Polarized neutron scattering from dynamic polarized nuclei.

Heinrich Stuhrmann

To be presented at the Conference on

Neutron Scattering and Complementary Techniques in the Research of Matter

At 9:00 the 12th June 2024

NB: *Polarised neutron scattering from dynamic polarised nuclei as we know it now would not have been possible without the help of a number of people and their institutions.*

As am not present in flesh (en chair et en os)
allow me to present myself:

Heinrich Stuhrmann, born on 29 May 1938,

In Schönwalde, Kreis Bartenstein, Ostpreussen.

My parents are from Ermland (Warmia).

This photo has been taken near my home at

29 rue Roger du Marais, 38430 MOIRANS, France

20 km northwest of Grenoble

Email: heinrich.stuhrmann@free.fr

10 May 2024

Early times of neutron scattering

In the absence of any polarization neutron scattering from hydogenous matter relies on the change of the scattering length b with the abundance of the hydrogen isotopes 1H and 2H. The scattering length of 2H is positive, like that of many other nuclei, whereas that if 1H is negative.
This property is exploited in most applications of neutron scattering from hydrogenous matter.

Using neutrons with the polarization p, the change of the **scattering length b** with the **polarization P** of the hydrogen isotopes is impressive. It is quite large with 1H and not negligible for 2H.

$$b(1H) = (\mathbf{-0.374} + \mathbf{1.456\ p\ P(1H)}) \text{ E-12 cm}$$
$$b(2H) = (\mathbf{+0.66} + 0.28\ p\ P(2H)) \text{ E-12 cm}$$

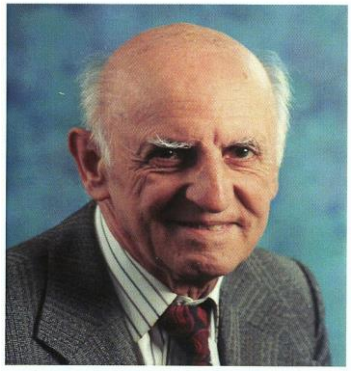
In the early times of neutron scattering very few researchers tried to use the spin spin interaction of neutrons with protons. **John Hayter, G. Jenkins and John White** belong to them. In their famous PRL paper (1974) on polarised neutron scattering from dynamic polarised protons they show the feasibility of the method and predicted its potential:
Selective depolarisation of nuclei whose Larmor frequencies differ either by their magnetic moments or because of their proximity to paramagnetic impurities in the crystal, lanthanum magnesium nitrate crystal.
Hayter, J. B., Jenkin, G. T. & White, J. W. (1974). Phys. Rev. Lett. **33**, 696-698.

How to control both neutron polarisation and nuclear polarisation

Polarised neutron beams (thanks to supermirrors from **Otto Schärpf**) are available at most neutron scattering centres.
The **inversion of the neutron polarization** is achieved by a neutron spin flipper.

Thus we have $-1 < p < 1$.

Schärpf, O. (1989). Physica B, **156&157**, 631-638,



Operational **polarised target facilities** at neutron sources exist at very few places like the Paul-Scherrer Institut (Switzerland) and the MLF neutron target at J-PARC (Japan).
The direction dynamic nuclear polarization (DNP) depends on the frequency of microwave irradiation.
Positive polarization below EPR, negative polarization above EPR.

Thus we have $-1 < P < 1$.

Dynamic nuclear nuclear polarisation (DNP)

The built up of nuclear polarization by **DNP** (**D**ynamic **N**uclear **P**olarisation) takes time. It is a slow process occurring in an external magnetic field B > 2T and temperatures T < 1K in the presence of a small amount of paramagnetic impurities, sources of nuclear polarization created by microwave irradiation.

In a first step, it affects the nuclear spins in the close vicinity of a paramagnetic impurity (unpaired electrons, for instance). The **microwave irradiation transfers the polarization** of the the electron spin exchange reservoir **to the close protons**.

In a second step the polarization of the close protons spreads out into the bulk. van den Brandt, B., Glättli, H., Grillo, I., Hautle, P., Jouve, H., Kohlbrecher, J., Konter, J.A., Leymarie, E., Mango, S., May, R.P., Michel, A., Stuhrmann, H.B. & Zimmer, O. (2006). Eur. Phys. J. **B 49**, 157-165.

All non-spinless nuclei are dynamically polarized, both 1H and 2H e.g. in hydrogenous matter.

A proton spin target is obtained by selective depolarization of deuterons.

A deuteron spin target is obtained by selective depolarization of protons.

A selective depolarization of 1H and 2H is achieved by an rf scan across the NMR lines of 2H and 1H, respectively.

The ways to use DNP in neutron scattering

The arsenal of techniques of nuclear polarization opens new horizons

In structural studies:

They rely on a high nuclear polarization obtained after prolonged microwave irradiation.
Low temperatures close to 0.1 K are obtained with a dilution refrigerator is needed. They allow for frozen spin targets of 1H and 2H. But they are rare.
Usually a temperature around T = 1K together with a higher magnetic field strength is preferred.

