High-throughput Experimentation: Designed Serendipity

Andy Thomas
Group Meeting 2015-2-10
1. Introduction
   • Serendipity in chemistry
   • Traditional Reaction Development
   • High-throughput experimentation (HTE)

2. Design of Experiments
   • Tagged approaches
   • Non tagged approaches
   • Methods for analysis of data

3. Catalyst Development

4. Synthesis: Small molecule Screening

5. Reaction Discovery
   • Immunoassays
   • GC/MS
   • DNA Templating
   • MALDI

6. Reaction Intermediates
   • Mass spectrometry

7. Overview of methods

8. Conclusions with future outlook
Serendipity in Science

Indigo

Alfred Baeyer
(1905 Nobel Prize)

\[
\text{Cyclohexane} \xrightarrow{H_2SO_4/\text{SO}_3} \text{Cyclohexane-1,2-dicarboxylic acid}
\]
slow process

Eugene Sapper

\[
\text{Cyclohexane} \xrightarrow{\text{HgSO}_4} \text{Cyclohexanecarboxylic acid}
\]
catalyst discovery

Dr. Albert Hofmann-1938

"I was cycling, cycling, but the time seemed to stand still"

Lyseric acid diethylamide

Ph_3P-CH_3

\[
\begin{align*}
\text{BuLi} & \quad \text{Bu} \\
\text{Ph}_3\text{P-CH}_3 & \quad \text{Ph}_3\text{P-CH}_3
\end{align*}
\]
not observed

1954 Georg Wittig
Nobel Prize 1979

\[
\begin{align*}
\text{Ph}_3\text{P-CH}_3 & \xrightarrow{\text{BuLi}} \text{Ph}_3\text{P}=\text{CH}_2 \\
\text{Ph}_3\text{P-CH}_3 & \xrightarrow{\text{BuLi}} \text{Ph}_3\text{P-CH}_3
\end{align*}
\]

Teflon

\[
\begin{align*}
\text{Heat} & \quad \text{Pull slow (Hard and Brittle material)} \\
\text{Pull Fast (PTFE or Gore-tex)} & \quad \text{not observed}
\end{align*}
\]
Traditional Reaction Development

Research directed toward the development of new systems.

Phase 1:
In the initial lead discovery phase effort is directed toward screening a wide variety of variables with the goal of identifying a novel catalyst system for the reaction of interest.

Phase 2:
This is typically followed by a lead optimization stage wherein a expansion of reactivity is sought through systematic variation of the components and reaction conditions

High-Throughput Experimentation

- High-throughput screening (HTE) is a method for experimentation used in drug discovery as well as biology and chemistry.

- High-throughput screening allows a researcher to quickly conduct millions of chemical, genetic, or pharmacological tests by using robotics.

- These tests are performed on small amounts of material.

- Organic Chemists can use HTE for:
  1. Reaction Development
  2. Diversity oriented synthesis
  3. Reaction Discovery

References:
- Maggie Bartlett, National Human Genome Research Institute
The Chemspeed SLTII Platform consists of a flexible, modular robotic platform possessing an X, Y and Z arm with rotating alpha-axis and automatic tool exchange within a unique hood with airlock.

- Reaction preparation will be performed by precise, gravimetric, overhead dispensing of solids and four-channel volumetric liquid transfer. The liquid transfer tool can also be used for taking and preparing samples, with the option for direct injection to analytical devices such as HPLC/GC.
- Solid phase Extraction and ultrasonification tools are included.
- Heating and cooling circuit is supplied via fluid from external cryostat with temperature control of -40 °C to 145 °C.
- Agitation is by orbital shaking up to 1400 rpm.
- Reactive gas connections are included with capability of pressures up to 100 bar inside reactors. Inert gas and vacuum connections are also supplied.

http://scs.illinois.edu/htsf/equipment/chemspeed.php
High-Throughput Experimentation-Approaches

**Approaches**

1. Combinatorial Chemistry
   - Prepare large numbers of compounds in a single process
   - Molecules with multiple points of diversity \((R_1, R_2, R_3)\) can generate \(N_{R_1} \times N_{R_2} \times N_{R_3}\) molecules

2. Two-Substrate-one catalyst screen
   - Parallel Screen of two component reactants with a single catalyst

3. Multisubstrate-one catalyst screen
   - Parallel Screen of different reactants(random) with a single catalyst system

4. Intermediates and transition states in reaction development
   - obtain mechanistic data

**What can we obtain with HTE?**

- Obtain lots of data on small amounts of material (mg or ng)
- Discovery new catalysts, ligands, and reactions
- Save time (1000’s of reactions can be performed in a single day, even with out robots!)

