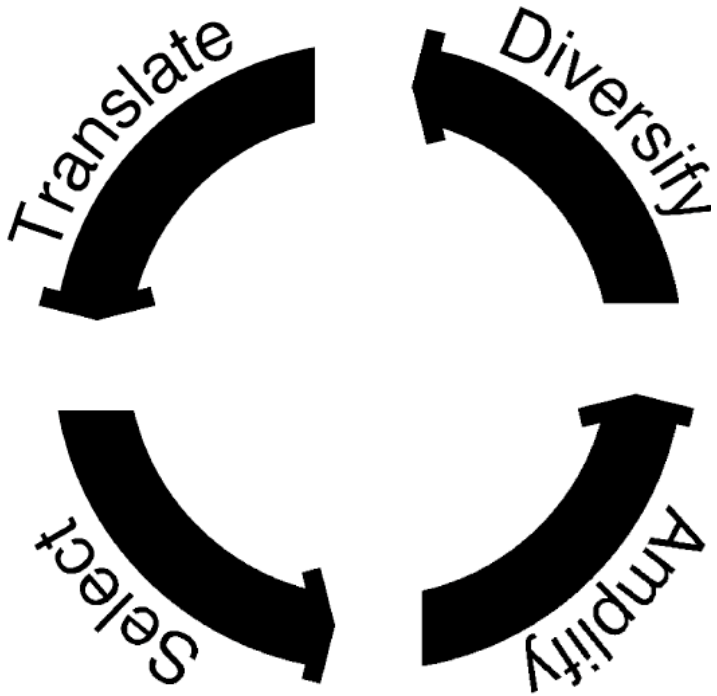
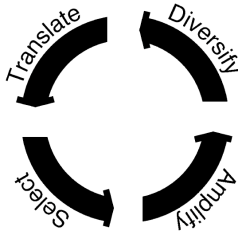
Chemistry from Nature

~ the Application of Evolutional Principals~



Literature Seminar 3
11/4/26
Yutaka Saga (D1)

Today's Contents



0. Introduction

1. Genetic Algorithms

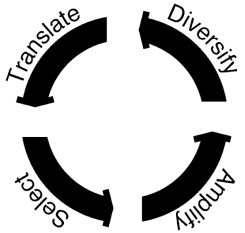
2. Dynamic Combinatorial Chemistry

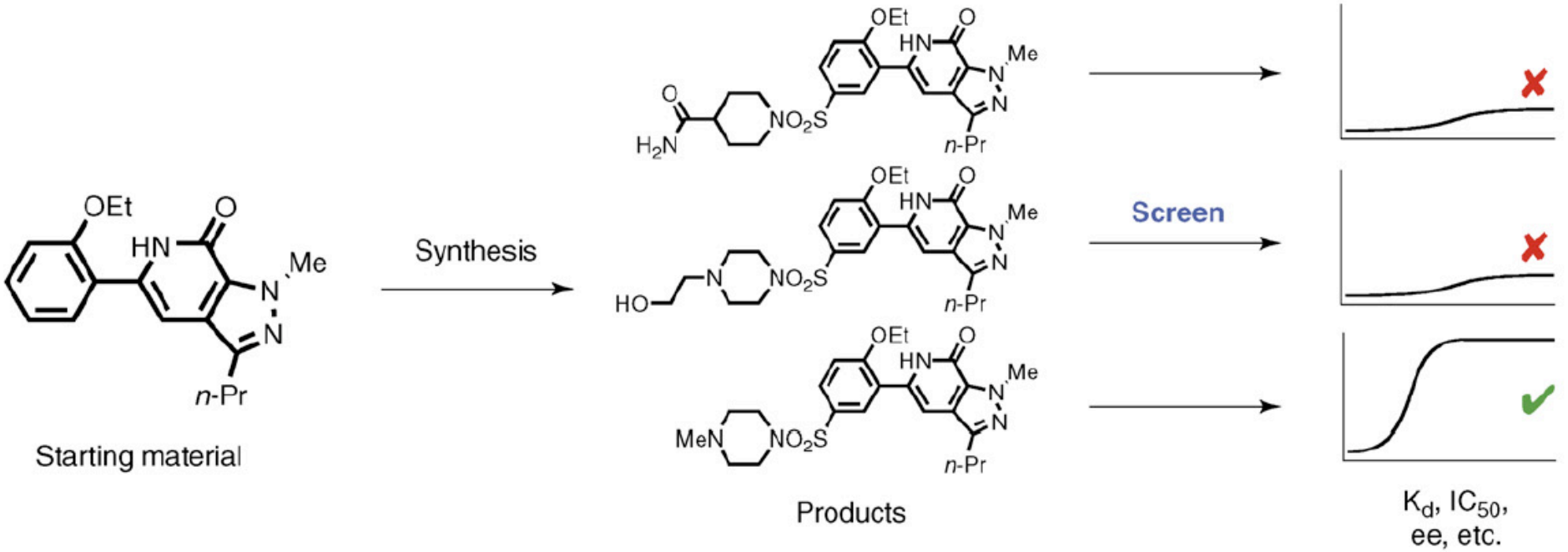
3. DNA-Templated Synthesis

4. Future Direction

0. *Introduction*

Traditional Chemical Approaches



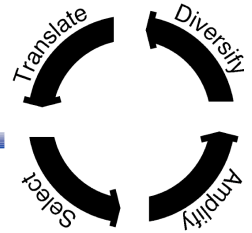


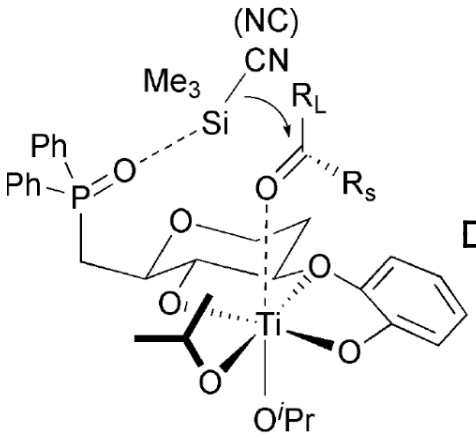
- **Chemists specifically have been interested in the discovery of *functional molecules (catalysts, drugs, materials etc).***

- **Iterated cycle**
  design, synthesis, activity assay, SAR(structure activity relationship)

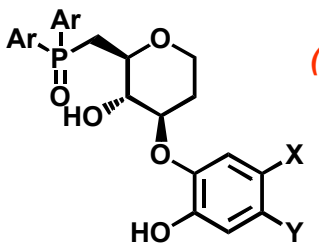
Z.J.Gartner *et al. Pure Appl. Chem.*, 2006, *78*, 1

Chemistry of GluCAPO & FujiCAPO





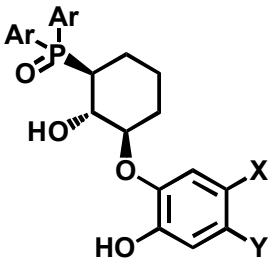
design



Screening

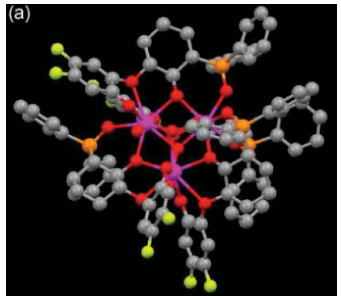
(metal, solvent, temp etc)

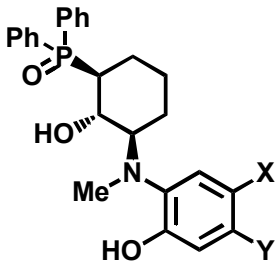
Cyanosilylation of ketons
Strecker Reactions
Conjugate Addition of CN to N-acyl Pyrroles
Ring-Opening of meso-Aziridines



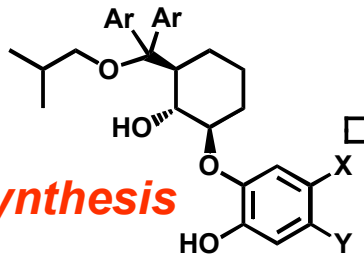
Ring-opening of meso-Aziridines
Conjugate Addition of CN to Enones
Diels-Alder Reactions
Protonation Reactions

SAR





Protonation Reactions

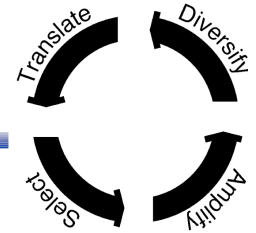


Synthesis

Conjugate Addition of CN to Enones

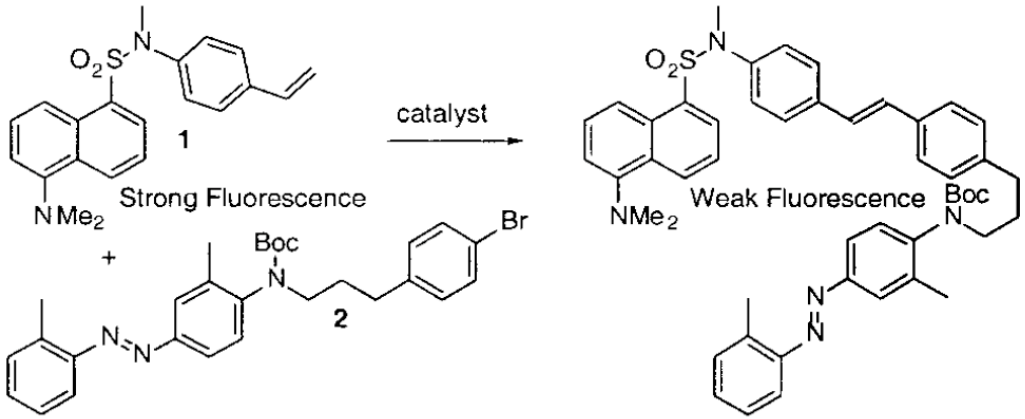
M. Shibasaki, M. Kanai. *Org. Biomol. Chem.*, 2007, *5*, 2027

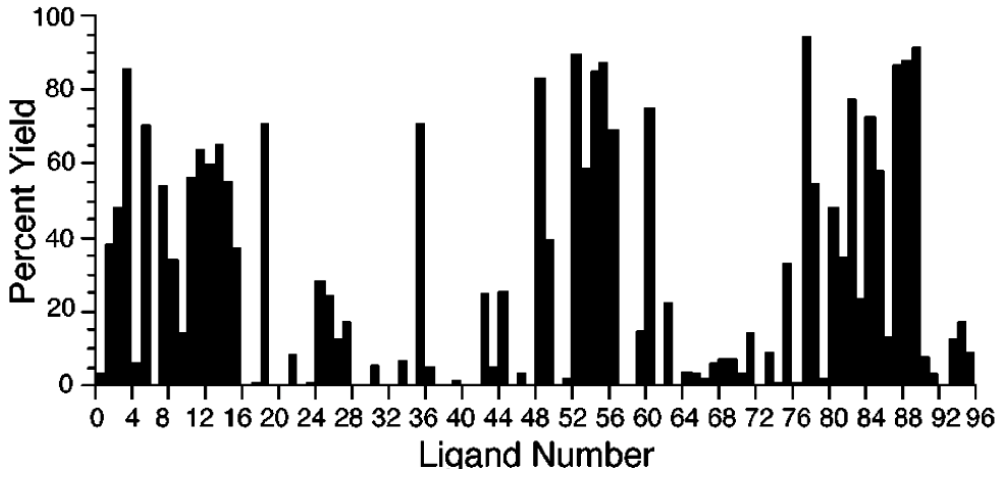
Overcoming the Limitations (1)



HTS (High-Throughput Screening) assays

HTS of Heck reactions by using FRET

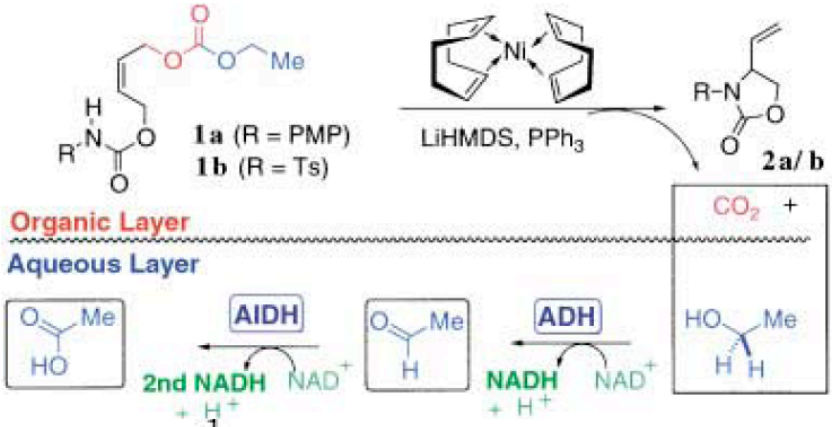


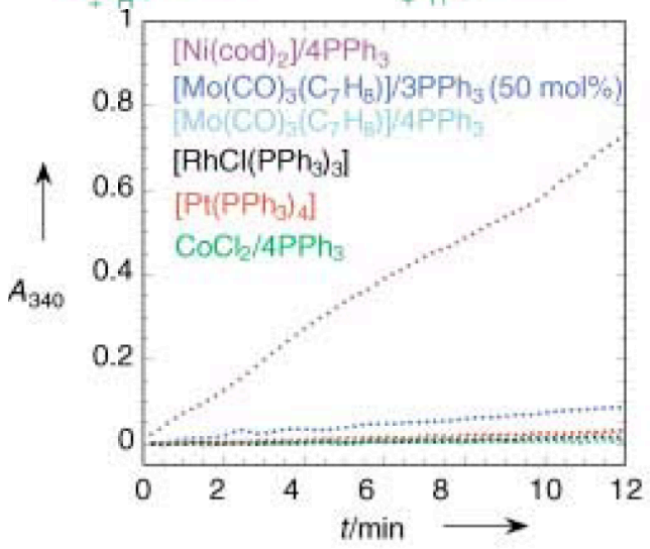


J. F. Hartwig *et al. JACS.*, 2001, *123*, 2677

With *HPLC, MS, colorimetric, IR* ,etc

In Situ Enzymatic Screening

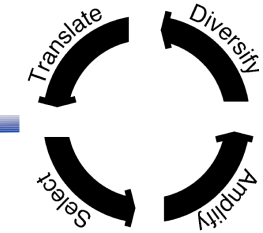




D.B. Berkowitz *et al. ACIE.*, 2002, *41*, 1603

J. F. Hartwig *et al. Curr. Opin. Chem. Biol*, 2003, *7*, 420

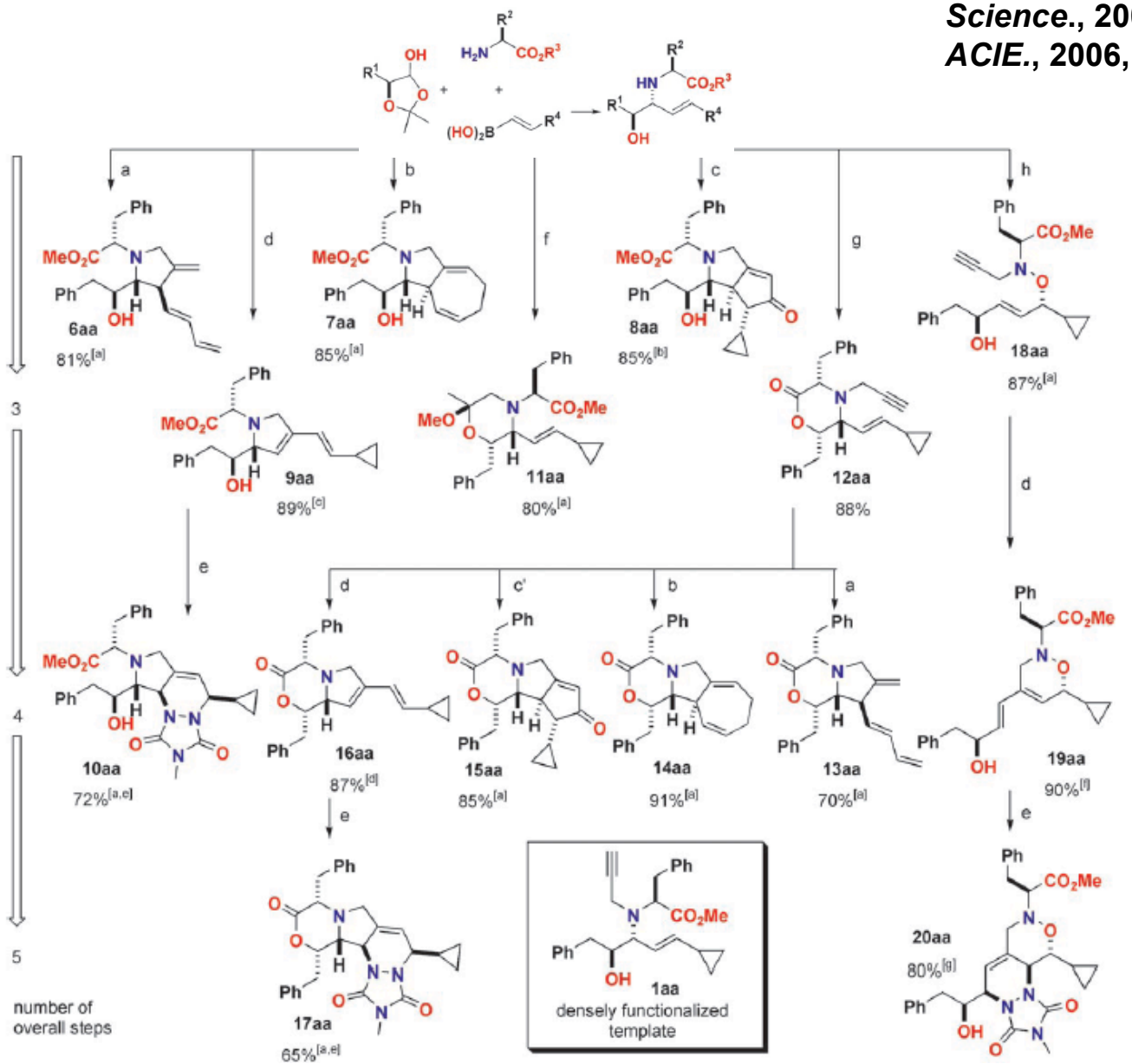
Overcoming the Limitations (2)



Diversity-Oriented Synthesis

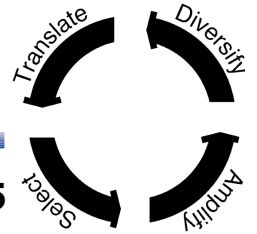
S.L. Schreiber *et al.*
Science., 2000, *287*, 1964
ACIE., 2006, *45*, 3635

Simple SM



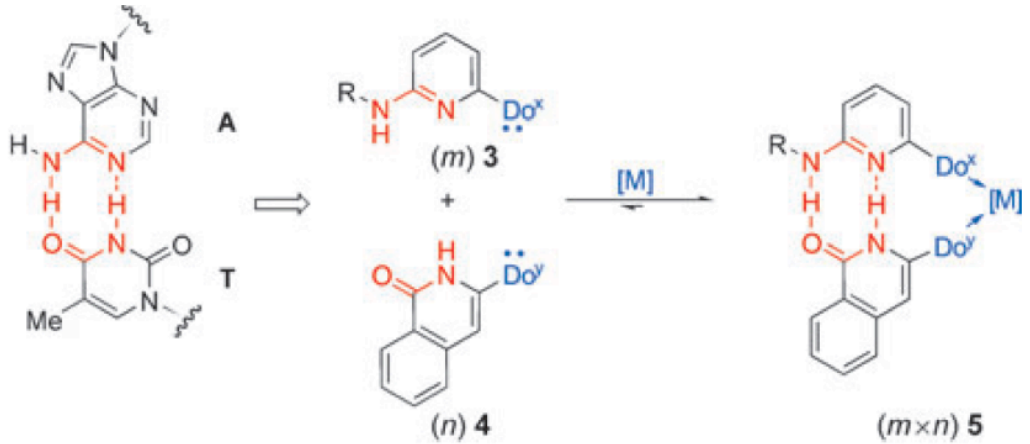
Complexity, Diversity

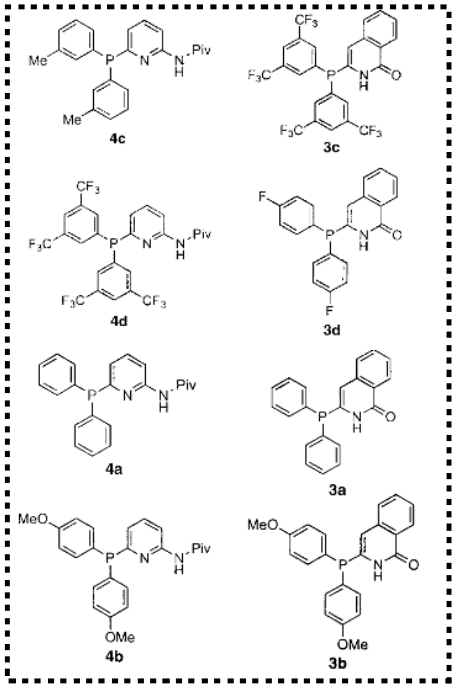
Overcoming the Limitations (3)



Self-Assembled Libraries

N.H. Reek *et al. Nat. Chem.*, 2010, *2*, 615
B.Breit *et al. ACIE.*, 2005, *44*, 1640
Dr. Shimizu's Lit. Seminar (D3)



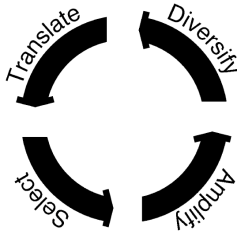


	L(3)			
L(4)	3a	3b	3c	3d
4a	2425 h^{-1}[b]	1040 h^{-1}	2732 h^{-1}	2559 h^{-1}
	94:6[c]	94:6	96:4	95:5
4b	2033 h^{-1}	1058 h^{-1}	1281 h^{-1}	1772 h^{-1}
	93:7	92:8	96:4	94:6
4c	3537 h^{-1}	1842 h^{-1}	1808 h^{-1}	2287 h^{-1}
	94:6	93:7	96:4	94:6
4d	7439 h^{-1}	2695 h^{-1}	7465 h^{-1}	**8643 h^{-1}**
	96:4	95:5	94:6	**96:4**

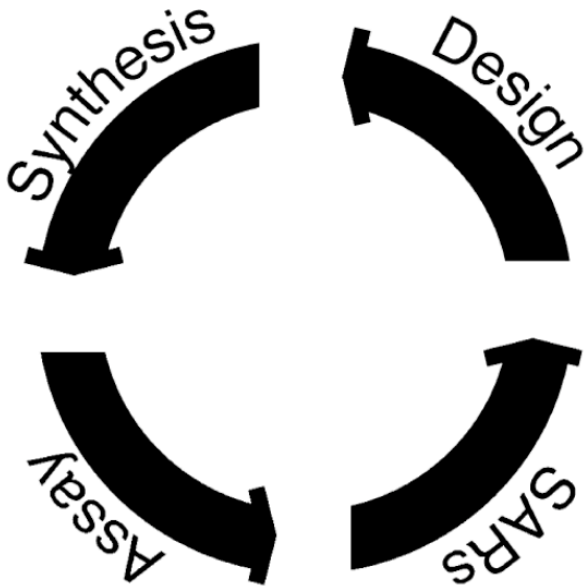
> large catalyst libraries
> just mixing components
> Not covalently bonded, with multiple weak interactions

Also see: T. Ooi *et al. Science.*, 2009, *326*, 120

Toward Evolutional Approaches (1)



Chemists' Approches



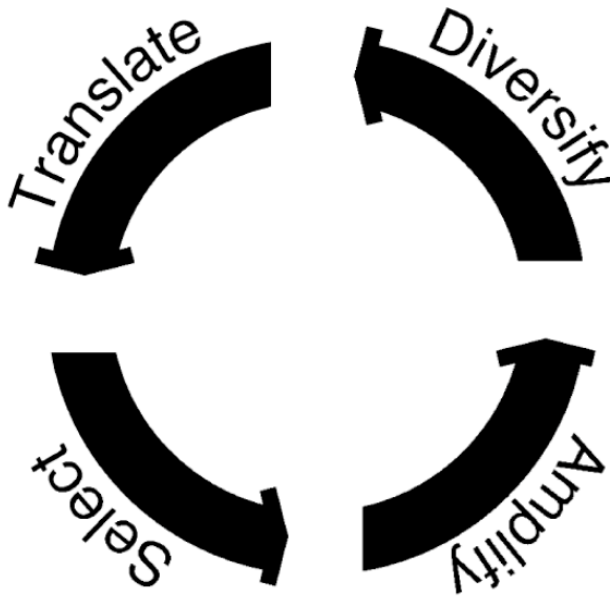
> ***limited** diversities and complexities*

> ***individually** evaluated (homogeneous)*

> ***spatial separation***

> ***high** conc*

Nature's Approches



> ***vast magnitude** of diversities and complexities*

> ***in one-pot** evaluated (heterogeneous)*

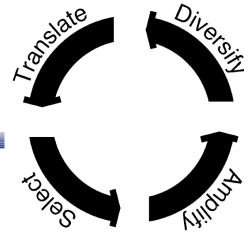
> ***without** spatial separation*

> ***very low** conc*

> ***new reaction discovery ???***

Z.J.Gartner *et al. Pure Appl. Chem.*, 2006, *78*, 1

Toward Evolutional Approaches (2)

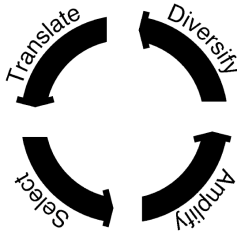


	Chemist's Approach	Nature's Approach
Identifying Hits	individually in spatially separated *assays*	one pot *selections*
Material Quantity	> ~10^{12} copies of each molecule	<~1,000 copies of each molecule
Sample Size	≤10^{6} members per screen (CAS < 10^{8})	≤10^{15} members per selection
Generality	whatever can be synthesized	??? nucleic acids & proteins

Z.J.Gartner *et al. Pure Appl. Chem.*, 2006, *78*, 1

1. Genetic Algorithms

GA (Genetic Algorithm)



