Supporting Information

Behaviors of self-delivery lidocaine nano systems affected by intermolecular interaction

Lei Tang,[*a,b] *Feng Qin,*[c] *Deying Gong,*[a,b] *LiLi Pan,*[c] *Changcui Zhou,*[a,b] *Rui Ling,*[a,b] *Wenxue Shen,*[a,b] *Qin Fan,*[c] *Wenhao Yi,*[c] *Fengbo Wu,*[c] *Xiaoai Wu,*[c] *Weiyi Zhang*[a,b] *and Jun Yang*[*a,b]

a Department of Anesthesiology, West China Hospital, Sichuan University, Chengdu 610041, P.R. China.

b Laboratory of Anesthesia and Critical Care Medicine, National-Local Joint Engineering Research Centre of Translational Medicine of Anesthesiology, West China Hospital, Sichuan Universit, Chengdu 610041, P.R. China.

c West China Hospital, Sichuan University, Chengdu 610041, China.

Corresponding Author

*E-mail for Lei Tang: manstein_1984@sina.com.

*E-mail for Jun Yang: yang215jun@163.com.

Claim:

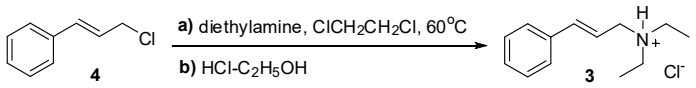
The animal experiments were performed in accordance with the guide for the care and use of medical laboratory animals (Ministry of Health, China). All animal procedures in this work were approved by the Institutional Animal Experimental Ethics Committee of Sichuan University (Chengdu, China, 13 Approval file No. 2015014A).

Table of Contents

SI.1 Synthesis and structure information

Method on the prapration of lidocaine salts.

HCl or HBr in gas form was slightly bubled into ehanol (500mL) with ice bath for 30min. These ethanol solution of HCl or HBr was saperately added into lidocaine 5.0g in 10mL ethanol at -5°C until pH < 2.0. The solvent was evapored. Ehyl actate 30mL was added, stired and filtered. The residue was washed by ehyl actate 10mL for 3 times and dried under vano to get white solid.



Synthesis of (E)-N,N-diethyl-3-phenylprop-2-en-1-aminium chloride (3)

Compound **4** (5.0g, 32.8mmol) was added into a flask and dissolved with 1,2-dichloroethane (50mL). Diethylamine (7.2g, 98.28mmol) was added dropwise at room temperature during 10min, and allowed to stir vigorously at 60℃ for 16h.

The reaction solution was cooled to room temperature, and washed with 20mL x 2 NaOH solution (1N in water). The organic layer was separated and removed in vacuo. The residue was purified by chromatography with CH_2Cl_2-CH_3OH as eluent, and mixed with HCl-ethanol 10mL to give slight yellow syrups as crude product. After mixed with ethyl acetate 10mL, this residue changed into solid, which was filtered and washed with ethyl acetate 5mL. The residue was dried under vacuo to

give product **3** as white powder, yield: 36%. Mp: 130.0~131.8 °C. ^{1}H NMR (400 MHz，$CDCl_3$)δ: 7.38~7.41 (m, 2H), 7.26~7.30 (m, 3H), 6.71 (d, *J* = 15.9Hz, 1H), 6.41 (dt, *J* = 15.9Hz, 7.4Hz, 1H), 3.77 (dd, *J* = 7.4Hz, 12Hz, 2H), 3.11 (q, *J* = 7.3 Hz, 4H), 1.40 (t, *J* = 7.3 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$)δ: 8.79, 46.14, 53.81, 116.36, 126.97, 128.75, 129.08, 134.77, 139.60.

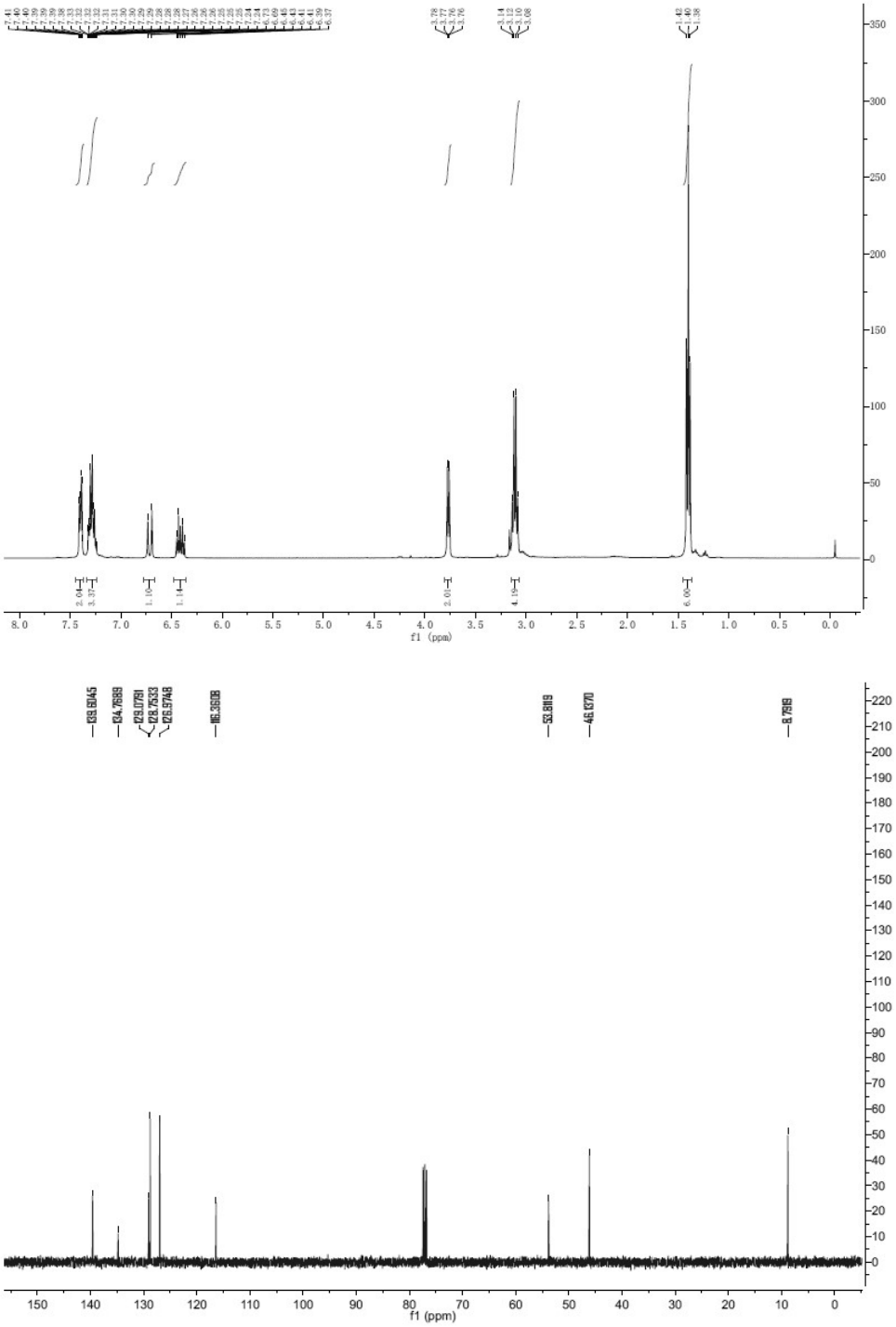


Figure S1 ^{1}H NMR, ^{13}C NMR of compound **3**

SI.2 TEM observation

General method on liquid sample preparation

The compound was dissolved in pure water (2mL) with vigorous magnetic stirring (1000 rpm) at 30℃ for 0.5 hour. Some compounds could not dissolve to be a clear solution. All the compound-water mixtures were filtered by needle filter (0.22μm) to get liquid samples. Almost all the operation before TEM observation should be avoided to get in touch with air, otherwise carbon dioxide in the air would probably react with lidocaine base and disturb the self-assemble of lidocaine salts.

pH determination of nano systems

Table S1 pH determination of lidocaine salts at 73.6mmol/L in water

Samples	**2a**	**2a** + CO_2	**2a** + CO_2 + N_2	**2b**
pH value	2.16	2.03	2.22	2.41

General method on TEM observation

The liquid sample for TEM observation were prepared by depositing a drop of solution (100μL) mentioned above onto a 400 mesh copper grid covered with thin amorphous carbon. A drop of phosphotungstic acid (2%, 100μL) was added for negative staining, which lasted for 10min. Then the sample would be observed by a Hitachi H-600 TEM (120KV) instrument.

Each sample was observed for at least twice. For each compound, we began observation from a relative lower concentration. If we could not find JMs at this concentration, we would observe this compound at a higher concentration until the sample was too thick to observe.

