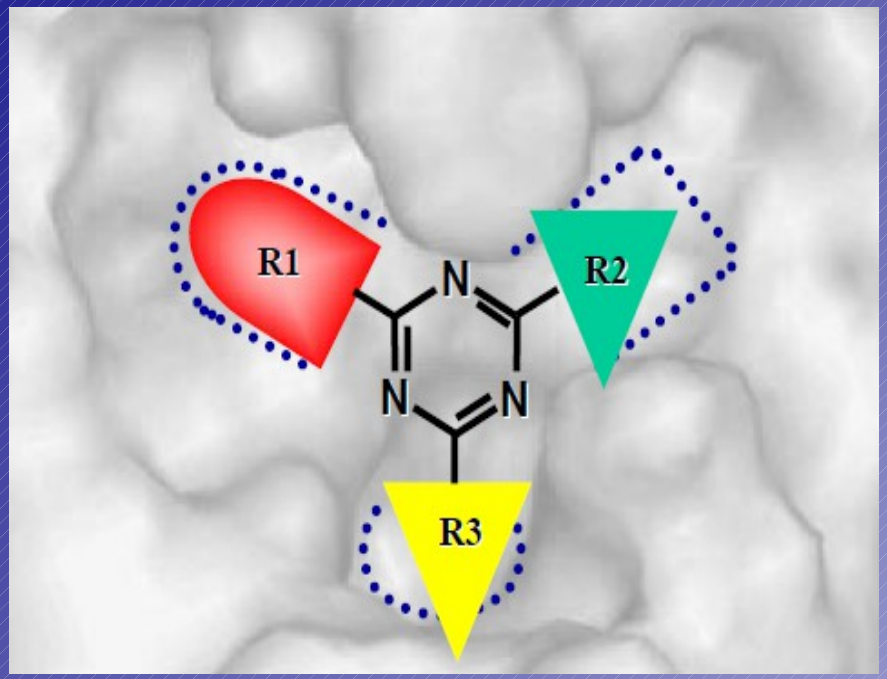
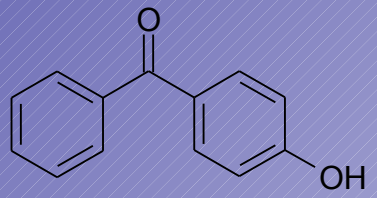
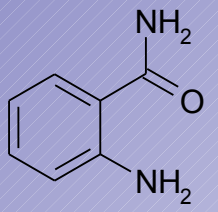
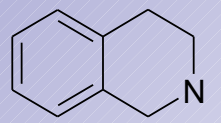
Fragment based drug design









Fragment based drug design

- Nový směr zaváděný v posledních 13 letech v předních farmaceutických společnostech nebo specializovaných high-tech. Firmách
- Využívá nejnovějších vědeckých poznatků a technologií z oblastí genetiky, molekulární biologie, proteinové krystalografie a/nebo NMR spektroskopie, bio-informatiky a počítačové chemie (modelování, docking)

Traditional design

- Traditional recipes and random discoveries
- Combinatorial chemistry and natural drugs
- Screening (ultra high-throuput screening, HTS), "hit" → "lead compound"
- Compounds library

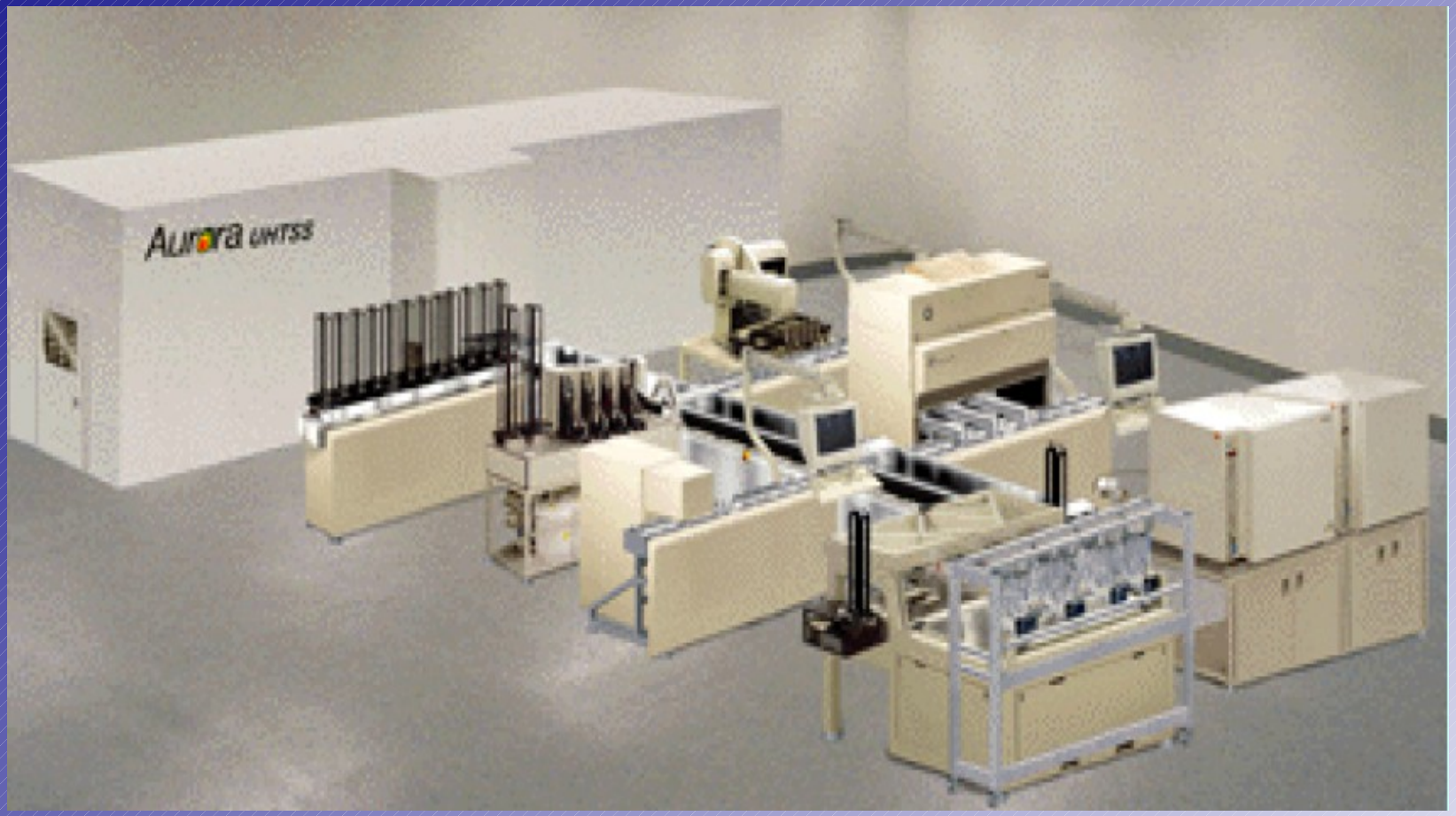
Typical drug properties

- Molecular mass 350-450
- Protein affinity, inhibition IC50 50 nM
- Five - rule
- Defined number of bond rotations
- Optimisation usually by increasing molecular mass

Five - rule

- Ch.A.Lipinski, 1997. Oral drugs
- Not more than 5 hydrogen donors (OH, NH)
- Not more than 10 (2x5) HB acceptors (N a O atomy)
- Molecular mass up to 500
- ClogP less than 5

