

A Visual Demonstration of Supramolecular Chemistry: Observable Fluorescence Enhancement upon Host-Guest Inclusion

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Supramolecular chemistry, or “chemistry beyond the molecule”, is a hot topic in current chemical research (1–9). It deals with highly organized structures involving two or more individual molecules held together only by intermolecular forces (1). It is easily distinguishable from molecular chemistry by the lack of covalent bonding interactions between the species involved. Supramolecular systems are becoming increasingly important in various areas of chemistry, including control and catalysis of chemical reactions (1, 2), organic synthesis (3), molecular recognition (1, 2, 4), design of materials for molecular-scale electronics (5), chemical separations (6), and bioorganic chemistry (4, 7). It is therefore useful and appropriate to provide an introduction to the concepts and applications of this interesting field of chemical research to advanced undergraduate students; this would be particularly suitable for an advanced organic or physical chemistry class.

Background and Overview of the Demonstration

In the demonstration described in this article, the concept of supramolecular chemistry is strikingly illustrated by a readily observable increase in fluorescence emission upon formation of a supramolecular structure. Since the demonstration depends on changes in the fluorescence of one of the molecules involved in the supramolecular structure, it could also be used to illustrate some of the more detailed aspects of molecular fluorescence, which would also be suitable for an advanced physical chemistry class.

This demonstration involves a specific type of supramolecular structure referred to as a host-guest inclusion complex, in which a smaller *guest* molecule is held within the internal cavity of a larger *host* molecule. These complexes fit the definition of *supramolecular*, as they do not involve covalent bonding between the guest and host, but are held together only by van der Waals interactions and/or hydrogen bonding. There is a wide range of large, cage-like organic molecules available with the necessary accessible internal cavities to allow them to serve as hosts (8, 9). Cyclodextrins (cyclic oligosaccharides obtained from starch) are by far the most commonly used host molecule because of their water solubility, range of cavity size, and well-defined internal cavity (10, 11). A generalized structure of β -cyclodextrin (which contains seven sugar units) is shown in Figure 1. Cyclodextrins can be thought of as “molecular buckets” because of their shape and ability to act like containers for guest molecules. Their host-guest inclusion properties have various useful applications,

including those described in general above. For example, cyclodextrins have been used to perform chemical separations based on differential inclusion ability for different guest molecules (10) and to provide increased guest solubility in aqueous solution (12).

In order to study host-guest complexes, some measurable property of the guest (or host) must change upon complexation. Techniques that have been used to measure such properties include NMR, UV-vis, and fluorescence spectroscopy (10). In the present case, the fluorescence intensity of the guest species, 8-anilino-1-naphthalenesulfonic acid (1,8-ANS, or simply ANS, shown in Fig. 1), changes significantly upon inclusion. This molecule is widely used as a fluorescence probe because of its extreme sensitivity to its local polarity (13). It is only weakly fluorescent in highly polar media such as water. However, it becomes extremely fluorescent in nonpolar environments, such as organic solvents.¹ Since the internal cavity of cyclodextrins provides a significantly less polar environment than water, an increase in fluorescence intensity (enhancement) will be observed upon inclusion of ANS into β -cyclodextrin; this was first reported more than 30 years ago (14). The formation of a host-guest complex between ANS and cyclodextrin, resulting in fluorescence enhancement, is illustrated in Figure 2, in which ANS is represented as a naphthalene ring (for simplicity) and the modified cyclodextrin is represented as a bucket shape. An equilibrium is established between the free and complexed ANS. In free aqueous solution, ANS does not fluoresce significantly, but when included inside the cyclodextrin cavity, the probe becomes highly fluorescent.

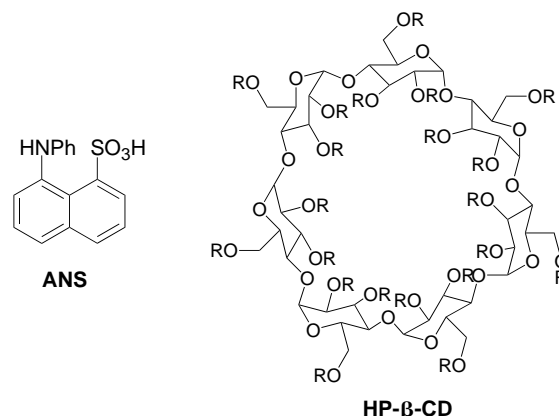


Figure 1. Chemical structures of the guest molecule ANS and the host molecule hydroxypropyl- β -cyclodextrin (R = H or $\text{CH}_2\text{CH}(\text{OH})\text{CH}_3$).

Note that the host-guest complex shown in Figure 2 is for illustrative purposes only, and is not intended to indicate exactly how the ANS and cyclodextrin would fit together to form the complex. A detailed thermodynamic study of the inclusion of a number of anilino-naphthalenesulfonate probes, including 1,8-ANS, into cyclodextrins has been published, with details on the actual geometry of the ANS-CD inclusion complexes (15).

