Designing Allosteric Inhibitors of Factor XIa.
Lessons from the Interactions of Sulfated Pentagalloylglucopyranosides

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SUPPLEMENTARY MATERIAL

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Synthesis and characterization of different sulfated pentagalloyl*β-D*-glucopyranoside variants.

Synthesis and characterization of sulfated pentagalloyl *β-D*-glucopyranoside (*β*-SPGG-2).[1,2] The polyphenolic precursor, pentagalloyl-*β-D*-glucopyranoside (PGG, **3a**), was obtained in two steps, esterification and catalytic hydrogenation as reported earlier.[2] Sulfated pentagalloyl *β-D*-glucopyranoside (*β*-SPGG-2, **4c**) (Scheme 1) was synthesized as reported earlier[2] by microwave-assisted synthesis followed by size exclusion and sodium exchange chromatographies. This inhibitor was characterized by ^{1}H NMR, ^{13}C NMR and found to be consistent with previous report.[15] UPLC-MS was further used to structurally characterize SPGG components and estimate its average molecular weight in a manner similar to pervious study.[2] Exact similar experimental conditions were applied to synthesize other SPGG derivatives except for the sulfation time (0.5, 1, 2, 4, 6, or 8 hrs) and the glucose unit which could be *α*-unit or *α,β*-unit instead of *β*-unit. The configuration of the anomeric carbon in each variant was determined by measuring the $[\alpha]_D^{20}$ in acetone (c=1%) of the corresponding polyphenolic precursor. Consistent with literature, the specific rotations of the precursors were found to be +25.2º for *β*-, +65.5º for *α*-, and +57.9º for *α,β*-derivative.[1]

General procedure for esterification of polyalcohol (*β-D*-glucopyranose). To a stirring solution of 3,4,5-tribenzyloxybenzoic acid (5 mmol) and DMAP (5 mmol) in anhydrous CH_2Cl_2 (20 mL), DCC (5 mmol) was added and the resulting mixture was stirred for 30 min at RT. The saccharide (*β-D*-glucopyranose (**1a**)) (1 mmol) was then added and the resulting reaction mixture was refluxed 24 hrs. After that, the reaction was cooled to RT and filtered through a pad of Celite. The organic phase was then washed with HCl (1 M, 15 mL), brine solution (15 mL), and H_2O (15 mL).The organic phase was dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography using hexanes/EtOAc mobile phase as described above. 20 mL fractions containing the desired product (protected PGG (**2a**)) were pooled together and concentrated *in vacuo* to afford the desired product as a white solid in yields of (85–90 %).

1, 2, 3, 4, 6-Penta-O-(3, 4, 5-tri-O-benzylgalloyl)-β-D-glucopyranose (Protected PGG, 2a). ^{1}H NMR ($CDCl_3$, 400 MHz): 7.38–7.08 (m, 85 H), 6.15 (d, 1 H, J = 8.12 Hz), 5.98 (t, 1 H, J = 9.68 Hz), 5.78–5.74 (dd, 1 H, J_1 = 9.80 Hz, J_2 = 8.20 Hz), 5.66 (t, 1 H, J = 9.64 Hz), 5.08–4.77 (m, 30 H), 4.71–4.68 (dd, 1 H, J_1 = 11.68 Hz, J_2 = 2.32 Hz), 4.39–4.35 (m, 1 H), 4.35–4.28 (dd, 1 H, J_1 = 11.8 Hz, J_2 = 5.88 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): 165.67, 165.61, 165.07, 165.01, 164.22, 152.67, 152.62, 152.54, 143.36, 143.30, 143.23, 142.74, 137.56, 137.38, 136.78, 136.51, 136.46, 136.37, 128.57, 128.55, 128.53, 128.48, 128.46, 128.41, 128.39, 128.31, 128.24, 128.17, 128.13, 128.05, 127.65, 127.60, 127.58, 124.62, 123.75, 123.64, 123.40, 109.56, 109.51, 109.43, 109.31, 109.27, 106.51, 93.08, 75.14, 73.36, 73.30, 71.47, 71.34, 71.26, 71.16, 71.11, 69.88, 63.23. MS (ESI) calculated for $C_{146}H_{122}O_{26}$, $[M + H]^+$, m/z 2293.53, found for $[M + H–C_{28}H_{23}O_5]^+$, m/z 1852.975.

