SUPPLEMENTARY MATERIALS

AB3-Loaded and Tumor-Targeted Unimolecular Micelles for Medullary Thyroid Cancer Treatment

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1. Synthesis and Characterization of PAMAM–PVL–PEG–OCH_3/KE108/Cy5 and PAMAM–PVL–PEG–OCH_3/OCT/Cy5

1.1 Materials

Poly(amidoamine) (1,4-diaminobutane; G4) dendrimer was purchased from NanoSynthons LLC (Mt. Pleasant, MI, USA). Valerolactone (VL) and stannous (II) octoate ($Sn(Oct)_2$) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Methoxy–PEG–OH (mPEG–OH, M_n=5 kDa) and OH–PEG–*N*–hydroxylsuccinimide (HO–PEG–NHS, M_n=5 kDa) were obtained from JenKem Technology (Allen, TX, USA). 4-Dimethylamino pyridine (DMAP) and 1,3–dicyclohexylcarbodiimide (DCC) were purchased from ACROS. Cy5 dye was obtained from Lumiprobe Corporation (Hallandale Beach, FL, USA). KE108 was purchased from Bachem Americas, Inc. (Torrance, CA, USA). Other reagents were purchased from Thermo Fisher Scientific (Fitchburg, WI, USA) and used as received unless otherwise stated.

1.2 Synthesis and characterization of PAMAM–PVL–OH

PAMAM–PVL–OH polymer was synthesized by ring–opening polymerization of the VL using dendrimer PAMAM–OH as the macro-initiator. A 50 ml two-neck flask equipped with an argon

gas inlet was charged with PAMAM–OH (20 mg, 1.4 μmol), VL (277 mg, 2.7 mmol) and $Sn(Oct)_2$ (1.1 mg, 2.7 μmol). The reaction was carried out at 120 °C for 24 h. After the resulting mixture was fully dissolved in THF, the solution was added dropwise into methanol to obtain a pale yellow precipitate. The precipitation process was repeated for 2 times and the final product was dried under vacuum overnight. The chemical structure was confirmed by ^1H NMR collected on a Varian Mercury Plus 300 NMR spectrometer using $CDCl_3$ as the solvents. (**Figure S1 (A)**). The FT–IR spectrum recorded on a Bruker Tensor 27 FT-IR spectrometer (**Figure S1 (B)**) also confirmed the chemical structure of PAMAM–PVL–OH. Molecular weights (M_n and M_w) and polydispersity indices (PDI) of all intermediate and final polymer products (**Table S1**) were determined by a gel permeation chromatographer (GPC) equipped with a refractive index detector, a viscometer detector, and a light scattering detector (Viscotek, USA). DMF with 0.1 mmol/L of LiBr was used as the mobile phase at a flow rate of 1 mL/min. The number of arms **(# arm**) per PAMAM–PVL–OH polymer was determined by comparing the molecular weights of PAMAM–OH and PMAMA–PVL–OH. Based on **Equation S1**, **# arm** per PAMAM–PVL–OH molecule was 33.

$$\#arm = \left(Mn_{PAMAM-PVL-OH} - Mn_{PAMAM-OH}\right)/Mn_{PVL} \qquad \textbf{Equation S1}$$

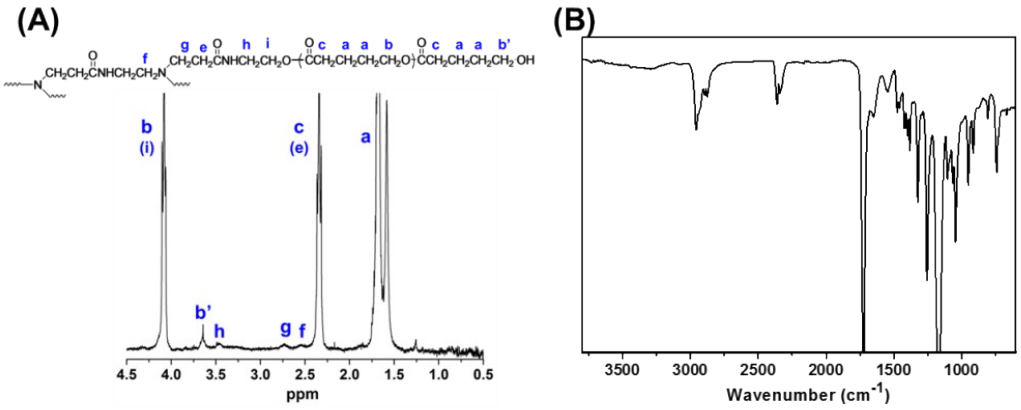

Figure S1. (A) ^{1}H NMR and (B) FT–IR spectra of PAMAM–PVL–OH.

