CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Online at: www.iajps.com *Review Article*

A VALUE AND CONTRIBUTORS TO INCREASED ENERGY POTENTIAL IN A CELL MEMBRANE ESPECIALLY BY LOW INTENSITY FOCUSED ULTRASOUND (LIFU): A REVIEW

Dr. Raymond L Venter
([1]BSC, [2]PhD Natural Medicine)
[1]Quantum University
[2]University of Southampton UK

Article Received: March 2021 **Accepted:** March 2021 **Published:** April 2021

Abstract:
Low-Intensity Focused Ultrasound (LIFU) can modulate region-specific brain activity in vivo in a reversible and non-invasive manner, suggesting that it could be used to treat neurological disorders such as epilepsy and Parkinson's disease. Although in vivo studies demonstrate that LIFU has bioeffects on neuronal activity, they only hint at possible mechanisms and do not fully explain how this technology accomplishes these effects. According to one theory, LIFU may cause local membrane depolarization by mechanically disrupting the neuronal cell membrane or activating channels or other membrane proteins. Proteins that detect membrane mechanical perturbations, such as those regulated by membrane tension, are prime candidates for activation in response to LIFU, resulting in the observed neurological responses. We examine how LIFU affects the activation of the purified and reconstituted in liposomes bacterial mechanosensitive channel MscL.
Additionally, two bacterial voltage-gated channels, KvAP and NaK2K F92A were investigated. Surprisingly, the findings indicate that ultrasound modulation and membrane perturbation do not result in channels but rather in pores at the membrane protein-lipid interface. However, apparent reductions in pore formation have been observed in vesicles containing high MscL mechanosensitive channel concentrations, implying that this membrane-tension-sensitive protein may increase membrane elasticity, presumably through channel expansion of the plane of the membrane independent of channel gating.

Corresponding author:
Dr. Raymond L Venter,
(BSC, Honorary Doctorate)
Quantum University
University of Southampton UK

QR code

Please cite this article in press Raymond L Venter., ***A Value and Contributors to Increased Energy Potential in A Cell Membrane Especially by Low Intensity Focused Ultrasound (LIFU): A Review.,*** *Indo Am. J. P. Sci, 2021; 08(04).*

INTRODUCTION:

Currently, treatment options for neurological and psychiatric diseases such as Alzheimer's, Parkinson's, and epilepsy, as well as neuropathic pain, are limited to pharmacological or invasive surgical interventions. While pharmacologic treatments can be tailored to target neurotransmitters, they lack the regional selectivity offered by specific device interventions. Additionally, pharmacotherapy is frequently associated with adverse effects and concerns about drug metabolism and clearance in individuals with impaired hepatic and renal function. On the other hand, neurosurgical interventions such as resections and Deep Brain Stimulation (DBS), which are now established clinical procedures and are being investigated in clinical trials for various neurological conditions, can target specific brain regions that are invasive and associated with morbidity. While novel transcranial magnetic stimulation is entirely non-invasive and capable of targeting deep brain structures, it has a limited spatial resolution. As a result, a low-cost, non-invasive neuromodulation procedure capable of precisely targeting deep brain structures in vivo is urgently needed. Researchers are increasingly interested in focused ultrasound (FUS) as a non-invasive neuromodulation strategy capable of targeting specific brain regions to circumvent these limitations.

Ultrasound was not investigated as a reversible neural suppressor and activator until the 1950s. In vitro research, FUS can effectively stimulate both neurons in culture and induce a short-latency excitatory response in a rodent brain-slice assay. In various small and large ex vivo animal models, rodents, nonhuman primates, and humans were used to test these findings. These studies appear to demonstrate compelling in vivo evidence of FUS's neuromodulatory capabilities, leading investigators to believe that, despite a somewhat variable success rate, FUS may be a candidate for transcranial neuromodulation for conditions such as Parkinson's disease and epilepsy. The lack of knowledge regarding (1) the mechanism underlying neuromodulation and (2) effective system parameters capable of successfully stimulating or suppressing nervous activity may account for the observed variability between studies and the limited success.

