

Probing the *hras*-1^Y i-motif with small molecules

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General Information

The following nucleic acids were purchased and HPLC purified from Integrated DNA Technologies, containing the corresponding sequences:

Unlabeled *hras*-1^Y: 5'- CGCCCGTGCCCTGCGCCCGCAACCCGA-3'
A647-labeled *hras*-1^Y: 5'-A647-CGCCCGTGCCCTGCGCCCGCAACCCGA-3'
Biotin labeled *hras*-1^Y: 5'-Biotin- CGCCCGTGCCCTGCGCCCGCAACCCGA-3'
FRET *hras*-1^Y: 5'-ATTO- CGCCCGTGCCCTGCGCCCGCAACCCGA-TAMRA-3'

Oligos used for SMM Screens (From Figure 1):

DNA G4

- AlexaFluor647-MYC:
5' -AlexaFluor648 AGGGTGGG GAGGGTGGGG
- AlexaFluor647-VEGF:
5' -AlexaFluor648 CGGGGCGG GCCGGGGGCGGGGT-3'
- AlexaFluor647-c-KIT:
5' -AlexaFluor648 AGGGAGGG CGCTGGGAGGAGGG-3'
- AlexaFluor647-KRAS:
5'-AlexaFluor648 AGGGCGGT GTGGGAAGAGGGAAGAGGGGGAGGCAG-3'

RNA G4

- AlexaFluor647-AKTIP:
5' -AlexaFluor648 GGGGUG GGGCGGGGCGGGG-3'

RNA Hairpins

- Cy5-labeled miR-21-hairpin RNA:
5'-Cy5-GGGUUGACUGUUGAAU- CUCAUGGCAACCC-3'
- Cy5-labeled Cy5-labeled TAR RNA:
5'-Cy5-GCAGAUUCUGAGCCUGGGAGCUCUCUGCC-3'.

Riboswitches

- Cy5-PreQ1 riboswitch (B. Subtilis):
5'-Cy5-AGA GGU UCU AGC UAC ACC CUC UAU AAA AAA CUA A-3'
- Cy5-SAM-II riboswitch:
5'-Cy5-UCG CGC UGA UUU AAC CGU AUU GCA AGC GCG UGA UAA AUG
UAG CUA AAA AGG G-3'

- Cy5-TPP riboswitch:
5'Cy5-CAG UAC UCG GGG UGC CCU UCU GCG UGA AGG CUG AGA AAU
ACC CGU AUC ACC UGA UCU GGA UAA UGC CAG CGU AGG GAA
GUG CUG-3'

Calf thymus DNA was purchased from ThermoFisher Scientific at 10mg/mL.

All small molecules were purchased from ChemBridge Hit2Lead®, then analyzed for purity via LC/MS, ¹H NMR, and ¹³C NMR. Several compounds were purified using HPLC at 10-95% Acetonitrile gradient in 0.1% THF-MilliQWater, and analyzed again prior to dissolution in D-DMSO to a concentration of 20 mM. See Table below for product ID.

Compound number	ChemBridge Product number	Compound number cont.	ChemBridge Product number cont.
1	27001422	13	27067624
2	16967942	14	16749936
3	94230832	15	95636859
4	62400924	16	82010209
5	SNJ-91	17	30918647
6	17359670	18	46168464
7	59261919	19	61479509
8	27297065	20	33020308
9	20968889	21	35564261
10	95636859	22	13051644
11	27263777	23	19216641
12	62080396		

Small Molecule Microarray

The small molecule microarray screening procedure was performed as previously published^[1] Various compounds can be immobilized to the slides by covalently bonding either an amine or primary alcohol on each compound to the isocyanate. 7024 compounds purchased from Chembridge and diluted to 10mM DMSO stocks were printed in duplicate on the slides, then were exposed to pyridine vapor overnight in a vacuum desiccator. The compound-appended slides were then exposed to a 1:20 polyethylene glycol:DMF solution to quench unreacted surface.

The Alexofluor647-*hras*-1^Y i-motif was diluted to 150nM in 50mM KCl, 50mM Tris-HCl, 0.05% Tween, pH 6.05 buffer and was annealed for 3 minutes at 90° C, followed by cooling to 23° C. The printed SMM slides were incubated at 23°C with the A647-*hras*-1^Y for 2h. Slides were then washed three times with Tris buffer and then with milliQ water and dried by centrifugation for 2 min at 4000RPM. The fluorescence of the slides was measured on an Innopsys Innoscan 1100 AL Microarray Scanner (650 nm excitation, 670 nm emission). 61 hits out of the 7024 compounds were discovered to bind to the i-motif, and 31 of the hits were selective (0-2 oligonucleotides hit) for *hras*-1^Y (0.86% hit rate).

Synthesis of 2-((2-amino-6-(4-(1-(pyrrolidin-1-yl)ethyl)phenyl) pyrimidin-4-yl)amino)ethan-1-ol

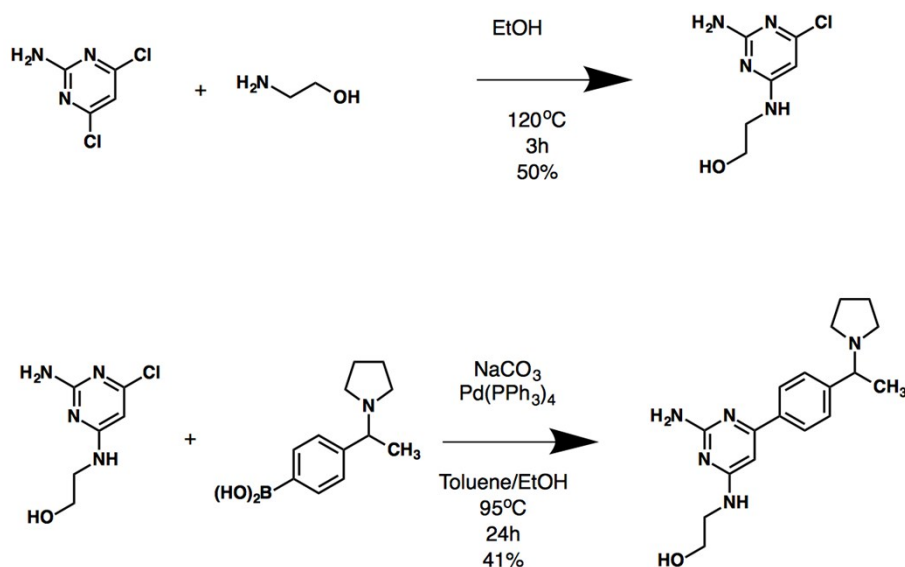


Figure S1: Synthetic scheme of 2-((2-amino-6-(4-(1-(pyrrolidin-1-yl)ethyl)phenyl)pyrimidin-4-yl)amino)ethan-1-ol

2-((2-amino-6-chloropyrimidin-4-yl)amino)ethanol (0.03g, 0.000159mmol) was suspended in a 2mL:1mL mixture of toluene to ethanol, and 0.5 mL of 2M Na₂CO₃ was added. Upon stirring, the solution was activated by tetrakis(triphenylphosphine) palladium(0) (0.0459g, 0.0000397mmol), then (4-(1-(pyrrolidin-1-yl)ethyl)phenyl)boronic acid (0.0418g, 0.00019mmol) was added to the solution. The reaction was heated to reflux. At 1.5h and 4.5h, the reaction was cooled to 23°C and excess tetrakis(triphenylphosphine)palladium(0) (0.00184g, 0.00159mmol) (Strem Chemicals) and (4-(1-(pyrrolidin-1-yl)ethyl)phenyl)boronic acid (0.0104g, 0.0475mmol) were added. The reaction was heated to reflux overnight. The reaction was diluted with water and extracted with ethyl acetate (2x 20mL), then briefly washed with 0.2M of NaOH. The combined organic layers were dried with sodium sulfate. The product was purified by HPLC at a 10-95% acetonitrile gradient in 0.1% THF-MilliQ Water. Pure **1** was isolated with 40.9% yield and 95% purity. ¹H NMR (400 MHz, MeOD) δ 7.79 (d, *J* = 8.3 Hz, ²H), 7.43 (d, *J* = 8.3 Hz, ²H), 6.25 (s, ¹H), 3.71 (s, ²H), 3.49 (s, ²H), 3.35 (s, ¹H), 2.65 (s, ²H), 2.43 (s, ²H), 1.80 (s, ⁴H), 1.44 (d, *J* = 6.6 Hz, ³H). ¹³C NMR (126 MHz, MeOD) δ 165.91, 164.78, 147.49, 138.80, 128.56, 128.05, 93.58, 62.13, 61.53, 54.03, 44.18, 24.16,

22.83. HRMS for $C_{18}H_{26}N_5O$ $[M+H]^+$ calc. 328.21318, found 328.21385 (error = -1.87 ppm).

1H NMR and ^{13}C NMR Spectrum of 2-((2-amino-6-(4-(1-(pyrrolidin-1-yl)ethyl)phenyl) pyrimidin-4-yl)amino)ethanol

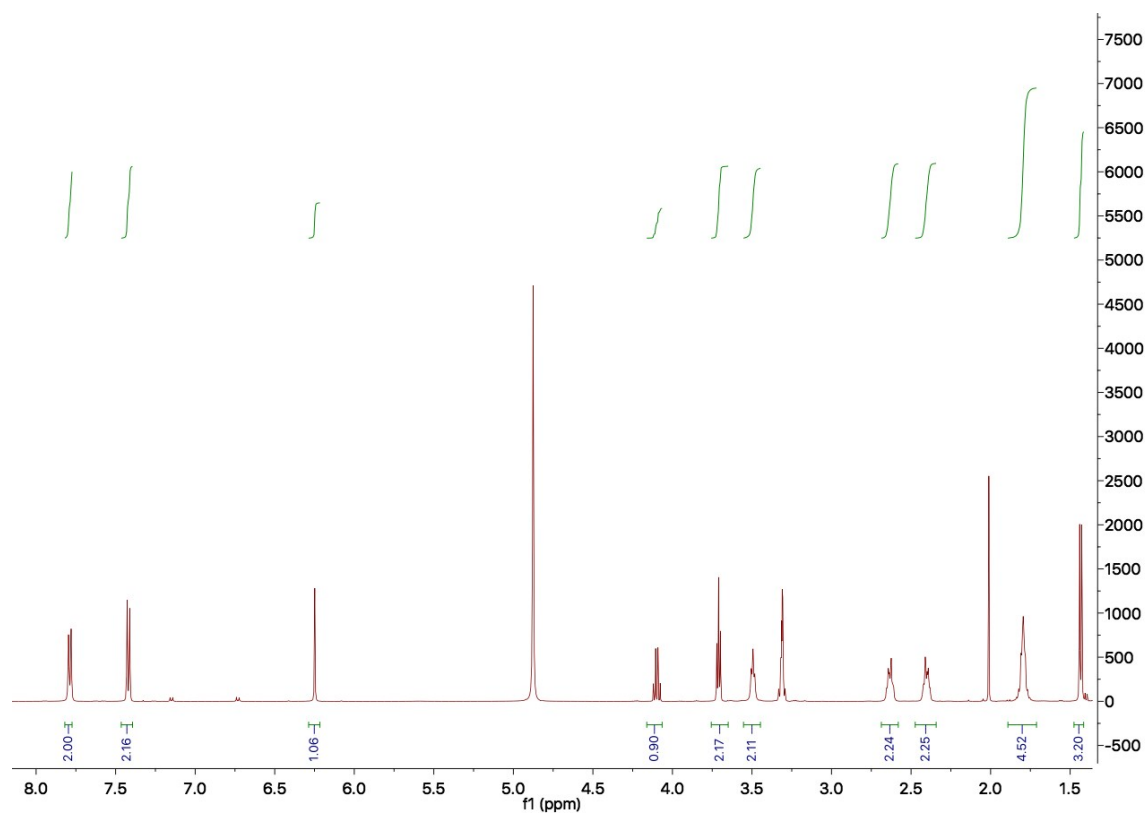


Figure S2: 1H NMR spectrum for 2-((2-amino-6-(4-(1-(pyrrolidin-1-yl)ethyl)phenyl) pyrimidin-4-yl)amino)ethanol (20mM, 400MHz, MeOD, 298K)

