Directed Evolution In Mammalian Cells

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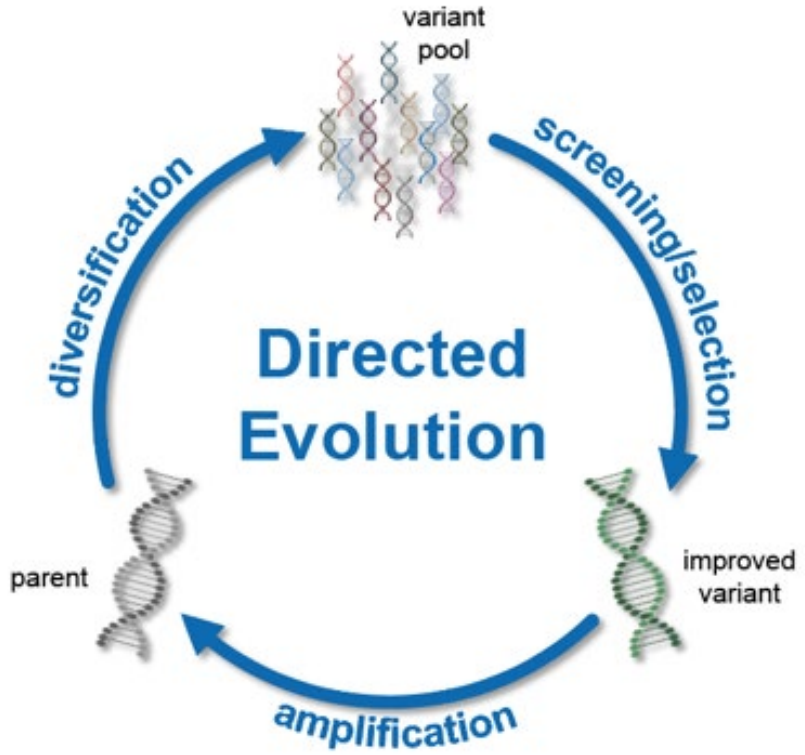
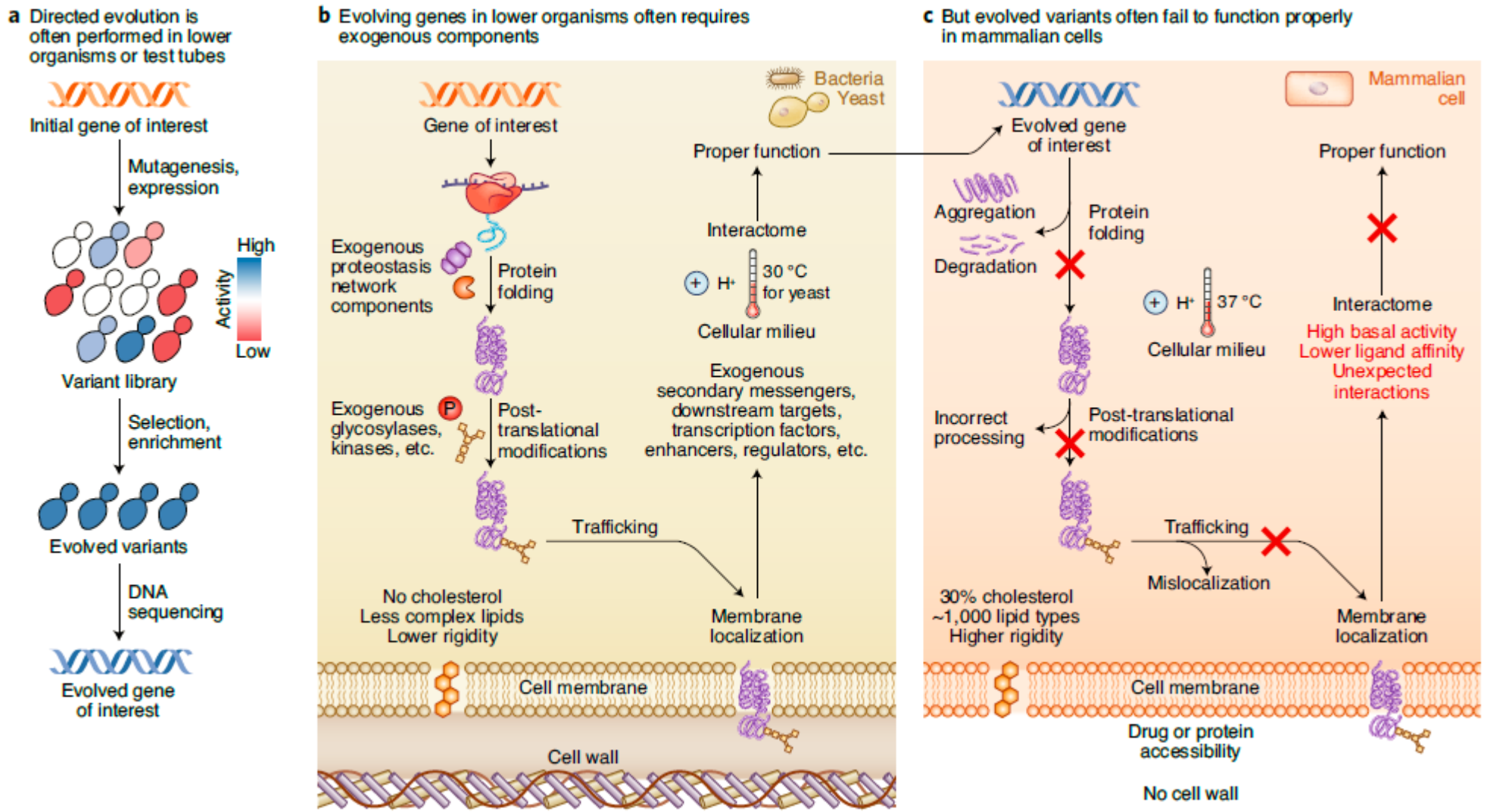
27.04.2021

Directed evolution (DE) is a method used in protein engineering that mimics the process of natural selection to steer proteins or nucleic acids toward a user-defined goal.

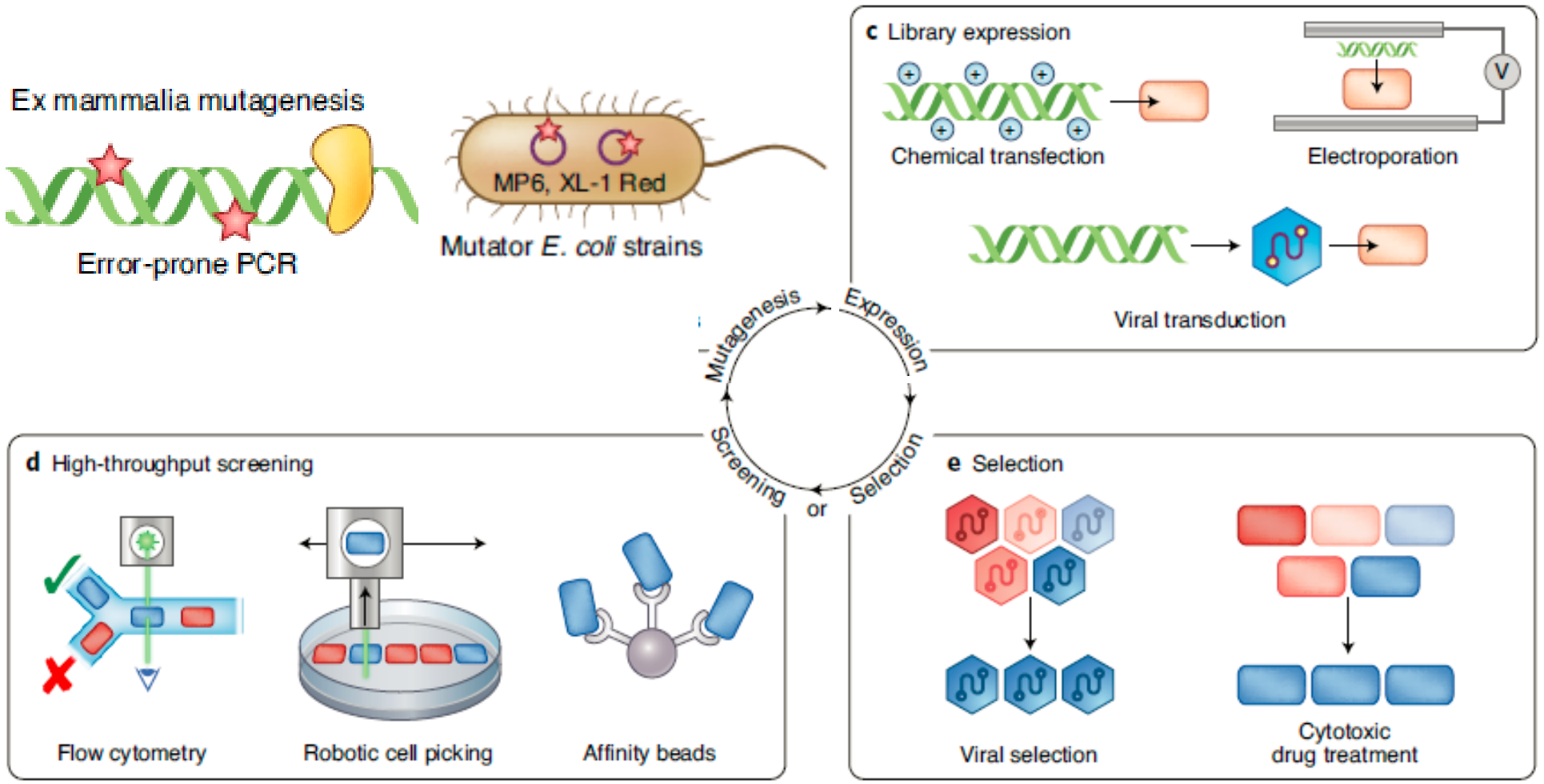


- Study the basic principles of evolution
- Generation of useful research tools (e.g. chemogenetics)
- Improving binding affinity of therapeutic antibodies (Affinity maturation) or the activity of de novo designed enzymes.
- Altering substrate specificity of existing enzymes
- ……

