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Akul Y. Mehta, Ph.D.
Department of Medicinal Chemistry
Virginia Commonwealth University

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Thursday, April 2, 2015

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2015 Drug Design & Delivery Symposium

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“Screening Strategies”
with Dr. David Swinney
Institute for Rare and Neglected Diseases Drug Discovery

www.acs.org/content/acs/en/events/upcoming-acs-webinars/drug-design-2015.html

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Co-founder and President, Carmot Therapeutics

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Chief Scientific Officer, Dalton Medicinal Chemistry

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Fragment-Based Drug Design Strategies

Daniel A. Erlanson
Carmot Therapeutics, Inc.
derlanson@carmot.us

ACS 2015 Drug Design and Delivery Symposium
Session 3
19 March 2015

What You Will Learn:

1) Why FBLD can be useful
2) How to find fragments – and avoid pitfalls
3) What you can do with fragments
From Fragment to Clinic: 30+ and Counting

Approved
- Vemurafenib (PLX-4032) Plexxikon B-Raf (V600E)

Phase 3
- ABT-199 Abbott Bcl-2
- MK-8931 Merck BACE1

Phase 2
- AT13387 Astex HSP90
- AT7519 Astex CDK1,2,4,5
- AT9283 Astex Aurora, Janus Kinase 2
- AUY-922 Novartis/Vernalis HSP90
- AZD5363 AstraZeneca/Astex AKT
- Indeglitazar Plexxikon PPAR agonist
- Linifanib (ABT-869) Abbott VEGF & PDGFR
- LY2886721 Lilly BACE1
- LY517717 Lilly/Protherics Fxa
- Navitoclax (ABT 263) Abbott Bcl-2/Bcl-xL
- PLX3397 Plexxikon FMS, KIT, and FLT-3-ITD

Phase 1
- ABT-518 Abbott MMP-2 & 9
- ABT-737 Abbott Bcl-2/Bcl-xL
- AT13148 Astex AKT, p70S6K
- AZD3839 AstraZeneca BACE1
- AZD5099 AstraZeneca Bacterial Topo II
- DG-051 deCODE LTA4H
- IC-776 Lilly/ICOS LFA-1
- JNJ-42756493 J&J/Astex FGFr
- LP-261 Locus Tubulin
- LY2811376 Lilly BACE1
- PLX5568 Plexxikon Kinase
- SGX-393 SGX Bcr-Abl
- SGX-523 SGX Met
- SNS-314 Sunesis Aurora
- Undisclosed Roche BACE1
- Undisclosed Vernalis/Servier Bcl-2

Practical Fragments 5 January 2015
Also Baker Nature Reviews Drug Discovery 2013 12(1) 5-7

HTS vs. Fragment Approaches:

Traditional HTS

[Diagram showing the process of traditional HTS]

Slide 17

Slide 18
“Chemical Space” is Unimaginably Large:

- \( \sim 10^{63} \) possible molecules with up to 30 C, N, O, S atoms

- Global screening collection \( \sim 10^8 \) different molecules

.: Even largest libraries sample an insignificant fraction
of diversity space

**Audience Survey Question**

ANSWER THE QUESTION ON SCREEN

How many possible molecules can be made with up to 11 non-hydrogen atoms (C, N, O, and F only)?

- \( \sim 100,000 \)
- \( \sim 1,000,000 \)
- \( \sim 10,000,000 \)
- \( \sim 100,000,000 \)
Smaller Number of Smaller Fragments:

- $1.11 \times 10^8$ molecules with up to 11 non-hydrogen atoms (MW < 160; C, N, O, and F only)
- $6.4 \times 10^5$ reported molecules of this size (0.06%)
- $9.77 \times 10^8$ molecules with up to 13 atoms
- $1.66 \times 10^{11}$ molecules with up to 17 atoms

Other Advantages of Fragments:

- Small fragments less likely to have interfering functionality ("molecular complexity")
- Smaller libraries allow more up-front attention to purity and drug-like properties
- Smaller libraries easier for universities and small companies to get started
- Fragments can tackle new classes of targets


What is a Fragment?

- **Lipinski’s “Rule of 5” for orally active drugs**
  
  < 500 Da (~38 heavy atoms)*
  < 5 H-bond donors
  < 10 H-bond acceptors
  < 5 octanol-water partition coefficient (logP)

- **Astex’s “Rule of 3” for fragments**
  
  < 300 Da (~23 heavy atoms)*
  < 3 H-bond donors
  < 3 H-bond acceptors
  < 3 ClogP

  Mean molecular mass of a non-hydrogen (or “heavy”) atom is 13.286 Da (Pfizer)

How Large (and Small) are Fragments?

