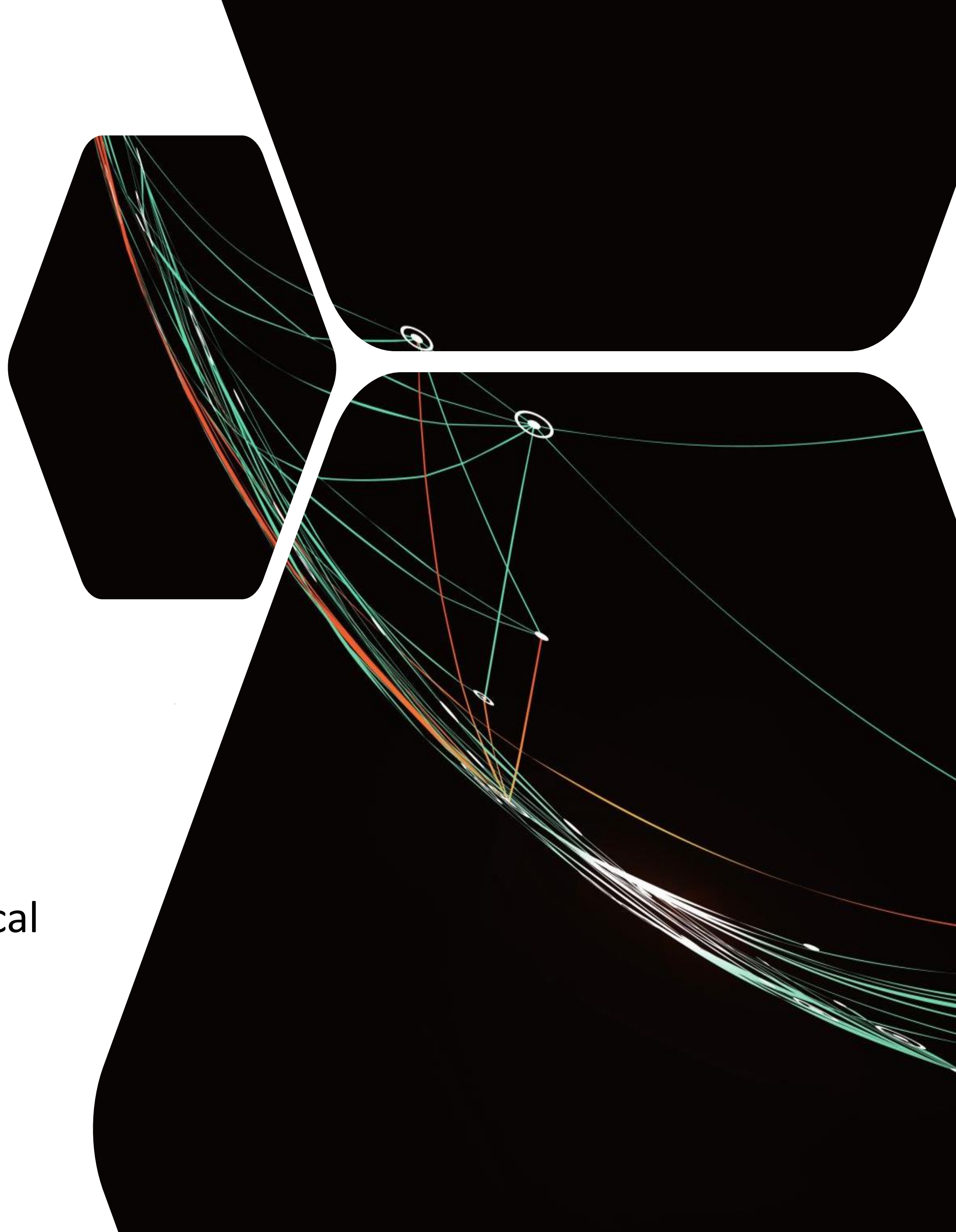
Using random perturbation experiments to constrain feedback in biomolecular networks



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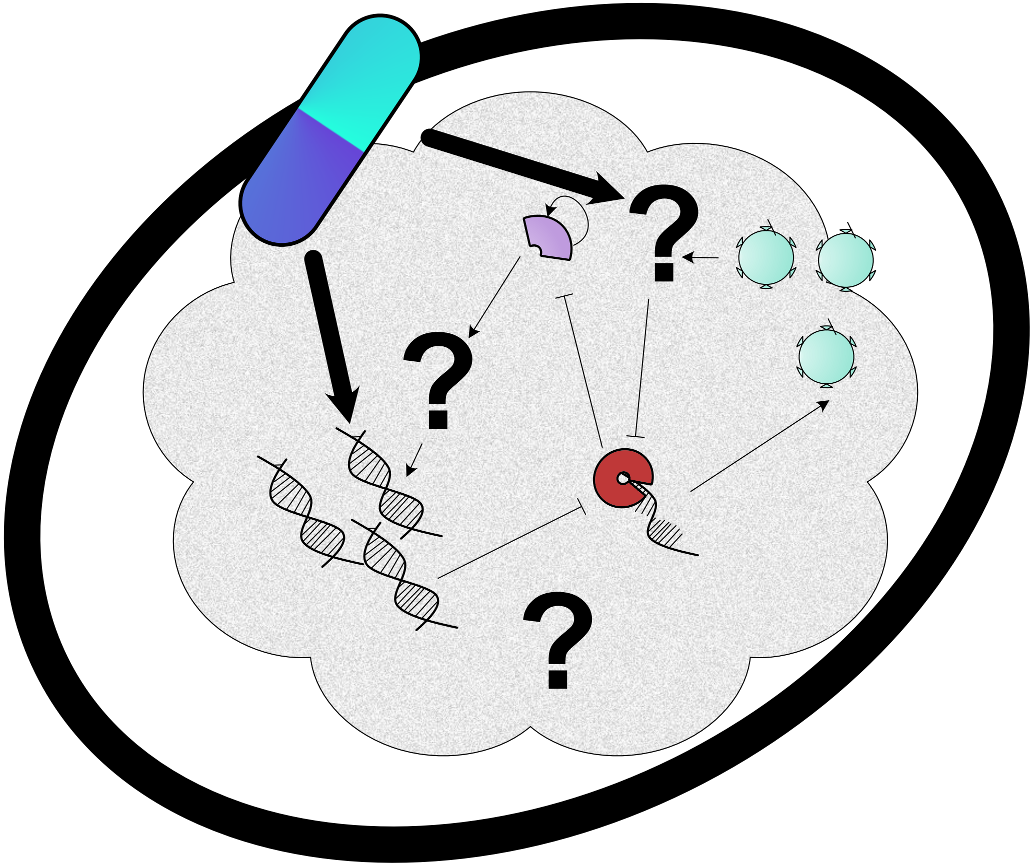
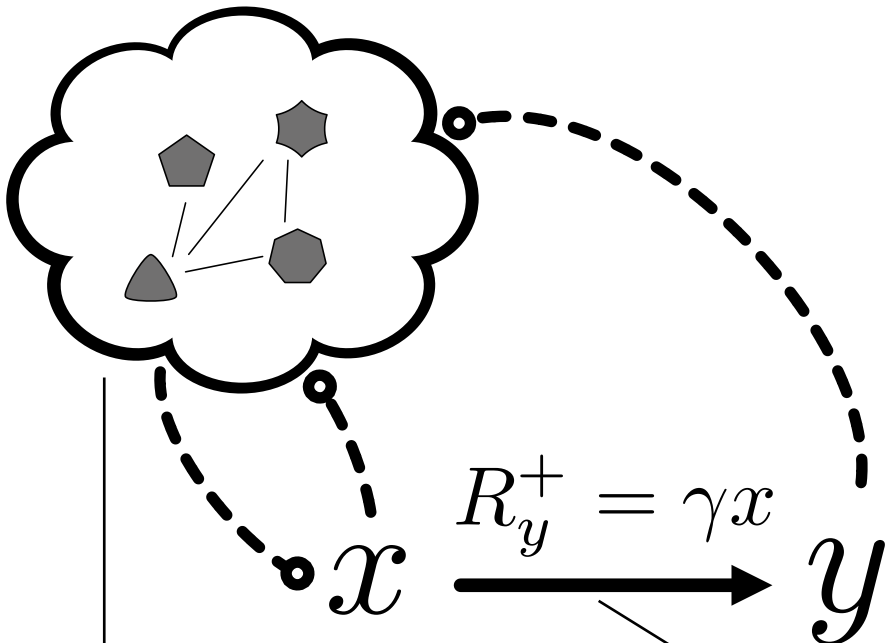
How do molecules in cells interact?

Hit something, see how molecular levels respond



Problem: drugs may have effectively “random” effect

Class of models: systems that contain a linear production rate

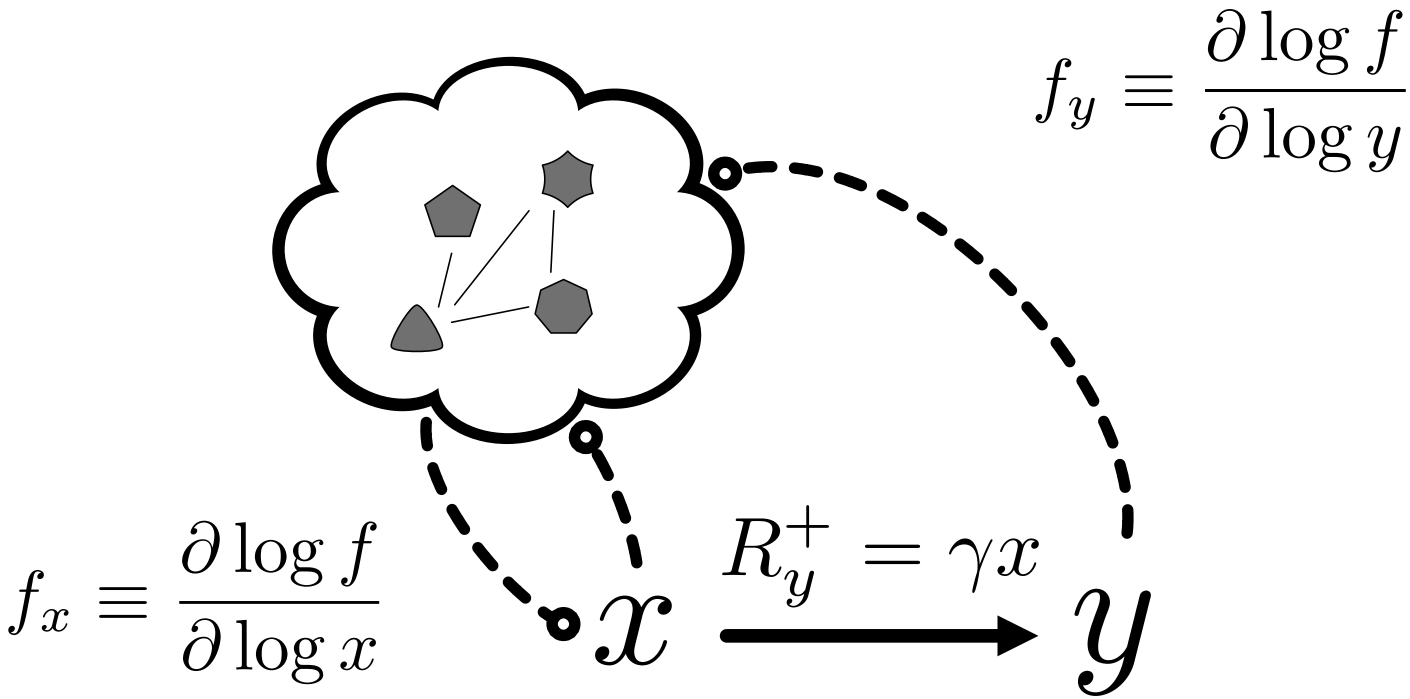


Unspecified dynamics, $f(x, y)$

$$x = \frac{\lambda}{\beta_x} f(x, y)$$

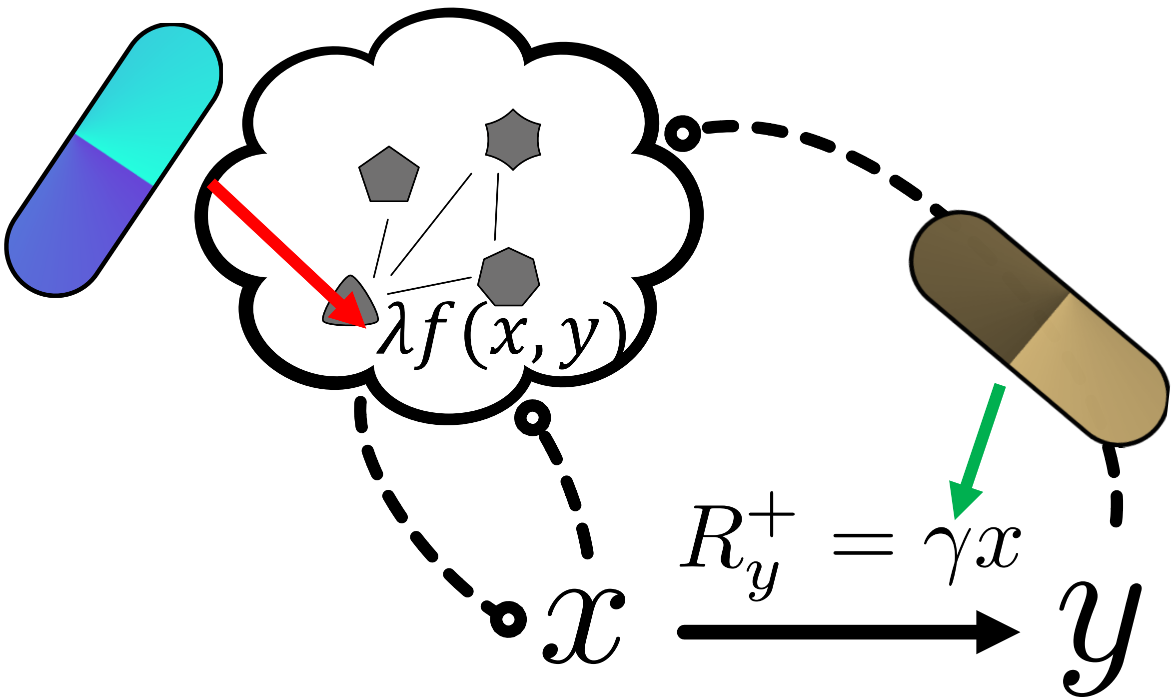
$$y = \frac{\gamma}{\beta_y} x$$

Can we infer the sensitivity of this system to feedback via perturbation responses?



Sign of sensitivity defines positive or negative feedback

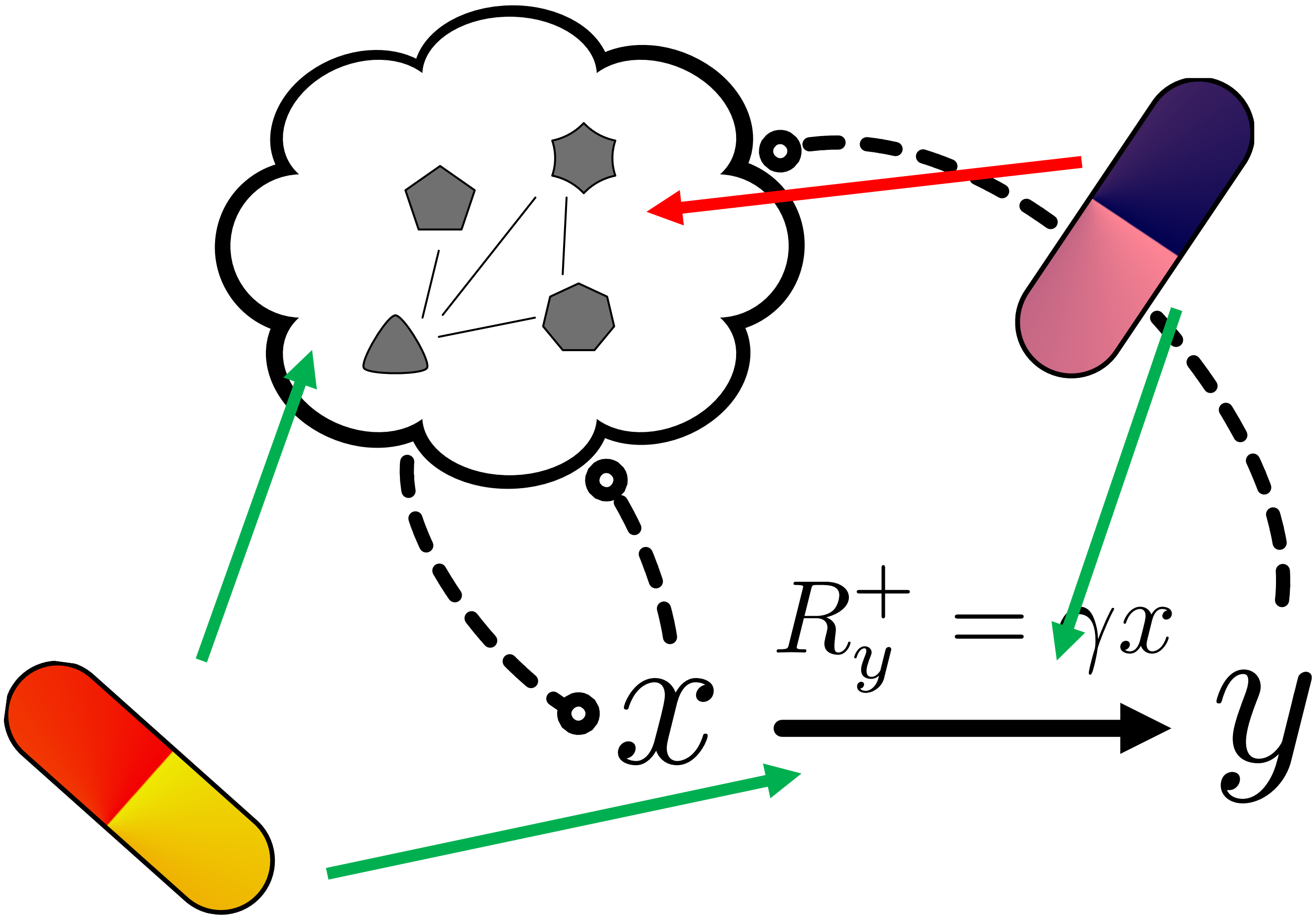
Yes with linear response analysis! But perturbations can only hit one target



