2021 Literature Report I

nature biotechnology

BRIEF COMMUNICATION

https://doi.org/10.1038/s41587-020-00794-3

Check for updates

Super-resolution RNA imaging using a rhodamine-binding aptamer with fast exchange kinetics

Murat Sunbul[1]✉, Jens Lackner[2], Annabell Martin[1], Daniel Englert[1], Benjamin Hacene[2], Franziska Grün[1], Karin Nienhaus[2], G. Ulrich Nienhaus[2,3,4,5]✉ and Andres Jäschke[1]✉

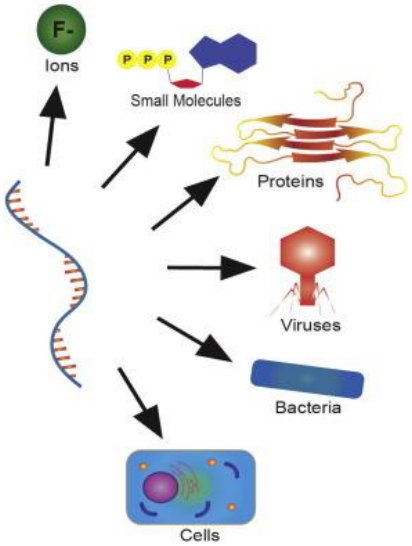
Reporter: Jin Li

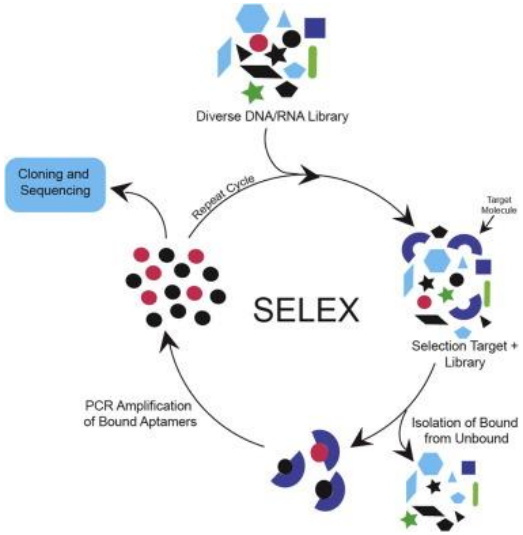
Date: 2021-03-04

基本概念

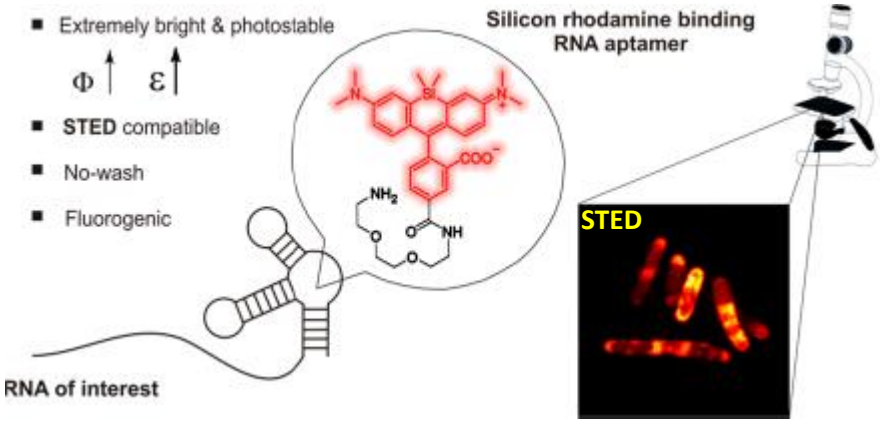
核酸适配体(Aptamer)：基于空间构象多样性与特定靶标结合的短的ssDNA或RNA。

