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Biothermal sensing of a torsional artificial muscle†

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Biomolecule responsive materials have been studied intensively for use in biomedical applications as smart systems because of their unique property of responding to specific biomolecules under mild conditions. However, these materials have some challenging drawbacks that limit further practical application, including their speed of response and mechanical properties, because most are based on hydrogels. Here, we present a fast, mechanically robust biscrolled twist-spun carbon nanotube yarn as a torsional artificial muscle through entrapping an enzyme linked to a thermally sensitive hydrogel, poly(*N*-isopropylacrylamide), utilizing the exothermic catalytic reaction of the enzyme. The induced rotation reached an equilibrated angle in less than 2 min under mild temperature conditions (25–37 °C) while maintaining the mechanical properties originating from the carbon nanotubes. This biothermal sensing of a torsional artificial muscle offers a versatile platform for the recognition of various types of biomolecules by replacing the enzyme, because an exothermic reaction is a general property accompanying a biochemical transformation.

Introduction

Biomolecule sensing artificial muscles are of great interest in many scientific fields, particularly in bioengineering and medical applications, because they can sense the existence of specific biomarkers and generate mechanical energy to regulate the homeostatic function of living organisms for various purposes.[1,2] Hydrogels are promising candidates for fabricating this type of artificial muscle by immobilizing antibodies,[3] DNA,[4] enzymes[5] or synthetic molecules[6] which enable the hydrogels to change their volume by interacting with specific biomolecules. However, because the swelling kinetics of a hydrogel are controlled by diffusion, which is proportional to the square of the size of the hydrogel,[7] the response speed is generally slow,[2,7,8] and dependent on the actual size. In other words, the response speed can be improved by making smaller and thinner hydrogels or by introducing porosity.[7,9] Unfortunately, both these approaches usually make hydrogel systems too fragile to be utilized as artificial muscle.[7]

To improve the response speed, mechanical properties and capability in biomedical applications at the same time, we focused on a multiwalled carbon nanotube (MWCNT) yarn-based torsional artificial muscle, which could provide fast and outstanding actuating performance results from dimensional changes of the yarn guests[10,11] and good biocompatibility compared with conventional carbon nanotubes.[12,13] This actuation platform would also be able to work reversibly in response to several stimuli, such as electrochemical, electrical, photonic, and chemical stimuli, depending on what materials the yarn contained as a functional guest.[11] Enzymatic smart systems have a number of advantages because they are highly selective, work under mild conditions, and are involved in catalyzing most reactions in cells and organisms. But most of them have some limitations that debase their versatility such as redox reaction dependent actuation that is available from only limited types of enzymes[5] or irreversibilities.[14] Due to these reasons, we considered utilizing the exothermic catalytic reaction of an enzyme because of its general properties accompanying biochemical transformation.[15,16]

Here, we demonstrate experimentally fast, mechanically robust, and biothermal sensing of a torsional artificial muscle system that is powered by the exothermic catalytic reaction of an enzyme in a human physiological environment (pH = 7.3 and T = 36 °C). To achieve this goal, we synthesized poly(*N*-isopropylacrylamide) (PNIPAm) hydrogel particles that have the capability to immobilize an enzyme from copolymerized *N*-succinimidyl acrylate (NSA) to fabricate a biscrolled MWCNT yarn

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†Electronic supplementary information (ESI) available: Experimental sections; a schematic drawing of the custom-built flow injection system; the change in the length of the yarn correlated with the wetting and drying of the entrapped PNIPAm–GOx particle *versus* time; the particle size distribution of PNIPAm–GOx hydrogel in a pure PBS solution and in a PBS solution containing 100 mM glucose at 36 °C; biothermal sensing torsional actuation of a carbon nanotube yarn (video). See DOI: 10.1039/c5nr07195j

that contained these enzyme-immobilized hydrogel particles as a functional guest material.[17] Glucose oxidase (GOx) was chosen as a reaction catalyst because it is a well-characterized enzyme and is widely utilized for many purposes in biomedical applications.

