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Published in:
Langmuir

DOI:
10.1021/acs.langmuir.5b02347

Publication date:
2015

Document Version
Peer reviewed version

Link to publication in Discovery Research Portal

Citation for published version (APA):
Brandani, G. B., Schor, M., Morris, R., Stanley-Wall, N., MacPhee, C. E., Marenduzzo, D., & Zachariae, U. (2015). The bacterial hydrophobin BslA is a switchable ellipsoidal Janus nanocolloid. Langmuir, 31(42), 11558-11563. DOI: 10.1021/acs.langmuir.5b02347

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The bacterial hydrophobin BslA is a switchable ellipsoidal Janus nanocolloid

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Abstract

BslA is an amphiphilic protein that forms a highly hydrophobic coat around *Bacillus subtilis* biofilms, shielding the bacterial community from external aqueous solution. It has a unique structure featuring a distinct partition between hydrophilic and hydrophobic surfaces. This surface property is reminiscent of synthesized Janus colloids. By investigating the behavior of BslA variants at water-cyclohexane interfaces through a set of multi-scale simulations informed by experimental data, we show that BslA indeed represents a biological example of an ellipsoidal Janus nanoparticle, whose surface

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interactions are, moreover, readily switchable. BslA contains a local conformational toggle, which controls its global affinity for, and orientation at, water-oil interfaces. This adaptability, together with single-point mutations, enables the fine-tuning of its solvent and interfacial interactions, and suggests that BslA could be a basis for biotechnological applications.

Introduction

The biofilm formed by *Bacillus subtilis* bacterial communities features an extremely non-wetting surface, which exceeds the water repellency of Teflon.[1,2] This behavior is mainly ascribed to a distinct layer predominantly formed by the protein BslA.[3,4] Its properties are underpinned by the recently determined crystal structure of BslA, in which a hydrophobic cap region, which exposes an unusually extended area of hydrophobic surface to external medium, is appended to a large polar domain[4] (Fig. 1A,B). By its geometrical and functional similarity to fungal hydrophobins – proteins that display high intrinsic surface activity and a capability to assemble into layers – BslA has been defined as a bacterial hydrophobin, although it differs in sequence and structure.[4,5]

The geometric separation into clearly defined hydrophobic and hydrophilic regions seen in BslA is reminiscent of non-spherical Janus particles (Fig. 1C), synthesized colloids that have two sides with different, often opposite surface properties.[6,7] Non-spherical Janus colloids have recently engendered intense interest among scientists interested in nanotechnology, as they can adopt a wide spectrum of orientations at interfaces, a characteristic that bears great promise for their use as versatile surfactants.[8] Similar to synthetic colloids, water-soluble biological proteins play an important role in the stabilization of emulsions.[9] This role hinges on the ability of nanoparticles to arrest demixing in an otherwise phase-separating mixture by occupying the interface between the immiscible components. In this way, they decrease the interfacial energy of the system, which ultimately provides the driving force for phase separation. The resulting emulsions are termed Pickering emulsions[10] or bijels.[11]

When a colloidal particle has no strong preference for one of the two solvents that form the interface, it experiences an attraction to the interface. The correspondent interfacial energy is equal to the product of the fluid-fluid surface tension and the area of interface covered by the particle.[10] We refer to this type of non-specific particle-interface interaction as the "Pickering effect", since it controls the adsorption of colloidal particles forming Pickering emulsions.[10] For particles larger than a few nm, the interfacial attraction exceeds Brownian forces, and adsorption becomes irreversible within the typical experimental or biological timescales.[10] While proteins are directed to the interface by the Pickering effect, their enhanced complexity can lead to richer behavior. For instance, when proteins adsorb to an interface, they often undergo large conformational changes up to unfolding.[9] As the interfacial energy of proteins is often smaller than that of the typically larger synthetic colloids, this energy can become comparable with the free energy change of refolding.

In this work, we investigated the adsorption of the BslA protein to a cyclohexane (c-hex)/water interface by multi-scale molecular dynamics simulations, closely linked to experiments and biologically relevant scenarios.[4,12] Our simulations provide reliable estimates of the adsorption free energy of wild-type (wt) BslA and enable us to rationalize a number of experimental observations.[4,12]

Importantly, we show that the two main forms of BslA found in its crystal structure interact with the interface in a markedly different manner. While both conformations are elliptical in shape, one of them adopts an upright orientation at the interface, whereas the other one tilts to enhance surface area coverage. These two different interfacial arrangements are analogous to different regimes experienced by colloidal ellipsoidal Janus particles.[13] They arise due to a distinct and finely tuned balance between Pickering and hydrophobic interactions in BslA. The fact that both of these conformations are present in the crystal structure of BslA is particularly remarkable, as this makes it possible to switch between the two by tuning external parameters or by varying the protein's local chemical environment and may thus enable the versatile use of BslA variants as readily adjustable surfactants or emulsifiers.

