

## Abstract

To engineer complex synthetic biological systems will require modular design, assembly, and characterization strategies. The RNA *polymerase arrival rate* (PAR) is defined to be the rate that RNA polymerases arrive at a specified location on a DNA molecule. Designing and characterizing biological modules in terms of RNA polymerase arrival rates provides for many advantages in the construction and modeling of biological systems.

PARMESAN is an *in vitro* method for measuring polymerase arrival rates using pyrrolo-dC, a fluorescent DNA base that can substitute for cytosine. Pyrrolo-dC shows increased fluorescence when in single-stranded versus double-stranded DNA. During transcription, RNA polymerase separates the two strands of DNA, leading to a change in the fluorescence of pyrrolo-dC. By incorporating pyrrolo-dC at specific locations in the DNA, fluorescence changes can be taken as a direct measurement of the polymerase arrival rate.

# PARMESAN: Fluorescence Assay for Polymerase Arrival Rates

Austin Che  
[austin@csail.mit.edu](mailto:austin@csail.mit.edu)

December 10, 2003



# Overview of presentation

Motivation

- Modularity

Polymerase Arrival Rates (PAR)

PARMESAN

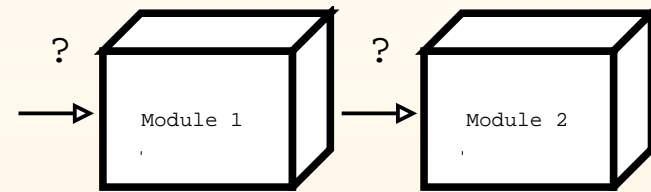
Experimental Results

Conclusions

# Motivation for measuring transcription events

## Engineering synthetic biological circuits

- *Modularity is essential*
- What should be used as the signal among modules?
  - ▷ *states of proteins (e.g. phosphorylation)*
  - ▷ *total protein levels*
  - ▷ *mRNA levels*



## Characterization of biological systems

- transfer curves

## Modeling biological systems

# Modularity as the overarching goal

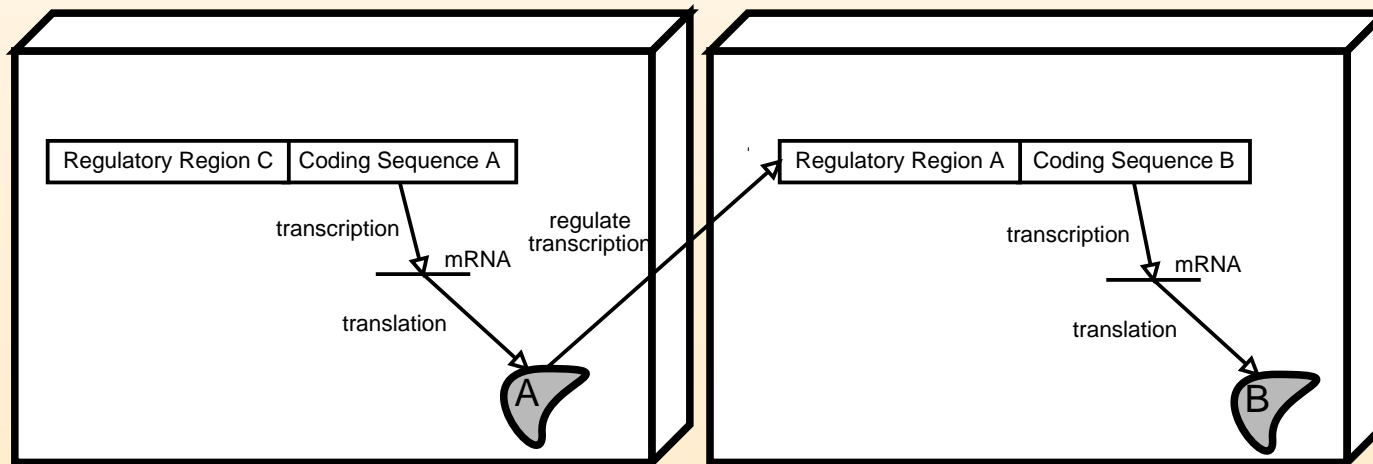
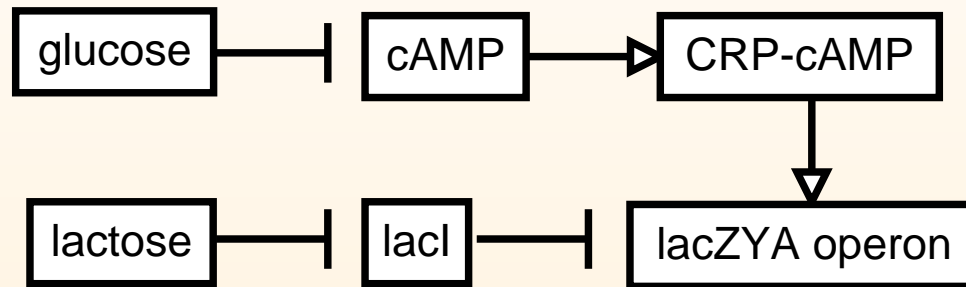
Design

