Supporting Information

Synthesis of FmocSal

2- Methoxy 5-(N-9-fluorenylmethoxycarbonyl)-aminobenzoic acid (4a). To the solution of methyl 2-methoxy-5-nitrobenzate (0.55 g, 2.59 mmol) in methanol (10 mL) there was added 2 N LiOH (2.6 mL, 5.4 mmol). The resulting solution was stirred at room temperature until no starting material remained (cal. 3 h). The solution was then acidified to pH = 4~5. Pd-C (0.2 g) was introduced, the resulting mixture was charged hydrogen by a balloon, and stirred at room temperature overnight. The catalyst was filtered and methanol was removed under reduced pressure. To the resulting aqueous solution there was added acetone (3 mL), NaHCO₃ (218 mg, 2.59 mmol mmol), and Fmoc-OSu (N-(9-Fluorenylmethoxycarbonyloxy)succinimide) (875 mg, 2.59 mmol). The resulting mixture was stirred at room temperature overnight, acetone was then removed. The aqueous solution was neutralized with 1 N HCl to pH = 4, and extracted with ethyl acetate (4 x 10 mL), the combined organic layers were washed with water, brine, dried over MgSO₄, and the solvent was removed under reduced pressure. Recrystallization (ethyl acetate, hexane) gave the pure product (0.89 g, 88% in three steps). H NMR (CDCl₃, 500 MHz): δ 10.74 (br, 1 H), 8.00 (br, 1 H), 7.88 (d, J = 2.8 Hz, 1 H), 7.79 (d, J = 7.5 Hz, 2 H), 7.63 (d, j = 7.1 Hz, 2 H), 7.43 (t, J = 7.4 Hz, 2 H), 7.34 (t, J = 7.4 Hz, 2 H), 7.05 (d, J = 9.6 Hz, 1 H), 6.75 (br, 1 H), 4.55 (d, J = 6.7 Hz, 2 H), 4.29 (t, J = 6.7 Hz, 1 H), 4.08 (s, 3 H); ¹³C NMR (CDCl₃, 125 MHz): δ 164.9, 154.3, 153.7, 143.8, 141.6, 132.7, 128.1, 127.4, 126.0, 125.2, 123.8, 120.3, 118.0, 112.8, 67.3, 57.3, 47.3; IR: 3411.8, 1657.3 cm⁻¹; HRMS for C₂₃H₁₉NO₅Na [M+Na]⁺ m/z: 412.1161 (calcd), 412.1197 (found).

Synthesis of SalAA Compound 1

A mixture of Boc-Lys(Z)-OSu (9; 47.8 g, 100 mmol) and methyl 5-amino-2-methoxybenzoate (10; 18.1 g, 100 mmol) in 500 mL of anhydrous CHCl₃ was stirred at room temperature under Ar and, after 30 minutes, a clear orange solution was observed. After 60 h, the reaction mixture was concentrated under reduced pressure to give a brown syrup that was partitioned between EtOAc and water. The aqueous layer was extracted twice more with EtOAc and the combined EtOAc layers were washed four times with water, once with 10% aqueous citric acid, twice with water, 3 times (carefully) with saturated aqueous NaHCO₃, and once with brine. The EtOAc layer was then dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to afford compound 11 (53.5 g, 98%); MS-ESI m/z [M+H]⁺: 544.3 (calcd), 544.3 (found), [M+Na]⁺: 566.3 (calcd), 566.3 (found).
A solution of 11 (26.7 g, 49.1 mmol) in a mixture of 490 mL of THF/MeOH (3:2) was treated with 98 mL of 2.0 M aqueous LiOH (196 mmol) and the resultant mixture was stirred at room temperature for 18 h. The reaction mixture was cooled in an ice bath and adjusted to pH 7.0 with 196 mL of cold 1.0 M aqueous HCl. The neutralized reaction was partially concentrated under reduced pressure to give an aqueous slurry that was extracted with EtOAc until TLC showed the extraction was complete. The combined EtOAc extracts were dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure to afford compound 12 (25.7 g, 99%) as a beige solid;

MS-ESI m/z [M+H]$^+$: 530.3 (calcd), 530.4 (found), [M+Na]$^+$: 552.2 (calcd), 552.3 (found).

