They did mind control on worms

Huang *et al.* Remote control of ion channels and neurons through magnetic-field heating of nanoparticles. *Nature Nanotech* 5, 602–606 (2010).

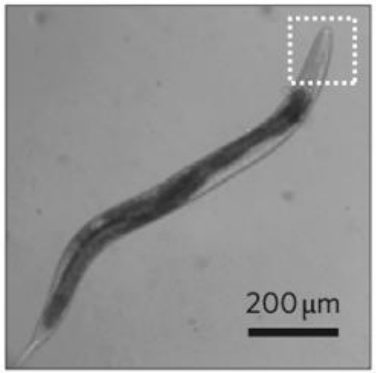
– Amparo Figueroa, Rares Fota, Andrew Gao, Carson Gause, Aishi Guha

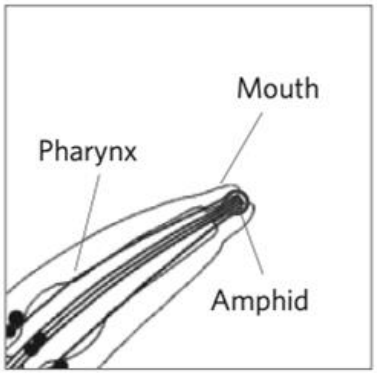
LETTERS
PUBLISHED ONLINE: 27 JUNE 2010 | DOI: 10.1038/NNANO.2010.125
nature nanotechnology

Remote control of ion channels and neurons through magnetic-field heating of nanoparticles

Heng Huang[1], Savas Delikanli[1], Hao Zeng[1], Denise M. Ferkey[2] and Arnd Pralle[1]*

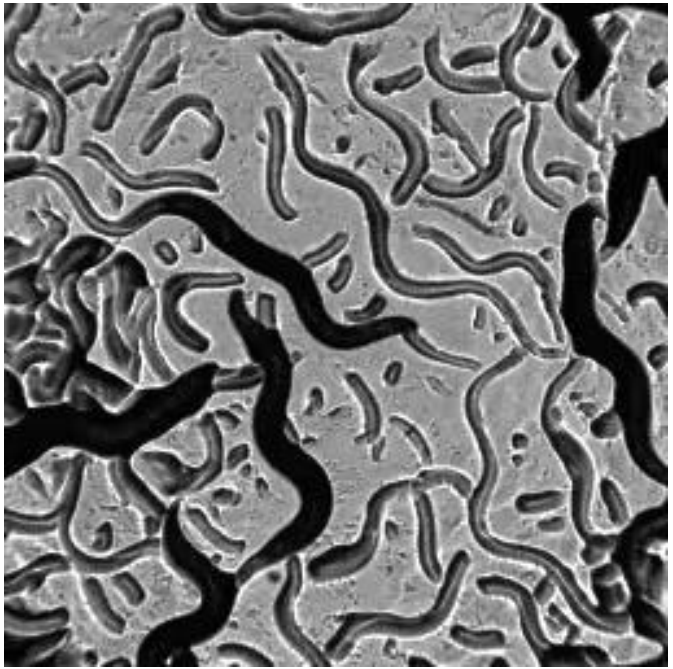
Recently, optical stimulation[1–3] has begun to unravel the neuronal processing that controls certain animal behaviours[4,5]. However, optical approaches are limited by the inability of visible light to penetrate deep into tissues. Here, we show an approach based on radio-frequency magnetic-field heating of nanoparticles to remotely activate temperature-sensitive cation channels in cells. Superparamagnetic ferrite nanoparticles were targeted to specific proteins on the plasma membrane of cells expressing TRPV1, and heated by a radio-frequency magnetic field. Using fluorophores as molecular thermometers, we show that the induced temperature increase is highly localized. Thermal activation of the channels triggers action potentials in cultured neurons without observable toxic effects. This approach can be adapted to stimulate other cell types and, moreover, may be used to remotely manipulate other cellular machinery for novel therapeutics.





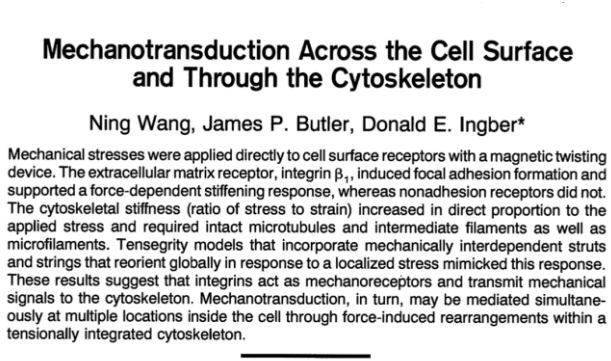
Would you still love me if I was a worm?

Would you still love me if I was a worm?



A field with a long history

Utilizing electromagnetic waves to ***simulate*** and ***stimulate*** neuron firing to control animal behavior has a long history.

