Structure-Based Design
Structure-Based Design (SBD)

State-of-the-art approach in the Computer-Aided Drug Design applied when 3D structure of the target protein is known (thanks to a rapid advance of protein purification and crystallography methods, robotics and automation).

**Applicability domain**
- projects in which 3D structure of the receptor (with a ligand) is known

**Examples of drugs designed by SBD:**
- carbonic anhydrase-II inhibitors (434 crystal structures in PDB, since 1990!!!)
- antivirotics: Indinavir - HIV protease inhibitor, Zanamivir – neuraminidase inhibitor
- kinase inhibitors (challenging due to target flexibility)
- domain selective ACE inhibitors → case study
**Ligand-based design**

3D structure of the target protein is **unknown**

- screening study
- hit(s)
- conformational search
  - superposition
  - pharmacophore
  - ligand optimization / database searching
  - scoring: \textit{rmsd}, QSAR, CoMFA, etc.
- assay
- lead compound / drug candidate

**Structure-based design**

3D structure of the target protein is **known**

- target purification & crystallization
- protein(-ligand) 3D structure
- molecular docking
  - pharmacophore
  - ligand optimization / database searching
  - scoring: \textit{generic function}, QM-MM etc.
- assay
- lead compound / drug candidate
Structure-Based Design: Workflow

3D-Protein Structure (PDB or in-house)
↓ completion (hydrogen atoms, missing sidechains, residues & loops)
↓ construction of H-bond network, solvation
↓ structure optimization (molecular mechanics, molecular dynamics)

3D-structure of active compound (model building / optimization)
↓ DOCKING (interactive / automatic)
↓ structure optimization (molecular mechanics, molecular dynamics)
↓ binding affinity estimation
↓ chemical synthesis
↓ experimental verification of binding affinity
SBD: Typical procedure

1. Detailed analysis of known ligand–protein complexes
   - binding site (where is?)
   - binding mode (H-bonds, metals, hydrophobic interactions)
   - pharmacophore: which are the most important residues
   - what are competitors doing (which compound classes are already patented)?

2. Rational design of a novel active molecule (creative act or database search)
   - feasible synthesis
   - metabolic stability, bioavailability, solubility, toxicity (ADMET → next week)
   - protonation state at the site of action

3. Building 3D-structure model
   - optimizing 3D-structure (Molecular Mechanics)
   - conformational search in water (global minimum + low energy conformers)
   - calculation of partial atomic charges (quantum chemistry)
SBD : Hydrogen bonds & Salt bridges

H-bond
neutral

Salt bridge
-COO······Lys-

Salt bridge
-COO······Arg-
(plus an H-bond)

Salt bridge
-NH₃⁺······H₂O······Asp-
water mediated
SBD : special interactions

metal···ligand interaction

\[(2\text{His, 1Glu})\textrm{Zn}^{2+}\cdotsOOC-R\]

charge···π-system interaction

\[R-NH_3^+\cdots\text{Trp-}\]
SBD : hydrophobic interactions

π-system···aliphatic ring

aliphatic chain···aliphatic chain
SBD: Typical procedure (continued)

4. Molecular Docking – computational technique for the exploration of the possible binding modes of a molecule to a given receptor, enzyme or other binding site:

- interactive (manual) – requires experienced chemist, hardly reproducible
- automatic (looking for a needle in a haystack...)
- rigid (fast, not too reliable) or flexible (slow, problematic)
- advanced docking methods:
  - pharmacophore pre-alignment (Praktikum)
  - placing fragments into preferred position and linking them together

5. Scoring
- direct scoring calculation of the binding free energy using force-field terms (or in combination with quantum mechanics → QM-MM methods)
- specialized scoring function containing empirical terms (e.g. polar surface area)
- QSAR models
**Molecular Modeling**: Computergestützte Verfahren in der modernen Arzneistoffentwicklung

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**SBD: Interactive molecular docking**

Coloring by atom types

Coloring: ligand → by atom types, protein → green, water → blue

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SBD: Automatic molecular docking

Identification of all (?) binding modes

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Optimization of the ligand–protein-interactions

\[
E_{\text{total}} = \sum_{\text{bonds}} K_r (r - r_{eq})^2 + \sum_{\text{angles}} K_\theta (\theta - \theta_{eq})^2 + \sum_{\text{tensions}} \frac{V_n}{2} [1 + \cos(n\phi - \gamma)] + \\
\sum_{\text{nb pairs}} \frac{q_i \cdot q_j}{4 \pi \varepsilon_0 D(r) r_{ij}} + \sum_{\text{nb pairs}} \left( \frac{A}{r_{ij}^{12}} - \frac{B}{r_{ij}^6} \right) + \\
\sum_{\text{H bonds}} \left( \frac{C}{r_{ij}^{12}} - \frac{D}{r_{ij}^{10}} \right) \cdot \cos^2 (\theta_{\text{Don–H…Acc}}) \cdot \cos^n (\omega_{H…\text{Acc–LP}}) + \\
\sum_{\text{metal pairs}} \frac{q_i^{CT} \cdot q_j^{CT}}{4 \pi \varepsilon_0 D(r) r_{ij}} + \sum_{\text{metal pairs}} \left( \frac{E}{r_{ij}^{12}} - \frac{F}{r_{ij}^{10}} \right) + \\
(E_{MC} + E_{LFS}) \cdot \prod_{\text{angles}} \cos^2 (\Psi_{\text{Lig–Met–Lig'}} - \Psi_{eq}) \cdot \frac{1}{n} \sum_{\text{ligands}} \cos^n (\omega_{\text{Met–Lig–LP}})
\]
Structure optimization: Molecular Mechanics

