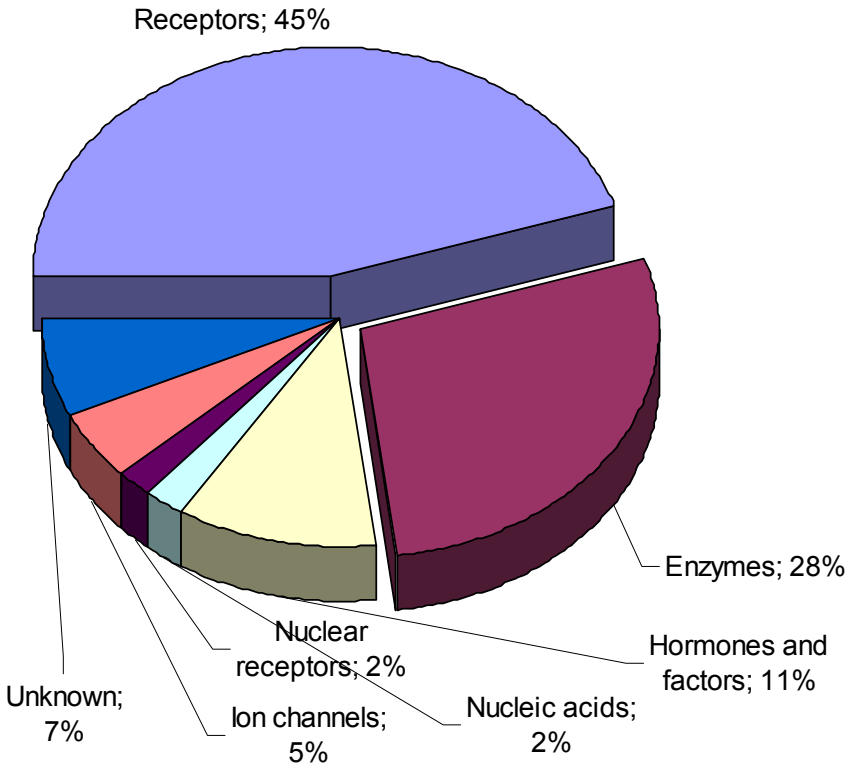
Small Molecule Drug Discovery: Opportunities and Challenges

Ulrich Stilz
Sanofi-Aventis Deutschland GmbH
Frankfurt

Overview

- Lead Generation ⇨ Exploring Target Space
- Lead Optimization ⇨ Reducing Attrition
- Case Study ⇨ Factor Xa
- Emerging Frontiers

Relevant Therapeutic Target Classes



In 2000, all marketed drugs were estimated to target less than 500 biomolecules.

5,000 and 10,000 potential targets on the basis of an estimate on disease relevant genes.

Major therapeutic target classes in pharmaceutical research subdivided into seven families.

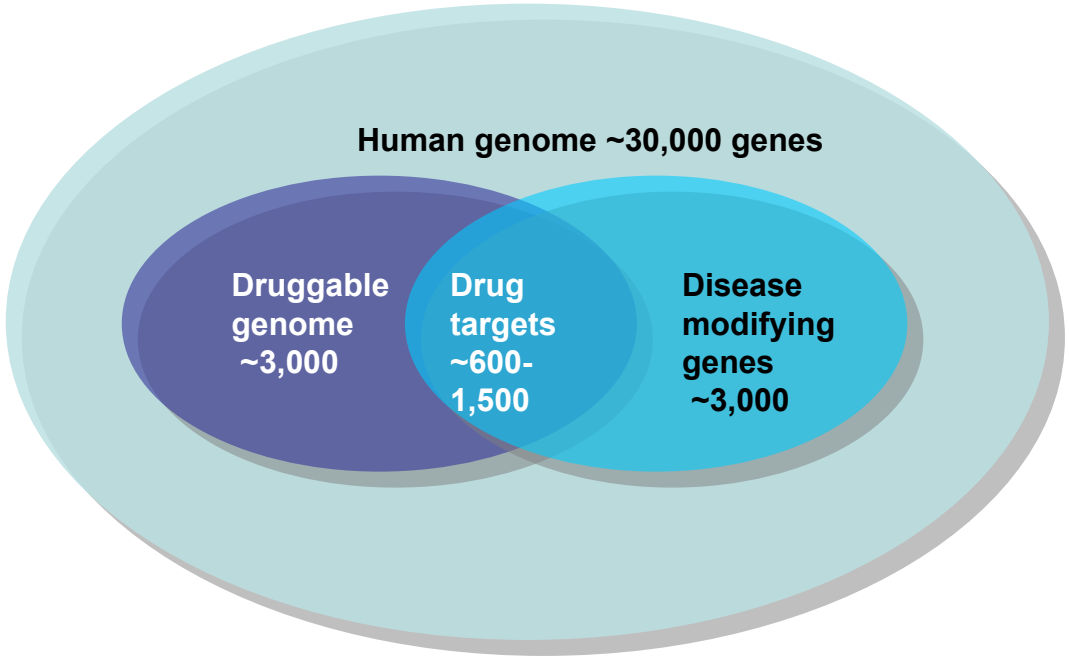
Enzymes and receptors most relevant. More than 600 genes encoding GPCRs are known.

Lit: Drews, J. Drug discovery: A historical perspective. *Science* **2000,** *287*, 1960–1964.

Biological Opportunity: Number of Drug Targets

Effective number of exploitable drug targets by intersection of number of genes linked to disease with the "druggable" subset of the human genome.

Total number of human genes: ~30,000
Estimated druggable targets: 3,051 (10-14%)
Estimation based on protein sequence similarity and properties of known drugs for a family.

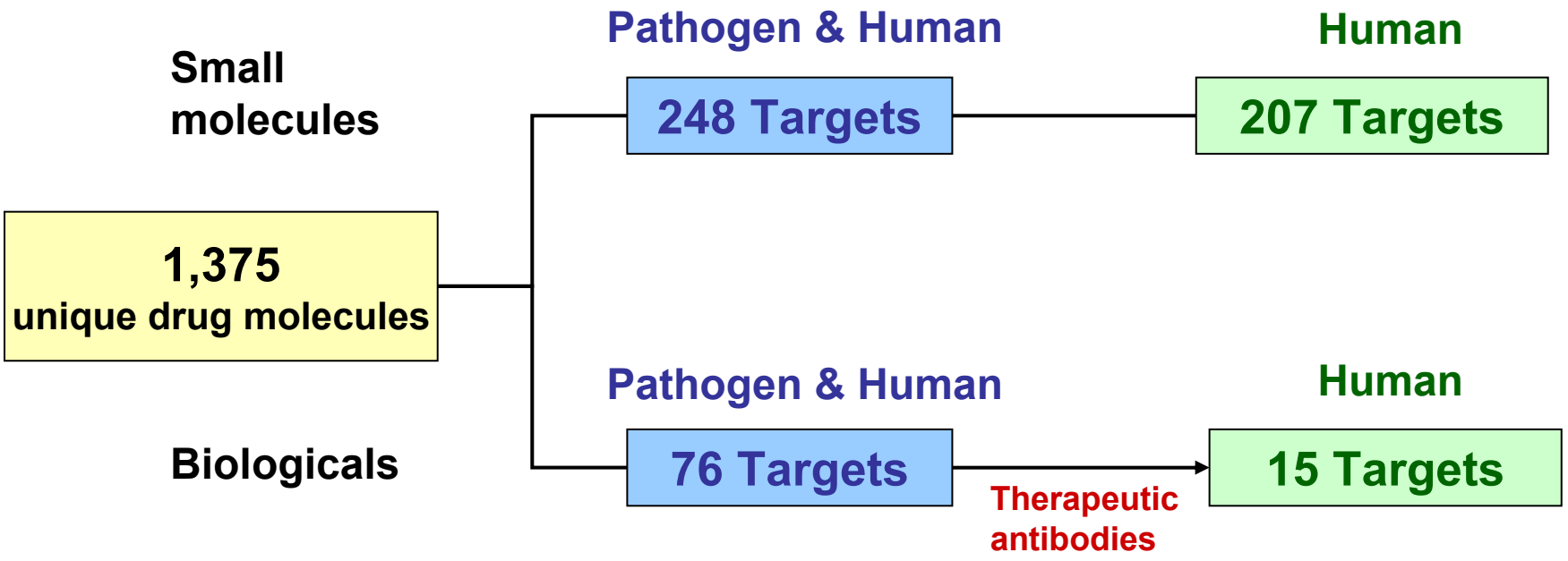


Lit: A.L. Hopkins, C.R. Groom. *Nature Rev. Drug Disc.* **2002**, *1*, 727-730.

Molecular Targets of Approved Drugs

Based on analysis of 1,357 unique drug molecules in 2005.

Drugs approved by FDA (FDA's Orange Book)



Lit: J.P. Overington, B. Al-Lazikani, A.L. Hopkins. *Nature Rev Drug Disc.* **2006**, *5*, 993-996.

Dual Approach to New Drugs

„No *either – or*, but what is better suited“

Small molecules

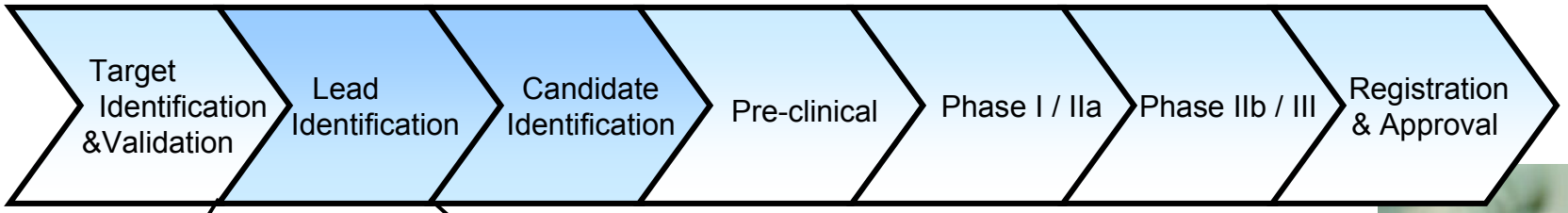
- Intra- and extracellular targets
- Oral administration preferred
- Acceptable cost of goods
- Natural products as source for novel antiinfectives
- Challenges through drug metabolism and DDI

Biotherapeutics

- Applicable to extracellular targets (proteins)
- High specificity
- Substitution therapy
- New Platforms emerging
- Parenteral application necessary
- Cost of goods is a potential issue
- Logistics challenges (i.e refrigeration)

Drug Discovery Value Chain

Exploring target space



Multiple lead finding approaches

Affinity
Selectivity
Risk assessment
Identify promising series

Key technologies

High-throughput screening
Advanced assay technology
Structural biology / X-ray crystallography
High-throughput library synthesis
Computational chemistry
- Virtual screening
- Structure-/ligand-based design

Medicinal chemistry
ADMET

Challenges

- Integrate heterogenerous approaches
- Increase success by promising lead
- Identify optimal starting point
- Analyse large volume of data
- Increase predictivity

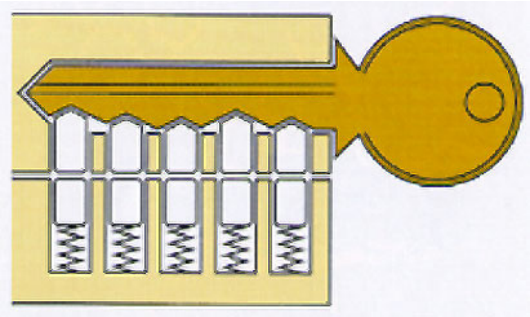
Emil Fischer: „Key-and-Lock“ Principle

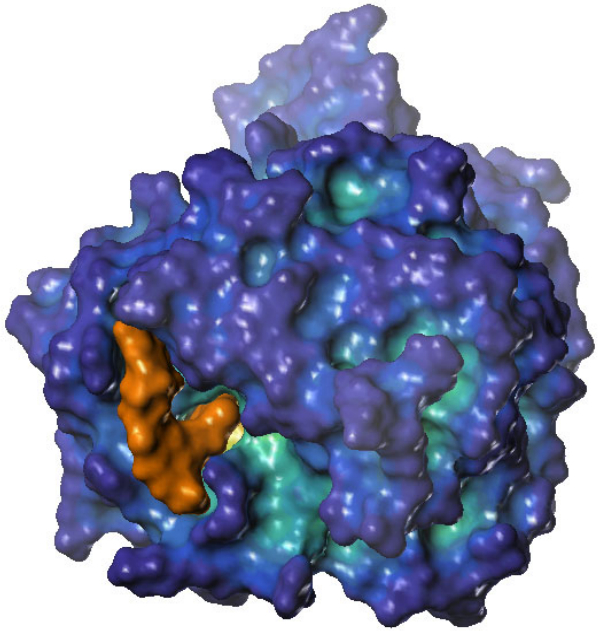
Emil Fischer
1852 - 1919

Um ein Bild zu gebrauchen, will ich sagen, dass Enzym und Glucosid wie Schloss und Schlüssel zueinander passen müssen, um eine chemische Wirkung aufeinander ausüben zu können.“

Berichte der Deutschen Chemischen Gesellschaft 1894.

For any biological and pharmacological effect, the ligand (*key*) must interaction with its biological target (*lock*).





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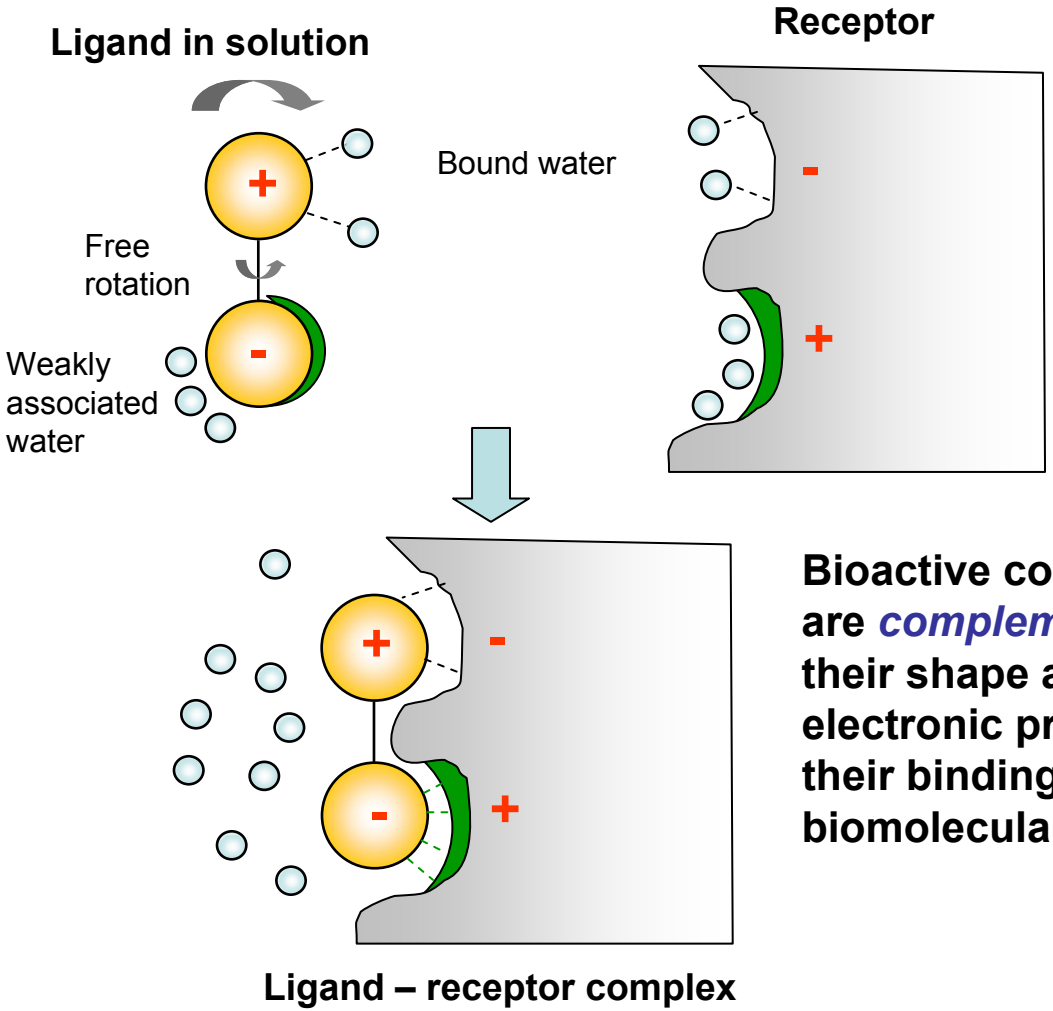
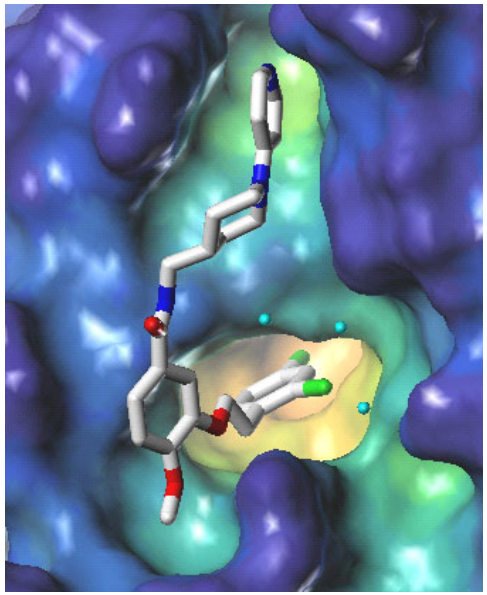
Ligand – Receptor Interactions



Electrostatic interactions

Hydrophobic interactions

Hydrogen-bonding interactions



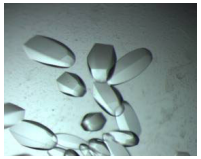
Bioactive compounds are *complementary* in their shape and electronic properties to their binding site in a biomolecular receptor.