General procedure for catalytic hydrogenation (*O*-Debenzylation*).* A solution of benzylated benzoyl derivative of glucopyranose (protected PGG (**2a**)) in CH_3OH/THF solvent mixture (15 mL) was hydrogenated over 20% of 10% $Pd(OH)_2$/C with H_2 (gas) (50 psi) at RT for 10 hrs. The $Pd(OH)_2$/C catalyst was then removed by filtration over Celite and the resulting filtrate was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. A flash chromatography using hexanes/EtOAc mixture as a mobile phase was utilized to purify the reaction crude to afford the corresponding polyphenolic intermediate (PGG (**3a**)) as a yellow–white solid in yields of (>92%).

1, 2, 3, 4, 6-O-Pentagalloyl-β-D-glucopyranose (PGG, 3a). ^{1}H NMR (acetone-d_6, 400 MHz): 8.32–7.98 (m, br, 15 H, phenolic–OH), 7.18 (s, 2 H), 7.12 (s, 2 H), 7.06 (s, 2 H), 7.02 (s, 2 H), 6.97 (s, 2 H), 6.33 (d, 1 H, J = 8.32 Hz), 6.01 (t, 1 H, J = 9.68 Hz), 5.68–5.59 (m, 2 H), 4.56–4.52 (m, 2 H),4.43–4.39 (dd, 1 H, J_1 = 12.68 Hz, J_2 = 4.68 Hz). ^{13}C NMR (acetone-d_6, 100 MHz): 166.40, 165.92, 165.70, 165.65, 164.98, 146.18, 146.06, 146.02, 145.98, 145.90, 139.81, 139.32, 139.04,

121.52, 120.75, 120.65, 120.04, 110.46, 110.36, 110.25, 110.20, 93.39, 74.03, 73.37, 71.82, 69.38, 62.86. MS (ESI) calculated for $C_{41}H_{32}O_{26}$, $[M + H]^+$, m/z 941.69, found for $[M + Na]^+$, m/z 963.157.

General procedure for microwave-assisted sulfation. To a stirring solution of polyphenolic intermediate (PGG (**3a**)) (1 mmol) in anhydrous CH_3CN (2 mL), the sulfating reagent, $N(CH_3)_3–SO_3$ complex (5 mmol/OH), was added. The resulting mixture was then microwaved at 90 °C for 2 hrs. The resulting crude product was cooled to RT and concentrated *in vacuo* at temperature less than 35 °C. All resulting polysulfated molecules were purified as described above using the size exclusion chromatography (G10). The sodium salt form of the isolated white fluffy powder of sulfated species (*β*-SPGG-2 (**4c**)) was generated in yields of (66–70%) by the sodium exchange chromatography followed by lyophilization as described above.

Sulfated Pentagalloyl β-D-glucopyranoside (β-SPGG-2, 4c). ^{1}H NMR (D_2O, 400 MHz): 8.11–7.40 (m, 10 H), 6.51–6.47 (m, 1 H), 6.11–6.18 (m, 1 H), 5.79–5.97 (m, 2 H), 4.85–4.60 (m, 3 H). ^{13}C NMR (D_2O, 100 MHz): 166.4, 165.7, 165.4, 164.7, 150.6, 150.5, 147.8, 147.4, 147.2, 145.7, 145.5, 122.4, 122.2, 122.2, 122.0, 121.0, 119.7, 119.0, 118.7, 115.3, 93.0, 74.5, 72.2, 71.6, 68.9, 63.5.

Similar chemical reactions were exploited to synthesize other SPGG derivatives. Following are the characterization data.

Sulfated Pentagalloyl β-D-glucopyranoside (β-SPGG-0.5, 4a). ^{1}H NMR (D_2O, 400 MHz): 7.91–7.73 (m, 4 H), 7.59–7.21 (m, 4 H), 7.12–6.70 (m, 2H), 6.45–6.40 (m, 1 H), 6.07–5.90 (m, 1 H), 5.89–5.76 (m, 2 H), 4.70–4.64 (m, 3 H). ^{13}C NMR (D_2O, 100 MHz): 166.3, 165.9, 165.7, 165.4, 150.3, 150.2, 147.8, 147.4, 145.3, 144.6, 143.1, 122.4, 122.1, 119.7, 119.2, 118.8, 117.2, 116.8, 115.5, 110.3, 93.0, 73.5, 71.8, 70.6, 68.5, 63.0.

Sulfated Pentagalloyl β-D-glucopyranoside (β-SPGG-1, 4b). ^{1}H NMR (D_2O, 400 MHz): 8.11–7.77 (m, 3 H), 7.59–7.19 (m, 5 H), 7.15–6.89 (m, 2 H), 6.45–6.40 (m, 1 H), 6.22 –5.90 (m, 1 H), 5.86–5.59 (m, 2 H), 4.70–4.60 (m, 3 H). ^{13}C NMR (D_2O, 100 MHz): 166.2, 165.6, 165.3, 164.6, 150.6, 150.5, 147.4, 145.3, 144.6, 143.2, 122.3, 122.0, 119.1, 118.8, 117.2, 116.8, 115.0, 110.2, 93.0, 73.5, 72.0, 71.0, 69.0, 63.20.