Compound 11 (26.7 g, 49.1 mmol) was added to a 1 L round bottom flask that was equipped with a ground glass stopper (secured by a Keck clamp) and treated with 385 mL of cold CH$_2$Cl$_2$/TFA (9:1) at 5 °C (CAUTION: Gas pressure buildup!). The resultant brick red solution was allowed to slowly warm to room temperature while venting several times to relieve gas pressure. After 24 h, the reaction mixture was diluted with twice its volume of CH$_3$CN and concentrated under reduced pressure without heating to a brown syrup. This syrup was dissolved in EtOAc and extracted (carefully) three times with saturated aqueous NaHCO$_3$. The pH of the combined aqueous extracts was adjusted to pH 8.0 with solid NaHCO$_3$ and extracted twice with EtOAc. The combined EtOAc layers were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to furnish compound 13 (24.8 g, 91%);

MS-ESI m/z [M+H]$^+$: 444.2 (calcd), 444.2 (found), [M+Na]$^+$: 466.2 (calcd), 466.3 (found).

Compounds 12 (1.06 g, 2.00 mmol) and 13 (1.12 g, 2.00 mmol) were dissolved in 60 mL of anhydrous CHCl$_3$ and treated with 1-hydroxybenzotriazole hydrate (0.54 g, 4.0 mmol), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (0.46, 2.4 mmol), and N-methylmorpholine (0.33 mL, 3.0 mmol). The resulting slurry was stirred at room temperature under an Ar atmosphere. After 24 h the reaction mixture, which appeared as an orange solution, was diluted with CH$_2$Cl$_2$ and extracted twice with water, twice with saturated aqueous NaHCO$_3$ and once with brine. The organic layer was dried over Na$_2$SO$_4$, filtered and concentrated in vacuo to afford a brown crusty foam that was purified by to flash chromatography on silica gel (50-75% EtOAc in hexanes) to afford 14 (1.71 g, 89%) as a white foam;

MS-ESI m/z [M+H]$^+$: 955.5 (calcd), 955.4 (found), [M+Na]$^+$: 977.4 (calcd), 977.8 (found).
A solution of 14 (0.33 g, 0.346 mmol) in 3.5 mL of THF/MeOH (3:2) was treated with aqueous 2.0 M LiOH (0.70 mL, 1.4 mmol) and stirred at room temperature for 8 h. The reaction mixture was cooled in an ice bath and adjusted to pH 7.0 with 1.4 mL of cold aqueous 1.0 M HCl. The neutralized reaction was partially concentrated in vacuo to give an aqueous slurry that was extracted with EtOAc until TLC showed the extraction was complete. The combined EtOAc layers were dried over Na$_2$SO$_4$, filtered and concentrated to afford compound 15 (0.321 g, 99%); MS-ESI m/z [M+H]$^+$: 941.4 (calcd), 941.6 (found), [M+Na]$^+$: 963.4 (calcd), 963.6 (found).

A mixture of 15 (0.798 g, 0.849 mmol), 1-hydroxybenzotriazole hydrate (0.224 g, 1.70 mmol), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (0.278 g, 1.70 mmol), and NH$_4$Cl (0.099 g, 1.7 mmol) was dissolved in 8.0 mL of DMF. DIEA (0.59 mL, 3.4 mmol) was added and the reaction mixture stirred at room temperature under an Ar atmosphere for 8 h. The reaction mixture was poured into 5 mL of 1.0 M aqueous HCl and extracted 3 times with EtOAc. The combined EtOAc extracts were washed with H$_2$O, brine, dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure to yield compound 16 (0.729 g, 91%), which was used without further purification in the next reaction to prepare 17; MS-ESI m/z [M+H]$^+$: 940.5 (calcd), 940.5 (found), [M+Na]$^+$: 962.4 (calcd), 962.6 (found).

