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REVIEW ARTICLE

Giant vesicles as cell models

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Tremendous progress has been made in recent years in understanding the working of the living cell, including its micro-anatomy, signalling networks, and regulation of genes. However, an understanding of cellular phenomena using fundamental laws starting from first principles is still very far away. Part of the reason is that a cell is an active and exquisitely complex system where every part is linked to the other. Thus, it is difficult or even impossible to design experiments that selectively and exclusively probe a chosen aspect of the cell. Various kinds of idealised systems and cell models have been used to circumvent this problem. An important example is a giant unilamellar vesicle (GUV, also called giant liposome), which provides a cell-sized confined volume to study biochemical reactions as well as self-assembly processes that occur on the membrane. The GUV membrane can be designed suitably to present selected, correctly-oriented cell-membrane proteins, whose mobility is confined to two dimensions. Here, we present recent advances in GUV design and the use of GUVs as cell models that enable quantitative testing leading to insight into the working of real cells. We briefly recapitulate important classical concepts in membrane biophysics emphasising the advantages and limitations of GUVs. We then present results obtained over the last decades using GUVs, choosing the formation of membrane domains and cell adhesion as examples for in-depth treatment. Insight into cell adhesion obtained using micro-interferometry is treated in detail. We conclude by summarising the open questions and possible future directions.

Introduction

The concept of a model system is fundamental to modern biology, the study of cells in culture being a prominent example. As a matter of fact, much of our current knowledge about cell biology originates from such model studies. In the context of adhesion, for example, the cell provides one of the adhesive interfaces and the other interface is provided by a planar substrate or bead surface coated with the relevant adhesion molecules.[1,2] Such substrates have been made progressively more bio-mimetic and have led to recent advances in our understanding of various phenomena including focal adhesion formation,[1,3] mechanosensing,[4] formation of the immunological synapse,[5,6] and influence of the micro-environment on tissue architecture and gene expression.[7] The planar substrate is compatible with powerful modern microscopic techniques such as TIRF (total internal reflection fluorescence), and at the same time provides considerable control over the properties of one of the surfaces. However, since the surface of the cell is still not controlled, detailed information like

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Insight, innovation, integration

Richard Feynmann, Nobel laureate in Physics and an iconic figure in modern science, famously wrote: "what I cannot create, I do not understand" – is this applicable to biology too? A whole new activity at the interface of biology and physics/engineering that calls itself "synthetic", "bottom-up" or "reconstitutive" biology is emerging with exactly such a point of view in mind. A striking example of reconstitutive biology is provided by giant unilamellar vesicles (GUVs), also known as giant liposomes, that are increasingly used as cell models suitable for probing membrane related phenomena like domains/rafts, adhesion *etc.* To reconstitute a whole cell in a liposome is a distant dream, perhaps of doubtful utility. However, as we demonstrate here with examples, well designed reconstitution of a specific function can lead to a quantitative understanding otherwise not achievable, and can grant us a certain degree of predictive power. Such a bio-mimetic approach complements traditional biology, bridging the gap between biochemistry and cell biology, and is an essential step towards a holistic picture.

molecular structure or affinity cannot be unambiguously assessed. In general, such cell studies are still too rudimentary to allow quantitative comparisons with theoretical approaches from a fundamental basis and as such lack predictive power.

An extreme example of the reductionist approach is biochemistry. It is clear that for a quantitative description of living cells from first principles, an essential step is a thorough understanding of the building blocks – the bio-macromolecules that make up the cell. However, biochemistry in solution, while of tremendous importance for initial identification and quantification, is of limited interest in the context of molecules that are habitually confined to a surface, membrane bound proteins being the prime example. This calls for a biophysical approach to the surface of a substrate and/or a colloidal bead consisting of confining molecules, and studying the interaction of two such surfaces. In this approach, near perfect control over the surface properties can be achieved and has yielded interesting insight into, for example, adhesion kinetics of single molecules.[8] In the context of membrane bound proteins however, several biologically important properties like *in-plane* mobility and flexibility, are accessible only when the proteins are integrated into a membrane.[9] It is therefore imperative to use model membranes to create even a rudimentary level of cell mimicry *in vitro* (see, for example ref. 10–12, for recent general reviews on use of semi-synthetic model systems to answer pertinent questions in biology).

Membrane based model systems take different forms, the best studied being monolayers, bilayers that are either supported or free standing, and vesicles of sizes ranging from tens of nano-meters to tens of micrometers. Among these, giant unilammelar vesicles, also called giant liposomes, have sizes close to typical cells. Similar to cells, they are flexible and of finite volume and area. Thus, they are particularly suitable for use not only as model membranes but also as cell models.†

† Note that the word "vesicle" also signifies naturally occurring intracellular bodies, which have a structure similar to small liposomes and serve various specific functions. Exocytic vesicles, for example, are involved in exocytosis.

A very fascinating endeavour, making elegant use of advanced GUVs, is the attempt to make self-replicating artificial cells with the long term goal of evolutionary experiments.[13] Other interesting applications concern the use of GUVs as minimal systems for cell-free protein synthesis[14] or micro-reactors in general (see ref. 15 and references therein). However, here we shall restrict ourselves to the use of GUVs as cell models to study membrane based phenomena. In such an approach a specific biological feature is reconstituted in a GUV and probed for its physical basis. GUVs have so far been used to shed light on the concept of "rafts" and its relation to membrane domains,[16,17] cell adhesion (see ref. 18 and 19 and references therein), endo and exocytosis,[20,21] antimicrobial peptides,[22] ion channels,[23] antibody binding[24] and more recently, cell division.[12]

In this review, we shall first briefly discuss the physical properties of the cell membrane which allow the cell to function as it does. Next, we shall introduce the general physics of model membranes, known to us from experiments on one or more kinds of model systems, but generally valid for all membrane systems. Afterwards we shall focus on giant unilamellar vesicles, their fabrication and finally, their use as test cells, restricting ourselves to detailed discussion of two such applications that have led to insight into the formation of domains and cell adhesion.

A closer look at membranes

The cell membrane and model membranes

The biological activity of the cell membrane is intimately linked to the physics of lipid bilayers, as realised by early pioneers in membrane physics[25–27] and vindicated by recent experiments. The plasma membrane of a living cell forms a barrier between its inside and the outer environment. The development of such a barrier was a crucial step in the development of life since it confined the proteins fabricated by a particular set of nucleic acid within a proto-cell.[28] Even at the stage of the proto-cell, the physical properties of a lipid membrane,

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namely flexibility, softness, fluidity and semi-permeable nature made it the obvious choice. The membrane of a modern eucaryotic cell is of course much more than a passive barrier. It participates actively in cell–cell communications, helps the cell to maintain homoeostasis, to attach itself to a substrate, to ingest nutrients and so on. The cell also has many internal organelles that are bound by membranes, which are structurally similar to the cell membrane, but they differ in their specific composition and have very different functions.

The membrane of the cell and its organelles are made chiefly of double-chained phospholipids, cholesterol and glycolipids, arranged as a bilayer with embedded proteins. The hydrophobic chains of the lipids face the inside of the membrane and the hydrophilic heads face the aqueous environment outside. The thickness is of the order of four to five nanometers: about two thousand times smaller than the radius of a typical mammalian cell. The two leaflets of the lipid bilayer that makes up the membrane of the cell are not symmetric. This asymmetry gives it special structural properties and is essential for some of its functions.[9]

In addition to the large variety of proteins embedded in or attached to the bilayer, the cell membrane also exhibits highly flexible polymeric sugars (the glycocalyx) on the outer surface and is linked to a mesh of cross-linked actin polymers on its inner surface. These two polymeric layers or shells can profoundly influence the structures and the function of the membrane. In particular, the relatively rigid actin shell, which is also intimately connected to the membrane, can have a very strong effect on the mechanics of the ensemble.[9]

The physical properties and hence the functions of the cell membrane are related to its composition.[9] Traditionally, biologists have viewed the lipid membrane as an inert matrix and have considered that all its functions must be tightly controlled by functional proteins which are, in turn, actively controlled by inter-cellular signalling pathways. However, it is becoming increasingly clear that many membrane phenomena attributed to active control may in fact arise passively, simply as a result of the physics of the membrane. In general, it can be expected that physical effects are always present – biological activity can either harness these effects to its own purpose, or find ways to suppress them.

