Supporting Information for:

Self-assembled protein-aromatic foldamer complexes with 2:3 and 2:2:1 stoichiometries

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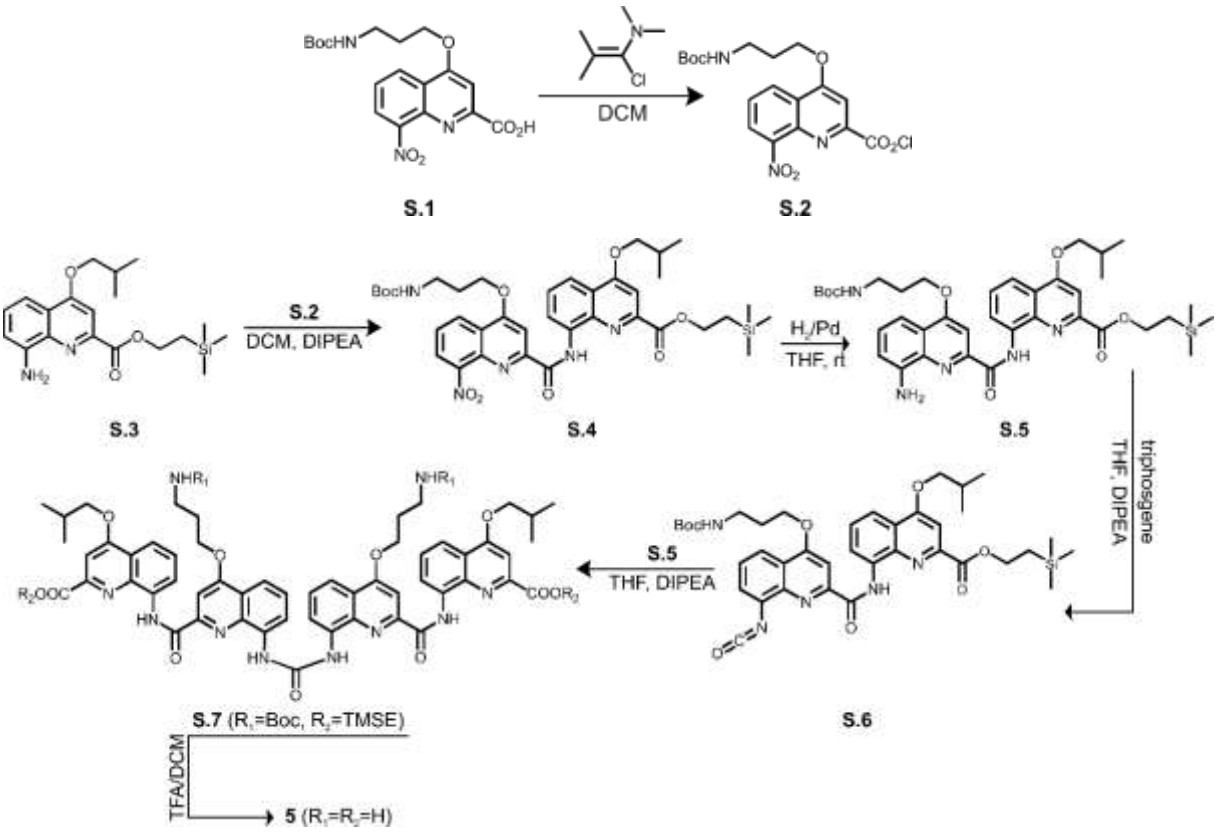
1. Synthetic Schemes



Scheme S1. Synthesis of compound **5**.

2. Experimental section

Spectroscopy of protein complexes. The HCAII used is the recombinant enzyme expressed and purified according to Reference 1. NMR and circular dichroism studies of HCA complexes were carried out as described in Reference 1.

General analytical procedures and materials. Unless otherwise noted, materials were obtained from commercial suppliers and use without further purification. Low loading Wang resin was purchased from Novabiochem. Ghosez reagent was purchased from Sigma Aldrich. N,N-diisopropylethylamine (DIPEA) was distilled over calcium hydride. All organic solvents were synthesis grade. Dry THF and DCM for solution phase synthesis and solid phase synthesis were dispensed from a solvent purification system equipped with packed dry neutral alumina columns. HPLC-grade acetonitrile and MilliQ water were used for RP-HPLC analyses and purifications. ^{1}H NMR spectra of new organic compounds were recorded at 300 MHz, and chemical shifts are reported in ppm and are calibrated against residual solvent signals of $CDCl_3$ (δ 7.26) or DMSO-d_6 (δ 2.50).Coupling constants are reported in Hz. ^{13}C NMR spectra were recorded at 75 MHz, and chemical shifts are reported in ppm and are calibrated against residual solvent signals of $CDCl_3$ (δ 77.16).Silica gel chromatography was performed using Merck Kieselgel Si 60. RP-HPLC analyses were performed on an analytical system using a RP-18 column (4.6 × 100 mm, 5 µm). The mobile phase was composed of 0.1% (vol/vol) TFA-H_2O (Solvent A) and 0.1% (vol/vol) TFA-CH_3CN (Solvent B), unless otherwise noted. Monitoring by UV detection was carried out at 214, 254 and 300 nm using a diode-array detector. Semi-preparative purification of foldamers was performed on a semi-preparative HPLC system using a C18 column (10 mm × 250 mm, 5 µm). Monitoring by UV detection was

carried out at 214 using a diode-array detector. Preparative recycling GPC (gel permeation chromatography) was performed on a LC-9130G NEXT equipped with two columns (20*600 mm): a JAIGEL 2.5H and a JAIGEL 3H (Japan Analytical Industry) at a flow rate of 7 mL/min with a mobile phase composed of 1% (vol/vol) ethanol in chloroform (HPLC grade). High resolution electrospray ionization time of flight (ESI-TOF) mass spectra were measured in the positive ion mode.

Compound 2 was prepared according to previously reported solid phase synthesis protocols,[2] and obtained in 80% purity. Purification by RP-HPLC: Gradient 40 to 80% Solvent B in 15min. Purified Yield: 33%. ^{1}H NMR (300MHz, DMSO-d_6): δ 1.13 (m, 6H), 1.40-1.48 (m, 2H), 1.59-1.63 (m, 2H), 2.29-2.35 (m, 2H), 2.40-2.49 (m, 1H), 2.75-2.81 (m, 1H), 2.88-2.98 (m, 1H), 3.20-3.27 (m, 2H), 3.91 (t, *J*=6.2, 2H), 4.19-4.26 (m, 1H), 4.34-4.39 (m, 1H), 4.45 (d, *J*=5.7, 2H), 4.51-4.64 (m, 2H), 4.78-4.98 (m, 2H), 6.53-6.56 (m, 2H), 6.77-6.80 (m, 1H), 6.82 (s, 1H), 6.87-6.89 (m, 2H), 7.18-7.29 (m, 3H), 7.43-7.48 (m, 3H), 7.58-7.63 (m, 2H), 7.72-7.96 (m, 11H), 8.02-8.04 (m, 2H), 8.43 (dd, *J*=7.6 *J*=1.3, 1H), 8.51 (s, 1H), 8.86 (dd, *J*=7.5 *J*=1.1, 1H), 9.18 (t, *J*=5.9, 1H), 11.57 (s, 1H), 11.67 (s, 1H), 11.76 (s, 1H), 12.22 (s, 1H), 12.29 (bs, 1H), 13.42 (bs, 1H). HRMS (ESI) calculated for $[C_{68}H_{65}N_{12}O_{16}S]^+$ 1337.4357; found 1337.4450

Compound 3 was prepared according to previously reported solid phase synthesis protocols,[2] and obtained in 95% purity. Compound was purified through HPLC purification; Gradient 30 to 80% Solvent B in 25min. Purified Yield: 42%. ^{1}H NMR (300MHz, DMSO-d_6): δ 1.29 (t, J=6.8, 6H), 2.27-2.31 (m, 2H), 2.40-2.46 (m, 1H), 2.52-2.60 (m, 4H), 2.74-2.78 (m, 2H), 2.84 (s, 3H), 2.91-2.98 (m, 2H), 3.06-3.13 (m, 2H), 3.56 (d, *J*=15.3, 1H), 3.73 (d, *J*=15.3, 1H),4.25-4.41 (m, 2H), 4.56-4.65 (m, 3H), 4.77-4.83 (m, 1H), 6.53 (s, 1H), 6.58 (bs, 1H), 6.76 (s, 1H), 7.23 (s, 1H), 7.35-7.44 (m, 2H), 7.63 (dd, *J*=8.0 *J*=8.0, 1H), 7.69-7.74 (m, 2H), 7.82-7.91 (m, 6H), 8,42 (dd, *J*=7.6 *J*=1.1, 1H), 8.89 (dd, *J*=7.7 *J*=1.3 , 1H), 9.90 (s, 1H), 11.64 (s, 1H), 11.69 (s, 1H), 12.14 (s, 1H). HRMS (ESI) calculated for $[C_{56}H_{56}N_9O_{15}]^+$ 1094.3890; found 1094.3923.

