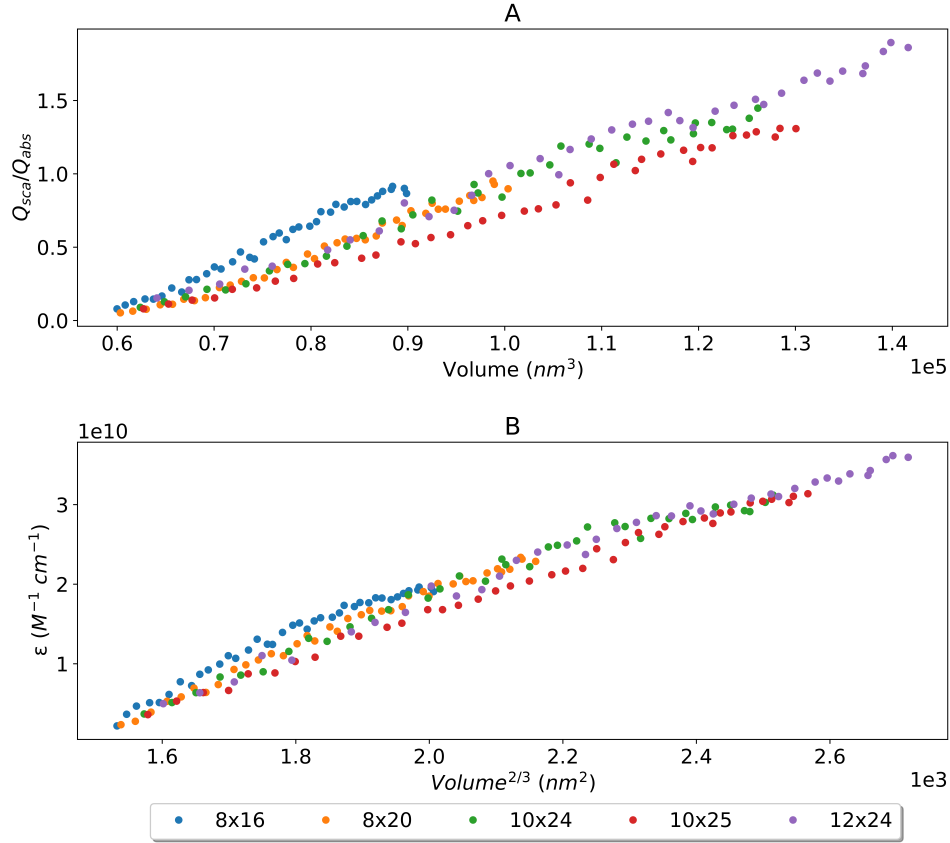


Figure 3.3.2: A nanostar with a core radius of 24 nm and various tip geometries (shown in legend as radius $\times$ length). As more tips are added, the overall volume increases, showing A) a linear relationship between molar absorptivity and volume^{2/3}, and B) a linear increase in the dominance of Q_{sca} on the extinction spectra. Reproduced with permission from Morton, W. et al [89]

of the unhybridised elementary structures. As seen in Figure 3.3.3, particles with larger cores shift less, which could be associated with an increasing number of electrons present in the core. A nanostar with a large core remains excited by higher energy wavelengths, with the smallest range of LSPRs out of the radii considered. As the spherical core grows larger, the LSPR effect of gold diminishes, and has a smaller wavelength shift, which causes the plateau seen in Figure 3.3.3 [108].

The dynamic range should be considered the difference between the largest resonance wavelength for a given tip geometry on a core, typically 5 tips, and the saturation resonance as many tips are added. A larger dynamic

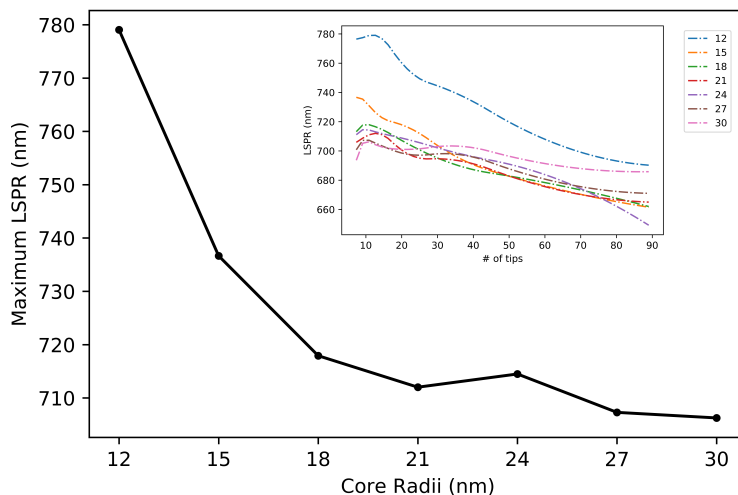


Figure 3.3.3: Change in LSPR for varying core radii with 5 tips. All tips have identical dimensions. The inset shows how LSPR varies with an increase in tips for spheres with different radii. Reproduced with permission from Morton, W. et al [89]

range would give more sensitivity towards the addition of tips and provide a more accurate estimation of the volumetric ratio between the core and tips. In the systems studied above, a core radius of 12 nm has a dynamic range of 88 nm, while a core radius of 30 nm only has a dynamic range of 20 nm. Therefore, the core radius of the nanostar will limit the accuracy of all nanostar models. This unfortunate limitation means smaller nanostars will be more accurately modelled, but it does not invalidate the modelling of larger nanostars. The limitation only implies that the number of tips must be discerned through alternative methods.

3.3.4 Application

After finding the limitations and relationships above, two test cases were prepared to see if it is possible to predict the average geometry of gold nanostars in two bulk solutions grown using small and large gold seeds. Various models will be created to determine which AuNS has the closest LSPR to experimental results. The core radius, tip length, and tip radius will be chosen based on the projection area measurement of 20 gold nanostars per solution using TEM images. For a moment, we will assume that the average tip length and radius are known, along with the radius of the core for

an experimentally synthesised solution of nanostars. A simple assumption can be made that only a fixed number of tips can fit onto the core of the nanostar due to spatial limitations of the tips base. Then, all that's needed is to add tips until the theoretical projection area matches that seen experimentally. Suppose the projection area is within 10% of that for the synthesised solution of particles. In that case, it is considered viable, and the volume and surface area of the nanostar are averaged over all viable nanostars. The core radius is established by identifying the largest perfect circle within the star's core. The circle that passes through the three longest tips determines the maximum radius, ensuring that a circle is formed. While this will favour the largest tip rather than the average tip size, it will only be used as an upper limit for choosing which tips to model. An example of how the geometric measurements are gathered can be seen in Figure 3.3.4. Experimental observables, such as the hydrodynamic radius and concentration of Au in the solution, along with the measured geometric properties used in the next step, can be seen in Table 1.

Table 3.1: *Experimentally observed properties of each nanostar solution*

Solution	Rh (nm)	Conc. (PPM)	Core Radius (nm)	Max Radius (nm)	Projection Area (nm ²)
Small	92	0.29	39	83	10.6×10^3
Large	111	0.60	52	113	18.6×10^3

Nanostars are modelled within the measured parameters, with tips at random orientations, and the average projection area is used as a success metric. Nanostars with similar projection areas are then modelled in DDSCAT to determine their LSPR.

Of the nanostars modelled, only one nanostar for each solution could reproduce the experimental LSPR, the geometry of which is seen in Table 2. However, should multiple nanostars have the same resonance and projected area, it would be up to the researcher to determine how best to treat the data (e.g. by averaging the properties of the nanostars or eliminating unreasonable possibilities). Figure 3.3.5 shows the extinction spectra of the modelled nanostars compared to those gathered from the experiment. The modelled LSPR of the small and large nanostars are 840 and 893 nm respectively. The resonance wavelengths match well, indicating an average tip size, and the ratio between core and tip volume have been identified. The modelled resonance of the nanostars was measured every 13 nm, and it

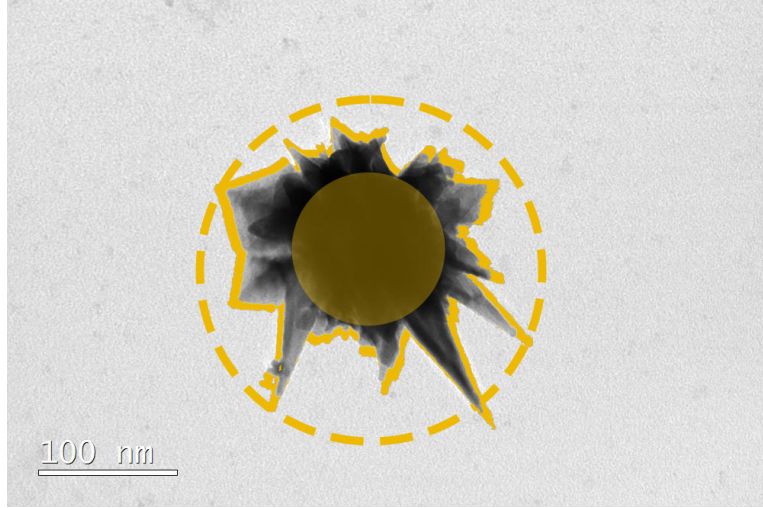


Figure 3.3.4: TEM image of Gold nanostar from the large growth solution, depicting the core and maximum enclosed sphere for calculating the maximum tip length. Additionally, the cross-sectional area is seen around the star to calculate a_{eff} . Reproduced with permission from Morton, W. et al [89]

is possible that a finer resolution might have resulted in better agreement. However, in most numerical modelling of nanoparticles, agreement within less than 10 nm is typically acceptable [109]. In comparing the Q_{abs} between the two nanostars, the larger one has a greater absorption efficiency by 11%, making it a better candidate for PTT.

