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Co-assembly of liposomes, Dendrimersomes, and Polymersomes with amphiphilic Janus dendrimers conjugated to Mono- and Tris-Nitrilotriacetic Acid (NTA, TrisNTA) enhances protein recruitment

Full-length article

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Metal-chelating ligands such as nitrilotriacetic acid (NTA) bind to polyhistidine-tagged (His-tagged) proteins. Lipids conjugated to NTA are widely used to decorate the surface of liposomes with proteins in cell biology applications. Multivalent NTA ligands such as tris-nitrilotriacetic acid (TrisNTA) display higher affinities than the monovalent NTA when co-assembled with phospholipids and cholesterol in liposomes. However, there is a limited number of available lipids conjugated to NTA and only few are commercially available. Additionally, their activity diminishes during storage or upon exposure to air. Here we report a library of five amphiphilic Janus dendrimers conjugated to NTA (JD-NTA) and three to TrisNTA (JD-TrisNTA). Both JD-NTA and JD-TrisNTA are indefinitely stable at room temperature in air and preliminary results demonstrate that they co-assemble with phospholipids and cholesterol into liposomes, with Janus dendrimers into dendrimersomes, and with block copolymers into polymersomes. The resulting hybrid liposomes co-assembled with JD-NTA display up to thirty-fold higher activity towards His-tagged fluorescent proteins when compared to lipid-NTAs. Hybrid liposomes co-assembled with JD-TrisNTA exhibit even higher binding affinity to His-tagged proteins and can function at much lower ligand concentration in hybrid liposomes than those containing JD-NTA. These preliminary results demonstrate the power of modular synthesis of JD-NTA or JD-TrisNTA to provide highly efficient new tools for biological reconstitution and synthetic cell biology as well as for nanomedicine.

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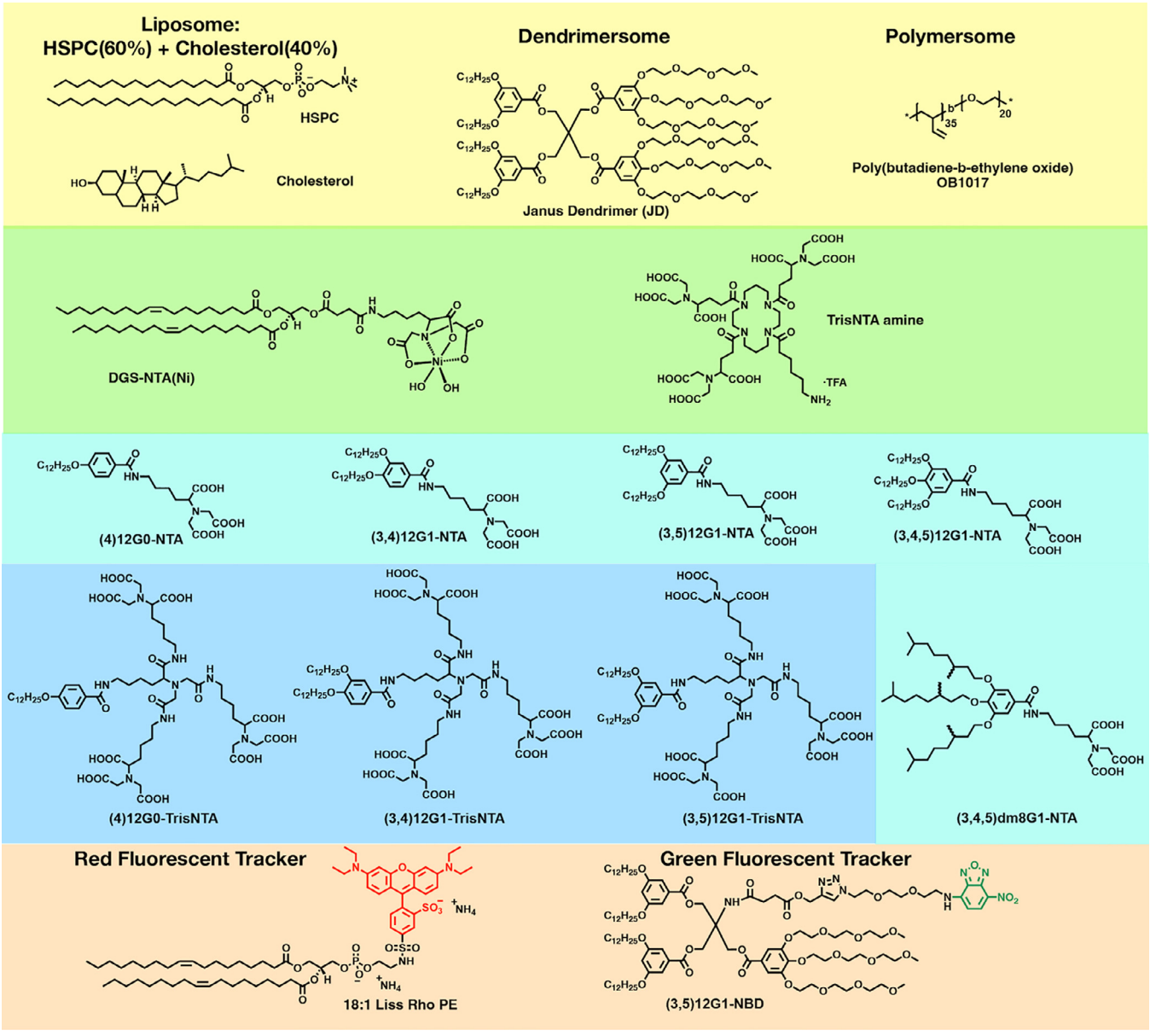

Fig. 1

The structures of components required to assemble liposome by co-assembly of HSPC (60%, w/w) with cholesterol (40%, w/w), dendrimersomes by self-assembly of Janus dendrimer (JD), and polymersomes by self-assembly of block copolymer poly(butadiene-b-ethylene oxide) known commercially as OB1017, are shown in the yellow marked box. The structure of the commercial NTA lipid Ni complex DGS-NTA(Ni) is shown in the green box. The new library of Janus dendrimers containing the NTA ligand is shown in the cyan box while the new library of the Janus dendrimers containing the TrisNTA ligand is shown in the blue box. The structures of red and green fluorescent trackers are shown in the orange box. Liposomes, dendrimersomes, and polymersomes were prepared by hydration of a film containing the proper components on a Telfon sheet in PBS (pH 7.4, 1X) with $NiSO_4$ (200 μM).

