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Self-Sorting and Coassembly of Fluorinated, Hydrogenated, and Hybrid Janus Dendrimers into Dendrimersomes

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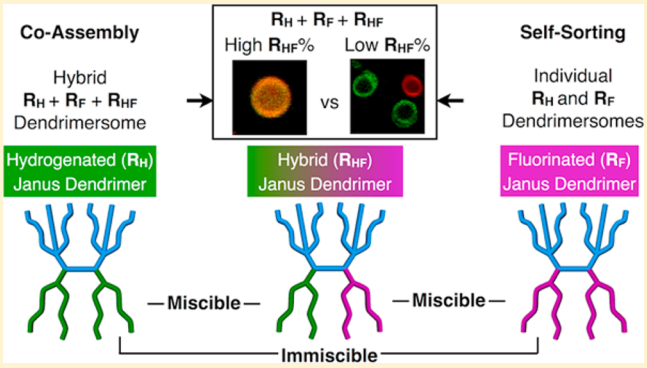
Supporting Information

ABSTRACT: The modular synthesis of a library containing seven self-assembling amphiphilic Janus dendrimers is reported. Three of these molecules contain environmentally friendly chiral-racemic fluorinated dendrons in their hydrophobic part (R_F), one contains achiral hydrogenated dendrons (R_H), while one denoted hybrid Janus dendrimer, contains a combination of chiral-racemic fluorinated and achiral hydrogenated dendrons (R_{HF}) in its hydrophobic part. Two Janus dendrimers contain either chiral-racemic fluorinated dendrons and a green fluorescent dye conjugated to its hydrophilic part (R_F-**NBD**) or achiral hydrogenated and a red fluorescent dye in its hydrophilic part (R_H-**RhB**). These R_F, R_H, and R_{HF} Janus dendrimers self-assembled into unilamellar or onion-like soft vesicular dendrimersomes (DSs), with similar thicknesses to biological membranes by simple injection from ethanol solution into water or buffer. Since R_F and R_H dendrons are not miscible, R_F-**NBD** and R_H-**RhB** were employed to investigate by fluorescence microscopy the self-sorting and coassembly of R_F and R_H as well as of phospholipids into hybrid DSs mediated by the hybrid hydrogenated-fluorinated R_{HF} Janus dendrimer. The hybrid R_{HF} Janus dendrimer coassembled with both R_F and R_H. Three-component hybrid DSs containing R_H, R_F, and R_{HF} were formed when the proportion of R_{HF} was higher than 40%. With low concentration of R_{HF} and in its absence, R_H and R_F self-sorted into individual R_H or R_F DSs. Phospholipids were also coassembled with hybrid R_{HF} Janus dendrimers. The simple synthesis and self-assembly of DSs and hybrid DSs, their similar thickness with biological membranes and their imaging by fluorescence and ^{19}F-MRI make them important tools for synthetic biology.



INTRODUCTION

Synthetic lipids[1] such as phospholipids and glycolipids self-assemble into vesicles that mimic biological membranes. The amphiphilic structure of the lipids contains a hydrophilic head and hydrophobic tails. Lipophilic groups such as alkyl chains and fluorophilic groups such as fluorocarbon fragments have been successfully incorporated into lipids as their hydrophobic tails[1b] and therefore form hydrogenated and fluorinated vesicles with different stabilities and permeabilities.[2] The immiscibility of hydrogenated and fluorinated components induced the phase separation of these domains.[2h] Fluorinated amphiphiles have been employed for biomedical applications including for drug and gene delivery.[2d,e] Fluorinated components including fluoropolymer nanoparticles also present potential functions such as ^{19}F magnetic resonance imaging (MRI) agents.[3] This requires stable and biocompatible fluorinated vesicles, which can be synthesized and self-assembled by simple methods. The miscibility of hybrid structures with both hydrogenated and fluorinated chains has been preliminarily investigated in

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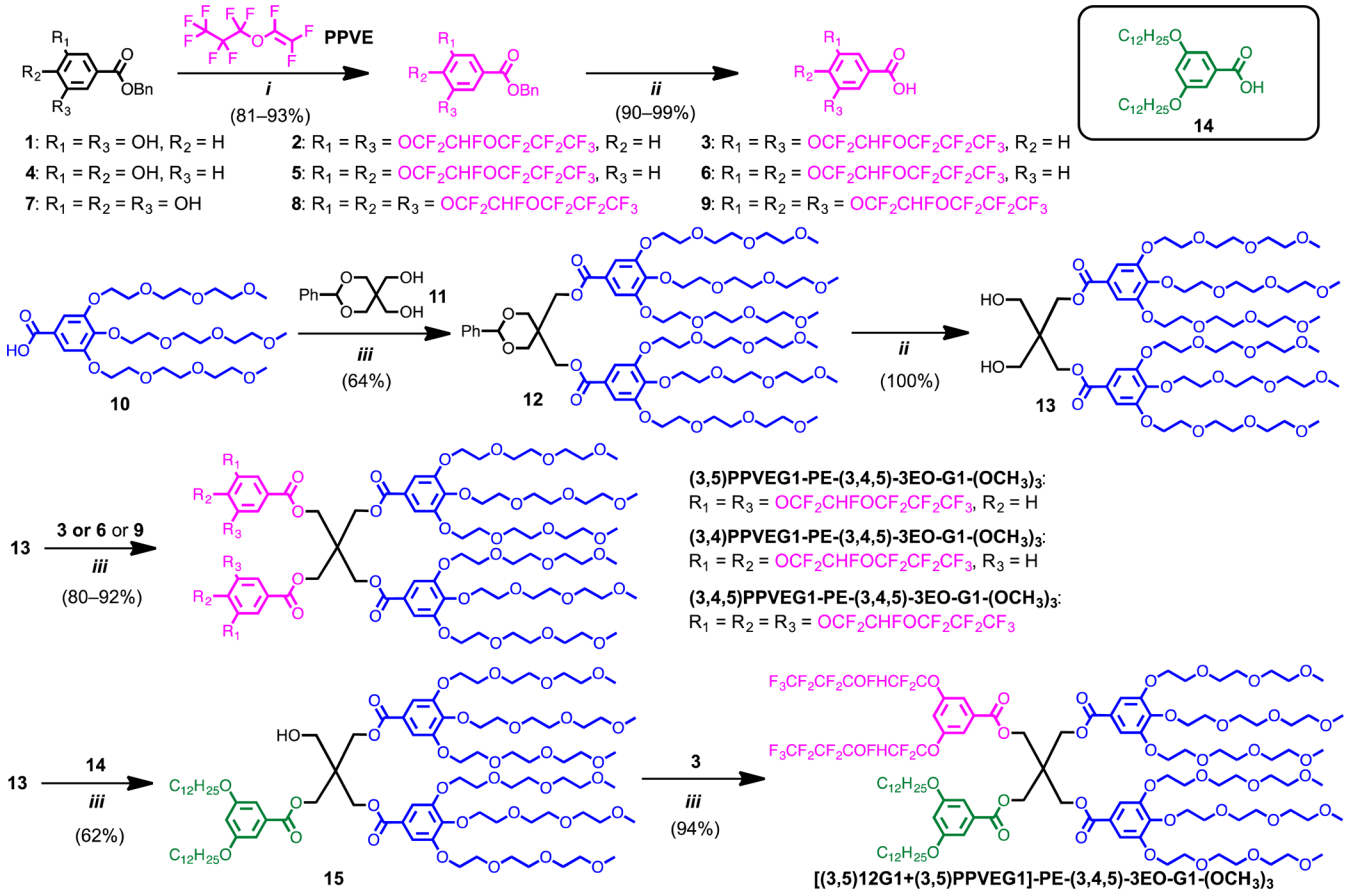
Scheme 1. Synthesis of R_F Janus Dendrimers and Hybrid R_{HF} Janus Dendrimer with Both R_F and R_H Chains[a]



