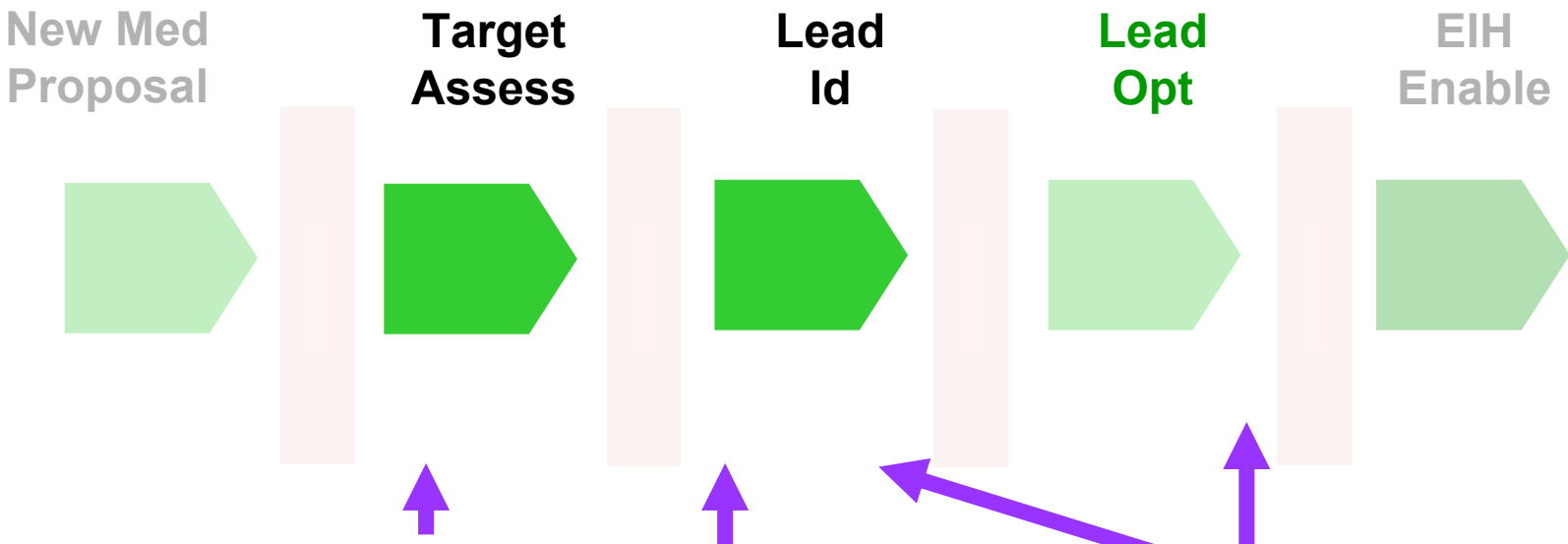
Current status and future prospects of structural biology in drug discovery

Michael Hennig

Biostructure in drug discovery



- Drugability of the target
- Selection of target sites
- Selectivity assessment (target family, species, bioinformatic, homology modeling)

- Focused Screening
- Fragment screening
- HTS hit profiling

- Assist chemistry in classical **structure based design** cycles

Key progress of past years: Structure information in early phase of project

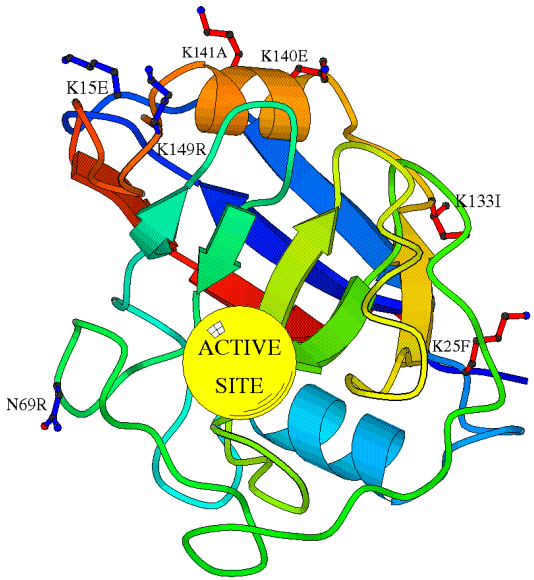
Key to early access to biostructural information

- **Organisational set-up**
  - Early involvement of biostructure expertise in target assessment
  - Start protein preparation as early as possible

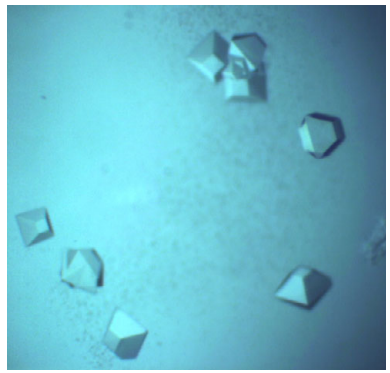
  - Dedicated protein labs for biostructural research
    - Priority setting that fits to structure group
    - Taylored protein for biostructure (use of tags, construct design, purity requirements, …..)
    - Close feedback loop and mutual understanding of protein and crystallization lab‘s (avoid scapegoat effect)

Key to early access to biostructural information

- **Protein production**
  - Start with several constructs in parallel in various expression systems
  - „Crystal-tailored“ protein
    - „Rational“ crystal engineering
    - Directed evolution/DNA shuffling
    - Use of antibodies & other binding proteins



10 mutants – one crystallizes

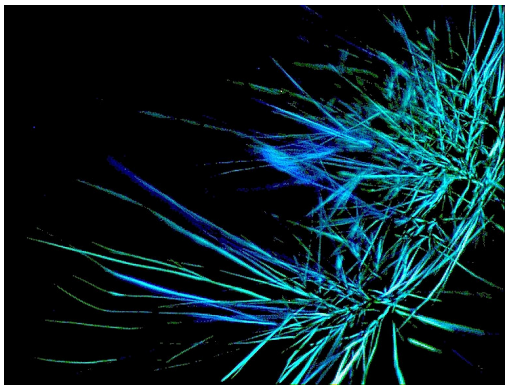


- diffract to better than 1.5 Å, $P4_12_12$
- we have always been successful, but how long do you try?

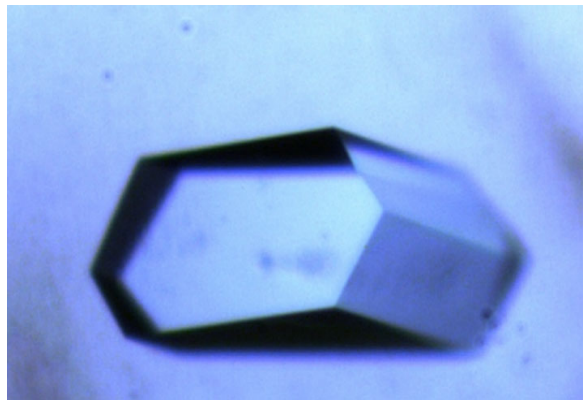
Key to early access to biostructural information

Crystallization

- Miniaturization to set-up many experiments with limited amount of protein (<100 nl), fluidic circuit systems
- Diverse set of buffers, precipitants, additives …….an unlimited experimental space
- Automation of liquid handling and crystal inspection



Optimization & set-up of robust system for xx complex structures still „manual“ work !!!!