In polarization rate studies:

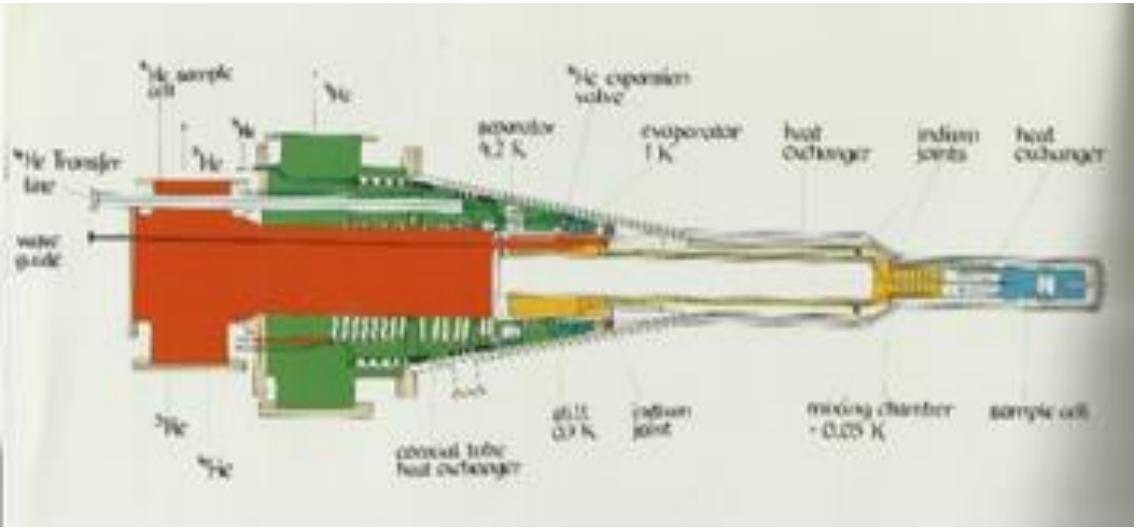
The studies of time dependent of proton polarization have been done typically at T = 1K. The sample is cooled by rapid evaporation of liquid helium.

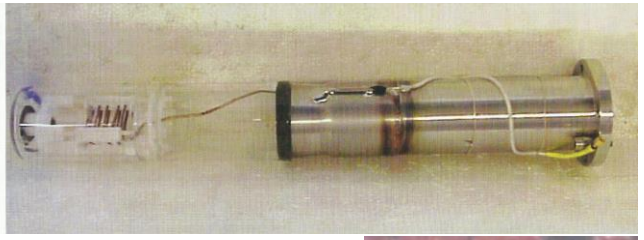
Contrast variation using DNP at T = 0.1 K

Dilution refrigerator from CERN, (Tapio Niinikoski). adapted by CERN to the constraints imposed by thermal neutron scattering.

Niinikoski, T. (2020). *The Physics of Polarized Targets*, Cambridge University Press.

The ^{4}He filled sample chamber is coupled to the ^{3}He/^{4}He mixing chamber by a heat exchanger. Temperature of 0.1 K reached within 5 hours, sample loading included. With appropriate target material the proton polarisation may be close to P = 1. With **ribosomes** in a mixture of **deuterated glycerol and heavy water** we obtained P(1H) = 0.7 and and P(2H) = 0.2.





Sample chamber

The implementation of the NMR coil has been done by Jinkui Zhao.

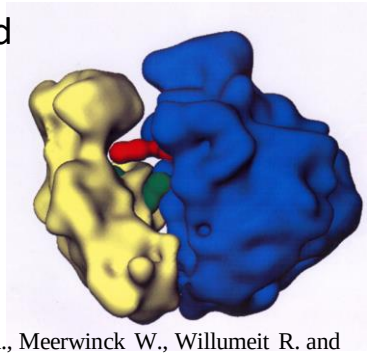
Contrast variation using DNP at T = 0.1 K

The polarised target station from **CERN** at the former GKSS, Geesthacht, running at 0.1K, allowed the localisation of tRNA in the 70S ribosome particle. P = 0.8. Regine Willumeit (GKSS) at work.

Neutron scattering was intensively used for structural studies of the **ribosome.**

*The **method of triangulation** developed by Engelman and Moore was used to locate selectively labelled proteins of the small ribosomal subunit, mainly. The constituents of the ribosomal subunt were ear marked by their content of either 1H or 2H.*

A more powerful labeling procedure is offered by dynamic nuclear polarisation. A proteited label, in this case the **transfer RNA,** is embedded in a fully deuterated **functional ribosome.** Its large subunit in blue,small subunit in yellow.



Ribosome model after Frank

Nierhaus K.H., Wadzack J., Burkhardt N., Jünemann R., Meerwinck W., Willumeit R. and Stuhrmann H.B. Proc. Natl. Acad. Sci. USA, **95**, 945-950 (Biochemistry) (1998).