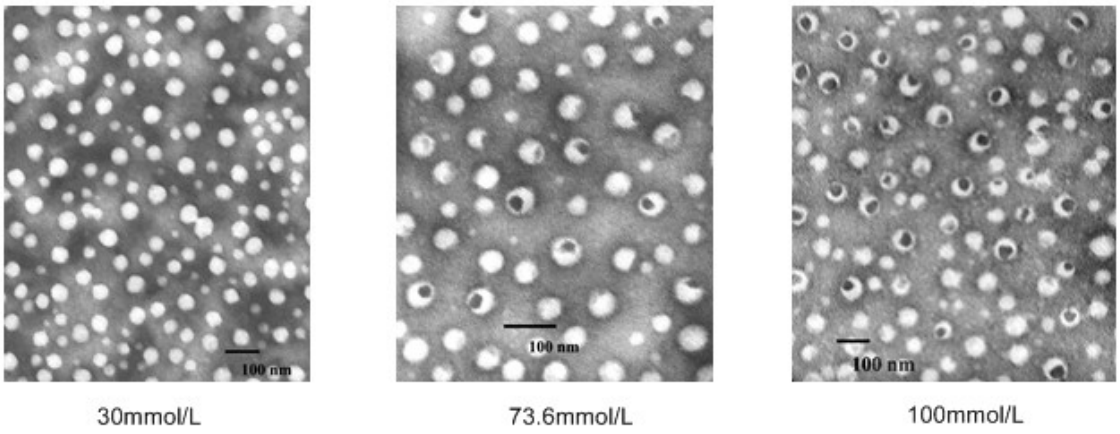


Figure S2 TEM images for **2a** in water on different concentration

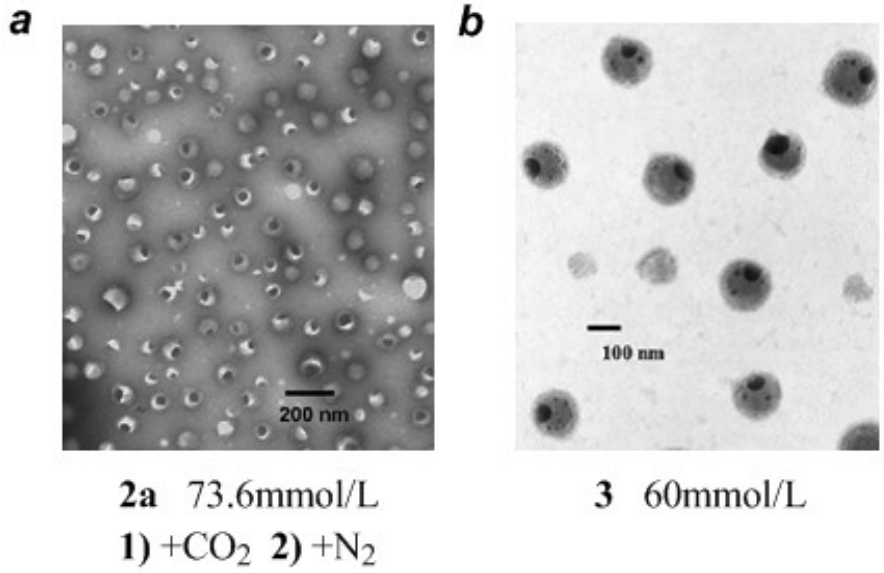


Figure S3 TEM images for **a) 2a** + CO_2 + N_2. **b) 3** in water.

pH value of nano systems self-assembled by **1a** at 73.6mmol/L was adjusted from 2.16 to 2.03 with hydrochloride acid (0.1N), which was equal to pH value of **1a** + CO_2 (Table S1). As shown in Figure S3, different from image of SMs-like particles, gourd-shaped JPs with almost same image of JPs self-assembled by **1a** (pH = 2.03) can be observed. It was suggested that image change lead by carbon dioxide addition was not caused by pH change but was directly relevant to carbon dioxide. In our opinion, the only reasonable explain for this result was influence of binary acid.

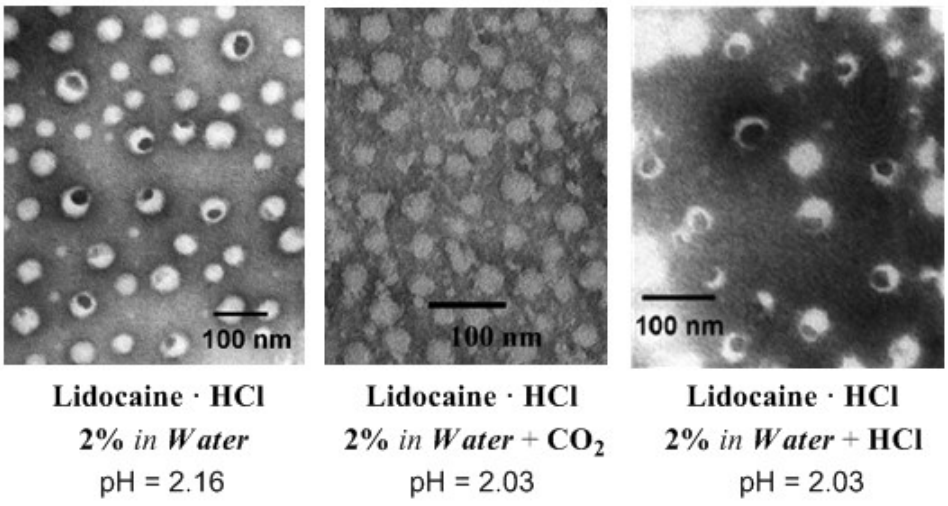


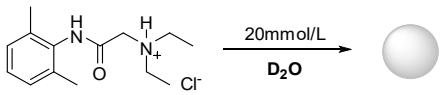
Figure S4 TEM images for **2a** at 73.6mmol/L in water.

SI.3 2D-NMR analysis

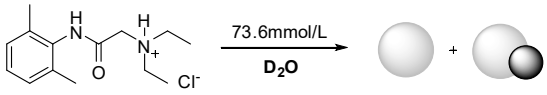
General method for sample preparation

The testing compound was dissolved in D_2O 0.5mL at the same concentration as TEM

observation, stirred at 1200rpm on room temperature. The solution was filtered by needle filter (0.22μm) to get the solution-like liquid samples.



As shown in Figure S2, lidocaine hydrochloride could only self-assemble into spherical micelles at 30mmol/L. At the same concentration, no signal could be found in ^{1}H-^{1}H NOESY spectrum. In our opinion, these spherical micelles were self-assembled by lidocaine hydrochloride in a usual manner for the supramolecular arrangement of small molecule amphiphilics: hydrogen atoms on different molecules were relative far away from each other.



According to the results of the 2D-NMR analysis for quaternary ammonium salts above, parallel-displaced π-π staking was thought to be of primary importance on the formation of these gourd-shaped Janus particles.

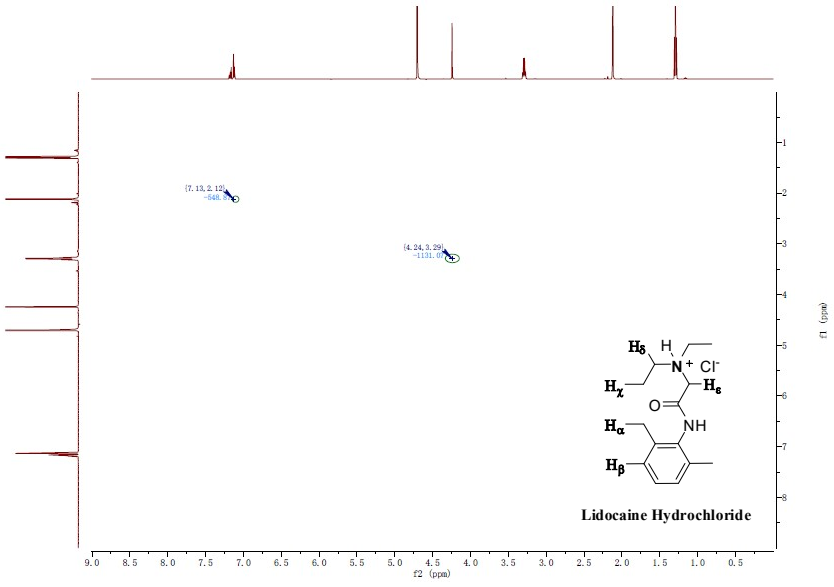


Figure S5 NOESY spectrum for **2a** in D_2O (73.6mmol/L)

As shown on Figure S2, at concentration of 73.6mmp/L, lidocaine hydrochloride could self-assemble into gourd-shaped JPs in water. The self-assembling of JPs by lidocaine hydrochloride in similar condition was thus considered to be correlated to π-π staking. In Figure S5 and S6, signal peak of H_α-H_β was observed clearly. This result verified the existence of parallel-displaced π-π

staking. Another signal peak of H_ε-H_δ could be found in NOESY spectrum. This result could also be explained: in this supramolecular system based on π-π staking, H_ε was closed enough to H_δ.

