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COMMUNICATION

A Cucurbit[8]uril Sponge

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This paper describes a convenient approach to quantitative removal of the synthetic host cucurbit[8]uril (Q8) from aqueous mixtures using a sepharose resin coated in memantine groups to selectively sequester Q8 in the presence of competing hosts and guests. The "Q8 sponge" can separate Q8 from Q6 and reverse the Q8-mediated dimerization of peptides.

Q8 and its homologues[1] are synthetic, water-soluble macrocycles that continue to earn increasing attention from the supramolecular, chemical biology, biotechnology, catalysis, and materials science communities[2] for their capacity to associate selectively with a broad range of guests in aqueous media with equilibrium dissociation constant (K_d) values ranging from millimolar to femtomolar.[3, 4] The structure of Qn hosts, comprising n repeating glycoluril groups linked by 2n methylene bridges (Fig. 1), promote the inclusion of nonpolar groups within the central cavity of the Qn and the stabilization of cationic groups on the guest by the carbonyl oxygens that line the two entries.

Among the Qn homologues, analogues,[5] and derivatives,[6-8] Q8 has received special attention for its ability to form specific ternary complexes with two guests bound inside the cavity.[9, 10] Q8 can induce the noncovalent homodimerization of amino acids, peptides, proteins, viologens, and other guests,[11, 12] as well as the heterodimerization of dications such as methyl viologen (MV), 2,7-dimethyldiazaphenanthrenium, or tetramethyl benzobis-(imidazolium) with relatively electron-rich aromatics such as naphthalene, and indole.[10, 13-15] Using Q8 as a so-called "handcuff" to dimerize species via the specific complexation of two guests has led to applications in molecular assembly,[13, 16] polymer assembly,[17] surface chemistry,[13, 18] and catalysis.[6, 19, 20]

Ternary complexes of Q8 can be reversed upon treatment with a competitive ligand, as demonstrated numerous times, including for example, molecular switches,[6] protein dimers,[21] and supramolecular polymers.[22, 23] In these and other potential applications, in particular catalysis, the complete removal of Q8 from the mixture would be desirable for the purpose of repetitive switching, reducing side-reactions, and simplifying analysis, but current approaches only add a competitive ligand to the mixture. Here we demonstrate that adding a solid resin coated in a competitive ligand to a ternary complex of Q8 completely reverses complex formation by enabling the selective removal of Q8 from solution. Moreover, we show that Q8 can be removed selectively in the presence of another Qn.

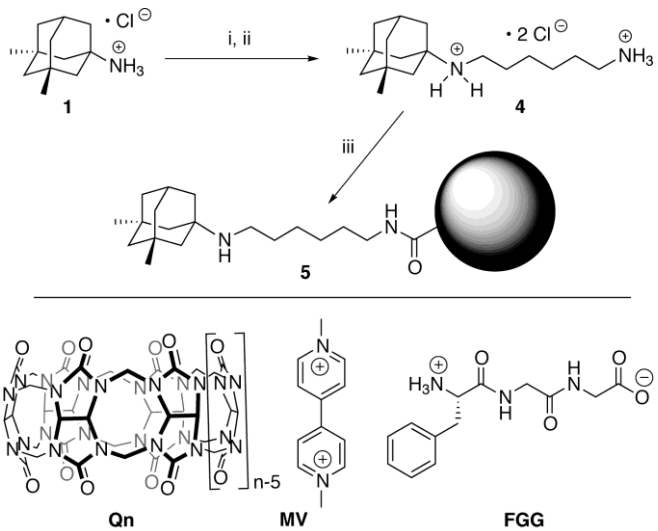

Fig. 1. (top) i) 6-(Boc-amino)hexyl bromide, Cs_2CO_3, DMF, r.t.; ii) 2.0 M HCl, ether, r.t.; iii) Sepharose-NHS resin, 0.2 M $NaHCO_3$, 0.5 M NaCl, pH 8.3, r. t. (bottom) Formulas for hosts and guests.

The Q8 sponge (**5**, Fig. 1) has three important design features: 1) the recognition group, memantine (1-amino-3,5-dimethyladamantane), which, as an ammonium ion, is known to bind to Q8 with a K_d value in the pM range;[3] 2) the linker, hexanediamine, which was designed to separate the recognition group from the solid support and to allow the sterically hindered secondary amino group proximal to the adamantane to remain basic (and thus protonated at pH 7) as part of the memantine recognition group, whereas the primary amino group is more accessible for coupling to the NHS-activated carboxylic acid groups on the surface of the resin; and 3) the resin, sepharose, which was chosen because Kim and coworkers successfully used this resin as part of an approach to isolate proteins from cell extract,[24] and thus this resin is known to accommodate cucurbit[n]uril interactions in aqueous media. Moreover, we found that the unmodified resin does not bind to Q8 (data not shown).