FUS's neuromodulatory capacity is currently explained as follows: pressure applied to the tissue causes conformational changes in the lipid membrane due to its elastic properties, modulating protein channels and mechanoreceptors embedded within the membrane. Protein channel modulation affects cellular excitability, action potential variation, and neurotransmitter release or uptake. As a result, several studies investigated the effect of ultrasound on ionic flux using ion-specific dyes. As a result of the observed ion permeation, channels have become candidates for conduits for the observed ion fluxes. According to one of these studies, the mechanical forces induced by the US in the membrane modulate channel activity. Numerous mechanosensitive (MS) channels are electro physiologically gated directly by membrane tension, and membrane forces modulate several channels that are typically gated by voltage or ligands. MS channels directly triggered by membrane tension should be susceptible to FUS if this theory is correct.

Numerous studies have used E. coli mechanosensitive channels to investigate the protein-lipid interaction and the effect of tension in lipid bilayers on protein conformational changes. One of these proteins is the Mechanosensitive Channel of Large Conductance or MscL. Rather than detecting membrane curvature or pressure across the membrane, it has been demonstrated that this channel detects membrane tension directly. MscL also contains the world's largest gated pore, estimated to be greater than 30 nm in diameter, which allows for the passage of relatively large molecules. As a result, physiological and biophysical approaches to studying the effects of membrane tension on protein conformation have become more accessible. MscL is an excellent model for studying the mechanical effects of low-intensity, low-frequency ultrasound on cells and the structural changes that occur at the molecular level due to these characteristics.

This study investigated the effects of low intensity focused ultrasound (LIFU) stimulation and varying parameters on MscL using a simplified in vitro proteoliposome model. Due to our setup, we can study the effects on a pure system composed entirely of protein and lipid, without regard for the cellular cytoskeleton or other structures. Our liposomes contain either the mechanosensor MscL or one of two non-mechanosensitive channels, NaK2K F92A or KvAP. We examined various acoustic intensities that are potentially most clinically relevant for in vivo animal and human applications, namely those that have been successful in previous in vivo ultrasound modulation experiments and are within the FDA regulatory limits for clinical ultrasound images. According to our findings, LIFU can modulate cell membranes and allow efflux through pores created at the protein-membrane interface, rather than gating the channels themselves. Additionally, when reconstituted

at high concentrations, the MscL mechanosensitive channel inhibits pore formation, presumably acting as an elastic, absorbing membrane force without undergoing complete channel gating.

REVIEW AND DISCUSSION:

Numerous in vivo studies have demonstrated that ultrasound can effectively stimulate nervous tissue, resulting in quantifiable ion fluxes and action potentials. A recent study reported that overexpression of MscL in neuronal cells was activated by acoustic force, strongly implying that when the cytoskeleton and extracellular matrix are present, ultrasound can induce membrane tension. Despite these recent advances, there remains a knowledge gap regarding the mechanisms underlying in vivo stimulation and the optimal ultrasound spatial, the temporal parameter for eliciting such stimulation. Different acoustic parameter sets can have various biological effects depending on the cell type and tissue structure. As a first step toward filling in these gaps in our knowledge, we investigated the mechanism of LIFU sonication by restricting our studies to a specific LIFU parameter set and a simplified in vitro proteoliposome system. We avoided the influence of other living-cell components such as the cytoskeleton by using only defined lipids and the MscL channel, both of which have well-characterized protein-lipid interactions. We also examined non-MS channels, which were initially thought to be controlled, and compared the results to those for MscL channels to determine whether efflux occurs via the MscL channel pore in this simplified system.

The LIFU parameters used in these studies were chosen by previous successful in vivo ultrasound modulation studies and FDA safety regulations for diagnostic imaging (Ispta = 720 mW/cm2) (AIUM Clinical Standards Committee 2004). Low ultrasound frequency was more beneficial in transcranial ultrasound stimulation due to lower acoustic beam attenuation and aberration through the skull bone. We chose the 0.5 MHz frequency for our studies based on these findings and the success of previous studies using this frequency in small animal and human models. The intensities used in this study are considered to be within the range of those found to be effective in previous in vivo transcranial ultrasound neuromodulation studies. According to our understanding, the use of continuous-wave and submersion of the hydrophone in this study may have resulted in reverberation within the small well volume. However, the intensities measured at the focal point within the well plate are considered safe for acoustic neuromodulation because they fall within the low-intensity range.

Previous in vivo LIFU modulation studies used pulsed stimulations, demonstrating that pulsed modulation is more effective than continuous stimulation. However, in vivo studies have revealed that LIFU stimulation is more effective as a function of both acoustic intensity and duration. Our findings that longer stimulation durations and continuous rather than pulsating stimulation significantly affect protein-membrane perturbation are consistent with previous findings.