Rapid technology progress

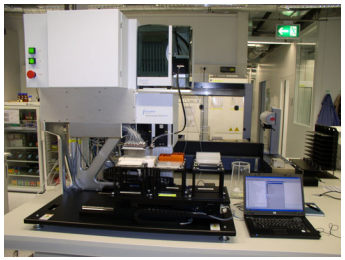
Multiple hundred protein ligand structures p.a.

SBS type plates

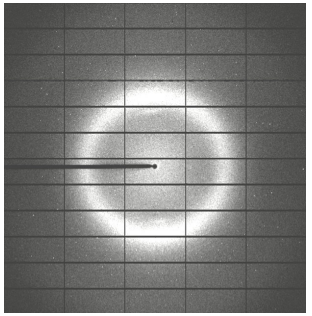
Crystal imaging



(> 1 Mio images p.a.)



Nanoliter crystallization

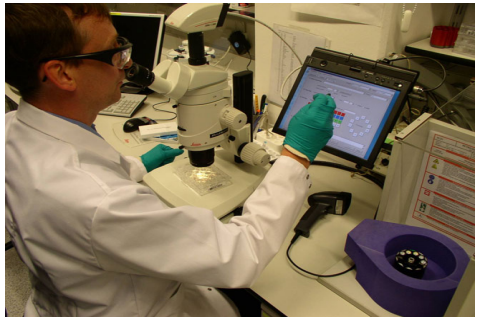


Ultra-rapid silicium detector (SLS)
datacollection in seconds to minutes

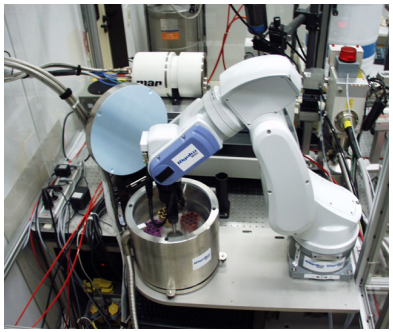
Structure Determination

Data handling

Miniaturization



LIMS
with touch screen PC interface



X-ray sample changing

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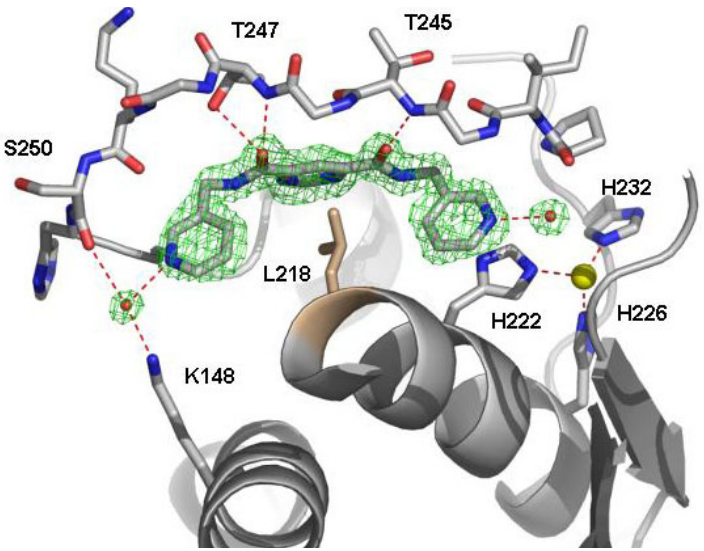
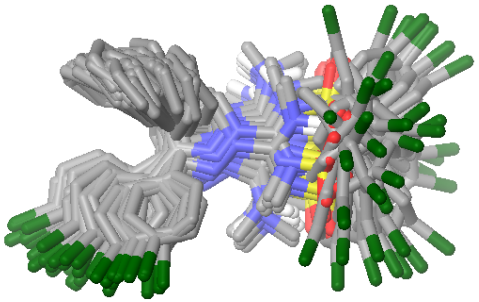
Biostructure in Industry



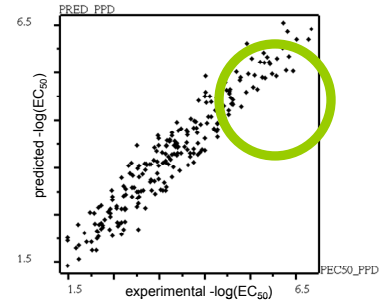
Boost Creativity

Priorization by molecular docking

Ligand alignment for QSAR studies

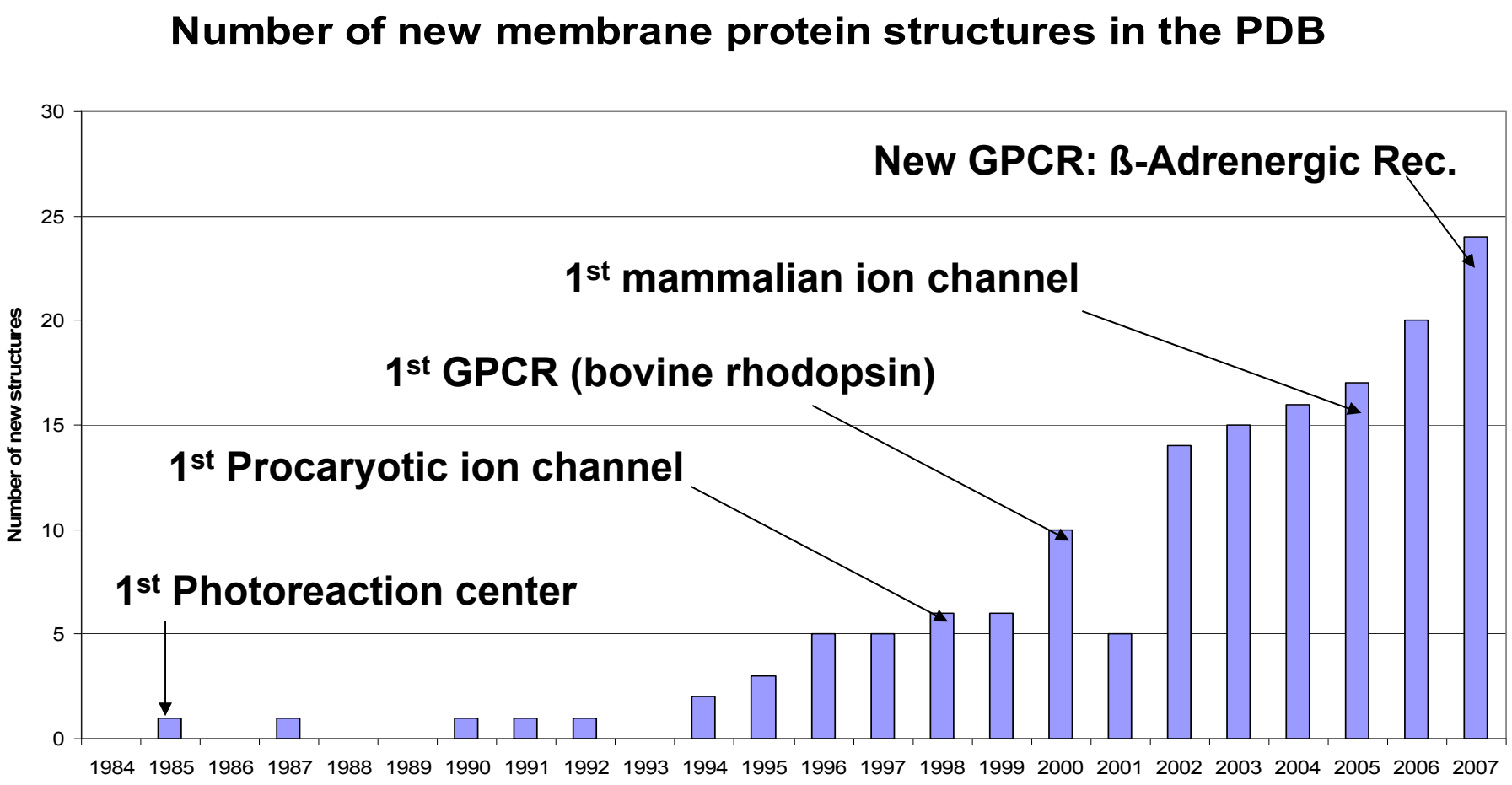


Experimentally validated alignment

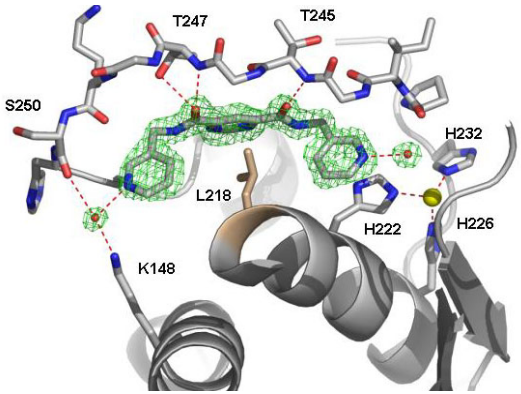


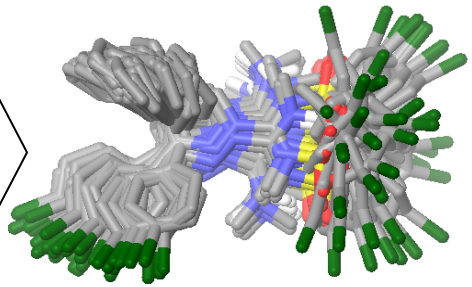
Predictive model for the prioritization of synthesis proposals

Next frontier: Membrane proteins



NMR based constraints for GPCR drug design





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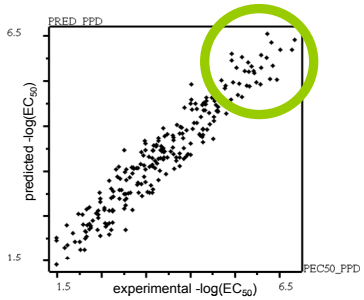
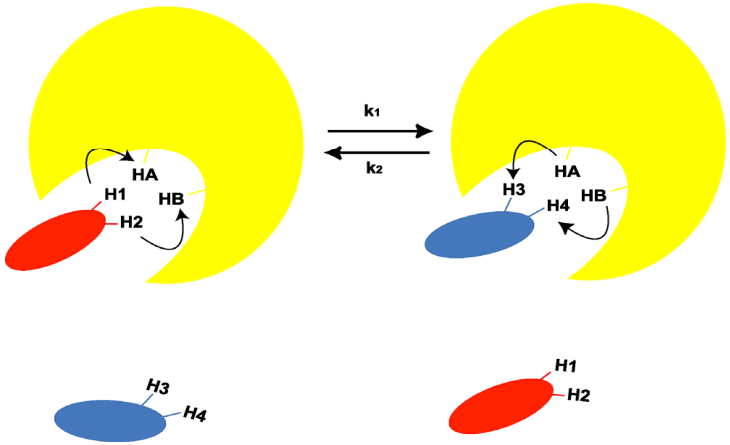
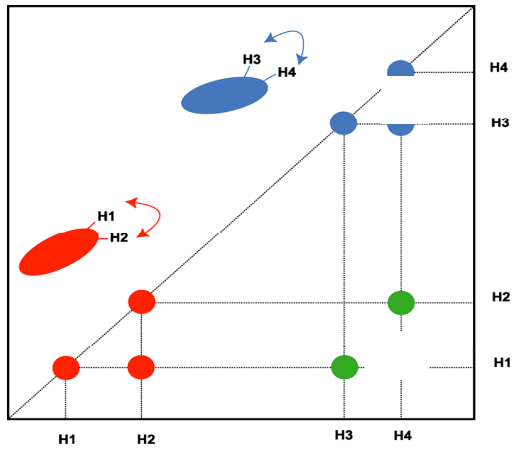


Predictive model for the prioritization of synthesis proposals





Orts. et al., *Angew. Chem. Int. Ed.* **47**, 2008, 7736; Orts. *et al.*, *Chemistry* **14**, 2008, 751; Bartoschek *et al.*, submitted.

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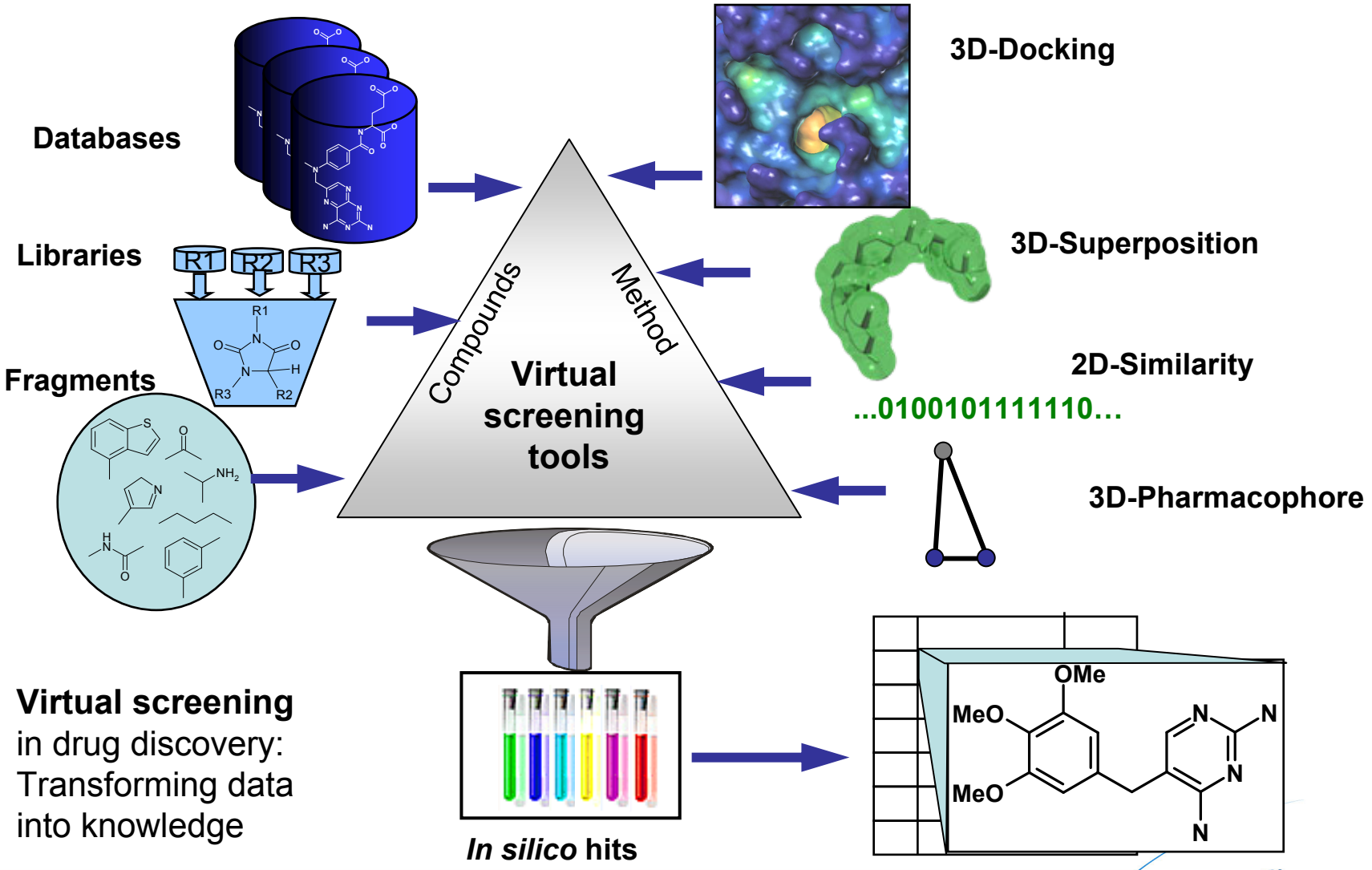
Different Approaches for Virtual Screening