Dynamic polarised targets at T = 1 K elsewhere

Nuclear spin contrast in Materials Science

Presently the most productive application of dynamic nuclear polarisation

Koghi M., Ishida M., Yshikawa Y., Ishimoto S. Kanno Y., Masaike A., Masuda Y., Morimoto K., J. Phys. Jpn, **56** 2681-2688 (1987)

Kumada, T., Noda, Y., Koizumi, S., Hashimoto, T. (2010). The Journal of Chemical Physics **133**, 054504

Noda, Y., Yamaguchi, D., Hashimoto, T., Shamoto, S., Koizumi, S., Yuasa, T., Tominaga, T., & Sone, T. (2013). Physics Procedia **42**, 52-57

T. Kumada, K. Akutsu, K. Ohishi, T. Morikawa, Y. Kawamura, M. Sahara, J. Suzuki,

N. Torikai, (2018) JPS Conference Proceedings, **22**, 011015

T. Kumada, K. Akutsu, K. Ohishi, T. Morikawa, Y. Kawamura, M. Sahara, J. Suzuki, N. Torikai, (2019) J. Appl. Cryst. **52**, 1054

T. Kumada, D. Miura, K. Akutsu-Suyama, K. Ohishi, T. Morikawa, Y. Kawamura, J. Suzuki, T. Oku, N. Torikai, T. Niizeki, (2022), J. Appl. Cryst. **55**, 1147

In neutron crystallography

J. Pierce, M.J. Cuneo, A. Jennings, L. Li, F. Meilleur, Jinkui Zhao, D.A.A. Myles, Methods in Enzymology (2020) **634**, 153-175

Why radical proteins

Radicals play an important role in living cells. Radical intermediates allow enzymes to perform a wide a wide variety of chemically challenging reactions.

Catalase is a redox enzyme which converts hydrogen peroxide at an incredibly fast, diffusion limited rate **into water and molecular oxygen**.

By offering **peroxyacetic acid**, a derivative of hydrogen peroxide with more steric hindrance, to bovine liver catalase the reaction differs in at least two points: first, the response is slow, and second, after some intermediate steps one of the amino acids, a **tyrosine, is converted into a tyrosyl radical**. It is assumed that one and only one of the 20 tyrosines of each of the four subunits of catalase becomes a tyrosyl radical.

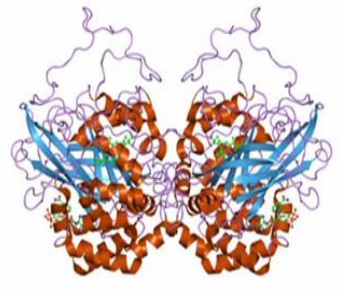
Ivancich A., Jouve, H. M., Sartor, B., & Gaillard J. Biochemistry **36**, 9356-9364 (1997)

Nuclear polarisation occurs near paramagnetic centres through the electron nuclear dipolar interaction decreasing with the third power of the distance between electron and nuclear moments (**magnetic nuclear spin diffusion barrier**). More distant bulk protons are polarized by dipolar interaction between nuclei (spin diffusion)

Where is the tyrosyl ?

But….

Is the magnetic proton spin diffusion **barrier tight enough** to hold polarised protons in sufficient number for a time long enough to be visible in polarised neutron scattering ?



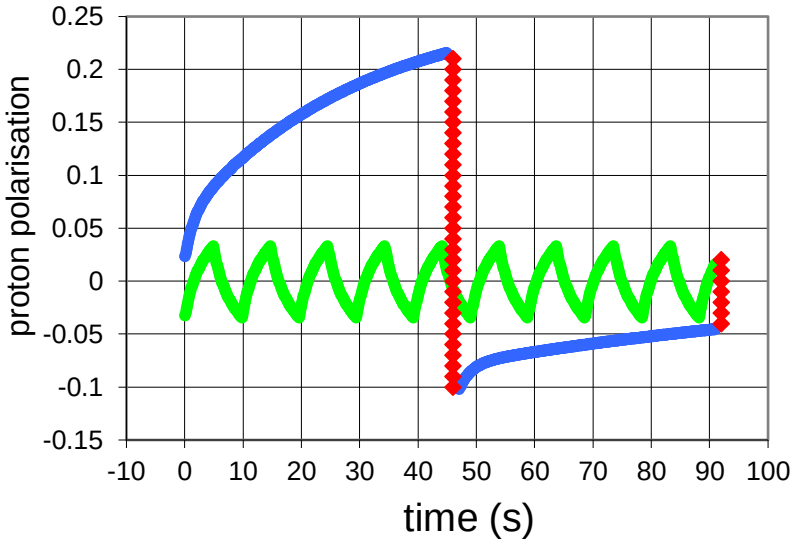
Time resolved polarized neutron scattering

Techniques and Equipment

Periodic change of the direction of polarisation

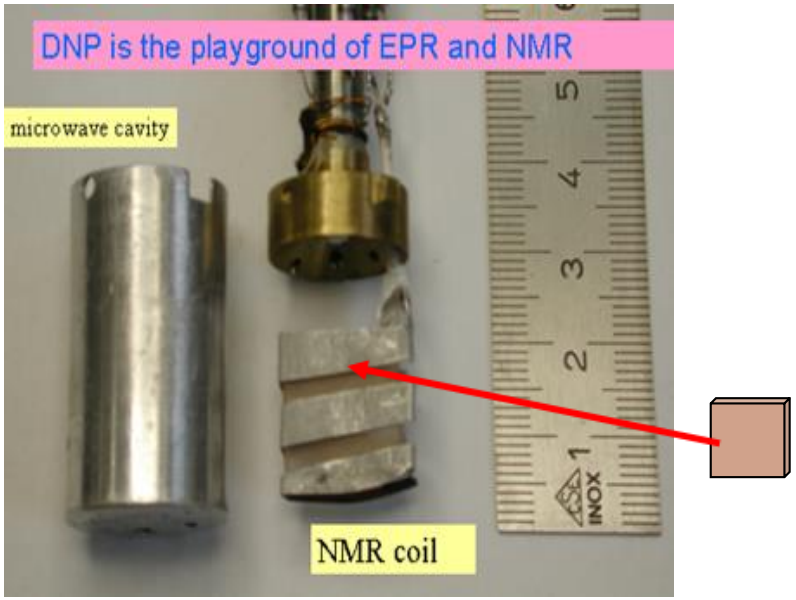
Periodic inversion of the proton polarisation by AFP