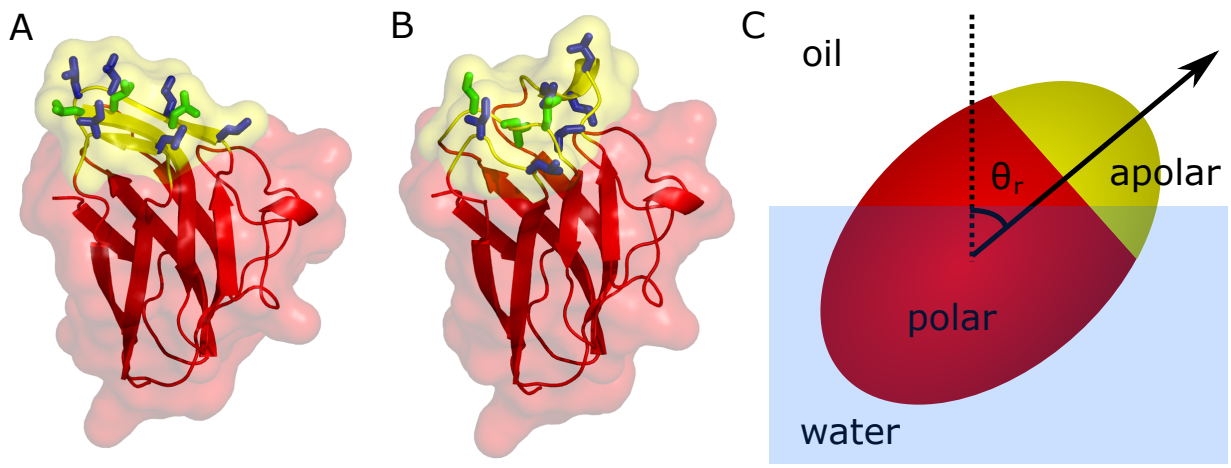


Figure 1: Crystal structure of the L_{out} (A) and L_{in} (B) conformations of BslA (PDB ID: 4bhu;[4] hydrophobic cap: yellow, polar (immunoglobulin) domain: red). Large hydrophobic residues in the cap are shown as blue (outer) or green sticks (inner strand) (C) Schematic of an ellipsoidal Janus colloid at a water-oil interface (θ_r, orientation angle; yellow, apolar; red, polar surface).

Model and methodology

All molecular dynamics simulations were performed in the NAP_zT ensemble with a constant area of the interface, P_z=1 bar and T=300 K, by using the software GROMACS 4.[14] The system was thermostated using the v_rescale algorithm,[15] while the pressure was maintained around its target value using the Parrinello-Rahman method.[16,17] The protein and solvents (water and cyclohexane) were represented with the polarizable MARTINI coarse-grained force field.[18,19] The MARTINI force field is well suited for the study of proteins at interfaces,[20] since the amino-acids in this force field have been parameterized based on their experimental water to cyclohexane partitioning free energies[18] and the water-oil surface tensions are in good agreement with experiments.[21] The coarse-grained topology of the protein has been defined from the BslA crystal structure (PDB ID: 4bhu[4]) using the MARTINI tools.[18] To obtain a realistic flexibility of the protein, we combined the MARTINI force field with an elastic network model (ELNEDYN,[22] see supplement for further details).

The free energy of adsorption of BslA to a water-cyclohexane interface (ΔG_{ads}) was reconstructed from a set of non-equilibrium force-probe simulations by making use of the

Jarzynski equality,[23] as implemented by Park and Schulten.[24] The center of mass of the protein was pulled toward the water phase relative to the center of mass of the oil phase at a speed of 0.1 Å/ns, using a spring constant of 10^4 kJ/mol nm^2. Within these settings, the stiff-spring approximation holds, and the cumulant expansion of the free energy is consistent with the result obtained from the Jarzynski equality.[24] This ensures a sufficient sampling of pulling simulations (a minimum of 64 independent runs for each structure) for a reliable free energy estimate.[24]

The two-dimensional free energy in Fig. 3A was obtained from a series of simulations in which the distance between the protein and the interface was restrained to a range of values by harmonic potentials. In each case we performed 12 simulations of 200 ns length with equilibrium distances from the interface uniformly spaced between 0 and 20 Å; the spring constant was set to 10^3 kJ/mol nm^2. This protocol ensured a good overlap of the neighboring probability distributions. The purpose of this umbrella sampling[25] was to enhance the exploration of the different protein orientations at the interface. The two-dimensional free energy along both the distance and the orientation angle θ_r can be then obtained by reweighing each observed configuration using the weighted histogram analysis method (WHAM).[26]

To enable the decomposition of $\Delta G_{\rm ads}$ into its protein-solvent (E_{PS}) and interfacial (E_I) energy contributions directly from equilibrium molecular dynamics simulations, we estimated E_{PS} as the sum of the water-to-cyclohexane transfer free energies of the amino acid side-chains exposed to oil.[27,28] The sum includes those residues whose side-chain centers of mass are located within the oil region, and it excludes the buried ones, defined as those having a solvent accessible surface area[29] lower than 30Å^2. The interfacial term is defined as $E_I = -\gamma S_I$, where γ is the water-cyclohexane surface tension and S_I is the area occupied by the protein coarse-grained beads within the interface. The particle radii and the interface width were both taken from the van der Waals radius of the coarse-grained water molecules (2.1 Å). We defined the location of the interface along the $\hat{z}$ direction as the position at which the density of water equals the density of oil. The energy contributions reported in Fig. 3B were

computed by averaging over the configurations explored during long equilibrium trajectories of BslA adsorbed at the interface (400 ns), whose duration extended substantially beyond the maximum autocorrelation time of protein reorientation (40 ns).