Results and discussion

A schematic illustration of the biothermal actuation is shown in Fig. 1a. The copolymerization of NSA, which provides a binding site that enables it to couple with the carboxyl group of the enzyme, was applied to make the gel interact to provide an exothermic event. As shown in Fig. 1b, the lower critical solution temperature (LCST) of the synthesized hydrogel particle had shifted and narrowed from the temperature range 26–36 °C (black squares) to 31–37 °C (red circles) after immobilization of the GOx. With this additional cross-linking effect, the size of gel changed from around 300 nm in the swollen phase to 110 nm in the contracted phase. The results from the isothermal calorimeter titration experiments (Fig. 1c) show that the catalytic reaction for oxidizing glucose by GOx was exothermic.

Fig. 2a–c show the fabrication methods for incorporating biomolecule-responsive aqueous hydrogel particles into an MWCNT yarn as a functional guest material. Despite the poor wettability of the well-aligned MWCNT sheets, this method helps to adsorb the PNIPAm particles onto the hydrophobic surface of MWCNTs while the hydrogel particle transited its phase at a temperature above the LCST (Fig. 2d). A GOx solution was then added on the host sheet's surface and a self-assembled enzyme immobilization occurred (Fig. 2e). Finally, the MWCNT sheets were twisted into a yarn that contained 81.92 ± 3.56 wt% of the functional guest material that could act as a biomolecule-responsive material.

The biomolecule-responsiveness and mechanical properties of the fabricated yarn were then measured. The catalytic activity of the yarn was determined by using UV/Vis spectroscopy, and the results show that the reaction followed Michaelis–Menten kinetics (Fig. 3a). The catalytic activity of the yarn was measured as 1.68 U cm^{-1}. Next, the responsiveness toward glucose was compared between an enzyme-immobilized yarn and an enzyme-free yarn in a 20 mM PBS solution with 7 mN of constantly loaded tensile force at room temperature (Fig. 3b). After the yarn was exposed to a 50 mM glucose solution, the enzyme-immobilized yarn extended about three times more than the enzyme-free yarn. The extension of the enzyme-free yarn in Fig. 3b occurred because the phase transition of the PNIPAm was affected by the injected glucose molecules[18] but as shown in Fig. 4f, this effect is not significant when torsional actuation was triggered by the exothermic reaction of the enzyme. In addition, the yarn

