Local heating of molecular motors using single carbon nanotubes

Yuichi Inoue¹ · Akihiko Ishijima^{1,2}

Received: 20 October 2015 / Accepted: 26 November 2015 / Published online: 6 January 2016

© International Union for Pure and Applied Biophysics (IUPAB) and Springer-Verlag Berlin Heidelberg 2015

Abstract Temperature globally affects all chemical processes and biomolecules in living cells. Elevating the temperature of an entire cell accelerates so many biomolecular reactions simultaneously that it is difficult to distinguish the various mechanisms involved. The ability to localize temperature changes to the nanometer range within a cell could provide a powerful new tool for regulating biomolecular activity at the level of individual molecules. The search for a nanoheater for biological research has prompted experiments with carbon nanotubes (CNTs), which have the highest conductivity of any known material. The adsorption of skeletal muscle myosin molecules along the length of single multi-walled CNTs (~10 μm) has allowed researchers to observe the ATP-driven sliding of fluorescently labeled actin filaments. In one study, red-laser irradiation focused on one end of a myosin-coated CNT was used to heat myosin motors locally without directly heating the surrounding water; this laser irradiation instantly accelerated the actin-filament sliding speeds from ~6 to ~12 $\mu\text{m/s}$ in a reversible manner, indicating a local, real-time heating of myosin motors by approximately $\Delta 12$ K. Calculation of heat transfer using the finite element method, based on the estimated temperature along a single CNT with a diameter of 170 nm, indicated a high thermal conductivity of ~1540 $\text{Wm}^{-1}\text{K}^{-1}$ in solution, consistent with values measured in vacuum in earlier studies. Temperature distribution indicated by half-decrease dis-

tances was ~3660 nm along the length of the CNT and ~250 nm perpendicular to the length. These results suggest that single-CNT-based heating at the nanometer- or micrometer-range could be used to regulate various biomolecules in many areas of biological, physical, and chemical research.

Keywords Carbon nanotube · Myosin · Laser-induced heating · Thermal conductivity

Introduction

Carbon nanotubes (CNTs), which are carbon allotropes with a cylindrical nanostructure and a large length/diameter ratio, were first described by Iijima (1991). CNTs with a diameter of ~1 nm are categorized as single-walled nanotubes. Multi-walled nanotubes can have up to several tens of graphitic shells with an ~0.34-nm separation between adjacent shells (Popov 2004). CNTs have unique physical characteristics, including their mechanical strength, electrical and thermal conductivity, and optical properties, which make them valuable in several research fields. In bioscience, CNTs are of particular interest in drug delivery systems, tissue-scaffold reinforcements, and cellular sensors (Cha et al. 2013).

In this review, we briefly outline recent biomolecular applications of CNTs at the subcellular level and describe how their thermal properties have been used to develop a new CNT nanoheater capable of changing temperatures locally to regulate protein activity in real time.

Thermal conductivity of carbon nanotubes

Single-walled nanotubes can carry a current three orders of magnitude higher than that carried by typical metals, such as

✉ Akihiko Ishijima
ishijima@fbs.osaka-u.ac.jp

¹ Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai, Miyagi 980-8577, Japan

² Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamadaoka, Suita, Osaka 565-0871, Japan

copper and aluminum (Hong and Myung 2007). Owing to this excellent electrical conductivity, CNTs have extremely high thermal conductivity, higher than that of any other known material—up to 7000 and 3000 $\text{Wm}^{-1}\text{K}^{-1}$ at room temperature for single-walled and multi-walled nanotubes, respectively (Table 1). Most studies have calculated single-walled nanotube thermal conductivity by molecular dynamic (MD) simulation. In contrast to the constant thermal conductivities of metals such as gold ($310 \text{ Wm}^{-1}\text{K}^{-1}$) and copper ($401 \text{ Wm}^{-1}\text{K}^{-1}$), the thermal conductivities calculated for single-walled nanotubes range from 120 to 7000 $\text{Wm}^{-1}\text{K}^{-1}$ and are dependent on factors such as chirality, potential, and boundary conditions (Berber et al. 2000; Osman and Srivastava 2001; Padgett and Brenner 2004; Lukes and Zhong 2007; Cao and Qu 2012). In addition to these factors, Mingo and Broido (2005) suggested that the calculated thermal conductivity for single-walled nanotubes depends strongly on length, and this theory was subsequently confirmed by the experimental measurement and analytical model of Pop et al. (2006). Multi-walled nanotube thermal conductivities have been determined via electrical measurements in a vacuum—with the exception of a study performed by Inoue et al. (2015). The thermal conductivity of multi-walled nanotubes is dependent on both the length and diameter of the nanotubes, such that it increases with increasing length (Yang et al. 2011), but decreases with increasing diameter (Kim et al. 2001; Fujii et al. 2005).

The thermal conductivity of CNTs is much higher than that of other components used in bioscience research, such as water ($\sim 0.6 \text{ Wm}^{-1}\text{K}^{-1}$), glass ($\sim 1.0 \text{ Wm}^{-1}\text{K}^{-1}$), and biomolecules such as proteins ($0.12\text{--}0.23 \text{ Wm}^{-1}\text{K}^{-1}$) (Lervik et al. 2010). Thus, CNTs are an ideal candidate nanomaterial for exploring heat transfer at the level of individual biomolecules.