Q8 sponge **5** was synthesized in two steps from commercially available memantine hydrochloride (**1**) (Fig. 1). Compound **1** was coupled to 6-(boc-amino)hexyl bromide **2** by stirring overnight in basic DMF solution, and the Boc group was removed in acidic ether solution to yield the diammonium chloride salt **4** in 33% overall recovery after reverse-phase HPLC purification. To complete the synthesis, **4** was coupled to NHS-activated sepharose

beads under basic conditions to obtain **5**.

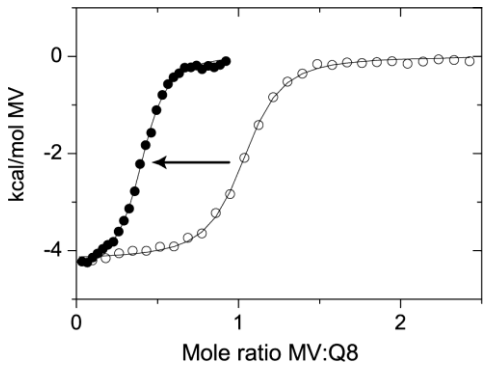

Fig. 2. Overlay of ITC data (27 °C, 10 mM sodium phosphate, pH 7.0) quantifying the concentration of a sample of Q8 before (open circles) and after (closed circles) the addition of sponge.

The capacity of **5** to remove Q8 from solution was quantified by isothermal titration calorimetry (ITC) (Fig. 2). In this method, a methyl viologen (MV) solution of known concentration‡ was titrated into a Q8 solution of unknown concentration, and the known 1:1 binding stoichiometry[10, 11] was used to determine the concentration of Q8 based on the observed equivalence point. This sample was treated with dry sponge **5**, and the residual solution concentration of Q8 was then measured again by ITC. From these experiments, the removal of Q8 from solution per gram of **5** was calculated to be 127 μmol/g. This approach to quantifying the functional loading of the resin is complementary to that described recently by Hennig and coworkers using a fluorescent dye.[25]

One of the unique and fascinating properties of Q8 is its ability to induce the reversible, noncovalent dimerization of small molecules in aqueous media. We have shown that Q8 can dimerize peptides containing an N-terminal aromatic residue (e.g., Phe-Gly-Gly) via hydrophobic inclusion of the nonpolar aromatic sidechain within the nonpolar cavity of Q8 and electrostatic attraction of the N-terminal ammonium group with ureido carbonyl oxygens on Q8.[26] Just as Q8 can induce the dimerization of Phe-Gly-Gly, we hypothesized that a Q8 sponge should be able to compete for Q8 binding, remove Q8 from solution, and thereby fully restore the peptides to their original environment before Q8 was introduced. To test this hypothesis, a solution containing a 1:2 mixture of Q8:Phe-Gly-Gly (i.e., the Q8•(Phe-Gly-Gly)$_2$ complex) was treated with a 4-fold molar excess of sponge **5**, mixed, and separated by centrifugation. The ^{1}H NMR spectral overlay of this sample before and after treatment with **5** (Fig. 3a vs. Fig. 3c) showed disappearance of Q8 signals and a return of the peptide signals to their original, unbound chemical shifts,[26] thus demonstrating removal of Q8 and reversal of peptide dimerization. The same reversal process can be achieved by treating the Q8•(Phe-Gly-Gly)$_2$ complex with memantine hydrochloride (**1**) (Fig. 3b), but the sponge **5** has the clear advantage that it can remove Q8 completely from the sample by centrifugation or filtration. Given the successful demonstration of Q8 to dimerize fully folded proteins terminated in phenylalanine,[21] we expect the Q8 sponge to find broader use in chemical biology.

In a second application we show that the Q8 sponge can be useful for separating mixtures of cucurbit[n]uril (Qn) homologues. The isolation of pure Qn homologues, analogues, derivatives, and uncyclized glycoluril oligomers remains a technically challenging

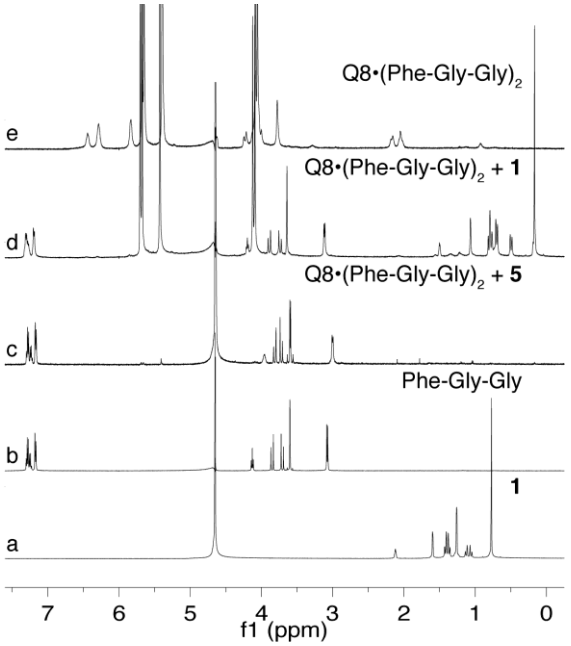

Fig. 3. Overlay of ^{1}H NMR spectra at 1 mM in D_2O of a) **1**; b) Phe-Gly-Gly; c) Q8•(Phe-Gly-Gly)$_2$ + 4 equiv. **5**; d) Q8•(Phe-Gly-Gly)$_2$ + **1**; and e) Q8•(Phe-Gly-Gly)$_2$.