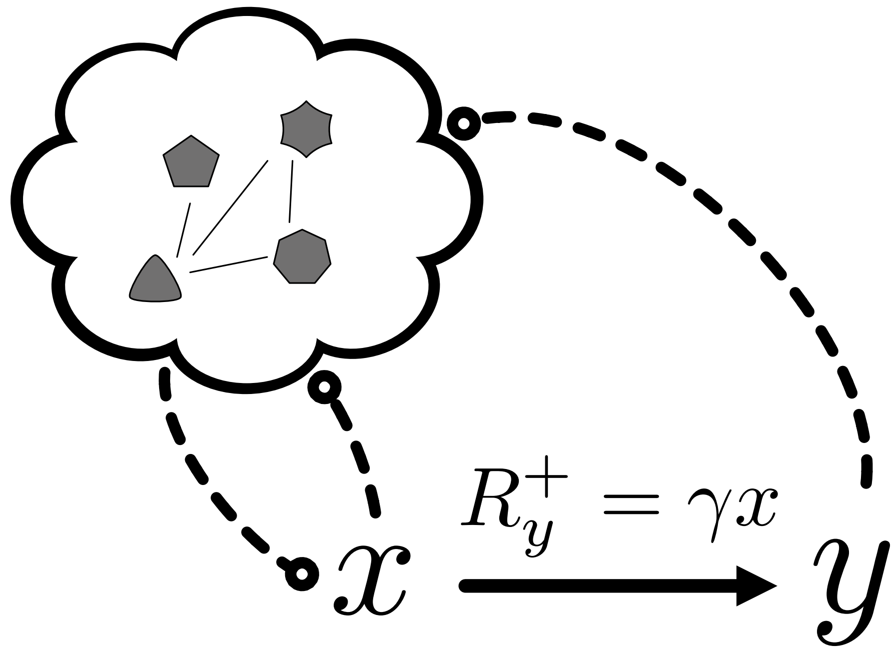
True even if size of perturbations is unknown

Kholodenko., et al. *PNAS* 99.20 (2002)

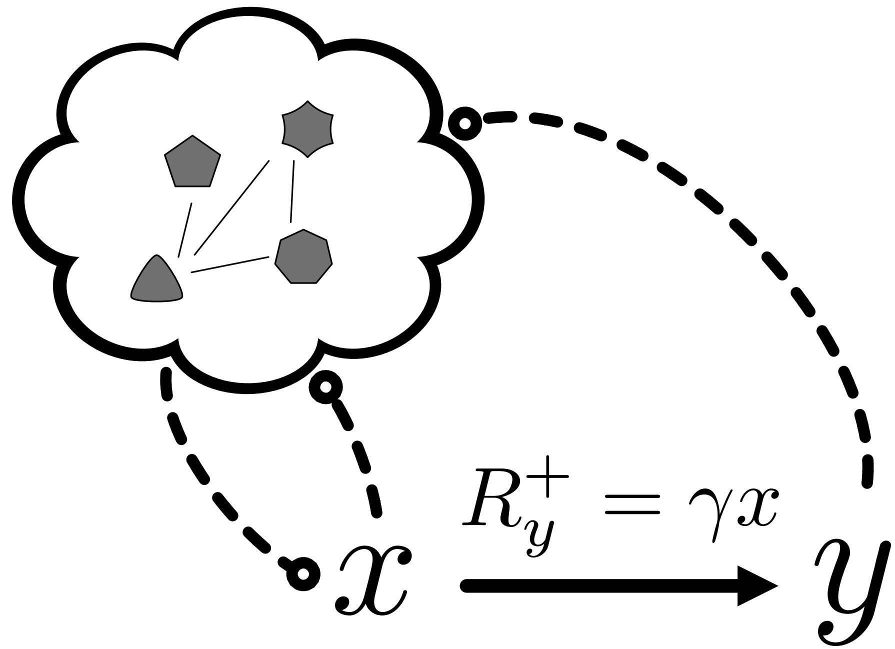
These methods do not account for drugs hitting both targets



Methods do not study correlations in response to set of perturbations



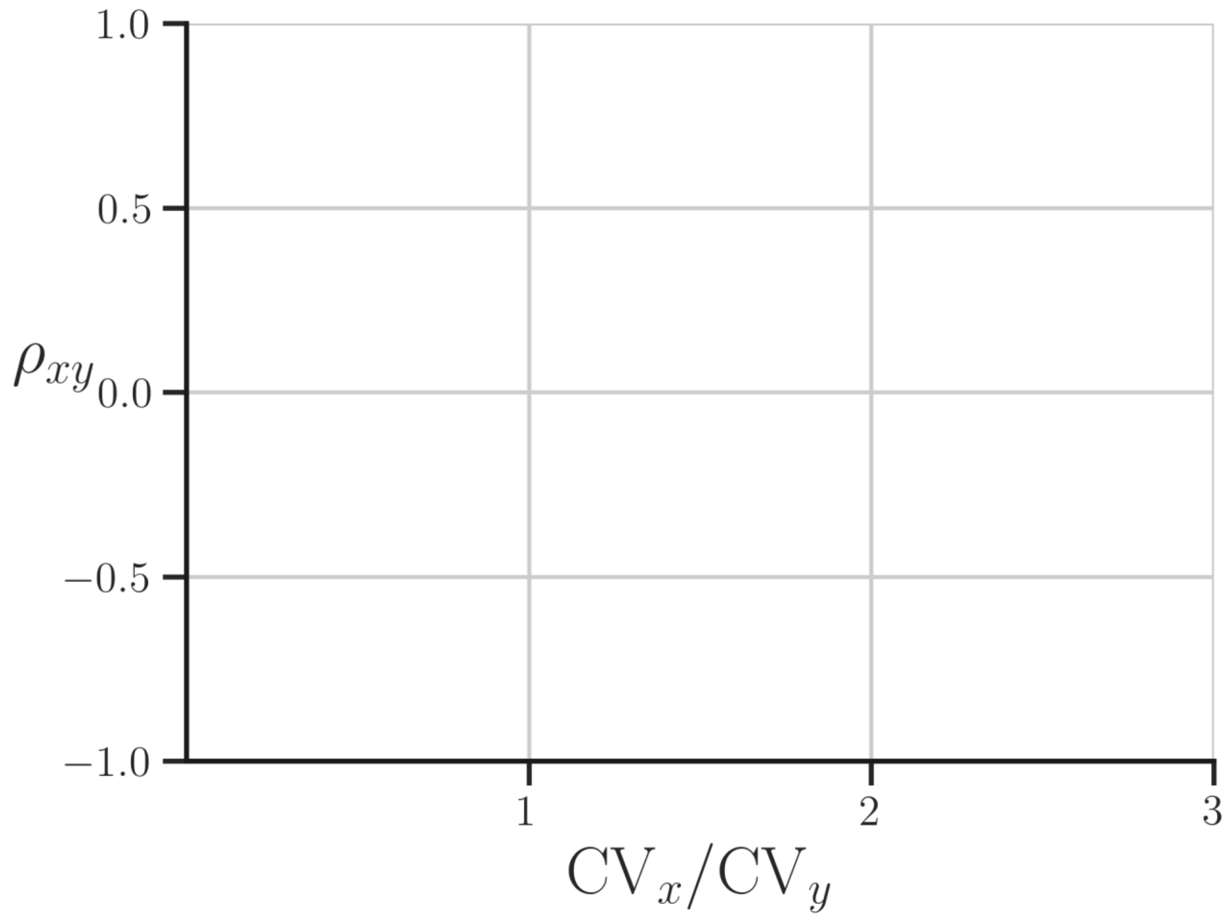
Let alone correlations to set of unknown perturbations



Can we develop a linear analysis of ‘random’ perturbations?

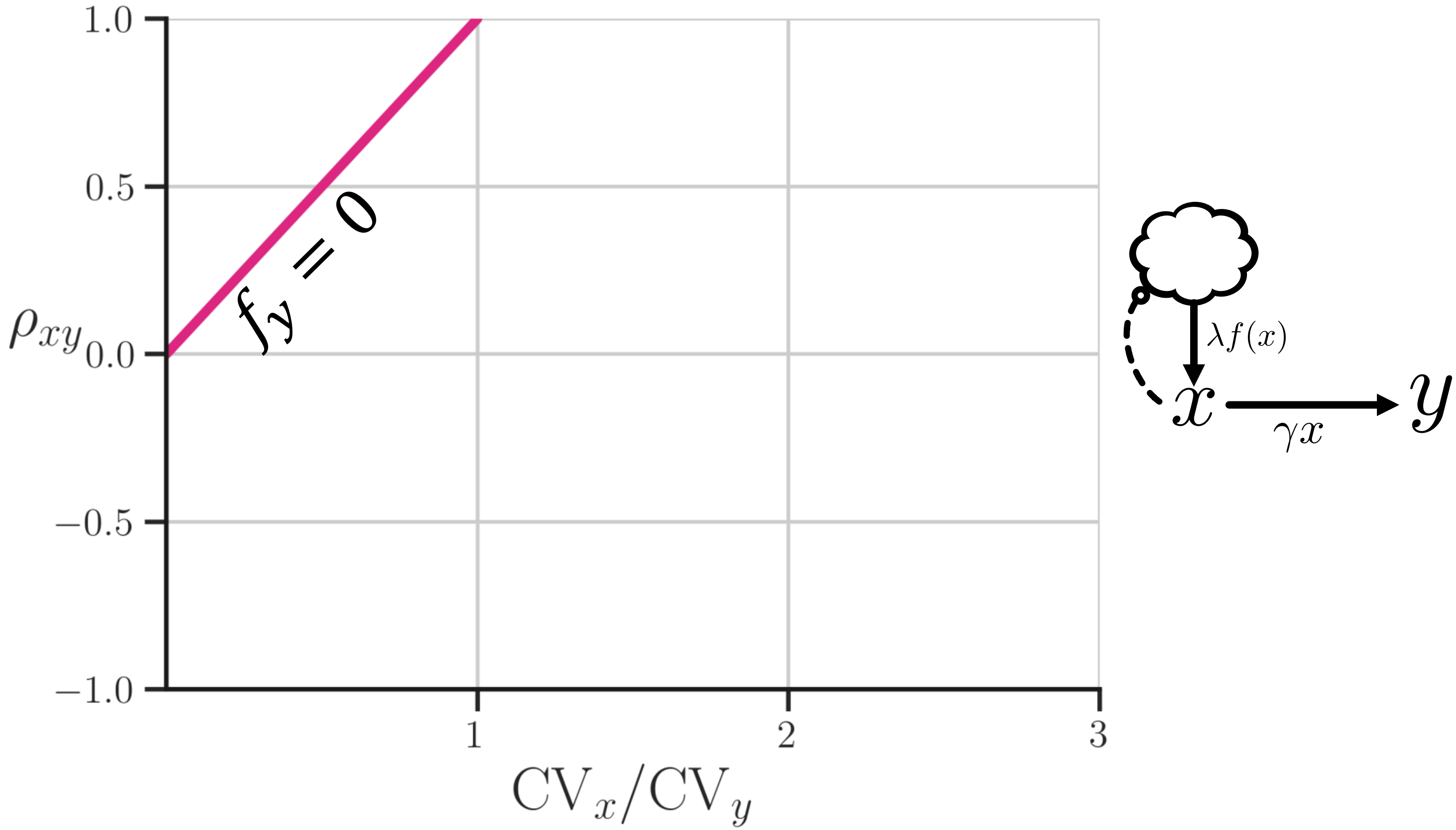
Linear analysis of random perturbation experiments yields constraints on feedback sensitivities

Correlation between levels across random perturbations

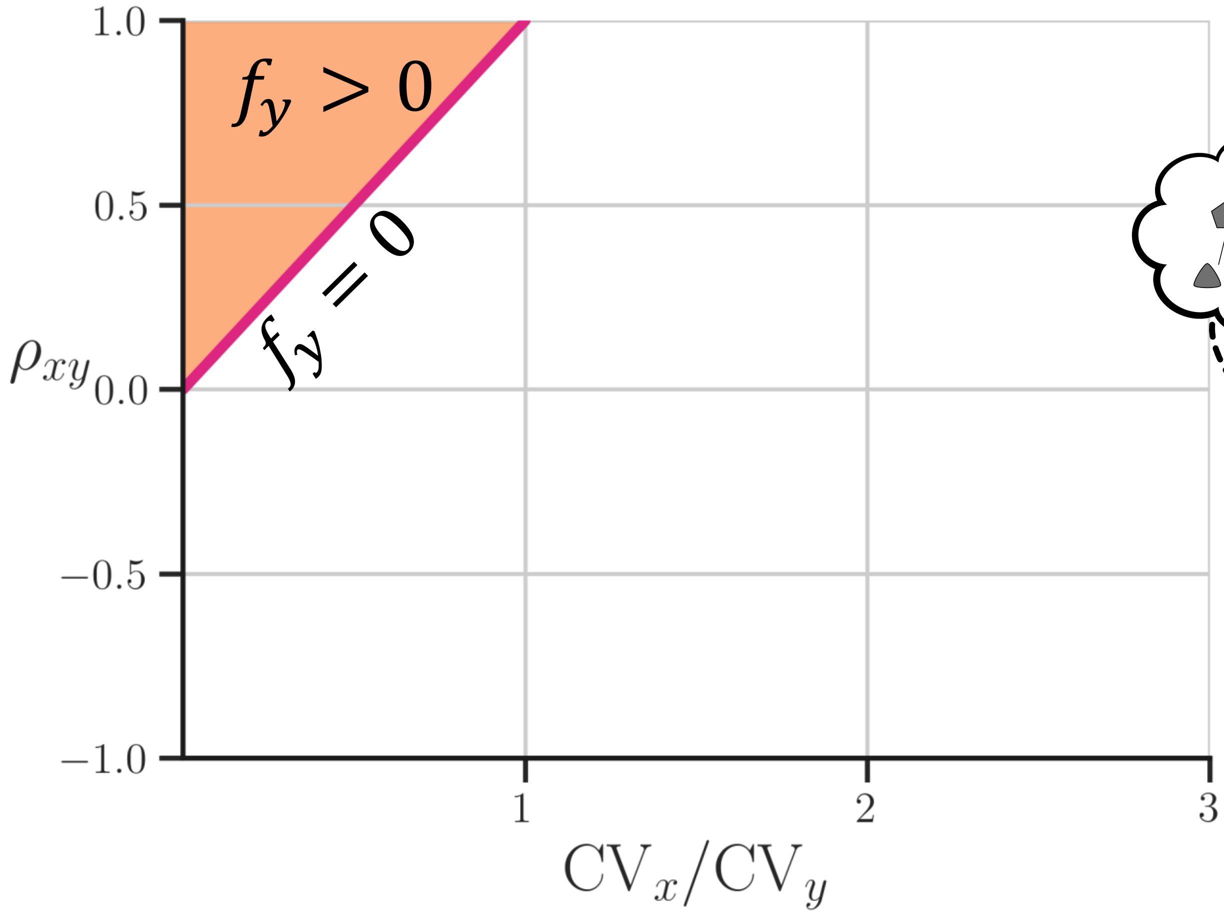


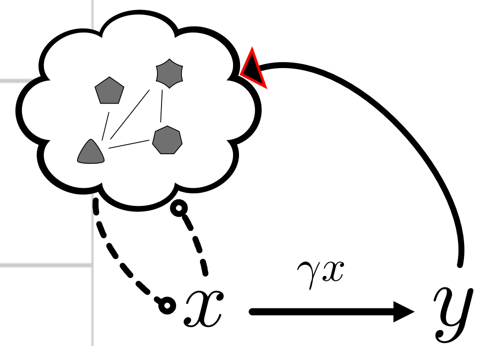
Ratio of coefficients of variation $\left(\frac{\sigma}{\mu}\right)$ of levels across perturbations