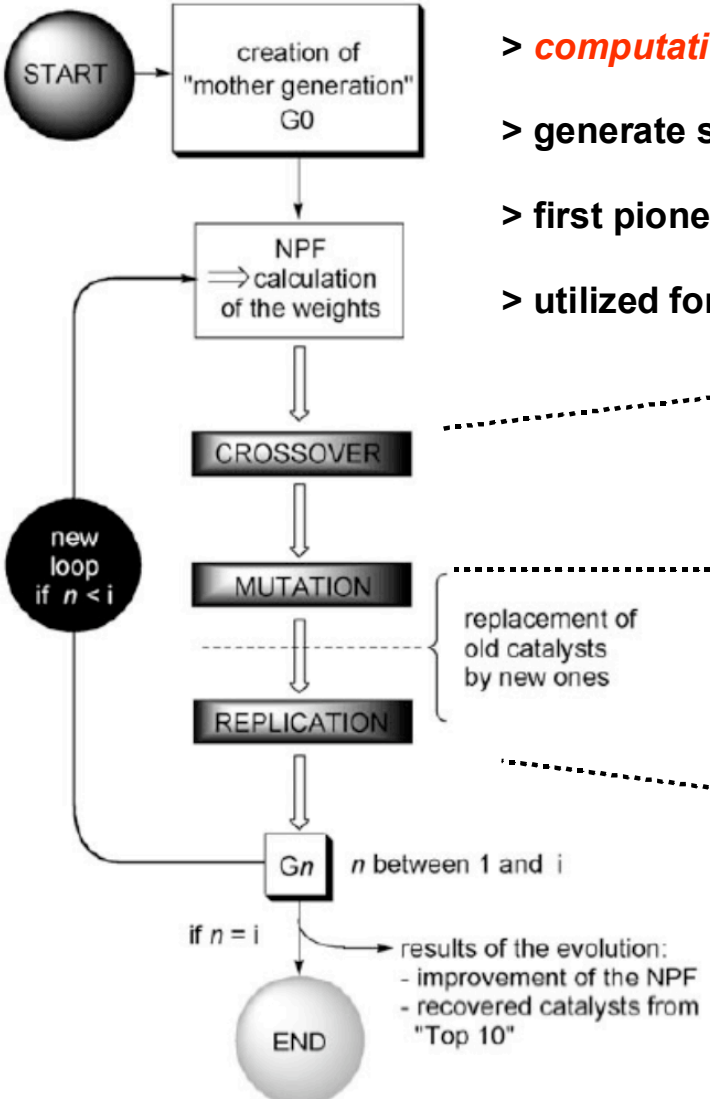
> a search heuristic that *mimics the process of natural evolution.*

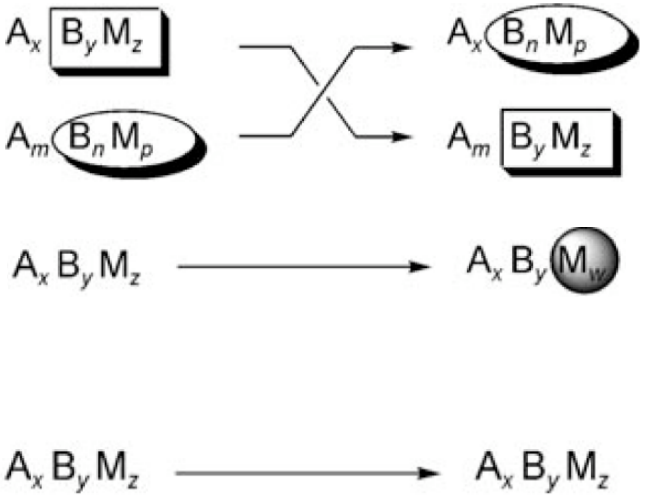
> *computationally mimic* Darwinian evolution

> generate solutions to optimization problems inspired by natural evolution

> first pioneered by Holland in the 1970s

> utilized for *numerous fields* (computer, mathmatics, biology, economics etc)





> adjusting several parameters for the evolution process (G_0, R, RE, RM, RR, NI etc)

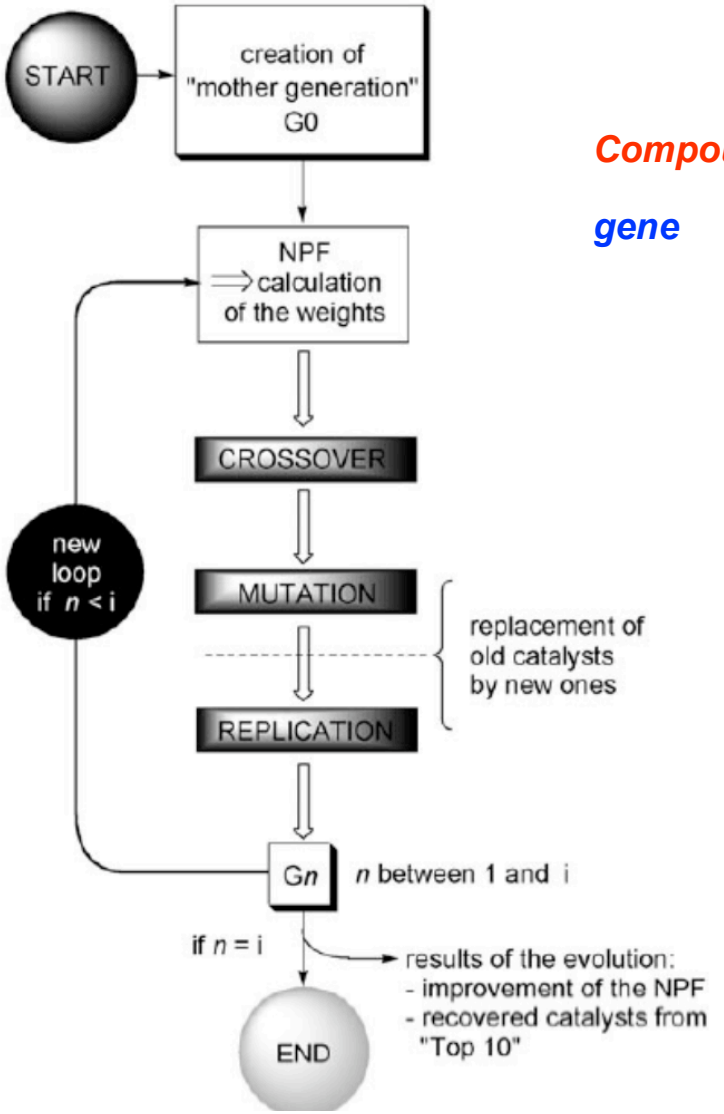
J.H.Holland *Adaptation in Natural and Artificial Systems* Univ.of Michigan Press, 1975.

L. Weber *et al. Curr. Opin. Chem. Biol*, 1998, *2*, 381

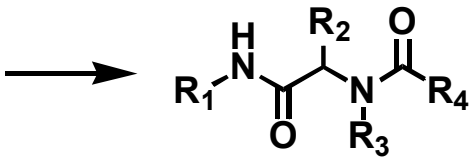
GA (Genetic Algorithm) for Japanese Game

- the player cultivating vegetables on a fictional, futuristic star system.
- the objective is to win vegetable contests.
- pest called Baboo undergo the evolution based on ‘genetic algorithm’

Ambitious Application of GA (1)



Compounds	*40*	×	*40*	×	*10*	×	*40*
gene	*0010*		*01100*		*011*		*010011*



160000

0010/ 01100/ 011/ 010011

target : serine protease thrombin

Crossover

0010/ 01100/ 011/ 010011 ⇨ **0010/ 01100/ 011/ 100011**

0010/ 01100/ 011/ 100011 ⇨ **0010/ 01100/ 011/ 010011**

Mutation

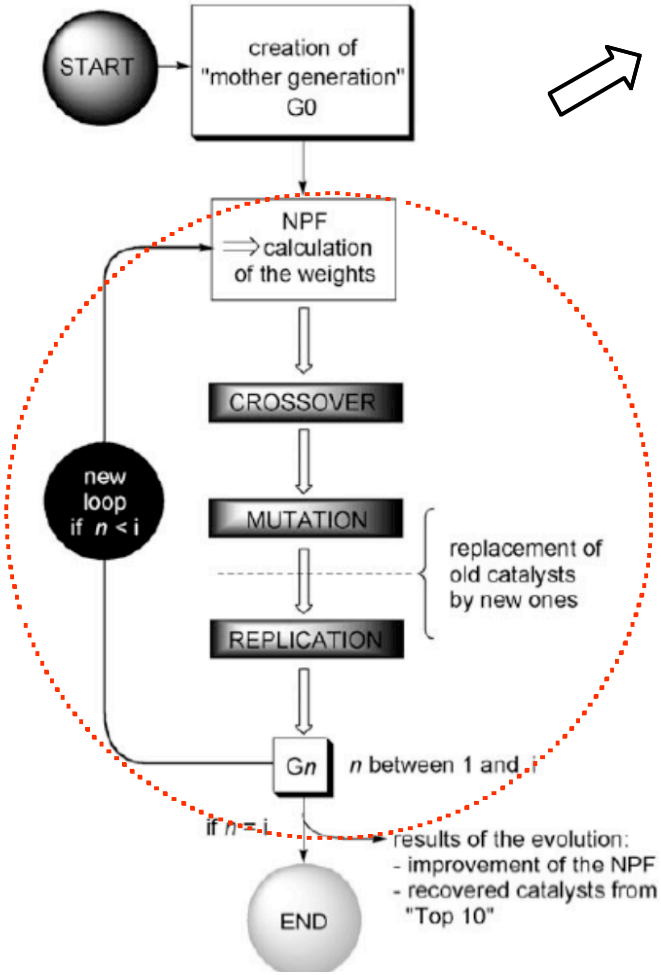
0010/ 01100/ 011/ 010011 ⇨ **0010/ 01100/ 011/ 000111**

Replication

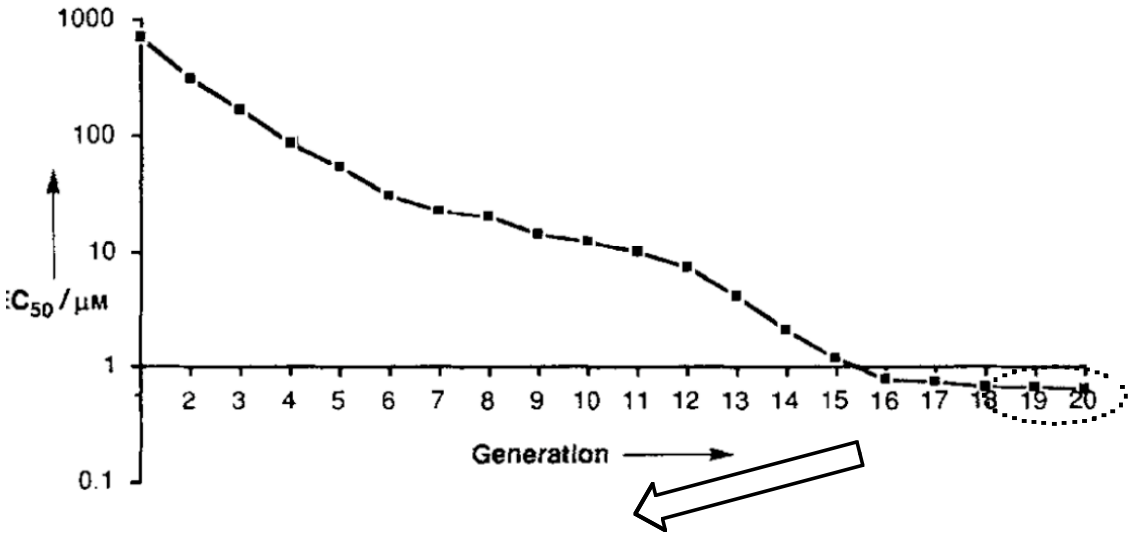
0010/ 01100/ 011/ 010011 ⇨ **0010/ 01100/ 011/ 010011**

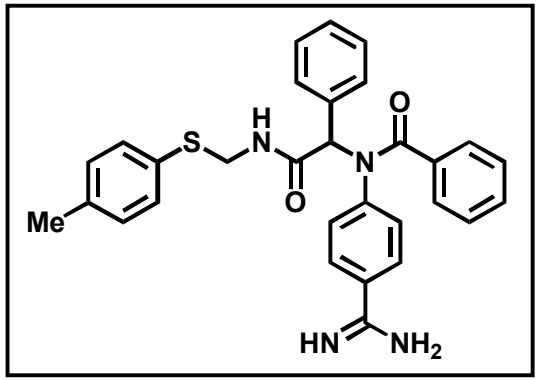
L. Weber *et al. ACIE*, 1995, *34*, 2280

Ambitious Application of GA (2)



best 20 coumpounds × 20 generations





> the best inhibitor exhibited K_i < 1 µM

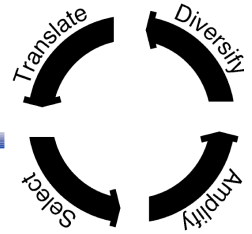
> ***only 400 reactions*** out of 160000 reactions

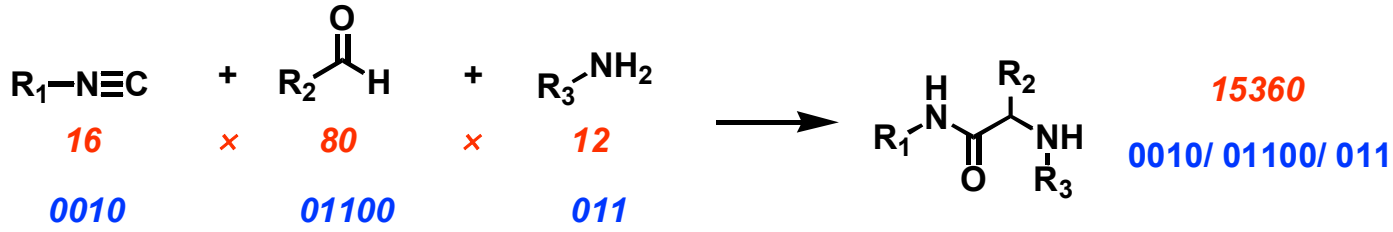
> totally adjusting GA principals for targeting

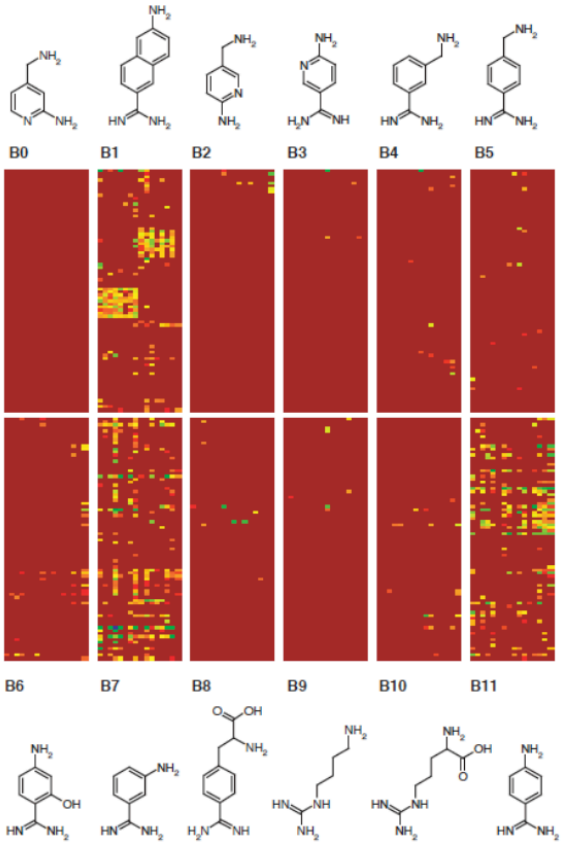
> but ***nonpeptidic*** (no structural biases)

L. Weber *et al. ACIE*, 1995, *34*, 2280

Ambitious Application of GA (3)







> chosing the amines *with a structural bias* toward binding pocket (hydrogen-donor, hydrogen-acceptor etc)

⇨ in a manner analogous to *proteins or nucleotic acids*

> 15360 reactions were actually performed to provide library.

> IC_{50} values were evaluated to be coded by spectral colors

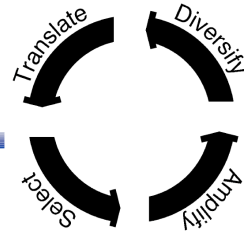
> IC_{50} < 1μM: 0.059 %, 1~10μM: 0.352 %, 10~100μM: 4.395 %

⇩

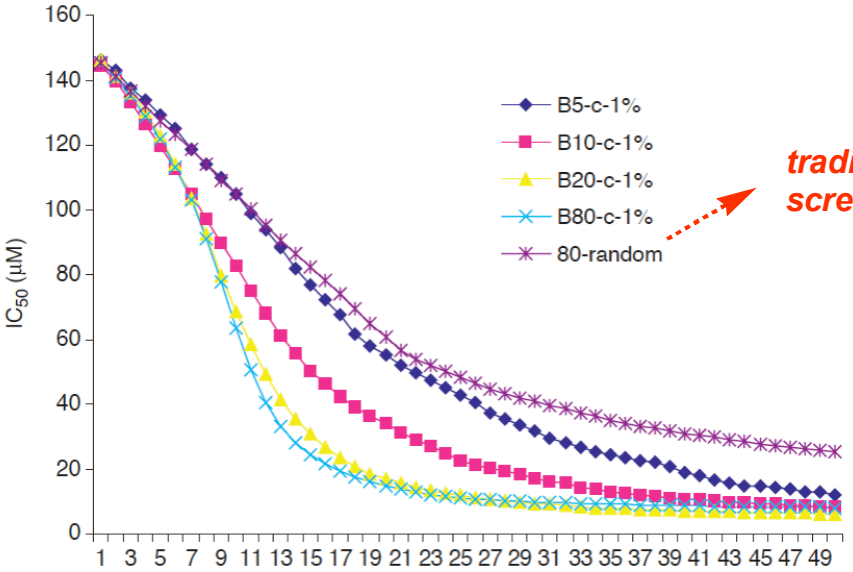
simulate GAs to *optimize the various parameters*

L. Weber *et al. Chem. Biol.*, 2000, *7*, 433

Ambitious Application of GA (4)

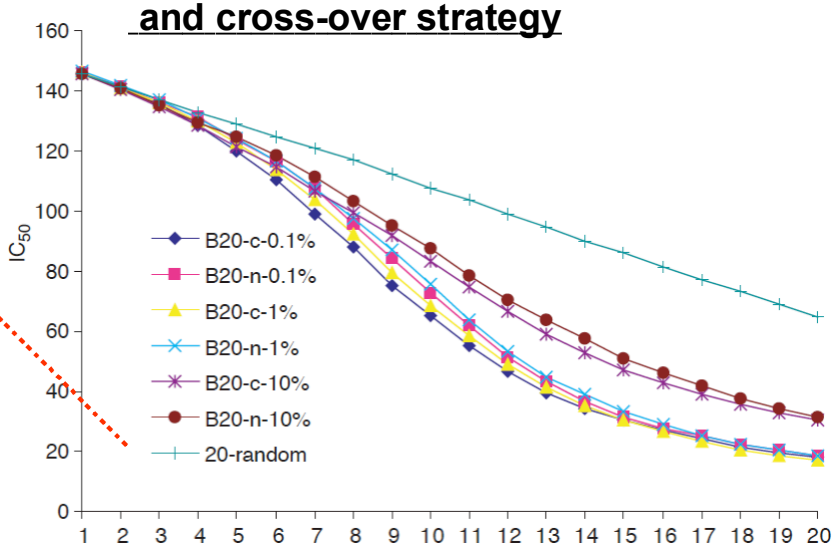


Optimization of generation size



traditional screening

Optimization of the mutation rates and cross-over strategy



> P (*the average activity*) =$(m_{GA}/N_{GA})/(m_{rondom}/N_{rondom})$
(m_{GA}, m_{rondom} = the slopes of the performance curves)

> P =23.6, 14.1, 10.3, 3.1 (N = 5, 10, 20, 80)
> P×N = 118, 141, 206, 248 (N = 5, 10, 20, 80)
> small N *learns more per individual*
> large N *learns faster*
> *judge the efficiency* of GAs or other methods

> c = crossover only between starting materials
n = DNA-like crossover at any bit

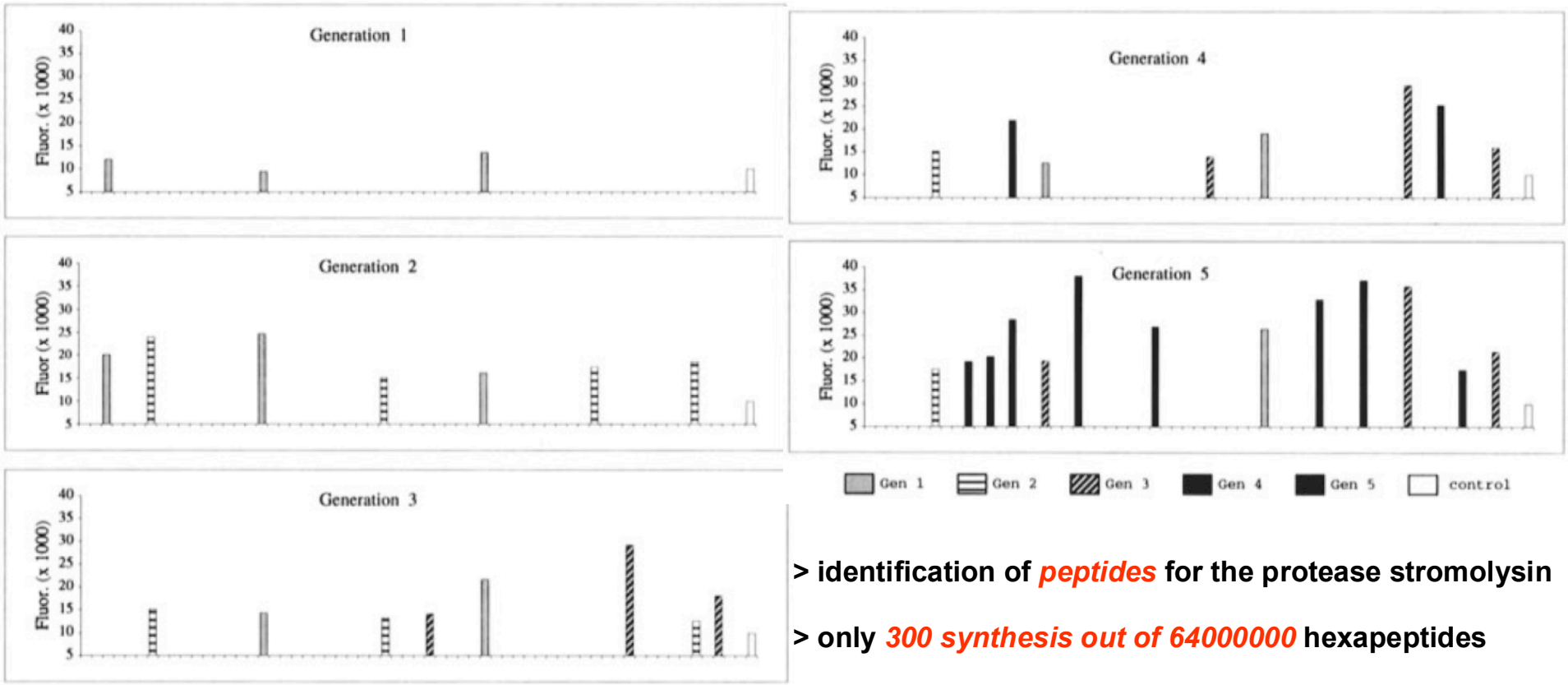
> *c excerts a little better peformance* despite of lower diversity

> *increasing the mutation rate deteriorated* the performance

→ *mutation destroyed* the acquired knowledges

L. Weber *et al. Chem. Biol.*, 2000, *7*, 433

Various Applications of GAs (1)

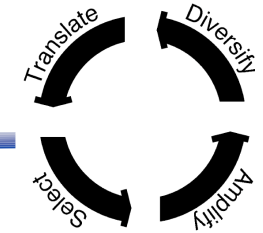


> identification of *peptides* for the protease stromolysin

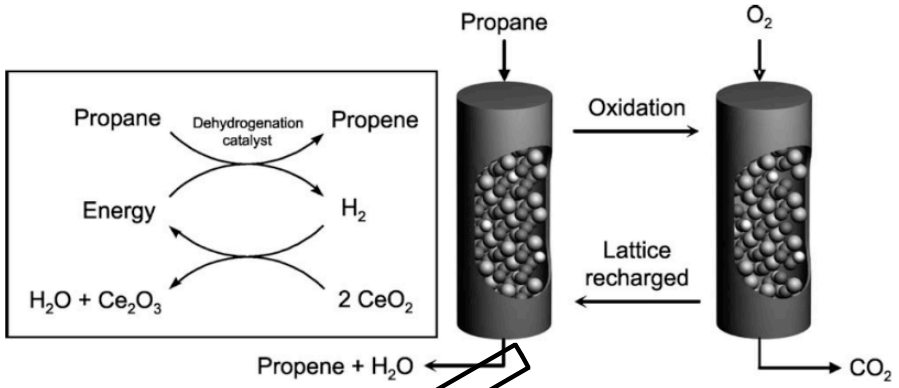
> only *300 synthesis out of 64000000* hexapeptides

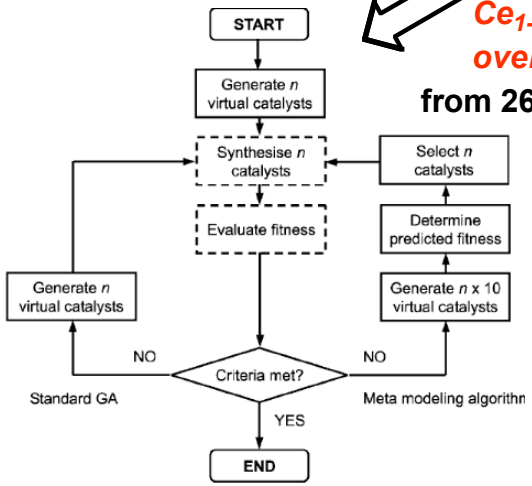
J. Singh *et al. JACS.*, 1996, *118*, 1669

Various Applications of GAs (2)

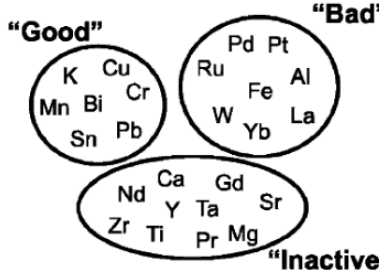


Selective Hydrogen Oxidation Catalysts via GAs

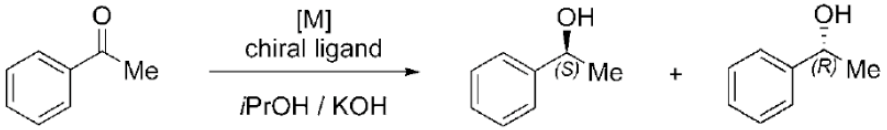




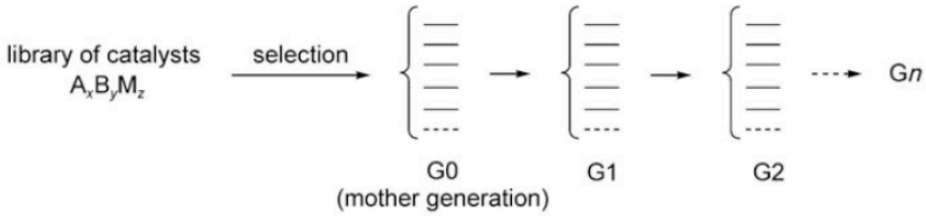
$Ce_{1-x-y}M^1_xM^2_yO_2$ dopants
over 17000 combinations
from 26 metals in 5 cat amounts

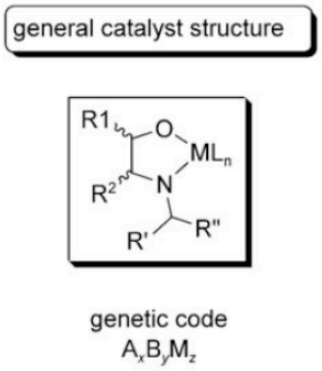


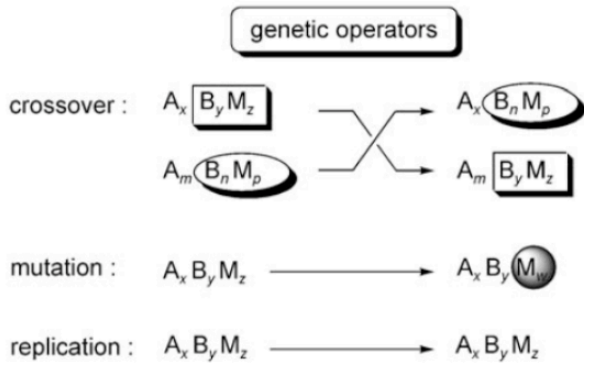
Asymmetric Hydrogenation Catalysts via GAs



A: chiral amino alcohol × 11 B: aldehyde × 30
C: metal (Ru, Ir) × 6





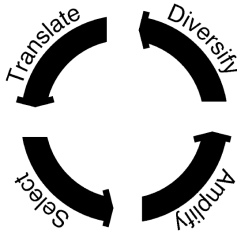


> the best catalyst found by evaluating only 10 % of the whole
> a time reduced to 30 % compared to the traditional methods
> development of double algorithm

J. Beckers *et al. Adv. Synth. Catal.*, 2008, *350*, 2237

O. Riant *et al. Chem. Eur. J.*, 2009, *15*, 6267

Features of GAs



Advantages

> mimicking the evolutional cycle in Nature
→ realizing *the diversity close to Nature*

> a small fraction of the *'virtual liblary'*
→ not time-consuming

> equally applied to *synthetic molecules* as to DNA or proteins

Disadvantages

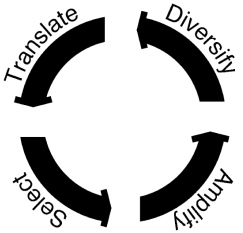
> the need to *synthesize and assay indivisual molecules*
→ What's the difference from traditional methods?