**Who uses HTE?**

- Mostly industry
- Starting to appear in academics

Molecular Tags:
• Immunoassays (Sandwich enzyme-linked immunosorbant assay [ELISA])
• DNA Templating (DNA templated organic synthesis)
• Laser desorption/ionization-time-of-flight mass spectrometry (MALDI)
• Fluorescence and Colourimetry (FRET can be used)

Tag free approaches:
• Optical methods of reaction analysis
• In situ enzymatic screening (ISES) of reactions
• GC/MS, HPLC, NMR

Intermediates and transition states in reaction development
• ESI-MS screening of ionic intermediates
• Self Selecting Catalysts

Outline

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Combinatory Chemistry: Epoxidation of Alkenes

192 catalysts in each well, (Each well contains a different metal)

Reaction Development

\[
\begin{align*}
\text{benzene} & + 30\% \text{ H}_2\text{O}_2 \text{ (aq)} \\
200 \text{ mM} & + 200 \text{ mM} \\
& \xrightarrow{1 \text{ mg metal library}} \\
& \xrightarrow{50 \mu\text{L CH}_2\text{Cl}_2 / \beta\text{BuOH 1/1}} \\
& \xrightarrow{15 \text{ h}} \\
& \text{phenyl epoxide}
\end{align*}
\]

2\textsuperscript{nd} screen

Beads (aminomethyl polystyrene)
100 ± 200 mesh particle size, 0.6 mmol g\textsuperscript{-1} loading

Combinatory Chemistry: Epoxidation of Alkenes Results

5760 ligands screened: Discovered three effective ligands

Best Catalysts with Iron

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>ee</th>
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<tbody>
<tr>
<td>18</td>
<td>4%</td>
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<td>19</td>
<td>7%</td>
</tr>
<tr>
<td>20</td>
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</table>

Reactions monitored by GC

Thermographic Selection of Effective Catalysts from an Encoded Polymer-Bound Library

Thermographic Selection of Effective Catalysts from an Encoded Polymer-Bound Library

Combinatory Chemistry: Solid Support

strong Fluorescence

no Fluorescence

strong Fluorescence

no Fluorescence

no Fluorescence

Förster Resonance Energy Transfer (FRET)

- FRET occurs when the emission band of one molecule overlaps with an excitation band of a second molecule.
- Upon excitation of the FRET donor that absorbs at a higher energy, quenching of its emission occurs by an acceptor.
- At an appropriate constant total concentration of free and associated FRET pairs, the emission of the FRET donor is inversely related to the mole fraction of associated molecules.
- Product yields for a reaction that forms a covalent bond can be determined fluorimetrically by using automated fluorescence plate readers.

Heck Reaction

![Heck Reaction](image)


Catalysts for Room-Temperature Heck Reactions

Figure 1. Yields by FRET for 96 reactions with different ligands.

