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Molecular protection of fatty acid methyl esters within a supramolecular capsule

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We describe the use of a supramolecular nano-capsule for selective protection of *cis*- and *trans*-C_{18} mono-unsaturated fatty-acid esters. In contrast to earlier studies revealing that protection of smaller esters is dictated by affinity, protection of these larger esters was found to be dependent on the packing motif of the guest.

Molecular encapsulation can either bring things together, or, keep things apart. Inspired by the power and selectivity of enzymes,[1] studies involving the former have dominated. Early work in "artificial enzymes" involved molecular hosts such as cyclodextrins and calixarenes,[2] and this thread of thought has continued apace with, for example, calixarenes.[3] Arguably however, in the last decade or so supramolecular hosts assembled via metal-ligand coordination[4] and hydrogen bonding[5] have proven to be the most popular strategy to encapsulation-controlled catalysis. Along this line of thinking our own group has used supramolecular hosts assembled via the hydrophobic effect to control molecular reactivity.[6]

In contrast, the idea of using molecular encapsulation to induce molecular separations has not been explored to the same extent. This is surprising as selective guest encapsulation can lead to powerful physical separations.[7] Moreover, kinetic resolutions utilizing molecular protection and the inhibition of reactivity has been demonstrated by both molecular[8] and supramolecular hosts.[9]

Regardless of the goal, promoting or preventing reactions by encapsulation requires a thorough understanding of the behavior of guests within nano-spaces. Toward this, we report here on the ability of the dimeric capsule formed by octa-carboxylate **1**[10] to control the protection of C_{18} fatty acid esters (Figure 1a and b). Driven by the hydrophobic effect, **1** forms stable dimers around non-polar guests in 2:1 and 2:2 host-guest stoichiometries,[11] and in previous work we have shown that the kinetic resolution of small esters encapsulated within $\mathbf{1}_2$ occurred via a Michaelis-Menton type mechanism and that guest egression was necessary for hydrolysis. Hence the highest affinity guests were protected the most.[9] Considering the multiple roles of fatty acids in biology,[12] and because their diverse yet similar structures represent a challenging recognition problem in its own right,[13] we were keen to probe the selective protection of C_{18} methyl esters. As we describe, for these larger guests protection is not controlled by affinity, but rather by guest binding motif to the capsule.

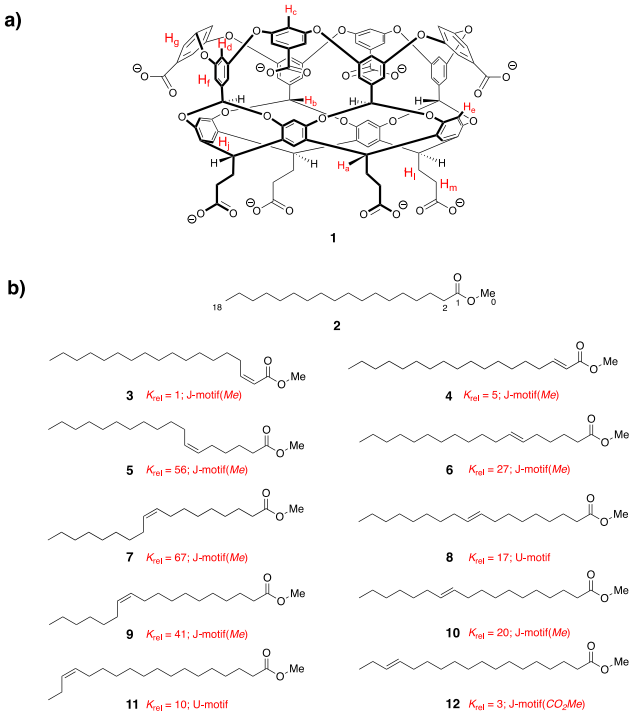

Figure 1: a) Octa-carboxylate **1** (sodium counter ions not shown) Proton designations are highlighted in red. b) Guests used in this study: methyl sterate **2**, and mono-unsaturated derivatives

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3–12. Relative (to guest 3) binding constants and binding motif are shown in red.[10]