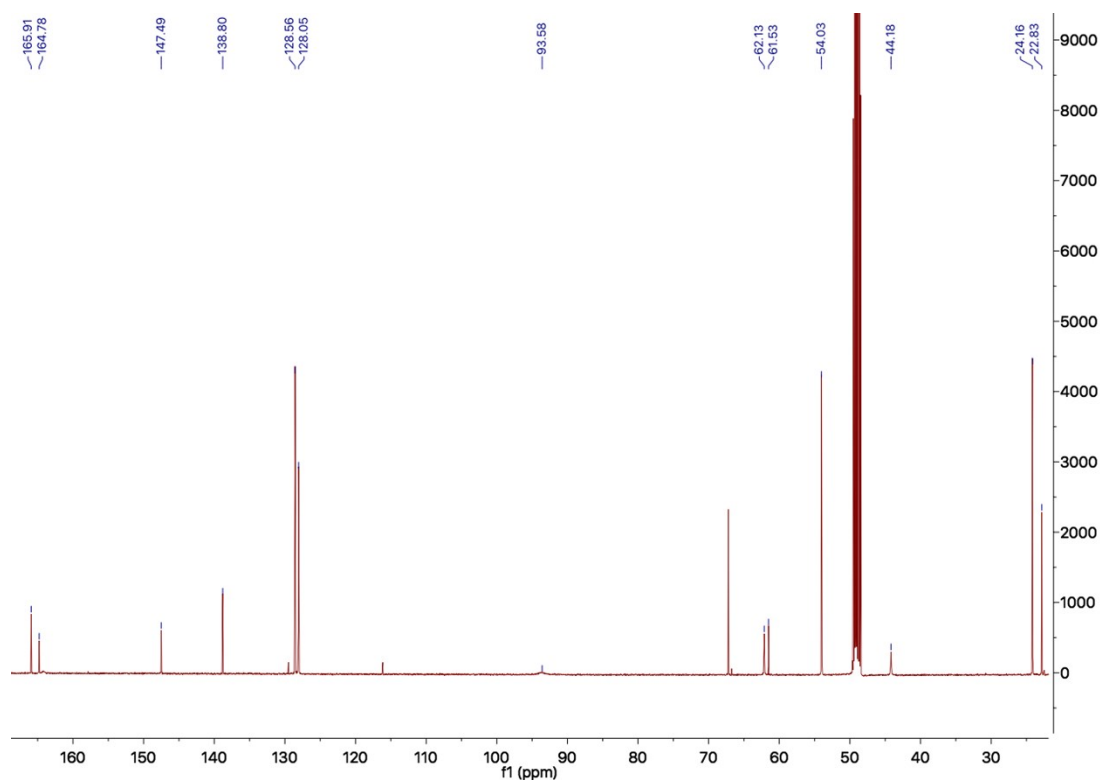


Figure S3: ^{13}C NMR spectrum for 2-((2-amino-6-(4-(1-(pyrrolidin-1-yl)ethyl)phenyl)pyrimidin-4-yl)amino)ethanol (50mM, 500MHz, MeOD, 298K)

Circular Dichroism Measurements

Unlabeled *hras-1^Y* was annealed at 95°C in 50mM KCl, 50mM Tris-HCl or Tris-Acetate buffer at varying pH for 3 minutes and cooled to room temperature prior to any experimentation. All experiments were run on a Model 420 Circular Dichroism Spectrometer by AVIV Inc. (Lakewood, NJ), and data were analyzed in GraphPad Prism.

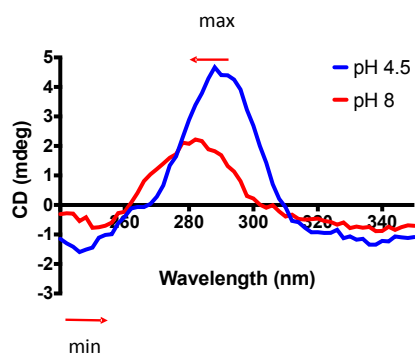


Figure S4: CD measurement of unlabeled *hras*-1^Y in 50mM KCl, 50mM Tris-HCl buffer at pH 4.5 and 8. The peak maximum at pH 4.5 was ~289nm and for pH8 it was ~282nm.

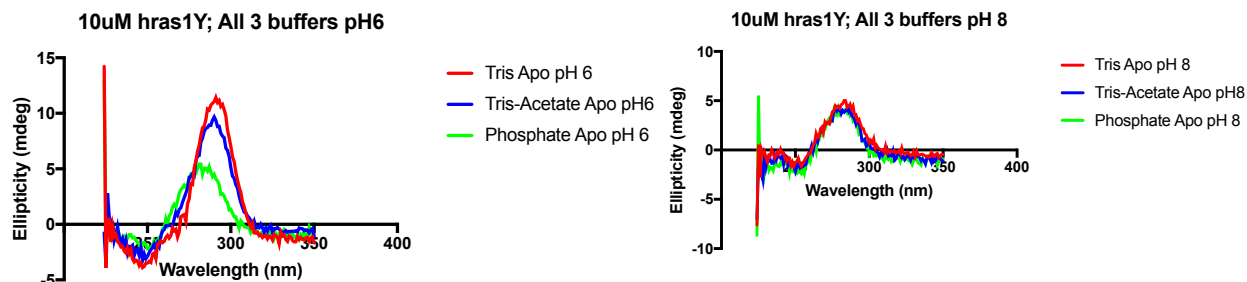


Figure S5: CD measurement of unlabeled *hras*-1^Y in 50mM KCl and 50mM of either Tris-HCl, Tris-Acetate or Phosphate buffer at pH 6 or pH 8.

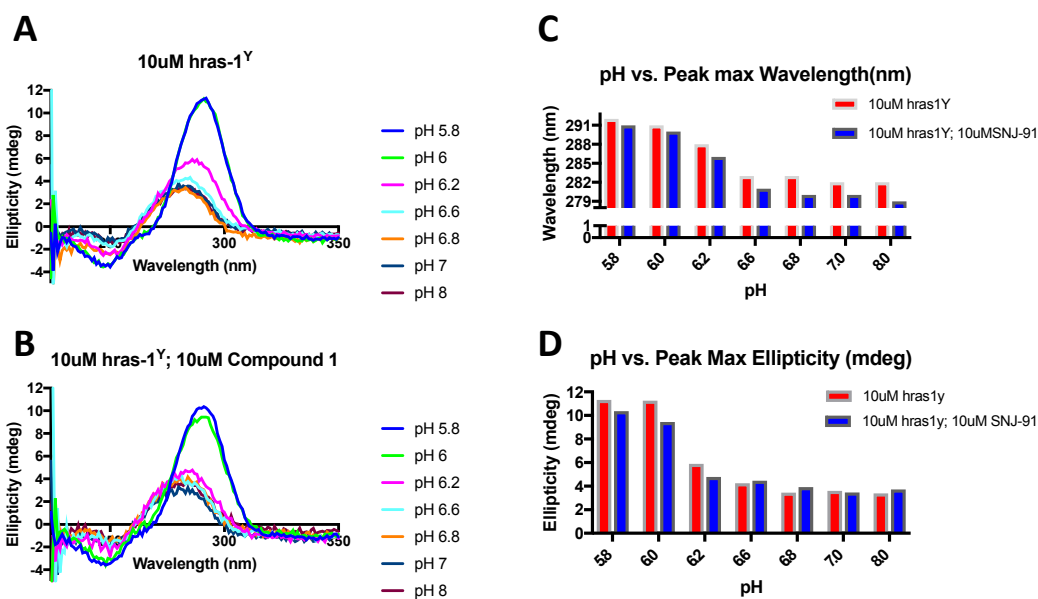


Figure S6: CD measurement of a.) unlabeled *hras*-1^Y in 50mM KCl and 50mM Tris-HCl, and varying pH buffer, b.) unlabeled *hras*-1^Y with 1 equivalent of compound present in the same buffer at varying pH's. Graphical representation of the compound induced peak changes from graph A to graph B for c.) peak max wavelength and d.) peak max ellipticity.

Surface Plasmon Resonance (SPR)

A Biacore 3000 (Biacore, Inc.; GE Healthcare Lifesciences) instrument was used for all SPR experimentation. All experiments were performed at 23°C. Gold plated Sensor Chip CM5 (GE Healthcare Lifesciences) were primed twice with SPR buffer (50mM KCl, 50mM Tris-HCl, pH 6, 2.5% DMSO, 0.005% Tween) prior to injection of reagents. A 0.4M EDC/ 0.1M NHS solution flowed over the chip at 5μL/min. Streptavidin

(ThermoFisher) was then immobilized to the chip to ~24000 Reference Units (RU) for both flow cells. The streptavidin-laced surface was then blocked with 10mM ethanolamine pH8 solution. A 1M NaCl/50mM NaOH solution then flowed at 50 μ L/min over the surface to remove any non-bound streptavidin. Biotin appended *hras*-1^Y oligos were purchased from Integrated DNA Technologies Inc (HPLC purified) and were annealed at 95°C for 3 minutes, then were cooled to room temperature for 1h. A 10 μ M oligo stock was injected at 2 μ L/min over the second flow cell. The flow cells were reference subtracted and had a 2000-3000 RU difference.

Each compound was diluted to either 300 μ M or 100 μ M, then diluted two-fold in the SPR buffer, and varied depending on each compound. Each dilution flowed over the flow cell at 30 μ L/min and was repeated two times.

The SPR data were analyzed using BIAevaluation Software (GE Healthcare Lifesciences). Each trace was either fit to the Langmuir model, or was evaluated using methods for non-specific binding. If there was non-specific binding present, each trace peak was recorded and fit into Prism (Graphpad). Then, the curve was analyzed One Site-Total to determine a K_D .

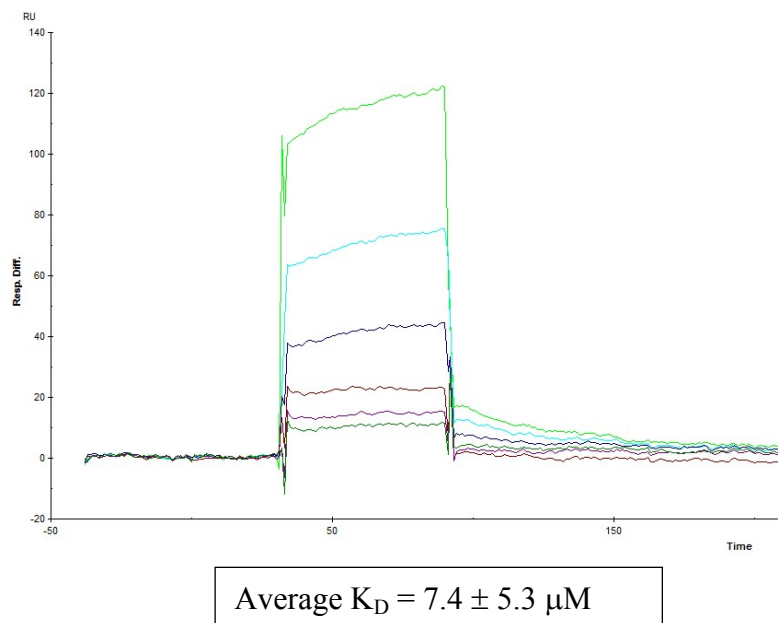


Figure S7: SPR data for compound 27001422 for Trial 1 showing the plots of each concentration stacked and the average K_D .

