Innate Immunity in the Purple Sea Urchin and Diversity of the *Sp185/333* System



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Strongylocentrotus purpuratus

Pacific ocean, west coast of North America

Near shore habitat in kelp forests

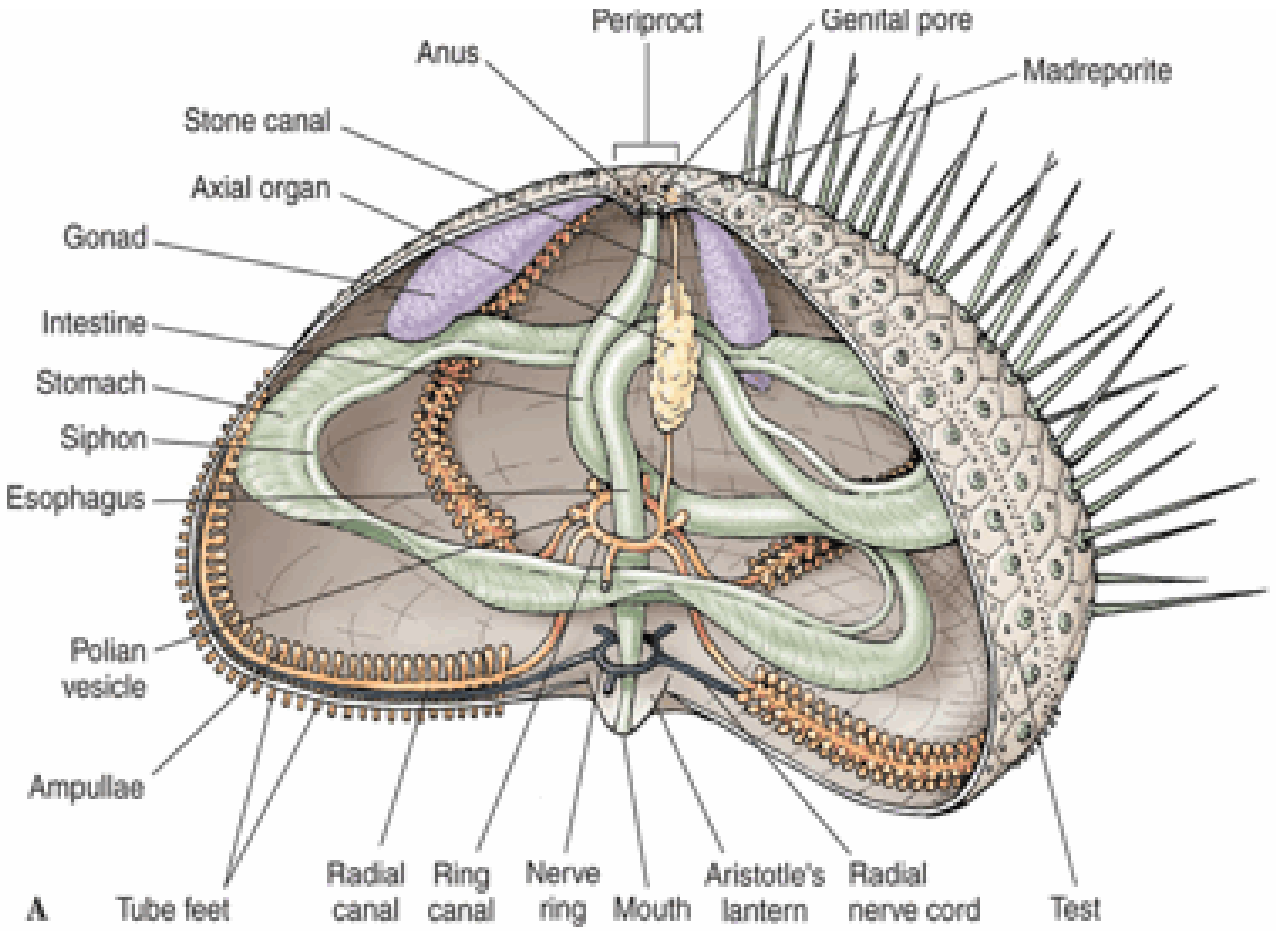
Good model for developmental biology

Genome sequenced in 2006





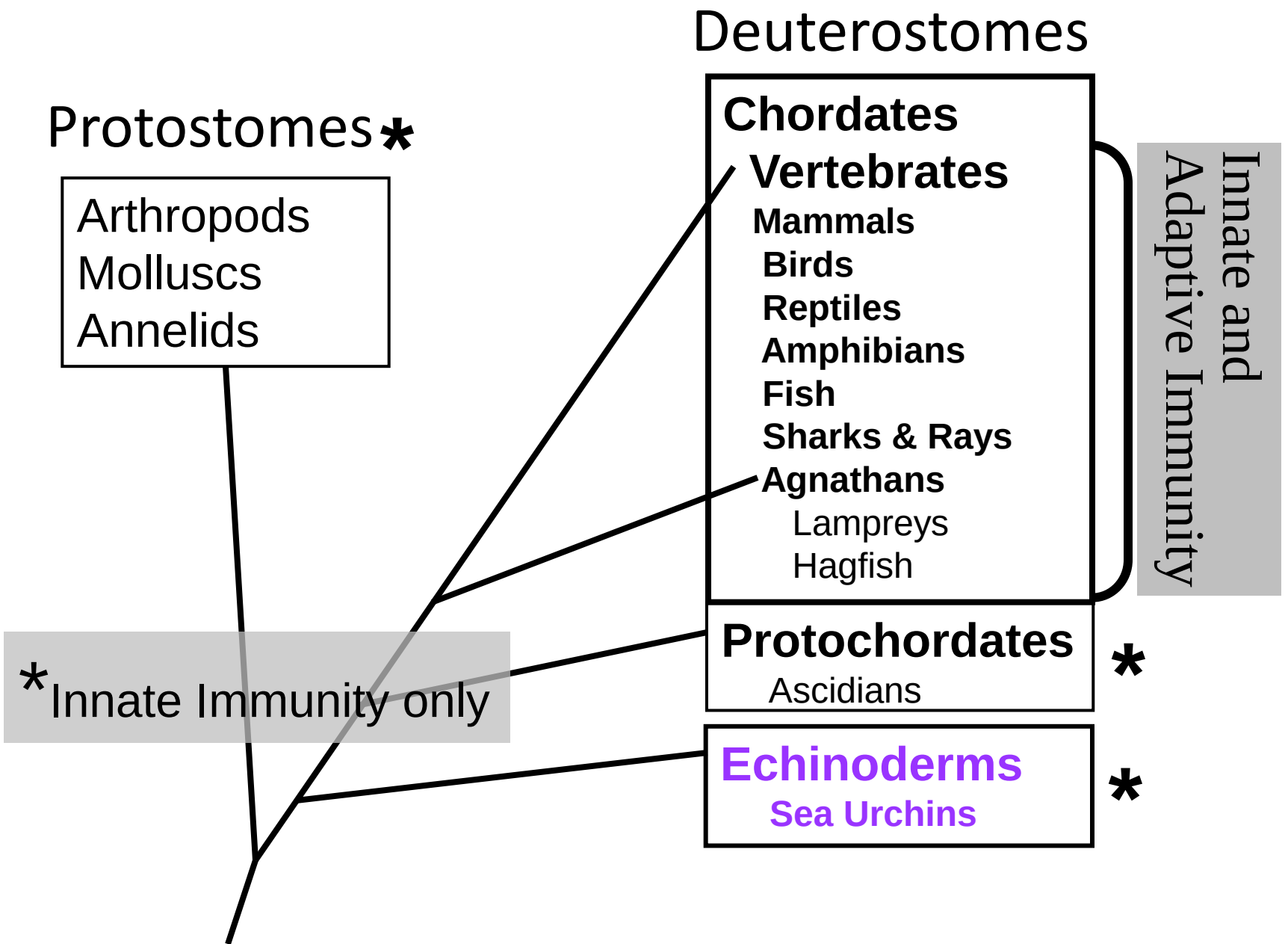
Sea urchin anatomy



Immune cells; coelomocytes

Eat, spawn, live ~50 years

Animal Phylogeny



Staying ahead of the pathogens

Problem:

Microbes typically have very short generation times

- diversification through rapid proliferation and/or gene exchange
- selection for virulence

Hosts generally have long(er) generation times

Solution:

To survive, hosts use molecular mechanisms to generate immune diversity within the lifespan of the individual

How is this accomplished without adaptive immunity?

Haldane, 1949

Sea urchin genome reveals a complex and sophisticated innate immune system

- Large gene families
  - Toll-like receptors, 253 gene models
    - expressed in phagocytes
  - NOD-like receptors, 203 gene models
    - expressed in gut
  - Scavenger receptors, 218 gene models
    - expressed in coelomocytes
  - *Sp185/333* system
    - 20-60 genes, small, clustered, diverse

Sodergren et al., 2006
Rast et al., 2006
Hibino et al., 2006
Buckley & Rast, 2012

The *Sp185/333* System Outline

- *Sp185/333* Genes
  - structure
  - sequence diversity
  - family structure
- SpShapeshifter Proteins
  - diversity
  - native protein function
  - recombinant protein
    - function
    - structure

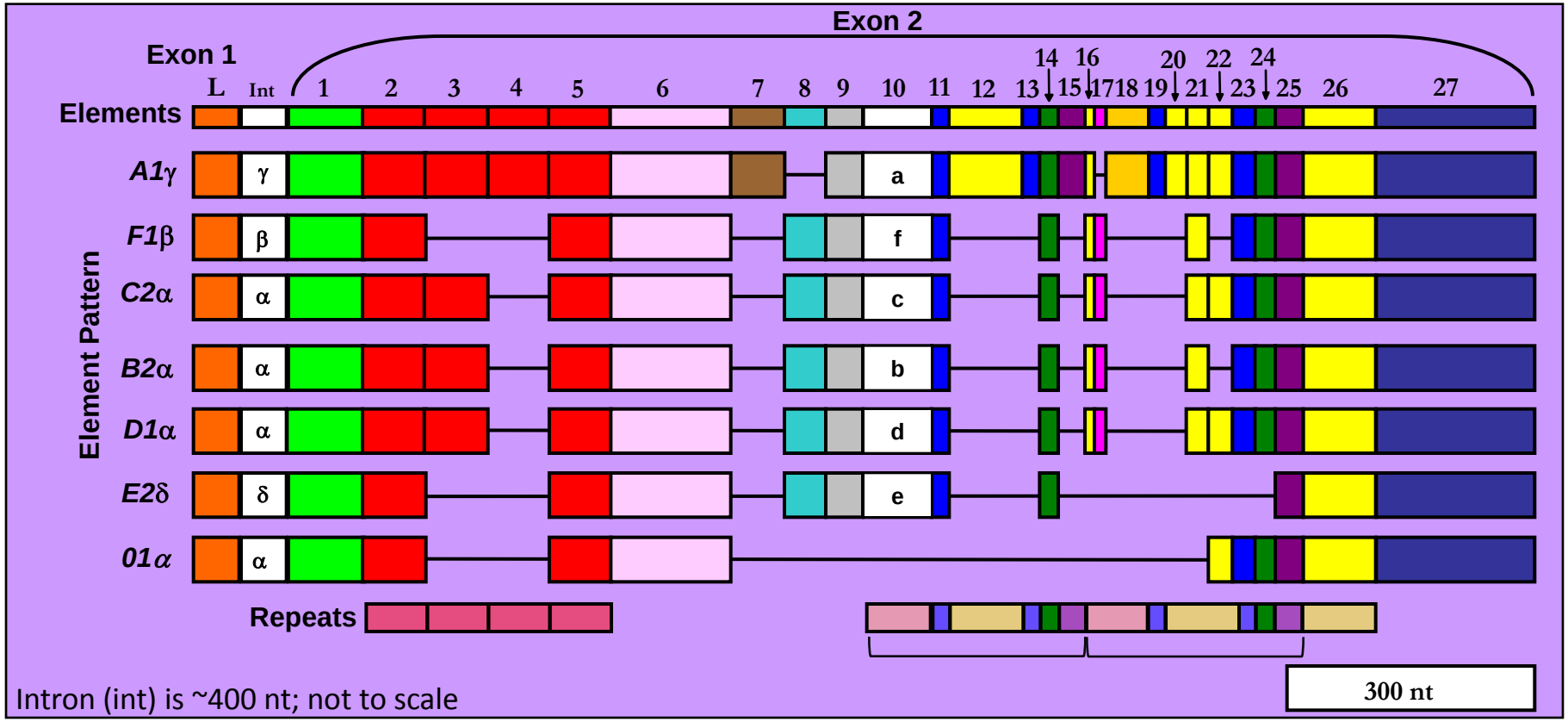
Sp185/333 gene structure



- genes are <2kb, 2 exons
- 1 pseudogene of 171 sequenced genes
- no gene fragments found

Terwilliger et al., 2006; 2007
Buckley & Smith, 2007
Oren et al., *unpub*

Sequence diversity of the *Sp185/333* genes

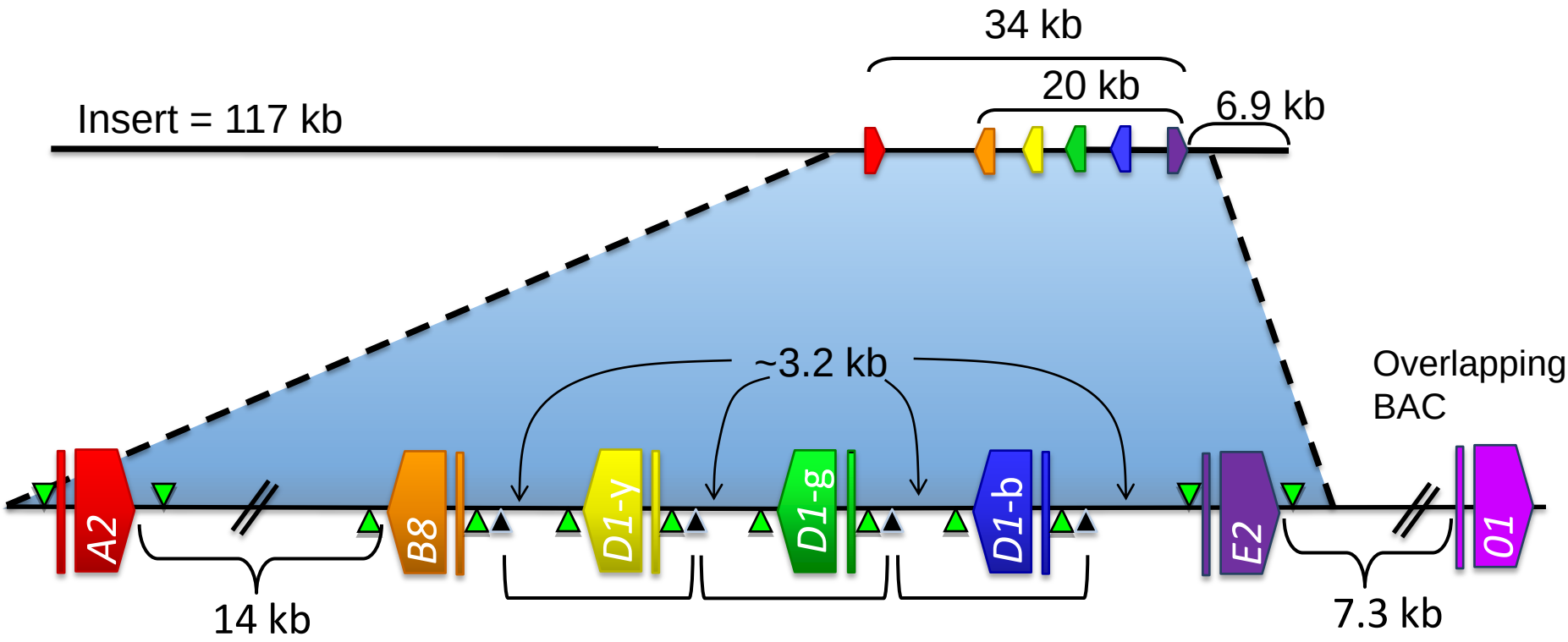


Element pattern names are defined by the
sequence of element 10
sequence of the intron

Buckley & Smith, 2007

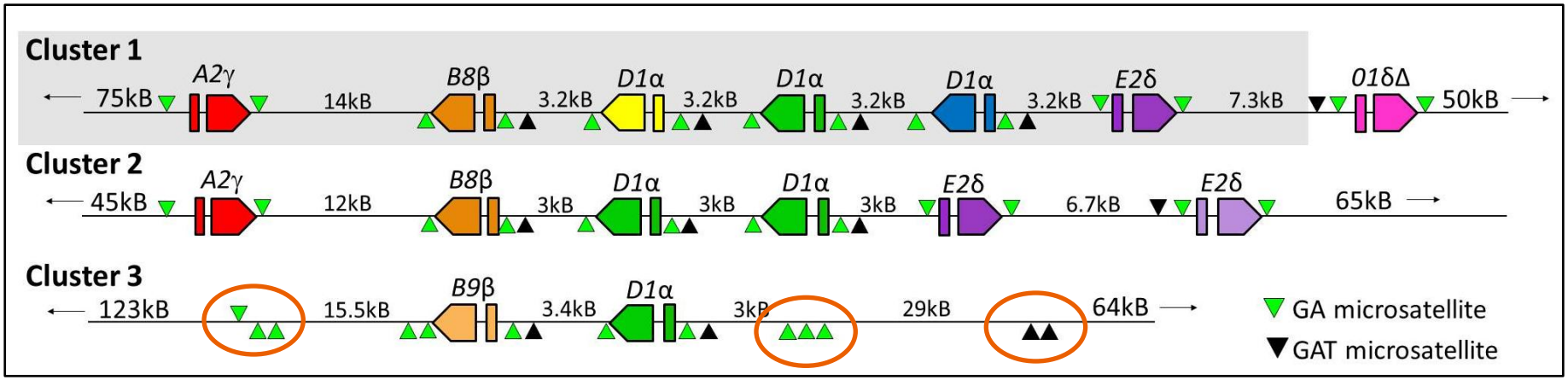
Gene family structure:

Seven ~~Six~~ linked *Sp185/333* genes in a BAC insert

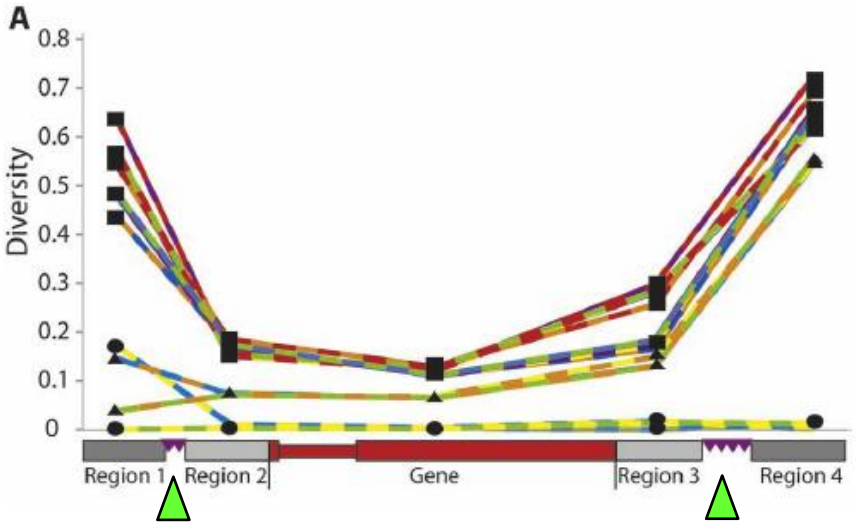


Miller et al., 2010

Three clusters of *Sp185/333* genes in different sea urchin BACs



Six genes in Cluster 1: Low sequence diversity between the GA microsatellites



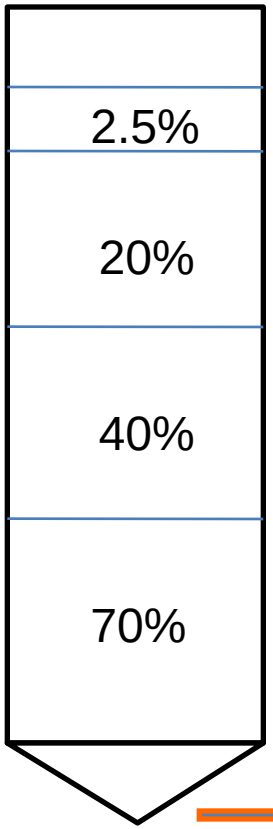
Miller et al., 2010

Oren, Barela Hudgell, Podini, Smith, *in prep*

How many *Sp185/333* genes are expressed in single phagocytes?

Enrichment of immune activated phagocytes by density centrifugation

Discontinuous Percoll fractions

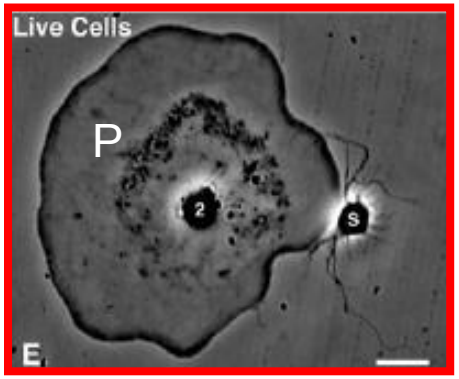


P,S → Polygonal cells = 75% – 85%
Small phagocytes = 10%

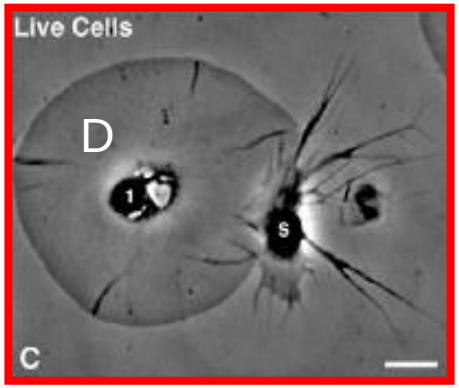
D,S → Discoidal cells = 90%
Small phagocytes = 5%

Vibratile cells = 66%
Red spherule cells = 20%

Red spherule cells = 85%
Vibratile cells = 10%



Brockton et al., 2008



90% cell viability based on propidium iodide exclusion

Majeske, Oren, et al., 2014

Limiting dilution, nested RT-PCR of single phagocytes

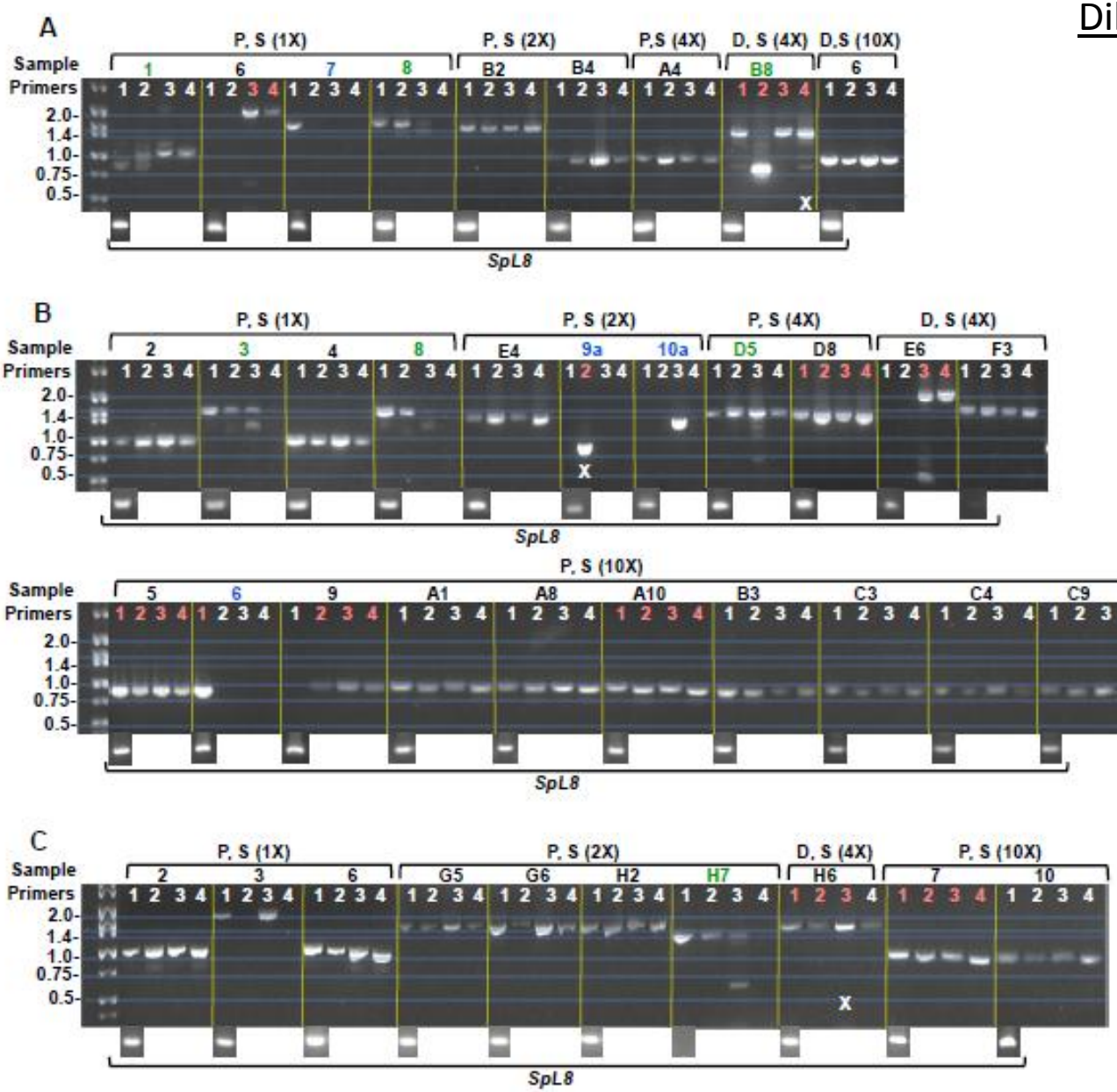
Sea urchin

11

17

17

26



Dilutions (cell/sample)