[a]Reagents and conditions: (i) *t*-BuOK (cat.), DMF, 0 °C for 2 h, then 23 °C for 24 h; (ii) H_2, Pd/C, DCM, methanol, 23 °C, 12 h; and (iii) 4-(Dimethylamino)pyridinium 4-toluenesulfonate, DCC, DCM, 23 °C, 12 h.

phospholipids and glycolipids.[4] However, very little is known about their assemblies.

Amphiphilic Janus dendrimers[5] containing both hydrophobic (hydrogenated lipophilic chains) and hydrophilic dendrons self-assemble into nanoscale vesicular dendrimersomes (DSs) by simple injection of their solution in a water miscible organic solvent into water and buffer and into micrometer-scale giant DSs by film hydration. The sizes of DSs can be predicted from the thickness of their bilayer in the bulk state and from the concentration of the Janus dendrimer.[5b] It is expected that hydrogenated chains can be replaced with fluorinated chains leading to a new family of fluorinated vesicles named fluorinated DSs that will follow the same self-assembly principles as hydrogenated DSs.

Hybrid vesicles coassembled from lipids and either block copolymers[6] or Janus dendrimers[5c] provided a platform for the incorporation of biological cell membrane components into polymersomes and DSs.[5c] Polymersomes[7] generate bilayers with a thickness of 8−50 nm and therefore are not compatible with biological membranes that have a thickness of ∼4 nm,[6c] while DSs overcame this biocompatibility issue.[5c] Hence the design and synthesis of stable and biocompatible fluorinated DSs and of their hybrid structures with biological membranes including phospholipids and glycolipids became achievable.

Here we report the design and synthesis of a library containing hydrogenated, environmentally friendly chiral-racemic fluorinated and hybrid hydrogenated-fluorinated amphiphilic Janus dendrimers. Fluorinated and hydrogenated Janus dendrimers conjugated in their hydrophilic part with complementary fluorescent dyes were also elaborated. This library allowed us for the first time to investigate the self-sorting and coassembly of fluorinated, hydrogenated and hybrid hydrogenated-fluorinated Janus dendrimers into fluorinated, hydrogenated, and hybrid hydrogenated-fluorinated DSs as well as hybrid vesicles coassembled from phospholipids and fluorinated Janus dendrimers.

RESULTS AND DISCUSSION

Design and Modular Synthesis of Amphiphilic Janus Dendrimers with Fluorinated, Hydrogenated, and Hybrid Fluorinated-Hydrogenated Chains. The chiral-racemic fluorinated dendrons employed in this study provide access to less ordered structures than those resulting from achiral hydrogenated or perfluorinated dendrons.[8,9] Perfluoropropyl vinyl ether (PPVE) used in their synthesis is an environmentally tolerable fluorinated component that has been incorporated into chiral-racemic fluorinated dendrons in a one-step reaction performed under very mild reaction conditions (Scheme 1).[9]

With a catalytic amount of potassium *tert*-butoxide (*t*-BuOK) as base in anhydrous dimethylformamide, PPVE chains were introduced as side groups to benzyl (3,5)-, (3,4)-, and (3,4,5)-hydroxyl benzoates (Scheme 1). Previous studies have demonstrated that linear perfluorooctyl alkyl groups enhance

