

A Synthetic Erectile Optogenetic Stimulator Enabling Blue-Light-Inducible Penile Erection**

Taeuk Kim, Marc Folcher, Marie Doaud-El Baba, and Martin Fussenegger*

Abstract: Precise spatiotemporal control of physiological processes by optogenetic devices inspired by synthetic biology may provide novel treatment opportunities for gene- and cell-based therapies. An erectile optogenetic stimulator (EROS), a synthetic designer guanylate cyclase producing a blue-light-inducible surge of the second messenger cyclic guanosine monophosphate (cGMP) in mammalian cells, enabled blue-light-dependent penile erection associated with occasional ejaculation after illumination of EROS-transfected corpus cavernosum in male rats. Photostimulated short-circuiting of complex psychological, neural, vascular, and endocrine factors to stimulate penile erection in the absence of sexual arousal may foster novel advances in the treatment of erectile dysfunction.

Precise spatiotemporal neuromodulation by light has significantly advanced our understanding of neuronal activities managing memory,^[1] learning,^[2] and olfactory processing,^[3] as well as our understanding of neuropathologies associated with fear,^[4] depression,^[5] and addiction.^[6] Optogenetics has also become a key technology that enables researchers in the emerging science of synthetic biology to rationally and predictably engineer mammalian cells for the precise stimulation of cellular behavior and metabolic activities in animal models. For example, optogenetic pacemakers were successfully used to modulate heart-beat frequency by programming a synchronized contraction of cardiomyocytes.^[7] Type-2 diabetes can be remedied by stimulating a subcutaneous designer cell implant to release insulin in response to

illumination in treated animals.^[8] Moreover, wireless-powered optogenetic designer cell implants stimulated by brain-wave-derived electroencephalography-based electronics capturing human mental states have enabled mind-controlled transgene expression in mice.^[9]

Erectile dysfunction is a multifactorial disorder affecting more than 150 million males worldwide^[10] and is associated with aging and a range of medical conditions, including hypertension,^[11] hypercholesterolemia,^[12] diabetes mellitus,^[13] cardiovascular disease,^[14] and depression.^[15] Penile erection is triggered by the central nervous system in response to tactile, olfactory, visual, auditory, and mental stimuli^[16] and requires adequate hormone supplies^[17] (e.g., testosterone from the testes and luteinizing hormone from the pituitary gland) to integrate a series of physiological events that culminate in the release of nitric oxide by the nerves and endothelial cells enmeshing and surrounding the *corpora cavernosa*.^[18] Nitric oxide activates soluble guanylyl cyclase (sGC), which produces a surge of the second messenger 3',5'-cyclic guanosine monophosphate (cGMP) to then trigger a decrease in intracellular calcium by the closing of voltage-gated calcium channels. This cascade process ultimately results in the relaxation of the *corpus cavernosum* smooth muscle, blood influx, and erection of the penis (Figure 1a).

The advent of oral cGMP-specific phosphodiesterase inhibitors (e.g., Viagra, Levitra, and Cialis), which counteract and delay the degradation of cGMP and thus reinforce and prolong an existing penile erection, has significantly improved the treatment success of erectile dysfunction.^[19] However, patients with erectile dysfunction after a radical prostatectomy are not responsive to phosphodiesterase inhibitors;^[20] the use of phosphodiesterase inhibitors is also contraindicated for patients on nitrate medication^[21] and inadvisable for those with hypotension or severely decreased liver function as well as those who have suffered a stroke, heart attack, or severe heart failure.^[22] Therefore, a remote-controlled synthetic device triggering the de novo synthesis and accumulation of cGMP in the *corpora cavernosa*, thereby decoupling penile erection from natural stimuli and neural-system control, would enable an erection on demand and would represent a major advancement in erectile-dysfunction therapy.

Capitalizing on synthetic-biology-inspired engineering principles, we have designed an erectile optogenetic stimulator (EROS) consisting of a modified and human-codon-optimized *Beggiatoa* sp. PS BlaC-derived guanylate cyclase^[23] that enables blue-light-responsive remote control of the intracellular cGMP pool in human cells. When rewiring the intracellular cGMP pool to a synthetic cGMP-specific biosensor GTA (pTK56; P_{hCMV}-GTA-pA), which acts as a transcription factor to induce transgene expression driven by