For the Janus ellipsoidal colloid model of BslA, the energy of the system is described by the following continuum approximation:[13]

$$E = \gamma(S_A \cos\theta_A + S_P \cos\theta_P - S_I), \tag{1}$$

where S_A and S_P are the apolar and polar surface areas, respectively, exposed to the oil phase, and S_I is the area occupied by the particle at the interface. Furthermore, γ is the water/oil surface tension, and θ_A and θ_P are the contact angles of the apolar and polar surfaces of the colloid, which serves as a measure of their hydrophobicity. The geometry of the colloid was defined to match that of BslA. The colloid longest axis was set to 52 Å, whereas the two shorter ones were set to 28 Å (the BslA inertia equivalent ellipsoid[30] has axes of $l_{x,y,z}$=55,33,26 Å). The BslA protein exhibits only a very small angle ($\sim 8^\circ$) between the direction of the longest protein axis and the position of the cap w.r.t. the protein center of mass; therefore, the ellipsoid was polarized along the longest axis, with the apolar side covering 18% of the total surface. We then performed Monte-Carlo simulations of the colloid adsorbed at a water-oil interface. At each step, a change of the particle's orientation and distance from the interface was attempted, and the move was accepted or rejected according to the Metropolis criterion with the energy defined by Eq. 1. Quantities reported in Fig. 4 were obtained from averaging over long equilibrium simulations. Each of the trajectories is at least 50 times longer than the autocorrelation time of reorientation of the colloid, and we discarded the first tenth of each simulation to allow for system equilibration.

Results

The crystallographic BslA decamer shows slight conformational differences between the protein subunits,[4] which fall into two major clusters (Fig. 1A-B). In total, the BslA cap contains eleven hydrophobic side-chains. The two clusters are characterized by surface outward- or inward-facing configurations of the hydrophobic cap leucine side-chains L77, L119, and L123 (see the supplement for more details on the two clusters). We therefore refer to these two representative structures as BslA-L_{out} and BslA-L_{in}, respectively. In addition, the BslA-L_{in} cap is chiefly random-coil, whereas the BslA-L_{out} cap displays a three-stranded β-sheet (Fig. 1A, B). In experiments, mutations in the central cap β-strand (L76, L77, L79[4] and G80[3]) strongly reduce the stability and water-repellency of *B. subtilis* biofilms.

We conducted coarse-grained molecular dynamics (CGMD) simulations[31] of BslA-L_{out} and BslA-L_{in} and compared wt-BslA with the single residue mutants L76K, L77K and L79K. In our initial simulations, each of the proteins was placed into the aqueous section of a $9 \times 9 \times 14$ nm box filled with 65% (volume) water and 35% c-hex. All variants spontaneously adsorbed to the c-hex/water interface on short time-scales of ∼50 ns (Fig. 2A), however at slightly different individual rates.

We next aimed at quantifying the free energy of adsorption of the BslA variants. The potential of mean force (PMF) along the interface normal, F(z), was calculated by using the Jarzynski equality[23] to reconstruct F(z) from an ensemble of non-equilibrium force-probe simulations. In these, BslA was detached from the interface and moved into the aqueous phase.[24]

Fig. 2B shows the adsorption free energy, ΔG_{ads}, of the variants as the convergence level of F(z) upon increasing their separation from the interface (z=0). wt-BslA-L_{out} has a ΔG_{ads} of ∼65 kcal/mol (Fig. 1B, magenta), which is sufficiently high to consider the adsorption irreversible on experimentally or biologically accessible timescales. This value is also close to the calculated surface activity of fungal hydrophobins.[32] In the L_{out} form, the mutants L77K and L79K show a considerably lower ΔG_{ads} of ∼49 kcal/mol (blue) and ∼50