Introduction

The polyhistidine-tag (His-tag) is a sequence of at least six histidine (His) residues that is used to decorate the terminals of proteins. His-tag decorated proteins bind to specific metal chelating groups. For example, nitrilotriacetic acid (NTA), a tetradentate ligand chelating with various metal ions such as Ni(II), Co(II), Co(III), Cu(II), Zn(II) [1–13] were widely used in affinity chromatography for protein purification [6],biosensor detection [3,5], fluorescence labeling [7], as well as in molecular and cell biology to bind protein [2,10-13].

The NTA conjugated lipid Ni-precomplex, 1,2-dioleoyl-sn–glycero-3-[(*N*-(5-amino-1-carboxypentyl)iminodiacetic acid)succinyl] (nickel salt) / 18:1 DGS-NTA(Ni) (Fig. 1, green box), is commercially available (Avanti Polar Lipids, Inc. $261/25 mg, accessed on Nov. 29, 2021) and is widely used in cell biology and synthetic cell biology to co-assemble with

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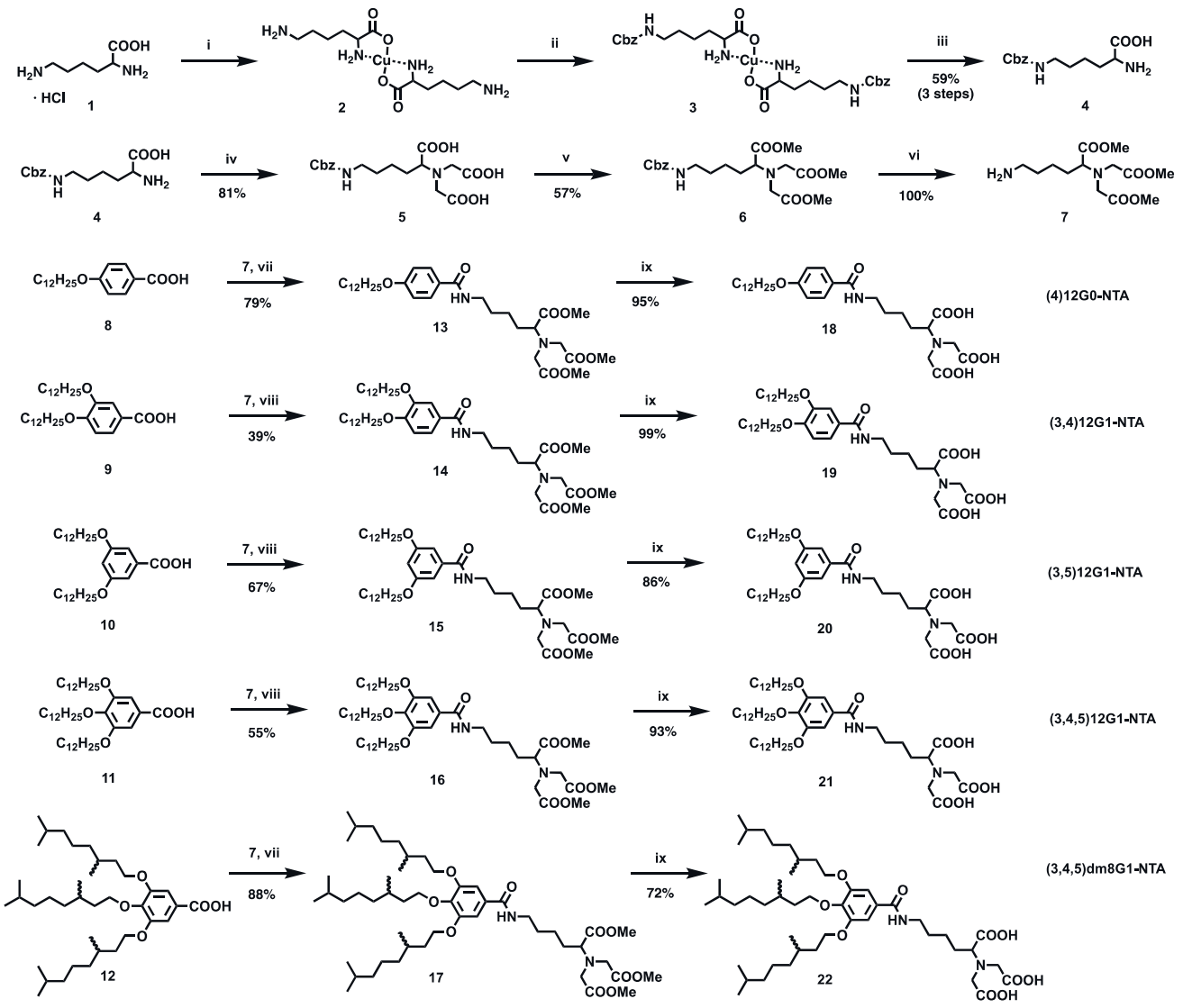