Assembly

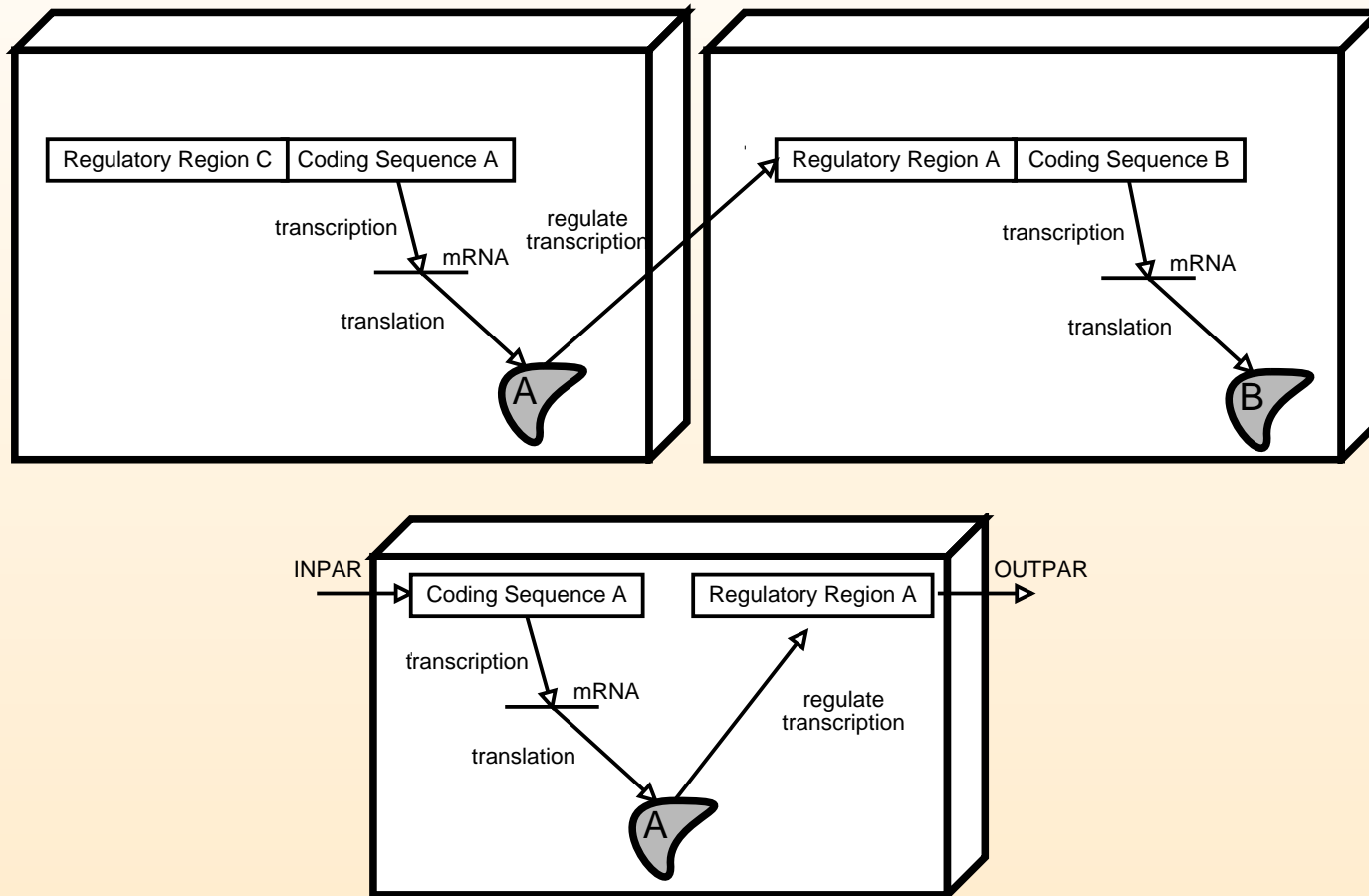
Characterization

- ▶ *Characterization method should facilitate design and assembly*

# Conventional modules cannot be characterized in a general way



# Redefine boundaries to allow common inputs and outputs

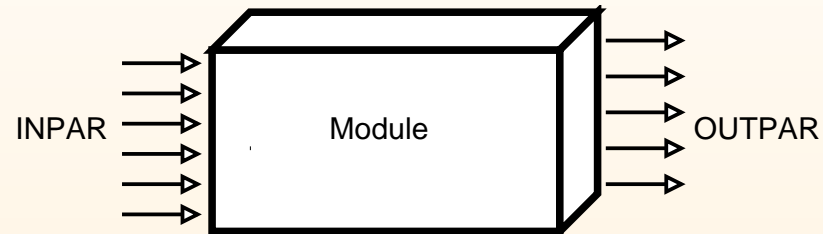


# Polymerase Arrival Rates (PAR) as common measurement unit

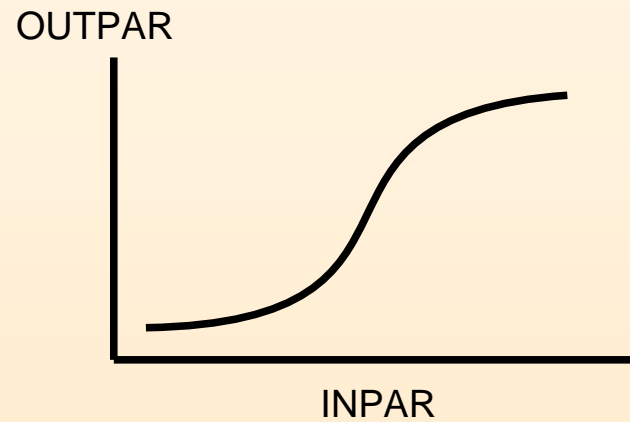


*PAR depends on many factors*

# General form for *modules*



*Modules can be specified completely by transfer curves*



# Does PAR satisfy the needs for module characterization?

- Abstraction
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  - + *Transfer curves completely describe a module*

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- Usefulness

- + *Modeling and engineering become straightforward*

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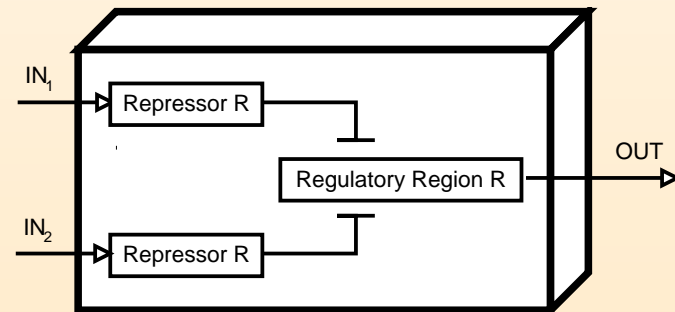
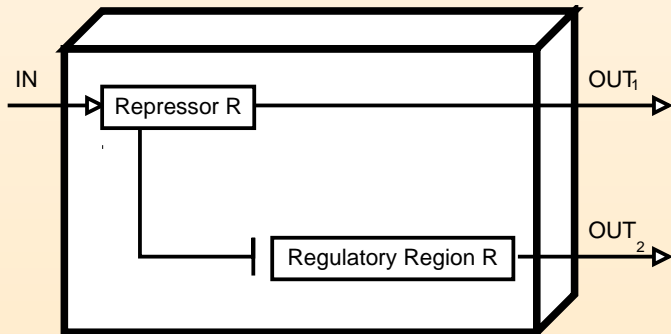
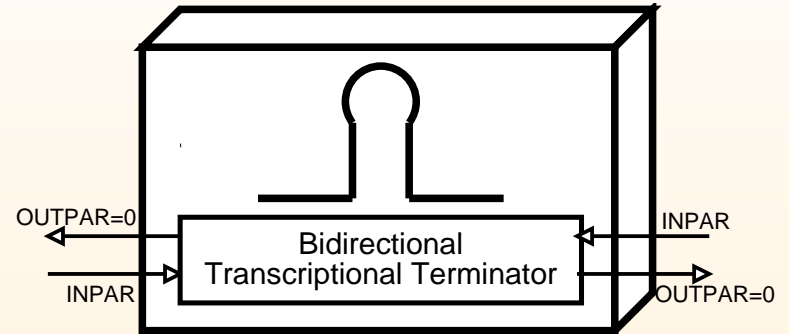
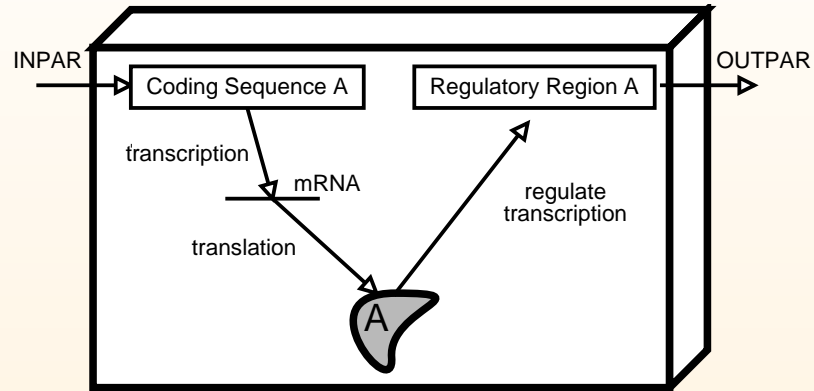
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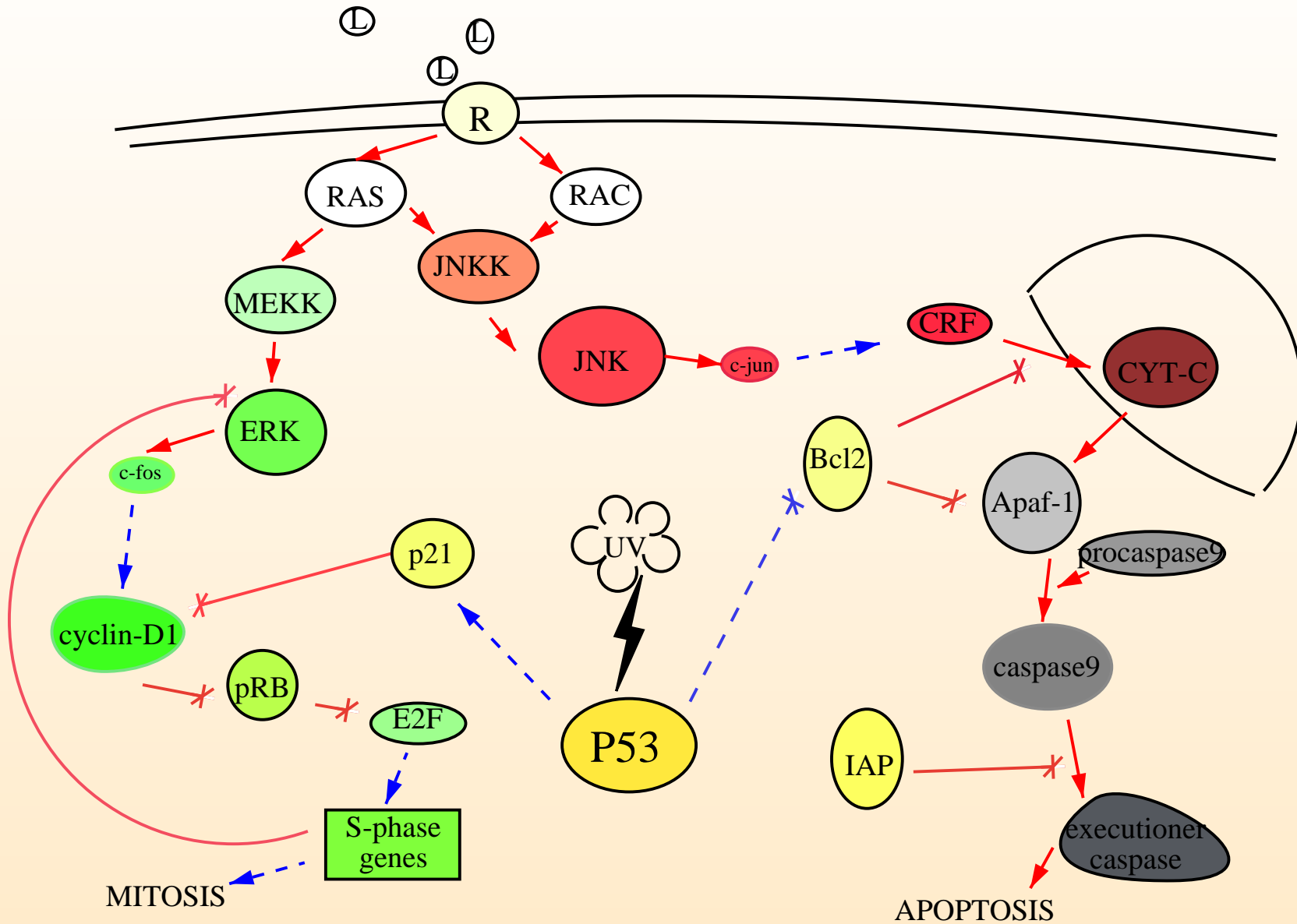
- Measurable