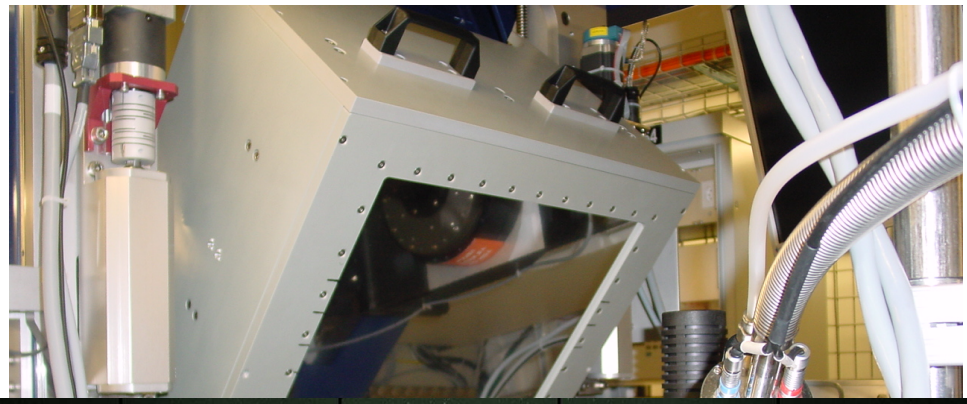


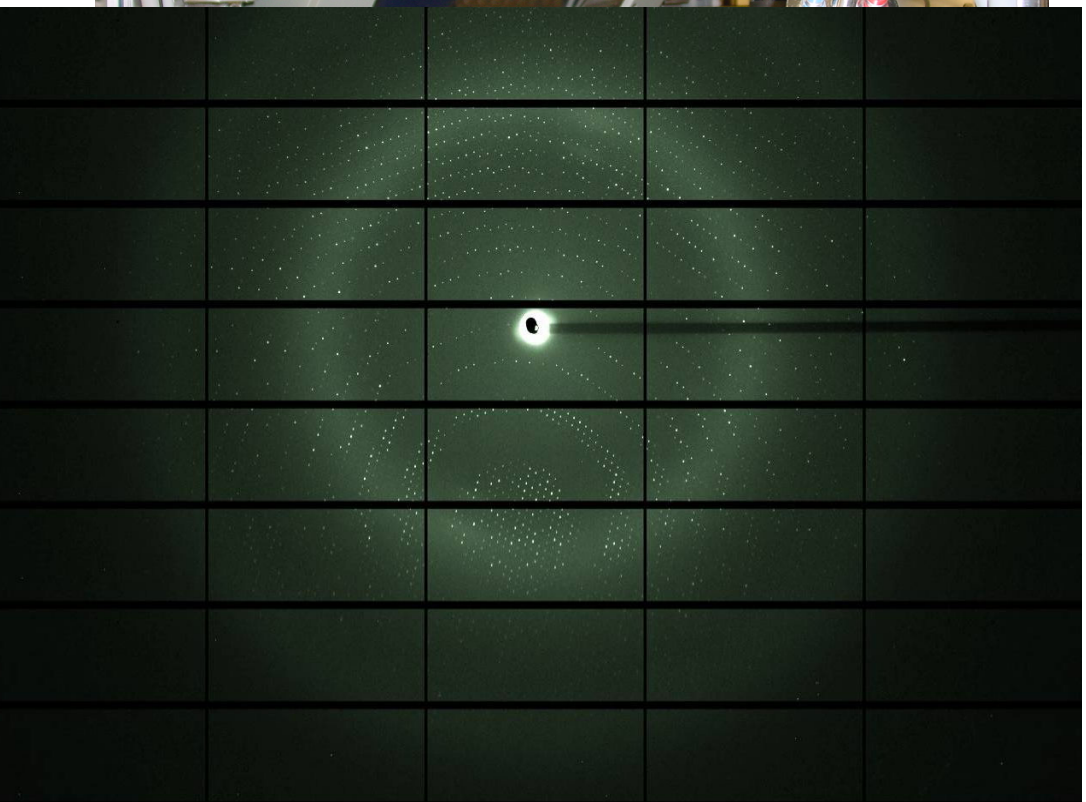
Key to early access to biostructural information

X-ray methods

- No real bottleneck anymore thanks to Se-Met and a rich source of homologous structures in the pdb
- Workflow and data capturing to keep track with increasing number of experiments
- Synchrotron access (Roche/SLS PX II- 40 days/year) and constant improvement in throughput and data quality
  - Sample changer
  - Pilatus detector

Pilatus detector



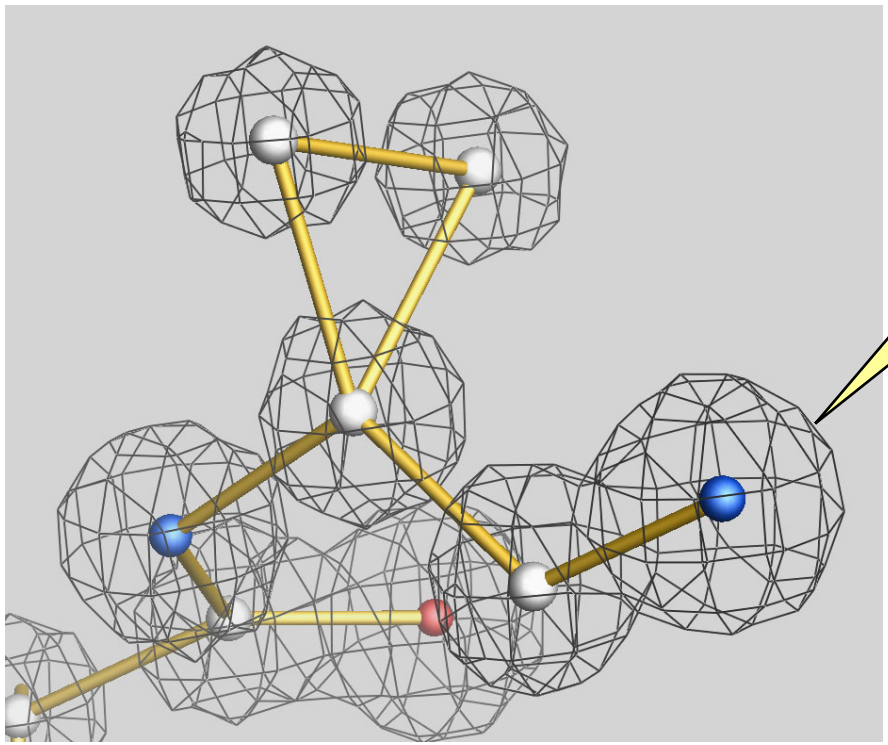


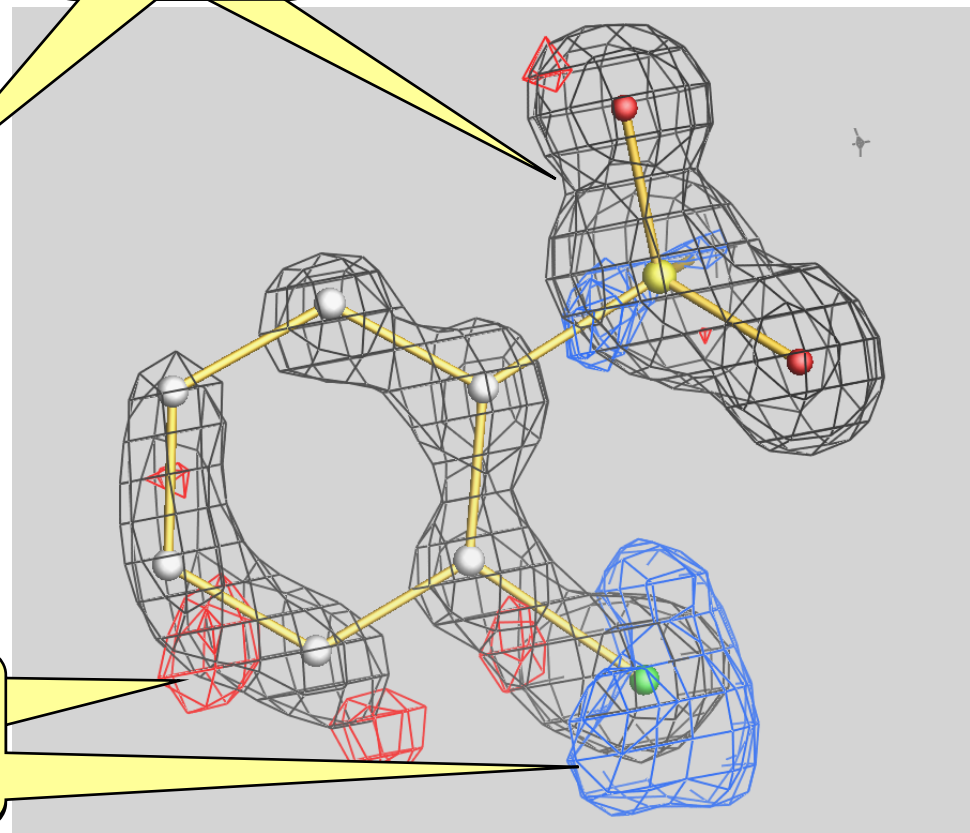
Properties:

Energy range 4 – 30 keV
Dynamic range higher than CCD
No dark current
No readout noise
Excellent point spread function
Short readout times: ms
Suppression of fluorescent background
Very good signal/noise ratio

….enables fine slicing
and data collection in a few seconds

0.95Å resolution measured on the Pilatus detector last week

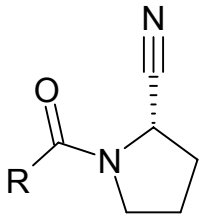




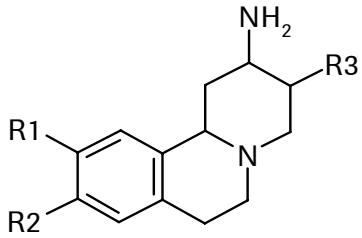
Structure based drug design at work

Example DPP-IV

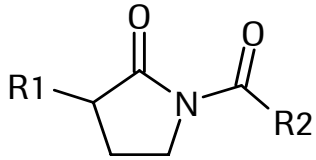
DPP-IV: SPR and X-ray in ligand characterization “pick the winner”



