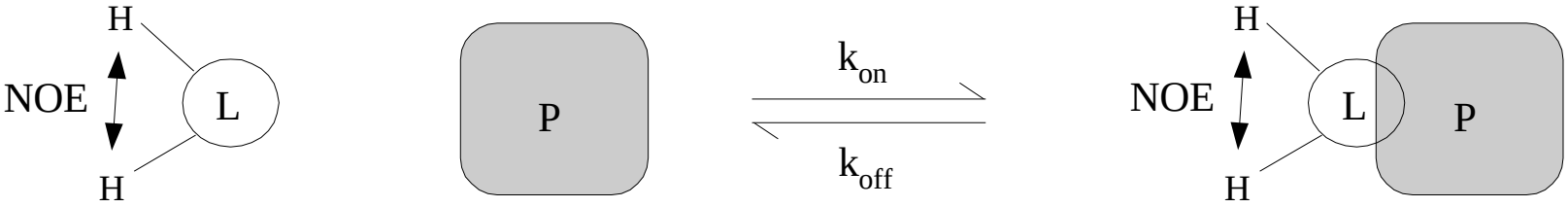
# NOE and dynamic processes - 'transferred' NOE




$\sigma_{IS} \gg k_{off}$ Cross relaxation acts indepently in the free and in the bound state.
The resulting NOESY spectrum is a popuation weighted superposition of the free and the bound state.

$\sigma_{IS} \ll k_{off}$ The chemical exchange causes an averaging of the cross relaxation rate:

$$\sigma_{IS} = x^{free} \sigma_{IS}^{free} + x^{bound} \sigma_{IS}^{bound}$$

# Complex formation and fast exchange

fast exchange on the chemical shift time scale $\Delta\omega \ll k_{ex}$

→ observation of the averaged chemical shift

$$\delta^{obs} = x^{free}\,\delta^{free} + x^{bound}\,\delta^{bound}$$

fast exchange on the time scale of transverse relaxation $k_{ex} \gg R_2$

→ observation of a averaged transverse relaxation rate

$$R_2^{obs} = x^{free}\,R_2^{free} + x^{bound}\,R_2^{bound}$$

fast exchange of the time scale of the cross relaxation rate $\sigma_{IS} \ll k_{ex}$

→ observation of the averaged cross relaxation rate

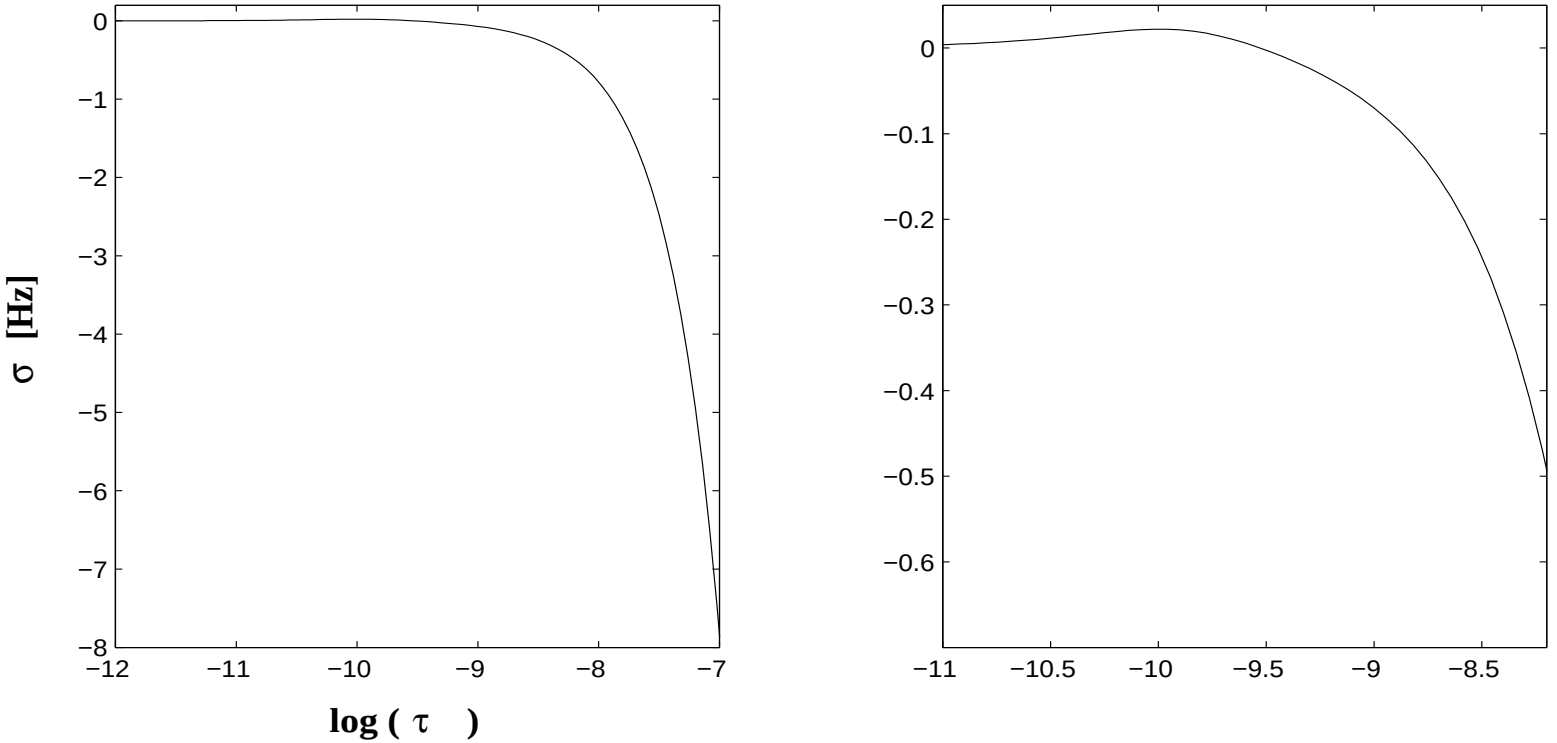
$$\sigma_{IS} = x^{free}\,\sigma_{IS}^{free} + x^{bound}\,\sigma_{IS}^{bound}$$

Dependence of the cross relaxation rate on the rotational correlation time

$$\sigma_{ij} = \frac{1}{10}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\hbar^4 \gamma_I^4}{r_{ij}^6} \tau_c \left\{ \frac{6}{1+4\omega_0^2\tau_c^2} - 1 \right\}$$

$\sigma_{IS} = 0$, if $\tau_c = \frac{1}{\omega_0}\sqrt{\frac{5}{4}}$ e.g. $\tau_c(\sigma_{IS})$ approx. $0.3\,ns$ at 600 MHz

