Self-Assembly

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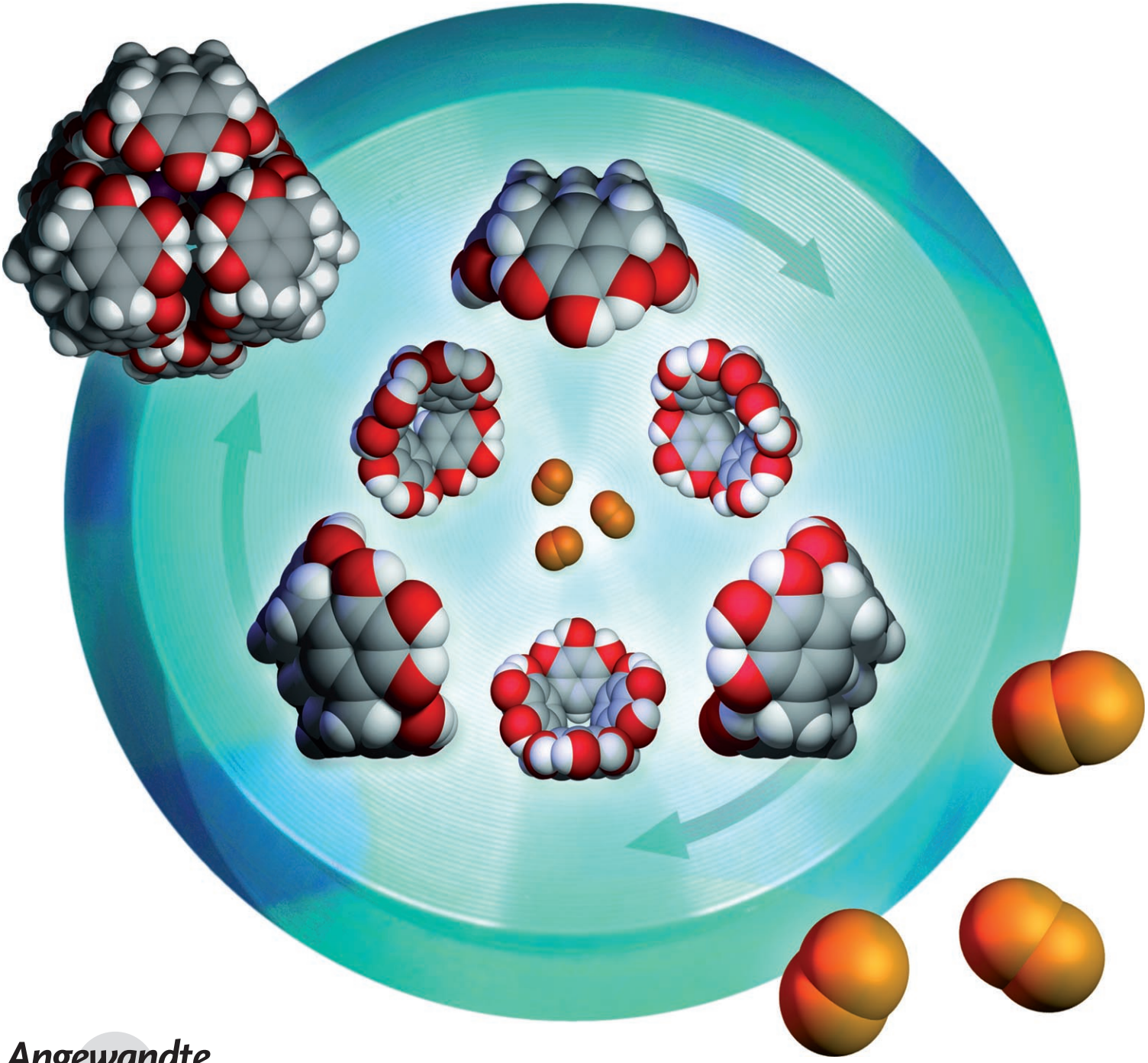
Loading Molecular Hydrogen Cargo within Viruslike Nanocontainers**