- + *PAR is a physical feature, independent of the measurement method*
- + *PAR is a quantitatively defined measure*
- *Difficult to measure and little data available*

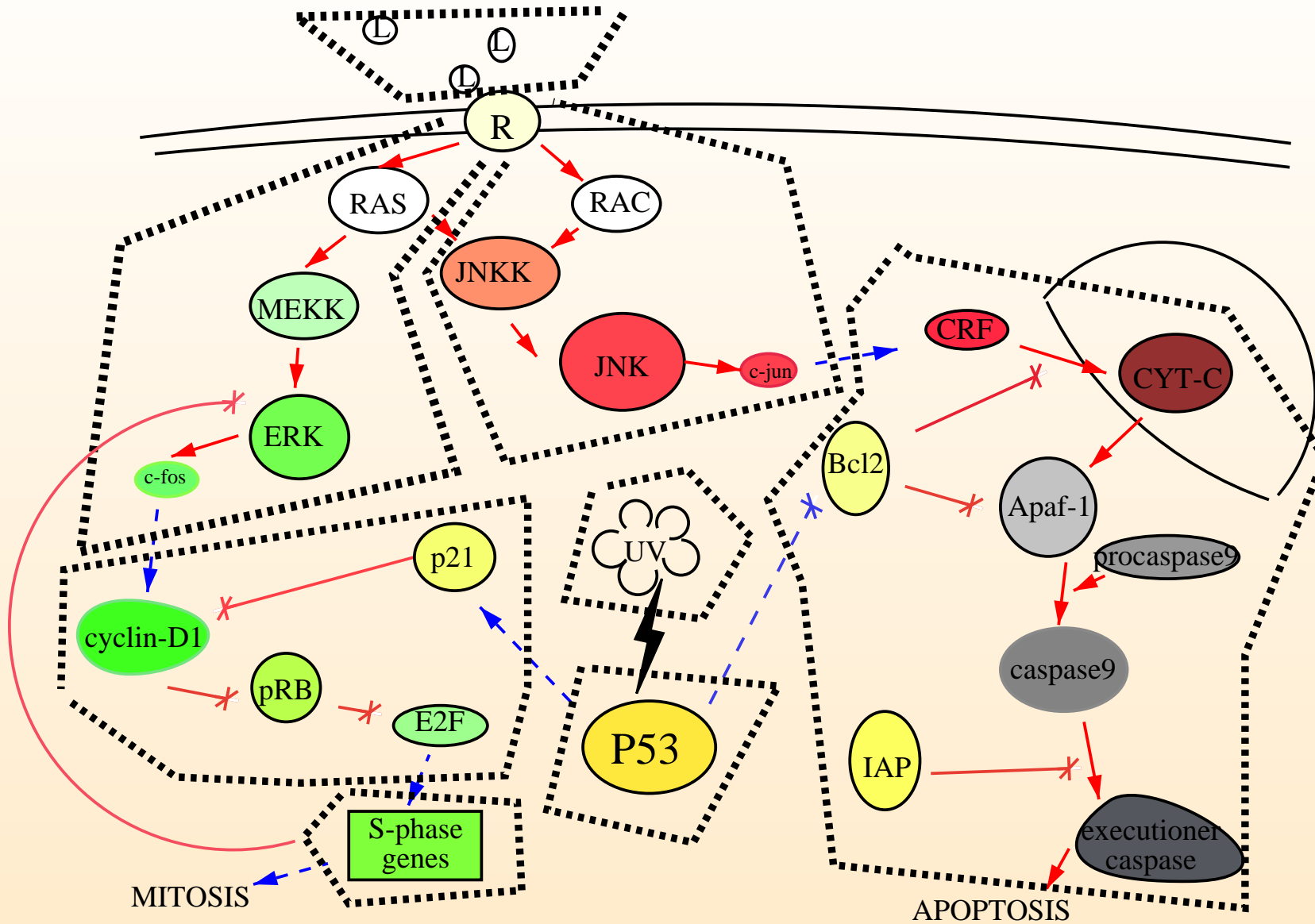
# Some example modules



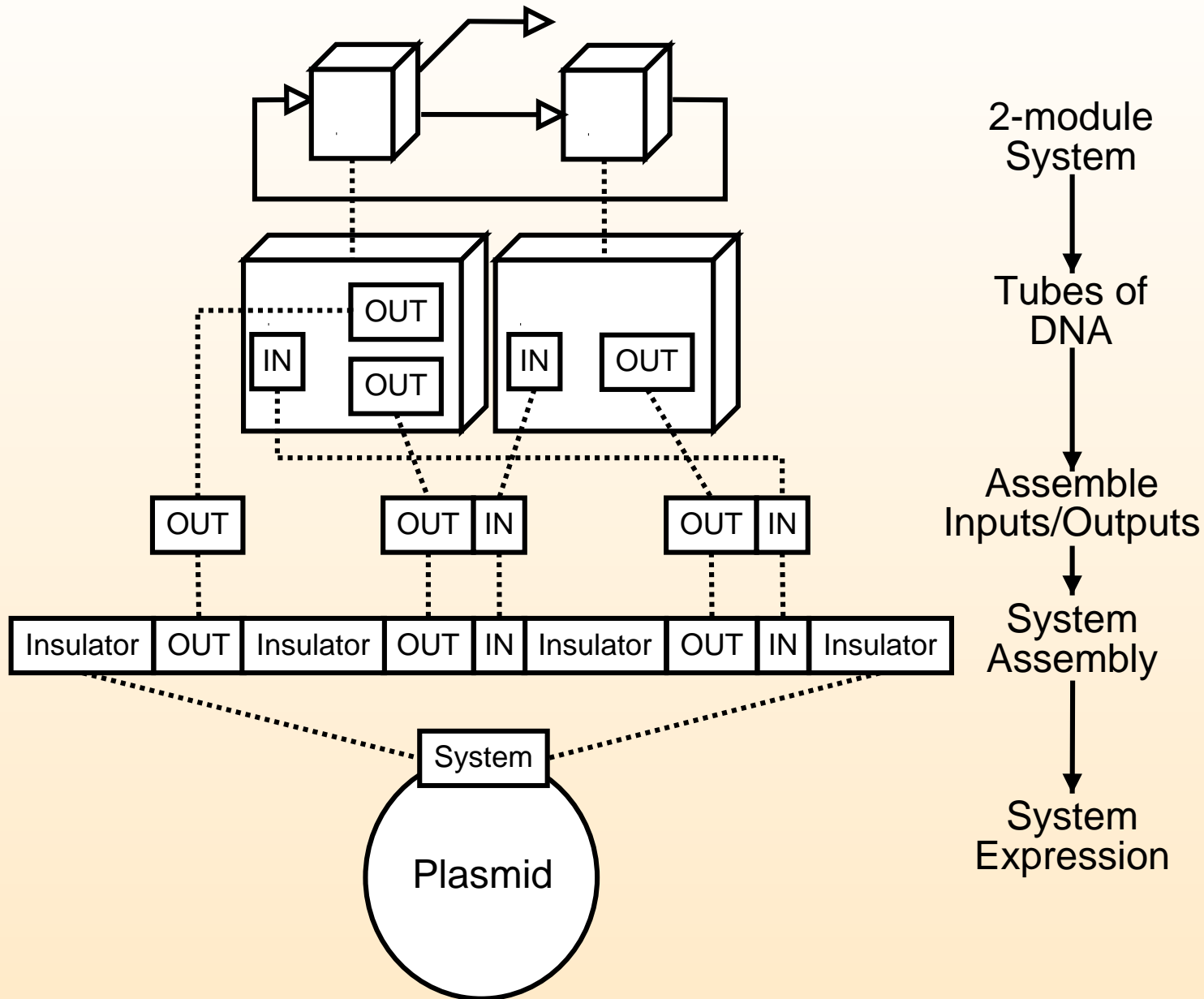
# Example: MAPK Signaling Pathway



# Modules in MAPK Signaling