$\nu_0$ = 600 MHz, $r_{ij}$ = 0.3 nm



small molecules

$\tau_c < 0.3\,ns$ , $MW < 500\,Da$

→ low cross relaxation rates

requires long mixing times in a NOESY experiment (ca. 0.5 - 2 s)

different sign of cross signals relative to the diagonal signals in a NOESY spectrum

larger molecules

$\tau_c > 1.5\,ns$ , $MW > 1500\,Da$

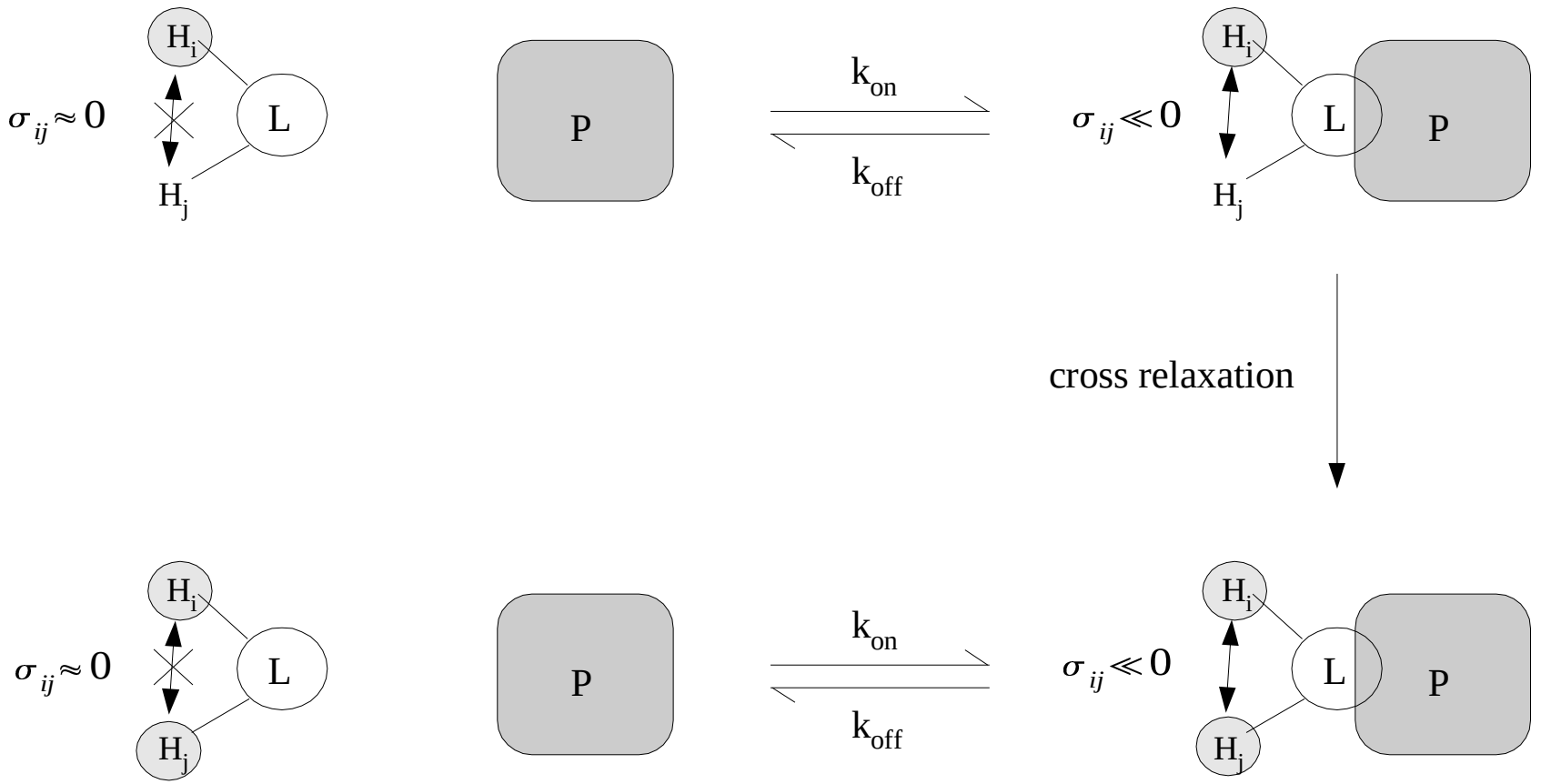
→ larger cross relaxation rates

short mixing times in a NOESY experiment (< 0.2)

same sign of cross signals relative to the diagonal signals in a NOESY spectrum

In the case of averaged cross relaxation rates the rate in the bound state can contribute the main part of the observed rate, even in the case of $x^{bound} << x^{free}$ :

$$\sigma_{IS} = x^{free}\,\sigma_{IS}^{free} + x^{bound}\,\sigma_{IS}^{bound}$$




In this case, cross relaxation acts only in the bound state

If

$$x^{free} \gg x^{bound} \quad \text{(e.g. 10:1)}$$

$$x^{free} \cdot \sigma^{free} \ll x^{bound} \cdot \sigma^{bound}$$

$$x^{free} \cdot R_2^{free} \gg x^{free} \cdot R_2^{bound}$$

is valid,

then mainly the NOE effects of the bound state are observed with line widths and resonance positions of the free state.

Therefore information of the bound conformation of a given ligand in the presence of a large protein is tranferred to the unbound NMR-favored state.

# Praxis of the tr-NOESY experiment

1. Determination / estimation of the cross relaxation rates of the given samll molecule in the unbound state

   -> Determinationof the cross relaxation rates by a series of NOESY experiments with variable mixing times

2. Preparation of the complex

   $\tau_c$ of the complex and the exchange rates determines the useful sample composition.

   With increasing molecular size (e.g. 100 kDa) the concentration of the protein can be reduced.

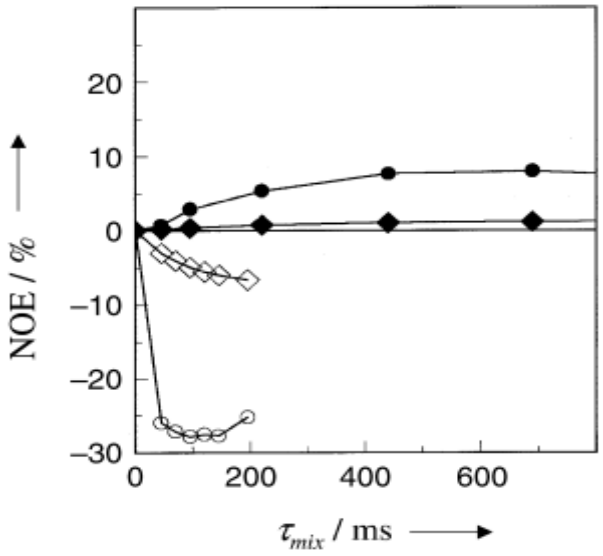
3. Conduction of the tr-NOESY experiment

   Again determination of the cross relaxation rates by running a series of NOESY experiments with variable mixing times.