High-Throughput Screening, HTS

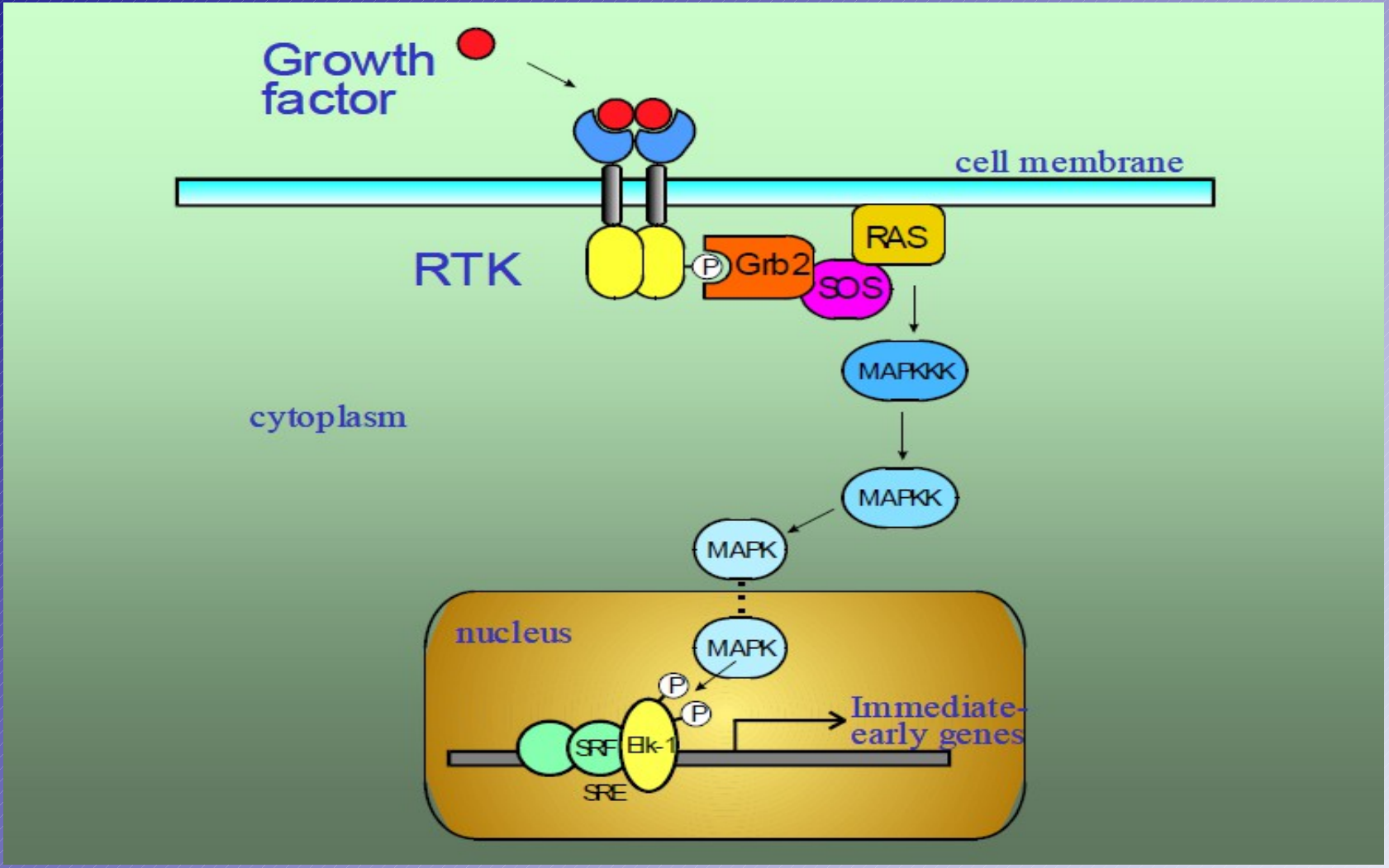


Up to one million compounds has to be scanned to find activity

Limity HTS

- Automated, costly method
- Less productive - high death ratio of hits
- Big libraries of chemical compounds – small drug-like space
- Did not reduce cost NCE/(milion $)
- For 5000 compounds only 5 clinical trials
- Only 5 - 20 % on the market
- Cost $800 mil.

Targets


Growth factor
cell membrane
RTK
P
Grb2
SOS
RAS
MAPKKK
cytoplasm
MAPKK
MAPK
nucleus
MAPK
P
P
SRF
Elk-1
SRE
Immediate-early genes

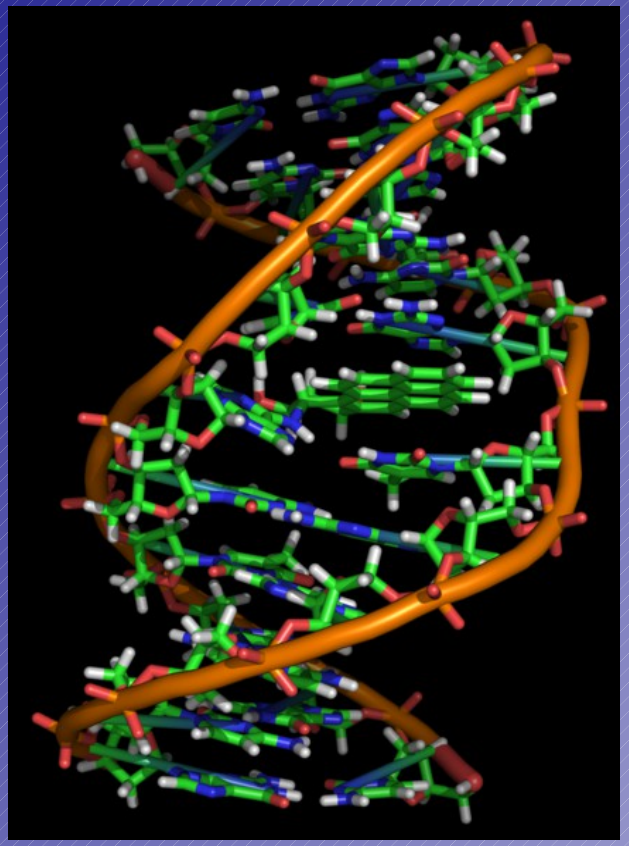
Targets

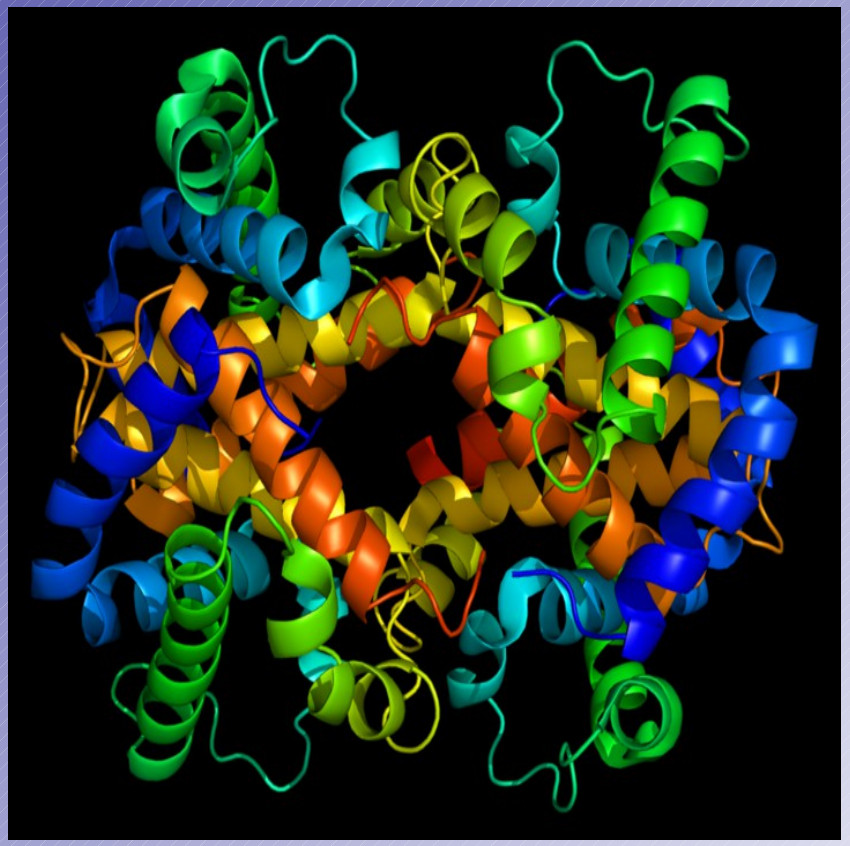
- Week binary complexes leads to stable multiprotein complexes
- Many targets can be found between multiprotein complexes for cell regulation and signalisation

Genomics

- „Human genome project“ human DNA.
- Leads to more protein targets
- Structural genomics - gene function determined from structure