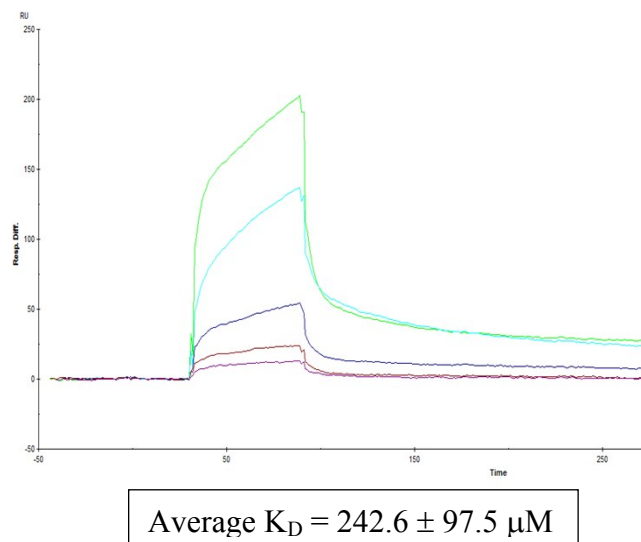


Figure S8: SPR data for compound 27067624 for Trial 1 showing the plots of each concentration stacked and the average K_D .

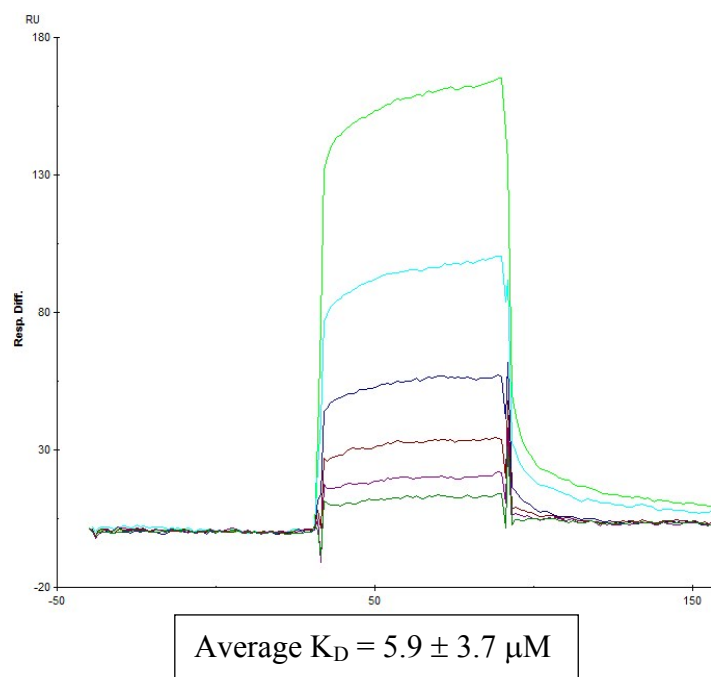


Figure S9: SPR data for compound 16967942 for Trial 1 showing the plots of each concentration stacked and the average K_D .

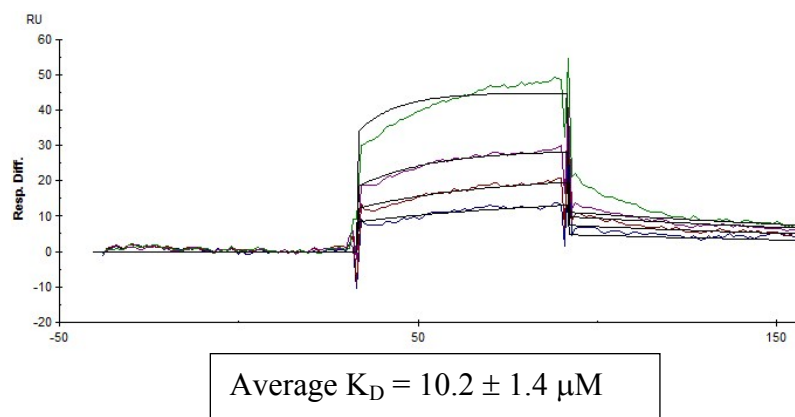


Figure S10: SPR data for compound 17359670 for Trial 1 showing the plots of each concentration stacked and the average K_D .

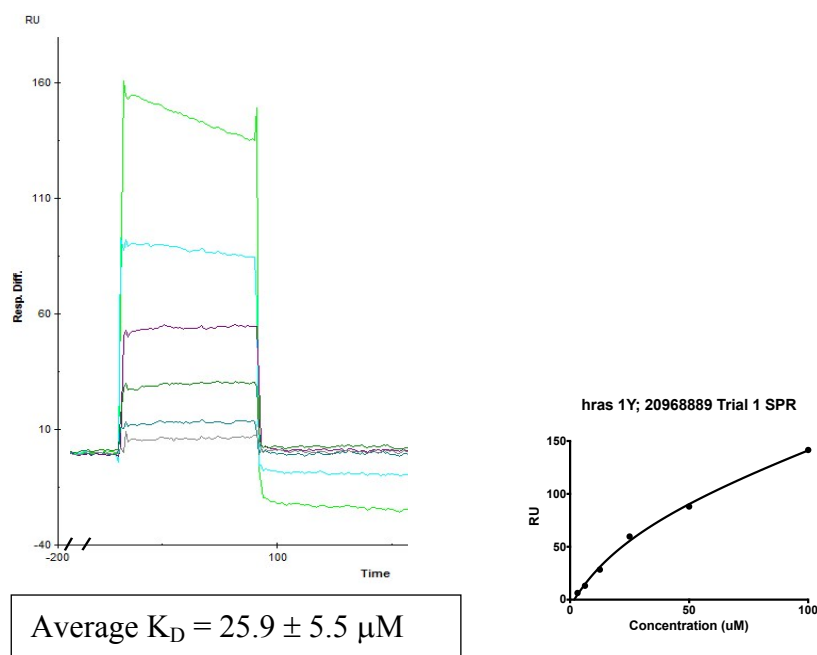
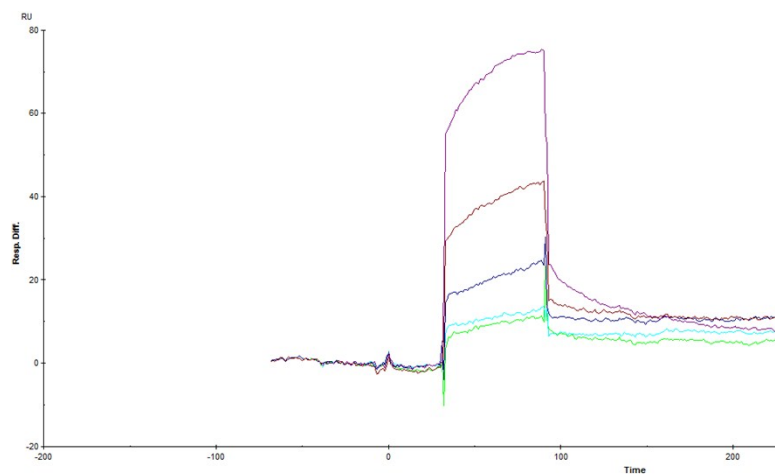
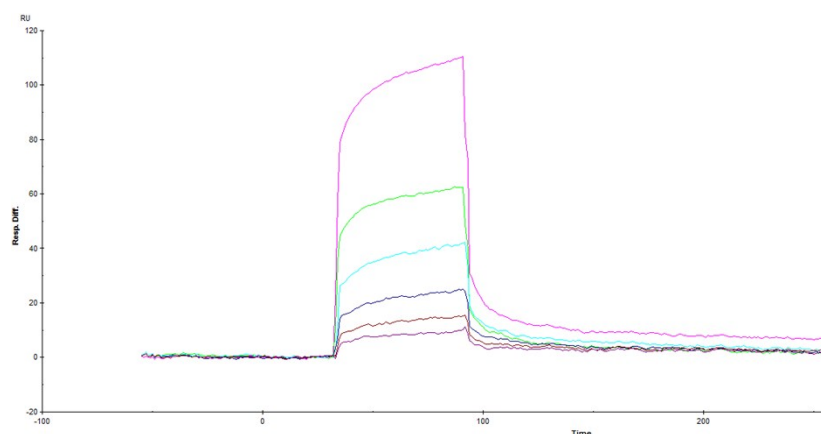


Figure S11: SPR data for compound 28068889 which shows the plots of each concentration stacked and the averaged K_D , and the Prism curve corresponding to each trial.



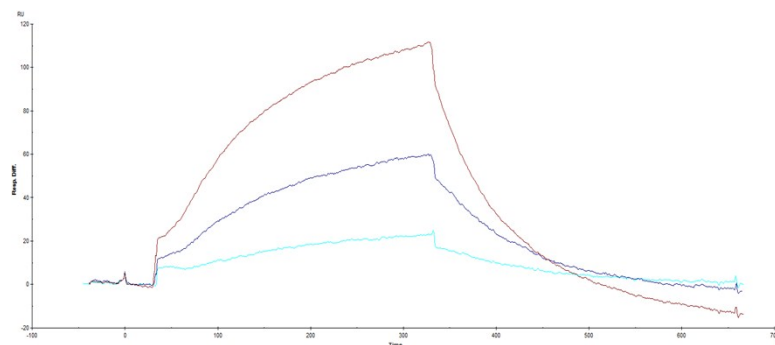
Average $K_D = 78 \pm 98 \mu\text{M}$

Figure S12: SPR data for compound 27263777 for Trial 1 showing the plots of each concentration stacked and the average K_D .



Average $K_D = 51 \pm 3.0 \mu\text{M}$

Figure S13: SPR data for compound 95636859 for Trial 1 showing the plots of each concentration stacked and the average K_D .



Average $K_D = 3350 \pm 1370 \mu\text{M}$

Figure S14: SPR data for compound 30918647 for Trial 1 showing the plots of each concentration stacked and the average K_D .

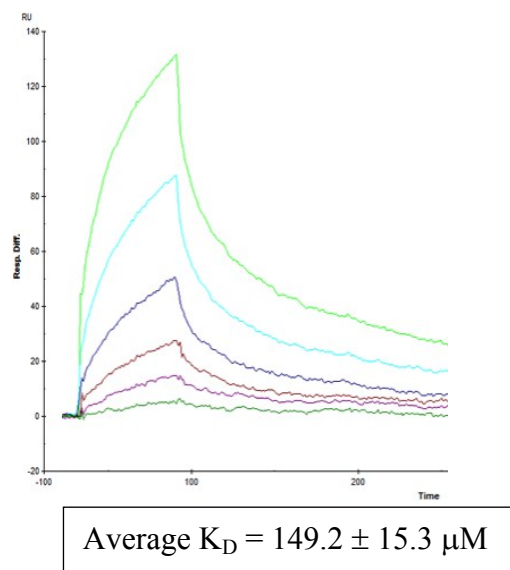


Figure S15: SPR data for compound 62080396 for Trial 1 showing the plots of each concentration stacked and the average K_D .

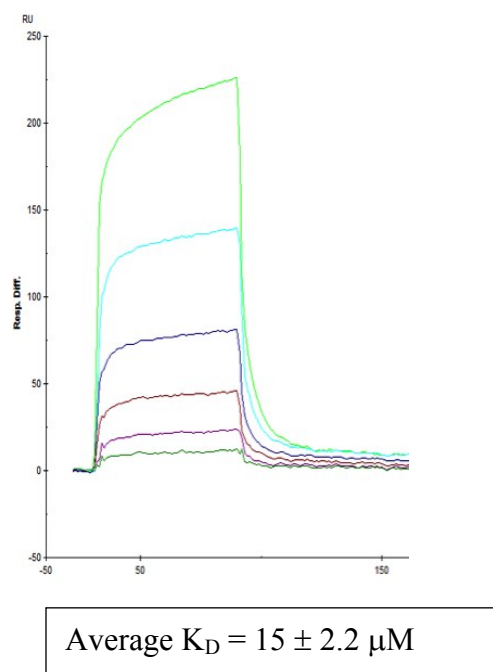


Figure S16: SPR data for compound 27297065 for Trial 1 showing the plots of each concentration stacked and the average K_D .