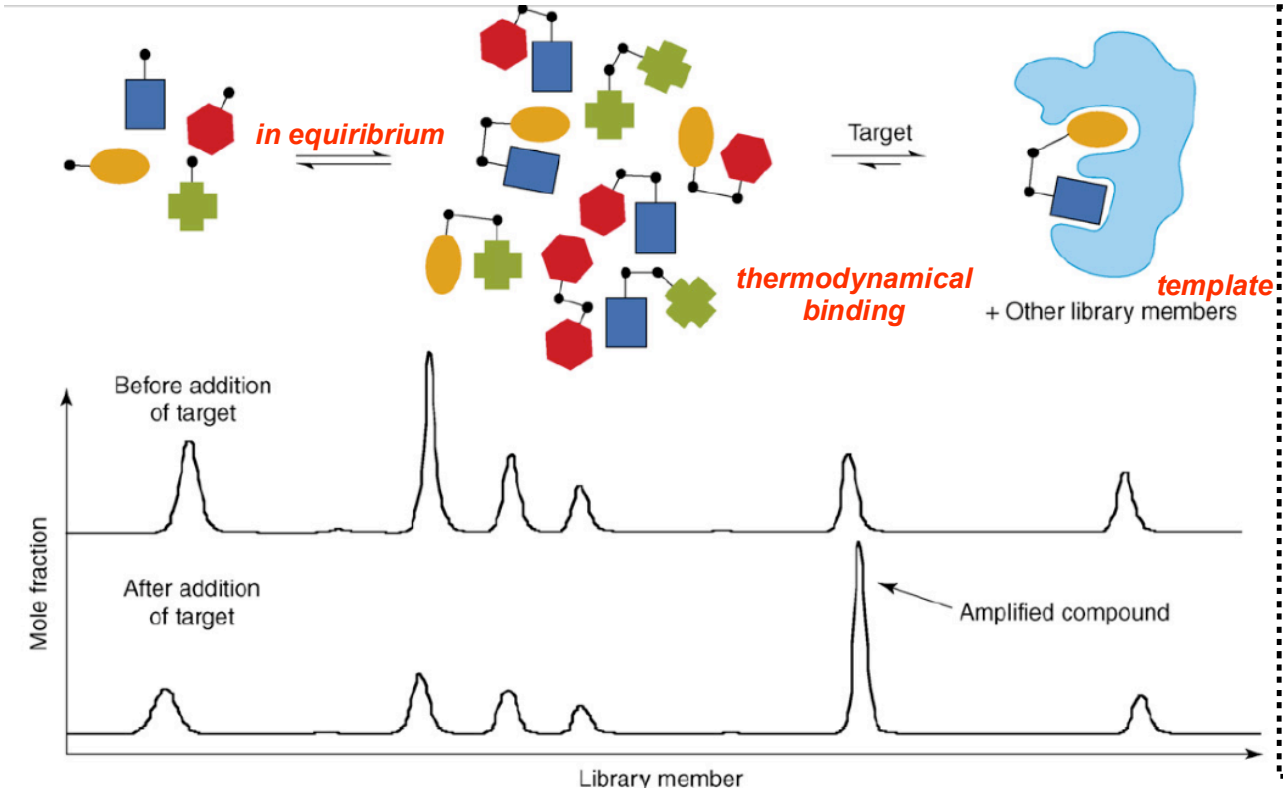
> *insufficient* size and complexity

> beneficial only for *the known developed systems*
→ known things of which *parameters set in advance*

2. Dynamic Combinatorial Chemistry

Dynamic Combinatorial Chemistry





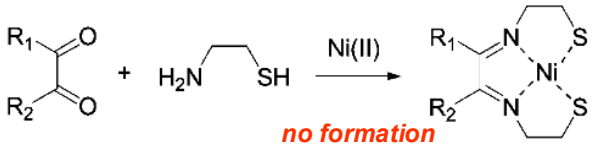
@ Fischer's study on carbohydrates in 19th century

⇩

@ Watson & Crick's DNA helix templates in 1950s

⇩

@ Busch's first example in 1960's



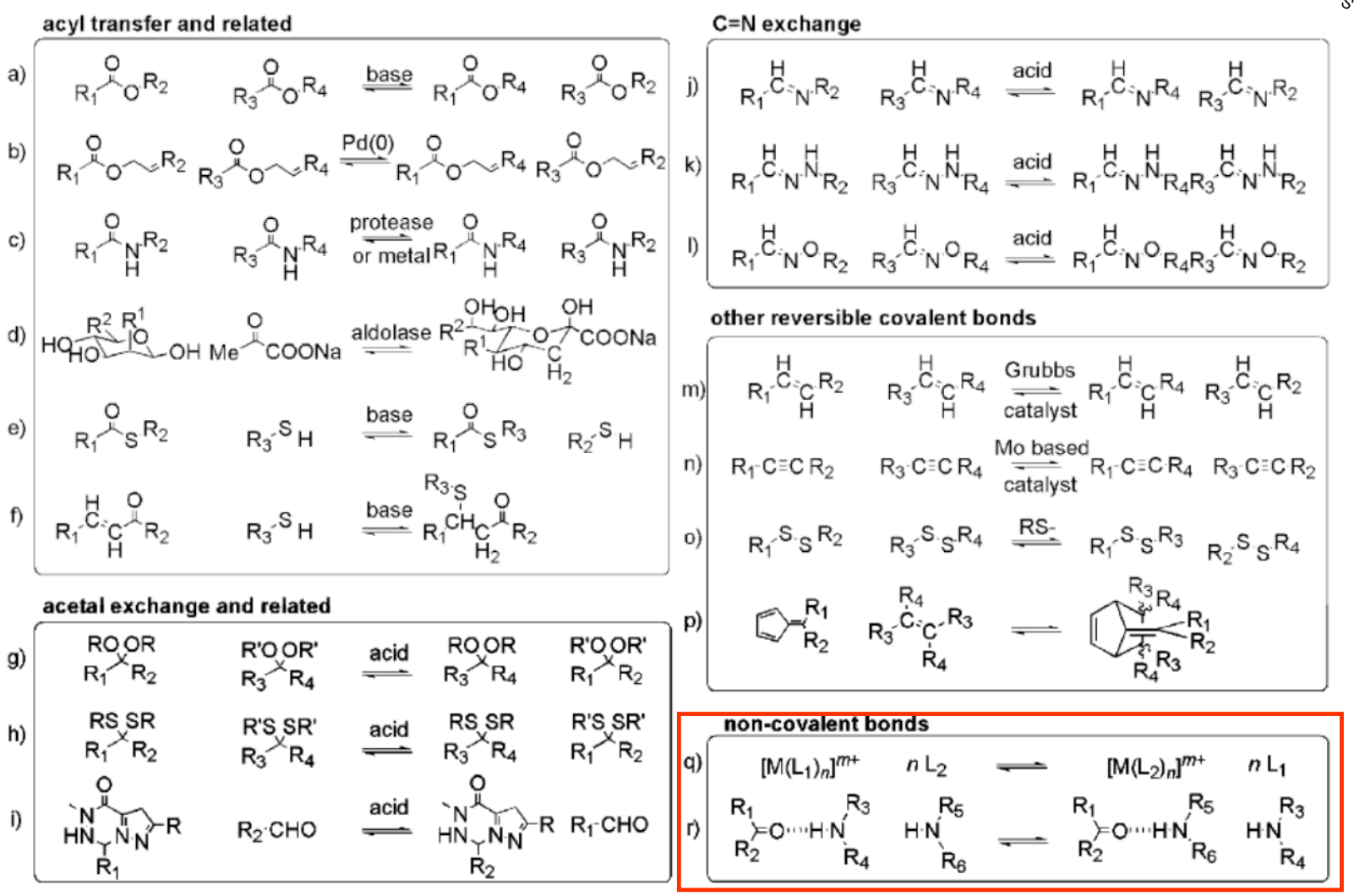
no formation witout metal

D. H. Busch *et al. JACS.* **1962**, *84*, 1762

> the composition is determined by the themodynamic stability, in equilibrium.
> upon the addition of templates, desired molecules can form the stable complex.
> not screen, but selection
→ evaluated in mixtures, not indivisually
> for the discovery of small molecules, catalysts, and materials

J.K.M. Sanders, S. Otto *et al. Chem Rev.*, 2006, *106*, 3652
D.R.Liu *et al. Curr. Opin. Chem. Biol.*, 2007, *11*, 259

Reversible Reactions Used for DCC



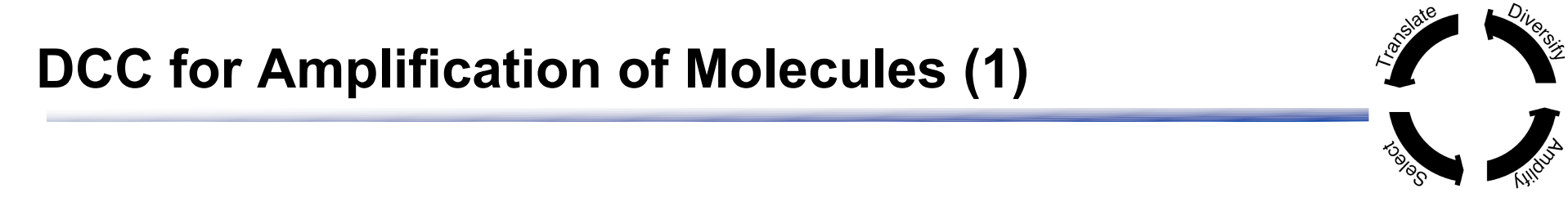
> weak and labile noncovalent bonds to achieve rapid equilibrium

vs

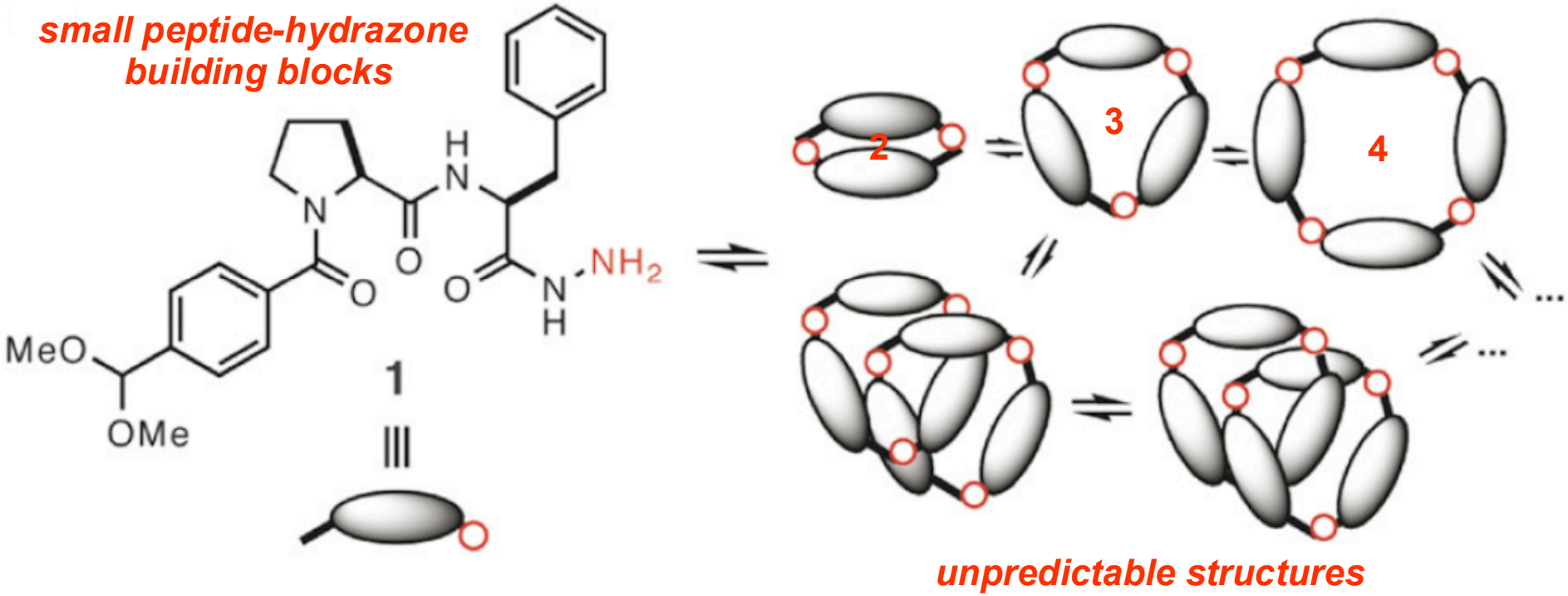
covalent bonds to ensure thermodynamic stability

J.K.M. Sanders, S. Otto *et al. Chem Rev.*, 2006, *106*, 3652

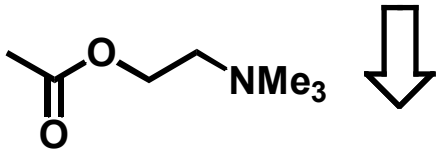
DCC for Amplification of Molecules (1)



small peptide-hydrazone building blocks



unpredictable structures



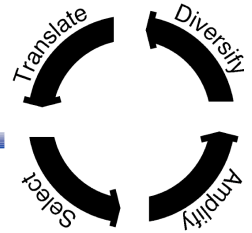
+ ACh

J. K. M. Sanders *et al. Org. Biomol. Chem.* **2003**, *1*, 1625

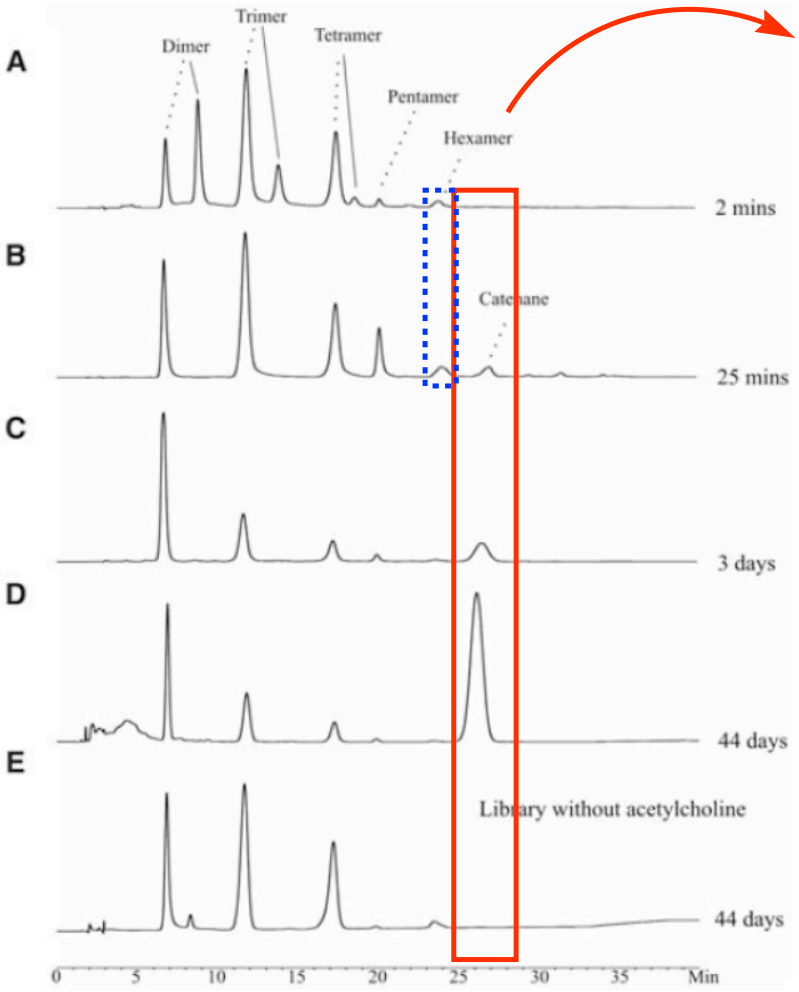
@ With the addition of ACh, accessing unpredictable structures ???

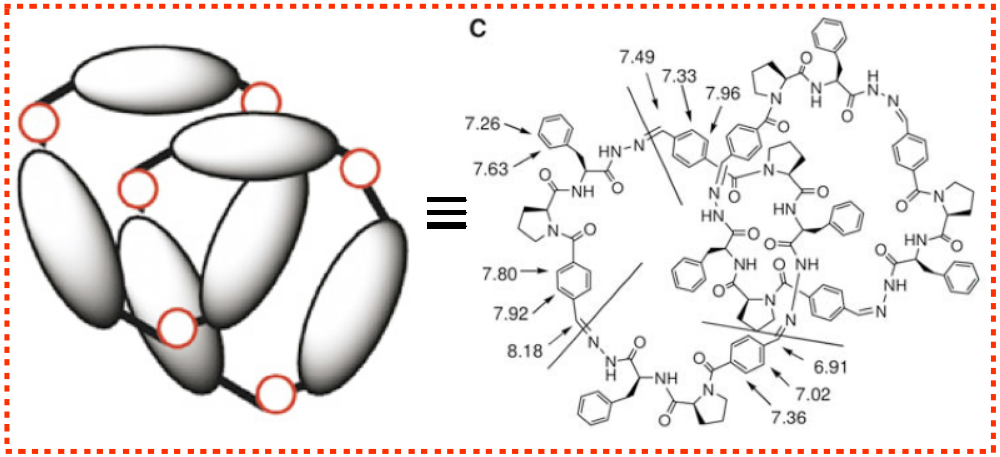
J.K.M. Sanders, S. Otto *et al. Science.*, 2005, *308*, 667

DCC for Amplification of Molecules (2)



HPLC traces





> *first kinetically favoured product was observed. (blue)*

> *afterwards thermodynamically favoured product was observed. (red)*
→ *[2]catenane*

> *[2]catenane was amplified at the expense of all the other materials.*

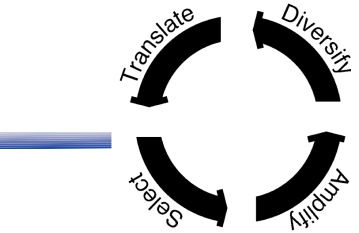
> *determined also by NMR analysis and MS analysis*

> *only one diastereoisomer in 67 % yield*

> *complex functions from small and simple SMs*

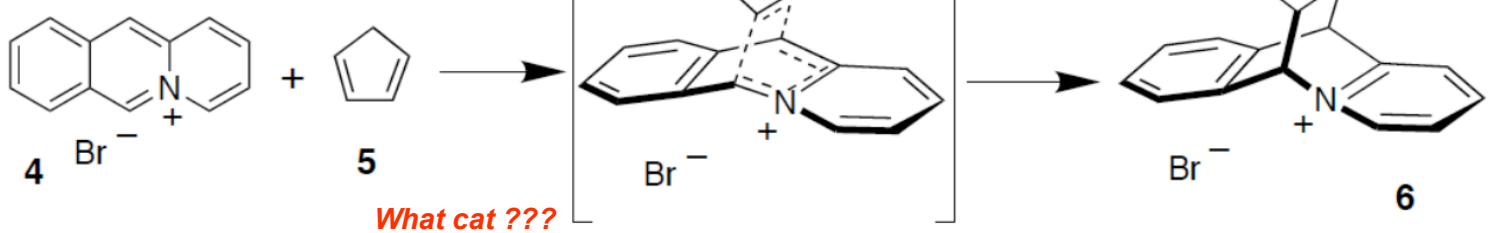
J.K.M. Sanders, S. Otto *et al. Science.*, 2005, *308*, 667

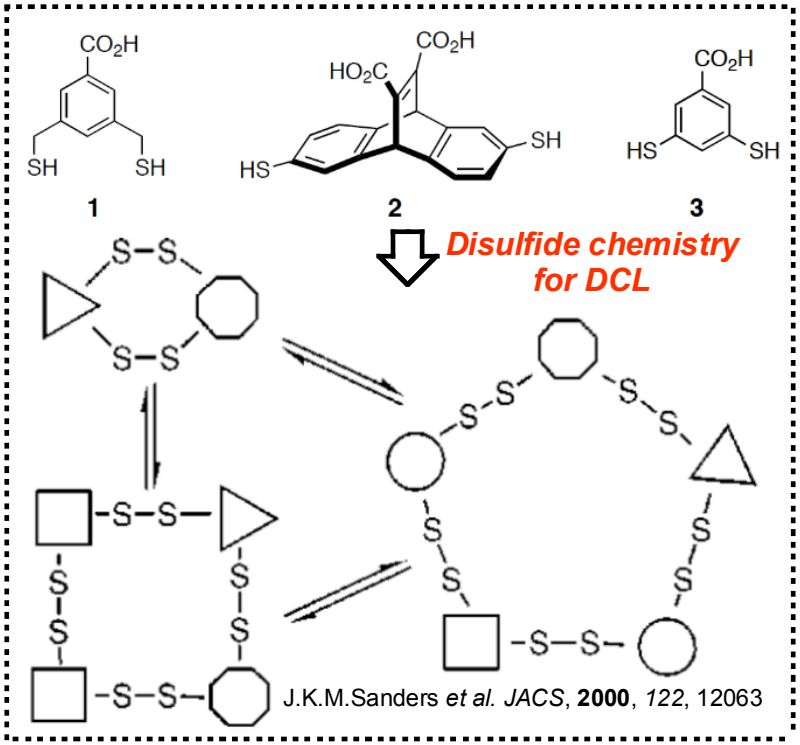
DCC for Catalyst Discovery (1)



DCL with anmonium ion

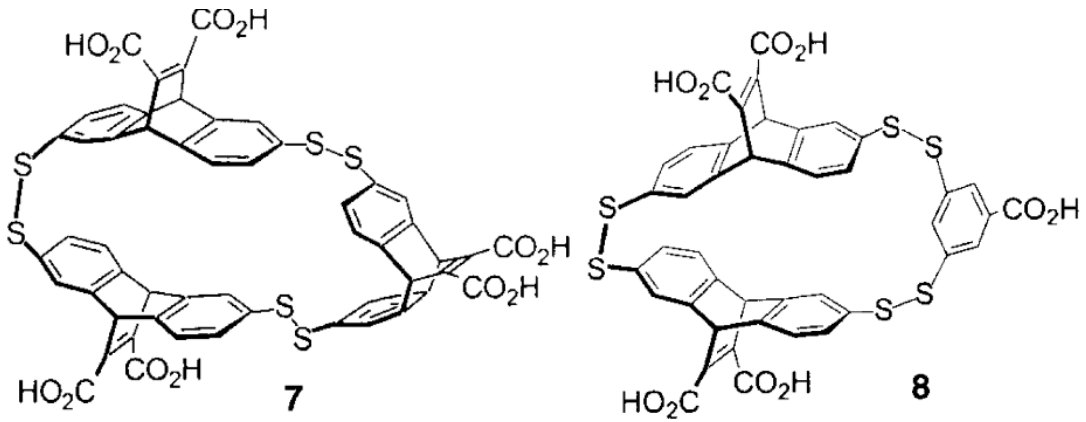
S. Otto *et al. Science.*, 2002, *297*, 590





