

Biophysical Characterization of a Riboflavin-conjugated Dendrimer Platform for Targeted Drug Delivery

Amanda B. Witte,¹ Christine M. Timmer,¹ Jeremy Gam,² Seok Ki Choi,^{2*} Mark M. Banaszak Holl,^{2,3,5,6} Bradford G. Orr,^{2,4} James R. Baker, Jr.^{2,5*}, and Kumar Sinniah^{1*}

¹*Department of Chemistry & Biochemistry, Calvin College, 3201 Burton St. SE, Grand Rapids, Michigan 49546, United States*

Department of ²Internal Medicine, Michigan Nanotechnology Institute for Medicine and Biological Sciences, ³Chemistry, ⁴Physics, ⁵Biomedical Engineering, and ⁶Macromolecular Science and Engineering, University of Michigan, Ann Arbor, Michigan 48109, United States

Table of Contents

1. Table S1. Summary of molecular weight, mean valency, yield, and purity of the RF-dendrimer conjugate (Page S2)
2. Figure S1. MALDI TOF mass spectra of RF-dendrimer conjugates G5-N(3)-RF_n **4–7**, and G5-N(10)-RF_m **10–12** (Page S3)
3. Figure S2. ¹H NMR (500 MHz) spectra of RF-dendrimer conjugates G5-N(3)-RF_n **4–7**, and G5-N(10)-RF_m **10–12** (Page S4-5)
4. Figure S3. Anal. HPLC traces of RF-dendrimer conjugates G5-N(3)-RF_n **4–7**, and G5-N(10)-RF_m **10–12** (Page S6)
5. Figure S4. UV/vis spectra of RF-dendrimer conjugates G5-N(3)-RF_n **4–7**, and G5-N(10)-RF_m **10–12** (Page S7)
6. Figure S5. A MALDI TOF mass spectrum (A), and a gel permeation chromatogram (B) for the purified G5 PAMAM dendrimer (Page S8)

Table S1. Summary of molecular weight, mean valency, yield, and purity of the RF-dendrimer conjugate.

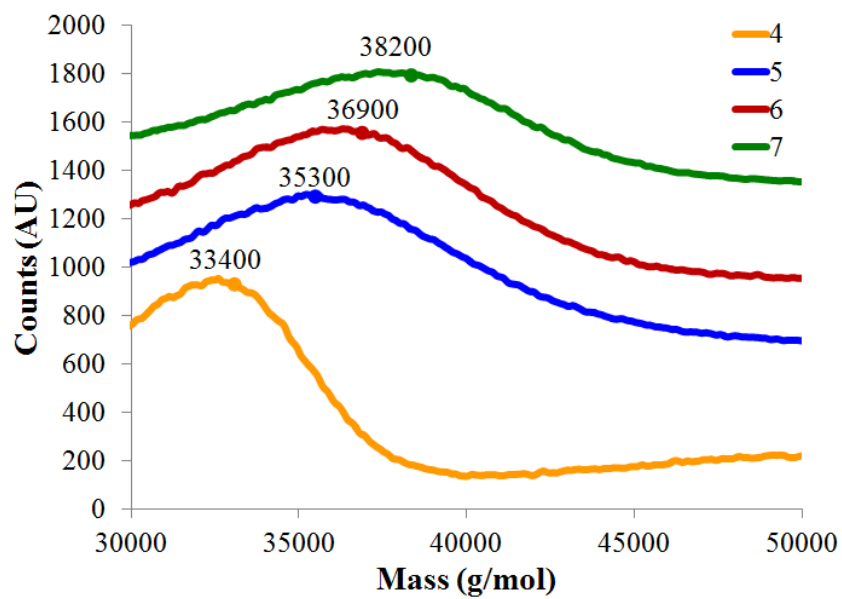
Conjugate	MW ^a	# of RF/G5 (n, or m)			Yield ^b	Purity (%) ^c
	MALDI	UV/vis	MALDI	¹ H NMR	weight	HPLC
G5-N(3)-RF _n -FITC _p	(g/mol)	n	n	n	(mg)	
4	33400	3.0	-	2.8	29	95
5	35300	6.0	5.0	4.5	28	97
6	36900	10.4	8.9	11.1	2b	98
7	38200	10.4	8.9	11.1	26	97
		(p = 1.6)	(p = 3.3)	(p = 2)		
G5-N(10)-RF _m	(g/mol)	m	m	m		
10	33700	5.2	-	4.0	25	99
11	36900	8.4	8.9	7.7	28	99
12	40300	9.2	17.1	8.4	30	99

^aMolecular weight (m/z) that refers to the mass value at or around the peak of each MALDI spectrum;

^bAmount of each conjugate isolated from the reaction from 30 mg of G5 PAMAM dendrimer (for the N(3) series, and 25 mg for the N(10) series); ^cPurity of each dendrimer conjugate was determined by analytical reversed phase HPLC at the detection wavelength of 285 nm (where RF has strong absorption).

