

## Modeling and Theory: general discussion

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DOI: 10.1039/C6FD90002J

**Sanat Kumar** began the discussion of the paper by Jayant K. Singh: You talked about gold and copper; it is apparent that you are missing the shape transition of gold. Is copper different? Is behaviour "universal" among this class of metals? Can we look at classes of metals and make a reduced model? Or, is the behaviour chemically specific?

**Jayant Singh** responded: The behaviour of copper is similar to that seen for gold, as far as the dewetting mechanism on graphene is concerned. We do observe a liquid to solid phase transition of gold nanoparticles within our simulation time frame. However, we could not capture the formation of triangular-shape gold nanoparticles. We are not sure if this is due to imperfections in the model, if there are any. Considering the similar behaviour seen for some metals on graphene, it seems plausible to make a reduced model for metals on a weakly interacting substrate such as graphene. Certainly this would be a worthwhile attempt.

**Guruswamy Kumaraswamy** remarked: Your images of the break-up of thin (0.5 nm) films suggest that a specific lengthscale characterizes the initial structure, immediately after the film breaks up to form holes. What physics governs this lengthscale? You have suggested that the Navier–Stokes equations could be used to describe the film break-up process. Would bulk constitutive equations such as the Navier–Stokes work in this regime of such thin films?

**Jayant Singh** responded: It is well known that thin film rupture leading to the formation of holes is mainly a spinodal process.<sup>1,2</sup> Usually, a thin film having a thickness lower than a critical thickness breaks up to form holes; however in our

case the holes do form initially (most likely due to spinodal instability) but merge to make bigger holes, and later the rim contracts leading to drop formation. This could be attributed to competitive inertial and viscous forces. However at higher thicknesses, such as 10 Å or higher which turns out to be greater than the critical thickness (for crossover behaviour), the viscous forces dominate which is seen in our work. The dewetting behaviour for thicknesses of <0.5 nm, as observed in our work, is also seen for other metallic films,<sup>3,4</sup> which is in good agreement with the experimental results.<sup>5</sup> The Navier–Stokes equations may be used to address the dewetting mechanism of the metallic thin film as shown recently by Nguyen *et al.*<sup>6</sup> The authors used a continuum model (CM) based on a long wave limit of the Navier–Stokes equation and molecular dynamics (MD) simulations to study the breakup of nanometer sized thin Cu rings. The results of the CM and MD were found to be in good agreement.

- 1 T. D. Nguyen, M. Fuentes-Cabrera, J. D. Fowlkes and P. D. Rack, *Phys. Rev. E*, 2014, **89**, 032403.
- 2 A. Atena and M. Khenner, *Phys. Rev. B*, 2009, **80**, 075402.
- 3 X. Li, Y. He, Y. Wang, J. Dong, and H. Li, *Sci. Rep.*, 2014, **4**, 3938.
- 4 Y. Li, C. Tang, J. Zhong and L. Meng, *J. Appl. Phys.*, 2015, **117**, 064304.
- 5 C. Favazza, R. Kalyanaraman and R. Sureshkumar, *Nanotechnology*, 2006, **17**, 4229.
- 6 T. D. Nguyen, M. Fuentes-Cabrera, J. D. Fowlkes, J. A. Diez, A. G. González, L. Kondic and P. D. Rack, *Langmuir*, 2012, **28**, 13960.

**Debdas Dhabal** addressed Jayant Singh: Upon heating at 1073 K, what is the reason behind the apparent lack of hole formation in the case of the 10 Å film? Whereas, hole formation is observed in the 5 angstrom film. Fig. 5 in your paper shows that in the case of the 10 Å film, the centre of mass (CM) velocity is going towards a negative *z*-direction up to 30 ps. Does this mean that it is trying to initiate hole formation but somehow it couldn't? If so, why?

**Jayant Singh** replied: As mentioned in response to the above question, the dewetting behaviour is clearly due to the competing forces. At higher thickness viscous forces dominate over inertial forces leading to the contraction behaviour without any hole formation.<sup>1</sup> This type of dewetting behaviour for thicknesses of <0.5 nm is also observed for other metallic films.<sup>2,3</sup>

The initial structures for the simulations are generated by placing the gold nanofilm on the surface of the graphene at a vertical distance of 0.3 nm. During the first 30 ps the film adjusts itself by moving towards the surface, which results in the negative velocity of the CM in the *z*-direction. Similar behaviour is seen for all film thicknesses. Hence, the movement of the CM in the negative *z*-direction does not indicate hole formation.

- 1 N. Péron, F. Brochard-Wyart and H. Duval, *Langmuir*, 2012, **28**, 15844.
- 2 X. Li, Y. He, Y. Wang, J. Dong, and H. Li, *Sci. Rep.*, 2014, **4**, 3938.
- 3 Y. Li, C. Tang, J. Zhong and L. Meng, *J. Appl. Phys.*, 2015, **117**, 064304.

**Daan Frenkel** commented: I notice that the position of the maximum of the free energy barrier for cluster nucleation (Fig. 8 in your manuscript) is almost independent of temperature, even though the temperature changes by more than 30%.

This is not the behaviour that one would expect on the basis of classical nucleation theory, and I wonder if there is a simple explanation for the observed behaviour.

**Jayant Singh** replied: The individual size of the cluster used in the study is 2 nm. The cluster size in Fig. 8 of the manuscript is the number of such particles in the cluster. The position of the maximum of the free energy barrier is not constant, and is found to vary slightly. For example, the critical cluster size is approximately 7, 8, 9, 7, and 7 at 673 K, 773 K, 873 K, 973 K, and 1073 K, respectively. Certainly, there appears to be some competing effects contributing to the unexpected behaviour of the critical cluster size. Unfortunately, with the current data from our simulation we cannot compare the nucleation behaviour observed in our case with classical nucleation theory. However, it will be investigated rigorously in our next work.

**Mohamed Laradji** remarked: Graphene sheets are characterized by a high bending elastic modulus. How are the dewetting kinetics affected by the bending modulus of the substrate?

**Jayant Singh** answered: It has already been shown in previous studies of bilayer systems that the elastic strain energy required to deform the top layer decreases when the bottom layer is changed from a rigid to viscoelastic layer.<sup>1-4</sup> Hence, we believe that a bending elastic modulus would certainly affect the dewetting behaviour of the gold film, which needs to be explored in future work.

1 S. Kumar and O. K. Matar, *J. Colloid Interface Sci.*, 2004, **273**, 581.

2 A. E. Hosoi and L. Mahadevan, *Phys. Rev. Lett.*, 2004, **93**, 137802.

3 D. Bandyopadhyay, A. Sharma and V. Shankar, *Europhys. Lett.*, 2010, **89**, 36002.

4 B. Ray, G. Biswas and A. Sharma, *Phys. Fluids*, 2012, **24**, 082108.

**Hari Om Sharanam Yadav** asked: What would be the effect of any additive such as some ligands or lattice defect on dewetting?

**Jayant Singh** responded: There is no simple answer as it depends on the order of roughness created by the lattice or additive. Lattice defects or the roughness of a substrate can change the dewetting behaviour significantly. For example, creating roughness such as pillar height and gaps on the surface may increase the dewettability of the substrate. Thus, detachment of the metallic droplet from the substrate can be tuned by patterning the substrate. We haven't yet looked into the role of such patterns in the current work. However, it has been explored for a Cu film on graphene.<sup>1</sup>

1 X. Li, Y. He, Y. Wang, J. Dong and H. Li, *Scientific Reports*, 2014, **4**, 3938.

**Priyadarshi Roy Chowdhury** communicated: What is the burn-in period of the simulations associated with your work mentioned in this paper?

**Jayant Singh** communicated in reply: Assuming the heating of the film is the 'burning period' for the dewetting study, we carried out the simulations for 2 ns for all the thicknesses considered in our study.

**Daan Frenkel** opened the discussion on the paper by Achille Giacometti:† As you correctly point out in your paper, it is usually impossible to make the pitch of

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† Achille Giacometti's paper was presented by Hima Bindu Kolli, University of Oslo, Oslo, Norway.

a cholesteric phase compatible with the periodic boundary conditions used in a simulation. Hence, simulations tend to probe cholesterics in a twist-free (*i.e.* stressed) state.

Can you estimate (*e.g.* using density functional theory (DFT)) how much this torsional stress would affect the location of the phase boundaries that you have computed?

**Hima Bindu Kolli** answered: Results on the cholesteric phase reported in the presentation stem from DFT calculations (see page 5, lines 20-25 of the provided paper). Depending on the shape of the particles (as given by  $r$  and  $p$ ), as well as on the packing fraction, estimates of the obtained pitches range from a few hundred to several thousands of units of length (Fig. 5). Hence, in most cases, these values are clearly significantly larger than the computational box and the torsional effect provided by the cholesteric twist cannot be probed by periodic boundary conditions, as remarked. The above estimates were obtained by using a DFT hinging upon a second-virial approximation. Increasing precision could be achieved by extending the calculation to account for the third virial coefficient, along the lines suggested in our previous work (Ref. 24 in the manuscript). We plan to do this in a future contribution.