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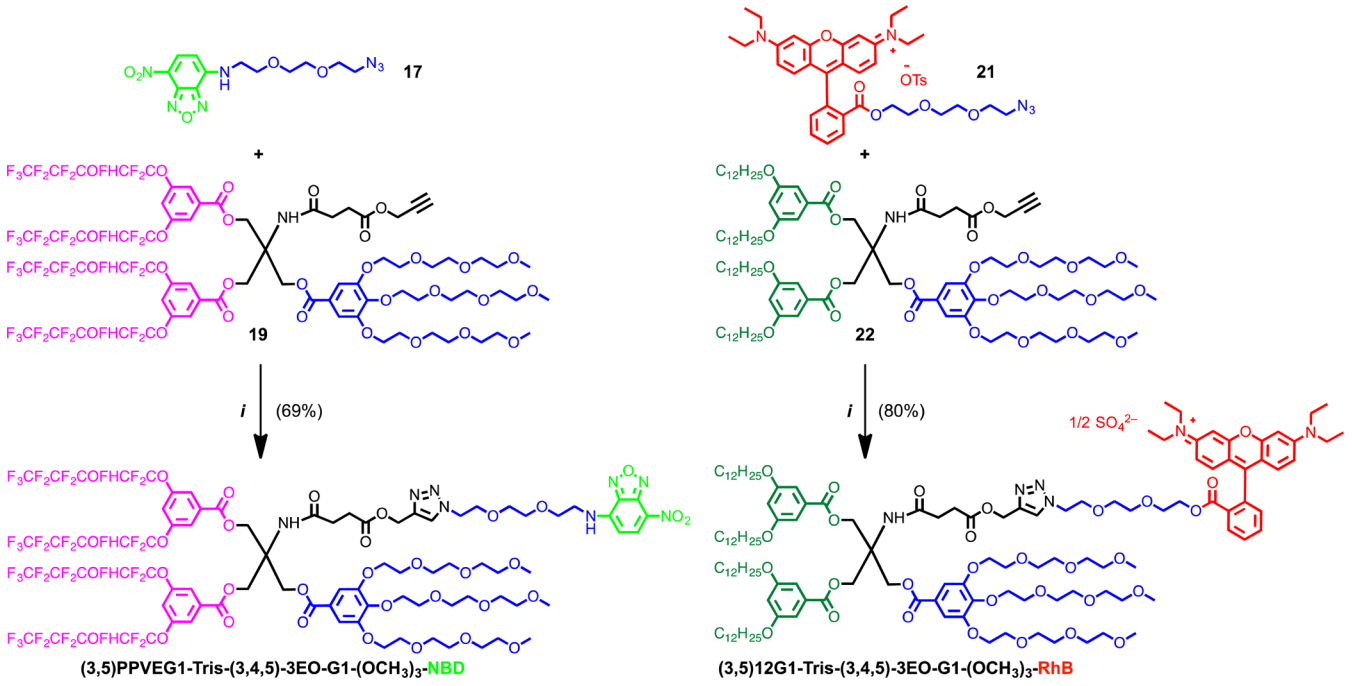
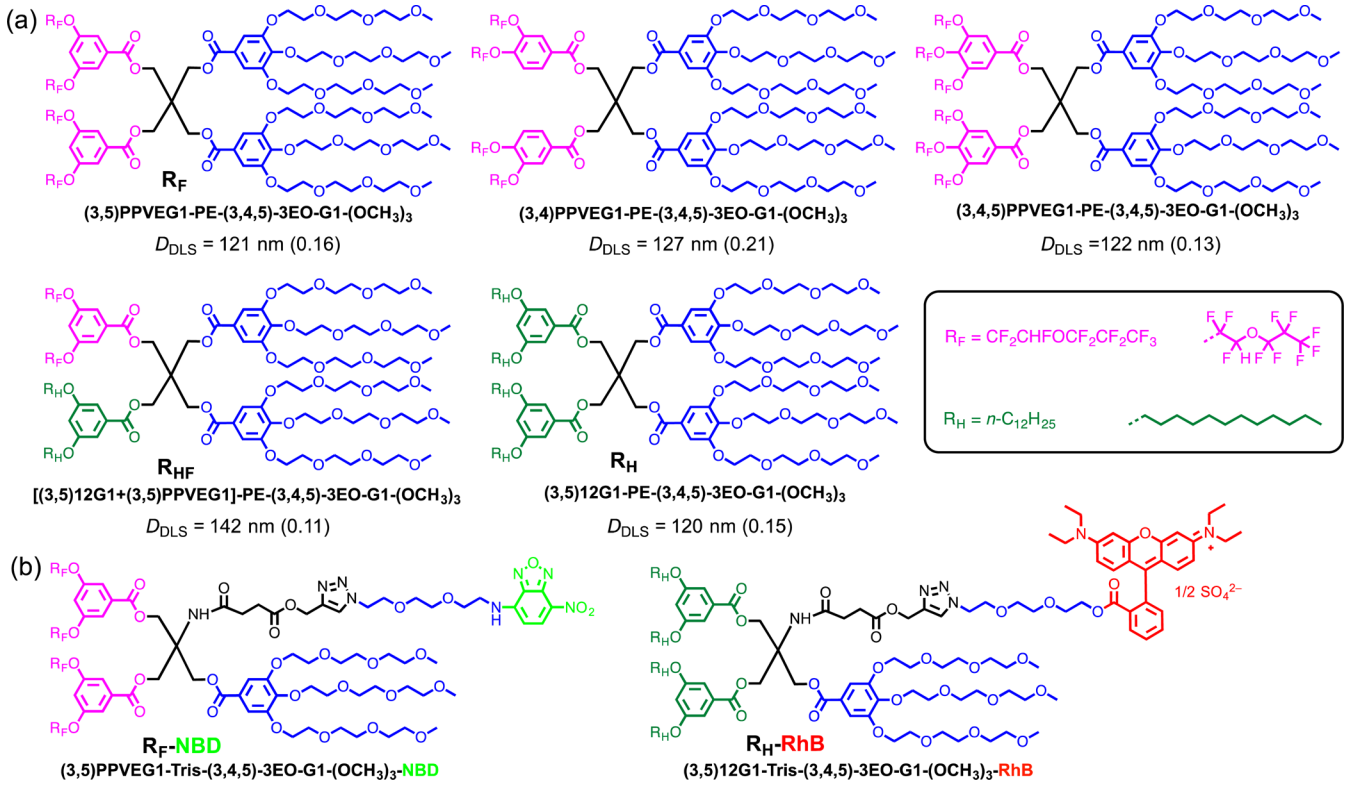
Scheme 2. Synthesis of NBD Conjugated Janus Dendrimer with R_F Chains and Rhodamine B Labeled Janus Dendrimer with R_H Chains[a]



[a]Reagents and conditions: (i) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF, water, 23 °C, 24 h.

Scheme 3. Library Containing Three Janus Dendrimers with R_F, One Hybrid with R_{HF}, One with R_H Chains, and Two Dye-Labeled Fluorescent Janus Dendrimers with R_F or R_H Chains[a]



[a]Diameters (D_{DLS}) and polydispersities (in between parentheses) indicated were obtained by DLS and refer to dendrimersomes obtained by injection of their ethanol solution into water (final concentration: 0.5 mg·mL^{-1}).

the stability of vesicles[2] and of other supramolecular assembles.[8] However, all linear perfluorooctyl based compounds degrade to the toxic and biopersistent perfluorooctanoic acid that was prohibited by the Environmental Protection

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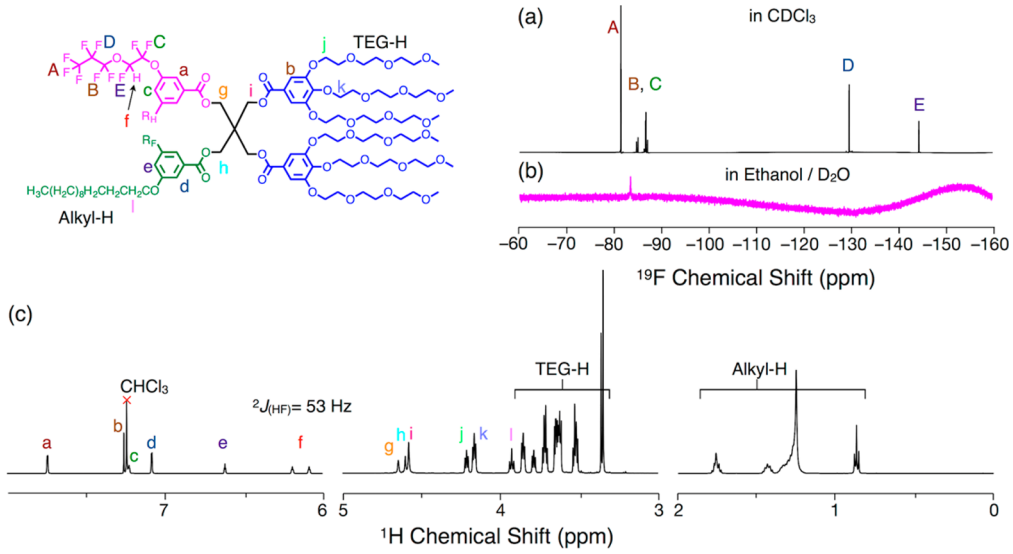