Table 3.2: Geometric properties of the modelled nanostars found to be representative of the experimental solution. Change in LSPR between experimental and theoretical results is used as a metric for success.

Solution	Δ LSPR (nm)	Tip Length x Radius (nm)	Volume (nm^3)	Surface Area (nm^2)
Small	3	39 x 14	4.23×10^5	6.5×10^4
Large	8	62 x 26	10.94×10^5	10.7×10^4

The results above estimate that the volume of the successful nanostar candidate is 50% of that which would be expected from just measuring a_{eff} . Nanostars with the same projection area were also found to have a 22% variation in volume, further suggesting that using a_{eff} alone is insufficient. Three times more growth solution was added to the 'large' solution, resulting in particles that have an estimated 2.6x increase in volume over the 'small' growth solution. Unreacted gold is common in some synthesis methods

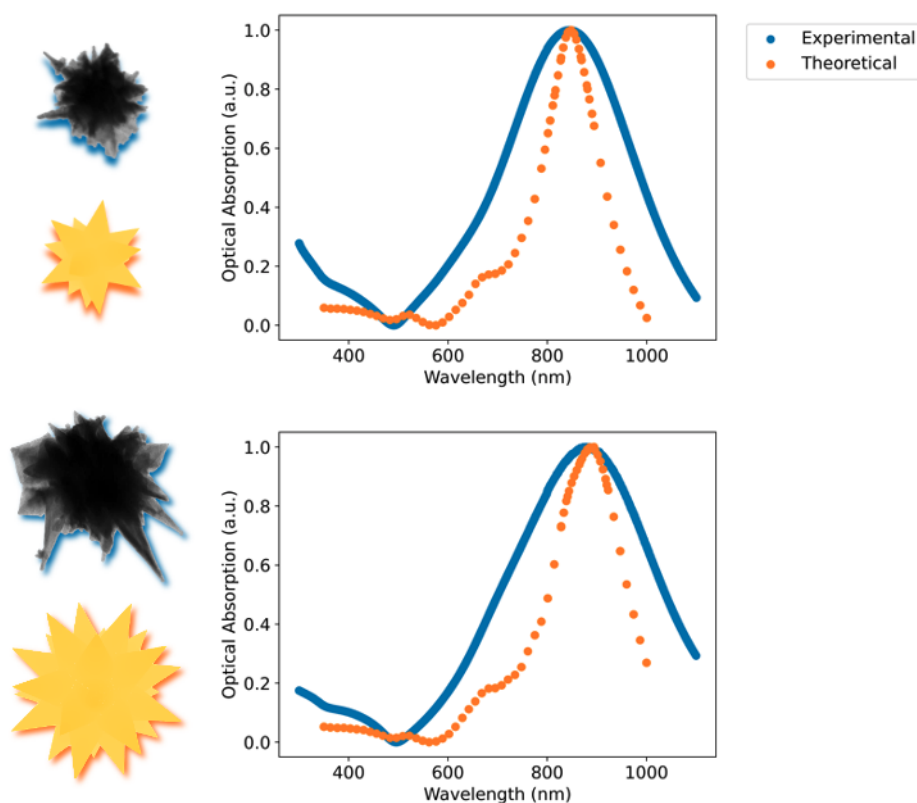


Figure 3.3.5: UV-Vis absorption spectra of the small (top) and large (bottom) nanostars, in comparison to the theoretical spectra of the modelled nanostars referenced in Table 2. Reproduced with permission from Morton, W. et al [89]

and may be responsible for the discrepancy. While unreacted gold makes calculating the exact concentration of a gold solution difficult, the method developed here is independent of this measurement.

3.4 Conclusion

The method outlined above is an improved alternative to traditional experimental methods used for estimating the size of nanostars. By utilising UV-Vis, TEM images, and DDA, the presented method can more accurately predict geometric properties such as particle volume, surface area, and tip count, enabling a comparison between the uptake of silica and gold nanostars with varying shapes. Without this method, there would be a limited ability

to accurately characterise the shape of the nanostars, as demonstrated in the previous chapter. If the TEM images are already available, the average volume and surface area of a bulk solution of nanostars can be determined using a single core on a computer with 8GB of RAM. Of course, the exact amount of memory, and the time it takes to measure the optical response at each wavelength is highly dependent on the size of the nanostar. However, for the nanostars in this study, no model took more than 24 hours to run. This method is therefore highly valuable for advancing research in this thesis and enhancing the characterisation of AuNS in literature.

4 Nanostars on Cell Surfaces

Notes

The following chapter is being prepared for a peer reviewed journal article as part of a series of papers investigating the use of silica nanostars for transport of drugs across the blood brain barrier: Alessandra Pinna, Ieva Ragaisyte, William Morton, Stefano Angioletti-Uberti, Alize' Proust, Rocco D'Antuono, Jakson Luke, Maximiliano Gutierrez, Maddalena Cerrone, Katalin Wilkinson, Robert J. Wilkinson, Ali A. Mohammed, McGilvery Catriona, Alexandra Porter (2023). Virus shaped silica nanostar for improving transport across the blood-brain barrier.

In the publication, I assisted with the microscopy analysis of nanostars, using the tools mentioned in Chapter 2. The molecular dynamics study in this chapter focuses on how the nanostar shape can impact cellular uptake. Although made from silica instead of gold, most of the results here were able to support the experimental conclusions from the work. Stefano Angioletti-Uberti provided guidance as a mentor for this project, but all of the following work, analysis, and writing was done by myself.

4.1 Background

The practical utility of nanostars in drug delivery and photothermal therapy, as explained in Chapter 1, relies on the cellular uptake of nanostars. In Chapters 2 and 3, gold nanostars were characterized by their shape. Chapter 4 refined a model for simulating the dynamics of nanoparticle uptake. Now, the information gained from each of the preceding chapters will culminate in the study of how lipid bilayer membranes interact with nanostars.

Uptake of nanoparticles in the previous chapter was focused on ligand-receptor interactions. For receptor mediated uptake, the formation of

ligand-receptor bonds releases chemical energy that balances the energy gain from bending the membrane around the nanoparticle. Here, the focus shifts towards thermal fluctuations of the membrane, which cause small oscillations in the Z-position of lipids in the membrane. Consider a nanoparticle diffusing towards a flat membrane; a thermal fluctuation moving the membrane closer to the particle elevates the likelihood of initial contact. Likewise, if a nanoparticle is already adsorbed to the membrane surface, thermal fluctuations raise the chances of the membrane encountering another binding site on the nanoparticle surface [110]. Not only do these fluctuations cause the membrane to interact with more of the nanoparticle, but they also help to reorient the nanoparticle into energetically favourable positions [111]. In reorientating the nanoparticle, the membrane can access areas of the surface with lower curvature, if any are present, or align unbound ligands to unbound receptors. These outcomes mitigate the energy gain from the Canham-Helfrich Hamiltonian for bending energy, reproduced from the last chapter here in Equation 4.1.1 [112].

$$E_{\text{bend}} = \int dA \left[\frac{\kappa}{2} (2H - C_0)^2 + \bar{\kappa} K \right] \quad (4.1.1)$$

This equation describes the energy required to bend the membrane per unit area, where H and K are the mean and Gaussian curvature of the surface, κ and $\bar{\kappa}$ are the bending and saddle-splay modulus, respectively, and C_0 is the spontaneous curvature. When the membrane is symmetric, meaning the same number of lipids in each leaflet, and there are no curvature inducing lipids, $C_0 = 0$, and $\bar{\kappa}$ is typically ignored, as in the membrane used here [113–116]. Experimental studies and previous models have shown that membranes wrapping nanoparticles increases the local curvature, H , where the bending modulus κ scales the penalty for bending [117, 118]. Since each lipid is attracted to surrounding lipids via their tails (Equation ??), out-of-plane movement creates a collection of oscillations similar to a ripple effect [111]. The magnitude and frequency of these oscillations are limited by κ , which acts as a restoring force that limits fluctuations in the Z-direction to the same order of magnitude as the membrane thickness [119, 120]. So, the bending modulus of the membrane determines the maximum fluctuation, which in turn limits the ability for the membrane to reach the next ligand.

