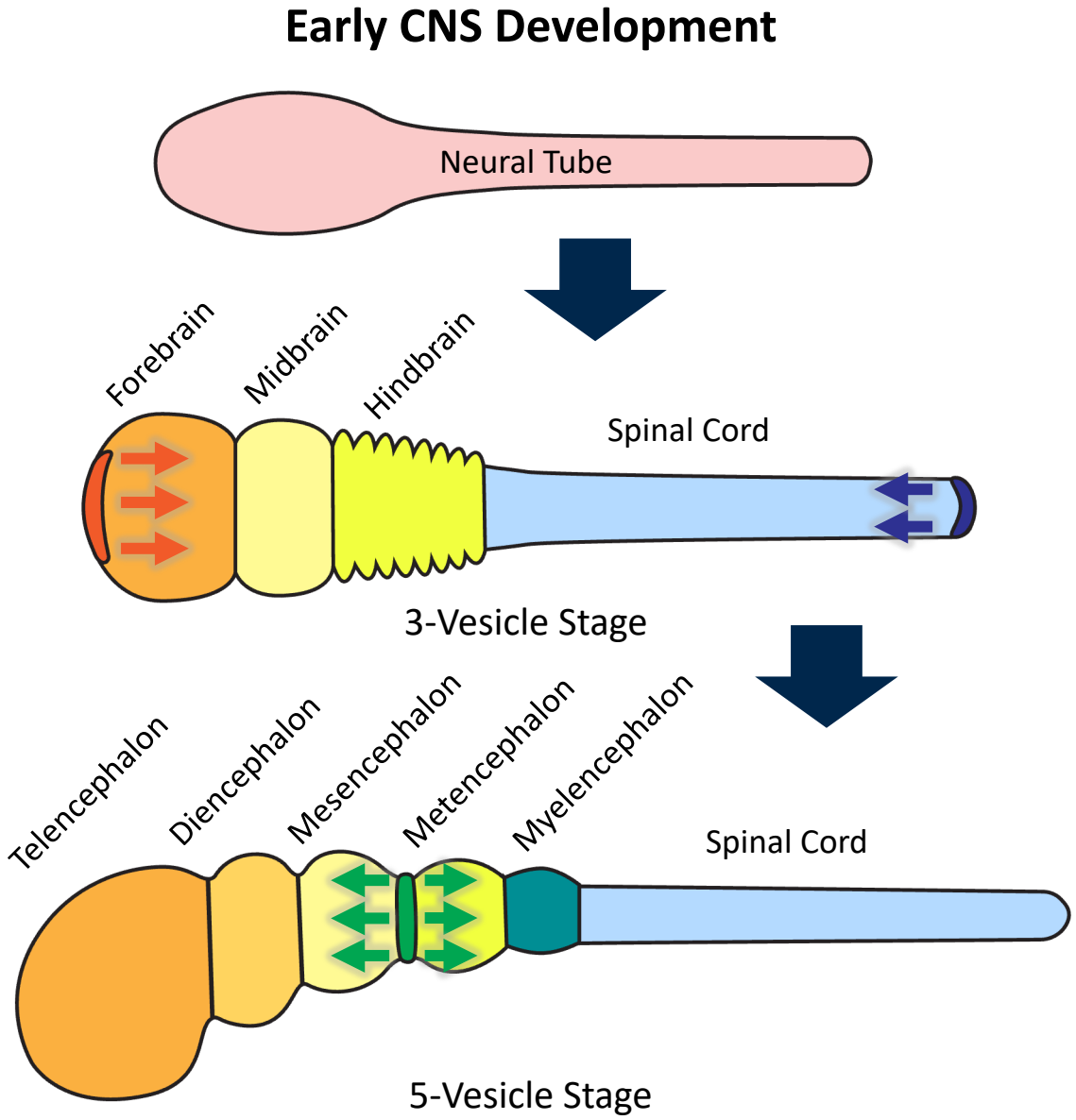
# Background

## Patterning in Biology

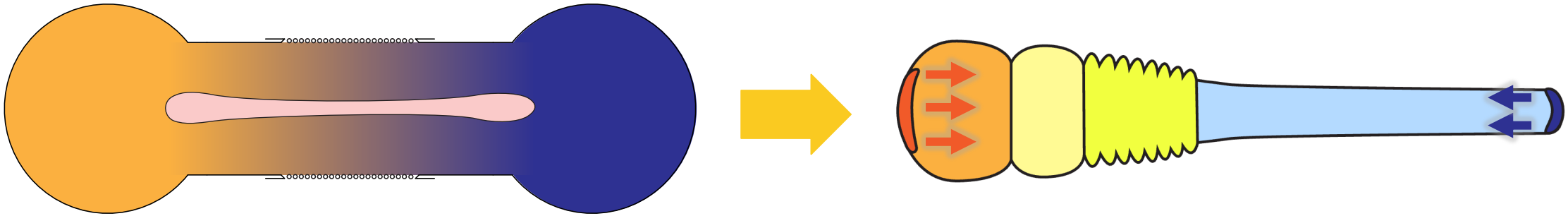
Generation of complex organizations through cell fate decisions

**Rostrocaudal (RC) Patterning**
of neural tube



# Background

Recapitulate CNS patterning using engineering tools



Achieve patterning

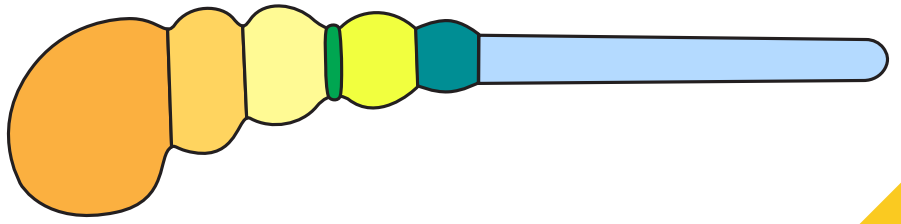
Patterning achieved

?

Microfluidics generate chemical gradient

Differential secretion of soluble factors

# Neural Cyst



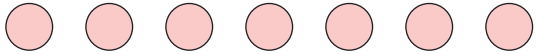
Model CNS development through a **continuous tube** model

+

- Capture **dynamics** between different regions
- Higher in vivo relevance

−

- Difficult to isolate and probe single region



Model CNS development through a **discrete cyst array** model

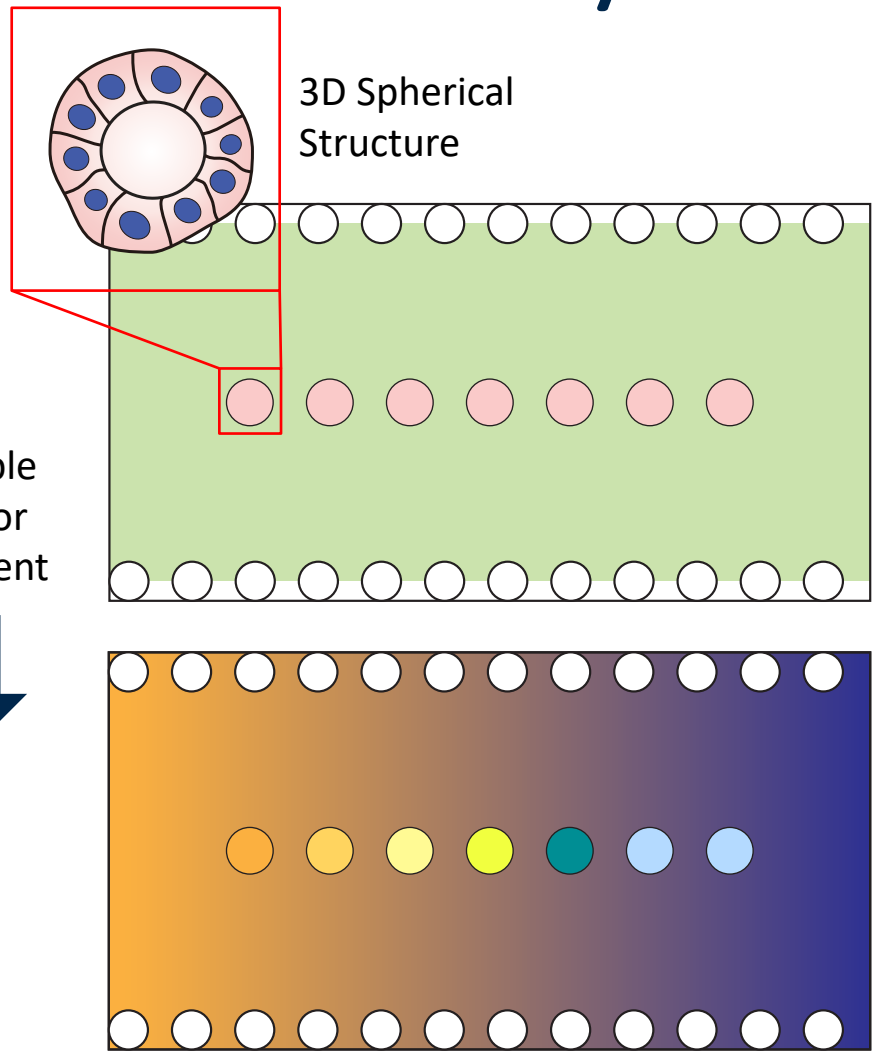
+

- **Modular** design with more geometric **control**
- Enables studies on **specific regions**