Figure 1. Excerpt of (a,b) ^{19}F NMR spectra (470 MHz) (a) R_{HF} Janus dendrimer [**(3,5)12G1+(3,5)PPVEG1**]-**PE-(3,4,5)-3EO-G1-(OCH$_3$)$_3$** in $CDCl_3$, and (b) self-assembled dendrimersomes prepared by injection of an ethanol solution of R_{HF} Janus dendrimer [**(3,5)12G1+(3,5)PPVEG1**]-**PE-(3,4,5)-3EO-G1-(OCH$_3$)$_3$** into D_2O. (c) Excerpt of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of R_{HF} Janus dendrimer [**(3,5)12G1+(3,5)-PPVEG1**]-**PE-(3,4,5)-3EO-G1-(OCH$_3$)$_3$**.

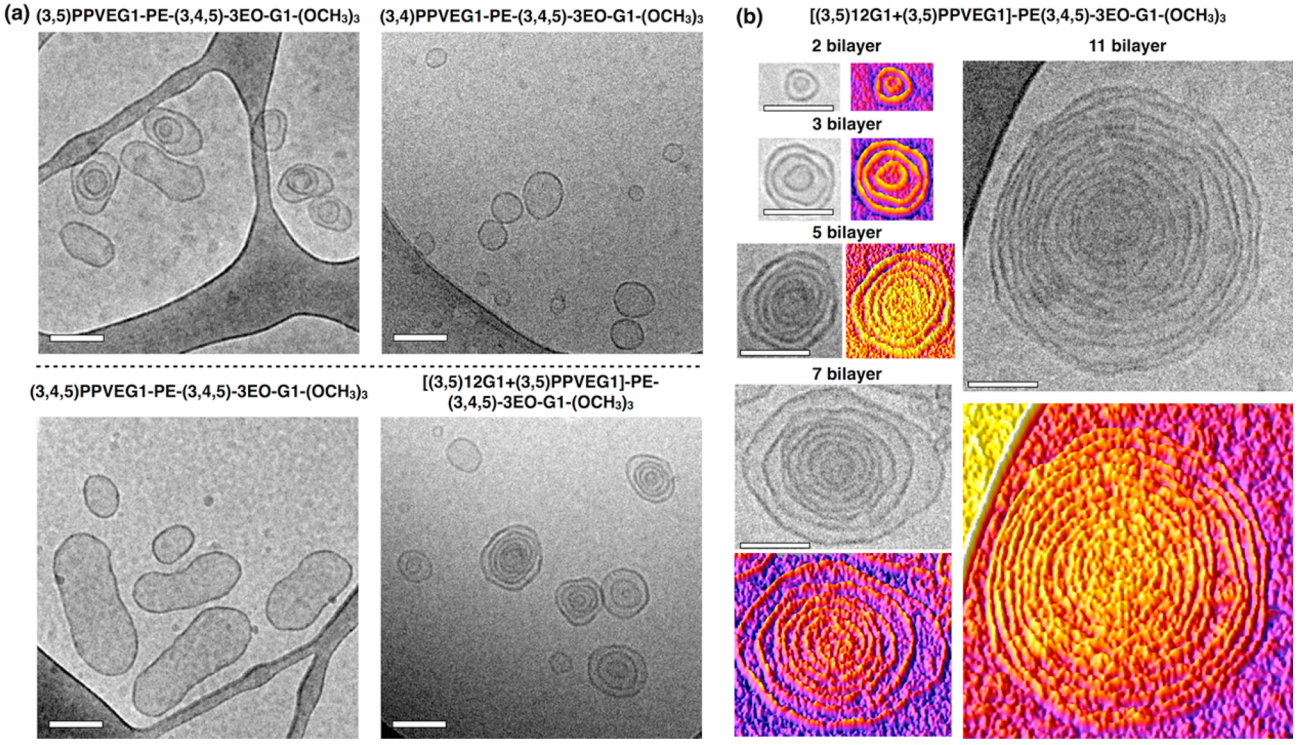

Figure 2. (a) Cryo-TEM images of DSs assembled by 0.5 mg·mL^{-1} (final concentration) of R_F and R_{HF} Janus dendrimers. (b) Selected cryo-TEM images of onion-like DSs self-assembled from 0.5 mg·mL^{-1} [**(3,5)12G1+(3,5)PPVEG1**]-**PE-(3,4,5)-3EO-G1-(OCH$_3$)$_3$** with hybrid R_{HF} chains and their 3D intensity-plotting images with different numbers of bilayers and diameters. Scale bar = 100 nm.

Agency.[9] The chiral-racemic fluorinated PPVE based dendrons employed in this study are prepared in one step, are soluble in ethanol rather than Freon-113 as the perfluorooctyl groups are, and degrade to environmentally friendly natural hydroxybenzoic acids and perfluoropropyl chains that are nontoxic and nonbiopersistent.[9]

Subsequent hydrogenation with palladium on carbon in a solvent mixture of dichloromethane and methanol gave the respective fluorinated (R_F)—COOH containing first generation dendrons (3,5)PPVE-G1 (**3**), (3,4)PPVE-G1 (**6**), and (3,4,5)PPVE-G1 (**9**) (Scheme S1 of the Supporting Information, SI). These R_F dendrons were conjugated to form half of a Janus dendrimer via esterification with half protected pentaerythritol (PE) as the core and 3,4,5-tris(methyl triethylene glycol)benzoic acid [(3,4,5)-3EO-G1-(OCH$_3$)$_3$] (**10**) as the hydrophilic fragments, which has been previously developed for the modular synthesis of amphiphilic Janus dendrimers that formed stable but soft DSs with narrow size