Based on the proteoliposome modulation results obtained using our LIFU system, we observed an increase in calcein efflux from liposomes reconstituted with MscL. We initially assumed that the increased efflux was due to LIFU-induced activation of MS channels, but we discovered that non-mechanically stimulated channels exposed to ultrasound had similar efflux values. Channel gating was ruled out because the calcein dye cannot pass through the NaK2K F92A and KvAP channel pores due to their size restrictions and ion selectivity. Increased efflux behaviour upon reconstitution of any of the three channels into our in vitro system could be explained by pore formation through the membrane, as Krasovitski predicted, or by protein-lipid interface disruption mechanisms. Krasovitski's proteoliposome simulation model, dubbed "bilayer ionophore," or BLS, explains the cellular membrane's ability to absorb LIFU mechanical energy and convert it to expansions and contractions of the intramembrane space. The BLS simulation model revealed that the severity of bio-effects on the cell membrane is dependent on the ultrasound parameters used and the strain-absorbing capacity of the leaflets' maximum area. According to this theory, the bio-effects could range from a mild and reversible excitation of the cell membrane to the formation of pores and even damage to membrane proteins and cytoskeletal fibres. The proteins inserted into the membrane will resist this expansion because the bilayer leaflets will separate – – helixes cannot quickly expand in this manner. In theory, insertion of more rigid membrane proteins, such as the channels studied here, could add strain to the leaflets, increasing the likelihood of lipid pore formation at or near the protein-lipid interface; however, it is unknown whether this occurs in some in vivo systems.

This property of proteoliposomes may be exploited: transmembrane peptides may be designed to enhance pore formation for targeted drug delivery. Liposomes, such as those we created, are prone to becoming stuck in cancerous and inflamed tissues and releasing their

contents is difficult. Additionally, LIFU can be directed to the problematic or diseased area to enhance drug release from liposomes containing the transmembrane peptide. This method could theoretically be used to design a targeted and triggered drug release device.

Although all three channels increased vesicle efflux, MscL channels appeared to respond differently to LIFU stimulation than other protein channels. Compared to liposomes containing lower protein concentrations, vesicles containing the highest MscL concentration demonstrated decreased calcein efflux rather than an increase. According to previous research, the MscL channel may expand in the membrane's plane before pore opening, resulting in the molecule being in a closed-expanded state. Due to the large opening pore (approximately 30 in diameter), this protein expansion may be more pronounced than in other channels. According to one interpretation of our findings, the MscL protein acts as a tension spring by achieving the closed-expanded state, relieving some strains within the membrane. True, such an idea has been attempted previously. Boucher and colleagues previously investigated how MS protein channels with stretching capabilities, colloquially referred to as membrane "spandex," can maintain bilayer tension within a specified range via a technique called closed-closed expansion. This two-state (expanded/contracted) simulated model was inspired and designed using the characteristics and expression levels of bacterial MscL channels. The model examined closed-closed expansion en route to opening or pre-open expansion states during the activation pathway, as well as overexpression in bacteria that act as stretch-tension buffers to prevent MscL channels from opening prematurely. MscL is predicted to expand up to 80% of its open area before gating, acting as tension-dampening spandex components. Under the right conditions, mechanosensitive channels could theoretically dampen the acoustic energy transformation onto the intramembrane spacing via conformational expansion, resulting in slow membrane tension relief. As a result, our findings appear to provide the first experimental evidence for Boucher's "spandex" model, assuming that LIFU induces membrane tensions, as a recent study with MscL suggested.

CONCLUSION:

Our findings establish a new paradigm for comprehending the underlying mechanisms of ultrasound stimulation. We observe an increase in calcein efflux through any of three membrane protein channels when they are reconstituted as proteoliposomes. Our findings do not support the hypothesis that channel opening increases efflux. Rather than that, they propose that "stiff" membrane proteins stifle the dynamics of LIFU-stimulated membranes, increasing the likelihood of pores forming at or near the protein-lipid interface. At its highest concentration, MscL partially inhibits additional calcein release; this is likely due to the channel's ability to expand within the plane of the membrane, relieving some of the tension. As a result of these unexpected findings, the mechanisms underlying LIFU stimulation of biological neuronal tissue gain a new dimension.