CNT toxicity in biological applications

Carbon nanotubes have a broad potential in biological applications, such as fluorescence imaging, drug delivery, and thermal ablation (Luo et al. 2013), but it is important to assess their toxicity before they are used on a wide scale in biological systems. One toxic biological effect which has been observed is decreased cell viability through membrane damage and subcellular organelle dysfunction; this has been reported by several authors in such cells as human macrophages (Cheng et al. 2009), human keratinocytes (Manna et al. 2005), and mesothelial cells (Pacurari et al. 2008). Although a concrete mechanism of CNT toxicity is still open to discussion, the CNT-induced generation of reactive oxygen species (ROS) may be a key factor to induce DNA damage and protein modifications (Pacurari et al. 2008; Liu et al. 2011). CNT toxicity can be strongly enhanced by laser irradiation, which generates both heat and ROS to kill cancer cells (Kam et al. 2005; Murakami

et al. 2012). In some biological applications, CNT toxicity can be minimized by reducing agglomerate CNT structures (Pauluhn 2010) and by processes that functionalize the CNT surface, such as the chemical formation of organometallic structures (Chen et al. 1998; Cui et al. 2004) or noncovalent coatings with phospholipid–polyethylene glycols or other amphiphilic surfactant molecules (Liu et al. 2007). Functionalization of CNTs also improves their solubility in water so they can be dispersed as individual tubes in a solution.

Measuring the function of single protein molecules on CNTs

Researchers have successfully achieved the absorption of single protein molecules onto a single CNT whereby the adsorbed protein successfully retained its enzymatic activity, i.e., DNA polymerase I (Olsen et al. 2013), cAMP-dependent protein kinase A (Sims et al. 2013), and lysozyme (Choi et al. 2013), as assessed by measuring electrical signals through the CNTs as field-effect transistors. This technique provides a new platform for the continuous, label-free readout of single-biomolecule functionality with a high sensitivity to single mutations (Choi et al. 2013). However, in this powerful system for purified proteins *in vitro*, interpretation of the electrical signals measured requires assumptions and several control experiments, unless the analyzed protein is a motor protein.

Motor proteins

Motor proteins convert chemical energy, such as the free energy of ATP hydrolysis, into mechanical work to drive intracellular transport or cellular movement. Skeletal muscle myosin, a typical motor protein, drives muscle contraction by interactions with actin filaments (Huxley 2000). The fundamental process of myosin-based motility can be reproduced on a glass surface, allowing the observation of individual fluorescently labeled actin filaments (Yanagida et al. 1984) and their sliding motion, which is propelled by glass-adsorbed myosin molecules, in the presence of ATP (Kron and Spudich 1986). *In vitro* motility assays have been used to examine the chemo-mechanical cycles of linear motors such as myosin and kinesin (Block 2007) and rotary motors such as F1-ATPase (Noji et al. 1997). Single-molecule techniques, which were originally developed for motor protein analysis, such as the visualization of single fluorescent molecules, the manipulation of proteins by microneedle or optical tweezers, and the detection of unitary steps and their force (Yanagida et al. 2008), have now grown into powerful techniques used in a wide range of bioscientific fields.

Table 1 Reported thermal conductivities^a of carbon nanotubes at room temperature

Carbon nanotubes	Wm ⁻¹ K ⁻¹	Diameter (nm)	Length (μm)	Method	Reference
Single-walled CNTs	6600	(~1)	0.0025	MD simulations	Berber et al. 2000
	7000	(~1)	0.020	MD simulations	Padgett and Brenner 2004
	120–160	(~1)	0.040	MD simulations	Lukes and Zhong 2007
	1700	(~1)	0.030	MD simulations	Osman and Srivastava 2001
	1580	1.7	2.4	MD simulations	Cao and Qu 2012
	500–5000	(~1)	1–10	MD simulations	Mingo and Broido 2005
	~3500	1.7	2.6	I–V measurements	Pop et al. 2006
	2500–3300	1.7	0.5, 1, 3, 10	Analytical model	
Multi-walled CNTs	400–3000	200, 80, 14	2.5	Electrical resistance	Kim et al. 2001
	400–2900	28, 16, 9.8,	3.7	Electrical resistance	Fujii et al. 2005
	110–210	66	4.4–12	Electrical resistance	Yang et al. 2011
	391 (Pristine)	90	4.9	Electrical resistance	Hayashi et al. 2013
	103 (with defect)	93	5.0		
	1540	170	~10	Myosin motors (in solution)	Inoue et al. 2015

CNTs Carbon nanotubes, MD molecular dynamic

^a Thermal conductivities are reported for various CNTs. For comparison, see The Engineering Toolbox (<http://www.engineeringtoolbox.com/>) which reports the thermal conductivities (in Wm⁻¹K⁻¹) for common materials, such as air (0.024), water (0.58), glass (1.05), iron (80), gold (310), and copper (401) at room temperature. Thermal conductivities have also been determined by MD simulation for various proteins, including myoglobin (0.13), green fluorescence protein (0.12), and two conformations of a Ca²⁺-ATPase protein (0.22–0.23) (Lervik et al. 2010).

