

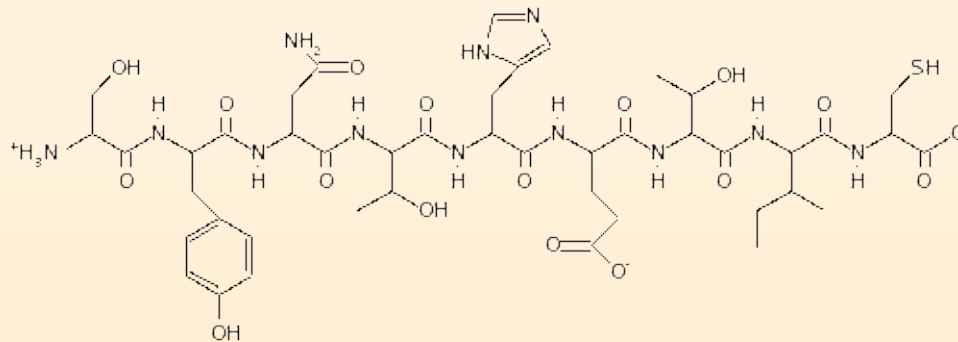
Assembly and Characterization of Synthetic Biological Systems

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BE442

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Synthetic Biology Working Group:

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

Making life better...one part at a time.

Requirements for building synthetic biological systems

Complexity necessitates modularity

- Reusable Biological Components
 - ▷ *Reuse existing, natural biological parts*
 - ▷ *Design new synthetic parts*
- Design
- Assembly
- Characterization

Parts Registry

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



BioBricks Data Book Parts Index

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MIT Registry Of Standard Biological Parts

- [Parts Index](#)
- [Assembly](#)
- [Part Lists](#)
- [Access Control](#)
- [Part Operations](#)
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BioBrick Devices are made from one or more BioBrick Parts. The available parts are defined under the BB Part Number links below. The first letter of the part number specifies the category: B-basic part such as a plasmid, C-protein coding sequence, E-reporter protein coding regions, R-regulatory region. Protein Coding Regions code for DNA binding proteins, Reporters, Sensors, Transporters, Structural, and other kinds of proteins.

BioBrick	Type	Description	Version	Status	Sequence
BBa_B0010	Terminator	T1 Transcriptional terminator	1	Planning	OK
BBa_B0011	Terminator	Terminator, Transcription (luxICDABEG bidirectional)	12	Available	OK
BBa_B0012		Terminator, Transcription (T7 TE)	10	Available	OK
BBa_B0013		Terminator, Transcription (T7 TE bidirectional)	9	Available	OK
BBa_B0014	Terminator		6	Available	OK
BBa_B0015	Terminator		1	Available	OK
BBa_B0020	Terminator	Reverse T1 Transcriptional Terminator	2	Planning	None
BBa_B0021	RBS	Reverse Terminator, from LuxICDABEG bidirectional	6	Planning	OK
BBa_B0022	Terminator	Reverse T7 TE Transcriptional Terminator	1	Planning	None
BBa_B0023	Terminator	Reverse T7 Bidirectional Transcriptional Terminator		Planning	None
BBa_B0030	RBS	RBS.1 (strong)	8	Available	OK
BBa_B0031		RBS.2 (medium)	6	Available	OK
BBa_B0032		RBS.3 (weak)	6	Available	OK
BBa_B0033		RBS.4 (weaker -- test)	6	Available	OK
BBa_B0034		RBS.5 (Elowitz RBS)	4	Available	OK
BBa_B0040		Spacer.1 (generic)	8	Available	OK
BBa_C0012		Repressor, LacI (RBS- LVA+)	11	Available	OK
BBa_C0040		Repressor, TetR (RBS- LVA+)	6	Available	OK
BBa_C0050		Repressor, HK022 cI (RBS- LVA+)	7	Available	OK
BBa_C0051		Repressor, Lambda cI (RBS- LVA+)	6	Available	OK
BBa_C0052		Repressor, 434 cI (RBS- LVA+)	6	Available	OK
BBa_C0053		Repressor, P22 c2 (RBS- LVA+)	6	Available	OK
BBa_C0060		Enzyme, aiiA (RBS- LVA+)	7	Available	OK
BBa_C0061		Enzyme, LuxI (RBS- LVA+)	6	Available	OK
BBa_C0062		Repressor/Activator, LuxR (RBS-)	6	Available	OK
BBa_D0001	not		1	Available	None
BBa_E0022	reporter	Reporter, CFP (RBS- LVA+)	14	Available	OK
BBa_E0032	reporter	Reporter, YFP (RBS- LVA+)	14	Available	OK
BBa_U010		cI(1) fused to tetP promoter	5	Available	OK