Experimental studies investigating the entry of nanostars into HeLa cells

have shown a dramatic increase in their speed and uptake percentage over spherical nanoparticles [121]. However, these results are in conflict with other studies that demonstrate how spiked structures, especially those with high aspect-ratio tips, have been shown to rupture membranes, trigger immune responses, or simply adsorb to the outside of cell membranes [76, 77, 103, 122, 123]. The mere wrapping of a nanoparticle by a membrane is sometimes insufficient for drug delivery; the nanoparticle must also be internalised by the cell [124]. The distinction between being trapped within the membrane or residing inside a vesicle in the cell cytoplasm could significantly influence the nanoparticle’s exocytosis time, the opposite process to endocytosis, and drug delivery efficacy [123].

In Chapter 2, nanostars were characterised by the largest fractional surface area that a membrane could contact, if the scale of fluctuations was 5 nm. While the membrane would not immediately contact the entire area, over time fluctuations would allow the membrane to explore that surface. If ligands are placed evenly around the nanoparticle, then having a larger fraction of the surface within a short distance from the membrane seems advantageous. However, the high curvature of the tips may make the shape challenging to wrap, due to the higher bending energy required [125–128]. The relationship between these two effects is likely the cause of discrepancies in cell viability when using nanostars. The work here aims to determine if the variety of cellular interactions with nanostars can be attributed to their shape.

4.2 Methods

4.2.1 System Parameters

The same membrane composition from Chapter 4 was used here, with a temperature so that $k_B T = \epsilon$, which was held using a Langevin thermostat. A zero-tension condition was enforced in the XY plane using a Nose-Hoover barostat, and a WCA cutoff of 1.5 was applied. Half of the lipid heads on the membrane are receptors that can attach to ligands on the nanoparticle surface with a valency $X = 1$. Rather than utilising a tabulated potential to model the ligand-receptor interactions, a Morse potential is used, as depicted in Equation 4.2.1. This decision was primarily driven by its availability as

an in-built bond style in LAMMPS and high tunability for inter-atomic interaction potentials.

$$V_{\text{Morse}}(r) = \epsilon_{\text{LR}}[1 - \exp^{-a(r-r_o)}]^2 \quad (4.2.1)$$

A potential depth of $\epsilon_{\text{LR}} = 30 \epsilon$ is used, unless stated otherwise, with an equilibrium length of $r_o = 1 \sigma$ and $\alpha = 1 \sigma$. There is a 50% chance to form a ligand-receptor bond, when an unbound pair is within 1.3σ . If the bond length exceeds 4σ , the pair dissociates. All simulations were executed with an equilibrated membrane of size $78 \times 78 \sigma$, containing 10,452 lipids. Simulations ran for $1e6 \tau$ with a timestep of 0.01τ , and properties were recorded every $1e4 \tau$.

4.2.2 Membrane

The membrane's thickness was determined by projecting the lipid heads from the top and bottom leaflet onto a surface divided into equally sized bins of width 0.5σ in the XY plane. Empty grid spaces were filled using the linear interpolation package from SciPy [83]. Leaflets were assigned based on the angle of the normal vector that aligns the tail's last bead to the lipid head. The Z position of each head within a bin was then averaged, and the thickness was calculated as the average difference between the Z positions in the leaflets for each bin.

A mesh was created to approximate the midplane of the membrane using the same binning method, except with the Z position of lipid tails rather than the lipid heads. Vertices and faces were generated via Poisson surface reconstruction, which smooths surfaces by adding additional points to eliminate discontinuities [129]. Upon forming the mesh, the mean curvature, H , was computed at each vertex using the quadratic fitting method developed by Panozzo et al [130]. By multiplying the curvature by the barycentric area, or the area around each vertex $\hat{a}$, in the mesh and summing over the N vertices, the discrete total curvature, H_{tot} , was obtained. This area scaling is vital as the surface reconstruction method introduces extra vertices around areas of high curvature.

$$H_{\text{tot}} = \sum_{n=1}^N H * \hat{a} \quad (4.2.2)$$

Both of the methods mentioned in this section can be seen in Figure 4.2.1.

4.2.3 Ligand Placement

Although an ongoing debate exists concerning the adsorption and distribution of ligands on nanoparticle surfaces, comparisons will be made based on randomly distributed ligands and homogeneously distributed ligand patches [131, 132]. Nanospheres will continue to be the comparative particle in this chapter. As discussed in Chapter 4, this comparison is common practice to assess the uptake of novel nanostructures against a well-studied reference.

The nanostars used in this study have a core radius of 7σ , a tip length of 5σ , a base radius of 3σ , and a variable number of tips. The maximum radius of the nanostars, here 12σ , will be equivalent to the radius of the comparative nanosphere, with the same number of ligands placed on both particles to isolate effects based on the nanostar's geometry.

4.2.4 Simulation Measurements

The wrapping fraction, W_F , is a widely used metric to compare nanoparticle uptake in molecular dynamics simulations, indicating the extent of the nanoparticle surface interacting with the membrane. The time required for the wrapping fraction to reach one is defined as the wrapping or uptake time, T_u , for the nanoparticle. However, the region near the nanostar's core may never interact with the membrane due to the high curvature and small inter-tip area. Since T_u is only a consideration in this study for nanostars that are fully wrapped, the wrapping fraction was calculated by normalising $\sum_{n=1}^L V_{\text{Morse}}$, where L is the total number of ligand-receptor bonds, to one over all timesteps. Once the vesicle is formed, the total number of ligand-receptor bonds remains constant, barring some fluctuations. While not exactly representative of the amount of surface in contact with the bilayer, it accurately describes the formation of bonds which lead to wrapping, and determines when the particle is fully internalised.

Internalisation was quantified by measuring the centre of mass (CoM) distance between the nanoparticle and the membrane section not in contact with the nanoparticle. A CoM separation larger than the radius of the nanoparticle and membrane thickness indicates that the nanoparticle has

been internalised, rather than trapped in the membrane. At the same time, smaller values suggest that the particle is likely trapped within the membrane.

Lastly, lipid displacement, $|\hat{x}|$ is measured by determining which lipid heads are involved in wrapping, and averaging their displacement over windows of 100τ throughout the simulation. By only studying the lipids involved with wrapping, the slowing of bound lipids can be studied easily.

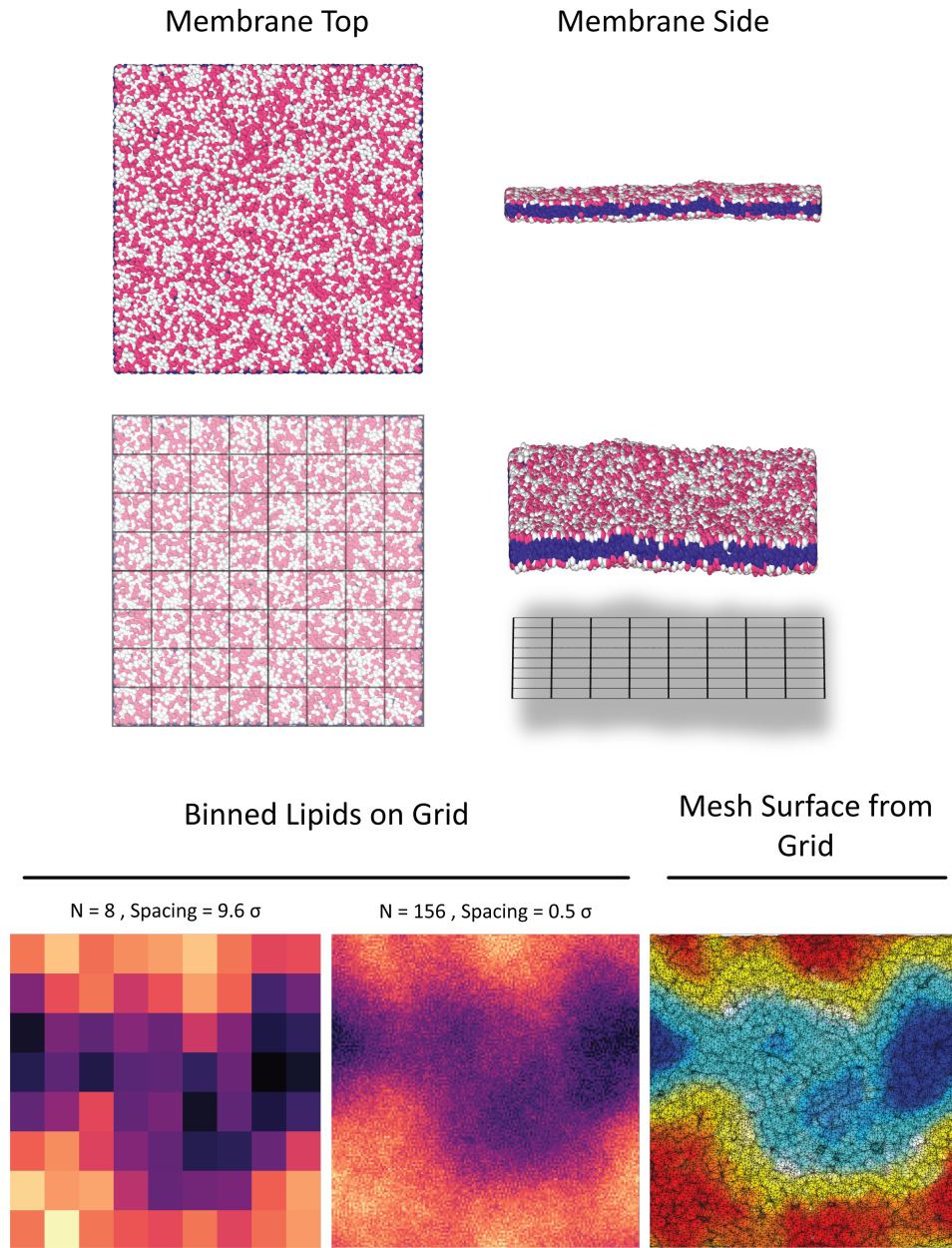


Figure 4.2.1: The coarse grained lipid bilayer is shown from the top and side profiles, and an example of splitting the membrane into equally spaced bins is shown. A membrane is shown being binned into a 8×8 , as well as 156×156 , array. The heatmaps show the relative height of the lipid tails, which are then used to generate a meshed surface where the curvature can be calculated.