Classical ways of directed evolution

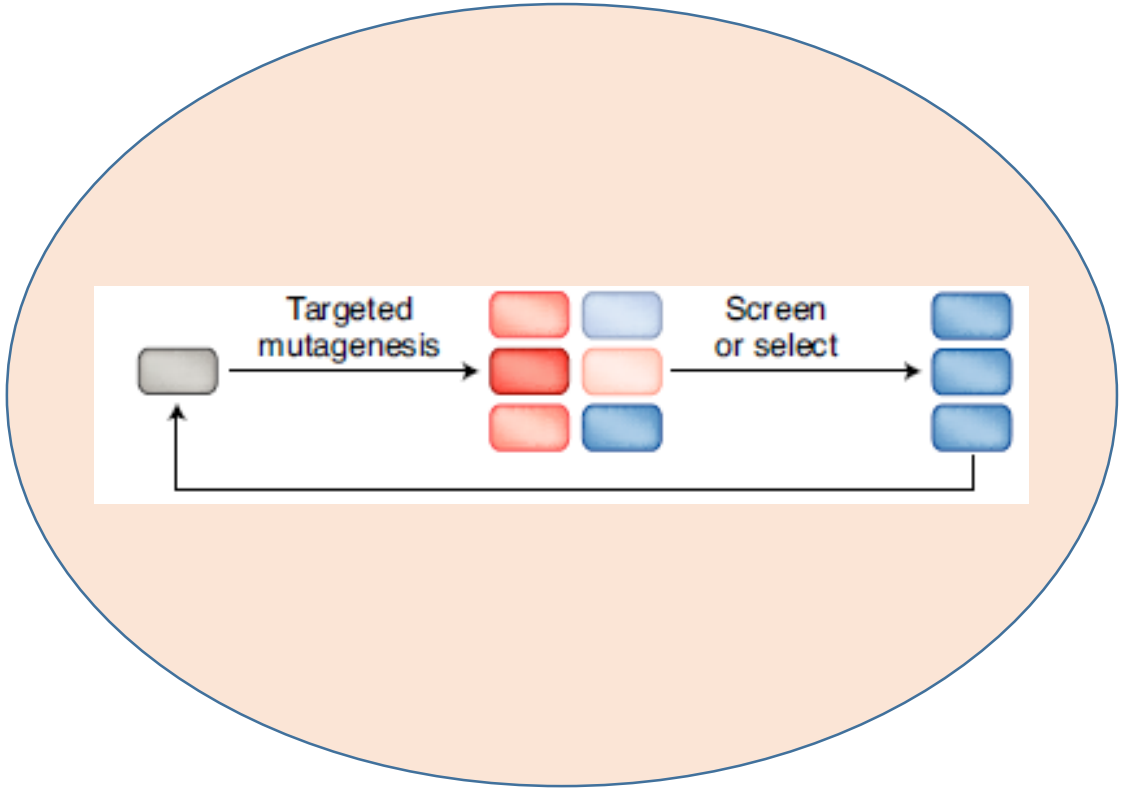


Samuel J. Hendel and Matthew D. Shoulders, Nature Methods, 2021



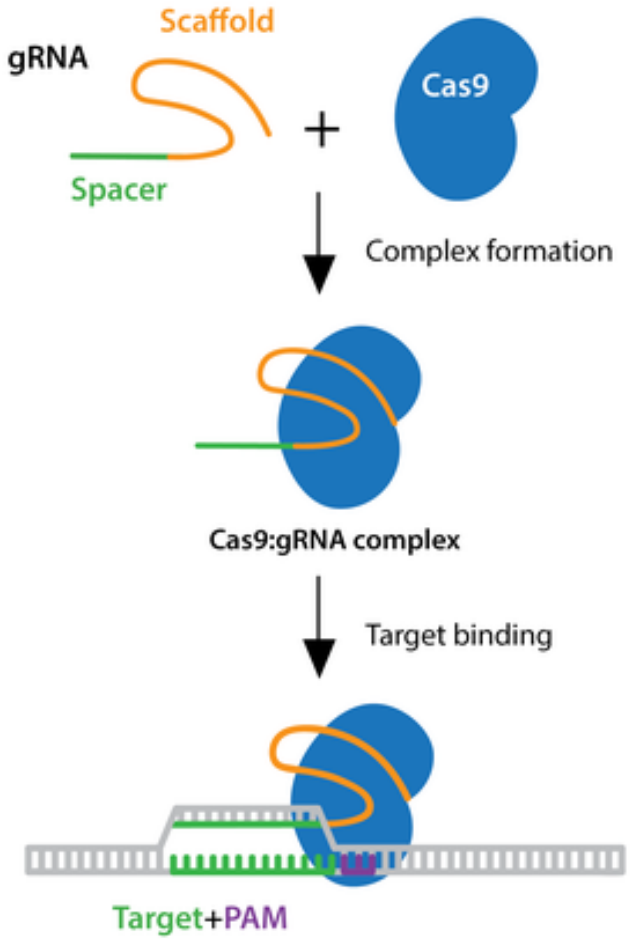
Samuel J. Hendel and Matthew D. Shoulders, Nature Methods, 2021

Integrated approaches of directed evolution in mammalian cells???

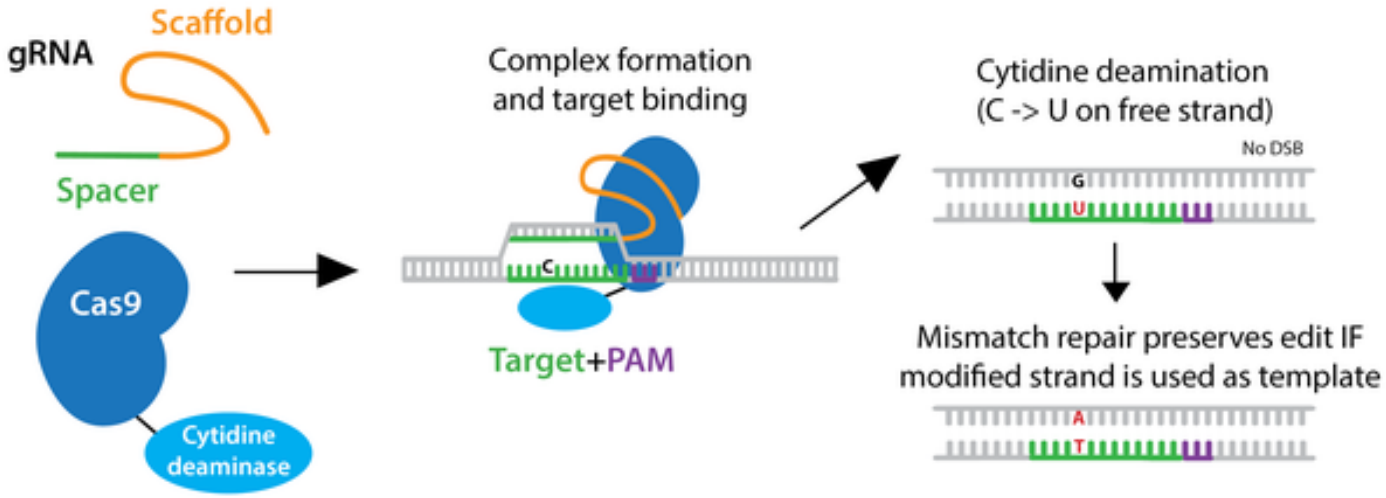


- CRISPR-CAS based approaches, e.g. CRISPR-X and EvolvR
- T7-RNA polymerase based approaches, e.g MutaT7, TRACE
- Virus based approaches, e.g. mPACE and VEGAS

