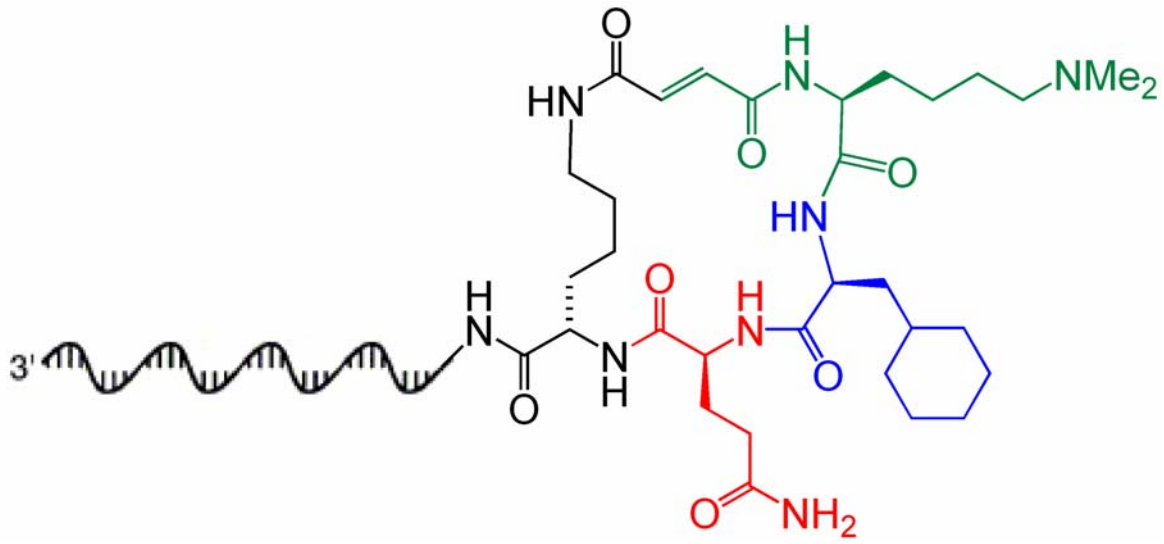
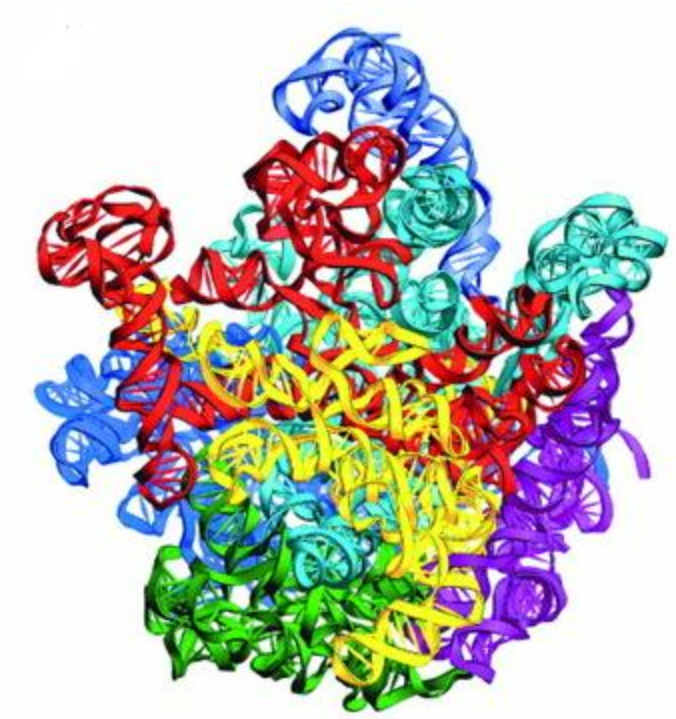
# *DNA-Templated Organic Synthesis*



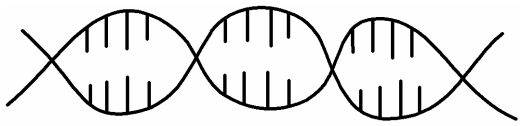
*Matthew Coulter*
*Organic-Biological Seminar*
*Department of Chemistry*
*University of Toronto*
*February 4, 2008*

# *DNA-Templated Organic Synthesis*

*David R. Liu*
*Professor of Chemistry and Chemical Biology*
*Harvard University*

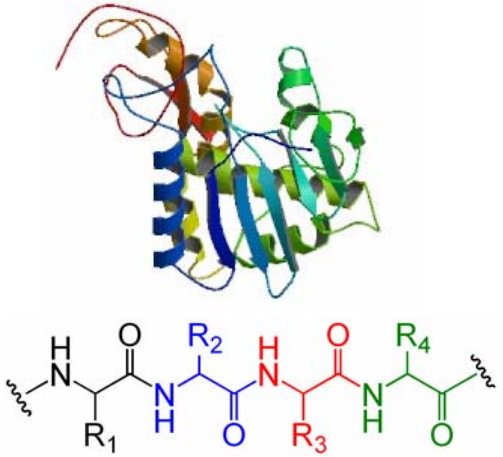
- *Small molecule library synthesis*
- *DNA assisted reaction discovery*

# DNA-Templated Synthesis



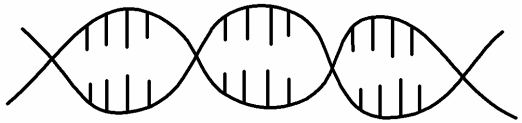
*Transcription*

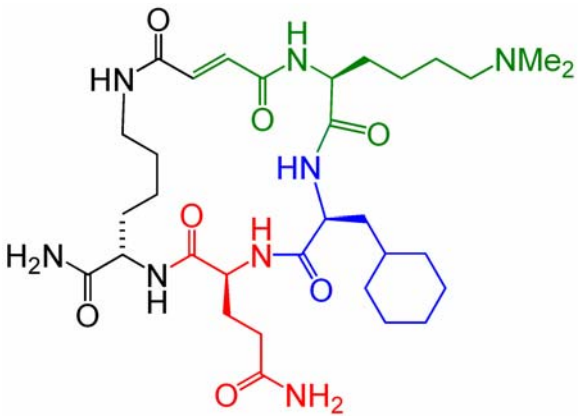
*Translation*




DNA

Protein






DNA

*Synthetic compound*

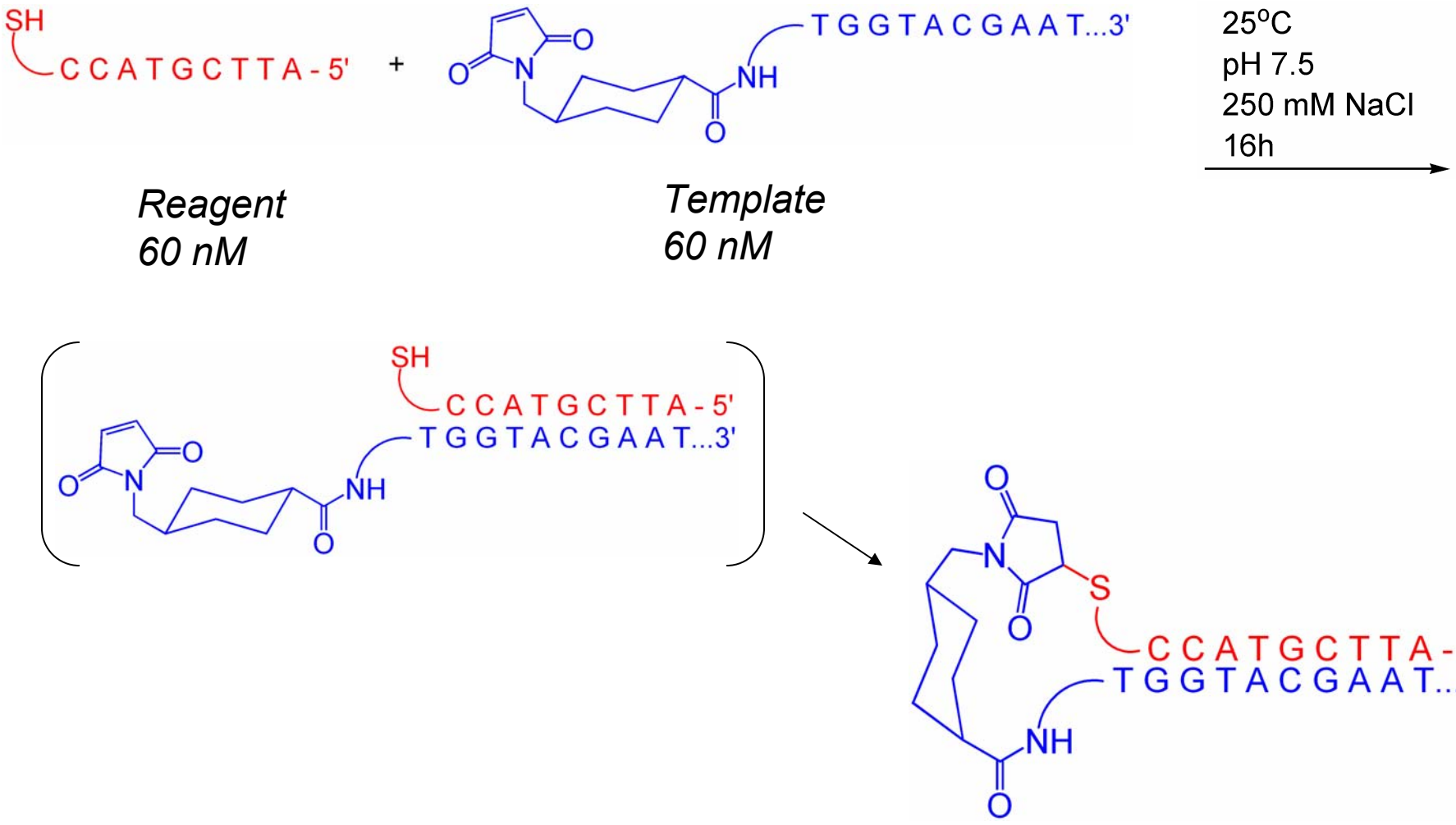
# DNA-Templated Organic Synthesis

| Conventional Screening Approach | DNA-Templated-Approach |
| --- | --- |
| • spatial separation generally required | • All molecules synthesized in one pot |
| • each compound analyzed individually | • All molecules analyzed simultaneously |
| • adequate material required for screen | • fmol - nmol scale |
| • workload increases with sample size<br>  • Synthetic compound library size $10^9 – 10^{13}$<br>  • *Modern high-throughput screening facility*<br>  • *9 – 9000 years* | • workload does not scale with sample size |
| • Tools: MS, NMR, HPLC etc. | • Tools: molecular biology |

Rozenman, M.M.; McNaughton, B.R., Liu, D.R. *Curr. Opin. Chem. Biol.* **2000**, *11*, 259-268.

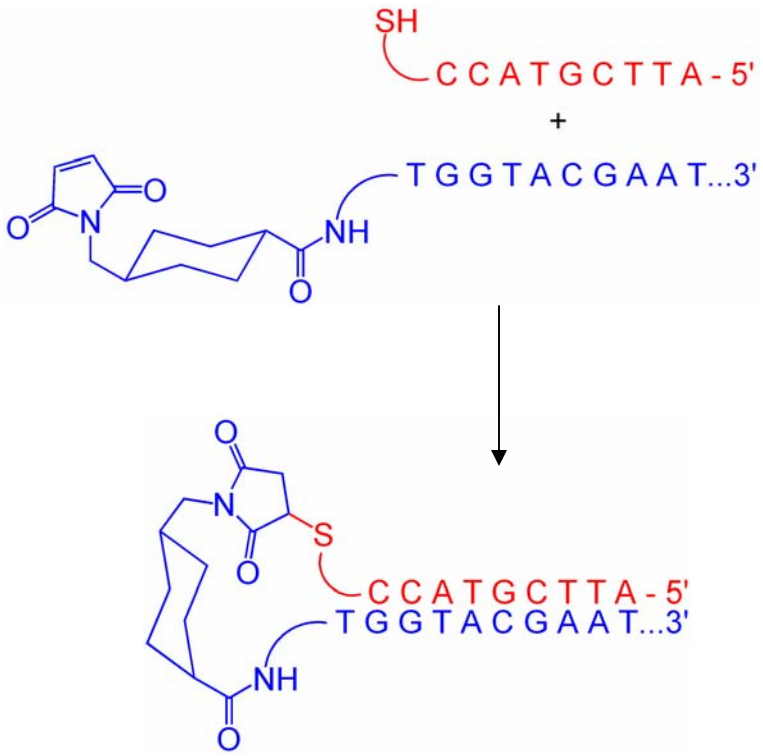
# Can DNA Encode Chemical Reactions?