4.3 Results & Discussion

4.3.1 Membrane Properties

The average thickness of the simulated membrane was determined to be $4.51 \pm 0.04 \sigma$, a value that also approximates the amplitude of thermal fluctuations [119]. This is of particular relevance when compared to experimentally derived measurements of lipid membrane thickness, typically around 5 nm [133]. Since the membrane thickness can vary experimentally depending on lipid composition, we assume here that $\sigma = 1$ nm for simplicity. Although the exact scaling relationship might exhibit some variability, having an understanding of this general scale is imperative. It serves as an essential bridge between the simulation and experimental realms, and underpins meaningful scientific discourse.

4.3.2 Distribution of Ligands on Nanostars

If ligands were placed randomly on the nanostar surface, the area closer to the core may be inaccessible due to the confined space between tips. Therefore, ligands were positioned on an increasing percentage of the tip length, ranging from 20-80%, for nanostars with 35 tips. Nanostars bearing ligands on only 20% of the tips displayed insufficient $\sum_{n=1}^L V_{\text{Morse}}$ to enable wrapping and were consequently excluded from subsequent analyses. For nanostars with a 40% tip coverage, implying that ligands were within 3σ of the maximum radius, the vesicle detached from the membrane during the simulation, as illustrated in Figure 4.3.1. Once completely wrapped, the CoM separation distance between the nanostars and the membrane was 21.32, 8.13, and 7.01σ for the tip coverages of 40%, 60%, and 80%, respectively. The closer the ligands were to the core, the more likely the nanostar would be trapped within the membrane.

The formation of a vesicle around a nanoparticle has four main regions. First ($\tau = 0$), initial contact is made and the membrane induces curvature to begin wrapping the nanoparticle. Next ($0 < \tau < 150$) a steady increase in bending energy is observed as wrapping continues, coming to a maximum when the membrane pinches to close the vesicle. The decrease in bending energy from $150 < \tau < 250$ mainly consists of reorganization of the membrane to optimize ligand-receptor bonds. Finally ($\tau > 250$), the membrane reaches

equilibrium, with the nanoparticle encapsulated in a vesicle [134].

After seeing that the nanostar with 40% tip coverage was not trapped in the membrane, a sphere with the same radius and number of ligands was tested for comparison. Ligands were placed in 35 patches across the surface of the sphere, providing a close comparison to the nanostar. Since both the nanostar and nanosphere have the same maximum radius, the vesicle formed after wrapping is the exact same size. Figure 4.3.1 shows that the nanostar with 40% tip coverage requires the same bending energy as the nanosphere. However, it takes longer to close the vesicle of the nanostar, spending more time at the peak membrane bending energy, an effect that will be explored later.

The simulations conducted with 60% and 80% of the tip covered in ligands demonstrated that the membrane bifurcates, forming a vesicle around the nanostar and entrapping it within the membrane. As evidenced by the graph in Figure 4.3.1, the energy penalty incurred to sustain a ruptured membrane appears to be offset by the formation of an increased number of ligand-receptor bonds.

The phenomena observed here is likely attributable to the specific membrane model used, and the high value of ϵ_{LR} . Other coarse-grained membrane models, where lipids consist of 4-16 beads rather than three, may present a larger energy penalty for membrane rupture. Nevertheless, as cited in the introduction, experimental studies have documented some nanostars' tendency to adsorb to the membrane or even become trapped within, suggesting potential validity in the findings presented here. Nanostars with a greater inter-tip area may have a larger concentration of binding sites near the core, but this is highly dependent on the surface composition of bound molecules, and requires further investigation [135]. Because the membrane can be so easily deformed, it was decided that the investigation of membrane rupture due to nanostar tips should be investigated in the future, rather than in this study.

To maintain a concentrated focus on the effect of nanostar internalisation by cell membranes, ligands will be situated on 40% of the tip in the remainder of the experiments carried out in this study.

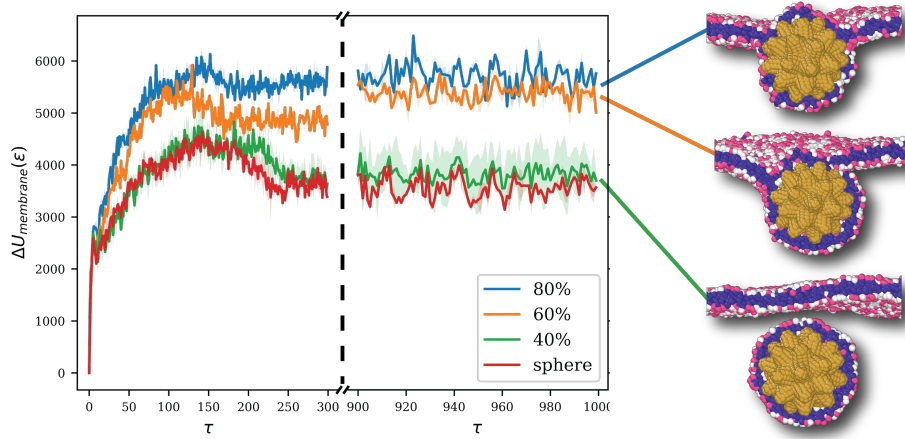


Figure 4.3.1: *The difference in potential energy (ΔU) of the lipid bilayer membrane as a function of time, from the state with a nanostar infinitely far from the surface to the wrapping state of the nanostar at time τ . Nanostars with ligands covering 80, 60, and 40% of the tip can be seen, along with an equivalently sized sphere. Although all are fully wrapped, two are trapped within the lipid bilayer due to membrane disruptions caused by the tips. A cross section of the final stage of the membrane, cut at the center of the nanostar, can be seen on the right hand side.*

4.3.3 Ligand Patches and Comparative Rates

Since nanostars used to model cell uptake will have high-density regions of ligands, referred to herein as ligand patches, a suitable spherical comparison is essential. In previous experiments, a random distribution of ligands on the surface of nanospheres was acceptable, but Figure 4.3.2 shows that this offers little control over ligand distribution. To generate accurate comparisons, ligand patches are applied to nanospheres as well, where the separation distance can then match those on nanostars.

Assuming ligands are homogeneously distributed over the nanosphere surface, their separation distance is likely to influence the wrapping time, T_u . A larger separation distance would require the membrane to undergo a greater bending penalty to connect to the next patch of ligands. When a critical separation distance is reached, the membrane may no longer be able to access new ligands. Thus, it is hypothesized that as the number of patches increases, the T_u will decrease, provided that $(\frac{1}{L} \sum_{n=1}^L \epsilon_{LR})$ is sufficient for wrapping the nanoparticle. The uptake rate of seven spheres, each with

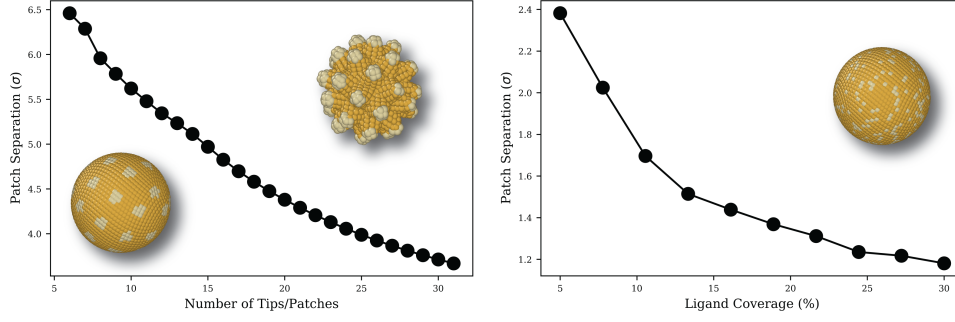


Figure 4.3.2: (Left) The distance between tips is more controllable, so spheres with patches have equivalent ligand distribution to nanostars. (Right) As the surface becomes increasingly crowded with ligands, the minimum distance between two randomly placed ligands should converge to 1σ . This holds true for spheres of differing radii, although it is not shown here.

different ligand patch sizes, was compared to a nanostar with 35 tips. Each nanoparticle has 280 ligands, though the patch size and separation distance differ, as presented in Table 4.1.