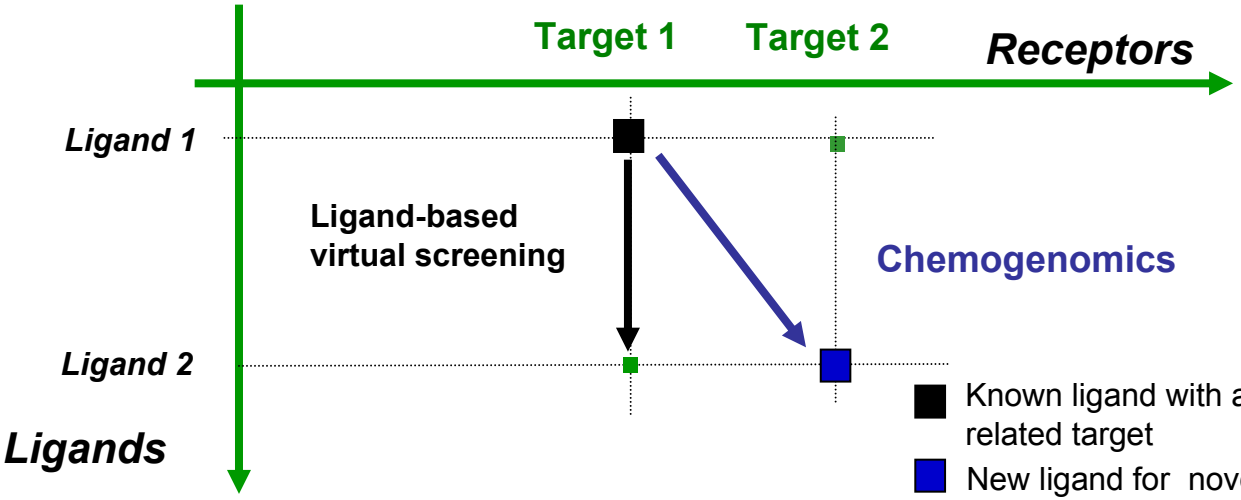


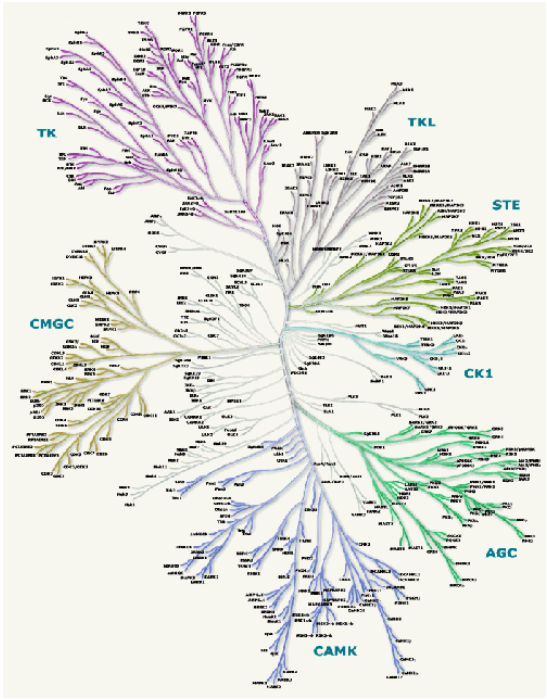
Virtual screening in drug discovery: Transforming data into knowledge

Lead Finding by Chemogenomics

- **Similarity principle** (Maggiora 1990)
  - *Similar* molecules have *similar* physicochem&biological properties
  - Applications: Analog searching, early lead optimization
  - Similarity is subjective and depends on context (2D vs 3D)
- **Chemogenomics** (Frye 1999, Murcko 2001)
  - *Similar* molecules interact with *similar* proteins
  - Applications: Use ligands of related target as starting point
  - Again, similarity is subjective and depends on context

"Similar Receptors Bind Similar Ligands"





Sugen Kinome Tree:
518 kinase families arranged by sequence similarity

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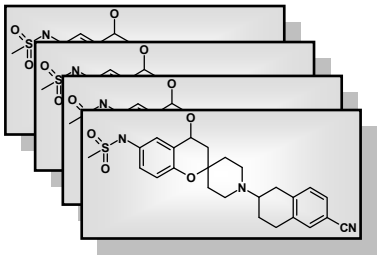
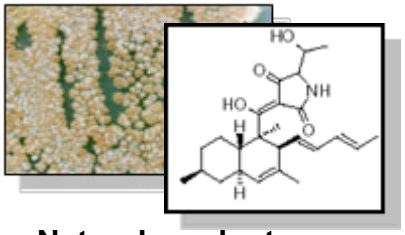
Various Sources for Leads in Drug Discovery

High-throughput library synthesis

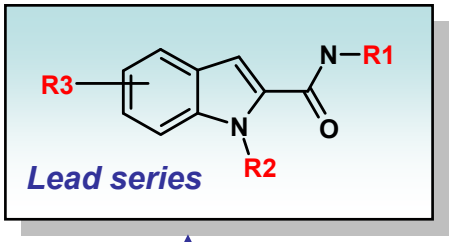


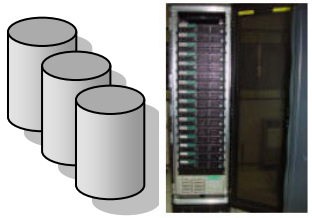
Priviledged motifs

Literature and patents

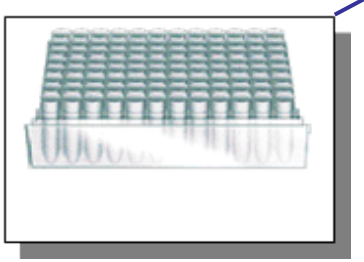


Natural products

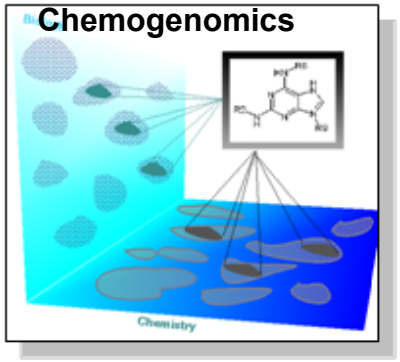


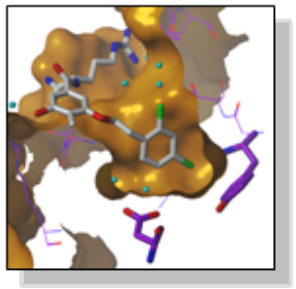


Virtual screening



High-throughput screening





Structure and ligand-based design

H_2N-xxx-**Arg**-**Gly**-**Asp**-yyy-OH

Natural ligands or peptide

HT Parallel Synthesis

- Robotic synthesis and purification platform for high throughput compound production
  - **Dedicated chemistry team**
    - Medicinal chemistry in parallel
  - **Automation team**
    - Robotics and analytics expertise

Therapeutic areas

Drug Design

Chemistry

Scaffolds
Library design
Rxn optimization
Medicinal Chemistry

Automation

Library production
HT Purification
Database management
Bottling, logistics

Libraries

Lead Finding
Optimization

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Microwave synthesis for libraries

- **Advantages**
  - Quicker reactions due to fast heating and higher temperatures
  - Frequently higher yields and less side products than classical heating
  - Solids like clays can be used as µW acceptors to get even higher local temperatures (surface reactions)
- **Limitations**
  - Reactions that develop gas are unsuitable (pressure buildup)
  - Not all solvents are suitable (but doping can help)
  - Scale up is still problematic

Tube sizes 4, 10, 35 ml

Different rack types

Control by PC and autosampler

3 machines i.e. 3 samples in parallel

total capacity 288 samples

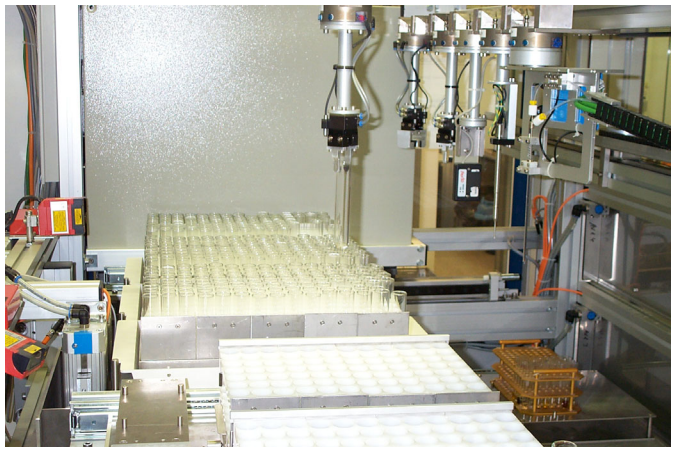
Reaction types run:

Suzuki-type coupling
Nucleophilic substitution on aromatic or vinylic halogen
Condensation reactions, heterocycle synthesis
Ester hydrolysis
N-alkylation
Amide coupling
Aminolysis of esters
Epoxide opening
Mitsunobu reaction
N-Acylation (e.g. urea formation)
PMB protecting group cleavage
Sulfonamide formation
Sonogashira coupling

High Throughput Library Purification - RP

- **Reverse HPLC**
  - **Typical conditions:**
    - 120 ml/min
    - 5 – 8 min run for 100 mg
    - 30 x 100 mm or 50 x 100 mm column
    - 5 µ Waters Sunfire and
    - 5 µ Varian Polaris
    - in-house redesigned splitter
    - customized fraction collectors
  - **500 samples / 24h**

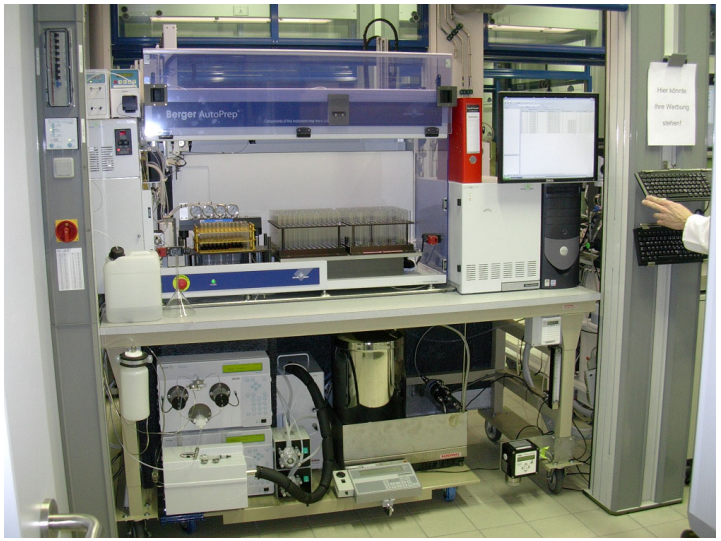
High capacity Labomatic fraction collectors (600 tubes) in RP Library Purification



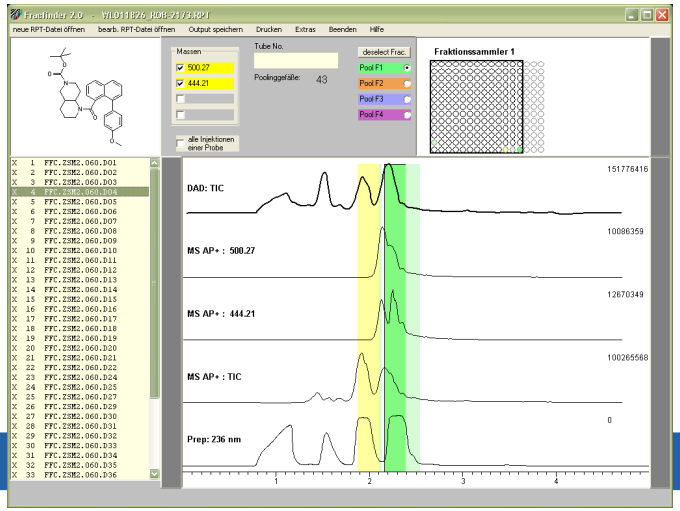
Pick & Place and Pooling & Distribution Robot

High Throughput Library Purification – supercritical carbon dioxide (SFC)

Analytical system



Preparative system



- **Inhouse FracFinder software combines analytical and preparative data to predict and control preparative collection**

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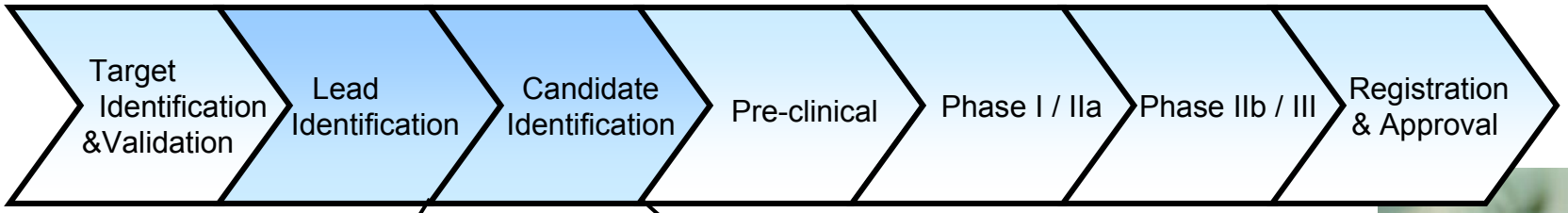
Drug Discovery Value Chain

Reducing attrition →



Multidimensional lead optimization
Affinity
Selectivity
ADME
Toxicology

Key technologies

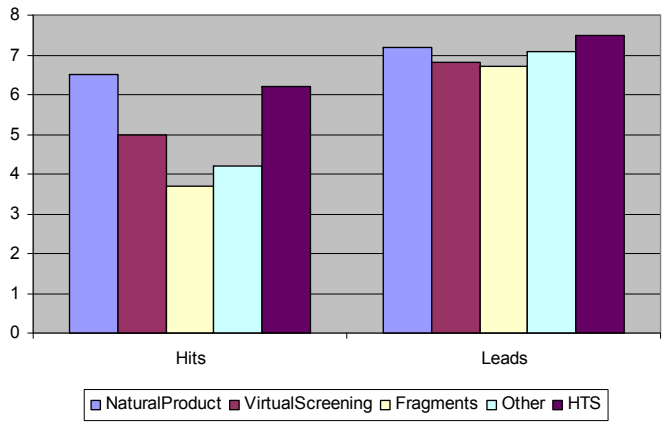
High-throughput screening
Advanced assay technology
Structural biology / X-ray crystallography
High-throughput library synthesis
Computational chemistry
- Virtual screening
- Structure-/ligand-based design

Medicinal chemistry
ADMET

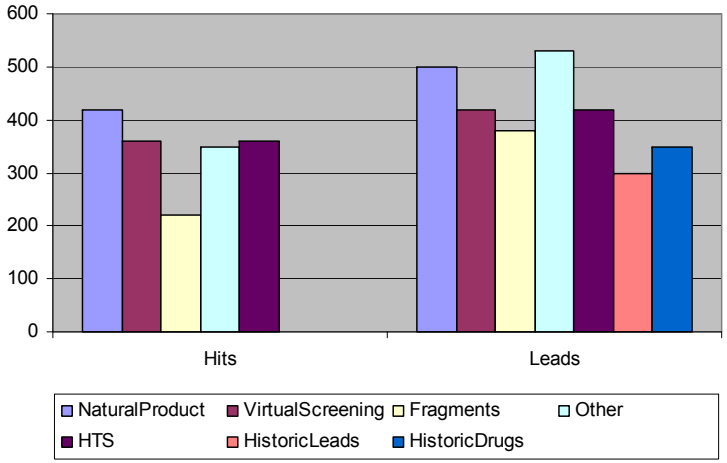
Challenges
- Reduce cycle times
- Optimize multiple parameters simultaneously
- Increase productivity and efficiency
- Transform data into knowledge

Lead Finding Strategy and Compound Property

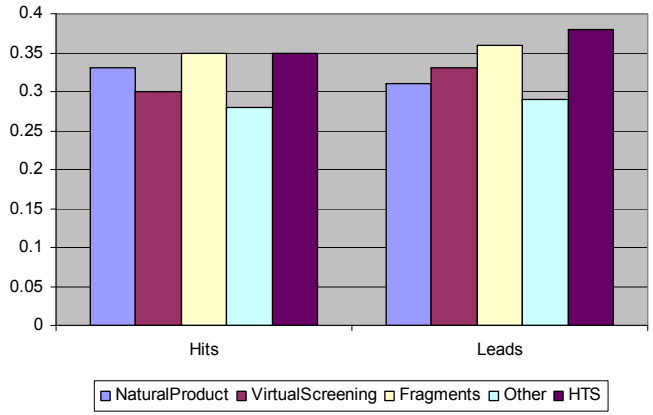
Potency



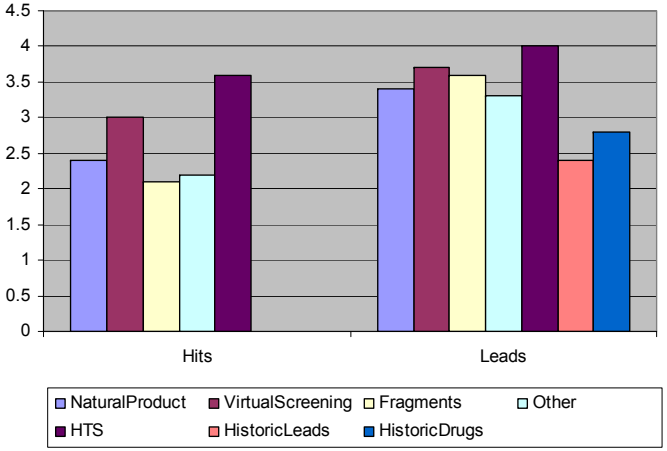
Molecular weight



Ligand efficiency



Lipophilicity (logP)



G.M. Keresü et al. Nature Rev. Drug Disc. 2009, 8, 203-212.

