Master's Thesis

Title: Cell Labeling by Colloidal Janus Nanoparticles

Name: Ms**.** Ilora Maiti

Roll. No.: 2023SMS002

Registration No.: 202302040101044

Supervisor: Prof. Nikhil Ranjan Jana

Program: MS (School of Materials Sciences)

Institution: Indian Association for the Cultivation of Science, Kolkata, India

Declaration

I hereby declare that the work presented in this project is the result of my original research, grounded in genuine scientific observations and findings. I affirm that no part of this work has been plagiarized from any source, and all data have been accurately represented without fabrication, manipulation, or misrepresentation. I have ensured that all references and sources of information are appropriately acknowledged and cited according to academic standards. This declaration is made in good faith and with a full awareness of the responsibilities associated with scholarly conduct.

……..	…….
Student's Signature	**Supervisor's Signature**
Date:	**Date:**

Abstract

Janus nanoparticles can label cell membranes significantly faster than respective isotropic nanoparticles, due to their surfactant-like property. In this study, we synthesized 60 nm Janus nanoparticles for extended cell membrane attachment. (see Figure 1) These nanoparticles comprise porous silica core terminated with asymmetrically conjugated hydrophilic dextrans on one side and hydrophobic decylamines at the other side. The attachment of Janus nanoparticles onto cell membrane has been explored and correlated with their colloidal property. Our findings suggest that Janus nanoparticles with anisotropic functionalization can label the cell membrane faster than the respective symmetric nanoparticles without any cytosolic entry and remains at the cell membrane for long time. Pores of these Janus nanoparticle can be loaded with drugs for cell delivery of drugs without the entry of nanocarrier into cell.

***Keywords*:** Janus nanoparticle, porous silica, drug delivery, cell labeling, endocytosis, healthcare.

Acknowledgement

It’s hard to believe that after spending so many months at the Indian Association for the Cultivation of Science, this chapter of my life is coming to an end. Looking back, I realize I had as much fun as the next student. The knowledge I gained during my MS degree from the School of Materials Sciences has enriched and enlightened me far beyond the scope of this thesis. I look forward to continuing down this path, as there is always much more to learn.

I owe a tremendous debt of gratitude to my thesis supervisor, Prof. Nikhil Ranjan Jana. I want to thank him not only for his financial support but also for his sharp insights and ability to tackle the most complex technical problems. I am grateful to have his overwhelming support as I embark on my career as a future scientist, and I hope to live up to his expectations in all my future endeavors.

I would also like to mention the people whose support was invaluable in bringing this project to completion. My sincere thanks go to Ms. Puja Garai, Ms. Nayana Mukherjee, Mr. Rajkumar Sahoo, Ms. Soumi Das, Dr. Abu Raihan Sarkar, Dr. Jayanta Dolai, and Dr. Debiprasad Roy for their innovation, dedication, and camaraderie, which have left an indelible mark on me.

I am grateful to my parents and my elder sister for their patience and encouragement throughout this process. Lastly, I want to thank my friends, Mr. Aiyush Saha, Mr. Sayan Maji, Mr. Syamantak Ray Sarkar, Ms. Riya Hazra, and others from the Materials, Chemical, and Physical departments for their assistance.

I am also thankful to all the faculty members of the School of Materials Sciences for providing me with the opportunity to pursue my project work, as well as to IACS for funding it.

Contents

1. Introduction

Janus particles, named after the Roman God, with faces directed towards the past and future are the particles whose surface chemical composition, physicochemical properties differ on two sides of the particle.[1-3] They exhibit two opposite but not mutually influential characteristics simultaneously. Depending on the ratio of anisotropicity, different materials have been engineered to show different effects in biological environment. Like- spherical, rod, disk etc. can be designed in polymeric, inorganic, and hybrid forms. They have a wide range of applications. In particular, the nanoscale asymmetry in a single particle and different physicochemical properties in its different parts offer surfactant like property, interesting assembly behavior and directional interaction/dynamics.[1-3] As a result, they have potential applications as micro/nanomotor,[4-8] emulsion stabilizer,[2] delivery carrier with specific advantages [8,9] and multifunctional theragnostic agents with enhanced performance.[1-3] like in amyloid fibrillogenesis,[4] sonodynamic therapy,[5] bacterial biofilm eradication,[7] treatment of multidrug-resistant bacterial infections,[8] drug delivery at the tumor site,[9] and enhanced brain delivery.[10]

Despite their unique advantages there are several shortcomings for biomedical applications. First, synthesis of colloidal Janus nanoparticles of less than 50 nm is challenging[11-14] but is necessary for cellular and subcellular targeting mechanisms.[15] Second, characterization of Janus structures at less than 50 nm regime is challenging due to requirements of sophisticated chemical characterization at such length scale. Third, cellular interaction with Janus nanostructures is not well understood and recent studies indicate that they can lead to interesting phenomena.[16-19] In particular, amphiphilic Janus nanoparticles shows cell membrane disruption,[16-18] anisotropically functionalized Janus nanoparticle offers extended cell surface attachment,[19] and amphiphilic polycationic Janus nanoparticle offers enhanced antibacterial activity.[19] These results suggest that Janus nanoparticles with anisotropic functionalization have wide range of application potentials that are yet to be explored. We design colloidal functional nanoparticle of 50 nm hydrodynamic size for cellular and sub cellular targeting and therapy.[20]