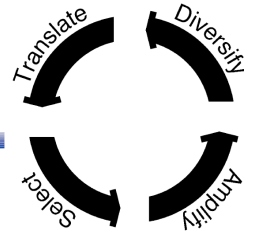
> disulfide chemistry for DCLs of macrocycles

> with high affinity for cationic hydrophobic molecules
→ the Diels-Alder reaction

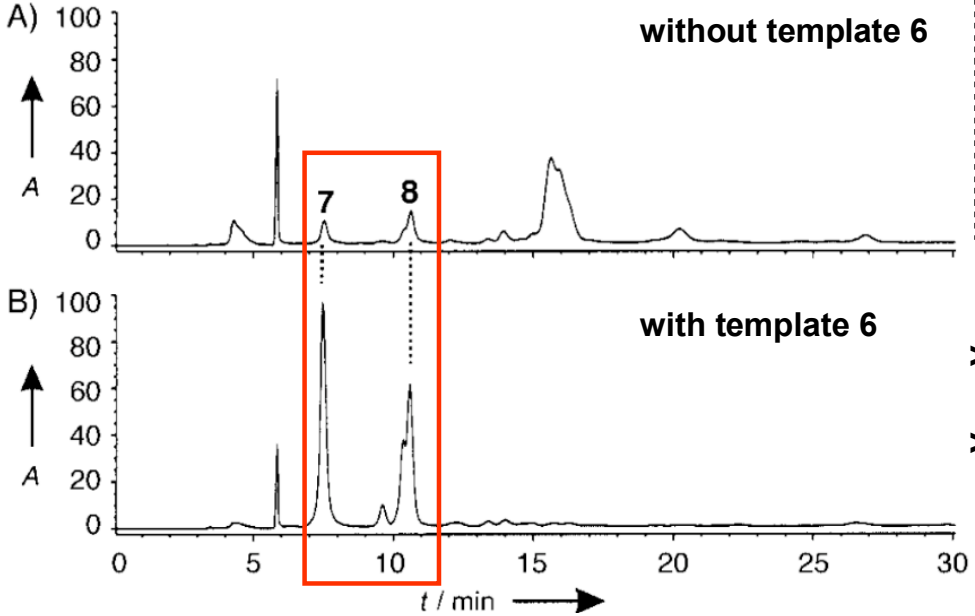


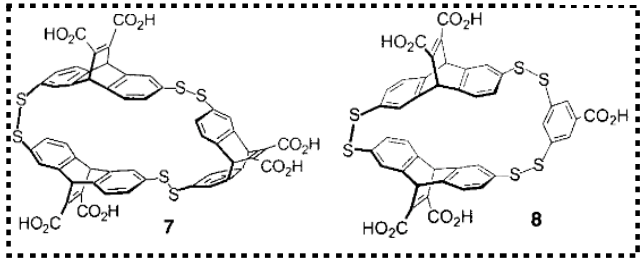
S. Otto *et al. ACIE.*, 2003, *42*, 1270

DCC for Catalyst Discovery (2)



HPLC analysis of a DCL





> *1, 2 and 3 mixed in water, under O_2*

> *With template, macrocycles 7 and 8 were obtained in the mixture.*

ITC analysis of the thermodynamic datas

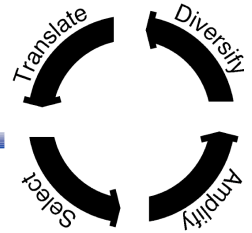
		7	8
4	K_1 [M^{-1}]	1.3×10^5	6.4×10^5
SM diene	$\Delta G°$ [kJ mol^{-1}]	−29.1	−33.1
	$\Delta H°$ [kJ mol^{-1}]	−23.7	−40.6
	$T\Delta S°$ [kJ mol^{-1}]	5.4	−7.5
6	K_1 [M^{-1}]	2.4×10^5	3.9×10^5
TM	$\Delta G°$ [kJ mol^{-1}]	−30.7	−31.9
	$\Delta H°$ [kJ mol^{-1}]	−25.8	−38.5
	$T\Delta S°$ [kJ mol^{-1}]	4.9	−6.6

> *8 binds more strongly with 4*
> *7 binds more strongly with 6*

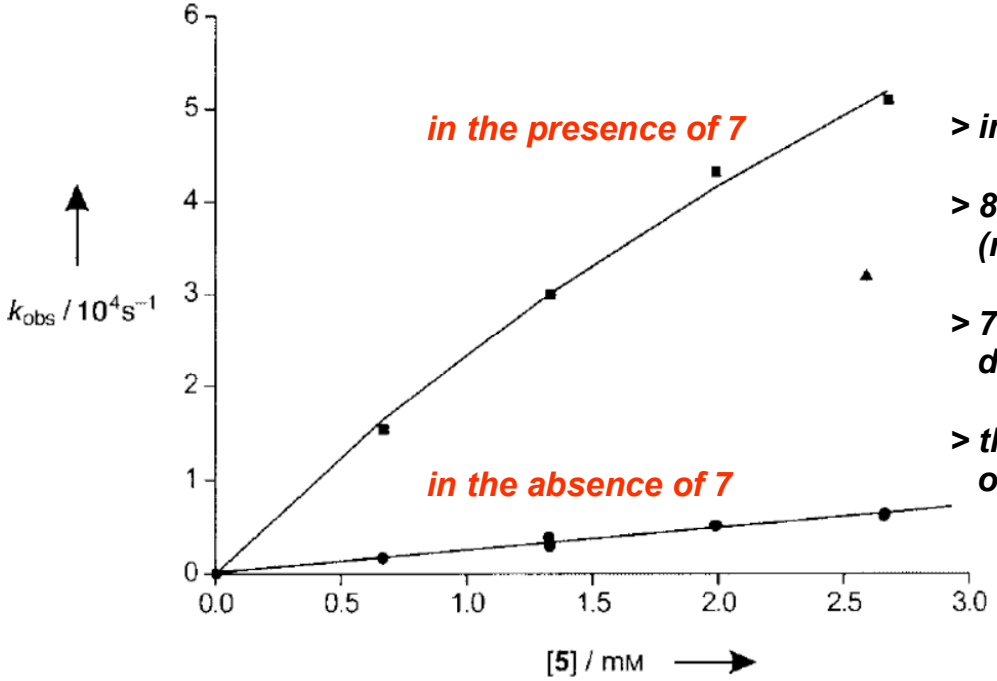
→ *incorporation of 5 into 7•4 complex*
→ *7 would be catalytically active*

S. Otto *et al. ACIE.*, 2003, *42*, 1270

DCC for Catalyst Discovery (3)



Observed rate constant for the Diels-Alder reaction

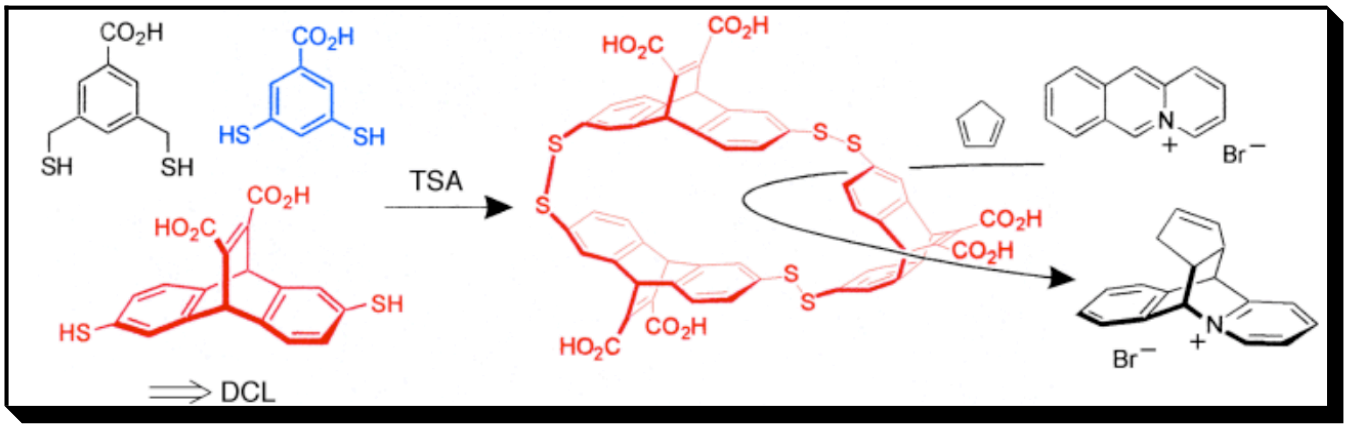


> *indeed 7 induced a modest acceleration*

> *8 turned out to be catallytically inactive (not shown)*

> *7 seemed to leave enough space to bind dienophile 5*

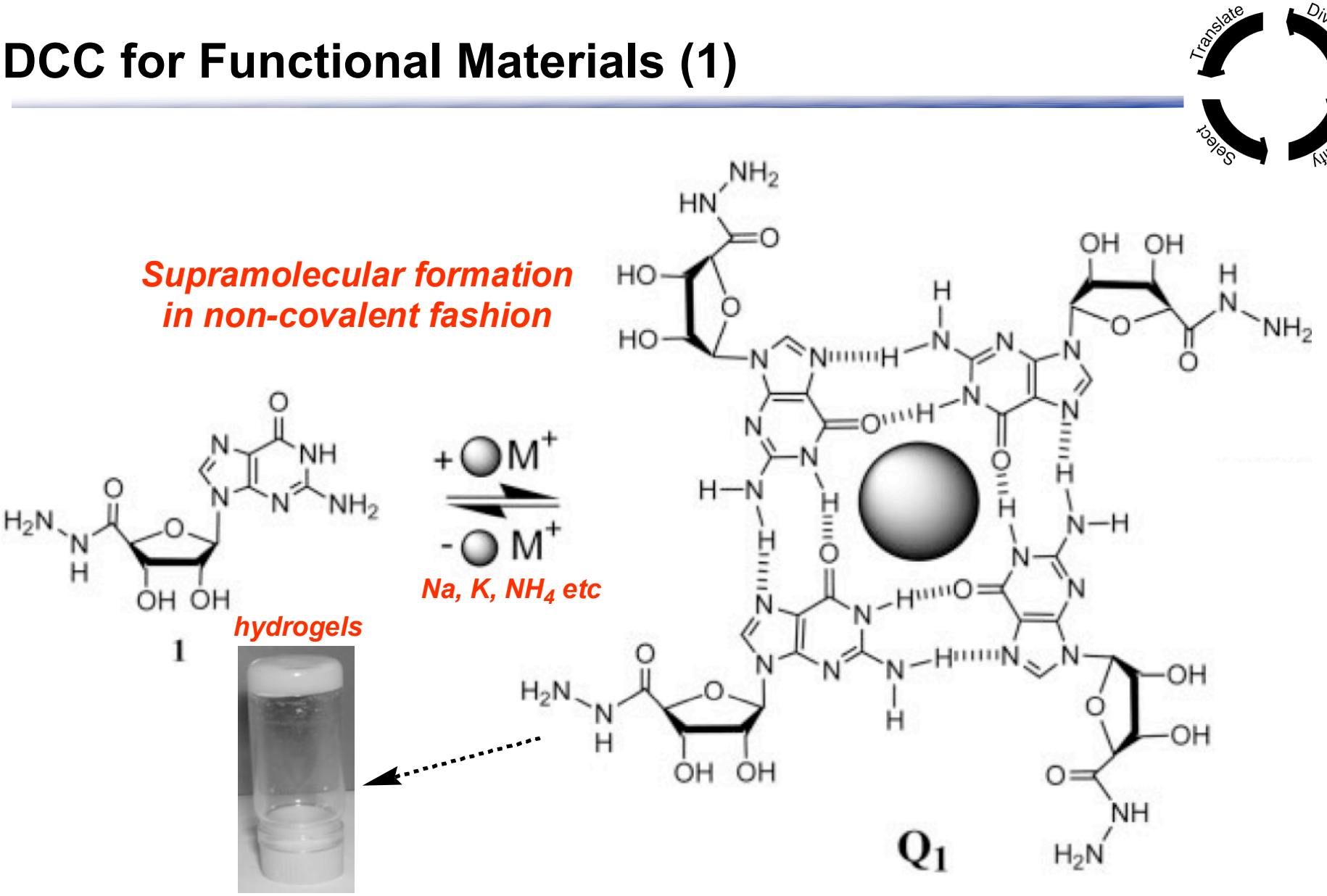
> *the efficiency of 7 was moderate compared with other catalysts*



@ In the mixture, the reacton reversibly discovered the efficient catalysts

S. Otto *et al. ACIE.*, 2003, *42*, 1270

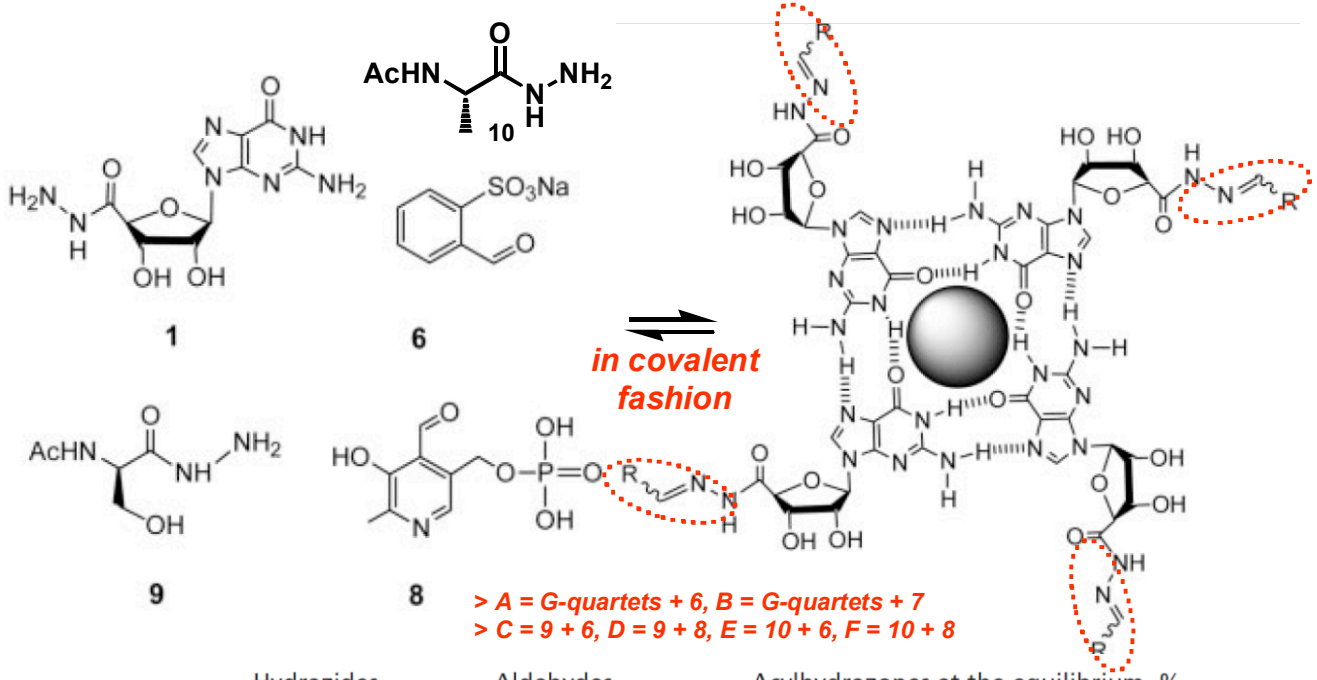
DCC for Functional Materials (1)



> undergoing the association into *G-quartets* in the presence of *cations*
> *with hydrogel formations*

J. M. Lehn *et al. PNAS.*, 2005, *102*, 17

DCC for Functional Materials (2)



> A = G-quartets + 6, B = G-quartets + 7
> C = 9 + 6, D = 9 + 8, E = 10 + 6, F = 10 + 8

	Hydrazides			Aldehydes		Acylhydrazones at the equilibrium, %					
Entry	1	9	10	6	8	A	B	C	D	E	F
1	1	1	0	1	1	8	39	42	11	—	—
2	1	0	1	1	1	9	37	—	—	40	12
3	0	1	0	1	1	—	—	15	85	—	—
4	0	2	0	1	1	—	—	50	50	—	—
5	1	1	0	0	1	—	87	—	13	—	—
6	1	0	1	0	1	—	96	—	—	—	4
7	1	1	0	1	0	48	—	52	—	—	—
8	1	0	1	1	0	51	—	—	—	49	—
9	0	1	1	1	1	—	—	25	25	25	25

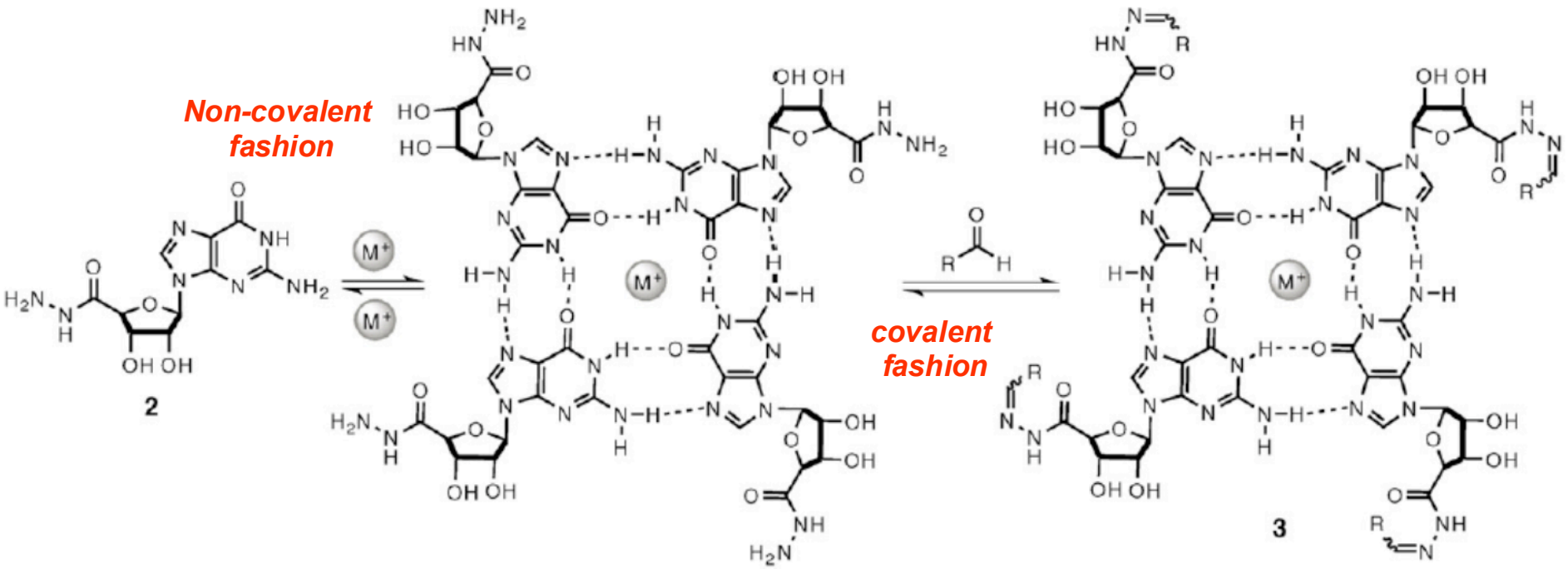
> *^{1}H NMR* determined the equilibrium

> At higher temp (~ 80 ºC), the selectivity got worse due to t*he melting of hydrogels*

> with the *thermodynamic gelation* event, 1 preferencially scavenged 8.

J. M. Lehn *et al. PNAS.*, 2005, *102*, 17

DCC for Functional Materials (3)



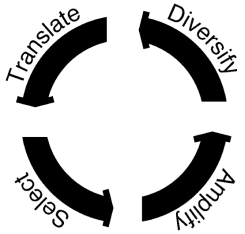
> the lability of *noncovalent interactions* + *reversible covalent* bonds
→ multistep self-assembly for DCLs

> with *themoreversible hydrogels*

> demonstrating DCC can be applied to a *supramolecular* scale
→ discovery of *materials* with desired bulk properties

J. M. Lehn *et al. PNAS.*, 2005, *102*, 17

Features of DCC



Advantages

> *reversible and thermodynamical* process in equilibrium

> desired = amplified, undesired = removed
→ *'automatically' selection + amplification*

> applications to small molecules, catalysts, materials

Disadvantages

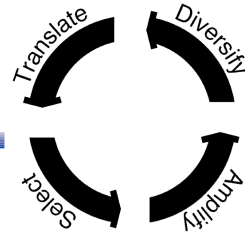
> *limited diversity* of available building blocks for appropreate targets

> difficulty of deconvoluting the complex mixtures

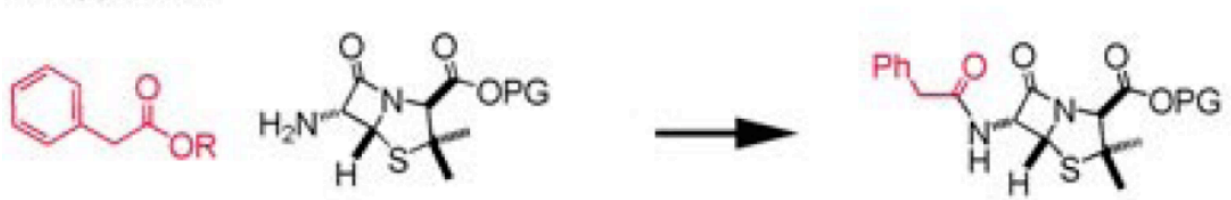
> Life can be thought of as '*a kinetic state of matter*'
→ systems biology and systems chemistry ???

3. DNA-templated Synthesis

DNA for Evolutional Approaches

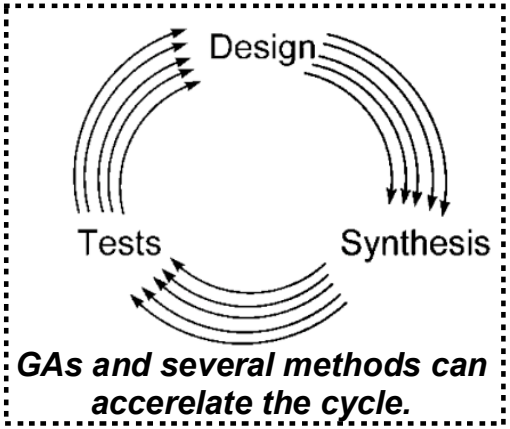


Chemists' approach to control reactivity



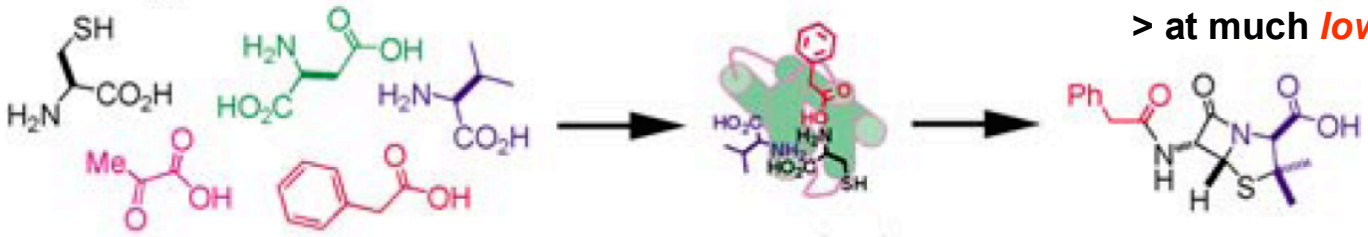
mM–M concentrations isolated in one vessel

one possible product



GAs and several methods can accerelate the cycle.