Fig. 2

Synthesis of the Library of Amphiphilic Janus Dendrimers Conjugated to NTA Ligand. *Reagents and Conditions*: (i) $CuSO_4 \bullet 5H_2O$, NaOH, water, 23 °C; (ii) CbzCl, $NaHCO_3$, 0–23 °C; (iii) EDTA, water, 100 °C, then 2 M NaOH; (iv) $BrCH_2COOH$, 2 M NaOH, 60 °C, then 2 M HCl; (v) MeOH, $TsOH \bullet H_2O$, 70 °C; (vi) H_2, Pd/C, DCM, MeOH; (vii) $SOCl_2$, then DCM, NEt_3; (viii) CDMT, NMM, THF; (ix) KOH, EtOH, THF, 90 °C, then 2 M HCl.

phospholipids and cholesterol and decorate their surface with binding sites for His-tagged proteins. This methodology allows to decorate the periphery of vesicles with proteins and other components. This immobilization method is "oriented" and very mild thus preserving the activity of the protein. However, in our experience a solution of commercial DGS-NTA(Ni) has limited stability over time, showing sensitivity to air and moderate-term cold storage. This is not unexpected since the double bonds of its alkyl tails will most probably oxidize in air. When monovalent NTA is transformed into TrisNTA [14–19], the affinity to His-tagged protein increases 1000-fold [17]. However, the synthesis of lipids conjugated to TrisNTA and of free TrisNTA is complex and the commercially available free TrisNTA containing a primary amine group is very expensive (Fig. 1, green box) ($361/100 μg, Sigma-Aldrich, accessed on Nov. 29, 2021) [14].

Amphiphilic Janus dendrimers (JDs) [20–33], their analogues conjugated with sugars, Janus glycodendrimers (JGDs) [22,34-42], and related structures with ionizable amines known as ionizable amphiphilic Janus dendrimers (IAJDs) [43,44] have been developed as biological membrane mimics [22–44] and as a new class of delivery systems for mRNA. JDs are constructed from hydrophobic and hydrophilic dendrons [20,22]. JDs and JGDs provide synthetic alternatives to natural lipids, glycolipids and amphiphilic block copolymers [20,22]. JDs and JGDs self-assemble into monodisperse and stable dendrimersomes and glycodendrimersomes by simple injection from ethanol or other water miscible solvents into water, buffer, or serum [20,22,28]. The resulting assemblies generate synthetic cell membranes with the similar thickness, membrane flexibility, and mechanical resistance as natural phospholipid cells [20,22,27,31-33]. Dendrimersomes and glycodendrimersomes encapsulate high concentrations of

hydrophobic organic compounds and their surface exhibit rafts-like domains with high activity to lectins [39,40,42]. Due to their high stability and similar thickness as that of liposomes assembled from natural phospholipids, cell-like hybrids with either bacteria or human cells membrane components and JDs and JGDs can be constructed [26,29]. An NTA conjugated JD was previously synthesized to functionalize the surface of dendrimersomes *via* bioaffinity interactions [28]. The NTA coated dendrimersomes showed excellent ability to bind His-tagged protein cargo [28]. Furthermore, a DNA aptamer was noncovalently attached to His-tagged SNAP proteins on the surface of dendrimersomes via NTA conjugated JD [28]. These positive preliminary results prompted the synthesis of a broader library of JD containing NTA, JD-NTA, and JD containing Tris-NTA, JD-TrisNTA reported here, to address the scope and limitations of JD-NTA and JD-TrisNTA for applications in cell biology and nanomedicine.

Methods

Materials

Hydrogenated soybean phosphatidylcholine (HSPC), 18:1 DGS-NTA(Ni), and 18:1 Liss Rhod PE were purchased from Avanti and stored at –20 °C. Block copolymer poly(butadiene-b-ethylene oxide) OB1017 was purchased from Polymer Source, Inc. (Dorval, Quebec, Canada, sample# P40463A-BdEO, PBd(1700)-PEO(1000), Mw/Mn = 1.04). Janus dendrimer (3,5)12G1-PE-(3,4,5)3EOG1-$(OCH_3)_6$ [20] and green tracker (3,5)12G1-NBD [45] were reported and synthesized by Percec Laboratory previously. The detailed synthesis of JD-NTA and JD-trisNTA will be discussed later.

Protein expression and purification

pET plasmids containing His_6-GFP or His_6-RFP were transformed into *E. coli* Rosetta 2 cells (Novagen) for protein expression. Cultures were grown in LB at 37 °C to an OD_{600} of 0.4, then temperature shifted to 16 °C for 20 min and induced using 0.5 mM IPTG at an OD_{600} between 0.5–0.7. Cultures were grown overnight at 16 °C. Cells were harvested by centrifugation and resuspended in PBS. Cells were lysed with three cycles of sonication and freeze-thaw and clarified by centrifugation. The supernatants were incubated with Ni-NTA agarose superflow resin (Qiagen) for 1 hr at 4 °C. After extensive washing, proteins were eluted in a buffer containing 150 mM NaCl, 25 mM Tris, 250 mM imidazole pH 8, 10% glycerol, 1 mM DTT), and then dialyzed overnight to remove imidazole. Stocks were aliquoted and snap frozen; stored at –80 °C.

Preparation of hybrid liposomes by film hydration

Stock solutions of HSPC and cholesterol were prepared in chloroform with 20 mg/mL concentration. Stock solutions of DGS-NTA(Ni) (2.5 mg/mL) and 18:1 Liss Rhod PE (1.0 mg/mL) were received in chloroform solution from Avanti. Stock solutions of JD-NTA and green tracker (3,5)12G1-NBD [45] were prepared in chloroform with 1 mg/mL concentration. Stock solutions of JD-TrisNTA were prepared in methanol with 1 mg/mL concentration. HSPC (0.567 mg, 28.3 μL), cholesterol (0.388 mg, 19.4 μL) with red (Rhod) or green (NBD) tracker (0.005 mg, 5 μL) and corresponding DGS-NTA(Ni), JD-NTA or JD-TrisNTA solution with required mol% were mixed together and placed on a roughened Teflon sheet (0.5 × 0.5 cm^2) in a 4 mL flat-bottomed vial. After all the solvent was evaporated and a film with HSPC, cholesterol, fluorescent tracker, and NTA molecules was dry, the vial was placed into a vacuum chamber for 12 h. The films were rehydrated in 250 μL of phosphate buffered saline (PBS, pH 7.4, 1X) with 200 μM of $NiSO_4$ at 50 °C for 24 h.