Figure S1. MALDI TOF mass spectra of RF-dendrimer conjugates G5-N(3)-RF_n **4–7** (A), and G5-N(10)-RF_m **10–12** (B).

(A)



(B)

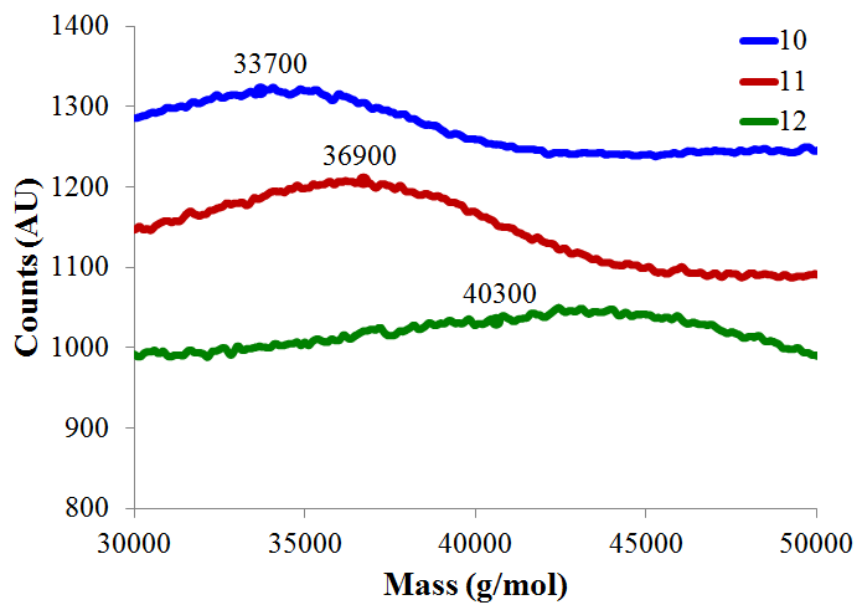
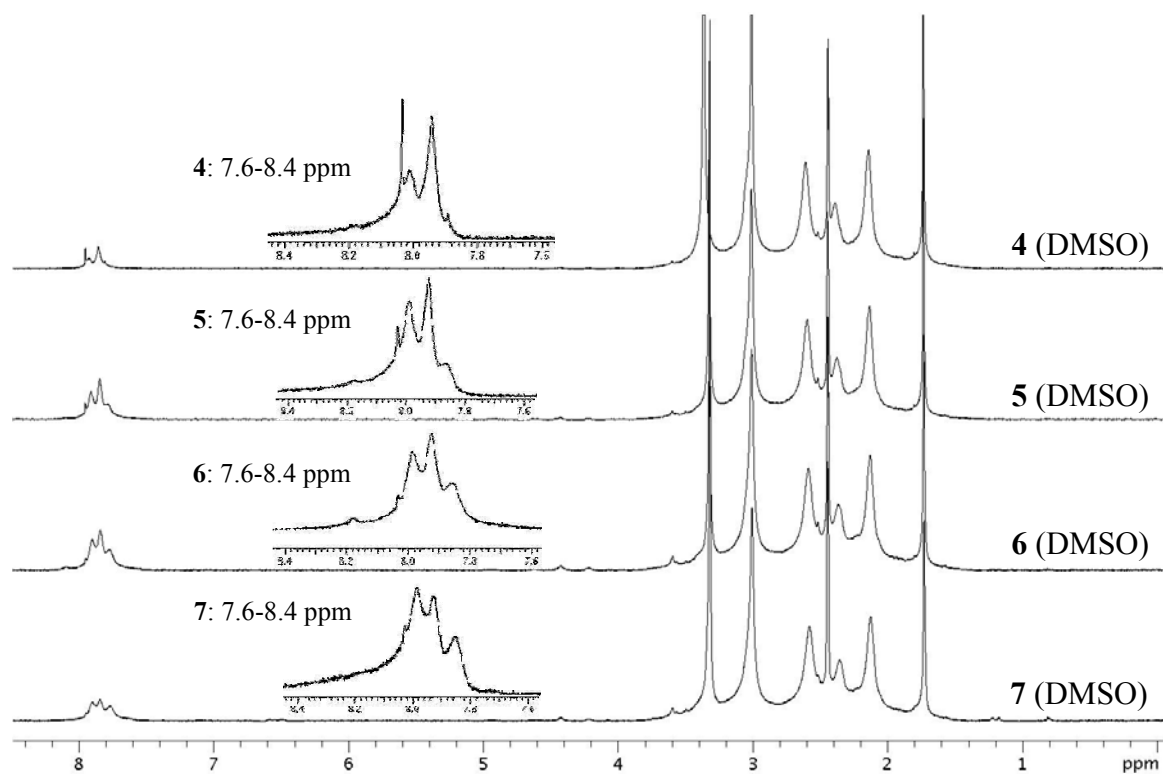