Nature's approach to control reactivity



nM–μM concentrations of many reactants in one solution

macromolecule-templated synthesis

selective product formation

> in *one-pot* fashion
> reactions are *randomly* performed
> at much *lower concentrations*

> ideally suited to perform the iterative operation (*translation, selection, amplification and diversification*)
→ power of *PCR* method

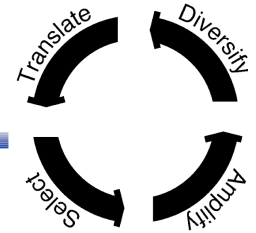
DNA as a tool for evolutional approaches

> with *high information density*
> with *chemical stability*
> with the *ease of manipulation*

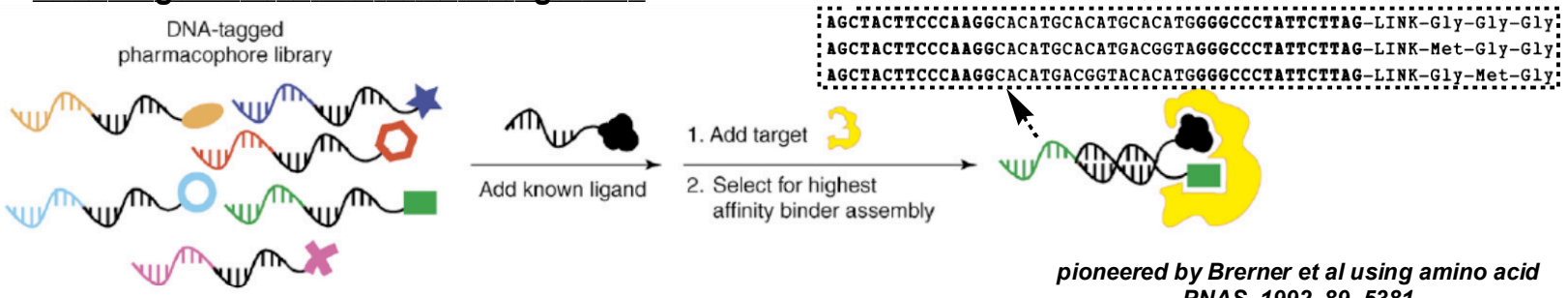
D. R. Liu *et al. ACIE.*, 2004, *43*, 4848

D. R. Liu *et al. Curr. Opin. Chem. Biol.*, 2007, *11*, 259

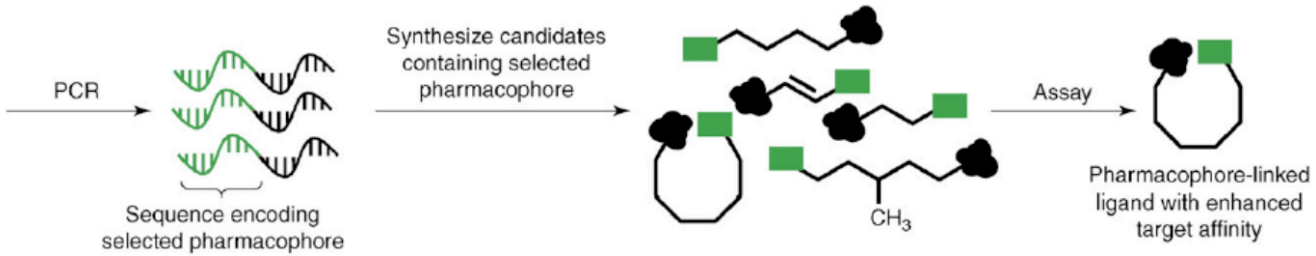
Several Methods



Encoding Chemical Libraries Using DNA > ***identification of DNA-limked small molecules***

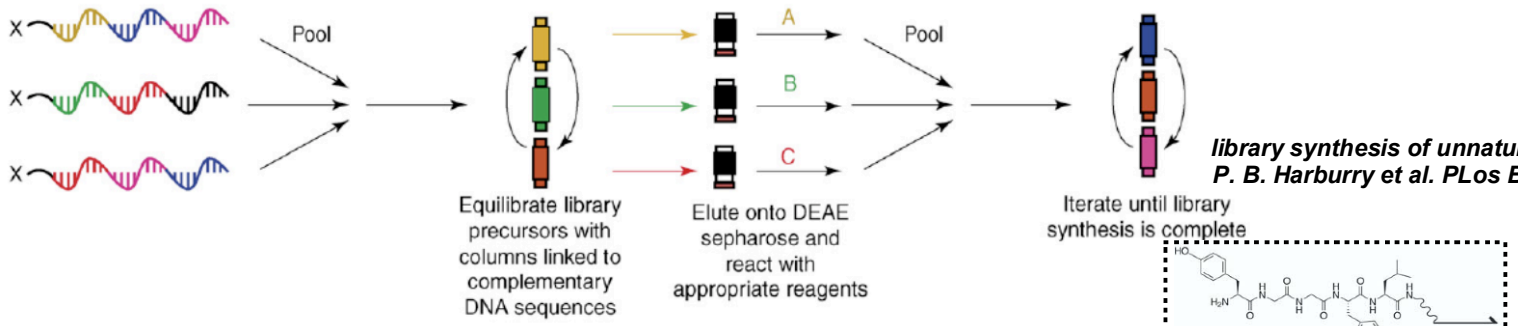


pioneered by Brerner et al using amino acid
PNAS. 1992, 89, 5381

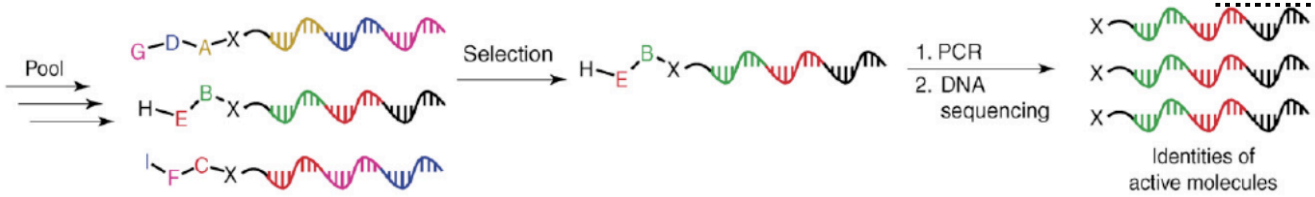


Also see:
D. Neri *et al. Nat. Biotechnol.*, 2004, *22*, 568

DNA Library > ***reaction of DNA-linked small molecules individually***

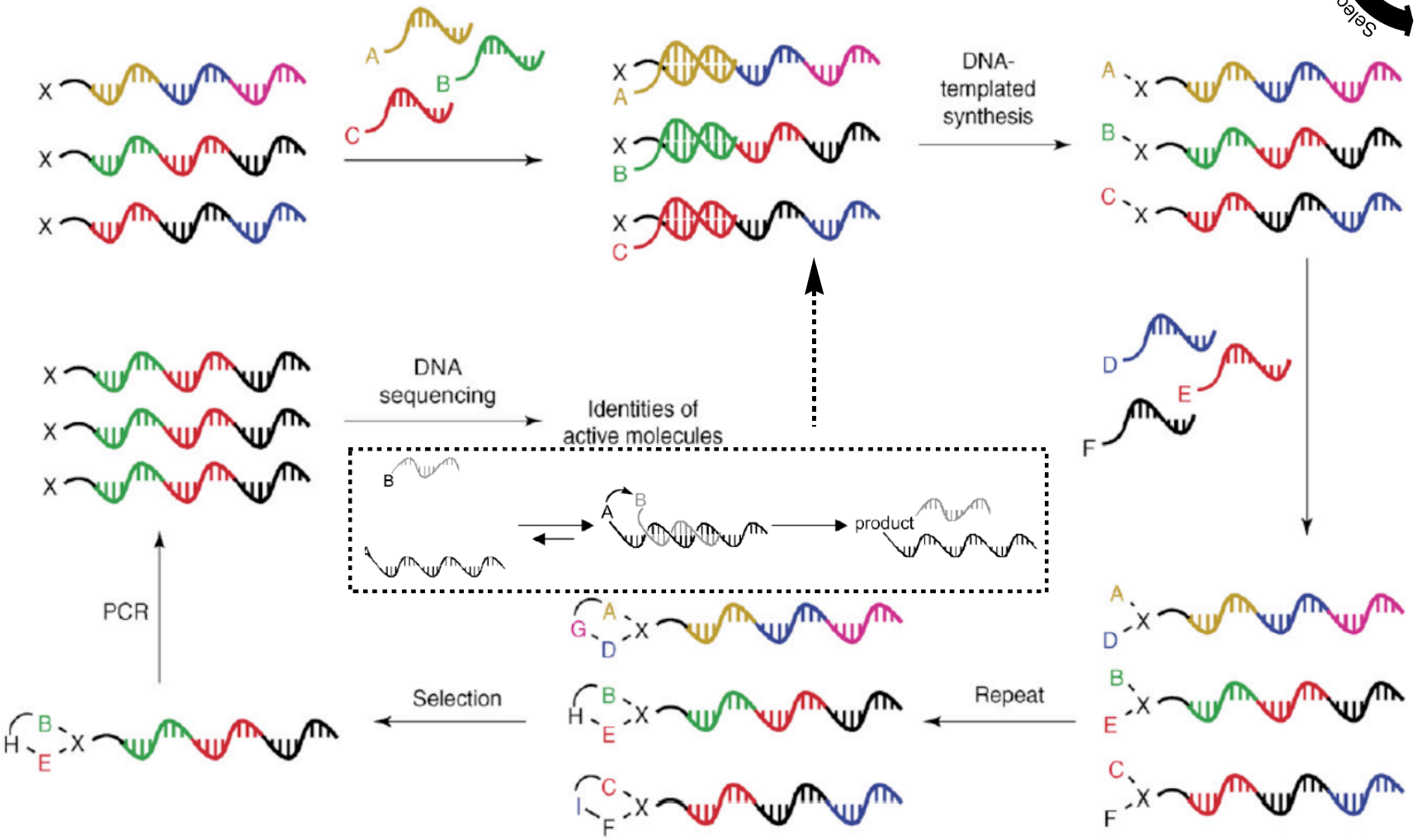


library synthesis of unnatural pentapeptides
P. B. Harburry et al. PLos Biol. 2004, 2, 1022



D.R.Liu *et al. Curr. Opin. Chem. Biol.*, 2007, *11*, 259

DNA-Templated Synthesis

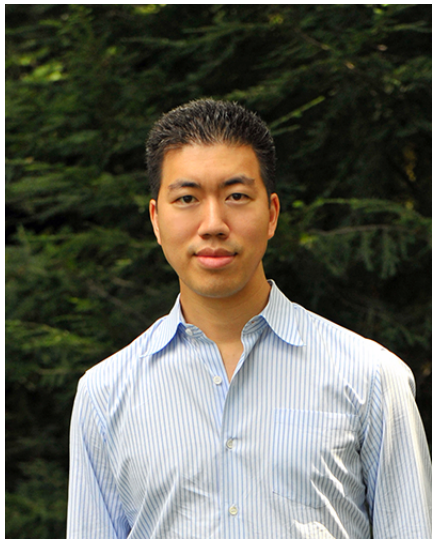


> *spatial separation is not required*
> *DNA annealing controlls reactivity*
> *→ all templates and reagents coexist in a single solution*
> Using complementary DNA-linked reagents *via Watson-Crick base-pairing*

D.R.Liu *et al. ACIE.*, 2004, *43*, 4848

D.R.Liu *et al. Curr. Opin. Chem. Biol.*, 2007, *11*, 259

David R. Liu as a Key Person



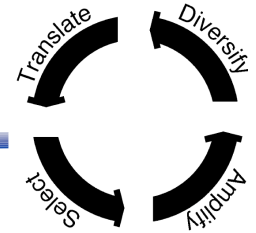
Training

1991-1994 (B.A. research)	Conducted research in synthetic organic chemistry, biochemistry, and molecular biology on 2,3-oxidosqualene cyclase (a key enzyme in steroid biosynthesis) under the guidance of Professor E. J. Corey at Harvard University.
1994-1999 (Ph.D. research)	Probed the mechanism of chorismate mutases using site-directed natural and unnatural amino acid mutagenesis; designed new tRNAs for unnatural amino acid mutagenesis; initiated the engineering and evolution of proteins and nucleic acids for the site-specific incorporation of unnatural amino acids into proteins in living cells. Research conducted under the guidance of Professor Peter G. Schultz at the University of California, Berkeley.

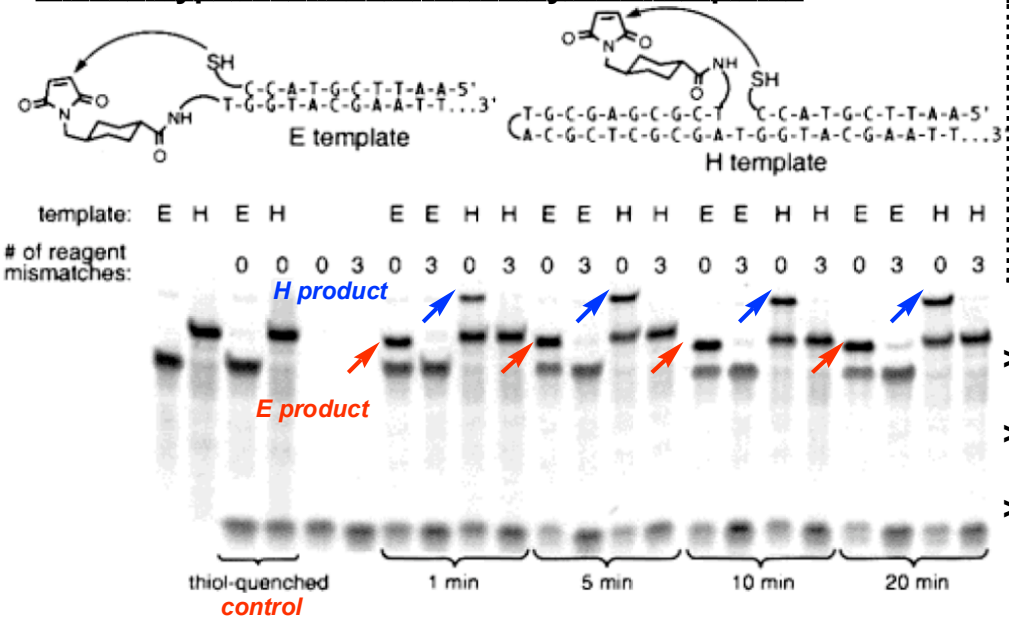
Positions

1999-2003	Assistant Professor of Chemistry and Chemical Biology, Harvard University
2003-2004	John L. Loeb Associate Professor of the Natural Sciences and Associate Professor of Chemistry and Chemical Biology, Harvard University
2007-2010	Harvard College Professor, Harvard University
2005-present	Senior Associate Member of the Broad Institute of Harvard and MIT (2010-present); Associate Member of the Broad Institute of Harvard and MIT (2005-2010)
2005-present	Professor of Chemistry and Chemical Biology, Harvard University and Investigator, Howard Hughes Medical Institute

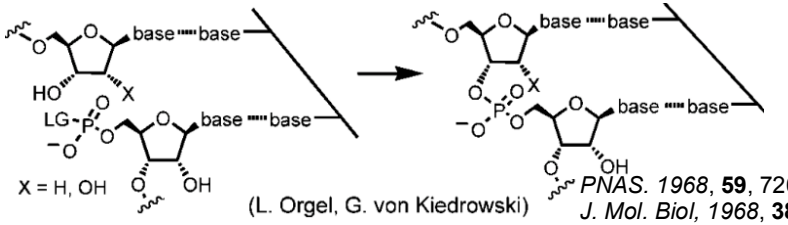
Pioneering Work (1)



Michael-type reactions directed by DNA templates



@ Early work: Synthesis of oligonucleotide analogue



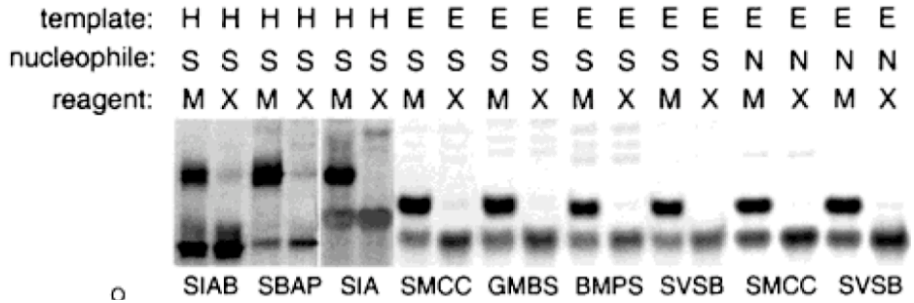
PNAS. 1968, **59**, 726;
J. Mol. Biol, 1968, **38**, 381

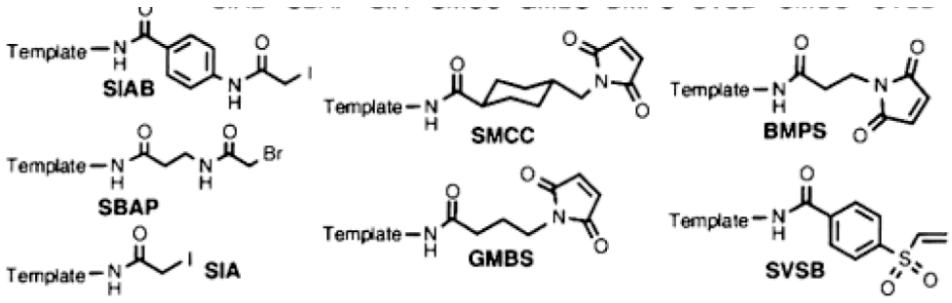
> ***H and E DNA architectures exhibited similar reactivities.***

> ***no products were observed by using mismatched DNAs.***

> ***DNA-templated reactions proceeded even the products differed from the natural backbones.***

Various reactions directed by DNA templates

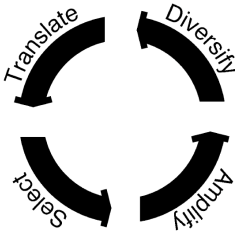




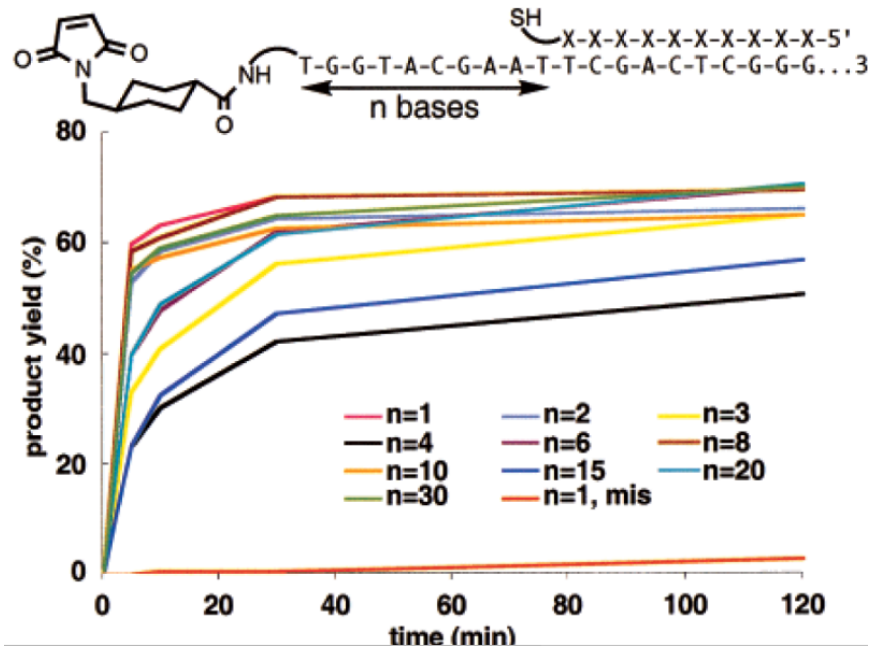
> ***Other reaction systems could be applied for (SN_2 substitutions, additions to $\alpha\beta$-unsaturated carbonils etc).***

D.R.Liu *et al. JACS.*, 2001, *123*, 6961

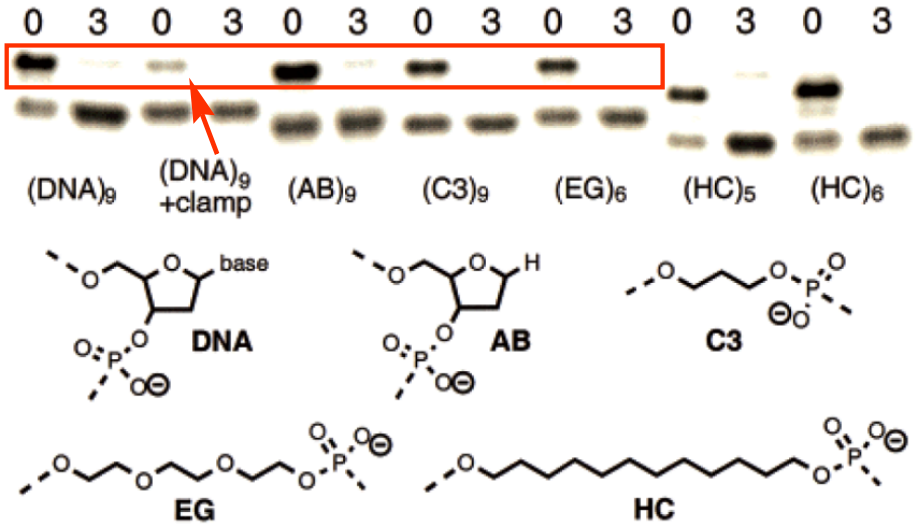
Pioneering Work (2)



Distant-independent (DTS) DNA-templated synthesis



Evaluation of possible contribution



> *possible contributions were evaluated.*
> *(conformational prefecences, interbase interactions, backbone hydrophilicity etc)*

> *demonstrating remarkable distance independence.*

> *Even in 30 bases distances, efficiently reacted.*
> *(through transition state 200 membered ring !!!)*

> *sharply contrast with traditional organic synthesis*

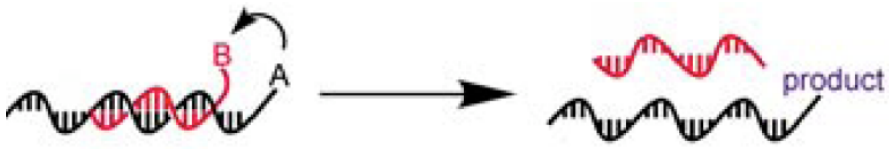
> *the backbone structural elements were not responsible.*

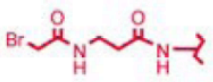
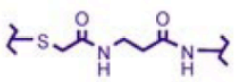
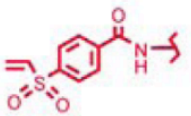
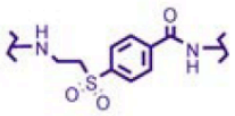
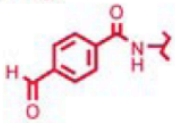
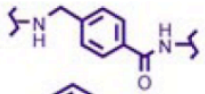
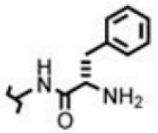
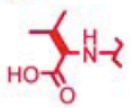
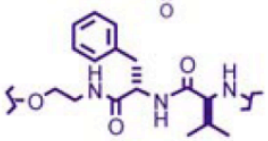
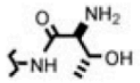
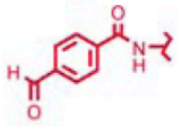
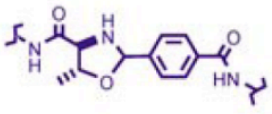
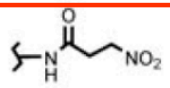
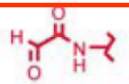
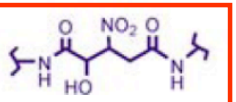
> *complementary DNAs to interventing region significantly reduced the products.*
> *→ the flexibility of the region is likely to crucial.*

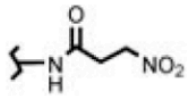
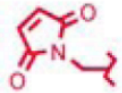
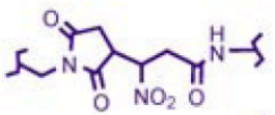
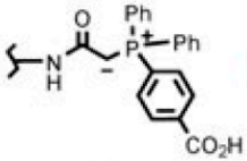
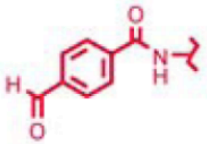
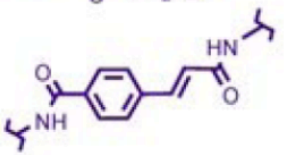
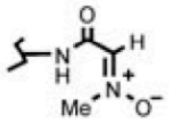
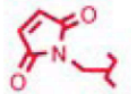
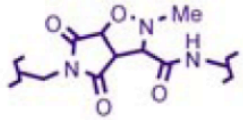
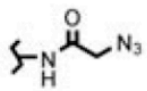
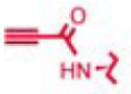
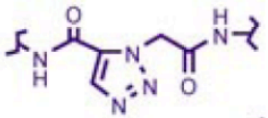
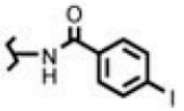
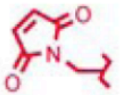
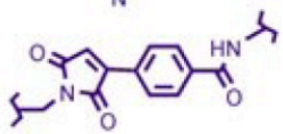
@ The fact that DNA annealing became the RDS would explane the distant independence.
(decreasing the conc. resulted in a marked reduce in the reaction rate)

D.R.Liu *et al. JACS.*, 2001, *123*, 6961

Expansion to the Reaction Generality



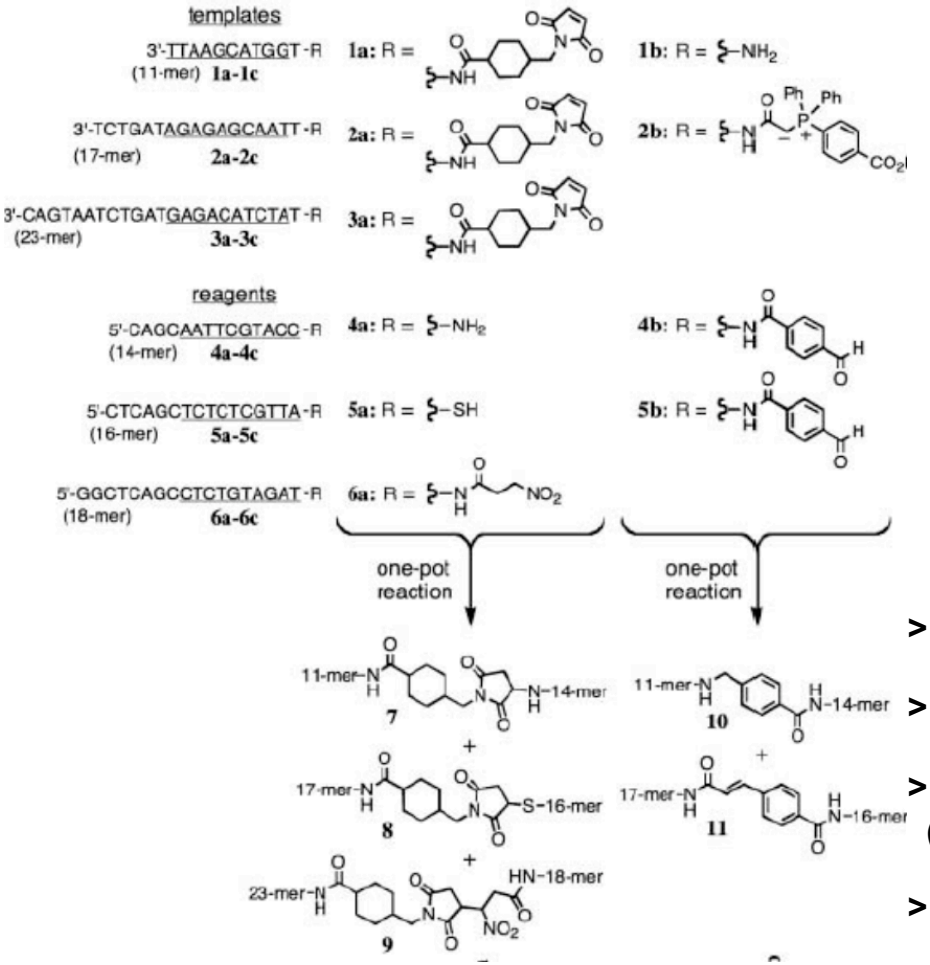
	A	B	product
S_N2[44]			
conjugate addition[44]			
reductive amination[43]			
amine acylation[43]			
oxazolidine formation[102]			
nitro-aldol (Henry)[43]			

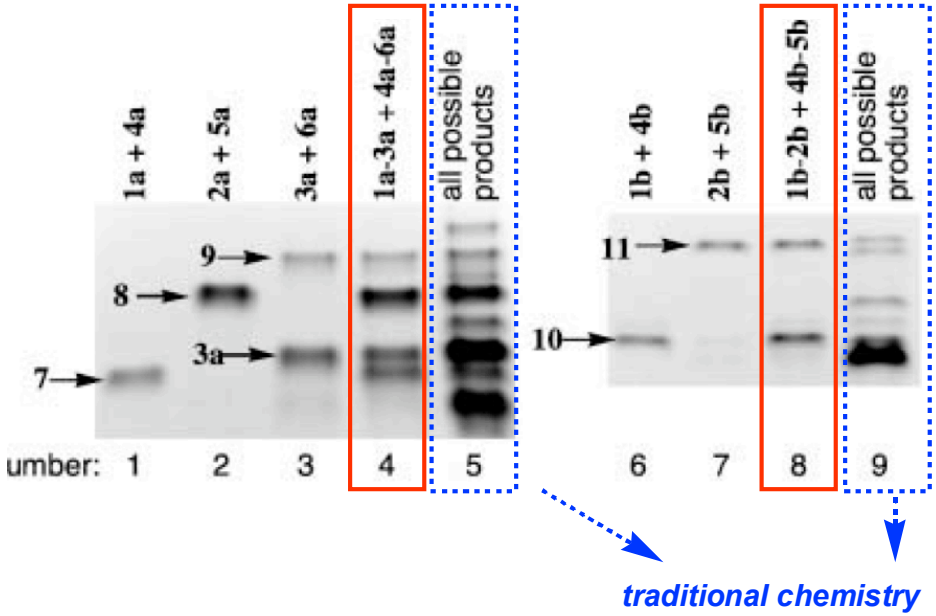
nitro-Michael[43]			
Wittig olefination[43]			
1,3-nitrone cycloaddition[43]			
Huisgen cycloaddition[46]			
Heck coupling[43]			

> the first useful C-C bond forming ractions (nitro-aldol, Wittig, Heck coupling etc)

D.R.Liu *et al. ACIE.*, 2002, *41*, 1796
D.R.Liu *et al. ACIE.*, 2003, *42*, 1370

New Modes of Controlling Reactivity (1)





> different type of reactions were conducted in one solution.