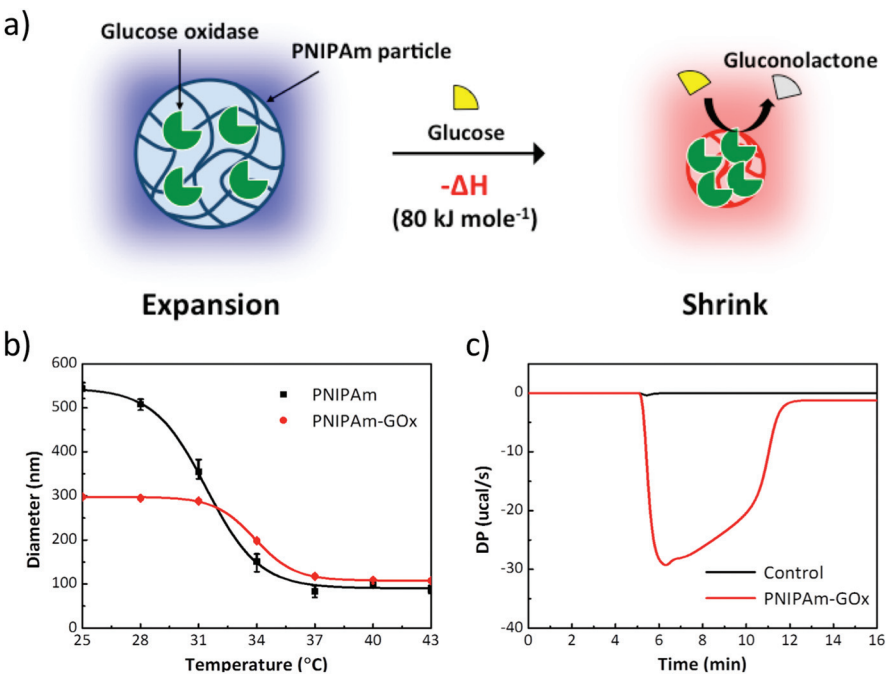

Fig. 1 The reaction mechanism and properties of a biomolecule-responsive PNIPAm–GOx particle. (a) Schematic drawing of the actuation mechanism of an enzyme-immobilized thermal response hydrogel particle. The volume of the particle changed, triggered by the exothermic catalytic reaction of the immobilized enzyme. (b) Change in the diameter of the poly(*N*-isopropylacrylamide) (PNIPAm) particle (black squares) and the GOx-immobilized PNIPAm particle (red circles) as a function of temperature (25–43 °C) when the particles were immersed in a 20 mM PBS solution (pH = 7.4). The fitted curve was calculated using the Boltzmann equation to represent the phase transition of the hydrogel particle. (c) Comparison of isothermal calorimetric titration experiments using a PBS solution as a blank control and 0.2 mg ml^{-1} PNIPAm–GOx in a PBS solution at 36 °C. 10 μl of 50 mM glucose in a PBS solution was injected into the calorimetric cell.

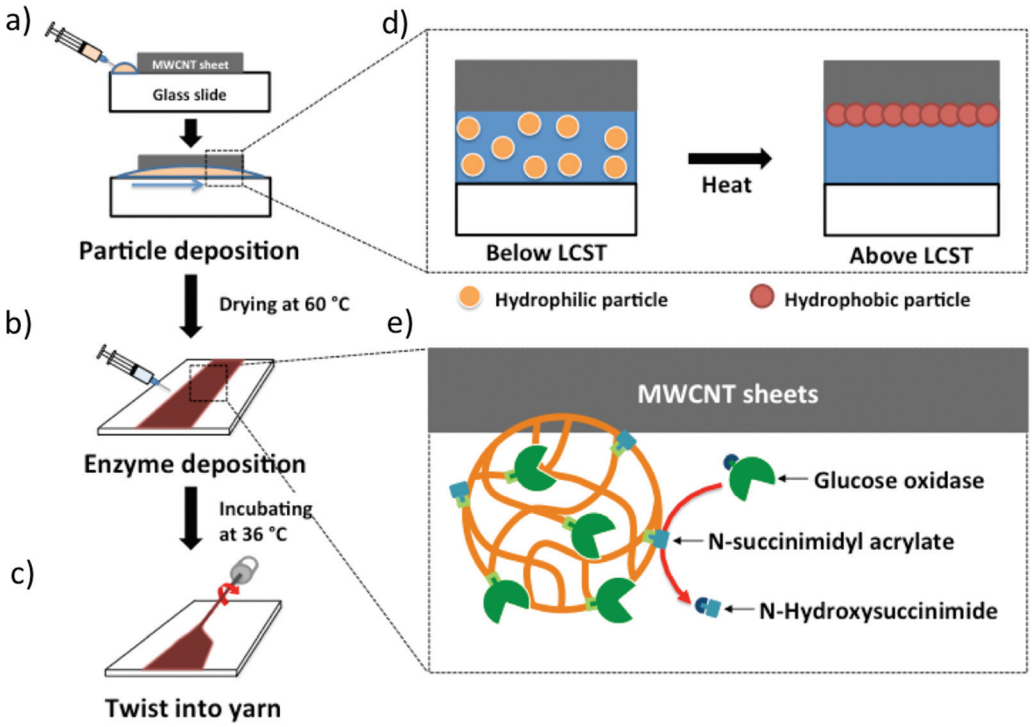

Fig. 2 Biomolecule-responsive biscrolled yarn fabrication. (a–c) Schematic of the floating aqueous solution based biscrolling procedure for entrapping PNIPAm–GOx particles into MWCNT yarns. (d) The mechanism of poly(NIPAm-NSA) particle absorption on the MWCNT surface. The hydrophobic interaction occurred due to the phase transition of PNIPAm at 60 °C. (e) The mechanism of self-assembled conjugation of the GOx to hydrogel particles. The introduced vinyl groups of a NSA residue react with the amine group of the enzyme.