   Additional T2 filter for suppressing the residual protein background

   (e.g. simple spin echo or 10 ms spinlock pulse before data acquisition)

Build up of cross peaks in a transferred-NOESY experiment




Build up of cross signals in a NOESY of a small oligosaccharide

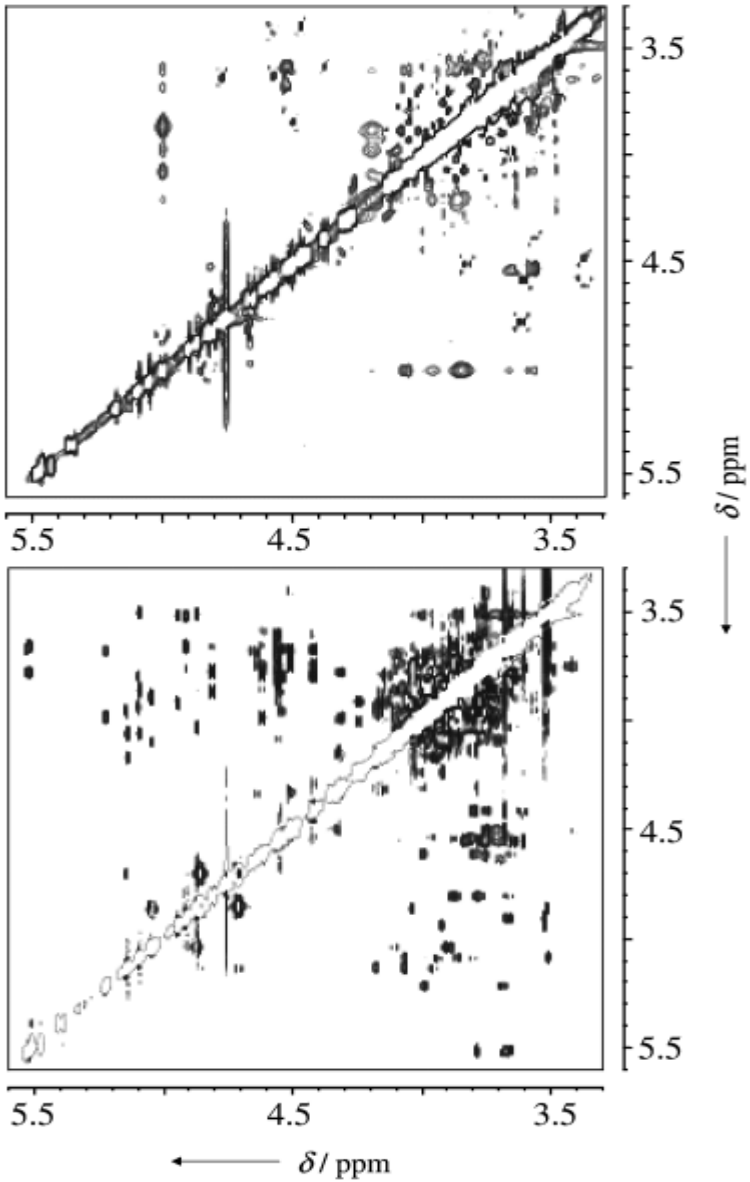
in the absence (filled symbols) and

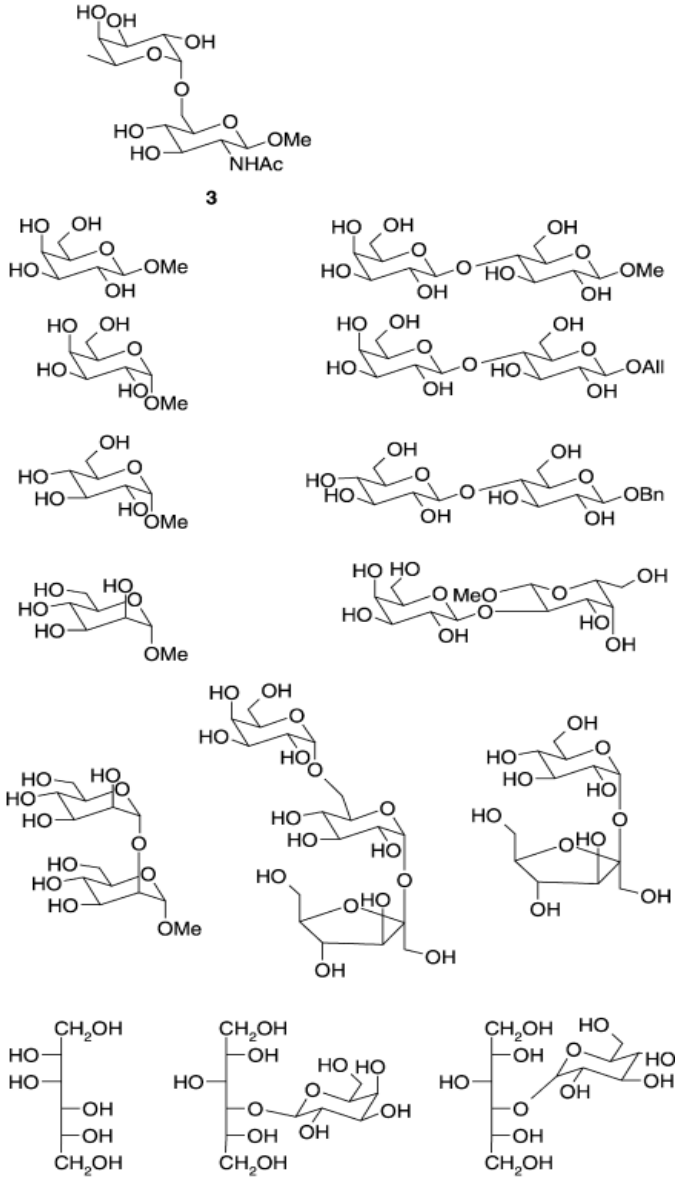
in the presence (open symbols) of a protein

# Transferred-NOESY spectrum of an oligosaccharide-library in the presence of a protein

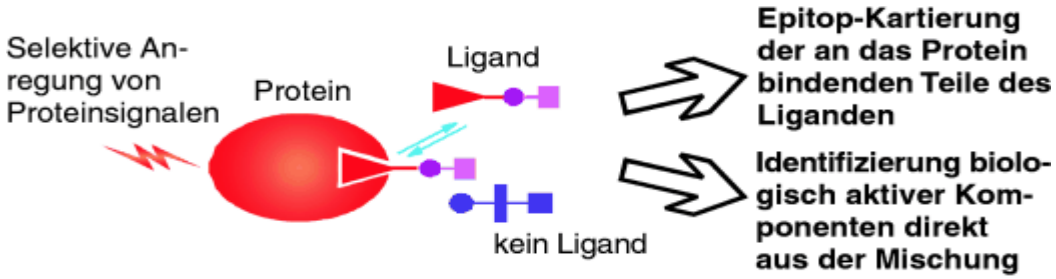
negative diagonal signals
negative cross signals

negative diagonal signals
positive cross signals



# The STD-experiment
# (STD = Saturation Transfer Difference)




Selective saturation of the protein signals: Irradiation in the methyl group region,