Preparation of hybrid dendrimersomes and hybrid polymersomes by film hydration

Stock solutions of Janus dendrimers or polymers were prepared in chloroform with 20 mg/mL concentration. Janus dendrimers (1.0 mg, 50 μL) with red tracker (Rhod) (0.005 mg, 5 μL) and corresponding JD-NTA solution with required weight% were mixed together and placed on a roughened Teflon sheet (0.5 × 0.5 cm^2) in a 4 mL flat-bottomed vial. Polymer (1.0 mg, 50 μL) and corresponding JD-NTA solution with required weight% were mixed together and placed on a roughened Teflon sheet (0.5 × 0.5 cm^2) in a 4 mL flat-bottomed vial. After all the solvent was evaporated and a film with Janus dendrimer or polymer and JD-NTA molecules was dry, the vials were placed into a vacuum chamber for 12 h. The films were rehydrated in 250 μL of phosphate buffered saline (PBS, pH 7.4, 1X) with 200 μM of $NiSO_4$ at 50 °C for 24 h.

Confocal fluorescence microscopy

Pre-assembled NTA-coated liposomes, dendrimersomes or polymersomes (10 μL) were mixed with His-GFP (1 μL, 53 μM) or His-RFP (1 μL, 76 μM) and incubated at 23 °C for 30 min. The incubated samples (3 μL) were diluted with 1:30 with PBS (87 μL), and a diluted liposome solution (15 μL) was pipetted into the custom gasket imaging chambers for microscopy.

Images were acquired using 488 nm (for GFP or NBD) and 561 nm (for Rhod or RFP) laser illumination on an Olympus IX81 inverted confocal microscope containing a Yokogawa X1 spinning disk head. Images were acquired using a 100 × 1.4 NA oil objective, an Andor iXon3 EM-CCD camera and MetaMorph acquisition software. Images containing a specific dye or tracer were collected at identical laser intensities and camera gain and exposed for the same period.

Image analysis

To quantify protein recruitment, confocal image stacks were analyzed using ImageJ. Midplane of vesicle were identified in Z- and objects were masked, and background subtracted to calculate integrated pixel intensities of fluorescent proteins. Measurements of recruitment were performed on a minimum of 20 vesicles. Error bars were calculated by standard error of the mean (SEM).

Results and discussion

Design and modular synthesis of JD-NTA

In order to expand and estimate the efficiency and the potential utility of different structures of JD conjugated to NTA, a library of JD-NTAs was designed and synthesized (Fig. 1). Their binding activities to His-tagged proteins after JD-NTA or DGS-NTA(Ni**)** were co-assembled into non-stealth liposomes [46–49] containing 60% (w/w) hydrogenated soybean phosphatidylcholine (HSPC) and 40% (w/w) cholesterol (Fig. 1, yellow box) was compared. The previously reported JD-NTA has a

hydrophobic 3,5-substitued dodecyl benzoic acid first generation AB_2 dendron (3,5)12G1 (Fig. 1, cyan box) [28]. Three additional hydrophobic minidendrons with 4-, 3,4- and 3,4,5- substituted dodecyl benzoic acid, and one hydrophobic minidendron 3,4,5-substitued 3,7-dimethyloctyl benzoic acid were conjugated to the NTA ligand (Fig. 1, cyan box).

To specify the nomenclature for molecular structures of JD-NTA, we employed the general classification developed by our laboratory for Janus dendrimers [22,50]. For example, (3,4)12G1-NTA specifies a 3,4-didodecyloxy benzoic minidendron, and in the (3,4)12G1, G1 denoted first generation dendron in the hydrophobic portion. (3,4,5)dmG1-NTA denotes a racemic 3,4,5-tri(3,7-dimethyloctyl) benzoic minidendron (3,4,5)dm8G1, while (4)12G0-NTA refers to the molecule that has 4-dodecyloxy benzoic minidendron (4)12G0; G0 denoted zero generation dendron because there is no branching point in its structure.

The modular synthesis of five JD-NTA molecules is shown in Fig. 2. This synthesis follows similar principles as the synthesis of (3,5)12G1-NTA [28] l-Lysine hydrochloride (1) was complexed with $CuSO_4$, followed by the protection of the N_6 terminal amino group with benzyl chloroformate (CbzCl) in 59% yield for three steps [51]. After an S_N2 reaction between N_6–Cbz-lysine (4) and 2–bromo acetic acid with NaOH as base in water, the tribasic acid (5) was obtained in 81% yield. A Fischer esterification was used to protect the three acid groups with methanol in the presence of *p*-toluenesulfonic acid (TsOH) with 57% yield. After Cbz deprotection *via* hydrogenolysis with palladium on carbon, the triester (7) with a free amino group was obtained [52]. The intermediate triester with a free amino group (7) was conjugated to the corresponding benzoic acids (8–12) to obtain the dendron-conjugated triesters (13–17) (39–88% yield) *via* either thionyl chloride, followed by amidation catalyzed by triethylamine, or by a direct amidation with 2–chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) and *N*-methylmorpholine (NMM). Finally, hydrolysis of dendron-conjugated triesters (13–17) with KOH in a mixture of tetrahydrofuran and ethanol followed by acidification with 2 M HCl aqueous solution, led to the target five JD-NTA molecules in 72–95% yield as white or light-yellow solids. Unlike the commercially available but unstable DGS-NTA(Ni), these five JD-NTA molecules are stable at room temperature in air for at least three years.