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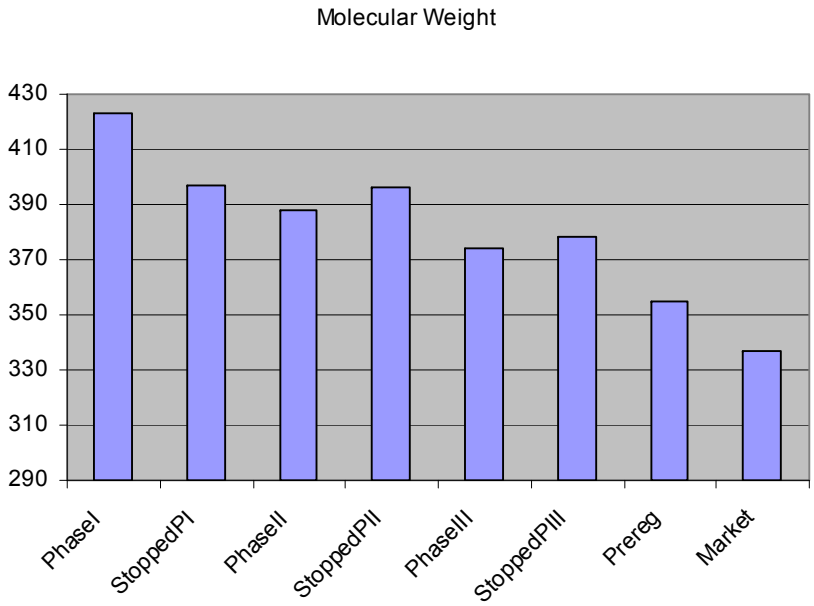
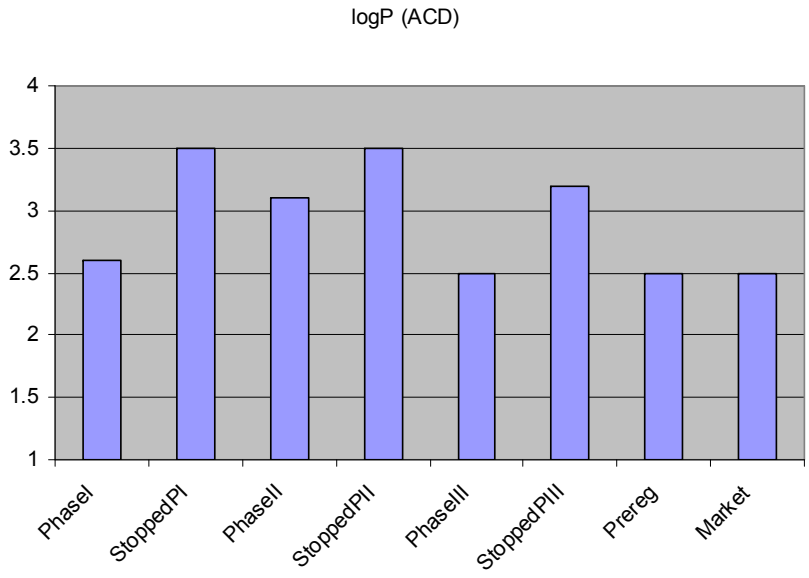
Physicochemical Properties of Marketed Drugs

Mean values for MW and logP for drug candidates in development phases.

Identification of trends in properties favoring a drug‘s passage through clinical development to market. Source: Database with status on ~1200 drug candidates.

Major trend: Molecular weight decreases, but more lipophilic drugs often discontinued.



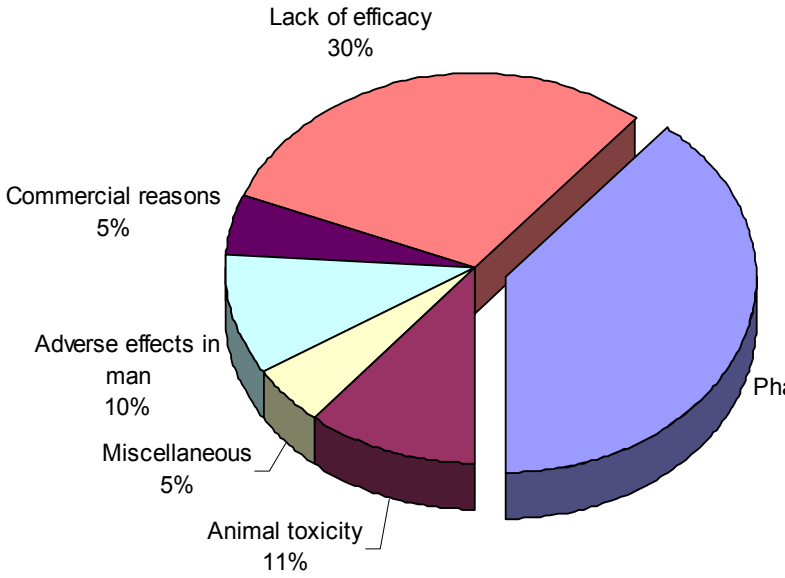


M.C. Wenlock et al. *J. Med. Chem.* **2003**, *46*, 1250-1256.

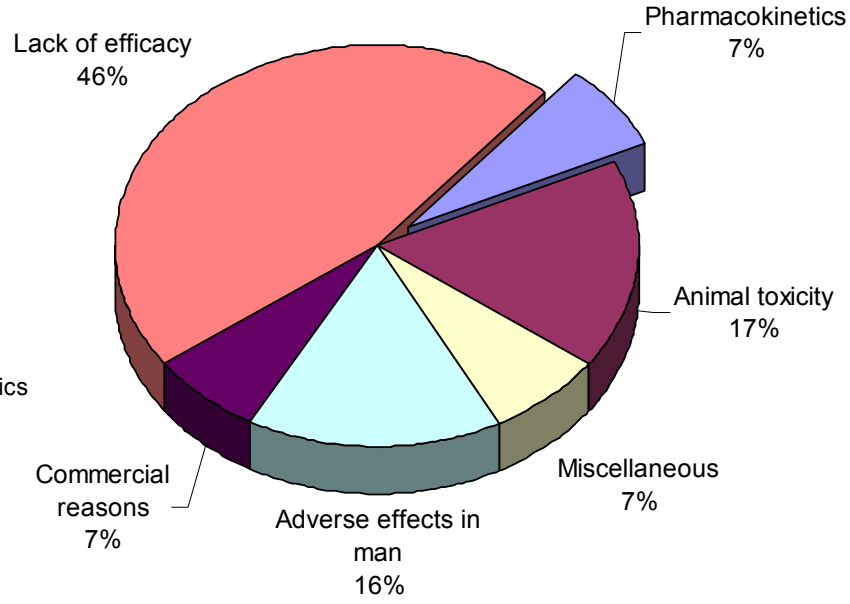
Reason for Failure of Drug Candidates

Historical survey reveals poor pharmacokinetics as reason for failure

198 NCEs



121 NCEs (excluding anti-infectives, more relevant)



T. Kennedy, *Drug Disc. Today* **1997**, 2, 436-444.
Prentis, R.A.; Lis, Y.; Walker, S.R. *Br. J. Clin. Pharmacol.* **1988**, 25, 387-396.

Lack of efficacy and pharmacokinetics connected in clinical studies
Pharmacokinetics addressed in lead optimization prior to development

Major Causes for Attrition in Drug Discovery (internal Data)

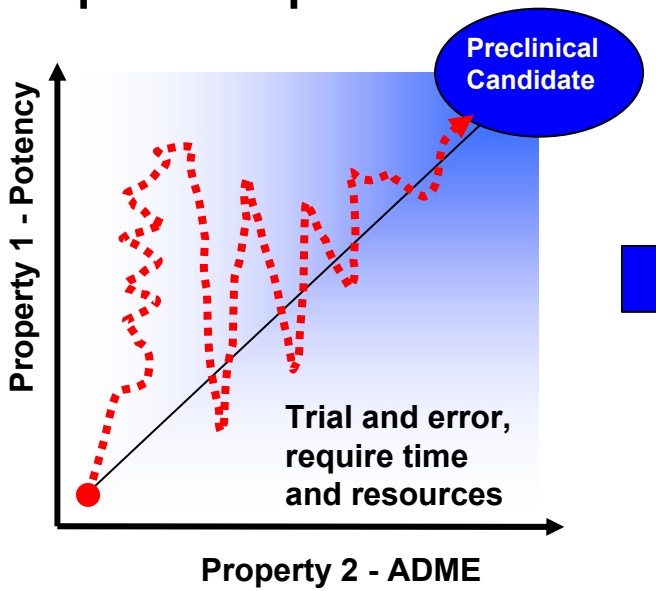
- **Significant trends**
  - ADME is relevant in earlier phases, while toxicity and efficacy dominate attrition in later phases
  - ADME is dominated by general cytochrome P450 interactions (CYP450 inhibition, induction, mechanism-based inhibition, metabolism)
  - Toxicity is dominated by cardiovascular toxicity and hepatotoxicity

Reducing Attrition Rates

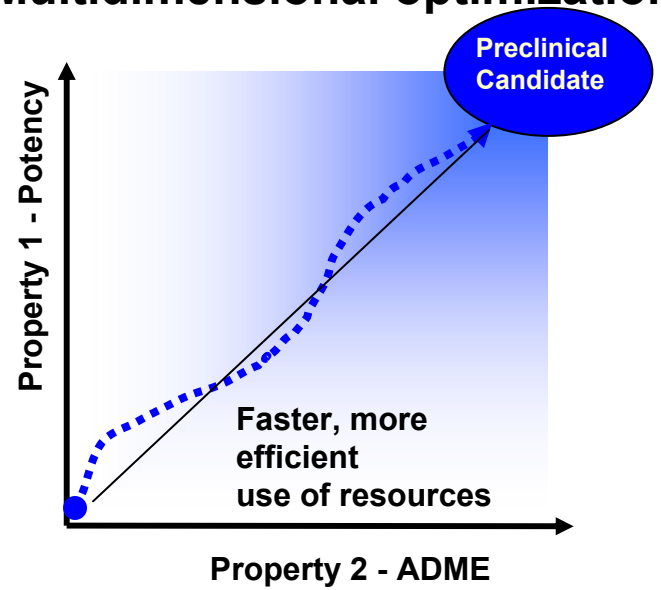
- **Major financial impact of late-stage attrition in development**
  - Minimizing risk of failure by rigorous quality assessment at key points in discovery
- **Risk assessment for promising lead series in optimization**
- **Knowledge-driven decision on lead-finding**
  - Assessment of multiple parameters for lead series:
  - Chemical properties, accessibility, physicochem., biology, ADME
  - Early awareness of series profile to identify issues for prioritization
  - Selection of series with best optimization potential
- **Early awareness of key liabilities**
  - Optimize within appropriate timeline and resources Backup lead series with significantly different profile as reserve
  - Timely react on unexpected failures due to toxicology

Multidimensional Compound Optimization

Sequential optimization



Multidimensional optimization



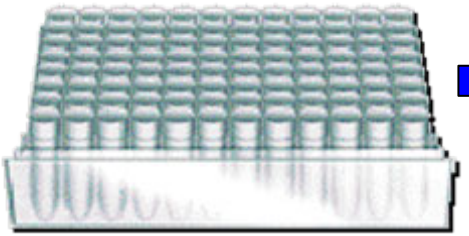
Sequential: Binding affinity optimized first, ADME properties later
Multidimensional: Simultaneously monitor changes on affinity and ADME
Challenge: Identify properties correlated with desirable biological profiles

Adapted from T.I. Oprea, *Molecules* **2002**, 7, 51-62.

⇒ From sequential to **multidimensional optimization** guided by parallel compound profiling and rational design

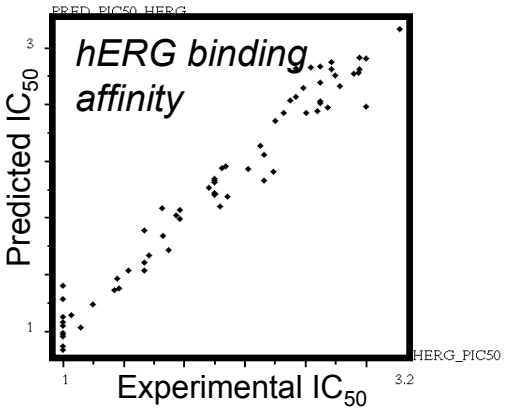
Multidimensional Compound Optimization

Experimental profiling

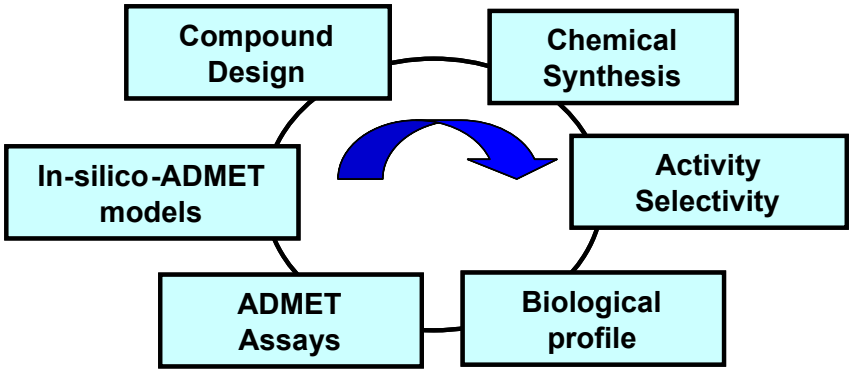


Compound	SAR1	SAR2	SAR3	SAR4	SAR5	SAR6	SAR7	SAR8
Affinity								
Selectivity								
Solubility								
Caco-2/TC7								
Metab. Labil								
hERG								
CYP Inhib								

Turning data into knowledge



Design cycle



⇒ **Combining *in-vitro* and *in-silico* tools** for profiling of compound series to guide chemical optimization cycle

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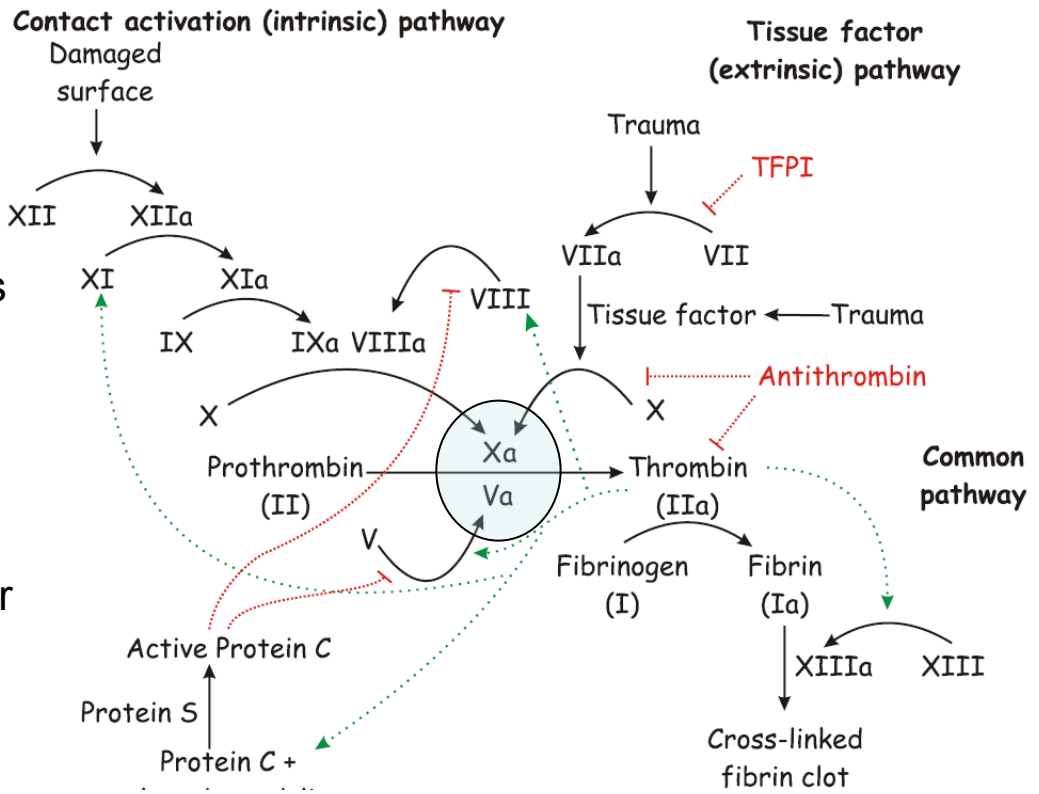
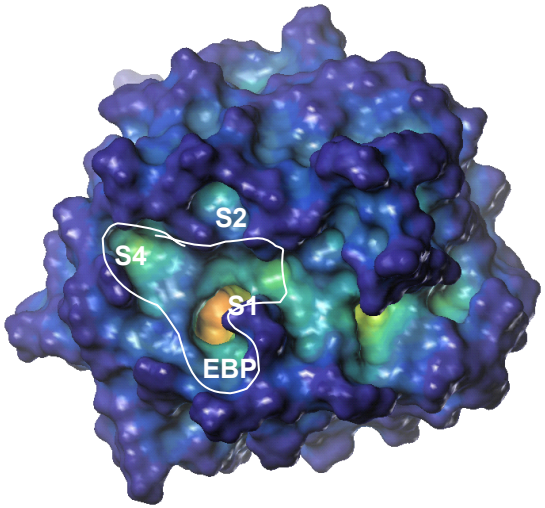
Thrombosis and Blood Coagulation Cascade

Thrombosis

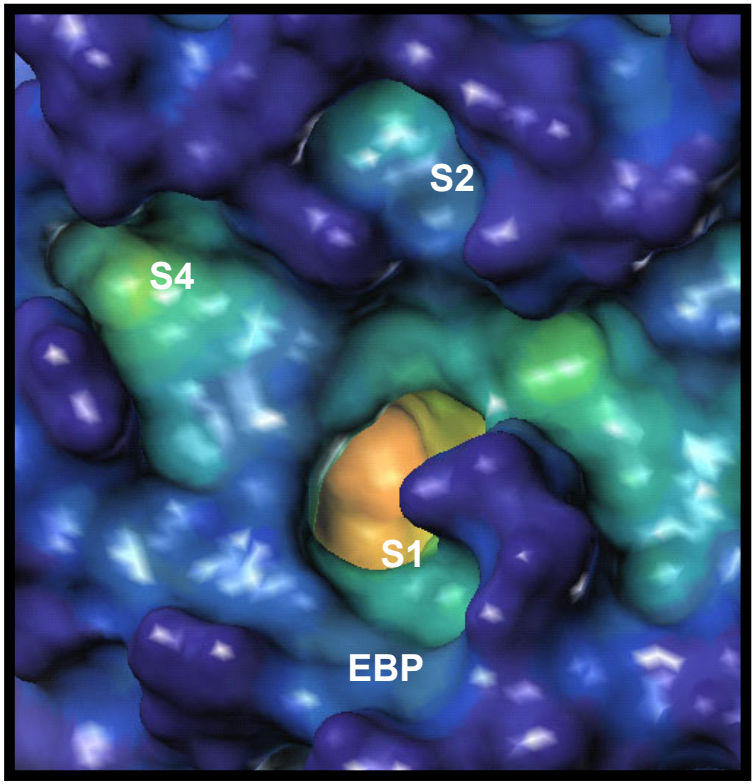
- **Serious medical problem**
- **50 % yearly mortality** by cardiovascular problems: heart attack, stroke, thrombosis
- **Coagulation cascade** highly amplified process
- **Conversion of fibrinogen to fibrin** and activation of platelets by thrombin receptor causing occlusive clot formation
- **Inhibition of thrombin production by factor Xa** effective for blocking thrombogenesis

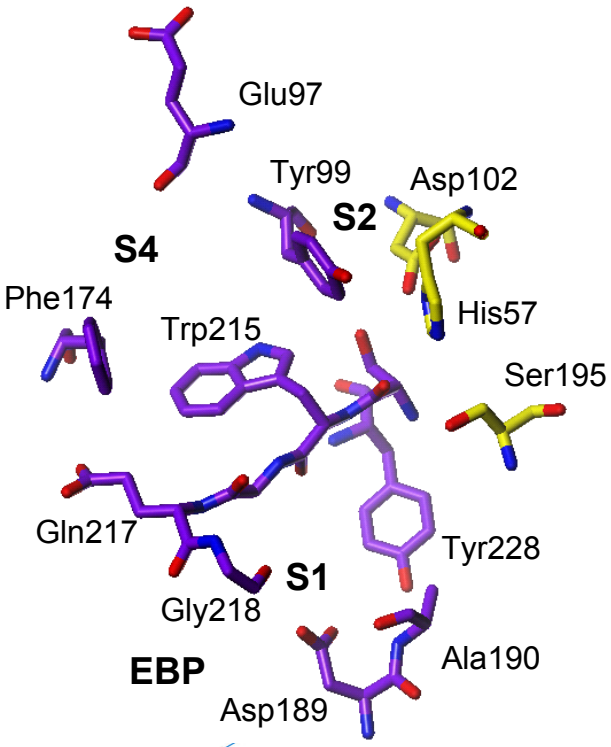


Factor Xa Topology of Inhibitor Binding Site



Blocking access to catalytic triad by small-molecule inhibitors in active site should be effective to reduce thrombogenesis.