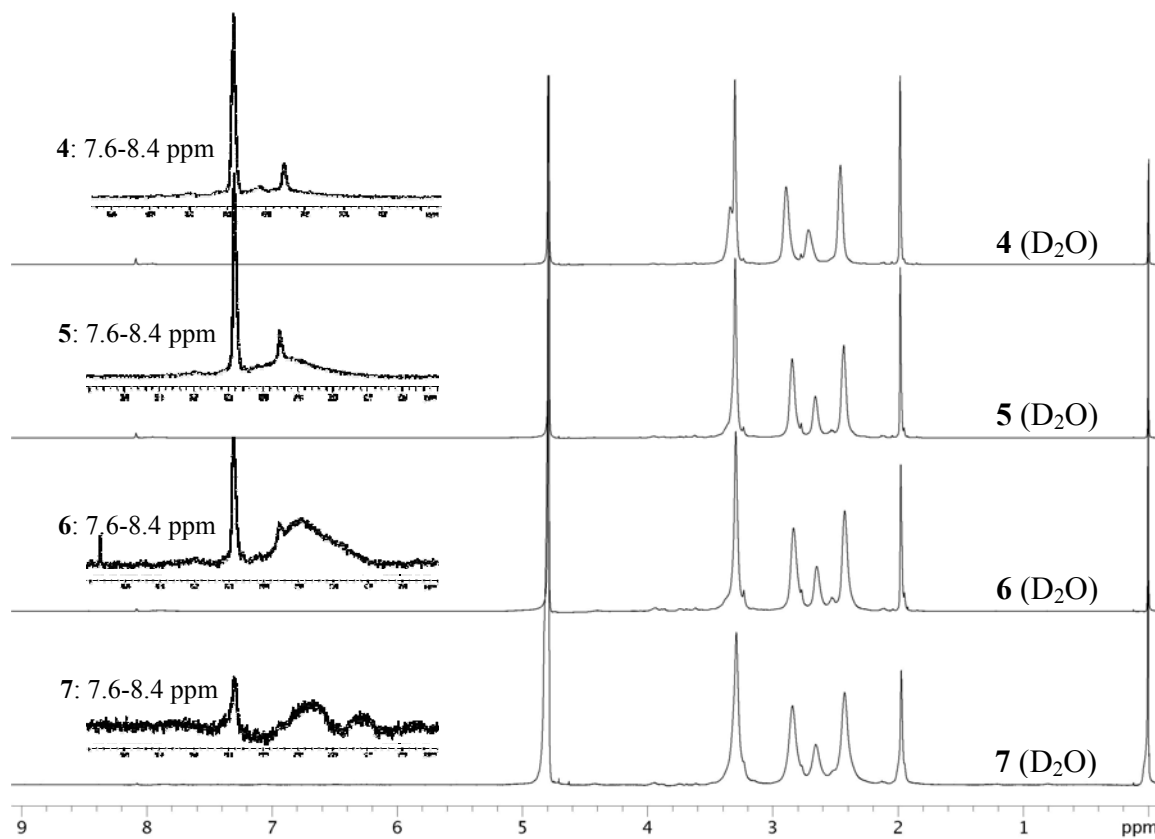


Figure S2. ^1H NMR (500 MHz) spectra of RF-dendrimer conjugates: (A) G5-N(3)-RF_n **4–7** (each taken in DMSO-*d*₆), (B) G5-N(3)-RF_n **4–7** (each in D₂O), and (C) G5-N(10)-RF_m **10–12** (each in D₂O).

