The Career of Peter G. Schultz

Nick Paras
MacMillan Group Meeting
January 10, 2001

The Career of Peter G. Schultz

Education: 1979—B.S., Caltech

1984—Ph. D., Caltech, advisor: Prof. Peter Dervan

Thesis Title: "Ground and Excited State Studies of 1,1-Diazenes/ Design of Sequence Specific DNA Cleaving Molecules"

1984—Postdoctoral Fellow, MIT, advisor: Prof. Christopher Walsh

Professional: 1985-1999—Professor, UC Berkeley (1987, 1989)

1985-present— P.I., LBL

1988-present— Founding Scientist/Chairman Scientific Advisory Board, Affymax Research Institute, Palo Alto

1993-present— Scientific Advisory Board, CV Therapeutics, Mountain View

1994-1998— Howard Hughes Medical Institute Investigator

1995-present— Founder and Director, Symyx Technologies, Palo Alto

1999-present— Professor, The Scripps Research Institute

1999-present— Director, Genomics Institute of the Novartis Research Foundation, La Jolla

2000-present—Founder and Director, Syrrx Inc., La Jolla

The Career of Peter G. Schultz: Major Research Interests

Catalytic Antibodies

Application of molecular diversity to problems in biomolecular recognition and catalysis, drug discovery, and materials science

Development of methods for incorporating unnatural amino acids and base pairs selectively into proteins and nucleic acids

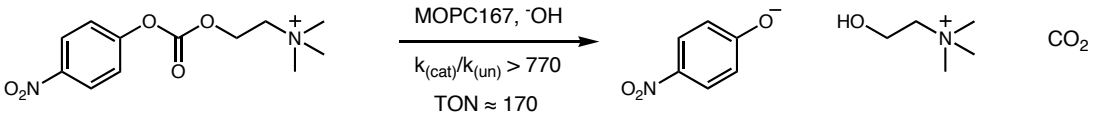
Single-molecule biological imaging

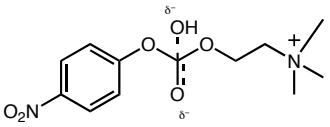
Functional genomics

Unifying theme: "A lesson from nature" => develop highly sophisticated methods for screening vast numbers of discrete compounds for novel or desired properties.

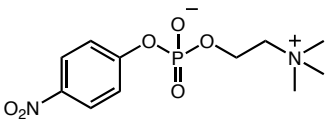
Lead References: *ACIEE*, 1999, 38, 35-54; *PNAS* 2000, 97, 5179-5184 (single molecule imaging); *ACIEE*, 1999, 96, 4780-4785 (expanded genetic code); *Science* 1998, 281, 533-538 (combi medchem); *PNAS* 1998, 95, 10523-10528 (function directed evolution); *Science* 1998, 279, 1712-1714 (combi materials); Science 1995, 269, 1835-1842 (catalytic antibodies).

PGS' First Report of Catalytic Antibodies:





Transition State

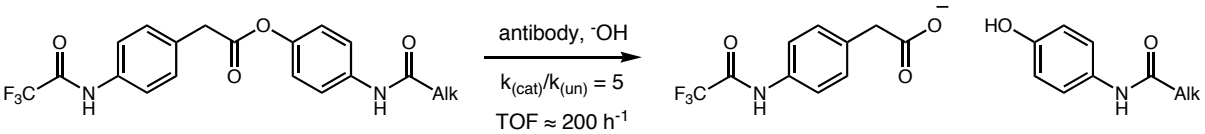


Transition State Analog

MOPC167 previously identified to bind to phosphate diester (*Biochem.,* 1978, 17, 1733.)

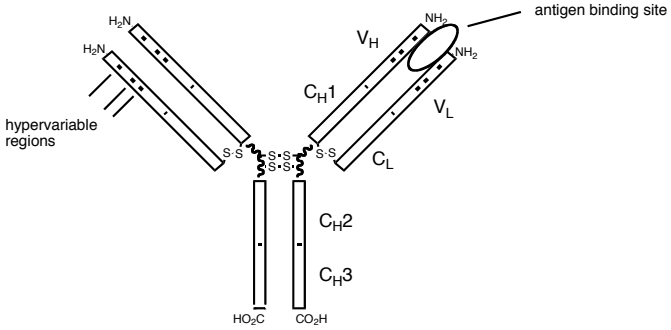
Pollack, Jacobs & PGS (*Science*, 1986, 234, 1570)

Lerner's First Report of Catalytic Antibodies:



Tramontano, Janda & Lerner (*Science*, 1986, 234, 1566)

More Antibodies than Gene Sequences: Anatomy of an Antibody



Symmetric peptide tetramer consisting of two identical heavy chains and two identical light chains

Antigen selectivity determined by composition and secondary structure of variable domains (V_H, V_L),

Except for 20-30 aa in hypervariable regions of variable domains, sequence is basically constant

Two of three constant domains in heavy chain (C_H2 & C_H3) determine action after antigen binding