The presented phase diagrams reported in Fig. 6 and 9 only deal with the screw-like phases. Here, unlike the cholesteric phase, the pitch of the phase is of the order of the pitch of the particle  $p$ , that is, a few units. In this case, the periodic boundary conditions are perfectly compatible with the phase, so we expect the estimates for the location of the phase boundaries reported in Fig. 6 and 9 to be very reliable. Of course these phase diagrams do not include the cholesteric phase explicitly. However, we have made progress in the direct simulation of the cholesteric phase by embedding the system between two parallel plane hard walls, as alluded to on page 13, lines 5–10. These preliminary results<sup>1</sup> indicate that i) the screw-like and the cholesteric phase are mutually exclusive; ii) the whole regions indicated by  $N^*$  in the phase diagram reported in Fig. 6 and 9, are cholesteric phases. There is also a recent preprint by Dussi and Dijkstra<sup>2</sup> that reports similar simulations obtaining a cholesteric phase for a different, but similar in spirit, particle model.

1 part of an oral communication entitled “Self-assembly of hard helices: cholesteric and screw-like, nematic and smectic, phases” given at the XII International Conference on Materials Chemistry, York, England, 20-23 July 2015.

2 S. Dussi and Marjolein Dijkstra, 2015, <http://arxiv.org/abs/1511.09318>.

**Nicholas Kotov** asked: What would be the difference in structure of the cholesteric phase as well as the phase diagram between hard and soft helices? Why and how is it different?

**Hima Bindu Kolli** responded: We would not expect significant qualitative differences between the structure of the cholesteric phase as formed by hard or soft helices. This should hold true in particular if one considers soft helices still interacting between themselves with short-range repulsive interactions. These expectations are based on past experience with soft short-range repulsive rod-like particles as compared to hard rod-like particles,<sup>1</sup> as well as on numerical simulations we are currently carrying out in which the bead–bead interaction is

changed from hard-sphere-like to soft repulsive (specifically, Weeks-Chandler-Andersen-like, *i.e.* the repulsive part of the properly shifted Lennard-Jones potential energy function). The addition of attractive interactions is again expected not to alter the main qualitative features of the structure of the cholesteric phase but they will have a role in modifying the value of the pitch as well as the location of the isotropic–cholesteric phase transition.

1 *e.g.* G. Cinacchi, L. De Gaetani and A. Tani, *Phys. Rev. E*, 2005, **71**, 031703.

**Vimala Sridurai** said: In liquid crystal systems with high chirality, Blue phases with microscopic cubic symmetry which are optically isotropic but show birefringence are obtained between the isotropic and the cholesteric phases.

So, were any such phases encountered in your studies? And one humble comment. While presenting the phase diagram, you mentioned various liquid crystalline phases like isotropic, nematic, smectic and so on. Typically in liquid crystal physics, the isotropic phase is not considered as a liquid crystalline phase. As liquid crystalline phases are mesophases between the isotropic and crystal phases.

**Hima Bindu Kolli** answered: In our simulations, performed using Periodic Boundary Conditions (PBCs) and not-large-enough systems, we could not find phases with long-scale periodicity, like the cholesteric phase and Blue Phases. In such simulations the isotropic phase was followed, at higher density, by a nematic phase with a uniform director. However, both second-virial Density Functional Theory (reported in the paper) and recent simulations where the PBC constraint was relaxed along one direction (Ref. 36 in the manuscript), showed that the liquid crystal phase following the isotropic phase is indeed cholesteric (see my above reply to Daan Frenkel's question). Likewise, we cannot exclude that, at least for some of our systems, Blue Phases can exist between the isotropic and the cholesteric phase. However, their identification may pose significant problems because of their 3-dimensional periodicity and especially their stability range, which is usually very narrow. We concur that this would be a very interesting study that is left for further investigation.

**Daan Frenkel** asked: The 'screw phase' of helical particles is fascinating and may have exciting dynamical properties. In particular, the translation–rotation coupling will be extremely large.

Hence, one could imagine that a helical 'tracer' particle with a dipole moment perpendicular to its axis could be 'propelled' by (rotating) micro-wave radiation. Are you aware of any studies of such effects and, if not, is this something that you could study by simulation?

**Hima Bindu Kolli** replied: We very much appreciate this comment. While we did not consider this very interesting possibility, nor are we aware of any studies of such helical tracer particles, an analysis of the dynamical properties would indeed be very exciting and is very high on our agenda. Imagine having a well equilibrated system of hard helices in a screw-nematic phase. Then imagine randomly selecting one of the helices and start spinning it about its own axis. This could be achieved, for instance, using the trick that you have suggested. According to our predictions on the translational–rotational coupling, we could

expect the helix to move like a “nut in a bolt”. How and how fast this motion would be achieved is inherently interesting.

**Charusita Chakravarty** communicated: 1. The papers show phase diagrams for hard helices. Soft spheres are well-studied. Can the authors comment on a way to add a simple softness parameter and the effect on the phase diagram? 2. While biological peptides may not fit the models here, well-developed methods for synthesising peptides and peptido-mimetics exist. Could these be adapted to fit the models studied here, especially to generate the smectic phases?

**Hima Bindu Kolli** communicated in reply: 1. Indeed, there are several issues related to this question. We did consider soft repulsive potentials in a few particular representative cases and found almost identical results. We expect this to be a general feature with possibly only minor changes in the exact locations of the phase boundaries. In addition, we also implemented successive umbrella sampling grand-canonical calculations, patterned after a similar calculation on sphero-cylinders,<sup>1</sup> with finite repulsive energy. This clearly introduces a dependence on temperature that can eventually be eliminated by sending this energy to infinity to recover the hard-core potential. This procedure is necessary to account for the extremely low insertion probability of hard helices at high density. A related interesting case, that we plan to tackle soon, hinges on the addition of a charge to hard helices within a DLVO description, that introduces a softness related to the Debye screening length of the DLVO potential.

2. Helical peptides can form liquid crystal phases; a well studied system is poly( $\gamma$ -benzyl-L-glutamate), which exhibits nematic and, if monodisperse, also smectic phases. This and other helical peptides, as well as other types of helical polymers, are possible candidates for the formation of the phases observed for our model helices. However, the existence of azimuthal correlations with the same periodicity of the peptide helix (less than one nm), which would be a signature of such phases, may be difficult to prove.

1 R. Vink, S. Wolfsheimer and T. Schilling, *J. Chem. Phys.*, 2005, **123**, 074901.

**Priyadarshi Roy Chowdhury** communicated: What is the iteration period of the simulation associated with your paper?

**Hima Bindu Kolli** communicated in reply: As mentioned in the paper (section II, page 5, top), a typical simulation has about  $3 \times 10^6$  MC steps of equilibration time, followed by an additional  $2 \times 10^6$  MC steps.

**Guruswamy Kumaraswamy** opened the discussion on the paper by Sudeep Punnathanam: What is the size of the critical nucleus compared to the size of the trough on the rough surface? Does this size ratio play a role in the enhancement of nucleation?

**Sudeep Punnathanam** answered: The size of the critical nucleus (defined as the number of particles in the nucleus) varies between 50 and 80. Assuming a spherical shape, this corresponds to a diameter of nearly 5 s. The surface is modeled as a sinusoidal wave with an amplitude of 15 s and a wavelength of 30 s.

**Daan Frenkel** posed two questions: 1. In your simulations, you find that the effect of nano-wrinkles on crystal nucleation only becomes significant if the range of the attractive particle–wall interaction is larger than approximately 20% of the protein diameter. Can you indicate if this regime is likely to be accessible in experiments? 2. In the simulations of van Meel *et al.* (Ref. 11 of your manuscript) capillary condensation played a key role in enhancing crystal nucleation. In your simulation, you observe enhancement of the particle protein concentration in the troughs of the wrinkles. It is not clear to me if this accumulation can be viewed as capillary condensation.

**Sudeep Punnathanam** replied: One of the conclusions from our simulations is that enhancement of protein nucleation due to nano-wrinkles can occur if there is sufficient attraction between the wall and the protein molecules. In this work this effect was simulated by increasing the range of the square well interaction. The effect of nano-wrinkles was felt only when the interaction range was 20% of the protein diameter. However, as you are rightly hinting at, this is too large a range for any realistic wall–protein interaction. Reflecting upon your question, another way to simulate an increase in the wall–protein interaction would have been to increase the well depth of the square well interaction while keeping the range at 0.076 times the protein diameter. Still, we felt that our conclusion is experimentally relevant since the range of attraction is limited to a single monolayer of protein molecules at the surface. This formation of a dense monolayer can also be caused by increasing the well depth of the square well. Whether this increase is greater at the troughs of the nano-wrinkles will need further study.

Our comparison of this work with that of van Meel *et al.* is based on the following similarity. van Meel observed capillary condensation, which is the formation of a liquid (a high density fluid region) inside pores, as the cause for enhanced nucleation. We observed formation of a dense fluid layer at the troughs which in turn enhances nucleation.

**Sanat Kumar** commented: It is possible that the critical nucleation size depends on the state that you come out of, namely gas *vs.* liquid. So, at a flat surface you might have nucleation from the gas phase nucleating out; on the other hand, in a pocket there might be liquid and the starting state is quite different. Does the critical nucleus size depend on where you start from? Is the pathway different and therefore is the critical nucleus different in the two cases?

**Sudeep Punnathanam** responded: The critical nucleus size as well as the nucleation barrier do depend on the initial state. A pocket of liquid will definitely lower the nucleation barrier. Such a situation was observed by van Meel *et al.*<sup>1</sup> where a pocket of liquid was formed inside nano-pores due to capillary condensation. In our simulations, there is a dense layer of proteins near the surface. This density is higher near the troughs of the wrinkled surface compared to the regions with zero curvature. This higher density is the reason for the enhanced nucleation rate on the nano-wrinkled surfaces.