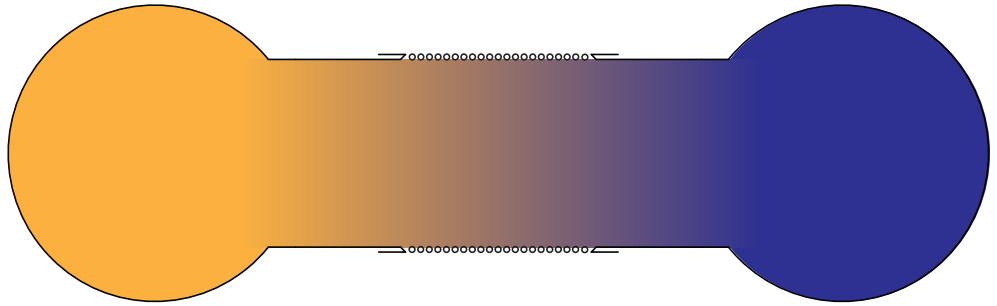
−

- Less in vivo relevance

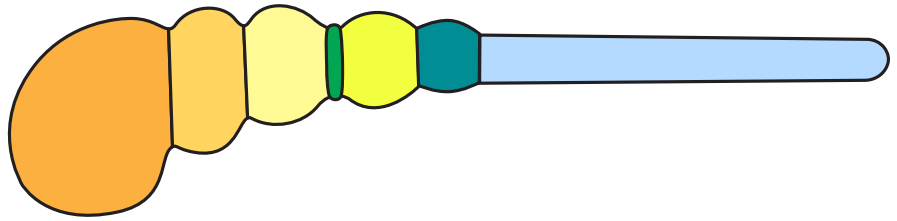
# Neural Cyst



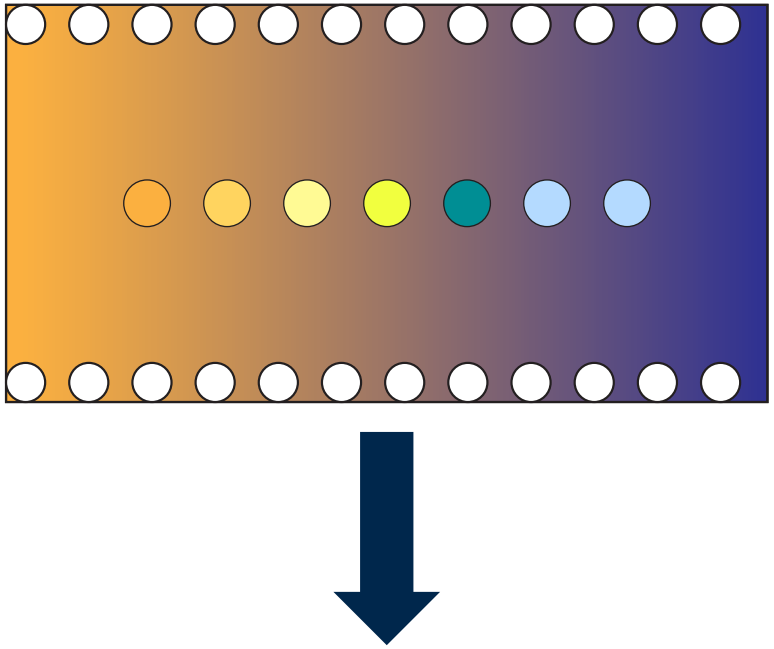

**CHIR (3µM)** +
FGF8 (200ng/mL) +
RA (1µM)



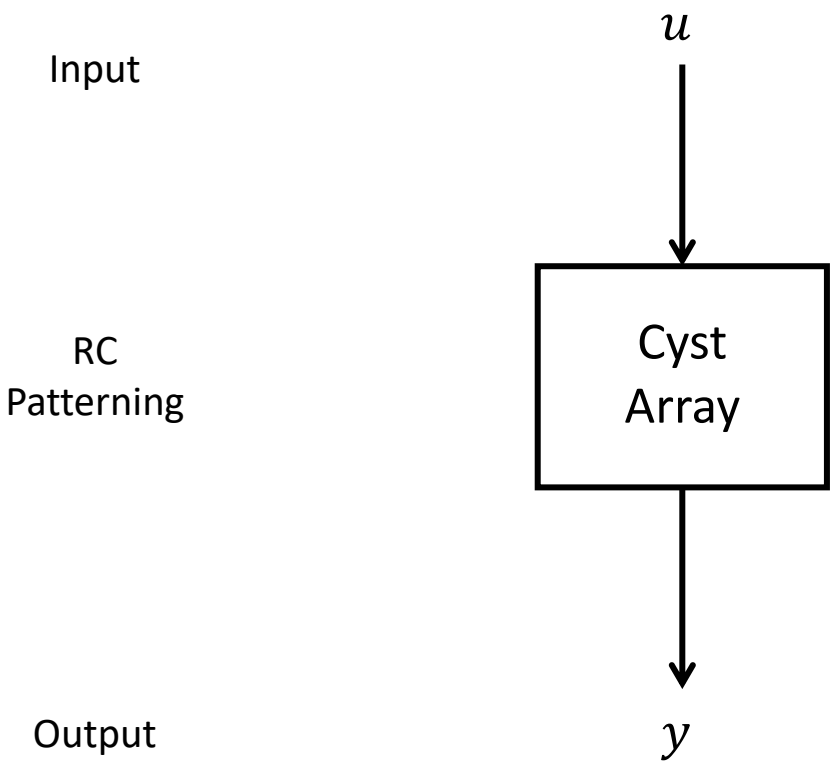
Recapitulate
Rostrocaudal
Patterning



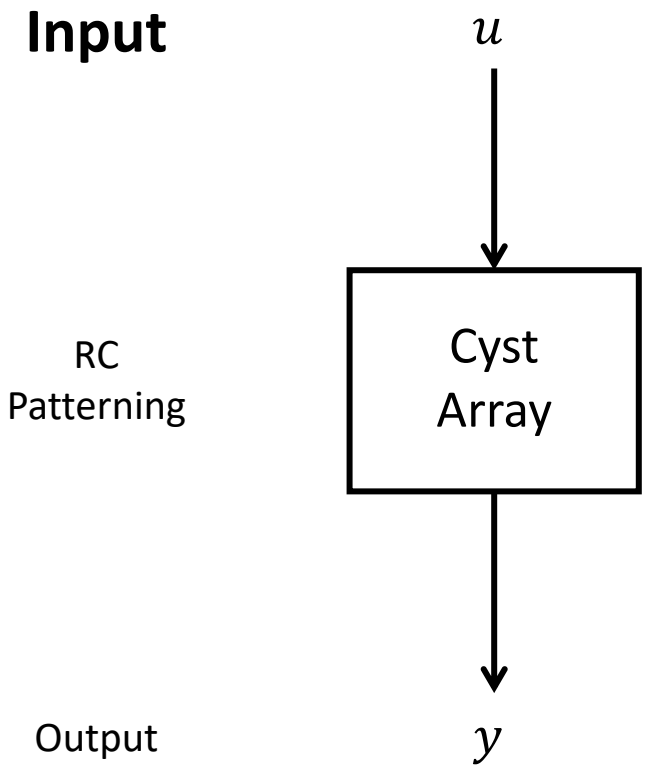
# Neural Cyst



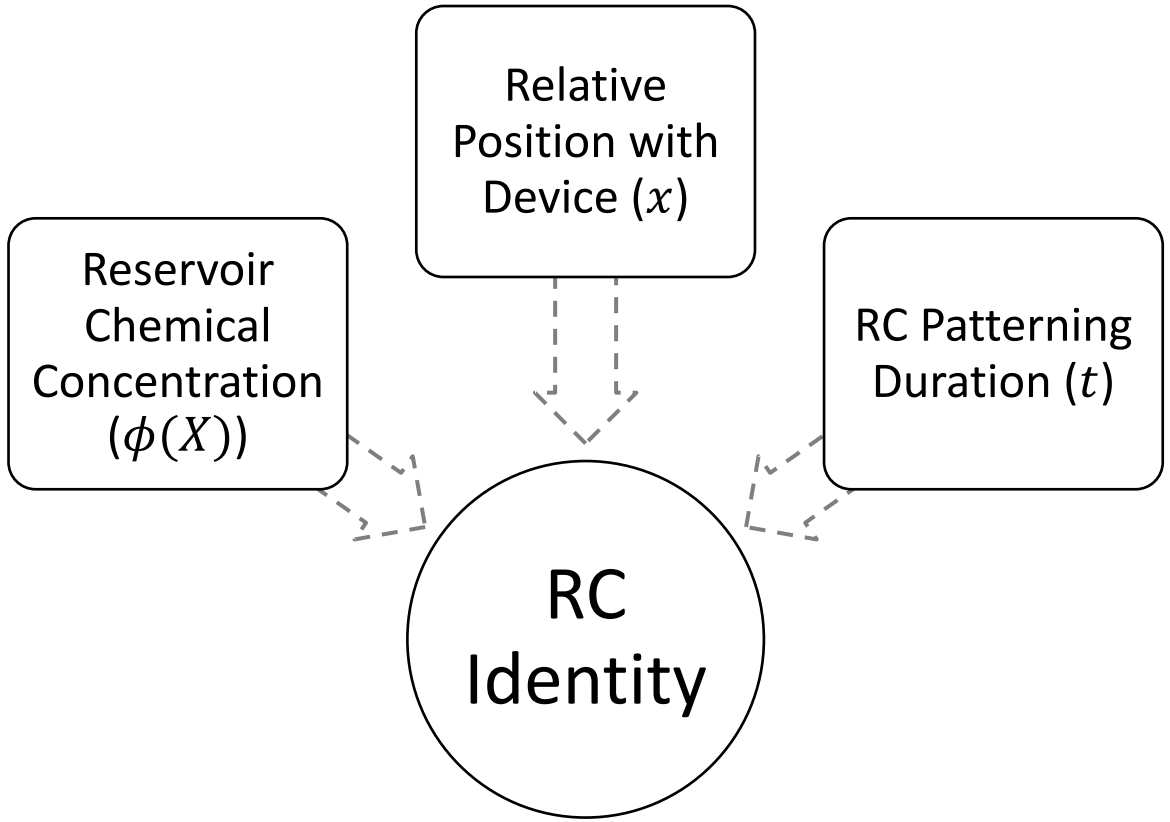
How can we model this patterning process?