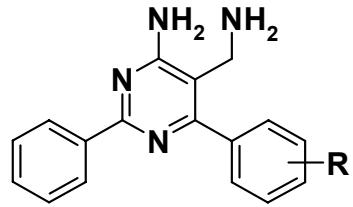
Cyanopyrrolidines
Literature



Benzoquinolizines
HTS



Pyrrolidinones
HTS

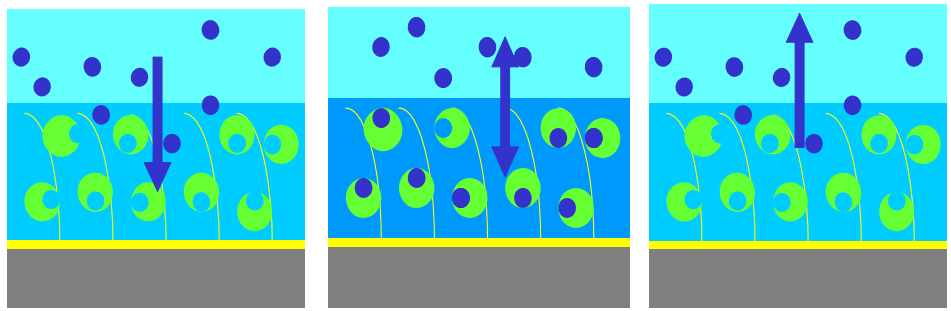


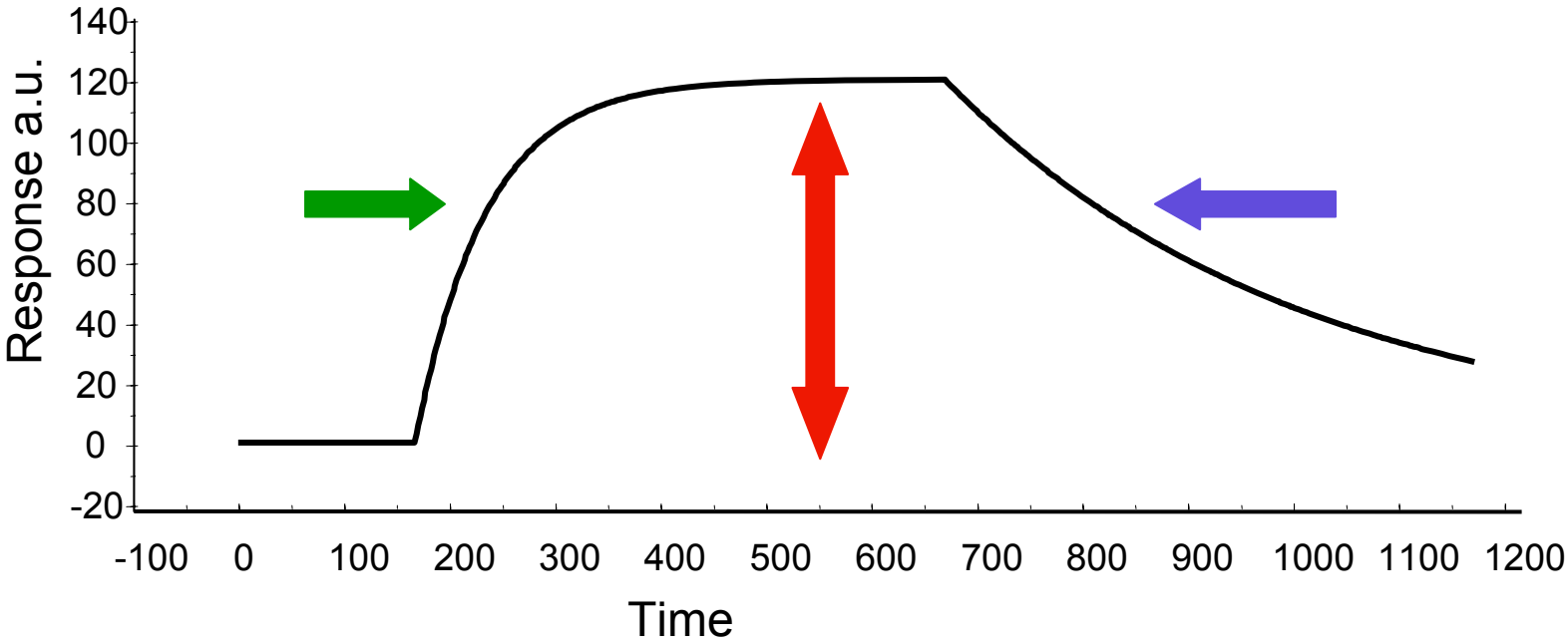
Aminomethyl-
pyrimidines
HTS

Questions to support characterization and prioretisation of hit cluster:

- Reversibility of binding?
- Active site binding – Specificity, stoichiometry of binding?
- Kinetic of association, recognition, kon
- Kinetic of dissociation, stability, koff
- Binding mode and potential of further optimization

SPR: A sensitive & information rich assay



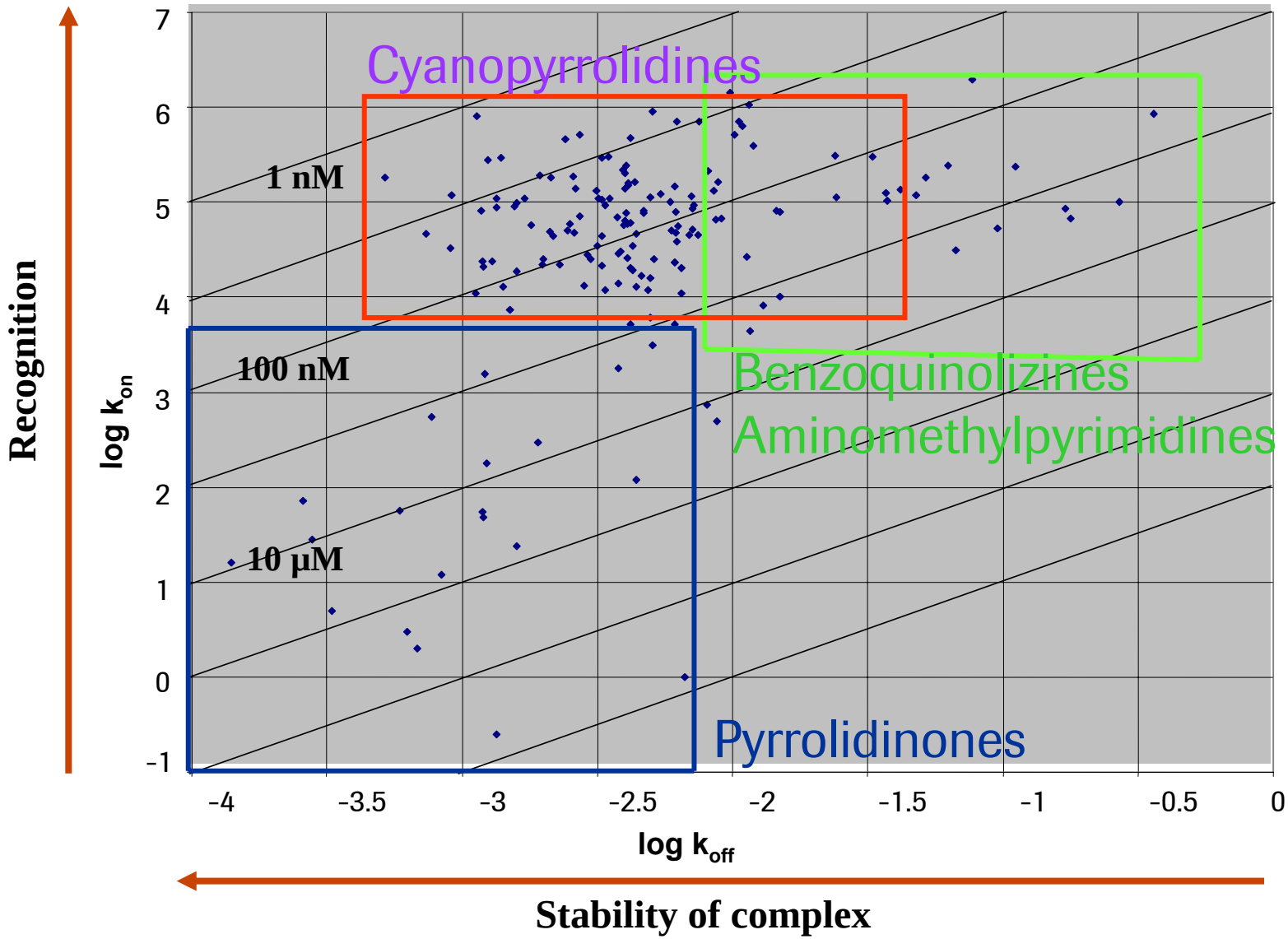


Association phase: **Kinetic of association, recognition, kon**

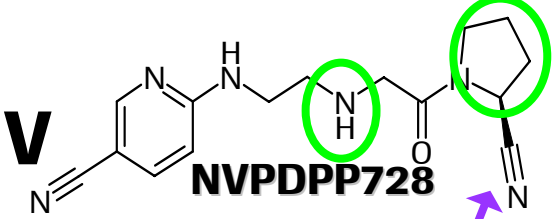
Equilibrium (saturation) phase: **Binding affinity, stoichiometry**

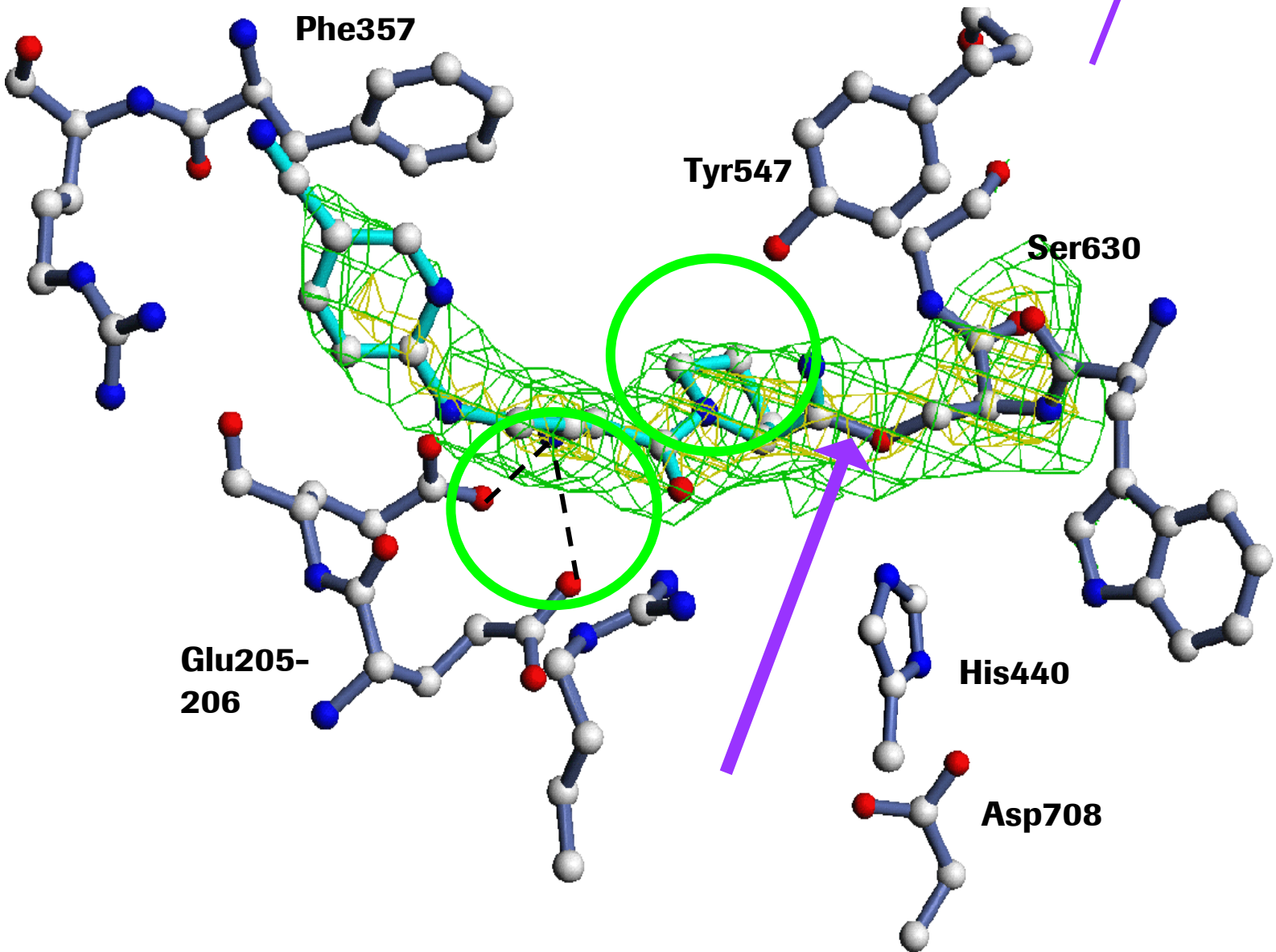
Dissociation phase **Reversibility, kinetic stability**, koff