1X = 1

2X = 0.5

4X = 0.25

10X = 0.1

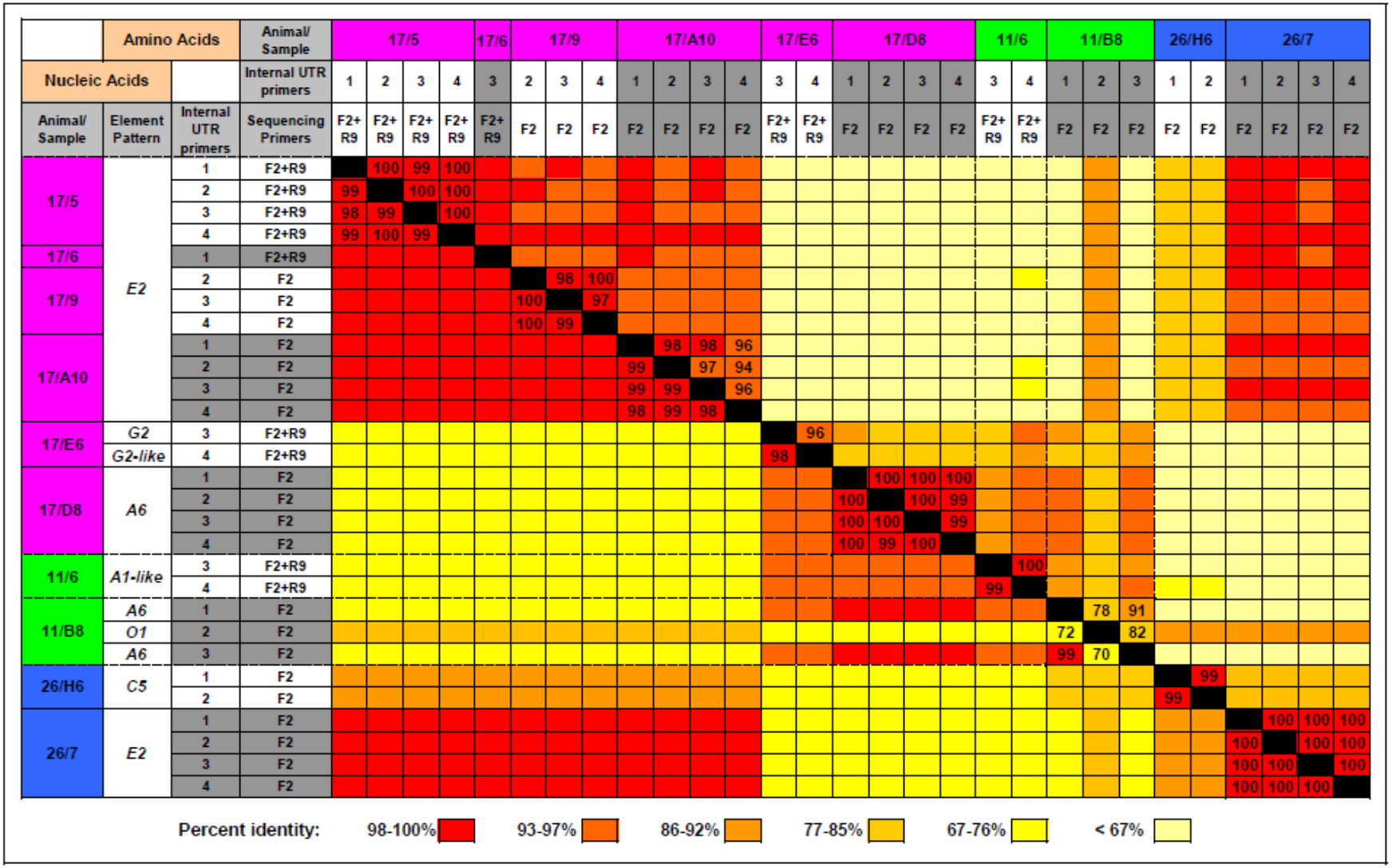
Sp185/333 Primers

One pair of external universal primers

Four pairs of internal primers (1-4)

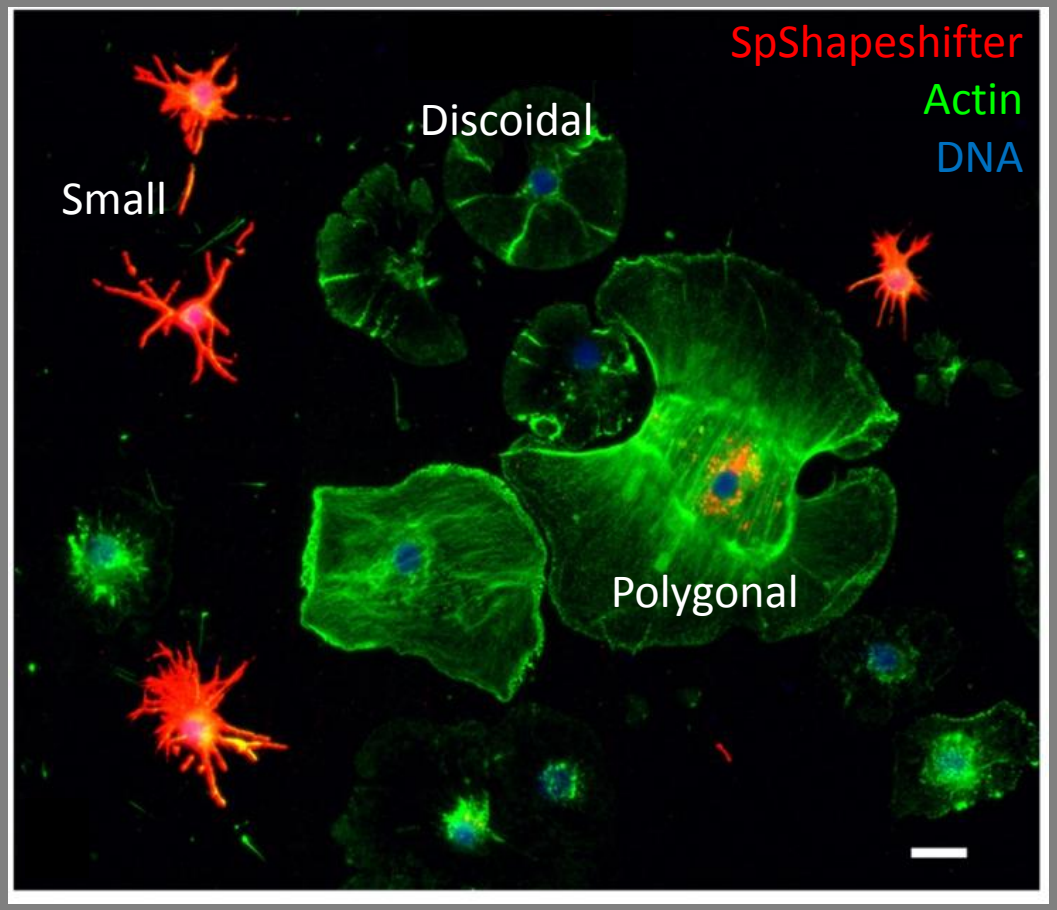
Majeske, Oren, et al., 2014

A single *Sp185/333* mRNA sequence per cell; single phagocytes express a single *Sp185/333* gene

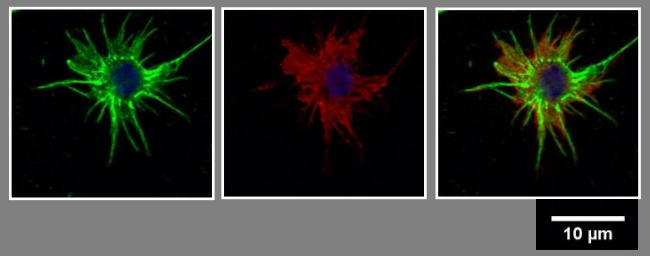


Majeske, Oren, et al., 2014

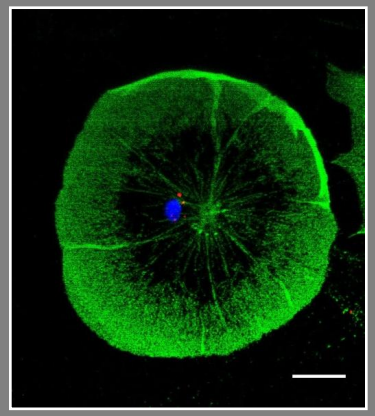
SpShapeshifter proteins are expressed by phagocytes



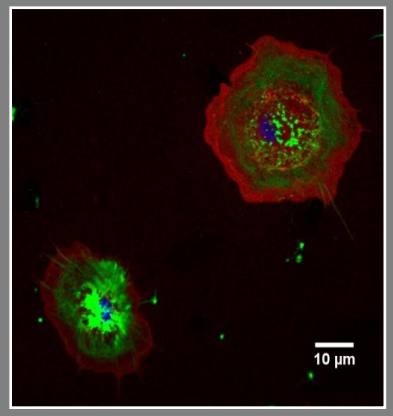
Small



Discoidal



Medium



transport vesicles
surface of small phagocytes

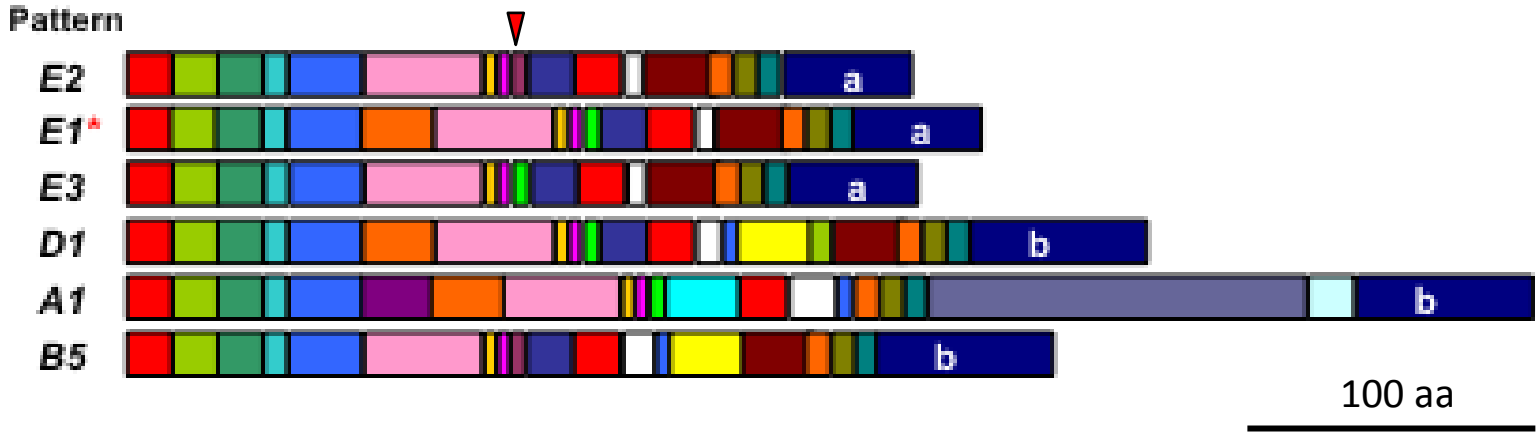
Brockton et al., 2008
Majeske, Oren, et al., 2014
Golconda & Smith, *unpub*

SpShapeshifter protein structure



- leader
- glycine-rich region
- RGD motif within the glycine-rich region
- histidine-rich region
- no amino acid sequence predictions for membrane association
- no cysteines

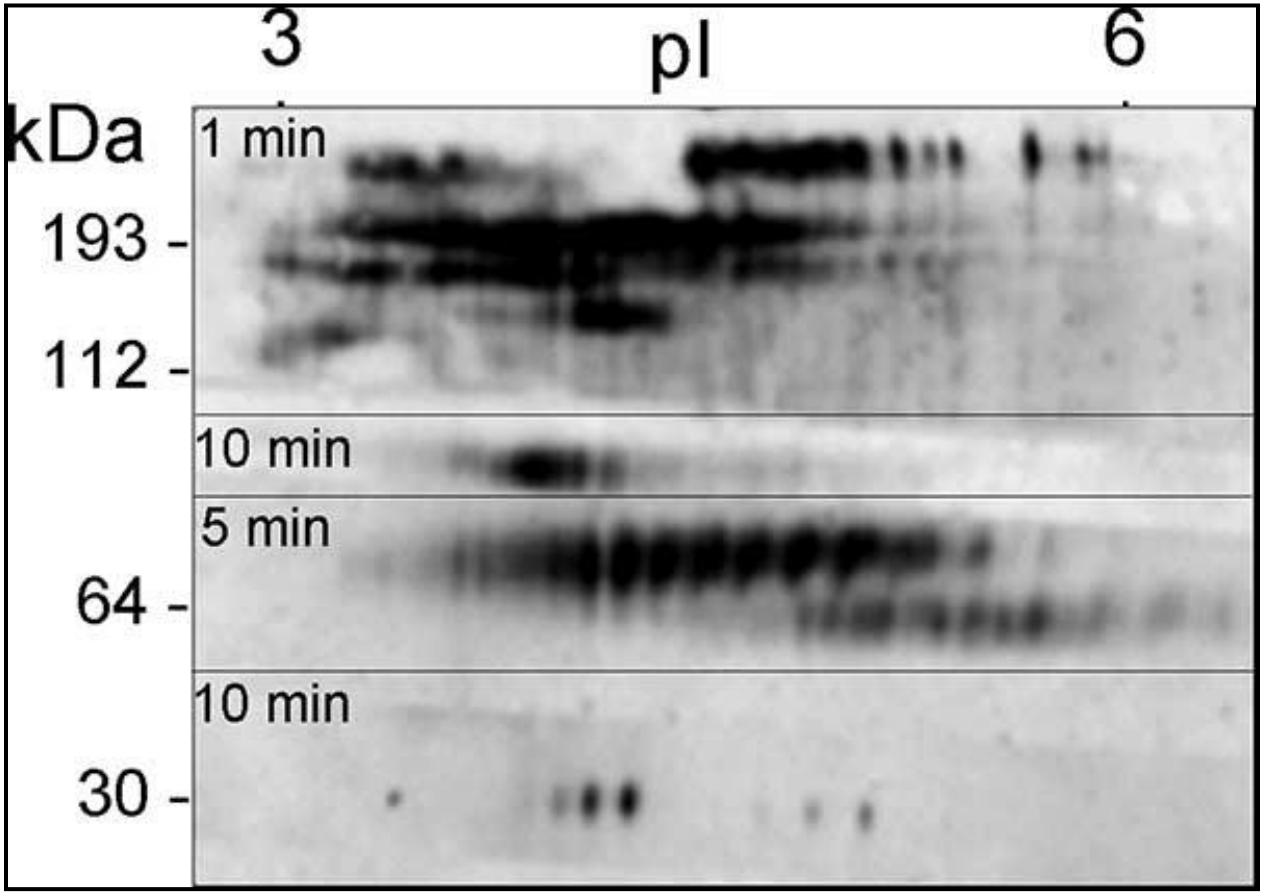
Diversity of the SpShapeshifter proteins



Diversity within and among animals

- mosaic of element patterns
  - broad size range
- sequence variations within elements among proteins
- mRNA editing
  - missense sequence
  - truncated proteins
- putative post translational processing

SpShapeshifter protein diversity in one animal

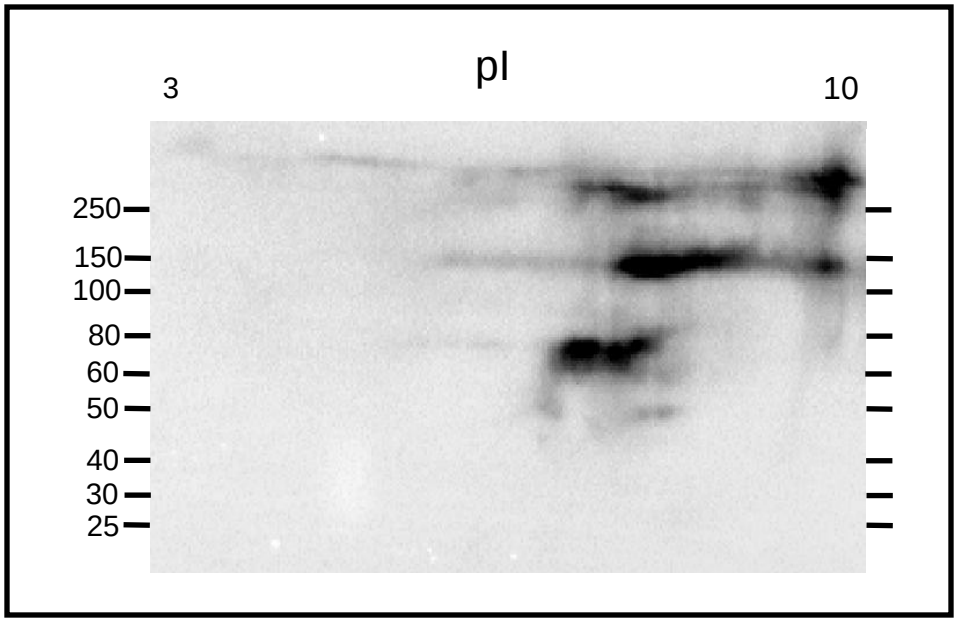


>260 SpShapeshifter spots

multimerization

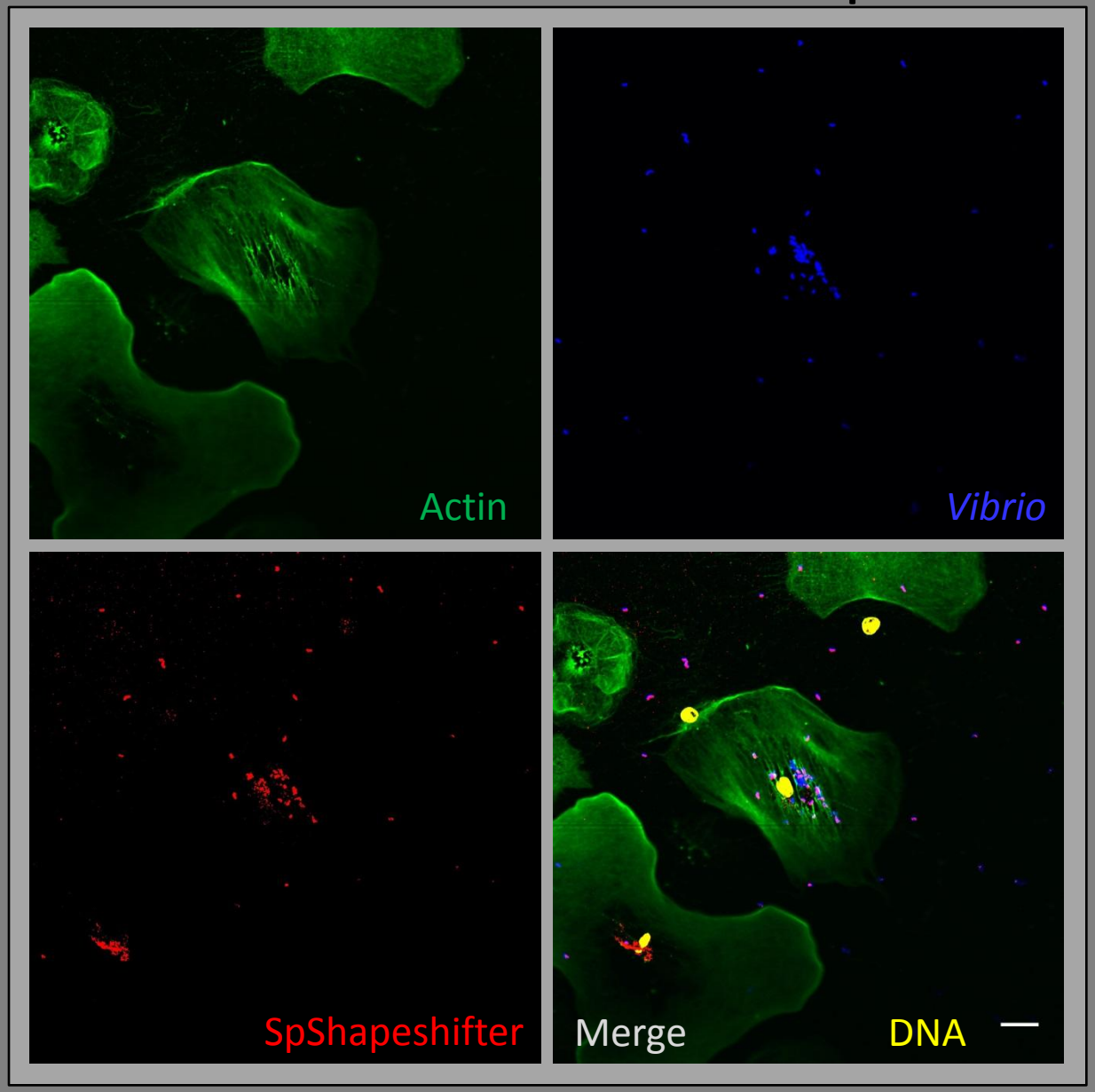
Dheilly et al., 2009

Native SpShapeshifter proteins can be isolated by nickel affinity based on the His-rich region