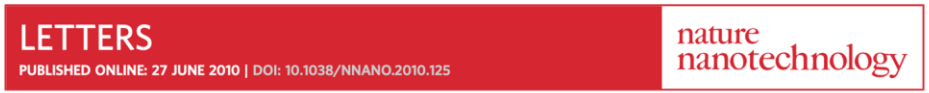


Mechanotransduction Across the Cell Surface and Through the Cytoskeleton

Ning Wang, James P. Butler, Donald E. Ingber*

Mechanical stresses were applied directly to cell surface receptors with a magnetic twisting device. The extracellular matrix receptor, integrin β_1, induced focal adhesion formation and supported a force-dependent stiffening response, whereas nonadhesion receptors did not. The cytoskeletal stiffness (ratio of stress to strain) increased in direct proportion to the applied stress and required intact microtubules and intermediate filaments as well as microfilaments. Tensegrity models that incorporate mechanically interdependent struts and strings that reorient globally in response to a localized stress mimicked this response. These results suggest that integrins act as mechanoreceptors and transmit mechanical signals to the cytoskeleton. Mechanotransduction, in turn, may be mediated simultaneously at multiple locations inside the cell through force-induced rearrangements within a tensionally integrated cytoskeleton.

1993



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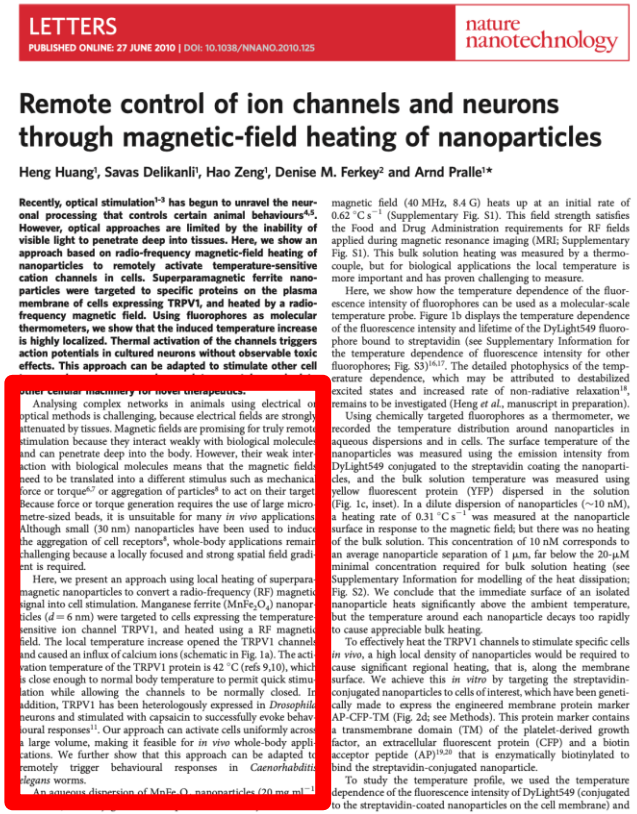
2010

An introduction to previous failure

The ***introduction*** of the 2010 Nature paper describes previous failed attempts to control neurons with electromagnetic waves.

Previous Work section



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Analysing complex networks in animals using electrical or optical methods is challenging, because electrical fields are strongly attenuated by tissues. Magnetic fields are promising for truly remote stimulation because they interact weakly with biological molecules and can penetrate deep into the body. However, their weak interaction with biological molecules means that the magnetic fields need to be translated into a different stimulus such as mechanical force or torque[6,7] or aggregation of particles[8] to act on their target. Because force or torque generation requires the use of large micro- to metre-sized beads, it is unsuitable for many *in vivo* applications. Although small (30 nm) nanoparticles have been used to induce the aggregation of cell receptors[9], whole-body applications remain challenging because a locally focused and strong spatial field gradient is required.

Here, we present an approach using local heating of superparamagnetic nanoparticles to convert a radio-frequency (RF) magnetic signal into cell stimulation. Manganese ferrite ($MnFe_2O_4$) nanoparticles ($d = 6$ nm) were targeted to cells expressing the temperature-sensitive ion channel TRPV1, and heated using a RF magnetic field. The local temperature increase opened the TRPV1 channels and caused an influx of calcium ions (schematic in Fig. 1a). The activation temperature of the TRPV1 protein is 42 °C (refs 9,10), which is close enough to normal body temperature to permit quick stimulation while allowing the channels to be normally closed. In addition, TRPV1 has been heterologously expressed in *Drosophila* neurons and stimulated with capsaicin to successfully evoke behavioural responses[11]. Our approach can activate cells uniformly across a large volume, making it feasible for *in vivo* whole-body applications. We further show that this approach can be adapted to remotely trigger behavioural responses in *Caenorhabditis elegans* worms.

An aqueous dispersion of $MnFe_2O_4$ nanoparticles (20 mg ml^{-1}

magnetic field (40 MHz, 8.4 G) heats up at an initial rate of 0.62 °C s^{-1} (Supplementary Fig. S1). This field strength satisfies the Food and Drug Administration requirements for RF fields applied during magnetic resonance imaging (MRI; Supplementary Fig. S1). This bulk solution heating was measured by a thermocouple, but for biological applications the local temperature is more important and has proven challenging to measure.

Here, we show how the temperature dependence of the fluorescence intensity of fluorophores can be used as a molecular-scale temperature probe. Figure 1b displays the temperature dependence of the fluorescence intensity and lifetime of the DyLight549 fluorophore bound to streptavidin (see Supplementary Information for the temperature dependence of fluorescence intensity for other fluorophores; Fig. S3)[16,17]. The detailed photophysics of the temperature dependence, which may be attributed to destabilized excited states and increased rate of non-radiative relaxation[18], remains to be investigated (Heng *et al.*, manuscript in preparation).