BioBricks++: Standardizing module assembly

Standard form for modules:

```
          AarI   BbvCI                               BbvCI
GCTGAGGGGCGCGCCACCTGCCCTCAGCT<--Part-->AGCTGAGTCTTCGGCCGGCCCCTCAGCGGTAGATAGATAGCCGCTGAGG
CGACTCCCCGCGGGGTGGACGGGAGTCGA<--Part-->TCGACTCAGAAGCCGGCCGGGAGTCGCCATCTATCTATCGGCGACTCC
BbvCI                                     BbsI                               BbvCI
```

Various operations are possible using standard techniques

Assembling two parts produces a new parts

Prefix part cut with BbsI and N.BbvCIA:

```

      AarI   BbvCI                               BbvCI
GCTGAGGGGCGCGCCACCTGCCCTCAGCT<--Part1-->AGCTGAGTCTTCGGCCGGCCCTCAGCGGTAGATAGATAGCCGCTGAGG
CGACTCCCCGCGCGGGTGGACGGGAGTCGA<--Part1-->TCGACTCAGAAGCCGGCCGGGAGTCGCCATCTATCTATCGGCGACTCC
BbvCI                                     ||                               BbsI                               BbvCI
                                     ||
                                     ||
                                     \
TGAGGGGCGCGCCACCTGCCCTCAGCT<--Part1-->
      CGA<--Part1-->TCGA
```

Suffix part cut with AarI and N.BbvCIB:

```

      AarI   BbvCI                               BbvCI
GCTGAGGGGCGCGCCACCTGCCCTCAGCT<--Part2-->AGCTGAGTCTTCGGCCGGCCCTCAGCGGTAGATAGATAGCCGCTGAGG
CGACTCCCCGCGCGGGTGGACGGGAGTCGA<--Part2-->TCGACTCAGAAGCCGGCCGGGAGTCGCCATCTATCTATCGGCGACTCC
BbvCI                                     ||                               BbsI                               BbvCI
                                     ||
                                     ||
                                     \
      AGCT<--Part2-->AGCTGAGTCTTCGGCCGGCCCTC
      <--Part2-->TCGACTCAGAAGCCGGCCGGGAGTCGCCATCTATCTATCGGCGACTCC
```

Assembled and ligated into a new plasmid:

```

      AarI   BbvCI                               BbvCI
GCTGAGGGGCGCGCCACCTGCCCTCAGCT<--Part1-->AGCT<--Part2-->AGCTGAGTCTTCGGCCGGCCCTCAGCGGTAGATAGATAGCCGCTGAGG
CGACTCCCCGCGCGGGTGGACGGGAGTCGA<--Part1-->TCGA<--Part2-->TCGACTCAGAAGCCGGCCGGGAGTCGCCATCTATCTATCGGCGACTCC
BbvCI                                     BbsI                               BbvCI
```

Reversing a part is easy

Part cut with BbsI and BbvCI:

```
          AarI   BbvCI                               BbvCI
GCTGAGGGGCGCGCCACCTGCCCTCAGCT<--Part-->AGCTGAGTCTTCGGCCGGCCCCTCAGCGGTAGATAGATAGCCGCTGAGG
CGACTCCCCGCGCGGGTGGACGGGAGTCGA<--Part-->TCGACTCAGAAGCCGGCCGGGGAGTCGCCATCTATCTATCGGCGACTCC
BbvCI                                     ||           BbsI                               BbvCI
                                     ||
                                     ||
                                     \
TCAGCT<--Part-->
CGA<--Part-->TCGA
```

Reversing Plasmid:

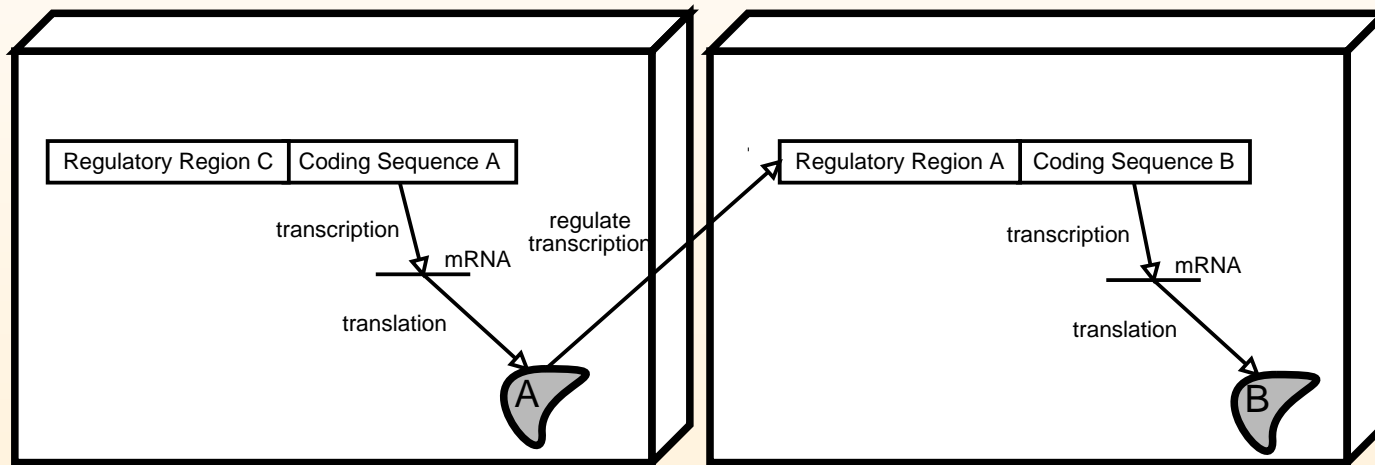
```
GCTGAGGGGCGCGCCACCTGCCCTC          TGAGTCTTCGGCCGGCCCCTCAGCGGTAGATAGATAGCCGCTGAGG
CGACTCCCCGCGCGGGTGGACGGGAGTCGA    CAGAAGCCGGCCGGGGAGTCGCCATCTATCTATCGGCGACTCC
```

Part ligated into reversing plasmid:

```
          AarI   BbvCI                               BbvCI
GCTGAGGGGCGCGCCACCTGCCCTCAGCT<--traP-->AGCTGAGTCTTCGGCCGGCCCCTCAGCGGTAGATAGATAGCCGCTGAGG
CGACTCCCCGCGCGGGTGGACGGGAGTCGA<--traP-->TCGACTCAGAAGCCGGCCGGGGAGTCGCCATCTATCTATCGGCGACTCC
BbvCI                                     ||           BbsI                               BbvCI
```