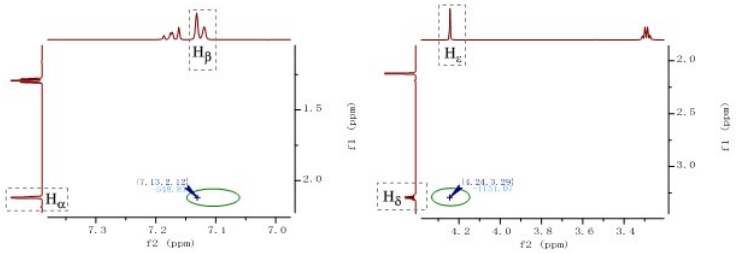


Figure S6 Amplified NOESY spectrum for **2a** in D_2O (73.6mmol/L)

Self-assembling mechanism of lidocaine hydrochloride in water was proposed by us in Figure S7. Both the spherical micelles and light-colored spheres of gourd-shaped Janus particles were self-assembled by single molecules with a distance from each other. No signal peaks on NOESY spectrum would be generated by these molecules. To be contrast, the black sphere of JPs was self-assembled by the molecules with the interaction of parallel-displaced π-π staking, and all the signals of NOESY spectrum were correlated to these molecules.

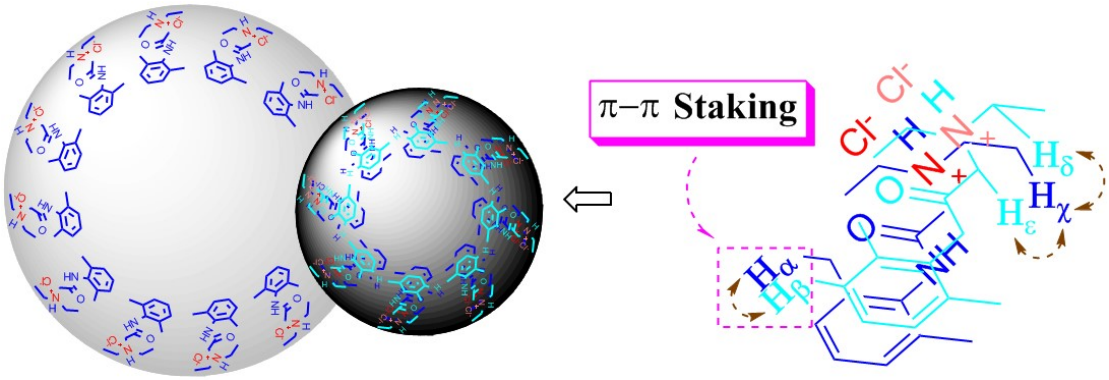
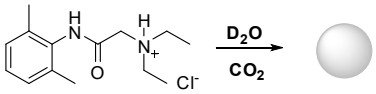


Figure S7 Proposed self-assemble mechanism for **2a** in D_2O (73.6mmol/L)



As shown in Scheme 1c in manuscript, when carbon dioxide was bubbled into the solution of lidocaine hydrochloride at 73.6mmol/L in water, gourd-shaped Janus particles were no longer detected, and only spherical micelles could be observed. NOESY spectrum indicated that parallel-displaced π-π staking was still existed, because signal peak of H_α-H_β could also be observed. Signal

peak of H_ε-H_δ was still existed. These two signals were in accordance with the arrangement manner of parallel-displaced π-π staking shown in Scheme 2. To be different, H_α was correlated to H_χ in this NOESY spectrum (Figure S8), which indicated that these two hydrogen atoms in different molecules were closed enough to each other.

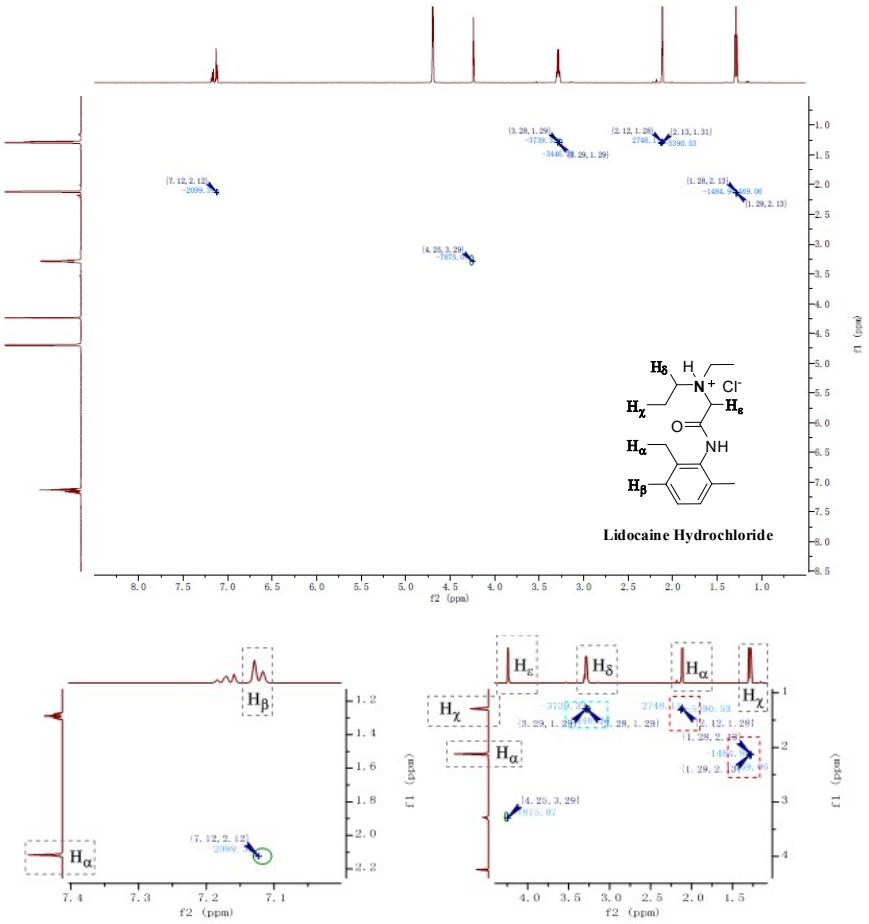


Figure S8 NOESY spectrum for **2a** in D_2O (73.6mmol/L) with **CO_2**

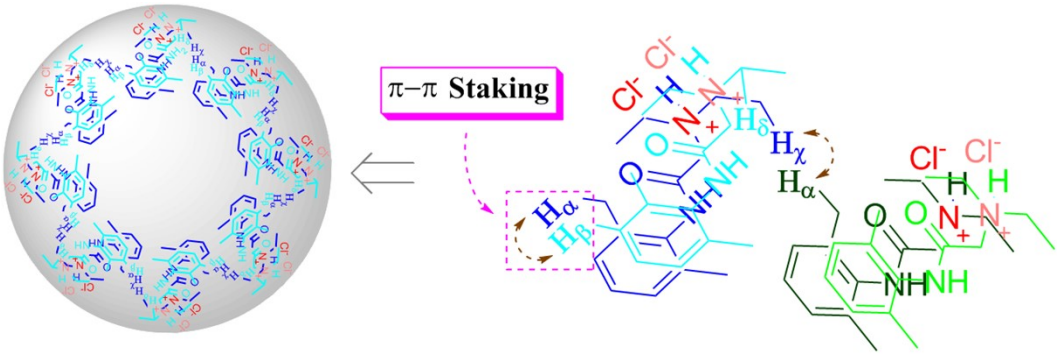
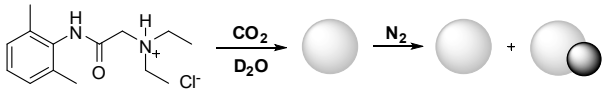


Figure S9 Proposed self-assemble mechanism for **2a** in D_2O (73.6mmol/L) with **CO_2**

Therefore, the only reasonable manner of supramolecular arrangement was summarized in

Figure S9. Compared to the arrangement manner on black sphere of JPs in Figure S7, molecules in these SMs-like particles were in a shorter distance to each other.



After N_2 gas was bubbled into sample of **2a-CO_2** to remove the carbon dioxide for 5min, gourd-shaped Janus particles as well as spherical micelles reappeared under TEM observation. Intermolecular ^{1}H-^{1}H NOESY spectrum showed that manner of supramolecular arrangement of this sample was similar to the manner of **2a** (73.6mmol/L) in water, as shown in Figure S10.

There were three groups of important signals:

First, H_α and H_β was correlated, which was in accordance with parallel-displaced π-π staking.