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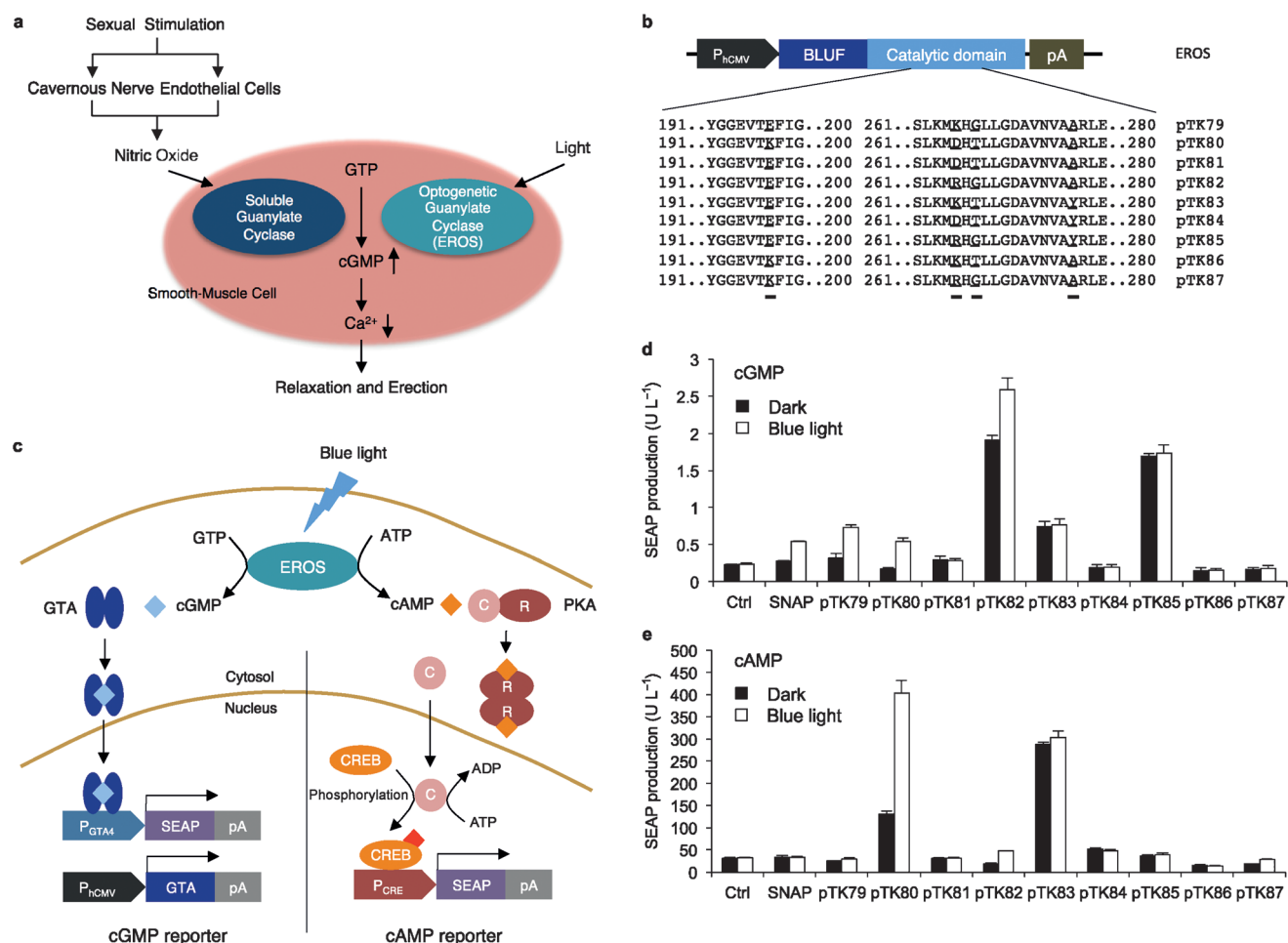