The versatile CRISPR-CAS system

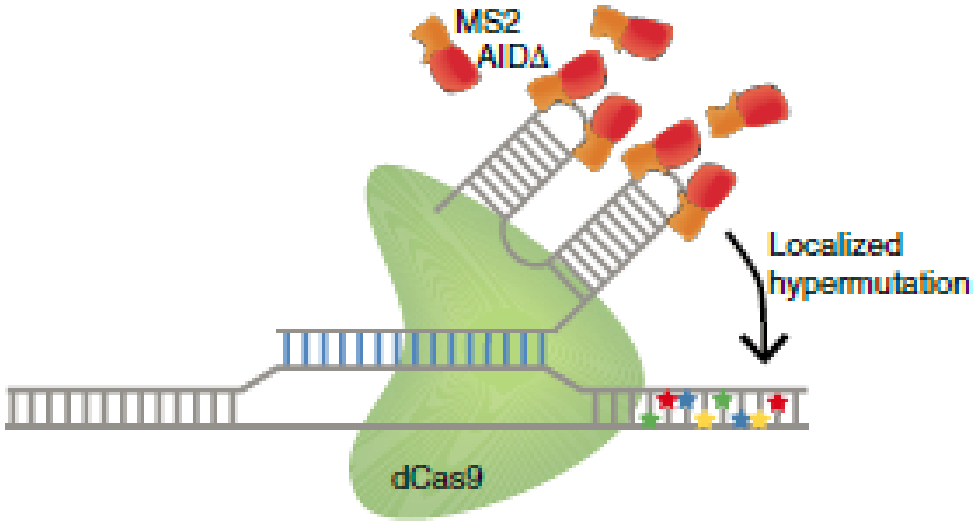


CRISPR Base Editing

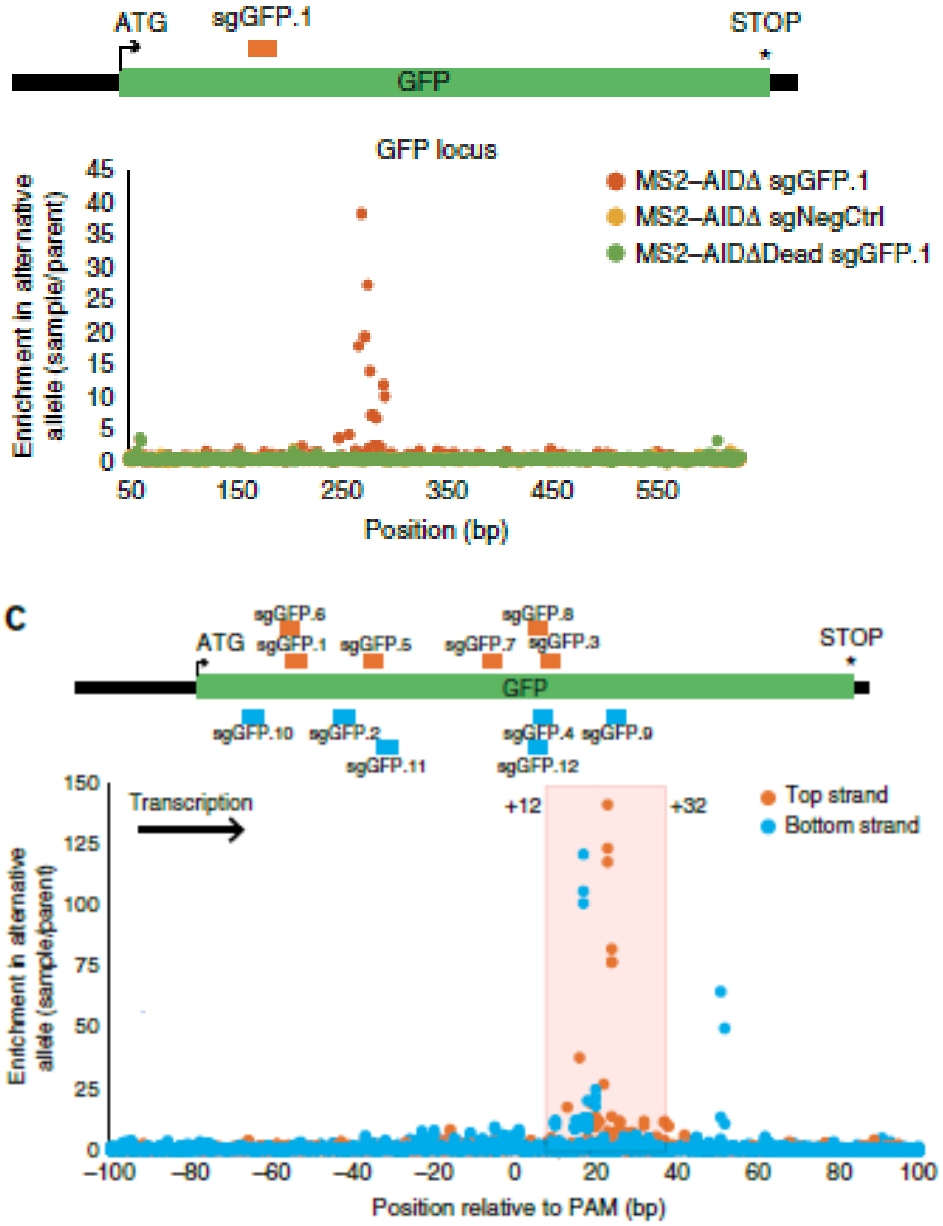


https://www.addgene.org/guides/crispr/

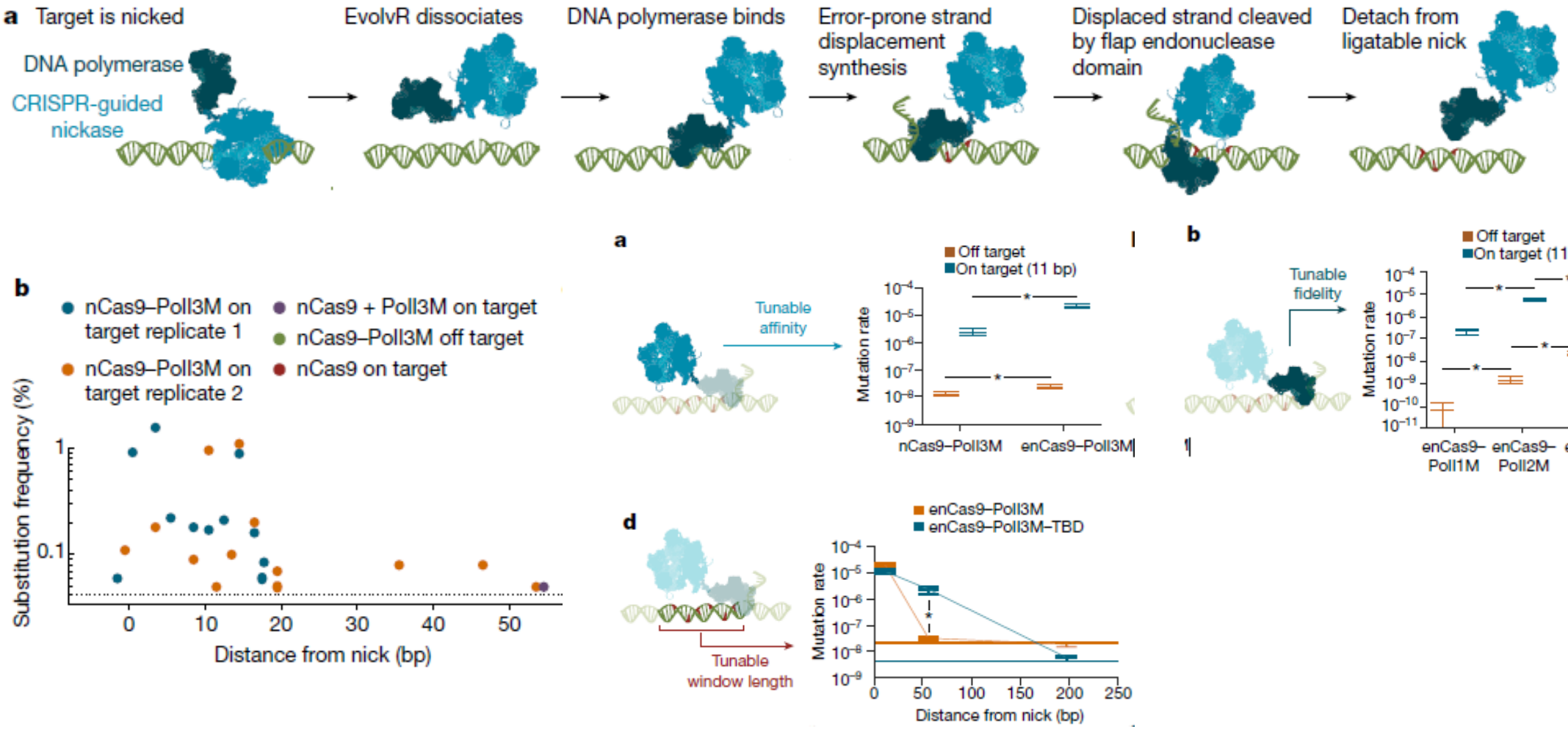
The CRISPR-X strategy



Gaelen T Hess & Michael C Bassik et al., Nature Methods, 2016

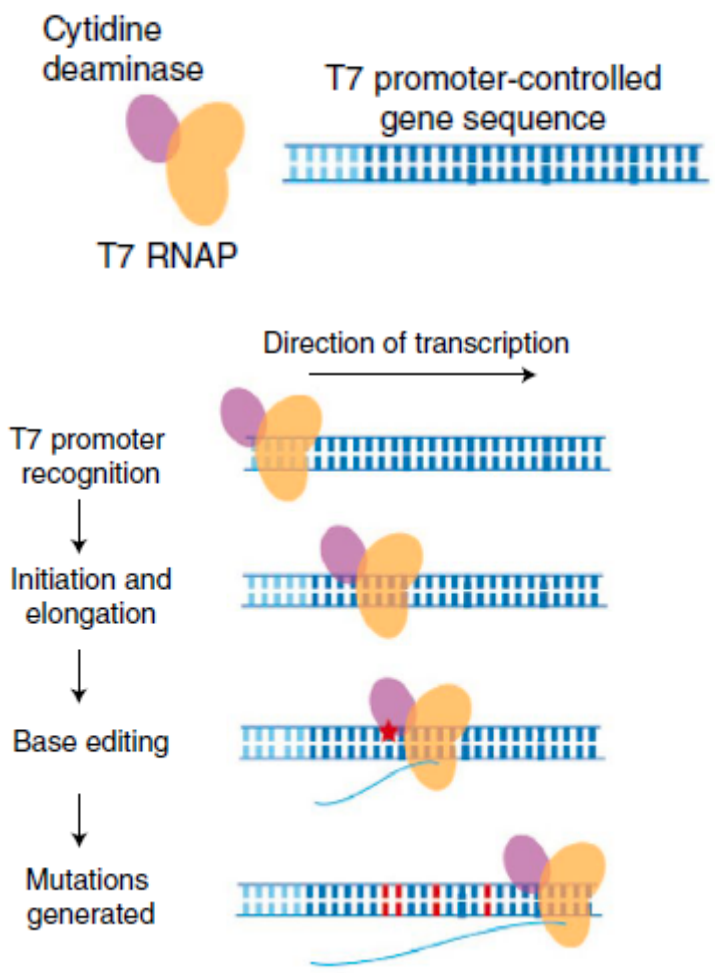


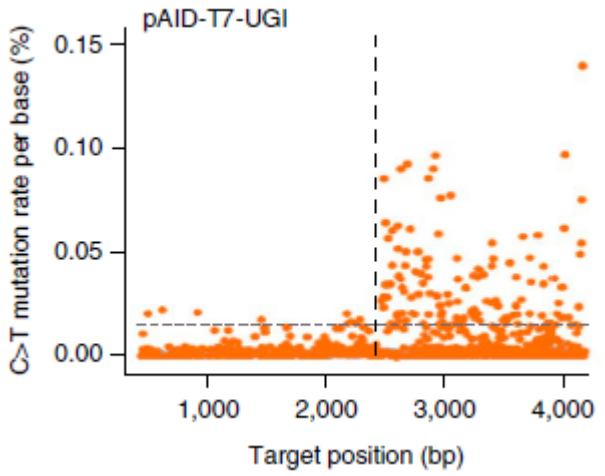
The EvolvR strategy

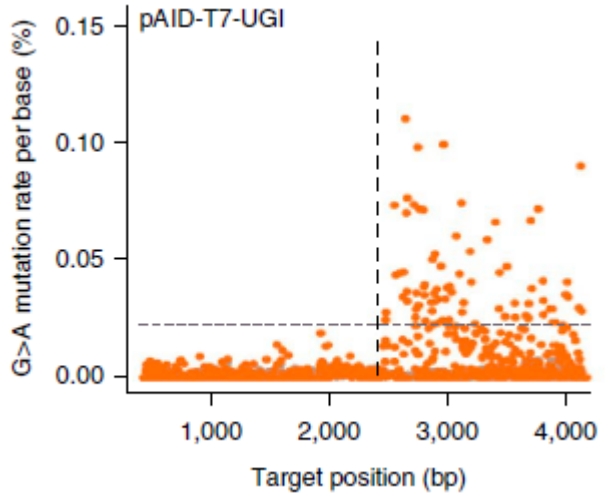


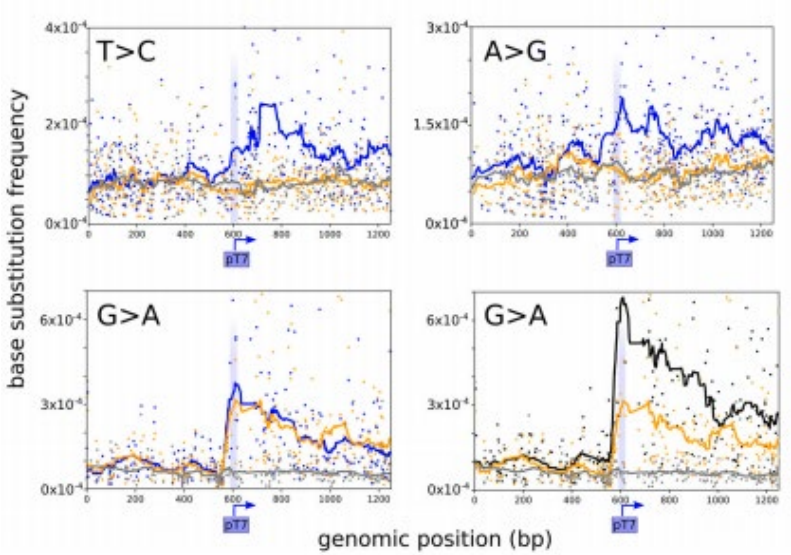
Shakked O. Halperin, David V. Schaffer & John E. Dueber et al., Nature, 2018