Sulfated Pentagalloyl β-D-glucopyranoside (β-SPGG-8, 4f). ^{1}H NMR (D_2O, 400 MHz): 8.18–7.45 (m, 10 H), 6.56–6.54 (m, 1 H), 6.21–6.10 (m, 1 H), 6.00–5.86 (m, 2 H), 4.84–4.75 (m, 3 H). ^{13}C NMR (D_2O, 100 MHz): 166.3, 165.6, 165.4, 164.7, 150.6, 150.5, 147.9, 147.5, 147.2, 145.8, 145.5, 122.4, 122.1, 122.0, 119.0, 118.8, 115.2, 93.0, 74.6, 72.3, 71.5, 68.7, 63.5.

Synthesis and characterization data of the decasulfated species (5, in Scheme 1) of SPGG variants.

Chemical Synthesis. Molecule **5** was performed in similar fashion to SPGG synthesis except 3,4,5-tribenzyloxy benzoic acid was replaced by 3,5-dibenzyloxy benzoic acid.

1, 2, 3, 4, 6-Penta-O-(3, 5-di-O-benzyloxybenzoyl)-α,β-D-glucopyranose (2d). ^{1}H NMR ($CDCl_3$, 400 MHz): 7.34–7.09 (m, 60 H), 6.78 (d, 1 H, J = 3.6 Hz), 6.70–6.69 (m, 1 H), 6.66–6.64 (m, 2 H), 6.63–6.62 (m, 2 H), 6.20 (t, 1 H, J = 9.92 Hz), 5.69 (t, 1 H, J = 10.12 Hz), 5.59–5.56 (dd, 1 H, J_1 = 10.16 Hz, J_2 = 3.76 Hz), 4.95–4.71 (m, 20 H), 4.63–4.59 (m, 1 H), 4.55–4.52 (m, 1 H), 4.37–4.33 (dd, 1 H, J_1 = 12.36 Hz, J_2 = 4.76 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): 165.72, 165.66, 165.11, 164.95, 164.23, 159.98, 159.88, 159.85, 159.80, 136.58, 136.30, 131.47, 130.89, 130.69, 130.54, 128.63, 128.59, 128.54, 128.14, 128.06, 127.64, 127.59, 127.55, 108.76, 108.71, 108.55, 108.39, 108.19, 107.96, 90.33, 70.91, 70.74, 70.56, 70.34, 70.30, 70.25, 70.17, 69.43, 62.86. MS (ESI) calculated for $C_{111}H_{92}O_{21}$, $[M + H]^+$, m/z 1761.91, found for $[M + Na]^+$, m/z 1784.975.

1, 2, 3, 4, 6-Penta-O-(3, 5-dihydroxybenzoyl)-α,β-D-glucopyranose (3d). ^{1}H NMR (acetone-d_6, 400 MHz): 8.5 (s, br, 20 H, phenolic–OH), 7.03 (d, 1 H, J = 2.08 Hz), 6.95 (d, 1 H, J = 2.08 Hz), 6.94 (d, 1 H, J = 2.16 Hz), 6.87 (d, 1 H, J = 2.08 Hz), 6.82 (d, 1 H, J = 2.12 Hz), 6.81 (d, 1 H, J = 2.16 Hz), 6.76–6.75 (m, 3 H), 6.73 (d, 1 H, J = 2.12 Hz), 6.69–6.29 (d & t & t & d, 2 H, J = 3.52 Hz & J = 1.88 Hz & J = 2.08 Hz & J = 8.28 Hz), 6.47–6.44 (m, 1 H), 6.42 (s, 1 H), 6.37 (s, 1 H), 6.35 (d, 1 H, J = 1.8 Hz), 6.10 & 5.97 (t & t, 1 H, J = 10.04 Hz & J = 9.68 Hz), 5.75 (t & m & dd, 2 H, J = 10.04 Hz & J_1 = 10.28 Hz, J_2 = 3.64 Hz), 4.66–4.51 (m, 1 H), 4.47–4.37 (m, 2 H). ^{13}C NMR (acetone-d_6, 100 MHz): 166.50, 166.30, 166.10, 166.03, 165.81, 165.75, 165.06, 159.75, 159.64, 159.49, 159.46, 159.42, 159.39, 132.75, 132.72, 131.97, 131.94, 131.91, 131.86, 131.55, 131.34, 125.42, 109.16, 109.16, 109.08, 109.0, 108.91, 108.86, 108.75, 108.66, 108.41, 93.51, 90.69, 73.89, 73.7, 72.07, 71.59, 71.57, 71.24, 69.62, 69.25, 63.06, 62.86. MS (ESI) calculated for $C_{41}H_{32}O_{21}$, $[M + H]^+$, m/z 861.69, found for $[M + Na]^+$, m/z 883.225.