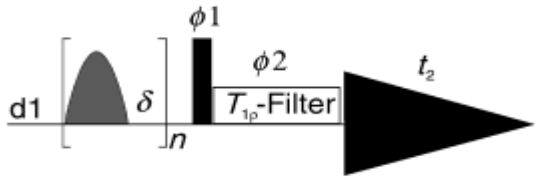
aromatic or amide proton region

(far away from ligand signals)

Spindiffusion averages the saturation over the protein

→ In the case of ligand binding the saturation can be transferred from the protein to the ligand

# The 1D STD NMR experiment




| | |
|---|---|
| ϕ1 | (x,-x,-x,x,y,-y,-y,y,-x,x,x,-x,-y,y,y,-y) |
| ϕ2 | ( 2(y,-y), 2(-x,x)) |
| $\phi_{rec}$ | (2(x),2(-x),2(y),2(-y),2(-x),2(x),2(-y),2(y)) |

The saturation is done by a series of bandselective pulses (e.g. gaussian shape) over a duration of approx. 2 seconds, e.g. 40 pulses with 50 ms pulse length.

For each scan the saturation is applied on-resonant and off-resonant (e.g. 40 ppm away) in an alterning fashion.

The on-resonant experiment gives the saturation experiment, the off-resonant spectrum serves as reference.

# Example for a 1D STD NMR experiment

Probing the binding of β-D-GalOMe to the protein $RCA_{120}$ (120 kDa)

| | |
|---|---|
| protein concentration | 50 µM (in relation to the binding sites) |
| concentration of β-D-GalOMe | 1.2 mM |

A) 1D reference spectrum of the protein

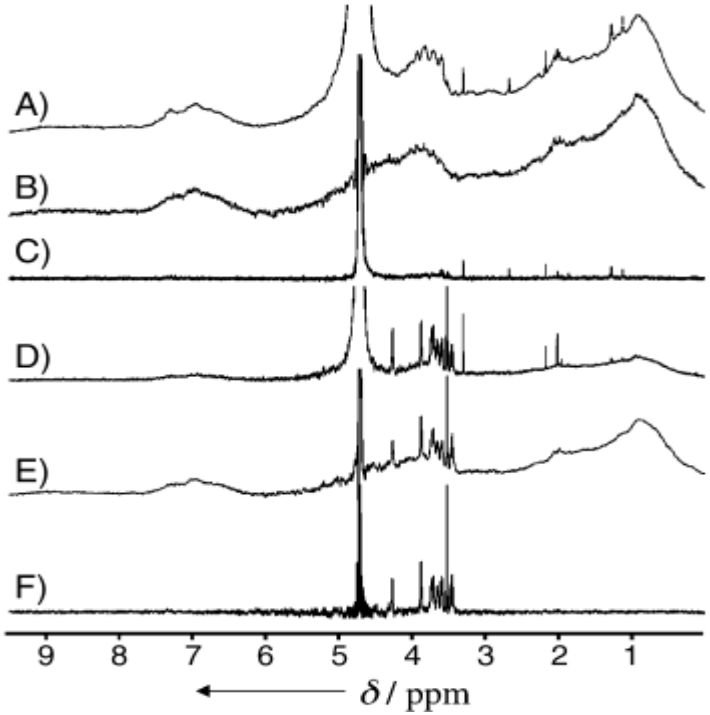
B) STD spectrum (reference spectrum - saturated spectrum)

C) 1D spectrum of the protein with a 30 ms $T_{1\rho}$ filter

D) 1D reference spectrum of the protein in the presence of the ligand

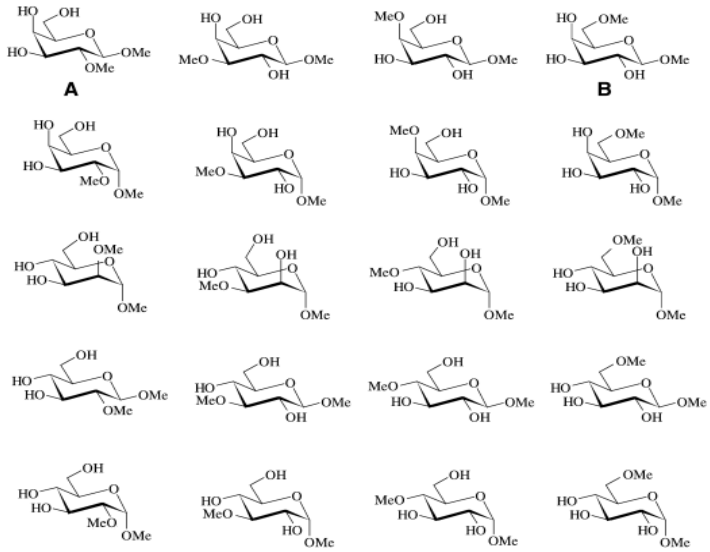
E) STD spectrum in the presence of the ligand

F) STD spectrum with the additional 30 ms $T_{1\rho}$ filter
in the presence of the ligand