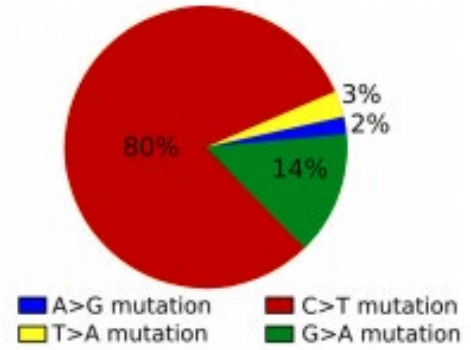
The T7-RNA polymerase based approaches











Haiqi Chen and Fei Chen et al., Nature Biotechnology, 2019
Aaron Cravens & Christina D. Smolke et al., Nature Communications, 2021

The state of the art: Virus-based directed evolution systems in mammalian cells

Cell

Sindbis Virus-based

Resource

VEGAS as a Platform for Facile Directed Evolution in Mammalian Cells

Justin G. English,[1,5,*] Reid H.J. Olsen,[1] Katherine Lansu,[1] Michael Patel,[2] Karoline White,[3] Adam S. Cockrell,[4] Darshan Singh,[1] Ryan T. Strachan,[1] Daniel Wacker,[1] and Bryan L. Roth[1,*]

AdenoVirus-based

JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Article

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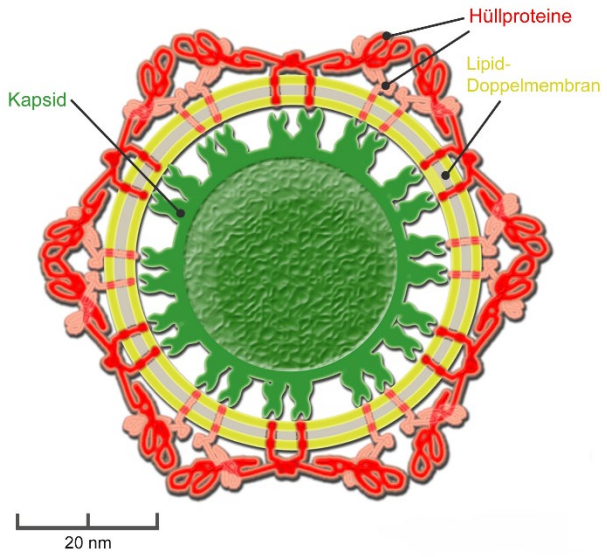
An Adaptable Platform for Directed Evolution in Human Cells

Chet M. Berman,[†,∥,#] Louis J. Papa, III,[†,#] Samuel J. Hendel,[†,#] Christopher L. Moore,[†] Patreece H. Suen,[†] Alexander F. Weickhardt,[†] Ngoc-Duc Doan,[†] Caiden M. Kumar,[†] Taco G. Uil,[§,⊥] Vincent L. Butty,[‡] Robert C. Hoeben,[§] and Matthew D. Shoulders*[,†]

Sindbis virus (SINV) is an enveloped RNA virus of the genus *Alphavirus* in the virus family *Togaviridae*.

It was first isolated from Culex mosquitoes in 1952 in the Nile river delta in Egypt. The first human cases were described in Uganda in 1961, South Africa in 1963, and Australia in 1967. However, clinical SINV infection in humans has almost exclusively been reported in Northern Europe where it is endemic and where large outbreaks occur intermittently.

https://www.ecdc.europa.eu/en/sindbis-fever/facts

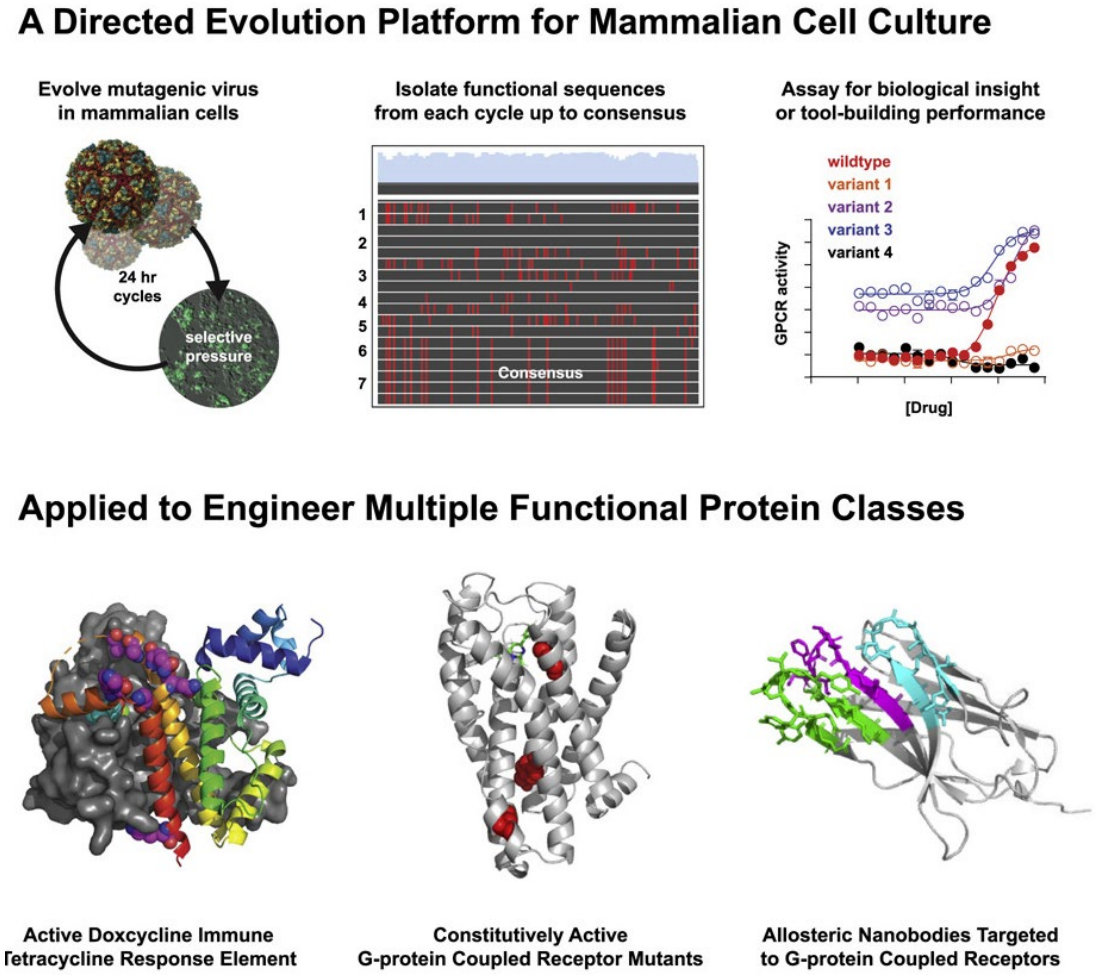


Fast replication (~ 24 hours per cycle)

RNA viruses, such as Sindbis, are **highly mutagenic**, with no known proof-reading capability.

Has its' **own** RNA-dependent **RNA replicase** targeted to the viral genome by cis-acting, **conserved sequences**. These sequences are required to initiate replication and RNA templates, even those from related viral families, cannot be replicated by the Sindbis virus replicase, resulting in **high selectivity** between the replicase and the Sindbis virus genome