REFERENCES:

1. Raymond L Venter., Role Of Bio-Resonance Focused Ultrasound On Stem Cell Proliferation And Growth: A Review.., Indo Am. J. P. Sci, 2021; 08(04).
2. Raymond L Venter., Environmental Energy For Cellular Growth And Repair Especially By Bio-Resonance Focused Ultrasound: A Literature Review., Indo Am. J. P. Sci, 2021; 08(04).
3. Raymond L Venter., Focused Ultrasound Involving The Usage Of Cell Resonance To Understand The Effect And Its Use As A Therapy For Disease Modification.,Indo Am. J. P. Sci, 2021; 08(03).
4. Gaitatzis, A. & Sander, J. W. The long-term safety of antiepileptic drugs. CNS drugs 27, 435–455 (2013).
5. Vonck, K. et al. Neurostimulation for refractory epilepsy. Acta neurologica belgica 103, 212–217 (2003).
6. Chkhenkeli, S. A. et al. Electrophysiological effects and clinical results of direct brain stimulation for intractable epilepsy. Clinical neurology and neurosurgery 106, 318–329 (2004).
7. Engel, J. et al. Practice parameter: Temporal lobe and localized neocortical resections for epilepsy Report of the Quality Standards Subcommittee of the American Academy of Neurology, in Association with the American Epilepsy Society and the American Association of Neurological Surgeons. Neurology 60, 538–547 (2003).
8. Fitzgerald, P. B. & Daskalakis, Z. J. The effects of repetitive transcranial magnetic stimulation in the treatment of depression (2011).
9. Fry, W. J. & Fry, F. J. Fundamental neurological research and human neurosurgery using intense ultrasound. IRE transactions on medical electronics Me 7, 166–181 (1960).
10. Wulff, V., Fry, W., Tucker, D., Fry, F. J. & Melton, C. Effects of Ultrasonic Vibrations on