Researchers have recently combined CNTs and motor proteins. Tsuchiya et al. (2010) constructed a container transport system, powered by myosin-VI that can move CNTs along an intracellular track of actin filaments. Multi-walled CNTs and semiconductor nanowires can be used as a one-dimensional (1-D) track for the myosin-based sliding of actin filaments (Siethoff et al. 2014) and the kinesin-based sliding of microtubules (Sikora et al. 2014). Motor proteins are compatible with CNTs and produce activity that is easily observed, such as actin-binding kinetics and enzyme-based motile activity. Thus, the use of motor proteins is ideal for experiments aimed at developing CNTs as novel nanoheaters for regulating the activity of individual biomolecules.

Observation of motor function on a single CNT

In the quest to develop nanoheaters to regulate motor protein activity, Inoue et al. (2015) performed an in vitro motility assay on individual multi-walled CNTs. The length (8.3 ± 4.2 μm) and diameter (174 ± 51 nm) of the multi-walled CNTs used in their assay are shown in Fig. 1a, b. The CNTs were dispersed with the detergent Tween-20 and coated with myosin molecules non-specifically. After unbound myosin molecules were removed from the system by repeated cycles of centrifugation and washing with buffer solution, the CNTs were used as myosin-coated CNTs. A myosin-coated CNT existing in solution as a single fiber on a glass surface was selected using scattered light intensity in a dark-field image

(Fig. 1b). The sliding motion of fluorescently labeled actin filaments on the myosin-coated CNT was clearly observed in the presence of ATP under fluorescence microscopy (Fig. 1c). This sliding movement, which was distinct from Brownian motion and the passive flow of solution, provided evidence that active myosin molecules were present on the CNT. The average speeds measured on the myosin-coated CNT (8.7 ± 3.5 μm/s; *n* = 32) were comparable to those measured on the glass surface in control experiments (6.0 ± 1.2 μm/s; *n* = 20), indicating that myosin motors retain their normal motile activity when adsorbed onto a CNT. Earlier studies with CNTs or semiconductor nanowires had reported sliding speeds far below the physiological speed—less than 20 % of that of kinesin (Sikora et al. 2014) and ~42 % of that of myosin (Siethoff et al. 2014). The normal level of motility shown by the CNT-adsorbed myosin molecule in this system (Inoue et al. 2015) is the first demonstration that a single CNT can be used as a platform to evaluate this protein's normal function as a myosin motor.

Laser-induced heating of biomolecules

To heat biological organisms in water, the water is often heated directly with infrared laser irradiation (~1480 nm), as in the targeted induction of gene expression in *Caenorhabditis elegans* (Suzuki et al. 2013). Since the minimal targeting size in the laser irradiation system is limited by the beam of the laser light, and 1480-nm light is relatively harmful to

Fig. 1 The sliding movement of single actin filaments along a myosin-coated carbon nanotube (CNT). A 170-nm CNT in vacuum is shown as a scanning electron microscopy image (**a**) and as a dark-field image (**b**). **c** Fluorescence images of a fluorescently labeled actin filament (*second from bottom*): *top* dark-field image of the myosin-coated CNT; *arrowheads in sequential images* leading edge of the actin filament. **d** Sliding speed of the actin filament, plotted against the length of the filament. *Filled circles* Speeds measured on the myosin-coated CNT ($8.7 \pm 3.5 \mu\text{m/s}$), *open squares* speeds on a myosin-coated glass surface without a CNT ($6.0 \pm 1.2 \mu\text{m/s}$). Figure is taken from Inoue et al. (2015)

biological tissue due to its high absorption by water, near-infrared (NIR) light is more suitable for non-invasive tissue irradiation. In contrast to the low absorption of the NIR light by water, CNTs can effectively absorb NIR light (wavelength 1064 nm, power 1536 mW) to induce a local temperature rise of up to 172 °C in mammalian tissue (Picou et al. 2010). Continuous NIR irradiation (wavelength 808 nm, power 50–500 mW) on single-walled CNTs effectively ablates cancer cells such as HeLa (Kam et al. 2005) and human lung-cancer NCI-H460 cells (Murakami et al. 2012). Intermittent laser irradiation (wavelength 1064 nm, power 1 W) of cancer cells labeled with multi-walled CNTs increases the cell temperatures to ~90 °C, killing the cancer cells without harming healthy

Fig. 2 Laser-induced activation of actomyosin sliding. **a** An optical microscope, used for both dark-field and fluorescence imaging, was combined with a 642-nm laser used to locally irradiate one end of a CNT. A shutter or a neutral density (ND) filter was used to induce controlled temporal changes in laser irradiation, while the movement of actin filaments sliding along a myosin-coated CNT was observed. **b** Actin filament sliding speed in response to laser irradiation. *Open blue circles* Speed of individual actin filaments along a myosin-coated CNT, *red filled circles* averaged speeds with standard deviations (*whiskers*). The average speed increased with increasing laser power (from 0 to 0.68, 1.4, and 2.7 mW) in a reversible fashion. Experiments were performed using myosin filaments at room temperature (20 °C). Figure is taken from Inoue et al. (2015)

cells in the surrounding tissue (Biris et al. 2009). Thus, targeting NIR-light irradiation to a single CNT may serve as a new tool to regulate local temperature and activate protein activity at a subcellular or even single-molecule level.

