Synthetic in vitro transcriptional oscillators

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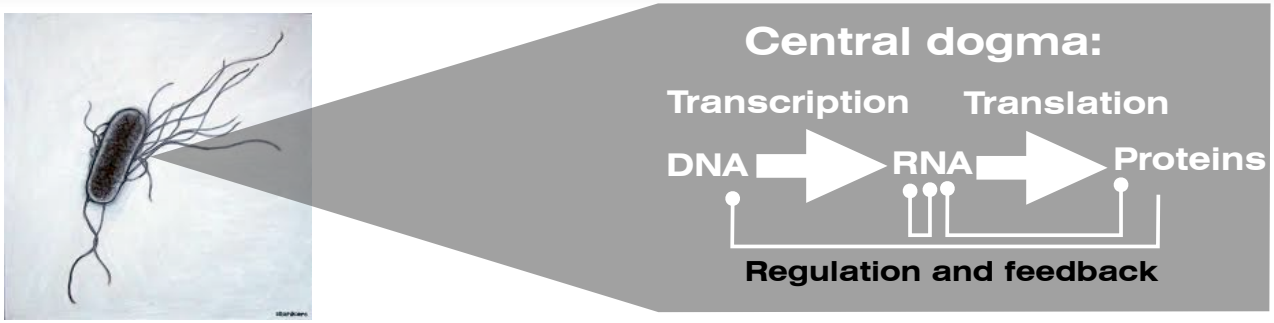
September 19, 2011

DNA 17

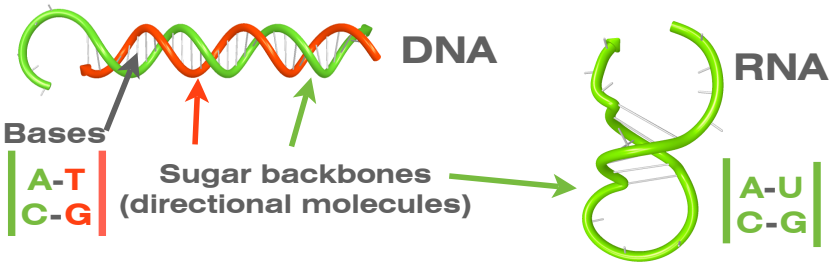
Outline

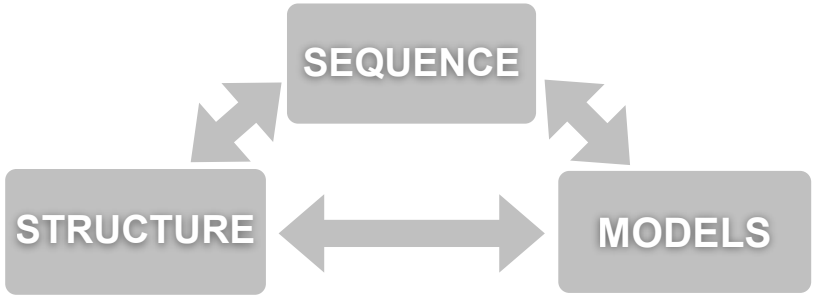
- Background on nucleic acids and proteins
- Transcriptional circuits
  - Design specifications
  - Examples
- Transcriptional oscillators
  - Two node architecture
  - Tuning
  - Challenges
  - Schedule

Background: Nucleic acids and proteins



NUCLEIC ACIDS (NA)



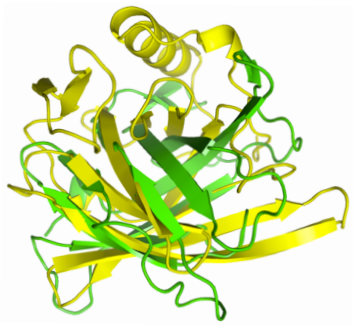


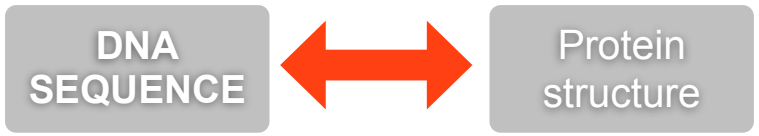
PROGRAMMABLE!

COMMERCIAL CUSTOM ORDERING, CHEAP

PROTEINS

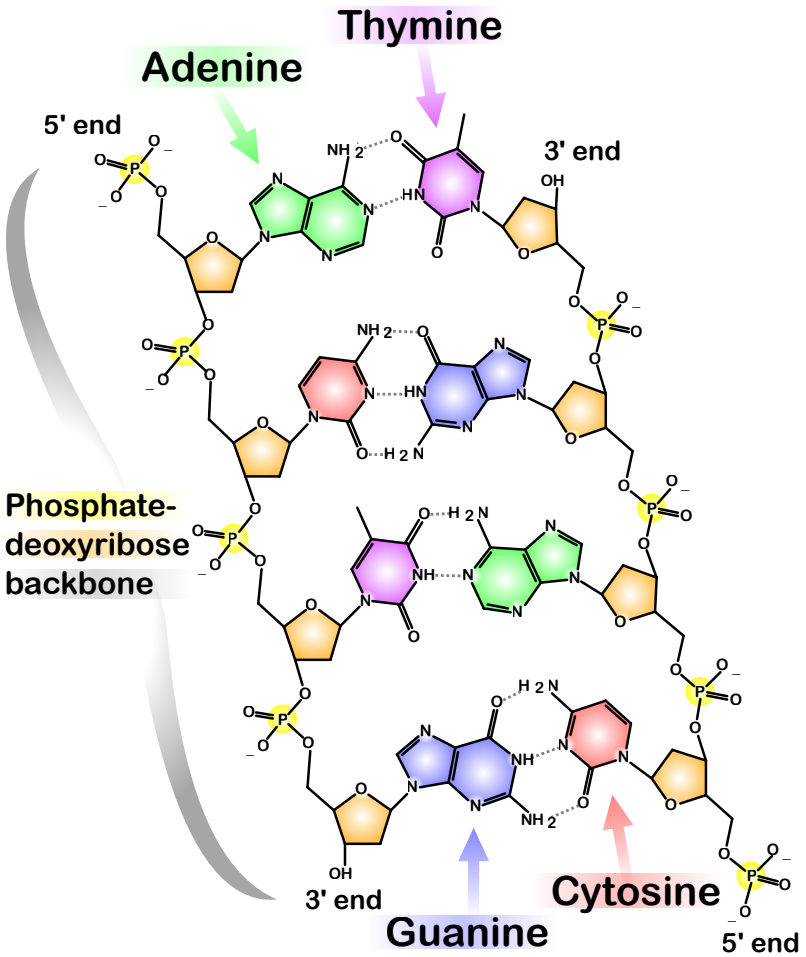
Complex spatial structures!





Not as easy
as for Nucleic Acids!

Background: Nucleic acids



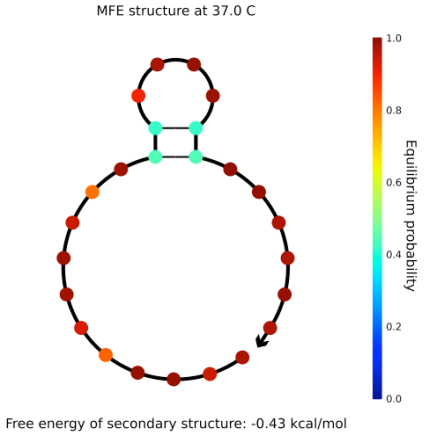
Sequence -> Secondary structure:

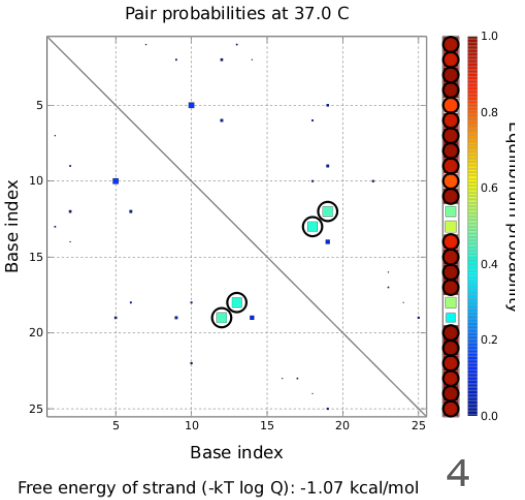
www.nupack.org MFold IDT Oligo Analyzer

DIFFERENT

- Thermodynamic parameters
- Algorithms

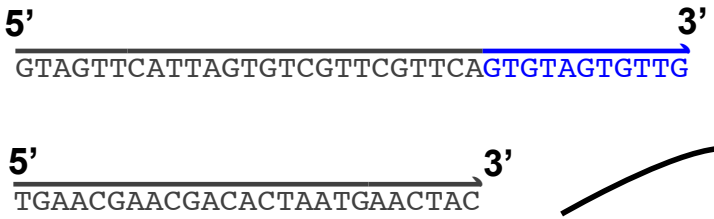
EXAMPLE: TGAACGAACGACACTAATGAACTAC
DNA, 37˚C, using Nupack