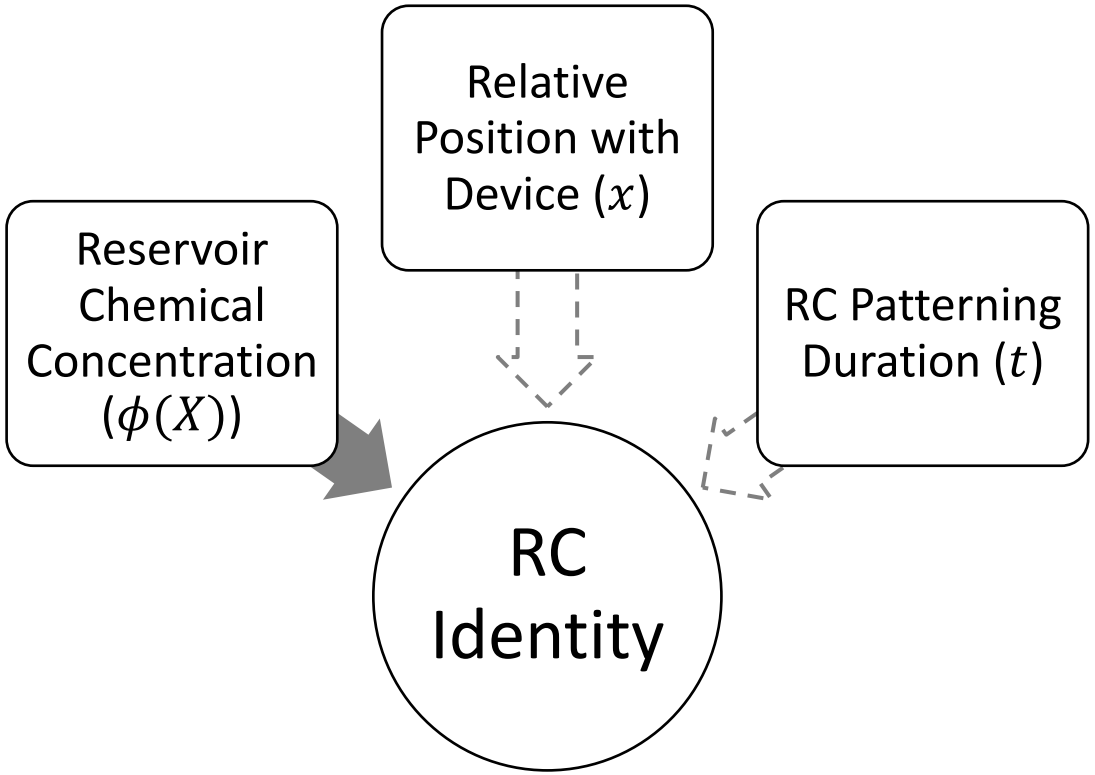
# Neural Cyst

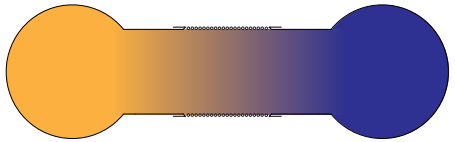


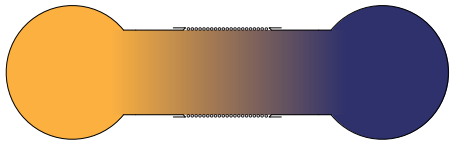

**Qualitative** understanding

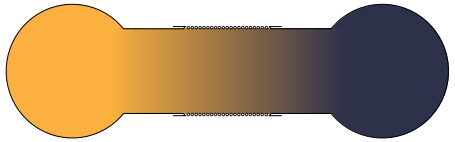


# Neural Cyst







Current Chemical Concentration:

**CHIR (3µM)**
FGF8 (200ng/mL)
RA (1µM)

**CHIR (4µM)**
FGF8 (200ng/mL)
RA (1µM)

**CHIR (6µM)**
FGF8 (200ng/mL)
RA (1µM)

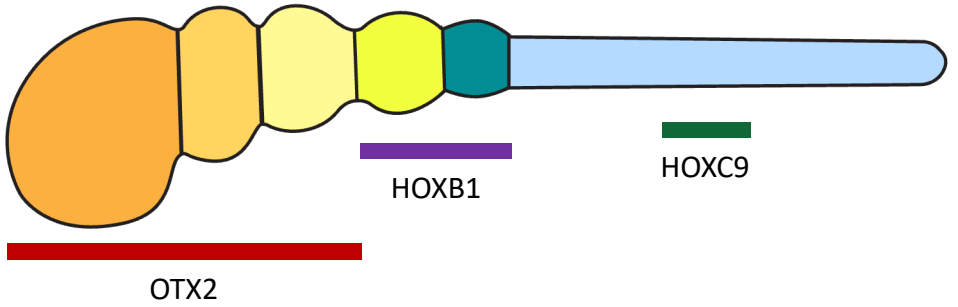
CHIR: CHIR99021
WNT pathway activator, closely associated with caudal fate