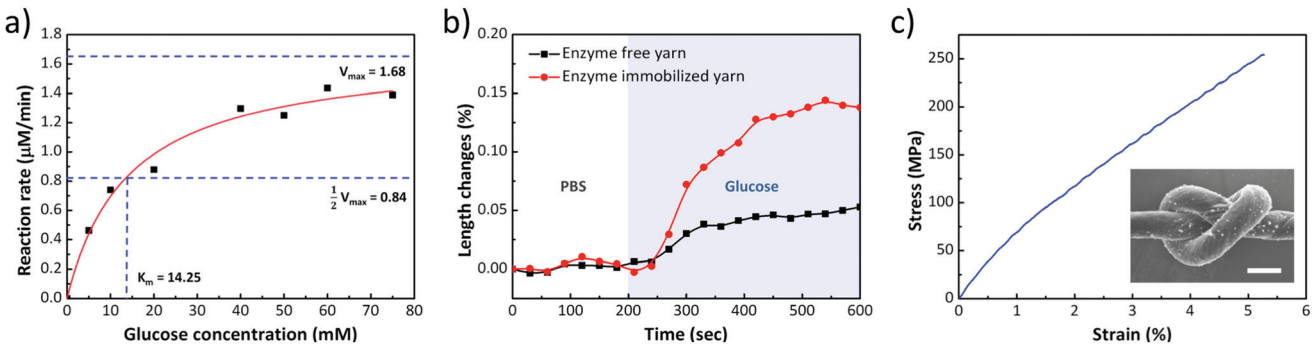

Fig. 3 Characteristics and properties of the PNIPAm–GOx biscrolled MWCNT yarn. (a) The rate of reaction of the enzyme (glucose oxidase)-immobilized biscrolled yarn as a function of the substrate (glucose) concentration at room temperature (25 °C). (b) Comparison of the change in the length of the enzyme-immobilized yarn (red) and enzyme-free yarn on injecting a 50 mM glucose solution. (c) Stress–strain curves of the enzyme-immobilized biscrolled yarn and a SEM image of a knotted enzyme-immobilized biscrolled yarn (inset). Scale bar = 50 μm. All experiments were measured with the yarn having an initial diameter of 50 μm and a length of 1 cm at room temperature (25 °C).

entrapped with up to 82 wt% of the enzyme-immobilized PNIPAm particle showed a tensile strength of 250 MPa with good flexibility (Fig. 3c), which is high enough to knit the yarn.

The torsional actuation configuration of a half-infiltrated two-tethered system was applied as described previously[11] (Fig. 4a). The thermally driven torsional actuation of a two-tethered system was tested to determine the potential temperature range of enzymatic actuation. The temperature range examined was determined by the catalytic activity of GOx. As shown in Fig. 4b, the rotation of the paddle occurred over the entire temperature range studied, which reveals that the LCST of the composite material was high. The immobilization of GOx did not have a significant effect on this phenomenon, but the rotation angle increased slightly.

To change the working solution sequentially in order to test the biothermal sensing actuation of the yarn-based artificial