His-Tagged protein affinity of nta containing liposomes and hybrid liposomes

Non-stealth liposomes containing NTA were prepared by film hydration. HSPC (60%, w/w) and cholesterol (40%, w/w) (Fig. 1, yellow box) with additional 2% mol of DGS-NTA(Ni) or JD-NTA was deposited on a Teflon sheet. A rhodamine red-fluorescent labeled lipid, 1,2-dioleoyl-sn–glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) 18:1 Liss Rhod PE (Fig. 1, orange box) (0.5%, w/w) was added as a red fluorescent tracker to visualize the liposomes by confocal microscopy. Hydration of the dried film on Teflon was performed in a phosphate-buffered saline at 50 °C for 24 h (PBS, pH 7.4, 1X) with additional $NiSO_4$ (200 μm), required for high affinity NTA-His-interactions. These Ni-NTA-containing hybrid liposomes were incubated with recombinant His-tagged green fluorescent protein

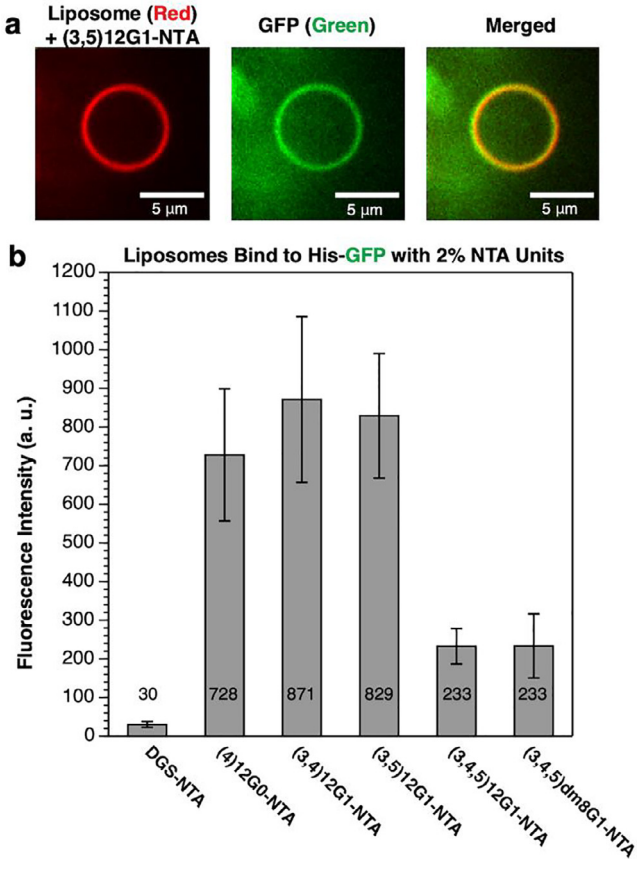

Fig. 3

(a) Recruitment of His-GFP on the membrane surface of the hybrid liposomes. Liposome (red color) was indicated by 0.5% (w/w) of 18:1 Liss Rhod PE as a red fluorescent tracker. GFP (green color) was located in the same position, confirmed by their merged images. (b) Comparisons of the recruitment of GFP on the surface of liposomes by 2% mol of DGS-NTA or JD-NTA. Error bars: ± standard error of the mean (SEM).

(His-GFP) [28] for 30 min before being diluted to remove unbound protein for imaging with confocal microscopy.

Typical confocal fluorescent images (Fig. 3a) show giant unilamellar hybrid liposomes in red channel containing 18:1 Liss Rhod PE (0.5%, w/w), a red fluorescent tracker. The green fluorescence of His-GFP overlaps spatially with the red fluorescence, indicating that the His-GFP binds on the liposome membrane *via* interaction with the NTA-Ni complex from DGS-NTA or JD-NTA.

Quantitative analysis of the green fluorescent intensity from liposomes or the hybrid liposome membranes was carried out to compare the recruitment of His-GFP (Fig. 3b). Liposomes co-assembled with 2% mol of DGS-NTA to His-GFP weakly. In contrast, liposomes co-assembled with JD-NTA molecules (3,4,5)12G1-NTA or (3,4,5)dm8G1-NTA showed approximately 6-fold higher recruitment of His-GFP cargo, and molecules (4)12G0-NTA, (3,4)12G1-NTA and (3,5)12G1-NTA displayed 27 to 30-fold higher recruitment of His-GFP cargo compared to DGS-NTA. The more modest activities of the two molecules with AB_3 3,4,5-substitied dendrons, (3,4,5)12G1-NTA or (3,4,5)dm8G1-NTA, can potentially be explained by the unfavorable conical shape of

