Rock the nucleus: significantly enhanced nuclear membrane permeability and gene transfection by plasmonic nanobubble induced nanomechanical transduction

Xiuying Li [a], Peiyuan Kang [a], Zhuo Chen [b], Sneha Lal [c], Li Zhang [c], Jeremiah J. Gassensmith [b,d], and Zhenpeng Qin * [a,d,e]

Abstract: Efficient delivery to the cell nucleus remains a significant challenge for many biomolecules, including anticancer drugs, proteins and DNAs. Successful delivery to the nucleus has to overcome two barriers, firstly crossing the cell membrane and then entering the cell nucleus. Here we report that the nanomechanical force from plasmonic nanobubbles increases nuclear membrane permeability and facilitates nuclear uptake of macromolecules that would not otherwise enter the nucleus. Importantly, we show that plasmonic nanobubble-induced nanomechanical transduction in combination with electroporation leads to significantly higher gene transfection and protein expression, compared to standard electroporation treatment. This novel nanomechanical transduction opens up a range of new opportunities and applications including for gene therapy and anticancer drug delivery.

The cell nucleus is a subcellular compartment where genetic information and the transcription machinery reside, and is also the target of numerous therapeutic agents. [1] For example, gene therapy aims at restoring dysfunctional or missing genes by delivering therapeutic genes into the cell nucleus. [2] Some anticancer drugs, such as doxorubicin, induce tumor cell apoptosis by oxidative DNA damage and DNA enzymes inhibition, *i.e.*, topoisomerase II in the nucleus. [1, 3] Upon entering the cell through endocytosis, many biomolecules, including anticancer drugs, proteins, and DNAs, need to escape the endosomal barrier and reach the nucleus to elicit their therapeutic effects. [4] Due to size limitations of the nuclear pore complex, it is extremely difficult for nanoparticles or macromolecules to enter the cell nucleus [5]. This challenge has been approached via numerous strategies in an effort to enhance nuclear delivery, for example, using ligand modified nanocarrier or biomolecule. [6] For instance, a nuclear localization signal (NLS) peptide-functionalized nanoparticle was reported to target and deliver doxorubicin into the cell nucleus. [7] Despite their advantages, nanoparticles must be small enough to enter the nucleus and needs to be functionalized with specific ligands to be recognized by a corresponding nuclear protein. [8] Moreover, it has been reported that direct conjugation of NLS peptide to linearized DNA did not enhance the nuclear entry. [9] Thus, a versatile method to enhance nuclear delivery and overcome limitations of current methods is highly desirable towards the improvement of the therapeutic and gene transfection.

Here we report a novel mechanism based on nanomechanical transduction to increase the nuclear membrane permeability and biomolecule delivery (Figure 1). Plasmonic nanoparticles efficiently absorb laser energy at plasmonic resonant wavelengths. [10] Under ultrashort laser pulse irradiation (ps, fs), plasmonic nanoparticles locally heat up to high temperatures, leading to transient nanoscale vaporization bubbles that grow and collapse on the order of nanoseconds. [11] The growth and collapse of nanobubbles lead to nanoscale mechanical effects and thus act as nanomechanical transducers, which converts optical energy into nanomechanical energy. Using this novel nanomechanical transduction mechanism, we demonstrated that single near-infrared laser pulse (750 nm, 28 ps) significantly increases the nuclear membrane permeability and allows nucleus uptake of macromolecules (dextran, 40 kDa), which would otherwise not have entered the nucleus. Importantly, the nanomechanical transduction in combination with electroporation significantly increased the gene transfection efficiency by 2.7-fold compared to electroporation alone. Finally, this nanomechanical transduction technique is highly localized, does not rely on specific functional ligands, and is promising for nuclear delivery of many biomolecules — especially DNAs and anti-cancer drugs.