Clustering of ligand classes in kon/koff plot

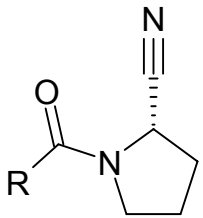


Covalent inhibitors of DPP-IV



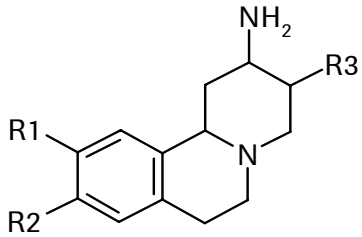


Use of SPR and X-ray in ligand characterization – **pick the winner**

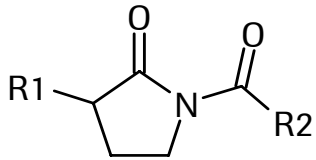


Cyanopyrrolidines
Literature

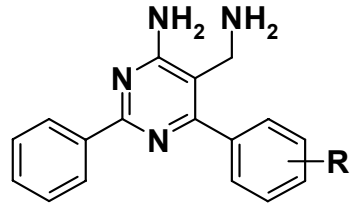
Covalent reversible inhibitor



Benzoquinolizines
HTS



Pyrrolidinones
HTS

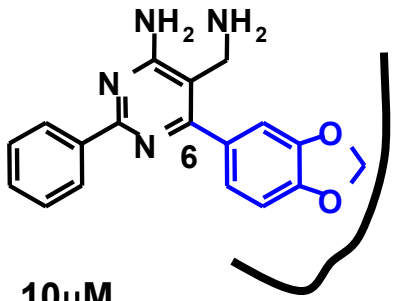


Aminomethyl
pyrimidines
HTS

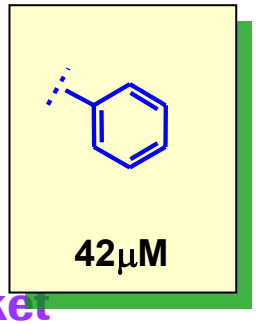
Explore hit by parallel synthesis

Structure to facilitate lead generation

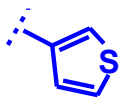
Starting point: hit from HTS - Aminomethylpyrimidines



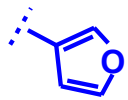
IC_{50} = 10μM



42μM



inact.



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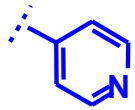

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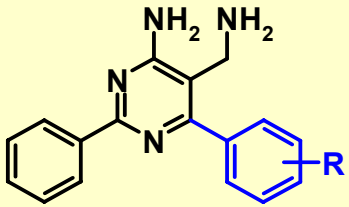
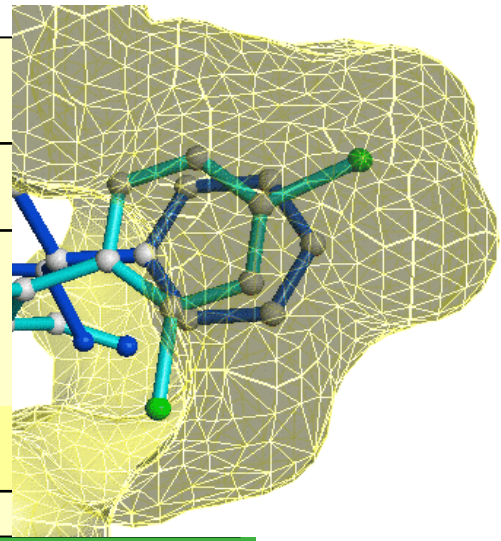
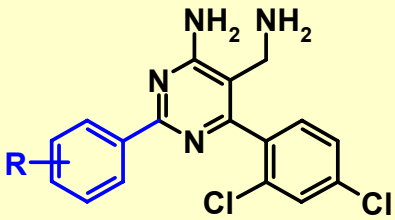
S1-pocket



	IC_{50} (μM)			
R =	Me	Cl	OMe	F
ortho	1.5	2.5	1.5	14
meta	20	31	80	40
para	1	1.4	47	18

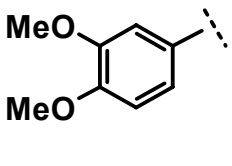


Optimization of activity & molecular properties

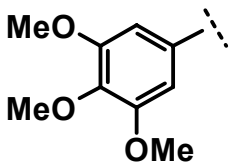


R = H: IC_{50} = 0.01μM

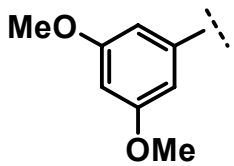
IC_{50} (μM)					
R =	Me	Cl	OMe	F	CF_3
ortho	1.75	-	-	-	-
meta	0.0009	0.24	0.34	0.0002	0.13
para	0.090	0.053	0.10	0.0002	0.18



0.023

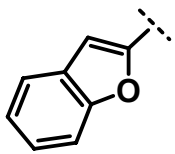


0.0002

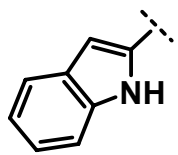


0.0001

IC_{50} (μM):



0.0007



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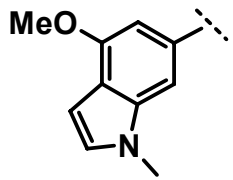

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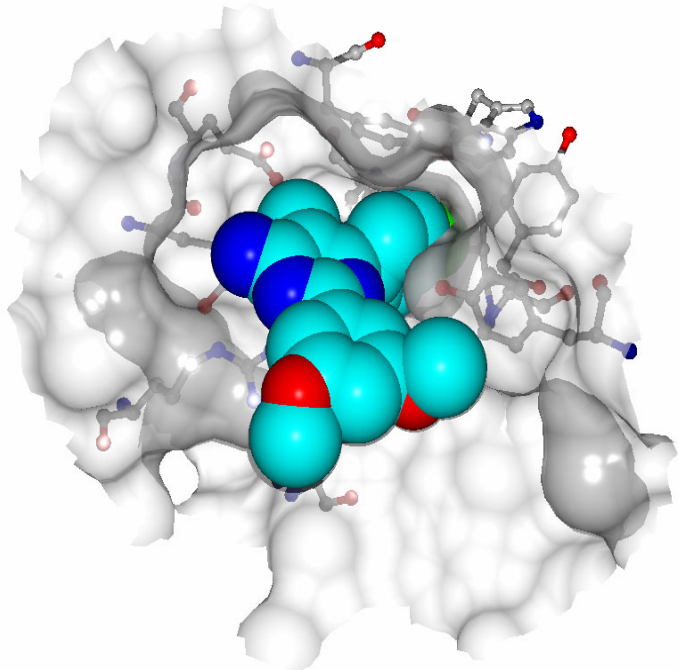
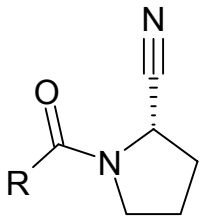
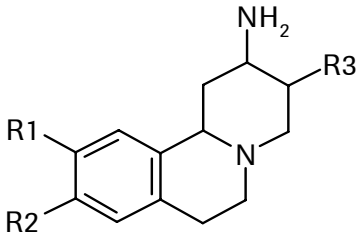


Use of SPR and X-ray in ligand characterization – **pick the winner**

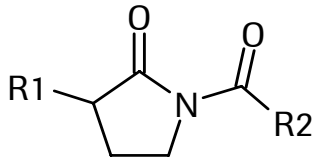


Cyanopyrrolidines
Literature

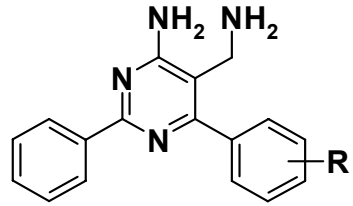


Benzoquinolizines
HTS

Very difficult synthesis, but….

