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Covalent ligands: Challenges and approaches for docking and design

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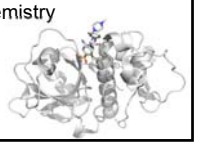
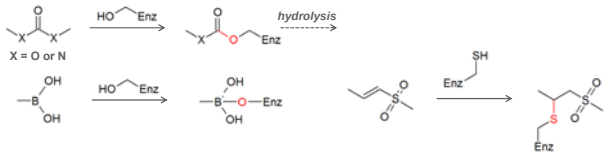


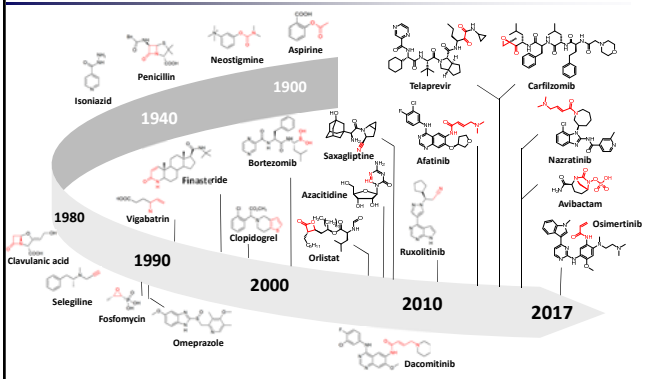
What is special about covalent ligands?

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



Binding of covalent ligands can be irreversible or reversible

The resurgence of covalent drugs



examples mostly from Singh et al., *Nature Rev. Drug Discov.* 2011, 10, 307-317

Why covalent ligands and drugs?

Reactivity can be modulated to obtain „targeted covalent inhibitors“

***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

Challenges for covalent drug development

A selection ...

adapted from Singh et al., *Nature Rev. Drug Discov.* 2011, 10, 307-317

Computational drug design	Chemistry	*In vitro* biology	Proteomics
• Identifying proteins with rare nucleophiles • Design ligands with appropriately positioned warhead • Modulating reactivity of warheads with quantum chemistry	• Development of chemistries that selectively target nucleophiles in binding sites • Design of scaffold-warhead combi-nations that bind and maintain good drug-like properties	• Enzymology to assess kinetics of binding and covalent bonding • Assessment of turnover rates of target proteins • Duration of action of drug after washout from cells	• Assessment of non-specific reactivity (e.g. glutathione) • Assessment of target occupancy and reactivity in cells • Assessment of reactivity towards plasma proteins

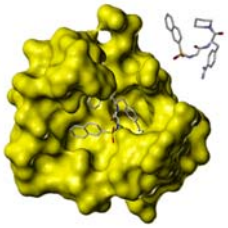
Docking for structure-based design of covalent ligands

Docking of covalent ligands

Docking programs were typically developed for noncovalent ligands.

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

⇨ in the past, covalent docking required many *ad-hoc solutions* and *manual interventions*



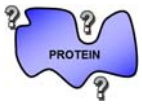
Fundamental problem:

Covalent bond formation requires quantum mechanical treatment

⇨ How can the need for QM calculations be circumvented with faster and simpler modelling approaches?

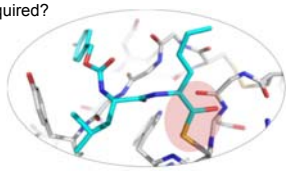
Docking of covalent ligands

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“**.

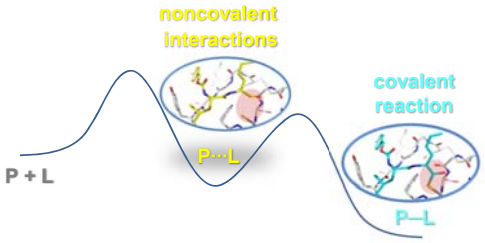
- Assumes equal energetics of covalent bond formation for all compounds!
- Problematic for advanced design or systems without prior knowledge!

Docking of covalent ligands

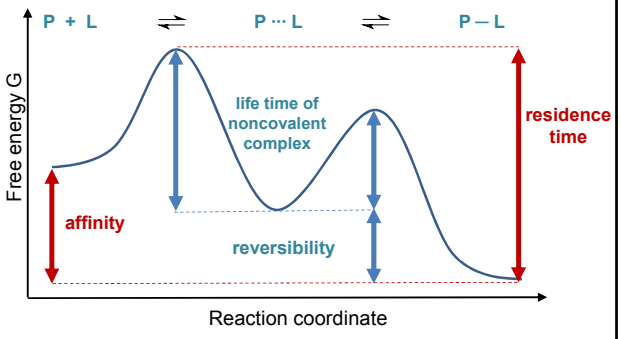
Problematic for advanced design or systems without prior knowledge:

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:



Reaction profile: thermodynamics and kinetics



How to approach the design computationally?