Nerve Tissues. Experimental Biology and Medicine 76, 361–366 (1951).
11. Tufail, Y., Yoshihiro, A., Pati, S., Li, M. M. & Tyler, W. J. Ultrasonic neuromodulation by brain stimulation with transcranial ultrasound. nature protocols 6, 1453–1470 (2011).
12. Tyler, W. J. et al. Remote excitation of neuronal circuits using low-intensity, low-frequency ultrasound. PLoS One 3, e3511 (2008).
13. Tufail, Y. et al. Transcranial pulsed ultrasound stimulates intact brain circuits. Neuron 66, 681–694 (2010).
14. King, R. L., Brown, J. R., Newsome, W. T. & Pauly, K. B. Effective parameters for ultrasound-induced in vivo neurostimulation. Ultrasound in medicine & biology 39, 312–331 (2013).
15. Kim, H. et al. Noninvasive transcranial stimulation of rat abducens nerve by focused ultrasound. Ultrasound in medicine & biology 38, 1568–1575 (2012).
16. Yoo, S.-S. et al. Focused ultrasound modulates region-specific brain activity. Neuroimage 56, 1267–1275 (2011).
17. Mueller, J., Legon, W., Opitz, A., Sato, T. F. & Tyler, W. J. Transcranial focused ultrasound modulates intrinsic and evoked EEG dynamics. Brain Stimul 7, 900–908, https://doi.org/10.1016/j.brs.2014.08.008 (2014).
18. Deffieux, T. et al. Low-intensity focused ultrasound modulates monkey visuomotor behavior. Current Biology 23, 2430–2433 (2013)
19. Legon, W. et al. Transcranial focused ultrasound modulates the activity of primary somatosensory cortex in humans. Nat Neurosci 17, 322–329, https://doi.org/10.1038/nn.3620 (2014).
20. Krasovitski, B., Frenkel, V., Shoham, S. & Kimmel, E. Intramembrane cavitation as a unifying mechanism for ultrasound-induced bioeffects. Proceedings of the National Academy of Sciences 108, 3258–3263 (2011).
21. Johns, L. D. Nonthermal effects of therapeutic ultrasound: the frequency resonance hypothesis. Journal of athletic training 37, 293 (2002).
22. Iscla, I. & Blount, P. Sensing and responding to membrane tension: the bacterial MscL channel as a model system. Biophys J 103, 169–174, https://doi.org/10.1016/j.bpj.2012.06.021 (2012).
23. Booth, I. R. & Blount, P. The MscS and MscL families of mechanosensitive channels act as microbial emergency release valves. J Bacteriol 194, 4802–4809, https://doi.org/10.1128/JB.00576-12 (2012).
24. Patel, A. J., Lazdunski, M. & Honore, E. Lipid and mechano-gated 2P domain K(+) channels. Curr Opin Cell Biol 13, 422–428 (2001).
25. Maingret, F., Patel, A. J., Lesage, F., Lazdunski, M. & Honore, E. Lysophospholipids open the two-pore domain mechano-gated K(+) channels TREK-1 and TRAAK. J Biol Chem 275, 10128–10133 (2000).
26. Brohawn, S. G., Su, Z. & MacKinnon, R. Mechanosensitivity is mediated directly by the lipid membrane in TRAAK and TREK1 K+ channels. Proc Natl Acad Sci USA 111, 3614–3619, https://doi.org/10.1073/pnas.1320768111 (2014).
27. Gu, C. X., Juranka, P. F. & Morris, C. E. Stretch-activation and stretch-inactivation of Shaker-IR, a voltage-gated K+ channel. Biophys J 80, 2678–2693, https://doi.org/10.1016/S0006-3495(01)76237-6 (2001).
28. Laitko, U. & Morris, C. E. Membrane tension accelerates rate-limiting voltage-dependent activation and slow inactivation steps in a Shaker channel. J Gen Physiol 123, 135–154, https://doi.org/10.1085/jgp.200308965 (2004).
29. Paoletti, P. & Ascher, P. Mechanosensitivity of NMDA receptors in cultured mouse central neurons. Neuron 13, 645–655 (1994).
30. Laitko, U., Juranka, P. F. & Morris, C. E. Membrane stretch slows the concerted step prior to opening in a Kv channel. J Gen Physiol 127, 687–701, https://doi.org/10.1085/jgp.200509394 (2006).
31. Moe, P. & Blount, P. Assessment of potential stimuli for mechano-dependent gating of MscL: effects of pressure, tension, and lipid headgroups. Biochemistry 44, 12239–12244 (2005).
32. Sukharev, S. I., Blount, P., Martinac, B., Blattner, F. R. & Kung, C. A large-conductance mechanosensitive channel in E. coli encoded by mscL alone. Nature 368, 265–268 (1994)
33. Cruickshank, C. C., Minchin, R. F., Le Dain, A. C. & Martinac, B. Estimation of the pore size of the large-conductance mechanosensitive ion channel of Escherichia coli. Biophys J 73, 1925–1931, https://doi.org/10.1016/S0006-3495(97)78223-7 (1997).
34. Ajouz, B., Berrier, C., Garrigues, A., Besnard, M. & Ghazi, A. Release of thioredoxin via the mechanosensitive channel MscL during osmotic downshock of Escherichia coli cells. J Biol Chem 273, 26670–26674 (1998).
35. Berrier, C., Garrigues, A., Richarme, G. & Ghazi, A. Elongation factor Tu and DnaK are transferred from the cytoplasm to the periplasm of Escherichia coli during osmotic downshock presumably via the mechanosensitive channel mscL. J Bacteriol 182, 248–251 (2000)