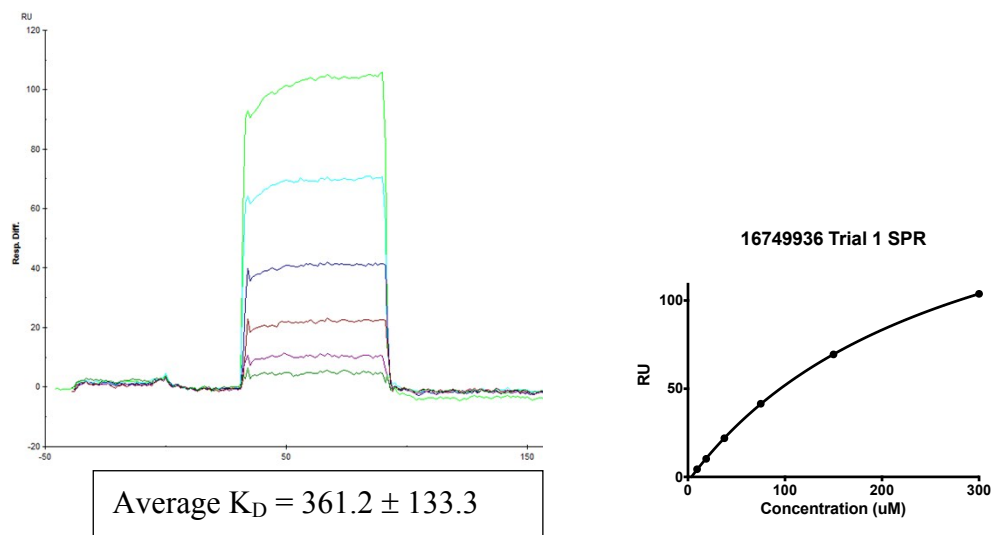


Figure S17: SPR data for compound 16749936 which shows the plots of each concentration stacked and the averaged K_D , and the Prism curve corresponding to each trial.

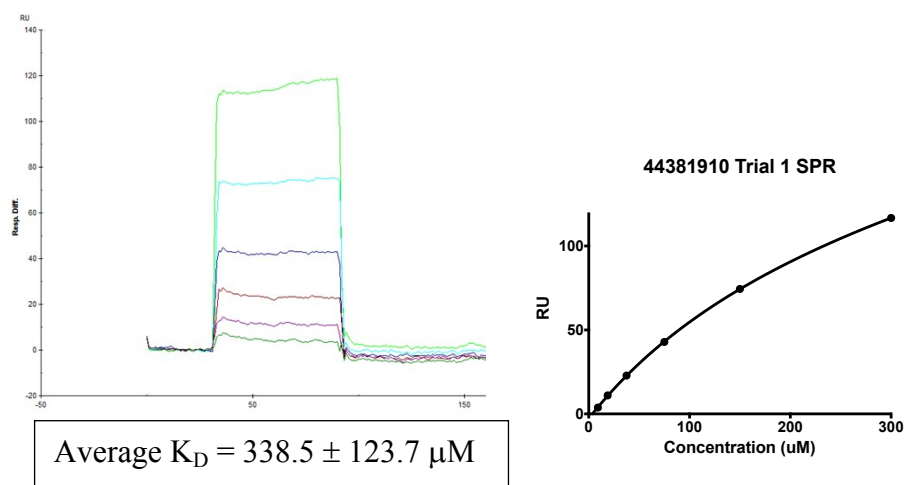


Figure S18: SPR data for compound 44381910 which shows the plots of each concentration stacked and the averaged K_D , and the Prism curve corresponding to each trial.

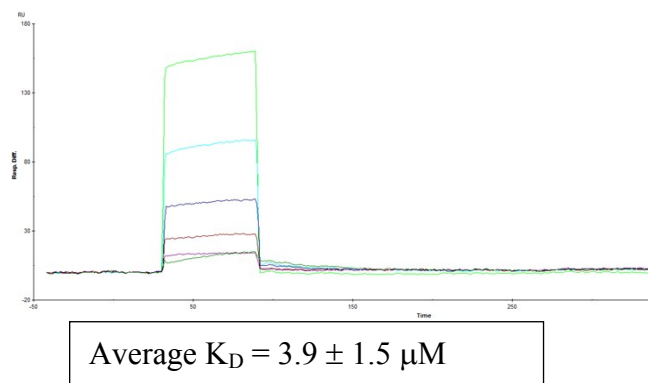


Figure S19: SPR data for compound SNJ-91 for Trial 1 showing the plots of each concentration stacked and the average K_D .

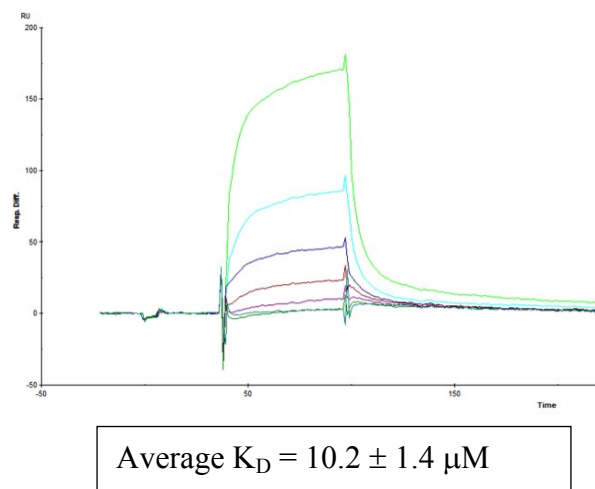


Figure S20: SPR data for compound 59261919 for Trial 1 showing the plots of each concentration stacked and the average K_D .

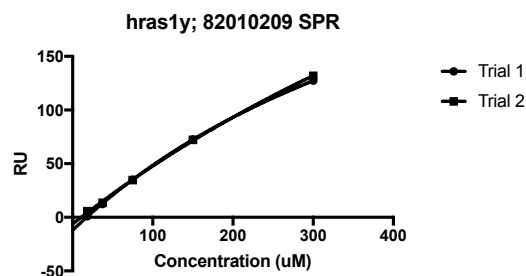
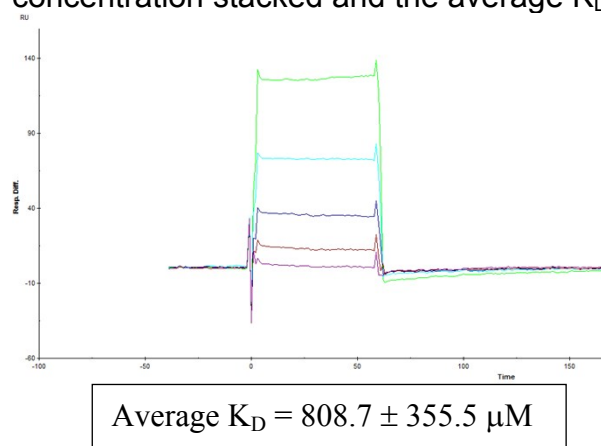


Figure S21: SPR data for compound 82010209 which shows the plots of each concentration stacked and the averaged K_D , and the Prism curve corresponding to each trial.

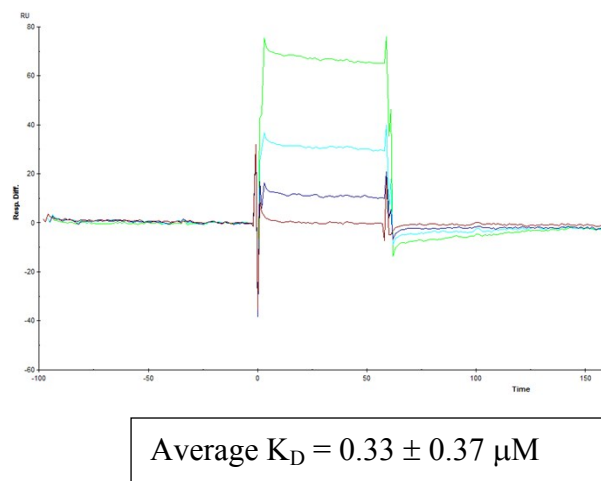


Figure S22: SPR data for compound 62400924 for Trial 1 showing the plots of each concentration stacked and the average K_D .

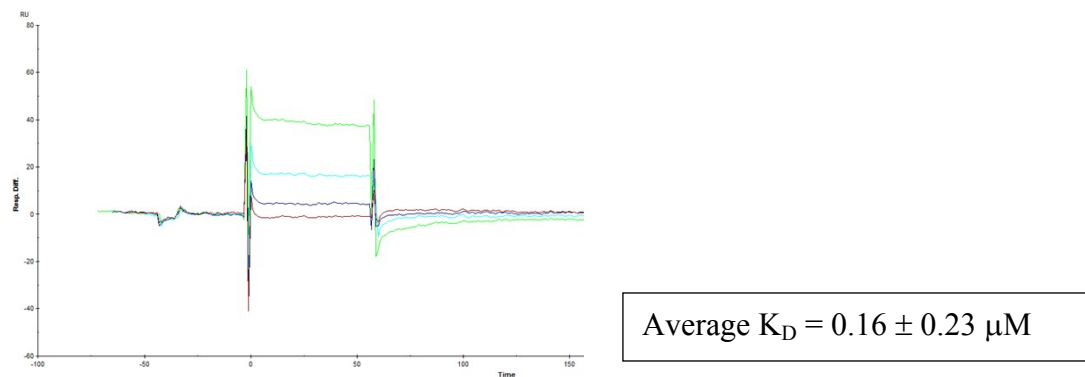


Figure S23: SPR data for compound 94230832 for Trial 1 showing the plots of each concentration stacked and the average K_D .

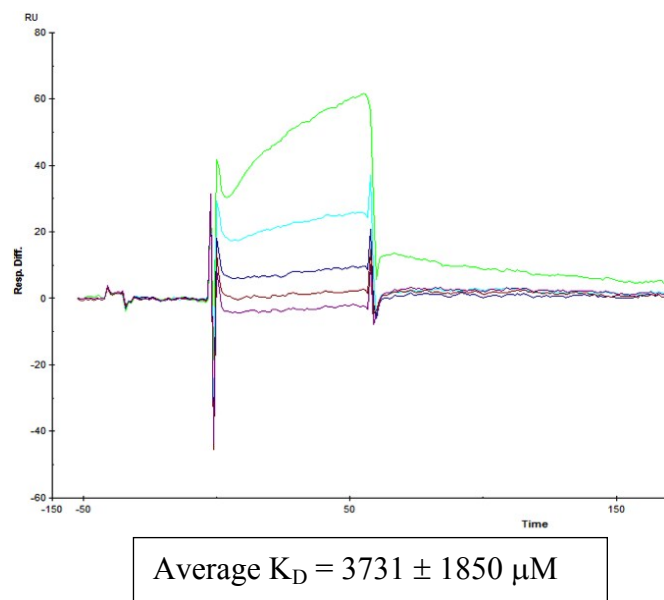


Figure S24: SPR data for compound 46168464 for Trial 1 showing the plots of each concentration stacked and the average K_D .

