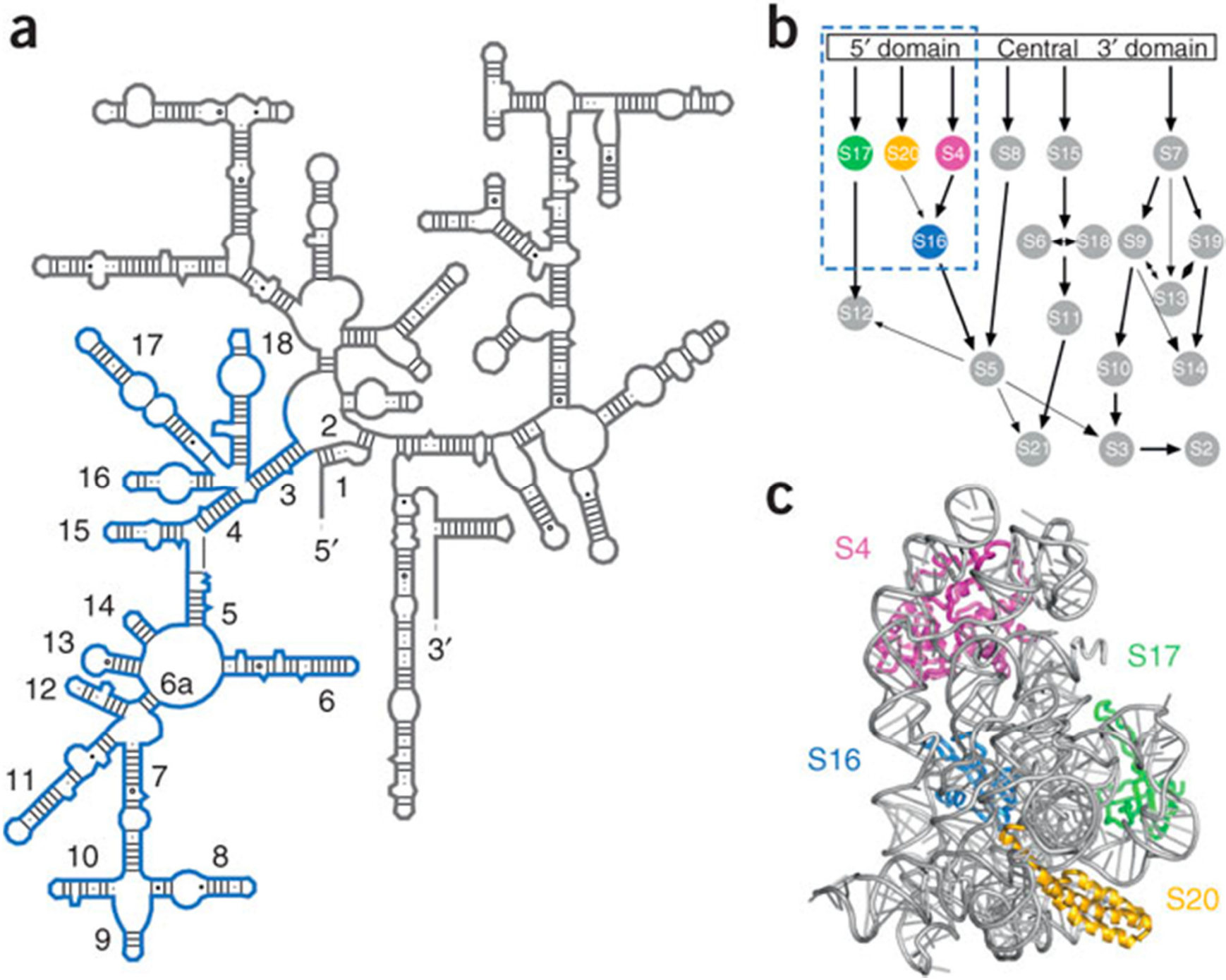


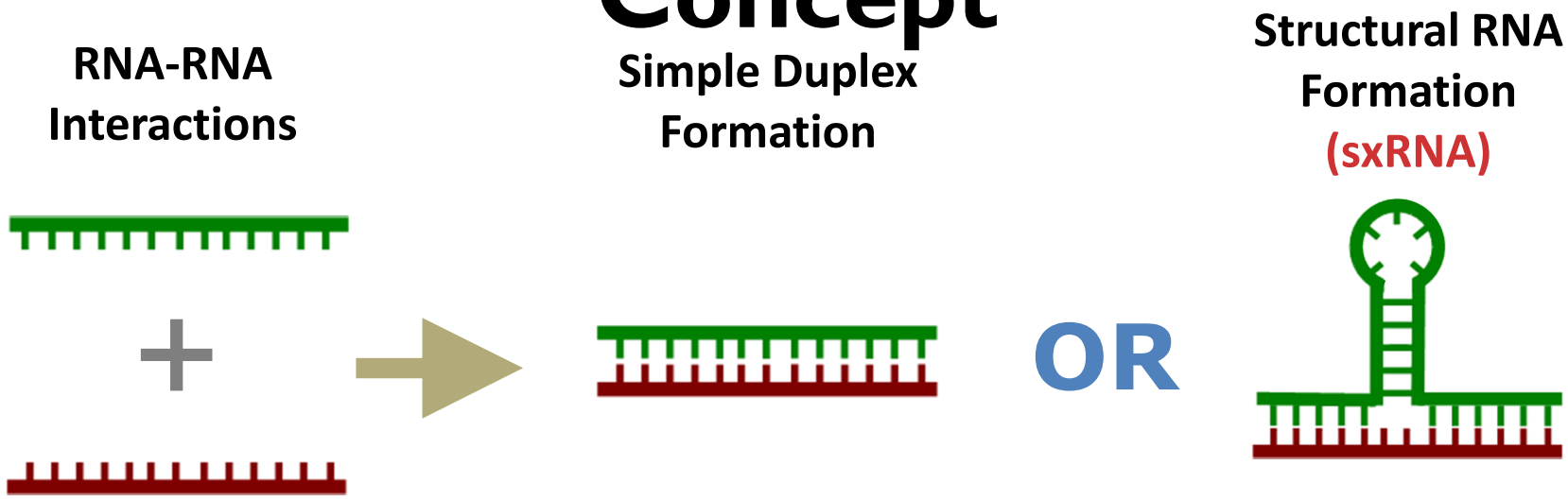
sxRNA: USING RNA-BASED, NANO-SWITCHES TO DETECT NOVEL NON-CODING RNA EXPRESSION

Scott A. Tenenbaum

SUNY- College of Nanoscale Science and Engineering, Nanobioscience

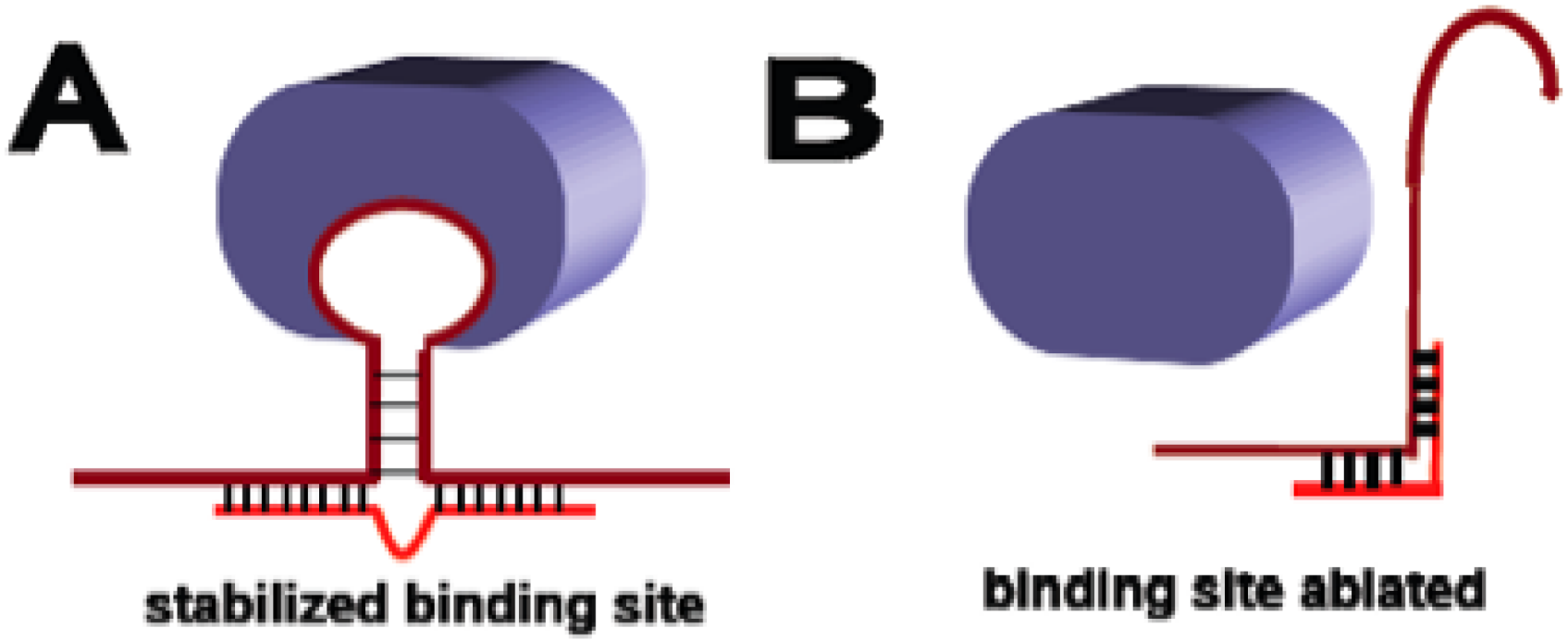

a
17
18
2
16
3
1
15
4
5′
14
5
13
3′
6a
12
6
11
7
10
8
9
b
5′ domain
Central
3′ domain
S17
S20
S4
S8
S15
S7
S16
S6
S18
S9
S19