Position on plot determines sign of f_y

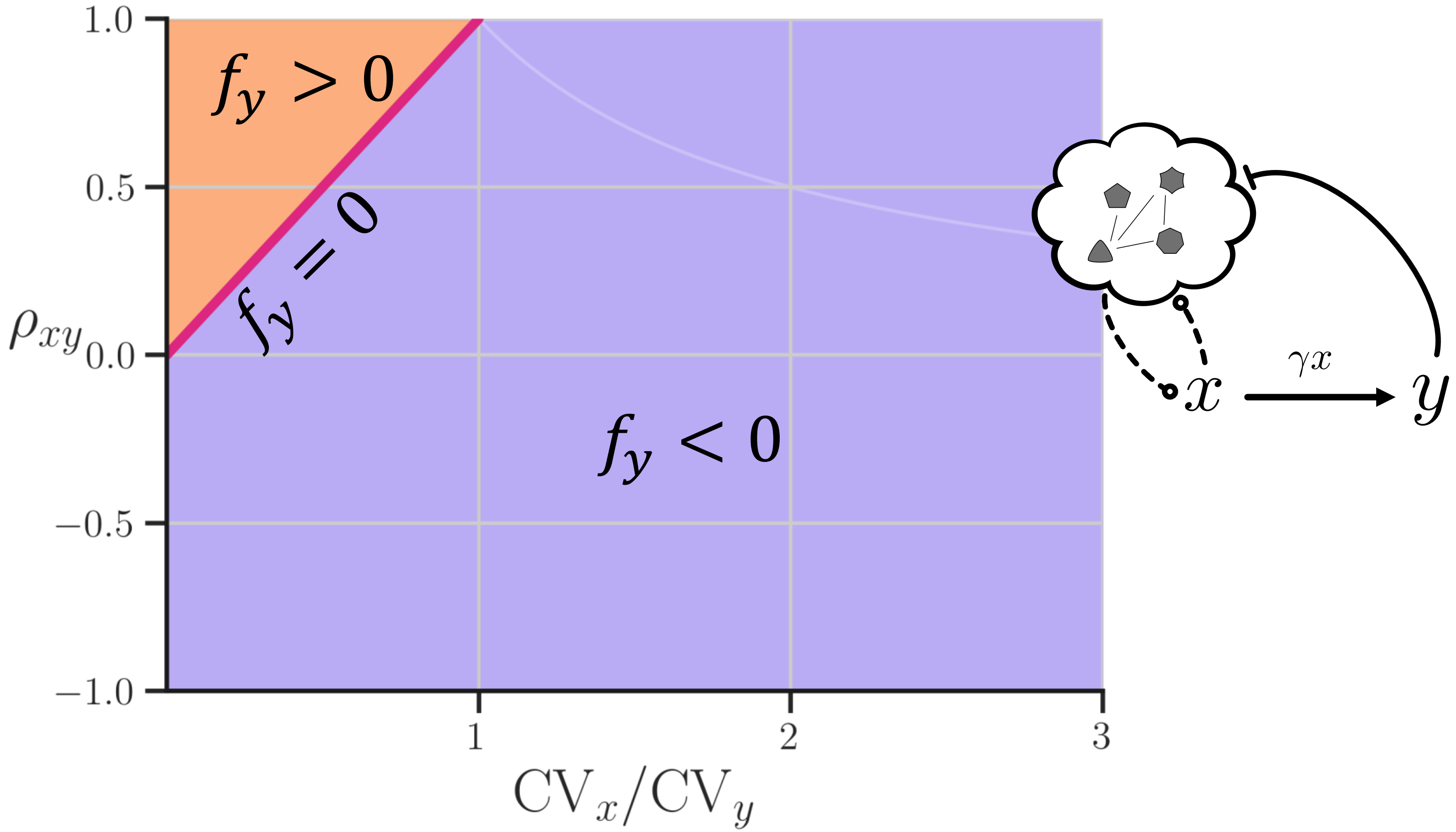


Position on plot determines sign of f_y

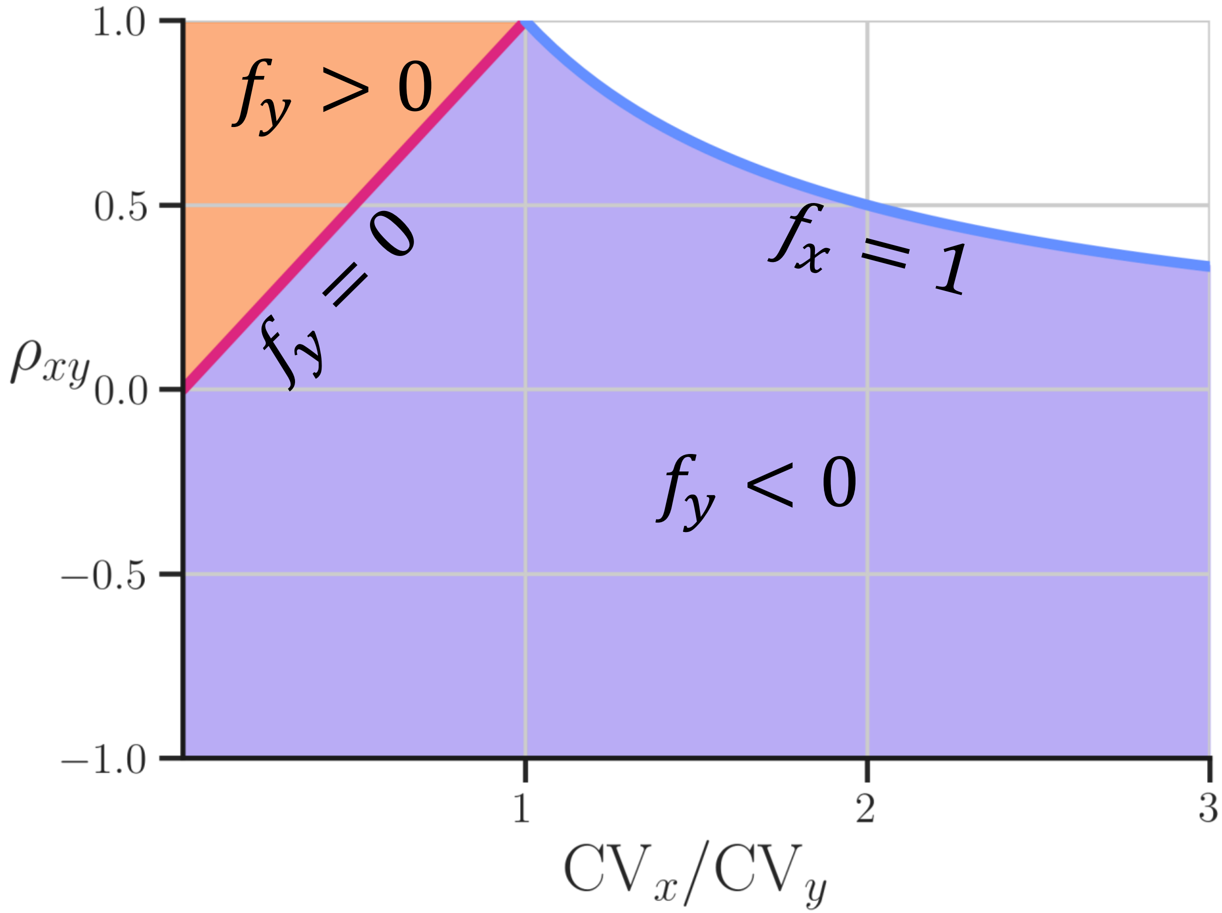




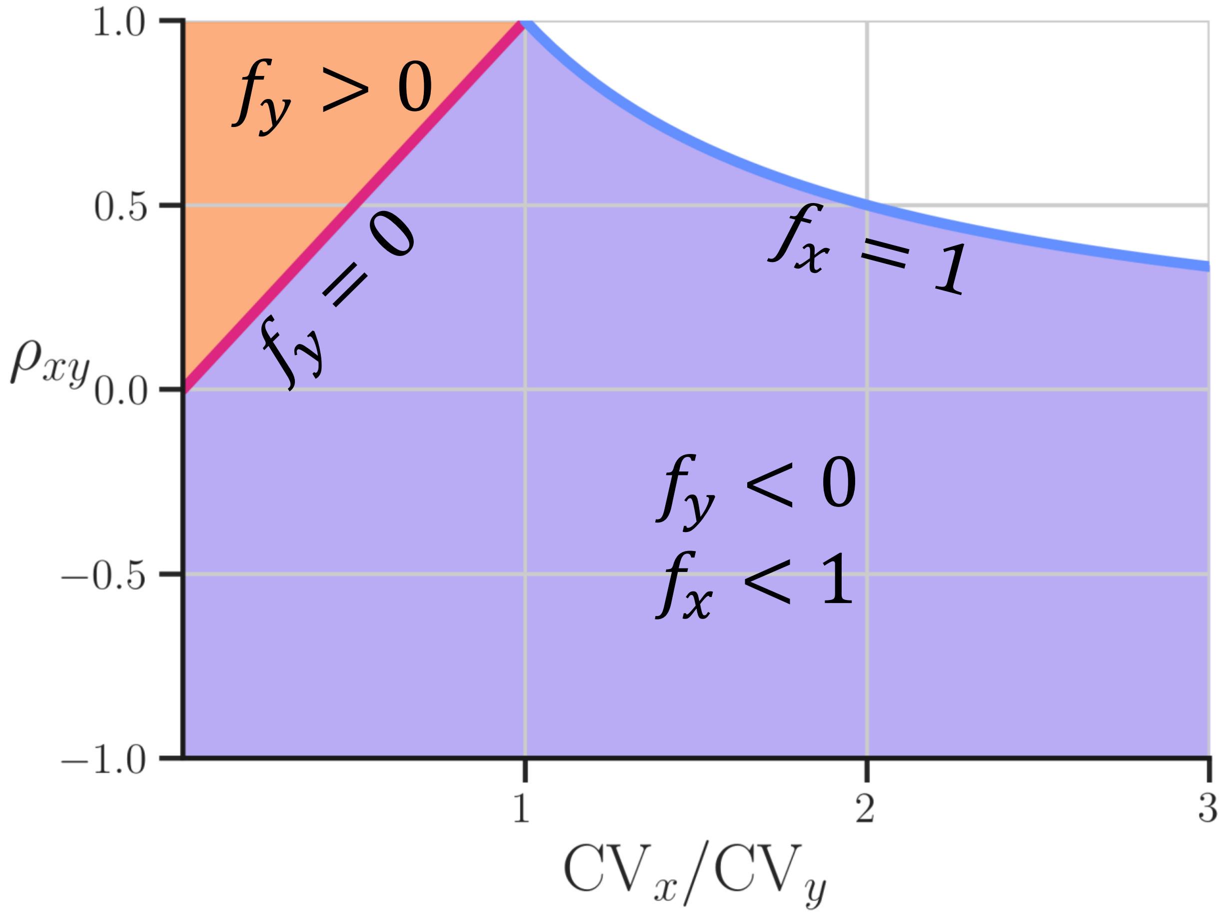
Position on plot determines sign of f_y



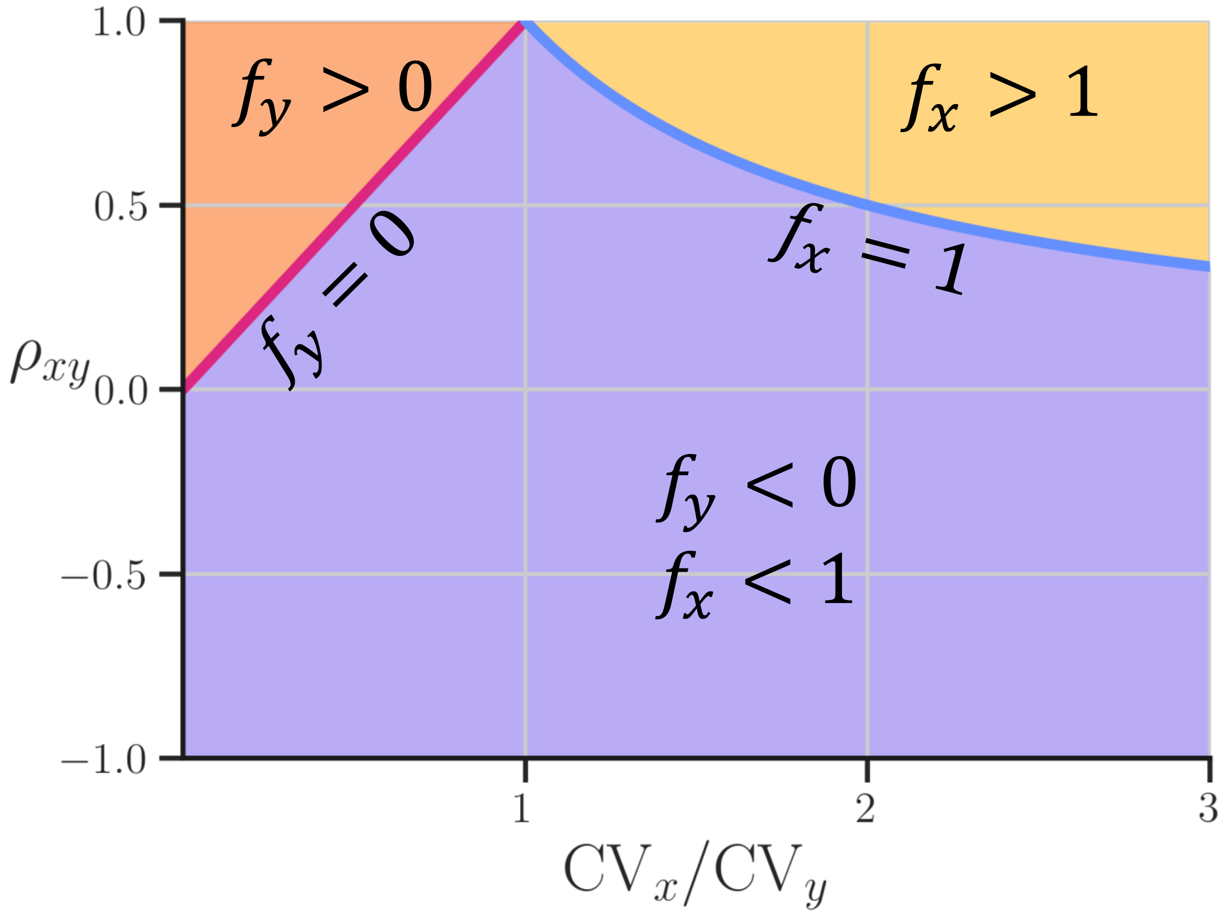
Position on plot determines sign of $1 - f_x$



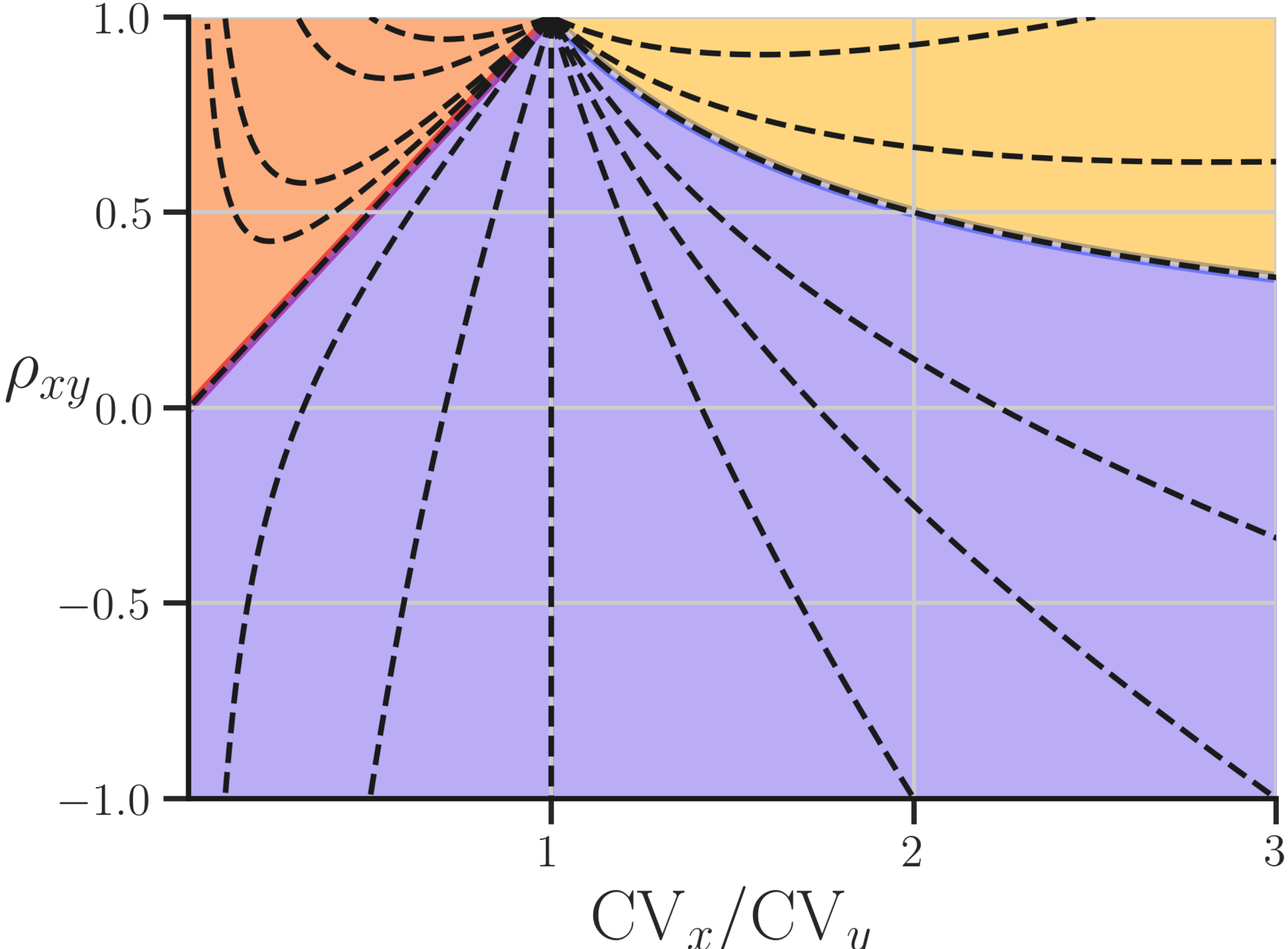
Position on plot determines sign of $1 - f_x$



Position on plot determines sign of $1 - f_x$



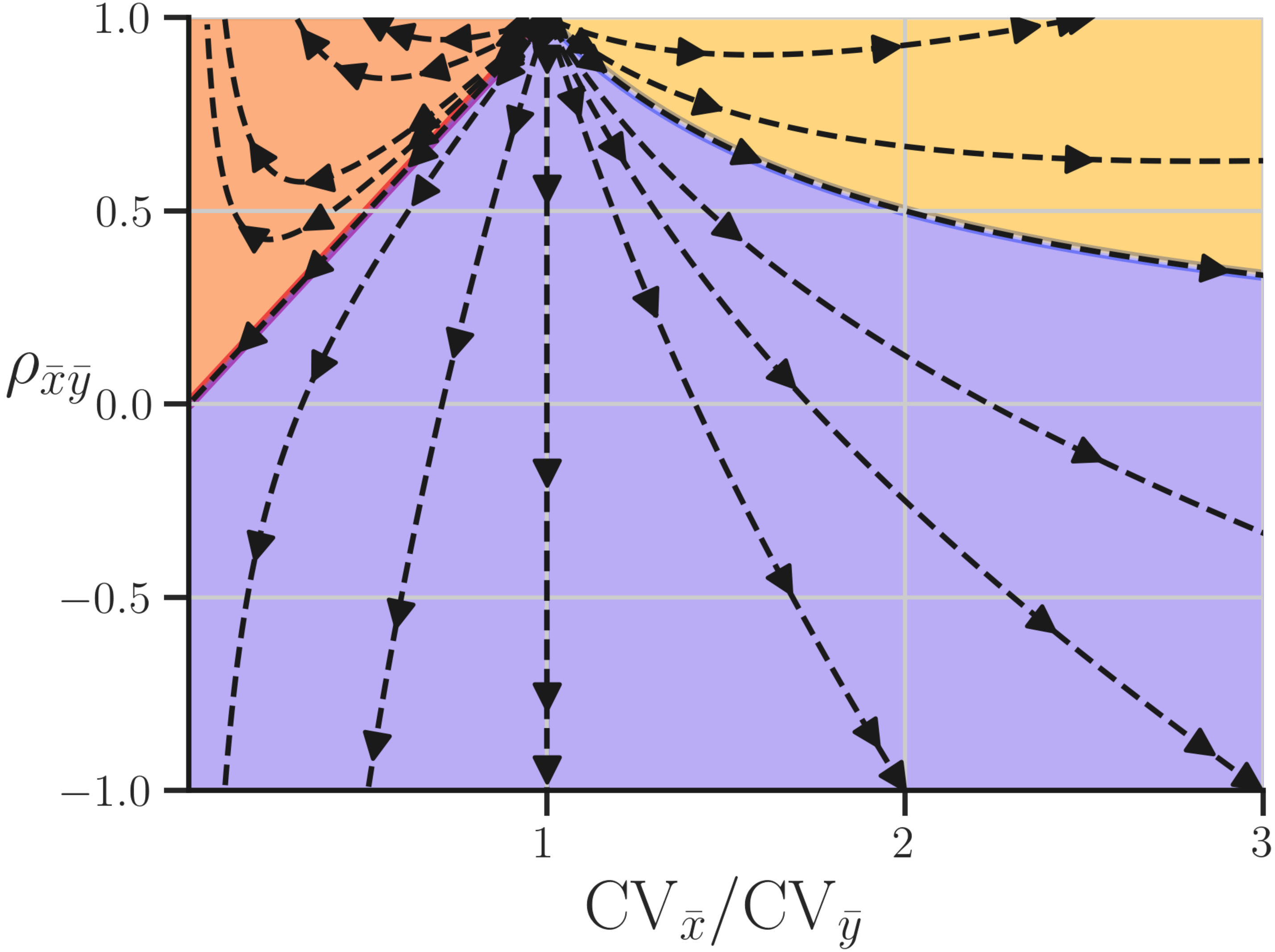
Systems land on curves based on relative feedback sensitivities

