NMR in Drug Discovery

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NMR: Principles

-Biophysical technique for resolving the three-dimensional structure of molecules in solution

-Based on the principle that some nuclei resonate at specific frequencies when placed in an external magnetic field

-Relative resonance frequency is called chemical shift

-Molecular structure is calculated from many ^{1}H-^{1}H distances measured by NMR

NMR: Requirements

-Pure protein (>95%) at a high concentration (~1mM)*

-Must be soluble and stable

-Ideally expressed in *E. coli** for easy labelling with ^{15}N and ^{13}C

-Upper limit of ~25kDa for routine analysis*

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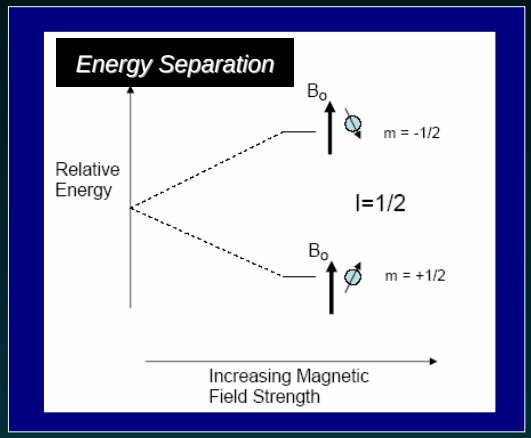
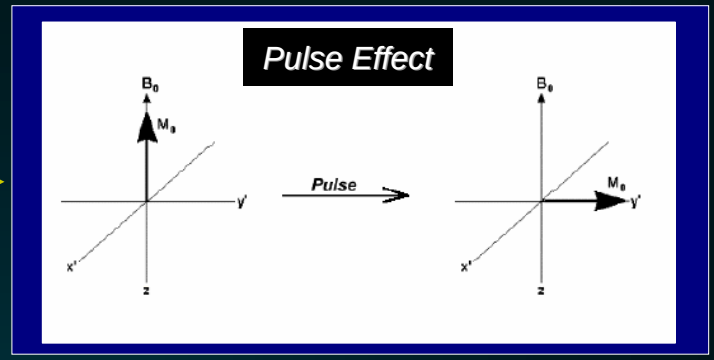
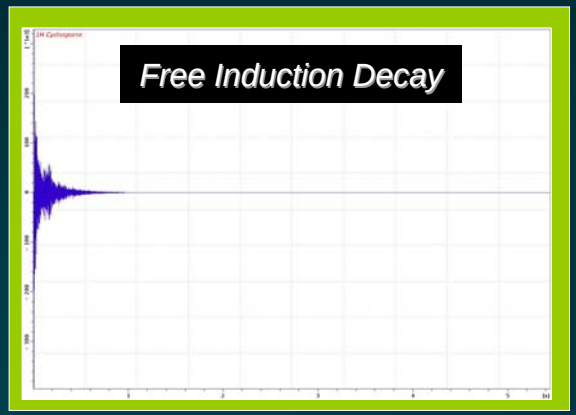
NMR: Introduction







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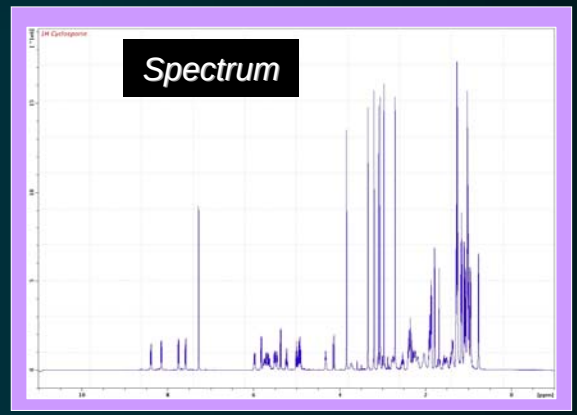

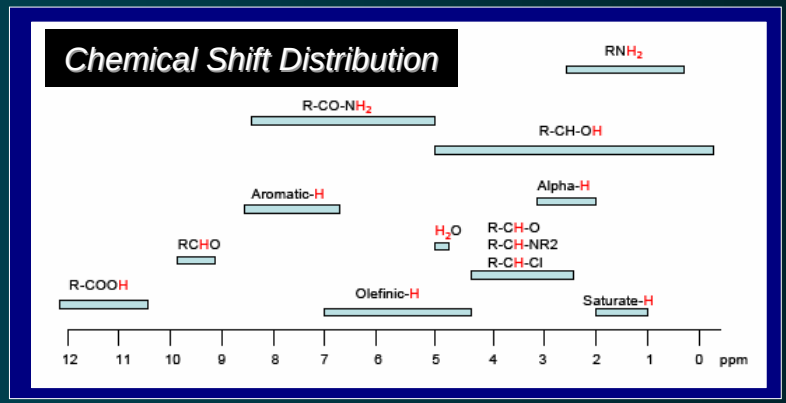

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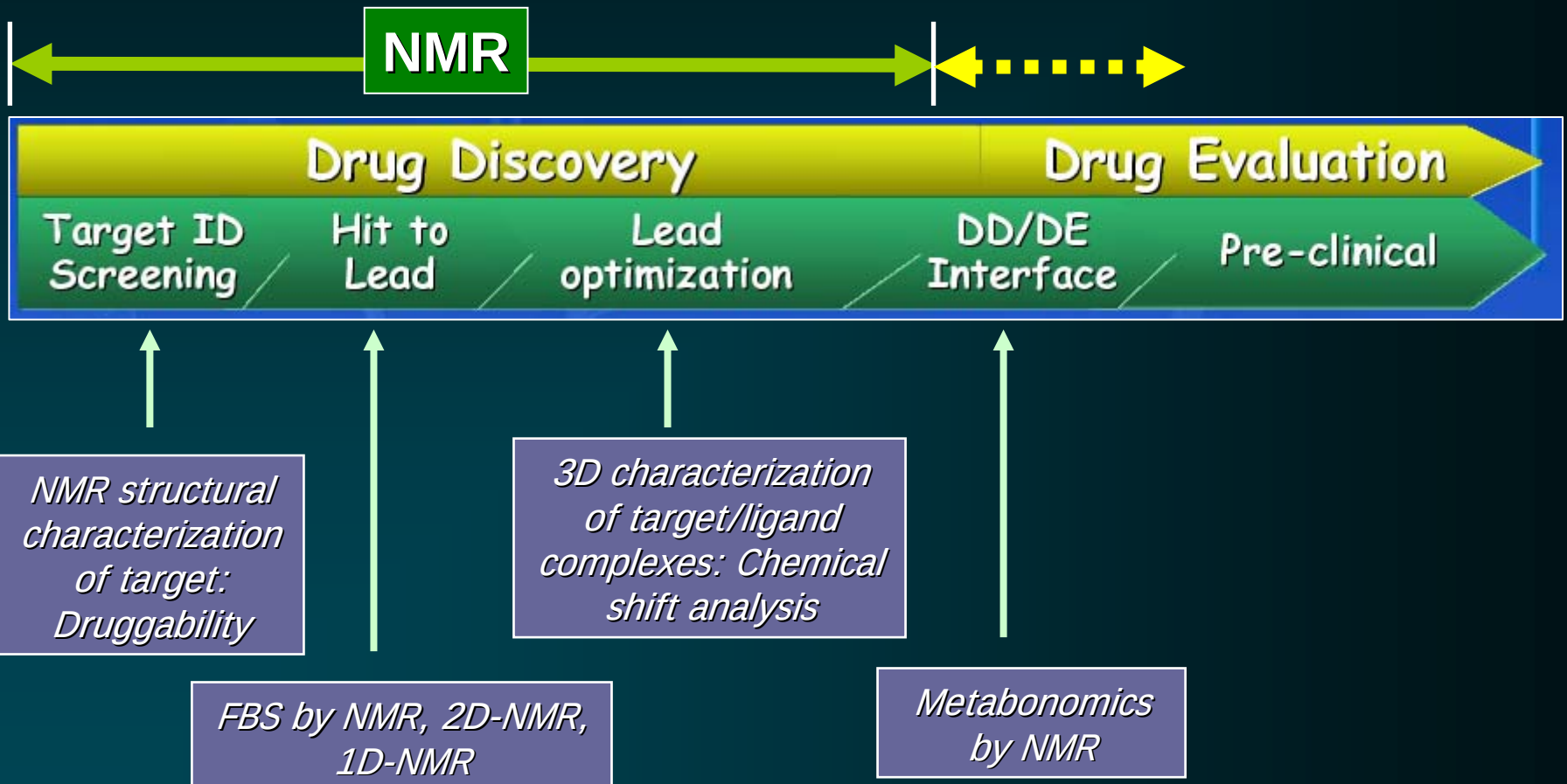
NMR Contributions to Drug Discovery



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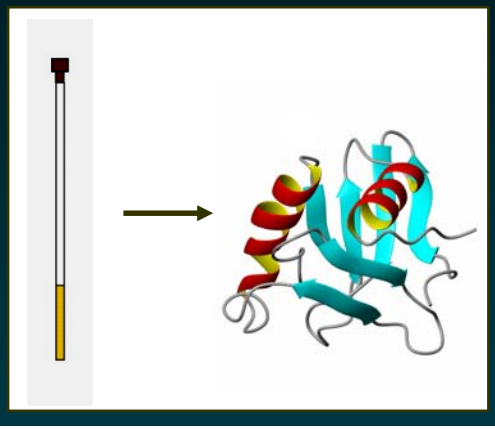
3D Structural Determination of the Proteins by NMR

- NMR experiments
- 3D structure determination of the protein

a



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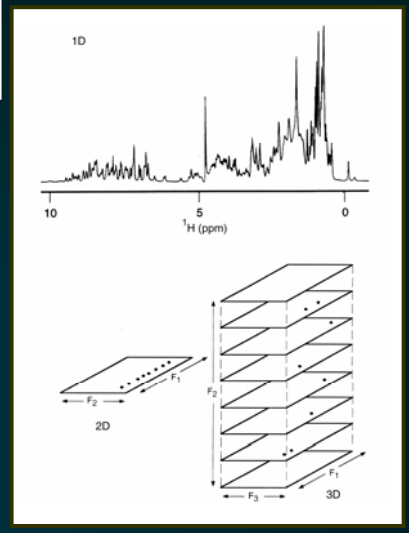

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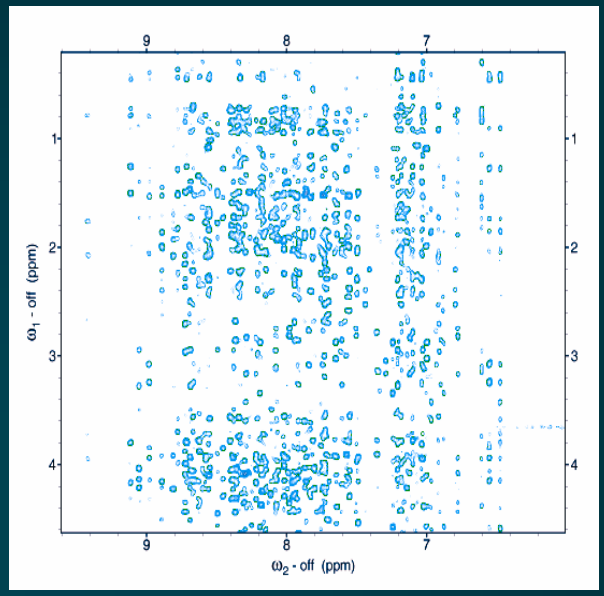

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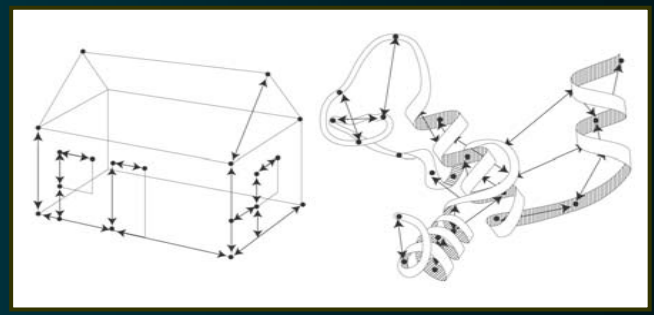

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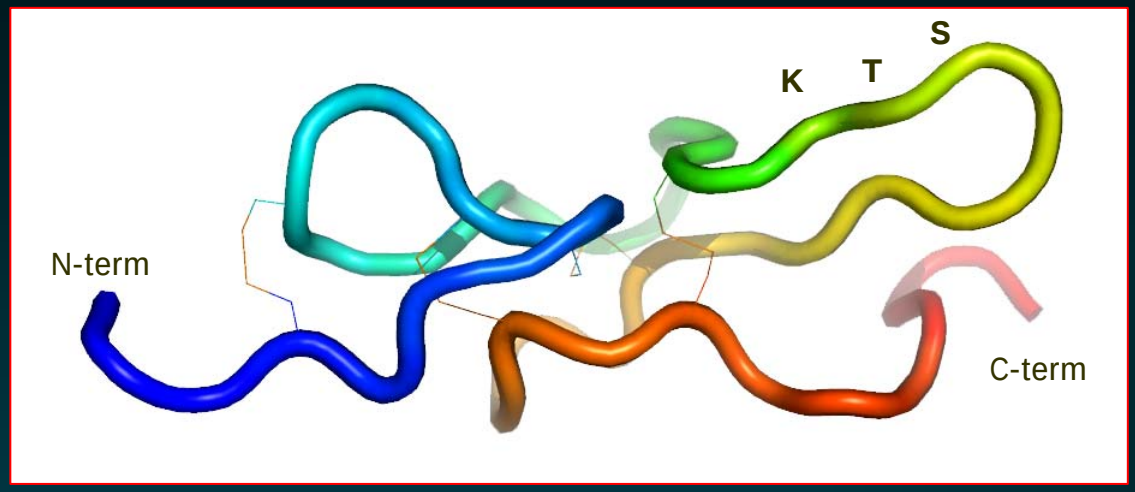
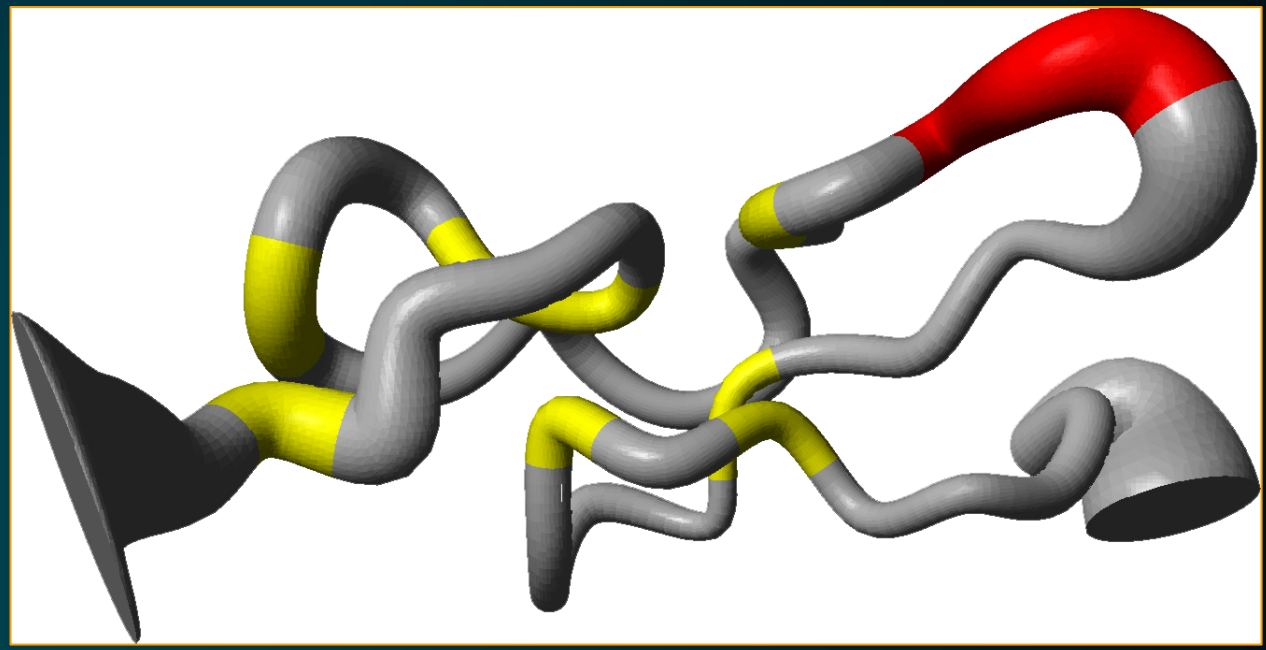
Jerdostatin: Structural Characterization of a Novel Disintegrin