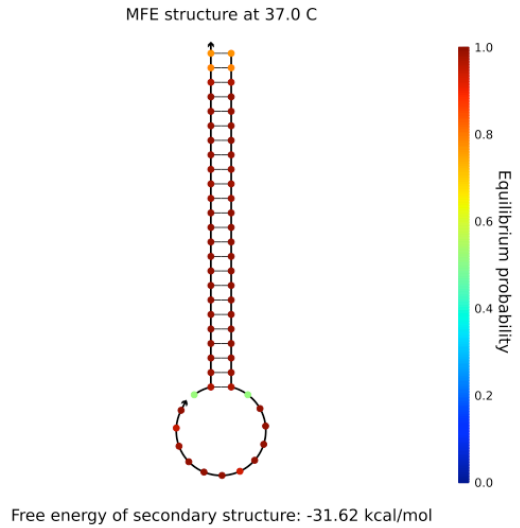


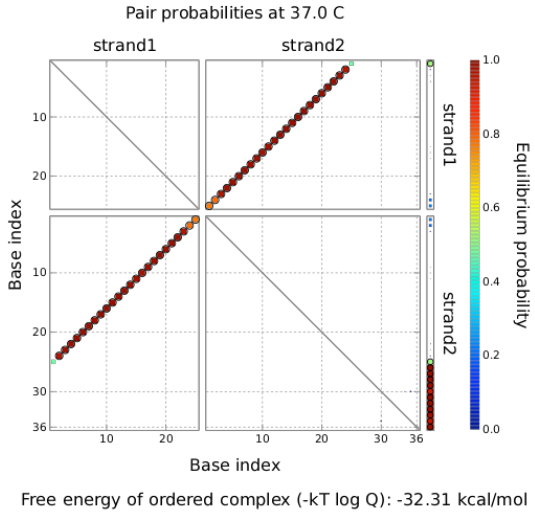


Background: Nucleic acids

HYBRIDIZATION:



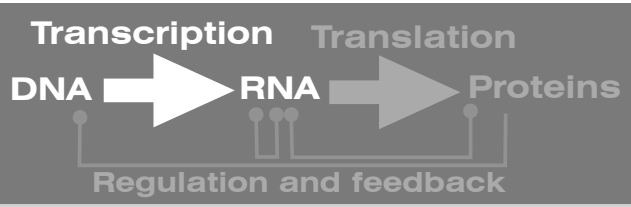




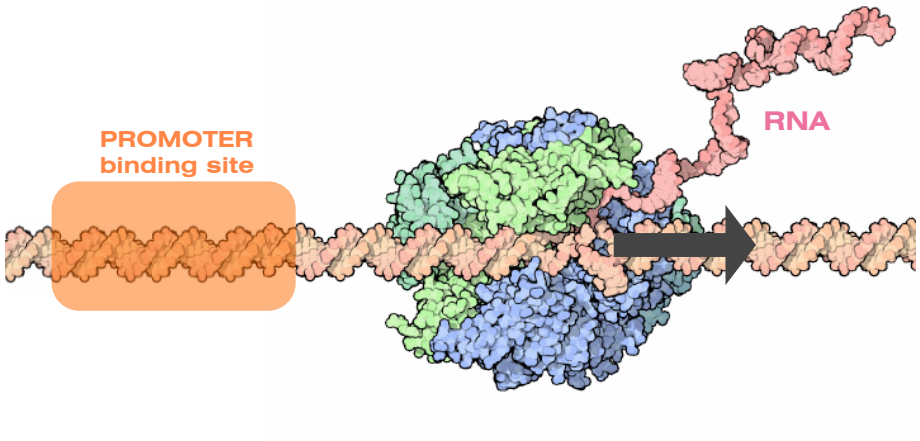
IMPORTANT:
DNA and RNA can hybridize (A-U, A-T), but hybridization parameters are not known

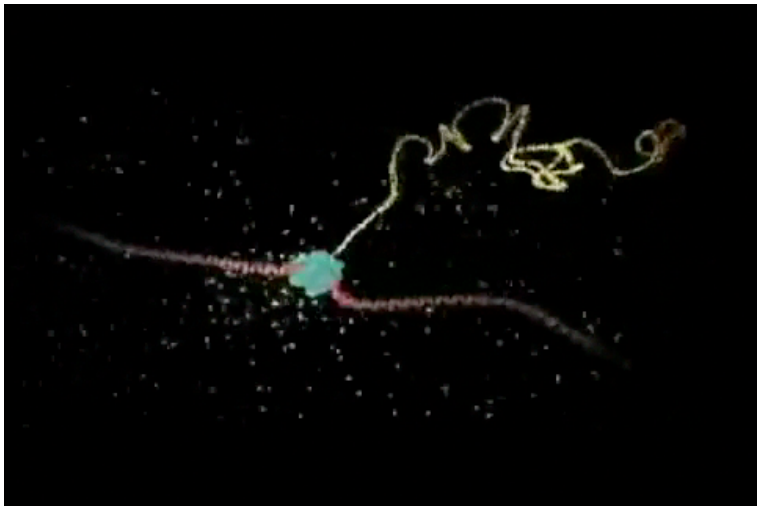
Representation:

Background: Transcription

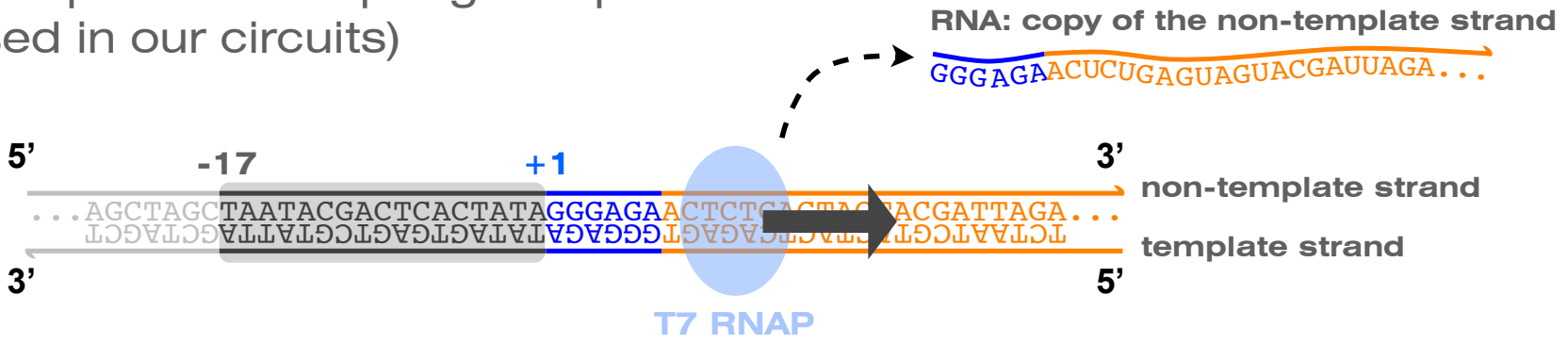


www.pdb.org



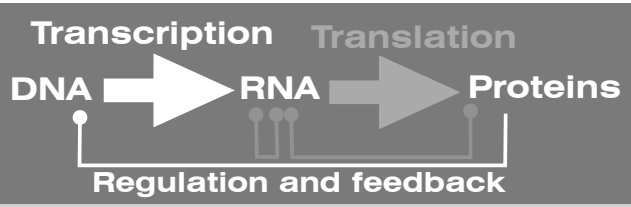


Example: Bacteriophage T7 promoter (used in our circuits)



Note: promoter efficiency (rate of RNA transcription) can be tuned by sequence modifications. See, for instance Imburgio et al. Biochemistry, 2000.

Background: Transcription control



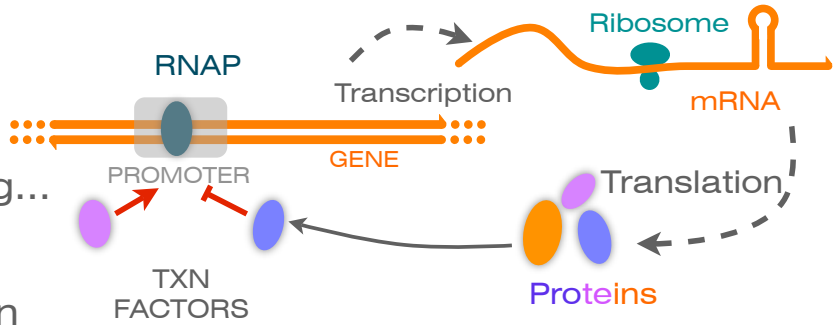
NATURAL SYSTEMS:

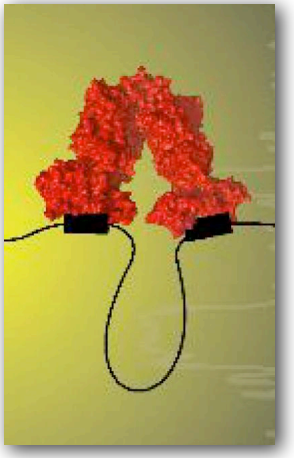
Transcription factors

Promoter occlusion, looping...

RNA folding

Cause abortive transcription

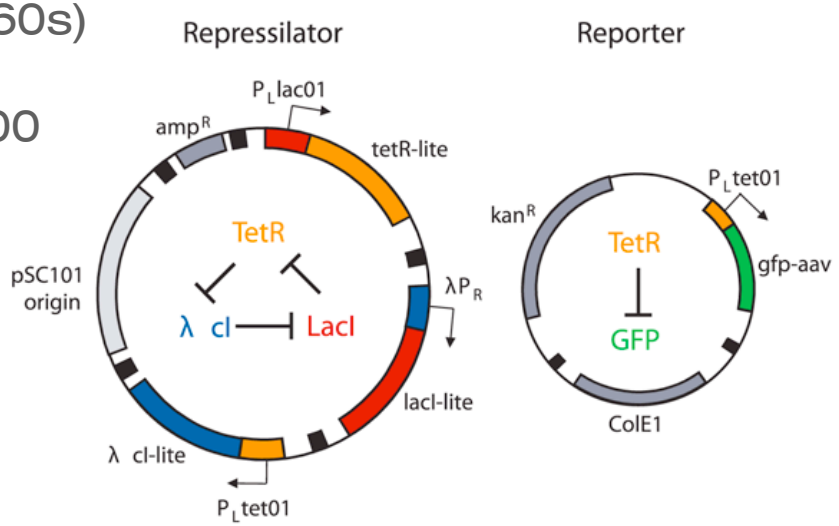




D. Rutkauskas & F. Vanzi, PNAS 2009

Transcriptional control of gene expression

- Well characterized (Jacob/Monod, 1960s)
- Widely used in synthetic biology
  Ex: Repressilator, Elowitz&Leibler, 2000

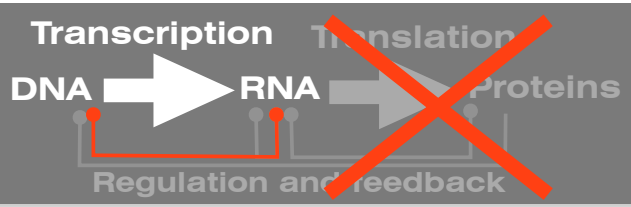


TRANSCRIPTION FACTORS...

