Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Sensing the impact of environment on small molecule differentiation of RNA sequences

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Contents

S1. Materials and Methods

The benzofuranyl uridine synthesis reactions were performed based on previous literature conditions.[1] Deionized water was obtained from an ELGA PURELAB Flex (Veolia Water Technologies) water purification system. Diethylpyrocarbonate (DEPC) treated water was used for RNAse free solutions. Water was treated with DEPC (1% v/v) overnight to remove all RNAse, followed by autoclaving at 120°C for 30 minutes to decompose the unreacted DEPC. Plate reader assays were run on a SpectraMax I3 (Molecular Devices) and used Corning 4514 384 well plates. The assays were run based on previous literature conditions.[1] Polyethylene glycol (PEG) 12,000 was purchased from Alfa Aesar and used without further purification. PEG was chosen as a molecular crowder because it has consistent effects across multiple RNA sequences.[2]

S2. RNA Training Set Sequences and Aminoglycoside Receptors

Table S2-1: RNA Training Set Sequences. The uridine replaced with benzofuranyl uridine is shown as (BFU).[1] The sequence length, GC content, and ΔG are also shown, with the ΔG calculated by m-fold.[3-5]

RNA ID	Sequence	Sequence Length	GC Content (%)	ΔG (kcal/mole)	Predicted Tm (°C)
IL A	GUCUGGACAC A(BFU)G CAGAGUACCUCUG AGA GUGUCCAGAC	39	56.4	-23.10	66.6
IL B	GUCUGGACAC AG(BFU) CAGAGUACCUCUG UAA GUGUCCAGAC	39	51.2	-22.70	65.2
IL C	GUCUGGACAC (BFU)CC CAGAGUACCUCUG ACA GUGUCCAGAC	39	69.7	-24.30	64.4
AIL A	GUCUGGACAC A(BFU)A CAGAGUACCUCUG A GUGUCCAGAC	37	54.1	-21.30	64.5
AIL B	GUCUGGACAC A(BFU)A CAGAGUACCUCUG GC GUGUCCAGAC	38	55.3	-22.80	66.9
AIL C	GUCUGGACAC AG(BFU) CAGAGUACCUCUG UA GUGUCCAGAC	38	52.6	-22.40	65.3
Bulge A	GUCUGGACAC (BFU)C CAGAGUACCUCUG GUGUCCAGAC	35	56.7	-21.80	66.4

Bulge B	GUCUGGACAC GC(BFU) CAGAGUACCUCUG GUGUCCAGAC	36	58.3	-20.80	63.5
Bulge C	GUCUGGACAC UG(BFU) CAGAGUACCUCUG GUGUCCAGAC	36	55.6	-20.80	66.1
Bulge D	GUCUGGACAC GA(BFU)ACAGAGUACC UCUG GUGUCCAGAC	37	54	-20.40	65.7
HP A	CAUGUGCUGGACAU GC(BFU)AAUGUCCAGC ACAUG	32	56.7	-24.10	64.0
HP B	CAUGUGCUGGACAU A(BFU)AUGAUGUCCAG CACAUG	33	52.6	-23.30	61.8
HP C	CAUGUGCUGGACAU ACAG(BFU)GAUGUCCA GCACAUG	34	55.2	-23.50	64.4
Stem A	GACAU GA(BFU)CUG CAGACUACGUCUG CAGAUC AUGUC	35	51.3	-29.80	65.3
Stem B	GACAU (BFU)CACGC CAGACUACGUCUG GCGUGA AUGUC	35	56.7	-30.90	68.1
Stem C	GACAUACGA(BFU)C CAGACUACGUCUG GAUCGUAUGUC	35	51.3	-29.30	67.1

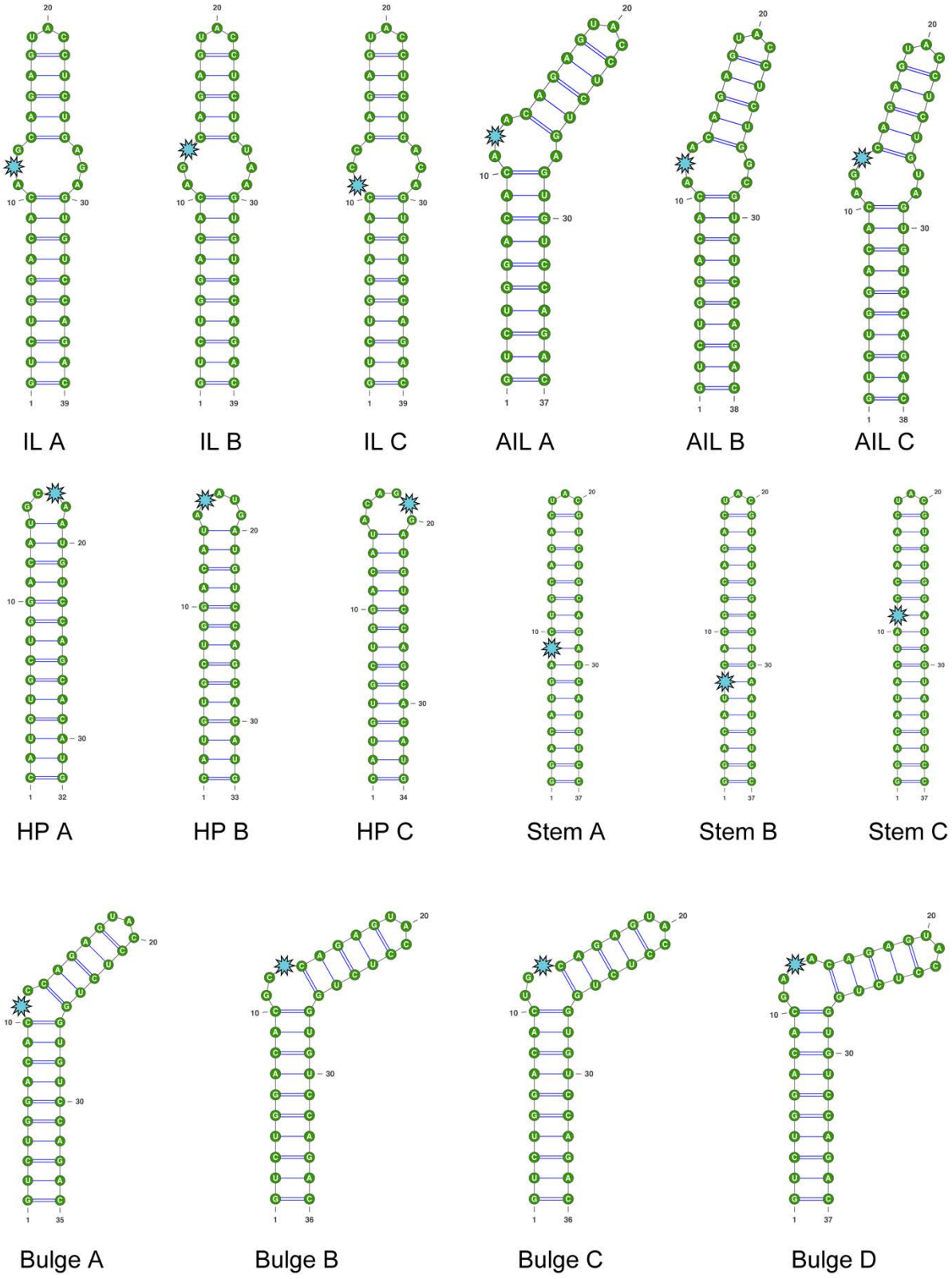


Figure S2-1. RNA training set secondary structure constructs. The benzonfuranyl uridine dye is shown for each structure (blue star).

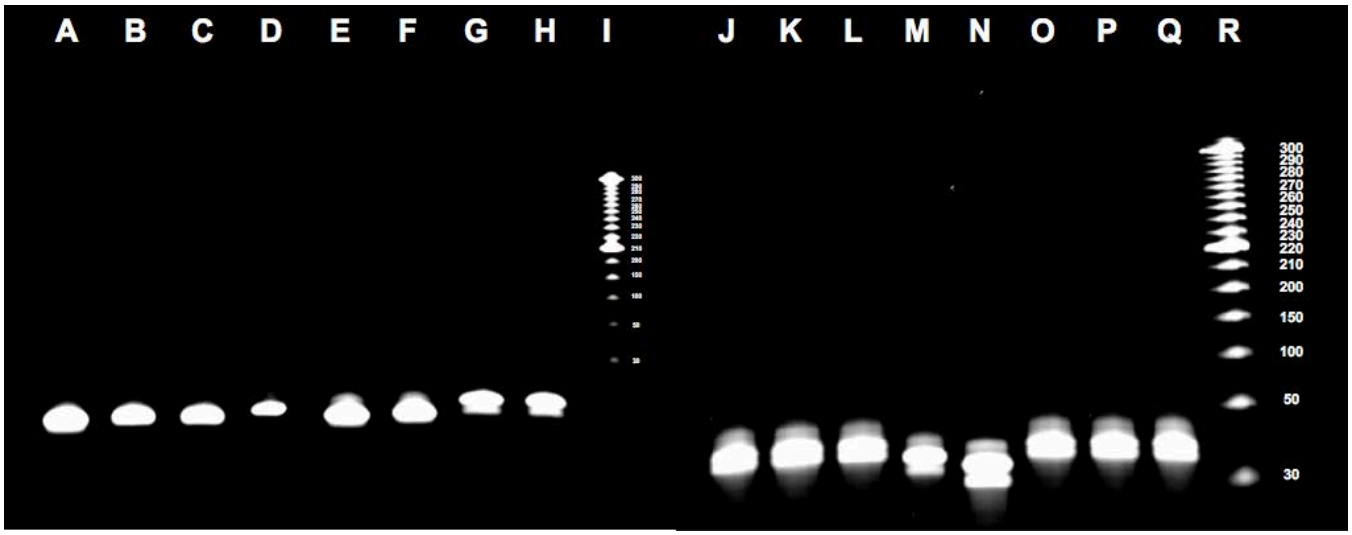
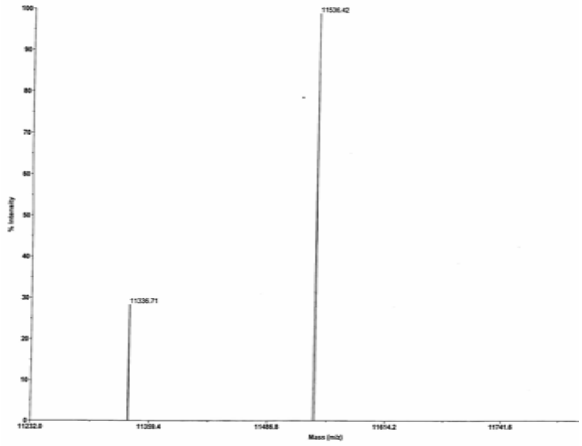


Figure S2-2. 15% PAGE gel run at a constant 180V for 2.5 hours in 1X Tris borate EDTA (TBE) buffer. RNA was stained with Diamond^{Tm} Nucleic Acid Dye (10,000:1 dilution) for 30 minutes. The columns from left to right; A) Stem A, B) Stem B, C) Stem C, D) HP A, E) HP B, F) HP C, G) Bulge A, H) Bulge B, I) Ladder, J) IL A, K) IL B, L) IL C, M) Bulge C, N) Bulge D, O) AIL A, P) AIL B, Q) AIL C, R) Ladder.

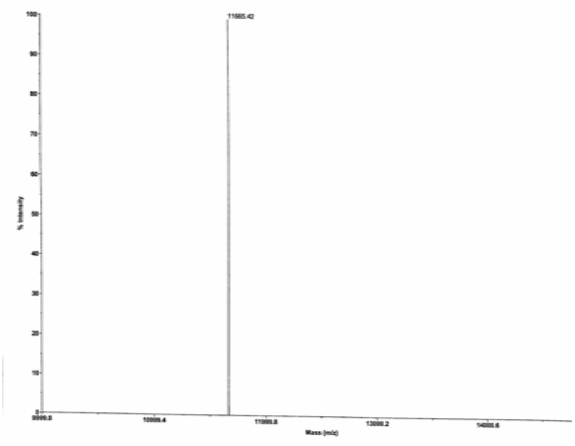
Matrix Assisted Laser Desorption Ionization –Time of Flight (MALDI-TOF)

MALDI-TOF was run for all 16 RNA sequences. 1 µL of saturated 3-hydroxypicolinic acid (3HPA) is placed on a gold MALDI plate and allowed to air dry for 5 minutes. Separately, 1 µL of 100 mM ammonium citrate was added to 1 µL of 200 µM RNA. When the 3HPA is dry, 1 µL of ammonium citrate: RNA solution was added to the plate and allowed to air dry. The plate was inserted into PerSeptive Biosystems Voyager DE-Pro spectrometer in negative-ion, delayed-extraction mode.

Stem A.

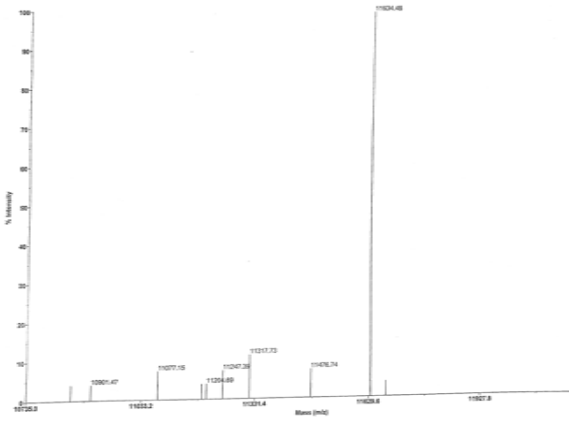


Stem B.

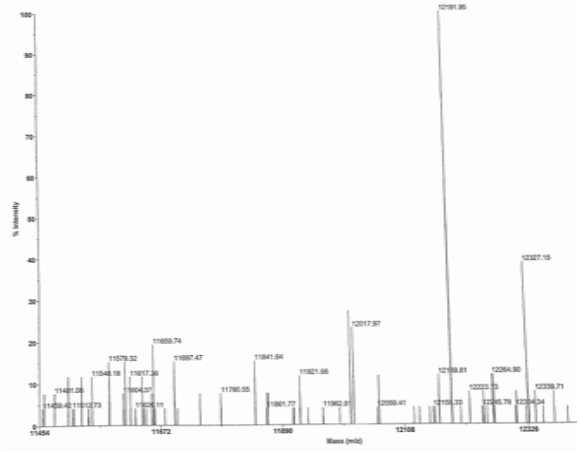


Stem A: Calculated MW: 11536.7 $[M-H]^+$ found: 11536.42
Stem B: Calculated MW: 11666.73 $[M-H]^+$ found: 11665.42

Stem C.

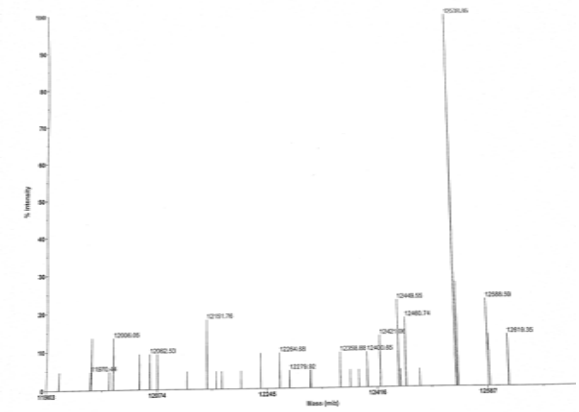


AIL A.

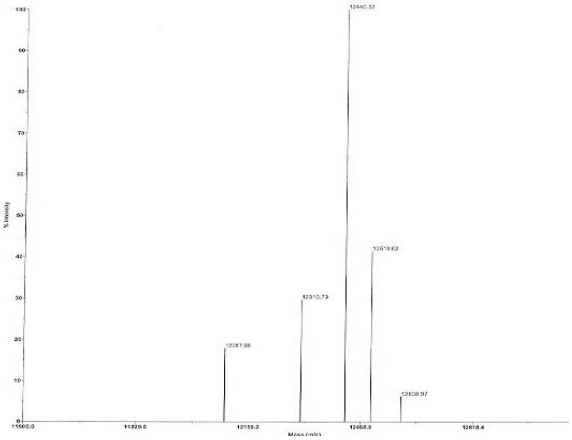


Stem C: Calculated MW: 11634.71 $[M-H]^+$ found: 11634.48
AIL A: Calculated MW: 12192.4 $[M-H]^+$ found: 12191.95

AIL B.

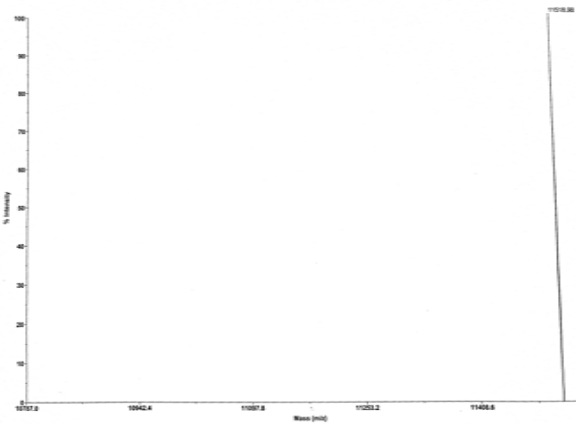


AIL C.

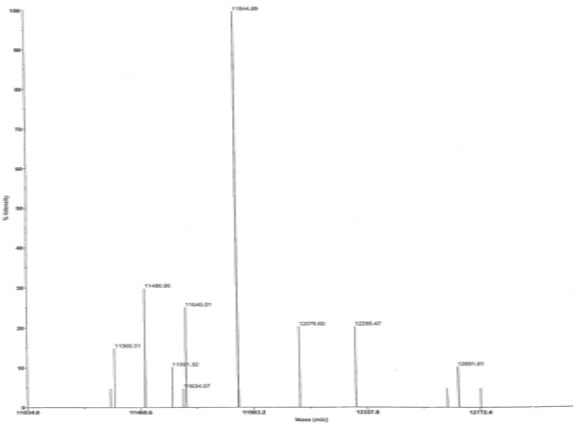


AIL B: Calculated MW: 12540.6 $[M-H]^+$ found: 12538.86
AIL C: Calculated MW: 12441.33 $[M-H]^+$ found: 12440.57

Bulge A.

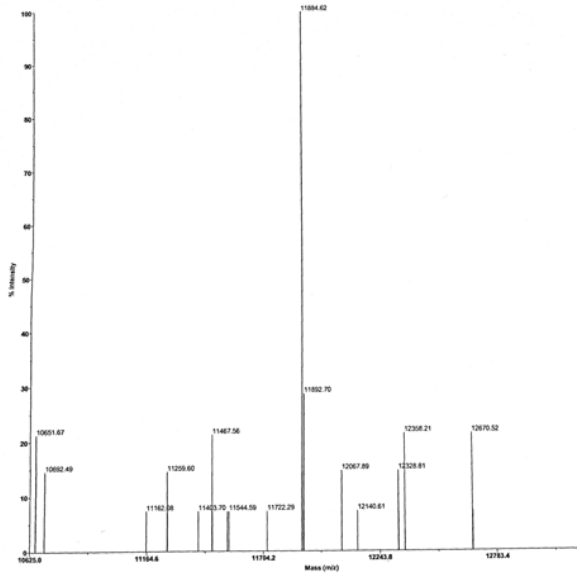


Bulge B.

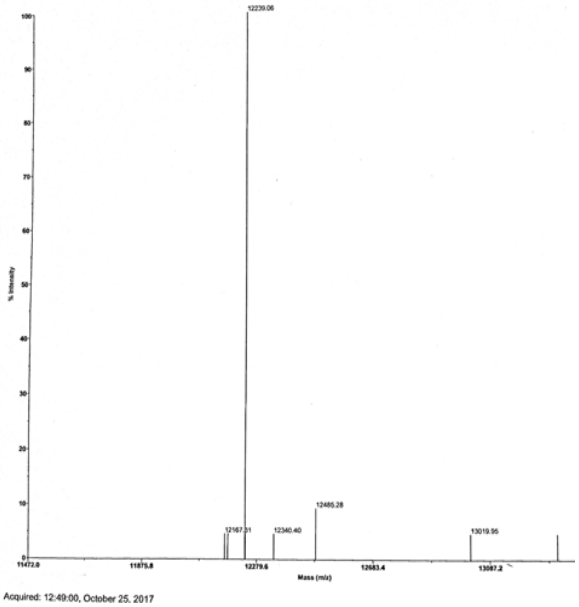


Bulge A: Calculated MW: 11517.73 $[M-H]^+$ found: 11518
Bulge B: Calculated MW: 11843.23 $[M-H]^+$ found: 11844.89

Bulge C.

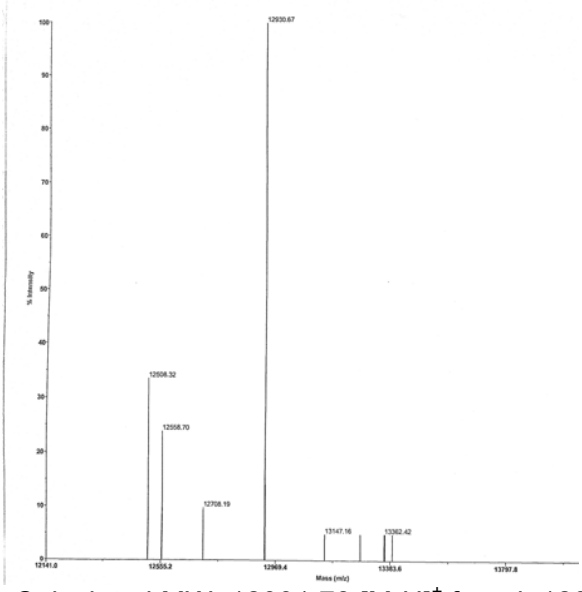


Bulge D.

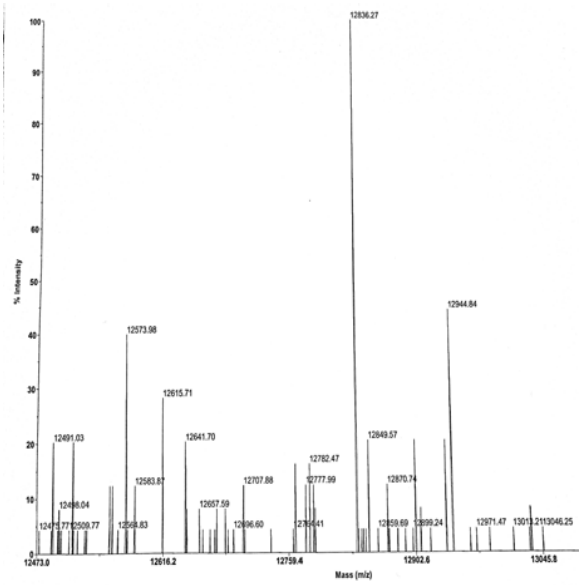


Bulge C: Calculated MW: 11883.2 $[M-H]^+$ found: 11884.62
Bulge D: Calculated MW: 12238.4 $[M-H]^+$ found: 12239.08

IL A.

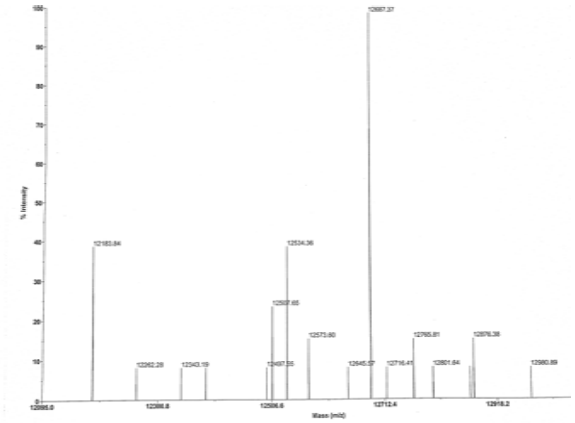


IL B.

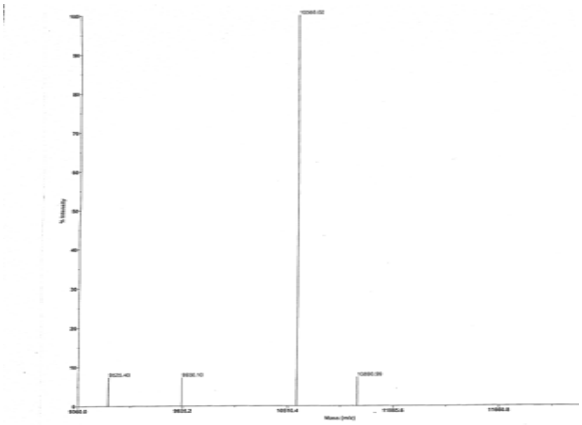


IL A Calculated MW: 12931.78 $[M-H]^+$ found: 12930.87
IL B: Calculated MW: 12836.53 $[M-H]^+$ found: 12836.27

IL C.

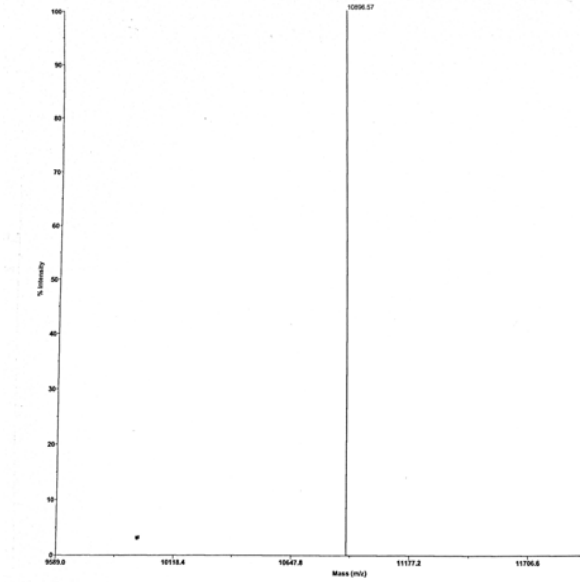


HP A.

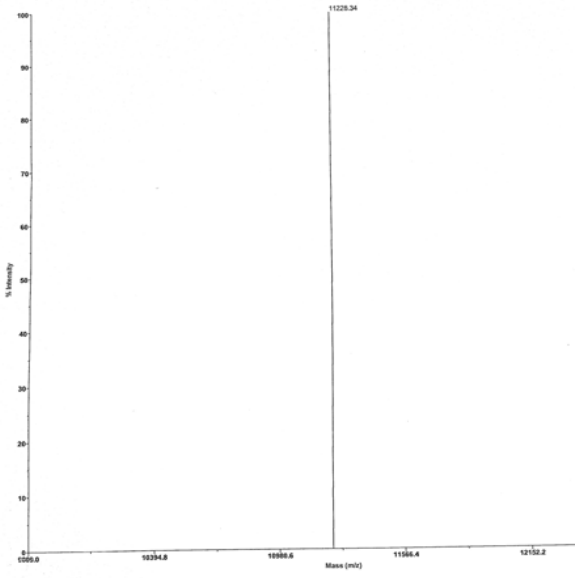


IL C: Calculated MW: 12687.77 $[M-H]^+$ found: 12687.37
HP A: Calculated MW: 10.561.23 $[M-H]^+$ found: 10560.10

IL C.



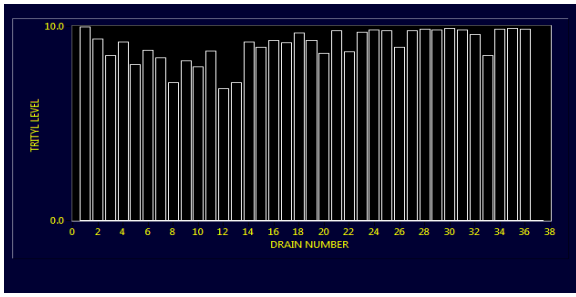
HP A.



HP B: Calculated MW: 10895.71 $[M-H]^+$ found: 10896.57
HP C: Calculated MW: 11227.63 $[M-H]^+$ found: 11228.34

Figure S2-3. MALDI-TOF spectras for the 16 individual RNAs. For each RNA, the calculated and experimental molecular weight (MW) are shown. Additional peaks in the spectra above were also seen in the control MALDI spectrum.

A.



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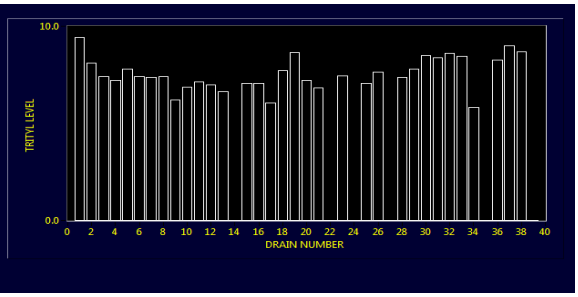
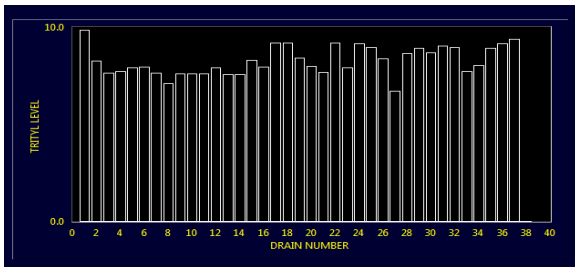


C.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DRAIN NUMBER

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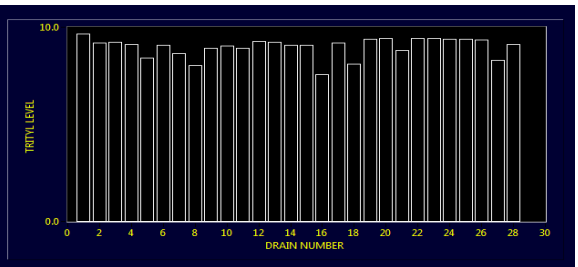
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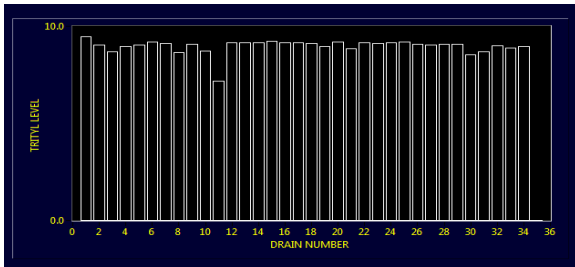
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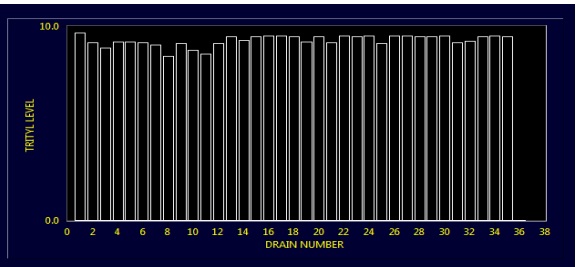
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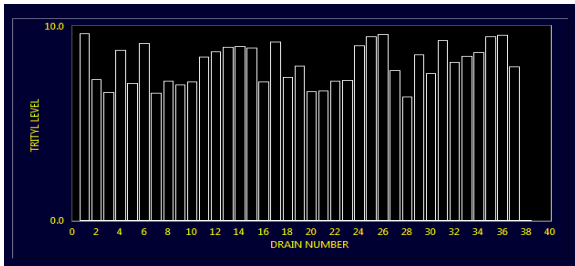
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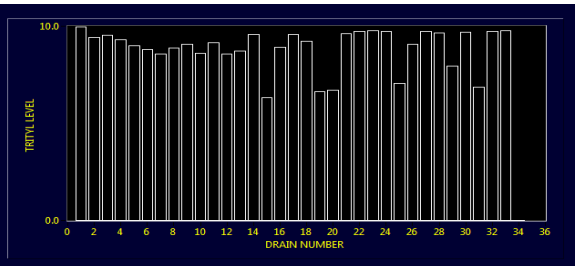
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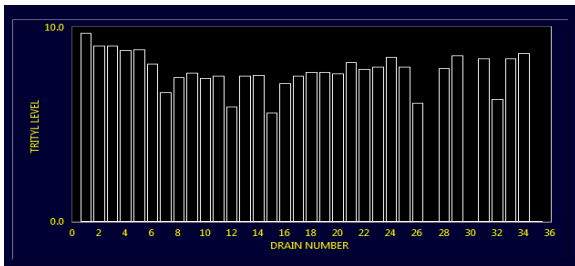
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DRAIN NUMBER

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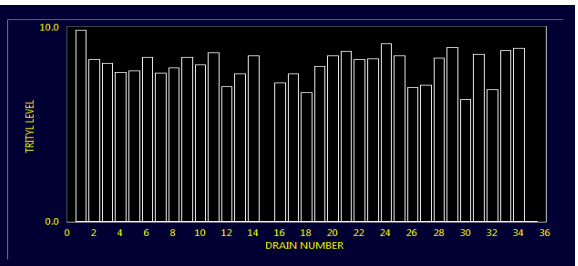
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DRAIN NUMBER

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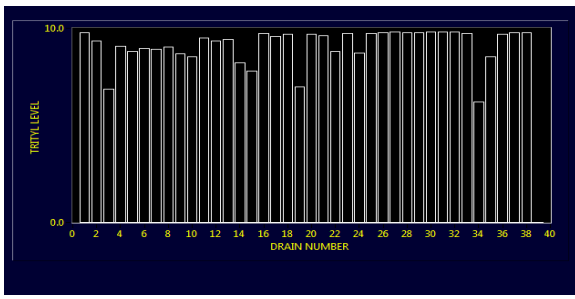
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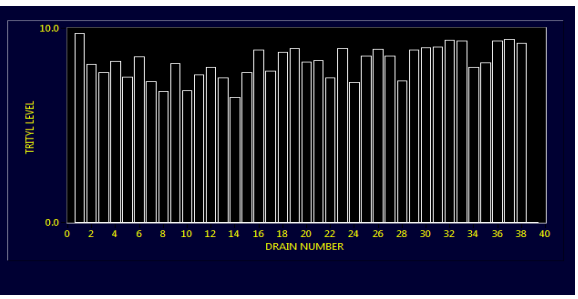
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
DRAIN NUMBER

L.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DRAIN NUMBER

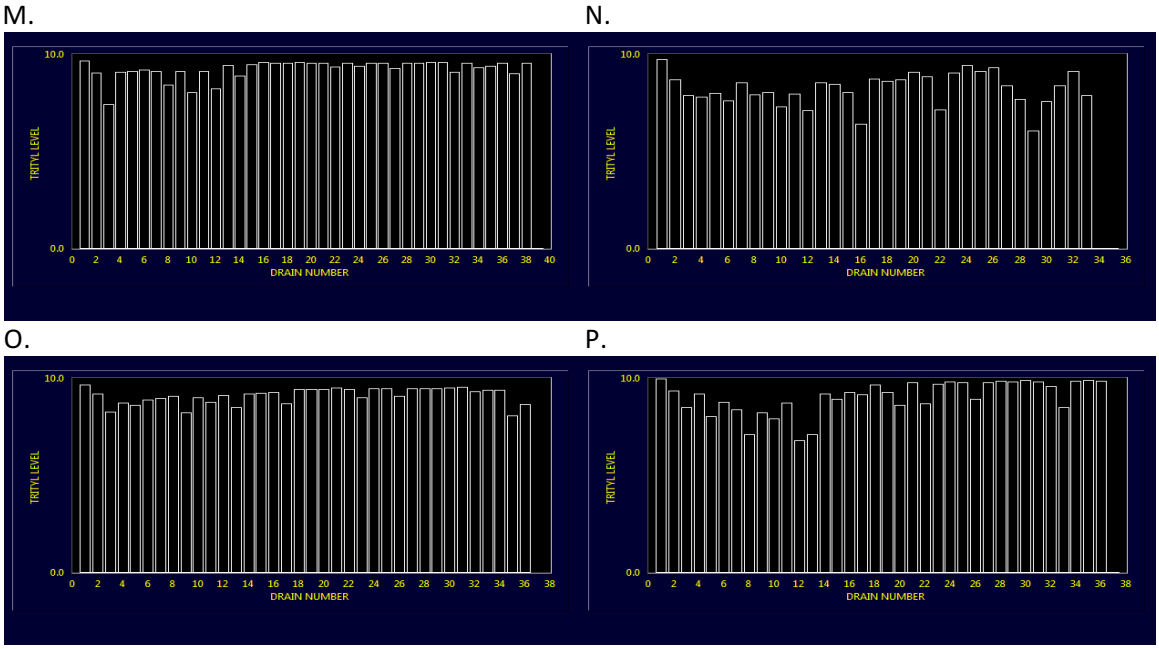


Figure S2-4. Trityl log from the solid phase synthesizer for the RNA constructs. A) AIL A, B) AIL B, C) AIL C, D) Bulge A, E) Bulge B, F) Bulge C, G) Bulge D, H) HP A, I) HP B, J) HP C, K) IL A, L) IL B, M) IL C, N) Stem A, O) Stem B, P) Stem C.

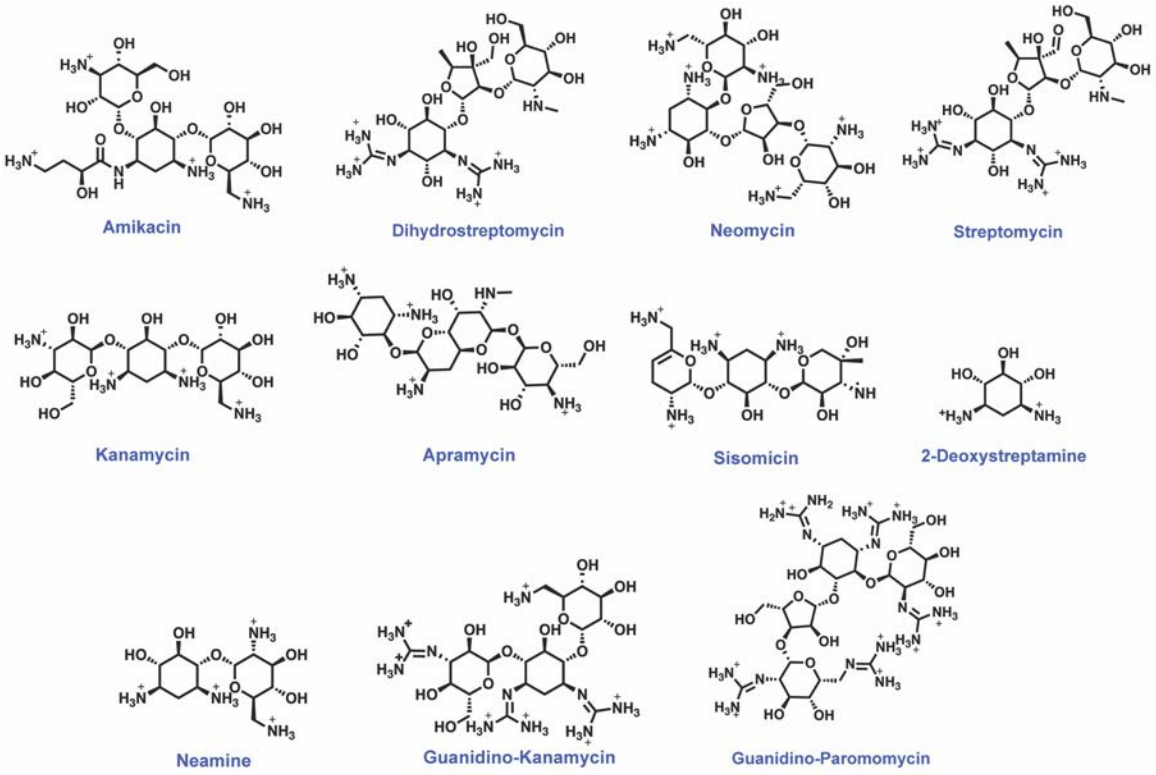


Figure S2-5. Aminoglycoside receptors used in the PRRSM assay. Aminoglycosides were purchased and used without further purification except for guanidino-kanamycin or guanidino-paromomycin, which were synthesized based on literature.[1]

S3. PRRSM PCA Plots under Different Environmental Conditions

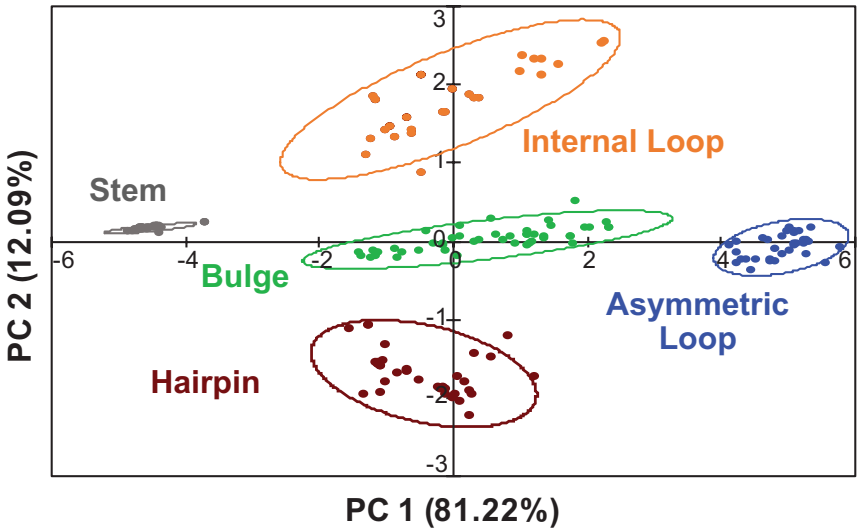


Figure S3-1. PCA plot of the RNA training set separated in the 5 secondary structure motifs in the standard buffer (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 7.3).

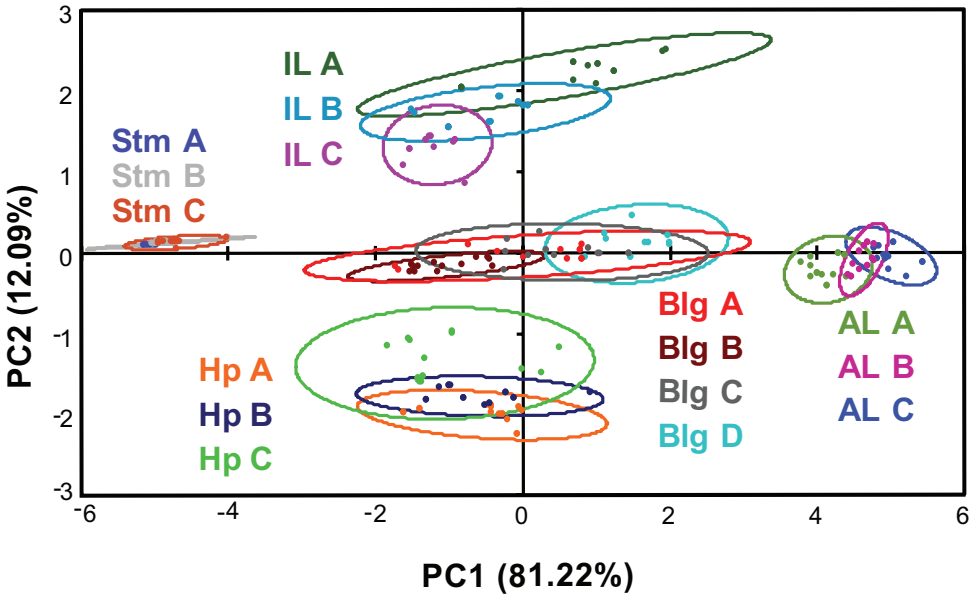


Figure S3-2. PCA plot of the 16 individual RNA training set in the standard buffer (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 7.3).

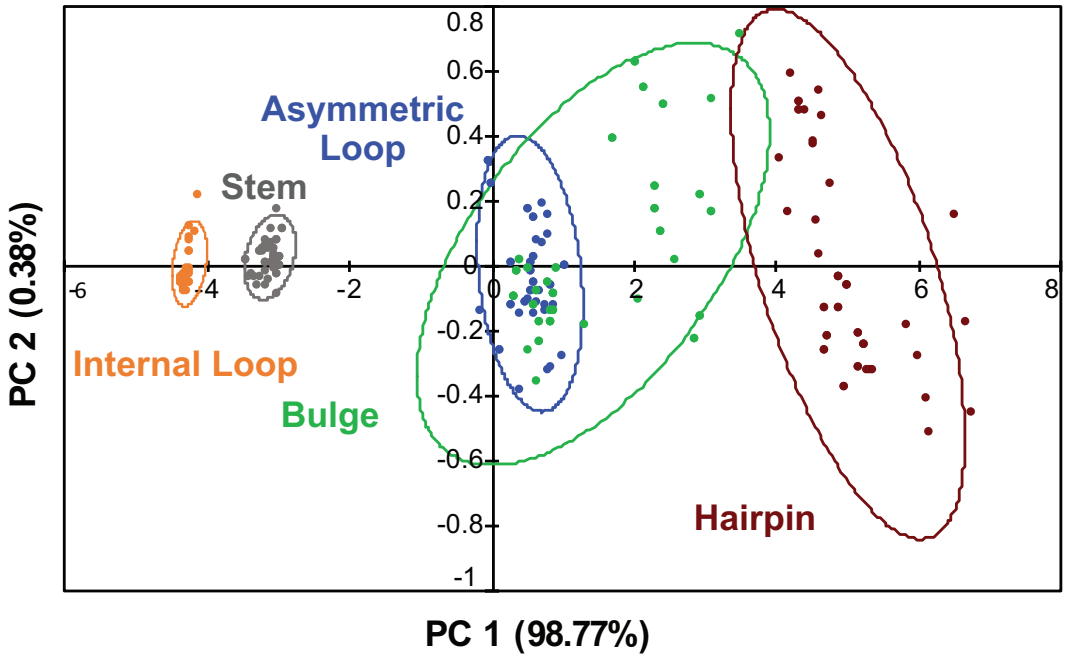


Figure S3-3. PCA plot of the RNA training set separated in the 5 secondary structure in the standard buffer at pH 5 (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 5).