S13
S12
S11
S5
S10
S14
S21
S3
S2
c
S4
S17
S16
S20

The Basic sxRNA Concept



- Structurally interacting RNA
  - A 3-way junction structure created from *trans*-interaction
  - An RNA sequence changes or "switches" the structure of the sxRNA in a designed manner
  - This results in the formation of a new or strengthened stem-loop structure

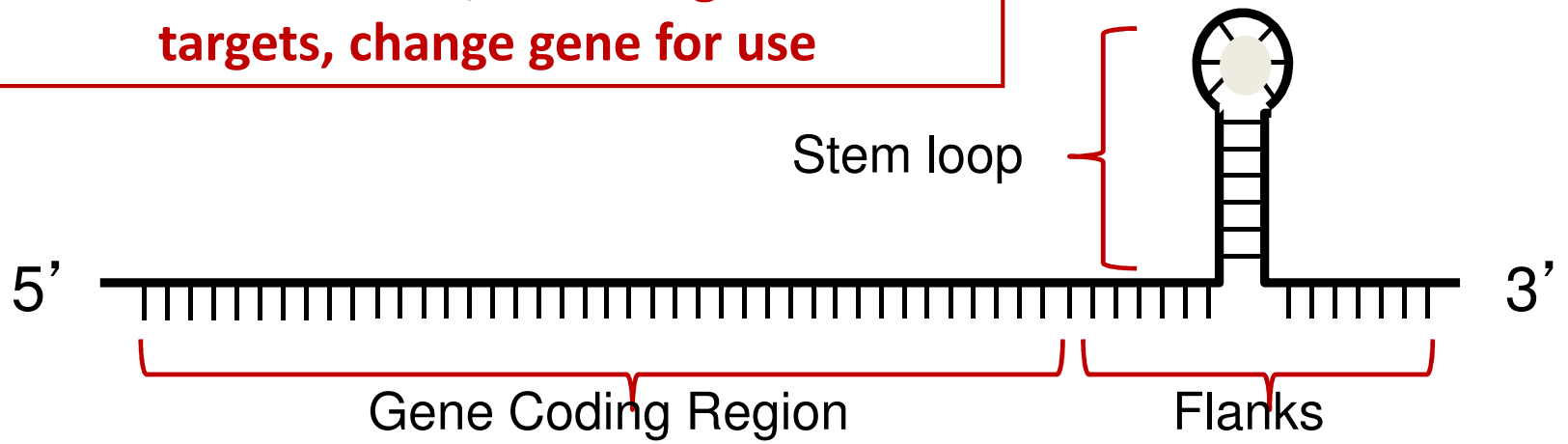
Structurally Interacting RNA (sxRNA)