Require translation

Complex process, need a lot of cellular machinery

Background: Transcription control



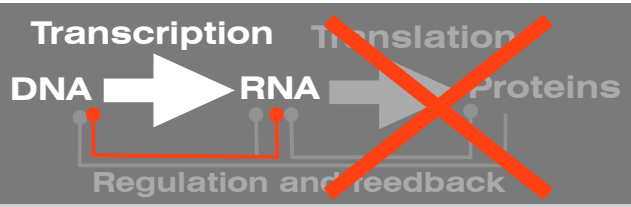
BIOCHEMISTRY of transcription has been studied thoroughly
Process that requires few components

TRANSCRIPTION REAGENTS:

- DNA with promoter
- RNA Polymerase
- Ribonucleotide tri-phosphates (rNTPs) (A, T, C and G)
- Buffer/salt to maintain pH (7-7.5) and correct ionic balance: typically Tris HCl, NaCl, Mg++
- "Assisting" enzymes: typically spermidine and pyrophosphatase
- Water

CAN WE DYNAMICALLY CONTROL TRANSCRIPTION IN THIS SIMPLE ENVIRONMENT?
... i.e. without translation...

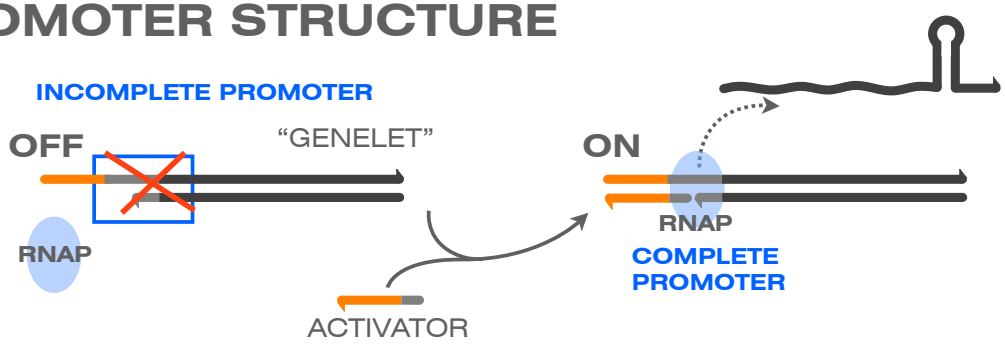
Background: Transcription control in vitro



IDEA 1: ALTERING THE PROMOTER STRUCTURE

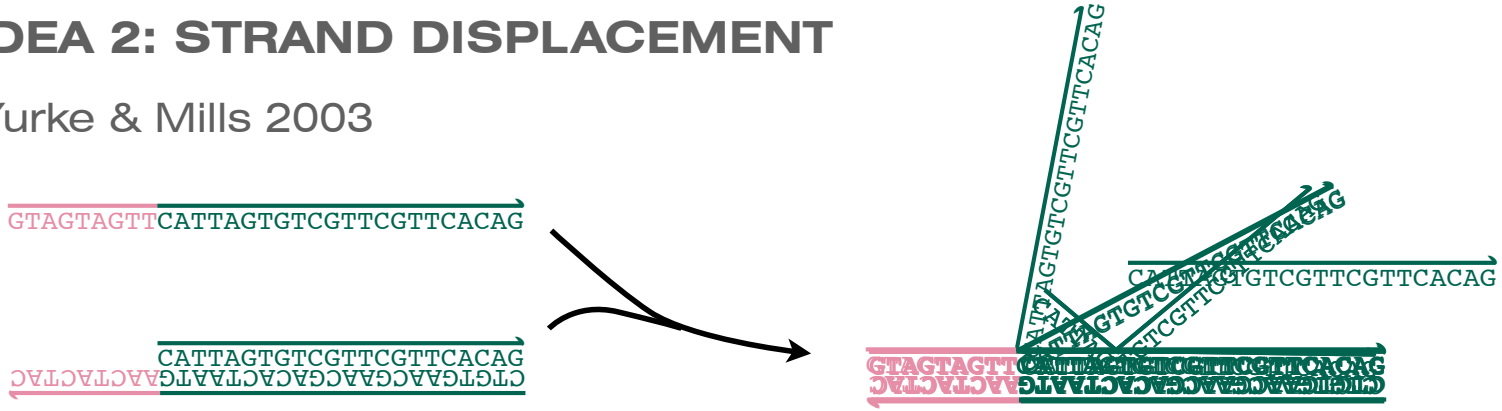
"GENELET" SWITCH

- short
- linear
- synthetic

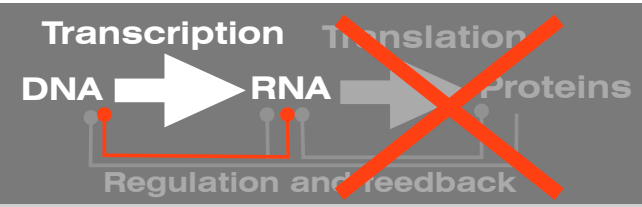


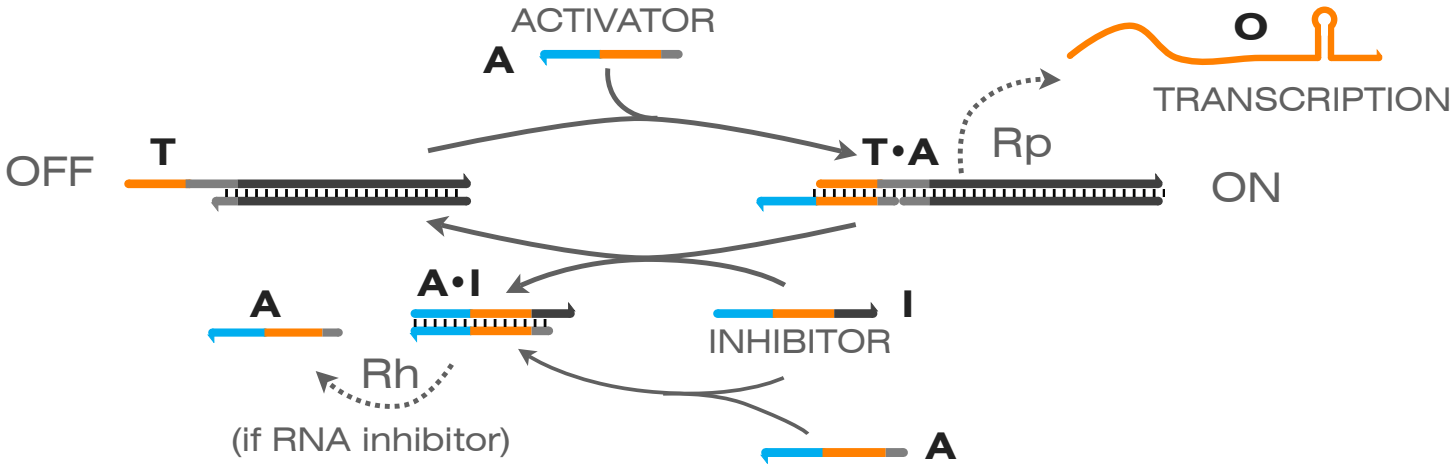
IDEA 2: STRAND DISPLACEMENT

Yurke & Mills 2003



Transcriptional circuits





- Biochemical networks with complex functionalities Kim, NIPS 2004
- Reduced number of components
- Models from first principles

Activation $\mathrm{T} + \mathrm{A} \overset{k_{TA}}{\rightarrow} \mathrm{T} \cdot \mathrm{A}$

Inhibition $\mathrm{T} \cdot \mathrm{A} + \mathrm{I} \overset{k_{TAI}}{\rightarrow} \mathrm{T} + \mathrm{I} \cdot \mathrm{A}$

Annihilation $\mathrm{A} + \mathrm{I} \overset{k_{AI}}{\rightarrow} \mathrm{A} \cdot \mathrm{I}$

Transcription: on $\mathrm{RNAP} + \mathrm{T} \cdot \mathrm{A} \underset{k^-_{ON}}{\overset{k^+_{ON}}{\rightleftarrows}} \mathrm{RNAP} \cdot \mathrm{T} \cdot \mathrm{A} \overset{k_{catON}}{\rightarrow} \mathrm{RNAP} + \mathrm{T} \cdot \mathrm{A} + \mathrm{O}$