Sodium 1, 2, 3, 4, 6-Penta-O-(3, 5-di-O-sulfonato-benzoyl)-α,β-D-glucopyranose (5). ^{1}H NMR (D_2O, 400 MHz): 7.38 (d, 2 H, J = 1.92 Hz), 7.36 (d, 2 H, J = 1.96 Hz), 7.32–7.27 (m, 9 H), 7.24 (d, 2 H, J = 1.76 Hz), 6.45 (d, 1 H, J = 7.88 Hz), 6.10 (t, 1 H, J = 9.28 Hz), 5.72 (t, 1 H, J = 8.52 Hz), 5.55 (t, 1 H, J = 8.88 Hz), 4.68 (s, br, 1 H), 4.32 (s, br, 2 H). ^{13}C NMR (D_2O, 100 MHz): 166.19, 165.45, 165.14, 165.01, 164.43, 152.29, 152.0, 131.35, 130.48, 130.32, 120.98, 120.61, 120.54, 120.42, 120.21, 120.11, 93.20, 73.84, 72.48, 71.97, 71.72, 70.09, 63.67. MS (ESI) calculated for $C_{41}H_{22}Na_{11}O_{51}S_{10}$ (sodium sulfates), $[M + Na]^{+1}$, m/z 1904.12 and calculated for $C_{113}H_{214}N_{12}O_{51}S_{10}$ (*n*-hexylammonium sulfates), $[M + (CH_3(CH_2)_5 NH_3)_{12}]^{+2}$, m/z 1438.815, found for $[M + (CH_3(CH_2)_5 NH_3)_{12}]^{+2}$, m/z 1438.707.

References

1. Khanbabaee, K.; Lötzerich, K. Efficient total synthesis of the natural products 2,3,4,6-tetra-*O*-galloyl-D-glucopyranose, 1,2,3,4,6-Penta-*O*-galloyl-β-D-glucopyranose and the unnatural 1,2,3,4,6-Penta-O-galloyl-α-D-glucopyranose. *Tetrahedron* **1997***, 53*, 10725–10732.
2. Al-Horani, R. A.; Ponnusamy, P.; Mehta, A. Y.; Gailani, D.; Desai, U. R. Sulfated pentagalloylglucoside is a potent, allosteric, and selective inhibitor of factor XIa. *J. Med. Chem.* **2013***, 56*, 867−878.

Table S1. UPLC-ESI-MS characterization of SPGG variants components and their corresponding average molecular weight.[a]

Molecule	*Mr*	*P1*	*P2*	*P3*	*P4*	*P5*	*P6*	*P7*
		m/z (proportions)						
		1207 (7 SO_3^-)	*1298 (8 SO_3^-)*	*1388 (9 SO_3^-)*	*1479 (10 SO_3^-)*	*1570 (11 SO_3^-)*	*1660 (12 SO_3^-)*	*1750 (13 SO_3^-)*
β-SPGG-0.5 (4a)	1923	3%	11%	28%	43%	15%	0%	0%
β-SPGG-1 (4b)	1940	4%	11%	23%	31%	23%	8%	0%
β-SPGG-2 (4c)	1962	5%	10%	19%	42%	17%	7%	0%
β-SPGG-4 (4d)	1975	5%	10%	20%	31%	15%	11%	6%
β-SPGG-6 (4e)	1960	8%	13%	20%	27%	18%	19%	6%
β-SPGG-8 (4f)	1982	3%	8%	18%	34%	24%	8%	5%
α-SPGG-8 (4g)	2071	3%	6%	11%	18%	15%	28%	18%
α,β-SPGG-8 (4h)	2090	2%	4%	7%	17%	19%	35%	16%

[a] *UPLC resolution of SPGG variants into seven peaks (p1−p7), which arise from variable sulfation of the PGG scaffold. The detailed compositional profile of these variants was determined using reversed-phase UPLC-ESI-MS analysis, as performed in our earlier work.[2] The profiles indicated the presence of doubly charged molecular ion peaks at 1207, 1297, 1388, 1478, 1569, 1661 and 1750 m/z, which corresponded to hepta-, octa-, nona-, deca-, undeca-, dodeca-, and trideca-sulfated species, respectively . Each of these peaks was a composite of multiple peaks, which implies the presence of several regio-isomers of identical sulfation level.*

