Charge-neutral urea cages as potent synthetic sulfate receptors in water

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Abstract: Strongly hydrated anions are challenging targets for molecular receptors. In nature, sulfate binding proteins use multiple neutral NH hydrogen bonds to surround the guest, resulting in sub-millimolar to sub-micromolar sulfate affinities. Synthetic receptors, however, have not yet achieved comparable efficiency and selectivity for sulfate solely by relying on hydrogen bonds or other ion-dipole interactions. Here we report charge-neutral macrotricyclic hexa-urea cages which exhibit strong sulfate affinities (6000 to 9000 M^{-1}) in water with exceptional selectivity over hydrophobic anions. Unlike sulfate binding proteins where a low-polarity microenvironment enhances sulfate binding, the urea cages achieve high sulfate affinities with the urea binding sites exposed to water. The work demonstrates that synthetic receptors operating solely by neutral hydrogen bond donors can emulate the efficiency and selectivity of naturally occurring systems.

Introduction

Molecular recognition in water that mimicks the function of proteins and enzymes remains challenging[1]. The high dielectric constant of water, strong solvation of polar groups, and poor solubility of organic scaffolds render the design of water-based molecular receptors difficult[2, 3]. To date, successful examples of water-based host-guest systems have predominantly resorted to hydrophobic effects and/or ionic interactions.[1] Classic examples include cyclodextrins/cucurbiturils[4] for hydrophobic organic guests and poly-charged cyclophanes[5] for oppositely charged guest species. By contrast, naturally occurring systems such as sulfate binding proteins[6] and chloride channels and transporters (ClCs)[7] have evolved to employ hydrogen bonds from charge-neutral motifs to selectively bind their highly hydrated anionic substrates. Such functionality is rarely seen in synthetic systems. Bambusurils[8] and biotinurils[9] are notable examples of neutral CH hydrogen bond-based anion receptors, which interact strongly with hydrophobic anions such as perchlorate but are not known to bind hydrophilic sulfate anions. Jeong's indolecarbazole foldamer[10] features aromatic NH binding sites and show modest halide affinities from 19 to 65 M^{-1} in water. Kubik's cyclopeptides[11] bind sulfate with amide NH sites in water showing affinities up to 3100 M^{-1}, but require over 10 synthetic steps and have comparable affinities for hydrophobic iodide anions[12]. Joliffe's macrocyclic squaramides can bind sulfate in DMSO-water mixtures with up to 70% water content but sulfate binding was not observed in 100% water[13, 14]. Molecular temples developed by Davis and co-workers can bind glucose up to an affinity of 18,000 M^{-1} in water but involve hydrophobic CH–aromatic contacts in addition to NH hydrogen bonds[15].

We recently developed a neutral macrobicyclic urea cage **1** capable of binding sulfate with up to 12 NH hydrogen bonds leading to sulfate binding in water (binding constant is 66 M^{-1} by NMR and 100 M^{-1} by ITC).[16] By using CTAB micelles to provide a low-polarity microenvironment, we have enhanced the apparent sulfate affinity of cage **1** to 990 M^{-1}.[16] In this work, we demonstrate that by judicious structural design to introduce macrotricyclic scaffolds, we have achieved sulfate binding in water at biologically relevant affinity levels without the need to use micelles.

Results and Discussion

Receptor design

Previously, sulfate binding to urea-based cage **1** was found to be exothermic[16] as opposed to the endothermic profile seen in sulfate binding to Kubik's amide-based cyclopeptide receptors[12]. This is attributed to stronger hydrogen bond donating strength of ureas than amides[17]. However, Kubik's cyclopeptide is much more entropically favourable in binding sulfate than cage **1**. These results lead us to suggest that cage **1** might be insufficiently pre-organized resulting in substantial entropic penalties from conformational reorganization upon binding sulfate. We thus chose to investigate charge-neutral macrotricyclic scaffolds in place of macrobicyclic scaffolds inspired by the effective aqueous anion binding achieved by macrotricyclic polycationic organic or metal-organic receptors.[18-22] Here we have designed charge-neutral macrotricyclic urea cages **2** and **3** (Fig. 1).