*K. Swaminathan Iyer, Marck Norret, Scott J. Dalgarno, Jerry L. Atwood, and Colin L. Raston**



Cells, viruses, and proteins are naturally occurring biological examples of systems that enclose functional entities within a protective boundary. Typically, viruses are assembled from repeating subunits to form highly symmetrical and homogeneous architectures. Capsids have emerged as platforms for synthetic manipulation with a range of applications from materials to medicine. Recent advances suggest that viruses can be used as molecular containers to deliver drugs by replacing the natural viral cargo by synthetic material.[1,2] The exchange of cargo is achieved by capsid dissociation and reassembly. However for many protein multimers, association and dissociation reactions fail to reach the same end point; hysteresis prevents one or the other reaction from equilibrating.[3] The ability to encapsulate chemical space by designing artificial viruslike architectures is a concept that has attracted considerable attention.[4–6] While spectacular advances have been made in the synthesis of nanoscale molecular capsules and the encapsulation of soluble guests in solutions, the challenge is in regulating the behavior of these synthetic capsules to mimic viruses. In particular, the challenge is in controlling the assembly–disassembly–reassembly process of the capsules efficiently and in the encapsulation of a nonsolvent molecular guest in solution without the practical inconvenience of changing the solvent(s) or undertaking chemical transformations.[7,8] Herein we demonstrate a strategic route to regulate the disassembly and reassembly of hexameric capsules in solution. Furthermore, we also show that the current technique offers an exciting route to load a nonsolvent molecular cargo (hydrogen gas) as guests within the capsules.[9,10]

Self-assembled molecular capsules, held together by noncovalent forces such as hydrogen bonding, metal–ligand interactions, or electrostatic interactions, have the potential to undergo reversible guest encapsulation, thereby providing a mechanical barrier between the guest and bulk solution.[11–13] The host capsules surround their guests, isolating them from the bulk solution and placing them in a distinctly different molecular environment. Such molecular capsules are capable of enantioselective molecular recognition, and allow the isolation and stabilization of reactive species.[14] Ideally, applying an external force should allow controlled disassembly of the capsule through disruption of the concerted noncovalent forces holding the assembly together. We are particularly interested in the controlled "assembly–disassembly–reassembly" of the cavitand *C*-alkylpyrogallol[4]arenes (general notation PgC_n). These molecules can self-assemble into hexameric capsules and have been characterized in the solid state, in solution, and in the gas phase.[15–18] Moreover, they contain an additional "upper-rim" hydroxy group relative to *C*-alkylresorcin[4]arenes resulting in a larger number of hydrogen bonds per hexamer, thereby increasing their structural integrity.[19]

Access to the internal space of self-organized molecular capsules requires formation of a portal through the rupture of numerous hydrogen bonds. The curvature of the cavitand molecules requires that the majority of the hydrogen bonds in the assembly are broken for this to occur, and it is therefore unlikely that these portals are formed without complete dissociation of at least one cavitand subunit.[16] The corresponding diffusion coefficients of the cavitands are relatively low in solutions at ambient conditions. Diffusion NMR spectroscopy is an effective method to monitor the structure, stability, and guest affinity of the above-mentioned hexameric capsules.[14] In chloroform, molecular capsules based on six *C*-alkylpyrogallol[4]arenes encapsulate six solvent molecules. It is also noteworthy that self-recognition is prevalent with a mixture of *C*-alkylresorcin[4]arenes and *C*-alkylpyrogallol[4]arenes resulting in homo-hexameric capsule assemblies consisting solely of the same cavitand.[14] However, two different *C*-alkylpyrogallol[4]arenes can form hetero-hexameric capsules in solution over time or after heating. Fluorescence resonance energy transfer analysis at nanomolar concentrations, where solvated monomers of the *C*-alkylpyrogallol[4]arene are likely, shows that the monomers exchange at a much slower rate than monomers of *C*-alkylresorcin[4]arene.[20,21] Hexamers based on *C*-alkylpyrogallol[4]arene are hence more stable, even at nanomolar concentrations, and necessitate the addition of a higher concentration of a cosolvent (methanol) for the complete disassembly of the hexameric capsules.[16] At ambient temperature, rupture of hydrogen bonds in hexameric capsules based on *C*-alkylpyrogallol[4]arene is slow on the NMR time scale. Thus, moving nonsolvent components both to and from the internal confines of the capsules (*endo-* ↔ *exo-*) at ambient temperature requires a dramatic enhancement of the corresponding diffusion coefficients of the cavitands.