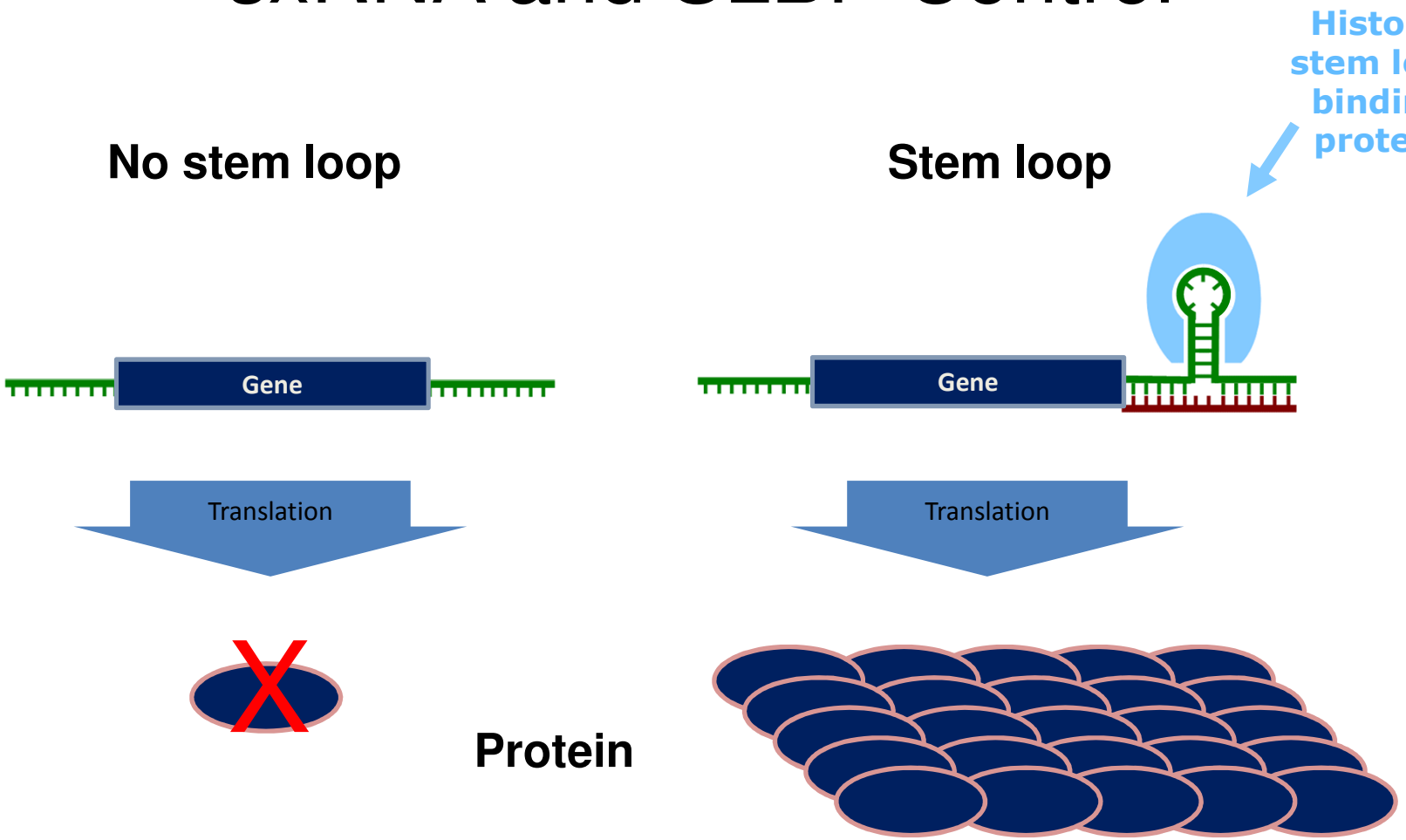
sxRNA Technology Platform Basics

- sxRNA molecule has three components
  - Stem loop – target of protein
  - Flanks – complementary to trigger RNA
  - Coding region – makes desired protein

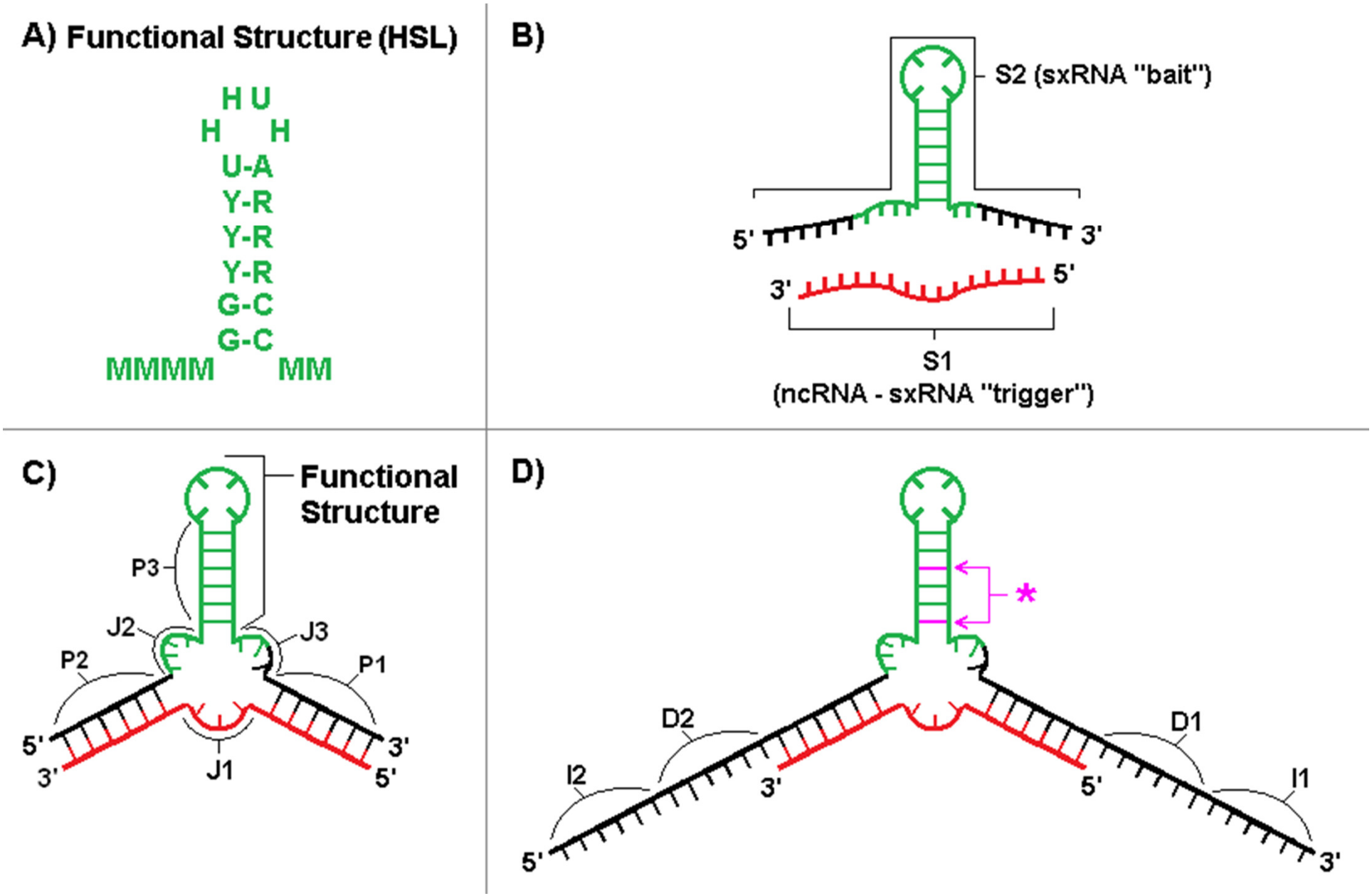
sxRNAs are modular, i.e. change flanks for targets, change gene for use

