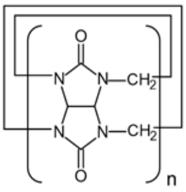
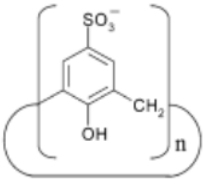
FINAL REPORT

Tuning of photoresponse by biocompatible molecular containers and electrolytes

For the rational design of tailor-made photoresponsive self-organized systems, it is of crucial importance to understand the effect of the microenvironment change on the rate of the excited-state deactivation processes as well as the major factors controlling the kinetics and thermodynamics of the spontaneous self-assembly of molecules. Macrocyclic compounds are widely used in nanotechnology to develop functional materials, molecular-scale devices, drug delivery vehicles, stimuli-responsive systems, and highly selective analyte sensing methods. Water-soluble cavitands have been receiving particular attention because of their ability to accommodate guest molecules enhancing thereby their solubility, stability and modifying photophysical or chemical characteristics. To exploit the advantageous properties and their great potential to serve as nanocontainers, we focused on the inclusion complex formation of cucurbiturils (CBn), the pumpkin-shaped rigid biocompatible macrocycles and the flexible negatively charged 4-sulfonatocalixarenes (SCXn). We examined the binding of natural alkaloids of pharmaceutical interest, surfactants, highly fluorescent and photochromic dyes. Special attention was devoted to the alteration of the photophysical characteristics upon confinement in CBn and SCXn cavitands. The most important results of the systematic studies are summarized as follows.

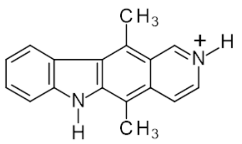


n = 7, 8
cucurbit[n]uril
CBn



n = 4, 6, 8
4-sulfonatocalix[n]arene
SCXn

1. Dimerization of protonated ellipticine in water and in the cavity of cucurbit[8]uril

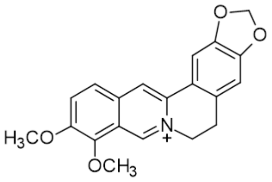


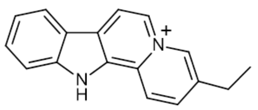
Protonated ellipticine (EH^+)

Our absorption and fluorescence spectroscopic measurements, as well as isothermal calorimetric titrations demonstrated that the protonated ellipticine (EH^+) associates to dimers even at micromolar concentrations. This knowledge is inevitably necessary for the correct interpretation of EH^+ binding to substrates and for the analysis of fluorescence decays of EH^+ in the presence of all types of additives. We emphasized that taking into account dimer formation is essential because otherwise a systematic error appears in the spectrophotometrically measured EH^+ concentrations and in all quantities derived therefrom.

EH^+ was found to produce three types of inclusion complexes with cucurbit[8]uril (CB8) depending on the host and guest concentrations. Gavvala and coworkers reported only 1:1 binding and could not reveal the reason of the substantial variation of the fluorescence decay parameters upon gradual increase of host concentration. Our results proved that not only one, but also two EH^+ was capable of encapsulation in CB8 in 37 μM EH^+ solution and the thermodynamics of the binding steps were revealed. When large excess of CB8 was employed in dilute (0.49 μM) EH^+ solution, sequential binding of two CB8 occurred to the monomer alkaloid. Significantly different fluorescence lifetimes were found for the various complexes. The CB8 concentration dependence of the kinetics of EH^+ fluorescence decay was attributed to the change of the binding stoichiometry. The distinct absorption and fluorescence spectra allowed the determination of the equilibrium constant of each individual binding process. Despite the 38-fold larger stability constant of the 1:1 complex, the inclusion of the first EH^+ was much less exothermic than the second binding step. Substantial entropy gain contributed to the driving force of 1:1 encapsulation, whereas 2:1 complexation was entropically highly unfavourable.

2. Kinetics and thermodynamics of the reversible binding of alkaloids in cucurbiturils

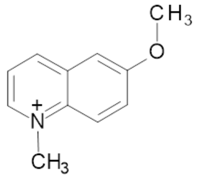

Berberine (B^+)


Flavopereirine (Fla)