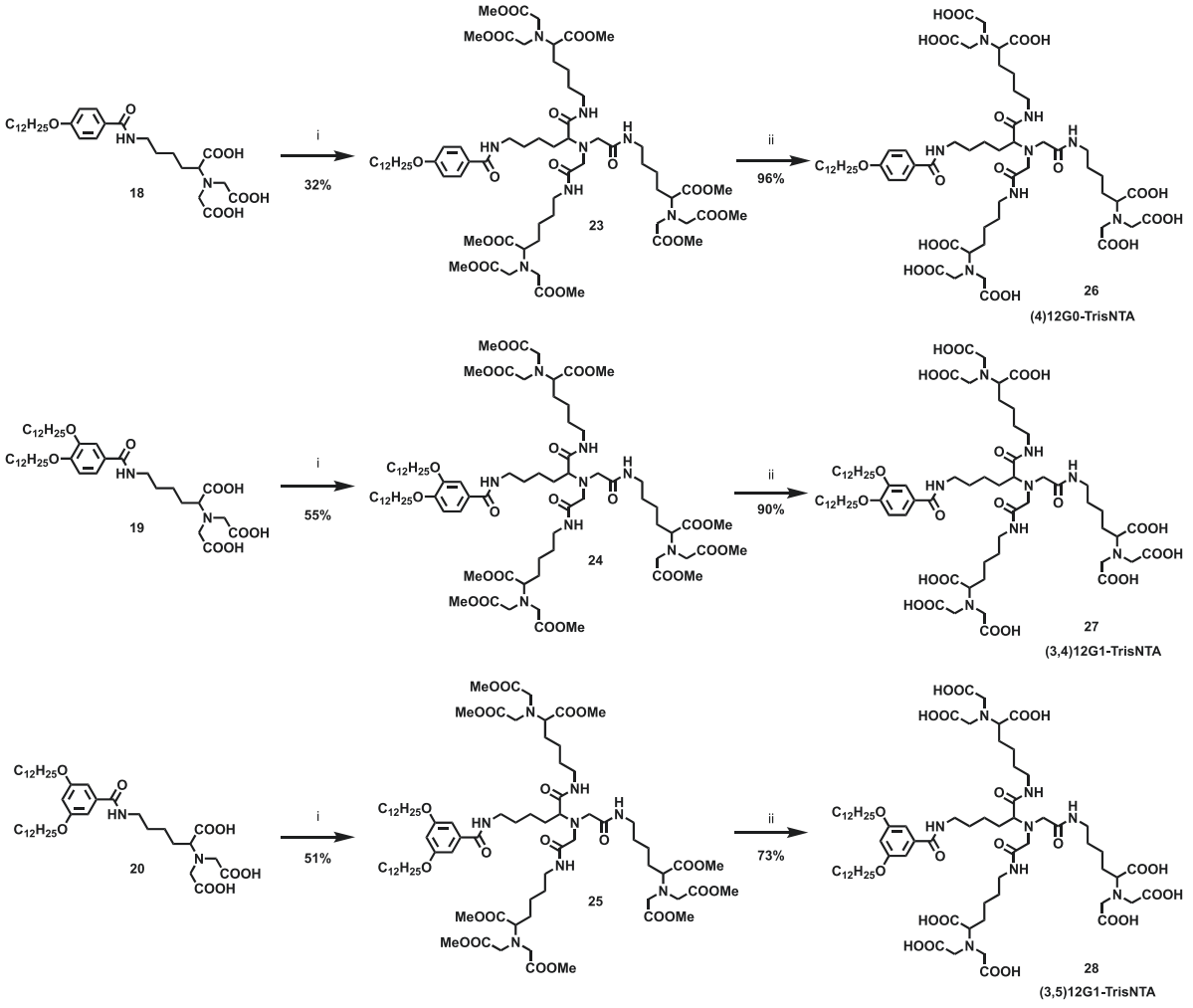

Fig. 4

Synthesis of the Library of Amphiphilic Janus Dendrimers Conjugated to TrisNTA Ligand. *Reagents and Conditions*: (i) 7, CDMT, NMM, THF, 48 h; (ix) KOH, EtOH, THF, 90 °C, 4 h, then 2 M HCl.

their minidendrons for incorporation into liposome bilayers. This finding suggested the need to synthesize TrisNTA containing JDs with the three hydrophobic minidendrons (4)12G0, (3,4)12G1, and (3,5)12G1.

Design and modular synthesis of JD-TrisNTA

(4)12G0-NTA, (3,4)12G1-NTA and (3,5)12G1-NTA were reacted with intermediate (7) *via* CDMT-NMM amidation in THF for 48 h to obtain the corresponding methyl nonaesters (23–25, 32–55% yield) (Fig. 4). Three target JD-TrisNTA molecules, (4)12G0-TrisNTA, (3,4)12G1-TrisNTA and (3,5)12G1-TrisNTA were obtained by hydrolysis of methyl nonaesters (23–25) and acidification in 90–96% yield. The synthetic route is simpler and more efficient when compared to the previous route for the synthesis of TrisNTA compounds *via t*–butyl protected nonaesters [14–19].

Increased JD-TrisNTA valency enhances his-tagged protein binding to hybrid liposomes

To investigate the relative affinities of His-tagged protein for JD-TrisNTA vs. JD-NTA in hybrid liposomes, we tested a recombinant His-tagged red fluorescent protein (His-RFP) and a green-fluorescent labeled Janus dendrimer (3,4)12G1-NBD [45] (Fig. 1, orange box). Co-assembly of JD-TrisNTA with HSPC and cholesterol in liposomes indicated clear recruitment of His-RFP (red color) on the membrane of hybrid liposome (green color). (Fig. 5a).

To determine the binding strength, we titrated the molar concentration of (3,4)12G1-NTA or (3,4)12G1-TrisNTA in hybrid liposomes from 1% to 0.5% and to 0.2% (mol% of NTA units, representing 0.33%, 0.17% and 0.067% of for TrisNTA). Binding of His-RFP was quantified from confocal fluorescent images (Fig. 5b). At 1% mol of NTA units, hybrid liposomes with either (3,4)12G1-NTA or (3,4)12G1-TrisNTA bind to His-RFP very strongly. In contrast, at 0.5% of NTA units in hybrid liposomes, (3,4)12G1-TrisNTA coated hybrid liposomes bound 50% higher His-RFP than (3,4)12G1-NTA coated hybrid liposomes. At even lower NTA concentrations, such as 0.2%, (3,4)12G1-TrisNTA coated hybrid liposomes showed 6-fold higher His-RFP recruitment than (3,4)12G1-NTA coated hybrid liposomes. These observations indicate JD-TrisNTA binds His-protein at much lower NTA ligand threshold concentration compared to JD-NTA in hybrid liposomes, likely as a result of increased valency.