Compound 4 was prepared as previously described.[3]

Compound S.2. To solution of **S.1**[4] (0.32 g, 0.82 mmol, 1.0 equiv.) in dry DCM, 1-chloro-N,N,2-trimethyl-1-propenylamine (325 µL, 2.46 mmol, 3.0 equiv.) was added via syringe. The reaction mixture was stirred for 2 h at room temperature under a nitrogen atmosphere, after which time the solvent was removed and the sample was dried under vacuum to give the acid chloride in quantitative yield. The sample purity was checked by ^{1}H NMR and the sample was directly used in the next step without further purification.^{1}H NMR (300MHz, $CDCl_3$): δ 1.43 (s, 9H), 2.20 (m, 2H), 3.43 (t, *J*=6.6, 2H), 4.39 (t, *J*=6.0, 2H), 4.75 (bs, 1H), 7.54 (s, 1H), 7.73 (dd, *J*=8.3 *J*=7.3, 1H), 8.13 (dd, *J*=7.5 *J*=1.2, 1H), 8.47 (dd, *J*=8.5 *J*=1.2, 1H)

Compound S.4. To solution of **S.3**[5] (0.25 g, 0.68 mmol, 1.0 equiv.) and DIPEA (610 µL, 3.5 mmol, 5.1 equiv.) in dry DCM (10 mL) was added dropwise a solution of **S.2** (0.32 g, 0.82 mmol, 1.2 equiv.) in dry DCM (10 mL) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 6 h, after which time it was quenched with saturated aqueous $NaHCO_3$ and successively washed with saturated aqueous $NaHCO_3$ (2x5 mL) and with saturated aqueous NaCl (1x5 mL). The combined aqueous fractions were back extracted with DCM (1x5 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified by silica gel chromatography using MeOH/DCM (5:95 vol/vol) to provide **S.4** (0.26 g, 52%) as a yellow solid. ^{1}H NMR (300MHz, $CDCl_3$): δ 0.08 (s, 9H), 1.15 (d, *J*=6.6, 6H), 1.26-1.32 (m, 2H), 1.45 (s, 9H), 2.22-2.37 (m, 3H), 3.42-3.48 (m, 2H), 4.06 (d, *J*=6.4, 2H), 4.46 (t, *J*=5.9, 2H), 4.71-4.77 (m, 3H), 7.57 (s, 1H), 7.61-7.68 (m, 2H), 7.95 (s, 1H), 8.01 (dd, *J*=8.3, *J*=0.9, 1H), 8.21 (dd, *J*=7.5, *J*=1.1, 1H), 8.51 (dd, *J*=8.4, *J*=1.0, 1H), 9.04 (dd, *J*=7.7, *J*=1.0, 1H), 11.88 (s,1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.38 (C), 162.95 (C), 162.79 (C), 162.53 (C), 156.05 (C), 154.14 (C), 149.21 (C), 147.89 (C), 139.91 (C), 139.48 (C), 134.98 (C), 127.73 (CH), 126.77 (CH), 125.65 (CH), 125.61 (CH), 123.31 (C), 122.28 (C), 118.76 (CH), 116.79 (CH), 101.45 (CH), 100.27 (CH), 79.60 (C), 75.22 (CH_2), 67.33 (CH_2), 64.83 (CH_2), 37.78 (CH_2), 29.43 (CH_2), 28.50 (CH_3), 28.29 (CH_3), 19.35 (CH_3), 17.67 (CH_2), -1.38 (CH_3). HRMS (ESI) calculated for $[C_{37}H_{48}N_{45}O_9Si]^+$ 734.3216; found 734.3202.

Compound S.7. A mixture of **S.4** (0.10 g, 0.14 mmol) dissolved in THF (10 mL) and 10% Pd/C (10 mg) was stirred overnight at room temperature under 1 bar atmosphere of hydrogen. The solution was filtered through celite and solvent was evaporated under vacuum. The product **S.5** was obtained quantitatively as a solid. Its purity was checked by ^{1}H NMR and was directly used in the next step without further purification. ^{1}H NMR (300MHz, $CDCl_3$) δ 0.15 (s, 9H), 1.17 (d, *J*=6.7, 6H), 1.22-1.28 (m, 2H), 1.46 (s, 9H), 2.15-2.23 (m, 2H), 2.29-2.36 (m, 1H), 3.42-3.48 (m, 2H), 4.06 (d, *J*=6.5, 2H), 4.41 (t, *J*=5.8, 2H), 4.57-4.62 (m, 2H), 4.82 (bs, 1H), 5.60 (bs, 2H), 6.98 (s, 1H), 7.00 (dd, *J*=7.5 *J*=1.1, 1H), 7.38 (dd, *J*=8.2 *J*=7.5, 1H), 7.50 (dd, *J*=8.3 *J*=1.2, 1H), 7.53 (s, 1H), 7.66 (dd, *J*=8.1 *J*=8.0, 1H), 7.77 (s, 1H), 7.95 (dd, *J*=8.5 *J*=1.2, 1H), 9.03 (dd, *J*=7.7 *J*=1.2, 1H), 12.79 (s, 1H). A mixture of **S.5** (0.090g, 0.128mmol, 1.0 equiv.) and DIPEA (47µL, 0.268mmol, 2.1 equiv.) in 4 mL of dry THF was stirred for a few minutes under a nitrogen atmosphere. Then a solution of triphosgene (0.038 g, 0.129 mmol, 1.01 equiv.) in 1mL of dry THF added via syringe. The reaction mixture was stirred vigorously for 20 min at room temperature then for 40 min at 40°C. To the reaction mixture a solution of **S.5** (0.090 g, 0.128 mmol, 1.0 equiv.) and DIPEA (47 µL, 0.268 mmol, 2.1 equiv.) in 4 mL of dry THF was added via syringe. The mixture was stirred for 48 h under a nitrogen atmosphere at 40°C. The reaction mixture was extracted with 5% aqueous of citric acid (2x5 mL) and with saturated aqueous NaCl (1x5 mL). The combined aqueous fractions were back extracted with THF (1x5 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified by GPC to provide **S.7** (41 mg, 22%). ^{1}H NMR (300MHz, $CDCl_3$) δ -0.25 (s, 9H), 1.26-1.30 (m, 8H), 1.49 (s, 9H), 2.19-2.28 (m, 2H), 2.33-2.42 (m, 1H), 3.10 (bs, 1H), 3.46-3.52 (m, 2H), 3.65 (bs, 1H), 4.02-4.20 (m, 2H), 4.46 (bs, 2H), 4.83 (bs, 1H), 7.16-7.22 (m, 1H), 7.23 (s, 1H), 7.45 (dd, *J*=8.4 *J*=1.1, 1H), 7.67 (m, 1H), 7.80 (s, 1H), 7.90 (dd, *J*=8.3 *J*=1.1, 1H), 8.38 (dd, *J*=7.5 *J*=1.1, 1H), 9.25 (dd, *J*=9.9 *J*=1.1, 1H), 9.52 (s, 1H), 11.68 (s, 1H). HRMS (ESI) calculated for $[C_{75}H_{97}N_{10}O_{15}Si_2]^+$ 1433.6668; found 1433.6658.

Compound 5. Compound **S.7** (41 mg, 29 mmol) was dissolved in 1.5 mL DCM. The solution was cooled in an ice bath and TFA was added dropwise (1.5 mL). The solution was stirred at room temperature for 6 h. The solvents were evaporated under vacuum. The crude product was purified by reverse phase HPLC chromatography. Gradient 10 to 100 % Solvent B in 30 min, to obtain **5** as a yellow solid (24 mg, 97 %) ^{1}H NMR (300MHz, DMSO-d_6) δ 1.25 (d, *J*=6.7, 6H), 2.22-2.38 (m. 3H), 3.14-3.18 (m, 2H), 4.10 (d, *J*=5.7, 2H), 4.56 (t, J=5.4, 2H), 7.21 (s, 1H*), 7.26 (t, *J*=8.1, 1H*), 7.40 (d, *J*=7.7m ,1H*), 7.75 (t, *J*=8.1, 1H), 7.81 (s, 1H), 7.93 (d, *J*=7.5, 1H), 8.40 (d, *J*=7.0, 1H), 9.08 (bs, 1H), 9.48 (bs, 1H), 11.66 (bs, 1H), 12.90 (bs, 1H). HRMS (ESI) calculated for $[C_{55}H_{58}N_{10}O_{11}]^{2+}$ 517.2138; found 517.2147.

*- signals overlapped with the broad signal of NH_2 from Q^{Orn} monomers.

3. Chromatographic data

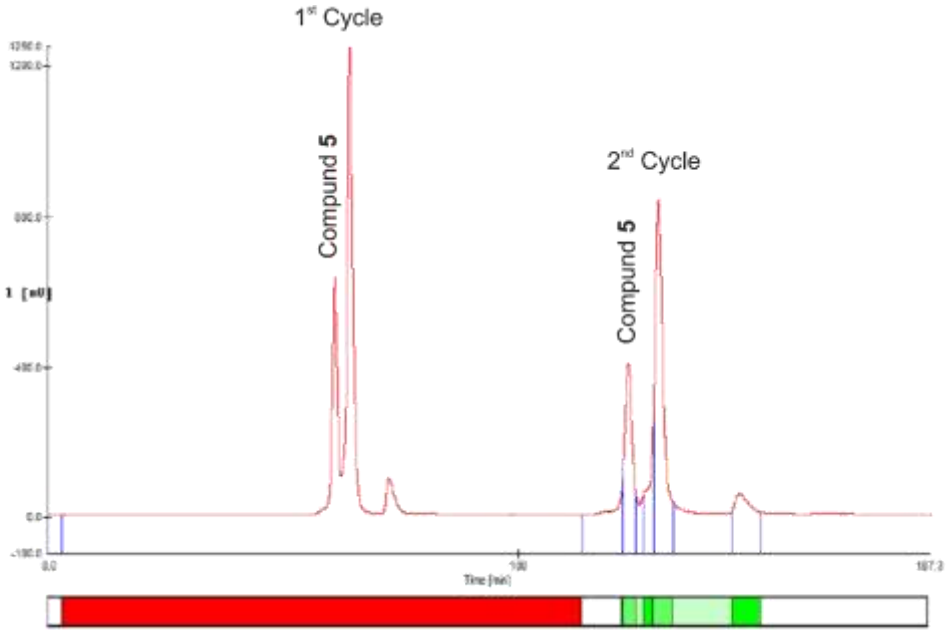


Figure S1. Chromatogram showing purification of compound **S7** using Gel Permeation Chromatography.