Octa-carboxylate **1** was synthesized as previously reported.[14] Of the eleven esters initially targeted, **2** and **5**–**10** were commercially available, whilst the remaining four (**3**, **4**, **11**, and **12**) were synthesized by Wittig chemistry.[10] Our previous studies revealed that as is seen in other capsules,[15] fatty acid transporters,[16] or bowl/toroidal-shaped hosts,[5a, 17] flexible chains in small spaces inevitably bind in U- or J-shaped motifs. Specifically, ^{1}H NMR signal Δδ value calculations between the free and bound states of guests **2**–**12** (COSY NMR), and the rule that the deeper an atom is located within the pocket the larger its Δδ value, revealed three guest motifs each with a reverse-turn occupying one "pole" of the capsule (Figure 2). In the J-motif(*Me*) the ester group resides at the equatorial region of the capsule whilst the terminal methyl and turn occupy the two poles. In the J-motif(*CO_2Me*) the positions of the ester head group and terminal methyl are reversed, whilst in the U-motif there is no evident energetic preference for either terminus of the guest to anchor into the polar region of $\mathbf{1}_2$.

Figure 2: From left to right, representative J-motif(*Me*), U-motif, and J-motif(*CO_2Me*) for esters **5**, **8** and **12**.

Determining the extent of protection necessitates a comparison of the rates of hydrolysis of the free and bound guests. For the former, **3–12** were insufficiently soluble in water and hence a 0.5 mM solution of 40:60 acetone-d_6/D_2O was used to ensure mono-dispersity. Unfortunately, even under these conditions **2** was not sufficiently soluble. Consequently, the hydrolysis rate of free **2** was not examined. Hydrolysis was monitored by ^{1}H NMR spectroscopy via integration of the signal from the ester methoxy group and the methanol side-product (ESI Figures S3-S12). In each case the large excess of base ensured *pseudo* first-order kinetics (ESI Figures S25–S34). For the 2:1 host-guest complexes, host and guest mixtures were initially formed in 10 mM $NaOH_{aq}$ to ensure deprotonation of **1**. Subsequently, to trigger hydrolysis the base concentration was increased to 150 mM and reaction monitored by ^{1}H NMR spectroscopy (ESI Figures S13-23). Depending on the complex, different signals were used for monitoring. For example, Figure 3 shows the stacked ^{1}H NMR spectra for the hydrolysis of encapsulated ester **10**. In this instance the ^{1}H NMR signal from the terminal methyl group of the bound ester and the resulting acid/carboxylate were well resolved. In some of the complexes however the relatively fast movement of the guest, and/or the tendency of the complex of the bound acid/carboxylate product to de-cap and form a 1:1 complex,[18] led to guest signal overlap and/or broadening. In these instances, integration of the signals from the "m" or "l" protons of the host relative to the terminal methyl of the product acid/carboxylate were used. Again, data fitting (ESI Figures S35-S45) demonstrated that hydrolysis of the bound guest followed *pseudo*-first order kinetics.

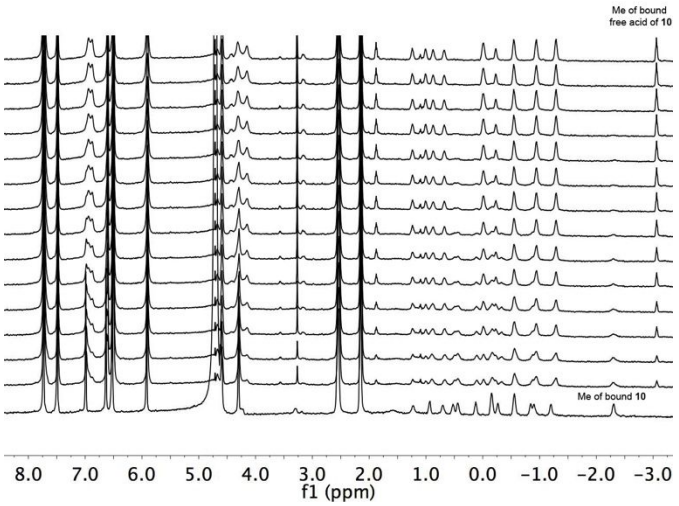

Figure 3: Stacked ^{1}H NMR spectra for the hydrolysis of encapsulated ester **10** at 10 min. intervals (D_2O, 25 ˚C, [Ester **10**] = 0.5 mM, [NaOH] =150 mM, [host] = 1 mM).