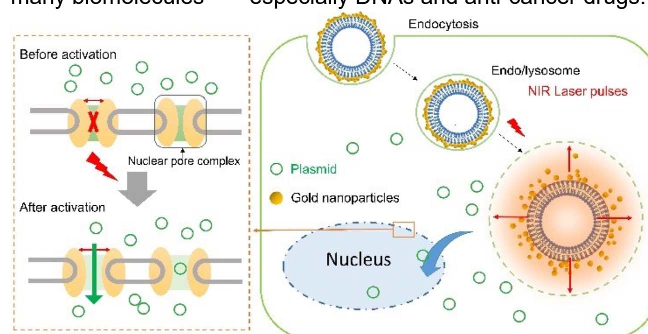


Figure 1. **Schematic of nanomechanical transduction enhanced nuclear entry of biomolecules**. Near-infrared laser pulse activates plasmonic nanoparticles to create plasmonic nanobubbles that grow and collapse within nanoseconds. The nanomechanical force from plasmonic nanobubbles increases the nuclear membrane permeability, thus leading to enhanced nuclear entry for biomolecules.

We first characterized the nanomechanical transducers that are capable of converting near-infrared laser pulse energy into nanomechanical forces. While many near-infrared absorbing

[a] Dr. X Li, P Kang, Prof. Z Qin
Department of Mechanical Engineering
University of Texas at Dallas
800 West Campbell Rd. Richardson, TX 75080, USA
E-mail: Zhenpeng.Qin@utdallas.edu
[b] Z Chen, Prof. J. J. Gassensmith
Department of Chemistry and Biochemistry
University of Texas at Dallas
800 West Campbell Rd. Richardson, TX 75080, USA
[c] S Lal, Prof. L Zhang
Department of Biological Sciences
University of Texas at Dallas
800 West Campbell Rd. Richardson, TX 75080, USA
[d] Prof. Z Qin, Prof. J. J. Gassensmith
Department of Bioengineering
University of Texas at Dallas
800 West Campbell Rd. Richardson, TX 75080, USA
[e] Prof. Z Qin
Department of Surgery
The University of Texas at Southwestern Medical Center
5323 Harry Hines Blvd., Dallas, TX 75390, USA

Supporting information for this article is given via a link at the end of the document.

WILEY-VCH

plasmonic nanoparticles have been reported, we followed previously reported methods to synthesize gold-coated plasmonic liposomes by a one-step deposition of gold nanoparticles directly onto the liposome surface. [12] Gold coating increased the liposome size by 10 – 15 nm (Supplementary Table S1). Figure 2A shows that small gold nanoparticles form discrete gold clusters surrounding the liposomal core. The plasmonic coupling between gold nanoparticles leads to a near-infrared absorption at around 750 nm for plasmonic liposomes, while non-coated liposomes did not show any resonant peak in the near-infrared wavelengths (Figure 2B). We measured the plasmonic nanobubble generation upon near-infrared pulsed laser activation using an optical pump-probe technique. The refractive index mismatch of vapor nanobubbles strongly scatters the probe laser beam, leading to a transient decrease in the transmitted laser intensity. The results show that increasing the pump laser energy above the nanobubble generation threshold leads to a greater drop in the transmitted intensity, while uncoated liposomes do not generate nanobubbles even at a high laser pulse fluence (150 mJ/cm^2, Figure 2C). The lifetime of the plasmonic nanobubbles ranges from 20—50 ns as a function of the laser pulse energy and is a transient event.