1 J. A. van Meel, R. P. Sear and D. Frenkel, *Phys. Rev. Lett.*, 2010, **105**, 205501.

**Sofia Kantorovich** remarked: Is there a non-monotonic dependence of the nucleation rate/size of nuclei on the size of the wrinkles?

**Sudeep Punnathanam** answered: In this study, we haven't investigated the dependence of nucleation rate on the size of the wrinkles. A non-monotonic dependence can be expected when the amplitude and the wavelength of the wrinkles become comparable to the size of the protein molecules. At such conditions, the nucleation rate would be much higher when there is a match between the lattice spacing of the growing crystal nuclei and the wavelength of the wrinkles.

**Daan Frenkel** asked: As was shown by Page and Sear<sup>1</sup> there is an optimal pore size for enhanced nucleation, namely under conditions where the nucleation barrier for crystal formation inside a pore is comparable to the barrier for 'break-out' of a crystal into the bulk.

1 A. J. Page and R. P. Sear, *Phys. Rev. Lett.*, 2006, **97**, 065701.

**Ajeet Srivastav** said: How do the nano-wrinkled surfaces facilitate the supersaturation and affect the activation barrier and critical size of the nucleus?

**Sudeep Punnathanam** responded: There is an enhancement in the density of the protein molecules near the troughs of the nano-wrinkled surfaces. This enhancement in density is strongest near the metastable vapor-liquid critical point. The higher density at the troughs leads to a lowering of the activation barrier for the critical nucleus as well as the number of particles required to form the critical nucleus.

**Jayant Singh** remarked: Does the wetting state affect the nucleation behaviour? In other words, how does the nucleation behaviour change if the proteins sit on the top of the rough substrate (a metastable state typically) as opposed to the results presented in the meeting.

**Sudeep Punnathanam** answered: The lengthscale of the roughness studied is much larger than the size of the proteins. Hence the critical nucleus is formed at the locations depicted in Fig. 5. If the roughness is of the order of the size of the protein molecules, compatibility between the periodicity of the roughness and the structure of the nucleating protein crystal would strongly affect the nucleation barrier. This aspect has been explored by van Meel *et al.*<sup>1</sup>

1 J. A. van Meel, R. P. Sear and D. Frenkel, *Phys. Rev. Lett.*, 2010, **105**, 205501.

**Priyadarshi Roy Chowdhury** communicated: Have you used other simulation models besides Monte Carlo? Or is the Monte Carlo model the best amongst other simulation models with respect to your presented paper?

**Sudeep Punnathanam** communicated in reply: We only used Monte Carlo simulation techniques. One can use NPT molecular dynamics to generate trajectories however each trajectory needs to be accepted or rejected using Metropolis criteria.



**Priyadarshi Roy Chowdhury** communicated: What is the iteration period of the Monte Carlo simulation results associated with your paper?

**Sudeep Punnathanam** communicated in reply: As mentioned in section 3.3, our simulations consisted of 100000 trajectories for equilibration and 4000000 trajectories for production. We defined a trajectory as a series of Monte Carlo moves consisting of on average 20 displacement, 1 insertion and 1 deletion move per particle.

**Sanat Kumar** began the discussion on the paper by Yogesh M. Joshi: Some of your data suggest that a gel forms in the time scale of 3 h (or some such small time). The charge conduction changes on the other hand are taking hundreds of hours. Are you suggesting the structure is changing on these slower time scales? What is the change? Is it big? What is this slower process? If it is ion bridging related then you can prove the hypothesis by using a higher valence counterion – maybe replace sodium with calcium. Do you expect the changes to take much longer? Maybe you can also vary the monovalent salt concentration to address this issue.

**Yogesh M. Joshi** answered: As rightly mentioned the gel formation takes place over a timescale of hours, while an increase in ionic conductivity continues over a timescale of hundreds of hours. It is proposed that even after gel formation, the stacks of Laponite plates continue to delaminate, and these delaminated plates participate in the network structure. Consequently, the network becomes progressively dense as a function of time, which also gets reflected in the time evolution of  $G'$  and  $G''$  over the mentioned timescales. To best of our knowledge, ion bridging has not been observed among Laponite particles in aqueous media. Multivalent cations, on the other hand, either counterions or those externally added, are known to have a profound effect on the gelation kinetics of clay suspensions in general and Laponite suspensions in particular. Typically the higher the valency of the cations, the more pronounced the shielding of the electrostatic repulsive interactions. Consequently, gel formation accelerates in the presence of multivalent cations. In our previous work we studied an effect of a monovalent salt on the aging behaviour of Laponite in great detail.<sup>1</sup>

1 A. Shahin and Y. M. Joshi, *Langmuir*, 2012, **28**, 15674–15686.

**B. L. V Prasad** asked: Is it possible to remove the sodium ions faster by increasing the temperature or following any other means? Does this lead to faster gelation?

**Yogesh M. Joshi** replied: An increase in temperature indeed enhances the ionic conductivity suggesting dissociation of the sodium ions, and therefore the delamination process, is faster at higher temperatures as observed by Dhavale *et al.*<sup>1</sup> An increase in temperature also leads to faster gelation.<sup>2</sup> To the best of our knowledge, we have not come across any other means by which the delamination process could be accelerated.

1 T. P. Dhavale, S. Jatava and Y. M. Joshi, *Soft Matter*, 2013, **9**, 7751–7756.

2 A. Shahin and Y. M. Joshi, *Langmuir*, 2012, **28**, 15674–15686.

**Jacques Jestin** questioned: What is the effect of the particle concentration? According to the orientation of the platelets, one should expect to see different percolation thresholds?

**Yogesh M. Joshi** responded: Our unpublished results show that an increase in the concentration of Laponite in the suspension reduces the gelation time, while it increases the fractal dimension associated with the critical gel state.

**Sanat Kumar** remarked: If you apply steady shear do you get gelation? I presume you realign the Laponite particles?

**Yogesh M. Joshi** responded: Laponite will eventually gel no matter whether its suspension is subjected to shear or not.<sup>1,2</sup> However, as rightly pointed out, the particles will align and therefore the system will not show a critical gel state as reported by Jatav and Joshi.<sup>3</sup>

1 A. Shukla and Y. M. Joshi, *Chemical Engineering Science*, 2009, **64**, 4668–4674.

2 Y. M. Joshi, A. Shahin and M. E. Cates, *Faraday Discuss.*, 2012, **158**, 313–324.

3 S. Jatav and Y. M. Joshi, *J. Rheol.*, 2014, **58**, 1535–1554.

**Erika Eiser** commented: I worked with Daniel Bonn on Laponite; one way to decouple timescales would be to strongly shear the samples and filter them, which also induces strong shear. This procedure leads to reproducible results. Have you tried such a procedure? In this way one could first let the sample reach its final delamination state and then only shear it. A second question is about the magnitude of the interactions holding the platelets edge-on-face together. Are these simply van der Waals interactions? In this context, does it matter whether you work in a nitrogen atmosphere or at ambient conditions?

**Yogesh M. Joshi** responded: Either applying strong shear or filtering the sample using a micron sized mesh indeed leads to reproducible results only if the time difference between the various experiments is very short compared to the time elapsed since preparation of a suspension. It has been reported by Shahin and Joshi<sup>1</sup> that a Laponite suspension undergoes irreversible aging wherein the application of strong shear cannot break the network to the individual particle scale such that the average size of the cluster that can be obtained after shear melting increases with time elapsed since preparation of the same.

The fact that the faces of the Laponite disks are negatively charged while the edges of the same are positively charged suggests the interactions holding the edges and faces together are electrostatic in nature.

Our experiments suggest that if the concentration of Laponite in the suspension is sufficiently large, whether one prepares and preserves the suspension under nitrogen or air does not matter. However for small concentrations of Laponite, a nitrogen atmosphere ensures the magnesium does not leach from the Laponite particles. The chemical stability limits for different Laponite concentrations after various days elapsed since preparation of the suspension have been reported by Joshi and coworkers.<sup>2,3</sup>

1 A. Shahin and Y. M. Joshi, *Langmuir*, 2010, **26**, 4219–4225.

2 S. Jatav and Y. M. Joshi, *App. Clay Sci.*, 2014, 97–98, 72–77.

**Sanat Kumar** remarked: Have you tried sonication as a means of separating the clay (Laponite) sheets?

**Yogesh M. Joshi** answered: We often perform sonication on a Laponite suspension. Sonication is observed to only marginally increase the rate of time evolution of ionic conductivity of a Laponite suspension. Consequently sonication does not seem to significantly influence the delamination process in a Laponite suspension.

**Erika Eiser** asked: The problem is the slow delamination process of clumps in the Laponite powder, which becomes increasingly more difficult for increasing concentrations. At the same time going to low concentrations, say 1 wt% or lower increases the gelation time making measurements more challenging. However, one possibility to accelerate the ageing process in these solutions is to add small amounts of salt. In this way better delamination and rejuvenation of the sample due to shear could be reached.

**Yogesh M. Joshi** responded: We agree that in a low concentration Laponite suspension, complete delamination may occur before a critical gel state is reached. We also agree that by adding salt, the time at which a critical gel state is reached could be altered. However, as mentioned before, shear cannot break all the inter-particle bonds among the Laponite particles.

