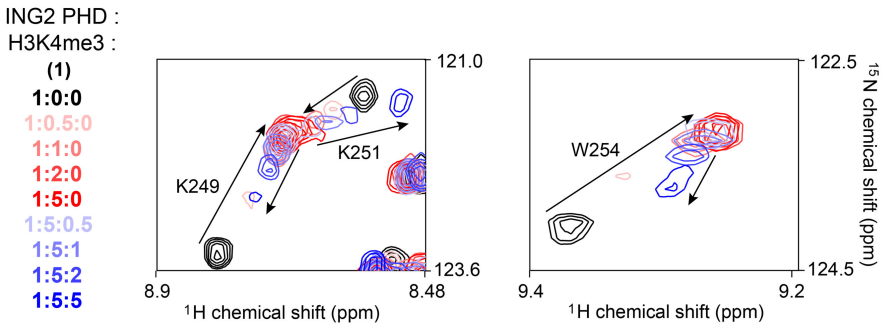


Figure S1, Related to Figure 3. Superimposed ^{1}H,^{15}N HSQC spectra of ING2 PHD collected as first H3K4me3 peptide and then (1) were titrated in. Spectra are color coded according to the protein:peptide:(1) molar ratio.

SI Experimental Procedures

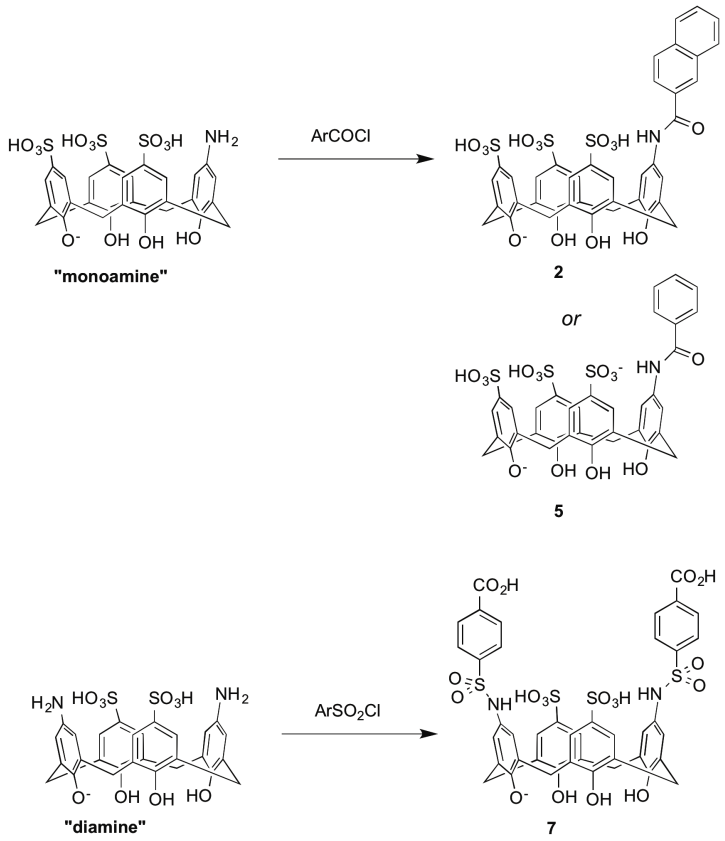
Synthesis - General

Calixarene **1** was purchased from TCI America and used as obtained. Lucigenin dye was purchased from Life Technologies and used as obtained. Low-resolution electrospray ionization mass spectrometry (LR-ESI-MS) was performed on a Finnigan LCQ MS, and High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was performed on a Thermo Scientific LTQ Velos Orbitrap. Calixarene (3), (4), (6), and 5-phenyl-25, 26, 27, 28-tetrahydroxy-11-17-23-trisulfonatocalix[4]arene (8) were made according to published procedures (Allen et al., 2014; Daze et al., 2012).

HPLC purifications

Synthetic peptides and compounds (2-7) were purified by RP-HPLC using a Shimadzu Prominence HPLC equipped with a preparative Apollo C18 column (Alltech, 5 μm, 22 x 250 mm) or a Thermo-Dionex HPLC/MS equipped with a Luna C-18 column (Phenomenex, 5 μm, 21.2 x 250 mm). Each instrument used a UV-Vis detector set to 280 nm. Compounds were purified by running a gradient from 90:10 0.1% TFA in H_2O:0.1% TFA in MeCN to 10:90 0.1% TFA in H_2O:0.1% TFA in MeCN over 35 minutes.

Synthesis of calixarenes (2), (5), and (7).



Compound (2)

Compound **monoamine** (25 mg, 3.7 x 10^{-5} mol) and 2-naphthoyl chloride (1.1 eq., 8 mg, 4.1 x 10^{-5} mol) were dissolved in 2.5 mL of 0.1 M phosphate buffer (pH = 8) and 100 uL of dimethylformamide then stirred at room temperature, overnight. The aqueous solution was extracted with dichloromethane (2 x 10 mL), and ethyl acetate (2 x 10 mL). The aqueous layer was evaporated to dryness under reduced pressure and the crude solid subjected to RP-HPLC purification, as outlined above. Fractions containing product were pooled and lyophilized to afford 9 mg (28% yield) as an off-white solid. NMR (300 MHz, D_2O, δ): 3.78 (br s, 2H), 4.0 (br s, 8H), 5.21 (d, 7.9 Hz, 1H), 6.85 (s, 2H), 7.02 (d, 8.2 Hz, 1H), 7.19 (d, 8.6 Hz, 1H), 7.25 (d, 8.6 Hz, 1H), 7.35 (s, 2H), 7.47 (s, 1H), 7.75 (s, 2H), 7.81 (s, 2H). LR-ESI-MS $[M–H]^–$ calculated for $C_{39}H_{30}NO_{14}S_3^-$ 832.1, found 832.3.