Table S2. Hydrolysis of the Chromogenic Substrate S-2366 by Human Factor XIa in the Presence of β-SPGG-8.[a]

Inhibitor	*Conc. (μg/mL)*	K_M *(mM)*	V_{MAX} *(mAU/min)*
β-SPGG-8 (4f)	0	0.24 ± 0.03[b]	76 ± 2
	0.05	0.25 ± 0.03	72 ± 2
	0.5	0.23 ± 0.02	69 ± 2
	5	0.25 ± 0.05	59 ± 3
	15	0.36 ± 0.10	40 ± 2
	30	0.33 ± 0.10	20 ± 2

[a] *K_M and V_{MAX} values of S-2366 substrate hydrolysis by human factor XIa were measured as described under Experimental Procedures. mAU indicates milliabsorbance units.*

[b] *Error represents ± 1 S.E.*

Table S3. Inhibition of Factor XIa by *β*-SPGG-8, *β*-SPGG-2, *β*-SPGG-1, and *β*-SPGG-0.5 in Presence of Increasing Concentrations of UFH (0–500 μM).[a]

SPGG variant	*UFH (uM)*	*FXIa IC_{50} (μg/mL)*	*HS*	*ΔY*
β-SPGG-8 (4f)	***0***	0.16 ± 0.01[b]	1.9 ± 0.3	95 ± 3
	5	0.22 ± 0.01	2.1 ± 0.2	98 ± 3
	50	0.34 ± 0.01	2.5 ± 0.2	96 ± 2
	250	0.60 ± 0.01	2.1 ± 0.2	100 ± 2
	500	1.17 ± 0.04	2.4 ± 0.4	103 ± 3
β-SPGG-2 (4c)	***0***	0.96 ± 0.03	1.6 ± 0.2	98 ± 3
	5	1.11 ± 0.04	1.7 ± 0.2	97 ± 3
	30	3.7 ± 0.1	1.7 ± 0.2	95 ± 3
	100	11.6 ± 2.8	1.3 ± 0.4	100 ± 6
	180	34.1 ± 5.0	2.3 ± 0.5	93 ± 3
	300	86.2 ± 1.8	6.7 ± 1.4	90 ± 4
β-SPGG-1 (4b)	***0***	1.01 ± 0.05	1.4 ± 0.2	93 ± 4
	5	1.97 ± 0.09	3.4 ± 0.5	100 ± 4
	30	4.39 ± 0.23	2.1 ± 0.5	93 ± 5
	100	10.24 ± 0.33	2.0 ± 0.2	96 ± 3
β-SPGG-0.5 (4a)	***0***	1.77 ± 0.05	2.5 ± 0.3	94 ± 3
	5	3.27 ± 0.05	3.4 ± 0.5	92 ± 3
	30	8.95 ± 0.26	4.6 ± 1.5	87 ± 4
	100	19.8 ± 0.84	3.1 ± 0.7	96 ± 5

[a] *The FXIa residual activity % values were obtained following analysis of direct inhibition of human factor XIa in appropriate TrisHCl buffers of pH 7.4 at 37 °C in presence of increasing concentrations of UFH. Inhibition was monitored by spectrophotometric measurement of residual enzyme activity. See details under Experimental Procedures.*

[b] *Errors represent ± 1 S.E.*

Table S4. Salt Dependence Studies of FXIa–DEGR Interactions with β-SPGG-2, UFH, and H8.[a]

Molecules	***[NaCl] (mM)***	K_D ***(μM)***	ΔF_{MAX} ***(%)***
β-SPGG-2	150	0.44 ± 0.10[b]	-16 ± 1
	100	0.31 ± 0.05	-17 ± 1
	50	0.25 ± 0.03	-16± 1
	25	0.11 ± 0.02	-17 ± 1
UFH	150	1.6 ± 0.5	-29 ± 2
	100	1.2 ± 0.2	-34 ± 3
	50	0.6 ± 0.2	-30 ± 2
	25	0.38 ± 0.10	-43 ± 3
H8	150	3.8 ± 0.7	-49 ± 6
	100	3 ± 0.7	-47 ± 7
	50	2 ± 0.1	-46 ± 1
	25	1.5 ± 0.1	-40 ± 2

[a] *Affinity was measured using the decrease in dansyl group fluorescence (λ_{EX} =345 nm and λ_{EM} ~547nm) as a function of ligand concentration in 50 mM TrisHCl buffer, pH 7.4, containing 150,100, 50, or 25 mM NaCl, and 0.1% PEG8000 at 37 °C. See Experimental Section for additional details.*