Table 4.1: Relative time to uptake a nanosphere with different ligand distributions, compared to a nanostar of the same maximum radius with 35 tips.

Patches	Ligands/Patch	Sep. Dist (σ)	$T_{\text{w-sphere}}/T_{\text{w-star}}$
56	5	2.75	0.78
40	7	3.23	1.01
35	8	3.46	1.11
28	10	3.81	1.61
20	14	4.38	1.72
14	20	5.11	NA
10	28	5.62	NA

Nanospheres with a ligand patch separation larger than 4.38σ , which is near the amplitude of membrane fluctuations of 4.51σ , could not be fully wrapped. This supports the hypothesis that the membrane fluctuations are insufficient to reach the next ligand patch. The data here also suggests that the nanostar's structure promotes wrapping in comparison to a spherical surface, by being uptaken faster than all but one distribution of ligands on the nanosphere. It is hypothesised that when interacting with a nanostar, the lipid bilayer experiences an increase in configurational flexibility, or degrees of

freedom, as compared to a nanosphere. These inter-tip spaces effectively act as relief areas that facilitate lipid reorganization during nanostar wrapping.

The membrane's increased adaptability during nanostar interaction can facilitate a more efficient wrapping process. In contrast, the uniform surface of a nanosphere may suppress membrane fluctuations, potentially resulting in slower and less efficient uptake.

4.3.4 Tips and Uptake

To test this hypothesis T_u was studied while increasing the number of tips on the nanostar from 10-40. It was ensured that the nanostar with 10 tips had a sufficient ($\frac{1}{L} \sum_{n=1}^L \epsilon_{LR}$) to be fully wrapped, by using a comparative nanosphere.

Figure 4.3.3 shows the final state of the nanostars with 10-25 tips. The tip-to-tip distance appears to limit the ability for the membrane to reach the ligands on the subsequent tips, similar to what is seen in Table 4.1.

For the nanostars that the membrane could wrap, the lipid displacement, $|\hat{x}|$, was measured as a function of wrapping fraction. The only lipids that were considered in this measurement, were those located in the vesicle at the end of the simulation, in order to isolate those interacting with the nanoparticles. From the results in Table 4.1, we expect to see if lipid displacement is greater with larger tip-to-tip separation distances.

A nanosphere with the same maximum radius ($R = 12 \sigma$) was simulated with 20%, 25%, 30%, and 35% ligand surface coverage, but the displacement showed little statistical difference with increasing ligand density. This lack of change is likely due to lateral rearrangement of the receptors being unnecessary in order to wrap, since there is a sufficiently high receptor density. It's hypothesised that at lower concentrations of receptors, there would be a more dramatic change in the rearrangement of the lipids in the vesicle.

The results shown in Figure 4.3.4 suggest that as more of the nanostar begins to wrap, the lipids slow down due to an increase in binding events which gives the required energy to wrap at the expense of decreasing the configurational entropy of the membrane.

Finally, to fully investigate the origin of the increased uptake speed, the total curvature of the membrane was measured at three different wrapping

fractions, which can be seen in Figure 4.3.5, 4.3.6, and 4.3.7. If the lipids truly have increased mobility, then they may also be able to rearrange, reducing the total curvature of the membrane and minimising the bending energy. The nanostar with 25 tips was used here, since it exhibited the most amount of lipid displacement in Figure 4.3.4.

In these figures, the total discrete curvature, H_{tot} is shown at a wrapping fraction, W_F , of 0.2, 0.5, and 0.8. Most of the curvature when $W_F = 0.2$ is still driven by normal membrane fluctuations, and so there is no discernible difference between the two particles. However, as wrapping increases H_{tot} remains lower for the nanostar, despite the nanostars having regions of higher curvature near the tips, as seen in the heatmap. This suggests that the membrane is able to relax in the area between tips, resulting in a smaller H_{tot} . The same procedure was carried out for each nanostar at $W_F = 0.8$, and the total curvature of the membrane seems to approach that of the sphere, represented as a dashed line in Figure 4.3.7.

The increased curvature present at the tips when $W_F = 0.8$ suggests that the membrane exerts a higher bending penalty for binding the ligands around the tip. To investigate the effects of this further, the minimum ϵ_{LR} should be studied for each particle.

4.3.5 Proneness

Endocytosis of nanoparticles can be characterized by two critical factors: wrapping kinetics, which dictate the speed of the uptake process, and what might be termed *proneness* — the thermodynamic favorability of the nanoparticle’s internalization. To test the proneness of both nanoparticles, ϵ_{LR} was lowered until the nanoparticle no longer fully wrapped within the simulation timeframe. Typical values used in previous publications range from $\epsilon_{\text{LR}} = 5\text{-}30 \epsilon$, but this is highly dependent on whether valency is enforced, the size of nanoparticles, number of ligands, and number of receptors [113, 134, 136]. Since both nanoparticles form a spherical vesicle of exactly the same size, the Canham-Helfrich Hamiltonian (Equation 4.1.1) suggests that $\epsilon_{\text{LR}}^{\text{min}}$ should be the same. Any differences would correspond to higher energy barriers, arising from changes in the local bending energy, between two partially wrapped states.

For a nanosphere of radius 12σ , the minimum ligand-receptor binding

energy necessary to facilitate wrapping is $\epsilon_{\text{LR}}^{\text{min}} = 18 \epsilon$. Contrasted to a nanostar with 35 tips, an identical total radius, and an equivalent number of ligands, which requires $\epsilon_{\text{LR}} = 24 \epsilon$.

This increase in required ϵ_{LR} for nanostars is attributable to their distinct geometry. Nanostars present an energy barrier during specific stages of wrapping, primarily due to their high-curvature tips, similar to deformable polymer nanospheres [134]. Wrapping initiation requires the membrane to conform to the first contact point on the tips, which have a substantially higher radius of curvature than a spherical particle. Therefore, the membrane must either incur a high bending penalty to access all of the ligands and continue wrapping, or the ϵ_{LR} must be high enough that not all ligands are needed. A similar challenge arises as the vesicle formation nears completion, in the partially wrapped state before reaching a fully wrapped state. In Figure 4.3.1, this was the point where the membrane took longer to close the vesicle for the nanostar than the nanosphere. Additionally, it is where the highest curvature was measured in Figure 4.3.6. As the membrane can interact with only a fraction of the tips during the initiation and final stages of wrapping, a higher ϵ_{LR} is necessitated to compensate for the high local curvature of the tips.