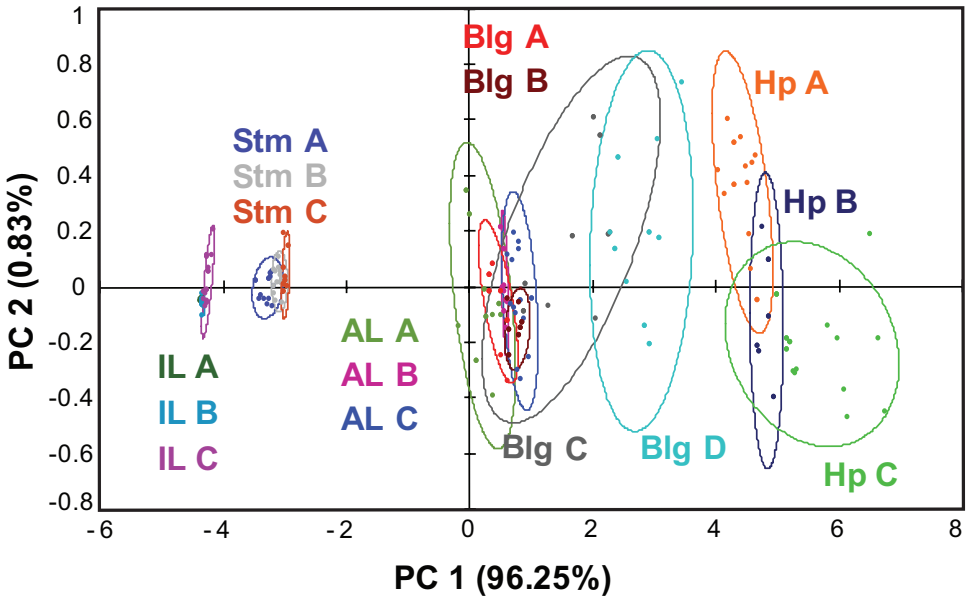


Figure S3-4. PCA plot of the 16 individual RNA training set of buffer at pH 5 (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 5).

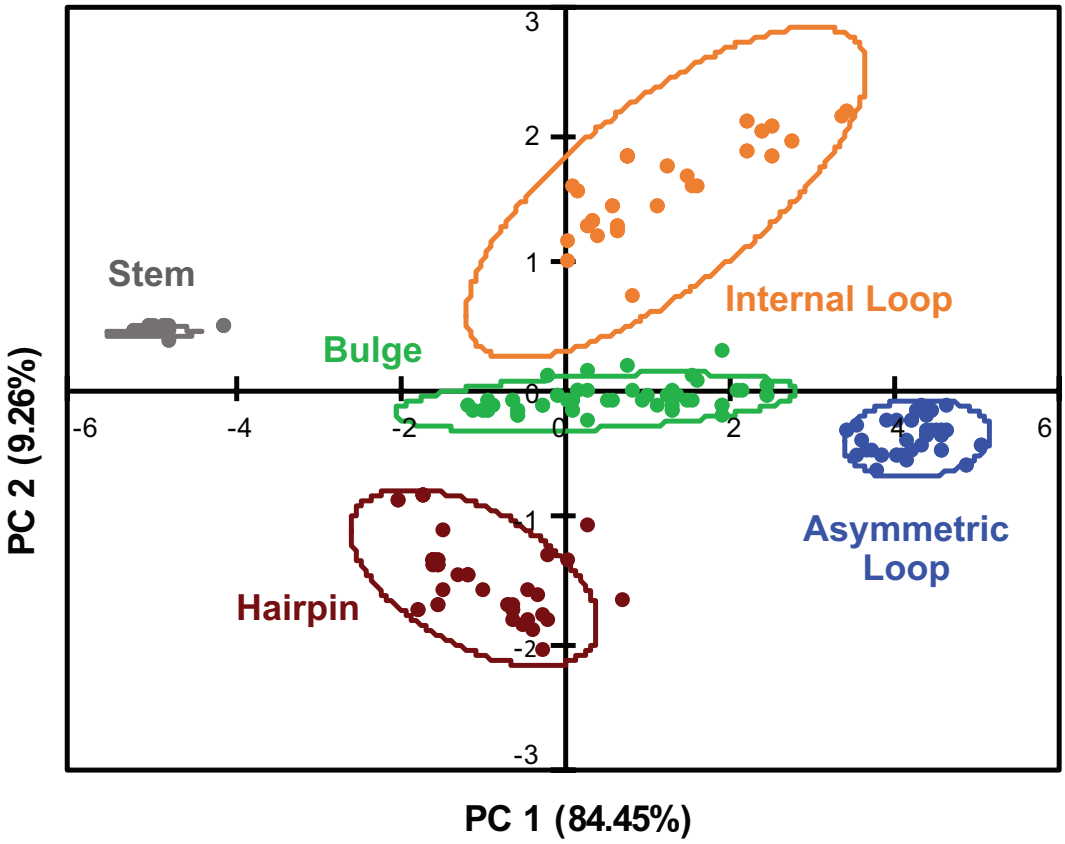


Figure S3-5. PCA plot of the RNA training set separated in the 5 secondary structure in the standard buffer at pH 6 (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 6).

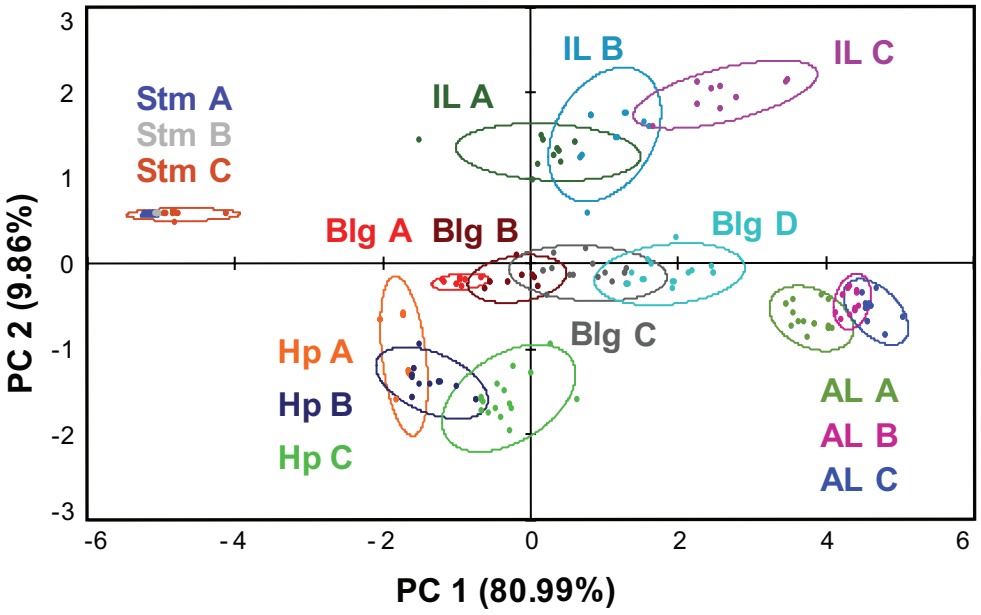


Figure S3-6. PCA plot of the 16 individual RNA training set of buffer at pH 6 (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 6).

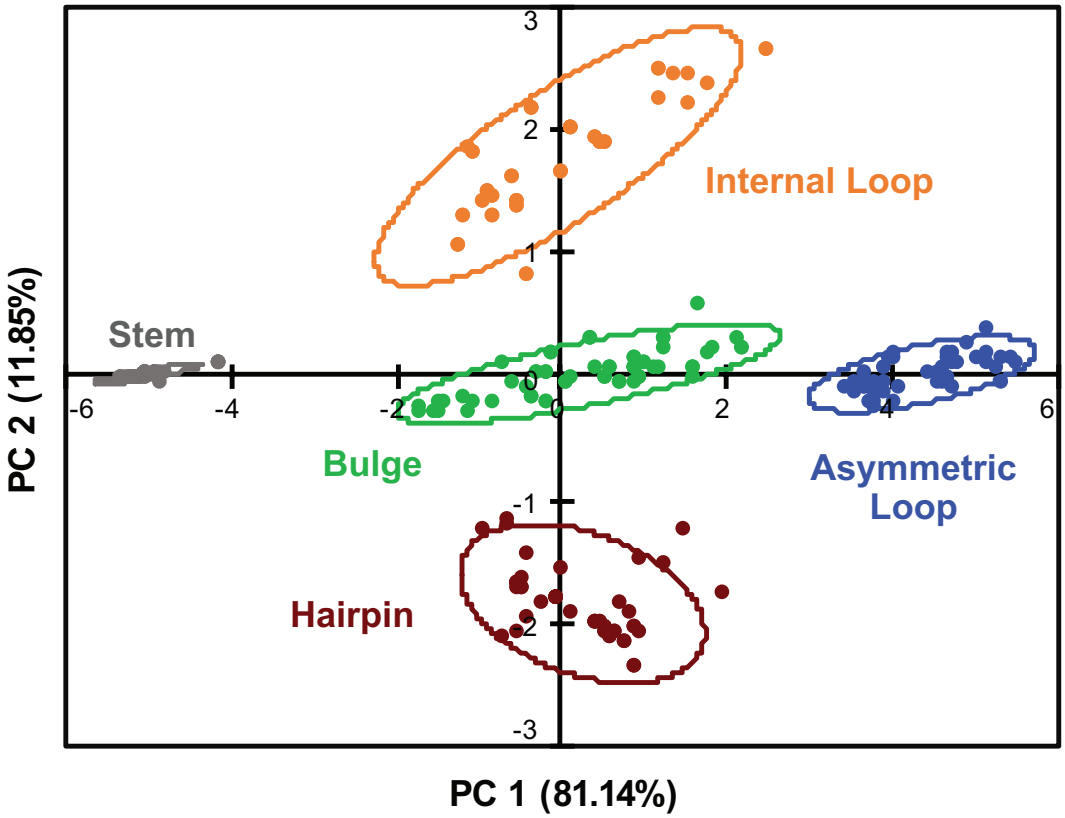


Figure S3-7. PCA plot of the RNA training set separated in the 5 secondary structure in the standard buffer at pH 8 (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 8).

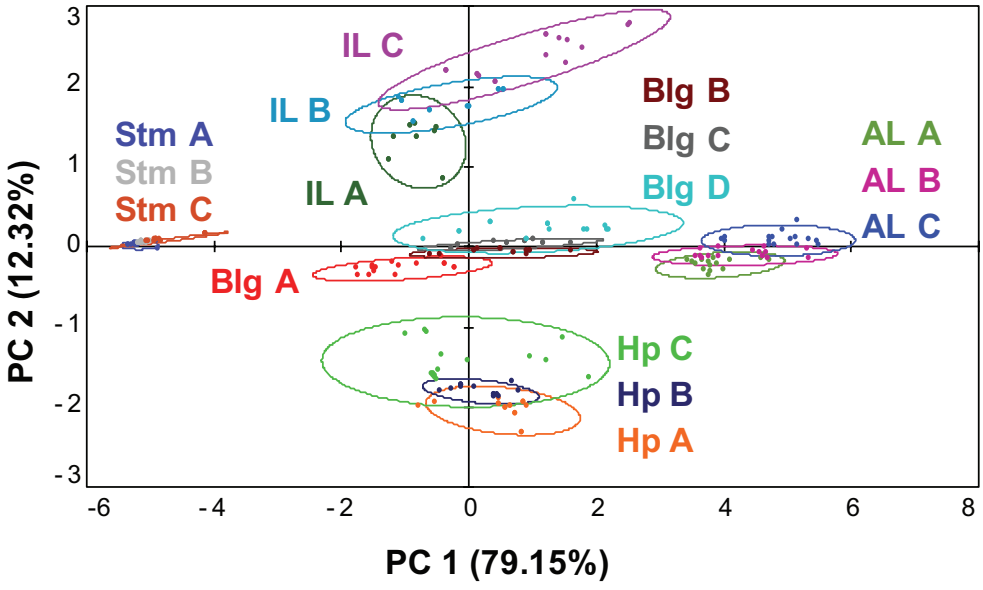


Figure S3-8. PCA plot of the 16 individual RNA training set of buffer at pH 8 (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 8).

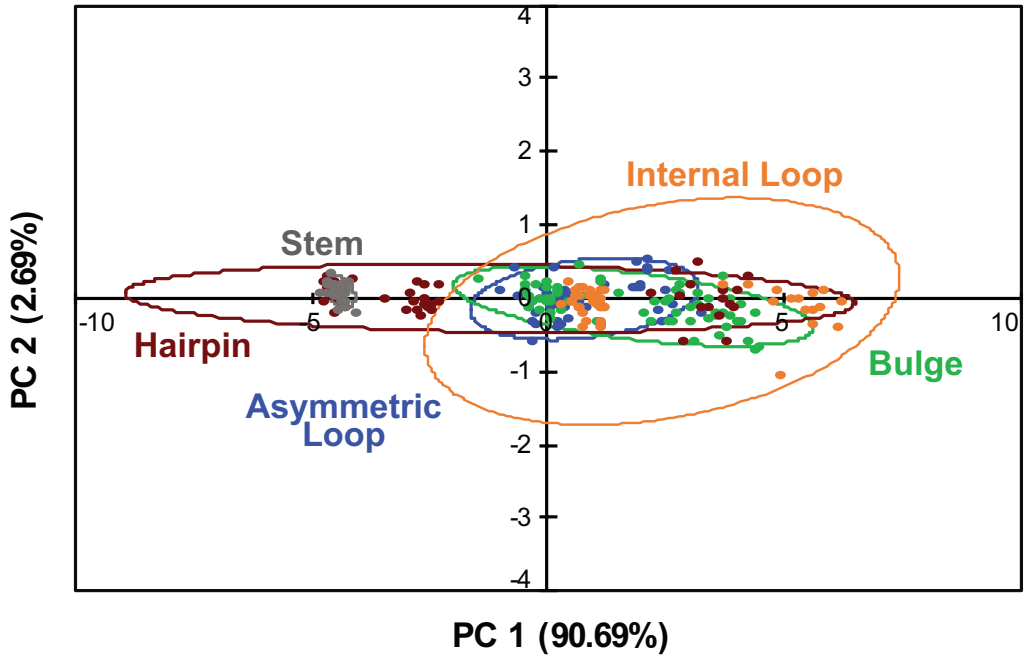


Figure S3-9. PCA plot of the RNA training set separated in the 5 secondary structure in the standard buffer with 140 mM NaCl (10 mM NaH_2PO_4, 140 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 7.3).

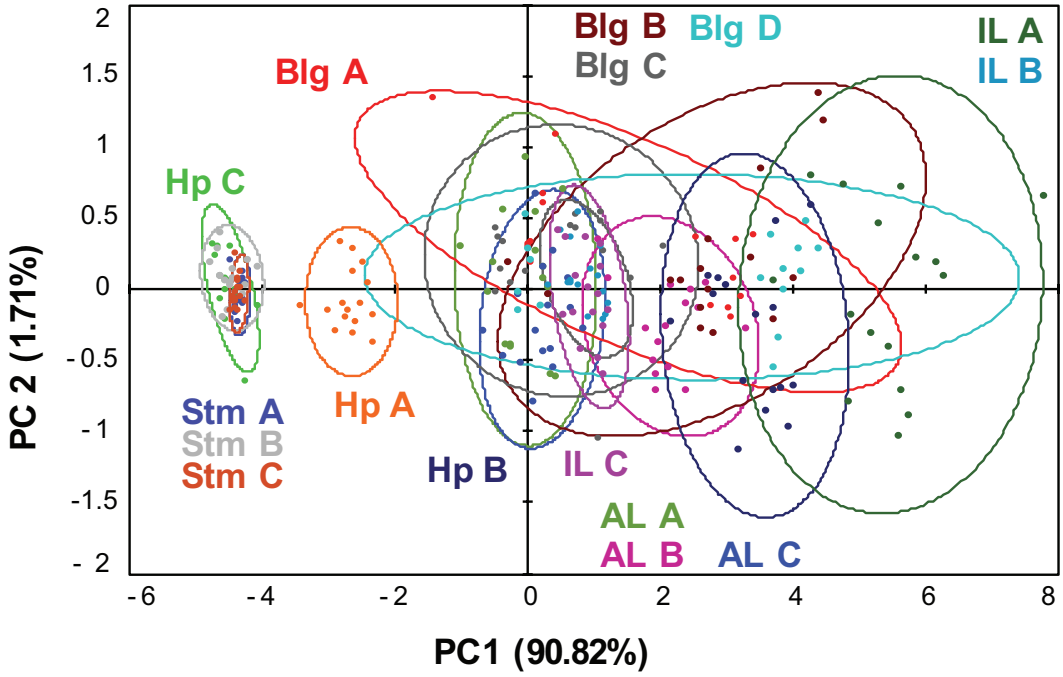


Figure S3-10. PCA plot of the 16 individual RNA training set of buffer with 140 mM NaCl (10 mM NaH_2PO_4, 140 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 7.3).

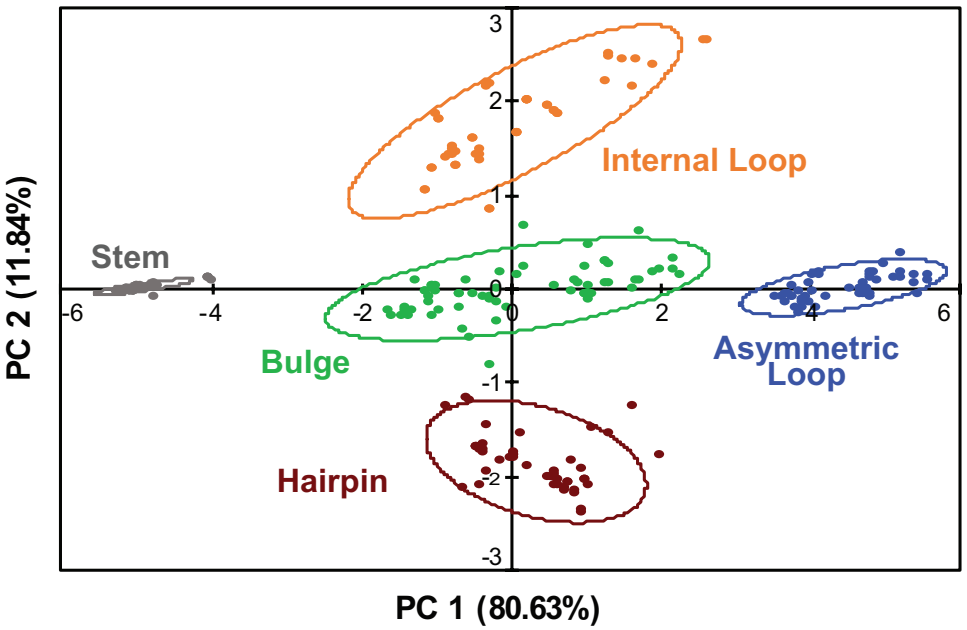


Figure S3-11. PCA plot of the RNA training set separated in the 5 secondary structure in the standard buffer with 0 mM $MgCl_2$ (10 mM NaH_2PO_4, 25 mM NaCl, 0.5 mM EDTA, pH 7.3).

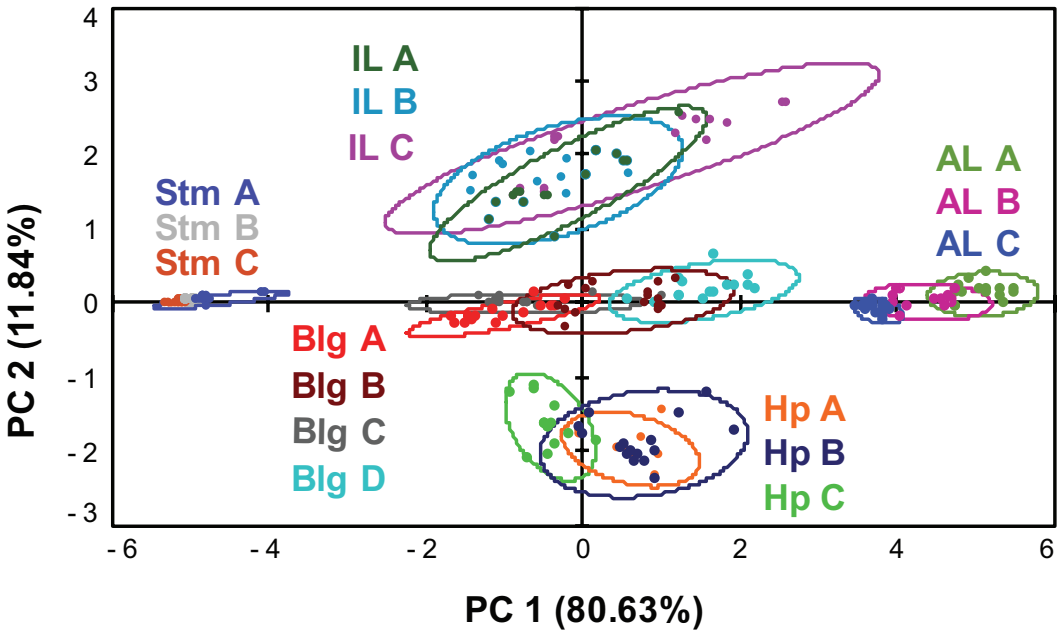


Figure S3-12. PCA plot of the 16 individual RNA training set of buffer without $MgCl_2$ (10 mM NaH_2PO_4, 25 mM NaCl, 0.5 mM EDTA, pH 7.3).

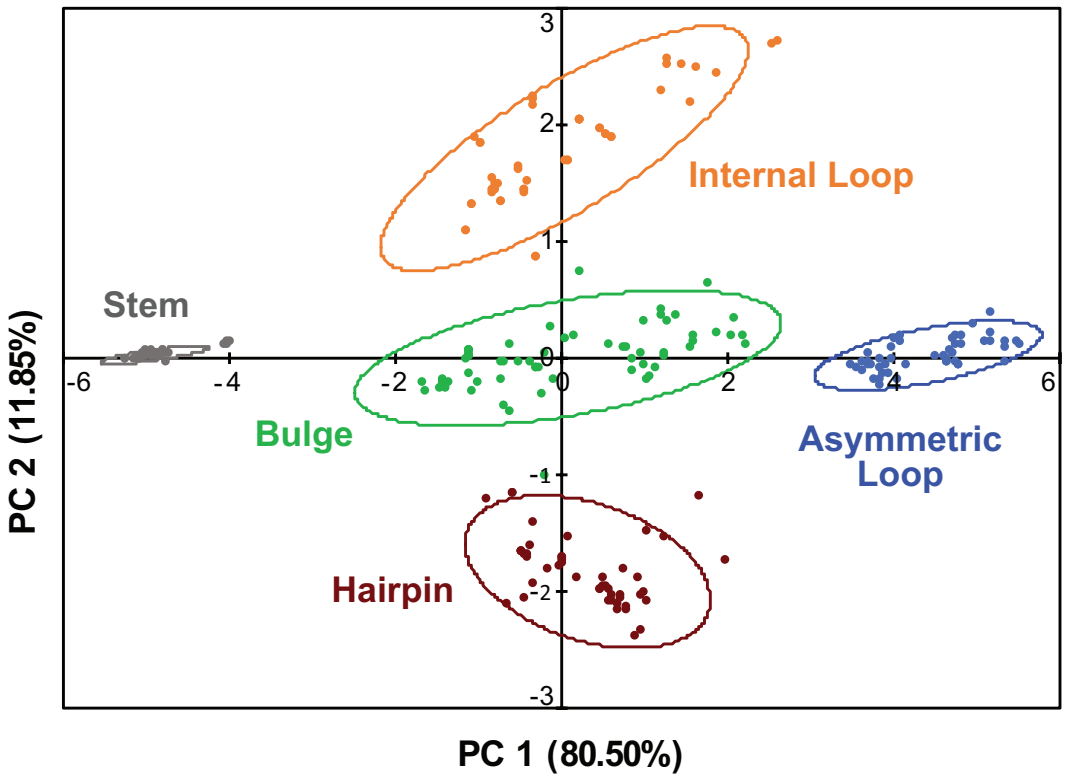


Figure S3-13. PCA plot of the RNA training set separated in the 5 secondary structure in the standard buffer with 10 mM $MgCl_2$ (10 mM NaH_2PO_4, 25 mM NaCl, 10 mM $MgCl_2$, 0.5 mM EDTA, pH 7.3).

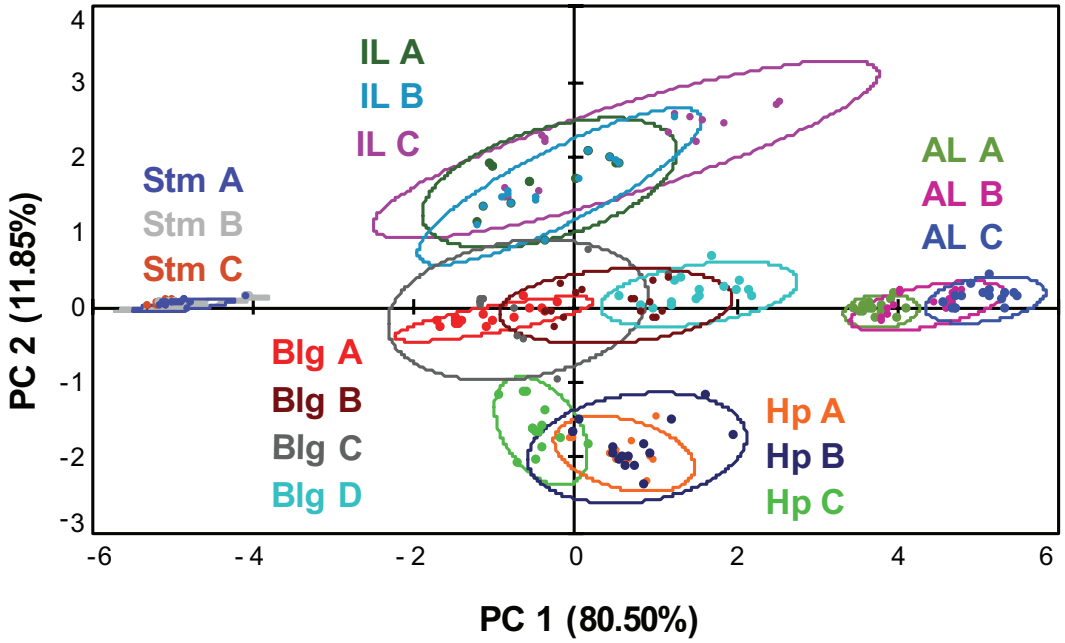


Figure S3-14. PCA plot of the 16 individual RNA training set of buffer with 10 mM $MgCl_2$ (10 mM NaH_2PO_4, 25 mM NaCl, 10 mM $MgCl_2$, 0.5 mM EDTA, pH 7.3).

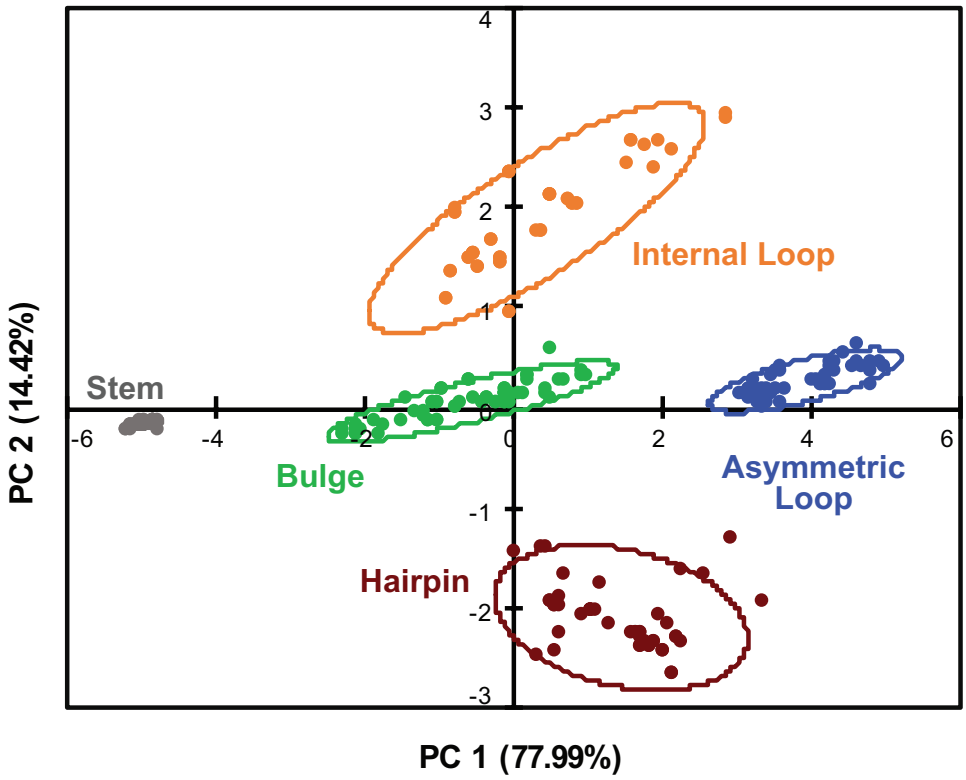


Figure S3-15. PCA plot of the RNA training set separated in the 5 secondary structure in Tris buffer (10 mM Tris, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 7.3).

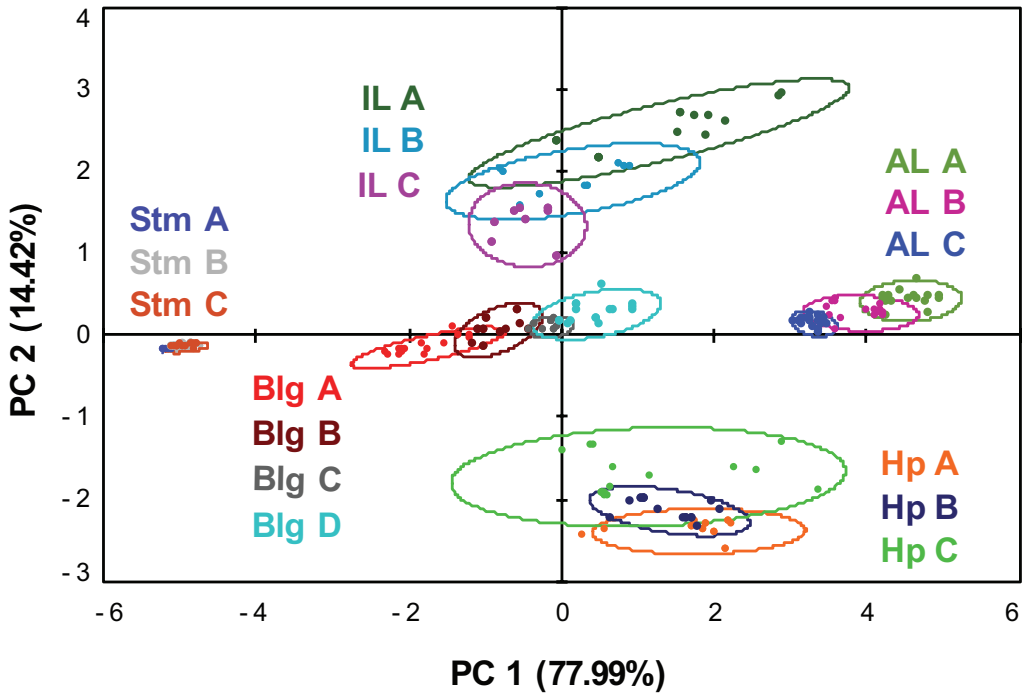


Figure S3-16. PCA plot of the 16 individual RNA training set of Tris buffer (10 mM Tris, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 7.3).

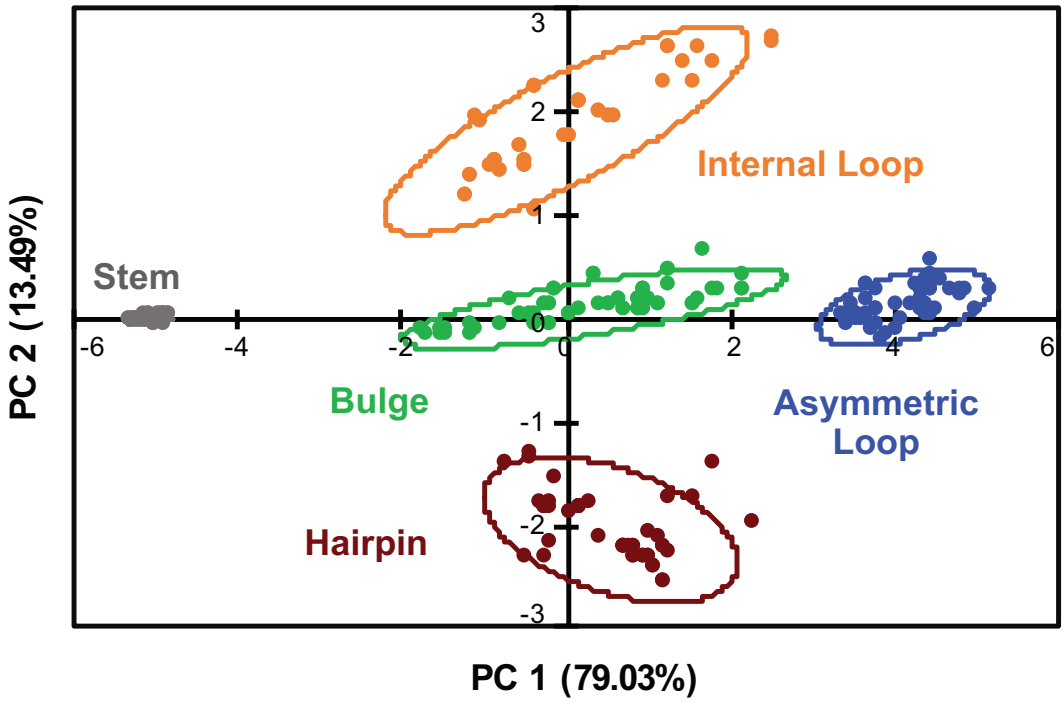


Figure S3-17. PCA plot of the RNA training set separated in the 5 secondary structure in the standard buffer at 37°C (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 7.3 at 37°C).

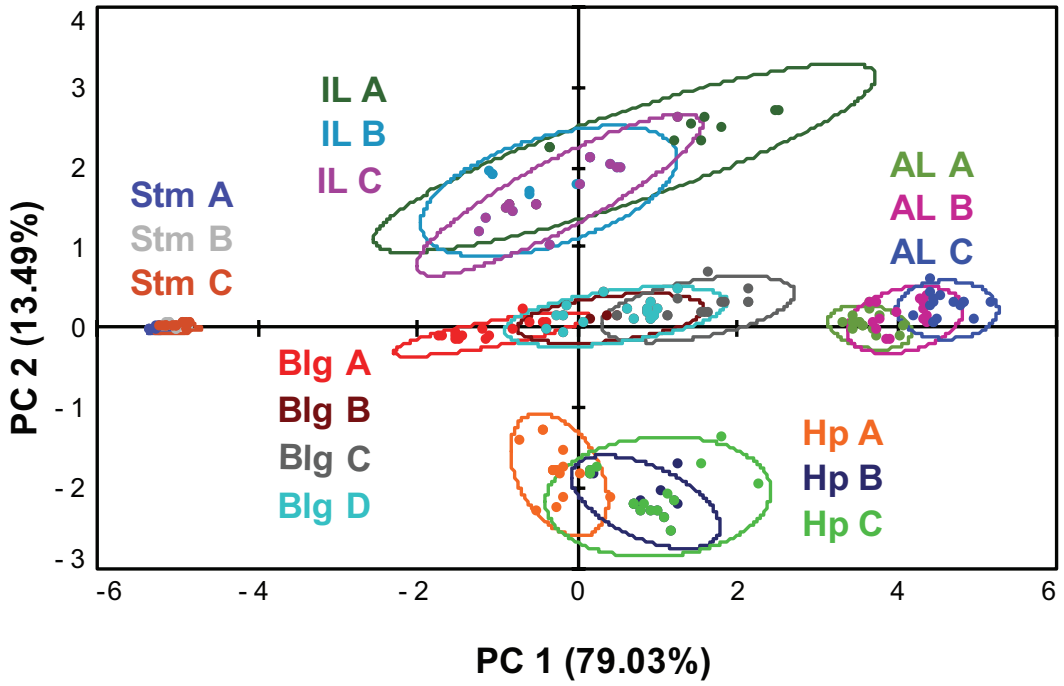


Figure S3-18. PCA plot of the 16 individual RNA training set in the standard buffer at 37°C (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 7.3 at 37°C).

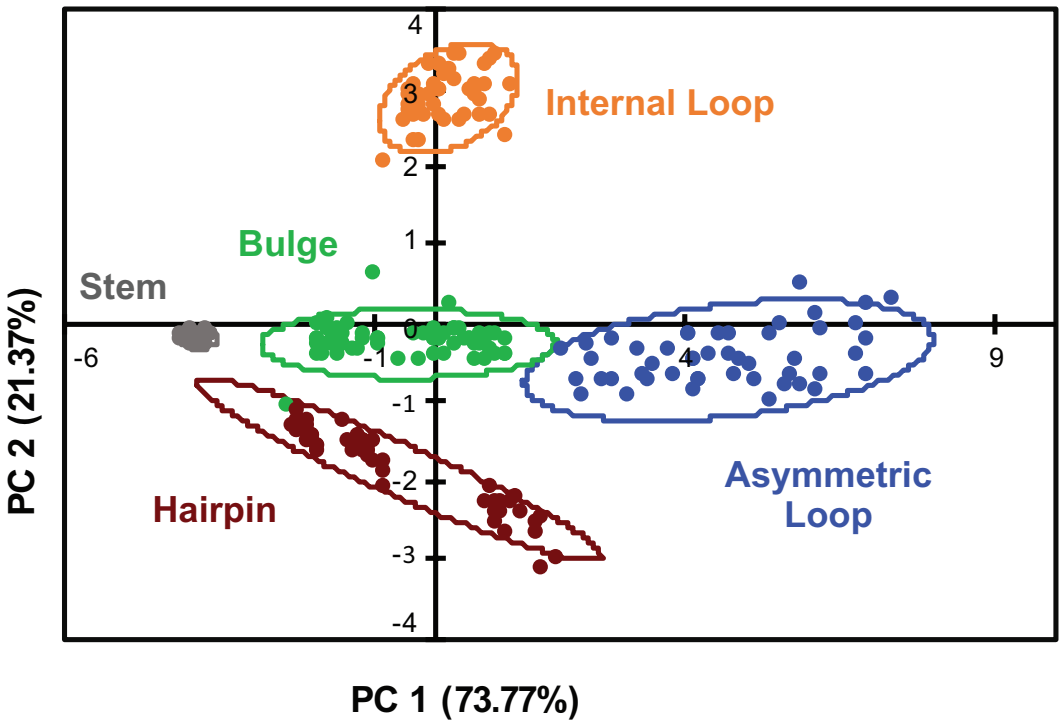


Figure S3-19. PCA plot of the RNA training set separated in the 5 secondary structure in the standard buffer with 8 mM PEG(10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, 8 mM PEG 12,000, pH 7.3).

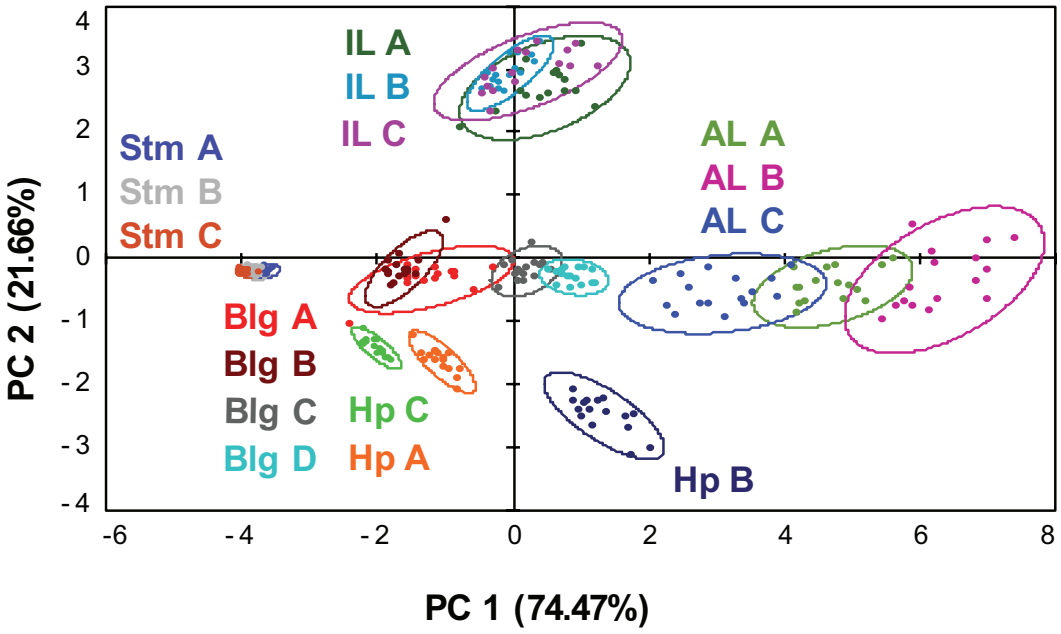


Figure S3-20. PCA plot of the 16 individual RNA training set in buffer with PEG (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, 8 mM PEG 12,000, pH 7.3).

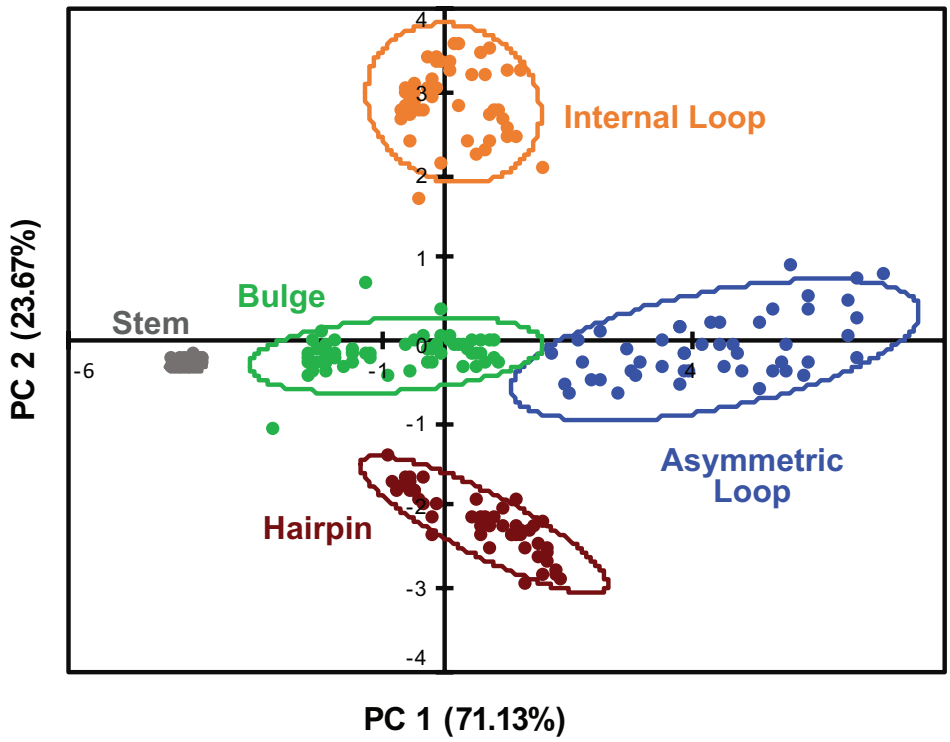


Figure S3-21. PCA plot of the RNA training set separated in the 5 secondary structure in the standard buffer with 8 mM PEG at 37°C (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, 8 mM PEG 12,000, pH 7.3 at 37°C).

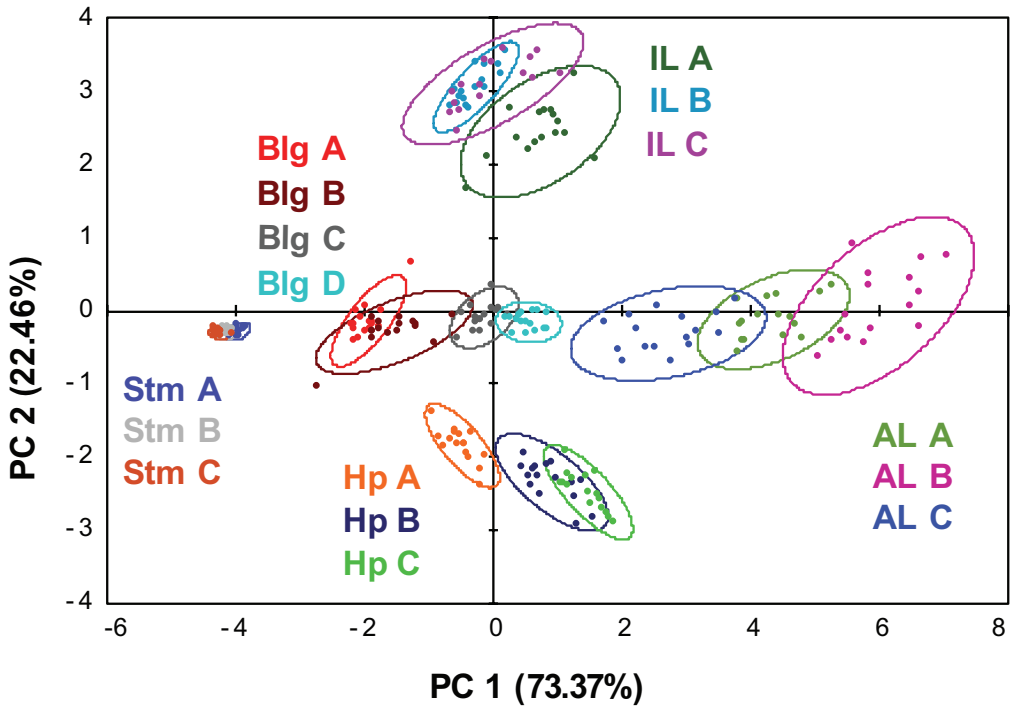


Figure S3-22. PCA plot of the 16 individual RNA training set in buffer with PEG at 37°C (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, 8 mM PEG 12,000, pH 7.3 at 37°C).