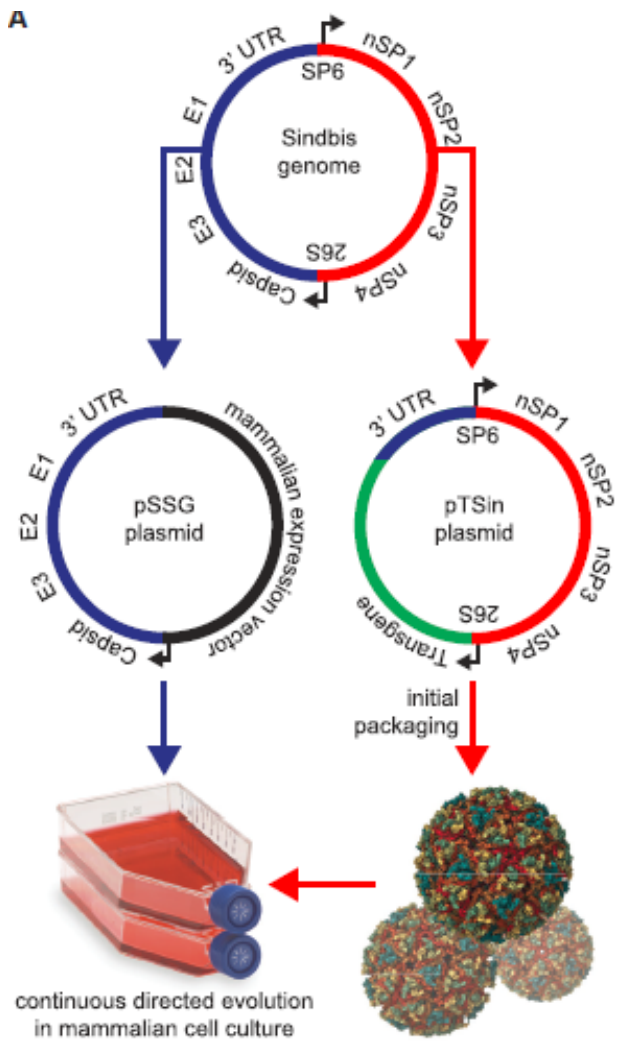
The VEGAS strategy

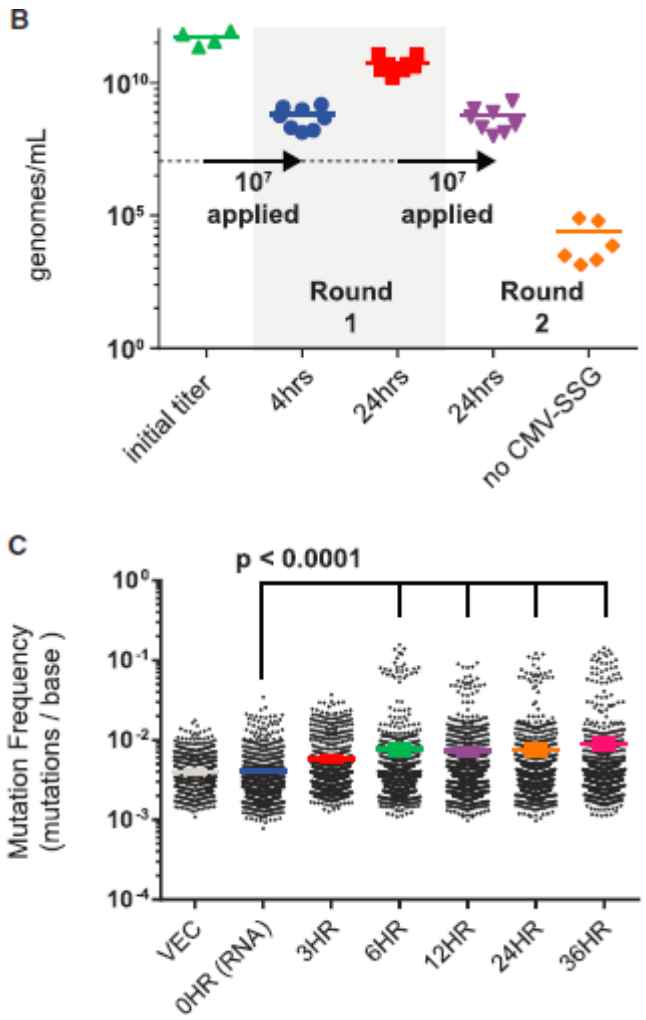


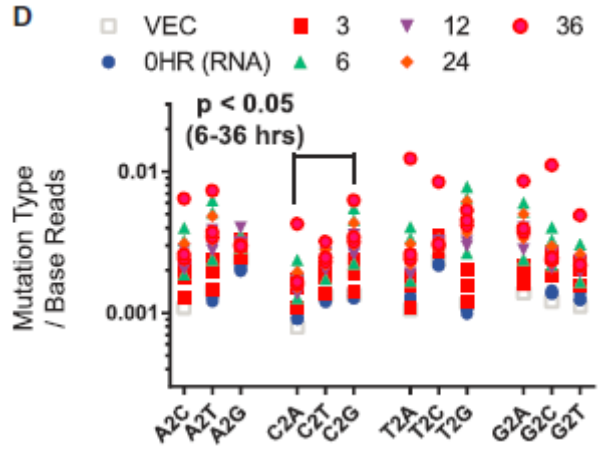
Highlights

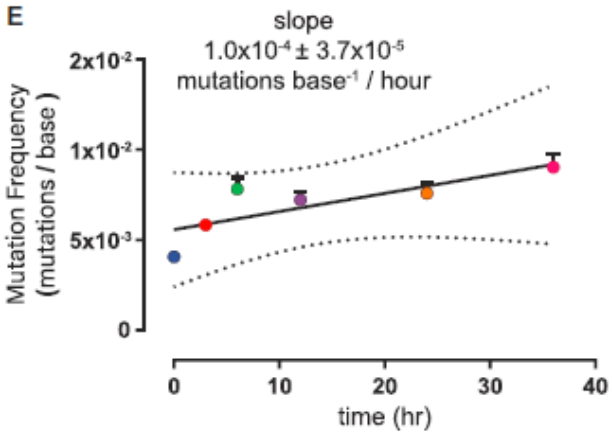
- One day per round of directed molecular evolution in mammalian cells
- Mutation rates of 10^{-3} from each round, surpassing many *in vitro* systems
- System does not require resetting or recycling of hits
- Three unique campaigns are presented, each succeeding in less than a week

Development of Sindbis virus for facile, mutagenic viral propagation in mammalian cell culture