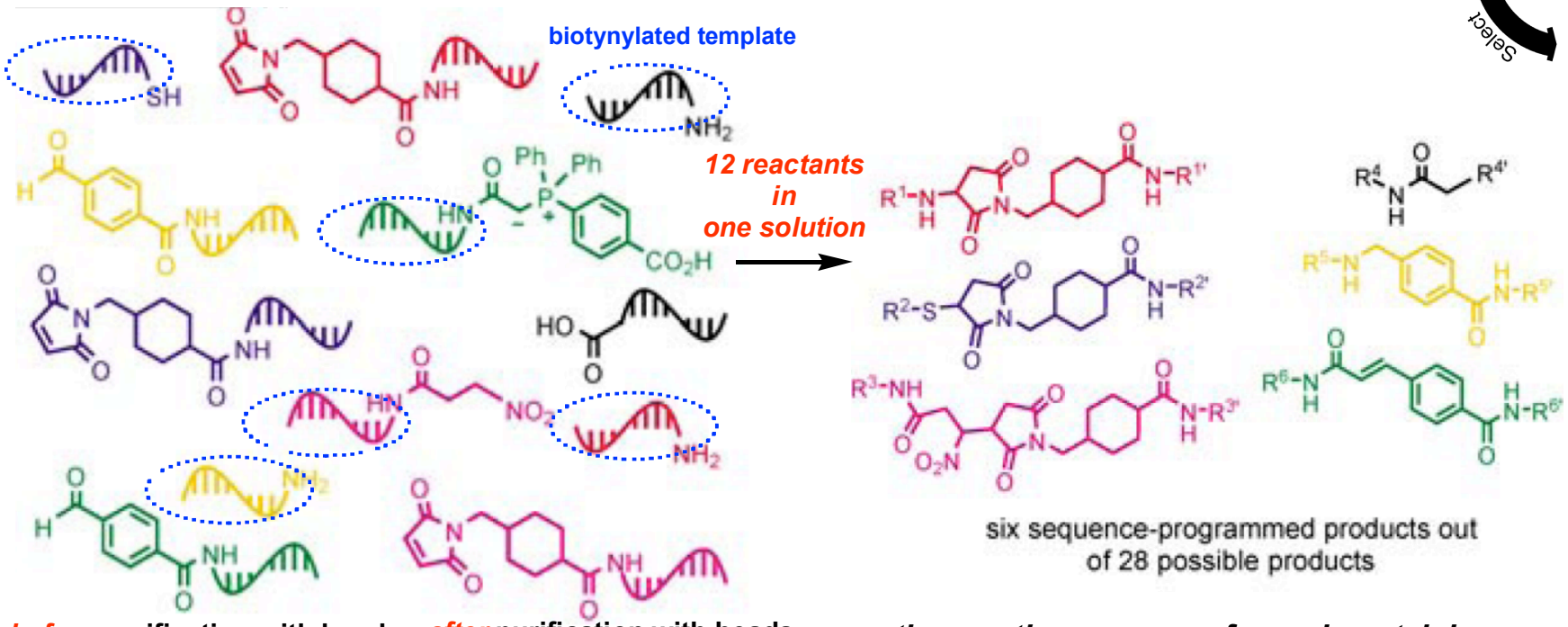
> the different length of DNAs enabled easier detection.

> selective 3 reactions took place to afford 7, 8, 9 exclusively. (from 9 possible reactions)

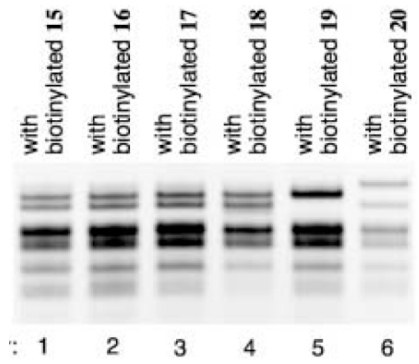
> other possible products were obtained in < 5 %.

D.R.Liu *et al. ACIE.*, 2002, *41*, 4140

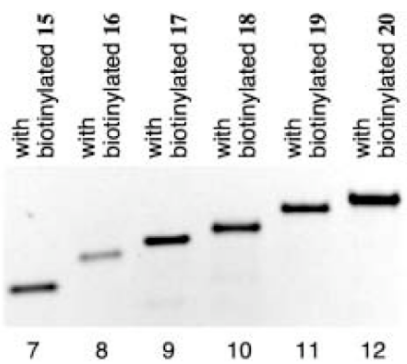
New Modes of Controlling Reactivity (2)



before purification with beads



after purification with beads



> *the reactions were performed containing 6 templates and 6 reactants in one solution.*

> *6 templates with biotinylation*

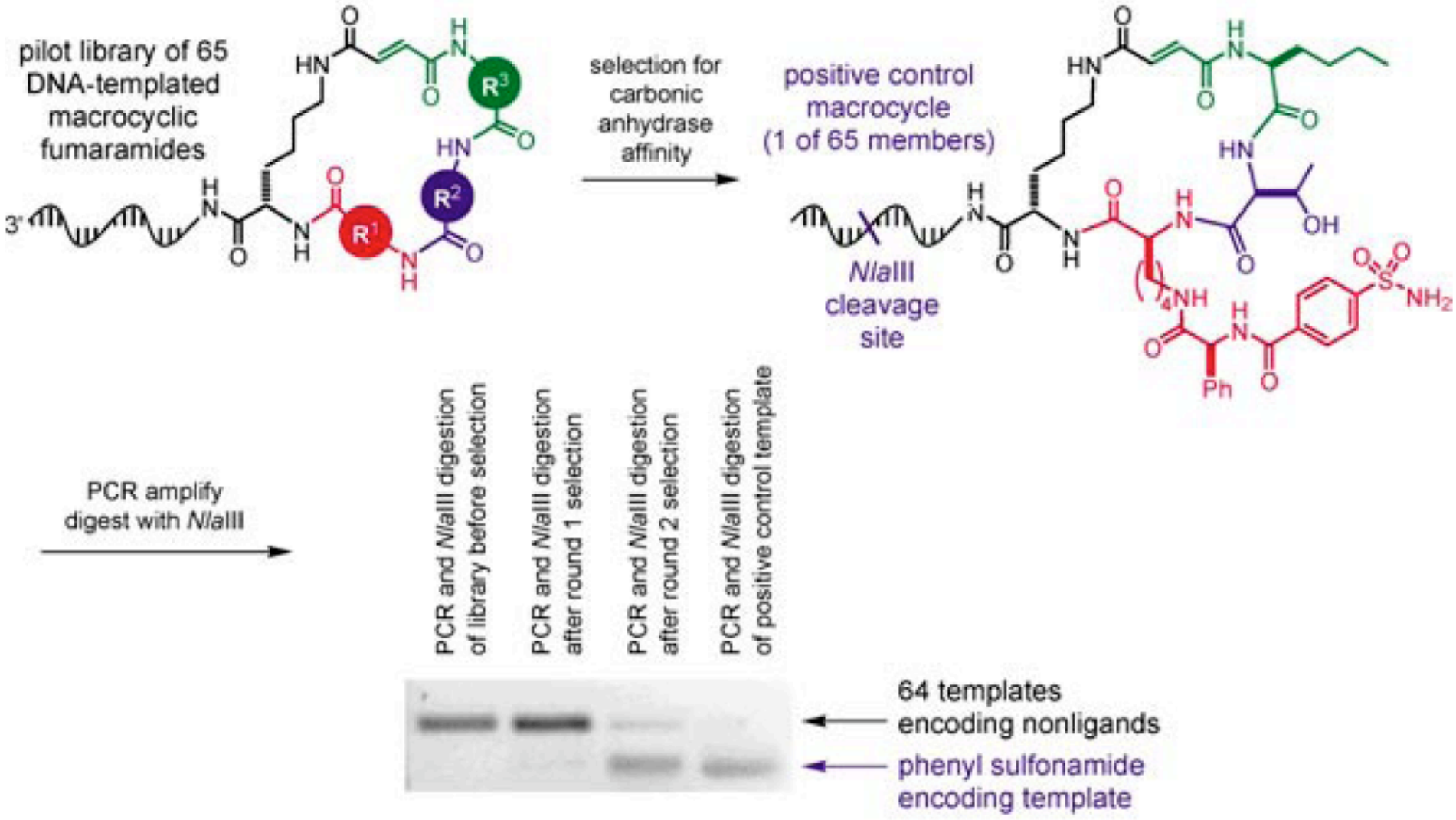
> *biotynylated templates could be purified with magnetic beads.*

> *only the single product was obtained pragrammed by DNA sequences.*

@ DTS exhibited the ability to direct different types of reactions in one solution, incompatible under traditional reaction conditions.

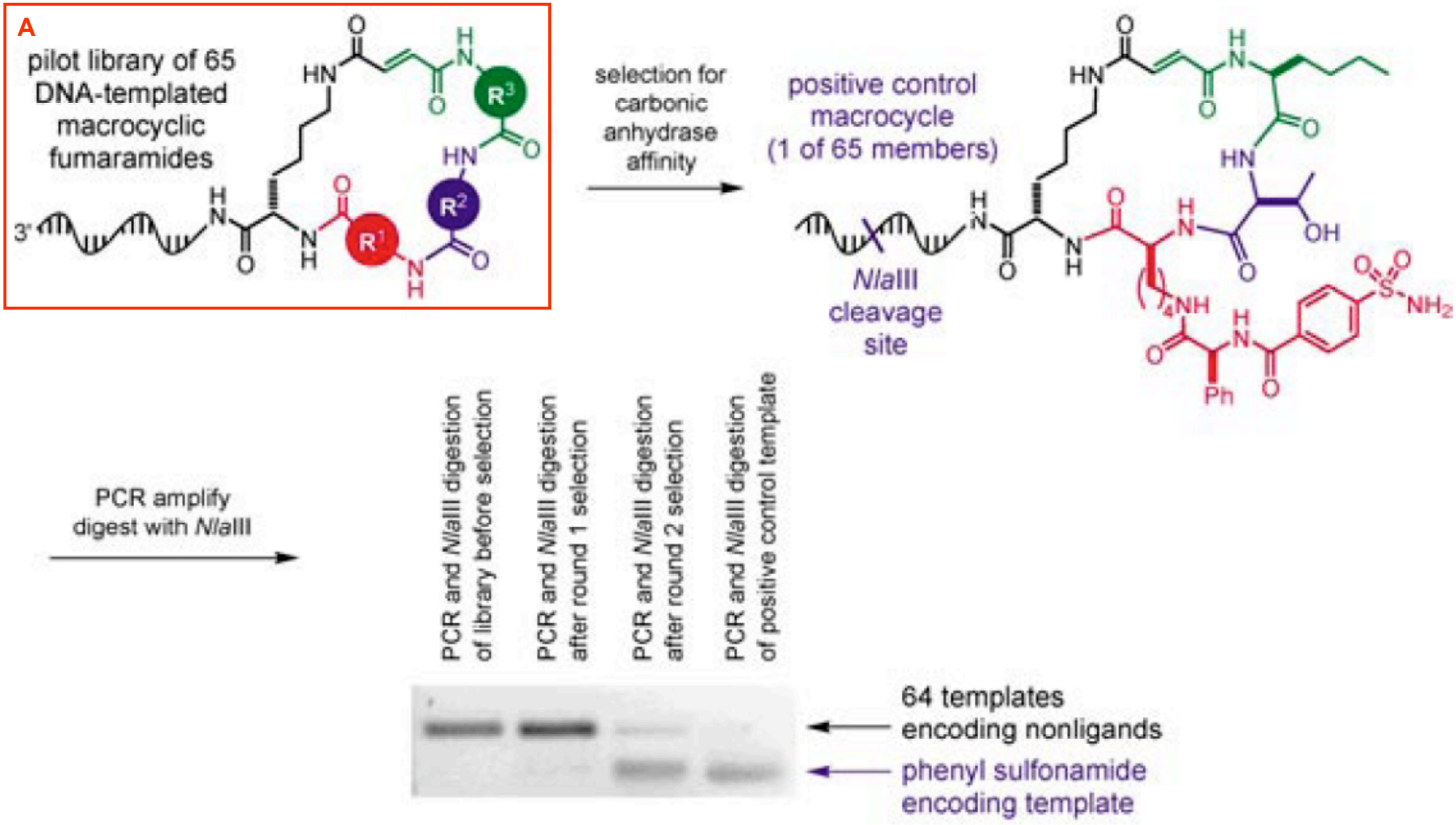
D.R.Liu *et al. ACIE.*, 2002, *41*, 4140

Applications of DTS ~ Selection of a Library (1)



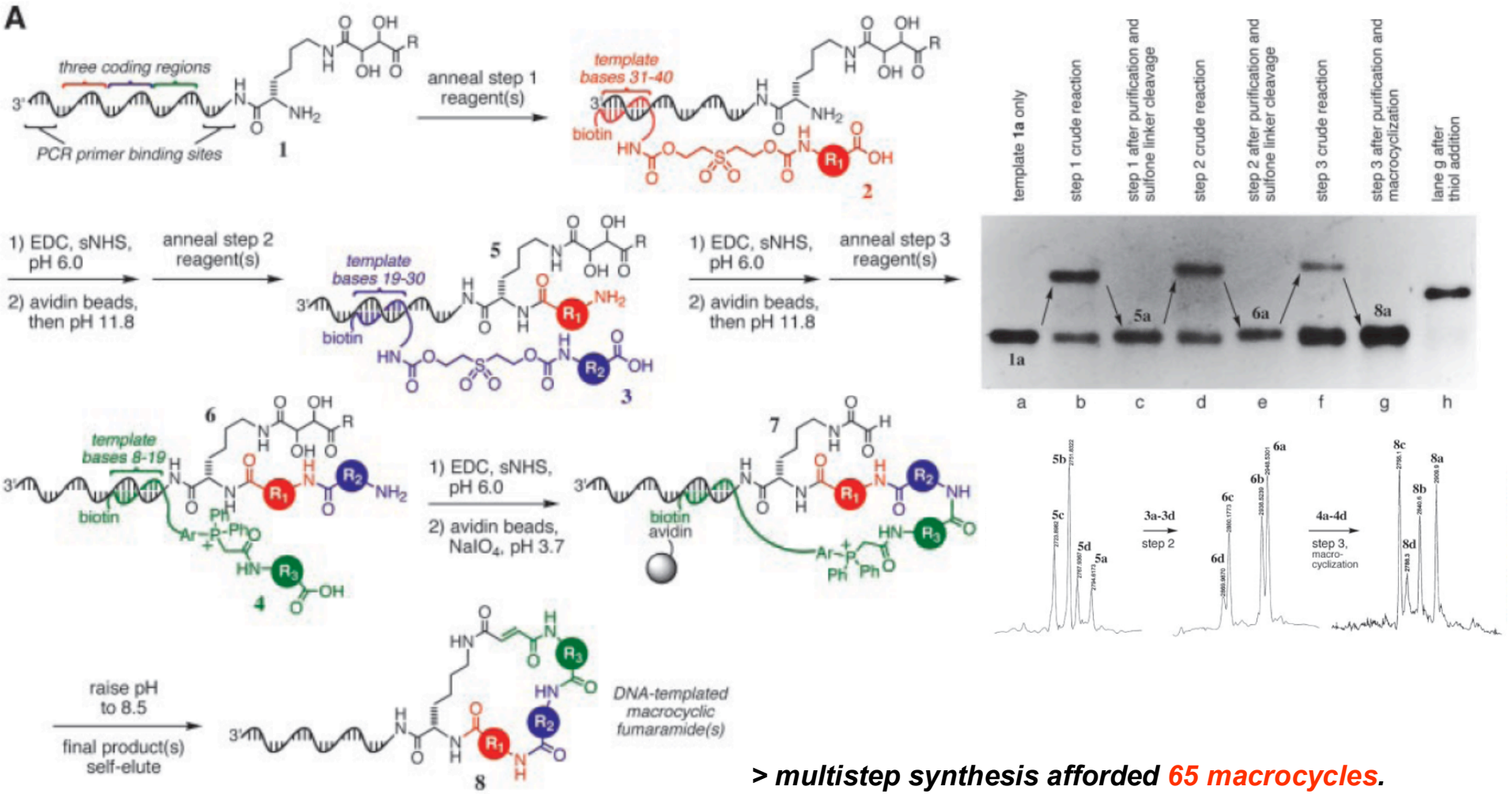
D.R.Liu *et al. Science.*, 2004, *305*, 1601

Applications of DTS ~ Selection of a Library (1)



D.R.Liu *et al. Science.*, 2004, *305*, 1601

Applications of DTS ~ Selection of a Library (2)



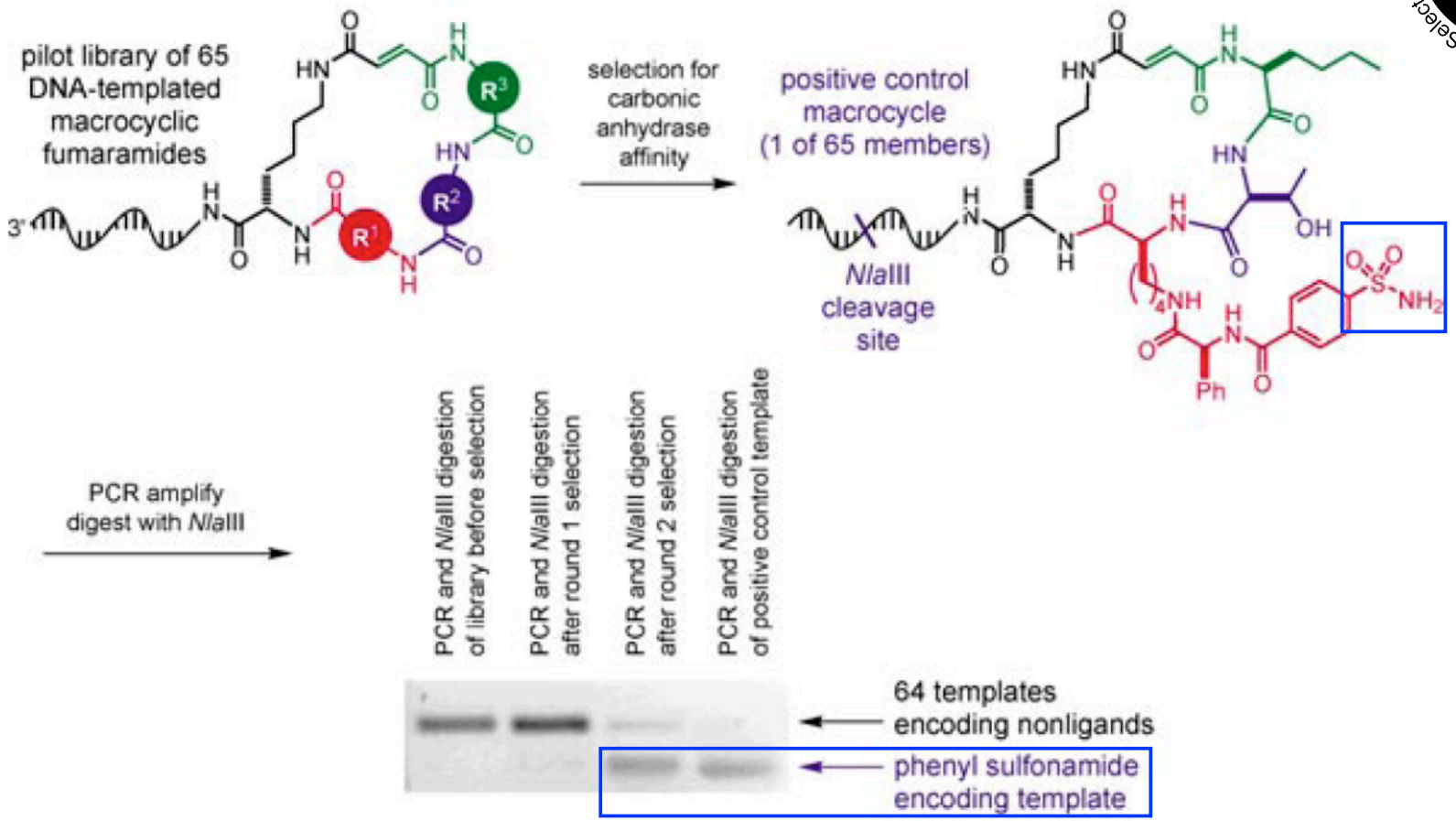
> multistep synthesis afforded 65 macrocycles.

> each step was monitored by PAGE and TOF-MS

D.R.Liu *et al. JACS.*, 2002, *124*, 10304

D.R.Liu *et al. Science.*, 2004, *305*, 1601

Applications of DTS ~ Selection of a Library (3)



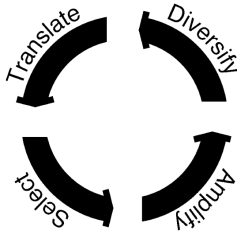
> a pilot liblary of 65 macrocycles was utilized for selective protein binding affinity (carbonic anhydrase).
> 2 selection rounds enriched the single molecule containing sulfonamide group.

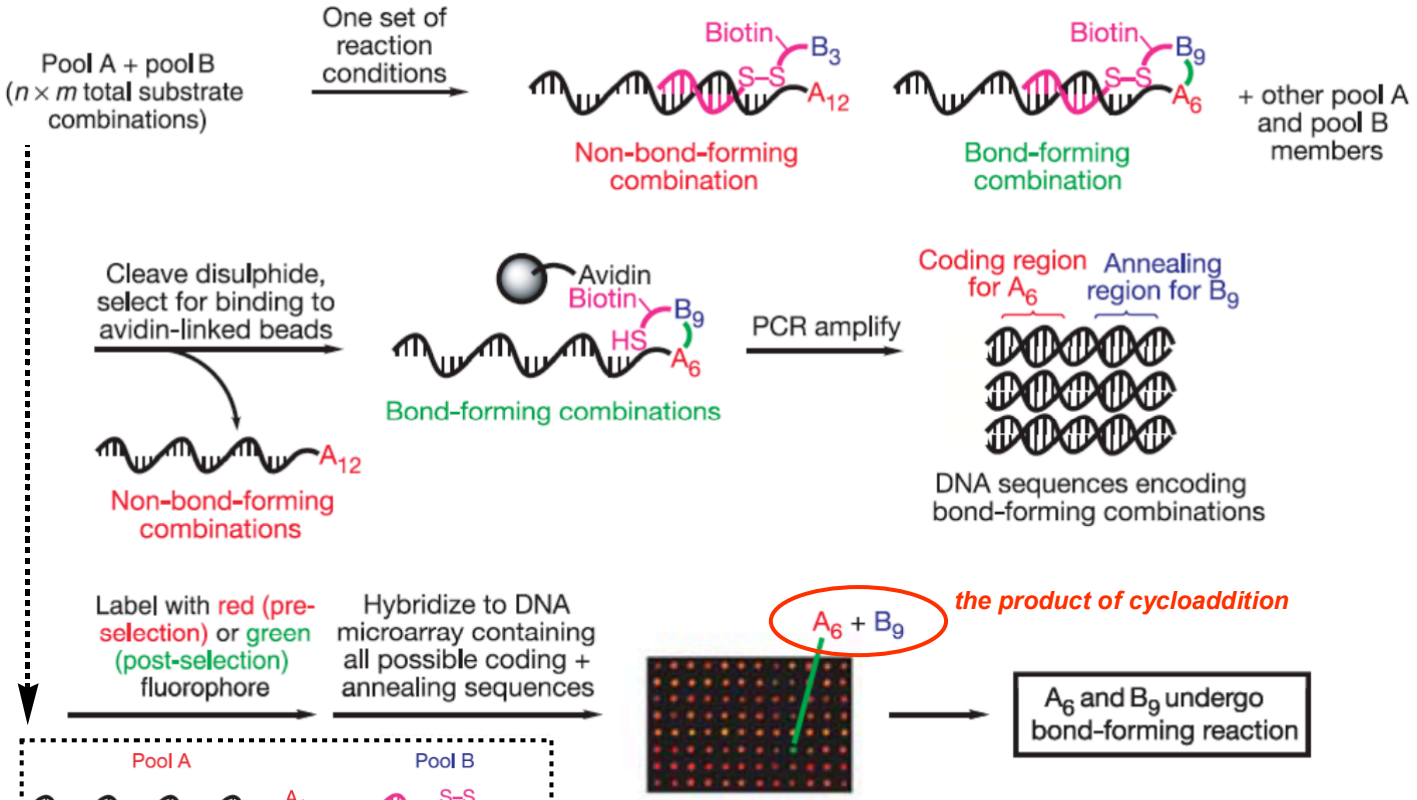
@ DTS coupled with in vitro selection enables the translation, selection, amplification of DNA encoding synthetic small molecules not biological molecules.

