Engineering a Model Cell for Rational Tuning of GPCR Signaling

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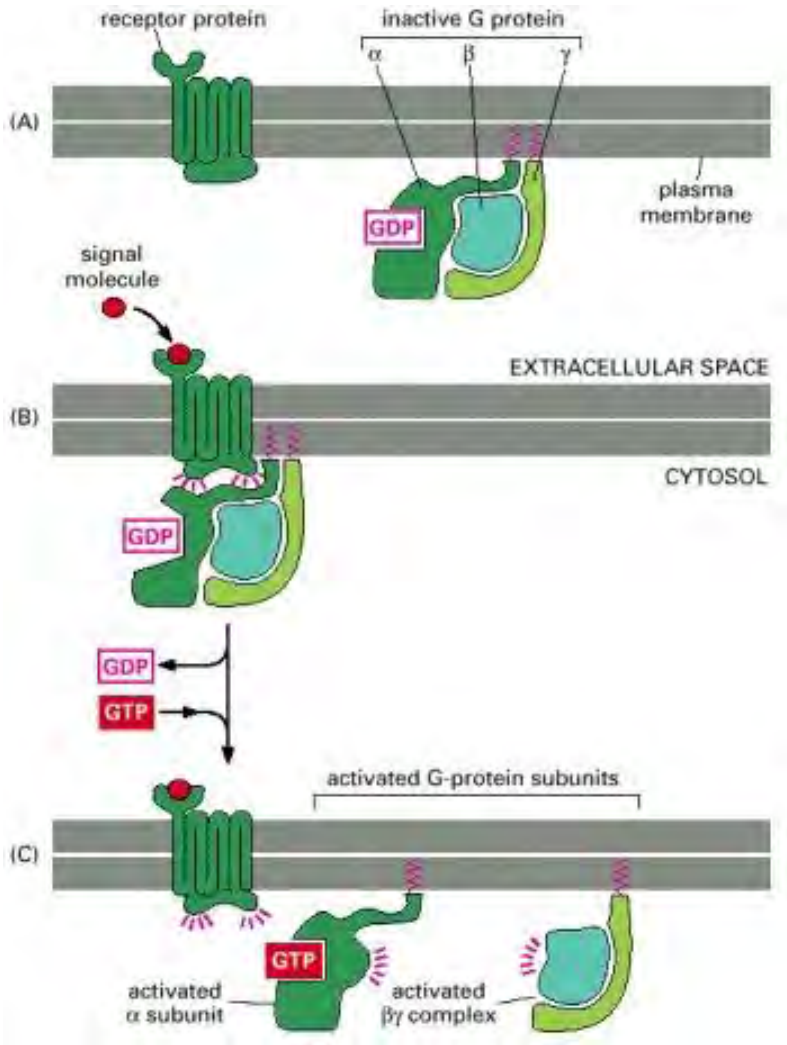
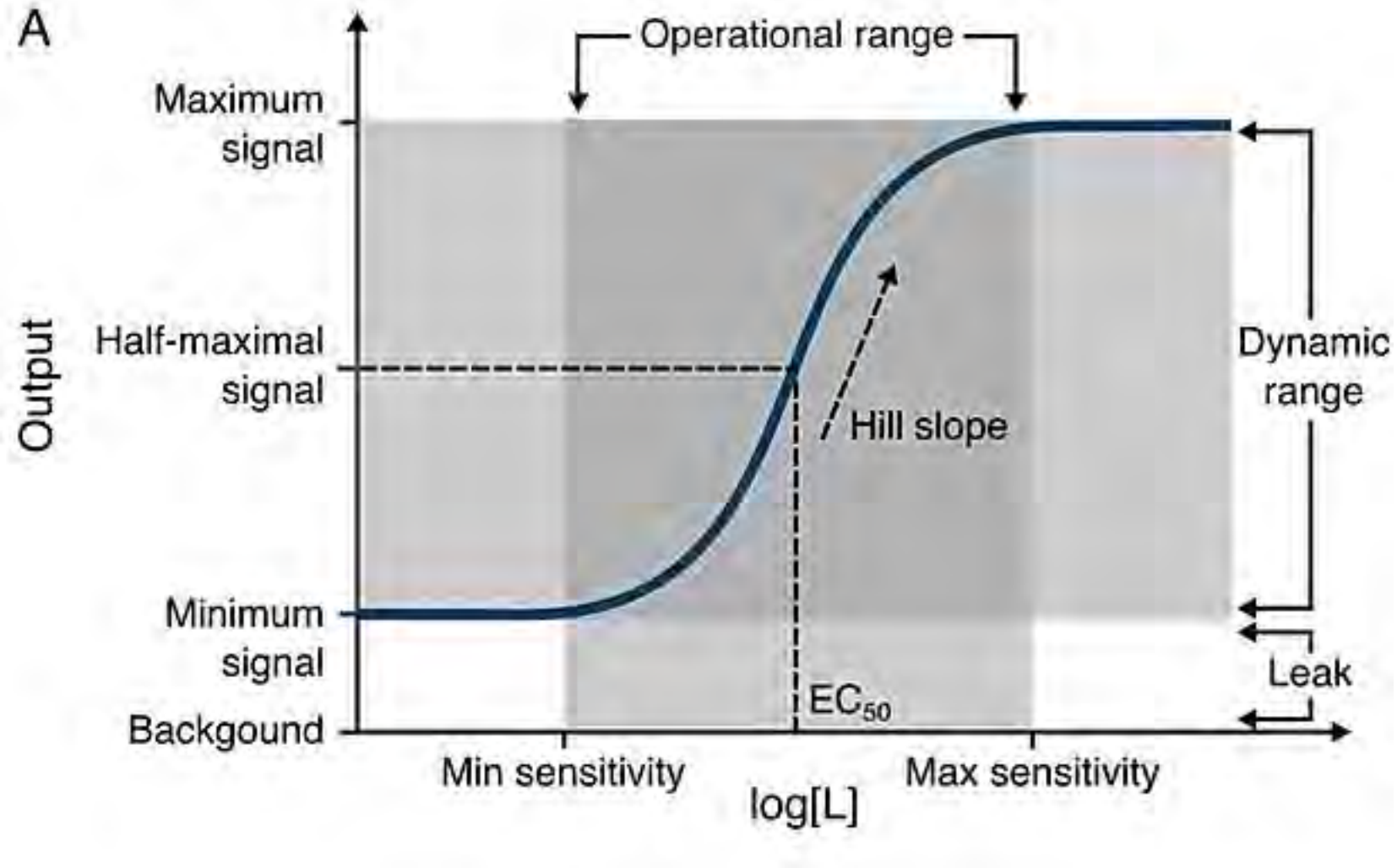
GPCR signalling:

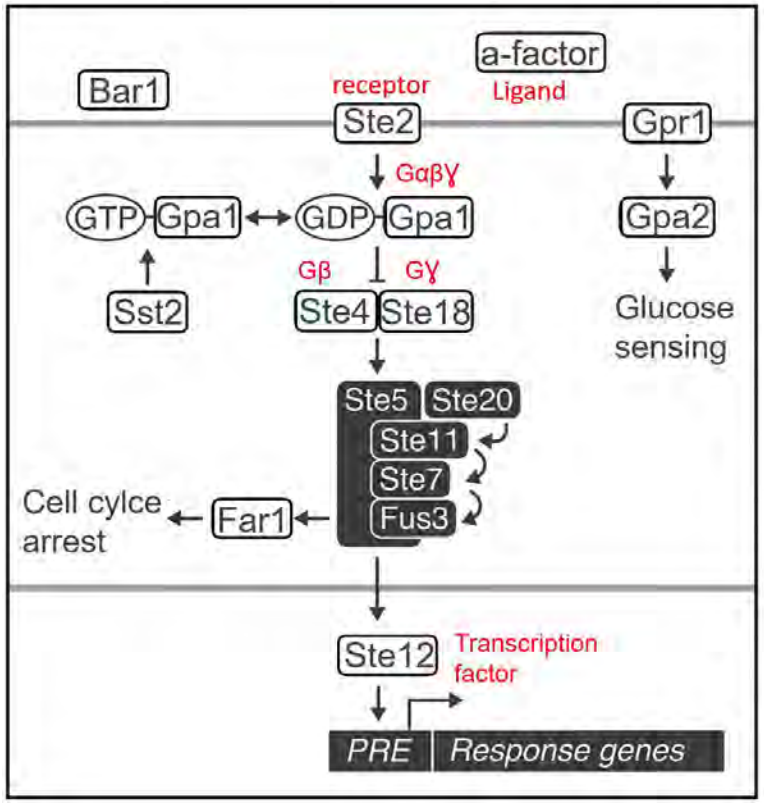
- Widely represented in eukaryotic lifeforms
- Largest family of signalling proteins in humans (>800 members)
- (indirect) target of almost half of all known drugs
- General mechanism, modular
- Very suited for biosensors



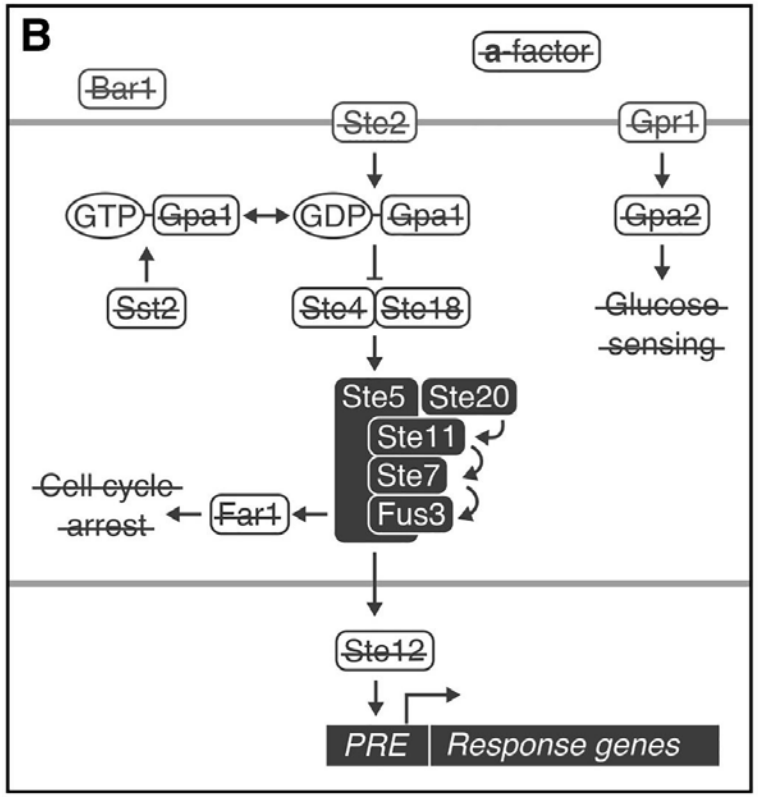
Characteristics of a sensor sensor



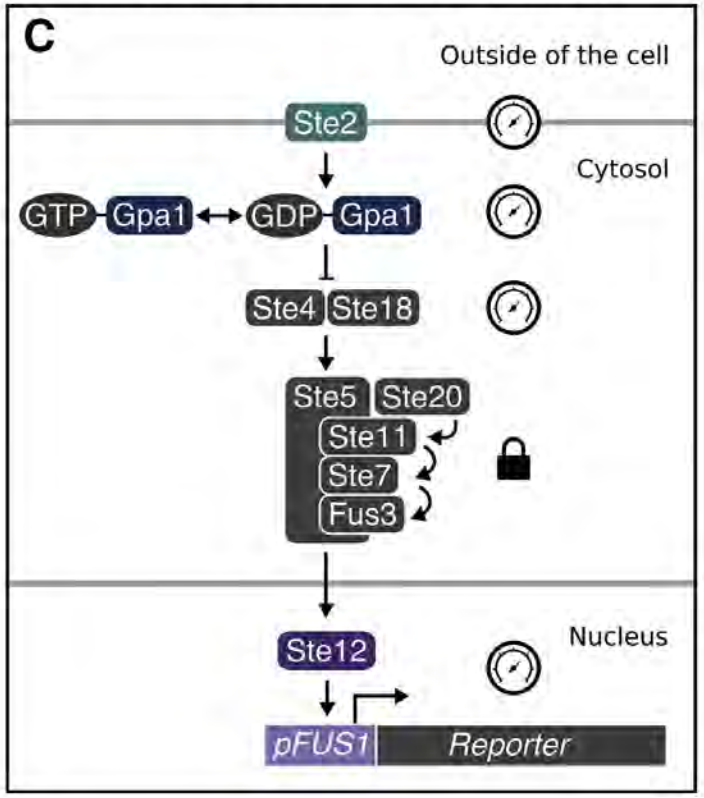
The pheromone response pathway of Saccharomyces cervisiae