It is virtually impossible to study the physics of the membrane using living cells as is since they are extremely complex entities where active and passive phenomena are inextricably mixed up. Studying a specific physical process and quantifying it in such a crowded and dynamic environment is extremely challenging. The way around is to first study the membrane in isolation, starting from a minimal single component lipid test cell. The strength of such model systems lies in their simplicity which is a prerequisite for well controlled experiments. Best results are achieved when the key components of a specific process can be isolated and transferred to a minimal system. This way, it is possible to identify how the cell exploits or counteracts fundamental physical laws to achieve optimal functionality. One important point however needs to be noted: that the cell membrane is in fact an out-of-equilibrium mixture that is kept very near the critical point (see ref. 25 for a recent review).

The simplest models for membranes are pure lipid monolayers and bilayers. Lipid bilayers exist either as membrane stacks, also called lyotropic liquid crystals, or as single membranes that are typically studied either as a free standing membrane or on a solid support. A free lipid membrane in water closes up on itself to form vesicles. Such vesicles may be unilamellar or multilamellar. Typically, unilamellar vesicles are named according to their size as GUV (giant unilamellar vesicles, radius >500 nm), LUV (large unilamellar vesicles, radius between 50 nm and 500 nm) or SUV (small unilamellar vesicles, radius <50 nm).‡ Being made of lipids, the principle constituent of the cell membrane, all these artificial membranes share, under suitable conditions, some of the physical properties of the cell membrane, such as in-plane diffusion.

However, all these model membranes are not necessarily good models for cell membranes. Membrane stacks are not good candidates for most purposes because their geometry is very different from that of the single bilayer of the cell membrane. Supported bilayers are not flexible, the free standing membranes too have a fixed geometry. A giant unilamellar vesicle, with its size comparable to an entire cell, can serve as a simple test cell. The simplest GUV consists only of a spherical lipid bilayer enclosing a buffer, but it already captures a key feature of cellular membranes: compartmentalisation. Such a membrane bound entity also mimics two other important features of the cell which are not mimicked by other kinds of confinement – for example in a liquid droplet or a bubble: non-stretchability (finite surface area) and flexibility (bending elasticity) of the cellular membrane. More complex GUVs made from lipid mixtures, carrying adhesion proteins and/or filled with artificial cytoskeleton provide more advanced test cells. The multitude of different modifications to the basic GUV that are now available, each capturing the essence of a different aspect of the cell, are summarised in Fig. 1.

Biophysics of model membranes

Lipids, whether presented as supported bilayers, bilayer stacks or vesicles, undergo structural phase transitions as a function of environmental parameters like temperature or humidity. The best studied example is that of diacyl-phospholipid (a lipid with phosphated head groups and two carbon chains) which is the major constituent of the cell membrane. At high temperatures and humidity, there is no positional order of either the chains or the head-groups within the bilayer and the membrane is said to be in the fluid or L_α phase. As the temperature (and/or humidity) is lowered, the chains and the heads get progressively ordered. The bilayer first enters a phase where the chains are ordered but not the heads – the gel or L_β phase. Finally, it may exhibit more than one crystalline phase, with both chains and heads ordered.[9] The transition temperature is a property of the specific lipid under study. In general, saturated chain lipids have a lower gel to fluid transition temperature.

If lipids with different phase transition temperatures are mixed in one membrane, separation into distinct coexisting

‡ Note however, the GUVs need to be >5 μm in size for the applications discussed here.

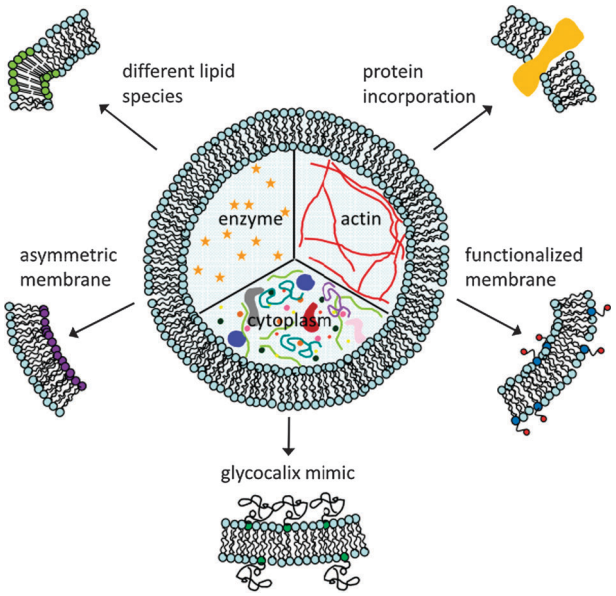

Fig. 1 A schematic representation of the different modifications to the basic GUV that have been achieved to date. Each of these modifications captures the essence of a different aspect of the cell. At the center three modifications to the interior of the vesicle are depicted, representing different encapsulations. The surrounding sketches depict different modifications to the membrane. Note that the figure is not to scale: the bilayer thickness is about 4 nm whereas the vesicle diameter is about 10 μm.

phases can be observed as a function of the temperature. For example, lipid membranes in gel phase, composed of ternary mixtures of cholesterol and lipids with saturated as well as unsaturated chains, readily phase separate below a miscibility transition temperature into liquid-ordered (Lo) and liquid-disordered (Ld) phases. Lipids are mobile in both phases, but exhibit greater chain ordering in the Lo phase. Readily available fluorescent amphiphilic molecules partition into one or the other phase and can be used as probes, allowing observation of the phase behaviour using fluorescence microscopy.[9] Similarly, different protein species partition into different lipid phases due to different miscibility properties. In particular, as beautifully recounted in ref. 27, the early work of pioneers like Sackmann[26] presaged micro-domain formation and control of protein function by hydrophobic mismatch, arguing from a purely physical point of view.

Recent experiments have vindicated those early predictions and have revolutionized the traditional picture of the cell membrane initially proposed in 1972 by Singer and Nicolson.[29] In their "fluid mosaic" model the lipids in the membrane are in a fluid state and all components are essentially free to diffuse around passively. Today, it is well accepted that the cell membrane is a structurally complex and dynamic entity, with nanoscopic, highly dynamic "rafts"[30–32] that are thought to have an impact on many membrane functions by locally controlling protein distribution and functional state *via* lipids.