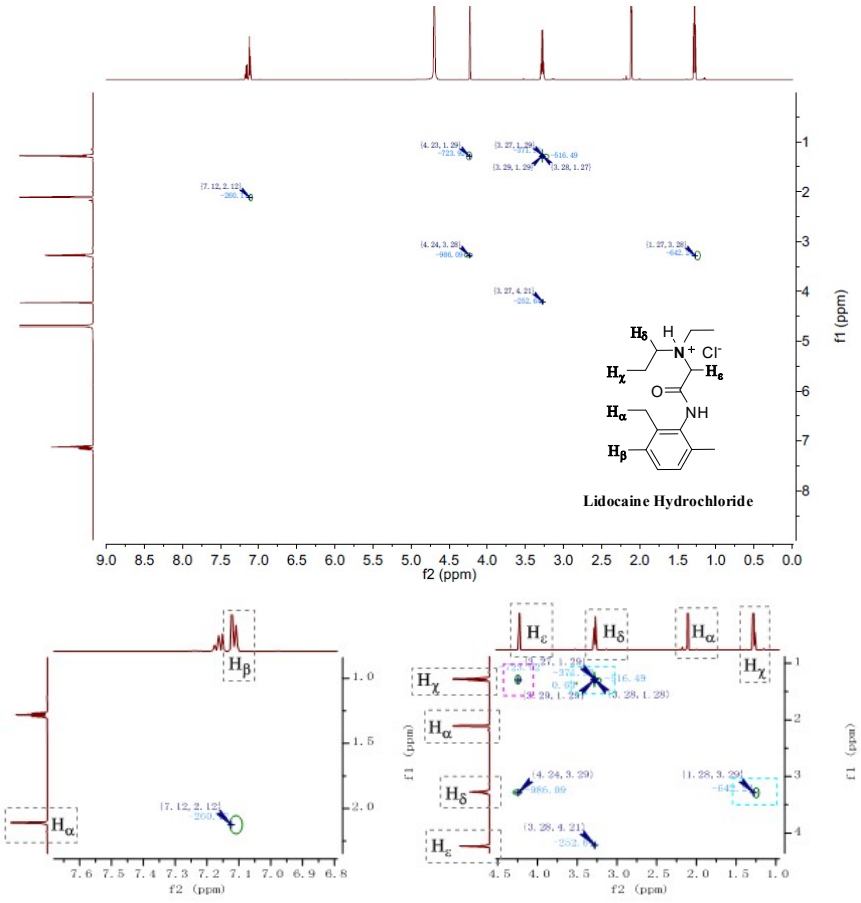


Figure S10 ^{1}H-^{1}H NOESY for **2a** in D_2O (73.6mmol/L) with **CO_2** and **N_2** bubbling

Second, signal of H_χ-H_δ could be found in this spectrum as well as spectrum of **2a-CO_2**, but cannot be observed on the spectrum of **2a** in water (Figure S11a and S11b).

Third, correlation signals of H_ε-H_χ can only be found on this spectrum (Figure S11c).

As results shown above, the intermolecular distance between H_ε to H_χ in this sample was shorter than the value in the sample of **2a** at 73.6mmol/L. H_χ was also closer to H_δ in different molecules in this sample compared to the their relative position in initial sample of **2a**. These results suggested that these two differences on intermolecular distance would not obviously prevent the formation of gourd-shaped Janus particles.

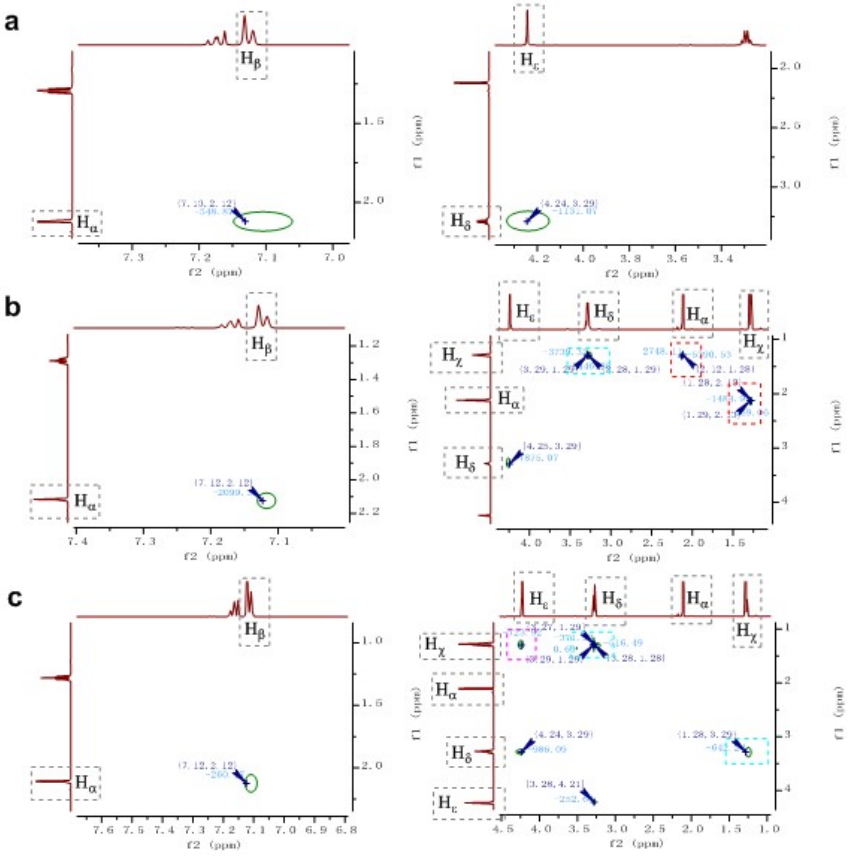


Figure S11 ^{1}H-^{1}H NOESY spectrum. **a) 2a** in D_2O (73.6mmol/L) **b)** with $\mathbf{CO_2}$ bubbling. **c)** with $\mathbf{N_2}$ bubbling.

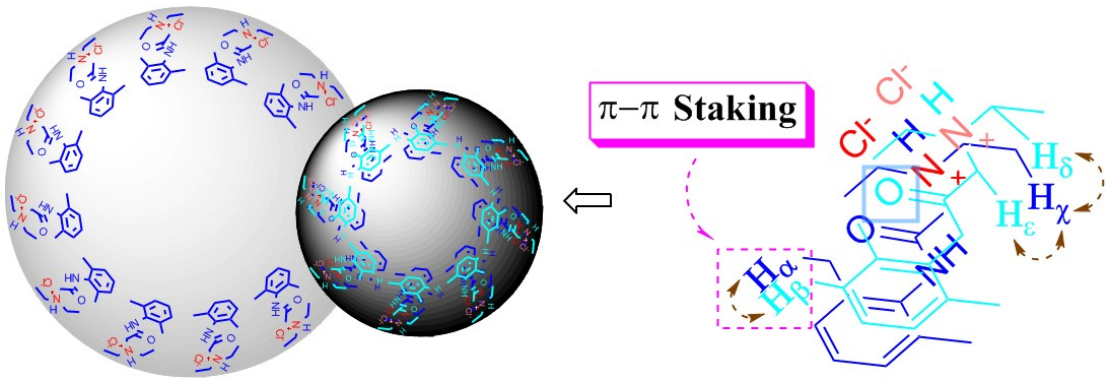
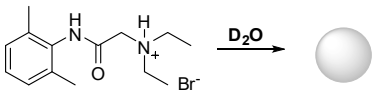


Figure S12 ^{1}H-^{1}H NOESY for **2a** in D_2O (73.6mmol/L) with $\mathbf{CO_2}$ and $\mathbf{N_2}$ bubbling

The manner of supramolecular arrangement similar as that of original JPs was thus summarized

in Figure S12 based on results above. The main difference was that H_χ, H_δ and H_ε in different molecules of **2a** in this sample were in shorter distance to each other than those in original sample of **2a** without any gas bubbling.



At the same concentration as lidocaine hydrochloride (73.6mmol/L) mentioned above, lidocaine hydrobromide cannot self-assemble into Janus particles in water. The only image that could be observed was spherical micelles (Scheme 1e), and the only signal that could be detected on NOESY spectrum was H_ε-H_δ (Figure S13). Parallel-displaced π-π staking did not exist.

