Dynamic Combinatorial Chemistry

in the identification of new host–guest interactions: proof of principle

Nick Paras
MacMillan Group Meeting
October 17, 2001

**Conventional combinatorial approach to identification of host–guest interactions**

**Combinatorial Library**

- molecular constituents
- real set
- collection of molecules
- covalent
- non-reversible
- neutral, uninformed
- systematic
- performed by synthesis in the absence of the target
- assayed by high throughput screening
- amplified by independent chemical synthesis

Conventional combichem used to identify molecules of interest ranging from drugs to novel catalysts.

---

**Dynamic combinatorial approach to identification of host–guest interactions**

**Dynamic or Virtual Combinatorial Library (DCL/VCL):**

- molecular or supramolecular constituents
- virtual set
- collection of components
- covalent or non-covalent
- reversible
- instructed
  - internally (self-recognition)
  - externally (species binding)
  - adaptive
- recognition-directed
- self-assembled
- assayed *in situ*
- amplified *in situ*

*a set of real or potential compounds which equilibrate under reaction conditions*

**Unifying features of POP research:**

- reversible associations
- selection of subunits
- selection of template
- analytical technique
- method for isolation

Dynamic combichem unifies synthesis, screening and amplification steps.
Dynamic combinatorial approach based on Le Châtelier's principle

\[ \text{M}_1 \cdots \text{M}_5 \cdots \text{M}_n \quad \text{T} \]

- initial concentrations of library members based on thermodynamic distribution
- addition of template
- equilibrium driven toward members which form favorable associations with template

Two kinds of templating

- **Casting.** A relatively small molecule is formed to fit a large receptor template (e.g. enzyme.)

- **Molding.** A large or even supramolecular assembly is formed to encapsulate a small molecule.

Reversible chemical reactions constitute basis of fluxionality

- tranesterification
- imine \((X = C)\), oxime \((X = OR)\), hydrazone \((X = NHR)\) formation
- hemiketal formation
- boronic ester formation
- disulfide formation
- olefin metathesis
- \(\text{cis-trans} \) isomerization

Also: Diels-Alder, conjugate addition, metal coordination, electrostatic interaction, bond rotation, ring inversion, tautomerism

Roots in supramolecular self-assembly:  
Trimericbipy-Fe cryptand templated for different counterions

Equilibration based on reversible Fe-bipy complexation.

Elementary examples: three member library based on π-bond isomerization

Selection: silica-bound arginine

Mutation: light source


Elementary examples: Miller’s DNA-binding Zn\textsuperscript{2+} salen complexes

\[
\begin{align*}
\text{Zn}^2+ & \quad \xrightarrow{2} \quad \text{Zn}^2+ \\
\end{align*}
\]

When eluted over an affinity column of immobilized poly-d(AT) DNA in the presence of Zn\textsuperscript{2+}, significantly decreased amounts of 4 were recovered.

"Informed" 3-member DCL used shows bias for homodimers in presence of template

- A-SS-A linked to fluorophore and screened against library of 3375 N-acetyl tripeptides.
- Ac-(D)-Pro-(L)-Val-(D)-Val-PS was found to bind favorably to A-SS-A (binding constant \(\sim 10^4-10^5\)).
- A mixture of the two monomers are dimerized in the presence and absence of template.

<table>
<thead>
<tr>
<th>Presence of tripeptide-PS:</th>
<th>A-SS-B</th>
<th>B-SS-B</th>
<th>A-SS-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence of tripeptide-PS:</td>
<td>43%</td>
<td>57%</td>
<td></td>
</tr>
<tr>
<td>Presence of tripeptide-PS:</td>
<td>15%</td>
<td>85%</td>
<td>10%</td>
</tr>
<tr>
<td>Solution phase</td>
<td>13%</td>
<td>85%</td>
<td>10%</td>
</tr>
<tr>
<td>Resin phase</td>
<td>2%</td>
<td>0%</td>
<td>75%</td>
</tr>
</tbody>
</table>

In the presence of cognate peptide, equilibrium shifts to favor homodimers. A-SS-A can be isolated in 97% purity by simple wash cycle.