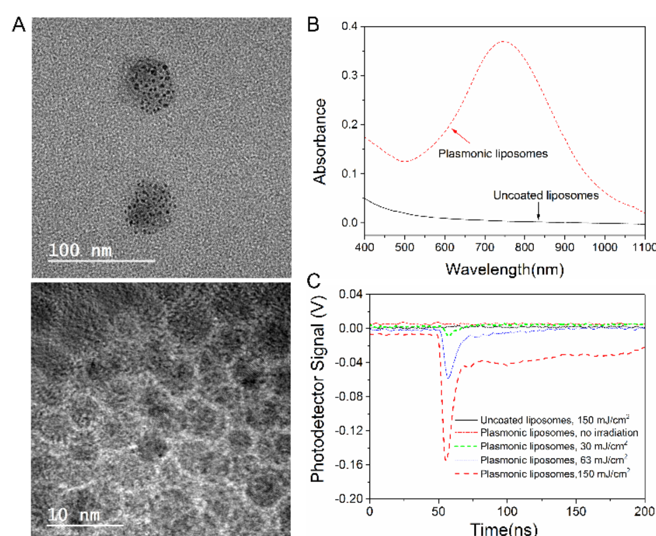


Figure 2. Characterization of plasmonic liposomes and detection of plasmonic nanobubbles. (A) TEM images of plasmonic liposomes; (B) UV-Vis spectra of plasmonic liposomes and uncoated liposomes; (C) Plasmonic nanobubble signal of plasmonic liposomes irradiated with single 750 nm laser pulse under different laser fluence.

Next, we assessed how the nanomechanical transduction affected the intracellular transport of macromolecules. The nuclear pore complex has an estimated cutoff size of 5 nm [13] and larger macromolecules do not readily enter the nucleus. To demonstrate this point, cells (Raw 264.7) were first treated by electroporation to allow trans-membrane uptake of FD-40 in the cytosol. Due to the large size (6 nm), FD-40 does not diffuse through the nuclear pores and thus can't enter the nucleus without external forces. [14] This is confirmed by the confocal imaging that shows the distribution of FD-40 in the cytosol after electroporation (Figure 3B), with very weak fluorescence signal in the nucleus region. To test the effect of the nanomechanical transduction, cells were incubated with plasmonic liposomes to allow the endosomal uptake of nanomechanical transducers. After a single near-infrared laser pulse (60 mJ/cm^2), the nanomechanical transduction leads to significant nuclear uptake of FD-40, up to 70% fluorescence intensity in the nucleus compared to the cytosol. In contrast, when using standard liposomes without gold coating or laser alone without nanoparticles, no mechanical transduction occurred and no change in the nucleus uptake of FD-40 was observed. These results suggest that nanomechanical transduction induced by plasmonic nanobubbles enhanced the permeability of nuclear membrane, thus enabling nuclear entry of macromolecules with size larger than the nuclear pore.

Finally, we tested whether the enhanced nuclear membrane permeability by nanomechanical transduction leads to higher gene transfection efficiency. As a testbed, we performed gene transfection in Raw 264.7 cells using stomatitis virus-encoded glycoprotein tagged with enhanced green fluorescence protein plasmid (pEGFP-VSVG) as a reporter gene. Gene transfer into macrophage cell lines is frequently impeded by extremely low transfection efficiencies [15] even with the use of electroporation. We first introduced pEGFP-VSVG into Raw 264.7 cells by electroporation and then allowed the cells to uptake the nanomechanical transducers (*i.e.* plasmonic liposomes), followed by single near-infrared laser pulse treatment (Figure 3). Qualitative confocal imaging suggests a higher level of EGFP expression with nanomechanical transduction treatment compared with electroporation alone (Figure 3F). Further quantitative measurement of EGFP showed a 2.7-fold increase in the EGFP expression as a result of the nanomechanical transduction (Figure 3G). As a comparison, cells with plasmonic liposomes but without near-infrared pulse activation didn't show significant improvement in EGFP expression compared with electroporation alone. Thus, the enhanced nuclear membrane permeability by nanomechanical transduction leads to improved plasmid accumulation in the nucleus and, as a result, significantly higher gene transfection efficiency.