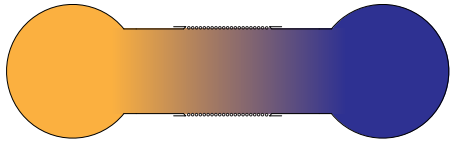
Rostral ⟷ Caudal

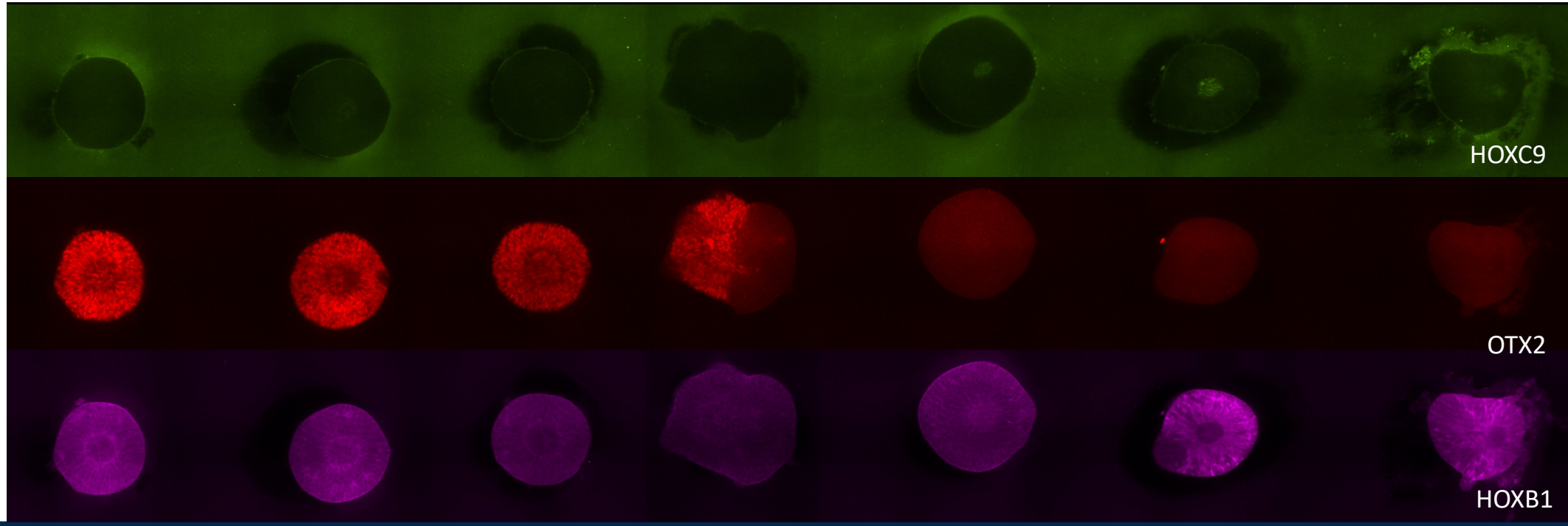
# Neural Cyst

**CHIR Concentration**

$\phi_{CHIR}(X) = 3\mu M$



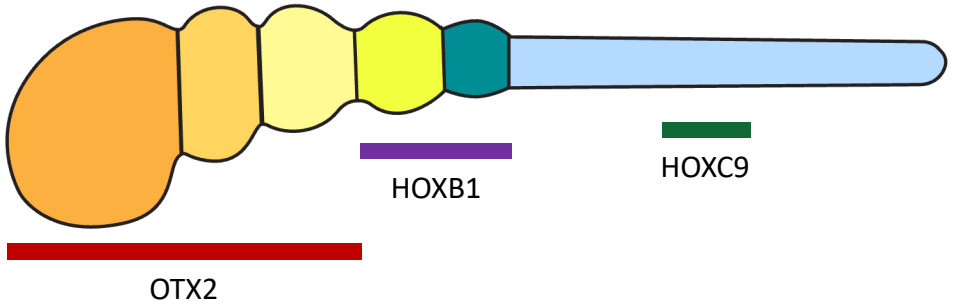



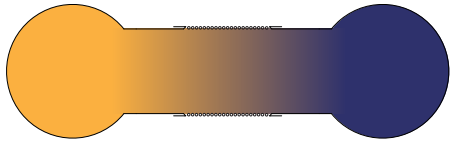


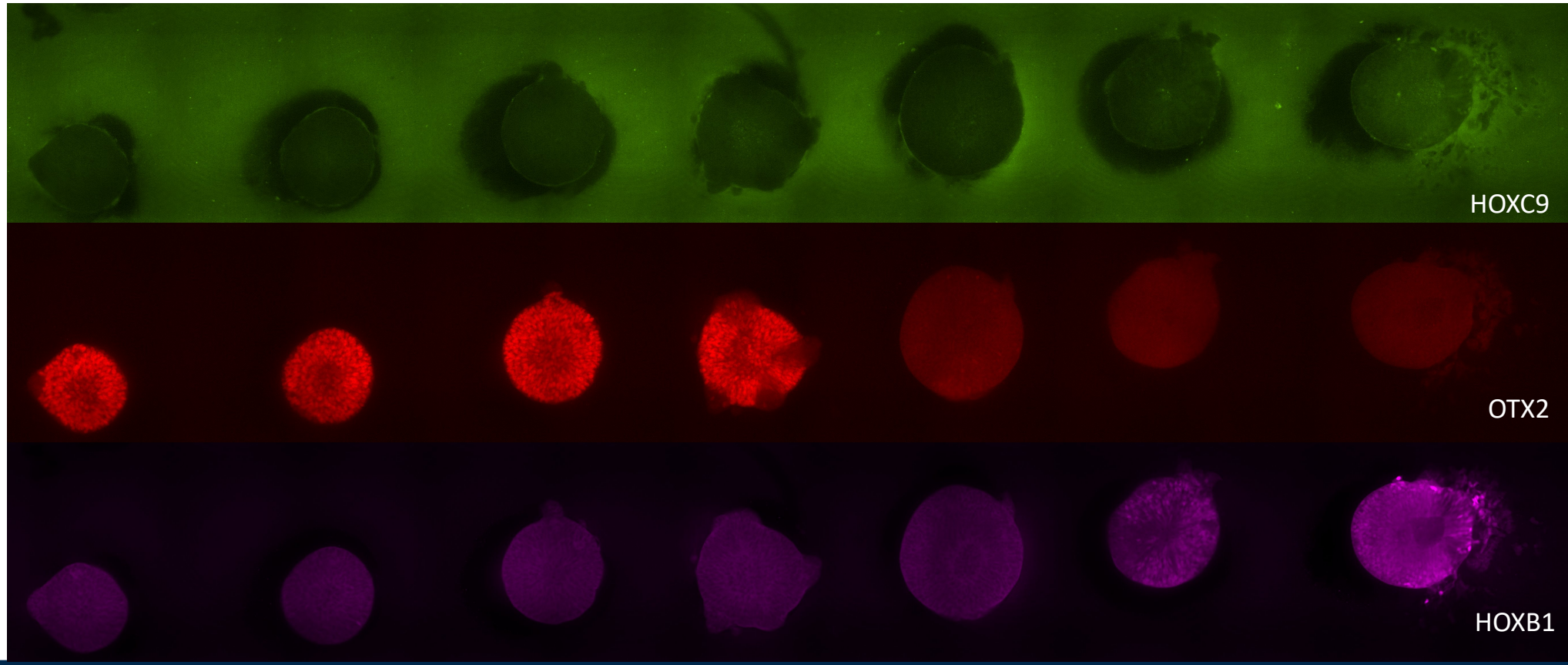
# Neural Cyst

**CHIR Concentration**

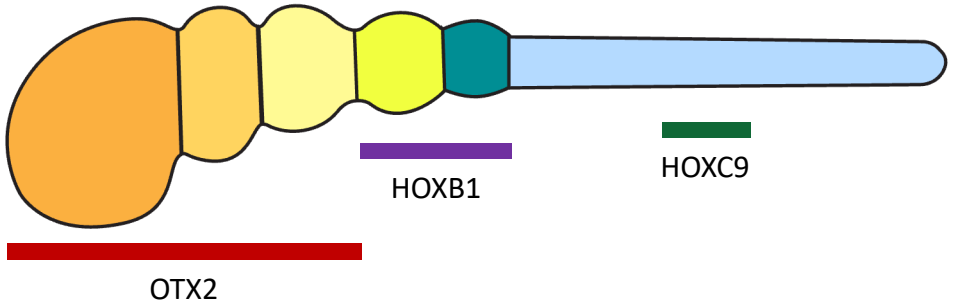
$\phi_{CHIR}(X) = 4\mu M$



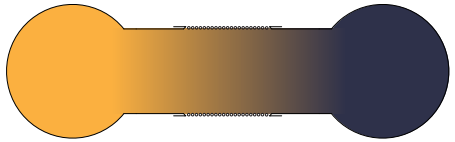


# Neural Cyst

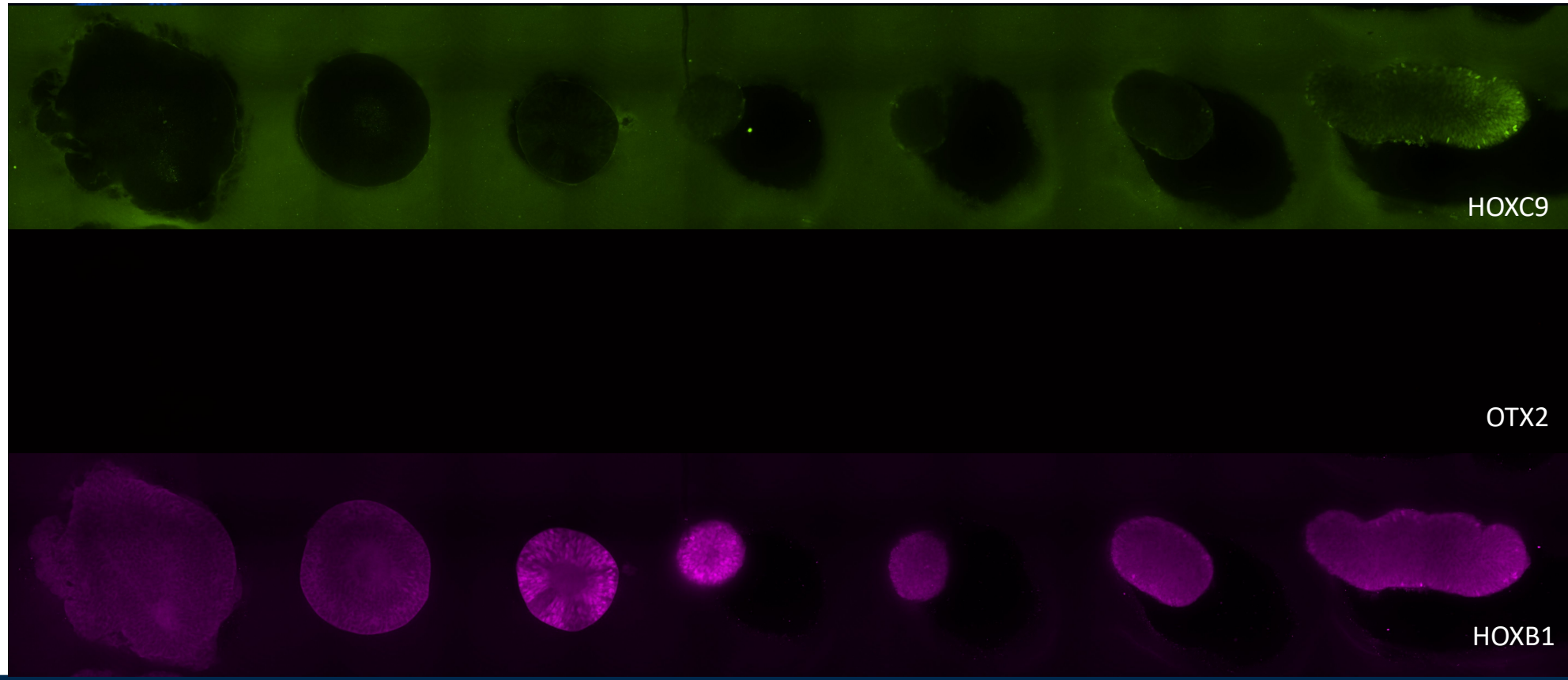



**CHIR Concentration**

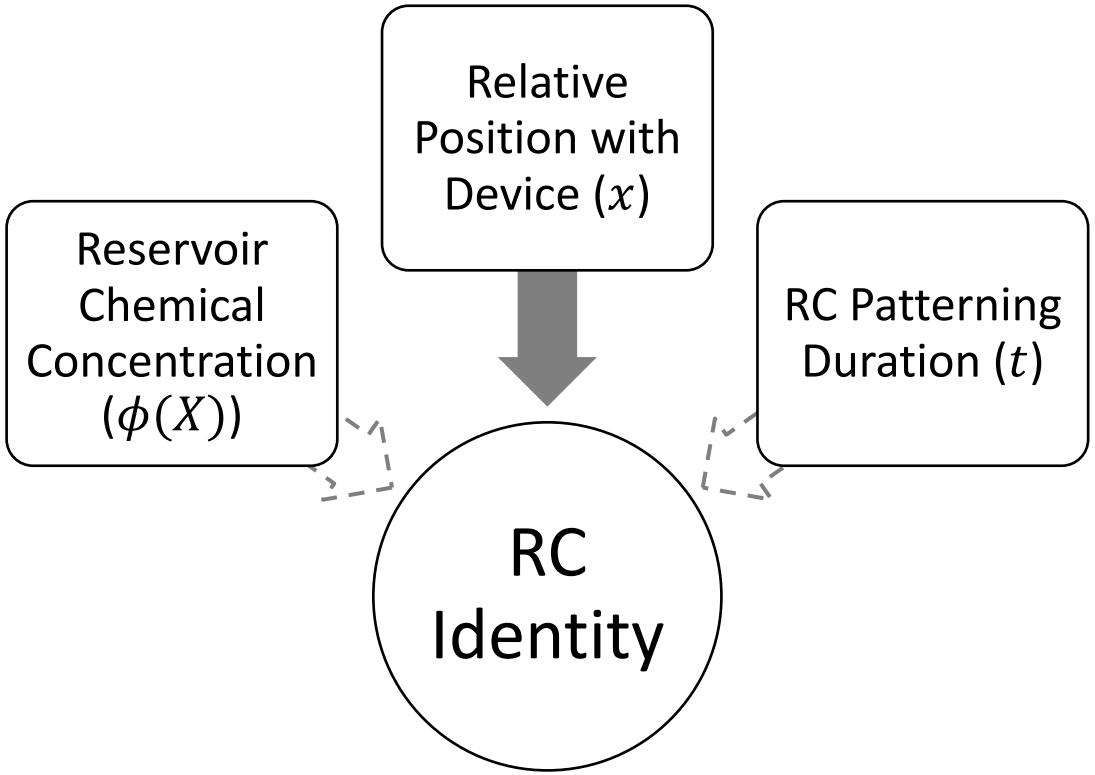
$\phi_{CHIR}(X) = 6\mu M$



Increasing CHIR shifts cyst identity caudally

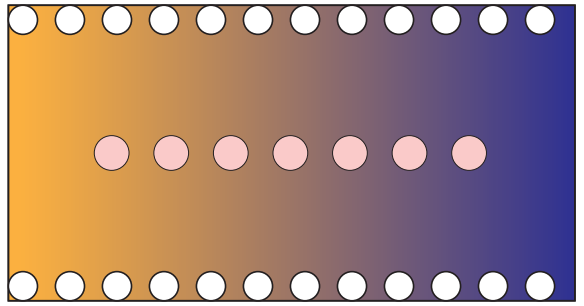


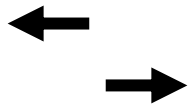
# Neural Cyst




Array printed at channel center

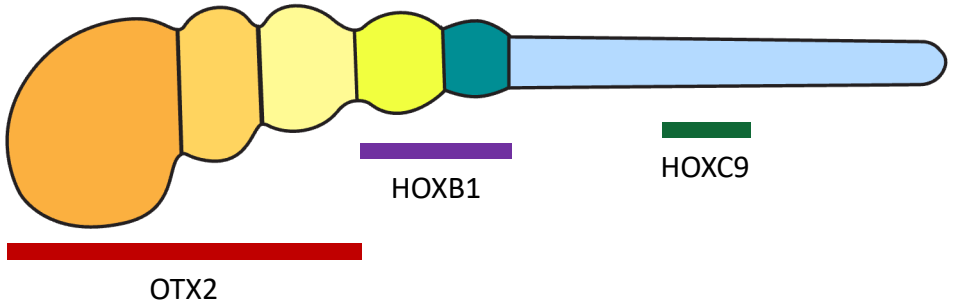
x = X/2





Shift printing location

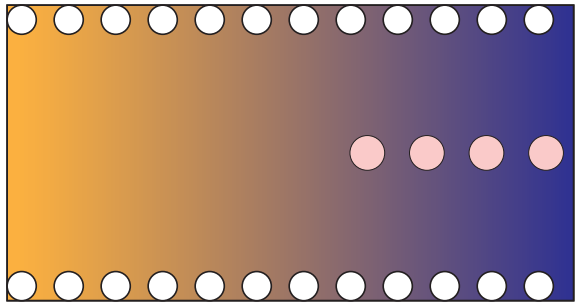