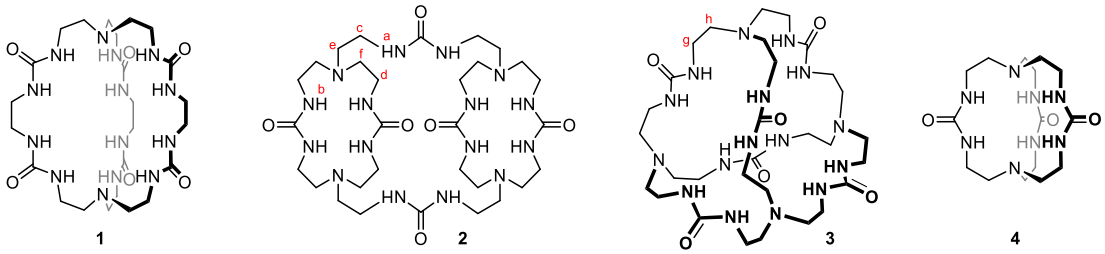

Fig. 1 Structures of previously reported lantern-type cages **1** and **4**, and macrotricyclic cages **2** and **3**.

Syntheses of receptors

A sulfate-templated [2+2] tripod-tripod coupling reaction between tris(2-aminoethyl)amine (TREN) and the carbonyl-imidazole derivative of TREN (TREN-3CDI) produced cage **2** in 80% yield; the same reactants form the [1+1] lantern-type cage **4** in the absence of the sulfate template[23]. Removal of the sulfate template by treatment with $BaCl_2$ followed by purification via extraction, silica gel filtration and recrystallization afforded cage **2**. Although the isolated yield of cage **2** is low (15 %) due to the adsorption of a portion of product on the silica gel, the overall synthesis proceeds in one-step from cheap commercially available reagents.

The synthesis of tetrahedral cage **3** was more challenging and after numerous attempts only milligram quantities of this cage in 90% purity by preparing and cyclizing a hexa-amine dendrimer **5** following a similar approach reported by Bowman-James.[24] The low yield of the final cage formation step even in the presence of the sulfate template is attributed to the flexibility of the TREN-based building units.

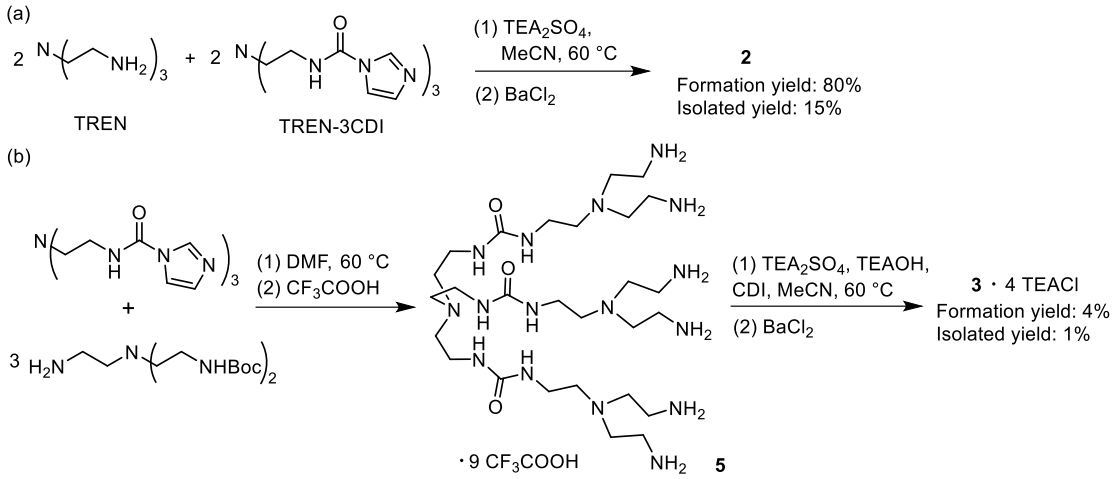

Fig. 2 Sulfate-templated syntheses of urea cages. (a) One-pot synthesis of D_{2h}-symmetric cage **2**; (b) Stepwise synthesis of tetrahedral cage **3**. TEA_2SO_4, tetraethylammonium sulfate.