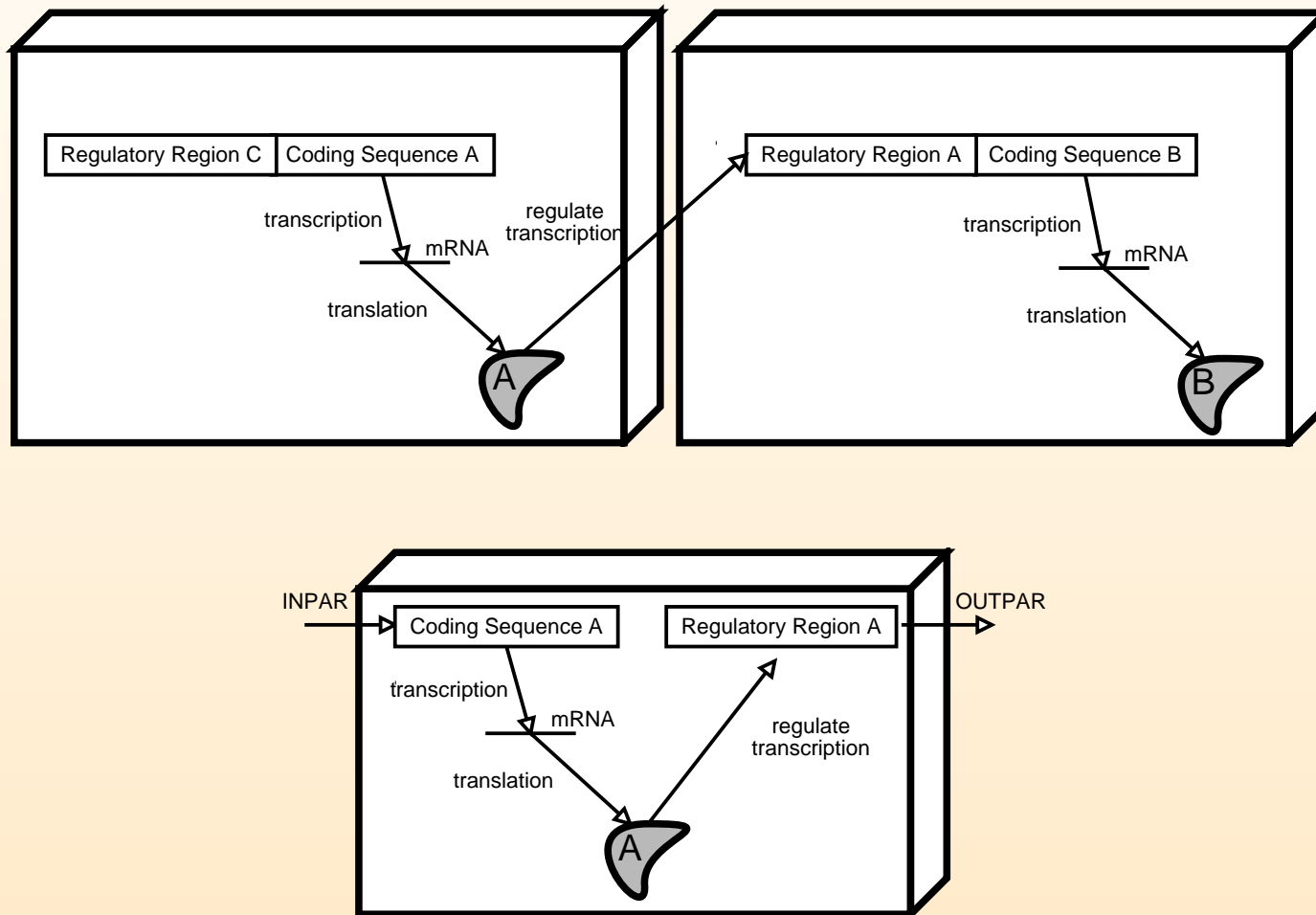
Modules are no use if left uncharacterized

Conventional modules cannot be characterized in a general way



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Conventional modules cannot be characterized in a general way



Polymerase Arrival Rates (PAR) as common measurement unit



PAR depends on many factors

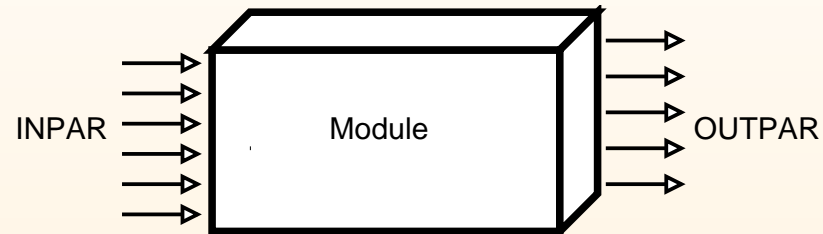
Advantages

- Polymerase Arrival Rate (PAR) as modular and universal input/output
- Everything is transcribed at some point
- PAR is a physical feature of the system
- PAR is independent of the method for measurement
- PAR is a quantitative measure
- Modeling and engineering become straightforward