Compound 16 (0.900 g, 0.957 mmol) was stirred at room temperature in 4.5 mL of CH$_2$Cl$_2$/TFA (3:1) for 1.5 h. Et$_2$O was added and the resulting precipitate was either isolated by filtration or centrifuged and the solvent decanted. The resultant solid was triturated with Et$_2$O and dried to provide the mono trifluoroacetate salt of 17 (0.750 g, 82%) as a white powder; MS-ESI m/z [M+H]$^+$: 840.4 (calcd), 840.4 (found), [M+Na]$^+$: 862.4 (calcd), 862.4 (found). A portion of this material was free based from its TFA salt by partitioning between saturated aqueous NaHCO$_3$ and EtOAc and used in the next step.
A mixture of 15 (0.321, 0.341 mmol) and 17 (0.286 g free base, 0.341 mmol) was dissolved in 15 mL of anhydrous CHCl₃, 1-Hydroxybenzotriazole (0.092 g, 0.681 mmol), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (0.079 g, 0.509 mmol) and N-methylmorpholine (0.056 mL, 0.509 mmol) were added and the resulting slurry was stirred at room temperature under Ar for 40 h. The reaction mixture, which appeared as a yellow solution, was diluted with CH₂Cl₂ and extracted twice with water, twice with saturated aqueous NaHCO₃, once with 10% aqueous citric acid and twice with brine. The CH₂Cl₂ layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure to afford 0.607 g of beige wax that was purified by flash chromatography on silica gel (0-3% MeOH in CH₂Cl₂) to provide 18 (0.411 g, 68%) as a beige solid; MS-ESI m/z [M+H]+: 1762.8 (calcd), 1762.9 (found), [M+Na]+: 1784.8 (calcd), 1784.7 (found).

Compound 18 (0.411 g, 0.233 mmol) was introduced to a 100 mL round bottom flask that was equipped with a ground glass stopper (secured by a Keck clamp) and treated with 5 mL of CH₂Cl₂/TFA (9:1) at 5 °C (CAUTION: Gas pressure buildup!). The resulting solution was allowed to warm to room temperature over 24 h while venting when needed to relieve gas pressure. The reaction mixture was diluted with CH₃CN and concentrated under reduced pressure without heating to a brown syrup. This residue was dissolved in CH₂Cl₂ and extracted three times with saturated aqueous NaHCO₃. The combined aqueous layers were extracted twice with CH₂Cl₂ and the combined CH₂Cl₂ extracts were dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a beige amorphous solid. This material purified by flash chromatography on silica gel (1-10% MeOH in CH₂Cl₂) to provide 19 (0.151 g, 39%) as a white solid; MS-ESI m/z [M+H]+: 1662.8 (calcd), 1662.7 (found), [M+Na]+: 1684.7 (calcd), 1684.8 (found).

A solution of 19 (2.09 g, 1.26 mmol) in 84 mL of MeOH in a Parr hydrogenation bottle was treated with 1.0 M aqueous HCl (6.30 mL, 6.30 mmol) and Ar was bubbled through the reaction solution for 15 minutes. 10% Pd/C (0.835 mg) was added and the reaction mixture was placed under hydrogen pressure (70 psig) on a Parr hydrogenator for 2 h at 25 °C. The reaction mixture was filtered through a pad of Celite and a 1-micron membrane filter and the residual methanol was driven off by co-distillation with acetonitrile (100 mL). The resulting product was slurried in MTBE (40 mL), filtered and dried under reduced pressure to furnish 1 as a white solid (1.50 g, 91%); MS-ESI m/z [M+H]+: 1126.6 (calcd), 1126.7 (found), [M+Na]+: 1148.5 (calcd), 1148.6 (found).
**Synthesis of SalAA Compound 2**