Table S1. GPC analyses of all polymers.

Polymers	M_n (g/mol)	M_w(g/mol)	M_w/M_n
PAMAM–OH	14277		
PAMAM–PVL–OH	99450	141616	1.424
PAMAM–PVL–COOH	102037	147035	1.441
PAMAM–PVL–PEG–OCH_3/NHS	257017	354945	1.381

1.3 Synthesis and characterization of PAMAM–PVL–COOH

The –OH groups of PAMAM–PVL–OH were converted into –COOH. PAMAM–PVL–OH (50 mg, 0.51 μmol), succinic anhydride (8.2 mg, 82 μmol) and DMAP (12.5 mg, 98 μmol) were dissolved in anhydrous DCM (10 mL) and the reaction was carried out at room temperature for 48 h. The resulting solution was added dropwise into cold diethyl ether to yield a pale yellow precipitate. The precipitate was then redispersed in DI water and the impurities were removed by dialysis against DI water using cellulose tubing (molecular weight cut-off (MWCO), 15 kDa) for 48 h. The final product was obtained after freeze-drying. The chemical structure of PAMAM–PVL–COOH was also confirmed by ^{1}H NMR (**Figure S2 (A)**).

1.4 Synthesis and characterization of PAMAM–PVL–PEG–OCH_3/NHS

mPEG–OH and NHS–PEG–OH were conjugated onto the PAMAM–PVL–COOH via esterification to form the multi-arm star amphiphilic block copolymer PAMAM–PVL–PEG–OCH_3/NHS. PAMAM–PVL–COOH, mPEG–OH, NHS–PEG–OH, DCC and DMAP were dissolved in DMF. The molar ratio of the reactants (PAMAM–PVL–COOH:mPEG–OH:NHS–

PEG–OH:DCC:DMAP) was set at 1:48:16:70:7. The reaction was carried out at room temperature for 24 h. After removal of the by-product, dicyclohexylcarbodiurea by filtration, the solution was added dropwise into cold diethyl ether to yield the crude product. The precipitate was then redispersed in DI water and the impurities were removed by dialysis against DMF for 48 h using cellulose tubing (MWCO, 15 kDa). The resulting polymer, PAMAM–PVL–PEG–OCH_3/NHS, was dried under vacuum. PAMAM–PVL–PEG–OCH_3 was prepared following a similar procedure. The ^{1}H NMR spectrum (shown in **Figure S2 (B)**) clearly demonstrated the successful synthesis of PAMAM–PVL–PEG–OCH_3/NHS. GPC analyses (**Table S1**) further confirmed the formation of PAMAM–PVL–PEG–OCH_3/NHS. The molecular weight of PAMAM–PVL–PEG–OCH_3/NHS was measured as 174,721 Da, which was significantly larger than that of PAMAM–PVL–COOH (54,721 Da). Similarly, the **# arm** per PAMAM–PVL–PEG–OCH_3/NHS was determined as 32 using **Equation S2**, which is in good agreement with previous reports on the number of arms in PAMAM-based multi-arm star amphiphilic block copolymers [1].

$$\#arm = \left(Mn_{PAMAM-PVL-PEG-OCH_3/NHS} - Mn_{PAMAM-PVL-COOH}\right)/Mn_{PEG} \qquad \textbf{Equation S2}$$

