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An unresolved LINC in the nuclear envelope

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Abstract

The nuclear envelope segregates the nucleoplasm from the cytoplasm and is a key feature of eukaryotic cells. Nuclear envelope architecture is comprised of two concentric membrane shells which fuse at multiple sites and yet maintain a uniform separation of 30–50 nm over the rest of the membrane. Studies have revealed the roles for numerous nuclear proteins in forming and maintaining the architecture of the nuclear envelope. However, there is a lack of consensus on the fundamental forces and physical mechanisms that establish the geometry. The objective of this review is to discuss recent findings in the context of membrane mechanics in an effort to define open questions and possible answers.

Introduction

The nuclear envelope (NE) is the physical barrier between the cytoplasm and the genome. It regulates gene expression by controlling the access of transcription factors to chromatin through passageways called nuclear pore complexes (NPCs) (Akhtar & Gasser, 2007). The physical properties of the NE are important for organizing chromatin domains that bind to envelope-anchored proteins (Hetzer et al., 2005) (Starr & Fridolfsson, 2010), for resisting cell generated mechanical forces (Neelam et al., 2015) and for regulating signaling pathways (Akhtar & Gasser 2007). The NE is a unique membranous structure because it contains two membranes: the outer nuclear membrane (ONM) and the inner nuclear membrane (INM) that are fused together at NPCs. The ONM is contiguous with the endoplasmic reticulum (ER), providing an avenue for the exchange of lipids and proteins between the two organelles. On the nucleoplasmic side, the NE is supported by a meshwork of intermediate filaments, called the nuclear lamina (Figure 1). The NE is connected to the cytoskeleton via the LINC complexes (for linker of nucleoskeleton to the cytoskeleton) that span across the two bilayers and presumably transfer forces from the cytoskeleton to the nucleoskeleton (Tapley & Starr, 2013)(Shimi, Butin-Israeli, & Goldman, 2012)(Stewart, Roux, & Burke, 2007)(Neelam et al., 2015)(Alam et al., 2015)(Gundersen & Worman, 2013)(Kutscheidt et al., 2014)(Luxton, Gomes, Folker, Vintinner, & Gundersen, 2010)(Lombardi et al., 2011)

CONFLICTS OF INTEREST

Mehdi Torbati, Tanmay P Lele, and Ashutosh Agrawal declare no conflict of interest.

ETHICAL STANDARDS

The authors for this article carried out no human studies.

The authors for this article carried out no animal studies.

(Lombardi & Lammerding, 2011)(Friedl, Wolf, & Lammerding, 2011) (Li et al., 2015) (Chancellor et al., 2010) (Lovett et al., 2013) (Wu et al., 2011).

The NE is an intriguing structure because of unique features related to its geometry and dynamic remodeling. For example, the two concentric bilayers (ONM and INM) maintain a uniform separation of 30–50 nm across different cell types which is called the perinuclear space (PNS) (Franke, Scheer, Krohne, & Jarasch, 1981). The proteins and mechanisms that maintain this spacing are not fully understood. During interphase, the ONM and INM undergo numerous fusion events to allow creation of new nuclear pores (NPs) (Hetzer 2010), yet the 30–50 nm spacing continues to be maintained in interphase. Fusing the membrane to form nuclear pores entails overcoming the forces that maintain NE separation to bring the two bilayers in close proximity. The physical mechanisms underlying this dynamic remodeling remain unknown. Once the NPs have been created, they exhibit a relatively uniform areal density at a preferred inter-NP distance. What physical factors determine the NP spacing remain elusive. The LINC complex and its constituent proteins have been implicated in maintaining all of these geometric features. In this review, we summarize and analyze the key findings related to the LINC complex and geometric features of the NE. We discuss these findings from a biophysical perspective. We refer the reader to excellent in-depth reviews by (Starr & Fridolfsson, 2010), (Sosa, Kutay, & Schwartz, 2013), (Chang et al., 2015) for a more detailed discussion on the biology of the LINC complex and the nuclear envelope.