Four relevant subpockets with effect on inhibitor binding affinity.

S1, S2, S4, EBP

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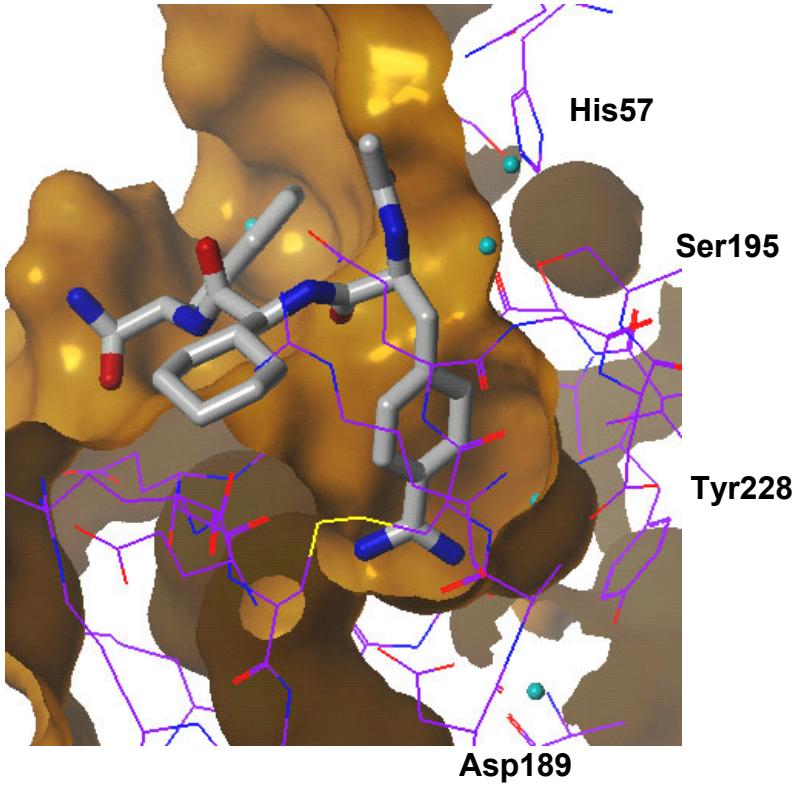
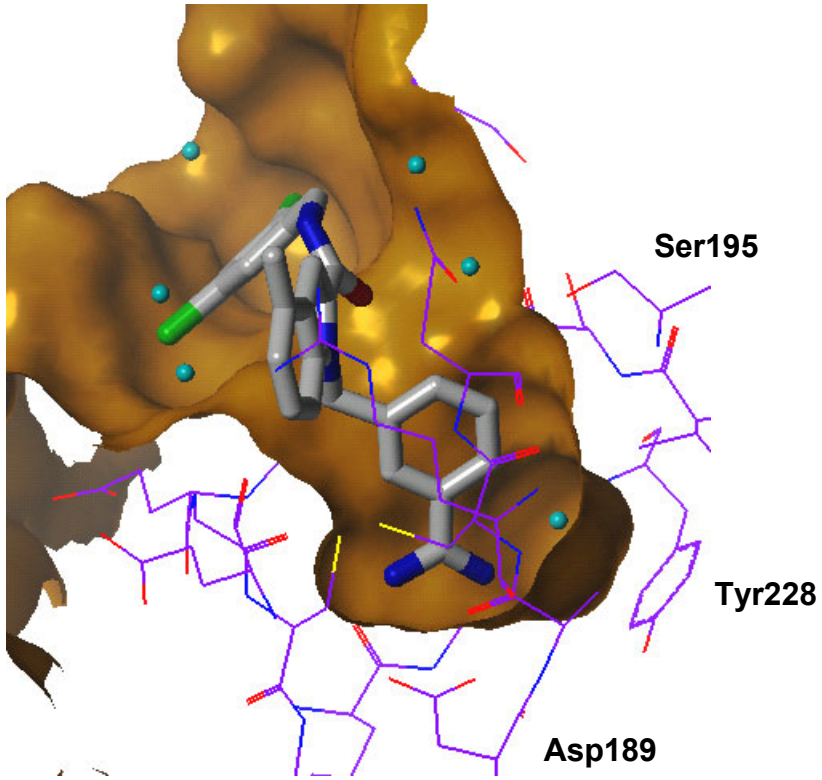
Searching for Factor Xa Lead Structures



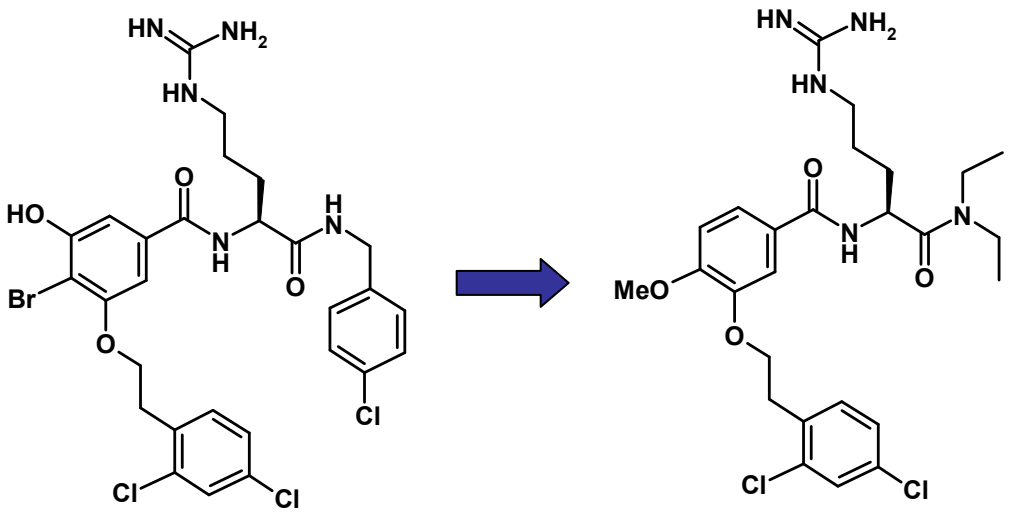
First FXa inhibitors: Peptides
Ostrem et al., *Biochemistry* **1998**, *37*, 1053-59

High affinity, but benzamidine in S1



Next generation: Small molecules
Matter et al., *J. Med. Chem.* **2002**, *45*, 2749-2769

3-Oxybenzamide as Novel Factor Xa Scaffold



Library hit

Second generation
(153 nM)

Screening combinatorial libraries
Non-peptide **3-Oxybenzamides**

Evaluation by combinatorial chemistry and structure-based design

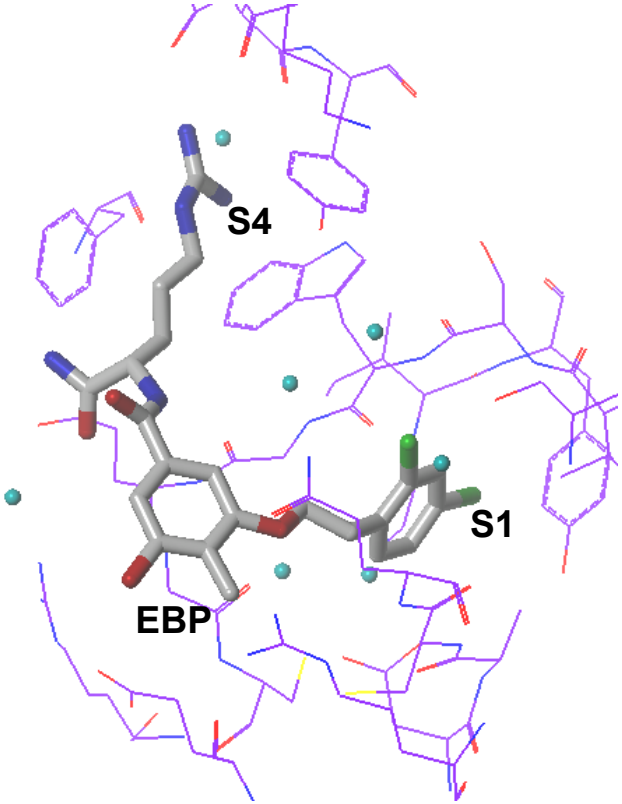
Nazaré et al., *Bioorg Med Chem Lett.* **2004,** *14,* 2801-2805

Hypothesis: Basic arginine in FXa S1 pocket
But: Conflicting SAR and docking studies

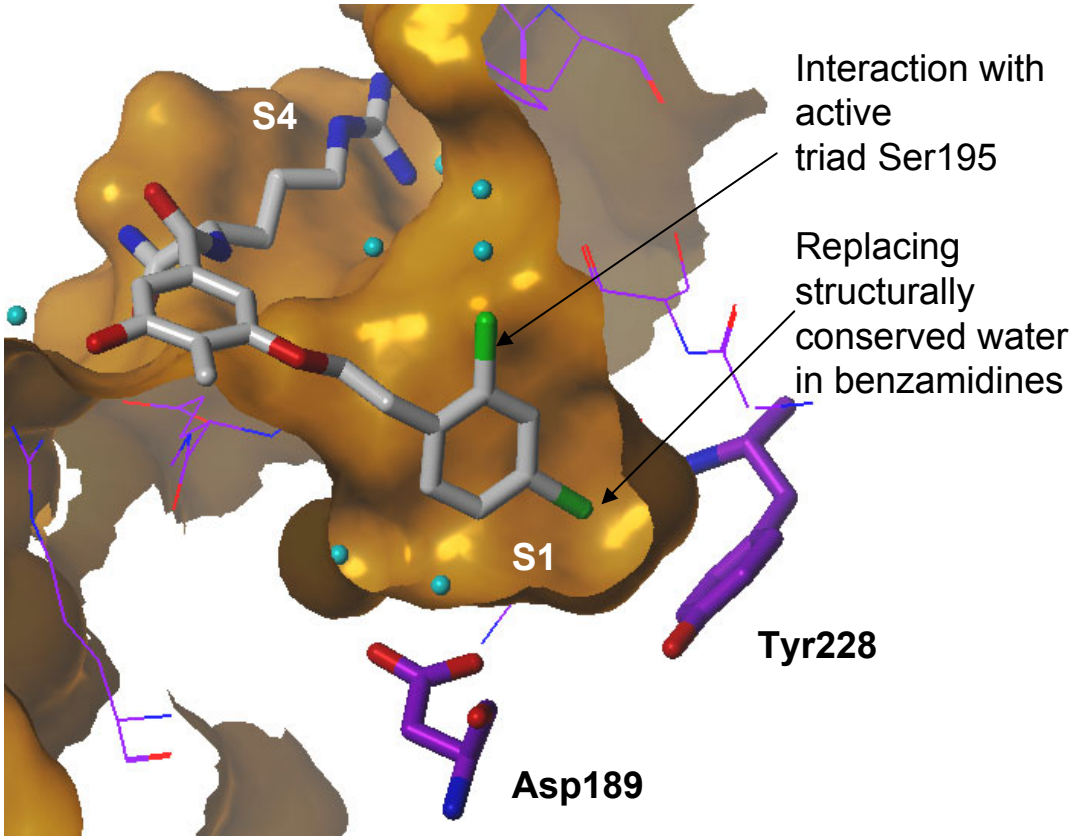
Literature: Evidence for *„reversed binding“* from thrombin

Tucker et al, *J. Med. Chem.* **1998**, *41*, 3210-3219

Reversed Binding Mode in Factor Xa



X-ray structure of Factor Xa complex (2.15 Å)



X-ray structure reveals reversed binding mode
Dichloro-phenyl situated in S1, arginine in S4

Matter et al., *J. Med. Chem.* **2005,** *48,* 3290-3312.

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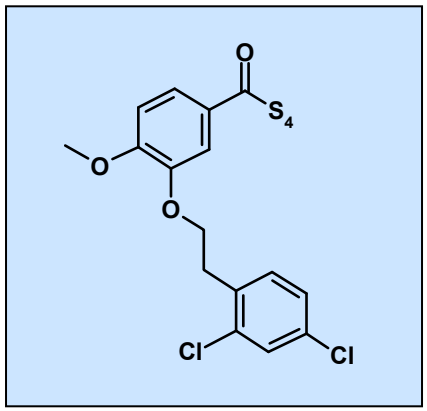
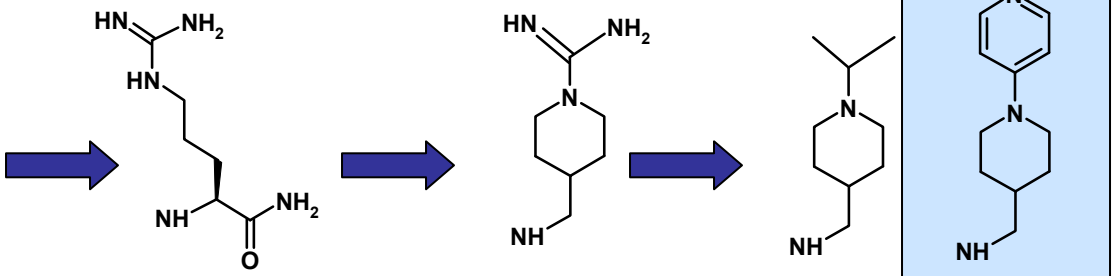
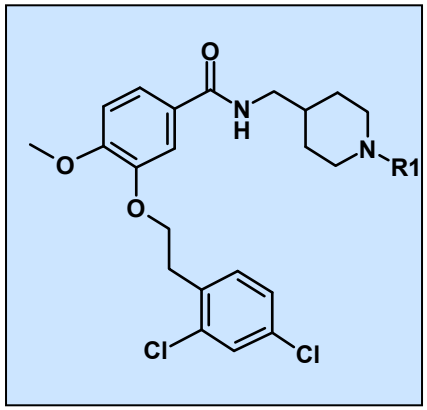
Structure-Activity Relationship of FXa