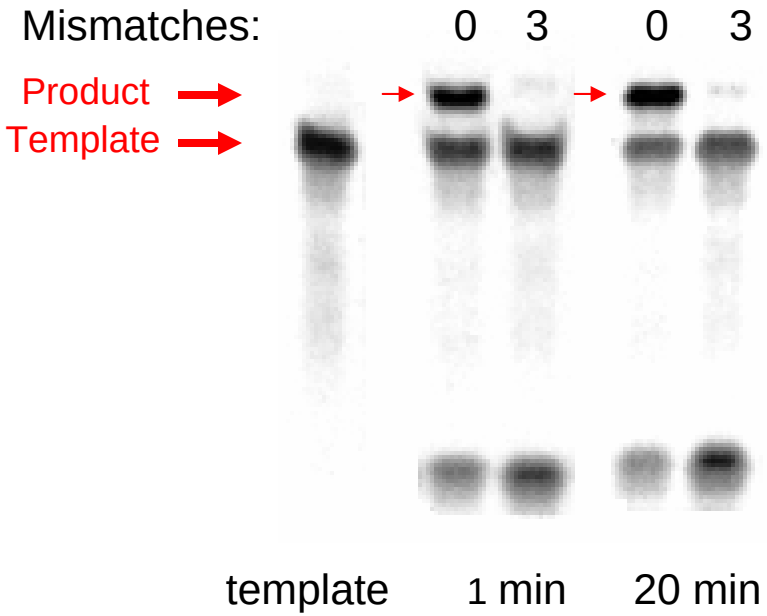
*Strategy: Link nucleophile and electrophile to separate strands of complementary DNA*




Gartner, Z. J.; Liu , D.R. *J. Am Chem Soc.* **2001**, *123*, 6961- 663.

# A Sequence Specific Synthetic Reaction



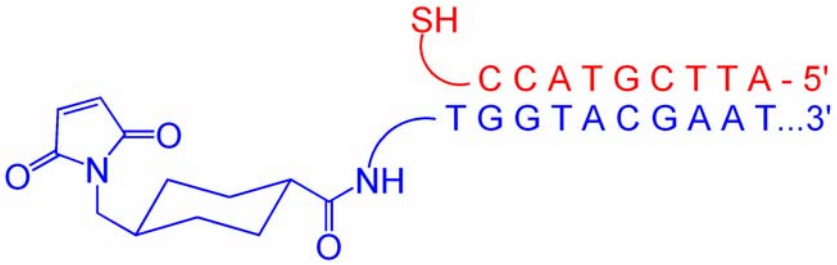




- *Bond formation occurs sequence specifically*
- *Very low background (non-templated) reactivity due to very low reagent concentrations*
- *DNA encoded reaction*

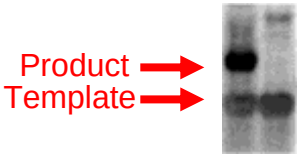
Gartner, Z. J.; Liu , D.R. *J. Am Chem Soc.* **2001**, *123*, 6961- 663.

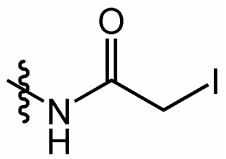
# Multiple Reaction Types Supported

*All substrates tested reacted sequence-specifically:*

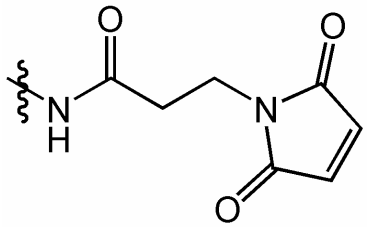


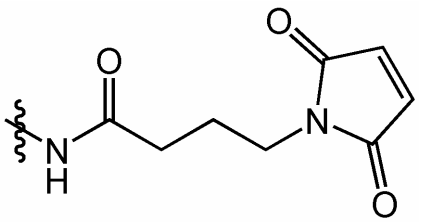

M = matched; X = mismatched

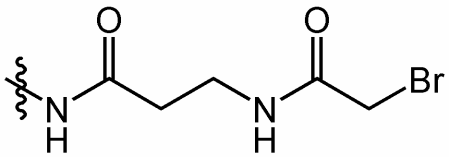


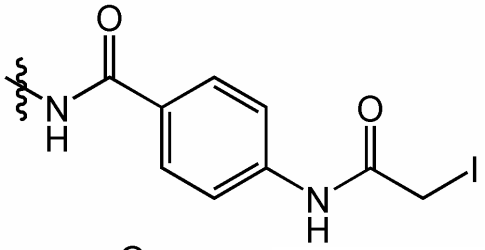



*1:1 template: reagent*
*pH 7.5, 25°C, 16h*

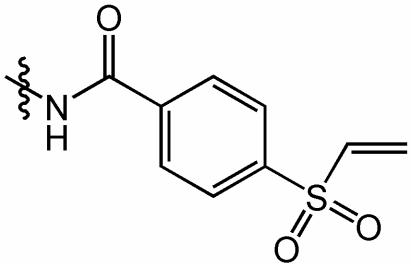




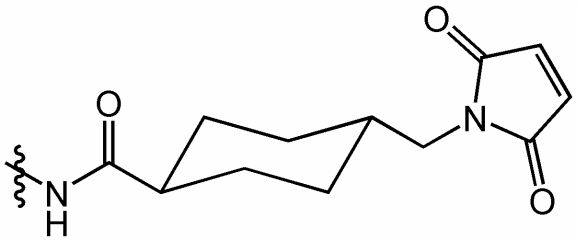






*1:1 template: reagent*
*pH 7.5, 25°C, 10 min*

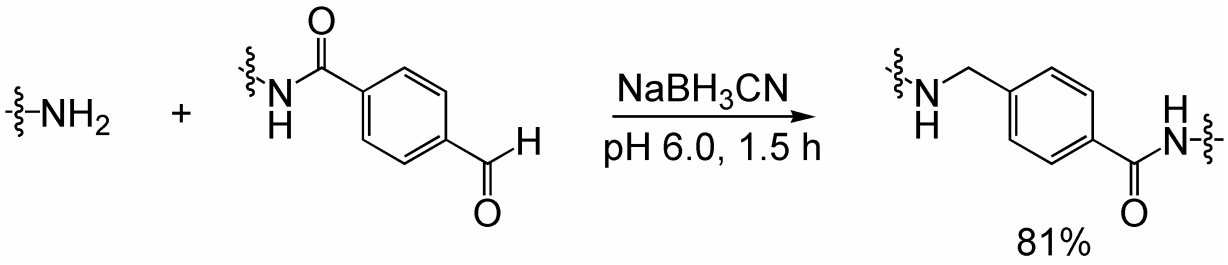


Gartner, Z. J.; Liu , D.R. *J. Am. Chem. Soc.* **2001**, *123*, 6961- 663.

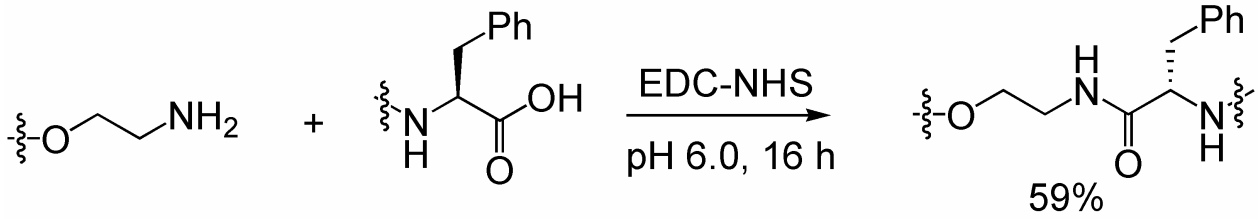
# Scope of DNA-Templated Reactivity

*Many standard reactions are encoded sequence specifically at nM concentrations of reactants:*

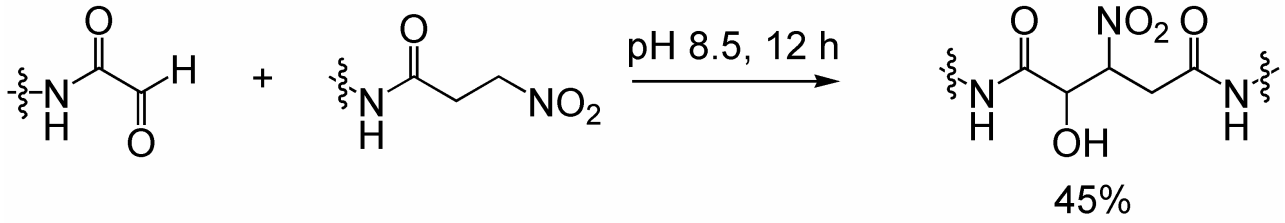

*Reductive amination:*




*Amide bond formation:*



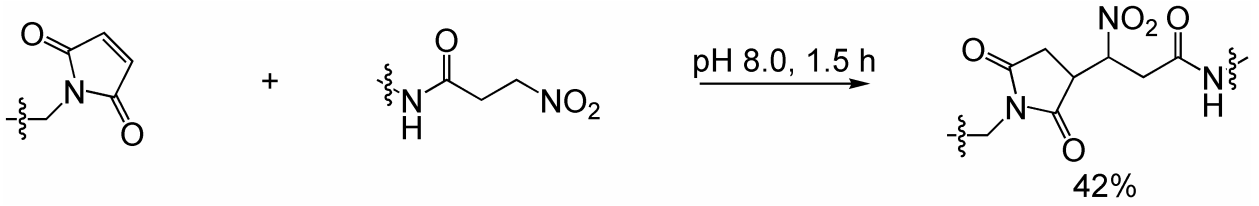

*Henry reaction:*



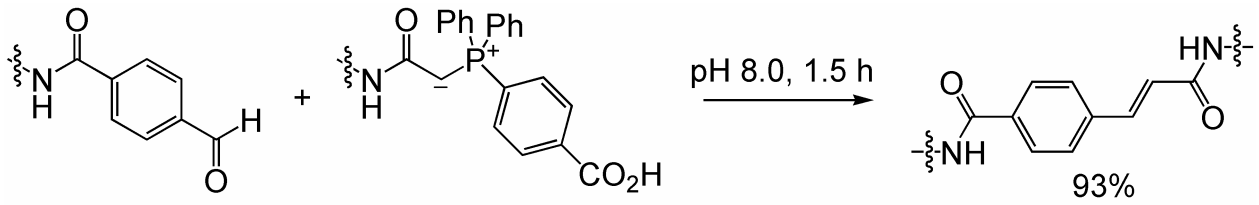

Gartner, Z. J.; Kannan, M.W.; Liu , D.R. *Angew. Chem. Int. Ed.* **2002**, *41*, 1796-1800.