LINC Complex and NE spacing

The key proteins in the LINC complex comprise the SUN (Sad1p, UNC-84) proteins in the INM that span the nuclear envelope (Figure 1) and the Nesprin family of proteins which contain the KASH domain in the ONM (Burke, 2012)(Sosa et al., 2012)(Wang et al., 2012) (Cain & Starr, 2015) (Starr & Fridolfsson, 2010) (Padmakumar et al., 2005) (Zhang et al., 2001). The two domains of KASH and SUN proteins bind to each other in the space between the ONM and INM. Nesprin proteins extend out into the cytoplasm and bind to F-actin filaments, vimentin intermediate filaments and microtubule motors (Figure. 1). SUN proteins bind to the lamina and other proteins in the INM. This allows the LINC complex to transfer forces across the nuclear envelope (Chang, Worman, & Gundersen, 2015).

Crisp et al. showed that depletion of the SUN1 and SUN2 proteins in HeLa cells led to a significant dilation of the spacing between the lipid bilayers from 45 nm to more than 100 nm (Crisp et al., 2006). The prime reason for this expansion was found to be the outward movement of the ONM (Figure 2). Any undulations in the INM are expected to be restricted because the INM is anchored to the lamina through other proteins like emerin (Hetzer, 2010).

In contrast to the findings of Crisp et al., a recent study by Cain et al. suggests a different picture in *C. elegans* (Cain et al., 2014). The authors found that the absence of functional SUN proteins in most *C. elegans* nuclei (with spherical shape) did not have any significant impact on the NE spacing. But muscle cells with elongated shape displayed an expansion in the NE spacing at the anterior and the posterior ends. Cain et al. assigned these apparently

contradictory observations to the higher mechanical strain at the anterior and the posterior ends, which they inferred from the overall shape of the nuclei. The average PNS spacing at the anterior and the posterior ends was found to be around 70 nm in wild-type nuclei and 210 nm in nuclei lacking functional SUN domain. Based on these findings, Cain et al. concluded that the LINC complexes are required to maintain NE spacing only in nuclei experiencing high mechanical strain (and hence, stress). In addition, Cain et al. deleted 306 amino acids of the UNC-84 linker domain to create shorter functional SUN domains (UNC-84 (Δ556–861). Surprisingly, this did not have noticeable impact on the NE spacing.

LINC complex and NP formation and distribution

While on the one hand the puzzle of NE spacing remains unsolved, how this NE spacing is significantly reduced in order to create new NPs also remains unaddressed. During interphase, NPs double in number (Winey, Yarar, Giddings, & Mastronarde, 1997), which entails the creation of new pores in an intact NE. While NE spacing is maintained at 30–50 nm, the bilayers have to come next to each other for fusion to occur. The optimal separation for a fusion reaction to take place is around 2–3 nm between the two bilayers, an order of magnitude smaller than the resting NE spacing (Lee & Schick, 2007). Numerous studies have implicated protein-induced bending of the membrane to be a prerequisite for pore formation (Rothballer, Schwartz, & Kutay, 2013)(Crisp et al., 2006)(Sosa et al., 2012) (Fichtman, Ramos, Rasala, Harel, & Forbes, 2010)(Jaspersen & Ghosh, 2012)(Talamas & Hetzer, 2011)(Funakoshi, Clever, Watanabe, & Imamoto, 2011)(Doucet & Hetzer, 2010) (Rothballer & Kutay, 2013). The list includes various nucleoporins such as Nup201/gp210 and POM121, ER proteins reticulons, and the LINC complex. Going beyond specific proteins, studies by D'Angelo et al. and Dulz et al. unraveled another key requirement for NP creation (Angelo et al., 2006)(Dultz & Ellenberg, 2010): in addition to the presence of pore-creating proteins, a concomitant increase in the membrane area is also needed to create new pores.

In the context of LINC complexes, bilayer fusion and pore distribution has been linked to SUN1 proteins. A study by Liu et al. suggests that SUN1 proteins cluster around the NPs, suggesting a potential role in bilayer fusion (Liu et al., 2007). An *in vitro* study by Talamas & Hetzer supports this idea (Talamas & Hetzer, 2011). Inhibition of SUN1 proteins arrests NP creation during interphase, but NP formation after mitosis is unaffected. Different domains of SUN1 have been shown to impact NP formation differently. Nuclei with SUN1 mutants lacking the PNS domain inhibited NPC insertion during interphase (Talamas & Hetzer, 2011). In contrast, nuclei with SUN1 mutants lacking both PNS and INM domains did not prohibit NPC insertion. Based on these findings, SUN1 is hypothesized to act in a manner similar to SNARE (soluble NSF attachment protein receptor where NSF stands for *N-ethyl-maleimide-sensitive* fusion protein) proteins or viral membrane fusion complexes. In addition to NPC assembly, SUN1 proteins have been suggested to influence NPC distribution in the NE. The study by Liu et al. shows clustering of NPCs in cells lacking SUN1 proteins (Liu et al., 2007).