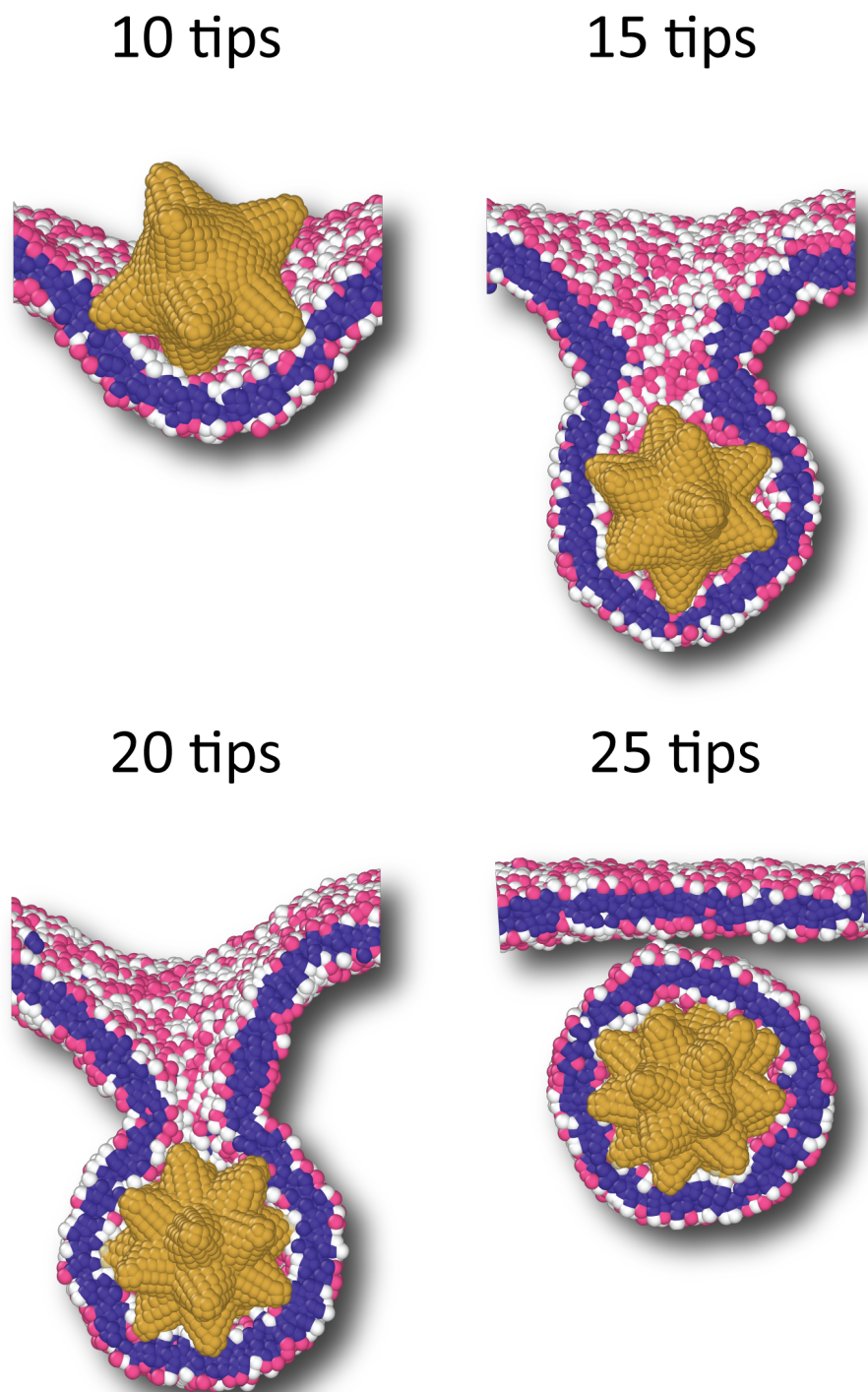


Figure 4.3.3: A cross section of the bilayer membrane that shows the final position of the nanostar for an increasing number of tips.

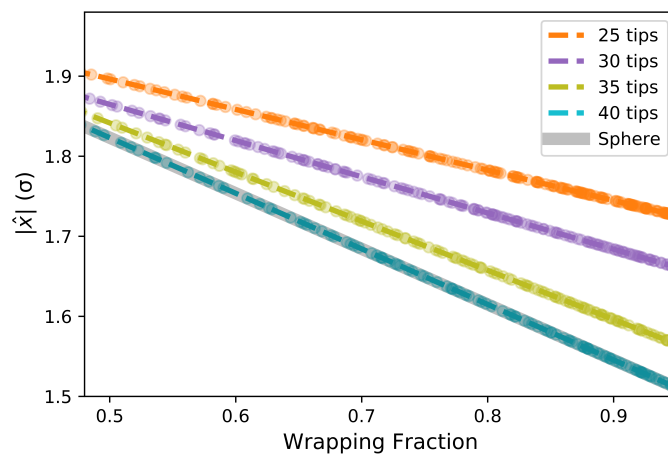


Figure 4.3.4: As the number of tips increases from 25 to 40, the nanostar more closely resembles a sphere, reducing the speed of lipids moving around the vesicle.

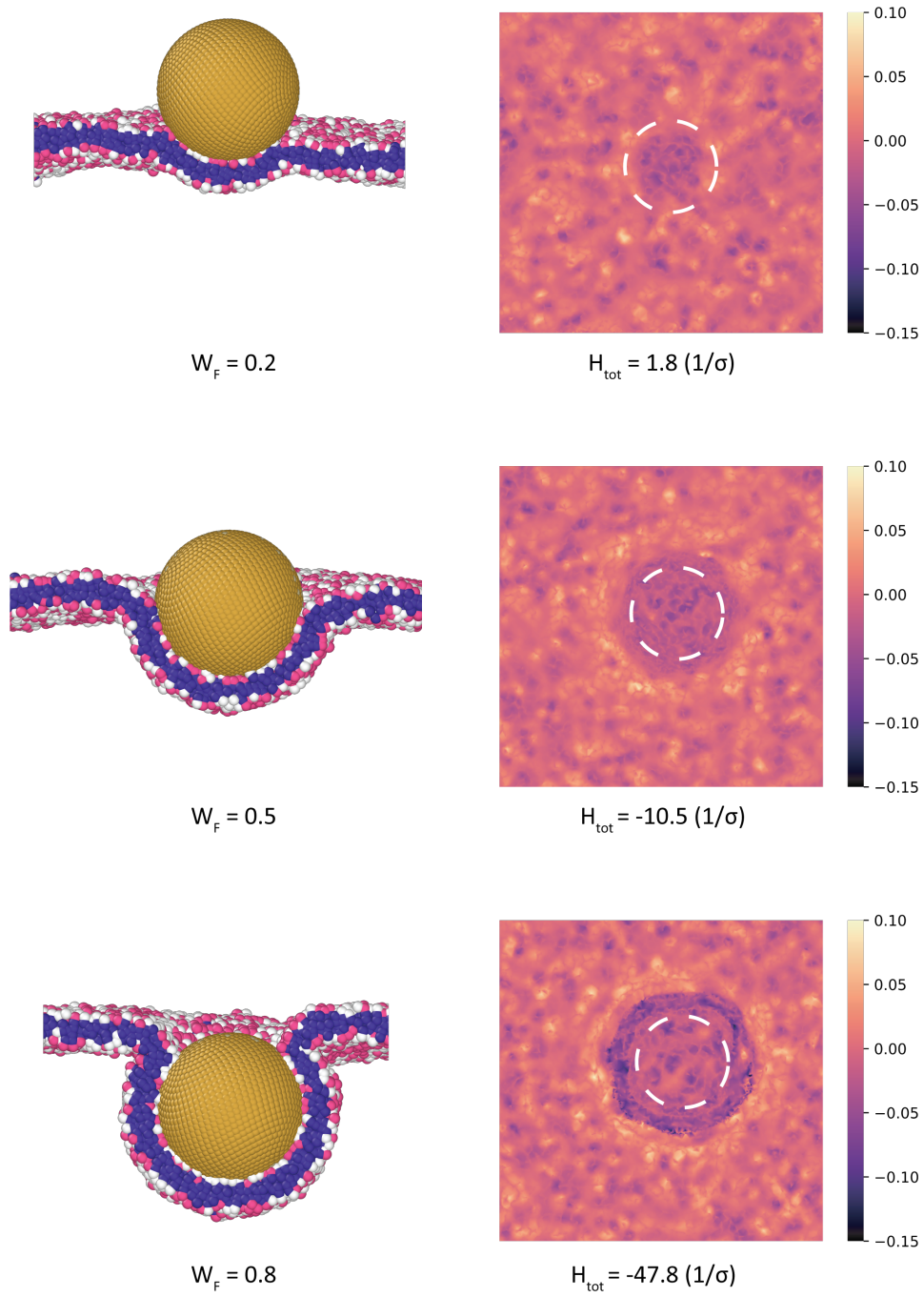


Figure 4.3.5: Snapshots of the simulation where a nanosphere of radius $= 12 \text{ nm}$ is wrapped by a membrane. A mesh of the membrane is generated, and the discrete curvature is found at every point shown and shown in the heatmap to the right. The projection of the nanosphere is outlined overlaying the map.