# Scope of DNA-Templated Reactivity

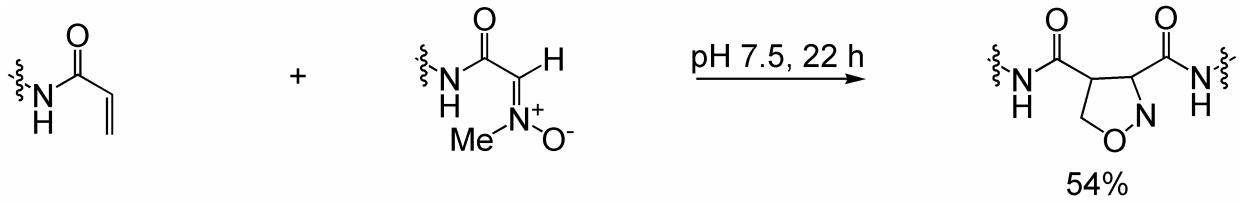
*Nitro-Michael:*



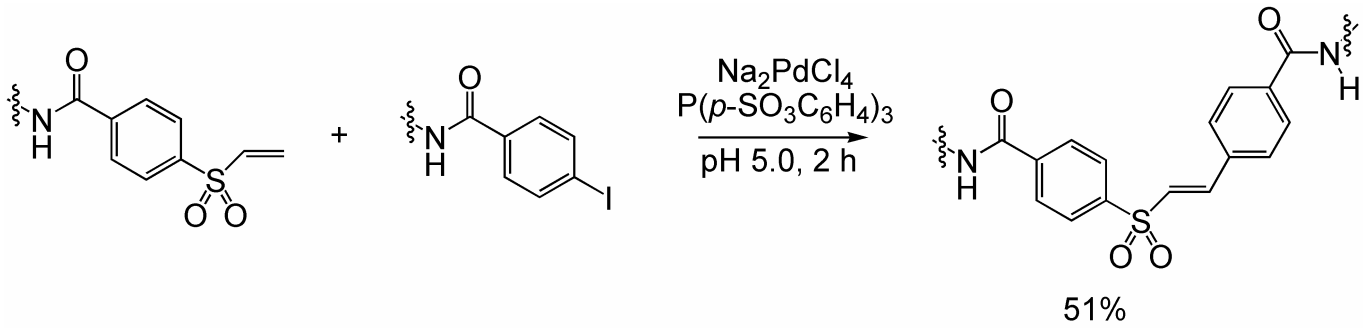

*Wittig Reaction:*




*1,3 dipolar cycloaddition:*



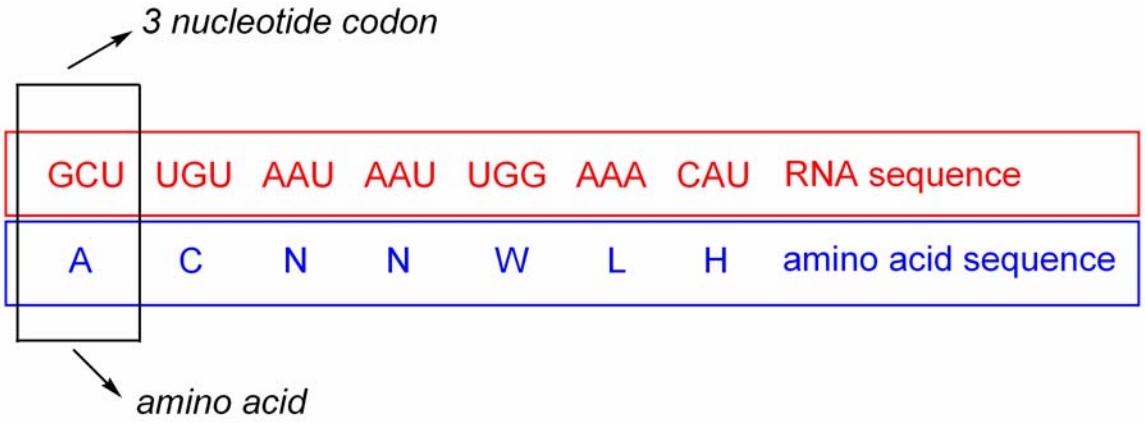

*Heck Reaction:*

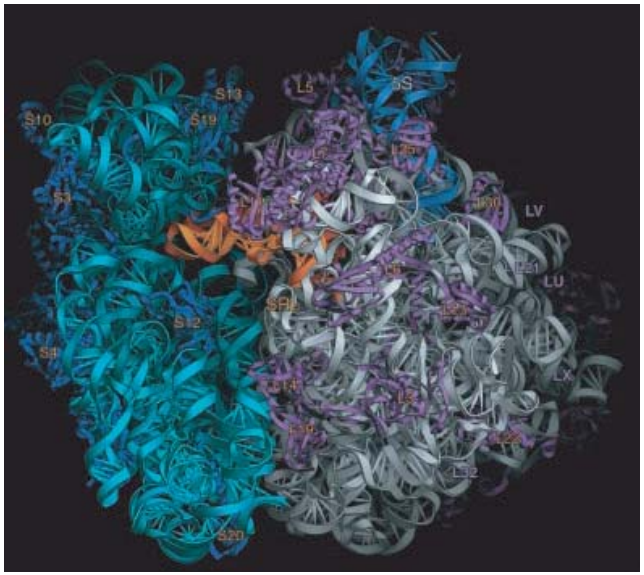



Gartner, Z. J.; Kannan, M.W.; Liu , D.R. *Angew. Chem. Int. Ed.* **2002**, *41*, 1796-1800.

# A Synthetic Code

- *A number of reactions are encoded sequence specifically*
- *Reactivity is controlled by hybridization of complimentary DNA*
- *Sequence specificity: supports the faithful translation of a particular nucleic acid sequence to a given amino acid sequence*
- *Ribosomes decipher the genetic code during protein synthesis*
- *Input – output in templated synthesis*



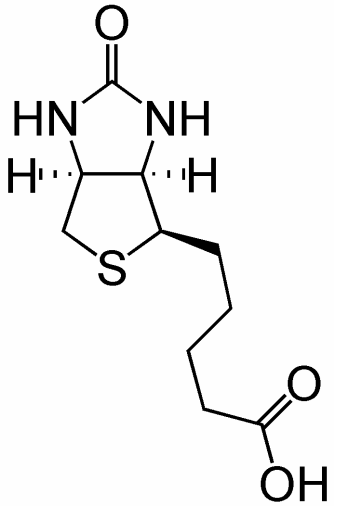



Yusupov et al. *Science*. **2001**, *292*, 883-896.

# Applications to Small Molecule Library Synthesis

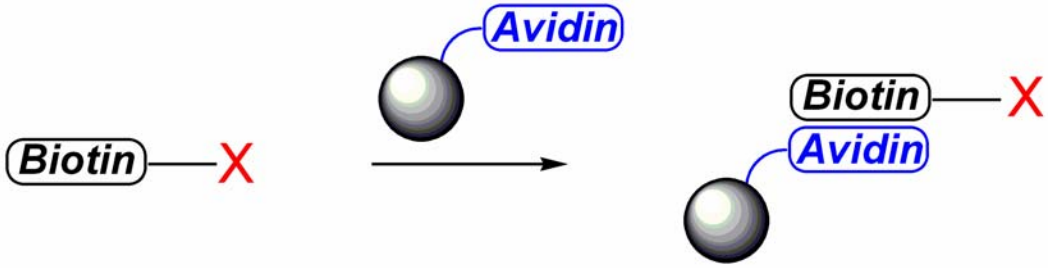
- Synthesize and evaluate a programmed small molecule library
  - *Multi-step DNA-templated synthesis*
  - *Select a single molecule based on a particular function*
  - *Identify member based on its associated DNA sequence*

# Affinity Purification



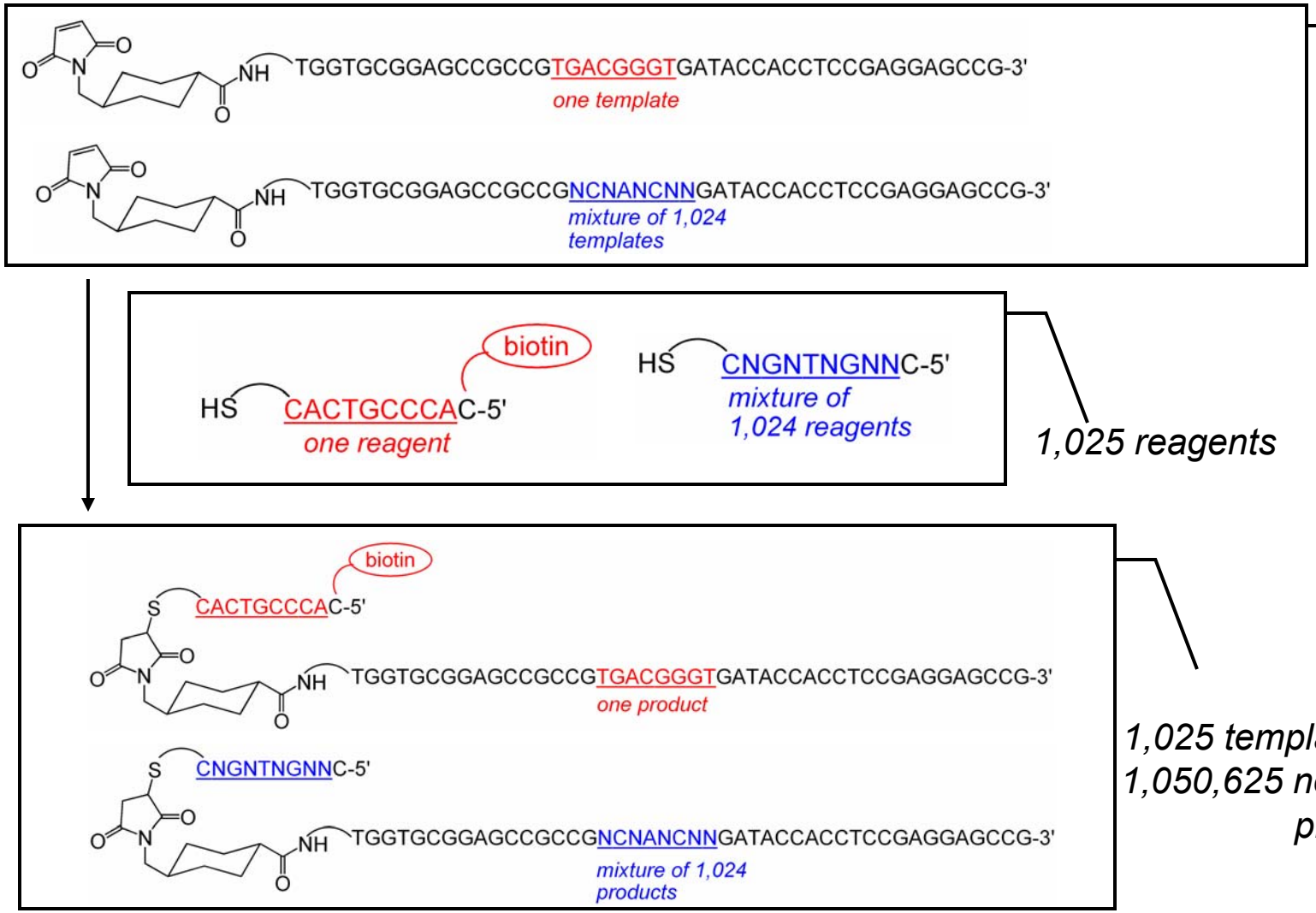

*Biotin*

- Biotin binds the proteins avidin and streptavidin very tightly ($K_d \sim 10^{-15}$)
- Avidin-biotin interaction used in affinity purification



# Selection and Identification of a Single Target

*Proof of Principle: Translation, Selection and Amplification of a Synthetic Library*




Gartner, Z. J.; Liu , D.R. *J. Am. Chem. Soc.* **2001**, *123*, 6961- 6963.

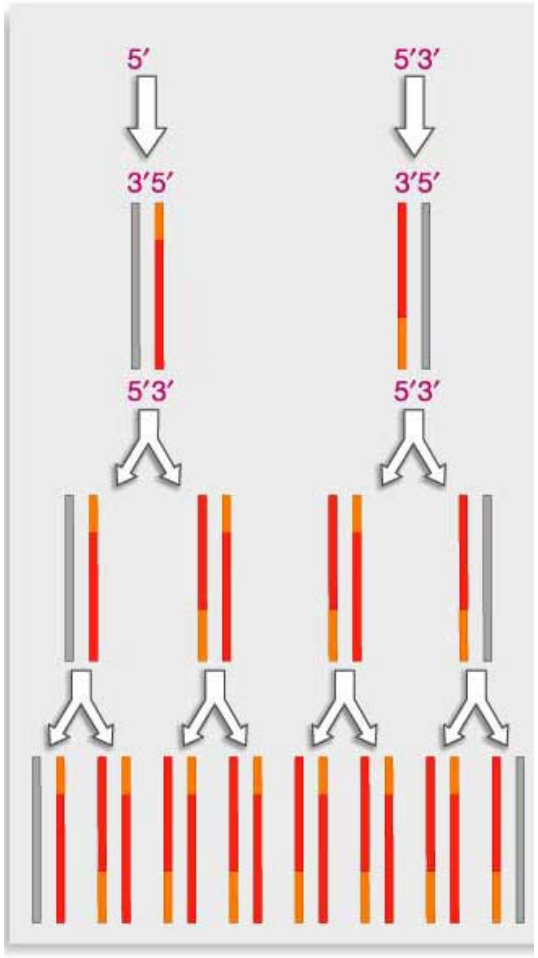
# Selection and Identification of a Single Target


*1,025 templated products of 1,050,625 non-templated products*

1) *In vitro selection - avidin beads*
2) *PCR amplification*

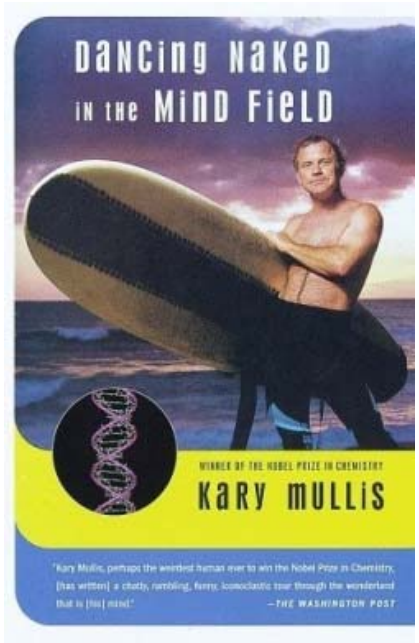
Gartner, Z. J.; Liu , D.R. *J. Am. Chem. Soc.* **2001**, *123*, 6961- 6963.