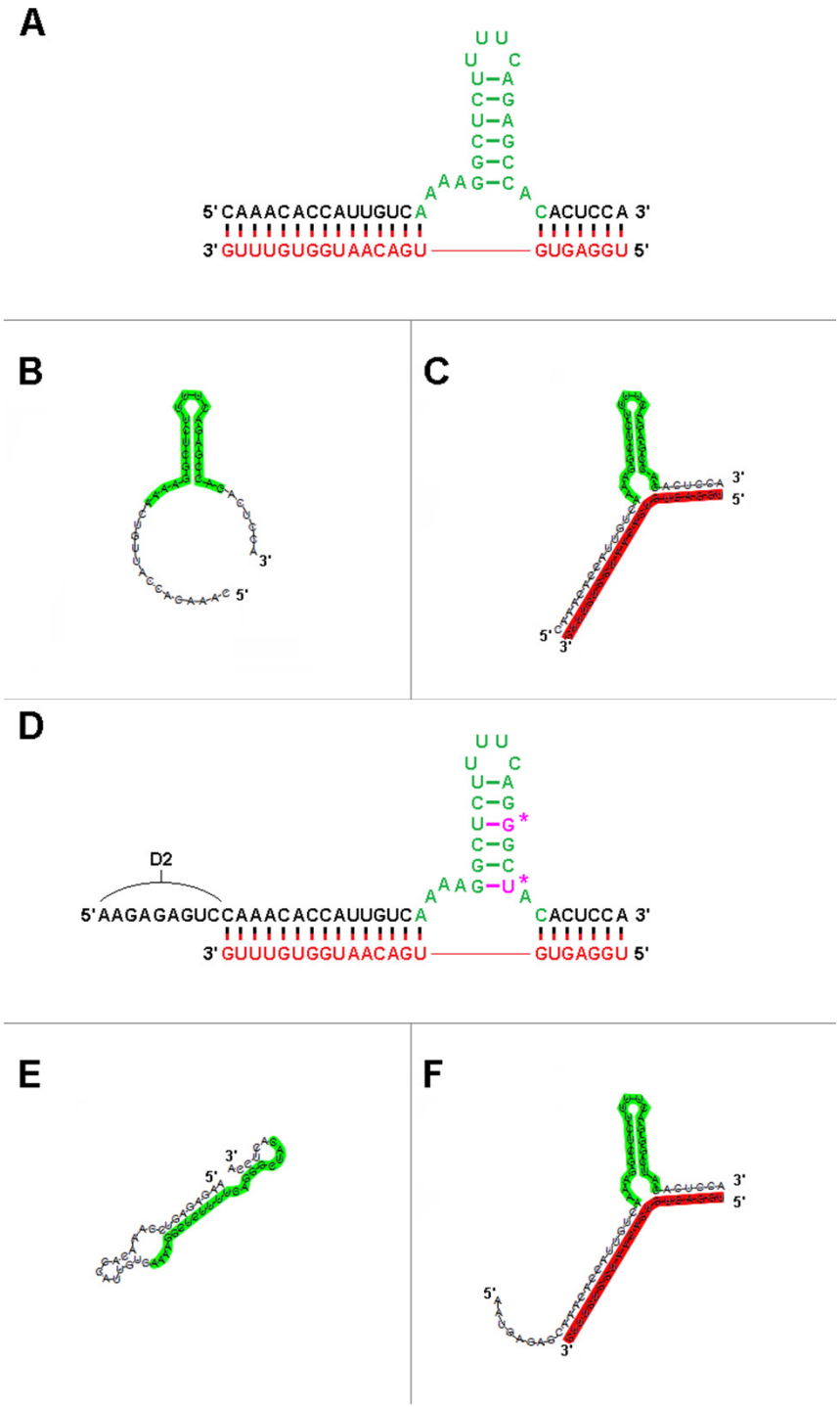


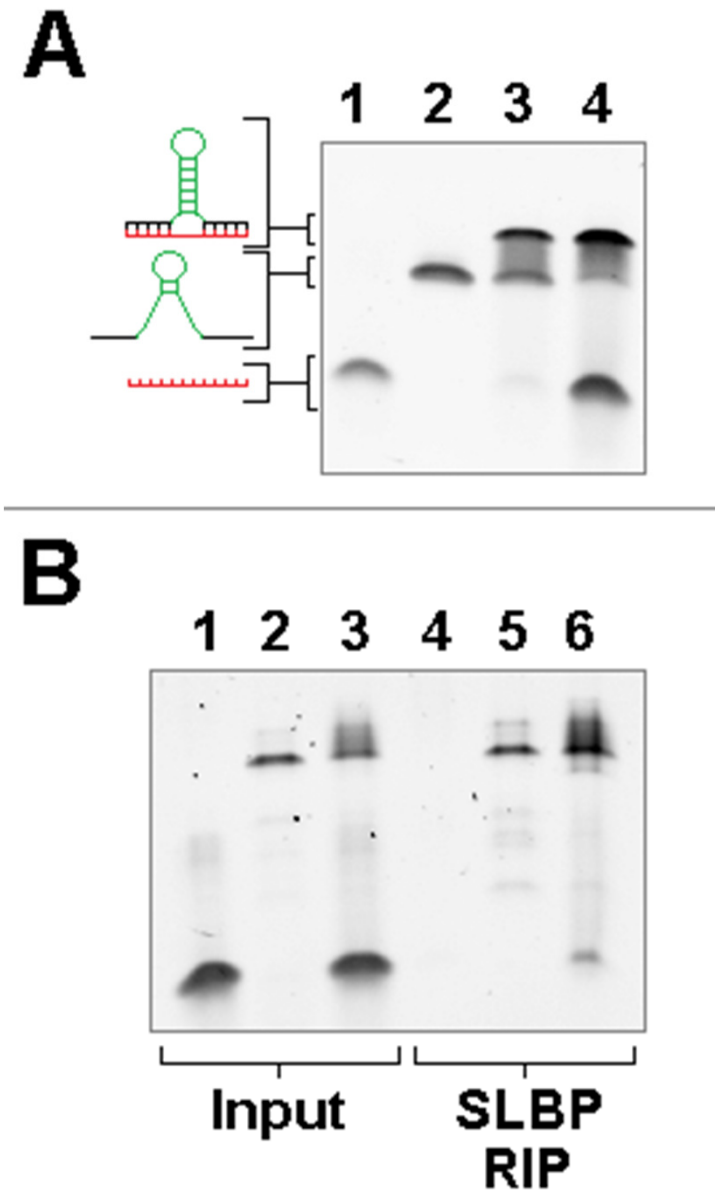
sxRNA and SLBP Control

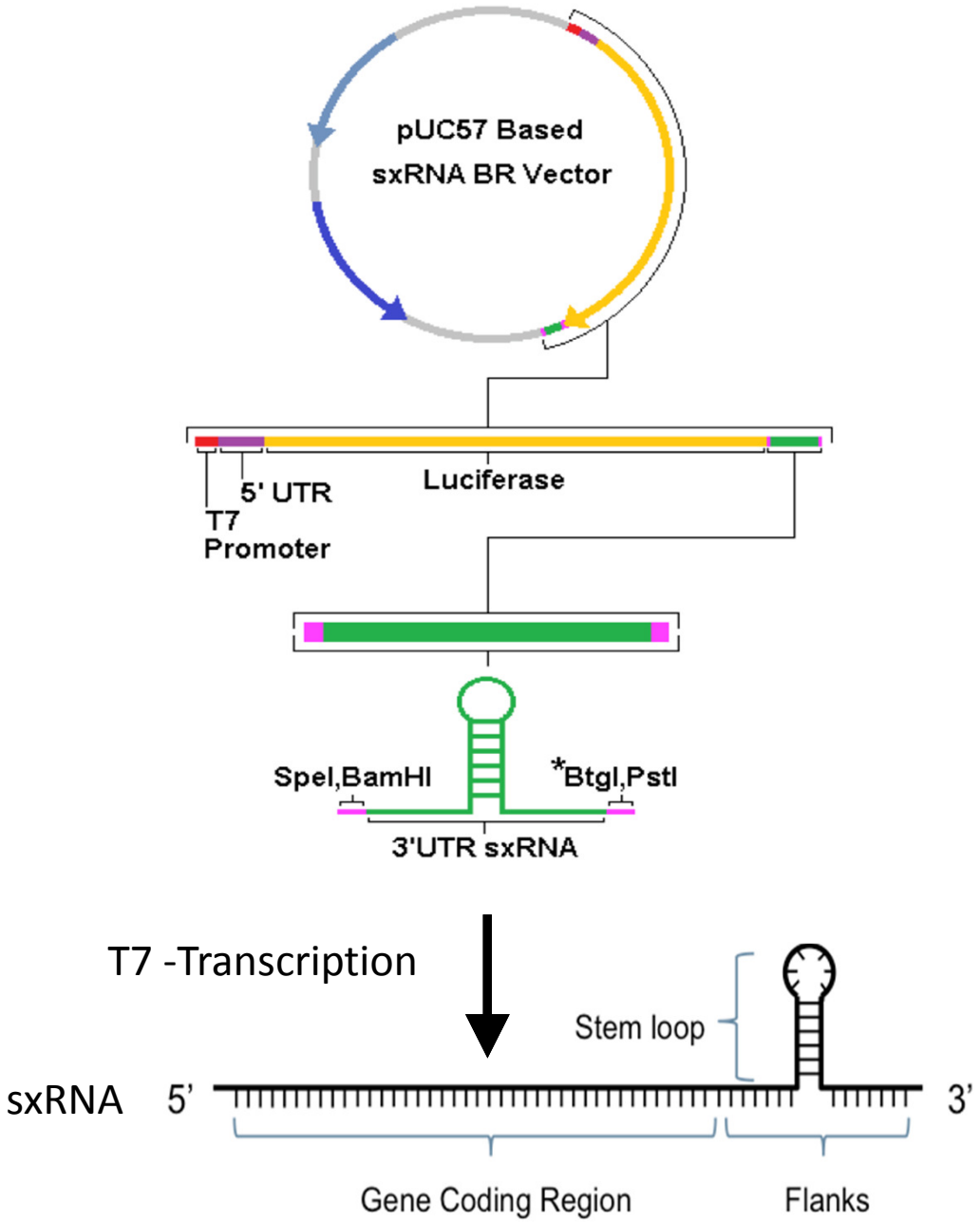