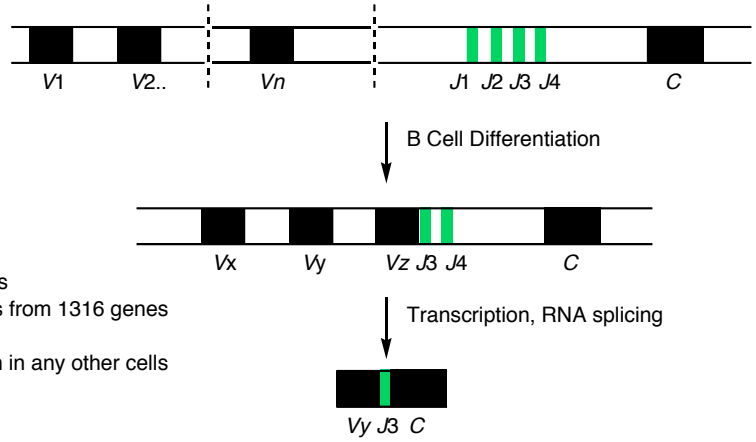
Origins of Diversity:

2-3 genes code each variabe domain (*V*, *D*, *J* on heavy chain; V, J on light chain)

Multiple copies of genes that code the variable domains are carried in the germline cells

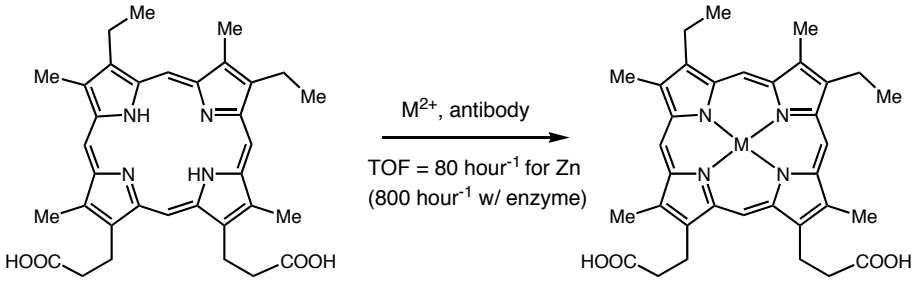
Example: 300 V x 4 J =1200 V_L domains
1000 V x 12 D x 4 J = 48000 V_H domains
1200 V_L x 48000 V_H > 5 x 10^7 antibodies from 1316 genes

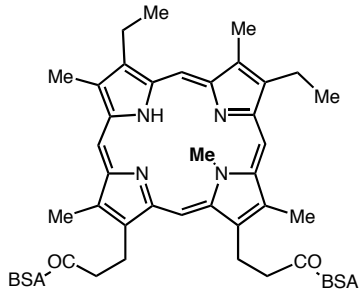
Somatic mutations are 1 x 10^6 more frequent than in any other cells



Molecular Biology of the Cell, 2nd ed. Alberts, et. al. Garland: New York; pp. 1011-1031.

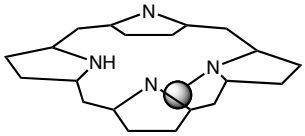
Scope of Antibody Catalysis: Enzymatic Reactions





Antigen

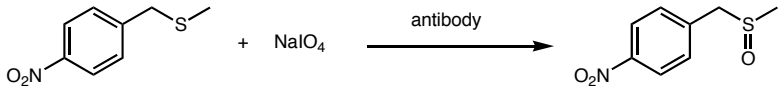
≡

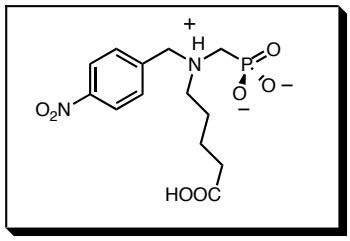


Raman spectroscopy shows that Me induces distortion of porphyrin analogous to transition state.

Cochran & PGS Science, 1990, 249, 781.
PGS, et.al. Biochem 1998, 37, 779.

Scope of Antibody Catalysis: Reactions with Inexpensive Cofactors

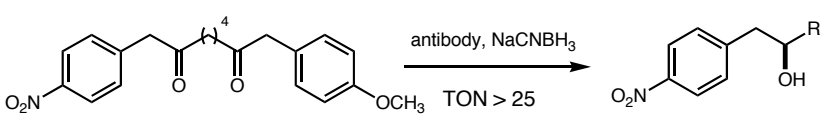




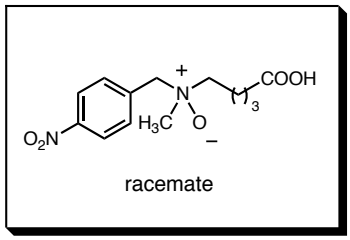
Oxidation Hapten

Hsieh, Stephans & PGS, *JACS*,1994, 116, 2167.

$NaIO_4$ and $NaCNBH_3$ substantially cheaper than enzymatic cofactors like NADH.