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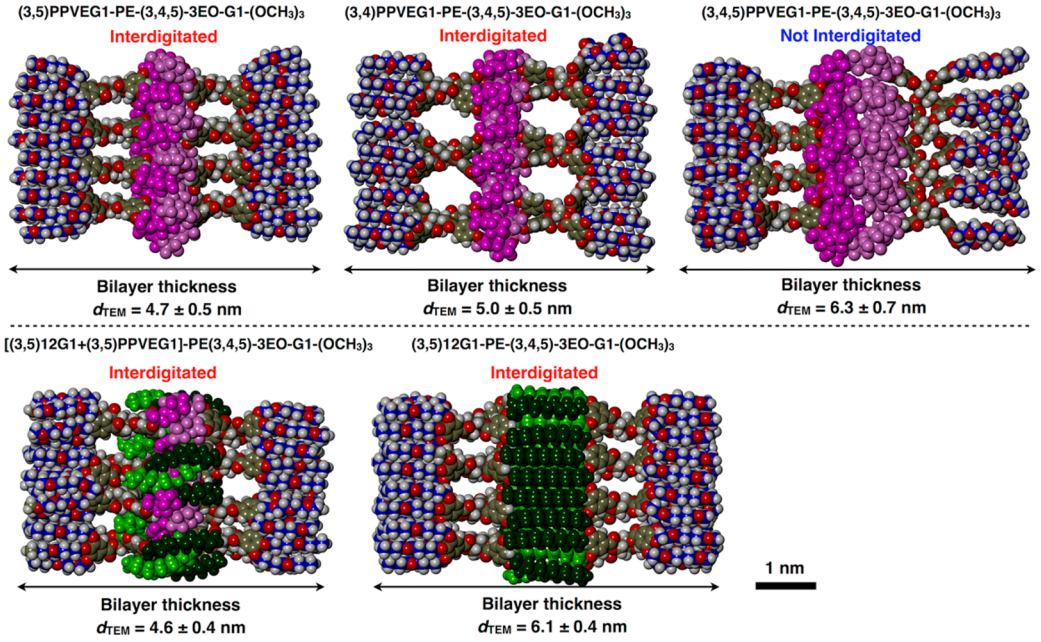

Figure 3. Molecular models of the bilayer thickness with and without interdigitation of fluorinated or hydrogenated chains calculated from cryo-TEM images.

distribution (Scheme 1).[5] A stepwise esterification strategy (Scheme 1) was employed for the synthesis of a hybrid Janus dendrimer containing both (3,5)PPVE-G1 (**3**) (R_F) and 3,5-bis(dodecyloxy)benzoic acid [(3,5)12G1] (**14**) (R_H) fragments. This hybrid compound was denoted as hybrid R_{HF} Janus dendrimer.

In order to elucidate the self-assembly and coassembly/self-sorting of R_F, R_H, and hybrid R_{HF} Janus dendrimers as individual and as a multicomponent system and to investigate their miscibility/immiscibility, two Janus dendrimers with R_F or R_H chains, and with different dyes attached to their hydrophilic parts, were designed and synthesized (Scheme 2, and Schemes S4–S5).

A Janus dendrimer with R_H chains was labeled with a rhodamine B (RhB) red fluorescent dye, and a Janus dendrimer with R_F chains was labeled with a 7-nitrobenzofurazan (NBD) green fluorescent dye. These azido triethylene glycol conjugated dyes were conjugated with the alkyne groups from the tris(hydroxymethyl)aminomethane (Tris) core via copper-catalyzed click chemistry[10] which was previously developed for the synthesis of carbohydrates (glycan) conjugated Janus glycodendrimers.[11]

The molecular structures of three R_F Janus dendrimers **(3,5)PPVEG1-PE-(3,4,5)-3EO-G1-$(OCH_3)_3$**, **(3,4)PPVEG1-PE-(3,4,5)-3EO-G1-$(OCH_3)_3$**, and **(3,4,5)PPVEG1-PE-(3,4,5)-3EO-G1-$(OCH_3)_3$**, one hybrid R_{HF} Janus dendrimer **[(3,5)12G1+(3,5)PPVEG1]-PE-(3,4,5)-3EO-G1-$(OCH_3)_3$** together with an R_H Janus dendrimer **(3,5)12G1-PE-(3,4,5)-3EO-G1-$(OCH_3)_3$**[5a−c] are presented in Scheme 3a, in which magenta denotes R_F chains, green denotes R_H chains, and blue denotes hydrophilic parts. The fluorescent R_F Janus dendrimer with NBD label **(3,5)PPVEG1-Tris(3,4,5)-3EO-G1-$(OCH_3)_3$-NBD** and R_H Janus dendrimer with RhB label **(3,5)12G1-Tris(3,4,5)-3EO-G1-$(OCH_3)_3$-RhB** were presented in Scheme 3b, with abbreviations as **R_F-NBD and R_H-RhB**.

The structures of the newly synthesized R_F and R_{HF} Janus dendrimers were confirmed by NMR, in particular a ^{1}H NMR chemical shift at 6.0–6.2 ppm, with a hydrogen–fluorine coupling $^2J_{(HF)}$ = 53 Hz (Figure 1c). The five different fluorine atoms in the PPVE chain were observed by ^{19}F NMR as five groups of signals, from *F*—CF_2 at the lowest field to *F*—C(O)H at the highest field (Figure 1a).

Self-Assembly of Amphiphilic Janus Dendrimers with Fluorinated, Hydrogenated, and Hybrid Chains into Nanoscale Unilamellar or Onion-Like DSs. Nanoscale DSs of R_F, hybrid R_{HF}, and of R_H Janus dendrimers were prepared by injection of their ethanol solution into water. Monodisperse peaks with narrow polydispersity (PDI) obtained from dynamic light scattering (DLS) analysis (Scheme 3a, Figure S1) indicated the formation of nanoscale assemblies. R_F, R_{HF}, and R_H Janus dendrimers shared a similar trend of increasing the size of their assemblies with increasing concentration of the Janus dendrimer (Figure S1).[5b] The vesicular morphologies of the assemblies were characterized by cryogenic-transmission electron microscopy (Cryo-TEM) (Figure 2a). **(3,4)PPVEG1-PE-(3,4,5)-3EO-G1-$(OCH_3)_3$** and **(3,4,5)PPVEG1-PE-(3,4,5)-3EO-G1-$(OCH_3)_3$** self-assembled into unilamellar DSs with single bilayers, while **(3,5)PPVEG1-PE-(3,4,5)-3EO-G1-$(OCH_3)_3$** and **[(3,5)12G1+(3,5)PPVEG1]-PE-(3,4,5)-3EO-G1-$(OCH_3)_3$** formed multilamellar DSs.[12] In samples of hybrid R_{HF} Janus dendrimer **[(3,5)12G1+(3,5)-PPVEG1]-PE-(3,4,5)-3EO-G1-$(OCH_3)_3$**, well-defined onion-like vesicles were observed, with numbers of bilayers from 2 to 11 (Figure 2b). The unilamellar vesicular assemblies from R_H Janus dendrimers were reported before.[7a] The thickness of the bilayers of DSs was calculated from cryo-TEM data (Figure 3).[8a,13] Cryo-TEM together with molecular modeling (Figure S2), demonstrated that R_F Janus dendrimers with (3,5)- and (3,4)-substituted PPVE chains and the hybrid R_{HF} Janus dendrimer showed interdigitation of their fluorinated fragments, with a membrane thickness in the range of 4.5 to 5 nm. These values are comparable to those those of the biological phospholipid-derived membranes (~4 nm) and thinner than that of the interdigitated R_H Janus dendrimer (6.1 nm).[5a] This is due to the shorter PPVE chain compared to the dodecyl alkyl