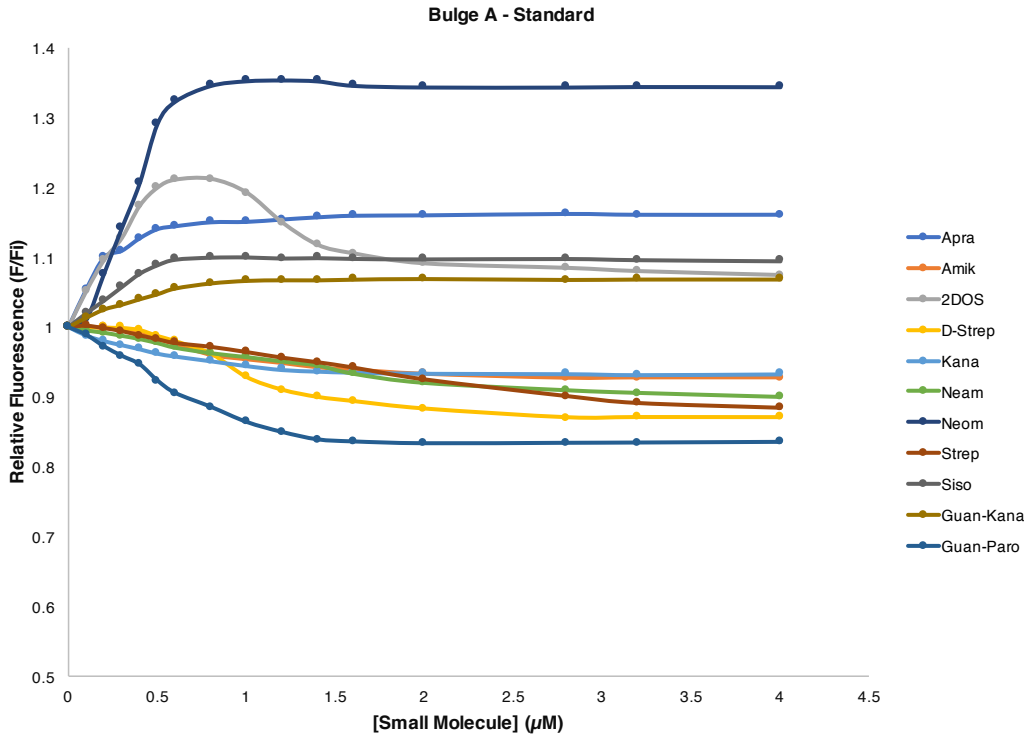
Leave-one-out cross validation was achieved using R-studio with both the carat and klaR packages. To begin with, data sets that have been analyzed by principal component analysis (PCA) are imported into R-studio, with a header 'Name' used to classify different RNA motifs. The data is then partitioned into two sets, either training or test sets. The training set is the data used in Bayesian prediction to recapitulate the experimental data, while the test set is data removed and was used as externally validated to determine the predictive power of the experimental data. The data sets are created utilizing the "createDataPartition" function, using the initial data set classified by the name. A partition fraction (p) argument is used to randomly separate the data used in the training set. Based on literature, 8-fold cross validation was utilized, so p was set to 0.875, but any partition from 0-1 can be used. Finally, a list argument must be stated as FALSE since the rows and columns of the data are not equal. The entire process is named "trainIndex" in order to quickly import the partition into future computations. After partitioning the data, the data sets are named in order to be used easily in future computations. The training set is named data_train and is correlated to the first partition (trainIndex), while the test set is the remaining data (-trainIndex) and is named data_test.

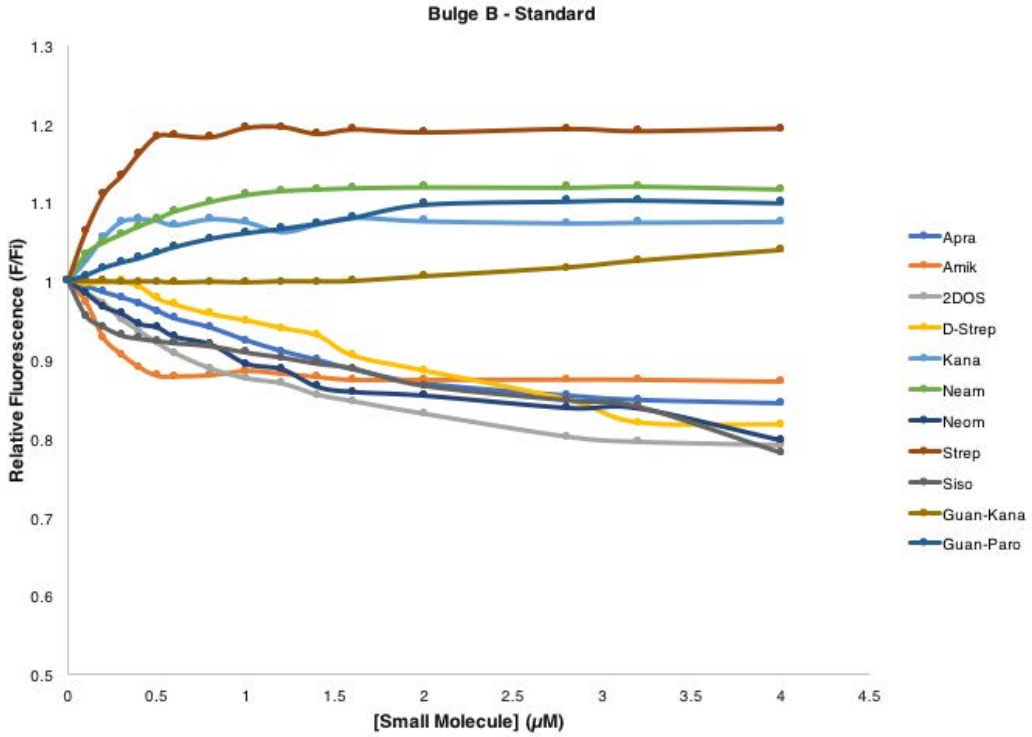
After partitioning, a Naïve Bayes algorithm (NaiveBayes function) is run on the training set to create a predictive model. For ease, the Bayesian function was named "model" and the only arguments necessary are the classifier ('Name'), and the data set (data_train). Afterwards the test set was externally validated utilizing the Bayesian model, with the RNA motifs as the basis of the prediction. Finally, the data is compiled from the functions above and exported as a confusion matrix and the predictive percentage for each individual sequence, as well as the average predictive percentage of the individual sequences to determine the overall predictive power of each environmental condition. The script used is below and has titles for all functions. All variables that can be changed are bolded.

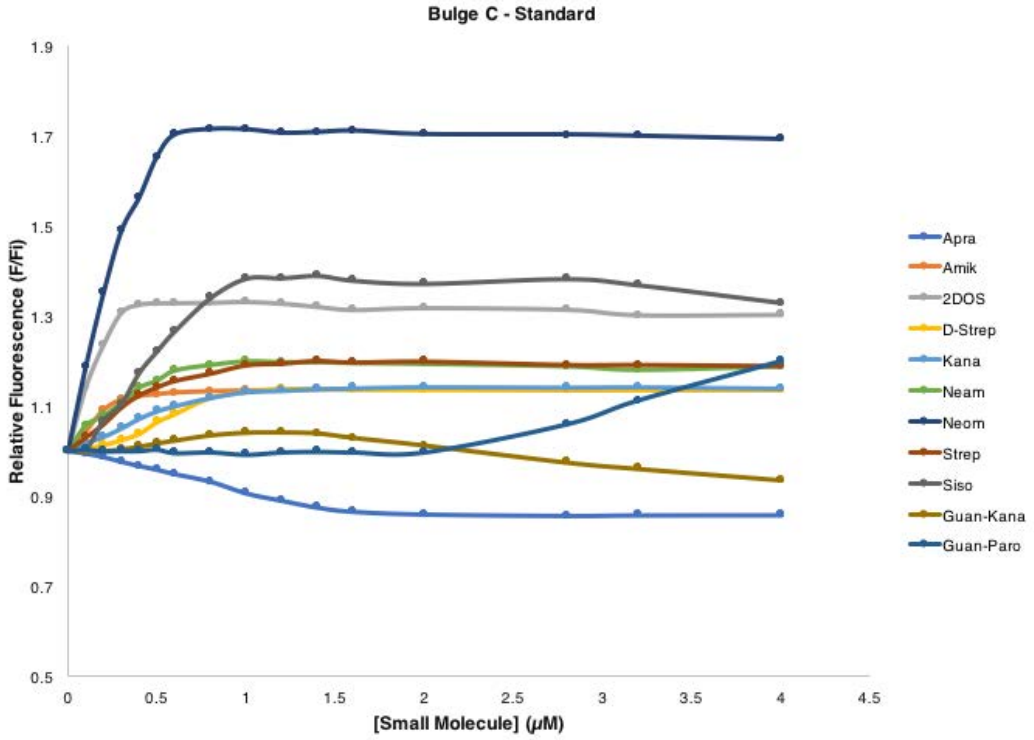
```
> Separating the data into two sets:
trainIndex <- createDataPartition('Data set'$ 'Name', p='0-1', list=FALSE)
> Naming the training set
data_train <- 'Data set'[ trainIndex,]
> Naming the test set
data_test <- 'Data set'[-trainIndex,]
> Running high performance naïve bayes algorithm
model <- NaiveBayes('Name'~., data=data_train)
> Analyzing the experimental data to the computational data to determine the precision of the model
predictions <- predict(model, data_test[2:9])
> exporting the predictions above for viewing
confusionMatrix(predictions$class, data_test$'Name')
```

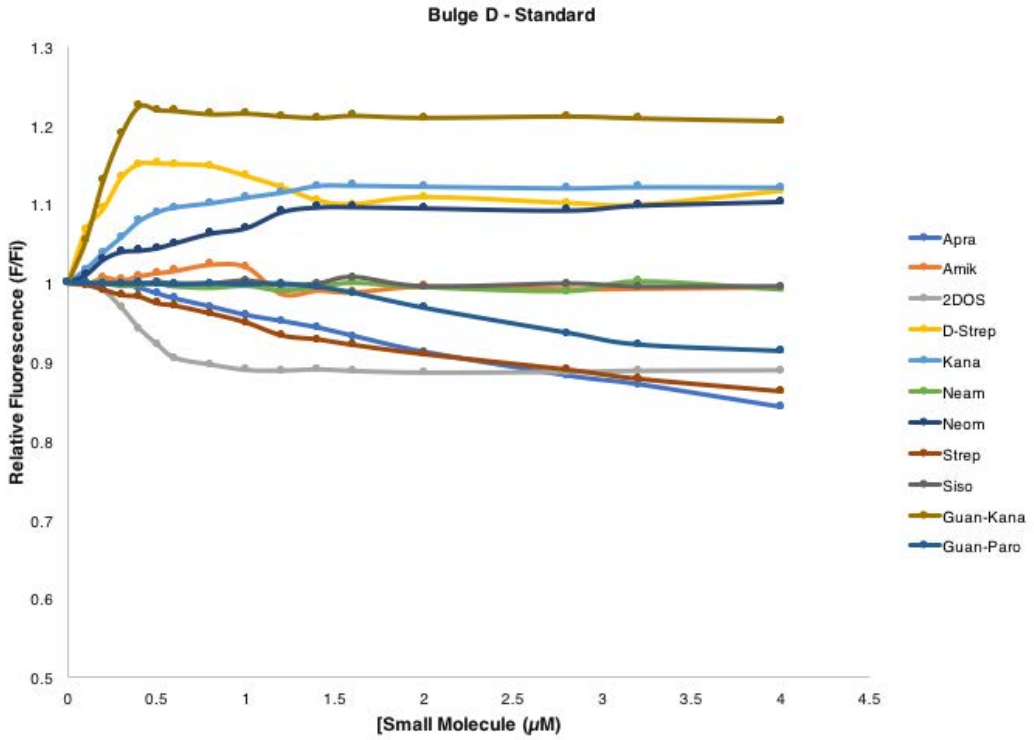
S4. Aminoglycoside Receptor: RNA Titration Graphs

pH 6, pH 8, 0 mM Mg, and Tris titration graphs have been removed because of minimal difference compared to previously published data[1], but are available upon request.






Bulge C - Standard
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
D-Strep
Kana
Neam
Neom
Strep
Siso
Guan-Kana
Guan-Paro


Bulge D - Standard
Relative Fluorescence (F/Fi)
[Small Molecule (μM)
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Kana
Neam
Neom
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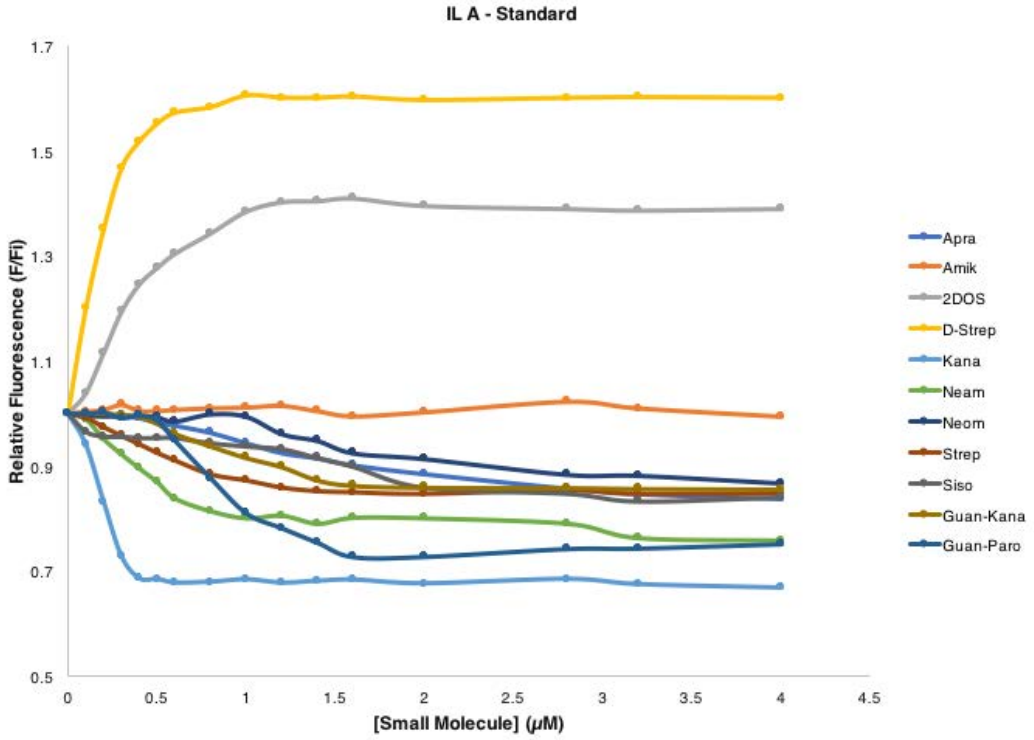
Siso
Guan-Kana
Guan-Paro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Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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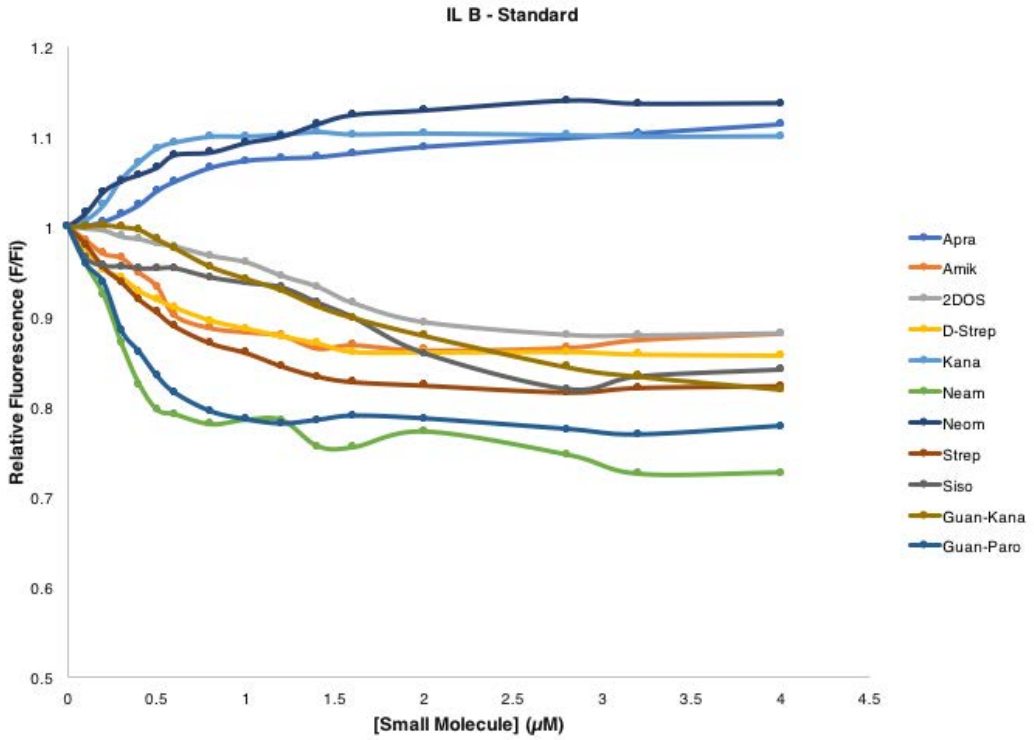
D-Strep
Kana
Neam
Neom
Strep
Siso
Guan-Kana
Guan-Paro


IL B - Standard
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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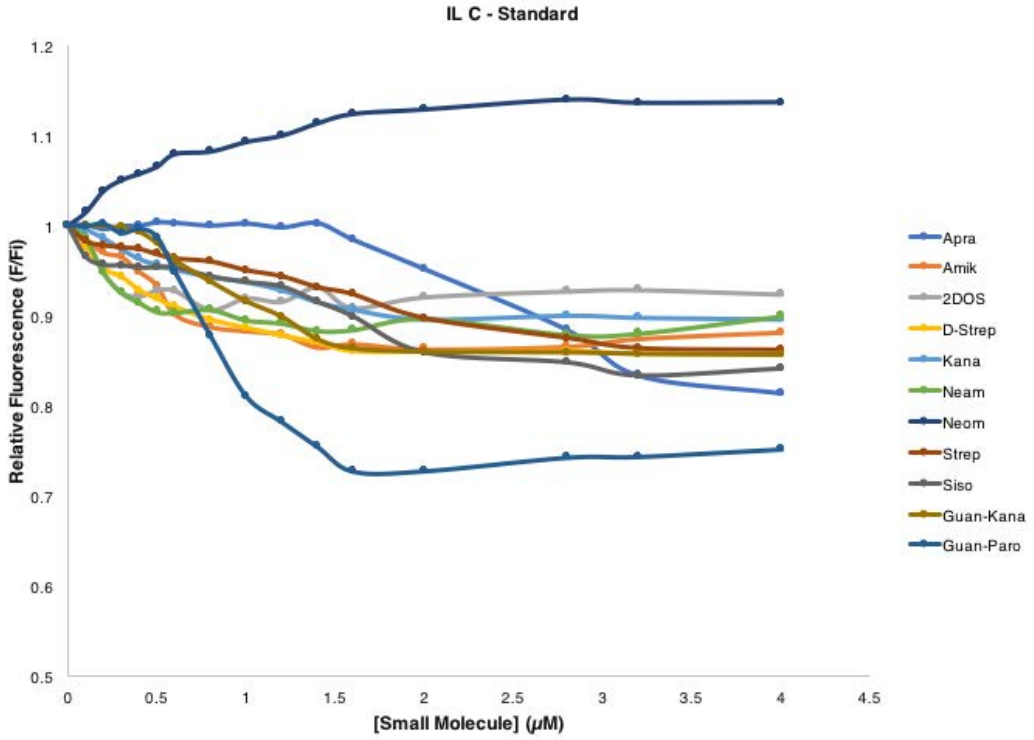
2DOS
D-Strep
Kana
Neam
Neom
Strep
Siso
Guan-Kana
Guan-Paro


IL C - Standard
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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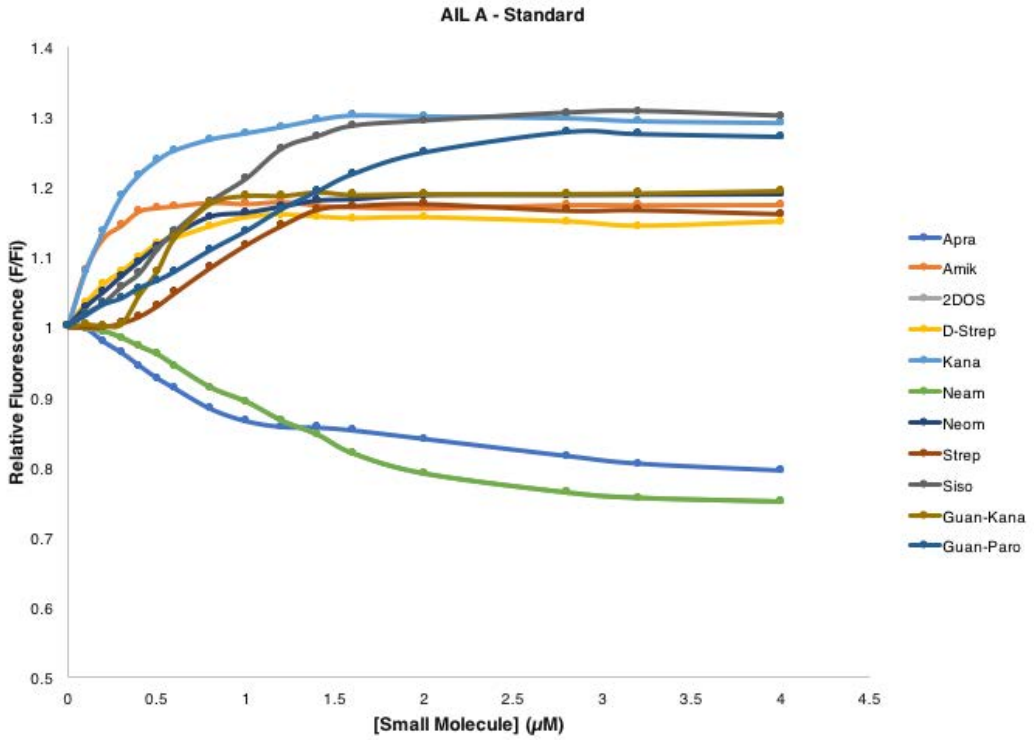
2DOS
D-Strep
Kana
Neam
Neom
Strep
Siso
Guan-Kana
Guan-Paro


AIL A - Standard
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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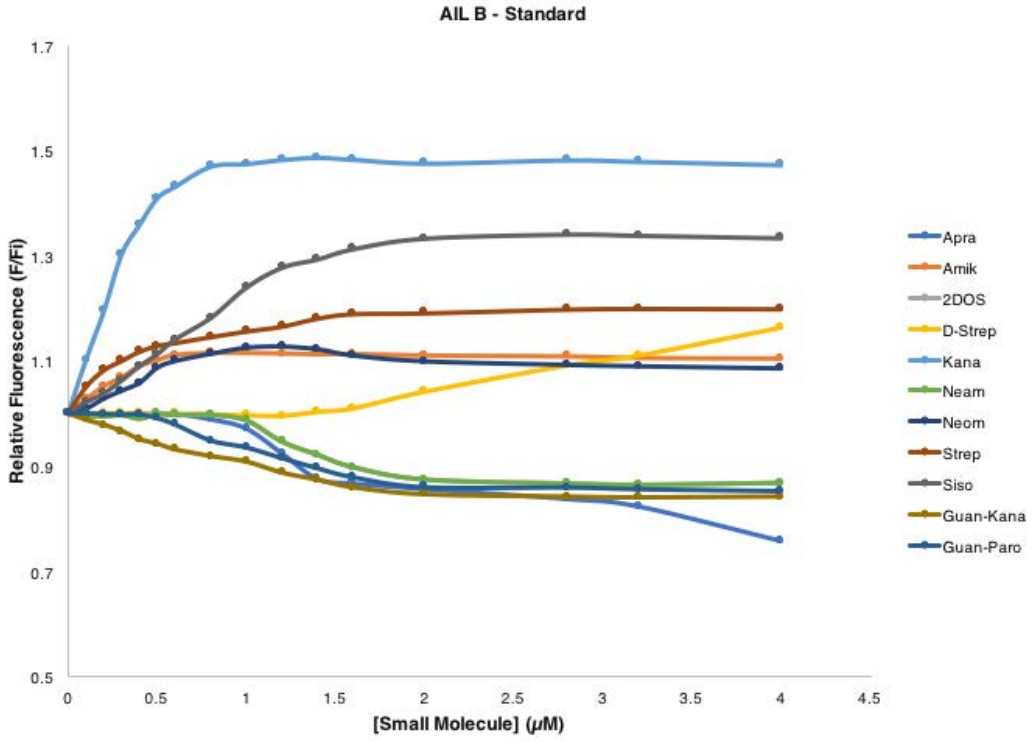
Amik
2DOS
D-Strep
Kana
Neam
Neom
Strep
Siso
Guan-Kana
Guan-Paro


AIL B - Standard
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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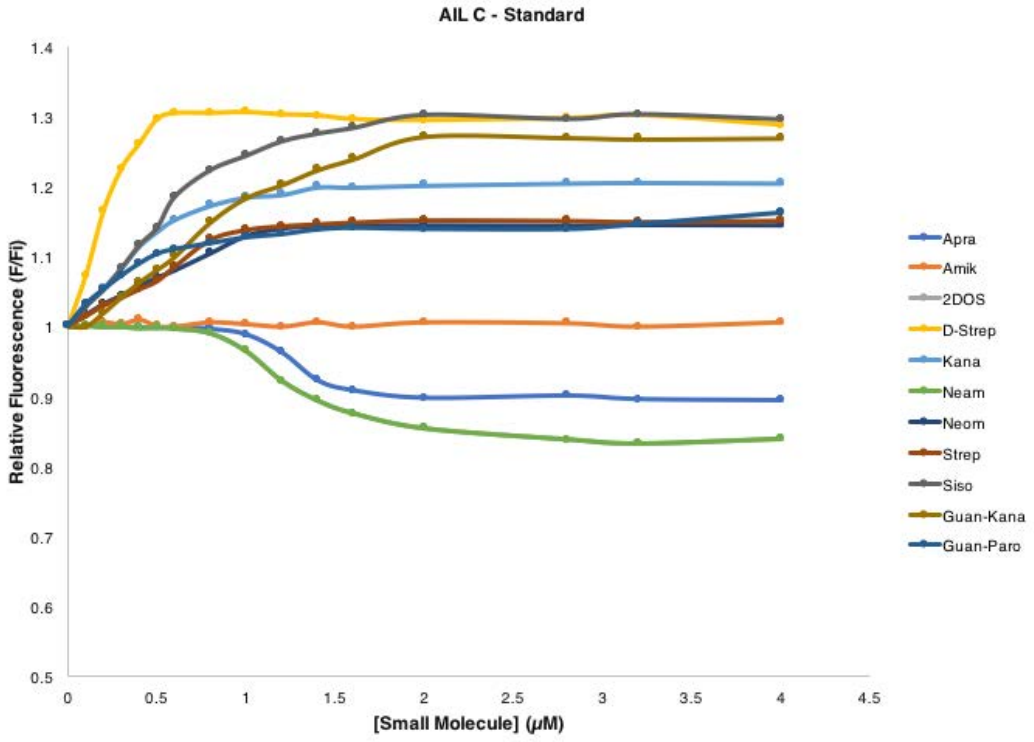
Amik
2DOS
D-Strep
Kana
Neam
Neom
Strep
Siso
Guan-Kana
Guan-Paro


AIL C - Standard
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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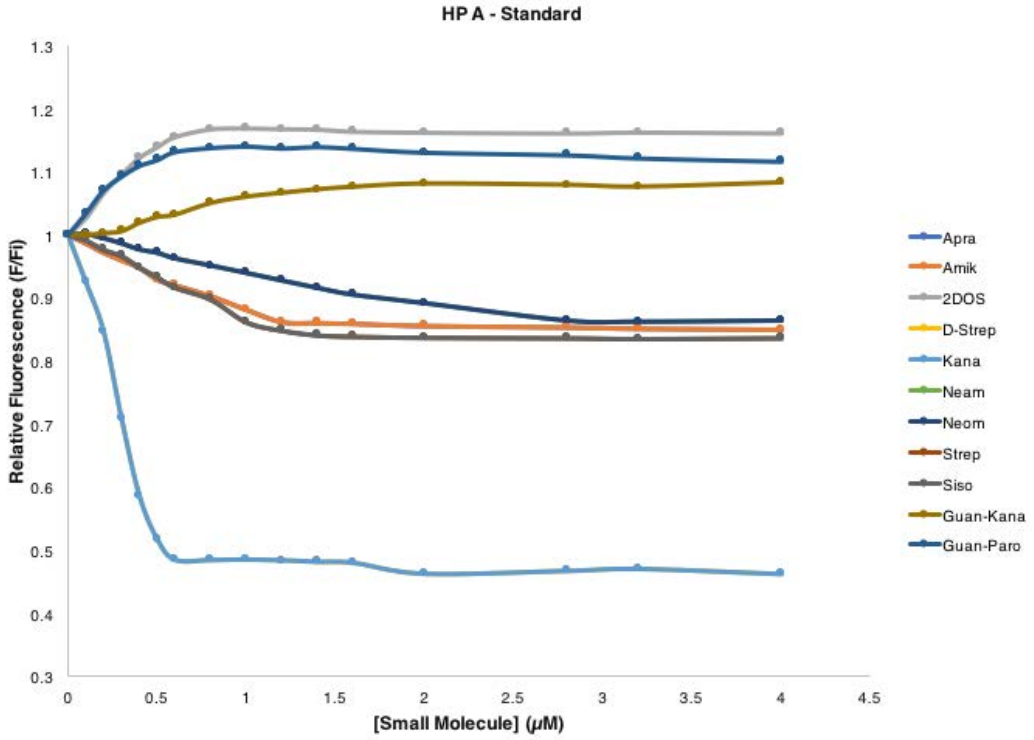
Amik
2DOS
D-Strep
Kana
Neam
Neom
Strep
Siso
Guan-Kana
Guan-Paro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Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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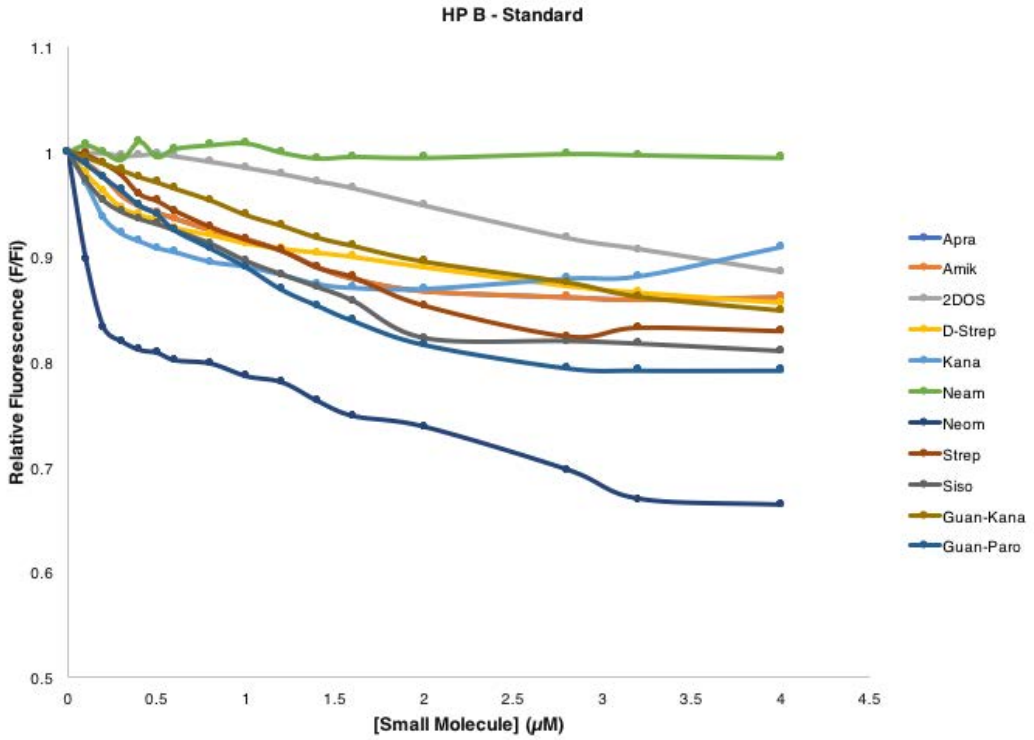
Amik
2DOS
D-Strep
Kana
Neam
Neom
Strep
Siso
Guan-Kana
Guan-Paro


HP B - Standard
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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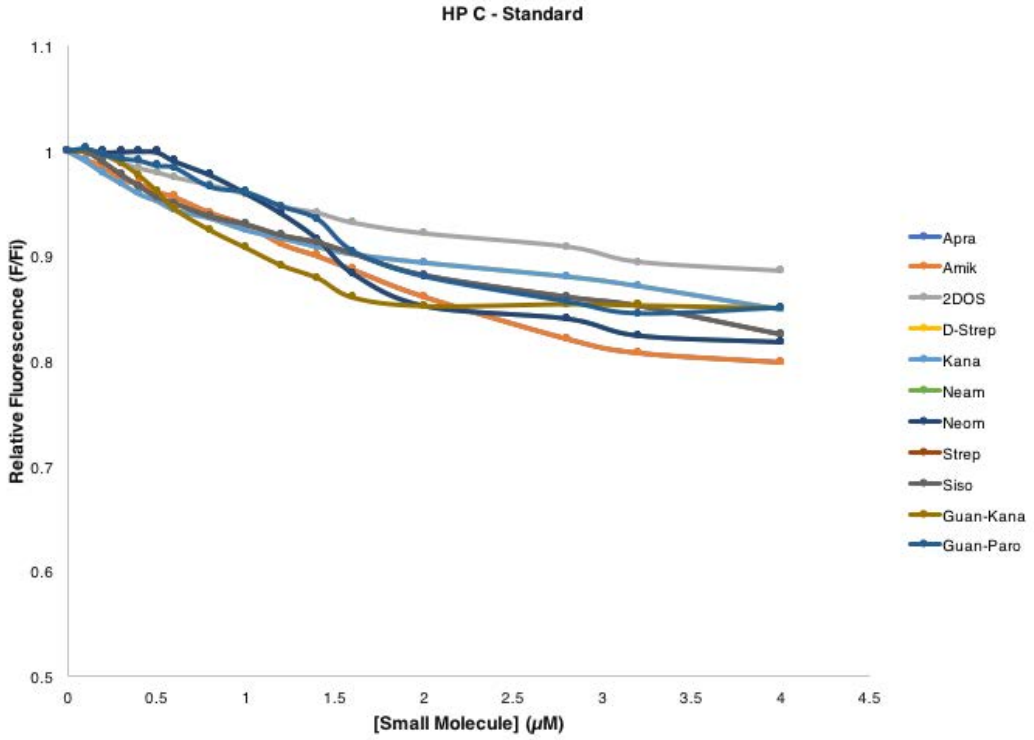
Amik
2DOS
D-Strep
Kana
Neam
Neom
Strep
Siso
Guan-Kana
Guan-Paro


HP C - Standard
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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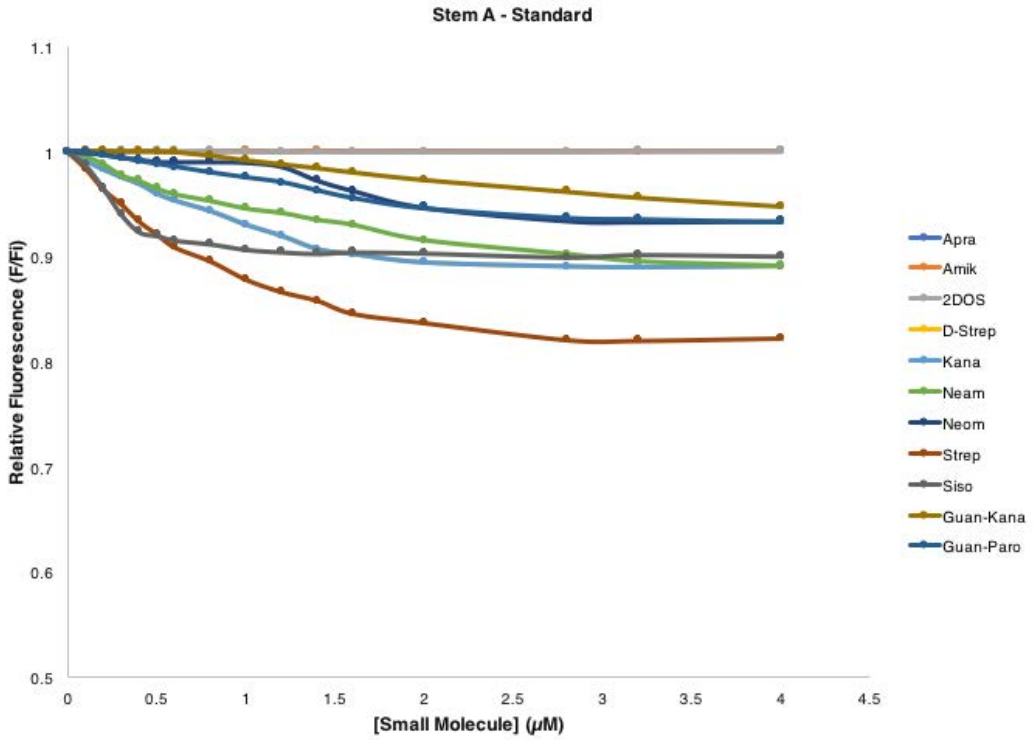
Amik
2DOS
D-Strep
Kana
Neam
Neom
Strep
Siso
Guan-Kana
Guan-Paro


Stem A - Standard
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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Amik
2DOS
D-Strep
Kana
Neam
Neom
Strep
Siso
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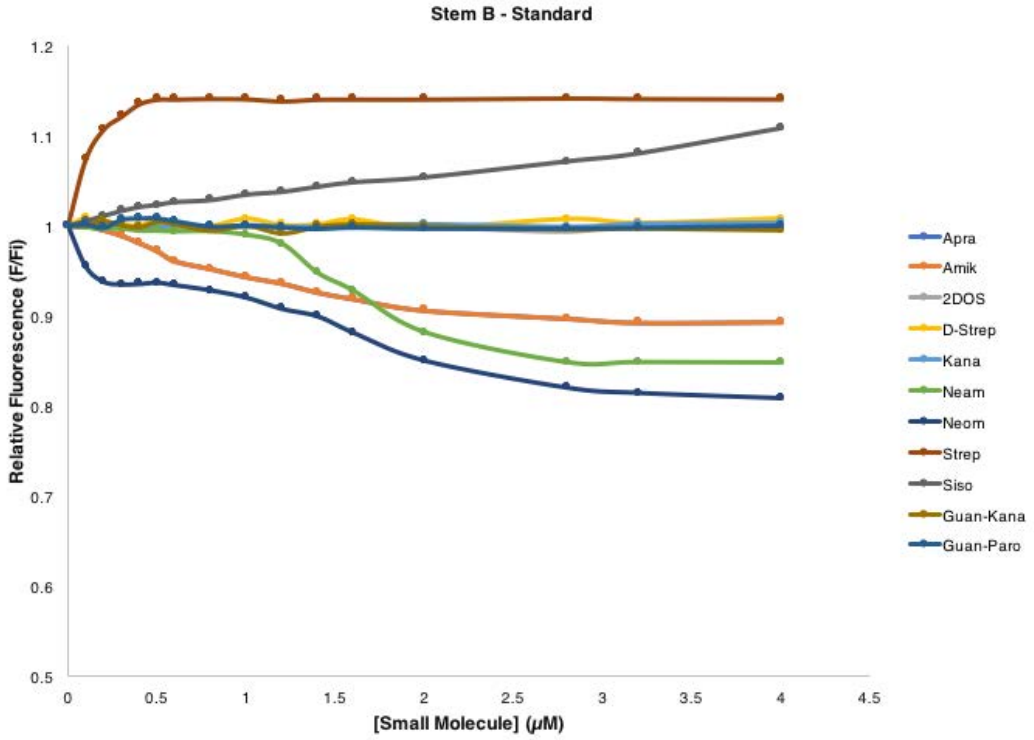
Guan-Paro



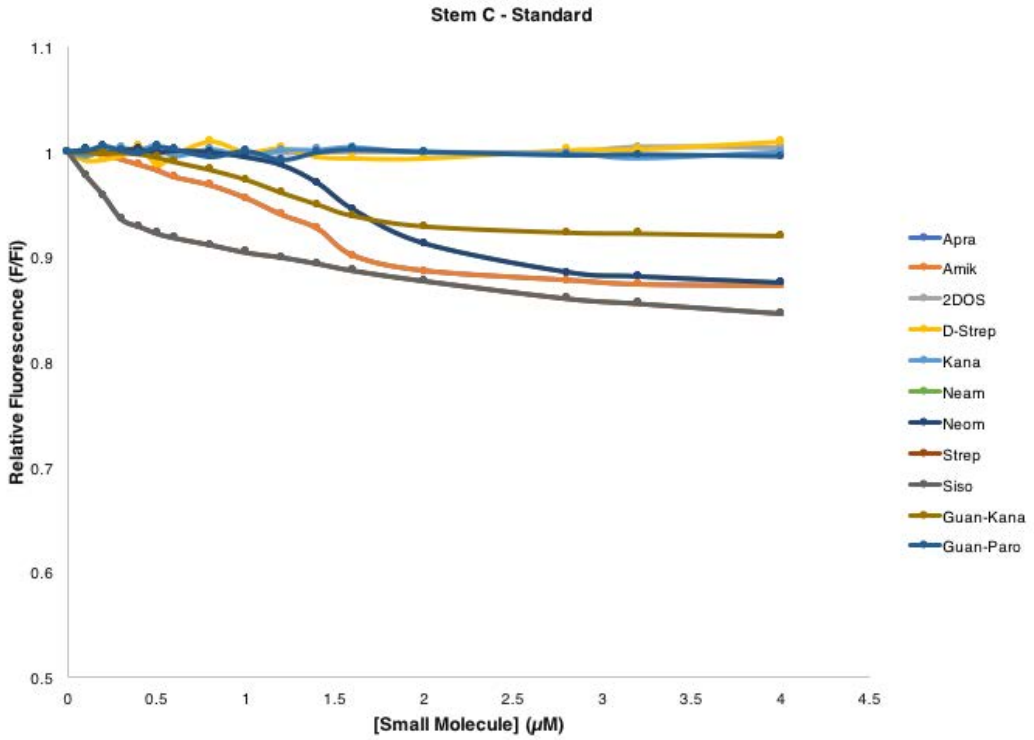
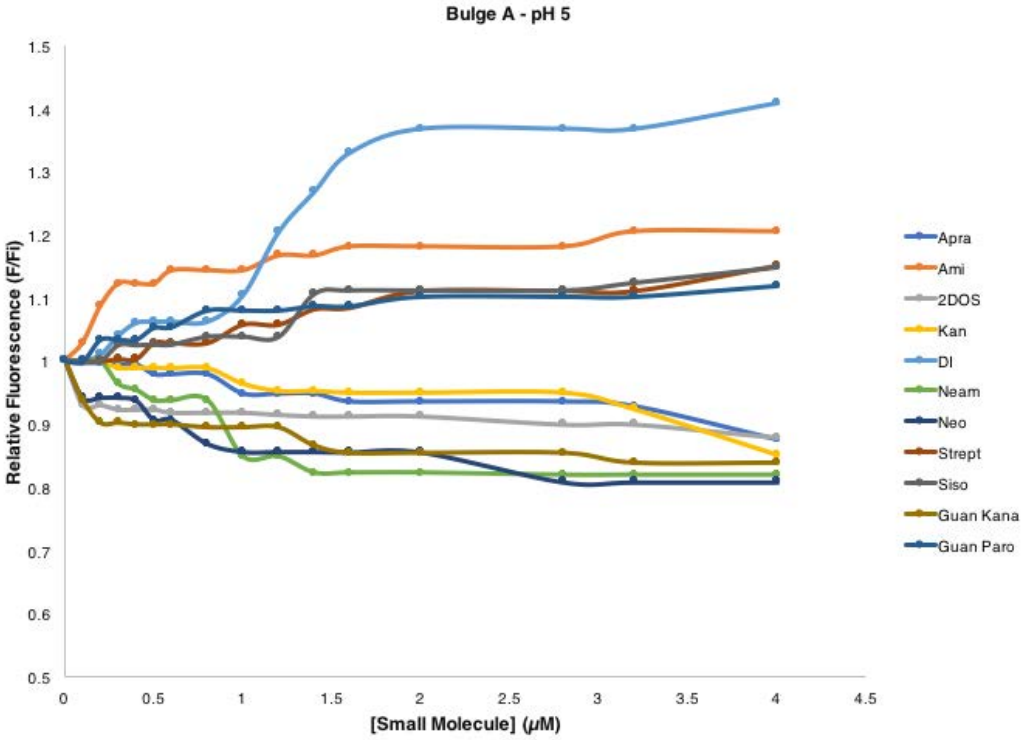
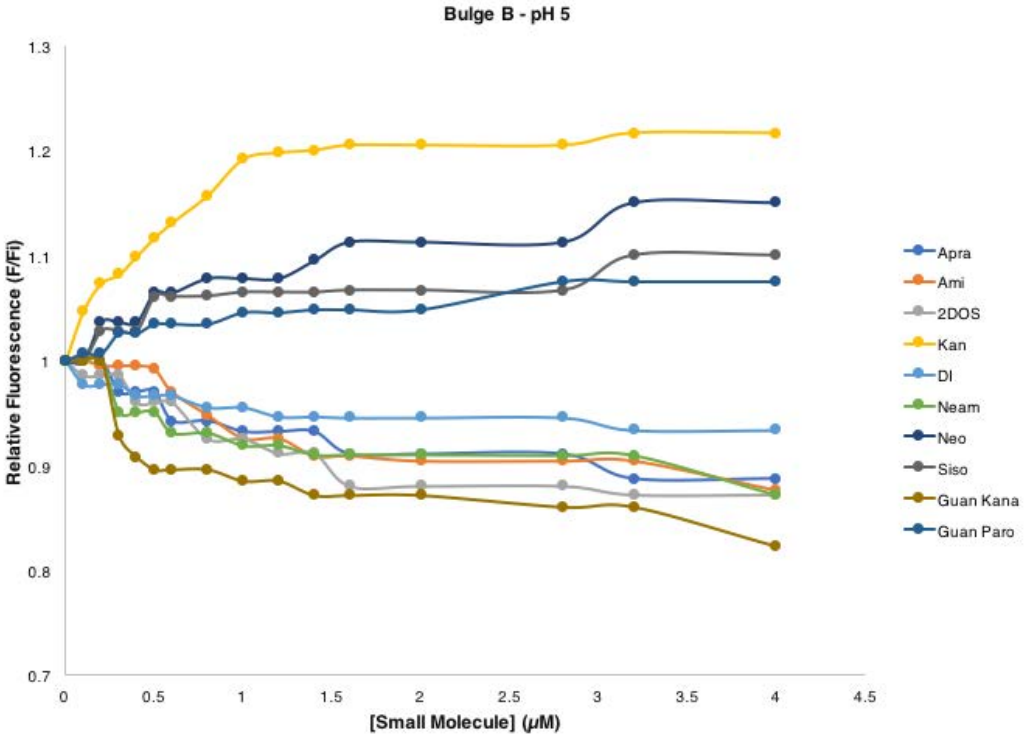
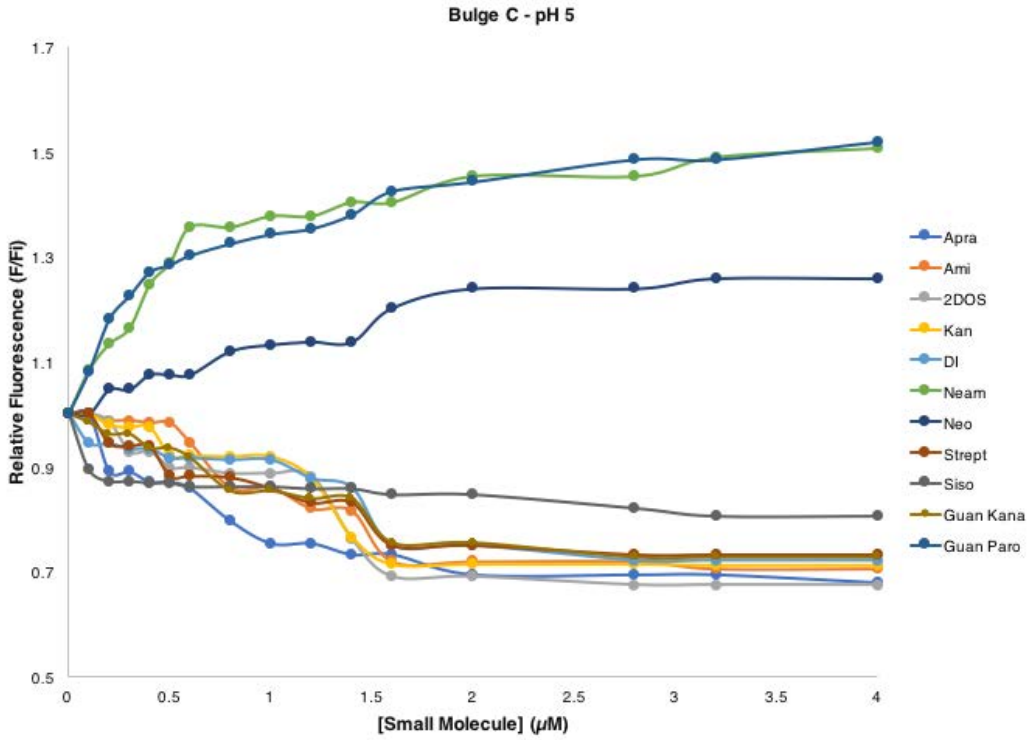


Figure S4-1. All RNA sequence titration curves to the small molecule receptors in standard buffer (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 7.3, 25°C). Each graph shows a single RNA sequence and the receptors titration curves. The error for all experiments were less than 10%. The error was calculated using the standard error of triplicates, and relative standard error was determined based on the mean of each RNA sequence.