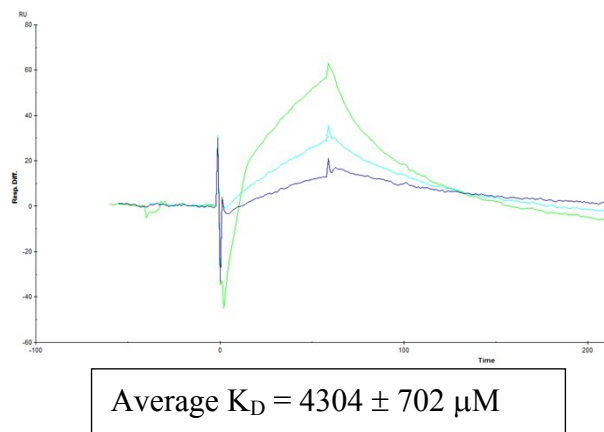


Figure S25: SPR data for compound 61479509 for Trial 1 showing the plots of each concentration stacked and the average K_D .

Fluorescence Titrations of Alexo Fluor 647 Labeled *hras-1^Y*

A647-*hras-1^Y* 10uM stocks were stored at -20°C . All compounds were stored at -20°C at 20mM concentrations. Experiments were performed on a Photon Technology 400 Fluorimeter and detected into Felix GX 4.2.2 software. Fluorescence emission measurements were measured in a Quartz SUPRASIL[®] cuvette (10x4 mm path length) purchased from Hellma[®] Analytics (Germany). All measurements were excited at 645 nm and data was collected in an emission range of 650-800 nm. Solutions were prepared in 50mM KCl, 50mM Tris-HCl buffer at pH6. Each sample contained 972.5 μL buffer, 25 μL DMSO (2.5%), and 2.5 μL A647-*hras-1^Y* (25nM). 200 μM , 2.5M, and 5M compound dilutions were prepared from the 20mM stock solutions. Fluorescence titrations were measured at varying concentrations, with a <1% change in DMSO, and <100 μL change in volume. Each analysis was performed in triplicate. Blank emission spectra were taken prior to the addition of *hras-1^Y*, which was reference subtracted from each spectrum for data analysis. To determine K_D , data points were taken from the fluorescence peak at 666 nm, and then normalized to account for the change in volume. The graphs were then fit through Prism (Graphpad) with One Site-Total or One Site-Total with Nonspecific binding.

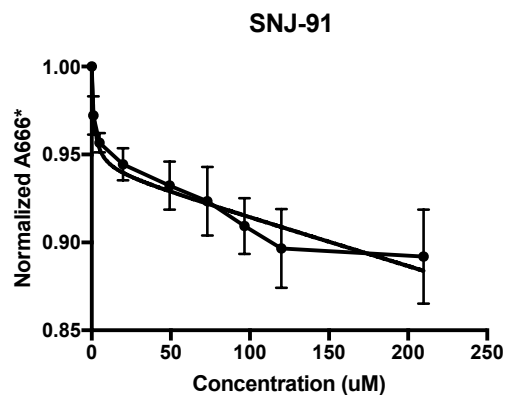


Figure S26: Fluorescence Titration data for compound SNJ-91 as an average of three replicates.

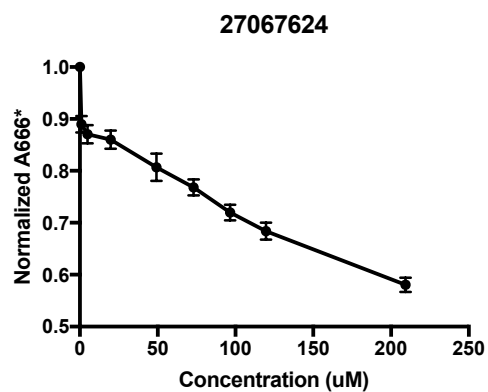


Figure S27: Fluorescence Titration data for compound 27067624 as an average of three replicates.

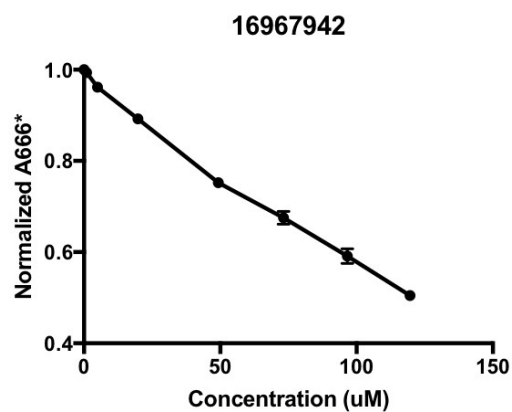


Figure S28: Fluorescence Titration data for compound 16967942 as an average of three replicates.

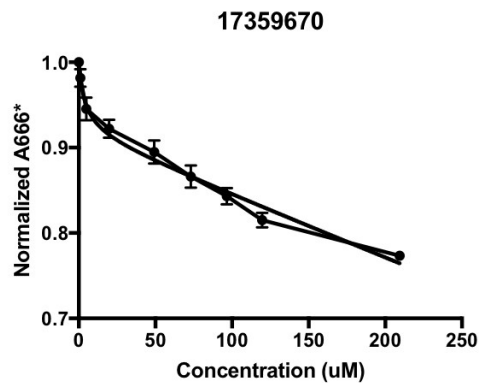


Figure S29: Fluorescence Titration data for compound 17359670 as an average of three replicates.

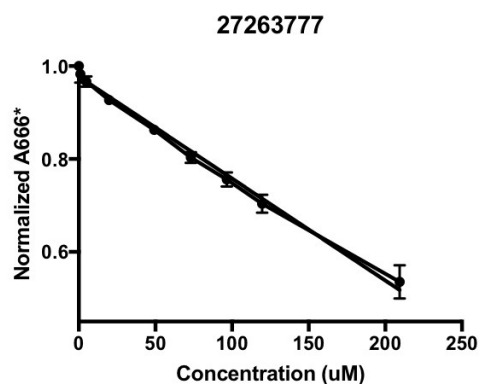


Figure S30: Fluorescence Titration data for compound 27263777 as an average of three replicates.

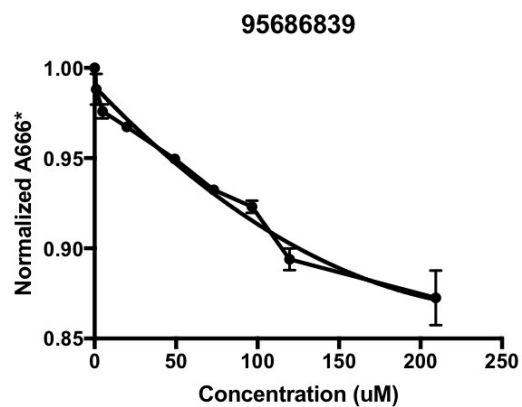


Figure S31: Fluorescence Titration data for compound 95636859 as an average of three replicates.

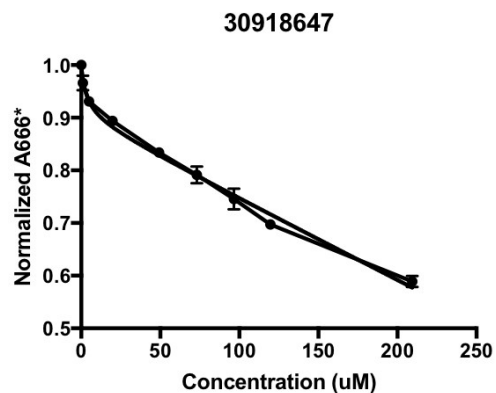


Figure S32: Fluorescence Titration data for compound 30918647 as an average of three replicates.

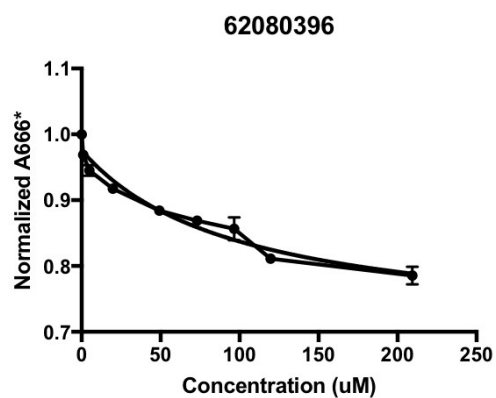


Figure S33: Fluorescence Titration data for compound 62080396 as an average of three replicates.

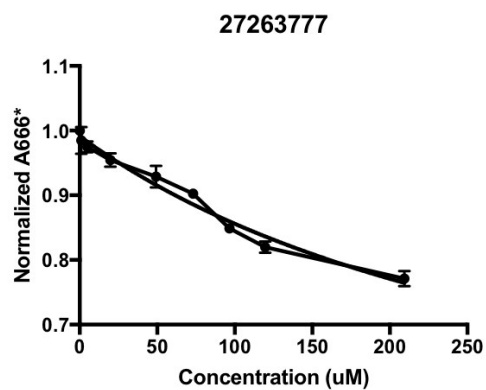


Figure S34: Fluorescence Titration data for compound 27297065 as an average of three replicates.

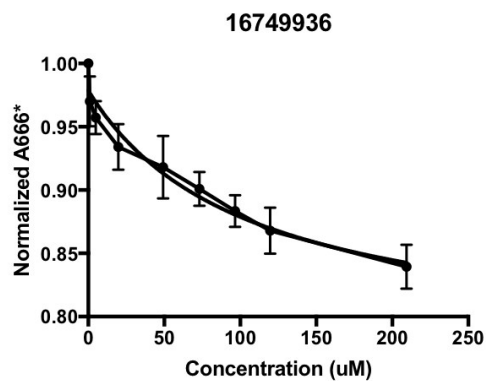


Figure S35: Fluorescence Titration data for compound 16749936 as an average of three replicates.

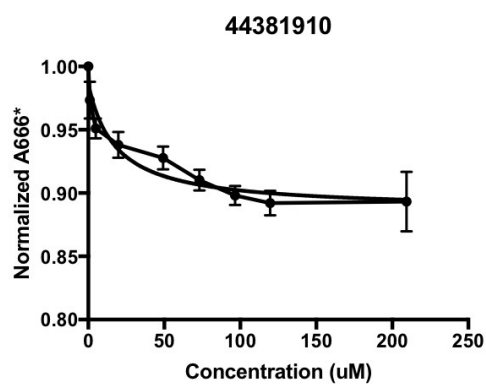


Figure S36: Fluorescence Titration data for compound 44381910 as an average of three replicates.

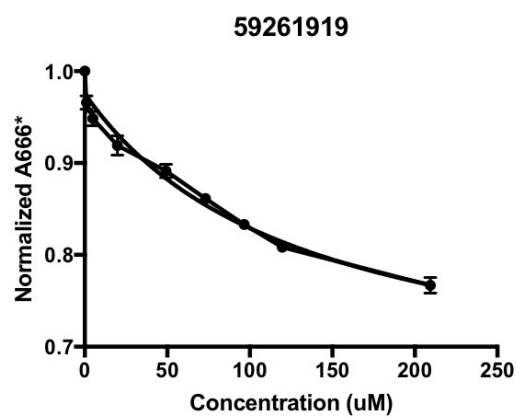


Figure S37: Fluorescence Titration data for compound 59261919 as an average of three replicates.

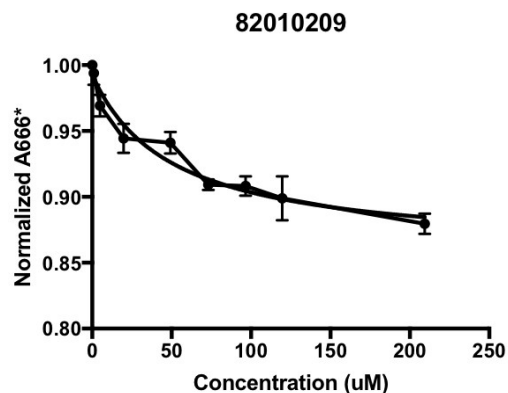


Figure S38: Fluorescence Titration data for compound 82010209 as an average of three replicates.