Pathway



# Assembly is simple with modules



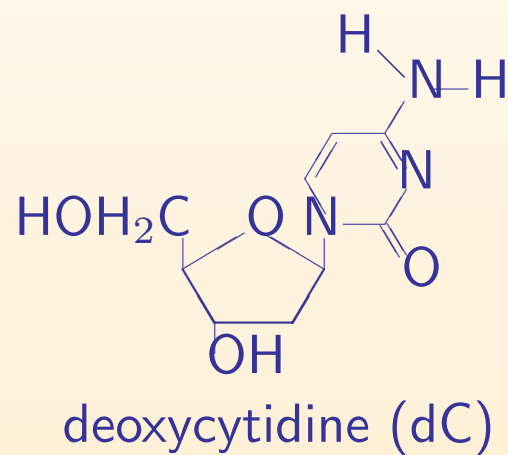
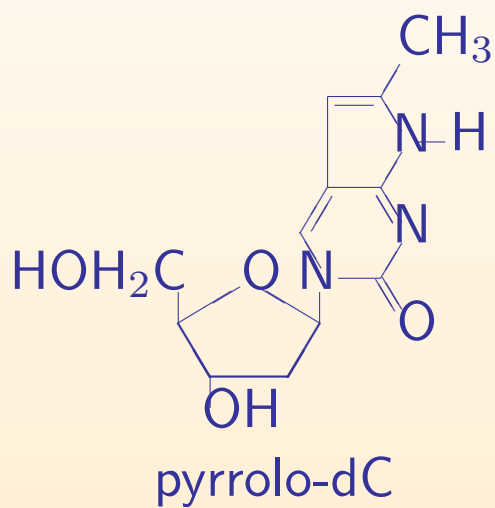
# PARMESAN: Proposed method to measure transcription rate

*in vitro* assay for Polymerase Arrival Rates

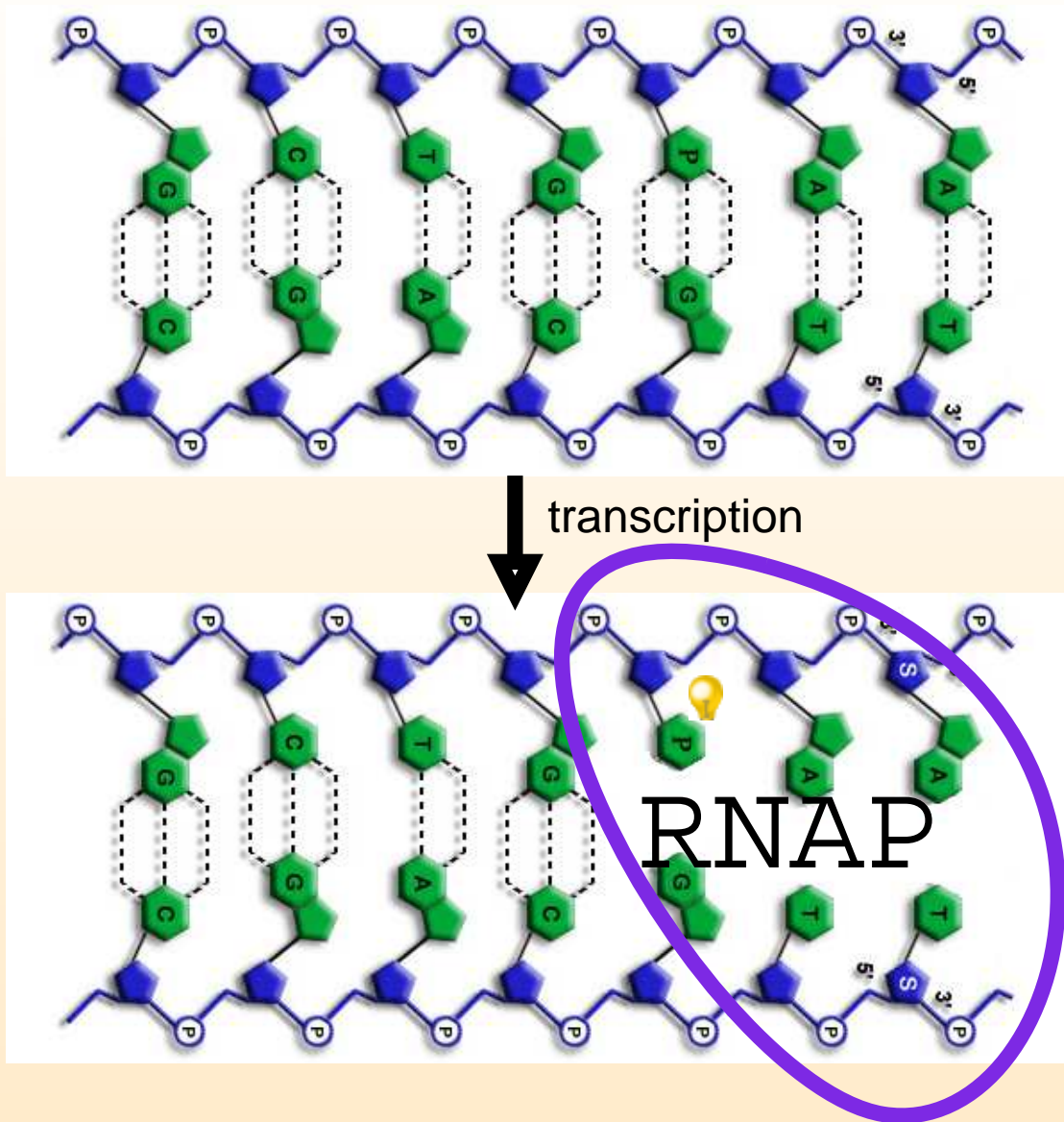
Hooks into the transcription process via a fluorescent probe