A physical view of nuclear envelope architecture

While the above mentioned studies reveal the role of various determinants and constituents of the nuclear envelope, some fundamental questions remain unaddressed from a biophysical perspective. We discuss some of these critical questions below.

What are the stresses in the nuclear membranes in cells?

While the work of Cain et al. has demonstrated that LINC disruption in *C. elegans* muscle nuclei increases the PNS, that higher stresses in the ONM cause increased spacing has not been directly demonstrated. A quantification of the effect of membrane stresses on NE spacing is still pending.

A recent study by Neelam et al. suggests an unusually strong ONM-INM integration in different cell types (Neelam et al., 2015). They sealed a micropipette tip (0.5-μm diameter) to the nuclear surface in well-spread cells with a specified suction pressure. Moving the micropipette away from the nucleus caused the nucleus to deform and move in the direction of micropipette motion. The suction force applied to the ONM did not peel the ONM away from the INM. This indicates that the ONM, INM and the lamina maintain mechanical continuity. Neelam et al observed a proportional increase in nuclear strain with increasing suction pressure. At high forces of ~20 nN (applied over a circular patch of 0.5 micron diameter; the normal stress on the ONM is then on the order of 40 kPa which is much larger than typical cellular traction stresses which are on the order of several hundred Pa (Alam et al., 2015)), the nucleus was pulled out of the cell. This result reflects the tight integration of the nuclear membrane with the rest of the nucleus. The membrane did not peel away from the nucleus even when the SUN linkages with the KASH domain proteins are disrupted in the absence of lamin A/C. This suggests that the SUN-KASH linkages and the linkages with the lamin A/C network are not the only source of mechanical anchorage of the ONM with the INM and the rest of the nucleus. Indeed, the nuclear envelope is an integral part of the nucleus.

The experiments by Neelam et al were performed over short time scales of a few seconds where elastic effects are predominant. The increase in the PNS spacing at the poles of ovoid shape nuclei as seen in the experiments by (Cain et al., 2014) may not require such large stresses if the process occurs over slower time scales which would allow binding and rebinding of linkages between the ONM and INM and/or remodeling of the membrane itself through addition of lipid molecules from the ER. Therefore the fact that the PNS increases in muscle cells but not in other cell types upon LINC disruption may not necessarily reflect the magnitude of the stresses on the nucleus in these different cells. An additional complexity is that the shape of the nucleus may not necessarily reflect the stress distribution on the nuclear surface, but rather a consequence of dynamic evolution of cell shape that exerts viscous stresses on slow time scales on the nucleus to elongate it (Li et al, 2014).

How stiff are the LINC complexes?

While LINC complexes are implicated in transferring forces from the cytoplasm to the nucleoplasm, their stiffness and load bearing capacity are not yet known. The study by (Cain

et al., 2014) revealed that the shortening of UNC-84, a SUN constituent protein, by 300 amino acids in C. *elegans* embryonic and muscle nuclei created functional LINC complexes but led to no detectable effect on the NE spacing. Based on these findings Cain et al. suggested two possibilities- i) first, that the LINC complexes with shortened UNC domain create localized dimples that might go undetected in imaging measurements, and ii) the mutant LINC complex is compliant and undergoes extension to match the NE spacing. The first option is less likely to be the case, based on membrane energetics. It costs energy to bend membranes and therefore, structures with high curvatures are energetically unfavorable. For this reason, application of a point force on a planar patch of membrane or a spherical vesicle generates a gentle curvature spread out over a large domain instead of a localized dimple (Derényi et al., 2002) (Agrawal & Steigmann, 2009). The second option suggests that the mutant LINC complexes are compliant, but the same conclusion cannot be drawn for the wildtype LINC complexes. Modification of the LINC complex could possibly compromise its stiffness. Thus, based on the existing data, it is not clear how compliant or stiff the wildtype LINC complexes are. To gain insights into this issue and the role of LINC complexes it is critical to understand the physical properties of LINC complexes as has been done in the context of other tether-type proteins (Bustamante et al., 2000) (Su & Purohit, 2009) (Su & Purohit, 2010).

