

Optimal Experimental Design for Estimating the Parameters of Akt-PH Domain Translocation and for Calibrating Protein Constructs for the Chemically Inducible Production of PIP₃

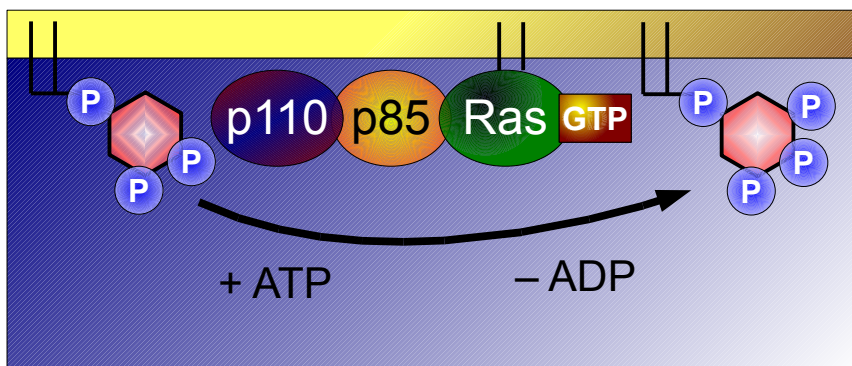
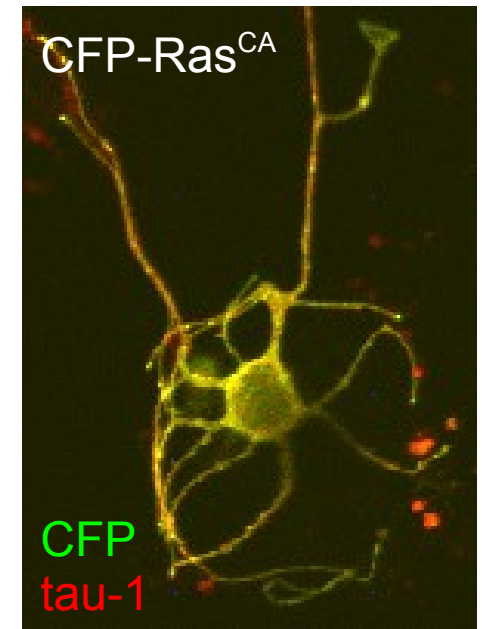
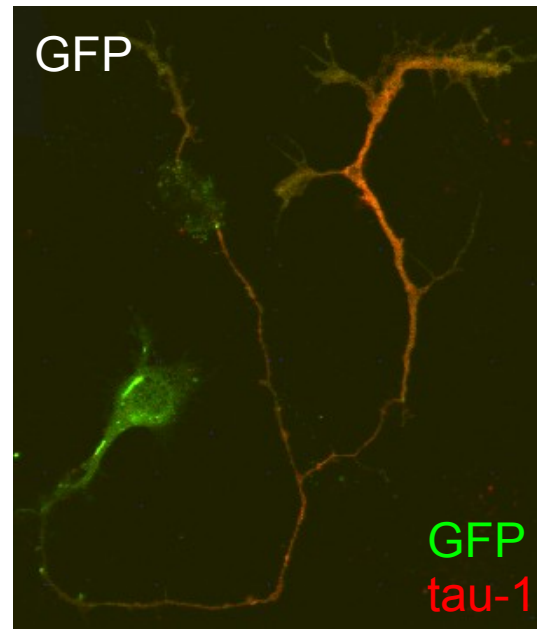
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PIP₃ is a signalling lipid in the plasma membrane