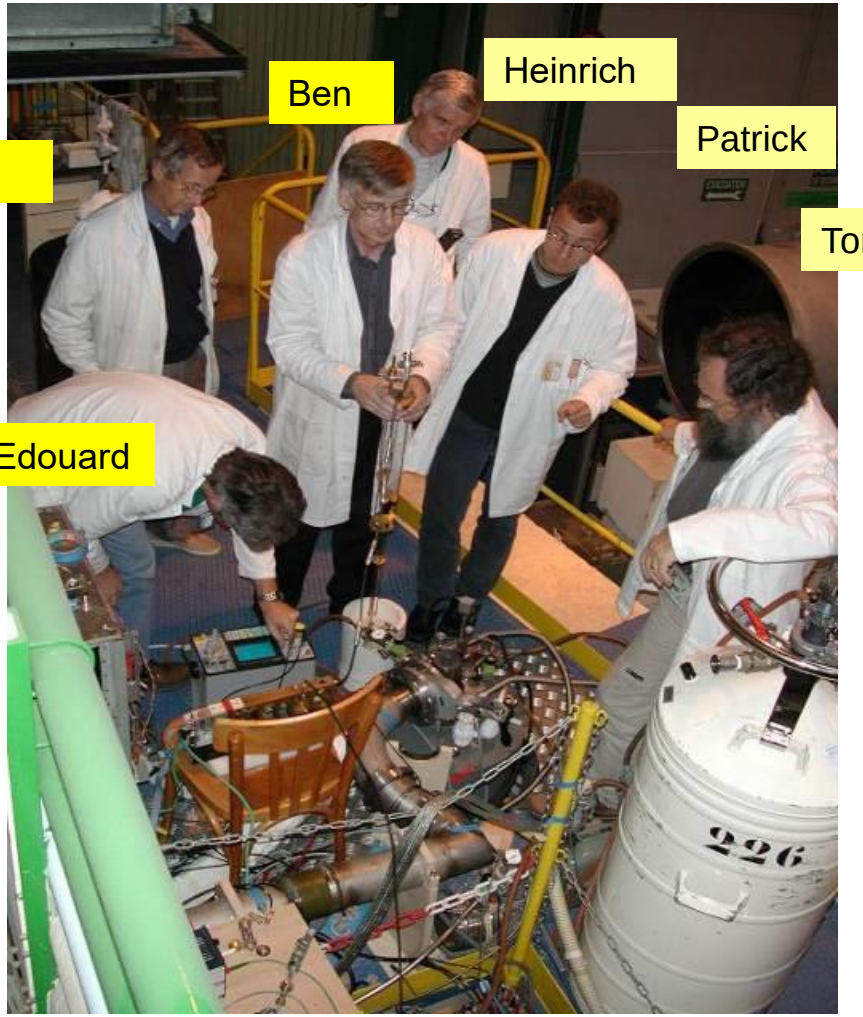
Repetition of the cycle: some hundred to some thousand times



The central part of the polarised target station of PSI

The sample fits into the NMR coil.





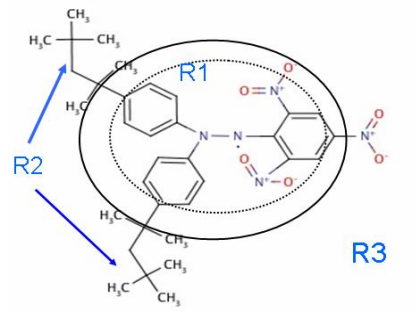
Loading the refrigerator.

The frozen sample is kept at liquid nitrogen temperature.