**Ranjini Bandyopadhyay** asked: Could you discuss the mechanism of the clay delamination process under the freezing–thawing cycle?

**Guruswamy Kumaraswamy** added: I am also curious about how the freeze–thaw cycle results in delamination of the Laponite stacks. I would have imagined that expulsion of the Laponite particles to the ice domain boundaries would result in (re)stacking of the plate like particles. Could you please comment on how your experimental protocol results in enhancing the delamination of Laponite stacks? Also, I would like to comment that it should be possible to use SAXS to characterize the Laponite aggregate structure through the freeze–thaw process. This might provide direct structural evidence for the delamination that you propose.

**Yogesh M. Joshi** answered: The delamination of Laponite stacks is proposed to occur as a function of time over the timescale of days. The newly delaminated clay plates participate in the gel/network structure. What we believe the freeze–thaw cycle does is to break the gel/network structure into primary components. We propose that upon increasing the temperature of the frozen Laponite suspension, in the vicinity of the melting point, ice surrounding the Laponite particles undergoes a phase transformation from solid to liquid. Since the relative permittivity of liquid water at the freezing point is 88 while that of ice is 3.2, the presence of crystallites along with water obstructs the electrostatic interactions, which is the very backbone of a structure. Consequently, after defrosting, the suspension acquires a liquid state of low viscosity. Consequently carrying out a freeze–thaw cycle at a later date since preparation of the suspension, as done in

the present experimental protocol, leads to a greater extent of delaminated Laponite particles in a suspension. We agree that SAXS can be used to provide direct structural evidence to confirm the proposed delamination mechanism.

**Guruswamy Kumaraswamy** commented: One of the reasons that the research community started working on Laponite was that it appeared to be an excellent model system for plate-like particles. It is commercially available, synthetic (and therefore, has controlled concentration), has moderate size polydispersity and a large aspect ratio. However, your work clearly indicates the challenges inherent in this system. Could you comment on the suitability of using Laponite as a model system to understand the phase behaviour of plate-like particles?

**Yogesh M. Joshi** replied: Certainly Laponite is not a model system to study the phase behaviour of plate-like particles due to their highly charged nature (under certain circumstances the particles even possess dissimilar charges). However, an aqueous Laponite suspension can certainly be considered as a model soft glassy material that shows spectacular aging and rejuvenation behaviour. Moreover, even though a large volume of literature exists on the Laponite suspension, there are several questions that are still puzzling. On the other hand, smectite clays in general and Laponite clay in particular are very popular additives used to modify the rheological behaviour in a number of industries that deal with water based paste-like products. Therefore it is important that we understand this system well.

**Vinothan N. Manoharan** commented: In Prof. Joshi's paper the results are interpreted using DLVO theory. But DLVO theory has several limitations: it is a mean-field theory; it assumes constant charge density; and to understand structure formation one has to assume pairwise additivity. Prof. Joshi, is there any reason to believe, *a priori*, that DLVO theory should give sensible results for Laponite suspensions? A broader question for all delegates: Has DLVO theory been "proven innocent" for modeling interactions in nanoparticle dispersions? In other words, is it more helpful than harmful to our understanding of structure formation?

**Jayant Singh** answered: It is evident from our recent work on silica deposition on a soft surface that DLVO cannot capture many aspects of nanoparticle structural formation. Was the use of DLVO harmful? I guess not as long as one is aware of the danger of approximation. This may be true for any mean-field theory based prediction.

**Nicholas Kotov** responded: The application of DLVO theory to nanoparticles reminds me of the story about Cinderella's sisters. They tried very hard to put on the shoe, but it did not fit any way they tried it. We can conclusively state that DLVO theory is not applicable to nanoparticles below 20 nm in diameter because nearly all key assumptions of DLVO theory are violated at this scale.<sup>1</sup> One of the central fundamental problems is the separation of interactions into electrostatic, van der Waals, hydrophobic and others. For nanoparticles at small separations (20 nm and less) these interactions are non-additive and become interdependent on each other. Atomistic molecular dynamic modeling can help to resolve this problem. Potentially other methods are possible, too.

1 C. A. Batista, R. G. Larson, N. A. Kotov, *Science*, 2015, **350**, 1242477.

**Yogesh M. Joshi** answered: We agree with the mentioned criticism that DLVO theory makes a number of assumptions, and therefore before employing DLVO theory to analyze any experimental data it is important to assess to what extent the assumptions are valid. Firstly regarding the Laponite particle, it is safe to assume that negative and positive charges are distributed uniformly across the faces and the edge, respectively. DLVO considers the addition of van-der Waals interactions and electrostatic interactions. The former is dominant only over inter-particle lengthscales of less than 1 nm. In this work we used DLVO theory to essentially make a point. We solved the theory only for a case when two plates approach each other in a parallel fashion. DLVO theory predicts that with an increase in counterion concentration the height of the repulsive barrier increases while its width decreases. Consequently, when two particles approach each other in a non-parallel fashion, with the edges being positive and the faces being negative, DLVO theory implicitly suggests that edge to face bond formation is faster with an increase in ionic conductivity as observed experimentally. Regarding the broader question on DLVO theory, we still feel that DLVO theory gives good qualitative information about the interparticle interactions. However it is important to judiciously examine the assumptions involved in DLVO theory with respect to the experimental system before interpreting the results.

**Nicholas Kotov** addressed Vinothan N. Manoharan and Yogesh M. Joshi: Thank you Vinothan Manoharan for bringing this issue up. The validity of DLVO at the nanoscale is the core of this meeting. Referring to my earlier analogy to the story of Cinderella, maybe it is a good time to turn this story around and say that DLVO is indeed the wrong shoe for this foot. The foundational assumptions of DLVO are not valid for many if not all nanoparticles especially when they have such an anisotropic shape as clay nanosheets.

**Vinothan N. Manoharan** answered: I agree. As Prof. Kotov notes in his recent review paper,<sup>1</sup> atomistic simulation is a tractable alternative to DLVO for small nanoparticles. For larger (10–100 nm) particles, it will be important to develop experimental tools that allow direct or indirect measurement of the effective potential between particles. We recently demonstrated an optical technique that allows us to measure the interaction between two 50 nm nanoparticles at separations close to contact.<sup>2</sup> Other techniques, such as fluid-cell electron microscopy, offer even higher resolution.

1 C. A. S. Batista, R. G. Larson and N. A. Kotov, *Science*, 2015, **350** (6527), 1242477.

2 S. Faez, Y. Lahini, S. Weidlich, R. F. Garmann, K. Wondraczek, M. Zeisberger, M. A. Schmidt, M. Orrit and V. N. Manoharan, *ACS Nano*, 2015, **9**, 12349.

**Yogesh M. Joshi** replied: Kotov and coworkers<sup>1</sup> described the following four fundamental assumptions of DLVO theory that are not valid at the nanometric lengthscale: 1. Contrary to assumption the sizes of the solvated ions and solvent molecules are not negligibly small over the lengthscales of 1 to 20 nm; 2. The additivity assumption of van der Waals and electrostatic interactions fails over the considered nanometric lengthscales; 3. The thickness of an interfacial layer on

a nanoparticle could be as large as the nanoparticle size itself raising a question over the assumption of uniform continua outside a particle; and 4. The assumption of nanoparticles having a simple shape. In the present work we consider a special case of two flat plates approaching each other in a parallel fashion over lengthscales of typically up to 7 nm with a Debye screening length of around 3 nm. On the other hand the sizes of the water molecule as well as  $\text{Na}^+$  ions are around 0.3 and 0.1 nm respectively, which clearly suggests that the first assumption of DLVO theory is not too bad if not good. Regarding the failure of the additivity assumption of van der Waals and electrostatic interactions, it has been observed that for the Laponite system van der Waals interactions are negligible beyond 1 nm of inter-surface distance of the plates.<sup>2</sup> Therefore for the lengthscales from 1 to 7 nm we are primarily accounting for only the electrostatic interactions between the faces of the particles. Consequently violation of the non-additivity assumption does not affect the present system over most of the considered region. The third issue is about a layer on the nanoparticle surface. Since the present system contains only water, salt and the Laponite particles, the layer on the face of the Laponite particle is essentially a Stern layer of the order of 0.1 nm thickness (which is followed by a diffuse layer in the solvent). Finally regarding the shape of a particle, it is well known that clays are plate-like particles (in the case of Laponite the diameter is around 30 nm). As mentioned before we consider only a case when two plates are parallel to each other. The electrostatic interaction between the two charged plates parallel to each other is a classic problem having an analytical solution, and examples of clays are often given as textbook problems. We understand that in all the above cases various assumptions may not be perfectly validated owing to the finite size of ions involved as mentioned by Kotov and coworkers. However isn't it the case that DLVO theory tends to show significant interactions for many systems only below 10 nm of inter-surface distance irrespective of the size of a particle.<sup>3</sup> Therefore we believe that there is nothing specific about Laponite that would violate the DLVO assumptions more than in the case of larger particles as the distances over which significant interactions are observed are similar in both. Finally, it is important to note that the present work uses DLVO theory for a specific configuration just to make a qualitative point. To conclude metaphorically, in the whole kingdom only Cinderella had the precisely same sized feet for the shoes. All the other girls, let alone her sisters, can still make use of oversized or undersized shoes as no perfect sized shoes are available with an understanding that there is indeed a mismatch, in some cases greater while in other cases smaller.