Sherman, Schrankel, Brown, Smith, *submitted*

SpShapeshifters are secreted and opsonize bacteria

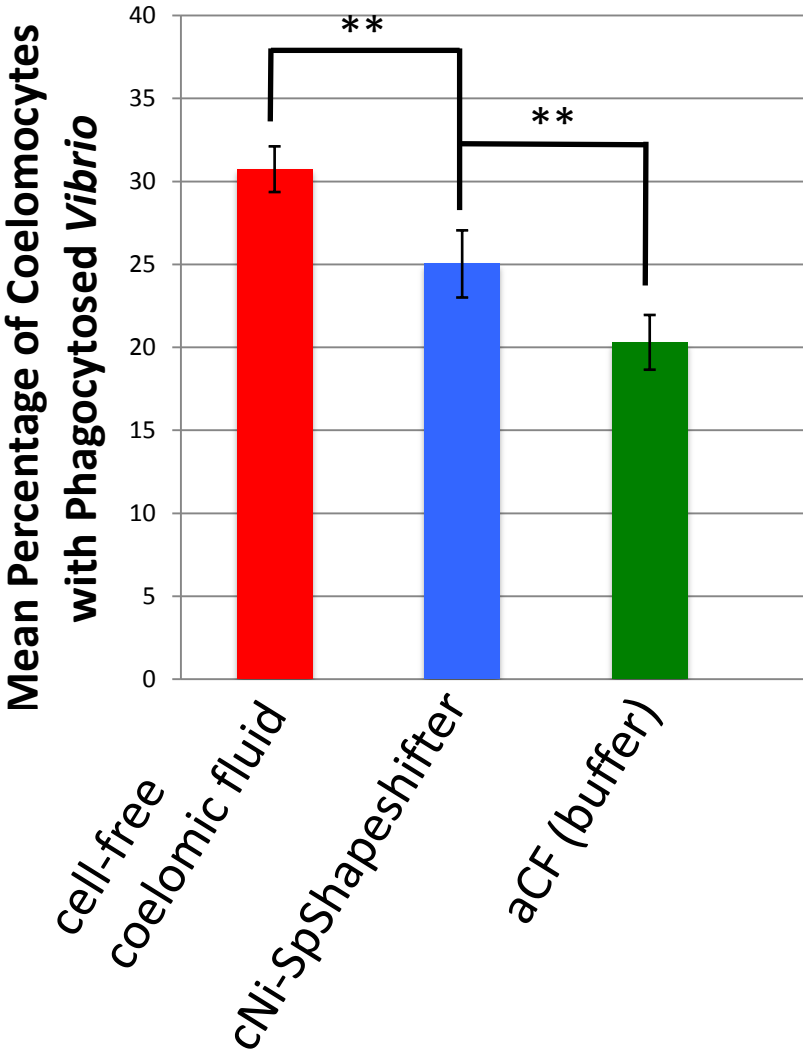


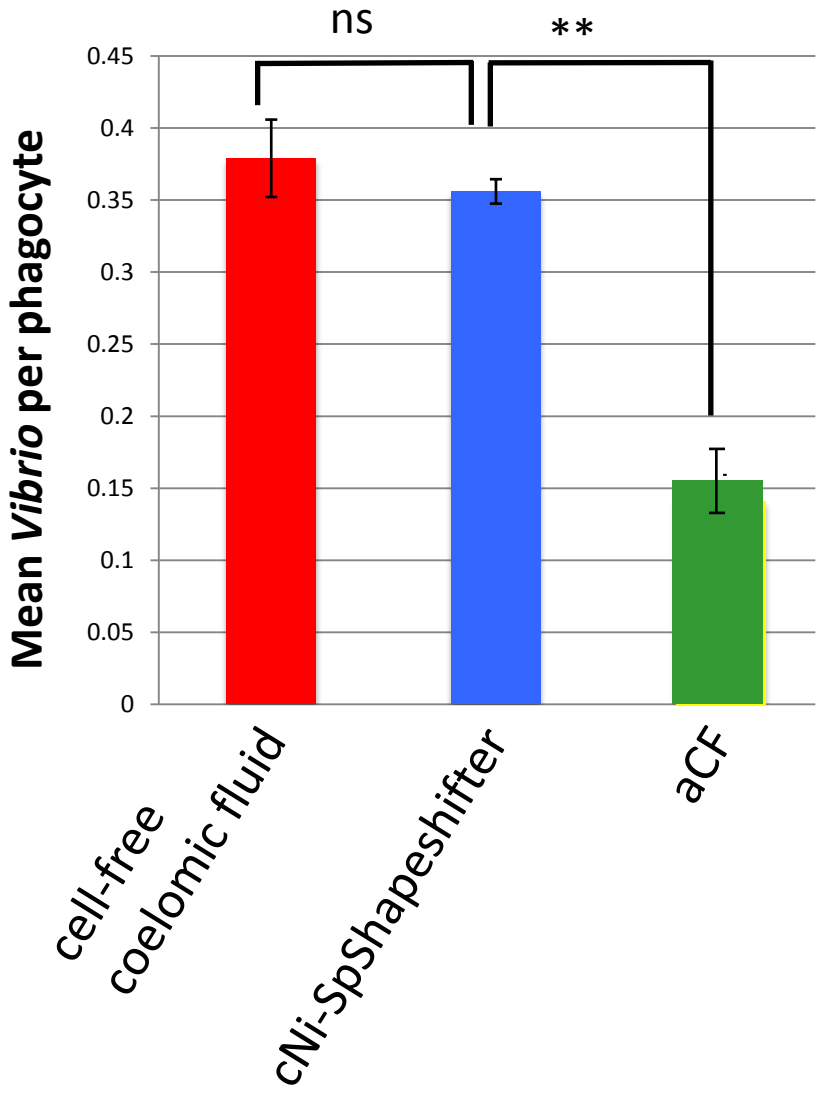
Chou & Smith, *unpub*



Chou & Smith, *unpub*

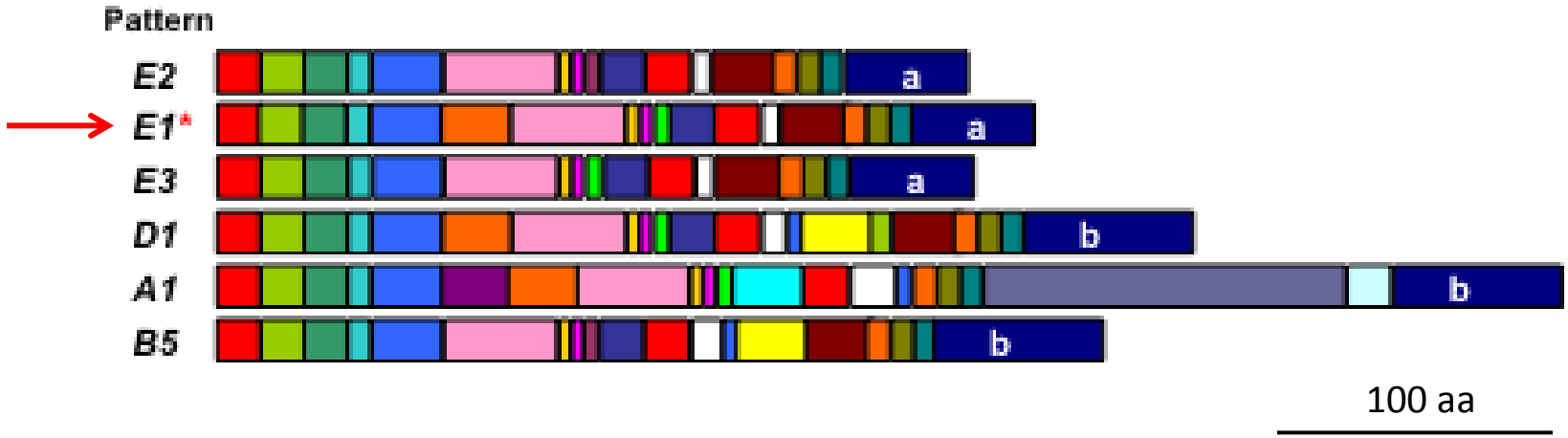
SpShapeshifter proteins opsonize *Vibrio* and induce phagocytosis





Chou & Smith, *unpub*

One recombinant SpShapeshifter protein can be expressed in *E. coli*



rSp0032 (= rSpTransformer)

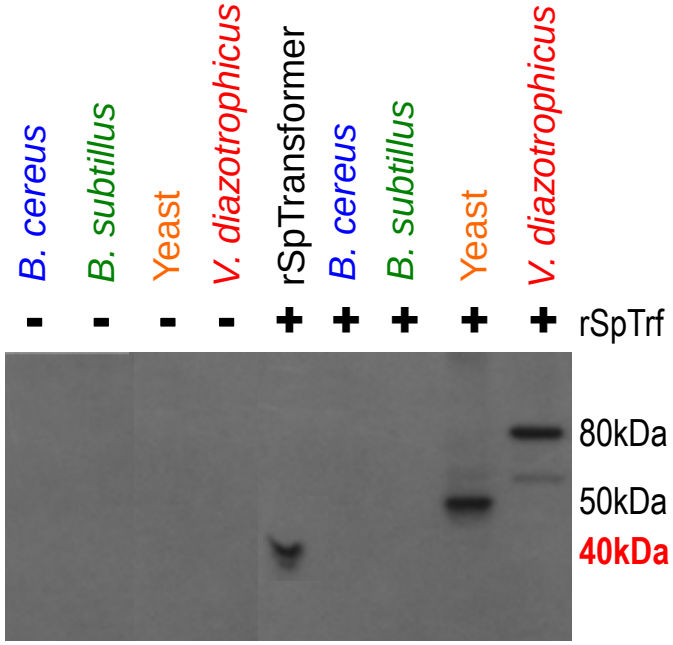
E1 element pattern

Could be expressed in bacteria

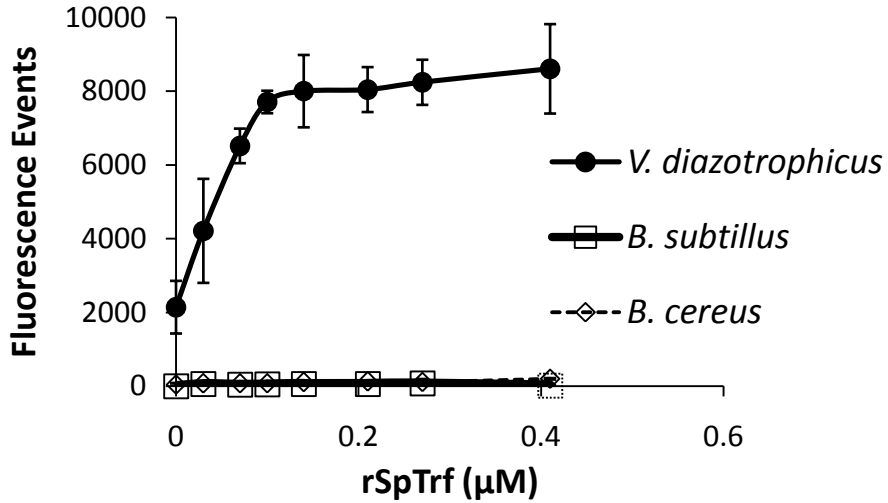
Not as lethal as other versions

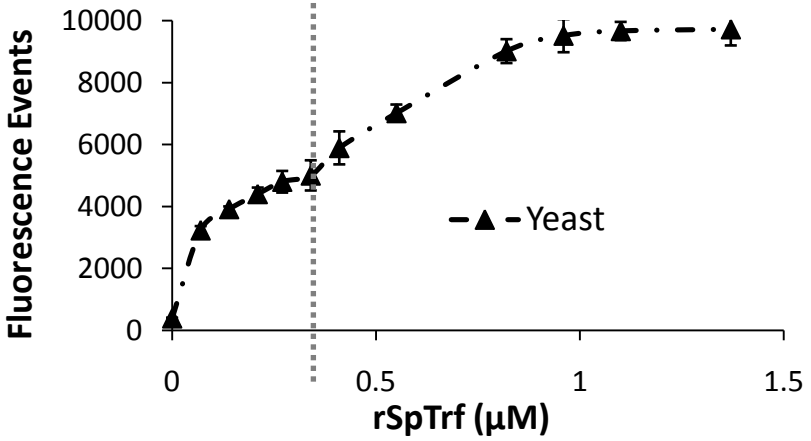
Lun, Schrankel, Chou, Sacchi, Smith, *unpub*

rSpTransformer binds to *Vibrio* and yeast but not *Bacillus*



Bacillus cereus (Marine Gm positive)
B. subtillus (Soil Gm positive)
Vibrio diazotrophicus (Marine Gm negative)
Baker's yeast



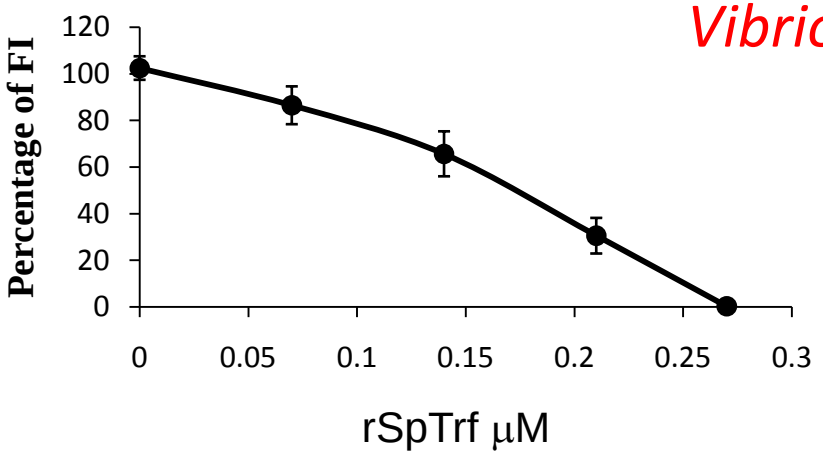


Lun, Schrankel, Chou, Sacchi, Smith, *unpub*

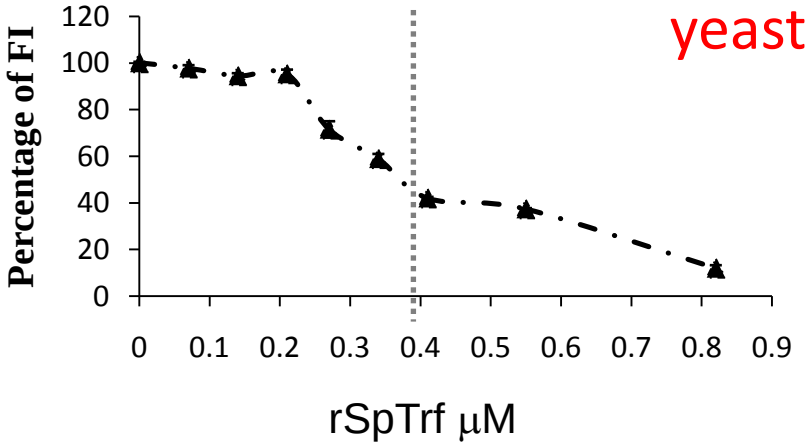
rSpTransformer binds specifically and with high affinity

Competition

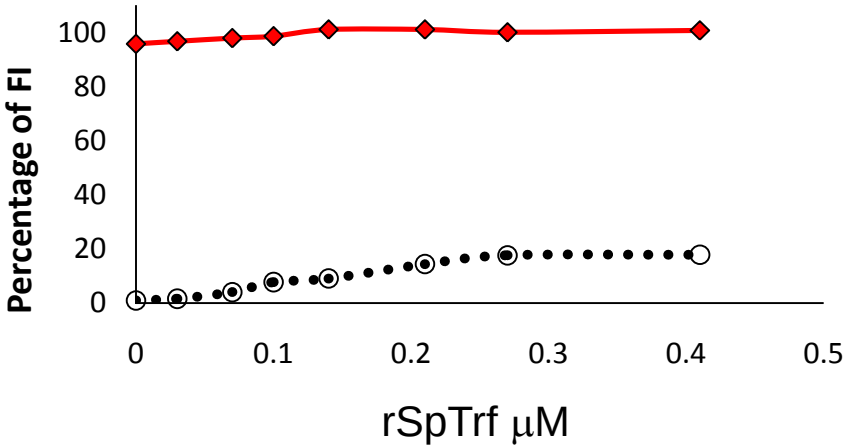
Vibrio

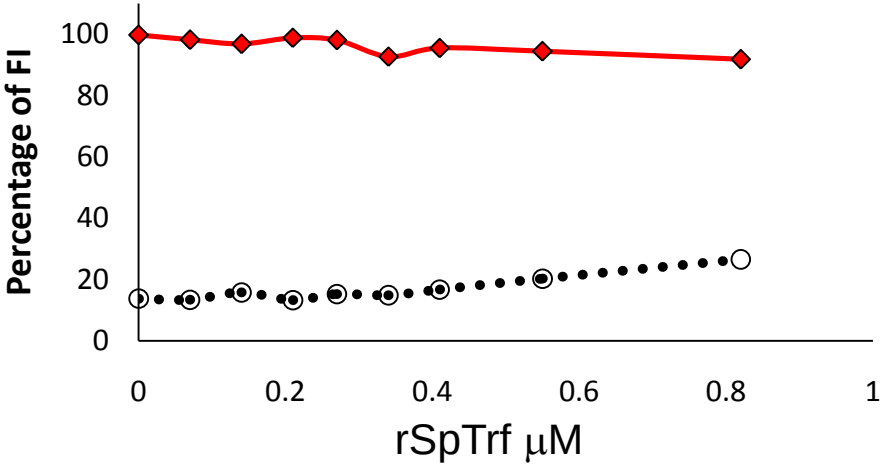


yeast



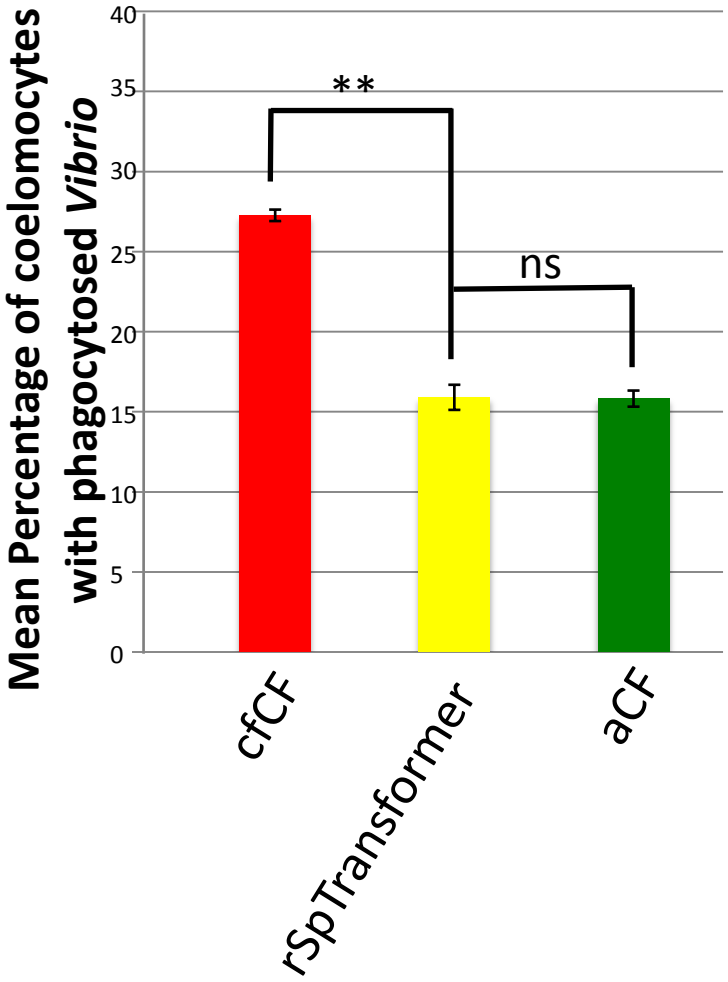
Displacement

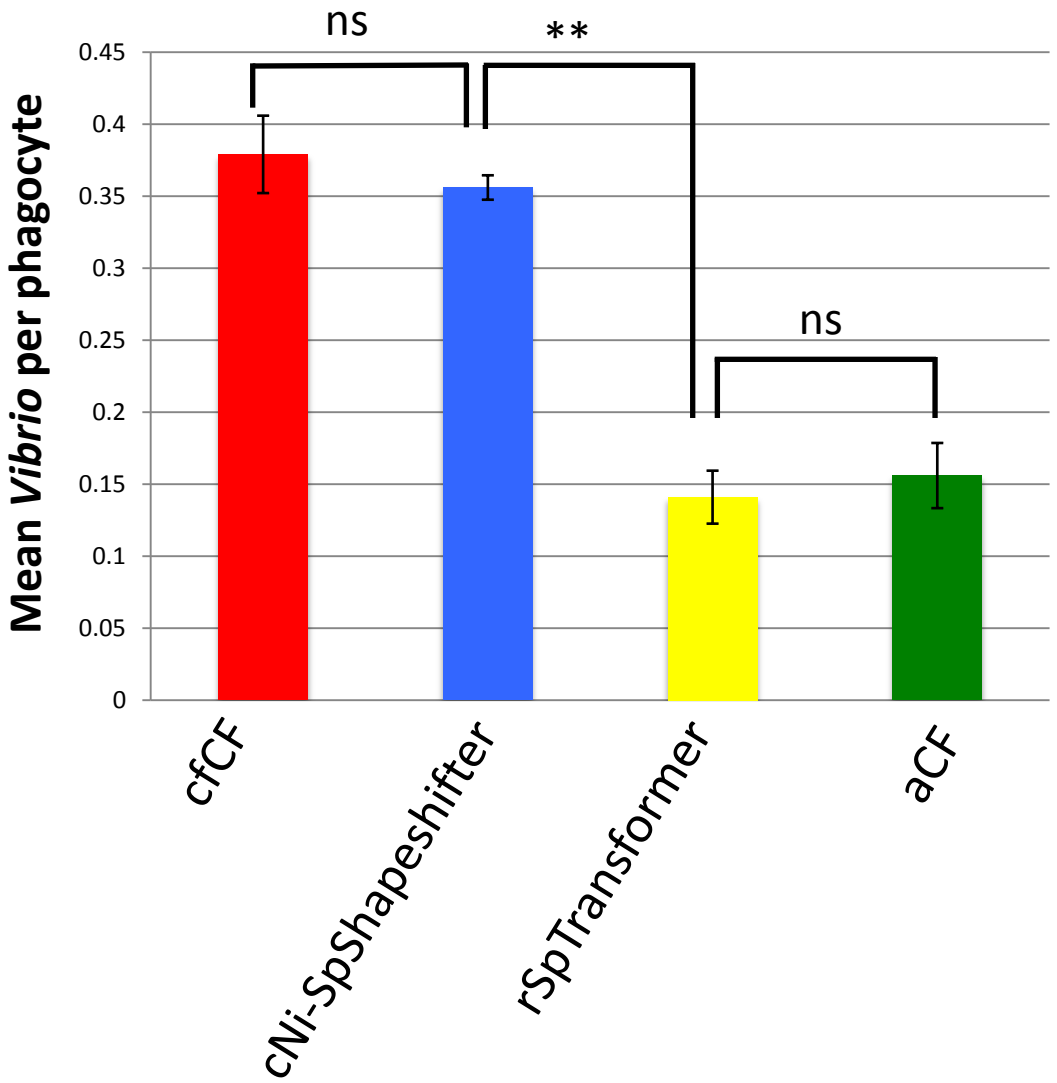




Lun, Schrankel, Chou, Sacchi, Smith, *unpub*

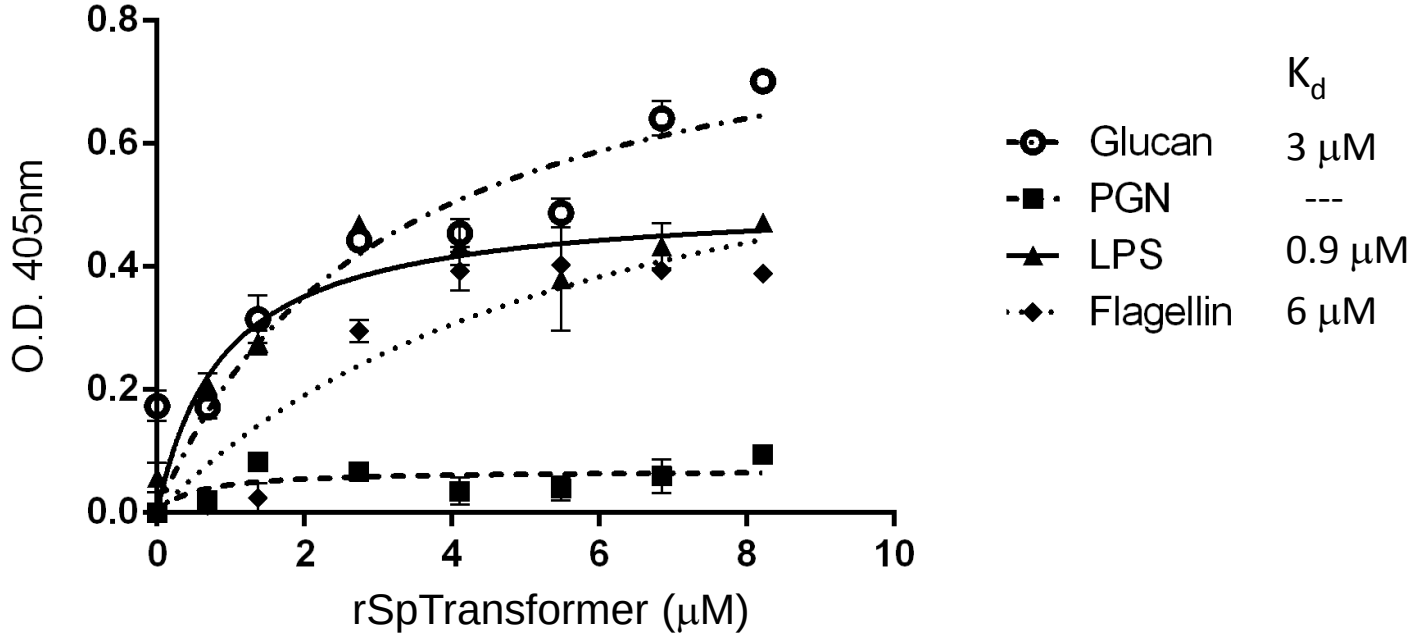
rSpTransformer does not augment phagocytosis of opsonized *Vibrio*