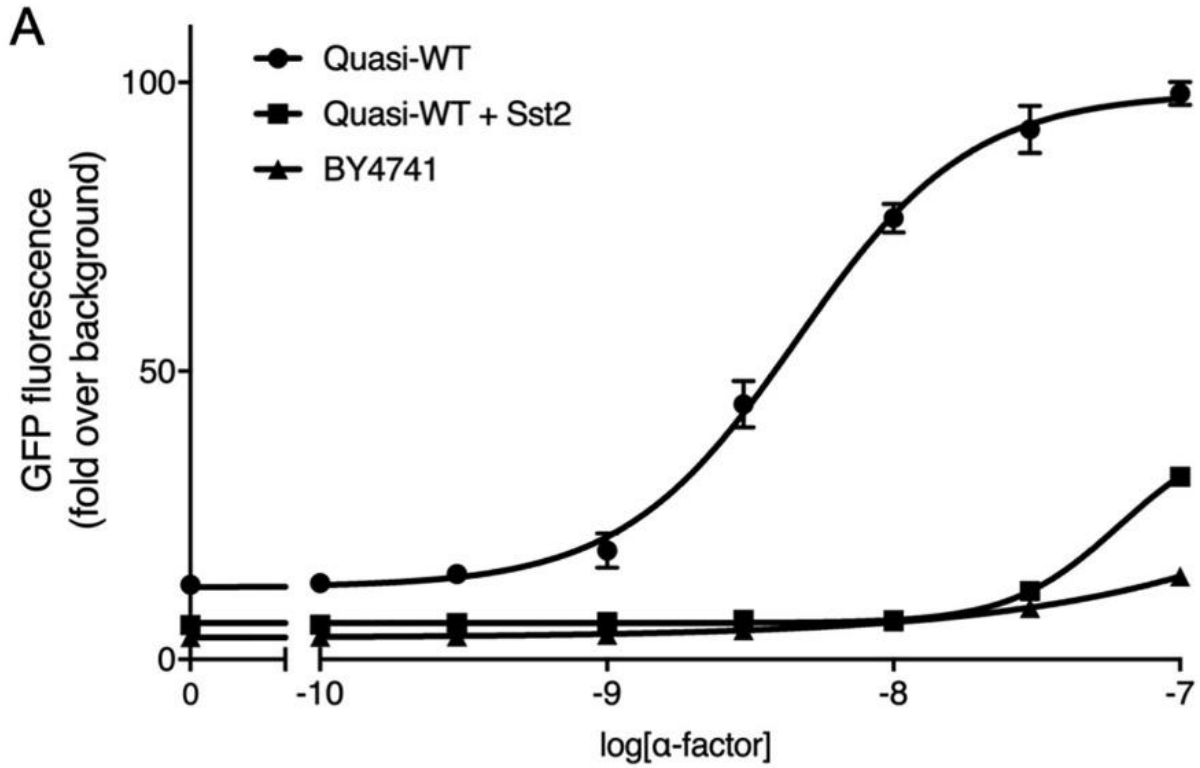


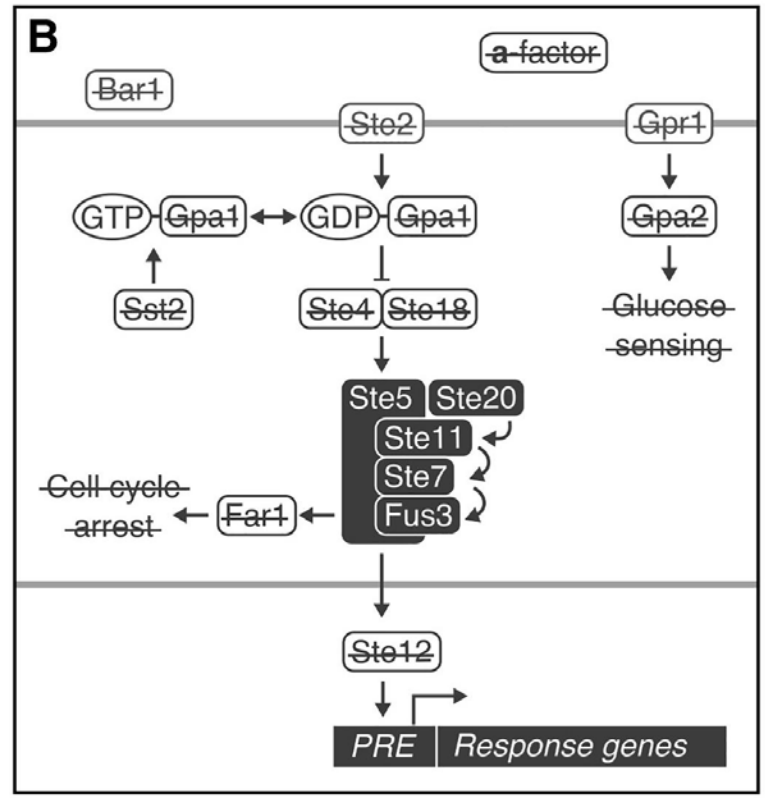
11 of the 15 genes deleted in the GPCR model strain