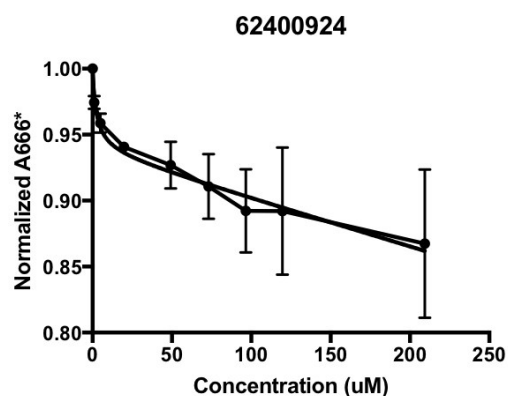


Figure S39: Fluorescence Titration data for compound 62400924 as an average of three replicates.

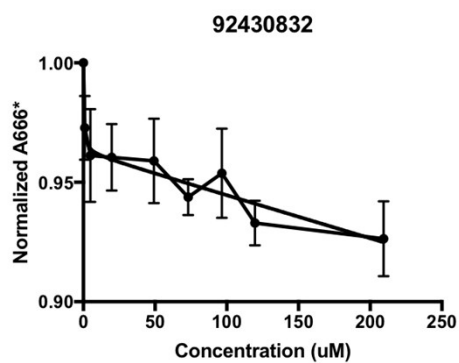


Figure S40: Fluorescence Titration data for compound 92430832 as an average of three replicates.

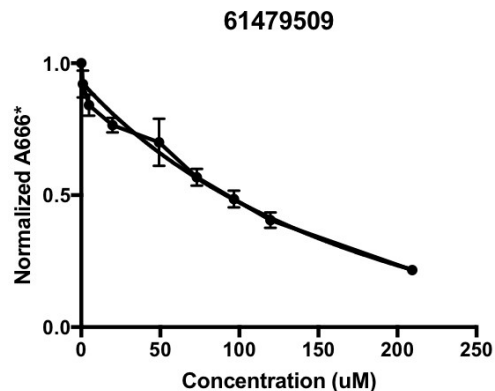


Figure S41: Fluorescence Titration data for compound 61479509 as an average of three replicates.

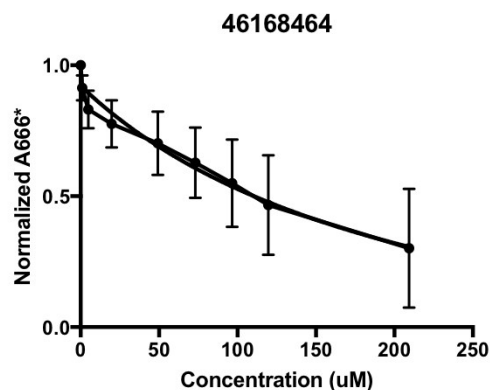


Figure S42: Fluorescence Titration data for compound 46168464 as an average of three replicates.

WaterLOGSY Nuclear Magnetic Resonance Experiments

All compounds were stored at 20mM concentrations. All experiments were performed on a 500MHz Bruker Topspin 3.0 NMR. Samples were prepared with 10% D₂O, 3% D-DMSO, 100μM N-methylvaline, and 100μM of compound suspended in 50mM KCl, 25mM D-Tris-HCl, pH6 or pH8 buffer. ¹H NMR and WaterLOGSY measurements were performed on the sample without unlabeled *hras*-1^Y DNA at 128 and 1024 scans respectively. 5 μM unlabeled *hras*-1^Y DNA was added to the sample and a WaterLOGSY experiment was performed with 2048 scans.

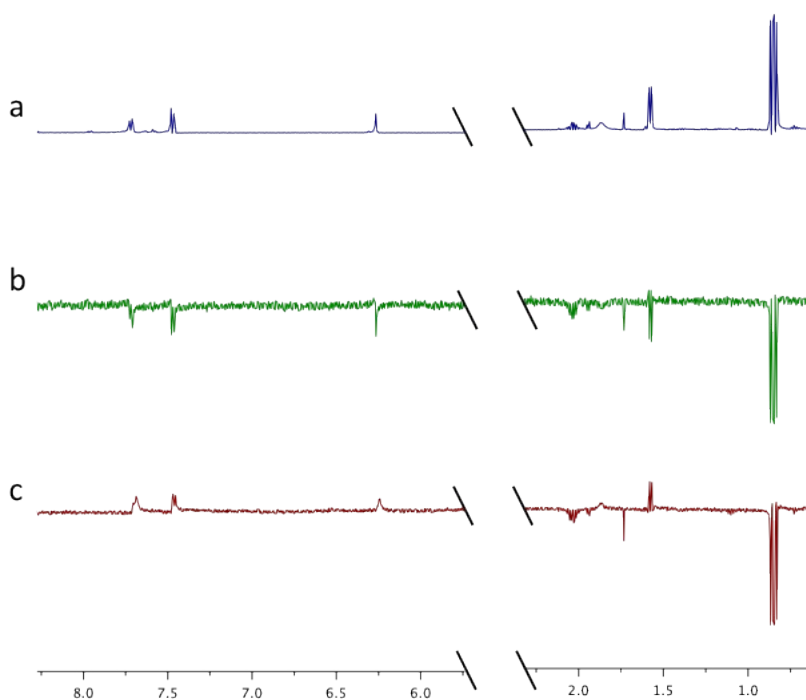


Figure S43: WaterLOGSY experiment at pH 6 for compound 27001422 with a.) ^1H NMR, b.) WaterLOGSY without unlabeled *hras-1*^Y, and c.) WaterLOGSY with unlabeled *hras-1*^Y (500MHz, H₂O/D₂O/salt, 298K)

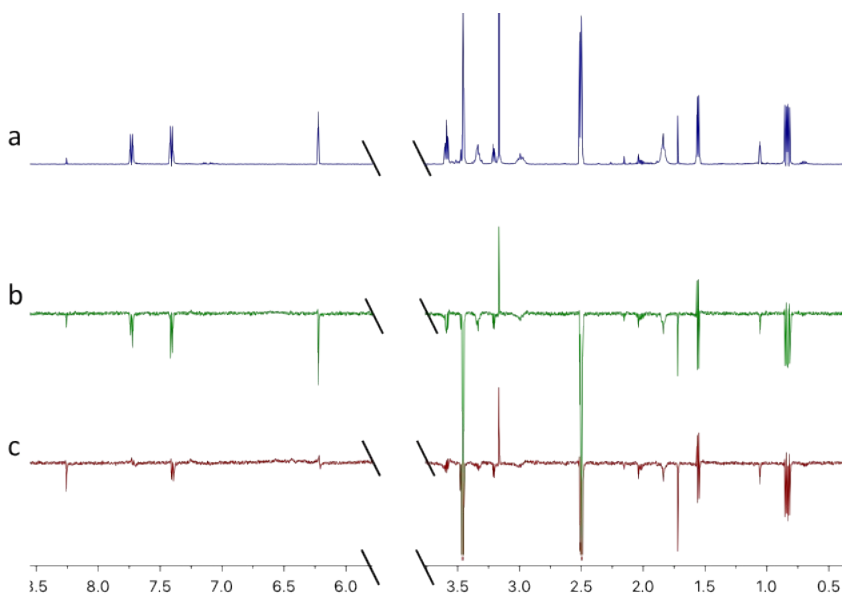


Figure S44: WaterLOGSY experiment at pH 8 for compound 27001422 with a.) ^1H NMR, b.) WaterLOGSY without unlabeled *hras-1*^Y, and c.) WaterLOGSY with unlabeled *hras-1*^Y (500MHz, H₂O/D₂O/salt, 298K)

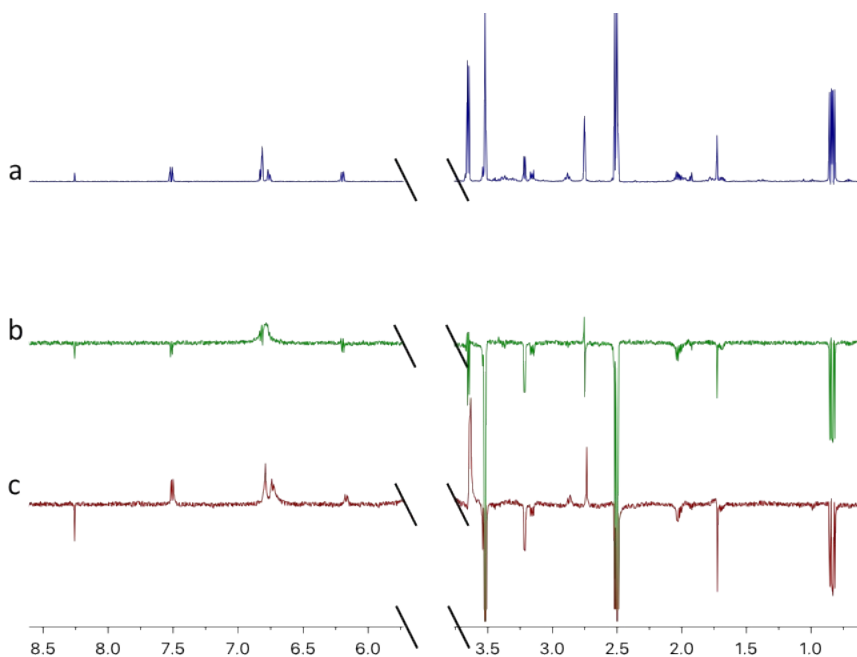


Figure S45: WaterLOGSY experiment at pH 6 for compound 16967942 with a.) ^1H NMR, b.) WaterLOGSY without unlabeled *hras-1^Y*, and c.) WaterLOGSY with unlabeled *hras-1^Y* (500MHz, H₂O/D₂O/salt, 298K)

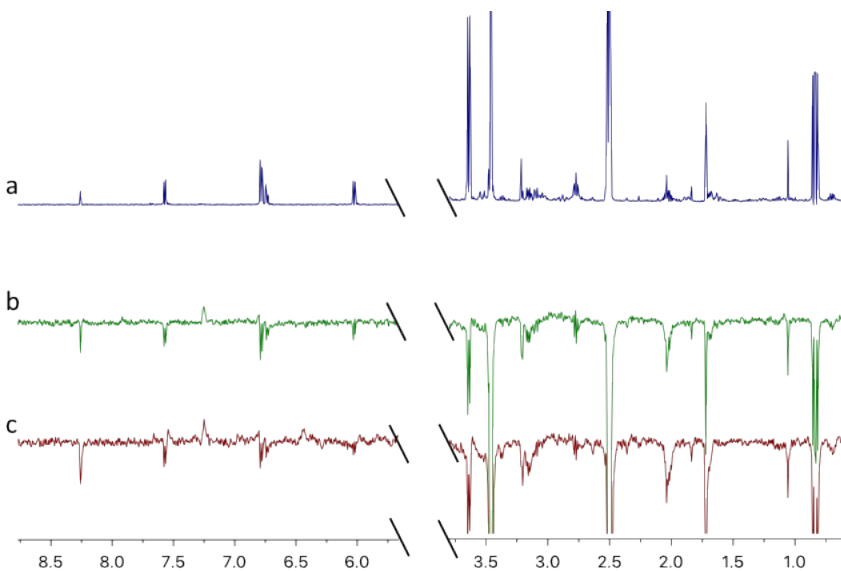


Figure S46: WaterLOGSY experiment at pH 8 for compound 16967942 with a.) ^1H NMR, b.) WaterLOGSY without unlabeled *hras-1^Y*, and c.) WaterLOGSY with unlabeled *hras-1^Y* (500MHz, H₂O/D₂O/salt, 298K)