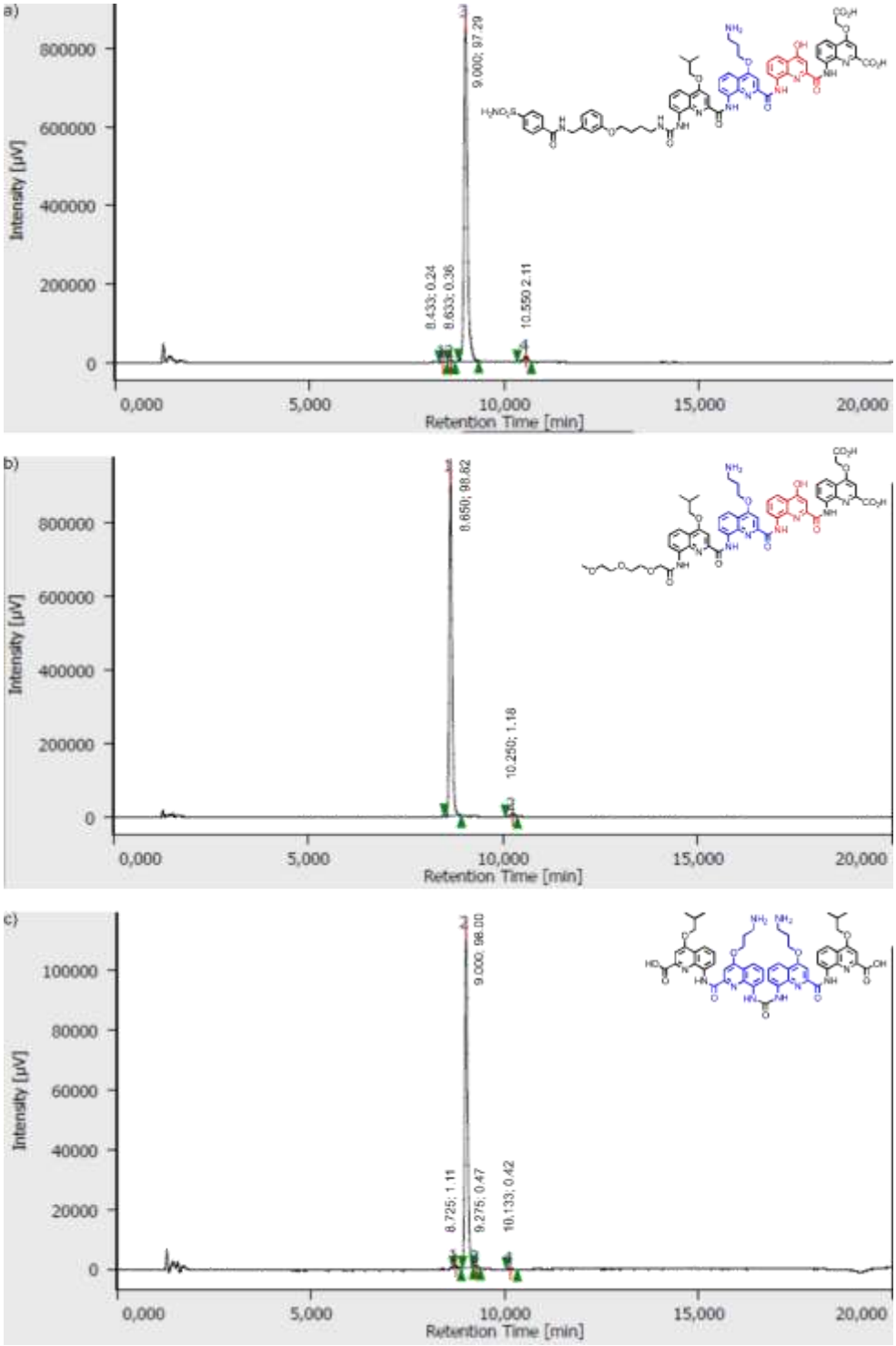


Figure S2. Analytical RP-HPLC profiles of purified **2** (a), **3** (b), and **5** (c). Conditions: From 5% to 100% of solvent B in 15 min then 100% solvent B.

4. ^{1}H NMR spectra of new compounds

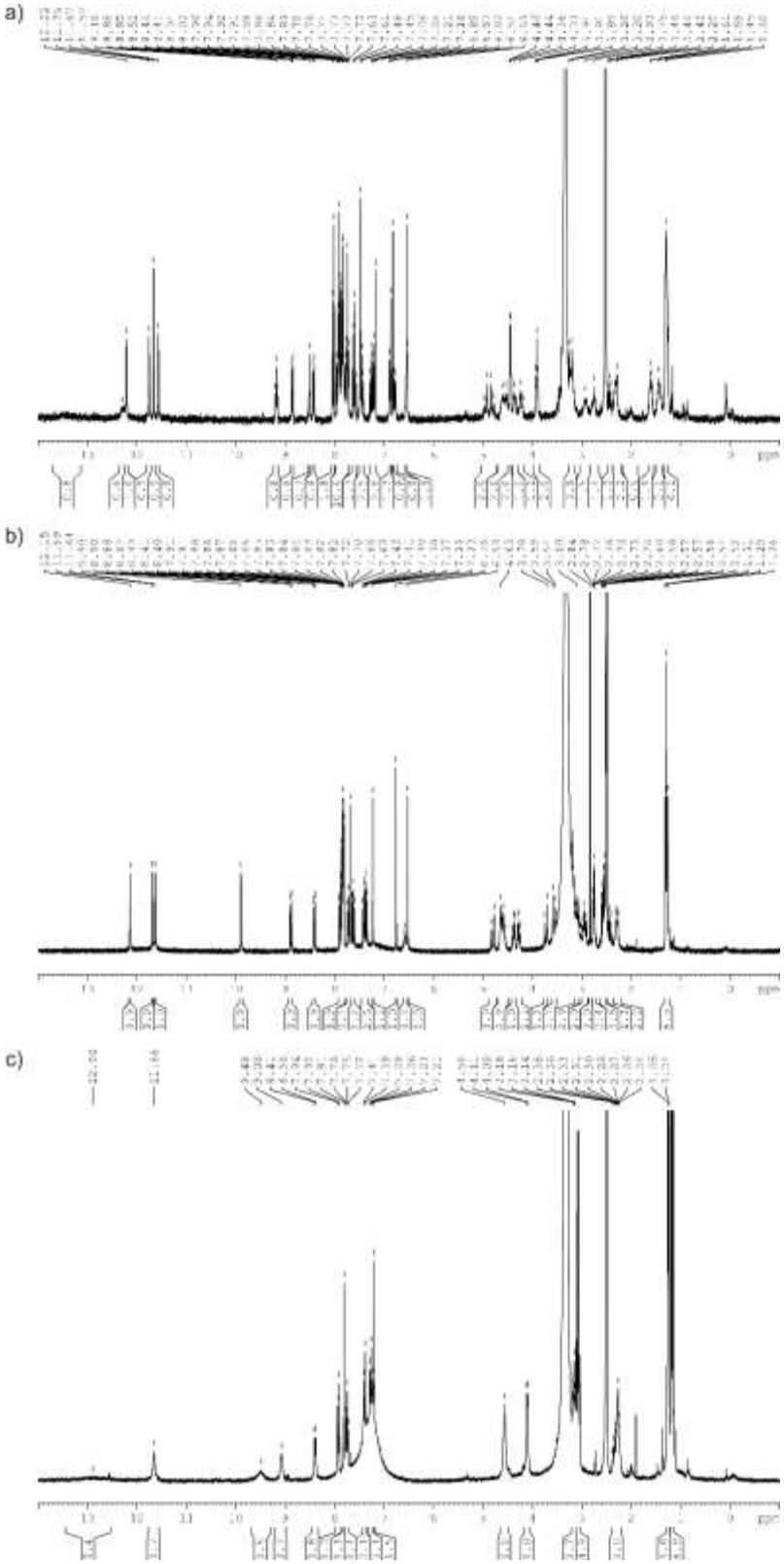


Figure S3. ^{1}H NMR analysis of compound a) **2** b) **3** and c) **5** in DMSO-d_6.