What is the effect of SUN-KASH binding/unbinding?—Another key aspect of the LINC complex structure is the binding affinity of the SUN and KASH proteins. First, the binding affinity will determine the maximum tensile force that the LINC complex can sustain and determine the nature of forces the LINC proteins apply on to the NE bilayers. In the bound state, they can apply direct forces that can restrict the motion of the bilayers. In the unbound state, due to their coiled coil domains, SUN proteins can generate a preferred spontaneous curvature in the membrane due to the mushroom effect (Lipowsky, 1997) (Lipowsky, 2007). The entropic repulsion between the mushroom-like coiled coil domains can bend the INM towards the ONM (Fig. 3). This can potentially be a factor that contributes to membrane fusion. The findings of Talamas and Hetzer support this mechanism (Talamas & Hetzer, 2011). Deletion of the PNS spanning SUN1 domain would reduce the bending of the INM towards the ONM, inhibiting NPC assembly.

How are the LINC complexes distributed?—In addition to the force transducing capabilities of a single LINC complex, it is equally important to know their spatial distribution. Are LINC complexes clustered near the NPs or are they uniformly distributed throughout the NE? The areal distribution of the LINC complexes would determine the effective force per unit area that LINC complexes can transfer to the NE. This, in turn, would determine the global impact of the LINC complexes on the geometry of the bilayers and the NE spacing. Due to a required high curvature in the pore region (due to fusion of bilayers) and the natural propensity of bilayers to minimize bending energy, the bilayers can be expected to expand out away from the pore domain (like a catenoid) (Fig. 4). To prevent this from happening, it is possible that LINC complexes are present in a higher density near the pores to supply the bending energy required to flatten the bilayer. Once the bilayers have been made flat, they can maintain their geometry and spacing in the absence of external forces. While SUN1 proteins have been shown to localize near NPCs (Liu et al., 2007),

whether other components of the LINC complex also cluster similarly has not yet been demonstrated. On a similar note, localization of curvature-inducing proteins in the pore region was conceptually shown to establish a uniform spacing between the bilayers (Agrawal and Steigmann, 2009). In addition to the geometry of the membranes, external forces from the actin cytoskeleton can also potentially influence the spatial distribution of LINC complexes (Versaevel et al., 2014).

How strong are entropic effects?—As the bilayers come closer during fusion, they begin to oppose out-of-plane thermal fluctuations of each other. Helfrich studied this effect in his seminal paper and showed that the interaction results in a steric hindrance or entropic pressure, which varies as $1/\Delta h^3$, where Δh is the bilayer separation (Helfrich, 1978). In recent studies Helfrich's assumptions have been revisited and the force has been shown to exhibit a more complex behavior. For extremely small separations, it scales as $1/\Delta h$, for intermediate distances it follows Helfrich's predictions and for larger separations, it shows an exponential decay (Freund, 2013)(Sharma, 2013)(Wennerström, Olsson, & Israelachvili, 2013)(Hanlumyuang, Liu, & Sharma, 2014). It would be important to understand how the fusion proteins overcome this force to gain insight into pore creation.

An additional entropic resistance may come from LINC complexes. A reduced NE spacing during fusion would force the LINC complex to shrink considerably. A protein that spans 30–50 nm space when confined to a few nanometer space should offer considerable entropic resistance to bilayer fusion unless it undergoes some major structural remodeling (see Fig. 3). However, so far, there is no experimental evidence to support this remodeling. Because of this entropic resistance, it becomes even more critical to quantify the spatial distribution of LINC complexes. If the LINC complexes are uniformly distributed in the NE, the entropic force would resist the creation of new pores everywhere in the NE. If the LINC complexes are localized near the NPs, the resistance would be higher near the existing pores, making fusion less energetically expensive far away from the pores. Thus, from the point of view of fusion, clustering of LINC complexes near the NP appears to be the preferred option.