→ the combined ability of synthetic chemistry and PCR method !!!

D.R.Liu *et al. Science.*, 2004, *305*, 1601

Applications of DTS ~ Reaction Discovery (1)





> identifying all substrate pairs capable of forming a covalent bond
→ n× m discrete pairs

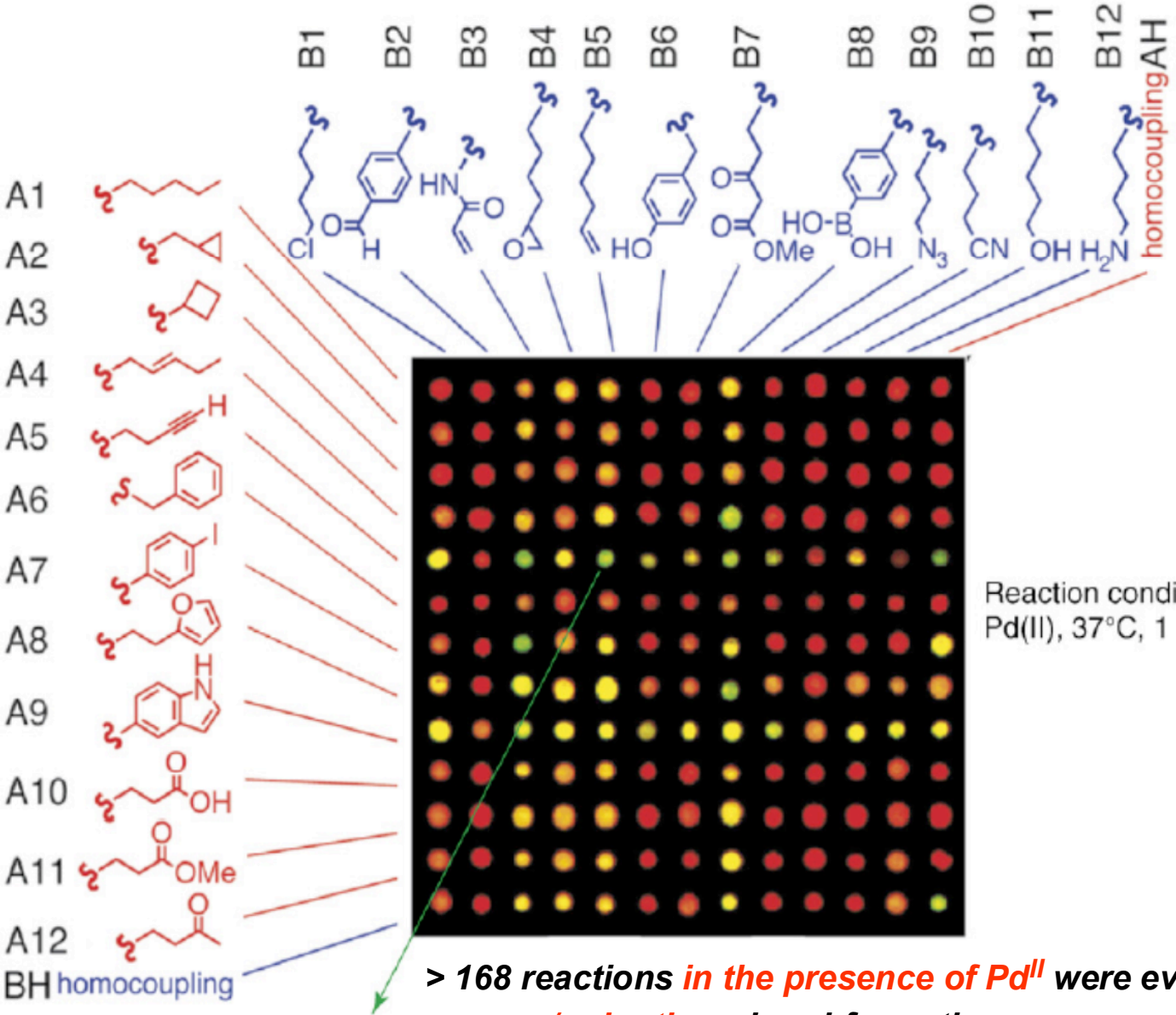
> also n× m discrete pairs were mixed in one solution

> first the cycloaddition of azide and alkyne in the presence of Cu was performed to test the ability.

> the successful detection despite of DNA degradation by Cu
→ the extreme sensitivity of PCR amprification

D.R.Liu *et al. Nature.*, 2004, *431*, 545

Applications of DTS ~ Reaction Discovery (2)



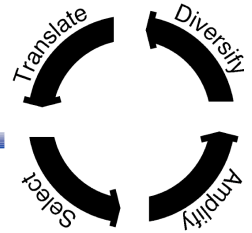
Reaction conditions:
Pd(II), 37°C, 1 h

> 168 reactions in the presence of Pd^{II} were evaluated.

> green/red ratio ⟶ bond-formation

D.R.Liu *et al. Nature.*, 2004, *431*, 545

Applications of DTS ~ Reaction Discovery (3)



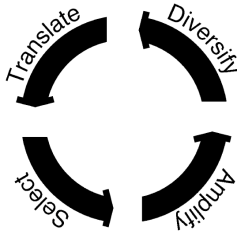
> characterizion of array positives

Substrates		Green/red fluorescence ratios 37°C	Green/red fluorescence ratios 25°C	DNA-templated yields (%) 37°C	DNA-templated yields (%) 25°C	Product consistent with observed mass
A5	B5	2.7	3.7	35	31	???
A5	B3	3.5	3.1	28	20	
A5	B8	1.6	1.9	36	34	
A5	Homocoupling	2.6	2.7	45	42	
A4	B8	3.0	2.8	57	39	
A8	B8	1.8	<1.2	30	10	
A8	B3	1.8	<1.2	19	<10	
A7	B3	3.6	<1.2	39	14	

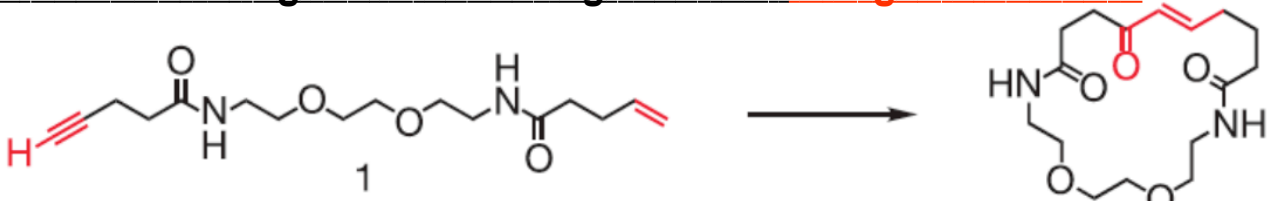
Heck

D.R.Liu *et al. Nature.*, 2004, *431*, 545

Applications of DTS ~ Reaction Discovery (4)



> demonstrating the '*discovering reaction*' in organic solvents



the first example !!!

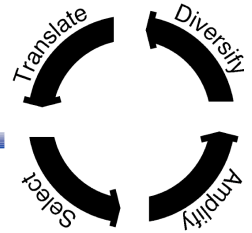
Entry	Metal(s)	Solvent	Conditions	Isolated yield
a	1 equiv. Na_2PdCl_4	1 M NaCl in H_2O	25 °C, 15 h	86%
b	5 mol% Na_2PdCl_4 1 equiv. $CuCl_2$	100 mM NaCl in H_2O	25 °C, 2 h	90%
c	5 mol% Na_2PdCl_4 1 equiv. $CuCl_2$	9:1 THF: H_2O	25 °C, 4 h	91%
d	15 mol% Na_2PdCl_4 1 atm O_2	9:1 THF: H_2O	25 °C, 14 h	73%
e	1 equiv. $CuCl_2$	100 mM NaCl in H_2O	25 °C, 4 h	0%
f	1 equiv. CuCl	100 mM NaCl in H_2O	25 °C, 4 h	0%

@ the value of searching a large number of substrate combinations for unexpected reactions

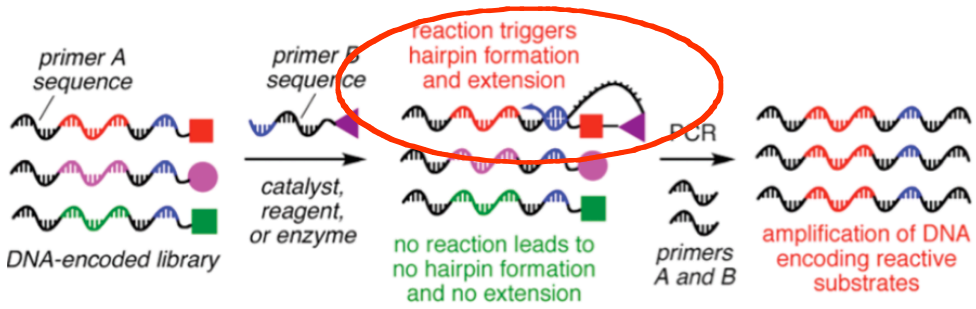
D.R.Liu *et al. Nature.*, 2004, *431*, 545

D.R.Liu *et al. JACS.*, 2007, *129*, 2230

Applications of DTS ~ Reaction Discovery (5)

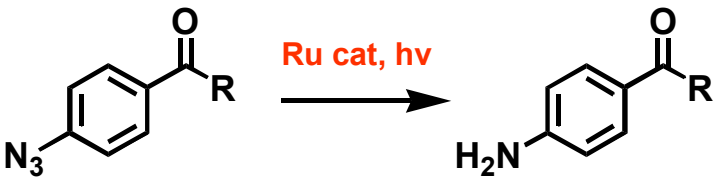


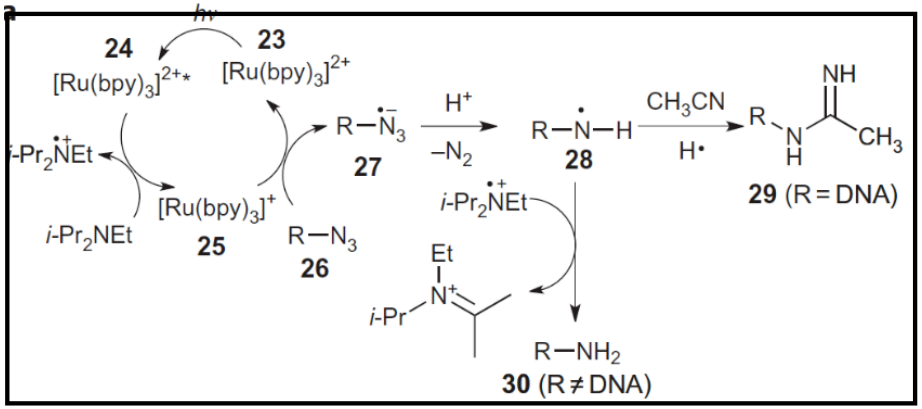
RDPCR (Reactivity-Dependent PCR)



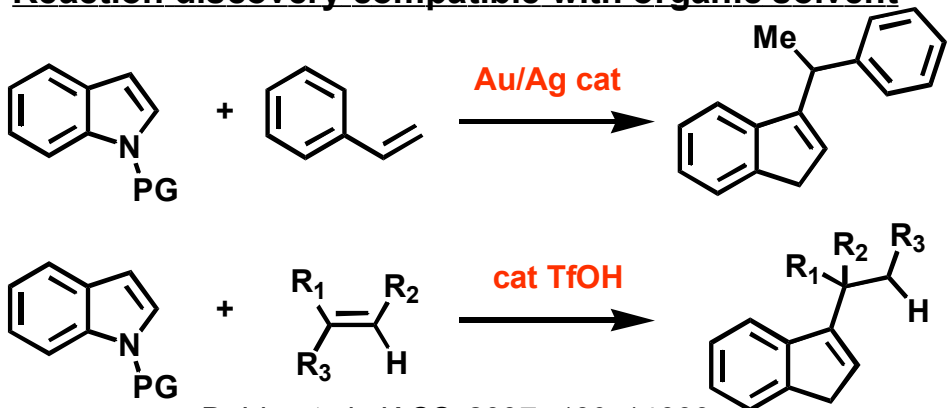
D, Liu *et al. JACS.* **2009**, *131*, 9189

Reaction discovery of mild azide reduction





Reaction discovery compatible with organic solvent



D, Liu *et al. JACS.* **2007**, *129*, 14933

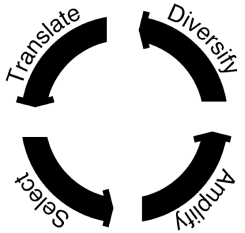
> in orgnic or aqueous solvent, open to air, at r.t

> compatiable with various functional groups (alcohols, phenpols, acids, alkenes, aldehydes etc)

> on oligonucleotides, oligo saccharides, protein

D, Liu *et al. Nat. Chem.* **2011**, *3*, 146

Features of DNA-Templated Synthesis



Advantages

> *genuine evolutional cycle* (selection, translation, amplification)

> with the *synthetic molecules* not amplifiable information (RNA etc)

> in a single solution
→ *the enormous magnitude of diversity, easiness, new reactivity*

> *reactivity controlled by DNA annealing*
→ *selectivity*

Disadvantages

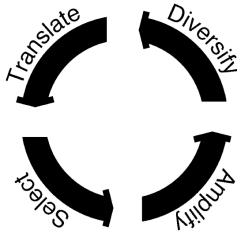
> the need to prepare DNA-linked reagents

> under conditions that *support DNA hybridization*
(in the aqueous solvent, moderate temp., neutral pH etc)

> just the combination of reactants in *'rediscovering reactions'*
→ the ability to develop *conceptually new methodologies ???*

4. Future Direction

Toward Future Methodologies



Design

Tests

Synthesis

Traditional chemical approaches

> reductionistic approaches

Design

Tests

Synthesis

New methods supported by chemical modifications (HTS etc)

> time-economic, easy screening
> beneficial for 'optimization screening'

1990

Translate

Diversify

Select

Amplify

Evolutional Approaces (GA, DCC, DTS)

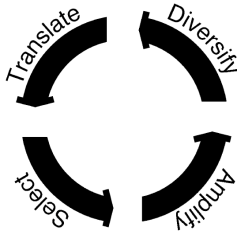
What is the next generation???

> new concept inspired by Nature
> huge diversity treated in the mixture
> new reactivity for 'rediscovering reactions'

2000

2010

Key Words



> ***kinetic and thermodynamic combined system***

> ***auto-catalytic***

> ***self-replicating***

> ***feedback***

> ***oscillation***

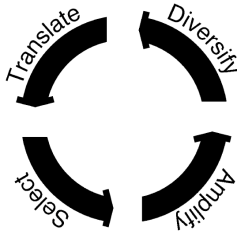
> ***systems chemistry***

> ***application to medicine***

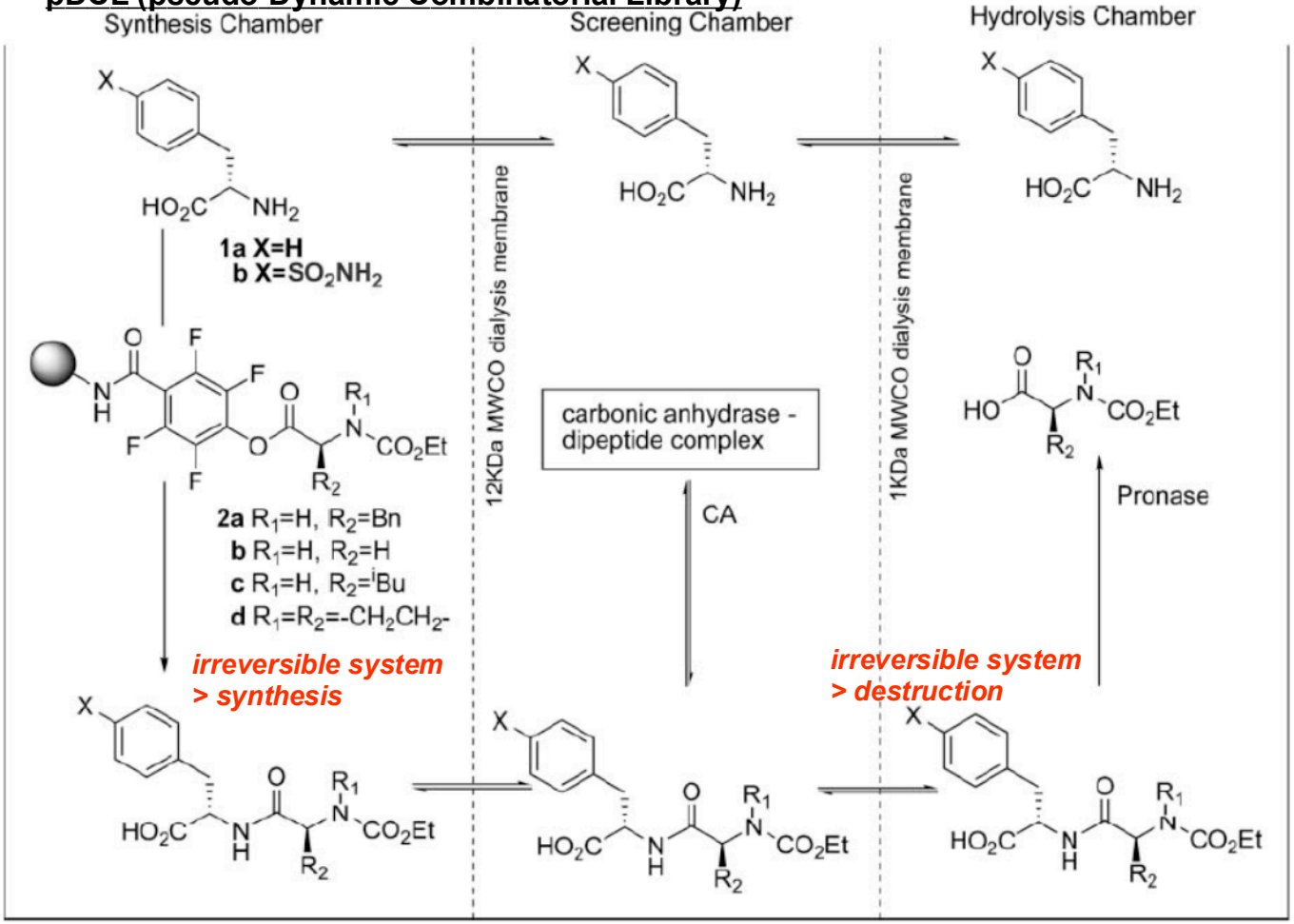
Also see: Mr. Sato's Lit. Seminar (B4)

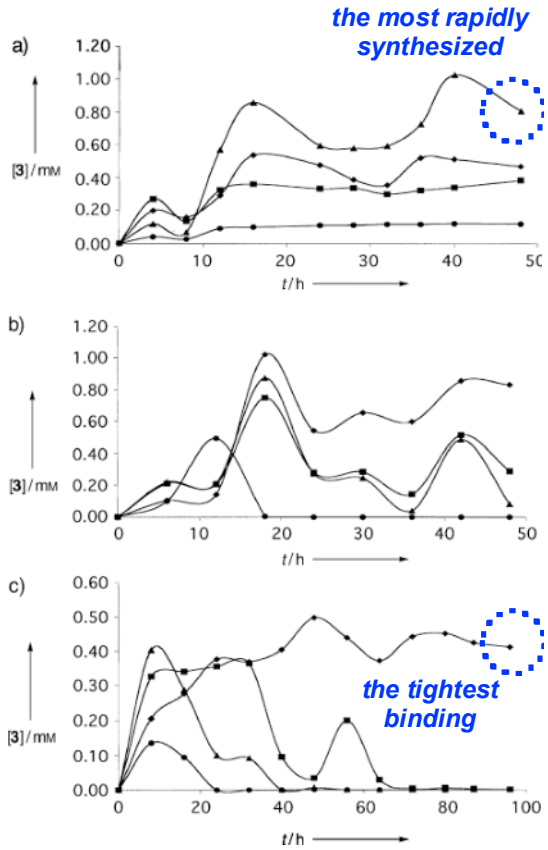
@ multiple component as a whole system

Molecular Networks under Kinetic Control



pDCL (pseudo-Dynamic Combinatorial Library)



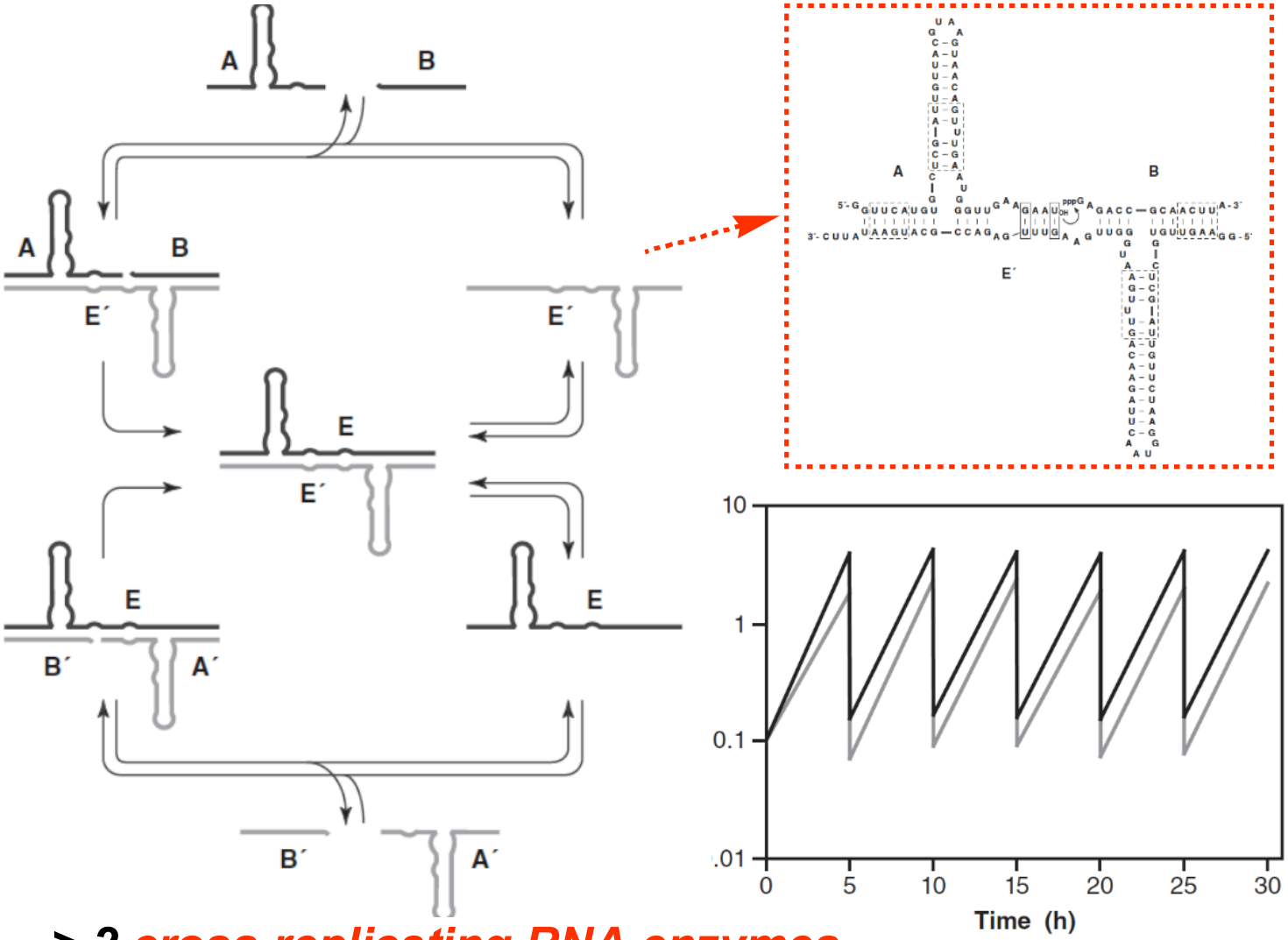


> biology: balance between several processes that are kinetically controlled

→ drug discovery ???