1 C. A. S. Batista, R. G. Larson and N. A. Kotov, *Science*, 2015, **350** (6527), 1242477.

2 D. Saha, R. Bandyopadhyay and Y. M. Joshi, *Langmuir*, 2015, **31**, 3012–3020.

3 J. N. Israelachvili, *Intermolecular and Surface Forces*, Academic Press, London, UK, 3rd edn, 2010.

**Madivala G. Basavaraj** asked: The analysis of interactions in a system of clay particles in terms of classical DLVO interactions seems an over simplification, especially because of the fact that the clay particles are “patchy” – that is – the rim and the face are differently charged. In such cases one has to worry about orientation dependent interactions in asymmetrically charged anisotropic colloids. How does one visualize a double layer in such cases? Would the screening of the double layer be different in different directions? Do you include

the contribution of  $\text{Na}^+$  ions that dissociate from clay particles into the calculation of the Debye screening length?

**Yogesh M. Joshi** responded: Firstly even though the rim and the faces have different charges, it is safe to assume the charge distribution to be uniform as well as continuous. Secondly as mentioned before we solve the DLVO theory only for a case when two Laponite particles approach each other in a face to face parallel fashion. We therefore do not solve DLVO theory for interactions between an edge and a face, when two particles approach each other in a nonparallel fashion. We only implicitly infer about such interactions. Similar to an electric double layer on the negatively charged faces of Laponite particles, there exists a double layer on the positively charged edges as well. However as mentioned before we did not analyze the interaction between the edges and faces using DLVO theory.

We indeed considered the concentration of sodium ions while computing the Debye screening length. On the other hand, the concentration of dissociated sodium ions also leads to surface charge density on the faces of the Laponite particles.

**Lynn Walker** commented: I worry that DLVO theory is dangerous in a system as complex as this one. My concern is that it might be leading to some erroneous interpretation of the gelation behaviour. I do recognize that it is the obvious zeroth order place to start, but some of the observations might suggest the need for more complex theory. This is more of a comment than a question.

Is it possible that some of the behaviour in these systems is governed by aggregate interactions rather than individual particle interactions? In a number of colloidal systems, there are observations of critical aggregates forming that have very different inter-aggregate interactions than the individual particles.

**Yogesh M. Joshi** responded: We agree that the DLVO theory makes a number of assumptions and it is important to carefully scrutinize the assumptions with respect to the experimental system. It is important to note that in this work we use DLVO theory essentially to make a point. We solve the theory only for a case when two plates approach each other in a parallel fashion. The DLVO theory predicts that with an increase in counterion concentration the height of the repulsive energy barrier increases while its width decreases. Consequently when two particles approach each other in a non-parallel fashion, with the edges being positive and the faces being negative, DLVO theory implicitly suggests edge to face bond formation is faster with an increase in ionic conductivity as observed experimentally. The very fact that gelation precedes with complete delamination of the stacks with the difference in timescale being at least one order of magnitude suggests that, to begin with, the stacks of Laponite particles undergo gelation. As time progresses, more plates delaminate and the size of a stack reduces. Furthermore, while the surface of the stacks or aggregates is formed by the faces and edges of the Laponite particles themselves, as rightly mentioned the inter-aggregate interactions could be different from those of the interparticle interactions.

**Siddharth Kulkarni** remarked: Why does the crossover between  $G'$  and  $G''$  get delayed with a decrease in angular frequency (Fig. 4)? Is this indicative of liquid-like behaviour?



**Yogesh M. Joshi** responded: In gel forming systems sampling a critical gel state, such as the aqueous Laponite suspension, the frequency dependence of time at which  $G'$  and  $G''$  cross each other depends on the fractal dimension of the critical gel state (or the unique value where all the iso-frequency  $\tan \delta$  curves meet). If the fractal dimension associated with a critical gel state is above 2, as is the case with the Laponite suspension studied in the present work, crossover between  $G'$  and  $G''$  will get delayed with a decrease in frequency. On the other hand if the fractal dimension of a critical gel state is below 2, crossover between  $G'$  and  $G''$  will get delayed with an increase in frequency.

**Priyadarshi Roy Chowdhury** communicated: In Fig. 2, the behaviour of free energy interactions obtained from DLVO theory, of the Laponite particles, having face to face interactions is shown. What would be the behaviour of these Laponite particles when they are moderately apart and far apart from each other from a theoretical point of view?

**Yogesh M. Joshi** communicated in reply: DLVO theory assumes electrostatic interactions and van-der Waals interactions to be additive. The latter are dominant only at very short length-scales (<1 nm). The interaction associated with the former on the other hand depends on the surface potential as well as the Debye screening length. In the present work the DLVO theory has been solved to obtain the interaction between two Laponite particles approaching each other in a parallel fashion. For this configuration, over moderate length-scales (less than 10 nm but more than 1 nm), a decrease in distance between the same will lead to an increase in repulsion as shown in Fig. 2. However at large distances between the plates (>10 nm), interaction among them will be negligibly small.

**Guruswamy Kumaraswamy** opened the discussion of the paper by Daan Frenkel: If we consider pathways to the ordered state, could we derive inspiration from protein folding where chaperones play an important role in the folding process? Are there any examples that you are aware of where such biomimetic strategies have been employed to form ordered structures from complex mixtures? Is there a way to obtain insight from simulations on how to design such chaperones for ordering?

**Daan Frenkel** answered: In the design of complex, self-assembling structures, the building blocks must obviously encode for the target structure, but as Dr Kumaraswamy points out: this is not enough. It is crucial that the self-assembly pathway does not terminate in a metastable 'off-target' structure, of which there are very many. Protein folding is an excellent example: many proteins would misfold if, in their nascent state, they were not be protected by a variety of chaperones. Similarly, in synthetic chemistry, protecting groups are commonly used to inhibit undesired ('off-pathway') reactions. Clearly, in complex self-assembly, the use of protecting groups or chaperone-like units is (or will be) of crucial importance. In existing DNA-brick structures, the DNA bricks on the surface are commonly made non-reactive by terminating them with poly-T chains. I am not aware of the use of DNA-brick based chaperones, but the potential is obvious.



**G V Pavan Kumar** asked: In your simulations on aggregates, the coordinate number will influence the lattice phonon modes. Can you simulate them?

**Daan Frenkel** replied: You are absolutely right that different coordination numbers will affect the phonon density-of-states of the self-assembled structures. At this stage, not much is known (at least not to me) about the phonon spectrum of DNA-brick structures. Of course, as the structures form in aqueous solution, most lattice vibrations are likely to be overdamped. But even so, the low-frequency phonon spectrum is of crucial importance for the elastic properties of the self-assembled structures. It would be interesting to investigate this, but I fear that the highly coarse-grained model is not sufficiently realistic to capture the phonon spectrum.

**Priyadarshi Roy Chowdhury** asked: Is it possible to achieve high co-ordination numbers in many component self assembly in spite of having high structural barriers and complexities?

**Daan Frenkel** answered: We were indeed surprised to find that, in simulations, we could self-assemble structures with high coordination numbers because at low super-saturations the nucleation barriers for such clusters are high. We feared that at higher super-saturation, many defects would be incorporated into the growing structures. The simulations showed that the situation was not as we feared: we found that even structures with a coordination number of 12 could self-assemble in a reasonable time and with some, but not very many, point defects. Whether this can be achieved in experiments remains to be seen.

**Sofia Kantorovich** said: Is it possible to introduce temperature activated patches, so that not only the combination of active sites per particle determines the final self assembled structure, but the latter can also be altered afterwards by changing  $T$ ?

**Daan Frenkel** replied: Thus far, the main tool that has been used to change functionality is the composition of the mixture from which the structures self-assemble.

However, there are techniques by which the functionality of patches can be made temperature-dependent. Examples can be found in the literature.<sup>1,2</sup>

But, of course, there is huge scope for new approaches to add active 'control' to complex self-assembly.

1 S. Angioletti-Uberti, B. M. Moggetti and D. Frenkel, *Nat. Mater.* 2012, **11**, 518–522.

2 W. B. Rogers and V. N. Manoharan, *Science*, 2015, **347**(6222), 639–642.

**Vinothan N. Manoharan** asked: In response to the question from Dr Kantorovich: Arvind Murugan and colleagues<sup>1</sup> conducted a theoretical study of how to make nanoparticle dispersions that can self-assemble into multiple different prescribed structures. Different structures can be assembled from the same mixture of components by changing, for example, the temperature. It should be possible to make such mixtures using DNA-labeled particles and strand displacement reactions. Michael Brenner's group<sup>2</sup> has also used simulations to

examine the assembly of maximally specific colloidal particles with high coordination number. Just as in Prof. Frenkel's work, the self-assembled structures contained defects, but those defects did not prevent the target structure from forming.

Experimentally, colloids with 'addressable complexity' can be made using grafted DNA strands, but it is difficult to make multiple species all having the same melting temperature. The melting temperature depends on the sequence of the grafted DNA strands as well as the grafting density, which is difficult to control precisely. An alternative approach is to use 'blunt-end stacking' interactions between grafted DNA sequences.<sup>3</sup> These interactions are specific to the shape of the DNA constructs, and they can have a different temperature dependence than base-pairing interactions. Prof. Frenkel, have you considered studying the self-assembly of particles that have both sequence-specific and shape-specific interactions?