# Neural Cyst



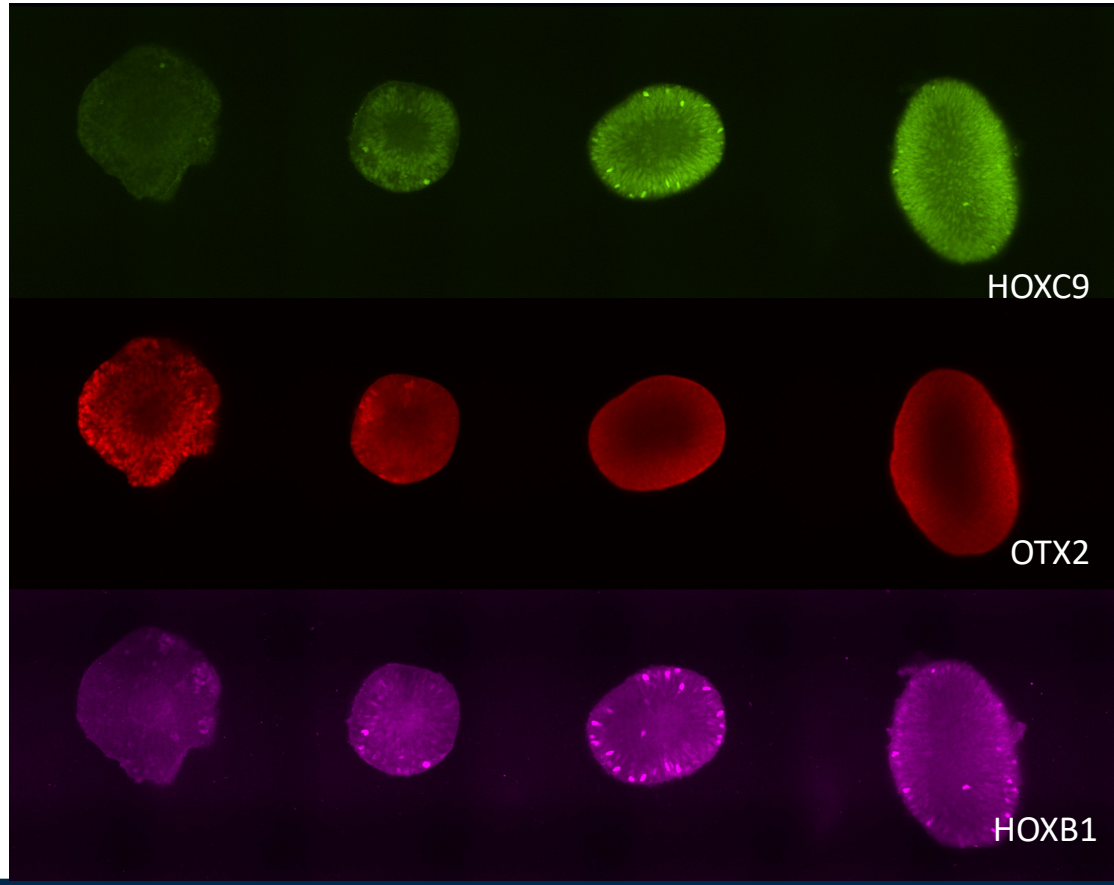

**Array Location**

$x = \frac{3}{4}X$ (shift caudally)

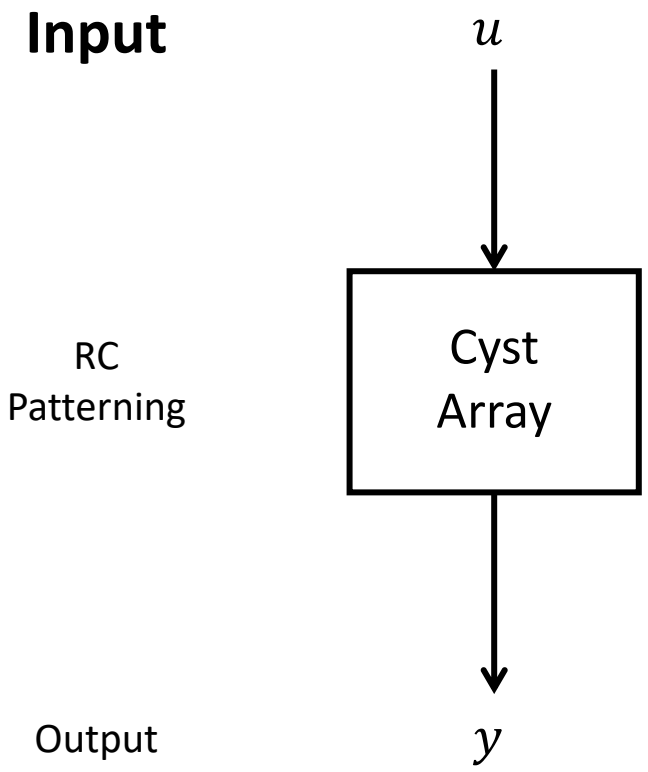
$\phi_{CHIR}(X) = 3\mu M$

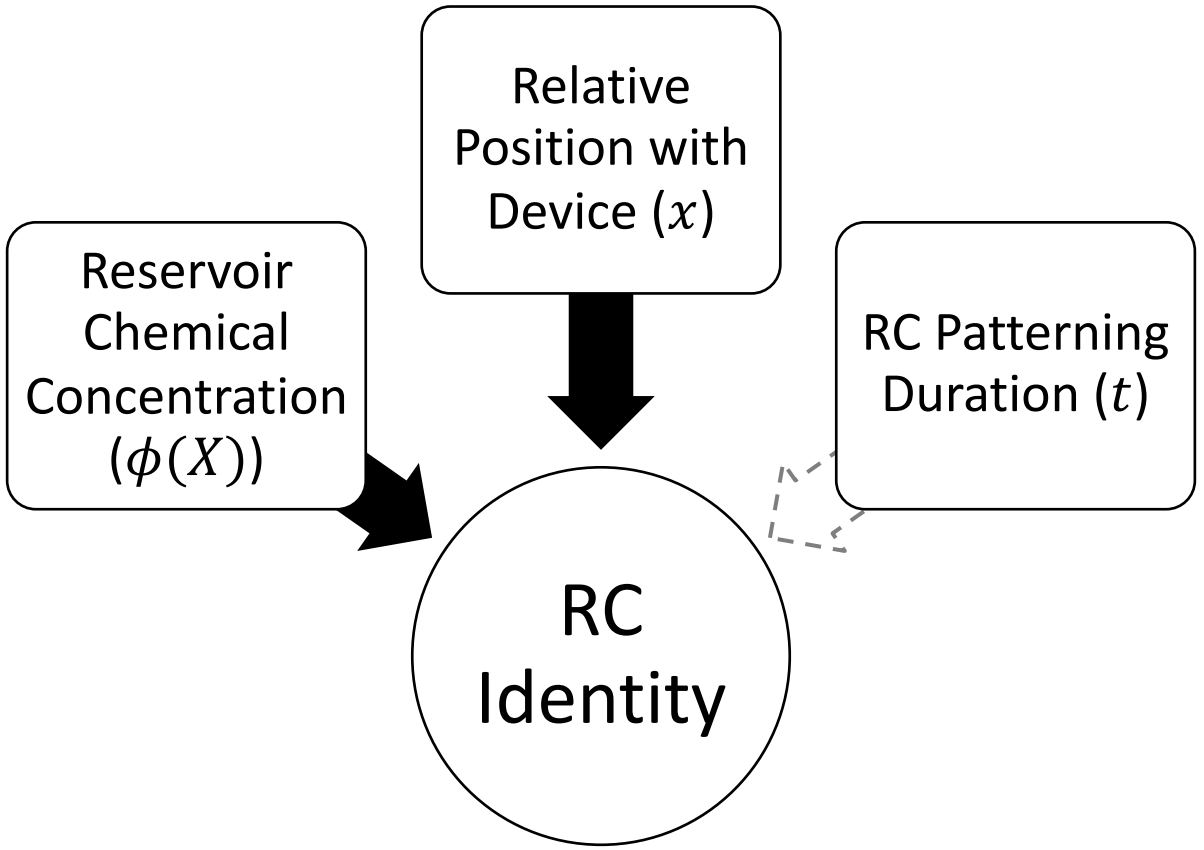


Moving array caudally results in more caudal identity



# Neural Cyst




**Quantitative** understanding

Fick's Law

$$\phi = g(t, x, \phi(X))$$

# Neural Cyst

**Fick's Law**

Fick's second law:

$$\frac{\delta\phi}{\delta t} = D\frac{\delta^2\phi}{\delta x^2}$$

$\phi$: concentration

$D$: diffusivity

With constant concentration source and diffusion length

$$\phi(x,t) = \phi_0 \mathrm{erfc}\left(\frac{x}{2\sqrt{Dt}}\right)$$

Approximation with first two terms of Taylor series expansion

$$\phi(x,t) = \phi_0\left(1 - 2\left(\frac{x}{2\sqrt{Dt\pi}}\right)\right)$$

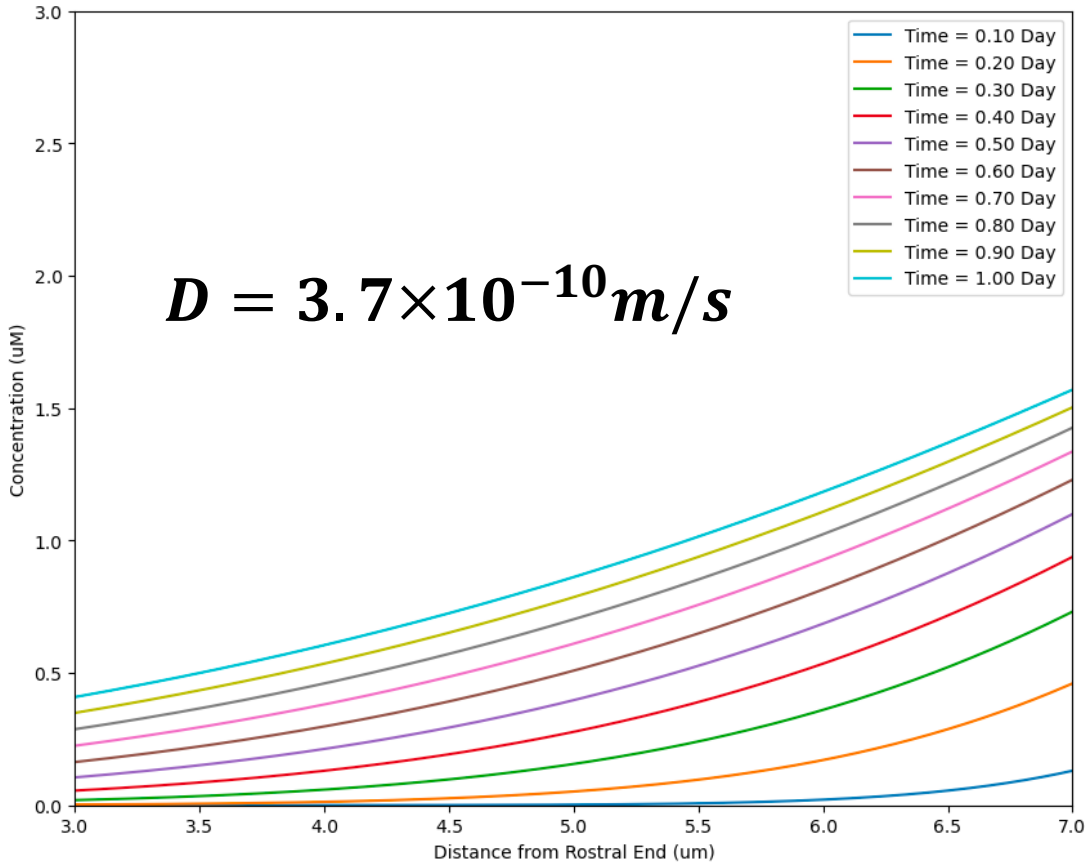
Solve D using exp. results
Steady linear distribution at t = 1 day