- Fluorescence is measured during transcription
- Fluorescence is directly related to the rate of polymerase arrivals

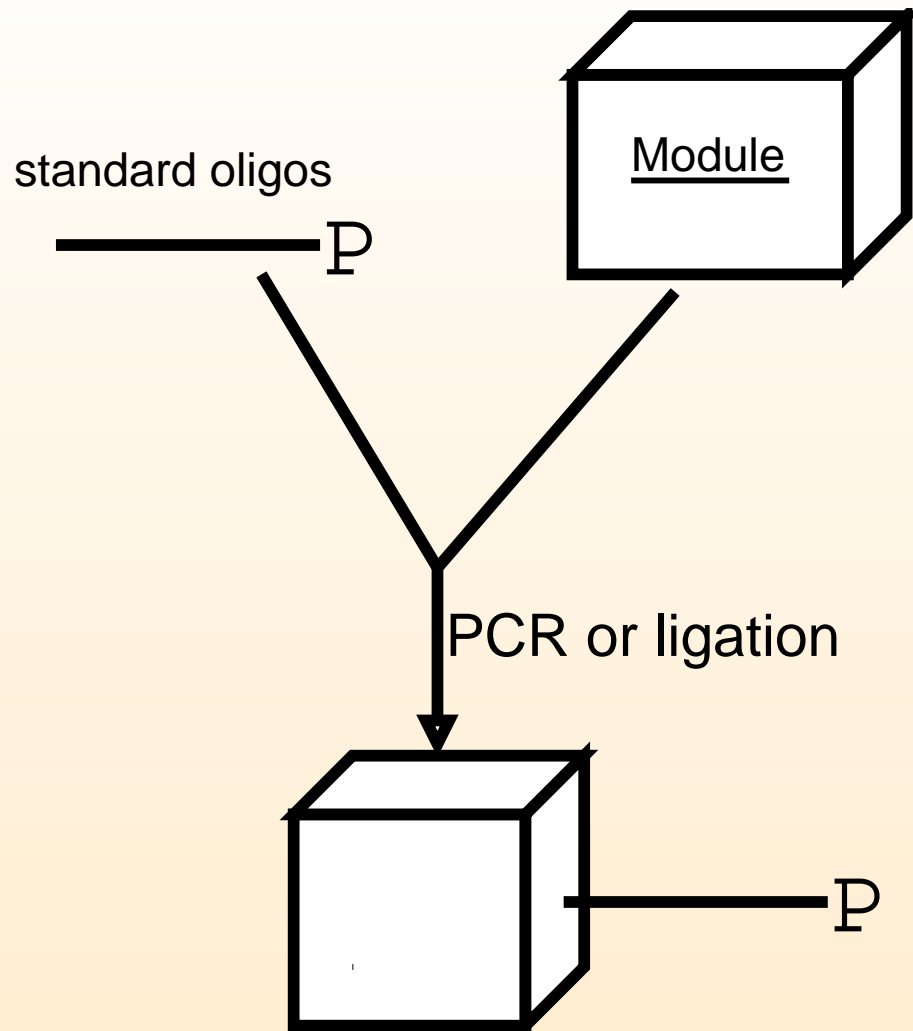
# Pyrrolo-dC is a fluorescent probe and can substitute for dC



# Pyrrolo-dC increases fluorescence during strand separation



# Incorporation of pyrrolo-dC in a generic fashion



*PAR can be measured at the point of incorporation of the pyrrolo-dC*

# Experimental Results

Experiments with single promoters

PCR incorporation of pyrrolo-dC on the *template strand*

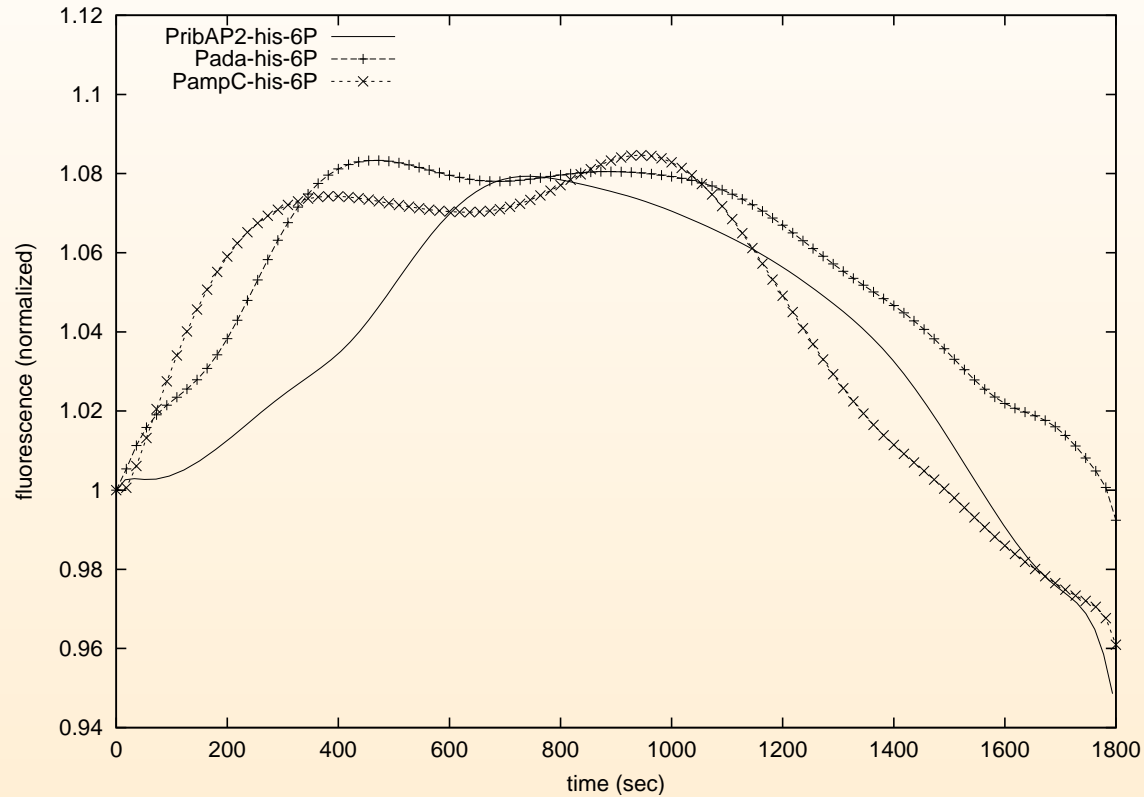
- Probe may not be effective on template strand due to RNA:DNA hybrid