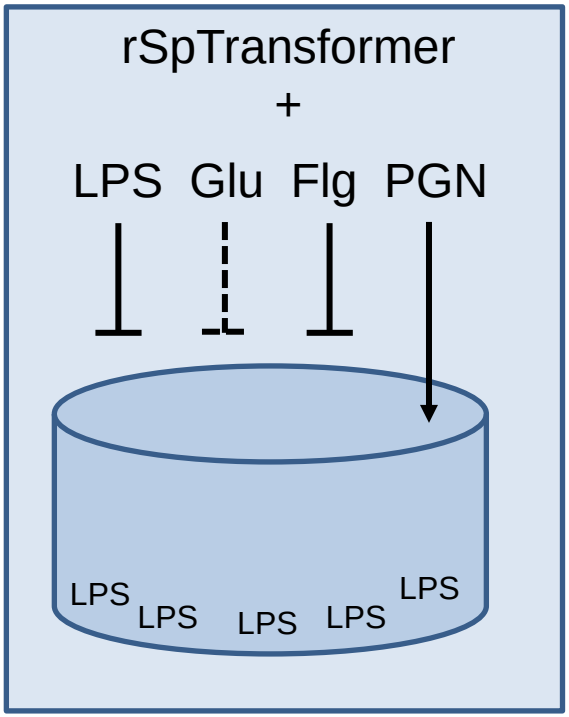
Chou, Smith, *unpub*

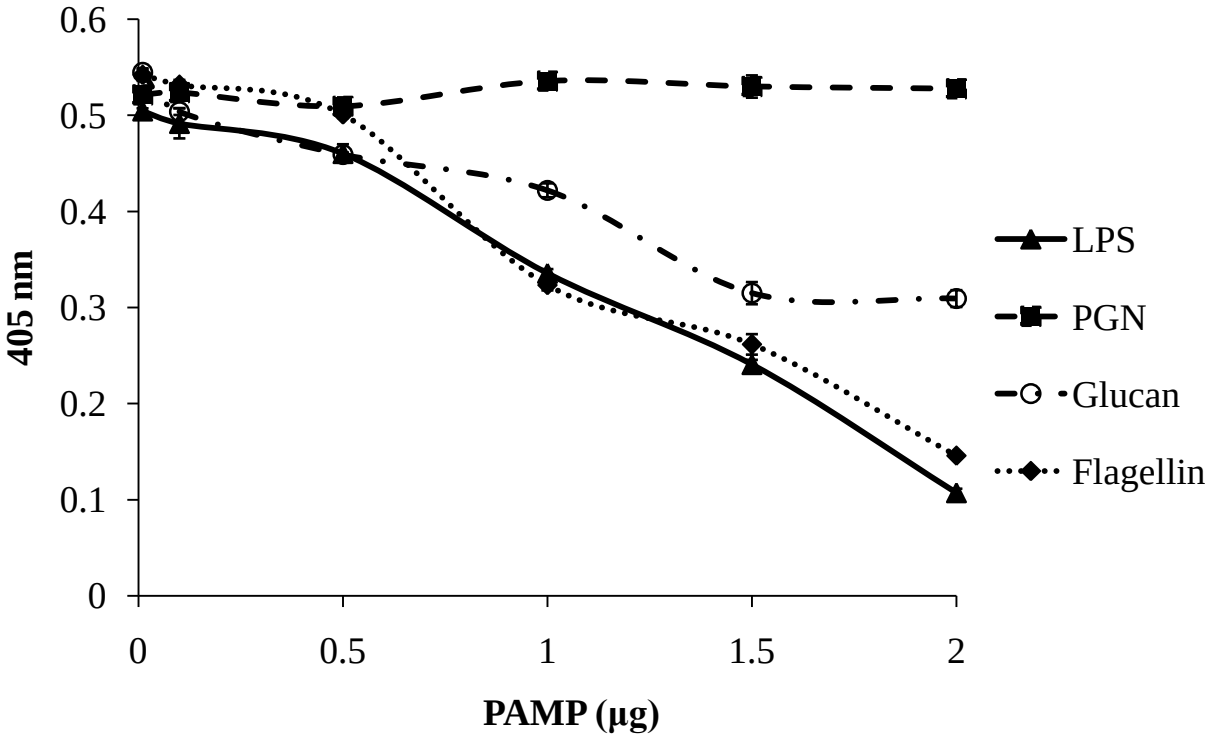
rSpTransformer binds to LPS, β,1-3,glucan and flagellin, but not PGN



Lun, Schrankel, Chou, Sacchi, Smith, *unpub*

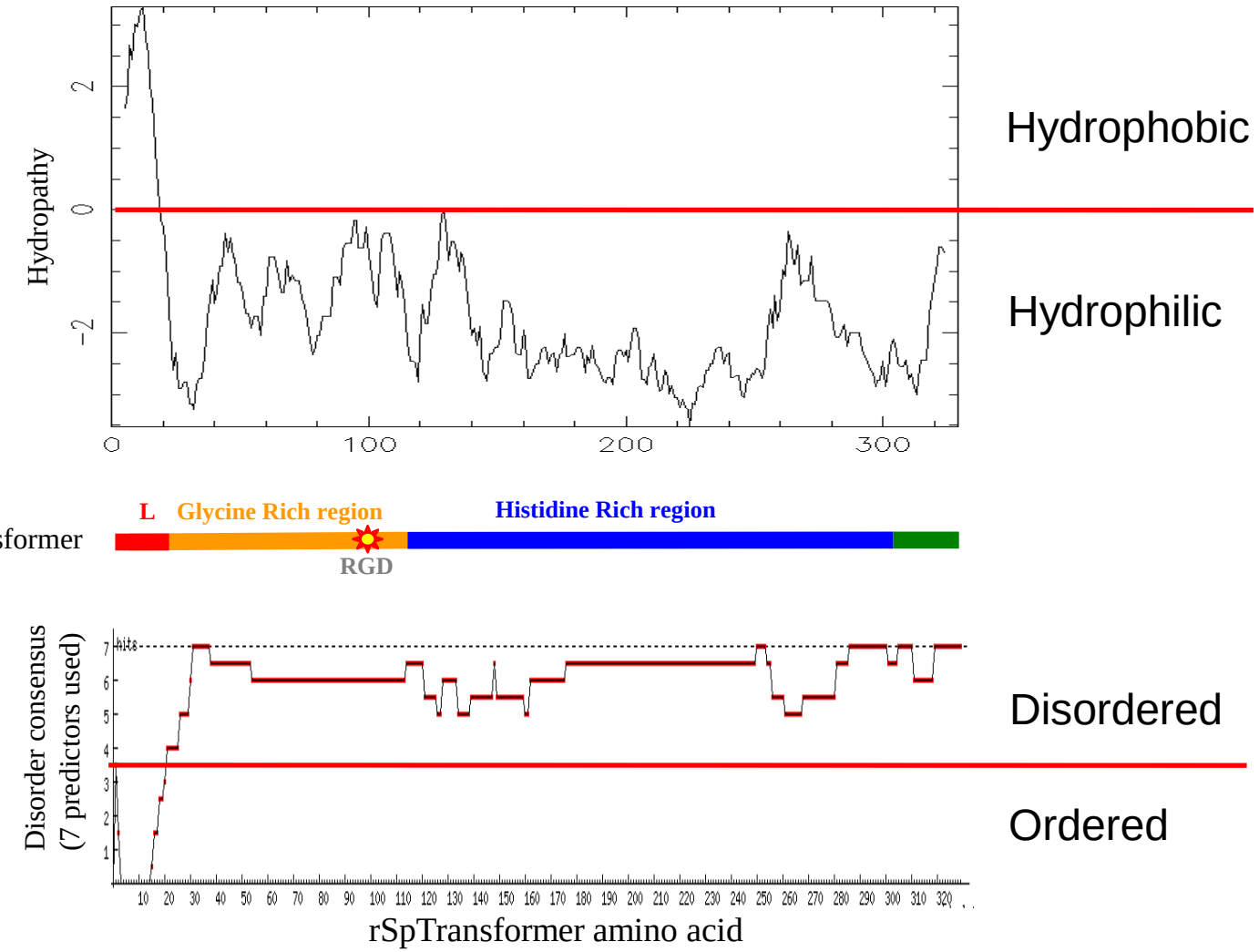
LPS, flagellin and β,1-3,glucan compete for rSpTransformer binding





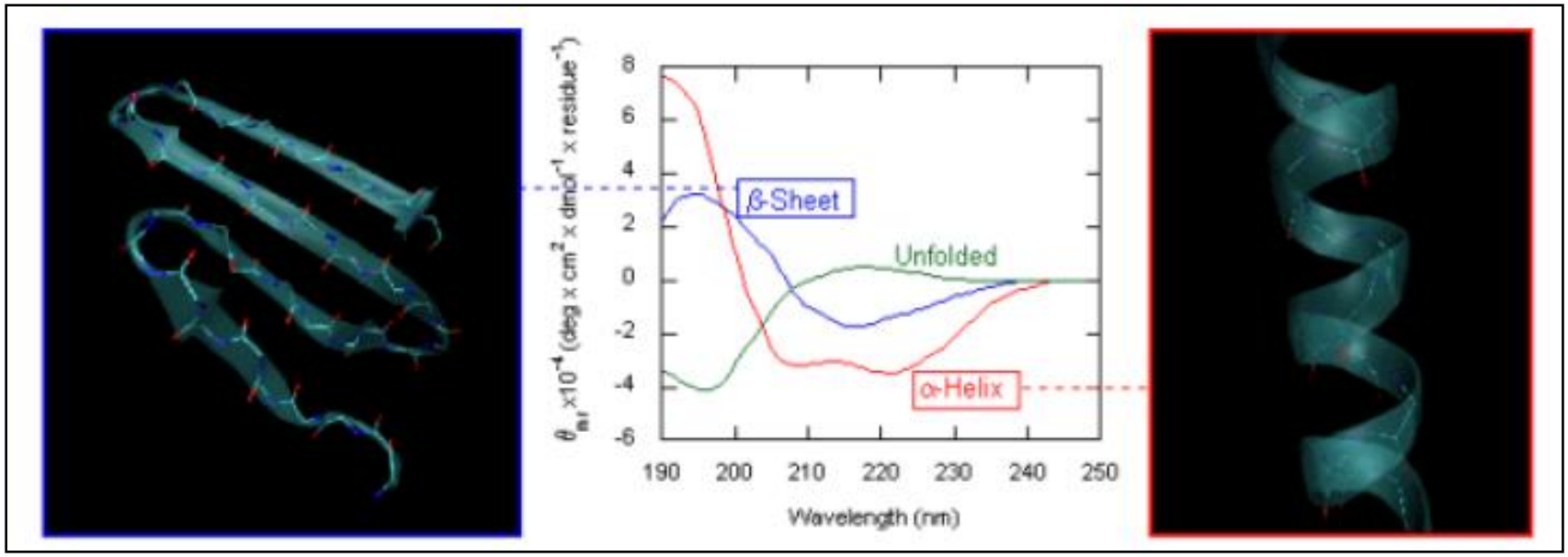
Lun, Schrankel, Chou, Sacchi, Smith, *unpub*

rSpTransformer is predicted to be hydrophilic and intrinsically disordered

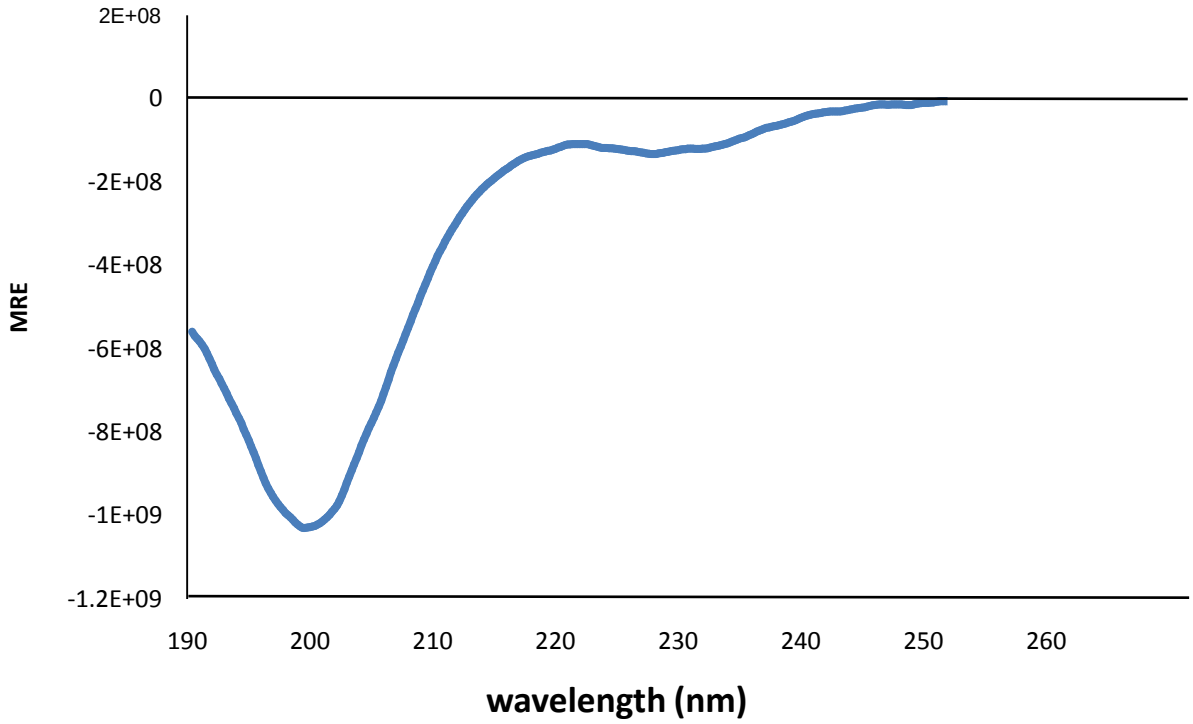


Lun, Bishop, Smith, *unpub*

Secondary structure of proteins by Circular Dichroism

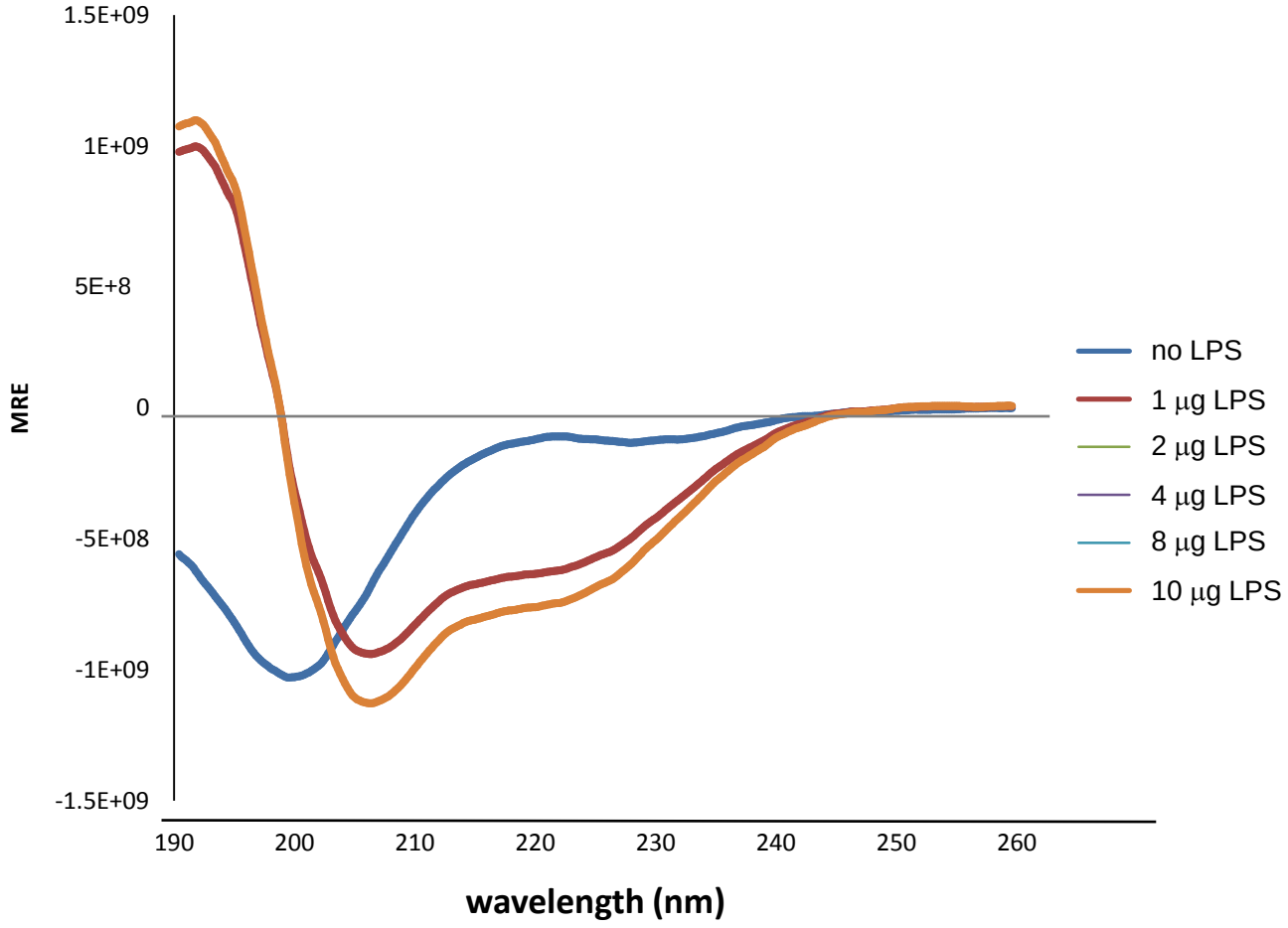


rSpTransformer structure is disordered



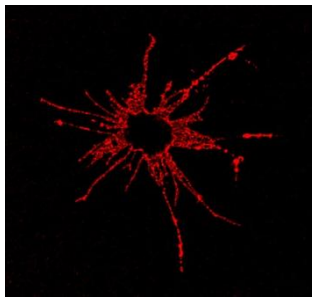
Lun, Bishop, Smith, *unpub*

rSpTransformer plus LPS; transforms from disordered to α helical



Lun, Bishop, Smith, *unpub*

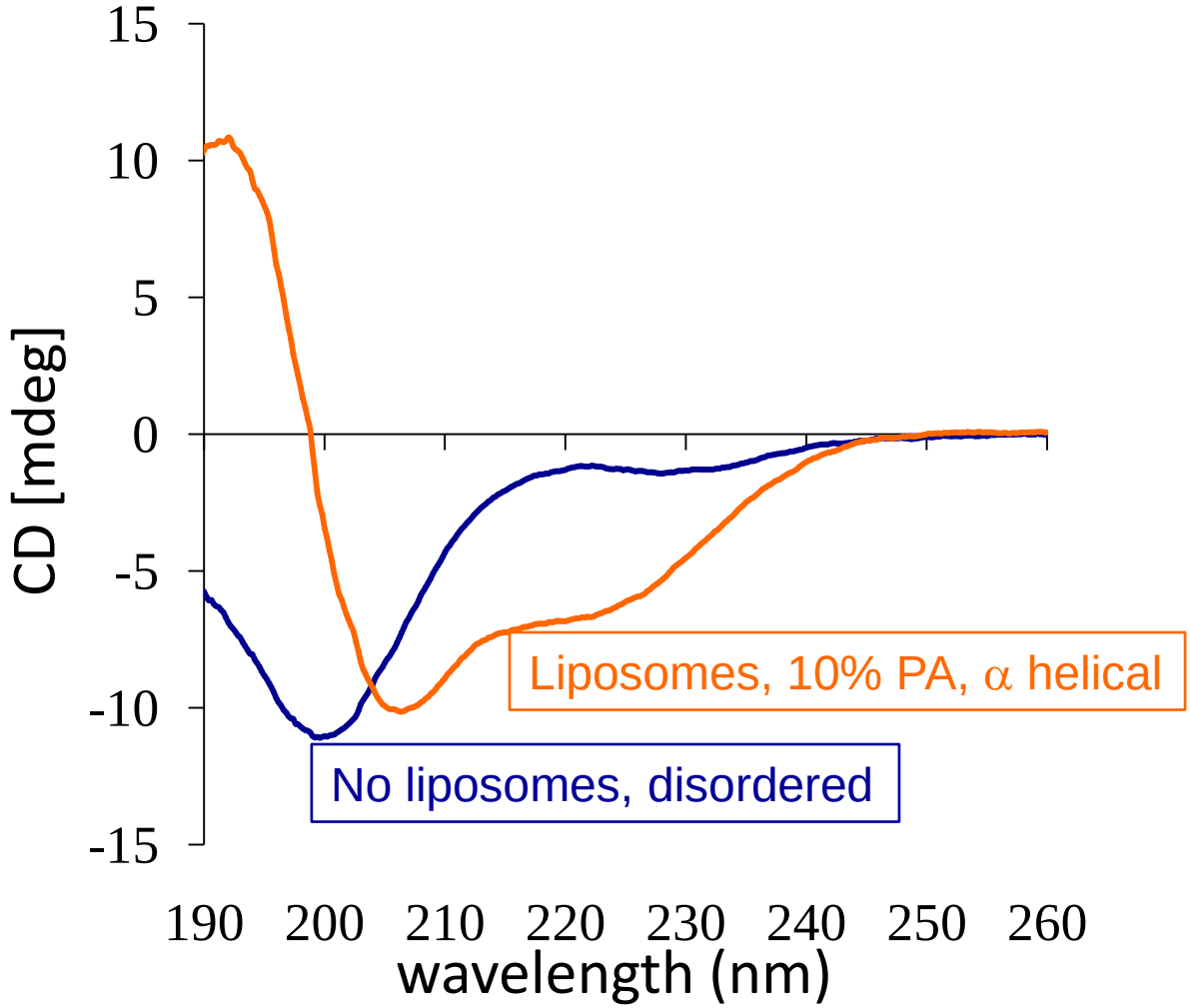
SpShapeshifter proteins on small phagocytes



Small phagocyte;
SpShapeshifter proteins
are on the membrane
from Brockton et al., 2008

Lun, Samuel, Boyd, Bishop, Gillmor, Smith, *unpub*

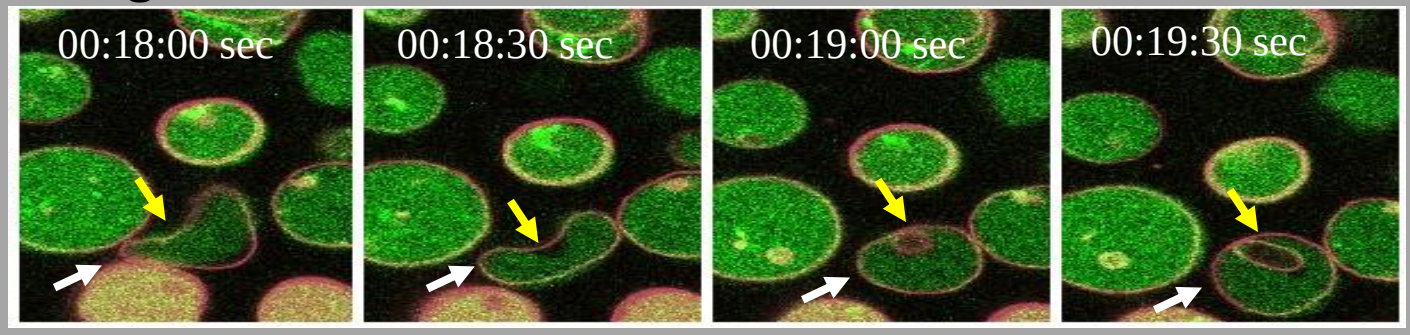
rSpTransformer binds to liposomes with 10% PA; transforms from disordered to α helical



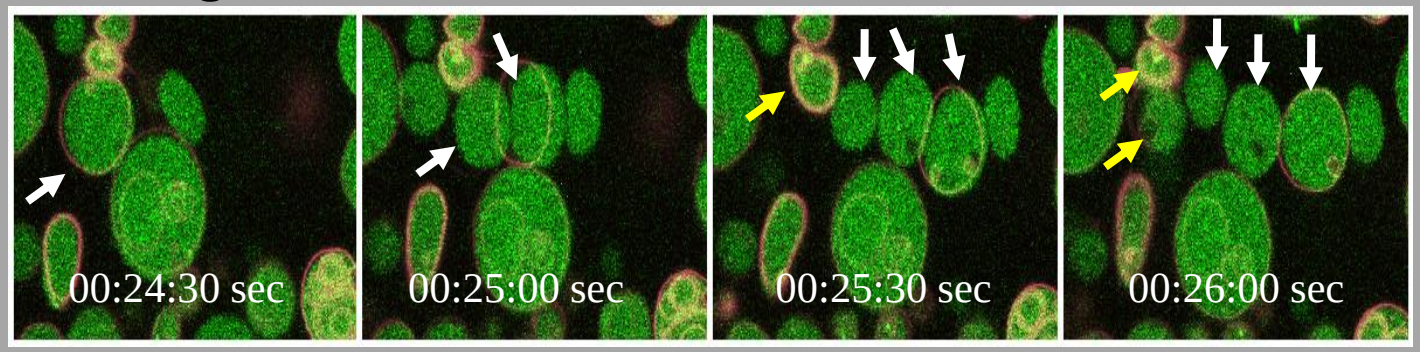
Lun, Samuel, Boyd, Bishop, Gillmor, Smith, *unpub*