The diffusion constant of lipids themselves in the cell membrane has been measured to be about 1 $\mu m^2\ s^{-1}$ and that of proteins to be about 0.1 to 0.01 $\mu m^2\ s^{-1}$. This means that in the interval of one second, a lipid, on the average will be approximately 2 μm away from its point of departure; for a protein, this number is 0.45 μm. The fluidity of the membrane not only allows the membrane bound proteins to be transported on the membrane but also helps heal the membrane after budding or exocytosis, and to integrate extra membrane on endocytosis. Thereby, the mobility of lipids in the cell membrane is not only modulated indirectly by proteins in the membrane *via* phase separation, but also directly *via* increased friction as observed in a model system. Lipids in a supported membrane of SOPC diffuse at 2.2 $\mu m^2\ s^{-1}$. This diffusion constant drops to 1.7 $\mu m^2\ s^{-1}$ upon binding of proteins to the free surface.[33] In a GUV without friction-generating contact to a substrate, lipid diffusion can be up to ten times faster whereas transmembrane proteins can be expected to diffuse more slowly.[34]

The mechanical properties of lipid bilayers are also related to their composition. For example, the bending modulus of a bilayer made purely of DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine), is 20 k_BT, whereas a mixture of cholesterol and DMCP (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) yields a value of 100 k_BT, much closer to the value of the cell membrane. k_B is the Boltzmann constant, and T is the room temperature in Kelvin; 1 $k_BT = 4.0 \times 10^{-21}$ J. The quantity k_BT is a "natural" unit of energy that allows an easy comparison with what can be achieved by thermal energy alone. Energy of a few tens or a few hundreds of k_BT can be provided simply by the random thermal motion of the molecules in the medium, and makes a lipid membrane susceptible to thermal fluctuations: in the same way as particles are subjected to Brownian motion. Embedding proteins in the membrane can change not only the bending modulus but also the spontaneous curvature.[9] Overall, the cell membrane is very soft (bending modulus of about a few tens to a hundred times k_BT). Such a low bending modulus also means that the energy cost of creating highly curved regions like necks of buds, microvilli, or cell-division furrows is very low. Another consequence is that the force exerted by a single molecular motor or by the polymerization of actin, is enough to pull out thin membrane tubes (see ref. 35, for example). Later we discuss that an interdependence of bending and local composition arises as a consequence of the membrane being flexible as well as multi-component and fluid.

GUV fabrication

Basic GUVs

Traditionally, GUVs were made by simple hydration of lipids. Dry lipid deposits were exposed to an aqueous solution and taken through thermal cycles. A key step forward was the introduction of electro-swelling in 1986[36] which dramatically increased the yield and reduced the preparation time, at the same time avoiding high temperatures that can potentially damage the biological components (Fig. 2a). Tremendous progress in GUV design has been achieved since then (see ref. 37 for a recent detailed review). The main effort has been directed at tightly controlling GUV size, tuning the preparation conditions towards the physiological range, and on achieving better cell mimicry by incorporating proteins into the vesicle membrane, filling of the GUV with passive as well as active components and designing asymmetric vesicle membranes.

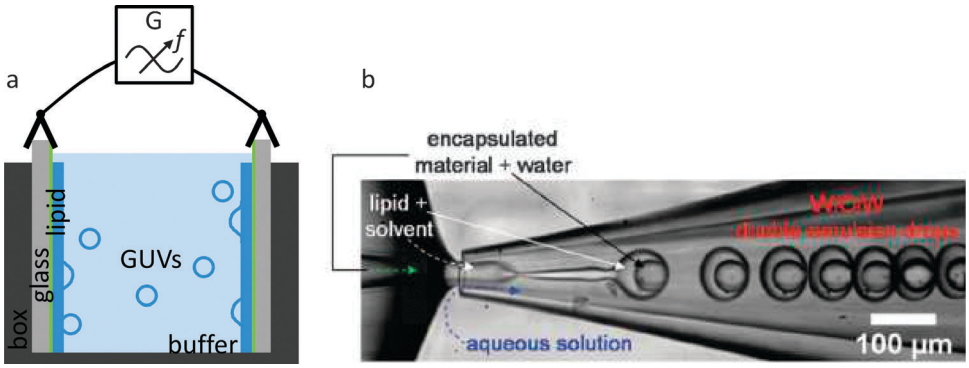

Fig. 2 Schematic diagram showing the preparation technique for two of the popular choices for GUV preparation. (a) Electro-swelling, where vesicles are formed when a current is passed between two conducting plates (for example, indium tin-oxide coated glass), on which lipids are first deposited from a solution. (b) Double-emulsion templating where a combination of co-flow and flow focusing realized in a microfluidics device produces droplets of water in oil in water. b: Reprinted with permission from ref. 40. Copyright (2008) American Chemical Society.

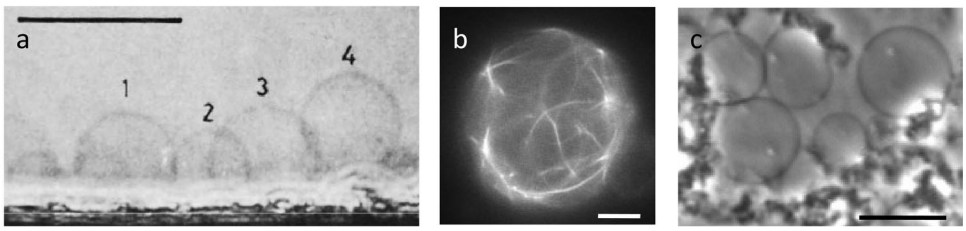

Fig. 3 (a) GUVs being prepared by electro-swelling visualised in bright-field mode.[36] GUVs are seen to be separating out from the bulk lipid phase deposited on an electrode. Four different vesicles in different stages of detachment are seen. Scale bar 50 μm, (b) GUV filled with cross-linked actin network visualised in fluorescence. The filamentous and cross-linked actin, labeled with phalloidin, is visible. The unmarked membrane of the GUV is not seen but it confines the actin network and is responsible for the characteristic spider-web architecture.[52] Scale bar 5 μm (c) GUVs prepared from an eucaryotic cell extract visualised in bright-field mode.[53] Scale bar 20 μm. (a) Reproduced by permission of The Royal Society of Chemistry. (b) and (c): Courtesy Laurent Limozin, CNRS and Stefan Semrau, MIT respectively.

The diameter of living cells typically range from 1 to 100 μm. Electro-swelling as pioneered by Angelova and Dimitrov is based on combined effects of lipid self organisation in aqueous solution and electro-osmosis: a lipid film is deposited on a homogeneous conductive surface and after evaporation of the solvent, an aqueous solution is added and an alternating electric field is applied. This procedure yields GUVs in a size distribution range of about 5 to 150 μm[38] (see Fig. 3a). One recent approach to achieve mono-disperse GUVs is based on electro-swelling, but replaces the flat electrode by a micro-structured silicon substrate that allows controlled deposition of lipid only in holes of defined diameter and depth.[38] The final GUV diameter can be adjusted by varying the hole diameter. With this strategy, populations of GUVs can be produced in the range of 5–20 μm or 10–30 μm.

Two alternative approaches that simultaneously create GUVs of a defined size and load them with high encapsulation efficiencies are microfluidic jetting[39] and double-emulsion templating.[40] In microfluidic jetting, a piezoelectric actuator pushes the plunger of a syringe fitted with a glass micronozzle (40 μm diameter) to produce and control a fluid jet aiming at a preformed planar lipid bilayer. The microfluidic jet deforms the lipid bilayer into a vesicle that is filled with solution from the jet and separates from the planar bilayer. The resulting GUVs in a range from 100 to 200 μm exhibit diameter variations of less than 10%.

GUVs produced by double-emulsion templating undergo a two step procedure. First, monodisperse double emulsions (water in oil in water, W/O/W) are generated using a glass microcapillary microfluidic device that combines a co-flow and a flow focusing geometry (see Fig. 2b). The co-flow geometry combines the inner phase (aqueous solution of model encapsulant) with the middle phase (solution of phospholipids dissolved in a mixture of toluene and chloroform) shortly before traversing the outer phase (aqueous solution of poly(vinylalcohol) and glycerol) and entering into the collection tube where the fluid stream breaks up into W/O/W droplets. Second, phospholipid vesicles are obtained from these emulsions by removing the solvent by evaporation or dialysis. Typical diameters of the vesicles can be tightly controlled within a range from 20 to 150 μm. A recent variation with a simplified geometry was proposed where the injection is effected by a capillary that drips the aqueous inner phase into an oil base, the droplets then pass through a lipid-in-oil solution acquiring a first monolayer coating and finally passing into the dispersing aqueous phase through an interfacial lipid monolayer, thus completing the bilayer.[41] The drawback of these techniques is that the non-aqueous phases may remain trapped in the chain region and thus change the membrane properties.