J. L. Gleason *et al. ACIE.*, 2004, *43*, 2432

Self-Replicating (1)



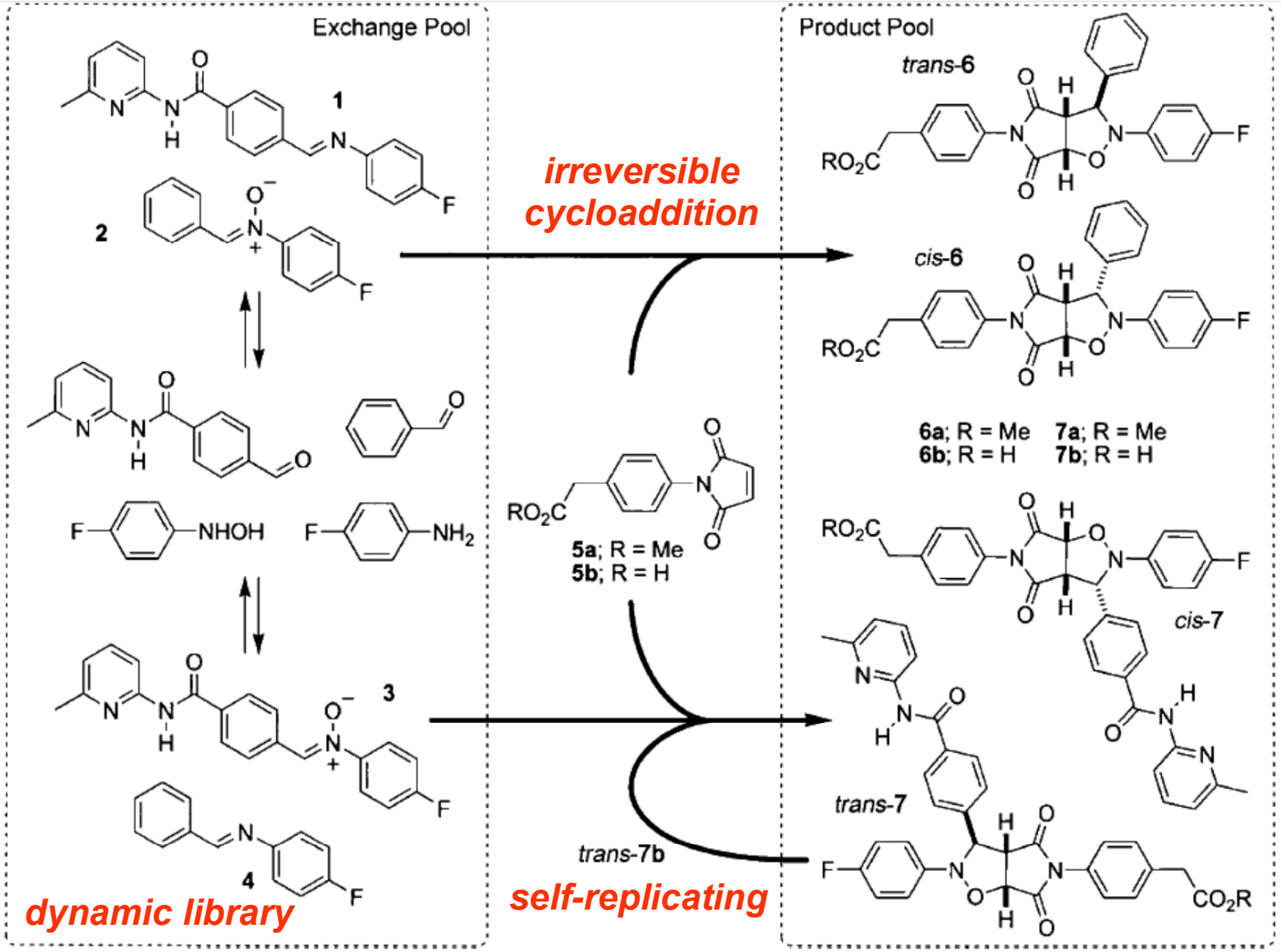
> 2 *cross-replicating RNA enzymes*

> *Self-sustained amplification* ***without other proteins***

G. F. Joyce *et al. Science.*, 2009, *323*, 1229

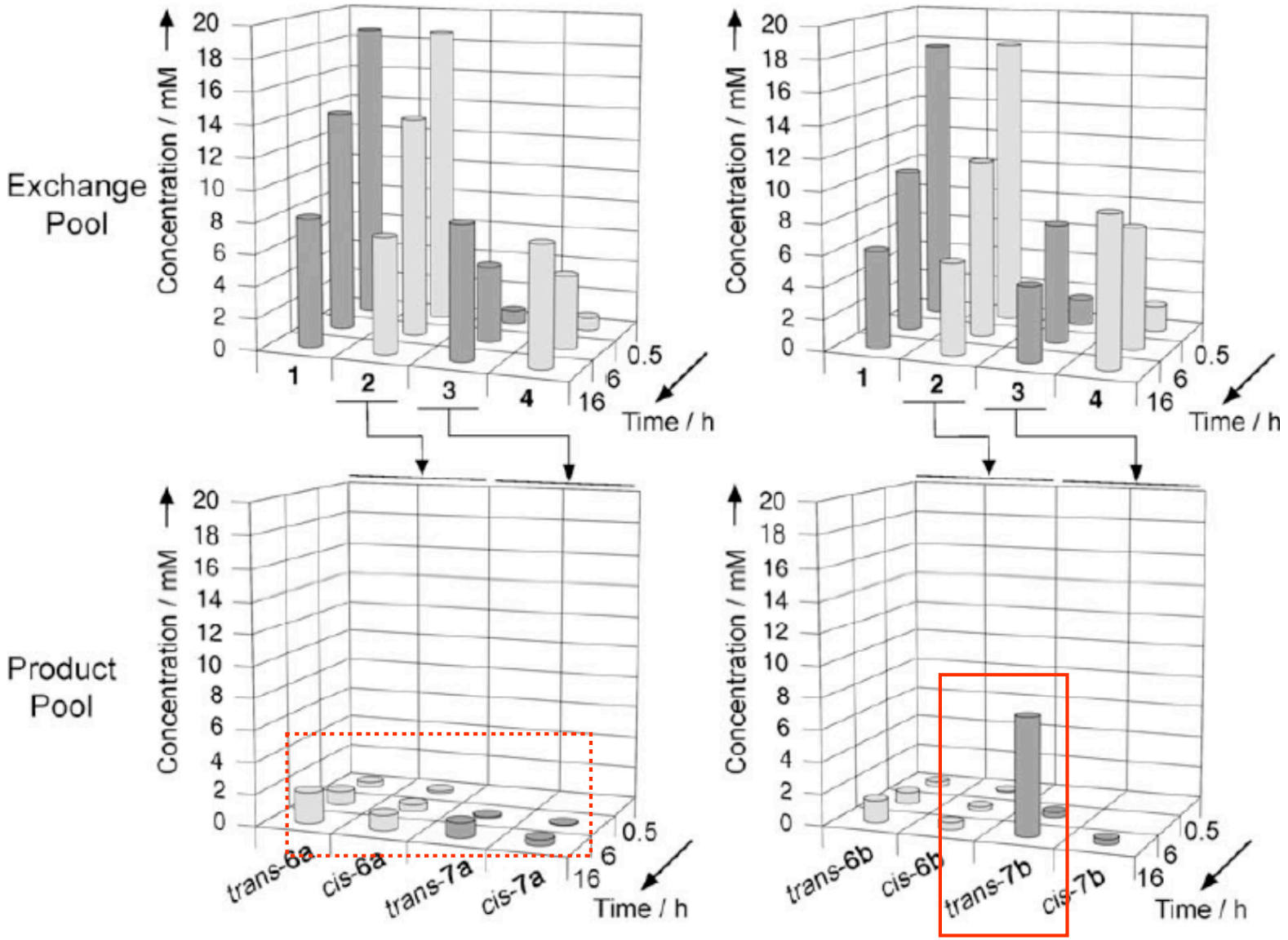
Also see: Mr. Sato's Lit. Seminar (B4)

Self-Replicating (2)



D. Philip *et al. ACIE.*, 2006, *45*, 6344
D. Philip *et al. ACIE.*, 2008, *47*, 9965

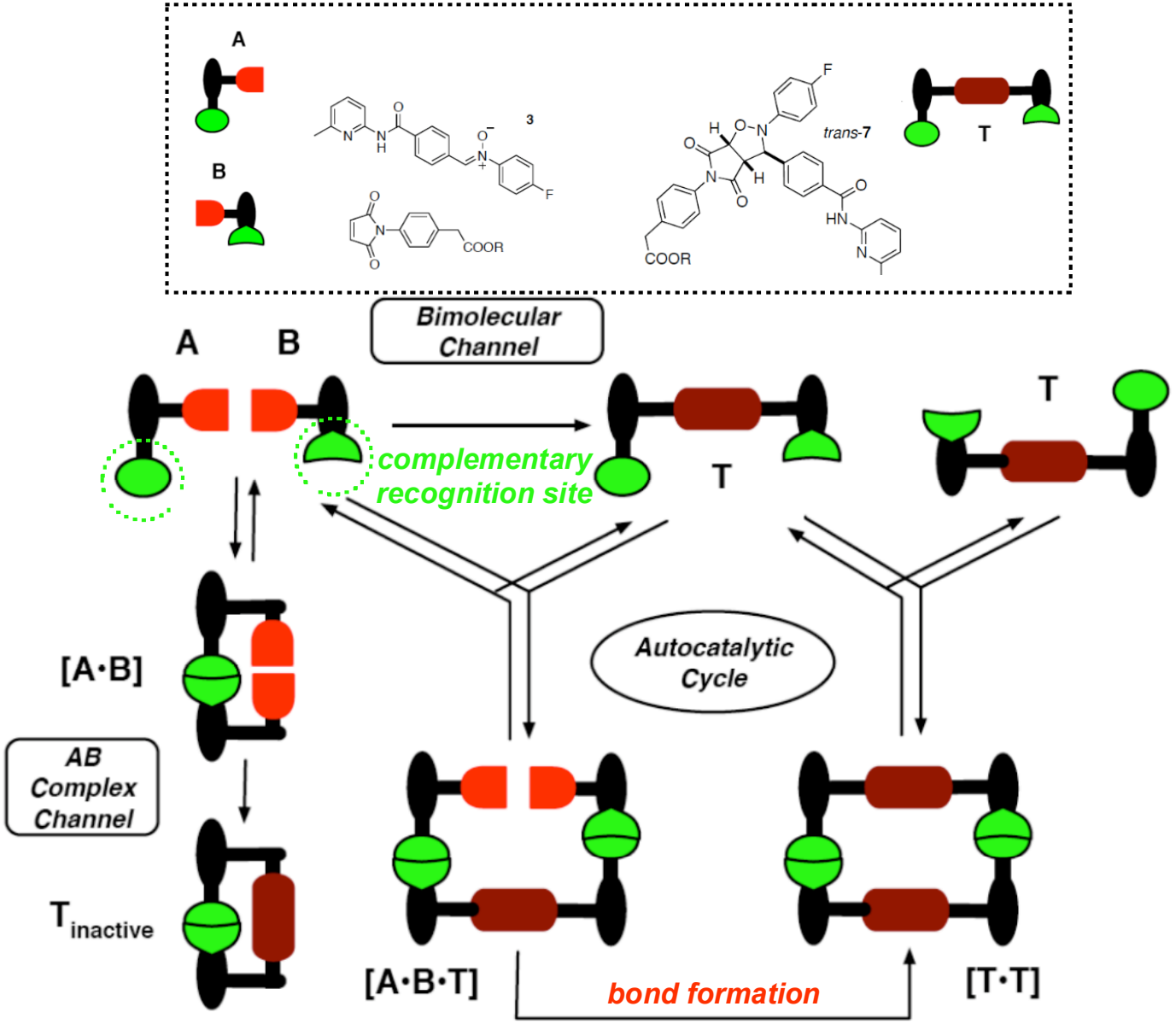
Self-Replicating (3)



> the catalytic [3•5•trans 7] complex accelerated own formation

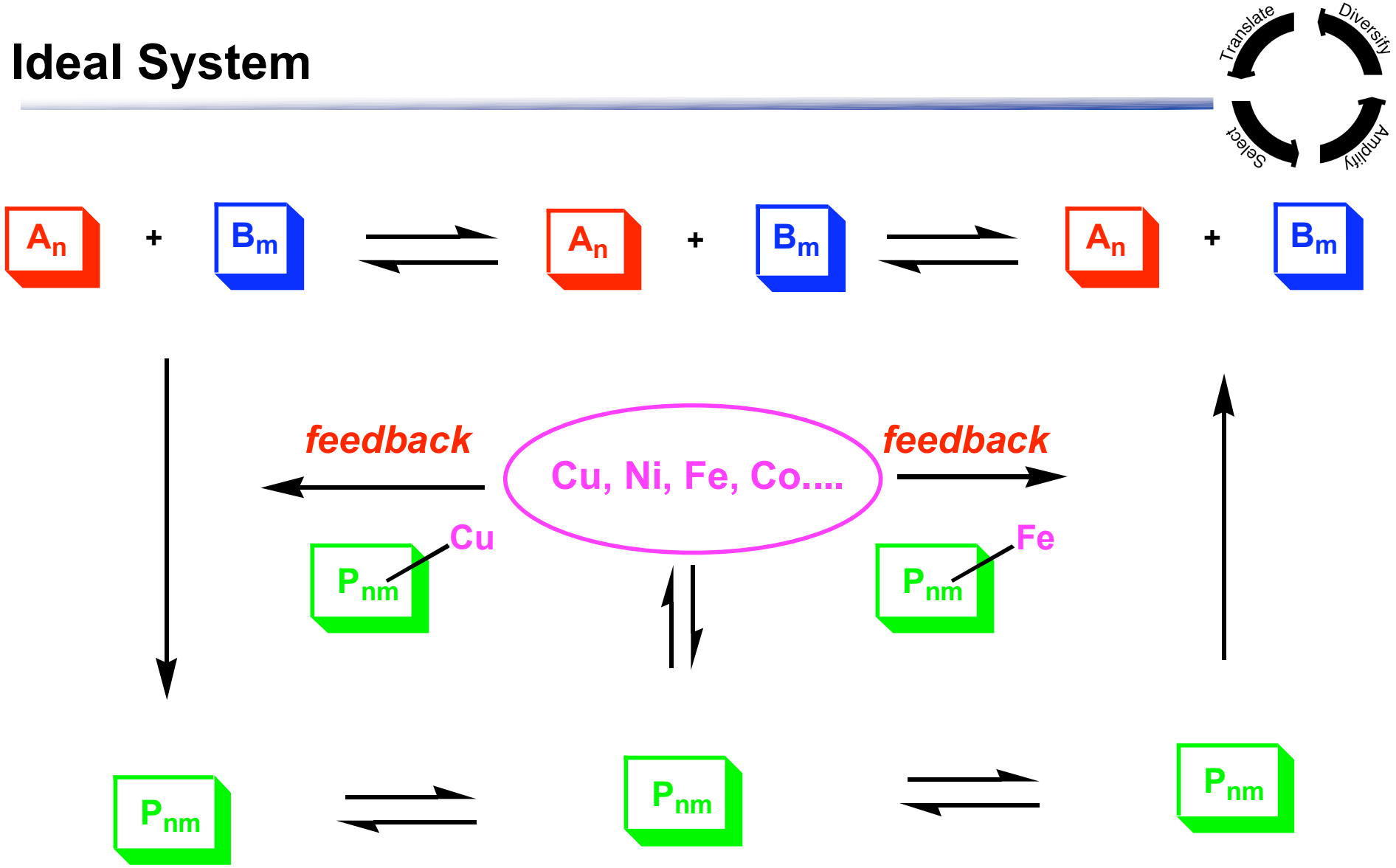
D. Philip *et al. ACIE.*, 2006, *45*, 6344 D. Philip *et al. ACIE.*, 2008, *47*, 9965

Self-Replicating (4)



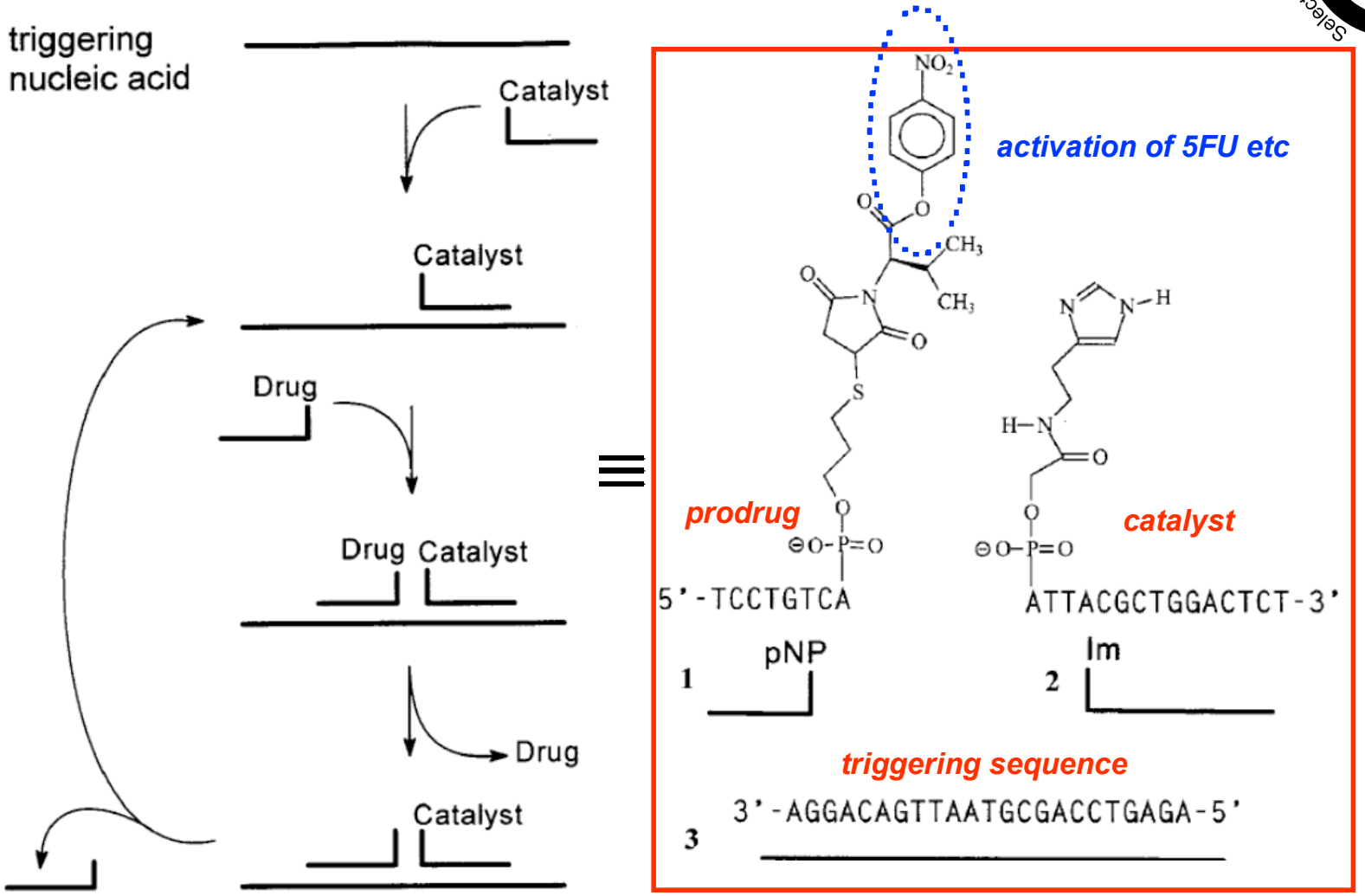
D. Philip *et al. ACIE.*, 2006, *45*, 6344 D. Philip *et al. ACIE.*, 2008, *47*, 9965

Ideal System



@ Can we construct the similar system as 'organic chemists of organometallics' ???

Application to Medicine (1)

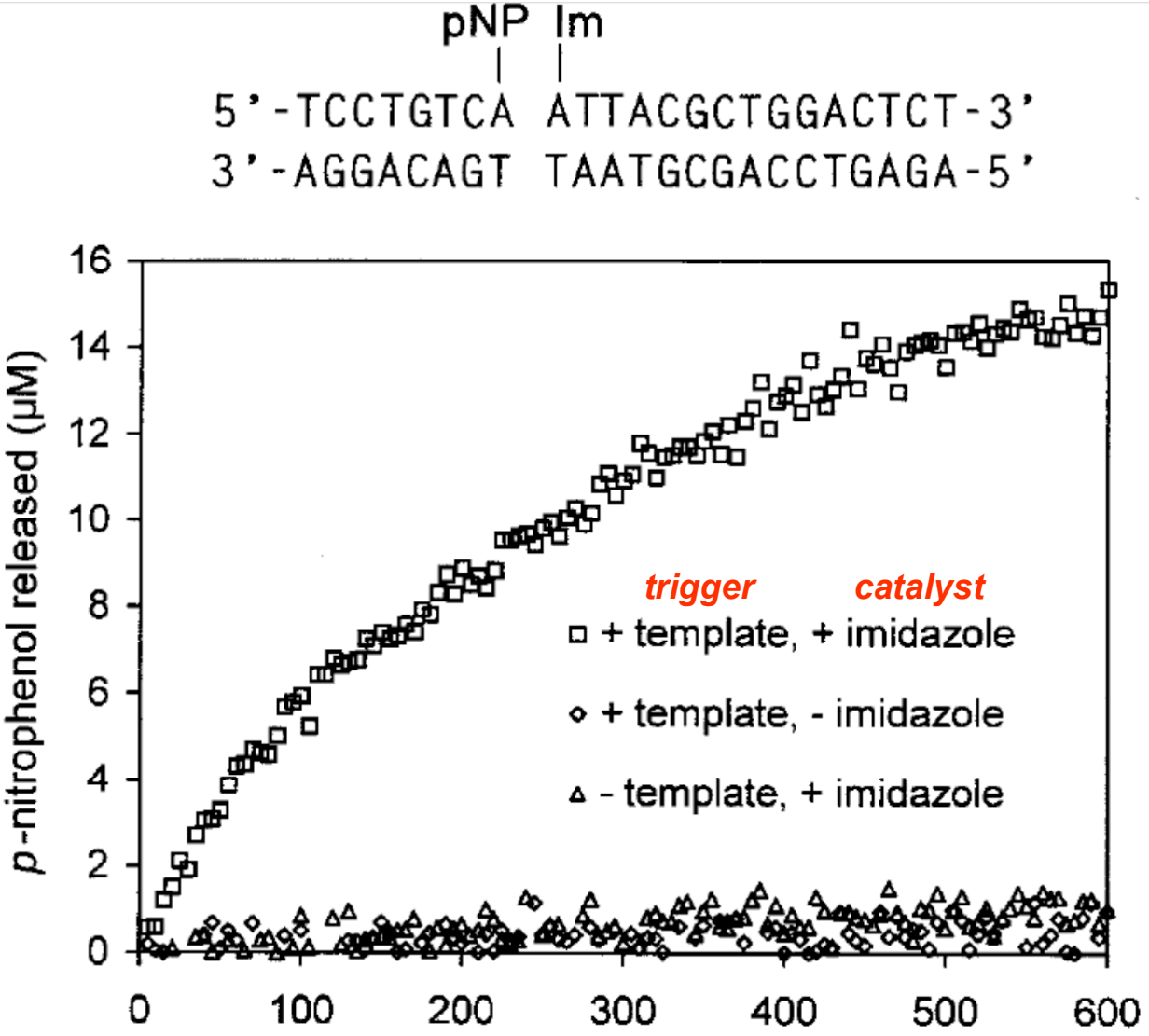


> *rational concept design for 'triggered catalytic drug release'*

> *the desease state (binding by catalyst) triggered the release of drugs*

J. S. Taylor *et al. PNAS.*, 2000, 21, 11159

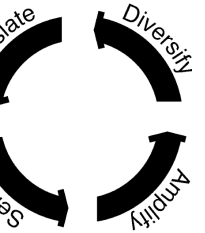
Application to Medicine (2)



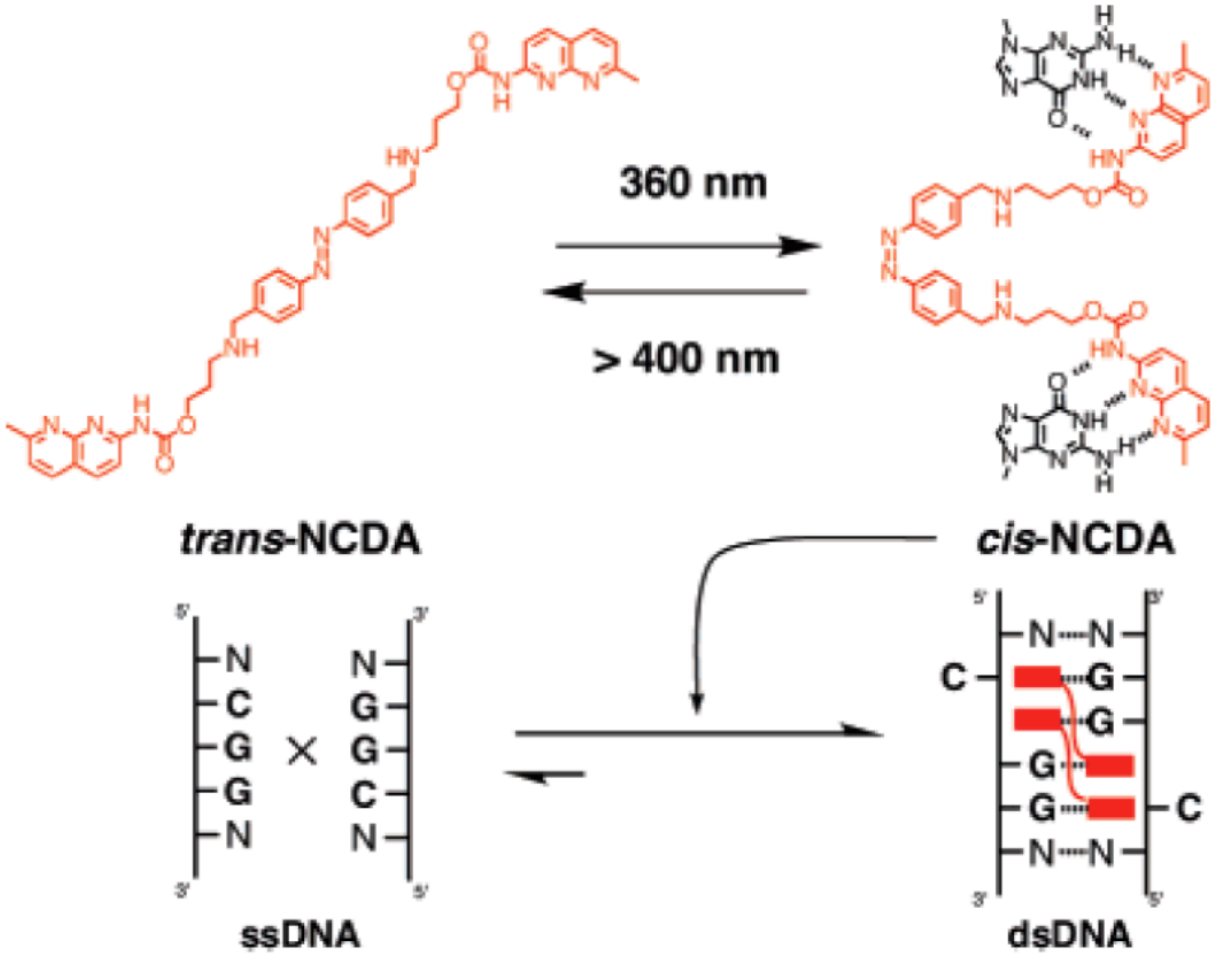
> *only in the presence of a catalyst and a trigger, drug release proceeded*
> *enzyme-like behavior following Michaelis-menten kinetics*
> *sensitive to mismatch sequences → SNP ???*

J. S. Taylor *et al. PNAS.*, 2000, 21, 11159

Application to Medicine (2)



Phostoswithcable molecular glue for DNA



@ Without using external switch, can we develop the original methodologies ???
→ New systems with 'oscillation' in time (rhythms) and space (patterns)

K. Nakatani *et al. JACS.*, 2007, 129, 11898