Ligation incorporation of pyrrolo-dC on either strand

Fluorescence Measurement Issues

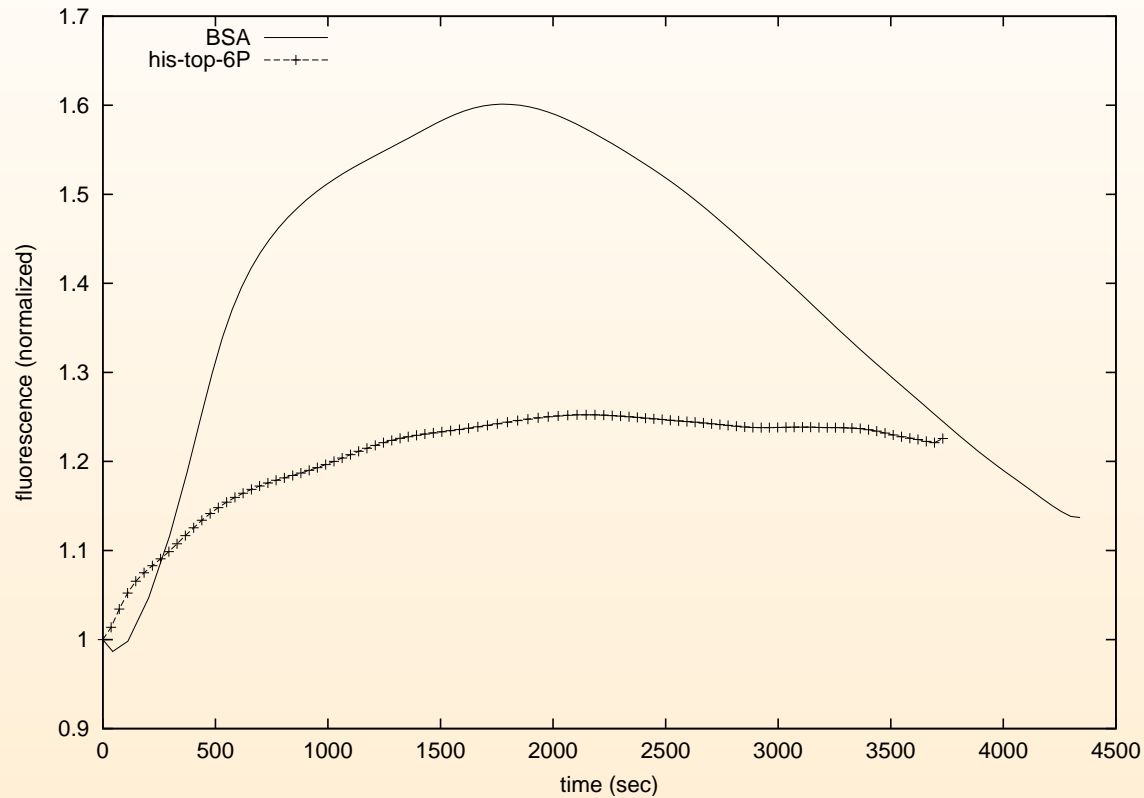
- Equipment
  - ▷ *Plates*
  - ▷ *Plate Reader*
- Sensitivity

# Fluorescence of some promoter constructs during transcription



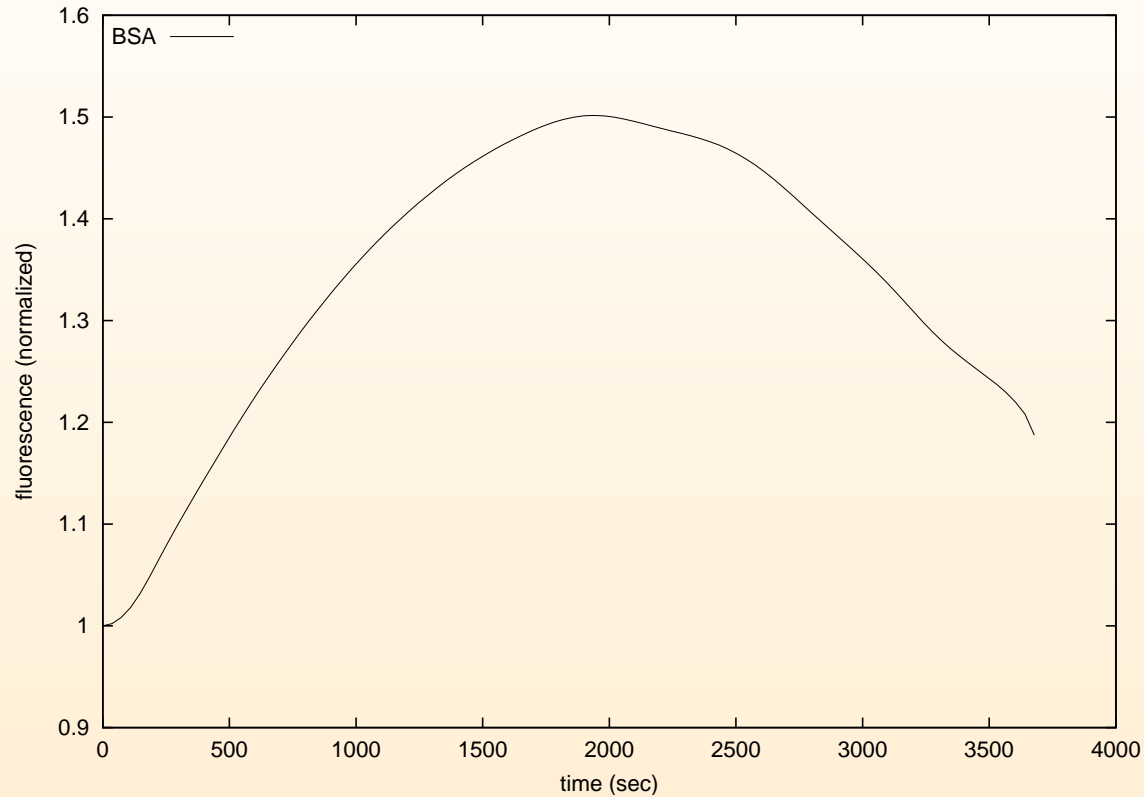
*Bell-shaped curves seen across many experiments.*