Docking strategies for covalent ligands

Docking → focus on binding mode prediction
docking scores approximate mainly ΔG_1
(~OK for known irreversible binders)

Most common technical solution for covalent docking:

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 ...

Docking reduced to s.c. conformational sampling

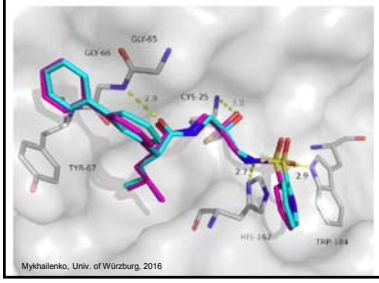
→ *amenable to protein modeling tools!*

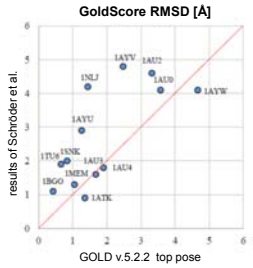
For details on available approaches see accompanying review: Sotriffer, *Mol. Inf.*. 2018, 37, 1800062

Covalent docking with direct linking approaches

How well does binding mode prediction work?

Test set: 13 covalent CathK-inhibitor PDB complexes (11-23 rotatable bonds)
from Schröder et al., *J. Med. Chem.* 2013, 56, 1478-1490

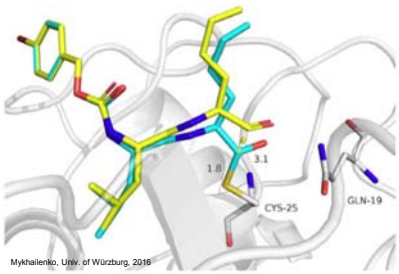




1BGO
X-ray structure
Docking pose (0.427 Å RMSD)

Generating the noncovalent association complex

Does noncovalent docking of the prereacted species yield productive poses?



Of the 13 CathK complexes:

7 show an RMSD < 2Å

AND

a C–S_{Cys25} distance < 3.7 Å

1SNK
X-ray structure
Docking pose (0.559 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 (Ai 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

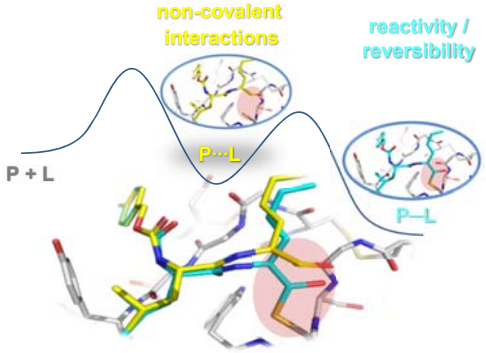
  ⇨ as usual, testing and validation required for a given target and ligand class
- Predictive virtual screening is possible
- Scoring possibilities remain very limited, in particular across warhead classes

  ⇨ design of customized covalent inhibitor requires stepwise application of multiple methods, including QM approaches

Design example: Fine-tuning of covalent inhibitors

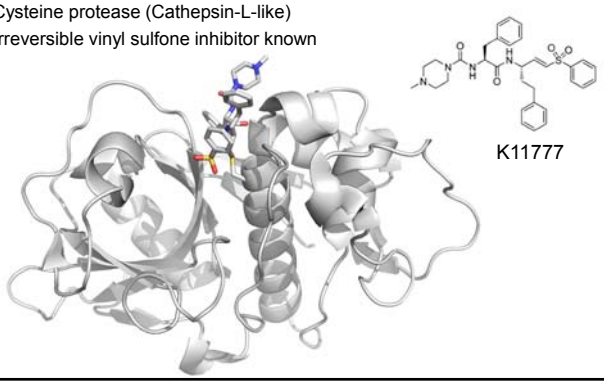
• Two steps: 1. non-covalent association 2. covalent reaction

recognition unit (scaffold) **warhead (electrophile)**



Model system: *Trypanosoma brucei* Rhodesain

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

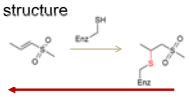


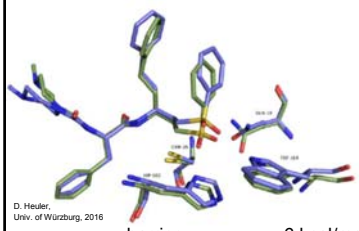
K11777

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
     - *bond breaking and minimization*
     - *QM/MM calculation*