Bulge A - pH 5
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Ami
2DOS
Kan
DI
Neam
Neo
Strept
Siso
Guan Kana
Guan Paro


Bulge B - pH 5
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Ami
2DOS
Kan
DI
Neam
Neo
Siso
Guan Kana
Guan Paro


Bulge C - pH 5
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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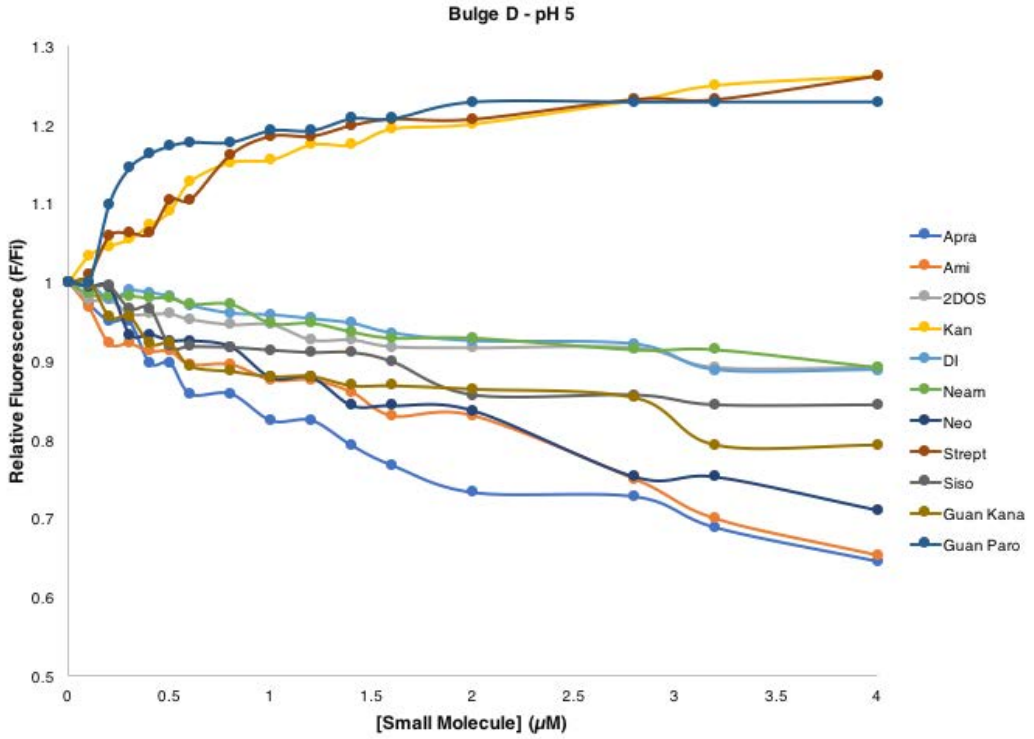
Ami
2DOS
Kan
DI
Neam
Neo
Strept
Siso
Guan Kana
Guan Paro


Bulge D - pH 5
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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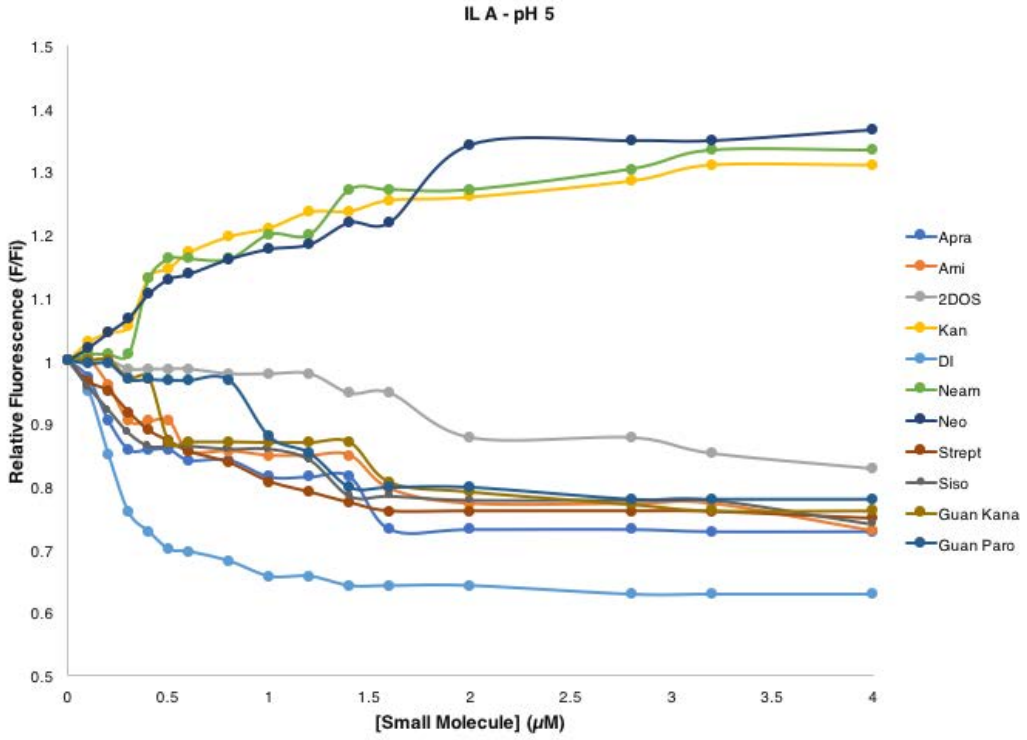
Ami
2DOS
Kan
DI
Neam
Neo
Strept
Siso
Guan Kana
Guan Paro


IL A - pH 5
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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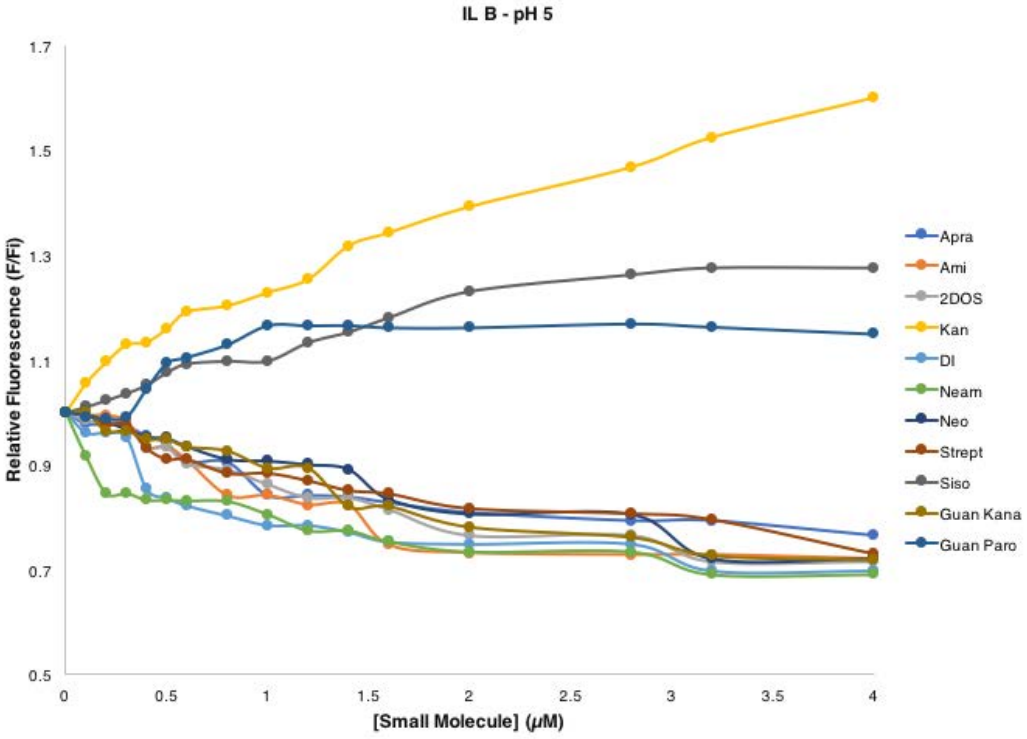
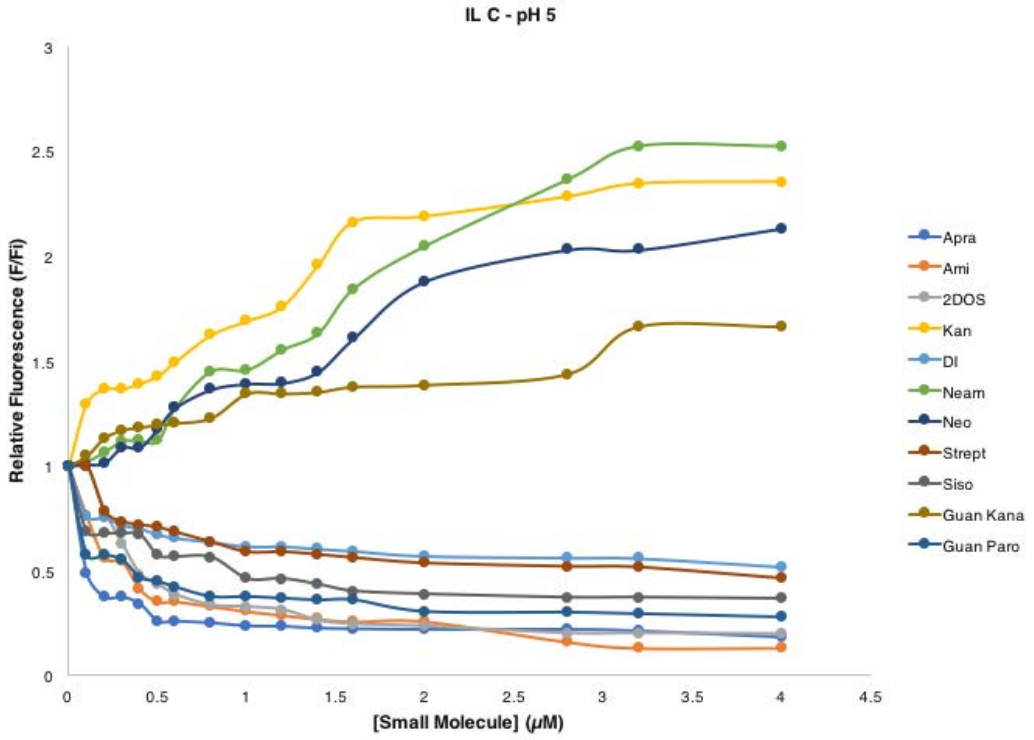
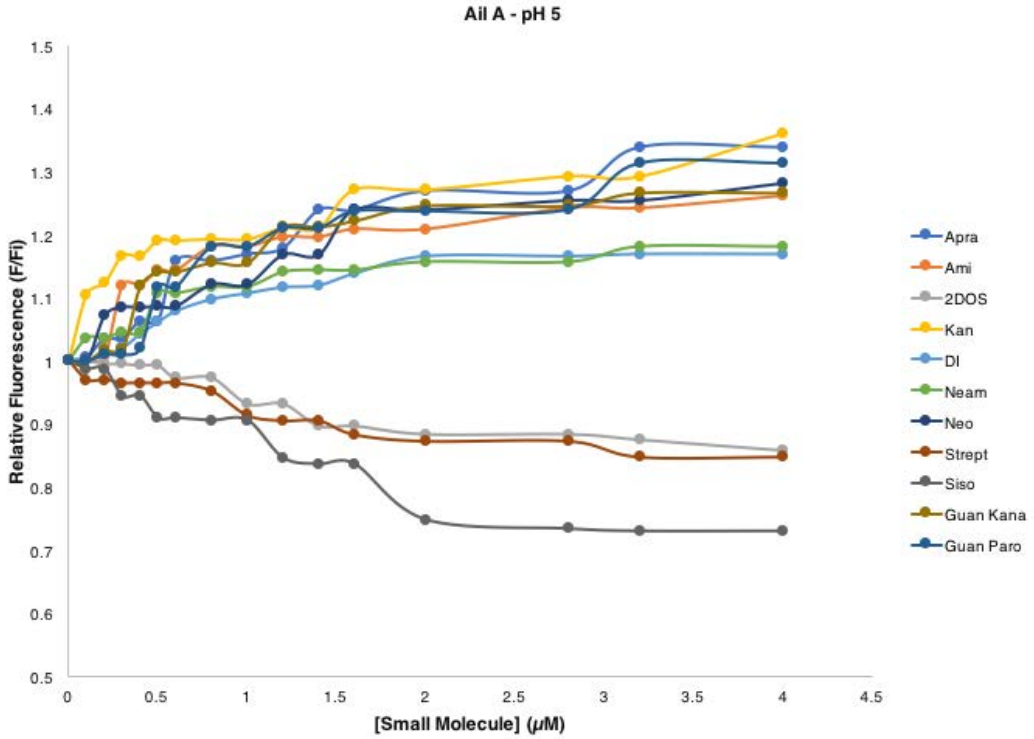
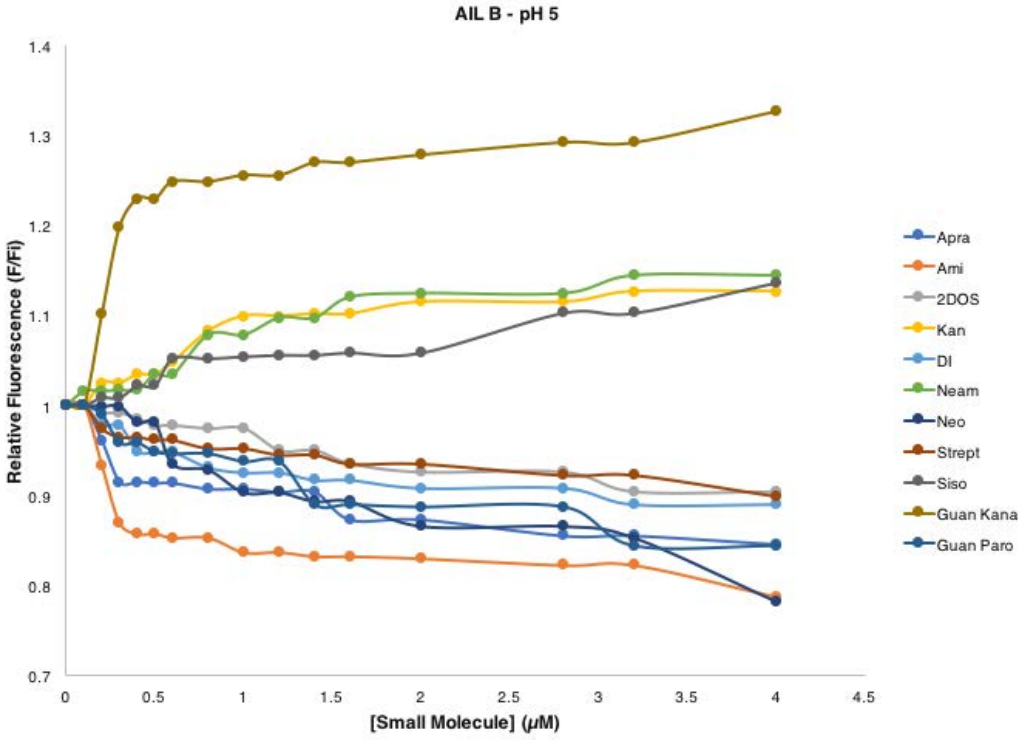
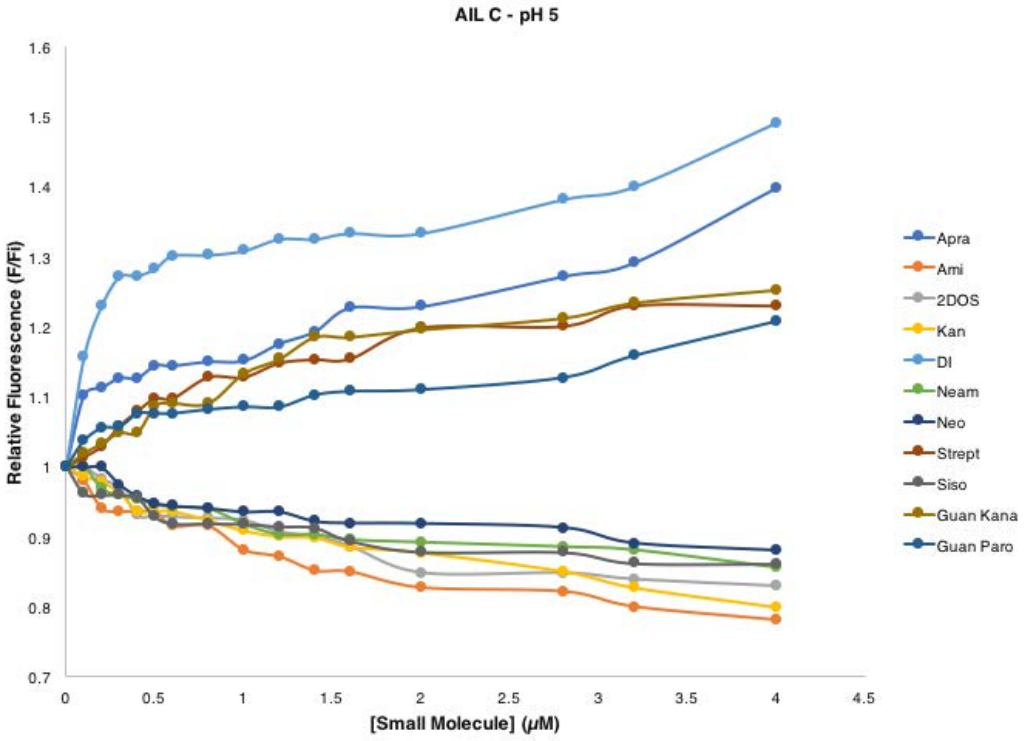
Ami
2DOS
Kan
DI
Neam
Neo
Strept
Siso
Guan Kana
Guan Paro

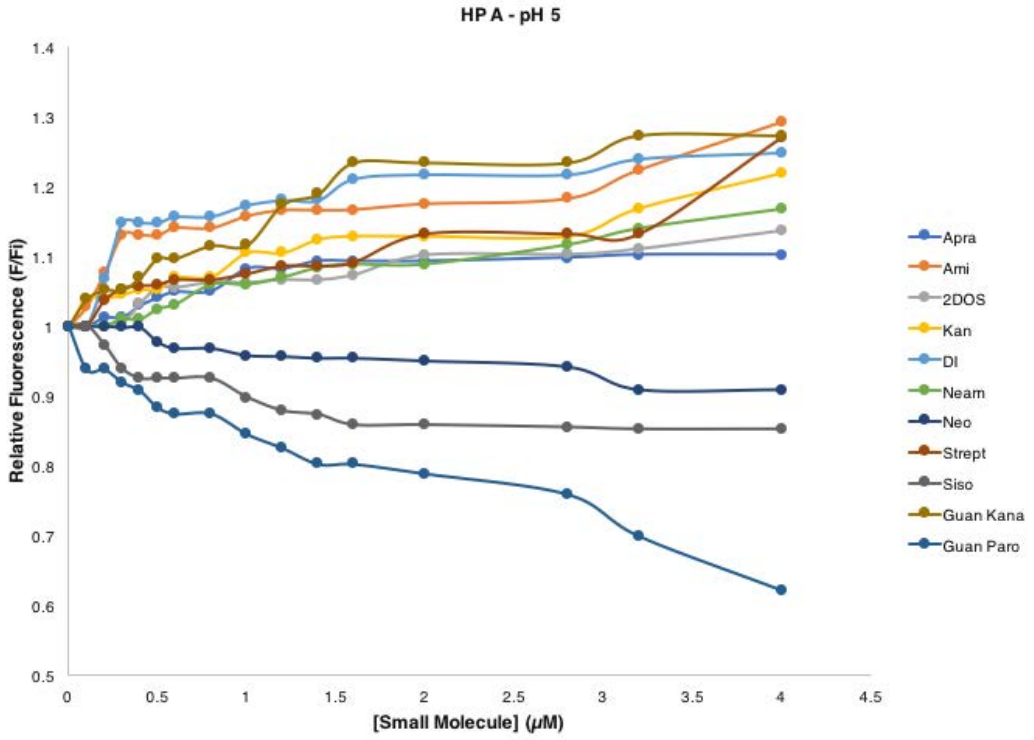

IL B - pH 5
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Ami
2DOS
Kan
DI
Neam
Neo
Strept
Siso
Guan Kana
Guan Paro

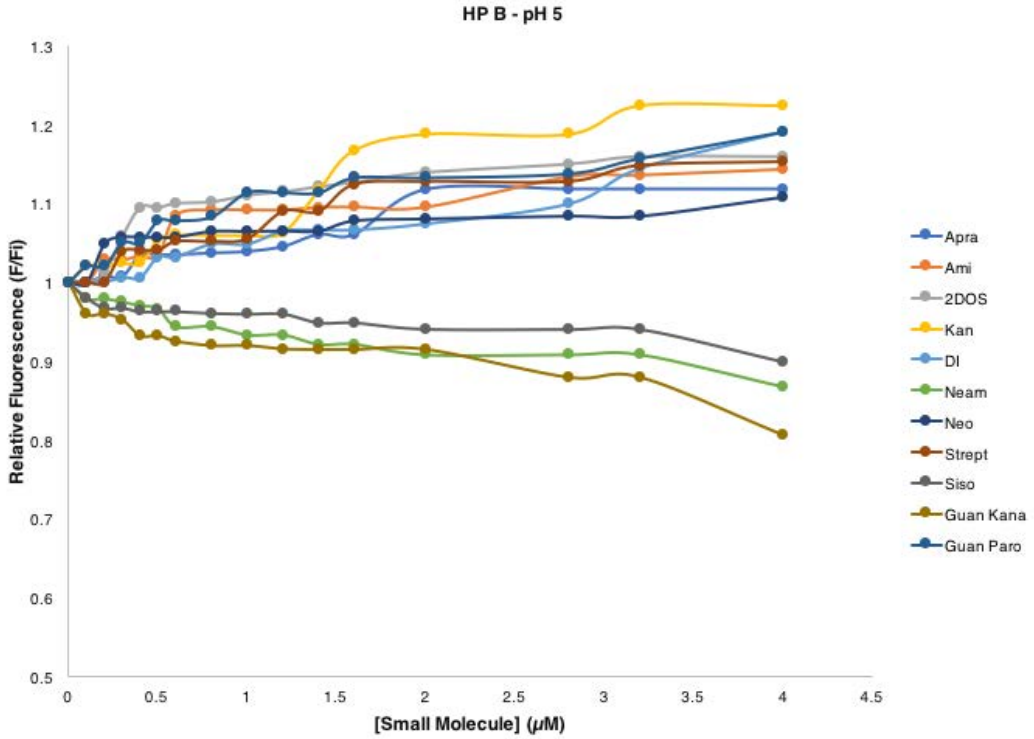

IL C - pH 5
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Ami
2DOS
Kan
Dl
Neam
Neo
Strept
Siso
Guan Kana
Guan Paro

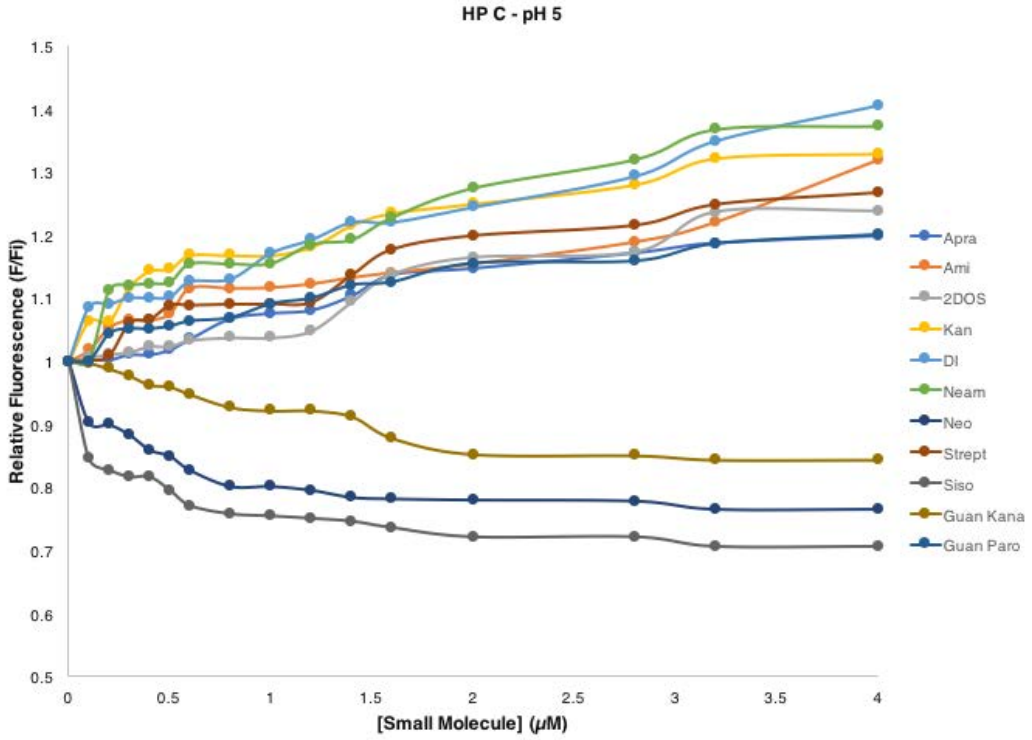

Ail A - pH 5
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Ami
2DOS
Kan
Dl
Neam
Neo
Strept
Siso
Guan Kana
Guan Paro

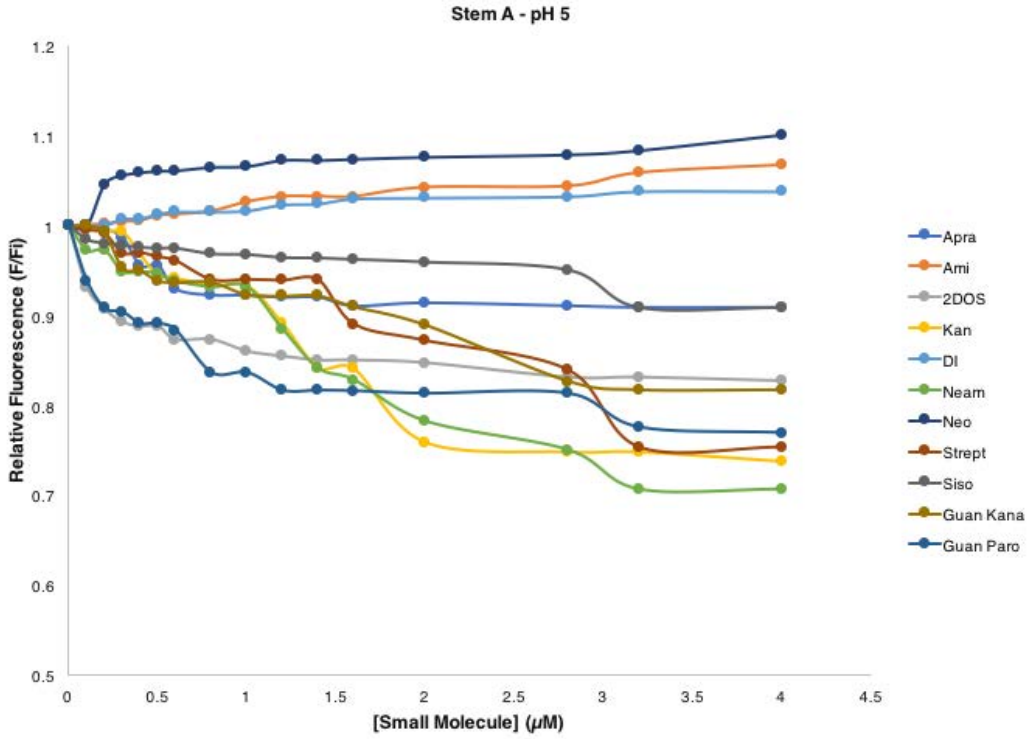

AIL B - pH 5
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Ami
2DOS
Kan
DI
Neam
Neo
Strept
Siso
Guan Kana
Guan Paro


AIL C - pH 5
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Ami
2DOS
Kan
DI
Neam
Neo
Strept
Siso
Guan Kana
Guan Paro


HP A - pH 5
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Ami
2DOS
Kan
Dl
Neam
Neo
Strept
Siso
Guan Kana
Guan Paro


HP B - pH 5
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Ami
2DOS
Kan
Dl
Neam
Neo
Strept
Siso
Guan Kana
Guan Paro


HP C - pH 5
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Ami
2DOS
Kan
DI
Neam
Neo
Strept
Siso
Guan Kana
Guan Paro


Stem A - pH 5
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Ami
2DOS
Kan
DI
Neam
Neo
Strept
Siso
Guan Kana
Guan Paro

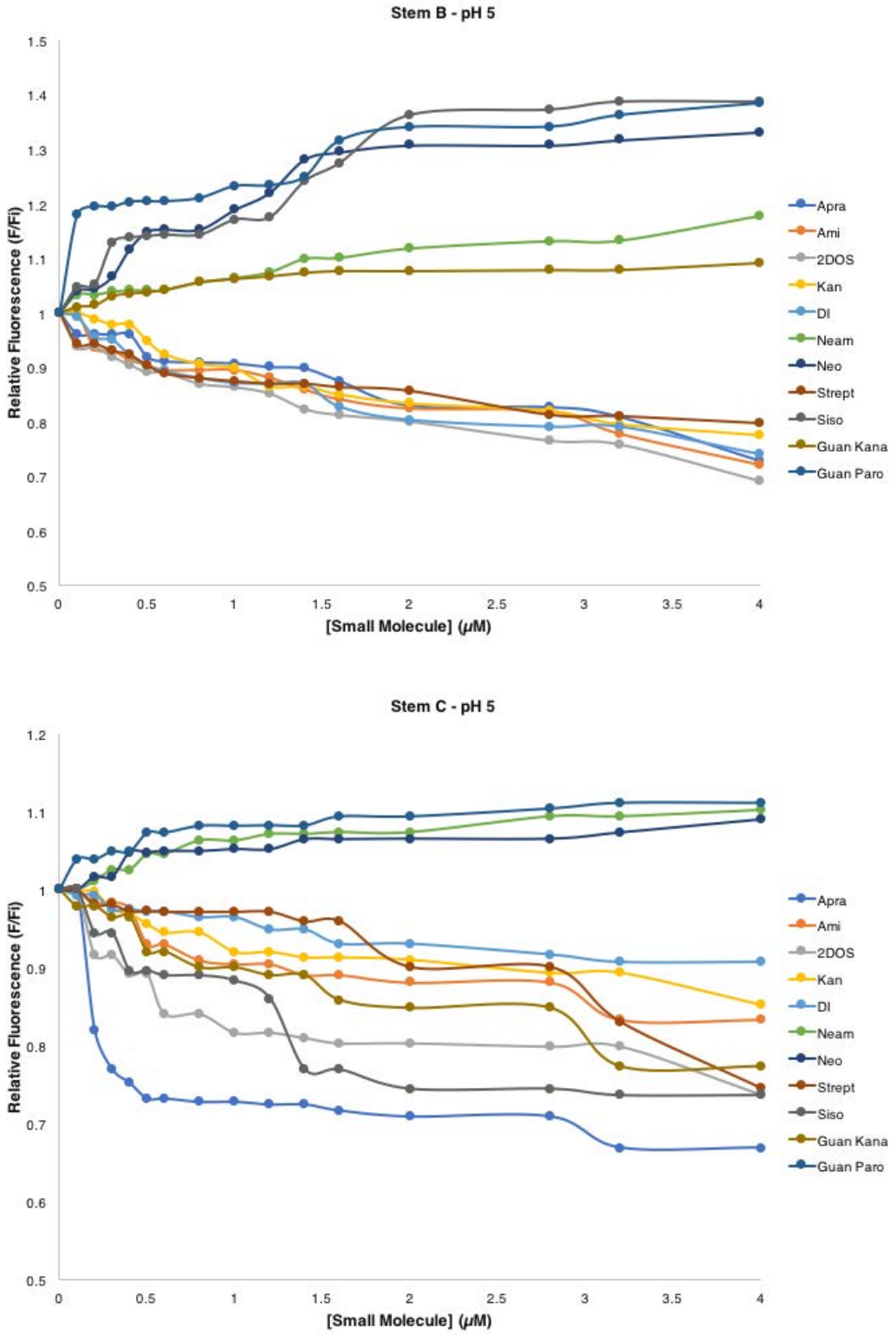
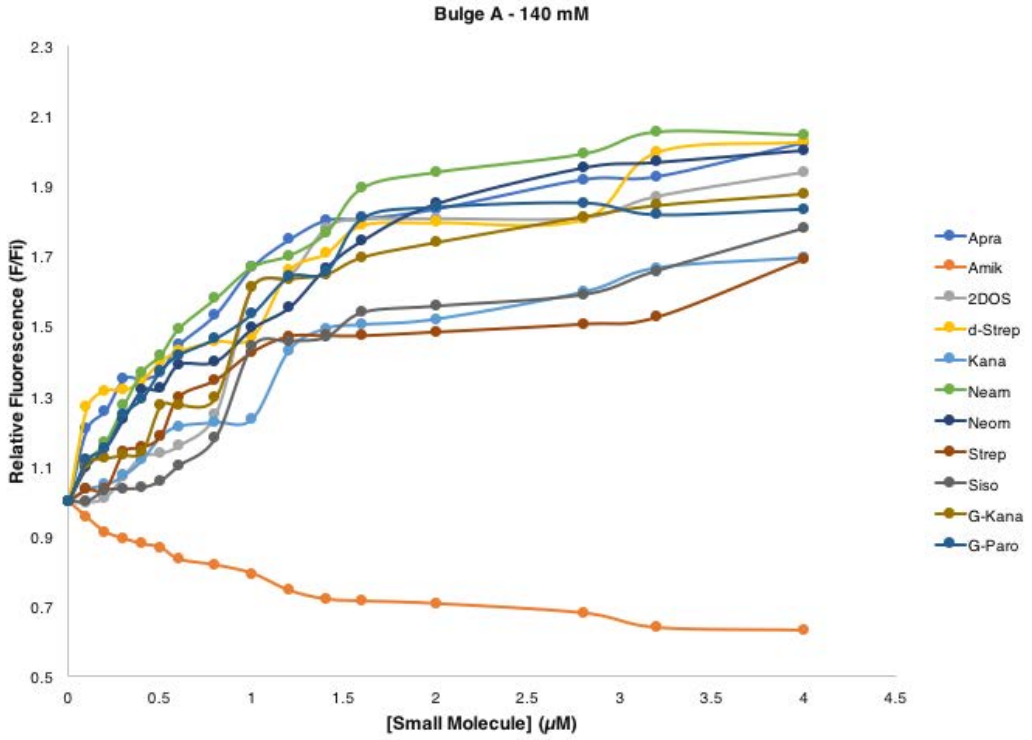
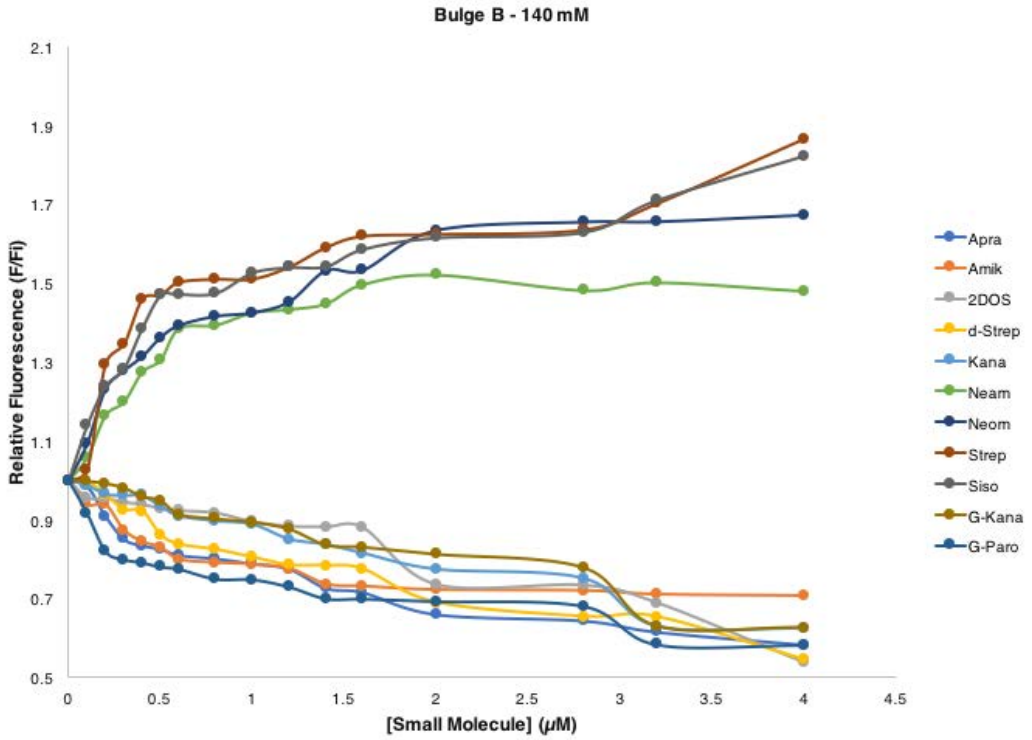
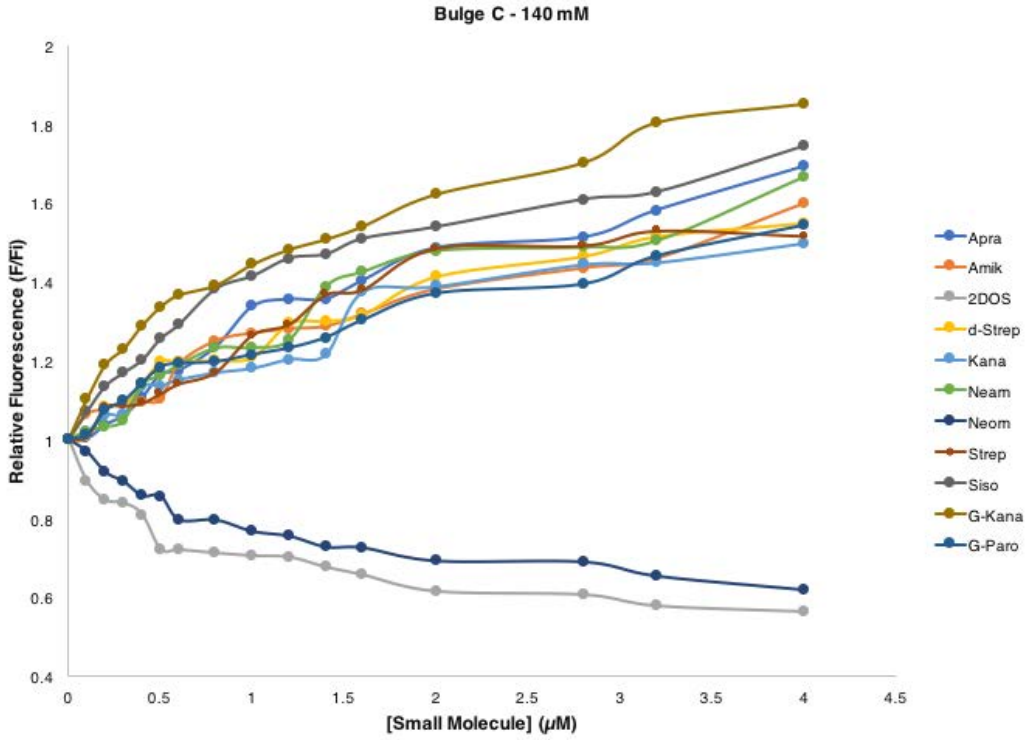
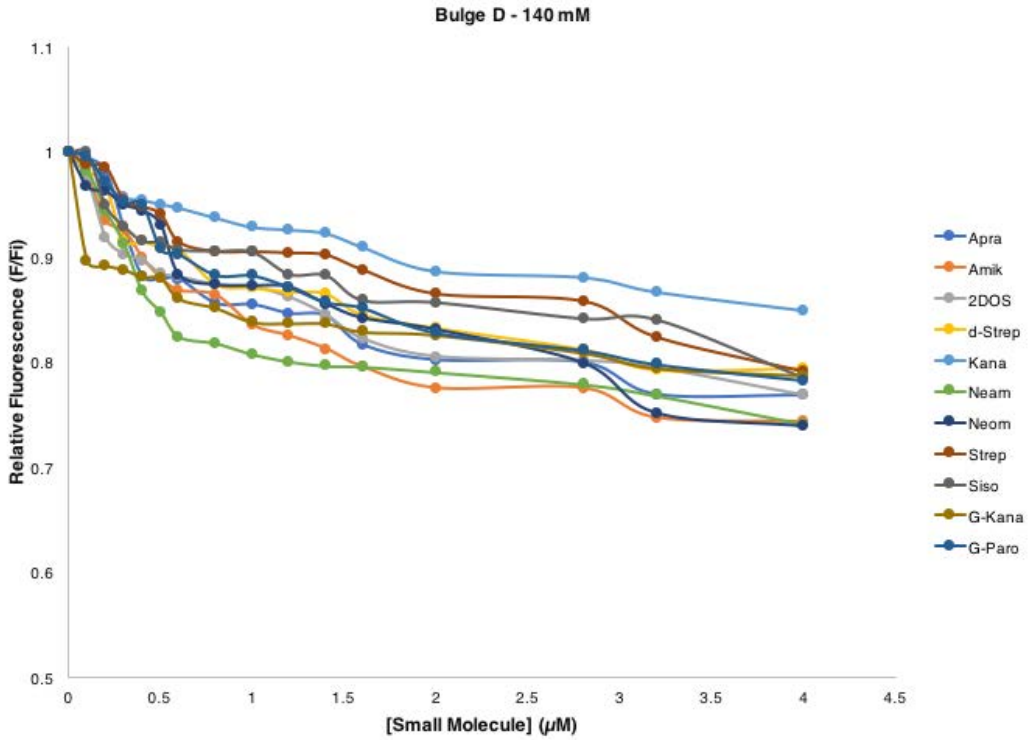


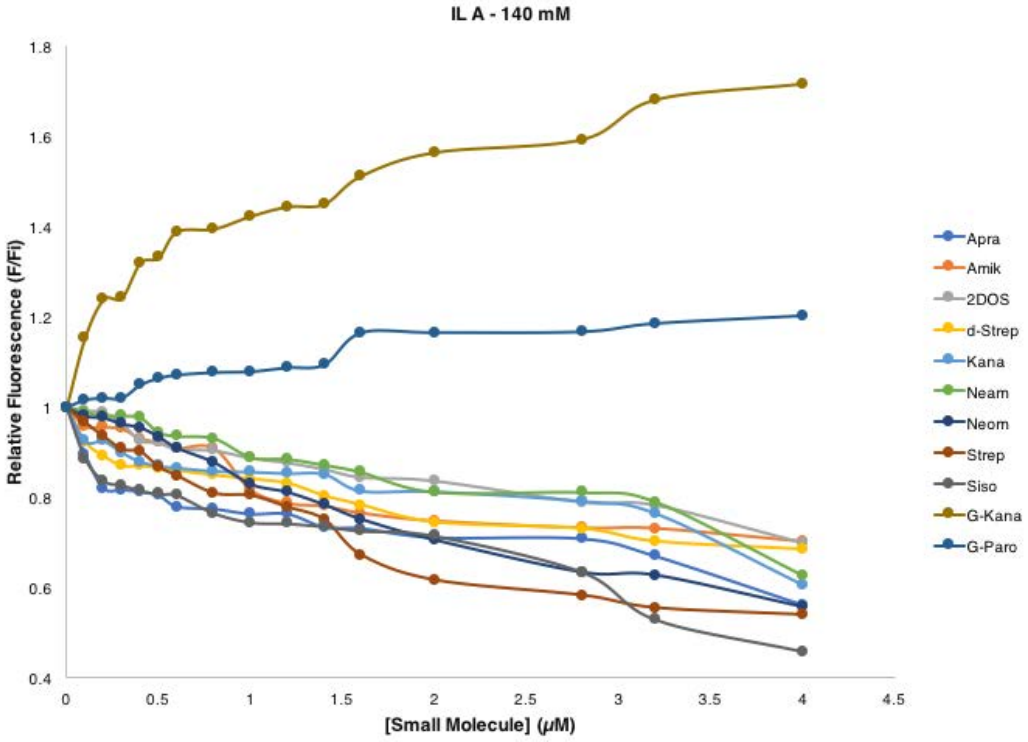
Figure S4-2. All RNA sequence titration curves to the small molecule receptors in buffer at pH 5 (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 5, 25°C). Each graph shows a single RNA sequence and the receptors titration curves. The error for all experiments were less than 10%. The error was calculated using the standard error of triplicates, and relative standard error was determined based on the mean of each RNA sequence.