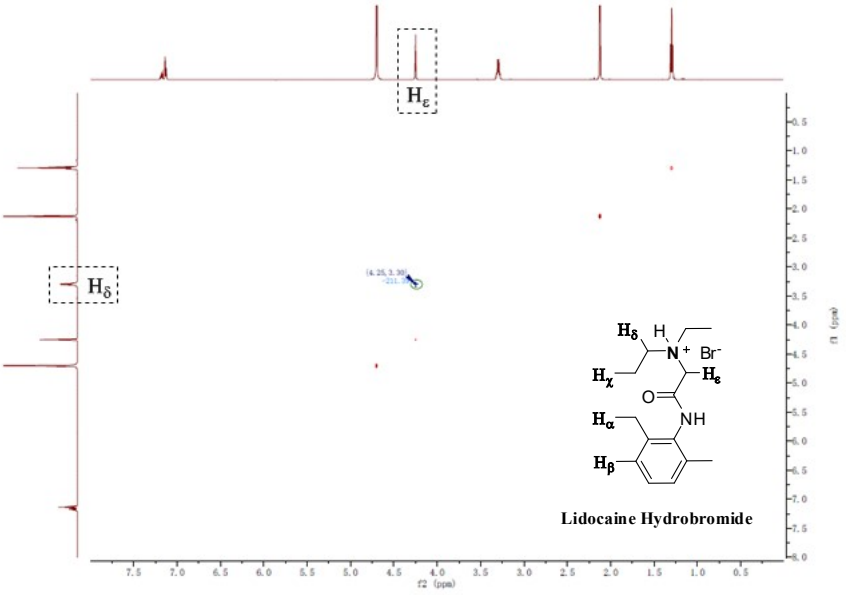


Figure S13 NOESY spectrum for **2b** (73.6mmol/L) in D_2O

Thus, the most reasonable supramolecular arrangement manner was shown in Figure S14: spherical micelles were self-assembled by single molecules in a more crowd manner than micelles formed by lidocaine hydrochloride.

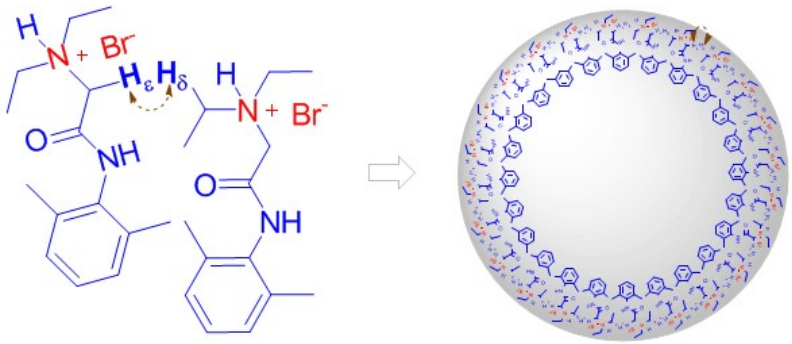
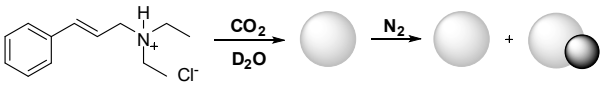
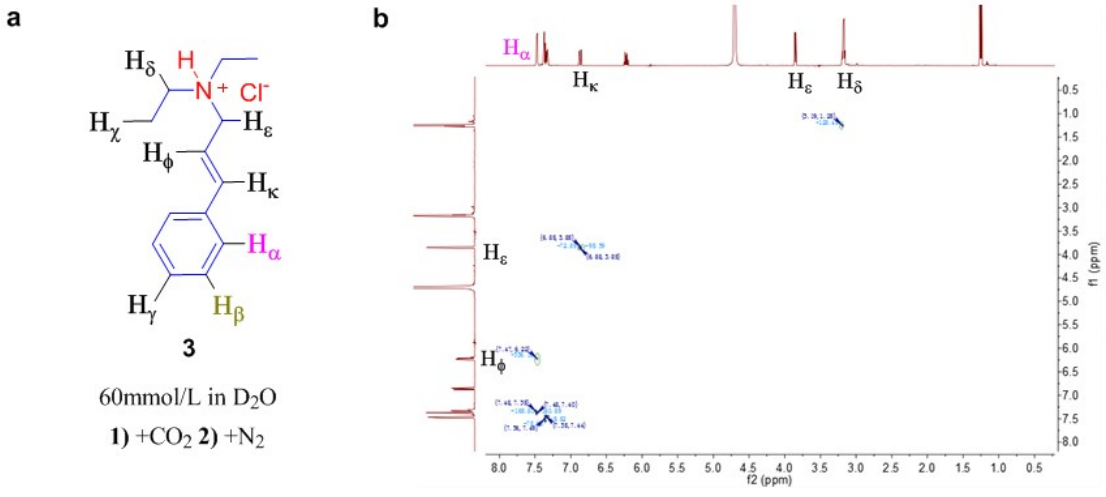


Figure S14 Proposed self-assemble mechanism for **2b** in D_2O



At concentration of 15mmol/L, **3** could only self-assemble into spherical micelles in water after CO_2 and N_2 bubbling. When water was replaced by D_2O, solution of **3** at same concentration was detected by intermolecular ^1H-^1H NOESY. No correlation signal was detected. These spherical micelles were therefore determined to be arranged by monomers of **3**.

The location of correlated signal peaks of H_α, H_β and H_γ on the aromatic ring of **3** (Figure S15a) was important on the analysis of intermolecular arrangement, otherwise π-π staking would be difficult to be proved. Signal peak of H_γ on ^1H NMR spectrum was determined due to the quantity of hydrogen atom first. H_α was next determined by the cleavage shape of signal peak, and the rest signal peak was therefore singed as H_β. H_κ, H_Φ, H_ε and H_δ were located by the consideration of chemical shift, shape of signal peak as well as coupling constant.



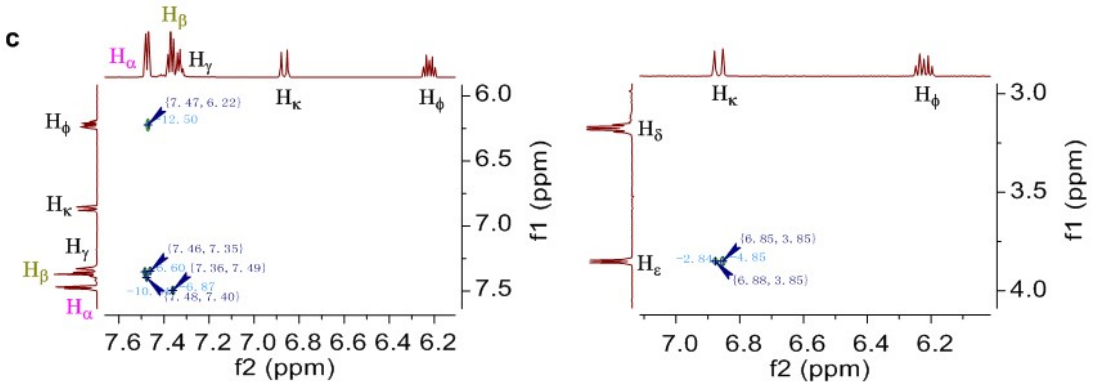


Figure S15 Proposed self-assemble mechanism for **3** in D_2O

Correlation signals of H_α-H_β as well as H_β-H_γ indicated parallel-displaced π-π staking. The result of H_ε correlated to H_κ was in accordance with our judgment on parallel-displaced π-π staking (Figure S15b and S15c). Correlation signal of H_α-H_Φ suggested that H_α was close enough to H_Φ in another molecule of **3**. According to analysis result of the intermolecular ^1H-^1H NOESY spectrum, π-π staking widely existed in this nano system. Similar to the JPs self-assembled by **2a**, the deep-colored spheres of JPs were believed to be consisted of dimmers with π-π interaction, and the light-colored spheres were arranged by monomers of **3** without intermolecular π-π interaction (Figure S16). The reasonable arrangement manner based on the short distance between H_α and H_Φ was exhibited in Figure S14, which might lead different dimmers of **3** closer to each other in these dark-colored spheres of JPs than different dimmers of **2a**.