In Situ Enzymatic Screening (ISES): Catalyst Discovery

Enzymatic Screening: Catalyst Discovery


<table>
<thead>
<tr>
<th>Entry</th>
<th>Ligand (L)</th>
<th>N-Protecting Group (R)</th>
<th>Slope[a] [mAbs/n]</th>
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<td>H (no protection)</td>
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<td>C₆H₄-p-NO₂ (PNP)</td>
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<tr>
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<td>C₆H₂-3,4,5-(OMe)₃</td>
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</tr>
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<tr>
<td>5</td>
<td>PPh₃</td>
<td>CO₂Bu (Boc)</td>
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<td>6</td>
<td>PPh₃</td>
<td>C₆H₄-p-OMe (PMP)</td>
<td>35</td>
</tr>
<tr>
<td>7</td>
<td>P(OMe)₃</td>
<td>C₆H₄-p-OMe (PMP)</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>P(2,6-di-OMe-C₆H₃)₃</td>
<td>C₆H₄-p-OMe (PMP)</td>
<td>7</td>
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<td>9</td>
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<td>dppf[c]</td>
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<td>12</td>
<td>P(C₆H₄-p-NMe₂)₃</td>
<td>C₆H₄-p-OMe (PMP)</td>
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</tr>
<tr>
<td>13</td>
<td>P(C₆F₅)₃</td>
<td>C₆H₄-p-OMe (PMP)</td>
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<tr>
<td>14</td>
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<td>16</td>
<td>P(tBu)₃</td>
<td>C₆H₄-p-OMe (PMP)</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Determining the Enantioselectivity of Chiral Catalysts by Mass Spectrometric Screening

Determining the Enantioselectivity of Chiral Catalysts by Mass Spectrometric Screening: Multiple at once

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   • DNA Templating
   • MALDI

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Nanomole-scale high-throughput chemistry for the synthesis of complex molecules

Nanomole-scale high-throughput chemistry for the synthesis of complex molecules

Nanomole-scale high-throughput chemistry for the synthesis of complex molecules

Nanomole-scale high-throughput chemistry for the synthesis of complex molecules

Rapid Assessment of Chemical Reactions via High throughput screening

<table>
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<tr>
<th>Entry</th>
<th>Additive</th>
<th>Yield of 3 (%)</th>
<th>Additive remaining (%)</th>
<th>SM remaining (%)</th>
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<td>A8</td>
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<td>9%</td>
<td>96%</td>
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</table>

<table>
<thead>
<tr>
<th>Entry</th>
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<th>Additive remaining (%)</th>
<th>SM remaining (%)</th>
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<td>0%</td>
<td>100%</td>
<td>100%</td>
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<td>21%</td>
<td>0%</td>
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</tr>
<tr>
<td>A16</td>
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<td>100%</td>
<td>65%</td>
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<td>A17</td>
<td></td>
<td>80%</td>
<td>83%</td>
<td>0</td>
</tr>
</tbody>
</table>

Yield 4, 75% Recovered SM, 0%
Yield 5, 75% Recovered SM, 0%
Yield 7, 24% Recovered SM, 19%
Yield 8, 42% Recovered SM, 34%

Rapid Assessment of Chemical Reactions via High throughput screening

High-Throughput Discovery of Catalytic Reactions: Hartwig

17 substrates in each well

Metals
1) Fe(acac)_2
2) FeCl_3
3) Mo(CO)_3(EtCN)_3
4) MoCl_5
5) Mn(acac)_2
6) W(CO)_3(MeCN)_3
7) Yb(OAc)_3
8) Cr(CO)_3(C_6H_5)_3
9) Co(OAc)_2
10) Ni(cod)_2
11) CuCl
12) Cu(OAc)_2
13) [Ru(p-cymene)Cl_2]_2
14) AuCl
15) NiCl_2-dme
16) none

Ligands

Took a few days to perform over 50,000 reactions

High-Throughput Discovery of Catalytic Reactions: Representative data

**Results**

High-Throughput Discovery of Catalytic Reactions: Rxn3 Optimization

Discovery of an $\alpha$-Amino C-H Arylation Reaction

**Reaction discovery via accelerated serendipity:** High-throughput combination and evaluation of benign substrates and catalysts

**Substrate Pool**
- **(A) aromatics**
- **(B) alkenes**
- **(C) alkynes**
- **(D) carbonyls**
- **(E) hetero-aromatics**
- **(F) nitriles**
- **(G) alcohols**
- **(H) amines**

**Large No. Random Reactions**
- 96 wells of non-reactive paired substrate combinations

**Initial Result - ‘Hit’**
- Catalyst
- GC-MS Hits
- t/min: 5
- Peak of significant intensity and molecular weight
- New bond formed