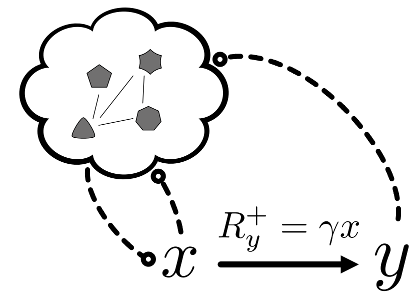


Curves shared by systems with same

$$\frac{f_y}{1 - f_x}$$

Position on ‘iso-feedback curves’ is from relative variation in parameters

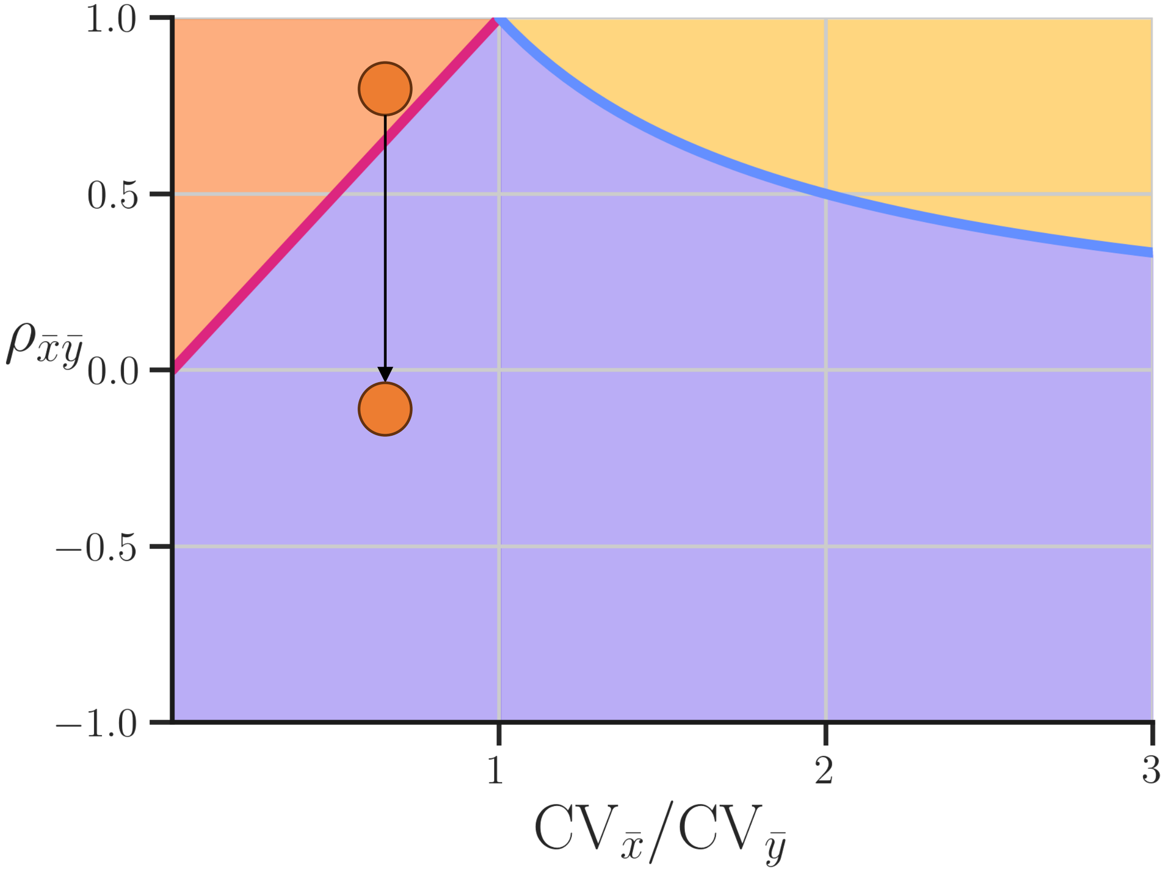


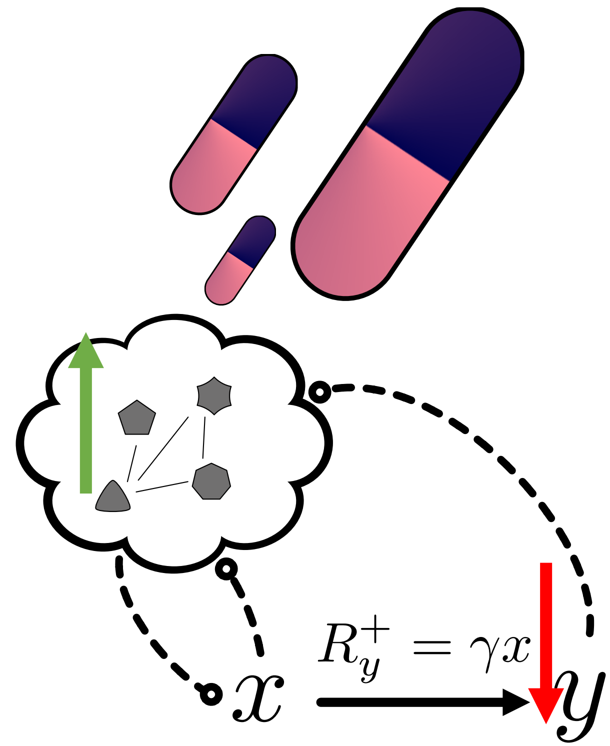


Arrows in direction of increasing $\frac{CV_\gamma}{CV_\lambda}$, from $0 \rightarrow \infty$

<u>The fine print:</u>

Bounds assume perturbations have uncorrelated effects on parameters





Finite perturbation effects: Real biological systems are non-linear

$$x = \frac{\lambda}{\beta_x} f(x, y)$$

$$dx \approx d\lambda + f_x dx + f_y dy$$

$$dx \approx d\lambda + f_x dx + f_y dy \boxed{+ O(dx^2, dy^2, dxdy)}$$

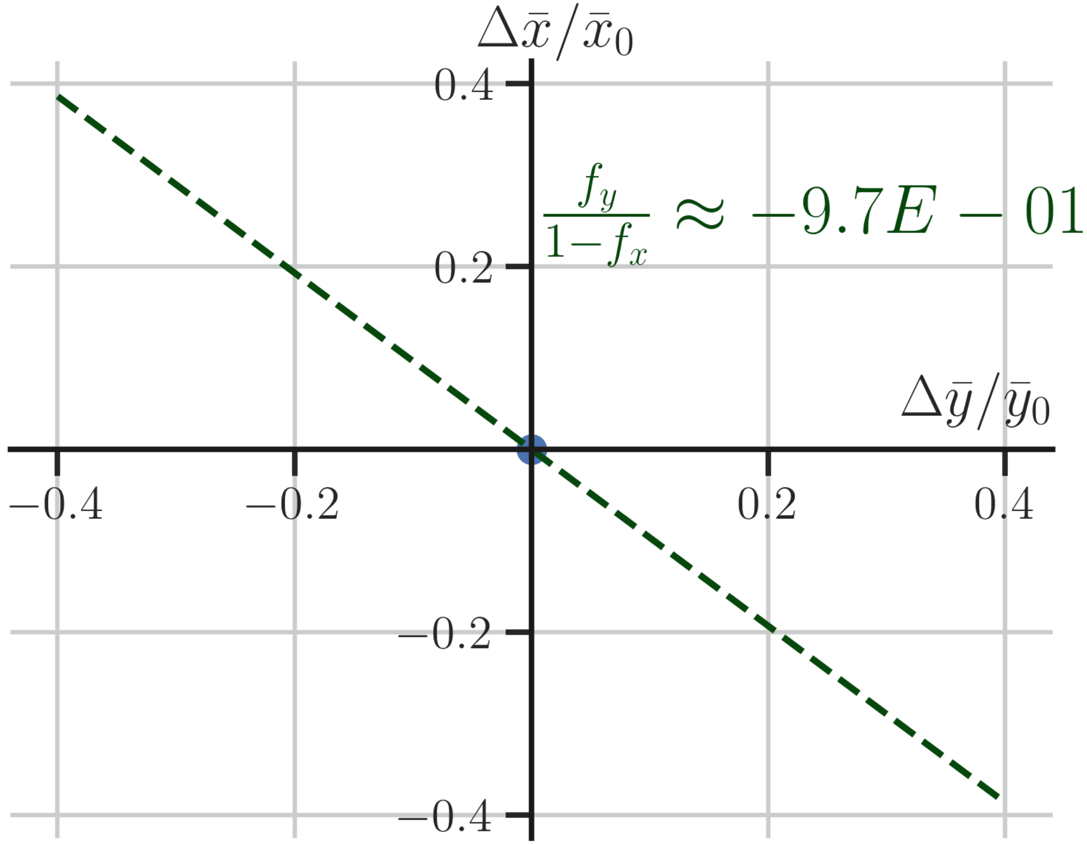
Finite perturbation effects: Real biological systems are non-linear AND stochastic

$$x = \frac{\lambda}{\beta_x} f(x, y)$$

$$\bar{x} = \frac{\lambda}{\beta_x} \overline{f(x, y)}$$

$$\bar{x} \approx \frac{\lambda}{\beta_x} \boxed{f(\bar{x}, \bar{y})}$$

Real biological systems deviate under finite perturbations to γ



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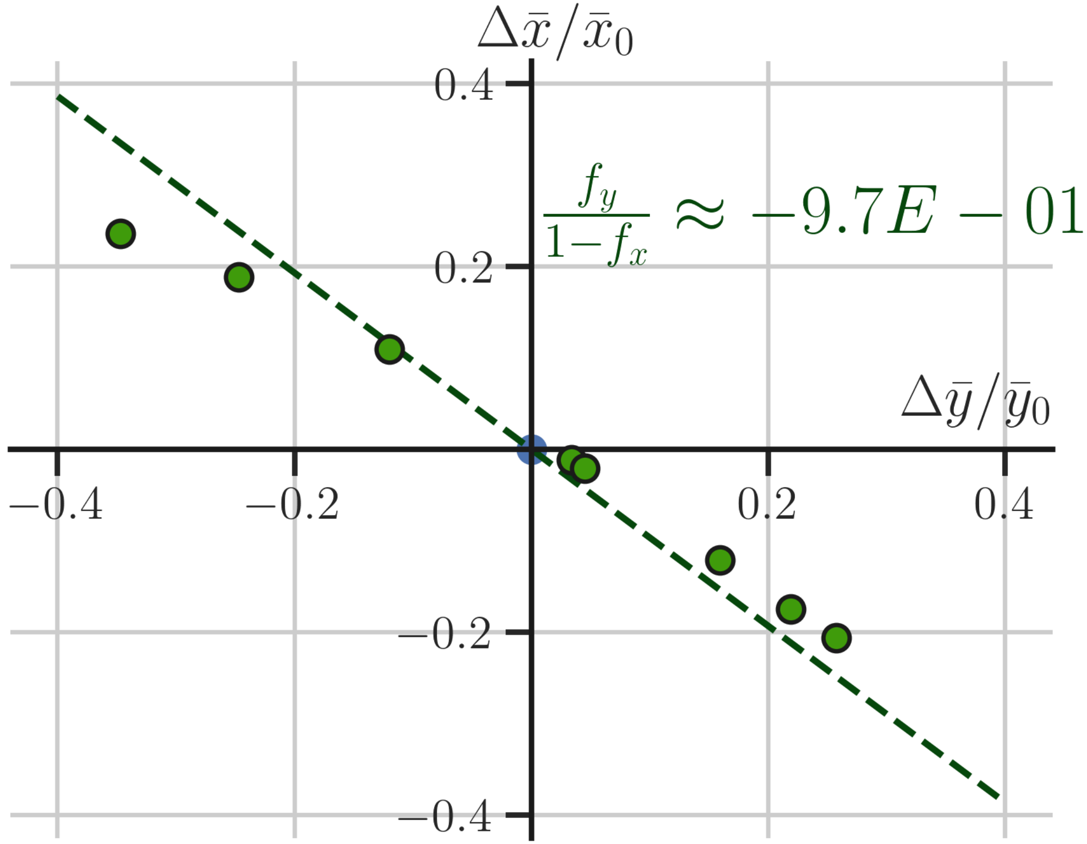
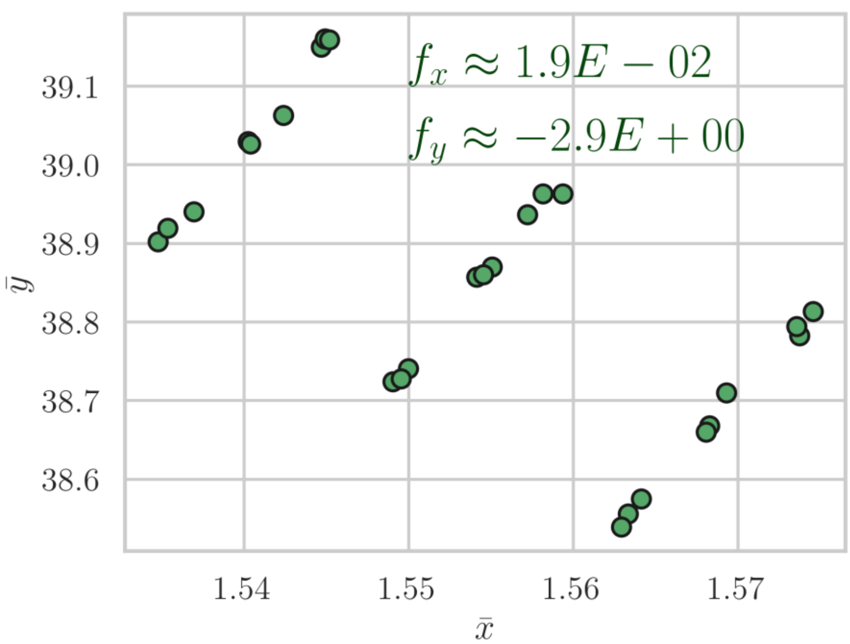
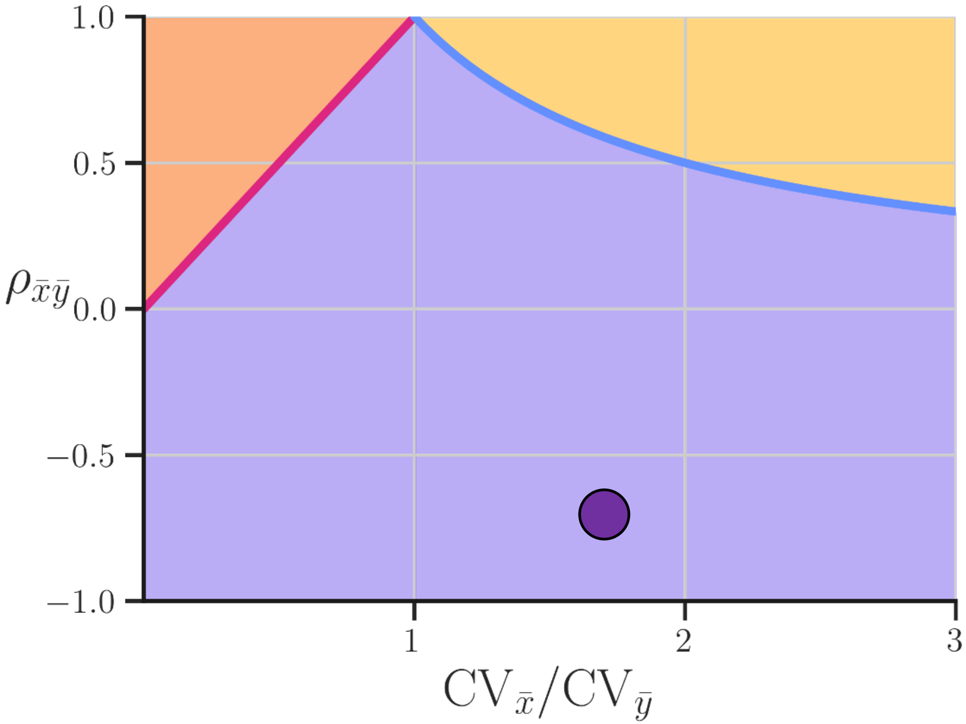


Do random perturbation constraints work?