(A)



(B)



(C)

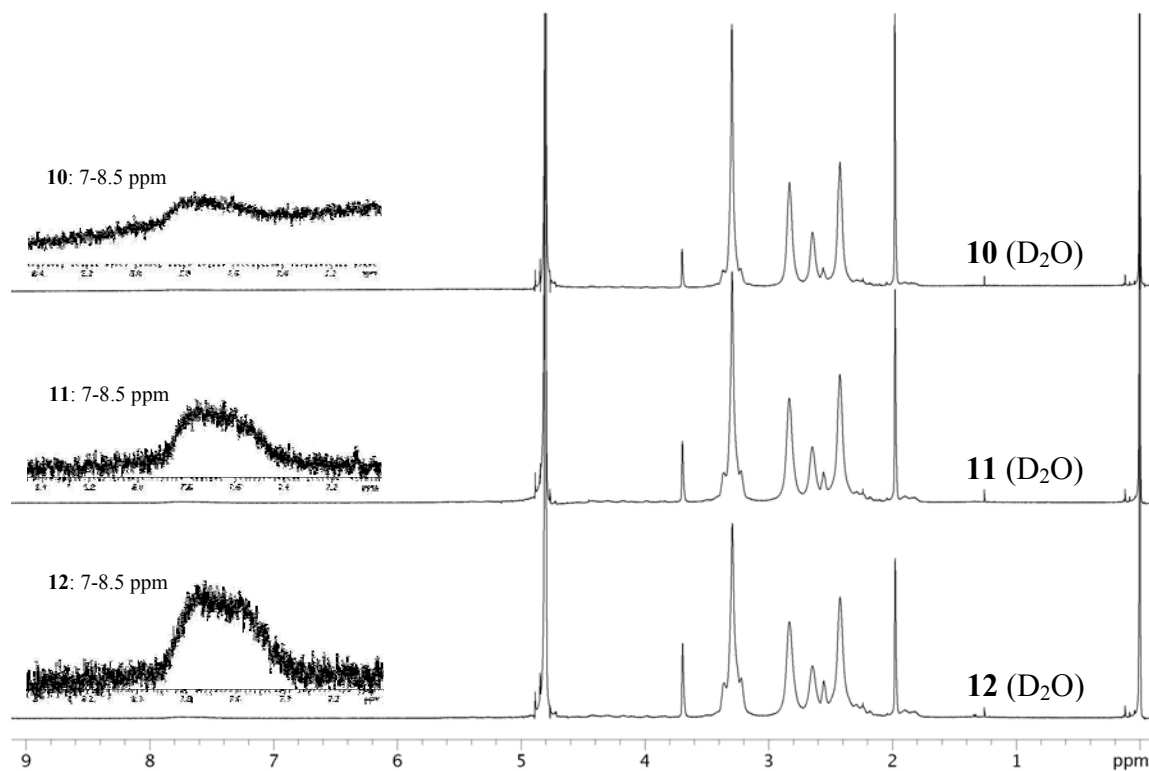
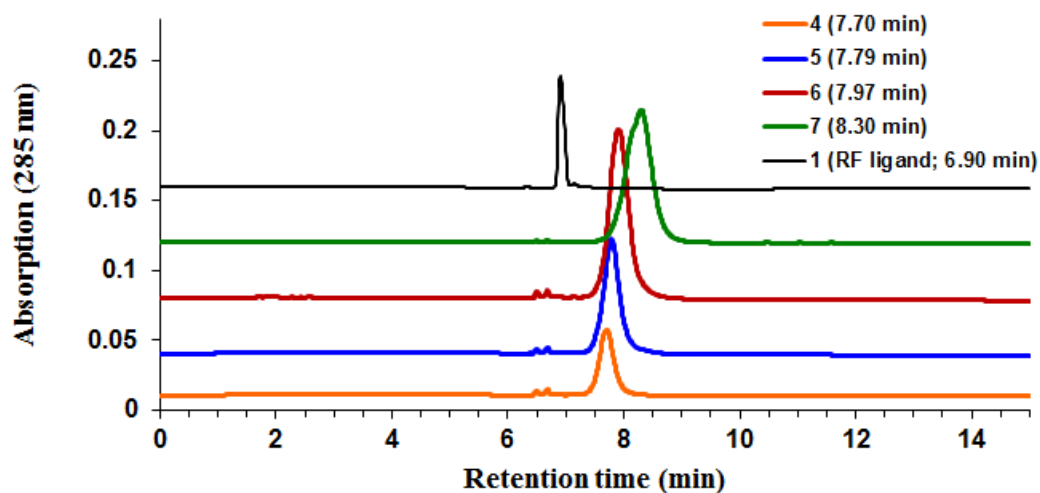
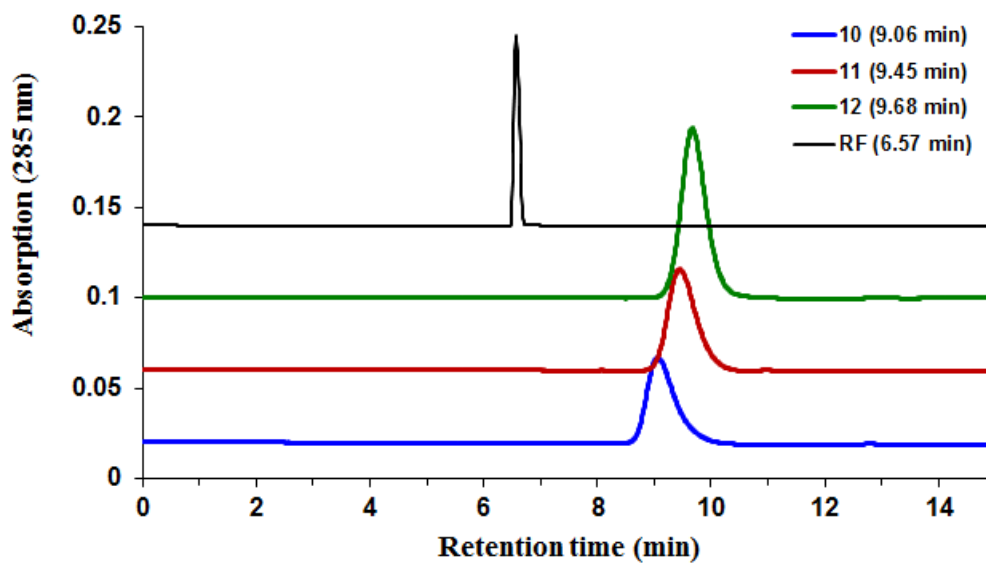