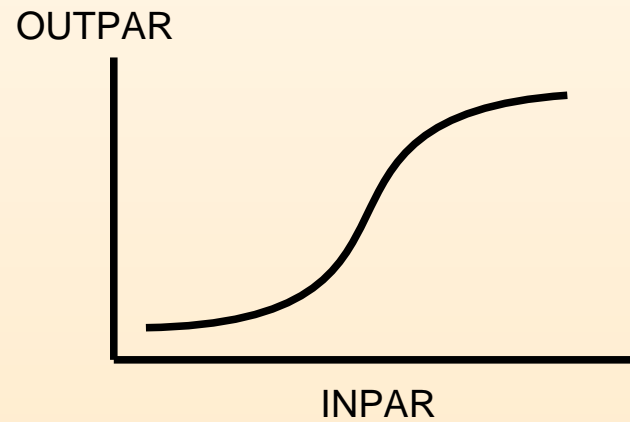
Disadvantages

- Difficult to measure
- Little data available
- No information about non-transcriptional events (protein-protein interactions)

General form for *modules*



Modules can be specified completely by transfer curves



PARMESAN: Proposed method to measure transcription rate

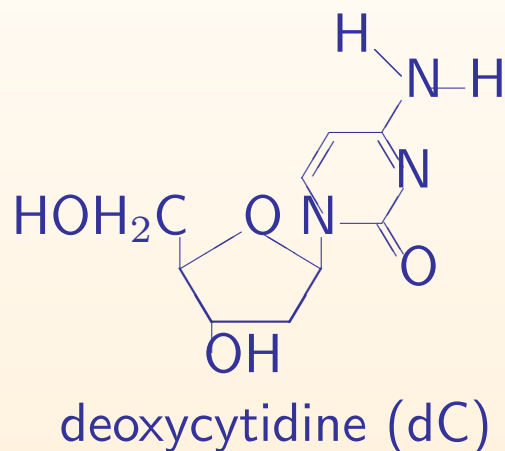
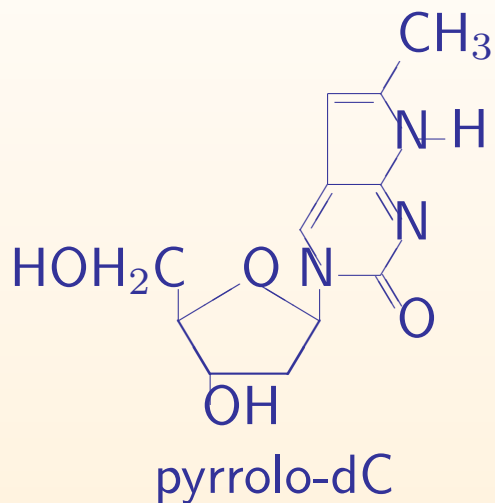
in vitro assay for Polymerase Arrival Rates