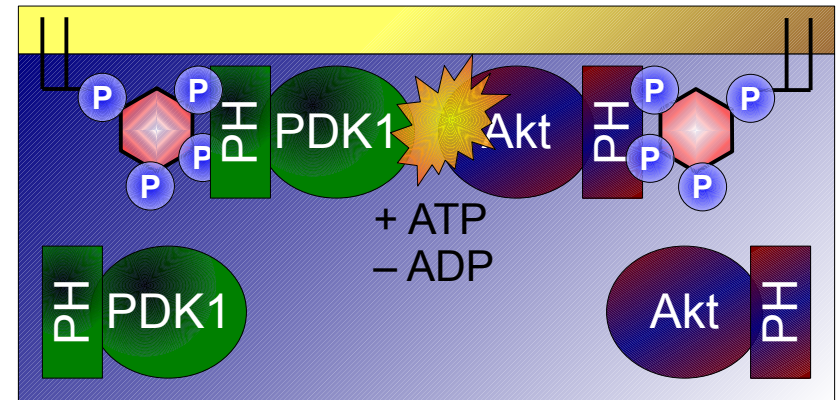
- rat E18 hippocampal neurons can be cultured *in vitro*
- poorly understood mechanism ensures formation of a single axon
- PIP₃ is involved in neuronal symmetry breaking



- PIP₃ signal produced by PI3-kinase
- stimulated by growth factors via Ras
- degraded quickly by PTEN, SHIP, ...
- disregulated in many cancers
- inhibitors of PI3K - wortmannin
- LY294002

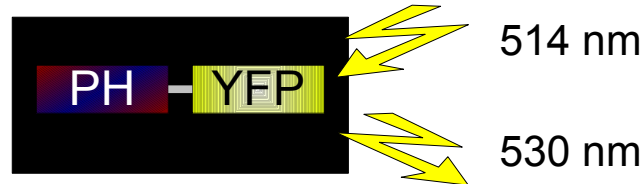
Binding of PH domains relays PIP₃ signals

- Pleckstrin homology (PH) domain found in more than a hundred human genes
- PH domain makes proteins translocate to PIP₃-enriched membranes
- Localization mediates different reactivity



Study PH-domain translocation using fluorescent protein constructs

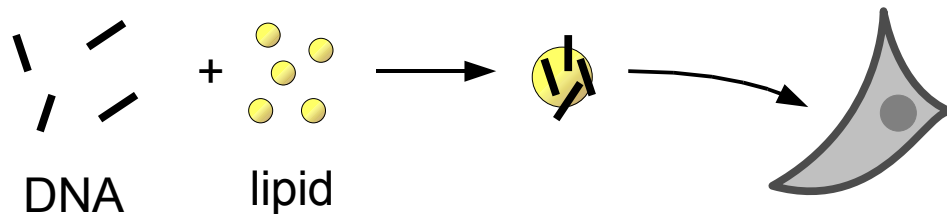
- genes can be composed from parts of other genes



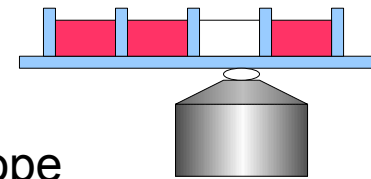
- image localization by confocal fluorescence microscopy