Advanced GUVs

With advances in the methods of preparation, the composition of the GUV membranes got more and more complex. One famous example is binary and ternary lipid mixtures to study

membrane heterogeneity in model systems.[42–46] This development peaked in the preparation of GUVs from natural lipid extracts.[47–49] These are especially interesting objects of research because they maintain the natural asymmetry of cell membranes which is difficult to achieve starting from solubilised synthetic lipids which mix homogenously. To our knowledge, only Chiantia *et al.*[50] and Pautot *et al.*[51] have succeeded in producing asymmetric GUVs starting from synthetic lipid mixes which are stable for at least 4 or 24 hours respectively.

Encapsulation of enzymes in lipid vesicles was first attempted by Sessa and Weissmann in 1970.[54] Since then, vesicles filled with biologically active compounds have been used as chemical micro-reactors,[55] delivery vehicles for pharmaceuticals,[56] and platforms for synthetic biological systems.[57,58] In addition to the already mentioned filling methods, various other strategies have been developed. Among these are electro-injection,[59] micromanipulation-injection procedures[60] or techniques based on adding the load to the solution during electro-swelling. This way, improved cell-models could be achieved that mimic specific features of real cells, such as the actin cytoskeleton[52,61–63] (for an example see Fig. 3b) or the protein rich, dense cytoplasm.[64,65]

Another important step towards cell mimicry by GUVs was the integration of fully functional integral proteins in the vesicle membrane. The crucial step towards this goal was made by Rigaud *et al.* in 1988 who succeeded in detergent assisted protein insertion in the membrane of large liposomes (LUV) produced by reverse-phase evaporation.[66] Based on this ground breaking work, two techniques were developed that allow protein incorporation into the GUV membrane. The first technique employs LUVs (preformed following the protocol by Rigaud *et al.*) which are additionally decorated with peptides. Upon mixing these LUVs with the target GUVs the peptide induces fusion between the vesicles resulting in protein transfer from the LUV to the GUV.[67] An alternative approach utilises again preformed large proteo-liposomes which get partially dried on an electrode to form a protein–lipid layer as the starting point for electro-swelling of GUVs.[68,69] The major drawbacks of these techniques are the risk of protein denaturation during the drying step and the presence of foreign molecules (fusion peptides) in the final GUV. Only recently, Varnier *et al.* succeeded in circumventing these drawbacks with a method free of detergents, fusion peptides or a dehydration step.[70] In this method, the target GUVs are produced by traditional electro-swelling while the small submicron carrier proteo-liposomes get assembled in the commercial Rapid Translation System. This cell-free protein expression system is based on T7 RNA polymerase and an optimised *E. coli* lysate.[71] Material transfer from the small proteo-liposomes to GUVs occurs spontaneously. A weak point in the production of membrane proteins using a bacterial cell-free over-expression system is the inability to introduce post-translational modifications. One solution was provided by Shaklee *et al.*[53] who incorporated palmitolated H-Ras into GUVs made of native endoplasmatic reticulum membranes with an eucaryotic cell extract based *in vitro* translation system (see Fig. 3c). Depending on the type of protein it can be necessary to swell the GUVs under physiological conditions. Especially, the requirement of high ionic strength demands special protocols.[48,53,72] In any such study involving incorporation of proteins into lipid bilayers, the complex miscibility properties of proteins and lipids have to be kept in mind.[73]

GUVs as a tool to look deeper into the cell

The GUV "test cells" can be studied using almost all of the techniques that are used for the corresponding measurements on cells. For example, advanced fluorescent studies on complex GUVs have afforded insight into lipid domains,[16,35,44,74] and use of reflection interference contrast microscopy (RICM)[75] has led to a better understanding of adhesion.[18,76–79] GUVs also lend themselves to mechanical studies using, for example, micro-pipette aspiration,[80] magnetic tweezers[62,77] or even atomic force microscopy,[81] offering insights into cell mechanics as well as force response of adhesive bonds. It is important to emphasise here that the coupling of the cell membrane with the cytoskeleton can influence both the structure and the mechanics of the composite shell. This has to be kept in mind for GUV studies. In the following, for the sake of brevity, we shall not describe commonly used observational techniques, concentrating instead on the insights obtained into the functioning of living cells through studies on GUVs, taking the case of membrane domains and cell adhesion as examples.

Insight into rafts, domains and membrane curvature

Domains in membranes: towards understanding of rafts?. The so called "raft" model has fascinated and frustrated biologists to about equal measure in the last few decades.[82] Originally two independent sets of experiments gave rise to the notion of membrane rafts – in the first, biologists found that certain proteins are associated with detergent-insoluble lipids and in the second, biophysical assays showed that certain kinds of lipid mixtures spontaneously phase separate. Specifically, it has been known for some time now that under suitable conditions, the so called raft-mixtures, which are ternary mixtures of lipids and sterols, readily undergo phase separation into Lo and Ld phases. This separation was initially believed to be relevant for the formation of rafts in the cell membrane. In recent years the consensus in the literature has been that the micron sized domains seen in synthetic model systems do not have any biological relevance, since in cell membranes the putative rafts are of the order of tens of nanometers in size and highly dynamic on a timescale of a few hundred nanoseconds.[30,82] However, recent experiments on vesicles made from plasma membranes of cells show the coexistence of two fluid phases[47] reminiscent of the behaviour of model raft membranes.[44] The membrane composition of these vesicles is equivalent to that of the membrane of an intact living cell but they lack a cytoskeleton. The domains observed in this system were micron sized. This opens up a host of questions – does the cytoskeleton play a role in keeping the raft size so small? Is raft formation and maintenance an active process requiring energetic input from the cell? Or are the rafts simply density fluctuations that arise because the cell membrane is maintained close to phase separation?

Compartmentalisation in the cytoplasm. Recently, the phenomenon of phase separation and its potential biological relevance

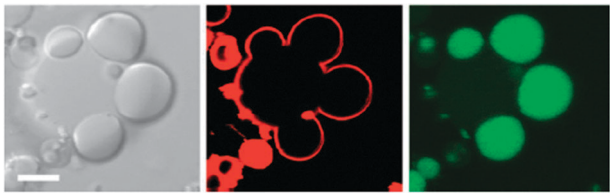

Fig. 4 Confocal micrographs illustrating the response of GUVs filled with a PEG/dextran mixture to increased osmotic stress. From left: transmitted light(DIC), lipid fluorescence, lectin SBA fluorescence in the dextran-rich phase. Scale bar 10 μm Reprinted with permission from ref. 84. Copyright (2008) American Chemical Society.

has also been studied inside GUVs. In this experimental setting, phase separation was induced in the enclosed artificial cytoplasm, rather than in the GUV membrane.[83] This model for cytoplasmic organisation consists of a GUV filled with a one-phase mixture of PEG and Dextran. Upon changes in either the temperature or the osmolarity (leading to changes in the concentration) phase separation can be induced in a controlled and reversible way. The authors showed that additional proteins in this cytoplasm-mimicking solution sort according to their function into the micro-compartments inside the GUV. For example, the carbohydrate binding lectin SBA accumulates in the dextran-rich phase. The authors suggest that in a living cell, proteins themselves would act as the phase-forming polymers resulting in multiple phases and micro-compartments without the need for intervening membranes. Extreme changes in osmolarity additionally introduced budding of the dextran-rich phase leading to polarised cell-models[84] (see Fig. 4). Since asymmetrically distributed proteins follow into those reversible buds, this represents a very exciting model system for endo- and exocytosis.