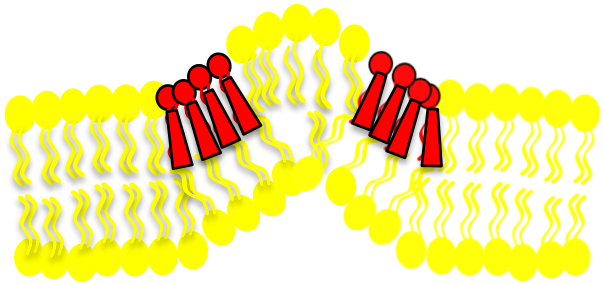
rSpTransformer alters liposome shape

Invagination



Budding





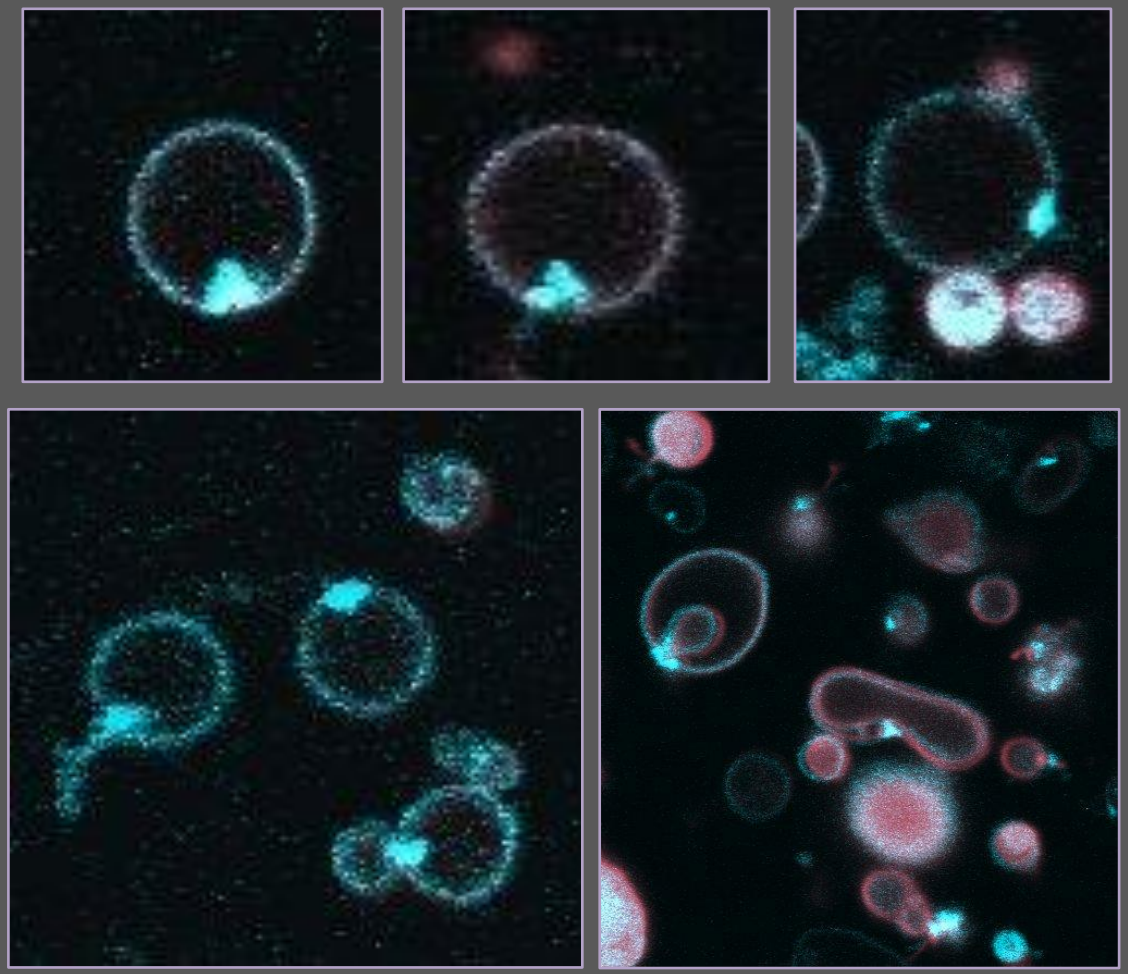
PA clusters may induce membrane curvature

Lun, Samuel, Boyd, Bishop, Gillmor, Smith, *unpub*

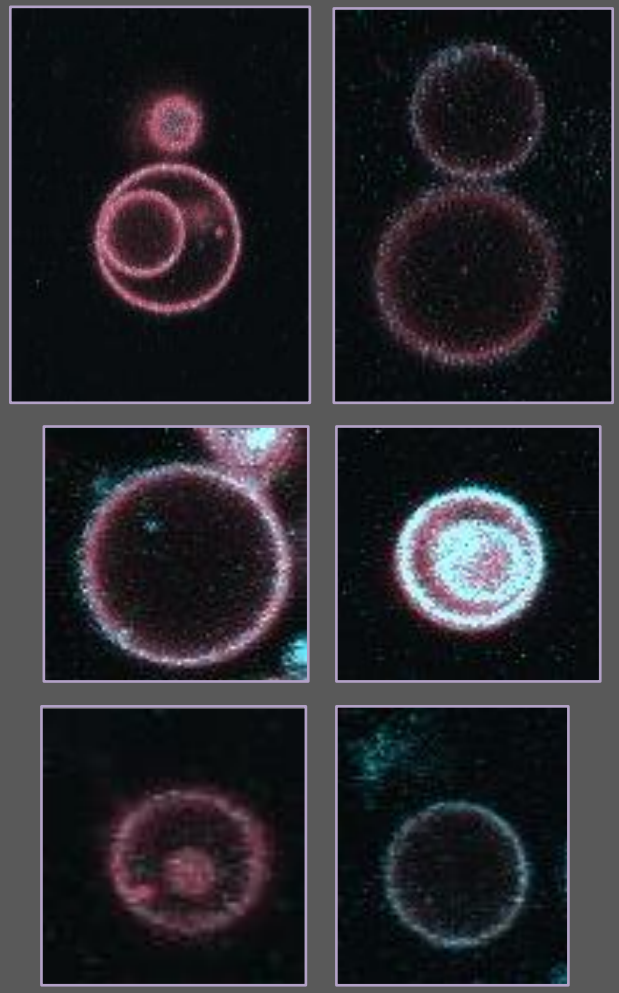
PA clusters in liposomes in the presence of rSpTransformer

10 μM rSpTransformer (30 min)

PA Dye I



No rSpTransformer

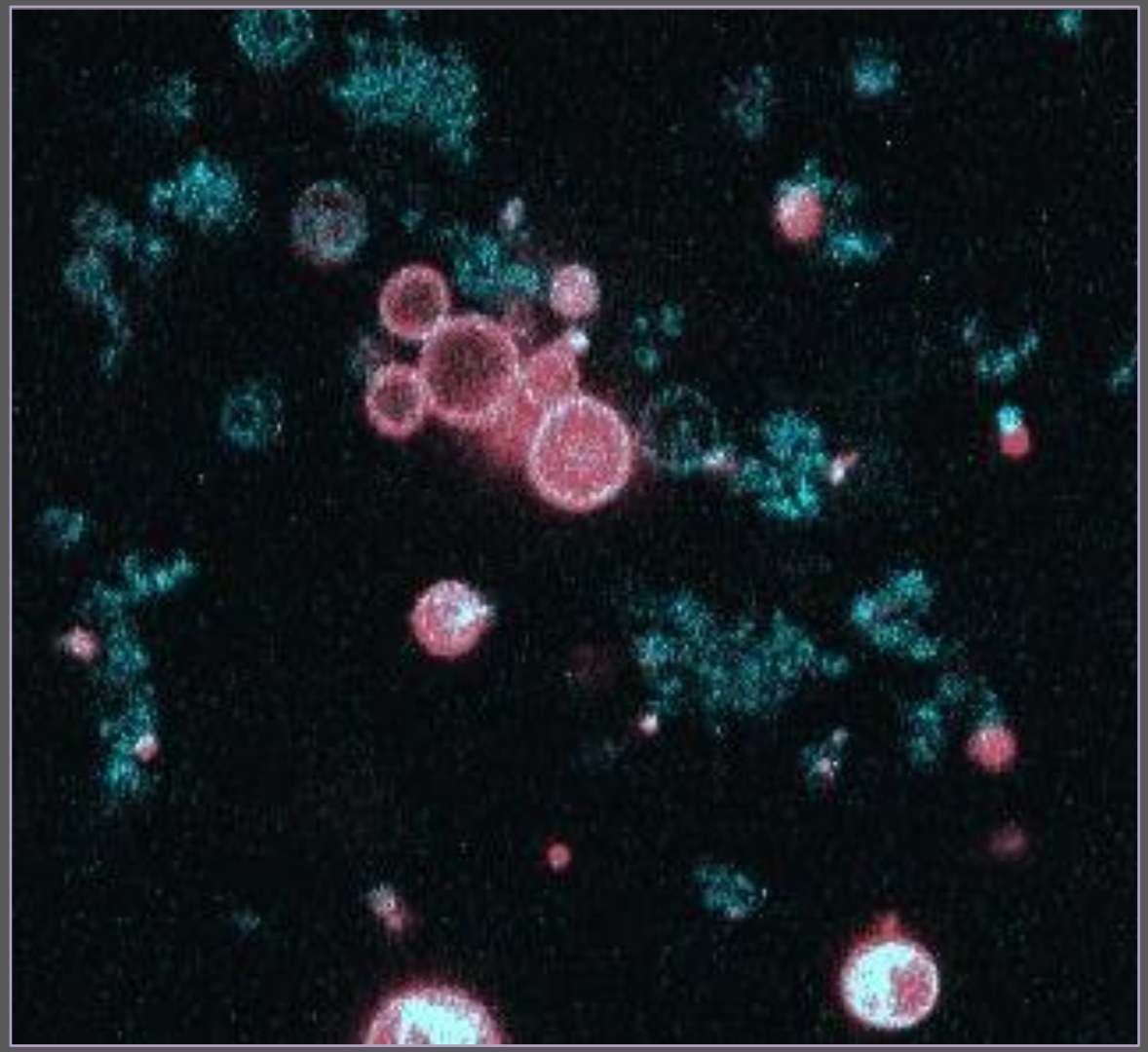


Lun, Samuel, Boyd, Bishop, Gillmor, Smith, *unpub*

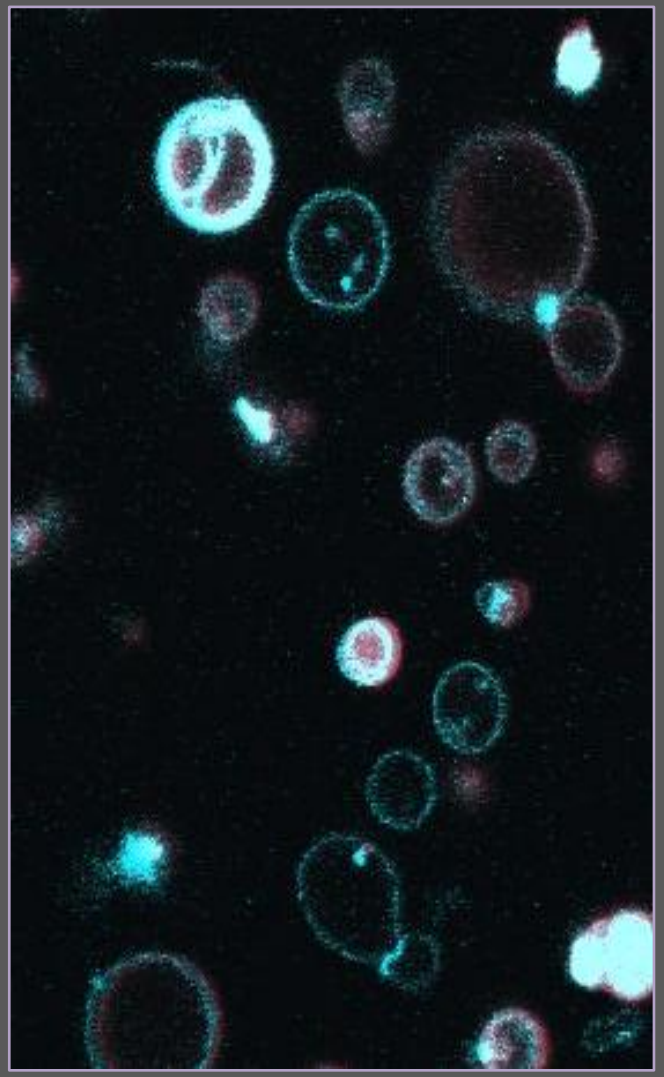
PA is extracted from liposomes in the presence of rSpTransformer

10 μM rSpTransformer (2 hrs)

PA Dye I

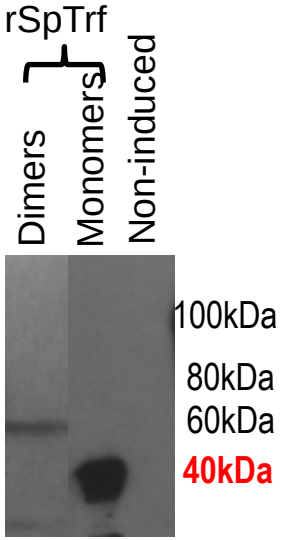


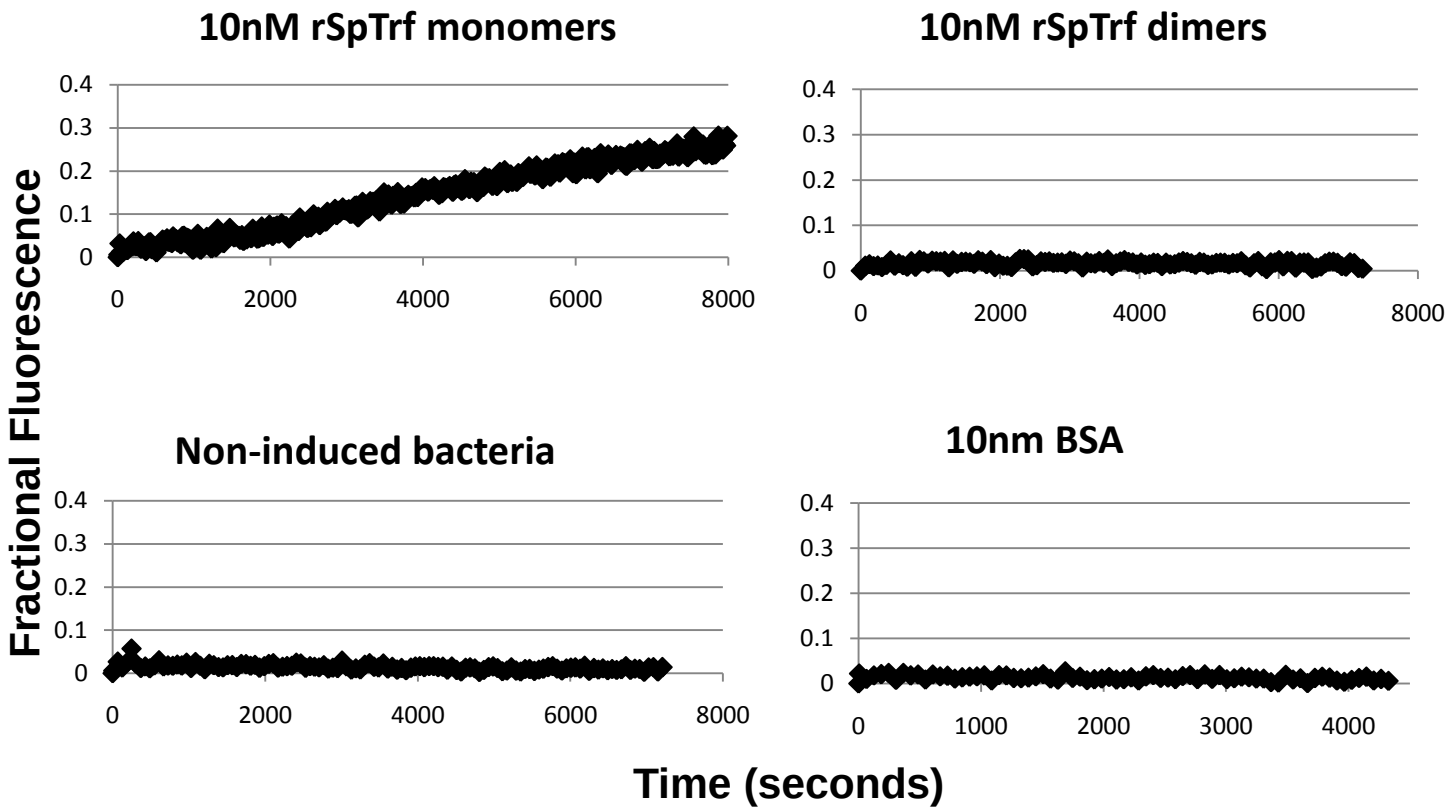
No rSpTransformer



Lun, Samuel, Boyd, Bishop, Gillmor, Smith, *unpub*

rSpTransformer induces liposome leakage





Liposome
10% PA

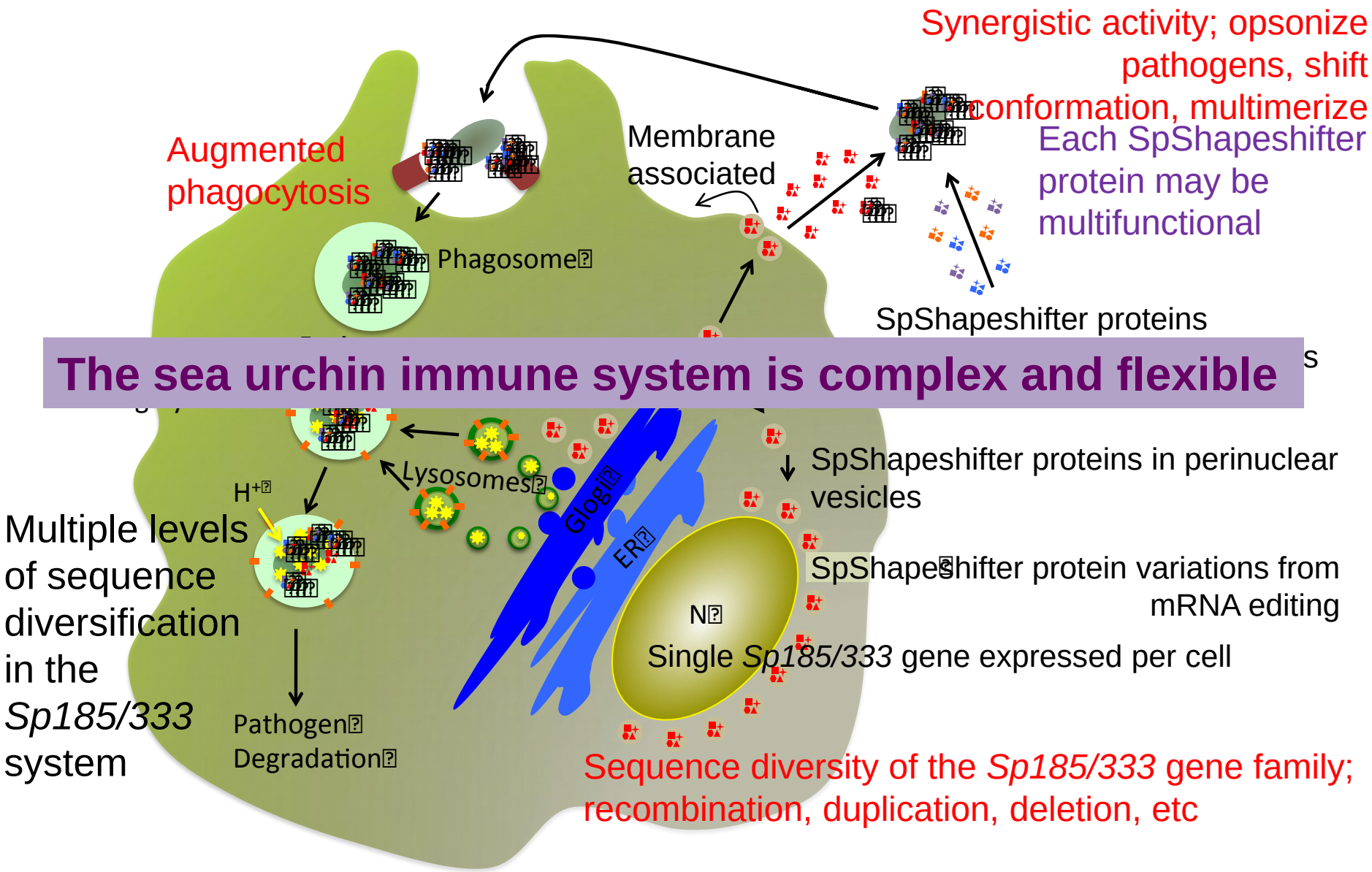
Dye plus
Quencher

rSpTransformer

Dye released from Quencher

Lun, Samuel, Boyd, Bishop, Gillmor, Smith, *unpub*

Summary: SpShapeshifter Diversity



from Chou & Smith, *unpub*

Acknowledgements

George Washington University

Biology

- David Terwilliger
- Katherine Buckley
- Chase Miller
- Catherine Schrankel
- Virginia Brockton
- Bonnie Lun
- Peter Chou
- Anthony Boyd
- Sandro Sacchi
- Audrey Majeske
- Matan Oren
- Megan Barela Hudgell

Chemistry

- Robyn Samuel
- Susan Gillmor

George Mason University

- Barney Bishop

Dickinson College

- John Henson

Chinese Academy of Sciences

- Limei Qiu

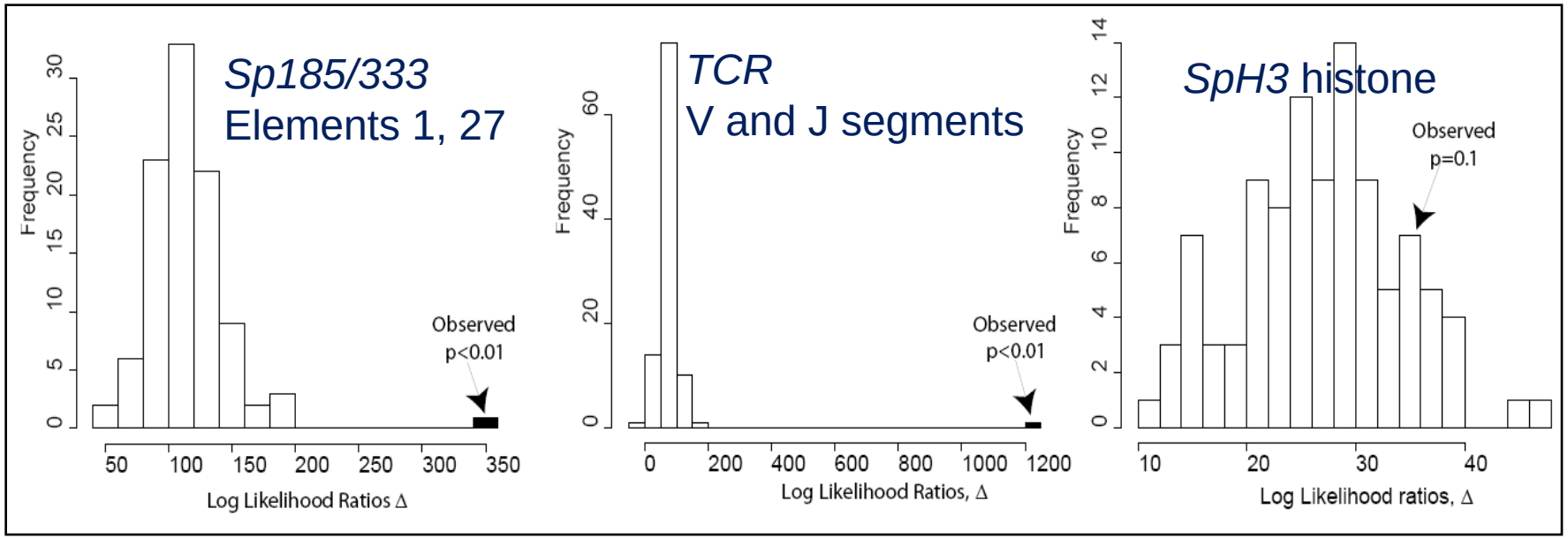


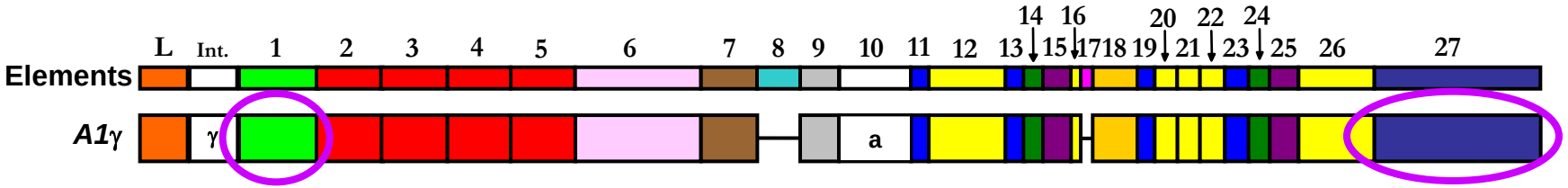
Sea Urchin Immunology is funded by the US National Science Foundation



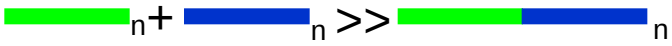
http://biology.columbian.gwu.edu/l-courtney-smith

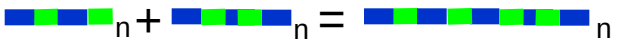
Gene recombination among elements





Incongruence (phylogenetic tree) length difference





Buckley et al., 2008a

Recombination may occur at any point within the gene

	L	Int a	Int b	1	6	6a	6b	26	27	27a	27b	H3.1	H3.2	TcRV	TcRJ
L		***	***	**	**	.	.	***	***	.	.	.	.	.	.
Int a	**		***	**	***	.	.	***	***	.	.	.	.	.	.
Int b	**	**		ns	***	.	.	***	***	.	.	.	.	.	.
1	**	**	*		***	.	.	***	***	.	.	.	.	.	.
6	*	**	**	**		.	.	***	***	.	.	.	.	.	.
6a	.	.	.	.	.		*	.	.	.	.	.	.	.	.
6b	.	.	.	.	.	*		.	.	.	.	.	.	.	.
26	**	**	**	**	**	.	.		***	.	.	.	.	.	.
27	**	**	**	**	**	.	.	*		.	.	.	.	.	.
27a	.	.	.	.	.	.	.	.	.		*	.	.	.	.
27b	.	.	.	.	.	.	.	.	.	**		.	.	.	.
H3.1	.	.	.	.	.	.	.	.	.	.	.		ns	.	.
H3.2	.	.	.	.	.	.	.	.	.	.	.	ns		.	.
TcRV	.	.	.	.	.	.	.	.	.	.	.	.	.		***
TcRJ	.	.	.	.	.	.	.	.	.	.	.	.	.	**	