Genomics

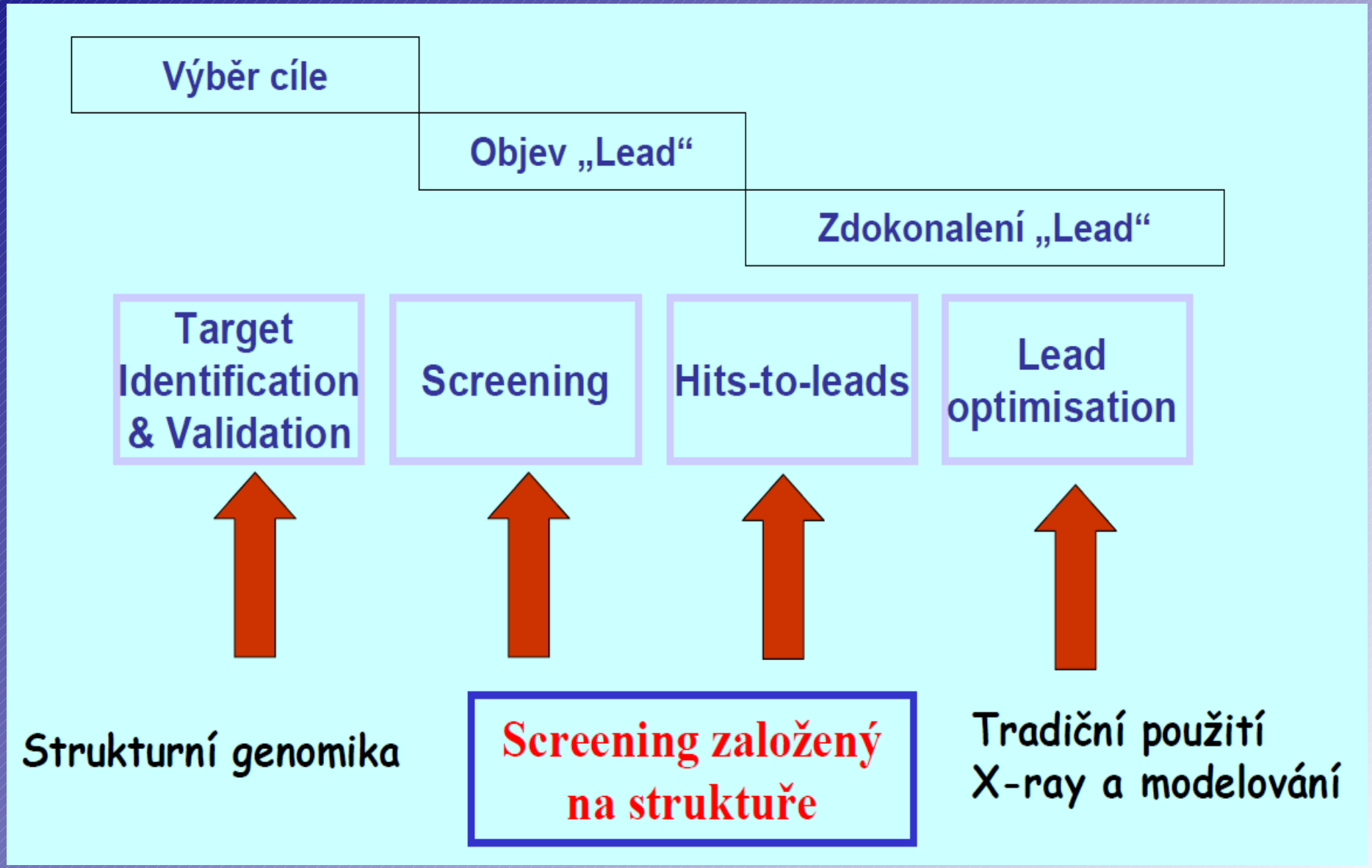




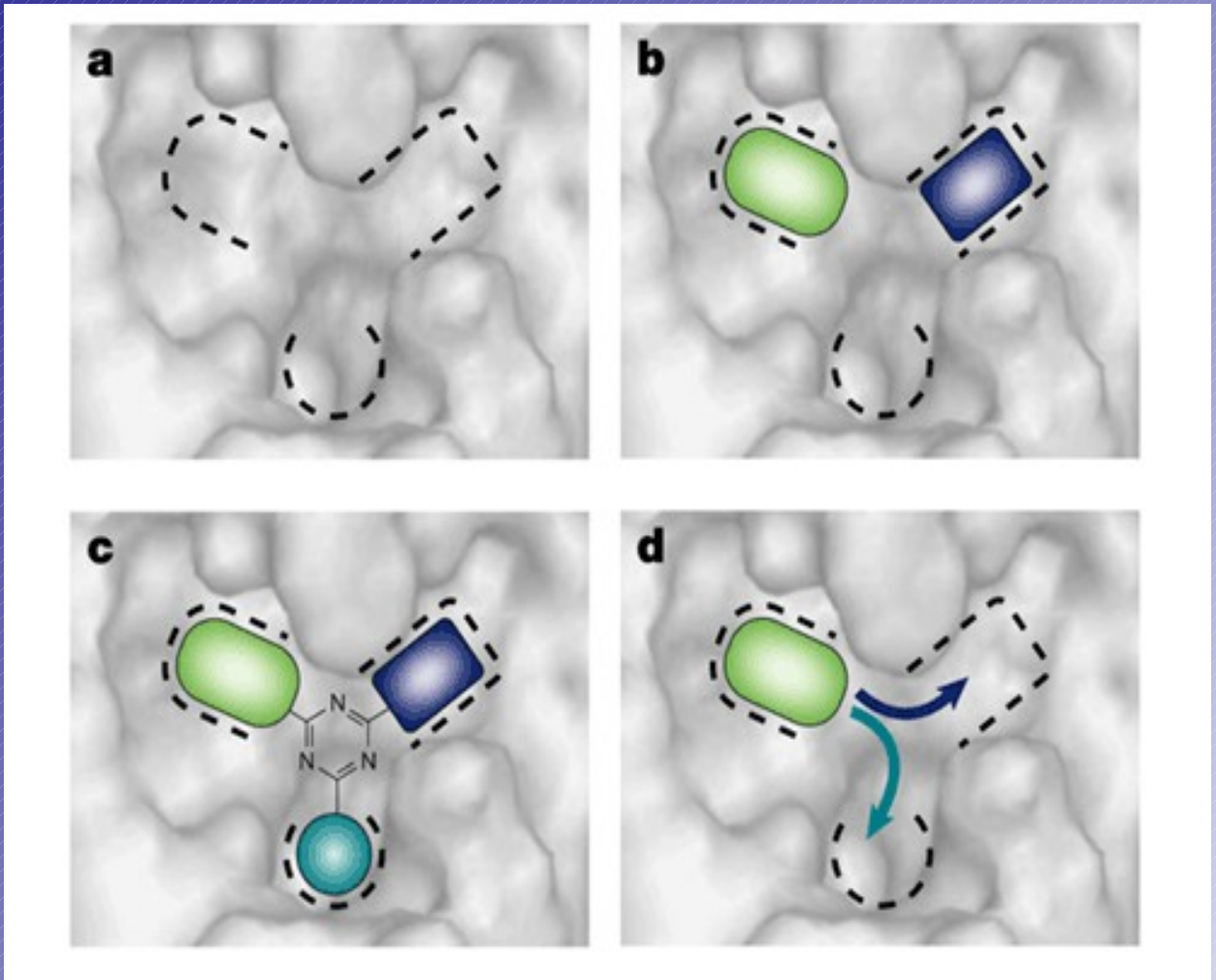
Target identification

- Functional genomics - huge number of potential protein targets
- Structural genomics searches relationship between sequence structure and biological function
- PDB is growing

Structural biology and drug discovery



Fragment based drug design



Fragment definition

- Men.islou.enina (Pravidlo 3. Men. ne. 300 Da. Typical 150-250. ClogP 3, not more than 3 HB donors and acceptors
- Medium affinity (100ĘM-1mM)
- HTS. Smaller library (Graffinity library - 20 000 fragments).
- Biophysical screening: NMR, X-ray, MS
- Binding sites

FBDD - advantages

- Smaller library covers bigger chemical space
- 100 fragmens 1 000 000 combinations
- Fragments can be identified by protein crystallography even if it has no biological activity
- Fragments can be found in silico - docking