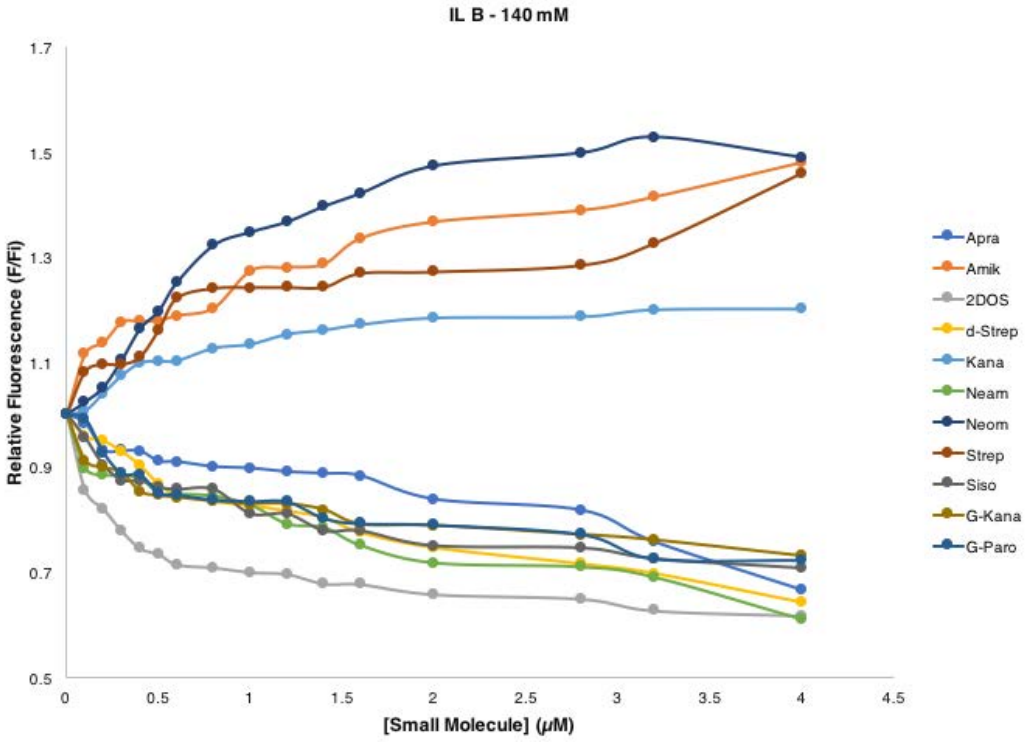

Bulge A - 140 mM
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

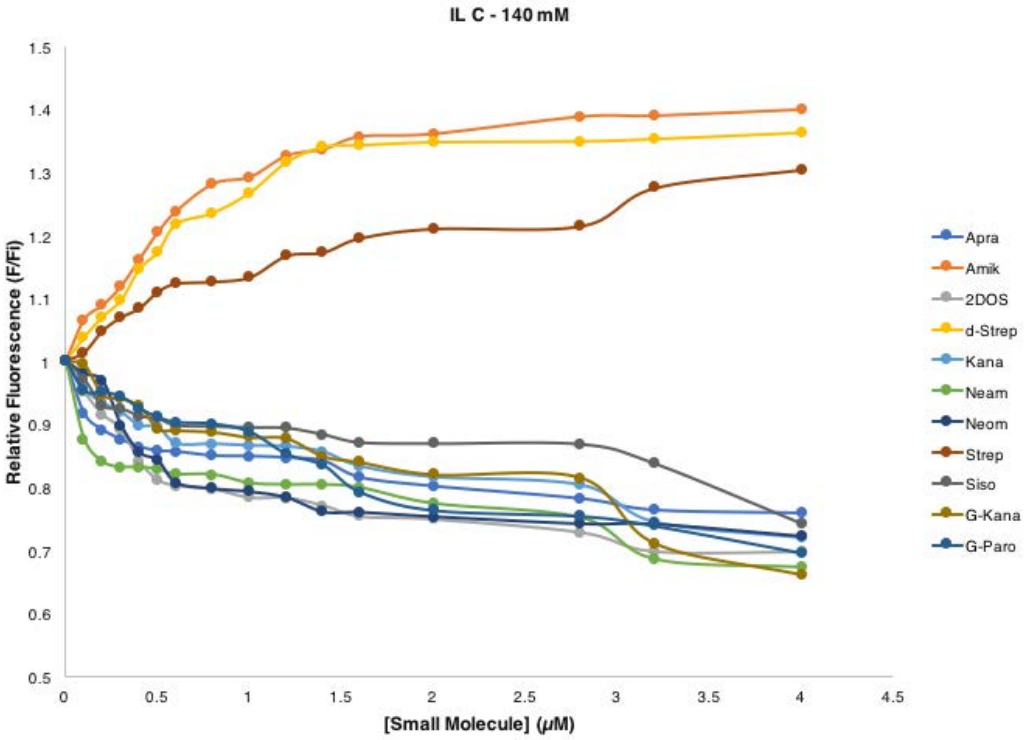

Bulge B - 140 mM
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

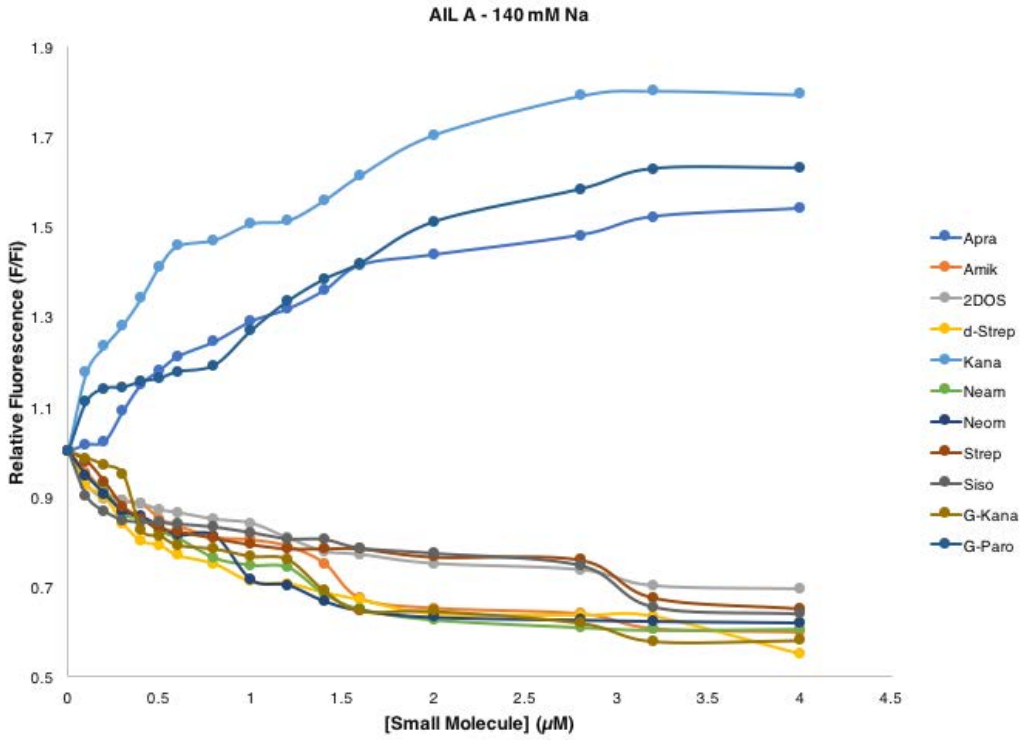

Bulge C - 140 mM
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

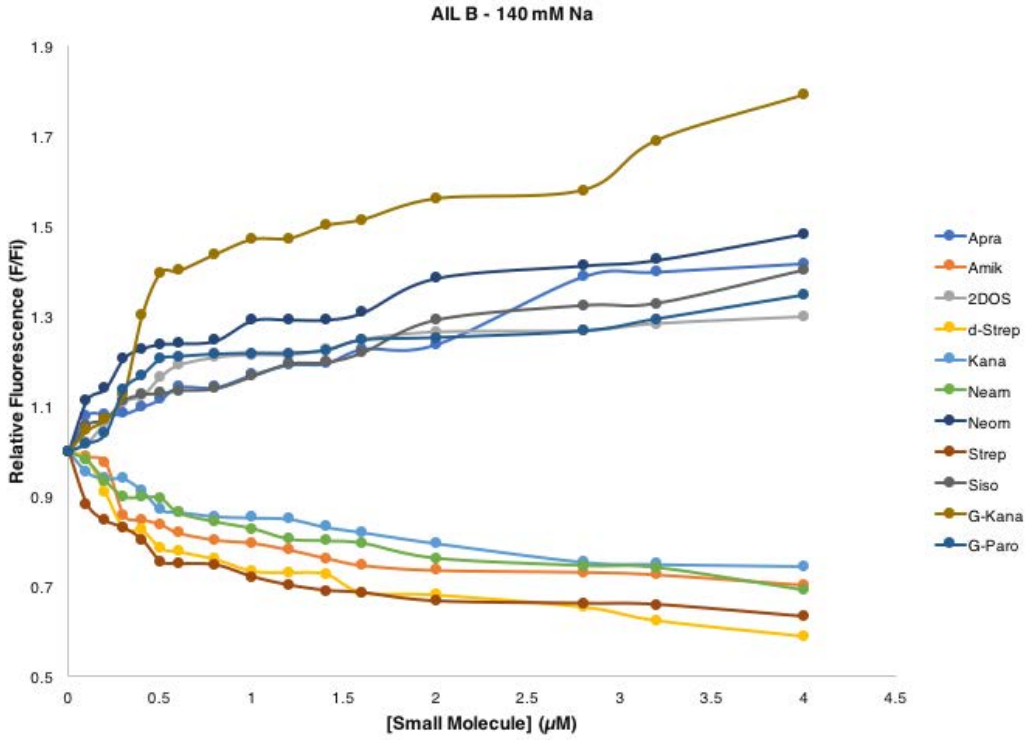

Bulge D - 140 mM
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

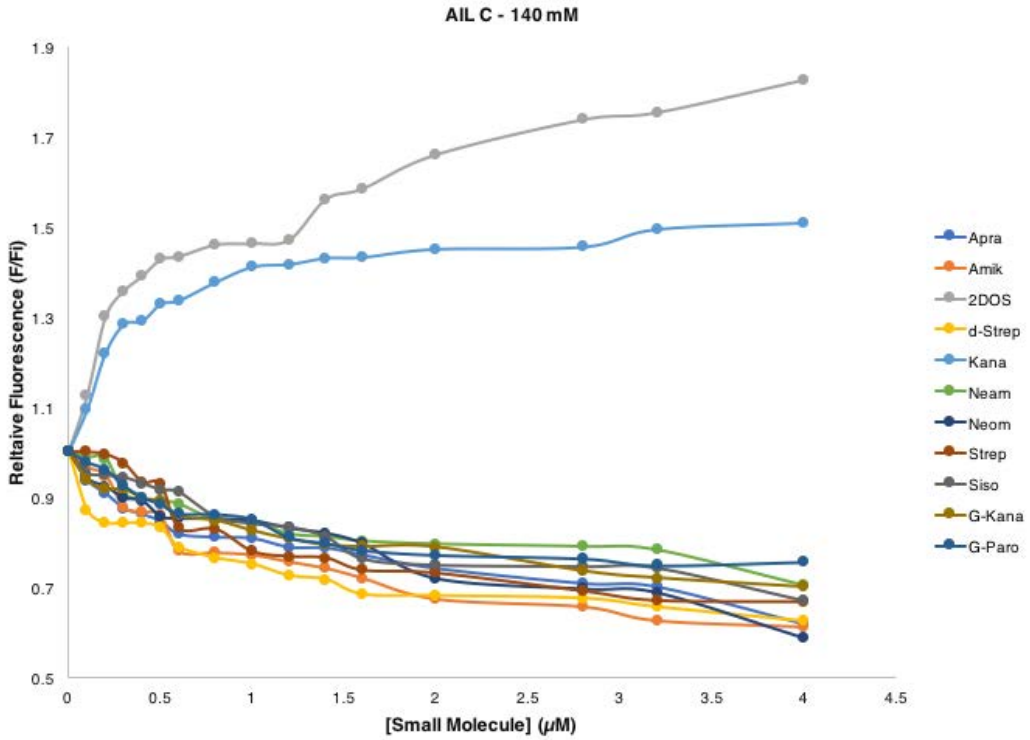

IL A - 140 mM
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

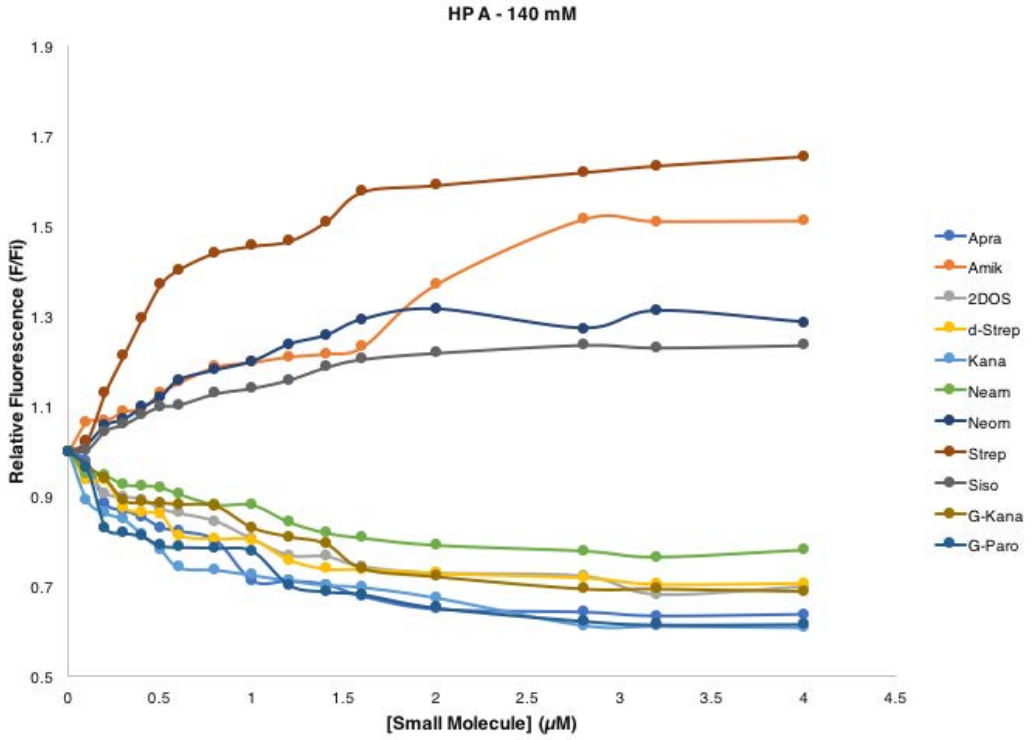

IL B - 140 mM
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

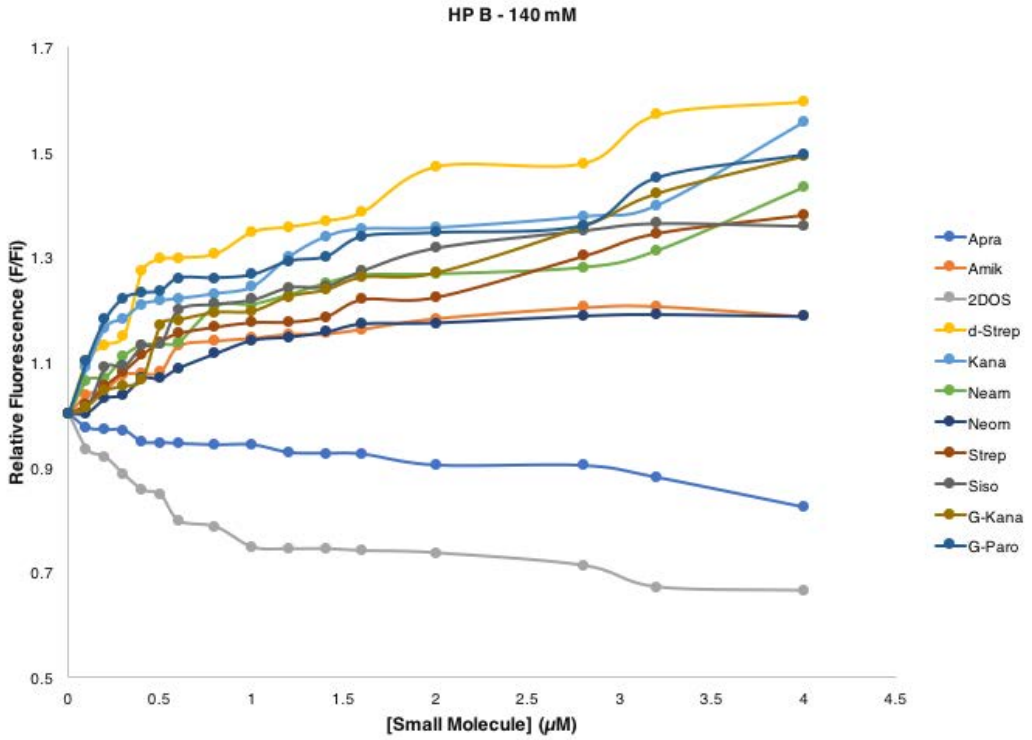

IL C - 140 mM
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

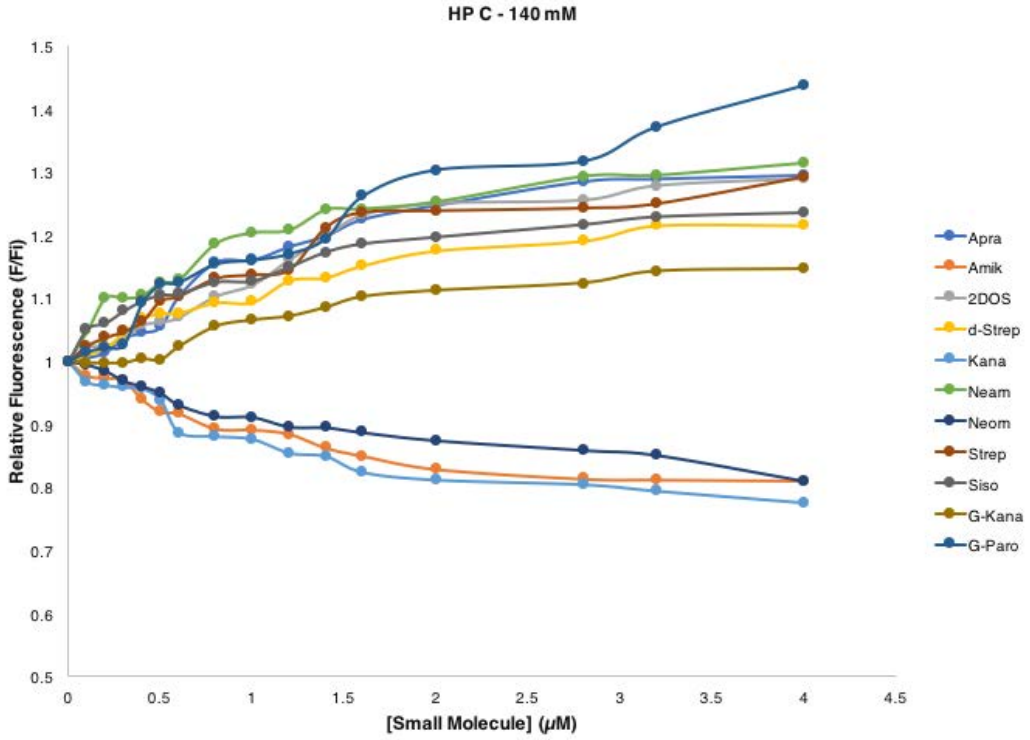

AIL A - 140 mM Na
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

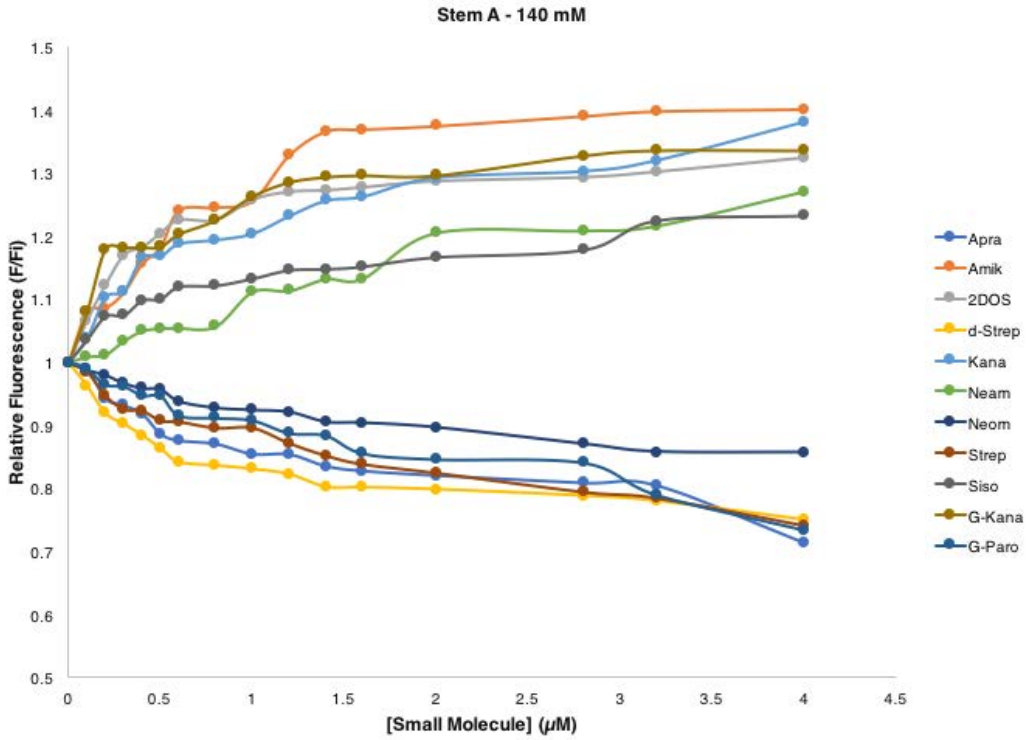

AIL B - 140 mM Na
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

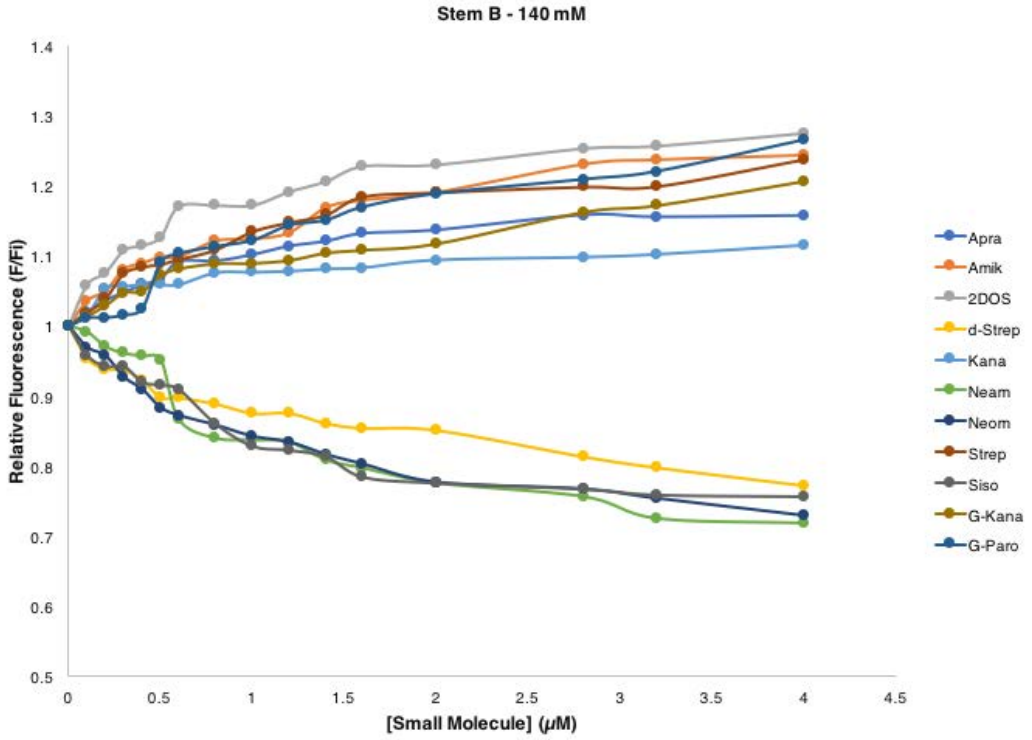

AIL C - 140 mM
Reltaive Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


HP A - 140 mM
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


HP B - 140 mM
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


HP C - 140 mM
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


Stem A - 140 mM
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro



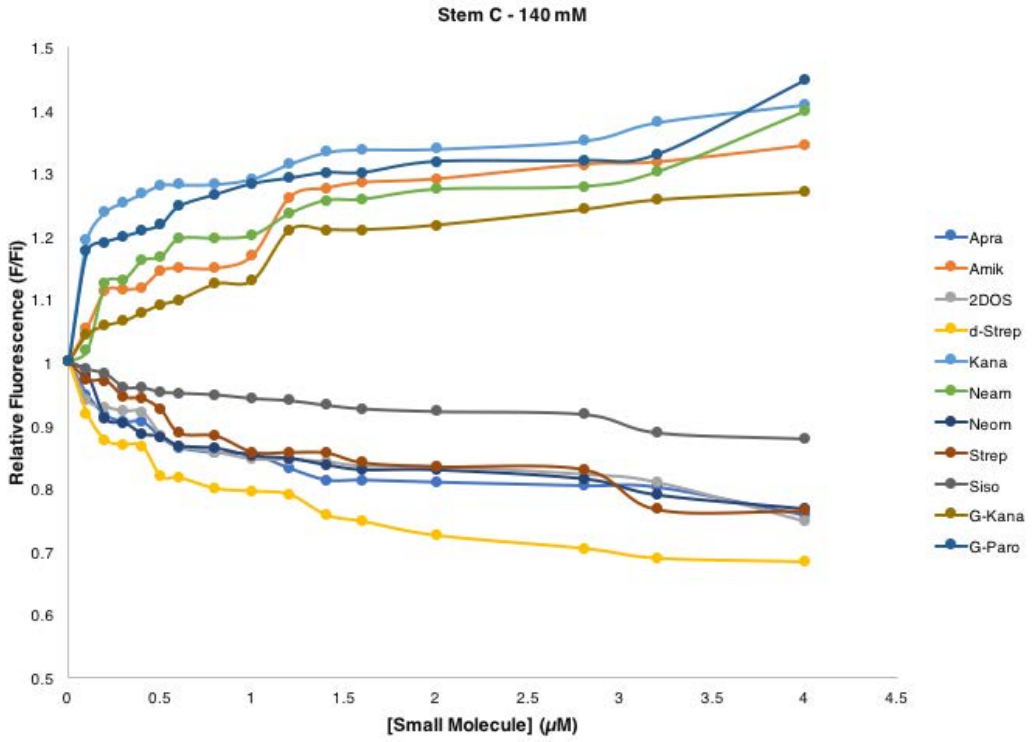
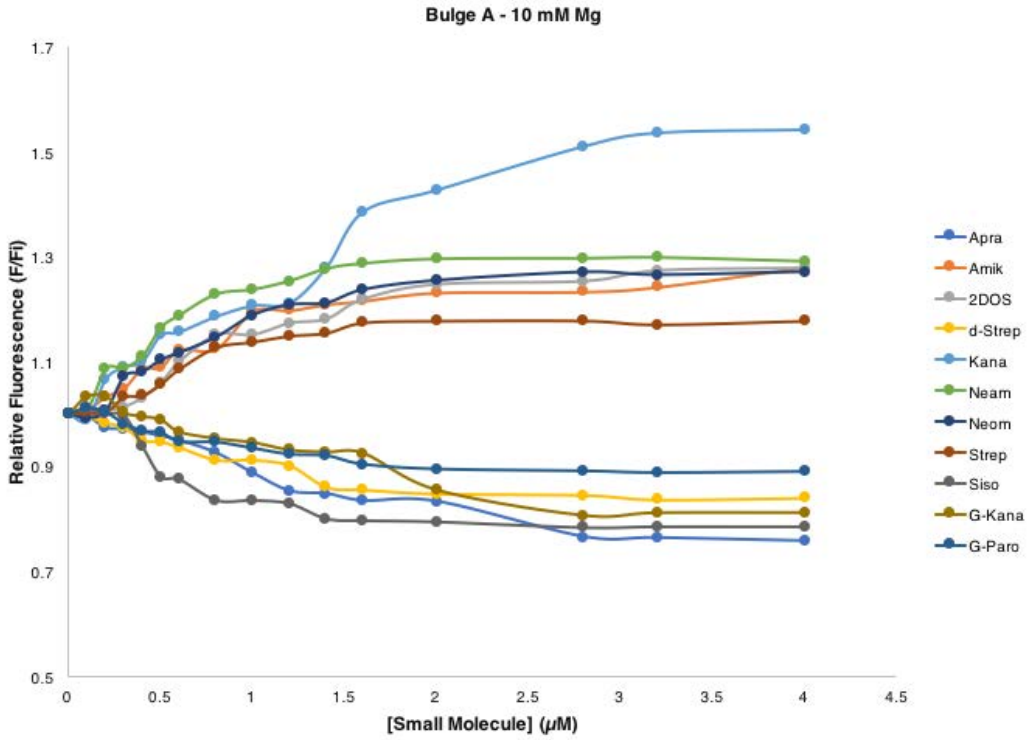
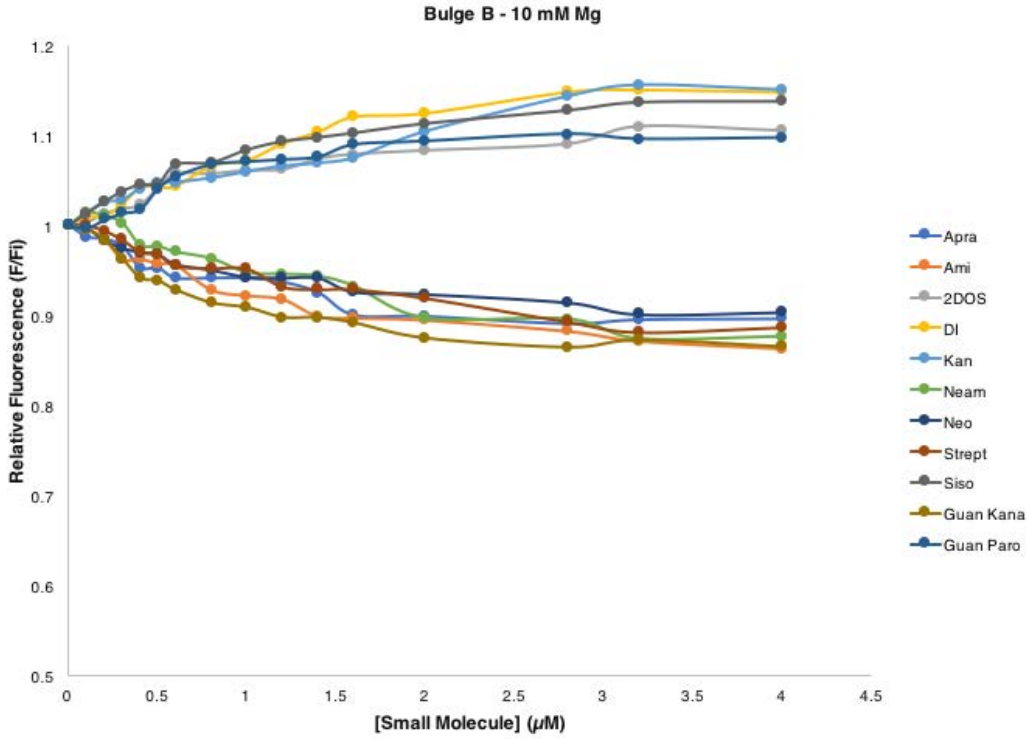
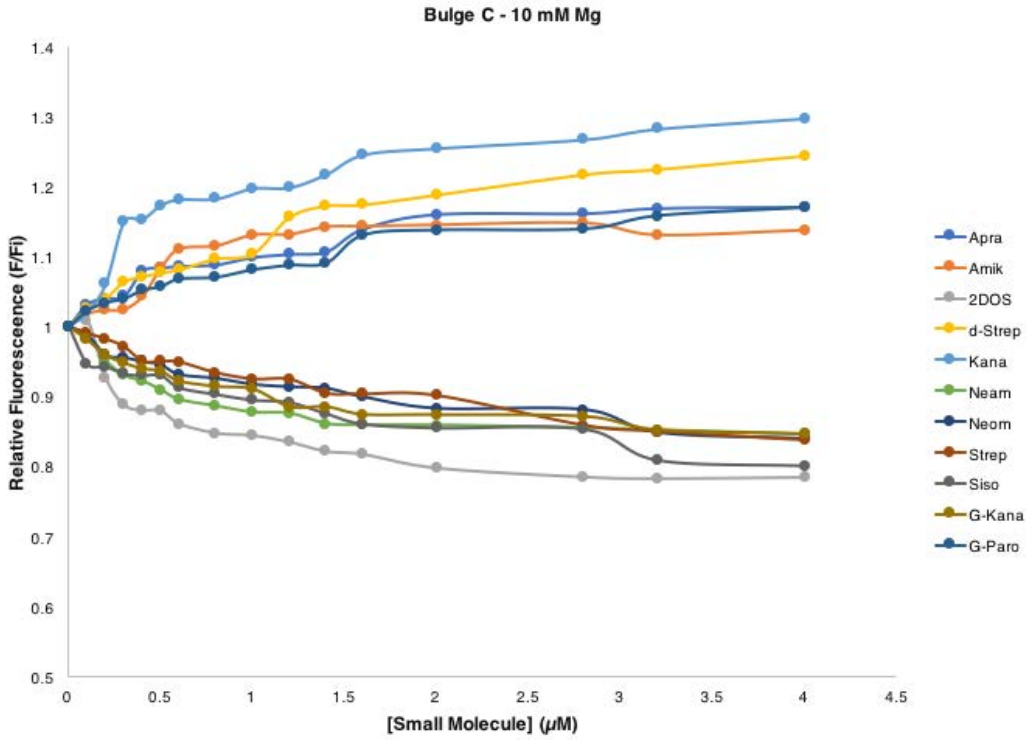
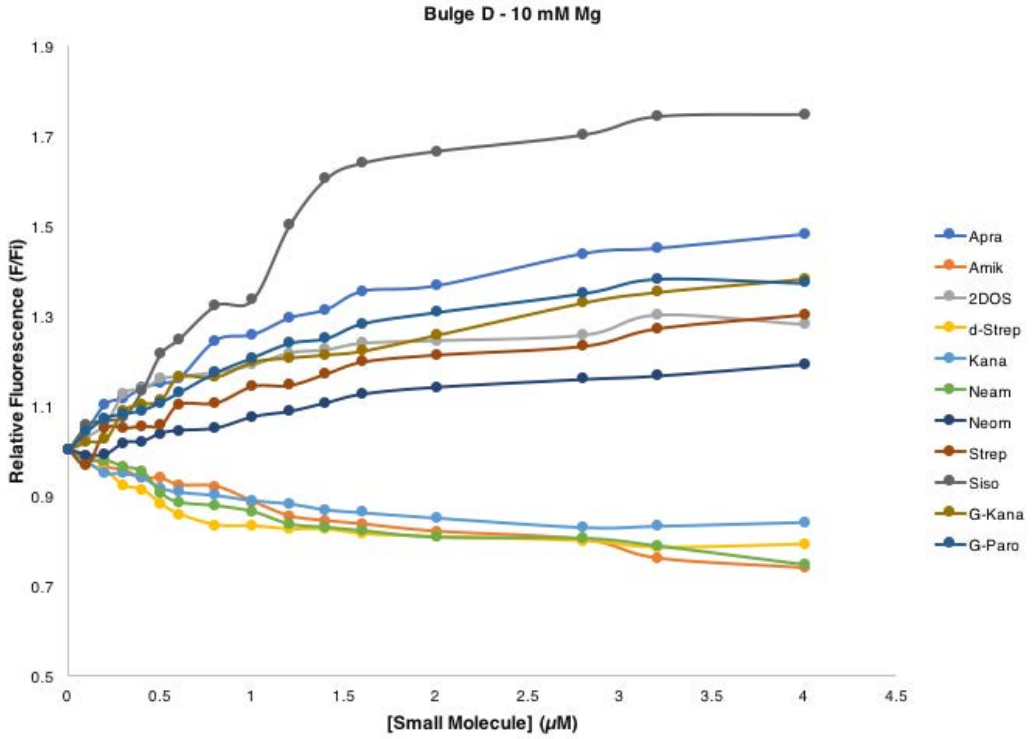


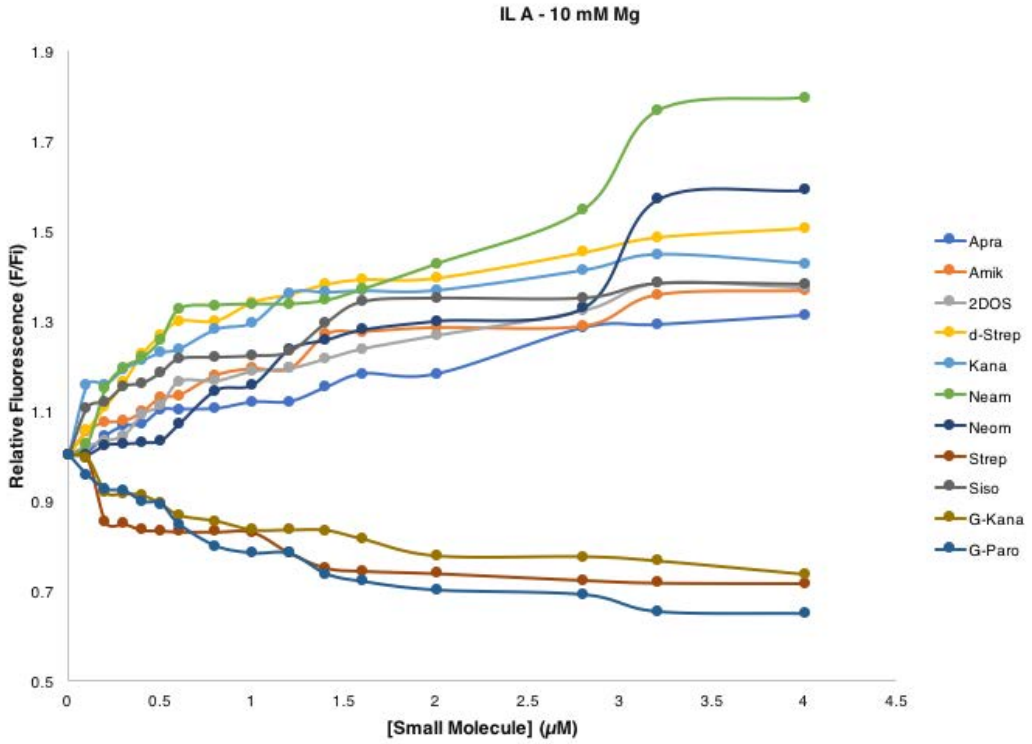
Figure S4-5. All RNA sequence titration curves to the small molecule receptors in buffer with 140 mM NaCl (10 mM NaH_2PO_4, 140 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 7.4, 25°C). Each graph shows a single RNA sequence and the receptors titration curves. The error for all experiments were less than 10%. The error was calculated using the standard error of triplicates, and relative standard error was determined based on the mean of each RNA sequence.