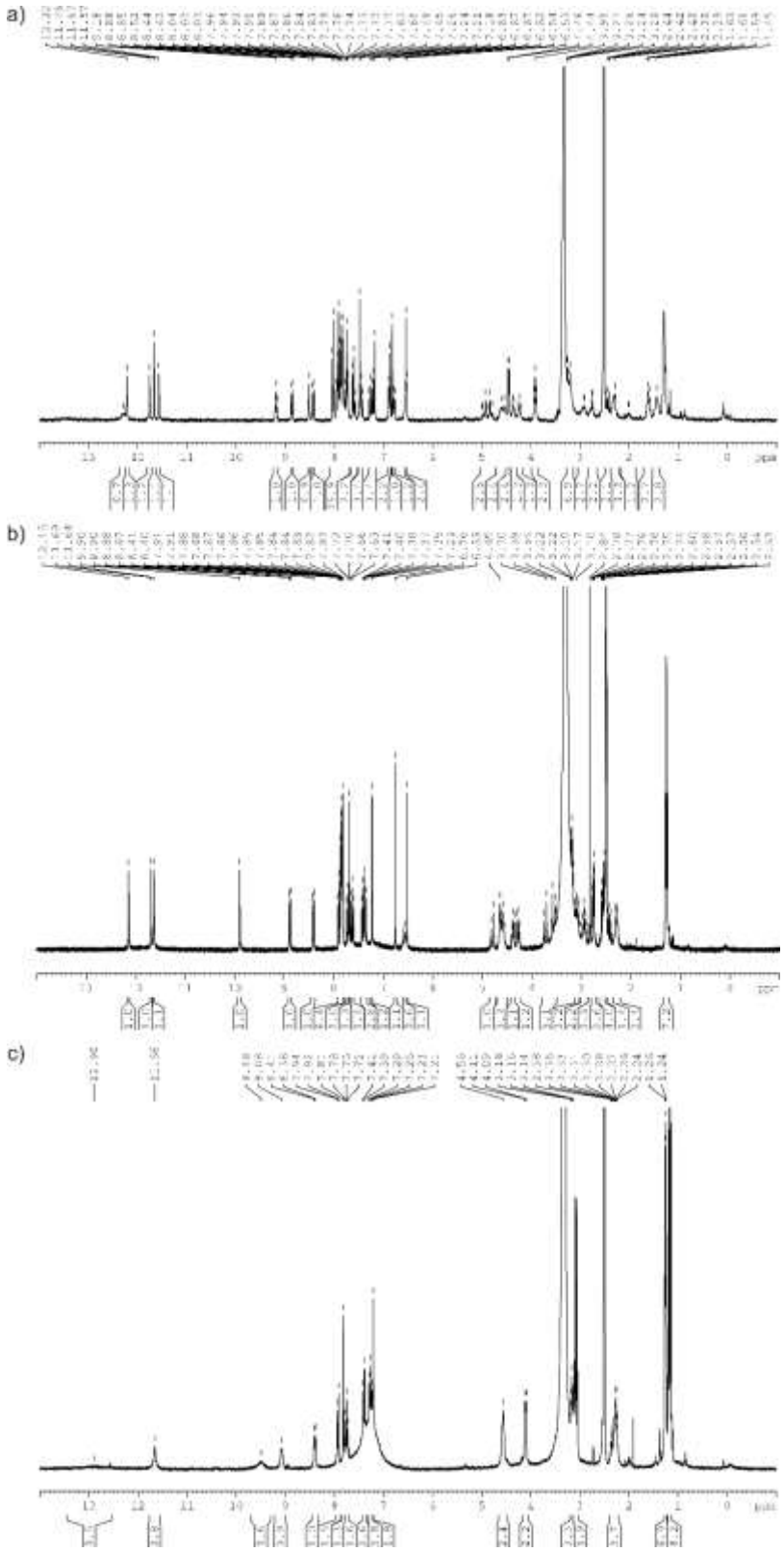


Figure S4. ^{1}H NMR analysis of compound a) **S.2** b) **S.4** and c) **S.5** in $CDCl_3$.

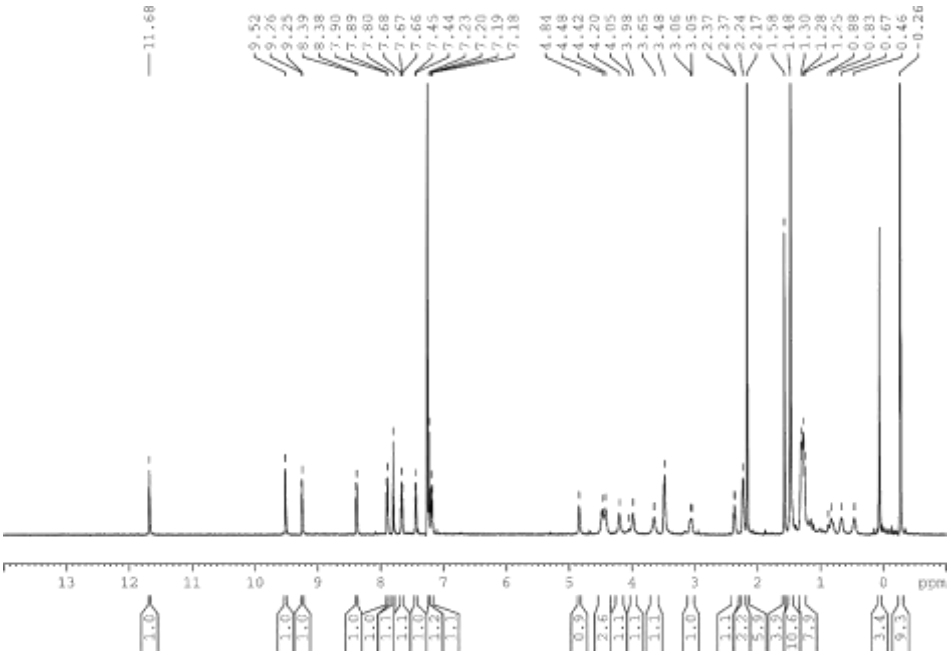


Figure S5. ^{1}H NMR analysis of compound **S.7** in $CDCl_3$.

5. HRMS (ESI) spectra

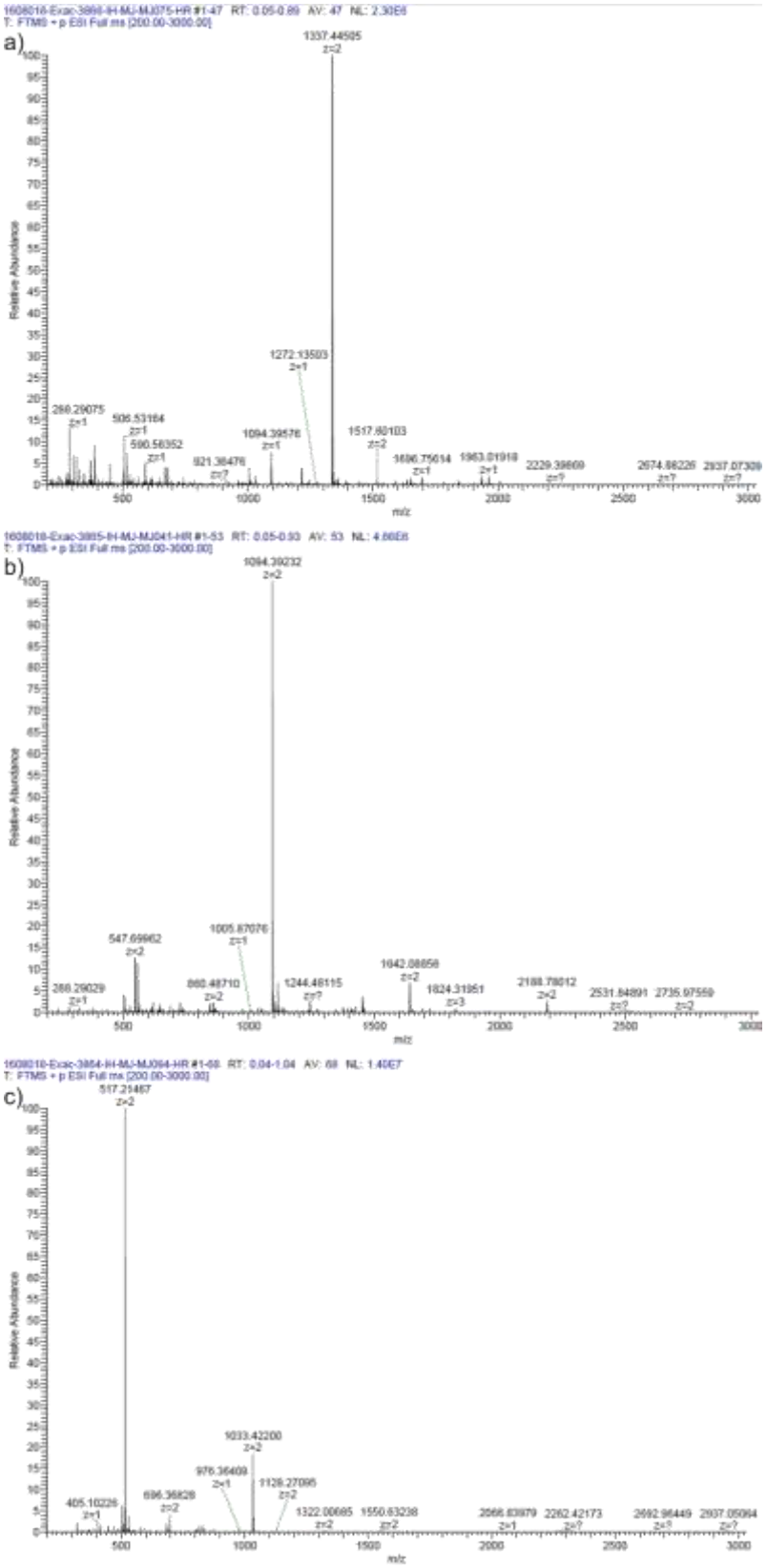


Figure S6. HRMS (Thermo Exactive Orbitrap) analysis in positive mode: m/z calculated for a) compound **2** $[C_{68}H_{65}N_{12}O_{16}S]^+$ 1337.43567; found 1337.44505; b) compound **3** $[C_{56}H_{56}N_9O_{15}]^+$ 1094.38904; found 1094.39232; c) compound **5** $[C_{55}H_{58}N_{10}O_{11}]^{2+}$ 517.21378; found 517.21467.