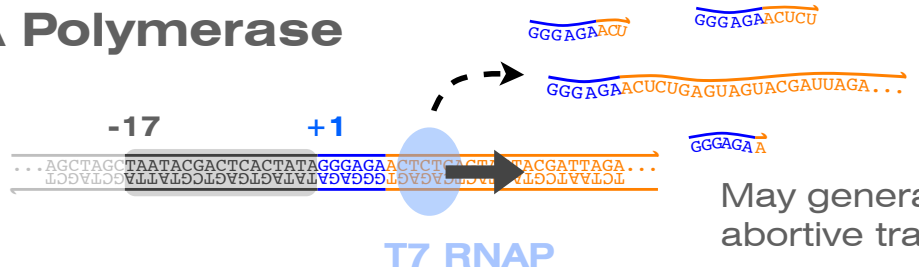
Transcription: off state $\mathrm{RNAP} + \mathrm{T} \underset{k^-_{OFF}}{\overset{k^+_{OFF}}{\rightleftarrows}} \mathrm{RNAP} \cdot \mathrm{T} \overset{k_{catOFF}}{\rightarrow} \mathrm{RNAP} + \mathrm{T} + \mathrm{O}$

Degradation $\mathrm{RNaseH} + \mathrm{A} \cdot \mathrm{I} \underset{k^-_{H}}{\overset{k^+_{H}}{\rightleftarrows}} \mathrm{RNaseH} \cdot \mathrm{A} \cdot \mathrm{I} \overset{k_{catH}}{\rightarrow} \mathrm{RNaseH} + \mathrm{A}.$

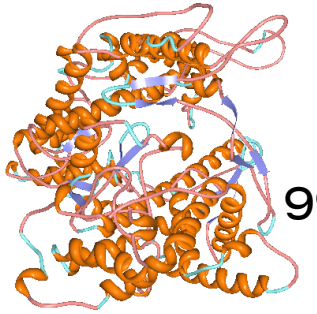
=> write ordinary differential equations

Transcriptional circuits

Production of RNA:
T7 RNA Polymerase



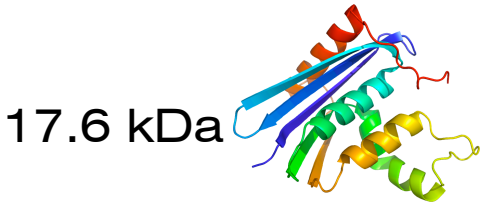
May generate abortive transcripts



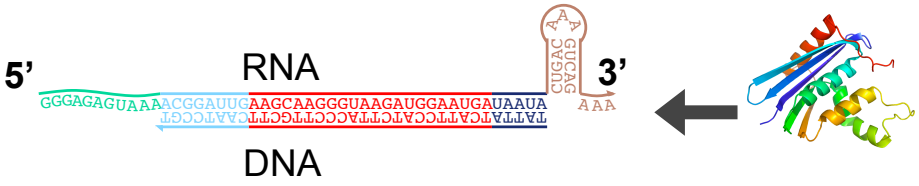
99 kDa

Degradation of RNA, in DNA-RNA hybrids:
RNAse H

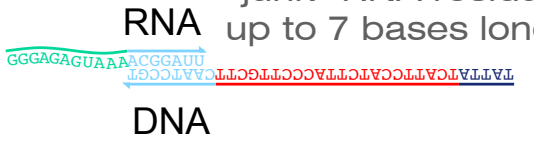
Cleaves the 3'-O-P bond of RNA in a **DNA/RNA** duplex to produce 3'-hydroxyl and 5'-phosphate terminated products



17.6 kDa



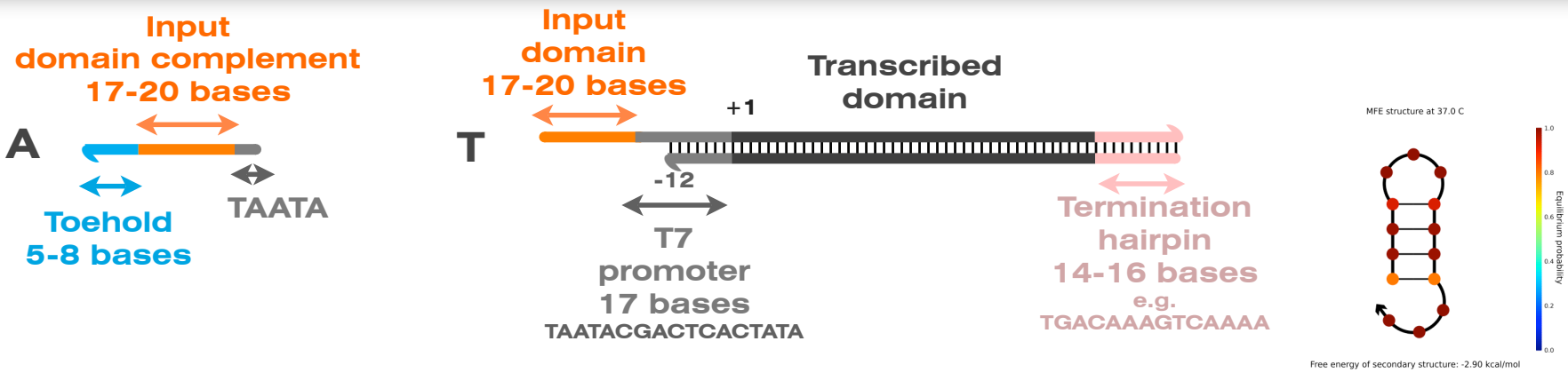
Partially degraded "junk" RNA residuals up to 7 bases long



NOTE: These enzymes are sold by many vendors (Ambion, Epicentre, Sigma, NEB...)
Enzymes are expressed in bacteria, extracted and stored in glycerol following vendor protocol.
Activity varies from batch to batch!

Genelets design specifications

See also the PhD thesis of J. Kim, Caltech, 2007



LENGTH AND SEQUENCE CHOICES:

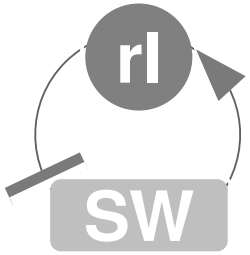
TOEHOLD
- Displacement speed
- Specificity

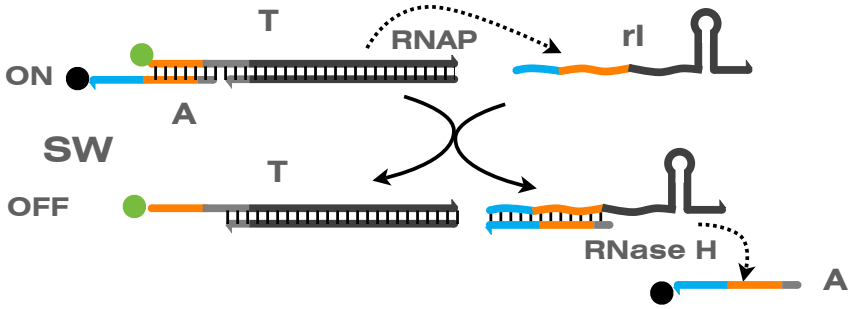
INPUT DOMAIN
- Binding specificity
- Minimize cross-talk
- Maintain domain short (better for synthesis cost)

PROMOTER NICKING
at -12
- Reduce crosstalk among activators (TAATA)
- Low off transcription

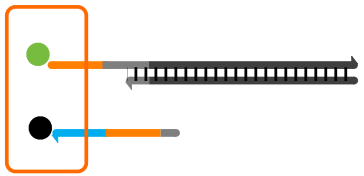
TERMINATOR
- RNAP may hang on to RNA and use it as a template, extending the transcript
- Strong structure followed by unbound bases reduces this phenomenon

Example: self-repression

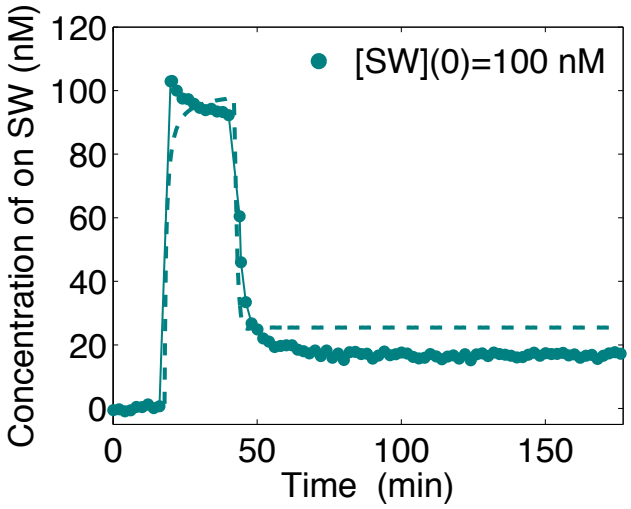




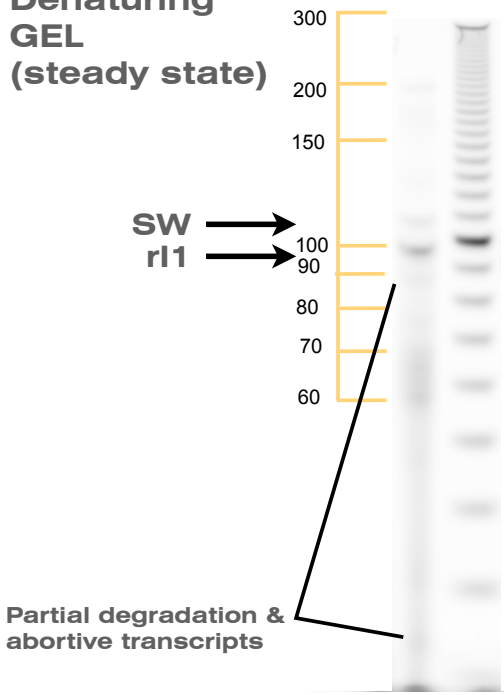
Monitor ON-OFF state of the switch over time