[b] *Error represents ± 1 SE.*

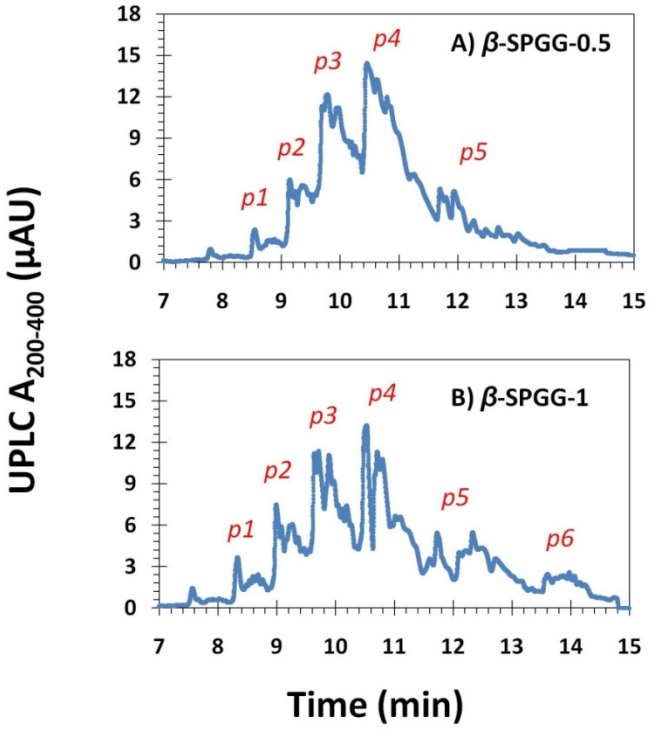

Figure S1. Representative UPLC profiles for *β*-SPGG-0.5 (A) and *β*-SPGG-1 (B). See Experimental part for further details.

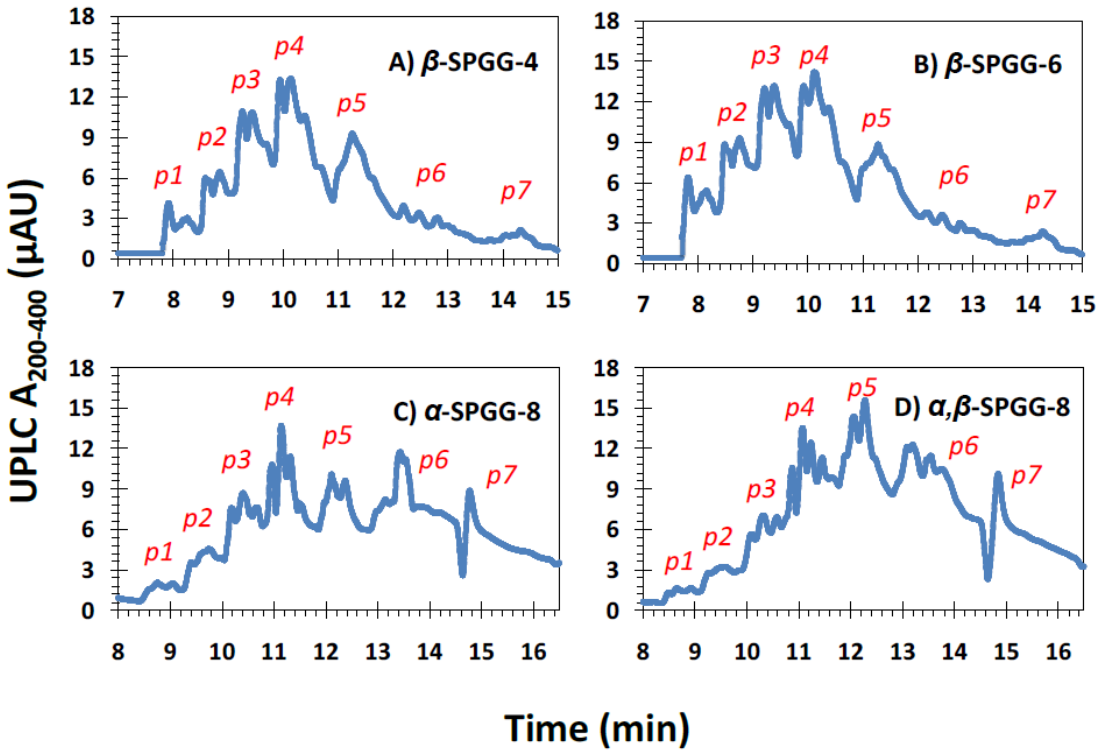

Figure S2. Representative UPLC profiles for *β*-SPGG-4 (A), *β*-SPGG-6 (B), *α*-SPGG-8 (C), *α,β*-SPGG-8 (D). See Experimental part for further details.