Many applications of cucurbiturils (CBn) are hindered by the lack of information on the rate of entry into and exit from their hydrophobic cavity. Therefore, we intended to unravel the effects of the various factors controlling the kinetics of their reversible binding processes. All experimental results could be rationalized by a one step binding equilibrium. Intermediate or exclusion complex formation was not observed. The substantial change of the fluorescence properties upon inclusion complex formation was exploited to monitor the embedment in real time by stopped-flow method. A highly accurate simple method was developed for the selective determination of the dissociation rate constant of cucurbit[7]uril (CB7) complexes. The rate constant of the encapsulation of alkaloid (berberine or flavopereirine) in the apolar core of CBn macrocycles was 2–3 orders of magnitude lower than that of the diffusion-controlled process. Measurements at various temperatures provided information on the activation parameters of the association and dissociation. Such quantities could not be determined previously. Large activation enthalpies were found suggesting that substantial structural change had to occur when the studied alkaloids squeezed through the tight carbonyl-rimmed portal of the macrocycle. We demonstrated that the combination of absorption and fluorescence spectroscopic studies with isothermal calorimetric titrations and stopped-flow measurements is a powerful way to reveal both the thermodynamics and the kinetics of inclusion complex formation. With the global analysis of the results obtained by various experimental techniques, we could provide the first comprehensive view on the dynamics of ternary complex formation. Highly stable 1:1 and 2:1 berberine–CB8 complexes were obtained due to the release of the high energy water molecules from the CB8 interior, and the second binding step proved to be almost 3 times more exothermic. The favourable entropy change contributed appreciably to the driving force of 1:1 encapsulation. Despite the larger enthalpy gain for the 2:1 complex formation, the ingression of the second berberine into CB8 cavity was considerably slower than 1:1 confinement. This exemplifies that the kinetic behaviour cannot be predicted on the basis of the thermodynamic parameters of host-guest binding.

3. Alteration of the fluorescence of 6-methoxy-1-methyl-quinolinium upon inclusion in macrocycles

To prove the essential role of the steric factors in the kinetics of embedment in CB7, host-guest complexation of 6-methoxy-1-methylquinolinium (C_1MQ) cation was studied. The rate of exchange between CB7-bound and free C_1MQ was too rapid for stopped-flow measurements but was comparable to the NMR timescale. The analysis of the line width of the NMR spectra showed that the rate constant of the inclusion is close to the diffusion-controlled limit of bimolecular reactions. This suggests that the relatively small C_1MQ can easily enter into CB7 without significant steric hindrance. The exit from CB7 occurred also significantly faster than found for the bulkier alkaloids.



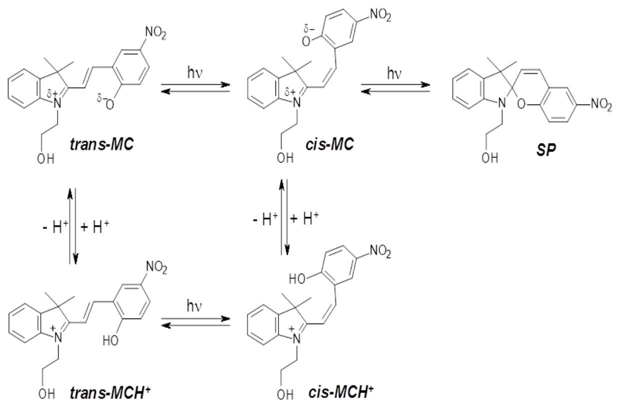
C_1MQ

We assigned the dual-exponential fluorescence decay kinetics of the free C_1MQ to two torsional isomers differing in the orientation of the methoxy moiety relative to the heterocyclic ring. Such an often overlooked phenomenon may also contribute to the intricate photophysics of methoxyquinoline type alkaloids.

Inclusion complex formation significantly slowed down the photoinduced electron transfer from iodide and azide to the singlet-excited C_1MQ, but did not preclude the reaction. These results are the first indication that long distance electron transfer may occur through the wall of the CB7 macrocycle. The enormous difference in the reaction rate constant for free and encapsulated C_1MQ in the electron transfer from chloride or bromide was exploited to sensitively detect the release of the guest from CB7.

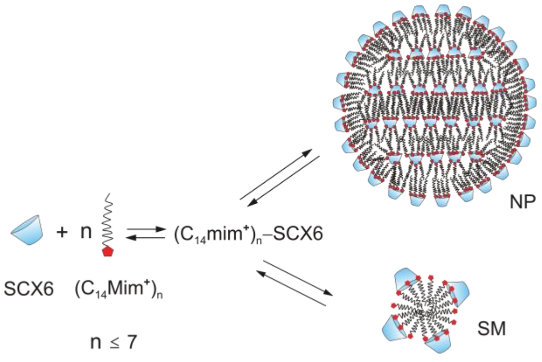
The encapsulation of C_1MQ in 4-sulfonatocalix[4]arene (SCX4) in the ground state resulted in an efficient fluorescence quenching due to electron transfer from the host to the excited guest. The marked difference in the fluorescence quantum yields for free and bound C_1MQ was used to detect the competitive complexation of the widely used herbicide, difenzoquat in SCX4. We demonstrated that C_1MQ is a valuable off–on fluorescent probe for the study of the binding affinity of nonfluorescent guests to SCX4. It can be used in a wide pH range because it does not contain acid or base susceptible moiety.