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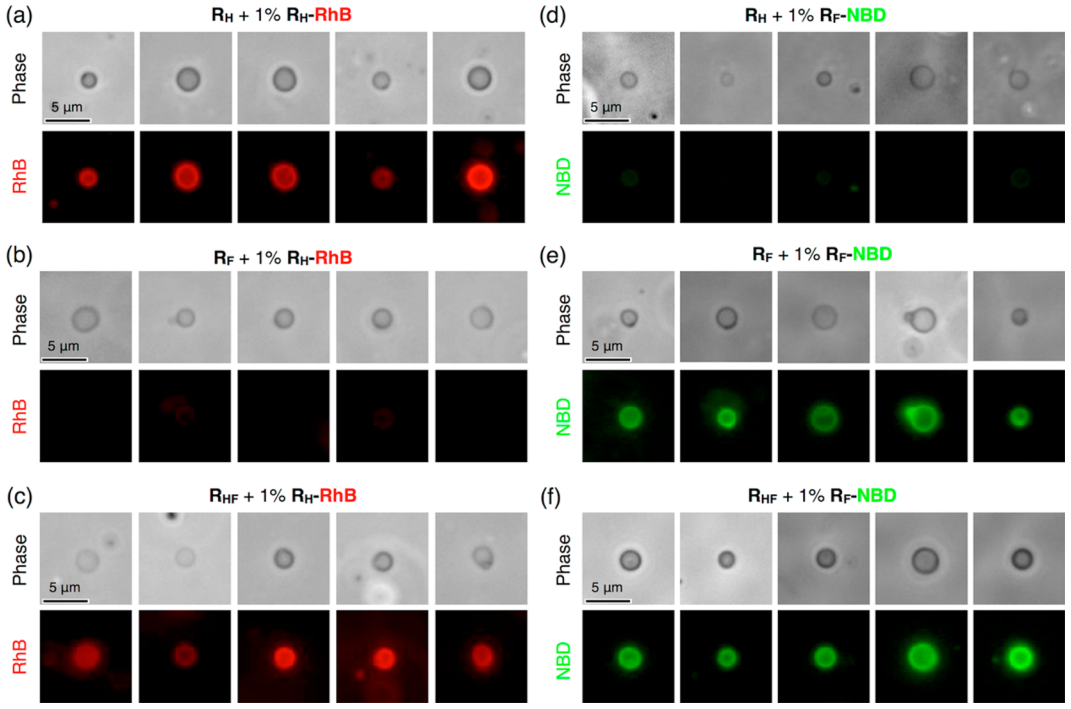

Figure 4. Representative microscopy images of giant unilamellar vesicles assembled from R_H, R_F, and hybrid R_{HF} Janus dendrimers with an additional 1% (w/w) **R_H-RhB** or **R_F-NBD**. Exposure time for fluorescence = 5 ms. Phase-contrast images and fluorescence images under red fluorescence channel or green fluorescence channel were taken by successive exposures on the same vesicle. Images in the same fluorescence channel were normalized to have the same values for the darkest and brightest pixels.

chain and/or the perfluorooctyl-based linear chains of the same length in the dendrons.[8a] In contrast, the (3,4,5)-substituted derivative showed a thicker bilayer (6.3 nm) indicative of no interdigitation. These results are consistent with the structures reported in a previous report on R_H based Janus dendrimers.[5b]

A representative ^{19}F NMR spectrum of the DSs self-assembled from hybrid R_{HF} Janus dendrimers exhibited a clear fluorine signal (Figure 1b) and suggests potential applications of these or related fluorinated DSs as ^{19}F MRI imaging agents capable of being loaded with drugs, proteins, nucleic acids, or even with other imaging agents to generate hybrid imaging tools.[5f]

The noncrystallization nature of R_F chains of these Janus dendrimers was also confirmed by a combination of differential scanning calorimetry (DSC) (Figure S3) and X-ray diffraction (XRD) (Figure S4) experiments. Below the phase transition temperature at −60 °C, all R_F and hybrid R_{HF} Janus dendrimers present a glassy phase. The R_H Janus dendrimer exhibited a 2D hexagonal columnar phase as previously reported.[5b] These noncrystallizable R_F chains help their self-assembly into soft vesicles.

Self-Sorting and Coassembly of Janus Dendrimers with R_F, R_H, and Hybrid R_{HF} Chains. In order to be analyzable by fluorescence microscopy, giant DSs (diameter >1 μm) in phosphate-buffered saline (PBS) from R_F, R_H, and hybrid R_{HF} Janus dendrimers were prepared by film hydration on a Teflon sheet (Figure 4),[5] via coassembly with 1% of the dye-labeled Janus dendrimers **R_H-RhB** with red fluorescence, and **R_F-NBD** with green fluorescence. **(3,5)PPVEG1-PE-(3,4,5)-3EO-G1-$(OCH_3)_3$** was selected as the fluorinated **R_F** molecule based on its structural similarity with the **R_H** and hybrid **R_{HF}** Janus dendrimers (Scheme 3). Due to the expected immiscibility between **R_H** and **R_F**, the **R_H** Janus dendrimer preferentially coassembles with **R_H-RhB** (Figure 4a), while the **R_F** Janus dendrimer coassembles with **R_F-NBD** (Figure 4e), leading to bright red or green fluorescence along the boundary of the vesicles. By contrast, since **R_F** and **R_H** are not miscible and therefore attempts to coassemble **R_F** with **R_H-RhB** (Figure 4b) and **R_H** with **R_F-NBD** (Figure 4d) produced vesicles that exhibit much weaker fluorescence. This miscibility problem was alleviated with the help of **R_{HF}** which enabled both dye-labeled Janus dendrimers **R_H-RhB** (Figure 4c) and **R_F-NBD** (Figure 4f) to be incorporated into the giant DSs formed from **R_{HF}** Janus dendrimers, resulting in intense red or green fluorescence (Figure 4c and 4f). A representative confocal scanning laser tomograph (Movie S1) of a giant DS formed from **R_F** Janus dendrimer and a reconstructed 3D projection (Movie S2) demonstrate their self-assembly into vesicular structures.