Screening

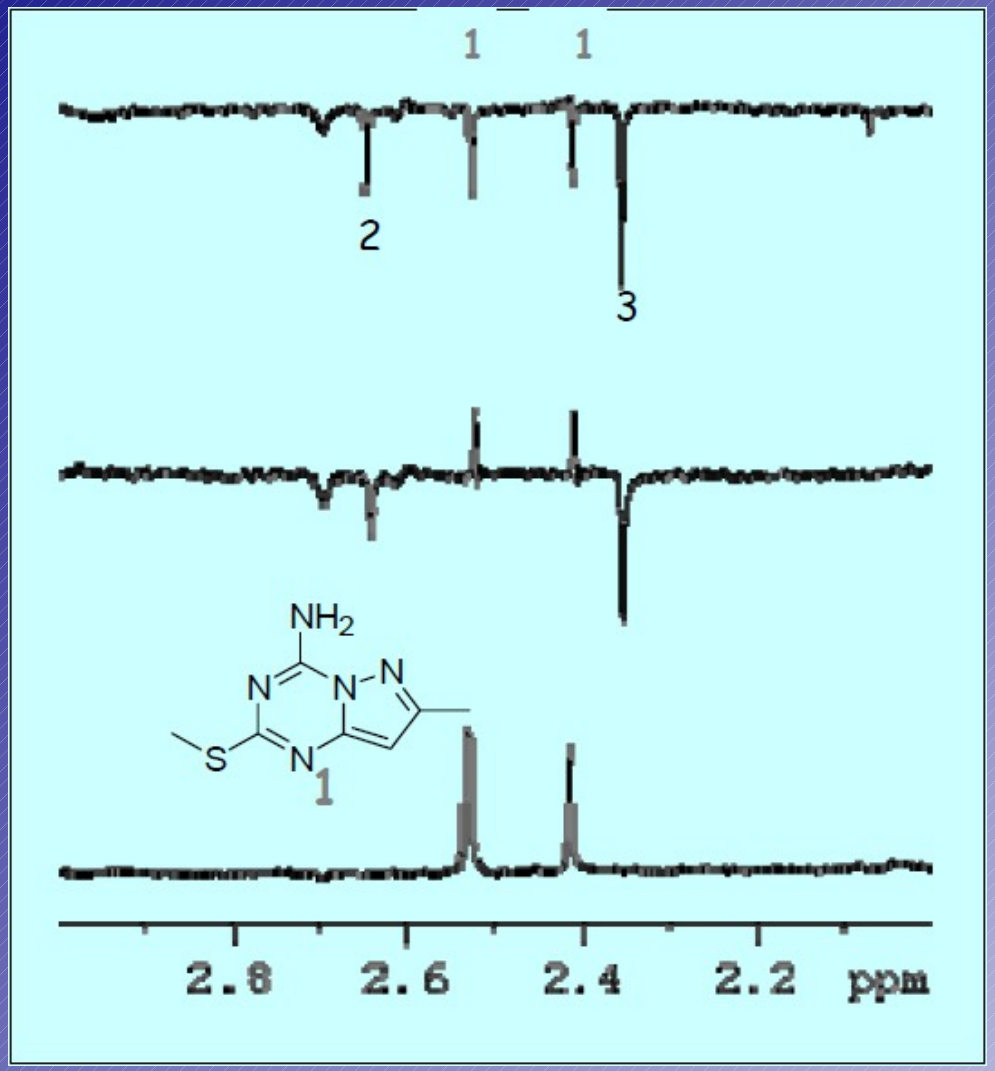
- Screening fragments against protein targets
- Following techniques are used

- rtg. crystallography
- NMR spektroskopy (Water LOGSY)
- Isothermal titration calorimetry
- Surface plasmon resonance
- Non covalent mass spectrometry

NMR Screening

- NMR most productive
- SAR using NMR, (SAR = structure activity relationship)
- Subjective interpretation
- Not automated
- Abbott laboratories, Novartis, Vertex Pharmaceuticals, Hoffmann-La Roche, Triad Therapeutics

NMR screening



cocktail of three fragments
0.5 mM

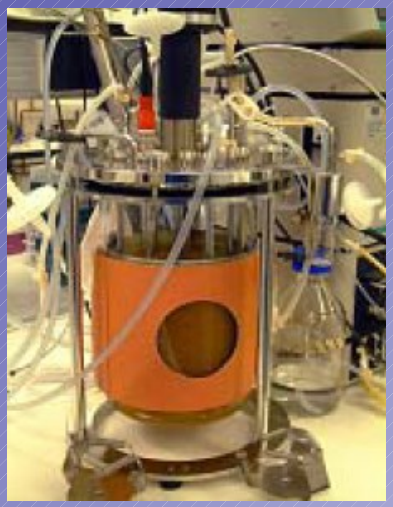
+ enzyme 20 mM

Only fragment 1 + enzyme (diference)

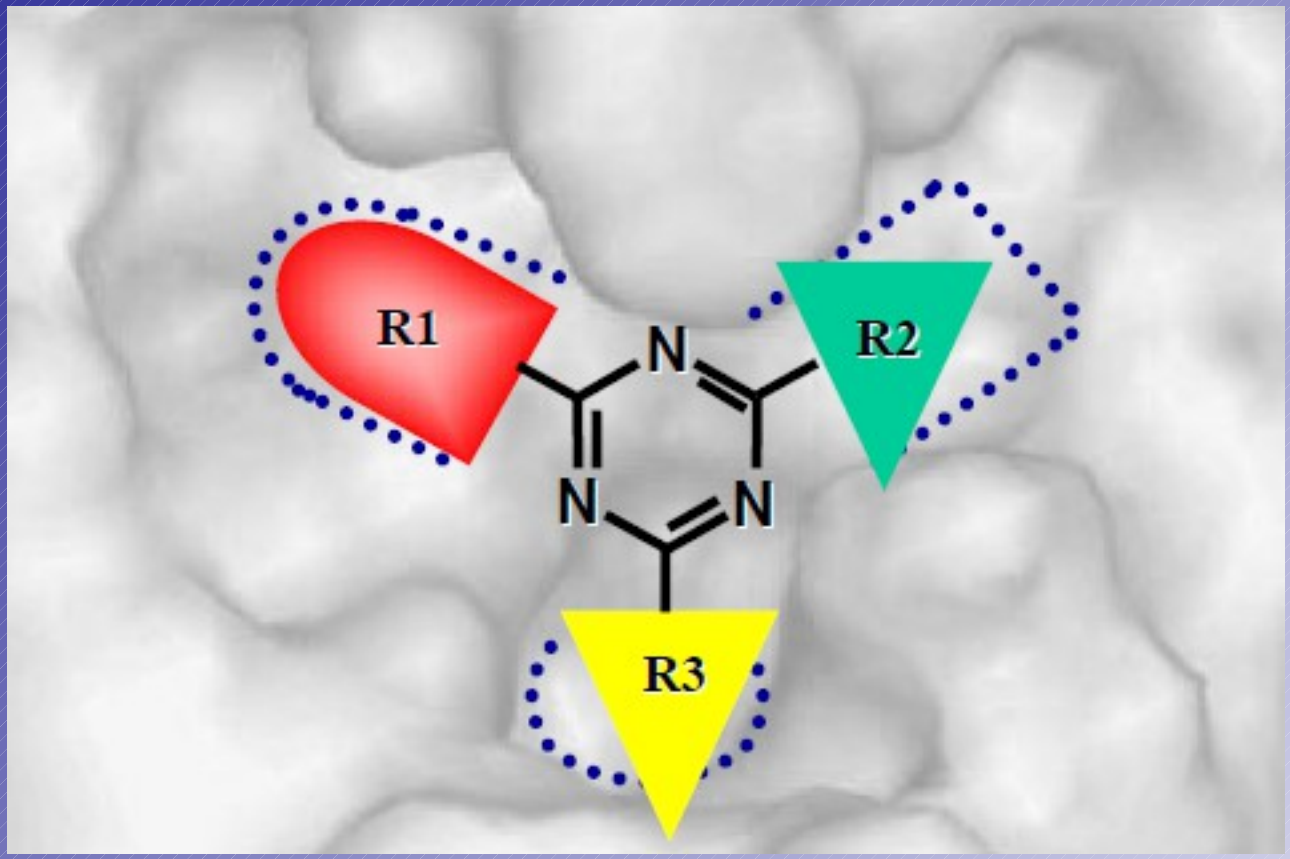
X-ray screening

- Best for protein - ligand interaction study
- Highly automated
- Fragment visualisation - possible in silico optimization
- Rejection of non specific affinity
- Synchrotron is required
- Astex Therapeutics (Technology), Abbott Laboratories, Structural GenomiX (SD CA)