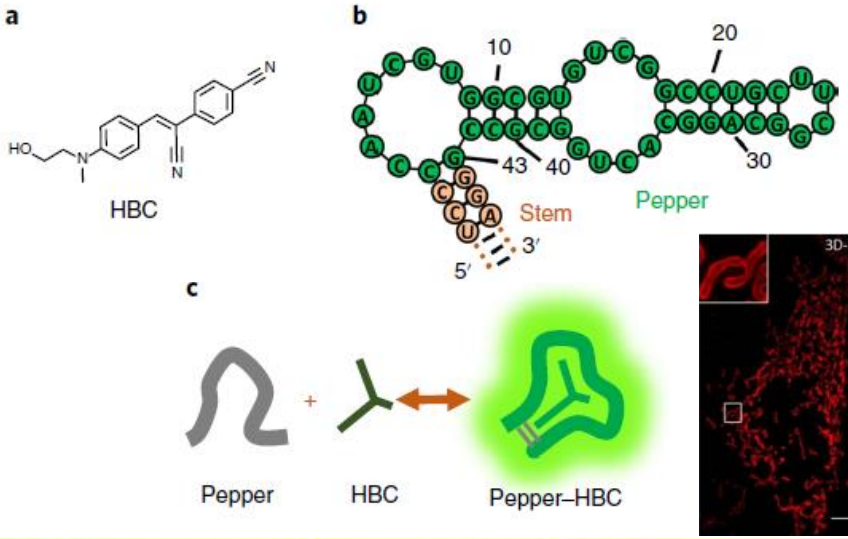
指数富集配体系统进化技术(systematic evolution of ligands by exponential enrichment，SELEX)



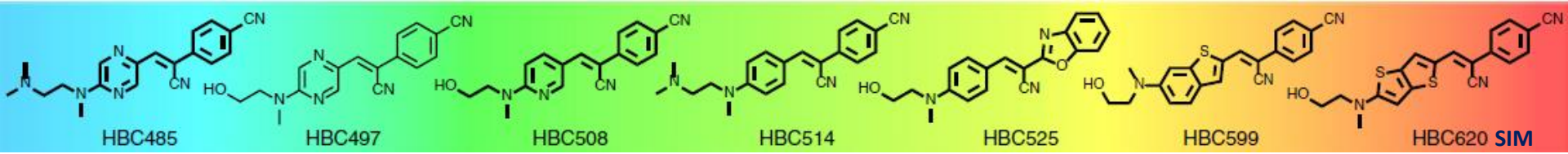


为什么做？





SiRA: A Silicon Rhodamine-Binding Aptamer for Live-Cell Super-Resolution RNA Imaging

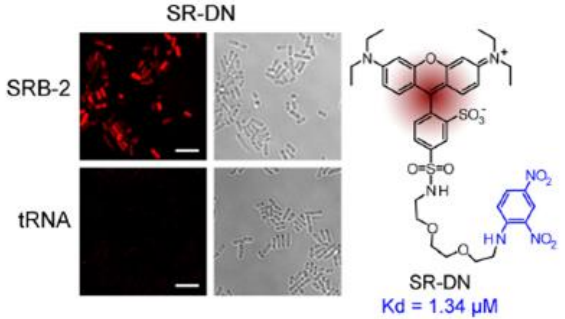


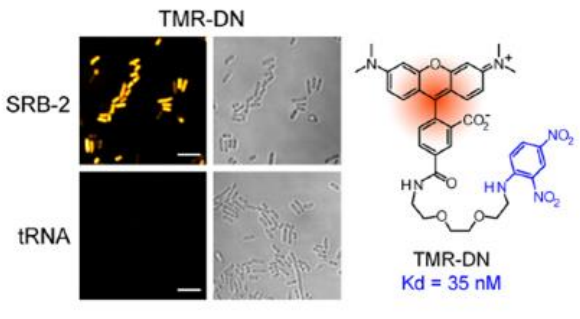
Visualizing RNA dynamics in live cells with bright and stable fluorescent RNAs

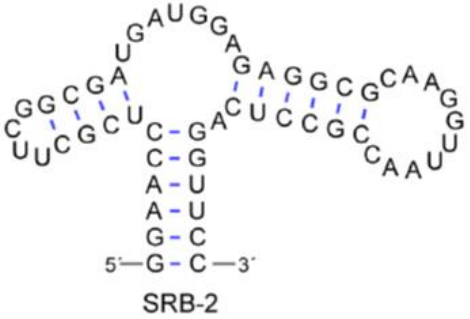
Andres Jäschke and G. Ulrich Nienhaus , *J. Am. Chem. Soc*. **2019**, 141, 7562; *Nature Biotechnology*, **2019**, 37, 1287