Using chemically targeted fluorophores as a thermometer, we recorded the temperature distribution around nanoparticles in aqueous dispersions and in cells. The surface temperature of the nanoparticles was measured using the emission intensity from DyLight549 conjugated to the streptavidin coating the nanoparticles, and the bulk solution temperature was measured using yellow fluorescent protein (YFP) dispersed in the solution (Fig. 1c, inset). In a dilute dispersion of nanoparticles (~10 nM), a heating rate of 0.31 °C s^{-1} was measured at the nanoparticle surface in response to the magnetic field; but there was no heating of the bulk solution. This concentration of 10 nM corresponds to an average nanoparticle separation of 1 μm, far below the 20-μM minimal concentration required for bulk solution heating (see Supplementary Information for modelling of the heat dissipation; Fig. S2). We conclude that the immediate surface of an isolated nanoparticle heats significantly above the ambient temperature, but the temperature around each nanoparticle decays too rapidly to cause appreciable bulk heating.

To effectively heat the TRPV1 channels to stimulate specific cells *in vivo*, a high local density of nanoparticles would be required to cause significant regional heating, that is, along the membrane surface. We achieve this *in vitro* by targeting the streptavidin-conjugated nanoparticles to cells of interest, which have been genetically made to express the engineered membrane protein marker AP-CFP-TM (Fig. 2d; see Methods). This protein marker contains a transmembrane domain (TM) of the platelet-derived growth factor, an extracellular fluorescent protein (CFP) and a biotin acceptor peptide (AP)[19,20] that is enzymatically biotinylated to bind the streptavidin-conjugated nanoparticle.

To study the temperature profile, we used the temperature dependence of the fluorescence intensity of DyLight549 (conjugated to the streptavidin-coated nanoparticles on the cell membrane) and

The physics Behind Failure

For each step, the paper documents ***the physics*** of why previous attempts failed.

Example:

- **Electrical** fields penetrate *shallowly*, but interact *strongly*
- **Magnetic** fields penetrate *deeply*, but interact *weakly*

Analysing complex networks in animals using electrical or optical methods is challenging, because electrical fields are strongly attenuated by tissues. Magnetic fields are promising for truly remote stimulation because they interact weakly with biological molecules and can penetrate deep into the body. However, their weak interaction with biological molecules means that the magnetic fields need to be translated into a different stimulus such as mechanical force or torque[6,7] or aggregation of particles[8] to act on their target. Because force or torque generation requires the use of large micrometre-sized beads, it is unsuitable for many *in vivo* applications. Although small (30 nm) nanoparticles have been used to induce the aggregation of cell receptors[8], whole-body applications remain challenging because a locally focused and strong spatial field gradient is required.

Why Mention Previous Failure?

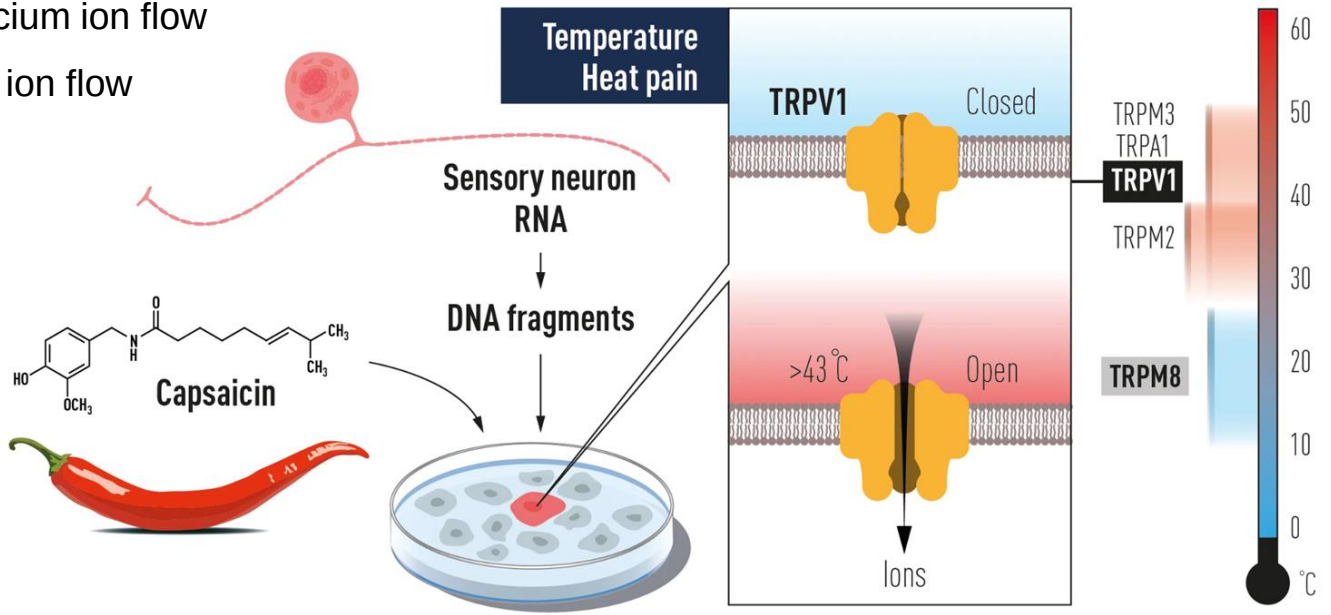
The context of previous failure both ***frames*** and ***justifies*** this paper to a ***broad*** audience.