Figure S3. Analytical HPLC traces of RF-dendrimer conjugates G5-N(3)-RF_n **4–7** (A), and G5-N(10)-RF_m **10–12** (B).

(A)



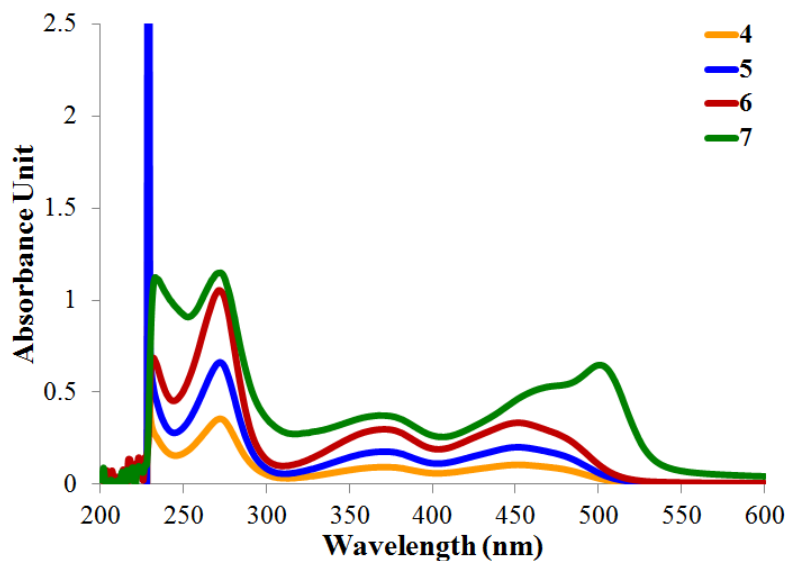
(B)