Anion binding in water

The sulfate-bound macrotricyclic cages **2** and **3** exhibit sharp, well-resolved triplet urea NH ^{1}H NMR signals in DMSO-d_6, in contrast to the broad urea NH signals of sulfate-bound macrobicyclic cage **1** under the same conditions[16]. This suggests that the conformational and/or host-guest exchange rates in sulfate-bound **2** and **3** are reduced compared to those in **1**. In addition, the average urea NH chemical shifts of the sulfate-bound cages follow the order of **3** (7.65 ppm) > **2** (7.09 ppm) > **1** (6.85 ppm) in DMSO-d_6. These proton chemical shifts measure the de-shielding effect of the bound sulfate on the NH protons via polarization of the NH bonds, thus indicating the strengths of hydrogen bonds for structurally related systems.

^{1}H NMR binding studies of sulfate to cages **2** and **3** in water support the above hypothesis. The urea NH signals of cage **2** undergo downfield shift upon adding Na_2SO_4 to **2** in 9:1 H_2O/D_2O (Fig. 3), characteristic of hydrogen bonded complex formation. The sulfate binding of cage **2** quickly saturates after one equivalent of sulfate. Again, the urea NH signals of sulfate-bound cage **2** are much sharper and more downfield shifted than those of sulfate-bound cage **1** in water. Fitting to a 1:1 binding isotherm gives a sulfate affinity of 6800 ± 200 M^{-1} (Fig. 4), a record value for any charge-neutral synthetic receptors in water (excluding biphasic systems). The greater sulfate-induced downfield shift of NH^a (those on the two central straps) than NH^b (those on the four terminal straps) is consistent with a bound sulfate locating at the center of the cage.

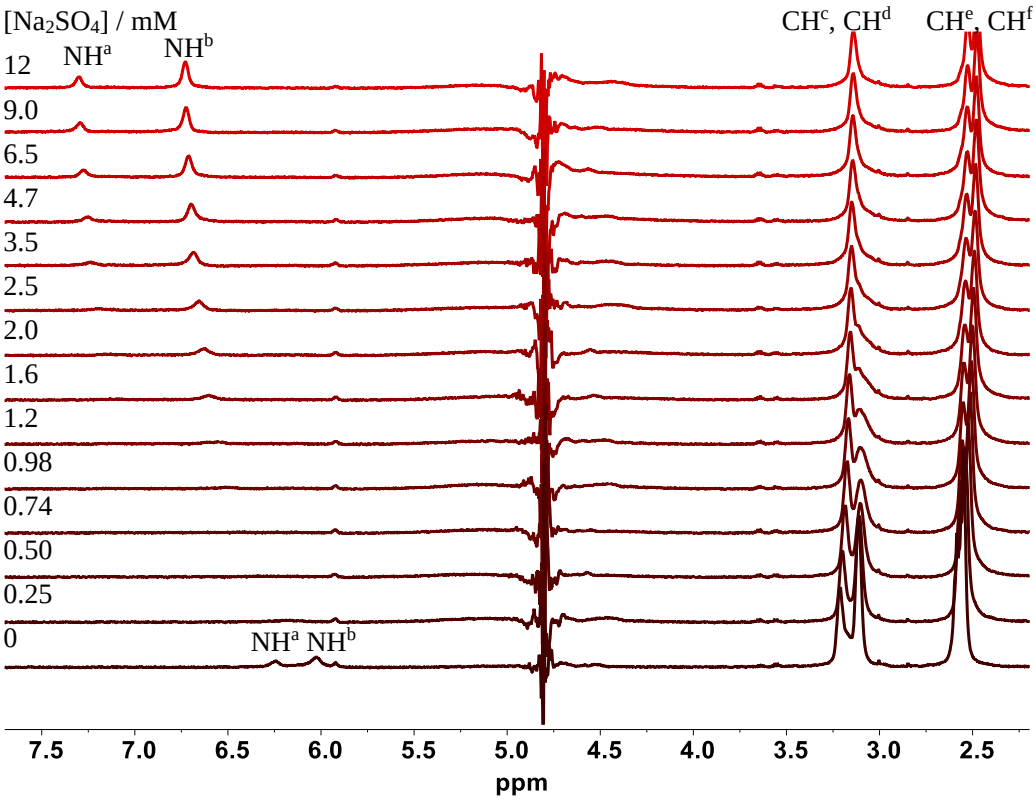

Fig. 3 The ^{1}H NMR (500 MHz) titration of **2** (1.0 mM) with Na_2SO_4 in 9:1 H_2O/D_2O at 298 K, showing urea NH signals. See Fig. 1 for NH labels.