The same model system was also studied from the theory side. Li *et al.* identified a wetting transition governed by the interplay of the interfacial tensions between the two phases and the GUV membrane as the mechanism behind the phase separation.[85] The difference in osmotic pressure that drives the phase separation in this experiment also has an interesting side effect. The excess membrane area of the shrinking GUV is stored in membrane nanotubes with diameters below the optical resolution limit. In such tubes which are stabilised by spontaneous curvature of the GUV membrane up to 15% of the GUV membrane can be stored. Thus, these nanotubes are suggested to constitute a means of lipid storage in cells.

Interplay of membrane curvature and domains. While the nature and function of 'rafts' is still elusive, considerable progress in understanding of phase-separation and its coupling to membrane curvature has led to insight into possible mechanisms important to cells. In a series of pioneering experiments,[16,44] Baumgart *et al.* showed that in accordance with theoretical expectations,[86] domains formed in a GUV made from the raft mixture spontaneously bulge-out in 3D and have a tendency to bud-off. The curvature of the buds is determined by an interplay between the line-tension at the phase boundary and the bending energy. It has been shown that such domains can arrange themselves into stripe and hexagonal patterns, putatively stabilised by membrane-mediated elastic interactions.[87] In some experiments the transition between these patterns has also been seen on gradual adhesion of the vesicle on a substrate.[88]

Not only does phase-separation lead to membrane curvature, the inverse is true as well, as shown by Roux *et al.* who pulled out tethers from GUVs made of raft-mixtures and saw the enrichment of the Ld phase in the highly curved tether.[74] Only recently, it was demonstrated that also the speed at which a tether is extracted serves as a means of lipid sorting. If the tether forms slowly, equilibration of chemical potentials between the extracted tether and the donor organelle is facilitated, and the extracted membranes show enrichment in liquid disordered lipids. If, however, membrane patches are extracted quickly within a range of trafficking speeds of intracellular cargo membranes, then equilibration is prevented and liquid ordered rather than liquid disordered membranes are extracted from the reservoir.[89]

A different way to promote membrane curvature is with the help of specific proteins. It has long been suggested that certain proteins may be able to introduce membrane curvature entirely passively, simply due to their shape. However, it is only recently, with the emergence of a description of the banana shaped BAR domain present in many shape-change inducing proteins that quantitative estimates of the link between membrane bending and protein binding has been possible.[90] A very interesting possibility concerning proteins is that membrane deformation can provide a means to adjust the hydrophobic mismatch between a bilayer and a membrane protein which in turn may regulate the activity of the protein. Thus proteins which are inactive in a planar membrane could become active when the bilayer is bent.

Another example for curvature associated proteins are dynamin-like proteins. They deform membranes into tubules by imposing a helical coat as they polymerise. The force generated by dynamin polymerisation, 18 pN, was measured in a micropipette/optical trap configuration.[17] Interestingly, it was demonstrated in the same study that dynamin not only imposes curvature on membranes, but also serves as a curvature sensor. In the low concentration regime, dynamin was able to bind only to already curved membrane tubes. This suggests that dynamin may be precisely recruited to the neck region of the membrane buds because of their high curvature, thus playing an important role in the late stages of vesiculation.

Insights into cell adhesion and cell mechanics

Basics of cell adhesion. Adhesion of cells to each other and to external substrates is a complex process essential for the existence of multicellular organisms. Whenever two cells connect to form a tissue (for example during wound healing) or detach (for example during embryogenesis) a large variety of specific adhesion molecules are strictly orchestrated by many signalling cascades. The adhesion/deadhesion process is thus tightly and actively controlled by the cell. Nevertheless, there is wide interest in elucidating the underlying processes from the physical first principles. This is especially pertinent in the early states of adhesion (up to ≈ 1 min) when physical laws are expected to dominate because the cell needs some time to

initiate an active response. In the context of adhesion, a very important point was made by Bell,[91,92] who realised that the binding constants or chemical affinity between two membrane bound molecules will be different from their affinity measured in solution. In the modern literature, the former is called the 2D affinity and the latter the 3D affinity. The 3D affinity is intrinsic to the ligand–receptor pair under consideration, whereas the 2D affinity is influenced by the precise geometry in which the molecules find themselves. In this context, the quantitative measurement of affinity with ligands and receptors bound to membranes is extremely important. While some recent measurements have been reported in live cells,[93] in the absence of perfect knowledge of even the receptor concentration on the cell surface, they necessarily remain semi-quantitative and open to different interpretations. Studies on adhesion of GUVs to model substrates, to be further discussed below, have however provided insight into the influence of the environment on the 2D affinity.

GUVs can mimic the cell feature essential for adhesion by carrying receptors that specifically bind to ligands on a complementary planar surface. The ligands are either immobilised on the substrate, thus mimicking cell-surface adhesion; or diffuse on a supported lipid bilayer, thus mimicking intercellular adhesion. In a typical experiment, the GUV is filled with a sucrose solution that has a higher density than the surrounding buffer to induce sedimentation towards the substrate due to gravity. Such a geometry also replicates typical cell adhesion studies dealing with the early stages of adhesion.[94–96] As in the case of cells, in addition to the specific ligand–receptor bond formation, other forces of physico-chemical origin are also important (see Fig. 5 for an overview). Foremost amongst these is the repulsion due to the presence of the glycocalyx in cells which in vesicles is mimicked by incorporation of lipids carrying a short passive polymer chain (typically polyethlene glycol).[76] In addition, when the two surfaces approach, depending on the physical-chemistry of the specific lipids and the medium used, other physical forces, such as van der Waals, Coulomb and hydration forces emerge. Moreover, the GUV membranes are usually soft with a bending modulus of several k_BT. Typically, GUVs are prepared under hypo-osmotic conditions leading to excess membrane area exhibiting thermal fluctuations (so-called Helfrich undulations) which contribute to repulsive forces. This kind of excess or "hidden" area in the form of folds and undulations are also present in cells.

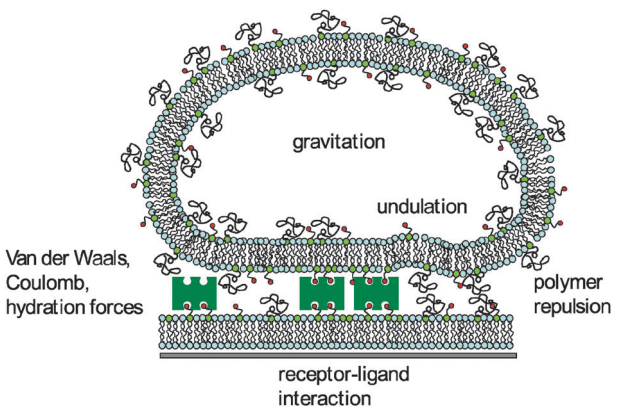

Fig. 5 A schematic representation of the physical forces that are relevant for GUV test cell adhesion. Note that the figure is not to scale. Typical diameter of a GUV is 10 to 30 μm, whereas the vesicle membrane and supported bilayer are 4 nm thick. Here avidin and biotin are illustrated as receptor–ligand pair. The size of the avidin as well as the depicted polymers, which are typically PEG-2000, is also about 4 nm.