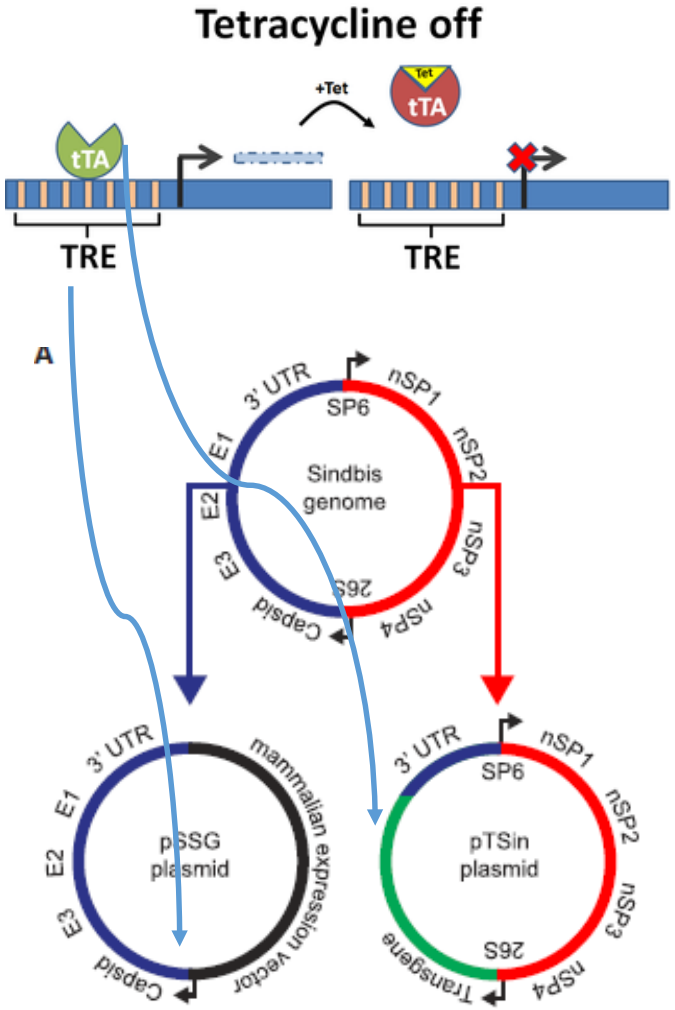


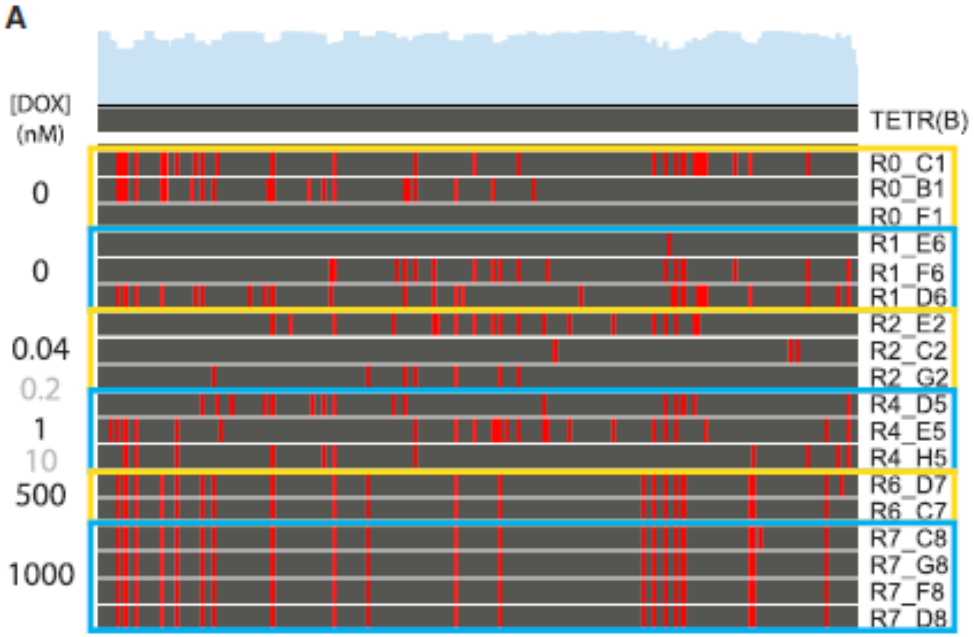


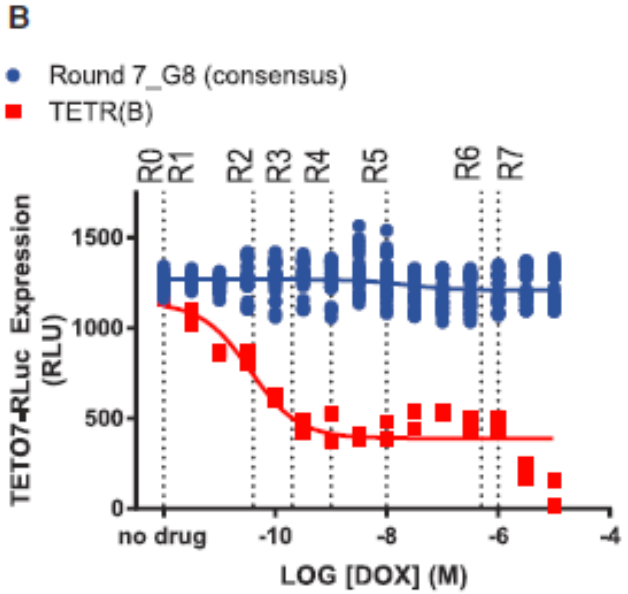


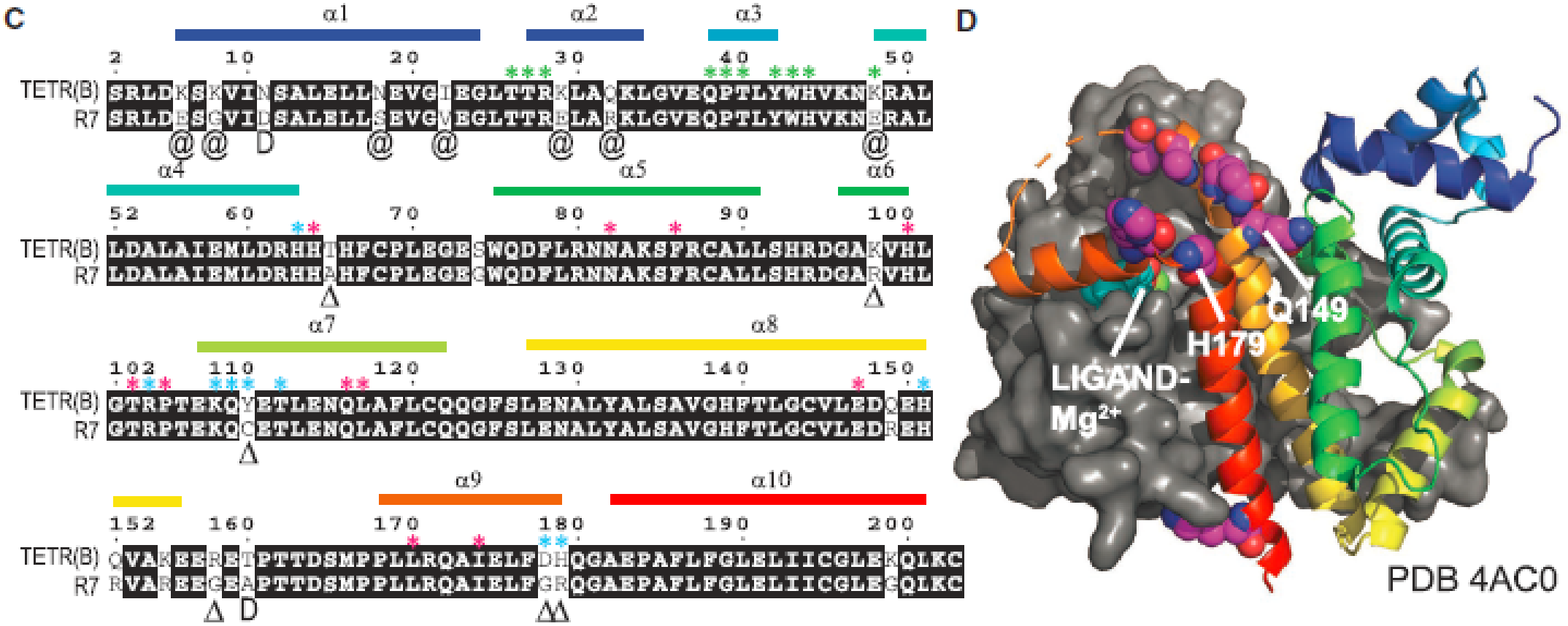
~ 1 mutation per 1,000 bases replicated or >10e9 total mutations per day at the observed viral production rates.

Proof of concept: directed evolution of a doxycycline (DOX)-resistant variant of the transcription factor tTA.





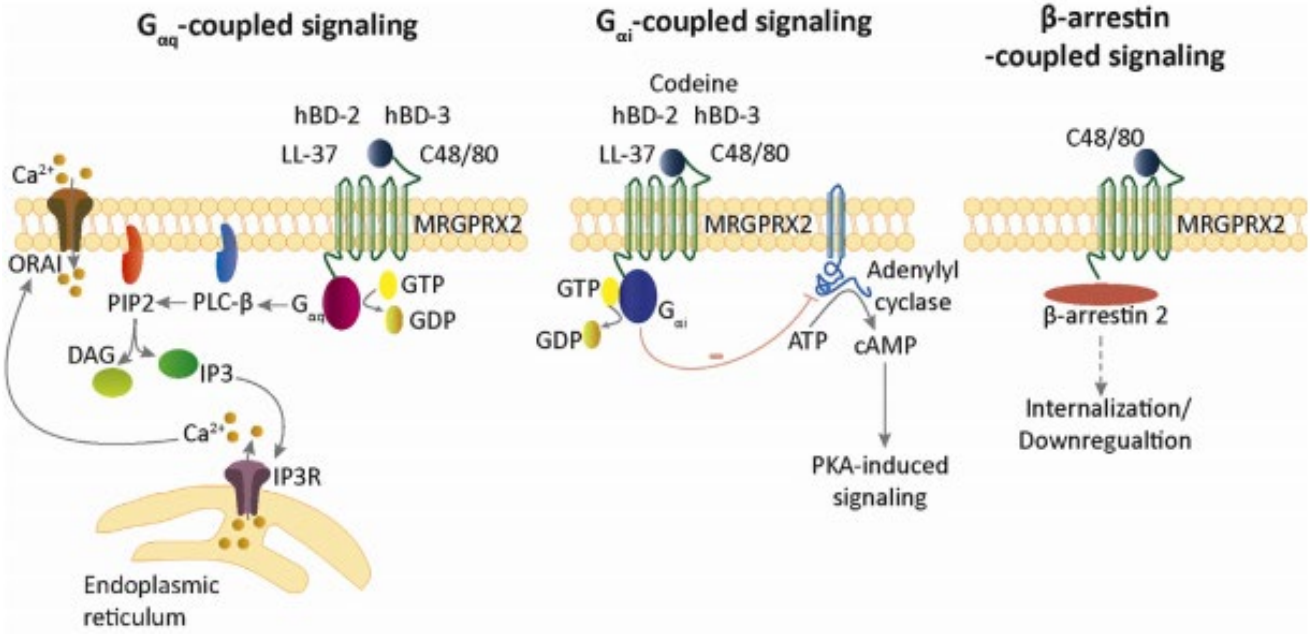




C. Peptide sequence alignment of TETR(B) and the R7 consensus. Exact residue (Δ), position (@), or subtype (D) substitutions previously published to enhance tTA activity in the presence of DOX. Residues (*) with direct involvement in DNA binding (green), ligand binding (magenta), and ligand entry (cyan).

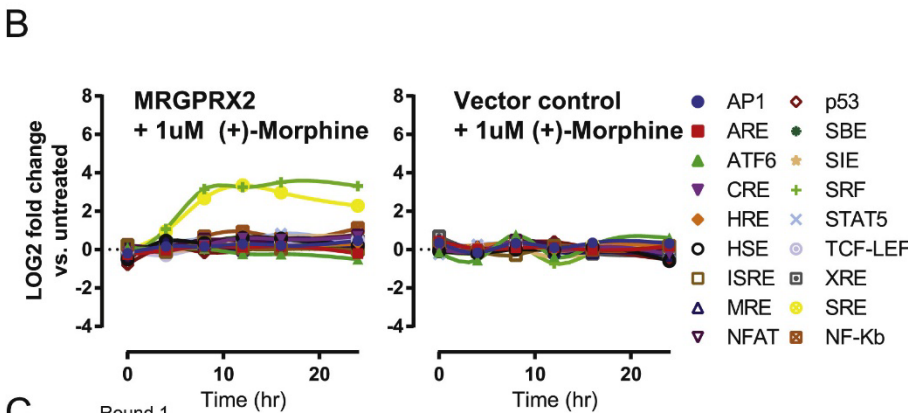
Application of VEGAS for directed evolution of GPCRs

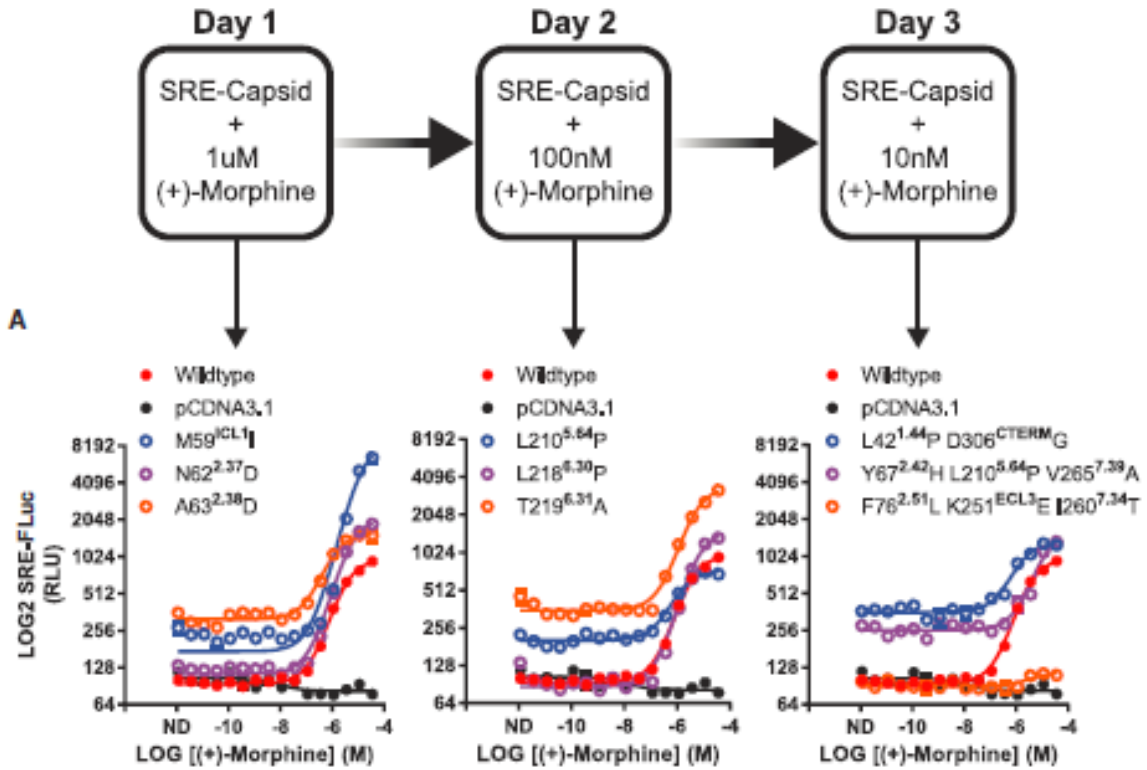
Mas-related G protein-coupled receptor X2 (MRGPRX2) is a primate-exclusive GPCR recently identified as an atypical opioid-recognition receptor, mainly expressed by neurons and mast cells.