- **Frame**: an *ongoing* and *widely spread* effort
- **Justification**: a *different method* that *corrects* the *failure* of previous attempts

TRPV1: How our body detects heat

TRPV1: a temperature sensitive ion channel

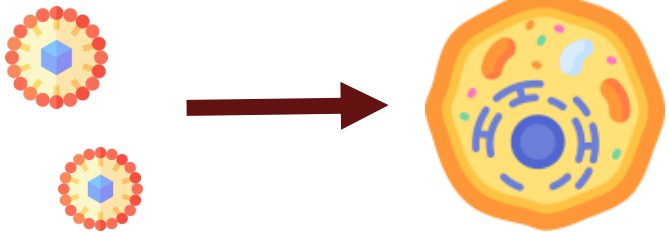
- Closed: No calcium ion flow
- Open: Calcium ion flow



The Nobel Committee for Physiology or Medicine, Mattias Karlén

Magnetic nanoparticles and nanoscale thermometers

Targeted magnetic nanoparticles



$MnFe_2O_4$ + streptavidin

Cell membrane with protein marker AP-CFP-TM

Nanoscale thermometers: fluorophores

- Dylight549, GFP, CFP, ANNINE6, YFP, fluorescein

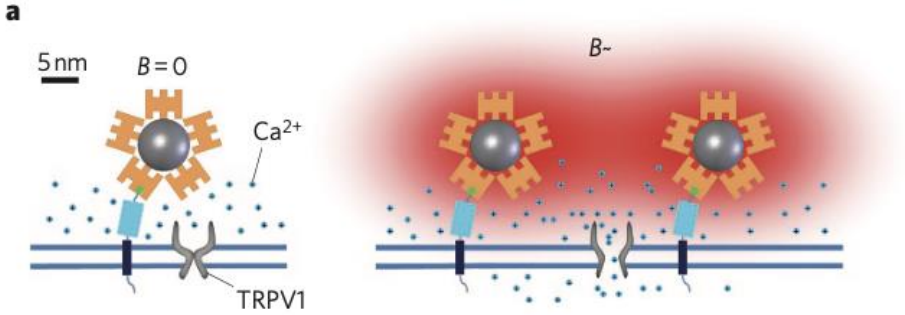
Methodologies and Media used

Aqueous Solution

Cells

C. elegans

Remote activation of TRPV1



RF field ↑,

temperature ↑,

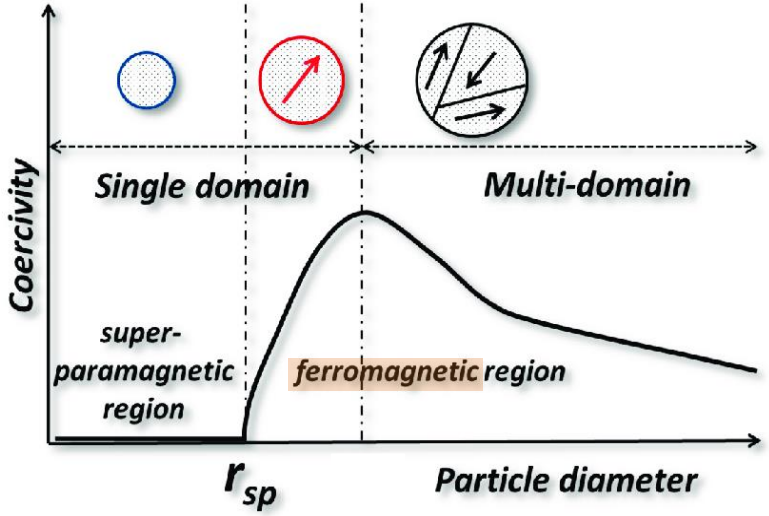
TRPV1 opens,

fluorescence ↓

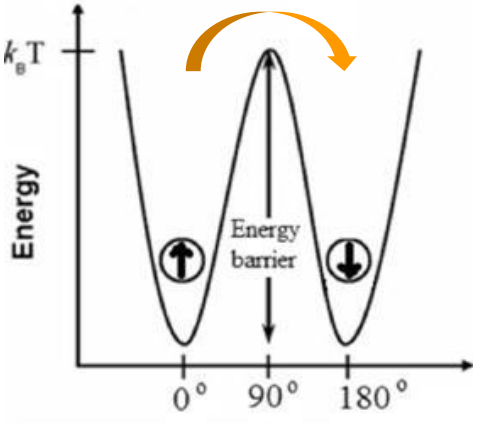
- Calcium concentration changes immediately
- Action potentials induced without cellular damage
- Stimulated behavioral response in C. elegans

Huang et al. 2010

Advantages of **superparamagnetic** nanoparticles



Zero average magnetization when no external magnetic field is applied ➡ No agglomeration, biologically safe, reversible

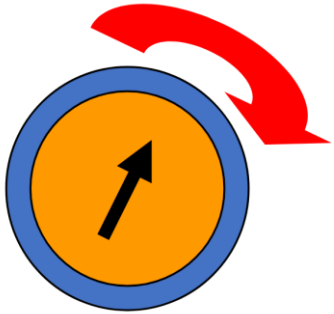


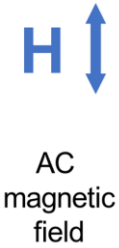
Higher susceptibility than paramagnets ➡ Weaker magnetic fields are enough to control the magnetization