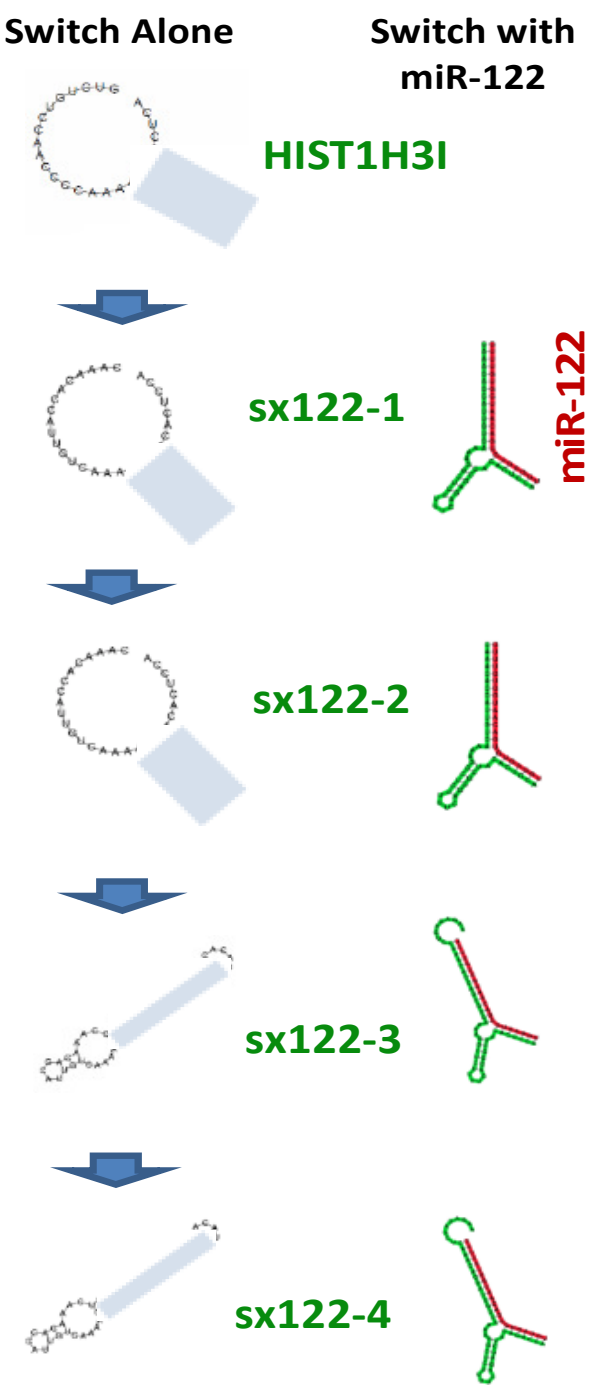
The presence of a stem-loop turns on protein production when the sxRNA complex attracts binding protein