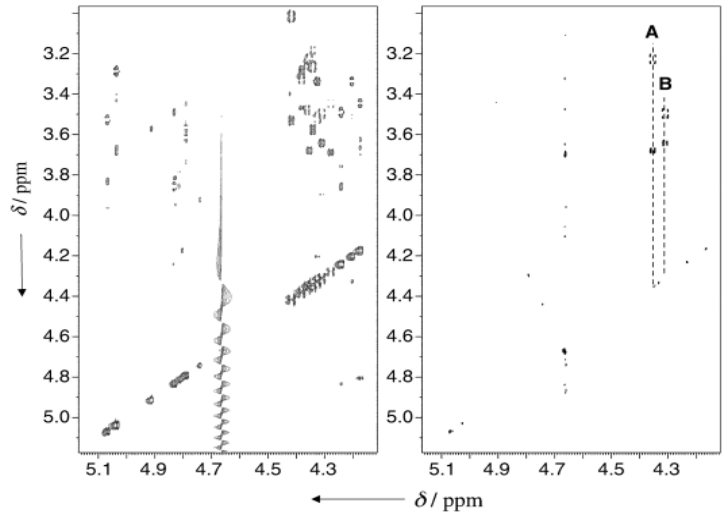


# Combination of the STD experiment with a 2D correlation experiment

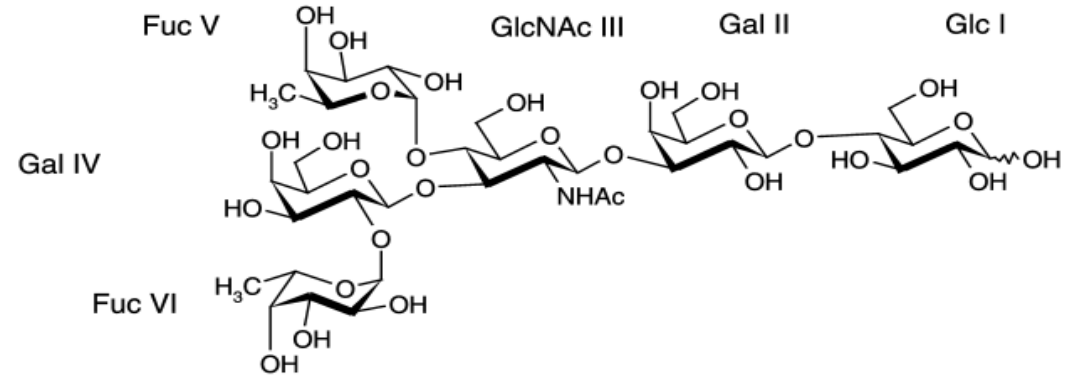
Testing the binding of a mixture of 20 arbitrary dimethylated monosaccharides to lectin of Sambucus nigra (SNA, Sambucus igra aglutinin)

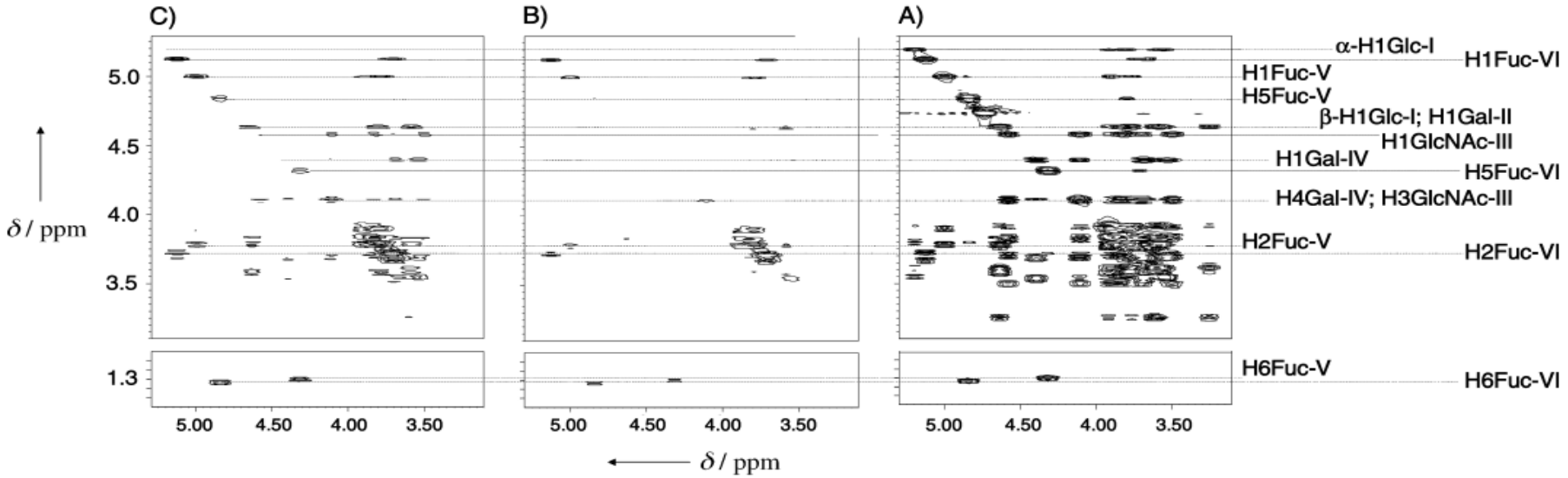



left: 2D TOCSY
right: 2D STD-TOCSY



Determination of the binding epitope of lacto-N-difucosylhexanose I, bound to AAA




STD-TOCSY
(low contour levels)

STD-TOCSY
(high contour levels)

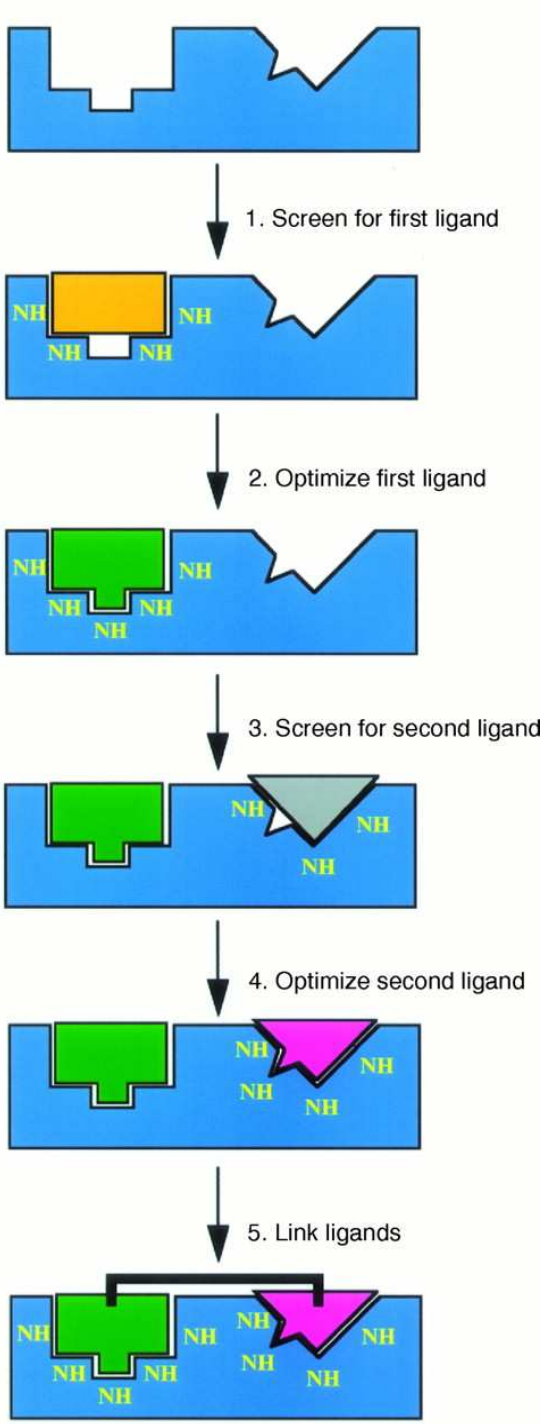
2D TOCSY of the free oligosaccharide

# Discovering high affinity ligands for proteins - SAR by NMR

Suzanne B. Shuker, Philip J. Hajduk, Robert P. Meadows & Stephen W. Fesik (1996), Science 274, 1531-1543

| | |
|---|---|
| Search for new pharmaceuticals | finding of active compounds by screening of large substance libraries and subsequent optimization of their properties by synthesizing and testing of structural related compounds |
| Method of the Abbott team | Search for a binding ligand using $^{1}H$, $^{15}N$ HSQC experiments<br>optimization of the binding ligand<br>search for a second binding ligand with a binding site near to the first binding site (structure of protein is known)<br>linking both ligands to a new molecule |