S. H. Bossman and H. Wang, Royal Society of Chemistry 2017

Magnetic nanoparticle heating mechanisms

Brownian relaxation: blocked magnetization





Neél relaxation: unblocked magnetization

Whole particle rotates

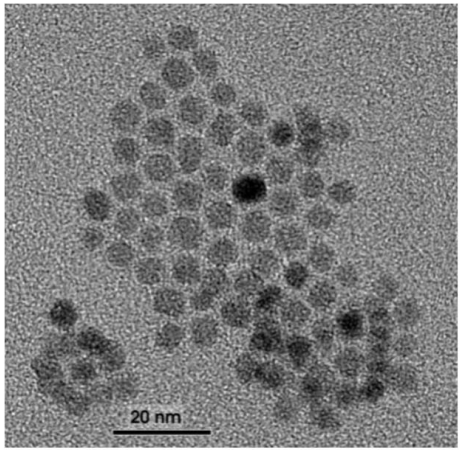
Only magnetization rotates

Heats up magnetic particles due to hysteretic losses

- Efficient energy conversion from field into heat
- Only the intended target tissue is heated

R. E. Rosensweig, JMMM 2002

Magnetic nanoparticle heating



Huang et al. 2010

Power loss of SPM nanoparticles in AC field:

$$P = \mu_0 \pi f {H_0}^2 \chi_0 \frac{2\pi f\tau}{1+(2\pi f\tau)^2}$$

- Heating rate is sensitive to particle size
- Size dispersion is detrimental (σ=0.05: heating rate/2)

Their nanoparticles:

- 6 nm $MnFe_2O_4$ + streptavidin → Neél relaxation dominates
- Size distribution not mentioned → Possible source of efficiency loss

Can nanoparticles stimulate tissue?

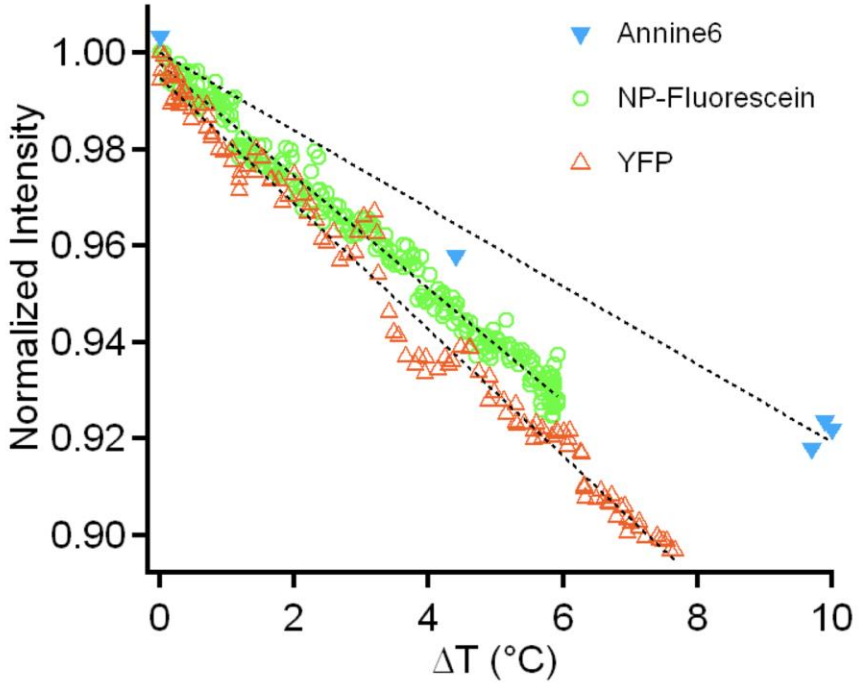
Claim: RF magnetic fields heating nanoparticles can create local stimuli inside tissue

Experimental evidence:

- Temperature change measured by fluorescence: solution, cell
- Activation of calcium channel through heat
- Behavior change of worms