Table 1 summarizes the rate constants for the free (k_{free}) and bound (k_{bound}) esters and the (protection) ratio of these. This data shows that with the exception of the conjugated **3** and **4**, within error all of the esters in the free state reacted at the same rate. In contrast, for the bound state the intrinsic rate of hydrolysis of the guest varied, with encapsulated **12** undergoing the slowest hydrolysis of the non-conjugated esters, and **6** reacting the fastest.

Table 1: Hydrolysis rates of free (k_{free}), encapsulated (k_{bound}) and the k_{free}:k_{bound} ratio for esters **2–12**

Guest	k_{free}[a,b] (× 10^{-3})	k_{bound}[a,c] (× 10^{-3})	k_{free}:k_{bound}
2	-[d]	13.37	-
3	2.71	0.61	4.44
4	4.23	1.94	2.18
5	16.65	11.18	1.49
6	17.04	13.38	1.27
7	16.04	11.57	1.39
8	16.72	2.73	6.12
9	14.12	13.14	1.07
10	15.03	11.69	1.29
11	15.38	6.80	2.26
12	14.19	2.45	5.80

[a] Average of two trials with error < 10%.
[b] 0.5 mM ester in 150 mM NaOH in 40% acetone-d_6/D_2O.
[c] 0.5 mM ester in 150 mM NaOH in D_2O.
[d] Guest not soluble under the conditions examined.

The k_{free}/k_{bound} ratios revealed a rather narrow range in the degree of protection. At the two extremes, ester **8** reacted 6×

more slowly when bound, whereas **9** was afforded no protection by encapsulation. Overall, this narrow range led to moderate kinetic resolutions. Thus, in competition experiments (ESI) between mixtures of well-protected **8** and poorly protected **7** (or **10**), it was found that upon complete hydrolysis of **7** (or **10**), 34% (32%) of **8** had also been hydrolyzed. We attribute this limited extent of protection to the large size of the esters; comprised of twenty-one non-hydrogen atoms, these guests are near the upper size limit for the capsule and hence in some cases the capsule may not be tightly closed (*vide infra*).

A plot of relative affinity of each ester for the capsule (K_{rel} = 1 for guest **3**) against the protection ratio (k_{free}/k_{bound}) is revealing (Figure 4). If reaction occurs outside the container then, as the free esters **5**–**12** all react at approximately the same rate, the esters with the highest affinity should be protected the most. This is not the case. As Figure 4 shows, there is no simple relationship between affinity and protection. Rather, the five most strongly binding esters are afforded the least protection. Excluding conjugated esters **3** and **4**, the five esters protected the least are **5**, **6**, **7**, **9**, and **10** (k_{free}/k_{bound} ~ 1.0–1.5). In contrast the three best protected guests are all weak-binding guests: **8**, **11**, and **12** (k_{free}/k_{bound} ~ 2.3–6.1). This supports the notion that in contrast to smaller ester guests that escape the capsule to undergo reaction via a Michaelis-Menton type mechanism,[9] hydrolysis of the more tightly bound guests examined here occurs inside the container.

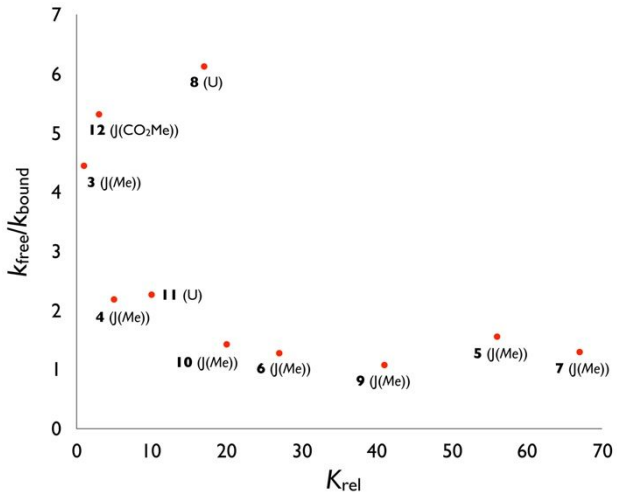

Figure 4: Plot of K_{rel} (= 1 for guest **3**) vs. k_{free}/k_{bound}. The bound guest motif is indicated: J-motif(*Me*), J-motif(*CO_2Me*). and U-motif.