6. Crystallography

Crystallization of foldamer-HCAII complexes and data collection

The recombinant enzyme was expressed and purified according to Reference 1. Prior to crystallization, foldamers **2**, **3** or **5** were solubilized in DMSO. HCA (0.3 mM) was preincubated 2 hours with 1.5 equiv. of **2**, or 1.0 equiv. of **2** and 0.5 equiv. of either **3** or **5** in Tris 50 mM pH 8.0, NaN_3 3 mM. Since the crystallizing conditions used for **HCA:1** complex were not successful, commercial screens were used. The first acceptable hint was obtained with Hampton Research Crystal Screen.

For the complex of HCA with foldamer **2** (ratio 1.0:1.5) drops consisted of 0.4 µl of complex solution and 0.4 µl of the precipitant solution containing 100 mM sodium acetate pH 4.6, 8% PEG 4000 and sodium azide 3 mM. The drops were equilibrated by vapor diffusion against the precipitant solution at room temperature, and crystals appeared after 2-3 days and grew to their final size within 2 weeks. They were cryoprotected in the precipitant solution supplemented by 28 % glycerol.

For the complex of HCA with foldamers **2** and **3** (ratio 1.0:1.0:0.5), the crystallizing solution was 100 mM sodium acetate pH 5.1, 28% PEG 4000 and sodium azide 3 mM. Crystals appeared in 24 hours and grew to their final size within one week. They were cryoprotected in the precipitant solution supplemented by 28 % glycerol

For the complex of HCA with foldamers **2** and **5** (ratio 1.0:1.0:0.5), the crystallizing solution was 200 mM ammonium sulfate, 100 mM sodium acetate pH 5.3, 24% PEG 4000 and sodium azide 3 mM. Crystals appeared after 2 days and grew to their final size within one week. They were cryoprotected in the precipitant solution supplemented by 37 % glycerol.

Data were collected at ESRF, on beam lines ID23-2 and ID30A-3 for HCA_2-$\mathbf{2}_3$ and HCA_2-$\mathbf{2}_2$-**3**, respectively, and at SOLEIL on beam line Proxima-2 for HCA_2-$\mathbf{2}_2$-**5**. All data were reduced with XDS.[6] Structures were solved by molecular replacement using the programs Phaser[7] and Molrep[8] and atomic coordinates of the native protein (PDB code 3KS3[9]) as a search model. Refinement was carried out using Refmac[10] and manual rebuilding using Coot[11]. The topology files used to build and refine the modified inhibitors have been generated using Prodrg.[12] The structures were validated using Molprobity[13] and PDB_REDO,[14] prior to deposition in the RCSB Protein Data Bank (entry codes 5L3O, 5L6K and 5LVS).

Table S1. Data-collection and refinement statistics

	HCA_2-**2**$_3$	HCA_2-**2**$_2$-**3**	HCA_2-**2**$_2$-**5**
X-ray source	ESRF- ID23-2	ESRF-ID30A-3	SOLEIL-Proxima-2
Wavelength (Å)	0.8726	0.9677	0.9763
Resolution (Å)(last shell)	51.06-1.98 (2.10-1.98)	78.64-1.70 (1.76-1.70)	50.0-1.42 (1.46-1.42)
Space Group	C2	$P2_1$	C2
Cell dimension (Å)	a = 158.88	44.07	158.73
	b = 54.48	84.87	54.81
	c = 84.92	77.22	84.70
β angle (°)	112.79	97.81	112.77
Asymmetric unit	2 HCA molecules	2 HCA molecules	2 HCA molecules
Unique reflexions	46107 (6993)	61725 (6064)	125435 (9854)
Multiplicity	4.0 (4.0)	4.4 (4.4)	13.7 (14.0)
Completeness (%)	98.1 (92.5)	99.7 (99.8)	98.9 (97.7)
I/σ(I)	7.9 (1.9)	11.3 (1.9)	24.2 (2.3)
Rmrg	0.125 (0.665)	0.096 (0.788)	0.063 (1.420)
Refinement			
Resolution (Å)	46.0-1.98 (2.03-1.98)	78.64-1.70 (1.74-1.70)	50.0-1.42 (1.47-1.42)
Rfact (%)	0.207 (0.330)	0.153 (0.267)	0.149 (0.461)
Rfree (%)	0.236 (0.311)	0.181 (0.283)	0.178 (0.470)
r.m.s. bonds (Å)	0.012	0.017	0.0140
r.m.s. angles (°)	1.62	1.86	1.85
No. of atoms			
Total	4761	5487	5064
ligand	320	326	256
Water	260	397	575
Zn	2	2	2
glycerol	48	24	78
DMSO	0	4	0
Na	0	1	0
PDB code	5L3O	5L6K	5LVS

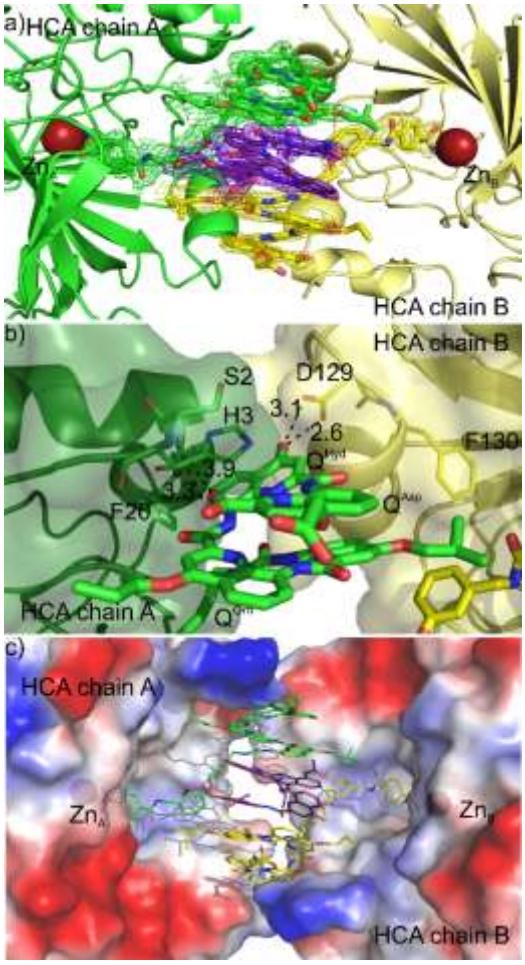


Figure S7. Crystal structure of HCA_2–**2**$_3$ complex (PDB# 5L3O), HCA molecules in green (chain A) and pale yellow (chain B), three Inh-Q^{Leu}-Q^{Orn}-Q^{Hyd}-Q^{Asp} molecules (**2**) (green, purple and yellow) and Zn^{2+} ions as red spheres; a) foldamer backbones shown in stick and contoured by 2 mFo-DFc density maps at 1σ level; b) polar contacts between: carboxylic group of Q^{Aps} (green stick) of foldamer A and amide group of S2 (green line) of HCA-A, side chain hydroxyl group of Q^{Hyd} (green stick) of foldamer A and side chain carboxylic group of D129 (yellow line) of HCA-B, π-π stacking interaction between quinoline ring of Q^{Hyd} (green stick) of foldamer A and side chain ring of H3 (green line) of HCA-A; c) charge-charge protein-protein interaction between both HCA chains ~~Put ZnA and B instead of I and II in panel c)~~

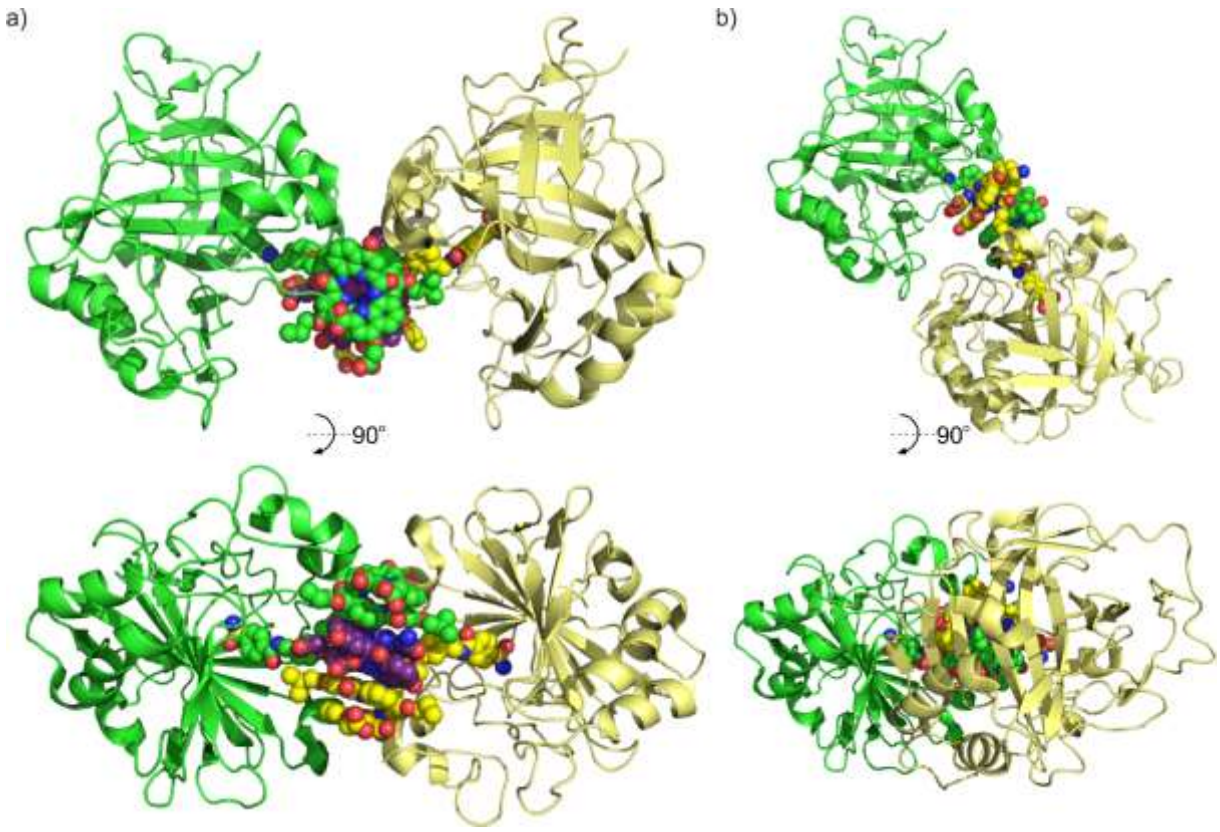


Figure S8. The crystal structure of a) HCA_2-**2**$_3$ (PDB# 5L3O) and b) HCA_2-1_2 (PDB# 4LP6), the orientation of HCA chain A (in green) is the same in both structures.