Lowest Energy Structure

Structure Ensemble



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NMR Applications in Drug Discovery
Fragment-Based Screening

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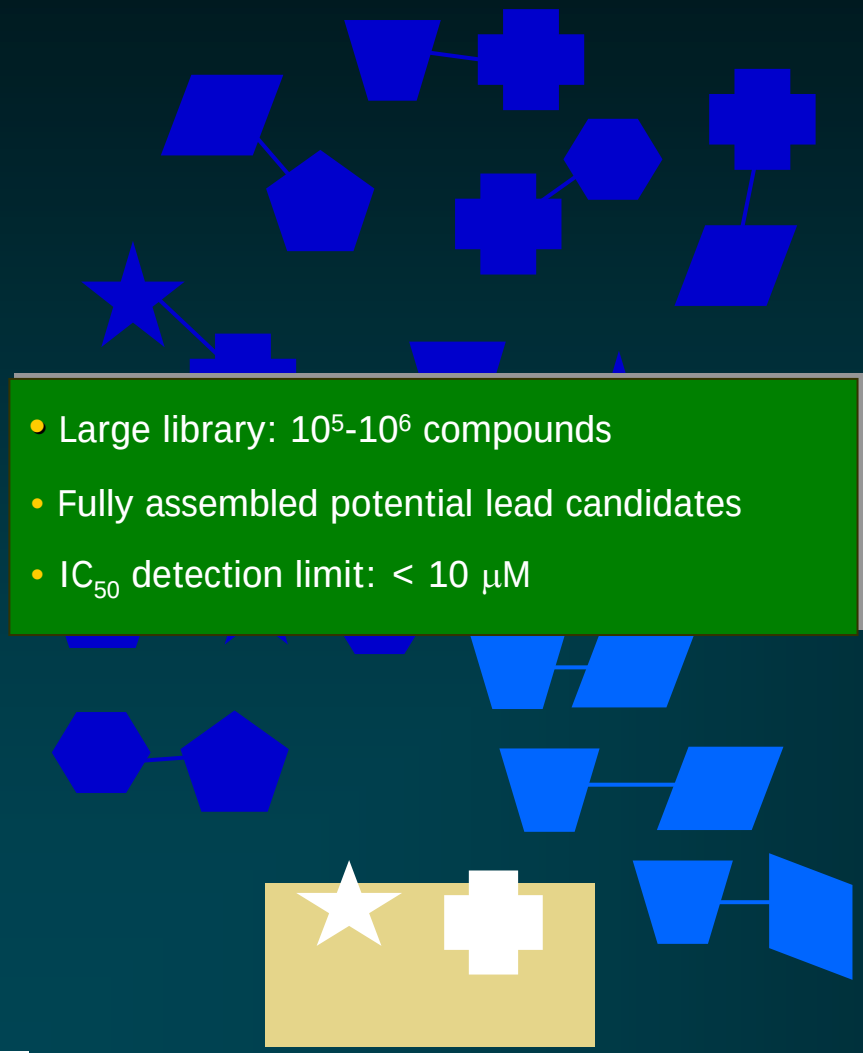
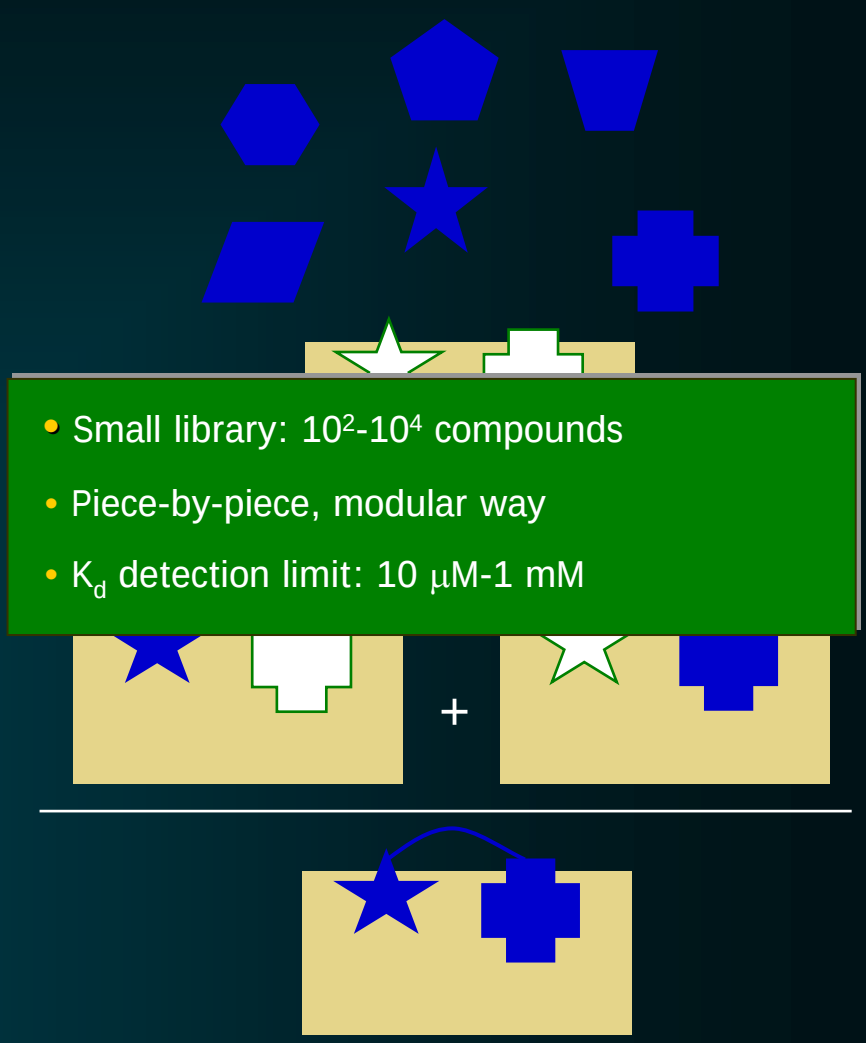
HTS vs Fragment-Based Screening



- Large library: 10^5-10^6 compounds
- Fully assembled potential lead candidates
- IC_{50} detection limit: < 10 μM



- Small library: 10^2-10^4 compounds
- Piece-by-piece, modular way
- K_d detection limit: 10 μM-1 mM

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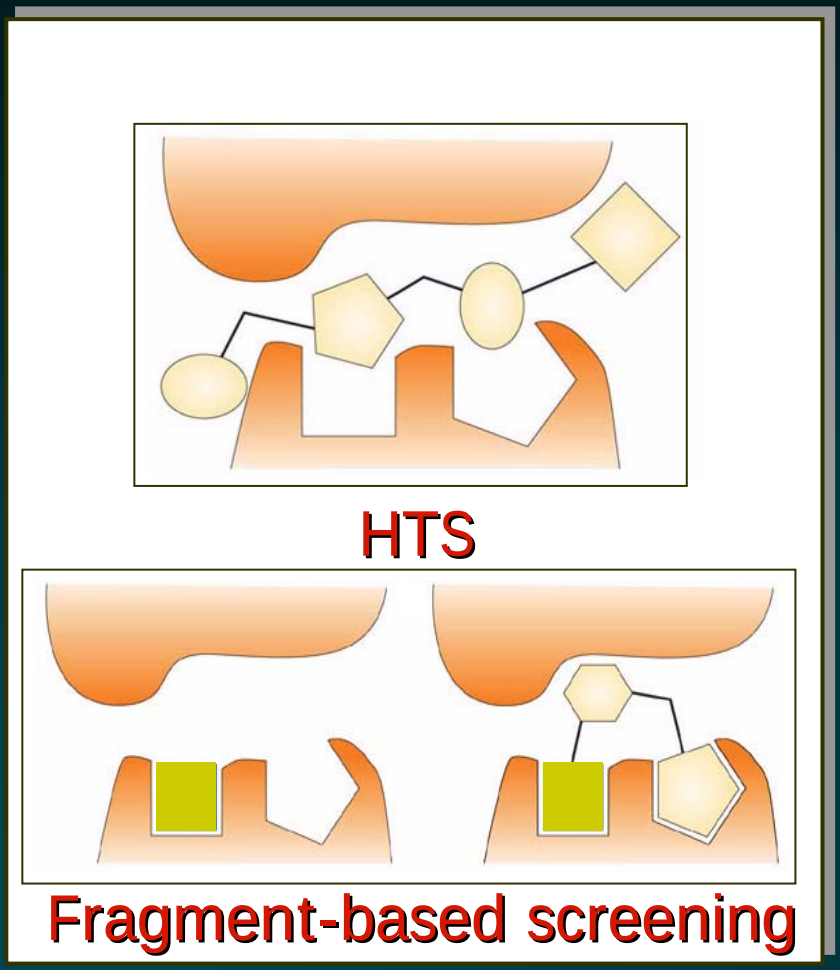
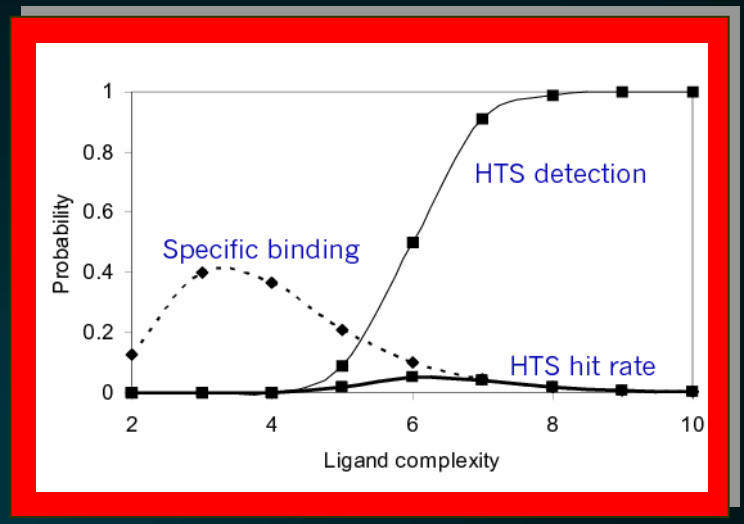
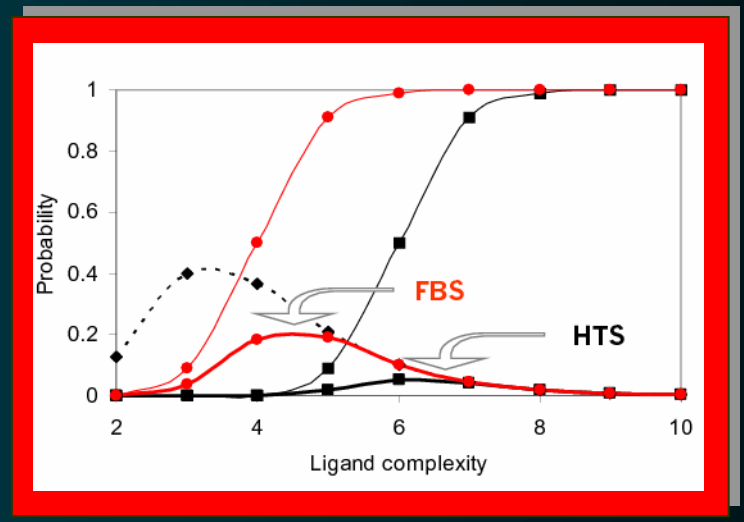
Fragment-Based Screening has a higher hit rate than conventional HTS

Matches and mismatches...







Rees *et al.*, *NRDD*, **3** (2004), 660

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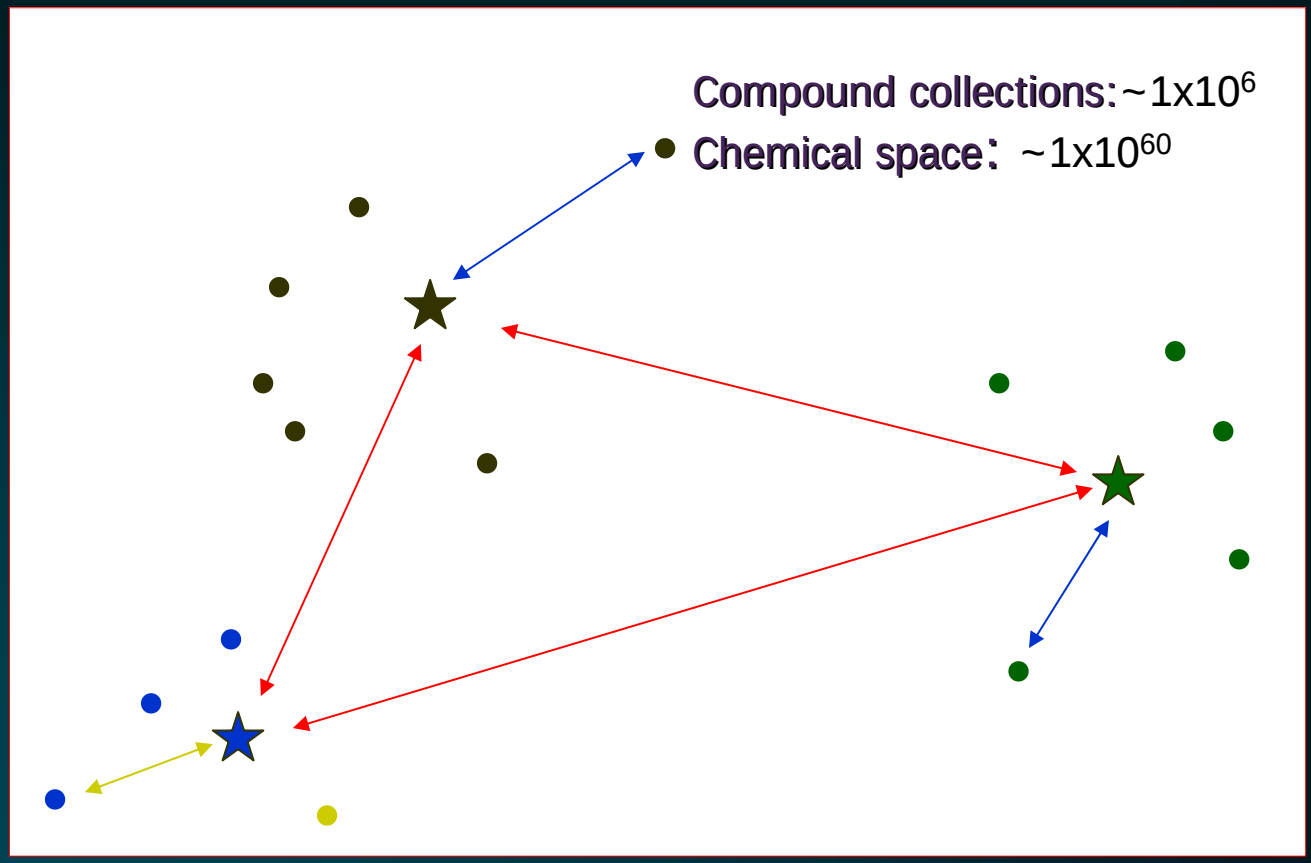
Measures of diversity & coverage
[Distance inversely related to similarity]