Single-CNT-based local heating of myosin motors

Rapid thermal activation of motor proteins can be achieved by directing 1053-nm laser irradiation (power 50–100 mW) onto a 2-D metal surface (Kato et al. 1999; Kawaguchi and Ishiwata 2001). Inoue et al. (2015) activated actin-filament sliding by locally applying NIR-laser irradiation to one end of a single myosin-coated CNT (Fig. 2) or to a planar aggregate of myosin-coated CNTs, suggesting that a single CNT

can efficiently convert NIR light into heat. The average speed before irradiation was $6.0 \pm 1.8 \mu\text{m/s}$ at 20°C ; the speed increased slightly to $7.8 \pm 2.5 \mu\text{m/s}$ with irradiation of 0.68 mW , and returned to $6.3 \pm 3.9 \mu\text{m/s}$ after irradiation had been stopped. This reversible increase in speed indicated that the temperature increase was localized close to the CNT, with negligible heating of the solution as a whole. When the laser power was increased first to 1.4 mW and then to 2.7 mW , the sliding speed accelerated to 8.2 ± 1.4 and $11.4 \pm 3.0 \mu\text{m/s}$, respectively, before returning to original speed upon cessation of irradiation. Using the temperature dependence of the sliding speed, which had been measured in a previous study (Baker et al. 2011). Inoue et al. (2015) estimated the temperature of the myosin motors as shown in Fig. 2b (right axis), indicating a temperature jump by up to $\sim 12 \text{ K}$. These results suggest that multi-walled CNTs are a powerful platform for activating motor proteins in real time by applying external heat, as well as for measuring the proteins' activities.

Repetitive acceleration of a single actin filament on a CNT

In addition to the long heating period of $\sim 20 \text{ s}$ required for single-CNT-based local heating, Inoue et al. (2015) also demonstrated that local heating can be achieved by intermittent laser irradiation of $\sim 0.2 \text{ s}$ (Fig. 3), consistent with a fast heat transfer of $< 1 \text{ s}$. Laser irradiations of both 1.4 (Fig. 3a) and 2.8 mW (Fig. 3b) increased the sliding speed of single actin filaments in a reversible manner. When the laser power was increased to $\geq 3 \text{ mW}$, the speed did not increase or was reduced irreversibly due to thermal denaturation of the myosin molecules at temperatures of $> 40^\circ\text{C}$ (Baker et al. 2011). Kymographs of changes in the sliding speed of a single actin filament at different powers of laser irradiation (1.4 – 2.8 mW ; (Fig. 3a, b) showed that the sliding speed accelerated markedly during the first and second irradiations, followed by more moderate acceleration during the third to sixth irradiations. These results suggest that the acceleration may depend on the distance from the irradiated area, with a temperature gradient forming along the longitudinal axis of the CNT.

The temperature profile along the single CNT was confirmed by plotting the temperature of the myosin molecules, estimated from the sliding speed of each actin filament (Fig. 2b) versus the mean distance from the irradiated spot (Fig. 4a). In this case, actin filaments could be propelled in either direction on the myosin-coated CNT depending on the directionality of each actin filament, demonstrating that the downward trend of temperature in Fig. 4a was due to neither the directionality of the sliding movement nor convective water flow (which was negligible with an experimental condition of 0 – 2.8 mW).

Fig. 3 Repetitive laser-induced changes in the sliding speed of a single actin filament. **a** Kymograph showing the position of a single actin filament (*Y*-axis) and the elapsed time (*X*-axis). The sliding speed was accelerated repeatedly by ~ 0.2 -s pulses of 1.4 -mW laser irradiation. **b** Kymograph generated with a 2.8 -mW laser. The experiments were performed using myosin filaments at room temperature (20°C). Figure is taken from Inoue et al. (2015)

Heat conduction by a single multi-walled CNT in solution

To explain the temperature profile along a single CNT, Inoue et al. (2015) calculated the heat transfer from the irradiated spot to the myosin-coated CNT and surrounding water by the finite element method (FEM) using a 3-D model (shown in Fig. 4b) consisting of a single CNT (length $15 \mu\text{m}$, diameter 170 nm) and a large amount of surrounding water (a cuboid of $20 \times 20 \times 30 \mu\text{m}^3$). Assuming that the end point (length $2 \mu\text{m}$) of the CNT works as a heat source by laser irradiation, these authors numerically determined the heat conduction from the end point into the rest of the CNT and the surrounding water using a boundary condition of constant temperature (20°C) at the 6 planes of the water cuboid. In this calculation, the thermal conductivity of both myosin and the actin filament was neglected due to the small size of these proteins and their similar thermal conductivity (0.12 – $0.23 \text{ Wm}^{-1}\text{K}^{-1}$; Lervik et al. 2010) to water ($\sim 0.6 \text{ Wm}^{-1}\text{K}^{-1}$). The convective water flow was also neglected in the subsequent FEM calculations because the effect of convection was confirmed to be negligibly small in both the experiment ($\leq 2.7 \text{ mW}$) and the preliminary calculation. The FEM calculation was repeated with