We have shown that nanoparticle size, nature of surface functional groups and number of functional groups per nanoparticle have significant impact on cell nanoparticle interaction, cell uptake mechanism and subcellular trafficking.[20] In this work, we have shown that nanoparticle terminated with decylamine groups

has unique interaction property with cell membrane that can be utilized for non-endocytic cell delivery with extended cell membrane anchoring. We have shown that covalent to electrostatic attachment ratio of a hydrophobic molecule can influence endocytosis or direct membrane penetration-based cell uptake mechanism.[16] The study suggests enormous impact to cell nanoparticle interaction via control of hydrophobic-hydrophilic balance at the nanoparticle surface.

2. Research Objectives

As previously reported, arginine-conjugated Janus particles demonstrated enhanced cellular uptake in CHO cells compared to the isotropic control particles. We plan to synthesize 60 nm Janus nanoparticles that are conjugated with dextran on one hemisphere and decylamine on the other using a Pickering emulsion-based masking technique. We anticipate hydrophobic decylamine-conjugated Janus nanoparticles will exhibit distinct bioactivity relative to previous Janus nanoparticles. We want to compare the colloidal stability and membrane affinity of Janus nanoparticles with control particles possessing isotropic functionalization and assess cell-labeling efficiency, surface anchoring duration, and cytotoxicity profiles in vitro.

3. Experimental Section

3.1 Materials

Tetraethylorthosilicate (TEOS), [3-(2-aminoethylamino)propyl]trimethoxysilane (AEAPS), tetramethylammonium hydroxide (25 wt %) (TMAH), octadecylamine, methyl morpholin N-oxide, octadecene, glutaraldehyde solution, decylamine, dextran (MW-6000), fluorescamine, sodium dodecyl sulfate (SDS), sodium cyano-borohydride ($NaCNBH_3$) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Sigma-Aldrich. Cetyltrimethylammonium bromide (CTAB) and N,N,N´,N´-tetramethylethylenediamine were purchased from Alfa Aesar. Ammonia (25 wt %) was purchased from Merck. 3- (4,5 Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Himedia.

3.2 Instruments

For hydrodynamic size and ζ potential measurement, a Malvern Nano ZS instrument was used. UV−visible absorption spectra were measured using a Shimadzu UV- 2550 UV−visible spectrophotometer, and fluorescence spectra was measured using a PerkinElmer LS 45. Fluorescence images were captured using Image-Pro Plus 7.0 software with an Olympus IX 81 microscope, Transmission electron microscopic (TEM) study was carried out using FEI Tecnai G2 F20 microscope by putting a drop of nanoparticle solution on a carbon coated copper grid.

3.3 Synthesis

3.3.1 Synthesis of nanoparticle incorporated porous silica particles

Synthesis is carried out using our previously reported method.[17] In brief, 2 mL hydrophilic solution of silica-coated iron oxide was taken in a 250 mL beaker. Then 45 mL distilled water and 5 mL 0.015 M CTAB solution were added under stirring condition. After 15 min of stirring 1.5 mL of 25 % NH_3 solution was added to this solution. Next, 0.5 mL ethanolic solution of tetramethyl orthosilicate (300 µL dissolved in 2.5 mL ethanol), 2 mL ethanolic solution of AEAPS (50 µL of AEAPS dissolved in 10 mL of ethanol) and 2 mL DMF were added stepwise at 5 min interval. The whole solution was kept under stirring condition for 3 h. After that excess ethanol was added for the precipitation of the particles.

Next, the particles were isolated by centrifuge and washed three times with ethanol and three times with water. For removing CTAB, the particles were dispersed in ethanolic solution of NH_4NO_3 (250 mg NH_4NO_3 dissolved in 25 mL ethanol) by sonication and the whole solution was heated to 80 ºC for 1 h under stirring condition. The process was repeated three times and finally the particles were dispersed in 5 mL of water and stocked for further use.

3.3.2 Preparation of silica particles stabilized wax microparticle

In a typical process, 20 mg of nanoparticle incorporated silica particle was dispersed in 15 mL water under magnetic stirring condition. The temperature was increased to 75 ºC and at that temperature 1 g of wax was added. After that 1 mg sodium dodecyl sulfate (SDS) was added to the solution. The resulting mixture

was kept for 10 min under stirring condition and then cooled to room temperature, giving rise to a solid wax microparticles with attached silica particles at their surface. These wax microparticles were filtered, washed with water for several times and dried under vacuum for 24 h. We get about 600-700 mg of particles for each batch.