为什么做？

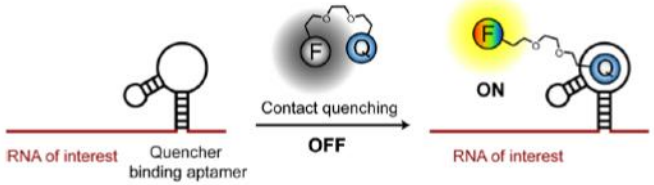
Previous Work:







Previous Work:

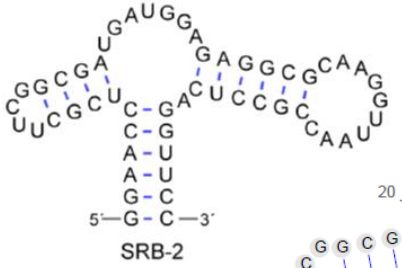


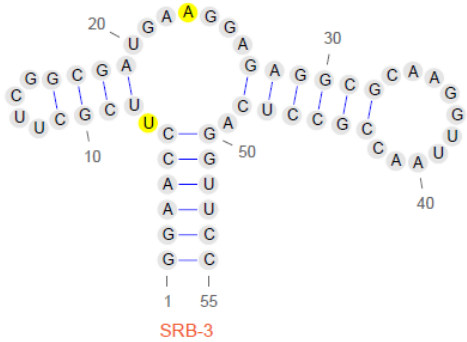
However,

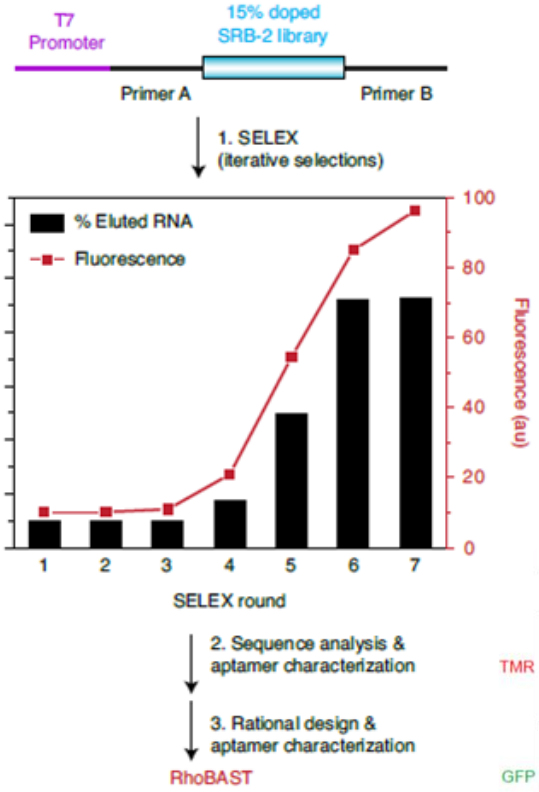
- **No SMLM**
- **Insufficient sensitivity**

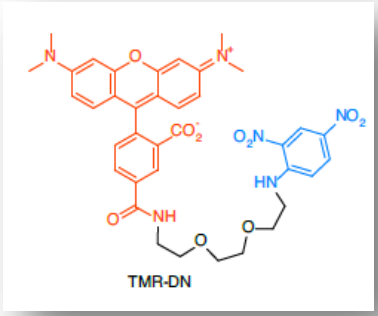
怎么做？rhodamine-binding aptamer

Previous Work:

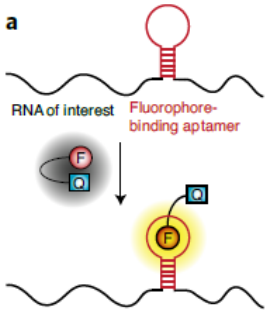


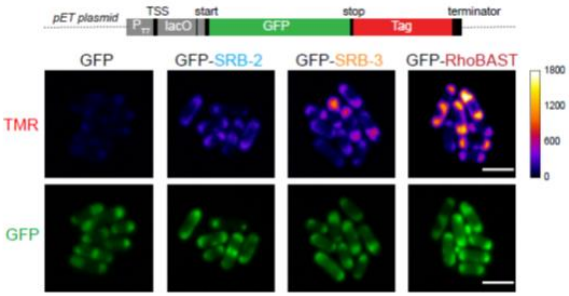






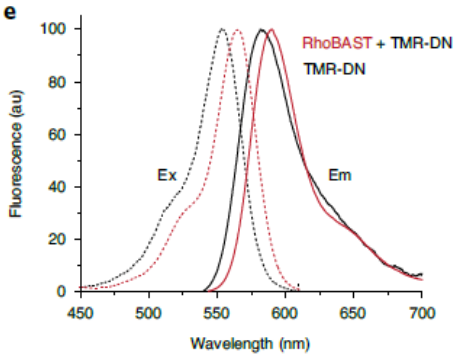
This Work:

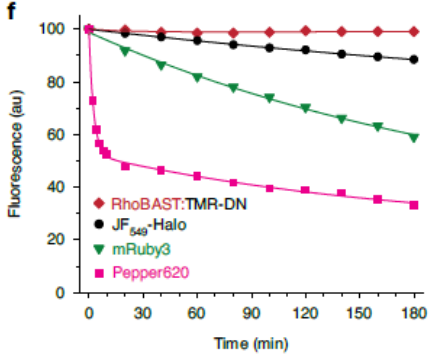


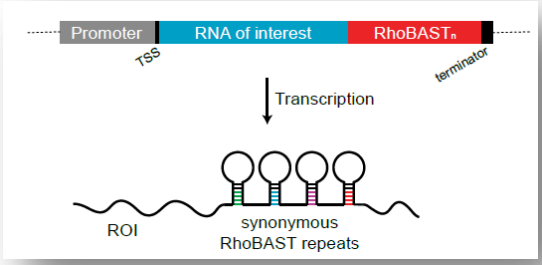


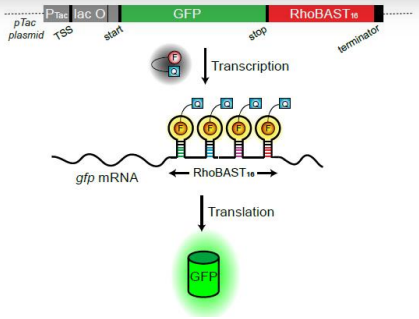
RhoBAST (Rhodamine Binding Aptamer for Super-resolution Imaging Techniques).

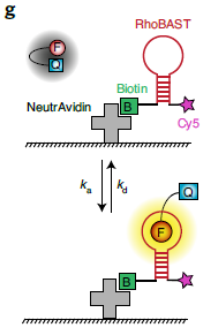
效果

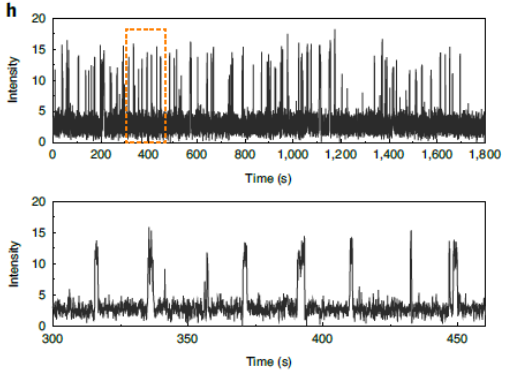

e
RhoBAST + TMR-DN
TMR-DN
Ex
Em
Fluorescence (au)
Wavelength (nm)