Astex facilities



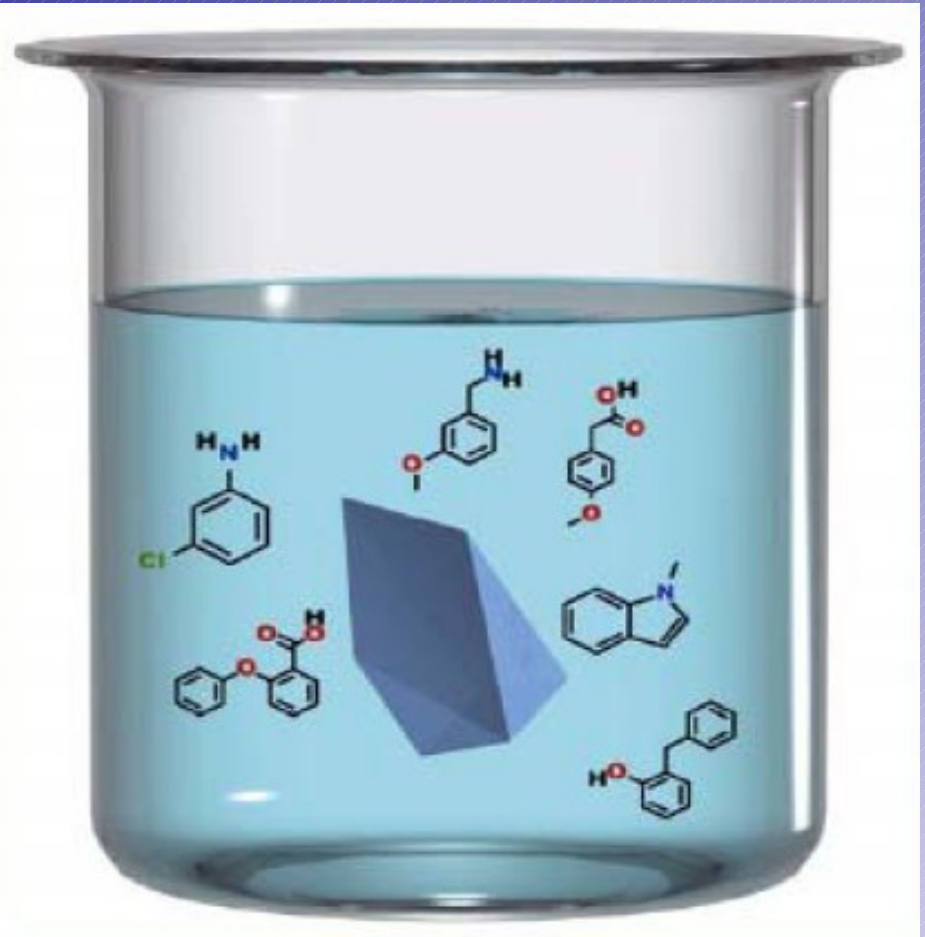
Strukturní screening fragmentů



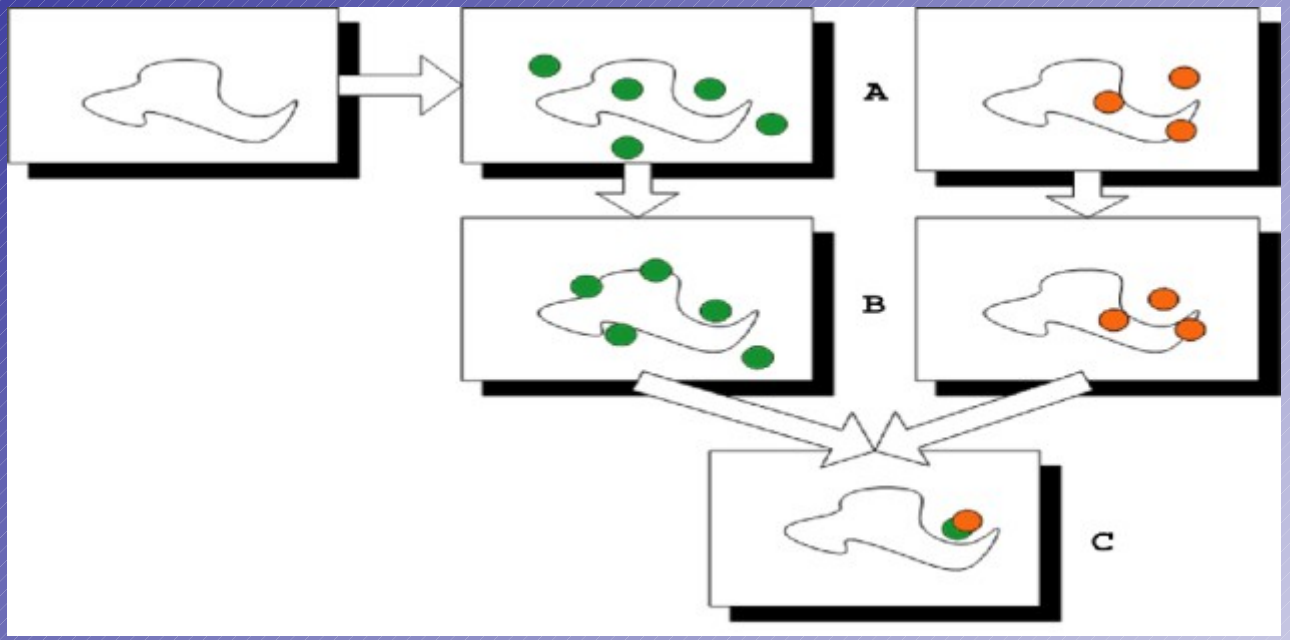
Structure based fragment screening

- 100R1x100R2x100R3 = 1,000,000 cmpds
- Higher hit rate
- Detect unique structures (mM)
- Precise structural data (validate/prioritise the hits)
- Rapid structure based optimisation
- Room to optimise
- High re-use -protein family specific scaffolds

X-ray screening

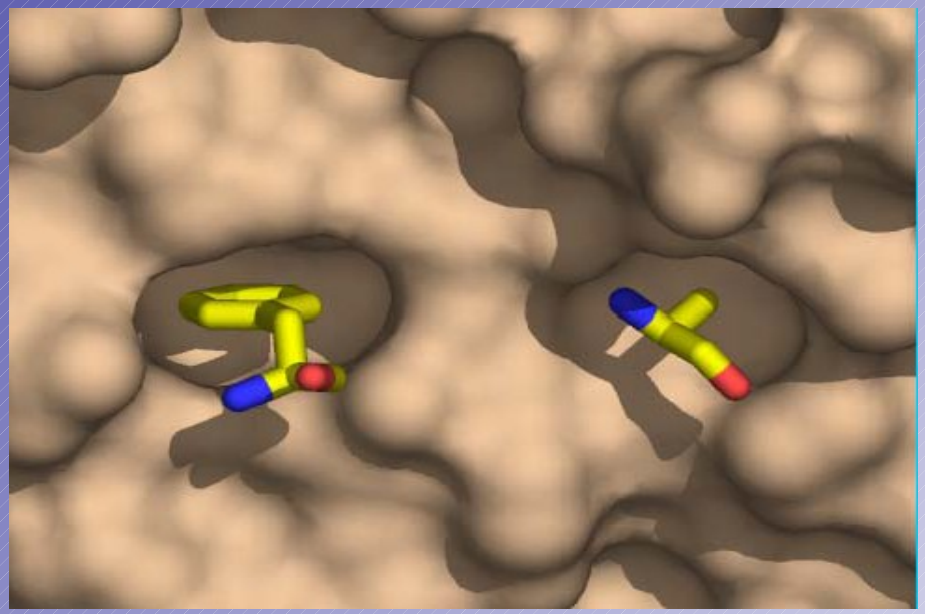


X-ray screening



X-ray screening

- 4-10 compounds 25-50mM concentration Crystal is submerged and it chose best fragment



Identification in electron density maps

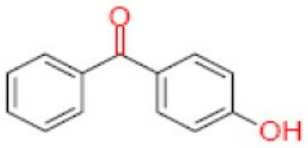
Cdk2 Hit Generation

Drug Fragment Set + targeted library (~600 cmpds)

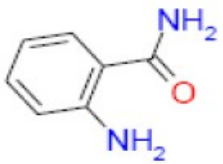
>25 confirmed hits

Structurally diverse - would not detect with HTS

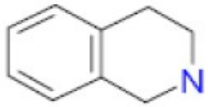
AT2202 29%

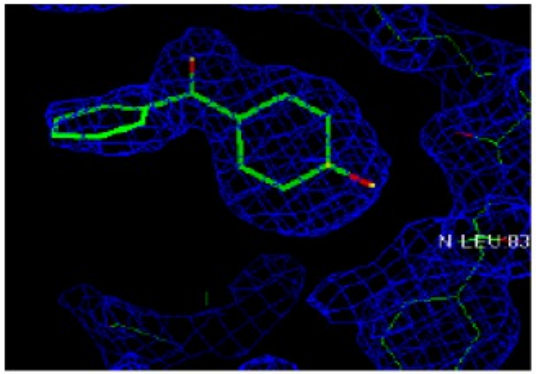


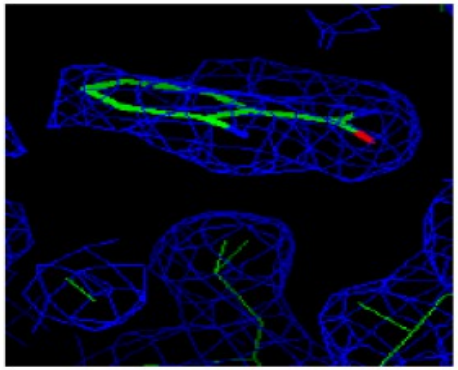
AT2282 28%

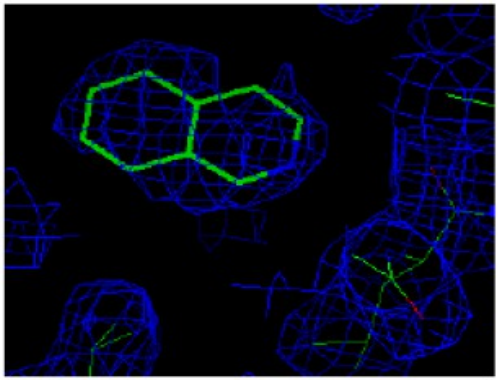


AT402 88%









(% inhibitions at 1mM)