Spinning disk processing (SDP; Figure 1) is a process intensification module recently used in the fabrication of nanomaterials with remarkable precision under continuous flow,[22] and we now establish its potential in controlling self-assembly processes. The key components of SDP herein include the use of a 100 mm diameter rotating disk with controllable speed and feed jets located at a radial distance of 5 mm from the center of the disk. SDP generates a very thin fluid film (1 to 200 μm) on a rapidly rotating disc surface (300 to 3000 rpm). The hydrodynamics of the film flow over the spinning disk is important in controlling the disassembly of the hexameric capsules. The thinness of the film contributes to many influential chemical processing characteristics, one being a very high surface area to volume ratio, resulting in more favorable interactions between the film and its surroundings. Thin layering permits uniform transfer rates throughout the entire reaction mixture. In addition, strong

[*] Dr. K. Swaminathan Iyer, Dr. M. Norret, Prof. C. L. Raston
Centre for Strategic Nano-Fabrication, School of Biomedical, Biomolecular and Chemical Sciences
The University of Western Australia
Crawley, WA-6009 (Australia)
Fax: (+61) 8-6488-8683
E-mail: clraston@chem.uwa.edu.au
Homepage: http://www.strategicnano.uwa.edu.au
Dr. S. J. Dalgarno, Prof. J. L. Atwood
Department of Chemistry, University of Missouri-Columbia
601 South College Avenue, Columbia, MO 65211 (USA)

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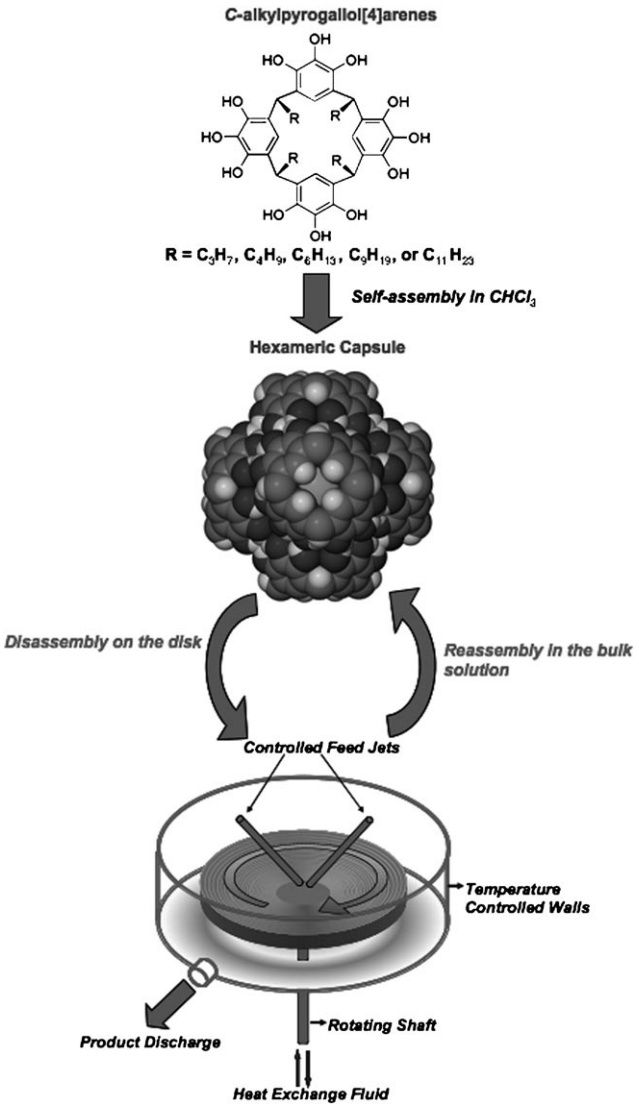