# Neural Cyst

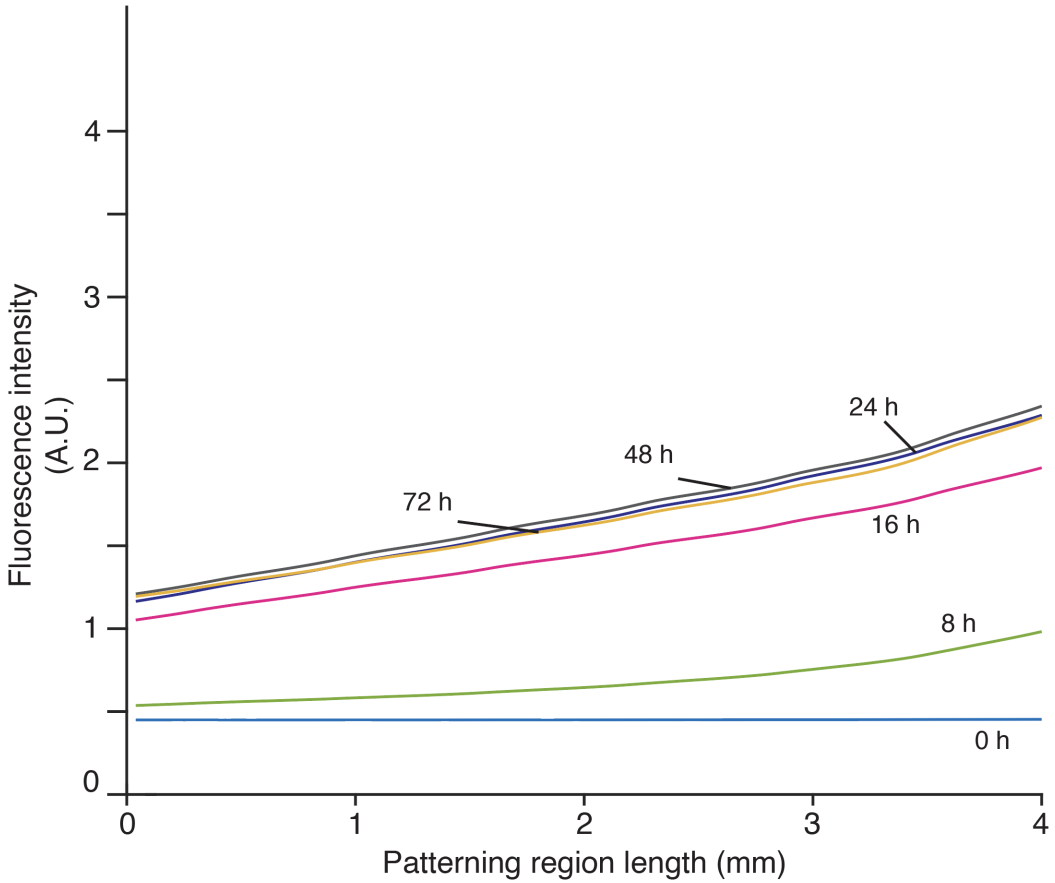
Model based on Fick's law recapitulate the dynamics of gradient in channel