Compound 14 (1.00 g, 1.047 mmol) was added to a 100 mL round bottom flask that was equipped with a ground glass stopper (secured by a Keck clamp) and treated with 5.7 mL of CH₂Cl₂/TFA (9:1) at 10 °C (CAUTION: Gas pressure buildup!). The resulting solution was allowed to warm to room temperature over 1.5 h while venting when needed to relieve gas pressure. The reaction mixture was cooled to 5 °C and diluted with 20 mL of MTBE and stirred for 1 h. The resulting white precipitate was isolated by filtration, rinsed 3 times with 20 mL portions of MTBE, dissolved in CH₃CN and concentrated under reduced pressure to give a tacky glass. This material was partitioned between CH₂Cl₂ (20 mL) and saturated aqueous NaHCO₃ (20 mL). The CH₂Cl₂ layer was extracted twice more with 20 mL portions of saturated aqueous NaHCO₃. The combined aqueous layers were extracted twice with 20 mL portions of CH₂Cl₂ and the combined CH₂Cl₂ extracts were washed twice with 20 mL portions of brine, dried (Na₂SO₄) and concentrated under reduced pressure to afford compound 20 (0.84 g, 94%) as a white foam; MS-ESI m/z [M+H]⁺: 855.4 (calcd), 855.5 (found), [M+Na]⁺: 877.4 (calcd), 877.5 (found).

Compounds 15 (1.03 g, 1.09 mmol) and 20 (0.840 g, 0.983 mmol) were combined with 1-hydroxybenzotriazole hydrate (0.266 g, 1.18 mmol) and N-methylmorpholine (0.162 mL, 1.47 mmol) in anhydrous CH₂Cl₂ (20 mL). The resulting solution was treated with N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (0.226 g, 1.97 mmol) and stirred at room temperature under Ar for 15 h. The reaction mixture was diluted with 20 mL of CH₂Cl₂ and extracted 3 times with 20 mL portions of H₂O. The combined aqueous layers were extracted twice with 20 mL of CH₂Cl₂ and the combined CH₂Cl₂ extracts were washed twice with 20 mL of 10% aqueous citric acid, twice with 20 mL of saturated aqueous NaHCO₃, twice with 20 mL of brine, dried (Na₂SO₄), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel eluting with 0-5% MeOH in CH₂Cl₂/MeOH to give compound 21 (1.05 g, 60%) as a white foam; MS-ESI m/z [M+H]⁺: 1777.8 (calcd), 1777.9 (found), [M+Na]⁺: 1799.8 (calcd), 1798.6 (found).

Compound 21 (0.570 g, 0.321 mmol) was dissolved in 3.4 mL of THF/MeOH (3:2) and treated dropwise over 2 min with 2 M aqueous LiOH (0.65 mL, 1.30 mmol). The reaction mixture was placed under Ar and stirred at room temperature for 9 h, diluted with 10% aqueous citric acid (60 mL) and extracted 3 times with 20 mL portions of CH₂Cl₂. The combined CH₂Cl₂ layers were washed twice with 20 mL portions of brine, dried (Na₂SO₄), filtered and concentrated under reduced pressure to furnish 22 (0.56 g, 99%) as an off-white solid; 1763.8 (calcd), 1763.2 (found), [M+Na]⁺: 1785.8 (calcd), 1785.0 (found).
Compound 22 (0.580 g, 0.329 mmol) was introduced to a 25 mL round bottom flask that was equipped with a ground glass stopper (secured by a Keck clamp) and treated with 3.0 mL of TFA/CH₂Cl₂ (1:2) at room temperature (CAUTION: Gas pressure buildup!). The resulting solution was stirred for 5 h at room temperature, venting when necessary to relieve gas pressure. The reaction mixture was concentrated under reduced pressure and the residue was triturated twice with anhydrous Et₂O. The resulting solid was dissolved in CH₂Cl₂ (25 mL), treated with saturated aqueous NaHCO₃ (10 mL) and stirred at room temperature for 1 h. A precipitate formed which was isolated by filtration and washed 3 times with CH₂Cl₂ and dried under reduced pressure to give 23 (0.686 g, 124%; wet?) as a white solid; MS-ESI m/z [M+H]⁺: 1663.7 (calcd), 1663.2 (found), [M+Na]⁺: 1685.7 (calcd), 1685.0 (found).