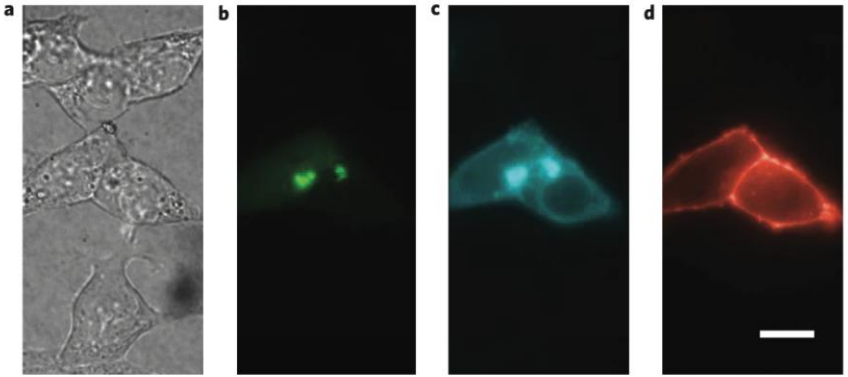
Fluorescence as a thermometer

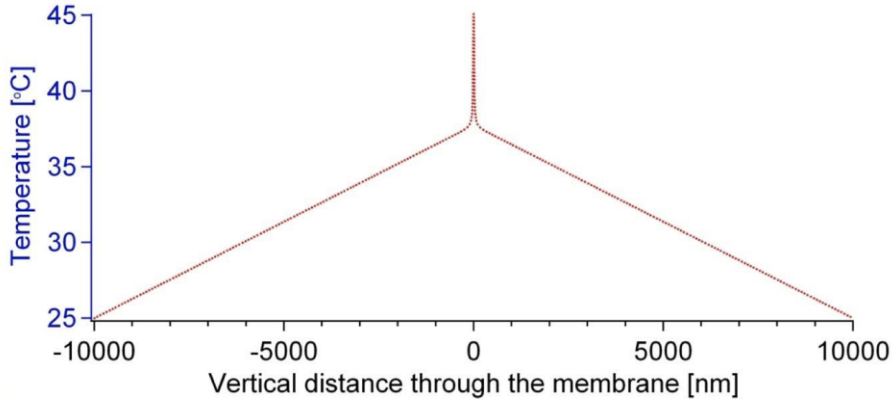
Fluorescence luminosity approximately linearly dependent on temperature