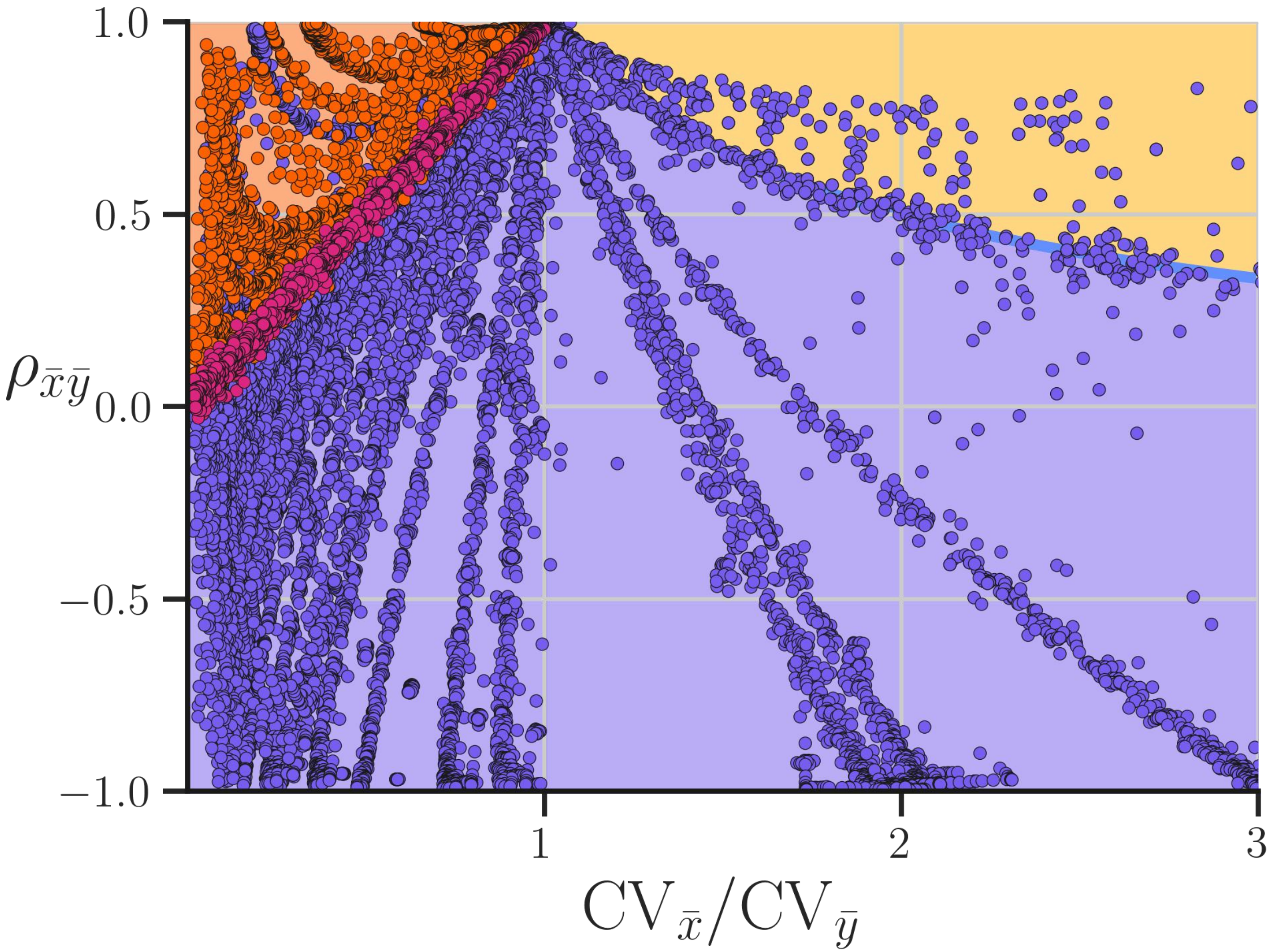
Stochastic simulations calculate exact values for observables and feedback under finite perturbation





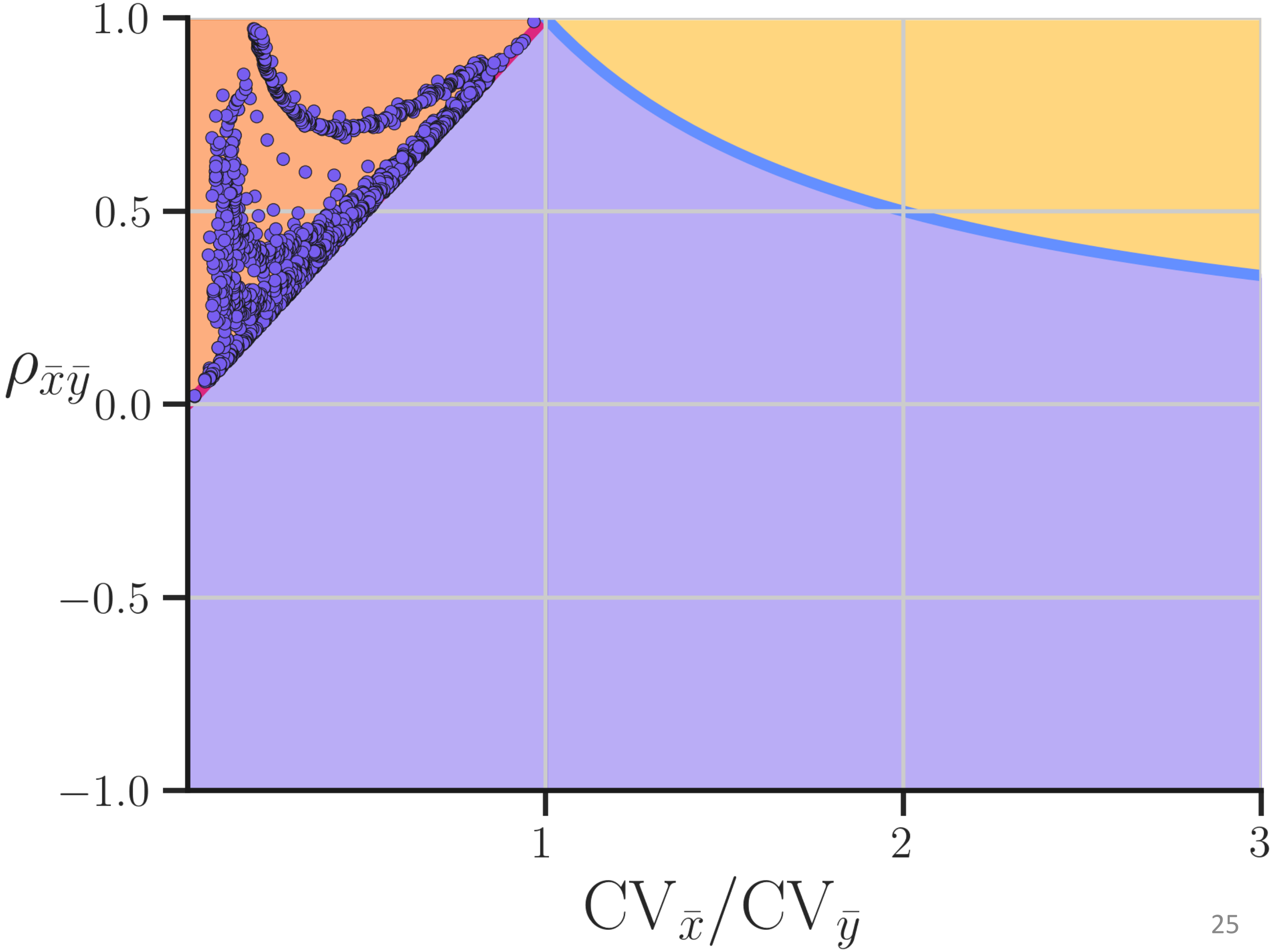
Mismatch of dot colour and background colour => inferred sensitivity category “incorrect”

Most sampled systems obeyed predicted constraints under finite perturbation



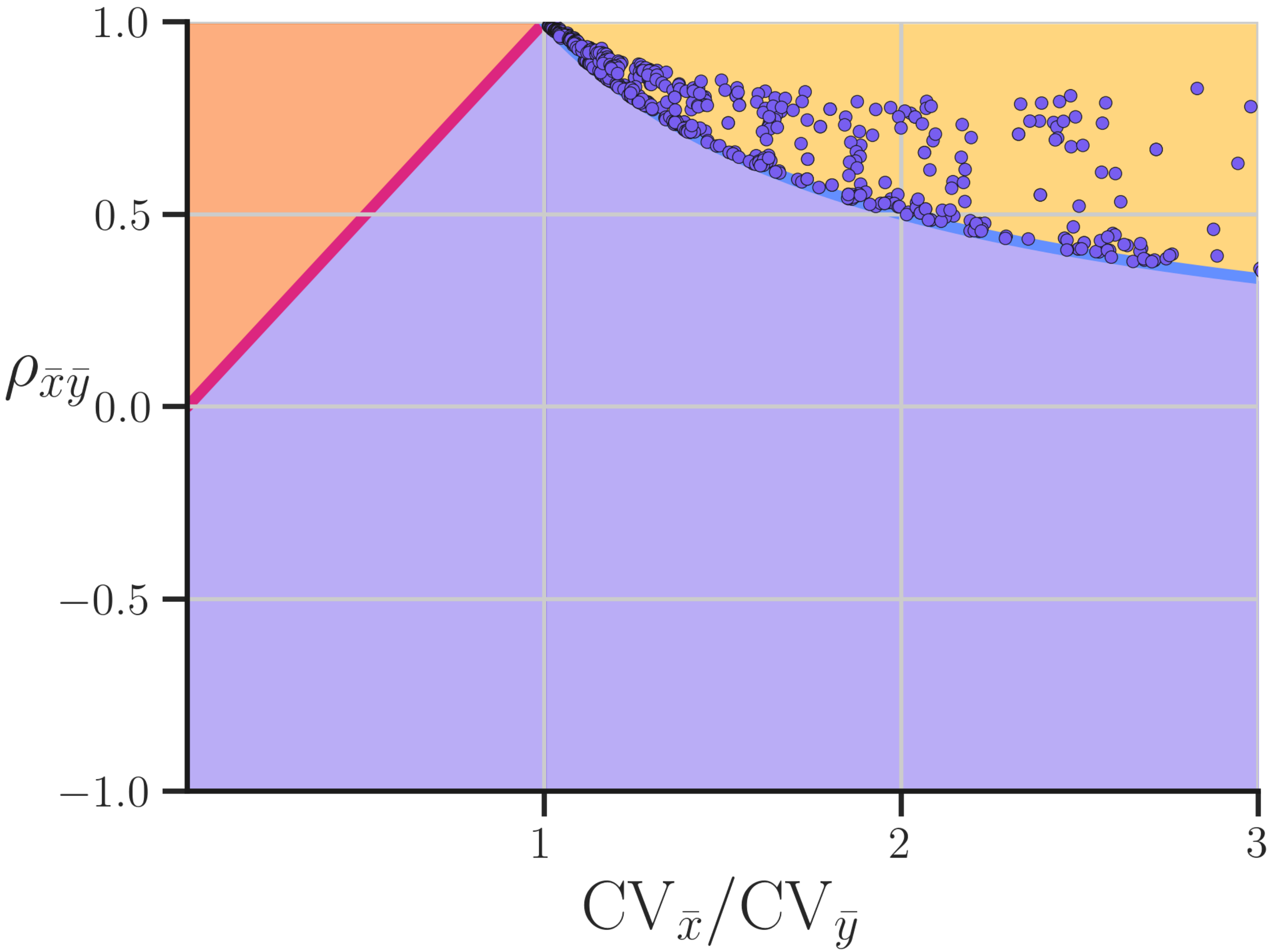
Parameters perturbed by 1-97%

Some systems with $f_y < 0$ behave like positive feedback systems



Curves look like positive feedback systems

Some systems with $f_y < 0$ cross $f_x \geq 1$ bound 'early'



Most violators away from singular point in $0.95 < f_x < 1$

Additional problem: what if sampling from ‘correlation-free’ perturbations

Statistically independent population

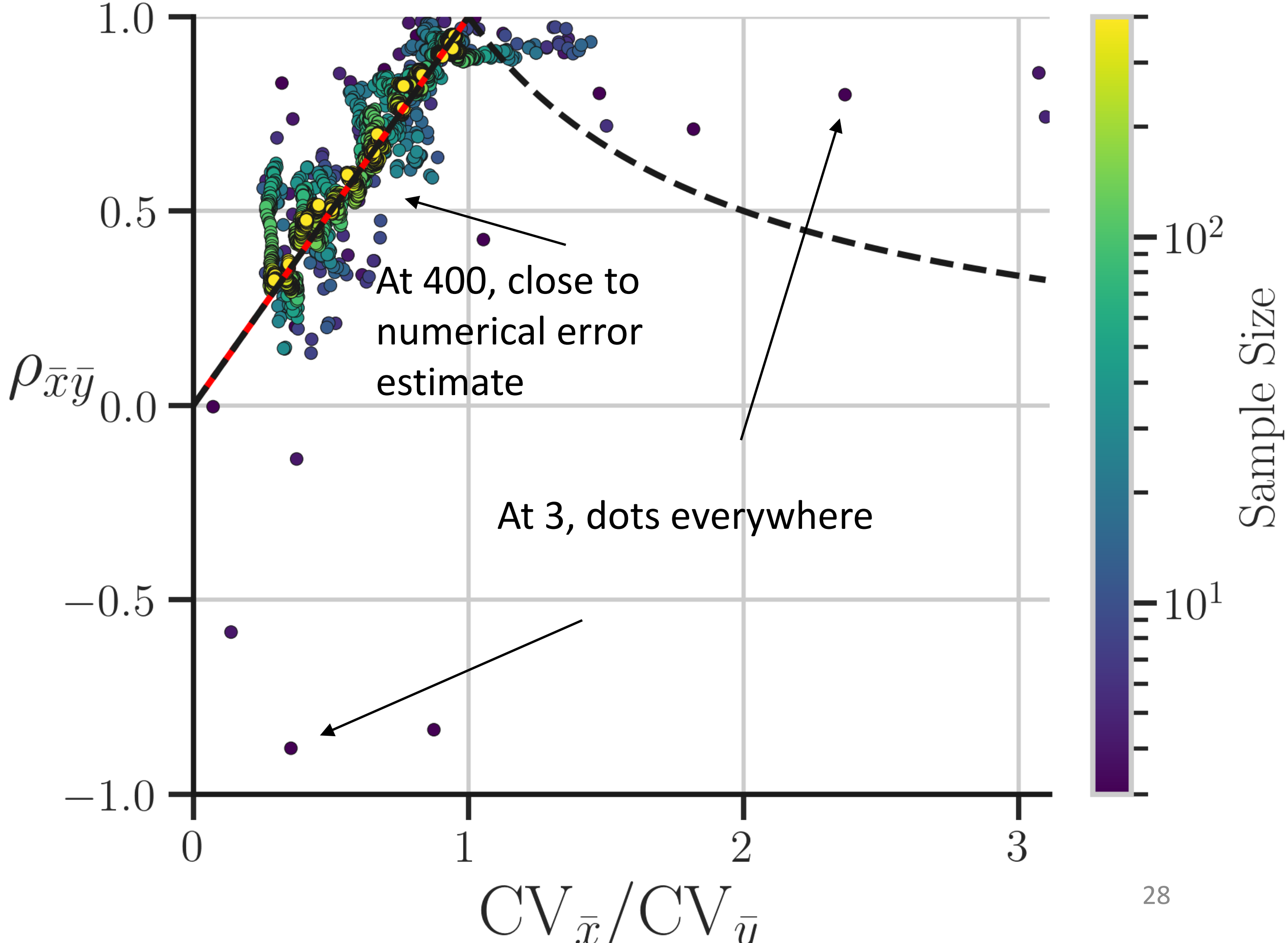
$$\rho_{\lambda\gamma} = 0$$

Sample

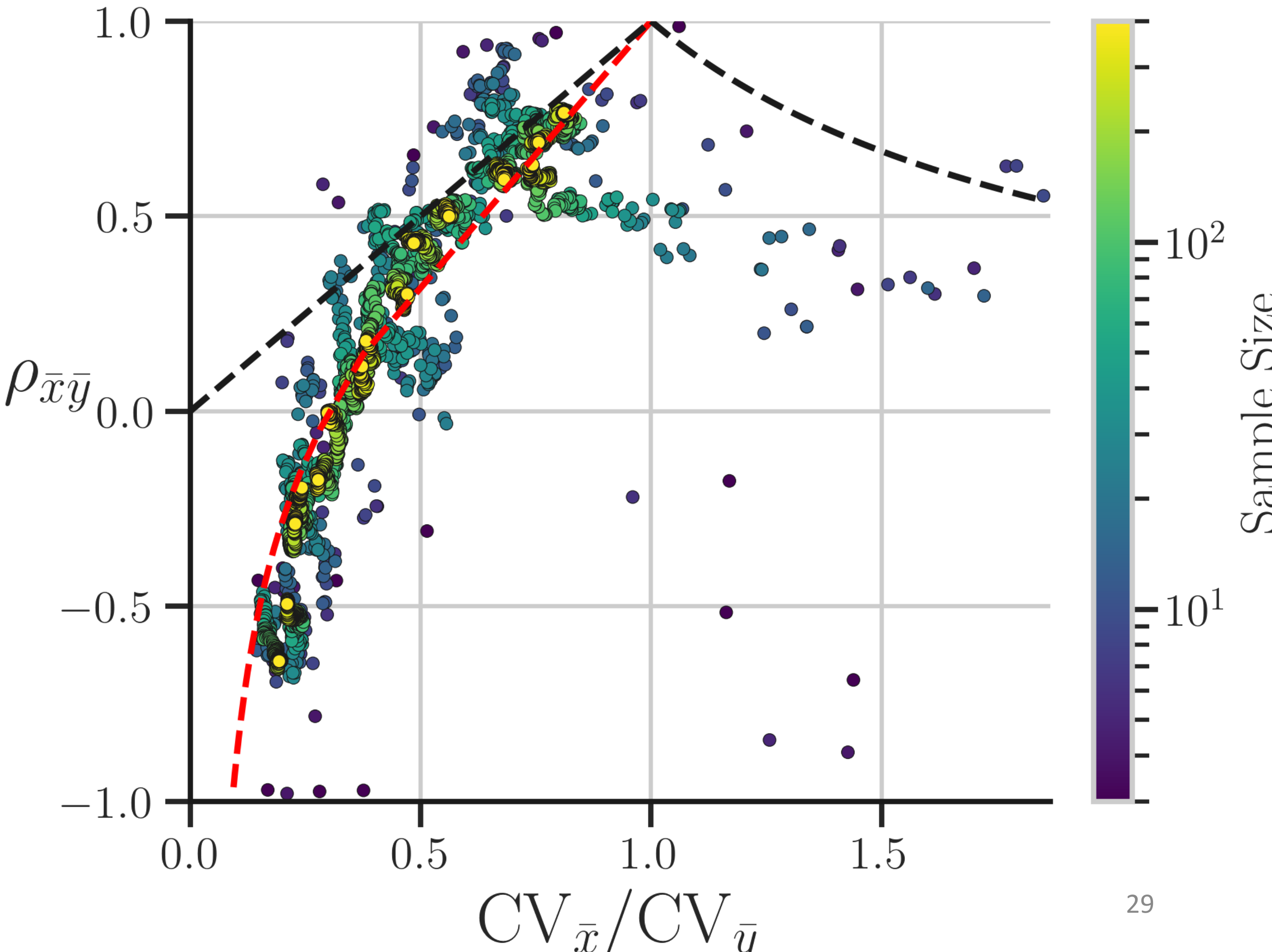
$$\rho_{\lambda\gamma} \neq 0$$

Finite sampling causes deviation from constraints due to sample perturbation correlations

No feedback: finite perturbation size not responsible!