A solution of 23 (0.686 g, presumed 0.329 mmol) in 100 mL of MeOH in a Parr hydrogenation bottle was treated with 1.0 M aqueous HCl (1.97 mL, 1.97 mmol) and Ar was bubbled through the reaction solution for 15 minutes. 10% Pd/C (0.137 mg) was added and the reaction mixture was placed under hydrogen pressure (60 psig) on a Parr hydrogenator for 16 h at 25 °C. The reaction mixture was filtered through a pad of filter aid (Hyflo Super-Cel) and the filtrate was concentrated under reduced pressure. The residue was purified by reversed phase HPLC on a 41.4×250 mm Varian Pursuit 5 Diphenyl column using a gradient over 20 min of 20-25% CH₃CN in H₂O containing 0.1% TFA at a flow rate of 60 mL/min. The fractions containing the desired product were combined and lyophilized to provide the TFA salt of 2 (0.180 g, 32%) as a white solid; MS-ESI m/z [M+H]⁺: 1127.6 (calcd) 1127.7 (found), [M+Na]⁺: 1149.6 (calcd), 1149.8 (found).

Synthesis of SalAA Compound 3

Compound 21 (0.500 g, 0.281 mmol) was introduced to a 10 mL round bottom flask that was equipped with a ground glass stopper (secured by a Keck clamp) and treated with 1.7 mL of TFA/CH₂Cl₂ (1:2) at 5 °C (CAUTION: Gas pressure buildup!). The resulting solution was magnetically stirred and allowed to warm to room temperature over 3 h while venting when needed to relieve gas pressure. The reaction mixture was concentrated under reduced pressure and the residue was triturated 3 times with MTBE. The resulting solid was dissolved in CH₂Cl₂ (30 mL), treated with saturated aqueous NaHCO₃ (20 mL). The CH₂Cl₂ layer was extracted twice with 20 mL portions of saturated aqueous NaHCO₃. The combined saturated aqueous NaHCO₃ layers were extracted twice with 30 mL of CH₂Cl₂. The combined CH₂Cl₂ layers were washed with brine, dried (Na₂SO₄) and concentrated under reduced pressure to yield 24 (0.431 g, 91%) as a white solid; MS-ESI m/z [M+H]⁺: 1677.8 (calcd), 1677.3 (found), [M+Na]⁺: 1699.7 (calcd), 1699.3 (found).
A solution of 24 (0.140 g, 0.0834 mmol) in 30 mL of MeOH in a Parr hydrogenation bottle was treated with 1.0 M aqueous HCl (0.42 mL, 0.42 mmol) and Ar was bubbled through the reaction solution for 15 minutes. 10% Pd/C (0.072 mg) was added and the reaction mixture was placed under hydrogen pressure (62 psig) on a Parr hydrogenator for 24 h at 25 °C. The reaction mixture was filtered through a pad of filter aid (Hyflo Super-Cel) and the filtrate was concentrated under reduced pressure. The residue was purified by reversed phase HPLC on a 41.4×250 mm Varian Pursuit 5 Diphenyl column using a gradient over 20 min of 23-28% CH₃CN in H₂O containing 0.1% TFA at a flow rate of 60 mL/min. The fractions containing the desired product were combined and lyophilized to provide the TFA salt of 3 (0.042 g, 29%) as a white solid; MS-ESI m/z [M+H]+: 1141.6 (calcd), 1141.7 (found), [M+Na]+: 1163.6 (calcd), 1163.7 (found).

Solid phase synthesis procedures for foldamers 4-8.