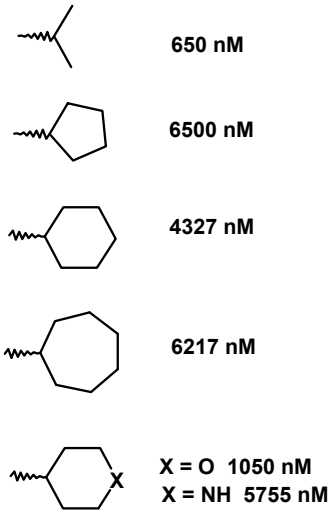
A

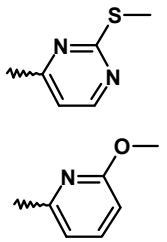


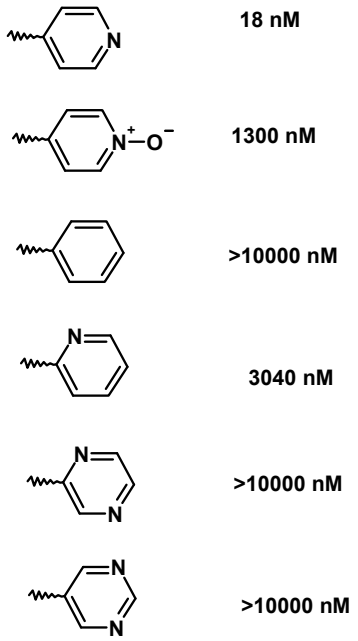


B









Nazaré et al., *Bioorg Med Chem Lett.* **2004,** *14,* 2801-2805

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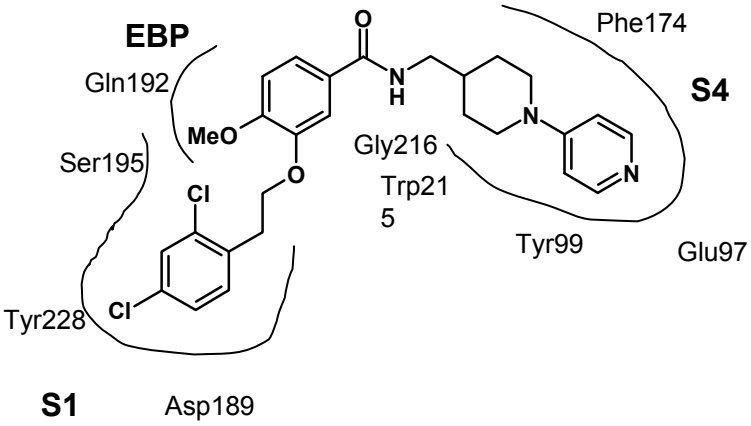
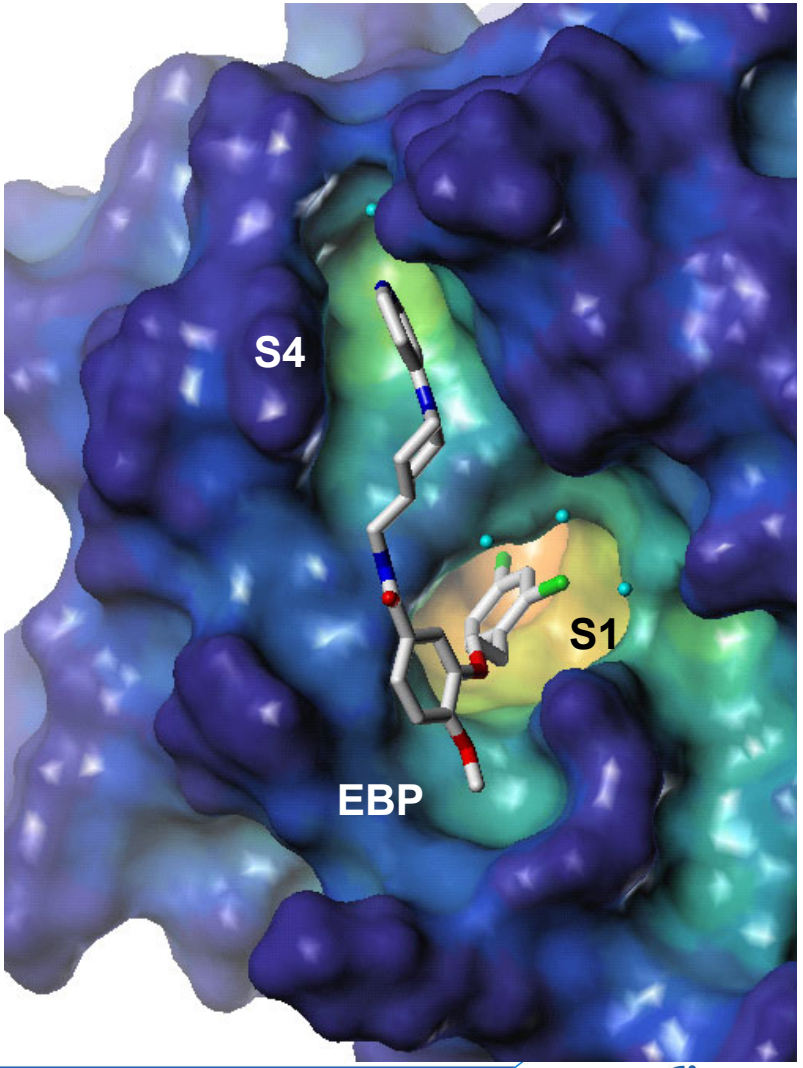
Detailed Interactions from X-Ray Structure

X-ray crystal structure of Factor Xa / Oxybenzamide Inhibitor Complex

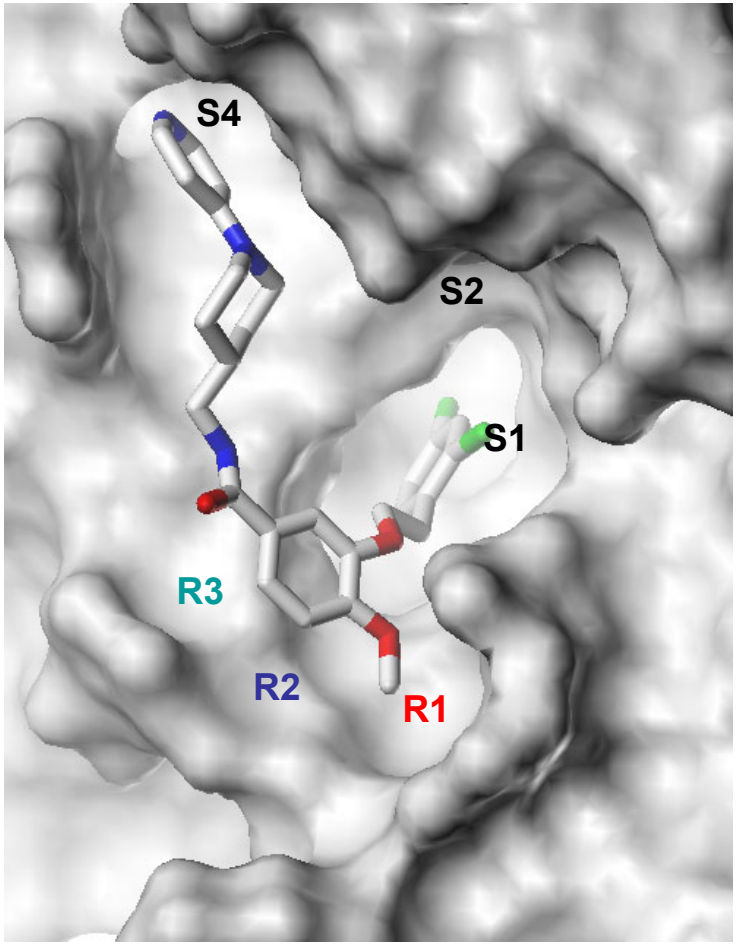


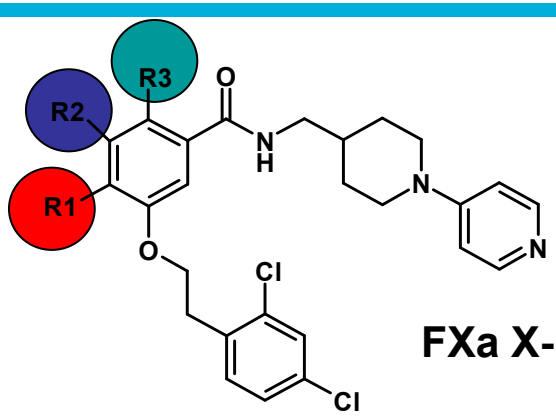
Ki: 18 nM
Resolution 2.7 Å



Matter et al., *J. Med. Chem.* **2005,** *48,* 3290-3312.

Structure-Activity Relationship of FXa Inhibitors



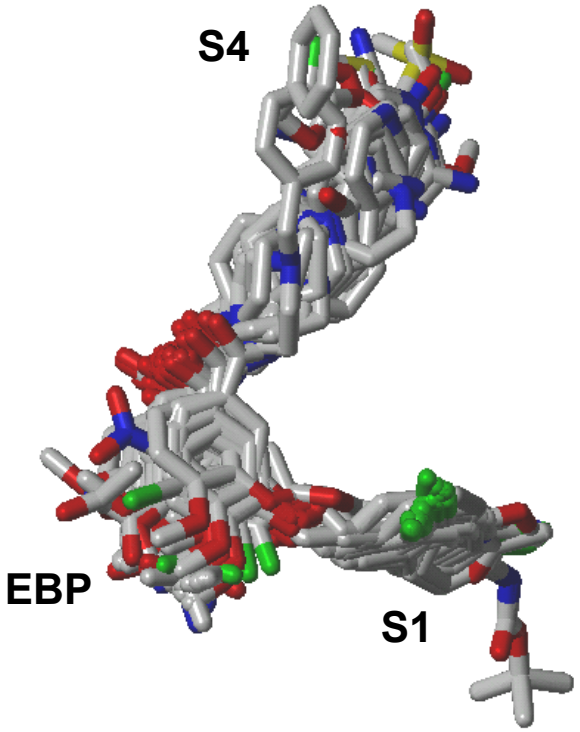


FXa X-ray structure

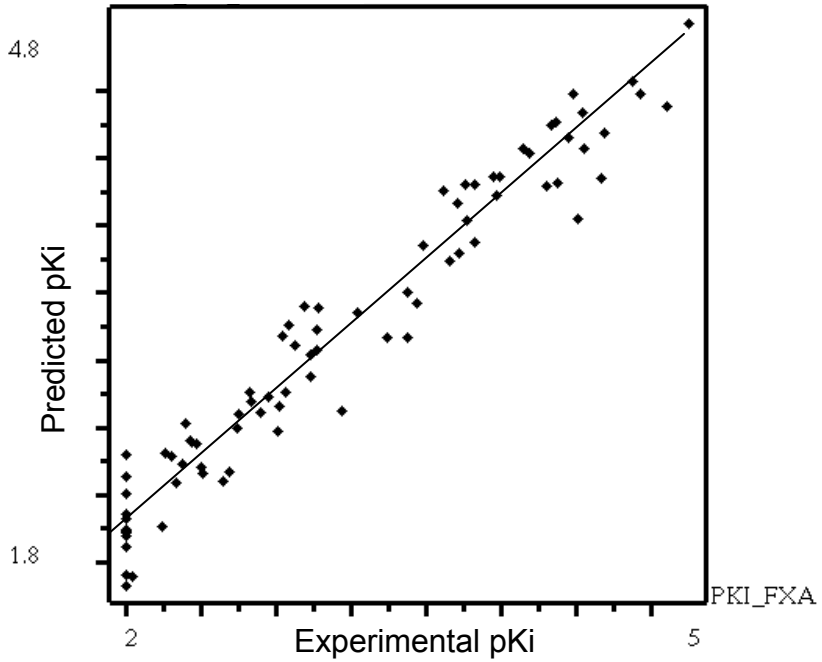
R1	R2	R3	FXa [nM]
OMe	H	H	18
SMe	H	H	28
OMe	OMe	H	50
F	H	H	50
Me	H	H	55
H	$CONH_2$	H	57
H	H	H	106
H	H	Cl	241
Br	OC(=O)Me	H	285

Nazaré et al., *Bioorg Med Chem Lett.* **2004,** *14,* 2801-2805.

3D-QSAR Models for 3-Oxybenzamides



Superposition of 80 **FXa** inhibitors after docking into binding site



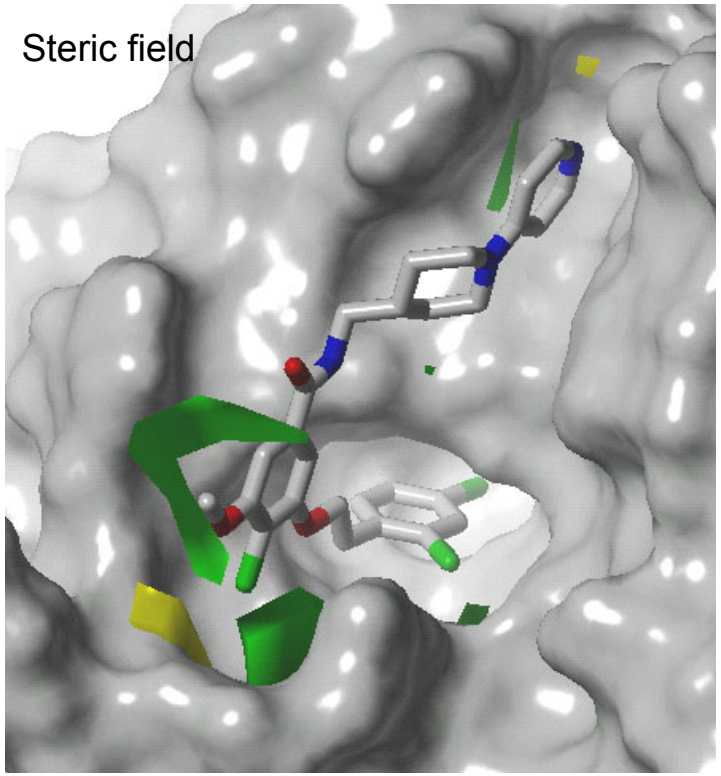
CoMFA (80 cpds, see above):
q^2: **0.741** (6 comp), r^2: 0.947
pred. r^2 0.732 (27 external cpds)

Matter et al., *J. Med. Chem.* **2005,** *48,* 3290-3312.

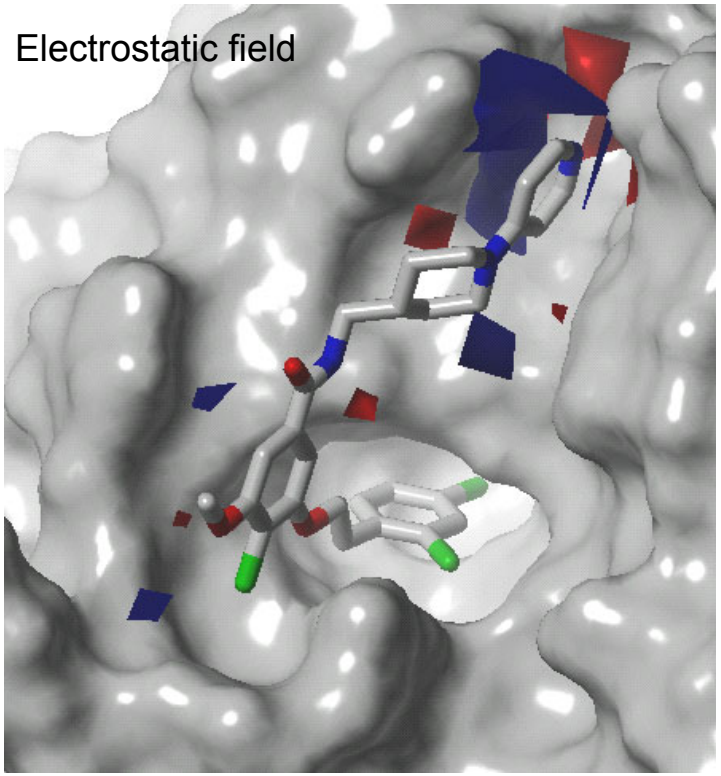
3D-QSAR Models for FXa Inhibitors

Chemical Interpretation of CoMFA Model

Steric field



Electrostatic field

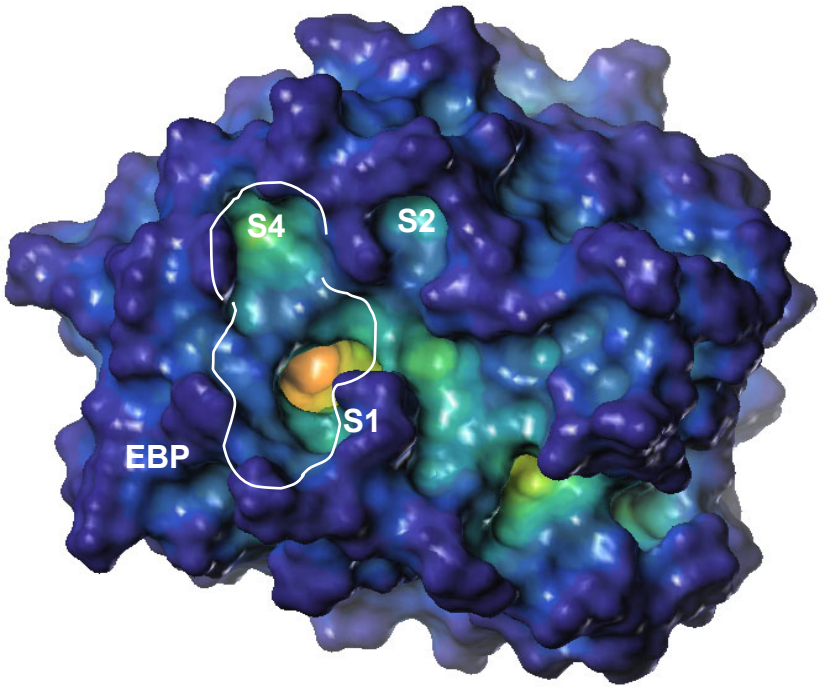


CoMFA (80 cpds): q^2: **0.741**, r^2: 0.947

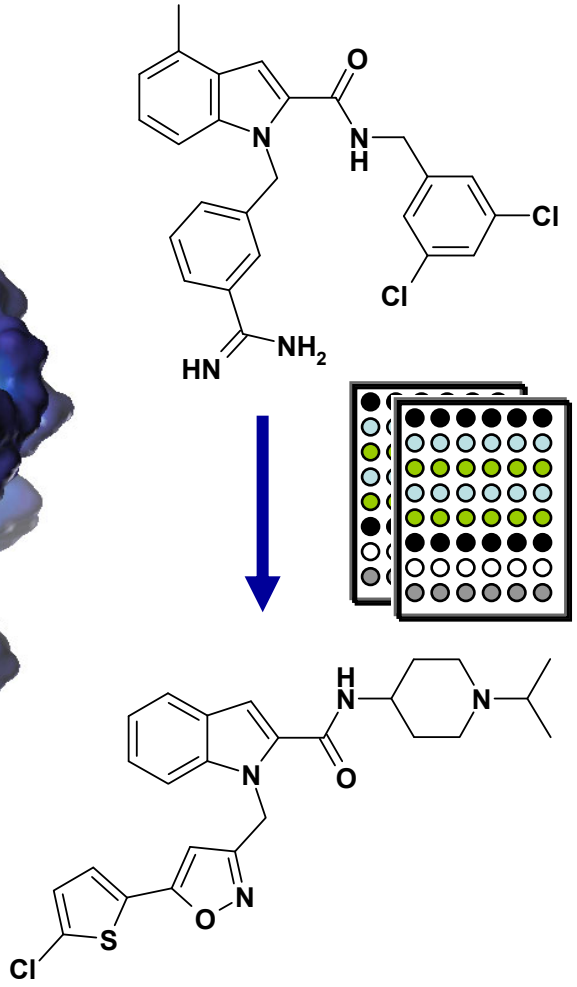
green: steric bulk favourable
yellow: steric bulk detrimental

blue: positive charge favourable
red: negative charge favourable

Indole-2-Carboxamide as Novel FXa Scaffold



Factor Xa X-ray Structure



Indole-3-carboxamide
with S1 benzamidine
FXa 25 nM

Matter et al., *J. Med. Chem.* **2002**, *45*, 2749-2769.