**Evaluation**
- New reaction?
- Is the reaction interesting? Important?

**Optimize**

**Accelerated Serendipity: GC Hit, Initial Result, Reaction Evaluation, Optimization and Outlook**

**GC Hit**
- \(\text{Me} \quad \frac{N}{Me} \quad \text{Ph}\)
- \(1,4\text{-DCB}\)

**Initial Result**
- \(\text{Me} \quad \frac{N}{Ph} \quad \text{CN}\)
- \(2, 11\%\)
- \(\text{Ir(ppy)}_2(\text{dtbbpy})\text{PF}_6 \cdot 0.5 \text{ mol}\%\)
- \(\text{Na}_2\text{CO}_3, \text{DMF}, 23^\circ\text{C}, 26 \text{ W Lamp}\)

**Evaluation**
- Unanticipated Transformation
- Valuable Product

**Optimization**
- \(\text{Optimization}\)
- \(\text{Ir(ppy)}_3 \cdot 1.0 \text{ mol}\%\)
- \(\text{NaOAc, DMA, 23}^\circ\text{C, 26 W Lamp, 85}\% \text{ yield}\)

**Outlook**
- Acyclic and Cyclic
- Benzylic Amines
- Important motif

---

Discovery of an α-Amino C-H Arylation Reaction: Experimental Design

2 substrates were combined in each well (171 different combinations)

Parameters:
- Metals: Ir, Pd, Ru, Au, Fe, etc.
- Solvent
- Base

(Use Chemspeed robotic plateform)

Substrate pool
Metal, Base, Solvent
26 W fluorescent light bulb

(authors do not disclose all of the screen parameters)

Figure S8. Examples of discoveries based on transition metal catalysis.

Discovery of an α-Amino C-H Arylation Reaction: Mechanism

Sandwich Immunoassay Method

1. Reactions with tagged substrates
   \[ A + B \rightarrow A' - B' \]

2. Immobilization of tagged substrates
   Solid-supported antibodies

3. Introducing signalling enzyme
   Antibody

Epitopes

Immunoassay: Development

- Current discussion on immunoassay development.
**Reaction Discovery using Immunoassay**

**Two-Substrate-one catalyst screen**

**Pros = Fast detection, (no automation)**  
**Cons = prior substrate prep., not amenable to certain reaction conditions**

DNA Templating: Reaction Discovery

**Substrates**

1. **Pool A** and **Pool B** are prepared.
2. Mix **A** and **B**.
3. (Watson-Crick Base Pairing)
4. Reaction conditions.
5. Cleave disulphide.
6. Use Avidin beads to bind biotin.
7. PCR amplify.
8. Label with red or green fluorophore.

---

DNA Templating: Reaction Discovery

Pros:
• Small scale (femtomole scale)
• Can visualize determine Rxns

Cons:
• Prep of DNA containing substrates
• each rxn goes through 7-8 steps
• Some Rxn conditions not compatible with DNA.

### DNA Templating: Reaction Discovery

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Green/red fluorescence ratios</th>
<th>DNA-templated yields (%)</th>
<th>Product consistent with observed mass</th>
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<td>37°C</td>
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<td>37°C</td>
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<td>3.6</td>
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- 

DNA Templating: Reaction Discovery

Label-assisted Mass Spectrometry for the acceleration of reaction discovery and optimization

Label-assisted Mass Spectrometry for the acceleration of reaction discovery and optimization

Label-assisted Mass Spectrometry for the acceleration of reaction discovery and optimization

Label-assisted Mass Spectrometry for the acceleration of reaction discovery and optimization