Figure 1. Design and validation of a blue-light-responsive synthetic erectile optogenetic stimulator in human cells. **a**) Pathways controlling penile erection. Sexual arousal by tactile, olfactory, visual, auditory, and mental stimuli programs nerve cells and endothelial cells enmeshing and surrounding the *corpus cavernosum* smooth-muscle cells to produce and release the intercellular signaling molecule nitric oxide (NO). NO activates the soluble guanylyl cyclase (sGC) inside the smooth-muscle cells of the *corpus cavernosum*, which produces a surge of the second messenger 3',5'-cyclic guanosine monophosphate (cGMP), triggers a decrease in intracellular calcium by the closing of voltage-gated calcium channels, and ultimately results in relaxation of the *corpus cavernosum* and penile erection. The synthetic erectile optogenetic stimulator (EROS) enables the direct blue-light-inducible conversion of GTP into cGMP, thereby decoupling penile erection from the physiological signaling pathways and triggering immediate photoinducible erection. **b**) Engineering of EROS variants. EROS consists of a blue-light sensor domain BLUF (blue-light-using FAD) that activates an adjacent catalytic nucleotide cyclase domain. Combinatorial site-directed mutagenesis of the EROS nucleotide cyclase domain (E197, K265, G267, and A277) modifies the blue-light sensitivity and alters the substrate specificity (GTP vs. ATP). The mutated target residues are underlined. **c**) Schematic illustration of mammalian cell-based assays scoring the blue-light-triggered guanylate versus adenylate cyclase activity of EROS variants. Guanylate cyclase activity is quantified by using a synthetic cGMP-specific biosensor that binds to a chimeric promoter (P_{GTA4}) upon interaction with cGMP, thereby acting as a transcription factor inducing the reporter gene SEAP. Adenylate cyclase activity is quantified by using a P_{CRE}-driven SEAP expression vector that taps into the cellular cAMP-signaling cascade (cAMP activates protein kinase A (PKA), and the catalytic subunit of PKA (C) is transferred to the nucleus, where it phosphorylates the cAMP response element-binding protein (CREB1), and CREB1-mediated activation of P_{CRE} promoters occurs. Both reporter-gene assays can also be used as optogenetic transcription-control devices to enable blue-light-responsive target gene expression in mammalian cells. **d,e**) Performance analysis of EROS variants: 1×10^4 HEK-293T cells were either cotransfected with the d) cGMP (pTK56, P_{hCMV}-GTA-pA; pTK551, P_{GTA4}-SEAP-pA) or e) cAMP (pCK53, P_{CRE}-SEAP-pA) assay components and a constitutive expression vector encoding a specific EROS variant (**b**; see Table S1 in the Supporting Information). Cells (co-)transfected with pTK56/pTK551 (**d**) and pCK53 (**e**) served as negative controls (Ctrl). Transfected cell populations were either treated with the sGC-inducing NO-releasing compound S-nitroso-N-acetylpenicillamine (SNAP, 2 h), kept in the dark, or illuminated for 12 h with blue light (470 nm, 5 s ON/15 s OFF) before SEAP levels were profiled in the culture.

chimeric GTA-specific promoters (pTK551; P_{GTA4}-SEAP-pA), blue-light-dependent intracellular cGMP levels can be scored by reporter-gene expression (Figure 1b). Likewise, undesired cAMP production by EROS derivatives can be profiled by tapping into the endogenous cAMP-triggered protein kinase A (PKA)/cAMP-responsive binding protein 1

(CREB1)-mediated signaling cascade by the use of P_{CRE}-driven reporter-gene expression constructs (pCK53; P_{CRE}-SEAP-pA; Figure 1b). Combinatorial site-directed mutagenesis targeting four residues (E197, K265, G267, and A277) within the EROS cyclase domain resulted in a variety of EROS derivatives (Figure 1c), whose blue-light responsive-

ness and cGMP-production specificity were profiled by differential cGMP-/cAMP-responsive reporter-gene expression in human cells (Figure 1d,e). Whereas most EROS variants showed an undesirable blend of high basal expression (pTK82, pTK83, pTK85), elevated cAMP-production capacity (pTK80, pTK82, pTK83), and insignificant blue-light responsiveness (pTK81, pTK83, pTK84, pTK85, pTK86, pTK87), the pTK79-encoded EROS variant (EROS) exhibited a unique combination of blue-light responsiveness, low leakiness, and exclusive cGMP production in the absence of cAMP synthesis (Figure 1d,e). Furthermore, blue-light-triggered activation of EROS resulted in illumination-time-dependent increases in SEAP production (Figure 2a) as well as intracellular cGMP levels (Figure 2b). The production of human placental secreted alkaline phosphatase (SEAP; Figure 2c) and cGMP (Figure 2d) was reversible over repeated illumination cycles. Furthermore, because the photoinducible EROS-triggered intracellular cGMP levels compared favorably with those reached with the sGC-inducing NO-releasing compound *S*-nitroso-*N*-acetylpenicillamine (SNAP), EROS (pTK79) was chosen for follow-up in vivo validation (Figure 1d).

Owing to the simple and compact one-gene-based design of constitutive EROS-encoding expression, vector transfection cocktails can be directly administered to reach resident cells of target tissues and program an intracellular cGMP surge by blue-light illumination. The transfection of naked DNA is a validated process with demonstrated results in muscle,^[24] skin,^[25] and liver.^[26] Therefore, we transfected the *corpora cavernosa* of male rats with the constitutive EROS-encoding expression vector (pTK79, P_{hCMV} -EROS-pA_{BGH}; 2 and 4 μ g per dose) and used pDSRed-Express-N1 as a negative control (Figure 3a). Twenty-four hours after treatment, the animals were placed in a metabolic cage that was retroilluminated on the bottom side with blue light by the use of a portable Philips goLITE BLU device licensed for the treatment of seasonal affective disorder (Figure 3b,c). Whereas none of the non-illuminated/illuminated EROS-devoid control animals and none of the non-illuminated EROS-treated animals exhibited any evidence of penile reflexes (Figure 3d,e), the EROS-treated rats showed different levels of erectile response within (55 ± 22) s upon exposure to blue light, ranging from tumescence of the tip of the glans (duration, (11 ± 3) s; Figure 3f) to erection (duration, (37 ± 2) s; Figure 3g), and