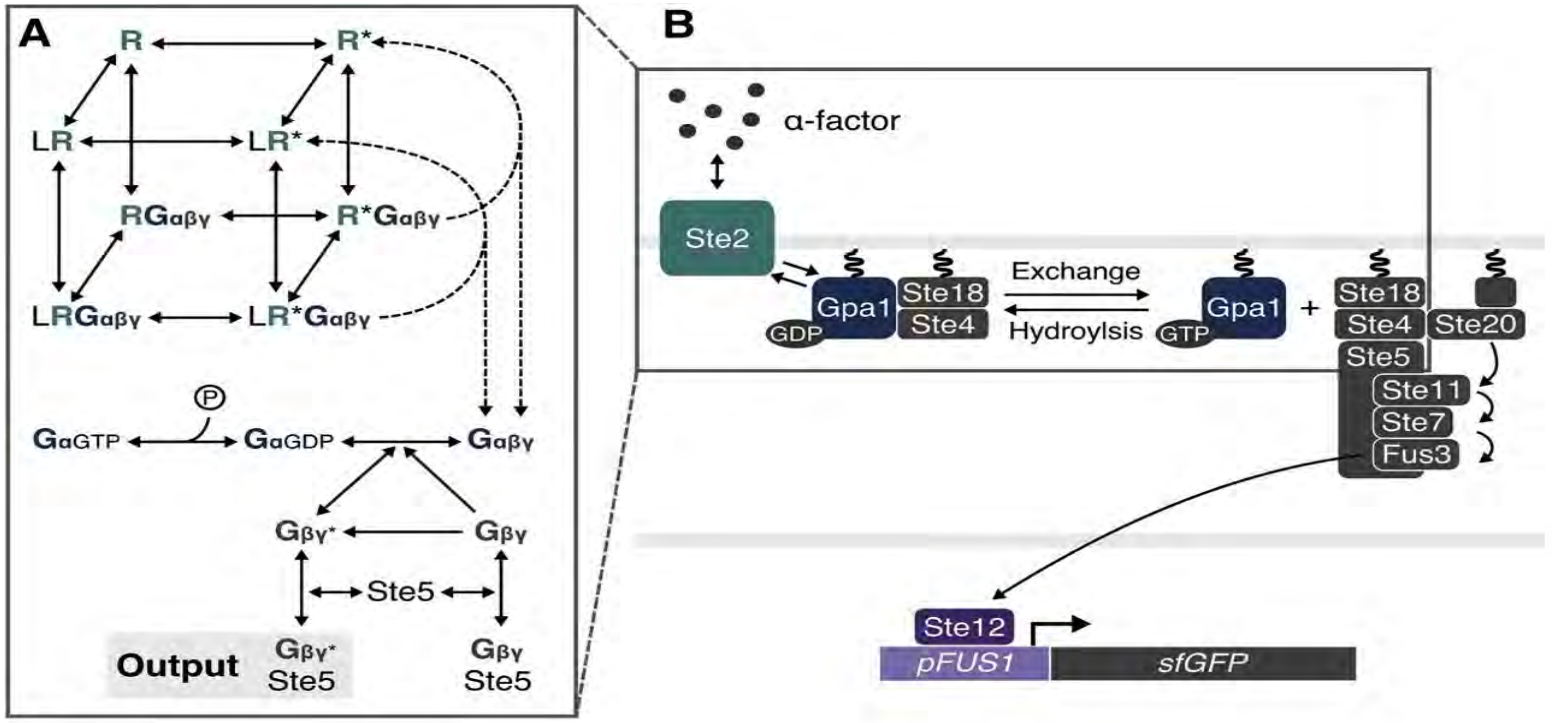
Refactored minimised GPCR pathway



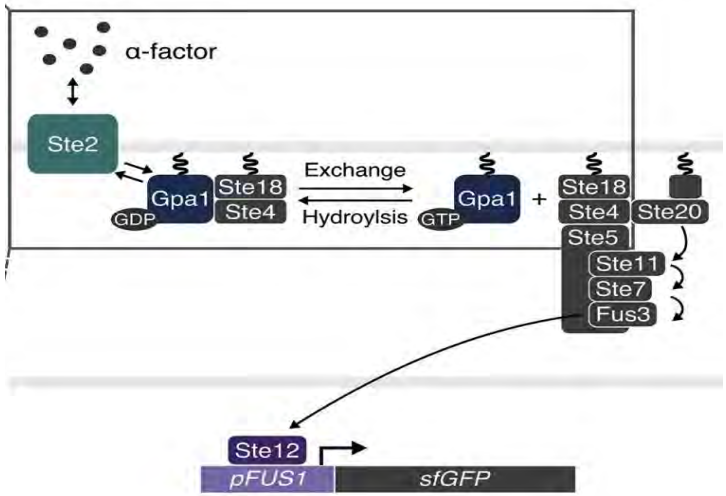

A
Quasi-WT
Quasi-WT + Sst2
BY4741
GFP fluorescence
(fold over background)
100
50
0
0
-10
-9
-8
-7
log[α-factor]


B
a-factor
Bar1
Ste2
Gpr1
GTP
Gpa1
GDP
Gpa1
Gpa2
Sst2
Ste4
Ste18
Glucose
sensing
Ste5
Ste20
Ste11
Ste7
Fus3
Cell cycle
arrest
Far1
Ste12
PRE
Response genes

