Nano-Science Center

Encoding in Chemistry and Biology

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Encoding in chemistry and biology

- Biological encoding (DNA)
- Chemical encoding
- Encoding by position
- Optical pattern encoding (barcodes)
- Color encoding
- Rf - encoding
- Magnetic encoding
- Spectroscopic encoding

- Ease of preparation
- Ease of handling
- Cost
- Feasibility
- Chemistry compatibility
- Speed of decoding
- Assay compatibility
- Reliability
**Split mix**

- Array synthesis
- Parallel synthesis
- Phage display

1. **Compartmentalize resin**
2. **Add molecular building block to every column**
3. **Wash, Mix and Split**
4. **Prepared for next building block**
5. **Repeat**
6. **Enzyme from Barley is added**
7. **Beads with substrate fluoresce: Isolate and determine structure**
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SPHERECODES

BARCODES (Tibotec, Ghent Uni.)

NANOPARTICLES

- Spherical encoding by diffusion, Miller et al.
- Laser etched barcodes, Leblans et al.
- Nanoparticle in situ encoding, Battersby et al.
- Infra red tags
- Binary fluorescence encoding

Micro-Particle-Matrix encoding

Discovery Partners Int.*
Encore International Corp.
Luminex*
Quantum dot*
Smart Bead Technologies Ltd.*
Spectra science corporation
Vitra Bioscience
Illumina
and many more ...
BARCODES (Tibotec, Ghent Uni.)

Code reads by confocal microscopy

Code reads problematic

Time consuming
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Red Labels

Mix 1 d

Green Labels

Red Labels

Green Labels

Cross talk
Obstruction of screen
500 μm beads and 1 μm resolution:
65449846 unique positions
$10^{14}$-$10^{15}$ absolute vectors to combine.
Vector combinations are $\sim$infinite

- 3 Orthogonal CCD detectors
  6*2D ..... 3*3D-coordinates
- Inter-particle vectors
  lengths and angles
  orientation independent parameters.
- 3 Particles: 6 vectors, 3 angles
- 4 Particles: 12 vectors, 12 angles etc.
MPM-encoding

Easy to prepare (random code)
Very high coding potential
Optical
Non interfering signal
Resin independent
Stable encoding
Reliable reading
Biochemistry and chemistry
Very fast decoding
Direct structure/activity information
Cleavable linkage

Stable:
Perfect beads
Narrow size distribution

Unstable:
vacuoles
airbubbles
irregular shapes
broad size distribution
aggregation

Sorbitan monolaurate:
500 mg / 30 g polymerization

16 mg / 30 g polymerization
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Solvent

Screening

Decoding

Sorting

Actuator

Beads in

Laser 477nm

Beads out
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Optimal recording speed is 9 images/s with ~50 ms exposure/3 images

- Telecentric raytracing:
- Focal plane 1 mm
- No distortion of image

Splits

Hits

PMT Signal: 33

Camera A Live: H00000000063A.bmp

Camera B Live: H00000000063B.bmp

Camera C Live: H00000000063C.bmp

Telecentric raytracing:
Focal plane 1 mm
No distortion of image
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Encoding 48 structures with 3 readings

<table>
<thead>
<tr>
<th>Split 1</th>
<th>Split 2</th>
<th>Split 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1B1C1</td>
<td>A1B1C2</td>
<td>A1B1C3</td>
</tr>
<tr>
<td>A1B3C1</td>
<td>A1B3C2</td>
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<td>A1B4C1</td>
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<td>A2B1C3</td>
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<td>A3B3C2</td>
<td>A3B3C3</td>
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<tr>
<td>A3B4C1</td>
<td>A3B4C2</td>
<td>A3B4C3</td>
</tr>
</tbody>
</table>
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Eight step synthesis: No problem to determine the structure
Selected for maximum background

Step
4
6
8
1) Over background?
2) Over local background?
3) Surrounding spots?
4) Locate max intensity
5) Go repeatedly to surrounding sphere
6) A gradient in all directions confirmed
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The 3D matrix from 2D images