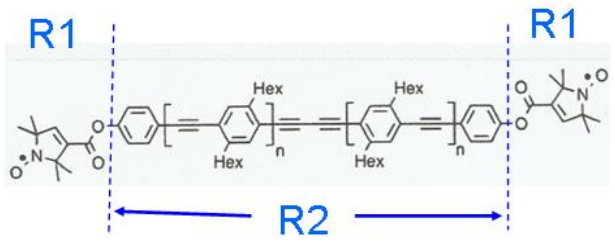
At the ORPHEE reactor, Saclay, France

Magnetic nuclear spin diffusion barrier exists

in radical molecules of medium size



DPPH, 2,2-di(tert-octylphenyl)-1-picryl-hydrazyl



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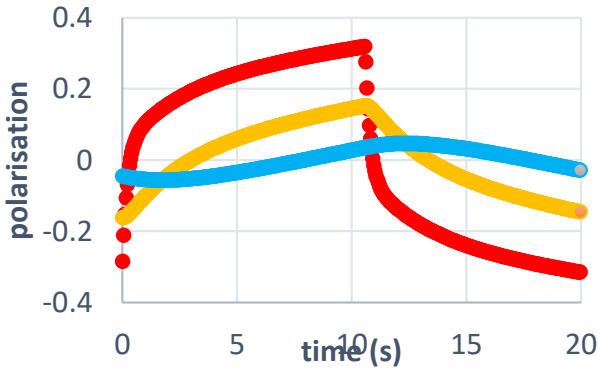
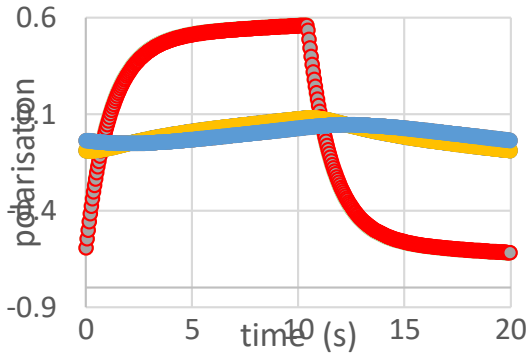




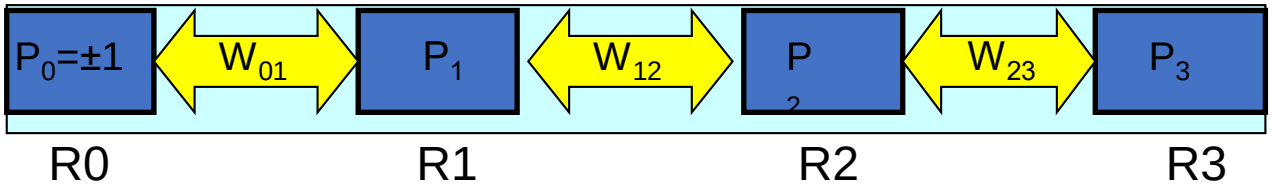
Three reservois (baths) **R1**, **R2** and **R3** define regions differing in the polarisation of their protons.

The evolution of proton polarisation in the reservoirs R1 (●●●●), R2 (●●●●) and R3 (●●●●, solvent)

Stuhrmann, H.B. (2015). Journal of Optoelectronics and Advanced Materials, **17**, 1417-1424.

Magnetic nuclear spin diffusion barrier exists

Data analysis



Three rate equations govern the dynamics of the four reservoirs R0 to R3 coupled in series:

$d P_1/dt = W_{01}/N_1 (P_0-P_1) - W_{12}/N_1 (P_1-P_2)$
$d P_2/dt = W_{12}/N_2 (P_1-P_2) - W_{23}/N_2 (P_2-P_3)$
$dP_3/dt = W_{23}/N_3 (P_2-P_3)$ to be solved by numerical methods

The time dependent scattering amplitude $A(\mathbf{Q},t)$

$$A(\mathbf{Q},t) = U(\mathbf{Q}) + P_1(t)V_1(\mathbf{Q}) + P_2(t)V_2(\mathbf{Q}) \quad \text{—} \quad P_3(t)\, V_3(\mathbf{Q})$$

solute: Close protons remote protons solvent: protons excluded by the molecular volume

is developed as a series Y_{lm} : $A_{lm}(Q) = \Sigma_n\, b_n\, j_l(Qr_n)\, Y^*_{lm}(\theta_n,\varphi_n)$

scattering intensity: $I(Q,t) = \Sigma\, \Sigma\, |\, A_{lm}(Q,t)\, |^2$ to be compared with S(Q,t) from experiment

A CATALASE bound tyrosyl radical

Larger molecules like **CATALASE** require a higher degree of differentiation:

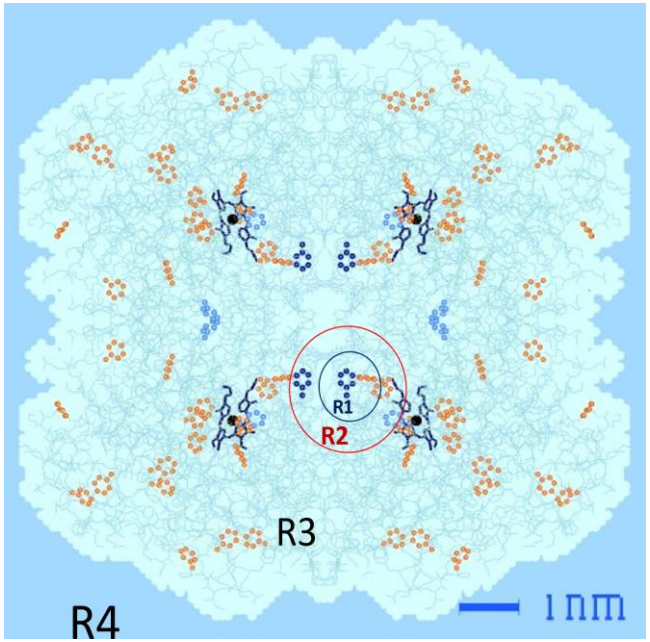
Hence we define four reservoirs **R1**, **R2**, **R3** and **R4**

Supposed close protons near Tyr369 (inside a sphere of 5 Å, R1) are shown as blue spheres

and those not so close (inside a hollow sphere, 5 < r < 10 Å, R2) are shown as green spheres