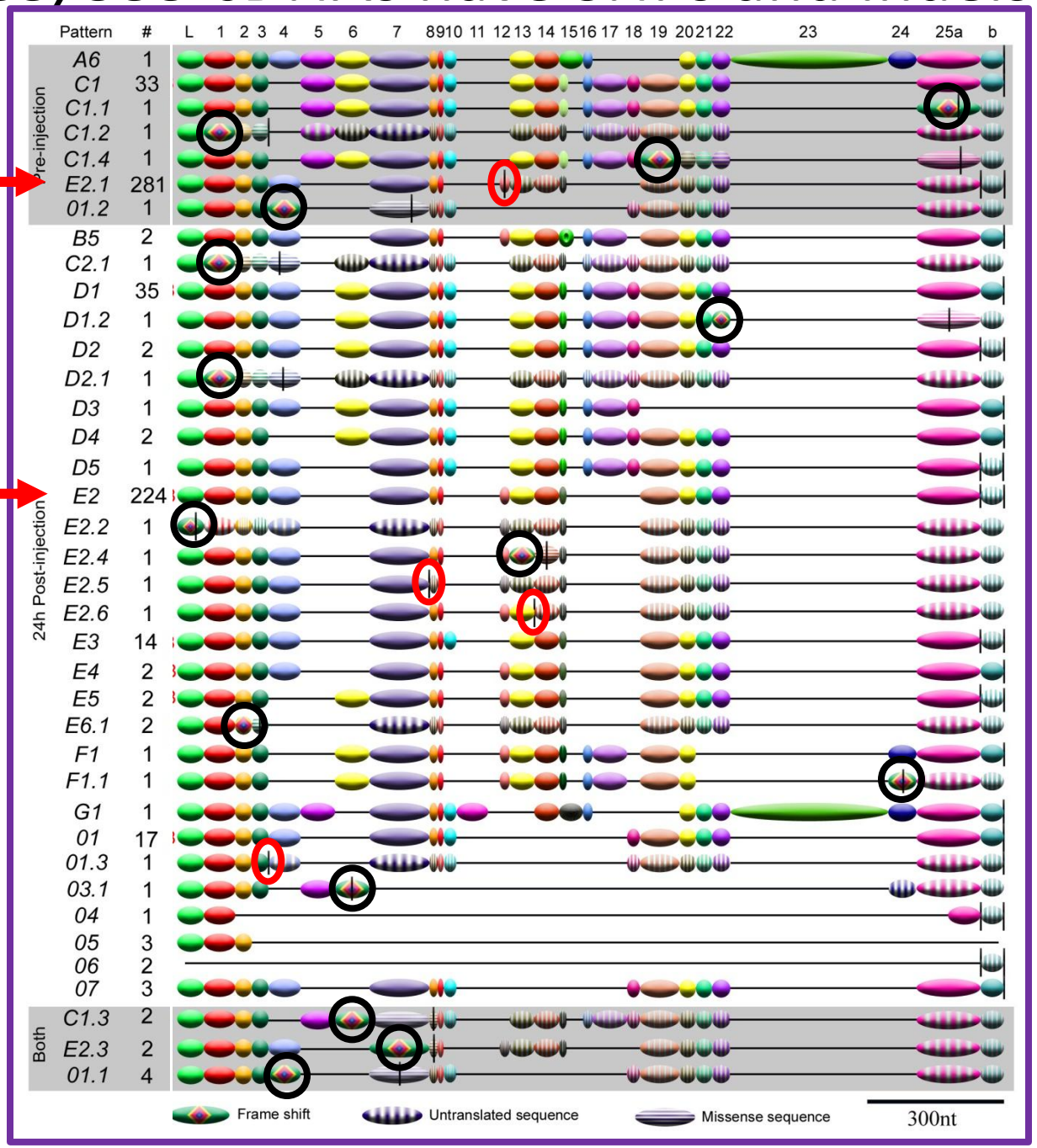
· = not done
a = 5' end
b = 3' end

Buckley et al., 2008a

Sp185/333 cDNAs have SNPs and Indels

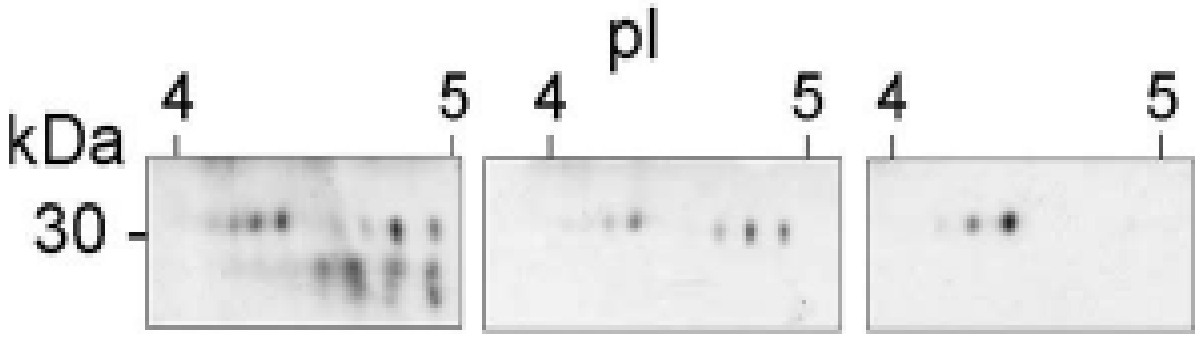
SNP, early stop codon

Indel, fram eshift



Terwilliger et al., 2007

Edited mRNAs express truncated SpShapeshifter proteins



Antibody against:

Gly-rich region

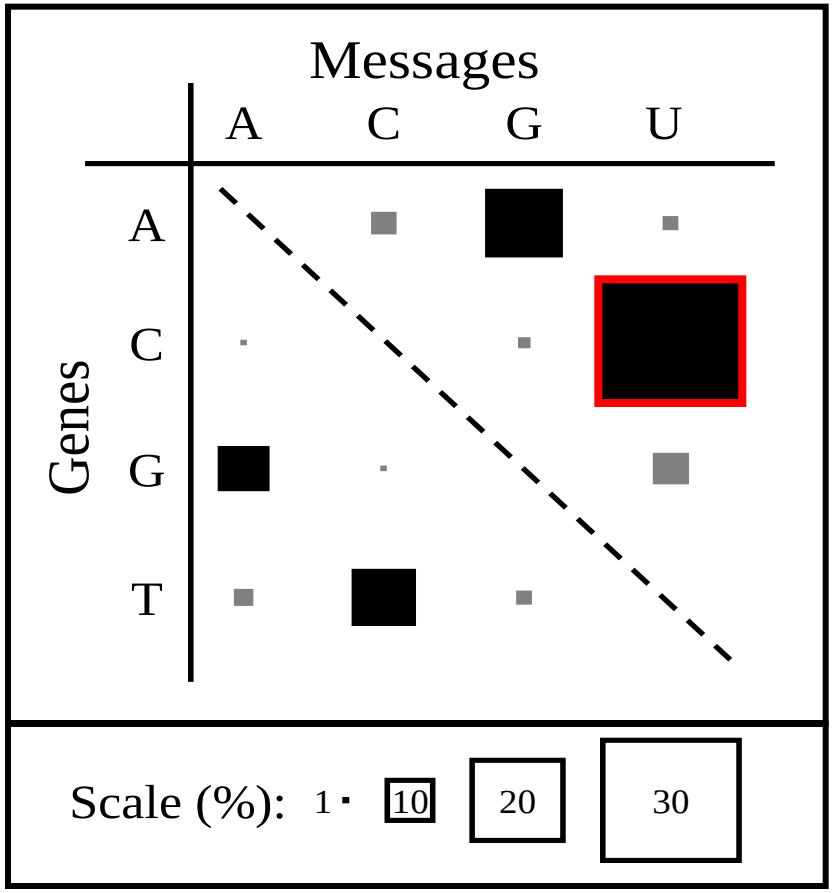
RGD region

C terminal region



Dheilly et al., 2009

C to U substitutions in the *Sp185/333* cDNAs compared to the genes – Looks like RNA editing



Transitions >> transversions

Most of the substitutions are C to U

Several cytidine deaminase gene models

TdT/Polμ gene model

Buckley et al., 2008b

Edited *Sp185/333* mRNAs

- SNPs, indels, missense sequence, early stop codons in half of the mRNAs
- 170 of 171 genes have perfect open reading frames
- Very few of the cDNAs match perfectly to a gene from the same sea urchin

Breaks the rule of transcription fidelity

Buckley et al., 2008b

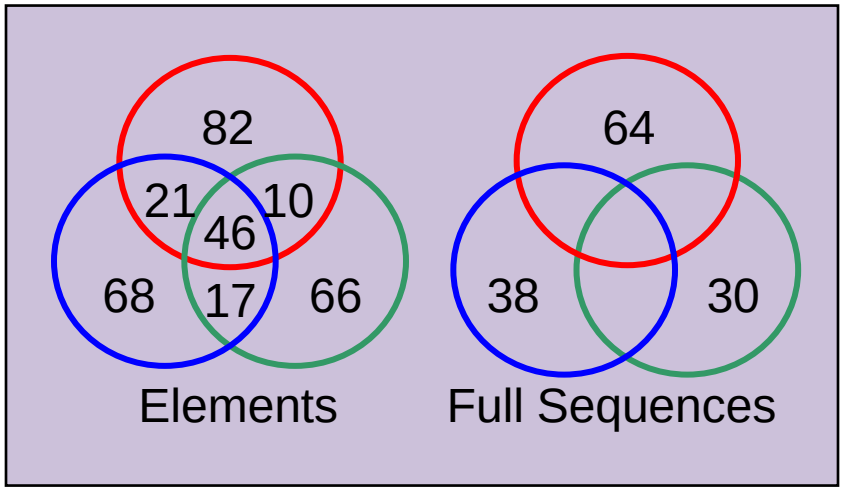
Sp185/333 genes from three sea urchins

- 171 sequenced genes

  one pseudogene

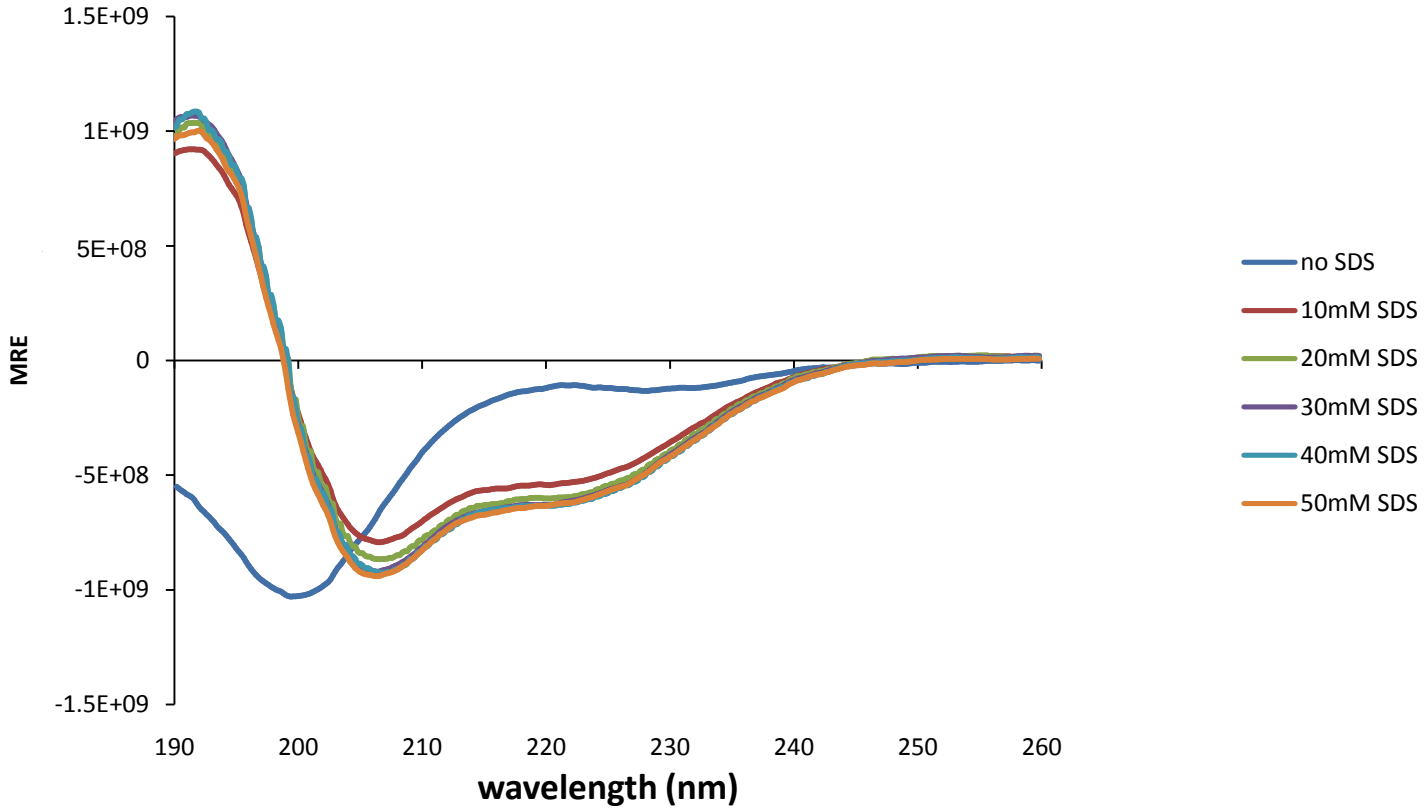
- no shared full length sequence

- some shared element sequences



Buckley et al., 2008a

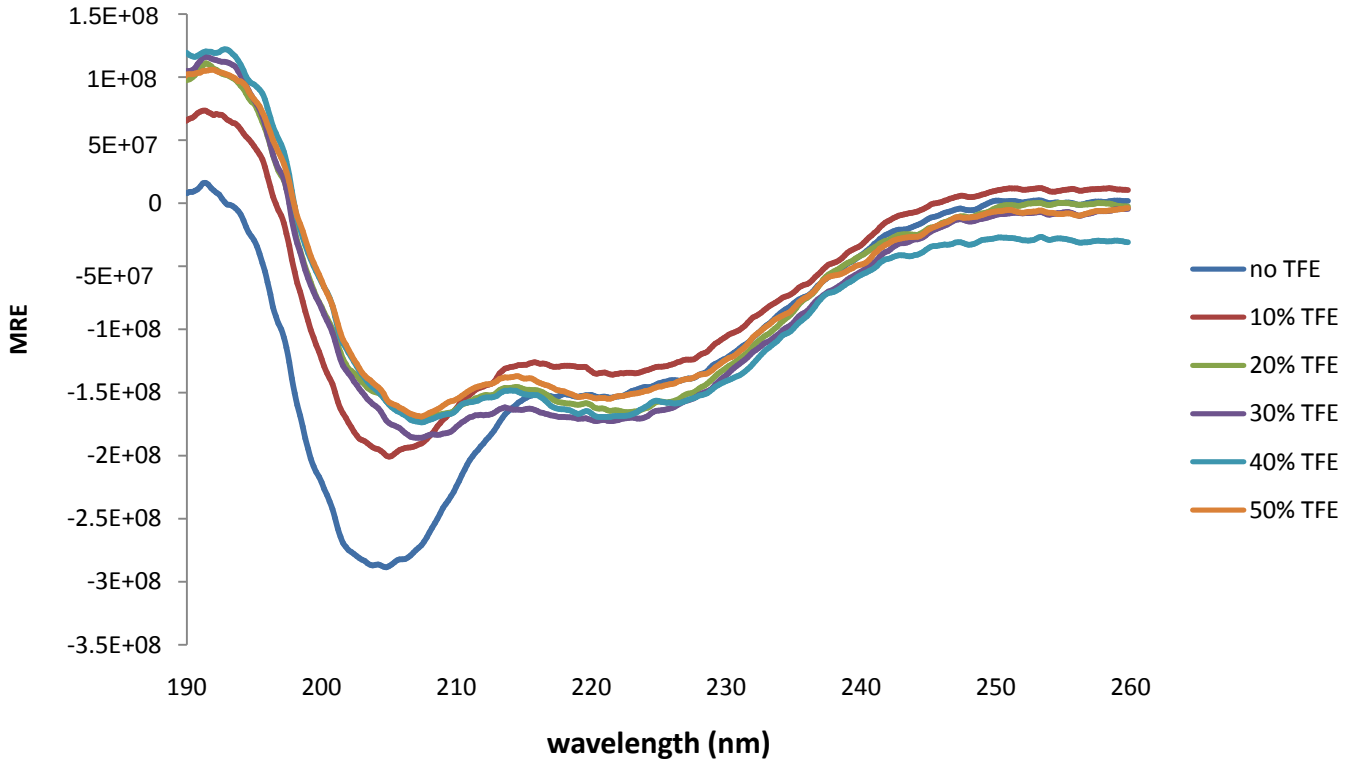
rSpTransformer in SDS



Secondary structure prediction:
disordered to mostly α helical

Bonnie Lun

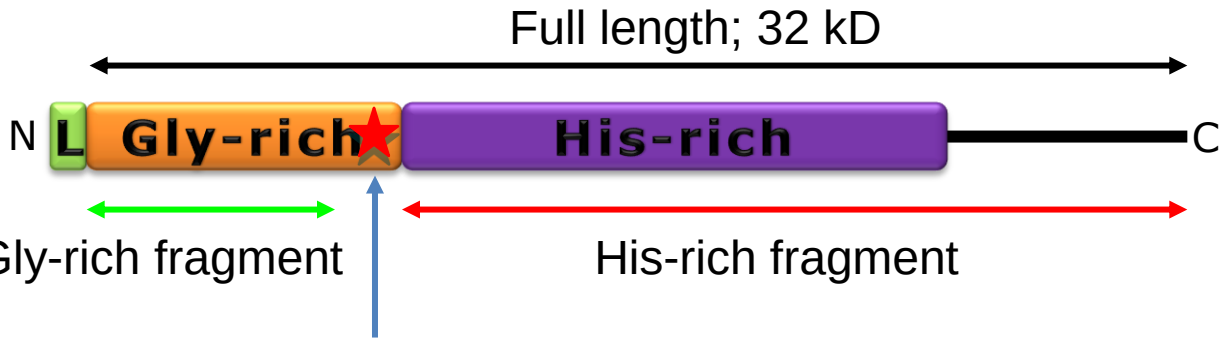
rSp0032 in trifluoroethanol



Secondary structure prediction:
Disordered, weak α helical

Bonnie Lun

rSpTransformer; full length and fragments



AHAQRDYNELRGN
KNGRERGQGRFGG
RPGGMQMGGSRQ
DGGPMGGRRFDGP
DSGAPQMDGRRQD
GGPMGGRRFDGPG
FGAPEMDGRRQNG
GPMGGRRFDGPGF
GGSRPDGAGGR….

10 kD

….RRHHGHRQGHPQDQAEEQPFG
QRNESSEEDGRPHPHHHRHHGHH
HRHHNQTEGHQGHNETGDQDQD
KLHDTRPFRYNHFGRKPFGDRPF
GRRNHTEGHQGHNETGDHPHRHH
NKT….

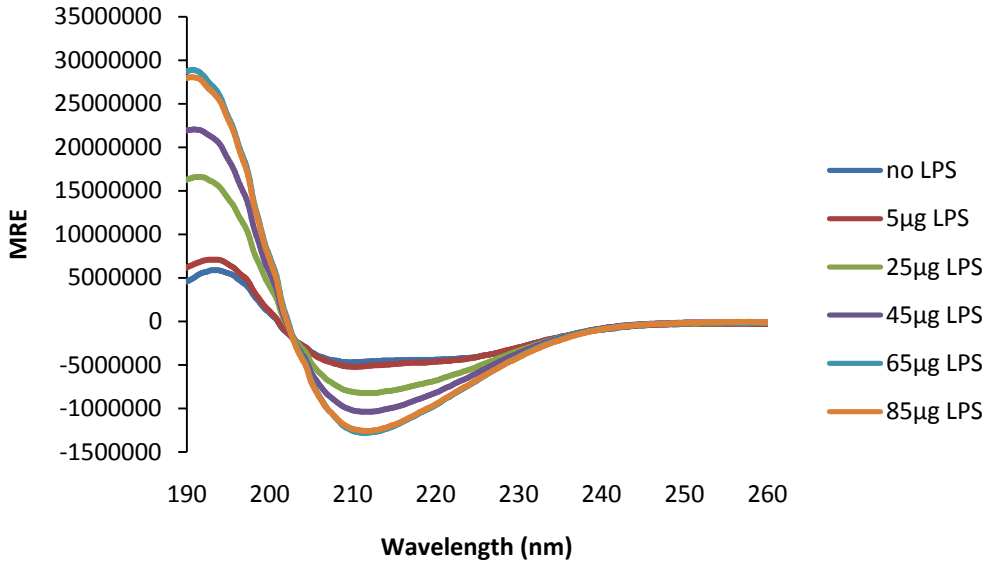
20 kD

rSpTranformer shifts secondary structure

Treatment	Full length	Gly-rich fragment	His-rich fragment
----	Disordered	Mostly α helical	Mostly α helical
SDS	α helical	Enhanced α helical	Enhanced α helical
TFE	weak α helical	β strand	β strand
LPS	α helical	β strand	α helical
PA:PC liposomes	α helical		
PA micelles	α helical		
PC micelles	disordered		
4-44^{o}C	No change	No change	No change