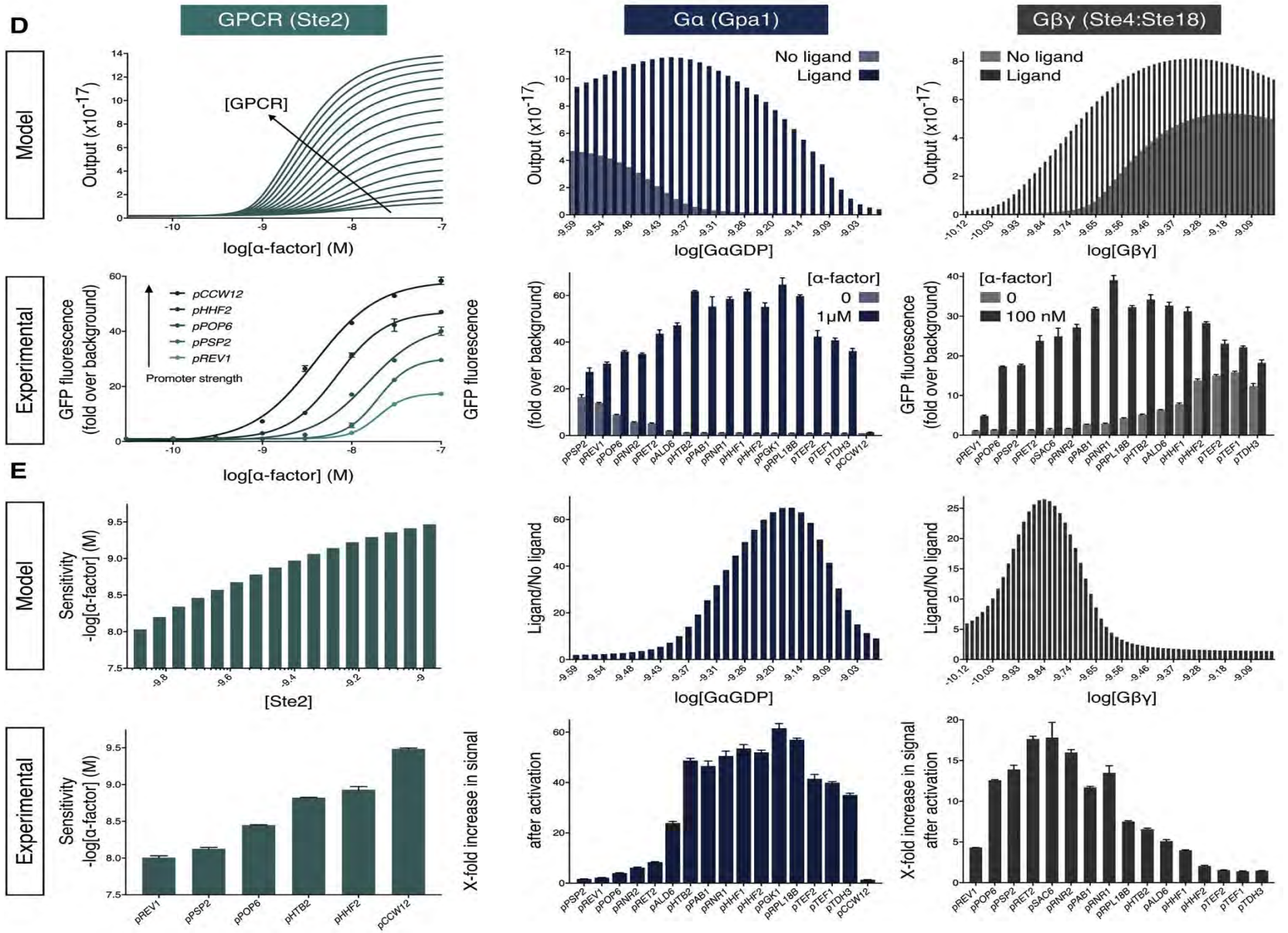
Mathematical Model



minimum Signal
maximum Signal

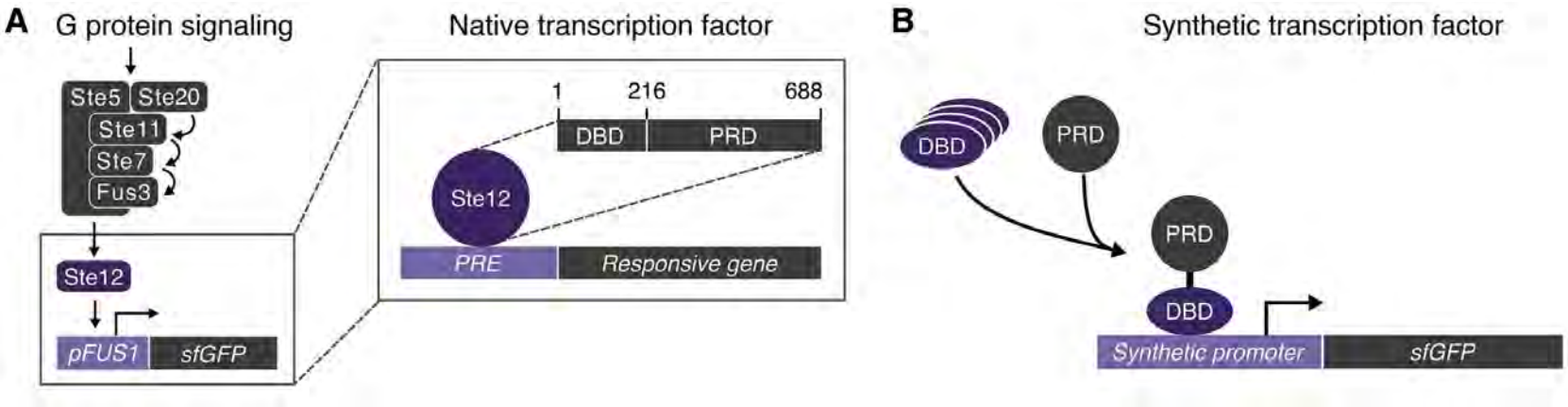


Sensitivity

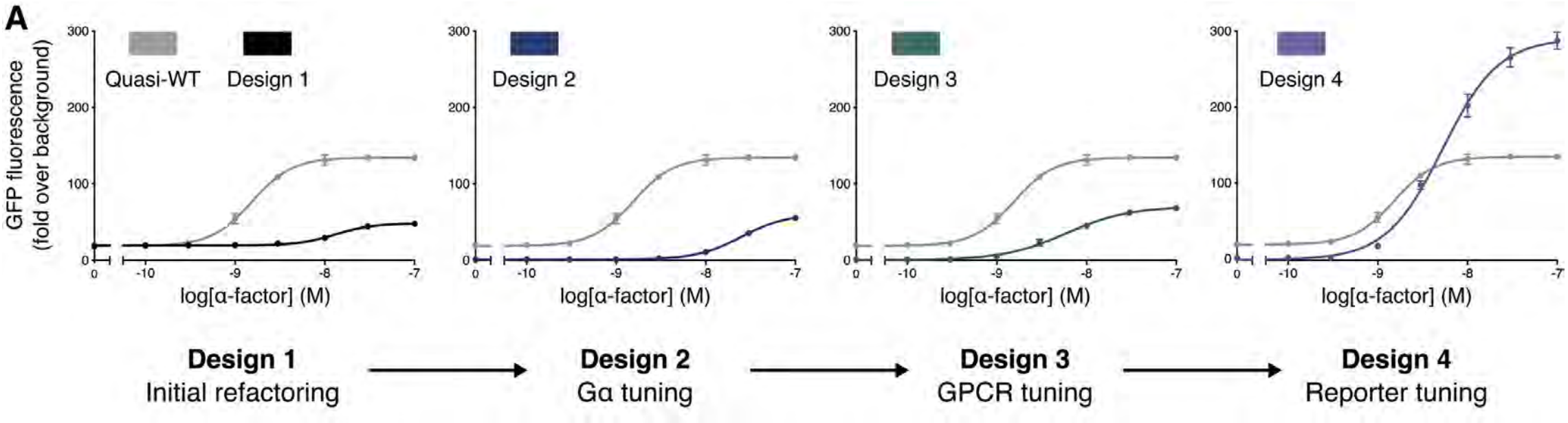


Maximum pathway output