# PCR Amplification




*Polymerase Chain Reaction*

- Kary Mullis
- 1993 Nobel Prize in Chemistry
- A method of amplifying a sequence of DNA
- 20 cycles: 1 x $10^6$ amplification of target sequence




*Exponential amplification of target sequence*

Queens University. *DNA Tech*. (n.d.) Retreived January 31, 2008 from http://campus.queens.edu/faculty/jannr/Genetics/images/dnatech/

# Biological Specificity: Selective Enrichment


*1,025 templated products of 1,050,625 non-templated products*

1) In vitro selection (avidin)
2) PCR amplification

5'-TGGTGCGGAGCCGCCG????????GATACCACCTCCGAGGAGCCG-3'

*Amplified DNA of selected molecules*

*Characterize:*
- Restriction digestion
- sequencing

*Tsp*45I

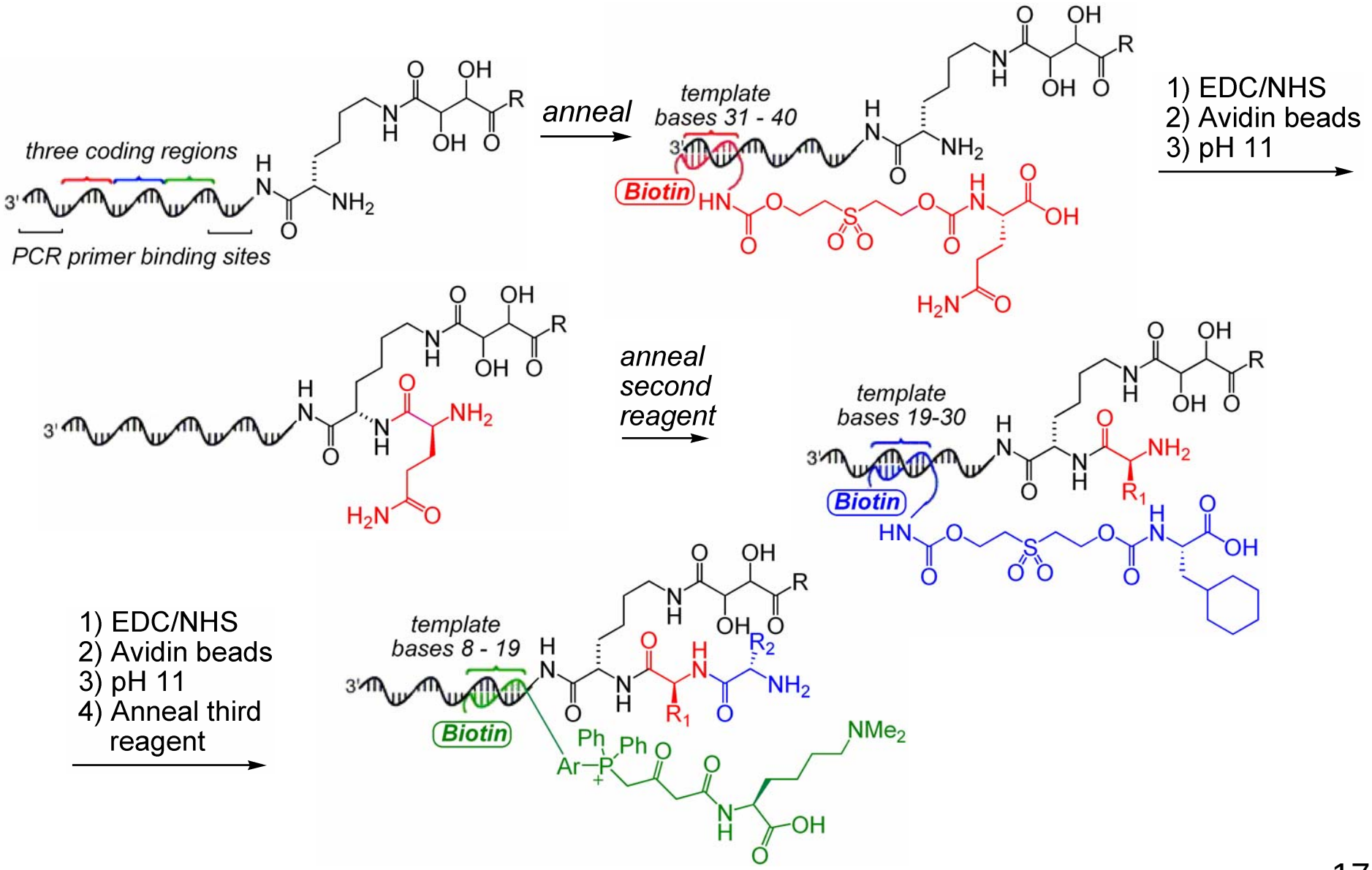
5'-TGGTGCGGAGCCGCCGTGACGGGTGATACCACCTCCGAGGAGCCG-3'

***Biotin linked product enriched 1000 fold after just one round of selection***



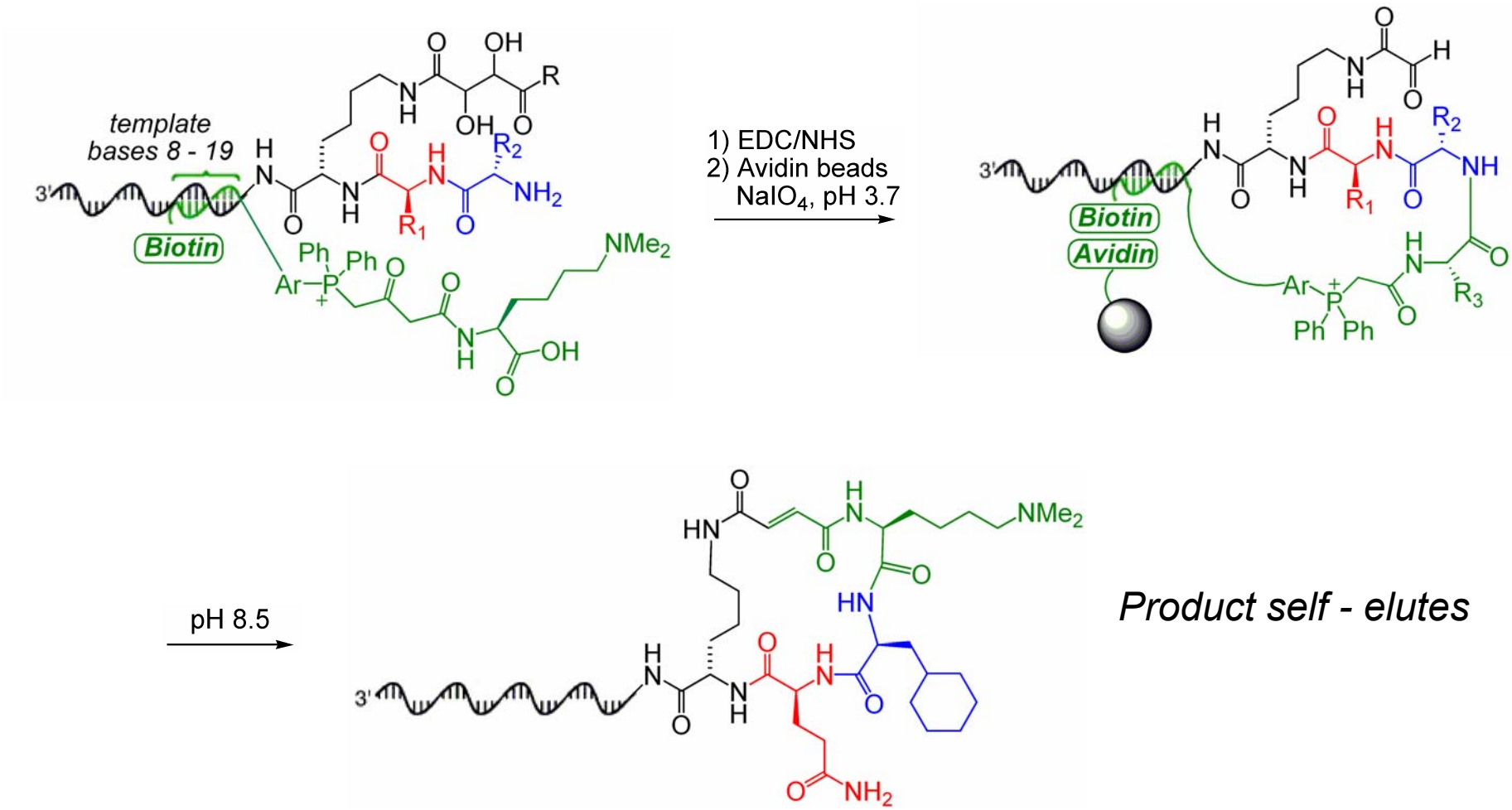

Gartner, Z. J.; Liu , D.R. *J. Am. Chem. Soc.* **2001**, *123*, 6961- 6963.

# Multistep DNA-templated synthesis




Gartner, Z. J.; Tse, B.N.; Grubina, R.; Doyon, J.B.; Snyder, T.M.; Liu , D.R. *Science.* **2004**, *305*, 1601- 1605.

# Multistep DNA-templated synthesis

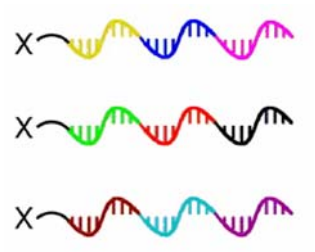



*Product self - elutes*

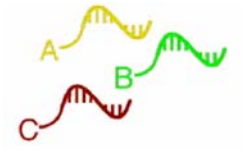
1 – 5%

Gartner, Z. J.; Tse, B.N.; Grubina, R.; Doyon, J.B.; Snyder, T.M.; Liu , D.R. *Science.* **2004**, *305*, 1601- 1605.