3 Images ~ 6 Point collections
Select relation e.g. 1 < 2 = 3
Least square fit alignment
Correlate all points
Reconstruct missing point or remove
Least squares fit
Correlate all points
Calculate average coordinates

X1, Y1, Z1
X2, Y2, Z2
X3, Y3, Z3
X3, Y3, Z3
Hit:
- Mic1: d1, d2, a1, d2, d3, a2, d3, d1, a3
- Mic2: d1, d4, a4, d4, d3, a5, d3, d4, a7
- Mic3: d3, d4, a7, d2, d5, a10, d6, d3, a9
- Mic4: d2, d5, a10, d5, d6, a11, d6, d2, a12

Split:
- Mic1: d1, d2, a1, d2, d3, a2, d3, d1, a3
- Mic2: d1, d4, a4, d4, d3, a5, d3, d4, a7
- Mic3: d3, d4, a7, d2, d5, a10, d6, d3, a9
- Mic4: d2, d5, a10, d5, d6, a11, d6, d2, a12

Rate of comparison: 100000 pairs of beads/min
Secondary matching:
Eliptic fitting
MonteCarlo-Simulated Anneling
Grid search

Long axis
Short axis
Perpendicular axis

Hit: Green
Split: Red
Neither swelling nor RI seem to be a problem and H$_2$O remains the best liquid for decoding on PEGA resin
### Table: Multivalent Ligand

<table>
<thead>
<tr>
<th>R₂</th>
<th>R₁</th>
<th>R₀</th>
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</thead>
<tbody>
<tr>
<td>val</td>
<td>Aze</td>
<td>Gln</td>
</tr>
<tr>
<td>His</td>
<td>Pro</td>
<td>gln</td>
</tr>
<tr>
<td>his</td>
<td>pro</td>
<td>NO₂Phe</td>
</tr>
<tr>
<td>Tha</td>
<td>NmeA</td>
<td>NO₂phe</td>
</tr>
<tr>
<td>3-Pya</td>
<td>NipCA</td>
<td>Cit</td>
</tr>
<tr>
<td>4-Pya</td>
<td>PipCA</td>
<td>3CNPhe</td>
</tr>
<tr>
<td>4-PipG</td>
<td>Oic</td>
<td>4CNPhe</td>
</tr>
</tbody>
</table>

**Diagram:**
- **Biotin**
- **Avidin**

**Text:**
- Multivalent ligand
- Strong binding
- Reduce loading
100 mg amino acid derivatives totally

\[ \text{H}_2\text{N-} \]

\[ \text{Fmoc-Gly-OH} \text{ 0.1 eqv} \]

\[ \text{Boc-Gly-OH} \text{ 2eqv} \]

\[ \text{MSNT, NEM} \]

\[ \text{TFA} \]

\[ \text{Ac}_2\text{O} \]

\[ \text{Fmoc-} \text{NH-} \text{NH} \]

\[ \text{Ac-NH-} \text{NH} \]

\[ \equiv \text{H}_2\text{N-} \]

\[ 20 \mu\text{M} \]

\[ \text{H}_2\text{N-} \text{NH-} \text{O-} \text{O} \]

\[ \text{H}_2\text{N-} \text{NH-} \text{O-} \text{O} \]

\[ \text{Fmoc-Gly-OH} \]

\[ \text{TBTU} \]

\[ \text{DHBT-OH} \]

\[ \text{R}_0 \sim 3.5 \text{ mg} \]

\[ \text{Mix and deprotect} \]

\[ 20\% \text{ Pip/DMF} \]

\[ \text{FmocN-} \text{NH-} \text{O-} \text{O} \]

\[ \text{H}_2\text{N-} \text{NH-} \text{O-} \text{O} \]

\[ \text{R}_0 \]

\[ \text{FmocN-} \text{NH-} \text{O-} \text{O} \]

\[ \text{TBTU} \]

\[ \text{DHBT-OH} \]

\[ \sim 3.5 \text{ mg} \]