Figure 1. Schematic representation of the self-assembly of *C*-alkylpyrogallol[4]arenes into hexameric capsules in chloroform followed by disassembly by spinning disk processing (SDP) and reassembly post-SDP in the product stream.

shearing forces create turbulence and breaks the surface tension of the film, making waves and ripples. These waves and ripples add to the vigor of mixing, enabling very high mass transfer rates in the film under plug flow conditions. The high shear rates coupled with the turbulent micromixing across the thin film provide a mechanism to disassemble the hexameric capsules based on *C*-alkylpyrogallol[4]arenes in a chloroform solution, followed by spontaneous reassembly of the *C*-alkylpyrogallol[4]arenes to the parent hexameric state as the solution leaves the disc.

The formation of hexamers of PgC_3 and PgC_9 were monitored using dynamic light scattering (DLS) as a technique to measure the hydrodynamic diameter of capsules; it indicates how the capsules diffuse within a fluid (Figure 2). The diameter obtained by DLS is that of a sphere with the same translational diffusion coefficient as a capsule, and this in turn depends on its size and surface characteristics. DLS was effective in differentiating between the homo-hexameric capsules of PgC_3 and PgC_9 in chloroform and their corresponding monomeric solvated *C*-alkylpyrogallol[4]arenes in DMSO. We note that mixing a solution of preformed capsules of PgC_3 in chloroform and a solution of preformed capsules of PgC_9 in chloroform resulted in a bimodal distribution with the peaks corresponding to the individual hexameric capsule sizes. Since the diffusion coefficients under ambient temperatures are relatively low, only homo-hexameric capsules are formed. Remarkably, mixing the same solutions using SDP results in a single DLS peak at 2.3 nm, which corresponds to an average of the preformed capsules in the absence of shearing. This result is consistent with the disassembly of the hexameric capsules into solvated monomers or to a solvated lower order oligomer, on the disk, with instantaneous reassembly into mixed hetero-hexameric capsules post-SDP. At the same time, this flash disassembly–reassembly process