Preparations begin long before experiment

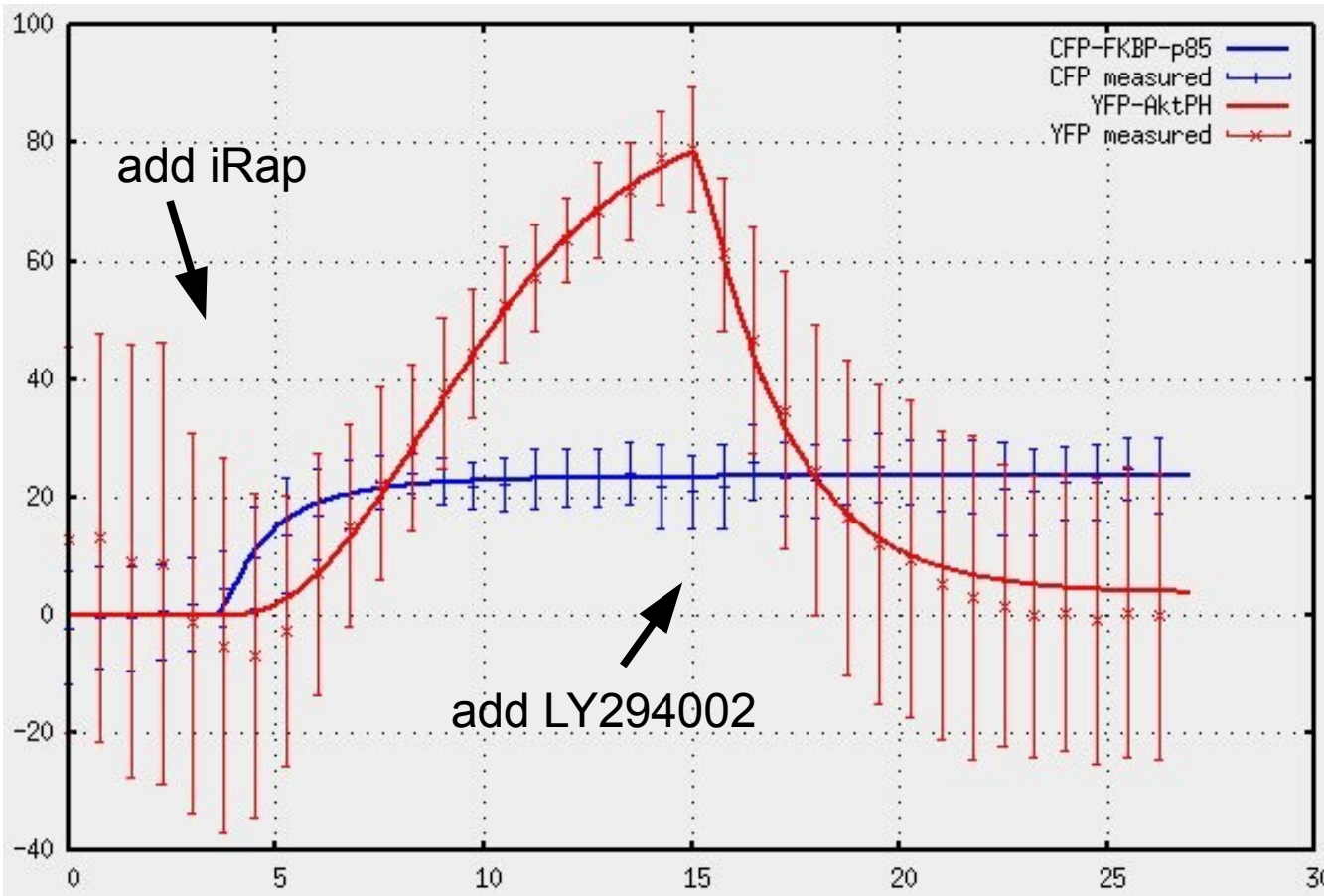
- Culture NIH3T3 cells in chambered glass slides until close to dense (1 day)
- Transfect cells with CFP-FKBP-p85, Lyn-linker-FRB, and YFP-Akt-PH for 18 hrs



- Serum starve cells for at least 1 hr
- Prepare shots of drug
- Warm up HeCd and Ar lasers
- Wash cells with clear extracellular buffer
- Mount on spinning disc laser confocal fluorescence microscope



Large errors result in poor parameter estimates



Φ	= 5	± 47
K_{LY}	= 0.6	± 4.7
α	= 0.47	± 0.61
k_a	= 0.02	± 0.18
ρ	= 0.1	± 1.6
k_r	= 0.4	± 5.8
τ	= 0.02	± 0.24
k_t	= 1	± 15
L_0	= 0.8	± 4.0
f_{p85}	= 30	± 152
PH_0	= 200	± 2300

measurement errors

- cell motility
- focus drift

cell to cell variability

- lipofection efficiency & ratio
- contact inhibition

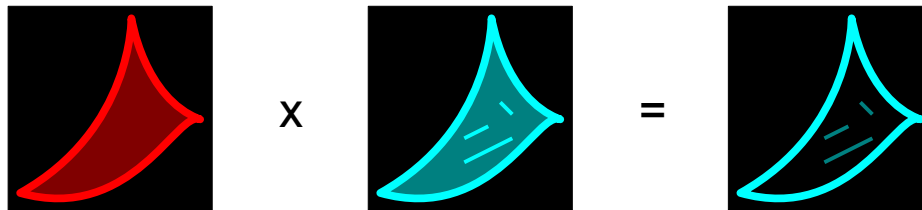
more problems

- varying intensity of lasers
- imperfect mixing

Improve by means of technique or of mathematics

Improve by means of technique

- seed fibroblasts at exact density and at time before experiment
- serum-starve cells at exact time before experiment
- practice for good mixing without disturbing fibroblasts
- use red membrane marker, e.g. tdimer-CAAX...