Fig. 4 Temperature distribution along a 170-nm CNT. **a** The calculated temperatures were plotted against the distance from the laser-irradiated spot. Speeds measured during 20-s cycles of laser irradiation (Fig. 2) were converted to the temperature of the myosin motors, as reported previously (Baker et al. 2011). The distance was measured from the center of the irradiated spot to the midpoint of the sliding observed on the CNT. Laser power: open circles 0 mW, blue triangles 0.68 mW, orange squares 1.4 mW, red circles 2.7 mW. Respective fitted lines were derived by calculations using **b** a 3-dimensional finite element method model in which a 170-nm CNT is surrounded by a large amount of water (a cuboid of $20 \times 20 \times 30 \mu\text{m}^3$). Heat conduction from an end point of the CNT, the heat source (length $2 \mu\text{m}$), into the rest of the CNT (length $15 \mu\text{m}$, diameter 170 nm) and into the surrounding water was numerically solved (see text) using a boundary condition of constant temperature (20°C) at the 6 planes of the water cuboid. Figure is taken from Inoue et al. (2015)

various values for CNT thermal conductivity (k) and the power of the heat source (Q) to find the best fit to the experimental data along a CNT (Fig. 4a; solid lines; $k = 1540 \pm 260 \text{ Wm}^{-1}\text{K}^{-1}$; $Q = 263 \pm 84 \mu\text{W}$). When the FEM calculation was repeated in a model that took the glass surface under the CNT into consideration (half of the water cuboid was replaced with glass as an extreme case), the thermal conductivity of the CNT did not decrease ($k = 2030 \pm 390 \text{ Wm}^{-1}\text{K}^{-1}$; $Q = 350 \pm 110 \mu\text{W}$), confirming the calculation. Therefore, a high thermal conductivity of $\geq 1540 \text{ Wm}^{-1}\text{K}^{-1}$ was estimated for the multi-walled CNT in solution, as determined based on the

activity of protein function. Previously reported thermal conductivities of CNTs, obtained by MD simulations or experiments in vacuum, are summarized in Table 1. Inoue et al.'s (2015) estimate of $\geq 1540 \text{ Wm}^{-1}\text{K}^{-1}$ is within the broad range of reported values for multi-walled CNTs at room temperature ($103\text{--}3000 \text{ Wm}^{-1}\text{K}^{-1}$). When the dependency of the thermal conductivity of the multi-walled CNT on both the length and diameter of the CNT was taken into consideration, the speculated value for the CNT in Inoue et al. (2015) (length $15 \mu\text{m}$, diameter 170 nm) is $800\text{--}1760 \text{ Wm}^{-1}\text{K}^{-1}$, which is consistent with the FEM calculation. The heat conduction in multi-walled CNTs is reported to be highly anisotropic, such that the in-shell thermal conductivity ($\lambda_{\text{in}} = 1800 \text{ Wm}^{-1}\text{K}^{-1}$ along the CNT) is much larger than the out-of-shell thermal conductivity ($\lambda_{\text{out}} = 0.05 \text{ Wm}^{-1}\text{K}^{-1}$ in cross-section), as determined by the thermal conductivities of pristine and the defective multi-walled CNTs (Hayashi et al. 2013). The similar values for the estimated thermal conductivity ($\geq 1540 \text{ Wm}^{-1}\text{K}^{-1}$) and the in-shell thermal conductivity ($\lambda_{\text{in}} = 1800 \text{ Wm}^{-1}\text{K}^{-1}$ along the CNT) may suggest that the single-CNT-based activation of motor proteins along the CNT is based on the anisotropic nature of heat conduction within multi-walled CNTs.

Conclusion and perspectives

In contrast to older laser induction systems that heat large numbers of cells or biomolecules, the single-CNT method offers far more precise control over local temperature and the function of biomolecules at a subcellular level. The reversible single-CNT-based activation of myosin motors revealed a local temperature jump of up to $\sim 12 \text{ K}$. The temperature range at which protein activity could be controlled could be expanded to $0\text{--}40^\circ\text{C}$ by combining the single-CNT-based heating with cooling of the entire solution by a temperature-control chip (Baker et al. 2011). FEM calculations for the localization of the temperature jump on a 170-nm-diameter CNT estimated the half-decrease distances to be $\sim 250 \text{ nm}$ perpendicular to the CNT and $\sim 3660 \text{ nm}$ along its length. This multi-walled CNT is estimated to have a thermal conductivity of $\geq 1540 \text{ Wm}^{-1}\text{K}^{-1}$ in solution, which ensures that it is an effective heat conductor for motor proteins and a wide range of other biomolecules. Using a thinner CNT (or smaller nanomaterials) would allow the study of single molecules using this single-CNT-based method.