## Fick's Law vs. Experimental Results

Fick's Law Model

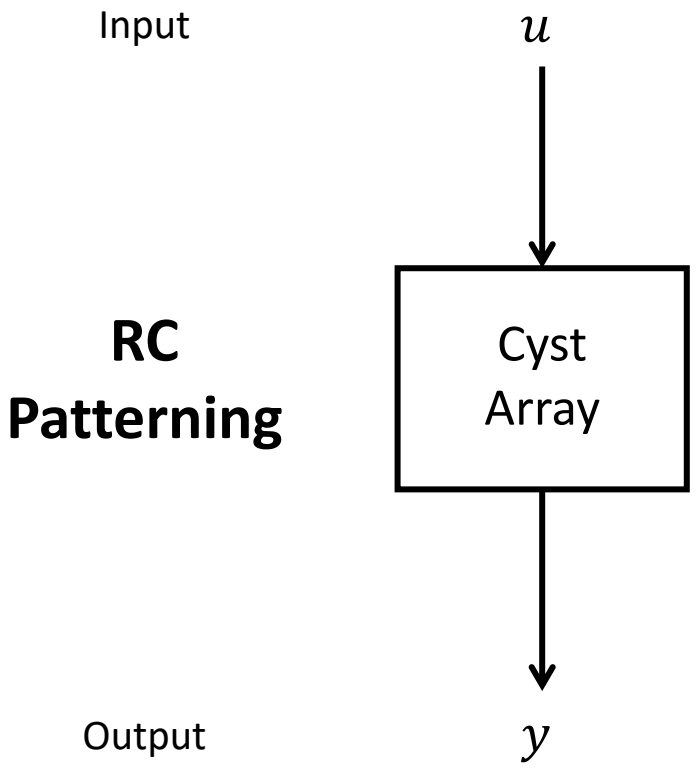



60kDa Dextran Diffusion




Rostral ⟷ Caudal

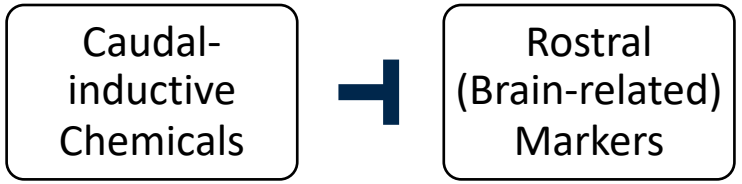
# Neural Cyst




$$\phi(x,t) = \phi_0(1 - 2\left(\frac{x}{2\sqrt{Dt\pi}}\right))$$

$$u = \int_0^T \phi \, dt$$

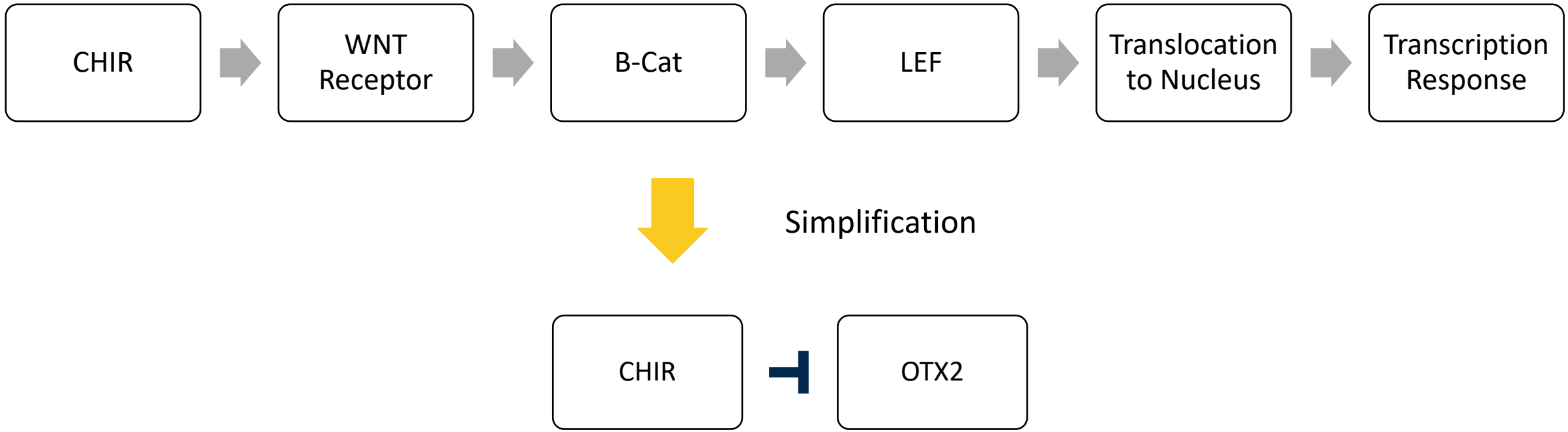
**Qualitative** understanding



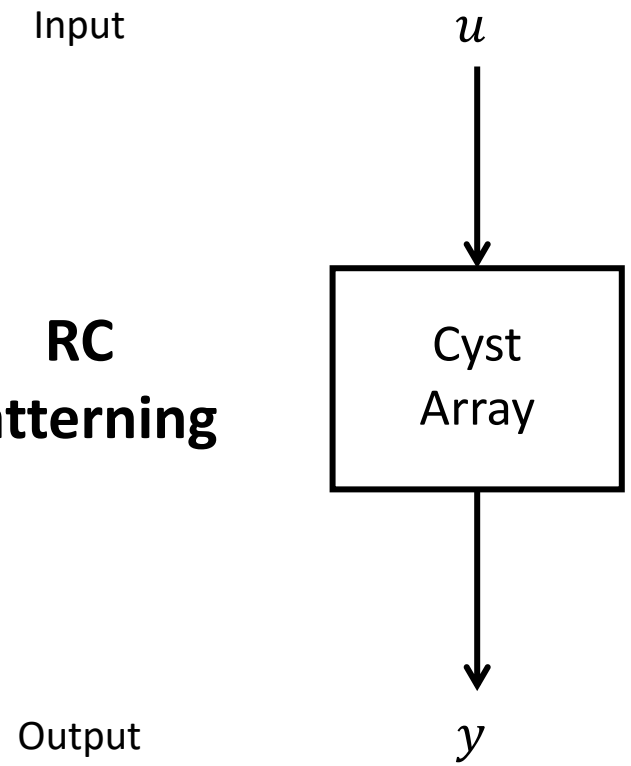
# Neural Cyst

## CHIR Inhibits Rostral Markers (e.g., OTX2)

in vivo knowledge



# Neural Cyst




$$\phi(x,t) = \phi_0(1 - 2\left(\frac{x}{2\sqrt{Dt\pi}}\right))$$

$$u = \int_0^T \phi \, dt$$

**Quantitative** understanding

Hill's Function

$$y = \frac{x^n}{x^n + K^n}$$

# Neural Cyst

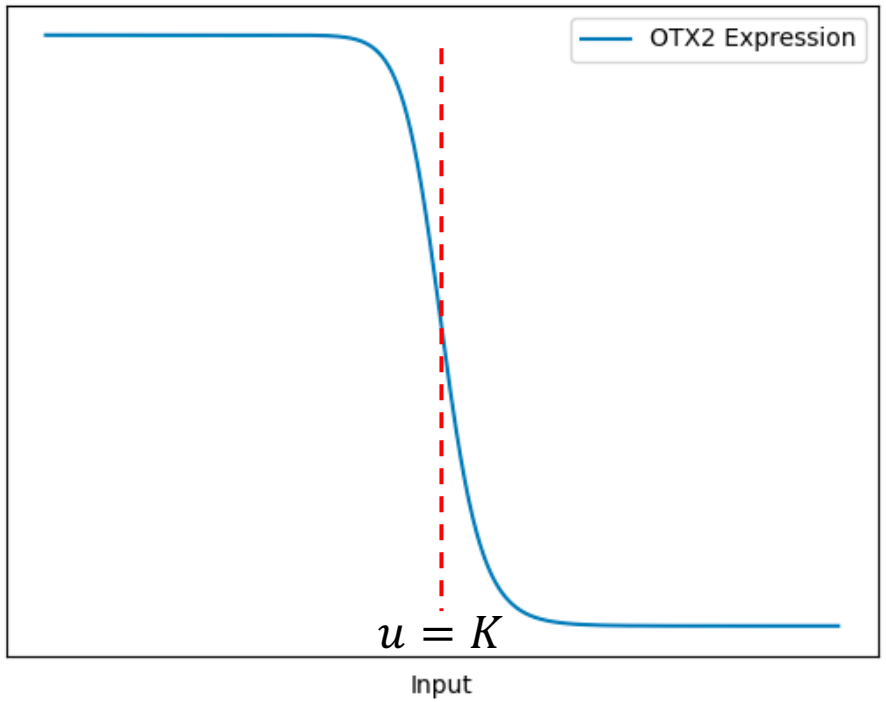
**Hill's Function**

Reverse Hill's function is used to model switch-like inhibition:

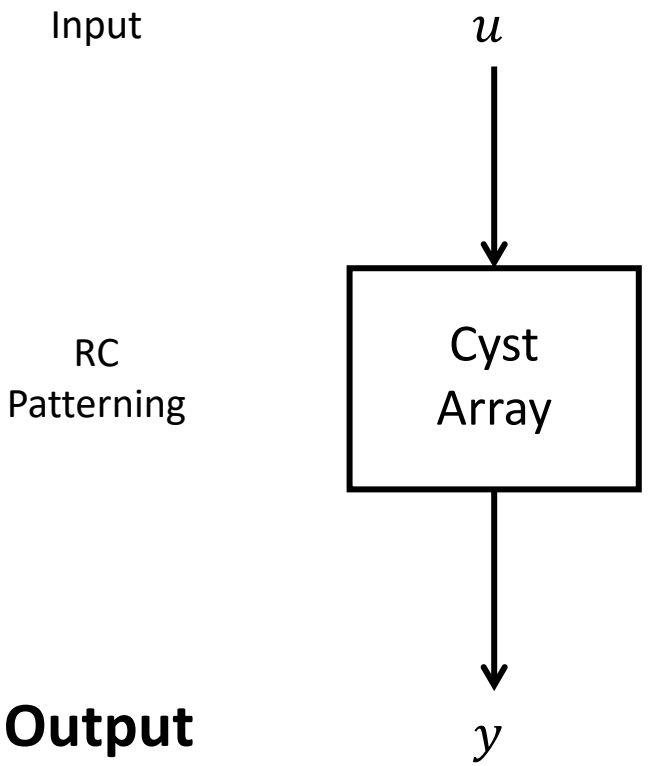
$$y = \frac{K^n}{u^n + K^n}$$

$K$: Inhibition threshold
$n$: degree of reaction
$u$: input
$y$: output, OTX2 expression level

Once Input reaches threshold, output will be rapidly inhibited



# Neural Cyst




$$\phi(x,t) = \phi_0(1 - 2\left(\frac{x}{2\sqrt{Dt\pi}}\right))$$

$$u = \int_0^T \phi \, dt$$

$$y = \frac{K^n}{u^n + K^n}$$

Image data under varying patterning condition

# Neural Cyst

## OTX2 Marker Intensity

After pre-processing

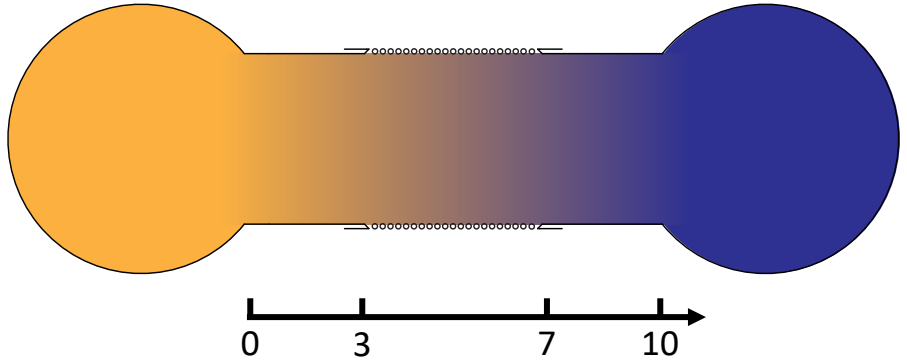
- Position calibration
- Intensity normalization

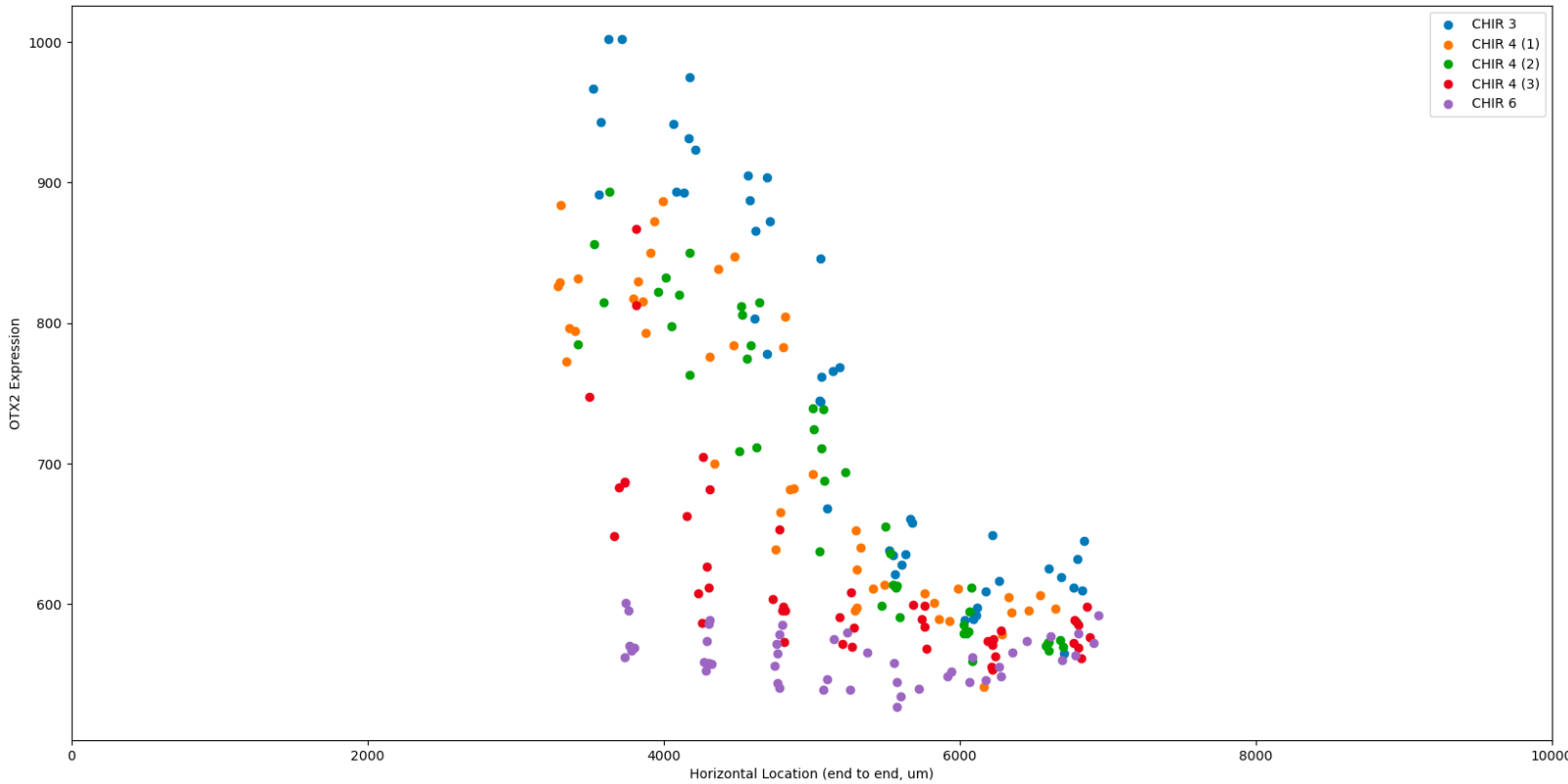
$$\phi(x,t) = \phi_0(1 - 2\left(\frac{x}{2\sqrt{Dt\pi}}\right))$$