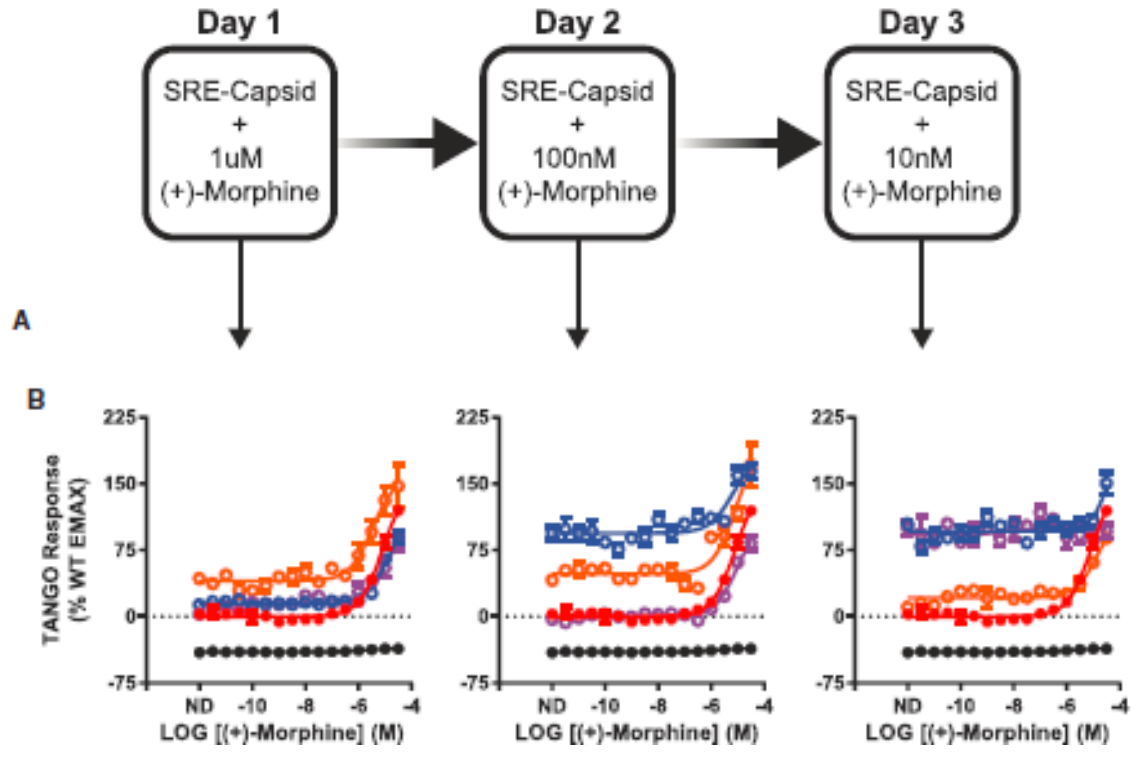


Helen Kuhn, Pavel Kolkhir, Andreas E. Kremer and Marcus Maurer et al.,, Journal of Allergy and Clinical Immunology, 2016

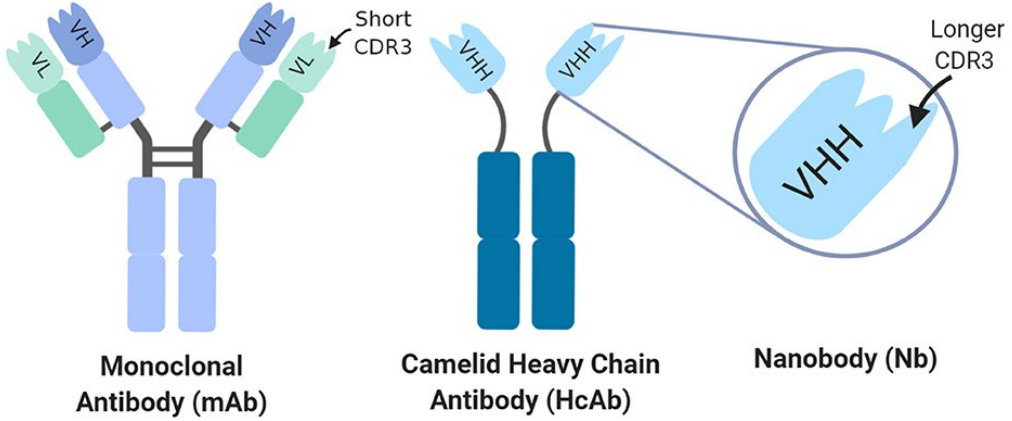
Goal: Use VEGAS to develop constitutively active mutations (CAMs) of MRGPRX2 activated by (+)-morphine.



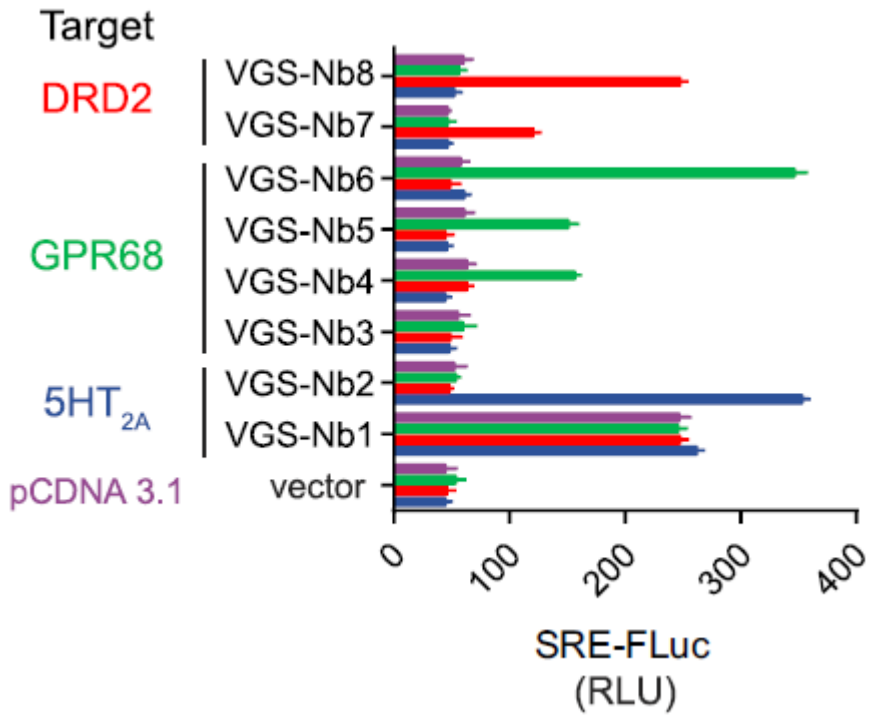

Day 1
SRE-Capsid + 1uM (+)-Morphine
Day 2
SRE-Capsid + 100nM (+)-Morphine
Day 3
SRE-Capsid + 10nM (+)-Morphine
A
Wildtype
pCDNA3.1
M59ICL1I
N62 2.37 D
A63 2.38 D
L210 5.64 P
L218 6.30 P
T219 6.31 A
L42 1.44 P D306 CTERM G
Y67 2.42 H L210 5.64 P V265 7.39 A
F76 2.51 L K251 ECL3 E I260 7.34 T
LOG2 SRE-F Luc (RLU)
8192
4096
2048
1024
512
256
128
64
ND -10 -8 -6 -4
LOG [(+)-Morphine] (M)

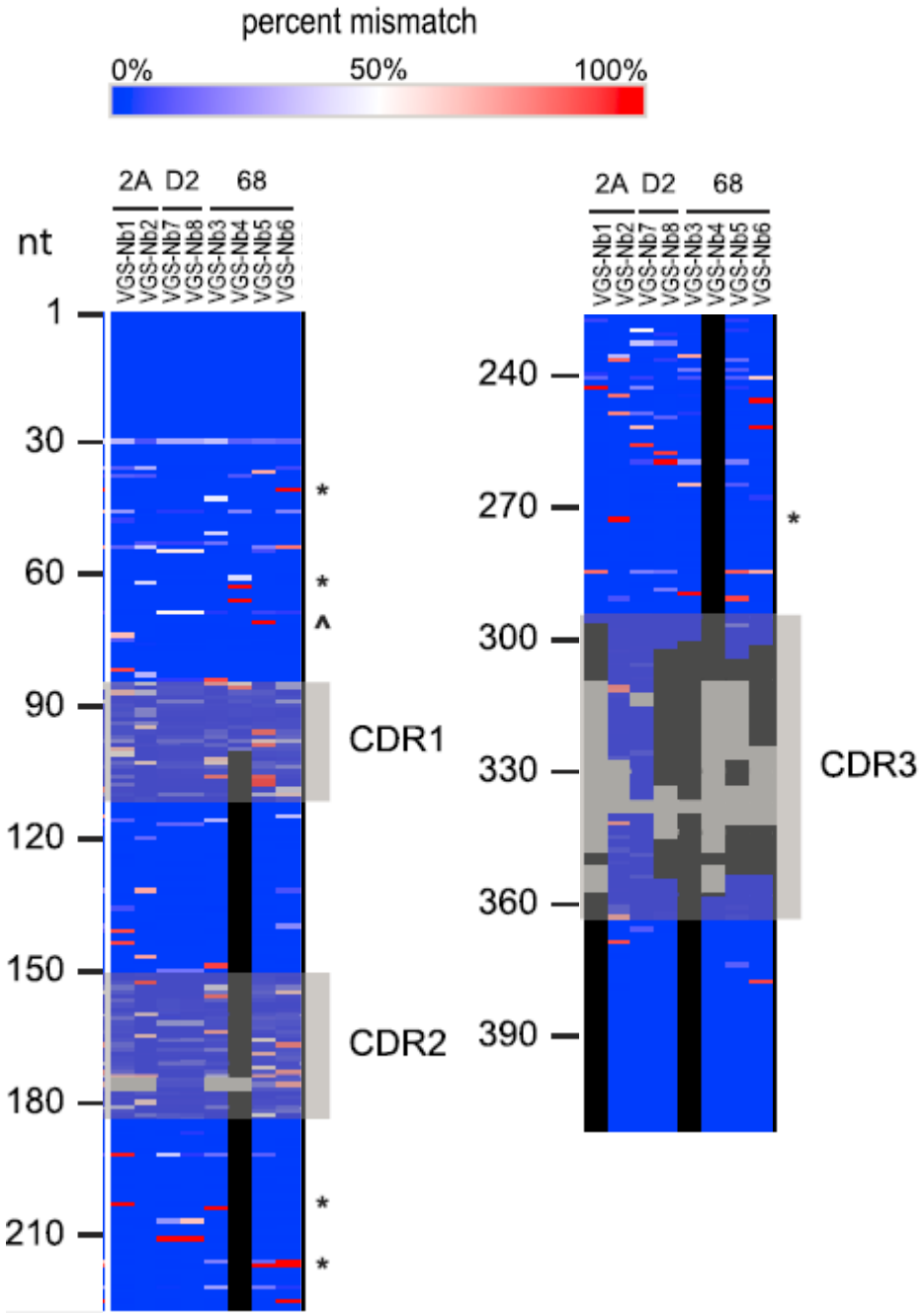

Day 1
SRE-Capsid + 1uM (+)-Morphine
Day 2
SRE-Capsid + 100nM (+)-Morphine
Day 3
SRE-Capsid + 10nM (+)-Morphine
A
B
TANGO Response (% WT EMAX)
225
150
75
0
-75
ND -10 -8 -6 -4
LOG [(+)-Morphine] (M)