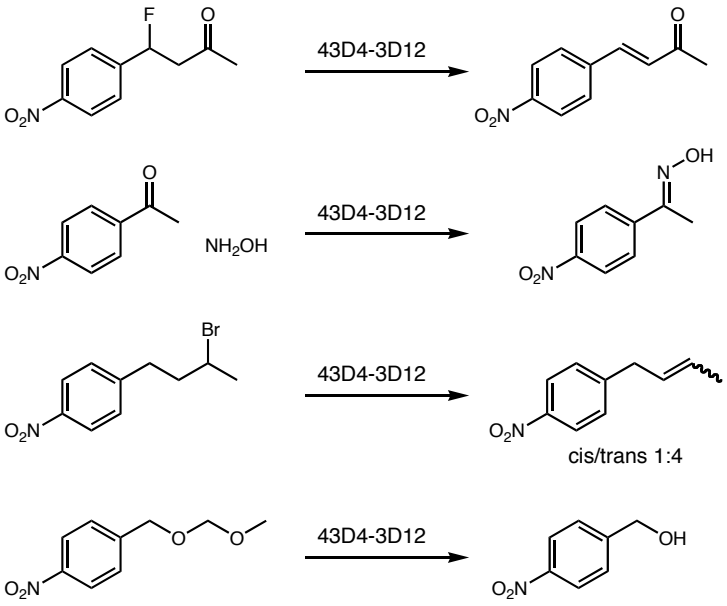
94% yield
96% ee
75:1 regioselective

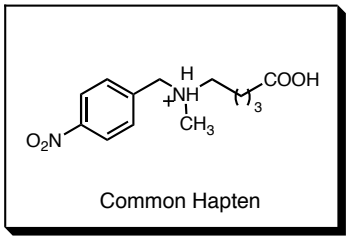


Reduction Hapten

Nakayama & PGS *JACS*, 1992, 114, 780.
Hsieh & PGS, et. al., *Science*, 1993, 260, 337.

Multiple Reactions from the Same Hapten



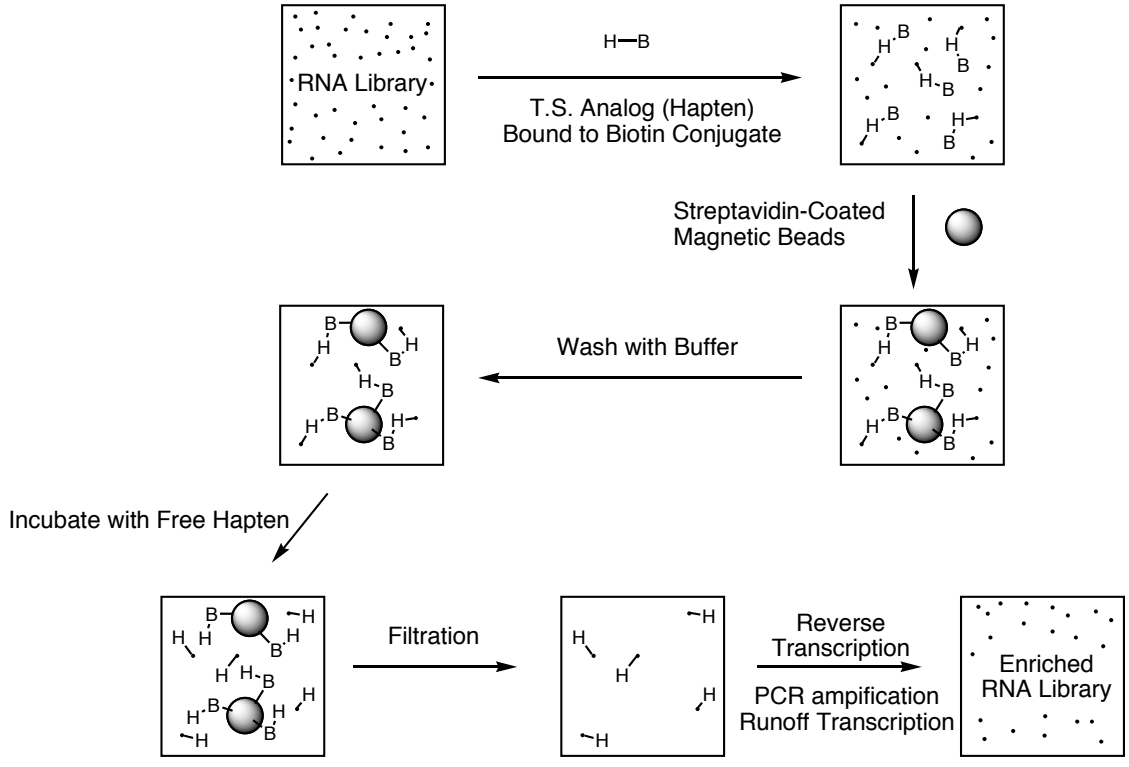


- Hapten induces a carboxylate residue in the active site of the cat. antibody.
- Any substrate bearing structural similarity to the identification region of the hapten can be subjected to general acid base catalysis.

PGS, et. al., *JACS*, 1998, 120, 5160.

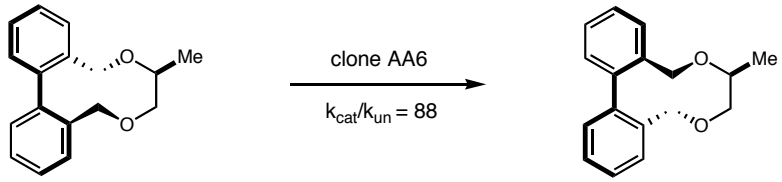
RNA as Amplifiable Biopolymers

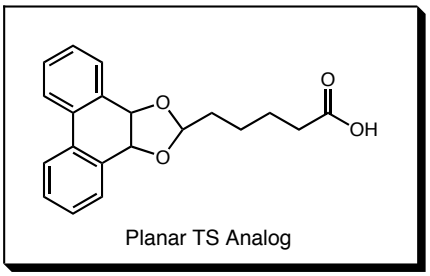
Isolation of Oligomers with Bias Toward Desired Activity



PGS, et. al., *JACS*, 1996, 118, 7012.
PGS, et. al., Science, 1994, 264, 1924.