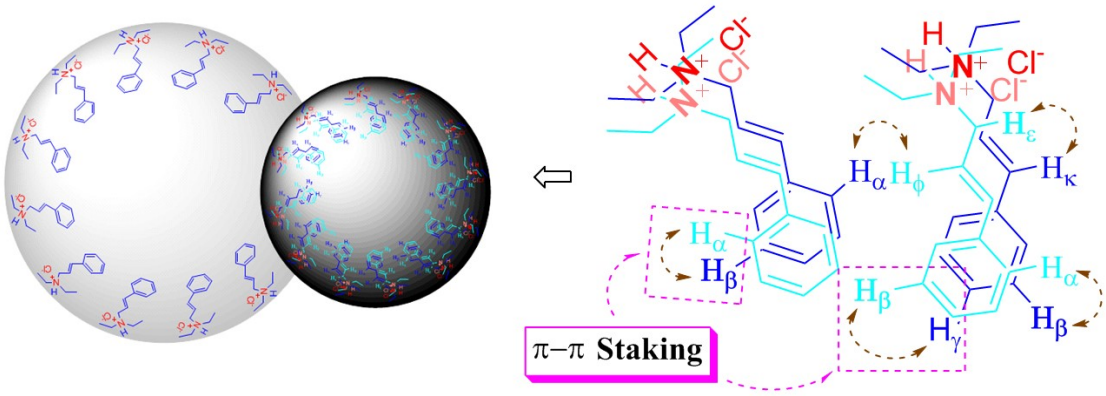
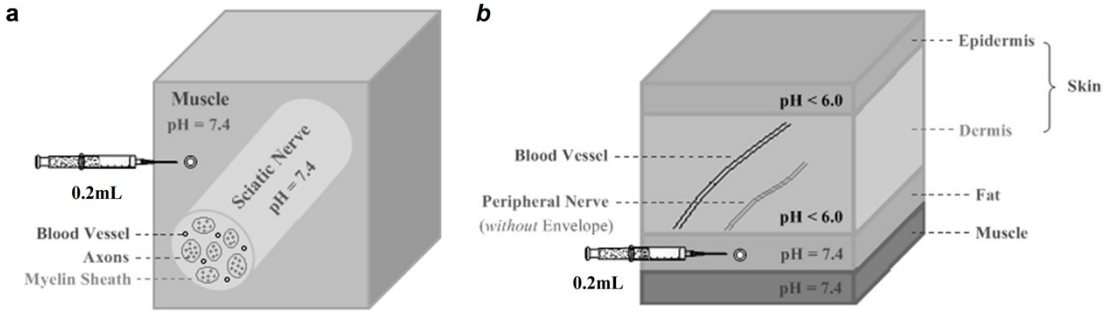


Figure S16 Proposed self-assemble mechanism for **3** in D_2O

In summary, dark-colored spheres of gourd-shaped Janus particles self-assembled by **2a** or **3** were arranged by dimmers of these amphiphilic molecules based on π-π staking, which were similar to JPs self-assembled by **1** as cationic lidocaine derivatives.

SI.4 Rats sensory block test

Two models were used in rats' sensory block test, as shown in Scheme S1.



Scheme S1 a) Model of rats' sciatic nerve block. **b)** Model of rats' intradermal administration.

Method on Rats' sciatic block

Sprague Dowley rats (male, Dossy Experimental Animal Company, Chengdu, China) weighted 180~200g were housed at room temperature and moiety of 40%~60%, in 12h light/12h dark cycle with free access to food and water. Animals were acclimated to experimental environments before tests. During acclimation, baseline of sensory was measured every day for three consecutive days, averaged, and recorded. Those with normal baselines were used, and randomized into groups (n = 8 for each group).

Rats received sciatic nerve block under sedation with inhaled 1.5%~2.0% isoflurane (v/v %) mixing with oxygen. 0.2 ml of test solution was injected through a 27-Gauge syringe that was inserted at the mid-point between trochanter and ischial tuberosity.[S1,S2] 100% successful rate was obtained with this method using common local anesthetics, and no signs of nerve injury was observed when saline was used in preliminary tests.

Sensation of the injected limb was evaluated after sciatic nerve block by revised hot plate test at 10min after injection, every 0.5 hours from 1 hour until the sensory block offset. The paw withdrawal latency (PWL) induced by heat stimulation represents the degree of sensation block. The baseline of PWL lasted no longer than 3s, and the cutoff time of PWL was set at 12s to avoid tissue injury. PWL > 6s was considered effective nociceptive blockade. The time for PWL to exceed 6s was the onset time of sensory block; the time for PWL to return below 6s was the offset of sensory block: the interval between onset and offset time was the duration of sensory blockade.

Systemic adverse effect was observed during the injection and observation period, and every day within 7 days after injection.

Method on Rats' infiltration sensory block

Sprague Dowley male rats weighted 180~220g were housed at room temperature and moiety of 40%~60%, in 12h light/12h dark cycle with free access to food and water. Animals were acclimated to experimental environments before tests. During acclimation, baseline of sensory was measured every day for three consecutive days, averaged, and recorded. Those with normal baselines were used, and randomized into groups (n = 8 for each group).

Rats received sciatic infiltration sensory block under sedation with inhaled 1.5%~2.0% isoflurane (v/v%) mixing with oxygen. A part of skin on rats' back was shaved off, and 0.2 ml of test solution was injected through a 27-Gauge syringe. The edge of swelling skin was marked (Figure S17).

A particular tool was prepared for sensation evaluation. The needle of the syringe (1mL) was attached to the probe of von Frey tool (26g) with adhesive tape (Figure S18). The needle tip was slightly blunted, so that the skin would not be penetrated by tested this tool. On each time point, the marked skin of every rat was tested with this processed tool 5 times. Rats that did not respond to more than 3 times of the stimuli were judged to be still effective. Sensation of the marked skin was evaluated with this tool beginning at 2 hours after injection, and continued to be tested at the time point of 1.5h, 2h, 2.5h and 3h. For the tested rats whose sensory block duration were longer than 3.5h, the next time point was 6h, and then every 1h until the sensory block offset. The strange choice of time point was based on the result of our pre-test, otherwise too many tests might interfere the results of pathology examination.

Figure S17 Images of rats to be tested for infiltration sensory block on skin.

Figure S18 Processed tool for sensory block test.

Results

Four groups of lidocaine salts were tested on rats' sciatic nerve block and rats' infiltration sensory block separately. As shown in Table S2, the duration of sensory block were different among sample of lidocaine hydrochloride, lidocaine hydrochloride + carbon dioxide and lidocaine hydrobromide. Result of lidocaine hydrochloride + CO_2 + N_2 was approximate to the result of lidocaine hydrochloride. Because the duration data might be of slight difference on the determination measured by different operators, all these operations were completed by the same researcher. The differences among sciatic nerve block duration of these three groups seem to be insignificant.

Table S2 Sensory block duration of lidocaine salts (73.6mmol/L)

Sample	Location	Sensory Block Duration (hours)								
		Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Mean
Lidocaine • HCl	Sciatic Nerve	2	2	2.5	2.5	2.5	2.5	2	2	2.3
	Skin	2.5	3	2.5	2.5	2.5	2.5	2.5	3	2.6
Lidocaine • HCl + CO_2	Sciatic Nerve	2.5	2.5	2.5	3	3	2.5	3	2.5	2.7
	Skin	3	3	3	2.5	3	2.5	2.5	2.5	2.8
Lidocaine • HCl + CO_2 + N_2	Sciatic Nerve	2.5	2	2.5	2.5	2	2.5	2	2.5	2.3
	Skin	2.5	3	3	2.5	2.5	2.5	2.5	3	2.7
Lidocaine • HBr	Sciatic Nerve	2	2	2.5	2.5	2	2.5	1.5	2	2.1
	Skin	8	8	8	8	8	7	8	7	7.8

Table S3 Stimulus response in different time point for sciatic nerve block of lidocaine salts

Time	Sample	Stimulus Response							
		Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8
1h	Lidocaine • HCl	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	Lidocaine • HCl+ CO_2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	Lidocaine • HBr	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
1.5h	Lidocaine • HCl	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	Lidocaine • HCl+ CO_2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

	Lidocaine • HBr	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
2h	**Lidocaine • HCl**	2/5	2/5	0/5	2/5	0/5	0/5	1/5	1/5
	Lidocaine • HCl+ CO_2	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
	Lidocaine • HBr	2/5	2/5	0/5	2/5	0/5	1/5	4/5	0/5
2.5h	**Lidocaine • HCl**	4/5	3/5	1/5	0/5	1/5	0/5	4/5	4/5
	Lidocaine • HCl+ CO_2	1/5	1/5	2/5	0/5	1/5	1/5	0/5	0/5
	Lidocaine • HBr	4/5	3/5	1/5	0/5	4/5	0/5	4/5	4/5
3h	**Lidocaine • HCl**	3/5	4/5	3/5	4/5	3/5	4/5	5/5	5/5
	Lidocaine • HCl+ CO_2	4/5	3/5	4/5	0/5	2/5	3/5	1/5	3/5
	Lidocaine • HBr	4/5	5/5	3/5	4/5	5/5	4/5	5/5	5/5
3.5h	**Lidocaine • HCl**	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
	Lidocaine • HCl+ CO_2	5/5	4/5	5/5	4/5	3/5	4/5	5/5	4/5
	Lidocaine • HBr	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

Table S4 Stimulus response in different time point for intradermal administration block of lidocaine salts