The 1st derivative of the force-field equation shows the direction of minimization.

Molecular Mechanics optimizations end up always in the nearest local minimum.
Structure optimization: Conformational search

Erzeugung einer Startkonformation mittels Zufallszahlengenerator

Erzeugung einer neuen Konformation

Minimieren

Lokales Minimum

Minimieren

Globales Minimum

Hier ist die Differenz zur letzten erzeugten Konformation zu klein und die Minimierung würde höchstwahrscheinlich wieder zur Startstruktur führen. Daher wird dieser (relativ) rechenintensive Schritt nicht ausgeführt und anstelle eine neue, andere Konformation erzeugt und minimiert.
Structure optimization: Molecular Dynamics

**Advantages:**
- strain relief
- simulation of induced-fit possible
- MD trajectory → information about dynamic stability of intermolecular interactions
- time-averaged (representative) structure
- explicit solvent

**Drawbacks:**
- risk of introducing artifacts
- time consuming
Structure optimization: Scoring

\[ E_{\text{Binding}} = E_{\text{Ligand-Protein}} + E_{\text{Ligand-Solvent}} - E_{\text{Internal Strain}} - E_{\text{Ligand-Desolvation}} - T\Delta S \]
Structure optimization: $E_{\text{Ligand–Protein}}$

- hydrophobic interactions
- displacing protein-bound water (entropic effect)
- salt bridges
- interactions with metals
- double H-bonds
- carbonyl groups: >C=O
- nitrogen atoms in aromatic rings: –N=
- bridging water molecules
- hydroxyl groups: –OH (3 H-bonds in water)
- charged groups (exception: salt bridges, metals)

Important is the difference between the strength of interaction with water (in aqueous environment) and with protein (in the binding site) $\rightarrow \Delta G$
Structure optimization: $E_{\text{Ligand-Solvens}}$

Solvent accessible binding site of the ACE (brown) with bound lisinopril (green) filled with water

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Structure optimization: $E_{\text{internal strain}}$

Vitamin $D_2$ in the glucocorticoid-receptor: unstrained

$E_{\text{int}} = 1.2$ kcal/mol

Glibenclamid in the Glucocorticoid-Receptor: strained

$E_{\text{int}} = 10.8$ kcal/mol
Structure optimization: $E_{\text{Desolvation}}$

neutral molecule (e.g. $17\beta$-estradiol): $E_{\text{Desolvation}} < 10 \text{ kcal/mol}$

singly charged molecule (e.g. acetazolamid): $E_{\text{Desolvation}} \approx 50-60 \text{ kcal/mol}$

multiply charged molecule (e.g. glyphosat): $E_{\text{Desolvation}} > 200 \text{ kcal/mol}$
Structure optimization: TΔS

Nonylphenol: 9 freely rotatable bonds → 6.3 kcal/mol

Rofecoxib: 3 freely rotatable bonds → 2.1 kcal/mol

TCDD: no freely rotatable bonds → 0.0 kcal/mol
Verification of the binding hypothesis

- Comparison of the crystal structure (1993) with the predicted binding mode (1983 published)
- Calculated binding affinity (QSAR: 22.6 nM) – experimental value (14.1 nM)
Structure Based Design - Conclusion

Advantages
- complete knowledge about the binding site → from (co)-crystal structure
- lower number of synthesized and tested compounds (rationally chosen structural changes)
- excellent complementarity with the binding site → good expected selectivity & safety
- virtual screening possible → novel ligands

Disadvantages
- none

Prerequisites
- 3D structure of target
- good conformational search algorithm, force-field parameters, robust docking protocol, scoring function, consensus of multiple methods

Might fail if
- big conformational changes at receptor site, depending on the ligand
- docking and scoring not accurate enough
Structure-Based Design – Case Study

Domain-Selective ACE Inhibitors
Ligand-Based Design of ACEI-s (pre-2003)

Enalaprilat

Captopril

Teprotide

snake venom

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Structure-Based Design

... applied when 3D structure of the target protein is known.

Crystal structure of the human angiotensin-converting enzyme–lisinopril complex

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Structure-Based Design

... applied when 3D structure of the target protein is known.