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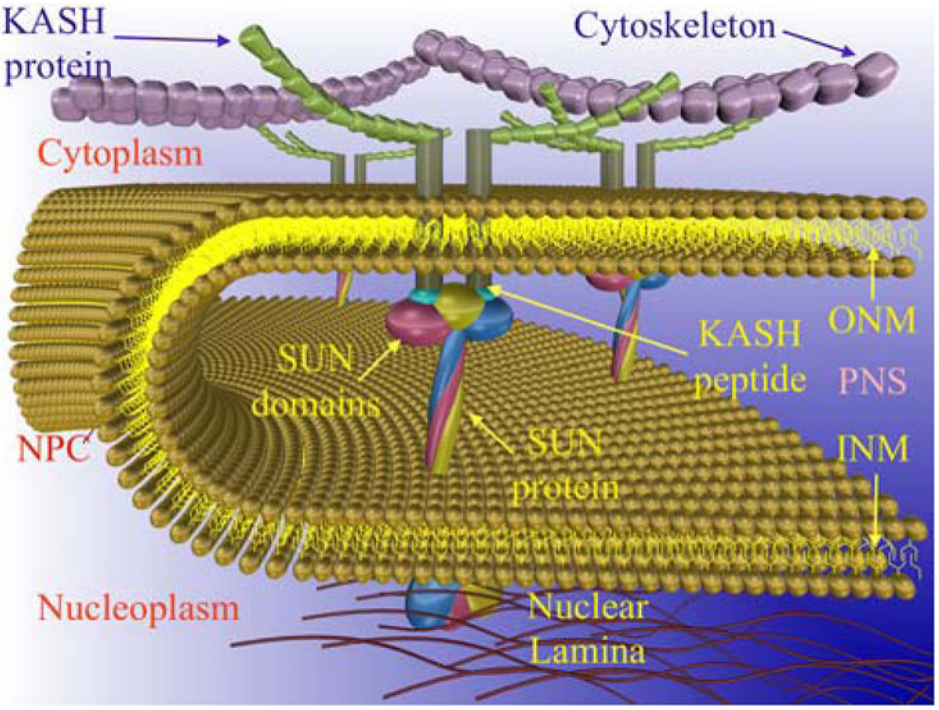

Figure 1.

Figure shows the outer nuclear membrane (ONM) and the inner nuclear membrane (INM) maintained at 45+/−5 nm (adapted from Chang et al., 2015). The SUN protein is a trimer that is embedded on the N terminal side in the INM and binds to KASH domain containing proteins embedded in the ONM. These link to the cytoskeleton. SUN and KASH proteins have been proposed to be responsible for maintaining the distance at 45 nm, although the mechanism is unclear.

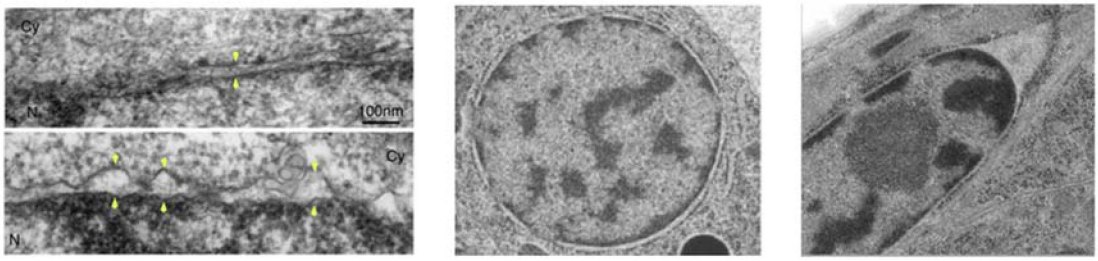

Figure 2.

Left: ONM expansion observed in HeLa cells with a disrupted LINC complex [(Crisp et al., 2006)]. Middle: Normal NE spacing in *C. elegans* nuclei lacking a functional LINC complex [(Cain et al., 2014)]. Right: Increase in NE spacing at the anterior and posterior ends of unc-84 (SUN) mutant muscle cell nuclei [(Cain et al., 2014)].