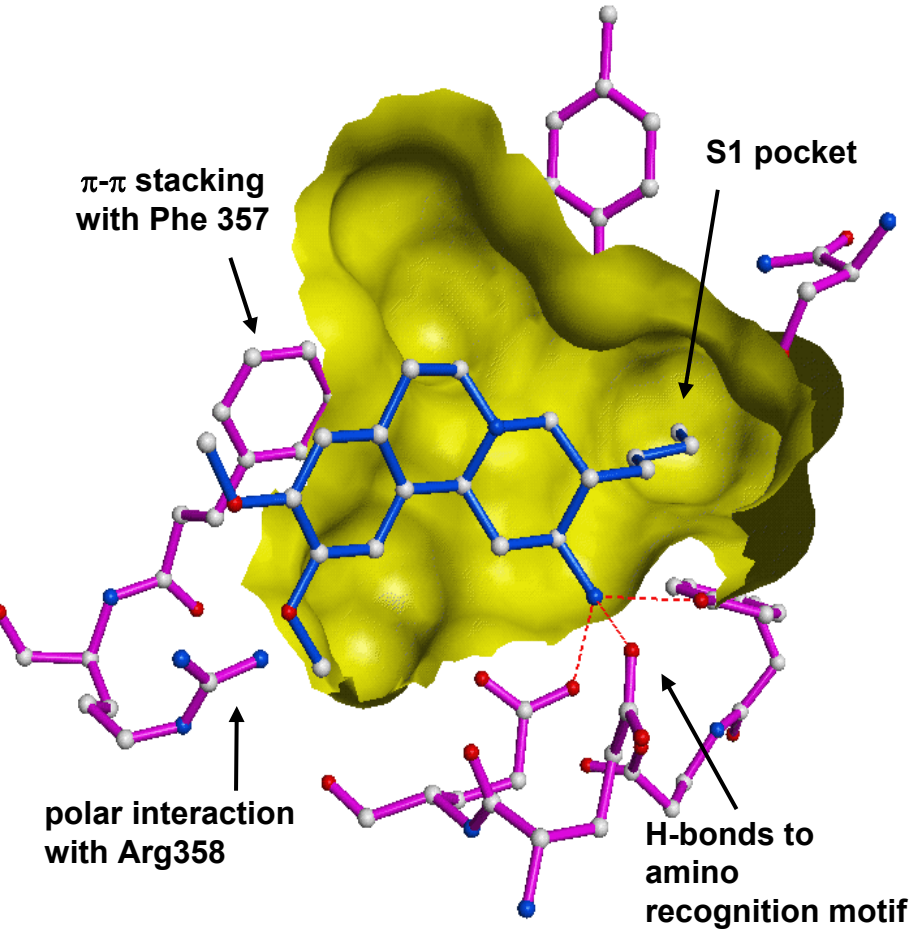


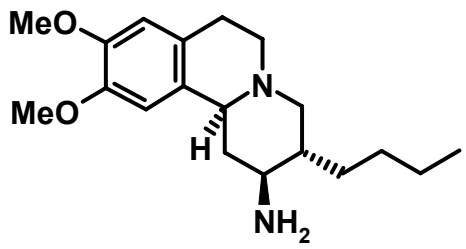
Pyrrolidinones
HTS



Aminopyrimidines
HTS

Binding mode of screening hit in DPP–IV



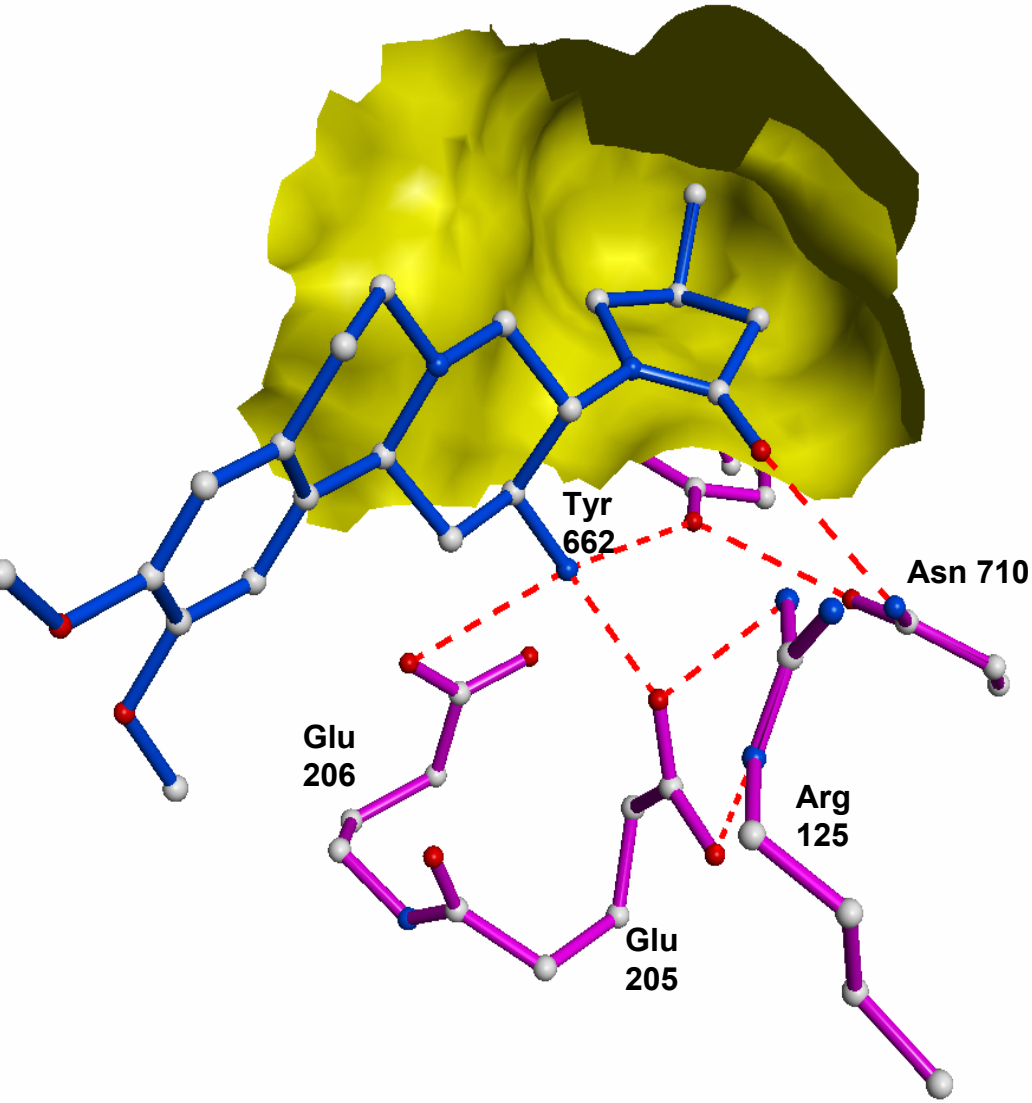


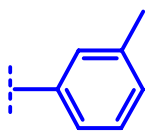
IC_{50} [nM]	500
solubility [mg/L] (LYSA, pH 6.5)	>414
logD (pH 7.4)	0.8
P_e [10^{-6} cm s^{-1}] (PAMPA)	2.5
Cl_{mic} [mL/min/mg protein] (rat; man)	4.7; 0.0
CYPs [μM] (2C9, 2D6, 3A4)	>50
OGTT* ($\Delta_{Glucose}$, 40 min)	**–16%**
Phospholipidosis in silico ($\Delta\Delta G_{am}$; kJ mol^{-1})	–6.47
hERG inhibition (10 μM)	45%

- n-butyl substituent not optimimal for S1 pocket

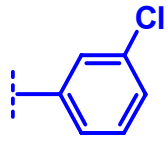
- drug-like profile but lack of affinity with target
- other weak points: hERG; amphiphilicity

Optimization of S1 pocket interaction

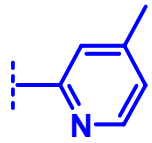




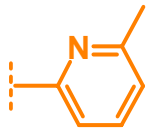
4.2 nM



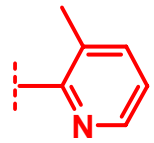
4.6 nM



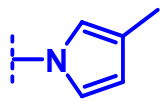
19 nM



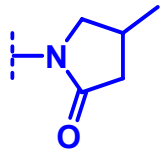
320 nM



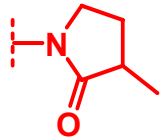
2500 nM



5.4 nM



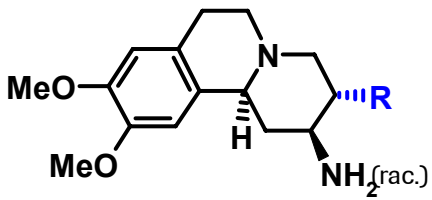
9.3 nM

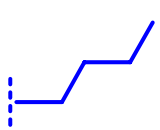


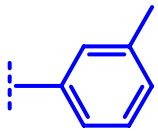
10000 nM

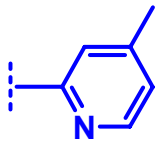
- large affinity gains (40- to 1800-fold) through small lipophilic substituents at optimal positions
- high sensitivity to polarity mismatch and steric repulsion

