The Career of Peter G. Schultz

Nick Paras
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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

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
1994-1996—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);
**PGS' First Report of Catalytic Antibodies:**

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

**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<sub>H</sub>, V<sub>L</sub>).

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

Two of three constant domains in heavy chain (C<sub>H2</sub> & C<sub>H3</sub>) determine action after antigen binding

Origins of Diversity:

2-3 genes code each variable 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<sub>x</sub> x 4 J = 1200 V<sub>L</sub> domains

1000 V<sub>x</sub> x 12 D x 4 J = 48000 V<sub>H</sub> domains

1200 V<sub>L</sub> x 48000 V<sub>H</sub> > 5 x 10<sup>7</sup> antibodies from 1316 genes

Somatic mutations are 1 x 10<sup>6</sup> more frequent than in any other cells

**Scope of Antibody Catalysis: Enzymatic Reactions**

![Chemical Structures and Diagrams]

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


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**Scope of Antibody Catalysis: Reactions with Inexpensive Cofactors**

![Chemical Structures and Diagrams]

Oxidation Hapten


NaIO₄ and NaCNBH₃ substantially cheaper than enzymatic cofactors like NADH.

Nakayama & PGS JACS, 1992, 114, 780.
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.

RNA as Amplifiable Biopolymers

Isolation of Oligomers with Bias Toward Desired Activity

PGS, et. al., JACS, 1996, 118, 7012.
**Catalytic RNA with Designed Activity**

A library of approximately $4 \times 10^{14}$ 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.

**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**


**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.


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**Synthesis and Analysis of a Carbamate Biopolymer Library**

Glass\(\text{NH}_2\) \(\xrightarrow{\text{carbonate monomer}}\) Glass\(\text{N}^\text{VOC}^\text{R}^\text{NH}_2\) 

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 fluorescein-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.

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$_2$O$_3$, SiO$_2$, CeO$_2$, EuF$_3$, Tb$_4$O$_7$, Ag, TiO$_2$, Mn$_3$O$_4$, Gd$_2$O$_3$, ZnO, and Y$_2$O$_3$

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

PGS, et. al., ACIEE, 1999, 38, 36-54.
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<sub>0.88</sub>S<sub>0.12</sub>)CoO<sub>3</sub>: magnetoresistive properties
Gd<sub>2</sub>Ga<sub>5</sub>O<sub>12</sub>/SiO<sub>x</sub>: blue phosphorescence
CuO based high temperature superconductors
Catalysts for organic reactions (polymerization, etc.)

PGS, et. al., ACIEE, 1999, 38, 36-54.

Analysis of Active Site-Binding of a Kinase Inhibitor

Cyclin-Dependent 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.

Construction of 2,6,9-Purine Libraries


Purvalanol as an Active and Selective Kinase Inhibitor

3 R groups varied independently in separate libraries.

Final library tested best candidates for cooperative effects.

Crystal structure of Purvalanol B inhibition complex at active site

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_{Gln}.
Pre-charged yeast tRNA_{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 ^13C labels.

Establishing an Orthogonal tRNA

E. coli GlnRS does not charge S. cerevisiae (yeast) tRNA_{Gln}.


Does E. coli GlnRS charge yeast tRNA_{Gln}?

**In vitro** incubation of two strains of yeast tRNA_{Gln} with E. coli GlnRS and ^3H-labeled-Gln.
Orthogonal tRNA aminoacylation was approximately 100,000 times slower than wild-type E. coli tRNA_{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_{Gln} DNA or that of supE, known to function in E. coli. The yeast tRNA_{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 aminoacylation of tRNA_{Gln} with any amino acid for ampicillin immunity. The yeast tRNA_{Gln}-transform did not survive in the presence of ampicillin, even when E. coli GlnRS was overexpressed in the plasmid.

Does yeast tRNA_{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.

Zeroing in on Unnatural Amino Acids

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.

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<th>AIC50, μM Rescue</th>
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<tr>
<td>C</td>
<td>60 Glu S31</td>
<td>15 Tyr</td>
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<td>Q</td>
<td>20 S39</td>
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Toxic glutamine alleles

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<th>Non-toxic glutamine alleles</th>
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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.