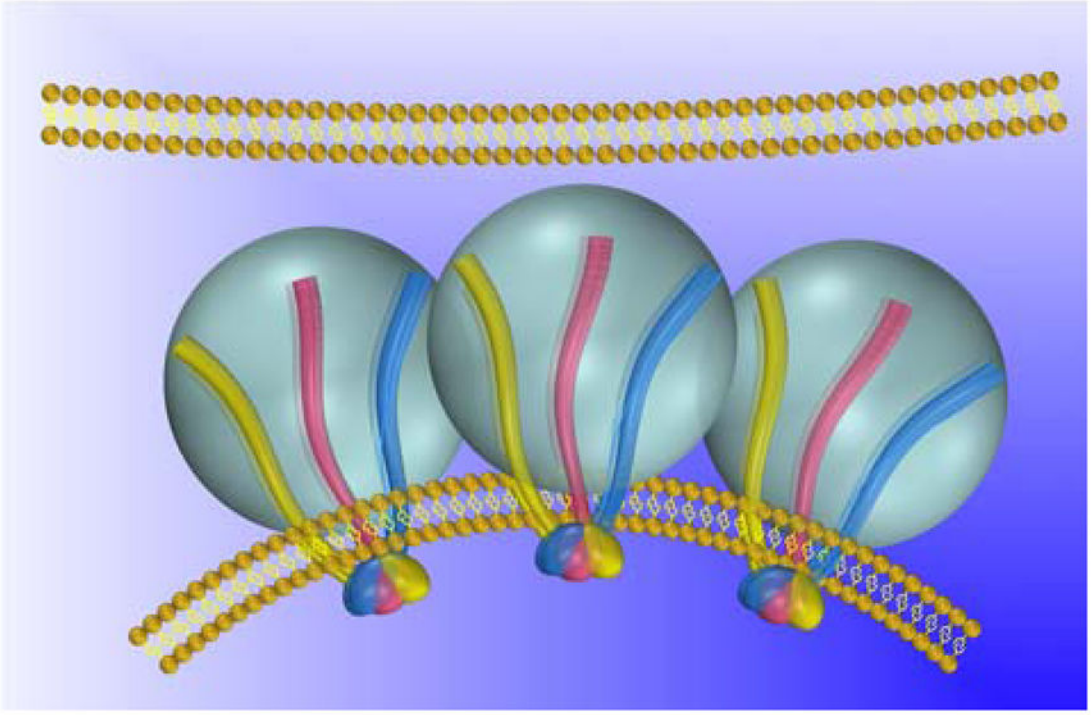

Figure 3.

Spontaneous curvature potentially generated by tethered proteins such as SUN1 due to the entropic repulsion between the coiled domains. The same entropic force can also prevent the bilayers from coming close together for fusion. Blue bubbles represent the excluded volume regions created by fluctuations of free SUN proteins.

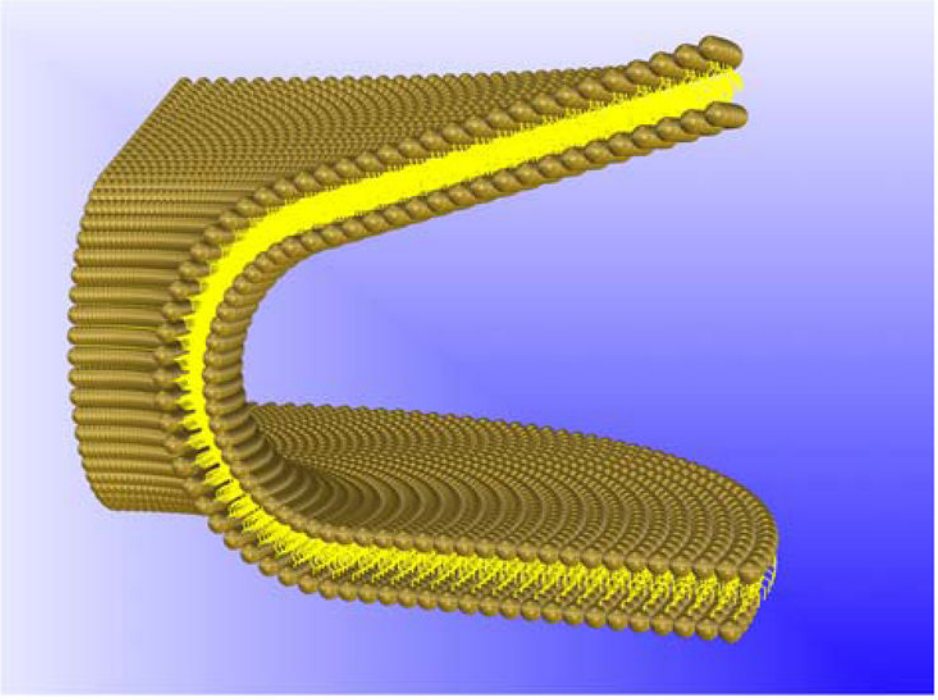

Figure 4.

The natural tendency of the bilayer to expand out near an existing pore in order to reduce the bending energy. A higher density of LINC complexes near the pores can provide the necessary force to flatten the bilayer.