Crystal Structure of the N Domain of Human Somatic Angiotensin I-converting Enzyme Provides a Structural Basis for Domain-specific Inhibitor Design

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Human somatic angiotensin I-converting enzyme (sACE) is a key regulator of blood pressure and an important drug target for combating cardiovascular and renal disease. sACE comprises two homologous metallopeptidase domains, N and C, joined by an inter-domain linker. Both domains are capable of cleaving the two hemoregulatory peptides angiotensin I and bradykinin, but differ in their affinities for a range of other substrates and inhibitors. Previously we determined the structure of testis ACE (C domain); here we present the crystal structure of the N domain of sACE (both in the presence and absence of the antihypertensive drug lisinopril) in order to aid the understanding of how these two domains differ in specificity and function. In addition, the structure of most of the inter-domain linker allows us to propose relative domain positions for sACE that may contribute to the domain cooperativity. The structure now provides a platform for the design of “domain-specific” second-generation ACE inhibitors.

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Keywords: angiotensin I-converting enzyme; cardiovascular disease; crystal structure; hypertension; inhibitor design


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ACE domains \textit{in vivo}

\begin{itemize}
\item AcSDKP \rightarrow N-domain \rightarrow degradation \rightarrow fibrosis
\item bradykinin \rightarrow both domains \rightarrow degradation
\item angiotensin I \rightarrow both but predominantly C-domain
\item unknown substrate \rightarrow C-domain (testis isoform) \rightarrow unknown product
\item angiotensin II \rightarrow blood pressure
\item male fertility
\end{itemize}

ACE domains

Superposition of N-domain and C-domain
Domain-selective ACEI-s: structure – activity relationship

\[
\begin{array}{cccccc}
K_i(C) & \text{Ki(N)} & \text{selectivity index} & K_i(C) & \text{Ki(N)} & \text{selectivity index} \\
[\text{nM}] & & & [\text{nM}] & & \\
3 & 10000 & 3300 & 4 & 60 & 15 \\
0.5 & 45 & 90 & 9 & 200 & 22 \\
20 & 450 & 22 & 60 & 8000 & 130 \\
0.8 & 0.8 & 1 & 65 & 9000 & 138 \\
\end{array}
\]

*Biochemistry (2004), 43: 8048-8054*

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Domain-selective ACEI-s: predicted binding mode

Table 2: Comparison of the Residues between the N- and C-Domains of Somatic Human ACE Delineating the $S_1'$ Subsite

<table>
<thead>
<tr>
<th>N-domain</th>
<th>C-domain</th>
<th>N-domain</th>
<th>C-domain</th>
<th>N-domain</th>
<th>C-domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gln 259</td>
<td>Gln 857</td>
<td>Thr 358</td>
<td>Val 956</td>
<td>Phe 438</td>
<td>Phe 1036</td>
</tr>
<tr>
<td>Ser 260</td>
<td>Thr 858</td>
<td>Asp 393</td>
<td>Asp 991</td>
<td>Tyr 498</td>
<td>Tyr 1096</td>
</tr>
<tr>
<td>Asp 354</td>
<td>Glu 952</td>
<td>Glu 431</td>
<td>Asp 1029</td>
<td>Tyr 501</td>
<td>Tyr 1099</td>
</tr>
<tr>
<td>Ser 357</td>
<td>Val 955</td>
<td>Phe 435</td>
<td>Phe 1033</td>
<td>Phe 505</td>
<td>Phe 1103</td>
</tr>
</tbody>
</table>

Biochemistry (2004), 43: 8048-8054

M. Smieško & A. Vedani — Departement Pharmazeutische Wissenschaften, Universität Basel, 2017
Domain-selective ACEI-s
RXPA380

Yellow : C-domain
Violet: N-domain

Biochemistry (2009), 48: 8409-8412
M. Smieško & A. Vedani — Departement Pharmazeutische Wissenschaften, Universität Basel, 2017
Domain-selective ACEI-s
N-domain selective: RXP407

Biochemistry (2009), 48: 8409-8412
M. Smieško & A. Vedani — Departement Pharmazeutische Wissenschaften, Universität Basel, 2017
Superposition of crystal poses of ACEI-s


SBD of domain-selective ACEI-s

Captopril
non-selective

Enalaprilat
non-selective

RXPA380
C-domain selective

RXP-407
N-domain selective
Knowing the 3D structure of both active sites

- fully utilize structure-based design methods in order to exploit differences in the binding sites of the two domains → maximize sub-site specific interactions

- the most advanced inhibitors can distinguish between two catalytic domains with a selectivity factor > 1 000 thanks to:
  
  C-domain → hydrophobic interactions in $S_2$ and $S_2'$
  
  N-domain → salt-bridge in $S_2$

- extending molecule of the first ACE inhibitor *Captopril* resulted in several new favorable interactions with the enzyme binding pockets improving selectivity toward ACE in general

- rigidification ($S_1'$), optimizing charge distribution, improve affinity by displacing solvent from the sub-sites