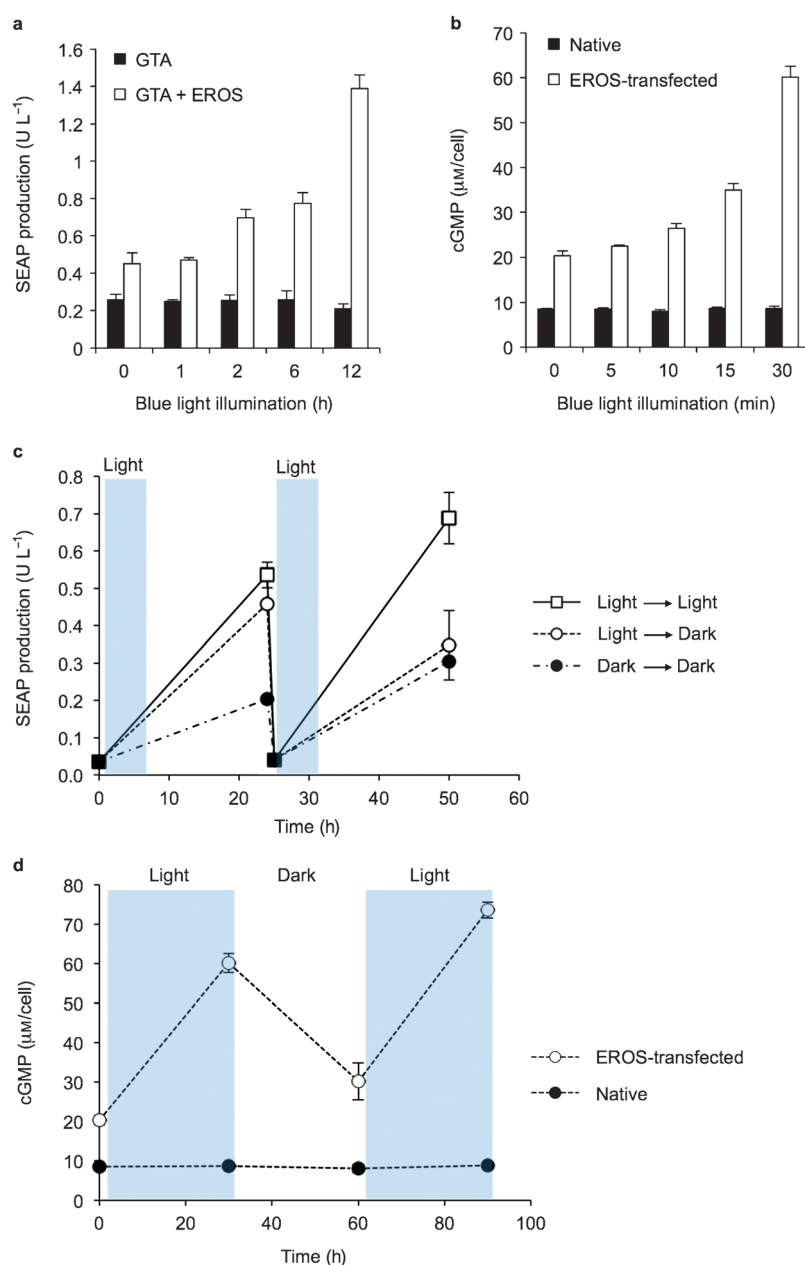


Figure 2. Characterization of blue-light-triggered EROS-mediated transgene expression. a) Illumination-time-dependent transgene expression. 1×10^4 HEK-293T cells were cotransfected with the constitutive EROS expression vector pTK79 (P_{hCMV} -EROS-pA) and the cGMP sensor components pTK56 (P_{hCMV} -GTA-pA) and pTK551 (P_{GTA4} -SEAP-pA) and illuminated (470 nm) for different periods of time (0–12 h). SEAP production was quantified in the culture supernatant after 24 h. b) Illumination-time-dependent intracellular cGMP levels. 1×10^4 HEK-293T cells were transfected with the constitutive EROS expression vector pTK79 (P_{hCMV} -EROS-pA) and illuminated (470 nm) for different periods of time (0–30 min) before intracellular cGMP levels were quantified. Mock-transfected HEK-293T served as a negative control. c) Reversibility of illumination-triggered EROS-mediated transgene expression. 2.5×10^4 HEK-293T cells were cotransfected with the constitutive EROS expression vector pTK79 (P_{hCMV} -EROS-pA) and the cGMP sensor components pTK56 (P_{hCMV} -GTA-pA) and pTK551 (P_{GTA4} -SEAP-pA), and SEAP expression kinetics were profiled for 48 h while alternating the blue-light illumination status between ON (light 6 h, 5 s ON/15 s OFF) and OFF (dark). d) Reversibility of illumination-triggered EROS-mediated intracellular cGMP levels. 1×10^4 HEK-293T cells were transfected with the constitutive EROS expression vector pTK79 (P_{hCMV} -EROS-pA), and intracellular cGMP levels were profiled for 1.5 h while alternating the blue-light illumination status between ON (light 30 min, 5 s ON/15 s OFF) and OFF (dark). Mock-transfected HEK-293T served as a negative control. The data are the means $\pm$ SD of triplicate experiments ($n=6$).