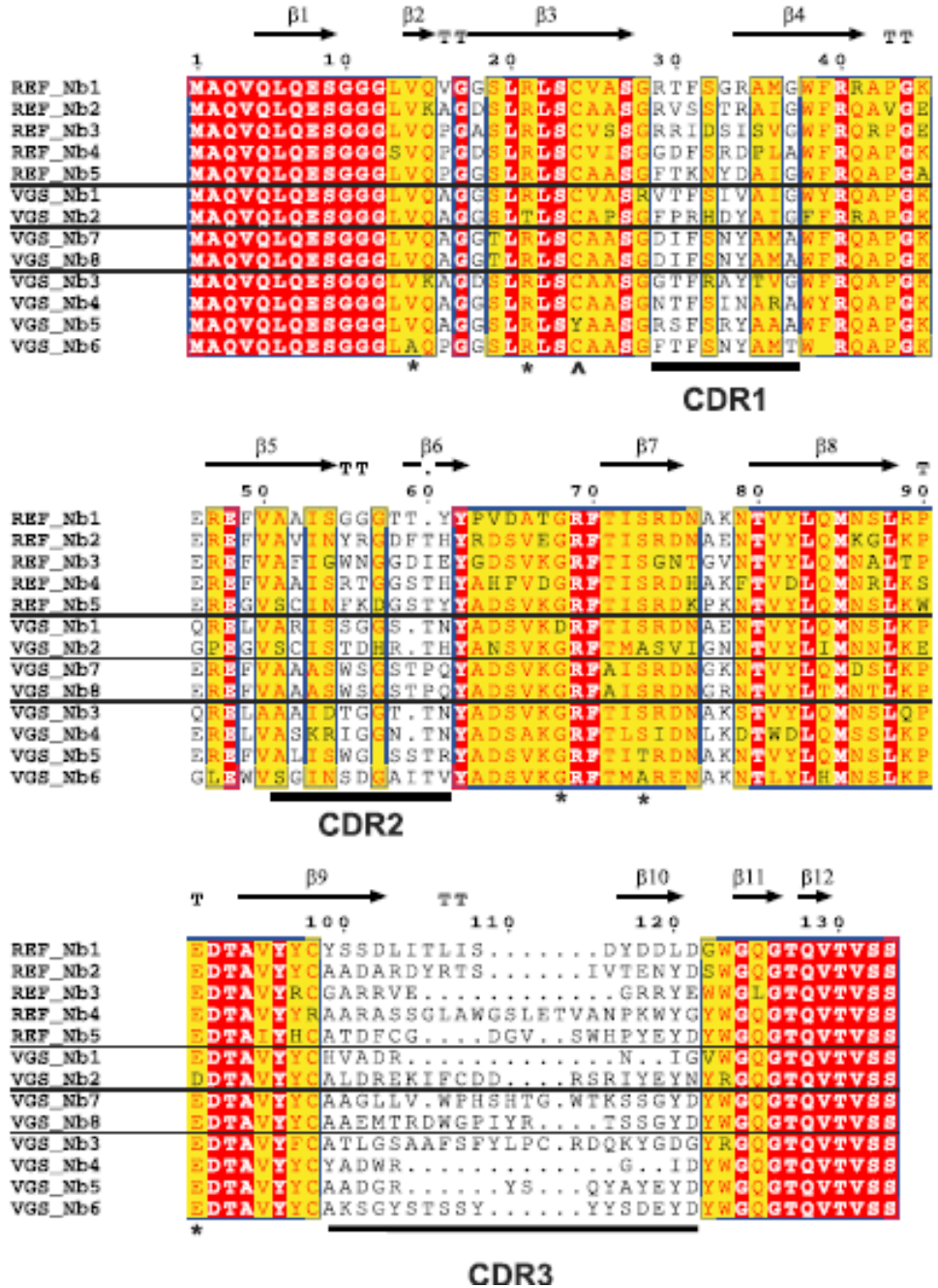
VEGAS for the Evolution of Active-State Nanobodies



Directed evolution of 5HT2A nanobodiy library to three GPCR targets




percent mismatch
0%
50%
100%
2A D2 68
nt
VGS-Nb1
VGS-Nb2
VGS-Nb7
VGS-Nb8
VGS-Nb3
VGS-Nb4
VGS-Nb5
VGS-Nb6
1
30
60
90
120
150
180
210
240
270
300
330
360
390
CDR1
CDR2
CDR3


REF_Nb1
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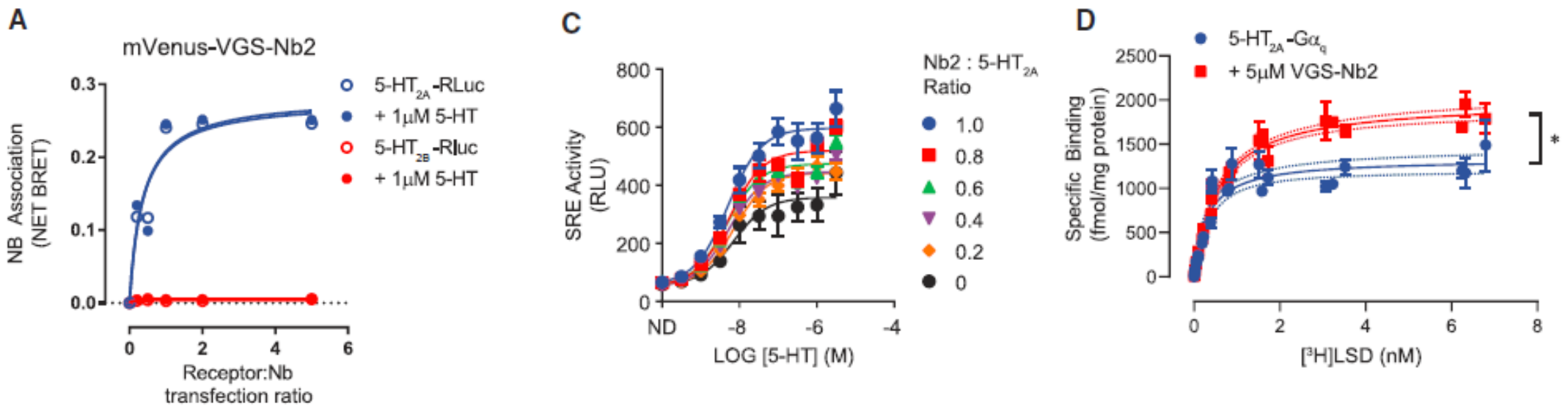
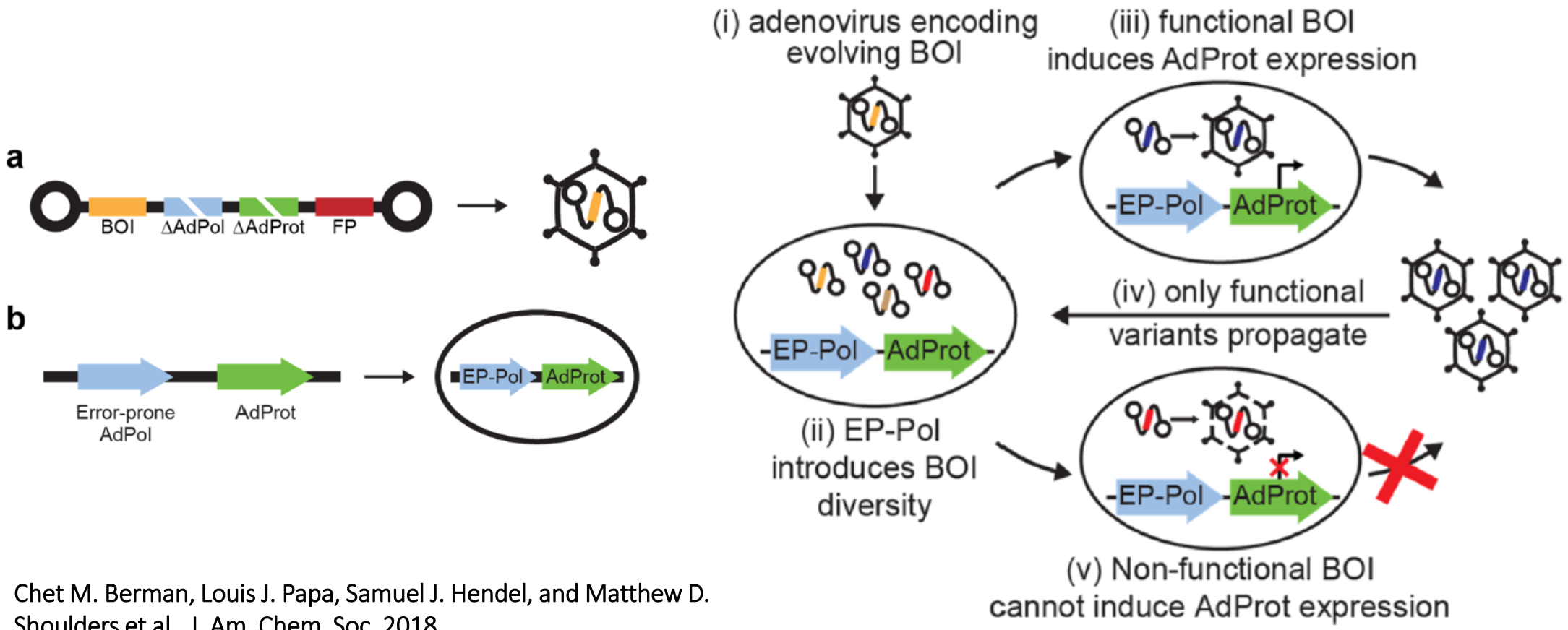
REF_Nb5
VGS_Nb1
VGS_Nb2
VGS_Nb7
VGS_Nb8
VGS_Nb3
VGS_Nb4
VGS_Nb5
VGS_Nb6
CDR1
CDR2
CDR3

Further characterization of evolved 5-TH2A nanobodies



The adenovirus-based directed evolution strategy



Chet M. Berman, Louis J. Papa, Samuel J. Hendel, and Matthew D. Shoulders et al., J. Am. Chem. Soc. 2018

Thank you for your attention!

Questions?