Combinatorial Chemistry

- Combinatorial chemistry is the systematic and repetitive covalent assembly of reactant molecules to create a diverse array of molecules with a common scaffold.

- Benefits of combinatorial chemistry
  - Economics
  - Speed
Lead Compounds from Combinatorial Chemistry

Bayer's CB-1 Anagonist

K_i = 4.6nM

K_i = 21nM

Pfizer's mGluR1 Antagonist

K_i = 9nM

K_i = 14nM

Dolle, R. E. et al, W. J. Comb. Chem. 2008, 10, 753
Tools for Combinatorial Synthesis

- Solid phase synthesis
  - Resins
  - Linkers
- Screening methods
Solid Phase Synthesis

http://nobelprize.org/nobel_prizes/chemistry/laureates/1984/
Mix and Split Synthesis

Challenges in Split Synthesis

- Time consuming deconvolution process

- The amount of material on one bead is insufficient for structural determination

- The chemical history of the compounds synthesized is lost
Encoding Strategies

- Positional encoding
- Graphical encoding
- Radiofrequency encoding
- Spectrometric encoding
- Chemical encoding
Chemical Encoding Strategy

- One bead two compound approach
- Peptide Nucleic Acid encoding
- DNA-encoding
One bead One Compound Encoding

Edman Sequencing

Anillinothiazoline amino acid

Phenylthiohydantoin-amino acid
Peptidomimetics

- Peptides do not make good drug candidate because they are rapidly proteolyzed and have poor bioavailability.

- Peptidomimetics allows access to compounds without undesirable characteristics of linear peptides.

- Cyclic peptoid provide ideal scaffolds as protein ligands.
  - Abiotic
  - Conformationally order

Conformational Basis for Permeability

Challenges In Peptoid Libraries Screening

- Peptoid sequence cannot be determined by Edman sequencing

- Cyclic peptoids fragment at multiple positions complicating interpretation of MS/MS spectrum

Chemical Encoding Strategy

- One bead two compound approach
- DNA-encoding
- Peptide Nucleic Acid encoding
One Bead Two Compound Encoding

Kwon, Y-U.; Kodadek, T.; *Chem. Commun.* **2008**, *5704*
Synthesis of Encoded Cyclic Peptoid Libraries

Kwon, Y-U.; et al. *Chem. Commun.* **2008**, *5704*
Coupling Reaction

\[
\text{NH}_2 \xrightarrow{\text{BrCH}_2\text{CO}_2\text{H}} \text{NH} \xrightarrow{\text{DIC, DMF}} \text{NH} \xrightarrow{\text{RNH}_2} \text{NH}
\]

\[\text{R-NH}_2:\]
- (Nhse)
- (Nmea)
- (Nall)
- (Nleu)
- (Nlys)
- (Nfffa)
- (Npip)

Encoded Cyclic Peptoid libraries

Cyclization Reaction

Mass Spectrum

Sequence Analysis

Chemical Encoding Strategy

- One bead two compound approach
- DNA-encoding
- Peptide Nucleic Acid encoding
DNA Encoded Libraries

**General structure**: 5'-NH₂(CH₂)₆PO₄-GCA GCT TCT XXX XXX CGA CCA TGG-3'  

48mer Oligonucleotide

X denotes different combinations of 6 bases for individual compounds

619 compounds library each with a specific coding sequence

Synthesis of DNA-Compound Conjugate

Synthesis of Oligonucleotide-Compound Conjugates

Library of Potential Albumin Binders

Human Serum Albumin (HSA)

- Most abundant protein in the circulatory system
- Principle function is transport of fatty acids
- It is also capable of binding a great variety of metabolites and drugs

Identification of HSA Binders

- Dimerization of DNA encoded compounds to cDNA
- Incubation of with Albumin immobilized on resin
- Washing with buffer solution
- PCR amplification using 5’-labeled primer
- Hybridization to DNA microarray

Selection HSA Binders

Potential HSA Binders

Enrichment Profile

\[
\text{Enrichment} = \frac{\text{Signal intensity of HSA selection}}{\text{Signal Intensity of Inactivated resin}}
\]

Promiscuous Binders for HSA

Consensus Structure of Identified Binders

- **622**
- **428**
- **536**
- **625**
- **630**
- **632**

- **X** must be a hydrophobic substituent
- **Y** can stand for a hydrophobic substituent
- Butanoyl moiety
- Hydrophobic groups in *para* position

Chromatographic Albumin Binding Assay

Chromatographic Albumin Binding Assay

Mass Analysis of Oligonucleotide-Compound Conjugate

Chemical Encoding Strategy

- One bead two compound approach
- DNA-encoding
- Peptide Nucleic Acid encoding
Peptide Nucleic Acid (PNA) Encoding

PNA Encoded Split Synthesis

Chemical Structure of PNA and DNA Oligomers

Peptide Nucleic Acid (PNA)

Synthesis of Peptide Nucleic Acid Monomers

Rapoport’s Reagent

Guanine Synthesis

Guanine Synthesis

Peptide Nucleic Acid Synthesis

Debaene, F.; *et al.* *Org. Lett.* *2003*, 5, 4445
Coupling Reaction

PNA Encoding

PG₂ : Boc, Mmt

Modified PNA Oligomers

**Solubility of Modified Oligomer**

![Chemical structures](image)

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<th>pH 5.5 Buffer</th>
<th>pH 7.0 Buffer</th>
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<tr>
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<td>PNA-Monomer</td>
<td>100%</td>
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% of PNA remaining in solution after sonication and centrifugation

Encoding Protease Libraries

Cathepsin B IC<sub>50</sub> 1-10 nM
Trypsin IC<sub>50</sub> 1-10 nM

PNA codon elements for diversity

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Summary

- PNA encoding strategies are being as means of tracking chemical history of library members

- By combining genetics with chemical synthesis new chemical entities can be unambiguously identified from combinatorial libraries

- For combinatorial chemistry to have maximum impact, a large range of bond-forming reaction need to be developed on solid phase
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