Time	**Sample**	**Stimulus Response**							
		Run 1	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Run 6**	**Run 7**	**Run 8**
1.5h	**Lidocaine • HCl**	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
	Lidocaine • HCl+ CO_2	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
	Lidocaine • HBr	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
2h	**Lidocaine • HCl**	2/5	0/5	2/5	2/5	0/5	1/5	1/5	0/5
	Lidocaine • HCl+ CO_2	1/5	0/5	0/5	2/5	1/5	2/5	1/5	2/5
	Lidocaine • HBr	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
2.5h	**Lidocaine • HCl**	2/5	1/5	1/5	2/5	1/5	2/5	2/5	1/5
	Lidocaine • HCl+ CO_2	1/5	1/5	2/5	1/5	0/5	2/5	2/5	2/5
	Lidocaine • HBr	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
3h	**Lidocaine • HCl**	4/5	2/5	3/5	5/5	4/5	4/5	5/5	1/5
	Lidocaine • HCl+ CO_2	2/5	1/5	2/5	4/5	1/5	5/5	4/5	4/5
	Lidocaine • HBr	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
3.5h	**Lidocaine • HCl**	4/5	4/5	5/5	5/5	5/5	4/5	4/5	5/5
	Lidocaine • HCl+ CO_2	4/5	3/5	4/5	5/5	5/5	4/5	5/5	5/5
	Lidocaine • HBr	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
7h	**Lidocaine • HCl**	-	-	-	-	-	-	-	-
	Lidocaine • HCl+ CO_2	-	-	-	-	-	-	-	-
	Lidocaine • HBr	1/5	1/5	2/5	0/5	1/5	2/5	0/5	1/5
8h	**Lidocaine • HCl**	-	-	-	-	-	-	-	-
	Lidocaine • HCl+ CO_2	-	-	-	-	-	-	-	-
	Lidocaine • HBr	1/5	2/5	2/5	2/5	1/5	4/5	0/5	4/5
9h	**Lidocaine • HCl**	-	-	-	-	-	-	-	-
	Lidocaine • HCl+ CO_2	-	-	-	-	-	-	-	-
	Lidocaine • HBr	3/5	5/5	4/5	5/5	3/5	5/5	3/5	5/5

However, with initial data of stimulus response (Table S3 and S4), we can find lidocaine hydrochloride was obviously different from lidocaine hydrochloride with carbon dioxide on sensory block duration: 1) Stimulus response was more frequently for **Lidocaine • HCl** than **Lidocaine • HCl + CO_2** at 2h and 2.5h**.** 2) In the group of **Lidocaine • HCl + CO_2**, rats' sensory function were not recover completely at 3.5h, which was absolutely different from the results in **Lidocaine • HCl** group.

SI.5 Slow release on local tissue measurement

General method on remaining test for sciatic nerve and muscular tissue:

After intraperitoneal pentobarbital, rats received sciatic nerve block with lidocaine hydrochloride, lidocaine hydrochloride with carbon dioxide and lidocaine hydrobromide at 73.6mmol/L. Then each rat was injected with 0.2ml solution. Tissues of sciatic nerve at injection site with adjacent 0.5g muscles and connective tissues were harvested. These tissues were crashed and grinded. Each tissue homogenates was added with formic acid solution (0.5% in water) to make them at the same weight of 6.0g. The homogenates were centrifuged at 4°C for 10000rpm/min during 10min, and 1.0g of the supernatant for each group was separately. 100μL of the supernatant for each group were added separately into acetonitrile 900μL and d_{10}-lodocaine solution (10ng/mL, 5μL), and centrifuged at 4°C for 10000rpm/min during 10min. 300μL of the supernatant for each group were used as an individual sample for LC-MS-MS test.The sample for 0 hour was similar with the operation above, and the only difference was that 0.2ml solution without injection was added directly into the mixture of crashed tissues and formic acid solution.

LC-MS-MS condition

The LC–MS–MS system consisted of an Agilent 1260 liquid chromatograph and a 6460 triple quadrupole mass spectrometer with an electrospray ionization source. Data were analyzed by MassHunter software (Agilent Corporation, MA, USA). Separation was on an Agilent Extend C18 column (100 mm × 3 mm, 3.5 μm) by isocratic elution with 0.05% formic acid/acetonitrile (84:16, v/v), at a flow rate of 0.3mL/min, and the total run time was 7 min for each injection.

Mass spectrometry conditions were set as follows: sheath gas flow 11.0L/min; sheath gas heater temperature 350℃, nebulizer pressure 45 psi, capillary voltage 3500V. Quantification was performed by multiple reactions monitoring (MRM) mode of the transitions at m/z 235.1 → 86.1

for lidocaine, and m/z 245.2 → 96.2 for d_{10}-lidocaine.

Table S5 Sciatic Nerve and Muscular Tissue Remain of Lidocaine • HCl (73.6mmol/L) after Injection

Time Point	Local Issue Remain (ng/mL)						Remain (%)
	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	
0	567801.86	578549.85	564871.59				100
0.5h	58959.46	42302.75	80082.47	35703.93	42394.99	50296.92	9.05
1h	13030.70	15186.71	12263.48	22316.60	19823.83	10402.10	2.72
1.5h	5294.95	7366.50	6580.24	5029.69	4763.49	6428.07	1.04
2h	2766.14	3114.57	3342.27	2323.15	3342.27	3160.39	0.53
2.5h	1223.65	1704.19	1151.13	1042.34	1444.18	1735.79	0.24
3h	689.38	732.86	774.38	647.12	540.88	666.42	0.12

Table S6 Sciatic Nerve and Muscular Tissue Remain of Lidocaine • HCl (73.6mmol/L) + CO_2 after Injection

Time Point	Local Issue Remain (ng/mL)						Remain (%)
	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	
0	473777.12	470152.38	458896.62				100
0.5h	32155.34	55099.92	32274.34	42115.94	57711.98	38889.64	9.20
1h	20432.97	13733.09	22887.58	25634.19	13994.34	25238.66	4.35
1.5h	8521.72	4937.17	8594.79	5043.00	4626.19	7506.06	1.40
2h	3008.79	3136.30	3745.69	3241.62	3889.64	3112.07	0.72
2.5h	1778.29	1480.43	2388.38	1898.74	1001.42	1704.19	0.37
3h	494.74	583.51	747.42	932.11	750.97	984.51	0.16

Table S7 Sciatic Nerve and Muscular Tissue Remain of Lidocaine • HBr (73.6mmol/L) after Injection

Time Point	Local Issue Remain (ng/mL)						Remain (%)
	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	
0	561397.57	547116.30	588070.21				100
0.5h	146109.10	138089.40	82582.65	69430.22	93934.99	145046.90	19.90
1h	8138.76	4959.54	8138.76	6024.36	4859.56	8880.80	1.21
2h	2338.83	3107.34	3863.26	2524.57	3389.27	2017.76	0.51
4h	1305.24	1394.20	985.36	938.63	1394.20	1015.22	0.21
6h	364.25	289.67	518.20	319.74	422.53	150.86	0.06

The results of lidocaine hydrochloride, lidocaine hydrochloride with carbon dioxide and lidocaine hydrobromide in sciatic nerve and muscular tissue were shown in Table S5 to Table S7. Sample of lidocaine hydrobromide (**2b**) showed slowest diffusion with in 30min after injection, but declined more quickly than the other two groups after 1h. After time point of 30min, lidocaine hydrochloride (**2a**) with carbon dioxide bubbled that afforded longest sciatic nerve block exhibited higher remaining percentage than the other two groups.

General method on *in vivo* remaining test for lidocaine salts on skin:

After intraperitoneal pentobarbital, rats received sciatic nerve block with lidocaine hydrochloride, lidocaine hydrochloride with carbon dioxide and lidocaine hydrobromide at 73.6mmol/L. Then each rat was injected with 0.2ml solution. 1.0g of skin tissues around the injection site was harvested.

These tissues were crashed and grinded in frozen condition separately. Each tissue homogenates was added with formic acid solution (0.5% in water) to make them at the same weight of 6.0g. The homogenates were centrifuged at 4°C for 10000rpm/min during 10min, and 1.0g of the supernatant for each group was separately. 100μL of the supernatant for each group were added separately into acetonitrile 900μL and d_{10}-lodocaine solution (10ng/mL, 5μL), and centrifuged at 4°C for 10000rpm/min during 10min. 300μL of the supernatant for each group were used as an individual sample for LC-MS-MS test. The sample for 0 hour was similar with the operation above, and the only difference was that 0.2ml solution without injection was added directly into the mixture of crashed tissues and formic acid solution. LC-Ms-Ms condition was same to that described in general method on remaining test for sciatic nerve and muscular tissue**.**

Results

The results were shown from Table S8 to Table S10 below. With obviously shorter block duration (2.7h) than sample of ldiocaine hydrobromide (**2b**, 7.8h), sample of lidocaine hydrochloride (**2a**) with carbon dioxide diffused more slowly than the other two groups. This result indicated that **2a** with carbon dioxide could remain in local tissue of skin for a long time, but difficult to release single molecules to block the target.