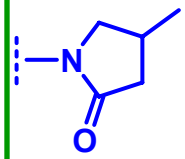
High affinity with diverse MDO profiles







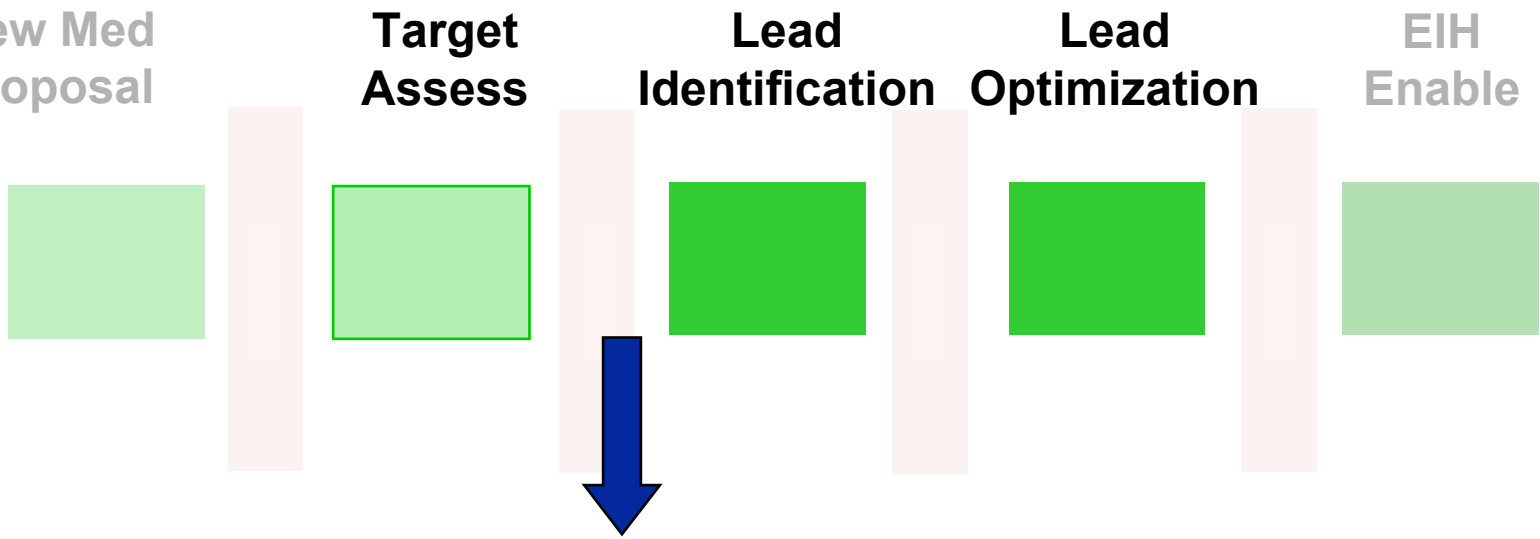




IC_{50} [nM]	500	4.6	19	9.3
logD (pH 7.4)	0.8	**1.3**	0.3	–0.2
P_e (PAMPA) [10^{-6} cm·s^{-1}]	2.5	3.4	2.4	**0.2**
Cl_{mic} (rat; man)	4.7; 0.0	1.3; 3.0	14.4; 0.0	8.0; 0.0
OGTT [$\Delta_{Glucose}$, 40 min]	–16%	**–41%**	**–62%**	**–42%**
PL in silico [$\Delta\Delta G_{am}$; kJ mol^{-1}]	**–6.47**	**–6.41**	**–6.02**	**–5.56**
hERG inh. (10 µM)	**45%**	**25%**	**29%**	**9%**
Rat SDPK Cl [ml/min/kg]	**57**	**87**	**118**	25
Vss [L/kg]	**43**	**42**	11.7	7.9
F [%]	38	56	50	94
$t_{1/2}$ [h]	10.4	6.9	1.4	4.9
brain/plasma	n.d.	**5.8**	**0.7**	**0.25**

- favourable MDO properties of screening hit are preserved
- improved in-vivo activity
- least amphiphilic lactam BZQ has minimal hERG inhibition and brain penetration

Fragment screening by biophysical methods



High Throughput Screening

Public Information

Focused Screening

Fragment based focused Screening

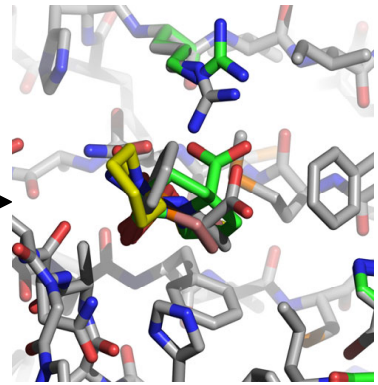
- Screening of X000 compounds, selected to have Mw < 300 etc. (rule of 3).
- Low affinity of interaction requires sensitive assay and chemistry efforts to become a “hit/lead”

Evolution of fragment screening at Roche Basel

„Early activities“, sparse fragment library (300 compounds), NMR and X-ray inhouse data collection

- 1997 Gyrase
  - Boehm et al., J.Med. Chem., 43, 2864 (2000)
- 2000 CyclophilinD
  - Schlatter et al. Acta Cryst. D61, 513 (2005)

…….



About 2003 – Switch from NMR to Biacore to filter hits, synchrotron radiation

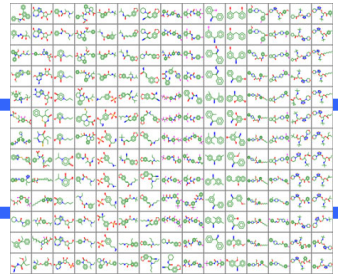
2003 BACE

- Kuglstatter et al., J. Med Chem. submitted

2004 – New fragment library (2200 compounds)

Roche fragment screening – Process today

Chemical diversity:

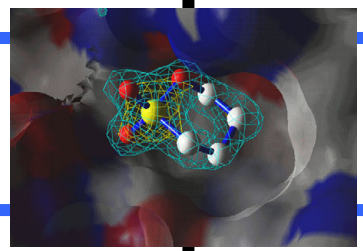


Fragment library

Screening of library by sensitive assay

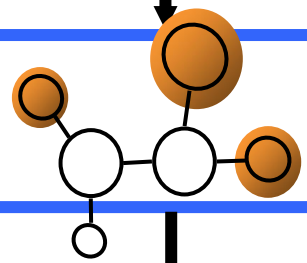
Hit finding and K_D, stoichiometry

Visualization of fragment binding:



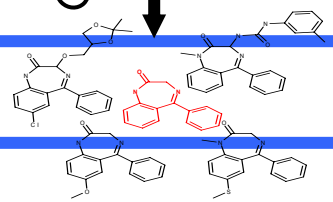
X-ray structure determination

Analysis of structure:



Computational chemistry

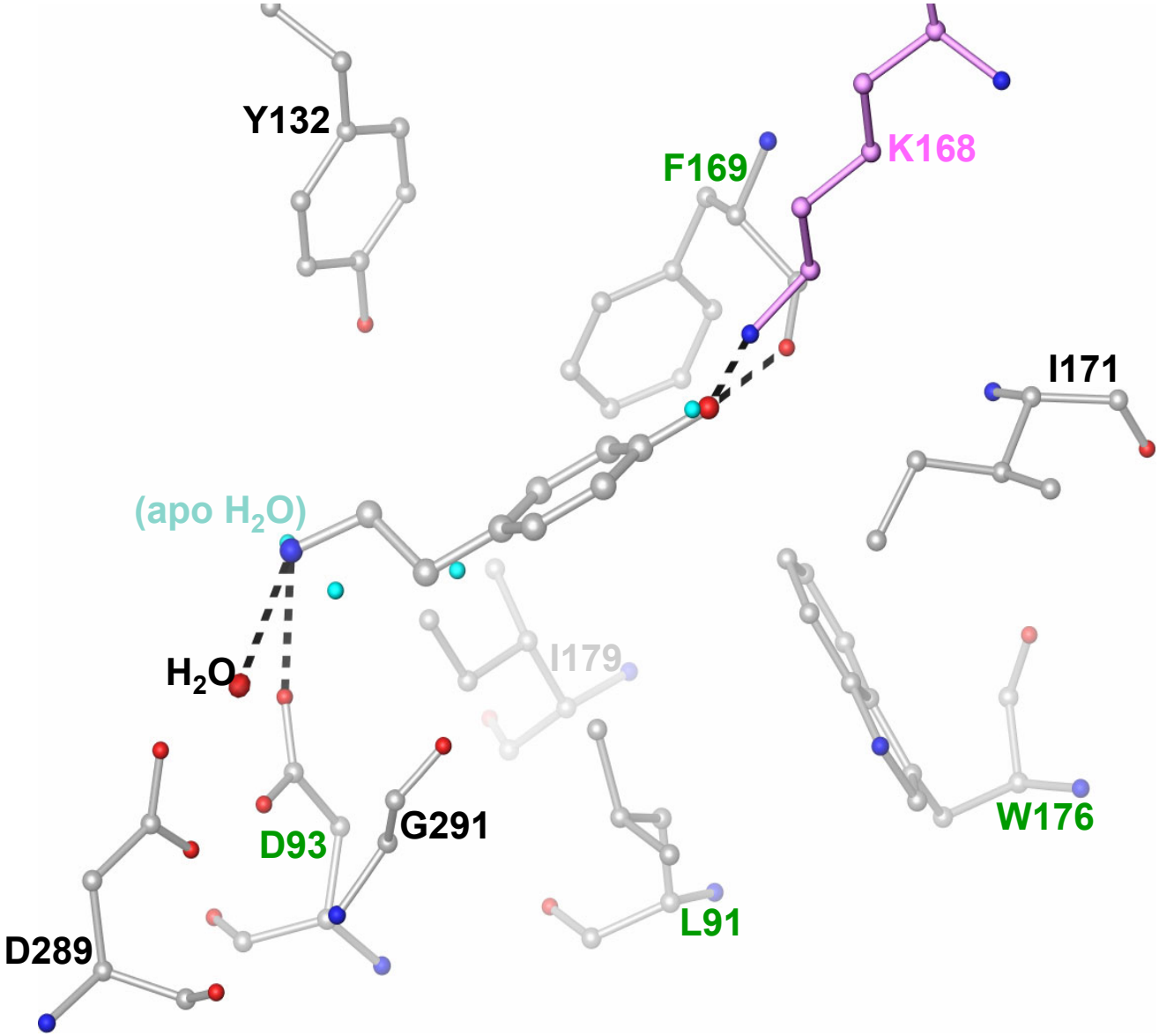
Explore fragments:

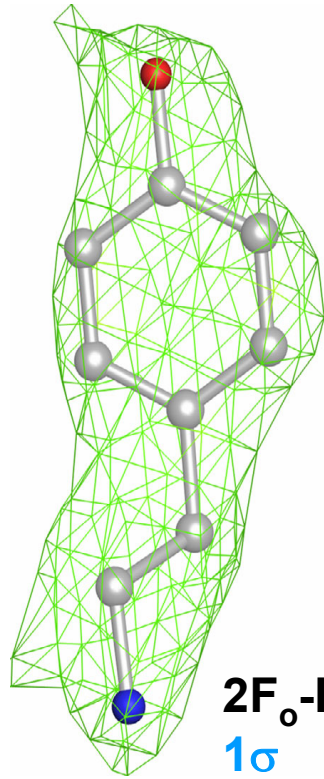


Hit expansion

Focused library

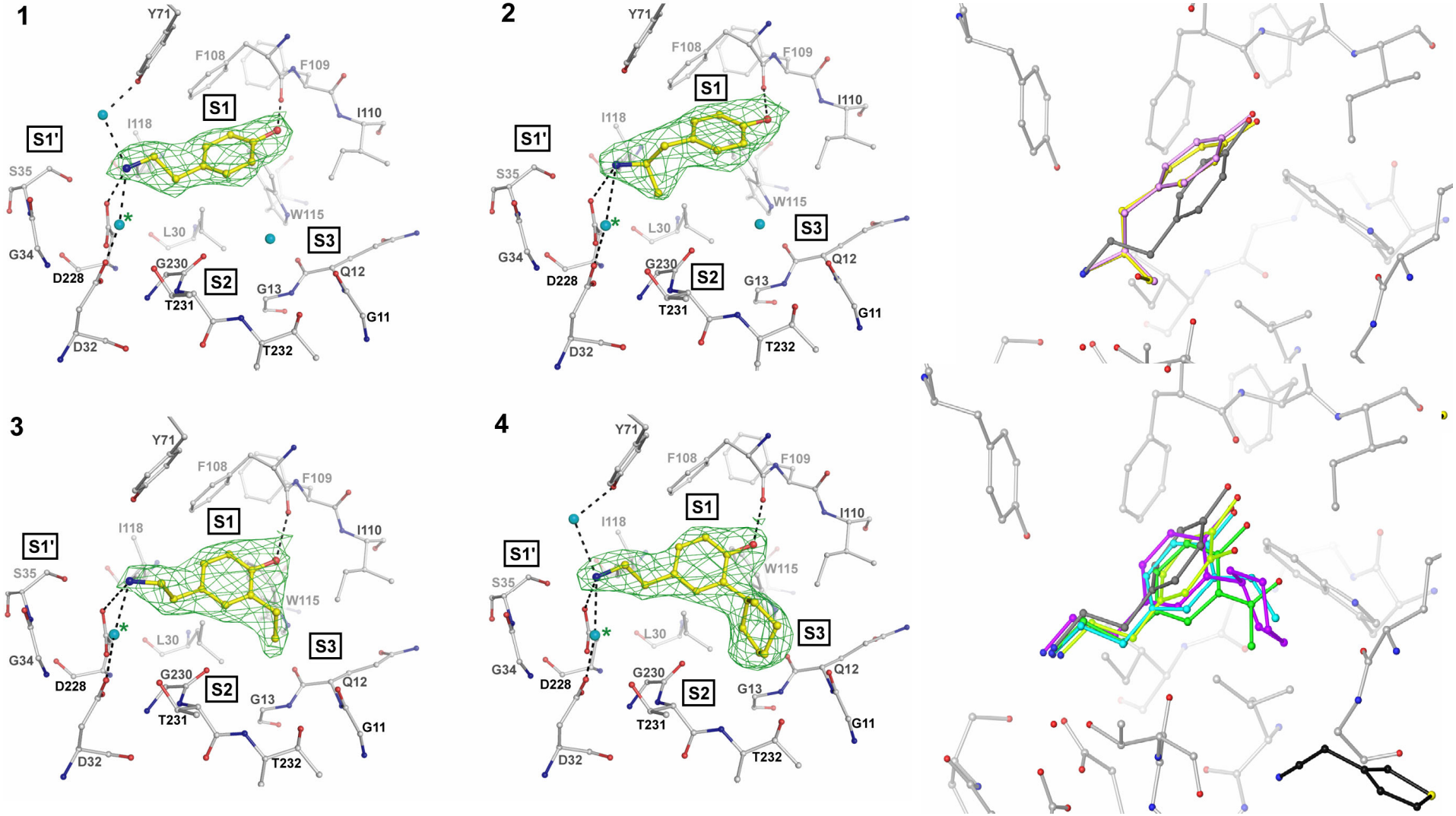
BACE 1 fragment screening hit:
Tyramine in S1 pocket



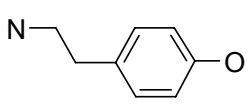


2.3Å,
$K_{D,1:1}$=2mM

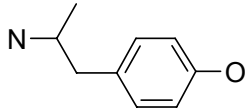
Initial chemistry exploration of the fragment hit



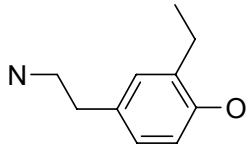
Initial chemistry exploration of the fragment hit



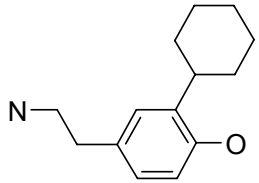
1, K_D = 2000µM
LE= 0.37



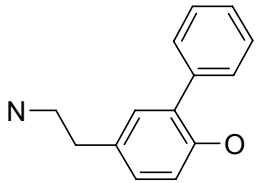
2, K_D = 800µM
LE= 0.38



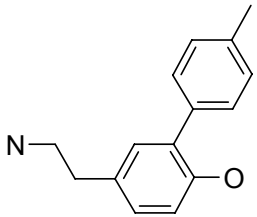
3, K_D = 660µM
LE= 0.36



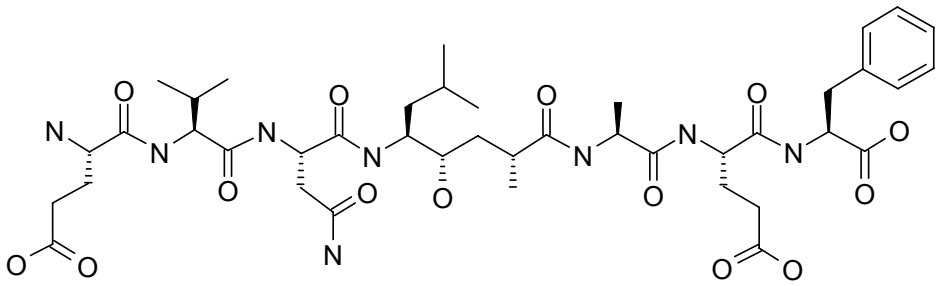
4, K_D = 220µM
LE= 0.31



5, K_D = 350µM
LE= 0.29



6, K_D = 60µM
LE= 0.35



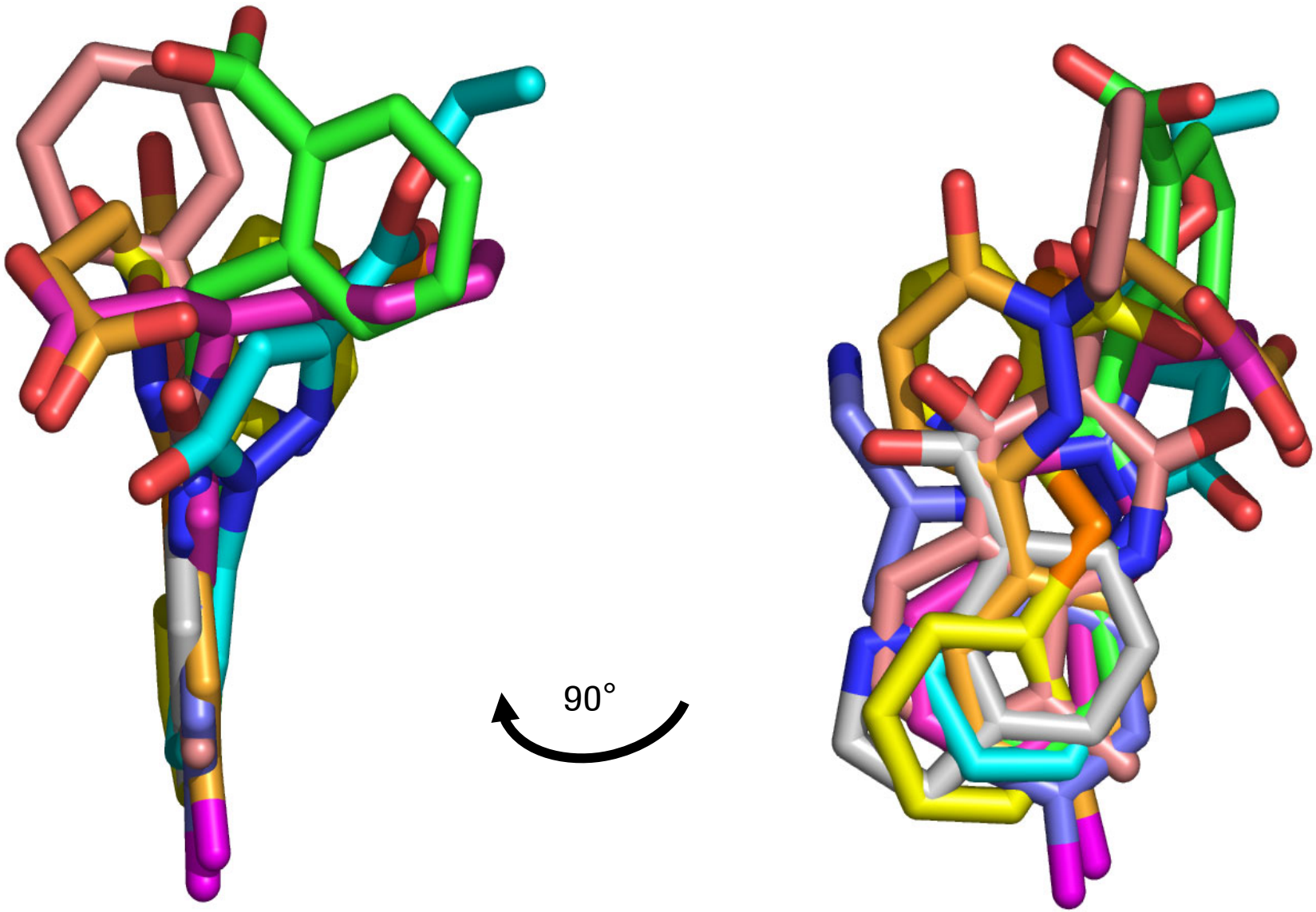
8, K_D = 0.04 µM, LE= 0.16

LE = Ligand efficiency in kcal mol-1 per non-H atom

Fragment Screening: What is it good for?