This study builds upon previous investigations on the mechanical stimulation of nucleus and reports a novel nanomechanical transduction mechanism to enhance nuclear membrane permeability. [16] There are some other simulation evidence suggesting that mechanical stimulation regulates the nuclear entry of endogenous biomolecule. [17] However the exact mechanism is still unknown, and there are several proposed mechanisms. One hypothesis is that mechanical stimulus transiently deforms the nuclear envelope to activate nuclear pore complexes. This in turn increases the nuclear membrane permeability and leads to enhanced nuclear entry of biomolecules (Figure 1). Further fundamental studies on the mechanical transduction are required to elucidate the exact mechanisms. The nanomechanical transduction is well tolerated by the cells as evidenced by the cell viability (Figure S2). As a critical cellular compartment, the nucleus plays numerous important roles in physiology and therapy. Regulation of nuclear delivery of biomolecules has many applications in understanding signaling pathway in cell nucleus, enhancing gene transfection [18] and drug therapeutic efficiency.

In conclusion, plasmonic nanoparticles can be used as intracellular nanomechanical transducers to enhance permeability of the nuclear membrane. Near-infrared laser pulses activate these nanoparticle transducers to generate short-lived plasmonic nanobubbles, which exerts a nanomechanical effect inside the cell close to the nucleus. The nanomechanical transduction increases the nuclear membrane permeability to allow accumulation of macromolecules that are larger than the nuclear pore complex. We further demonstrated that nanomechanical transduction significantly improves gene transfection efficiency in Raw 264.7 cells, which are notoriously