Catalytic RNA with Designed Activity





A library of approximately 4 x 10^{14} oligonucleotides was generated.

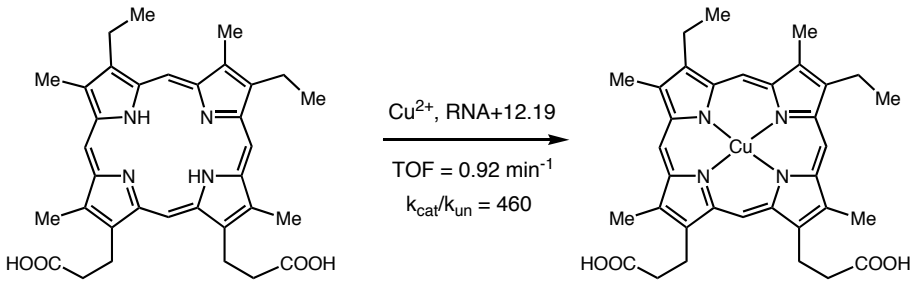
The compounds were selected by affinity chromatography on solid-support bearing the TS analog.

After 7 rounds of selection and amplification 20 active clones were sequenced, 16 of which were AA6.

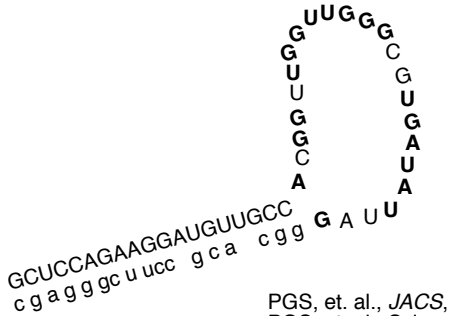
This represents the first production of an unnatural RNA with specifically designed catalytic function.

PGS, et. al., Science, 1994, 264, 1924.

RNA Enriched by TS-Affinity Selection Functions as a Catalyst



- An initial library of approximately 10^{15} oligonucleotides with 50 randomized positions was generated.
- Primary structure of RNA easily established by sequencing of complimentary DNA.

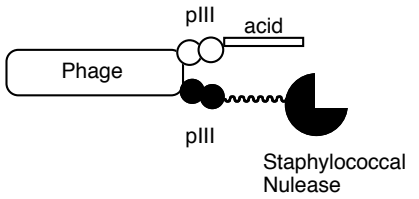


Predicted Secondary Structure of RNA+12.19
(*Nuc. Ac. Res.* 1991, 19, 2707)

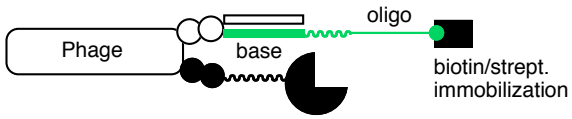
PGS, et. al., *JACS*, 1996, 118, 7012.
PGS, et. al., Science, 1994, 264, 1924.

PGS, et. al., *JACS*, 1996, 118, 7012.

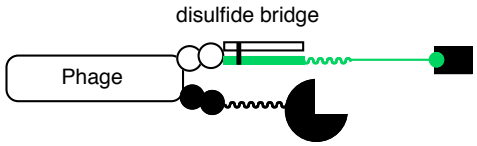
Phage Display: Non-Immunogenic Enzyme + Blueprint Amplification



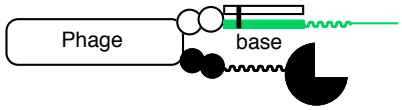
A phage carrying a gene for Staphylococcal Nuclease (SNase) and an "acid" chain expresses the peptides attached to pIII protein in its outer coating.



The "acid" chain forms a non-covalent heterodimer with a "base" chain covalently bound to an oligonucleotide link to solid support.



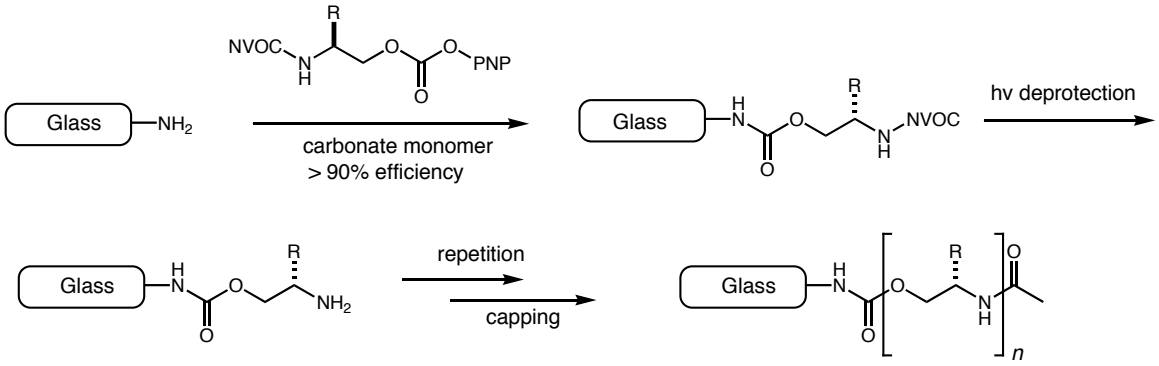
A covalent disulfide bridge between the two peptide chains is chemically induced in order to guard against crossover catalytic activity.