Hooks into the transcription process via a fluorescent probe

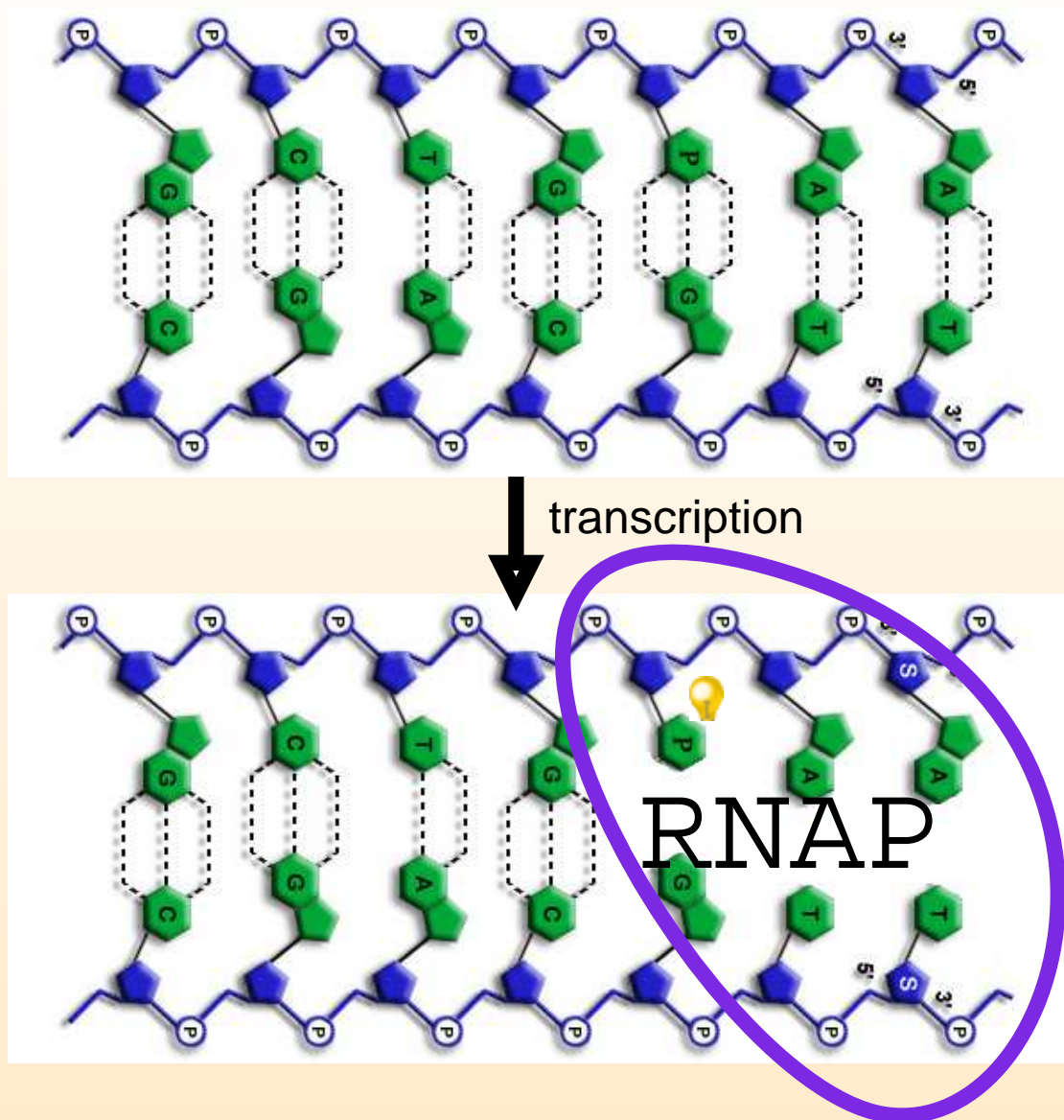
- Fluorescence is measured during transcription
- Fluorescence is directly related to the rate of polymerase arrivals



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



Pyrrolo-dC increases fluorescence during strand separation



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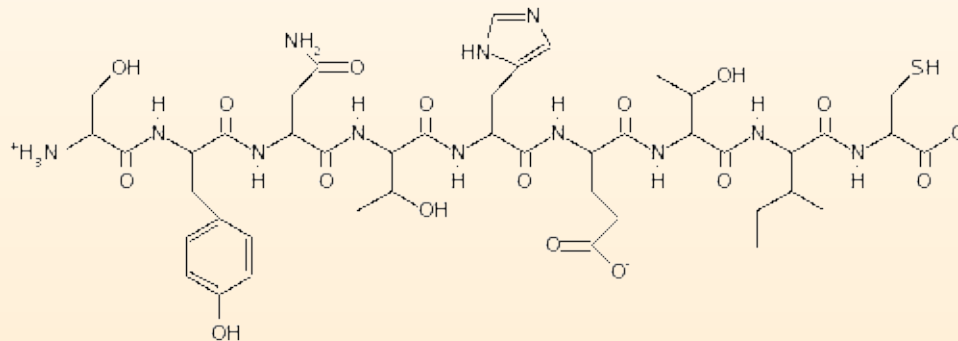
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- Design
 - ⇒ Assembly
 - ⇒ Characterization

Design something yourself!

Synthetic Biology IAP 2003: Engineered Genetic Blinkers

Synthetic Biology IAP 2004: Engineered Genetic **Polka Dots**

Apply by 12/5/03. More information at <http://polkadots.mit.edu/>



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