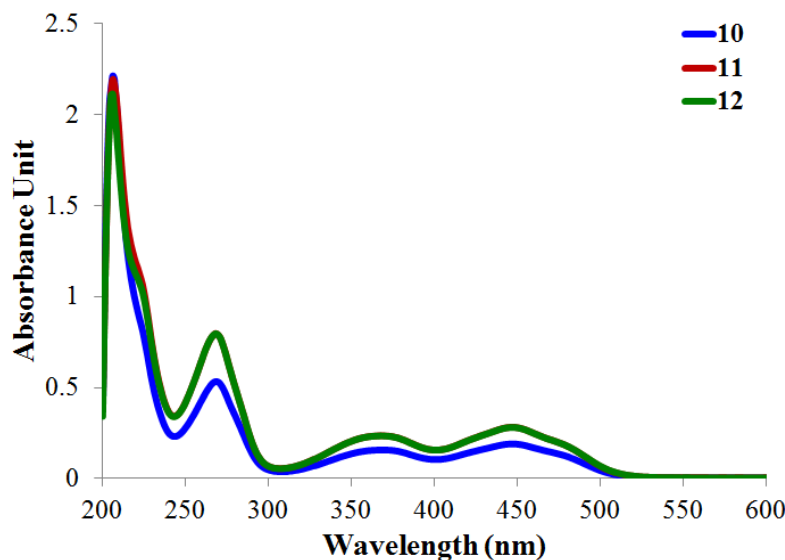
HPLC: **4** (t_r = 7.70 min; purity = 95%); **5** (t_r = 7.79 min; purity = 97%); **6** (t_r = 7.91 min; purity = 98%); **7** (t_r = 8.30 min; purity = 97%); **10** (t_r = 9.06 min; purity = 99%); **11** (t_r = 9.45 min; purity = 99%); **12** (t_r = 9.68 min; purity = 99%).

Figure S4. UV/vis spectra of RF-dendrimer conjugates G5-N(3)-RF_n **4–7** (A), and G5-N(10)-RF_m **10–12** (B).

(A)



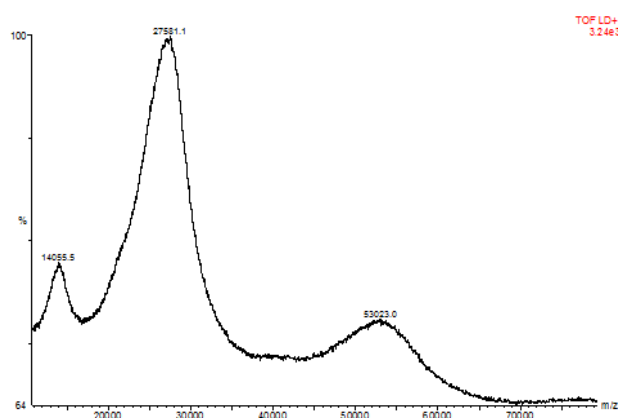
(B)



UV/vis (0.1 mg/mL; PBS, pH 7.4): **4–6**: $\lambda_{\text{max}} = 449 \text{ nm}$ ($\epsilon = 12100 \text{ M}^{-1}\text{cm}^{-1}$ calculated on the basis of RF), 272 nm ($\epsilon = 10300 \text{ M}^{-1}\text{cm}^{-1}$ calculated on the basis of RF); **7**: $\lambda_{\text{max}} = 491 \text{ nm}$ ($\epsilon = 100000 \text{ M}^{-1}\text{cm}^{-1}$ calculated on the basis of FITC); **10–12**: $\lambda_{\text{max}} = 449 \text{ nm}$ ($\epsilon = 12100 \text{ M}^{-1}\text{cm}^{-1}$ calculated on the basis of RF), 374 nm ($\epsilon = 10300 \text{ M}^{-1}\text{cm}^{-1}$ calculated on the basis of RF).

Figure S5. (A) A matrix assisted laser desorption ionization time of flight (MALDI TOF) mass spectrum of a generation 5 (G5) PAMAM dendrimer used for the conjugation with riboflavin ligands. Please note that this dendrimer batch refers to a purified form from a commercial batch as described in the experimental section. The molecular weight (MW) of the dendrimer at its major peak is 27,600 gmol^{-1} . The two additional peaks shown in the spectrum represent differently charged states of the dendrimer: doubly charged ion = $[M+2H]^{2+} = 14,055$; singly charged molecular ion = $[M+H]^+ = 27,581$; protonated dimer = $[2M+H]^+ = 53,203$; (B) A gel permeation chromatogram for the purified G5 PAMAM dendrimer: $M_n = 26,270 \text{ gmol}^{-1}$, $M_w = 26,550 \text{ gmol}^{-1}$, polydispersity index (PDI) = $(M_n \div M_w) = 1.010$.

(A)



(B)