Ca^{2+} is added which activates the SNase, cleaving the phage from support.

PGS, et. al., *PNAS,* 1998, 95, 10523.

Synthesis and Analysis of a Carbamate Biopolymer Library



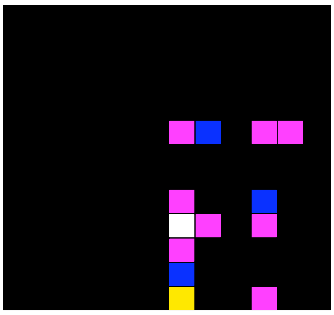
256 discrete, spatially segregated oligocarbamates were made with binary masks of 8 coupling steps.
(Synthesis of peptide library in this fashion previously described. PGS group also developed oligourea and cyclic urea libraries.)

Oligomer library was treated with a solution of monoclonal antibody followed by fluorescin-conjugated secondary antibodies.

Analysis by scanning epifluorescent microscopy showed sites of tightly binding polymer.

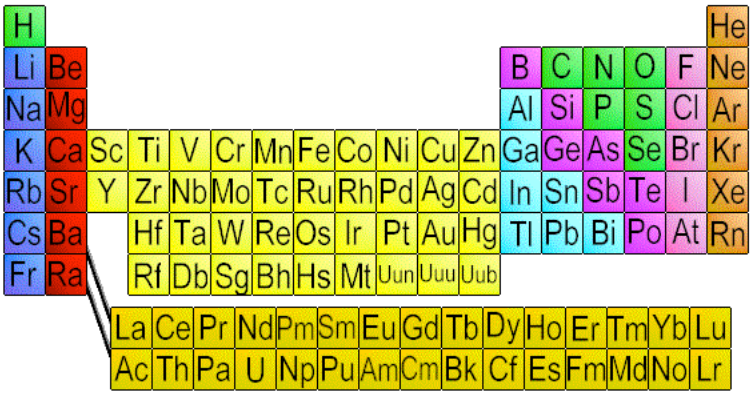
Independent resynthesis of both hits and misses and solution assays were in agreement with parallel data.

1 compound/ .0064 cm^2 →



PGS, et. al., *Science,* 1993, 261, 1303.

Extension of Combinatorial Synthesis to Inorganic Materials



Many subtle interactions of material components, dopants, and surface interfaces are poorly understood.

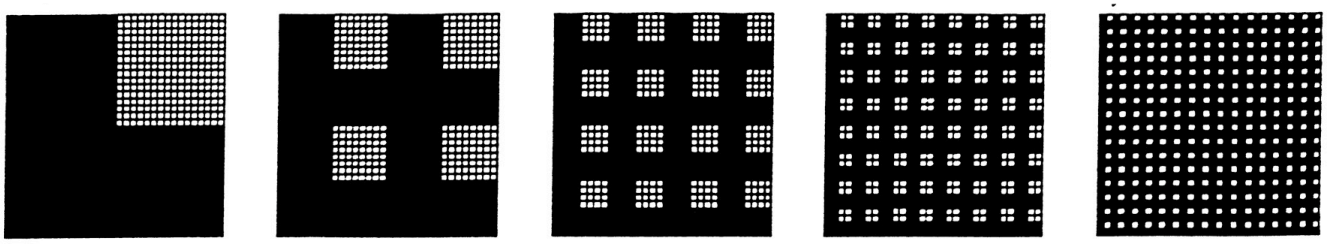
Combinatorial approaches could allow for rapid identification of unpredictable novel inorganic compounds.

PGS, et. al. reported the first use of combinatorial chemistry in materials science: *Science,* 1995, 268, 1738.

Generating a Library of Materials: ID of a Blue Photoluminescent Material

Photomask techniques for selective deposition of inorganic materials already exist (microchip industry)

Methods of deposition include: sputtering, pulsed laser deposition, and scanning fluid delivery systems.



Schultz and co-workers used the above photomasks to selectively deposit:

Ga_2O_3, SiO_2, CeO_2, EuF_3, Tb_4O_7, Ag, TiO_2, Mn_3O_4, Gd_2O_3, ZnO, and Y_2O_3

thereby generating a library of 4^5 (1024) different material compositions.

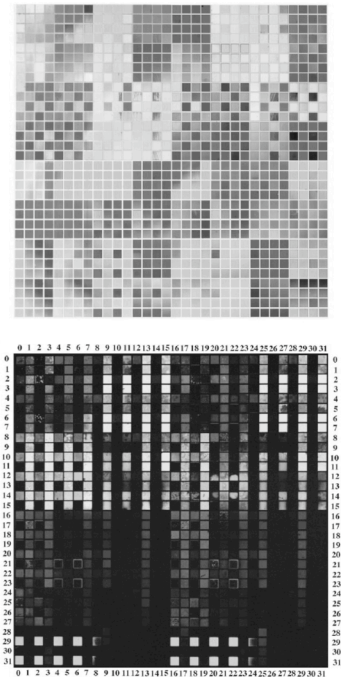
PGS, et. al., *ACIEE*, 1999, 38, 36-54.
PGS, et. al., *Science*, 1998, 279, 1712-1714.