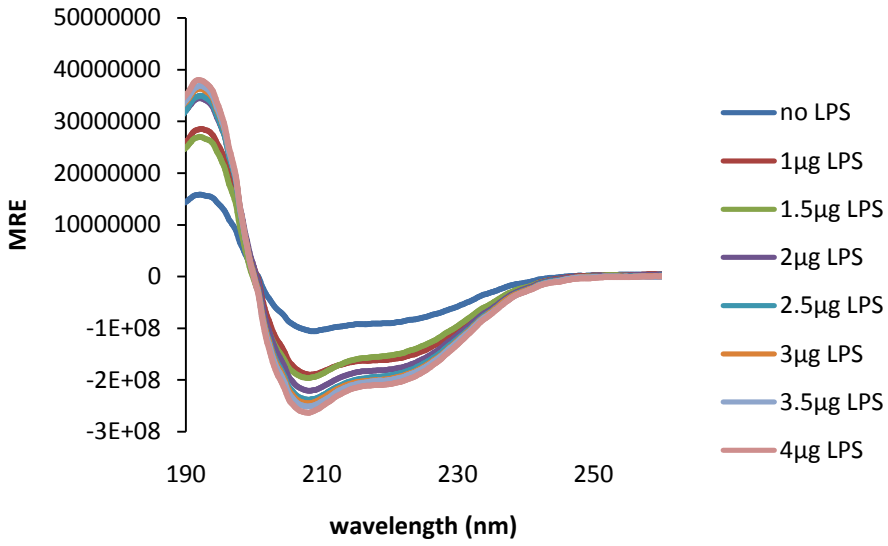
rSpTransformer fragment binding to LPS

glycine-rich fragment



Secondary structure prediction:
α helical to β strand

histidine-rich fragment

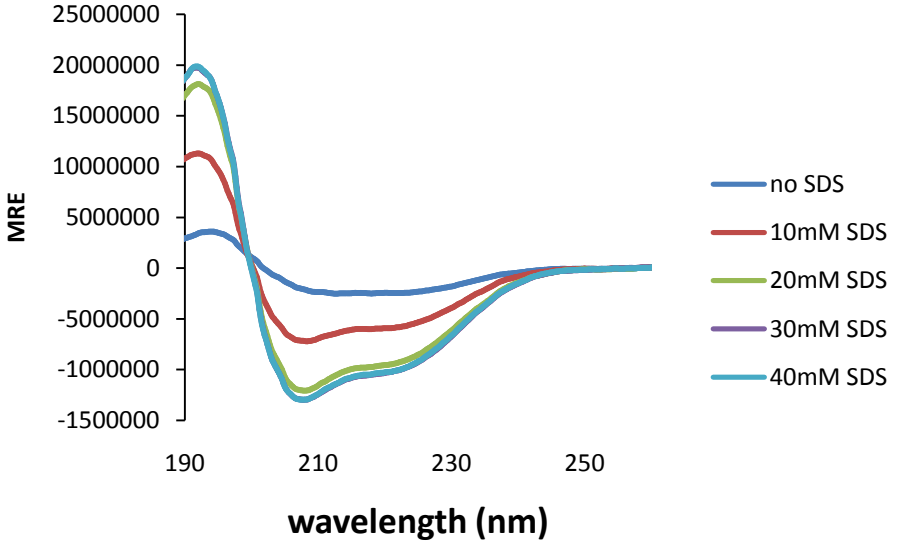


Secondary structure prediction:
Enhanced (mostly) α helical

Bonnie Lun

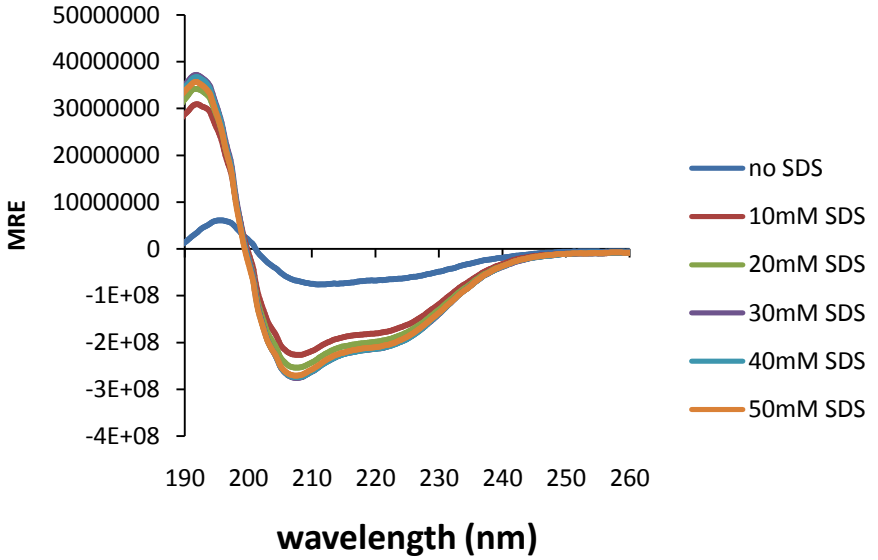
rSpTransformer fragments plus SDS

glycine-rich fragment



Secondary structure prediction:
Enhanced α helical

histidine-rich fragment

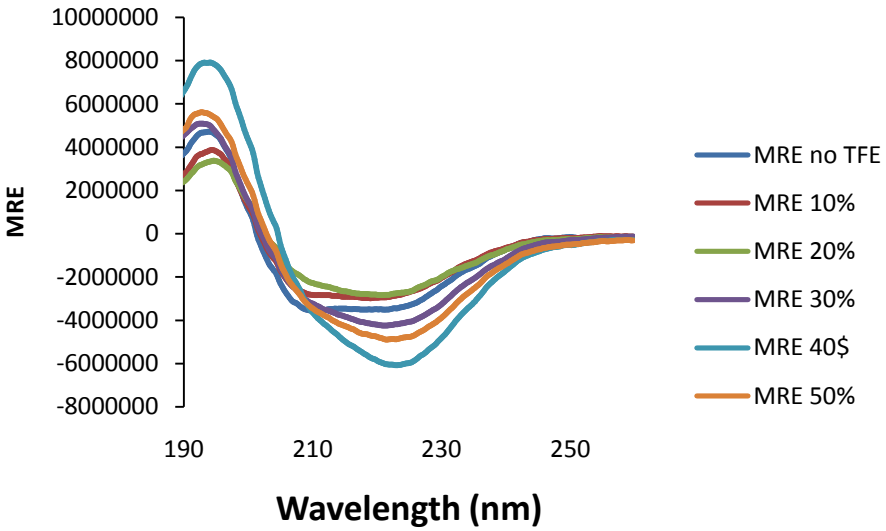


Secondary structure prediction:
Enhanced α helical

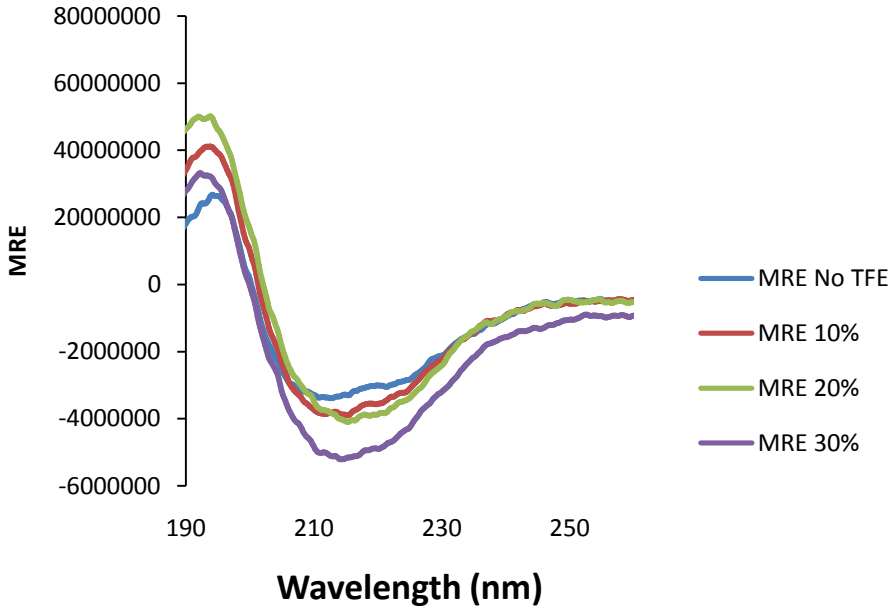
Bonnie Lun

rSpTransformer fragments in TFE

glycine-rich fragment



histidine-rich fragment

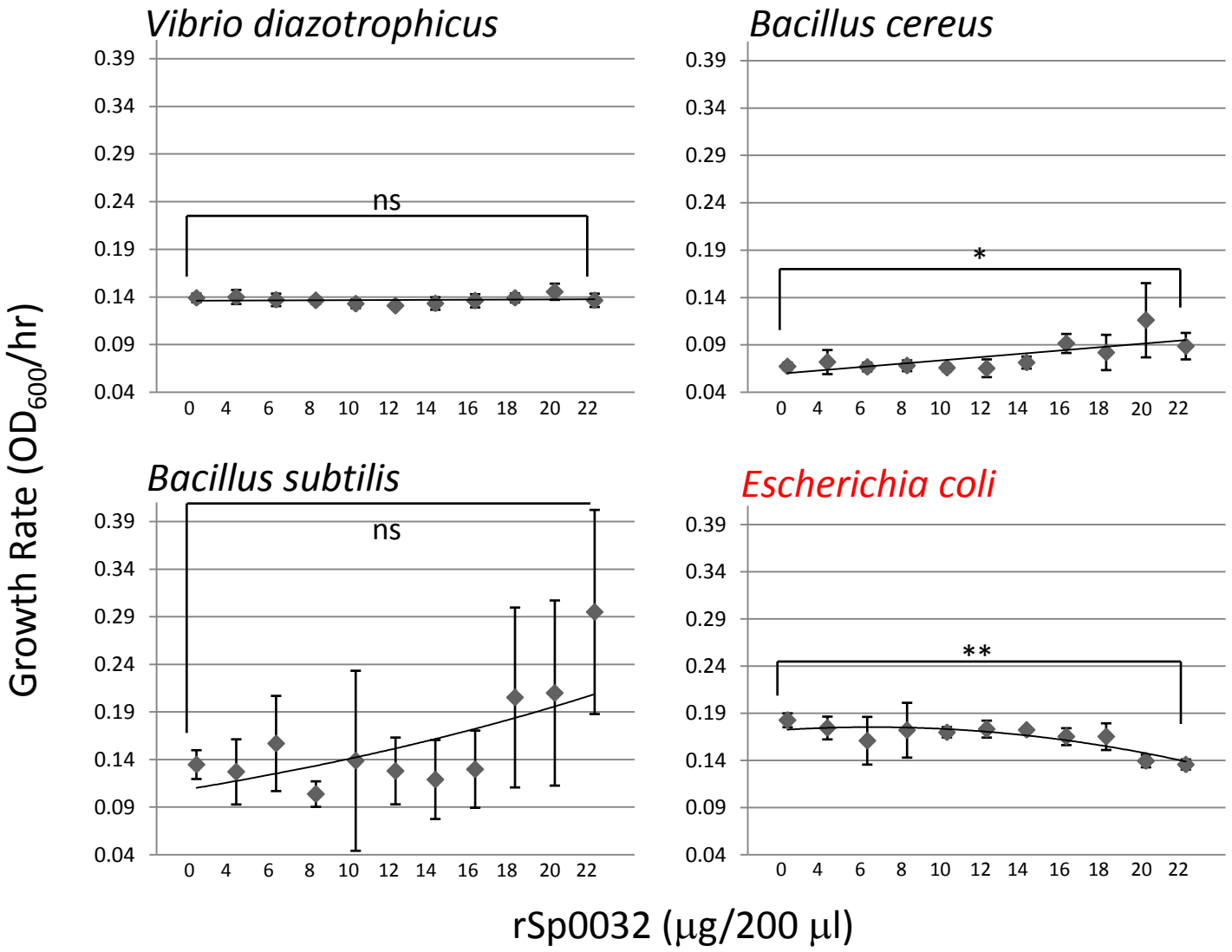


Secondary structure prediction:
α helical to β strand

Secondary structure prediction:
~α helical to β strand

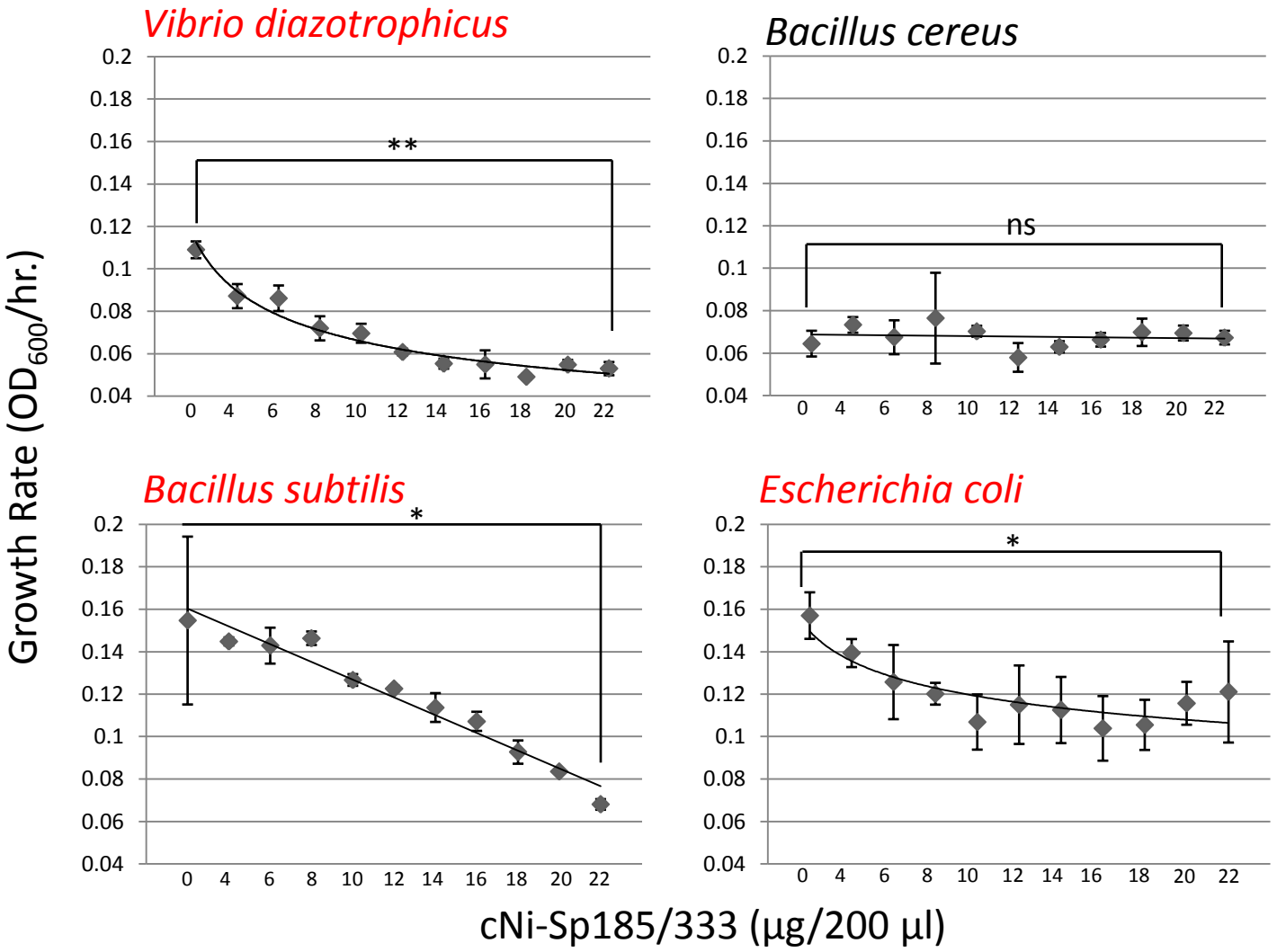
Bonnie Lun

rSpTransformer retards the growth of *E. coli*



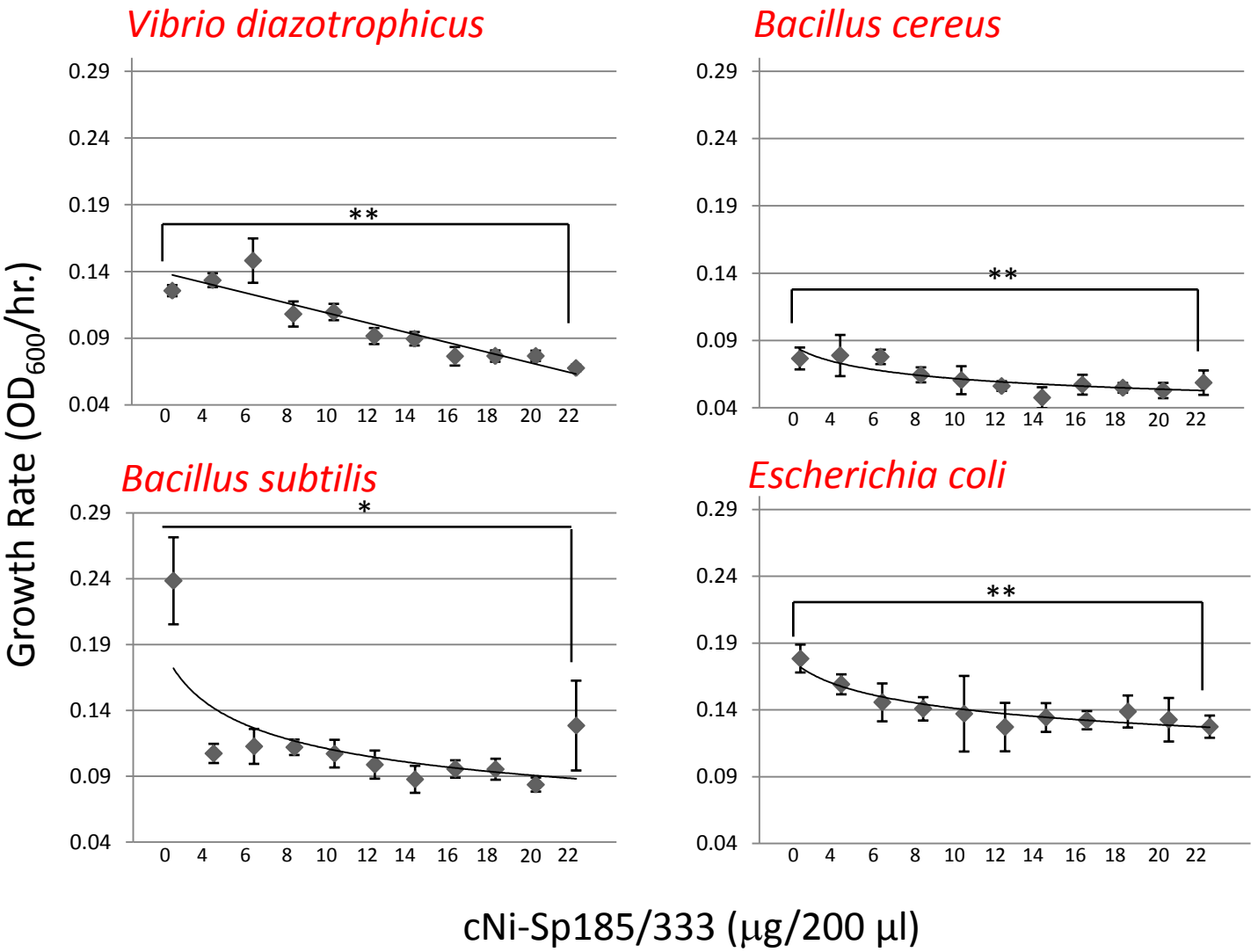
Peter Chou

Native SpShapeshifter proteins variably retard bacterial growth (sea urchin A)



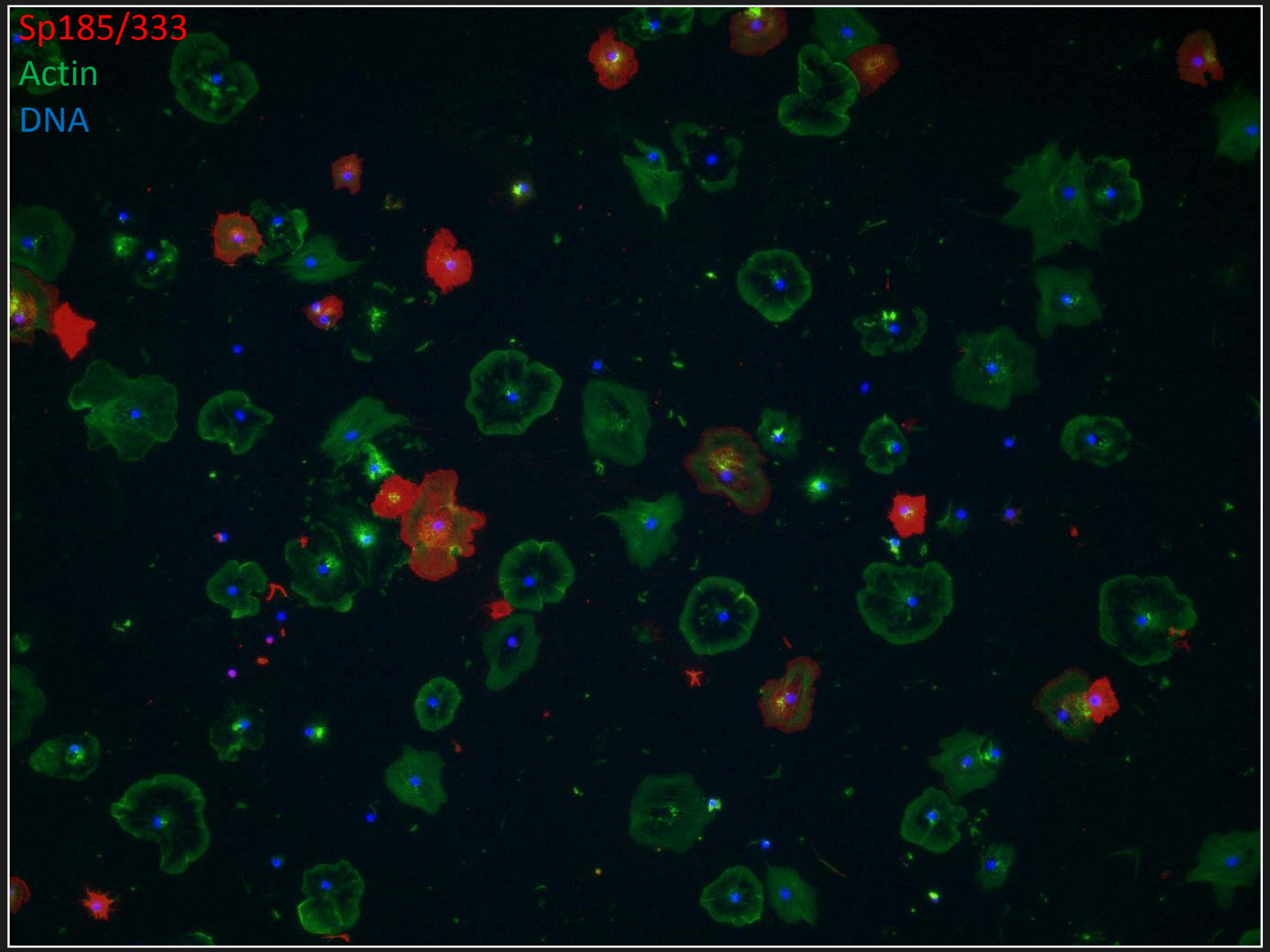
Peter Chou

Native SpShapeshifter proteins variably retard bacterial growth (sea urchin C)



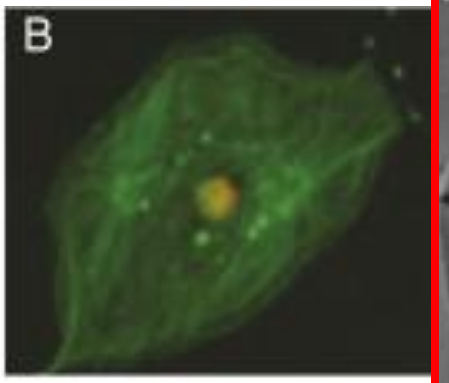
Peter Chou

SpShapeshifter proteins in phagocytes

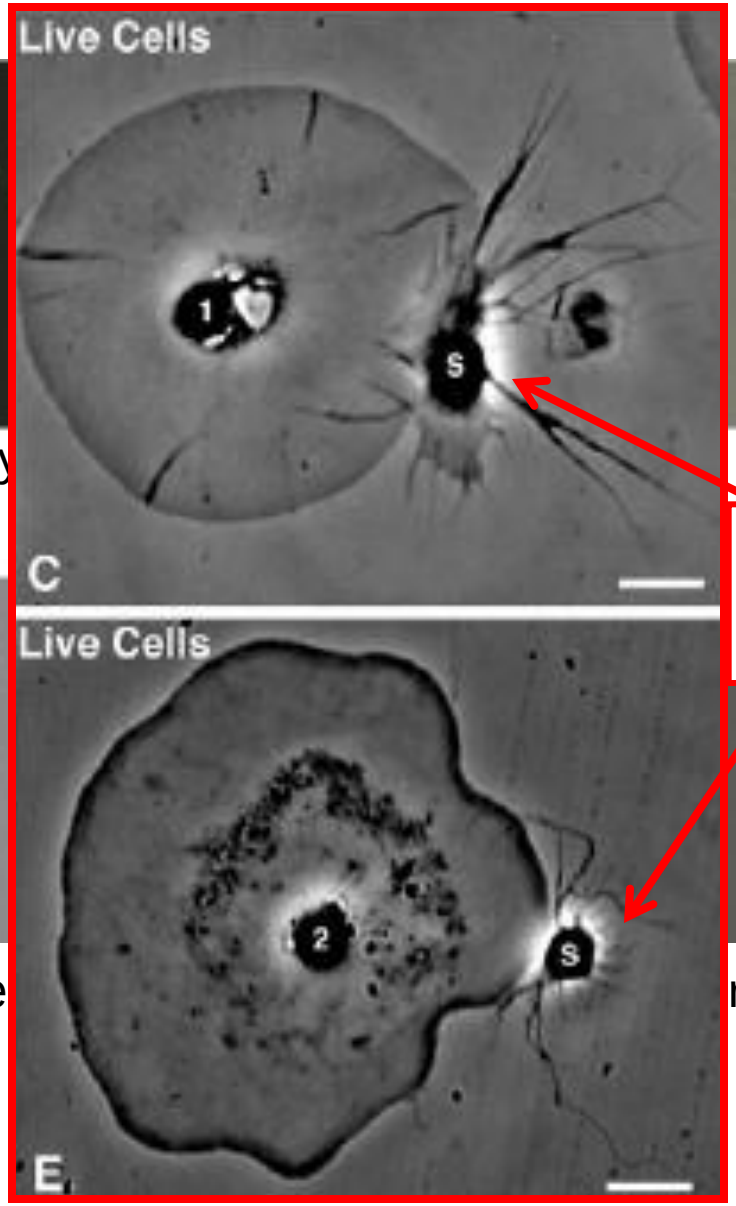


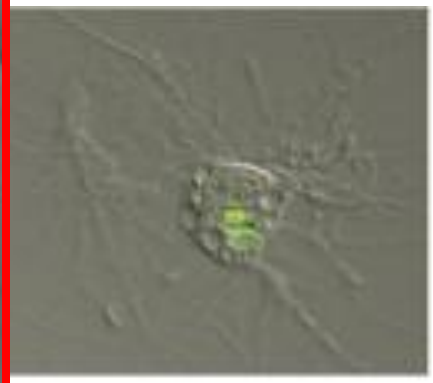
Preethi Golconda

What genes do coelomocytes express?