Selection rules: 1D and 2D filters to select cpds with specific physicochemical properties (MW, functional groups, clogP, H donors/acceptors), diversity based on Tanimoto distances, 3D pharmacophoric analysis, etc…

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Target-based Screening

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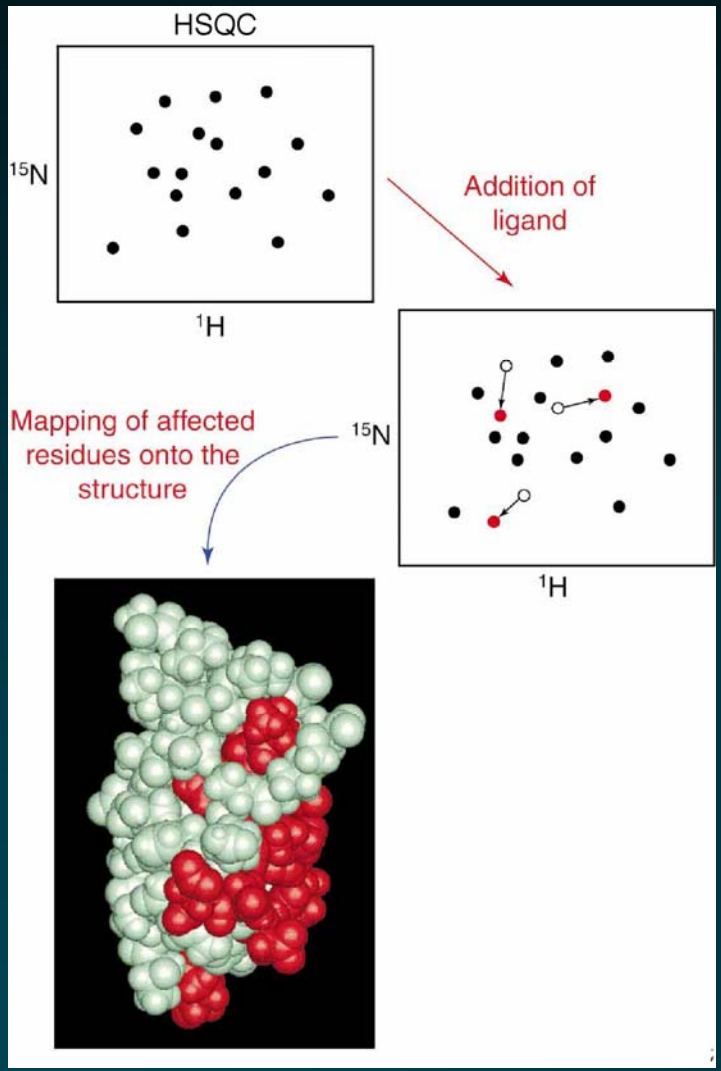
Chemical Shift Mapping: What is it?



Advantages

- Extremely weak binding can be probed (up to 10 mM)/K_d information
- Quality of starting points (real binders, physicochemical properties, pK_d/hvatom)
- Easy to demonstrate competitive binding
- Structural information even in the absence of sequence-specific assignment

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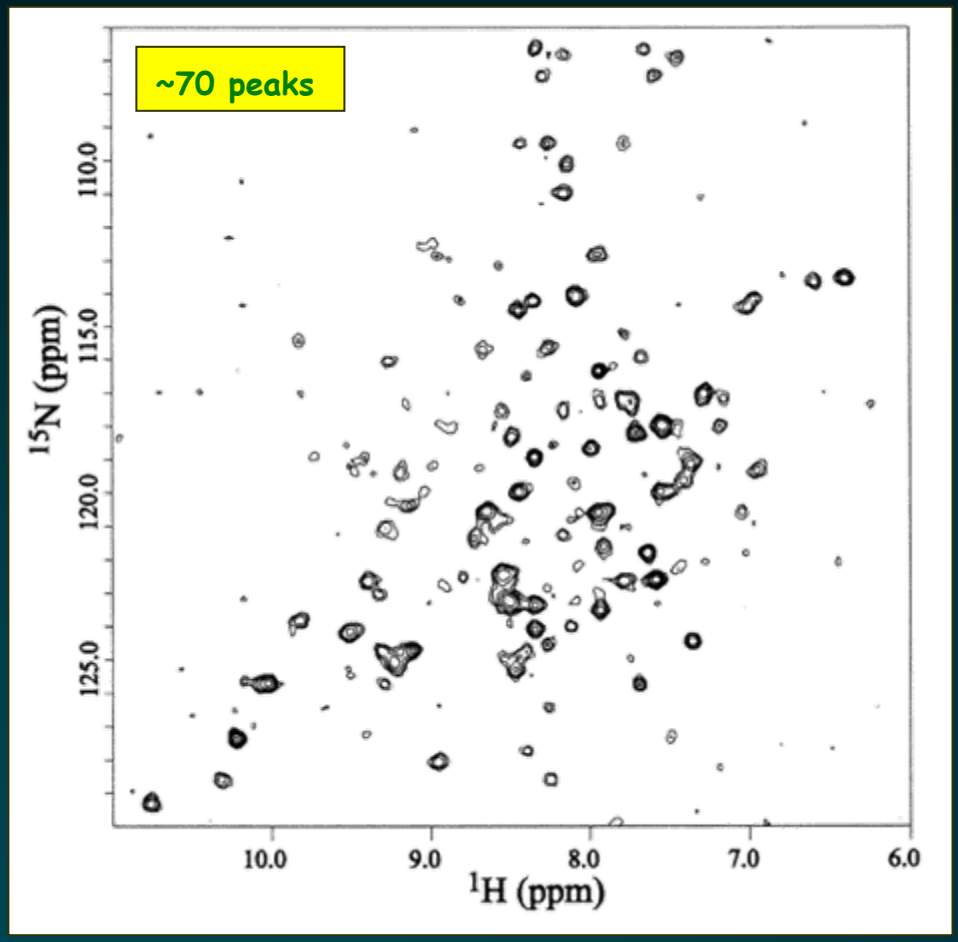
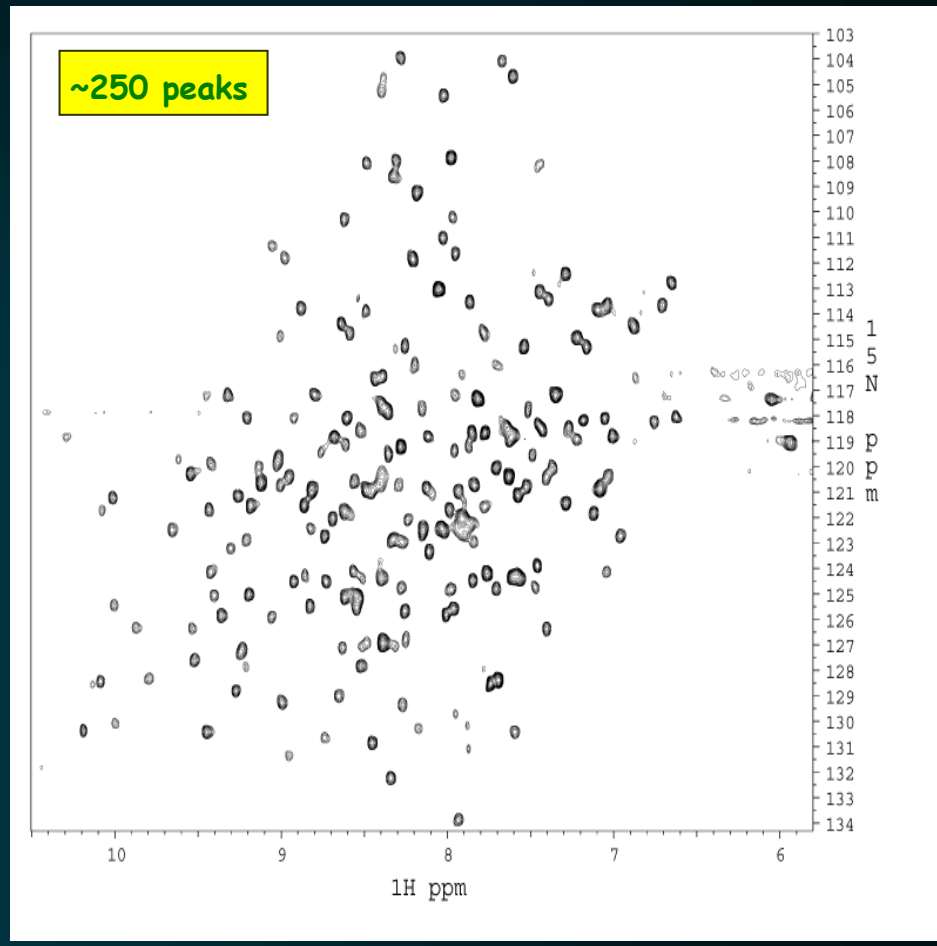
Chemical Shift Mapping: Protein Chemistry





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Ligand-based Screening

Methods that rely on the detection of an altered hydrodynamic property

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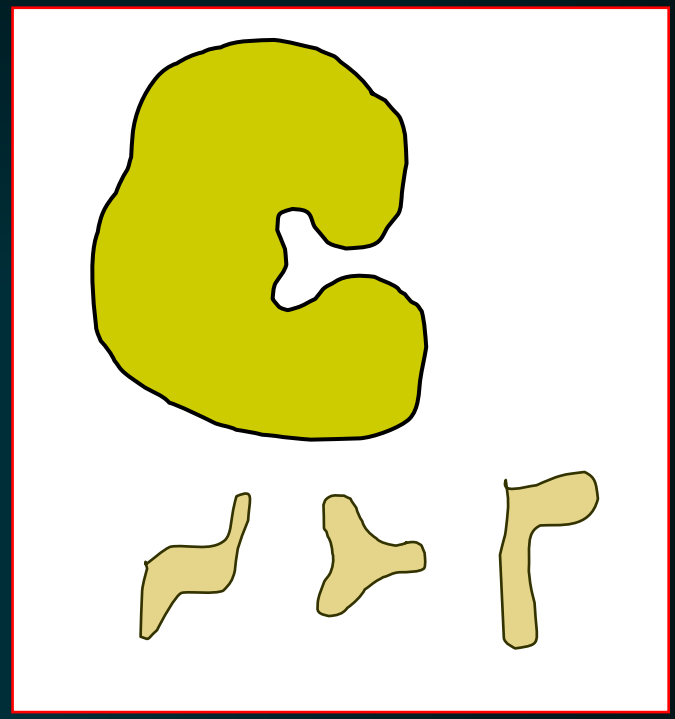
Ligand Observe: Employ Property Differences

- Big molecules (proteins)
  - Slow translational diffusion
  - Slow tumbling
  - Fast relaxation
  - Broad linewidths
  - *Negative* NOE
- Small molecules (ligands)
  - Rapid translational diffusion
  - Fast tumbling
  - Slow relaxation
  - Narrow linewidths
  - *Positive* NOE



A ligand which binds to a protein in fast exchange diffuses less rapidly, and relaxes less slowly

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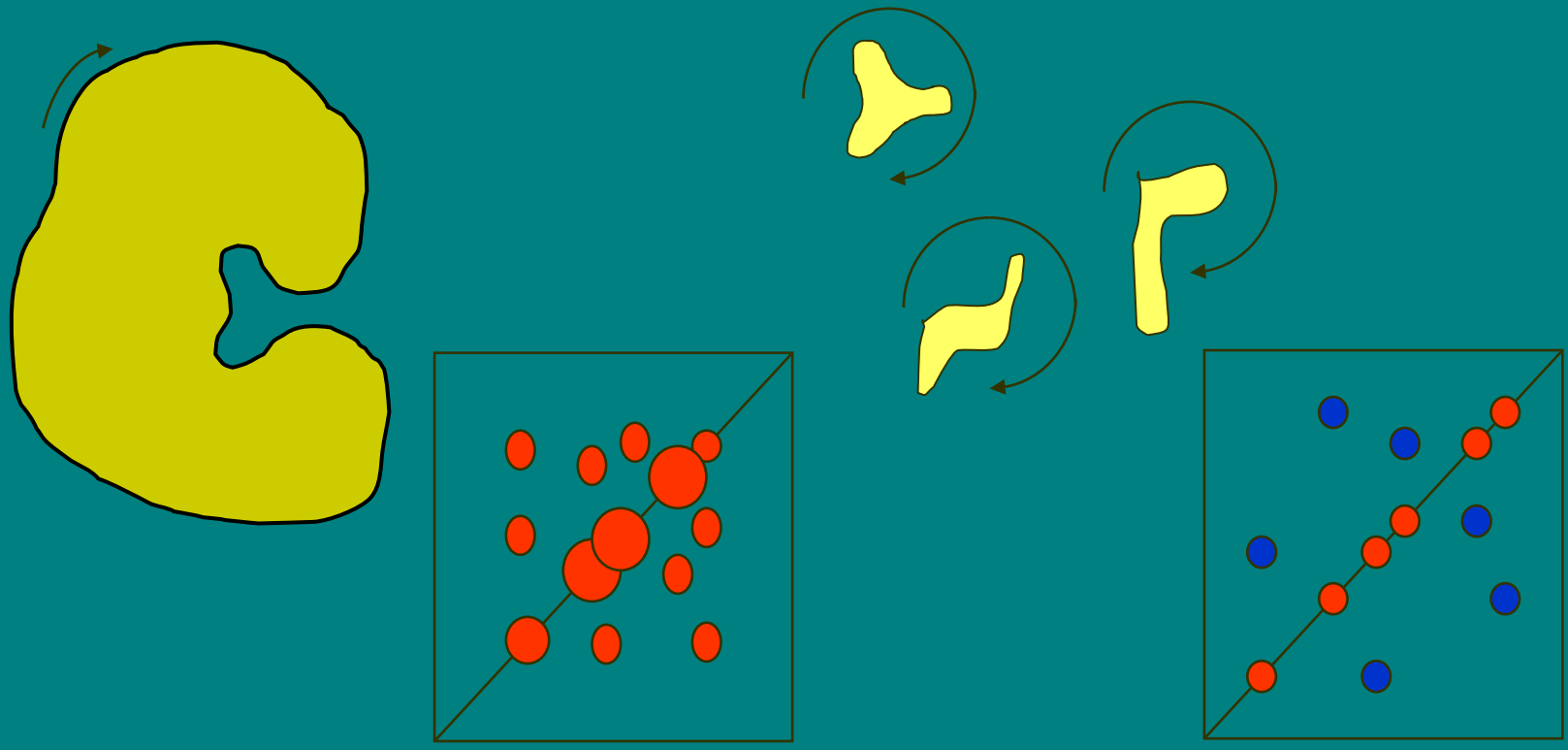
Hajduk *et al.*, *JACS* **119**, 12257 (1997)

Transferred NOESY

Proteins - slow tumbling
Negative NOE
Positive crosspeaks in NOESY

Small molecules - fast tumbling
Positive NOE
Negative crosspeaks in NOESY



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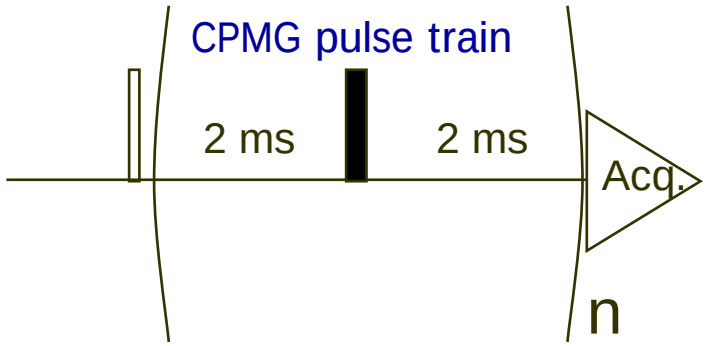
Ligand observe