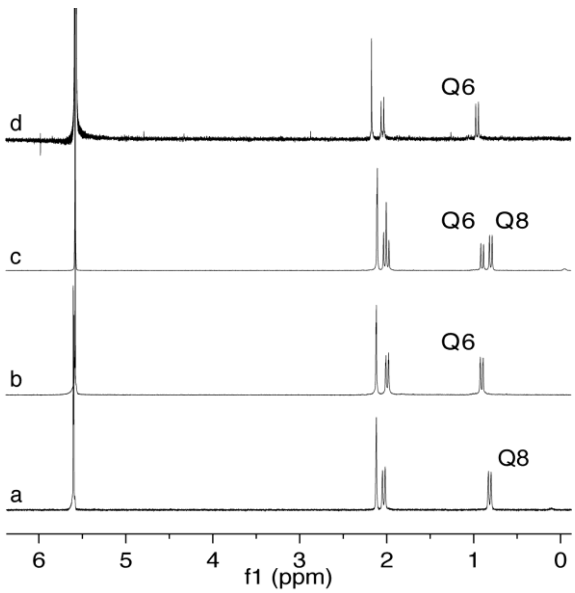

Fig. 4. Overlay of ^{1}H NMR spectra in 35% (w/v) DCl in D_2O of a) 1.0 mM Q8; b) 1.0 mM Q6); c) mixture of 1.0 mM Q8 1.0 mM Q6; and d) a mixture of 1.12 μmol Q6 and 0.98 μmol Q8 after treatment with 4.3 μmol **5** (see supporting information for details of this experiment).

process typically accomplished by iterative rounds of selective precipitation, recrystallization, and/or ion-exchange chromatography.[27] We hypothesized that a Q8-specific sponge, such as **5**, could selectively remove Q8 from a Qn mixture via selective interaction of Q8 with the memantine groups on the

surface of the sponge.[3] To demonstrate this concept, Q8 sponge **5** was added to a mixture of Q8 and Q6 (two prevalent but minimally soluble Qn homologues). ^{1}H NMR spectroscopy (Fig. 4) revealed the selective disappearance of Q8 signals upon treatment with **5**, and thus removal of Q8 from the mixture. Therefore, **5** can selectively and quantitatively separate Qn homologues.

The majority of the cost of this approach to separating Qn-related species is derived from the commercial sepharose resin. It is quite reasonable to consider more cost-effective materials comprising the same ligand attached to other aqueous-compatible resins, such as common chromatography matrices. Moreover, the Q8-sponge **5** (as with most affinity matrices) is recyclable by treatment with a competing ligand. To demonstrate recyclability, we treated a sample of used **5** (i.e., **5**•$Q8_n$) repeatedly with a 20 mM sample of memantine hydrochloride (**1**). The resulting recycled sponge was able to completely remove Q8 from a mixture of Q8 and methyl viologen just as effectively as a freshly synthesized sample of **5** (Fig. S4).[28]

Conclusions

In summary, this paper demonstrates novel and potentially general approaches to the complete reversal of Qn-mediated processes and to the separation of Qn homologues using a resin coated in strong-binding guests. The Q8 sponge is easy to make, fast and convenient to use, highly effective, and reusable. It is no surprise that an ultrahigh-affinity ligand should compete effectively for a receptor. By attaching the ligand to a solid support, however, the receptor-mediated process can be reversed completely, including removal of the receptor from solution. This concept was demonstrated here using Q8 sponge **5** to reverse the dimerization of aromatic peptides rapidly and under ambient, aqueous conditions, effecting a complete return of the peptides to their original, unbound state. Moreover, the use of a Q8-sponge to separate Qn homologues, as demonstrated here, presages a more general approach to the selective isolation of other target homologues, analogues, derivatives, and/or uncyclized glycoluril oligomers by linking their cognate guests to a solid-support, or by removing all known cucurbit[n]uril-related species in order to discover the rare, species that remain.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental details and spectral data are available.

‡ The concentration of methyl viologen was determined based on a molar absorptivity of 20,400 M^{-1} cm^{-1} at 257 nm.

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28. We note that this method elutes Q8•**1** complex rather than pure Q8. We have not attempted alternative methods of elution.