1. **Learn more about your target – BACE-1**
   - S1 pocket shows best drugability
   - Structures indicate flexibility of active site conformation
   - Water mediated Asp-binding feasible
   - Explore chemical space of binding sites

Overlay of fragments in S1 pocket



Fragment Screening: What is it good for?

1. Learn more about your target – BACE-1

- S1 pocket shows best drugability
- Structures indicate flexibility of active site conformation
- Water mediated Asp-binding feasible

2. Explore new chemical space by

- Replacement of parts of known ligands
- Fragment growth
- Fragment linkage to larger molecules

Fragment Screening: Challenges

Target feasibility

- Protein suitable for biophysical methods (globular proteins, low/no feasibility of membrane proteins)
- “Suitable” protein in mg amounts

Technology prerequisites

- Robust crystallization system (ligand free, soaking or co-crystallization)
- Sensitive, robust assay instruments, access to synchrotron, workflow for HT crystallography and tight interaction with other methods

Mind-set

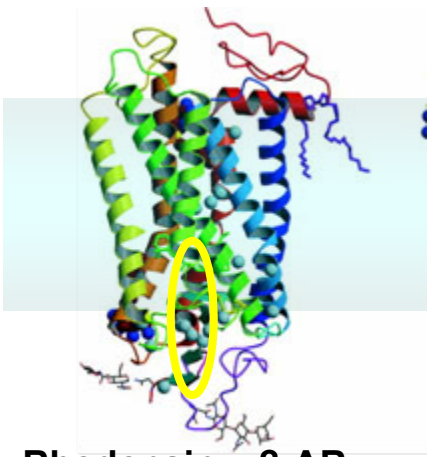
- low affinity compounds as starting points for chemistry

Structural biology today

- **Trends and challenges**
  - Key is early support in projects - protein production, multiple starting points (constructs, expression systems….)
  - More and more structures, but increased complexity for data analysis (Proasis)
  - Complement X-ray with other methods like SPR, AUC, NMR… & for biol. Systems electron microscopy, SAXS, ….
  - Off-target structure based drug (anti)design – P450 enzymes, hERG
  - Still several key drug targets without structural information
    - Multi protein complexes (when domain extraction fails)
    - Complexes of functional protein complexes (to address protein/protein interaction)
    - Membrane proteins

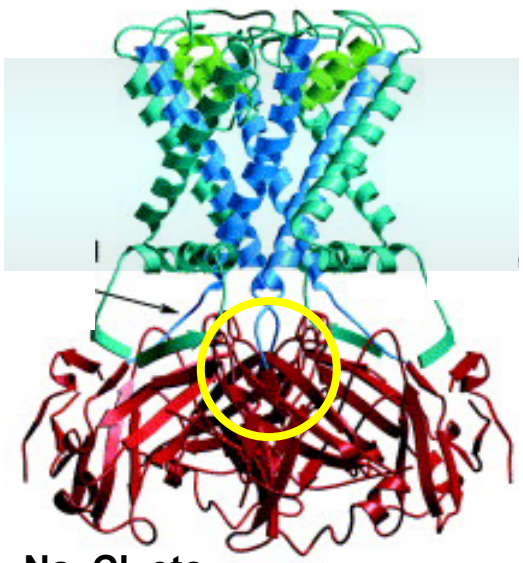
>> 50% drug targets are membrane proteins

GPCRs (45%)



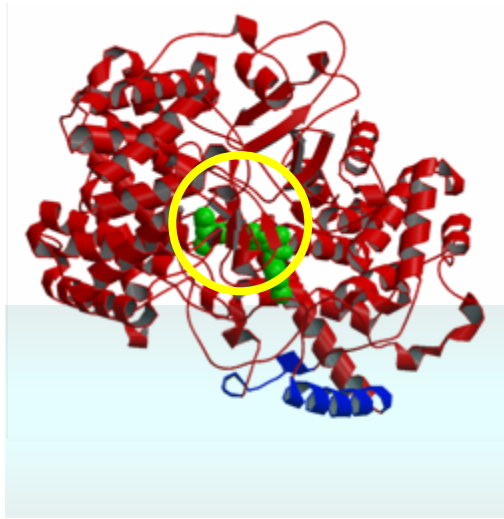
Rhodopsin , β-AR

Ion channels (5%)



Na, Cl, etc.

Enzymes (28%)



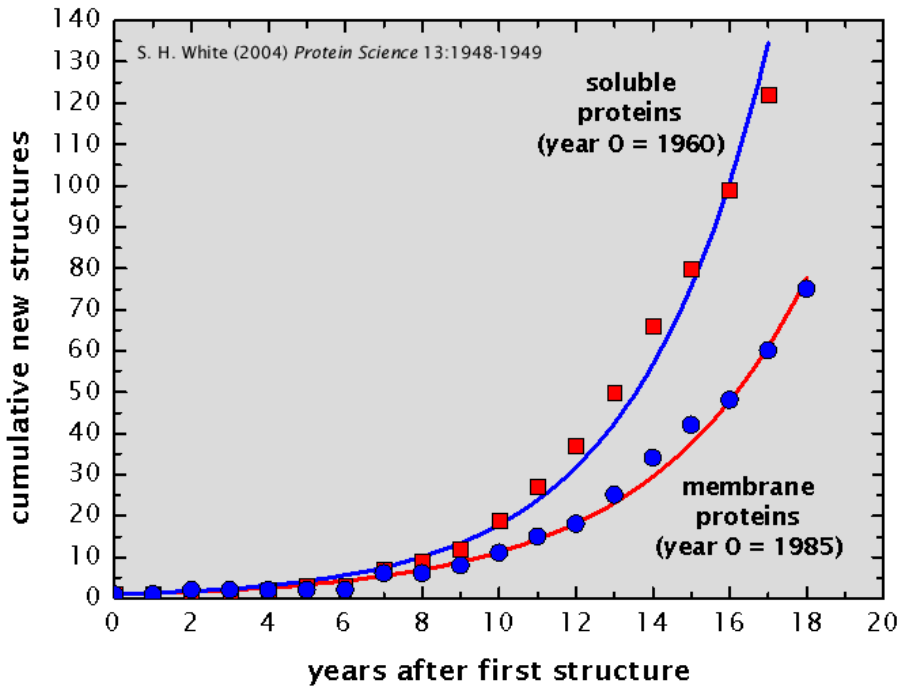
OSC, MaoB, CPT, COX, LTCS4, etc.

Neurotransmitter transporter
Cation transporter (Mg, Zn,..)
Etc.

Lower **Feasibility** **Higher**

> 50% drug targets are membrane proteins

….but there is progress!



- Structures of membrane bound enzymes are challenging, but possible

Roche Basel structures:

OSC, Thoma et al., Nature 432 (2004), MAOB, unpublished (2003),
CPT, Rufer et al. Structure 14 (2006)

GPCR's are not in line with industry requirements for project support, but there is progress

First GPCR structure with protein expressed in Sf9 cells!

doi:10.1038/nature06325 nature

ARTICLES

Crystal structure of the human β_2 adrenergic G-protein-coupled receptor

Søren G. F. Rasmussen[1]*, Hee-Jung Choi[1,2]*, Daniel M. Rosenbaum[1]*, Tong Sun Kobilka[1], Foon Sun Thian[1], Patricia C. Edwards[3], Manfred Burghammer[4], Venkata R. P. Ratnala[1], Ruslan Sanishvili[5], Robert F. Fischetti[5], Gebhard F. X. Schertler[3], William I. Weis[1,2] & Brian K. Kobilka[1]

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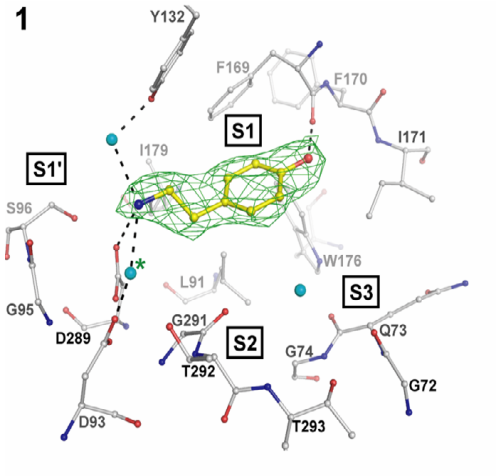

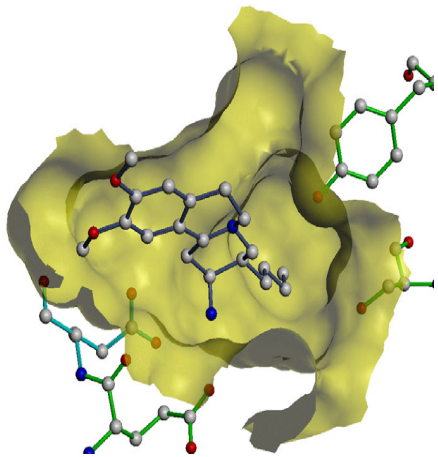

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Bernd Löffler
Jens-Uwe Peters
Thomas Lübbers
Patricio Mattei
Robert Narquizian
Luca Gobbi
Pierre Wyss
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Synchrotron staff at PSI-SLS

…… many more colleagues at Roche Basel Discovery!

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