# A DNA-Templated Library of Synthetic Molecules




***Starting template library***

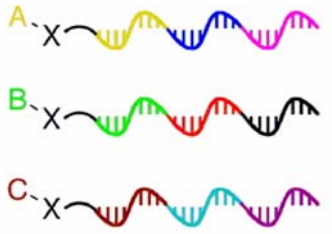



***Reagent library (1)***

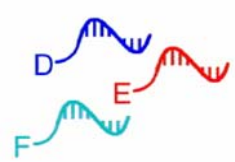
*Sequence specific annealing*


*DNA-templated synthesis*

***First Round***



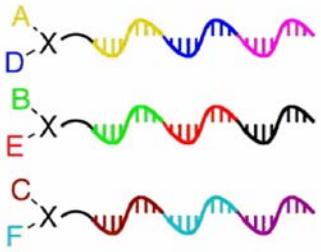

***Coded <u>first</u> round products***




***Reagent library (2)***

*Sequence specific annealing and synthesis*

***Second Round***




***Coded <u>second</u> round products***

***Reagent library (3)***

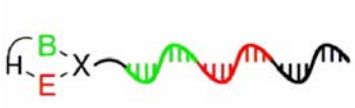
*Sequence specific annealing and synthesis*

***Third Round***


***Coded <u>third</u> round products***

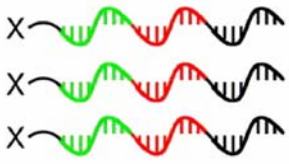
***Selection***

***ie. receptor binding***




***Functional, selected, encoded product***

***PCR amplification***



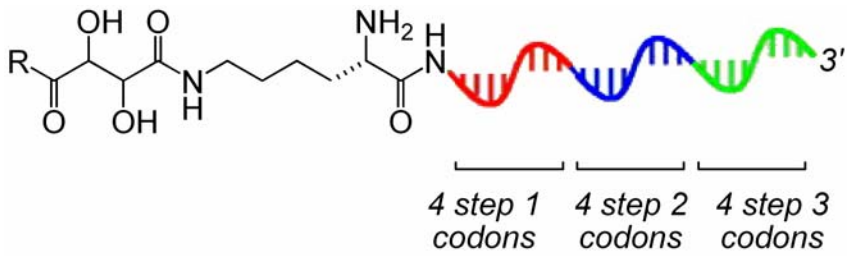

***DNA sequencing***


***Identify selected molecule***

Gartner, Z. J.; Tse, B.N.; Grubina, R.; Doyon, J.B.; Snyder, T.M.; Liu , D.R. *Science.* **2004**, *305*, 1601- 1605.

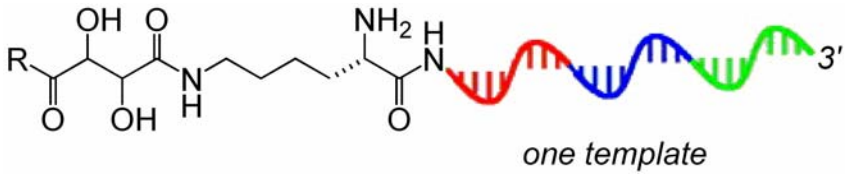
# Programmed Synthesis: A DNA Templated Library

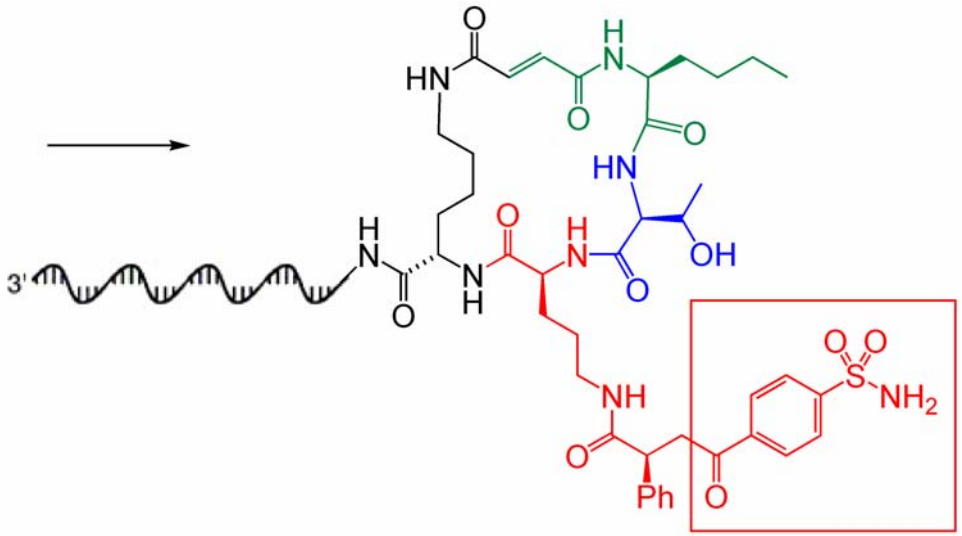
*65 DNA templates coding for 65 templated products were synthesized:*




*64 templates*

*64 templated macrocycles*

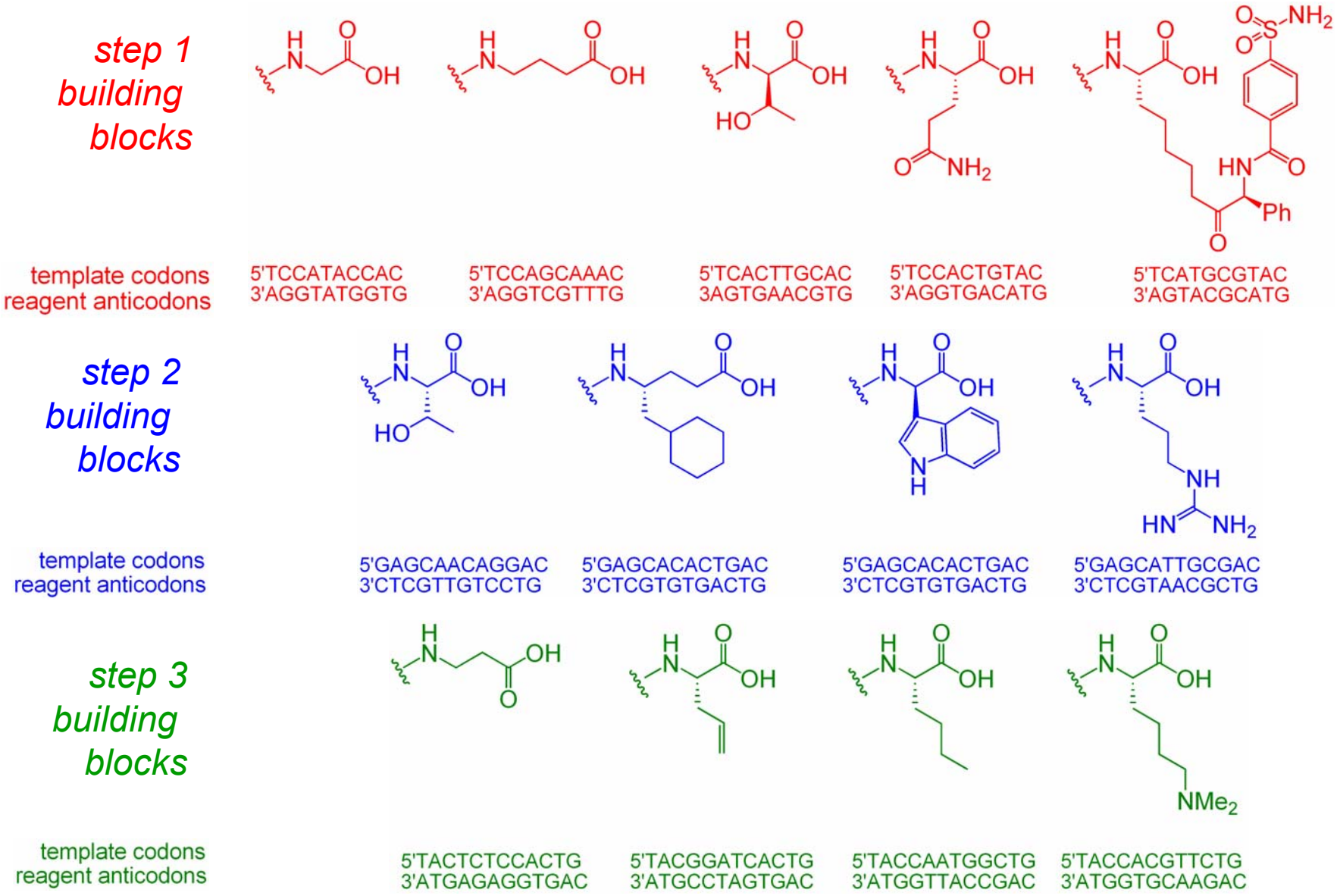



*1 positive control macrocycle*

Gartner, Z. J.; Tse, B.N.; Grubina, R.; Doyon, J.B.; Snyder, T.M.; Liu , D.R. *Science.* **2004**, *305*, 1601- 1605.

# A DNA-Templated Library

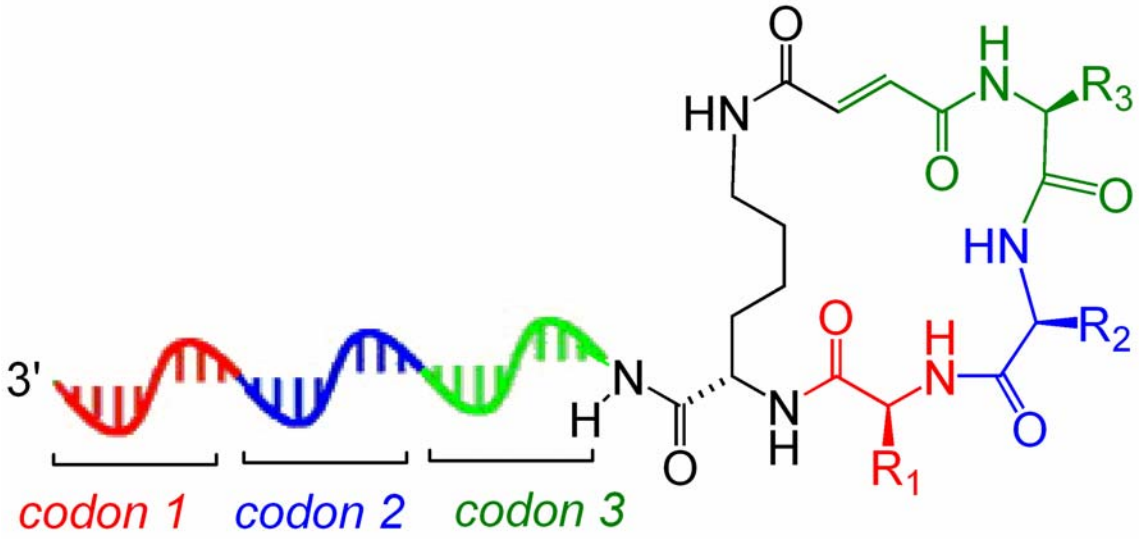



Gartner, Z. J.; Tse, B.N.; Grubina, R.; Doyon, J.B.; Snyder, T.M.; Liu , D.R. *Science.* **2004**, *305*, 1601- 1605.

# A DNA-Templated Library

- *65 macrocycles synthesized simultaneously in one pot*
  - *Presence of templated intermediates confirmed after each step*

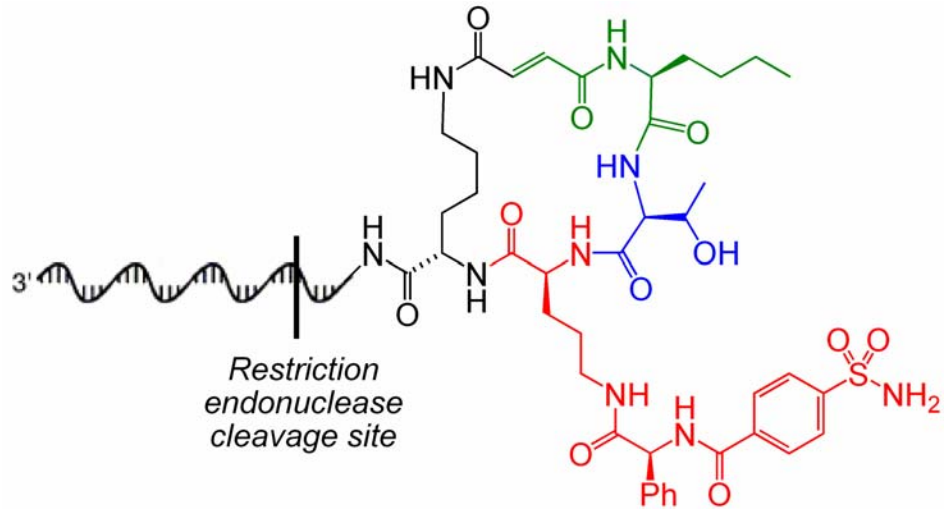
- *Each completed macrocycle associated with a molecule of DNA which*
  - *Directed sequence specific synthesis*
  - *Contains amplifiable sequence which identifies molecule*



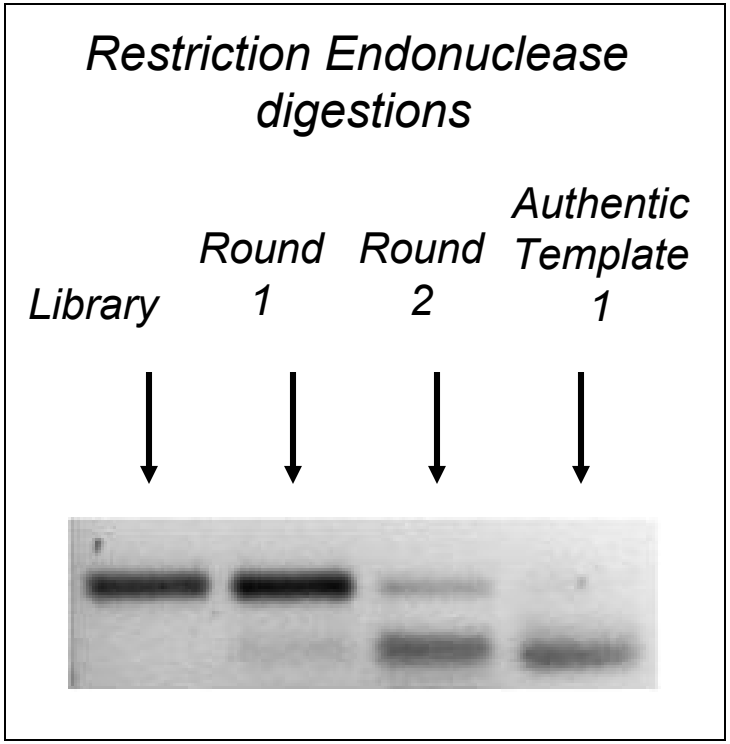

Gartner, Z. J.; Tse, B.N.; Grubina, R.; Doyon, J.B.; Snyder, T.M.; Liu , D.R. *Science.* **2004**, *305*, 1601- 1605.