The generic forces of physical origin, collectively called the "unspecific" forces, can be mathematically described in terms of an effective interaction potential. Bruinsma *et al*. applied a superposition approximation to calculate the effective unspecific potential resulting from gravitation, van der Waals and fluctuations.[97] They found a double well potential with one sharp and deep minimum close to the surface ($h \approx$ 5–10 nm, van der Waals dominated) and a broad shallow minimum further away ($h \approx$ 100 nm, Helfrich repulsion dominated). The deep unspecific minimum has to be suppressed in order to isolate and study the specific contributions to adhesion. In nature, the presence of glycocalyx largely suppresses the unspecific interactions – similarly, in model systems, the presence of the glycocalyx mimetic lipo-polymers can ensure this.[76] Specific interactions give rise to an additional local minimum in the interaction potential. The position of this minimum depends on the length and flexibility of the receptor–ligand pair used.[98,99] The final equilibrium adhesion state corresponds to a configuration of the membrane that minimises its free energy, taking into account the interaction potential described above as well as the energy corresponding to the adhesion induced deformation of the membrane.[18,97,100] Recently, it has been shown that the entropy of mixing of the receptors and ligands is also important.[101]

Observation techniques. In the context of cell adhesion, there is currently a lot of emphasis on imaging proteins – usually *via* sophisticated fluorescence microscopy. While such an approach does yield very interesting data, it does not necessarily give any information on the location of bonds or on the conformation of the membrane. Reflection Interference Contrast Microscopy (RICM) (see ref. 75 and references therein) is a technique that can "measure" adhesion, in the sense that it can quantify inter-surface distances and membrane fluctuations. Use of RICM for cells is still limited because of the problems associated with interpretation of signal from an optically complex body like a cell. RICM has however been successfully applied to experimentally investigate GUV adhesion in detail.[19,97]§ It is particularly suitable for exploring the dynamic aspects of membranes close to a surface, and is proving indispensable for advancing our understanding of membrane based biomaterials using tools of soft matter. The total control over GUV composition and hence the optics of the system also helped in advancing the image analysis in RICM.[75,102] The absolute distance in the vertical direction can now be measured with an accuracy that, depending on the signal to noise ratio for the given camera, can be as low as 1 nm[33] for a tightly adhered vesicle (where long signal integration times are possible). If the focus is on

§ Few studies on GUV adhesion using other techniques such as TIRF-M or quartz crystal micro-balance have also been reported though the use of these techniques remains rather limited due to difficulties in quantitative interpretation.

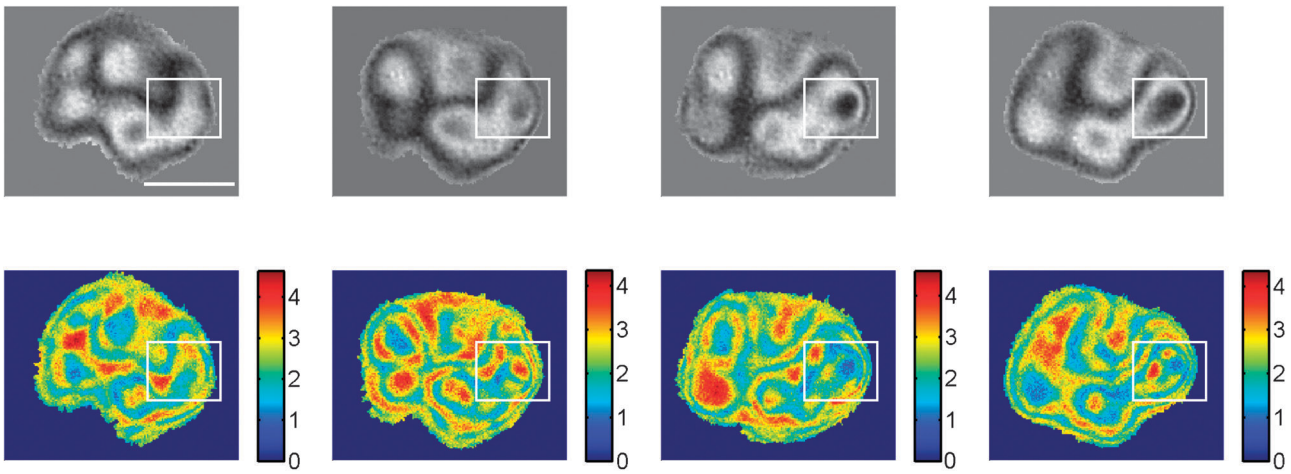

Fig. 6 Development of a nucleation centre in early GUV adhesion as observed with Dy-RICM. Top row: RICM micrographs of a GUV at 0, 2, 4 and 6 seconds. The ever-shifting shades of grey, going from black to white, are characteristic of an un-adhered, fluctuating membrane. The formation of a binding patch is seen within the white rectangle where a dark spot is seen to be developing over time. Bottom row: Corresponding fluctuation maps prepared by analysis of fluctuation data gathered using dynamic RICM. The absolute values of the fluctuation amplitude at any given spot can be compared to the value expected solely from camera noise. It is found that the putative adhesion patch, indeed exhibits reduced fluctuations. Intriguingly, just before the fluctuations are frozen, a "hot-spot" of increased fluctuations is detected. An analogous observation was made on cells[96] and can be explained on a purely thermodynamic basis.[105] Eventually, the adhesion spot seen as dark in RICM grows and, depending on the concentrations of ligands and receptors used, may resemble the mature adhesion disc seen in Fig. 7. Scale bar 5 μm.

extraction of dynamic data, a precision of about 5 nm can be achieved at a frame rate of 10 Hz with sensitive CCD cameras. These advances in RICM now need to be integrated into analysis of living cells.

Even in the relatively simple system of GUVs, where the adhesion dynamics and the final adhesion state are modulated solely by the strength of the interaction, the ligand mobility, and the ligand–receptor concentration a surprisingly rich behaviour has been observed using RICM and its modifications. The range of distance over which RICM is applicable to GUV studies has been increased by the use of dual or multiple wavelengths;[102,103] such analysis also enabled better quantification of membrane fluctuations which can be measured with an accuracy given by the intensity dependent camera shot noise. Quantification of fluctuations is also at the heart of dynamical RICM (Dy-RICM)[77,79,104,105] – a technique that uses suppression of fluctuations as a signature of binding (see Fig. 6). While the lateral resolution of RICM is, as any wide-field technique, limited by the Rayleigh criterion, in Dy-RICM analysis, much smaller pinning centres can become visible. Today many experimental works are available on the steady state behaviour of adhesion and our understanding is rather advanced; the dynamics, being much harder to record as well as interpret, is still poorly quantified.

Adhesion *via* strong bonds and abundant linkers. Conceptually, the simplest adhesion scenario that can be imagined is when the ligands as well as the receptors are present in large numbers and the interaction is strong. Such a system was realised by several groups using biotin and avidin as the ligand–receptor pair.[76,79,106–108] The intrinsic binding strength (enthalpy) for this bond is about 35 k_BT ($=140\times10^{-21}$ J. For comparison: a covalent carbon–carbon single bond has a strength of 580×10^{-21} J.) While such strong binding is not realistic for natural cell adhesion molecules, this system provides an easy to handle test case. On coming in contact with the substrate, the GUV membrane quickly spreads on the substrate, forming a dense array of bonds, visible in RICM as a dark adhesion zone,[76,79,107] as in the example shown in Fig. 7 and 8. Since there are always enough ligands and receptors, no net transport (diffusion) of either takes place – little difference is therefore expected between the mobile and immobile case.[109] In the absence of a role for diffusion, the time scale is set by hydrodynamics. Typically, three stages of spreading are seen: (i) the nucleation stage where the membrane, initially bound weakly in the shallow minimum of the interaction potential nucleates an adhesion patch at a distance corresponding to the deeper minimum. If this patch is large enough, it can grow; (ii) the growth stage where the adhesion patch grows pulling more and more of the membrane from the shallow to the deep minimum; (iii) the saturation stage where the growth of the adhesion patch slows down because all the membrane available is used up for adhesion. In the presence of a thick polymer cushion, the essential behaviour remains the same while the state of adhesion (strongly, weakly or not adhered) can be tuned by the thickness of the polymer layer.[110] More recent work with sparsely grafted polymers show that the polymers themselves are deformed and entangled by adhesion generated forces.[108] The vesicle spreading behaviour can be explained in the light of general scaling laws[109] and/or a more specific theory which provides semi-quantitative agreement with experiments.[110] Additional subtleties may arise when the ligands and receptors are not freely accessible to each other as a consequence of the specific manner in which they are bound to the substrate – as may in fact be the case *in vivo*.[107] Whether or not the ligands on the substrate are free to diffuse, this discussion holds as long as there is abundance of both ligands and receptors. However, in case there is a scarcity of one of the binding species (ligand or receptor), diffusion of the rare species is expected to dominate: however, such a scenario is