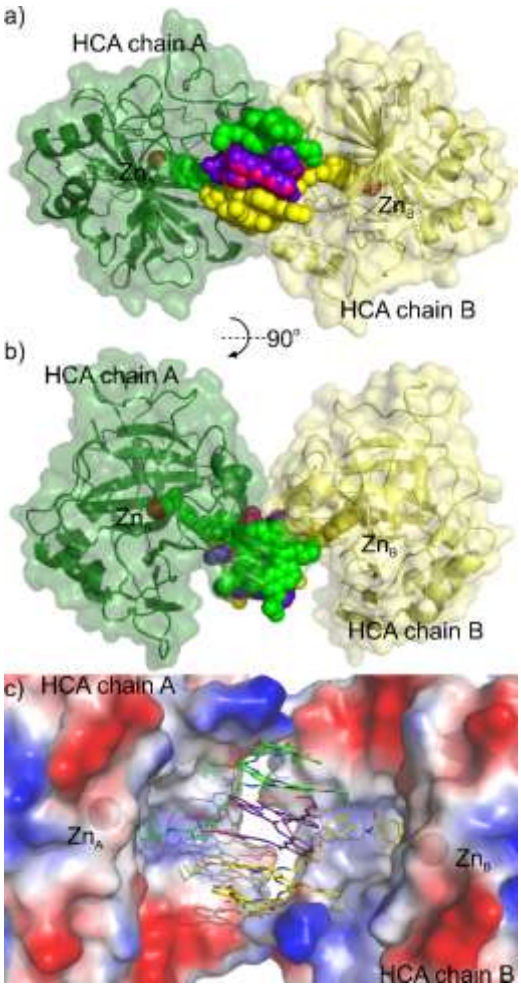


Figure S9. Crystal structure of the HCA_2-**2**$_2$-**3** complex (PDB# 5L6K). a) Asymmetric unit showing two HCA molecules in green and yellow and three foldamer helices in CPK representation. The green and yellow foldamers (**2**) have their ligand bound to an HCA active site. Native Zn^{2+} ions are shown as red spheres. A third foldamer (**3**, purple and magenta for the two orientations) is sandwiched between the first two. b) Top view of the complex showed in a). c) Charge-charge protein-protein interaction between both HCA chains and three foldamer helices in line representation. The green and yellow foldamers (**2**) have their ligand bound to an HCA active site. Native Zn^{2+} ions are shown as red spheres. A third foldamer (**3** purple) is sandwiched between the first two.

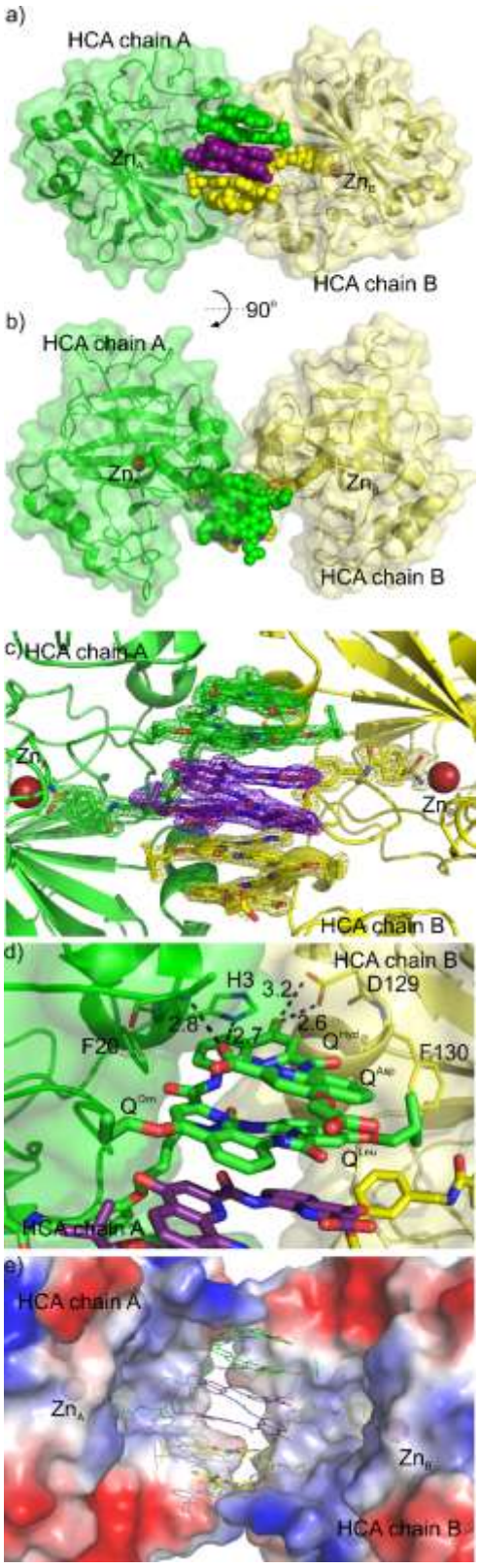


Figure S10. Crystal structure of the HCA_2-**2**$_2$-**5** complex (PDB# 5LVS). a) Asymmetric unit showing two HCA molecules in green and yellow and three foldamer helices in CPK representation. The green and yellow foldamers (**2**) have their ligand bound to an HCA active site. Native Zn^{2+} ions are shown as red spheres. A third foldamer (**5**, purple) is sandwiched between the first two. b) Top view of the complex showed in a). c) Foldamer backbones contoured by 2mFo-DFc density maps at 1s level, d) Some relevant contacts of a foldamer helix of compound **2** and neighboring protein surfaces (distances in Å). The imidazole of H3 stacks on a quinoline ring; the imidazole and amide NH groups hydrogen bond to the terminal quinoline carboxylate. F20 (in the back) engages in edge-to-face aromatic contacts with a quinoline, e) charge-charge protein-protein interaction between both HCA chains

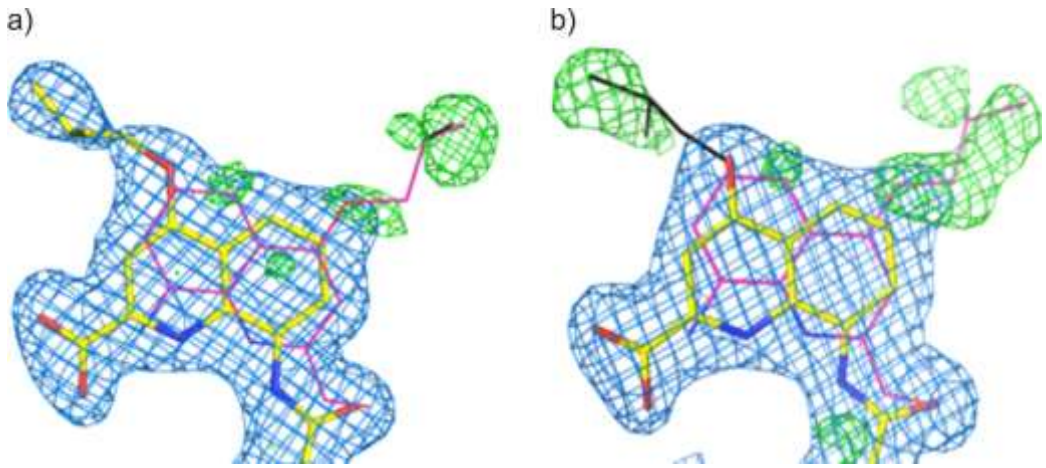


Figure S11. Superimposition of HCA_2-**2**$_3$ structure onto that of HCA_2-**2**$_2$-**5**, in the vicinity of the two quinolines of **5** decorated with leucine like proteinogenic side chains. Carbon atoms of the HCA_2-**2**$_2$-**5** and superimposed HCA_2-**2**$_3$ structures are colored yellow and magenta respectively. The 2mFo-DFc electron density maps (contoured at 1.3 rms) are shown in blue, the mFo-DFc ones (contoured at 3 rms) in green. They correspond to the final model of HCA_2-**2**$_2$-**5** structure. In both Figures, a residual density in the mFo-DFc map suggest that compound **2,** with a leucine like proteinogenic side chain matching the residual density, is present in low proportion. In Figure **b**, the leucine like side chain of **5** is highly disordered and could not be modeled with reliability (a possible rotamer is shown in black)