We have recently shown that modified β -cyclodextrins, in which some or all of the 21 hydroxyl hydrogens have been chemically replaced by other groups, provide extremely large enhancements of ANS fluorescence (16). For example, ANS fluorescence was found to be enhanced by factors of 8.4, 100, and 180 upon addition of 10 mM β -cyclodextrin, methyl- β -cyclodextrin, and hydroxypropyl- β -cyclodextrin (HP- β -CD), respectively. In the latter two cases, the enhancements are so large that they are clearly visible to the naked eye, using a simple hand-held UV lamp as the excitation source.

In this article, we describe a simple yet dramatic demonstration of supramolecular host-guest inclusion based on this extraordinarily large observed fluorescence enhancement of ANS by modified β -cyclodextrins. These cyclodextrins are by far the best for this demonstration, since they provide a much greater (and hence easily observable) enhancement than does either β - or γ -cyclodextrin.

Experimental Procedure

Reagents and Equipment

8-Anilino-1-naphthalenesulfonic acid (ANS, Aldrich, 97%)²

Hydroxypropyl- β -cyclodextrin (HP- β -CD, Aldrich)³

Any hand-held long-wavelength UV lamp, such as those used for viewing thin-layer chromatography (TLC) plates

Preparation for the Demonstration

The following two solutions are prepared in distilled water: 4.0×10^{-4} M ANS (0.012 g in 100 mL) and 2.0×10^{-2} M HP- β -CD (3.1 g in 100 mL). Brief sonication of the ANS solution will aid in dissolution. Fifty milliliters of each solution is then transferred to two separate 100 mL beakers. The HP- β -CD solution is stable and can be stored for many weeks; the ANS is photodegradable and should be used within a week (the solution becomes noticeably darker upon degradation). It will last longer if kept in the dark.

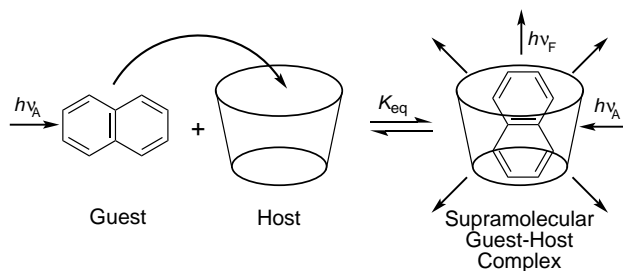


Figure 2. A pictorial representation of the formation of the host-guest complex of a naphthalene-based fluorescent probe, such as ANS, and a cyclodextrin.

Performing the Demonstration

This demonstration works best in a dimly-lit room (there is no need for total darkness). Place the UV lamp upside down on the table top⁴ and turn it on. Hold the beaker containing the HP- β -CD solution over the UV lamp to demonstrate the lack of observable fluorescence; then do the same with the ANS solution. The students *maybe* able to discern a weak, pale yellow-green fluorescence from this solution. Emphasize that the guest and host molecules separately are practically nonfluorescent. Next, place the beaker containing the ANS solution on the UV lamp and pour the HP- β -CD solution directly into it. A very bright blue fluorescence will immediately be observable to all of the students. The effect is quite impressive and persists as long as the UV illumination is maintained.

The sensitivity of the probe fluorescence to its environment can be further demonstrated by preparing solutions of ANS in a range of solvents of varying polarity. For example, a good set would be methanol, ethanol, acetonitrile, and cyclohexane. (The concentration of ANS is not important in these solutions; simply prepare them to exhibit strong fluorescence under the UV illumination used.) The color of the ANS fluorescence from each of these solutions will be discernibly different; this provides an excellent visual illustration of the Lippert relationship between the frequency of fluorescence and the solvent polarity (17). Comparison of the color of the fluorescence from the solution containing the ANS-CD host-guest complex with that of ANS in each of the solvents should allow for an estimation of the polarity of the cyclodextrin cavity. The results will show that the color of the inclusion complex matches closely that of ANS in ethanol, indicating that the polarity inside the cyclodextrin cavity is similar to that of ethanol. If a fluorescence spectrometer is available, then this comparison can be done more quantitatively by measuring the fluorescence spectrum of each of these solutions and determining the wavelength maximum, $\lambda_{F,max}$, in each case. The fluorescence spectra and values of $\lambda_{F,max}$ for ANS in the CD inclusion complex, as well as in a large number of solvents, have been reported previously (16) and show clearly that the polarity of the microenvironment within the cyclodextrin cavity is very similar to that of an ethanol solution. This demonstrates how the polarity sensitivity of the probe can be used to study the properties of the host.

Safety Precautions

UV light can cause permanent eye damage. Avoid looking directly at the UV light source. The demonstrator should wear good-quality anti-UV safety glasses or a UV face shield. UV light can also cause skin damage. The demonstrator should minimize exposure of the hands and arms; long sleeves and proper gloves can completely prevent skin exposure. The experiment as described should pose no danger to the audience, if care is taken not to shine the UV light directly at them. There are two alternative UV light sources, which may make the demonstration even safer. One is a UV light cabinet with an internal space (covered by retractable black cloth), such as is often used for viewing TLC plates. Although this prevents the eyes of both the demonstrator and the audience from being directly exposed to the UV light source, we found that using such a UV source was much more awkward and rendered

the demonstration less impressive. The second possibility would be the use of a UV transilluminator, which is designed for illuminating gel plates from underneath and is found in many biochemistry laboratories. Although this is essentially the same arrangement as using a hand-held UV light upside down, these transilluminators have a clear plastic viewing shield, which would completely filter the UV light from the audience.