astex TECHNOLOGY

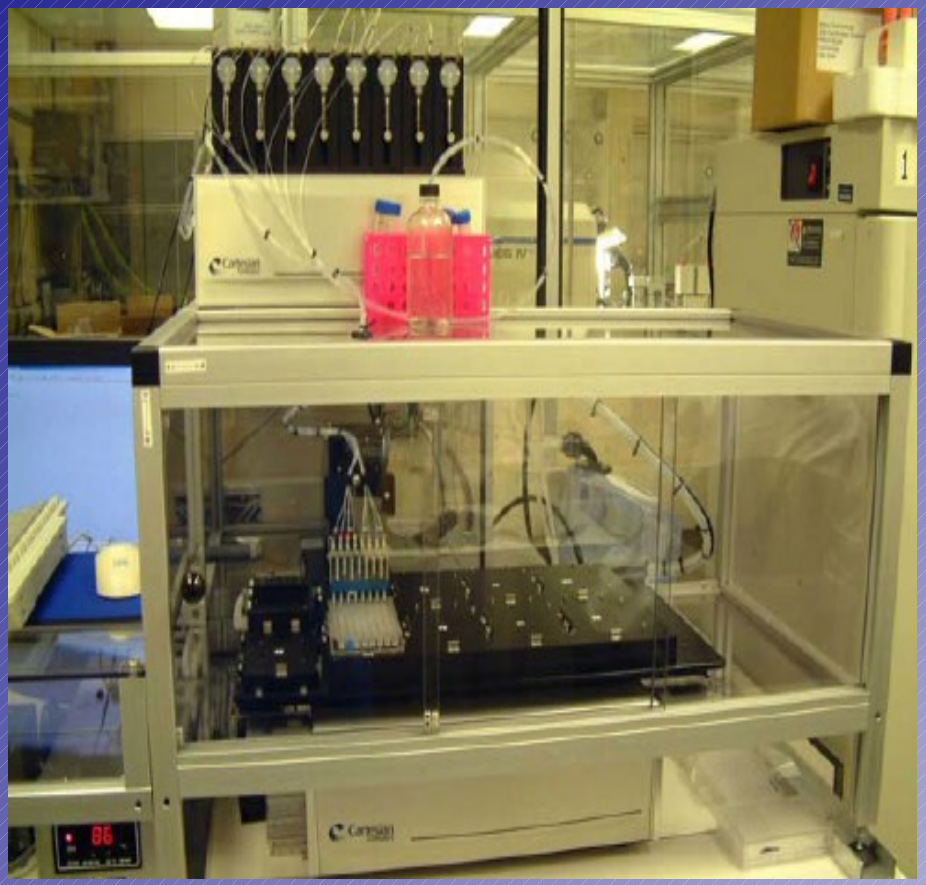
X-ray process definition

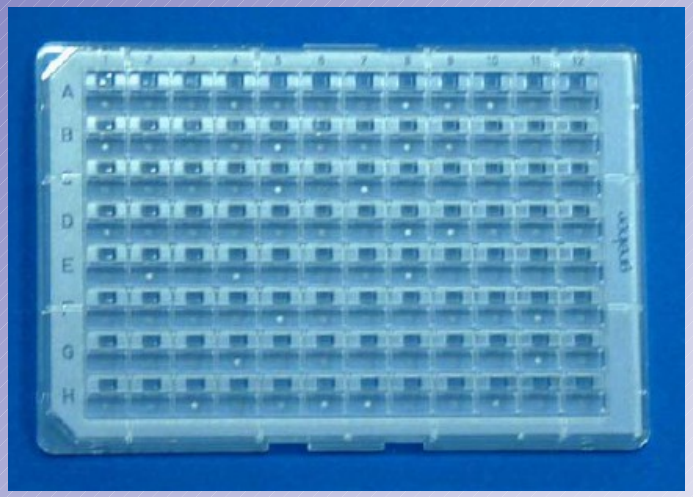
- 10-100 mg purified protein proteinových, 100
- Every crystal soaksz 5-10 fragments.
- Crystal is mounted and measured (flash cooling, cryo-crystallography)

X-ray process definition

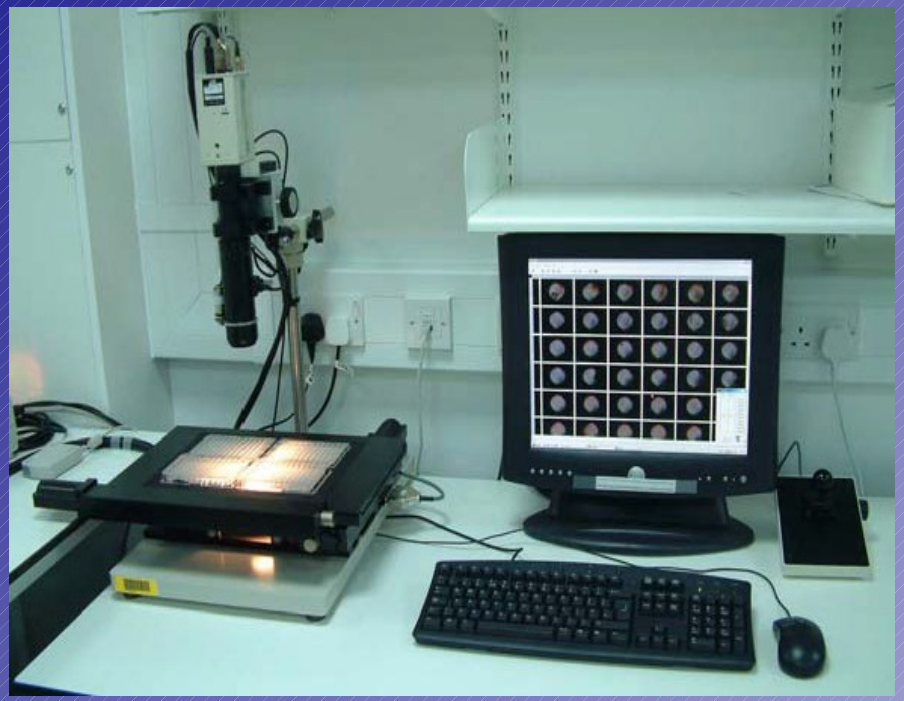
- Automated high throughput screening on synchrotron (in Argonne National Lab. X-ray screen 1000 compounds 24-48 h)
- Reasonable speed also on new diffractometersFragment i (54 crystals/80h on Rigaku FR-E Superbright).
- Fragment is shown on difference map

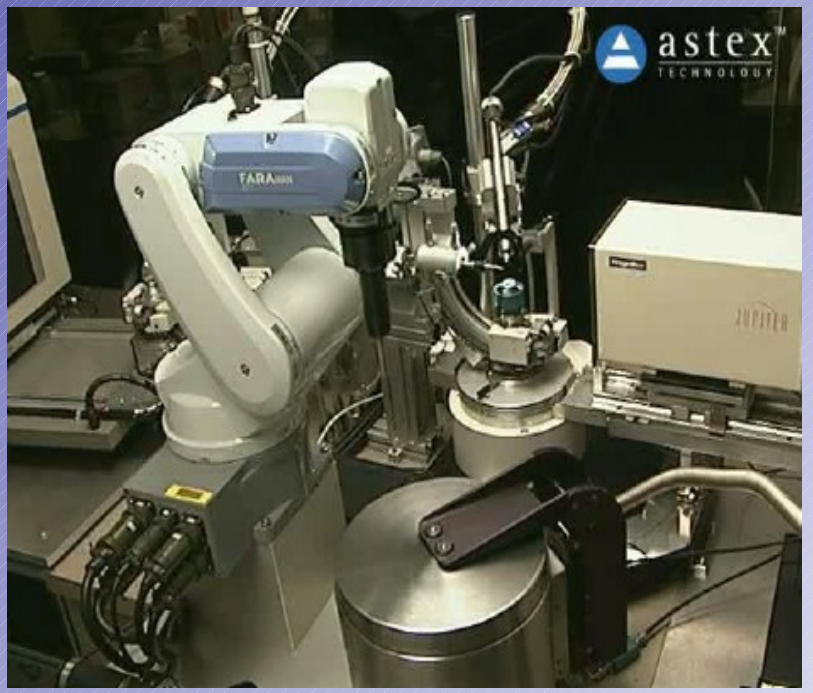
Crystallization protocol (PixSys)