Foldamers 5-8 were synthesized by solid phase methods on Fmoc-PAL-PEG-PS resin (Applied Biosystems) from commercially available Fmoc-5-amino-2-methoxybenzoic acid (Fmoc-Sal), Fmoc-L-Lys(Boc)-OH and Fmoc-D-Cit-OH, which were used without further purification. Foldamer 4 was similarly synthesized on preloaded Fmoc-β-alanine Wang resin (Novabiochem). The starting resin was washed twice with DMF and Fmoc deprotection was accomplished with two 15 minute treatments of 20% piperidine in DMF, followed by five further DMF washes. The coupling of Fmoc-Sal to the resin-bound amine was carried out in DMF using 2.2 molar equivalents of Fmoc-Sal and DIEA and 2.0 molar equivalents of HATU and HOAt. The resin was subsequently washed twice with DMF and deprotected with two 15 minute treatments of 20% piperidine in DMF, followed by 5 further DMF washes. The coupling of the Fmoc-amino acid to the resin-bound Sal amino group was performed with 5.5 molar equivalents of the Fmoc-amino acid and DIEA and 5.0 molar equivalents of HATU and HOAt in DMF. This sequence of wash, deprotect, wash, couple, wash, deprotect, wash, couple was repeated until the fully protected resin-bound SalAA foldamer was assembled. The final resin was washed twice with DMF, and deprotected using two 15 minute treatments of 20% piperidine in DMF, followed by 5 DMF washes, 2 isopropanol washes, 3 dichloromethane washes and the resulting resin dried thoroughly. Cleavage from the resin and concomitant Boc deprotection was achieved with 95% trifluoroacetic acid with 5% triisopropylsilane. An extended reaction time (15 h) was required to obtain high yields for the coupling of Fmoc-amino acids to the N-terminus of resin-bound Sal. In contrast, the coupling of Fmoc-Sal to the N-terminus of a resin bound amino acid typically required 5 hours. SalAA foldmers 2-8 were purified to homogeneity by reversed phase HPLC using a gradient of water/acetonitrile containing 0.1% TFA and a 41.4×250 mm Varian Pursuit 5 Diphenyl column and subsequently lyophilized. The purity and structure of the compounds was verified by analytical HPLC and the products had the correct masses based on electrospray mass spectrometry (Applied Biosystems API 2000). 4, m/z (MH+): 1198.3, (MNa+): 1220.3, HPLC (UV TWC) purity: 96.1%; 5, m/z (MH+): 998.2, (MNa+): 1020.0, HPLC (UV TWC) purity: 99.4%; 6, m/z (MH+): 1040.4, (MNa+): 1062.2, HPLC (UV TWC) purity: 99.9%; 7, m/z (MH+): 1242.6, (MNa+): 1264.4, HPLC (UV TWC) purity: 98.6%; 8, m/z (MH+): 849.8, (MNa+): 871.5, HPLC (UV TWC) purity: 99.0%.

Equilibrium Analytical Ultracentrifugation

Equilibrium analytical ultracentrifugation was used to characterize the oligomerization state and affinity for (Lys-Sal)-CONH₂ (1), using a Beckman XL-I analytical ultracentrifuge at 25 °C. 250 μM (Lys-Sal)-CONH₂ has been centrifuged at respectively 30, 35, 40, 45, 48 KRPM and the concentration distributions vs. rotor diameter were collected at 318 nm. Data obtained were globally fitted by nonlinear least-squares curve by IGOR Pro (Wavemetrics) as previously described. The solvent density (1.006 g/mL) was calculated using program Sednterp, and the partial specific volume was estimated as 0.8 mL/g. The aqueous solution molar extinction coefficients for foldamer at 318 nm (9900 M⁻¹ cm⁻¹) was calculated based on UV measurement. All these values were kept constant during global fitting. The fitting suggests a strong dimer-hexamer equilibrium; other equilibrium models failed to improve the quality of the fit.

Figure S1. AUC of Sal-AA foldamer 1, (Lys-Sal)-CONH₂.
Figure S2. UV-vis titration of H$_2$N-(Lys-Sal)$_3$-CONH$_2$ in Tris buffer pH 7.4. Conditions were as described in Figure 2.