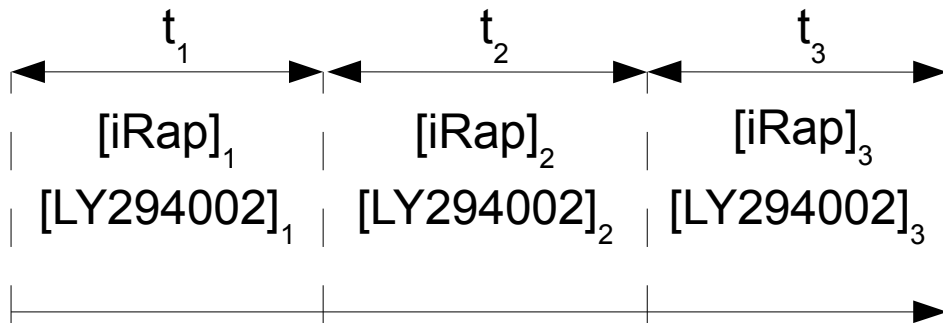
- ... or clone RFP into Lyn-linker-FRB, e.g. Lyn-RFP-FRB

Improve by means of mathematics

- get rid of fluorescence calibration by finding baselines and max
- find the optimal experiment that gives best parameter certainty

Experimental design optimized using VPLAN

- measure CFP and YFP every 45 seconds
- optimize buffer composition on three intervals
- optimize time points for buffer replacements



constraints

$$\sum t_i = 30 \text{ min}$$

$$7.5 \text{ min} \leq t_i \leq 15 \text{ min}$$

$$0 \text{ uM} \leq [iRap] \leq 5 \text{ uM}$$

$$0 \text{ uM} \leq [LY294002] \leq 25 \text{ uM}$$

Optimize of time intervals using VPLAN

- piecewise constant control u_{time} for the speed of time
- multiply all differential functions by u_{time}
- integrate u_{time} , resulting in a differential state $clock$
- evaluate constraint function $clock = 30$ at $t = 30$



Optimal experiment promising...

Optimal experimental design

- 25 uM LY294002 and 5 uM iRap for 7.5 min
- 5 uM iRap for 15 min
- 25 uM LY294002 for 7.5 min

A-criterion predicted to drop 9-fold

- variance drops linearly with measurements
- optimal one saves nine initial experiments

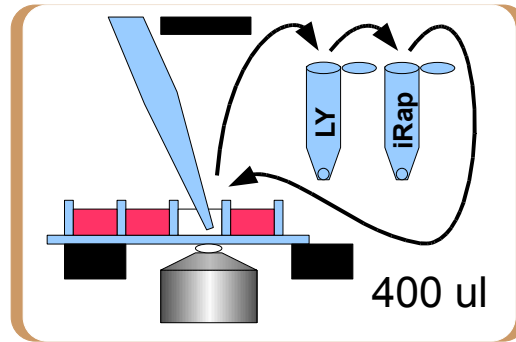
parameter	initial	optimal
Φ	$= 5$	± 29
K_{LY}	$= 0.6$	± 2.8
α	$= 0.47$	± 0.41
k_a	$= 0.02$	± 0.06
ρ	$= 0.1$	± 0.9
k_r	$= 0.4$	± 3.0
τ	$= 0.02$	± 0.08
k_t	$= 1$	± 8
L_0	$= 0.8$	± 1.8
f_{p85}	$= 30$	± 65
PH_0	$= 200$	± 520