Relaxation-edited NMR Experiment

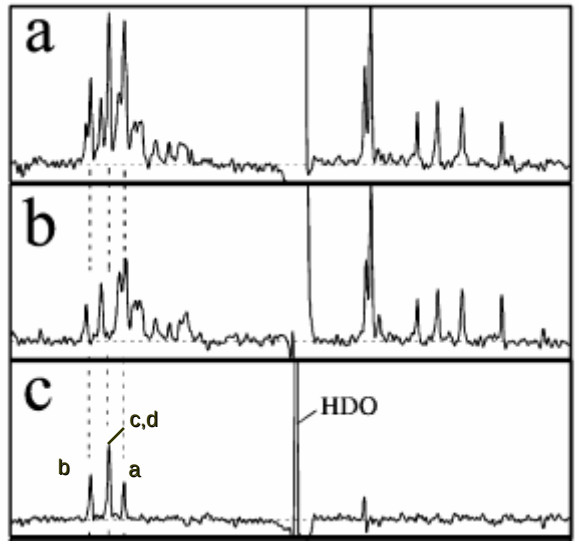


n=100; 400 ms spin lock time

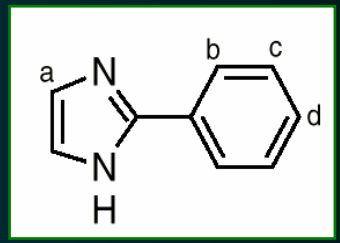
A: Transverse relaxation-edited ^{1}H-NMR spectrum of a mixture of 9 compounds in the absence of FKBP

B: Transverse relaxation-edited ^{1}H-NMR spectrum of the 9 compounds in the presence of FKBP after subtracting a similar spectrum recorded on just FKBP

C: Difference spectrum shows binding compound



Ligand observe



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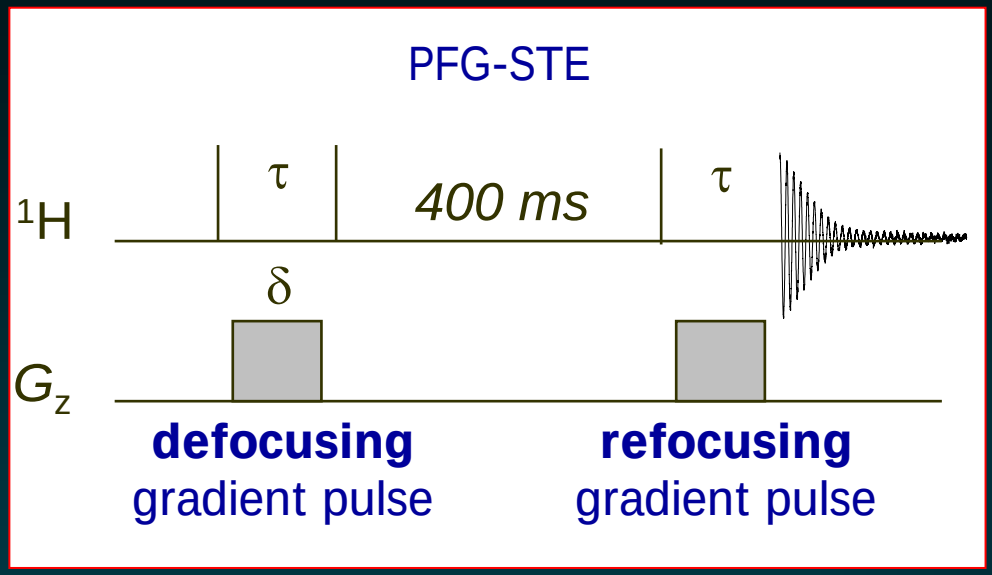
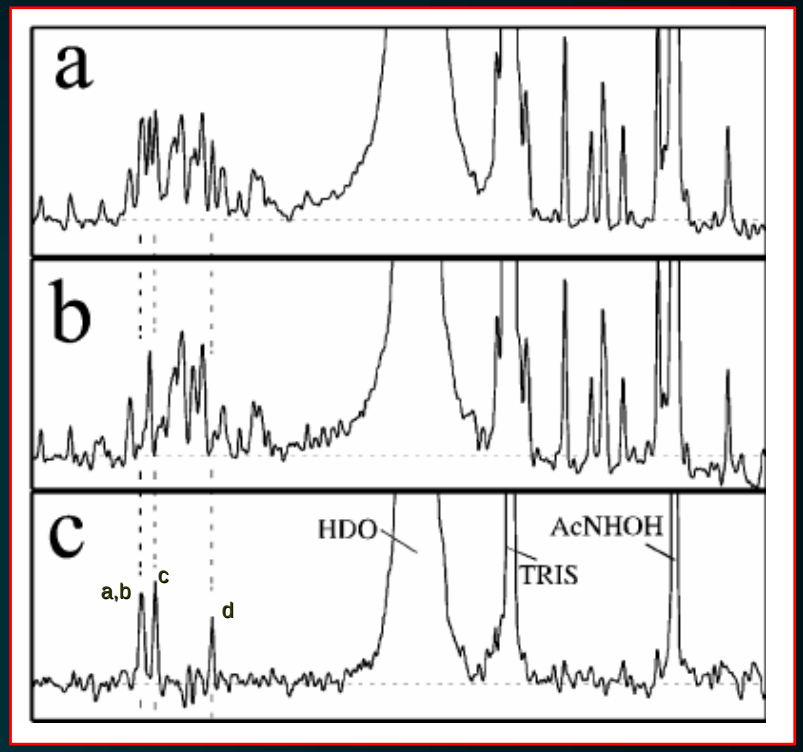
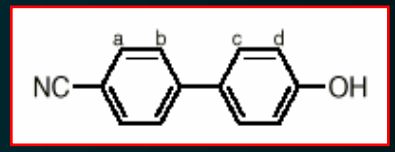
Diffusion-edited NMR Experiment



Ligand observe





A: Diffusion-edited ^{1}H-NMR spectrum of a mixture of 9 compounds in the absence of protein recorded with low gradient strength

B: Diffusion-edited ^{1}H-NMR spectrum of the 9 compounds in the presence of protein recorded with low gradient strength after subtracting a similar spectrum recorded with high gradient strength to remove the protein signals

C: Difference spectrum shows binding compound

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Ligand-based Screening

Methods that rely on transfer of an NMR signal between target and ligand

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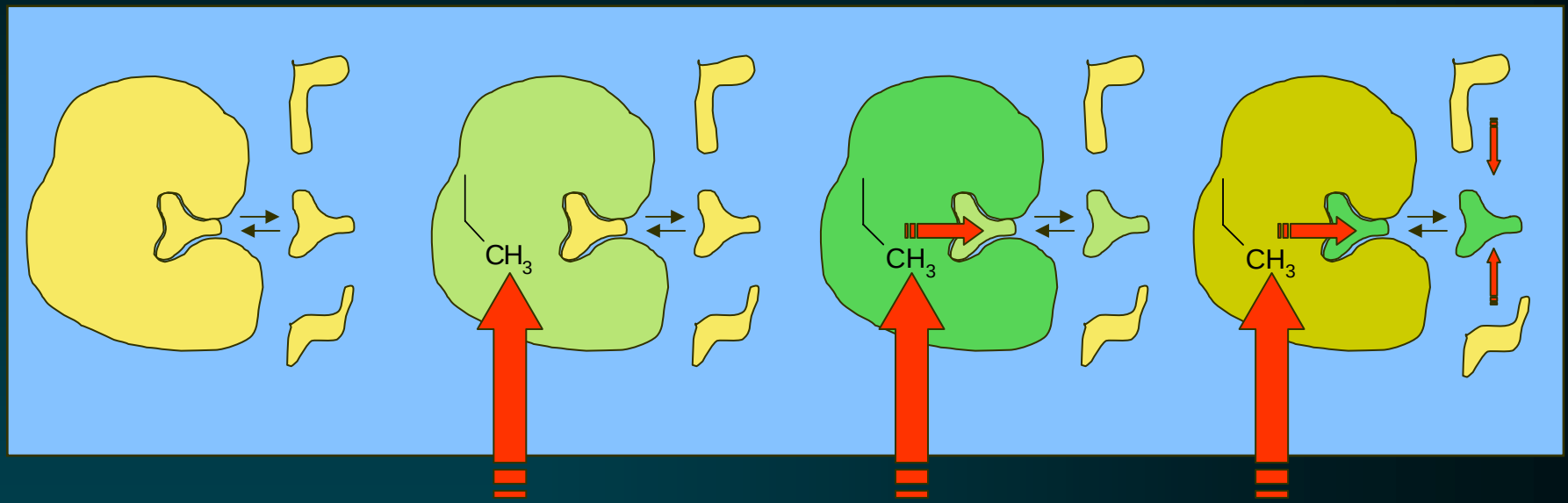
STD - Saturation Transfer Difference



Selective irradiation of protein methyls

Saturation transfer to bound ligand

Mayer and Meyer, *Angew. Chem. Int. Ed.*, **38** (1999), 1784

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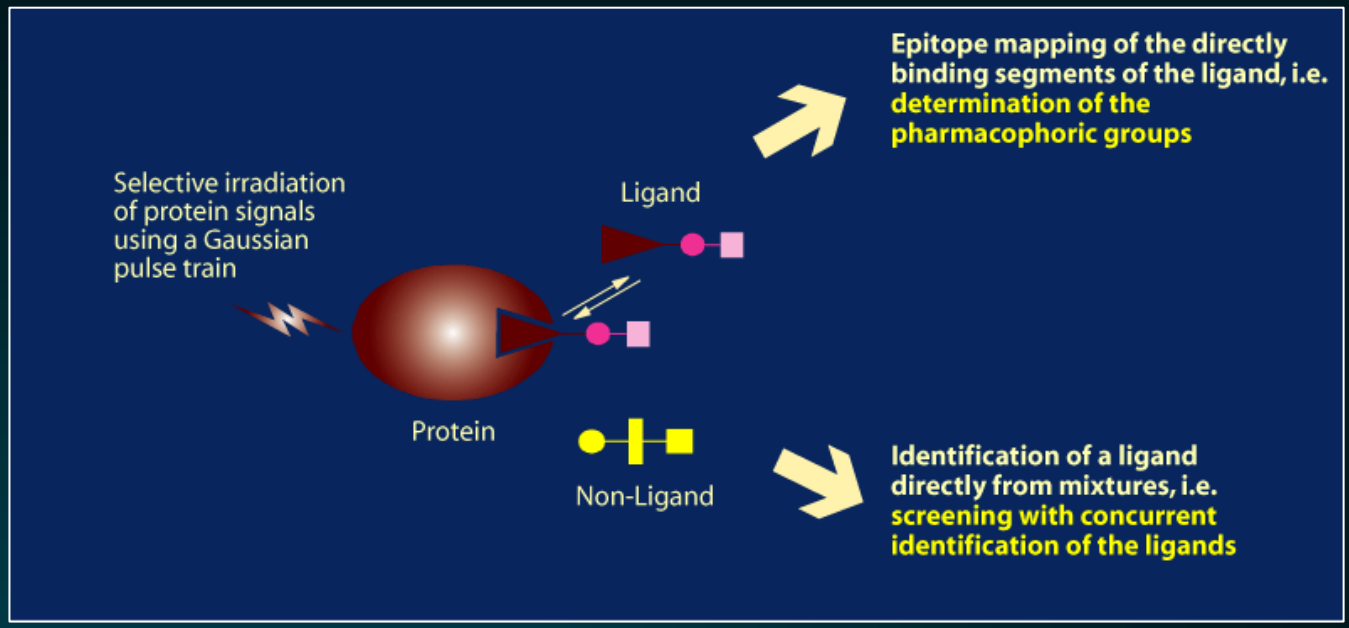
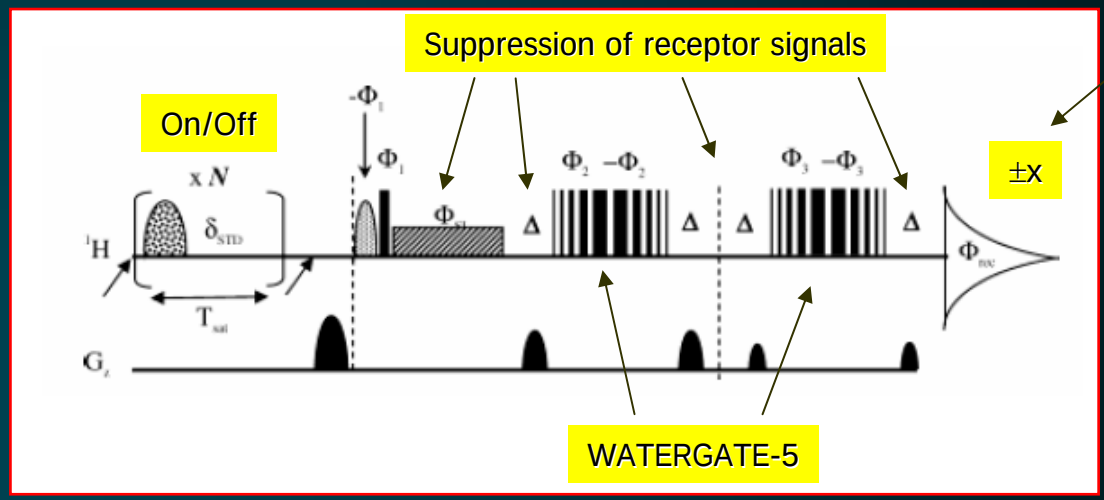
STD: Epitope Recognition and Pulse Scheme





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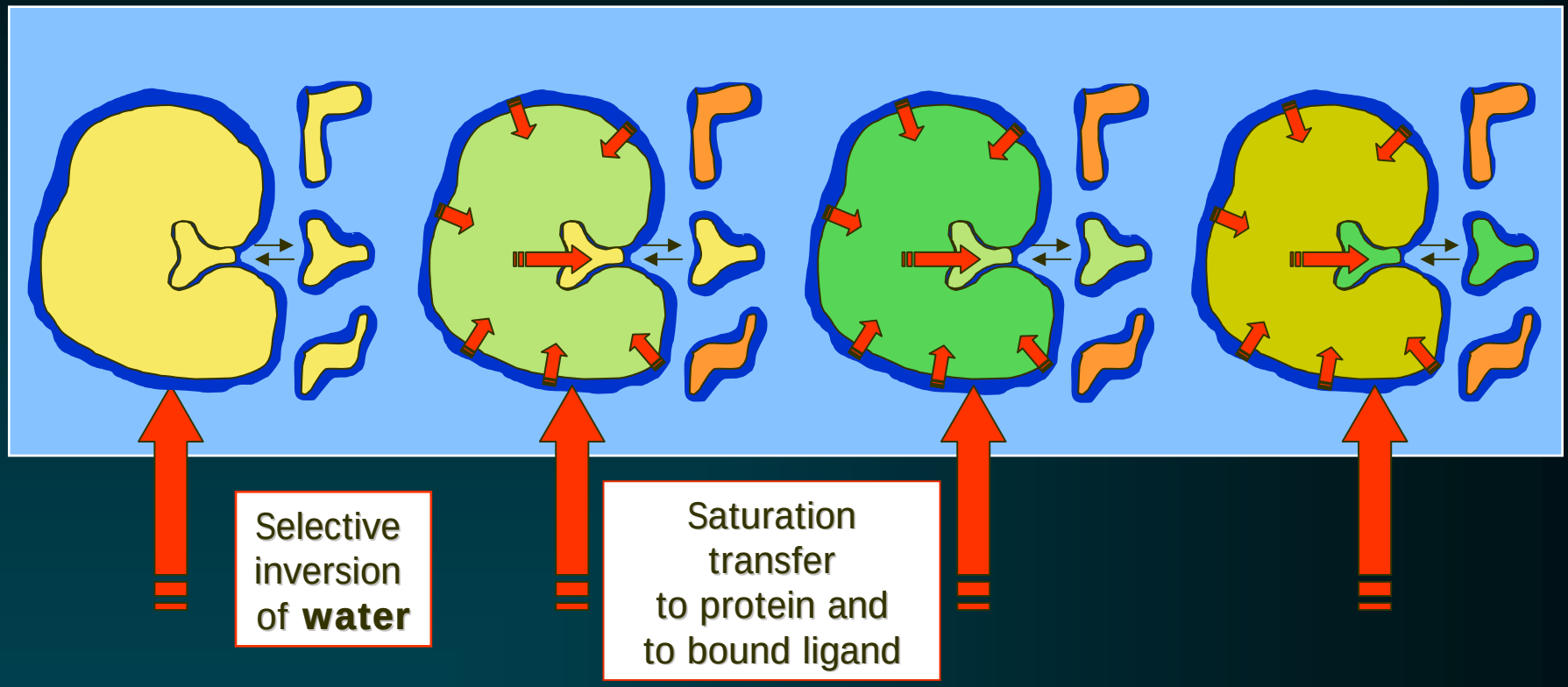
Water-LOGSY