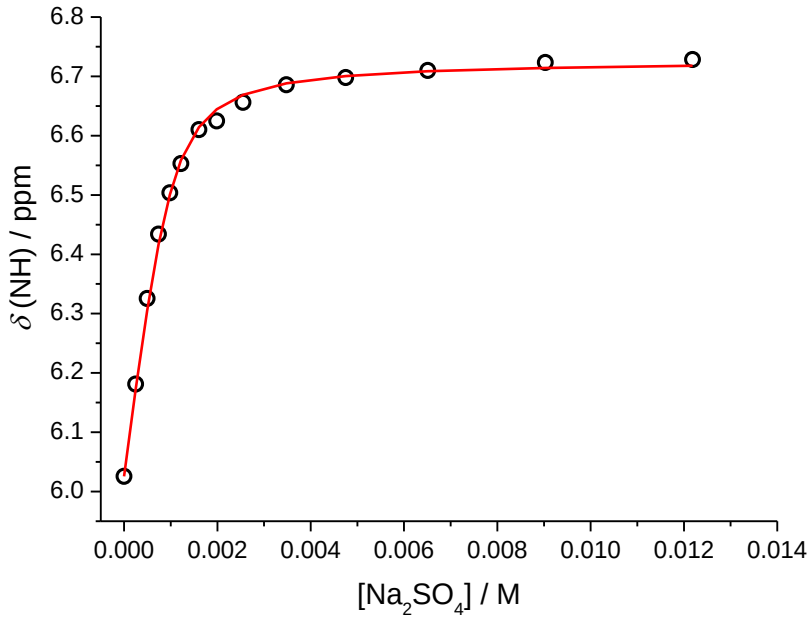

Fig. 4 Fitting of the urea NH (H^b) of **2** to a 1:1 binding model. *K* values of 6629 and 6906 were determined from two independent measurements, giving mean ± SD as 6800 ± 200 M^{-1}.

Unlike the D_{2h}-symmetric cage **2**, the sulfate exchange rate for **3** was observed to be slow on the NMR timescale in 9:1 H_2O/D_2O (Fig. 5), showing separate signals for the sulfate-free and sulfate-bound **3**. The sulfate binding constant of cage **3** was determined to be even higher than for **2** (8400 ± 800 M^{-1}, Fig. 6) potentially due to better shape complementarity between from the dynamic T_d symmetry of cage **3** and the sulfate guest.