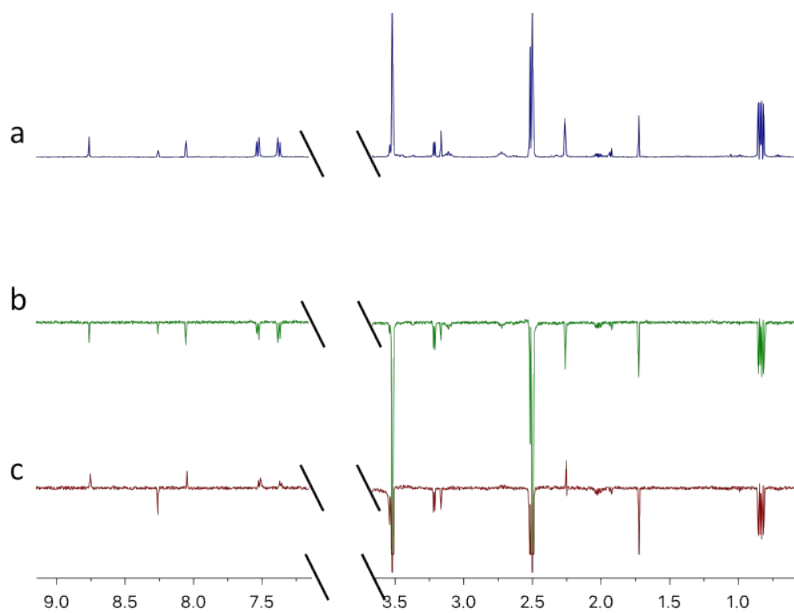


Figure S47: WaterLOGSY experiment at pH 6 for compound 17359670 with a.) ^1H NMR, b.) WaterLOGSY without unlabeled *hras-1*^Y, and c.) WaterLOGSY with unlabeled *hras-1*^Y (500MHz, H₂O/D₂O/salt, 298K)

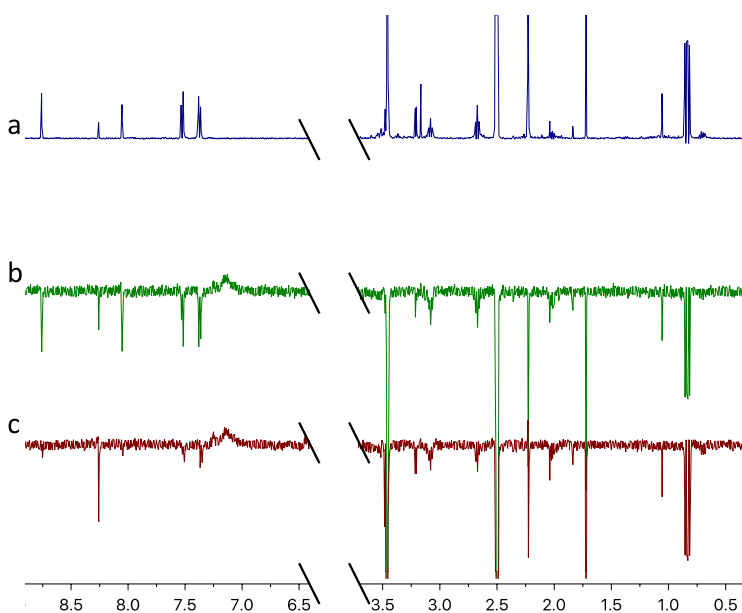


Figure S48: WaterLOGSY experiment at pH 8 for compound 17359670 with a.) ^1H NMR, b.) WaterLOGSY without unlabeled *hras-1*^Y, and c.) WaterLOGSY with unlabeled *hras-1*^Y (500MHz, H₂O/D₂O/salt, 298K)

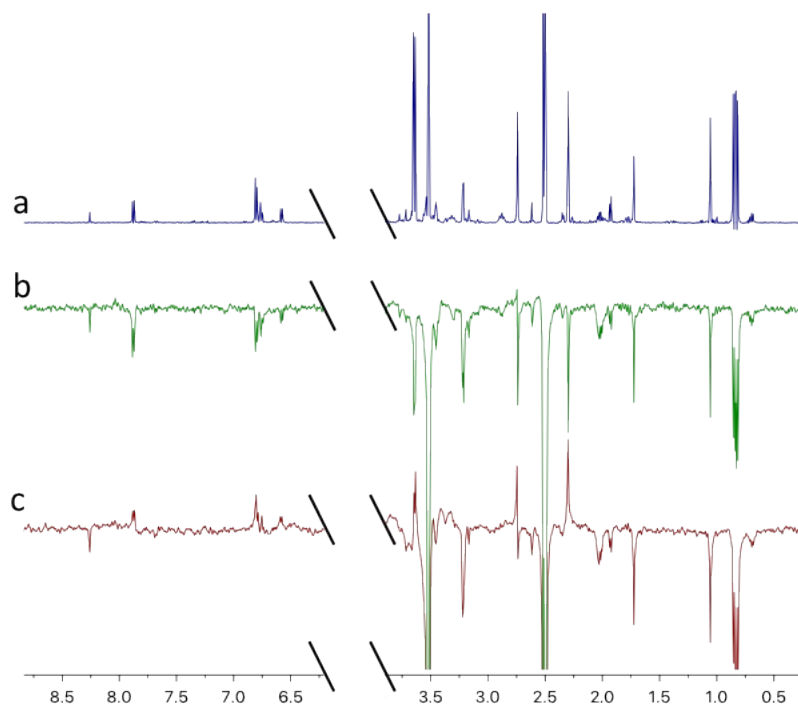


Figure S49: WaterLOGSY experiment at pH 6 for compound 62400924 with a.) ^1H NMR, b.) WaterLOGSY without unlabeled *hras-1^Y*, and c.) WaterLOGSY with unlabeled *hras-1^Y* (500MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}/\text{salt}$, 298K)

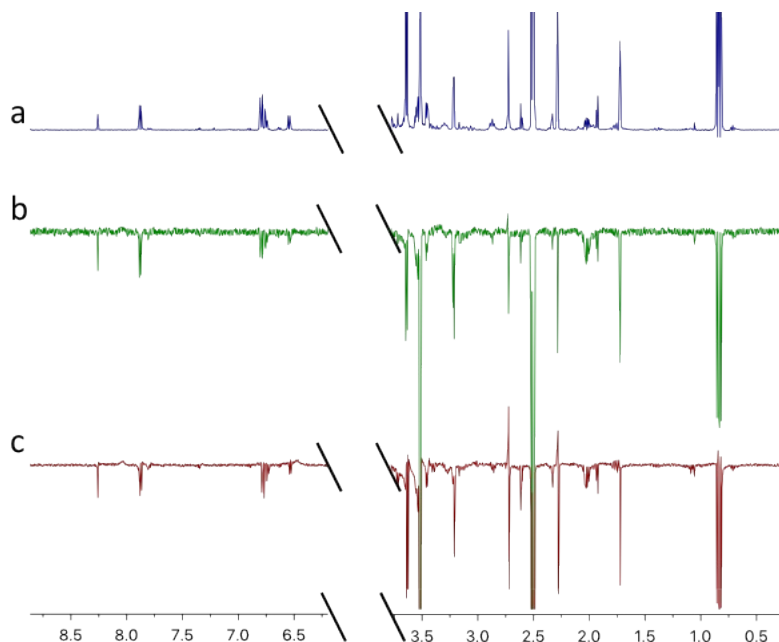


Figure S50: WaterLOGSY experiment at pH 8 for compound 62400924 with a.) ^1H NMR, b.) WaterLOGSY without unlabeled *hras-1^Y*, and c.) WaterLOGSY with unlabeled *hras-1^Y* (500MHz, $\text{H}_2\text{O}/\text{D}_2\text{O}/\text{salt}$, 298K)

Chemical Shift Perturbations

Unlabeled *hras*-1^Y was annealed at 95°C for 3 minutes prior to experimentation. Compounds were diluted in 10% D₂O, and a 50mMKCl, 25mM D-Tris/D-Acetate, pH 5.99 buffer resulting in a 3% D-DMSO, 0.6mM stock. All ¹H NMR experiments were run at 512 scans on a 500MHz Bruker Topspin 3.0 NMR. Each sample was prepared with 10% D₂O, 3% D-DMSO, in 50mM KCl, 50mM D-Tris/D-Acetic Acid, pH 6 buffer, and 50 μM unlabeled *hras*-1^Y. After each NMR, the sample was removed and compound from the 0.6 mM compound stock (containing 10% D₂O) was titrated into the sample at varying equivalence (see Figure S55), and then placed back into the same NMR tube for immediate experimentation.

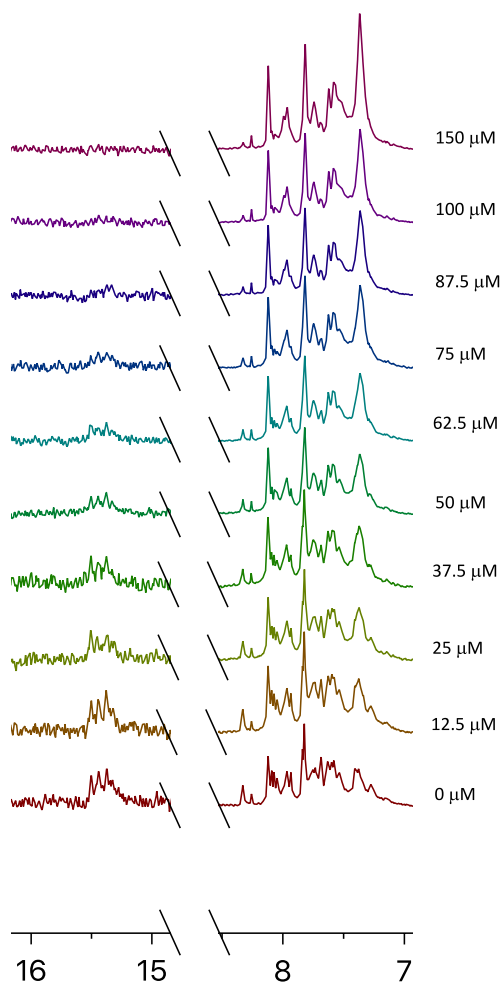


Figure S51: Chemical shift perturbation experiment for compound SNJ-91 (500MHz, H₂O/D₂O/salt, 298K)

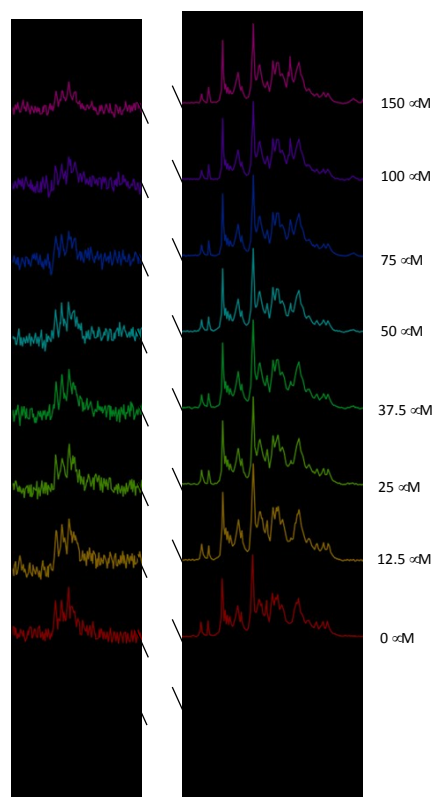


Figure S52: Chemical shift perturbation experiment for compound 16967942 (500MHz, H₂O/D₂O/salt, 298K)