- *Protein 1 - 10 uM*
- *Mixture of 4 - 10 compounds at 200 uM each*

Dalvit *et al.*, *J. Biomol. NMR,* **18** (2000), 5

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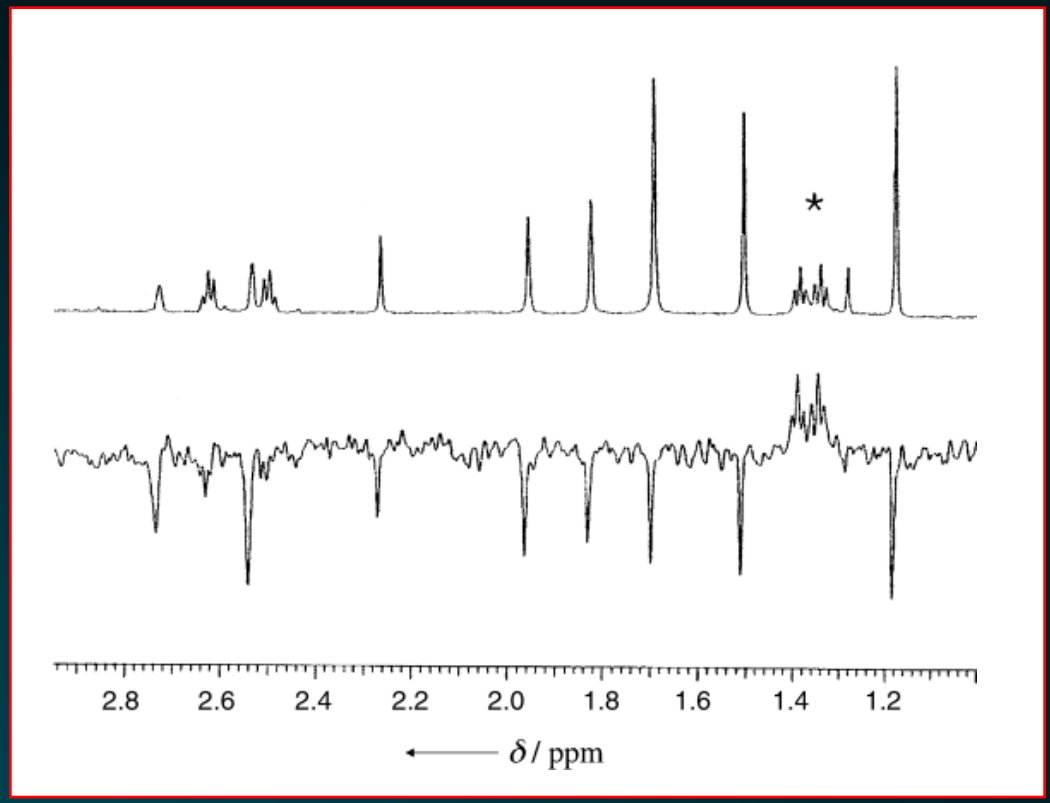
Water-LOGSY: Discriminate Binding by Sign



-Lower spectrum: Water-LOGSY spectrum of a library of ten compounds in the presence of cdk2

-Positive signals: Indole derivative with binding affinity for cdk2

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Ligand-based Screening

Methods that do not fall into either category

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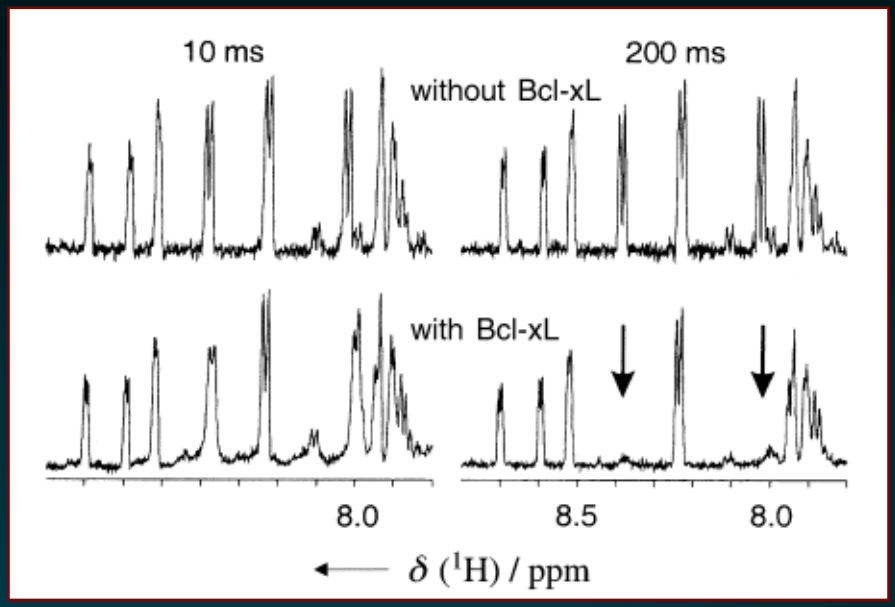
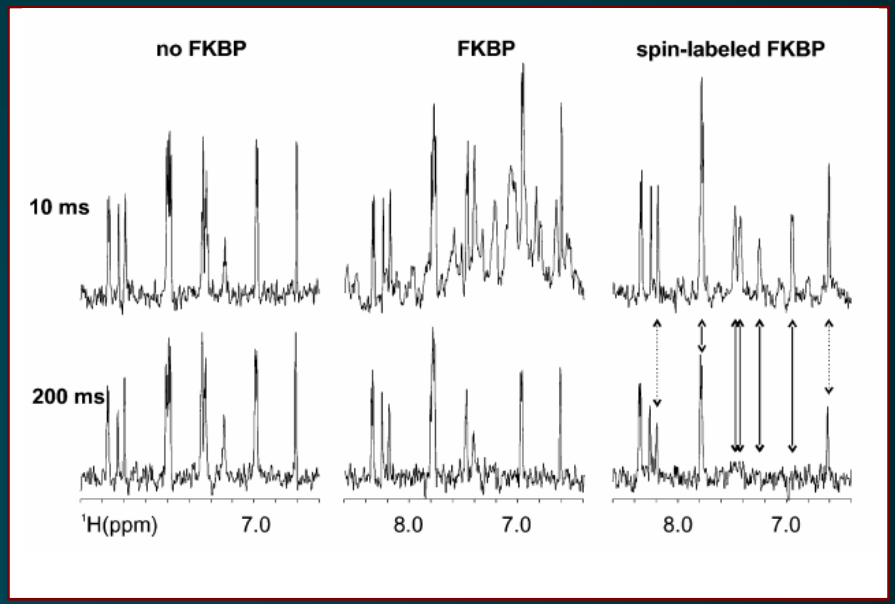
Spin Labels for Ligands and Proteins



Reference ^{1}H NMR spectrum, no protein present

Compound mixture in the presence of the protein and saturating amounts of a spin-labeled first ligand



$T_{1\rho}$ relaxation experiments of two binding and four non-binding compounds without FKBP, unlabeled FKBP and spin-labeled FKBP (lysines residues with TEMPO)

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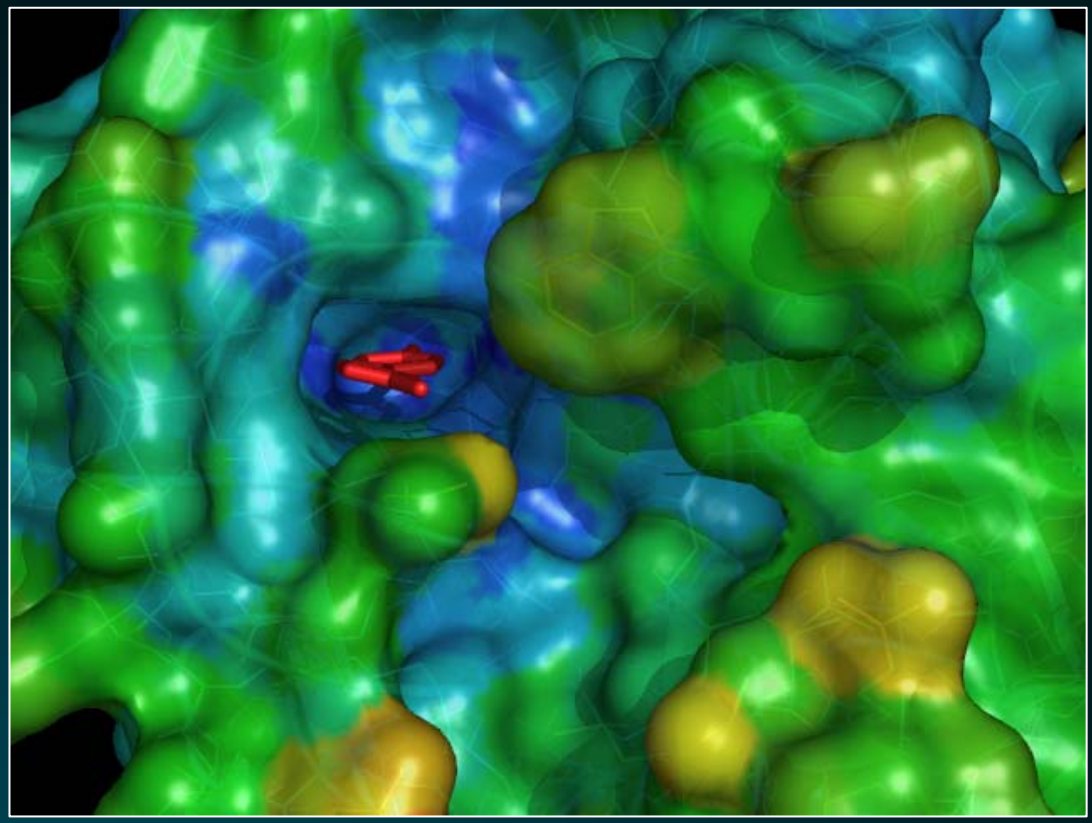
Lead Optimization by NMR



- In the pharmaceutical environment, NMR is also used for structure determination of proteins and protein complexes, although very often, structures are solved by X-ray (particularly for high-affinity binders)
- Still, NMR can provide high quality structural information for complexes of weakly bound compounds, and can be used to characterise proteins that do not crystallise

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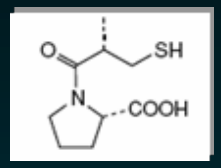
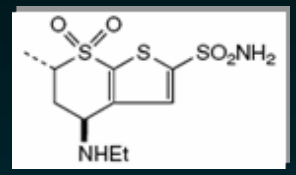
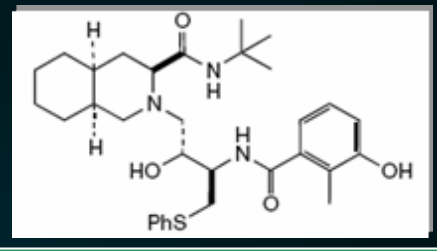
Drugs Derived from Structure-Based Approaches



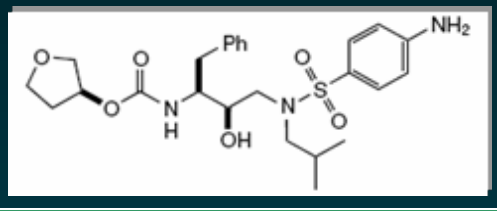
Capoten/Hypertension/BMS



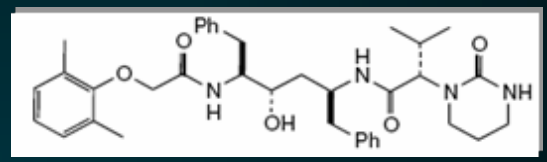
Trusopt/Glaucoma/Merck



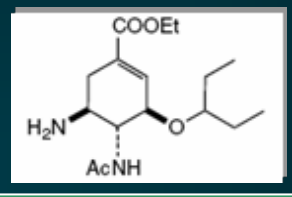
Viracept/HIV-AIDS/Agouron(Pfizer) & Lilly