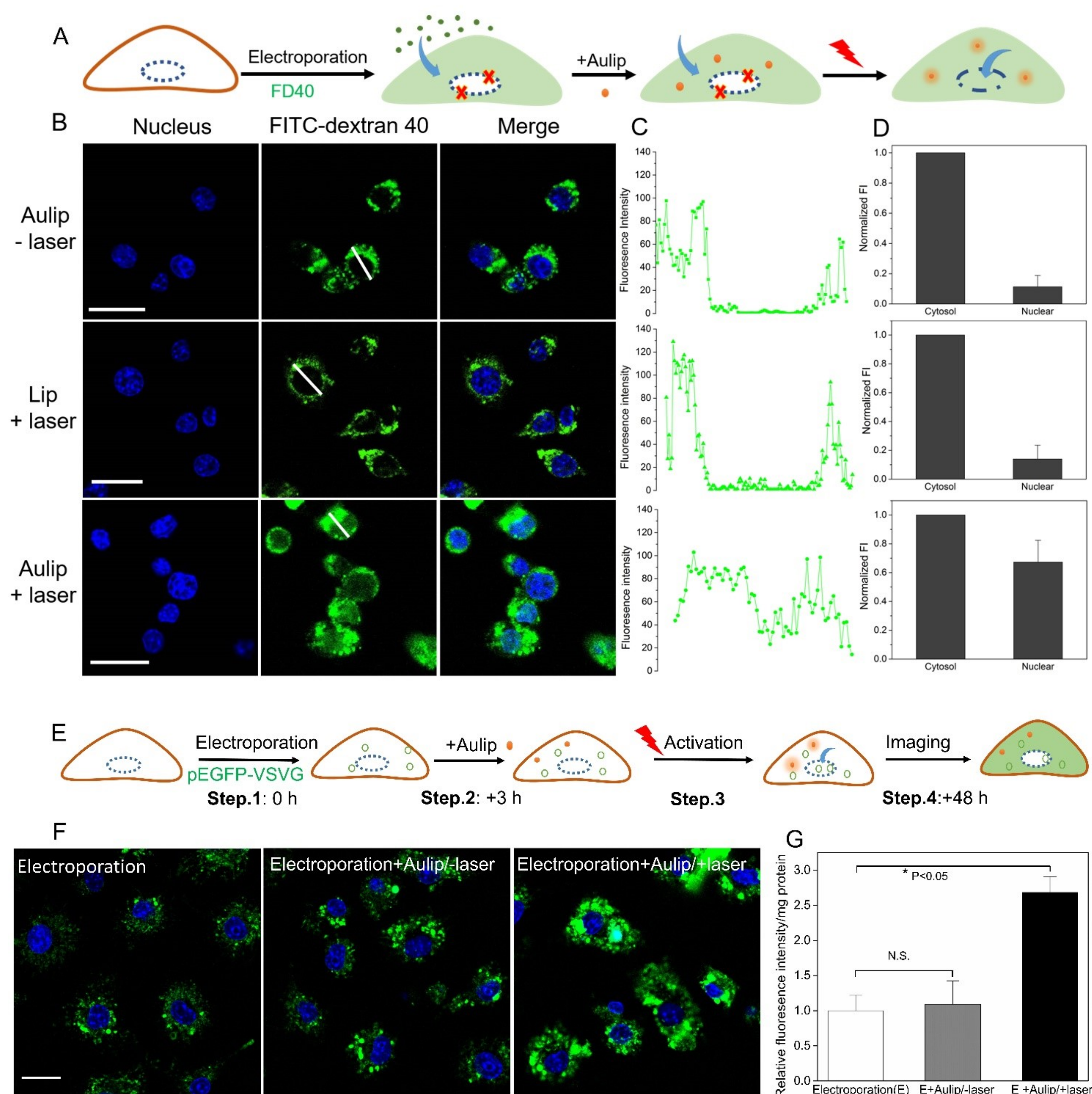


Figure 3. Nanomechanical transduction by plasmonic nanobubbles leads to enhanced nucleus membrane permeability and significantly improved gene transfection. (A) Schematic of the experimental procedure for macromolecule nuclear membrane permeability. Fluorescein isothiocyanate–dextran (FITC-dextran, 40 kDa, or FD-40) was introduced into cells by electroporation. Gold-coated plasmonic liposomes (Aulip) were loaded into the cells 3 h after electroporation. The endocytosed intracellular plasmonic liposomes were activated by single laser pulse (750 nm, 60 mJ/cm^2) to generate nanomechanical force. (B) Confocal images of cells for experimental groups including: plasmonic liposomes alone (top), with uncoated liposomes (Lip) and laser pulse (middle), and with plasmonic liposomes and laser pulse (bottom). (C) Fluorescent intensity plots of FD-40 across the cell (white lines in the confocal image). (D) Average fluorescent intensity in the cytosol and nucleus. (E) Schematic of the experimental procedure for gene transfection; (F) Confocal images of Raw 164.7 cells 48 h after gene transfection. Blue: nucleus; Green: EGFP-VSVG, (G) Relative transfection efficiency of EGFP-VSVG determined by fluorescence microplate reader. Values indicate the mean ± SD of the experiments, n=3. Scale bar, 20 μm.

difficult to transfect. [19] This technique is nanomechanical force based, and highly localized, doesn't rely on specific nuclear receptors, and can be applied to a broad range of biomolecules and cells. We anticipate that this technique will be useful for nuclear delivery of anti-cancer drugs and DNAs. Future studies include further understanding the mechanisms and integrate with common liposome vectors for gene delivery.

Experimental Section

Experimental details are described in Supplementary information.

Acknowledgements

XL wants to thank Prof. Yihong Wang from UT southwestern for kindly providing us with Raw 264.7 cells, and Dr. Peter van Hoogevests from The Phospholipid Research Center, Heidelberg, German, for helpful discussions. This work was supported by postdoc research grant from The Phospholipid Research Center, Heidelberg, German (1603574), CPRIT (Z.Q.: RP160770 and J.J.G.: RP170752) and NSF (Z.Q.: 1631910, J.J.G.: 1654405) grants.