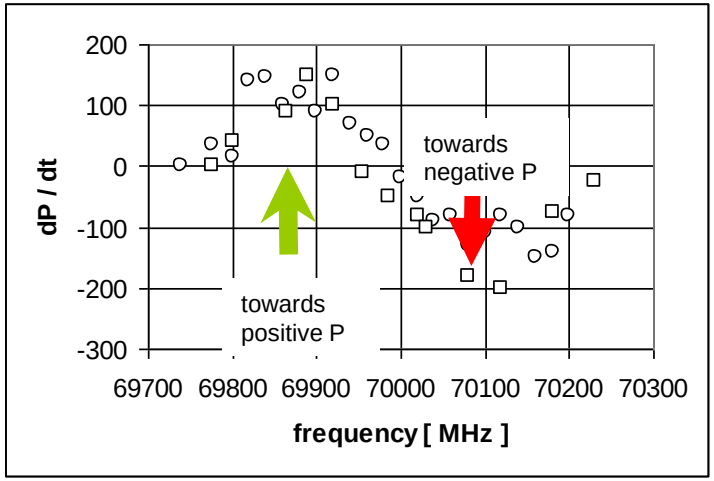
$d P_1/dt = W_{01}/N_1 (P_0-P_1) - W_{12}/N_1 (P_1-P_2)$
$d P_2/dt = W_{12}/N_2 (P_1-P_2) - W_{23}/N_2 (P_2-P_3)$
$dP_3/dt = W_{23}/N_3 (P_2-P_3) – W_{34}/N_3 (P_3-P_4)$
$dP_4/dt = W_{34}/N_4 (P_3-P_4)$ solved by numerical methods

The structure of the bovine liver catalase has been determined by Fita and Rossman (see PDB). In the Fourier space we have the amplitudes V(Q) containing N of protons in each reservoir. The variables to be determined are mainly the transition probabilities W.



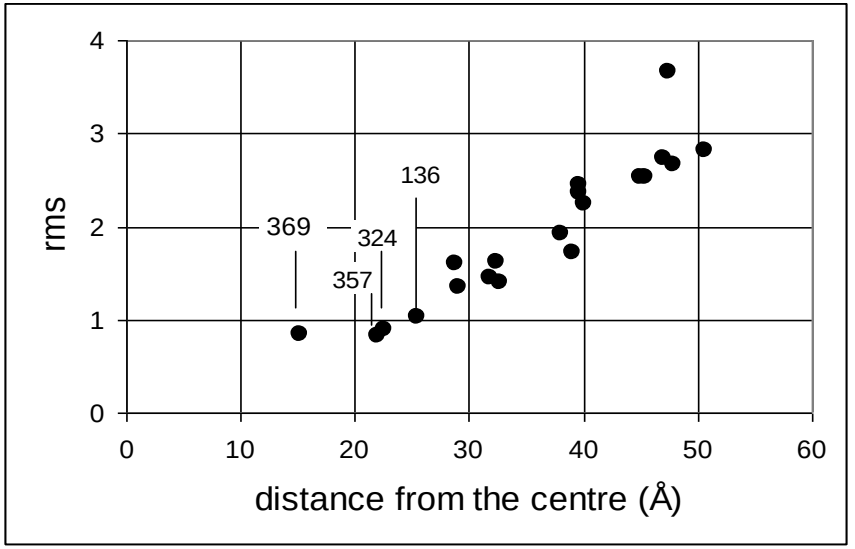
The time dependent amplitude is $A(\mathbf{Q},t) = U(\mathbf{Q}) + P_1(t)V_1(\mathbf{Q}) + P_2(t)V_2(\mathbf{Q}) + P_3(t)V_3(\mathbf{Q}) - P_4(t)\, V_4(\mathbf{Q})$

How to find the tyrosine which has been converted to tyrosyl radical



Each of the 20 tyrosines in one the four identical subunits of the catalase molecule is considered as a potential radical site. The neutron scattering intensity I(Q,t) is calculated for each configuration. The deviation (rms) of the calculated intensity function from the experimental intensity S(Q,t) is presented as a function of the distance of test candidate from the centre of the catalase molecule

The direction of the dynamic proton polarisation has been changed each five seconds. During each half-cycle 100 pictures of S(Q,t) were recorded.



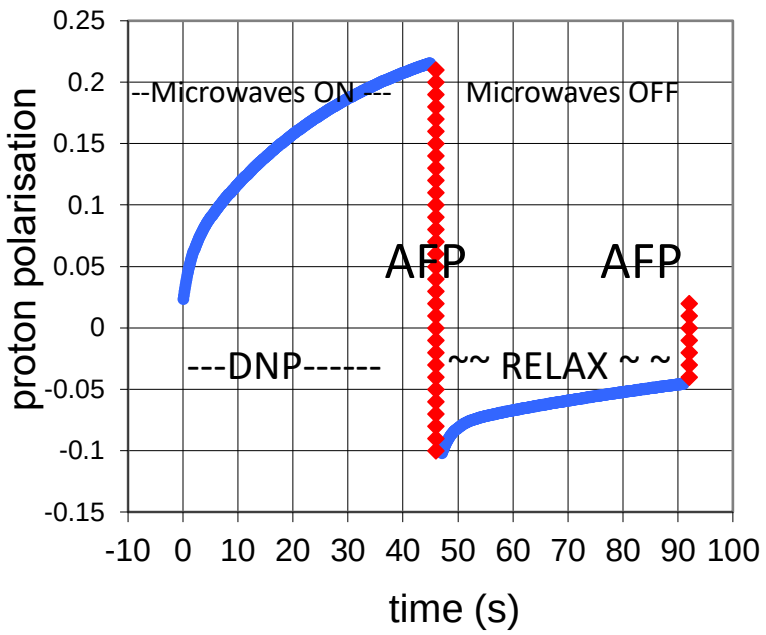
The best agreement with experimental data is found with tyr-369 in agreement with EPR. 369 means 369th amino acid of the peptide chain of a catalase subunit. q.e.d.