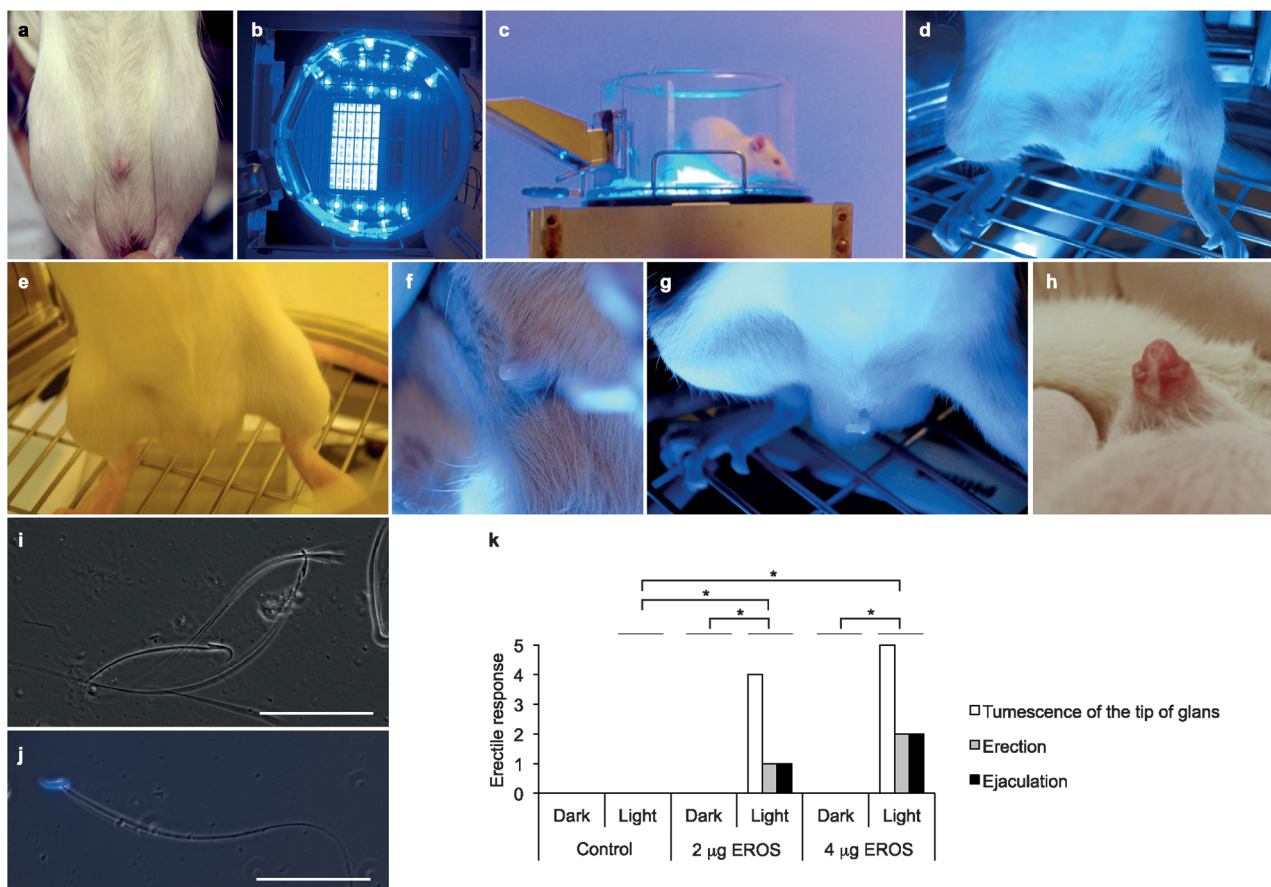


Figure 3. Blue-light-triggered penile erection. a) The *corpora cavernosa* of male rats were either transfected with the constitutive EROS-encoding vector (pTK79) or a negative control vector (pDSRed-Express-N1). b,c) Twenty-four hours after treatment, the animals were placed in a metabolic cage that was retroilluminated from the bottom with blue light (470 nm) emitted by a Philips goLITE BLU portable device licensed for the treatment of seasonal affective disorder (b, top view; c, side view). d–h,k) Blue-light-triggered erectile responses. Whereas none of the animals in the negative or non-illuminated control groups showed any evidence of erectile responses (d, pDSRed-Express-N1-transfected, illuminated; e, EROS-transfected, non-illuminated), the EROS-treated rats showed different levels of photostimulated erectile response within (55 ± 22) s upon exposure to blue light, ranging from f) tumescence of the tip of the glans (duration, (11 ± 3) s) to g) erection (duration, (37 ± 2) s) and h) ejaculation. i) Phase-contrast and j) fluorescence micrographs confirming the presence of sperm in the ejaculates (scale bar: 50 μ m). k) Quantitative analysis of photoinduced erectile responses of male rats. Data are absolute values; statistics were performed by the use of the one-tailed Fisher exact test; $n = 13$ rats; $*P < 0.05$.

ejaculation (Figure 3h). For further quantification of erectile responses, we performed isometric force measurements with isolated *corpus cavernosum* of EROS-treated rats. These experiments confirmed that *corpus cavernosum* relaxation was indeed triggered by blue light, that the relaxation intensity was independent of the illumination time, that EROS-mediated relaxation could be repeatedly induced by illumination, and that the administration of the phosphodiesterase 5 inhibitor sildenafil (Viagra) significantly increased blue-light-induced EROS-triggered relaxation (Figure 4).

Because the current phosphodiesterase-inhibitor-based blockbuster drugs, such as Viagra, Levitra, and Cialis, do not trigger *de novo* erection but only sustain it by delaying the degradation of cGMP, gene-based therapies have come into the limelight as treatment opportunities for erectile dysfunction. Because of its straightforward accessibility and reduced blood flow during the flaccid state, which limits undesired systemic delivery, the penis is an ideal target for the direct delivery of naked DNA encoding erection-modulating fac-

tors. Nonlimiting examples of successful proof-of-concept studies have used the transfection of DNA encoding for nitric oxide synthases,^[27] neurotropic factors,^[28] and calcium-sensitive potassium channels; calcium-sensitive potassium channels promoting smooth-muscle relaxation, such as hMaxi-K,^[29] are currently being tested in clinical trials for the treatment of erectile dysfunction.^[30] Collectively, the current treatment strategies focus either on restoring erection-promoting pathways or maintaining an established erection but fail to provide a trigger-inducible erection on demand. EROS decouples penile erection from physiological control, bypasses the causes for erectile dysfunction, and provides trigger-inducible erection on demand by simple illumination with a portable commercial light-therapy device.

Keywords: erectile dysfunction · gene expression · gene technology · optogenetic therapy · synthetic biology