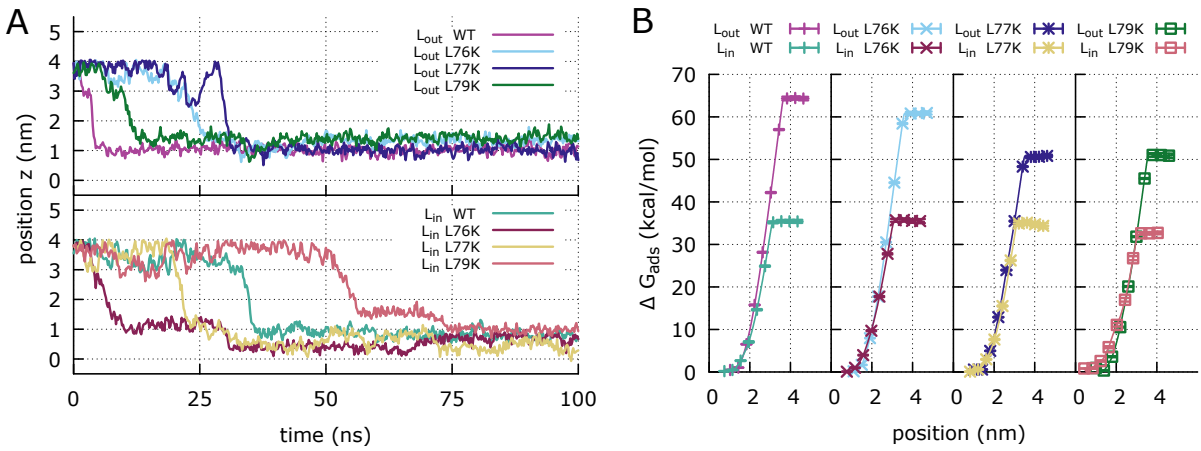


Figure 2: (A) Center of mass positions of BslA variants relative to the interface showing spontaneous adsorption to a c-hex/water interface from the aqueous phase. (B) Free energy of adsorption of BslA variants as reconstructed from force-probe CGMD simulations. The error bars, calculated as the standard deviation at each position we evaluated, are below 1 kcal/mol in each case.

kcal/mol (green), respectively, whereas the mutation L76K decreases ΔG_{ads} only slightly to ~61 kcal/mol (cyan). By contrast, the L_{in} form lowers ΔG_{ads} of wt-BslA and all cap mutants to only 35-36 kcal/mol. Because L_{out} is the conformation with the highest adsorption free energy, we conclude that BslA is adsorbed at interfaces in the L_{out} form, both in vivo and in vitro. This conclusion is supported by the observation that the pattern of BslA mutant film stability[4] and timescales in adsorption kinetics (Fig. S4) in experiments follows that of ΔG_{ads} for L_{out}, whereas ΔG_{ads} for BslA-L_{in} is insensitive to cap mutations.

Because most of the mutations and conformational changes impacting on BslA surface activity affected its cap, we expected changes in its surface hydrophobicity to be a main contributor to the ΔG_{ads} difference. Indeed, the magnitude of the BslA cap hydrophobic dipole[27] is correlated with the changes of the free energy of adsorption (Fig. S3). However, when the hydrophobic dipole is lowered below a certain value, further decrease has very little effect on ΔG_{ads}.

As previously discussed, the Pickering effect is another important factor favoring the adsorption of any protein or nanocolloid to a water/oil interface, as the interfacial energy

decreases when part of the interface is occupied by the particle. If BslA is viewed as a Janus ellipsoidal colloid, the location of the hydrophobic cap at one of the edges of the longest inertia axis can create a unique competition between particle-solvent and interfacial energies.[13] When the protein is adsorbed in an "end-on" state, with the main axis perpendicular to the interface, it can efficiently partition the hydrophobic cap into the oil phase, and the hydrophilic body of the protein into the water phase. In contrast, a "side-on" adsorption, with the main axis parallel to the interface, increases the area occupied by the protein at the interface, and optimizes the interfacial contribution to the free energy at the expense of protein-solvent interactions.