... but challenging

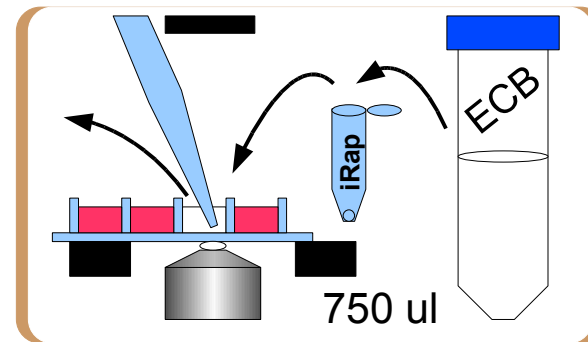
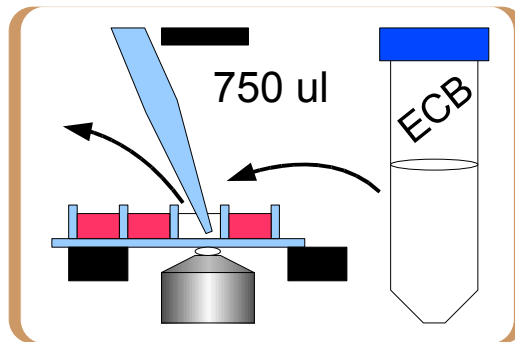
How to do it



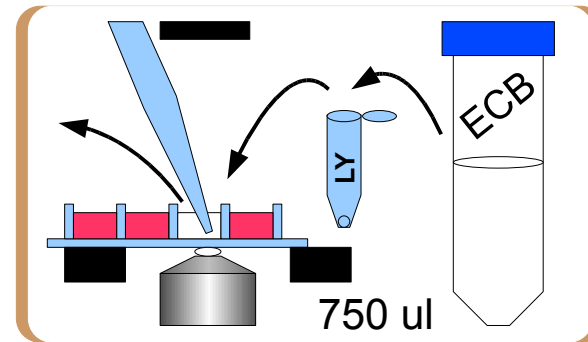
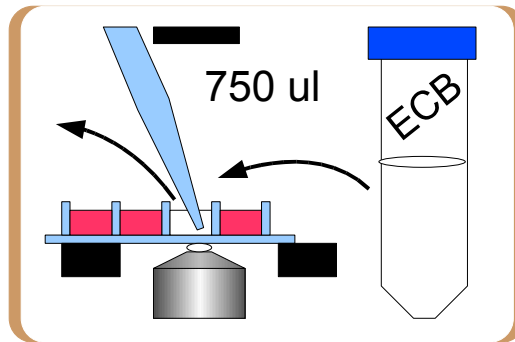
T = 0 min