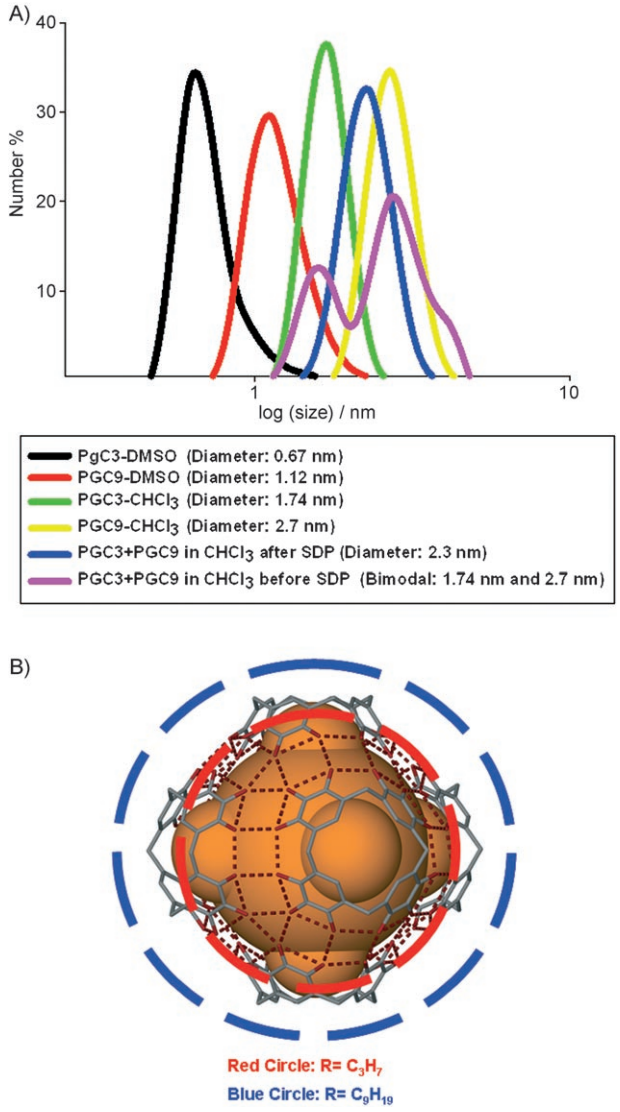

Figure 2. a) Average size distribution of PgC_3 and PgC_9 in DMSO and chloroform together with the size distribution of their equimolar mixtures pre- and post-SDP, as obtained by DLS. b) A schematic representation of the hexameric capsules and their corresponding hydrodynamic diameters (dashed circles).

can be used to load a range of molecular cargos within the capsules, demonstrated below for hydrogen molecules.

A recent report highlights that waves generated in a fluid film over a moderately spinning disk enhances the adsorption of gas molecules into a liquid.[23] The flow is accompanied by nonlinear waves, which strongly influence the diffusion boundary that develops beneath the surface of the film. The progressive waves generated in the region of active micromixing in the film of solution in the present study provides a method of dramatically increasing the concentration of hydrogen in solution, and thus reassembling capsules are able to entrap molecular hydrogen. ^{1}H NMR spectroscopy was used to monitor the molecular capsules both pre- and post-SDP. An additional peak at $\delta = 4.65$ ppm for all the PgC_n capsules was consistently present post-SDP (Figure 3). Entrapment of hydrogen in clatharate hydrates at 120 bar and 270 K results in a ^{1}H NMR peak at $\delta = 4.3$ ppm corresponding to hydrogen in small cavities and a signal at $\delta = 0$ ppm corresponding to the hydrogen in large cavities.[24] The former is consistent with our results of entrapment of hydrogen guests in the small cavities of the hexameric *C*-alkylpyrogallol[4]arenes capsules, along with solvent molecules, noting that the internal volume of the capsule is approximately 1250 Å^3.

It has also been reported that the optimal packing coefficient (PC) of neutral guests in capsules is close to 0.55; that is, chloroform entrapped in the *C*-alkylresorcin[4]arene hexamer is more dilute than bulk chloroform.[25] This implies that increased space available for entrapped species results in entropic stabilization of the hexameric assemblies. Hydrogen cannot be incorporated into the capsules by bubbling the gas through a solution in chloroform, and there is no evidence from ^{1}H NMR spectroscopy of hydrogen gas in the bulk solution (*exo*-capsule). The estimated average concentration of the gas molecules in the hexameric capsules as derived from the ^{1}H NMR peaks is up to 30 mol %, which is equivalent to 0.01 wt %. Not all capsules may contain molecular hydrogen, and the concentration herein is a measure of the average concentration of the gas retained within the capsules. Additionally, heating or exposure to air results in diffusion of the gas from the capsules and thus from solution, with concomitant loss of the ^{1}H NMR spectrum signal at $\delta = 4.65$ ppm. This result is indeed consistent with previous reports showing that guest exchange (*endo-* ↔ *exo-*) in *C*-alkylpyrogallol[4]arenes hexamers is slow on the NMR time scale for most entrapped species.[16]

In conclusion, we have established that entrapment of a nonsolvent guest, in particular molecular hydrogen, in self-organized molecular capsules is possible under ambient conditions through a controlled "disassembly–reassembly" process, facilitated using spinning disk processing. This type of application of SDP with its practical convenience has potential as a versatile method for assessing and controlling interactions at the molecular and macromolecular level. Furthermore, it can facilitate a large gamut of guest–host exchange within both synthetic and natural containers and potentially replace RNA from capsids. The continuous flow technology can be used to load molecular cargos within nanocontainers on a large scale. In the present case this includes the transfer of *exo*-capsule species to the interior of the capsule. Understanding the complexity of the current system arising from the number of statistical possibilities of disassembly of the hexamer into lower order oligomers is a major challenge, which will require, for example, exploring the effect of different shear rates.