Table S1. Summary of ITC and UV-vis $pK_{\text{hex-mon}}$, $K_{\text{hex-mon}}$, and $P_{\text{mut}}$ calculated values from fits.
were then expressed as a plot of Heat\textsubscript{obs} which is contributed by the heat exchange from hexamer-monomer disassociation (\(\Delta H_{\text{hex}}\)) plus that from the dimer (\(\Delta H_{\text{dim}}\)).

\[
\text{Heat}_{\text{obs}} = (\Delta H_{\text{hex}} \times 6[M_{\text{initial}}])/(K_{\text{dim}}^m K_{\text{hex}})/[T_{\text{final}}] - \Delta H_{\text{hex}} \times 6[M_{\text{final}}]/(K_{\text{dim}}^m K_{\text{hex}})/[T_{\text{final}}] + (\Delta H_{\text{dim}} \times 2[M_{\text{initial}}]^2)/K_{\text{dim}}/[T_{\text{initial}}] - \Delta H_{\text{dim}} \times 2[M_{\text{final}}]^2/K_{\text{dim}}/[T_{\text{final}}] \quad \text{Eqn. 1C}
\]

in which the subscripts for T and M refer to the concentration in stock solution versus the concentration in the cell after a given number of injections. The terms \(\Delta H_{\text{hex}} \times 6[M_{\text{initial}}]/(K_{\text{dim}}^m K_{\text{hex}})/[T_{\text{initial}}]\) and \(\Delta H_{\text{dim}} \times 2[M_{\text{initial}}]^2/K_{\text{dim}}/[T_{\text{initial}}]\) are constants throughout a given titration, as they depend only on the stock solution concentration and other constants. Therefore Equation 1C can be rewritten as:

\[
\text{Heat}_{\text{obs}} = - \Delta H_{\text{hex}} \times 6[M_{\text{final}}]/(K_{\text{dim}}^m K_{\text{hex}})/[T_{\text{final}}] - \Delta H_{\text{dim}} \times 2[M_{\text{final}}]^2/K_{\text{dim}}/[T_{\text{final}}] + C \quad \text{Eqn. 1D}
\]

in which \(K_{\text{dim}}, K_{\text{hex}}, \Delta H_{\text{hex}}\) and \(\Delta H_{\text{dim}}\) are dependent variables that were obtained by non-linear least squares fitting to the equation. The values obtained for 1 were: \(\Delta H_{\text{hex}} = 14100 \pm 800, \Delta H_{\text{dim}} = 7800 \pm 980; pK_{\text{dim}} = 2.97 \pm 0.02; pK_{\text{hex}} = 9.80 \pm 0.07.\)

For compounds other than 1 it was not possible to uniquely define all four parameters, probably because the assembly was even more cooperative than for 1 (i.e., the amount of the intermediate dimer was minimal). For these compounds a cooperative monomer-hexamer scheme provided a good fit to the data according to Equation 2C.

\[
[T] = [M] + 6[H] \quad \text{Eqn. 2A}
\]

\[
[T] = [M] + 6[M]^6/(K_0) \quad \text{Eqn. 2B}
\]

\[
\text{MRE}_{\text{obs}} = \text{MRE}_{\text{hex}} \times 6[M]^6/(K_0)/[T] + \text{MRE}_{\text{mon}} \times [M]/[T] + C \quad \text{Eqn. 2C}
\]
in which $K_{\text{hex-mon}}$ refers to the equilibrium constant for the dissociation of a hexamer to a monomer.

The stoichiometry of the interaction between compound 1 and fondaparinux was also determined by ITC. Fondaparinux sodium, Arixtra® (GlaxoSmithKline), injections were provided as single-dose, prefilled syringes. Each syringe contained a sterile solution of 2.5 mg of fondaparinux sodium in 0.5 mL of an isotonic solution of NaCl and water. Fondaparinux was titrated into compound 1 at different concentrations: 150 μM, 105 μM, and 75 μM fondaparinux was titrated into 50 μM, 35 μM, and 25 μM compound 1, respectively.

**aPTT:**
Activated partial thromboplastin time was measured in human plasma on an ACL Elite using synthasil and CaCl₂ to induce clotting as per manufactures instructions (Beckman Coulter). UFH, 0.5 units/ml, was added to citrated plasma to increase clotting time five fold. UFH reversal was measured following addition of serially diluted compound. EC₅₀ was calculated as the amount of compound needed to return clotting times to 50% that of normal.

**REFERENCES**

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