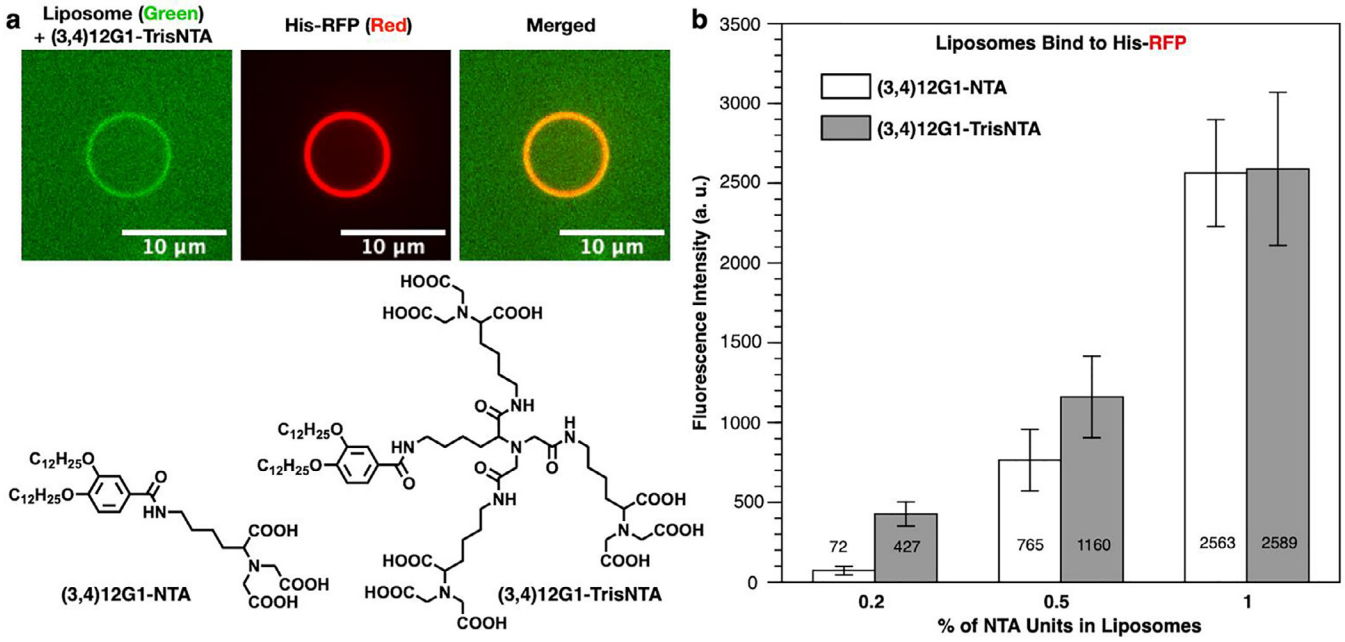

Fig. 5

(a) Recruitment of His-RFP to the membrane of JD-TrisNTA containing hybrid liposomes. Liposomes contain 0.5% (w/w) of (3,4)12G1-NBD as a green fluorescent tracker. Merge confirms colocalization. (b) Comparisons of recruitment of His-RFP on the surface of liposome by 0.2–1% mol of NTA units from (3,4)12G1-NTA or (3,4)12G1-TrisNTA. Error bars: ± SEM.

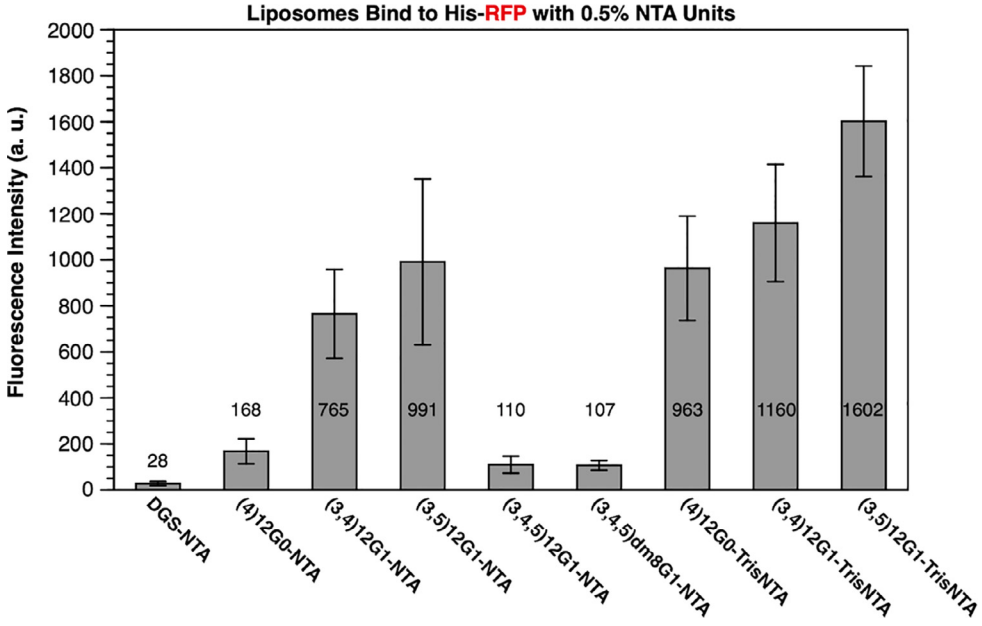

Fig. 6

Comparisons of recruitment of His-RFP on the surface of liposome by 0.5% mol of NTA units from JD-NTA or JD-TrisNTA. Error bars: ± SEM.