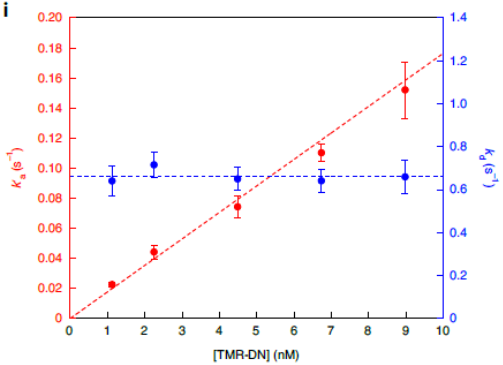

f
RhoBAST:TMR-DN
JF549-Halo
mRuby3
Pepper620
Fluorescence (au)
Time (min)

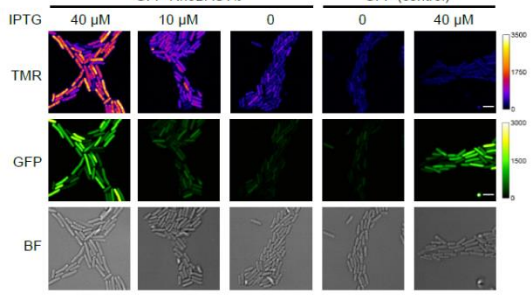

Promoter
RNA of interest
RhoBASTn
TSS
terminator
Transcription
ROI
synonymous
RhoBAST repeats


PTac
lac O
GFP
RhoBAST16
pTac plasmid
TSS
start
stop
terminator
Transcription
gfp mRNA
RhoBAST16
Translation
GFP