cf. Fig. S5.2 in Schirmeister et al., *JACS* 2016, 138, 8332

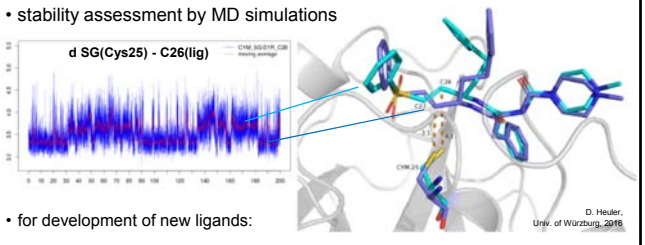
D. Heuler, Univ. of Würzburg, 2016

barrier 6 kcal/mol

reaction energy -23 kcal/mol K11777 irreversible!

Addressing the non-covalent association complex

- stability assessment by MD simulations



D. Heuler, Univ. of Würzburg, 2016

- for development of new ligands:
  - use modeled protein structure from non-covalent complex for docking
  - ensure sufficient stability of ligand candidates (MD of docking poses)

Addressing the non-covalent association complex

- classical **non-covalent** docking to reverse-engineered protein (targeting „pre-reaction state“)

FlexX

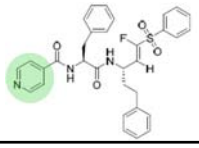
cf. Fig. S7.3 in Schirmeister et al., *JACS* 2016, 138, 8332

cf. Fig. S8.2 in Schirmeister et al., *JACS* 2016, 138, 8332

- combined with **covalent** docking to protein from covalent complex (assessing „post-reaction state“)

DOCKTITE

cf. Fig. S7.4 in Schirmeister et al., *JACS* 2016, 138, 8332

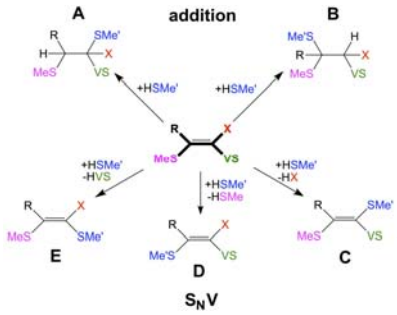


cf. Fig. S7.5 in Schirmeister et al., *JACS* 2016, 138, 8332

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:

for X=Hal, R=H

addition less exothermic than for K11777 warhead; X=Br, Cl ΔE ~ -6 kcal/mol

⇩

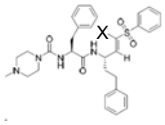
reversible
with appropriate substituents

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

Fine-tuning the covalent reaction

2. Fine-tuning the covalent reaction

- B) QM/MM: influence of the enzyme environment



likely reversible

X = F:

barrier 7 kcal/mol
reaction energy -16 kcal/mol

X = Cl:

barrier 12 kcal/mol
reaction energy -11 kcal/mol

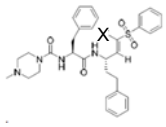
X = Br:

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

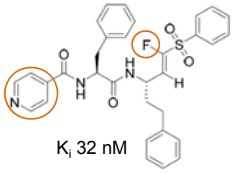
X = F, Cl, Br:
reversible

covalent reaction with Cys25 (proven by MS)

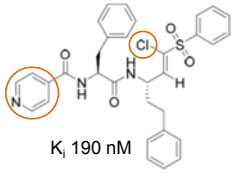
partial recovery for X=Br is due to slow elimination of HBr, which makes the inhibition ultimately irreversible

Schirmeister et al., JACS 2016, 138, 8332

Combining improved warhead and recognition unit



K_i 32 nM



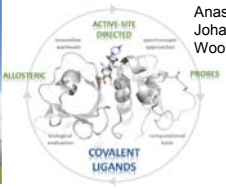
K_i 190 nM

	EC_{50}	
3.0 µM	*T. brucei*	3.1 µM
>100 µM	J774.1	>100 µM
>500 µM	HELA	>500 µM

With H (instead of F, Cl): irreversible! K_i = 3.7 nM, $k_{2nd} = 1.9 \cdot 10^{-6}$ $M^{-1}s^{-1}$

EC_{50} 1.7 µM / 8.6 µM / 11 µM
T.brucei / J774.1 / HELA

Acknowledgement



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