Astex facilities – X-ray analysis

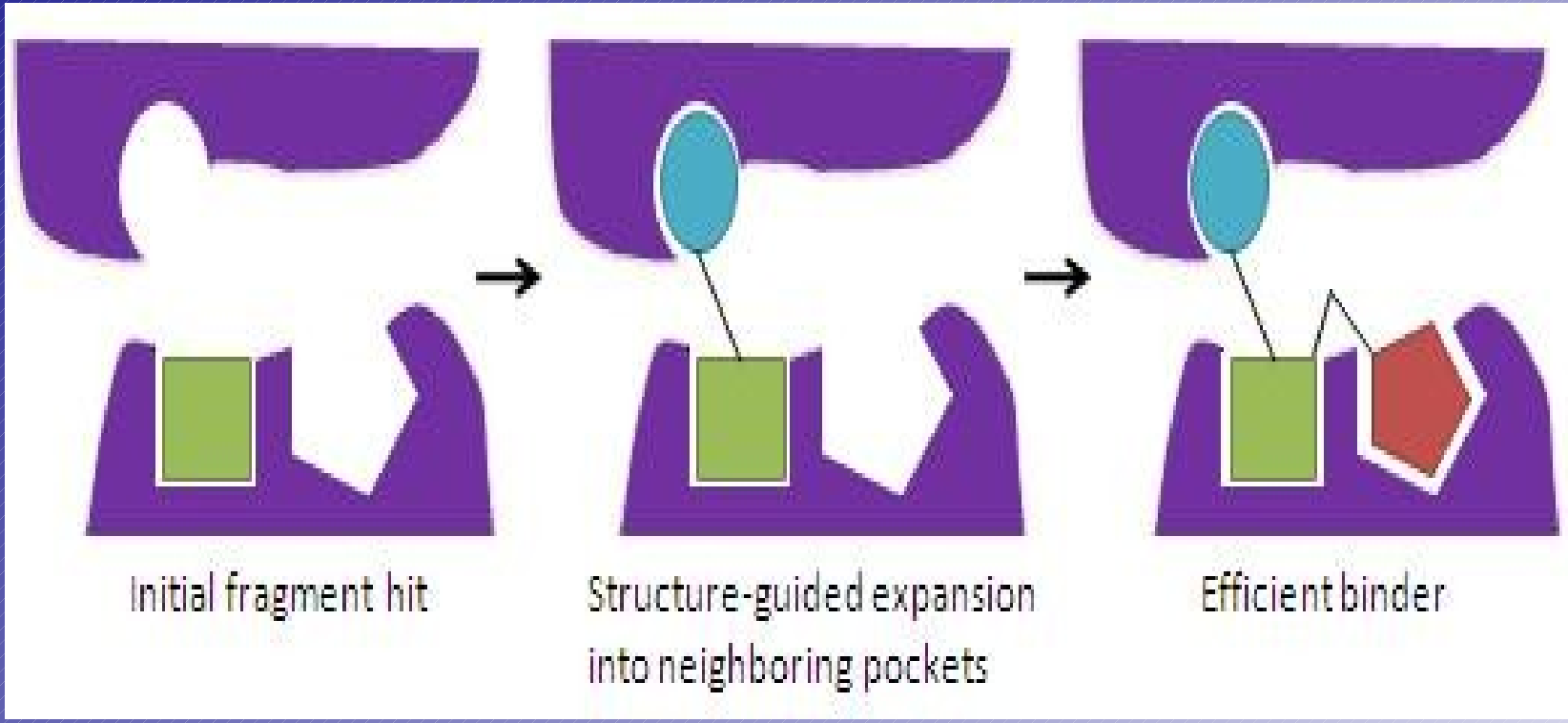




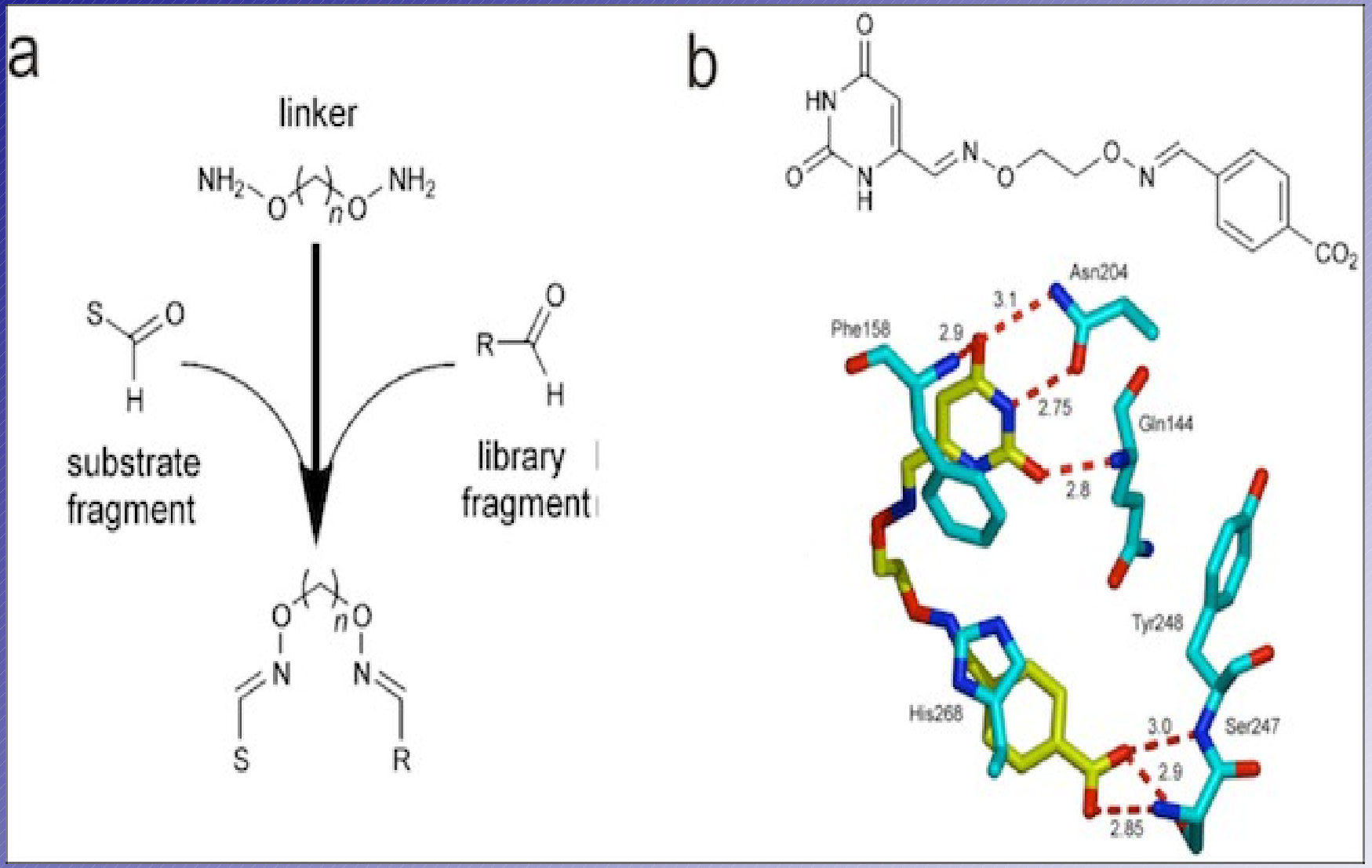
Fragment connection and optimization

- Two fragments in two places and connecting molecule (spacer)
- Self-assembly – template method, Protein catalyses synthesis its own inhibitor
- Binding sites mapping SAR (probe analogy GOLD).
- Connecting fragments increase entropy
- The potency is increased by 3-5 orders of magnitude Fragment→lead.