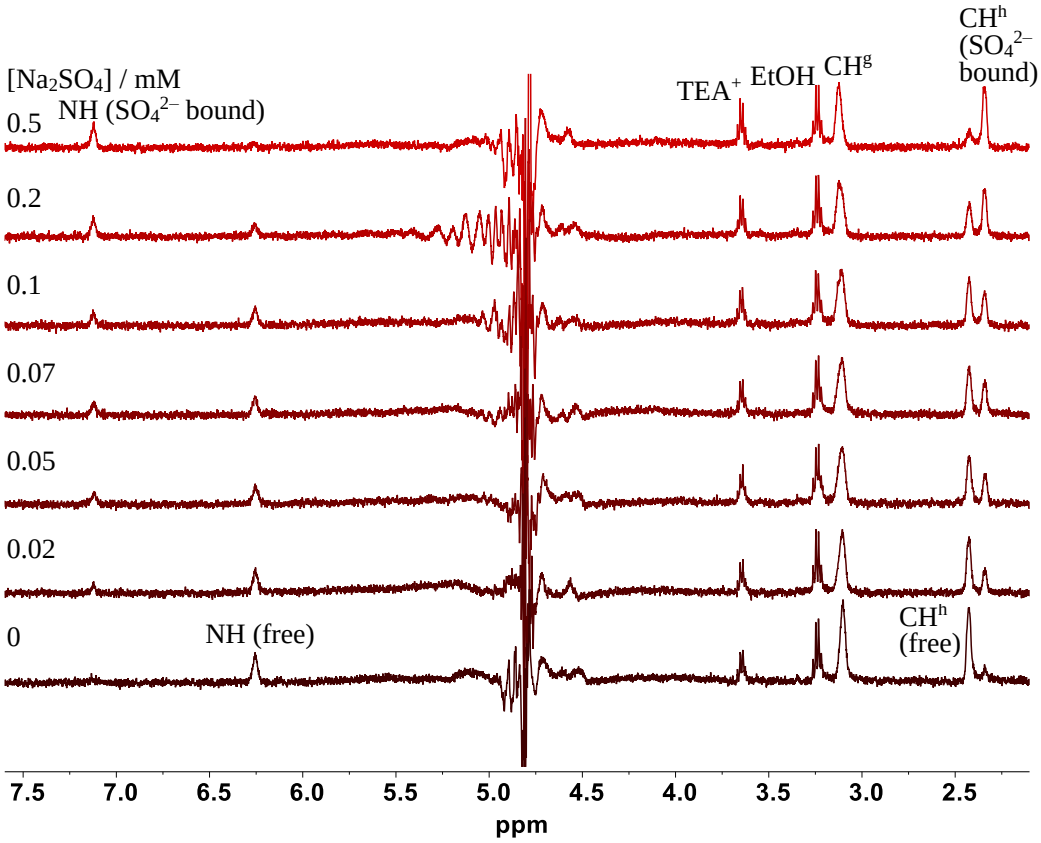

Fig. 5 The ^{1}H NMR (500 MHz) titration of **3** (0.1 mM, with 4 equivalents of TEACl) with Na_2SO_4 in 9:1 H_2O/D_2O at 298 K. EtOH was added as an internal reference.

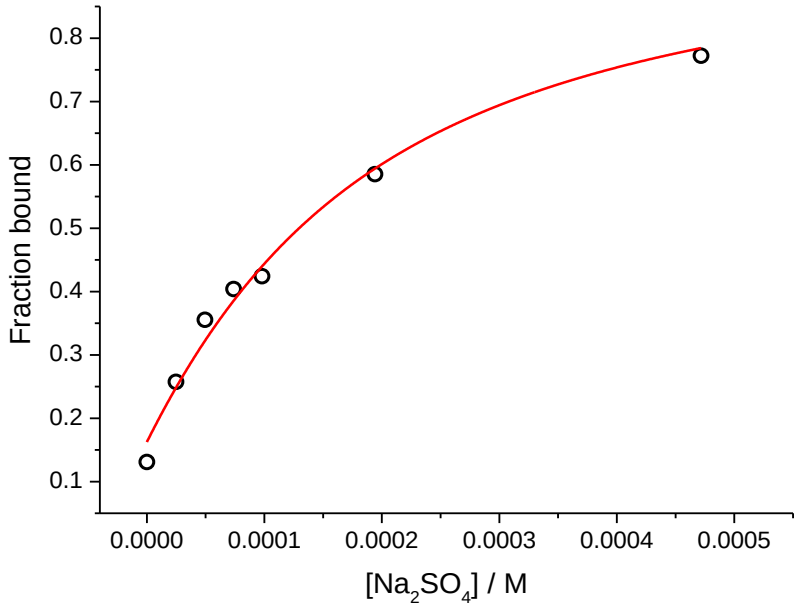

Fig. 6 Fitting of the data to a 1:1 binding model. The bound fraction was determined using integrations of the CH signals at 2.34 (free) and 2.42 (SO_4^{2-} bound) ppm. We have accounted for the sulfate impurity present in the solution before the addition of Na_2SO_4. The binding constant (K) of **3** towards SO_4^{2-} was determined to be 8400 M^{-1} in 9:1 H_2O/D_2O.

As shown in Table 1, cage **2** shows a strong selectivity for sulfate against less hydrated anions, e.g., a sulfate/iodide binding constant difference of 450-fold, in contrast to Kubik's cyclopeptides[12] which have similar sulfate and iodide affinities in water, and CH hydrogen bond-based receptors[8, 9] which are selective for weakly hydrated anions. The second most strongly bound anionic guest among the tested anions is oxalate ($C_2O_4^{2-}$), a highly hydrophilic anion whose shape fits into the long cavity of cage **2**. Chaotropic anions ReO_4^-, NO_3^- and ClO_4^- show the weakest affinities (< 5 M^{-1}) for **2**.

Table1 Binding constants (*K*) of cage **2** for various anions in 9:1 H_2O/D_2O at 298 K.