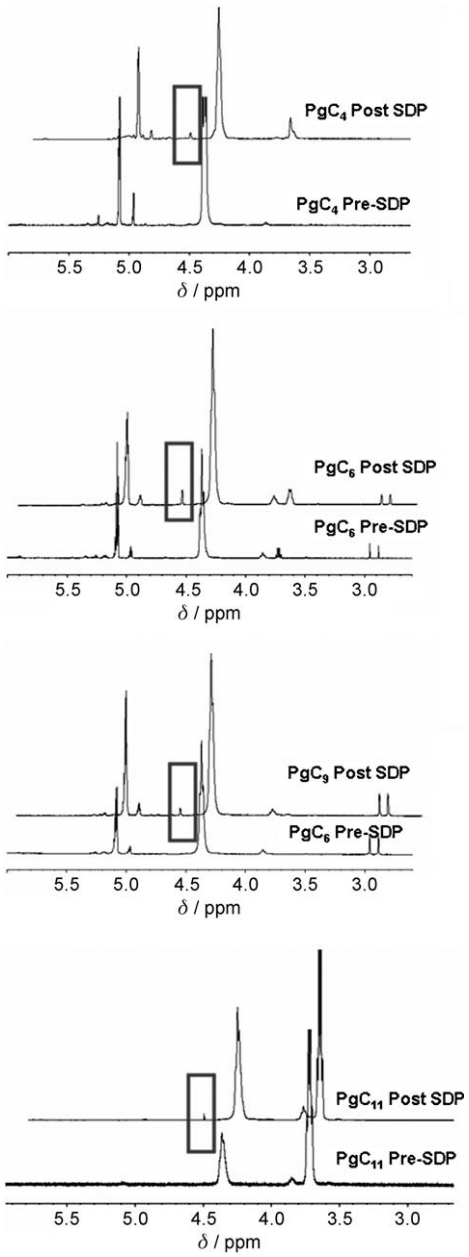

Figure 3. ^{1}H NMR spectra of PgC_4, PgC_6, and PgC_9 in chloroform and PgC_{11} in deuterated chloroform showing an additional peak corresponding to entrapped hydrogen at $\delta = 4.65$ ppm post-SDP.

Experimental Section

All chemicals ($CHCl_3$, $CDCl_3$, and DMSO) were purchased from Aldrich and were used without further purification. *C*-alkylpyrogallol[4]arenes were synthesized by using standard literature procedures.[26] Typically, the "assembly–disassembly–reassembly" proce-

dure involved preparing a solution (5 mg mL^{-1}) of the *C*-alkylpyrogallol[4]arenes in $CHCl_3$ (PgC_3, PgC_4, PgC_6, and PgC_9) or $CDCl_3$ (PgC_{11}). A Protensive 100 series SDP was used with integrated feed pumps to direct the reactants onto the rotating disk. The above solutions were delivered onto the disk surface by using one feed jet at 1.0 mL s^{-1} by using continuous flow gear pumps (MicroPumps). A constant flow of hydrogen gas at an outlet pressure of 15–20 psi was maintained through the second feed jet. The grooved steel disk had a diameter of 100 mm and was manufactured from 316 stainless steel with 80 concentric engineered grooves equally spaced at a depth of 0.6 mm. The optimal disk rotation speed for maximum mixing was 2500 rpm.[22] The samples were collected from the outlet for analysis. ^{1}H NMR spectra were collected on a Bruker AV500 instrument in a 5 mm NMR tube. The spectra were recorded in $CHCl_3$ (PgC_3, PgC_4, PgC_6, and PgC_9) or $CDCl_3$ (PgC_{11}) and were referenced to solvent signals ($CHCl_3$ at $\delta = 7.26$ ppm and $CDCl_3$ at $\delta = 7.26$ ppm). Solutions were analyzed for mean diameter by using dynamic light scattering (Zetasizer Nano ZS series; Malvern Instruments) with 532 nm wavelength laser and measurement angle 173° (backscatter detection) at 25 °C. An average of 300 readings were taken for each solution.

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