It should be noted that the single-CNT method is not limited to the reversible activation of protein function. The reversible mode becomes irreversible simply by extensive heating at $>40^\circ\text{C}$, which inactivates most proteins irreversibly through thermal denaturation, as demonstrated for ablating cancer cells (Kam et al. 2005; Biris et al. 2009; Murakami et al. 2012). Inoue et al. (2015) used a constant concentration of oxygen

scavengers for continuous fluorescence imaging (Harada et al. 1990). Because laser irradiation of CNTs is known to generate ROS, leading to cellular changes such as protein deactivation, DNA damage and lipid peroxidation, and multi-nuclei formation (Manke et al. 2013), the use of oxygen scavengers may offer another method to regulate protein activity by inhibiting ROS and blocking the irreversible deactivation of proteins. The flexible control of protein function offered by the single-CNT method, in both reversible and irreversible modes, either by the activation or deactivation, will be useful in many areas of biological research, including biophysics and biomedicine.

Acknowledgments This work was supported by CREST from Japan Science and Technology Agency (A.I.); KAKENHI on Innovative Areas from Ministry of Education, Culture, Sports, Science and Technology / Japan Society for the Promotion of Science (A.I.); KAKENHI from Ministry of Education, Culture, Sports, Science and Technology / Japan Society for the Promotion of Science (A.I., and Y.I.); the FIRST program from Japan Society for the Promotion of Science (A.I. and Y.I.). This work was also supported in part by the Management Expenses Grants for National Universities Corporations from Ministry of Education, Culture, Sports, Science and Technology.

Compliance with ethical standards

Conflict of interest Akihiko Ishijima declares that he has no conflict of interest.

Yuichi Inoue declares that he has no conflict of interest.

Ethical approval All applicable international, national, and institutional guidelines for the care and use of animals were followed.

Author contributions Yuichi Inoue and Akihiko Ishijima collaborated on all aspects of this review, including manuscript preparation.

References

- Baker MA, Inoue Y, Takeda K, Ishijima A, Berry RM (2011) Two methods of temperature control for single-molecule measurements. *Eur Biophys J* 40:651–660. doi:10.1007/s00249-010-0667-y
- Berber S, Kwon YK, Tomanek D (2000) Unusually high thermal conductivity of carbon nanotubes. *Phys Rev Lett* 84:4613–4616. doi:10.1103/PhysRevLett.84.4613
- Biris A, Boldor D, Palmer J, Monroe WT, Mahmood M, Dervishi E, Xu Y, Li Z, Galanzha EI, Zharov VP (2009) Nanophotothermolysis of multiple scattered cancer cells with carbon nanotubes guided by time-resolved infrared thermal imaging. *J Biomed Opt* 14:021007. doi:10.1117/1.3119135
- Block SM (2007) Kinesin motor mechanics: binding, stepping, tracking, gating, and limping. *Biophys J* 92:2986–2995. doi:10.1529/biophysj.106.100677
- Cao A, Qu J (2012) Size dependent thermal conductivity of single-walled carbon nanotubes. *J Appl Phys* 112:013503. doi:10.1063/1.4730908
- Cha C, Shin SR, Annabi N, Dokmeci MR, Khademhosseini A (2013) Carbon-based nanomaterials: multifunctional materials for biomedical engineering. *ACS Nano* 7:2891–2897. doi:10.1021/nn401196a
- Chen J, Hamon MA, Hu H, Chen Y, Rao AM, Eklund PC, Haddon RC (1998) Solution properties of single-walled carbon nanotubes. *Science* 282:95–98. doi:10.1126/science.282.5386.95