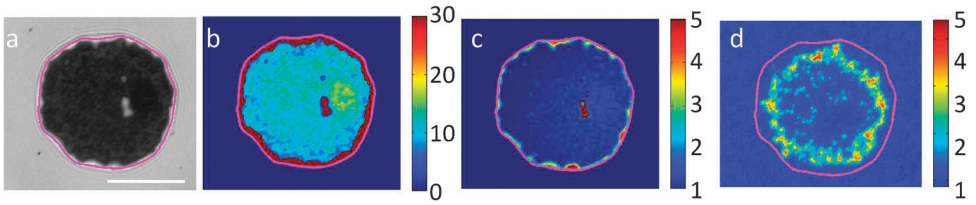

Fig. 7 (a) RICM micrograph of a GUV adhering *via* biotin–avidin. The adhered bottom of the GUV is visible as a dark area surrounded by a few fringes. A small white blister of the non-adhered membrane is visible within the adhesion disc. Shades of grey visible on closer inspection of the adhesion zone correspond to different membrane to substrate distances arising from different bond organisation. (b) The membrane to substrate distance as calculated from the RICM image (height colour scale bar: in nm). Note the relative predominance of dark blue close to the edge compared to the centre. This shows that the membrane is closer to the substrate in a zone along the periphery. (c) The corresponding map of membrane fluctuations reveals that the membrane is effectively pinned everywhere in the adhesion zone except in the blister and along the edge of the contact zone (scale noise). (d) The corresponding distribution of receptors was visualised in fluoresce. Here, the receptor distribution is presented in a colour coded map where one corresponds to the initial concentration on the bilayer (scale relative accumulation). The regions that are dense in receptors, along the periphery, are also the zone where the membrane is more tightly bound. Scale bar 10 μm. (Further details on this result can be found in ref. 79.)

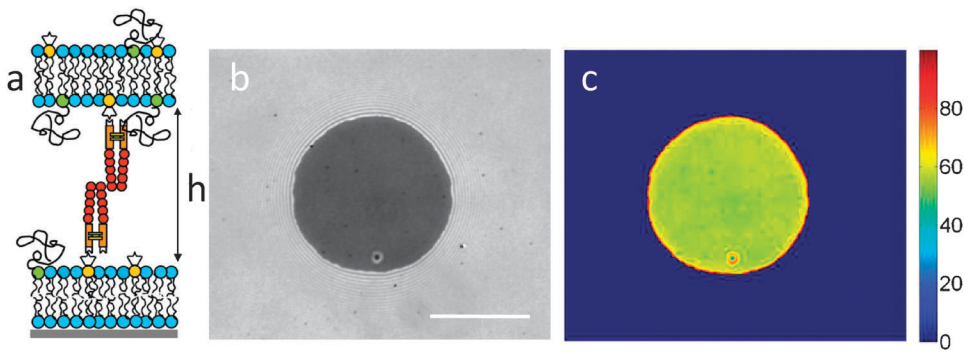

Fig. 8 The binding geometry of E-cadherin molecules, as depicted in (a), was determined from the RICM micrograph (b) of a GUV adhering to a supported lipid bilayer *via* E-cadherin. The membrane to substrate distance from which the geometry was inferred is depicted in (c) (height colour scale: in nm).[33] Scale bar 10 μm.

yet to be observed in its pure form since other phenomena like jamming or hydrodynamics often obscure this stage.[79,106]

Adhesion *via* weak bonds: immobilised ligands. The next level of sophistication in the model is the case of weak bonds. This case is important since the interactions between adhesion proteins in nature is typically weak with bond energies of the order of 2 to 10 k_BT. Such a system was realised with E-selectin molecules grafted on the support and lipids bearing the counter-receptors Sialyl Lewis X (SleX) on the vesicle.[78] This model closely mimics the weak initial adhesion of rolling leukocytes in the blood stream to the endothelium. Upon release of cytokines, the SleX on the leukocytes binds to the E-selectin expressed by the endothelial cells and thus effects a temporary arrest of the leukocytes. Using the model system, it was shown that adhesion can be modified by tuning the selectin concentration.[78] At high concentrations of E-selectin (keeping SleX concentration constant), as in the strong-linker case, a dense array of bonds identifiable as a dark adhesion zone spanning the whole of the available membrane to substrate contact area is formed. As the concentration is lowered, less and less of the available membrane adheres, instead the excess membrane fluctuates close to the substrate. Thus there is a lowered occupancy of available selectin as their concentration decreases. At the same time, the dynamics of the growth phase is drastically slowed down. These trends can be explained in the light of Bell's hypothesis[91] that geometry modifies 2D affinity. In fact, here, the binding state of an immediate neighbour influences both binding and unbinding rates and thus the effective 2D affinity.[78,111]

Adhesion *via* diffusing ligands and receptors. The case where both receptors and ligands are mobile was first studied with the homologous glycoprotein CsA,[112] a cell adhesion protein from *Dictyostelium* that resembles ICAM1. The receptor concentration was kept low to correspond to the concentrations realistically found in real cells – about a hundred receptors per square microns. Here it was shown that the binding molecules can aggregate into dense bond arrays. As before, at high receptor concentrations, all the available membranes adhered whereas at low receptor concentrations, only small patches of adhesion were seen. This early work already demonstrated the validity of the two-well potential model discussed above and set the scene for further understanding of the aggregation process.

In depth studies on mobile ligands were done on a model system where integrins were incorporated into a supported bilayer and the corresponding RGD ligands were incorporated into the GUV.[77,113,114] Integrins are a family of ubiquitous cell adhesion molecules that can mediate both cell to extra cellular

matrix (fironectin/RGD, laminin *etc.* as ligand which are immobilised on the matrix) and cell to cell (*e.g.* ICAM1 diffusing on the surface of endothelial cells binding to the integrin LFA1 on the membrane of leukocytes) adhesion. While integrin mediated adhesion is "strong" compared to the E-selectin mediated adhesion seen above, it is no where as strong as the biotin–avidin bond. In the model system, the integrins could be mobile or immobile depending on the specific bilayer preparations method.[114] This system is the only one to date where the same biochemistry was used for the mobile and the immobile case, and therefore provides a powerful platform for elucidating the role of mobility in adhesion. In both cases, the adhesion was partial. On immobile integrins, the adhesion clusters where submicroscopic and only identifiable using Dy-RICM or showed up as pinning centres when a pulling force was applied. When the integrins where mobile, as in the case of CsA, the binding molecules aggregated to form micron size clusters. Intriguingly, in addition to the dense bond cluster already described, a dilute bond cluster, showing up as frozen fluctuations in Dy-RICM but looking bright in RICM was identified. Under force, the dilute cluster compacted into a dense array. The over-all adhered area increased under repeated force application/release cycles. This could be understood in terms of a thermodynamic model that accounts for not only the bond energy but also the overall entropy of binders and bonds in the system.[77] This behaviour is reminiscent of mechano-transduction seen in living cells.[115] In numerous studies in the last decade, living cells, when subjected to an external force have been shown to strengthen the adhesion. Whereas no specific "force transduction" protein has yet been identified, it is generally believed that such (a) protein(s) will be identified in the future. Furthermore, the presence of the cytoskeleton is thought to be indispensable for mechano-transduction. In the model system however, adhesion strengthening under force was detected in a cytoskeleton free system – arising purely out of the physics of the two membranes. We believe that this result calls for a rethinking on the putative mechanism of the on-set of mechano-transduction.