$$u = \int_0^T \phi \, dt$$

$$\boldsymbol{y = \frac{K^n}{u^n + K^n}}$$

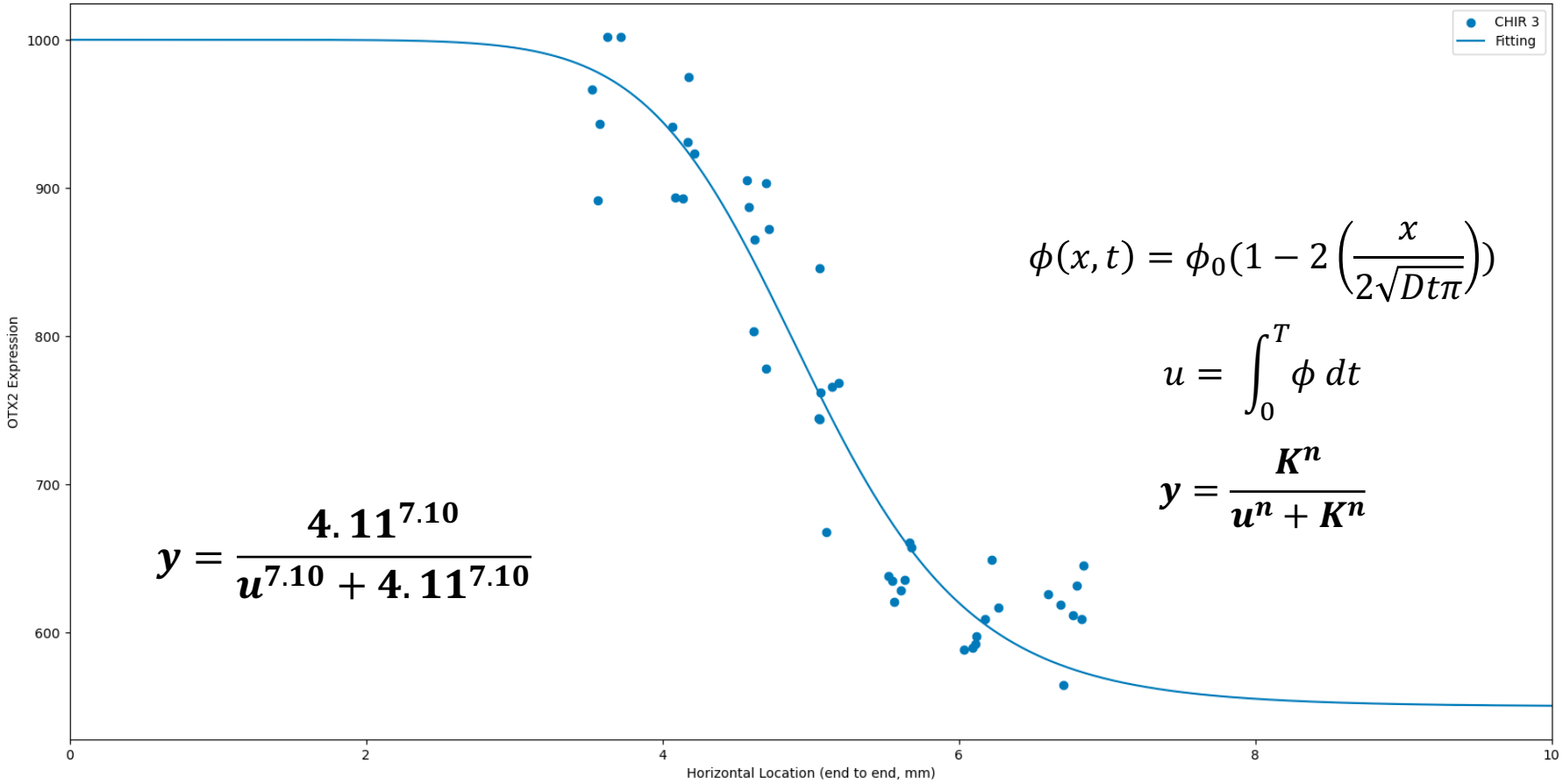
Regression



# Neural Cyst

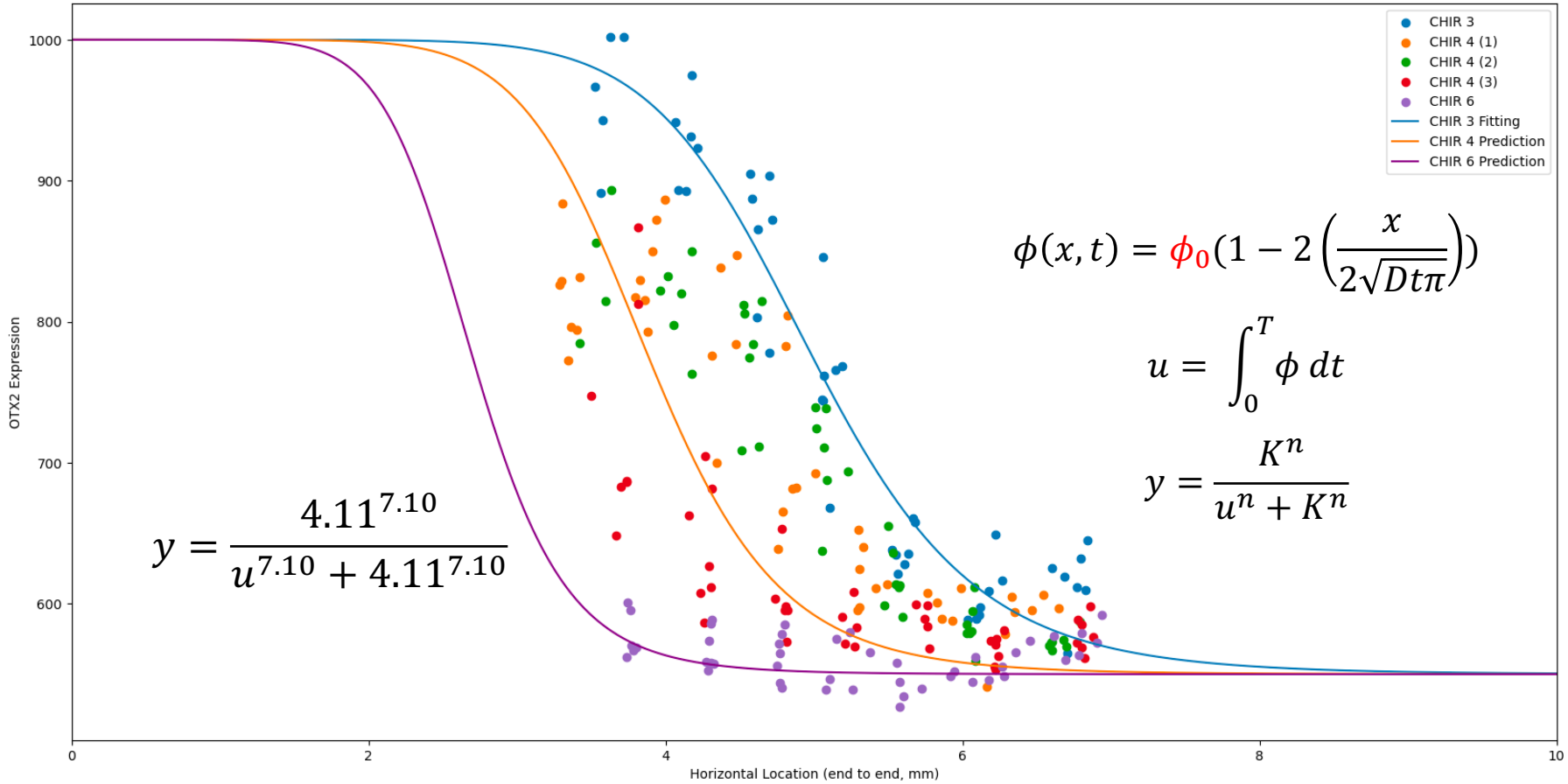
**Fitting based on $\phi_{CHIR}(X) = 3\mu M$**



# Neural Cyst

**Model prediction at varying $\phi_0$**

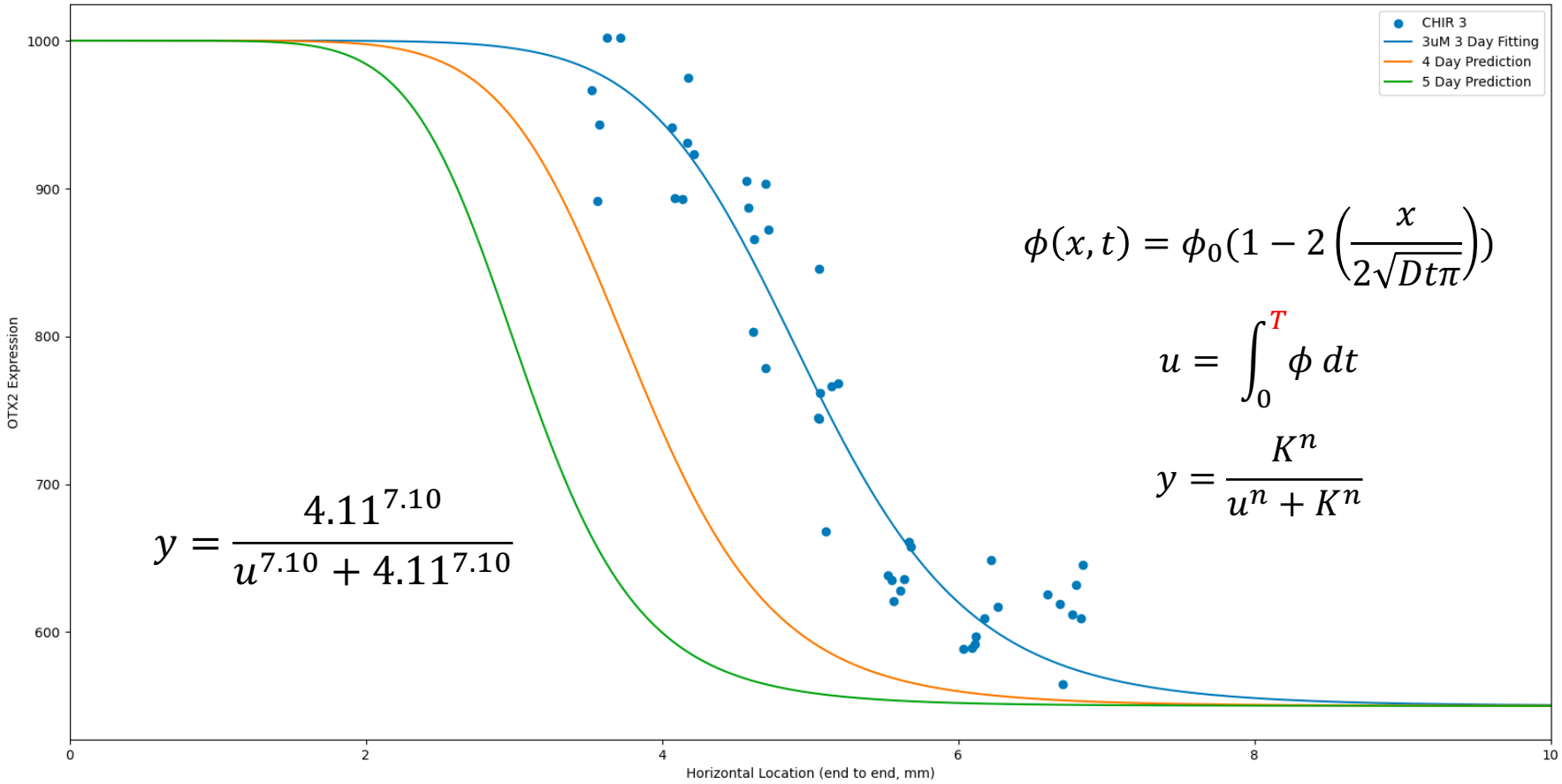
Model does a good job predicting cyst RC identity



# Neural Cyst

**Model prediction at varying $T$**

Need to verify via experiments




$$\phi(x,t) = \phi_0(1 - 2\left(\frac{x}{2\sqrt{Dt\pi}}\right))$$

$$u = \int_0^T \phi \, dt$$

$$y = \frac{K^n}{u^n + K^n}$$

$$y = \frac{4.11^{7.10}}{u^{7.10} + 4.11^{7.10}}$$