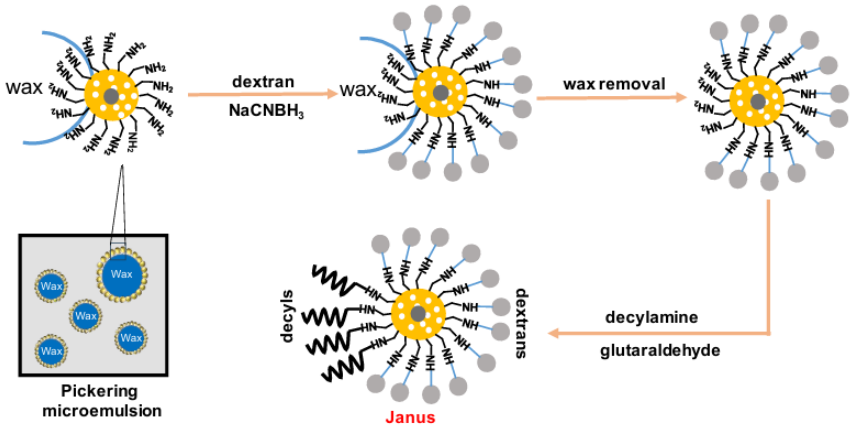

Figure 1. Schematic representation for the synthesis of Janus nanoparticle via one side attachment of decyl groups.

3.3.3 Preparation of Janus nanoparticles

Solid wax microparticles were used for functionalization with dextran by cyanoborohydride based conjugation chemistry.[19] Briefly, 600 mg wax microparticles was dispersed in 5 mL water and pH was adjusted to 9 by adding N,N,N´,N´ tetramethyl ethylenediamine (TMEDA). Next, 6 mg of dextran was dissolved separately in 500 µL water and mixed to the above solution followed by addition of $NaCNBH_3$ solution (18 mg dissolved in 200 µL water) and the solution was kept under magnetic stirring for 6 h. Next, solid particles were isolated from mixture via filtration and washed with water several times to remove the excess dextran. Solid particles were treated with cyclohexane-chloroform and sonicated for wax dissolution. The silica particles were then dispersed in 2 mL water-ethanol and used for decylamine functionalization. Decyl functionalization of silica particles was performed using decylamine via glutaraldehyde-based conjugation chemistry. In brief, 10 µL glutaraldehyde was dissolved in 250 µL ethanol and 20 µL decylamine

was separately dissolved in 250 µL ethanol. Next, two solutions were mixed, stirred for 30 min and then whole mixture was added to dextran functionalized silica particle (2 mL) along with continued stirring. After 30 min, 25 mg $NaCNBH_3$ was added and the stirring was continued for 6 h. Resultant particles are isolated by centrifuge and washed twice with ethanol and twice with water to remove excess glutaraldehyde/decylamine and finally dissolved in 1 mL (about 1 mg/mL) water for further use.

3.3.4 Preparation of control nanoparticles

We have synthesized a control silica nanoparticle having isotropically functionalized hydrophobic decyl groups. 5 mg primary amine terminated silica particles were dispersed in 5 mL water-ethanol mixture. In a separate vial, glutaraldehyde solution (10 µL glutaraldehyde dissolved in 250 µL ethanol) and decylamine solution (20 µL decylamine dissolved in 250 µL ethanol) was prepared and mixed together and after 30 min of mixing, it was added to the 5 mL nanoparticle dispersion under magnetic stirring condition. After 30 min, $NaCNBH_3$ solution (25 mg dissolved in 200 µL of water) was added and the stirring was continued for 6 h. The prepared particles were isolated by centrifuge and washed twice with ethanol and twice with water. Further, resultant control particles were dispersed in water.

3.4 Estimation of the primary amine

Fluorescamine-based titration method has been used to quantify the primary amines present in Janus particle as well as in control particles.[20] First, fluorescamine test was performed using glycine as a standard in the range of 20−400 µM. Typically, 100 µL of glycine solution was added to 800 µL of borate buffer solution of pH 9. Then 100 µL of acetone solution of fluorescamine (1×10^{-2} M) was mixed and fluorescence was measured at 493 nm (by exciting at 400 nm). A linear calibration curve was obtained by plotting the fluorescence intensity with respect to the concentration of glycine.

The linear equation was obtained as follows: $Y = 9.29*10^{-4}\ X - 63.62$ with $R2 = 0.99$

(Y is the glycine concentration in µM and X is the fluorescence intensity).

Similarly, fluorescamine test was performed for the Janus particle and isotropic control particles. The fluorescence intensity was measured at 493 nm and using the calibration curve described above, primary amines present was calculated.

3.5 Cell labeling study

Chinese hamster ovary (CHO) cells are commonly used as a model cell type for in vitro studies. We have conjugated nanoparticle with fluorescein using NHS-fluorescein for cell labeling study. Cells were cultured in DMEM medium with 10 % heat-activated fetal bovine serum (FBS) and 1% penicillin streptomycin at 37 °C and 5 % CO_2 atmosphere.