Focussed libraries (S1,S4)

Indole-3-carboxamide
with neutral S1 group
FXa 3 nM

Nazaré et al. *BMCL* **2004**, *14*, 4191-4195.
Nazaré et al., *J. Med. Chem.* **2005**, *48*, 4511-4525.

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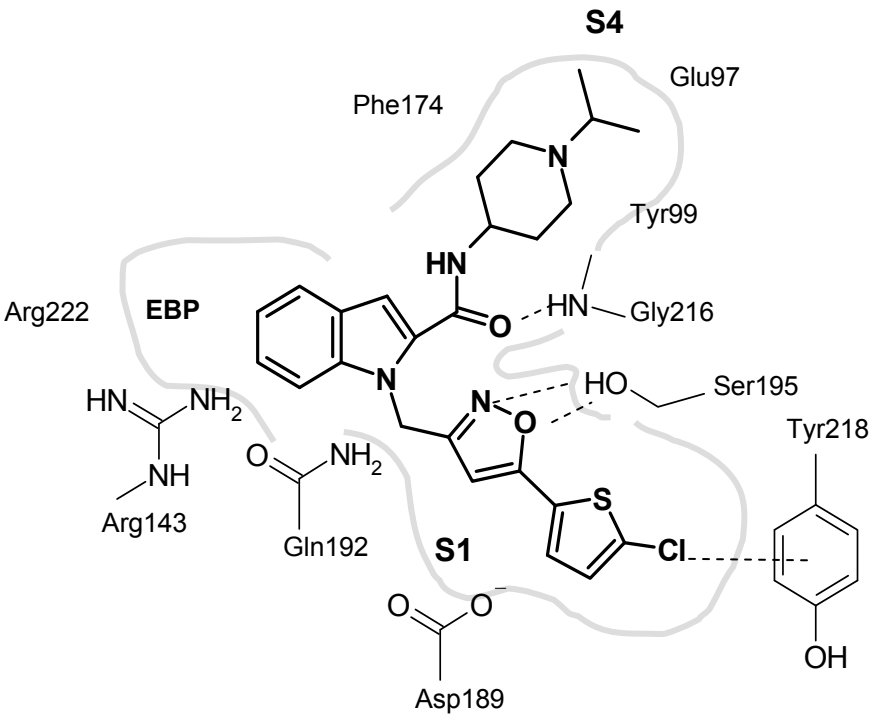
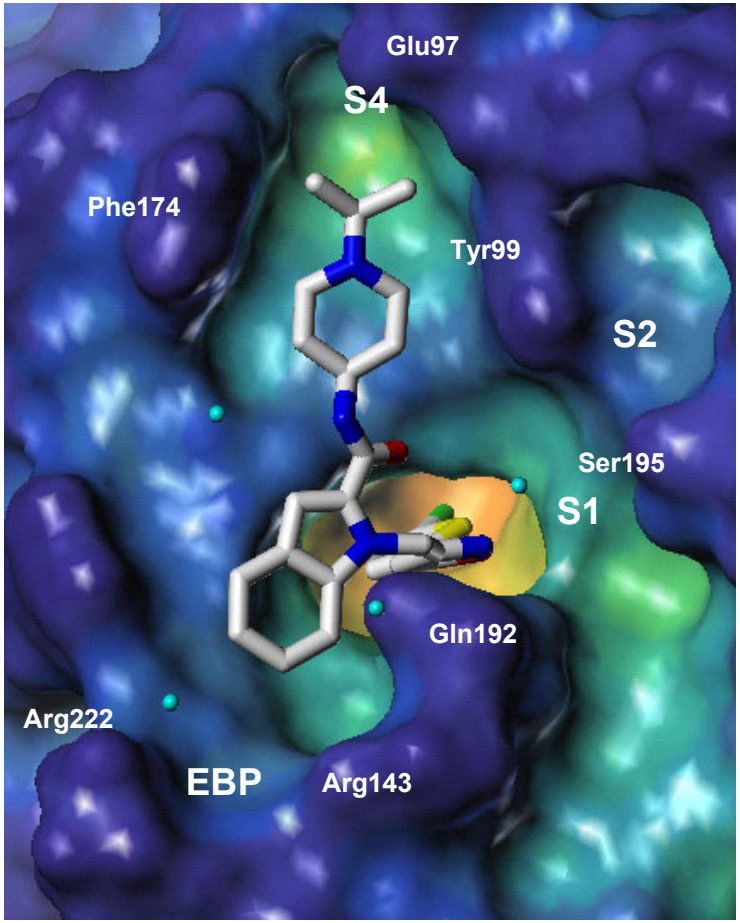
X-Ray of FXa / Indole-2-Carboxamide



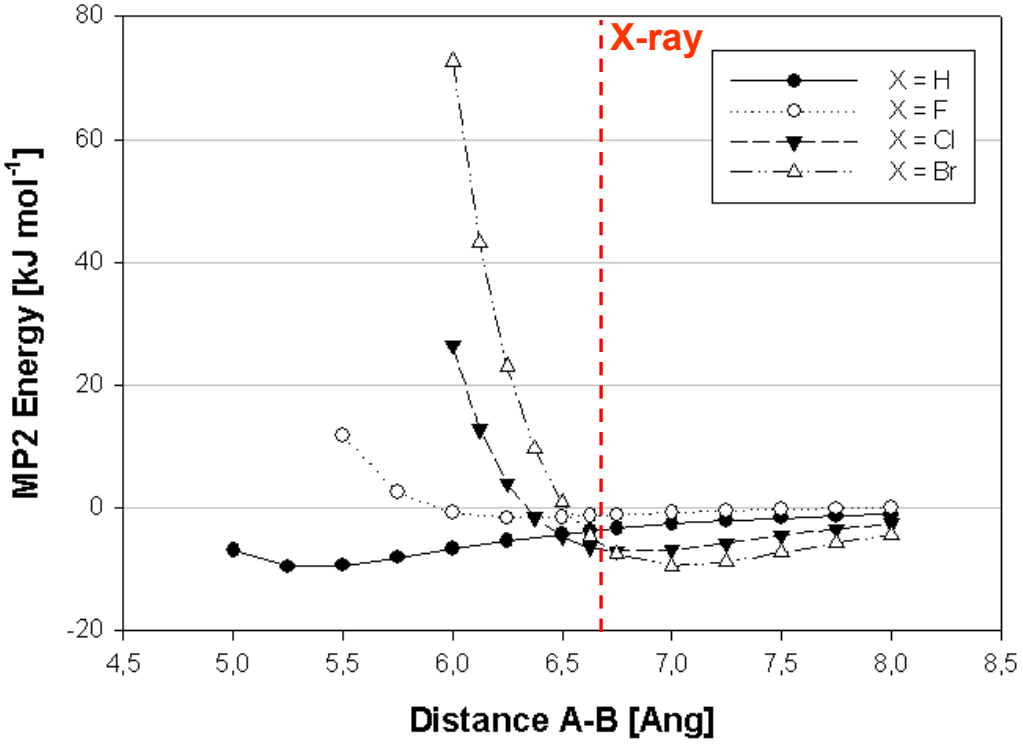


Ki: 3 nM, Resolution 2.2 Å

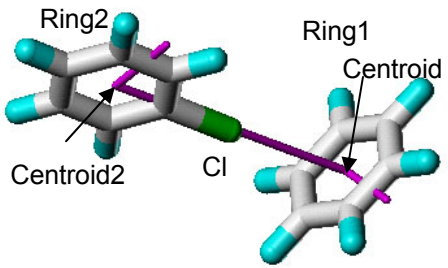
Nazaré et al., *J. Med. Chem.* **2005**, *48*, 4511-4525.

Nature of Chloro-Aromatic Interactions

Results from *ab-initio* calculations agree to experimental fXa affinity differences (ΔΔG). Major source of attraction: long-range interactions such as electrostatics and dispersion. At shorter distances, the influence of electrostatic interactions increases.



Model system for *ab-initio* calculation



d1: Cl – Centroid (3.497 Å)
d2: Centroid – Centroid2 (6.628 Å)
Angle: Plane Ring1 – Ring2 (100.9°)

H. Matter et al. *Angew. Chem. Int. Ed.* **2009**, *48*, 2911-2916

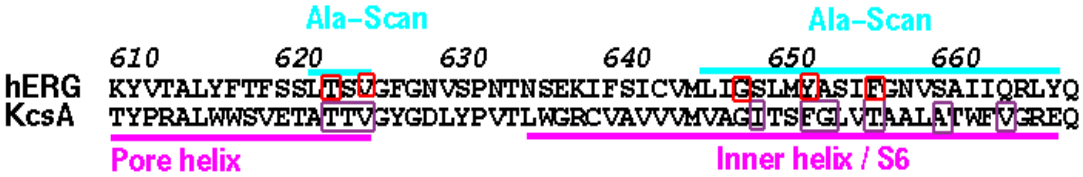
Towards a hERG Homology Model

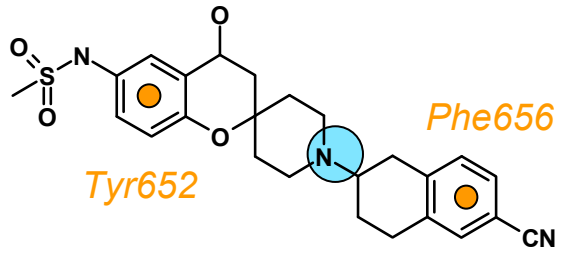
Similarity to KcsA channel (52.6 %)

- KcsA X-ray: D.A. Doyle et al. *Science* **1998**, *280*, 69

Binding site hypothesis

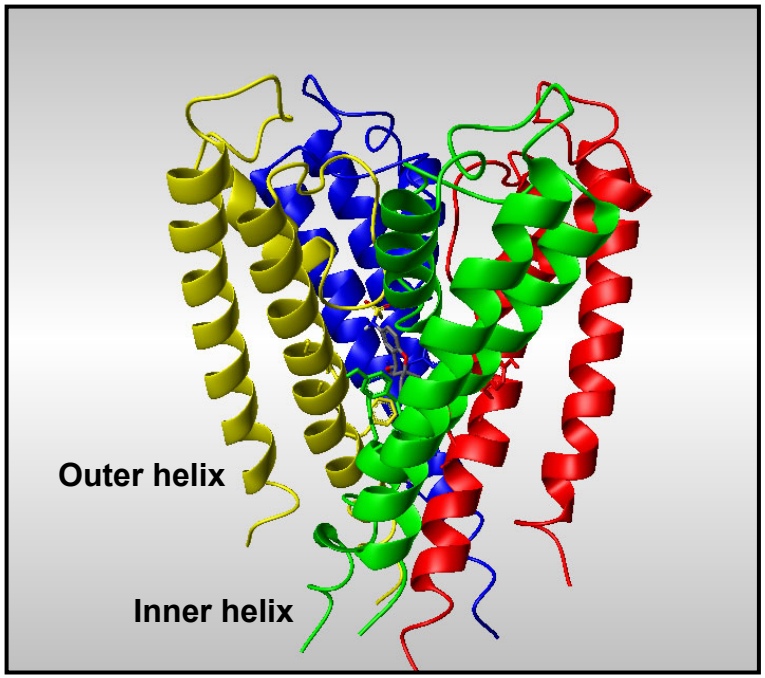
- Ala scanning: J.S. Mitcheson, *PNAS* **2000**, *97*, 12329
- Key residues Phe656, Tyr652, Gly648, from MK-499





MK-499, class-III antiarrhythmic drug

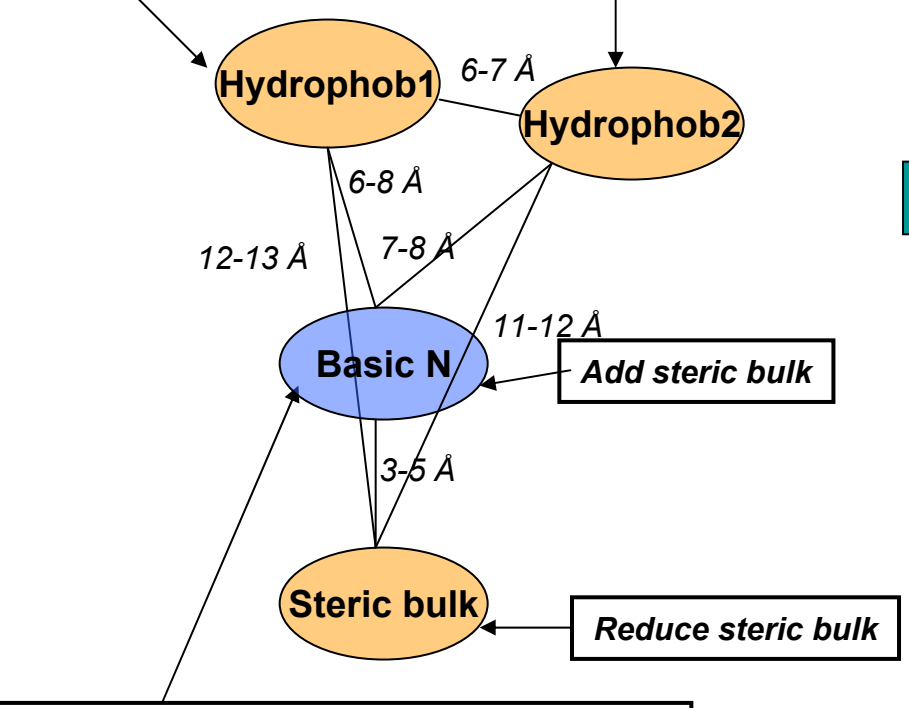
Homology Modelling from KcsA

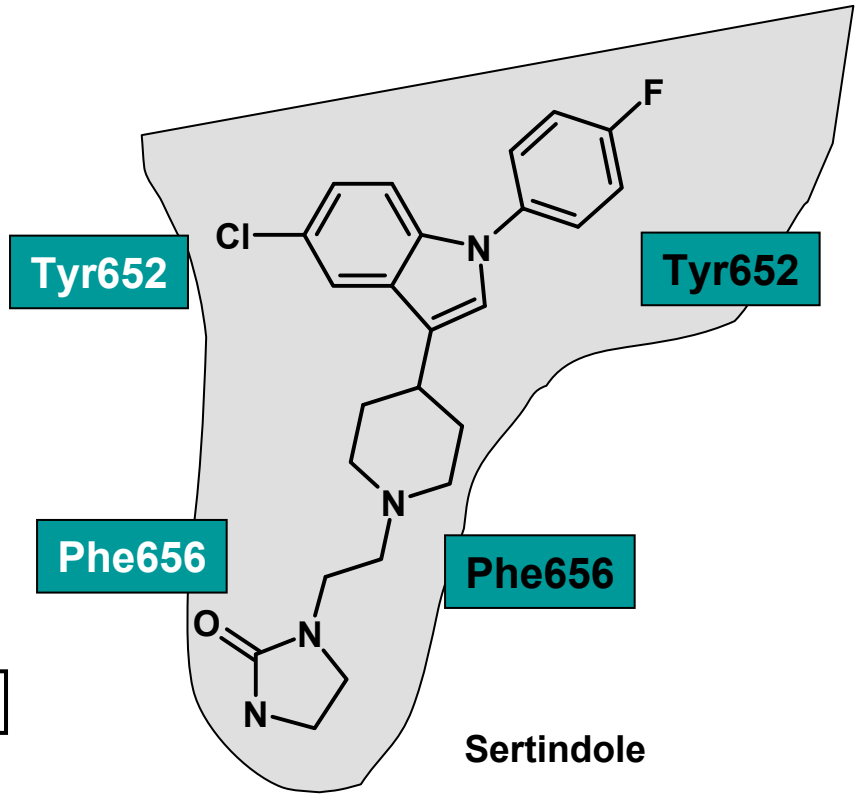


Blockage of IKr / hERG in myocardium
like other methansulfonanilides, e.g. dofetilide, sotalol.
Risk of polyarrhythmia limits therapeutic value.