# Fluorescence of proteins (BSA) on high-binding plates



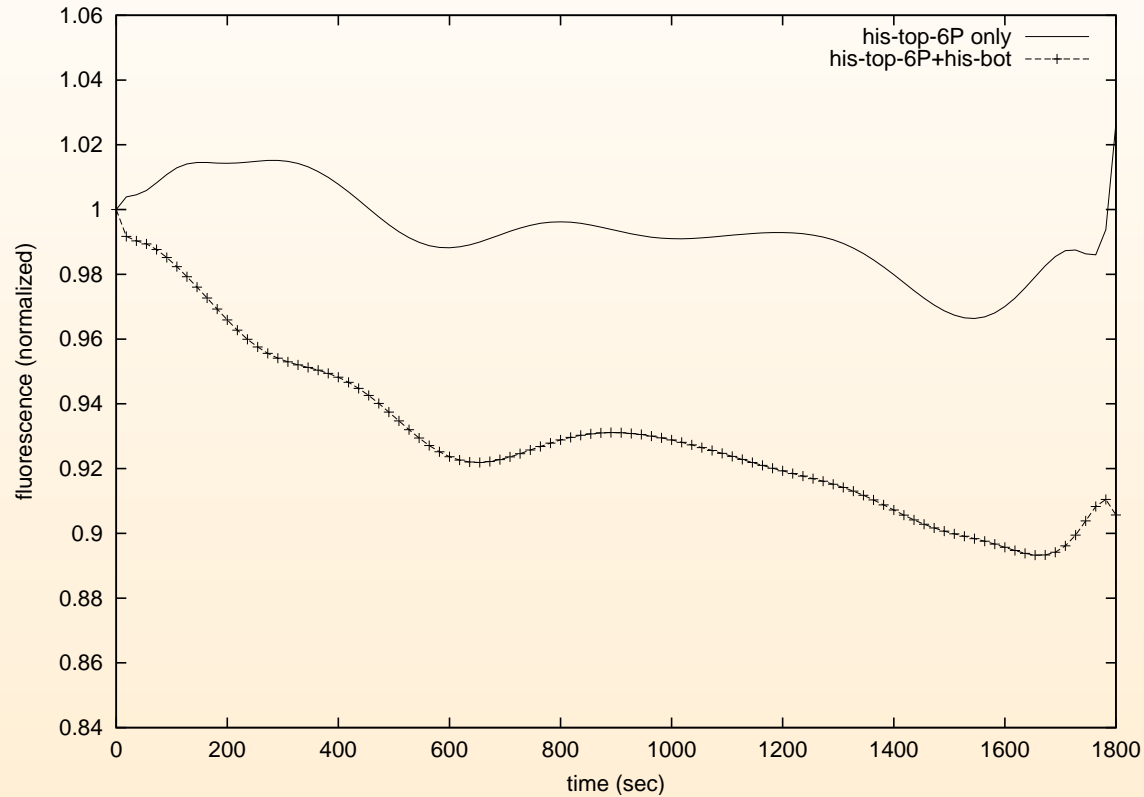
*The excitation/emission wavelengths 360nm/460nm should be far from any intrinsic fluorescence!*

# Fluorescence of BSA on low-binding plates



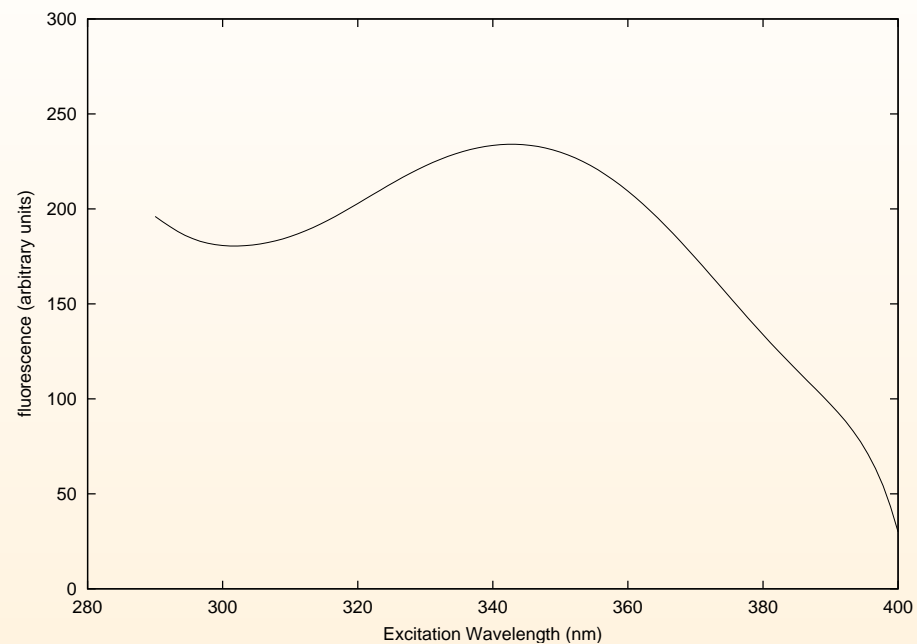
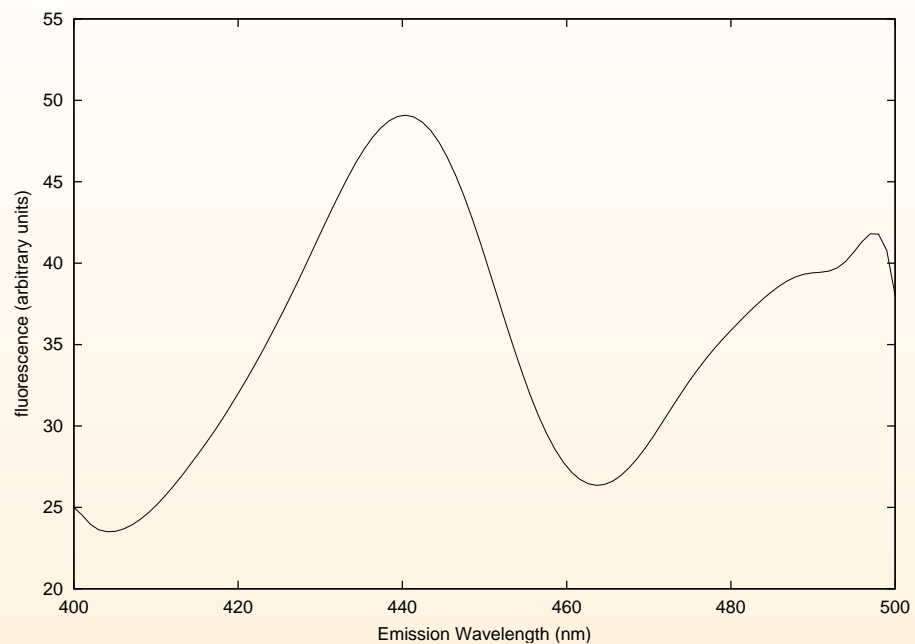
*Same type of curve for low-binding plates*