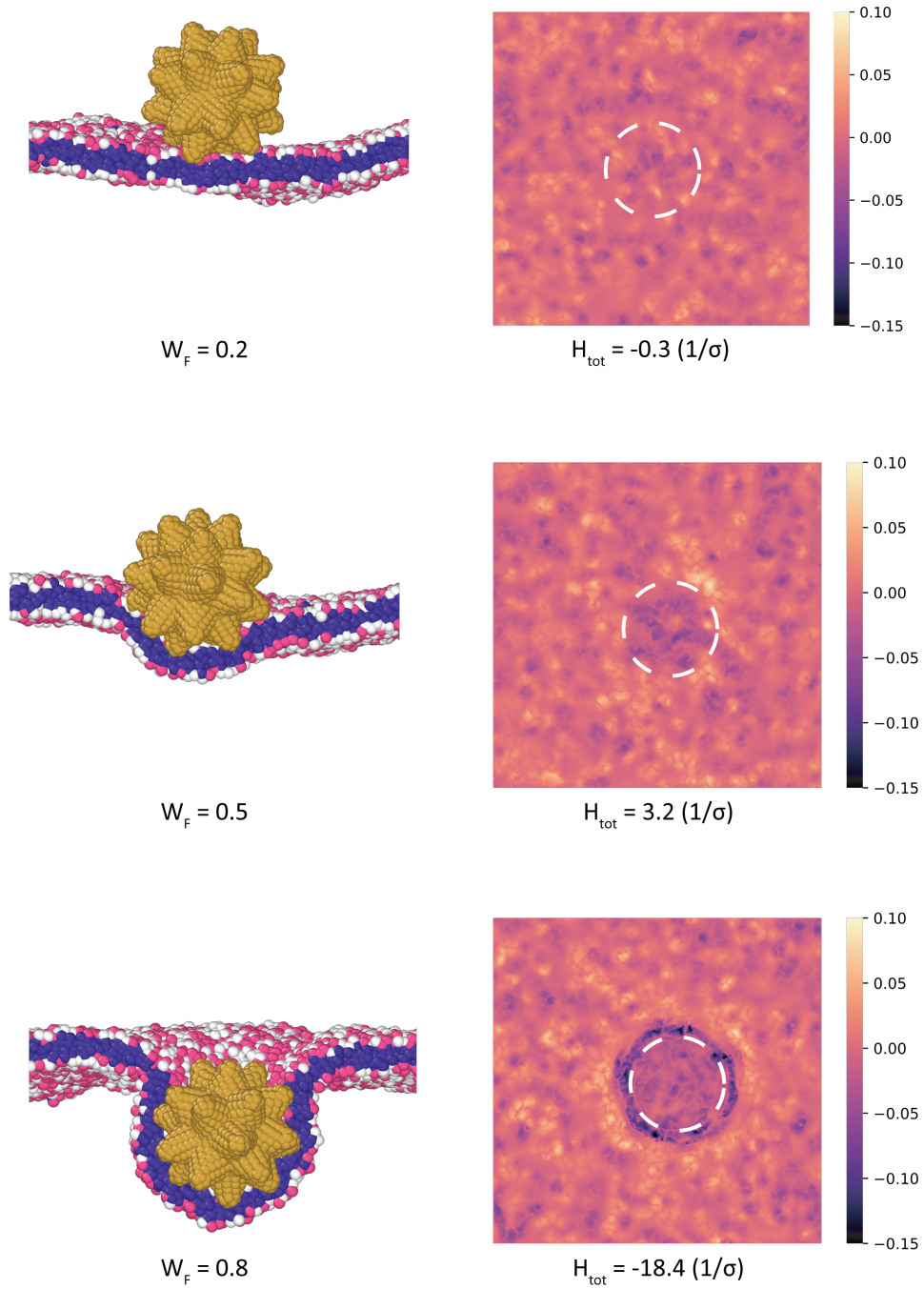


Figure 4.3.6: Snapshots of the simulation where a nanostar with 25 tips is wrapped by a membrane. A mesh of the membrane is generated, and the discrete curvature is found at every point shown and shown in the heatmap to the right. The projection of the nanostar's total radius is outlined overlaying the map.

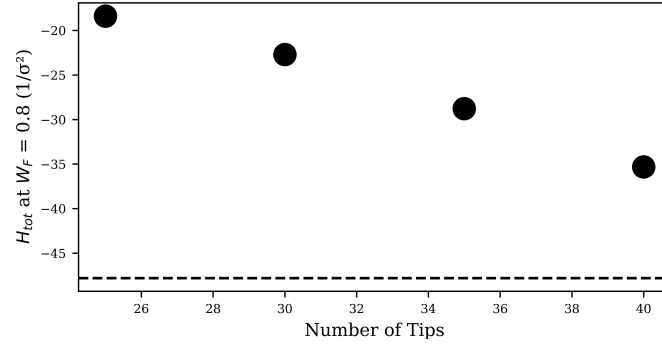


Figure 4.3.7: H_{tot} of the membrane for nanostars with increasing tips when the wrapping fraction reached 0.8. As the number of tips increase, the nanostar more closely resembles a sphere, and the curvature approaches the limit as shown by the dashed line.

4.4 Conclusions

The results from this chapter indicate that there are situations where nanostars could be uptaken faster than nanospheres of comparable radii. This increased speed of uptake is primarily attributed to the enhanced configurational flexibility of the vesicle by the inter-tip regions of the nanostar. However, the uptake is highly dependent on the strength and density of the ligand species on the surface. An experimental investigation into the surface species on nanoparticles would greatly advance future studies into the efficacy of nanostars.

For now, the function of nanostars can be tuned using the understanding of how shape impacts their membrane interactions. Nanostars developed for imaging of the cell membrane should have a large tip-to-tip separation distance. For targeted drug-delivery, the tip-to-tip separation distance should match the thermal fluctuations of the target cell. Unfortunately, the exploitation of this benefit is highly dependent on consistent and uniform synthesis methods, of which only very few are known for gold nanostars.

Given these characteristics, nanostars emerge as promising candidates for multivalent targeting strategies, using a population of multiple ligand species comprising both strong and weak binders [137]. The strong binders could circumvent kinetic barriers, while weak binding sites might further increase lipid mobility around the nanostar, enhancing its overall uptake efficiency.

Future work should investigate the impact of long and slender tips on membrane perforation. The current three-bead model may be ill-suited for such a study. Just two beads representing the lipid tail may be too easy to splay apart. Generally, simulations concerning inter-membrane interactions should use more detailed models. Future work could consider more detailed lipid models to capture these subtler aspects of nanoparticle-membrane interactions.

5 Conclusions

5.1 Overview

The work in this thesis set out to use experimental and theoretical techniques to better inform the synthesis of gold nanostars for photothermal therapy. In developing a therapeutic nanostar, the largest obstacle is delivery to the target cell [19]. The nanostar loses its usefulness if the feature that helps it heat better than nanospheres also prevents it from entering the target cell. By focusing on determining the shape of nanostars in Chapters 2 and 3, the cellular uptake could be studied in Chapter 5. While no perfect candidate for photothermal therapy was found in this thesis, the research and methodology can begin to narrow the selection criteria. For example, from Chapter 3 in Figure 3.3.2, we can see that a lower volume of tips will increase the absorption cross-section of a nanostar. However, in Table 4.1 from Chapter 5, we can see that many tips are needed to ensure the cellular membrane can uptake the nanostar. So, a nanostar with many small tips would be an excellent candidate for photothermal therapy. The exact shape and number would depend on the wavelength of the localised surface plasmon resonance, which should be between 625 - 1200 nm, to reach the largest penetration depth in the body [15]. But, this could be modelled using discrete dipole approximation and the nanostar structures developed herein. From Chapter 2, we can hypothesise that a nanostar with these physical characteristics would have a good predictability of volume from transmission electron microscopy.

The synthesis methods currently available don't allow for such fine control, but this information can help to guide the selection of viable methods. For example, the silver seeded method can result in nanostars with a few large tips and good control over the core radius, whereas the one-pot 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) method can give fine control over the number and length of tips, at the expense of control

over the core radius [138, 139].

5.2 Summary

The following accounts for the summary of findings from the research conducted within this thesis:

- Nanostars with long and thin tips are more likely to have a larger overestimation of volume when characterised by transmission electron microscopy alone. (Chapter 2)
- When a nanostar can lie on a plane, with a high fraction of its surface area in contact, the volume is likely to be overestimated when characterised by transmission electron microscopy alone. (Chapter 2)
- As the number of tips on a nanostar increases, it is more likely to scatter light than absorb it. (Chapter 3)
- As the variability in tip dimensions increases, the ultraviolet-visible absorption spectra will become broader. (Chapter 3)
- A nanostars localised surface plasmon resonance is determined by the volume fraction of tips, the core radius, and the tip dimensions. The core radius and tip length can be found from transmission electron microscopy imaging. (Chapter 3)
- Discrete dipole approximation can be used to estimate the tip radius, and the number of tips needed to generate the average nanostar shape, representative of a bulk solution. (Chapter 3)
- Purely distance based pair-potentials can result in uncontrolled valency, or number of binding partners, between ligands and receptors in molecular dynamics simulations. (Chapter 4)
- There is a larger kinetic energy barrier to commence and finish wrapping a nanostar due to the high local curvature of the tips.* (Chapter 5)
- A tip-to-tip separation distance smaller than the thermal fluctuations of the target cell membrane are needed for a cell to uptake the nanostar.* (Chapter 5)

- The nanostar with a tip-to-tip separation distance closest to the size of membrane fluctuations will be wrapped by a membrane faster than those with smaller tip-to-tip separation distances, assuming they share the same total radius.* (Chapter 5)

* under the assumption that the nanostar is endocytosed via ligand-receptor interactions, and that ligands are localised on the nanostar tips.

5.3 Limitations

While the scope of the study in this thesis was broad, many sections could benefit from more time and research. Based on the wide range of nanostar structures, no new synthesis techniques are necessarily needed. However, the structural prediction method outlined in Chapter 3 would have benefited from verification using alternative synthesis methods. There are perhaps more limitations in the method, other than the limit from large nanoparticles, which would be apparent with different nanostars. It would then be beneficial to take the predicted geometries, and compare the results to the analytical model in Chapter 2.

The molecular dynamics simulations performed here were designed to isolate the effect of shape on nanostar endocytosis, but not surface modifications. It is unknown whether gold itself would have been in contact with a membrane *in vitro*, or if a protein corona around the nanostar would play a crucial role in its cellular uptake. Perhaps, the nanostar shape may result in minimal binding of proteins to the surface, or the extra surface area may have the opposite effect. An investigation, where different concentrations of dyed surface modifiers (such as PEG) were mixed with a solution of nanostars and then imaged, might have given more insights to include in the model from Chapter 5. However, this would have required the use of a more detailed molecular dynamics parameterisation, which would have either needed to be adapted from previous works or developed.

In short, laboratory time to experiment with nanostars is the common factor lacking throughout this thesis. It's crucial that when performing theoretical research, a strong foundation of experimental work can be used to verify results.