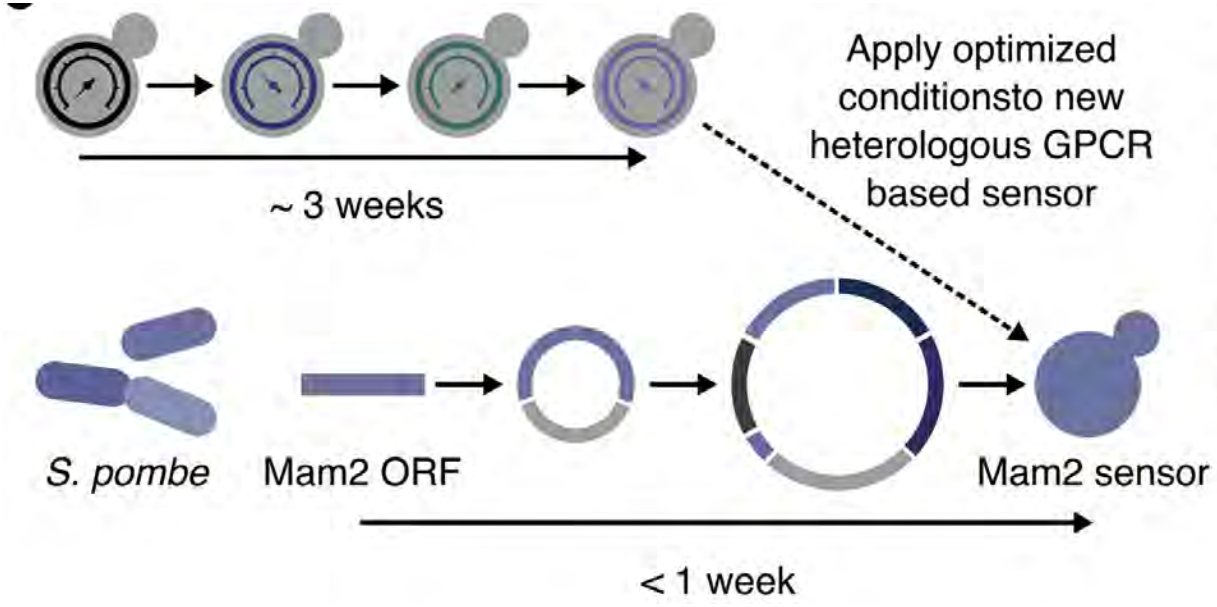
- Idea: increase pheromone-responsive transcription factor Ste12
- Problem: leads to poor cell growth
- Why: high basal activation of mating response genes
- Solution: synthetic transcription factor