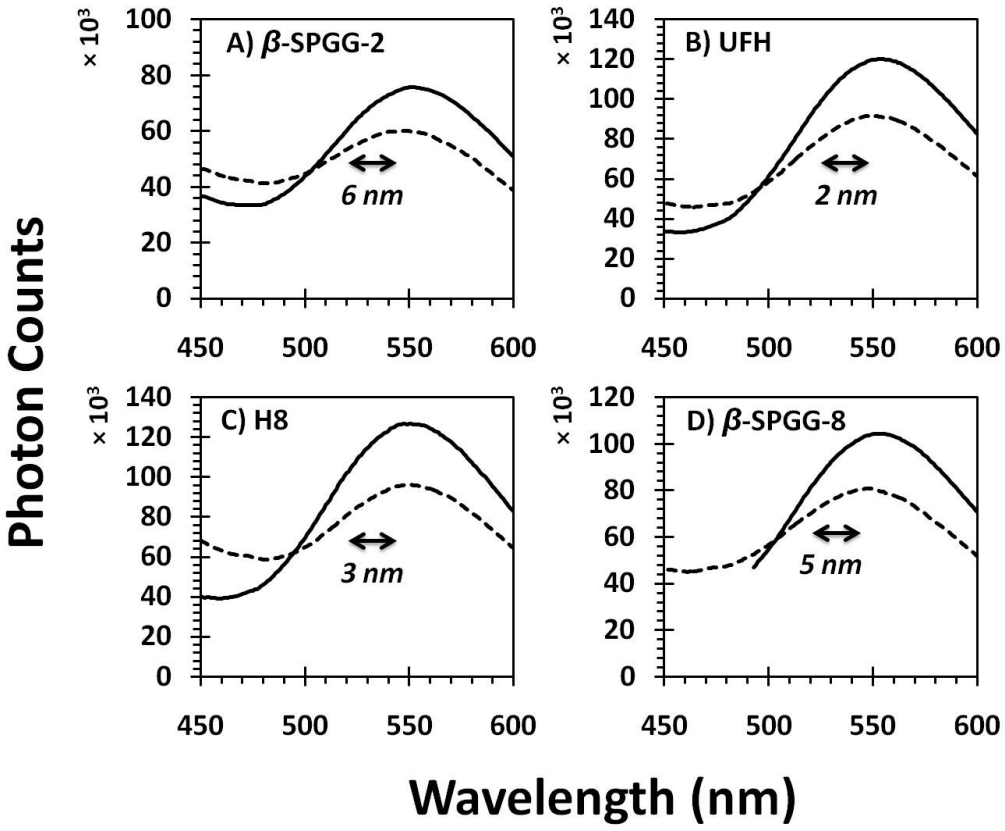

Figure S3. Changes in the fluorescence emission spectra of dansylated FXIa (FXIa–DEGR) (λ_{EX} = 345 nm) induced by the binding of *β*-SPGG-2 (A), UFH (B), H8 (C), and *β*-SPGG-8 (D). Solid lines represent the emission spectra of FXIa–DEGR in buffer. Dash lines represent the emission spectra of saturated complexes of FXIa–DEGR and corresponding ligands. Spectra were recorded in 50 mM TrisHCl buffer of pH 7.4 containing 150 mM NaCl and 0.1% PEG8000 at 37 °C.