1 A. Murugan, Z. Zeravcic, M. P. Brenner and S. Leibler, *PNAS*, 2015, **112**, 54.

2 Z. Zeravcic, V. N. Manoharan and M. P. Brenner, *PNAS*, 2014, **111**, 15918.

3 S. Woo and P. W. K. Rothmund, *Nature Chem.*, 2011, **3**, 620.

**Daan Frenkel** replied: In my talk, I did not really touch upon the 'multifarious' structures considered by Murugan *et al.*, but such systems that use the same building blocks to encode for a (large) number of different target structures are clearly extremely important. They are used in biology and there seems to be little doubt that they can be used in DNA brick assembly. However, in the work reported here, we did not consider this option.

The second suggestion (blunt-end stacking) is indeed a technique to expand the 'alphabet' of interactions. It is therefore likely to become increasingly important as we move to larger structures. Blunt-end stacking exploits shape complementarity and that is a good way to add selectivity to the interaction between different building blocks. I agree completely that the potential of blunt-end stacking deserves to be explored numerically in a generalised coarse-grained model.

**Alison Edwards** asked: It is refreshing to see published the initial hypothesis on which a study has been undertaken – especially when the results are contrary to the hypothesis. In the context of A. L. MacKay's classic<sup>1</sup> on a dense non-crystallographic packing of spheres based on 12-fold coordination, I wonder whether you have pondered the energetic consequences that such a 12-fold 'non-crystallographic' regime might facilitate in terms of optimizing entropy during nucleation which could allow a particle to expand out prior to collapse (or not) to an ordered CCP packing which would correspond to the tetrahedral preference you held at the beginning? Might your work demonstrate that nucleation as icosahedra in the first instance is a worthwhile hypothesis even for the particles which are ultimately found to be tetrahedral?

1 A. L. MacKay, *Acta Cryst.*, 1962, **15**, 916.

**Daan Frenkel** responded: In crystal nucleation, the Mackay solids play the role of the widely ignored elephant in the room. Yes, it is true that Ostwald's 'rule of stages' argues that the crystal phase that nucleates need not be the most stable

phase, but the phase that is closest in stability to the parent phase (a statement that, in its generality is also not quite correct). However, Mackay realised that finite structures might nucleate ('Mackay solids') that have a symmetry that is incompatible with three-dimensional periodicity (the paper by Mackay also contains a tantalising reference to a comment made by J. D. Bernal about extended, non-periodic structures). Could DNA brick structures also nucleate *via* intermediate ordered structures that do not have the symmetry of the target structure? It seems more difficult than for systems that have only one or two distinct building blocks. However, it should be possible to design bricks that could nucleate *via* two (or more) competing routes. I am not aware of work in this direction.

**Mukta Tripathy** said: The manuscript shows that at high coordination numbers the free energy barrier to forming self-assembled structures becomes quite high (20–25  $kT$ ). In spite of such high barriers, you report few defects. Could this be because the excess free energy of a defect is large compared to the thermal energy (perhaps because of the specificity of the interactions)?

More generally, are there good simulation techniques to estimate the excess free energy of a defect?

**Daan Frenkel** replied: To answer the second question first, there are good numerical techniques to compute the free energy (and hence the equilibrium concentration) of vacancies and interstitials in 'atomic' crystals.<sup>1,2</sup> These techniques can be extended to molecular crystals and structures with addressable complexity. In that case, we should also consider another type of (double) point defect: an exchange between two bricks. However, insufficient equilibration during growth is likely to be the main source of point defects in DNA brick structures. The free energy cost of all these defects is likely to be high – but out of equilibrium they may still form.

1 C. H. Bennett and B. J. Alder, *J. Chem. Phys.*, 1970, **54**, 4796.

2 S. Pronk and D. Frenkel, *J Phys. Chem. B*, 1970, **105**, 6722–6727.

**Jayant Singh** asked: Considering the complexity of the system, it would be useful if a comment is given on difficulties in the simulation, and in particular the necessity of a biasing method in such simulations. In other words, is there scope or future direction in the development of better methodologies for such systems.

**Daan Frenkel** answered: The biasing methods that we use are relatively simple and can certainly be improved. Hence there is huge scope for methodological improvement.

**Priyadarshi Roy Chowdhury** communicated: Could you elaborate on the 'narrow temperature window' associated with the many component self assembly with special emphasis to the nucleation behaviour.

**Daan Frenkel** communicated in reply: The temperature window for successful nucleation is bounded at high temperatures by the regime where the target structures are unstable: small structures can only form fleetingly and will then

decompose. At lower temperatures, non-selective interactions (*e.g.* due to not fully complementary DNA strands) will become strong enough to bind bricks together. As a result such bricks will bind in the wrong place. The phenomenon is similar to self-poisoning during crystal growth. The temperature where this happens depends on the strength (and the number) of non-specific interactions. For DNA bricks, there is a window where self-assembly favours the target structure. However, one can easily envisage linker molecules other than DNA for which there is no window for successful aggregation.

**Priyadarshi Roy Chowdhury** communicated: In order to co-relate the experimental results with those of theoretical interpretations, in a very realistic way, which model do you think is the best at present for studying the nucleation behaviour?

**Daan Frenkel** communicated in reply: To get a quick impression of the typical height and location of the nucleation barrier, cheap, coarse-grained models are arguably best. However, any model that aims at quantitative predictions needs a more realistic description of the DNA structure and dynamics. Possibly, the OxDNA model ([dna.physics.ox.ac.uk](http://dna.physics.ox.ac.uk)) could be used as a compromise between cost and accuracy.

**Oleg Gang** remarked: How do you see the relationship between thermodynamic entropy and Shannon entropy?

Can you imagine an algorithmic approach for the position of the particles, *i.e.* instead of the  $x$ ,  $y$ ,  $z$  of particles, to have a function or recursive relationship? Would it be more concise in terms of the amount of information for self-assembly in comparison with conventional methods (interaction, shapes) or not? In this respect, more generally: how one can define a minimum amount of information that is required to make a structure from particles? And the next question: how to pass that information to the system efficiently in order to prescribe the self-assembly process?

**Nicholas Kotov** said: Bulding on Oleg Gang's original question about the information needed to be encoded in each block to allow for specific self-assembly to occur, it seems to me that the seemingly simple nanostructures with relatively low contents of coded information, that is molecular and nanoscale engineering, can produce structures of high complexity. Their geometry can be competitive with those observed in biology. Examples can be multiple, but tobacco-mosaic-virus-like assemblies of inorganic nanoparticles with a core and helical shell with specific symmetry come to my mind first. The information that is needed to be encoded into CdTe nanoparticles was quite minimal compared to what we would expect from a self-assembled viral particle. This brings me to the fact that we do not really have good tools to assess and predict the assembly patterns of nanoparticles in solutions. Besides computational hardware that is constantly improving, we possibly need to plug new math into the description of self-organization phenomena.

**Vinothan N. Manoharan** addressed Daan Frenkel and Oleg Gang: In response to Dr Gang's question about the connection between information and DNA-

mediated self-assembly and to Prof. Frenkel's comment about the connection to neural networks: the paper by Murugan and coworkers<sup>1</sup> discusses the relation between the ability of a nanoparticle suspension to self-assemble into multiple structures and the ability of associative neural networks to store memories, within the context of the Hopfield model.

1 A. Murugan, Z. Zeravcic, M. P. Brenner and S. Leibler, *PNAS*, 2015, **112**, 54.

**Priyadarshi Roy Chowdhury** opened a general discussion on the paper by Robert Botet by communicating: § How can simulation be done in a better way so that the co-relation between experimental and theoretical results becomes much more realistic?

**Robert Botet** communicated in reply: The major improvement to do is to include the liquid phase in the Monte-Carlo simulations. Indeed, the liquid phase is expected to be a kind of reservoir of particles unfit for the expected crystalline phases. This idea is only an assumption at this stage and should be checked. The issue is to compute the correct free energy of the liquid phase for a polydisperse system at a given volume fraction.

**Priyadarshi Roy Chowdhury** communicated: Which simulation model do you consider to give a reliable and comprehensive explanation of molecular reaction dynamics?

**Robert Botet** communicated in reply: I probably do not grasp the question. The simulations presented in paper 8522 are about regular Monte-Carlo (*i.e.* performing random exchanges of system states according to the thermodynamic rules) and not reaction dynamics. For the latter, one may refer to the book by R. Levine.

**Vandana Shinde** communicated: 1. Can we find the % amount of FCC and BCC crystalline structures in the system? 2. Is this theory applicable for all types of binary nanostructure crystalline systems?

**Robert Botet** communicated in reply: 1. Yes, since the proportion of FCC and BCC is allowed to vary during a numerical simulation, the final state provides a value for the respective volume proportions. 2. This approach is quite general, providing the final system is, in some way, an equilibrated system (in the thermodynamic sense). Actually, the theory is very close to the original Monte-Carlo idea: one follows the biased random walk of the representative point of the system in the configuration space. The main issue is that one has to 'guess', before starting a simulation, which crystalline phases could be present. If one forgets a phase, only a part of the configuration space is investigated, which may lead to the wrong final state.

**Nicholas Kotov** opened a general discussion on the paper by Pedro A. Sánchez: One can, in principle, manipulate magnetic particles inside the body using external magnetic fields.