Table S8 Skin Remaining of Lidocaine • HCl (73.6mmol/L) after Injection

Time Point	Local Issue Remain (ng/mL)						Remain (%)
	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	
0	610168.61	533725.80	625221.60				100
0.5h	128079.50	123026.80	127256.70	113880.60	116512.80	158257.20	21.68
1h	26039.80	16753.57	16405.33	15674.83	27876.01	20208.63	3.48
1.5h	8148.82	12516.08	7700.61	15438.93	11152.68	16753.57	2.03
2h	3819.91	6373.63	6346.56	5491.82	7558.61	6854.81	1.03
2.5h	4261.91	2951.89	4563.68	2574.46	1381.46	5488.66	0.60
3h	1518.09	1075.86	1203.21	982.23	885.42	1635.25	0.21

Table S9 Skin Remianing of Lidocaine • HCl (73.6mmol/L) + CO_2 after Injection

Time Point	Local Issue Remain (ng/mL)						Remain (%)
	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	
0	577539.74	505497.00	530931.20				100
0.5h	225583.20	247934.60	271296.10	175228.00	226394.10	241992.40	43.01
1h	106686.47	91459.62	99525.79	77099.42	70194.50	87086.16	16.48
1.5h	28821.82	46670.18	36318.03	27742.56	36788.06	27724.97	6.32
2h	17695.21	18613.43	22595.16	14913.85	20541.44	16913.10	3.45
2.5h	8350.94	10401.69	12999.64	9312.39	14859.56	10010.20	2.04
3h	1823.82	842.48	1402.26	1641.59	725.69	1754.66	0.25

Table S10 Skin Remaining of Lidocaine • HBr (73.6mmol/L) after Injection

Time Point	Local Issue Remain (ng/mL)						Remain (%)
	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	
0	695168.73	655835.61	603261.56				100
0.5h	167222.20	187304.90	126998.50	181520.60	206720.60	163402.10	26.43
1h	23537.16	42760.18	50469.44	22393.39	44648.26	29523.06	5.46
2h	10445.14	9059.94	11960.98	12016.41	10588.81	18870.80	1.87
4h	8459.24	8552.35	8916.86	7093.57	8601.91	8770.63	1.29
6h	5774.90	3644.60	4172.27	3693.27	5180.10	4781.96	0.70
8h	1226.98	2664.54	2180.26	2112.44	2319.44	1646.23	0.31

SI.6 Score of pathological injury

Table S11 Pathological injury scoring of lidocaine salts (73.6mmol/L) on skin

Sample	Location	Pathological Injury Scoring								
		Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Mean
Lidocaine • HCl	Sciatic Nerve	0	0	0	0	0	0	0	0	0
	Skin	1	2	1	1	2	2	2	1	1.5
Lidocaine • HCl + CO_2	Sciatic Nerve	0	0	0	0	0	0	0	0	0
	Skin	2	1	1	1	2	1	1	1	1.25
Lidocaine • HCl + CO_2 + N_2	Sciatic Nerve	0	0	0	0	0	0	0	0	0
	Skin	1	2	1	1	1	2	2	1	1.4
Lidocaine • HBr	Sciatic Nerve	0	0	0	0	0	0	0	0	0
	Skin	2	1	1	1	1	2	2	2	1.5

Rats' sciatic nerve

Rats injected by the solutions of lidocaine salts were killed 7 days after injection. The sciatic nerve was collected as 3 parallel samples with same distance from injection site for each nerve and prepared as histopathologic section with HE stain. The neuropathic injury will be scored based on the images shown in Figure S19.

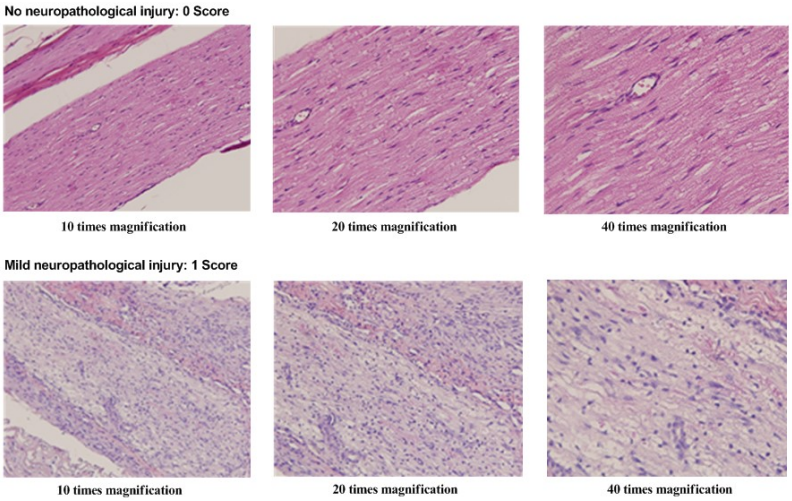


Figure S19 Principles of neuropathological injury scoring

Rats' skin

Rats injected by lidocaine salts were killed 7 days after injection. The skin around the injection site was collected and prepared as histopathologic section with HE stain. The neuropathic injury will be scored based on the following images shown in Figure S20. The results were shown in Table S11.

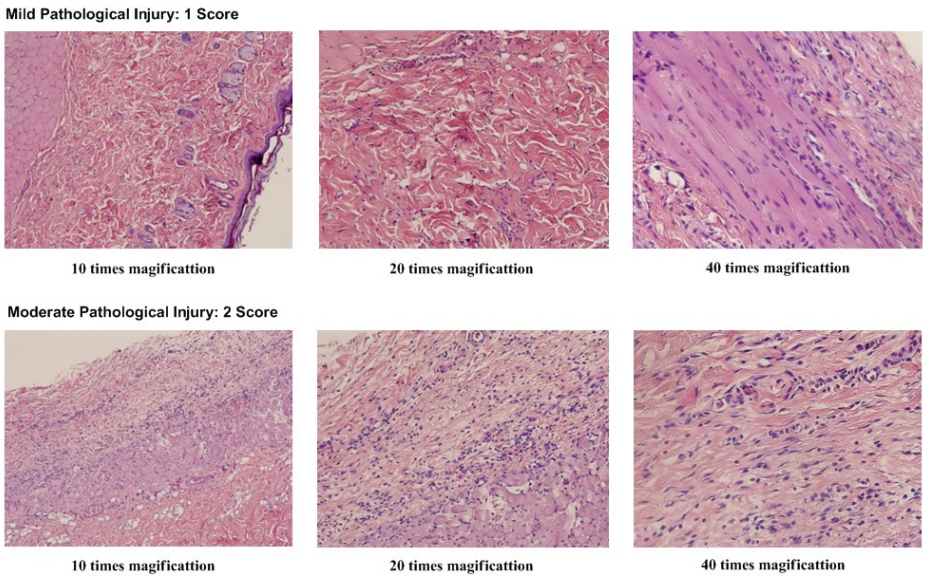


Figure S20 Principles of pathological injury scoring

SI.7 *in vitro* delivery determination on sciatic nerve

8 Sprague Dowley rats (Dossy Experimental Animal Company, Chengdu, China) weighted 180~220g were killed after sedation with inhaled 1.5%~2.0% isoflurane (v/v%) mixing with

oxygen. For each rat, two sciatic nerves weighted about 2g were intercepted rapidly and stored them in crashed ice separately. 16 sciatic nerves were obtained, and 12 of them with approximate thickness were selected for the next step. Sciatic nerves bifurcated was abandoned.

All of these 12 sicatic nerves were washed with pure water to remove the blood residue, dried by filter paper and cut into 1.10g intact standard samples. These standard samples were put into 12 glass bottles individually solution of lidocaine salts (73.6mmol/L) at 40°C approximate to the internal temperature of rats for 0.5 hour. Then these 12 samples was removed from the **1e** solution, washed with pure water, dried by filter paper, crashed, grinded with ice water 0.5mL and added with QX-314 as internal standard (0.01μg) separately in 1min. Each tissue homogenates was added with formic acid solution (0.5% in water) to make them at the same weight of 2.0g. The homogenates were centrifuged at 5°C for 4000rpm/min during 15min, and 1.6g of the supernatant for each group was separately as tested samples.

Results:

As shown on Table S12, there was no significant difference among deliver results for different samples of lidocaine salts.

Table S12 Deliver test of **lidocaine salts** (73.6mmol/L) on sciatic nerve *in vitro*

Solution	Percentage of lidocaine salts (%)								
	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Mean
Lidocaine • HCl	36.18	39.38	35.71	38.16	35.26	41.49	37.83	38.72	37.84
Lidocaine • HCl + CO_2	36.98	37.08	33.96	31.18	34.83	33.25	38.71	36.34	35.29
Lidocaine • HBr	36.74	31.56	32.27	35.57	33.28	38.53	28.42	31.76	33.52

SI.8 LD_{50} measurement

Sixteen drug-naive healthy male Kunming rats were randomly selected for each group of lidocaine salts. LD_{50} (the dose at which 50% of animals die) measurement for intravenous injection was established with up-and-down sequential allocation for six crossovers.[S3] The dosage began from 16mmol, and rised 1.25 times for the next test point.

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[S3] Liu, L.Q. et. ac. *J. Med. Chem.* **2020**, 63, 7857−7866.