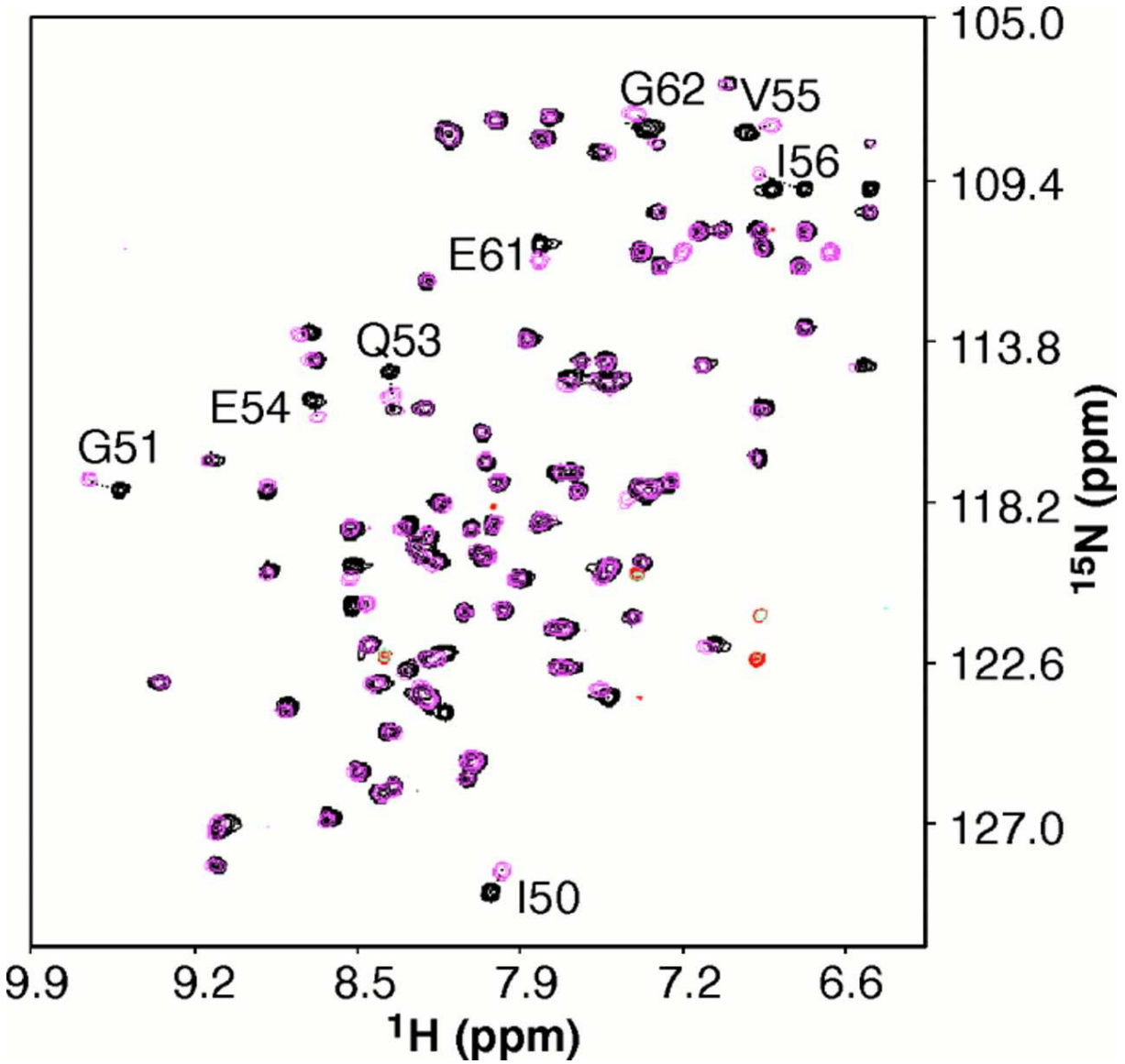
schematic procedure of the SAR-by-NMR method



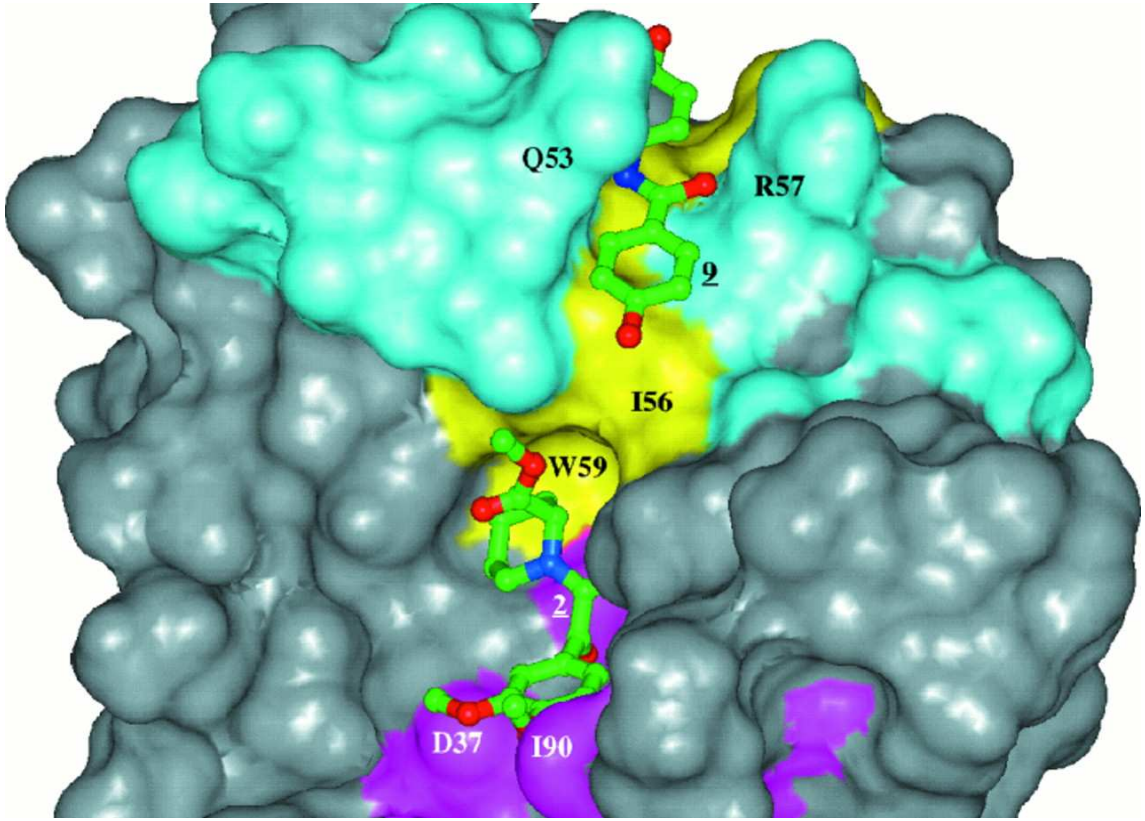
$^{1}H$, $^{15}N$ HSQC of FKBP in

the presence of a binding ligand (magenta)

the absence of a binding ligand (black)