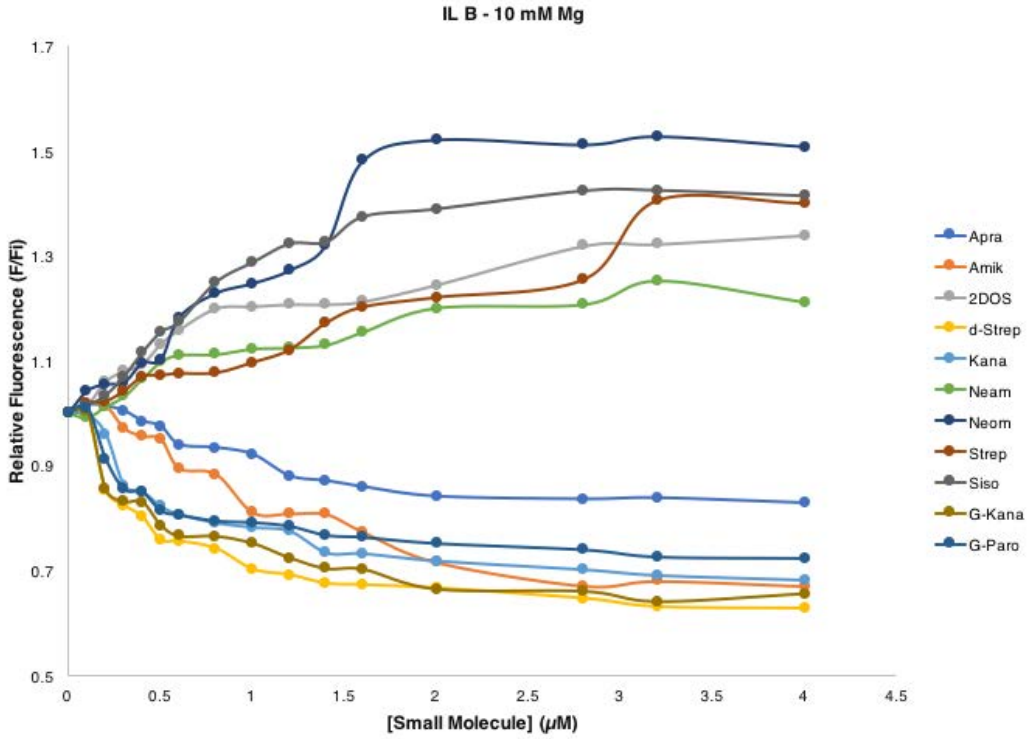

Bulge A - 10 mM Mg
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

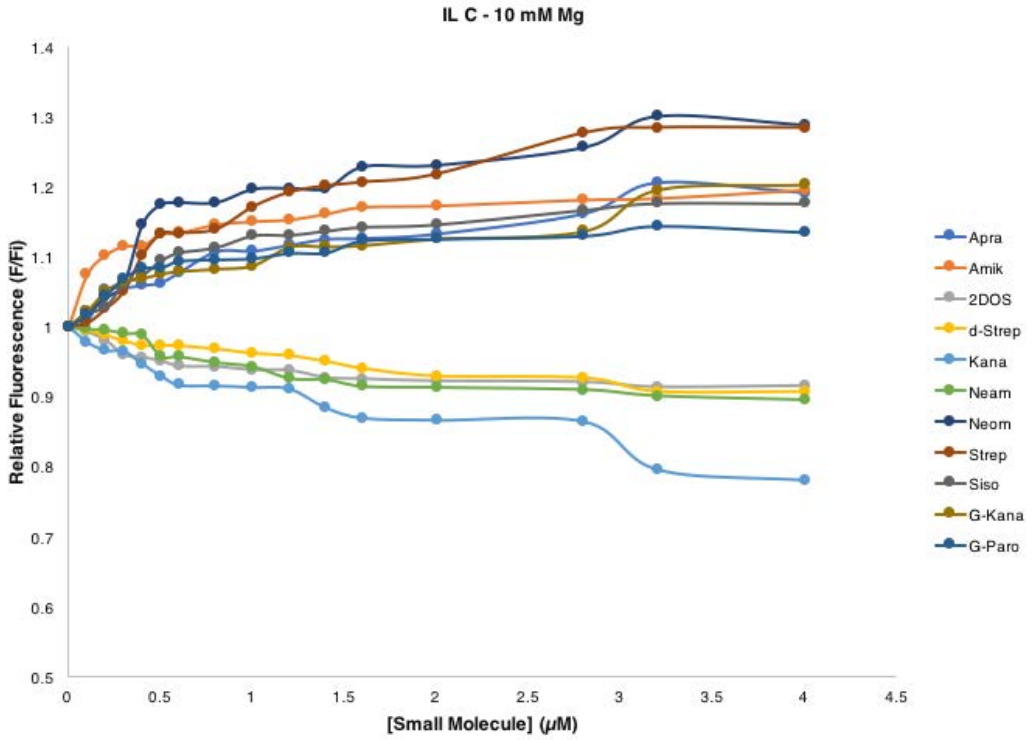

Bulge B - 10 mM Mg
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Ami
2DOS
DI
Kan
Neam
Neo
Strept
Siso
Guan Kana
Guan Paro

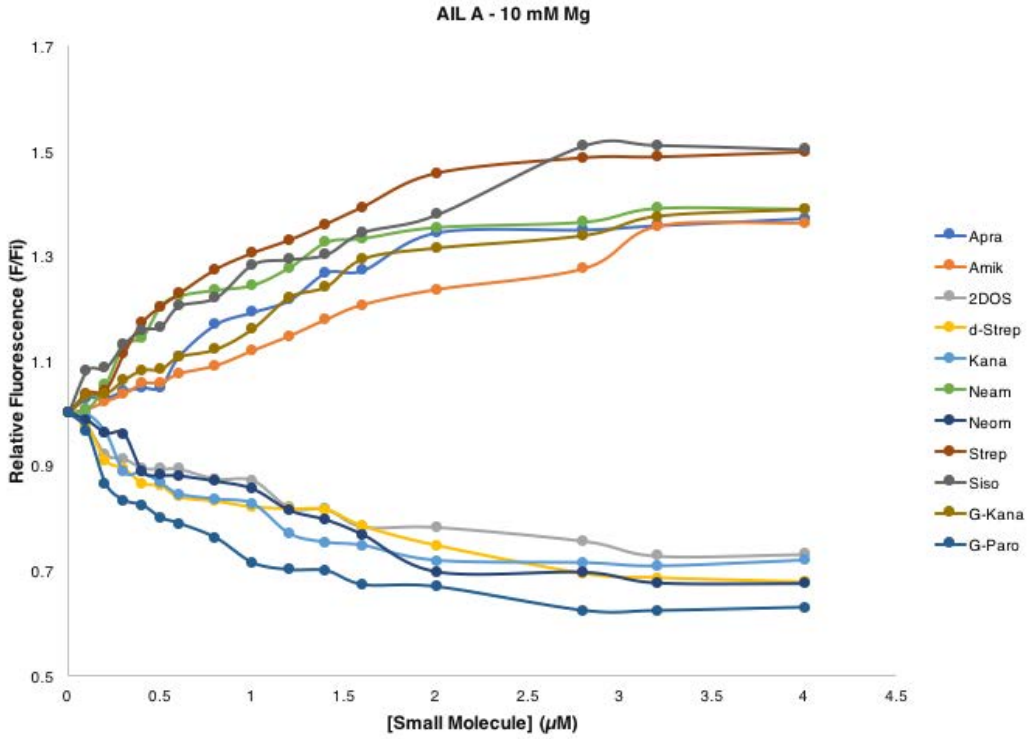

Bulge C - 10 mM Mg
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

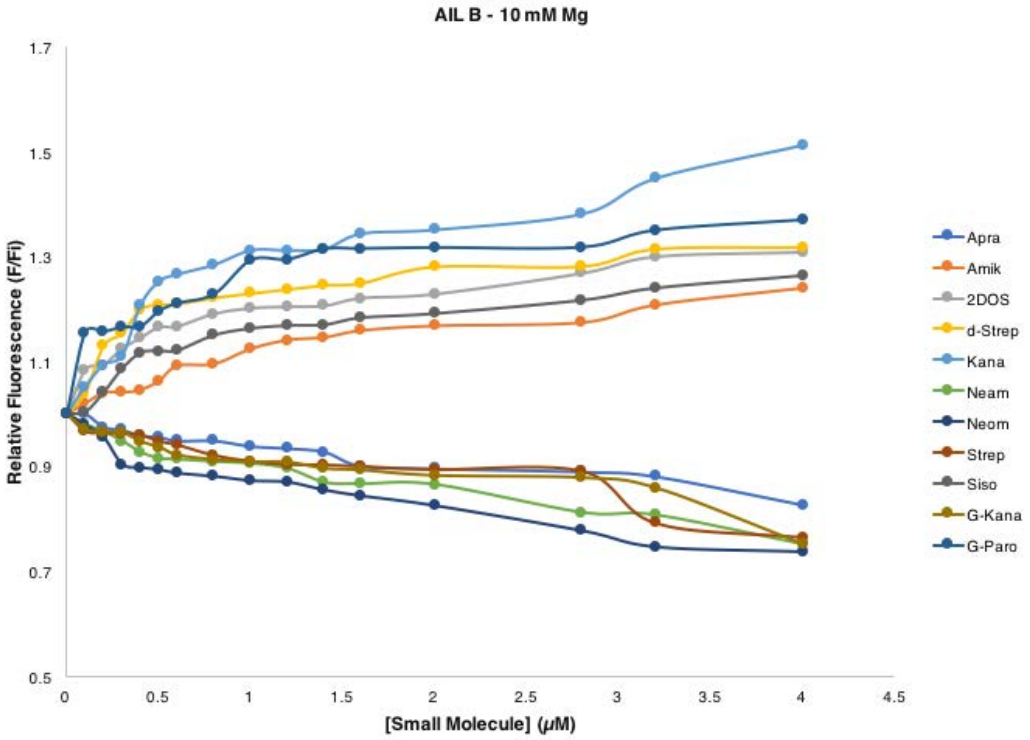

Bulge D - 10 mM Mg
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

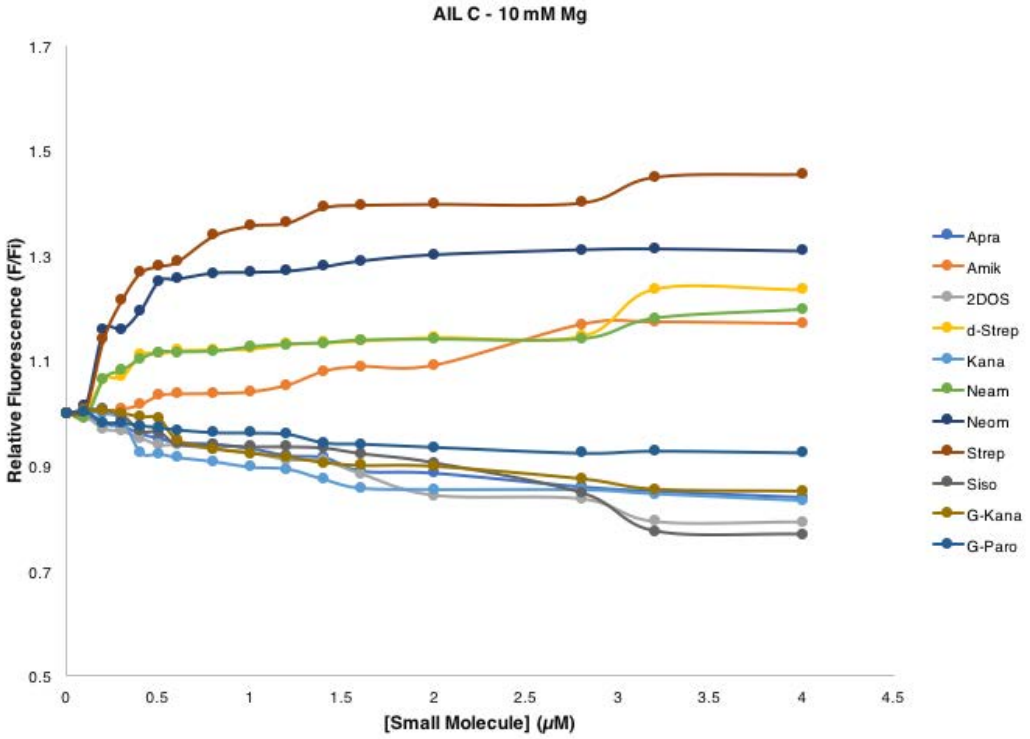

IL A - 10 mM Mg
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

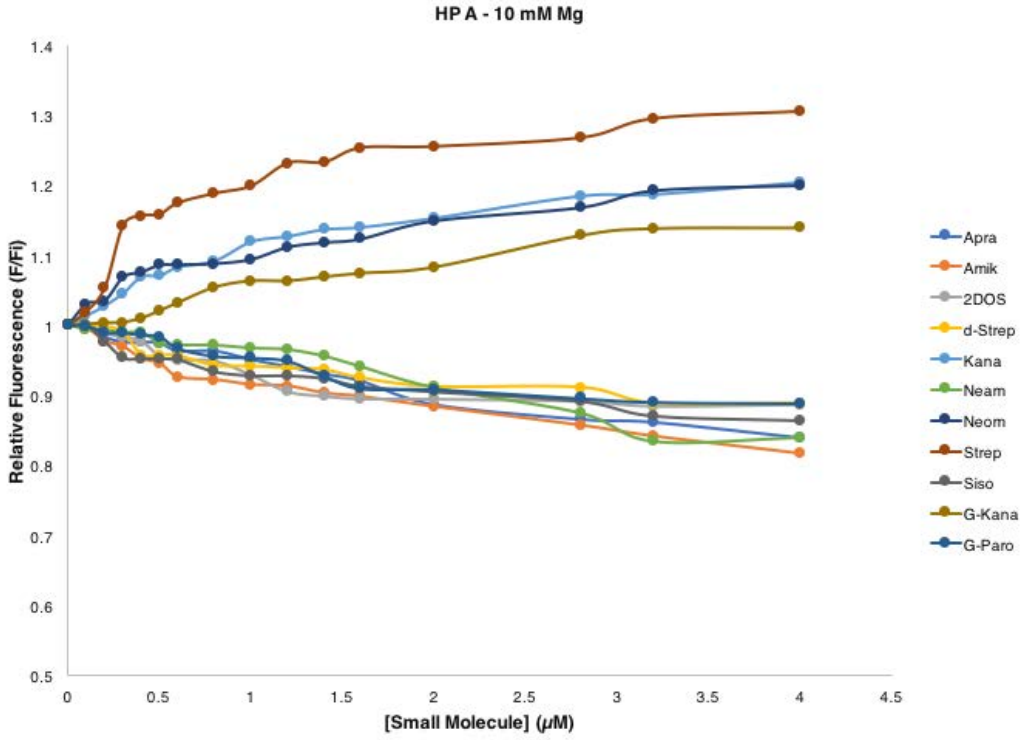

IL B - 10 mM Mg
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

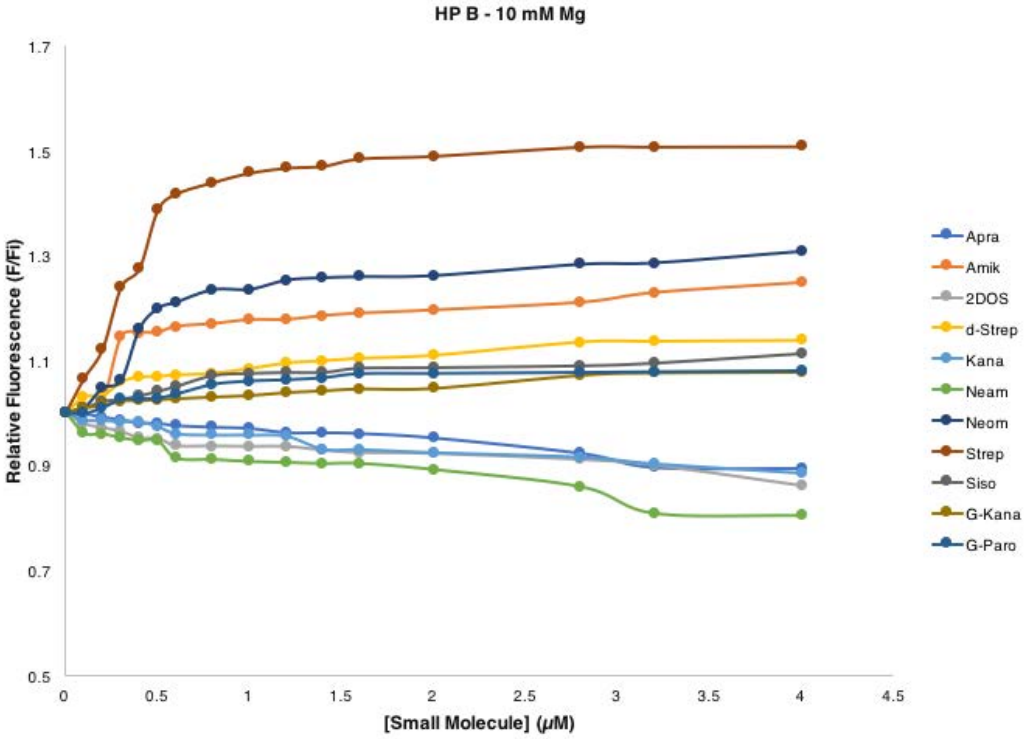

IL C - 10 mM Mg
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

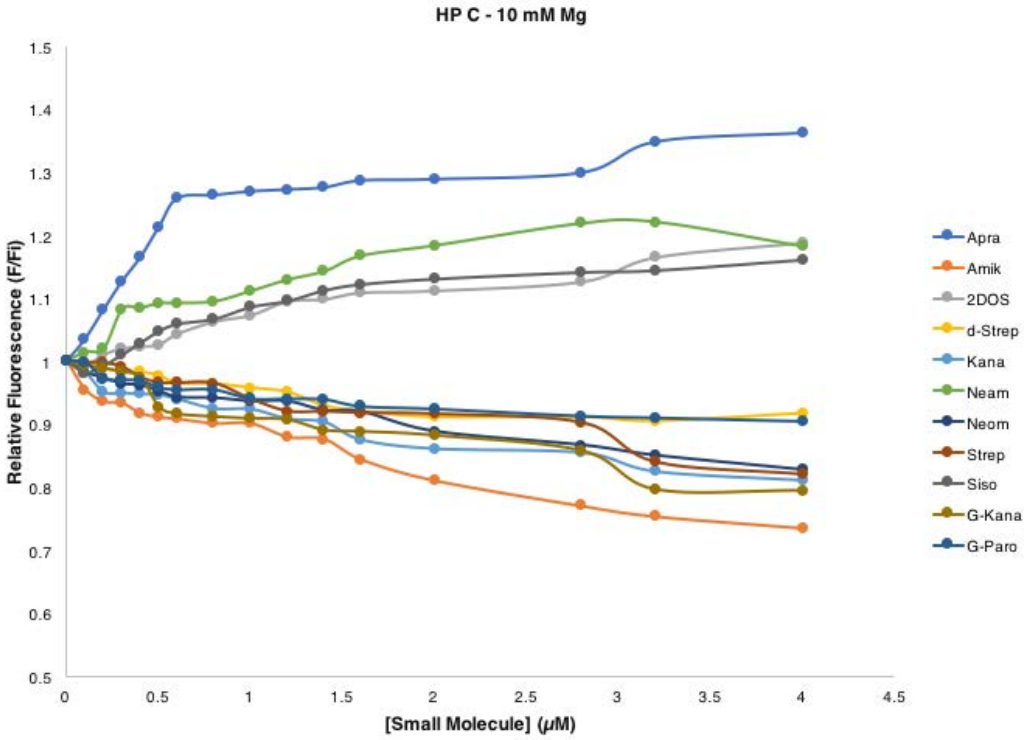

AIL A - 10 mM Mg
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

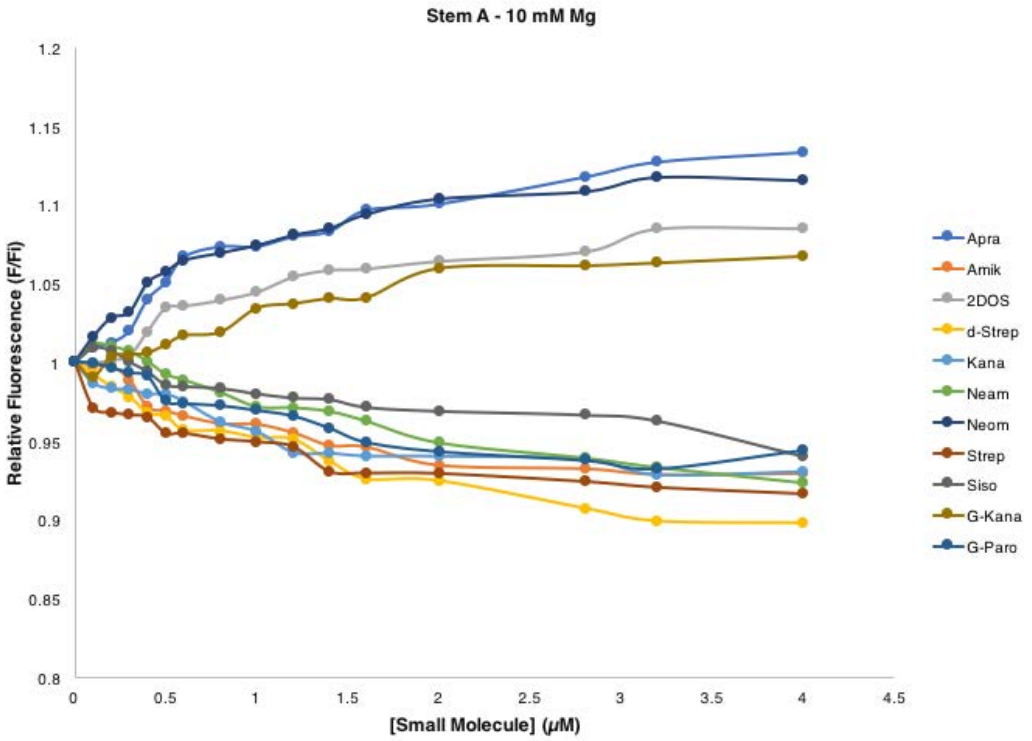

AIL B - 10 mM Mg
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


AIL C - 10 mM Mg
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


HP A - 10 mM Mg
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


HP B - 10 mM Mg
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


HP C - 10 mM Mg
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


Stem A - 10 mM Mg
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
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Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

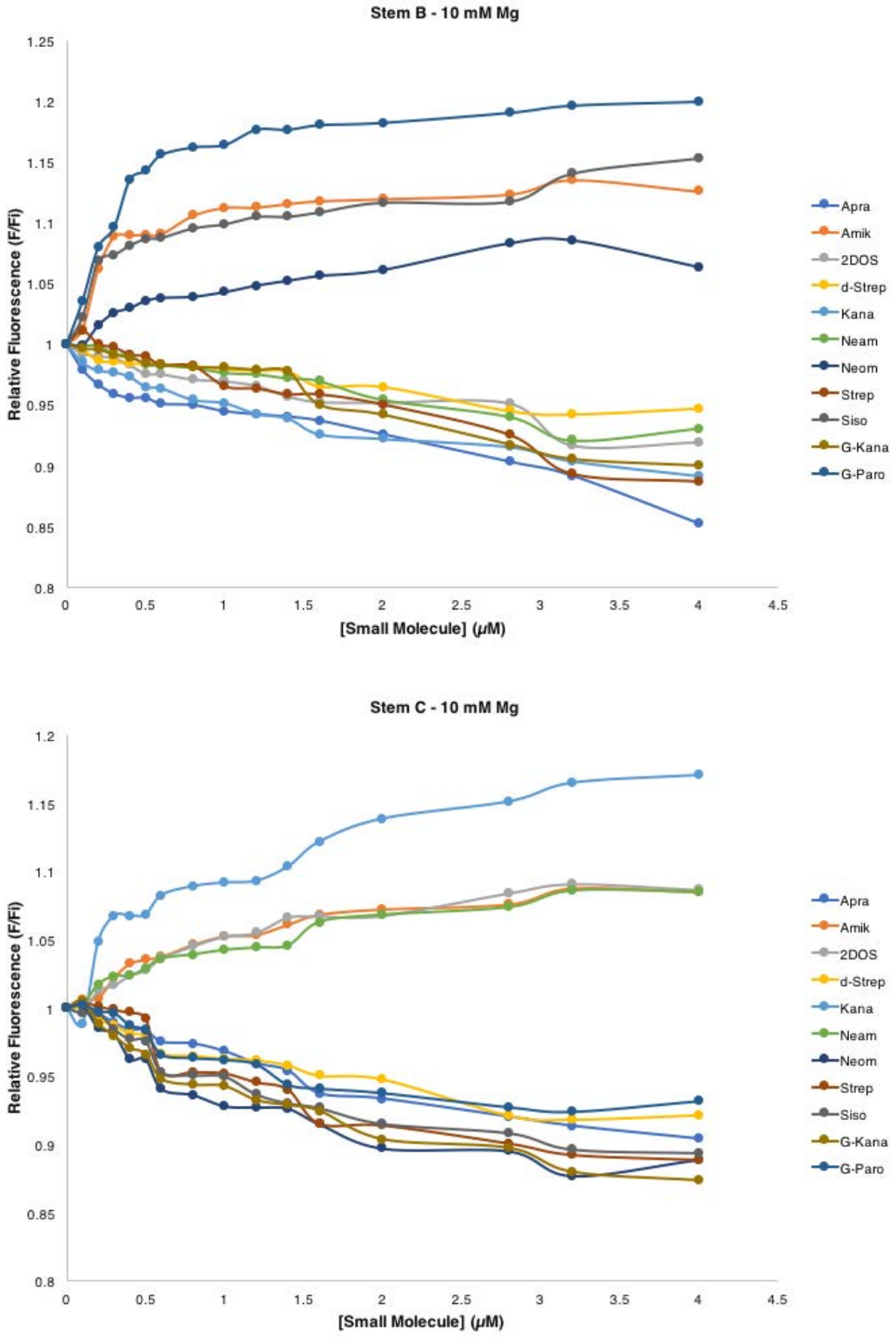
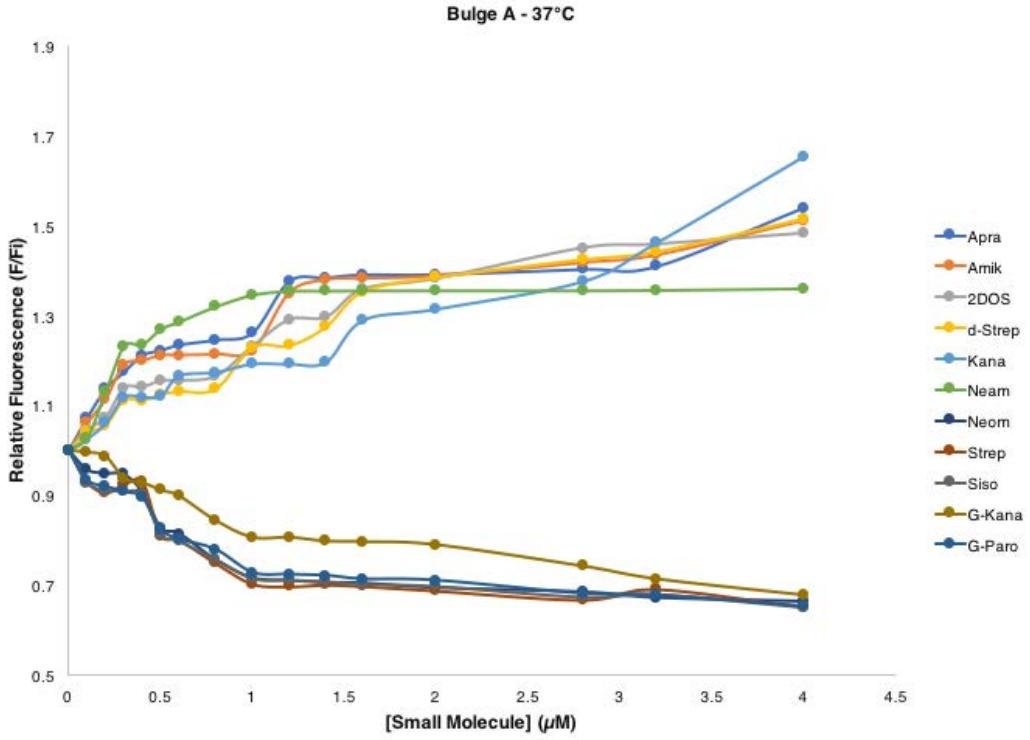
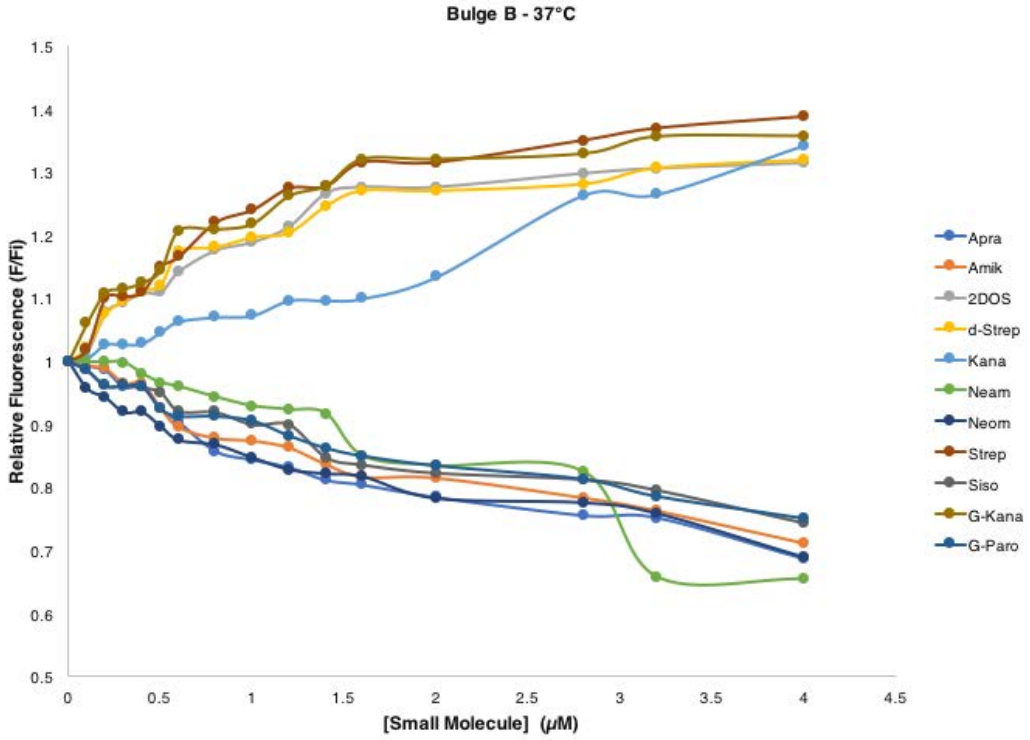


Figure S4-7. All RNA sequence titration curves to the small molecule receptors in buffer with 10 mM $MgCl_2$ (10 mM NaH_2PO_4, 25 mM NaCl, 10 mM $MgCl_2$, 0.5 mM EDTA, pH 7.4, 25°C). Each graph shows a single RNA sequence and the receptors titration curves. The error for all experiments were less than 10%. The error was calculated using the standard error of triplicates, and relative standard error was determined based on the mean of each RNA sequence.


Bulge A - 37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


Bulge B - 37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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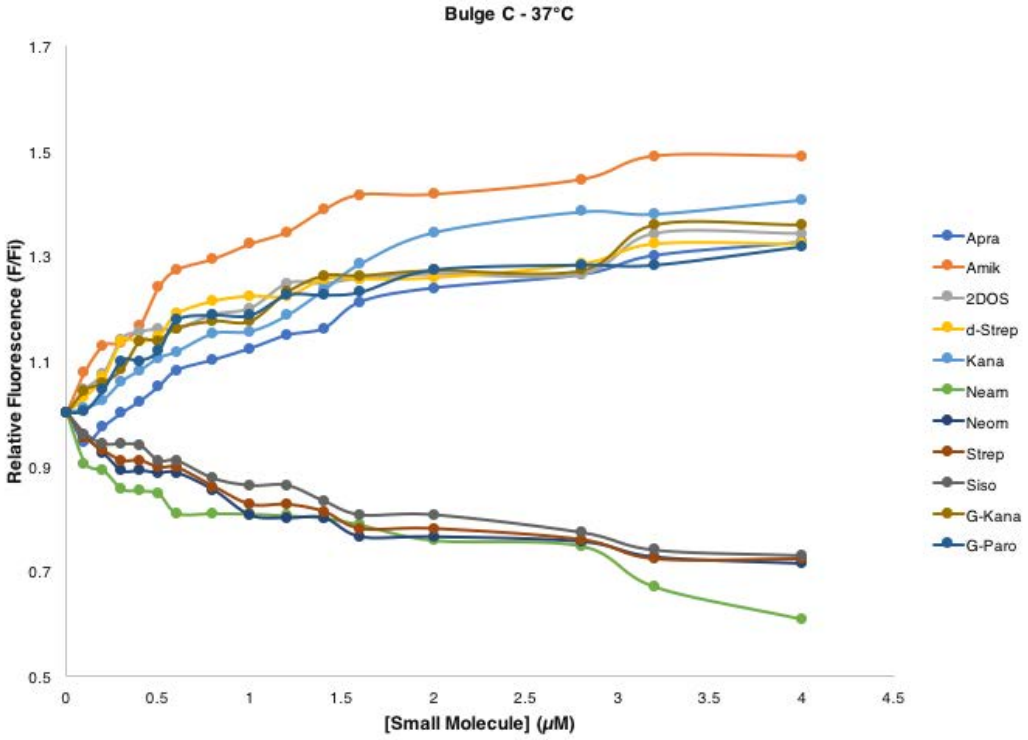
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


Bulge C - 37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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Amik
2DOS
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Kana
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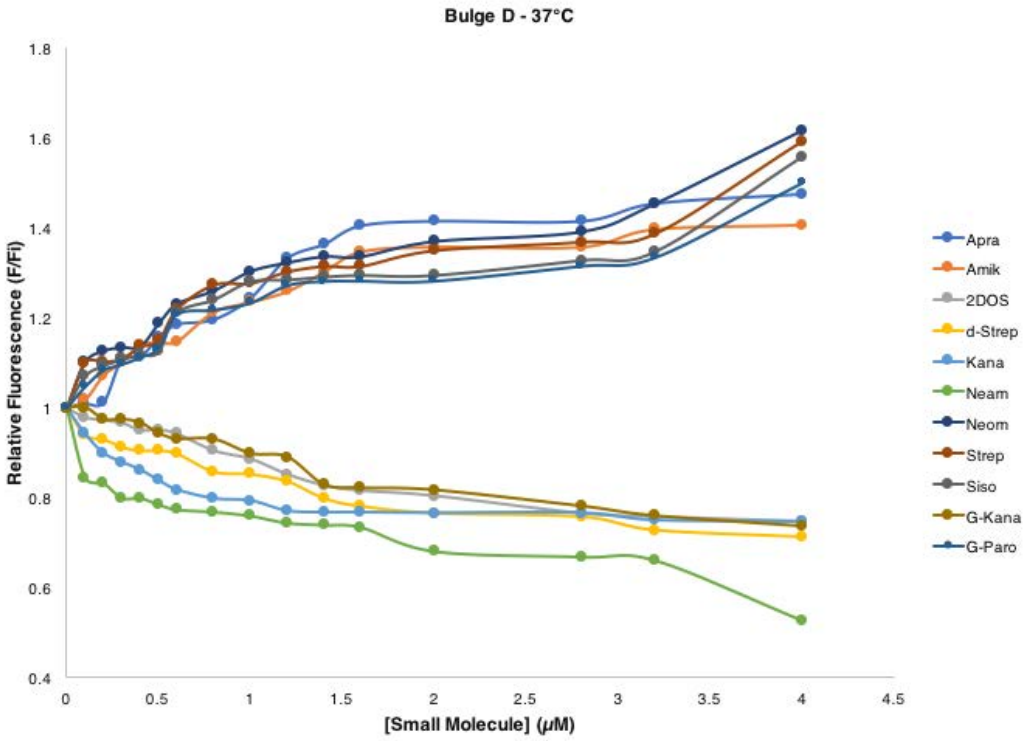
Neom
Strep
Siso
G-Kana
G-Paro


Bulge D - 37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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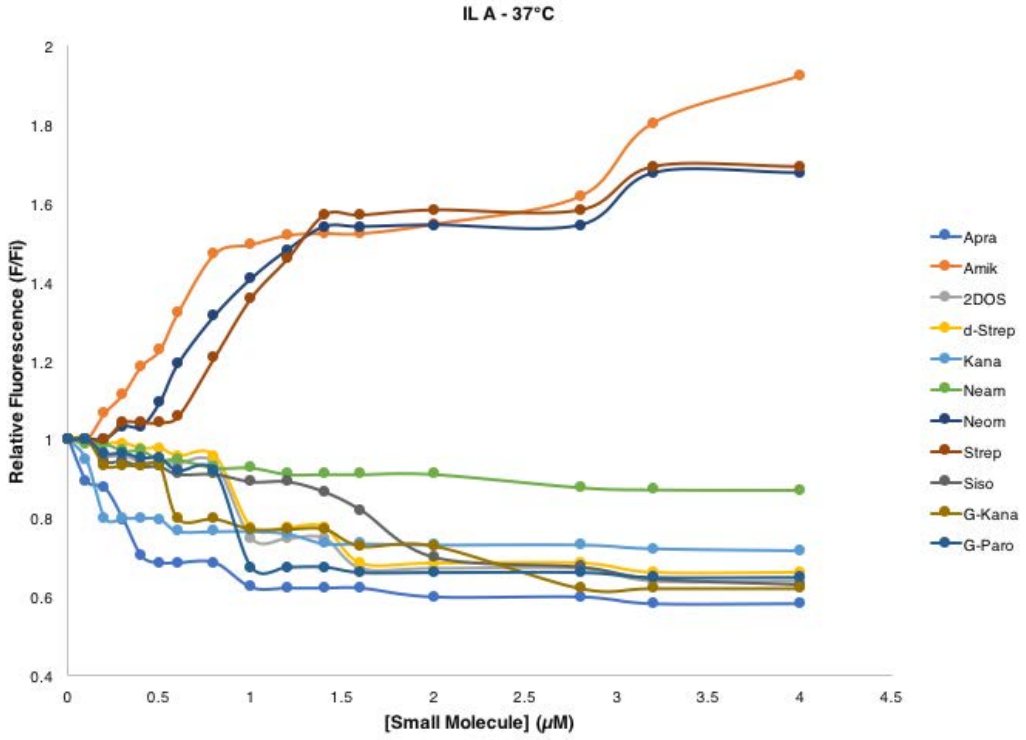
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro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Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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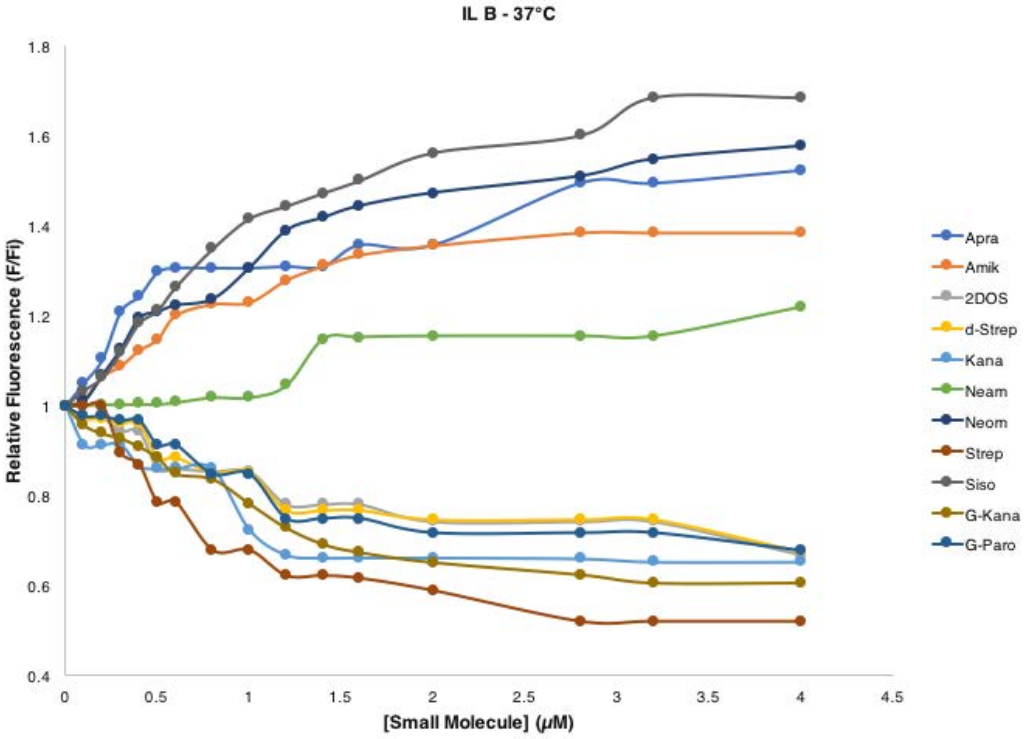
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


IL B - 37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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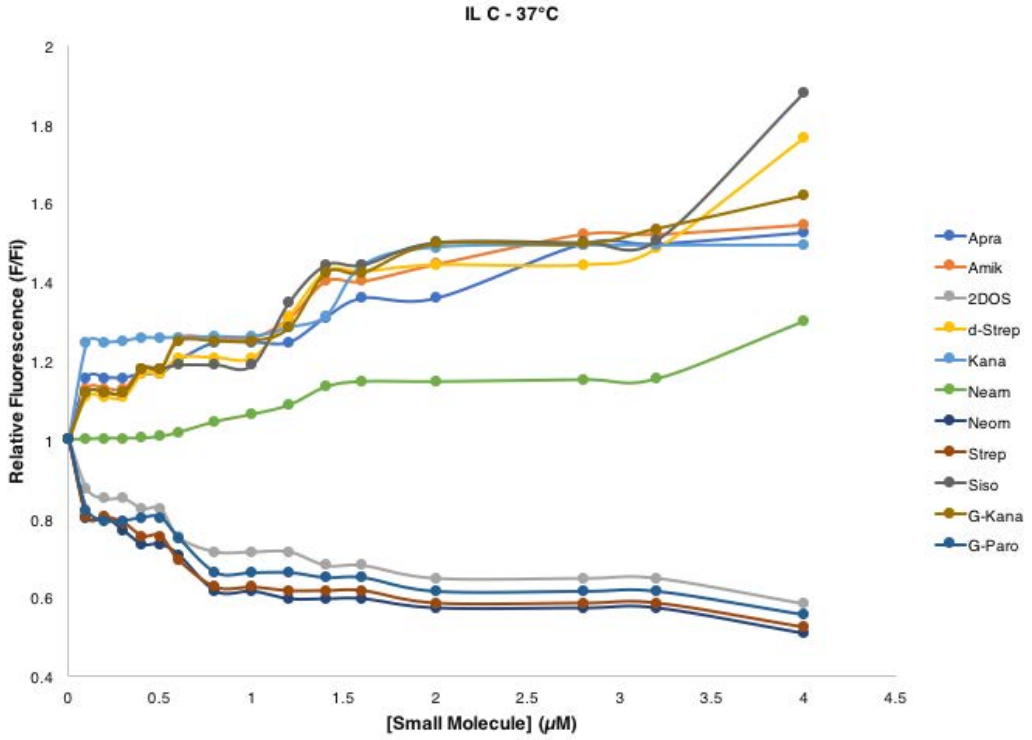
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


IL C - 37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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Strep
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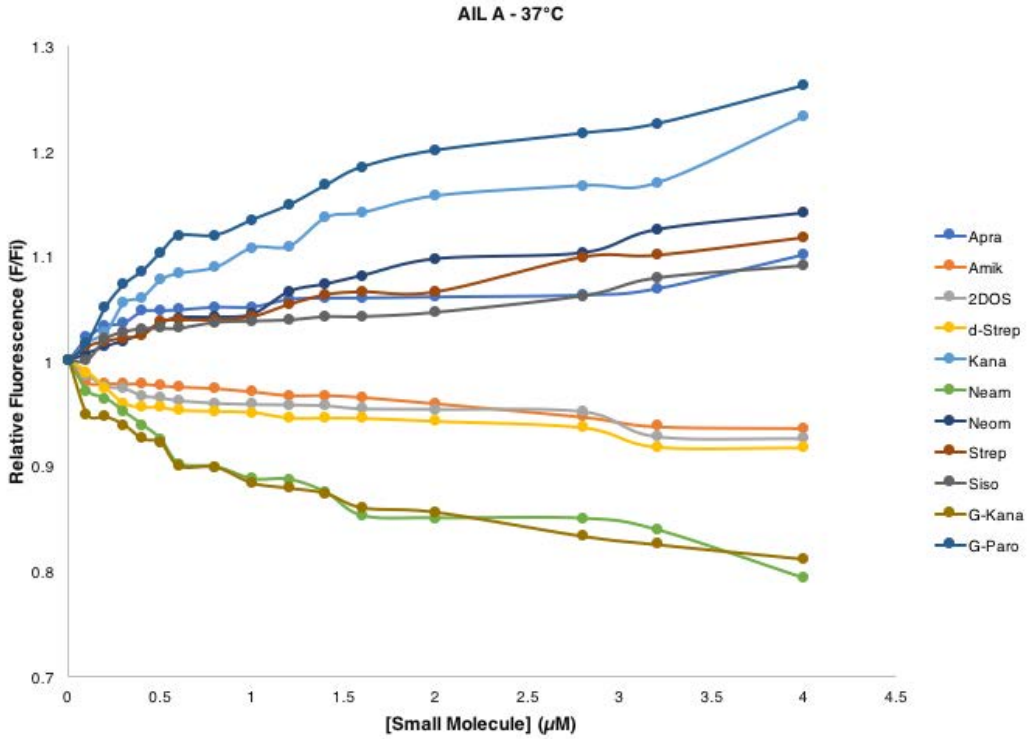
G-Kana
G-Paro


AIL A - 37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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2DOS
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Kana
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Neom
Strep
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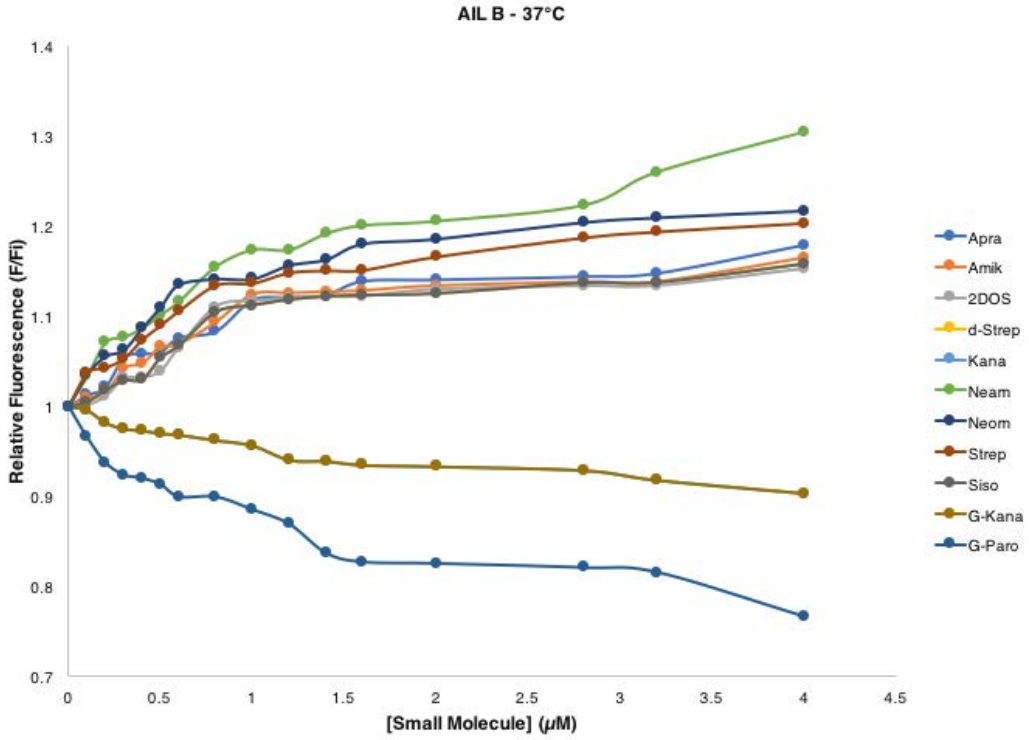
G-Paro


AIL B - 37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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2DOS
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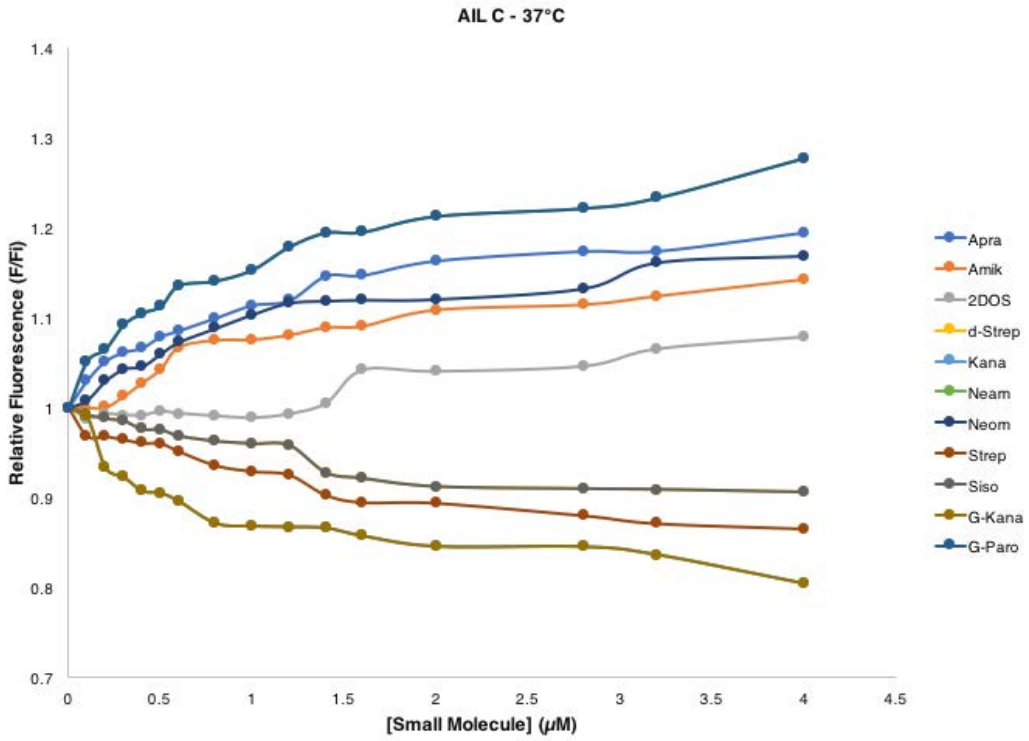
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