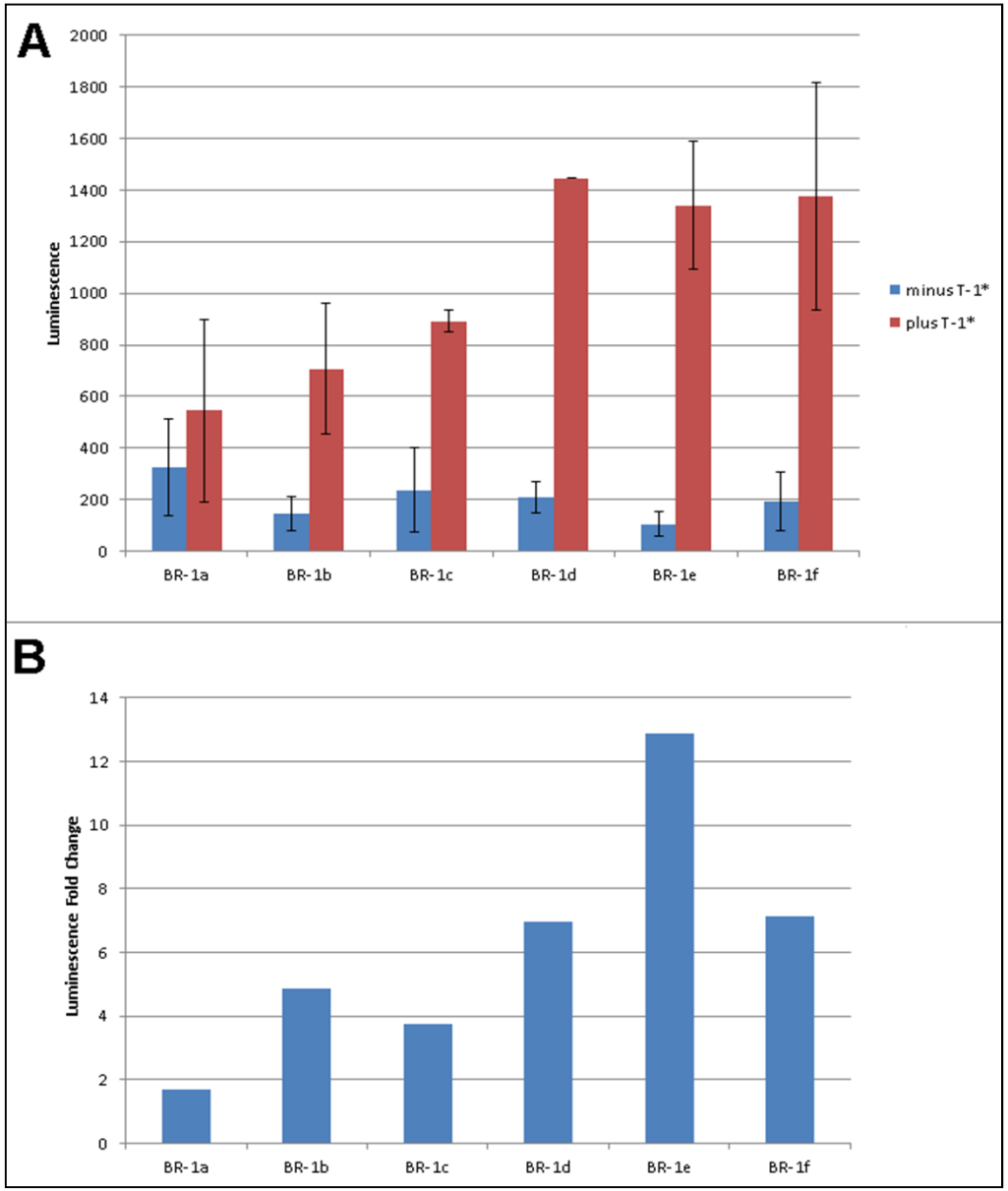

A) Functional Structure (HSL)
H U
H H
U-A
Y-R
Y-R
Y-R
G-C
G-C
MMMM MM
B)
S2 (sxRNA "bait")
5'
3'
3'
5'
S1
(ncRNA - sxRNA "trigger")
C)
Functional Structure
P3
J2
J3
P2
P1
5'
3'
J1
3'
5'
D)
*
D2
D1
I2
I1
3'
5'
5'
3'