Cells were cultured overnight in a 24-well plate with 500 µL of DMEM medium and 70 µL of particle dispersion was added followed by 4 to 8 h incubation. After incubation, cells were washed with PBS buffer solution and washed cells were used for imaging study or further incubated with fresh medium for 8 to 24 h for long-term localization study.

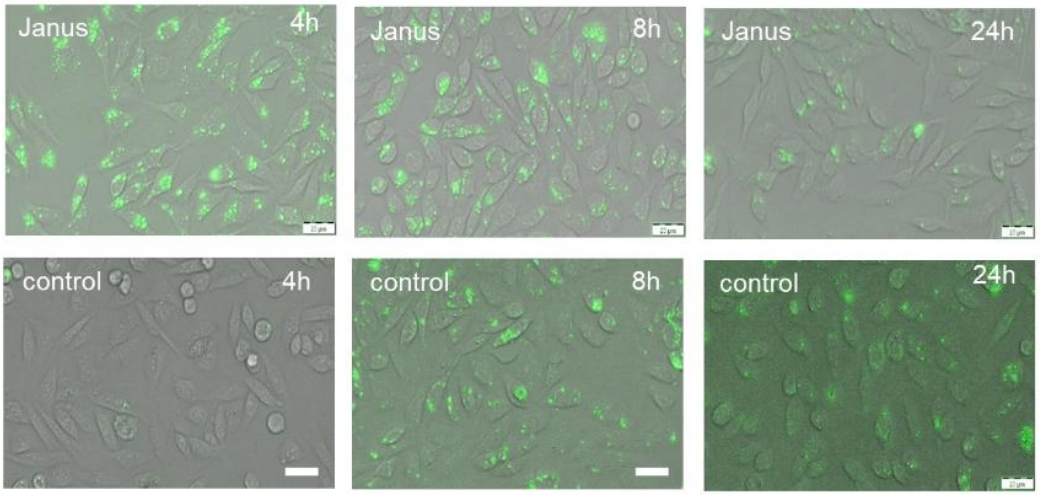

Fig 2. Enhanced membrane attachment of Janus nanoparticles in CHO cells than those by controls at 4h, 8h, and 24 h (from left to right).

4. Results

4.1 Functionalized Janus nanoparticle via Pickering microemulsion approach

Pickering microemulsion approach has been used for the synthesis of Janus nanoparticles.[20] Pickering microemulsion consists of liquid droplets stabilized by solid particles, rather than traditional surfactants.[20]

We tried stabilizing silica nanoparticle with wax microdroplets as a Pickering microemulsion. First, Au/iron oxide nanoparticle doped porous silica nanoparticles of 60 nm average size is prepares using our reported method.[17] These nanoparticles are terminated with primary amines that can be used for conjugation chemistry. These nanoparticles are used as stabilizer for Pickering microemulsion of wax. Typically, solid wax is mixed with aqueous dispersion of silica nanoparticle and heated at 75 ºC to convert solid wax into liquid wax. Under the stirring condition, liquid wax is converted to microdroplets terminated silica particles as stabilizers. Next, the mixture is cooled to room temperature, giving rise to a solid wax microparticles with attached silica particles at their surface (Figure 3). Wax side of primary amines of silica particles are blocked and rest of the outside amines are conjugated with dextran.

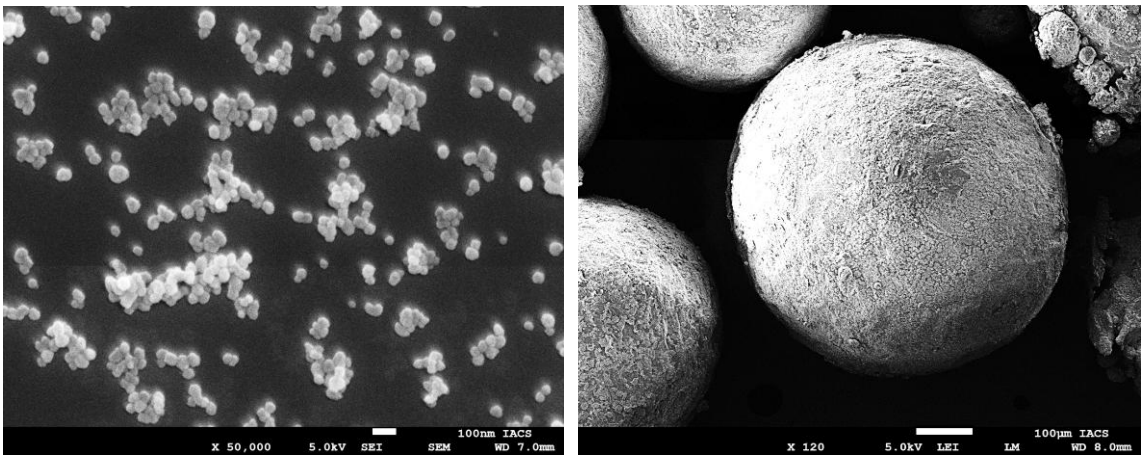

Figure 3: SEM image of silica nanoparticle stabilized single wax microparticles (left) and higher resolution SEM image of wax microparticles surface (right).