# Functional Selection and Identification of a One Macrocycle

- *Entire library subjected to two rounds of selection for binding to carbonic anhydrase*
- *Selected DNA amplified via PCR*
- *Digested with restriction endonuclease which cleaves only positive control template*




*1*




Gartner, Z. J. et al. *Science.* **2004**, *305*, 1601- 1605.

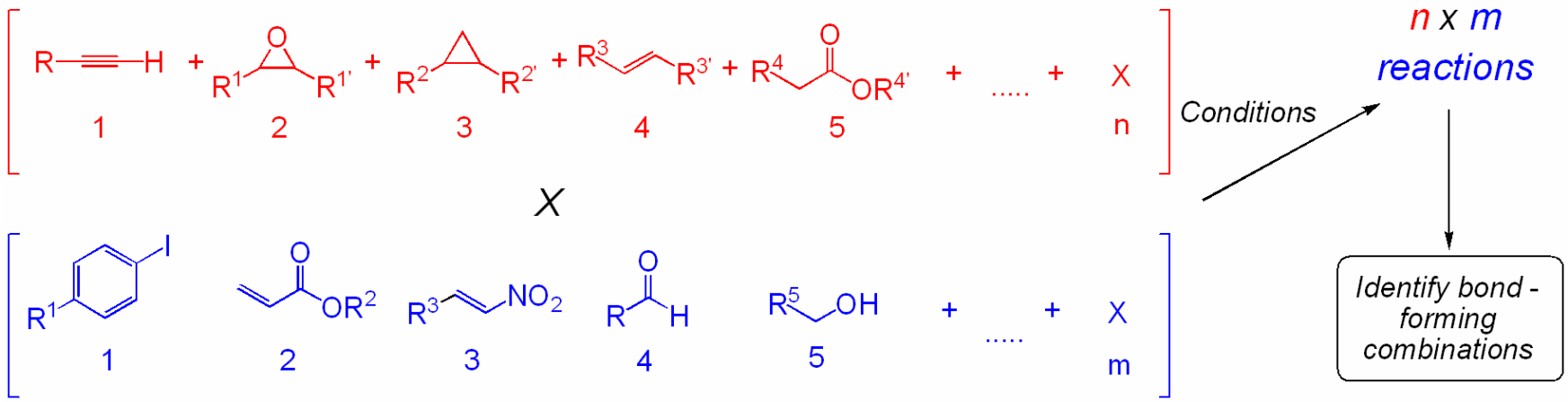
- *One molecule selected based on function*
- *No spatial separation or segregation of library members required*
- *Only small amounts of material required: PCR amplification*

# DNA Assisted Reaction Discovery

# Reaction Discovery Systems

- *Reaction discovery in organic synthesis generally begins with a targeted transformation*
  - *Optimization follows initial discovery or hit*

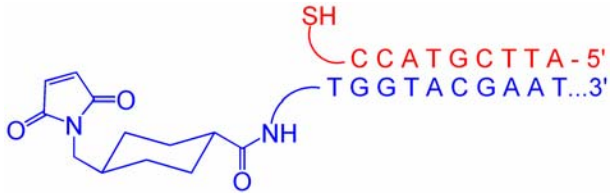
- *An un-biased, one-pot, high throughput probe of reactivity between many functional groups under a given set of reaction conditions is a challenge in organic synthesis*

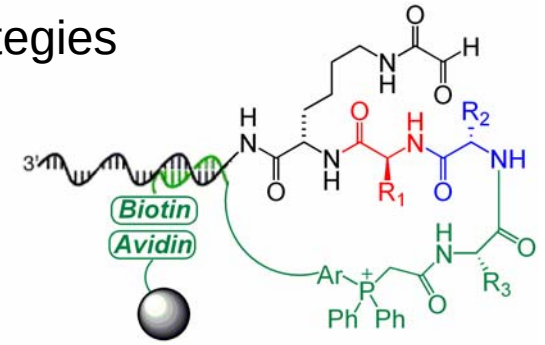



Kanan, M.W.; Rozenaman,M.M.; Sakural, K.; Snyder, T.M.; Liu, D.R. *Nature.* **2004**, *431*, 545-549 .

# DNA-Assisted Reaction Discovery

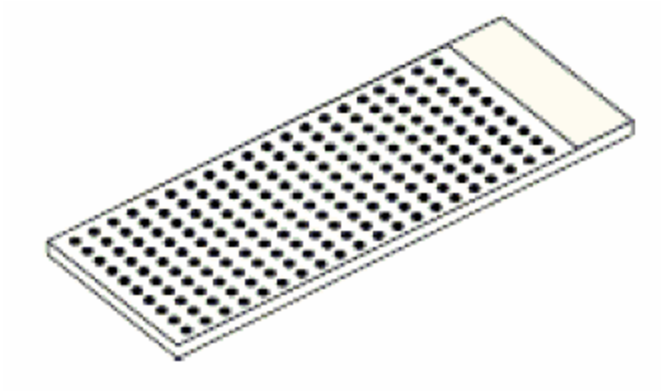
| *Criteria for a reaction discovery system* | *DNA-Templated Synthesis* |
|---|---|
| • Organize many reagents into defined substrate pairs in one pot | • Very low reagent concentrations and sequence specific annealing provide control over reactivity |
| • Facile separation of reactive from unreactive substrate pairs | • Bulk selection and purification strategies |
| • Identification of reactive substrate combinations | • DNA Microarrays |



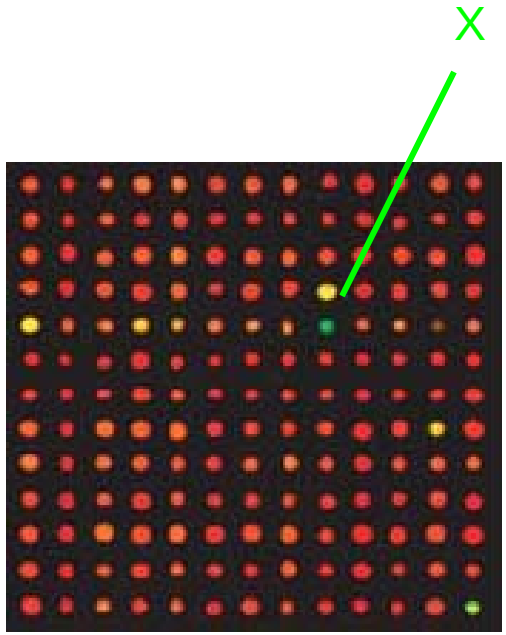

Kanan, M.W.; Rozenaman,M.M.; Sakural, K.; Snyder, T.M.; Liu, D.R. *Nature.* **2004**, *431*, 545-549 .

# DNA Microarrays



*1) Hybridize mixture of labeled <u>unknown</u> DNA to chip*


*2) Wash away non-binding DNA*

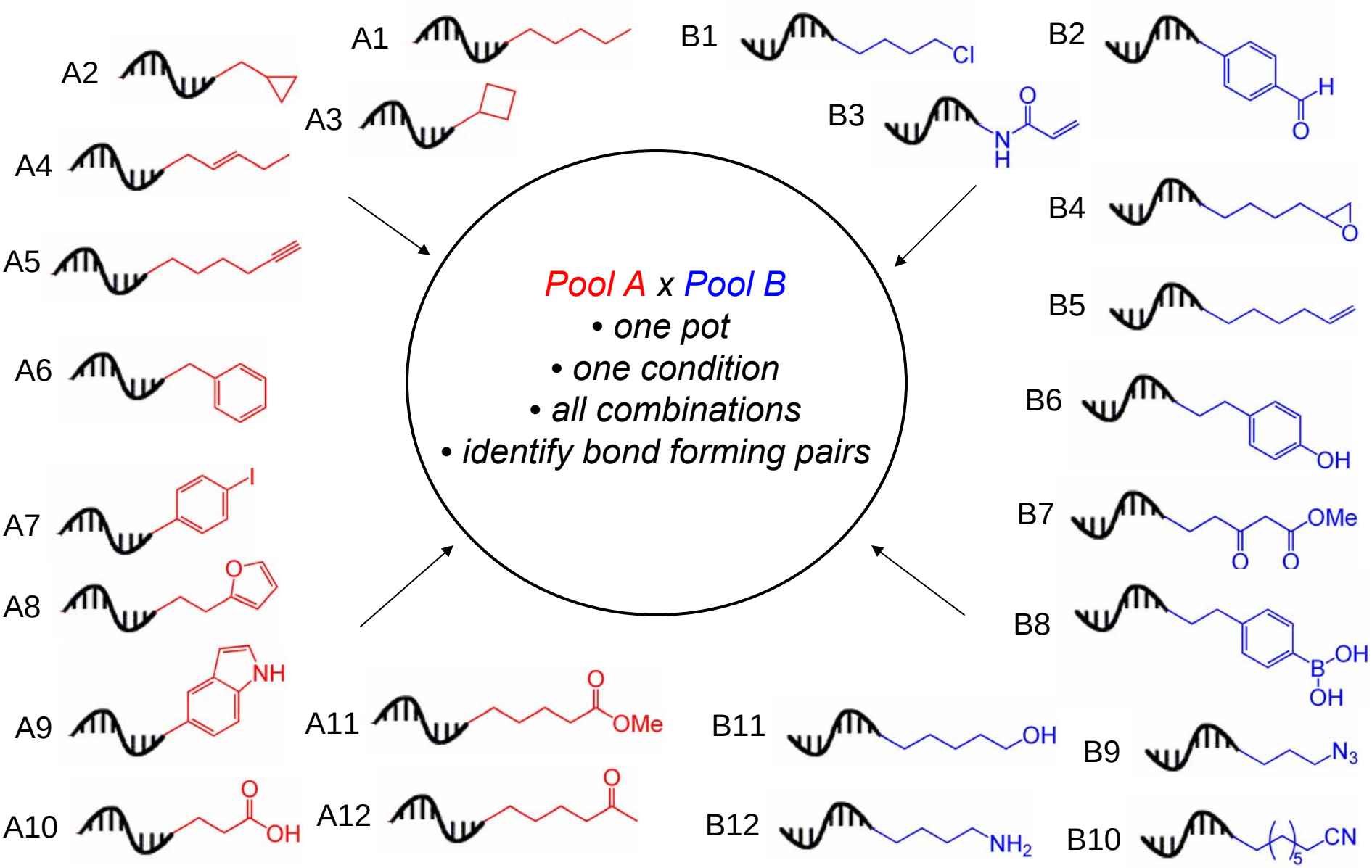




- *Often used in genomics studies*
- *Each spot contains DNA of unique, <u>known</u> sequence*

*3)Identify unknown DNA based on hybridization to chip*


Duggan, D.J., Bittner,M; Chen, Y.; Meltzer, P.; Trent. J.M. *Nature Genet.* **1999**, *21*, 15-19.
Kanan, M.W.; Rozenaman,M.M.; Sakural, K.; Snyder, T.M.; Liu, D.R. *Nature.* **2004**, *431*, 545-549.