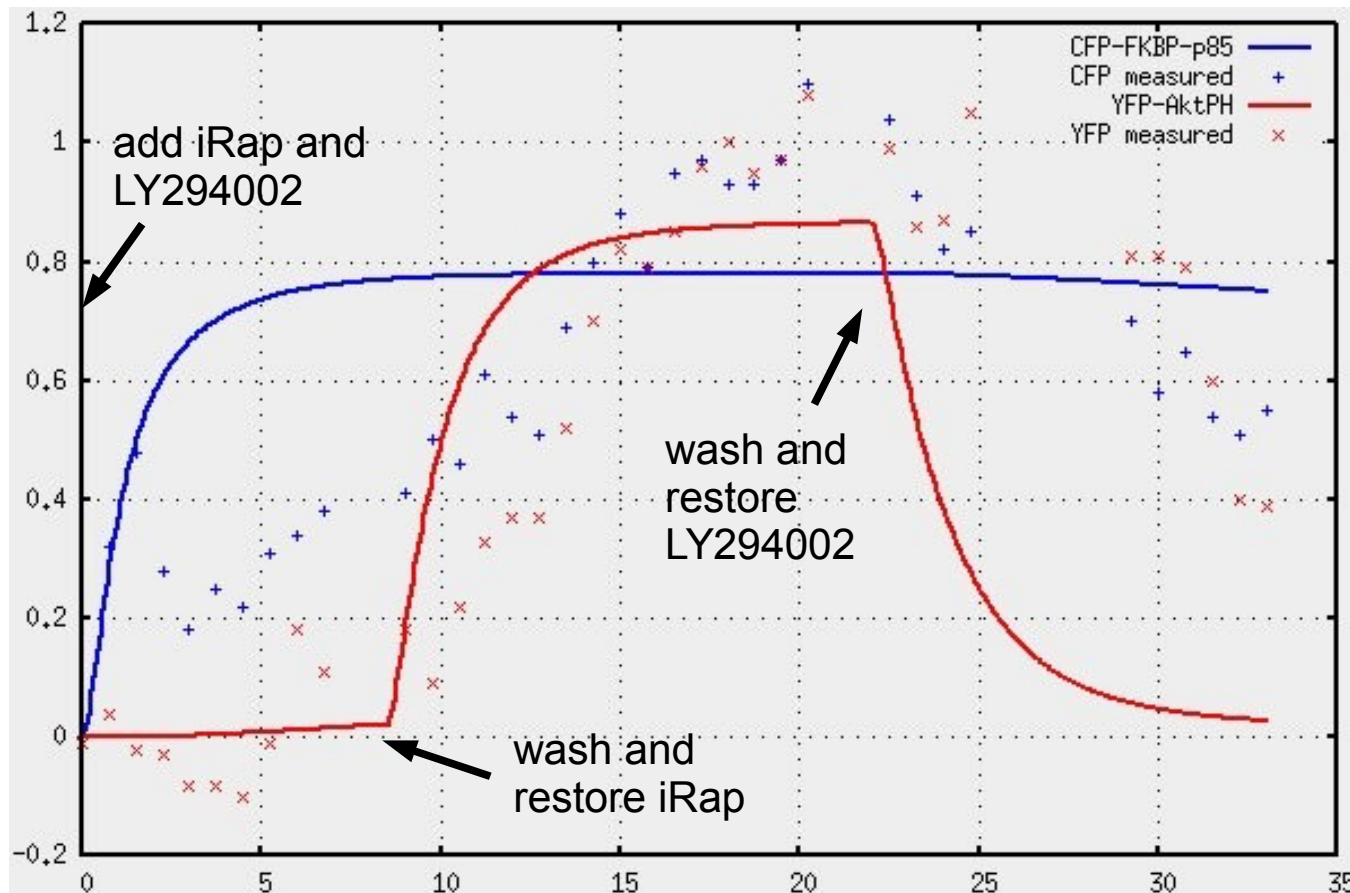
T = 7.5 min



T = 22.5 min



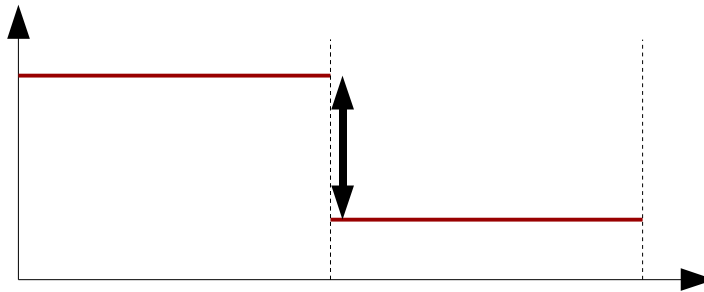
Optimal experiment feasible after training



- as obvious from the movie, no full translocation occurred
 - transfected with 4 constructs → membrane anchor?
 - starved in DMEM for 10 hrs and moved around a lot
 - system was driven within predictive range of model

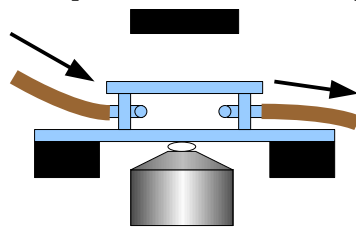
Conclusions

Mathematical constraint against stressful buffer changes



concentration of every
buffer component
 $u(t_i) \geq \alpha u(t_{i-1}), \alpha = 10\%$

Experimental approach: build a flow-chamber



use continuous piecewise linear
controls for driving pumps

Optimal experiment is interesting, challenging, but feasible

- Obtain more measurements within the next two or three weeks
- Will optimal experiment save 8 experiments of the initial design?

Acknowledgments

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