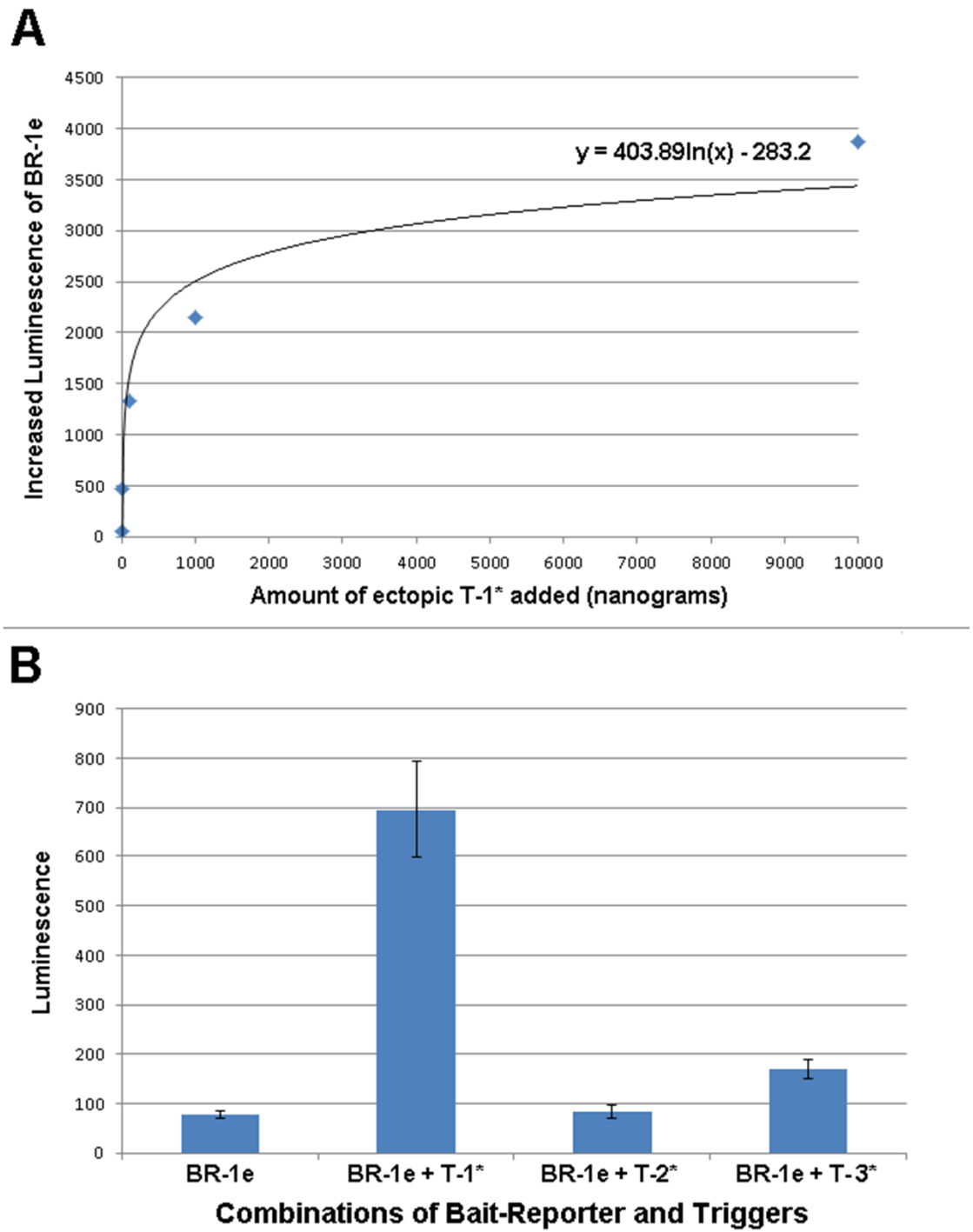

A
5' CAAACACCAUUGUCA
CACUCCA 3'
3' GUUUGUGGUAACAGU
GUGAGGU 5'
B
3'
5'
C
3'
5'
5'
3'
D
D2
5' AAGAGAGUCCAAACACCAUUGUCA
CACUCCA 3'
3' GUUUGUGGUAACAGU
GUGAGGU 5'
E
3'
5'
F
3'
5'
5'
3'

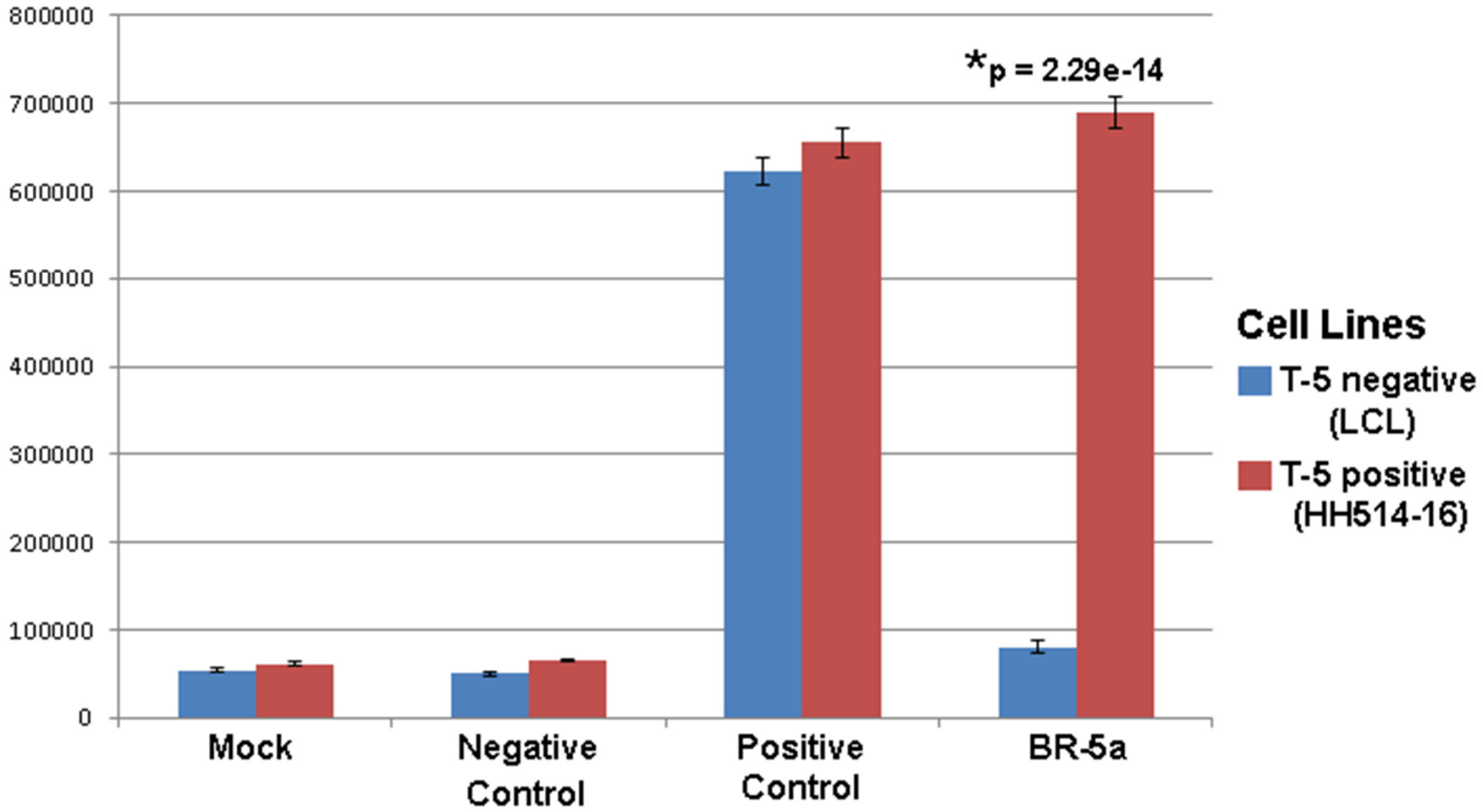

A
1 2 3 4
B
1 2 3 4 5 6
Input
SLBP
RIP


pUC57 Based
sxRNA BR Vector
5' UTR
T7
Promoter
Luciferase
SpeI,BamHI
*BtgI,PstI
3'UTR sxRNA
T7 -Transcription
Stem loop
sxRNA
5'
3'
Gene Coding Region
Flanks