ANS is an irritant; it may target the central nervous system and cause liver and kidney damage. Proper precautions should be taken and appropriate protection used when preparing and using this solution.

Discussion

Before the demonstration, the basic concepts of host-guest inclusion phenomena, as described earlier, should be discussed, with emphasis appropriate to the particular class (e.g., physical or organic) involved. It may be useful here to provide the students with a graphic demonstration of host-guest complexation (with reference to Fig. 2). Use a waste basket to represent the cyclodextrin host and an appropriately sized ball (or any other reasonable object) to represent the guest, and show how the guest fits inside the host to generate a host-guest complex. In addition, it may be useful to provide a brief introduction to the absorption and emission of light by molecules (18) if this has not already been done in the course.

After the demonstration, the students should be asked to describe and discuss the event that caused the large observed increase in the ANS fluorescence (inclusion of the ANS probe as a guest in the internal cavity of the cyclodextrin host) and why the ANS was more fluorescent inside the cyclodextrin cavity than in solution (polarity of the local environment is much lower in the cavity than in the bulk water; ANS is much more fluorescent in a nonpolar medium). Further discussion could involve potential applications of such a system, for example as a molecular sensor (i.e., a device for signaling the presence of this particular molecule). Although such a device would not be very useful in this case (ANS is not a molecule of widespread interest), similar enhancement behavior by cyclodextrin has been reported for probes of environmental interest, including PCBs (19). In such cases, a useful molecular sensor could be constructed, with practical applications in environmental science. Generation of such a discussion of the observed phenomenon and its potential applications could provide for a very interesting and stimulating class.

It has been reported in the literature that the presence of the cyclodextrin cavity is essential for the observation of enhancement of probe fluorescence by these molecules. This has been shown by performing analogous experiments using linear oligosaccharides instead of cyclodextrins. In all such cases, linear oligosaccharides gave no observable fluorescence enhancement. For example, the fluorescence of the probe TNS (6-*p*-toluidinonaphthalene-2-sulfonate, a polarity-sensitive probe related to ANS) was shown to be enhanced significantly in the presence of β - and γ -cyclodextrin, but relatively unaffected by the presence of maltoheptaose, the linear analog of β -cyclodextrin (20). The significance of this observation should be discussed with the class: it provides conclusive proof that it is inclusion inside the cyclodextrin cavity which results in the observed enhancement of the probe fluorescence. If appropriate linear oligosaccharides are available to the demonstrator, addition of these to the ANS

solution (using the same concentrations as for the cyclodextrin) could also be included in the demonstration. The observable ANS fluorescence will not be significantly affected by the addition of these compounds, demonstrating the requirement of host-guest inclusion (i.e., a host with a suitable internal cavity) for the enhancement of probe fluorescence.

Notes

1. The polarity dependence of the fluorescence intensity (or quantum yield) of ANS is fairly complicated. While the fluorescence energy (reciprocal wavelength) is well correlated to the bulk solvent polarity (resulting in a blue shift in the fluorescence with decreasing polarity, easily observable in this demonstration), the fluorescence quantum yield is more sensitive to water than would be expected strictly from polarity considerations. A number of mechanisms for this extreme sensitivity have been proposed, including triplet state formation, photoionization, and specific solute-solvent interactions. An excellent discussion and evaluation of these proposed mechanisms is given in ref 13, which concludes that specific solvent-solute interactions are most important. For the purpose of discussions of this demonstration, the greatly decreased fluorescence of ANS in water can simply (and generally) be attributed to the increased rate of various nonradiative decay processes available to the fluorescent state, which compete with fluorescence.

2. While a wide range of polarity-sensitive probes that *may* work in this demonstration is available, it is not expected that any other probe will result in a demonstration more impressive than that using ANS, with the possible exceptions of TNS (21), which is well known to be at least as sensitive as ANS, and other anilino-naphthalenesulfates. However, if it is necessary to use another probe instead of ANS (owing to availability, for example), it would have to be fully tested first, since even if fluorescence enhancement does occur, it may not be as large as that in the case of ANS (a factor of 180) and thus may not provide a very impressive visual demonstration.

3. This chemically modified β -cyclodextrin is available with varying degrees of average molar substitution (average number of substitutions per monomer unit). We used the product with average molar degree of substitution = 1.0. Alternatively, methyl- β -cyclodextrin, also available from Aldrich, could be used; it is less expensive, but gives a smaller enhancement of ANS fluorescence and hence a less dramatic demonstration.

4. Alternatively, the UV lamp could be clamped upright on the bench top, pointing in a direction away from both the audience and the demonstrator. The beakers could then be simply placed next to it, as described in ref 22. While this will reduce exposure of the demonstrator to the UV light, the demonstration is less effective than if the solutions are illuminated from beneath as described in the article.

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