Screening a Library of Materials: ID of a Blue Photoluminescent Material

Scanning microscopy and spectroscopy are techniques commonly employed for screening of material libraries for desired properties; infrared thermography used for detecting catalytic activity.

The library depicted at right under normal (top) and UV light (bottom) was subjected to scanning spectroscopy to measure luminescence of each material.

Inclusion of an internal standard of known luminescence allowed quantitative determination of luminescence.

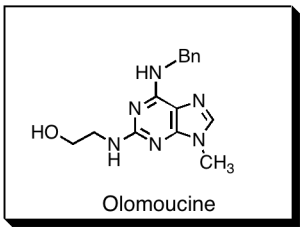


Examples of New Materials Obtained through Combi:

- $(La_{0.88}S_{0.12})CoO_3$: magnetoresistive properties
- $Gd_3Ga_5O_{12}/SiO_x$: blue phosphorescence
- CuO based high temperature superconductors
- Catalysts for organic reactions (polymerization, etc.)

PGS, et. al., *ACIEE*, 1999, 38, 36-54.
PGS, et. al., *Science*, 1998, 279, 1712-1714.

Analysis of Active Site-Binding of a Kinase Inhibitor



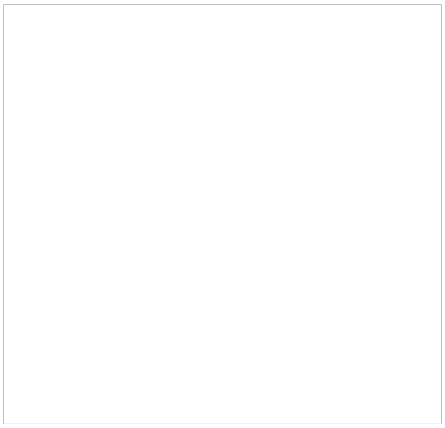
Olomoucine

Cyclin-Depependent Kinases represent a cancer therapeutic target.

Olomoucine binds human CDK2 at 160 degree angle from the native substrate.

Novel binding mode of Olomoucine suggested a library of 2,6,9-substituted purines as opposed to ribose chain modification.

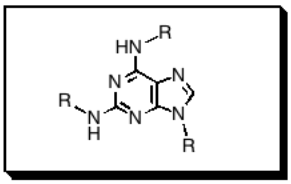
Binding conformation of 3 inhibitors (Olomoucine in white)

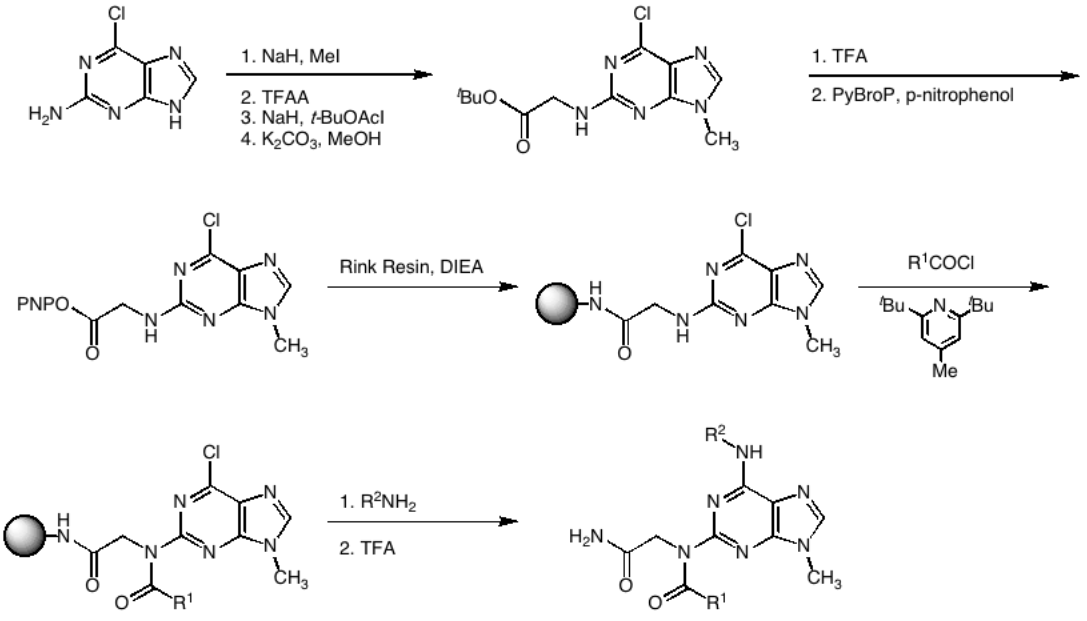


Inhibitor vs. ATP in binding conformation

PGS, et. al., *Science,* 1998, 281, 533.

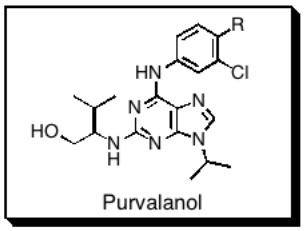
Construction of 2,6,9-Purine Libraries





PGS, et. al., *JACS,* 1996, 118, 7430.