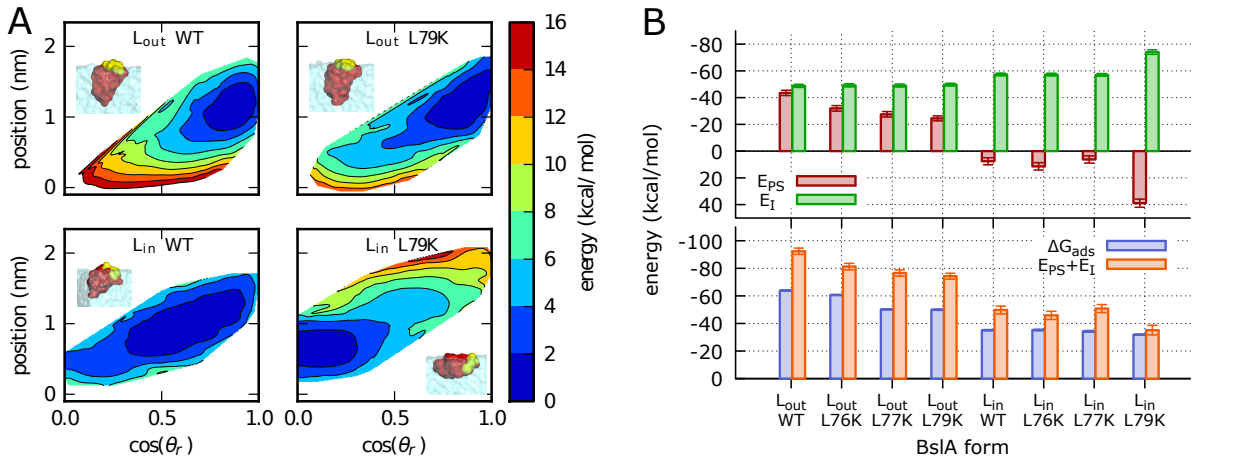


Figure 3: (A) 2D free energy landscape of BslA at the c-hex/water interface as function of the BslA center-of-mass relative to the interface and the cosine of its orientation angle, θ_r. (B) Estimate of the protein-solvent (E_{PS}) and interfacial (E_I) energies at the interface from CGMD simulations (top), and comparison with ΔG_{ads} determined by force-probe simulations (bottom).

By using CGMD and umbrella sampling,[26] we reconstructed the free energy of four of the BslA variants at the interface as a function of their orientation θ_r, defined as the angle between the direction of the main inertia axis and the normal to the interface. Fig. 3A shows that the free energy minimum of wt-BslA-L_{out} reflects an end-on orientation, where the hydrophobic cap (yellow) is immersed in cyclohexane, whereas the immunoglobulin domain (red) is surrounded by water (Fig. 3A, top left). A similar orientation is observed for the

L79K mutant, although a wider variation in θ_r is seen at the free energy minimum (Fig. 3A, top right). By contrast, the free energy minimum for L79K-BslA-L_{in} reflects a side-on orientation (Fig. 3A, bottom right). Notably, the cap structural change from wt-BslA-L_{out} to wt-BslA-L_{in} leads to a much broader distribution of orientations at the phase boundary (Fig. 3A, bottom left). The protein main axis is on average substantially more tilted, and both side-on and end-on orientations can be accessed at the thermal equilibrium.

Indeed, as suggested earlier, the transition of BslA from a end-on to a side-on orientation at the interface can be explained as the result of the optimisation of different energy contributions to the full free energy of adsorption. We estimated protein-solvent (E_{PS}) and interfacial (E_I) contributions to ΔG_{ads} for the different BslA forms in equilibrium at the interface (see methods section for the computation of these terms). Fig. 3B shows that the sum of these two contributions is in semi-quantitative agreement with the estimate of ΔG_{ads} obtained from the Jarzynski equality. Moreover, the analysis shows that only the L_{out} form of BslA, which adopts an end-on orientation, can efficiently partition its residues into the oil phase to minimize the energy of the system. By contrast, the adsorption of BslA-L_{in} is dominated by the interfacial energy term, which is underpinned by the increase in area covered at the interface. Notably, this result demonstrates that E_{PS} and E_I are sufficient to describe the full interaction of the BslA variants with the interface.

To investigate how the geometry and the variations in BslA cap hydrophobicity contribute to the observed tilting transition at the interface and to study the balance between the hydrophobic and Pickering effects, we further coarse-grained BslA to a Janus ellipsoidal colloid polarized along its main axis (see methods for the details of the colloid geometry), with the energy of the colloid-interface system described by equation 1. Within this continuum model, colloid-solvent and interfacial interactions neatly decouple: the former are accounted for by the first two terms (henceforth denoted collectively as E_{PS}), the latter by the third term (γS_I).

We performed Monte-Carlo simulations with the Janus ellipsoid at the oil/water interface,

enabling us to separate the effects of the two different driving forces for adsorption. We set the polar contact angle to $\theta_P = 60°$, whereas the cap apolar contact angle was varied between $\theta_A = 90°$ and $\theta_A = 180°$. The variation in θ_A represents the change in surface chemistry of the BslA cap as a result of the mutations and the conformational change. Fig. 4 shows that a transition takes place from an end-on, upright, orientation (where the angle between the ellipsoidal long axis and the interface normal, θ_r, is $\sim 0°$) to a side-on, tilted, orientation ($\theta_r \sim 90°$), as the hydrophobicity of the cap is decreased from $\theta_A = 180°$ to $\theta_A = 90°$, (Fig. 4A). Consistent with our CGMD simulations of BslA, the upright orientation is favored at high cap hydrophobicity (as for BslA-L_{out}), as it optimizes the solvent interactions with the nanoparticle, whereas the tilted orientation increases the interfacial area covered by the particle, hence maximizing the Pickering effect (as for BslA-L_{in}) (Fig. 4B). This finding is also consistent with the tilting transition observed in similar colloidal systems using alternative computational methods.[13,33]