- Cheng C, Müller KH, Koziol KK, Skepper JN, Midgley PA, Welland ME, Porter AE (2009) Toxicity and imaging of multi-walled carbon nanotubes in human macrophage cells. *Biomater* 30:4152–4160. doi:10.1016/j.biomaterials.2009.04.019
- Choi Y, Olsen TJ, Sims PC, Moody IS, Corso BL, Dang MN, Weiss GA, Collins PG (2013) Dissecting single-molecule signal transduction in carbon nanotube circuits with protein engineering. *Nano Lett* 13:625–631. doi:10.1021/nl304209p
- Cui D, Ozkan CS, Ravindran S, Kong Y, Gao H (2004) Encapsulation of pt-labelled DNA molecules inside carbon nanotubes. *Mech Chem Biosyst* 1:113–121. doi:10.3970/mcb.2004.001.113
- Fujii M, Zhang X, Xie H, Ago H, Takahashi K, Ikuta T, Abe H, Shimizu T (2005) Measuring the thermal conductivity of a single carbon nanotube. *Phys Rev Lett* 95:065502. doi:10.1103/PhysRevLett.95.065502
- Harada Y, Sakurada K, Aoki T, Thomas DD, Yanagida T (1990) Mechanochemical coupling in actomyosin energy transduction studied by in vitro movement assay. *J Mol Biol* 216:49–68. doi:10.1016/S0022-2836(05)80060-9
- Hayashi H, Ikuta T, Nishiyama T, Takahashi K (2013) Enhanced anisotropic heat conduction in multi-walled carbon nanotubes. *J Appl Phys* 113:014301. doi:10.1063/1.4772612
- Hong S, Myung S (2007) Nanotube electronics: a flexible approach to mobility. *Nat Nanotechnol* 2:207–208. doi:10.1038/nnano.2007.89
- Huxley AF (2000) Mechanics and models of the myosin motor. *Phil Trans R Soc Lond B* 355:433–440. doi:10.1098/rstb.2000.0584
- Iijima S (1991) Helical microtubules of graphitic carbon. *Nature* 354:56–58. doi:10.1038/354056a0
- Inoue Y, Nagata M, Matsutaka H, Okada T, Sato MK, Ishijima A (2015) Single carbon nanotube-based reversible regulation of biological motor activity. *ACS Nano* 9:3677–3684. doi:10.1021/nn505607c
- Kam NW, O'Connell M, Wisdom JA, Dai H (2005) Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction. *Proc Natl Acad Sci USA* 102:11600–11605. doi:10.1073/pnas.0502680102
- Kato H, Nishizaka T, Iga T, Kinoshita K Jr, Ishiwata S (1999) Imaging of thermal activation of actomyosin motors. *Proc Natl Acad Sci USA* 96:9602–9606. doi:10.1073/pnas.96.17.9602
- Kawaguchi K, Ishiwata S (2001) Thermal activation of single kinesin molecules with temperature pulse microscopy. *Cell Motil Cytoskeleton* 49:41–47. doi:10.1002/cm.1019
- Kim P, Shi L, Majumdar A, McEuen PL (2001) Thermal transport measurements of individual multiwalled nanotubes. *Phys Rev Lett* 87:215502. doi:10.1103/PhysRevLett.87.215502
- Kron SJ, Spudich JA (1986) Fluorescent actin filaments move on myosin fixed to a glass surface. *Proc Natl Acad Sci USA* 83:6272–6276. doi:10.1073/pnas.83.17.6272
- Lervik A, Bresme F, Kjelstrup S, Bedeaux D, Miguel Rubi J (2010) Heat transfer in protein-water interfaces. *Phys Chem Chem Phys* 12:1610–1617. doi:10.1039/b918607g
- Liu Z, Sun X, Nakayama-Ratchford N, Dai H (2007) Supramolecular chemistry on water-soluble carbon nanotubes for drug loading and delivery. *ACS Nano* 1:50–56. doi:10.1021/nn700040t
- Liu HL, Zhang YL, Yang N, Zhang YX, Liu XQ, Li CG, Zhao Y, Wang YG, Zhang GG, Yang P, Guo F, Sun Y, Jiang CY (2011) A functionalized single-walled carbon nanotube-induced autophagic cell death in human lung cells through Akt-TSC2-mTOR signaling. *Cell Death Dis* 19:e159. doi:10.1038/cddis.2011.27
- Lukes JR, Zhong H (2007) Thermal conductivity of individual single-wall carbon nanotubes. *J Heat Transf* 129:705–716. doi:10.1115/1.2717242