Purvalanol as an Active and Selective Kinase Inhibitor



3 R groups varied independently in separate libraries.

Final library tested best candidates for cooperative effects.

Kinase	Purv. A (IC50 nM)	Purv. B (IC50 nM)
cdc2-cyclin B	4	6
cdc2-cyclin B	40	50
cdk2-cyclin A	70	6
cdk2-cyclin E	35	9
cdk4-cyclin D1	850	>10,000
cdk5-p35	75	6
erk1	9,000	3,333
Protein kinase C	>10,000	>100,000
Protein kinase C 1	>10,000	>100,000
Protein kinase C 2	>10,000	>100,000
Protein kinase C	>10,000	>100,000
Protein kinase C	>100,000	>100,000

Crystal structure of Purvalanol B inhibition complex at active site

PGS, et. al., *Science,* 1998, 281, 533.

First Steps Toward an Organism with Expanded Genetic Code

Hijacking E. coli protein synthesis machinery

Step 1: Generate an orthogonal tRNA that will not be aminoacylated by any native aaRS.

E. coli aaRSs must not charge *S. cerevisiae* (yeast) $tRNA_2^{Gln}$.
Pre-charged yeast $tRNA_2^{Gln}$ must function in the *E. coli* ribosome.

Step 2: Generate a mutant aaRS that acylates the new tRNA with any amino acid.

Step 3: Generate a mutant that acylates the new tRNA with only an unnatural amino acid.

Positive and negative selections

Incidental: Develop new method to monitor uptake of unnatural aa which does not require a specific functional group or ^{13}C labels.

Liu & PGS, *PNAS,* 1999, 96, 4780.

Establishing an Orthogonal tRNA

E. coli GlnRS does not charge *S. cerevisiae* (yeast) $tRNA^{Gln}$.

Whelihan & Schimmel, *EMBO J.*, 1997, 16, 2966-2974.

Does *E. coli* GlnRS charge yeast $tRNA_2^{Gln}$?

In vitro incubation of two strains of yeast $tRNA_2^{Gln}$ with *E. coli* GlnRS and ^3H-labeled-Gln.
Orthogonal tRNA aminoacylation was approximately 100,000 times slower than wild-type *E. coli* $tRNA_2^{Gln}$.

In vivo a strain of *E. coli* requiring Gln suppression at an essential codon for growth on lactose minimal was transformed with either the yeast $tRNA_2^{Gln}$ DNA or that of *supE*, known to function in *E. coli*.
The yeast $tRNA_2^{Gln}$-transform did not survive, even when *E. coli* GlnRS was overexpressed in the plasmid.

Another negative selection was performed using a modified b-lactamase gene which would require the amino-acylation of $tRNA_2^{Gln}$ with any amino acid for ampicillin immunity.
The yeast $tRNA_2^{Gln}$-transform did not survive in the presence of ampicillin, even when *E. coli* GlnRS was overexpressed in the plasmid.

Does yeast $tRNA_2^{Gln}$ function in the *E. coli* ribosome?

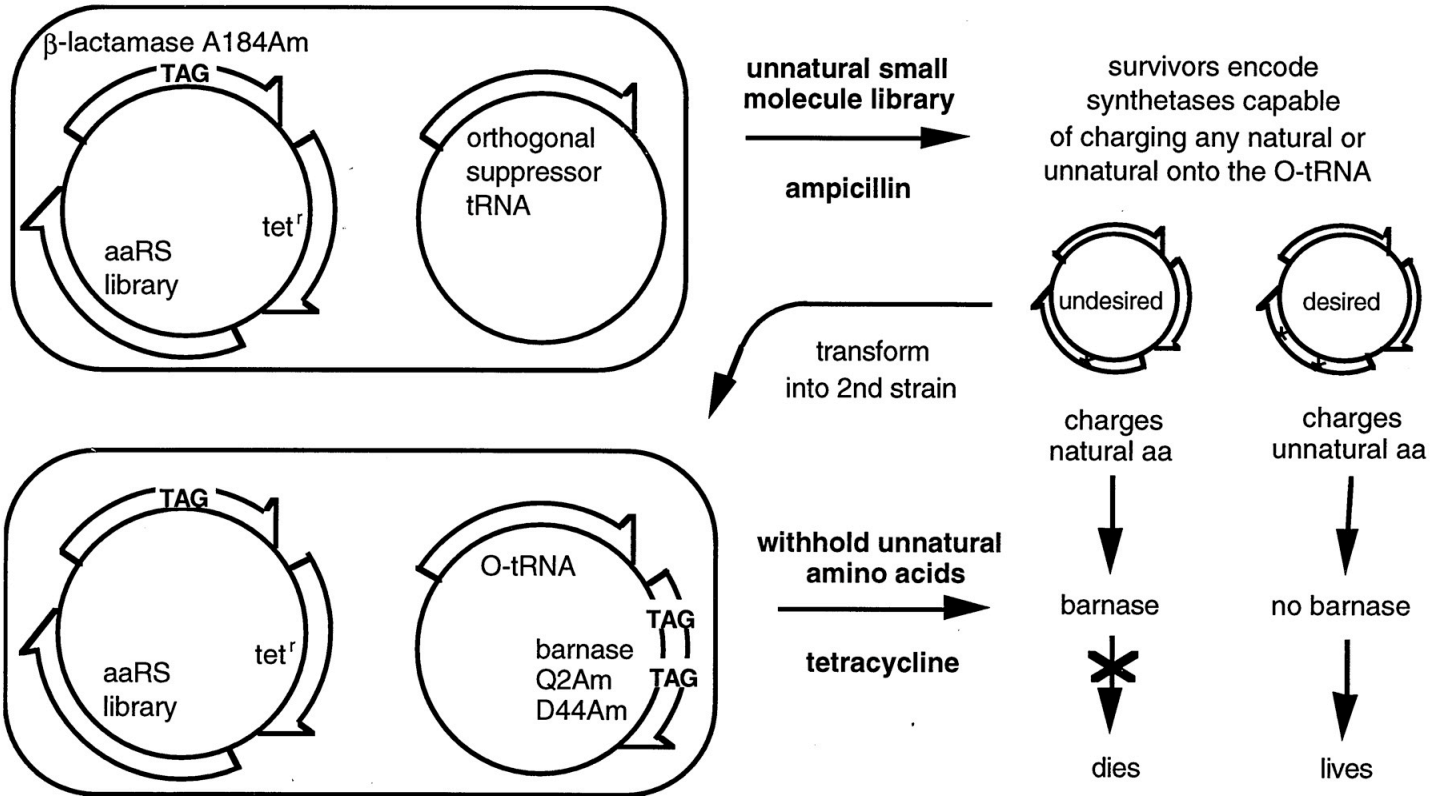
When yeast tRNA was chemically preacylated with valine it provided significat quantities of full length protein.
Efficiency at 57-74% of normal translation.