High-Throughput Discovery of Catalytic Reactions: MALDI

High-Throughput Discovery of Catalytic Reactions: MALDI

<table>
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<tr>
<th>Entry</th>
<th>Catalyst (mol%)</th>
<th>Additive (equiv.)</th>
<th>Solvent, temperature (°C)</th>
<th>Conversion (%)</th>
<th>d.r.</th>
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<td>1</td>
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<td>MeOH (5)</td>
<td>THF, 20</td>
<td>56</td>
<td>85:15</td>
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<tr>
<td>2</td>
<td>Pd(OAc)₃ (5)</td>
<td>MeOH (5)</td>
<td>Toluene, 20</td>
<td>&lt;2</td>
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<tr>
<td>3</td>
<td>Pd(PCy₃)₂ (5)</td>
<td>MeOH (5)</td>
<td>Toluene, 20</td>
<td>35</td>
<td>85:15</td>
</tr>
<tr>
<td>4</td>
<td>Pd(PPh₃)₄ (5)</td>
<td>MeOH (5)</td>
<td>Toluene, 60</td>
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<td>BnOH (2)</td>
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<td>(4-MeOC₆H₄)₃P (20)</td>
<td>4-FC₆H₄CH₂OH (2)</td>
<td>Toluene, 60</td>
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High-Throughput Discovery of Catalytic Reactions: MALDI

## Conclusions and Overview of the Methods

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<tr>
<th>Strategy</th>
<th>Author</th>
<th>No specialist skills required</th>
<th>No specialist equipment required</th>
<th>General applicability</th>
<th>Key advantages</th>
<th>Key limitations</th>
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</thead>
</table>
| Multidimensional. Undirected: two substrate-one catalyst | MacMillan\textsuperscript{24} | ✓✓✓                           | ✓✓✓                             | ✓✓✓                   | Simple analysis (H)  
Simple set-up                                                                 | Serial analysis (M)                                   |
| Multidimensional. Substrate directed: two substrate-one catalyst | Beeler and Porco \textsuperscript{26,27,29,30} | ✓✓✓                           | ✓✓✓                             | ✓✓✓                   | Simple analysis (H)  
Simple set-up  
Large number of hits                                                      | Serial analysis (M)  
Limited novelty discovered                                |
| Multidimensional. Undirected: multisubstrate-one catalyst | Hartwig\textsuperscript{33} | ✓✓✓                           | ✓✓✓                             | ✓✓✓                   | Streamlined analysis (M)  
Simple set-up  
Multiple reactions per well                                               | Analysis of mixtures (H)  
Difficult to identify sensitive reactivity               |
| Sandwich immunoassays                         | Taran\textsuperscript{13-47} | ✓                             | ✓                               | ✓✓                   | Full optimization using assay is feasible  
Parallel reaction analysis                                                   | Extensive pre- and post-screen work                    |
| DNA templating                                | Liu\textsuperscript{49-54}  | ✓                             | ✓                               | ✓✓                   | Scale  
All reactions in one well                                                  | Extensive pre- and post-screen work                    |
| Matrix-assisted laser desorption/ionization   | Kozmin and Mrksich\textsuperscript{56} | ✓✓✓                           | ✓✓                             | ✓✓                   | Very fast and simple analysis (M,H)                                           | Solubility of tagged substrates                      |
| Self-assembled monolayer desorption/ionization | Kozmin and Mrksich\textsuperscript{57} | ✓✓                             | ✓✓                             | ✓✓                   | Very fast and simple analysis (M, H)                                           | Compatibility of self-assembled monolayers with reaction conditions |
| Fluorescence                                  | Several                     | ✓✓✓                           | ✓✓✓                             | ✓✓                   | Parallel visual analysis  
Often quantifiable                                                             | Difficult to apply in undirected screens for discovery |
| In situ enzymatic screening                  | Berkowitz\textsuperscript{85-86} | ✓✓                             | ✓✓                             | ✓✓                   | Parallel visual analysis  
Quantifiable                                                                   | Requires alcohol product or by-product                 |
| Electrospray ionization mass spectrometry of intermediates | Pfaltz\textsuperscript{89,91,96,97} | ✓✓✓                           | ✓✓✓                             | ✓✓                   | Provides direct measurement of catalyst activity and/or selectivity           | Requires ionic intermediates  
Difficult to find novel reactivity                                        |
| Self-selecting catalysts                     | Several                     | ✓✓                           | ✓✓✓                             | ✓✓                   | Self-assembly of catalysts  
— no synthesis                                                                 | Requires transition-state analogue  
Difficult to find novel reactivity                                         |