- Luo E, Song G, Li Y, Shi P, Hu J, Lin Y (2013) The toxicity and pharmacokinetics of carbon nanotubes as an effective drug carrier. *Curr Drug Metab* 14:879–890. doi:[10.2174/138920021131400110](https://doi.org/10.2174/138920021131400110)
- Manke A, Wang L, Rojanasakul Y (2013) Mechanisms of nanoparticle-induced oxidative stress and toxicity. *Biomed Res Int* 2013:942916. doi:[10.1155/2013/942916](https://doi.org/10.1155/2013/942916)
- Manna SK, Sarkar S, Barr J, Wise K, Barrera EV, Jejelowo O, Rice-Ficht AC, Ramesh GT (2005) Single-walled carbon nanotube induces oxidative stress and activates nuclear transcription factor-kappaB in human keratinocytes. *Nano Lett* 5:1676–1684. doi:[10.1021/nl0507966](https://doi.org/10.1021/nl0507966)
- Mingo N, Broido DA (2005) Length dependence of carbon nanotube thermal conductivity and the “Problem of long waves”. *Nano Lett* 5:1221–1225. doi:[10.1021/nl050714d](https://doi.org/10.1021/nl050714d)
- Murakami T, Nakatsuji H, Inada M, Matoba Y, Umeyama T, Tsujimoto M, Isoda S, Hashida M, Imahori H (2012) Photodynamic and photothermal effects of semiconducting and metallic-enriched single-walled carbon nanotubes. *J Am Chem Soc* 134:17862–17865. doi:[10.1021/ja3079972](https://doi.org/10.1021/ja3079972)
- Noji H, Yasuda R, Yoshida M, Kinosita K Jr (1997) Direct observation of the rotation of F1-ATPase. *Nature* 386:299–302. doi:[10.1038/386299a0](https://doi.org/10.1038/386299a0)
- Olsen TJ, Choi Y, Sims PC, Gul OT, Corso BL, Dong C, Brown WA, Collins PG, Weiss GA (2013) Electronic measurements of single-molecule processing by DNA polymerase I (Klenow fragment). *J Am Chem Soc* 135:7855–7860. doi:[10.1021/ja311603r](https://doi.org/10.1021/ja311603r)
- Osman MA, Srivastava D (2001) Temperature dependence of the thermal conductivity of single-wall carbon nanotubes. *Nanotechnology* 12: 21–24. doi:[10.1088/0957-4484/12/1/305](https://doi.org/10.1088/0957-4484/12/1/305)
- Pacurari M, Yin XJ, Zhao J, Ding M, Leonard SS, Schwegler-Berry D, Ducatman BS, Sbarra D, Hoover MD, Castranova V, Vallyathan V (2008) Raw single-wall carbon nanotubes induce oxidative stress and activate MAPKs, AP-1, NF-kappaB, and Akt in normal and malignant human mesothelial cells. *Environ Health Perspect* 116: 1211–1217. doi:[10.1289/ehp.10924](https://doi.org/10.1289/ehp.10924)
- Padgett CW, Brenner DW (2004) Influence of chemisorption on the thermal conductivity of single-wall carbon nanotubes. *Nano Lett* 4: 1051–1053. doi:[10.1021/nl049645d](https://doi.org/10.1021/nl049645d)
- Pauluhn J (2010) Subchronic 13-week inhalation exposure of rats to multiwalled carbon nanotubes: toxic effects are determined by density of agglomerate structures, not fibrillar structures. *Toxicol Sci* 113:226–242. doi:[10.1093/toxsci/kfp247](https://doi.org/10.1093/toxsci/kfp247)
- Picou L, McMann C, Elzer PH, Enright FM, Biris AS, Boldor D (2010) Spatio-temporal thermal kinetics of in situ MWCNT heating in biological tissues under NIR laser irradiation. *Nanotechnology* 21: 435101. doi:[10.1088/0957-4484/21/43/435101](https://doi.org/10.1088/0957-4484/21/43/435101)
- Pop E, Mann D, Wang Q, Goodson K, Dai H (2006) Thermal conductance of an individual single-wall carbon nanotube above room temperature. *Nano Lett* 6:96–100. doi:[10.1021/nl052145f](https://doi.org/10.1021/nl052145f)
- Popov VN (2004) Carbon nanotubes: properties and application. *Mater Sci Eng R Rep* 43:61–102. doi:[10.1016/j.mser.2003.10.001](https://doi.org/10.1016/j.mser.2003.10.001)
- Siethoff LT, Lard M, Generosi J, Andersson HS, Linke H, Månsson A (2014) Molecular motor propelled filaments reveal light-guiding in nanowire arrays for enhanced biosensing. *Nano Lett* 14:737–742. doi:[10.1021/nl404032k](https://doi.org/10.1021/nl404032k)
- Sikora A, Ramón-Azcón J, Kim K, Reaves K, Nakazawa H, Umetsu M, Kumagai I, Adschiri T, Shiku H, Matsue T, Hwang W, Teizer W (2014) Molecular motor-powered shuttles along multi-walled carbon nanotube tracks. *Nano Lett* 14:876–881. doi:[10.1021/nl4042388](https://doi.org/10.1021/nl4042388)
- Sims PC, Moody IS, Choi Y, Dong C, Iftikhar M, Corso BL, Gul OT, Collins PG, Weiss GA (2013) Electronic measurements of single-molecule catalysis by cAMP-dependent protein kinase A. *J Am Chem Soc* 135:7861–7868. doi:[10.1021/ja311604j](https://doi.org/10.1021/ja311604j)
- Suzuki M, Toyoda N, Shimojima M, Takagi S (2013) Infrared laser-induced gene expression in targeted single cells of *Caenorhabditis elegans*. *Dev Growth Differ* 55:454–461. doi:[10.1111/dgd.12061](https://doi.org/10.1111/dgd.12061)
- Tsuchiya Y, Komori T, Hirano M, Shiraki T, Kakugo A, Ide T, Gong JP, Yamada S, Yanagida T, Shinkai S (2010) A polysaccharide-based container transportation system powered by molecular motors. *Angew Chem Int Ed Engl* 49:724–727. doi:[10.1002/anie.200904909](https://doi.org/10.1002/anie.200904909)
- Yanagida T, Nakase M, Nishiyama K, Oosawa F (1984) Direct observation of motion of single F-Actin filaments in the presence of myosin. *Nature* 307:58–60. doi:[10.1038/307058a0](https://doi.org/10.1038/307058a0)
- Yanagida T, Iwaki M, Ishii Y (2008) Single molecule measurements and molecular motors. *Philos Trans R Soc Lond B Biol Sci* 363:2123–2134. doi:[10.1098/rstb.2008.2265](https://doi.org/10.1098/rstb.2008.2265)
- Yang J, Yang Y, Waltermire SW, Gutu T, Zinn AA, Xu TT, Chen Y, Li D (2011) Measurement of the intrinsic thermal conductivity of a multiwalled carbon nanotube and its contact thermal resistance with the substrate. *Small* 7:2334–2340. doi:[10.1002/sml.201100429](https://doi.org/10.1002/sml.201100429)