36. Lee, W. & Garra, B. How to interpret the ultrasound output display standard for higher acoustic output diagnostic ultrasound devices - Version 2. Journal of Ultrasound in Medicine 23, 723–726 (2004).
37. Iscla, I. et al. Improving the design of a MscL-based triggered nanovalve. Biosensors 3, 171–184 (2013)
38. Yang, L. M. et al. Engineering a pH-Sensitive Liposomal MRI Agent by Modification of a Bacterial Channel. Small 14, e1704256, https://doi.org/10.1002/smll.201704256 (2018).
39. Kocer, A., Walko, M., Meijberg, W. & Feringa, B. L. A light-actuated nanovalve derived from a channel protein. Science 309, 755–758, https://doi.org/10.1126/science.1114760 (2005).
40. Derebe, M. G. et al. Tuning the ion selectivity of tetrameric cation channels by changing the number of ion binding sites. Proc Natl Acad Sci USA 108, 598–602, https://doi.org/10.1073/pnas.1013636108 (2011).
41. Lam, Y. L., Zeng, W., Sauer, D. B. & Jiang, Y. The conserved potassium channel filter can have distinct ion binding profiles: structural analysis of rubidium, cesium, and barium binding in NaK2K F92A. J Gen Physiol 144, 181–192, https://doi.org/10.1085/jgp.201411191 (2014).
42. Zheng, H., Liu, W., Anderson, L. Y. & Jiang, Q. X. Lipid-dependent gating of a voltage-gated potassium channel. Nat Commun 2, 250, https://doi.org/10.1038/ncomms1254 (2011).
43. Perozo, E., Kloda, A., Cortes, D. M. & Martinac, B. Physical principles underlying the transduction of bilayer deformation forces during mechanosensitive channel gating. Nature Structural & Molecular Biology 9, 696–703 (2002).
44. Legon, W., Rowlands, A., Opitz, A., Sato, T. F. & Tyler, W. J. Pulsed ultrasound differentially stimulates somatosensory circuits in humans as indicated by EEG and FMRI. PLoS One 7, e51177, https://doi.org/10.1371/journal.pone.0051177 (2012).
45. Ye, J. et al. Ultrasonic Control of Neural Activity through Activation of the Mechanosensitive Channel MscL. Nano Lett 18, 4148–4155, https://doi.org/10.1021/acs.nanolett.8b00935 (2018).
46. Gavrilov, L. R., Tsirulnikov, E. M. & Davies, I. A. I. Application of focused ultrasound for the stimulation of neural structures. Ultrasound in Medicine and Biology 22, 179–192, https://doi.org/10.1016/0301-5629(96)83782-3 (1996).
47. O'Reilly, M. A., Huang, Y. & Hynynen, K. The impact of standing wave effects on transcranial focused ultrasound disruption of the blood–brain barrier in a rat model. Physics in medicine and biology 55, 5251 (2010).
48. Hashizume, H. et al. Openings between Defective Endothelial Cells Explain Tumor Vessel Leakiness. The American Journal of Pathology 156, 1363–1380, https://doi.org/10.1016/s0002-9440(10)65006-7 (2000).
49. Sercombe, L. et al. Advances and Challenges of Liposome Assisted Drug Delivery. Front Pharmacol 6, 286, https://doi.org/10.3389/fphar.2015.00286 (2015)
50. Nehoff, H., Parayath, N. N., Domanovitch, L., Taurin, S. & Greish, K. Nanomedicine for drug targeting: strategies beyond the enhanced permeability and retention effect. Int J Nanomedicine 9, 2539–2555, https://doi.org/10.2147/IJN.S47129 (2014).
51. Anishkin, A., Chiang, C. S. & Sukharev, S. Gain-of-function mutations reveal expanded intermediate states and a sequential action of two gates in MscL. Journal of General Physiology 125, 155–170, https://doi.org/10.1085/jgp.200409118 (2005).
52. Betanzos, M., Chiang, C. S., Guy, H. R. & Sukharev, S. A large iris-like expansion of a mechanosensitive channel protein induced by membrane tension. Nat Struct Biol 9, 704–710, https://doi.org/10.1038/nsb828 (2002).
53. Boucher, P. A., Morris, C. E. & Joos, B. Mechanosensitive closed-closed transitions in large membrane proteins: osmoprotection and tension damping. Biophys J 97, 2761–2770, https://doi.org/10.1016/j.bpj.2009.08.054 (2009).
54. Sukharev, S., Durell, S. R. & Guy, H. R. Structural models of the MscL gating mechanism. Biophysical journal 81, 917–936 (2001).
55. Shi, N., Ye, S., Alam, A., Chen, L. & Jiang, Y. Atomic structure of a Na+- and K+ -conducting channel. Nature 440, 570–574, https://doi.org/10.1038/nature04508 (2006).
56. Jiang, Y. et al. X-ray structure of a voltage-dependent K+ channel. Nature 423, 33–41, https://doi.org/10.1038/nature01580 (2003).
57. Jiang, Y., Ruta, V., Chen, J., Lee, A. & MacKinnon, R. The principle of gating charge movement in a voltage-dependent K+ channel. Nature 423, 42–48, https://doi.org/10.1038/nature01581 (2003).
58. Yang, L.-M. et al. Three routes to modulate the pore size of the MscL channel/nanovalve. ACS nano 6, 1134–1141 (2012).