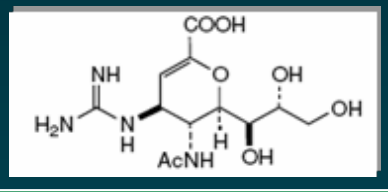
Agerenase/HIV-AIDS/Vertex & GSK



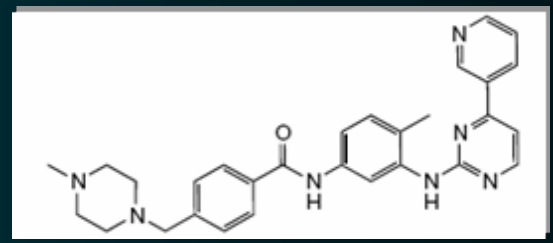
Aluviran/HIV-AIDS/Abbott



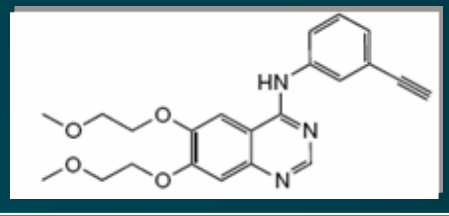
Tamiflu/Influenza/Gilead & Roche



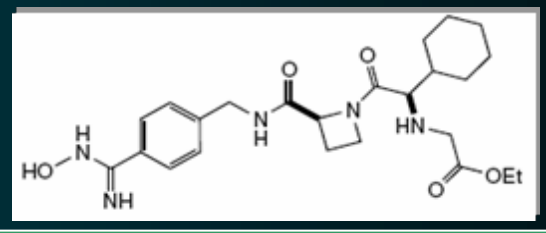
Relenza/Influenza/Monash Univ. & GSK



Gleevec/CML/Novartis



Tarceva/NSCLC/OSI & Genentech



Exanta/Venous Thromboembolic Events/AstraZeneca

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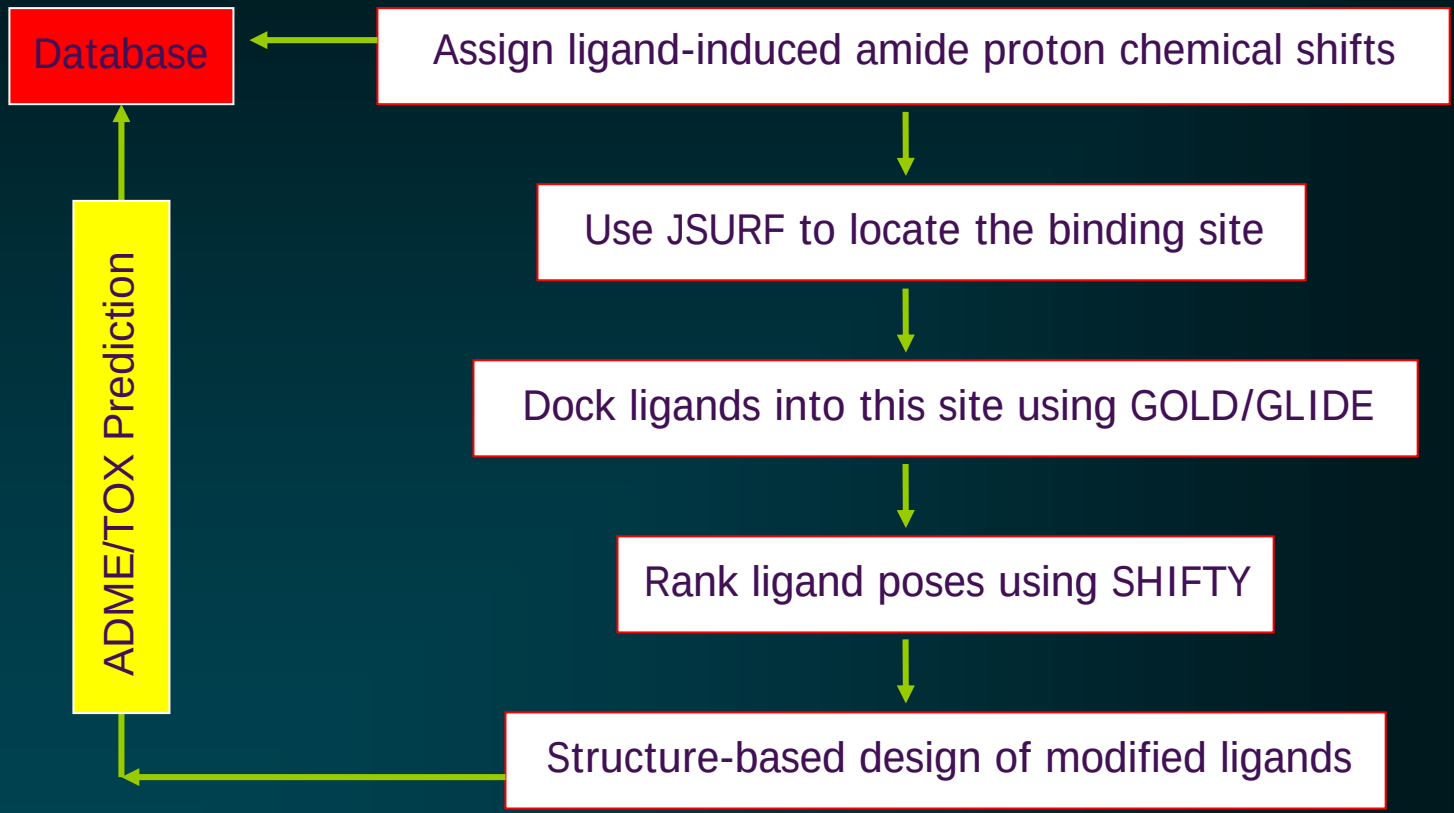
Structure-Based Design using Chemical Shift Data



AstraZeneca Computational Chemistry/CIPF Structural Biology
(Martin Packer & Antonio Pineda-Lucena)

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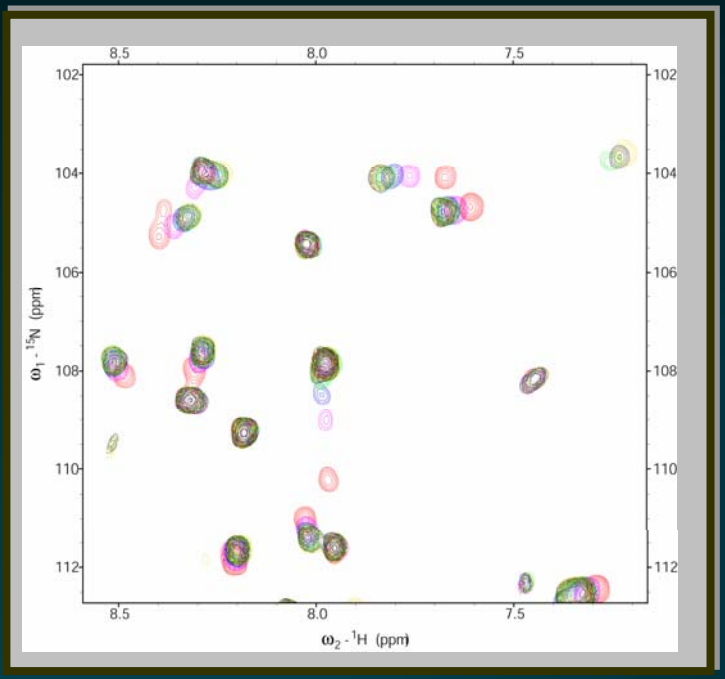
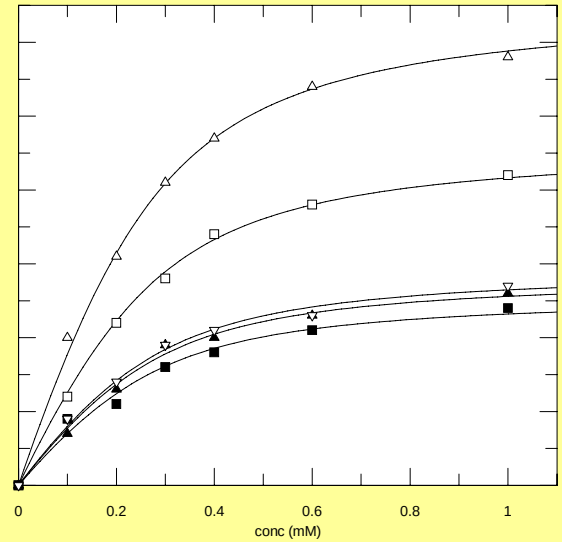
Analysis of Chemical Shift Mapping Experiments
K_d Analysis

Chemical shift mapping experiment



Fitting of experimental data



- *Analysis requires an **automatic routine for peak picking**, and the ability to carry the assignment from one spectrum to another*
- *Critical to **identify** and **assign all the amino acids** involved in the **interaction** with the ligand*
- *In many cases, the most important amino acids are **absent in the free form** of the enzyme (eg, residues in loop which only become apparent in the spectrum once the protein binds to a ligand)*

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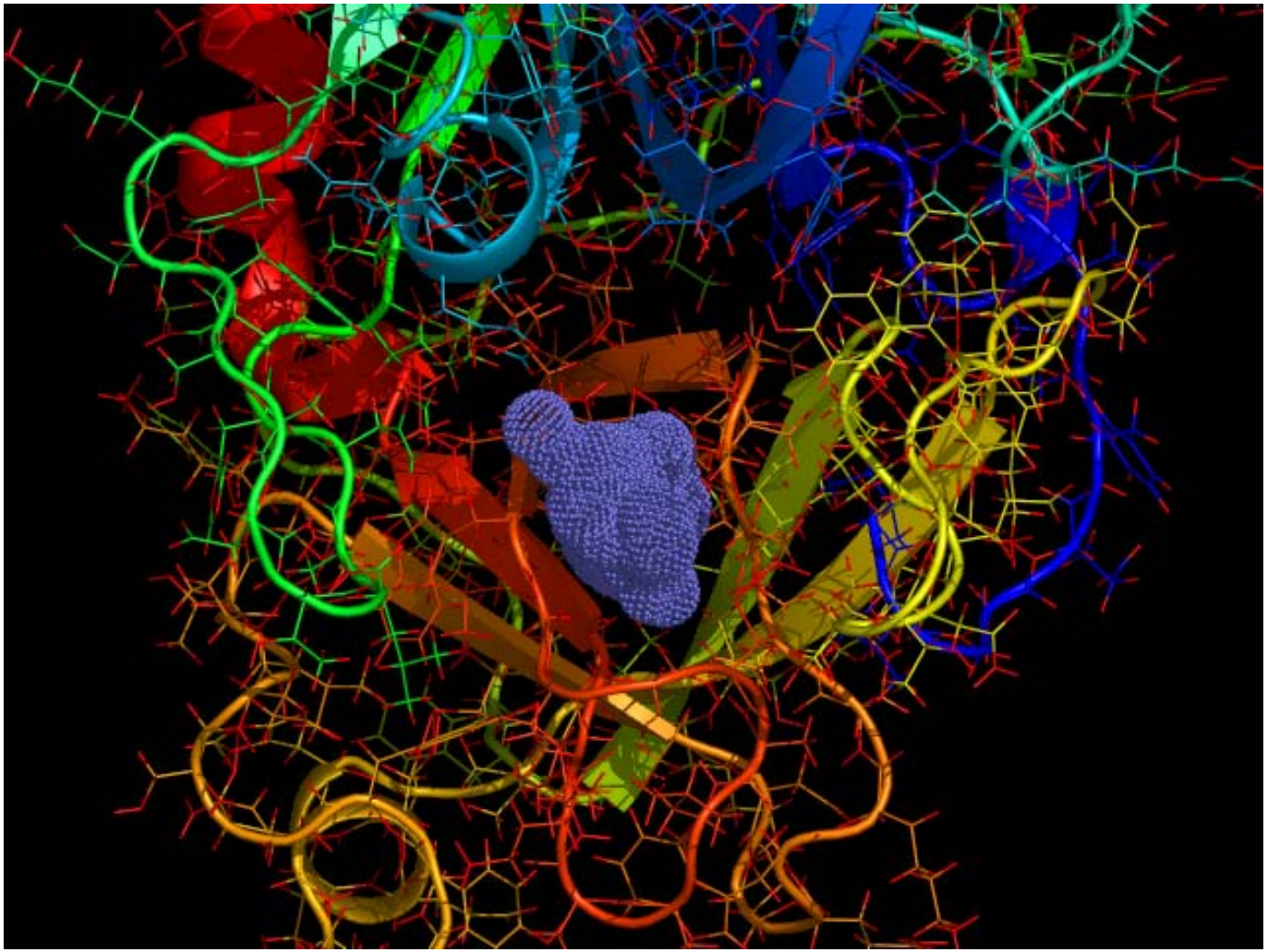
Jsurf Analysis of the Interaction of Benzamidine with a Protease



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Fragment-based Screening Campaign

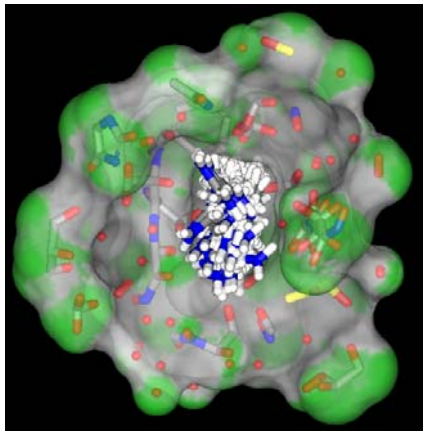
1.- Assign ligand-induced amide proton chemical shifts

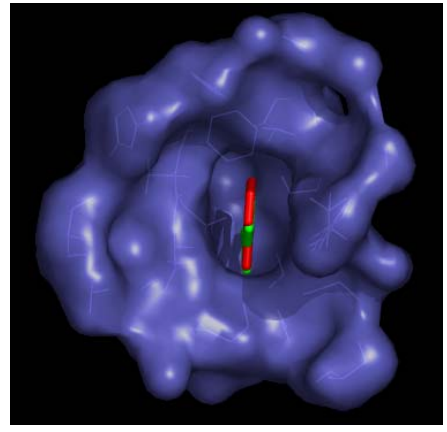
2.- Use JSURF to locate binding site

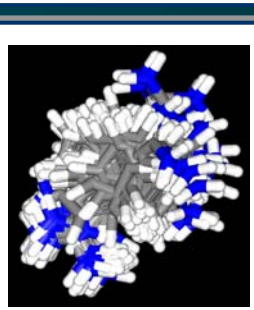
3.- Dock ligands into this site with GOLD/AUTODOCK

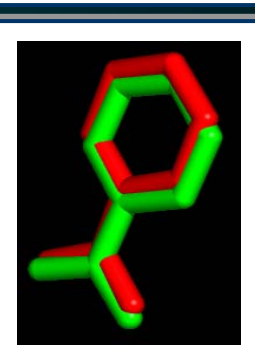
4.- Use SHIFTY to score docked poses by calculating proton chemical shifts for each pose

5.- Structure-based design of modified ligands









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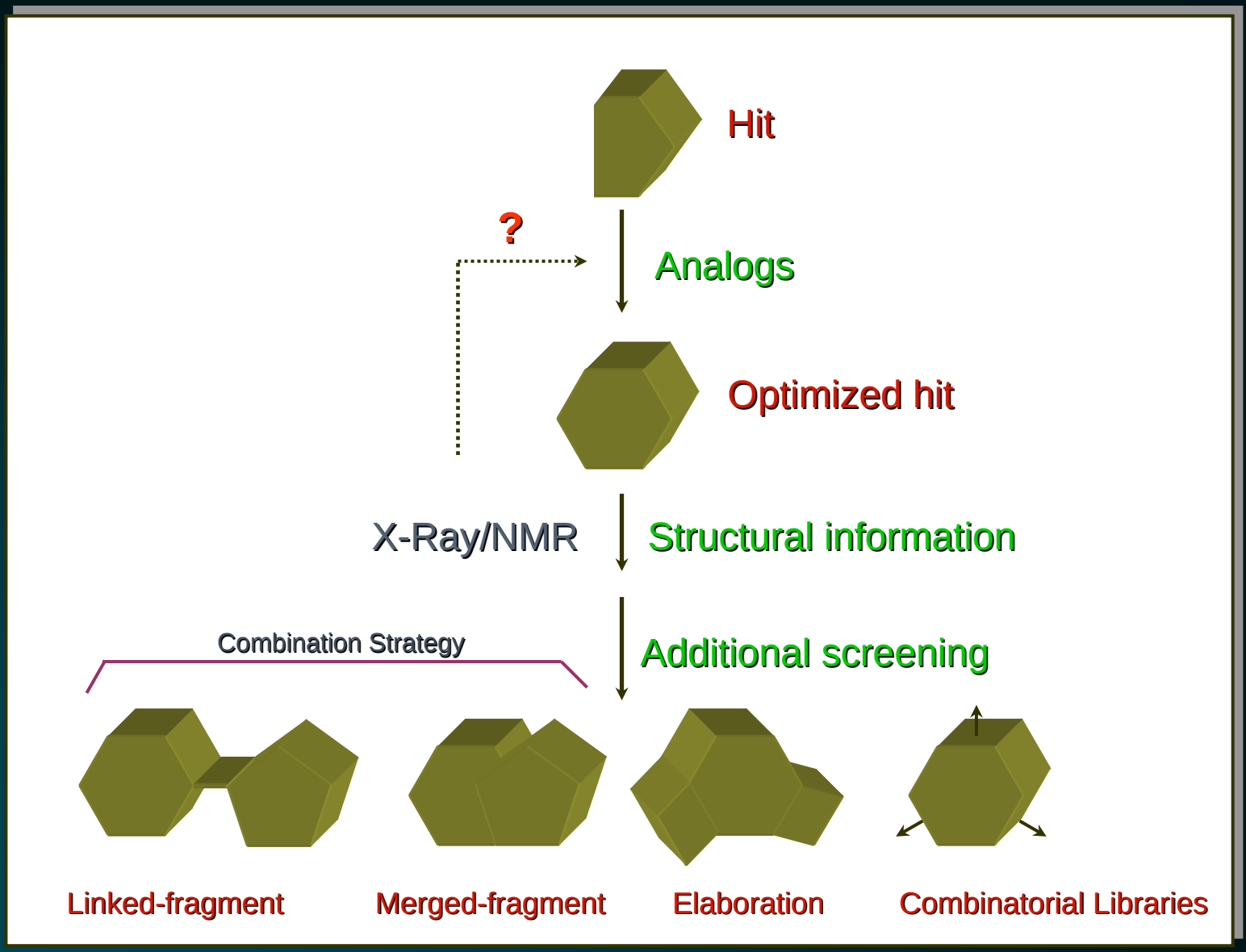
From Hit Identification to Lead Generation