Time trace: spectrofluorimeter



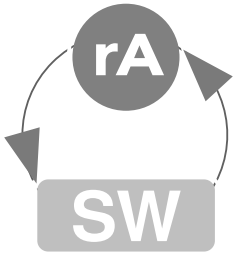
Denaturing GEL (steady state)

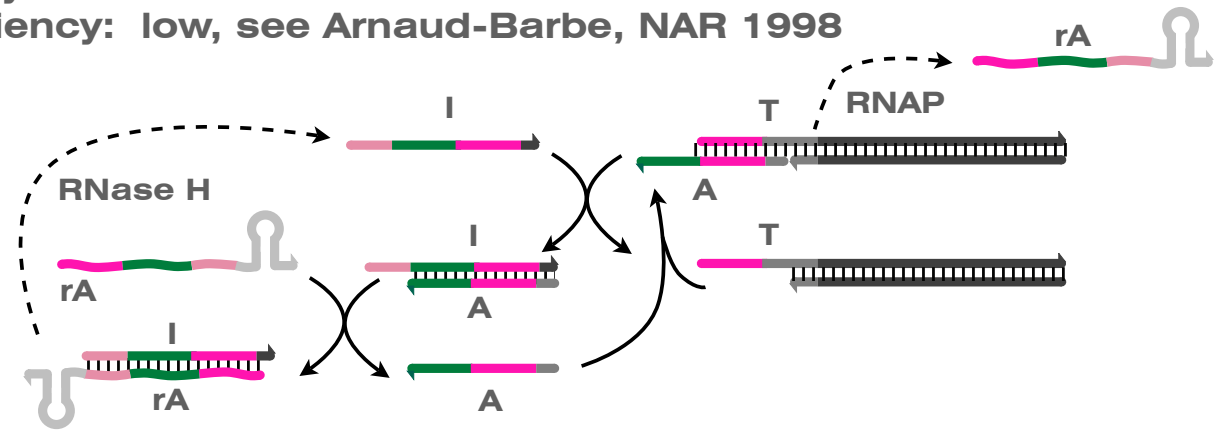


Example: self-activation

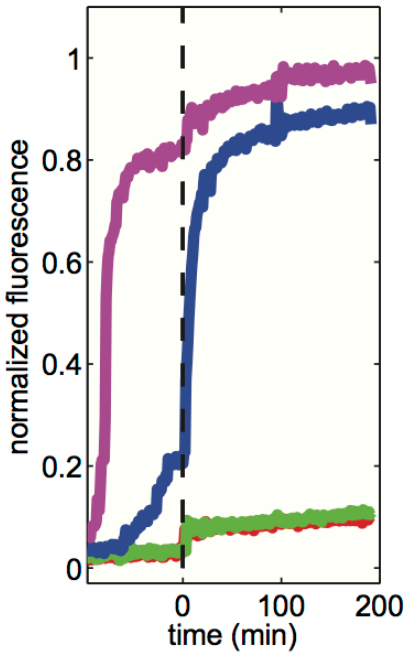
Data from Subsoontorn, Kim & Winfree, ArXiv 2011

Direct activation: DNA only
DNA-RNA templates efficiency: low, see Arnaud-Barbe, NAR 1998

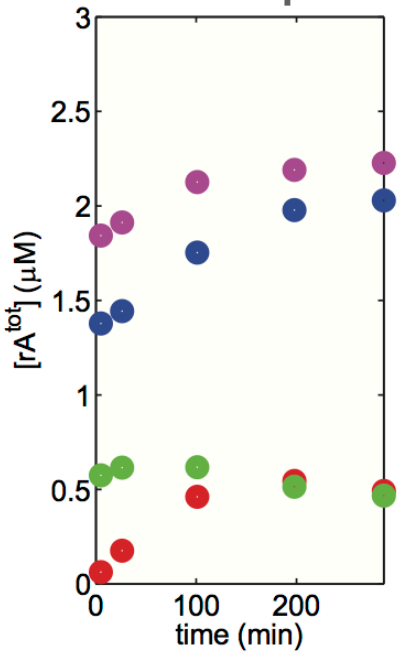




Fluorimeter traces



Gel samples

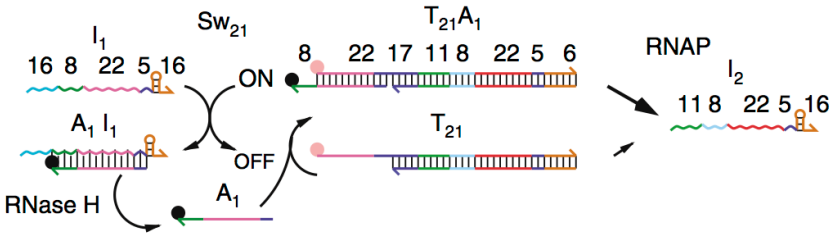


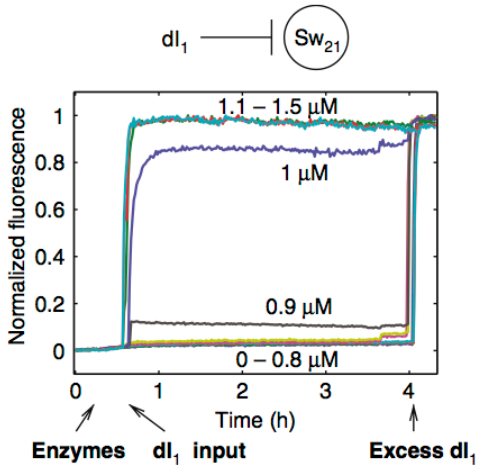
- **Needs initial kick of RNA for activation**
- **Behaves like a memory element**

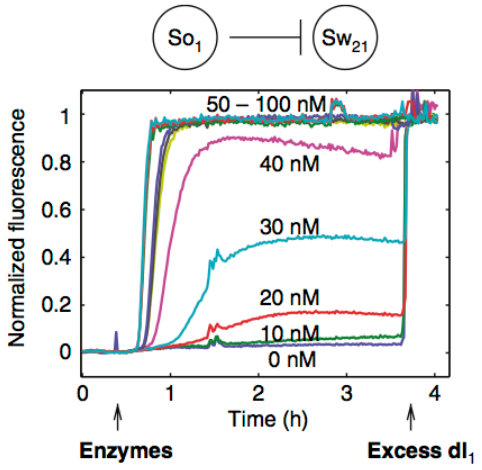
[rA1](0) 0 nM
[rA1](0) 550 nM
[rA1](0) 1350 nM
[rA1](0) 1750 nM

Example: interconnecting genelets

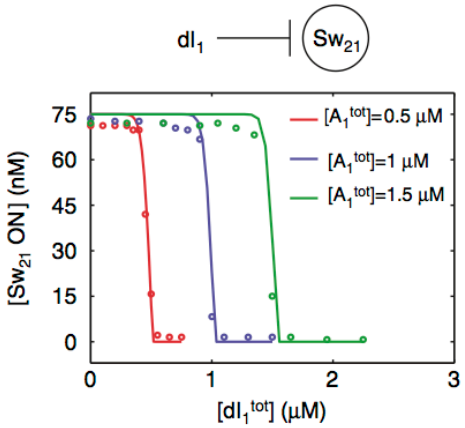
Figures and data from Kim & Winfree, Mol Sys Bio 2006