Switch Alone
Switch with miR-122
HIST1H3I
sx122-1
miR-122
sx122-2
sx122-3
sx122-4


A
Luminescence
2000
1800
1600
1400
1200
1000
800
600
400
200
0
BR-1a
BR-1b
BR-1c
BR-1d
BR-1e
BR-1f
minus T-1*
plus T-1*
B
Luminescence Fold Change
14
12
10
8
6
4
2
0
BR-1a
BR-1b
BR-1c
BR-1d
BR-1e
BR-1f


A
Increased Luminescence of BR-1e
4500
4000
3500
3000
2500
2000
1500
1000
500
0
y = 403.89ln(x) - 283.2
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
Amount of ectopic T-1* added (nanograms)
B
Luminescence
900
800
700
600
500
400
300
200
100
0
BR-1e
BR-1e + T-1*
BR-1e + T-2*
BR-1e + T-3*
Combinations of Bait-Reporter and Triggers


800000
700000
600000
500000
400000
300000
200000
100000
0
*p = 2.29e-14
Mock
Negative Control
Positive Control
BR-5a
Cell Lines
T-5 negative (LCL)
T-5 positive (HH514-16)

sxRNA Potential Applications

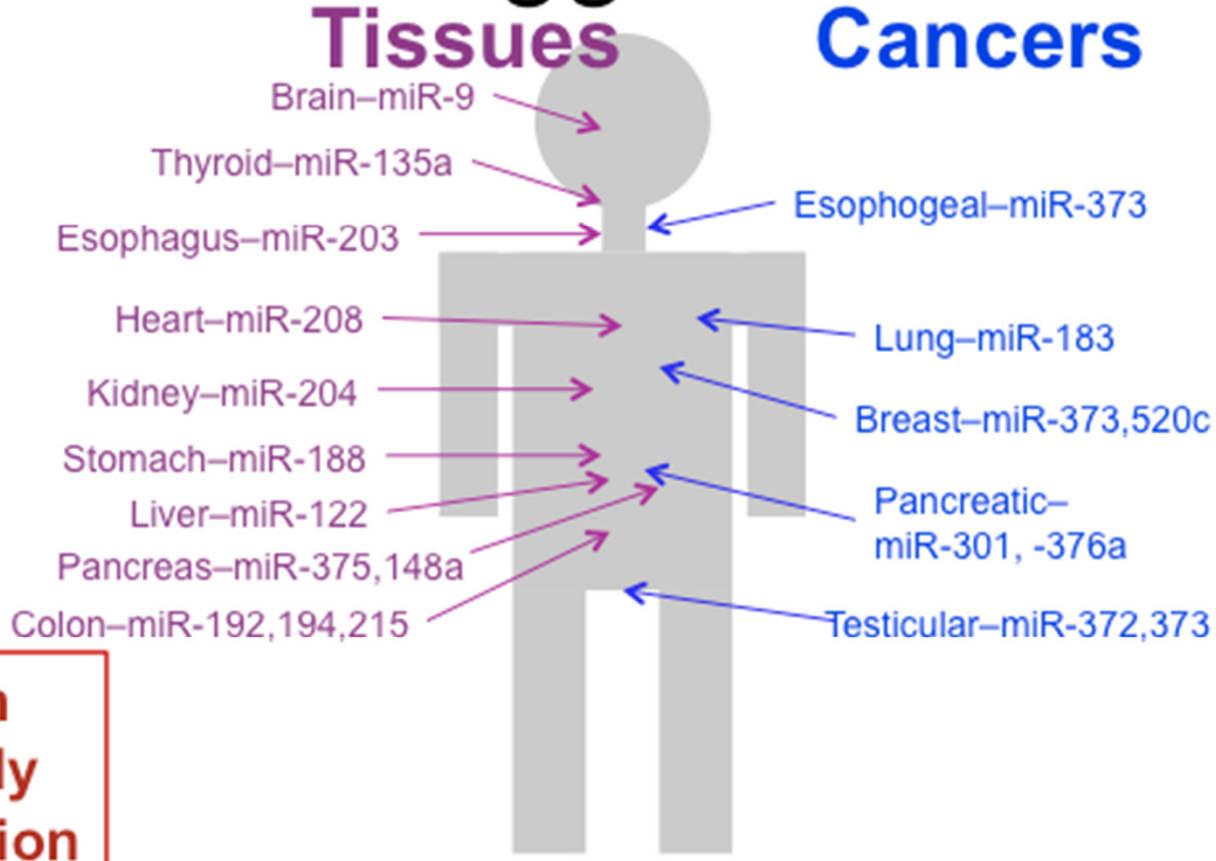
- **Molecular tool**
  - Stem cell quality control
  - miRNA expression in cancer
- **Genetic disorders**
  - Cystic Fibrosis
  - Duchenne muscular dystrophy
  - Glycogen storage diseases
  - Polycystic kidney disease
  - Celiac disease
  - Charcot-Marie-Tooth disease
  - Hemophilia
  - Sickle-cell disease
- **Tissue/Stem Cell Engineering**
  - Kidney repair
  - Liver repair
  - Heart
- **Cancer**
  - Breast
  - Lung
  - Colon
  - Pancreatic
  - Thyroid
  - Bladder
- **Antiviral, Vaccine, Imaging**

miRNA as Triggers

miRNA Benefits

- Unique sequences
- Highly conserved
- Different expression profiles
  - Cell types
  - Tissue types
  - Disease states
  - Cell development

miRNA expression patterns vary greatly with unique expression in tissues and cancers



Funding


HOCUS
LOCUS


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Science and Engineering


SUNY RF
The Research
Foundation for
The State University of New York


ENYA
Eastern New York Angels


National Institutes
of Health


The RNA Institute
College of Arts and Sciences
University at Albany State University of New York


University at Albany
State University of New York