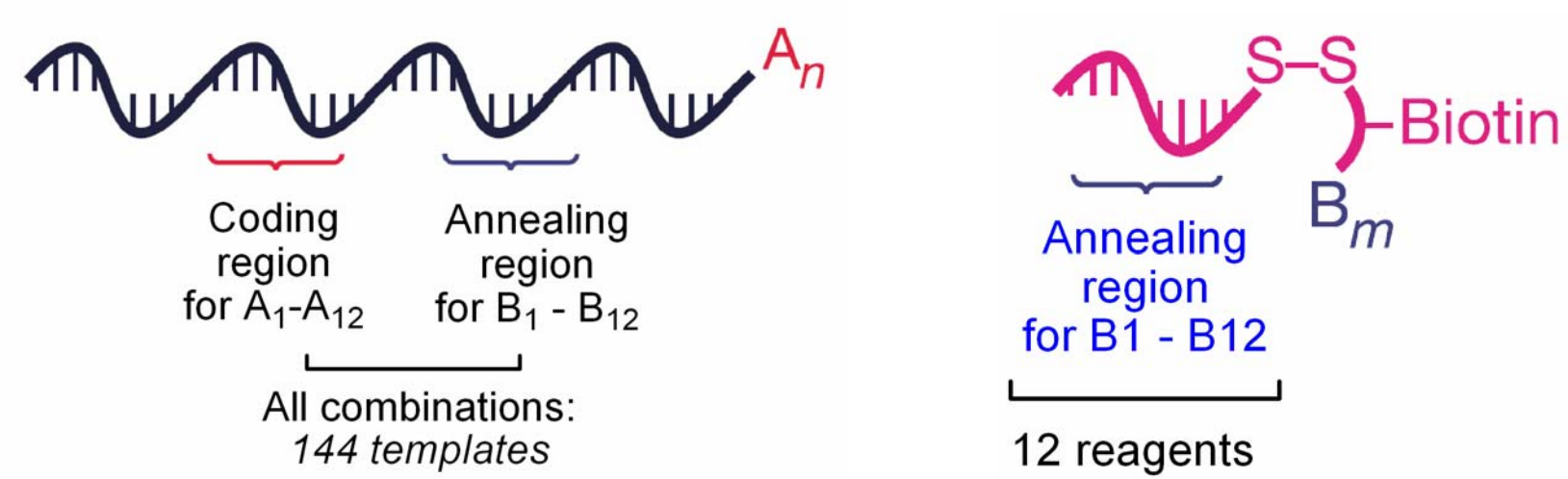
# Substrates for Reaction Discovery



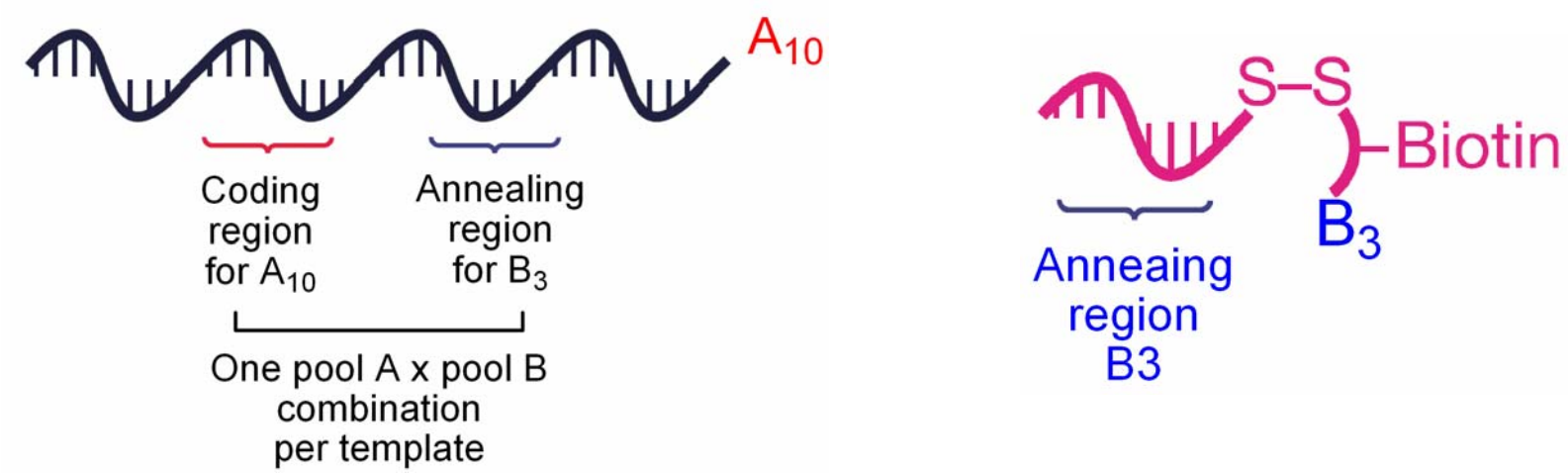

Kanan, M.W.; Rozenaman,M.M.; Sakural, K.; Snyder, T.M.; Liu, D.R. *Nature.* **2004**, *431*, 545-549 .

# Adapting Templated Synthesis to DNA Microarrays

*Library of templates encoding all possible pool A x pool B combinations:*

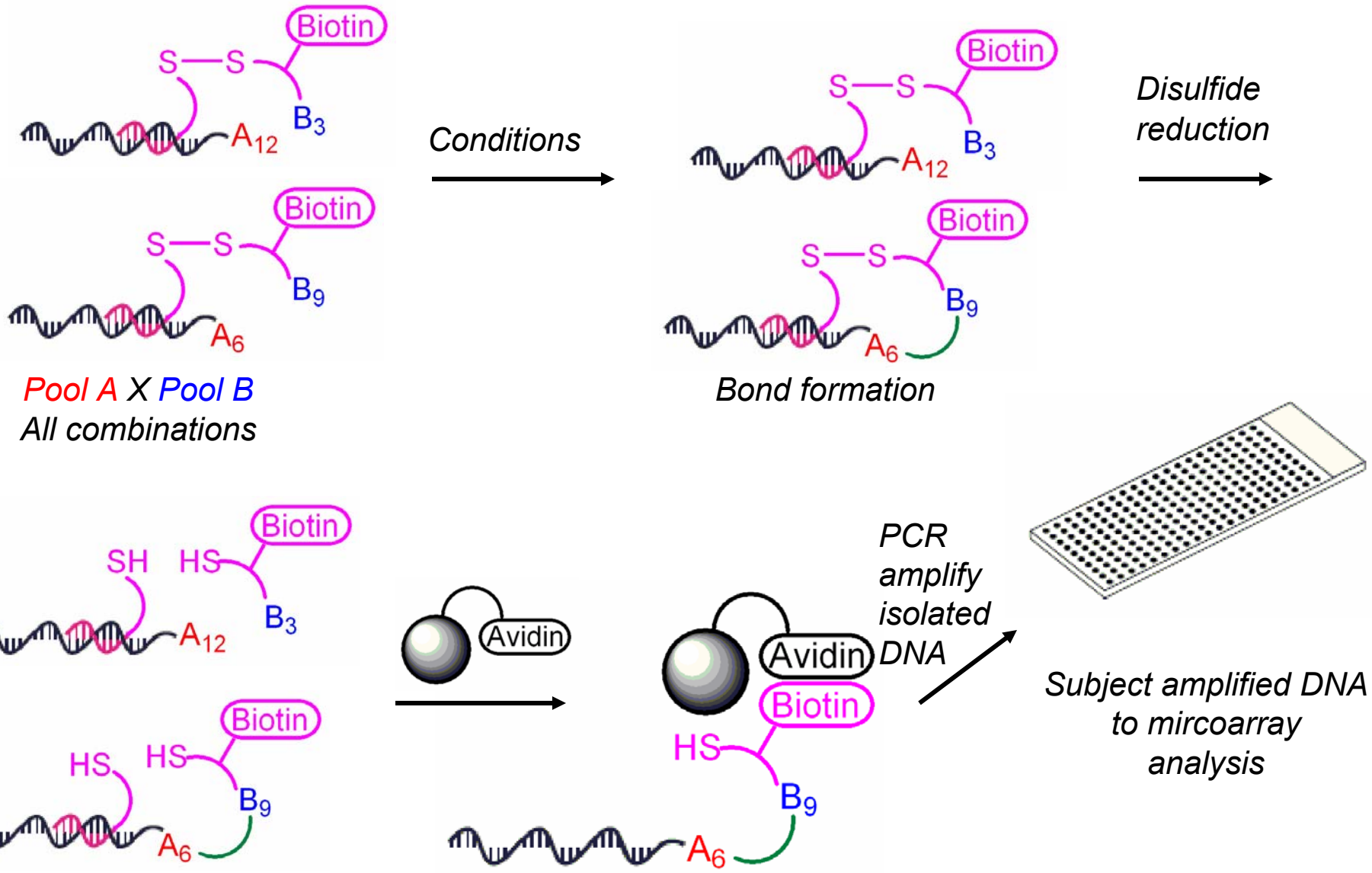



*Individual templates code for one substrate combination:*




Kanan, M.W.; Rozenaman,M.M.; Sakural, K.; Snyder, T.M.; Liu, D.R. *Nature.* **2004**, *431*, 545-549 .

# Adapting Templated Synthesis to DNA Microarrays




Kanan, M.W.; Rozenaman,M.M.; Sakural, K.; Snyder, T.M.; Liu, D.R. *Nature.* **2004**, *431*, 545-549 .

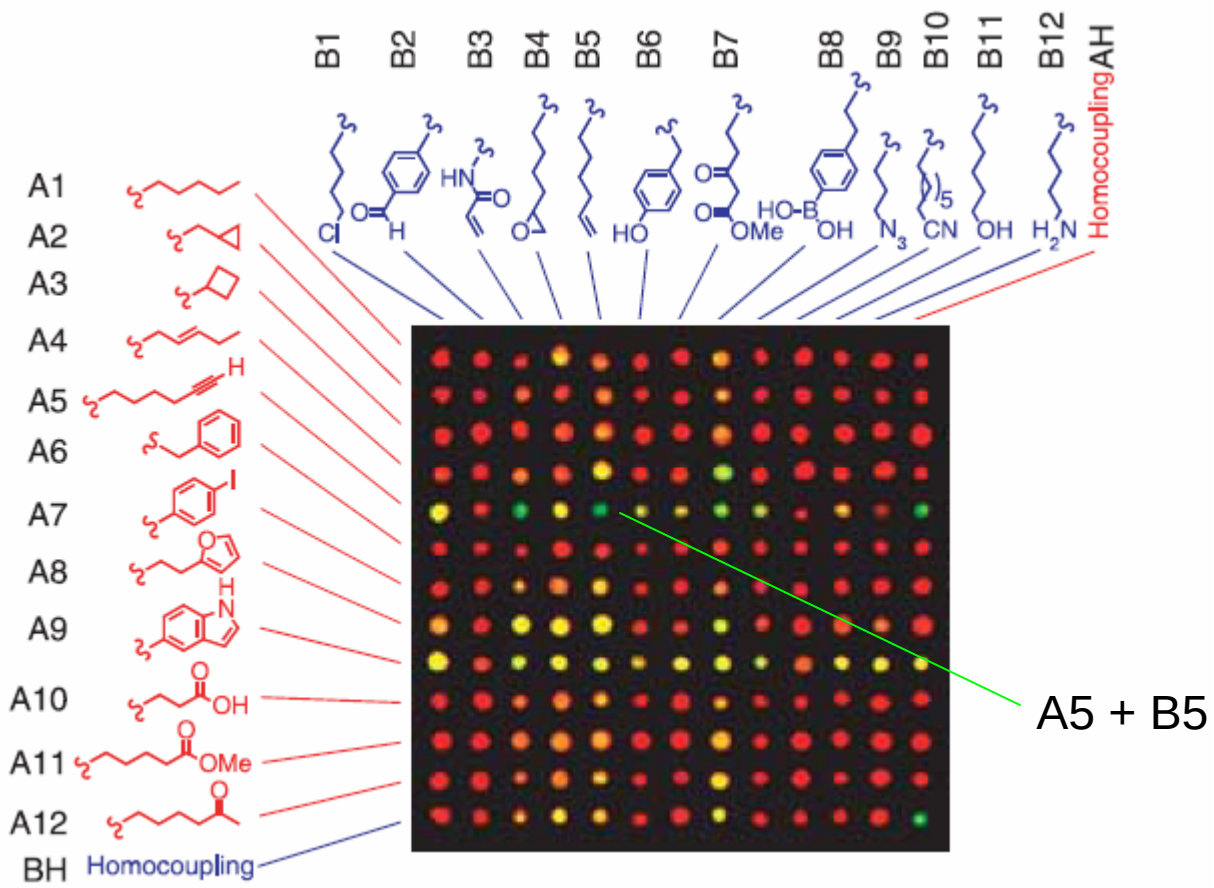
# Reaction Discovery: A New Oxidative Coupling