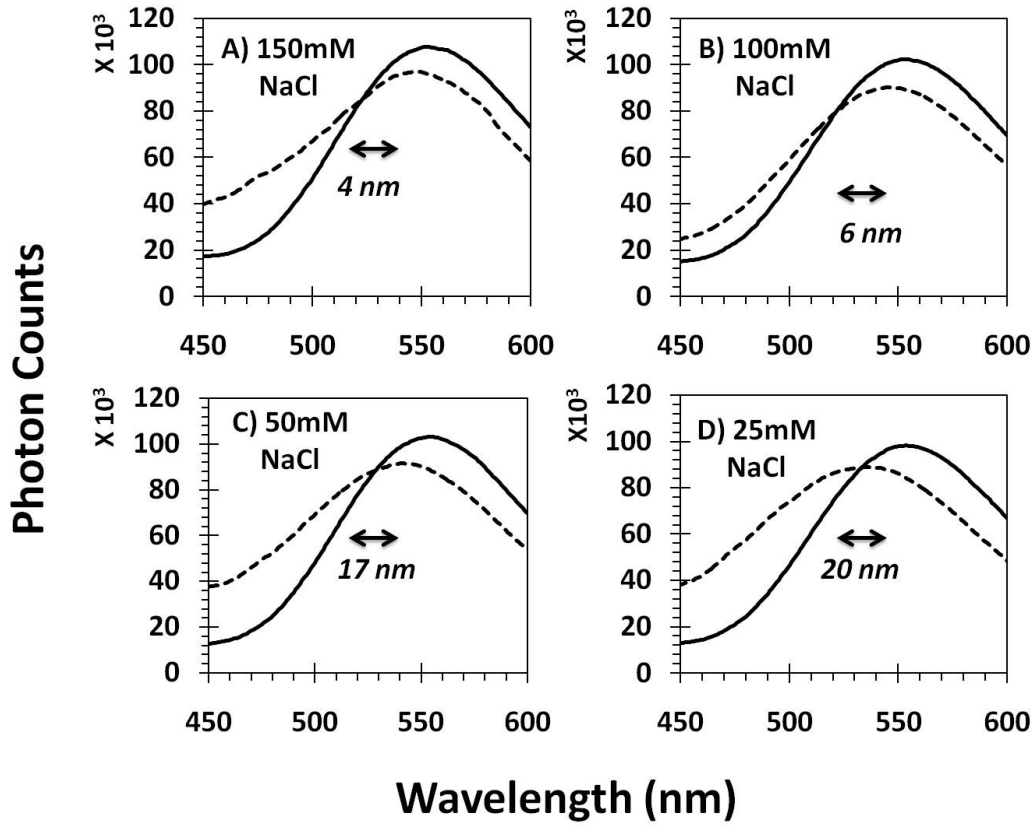

Figure S4. Changes in the fluorescence emission spectrum of dansylated factor XIa (FXIa–DEGR) (λ_{EX} = 345 nm) induced by the binding of β-SPGG-2 at different salt concentrations of 150 (A), 100 (B), 50 (C), and 25 mM (D). Solid lines represent the emission spectra of FXIa–DEGR in buffer. Dash lines represent the emission spectra of saturated complexes of FXIa–DEGR with β-SPGG-2. Spectra were recorded in 50 mM TrisHCl buffer of pH 7.4 containing 25–150 mM NaCl and 0.1% PEG8000 at 37 °C.

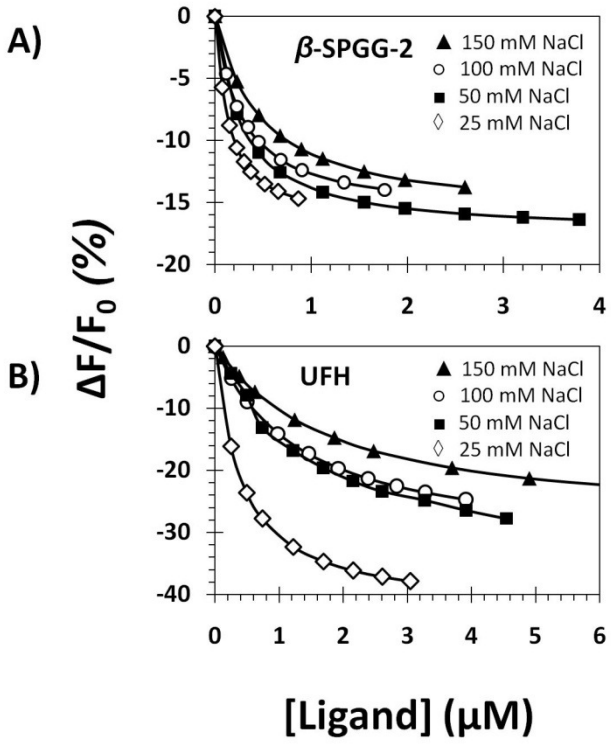

Figure S5. Spectrofluorimetric measurement of the FXIa–DEGR affinity of *β*-SPGG-2 (A) and UFH (B) at pH 7.4 and 37 °C and different salt concentrations of 150 (▲), 100 (○), 50 (■), and 25 mM (◊). The binding of *β*-SPGG-2 or UFH to FXIa–DEGR resulted in a saturable decrease in the dansyl group fluorescence at ~547 nm (λ_{EX} = 345 nm), which was fitted to the quadratic binding Eq. 2 to calculate the observed K_D. Solid lines represent the nonlinear regressional fit. Experiments were performed in 50 mM TrisHCl buffer of pH 7.4 containing 150 mM NaCl and 0.1% PEG8000. See Experimental procedures for details.