Drug development

Once a target has been identified (e.g. an enzyme), this is the conventional way:

1) Develop a sensitive activity assay (e.g. a substrate that looses or gains fluorescence in the enzymatic reaction)
2) High-throughput screening (e.g. 1 mio. compounds)

Drawbacks:

1) False positives (e.g., compounds may precipitate or denature the enzyme)
2) No inhibitor may be found
3) Structure-activity relationship (SAR) requires synthesis of many compounds, if 3D structure of target and ligand-binding mode is unknown
NMR in drug design I

- The $^{15}$N-HSQC spectrum displays one cross-peak for every amide, providing lots of site-specific probes.

- Binding of a ligand changes the chemical environment of some of the amides, causing chemical shift changes. This gives an idea, where the ligand binds.

E.g. Science 274, 1531 (1996)
Footnote: NMR signals broaden with increasing molecular weight
Footnote: complete structures of complexes can be determined by NMR

Pulse sequences can be designed so that intermolecular NOEs between $^{13}$C-labelled protein and DNA are selectively observed.

**NOESY spectrum showing intra-protein NOEs**

**NOESY spectrum showing intermolecular NOEs**

Red lines depict intermolecular NOEs

EMBO J. 9, 3085 (1990)
NMR in drug design II

Full structure determination

Intermolecular protein-ligand NOEs (purple lines) define the position of the ligand in the active site of the West Nile virus protease. In this example, the crystal structure of the protein is displayed. The ligand was docked using the NOEs and computational energy minimization.
Screening of chemical compound libraries by NMR

The NMR spectrum of a cocktail of compounds is recorded in the absence of the target protein (a) and in its presence (b). The protein signals are not observable, because the molecular weight of the protein is very high, resulting in extremely broad NMR signals that are indistinguishable from the baseline. The difference between spectrum (a) and (b) is shown in (c). It displays only three peaks (apart from the solvent signal in the centre). These three peaks belong to the compound the NMR spectrum of which is shown in (d). This compound obviously binds to the protein – its signals become as broad as those of the protein when it is bound and therefore disappear from the NMR spectrum in (b). If none of the compounds present in the cocktail binds to the protein, the difference between (a) and (b) is empty (example shown in (e)).

Even weak binding can be detected.
NMR in drug design IV

The compound is in fast exchange between free and bound state. Using an excess of compound, the protein signal is too small to be observed. Does the compound bind?

A) The high molecular weight of the protein broadens the peaks of the compound a little.

B) The paramagnetism of TEMPO (tetramethyl-piperidine-oxyl, shown in C) broadens the signals of a weakly binding compound dramatically.
Second-site screening: Compound 2 binds. A derivative of compound 2 containing a TEMPO group (2*) dramatically broadens the signals of any other compound binding nearby.

Identification of a second-site ligand. A mixture of eight aromatic compounds + spin-labelled compound 2* was used.
Left spectrum: without protein
Right spectrum: with protein
One of the aromatic compounds binds at a site different from 2*.
Irradiation of the protein at a resonance where no ligand signals are present leads to a selective and very efficient saturation of the entire protein by spin diffusion. Saturation is transferred to the binding parts of the ligand by intermolecular saturation transfer. Here, groups represented by the large proton are in close contact with the protein, while the medium-sized proton symbolizes a group with less interaction. The smallest proton represents a group with almost no contact with the protein, thus receiving minimal saturation. Therefore, the degree of saturation of the individual protons of a small ligand molecule reflects the proximity of these to the protein surface.

Spin-diffusion: transfer of magnetization from spin A to spin C via an intermediate spin B (A → B → C) by NOE
Saturation transfer: saturating the NMR signal of a spin by selective irradiation leads to saturation of spins in its vicinity (again by NOE)
(A) Reference 1D NMR spectrum of the 120-kDa lectin RCA120 (50 μM), displaying the very broad lines normal for a protein this size. The few sharp resonances arise from low-molecular-weight impurities.

(B) Corresponding STD NMR spectrum showing that, by irradiating at -2 ppm, the entire protein is saturated uniformly and can therefore be efficiently used for the STD NMR technique. One can also see that the impurities contained in the spectrum are effectively subtracted and therefore do not give rise to signals in the difference spectrum.