Poll on *Practical Fragments* May 2012:

“What is the largest number of atoms you would allow in a fragment?”

46 responses

Poll on *Practical Fragments* May 2013

“What is the smallest number of atoms you would allow in a fragment?”

75 responses

Most fragments have between 5 and 20 non-hydrogen atoms
Small Fragments Give High Hit Rates!

![Graph showing the percentage of compounds with specified heavy atom count.](image)

**Fig. 6.** Percentage of compounds with specified heavy atom count (i.e., the number of non-hydrogen atoms) for compounds in Astex screening libraries from 2001 to 2007 compared with X-ray hits.


---

Pitfalls in Fragment Screening

Davis & Erlanson
*Bioorg Med Chem Lett* 2013 2844
http://dx.doi.org/10.1016/j.bmcl.2013.03.028

Learning from our mistakes:
the 'unknown knowns' in fragment screening

- Solubility
- Reactive molecules (electrophiles, oxidizers, etc.)
  - not always obvious (PAINS, May 28)
  - at high concentrations, low-level impurities can be more problematic
- Aggregators
Compounds Can Form Aggregates that Non-specifically Inhibit:

- Even approved drugs can inhibit nonspecifically at micromolar concentrations

Strong Aggregate Formers

- Add non-ionic detergent to the assay (Triton X-100, Tween-20, CHAPS, others)
- Increase protein concentration – this should have no effect on genuine binders (within limits)
- Characterize the mechanism of inhibition (competitive, noncompetitive, or uncompetitive): competitive inhibitors are normally not promiscuous
- Centrifuge your samples and retest them – this can sometimes remove aggregators
- Examine your samples with DLS or flow cytometry – aggregators can sometimes be directly observed as 50-1000 nm particles
- Look closely at your dose-response curve - unusually steep slopes can signal aggregation


**Audience Survey Question**

ANSWER THE QUESTION ON SCREEN

Which two of these fragments are aggregators?

- All of the above
- 2 and 3
- 2 and 4
- 3 and 4
- None of the above

---


---

**Aggregators Can’t Be Recognized A Priori:**

- 2
  - IC\textsubscript{50} = 65 µM
- 3
  - 40 µM
- 4
  - 226 µM

---


---
The first principle is that you must not fool yourself— and you are the easiest person to fool.

So you have to be very careful about that.

---

Richard Feynman
1974 Caltech commencement address

---

So How Do You Find Fragments?

- NMR
  - Protein detected (ie, SAR by NMR) *Structural information*
  - Ligand detected (ie, STD, TINS)
- X-ray crystallography *Structural information*
- Surface plasmon resonance (SPR) *Increasingly common*
- Functional screening
  - High concentration screening *Keep eyes open!*
- Computational *Especially as filter*
- Thermal shift
- Isothermal titration calorimetry
- Mass spectrometry: non-covalent or covalent
- Affinity chromatography / capillary electrophoresis

*Combinations of above*
What Methods are People Using?

% of respondents using technique

- 2.4 techniques used on average (2011)
- 3.6 techniques used on average (2013)

Polls on Practical Fragments September 2011 and December 2013, 97 responses (each)

Ligand Efficiency (LE):
Binding Energy Per Non-hydrogen Atom

Binding energy:
\[ \Delta G = -RT \ln(K) \]

LE = \[ \frac{\Delta G}{\text{(# heavy atoms)}} \]

Some Predictability in Fragment Optimization:

Each atom adds ~0.3 kcal/mol (or)
Each 10x increase in potency adds ~64 Da


Fragment Growing: Hsp90 Clinical Compound from Astex

AT13387
Phase 1: solid tumors
Phase 2: GIST

But Don’t Assume Binding Mode Remains The Same!


The Dream of Fragment Linking:

$$\Delta G_{\text{linked fragments}} = \Delta G_{\text{(Frag A)}} + \Delta G_{\text{(Frag B)}} + \Delta G_{\text{linker}}$$

$$\therefore K_D(\text{linked fragments}) = K_D(\text{Frag A}) \times K_D(\text{Frag B}) \times L(\text{linker coefficient})$$

Huge combinatorial advantage:
$$10^4 \times 10^4 = 10^8 \text{ virtual compounds}$$
The Dream of Linking: Synergy SAR by NMR to Develop an MMP-2 Inhibitor

\[ \text{Kd} = 20 \mu M \quad \text{LE} = 0.43 \]

15 heavy atoms
MW 195

\[ \text{Kd} = 17,000 \mu M \quad \text{LE} = 0.48 \]

5 heavy atoms
MW 75

\[ \text{lC}_{50} = 0.025 \mu M \quad \text{LE} = 0.49 \]