4. Photochromism in the cavity of 4-sulfonatocalixarenes

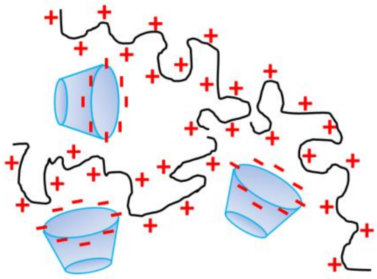


We revealed how the binding to the highly negative flexible SCXn cavitands changes the reversible interconversion between spiro (SP) and merocyanine (MC) forms of the frequently used N-(2-hydroxyethyl)-3',3'-dimethyl-6-nitrospiro[2H-1-benzopyran-2,2'-indoline] photochromic compound. To the best of our knowledge, photochromism was not studied previously in SCXn cavity. The equilibrium constants of 1:1 binding were independent of the size of the macrocycle, and about 7-fold more stable associates were produced at pH 2.3 than in slightly alkaline solution. The complexation diminished the acidity of protonated merocyanine and precluded its photoinitiated transition to spiro isomer, but did not affect the reactions in basic media. The association with SCX4 slowed down the thermal back reaction in the dark to a larger extent than the confinement to 4-sulfonatocalix[8]arene (SCX8). Both the activation energy and the Arrhenius A factor were significantly smaller when the less spacious, more rigid macrocycle served as a host.

5. Self-assembly promoted by 4-sulfonatocalixarenes

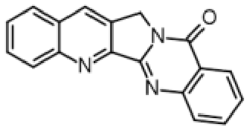


Our long-term objective is to design tailor-made stimuli-responsive self-assembled systems capable of drug delivery. To this end, the first step is to understand the major factors influencing the self-organization of host−guest complexes. As a model system, the association processes were studied in the solution of SCXn and 1-alkyl-3-methylimidazolium (C_nmim^+) type of amphiphiles. We demonstrated that SCXn induces spherical nanoparticle (NP) formation when $C_n$mim$^+$ possesses dodecyl, tetradecyl, or hexadecyl substituent (n = 12, 14, 16). The stability of the NPs increased with the lengthening of aliphatic chain of $C_n$mim$^+$. Systematic studies with the tetradecyl derivative (C_{14}mim$^+$) showed that supramolecular micelle (SM) can also be produced in the presence of NaCl when the cavitand contained 6 or 8 sulfonatophenol units (SCX6 or SCX8). The best highly reversible, temperature-responsive behaviour was achieved using C_{14}mim$^+$ cation and SCX6. The conditions of the NP−SM transition could be tuned by the alteration of C_{14}mim$^+$:SCX6 mixing ratio and NaCl concentration. The effect of experimental conditions on the enthalpy of the association processes was revealed by isothermal calorimetric titrations. The reversible NP−SM transformation may find applications in systems possessing thermally switchable optical properties and in assemblies capable of controlled release of substrates.



The biocompatible SCXn macrocycles were found to serve simultaneously as a crosslinking agent and a macrocyclic receptor in the presence of a biocompatible polysaccharide, chitosan in acidic solution. Due to their multianionic character, SCXn molecules established electrostatic interactions with the protonated amino groups of chitosan promoting thereby nanoparticle formation. The most stable nanoparticles with 160 nm diameter and narrow size distribution were obtained at pH 4 using SCX8 and low molecular weight chitosan. These particles encapsulated coralyne, an isoquinoline alkaloid with more than 90 % entrapment efficiency. A loading ratio of [coralyne]/[SCX8] = 1.7 was achieved without any stability loss. A significant advantage of SCXn utilization for the initiation of self-organization of the cationic polymer into NPs is that these biocompatible macrocycles keep polyanionic character and as a consequence, the crosslinking ability even in strongly acidic conditions.

6. Effect of hydrogen bonding and metal cations on the fluorescence of luotonin A



Luotonine A (LuA)

We plan to investigate the effect of metal ions on the inclusion complex formation of luotonin A (LuA). As a first step, we revealed how the solvent polarity, strong hydrogen bonding and metal ions affect the kinetics of excited-state deactivation. Despite the diverse biological activities of LuA, its time-resolved fluorescent behaviour was unknown. We found that the rate constant of the radiationless deactivation from the singlet-excited state diminished by more than one order of magnitude when the solvent polarity was changed from toluene to water. Dual emission was found in polyfluorinated alcohols of large hydrogen bond donating ability due to photoinitiated proton displacement along the hydrogen bond. In CH_2Cl_2, luotonin A produced both 1:1 and 1:2 hydrogen-bonded complexes with hexafluoro-2-propanol in the ground state. Photoexcitation of the 1:2 complex led to protonated luotonin A. Chelate formation was observed with metal ions in acetonitrile. The stability of the complexes diminished in the series of $Cd^{2+} > Zn^{2+} > Ag^{+}$, and upon competitive binding of water to the metal cations. Coordination of water to the metal ions influenced the stability of chelates much more significantly than hydrogen bonding with the ligand. The complexation with Ag^{+} caused efficient fluorescence quenching.

The results of this project were published in 14 scientific papers, whose total impact factor is 40. These articles have already received 48 independent citations despite the short time passed since their publication. Moreover, the outcomes of research were presented in 12 international and 16 national scientific meetings. Invited lectures were delivered in Australia, Japan, China, Thailand and Croatia. The OTKA/NKFIH grant significantly contributed to the successful collaborations with French, Japanese, and Chilean researchers.