(C) 1D NMR spectrum recorded with a $T_1$ filter, consisting of a 30-ms spin-lock pulse, to eliminate the broad resonances of the protein. Only those resonances of the low-molecular-weight impurities remain in the spectrum.

(D) Reference 1D NMR spectrum of RCA120 in the presence of 1.2 mM GalOMe, without the $T_1$ filter.

(E) Corresponding STD NMR spectrum showing that GalOMe yields signals and therefore binds to the receptor.

(F) STD NMR spectrum as in (E) but with the $T_1$ filter eliminating all protein background signals.

**STD NMR:** saturation transfer difference. A 1D NMR spectrum acquired *without* selective irradiation is subtracted from a 1D NMR spectrum acquired *with* selective irradiation that hits only protein signals.

$T_1$ filter: the NMR signal is acquired only after a delay during which the magnetization is kept transverse. This suppresses broad NMR signals, because they correspond to faster-relaxing magnetization.
NMR in drug design VII

WaterLOGSY

WaterLOGSY is the most sensitive screening technique by NMR.

The protein is shown with the buried cavities and the active binding site. The ligand is shown in the bound and free states. Excitation of bulk water (circles) is shown with a solid arrow and some of the different magnetization transfer pathways are shown with curved lines.

In a WaterLOGSY experiment, the NMR resonance of the water is selectively excited and the magnetization is allowed to be transferred by NOE to all other protons. The water magnetization is transferred by NOE to the protein and from there to the ligand (spin-diffusion). The NOE between water and macromolecules has the opposite sign from the NOE between water and small molecules. The ligand is used in very large excess over the protein so that the protein is not observable in the NMR spectrum.

J. Biomol. NMR 21, 349 (2001)
WaterLOGSY spectrum for ligands E and F (200 µM) in the presence of 10 µM of target protein. Positive and negative signals identify binding and non-interacting molecules, respectively.
NMR in drug design VIII

Transferred NOE

Determines the conformation of the bound ligand.

Dilemma: the signal of the bound ligand is very broad (high molecular weight complex)

Trick: if the ligand exchanges between bound and free state, the average signal can be quite narrow if the ligand is in large excess. Magnetization transfer by NOEs is much faster in the bound state, i.e. the NOEs observed between the averaged signals arise almost exclusively from the bound state.

Example: peptide ESKATNATLDPR bound to thrombin (15-fold excess of peptide). The NOESY spectrum shows (almost only) cross-peaks between peptide signals. They were used to determine the conformation of the bound peptide. The structure can be docked into the (independently determined) crystal structure of the protein.

Biochemistry 31, 11551 (1992)
Summary NMR methods for drug design:

1: follow chemical shift changes in HSQC spectrum
2: full structure determination of the protein-ligand complex (intermolecular NOEs)
3: identify ligands that bind to protein target by the line-broadening due to high MW of complex
4: identify target-binding ligands with increased sensitivity using paramagnetically tagged proteins
5: saturation transfer difference (STD): identifies target-binding ligands (even parts thereof)
6: WaterLOGSY: identify target-binding ligands with increased sensitivity using water magnetization
7: transferred NOEs: conformation analysis of the bound ligand
8: fragment-based drug design: discovering second-site ligands by screening compound libraries in the presence of the compound occupying the first binding site (especially good if first compound is tagged with TEMPO)
Reducing the size of the chemical library

**SHAPES strategy**

32 different frameworks describe ~50% of all existing drugs.
Use at least one nitrogen or oxygen to improve solubility.
Use 30 most common side chains.
Synthetic simplicity.

→ library of 132 compounds

Chemistry & Biology 6, 755 (1999)
Fragment-based drug design starting from organic solvents

- Soak single crystal of target enzyme with small molecules (e.g. acetonitrile, ethanol, hexenediol, isopropanol, DMF, acetone)
- Solve crystal structure
- Construct larger molecules that fill all sites capable of binding small molecules

Example: elastase structures with different small molecules (top)
A) Different ligands in the active site. The purple and green ligands only fill two of the pockets identified by small molecules.
B) This ligand fills three of the pockets identified by small molecules.

Drawback: crystal contacts interfere