21 heavy atoms
MW 282

\[ \text{lC}_{50} = 0.00078 \mu M \quad \text{LE} = 0.37 \]

34 heavy atoms
MW 505

Superadditivity from Extremely Weak Fragments

\[ \Delta \text{G}_{\text{int}} < -3.3 \text{ kcal/mol} \]

\[ \text{Compound 1a} \]
\[ K_i = 0.002 \mu M \quad \text{LE} = 0.49 \]
\[ \Delta \text{G} = -11.8 \text{ kcal/mol} \]

\[ \text{Compound 1g} \]
\[ K_i = 58 \mu M \quad \text{LE} = 0.58 \]
\[ \Delta \text{G} = -5.8 \text{ kcal/mol} \]

\[ \text{Compound 1d} \]
\[ K_i > 10,000 \mu M \quad \text{LE} < 0.19 \]
\[ \Delta \text{G} > -2.7 \text{ kcal/mol} \]


Growing versus Linking

% of responses, linking or growing

<table>
<thead>
<tr>
<th>Condition</th>
<th>Fragment linking</th>
<th>Fragment growing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never tried</td>
<td>45%</td>
<td>35%</td>
</tr>
<tr>
<td>Didn't work</td>
<td>10%</td>
<td>15%</td>
</tr>
<tr>
<td>Worked marginally</td>
<td>15%</td>
<td>10%</td>
</tr>
<tr>
<td>Worked OK</td>
<td>20%</td>
<td>25%</td>
</tr>
<tr>
<td>Worked well</td>
<td>10%</td>
<td>5%</td>
</tr>
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Poll on Practical Fragments July-August 2014: 69 responses (linking), 56 responses (growing)
http://practicalfragments.blogspot.com/2014/09/fragment-growing-vs-fragment-linking.html

Fragment Linking for LDHA: AstraZeneca

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<th>Compound</th>
<th>KD</th>
<th>LE</th>
<th>Enzyme IC50</th>
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<tr>
<td>12</td>
<td>770</td>
<td>0.28</td>
<td>&gt; 500 µM</td>
</tr>
<tr>
<td>20</td>
<td>210</td>
<td>0.33</td>
<td>&gt; 500 µM</td>
</tr>
<tr>
<td>24</td>
<td>160</td>
<td>0.25</td>
<td>&gt; 500 µM</td>
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Fragment linking? Abbott’s Bcl Family Inhibitors

\begin{align*}
K_d &= 300 \mu M \\
LE &= 0.30 \\
K_d &= 4300 \mu M \\
LE &= 0.29 \\
K_d &= 6000 \mu M \\
LE &= 0.27 \end{align*}

\begin{align*}
K_d &= 300 \mu M \\
LE &= 0.30 \\
K_d &= 4300 \mu M \\
LE &= 0.29 \\
K_d &= 6000 \mu M \\
LE &= 0.27 \\
K_i &= 0.036 \mu M \\
LE &= 0.27 \\
K_i &= \text{<} 0.0005 \mu M \\
LE &= \text{>} 0.20 \\
K_i &= 0.048 \mu M \\
LE &= 0.20 \end{align*}

Resources – books:

2006

Fragment-based Approaches in Drug Discovery

2008

Fragment-Based Drug Discovery

2011

Methods in ENZYMOLOGY

2012

Library Design, Search Methods, and Applications of Fragment-Based Drug Design

2012

Fragment-Based Drug Discovery and X-Ray Crystallography

2015

Fragment-Based Methods in Drug Discovery

Coming soon in 2015!

RSC Drug Discovery Series No. 47
Fragment-Based Drug Discovery
Edited by Steven Howard and Chris Abell

Resources from the Web

- **Practical Fragments** ([http://practicalfragments.blogspot.com/](http://practicalfragments.blogspot.com/))
- **LinkedIn** ([http://www.linkedin.com/groups?gid=121172](http://www.linkedin.com/groups?gid=121172))
- **Carmot Therapeutics** ([www.carmot.us](http://www.carmot.us))

Please contact me (derlanson@carmot.us)

Upcoming Events in 2015

- Fragments 2015, Cambridge, UK, March 22-24
- CHI’s Protein-Protein Interactions and Fragment-Based Drug Discovery, San Diego, CA, April 21-23
- NovAlix’s Biophysics in Drug Discovery, Strasbourg, France, June 9-12
- OMICS Group’s Drug Discovery and Designing, Frankfurt, Germany, August 11-13
- Pacifichem 2015, Honolulu, Hawaii, December 15-20
  [http://www.pacifichem.org/technical-program/abstracts/](http://www.pacifichem.org/technical-program/abstracts/)

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Department of Medicinal Chemistry
Virginia Commonwealth University

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