Systems with feedback show similar reliance on sample size



Finite perturbation effects will contribute divergence

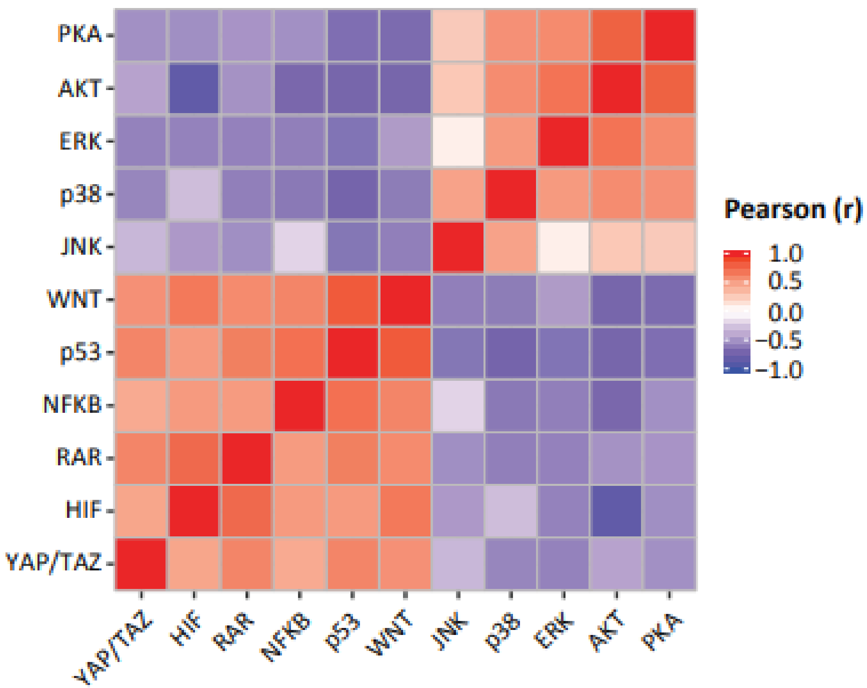
This work (and a lot of Physics) in one sentence

Linear analysis works, except when it doesn’t

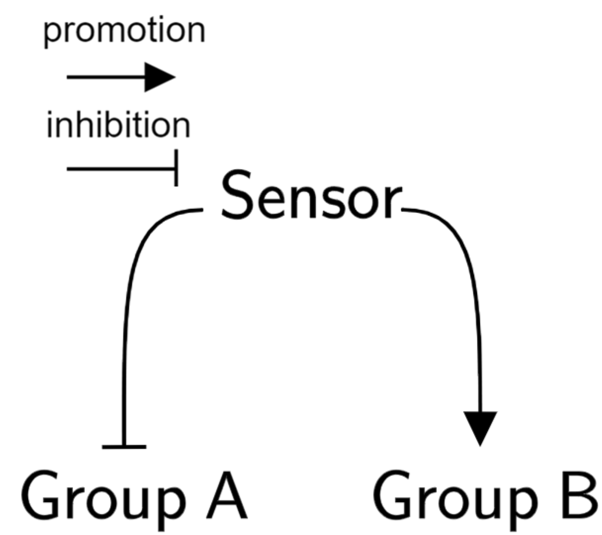
Predicting feedback via random perturbation is possible, but can fail:

- Finite perturbation effects for non-linear AND stochastic systems
- Finite sampling effects if experiment is subsample of a correlation free set

Large scale 'random' perturbation experiments are a reality: Physics can play role in modelling



Correlations of pathway activity changes in response to 122 drugs



"Naïve" model of data

Kaufman, Tom, et al. *Nature Communications* 13.1 (2022).

Lands and Treaty acknowledgement

Research performed on treaty lands of the Mississaugas of the Credit; ancestral lands of the Anishinabek, Huron-Wendat, Haudenosaunee, and Chippewa peoples; and on the lands of the Wolastoqey Nation.

<u>Research funding is produced by value extraction from these lands—often in systemic violation of the treaties which give us settlers the right to use it</u>

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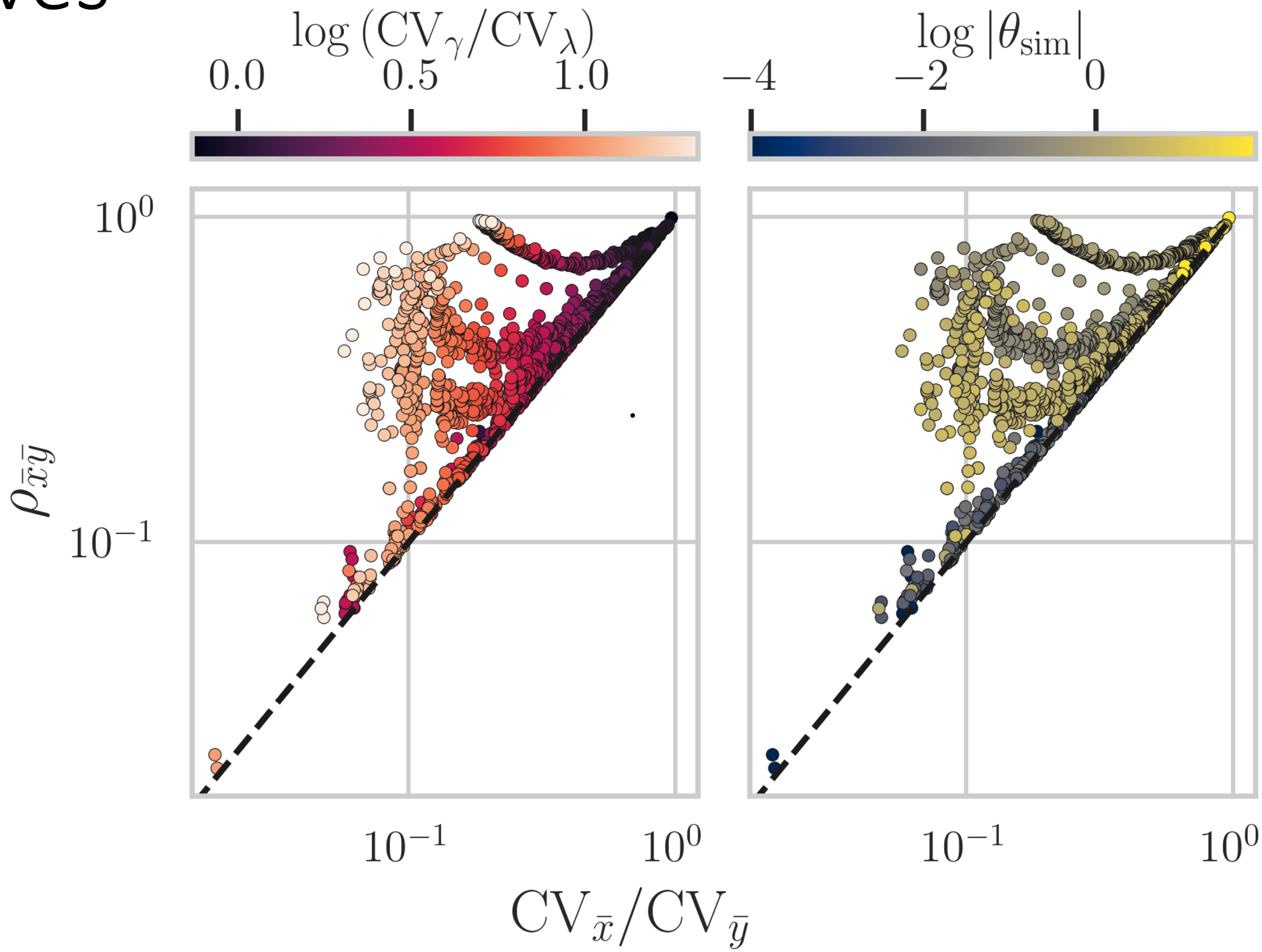
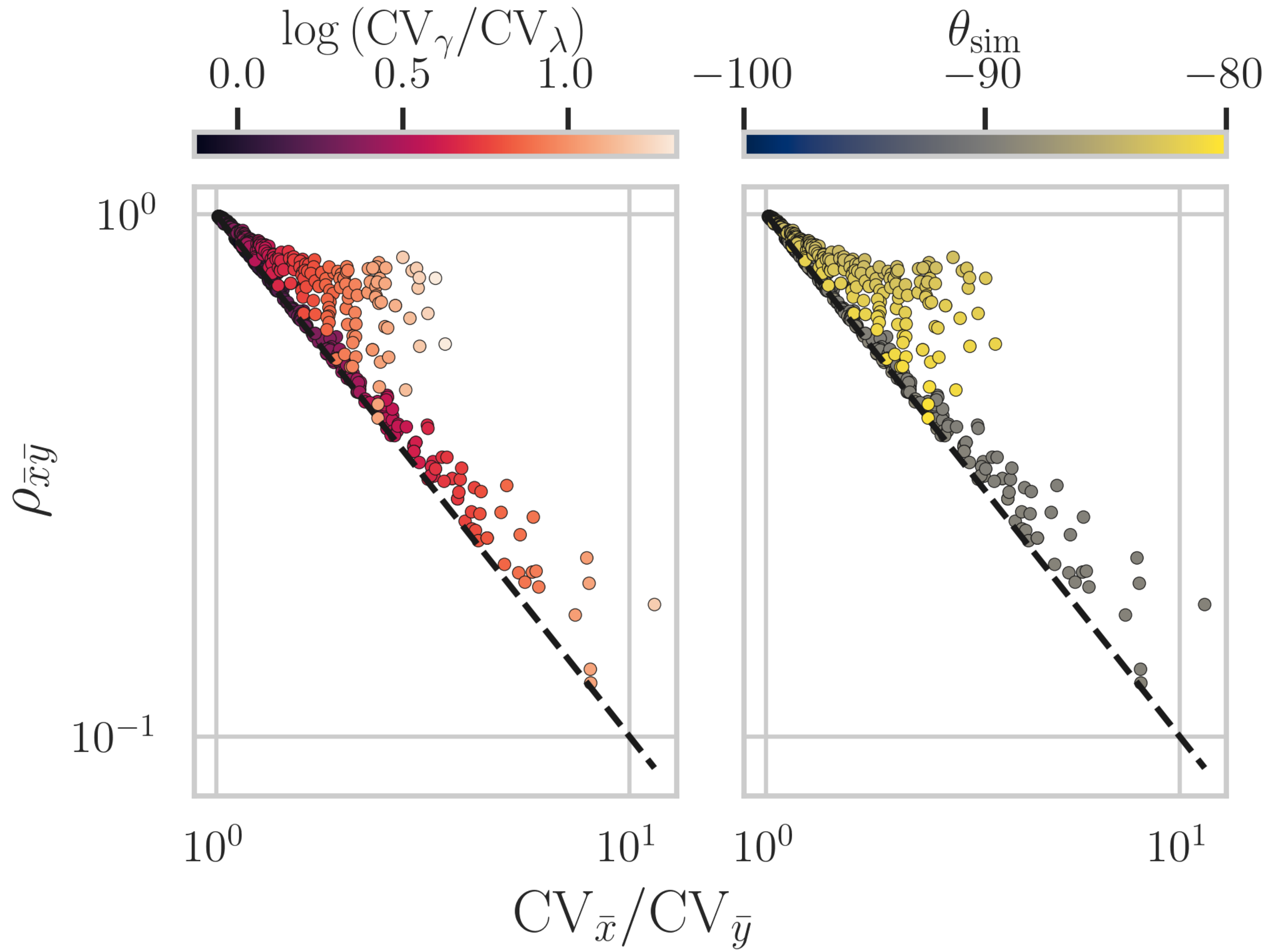
Appendix

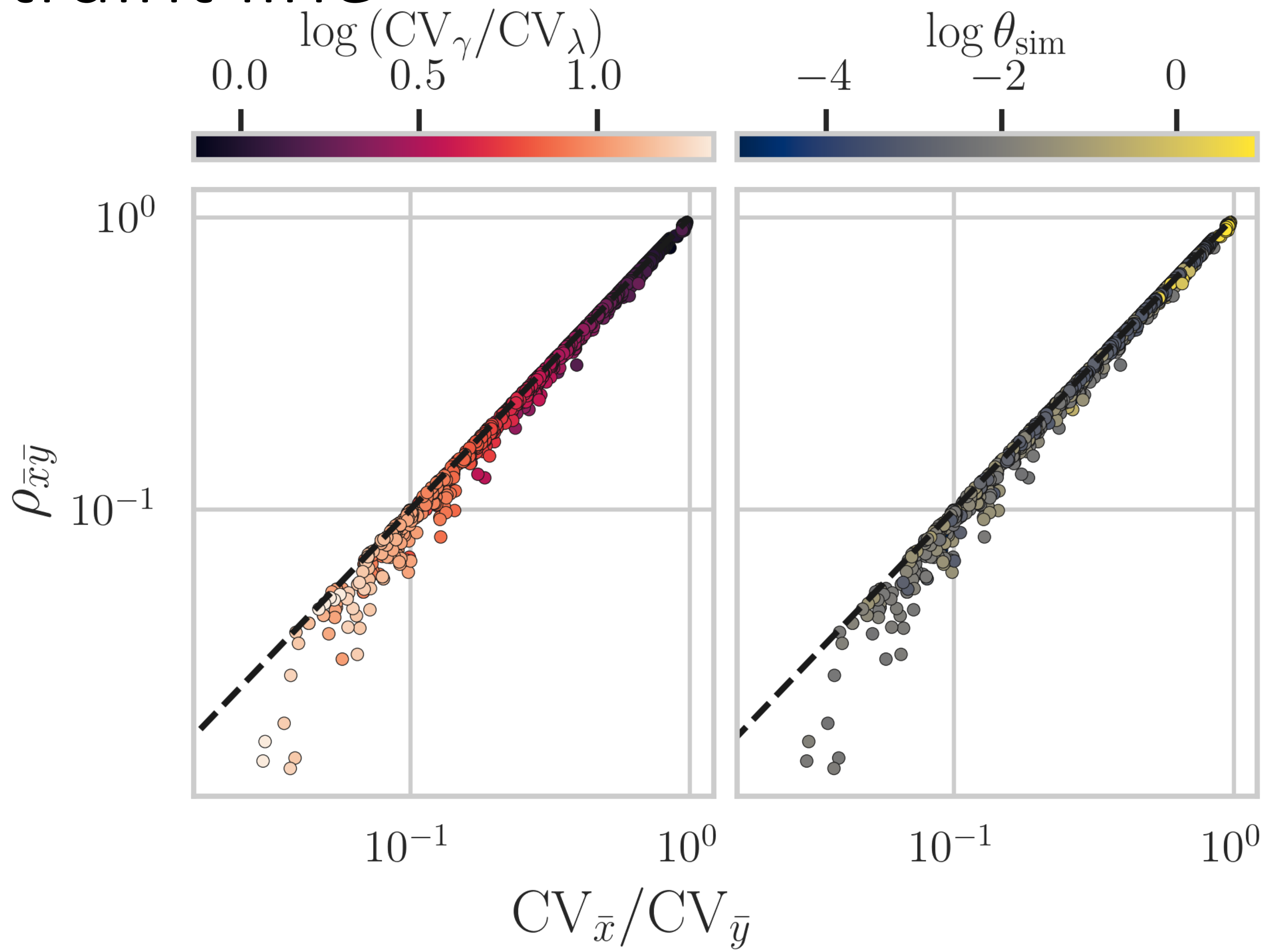
Some systems with $f_y < 0$ that violate constraint follow "positive feedback"-like curves



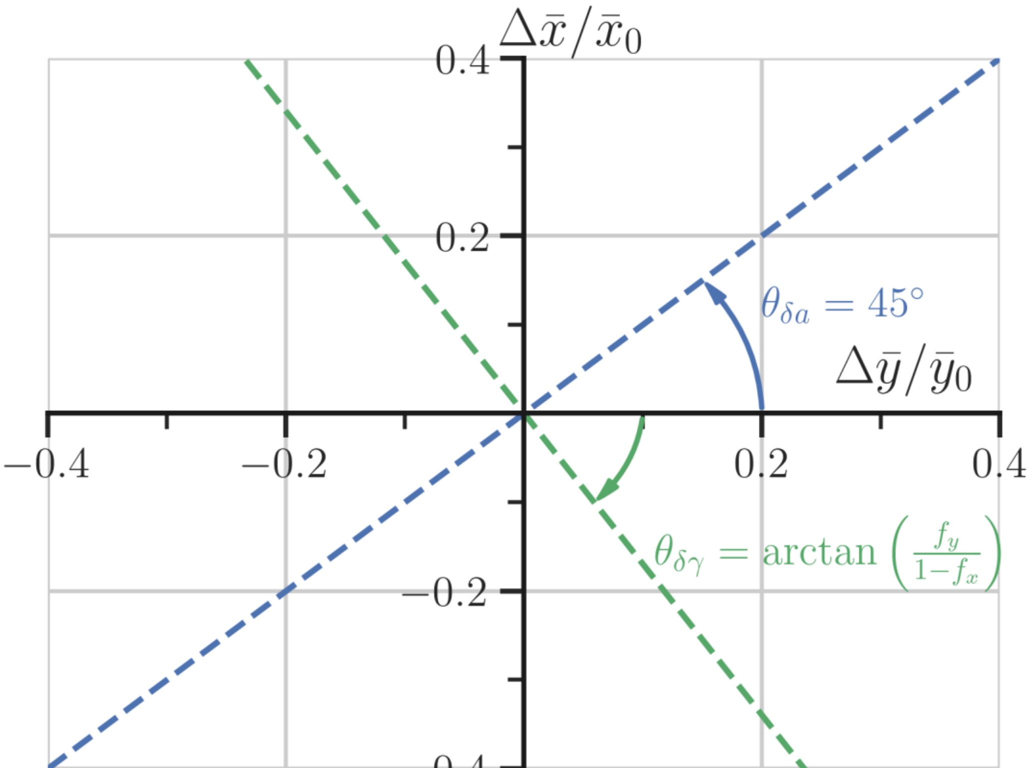
Systems that violate the f_x constraint have a sensitivity approaching the critical value