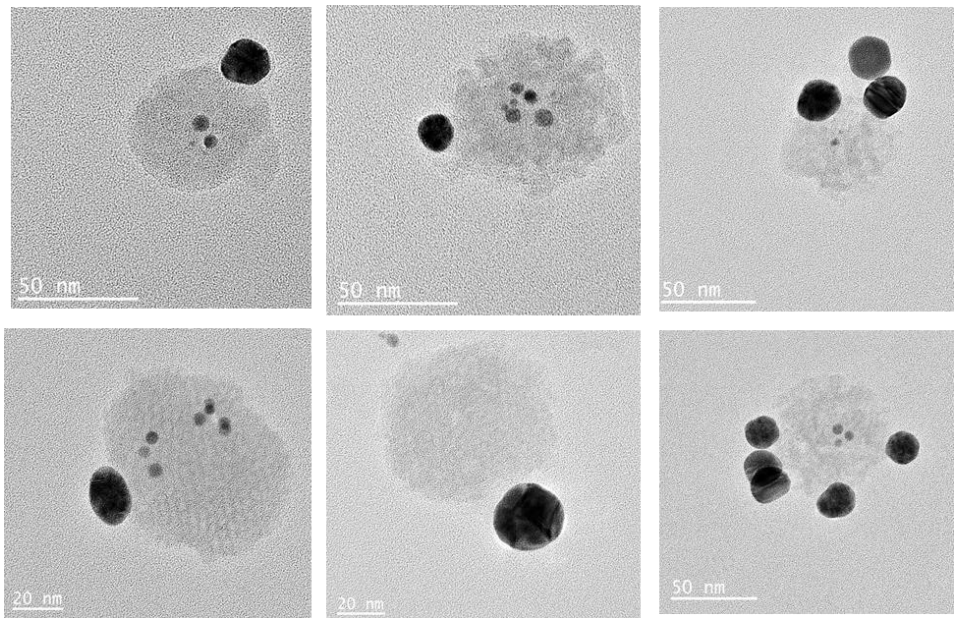

Figure 4: Evidence of accessibility of one side of silica particles and to identify Janus structure via electron microscopy. Dispersion of silica particle stabilized wax microparticles is incubated with gold nanoparticle (20 nm) and then wax is removed. All the TEM images show the gold nanoparticles attached to one side of silica particle.

Evidence of accessibility of one side of the silica nanoparticles, when present as Pickering microemulsion stabilizer, has been investigated via electron microscopy. When incubated with anionic gold nanoparticle (20 nm) and then wax is removed it has been found that 20 nm golds are attached only at one side of the silica particle. This one side attachment suggest that wax side of primary amines are not accessible for reaction and dextran functionalization occurs only at the other side. After dextran functionalization, wax is removed to uncover the amine groups of the previously masked side and then decyl functionalization has been performed via glutaraldehyde-based conjugation with decylamine. Control nanoparticle was prepared via isotropic functionalization of decylamine in absences of Pickering microemulsion and concentration of decylamine was adjusted to minimize the nanoparticle agglomeration.

Primary amines present at the silica nanoparticles surface has been estimated at each step of conjugation chemistry via fluorescamine test.[21] Results show that colloidal silica nanoparticles of 2.0 mg/mL have amine concentration of 116 µM that becomes 38 µM after dextran conjugation at one side and further lowered to 2 µM for Janus nanoparticle. Similarly, control nanoparticle with isotropically conjugated decylamine has

50 µM. These lowering of primary amine concentration at each step suggest they are reacted with dextran and decylamines.

Moreover, these findings indirectly suggests that the number of decyls in control particle is about 3 times than in Janus nanoparticle. Figure 5a shows the representative TEM image of Au nanoparticle doped silica particles at two different magnification that are primarily used for this study. The average size of the particles is 50-60 nm. Hydrodynamic size of the particles has been determined from DLS measurement. Both Janus nanoparticles and control nanoparticle show relatively larger sizes in the range of 50-300 nm due to their aggregation tendency. (Figure 5b) Janus nanoparticle have low positive charge as observed from Zeta potential and similar positive charge is also observed for to control nanoparticles. (Figure 5c) Such positive charge is coming due to remaining primary amine groups that results cationic charge via protonation.