Raising the bar: template directed amplification of a carbonic anhydrase (CA) inhibitor

known inhibitor of carbonic anhydrase II

• Purpose: to make a VCL of imines in the presence of CAII and look for amplification of known inhibitor motif

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bond equilibration under physiological conditions</td>
<td>Transimination, pH 6</td>
</tr>
<tr>
<td>Switch off equilibration process after templating</td>
<td>NaBH₃CN reduction of imines</td>
</tr>
<tr>
<td>Minimize uninformed thermodynamic bias</td>
<td>Only aryl aldehydes; keep divergent functionality away from bond forming site</td>
</tr>
<tr>
<td>Characterize library</td>
<td>HPLC/MS</td>
</tr>
</tbody>
</table>


Components of Lehn's carbonic anhydrase-templated iminium VCL
Results of Lehn's carbonic anhydrase-templated iminium VCL

Pseudo-trisaccharide identification and amplification via selective binding to sepharose bound Con A

Substrate Analogs

Flexible auxiliaries function in role of central mannose.
Disulfide bonds allow for interconversion between dimers.
Shallow enzyme binding pocket forgives obvious linker differences.

With $R = \text{H}, \text{OH}$ and $R' = \text{H CH}_2\text{OH}$ and tether lengths of 2 or 3 methylenes, a real library of 6 carbohydrate dimers was formed.
Two approaches toward identification and isolation:

6 initial homodimers → immobilized template, pH 7.4 → adaptive equilibration → acidification isolation → on support: only mannose containing dimers, primarily homodimer (2.1:1) → in solution: depression of mannose homo and hetero dimers

6 initial homodimers → pH 7.4 → thermodynamic equilibration → acidification template-assisted retrieval → on support: only mannose containing dimers, primarily homodimer (1.5:1) → in solution: depression of mannose homo and hetero dimers

• Addition of template during equilibration conditions allows for amplification of favorable ligands. (Adaptive effect)

• Addition of template to pre-equilibrated library is less selective but obviates need for compatibility with rxn conditions.

Double-level orthogonal dynamic combinatorial libraries:
A general scheme for ion coordination/transimination

\[ M^{\text{oxd}} + LX_2 + LY_2 \]

• Oxidation state of metal center functions as ligand on/off switch.

• pH and amine concentration (or oxime or hydrazine) regulate transimination.


Double-level orthogonal dynamic combinatorial libraries.
Reduced to practice: ligand lability of Co^{2+} and Co^{3+} complexes

\[ \text{Co(L}_{11}\text{)}^{2+} \rightarrow \text{Co(L}_{12}\text{)}^{2+} \rightarrow \text{Co(L}_{11}\text{)(L}_{22}\text{)}^{2+} \rightarrow \text{Co(L}_{22}\text{)}^{2+} \]

rate_1 \approx 1,300 \text{ M}^{-1}\text{s}^{-1}

DCL of symmetrical complexes

- Co^{3+} exchange 1/2 life @ 25 °C \approx 1 \text{ month} \implies \text{negligible}
- Interestingly, metal ligand exchange based on excess ligand is also slower (~15 M^{-1}s^{-1})
- Complete scrambling is possible via hydrazone exchange at pH 3, 60 °C


Pseudo-peptide cyclic oligomers

Proline used for geometrical constraint (β-turn enforcement)

- DCL at equilibrium, without template favors formation of cyclic oligomers with 2-5 repeating subunits. (a)
- On addition of 18-crown-6, HPLC trace is dominated by species 6 which is the monomer unit 1 in deprotected form. (b)
- MS dominated by 6 + 18-crown-6 + H^+.
- Original equilibrium quantities can be restored by the addition of KBr. (c)

Amplification and induced fit of pseudo-peptide cyclic oligomers

- Equilibrium shifted toward trimer on addition of inorganic salts
- No change observed with NR₄⁺ iodides or KI, RbI, CsI

- NMR of isolated trimer and trimer in the presence of lithium shows dramatic shifts throughout entirety of oligomeric structure.

Molecular amplification of pseudo-peptide cyclic oligomers

Kubik's peptide

hydrazone analog

Substrates:

\[ \text{Binding constant} \]

<table>
<thead>
<tr>
<th>Kubik's peptide:</th>
<th>N-methyl quinuclidium salts</th>
<th>acetyl choline</th>
</tr>
</thead>
<tbody>
<tr>
<td>42200 M⁻¹</td>
<td>11000 M⁻¹</td>
<td></td>
</tr>
</tbody>
</table>

| Binding constant hydrazone: | 150 M⁻¹ | 230 M⁻¹ |

- Kubik's trimeric cyclic peptide is known to have binding affinity for quaternary ammonium ions: quinuclidium and acetyl choline.