Can any GlnRS work with the mutant yeast tRNA?

In vitro and in vivo studies show that wild-type yeast GlnRS can acylate mutant yeast tRNA with Gln.
The efficiency of amino-acylation is approximately 20% that of the wild-type yeast tRNA.
Yeast GlnRS has no ability to acylate *E. coli* tRNA.

Liu & PGS, *PNAS,* 1999, 96, 4780.

Zeroing in on Unnatural Amino Acids



Liu & PGS, *PNAS,* 1999, 96, 4780.

Monitoring Unnatural Amino Acid Uptake

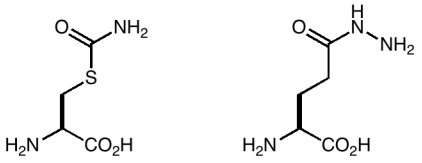
Library of 138 unnatural amino acids assayed for cytotoxicity.

16 of 22 toxic amino acids could be made non-lethal by an excess of a natural amino acid.

Basis of rescue thought to be competition for transport mechanisms.

22 of 138 screened unnatural aa's and rescue aa's.

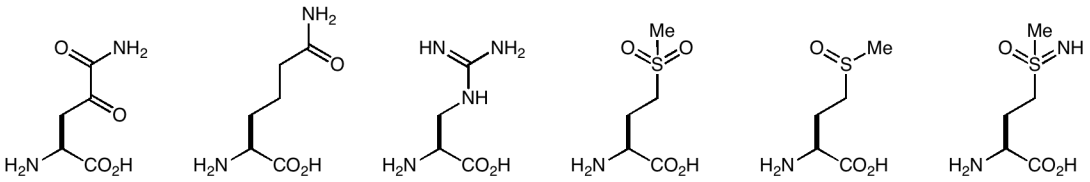
AA	IC50, μM	Rescue	AAIC50, μM	Rescue
C	60	Glu	S31 15	Tyr
Q	20		S39 <8	
V	50	Ala	S47 20	Gln
W	<8	Pro	S50 60	Glu
BB	400		S60 200	Gln, Glu
S2	500	Leu	S78 100	Tyr
S5	50	Lys	S83 20	Glu
S15	<8		S88 <8	
S26	40	Arg	S89 <8	Met
S27	200	Gln	S90 60	Thr
S30	<8	Ala	S94 <8	



S27 S47

Toxic glutamine alleles

Qualitative assay for measuring aa uptake: competition to rescue from lethal doses of toxic alleles.

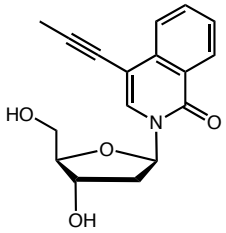


Non-toxic glutamine alleles

Liu & PGS, *PNAS,* 1999, 96, 4780.

Reports of Unnatural Base Pairs

Hydrophobic Bases:

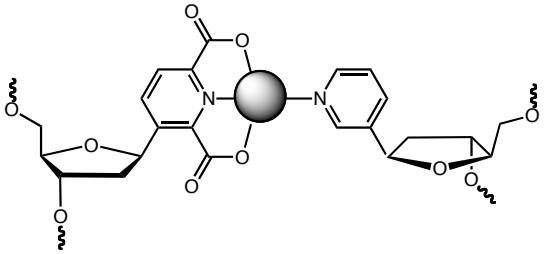


Self-pairing base recognized by *E. coli* DNA polymerase.

PGS, et. al., *JACS* ,1999, 121, 11585.

Third-Party Mediated Bases:

Cu(II) mediates the Dipic/Py base pair.



PGS, et. al., *JACS,* 2000, 122, 10714.