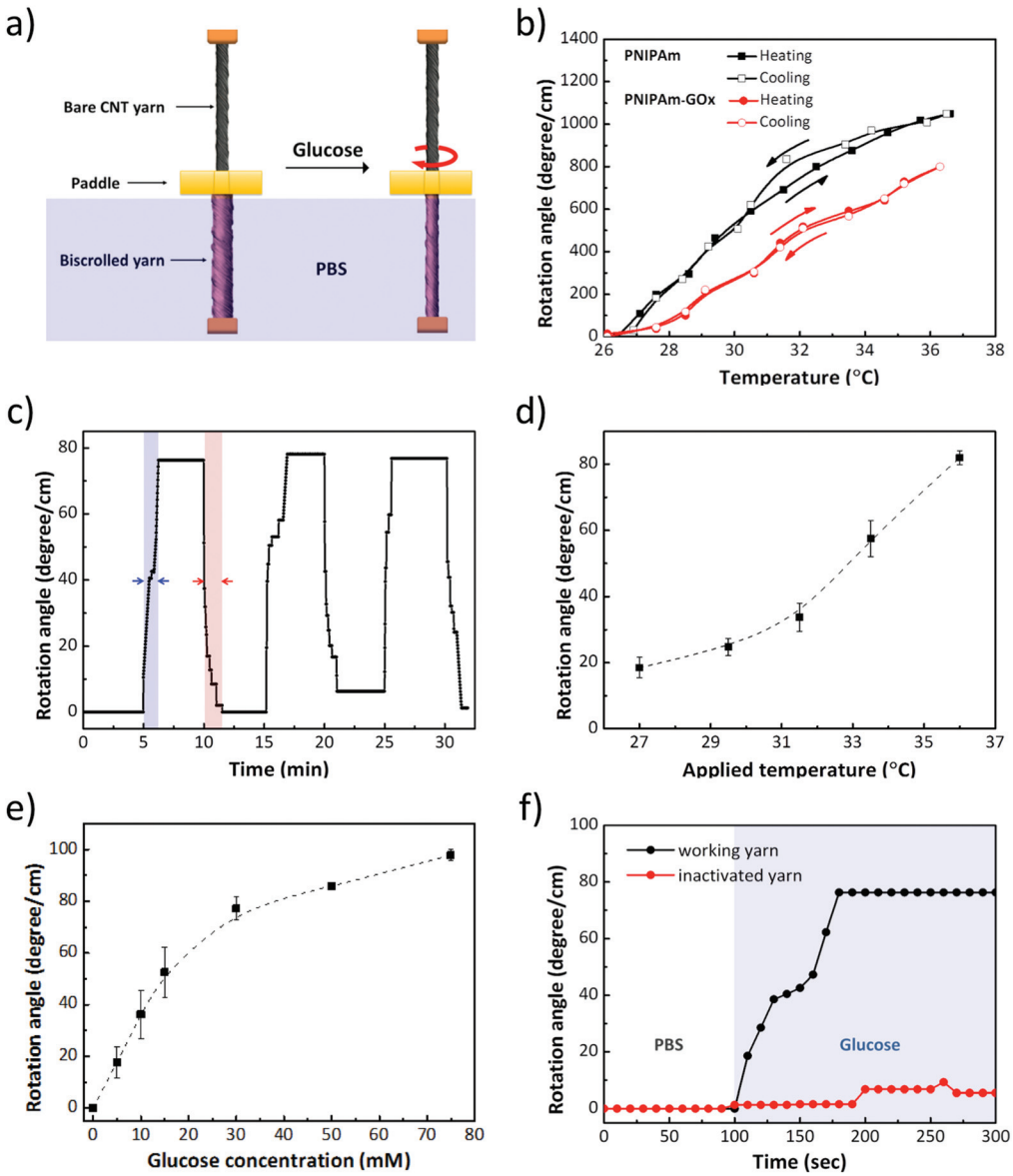

Fig. 4 Biomolecule responsive torsional actuations. (a) Schematic illustration of configuration for two-end-tethered, homochiral, half-guests contained a yarn torsional artificial muscle. (b) Rotation angle of the torsional actuation yarns as a function of temperature, the fabricated yarn entraps PNIPAm particles (black) or PNIPAm–GOx particles (red). (c) Reversible torsional actuation of biothermal sensing of a torsional artificial muscle *versus* time at 36 °C. (d) Rotation angle of glucose responding actuation for PNIPAm–GOx yarn *versus* applying temperature. The 50 mM glucose containing PBS solution is injected as a reactant. (e) Rotation angle of glucose responding actuation for the PNIPAm–GOx yarn *versus* applying concentration of glucose at 36 °C. (f) Rotation angle of glucose responding to actuation of the torsional actuation yarn *versus* times. 50 mM glucose containing PBS solution is injected. The torsional actuation yarns contain active enzyme (black) or deactivated enzyme (red).

muscle, the yarn was installed in a custom-built flow injection system, as shown in Fig. S1.† On sequential injections of a 50 mM glucose solution and a pure PBS solution, the yarn-based artificial muscle showed a reversible and fast actuation (Fig. 4c). Our biosensing actuator has high mechanical strength (~250 MPa) and fast response time (~60 s). The biscrolling method induces the fast actuation properties of hydrogel compared with those based on a hydrogel film (~24 h),[4] a Ppy film (~1200 s),[5] and a hydrogel composited silicone actuator (~1 hour).[19] Additionally, unlike other conventional hydrogels and polymer based biosensing actuators, our results show that high mechanical strength is achieved. The extraordinarily fast response time is attributed to the benefits that originate from using these materials and their structures. Fig. S2† shows that dipping the yarn in a PBS solution made the yarn contract within 1 min, and *vice versa* extension in the air. This result demonstrates that not only the speed of changing the particle volume but also the speed of entering the internal space of the yarn by water molecules increased. It can be considered that the diffusion capability improved because of the

capillary effect in the nanoscale corridors of the twisted yarn[20] and the shorter diffusion distance of the corresponding hydrogel by reducing the size of the hydrogel. Most of all, these benefits that originate from the structural aspects can also improve the speed of diffusion of the substrate biomolecules at the same time.[7]