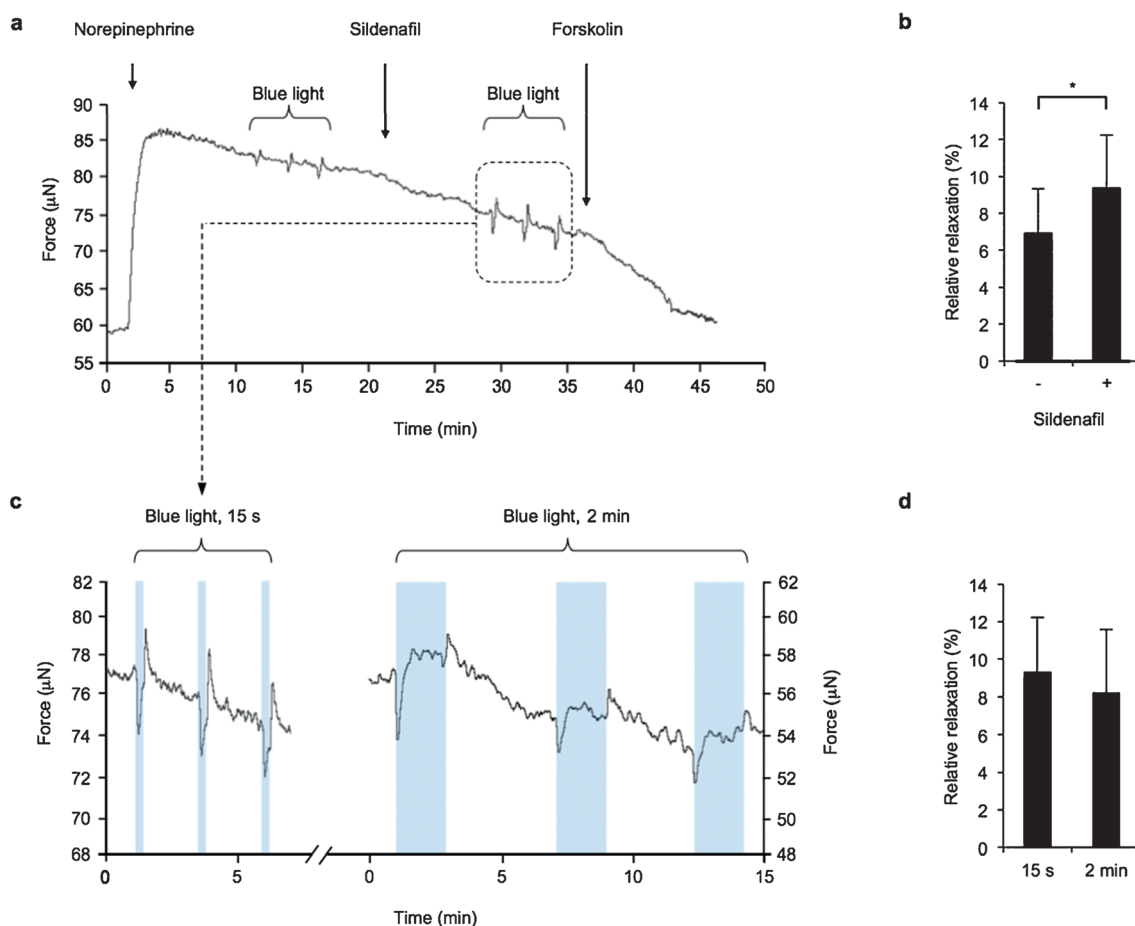


Figure 4. Isometric force measurement of *corpus cavernosum* relaxation by blue-light illumination. a) Isometric force measurement of the *corpus cavernosum* of EROS-treated rats exposed to norepinephrine to induce full contraction, sequentially illuminated three times for 15 s with blue light, treated with the phosphodiesterase 5 inhibitor sildenafil (Viagra, 0.1 μM), and again sequentially illuminated three times for 15 s with blue light. b) Blue-light-responsive *corpus cavernosum* relaxation in the presence (+) and absence (–) of sildenafil (0.1 μM). c) EROS-mediated relaxation in the presence of sildenafil with illumination times of 15 s (expansion of a region of the graph in a) as compared to the results of isogenic relaxation experiments with blue-light-illumination times of 2 min. d) *Corpus cavernosum* relaxation in the presence of sildenafil in response to blue-light-illumination periods of 15 s and 2 min. The data show representative relaxation trajectories or are the means $\pm$ SD; statistics were performed by the use of a one-tailed t test; $n=6$; $*P<0.05$.

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- [1] S. Ramirez, X. Liu, P. A. Lin, J. Suh, M. Pignatelli, R. L. Redondo, T. J. Ryan, S. Tonegawa, *Science* **2013**, *341*, 387–391.
- [2] C. Schroll, T. Riemensperger, D. Bucher, J. Ehmer, T. Voller, K. Erbguth, B. Gerber, T. Hendel, G. Nagel, E. Buchner, A. Fiala, *Curr. Biol.* **2006**, *16*, 1741–1747.
- [3] D. Bellmann, A. Richardt, R. Freyberger, N. Nuwal, M. Schwarzel, A. Fiala, K. F. Stortkuhl, *Front. Behav. Neurosci.* **2010**, *4*, 27.
- [4] a) X. Liu, S. Ramirez, P. T. Pang, C. B. Puryear, A. Govindarajan, K. Deisseroth, S. Tonegawa, *Nature* **2012**, *484*, 381–385; b) J. P. Johansen, H. Hamanaka, M. H. Monfils, R. Behnia, K. Deisseroth, H. T. Blair, J. E. LeDoux, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 12692–12697.