When both **R_H-RhB** and **R_F-NBD** were coassembled with R_{HF} Janus dendrimers, their giant DSs showed an expected decrease in green emission (Figure 5) (significant p-value <0.001) due to intermolecular fluorescent resonance energy transfer (FRET)[14] from the green dye to the red dye over a short distance (1−10 nm). This FRET experiment indicates that immiscible **R_H-RhB** and **R_F-NBD** Janus dendrimers mix well in giant DSs with the help of **R_{HF}** Janus dendrimers without phase separation.

The phase diagram of the three-component assemblies generated from **R_H**, **R_F**, and hybrid **R_{HF}** Janus dendrimers was investigated by confocal microscopy performed on giant DSs (Figure 6). The ratio of **R_H** and **R_F** was maintained at 1:1. The proportion of **R_{HF}** could be decreased to 50% and subsequently to 40% without phase isolation between the **R_H** and **R_F** components (Figure 6a−c).

At 34% of the **R_{HF}** component, the red and green colors from the dyes started to separate into a core−shell structure (Figure

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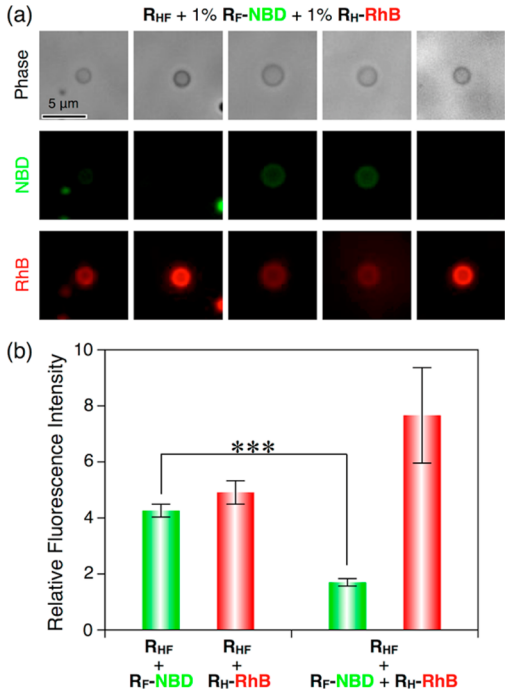

Figure 5. (a) Representative microscopy images of giant unilamellar vesicles assembled from hybrid $\mathbf{R_{HF}}$ Janus dendrimer with an additional 1% (w/w) $\mathbf{R_H}$-**RhB**, and 1% (w/w) $\mathbf{R_F}$-**NBD**. Exposure time for fluorescence = 5 ms. Phase-contrast images and fluorescence images under red fluorescence channel and green fluorescence channel were taken by successive exposures on the same vesicle. Images in the same fluorescence channel were normalized to have the same values for the darkest and brightest pixels. (b) Relative fluorescence intensity of giant vesicles in Figure 4c,f and part a, respectively. Green and red columns represent green and red fluorescence intensities of vesicles generated from hybrid $\mathbf{R_{HF}}$ Janus dendrimer with additional $\mathbf{R_H}$-**RhB** or $\mathbf{R_F}$-**NBD** (left two columns), and of vesicles obtained from hybrid $\mathbf{R_{HF}}$ Janus dendrimer with both $\mathbf{R_H}$-**RhB** and $\mathbf{R_F}$-**NBD** (right two columns). Relative intensity was calculated from the brightness histogram analysis by dividing the maximum value of each vesicle by the background. Error bars indicate standard error (SEM) of the mean based on data derived from five vesicles. ***denotes *p*-value <0.001.

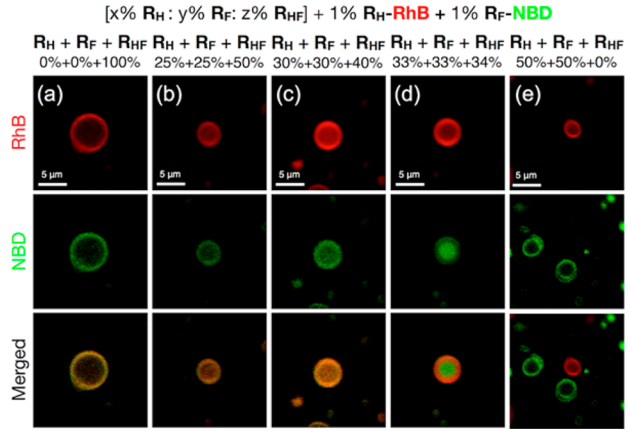

Figure 6. Representative confocal fluorescent microscopy images of giant vesicles assembled from mixtures of R_H, R_F, and hybrid R_{HF} Janus dendrimers with proportions (in wt %) of (a) $\mathbf{R_H}$ = 0%, $\mathbf{R_F}$ = 0%, $\mathbf{R_{HF}}$ = 100%; (b) $\mathbf{R_H}$ = 25%, $\mathbf{R_F}$ = 25%, $\mathbf{R_{HF}}$ = 50%; (c) $\mathbf{R_H}$ = 30%, $\mathbf{R_F}$ = 30%, $\mathbf{R_{HF}}$ = 40%; (d) $\mathbf{R_H}$ = 33%, $\mathbf{R_F}$ = 33%, $\mathbf{R_{HF}}$ = 34%; and (e) $\mathbf{R_H}$ = 50%, $\mathbf{R_F}$ = 50%, $\mathbf{R_{HF}}$ = 0%. All mixtures contain an additional 1% (w/w) of $\mathbf{R_H}$-**RhB** and 1% (w/w) $\mathbf{R_F}$-**NBD**. Red and green fluorescent images were merged into one to demonstrate their coassembly or self-sorting.