Keywords: Gene transfection • light responsive nanoparticles • nuclear delivery • laser pulse • plasmonic nanobubbles

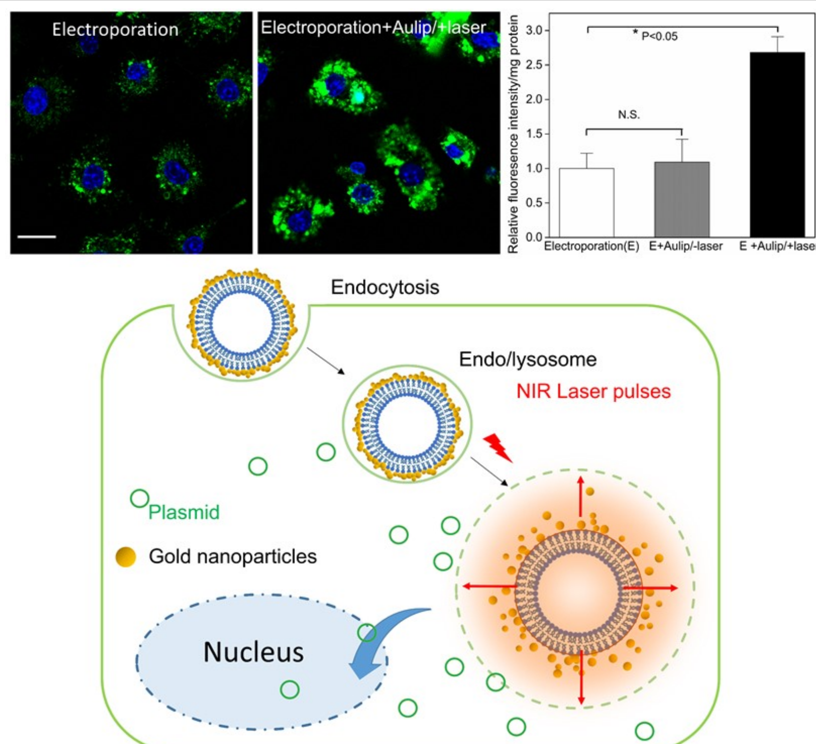
References

[1] J. Castro, M. Ribo, A. Benito, M. Vilanova, *Curr Med Chem* 2013, 20, 1225-1231.
[2] D. B. T. Cox, R. J. Platt, F. Zhang, *Nat Med* 2015, 21, 121-131.
[3] aH. Mizutani, S. Tada-Oikawa, Y. Hiraku, M. Kojima, S. Kawanishi, Life Sci 2005, 76, 1439-1453; bZ. Chen, N. Li, L. Chen, J. Lee, J. J. Gassensmith, *Small* 2016. 12, 4563-4571.
[4] aF. H. Meng, R. Cheng, C. Deng, Z. Y. Zhong, *Mater Today* 2012, 15, 436-442; bJ. Ma, Y. Zhang, K. Tang, H. Zhang, X. Yin, Y. Li, P. Xu, Y. Sun, R. Ma, T. Ji, J. Chen, S. Zhang, T. Zhang, S. Luo, Y. Jin, X. Luo, C. Li, H. Gong, Z. Long, J. Lu, Z. Hu, X. Cao, N. Wang, X. Yang, B. Huang, *Cell Research* 2016, 26, 713-727.
[5] aM. Sun, B. Sun, Y. Liu, Q. D. Shen, S. Jiang, Sci Rep 2016, 6, 22368; bA. Bestembayeva, A. Kramer, A. A. Labokha, D. Osmanovic, I. Liashkovich, E. V. Orlova, I. J. Ford, G. Charras, A. Fassati, B. W. Hoogenboom, *Nat Nanotechnol* 2015, 10, 60-64.
[6] aO. Cohen, R. Granek, Nano letters 2014, 14, 2515-2521; bY. Fan, C. Li, F. Li, D. Chen, Biomaterials 2016, 85, 30-39; cT. Schroder, N. Niemeier, S. Afonin, A. S. Ulrich, H. F. Krug, S. Brase, *J Med Chem* 2008, 51, 376-379; d W. Y. Li, Y. X. Wang, L. N. Chen, Z. X. Huang, Q. L. Hu, J. Ji, Chem. Commun. 2012, 48, 10126-10128; eS. Y. Lin, N. T. Chen, S. P. Sum, L. W. Lo, C. S. Yang, *Chem Commun* 2008, 4762-4764.
[7] L. M. Pan, Q. J. He, J. N. Liu, Y. Chen, M. Ma, L. L. Zhang, J. L. Shi, *J Am Chem Soc* 2012, 134, 5722-5725.
[8] aA. Lange, R. E. Mills, C. J. Lange, M. Stewart, S. E. Devine, A. H. Corbett, *J Biol Chem* 2007, 282, 5101-5105; bP. S. Tang, S. Sathiamoorthy, L. C. Lustig, R. Ponzielli, I. Inamoto, L. Z. Penn, J. A. Shin, W. C. W. Chan, *Small* 2014, 10, 4182-4192.
[9] aM. Tanimoto, H. Kamiya, N. Minakawa, A. Matsuda, H. Harashima, Bioconjug Chem 2003, 14, 1197-1202; bS. N. Tammam, H. M. E. Azzazy, A. Lamprecht, *J Control Release* 2017, 253, 30-36.