- In a DCL which favors the dimer over trimer (88:11) of subunit mPro, the 230 M⁻¹ binding affinity to AcCh reversed the preference to 14:86.

Expensive toys: analysis of a DCL of pseudopeptide oligomers by ESI-FTICR-MS/MS

Expected array of oligomeric species

Analysis of a DCL of pseudopeptide oligomers
Tetramer: the simplest non-degenerate case

Two possible orders for V₃L₂ oligomer:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
</tr>
</thead>
</table>

Mass fragmentation pattern for 1: V−V  V−L  V−L  L−L  1 : 2 : 1
Mass fragmentation pattern for 2: V−L  V−L  V−L  V−L  0 : 4 : 0

Net fragmentation pattern: 1 : 6 : 1

- Similar, but more complex, analyses can be performed on larger oligomers and DCL systems.
- Deviation from ideal ratio can give incite into connectivity as well as composition.
Low-tech/high-concept analysis of DCL
Dynamic deconvolution strategy based on enzyme inhibition

Step 1: Selection of template/assay

- Acetylcholinesterase activity and inhibition can be easily monitored spectrophotometry

Step 2: Construction of a suitable DCL

acylhydrazines:
- \[ \text{Me}_3\text{N}O\text{NH}_2 \times 2\text{HCl} \]
- \[ \text{Ph}_2\text{CH}_2\text{NH}_2 \times \text{HCl} \]
- \[ \text{Ph}_2\text{CH}_2\text{NH}_2 \times \text{HCl} \]

monoaldehydes:
- \[ \text{Ph}_2\text{CHO} \]
- \[ \text{Ph}_2\text{CHO} \]
- \[ \text{Ph}_2\text{CHO} \]

Dialdehydes: (linkers)
- \[ \text{Ph}_2\text{CHO} \]
- \[ \text{Ph}_2\text{CHO} \]
- \[ \text{Ph}_2\text{CHO} \]

- All constituents are water soluble and showed negligible inhibition as free hydrazines or aldehydes
- Up to 66 possible different species from a small set (13) components

Low-tech/high-concept analysis of DCL
Dynamic deconvolution strategy based on enzyme inhibition

- Each bar corresponds to omission of a given component.
- Hydrazine 4 and dialdehyde 1 seem to be most important in inhibition.
- New receptor compares favorably to known inhibitors of acetylcholinase

Effect of templating on vancomycin dimerization

- Dimerization of vancomycin leads to increase potency.
- Dimerization with various tether lengths in the presence of template should be faster and select for more effective binders.
- Clear preference was found for short tether lengths when equilibration was carried out in presence of template.
- Analogs with up to 12x activity against susceptible strains and up to 100x activity against resistant strains were identified.

Homodimerization of \( m = 1 \) and \( m = 3 \) substrates, via olefin metathesis

---

Is DCC doomed from the start?

A theoretical analysis

Assumptions:

- Binding affinities among a random population of aptamers are reasonably described as being normally distributed in \( \log K \).
- Any reasonably defined population of a noncovalent association will have a maximum typical stability range of 5-6 orders of magnitude in the equilibrium constant, resulting in a standard deviation of about 1 \( \log K \) unit.
- The mean will be determined by the inherent features of the population.
- The standard deviation, however is presumably controlled by the range of forces available from non-covalent interactions

Conclusions:

- In a random population, the mean binding constant can only be increased to a limited degree (ca. 2 orders of magnitude) by addition of a template.
- Iterative templating to get around this problem will be plagued by exponentially decreased yields.
- Selection and amplification will be required for true chemical evolution.
- DCC may be useful in generating lead compounds, but never in generating practical quantities of desired binders.
Summary

• Dynamic combinatorial libraries provide access to large numbers of real and virtual compounds with little synthetic effort.

• DCC research is still in the proof of principle stage.

• New reversible molecular associations are being explored.

• New methods for the analysis of increasingly complex DCLs are being developed.

• The goal of DCC research is to rapidly define new host-guest interactions important in biomedical applications and catalyst discovery.