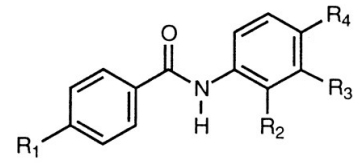


Determination of the ligand orientation by observation of intermolecular NOEs

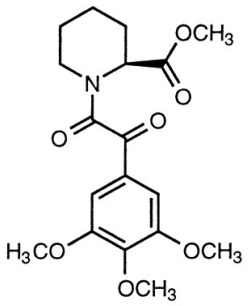



Color coding represents the changes of chemical shifts of FKBP in the presence of the ligands

Optimization of the affinity by linking of both ligands

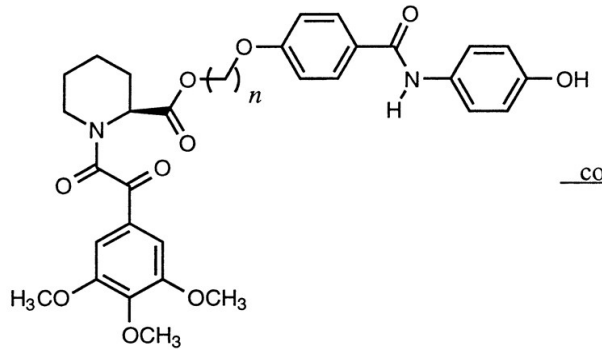



| compound | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $K_d$ |
|---|---|---|---|---|---|
| **3** | OH | OH | H | H | 0.8 mM |
| **4** | H | OH | H | H | 2.1 mM |
| **5** | OH | H | H | H | 0.6 mM |
| **6** | H | H | OH | H | 1.4 mM |
| **7** | H | H | H | OH | 0.5 mM |
| **8** | H | H | H | H | 9.5 mM |
| **9** | OH | H | H | OH | 0.1 mM |

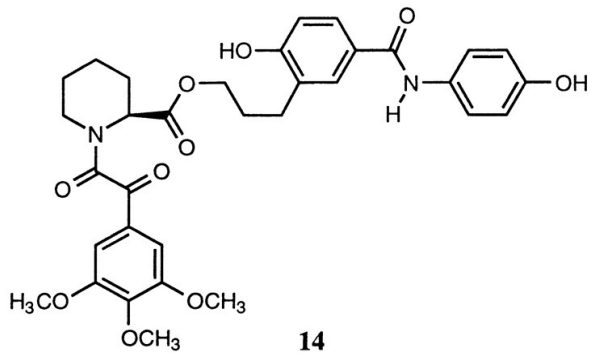



**2**

$K_d = 2\ \mu M$

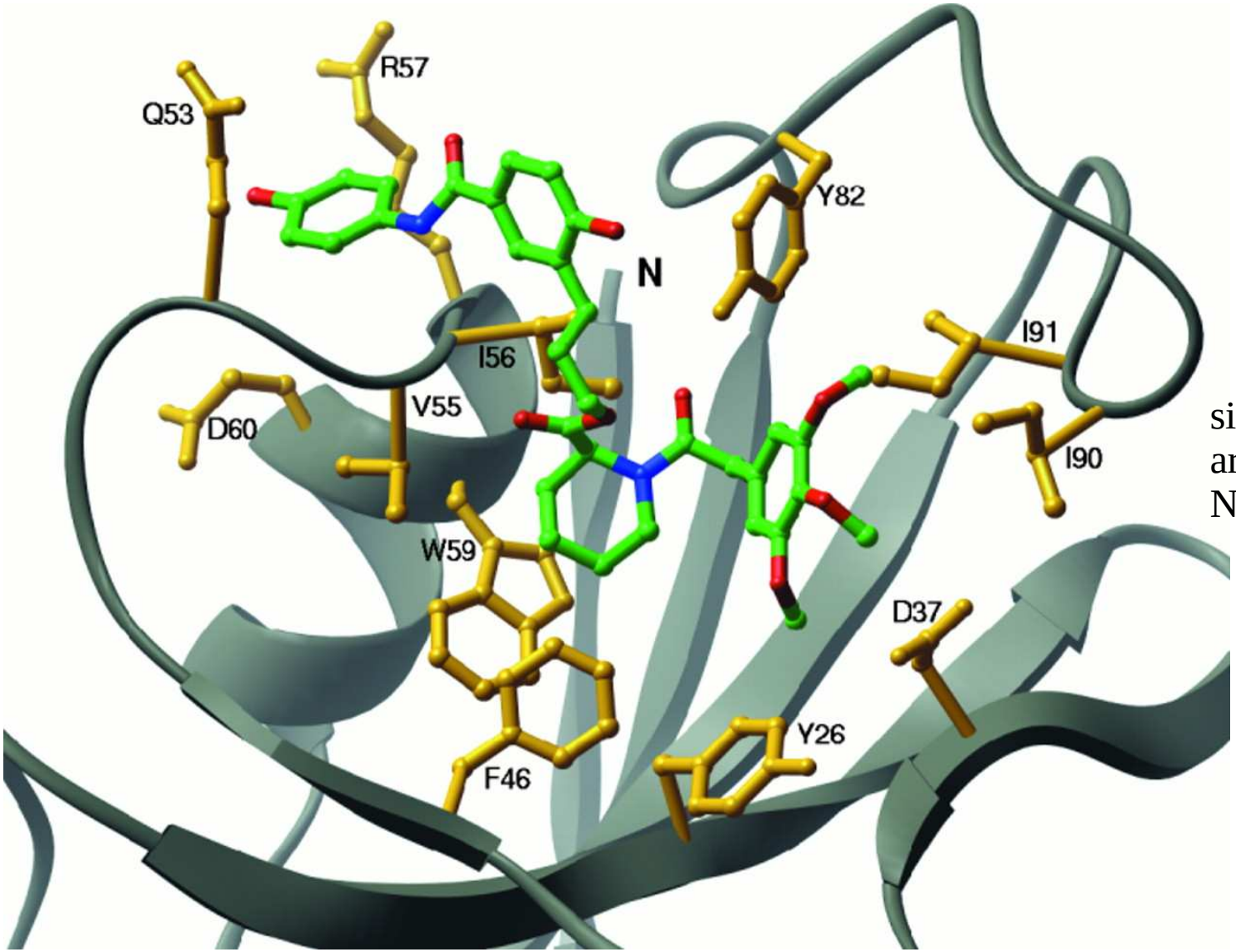



| compound | *n* | $K_d$ |
|---|---|---|
| **10** | 3 | 19 nM |
| **11** | 4 | 32 nM |
| **12** | 5 | 49 nM |
| **13** | 6 | 228 nM |
| **14** | - | 49 nM |