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§ The paper by Robert Botet was not presented at the meeting, but questions and responses have been provided afterwards.

**Pedro A. Sánchez** answered: Yes, that is, for instance, the idea behind the use of magnetic nanoparticles for hyperthermia treatments against cancer tumors, in which the particles are forced to quickly fluctuate under the action of oscillating external fields, heating the surrounding environment and destroying the cancer cells. Magnetic microswimmers have also been proposed as propellers for drug delivery microrobots driven by external magnetic fields.

**B. L. V Prasad** asked: A few nanoparticles assemble into a brush like structure, how do the brushes interact with each other? Does this influence the final structures being formed?

**Pedro A. Sánchez** answered: The interaction between two brushes is expected to be really complex due to the interplay between the bonding constraints and the steric and magnetic forces. Therefore, it is really hard to foresee the resulting structures without further studies. This is an interesting topic that we will surely address in the future.

**Ajeet Srivastav** commented: The magnetic anisotropies and the dipole–dipole interactions should be considered when designing the magnetic field interactions.

**Pedro A. Sánchez** responded: The design complexity of the external field depends on the scale at which one wants to control the structure of the brush. If one is interested only in determining the overall height of the brush, then it is enough to apply a homogeneous field perpendicular to the grafting surface with a proper intensity. The control of the structure at the scale of individual particles is much more complicated and it is even possible that some arbitrary configurations will never be accessible.

**Daan Frenkel** asked: In your presentation you discuss one possible application of the field-induced stretching of filaments of magnetic colloids, namely the design of switchable filters. Have you considered using such filaments in conjunction with a periodically modulated, rotating field to make magnetic ‘cilia’ that could be used to pump liquids?

**Pedro A. Sánchez** answered: That is actually one of the most largely studied applications of chains of magnetic colloids and nanoparticles to date, even though the amount of research groups around the world working on the topic is still relatively small. Both simply self-assembled chains and polymer crosslinked chains of magnetic particles, which are what we call magnetic filaments, have been considered for this functionality, either grafted to other small bodies to form a magnetically propelled microswimmer or grafted to the wall of a channel to pump the background fluid. In the latter case, that is closer to the brush structure discussed in our paper, there should be a minimum distance between the grafted chains in order to obtain an effective pumping. Therefore, the related studies consider only grafting densities of filaments that are significantly lower than the ones analyzed in our work.

**Nirmalya Bachhar** remarked: What happens if you change/tilt the direction of the magnetic field? Would you expect a different kind of shape?

**Pedro A. Sánchez** responded: We have not studied these conditions yet, but we can guess what will be the overall response of the brush structure. In any case, in the limit of high field intensities the filaments experience a straightening and tend to remain parallel to the field direction. When the field is perpendicular to the grafting surface, the brush reaches its maximum height, given by the contour length of the filaments. If the field is not perpendicular, the filaments will still tend to remain parallel to the field, resulting in a lower maximum vertical height of the brush. A more difficult to predict question is what will happen to the structure at the scale of individual particles. The tilting of the filaments, following the field direction, will reduce the distance between neighboring chains, maybe favoring the appearance of certain interchain connections, but probably also increasing the repulsions. Therefore, it is hard to predict the small scale structure without further analysis and eventual simulations.

**Priyadarshi Roy Chowdhury** communicated: What limitations arise if the temperature is kept in the low range rather than using an extended range with respect to the filament brush system?

**Pedro A. Sánchez** communicated in reply: At low temperatures, the structure of the magnetic brush experiences larger changes by switching the external magnetic field on and off. Without the field, the filaments bend towards the grafting surface to form a fully connected network and the average brush height reaches its minimum. In this situation, the structure of the filament network is mainly determined by the dipole–dipole interactions. When a field perpendicular to the grafting surface is switched on, the filaments tend to align with the field, remaining in a straight conformation. This hinders the connections between neighboring filaments. Therefore, at low temperatures the overall impact of the field is higher and the network of connected filaments tends to disappear for strong fields.

**Oleg Gang** opened a general discussion on the paper by P. B. Sunil Kumar:<sup>‡</sup> What is the main origin of the different phases that you observed?

**Mohamed Laradji** answered: The clustering of the nanoparticles is the result of effective membrane-mediated interactions between them. In particular, at high adhesion strengths, the nanoparticles locally induce a negative Gaussian curvature of the membrane, preventing them from clustering in chains, leading to star-shaped clusters.

**Sudeep Punnathanam** asked: Did you compute the surface diffusivity of the nanoparticles on the lipid surface? What is its role in the formation of ordered structures both in the low adhesion case as well as the high adhesion case?

**Mohamed Laradji** replied: We calculated the diffusion coefficient of a single nanoparticle on the lipid membrane and found that the diffusivity slightly decreases with increasing adhesion strength. We did not however compute the

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<sup>‡</sup> The paper by P. B. Sunil Kumar was presented by Mohamed Laradji, University of Memphis, Memphis, USA.

diffusivity of an ensemble of nanoparticles on the membrane. We anticipate that it will decrease with increasing the number density of the nanoparticles. However, the relationship between the diffusivity and the type of assembly is not known at the moment. We will compute it in the near future.

**Rajdip Bandyopadhyaya** asked two questions: 1. Have you conducted simulations where the membrane surface fluctuates or modulates spontaneously, creating zones of positive and negative curvature? Will this lead to transient nanoparticle assemblies? 2. What is the motivation for starting with anisotropic nanoparticles? Rather, if you start with spherical particles one can maybe compare the resulting simulated structures with experimental work in drug delivery, where spherical nanoparticles adsorb on lipid membranes (on the outer cell-boundary) before internalization into the cells.

**Mohamed Laradji** answered: 1. The membrane undergoes spontaneous thermally induced fluctuations with different local curvatures. We have performed simulations of planar membranes with different values of lateral tension. We found that for high values of tension, the adhesion of the anisotropic nanoparticles is hindered by the fact that the membrane is mainly flat. Small local fluctuations in curvature allow for the adhesion of the nanoparticles and their self-assembly. For low tension, the amount of thermally induced fluctuations increases, leading to an increase in the amount of self-assembled clusters. Once formed, the clusters tend to be very robust and stable. 2. We have indeed performed some simulations of spherical nanoparticles. In particular, we recently looked at the adhesion, wrapping and endocytosis of spherical nanoparticles.<sup>1</sup> We are also currently looking in detail at the self-assembly of spherical nanoparticles in bilayers. We already know that there exist spherical nanoparticles with a wide range of sizes and adhesion strengths which self-assemble into linear chains. They may also self-assemble into compact clusters with triangular symmetry. The stability of these structures is under investigation.

1 E. J. Spangler, S. Upreti and M. Laradji, *J. Chem. Phys.*, 2016, **144**, 044901.

**Vinothan N. Manoharan** commented: Prof. Bandyopadhyaya asked about the assembly of spherical nanoparticles at a membrane interface. My group has done experiments on a much simpler system consisting of spherical colloidal particles on the surface of a spherical droplet.<sup>1</sup> We found that the particles form quasi-one-dimensional ribbons (see Fig. 1), similar to the assemblies seen in Prof. Kumar's system of anisotropic particles on the surface of a vesicle. The difference is that in our system, the particles do not alter the curvature, and there are isotropic attractive interactions between the particles. The reason for the quasi-1D assembly was first proposed by Schneider and Gompper in a theoretical paper from 2005:<sup>2</sup> the formation of ribbons minimizes the elastic energy associated with stretching the assemblies onto a surface with positive Gaussian curvature. So in addition to the mechanism shown by Prof. Kumar for the formation of chain-like assemblies from anisotropic particles, there is another mechanism for the assembly of anisotropic assemblies from isotropic particles.

1 G. Meng, J. Paulose, D. R. Nelson and V. N. Manoharan, *Science*, 2014, **343**, 634.

2 S. Schneider and G. Gompper, *Europhys. Lett.*, 2005, **70**, 136.



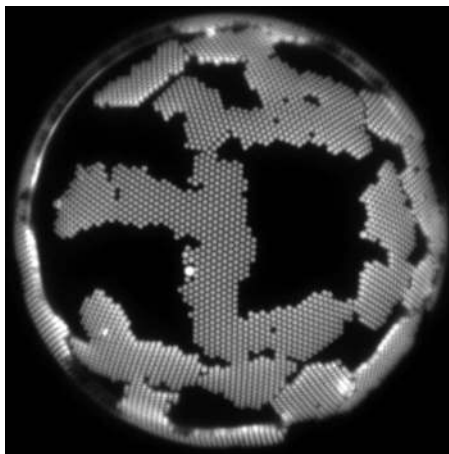


Fig. 1 Confocal microscope image of 1-micrometer, attractive colloidal particles assembling into quasi-one-dimensional crystals ('ribbons') on the surface of a spherical water droplet. See G. Meng, J. Paulose, D. R. Nelson and V. N. Manoharan, *Science*, 2014, **343**, 634. Image courtesy of G. Meng.

**Sunil Kumar** responded: I agree that there could be multiple mechanisms acting to form the one dimensional assembly. In our simulations, there is not explicit attractive interactions between the particles. Particle assembly is through membrane mediated interactions. We see sidewise attraction between pairs of particles and then these pairs attracting sidewise form bigger clusters *etc.* We also show that, depending on the parameters of the simulation, there is a preferred angle between a pair of particles, indicating that pairwise interactions are the dominant reason for the shape of the assemblies formed.