- [5] a) H. E. Covington III, M. K. Lobo, I. Maze, V. Vialou, J. M. Hyman, S. Zaman, Q. LaPlant, E. Mouzon, S. Ghose, C. A. Tamminga, R. L. Neve, K. Deisseroth, E. J. Nestler, *J. Neurosci.* **2010**, *30*, 16082–16090; b) R. D. Airan, L. A. Meltzer, M. Roy, Y. Gong, H. Chen, K. Deisseroth, *Science* **2007**, *317*, 819–823.
- [6] J. P. Britt, A. Bonci, *Curr. Opin. Neurobiol.* **2013**, *23*, 539–545.
- [7] T. Bruegmann, D. Malan, M. Hesse, T. Beiert, C. J. Fuegemann, B. K. Fleischmann, P. Sasse, *Nat. Methods* **2010**, *7*, 897–900.
- [8] H. Ye, M. Daoud-El Baba, R. W. Peng, M. Fussenegger, *Science* **2011**, *332*, 1565–1568.
- [9] M. Folcher, S. Oesterle, K. Zwicky, T. Thekkottil, J. Heymoz, M. Hohmann, M. Christen, M. Daoud-El Baba, P. Buchmann, M. Fussenegger, *Nat. Commun.* **2014**, *5*, 5392.
- [10] J. B. McKinlay, *Int. J. Impotence Res.* **2000**, *12* Suppl 4, S6–S11.
- [11] K. P. Nunes, H. Labazi, R. C. Webb, *Curr. Opin. Nephrol. Hypertens.* **2012**, *21*, 163–170.
- [12] R. A. Fraga-Silva, F. P. Costa-Fraga, Y. Faye, M. Sturny, R. A. Santos, R. F. da Silva, N. Stergiopulos, *J. Sex. Med.* **2014**, *11*, 1173–1181.
- [13] R. N. Jangir, G. C. Jain, *Curr. Diabetes Rev.* **2014**, *10*, 147–157.
- [14] Y. Pauker-Sharon, Y. Arbel, A. Finkelstein, A. Halkin, I. Herz, S. Banai, D. Justo, *Urology* **2013**, *82*, 377–380.
- [15] S. S. Huang, C. H. Lin, C. H. Chan, W. Lohel, T. H. Lan, *Psychiatry Res.* **2013**, *210*, 601–606.
- [16] K. E. Andersson, *Pharmacol. Rev.* **2011**, *63*, 811–859.
- [17] a) J. Buvat, G. Bou Jaoude, *World J. Urol.* **2006**, *24*, 657–667; b) S. G. Stoleru, A. Ennaji, A. Cournot, A. Spira, *Psychoneuroendocrinology* **1993**, *18*, 205–218.