Systems with $f_y > 0$ that violate constrain have weak feedback and appear near constraint line



Hit one parameter: “linear response” constrains feedback



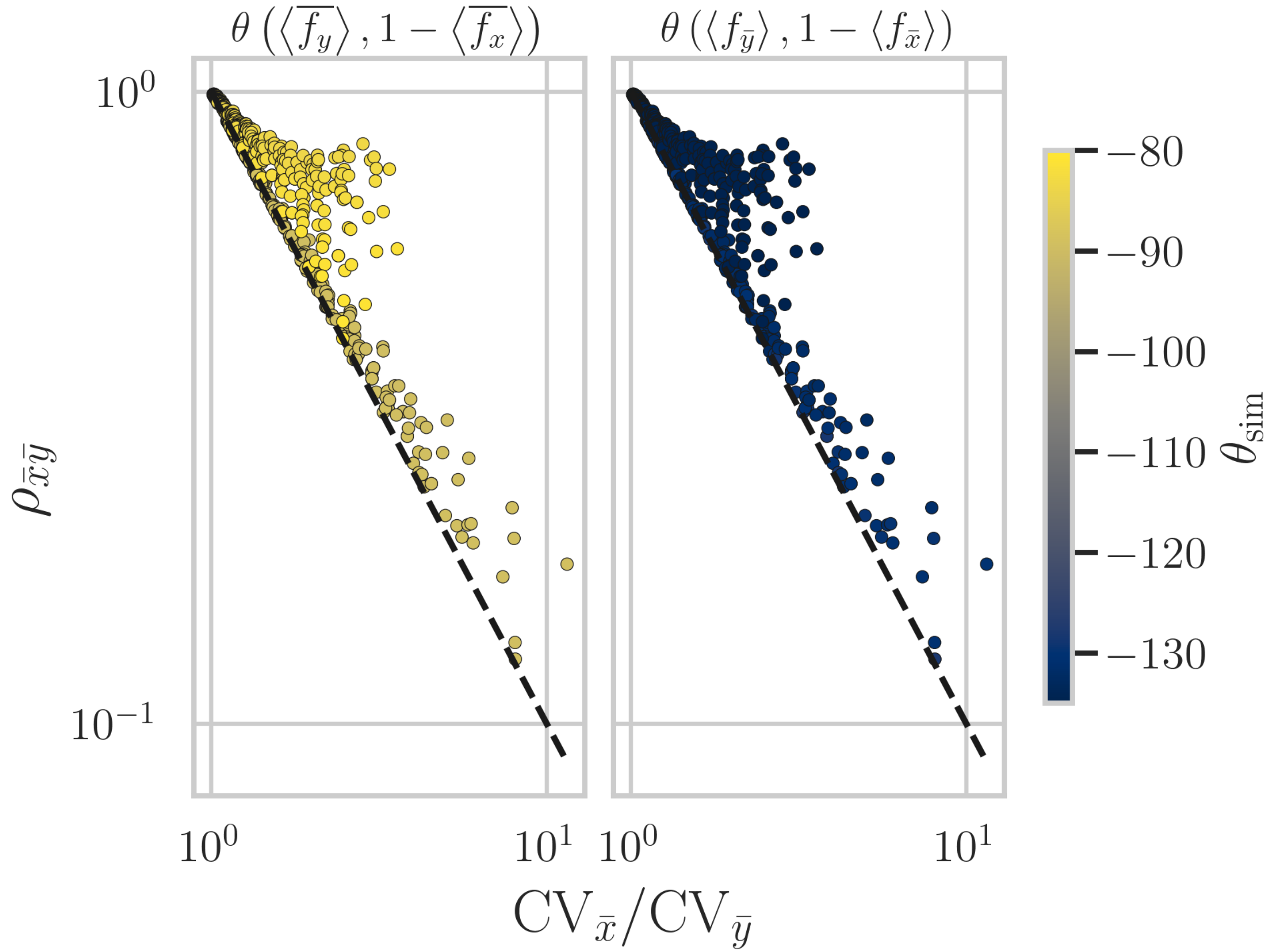
Feedback “sensitivity”

$$f_x \equiv \frac{\partial \log f}{\partial \log x} \qquad f_y \equiv \frac{\partial \log f}{\partial \log y}$$

$\boldsymbol{\theta_{\delta\gamma}}$	$\boldsymbol{f_y < 0}$	$\boldsymbol{f_y = 0}$	$\boldsymbol{f_y > 0}$
$\boldsymbol{f_x < 1}$	(-90,0)	0	(0,45)
$\boldsymbol{f_x = 1}$	-90	Unstable combinations	
$\boldsymbol{f_x > 1}$	(-135,-90)		

How to account for ‘random’ perturbations?

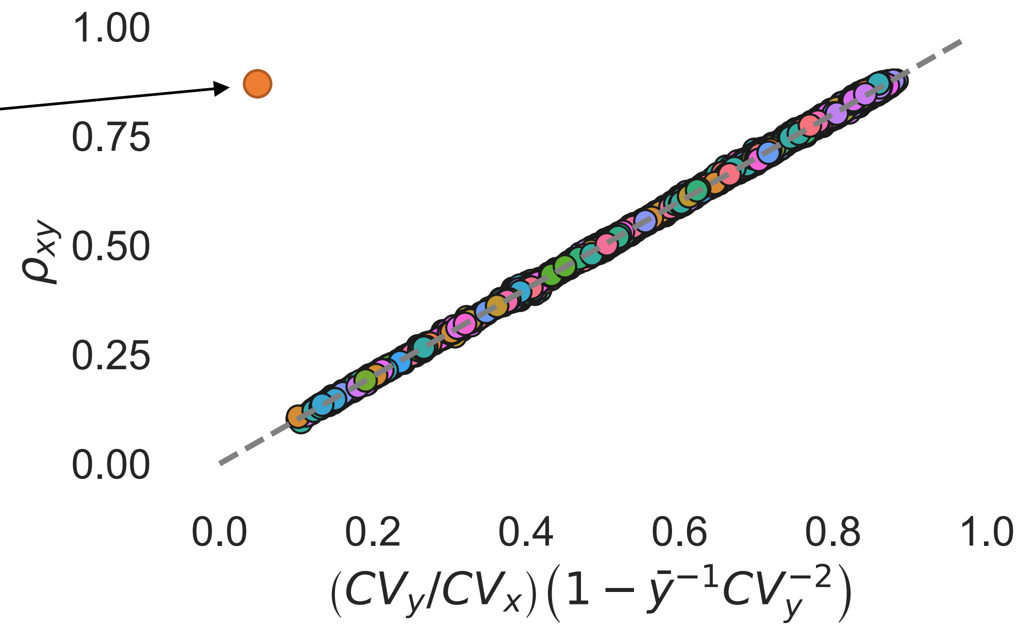
"Violations" in f_x constraint appear related to calculation of sensitivity

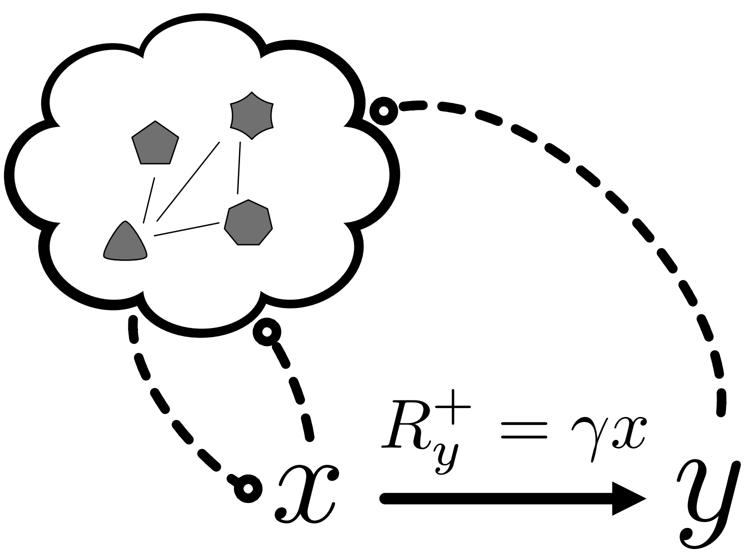


Incompletely specified stochastic systems still obey certain constraints on their correlation

Model: Stochastic Gene Expression

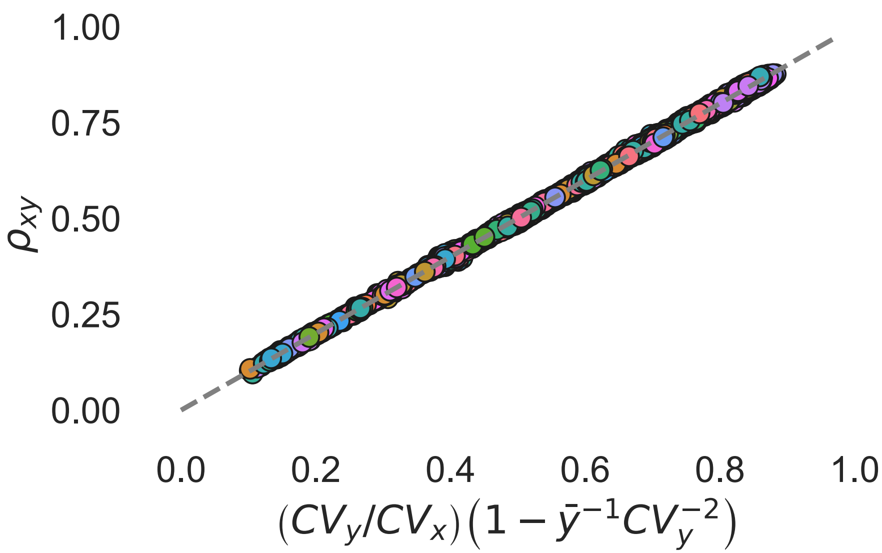
If a measurement lands here, system inconsistent with this class of model!



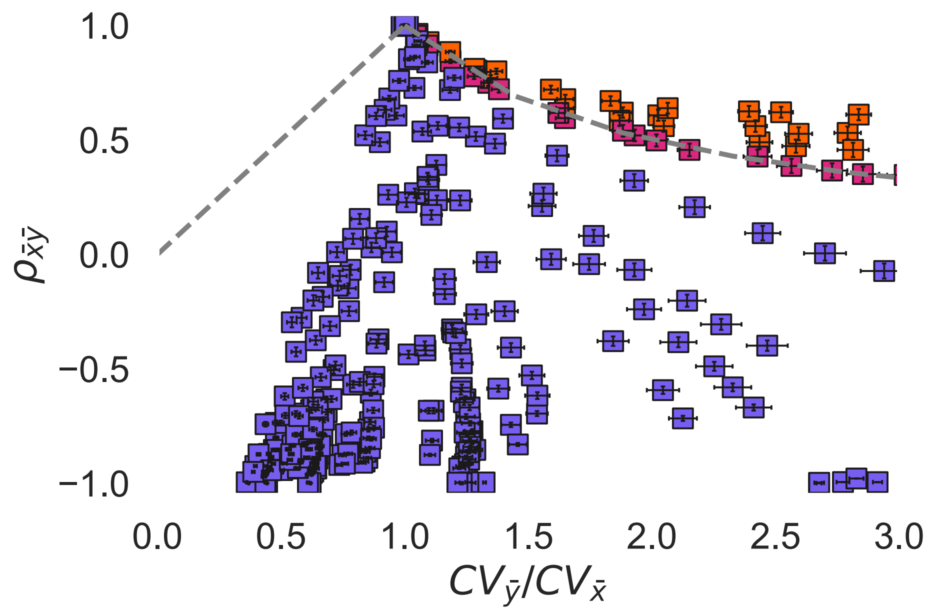


Hilfinger, Andreas, et al. *Cell Systems* 2.4 (2016).

Perturbation response correlations are linked to variation of molecular averages by physical model of system

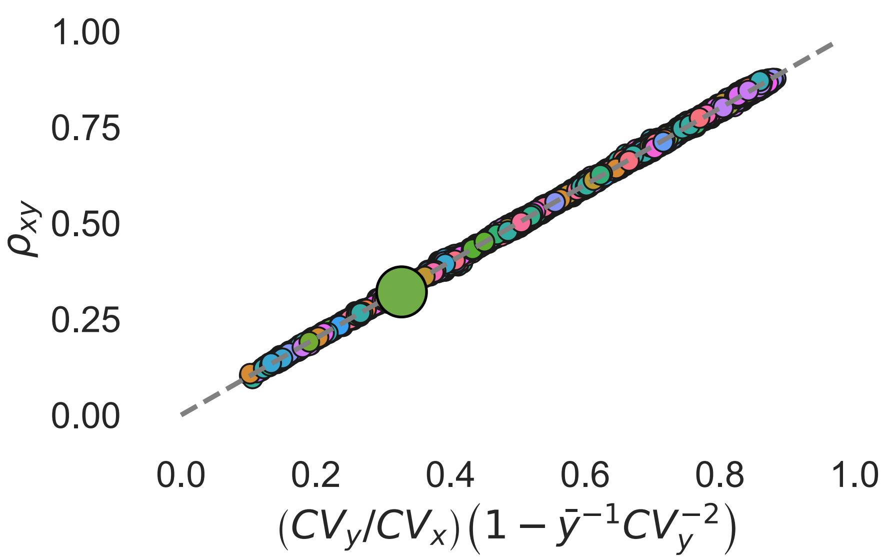


Verify consistency with linear translation



Infer feedback assuming linear translation model

Perturbation response correlations are linked to variation of molecular averages by physical model of system



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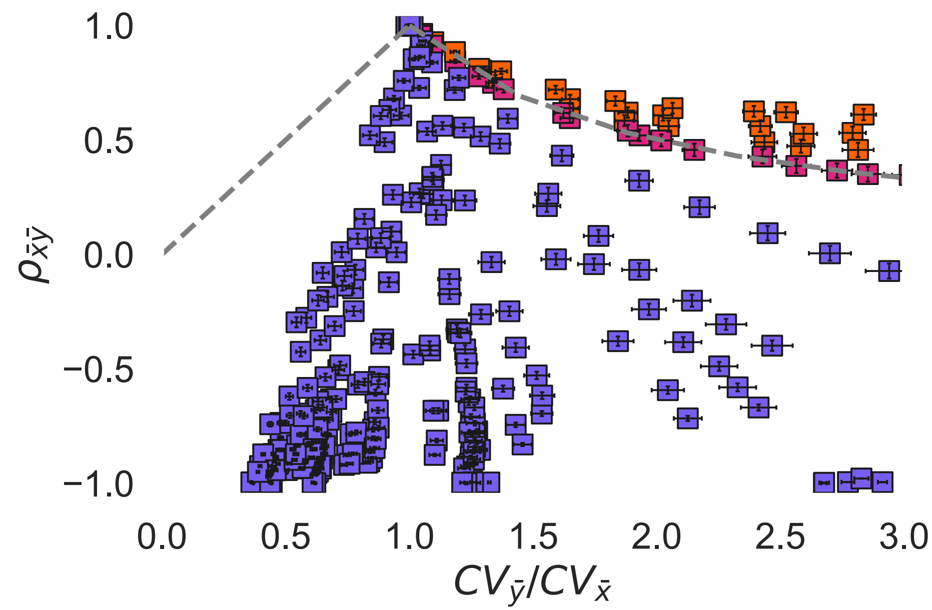

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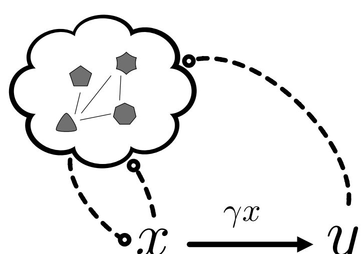

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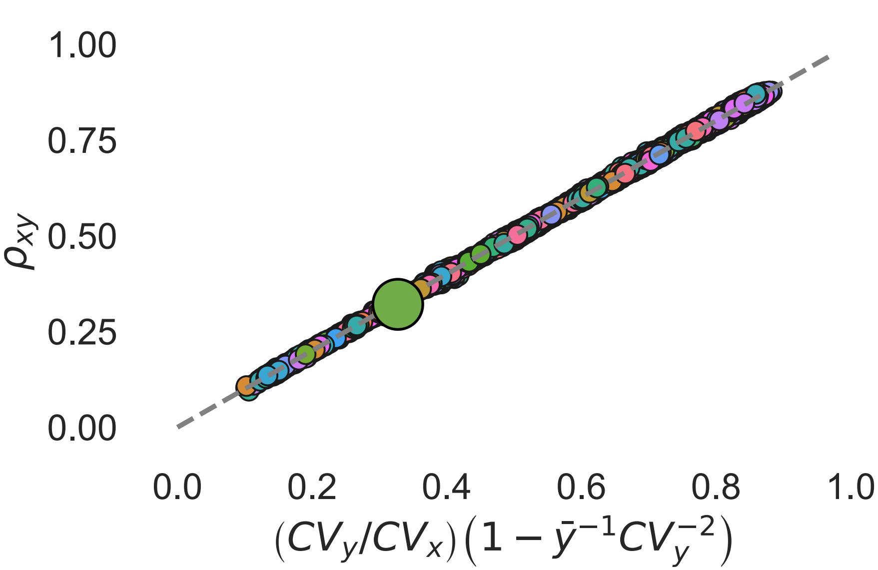