Because we used heat to trigger the PNIPAm phase transition, the fast response of our system showed that the high thermal conductivity of the MWCNT[18,21,22] could help enhance the spread of heat over the entire area of the yarn homogeneously.[18] Despite an enzyme-controlled phase transition of the PNIPAm gel that was reported previously,[23] the gel expanded after the substrate biomolecule had been injected, even if the enzyme reaction was an exothermic event. This was explained by the fact that the concentration of the substrate and the products are consumed and generated by the enzyme reaction, respectively. The LCST of the gel was affected so that the volume change only occurred. Fig. S3† shows that the phase transition of our PNIPAm–GOx particle showed the same expansion in volume. This can occur when the rate of heat loss is greater than the rate of heat generation. Biscrolling PNIPAm–GOx particles with MWCNT sheets overcome this heat loss problem because of the increased enzyme loading at high concentration originating from the higher surface area and also the compressive forces on the twisted yarn.[17,20] In addition, covalently bonded enzymes and hydrogels can communicate more efficiently in transporting this energy at the molecular level.[23,24]

Fig. 4d shows that the torsional actuation had a nonlinear characteristic and reached a maximum at a temperature around 36 °C, which follows the kinetics of GOx. A plot of the rotation angle as a function of glucose concentration at 36 °C (Fig. 4e) shows Michaelis–Menten-like kinetics, similar to the results from the particle as shown in Fig. 3a. In addition, the artificial muscle yarn was inactivated on exposure to hot water (90 °C) for 10 min, a temperature at which an enzyme should be deactivated. After this treatment, the rotation properties significantly decreased (Fig. 4f).

The clockwise torsional actuation of the biothermal sensing artificial muscle occurred on injection of glucose solution (Movie S1†). Considering that the PNIPAm–GOx/MWCNT yarn is a twisted S-type yarn, this result shows that the PNIPAm particles had shrunk, because the temperature inside of the yarn had increased above the LCST. This means that this actuation event was triggered by an exothermic event. The relationship between the actuation performance at the working temperature and the substrate concentration showed a similar tendency with an immobilized enzyme, providing evidence that confirms that this exothermic event originated from the enzyme reaction. In addition, the fact that the yarn-based artificial muscle lost its activity, as the immobilized enzymes were inactive, also shows that this biomolecule-responsive actuation was enzyme dependent.

In conclusion, we have successfully demonstrated a fast and mechanically robust biomolecule-responsive torsional artificial muscle based on an enzyme-immobilized PNIPAm-entrapped MWCNT yarn. This fast response is attributed to an enhanced diffusion speed from reducing the size of the PNIPAm hydrogel and the short diffusion length in the corridor structure of the yarn that provides the capillary force, as well as from an enhanced efficiency in energy transportation by covalent bonding of the enzyme on the hydrogel. Our newly developed liquid-based biscrolling method enabled us to entrap 82 wt% of the aqueous functional microsized hydrogels onto host sheets without any additional treatment, which made the host hydrophilic while maintaining the mechanical benefits of the superhydrophobic host materials. Thus, we have overcome the inverse proportional relationship between the response speed and the mechanical properties. Because of the exothermal catalytic reaction, nonspecific events for most biochemical reactions can be used to trigger the actuation, and this system can be utilized as a versatile platform where biomolecule-responsive actuation is required, such as Bio-MEMS, drug delivery systems, and implantable biosensor systems which can be mostly utilized for biomedical applications.

Abbreviations

MWCNT	Multiwalled carbon nanotube
PNIPAm	Poly(*N*-isopropylacrylamide)
NSA	*N*-Succinimidyl acrylate
GOx	Glucose oxidase
LCST	Lower critical solution temperature

Competing interests

The authors declare no competing financial interests.

Author contributions

S-H. L. and S. J. K. conceived the idea and designed the experiments. S-H. L. and T. H. K fabricated the biscrolled yarn and performed the experiments. S-H. L. analyzed the data and wrote the manuscript. M. D. L. synthesized the spinnable CNT forests. S-H. L., R. H. B., and S. J. K. revised the manuscript. All authors discussed the results and commented on the manuscript.

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