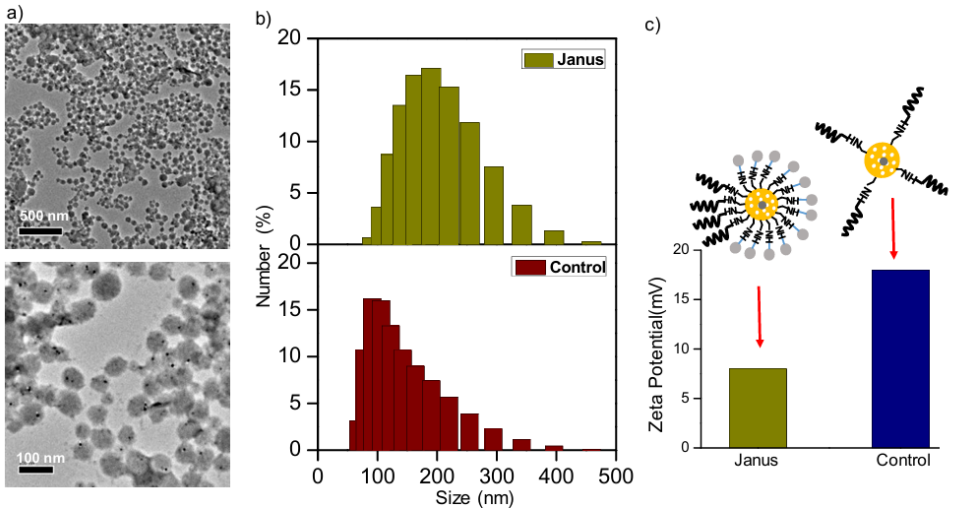

Figure 5. a) TEM image of Au nanoparticle doped silica particles at two different magnification that are primarily used for this study. b) Typical hydrodynamic size of Janus nanoparticles vs control nanoparticle with isotropic decyl functionalization. c) Zeta potential of Janus nanoparticle as compared to control nanoparticle with isotropic decyl functionalization.

4.2. Lower colloidal stability of Janus nanoparticles

We have extensively investigated the colloidal property of Janus silica nanoparticle and compared with control silica nanoparticle. Colloidal nanoparticles are prepared in water or ethanol and next their dispersion stability has been studied with time. It has been observed that Janus nanoparticles started to precipitate

after 2h both in water and in ethanol. (Figure 6) In contrast control nanoparticles remain dispersed till for 24h. This result clearly suggests lower colloidal stability of Janus nanoparticle.

We have further studied the particle aggregation via SEM imaging of Janus nanoparticle. Dilute solutions are used for sample preparation in order to avoid drying induced particle aggregation. Results show that Janus nanoparticles form smaller assembly involving 4-20 nanoparticles. Considering the presence of hydrophobic decyl groups at the particle surface particle-particle interaction is expected for both Janus and control nanoparticle. Moreover, considering the presence of 3 times more decyl groups, more aggregation is expected in control nanoparticle.

Although, we happen to observe more aggregation in Janus nanoparticle as evident by the higher hydrodynamic size shown in DLS measurement (Figure 5b). This was further anticipated by the increased precipitation of Janus nanoparticles shown in Fig 6 than that of controls. Presence of decyl groups in one side of Janus nanoparticle offers surfactant like property that drives particle-particle interaction similar to micelle formation via surfactant assembly.

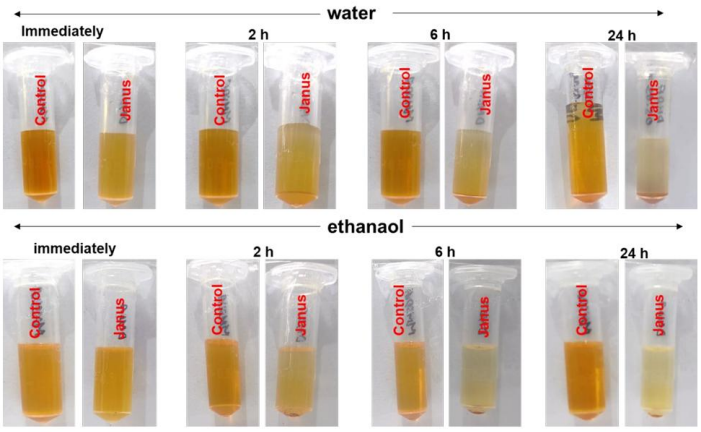

Figure 6. Evidence of lower colloidal stability of Janus nanoparticle as compared to control nanoparticle with isotropic decyl functionalization. Colloidal nanoparticles are prepared in water or ethanol with 1.0 mg/mL and digital images are captured till for 24h, showing that Janus nanoparticles started to precipitate after 2 h.

4.3 Extended cell membrane attachment of Janus nanoparticles

We have investigated the consequence of this lower colloidal stability of Janus nanoparticle in their interaction with biological interface. We have selected live cell for the study as the cell has lipophilic

membrane to interact. Cell-nanoparticle interaction is the key for biomedical application of Janus nanoparticle.