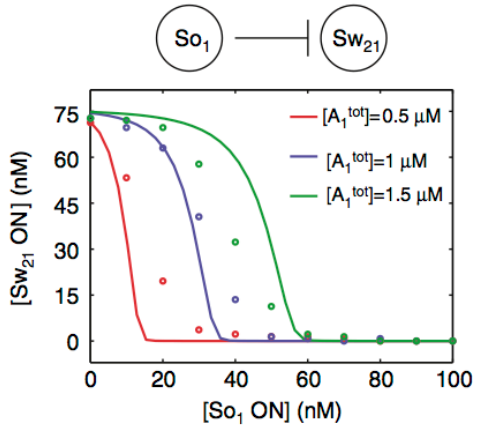






FLUORIMETER TIME TRACES



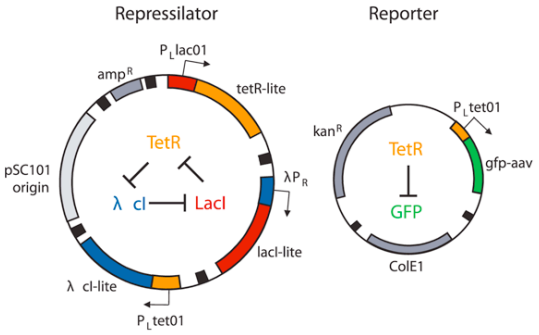


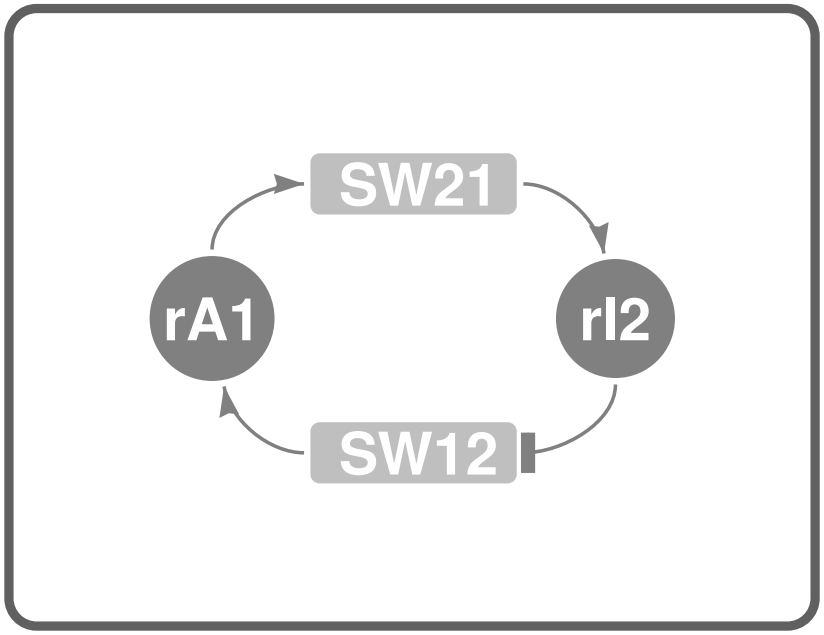
STEADY STATES

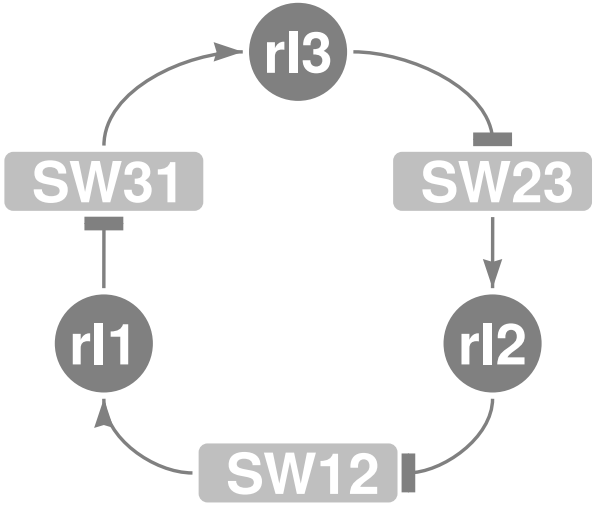
~ Hill functions with high Hill coefficient
Ultrasensitivity (see oscillator)

Transcriptional oscillators

Motivation?
Repressilator
(Elowitz & Leibler, 2000)



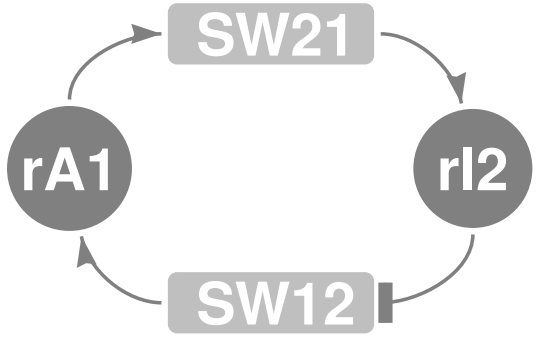




Two node oscillator: model problem

Kim & Winfree, Mol Sys Bio 2011

Simple intuition for the dynamics



$$\frac{d[rA1]}{dt} = k_p[SW12] - k_d[rA1]$$

$$\tau\frac{d[SW21]}{dt} = [SW21^{tot}]\frac{\frac{[rA1]^m}{KA^m}}{1+\frac{[rA1]^m}{KA^m}} - [SW21]$$

$$\frac{d[rI2]}{dt} = k_p[SW21] - k_d[rI2]$$

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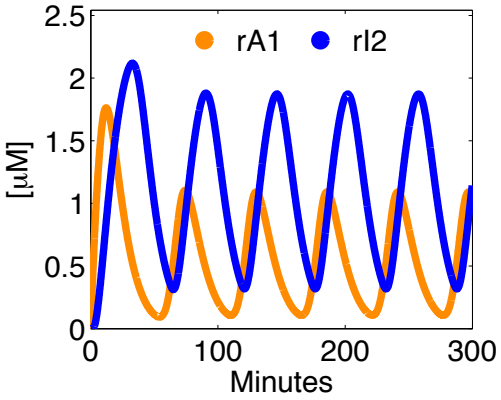
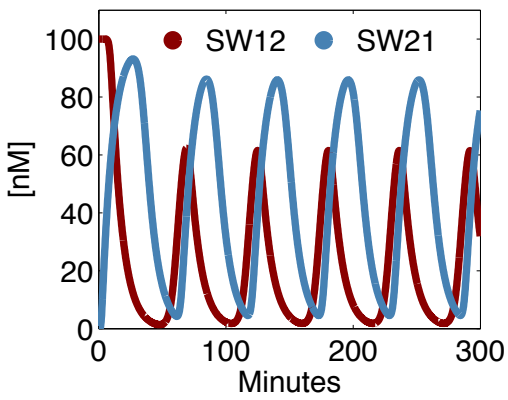
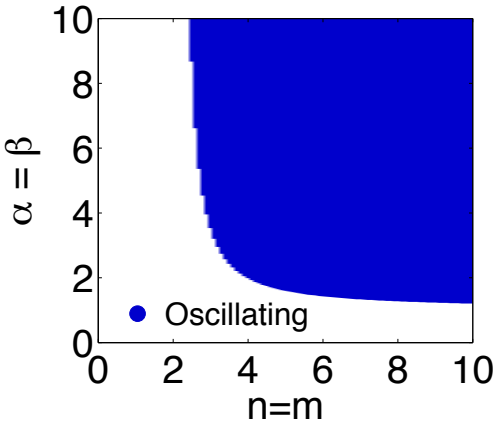




m=n=5 $k_p = 0.05/s$, $k_d = 0.002/s$

KA=KI=0.5 μM $\tau = 500\,s$

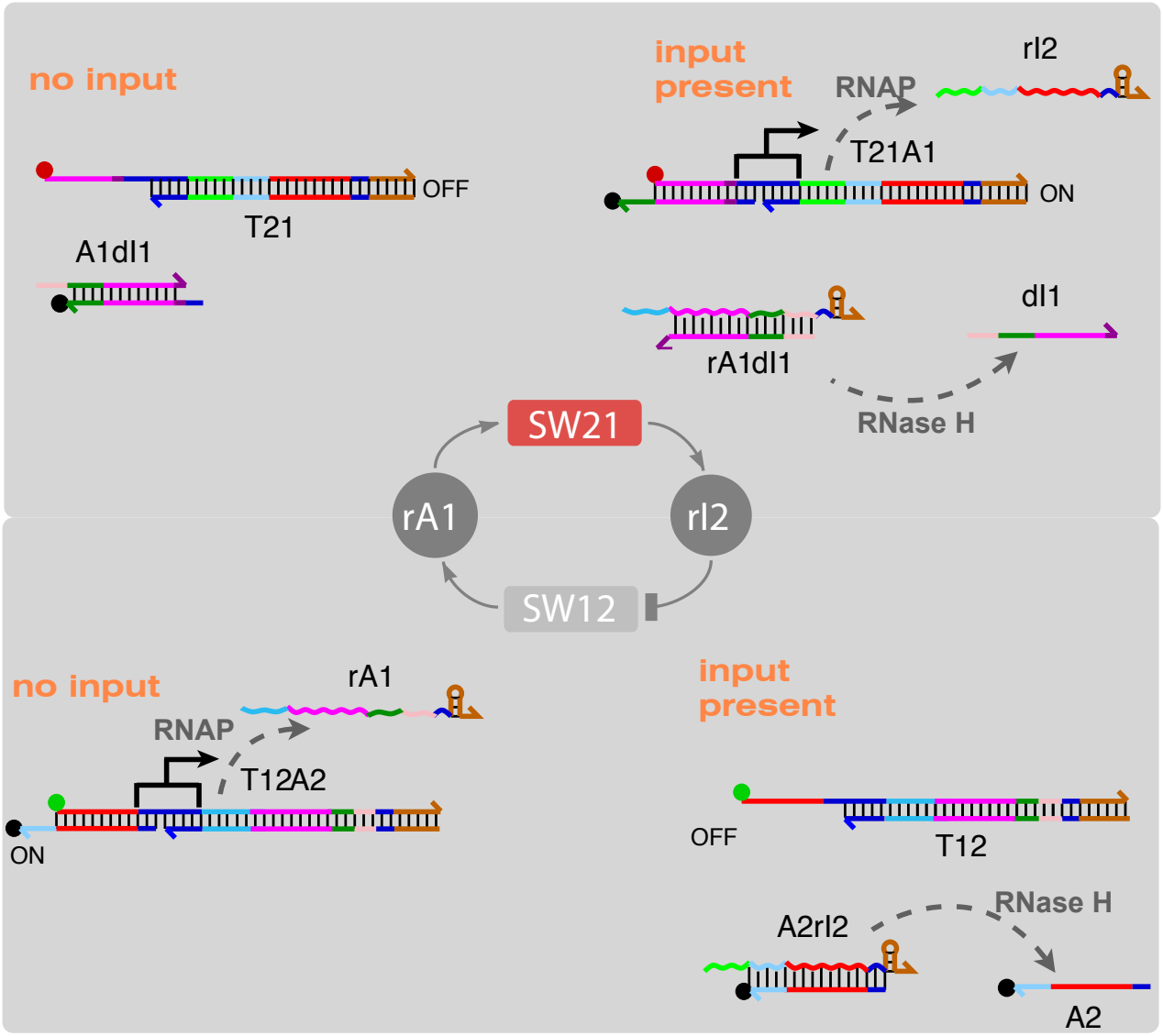
$[SW21^{tot}] = [SW12^{tot}] = 100\,nM$

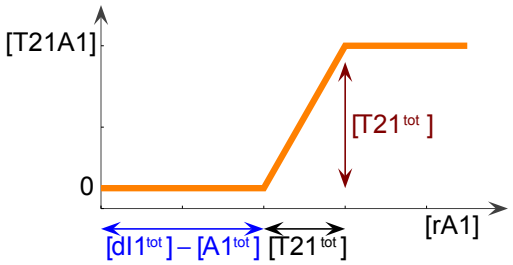


$\alpha = (kp/kd)[SW12^{tot}]/KA$

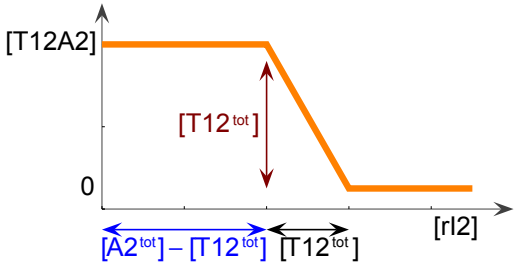
$\beta = (kp/kd)[SW21^{tot}]/KI$

Two node oscillator





Ultrasensitive switches
~ high Hill coefficients

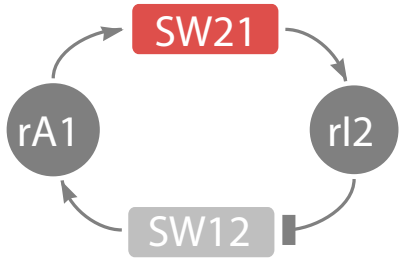


Oscillations

OPERATING POINT:

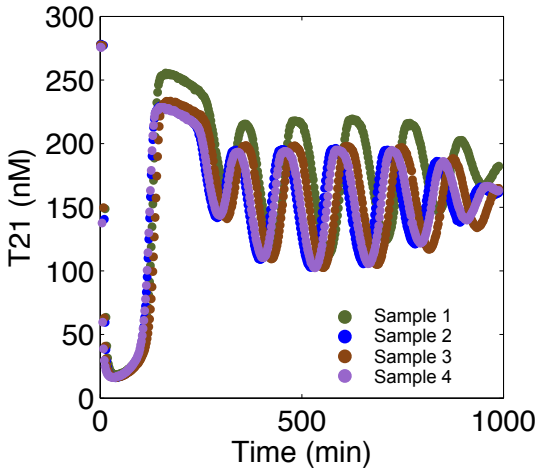
T21 250 nM
T12 120 nM

A1 250 nM
dI1 700 nM
A2 500 nM

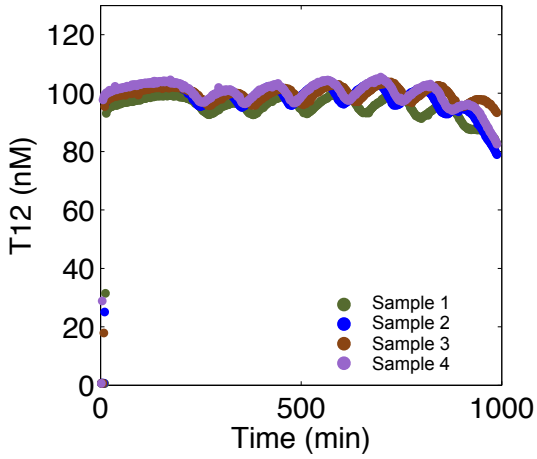


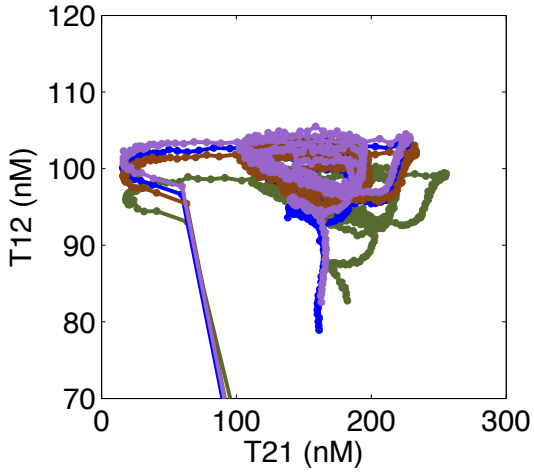
T21 genelet state oscillates more strongly

TYE665/TexasRed

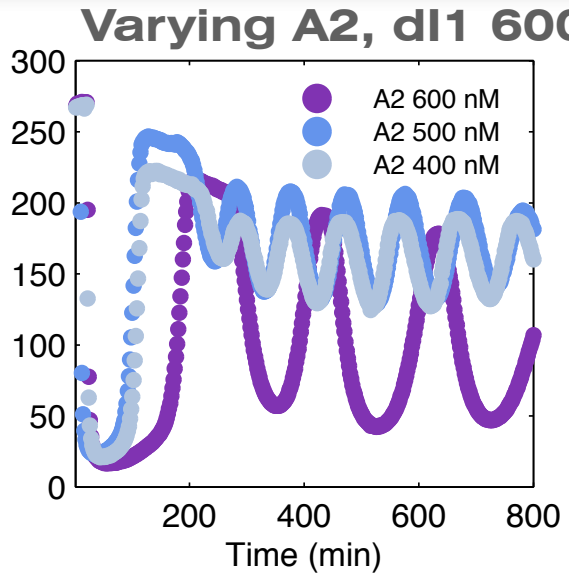


TYE563/TAMRA





Tuning: Varying the thresholds



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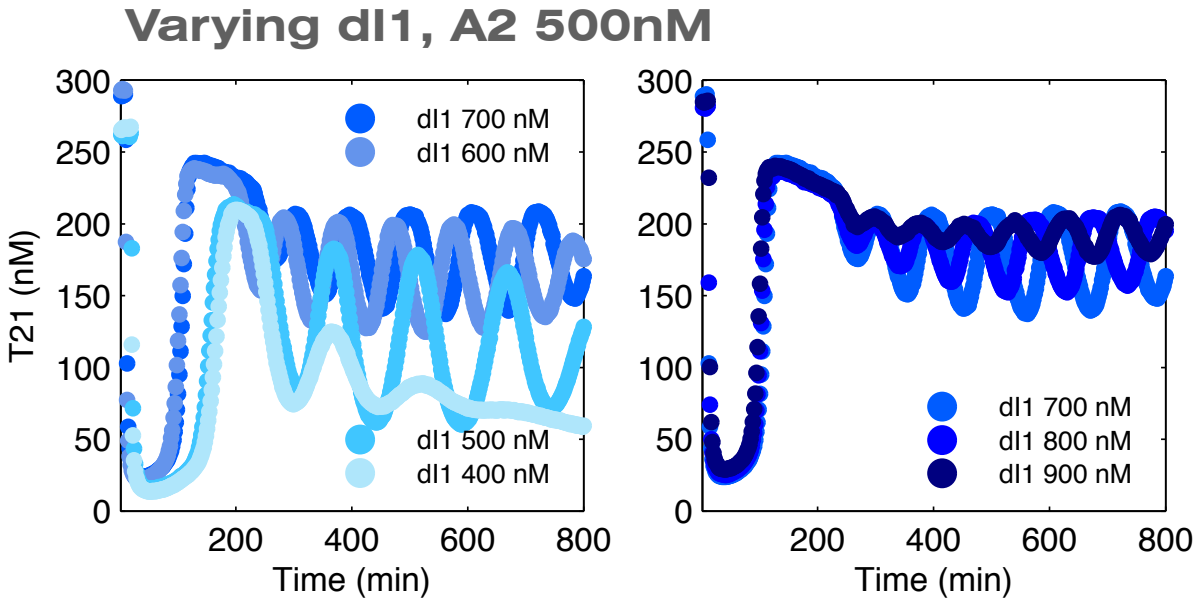
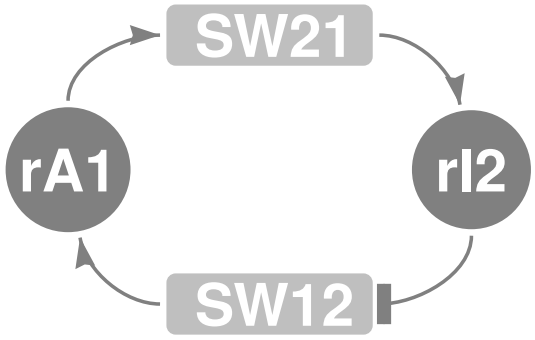
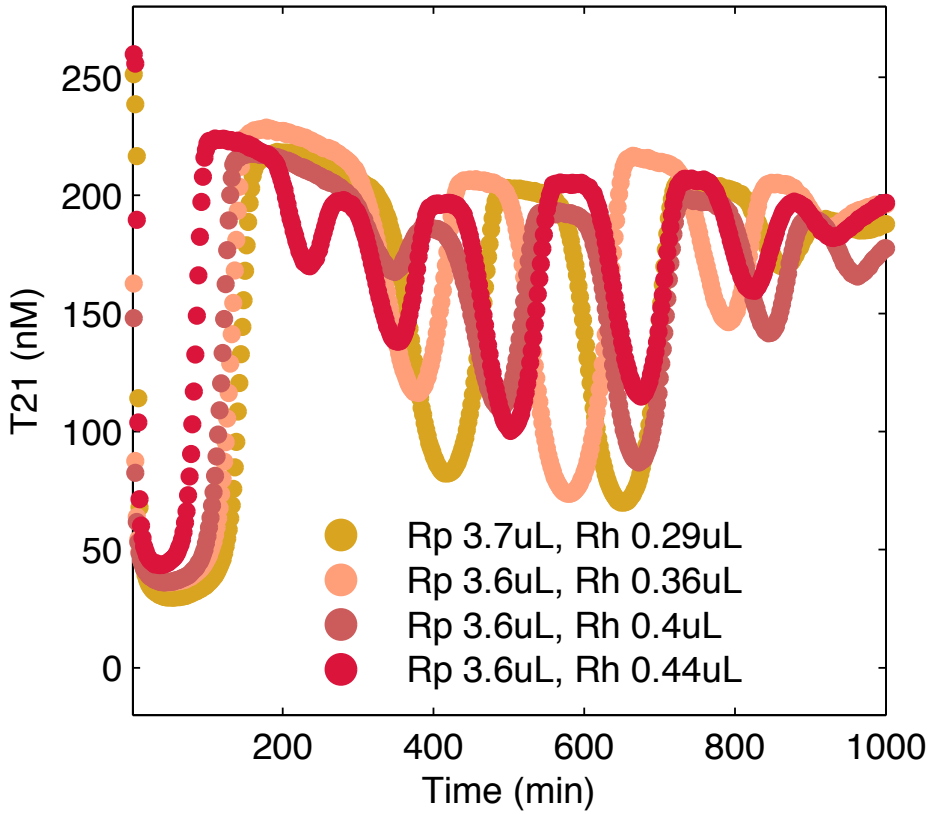




Tuning: Varying the enzyme concentrations

NOTE: enzyme amounts need to be optimized depending on the vendor and on the batch!