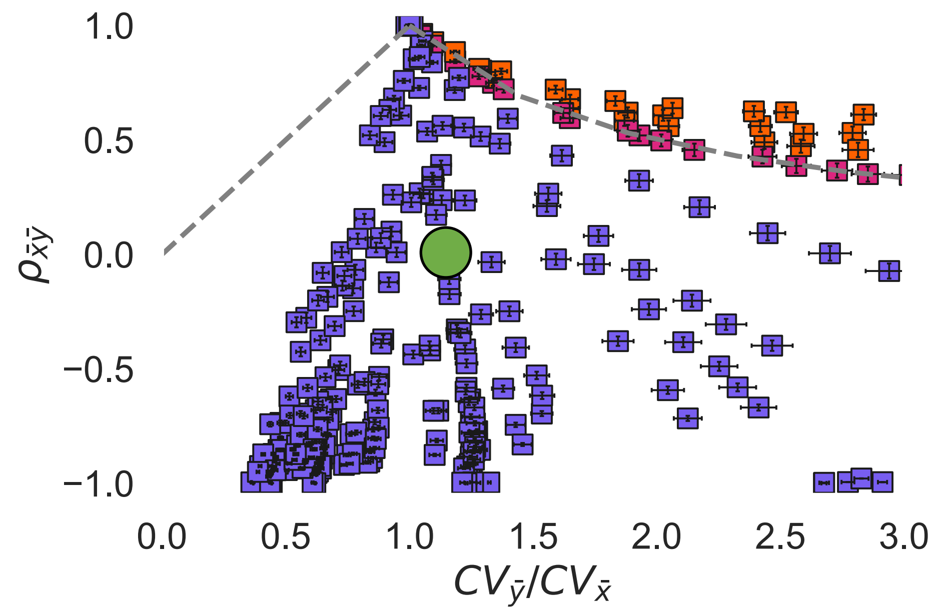

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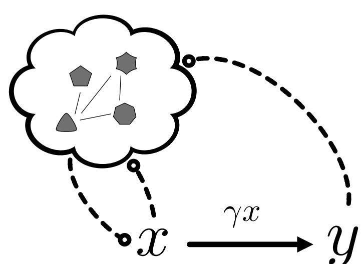
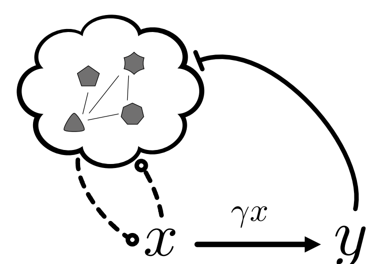
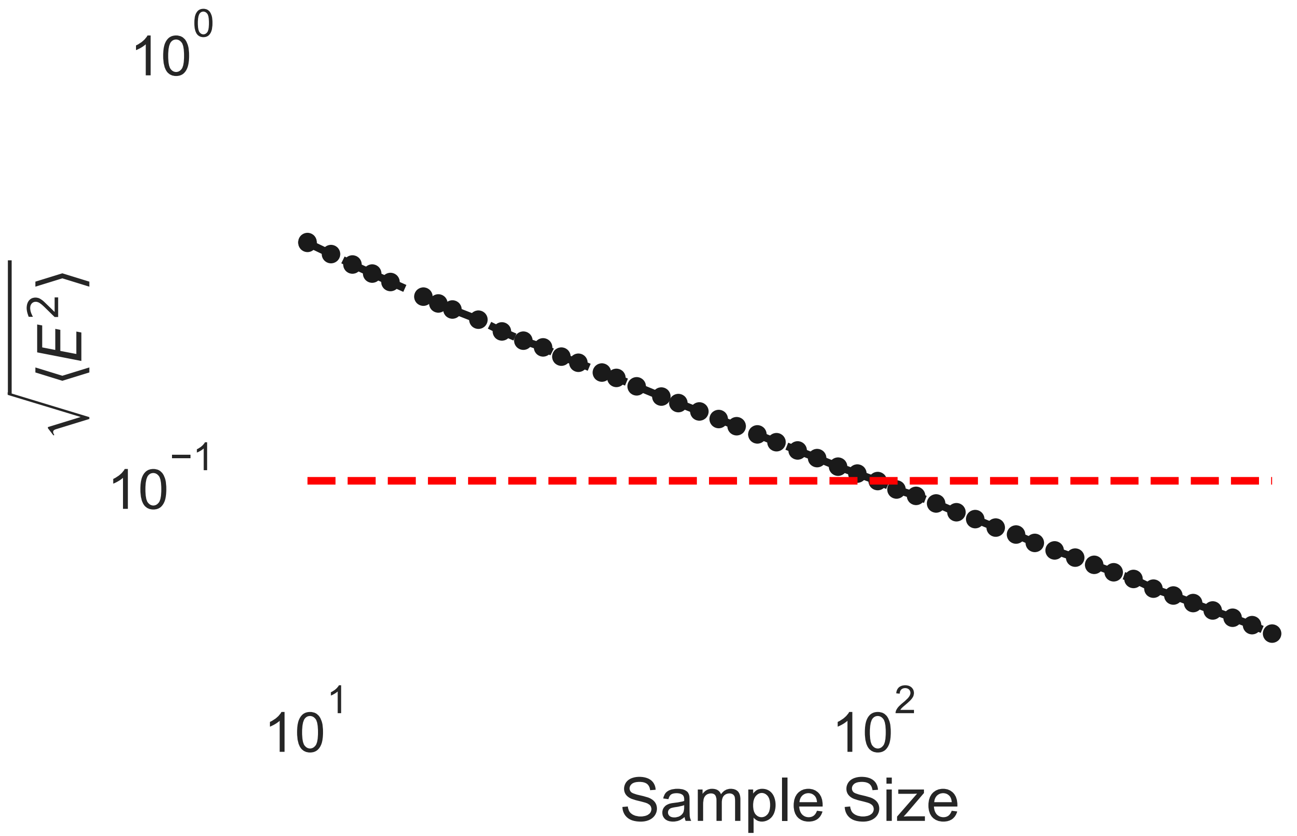


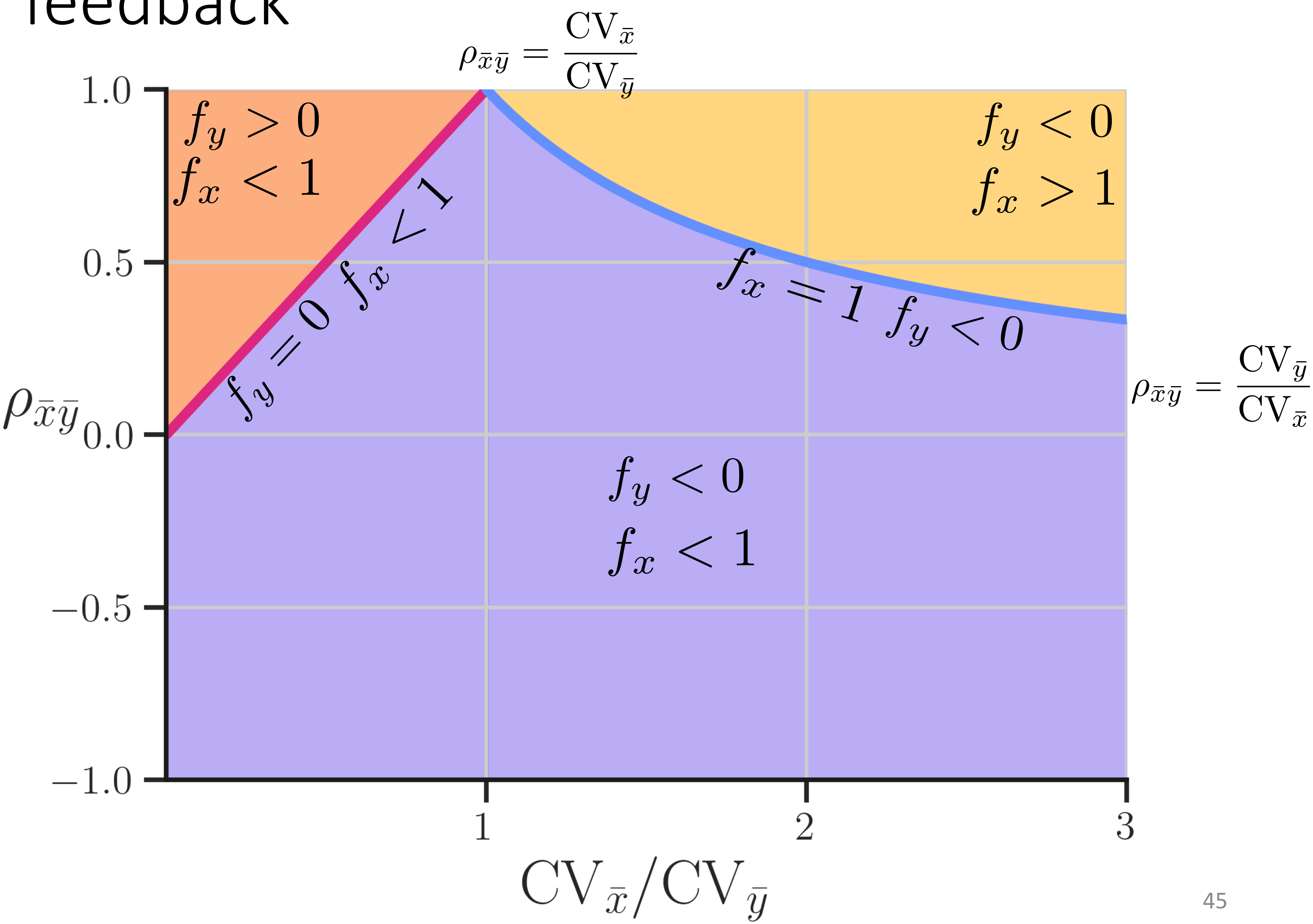


Reducing 'error' below 10% requires ~100 samples

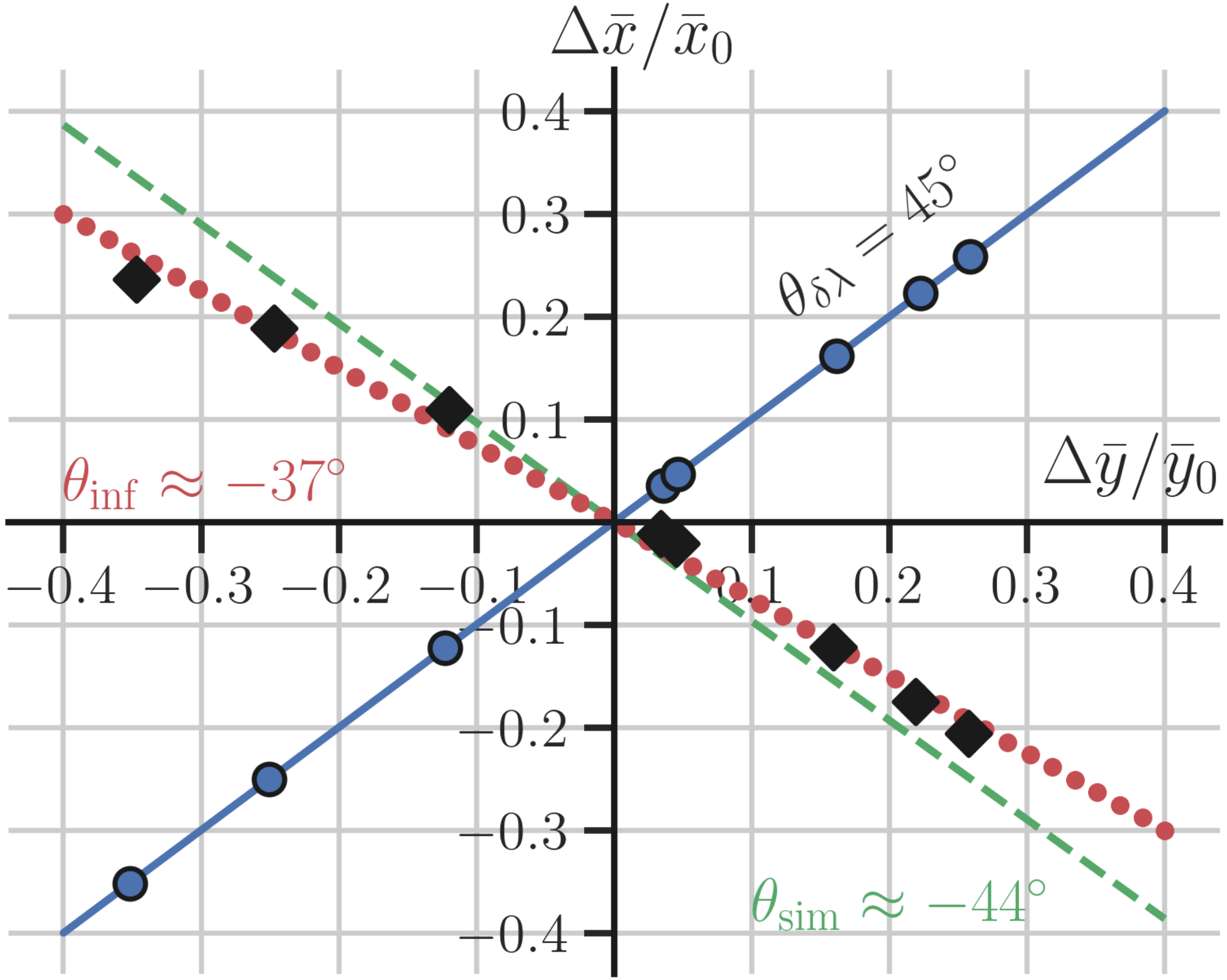
$$\rho_{\bar{x}\bar{y}} = \frac{\mathrm{CV}_{\bar{x}}}{\mathrm{CV}_{\bar{y}}}(1+E)$$ Function of sampling covariance between λ, γ



Two constraints for f_y and f_x determine "sign" of feedback

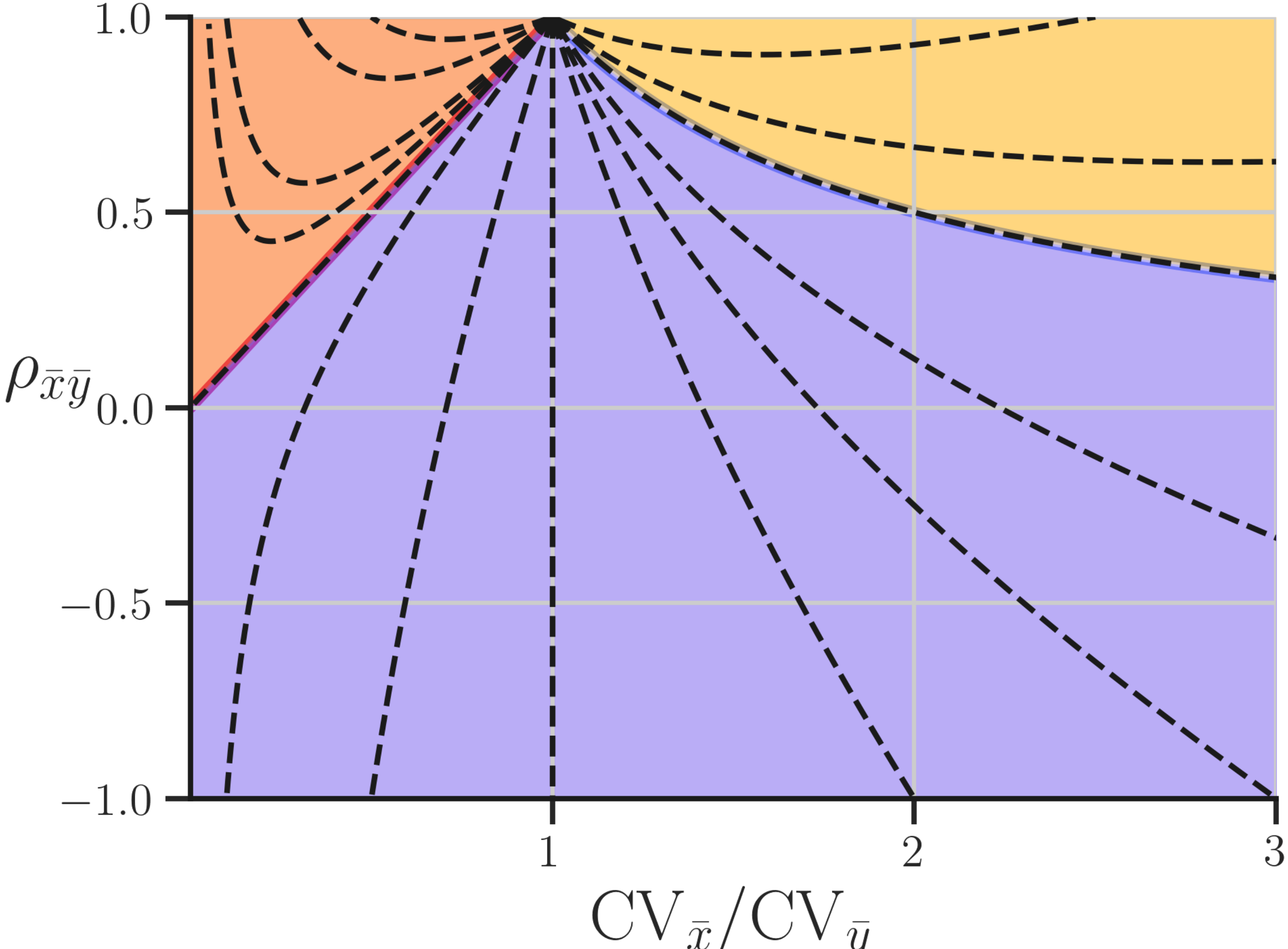


Non-linear, stochastic systems deviate for finite perturbations to γ



Systems land on curves based on relative feedback sensitivities

All curves start at (1,1)



Curves shared by systems with same

$$\frac{f_y}{1-f_x}$$

End at CV ratio equal to

$$\left|\frac{f_y}{1-f_x}\right|$$

With a correlation of

$$\operatorname{sgn}(f_y)\operatorname{sgn}(1-f_x)$$