**14**

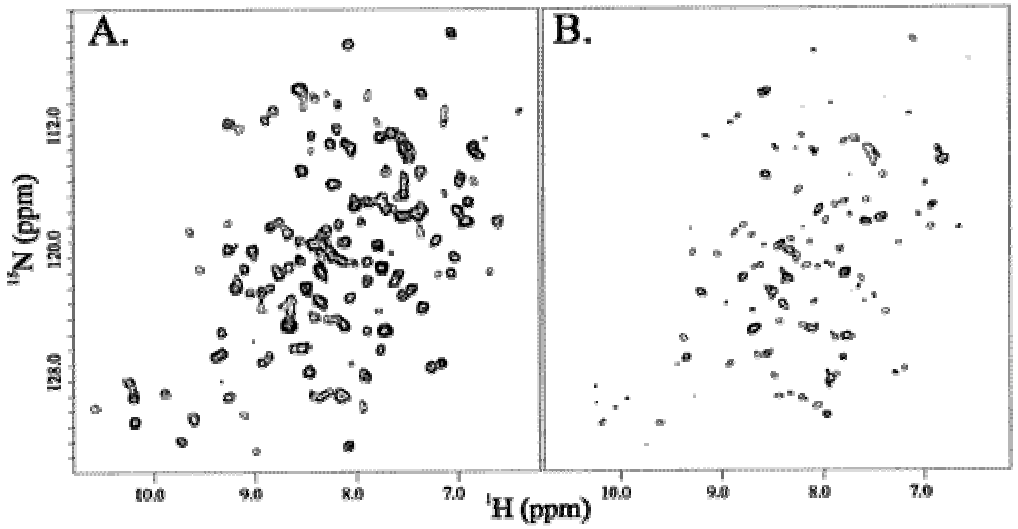
Structure determination of the new complex using NMR spectroscopy




side chains shown in yellow are involved in intermolecular NOEs

Extension of the HSQC screening by using modern cryo probes
(P.J.Hajduk et al. (1999), J. Med.Chem 42, 2315-2317)

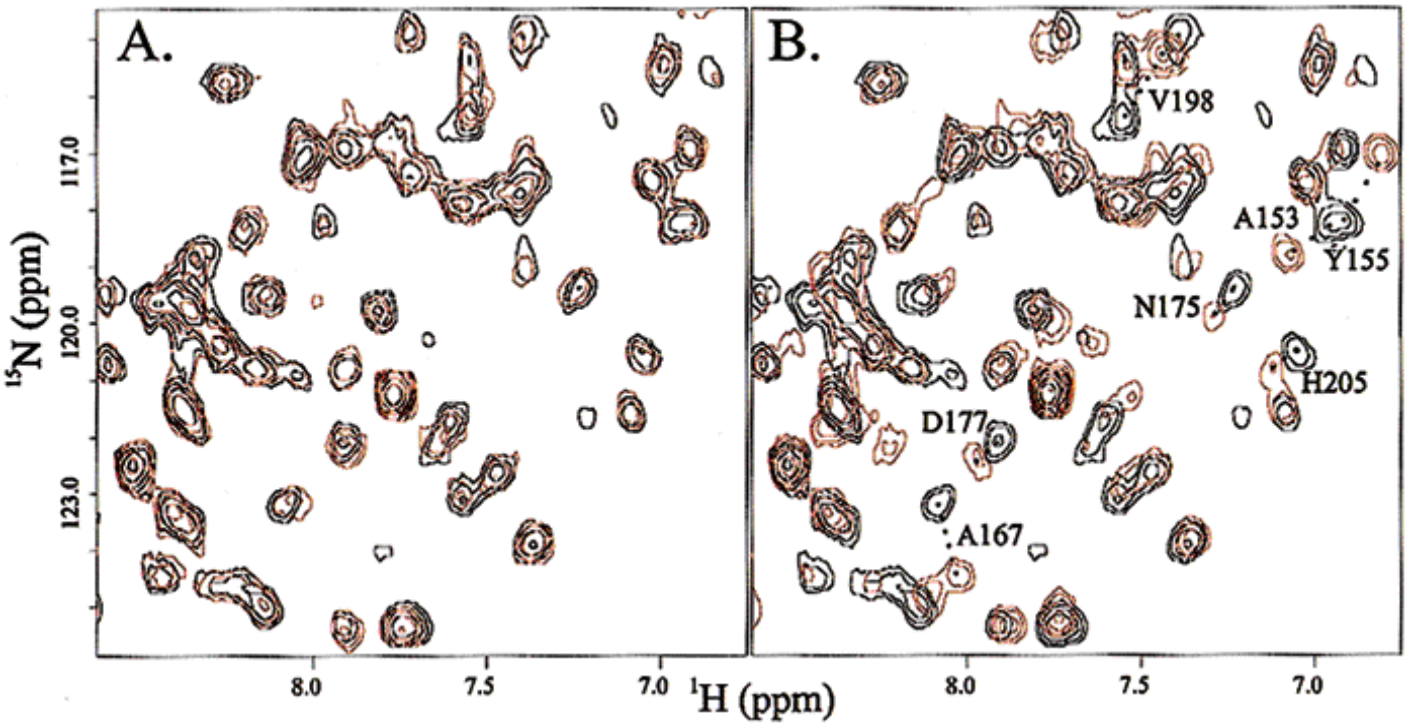
$^{1}H$, $^{15}N$ HSQC of the catalytic domain of stromelysin, 50 µM, 10 min for the NMR experiment
(NS = 8, TD1 = 64, 500 MHz)




cryoprobe regular probe

→ S/N improvement by a factor of 2.4

$^{1}H$, $^{15}N$ HSQC of the catalytic domain of stromelysin, 50 µM, 10 min for the NMR experiment (NS = 8, TD1 = 64, 500 MHz)




in the presence of a mixture of
100 (!) non binding molecules (each 50 µM)

in the presence of a mixture of
100 (!) non binding molecules (each 50 µM)
plus one known inhibitor

# (High) Through-put HSQC screening

Duration of one NMR experiment
(automatic sample changer)
→ ca. 10 min plus preparation (Shimming ...)
ca. 100-150 experiments per day

| | | |
|---|---|---|
| Number of testing ligands | 10 | ca. 20000 compounds per month |
| in one NMR sample | 100 | ca. 200000 compounds per month |

Required $^{15}N$ labeled protein

MW = 15 kDa, 50 µM in a 500 µl sample
→ 0.375 mg per NMR sample
ca. 40 mg / day