AIL C - 37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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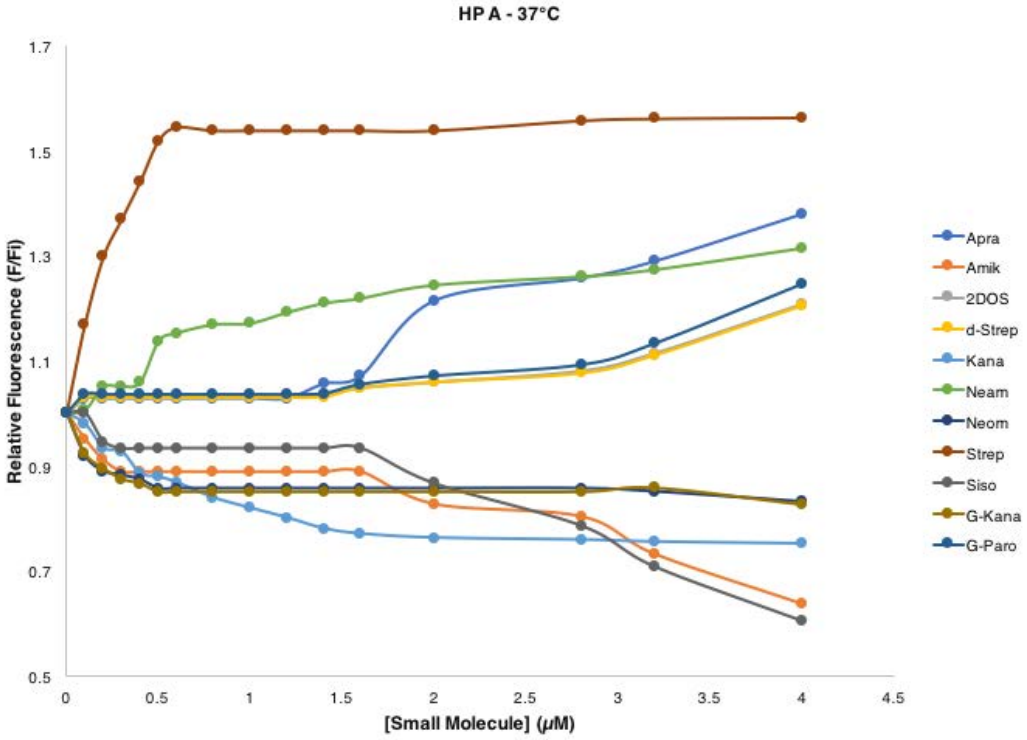
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


HP A - 37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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2DOS
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Kana
Neam
Neom
Strep
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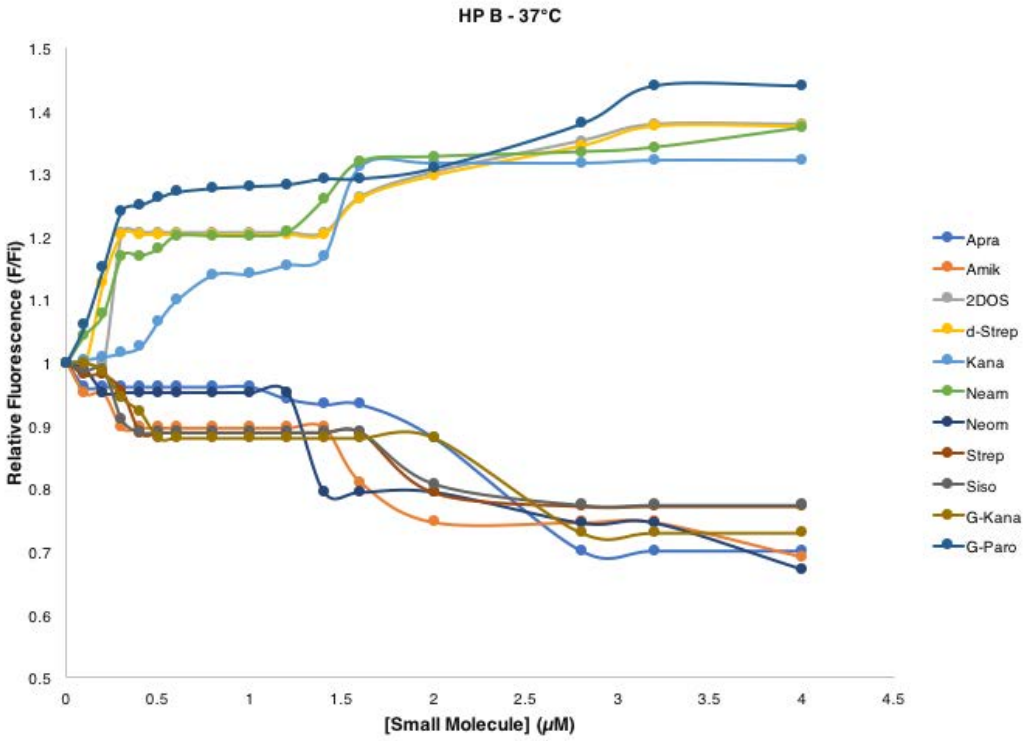
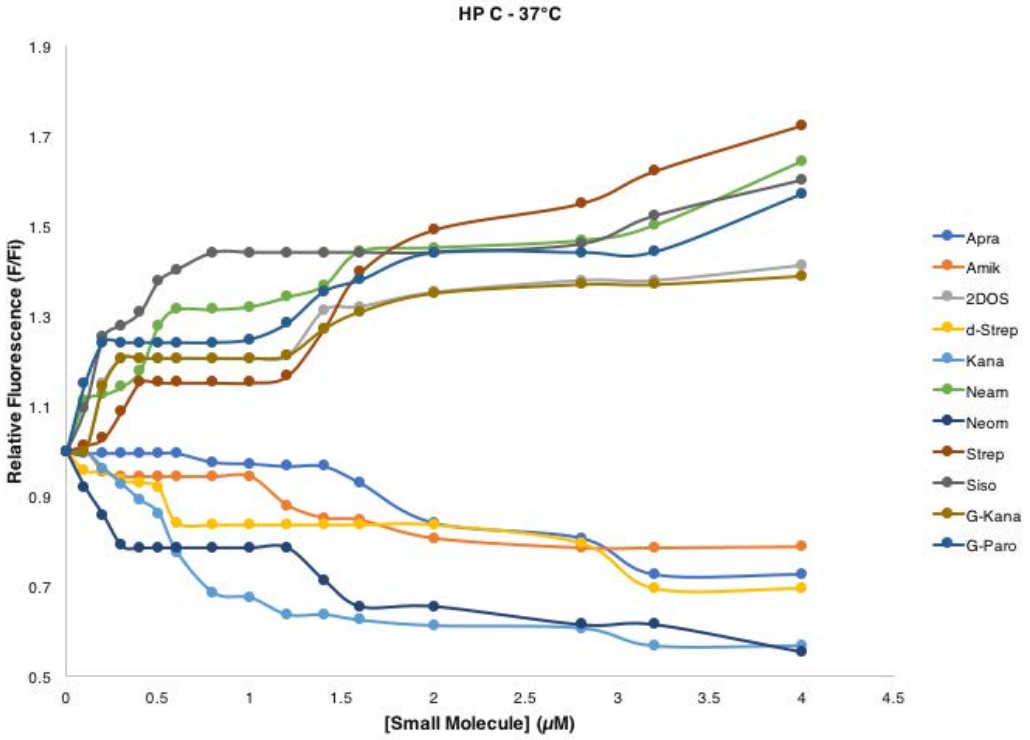
G-Kana
G-Paro


HP B - 37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


HP C - 37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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Amik
2DOS
d-Strep
Kana
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Neom
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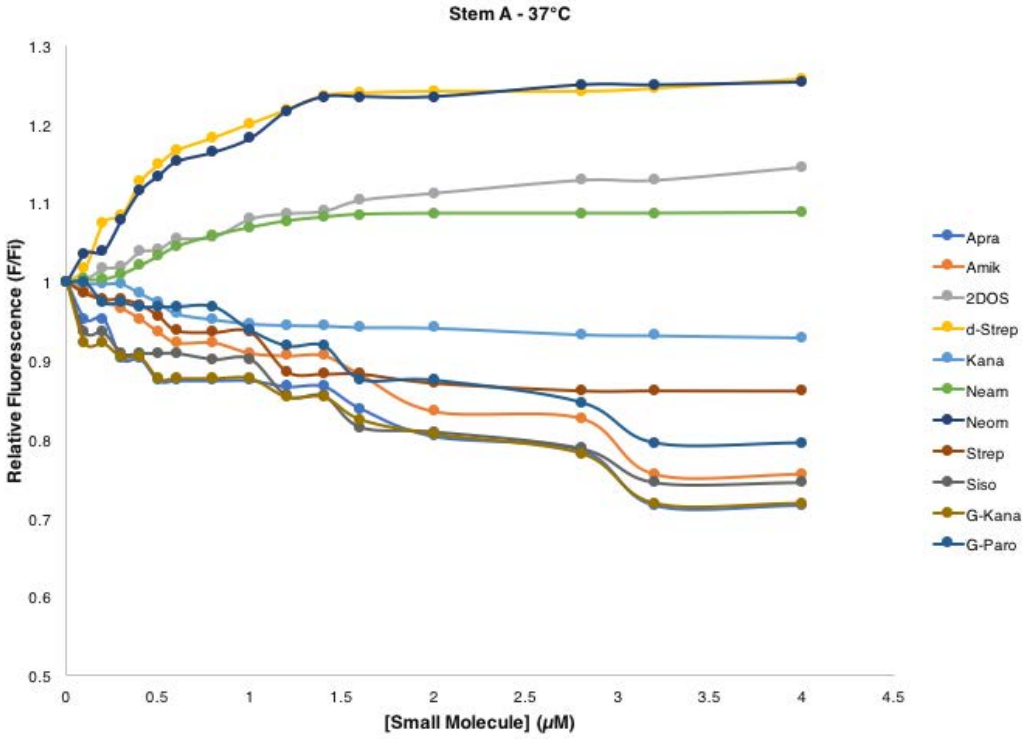
Siso
G-Kana
G-Paro


Stem A - 37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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2DOS
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Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

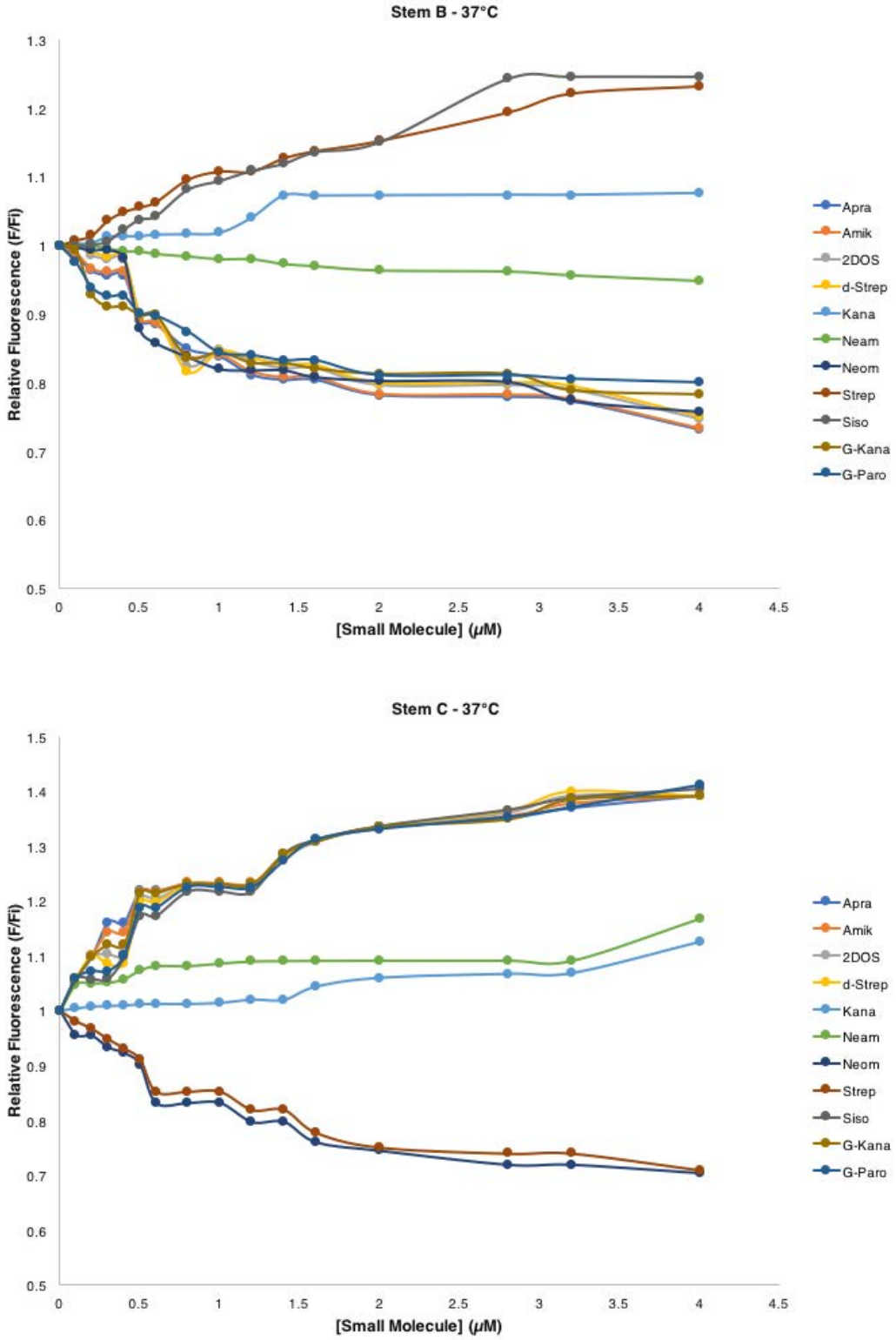
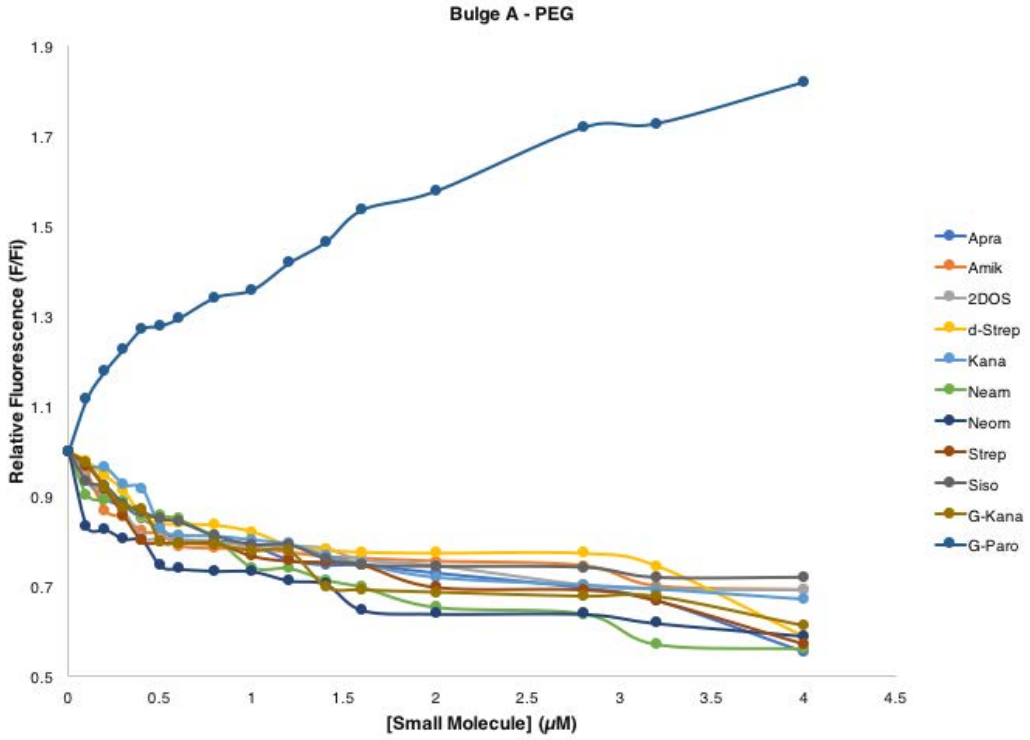


Figure S4-9. All RNA sequence titration curves to the small molecule receptors in standard buffer at 37°C (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, pH 7.4, 37°C). Each graph shows a single RNA sequence and the receptors titration curves. The error for all experiments were less than 10%. The error was calculated using the standard error of triplicates, and relative standard error was determined based on the mean of each RNA sequence.


Bulge A - PEG
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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Amik
2DOS
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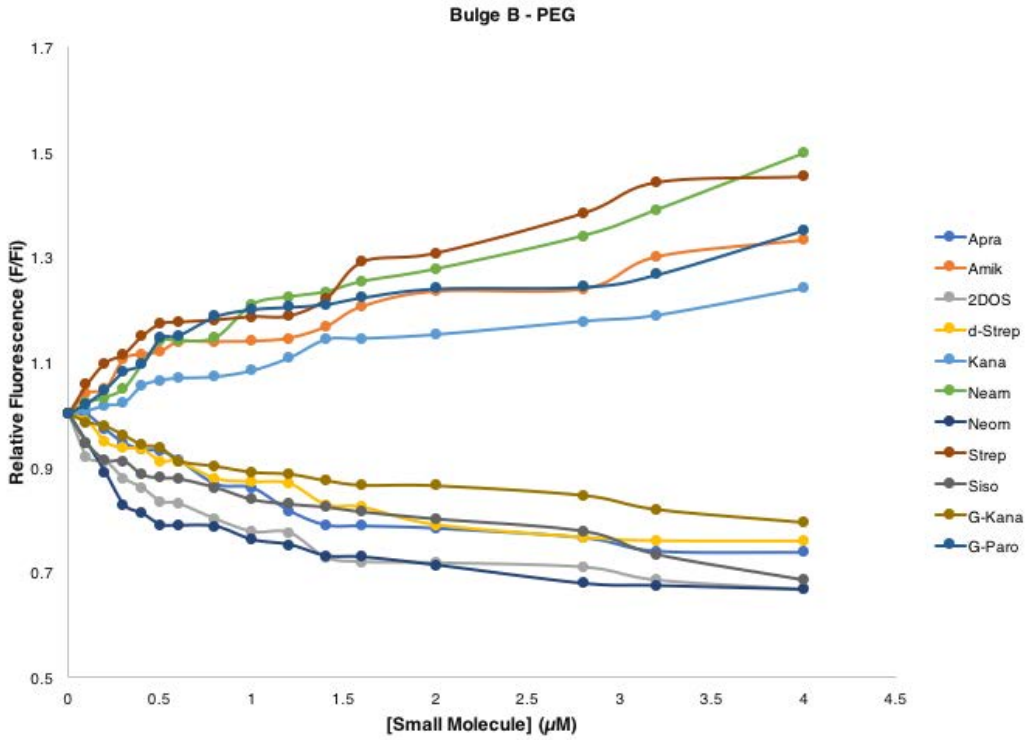
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


Bulge B - PEG
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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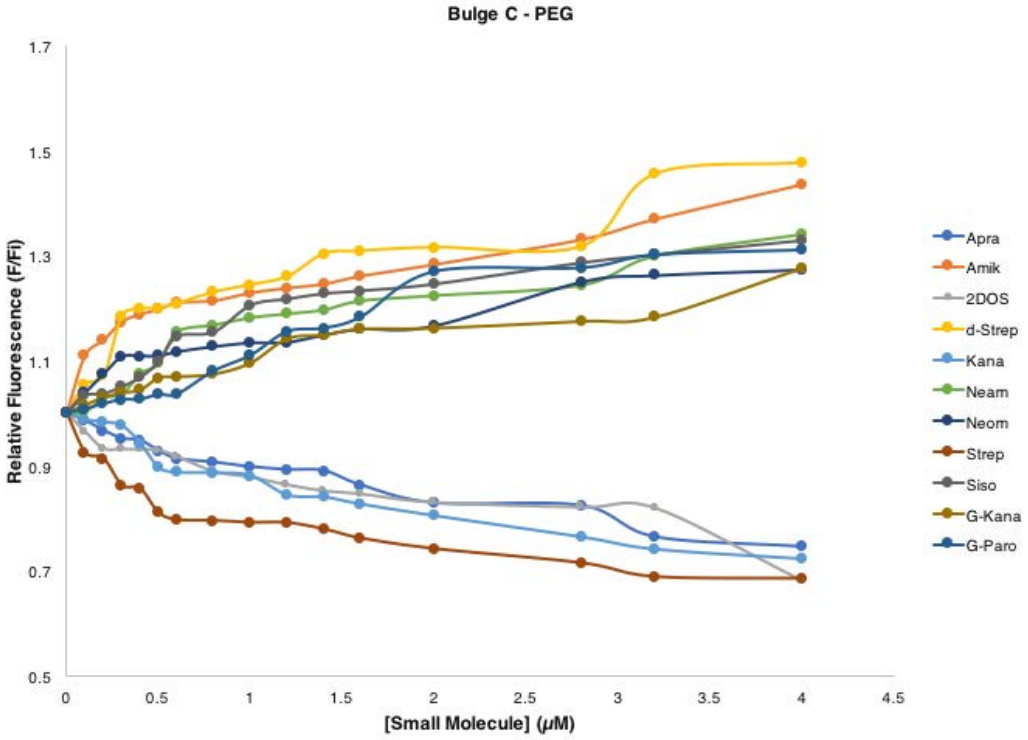
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


Bulge C - PEG
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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Amik
2DOS
d-Strep
Kana
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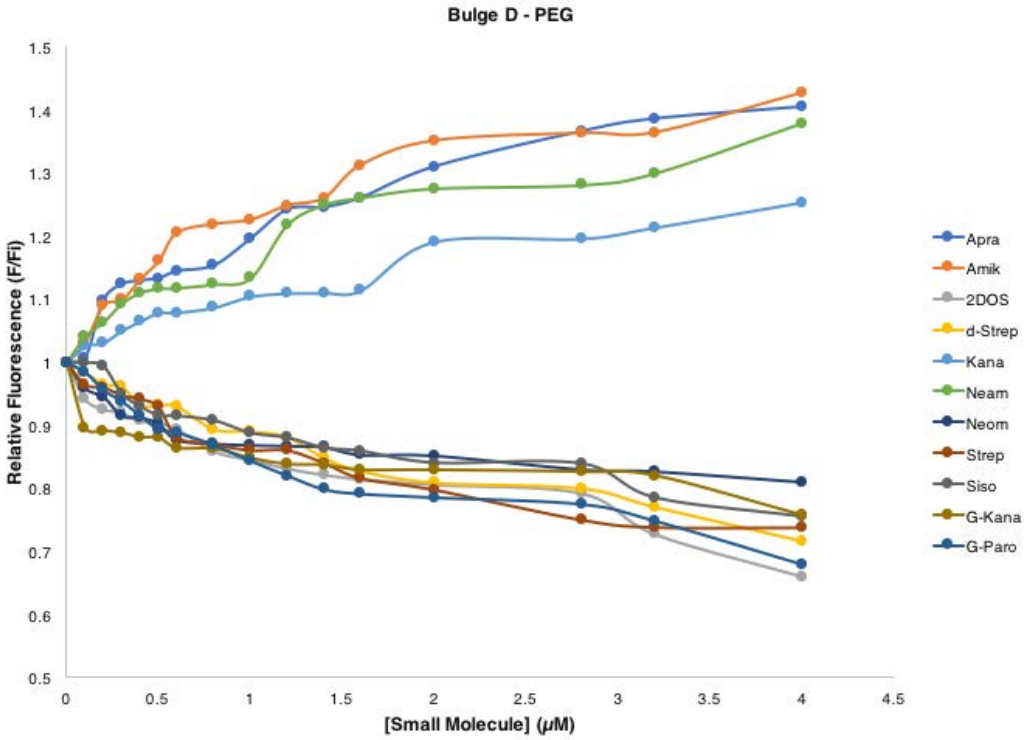
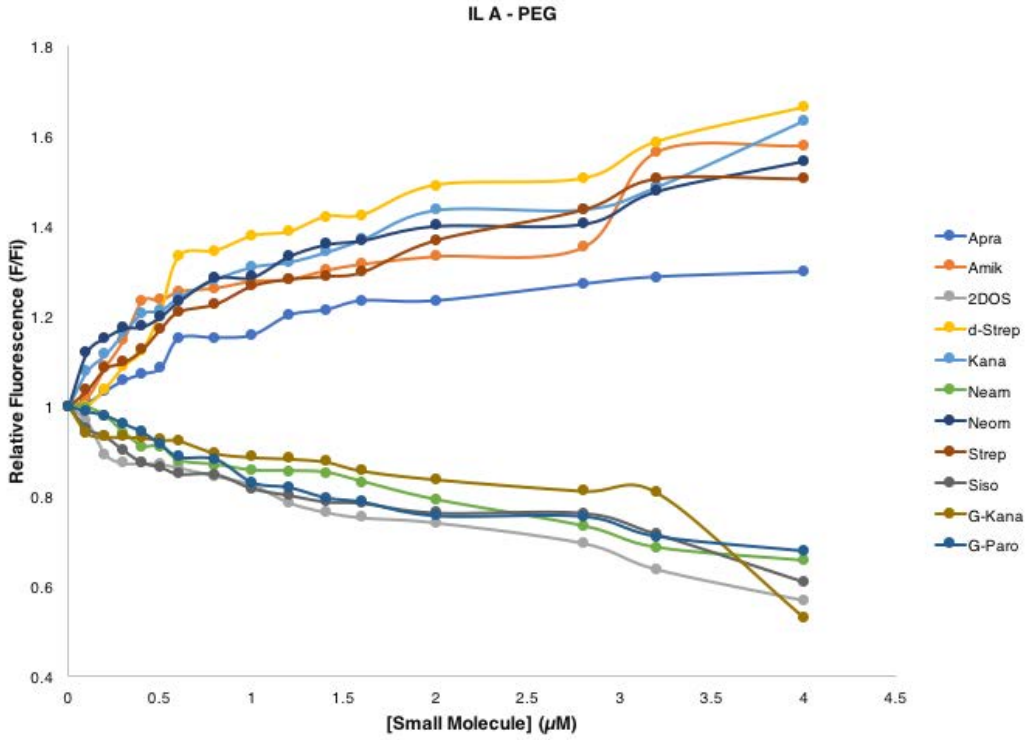
Neom
Strep
Siso
G-Kana
G-Paro


Bulge D - PEG
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


IL A - PEG
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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Kana
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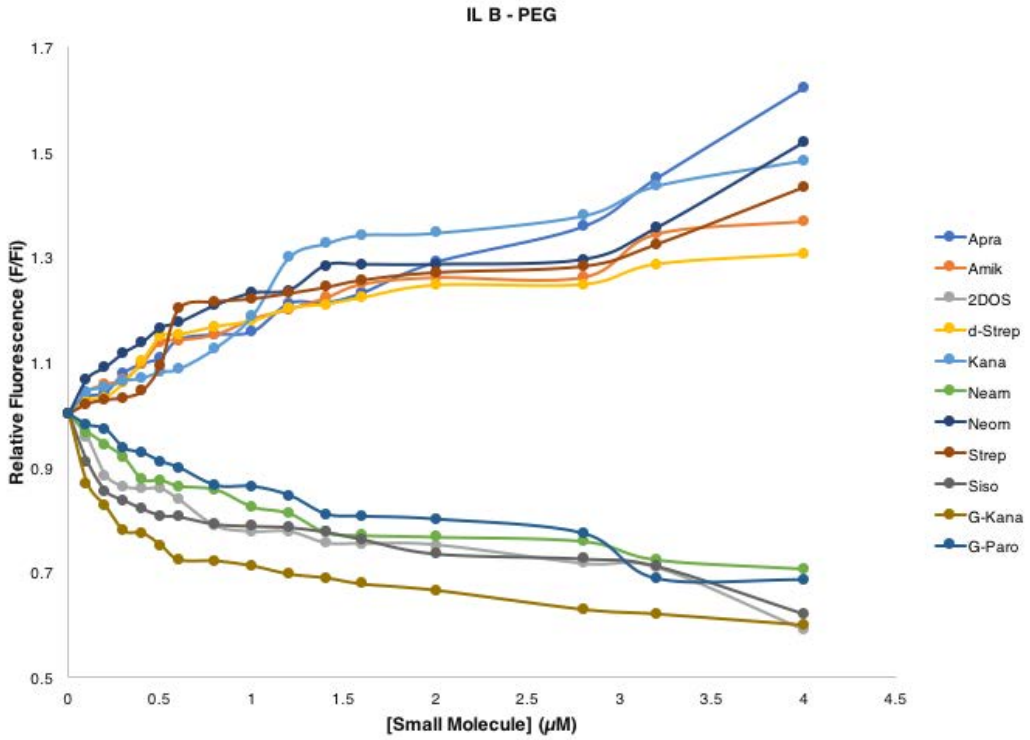
Neom
Strep
Siso
G-Kana
G-Paro


IL B - PEG
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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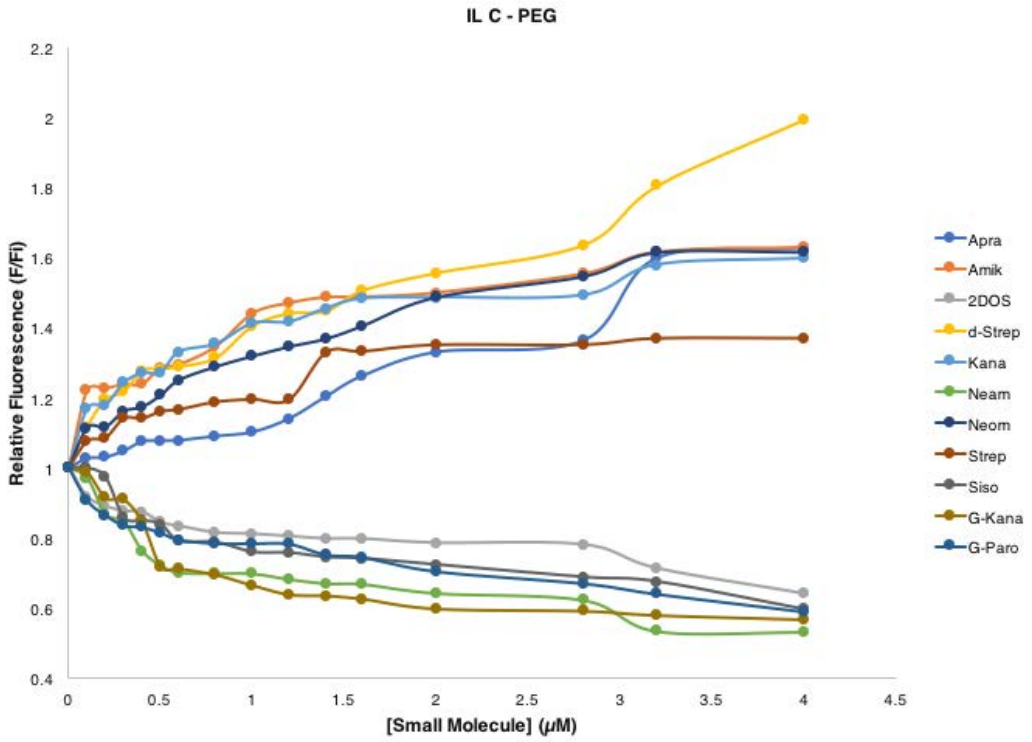
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


IL C - PEG
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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Kana
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Neom
Strep
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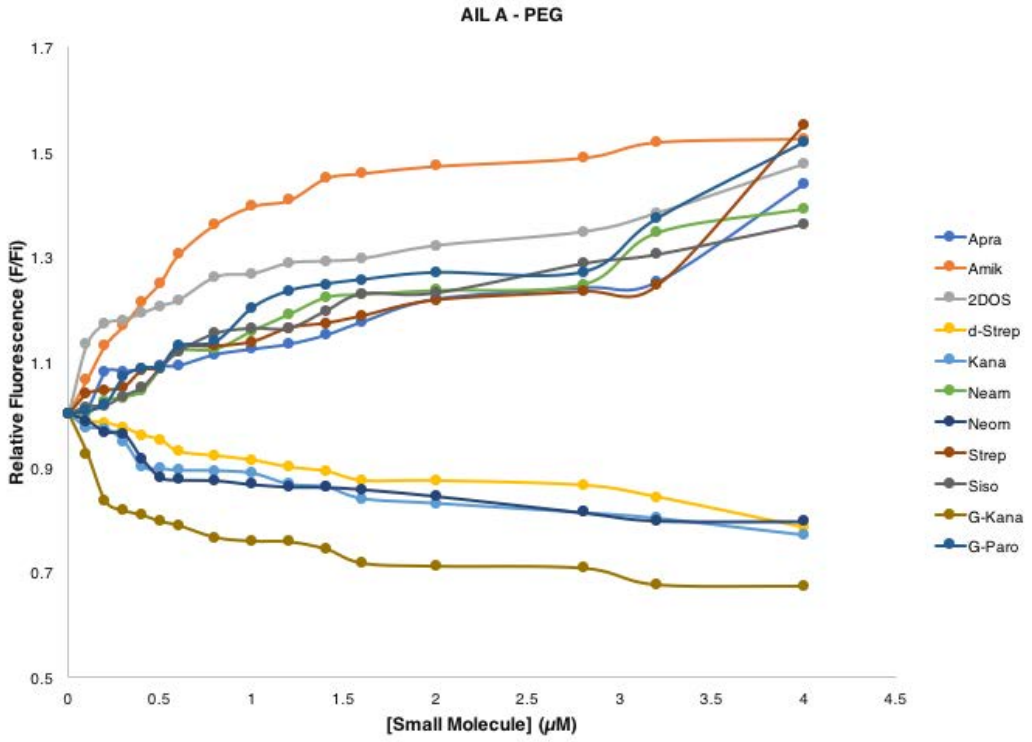
G-Kana
G-Paro


AIL A - PEG
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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Amik
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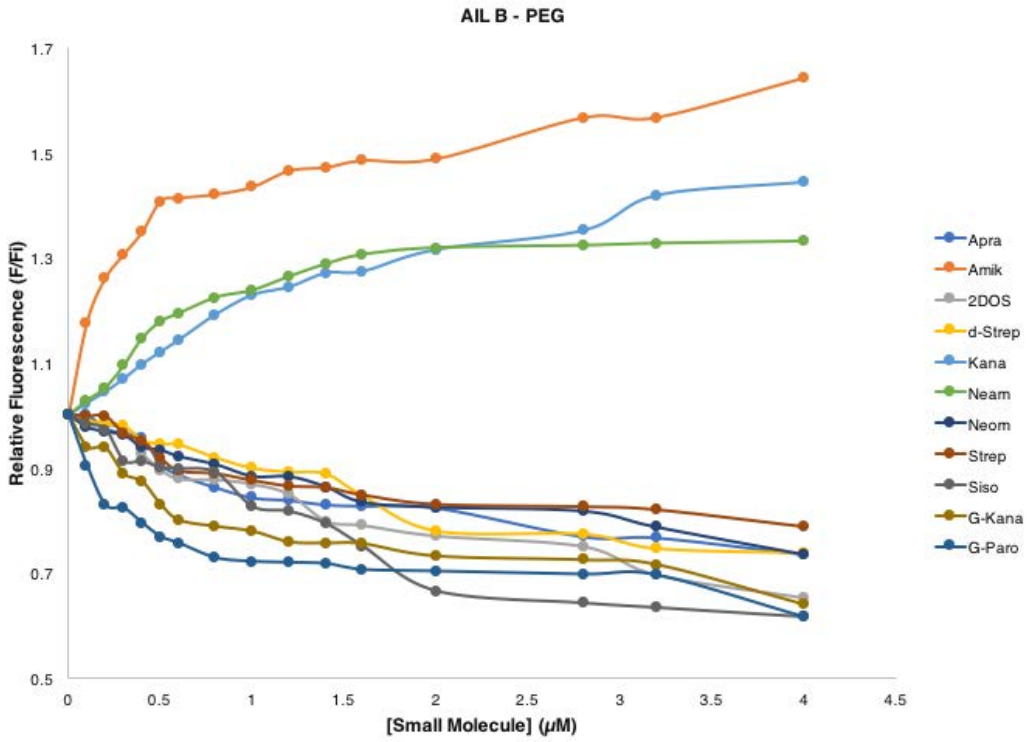
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


AIL B - PEG
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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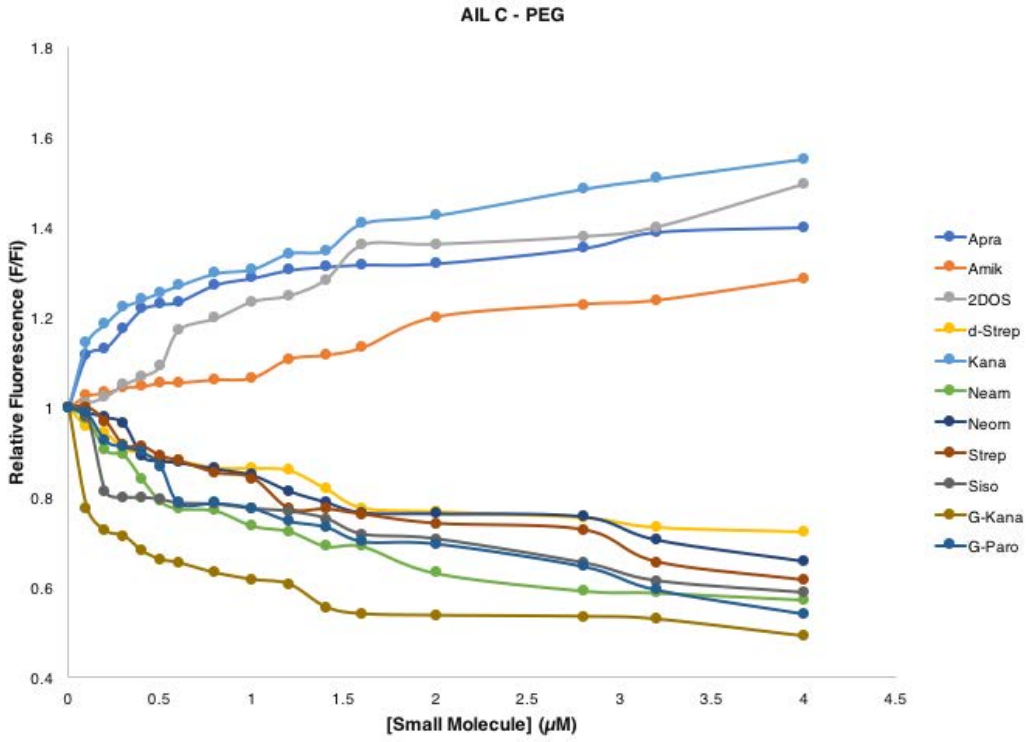
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


AIL C - PEG
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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Amik
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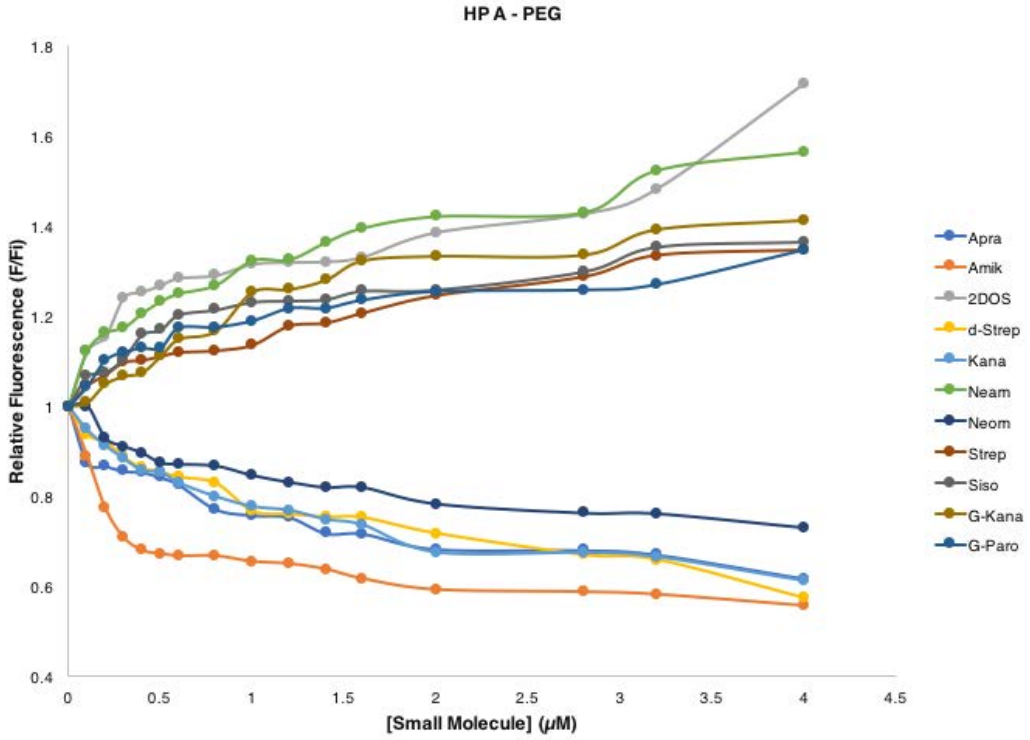
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


HP A - PEG
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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Kana
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Neom
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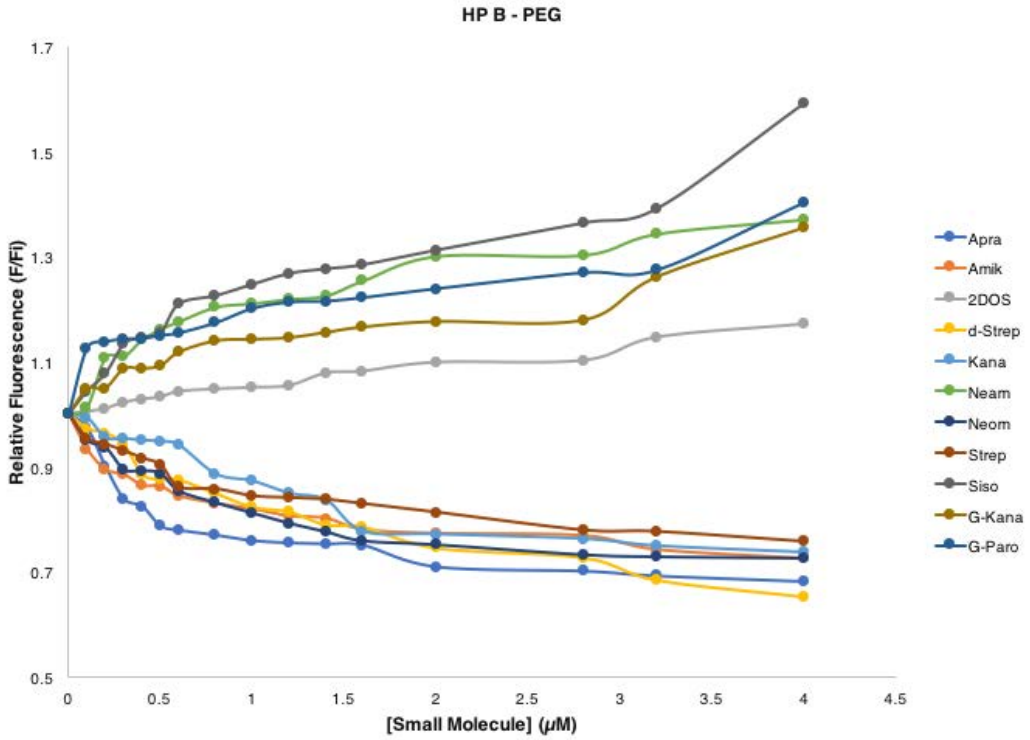
Siso
G-Kana
G-Paro


HP B - PEG
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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Kana
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Neom
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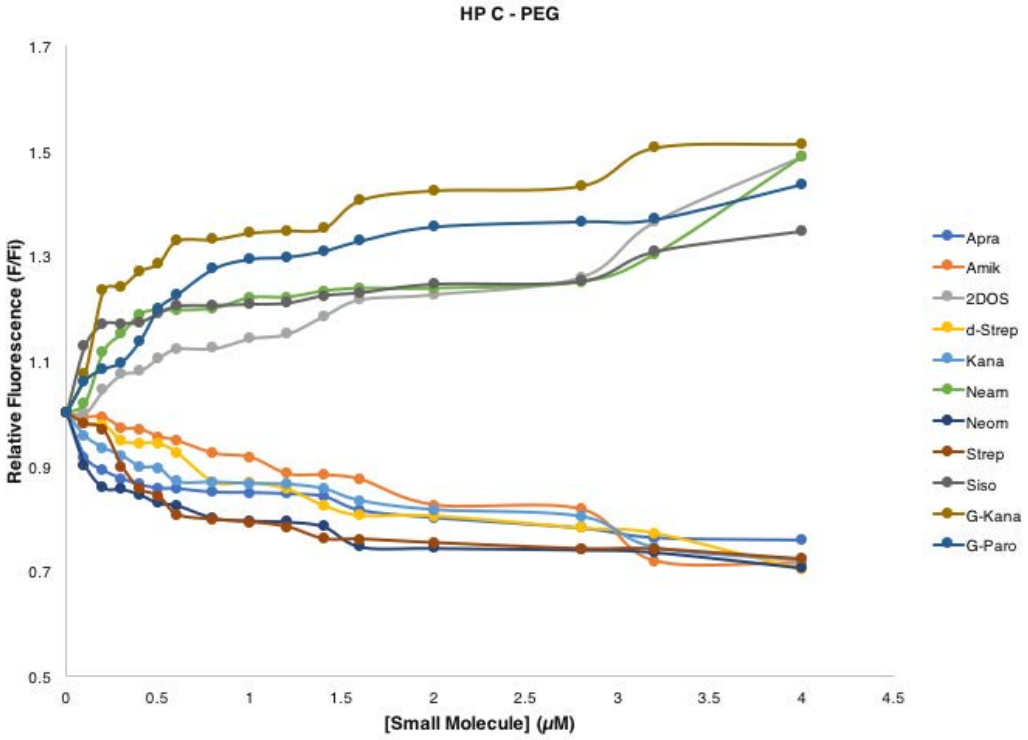
Siso
G-Kana
G-Paro


HP C - PEG
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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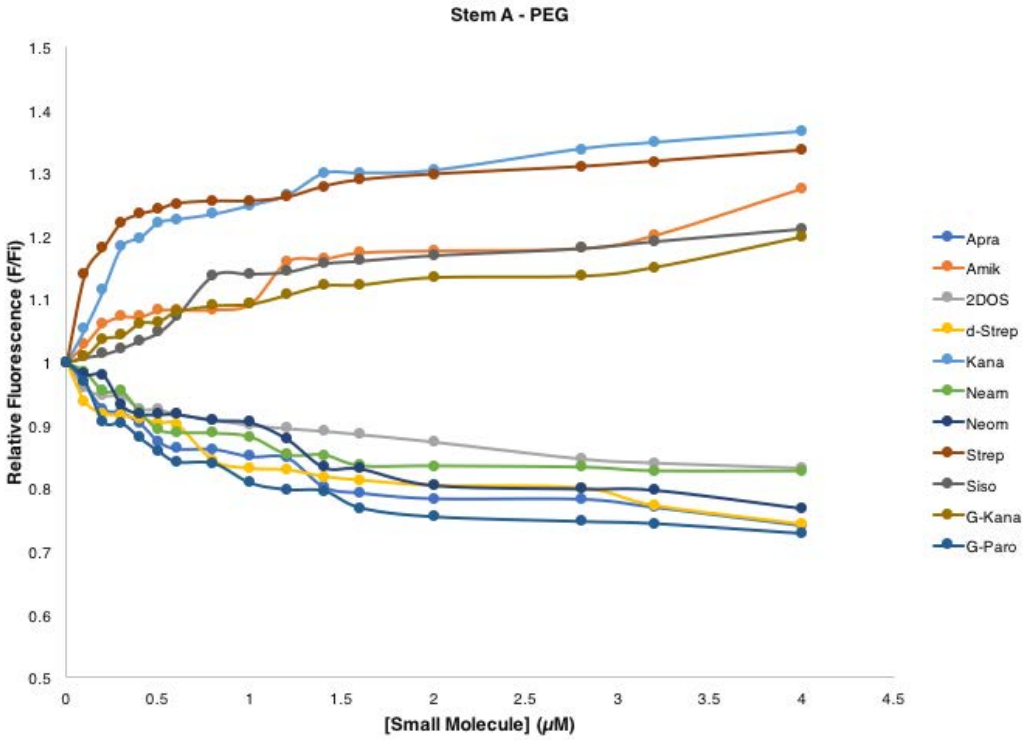
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