[10] aZ. P. Qin, J. C. Bischof, *Chem Soc Rev* 2012, 41, 1191-1217; bD. Lapotko, *Opt Express* 2009, 17, 2538-2556.
[11] E. Y. Lukianova-Hleb, Y. S. Kim, I. Belatsarkouski, A. M. Gillenwater, B. E. O'Neill, D. O. Lapotko, *Nat Nanotechnol* 2016, 11, 525-532.
[12] S. J. Leung, X. M. Kachur, M. C. Bobnick, M. Romanowski, *Adv Funct Mater* 2011, 21, 1113-1121.
[13] K. E. Knockenhauer, T. U. Schwartz, Cell 2016, 164, 1162-1171.
[14] B. Naim, V. Brumfeld, R. Kapon, V. Kiss, R. Nevo, Z. Reich, *J Biol Chem* 2007, 282, 3881-3888.
[15] X. Zhang, J. P. Edwards, D. M. Mosser, *Methods Mol Biol* 2009, 531, 123-143.
[16] aD. Lietha, Structure 2016, 24, 1223-1225; bA. C. Cardoso, A. H. M. Pereira, A. L. B. Ambrosio, S. R. Consonni, R. R. de Oliveira, M. C. Bajgelman, S. M. G. Dias, K. G. Franchini, Structure 2016, 24, 1301-1310.
[17] A. Garcia, J. F. Rodriguez Matas, M. T. Raimondi, *Integr Biol (Camb)* 2016, 8, 1011-1021.
[18] L. Li, X. Li, Y. Z. Wu, L. J. Song, X. Yang, T. He, N. Wang, S. L. X. Yang, Y. Zeng, Q. J. Wu, Z. Y. Qian, Y. Q. Wei, C. Y. Gong, *Theranostics* 2017, 7, 1633-1649.
[19] J. P. Carralot, T. K. Kim, B. Lenseigne, A. S. Boese, P. Sommer, A. Genovesio, P. Brodin, *J Biomol Screen* 2009, 14, 151-160.

WILEY

Entry for the Table of Contents

COMMUNICATION



*Xiuying Li [a], Peiyuan Kang [a], Zhuo Chen [b], Sneha Lal [c], Li Zhang [c], Jeremiah J. Gassensmith [b,d], and Zhenpeng Qin * [a,d,e]*

Page No.1 – Page No.4

Rock the nucleus: significantly enhanced nuclear membrane permeability and gene transfection by plasmonic nanobubble induced nanomechanical transduction

Efficient delivery to the cell nucleus remains a significant challenge for many biomolecules. Herein, we reported that nanomechanical force from plasmonic nanobubbles increases permeability of nuclear membrane and facilitates nuclear uptake of macromolecules that would not otherwise enter the nucleus. The nanomechanical transduction in combination with electroporation leads to significantly higher gene transfection and protein expression, compared to standard electroporation treatment.

WILEY-VCH