# Annealing kinetics of two complementary oligos



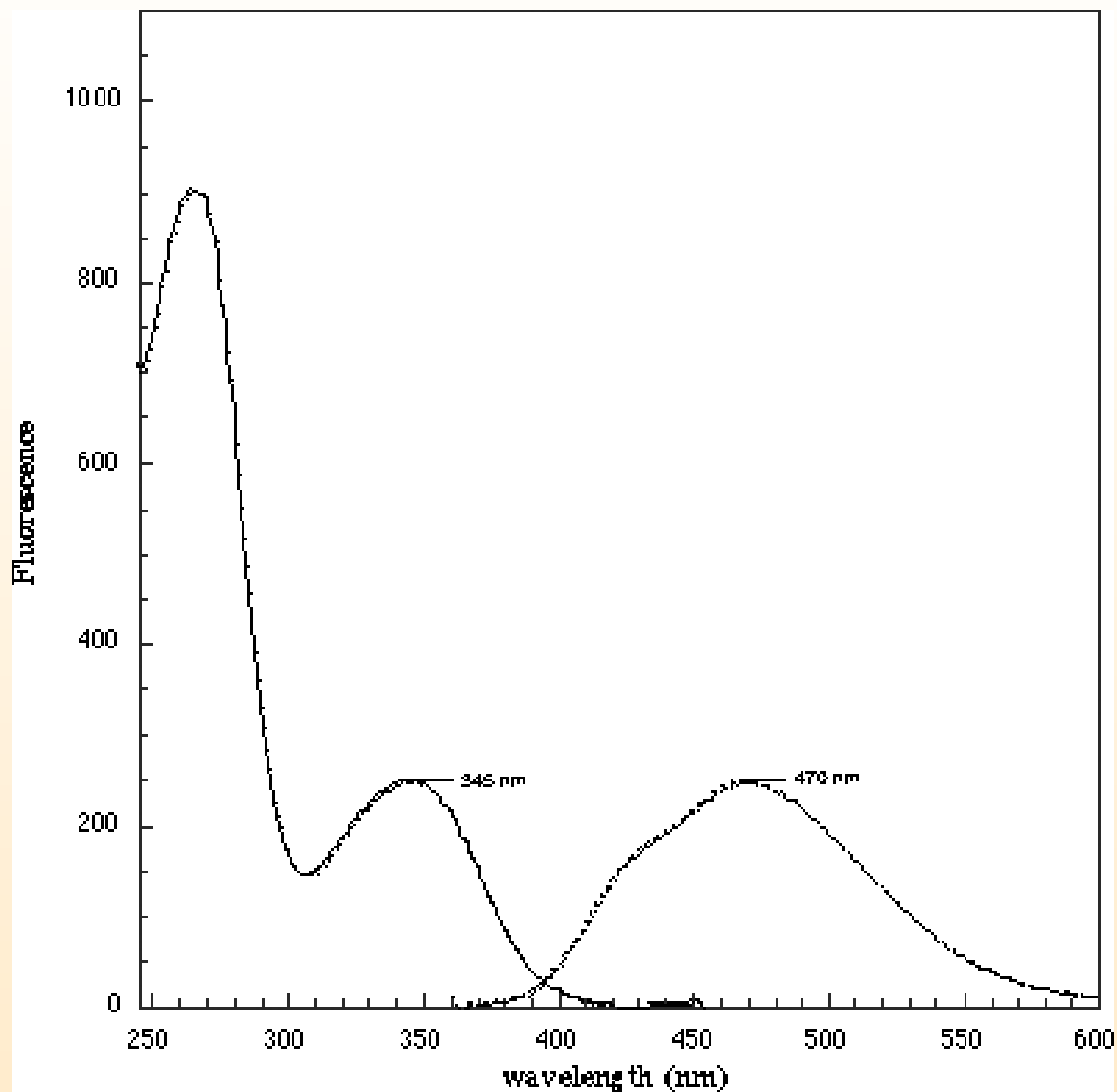
*Fluorescence decreases as two oligos containing pyrrolo-dC anneal to each other.*

# Measured spectrum of pyrrolo-dC



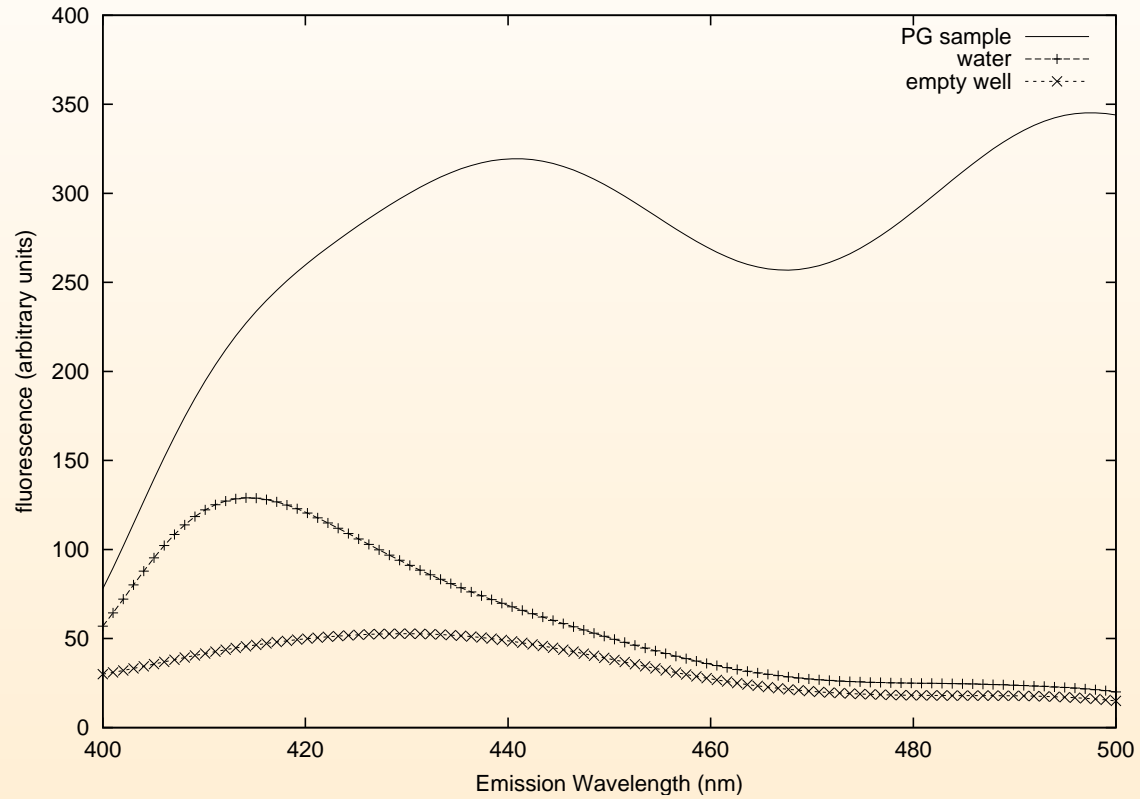
*Spectrum measured on a Tecan Safire plate reader (Ting lab)*

## Published spectrum of pyrrolo-dC



*from Glen Research*

# Fluorescence sensitivity compared to our plate reader



*signal-to-noise 7 on Tecan Safire vs. 1.5 on our reader*

# Transcription Experiments

	(a) Empty	(b) -RNAP	(c) +RNAP (-mix)	(d) +RNAP (+mix)	ratio (d/b)
Plac	809	821	812	671	0.82
Plac-P	845	757	742	720	0.95
Plac-5P-TE	821	775	739	772	1.00
PribAP2	845	949	943	699	0.74
PribAP2-P	842	821	748	748	0.91
PribAP2-5P-TE	851	742	739	726	0.98
Empty	861	912	964	949	1.04

# Comparison with other methods

Classical uses of reporter proteins or mRNA levels

- doesn't measure PAR as described here
- have many more complicating factors

2-aminopurine (2-AP) has similar properties to pyrrolo-dC

- has been used much more than pyrrolo-dC
- lower fluorescence wavelengths, leading to higher background

No radioactivity needed

Relatively simple in theory

Measurement is non-destructive so continuous, real-time measurements are possible

Measurements can be done in a modular fashion

- Oligos with pyrrolo-dC only need to be synthesized once

# Future Work

## Differences between the template and the non-template strand

- RNA:DNA hybrid during transcription can quench fluorescence

## Reaction conditions

- Buffers and concentrations used have shown effects on fluorescence and transcription

## Fluorescence measurements

- Real-time kinetics
- pyrrolo-dC characterization

## More complex modules

- transcription regulators

# Conclusions

Polymerase Arrival Rates are a useful way of characterizing modules

- Is an absolute and physically well-defined property
- A universal measure that allows for arbitrary connections among modules

PARMESAN is an *in vitro* assay for polymerase arrival rates

Experimental results have been disappointing

- Better fluorescence reader may solve some issues



# Acknowledgments

*Thanks!*

**Drew Endy**  
Sriram Kosuri

Biology and Biological Engineering

**Tom Knight**  
Randy Rettberg  
Reshma Shetty

EECS (CSAIL)

<http://www.syntheticbiology.org/>