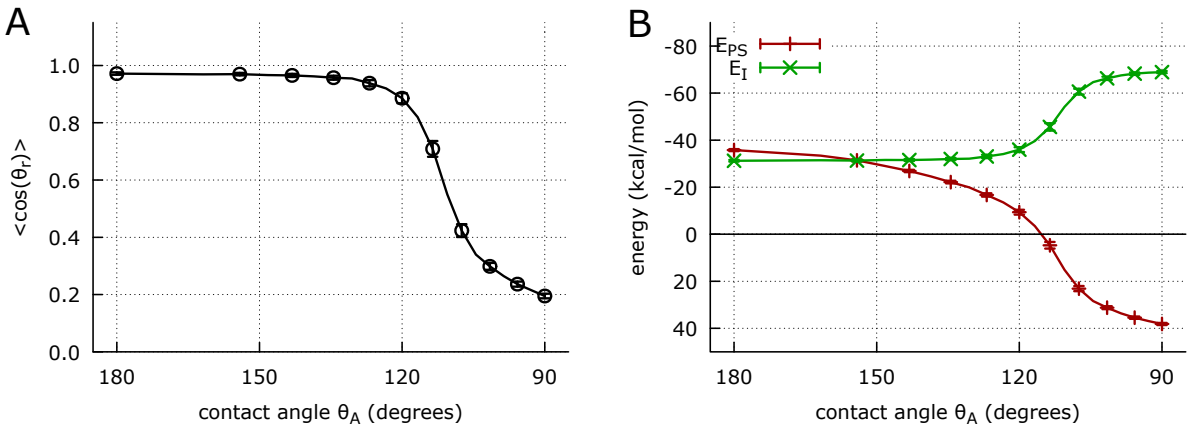


Figure 4: Monte-Carlo simulations of a BslA-like Janus ellipsoid adsorbed at an interface. The average cosine value of the orientation angle θ_r, defined as the angle between the colloid long axis and the normal to the interface, demonstrates that upon increasing hydrophobicity (varying θ_A from 180° to 90°) a transition from an upright to a tilted orientation is seen (A). The averages of the particle-solvent interaction energy E_{PS} and the interfacial energy E_I as functions of the apolar cap contact angle (B) show that the end-on regime is dominated by particle-solvent interactions, whereas the side-on orientation is dominated by the Pickering effect. Error bars in (B) were determined as standard deviation, but remain lower than 1 kcal/mol at each data point.

Conclusions

The results described above have important implications for the biological role of BslA and its potential use in nanotechnology. Firstly, our data define BslA as a nanoscale biological Janus ellipsoid, in which a finely tuned balance between particle-solvent and interfacial energies enables small conformational changes or mutations to control the overall orientation of the protein at the oil/water interface. Much of the interface behavior of BslA can be described by a simple Janus model, usually applied only to larger colloids.[8,13] Of particular relevance for its potential use in nanotechnology however is the fact that, to our best knowledge, BslA is one of the first examples of a Janus bio-nanocolloid with *switchable* surface properties [1].

Indeed, our simulations, based on the known crystal structures of BslA,[4] suggest that BslA possesses a local conformational switch in its hydrophobic cap region, which controls its global attraction energy to a water/oil interface. As the two configurations are seen in the same crystal unit, they can be expected to differ only moderately in energy and to reflect the intrinsic conformational variability of BslA.[34] This is in stark contrast to the unfolding seen in other proteins upon surface adsorption.[9] Experimental evidence for a subtle conformational switching process in BslA has recently also been obtained.[12] Large hydrophobic regions are usually unstable at the surface of soluble proteins as during folding, they normally organize into the shielded protein hydrophobic core by hydrophobic collapse, exposing mainly polar residues to water.[35] Interestingly, all previously characterized similar proteins stabilize their large external hydrophobic regions by forming an extended network of rigid disulfide bonds.[5] The alternation of large hydrophobic side-chains from an outward- to an inward-facing configuration thus defines a new way in which a protein stabilizes and presents an extended hydrophobic surface to an interface, while remaining water-soluble and stable in aqueous solution.

[1]Note that we use here the word "switchable" to mean that it is possible to convert one state onto the other when the protein adsorbs to, or desorbs from, the interface. However, it would be of great interest for nanotechnology applications to identify a practical route to control this switch while the protein is adsorbed at the interface.

Further, we show that single point mutations in the central β-strand of the cap region not only reduce the free energy of adsorption of BslA, but also alter its orientation at oil/water interfaces. It has been shown previously that purified BslA_{42-181} spontaneously aggregates into a stable protein film at air-water interfaces *in vitro*.[4] Electron microscopic images of the BslA film have demonstrated that the most stable assembly of BslA surface layers correlates with a high degree of order between individual proteins.[12] In our simulations, the outward facing conformation (wt-BslA-$\mathrm{L_{out}}$) exhibits the most clearly defined upright orientation at the interface in combination with the highest adsorption free energy. The deviation from clearly defined upright orientations we show here for mutant proteins is thus likely to be an important factor in the relative loss of mutant BslA layer stability *in vitro*, and of the observed wettability of the mutant biofilms *in vivo*.[3,4] A better structural understanding of the stability of the hydrophobic protective layers around biofilms is essential to aid the design of agents that disrupt this huge permeation barrier to water-soluble biocides such as antibiotics.[1,36]

Importantly, our results show that BslA is a biological nanocolloid which displays, both, a finely tuned balance between Pickering and solvent interactions at hydrophilic/hydrophobic interfaces and a rich conformational behavior. As such, BslA constitutes an example of a Janus colloid on the nanoscale with readily tunable surface interactions, and thus could form the basis of a highly versatile biologically derived surfactant or emulsifier in biotechnology applications, easily adaptable to a given interface. Furthermore, as we have demonstrated, the interactions of BslA with the surface can be easily pre-determined from its near-ideal behaviour as a Janus ellipsoid.