- [18] N. Toda, K. Ayajiki, T. Okamura, *Pharmacol. Ther.* **2005**, *106*, 233–266.
- [19] A. L. Burnett, *Am. J. Cardiol.* **2005**, *96*, 29M–31M.
- [20] E. K. Hong, H. Lepor, A. R. McCullough, *Int. J. Impotence Res.* **1999**, *11 Suppl 1*, S15–22.
- [21] B. G. Schwartz, R. A. Kloner, *Circulation* **2011**, *123*, e609–611.
- [22] M. D. Cheitlin, A. M. Hutter, Jr., R. G. Brindis, P. Ganz, S. Kaul, R. O. Russell, Jr., R. M. Zusman, *J. Am. Coll. Cardiol.* **1999**, *33*, 273–282.
- [23] M. H. Ryu, O. V. Moskvina, J. Siltberg-Liberles, M. Gomelsky, *J. Biol. Chem.* **2010**, *285*, 41501–41508.
- [24] J. T. Vilquin, P. F. Kennel, M. Paturneau-Jouas, P. Chapdelaine, N. Boissel, P. Delaere, J. P. Tremblay, D. Scherman, M. Y. Fiszman, K. Schwartz, *Gene Ther.* **2001**, *8*, 1097–1107.
- [25] J. J. Drabick, J. Glasspool-Malone, A. King, R. W. Malone, *Mol. Ther.* **2001**, *3*, 249–255.
- [26] H. Maruyama, N. Higuchi, Y. Nishikawa, S. Kameda, N. Iino, J. J. Kazama, N. Takahashi, M. Sugawa, H. Hanawa, N. Tada, J. Miyazaki, F. Gejyo, *J. Gene Med.* **2002**, *4*, 333–341.
- [27] a) H. C. Champion, T. J. Bivalacqua, A. L. Hyman, L. J. Ignarro, W. J. Hellstrom, P. J. Kadowitz, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 11648–11652; b) T. R. Magee, M. Ferrini, H. J. Garban, D. Vernet, K. Mitani, J. Rajfer, N. F. Gonzalez-Cadavid, *Biol. Reprod.* **2002**, *67*, 1033–1041; c) M. B. Chancellor, S. Tirney, C. E. Mattes, E. Tzeng, L. A. Birder, A. J. Kanai, W. C. de Groat, J. Huard, N. Yoshimura, *BJU Int.* **2003**, *91*, 691–696.
- [28] N. E. Bennett, J. H. Kim, D. P. Wolfe, K. Sasaki, N. Yoshimura, W. F. Goins, S. Huang, J. B. Nelson, W. C. de Groat, J. C. Glorioso, M. B. Chancellor, *J. Urol.* **2005**, *173*, 1820–1824.
- [29] G. J. Christ, N. Day, C. Santizo, Y. Sato, W. Zhao, T. Sclafani, R. Bakal, M. Salman, K. Davies, A. Melman, *Am. J. Physiol. Heart Circ. Physiol.* **2004**, *287*, H1544–1553.
- [30] A. Melman, N. Bar-Chama, A. McCullough, K. Davies, G. Christ, *Hum. Gene Ther.* **2006**, *17*, 1165–1176.

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