Fragment connection and optimization



Fragment connection and optimization



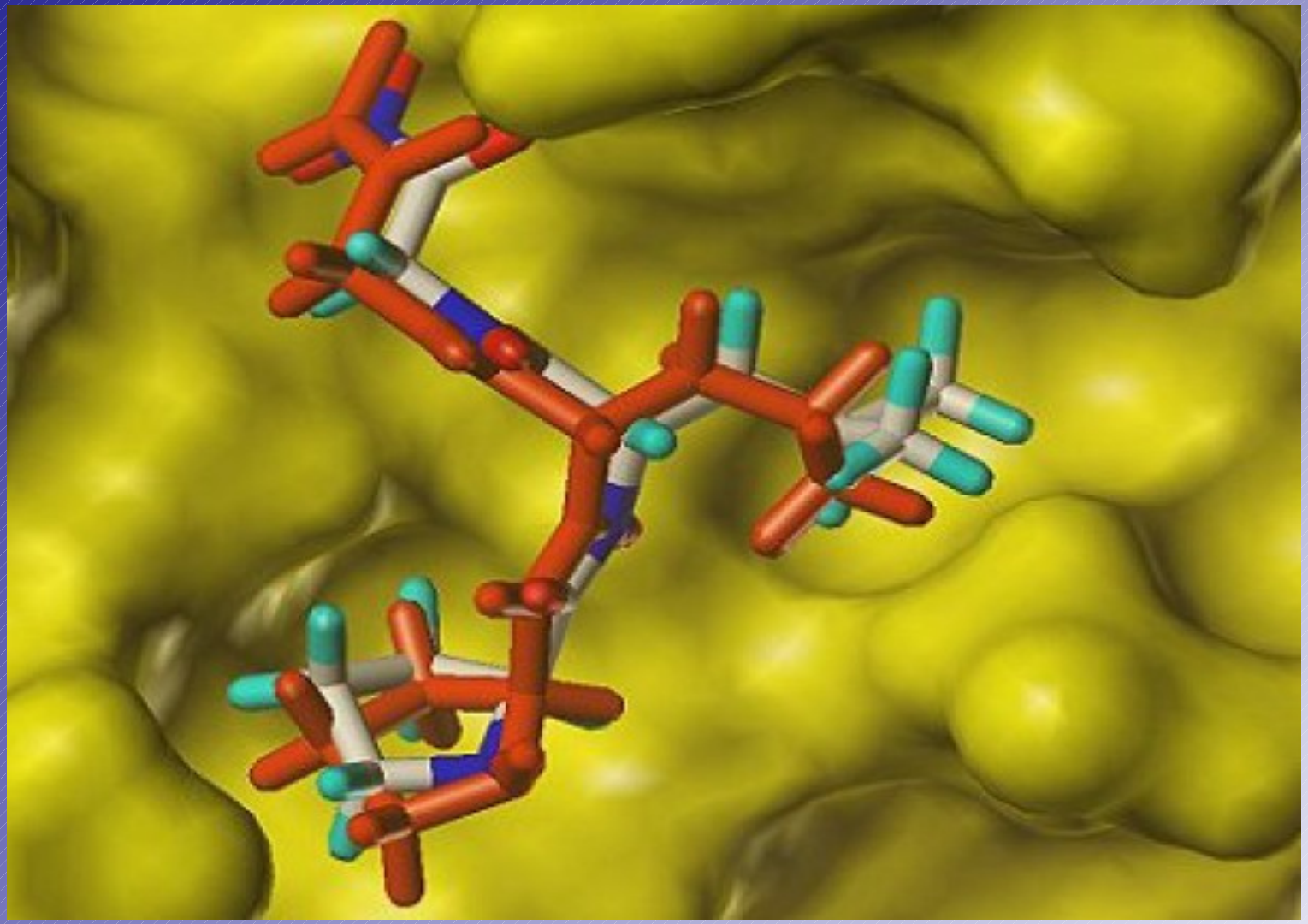
Optimalizace

- Znalost způsobu, jak se fragment váže na proteinový cíl umožňuje fragment zvětšovat.
- Používají se počítačové metody
- Docking
- „Experimentální“ metody Isostar, Superstar, Gold suite

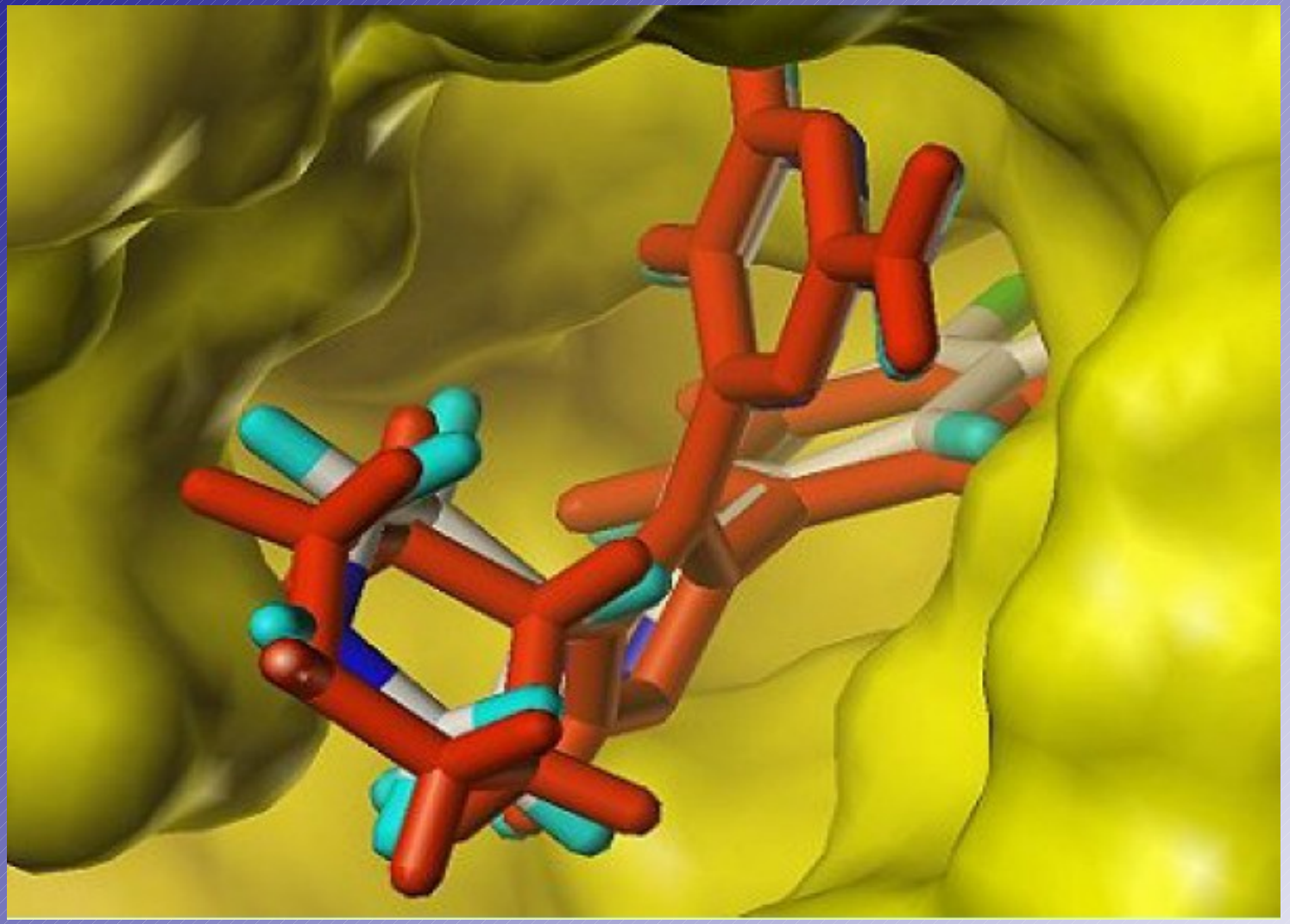
Gold - docking

- Protein-ligand docking
- GlaxoSmithKline, U.Sheffield, CCDC
- Complete flexibility of the ligand, the partial flexibility protein
- Energy-based functions Isostar
- Discrimination between active and inactive compounds
- Goldmine – decriptors

Gold - docking



Gold - docking



Advantages and disadvantages

- The small fragment has a greater chance that better incorporated into the binding site of the target protein than the whole molecule.
- The fragments have a higher binding energy per unit weight.
- Screening of small fragments leads to a greater number of "hits". The number of fragments is at 100 - 1000 (as compared to a million in HTS).

Advantages and disadvantages

- "Leads" from fragments have lower mortality rates. 70% of hits from HTS fails, 80% FSDD hits are successful.
- Allows you to discover the "lead" where HTS failed
- It is possible to obtain a lead outside the standard database and thereby obtain a patentable compound