Typically, cells are incubated with nanoparticle for different time period and then washed cells are used for imaging under bright field or fluorescence mode and merged images are shown in Figure 2. Nanoparticle are conjugated with fluorescein for their tracking under fluorescence microscope.

There are two important observations that we have identified. First, Janus nanoparticles label cells faster that control nanoparticle. For example, Janus nanoparticles can label cells within 4h as compared to 8h requirement for control nanoparticles. (Figure 2) Additionally, extended attachment of Janus nanoparticle at cell membrane without entry into cytosol. For example, Janus nanoparticle can stay at the membrane for 24 hours. In contrast control nanoparticle can significantly enter into cytosol. These results clearly suggest the enhanced membrane attachment of Janus nanoparticles as compared to control nanoparticle with isotropic functionalization of decyl groups.

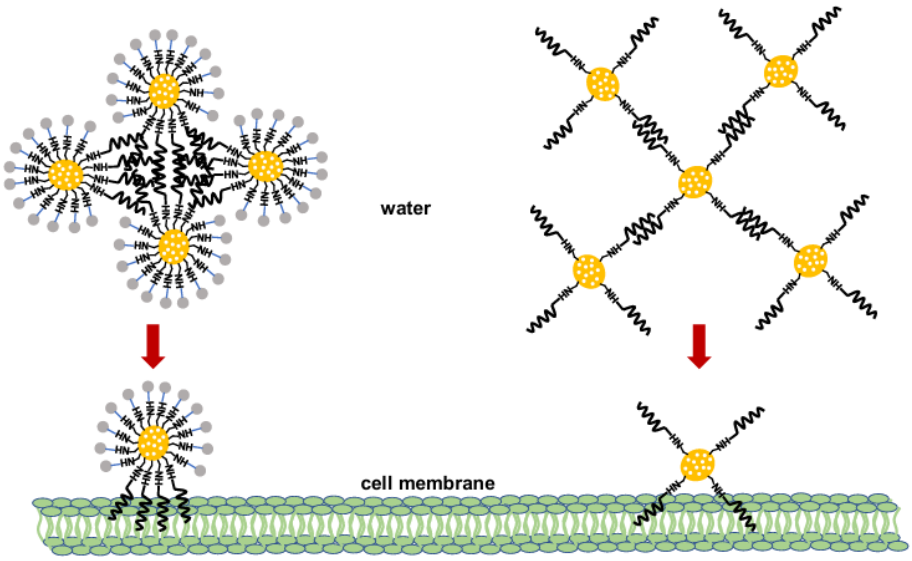

Figure 7. Proposed mechanism of self-assembly of Janus nanoparticles using decyl groups and their binding with cell membrane through decyl groups. Presence of decyl groups at one side induces the surfactant-like nature that leads to nanoparticle assembly via decyl groups and stronger attachment to cell membrane. In contrast isotropic decyl functionalization leads to poor decyl decyl interaction that leads to poor particle assembly and poor interaction with cell membrane.

Based on these observations, we have proposed a mechanism of enhanced particle-particle interaction due to Janus structure that leads to their poor colloidal stability and extended attachment to cell membrane. (Figure 7) Janus nanoparticles use their decyl groups for binding with another Janus nanoparticle or cell membrane. Presence of decyl groups at one side induces the surfactant-like nature that leads to nanoparticle assembly via decyl groups or stronger attachment to cell membrane whereas isotropic decyl functionalization leads to poor decyl-decyl interaction that leads to poor particle assembly and poor interaction with cell membrane.

5. **Conclusion**

This work emphasizes the impact of functional anisotropy on the interactions between nanoparticles and cell membranes. The Janus structure exhibits surfactant-like behavior, which promotes nanoparticle-nanoparticle interactions through decyl groups. This interaction reduces their colloidal stability and encourages attachment to the lipophilic cell membrane. In a comparative study, it was found that Janus nanoparticles can label cell membranes more quickly than symmetric nanoparticles and remain attached to the membrane for an extended period. Upon thorough examination, it can be concluded that Janus nanostructures possess unique physical properties compared to conventionally used isotropically functionalized nanoparticles, resulting in distinct interactions with biological interfaces.