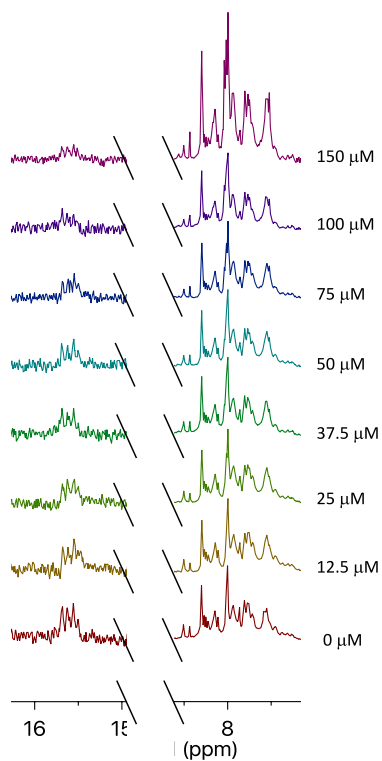


Figure S53: Chemical shift perturbation experiment for compound 62400924 (500MHz, H₂O/D₂O/salt, 298K)

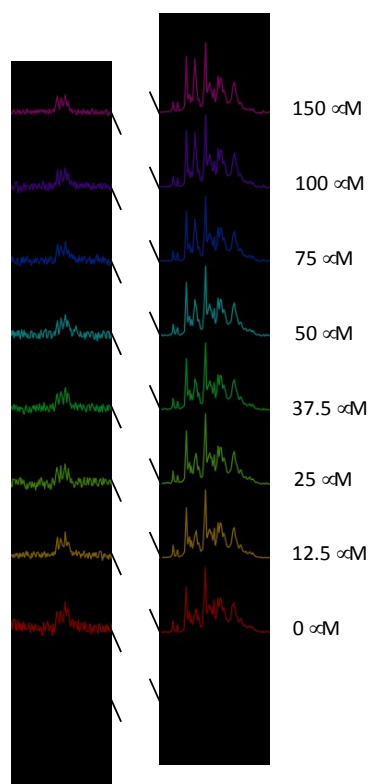


Figure S54: Chemical shift perturbation experiment for compound 94230832 (500MHz, H₂O/D₂O/salt, 298K)

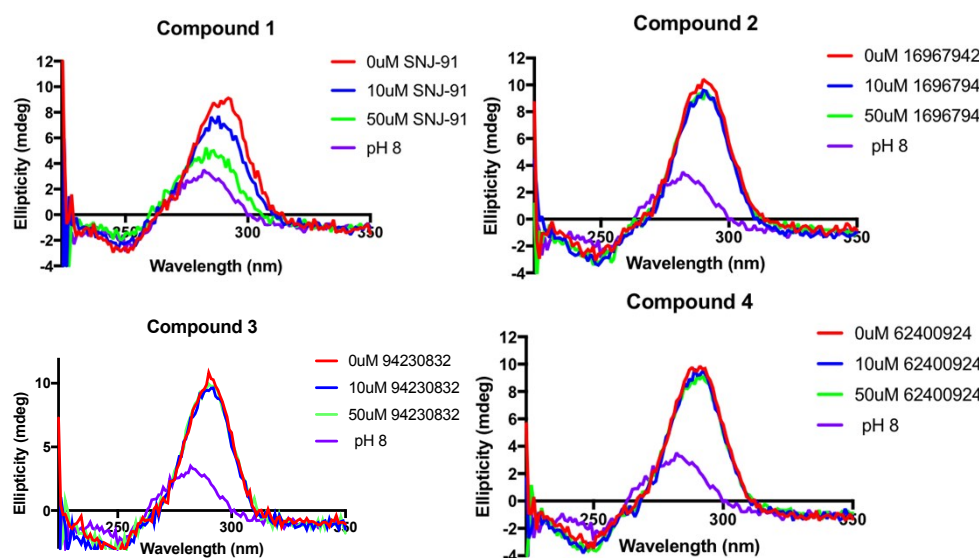


Figure S55: Circular dichroism for compound 1 (SNJ-91), compound 2 (16967942), compound 3 (94230832), and compound 4 (62400924) with 0 equivalence (0 μ M), 1 equivalence (10 μ M), and 5 equivalence (50 μ M) of SNJ-91. Each sample contained 3% DMSO and maintained 10 μ M of *hras-1*^Y.

FRET of *hras-1^Y* and SNJ-91

Experiments were performed on a Photon Technology 400 Fluorimeter and detected into Felix GX 4.2.2 software. Fluorescence emission measurements were measured in a Quartz SUPRASIL[®] cuvette (10x4 mm path length) purchased from Hellma[®] Analytics (Germany). All measurements were excited at 488 nm and data was collected in an emission range of 500-650 nm, identical to Migletta *et. al.*^[3] *hras-1^Y* was annealed at 90°C and let cool for 1h. Then, 50nM was suspended into FRET buffer (50mM KCl, 50mM Tris-Acetate, pH 6 or pH8 buffer with 5% DMSO) totaling 1mL of sample. Each compound was diluted in the FRET buffer (without added DMSO, and with 50nM FRET-*hras-1^Y*) to 1mM and was titrated into the sample at varying concentrations. After every titration, the sample was allowed to incubate at 23°C for 10 minutes prior to excitation.

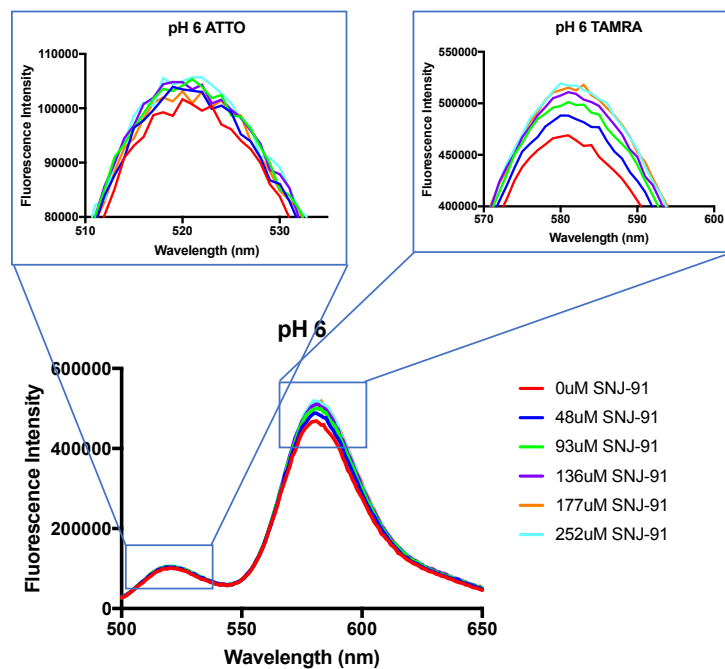


Figure S56: FRET experiment with 50nM *hras-1^Y* and titrated concentrations of SNJ-91.

Modeling Methods

Docking was done using the crystal structure of the d(ACCCT) i-motif (PDB code 1cn0).^[4] The terminal adenine and thymine residues were deleted. The central core of the i-motif is not symmetrical, but adopts a liferaft-like curved structure (Figure S58) with the phosphate groups on one side projecting outward to give a flat surface, and the phosphate groups on the other side projecting upwards to form a groove. The width of this groove is very close to the widened minor groove of B-DNA upon binding side-by-side ligands such as distomycin or other lexitropsins^[5,6] but the groove itself is shallower. This seemed the most logical binding site for small molecules interacting with the i-motif core.

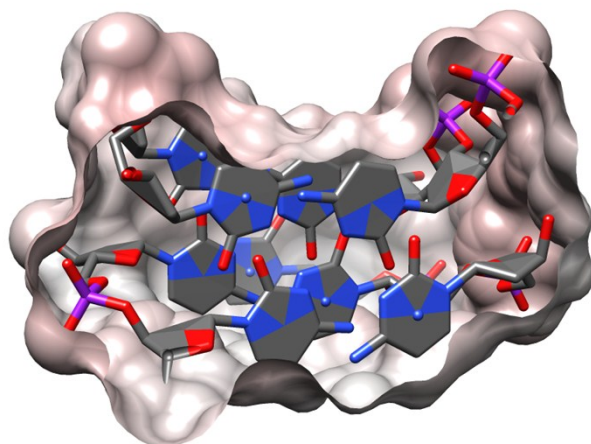


Figure S57: Cutaway view of the i-motif surface, looking down the main axis of the $[C\bullet CH]^+$ core. Bases and sugars are shown as filled rings, and the surface is colored by electrostatic potential. Figure rendered with Chimera.^[7,8]

An ensemble of structures for compounds **1** (Figure 7a and 7b) and **2** (Figure S59) was generated in LigPrep^[9] by enumerating ionization states over pH 6-8, stereoisomers, and low-energy unsaturated ring conformations. Structures were docked to the i-motif core with rDock, a docking program developed for nucleic acid targets as well as proteins.^[10] Fifty docking runs were performed for each structure, using the standard docking protocol and the SF5 scoring function that includes a desolvation potential.

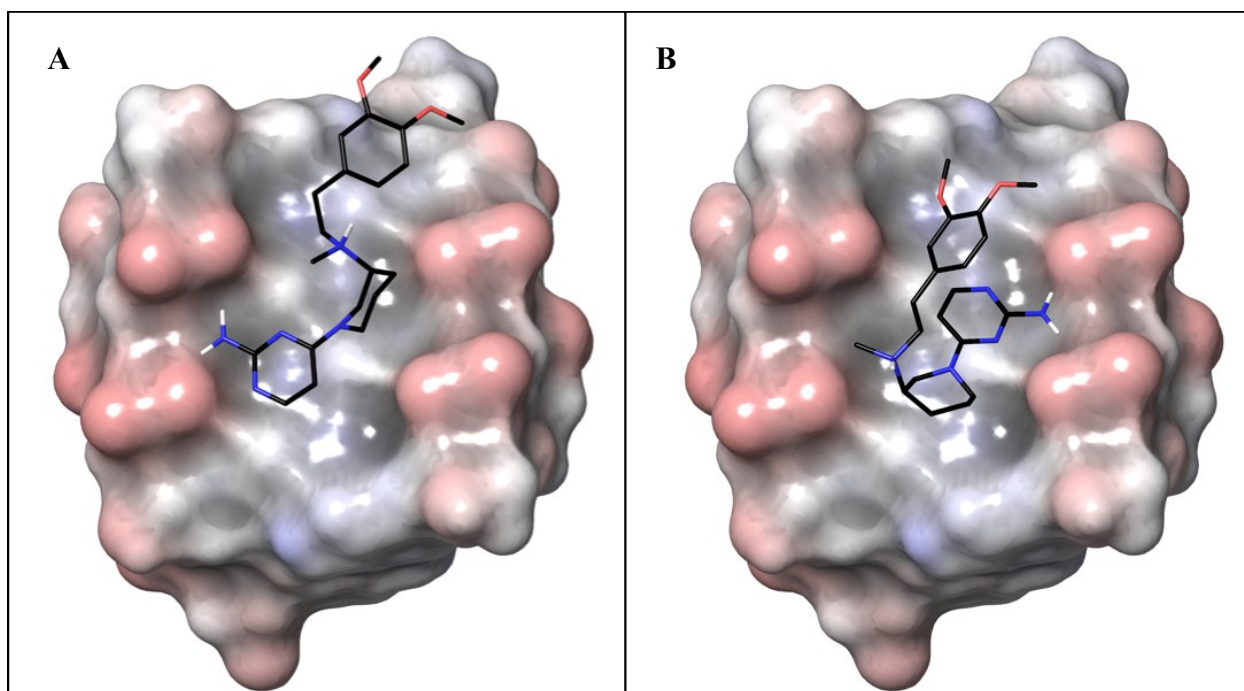


Figure S58: Two ensemble structures for compound **2** docked to the energy minimized structure of *hras*-1^Y (see methods above) where the unnatural nucleobase in the compound is hydrogen bonded to the phosphate backbone.

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