Compound (5)

Compound **monoamine** (20 mg, 2.9 x 10^{-5} mol) and benzoyl chloride (1.1 eq., 4 uL, 3.13 x 10^{-5} mol) were dissolved in 2.5 mL of 0.1 M phosphate buffer (pH = 8) and stirred at room temperature, overnight. The aqueous solution was extracted with dichloromethane (2 x 10 mL), and ethyl acetate (2 x 10 mL). The aqueous layer was evaporated to dryness under reduced pressure and the crude solid subjected to RP-HPLC purification, as outlined above. Fractions containing product were pooled and lyophilized to afford 11 mg (48% yield) as an off-white solid. NMR (300 MHz, D_2O, δ): 3.91 (br s, 4H), 4.07 (br s, 4H), 5.33 (s, 2H), 7.07 (m, 4H), 7.42 (s, 2H), 7.68 (s, 2H), 7.71 (s, 2H). LR-ESI-MS $[M–H]^-$ calculated for $C_{35}H_{28}NO_{14}S_3^-$ 782.1, found 782.3.

Compound (7)

Compound **diamine** (20 mg, 3.3 x 10^{-5} mol) and *p*-carboxybenzenesulfonyl chloride (1.9 eq., 13.6 mg, 6.3 x 10^{-5} mol) were dissolved in 2.5 mL of 0.1 M phosphate buffer (pH = 8) and stirred at room temperature, overnight. The aqueous solution was extracted with dichloromethane (2 x 10 mL), and ethyl acetate (2 x 10 mL). The aqueous layer was evaporated to dryness under reduced pressure and the crude solid subjected to RP-HPLC purification, as outlined above. Fractions containing product were pooled and lyophilized to afford 6 mg (16% yield) as an off-white solid. NMR (300 MHz, D_2O, δ): 3.84 (br s, 8H), 6.72 (d, 8.3 Hz, 4H), 6.88 (d, 8.3 Hz, 4H), 6.89 (s, 4H), 7.44 (s, 4H). HR-ESI-MS $[M–2H]^{2–}$ calculated for $C_{42}H_{32}N_2O_{18}S_4^{2–}$ 490.0271, found 490.0272.

Peptide Synthesis

Peptides were made using a CEM Liberty 1 microwave peptide synthesizer, using standard Fmoc-solid phase synthesis employing HBTU as coupling agent and DIEA as activator base. Fmoc-Lys(me3)-OH was purchased from GL Biochem and all other reagents were purchased from Sigma-Aldrich or ChemImpex. Peptides were made as H3 1-12 sequences (H-ARTKQTARK(me_3)STGY-

NH_2, H-ARTK(me_3)QTARKSTGY-NH_2 or H-ARTKQTARKSTGY-NH_2), with a N-terminal free amine, C-terminal amide and tyrosine added to the C-terminus to aid in UV detection for purification and concentration determination. Peptides were purified by HPLC as described above, and identity confirmed by ESI-MS. Concentration of peptide stocks was determined by dissolving the lyophilized solid into water and using the extinction coefficient of tyrosine at 280 nm (Spectramax M5, Molecular Devices).

Fluorescence Displacement Assay - General

Calixarene hosts were made in stock solutions from weighed solid and dissolved in dH_2O. All further dilutions were done using this stock. Lucigenin (LCG) was made from a stock 5 mM solution in dH_2O, and protected from light. 0.2 M phosphate buffer at pH 7.4 was made from the corresponding salts and used as is, 10 µL amounts were used in every well to furnish a final buffer concentration of 10 mM. Peptide solutions were freshly prepared and concentrations confirmed as outlined above. All titrations were performed in 96 well NUNC optically clear-bottomed, black-walled plates and read using a Spectramax M5 (Molecular Devices) plate reader.

Determination of K_{assoc} between calixarene and LCG

Using a calixarene stock solution, varying concentrations of calixarenes were made (0.05 µM-50 µM). The first well was a blank solution that contained 10 µL 0.2 M phosphate buffer, 20 µL (2.5 or 5 µM) LCG and 170 µL dH_2O. Subsequent wells (2-12) contained the same amounts except: 150 µL dH_2O and 20 µL calixarene (in increasing concentration). The plate emission was read (100 reads/well) between 445-600 nm, excitation set to 369 nm. The λ_{max} of the fluorescence readout was selected (485 nm) and this data was fit with the program Equilibria (available online at: http://www.sseau.unsw.edu.au) in order to determine the 1:1 binding constant.

Determination of K_{assoc} between calixarene and peptide

Similar to above, the first well in each row was a blank containing 20 µL (2.5 or 5 µM) LCG, 20 µL (5 or 12.5 µM) calixarene, 10 µL 0.2 M phosphate buffer and 150 µL dH_2O. Titration was performed as above except, using varying peptide concentrations (20 µL, 5 µM to 7 mM) and 130 µL dH_2O. Fitting was done as outlined above in the program Equilibria, (Young and Jolliffe, 2012) provided that LCG and calixarene concentrations remain constant and using the determined K_{assoc} between calixarene and LCG as an input.

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