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\[ \text{Fmoc-} \text{NH-} \text{NH} \]

\[ \text{H}_2\text{N-} \text{NH-} \text{O-} \text{O} \]

\[ \text{R}_0 \]

\[ \text{Fmoc-} \text{NH-} \text{NH} \]

\[ \text{TBTU} \]

\[ \text{DHBT-OH} \]

\[ \text{R}_0 \sim 3.5 \text{ mg} \]

\[ 1) 20\% \text{ Pip/DMF} \]

\[ 2) \text{Ac}_2\text{O} \]

\[ 3) 95\% \text{TFA} \]

\[ 343 \]
Encoded beads with biotin/ROX-avidin at 477/515 nm
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Hits on library background / BSA 2 mM

Decoding

30 beads decoded giving 29 structures
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Resynthesized peptides

HPQ

Less active

10
13
17
23
14
25
31
34
19
37
38
39
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- Avidin immobilized on CM5
- Tetravalent binding
- Lipophilic interactions
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\[ K_D = 1.21 \times 10^{-2} \text{ (M)} \]

\[ K_D = 1.4 \times 10^{-5} \text{ (M)} \]
<table>
<thead>
<tr>
<th>Structure</th>
<th>Amino Acid 1</th>
<th>Amino Acid 2</th>
<th>Amino Acid 3</th>
<th>Amino Acid 4</th>
<th>ESI-MS m/z (Calc.)</th>
<th>Kd /M</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Aze</td>
<td>Phe(4-CN)</td>
<td>Gly</td>
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<td>510.2091 (510.2101)</td>
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<tr>
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<tr>
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<td>Gly</td>
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<td>560.1815 (560.1815)</td>
<td>8.73E-05</td>
</tr>
</tbody>
</table>

**Cyclic: Binding residues**

- c-Ala-his-Oic-Phe(NO2)-Phe-Pro-Ala-Glu-Lys
- c-Ala-his-Oic-phe(NO2)-Phe-Pro-Ala-Glu-Lys
- c-Ala-His-pro-Phe(4-CN)-Phe-Pro-Ala-Glu-Lys
- c-Ala-His-PipCa-Phe(4-NO2)-Phe-Pro-Ala-Glu-Lys
- c-Ala-his-Oic-Phe(CN)-Phe-Pro-Ala-Glu-Lys
- c-Ala-His-Pro-Gln-Phe-Pro-Ala-Glu-Lys
- c-: Cyclization between Glu side-chain and N-terminal Ala
Ala-His-PipCa-Phe(NO$_2$)-Phe-Pro-Ala-Glu-Lys

$K_D = 2.67 \times 10^{-7}$

Ala-His-pro-Phe(CN)-Phe-Pro-Ala-Glu-Lys

$K_D = 5.34 \times 10^{-6}$

Ala-his-Oic-Phe(CN)-Phe-Pro-Ala-Glu-Lys

$K_D = 5.18 \times 10^{-7}$

Binding is not additive
Binding is not increased significantly by cyclization
Novonordisk A/S
hGH production and Purification (Protein A)

Jakob E. Rasmussen
Nano-Science Center

Ligands for human growth hormone (hGH)

Blue: receptor
Brown: hGH

2715 BB’s

Virtual screening

Hotspot

<table>
<thead>
<tr>
<th>(R^2_{n\text{[a]}})</th>
<th>Structure</th>
<th>(R^2_{n})</th>
<th>Structure</th>
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</thead>
<tbody>
<tr>
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<tr>
<td>2</td>
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<td><img src="image11.png" alt="Structure 11" /></td>
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<td>18</td>
<td><img src="image18.png" alt="Structure 18" /></td>
</tr>
</tbody>
</table>
3-Residue virtual library 328515 ligands totaly: 30000 selected by prefiltering

This was further reduced to 2178 compounds redundancy elimination
Selection of R1 R2:
Aromatic, basic, acidic, lipophilic, HB
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The “One Bead SOME Compounds” Solid Phase Assay

2178 compounds on 50000 beads
All structures are correlated with activity
Purification of hGH on affinity column