It is well documented that despite nominally having a charge of 8–, host **1** binds small inorganic anions in its non-polar pocket.[19] Additionally, $\mathbf{1}_2$ is known to allow the entry of small, hydrophilic guests via a rapid (relative to capsule disassembly) "breathing" mechanism.[20] Furthermore, it has recently been found that the acidity of thiols within $\mathbf{1}_2$ is highest when the S–H is located at or near the equator of the capsule and can be more strongly solvated.[6a] Figure 4 also shows that three of the four most protected esters (**8**, **11**, and **12**) adopt U- or J-motifs(*CO_2Me*), whereas the weakly protected esters all adopt J-motifs. In other words, the long residency time of these guests mean that the entry of hydroxide into the capsule becomes key, and an ester group located near to the more solvent-exposed equatorial region of the capsule (i.e., those in a J-motif) experiences little molecular shielding by the capsule. In contrast, when the ester group of the guest is positioned deep in the pocket of one of the "hemispheres" the guest is relatively well protected.

In comparing pairs of *cis* and *trans* isomers (Table 1) it is evident that the most extreme difference can be found for pairs **7** and **8** (*trans* to *cis* protection ratio = 3.57) whilst the second most extreme difference is for pairs **11** and **12** (*trans* to *cis* protection ratio = 2.56). These two examples represent cases where the double bond is near the center or end of the chain, and the relatively large differences may reflect the fact that the rigid double bond is necessarily located near the narrower regions of the capsule. It is also interesting to compare the five pairs of *cis* and *trans* isomeric pairs; if the double bond is located between the ester group and the reverse turn of the motif then the *cis* is protected more than the *trans* (**3**/**4** and **5**/**6**), whereas if the double bond is located between the turn and the terminal methyl group (**7**/**8**, **9**/**10** and **11**/**12**) the reverse is true.

We considered the possibility that the different positions of ester groups within $\mathbf{1}_2$ might lead to different mechanisms of hydrolysis. Normally the esters examined would be expected to undergo a $B_{AC}2$ mechanism. However, the alternative $B_{AL}2$ mechanism does become significant in esters possessing a very bulky acyl group and an alkoxy methyl; simple steric incumbrance forces attack of the methyl rather than the carbonyl. Hence, we considered it a distinct possibility that if J-motif(*Me*) esters have their alkoxy methyl group protruding out of the capsule somewhat, hydrolysis might occur by a $B_{AL}2$ mechanism. To examine this possibility, we carried out the hydrolysis of five selected esters using excess $Na^{18}OH$ in $H_2{}^{18}O$ and used electrospray MS analysis to examine the carboxylate products. The five esters selected (**6**, **7**, **8**, **11** and **12**) covered the range of observed binding constants and motifs. These studies revealed that in all cases (ESI Figures S48–S53), hydrolysis led to incorporation of the heavy oxygen into the carboxylate product indicating a universal $B_{AC}2$ mechanism. Thus, ester groups at the equatorial region of the capsule undergo the same overall hydrolysis mechanism as when the ester group of the guest is deeply buried.

In summary, we have shown that the molecular protection of long-chain fatty acid esters by the capsule $\mathbf{1}_2$ is dictated by the motif that the guest adopts within the container. This is in contrast to the molecular protection of smaller esters, reaction of which occurs inside the host. In combination these studies reveal a complex chemical landscape involving the interplay of substrate structure and host. We are continuing to evaluate this landscape in order to fully understand reaction and protection strategies with these types of water-soluble hosts.

Acknowledgements

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Conflicts of interest

There are no conflicts to declare.

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