Acknowledgement

We thank Rastko Sknepnek for critically reading the manuscript. This work was partially supported by EPSRC grant [EP/J007404/1] and BBSRC grants [BB/L006979/1; BB/I019464/1; BB/L006804/1]. GBB acknowledges a Principal's Career Development PhD scholarship of

the University of Edinburgh.

Supporting Information Available

Including a detailed description of the methods employed in this work. Additionally, we report the analysis of the BslA cap hydrophobic dipole and experimental results on the kinetics of adsorption of BslA at a water/oil interface.

This material is available free of charge via the Internet at `http://pubs.acs.org/`.

References

(1) Epstein, A. K.; Pokroy, B.; Seminara, A.; Aizenberg, J. Bacterial biofilm shows persistent resistance to liquid wetting and gas penetration. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 995–1000.

(2) Cairns, L. S.; Hobley, L.; Stanley-Wall, N. R. Biofilm formation by Bacillus subtilis: new insights into regulatory strategies and assembly mechanisms. *Mol. Microbiol.* **2014**, *93*, 587–98.

(3) Kobayashi, K.; Iwano, M. BslA(YuaB) forms a hydrophobic layer on the surface of Bacillus subtilis biofilms. *Mol. Microbiol.* **2012**, *85*, 51–66.

(4) Hobley, L.; Ostrowski, A.; Rao, F. V.; Bromley, K. M.; Porter, M.; Prescott, A. R.; MacPhee, C. E.; van Aalten, D. M. F.; Stanley-Wall, N. R. BslA is a self-assembling bacterial hydrophobin that coats the Bacillus subtilis biofilm. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 13600–5.

(5) Linder, M. B.; Szilvay, G. R.; Nakari-Setälä, T.; Penttilä, M. E. Hydrophobins: the protein-amphiphiles of filamentous fungi. *FEMS Microbiol. Rev.* **2005**, *29*, 877–96.

(6) Jiang, S.; Chen, Q.; Tripathy, M.; Luijten, E.; Schweizer, K. S.; Granick, S. Janus particle synthesis and assembly. *Adv. Mater. (Weinheim, Ger.)* **2010**, *22*, 1060–71.

(7) Sciortino, F.; Giacometti, A.; Pastore, G. Phase Diagram of Janus Particles. *Phys. Rev. Lett.* **2009**, *103*, 237801.

(8) Kumar, A.; Park, B. J.; Tu, F.; Lee, D. Amphiphilic Janus particles at fluid interfaces. *Soft Matter* **2013**, *9*, 6604.

(9) McClements, D. J. Protein-stabilized emulsions. *Curr. Opin. Colloid Interface Sci.* **2004**, *9*, 305–313.

(10) Aveyard, R.; Binks, B. P.; Clint, J. H. Emulsions stabilised solely by colloidal particles. *Adv. Colloid Interface Sci.* **2003**, *100-102*, 503–546.

(11) Cates, M. E.; Clegg, P. S. Bijels: a new class of soft materials. *Soft Matter* **2008**, *4*, 2132.

(12) Bromley, K. M.; Morris, R. J.; Hobley, L.; Brandani, G.; Gillespie, R. M. C.; McCluskey, M.; Zachariae, U.; Marenduzzo, D.; Stanley-Wall, N. R.; MacPhee, C. E. Interfacial self-assembly of a bacterial hydrophobin. *Proc. Natl. Acad. Sci. U. S. A.* **2015**,

(13) Park, B. J.; Lee, D. Configuration of nonspherical amphiphilic particles at a fluid–fluid interface. *Soft Matter* **2012**, *8*, 7690.

(14) Hess, B.; Kutzner, C.; van der Spoel, D.; Lindahl, E. GROMACS 4: Algorithms for Highly Efficient, Load-Balanced, and Scalable Molecular Simulation. *J. Chem. Theory Comput.* **2008**, *4*, 435–447.

(15) Bussi, G.; Donadio, D.; Parrinello, M. Canonical sampling through velocity rescaling. *The Journal of chemical physics* **2007**, *126*, 14101.

(16) Parrinello, M.; Rahman, A. Polymorphic transitions in single crystals: A new molecular dynamics method. *Journal of Applied physics* **1981**, *52*, 7182–7190.

(17) Nosé, S.; Klein, M. L. Constant pressure molecular dynamics for molecular systems. *Molecular Physics* **1983**, *50*, 1055–1076.

(18) de Jong, D. H.; Singh, G.; Bennett, W. F. D.; Arnarez, C.; Wassenaar, T. A.; Schäfer, L. V.; Periole, X.; Tieleman, D. P.; Marrink, S. J. Improved parameters for the martini coarse-grained protein force field. *J. Chem. Theory Comput.* **2012**, *9*, 687–697.