Huang et al. 2010

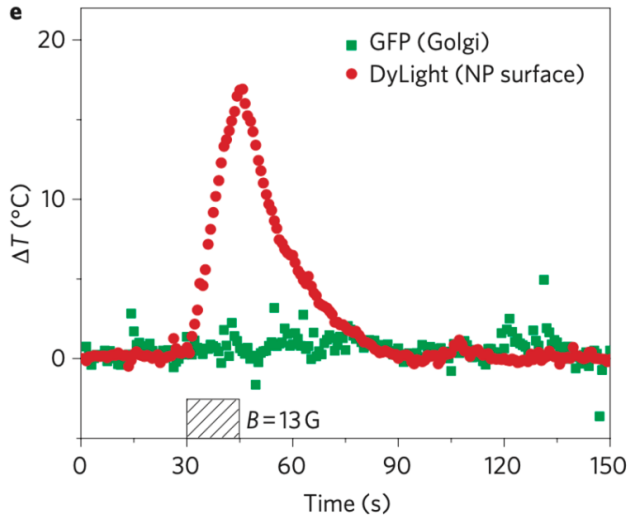
Nanoparticles heat locally





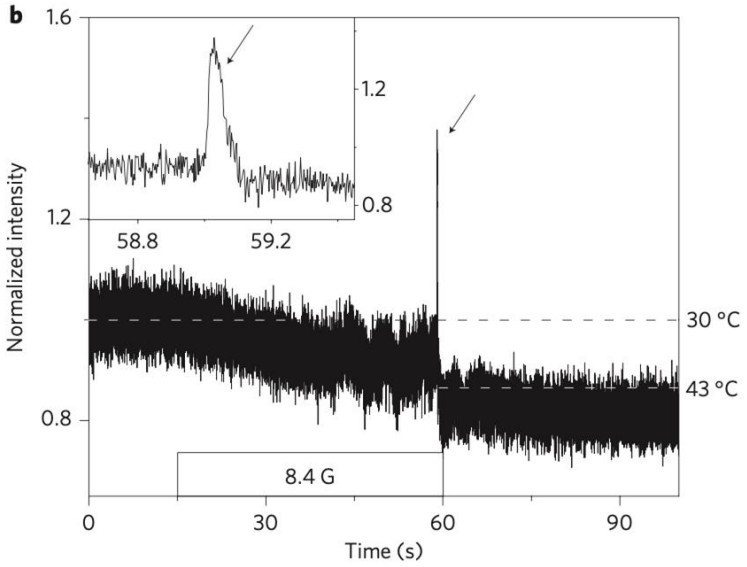
Simulation

Measurements



Huang et al. 2010

Activating ion channels

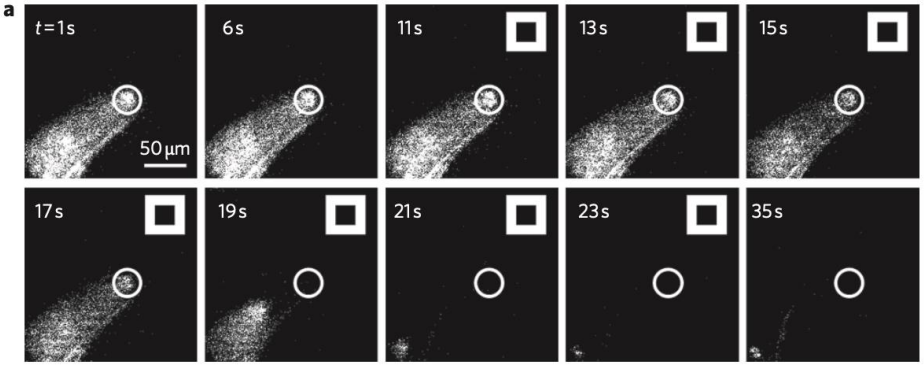


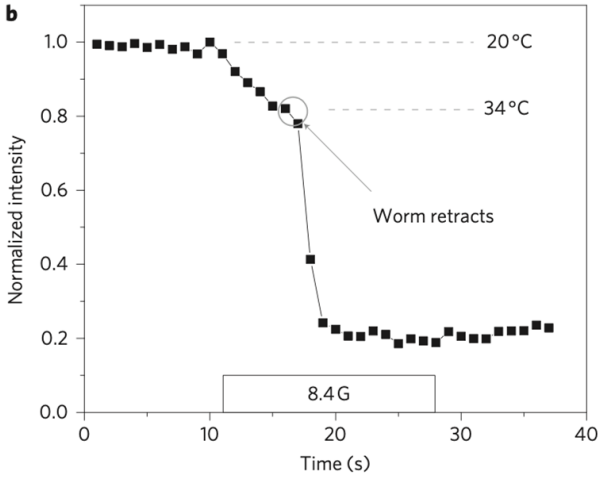
NP	TRPV1	RF field	Ca^{2+} Influx	Total
+	-	+	0	20
-	+	+	0	22
+	+	-	0	17
+	+	+	44	59

Why not 100% effective?

Huang et al. 2010

Stimulating C. elegans behavior





Stimulus was added 5 seconds after spontaneous reversal, observed for 30 seconds.

Average period of spontaneous reversal ~60 seconds

NP	RF field	*"Forward"*	*"Pause"*	*"Reversal" after "Pause"*	Total
+	+	6 (15%)	34 (85%)	27 (68%)	40
-	+	34 (85%)	6 (15%)	4 (10%)	40
-	-	29 (88%)	4 (12%)	3 (9%)	33

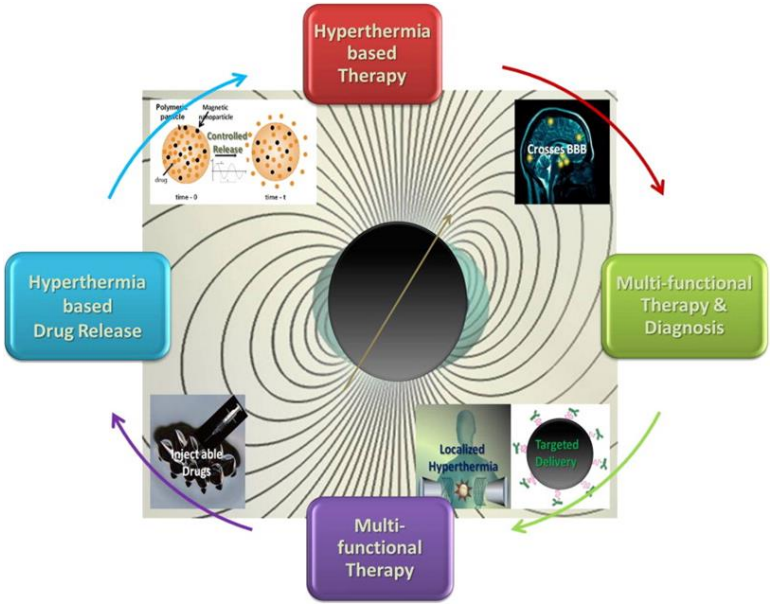
34 out of 40 worms “paused”, 27 “reversed”. Why not all?

Ability to target single cells?:

Unknown neural pathway for this behavior

Huang et al. 2010

Citation Evaluation



663 Citations since publication (https://app.dimensions.ai/details/publication/pub.1022012087)

- Most citations focused on promising applications of hyperthermia using magnetic nanoparticles (MNPs)
  - Heat-based treatments
  - Controlled drug delivery

(Kumar, C. S. S. R. & Mohammad, F. Magnetic nanomaterials for hyperthermia-based therapy and controlled drug delivery. *Advanced Drug Delivery Reviews* 63, 789–808 (2011).)

Representative Articles

- Improvements to NP delivery to brain using rats

  (Huang, Y. *et al. ACS Applied Materials and Interfaces* **8**, 11336–11341 (2016).)

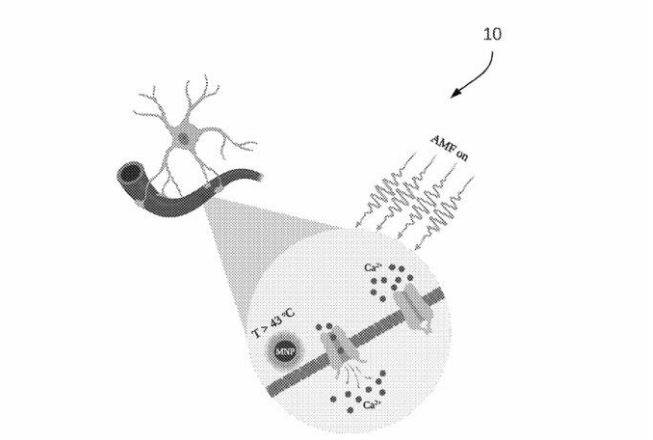
- Studies to optimize MNP heating for cancer therapies

  (1.Carrião, M. S. & Bakuzis, A. F. *Nanoscale* **8**, 8363–8377 (2016).

- Improvements in methods of mapping intracellular temperature

  (Okabe, K. *et al. Nat Commun* **3**, 705 (2012).)