1.5 Synthesis and characterization of PAMAM–PVL–PEG–OCH_3/KE108/Cy5 and PAMAM–PVL–PEG–OCH_3/OCT/Cy5

KE108 peptide and Cy5 dye were conjugated onto the PAMAM–PVL–PEG–OCH_3/NHS via an amidization reaction. PAMAM–PVL–PEG–OCH_3/NHS was first reacted with KE108 peptide (PAMAM–PVL–PEG–OCH_3/NHS: KE108 = 1:6, mol:mol) in DMF for 8 h. Thereafter, Cy5 dye (PAMAM–PVL–PEG–OCH_3/NHS: Cy5 = 1:2, mol:mol) was added into this reaction mixture which was stirred for another 16 h. Then the impurities were removed by dialysis against DI water for 48 h using cellulose tubing (MWCO, 15 kDa). The resulting polymer was obtained after lyophilization. The ^{1}H NMR confirmed the formation of PAMAM–PVL–PEG–OCH_3/KE108/Cy5 (**Figure S2 (C)**). The PAMAM–PVL–PEG–OCH_3/OCT/Cy5, PAMAM–PVL–PEG–OCH_3/KE108 and PAMAM–PVL–PEG–OCH_3/Cy5 block copolymers were also prepared following a similar procedure.

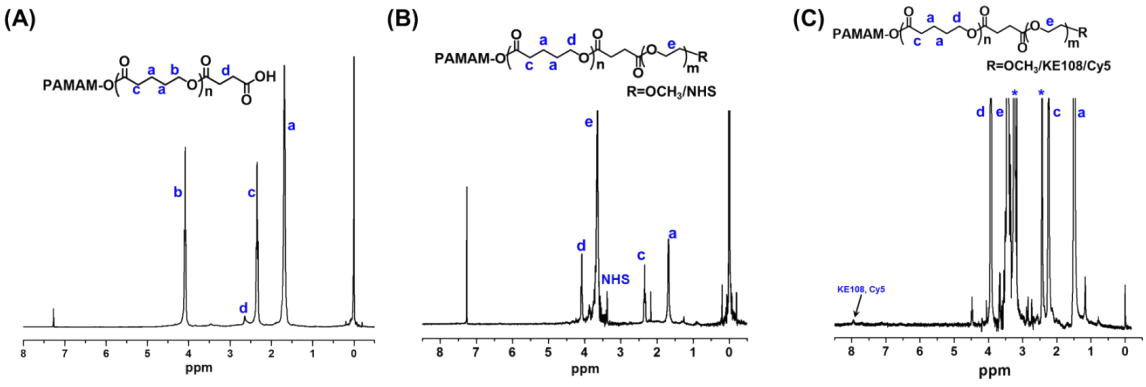

Figure S2. ^{1}H NMR spectra of (A) PAMAM–PVL–COOH, (B) PAMAM–PVL–PEG–OCH_3/NHS, and (C) PAMAM–PVL–PEG–OCH_3/Cy5/KE108.

The morphology of the unimolecular micelles was studied by dynamic light scattering (DLS, ZetaSizer Nano ZS90, Malvern Instruments, USA) and transmission electron microscopy (TEM, FEI Tecnai G^2 F30 TWIN 300 KV, E.A. Fischione Instruments, Inc. USA).

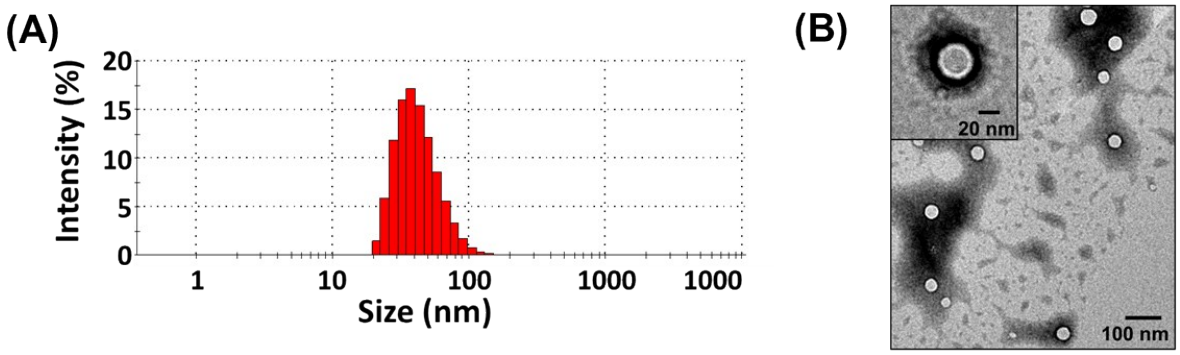

Figure S3. (A) DLS analysis and (B) TEM image of the unimolecular micelles.