Towards hERG Structure-Activity Relationship

Add negatively charged groups:
-OH, -SO_2NH_2, SO_2Me, COOH
Replace with (polar) heterocycles





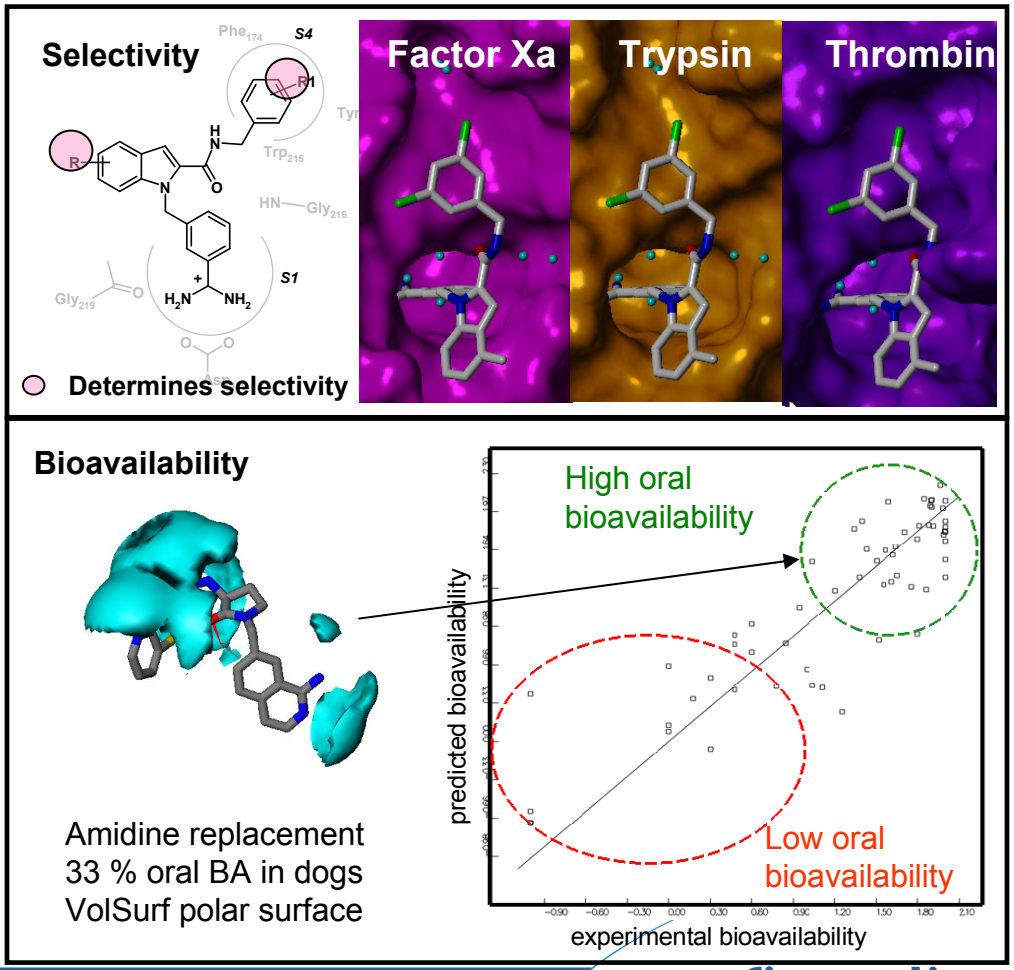
Sertindole

Reduce negative electrostatic potential:
Lower pKa, reduce basicity for nitrogen.
Substitute by amides, O, C, electron-withdrawing groups, heterocycle in linker

Multidimensional Optimization of FXa Inhibitors

Design of selective and orally available inhibitors

- Multiple **X-ray structures** of FXa / ligand complexes
- Tailored **scoring functions** for compound design
- **QSAR** to predict **affinity**, **selectivity, hERG, cytochrome P450s** inhibition
- **QSAR** to predict **oral bioavailability** for different species
- Combined use for **multidimensional compound optimization**



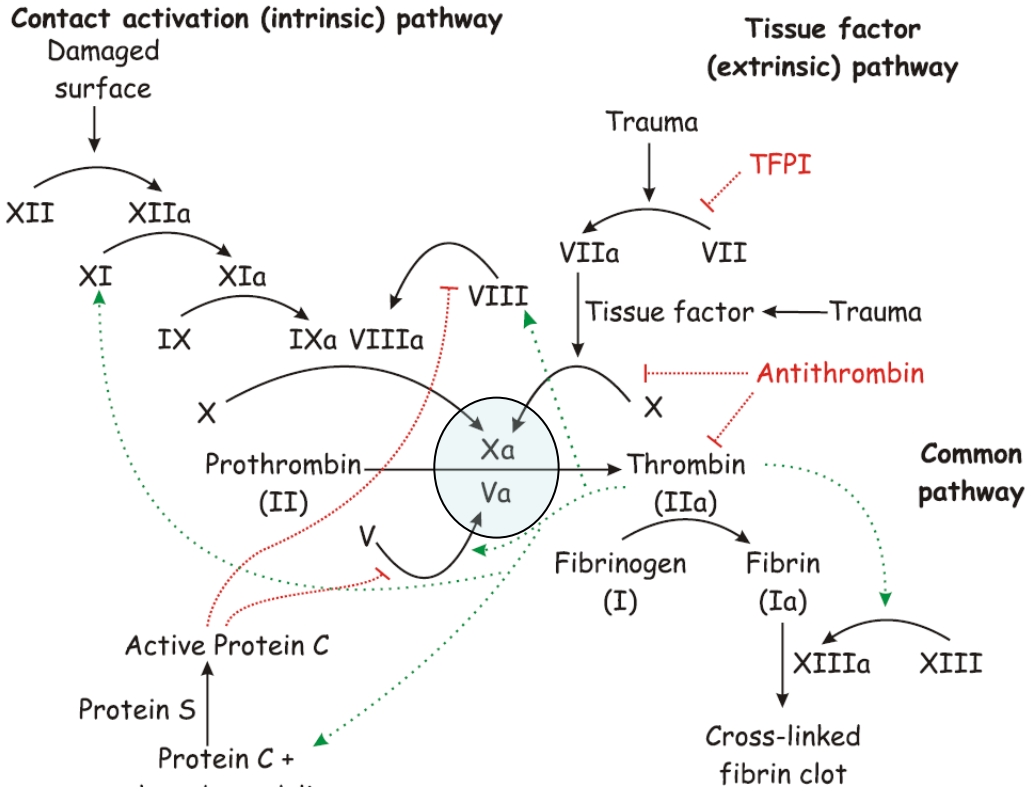
Factor Xa and Blood Coagulation Cascade

Factor Xa as Drug Target

- Single extracellular protein involved in regulation mechanism
- Example of single interaction in known pathway

Drug Design

- Capabilities are at hand to design and synthesize optimized ligands although still challenging

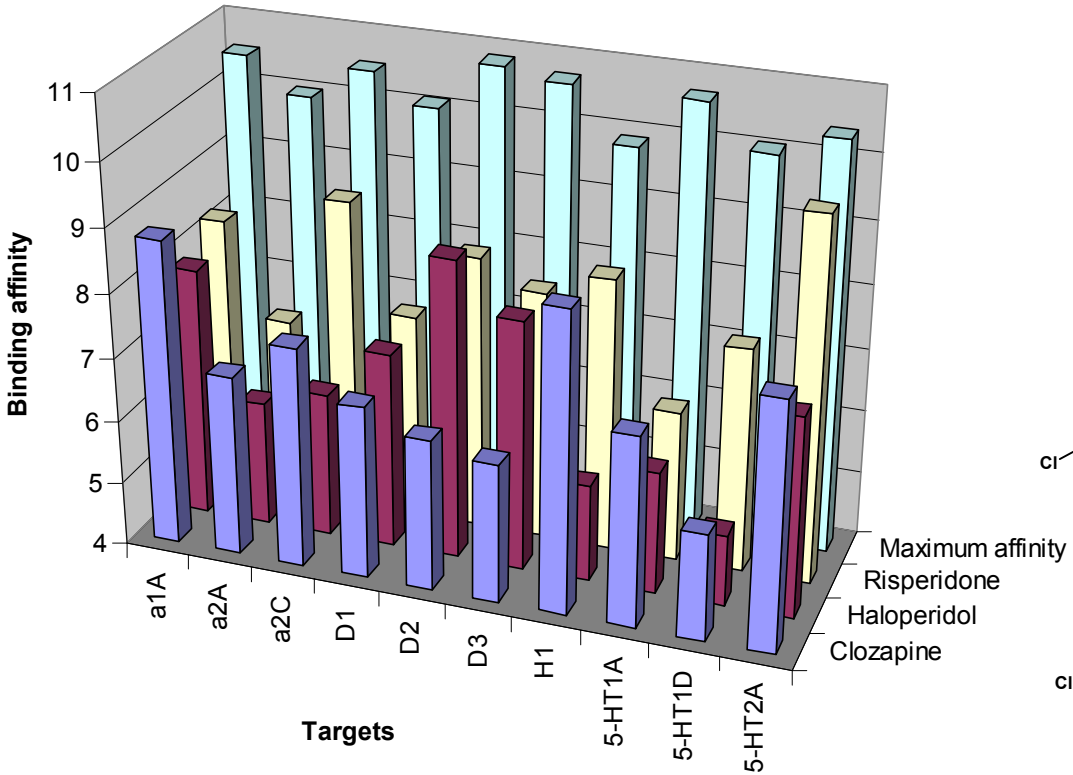


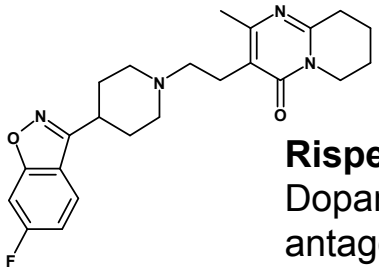
Polypharmacology

Binding affinity profiles for three antipsychotic drugs on aminergic GPCRs.

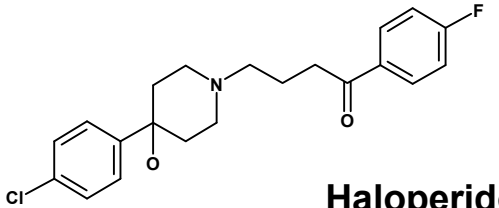
Many effective drugs for human diseases bind to more than one receptor.

How is drug promiscuity / polypharmacology linked to disease mechanism ?

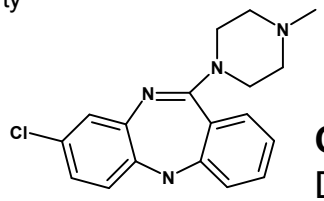




Risperidone (1993):
Dopamine D2 / 5-HT2A antagonist



Haloperidol (1959):
Dopamine D2 antagonist



Clozapine (1972):
Dopamine D4 antagonist

Lit.: Mestres, J. et al. *TIPS* **2009**, *30*, 470-474.

sanofi aventis
Because health matters

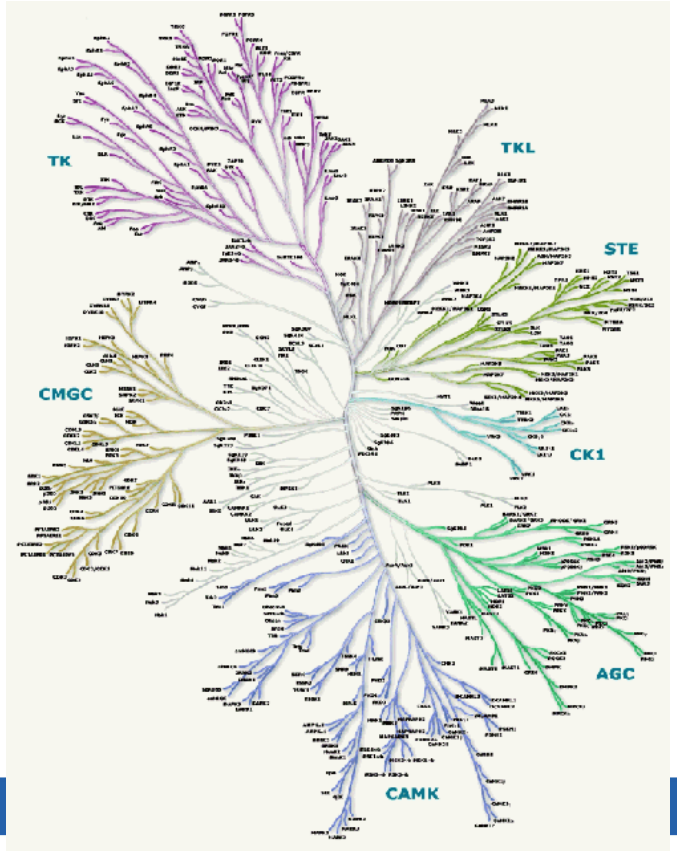
Selectivity Profiles in Diseases

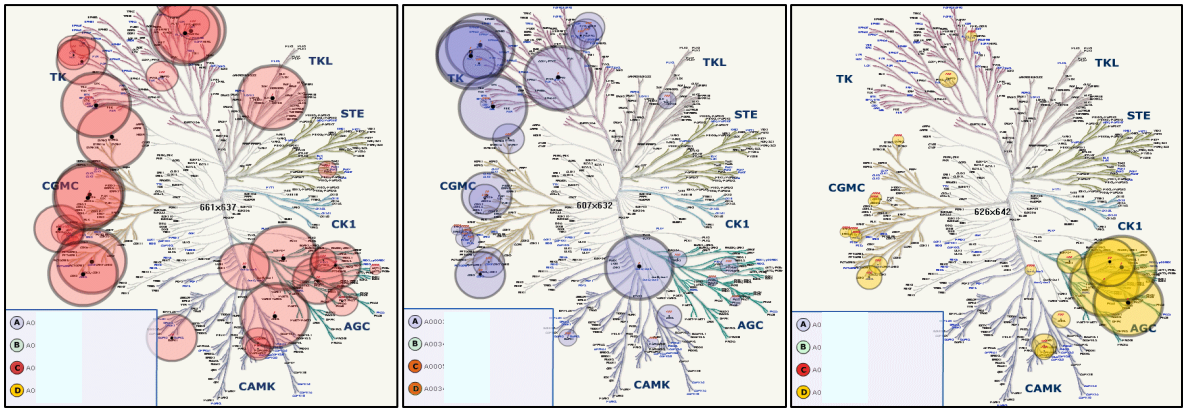
Sugen Kinome Tree: 518 kinases arranged by sequence similarity.

Selectivity for kinase ligands sometimes difficult to obtain.

How translates selectivity to outcome in disease model?

Clinical implication of inhibitor selectivity profile on human disease?

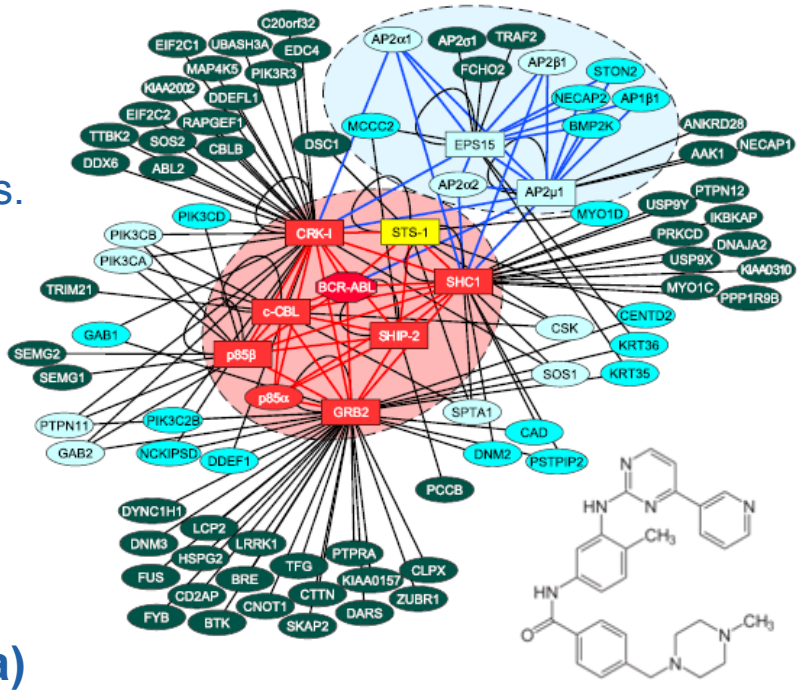




Different kinase selectivity profiles of three different kinase inhibitor compounds.

Molecular Networks of Drug Targets

- **Reductionist view**
  - Drugs act in cells on single proteins.
- **System biology view**
  - Proteins organized in multiprotein complexes.
  - Dynamical changes of their organization.
- **Most drugs do not interact specifically**
  - Interaction with many targets in complexes.
  - Side effects linked to pathways in other diseases.
  - Drugs disturb network at different levels:
    - Expression, modification, protein–protein int.,metabolites
- **Example: Bcr-Abl kinase (Myeloid leukemia)**
  - Interaction to 7 proteins in different pathways.
    - Grb2, Shc1, Crk-I, c-Cbl, p85, Sts-1, SHIP-2
  - Inhibitors disrupt this network differently.
    - Perturbation leads to „remodeling“ of interaction space.



Single protein-protein interaction network for Bcr-Abl.

Bcr-Abl inhibitor **Imatinib**

Adapted from: Superti-Furga, G. et al. *PNAS* **2009**, *106*, 7414-7419.

From Genes and Proteins to Networks, Dynamics and Disease Biology

- Decipher connectivity and dynamics of protein networks and build “system-level” understanding of health and disease state
- Study network modulation at multiple network nodes as a result of individual genetic background and therapeutic intervention
- Link compound profiles / network changes to clinical outcome

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