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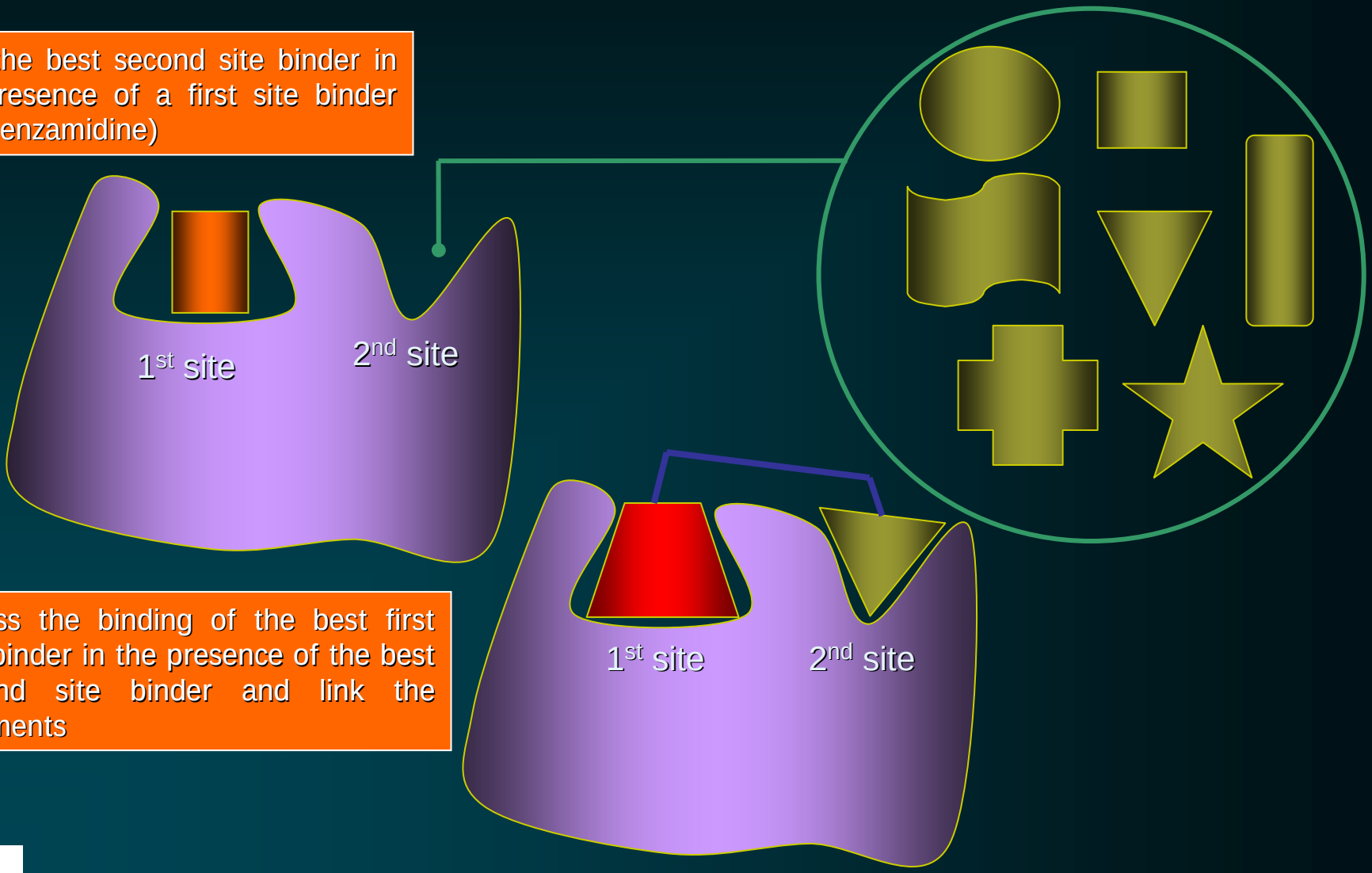
NMR Screening Campaign for Second Site Binders

Find the best second site binder in the presence of a first site binder (eg, benzamidine)



Assess the binding of the best first site binder in the presence of the best second site binder and link the fragments

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Summary

- *Fragment-based screening works best when you can rapidly increase the size of your molecule based on some rational means*
  - *e.g two fragments that bind to proximal sites almost guarantee a nM compound when linked properly*
- *Second site screening and linking are critical – much harder than finding 1st site ligands*
  - *substantially weaker affinities*
  - *presence of the first site blocker can interfere with the measurements*
- *Rapid explosion around a chemical series using high-throughput organic synthesis can be very successful*
  - *but only when we know (based on structure) where to modify and how far to extend out*
- *Fragment-based optimization is now finding significant application*
  - *even 1st site ligands can be valuable information to a mature project when coupled with structure and superimposed on the current series*
  - *Always surprises as to what groups bind where that can be incorporated*
  - *Not utilizing the exact fragment found by NMR, but using information derived from that fragment to impact a mature chemical series.*

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Lines of Research

- *The structural characterisation of proteins and peptides of pharmaceutical interest, especially in areas of cellular invasion and metastasis.*

- *The identification of modulators of the biological activity of targets of therapeutic interest. Design based on the structure of high-affinity inhibitors. Validation of compounds identified through HTP or virtual screening.*

- *Structural genomics with emphasis on the characterisation of proteins or protein domains related to pathologies.*

- *Control of stem cell fate by small molecules: Cell-type characterization and mode-of-action determination based on metabonomics.*

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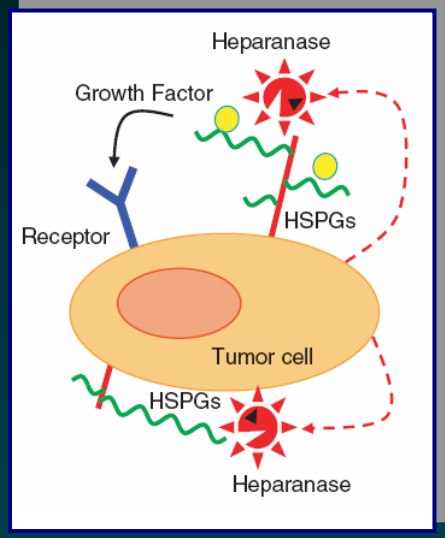
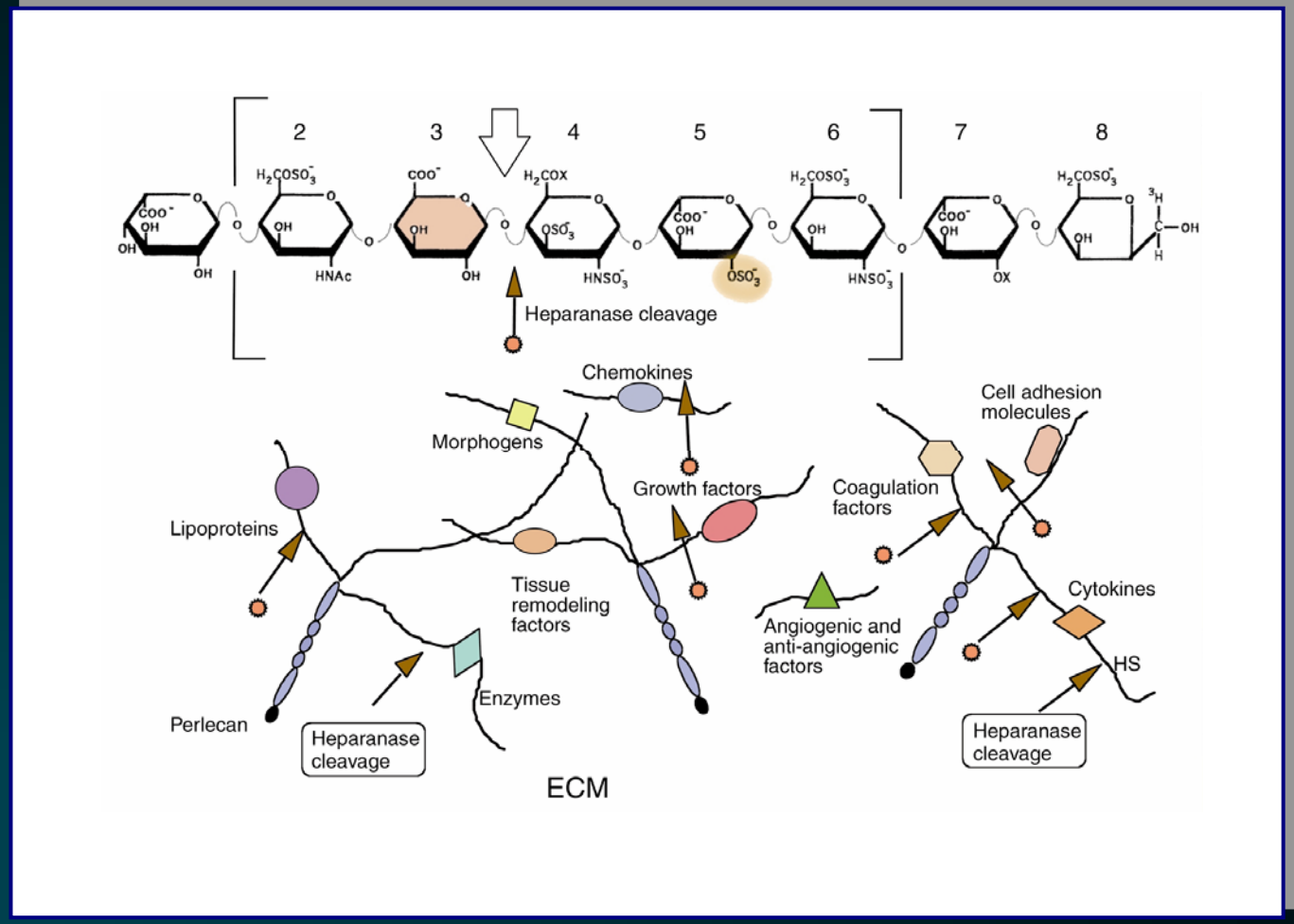
Heparanase: Biochemical Activity





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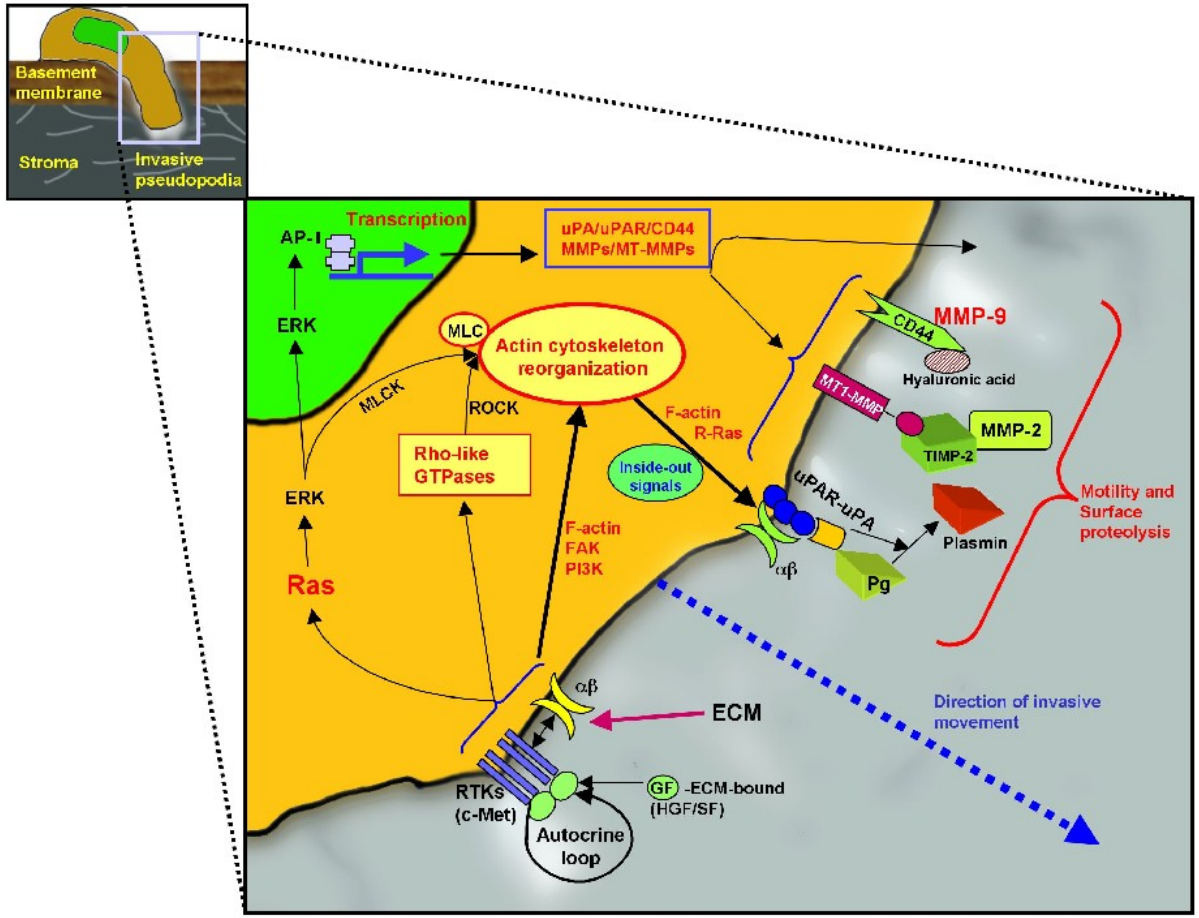
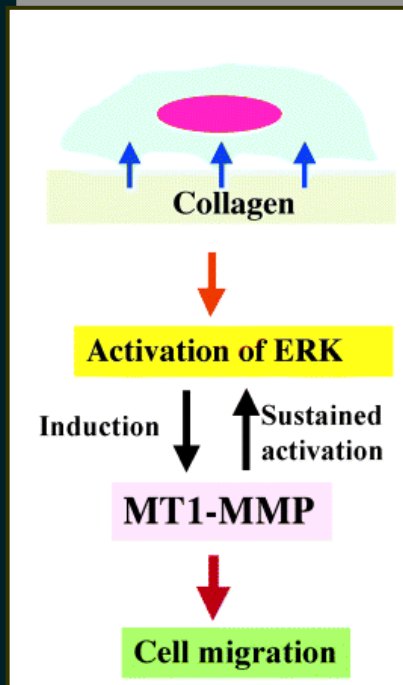
MMPs and Metastasis





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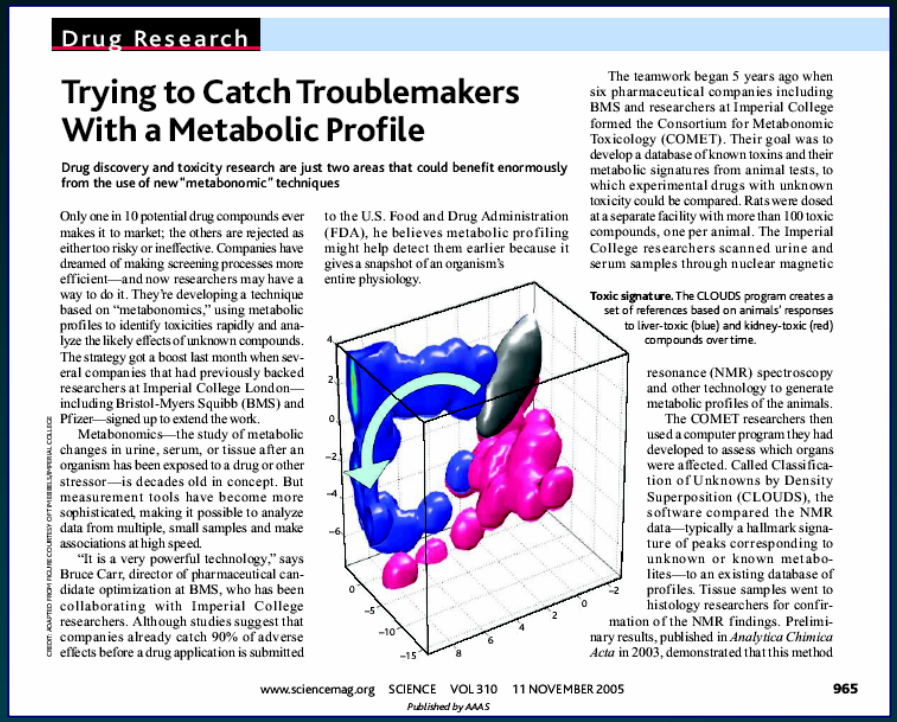
Toxicity Pre-evaluation: NMR Profiling



Drug Research

Trying to Catch Troublemakers With a Metabolic Profile

Drug discovery and toxicity research are just two areas that could benefit enormously from the use of new "metabonomic" techniques

Only one in 10 potential drug compounds ever makes it to market; the others are rejected as either too risky or ineffective. Companies have dreamed of making screening processes more efficient—and now researchers may have a way to do it. They're developing a technique based on "metabonomics," using metabolic profiles to identify toxicities rapidly and analyze the likely effects of unknown compounds. The strategy got a boost last month when several companies that had previously backed researchers at Imperial College London—including Bristol-Myers Squibb (BMS) and Pfizer—signed up to extend the work.