Zimmer, O., Jouve, H.M., & Stuhrmann, H.B. (2016). IUCr J. **3**, 326-340

Polarized neutron scattering from AFP modulated proton polarisation *

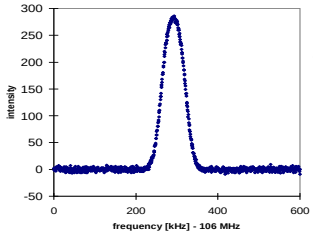
Submitted to IUCrJ on 30 October 2023, under review

a new time table



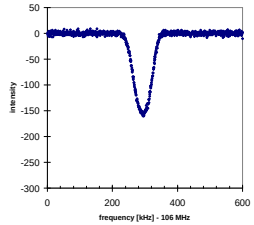
At constant microwave frequency

A rf sweep across the **NMR line** is used for both, the measurement of the proton polarisation and the reversal of the proton polarisation. It is the power of the rf and the sweep speed that make the difference. A fast sweep of a few milliseconds with very low power, which hardly affects the proton polarisation, is used for the measurement of the latter with the so-called Q-meter method. A much higher rf field amplitude and a slower sweep of typically 0.3 s are required to achieve the condition of adiabaticity resulting in an efficient reversal of the proton polarisation by **AFP**.



Proton polarisation seen by NMR

after AFP



* After P.Hautle, O.Zimmer, H.M. Jouve, H.B. Stuhrmann, Submitted to IUCrJ on 30/10/2023, a short version adapted for oral presentation

Polarized neutron scattering from AFP modulated proton polarisation

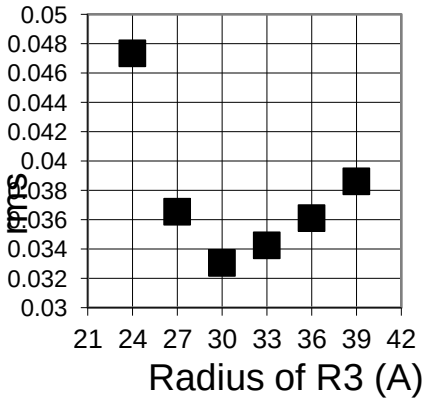
The **interplay between DNP and AFP** might reveal new features of the catalase model. Are the four hemgroups for something? To test the hypothesis of its role as nuclear spin diffusion barrier, we introduce a spherical boundary including the four iron atoms C of the catalase molecule. Using a model consisting of five reservoirs

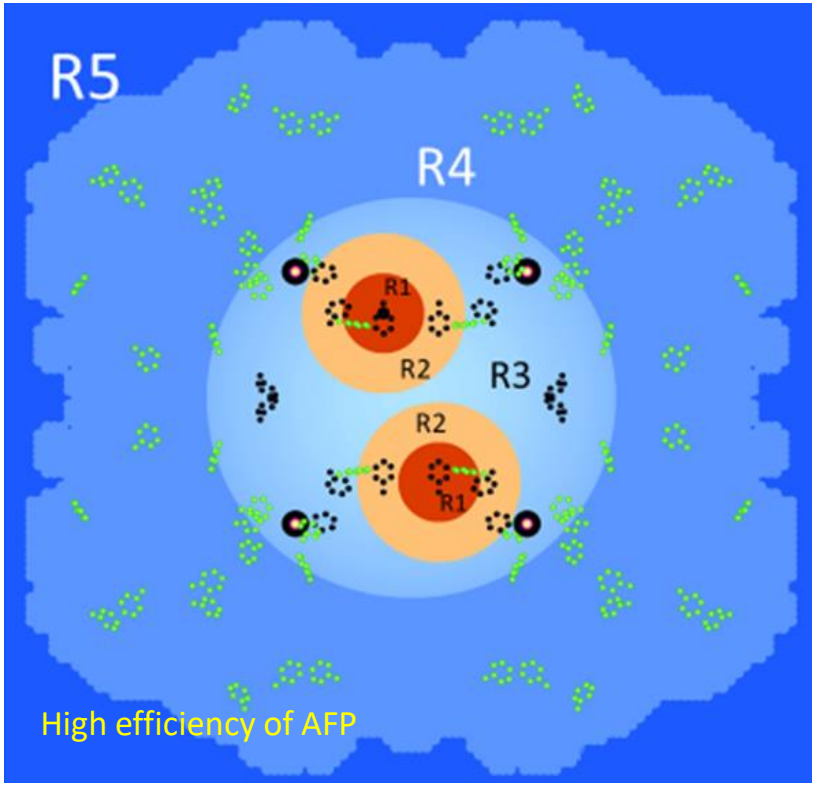
R1, R2,R3, R4, and R5

resulting in the time dependent amplitude

$$A(\mathbf{Q},t) = U(\mathbf{Q}) + P_1(t)V_1(\mathbf{Q}) + P_2(t)V_2(\mathbf{Q}) + P_3(t)V_3(\mathbf{Q}) + P_4(t)\,V_4(\mathbf{Q}) - P_5(t)\,V_5(\mathbf{Q})$$

The best fit of the calculated I(Q,t) with the experimental S(Q,t) is obtained with **R3** having a **radius of 30 Å**. The surface of R3 approximates the spin diffusion barrier.