Adhesion mediated by mobile ligands was also studied in the case of strong biotin–avidin bonds. A very interesting, but unanticipated interplay of receptor concentration and receptor diffusion was observed.[33,79] As is normal for mobile ligands, the bonds aggregate and get immobilised. In addition, the fluidity of the membranes is reduced locally due to adhesion, leading to the formation of large domains of elevated receptor concentration within which the receptors were effectively jammed. The interplay of the two effects modulated the shape of the adhesion zone that ranged from a homogeneous disc to a ring along the perimeter of the membrane-to-membrane contact zone (see Fig. 7).[79] The latter incidentally is strongly reminiscent of the bull's-eye structure of the immune synapse.[6] Interestingly, the two geometries lead to different effective binding affinities. This is because the closing of the ring switches the interior of the adhesion disc from one thermodynamic ensemble to another: initially the bilayer containing the receptors is an infinite source of mobile receptors but closing of the ring fixes the number of receptors sealed within even though the unbound receptors remain mobile. Interestingly, under these conditions, the initially strong neutravidin–biotin bond is in fact weak.[79]

Mobile weak bonds were studied taking the cell-adhesion molecule E-cadherin as the model ¶, in the limit of excess binders. Here the final state again corresponds to a dense array of bonds. Interestingly, unlike the case of biotin–avidin, where the linkers are short, the long E-cadherin bonds do not alter the mobility of the lipids in the bound membrane. With the help of this model system, we were able to add to the on-going debate on the structural basis for cadherin binding since the nano-metric resolution of RICM enabled us to measure the inter-membrane distance with high precision (see Fig. 8). Thus, we were able to resolve its binding configuration by comparison to structural data known from crystallography. We concluded that E-cadherin binds only with the outermost of its five extra-cellular binding domains.[33]

Cell mechanics. The mechanical properties of the cell are dominated by that of the cytoskeleton in general and, at the scale of deformation of the order of microns: the length-scale relevant for cell functions such as motility and budding, by the actin cortex in particular. There has been a growing interest in encapsulation of polymerisable actin in cell-models to probe the mechanical properties of confined filamentous actin. Pioneering experiments came from the group of Erich Sackmann[52,61,62] where GUVs, made of a phospholipid matrix and incorporated ion-channels were prepared by electro-swelling with G-actin inside. Subsequent transport of magnesium ions to the interior of the vesicle induced polymerisation of the actin and gave rise to a thin shell, reminiscent of the actin cortex. An even earlier study had already reported that liposomes can undergo morphological changes when the actin encapsulated inside polymerises and forms bundles due to the presence of excess calcium.[116] A more realistic cross-linking of actin was affected using the natural actin binding proteins α-actinin and formin. In solution, the former cross-links actin to form a network whereas the latter forms bundles. Inside GUVs, α-actinin provoked formation of spider-web like networks (see Fig. 3b), rings or bundles depending on the conditions, and formin, produced super-coiled helices.[52] The multitude of architectures could be explained in terms of the theory of semi-flexible polyelectrolytes and by considering the topology of the linkers.

The simpler system with actin polymers on the outside of the vesicle has elucidated the locomotion of certain bacteria. Such studies were inspired by earlier experiments that demonstrated that a colloidal bead can be driven forward by actin polymerisation.[117] Subsequently, it was shown that vesicles too can be pushed forward by actin polymerisation, and that this is accompanied by pronounced shape changes.[118] The dream of producing a motile actin-filled vesicle propelled from the inside is now one step closer with the incorporation of myosin as well as actin inside.[119]

In addition to structural studies, micro-mechanical properties of the artificial actin cortex were probed using pulling

¶ In these experiments, the five extracellular E-cadherin domains necessary for binding were fused to the Fc region of a human IgG exhibiting a hexahistindin tag. The chimeric protein could be bound to a SLB containing nickel chelating lipids via the histindin tag.

forces generated by magnetic tweezers.[62] The visco-elastic parameters measured compare well with values expected from measurements on living cells on one hand and on actin gels on the other hand. A recent study that looked at adhesion of GUVs with an actin cortex is a first step towards quantitative understanding of the mechanical aspect of the role of the cytoskeleton in adhesion.[120]

Conclusion

Learning about the functionality of a real cell by studying biomimetic test cells has been given by many different names: reconstitution biology, synthetic biology, bottom-up biology, model systems, mimetic systems, minimal system, cellular deconstruction *etc*. What is now increasingly clear is that such a reductionist approach complements traditional biology and is an essential step towards building a holistic, integrated picture. Numerous examples exist including supported bilayers for reconstitution of the immune synapse,[6] cytoskeletal reconstitution in confined geometries,[121] cell adhesion assays with specially designed substrates,[3] colloidal beads to mimic leukocyte rolling,[122] measurement of bond strengths and ligand–receptor on–off rates with beads and surfaces[8] *etc*. Here, we have focused on a few topics, namely: domain formation, membrane curvature, adhesion and cell mechanics and have reported results obtained in these contexts with cell free systems consisting of giant liposomes. These simplified systems have helped to bridge the gap between the quantitative world of soft matter systems and the reality of the complex, living world. A typical example of application of test cells discussed here is in a field that virtually exploded a decade ago, that of "rafts" in model systems. However, as is often the case with biological systems, insight has only led to deeper questions. It now seems clear that the cell must have an yet to be understood trick up its sleeve to keep the membrane in the state that provokes constant formation and breakage of tiny domains, which of course leads to the question "why?"

A clue to the possible answer lies in the second application of test cells discussed here, adhesion. It was hypothesised in 1978[91] and recently backed up by theoretical and experimental analysis of GUV data[78,111] that the effective 2D affinity and reaction rates on fluctuating membranes depend on the local concentrations of ligands and receptors. Can raft formation then be a way to influence reaction rates and affinity? Membrane domains or adhesion molecule clustering are found not only in the context of rafts but also in a more stable form in integrin clustering to form focal adhesions or cadherin clustering to form desmosomes. The early stage of clustering may profoundly influence adhesion. Only experiments with well constructed model systems, amenable to rigours comparison with a first principle theory can reveal quantitatively how clustering influences affinity and reaction rates. It is often argued that adhesion and mechano-sensing is affected by active processes and that the cytoskeleton directs the whole adhesion process. Yet, cell free models without a cytoskeleton can mimic even complex and seemingly active phenomenon such as force induced strengthening.[77] This field now cries out for comparative cell/GUV studies.

For each use of GUVs as test cells discussed here, a specific aspect of the living cell was mimicked in order to answer a well posed question. To reconstitute a whole cell in a liposome is not only a very distant dream but is arguably of doubtful utility. However, as demonstrated with examples in this article, well designed reconstitution of a specific function can lead to a quantitative understanding of the physical-chemistry of the process and grants us a certain degree of predictive power. The focus of this article has been on mimicking cellular scale features and phenomena: a choice that reflects current activity. Two future directions can be imagined: scaling down or scaling up. While some work on mimetic systems that seeks to reconstitute and understand intra-cellular organelles is already available,[123] to our knowledge, liposomes are yet to be used to model tissues. The future will probably see a proliferation of experiments seeking to model cells as well as organelles or tissues.

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