Metabonomics—the study of metabolic changes in urine, serum, or tissue after an organism has been exposed to a drug or other stressor—is decades old in concept. But measurement tools have become more sophisticated, making it possible to analyze data from multiple, small samples and make associations at high speed.

"It is a very powerful technology," says Bruce Carr, director of pharmaceutical candidate optimization at BMS, who has been collaborating with Imperial College researchers. Although studies suggest that companies already catch 90% of adverse effects before a drug application is submitted to the U.S. Food and Drug Administration (FDA), he believes metabolic profiling might help detect them earlier because it gives a snapshot of an organism's entire physiology.

The teamwork began 5 years ago when six pharmaceutical companies including BMS and researchers at Imperial College formed the Consortium for Metabonomic Toxicology (COMET). Their goal was to develop a database of known toxins and their metabolic signatures from animal tests, to which experimental drugs with unknown toxicity could be compared. Rats were dosed at a separate facility with more than 100 toxic compounds, one per animal. The Imperial College researchers scanned urine and serum samples through nuclear magnetic resonance (NMR) spectroscopy and other technology to generate metabolic profiles of the animals.

Toxic signature. The CLOUDS program creates a set of references based on animals' responses to liver-toxic (blue) and kidney-toxic (red) compounds over time.

The COMET researchers then used a computer program they had developed to assess which organs were affected. Called Classification of Unknowns by Density Superposition (CLOUDS), the software compared the NMR data—typically a hallmark signature of peaks corresponding to unknown or known metabolites—to an existing database of profiles. Tissue samples went to histology researchers for confirmation of the NMR findings. Preliminary results, published in *Analytica Chimica Acta* in 2003, demonstrated that this method

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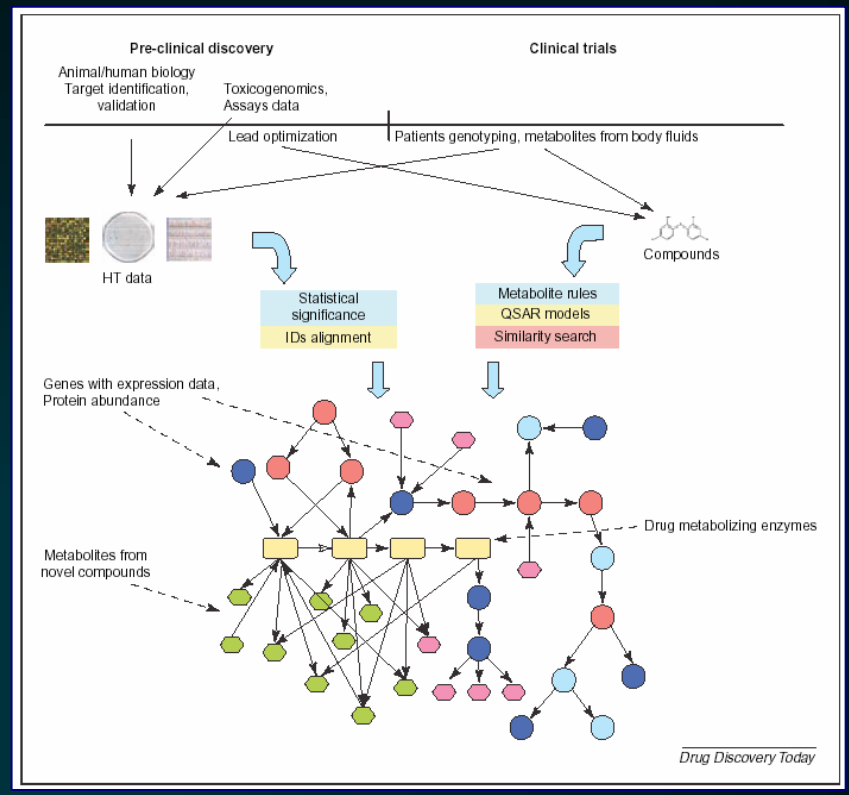

- *Metabonomics: Study of metabolic changes in urine, serum, or tissue after an organism has been exposed to a drug*
- *Biofluids analysis by NMR has already been shown to predict accurately liver and kidney toxicity*
- *COMET: COnsortium for MEtabonomic Toxicology. Academy/Industry partnership*

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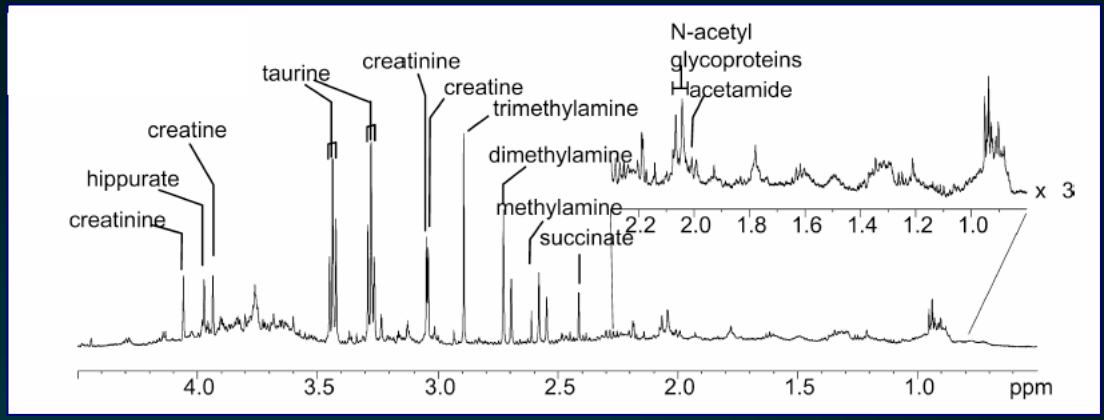
Toxicity Pre-evaluation: "In-cell" Profiling by NMR



- *"In-cell" NMR has a lot of potential applications:*
  - *Identification of novel Biomarkers (e.g., B-CLL project)*
  - *Characterization of interactions between apparently different cell-signalling pathways (e.g., combination drugs)*
  - ***Analysis (systems biology approach) of metabolic pathways after being exposed to a drug (e.g., detection of undesired interactions)***

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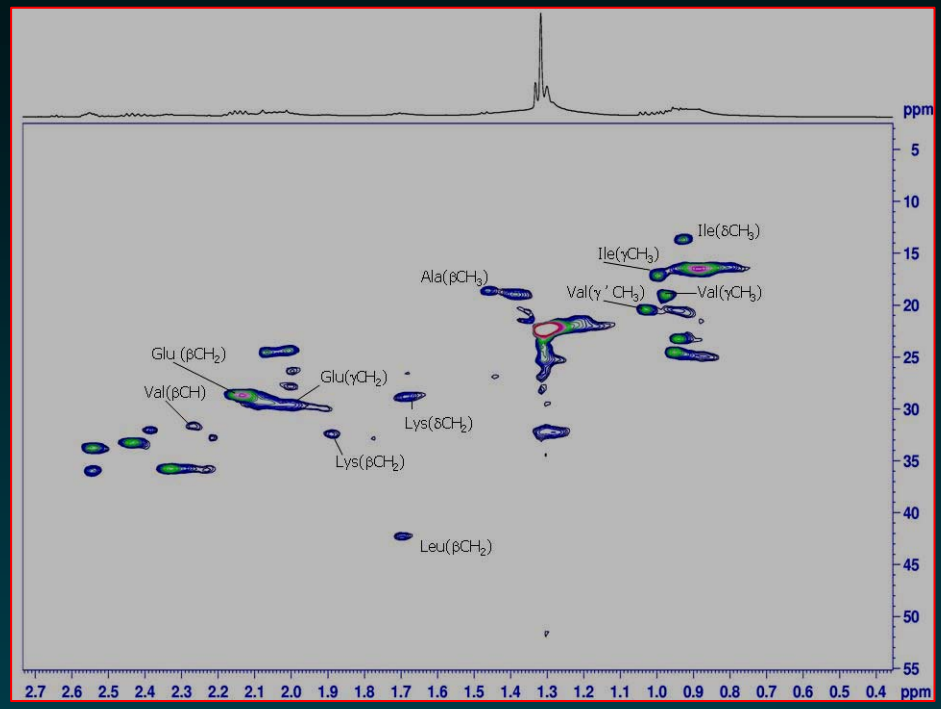
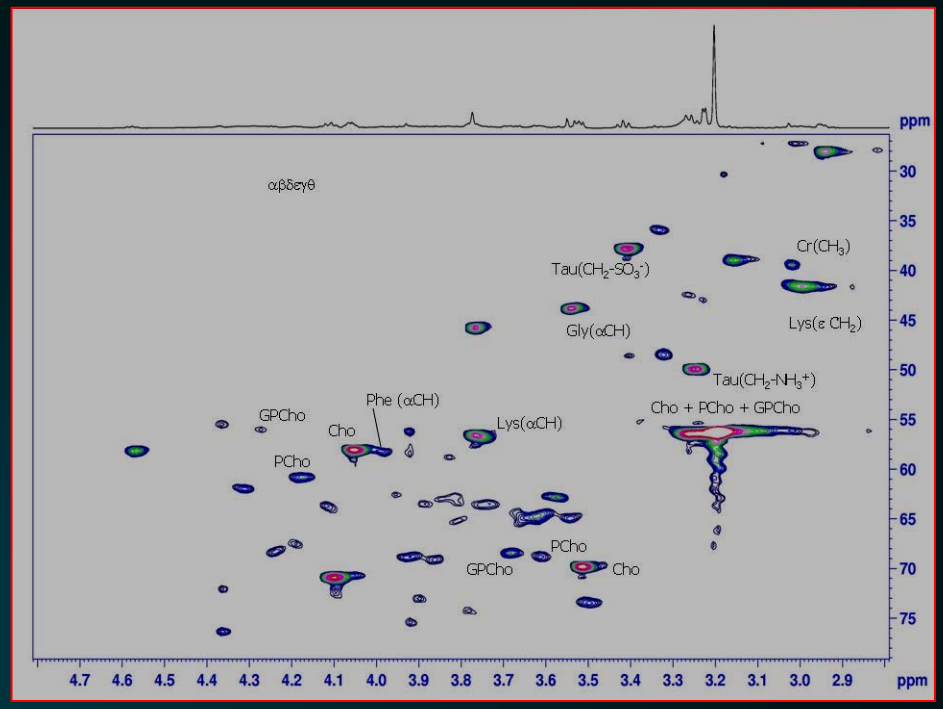
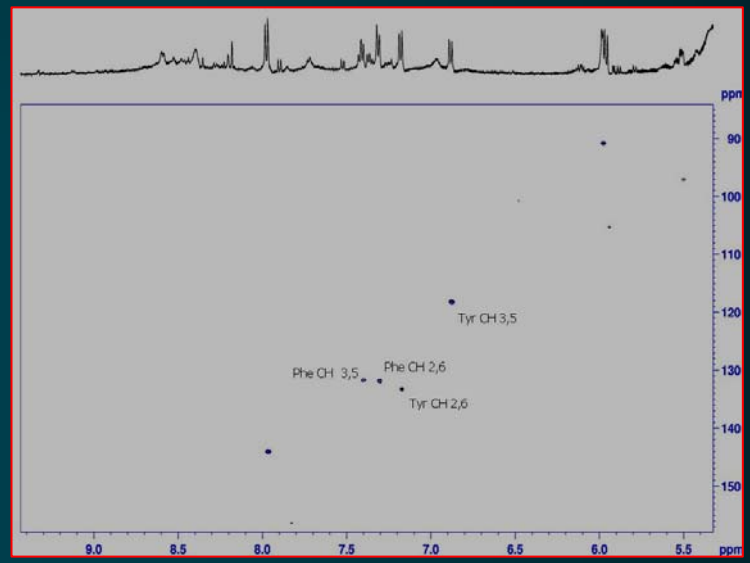
^{13}C-HSQC: Natural Abundance







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BRUKER BIOSPIN

Structural Genomics: Hsp90 Cofactors

Target 1: Tah1, Epigenetic Gene Regulation

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Navigating the Chaperone Network: An Integrative Map of Physical and Genetic Interactions Mediated by the Hsp90 Chaperone

Resource

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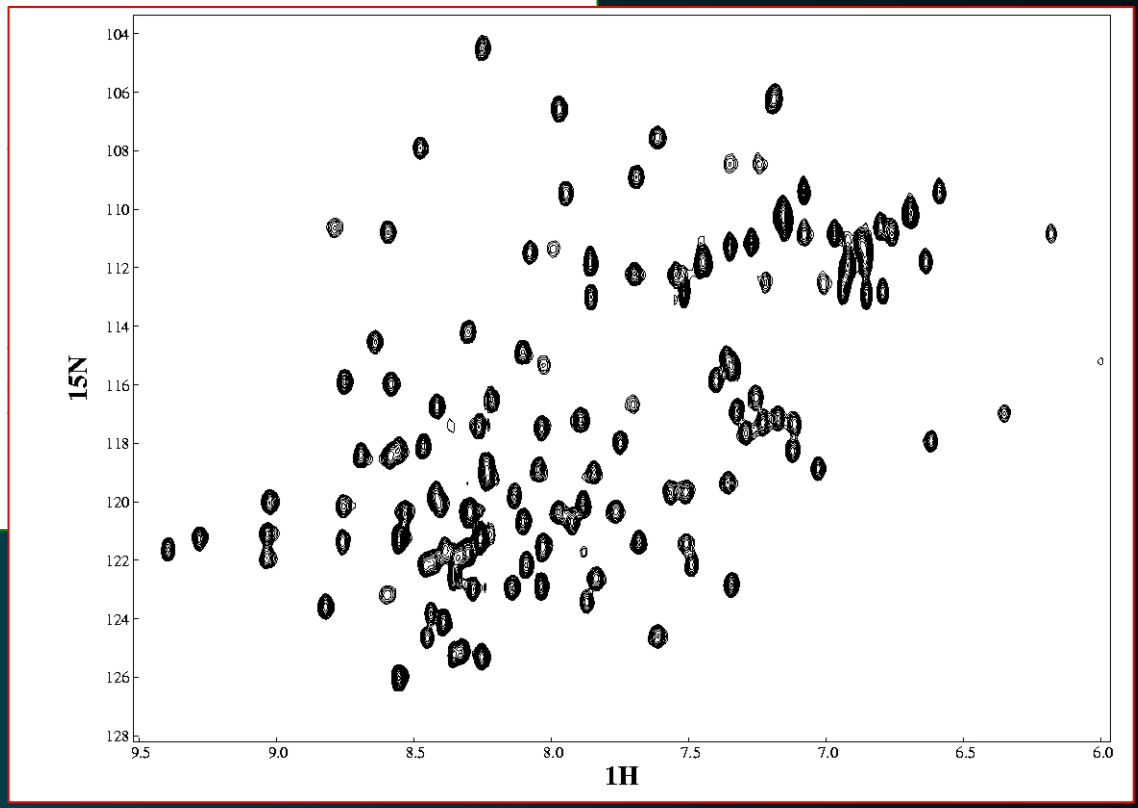

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Who are we?

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€ CIPF, MEC, FMMA, GVA

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