Method for Treating Neurodegenerative Disorders

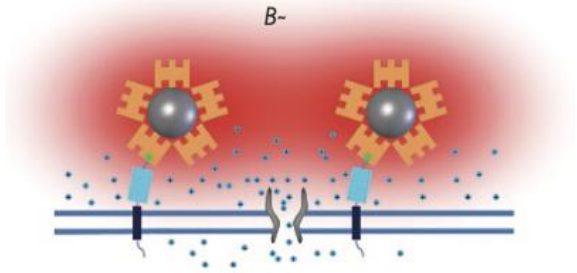


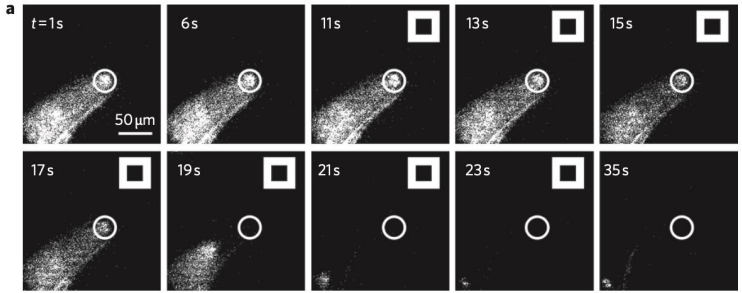
- Minimally invasive treatment method for Alzheimer's & Parkinson's
- Promising alternative to deep brain stimulation using electrodes
- Inventors utilize same mechanism as Huang *et al.*
  - Targets TRPV channels to stimulate Ca^{2+} ion exchange

K. Vafai and E. Kosari, "Method and system for thermal stimulation of targeted neural circuits for neurodegenerative disorders," US11147982B1, Oct. 19, 2021 [Online]. Available: ttps://app.dimensions.ai/details/patent/US-11147982-B1

Conclusions

- Establish **fluorescence** as a nanoscale thermometer
- Authors demonstrate remote control of ion channels in cells using RF magnetic-field heating of **nanoparticles**
- Limitation: **Heating not localized** in C. elegans
- Implications and impact: **Novel therapeutics**



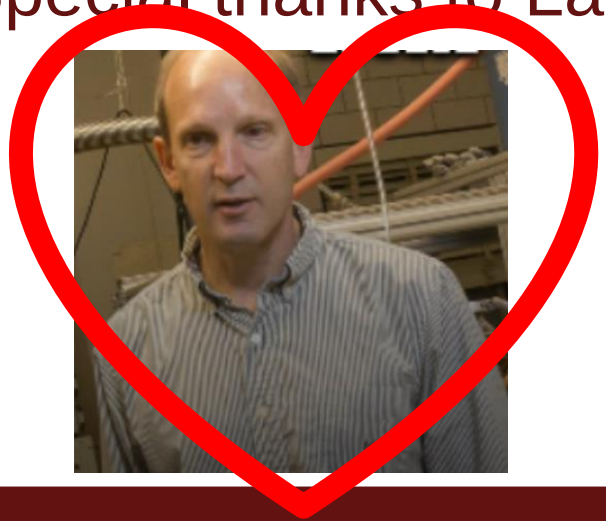


Huang et al. 2010

Thank you!

Thank you!

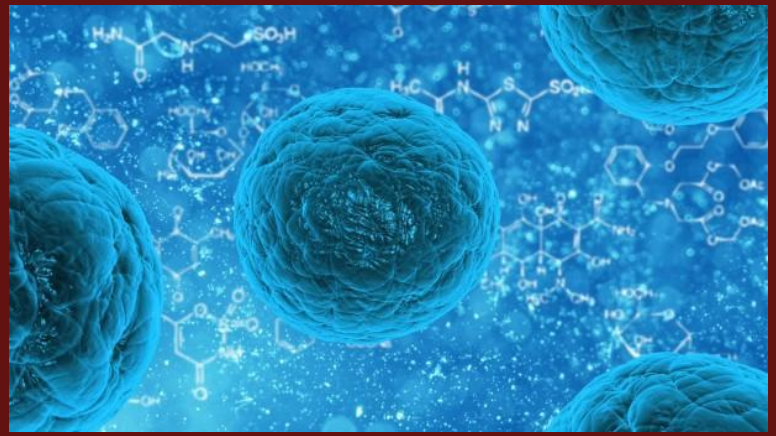
Special thanks to Lance



Quiz

Which of these cells did the authors not use?

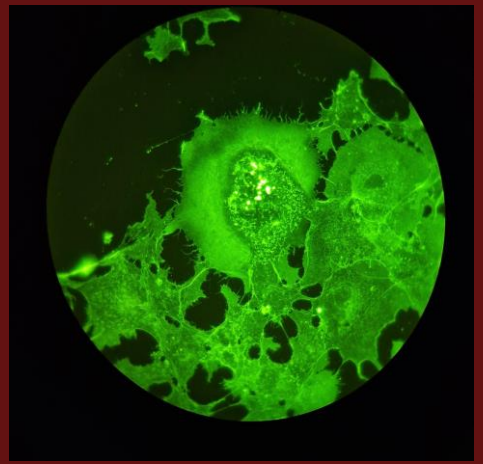
A. Human Embryonic Kidney Cells
B. Rat Hippocampal neurons
C. C. elegans neurons
D. Mice Dorsal Root Ganglions



Quiz

What happened to the fluorescence as the local temperature increased?

A. Intensity increased
B. Intensity remained constant
C. Intensity decreased
D. Mmm worms



Videos :)