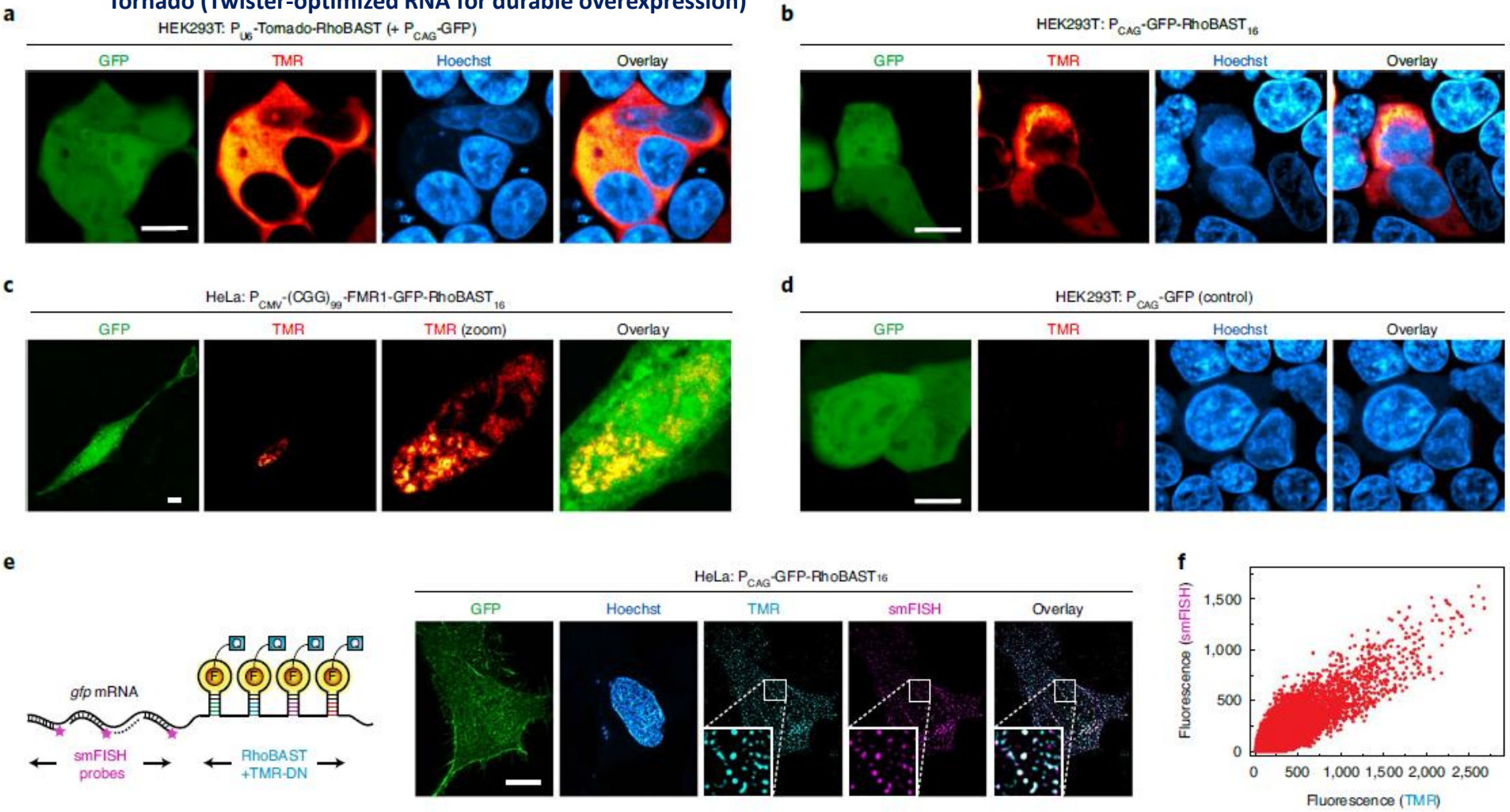

g
RhoBAST
Biotin
NeutrAvidin
Cy5

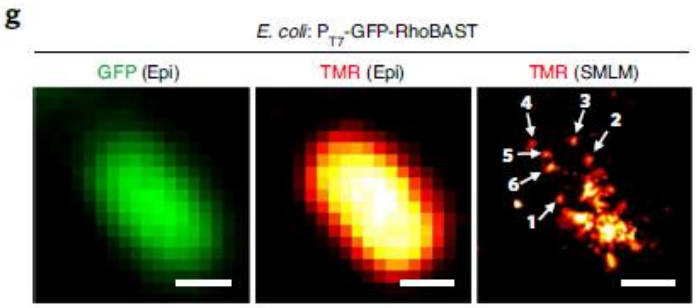

h
Intensity
Time (s)

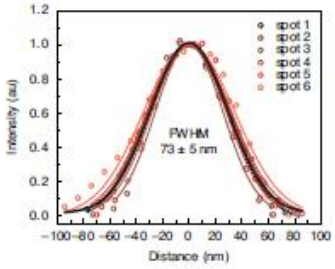

i
[TMR-DN] (nM)

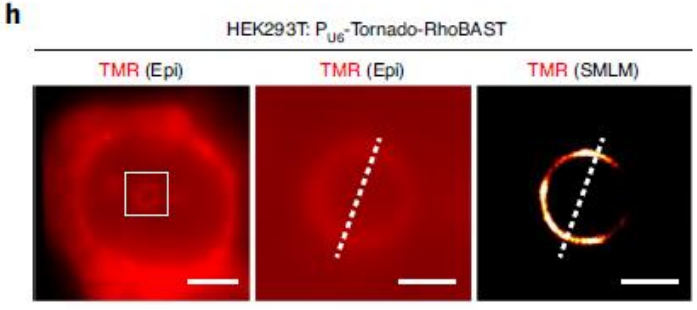

GFP-RhoBAST16
GFP (control)
IPTG
40 μM
10 μM
0
0
40 μM
TMR
GFP
BF

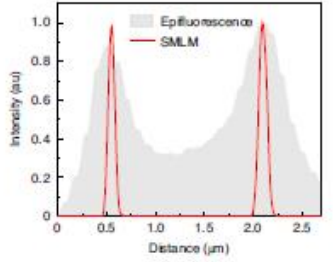
Tornado (Twister-optimized RNA for durable overexpression)