(19) Yesylevskyy, S. O.; Schäfer, L. V.; Sengupta, D.; Marrink, S. J. Polarizable water model for the coarse-grained MARTINI force field. *PLoS computational biology* **2010**, *6*, e1000810.

(20) Euston, S. R. Molecular dynamics simulation of protein adsorption at fluid interfaces: a comparison of all-atom and coarse-grained models. *Biomacromolecules* **2010**, *11*, 2781–2787.

(21) Marrink, S. J.; Risselada, H. J.; Yefimov, S.; Tieleman, D. P.; de Vries, A. H. The MARTINI force field: coarse grained model for biomolecular simulations. *J. Phys. Chem. B* **2007**, *111*, 7812–7824.

(22) Periole, X.; Cavalli, M.; Marrink, S.-J.; Ceruso, M. a. Combining an elastic network with a coarse-grained molecular force field: structure, dynamics, and intermolecular recognition. *J. Chem. Theory Comput.* **2009**, *5*, 2531–2543.

(23) Jarzynski, C. Nonequilibrium Equality for Free Energy Differences. *Phys. Rev. Lett.* **1997**, *78*, 2690–2693.

(24) Park, S.; Schulten, K. Calculating potentials of mean force from steered molecular dynamics simulations. *J. Chem. Phys.* **2004**, *120*, 5946–5961.

(25) Torrie, G. M.; Valleau, J. P. Nonphysical sampling distributions in Monte Carlo free-energy estimation: Umbrella sampling. *Journal of Computational Physics* **1977**, *23*, 187–199.

(26) Souaille, M.; Roux, B. Extension to the weighted histogram analysis method: combining umbrella sampling with free energy calculations. *Comput. Phys. Commun.* **2001**, *135*, 40–57.

(27) Eisenberg, D.; Weiss, R. M.; Terwilliger, T. C.; Wilcox, W. Hydrophobic moments and protein structure. *Faraday Symp. Chem. Soc.* **1982**, *17*, 109.

(28) Radzicka, A.; Wolfenden, R. Comparing the polarities of the amino acids: side-chain distribution coefficients between the vapor phase, cyclohexane, 1-octanol, and neutral aqueous solution. *Biochemistry* **1988**, *27*, 1664–1670.

(29) Chothia, C. The nature of the accessible and buried surfaces in proteins. *Journal of molecular biology* **1976**, *105*, 1–12.

(30) Moller, B. Y. J. J.; Schrauber, H.; Muller, J. J. The inertia-equivalent ellipsoid: a link between atomic structure and low-resolution models of small globular proteins determined by small-angle X-ray scattering. *J. Appl. Crystallogr.* **1992**, *25*, 181–191.

(31) Monticelli, L.; Kandasamy, S. K.; Periole, X.; Larson, R. G.; Tieleman, D. P.; Marrink, S.-J. The MARTINI coarse-grained force field: extension to proteins. *J. Chem. Theory Comput.* **2008**, *4*, 819–834.

(32) Cheung, D. L.; Bon, S. A. F. Stability of Janus nanoparticles at fluid interfaces. *Soft Matter* **2009**, *5*, 3969.

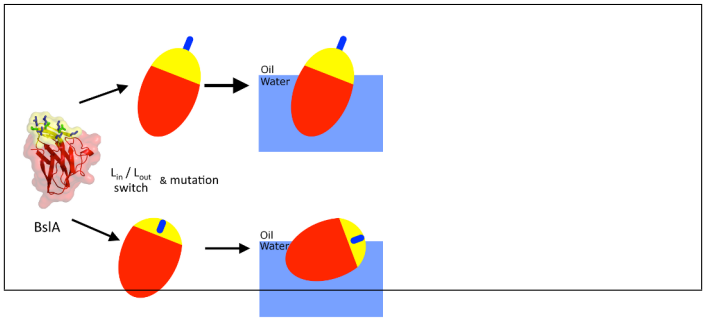
(33) Luu, X.-C.; Yu, J.; Striolo, A. Ellipsoidal Janus nanoparticles adsorbed at the water-oil interface: some evidence of emergent behavior. *J. Phys. Chem. B* **2013**, *117*, 13922–9.

(34) Jacobson, M. P.; Friesner, R. A.; Xiang, Z.; Honig, B. On the Role of the Crystal Environment in Determining Protein Side-chain Conformations. *J. Mol. Biol.* **2002**, *320*, 597–608.

(35) Zhou, R.; Huang, X.; Margulis, C. J.; Berne, B. J. Hydrophobic collapse in multidomain protein folding. *Science* **2004**, *305*, 1605–9.

(36) Mah, T.-F. C.; O'Toole, G. A. Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol.* **2001**, *9*, 34–39.

Graphical TOC Entry



Keywords: hydrophobin, Janus colloids, surfactant proteins, adsorption at interfaces, conformational switch, molecular dynamics, free energy