Anion	*K* for cage **2** (M^{-1})	Anion	*K* for cage **2** (M^{-1})
SO_4^{2-}	6800	Malonate^{2-}	40
$C_2O_4^{2-}$	260	I^-	14 [a]
CO_3^{2-}	230	ReO_4^-	5 [a]
Cl^-	83	NO_3^-	4 [a]
HPO_4^{2-}	72	ClO_4^-	2 [a]
Br^-	53	-	-

[a] Determined by competition titrations because these anions gave weak or negligible ^{1}H NMR responses in **2**.

Attempts to obtain single crystals of the new cages have not been successful. We have thus performed a preliminary modelling of the sulfate-bound cages. The structures optimized at ωB97X-D/6-31G(d) level of theory show sulfate forming hydrogen bonds with the 12 NH sites in the two cages (Fig. 7). In the tetrahedral cage **3**, the geometry of six urea groups around sulfate is similar to the *T*-symmetric 6:1 urea/sulfate complex computationally minimized by Hay,[25] suggesting that this cage is optimal for sulfate binding. The sulfate-bound cage **2**, in comparison, deviates from the ideal *T*-symmetric binding geometry, as eight NH sites from the small cyclic units are crowded on two ends of the receptor. The sulfate guest bound to **2** is solvent accessible, which likely reduces the enthalpic cost of sulfate dehydration compared with sulfate binding to **3**, resulting in similar sulfate affinities of the two receptors despite **3** forming stronger hydrogen bonds with sulfate (with an average NH···O=S distance of 1.86 Å, from optimized structure) than **2** (with an average NH···O=S distance of 2.02 Å, from optimized structure).

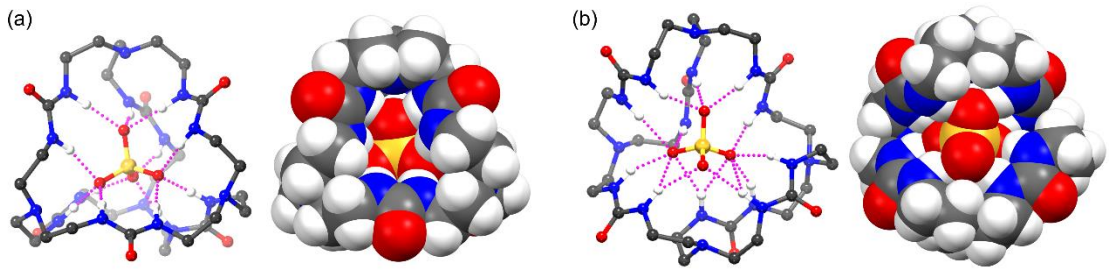

Fig. 7 The SO_4^{2-} complexes of **3** (a) and **2** (b) optimized at ωB97X-D/6-31G(d) level of theory, shown in ball-and-stick models (left) and space-filling models (right). Pink dashed lines in ball-and-stick models represent hydrogen bonds. Non-acidic hydrogen atoms have been omitted.

Conclusions

We have developed charge-neutral macrotricyclic urea cages capable of binding sulfate with record affinities in the range of 6000 to 9000 M^{-1}. Although the synthesis of the "perfect" sulfate-binding cage **3** is low yielding, the lower-symmetry cage **2** can be synthesized in one step from low-cost reagents and exhibits potent sulfate binding at 6800 M^{-1} with a strong selectivity against less hydrated anions in water. The sulfate affinities of the two cages, despite not reaching the sub-micromolar level exhibited by the sulfate binding protein from *S. Typhimurium*[6], match or surpass the potency of several sulfate transport proteins (e.g., the SLC26A2 protein with sulfate affinity in the range of 10^2–10^4 M^{-1} depending on the chloride concentration[26]). Remarkably, the guest-binding capability of our urea cages was achieved sole by hydrogen bonds, without assistance from ion pairing or hydrophobic effects. Our work demonstrates that with judicious structural design, molecular recognition at biologically relevant affinity levels can be achieved by using neutral NH hydrogen bond donors in water. With a potential of further boosting the affinity by placing the NH sites within a protein-like low-polarity microenvironment, such systems may be used to modulate anion concentrations in biological systems to be used as physiology research tools or therapeutics.[27]

Acknowledgements

We acknowledge the support from the Australian Research Council (DE220101000), the National Natural Science Foundation of China (92356308), Xiamen University and the University of Queensland.

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