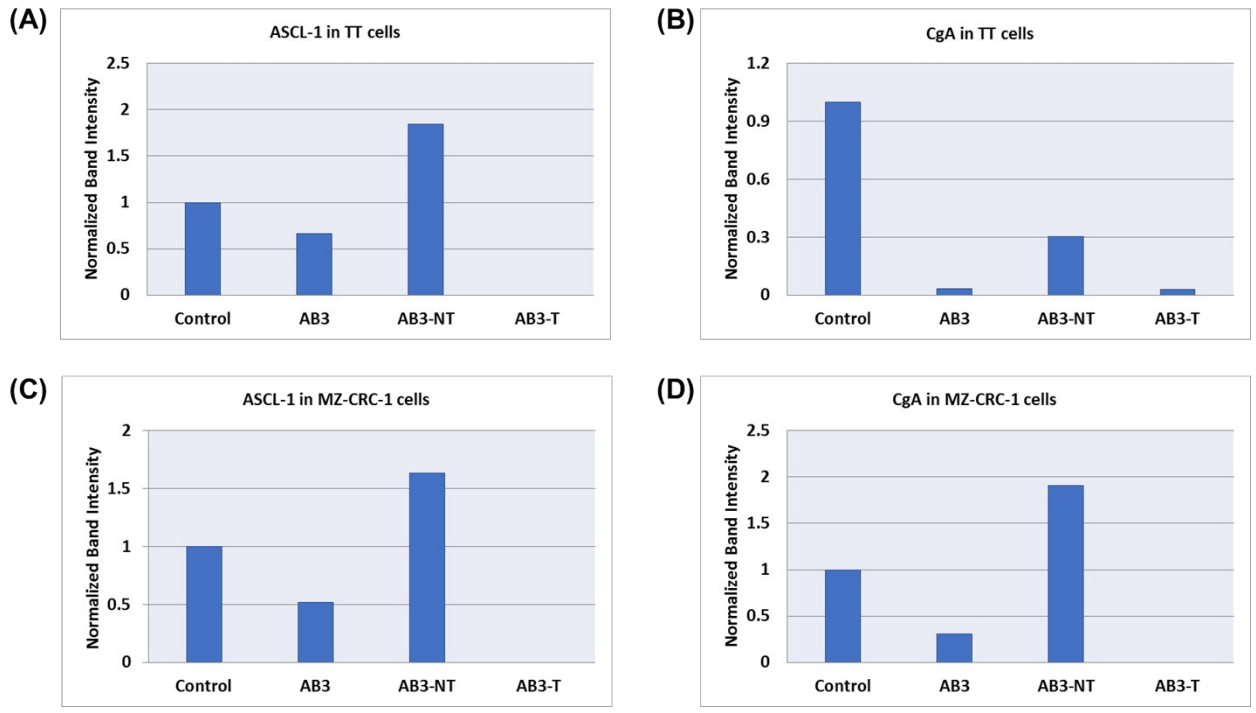

Figure S4. Densitometric analysis of Western blot results of ASCL-1 and CgA protein expression in TT (A and B) and MZ-CRC-1 (C and D) cells. Cells were treated with cell culture medium (Control), free AB3, and AB3-loaded non-targeted (AB3-NT) and AB3-loaded targeted (AB3-T)

micelles. ASCL-1 and CgA protein expression intensities were normalized to β-actin and plotted as bar graphs.

Reference

1. J. Guo, H. Hong, G. Chen, S. Shi, Q. Zheng, Y. Zhang, C. P. Theuer, T. E. Barnhart, W. Cai and S. Gong, *Biomaterials*, 2013, **34**, 8323-8332.