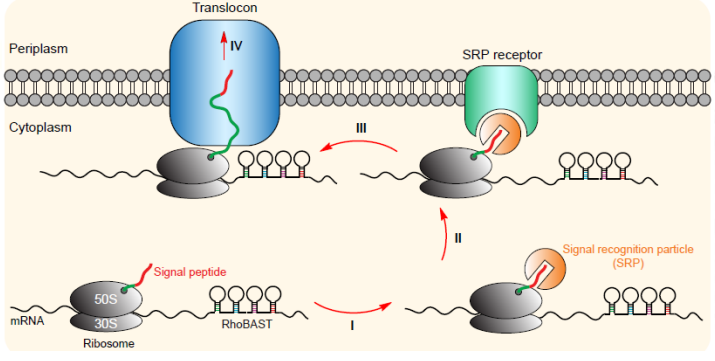


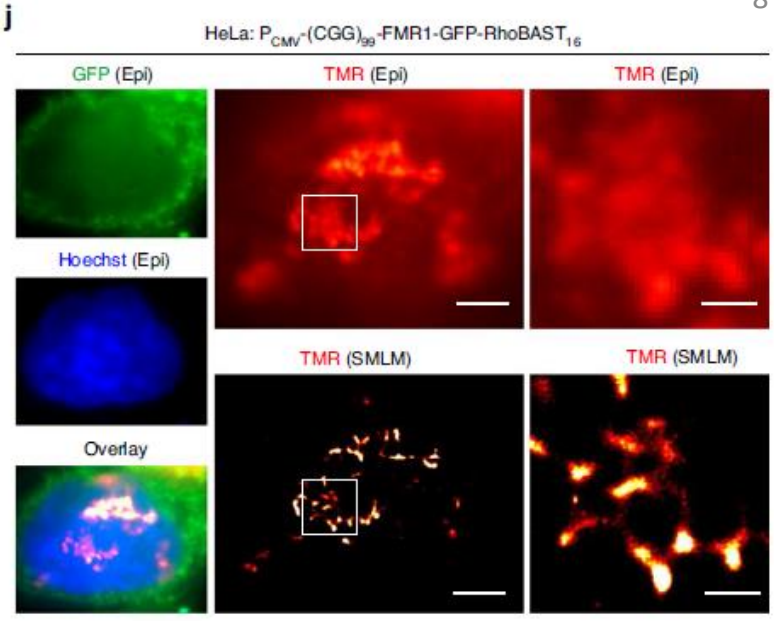

g
E. coli: P T7 -GFP-RhoBAST
GFP (Epi)
TMR (Epi)
TMR (SMLM)
1
2
3
4
5
6

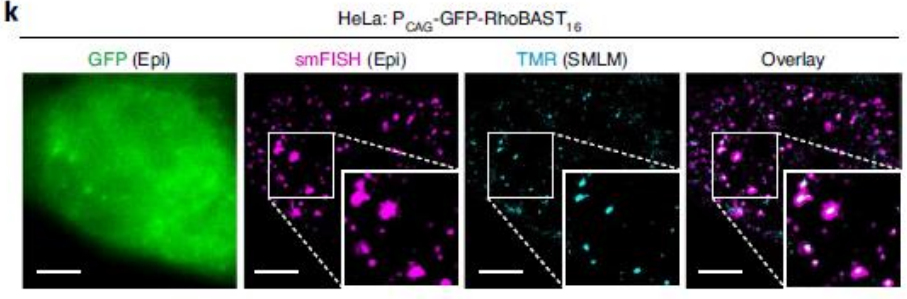

spot 1
spot 2
spot 3
spot 4
spot 5
spot 6
FWHM
73 ± 5 nm
Intensity (au)
Distance (nm)


h
HEK293T: P U6 -Tornado-RhoBAST
TMR (Epi)
TMR (Epi)
TMR (SMLM)


Epifluorescence
SMLM
Intensity (au)
Distance (μm)


Translocon
Periplasm
IV
SRP receptor
Cytoplasm
III
II
Signal peptide
Signal recognition particle
(SRP)
50S
30S
mRNA
RhoBAST
Ribosome
I


j
HeLa: P CMV -(CGG) 99 -FMR1-GFP-RhoBAST 16
GFP (Epi)
TMR (Epi)
TMR (Epi)
Hoechst (Epi)
TMR (SMLM)
TMR (SMLM)
Overlay


k
HeLa: P CAG -GFP-RhoBAST 16
GFP (Epi)
smFISH (Epi)
TMR (SMLM)
Overlay

Thanks for your attention!