Polygonal Phagocy

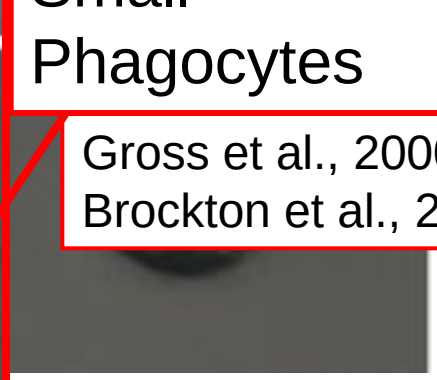




phagocytosis



Red Spherule Ce



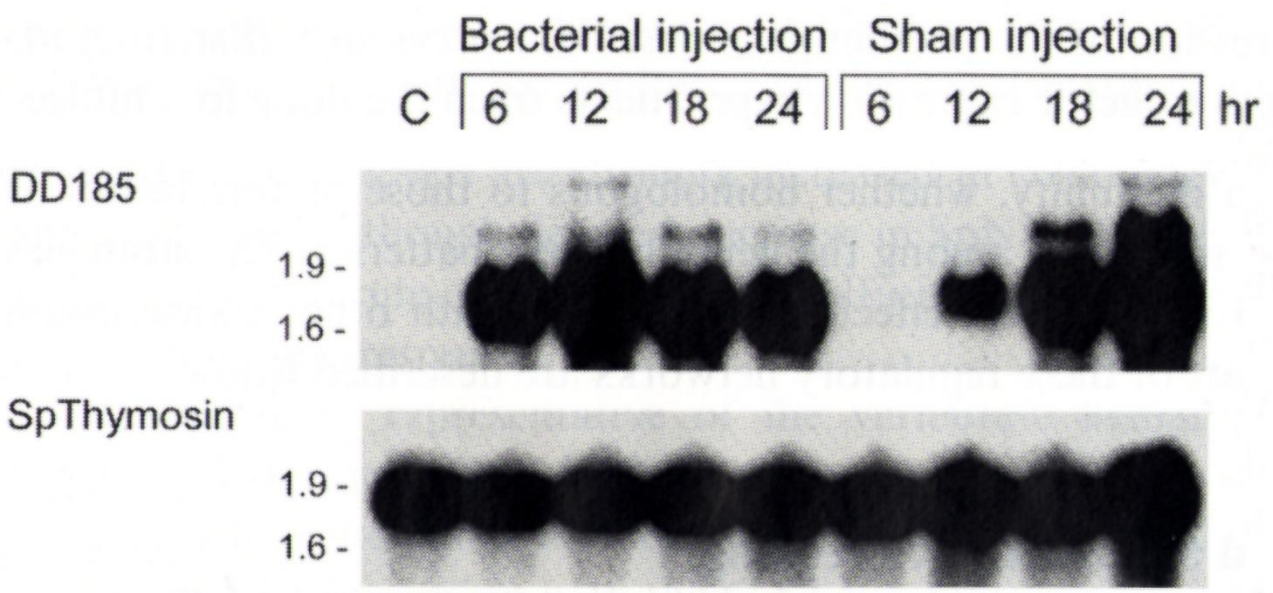
rless Spherule Cell

Small Phagocytes

Gross et al., 2000
Brockton et al., 2008

Hibino et al., 2006

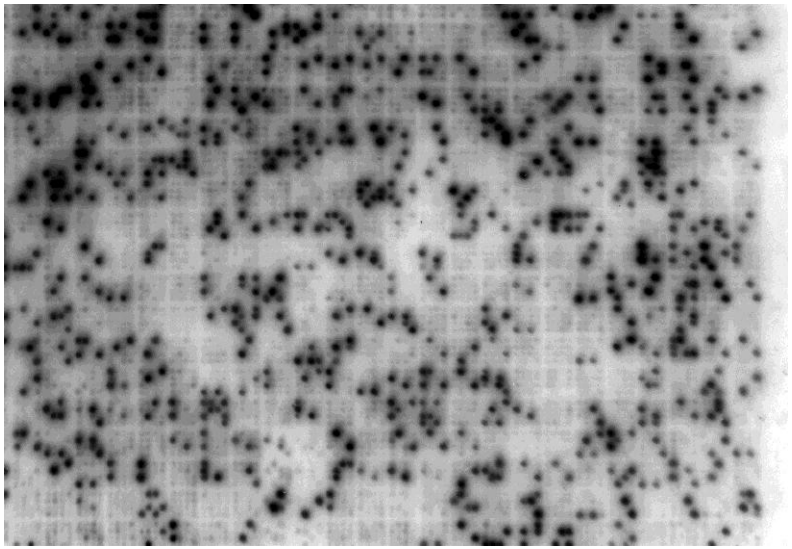
Immune challenge induces *Sp185/333* expression by coelomocytes



Rast et al., 1999

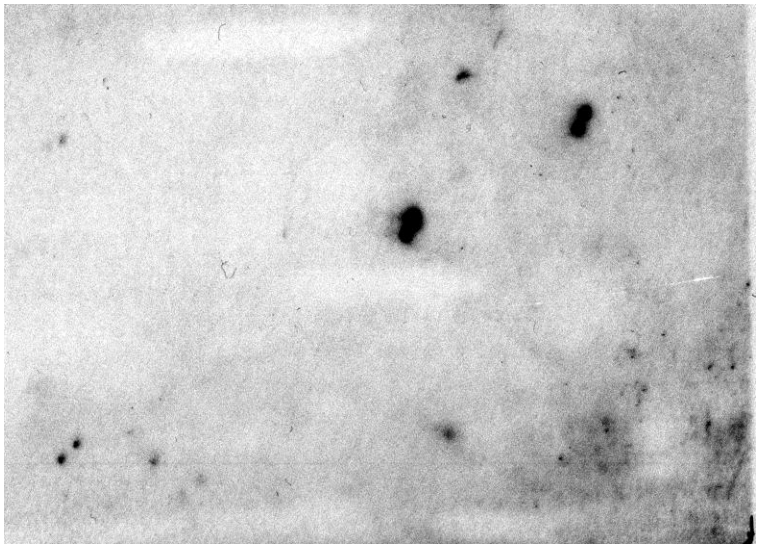
Sp185/333 gene expression is induced by LPS and bacterial challenge

~5,925 / 91,920 = 6.45%



Arrayed cDNA library from coelomocytes activated by bacterial challenge

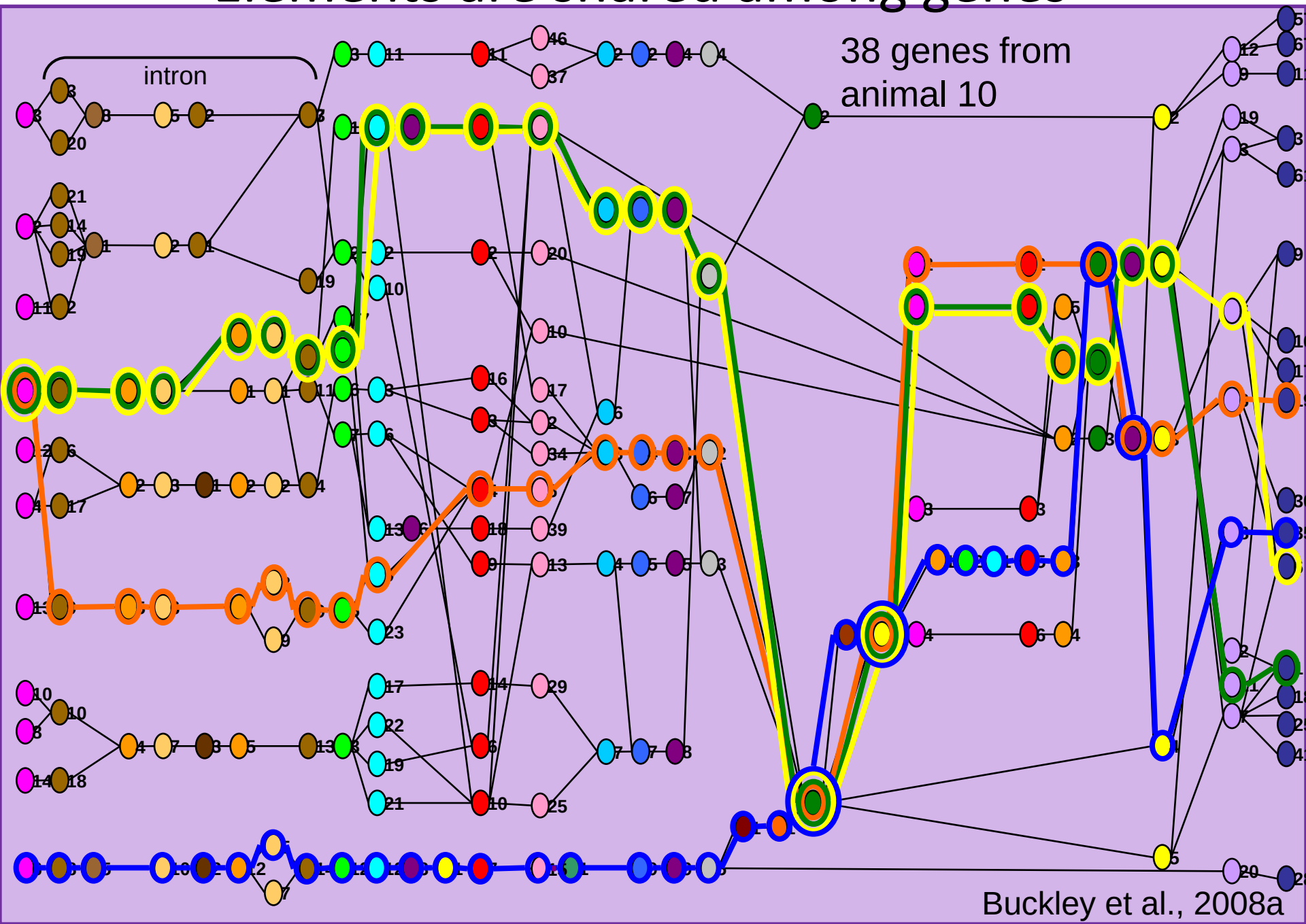
79 / 91,920 = 0.086%

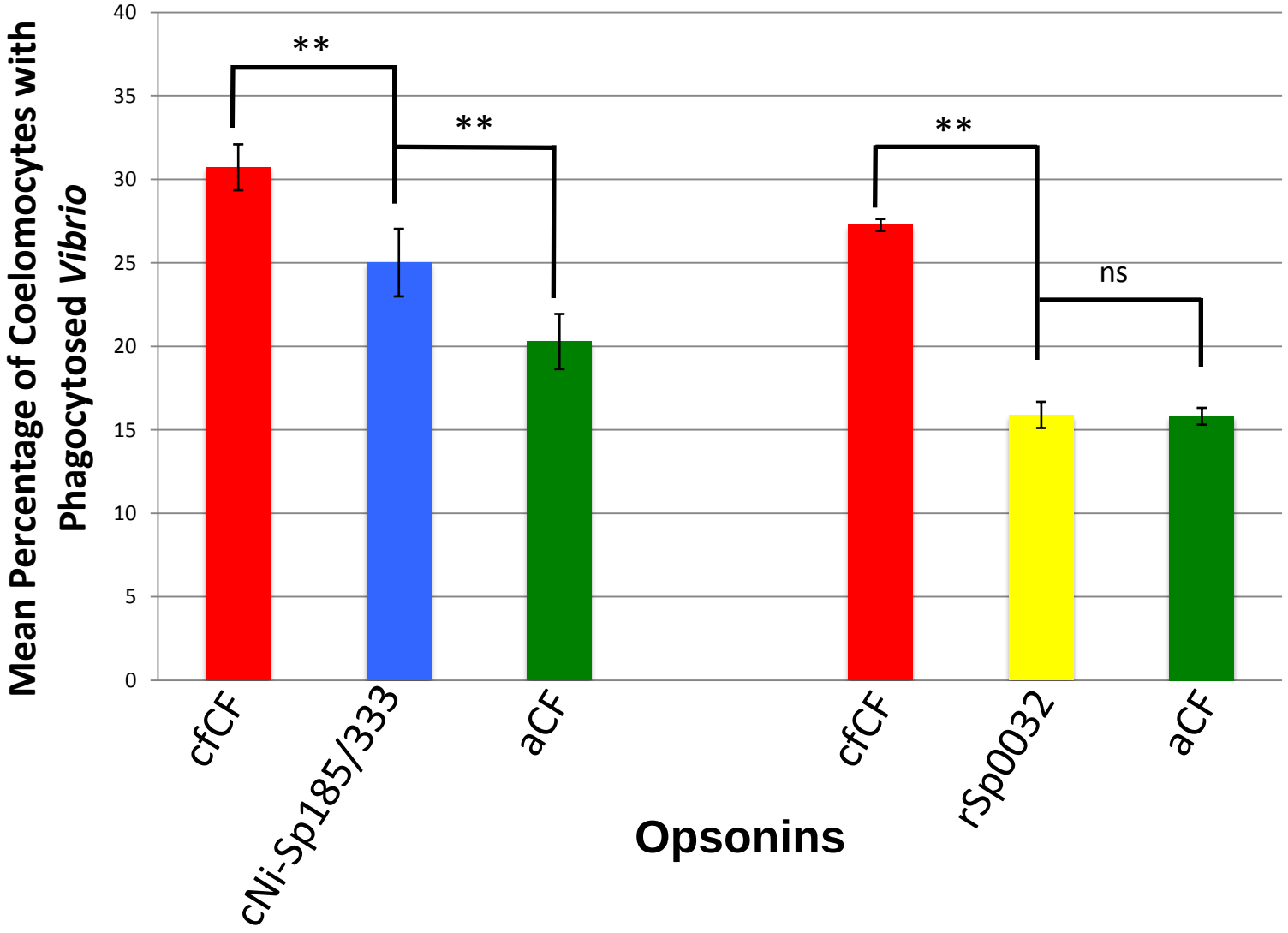


Arrayed cDNA library from non-activated coelomocytes

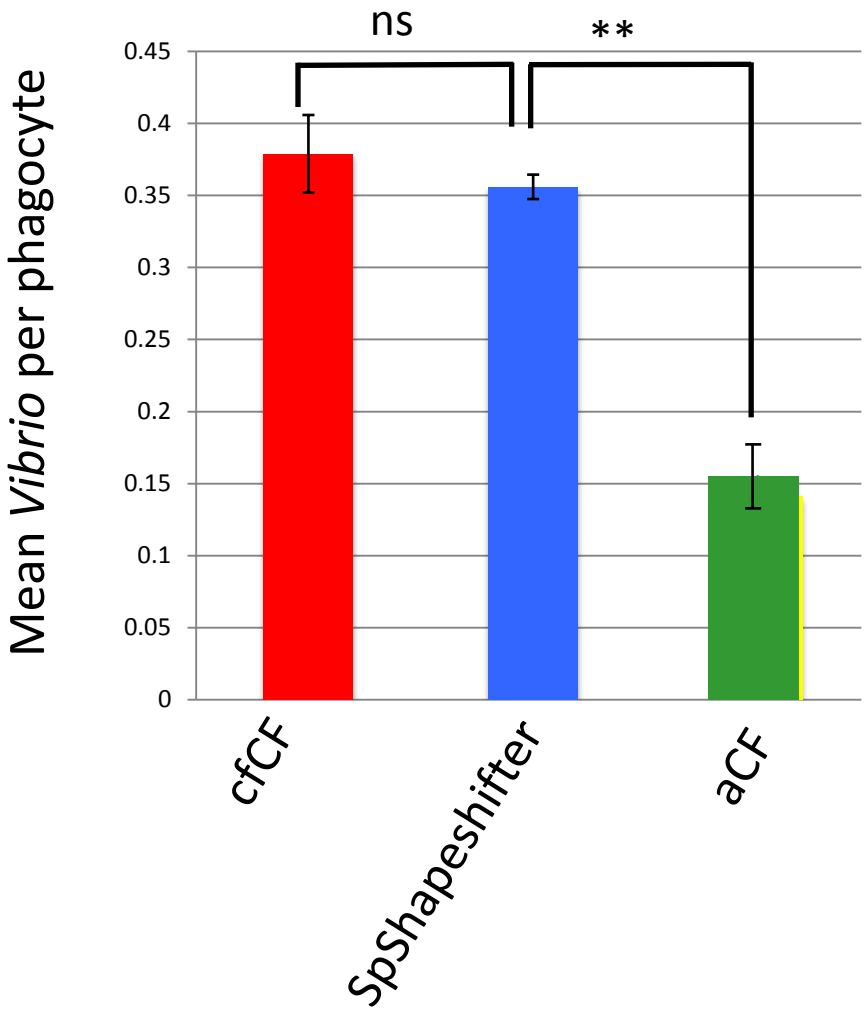
Nair et al., 2005

Elements are shared among genes




Mean Percentage of Coelomocytes with Phagocytosed *Vibrio*
40
35
30
25
20
15
10
5
0
**
**
**
ns
cfCF
cNi-Sp185/333
aCF
cfCF
rSp0032
aCF
Opsonins

Phagocytosis per individual phagocyte is increased for *Vibrio* opsonized with cNi-SpShapeshifter proteins

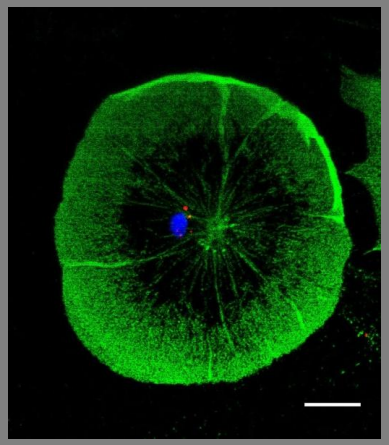


Peter Chou

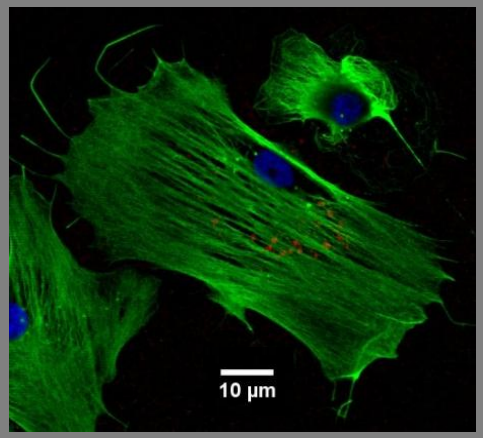
SpShapeshifter proteins are expressed by phagocytes

SpShapeshifter
Actin
DNA

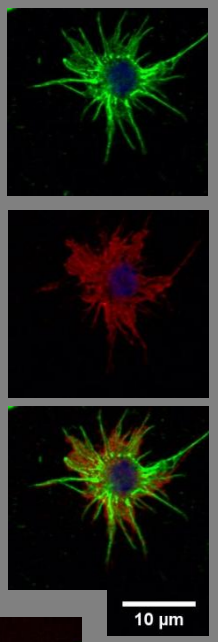
Discoidal



Polygonal



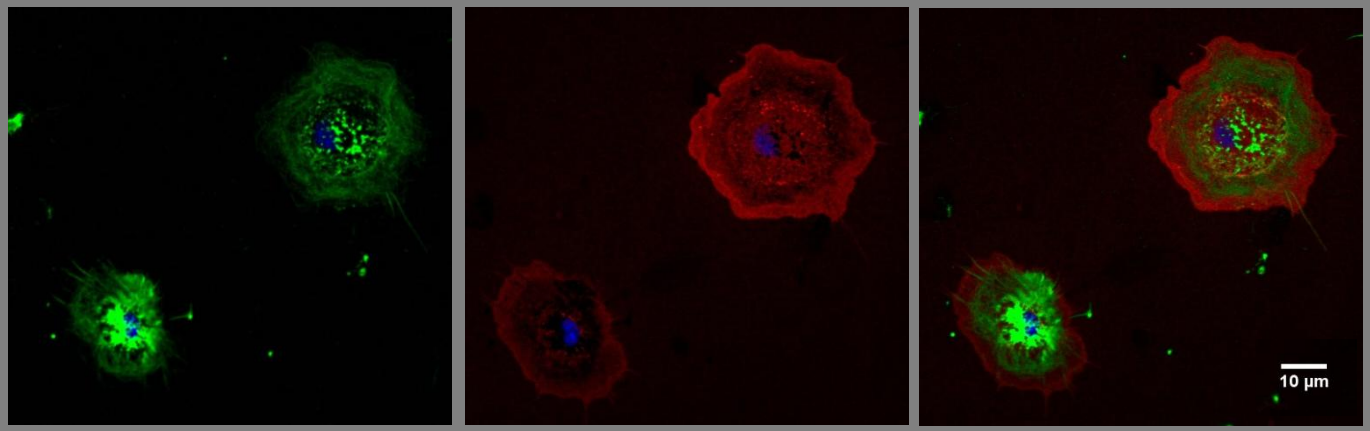
Small



Cell surface

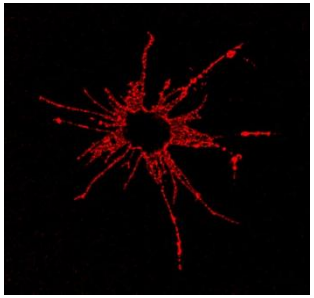
Perinuclear vessicles

Medium



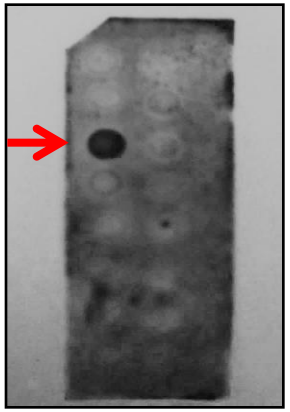
Peter Chou
Preethi Golconda

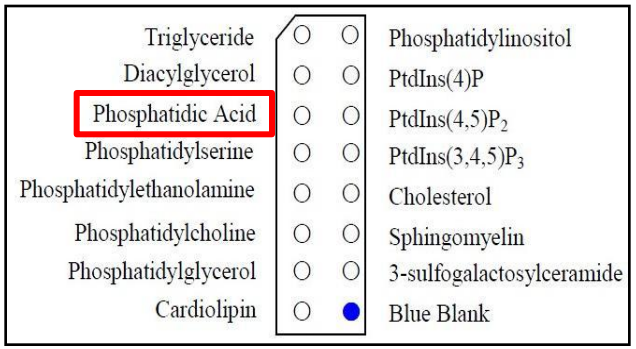
rSpTransformer binds to phosphatidic acid



Small phagocyte

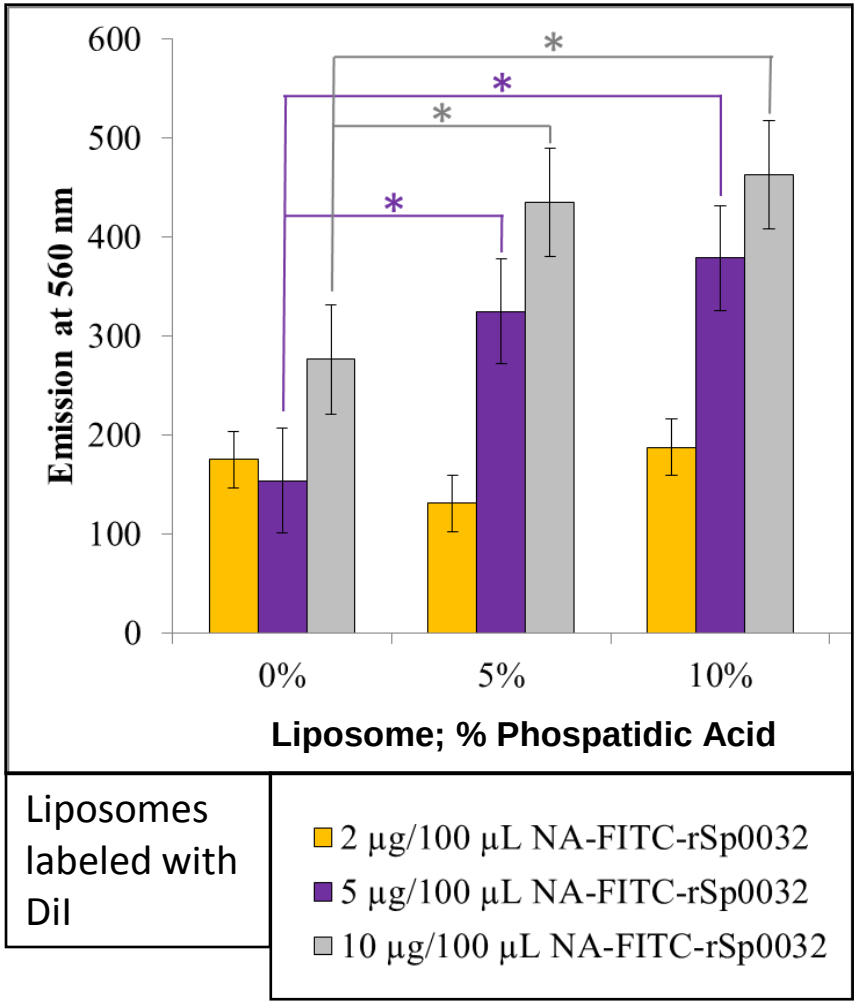
Image by John Henson





Bonnie Lun

FRET



Anthony Boyd

Multiple levels of sequence diversification in the *Sp185/333* system

Genes

multiple per genome, clustered

recombination, duplication, deletion

single gene expressed per phagocyte

mRNA

editing, amplifies protein sequence diversity

Proteins

Putative post translational modifications

secretion, opsonins, induce phagocytosis

disordered, shift to ordered upon binding to targets

synergistic function in complexes to target pathogens

The immune system is sophisticated and flexible