Stem A - PEG
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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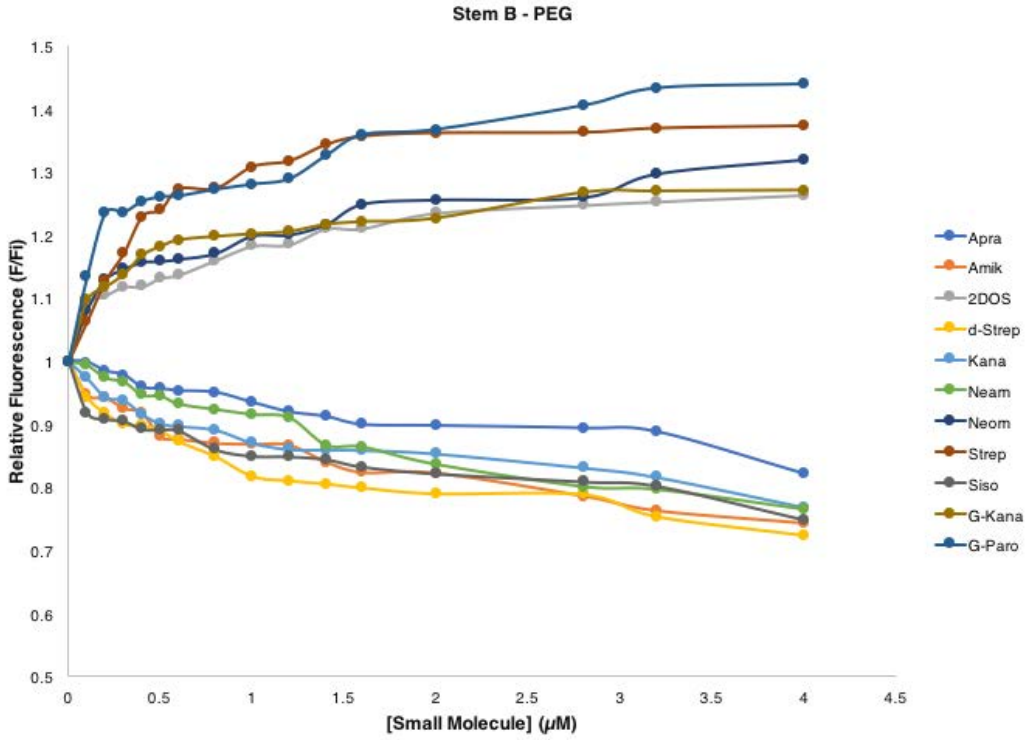
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro



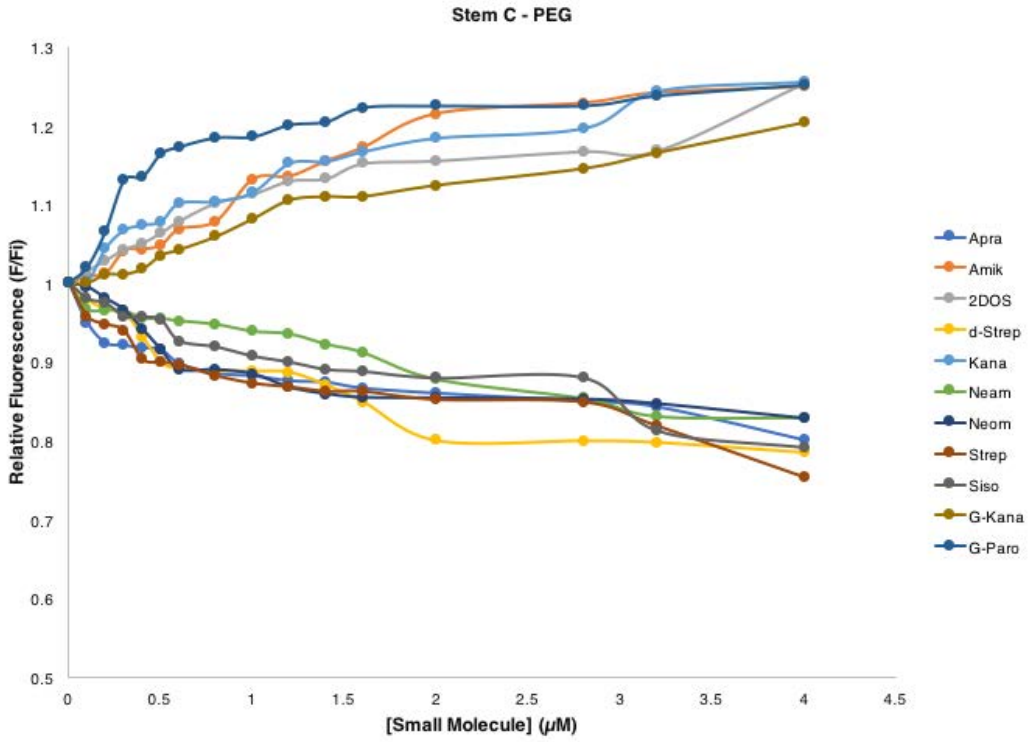
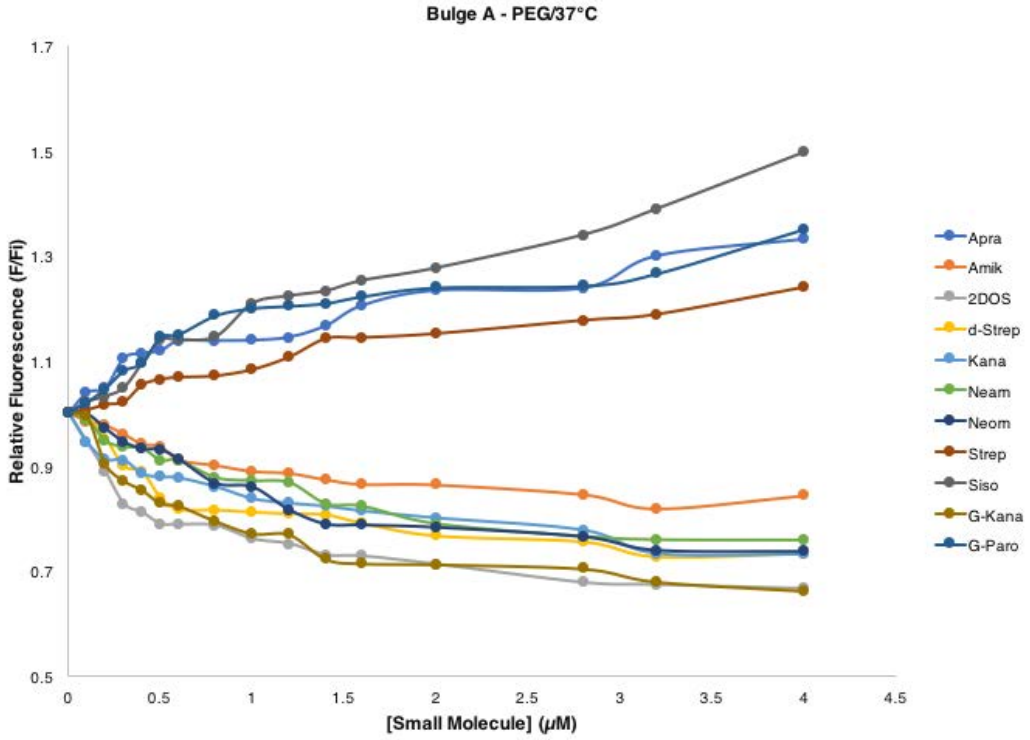


Figure S4-10. All RNA sequence titration curves to the small molecule receptors buffer with PEG 12,000 (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, 8 mM PEG 12,000 pH 7.4, 25°C). Each graph shows a single RNA sequence and the receptors titration curves. The error for all experiments were less than 10%. The error was calculated using the standard error of triplicates, and relative standard error was determined based on the mean of each RNA sequence.


Bulge A - PEG/37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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Amik
2DOS
d-Strep
Kana
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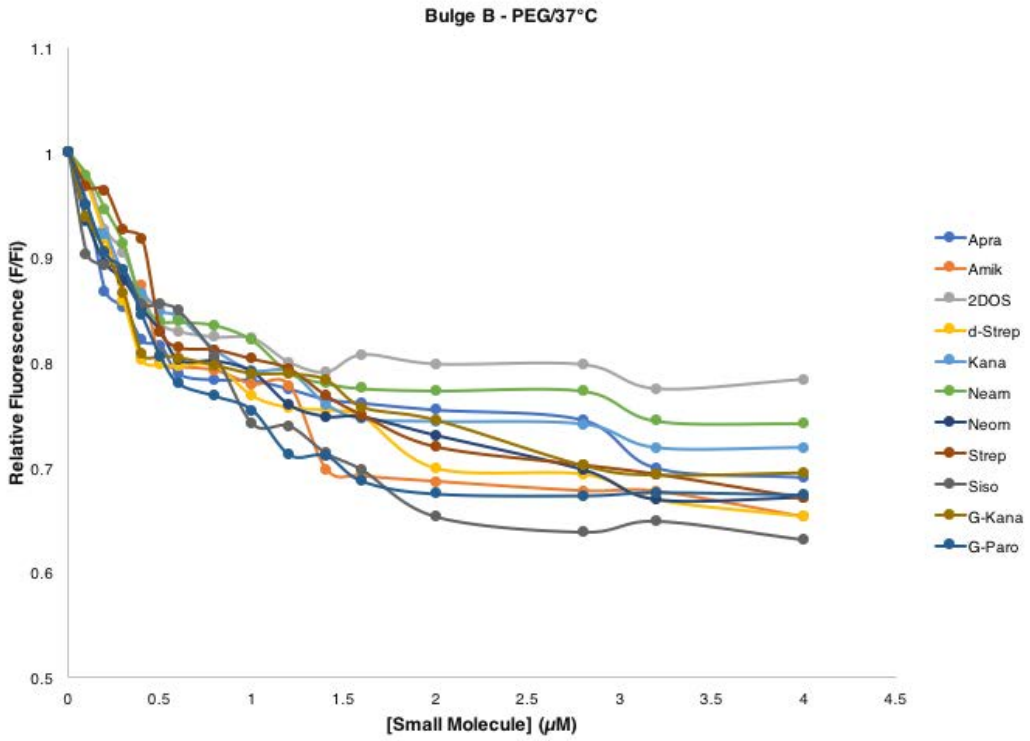
Neom
Strep
Siso
G-Kana
G-Paro


Bulge B - PEG/37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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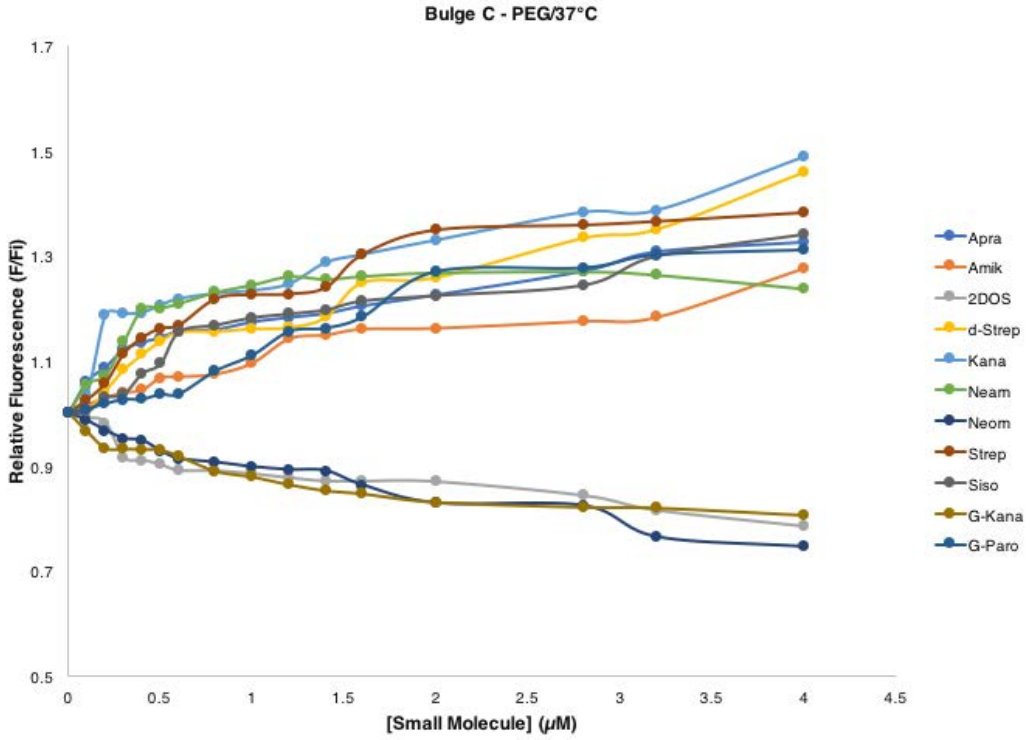
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


Bulge C - PEG/37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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Amik
2DOS
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Kana
Neam
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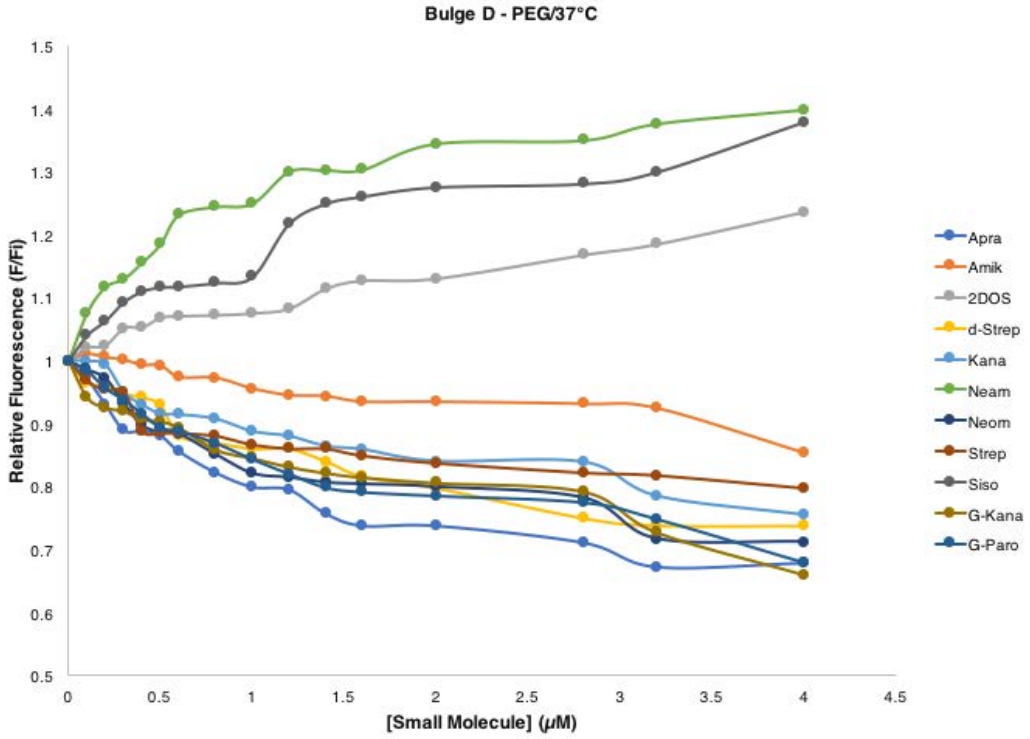
Strep
Siso
G-Kana
G-Paro


Bulge D - PEG/37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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Amik
2DOS
d-Strep
Kana
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Neom
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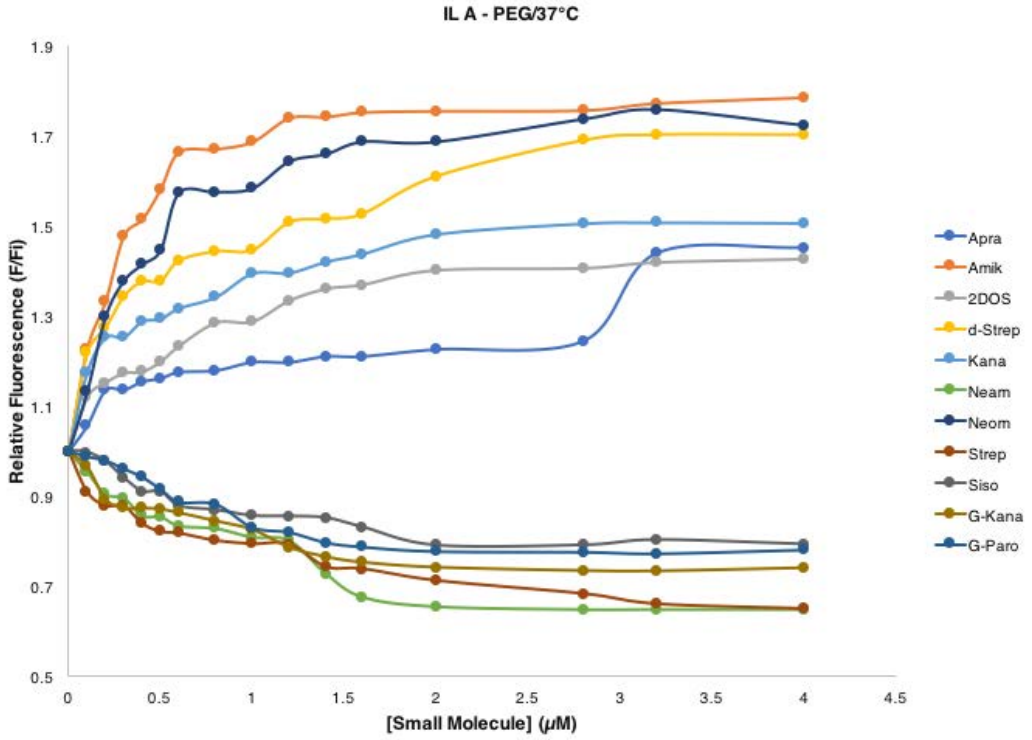
Siso
G-Kana
G-Paro


IL A - PEG/37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
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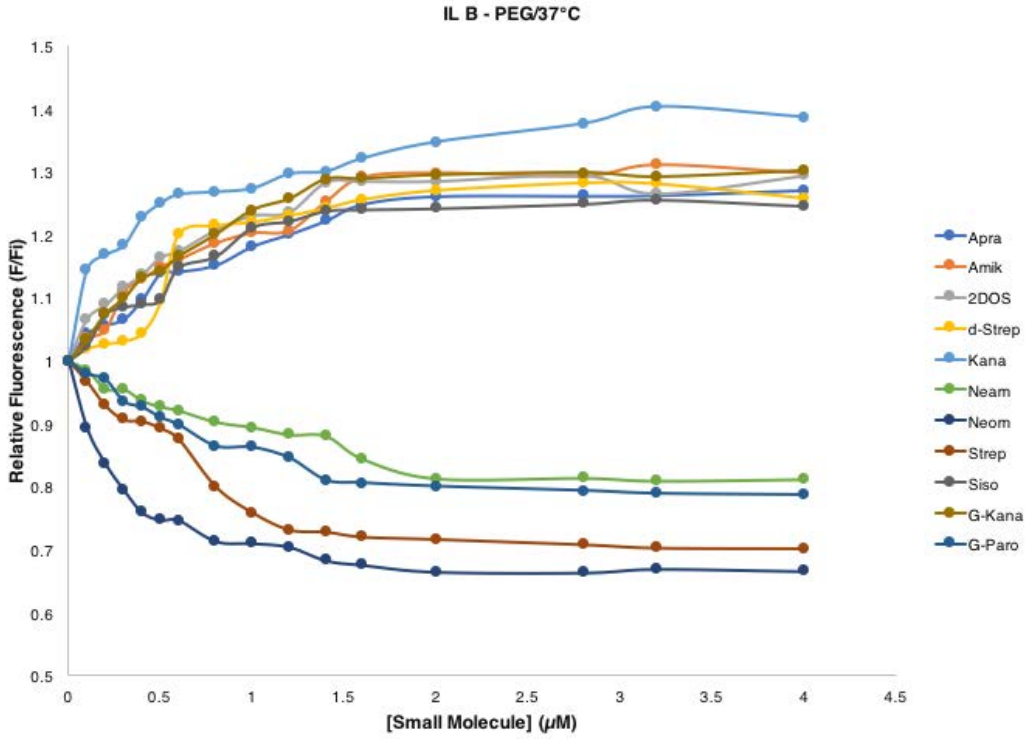
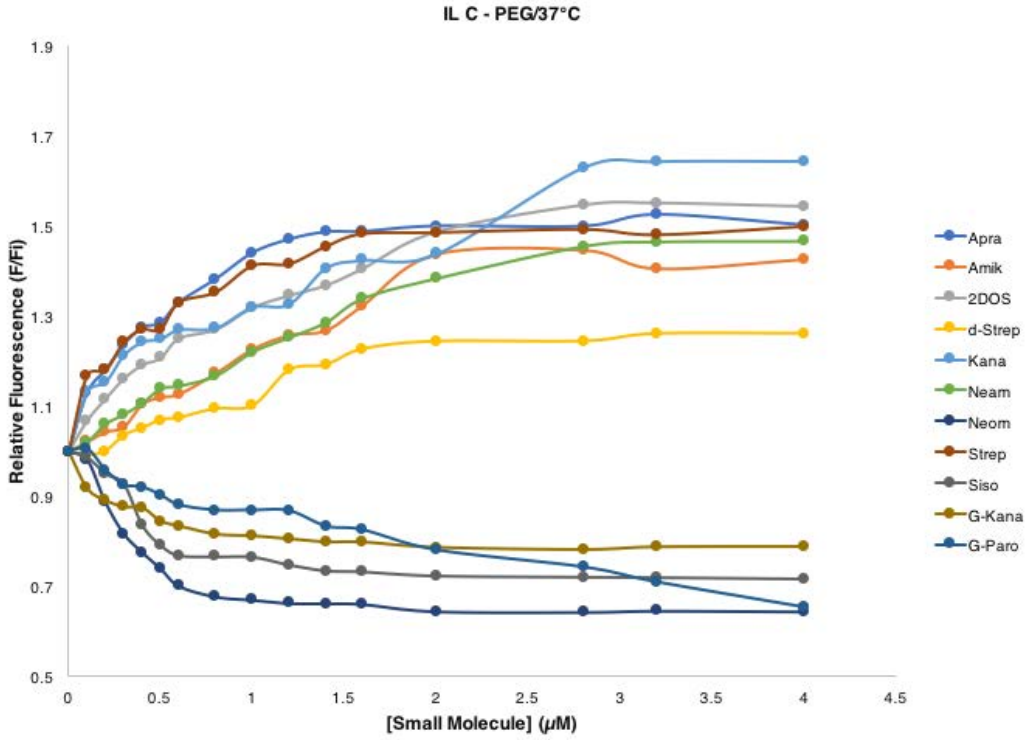
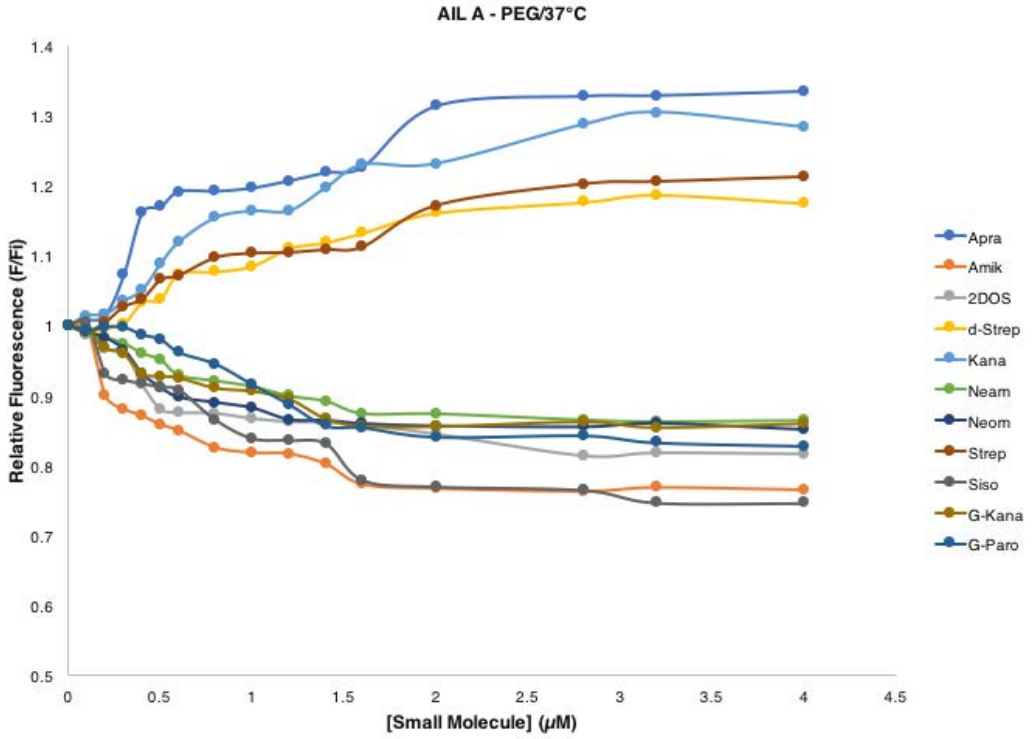
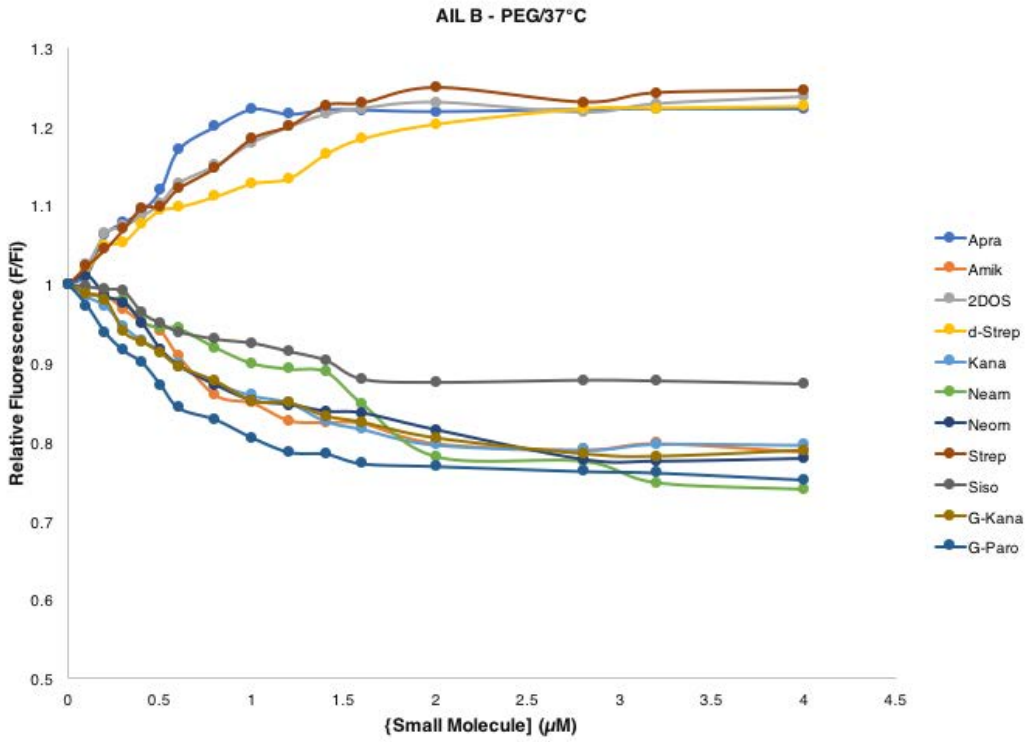
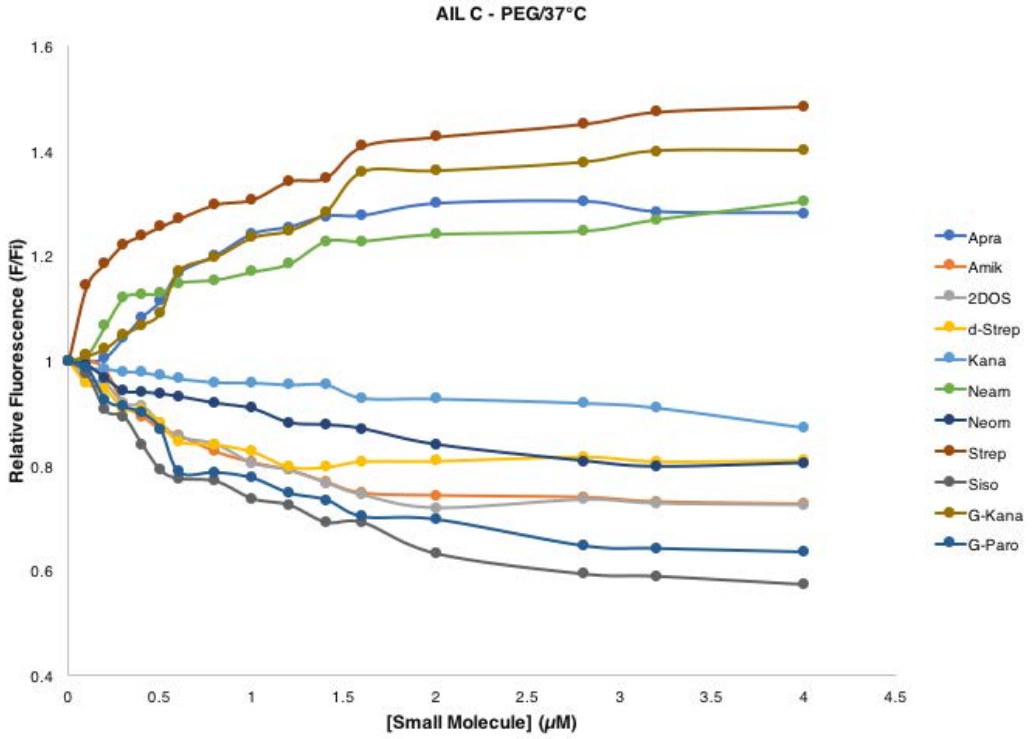
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

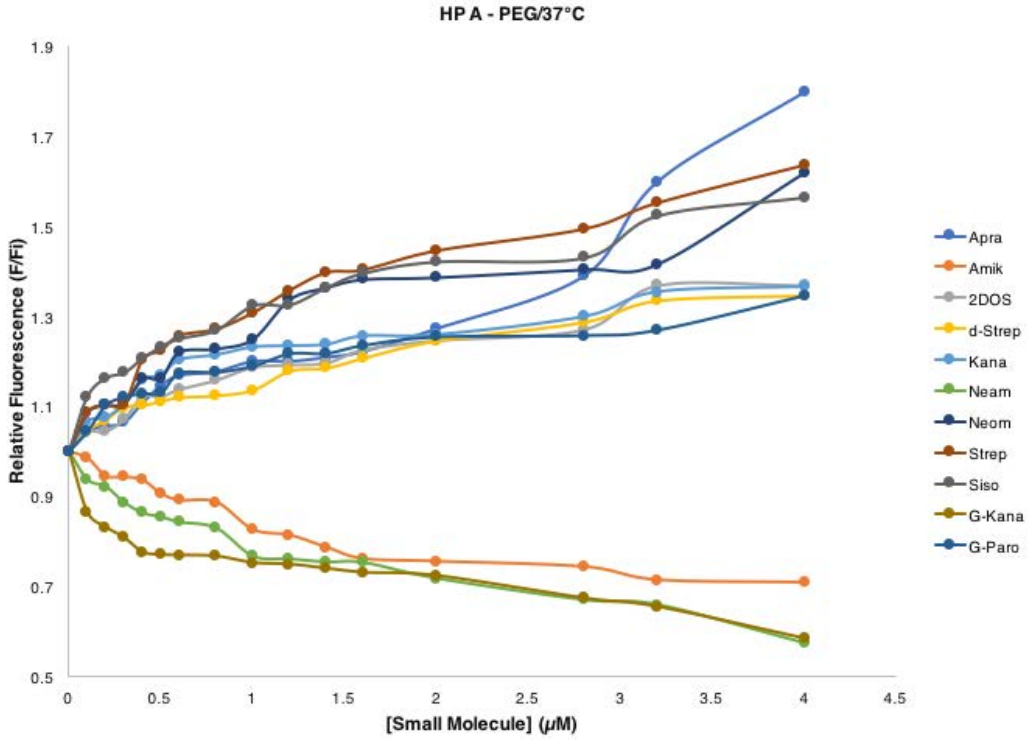

IL B - PEG/37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

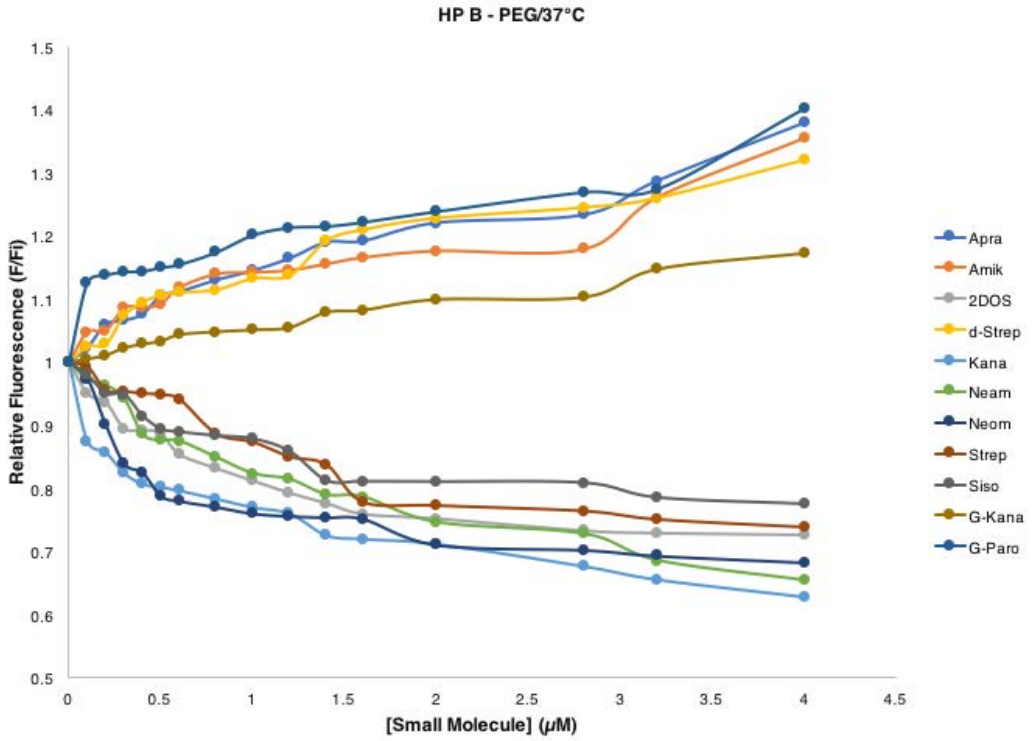

IL C - PEG/37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

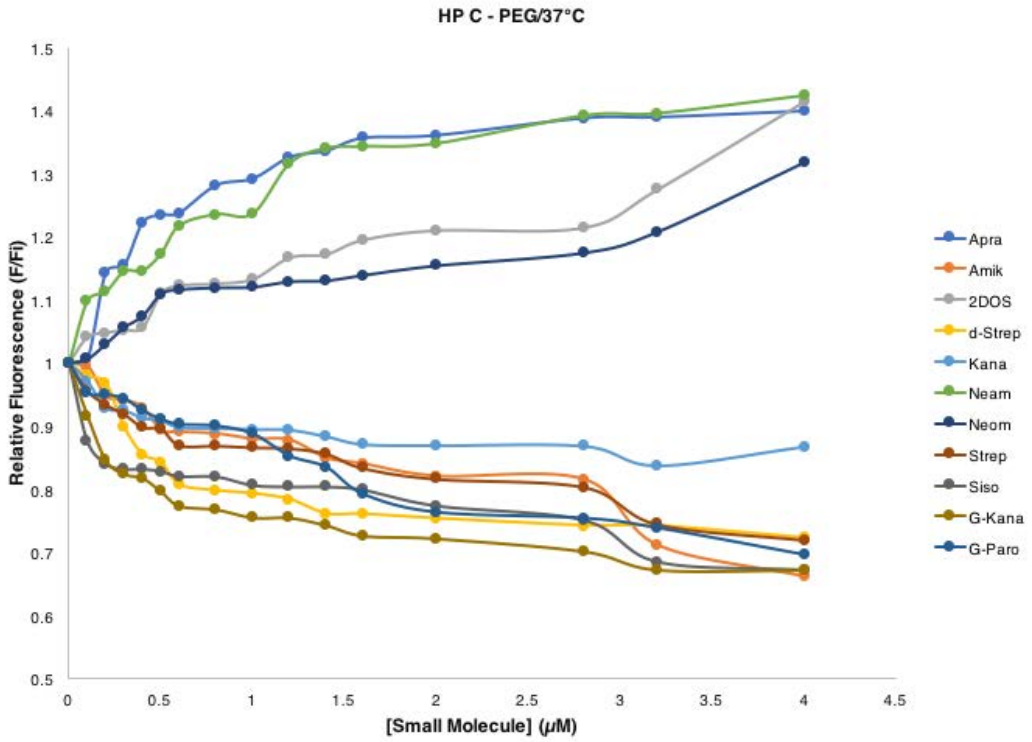

AIL A - PEG/37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

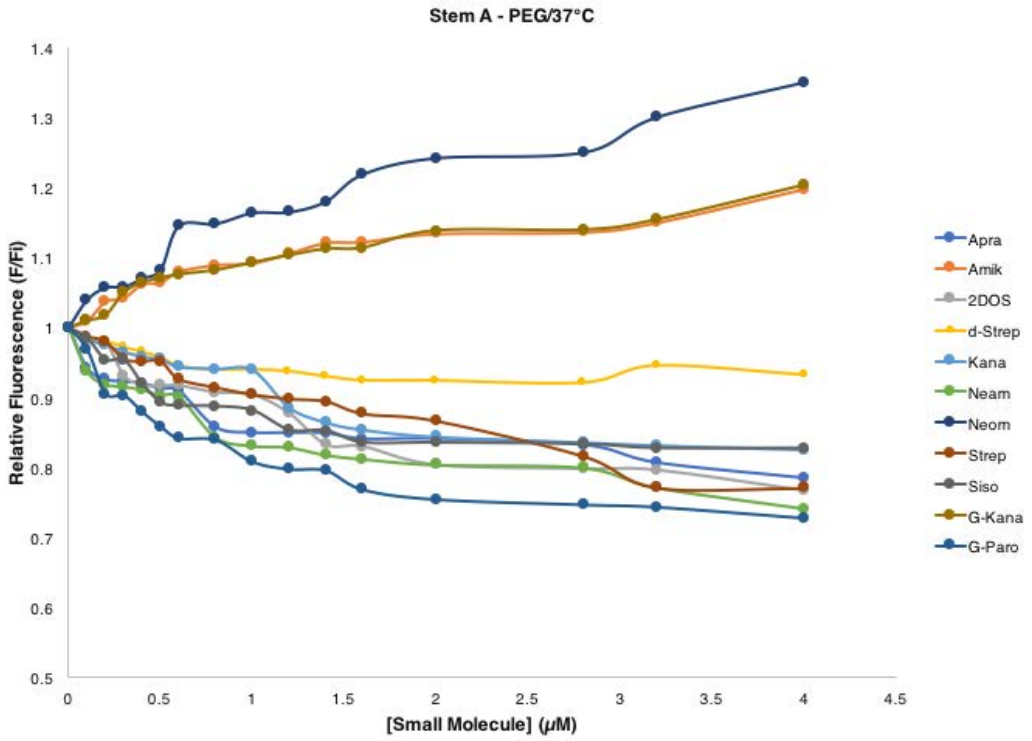

AIL B - PEG/37°C
Relative Fluorescence (F/Fi)
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
{Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro

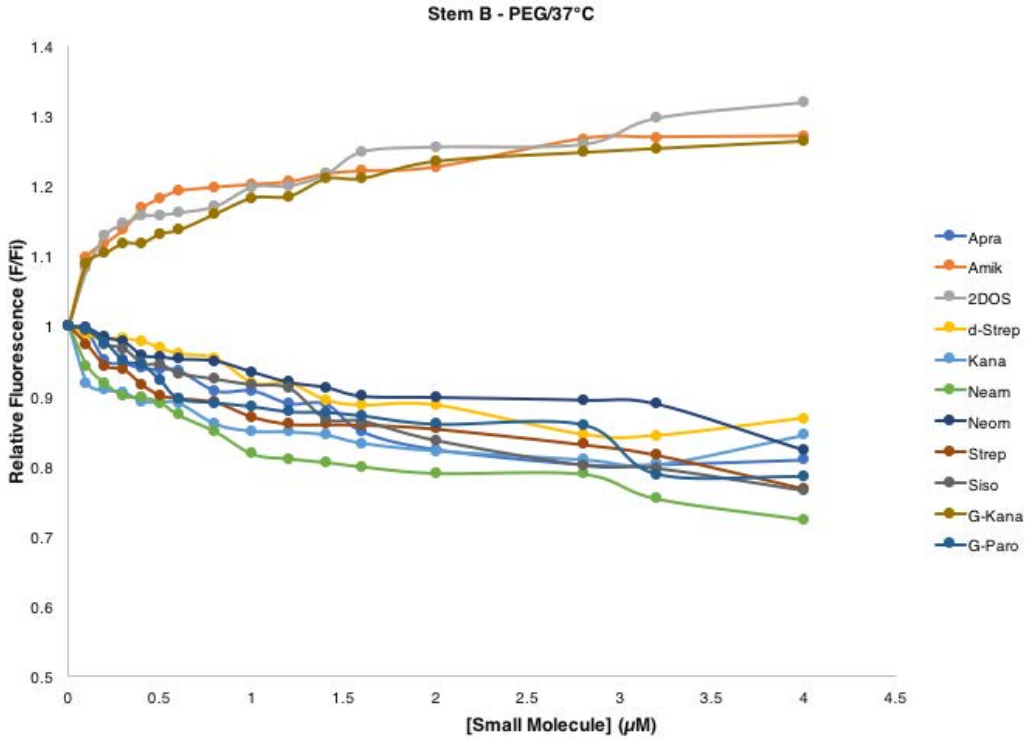

AIL C - PEG/37°C
Relative Fluorescence (F/Fi)
1.6
1.4
1.2
1
0.8
0.6
0.4
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


HP A - PEG/37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


HP B - PEG/37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


HP C - PEG/37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro


Stem A - PEG/37°C
Relative Fluorescence (F/Fi)
[Small Molecule] (μM)
Apra
Amik
2DOS
d-Strep
Kana
Neam
Neom
Strep
Siso
G-Kana
G-Paro



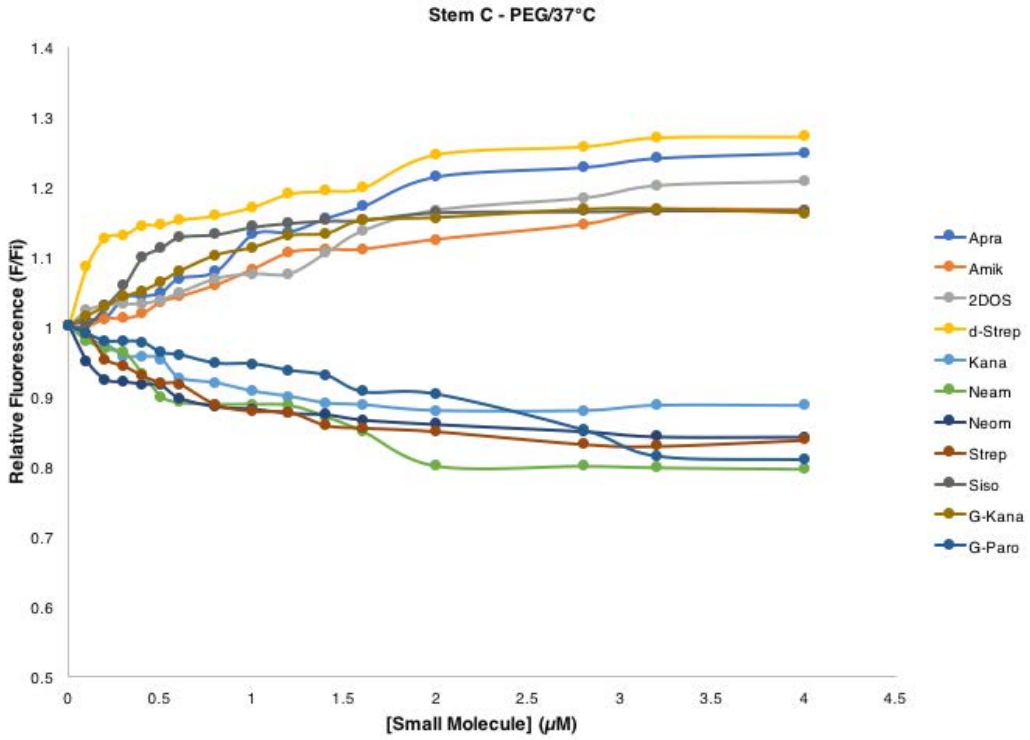


Figure S4-11. All RNA sequence titration curves to the small molecule receptors buffer with PEG 12,000 at 37°C (10 mM NaH_2PO_4, 25 mM NaCl, 4 mM $MgCl_2$, 0.5 mM EDTA, 8 mM PEG 12,000 pH 7.4, 37°C). Each graph shows a single RNA sequence and the receptors titration curves. The error for all experiments were less than 10%. The error was calculated using the standard error of triplicates, and relative standard error was determined based on the mean of each RNA sequence.

S5. References

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