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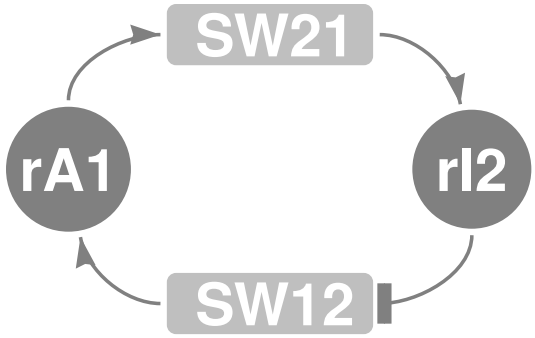
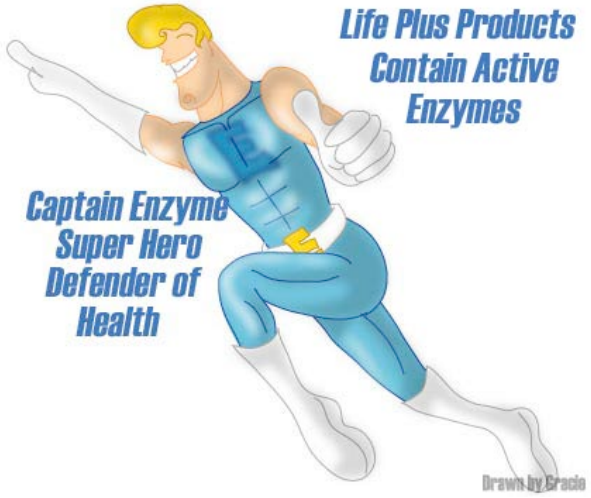


Challenges

REPRODUCIBILITY of oscillations is affected by:

- DNA pipetting accuracy (small volumes)
- Enzymes pipetting accuracy (glycerol)
- Enzymes activity

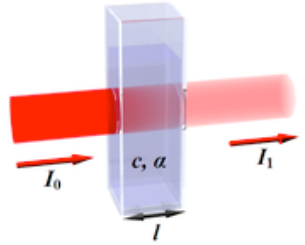


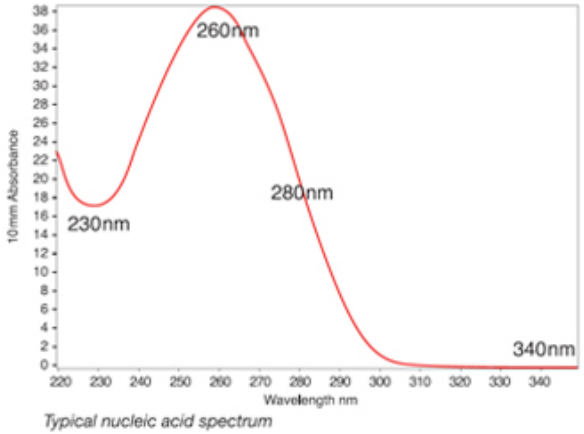
Timeline/experiments for the day

1. QUANTITATION OF OLIGOS

Absorbance measurement at 260 nm

$$A_\lambda = -\log_{10}(I_1/I_0)$$

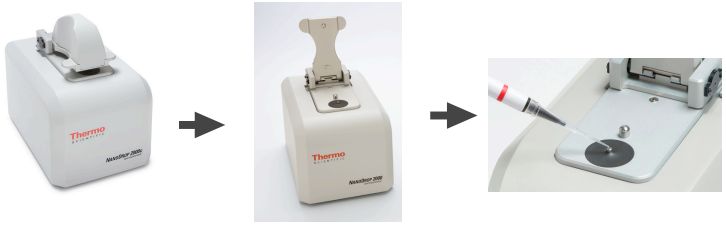


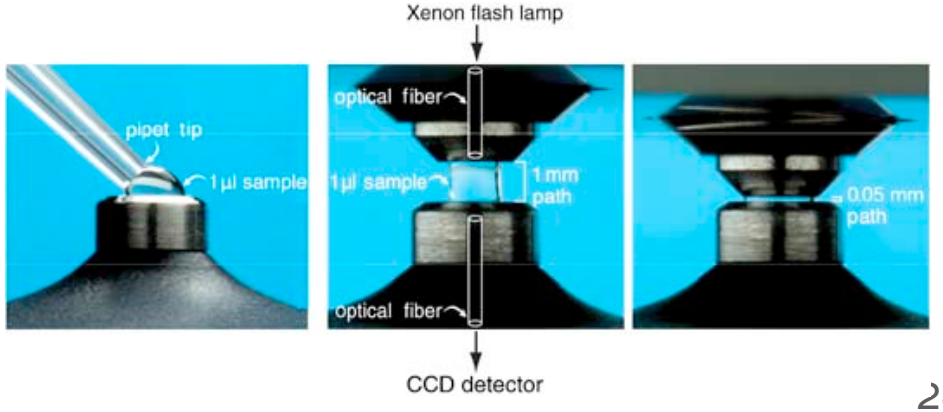


Typical nucleic acid spectrum

Biophotometer

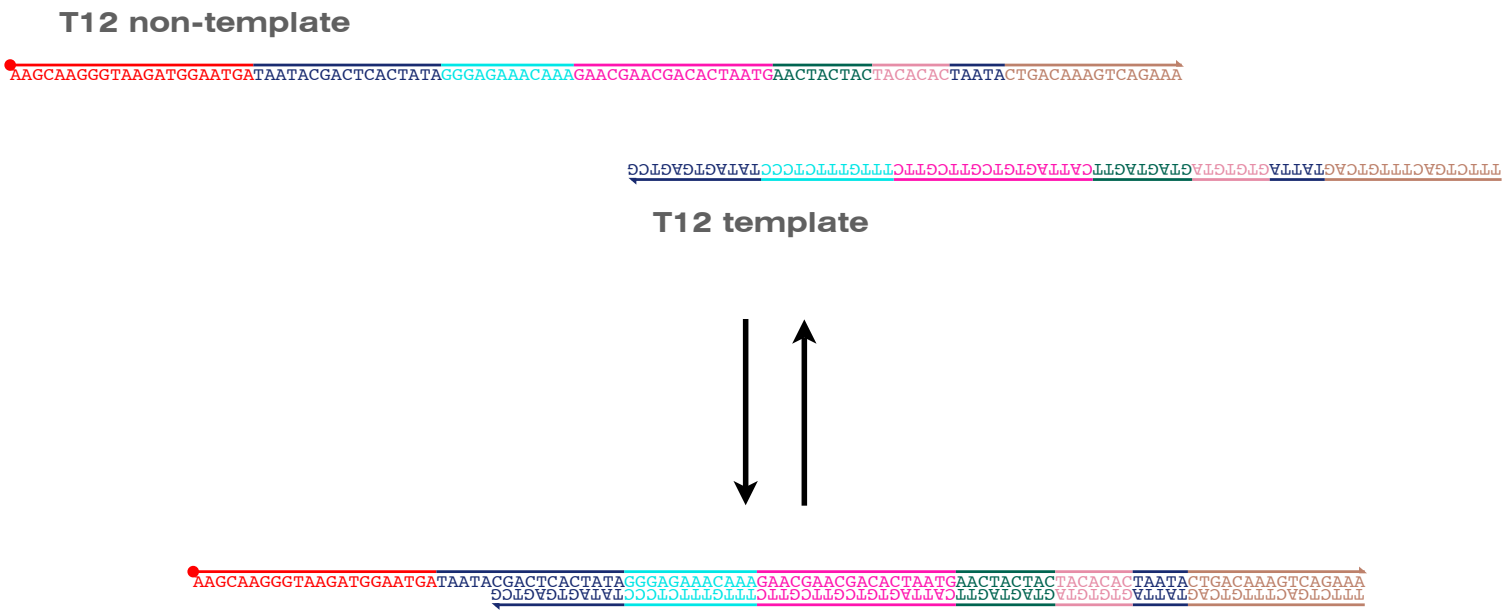
Nanodrop





Timeline/experiments for the day

2. ANNEALING GENELETS



THERMAL CYCLES:

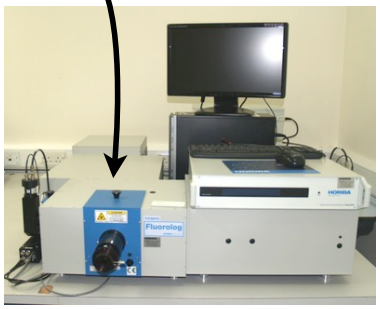
Initial denaturation at 90˚C
Then decrease to 20˚C with steps of ~ 1˚C up/down

REVERSIBLE PROCESS - to achieve the minimum folding energy!

Timeline/experiments for the day

3. OSCILLATOR EXPERIMENT

- mix DNA, buffer
- cover with oil (hexadecane)



- equilibrate at 37C in fluorometer chamber
- add enzymes

... measure fluorescence for ~ 15 hours