*Pool A*
*x*
*Pool B*

2 pmol
total material

$Na_2PdCl_4$

$H_2O$, pH 7.0




A5 + B5

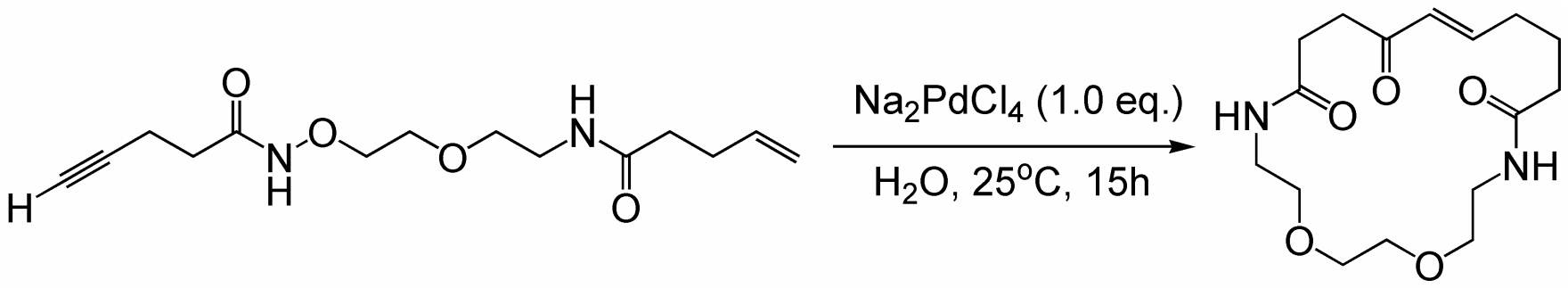
A5

B5

Kanan, M.W.; Rozenaman,M.M.; Sakural, K.; Snyder, T.M.; Liu, D.R. *Nature.* **2004**, *431*, 545-549 .

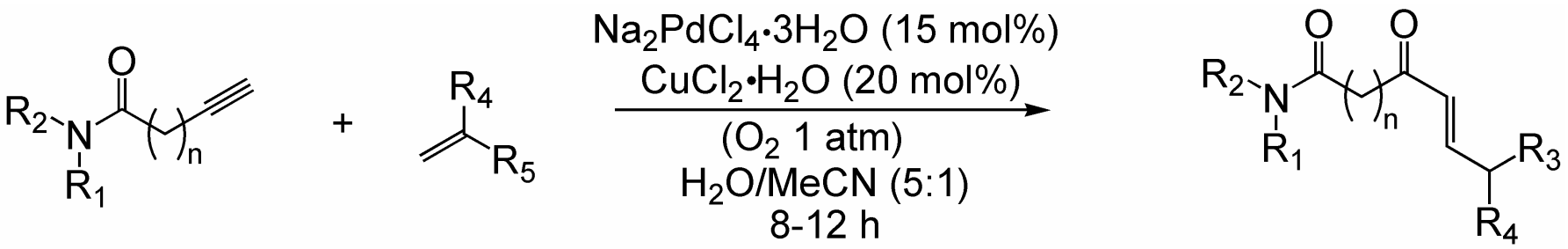
# A Novel Pd Catalyzed Oxidative Coupling

*Multi-milligram scale-up:*




86%
isolated yield

*This reaction was further developed in the synthesis of α,β-Unsaturated ketones:*




n = 2 or 3

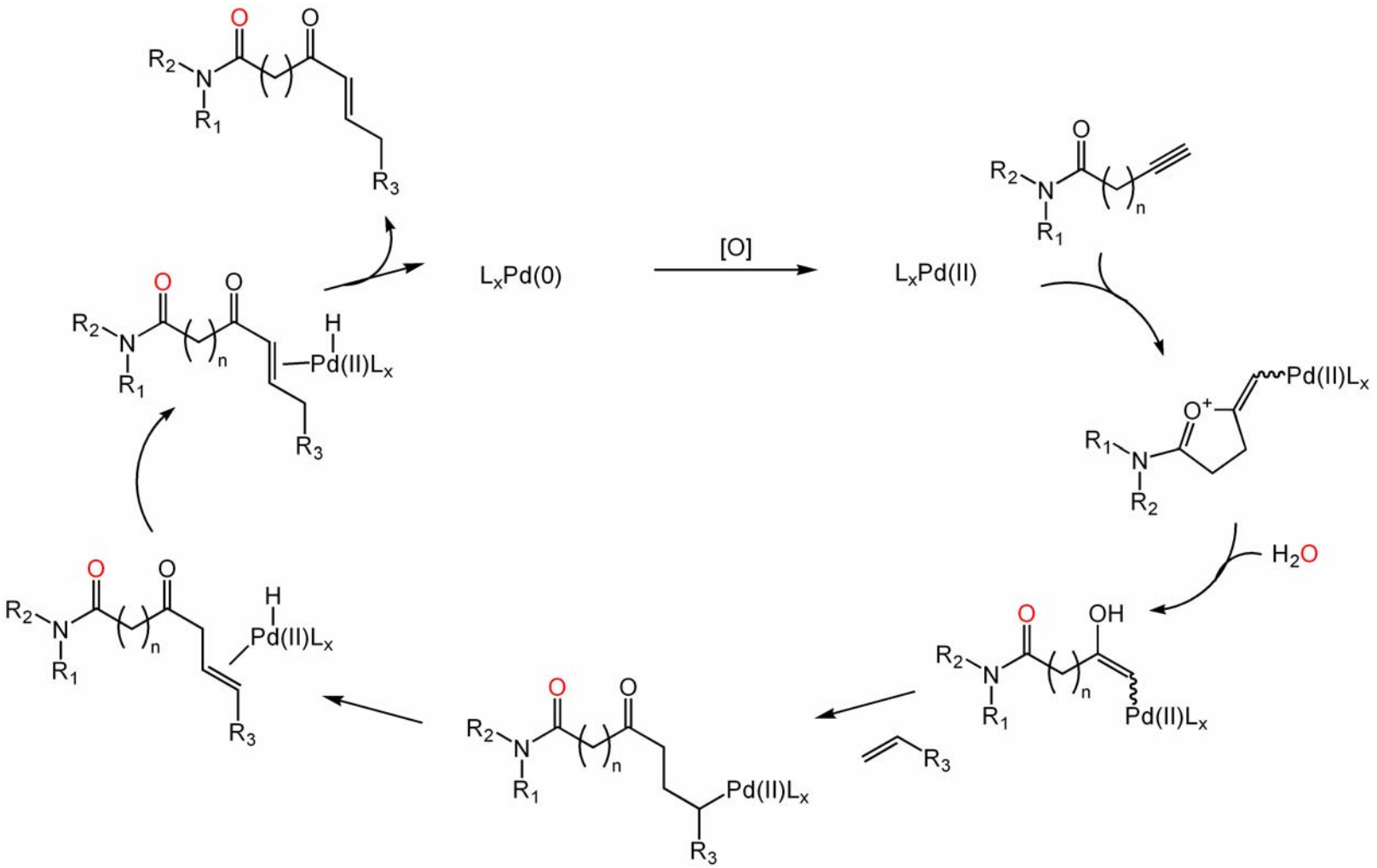
1.5 eq

53 - 80 %


Momiyama, M.W.; Kanan, M.W.; Liu, D.R. *J. Am. Chem. Soc.* **2007**, *129*, 2230-2231 .
Kanan, M.W.; Rozenaman,M.M.; Sakural, K.; Snyder, T.M.; Liu, D.R. *Nature.* **2004**, *431*, 545-549 .

# Mechanism of Oxidative Coupling

*Proposed mechanism:*



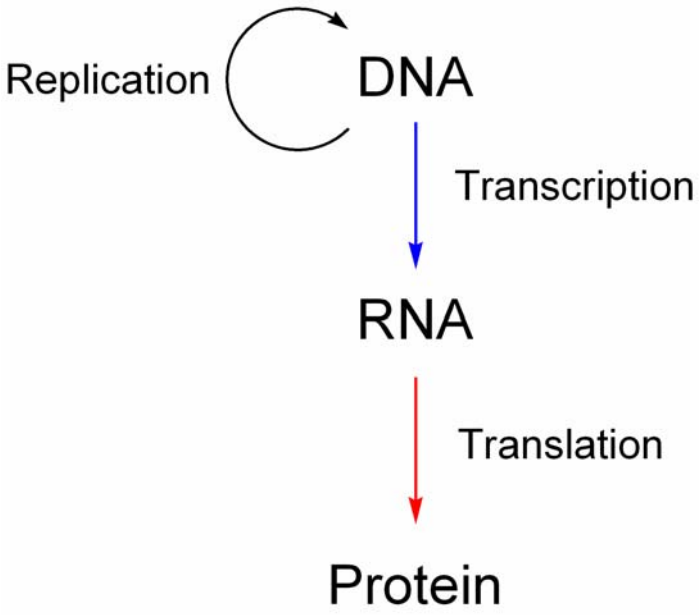

Momiyama, M.W.; Kanan, M.W.; Liu, D.R. *J. Am. Chem. Soc.* **2007**, *129*, 2230-2231 .

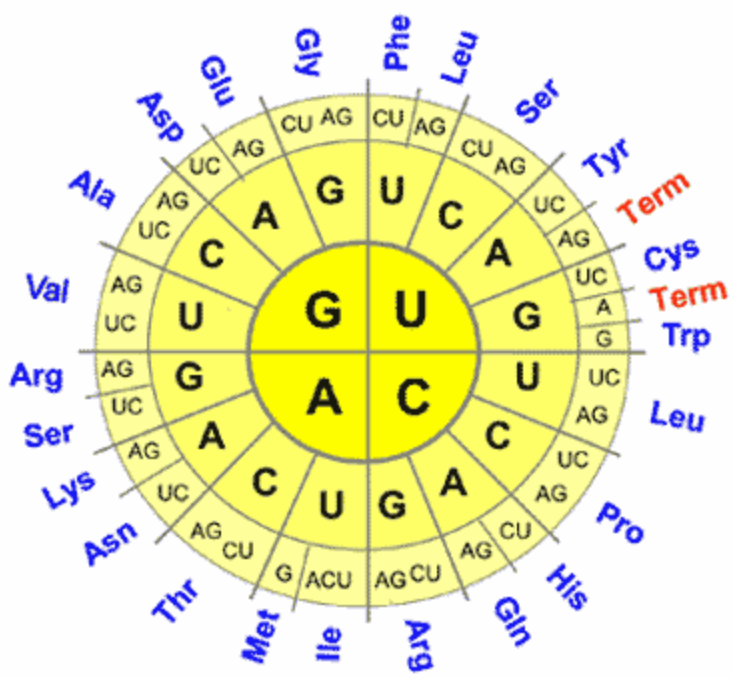
# Summary

- *DNA-templated organic synthesis enables sequence specific multi-step synthesis*
  - *One pot synthesis and selection of a library of programmed macrocylces*
- *Selected molecules can be identified based on associated DNA*
- *Many random combinations of substrates can be assessed for reactivity simultaneously*
- *Development of new synthetic methodology*

# Coded Synthesis

*Nature's templated synthesis:*




- *Application of coded reactivity to chemical synthesis*
- *New tools for exploring organic chemistry*

# Acknowledgements


- *Professor Vy M. Dong*
- *The Dong Group Members*


THE DONG RESEARCH GROUP