6. References

1) Zhang, J.; Grzybowski, B. A.; Granick, S. Janus Particle Synthesis, Assembly, and Application. *Langmuir* **2017,** 33, 6964– 6977.
2) Jishkariani, D.; Wu, Y.; Wang, D.; Liu, Y.; Van Blaaderen, A.; Murray, C. B. Preparation and Self-Assembly of Dendronized Janus Fe_3O_4-Pt and Fe3O4-Au Heterodimers. *ACS Nano* **2017**, 11, 7958– 7966.
3) Kadam, R.; Ghawali, J.; Waespy, M.; Maas, M.; Rezwan, K. Janus Nanoparticles Designed for Extended Cell Surface Attachment. *Nanoscale 2020*, **12,** 18938-18949.
4) Hao, M.; Chen, Y.; Leisen, J.; Whitworth, T. J.; Xia, Y. Multifunctional Janus Nanoparticles Capable of Anchoring to the Cell Membrane and Serving as “Cellular Backpacks” for Advanced Theranostics. *JACS* **2025**, 147 (15), 12973-12981.
5) Lee, K.; Yu, Y. Lipid Bilayer Disruption by Amphiphilic Janus Nanoparticles: The Role of Janus Balance. *Langmuir* **2018**, 34, 12387– 12393.
6) Nguyen, D.; Wu, J.; Corrigan, P.; Li, Y. Computational Investigation on Lipid Bilayer Disruption Induced by Amphiphilic Janus Nanoparticles: Combined Effect of Janus Balance and Charged Lipid Concentration. *Nanoscale* **2023**, 15, 16112– 16130.
7) Wiemann, J. T.; Nguyen, D.; Bhattacharyya, S.; Li, Y.; Yu, Y. Antibacterial Activity of Amphiphilic Janus Nanoparticles Enhanced by Polycationic Ligands. *ACS Appl. Nano Mater*. **2023**, 6, 20398–20409.
8) Nguyen, D.; Bhattacharyya, S.; Richman, H.; Yu, Y.; Li, Y. Targeting the Weak Spot: Preferential Disruption of Bacterial Poles by Janus Nanoparticles. *Nano Letters* **2024**, 24 (49), 15886-15895.
9) Liu, W.; Wang, W.; Dong, X.; Sun, Y. Near-Infrared Light-Powered Janus Nanomotor Significantly Facilitates Inhibition of Amyloid-β Fibrillogenesis. *ACS Appl. Mater. Interfaces* **2020,** 12, 12618–12628.
10) Žiemytė, M.; Escudero, A.; Díez, P.; Ferrer, M. D.; Murguía, J. R.; Martí-Centelles, V.; Mira, A.; Martínez-Máñez, R. Ficin−Cyclodextrin-Based Docking Nanoarchitectonics of Self Propelled Nanomotors for Bacterial Biofilm Eradication. *Chem. Mater.* **2023**, 35, 4412−4426.

11) Chen, W.; Jiang, R.; Sun, X.; Chen, S.; Liu, X.; Fu, M.; Yan, X.; Ma, X. Self-Fueled Janus Nanomotors as Active Drug Carriers for Propulsion Behavior-Reinforced Permeability and Accumulation at the Tumor Site. *Chem. Mater*. **2022**, 34, 7543– 7552.

12) Cao, W.; Liu, Y.; Ran, P.; He, J.; Xie, S.; Weng, J.; Li, X. Ultrasound-Propelled Janus Rod Shaped Micromotors for Site Specific Sonodynamic Thrombolysis. *ACS Appl. Mater*. *Interfaces* **2021**, 13, 58411– 58421.

13) Debnath, K.; Pal, S.; Jana, N. R. Chemically Designed Nanoscale Materials for Controlling Cellular Processes. *Acc. Chem. Res*. **2021**, 54 (14), 2916– 2927.

14) Chakraborty, A.; Jana, N. R. Clathrin to Lipid Raft-Endocytosis via Controlled Surface Chemistry and Efficient Perinuclear Targeting of Nanoparticle. *J. Phys. Chem. Lett.* **2015**, 6, 3688–3697.

15) Mandal, S.; Jana, N. R. Quantum Dot-Based Designed Nanoprobe for Imaging Lipid Droplet. J*. Phys. Chem. C* **2017**, 121, 23727–23735.

16) Ray, R.; Pal, S.; Das, S.; Jana, N. R. Direct Membrane Penetration and Cytosolic Delivery of Nanoparticles via Electrostatically Interfaces Bound Amphiphiles. *ACS Appl. Mater.* **2024**, 16, 15819-15831.

17) Sinha, A.; Jana, N. R. Nanoparticle-Incorporated Functional Mesoporous Silica Colloid for Diverse Applications. Eur. *J. Inorg. Chem***. 2012**, 4470-4478.

18) Garai, P.; Ghosh, S.; Jana, N. R. Nanoporous Silica Nanoparticles with Janus Functionalization for Enhanced Cell Uptake and Drug Delivery. *ACS Appl. Nano Mater***. 2024**, 7, 12207-12213.

19) Basiruddin, S.; Maity, A. R.; Jana, N. R. Glucose/Galactose/Dextran-Functionalized Quantum Dots, Iron Oxide and Doped Semiconductor Nanoparticles with < 100 nm Hydrodynamic Diameter. *RSC Adv.* **2012,** 2, 11915–11921.

20) Wu, J.; Ma, G.-H. Recent Studies of Pickering Emulsions: Particles Make the Difference. *Small,* **2016**, 12, 4633–4648. 21.

21) Hakanson, R.; Larsson, L.-I.; Sundler, F. Fluorescamine: A Novel Reagent for the Histochemical Detection of Amino Groups. *Histochemistry* **1974**, 39, 15−23.