7 LC-MS analysis of HCA_2-2_2-3 crystals

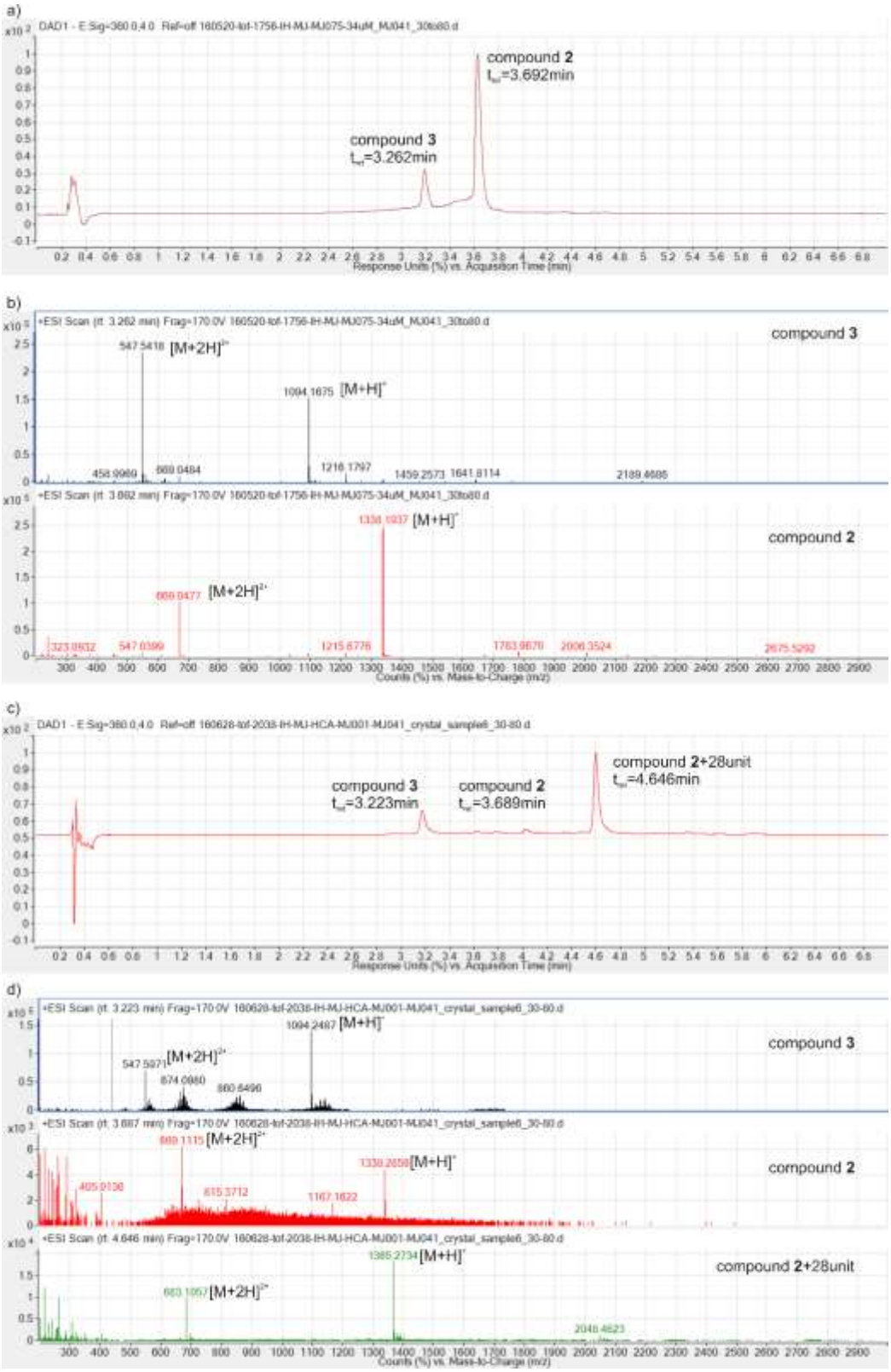


Figure S12. LC-MS analysis of HCA_2-**2**$_2$-**3** crystals, a) chromatographic analysis, recorded at 360nm, of a reference solution containing compound **2** (1 equiv.) and compound **3** (0.5 equiv.); b) MS analysis of peaks selected on a); c) chromatographic analysis, recorded at 360nm, of crystals of HCA-**2**$_2$-**3** dissolved in DMSO-d_6; d) MS analysis of peaks selected on c). The mass corresponding to the mass of compound **2** +28 was assigned to a formylation of the sulfonamide moiety by the formic acid used in LC-MS analysis.

8. NMR and CD solution studies of HCA complexes

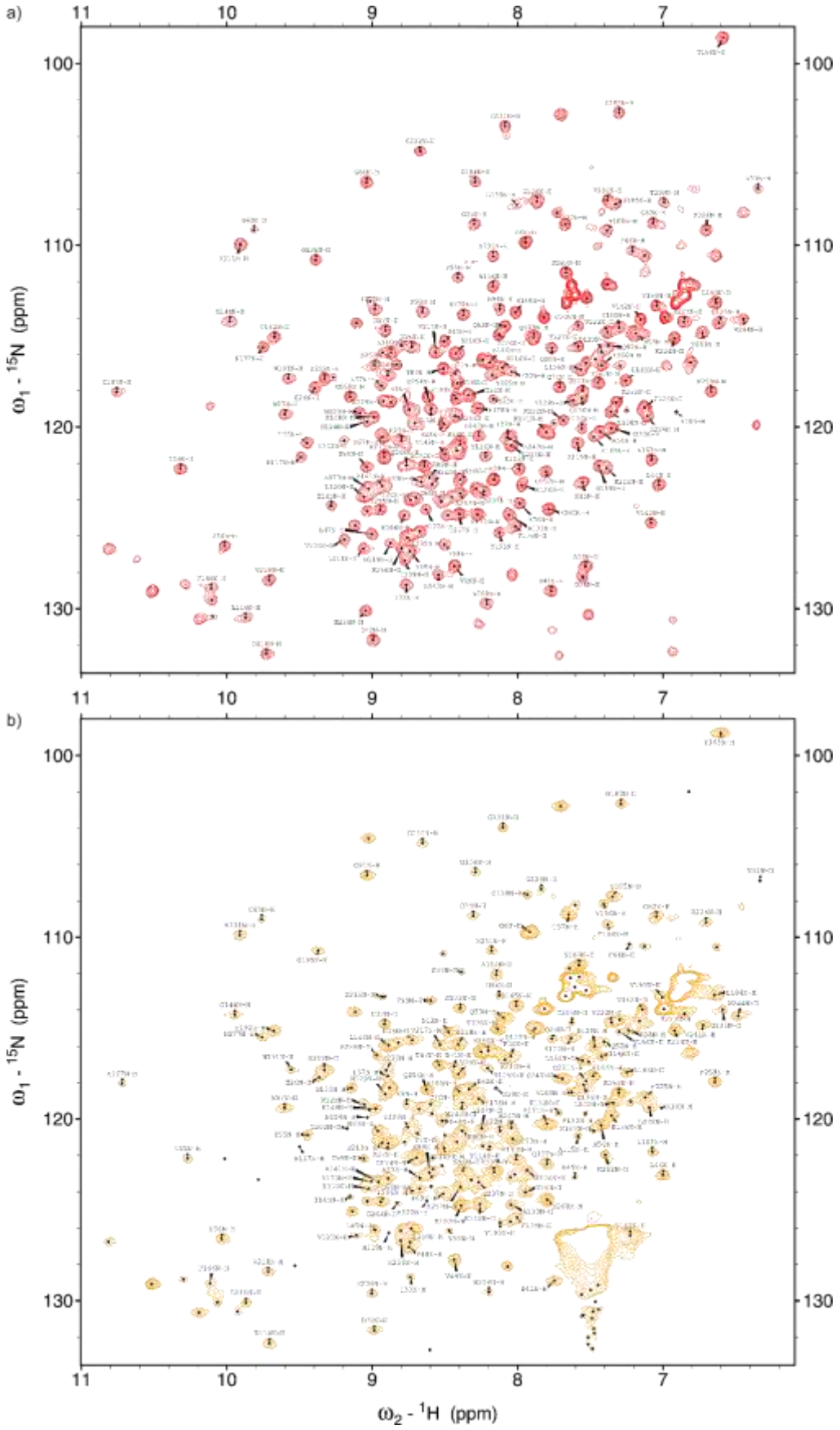


Figure S13. ^{1}H,^{15}N HSQC spectra of 500μM [^{15}N]-HCA in 50mM phosphate buffer pH=4.6 with 1.3 equiv. of compound **4** (a) and 1.5 equiv. of compound **2** (b).