**Rajdip Bandyopadhyaya** replied: It is nice to learn of contrasting examples of assembly of nanoparticles on a membrane surface – in one case it is repulsive anisotropic particles (Sunil Kumar and Mohammed Laradji's paper in this Faraday Discussion volume) influencing local membrane curvature to bring about chain-like or splay aggregation of nanoparticles, while in the example of this comment<sup>1</sup> it is that of attractive, spherical nanoparticles forming much larger global-scale branched-ribbon-like patterns. It will be interesting to explore other possible assembly-patterns theoretically and validate them through experiments, a concerted experimental plus simulation study, which will be a logical next step from these two papers.

1 G. Meng, J. Paulose, D. R. Nelson and V. N. Manoharan, *Science*, 2014, **343**, 634.

**Madivala G. Basavaraj** addressed Mohamed Laradji and Sunil Kumar: One way to realize the simulation results you have presented *via* experiments is by using a system of cationic vesicles and oppositely charged rods through electrostatic attraction. Would there be any shape transitions in the vesicles due to the fact that particles are charged, which would accompany the release of counterions? Have you considered the effect of such counterions? Would the presence of particles in the interior of the vesicles change the phase behaviour?

**Mohamed Laradji** answered: The proposal seems very reasonable. The nanoparticles used in the simulations are crescent-shaped. The aggregation of the anisotropically curved nanoparticles in our simulations is different from that of rods. For example, aster-shaped aggregates do not form when rods adhere to vesicles. Counterions are not explicitly accounted for in our simulations. As alluded, we do expect that aggregation of the anisotropically curved nanoparticles on the interior side of the vesicle to be different from the case where they are on the outer side of the vesicle. In particular, the binding of the nanoparticles to the vesicle inner side will occur at a higher binding threshold than that to the outer side. This is currently being investigated by us.

**Sunil Kumar** responded: Yes, electrostatic attraction would change the shape of the vesicles. Please see our recent work “Curvature inducing macroion condensation driven shape changes of fluid vesicles” on this.<sup>1</sup> In the current manuscript we do not consider any explicit electrostatic interactions. The shape of the vesicles can depend on the presence of particles inside the vesicles as this will restrict the volume change. We are currently working on this problem.

1 K. K. Sreeja, J. H. Ipsen, P. B. Sunil Kumar, *J. Chem. Phys.*, 2015, **143**, 194902.

**Daan Frenkel** addressed Sunil Kumar and Mohamed Laradji: Your curved nanoparticles reproduce many of the functions of BAR proteins.

As you know there are also proteins that stabilise phospholipid tubes between vesicles. Can this behaviour be captured by your coarse-grained model?

**Mohamed Laradji** answered: As stated in the introduction of our article, our work is in part motivated by BAR proteins. It is very possible that the anisotropic nanoparticles considered by us will indeed stabilize nanoscale tubes between vesicles or a single vesicle to a planar membrane. This is a very interesting point which we will certainly investigate in the near future.

**Sunil Kumar** replied: Yes, BAR proteins are believed to be involved in the formation of lips tubes and the ramified shapes of organelles. Though the exact mechanism by which they achieve this is still unclear, curvature induction and curvature sensing are supposed to play important roles in the ability of BAR proteins to deform the membrane. In our simulations, we do see stabilisation of highly curved regions, like ridges, on the membrane. However, to see tube formation we need to go into much larger system sizes. Please note that earlier Monte Carlo Simulations, which treat these inclusions as an anisotropic curvature inducing field, have seen tube formation.<sup>1</sup>

1 N. Ramakrishnan, P. B. Sunil Kumar and J. H. Ipsen, *Biophys. J.*, 2013, **104**,1018.

**Rajdip Bandyopadhyaya** addressed Daan Frenkel, Mohamed Laradji and Pedro A. Sánchez: Steve Granick mentioned the Science paper on constructing a periodic table of different protein complexes.<sup>1</sup>

Similarly, what is the possibility of nanoparticle-aggregate motifs? How many parameters influence this problem? What is the role of the individual nanoparticle and where does the effect of particle–particle interaction end? What

diverse topological structures are possible? For example, maybe the number of amino-acid units and pH define protein aggregates. Likewise what are the minimum number of parameters required to capture nanoparticle aggregates, in the absence of any external field? Is it possible to develop some finite number of measures of resulting nanoparticle aggregate-structures, so as to capture all structures and classify them systematically? Otherwise a large set of different aggregates is possible.

1 S. E. Ahnert, J. A. Marsh, H. Hernández, C. V. Robinson and S. A. Teichmann, *Science*, 2015, **350**, DOI: 10.1126/science.aaa2245.

**Daan Frenkel** replied: I should stress that our models are, at present, highly simplified and that therefore we cannot really assess the effect on the aggregates of the shape of the DNA bricks (or the constructs with a higher coordination number that we discuss in our paper). Apart from steric factors, the interaction between DNA bricks is determined exclusively by the complementary DNA sequences that link neighbouring particles together. As was shown in Ref. 4, DNA bricks can self-assemble into a variety of topologically distinct structures. These can be characterised by their topological genus. However, the topological genus is but one parameter to characterise DNA-brick structures.

**Mohamed Laradji** responded: These are excellent questions. I think that others should interject in answering them. I would guess that the shape of the nanoparticles, and the way they are decorated should affect very strongly the details of their aggregation.

**Pedro A. Sánchez** answered: In the simplest (but still reasonably representative) model of magnetic nanoparticles self-assembly, corresponding to monodisperse hard spheres with point dipoles located at their centers, the parameters that determine the self-assembly motifs are the ratio between the dipolar moment and the temperature, and the particle concentration. The latter determines the appearance of frustrated configurations in the aggregates. Even considering just the ground states of the system, the possibilities change strongly with any added parameter that we may want to consider, like the polydispersity of the particles or any shape anisotropy.

In an exercise of maximum abstraction, I would say that the minimum general factors that should determine the motifs in aggregates of nanoparticles are:

(1) The range of particle pair interactions (steric, patchy: short ranged; electrostatic: long ranged but usually screened; magnetic: long ranged, not screened ...); (2) The relative strength of the particle pair interactions with respect to the thermal fluctuations; (3) The anisotropies of the particle pair interactions; (4) The constraints (walls, density ...). Of course this exercise is just a starting point without any predictive power

**Oleg Gang** questioned: To what degree are the local membrane deformations important?

**Mohamed Laradji** replied: Clustering of the nanoparticles is the result of membrane deformations induced by the nanoparticles.

**Sanat Kumar** addressed Mohamed Laradji and Sunil Kumar: You use coarse-grained surfactants in your simulations to form the membrane. Cacciuto's work uses an abstraction for the membrane – in that he treats an elastic sheet with bending rigidity incorporated. In terms of the minimal information needed, what do you need to put in terms of the membrane? Do you need surfactants or is a sheet-like description enough? In the spirit of discussion, can I coarse grain the surfactant away? What is the minimum model to do what you're doing?

**Mohamed Laradji** responded: Indeed, Saric and Cacciuto's work<sup>1</sup> treats the membrane as an elastic sheet through a triangulated surface model. The model to be used depends on the ratio between the lengthscales of the nanoparticles of interest and the thickness of the lipid bilayer. In our work, we focus on elongated and curved nanoparticles with an arclength only a few times that of the bilayer thickness. The formation of aster-shape clusters at high adhesion strength, which we observe in our simulations is the result of the nanoparticles inducing regions with negative Gaussian curvature which is the result of an induced inhomogeneity in the properties of the lipid bilayer, including an increased lipid density of the vicinal leaflet and increased chain order parameter of the lipids close to the nanoparticles. Meanwhile the lipids in the distal leaflet are not as affected by the nanoparticle. These effects cannot properly be accounted for by an elastic model. We recently investigated the interaction and endocytosis of small spherical nanoparticles with tensionless lipid bilayers<sup>2</sup> using the same lipid model and found that the nanoparticles can be partially wrapped by the tensionless bilayer. This is in contrast to results based on the treatment of the bilayer as an elastic sheet with zero thickness.<sup>3</sup> We showed in this work that the finite range of the interaction (while being shorter than the thickness of the bilayer) and inhomogeneities of the mechanical properties of the bilayer, induced by the nanoparticle lead to partial wrapping. When the size of the nanoparticle is much larger than the bilayer thickness, we retrieve the results of the elastic theory, which treats the bilayer as an infinitesimally elastic sheet.<sup>3</sup> Therefore, if the interest is on small nanoparticles, then a model similar to ours is needed. For studies of large nanoparticles, an elastic sheet model for the bilayer might be sufficient.

1 A. Saric and A. Cacciuto, *Phys. Rev. Lett.*, 2012, **108**, 118101.

2 E. J. Spangler, S. Upreti and M. Laradji, *J. Chem. Phys.*, 2016, **144**, 044901.

3 M. Deserno and T. Bickel, *Europhys. Lett.*, **62**, 767.

**Sunil Kumar** responded: This will depend on the scale of deformation involved. The thickness of the bilayer lipid membrane is of the order of 5 nm. If one is considering particles which are much bigger than this length (the radius of curvature of the induced deformation being much higher than the smallest length of the anisotropic particle) and there is no lipid flip flop, then an elastic sheet approximation is valid. Also please note that the elastic sheet model is much harder to use for dynamics.