Covalent ligands: Challenges and approaches for docking and design

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What is special about covalent ligands?

Covalent bond formation between protein residue (nucleophile) and ligand (electrophile)

Binding of covalent ligands can be irreversible or reversible

Pros – possible advantages:
- higher potency and ligand efficiency through covalent binding
- longer residence time, resulting in prolonged duration of action
- targeting formerly untractable targets („drug the undruggable“)
- selectivity over closely related targets if unique nucleophile present

Cons – potential problems:
- chemical reactivity might lead to
  - undesired modification of off-targets
  - various forms of toxicity (in particular with irreversible binders)
  - haptenization of proteins which may elicit an immune response

Docking of covalent ligands

Docking programs were typically developed for noncovalent ligands.

- force fields or empirical potentials
- no handling of covalent reactions

Fundamental problem:
Covalent bond formation requires quantum mechanical treatment

How can the need for QM calculations be circumvented with faster and simpler modelling approaches?
Docking of covalent ligands

Challenges depend on the context:

• Is the binding site known?
• Is the target amino acid and its reactivity known?
• Is the type of warhead (electrophile) known?
• Are affinity and/or reactivity estimates required?

Most simple and most common case:

- target amino acid (nucleophile) known
- class of electrophile(s) is given

- elucidate putative binding mode; rank ligands by suitability to fit into the pocket after covalent "linking".
- Assumes equal energetics of covalent bond formation for all compounds!
- Problematic for advanced design or systems without prior knowledge!

Problematic for advanced design or systems without prior knowledge:

1) No rational warhead selection possible
2) No assessment of different (potential) target sites
3) No insight about most influencing factors

Ideal design tool would consider the full two-step binding process:

P + L → noncovalent interactions → P – L → covalent reaction

Docking strategies for covalent ligands

Most common technical solution for covalent docking:

Docking → focus on binding mode prediction

docking scores approximate mainly ΔG_1 (~OK for known irreversible binders)

Direct linking approaches

- bond pre-formed prior to actual docking
- ligand and protein atoms are connected after superpositioning or tethering
- requires special ligand preparation step

examples: AutoDock, DOCK, FlexX, ICM, GOLD ...

→ amenable to protein modeling tools!

How to approach the design computationally?

Docking with direct linking approaches

Generating the noncovalent association complex

Does noncovalent docking of the prereacted species yield productive poses?

Of the 13 CathK complexes:
7 show an RMSD < 2 Å
AND
a C–S<sub>25</sub> distance < 3.7 Å

1SNK X-ray structure
Docking pose (0.59 Å RMSD)

noncovalent docking can produce reasonable poses

illustrates importance of recognition unit for complex formation

feasibility of docking method without covalent bond formation:

SCAR = “steric clashes alleviating receptor” method (A et al., JCIM 2016, 56, 1563)
Performance and limitations of covalent docking

• Covalent docking and virtual screening is now technically readily accessible
• Pose prediction:
  test set of 76 covalent complexes (13 Michael acceptors and 63 β-lactams):
  top pose RMSD < 2 Å in roughly 40-65% of the cases
  No large-scale comparative analysis of covalent docking programs available yet
  as usual, testing and validation required for a given target and ligand class
• Predictive virtual screening is possible
• Scoring possibilities remain very limited, in particular across warhead classes
  design of customized covalent inhibitor requires stepwise application of multiple methods, including QM approaches

Model system: Trypanosoma brucei Rhodesain

• Target against human African Trypanosomiasis (sleeping sickness)
• Cysteine protease (Cathepsin-L-like)
• Irreversible vinyl sulfone inhibitor known

How to develop a customized covalent inhibitor?

1. Addressing the non-covalent association complex
   • problem: experimentally hardly accessible
     model building starting from covalent complex structure
     - QM/MM calculation
     - bond breaking and minimization
   • stability assessment by MD simulations

Addressing the non-covalent association complex

• classical non-covalent docking to reverse-engineered protein (targeting „pre-reaction state“)
• combined with covalent docking to protein from covalent complex (assessing „post-reaction state“)

Design example: Fine-tuning of covalent inhibitors

• Two steps:
  1. non-covalent association recognition unit (scaffold)
  2. covalent reaction warhead (electrophile)

Addressing the non-covalent association complex

• use modeled protein structure from non-covalent complex for docking
• ensure sufficient stability of ligand candidates (MD of docking poses)
How to develop a customized covalent inhibitor?

2. Fine-tuning the covalent reaction
   - A) QM for model reaction in solution (B3LYP/TZVP/COSMO(ε=78.39))

   Calculations show:
   addition less exothermic than for K11777 warhead;
   X=Br, Cl; ∆E ~ -6 kcal/mol
   reversible with appropriate substituents

   Schneider et al., New J Chem 2015, 39, 5841; Schirmeister et al., JACS 2016, 138, 8332

   2. Fine-tuning the covalent reaction
   - B) QM/MM: influence of the enzyme environment

   X = F:
   barrier 7 kcal/mol
   reaction energy -16 kcal/mol

   X = Cl:
   barrier 12 kcal/mol
   reaction energy -11 kcal/mol

   X = Br:
   barrier 13 kcal/mol
   reaction energy -10 kcal/mol

   Schirmeister et al., JACS 2016, 138, 8332

Testing for reversibility

Recovery of enzyme activity in dilution assay

X = H: irreversible
X = F, Cl, Br: reversible

covalent reaction with Cys25 (proven by MS)

partial recovery for X=Br is due to slow elimination of HBr, which makes the inhibition ultimately irreversible

Schirmeister et al., JACS 2016, 138, 8332

Combining improved warhead and recognition unit

<table>
<thead>
<tr>
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<th>EC50</th>
<th>Ki</th>
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<tbody>
<tr>
<td>T. brucei</td>
<td>3.0 µM</td>
<td>32 nM</td>
</tr>
<tr>
<td>J774.1</td>
<td>&gt;100 µM</td>
<td>&gt;190 nM</td>
</tr>
<tr>
<td>HEPA</td>
<td>&gt;500 µM</td>
<td>&gt;100 µM</td>
</tr>
</tbody>
</table>

With H (instead of F, Cl):
irreversible!

K = 3.7 nM, k_{off} = 1.9·10^{-6} M^{-1}s^{-1}

EC50 1.7 µM / 8.6 µM / 11 µM
T. brucei / J774.1 / HEPA

Acknowledgement

Dmytro Mykhailenko
Dominik Heuler

Universität Mainz
Tanja Schirmeister
Jochen Kesselring
Sascha Jung
Thomas Schneider

Bernd Engels
Anastasia Weickert
Johannes Becker
Wook Lee

Tanja Schirmeister
Johannes Becker
Sascha Jung
Thomas Schneider