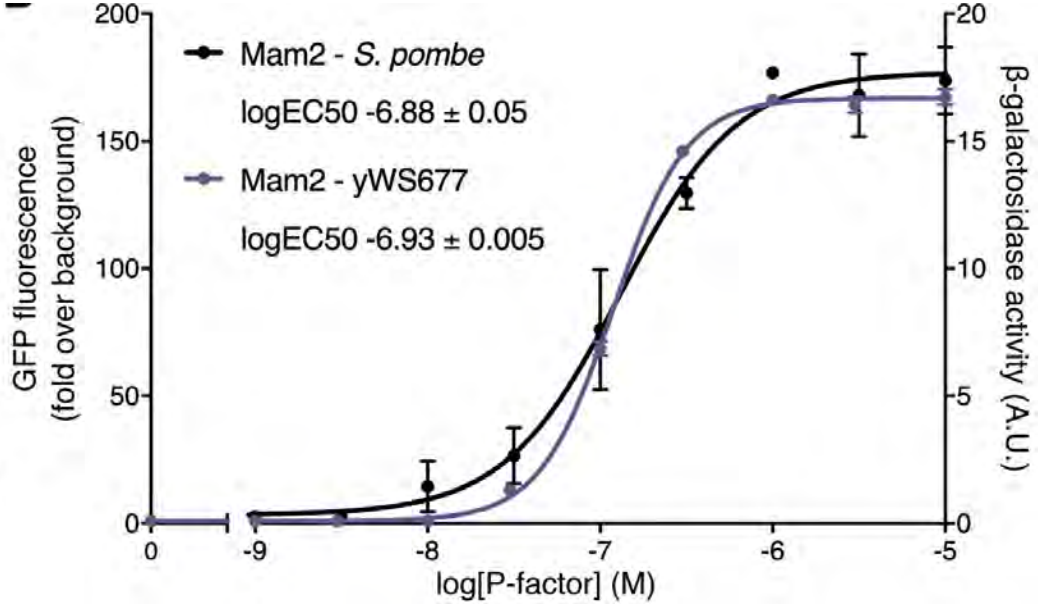


Putting it all together



Initial demonstration

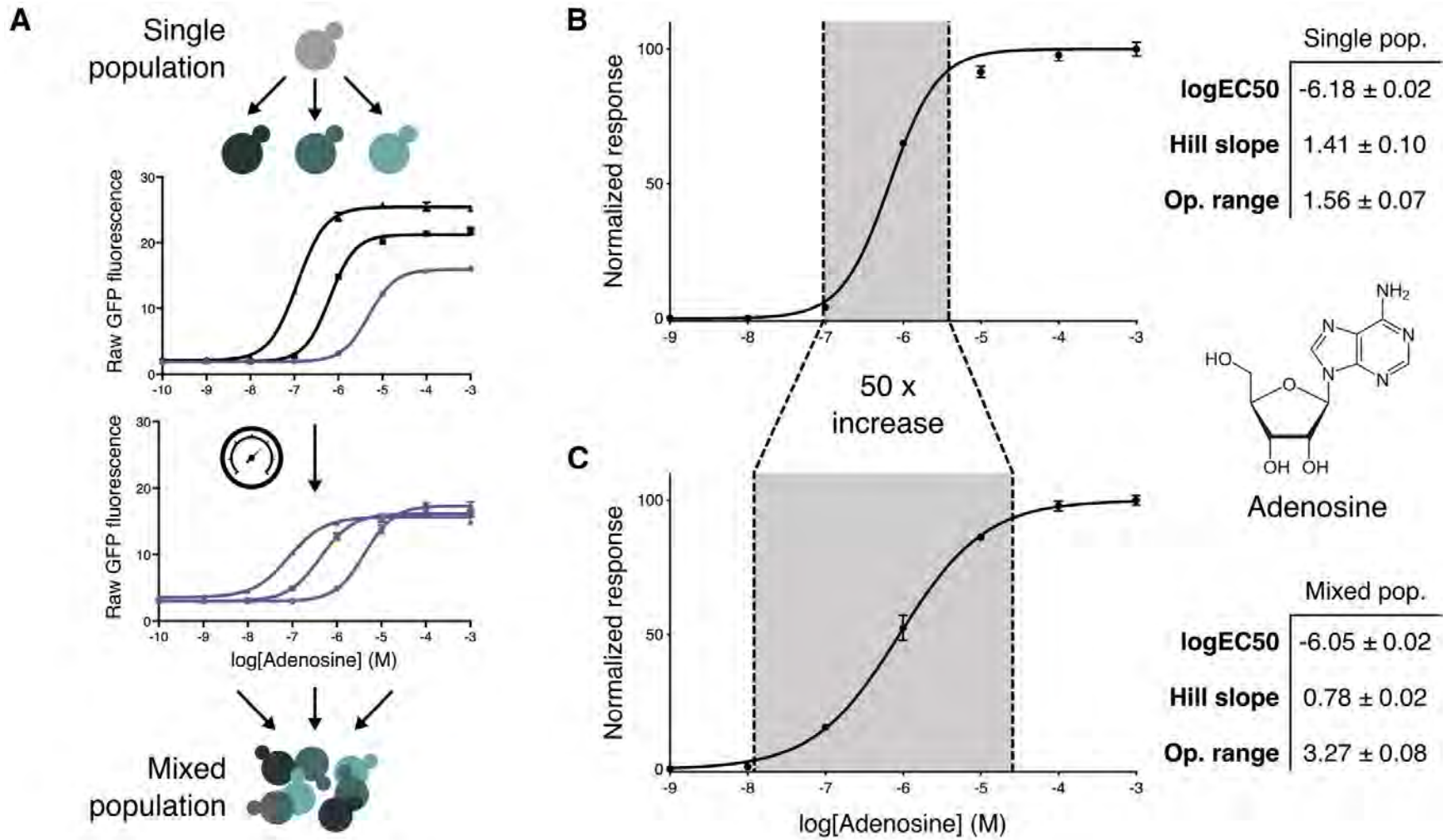


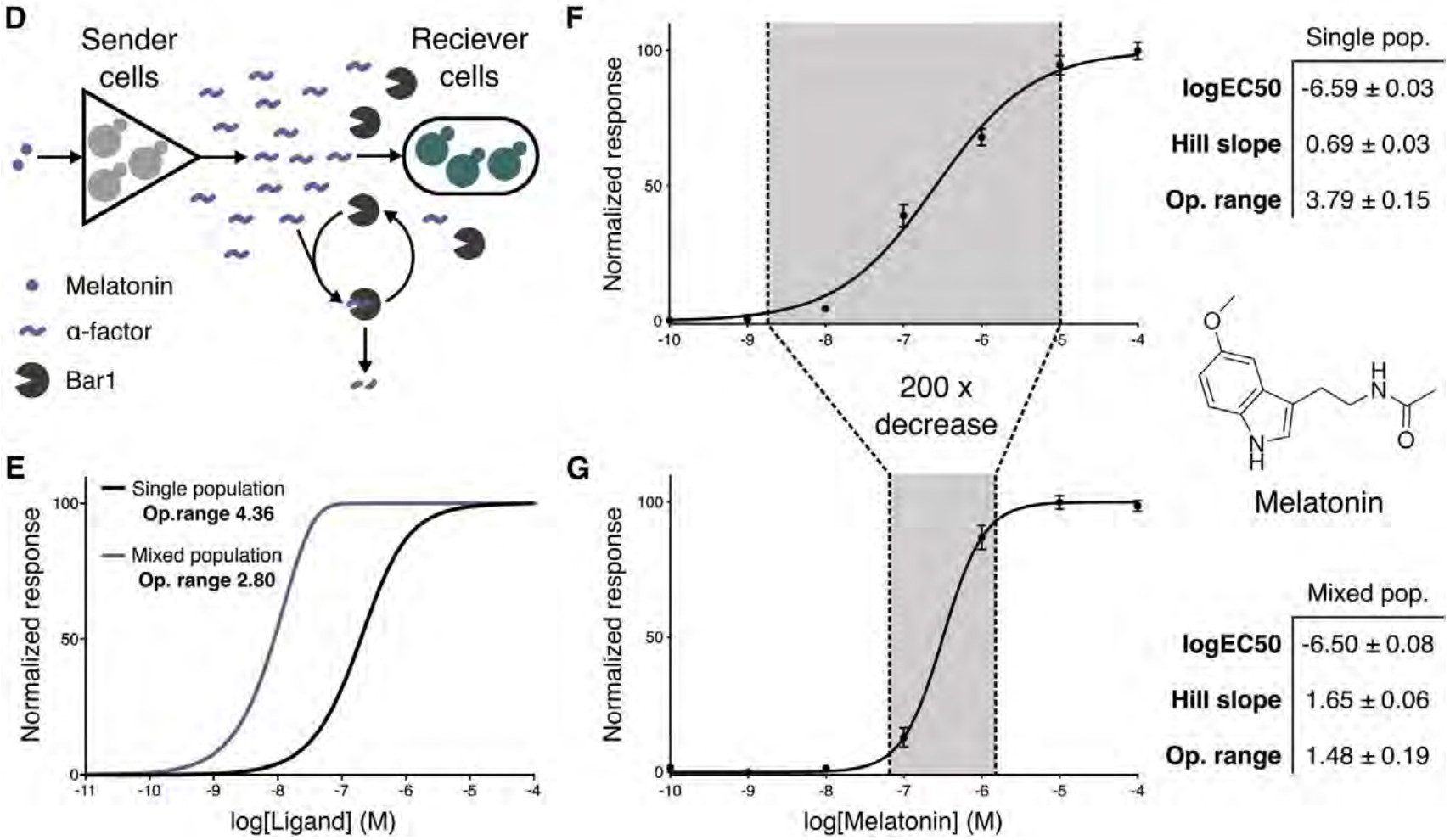


Extending and narrowing operational range

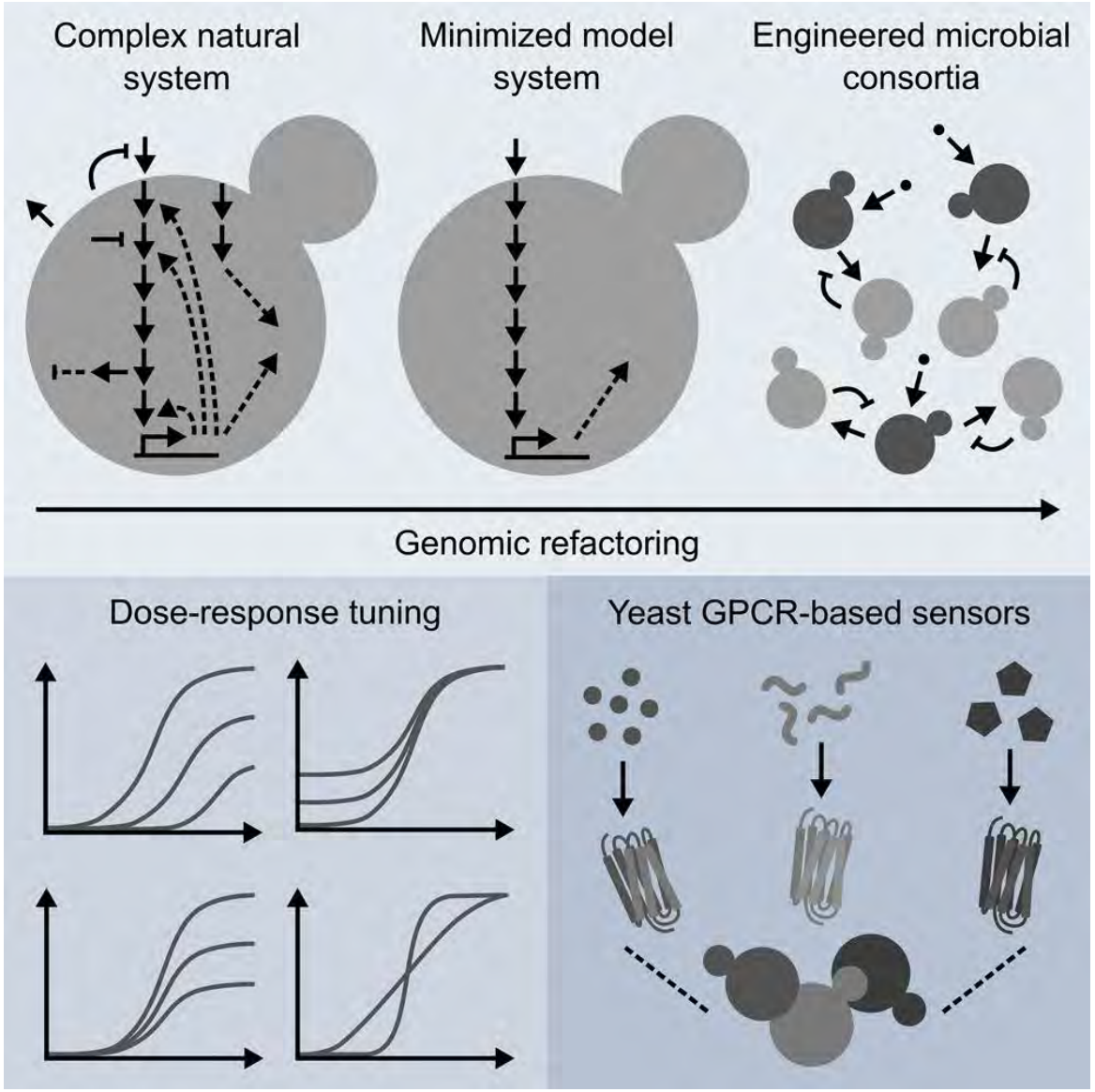
- How to tune the Hill slope of the dose-response curve?
- Usually done by introducing feedback loops
- This is what we tried to avoid
- Even if we wanted, we could not reintroduce it easily because the autoregulatory feedback of the Ste12 promotor is missing

→ Use population consortia


A
Single population
Raw GFP fluorescence
log[Adenosine] (M)
Mixed population
B
Normalized response
50 x increase
C
Normalized response
log[Adenosine] (M)
Single pop.
logEC50 -6.18 ± 0.02
Hill slope 1.41 ± 0.10
Op. range 1.56 ± 0.07
Adenosine
Mixed pop.
logEC50 -6.05 ± 0.02
Hill slope 0.78 ± 0.02
Op. range 3.27 ± 0.08


D
Sender cells
Reciever cells
Melatonin
α-factor
Bar1
E
Single population
Op.range 4.36
Mixed population
Op. range 2.80
Normalized response
log[Ligand] (M)
F
Normalized response
200 x decrease
G
Normalized response
log[Melatonin] (M)
Single pop.
logEC50 -6.59 ± 0.03
Hill slope 0.69 ± 0.03
Op. range 3.79 ± 0.15
Melatonin
Mixed pop.
logEC50 -6.50 ± 0.08
Hill slope 1.65 ± 0.06
Op. range 1.48 ± 0.19

Summary


Complex natural system
Minimized model system
Engineered microbial consortia
Genomic refactoring
Dose-response tuning
Yeast GPCR-based sensors

Questions?

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