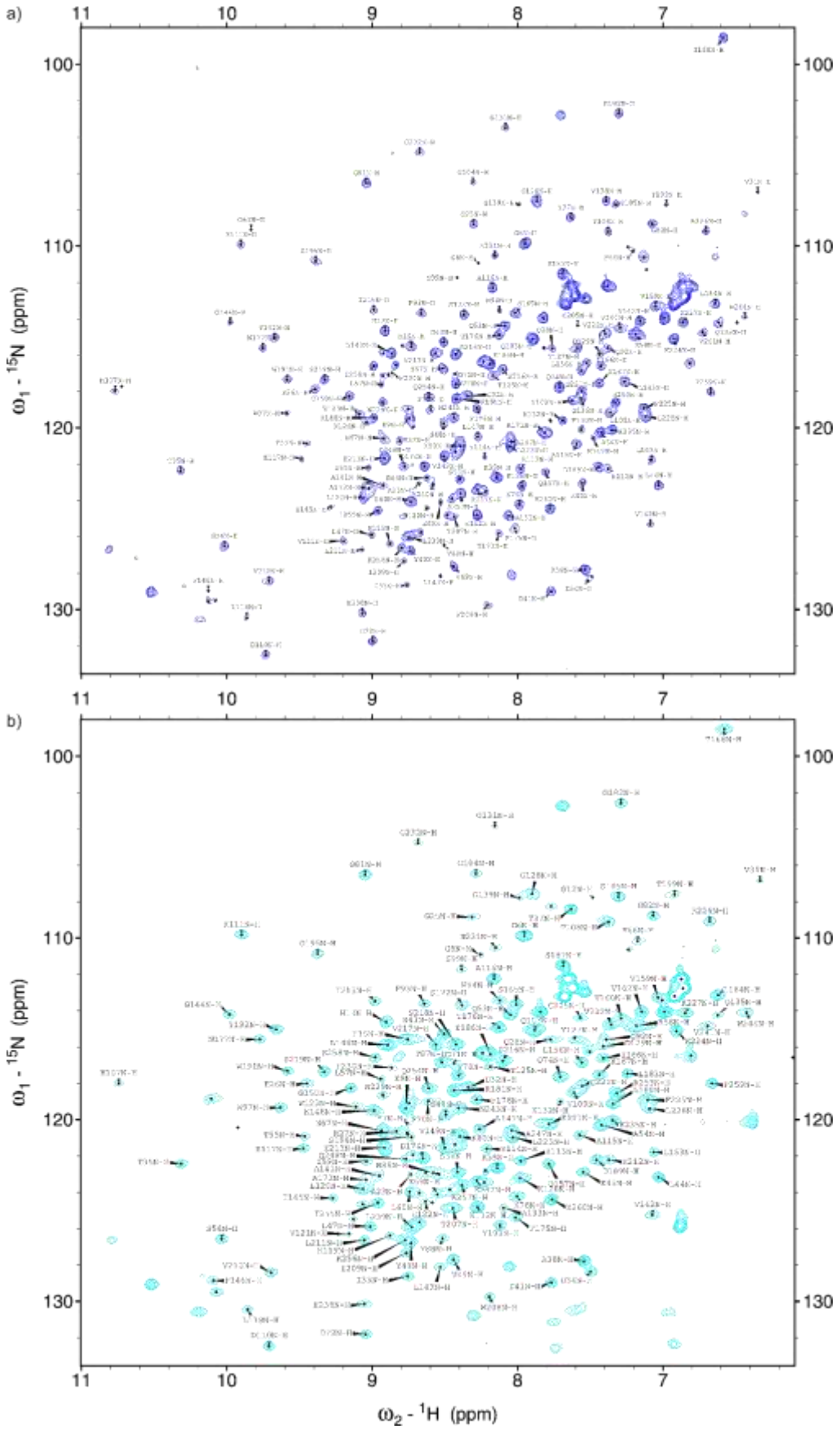


Figure S14. $^{1}H,^{15}N$ HSQC spectra of 500μM [^{15}N]-HCA in 50mM phosphate buffer pH=7.4 with 1.3 equiv. of compound **4** (a) and 1.5 equiv. of compound **2** (b).

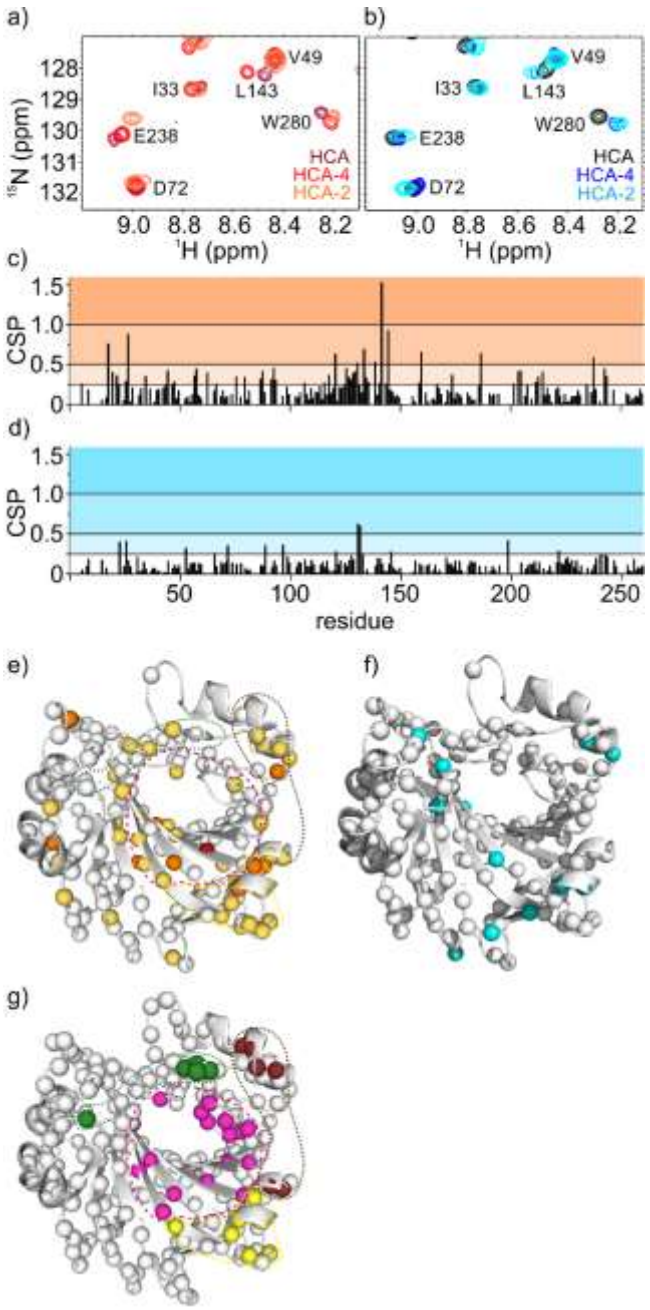


FIGURE S15. Intermolecular contacts identified by NMR spectroscopy in phosphate buffer. Part of ^{1}H-^{15}N HSQC spectra of [^{15}N]HCA (500 μM), either free (purple or black), or in presence of **4** (1.5 equiv., red or blue), or of **2** (1.5 equiv., orange or cyan) at pH=4.6 (a) and pH=7.5 (b), respectively. CSPs of HCA-**2** compared to HCA-**4** calculated as a root mean square deviation $((\Delta\delta_H)/0.14)^2+(\Delta\delta_N)^2)^{0.5}$ at pH=4.6 (c) and pH=7.5 (e) Amide nitrogen atoms (spheres colored as in c) observed in the HSQC spectrum of HCA-**2** in pH=4.6 (shown on chain A of PDB# 5L6K) (f) Amide nitrogen atoms (spheres colored as in d) observed in the HSQC spectrum of HCA-**2** in pH=7.5 (shown on chain A of PDB# 5L6K) (g)) Amide nitrogen atoms (spheres) of HCA residues of the HCA_2-$\mathbf{2}_3$ crystal structure located within 6 Å from: the sulfonamide inhibitor and linker (magenta); the foldamer helix (green – chain A, and yellow – chain B); the other HCA chain (chocolate).

Table S2. Molecular size of HCA, HCA–**2**, and HCA–**4** in solution by using 1D ^{1}HN NMR T2 relaxation measurements to estimate the correlation time

Sample	Conc. [μM]	Buffer[a]	pH	$^{1}H^{N}$ T_2 [ms]	τ_c [ns][b]
HCA-2	500	Phosphate	4.6	7.2±0.2	31.3
HCA-2	100	Phosphate	4.7	9.1±0.2	23.2
HCA-2	500	Phosphate	7.4	8.9±0.2	25.1
HCA-4	500	Phosphate	7.4	9.9±0.4	22.8
HCA-2	90	Phosphate	8.1	12.4±0.3	18.0
HCA-2	85	Tris	8.1	11.8±0.4	19.0
HCA-2	85	Tris:NaOAc	4.7	9.8±0.1	22.8

[a] Phosphate, 50 mM sodium phosphate; Tris, 50 mM Tris; Tris:NaOAc, 50mM Tris (pH=8.0) 50%v/v:100mM NaOAc (pH=4.6) 50%v/v, [b] Estimate of correlation time (τ_c) by using the equation $\tau_c=(4.45\cdot T_2)$

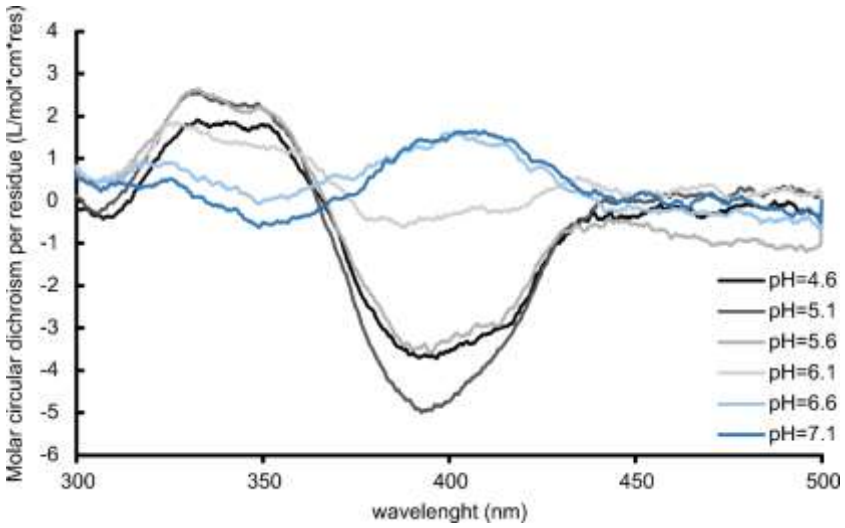


FIGURE S16. Circular dichroism spectra of HCA-**2** in phosphate buffer (50 mM sodium phosphate) at a function of pH

9. References

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