Finally, we tested the entire library at 0.5% mol of NTA within liposomes (Fig. 6). Hybrid liposomes containing (3,4)12G1-NTA and (3,5)12G1-NTA presented the highest relative affinities compared to the DGS-NTA molecule. These results mirrored the result with His-GFP (Fig. 3b)

The concentration of NTA unit used in the experiment described here (0.5%) is lower than in Fig. 3b (2%), since we demonstrated that at a lower concentration, the relative activities of JD-NTA and JD-TrisNTA can be discriminated (Fig. 5). The activity of (4)12G1-NTA is significantly lower than those of (3,4)12G1-NTA and (3,5)12G1-NTA, while the activities of (3,4,5)12G1-NTA and (3,4,5)dm8G1-NTA remained the lowest among the five JD-NTAs. These results indicated that 3,4- and 3,5- substituted hydrophobic dendrons (3,4)12G1 and (3,5)12G1 may have the best compatibility to co-assemble with liposomes due to interdigitation of their alkyl chains [21]. Importantly,

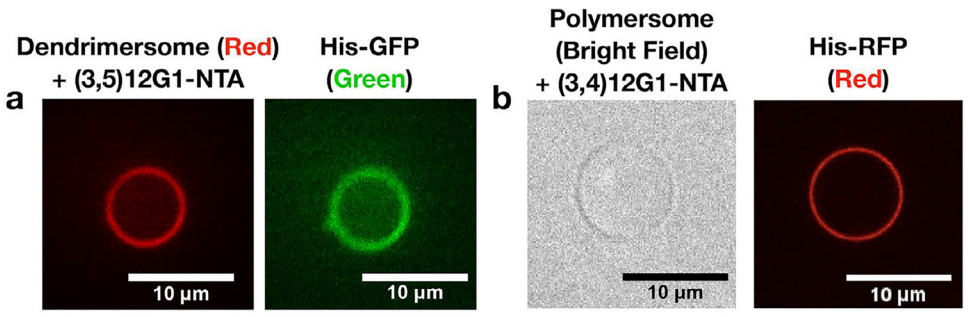

Fig. 7

(a) Recruitment of His-GFP to the membrane of JD-NTA (4%, w/w) containing hybrid dendrimersomes. Dendrimersomes contain 0.5% (w/w) of 18:1 Liss Rhod PE as a red fluorescent tracker. (b) Recruitment of His-RFP to the membrane of JD-NTA (5%, w/w) containing hybrid polymersomes. Bright field was used to visualize the polymersomes.

hybrid liposomes containing JD-TrisNTA showed higher values than their single NTA analogues with the identical hydrophobic minidendrons. Among all the molecules, (3,5)12G1-TrisNTA exhibited the highest affinity for His-RFP among the three JD-TrisNTAs.

Co-Assembly of dendrimersomes and polymersomes with JD-NTA and protein recruitment

In addition of the hybrid liposomes, we prepared the hybrid dendrimersomes [28] or hybrid polymersomes with selected JD-NTA. Janus dendrimer (JD) (3,5)12G1-PE-(3,4,5)3EOG1-$(OCH_3)_6$ (Fig. 1, yellow box) [20] was selected to prepare very stable giant vesicles, and block copolymer OB1017 (Fig. 1, yellow box) was selected to generate polymersomes [53]. Preliminary results on the recruitment of His-tagged proteins with hybrid dendrimersomes and polymersomes are presented in Fig. 7. The green or red fluorescent signal from the His-tagged protein were clearly showed on the surface of these dendrimersomes or polymersome vesicles. These preliminary results demonstrated that JD-NTA reported here can be utilized as a universal ligand for synthetic vesicles to bind to His-tagged proteins for various applications in cell biology and nanomedicine [54–62].

Conclusions

In summary, we report the synthesis of two libraries of amphiphilic JDs conjugated to NTA and TrisNTA that co-assemble with phospholipids and cholesterol, to generate hybrid liposomes extremely active towards binding His-tag proteins. JD-TrisNTA was synthesized starting from JD-NTA in just two steps and presents higher activities toward His-tagged fluorescent proteins at much lower ligand concentration than the liposomes containing the same concentration of JD-NTA incorporated in hybrid liposomes. All JD-NTA and JD-TrisNTA are stable at room temperature and insensitive to air exposure. In contrast, commercial unsaturated lipid NTA derivatives are sensitive to oxygen and cannot be stored for long periods of time, even frozen. Even more important, the activity of hybrid liposomes containing JD-NTA towards His-tagged fluorescent proteins can be twenty-fold to thirty-fold higher than that of liposomes containing the same NTA concentration derived from the commercially available lipid NTA DGS-NTA. The mechanism of the much higher activity of hybrid liposome assemblies constructed with JD-NTA is not known at this time. Most likely this is due to the different dynamics of the JD-NTA on the surface of liposomes and/or formation of supramolecular NTA assemblies because the reactivity of the individual NTA ligand must be identical both in JDs and in lipids. Regardless, these JD-NTA and JD-TrisNTA, most probably, will provide complementary tools to those of the commercially available lipid-NTA for applications in molecular biology, cell biology and nanomedicine [54–62]. JDs [20–33], JGDs [22,34-42] and IAJDs [43,44] are water complex self-organizable systems that are complimentary to related complex systems including liquid crystals that self-organize in bulk, including Frank-Kasper and chiral Frank-Kasper and other helical chiral phases [24,50,63-171]. Since JDs, JGDs and IAJDs self-assemble and co-assemble into monodisperse assemblies in water, they represent and interesting class of supramolecular polymers whose mechanism of supramolecular polymerization and self-organization is less investigated and understood since diffraction methods for their structural analysis were not yet elaborated except for very few particular cases [39,40,42]. However, due to their applications as models of biological membranes [20,26-29,31,33] and for the delivery of mRNA [43,44] we expect that the lyotropoic research field will develop and join in the near future the already very active field of surfactants [140–142], lipids [159–166] and other amphiphiles, in spite of the structural analysis difficulties mentioned above [39,42]. These research developments will facilitate a merger between Frank-Kasper phases and liquid crystals in the bulk of soft matter state, known also as thermotropic, with the corresponding lyotropic state that is widely available also in self-organized living matter, but it is less elucidated. Ultimately, merging principles of self-organized soft matter with those of living matter, that we believe are similar [64], is one of the greatest challenges that will produce an important step forward in self-organized molecular and supramolecular sciences [63,64,167-171].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.giant.2021.100089.

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