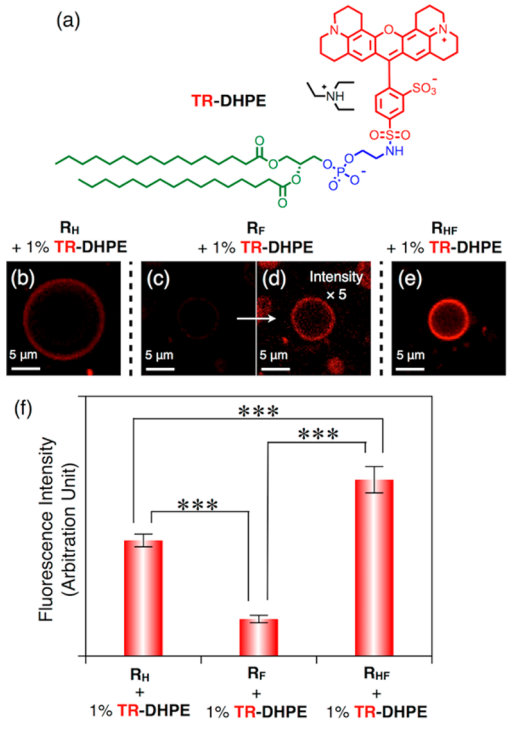

Figure 7. (a) Chemical structure of Texas Red (TR)-labeled phospholipid TR-DHPE, DHPE = 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine. (b–e) Representative confocal fluorescent microscopy images of giant unilamellar vesicles assembled from (b) $\mathbf{R_H}$, (c,d) $\mathbf{R_F}$, and (e) hybrid $\mathbf{R_{HF}}$ Janus dendrimers with 1% (w/w) of Texas Red (TR)-labeled phospholipid TR-DHPE, DHPE = 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine. Images (c) and (d) show the same area, but the intensity has been multiplied by 5-fold in (d) to better display vesicle formation. (f) Fluorescence intensity of giant vesicles. Error bars indicate standard error (SEM) of the mean based on data derived from 25 vesicles of each sample. ***denotes *p*-value <0.001.

6d). Without $\mathbf{R_{HF}}$ in the mixture (0% of $\mathbf{R_{HF}}$), $\mathbf{R_F}$ and $\mathbf{R_H}$ self-assembled into individual green and red giant vesicles, indicating the self-sorting of $\mathbf{R_F}$ and $\mathbf{R_H}$ into incompatible perfluorinated and perhydrogenated incompatible DSs (Figure 6e). Self-sorting[15] of immiscible fluorinated and hydrogenated components during their attempts to coassemble was previously demonstrated with a hydrogenated–fluorinated peptide system.[15a] Here we demonstrate and visualize self-sorting of vesicular DSs self-assembled from individual $\mathbf{R_F}$ and $\mathbf{R_H}$ Janus dendrimers.

Coassembly of Janus Dendrimers with Phospholipid. Finally, for potential biomedical applications of these $\mathbf{R_F}$ and $\mathbf{R_{HF}}$ DSs, the coassembly of $\mathbf{R_H}$, $\mathbf{R_F}$, and $\mathbf{R_{HF}}$ Janus dendrimers with phospholipids was conducted by using a phospholipid labeled with the red dye, Texas Red (TR), denoted phospholipid **TR-DHPE**, where DHPE = 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (Figure 7a).

Confocal images demonstrated that **TR-DHPE** could be incorporated into vesicles generated from $\mathbf{R_{HF}}$ derived Janus dendrimers (Figure 7e, Movie S3) even more efficiently than in vesicles assembled from $\mathbf{R_H}$ Janus dendrimers (Figure 7b,f) with higher intensity of fluorescence emission. As expected,

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giant DSs from $\mathbf{R_F}$ Janus dendrimer showed quite weak fluorescence (Figure 7c,d,f). These coassembly experiments are consistent with the previous coassembly with dye-labeled Janus dendrimers and reveal that different fluorinated vesicles, miscible or immiscible with the biological membrane, can be used for different purposes. For example, DSs from $\mathbf{R_F}$ Janus dendrimers can be used for remote lipophilic and hydrophilic drug loading due to low diffusion within biological membranes. In contrast, DSs from $\mathbf{R_{HF}}$ Janus dendrimers may coassemble with multiple components from biological membranes, including glycolipids, glycoproteins, and transmembrane proteins, to build biocompatible hybrids for immunology and targeted delivery. It is expected that these fluorinated components may coassemble with bacterial and human cell membranes as demonstrated recently with $\mathbf{R_H}$ Janus dendrimers.[5c] Therefore, the building block reported here provides an important new toolbox for the area of synthetic biology.

CONCLUSIONS

A library containing achiral hydrogenated ($\mathbf{R_H}$), chiral-racemic fluorinated ($\mathbf{R_F}$), hybrid hydrogenated-fluorinated ($\mathbf{R_{HF}}$), hydrogenated conjugated in the hydrophilic part with a red fluorescent dye ($\mathbf{R_H}$-**RhB**), and a fluorinated conjugated in the hydrophilic part with a green fluorescent dye ($\mathbf{R_F}$-**NBD**) is reported. These $\mathbf{R_H}$, $\mathbf{R_F}$, and $\mathbf{R_{HF}}$ Janus dendrimers are soluble in ethanol and self-assemble into nanometer-sized unilamellar and multilamellar onion-like DSs by injection of their ethanol solution into water or buffer and by hydration into micrometer size DSs. The thickness of these narrow size distribution DSs is similar to that of biological membranes. Self-assembly and coassembly of these Janus dendrimers were monitored by fluorescence microscopy to reveal self-sorting and self-assembly of the immiscible $\mathbf{R_H}$ and $\mathbf{R_F}$ Janus dendrimers into hydrogenated and fluorinated DSs. Coassembly of $\mathbf{R_H}$ with $\mathbf{R_F}$ and $\mathbf{R_{HF}}$ as a function of composition provided hybrid hydrogenated-fluorinated DSs when a high concentration (>40%) of $\mathbf{R_{HF}}$ was employed, and self-sorting into individual $\mathbf{R_H}$ or $\mathbf{R_F}$ DSs when low proportions of the $\mathbf{R_{HF}}$ component were used. The simple synthesis and self-assembly, the similar thickness to biological membranes and the imaging capabilities via fluorescence and ^{19}F MRI of these DSs make these building blocks and assemblies interesting tools for supramolecular science, medicine and synthetic biology,[16] including hybrid hydrogenated-fluorinated cell-like assemblies.[5c]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b08069.

Synthetic procedures with complete structural and self-assembly analysis, sample preparation, and experimental protocol (PDF)
Movie (AVI)
Movie (AVI)
Movie (AVI)

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Notes

The authors declare no competing financial interest.

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