5.4 Perspective

With this work in place, the impact and measurement of geometric properties of nanostars are now better understood. While there is always room for improvement and further research, the direction of that research must be analysed. Will our collective understanding of gold nanostars reach a sufficient understanding to justify their use in medical treatments? More importantly, will this happen before they become irrelevant compared to novel techniques? Improving nanoparticle systems does not require the synthesis of novel particle shapes but a more coherent focus on the delivery and targeting of cells. Advancements in ligand and protein design have ushered in a new era, where cellular processes can be better controlled through biological species on nanoparticle surfaces. Now is the time to capitalise on this new scientific wave.

5.5 Ongoing Work

The work in Chapters 2 and 5 is directly linked to an ongoing collaboration studying the passage of silica nanostars through the blood-brain barrier (BBB). While this is a shift from the focus of using gold, all of the results in Chapter 5 are determined by shape rather than chemical composition. The advantage of silica nanostars is that drugs can be loaded in the volume of the nanostar, meaning the outside surface can be optimised for targeted delivery. Studying the passage of nanostars through a complex cell network is exciting research and precisely the challenge this thesis has built towards understanding. The project has been multidisciplinary, and my focus has been on reconstructing and analysing angle-resolved TEM images of silica nanoparticles, along with developing computational models that can explain experimental findings. Since silica is not plasmonically active, the method developed in Chapter 3 cannot be used in the described project.

I took a selection of gold and silica nanostars with me to CEITEC at Masaryk University in Brno, Czech Republic, where we have been using cryogenic electron microscopy facilities to investigate the uptake of these nanostars into lipid vesicles. Due to a temporary shutdown of the facilities, and the ongoing energy crisis, the work has been stalled several times. Some images have been acquired, but nanostar concentration in the lipid solution

has yet to be appropriately tuned. Figure 5.5.1 shows a brief glimpse into the work.

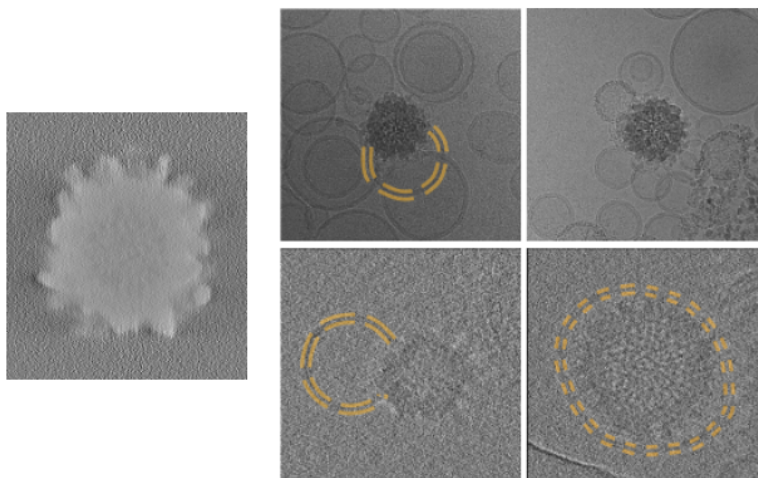


Figure 5.5.1: *Left: Angle-Resolved TEM image of silica nanostar. Right: 1:1 Molar Ratio of POPC:POPG lipid vesicles interacting with silica nanostars. Lipid vesicles beginning to wrap the nanostars are outlined in yellow for clarity.*

5.6 Future Work

The methods and understanding developed through this body of work can be applied to new and exciting fields. Two potential avenues of research directly relate to Chapters 2 and 5, which excite and inspire me to continue researching the topics. Before revealing these topics, a comparison must be made between the nanostar shape and the term 'virus-like nanoparticles'. Indeed, nanostars with a uniform distribution of tips resemble the same structure that has ironically hampered the production of this thesis; a virus with spike proteins. Silica nanostars can form uniform distributions with almost no deviation in tip radius or length. Therefore, these nanoparticle and coarse-grained simulations could offer a new route for research into the dynamics of viruses in cell systems.

The first avenue of research involves investigating the movement of nanostars, or viruses, across the surface of the membrane. Once one tip is bound to the membrane, the tip length and density will likely influence

whether the nanostar diffuses on the surface, walks - by creating a new set of bonds as the star rotates, breaking the previous bonds in the process, or rolls when two membranes come close together. This research could then be extended from cellular membranes to mucosal systems modelled as hydrogels or aggregates of similar structures.

The second avenue of research would investigate how the wrapping speed of these nanostars changes with different ligand species on the surface. A population of binding sites that are strong binders, slow to dissociate, and weak binders, fast to dissociate, may decrease the wrapping time of such complex structures. The thermodynamics of using multiple ligand species, or multivalent binding, has been studied extensively. However, the kinetics of the process has yet to be investigated.

The combination of the two research directions could characterise the likelihood of a nanostar, representing a virus stripped of most biological interactions, to move through the body or endocytose into a specific cell species. I intend to continue this work in my spare time while encouraging others to further our collective understanding of biological interactions with nanoparticles.

6 Appendix

Publications and Presentations

First Author:

Morton, W., Joyce, C., Taylor, J., Ryan, M., Angioletti-Uberti, S., & Xie, F. (2023). Modeling Au Nanostar Geometry in Bulk Solutions. *Journal of Physical Chemistry C*, 127(3), 1680–1686. <https://doi.org/10.1021/acs.jpcc.2c07520>

Second Author:

Xu, J., Morton, W., Jones, D., Tabish, T. A., Ryan, M. P., & Xie, F. (2022). Significant quantum yield enhancement for near infrared fluorescence dyes by silica templated silver nanorods. *Applied Physics Reviews*, 9(3). <https://doi.org/10.1063/5.0082187>

The work presented in this thesis was presented at the following:

- Department of Materials Postgraduate Research Day 2021 as a Poster Presentation
- Thomas Young Centre Lunchtime Seminar as a Talk
- Department of Materials Postgraduate Research Day 2022 as a Talk (Received a prize for Best Public Presentation)
- CCP5 2022 Summer School as a Poster Presentation

Scholarships and Awards

- Department of Materials PhD Research Scholarship (2019)
- Thomas Young Centre Junior Researcher Grant (2021)

Teaching Activity

Due to a shortage of instruction at the start of the pandemic, I began acting as a teaching assistant for many classes within the Department of Materials and Department of Bioengineering. In total I was a GTA for 12 courses across both departments, averaging 20 hours a week of GTA work for a year. The extra effort earned me the GTA of the year award for the Department of Materials in 2021.

Research Community Contribution

Each publication made involving work done throughout the timeline of this thesis is available for open access. All of the code to replicate results in published manuscripts are available for download to the public. It is my intention to take all of the analysis code written for this thesis, and submit it to MDAnalysis to improve LAMMPS compatibility.

Previous Thesis Topic & Progress Made

Characterising Super Sensitive Sensor Arrays

A non-negligible amount of time was spent investigating the development of super-sensitive biosensors. While the research was discontinued, here is a brief synopsis of the initial goals and results.

The reality of what was originally being developed was a substrate capable of large fluorescence enhancement by generating highly localised electric fields. Such surfaces could theoretically detect fluorescent markers at much lower concentrations, allowing for early disease detection. My design was a layered substrate with a periodic array of metallic gratings underneath the surface to expose a flat substrate to potential binding molecules. By burying the plasmonic elements in the material, the hope was to have a uniform surface for molecules to adsorb to without worrying about aggregation around specific features. Substrates would first be modelled and then created using the Thin Film Lab.

My training involved becoming proficient at sputter coating thin films of metals, maintenance and calibration of sputter coating systems, depositing uniform layers of polystyrene beads on the surface of glass, reactive ion

etching to remove surface impurities and control the size of polystyrene beads, and modelling electromagnetic properties using the Fourier Difference Time Domain technique. My hope was to one day become a lab manager for the Thin Film lab, since I had learned many of the necessary skills to do so when Sarah Fothergill and I were the sole users and operators of the lab over Covid (as part of a pandemic response grant - working with Cambridge Life Sciences).

The first stage in the project involved the modelling of substrate candidates to fabricate. I developed a genetic algorithm that could take in multiple parameters, such as the thickness of substrate material, grating material, shape, size, or separation distance, and alter these parameters until reaching a substrate with a considerable enhancement on the surface at a given wavelength. Initial tests were carried out in 2D as a proof of concept, where the algorithm consistently found the best-performing surface within 30-40 generations.

The next stage involved finding a robust surface resilient to fabrication errors. Developing a grating with a specific thickness and width proved challenging to do repeatably. A phase space was modelled to determine if a geometry offered minimal deviations in enhancement with fluctuations in thickness and width.

A lesson I was quickly learning: high electric field enhancement results in high errors. Enhancement is strongly correlated to localisation, meaning that if the substrate exhibited a larger maximum enhancement, it was likely more spatially confined. Fewer molecules could adsorb to high-enhancing areas, and there would be a more significant standard deviation of signal intensity at lower concentrations. The standard deviation is directly related to the limit of detection, and even if the sensor is designed to show the presence of markers, it nonetheless must reach a certain standard for use in the medical community. Analytical models were then developed for periodic substrates with different sizes, shapes, and electric field distributions to predict the minimum detection limit.

It was at this point that the research came to an end, and I was unable to further validate the analytical models I had developed.