The phenyl groups of tyrosine are shown. Those of tyr-369 define the centres of **R1**. Catalase structure from I. Fita and M.G. Rossmann. (1985). J. Mol. Biol. **185**, 21-37

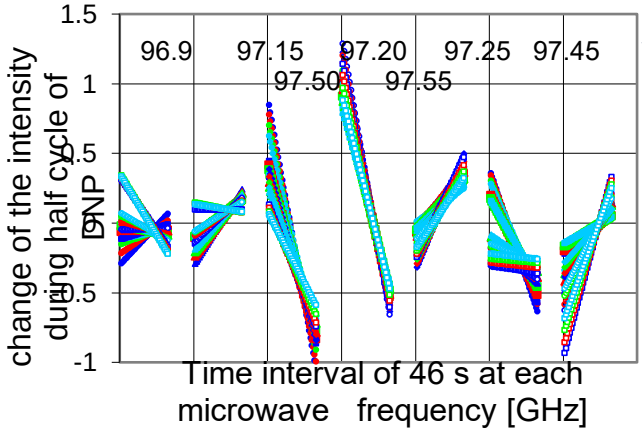
Polarized neutron scattering from AFP modulated proton polarisation

The dispersion of DNP

Time-resolved neutron scattering from tyrosyl doped catalase has been measured at 6 microwave frequencies close to the EPR profile of tyrosyl, i.e.

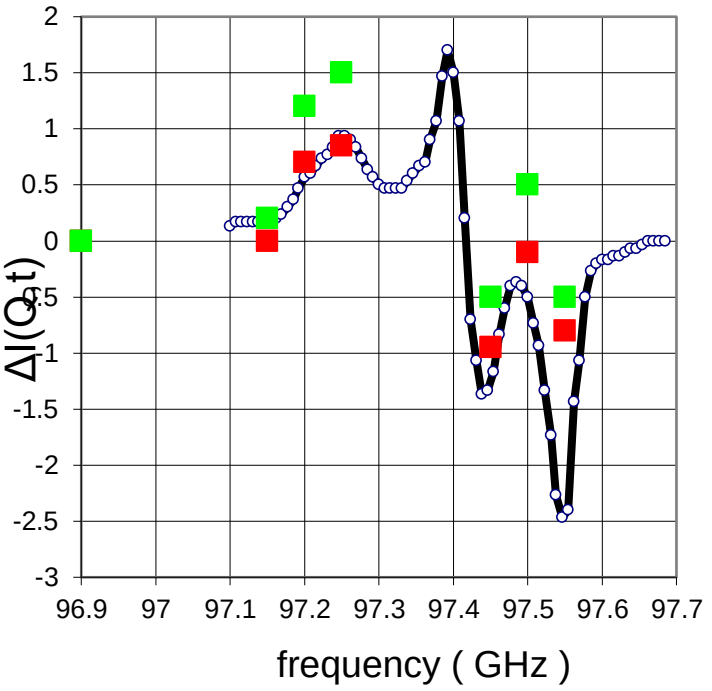
- below the EPR of tyrosyl: 97.15 GHz, 97.20 GHz, and 97.25 GHz
- above the EPR of tyrosyl: 97.45 GHz, 97.50 GHz, and 97.55 Ghz and a seventh one at 96.8 GHz assumed to be off-resonance.

ΔI(Q,t) is about a thousand times smaller than I(Q,t) and so is S(Q,t). Noise is a major problem.



At each micromave energy [GHz] the spread of the slopes reflects the noise of the experimental data.

mean value of each set of slopes



—◦—◦—◦ EPR line, T=10K, 244.996 GHz Ivancich et al

ΔI(Q,t) mean change during half-cycle of DNP

Maximum polarisation of close protons in R1 correlates with the EPR line.

Polarized neutron scattering from AFP modulated proton polarisation

Analysis of data from AFP modulated proton polarisation

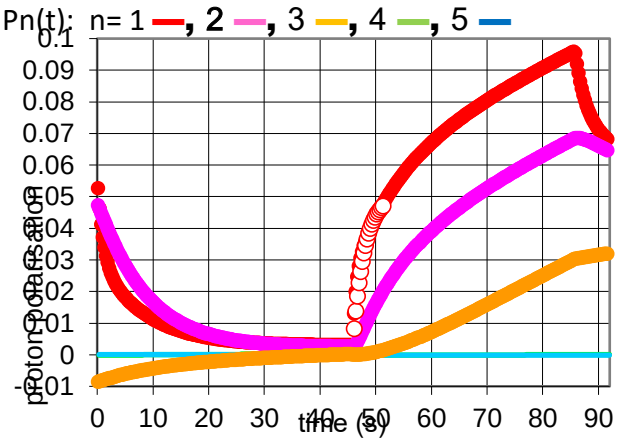
Essentially the same as for the model of four reservoirs,

Except for the discontinuty of the intensity due to AFP (efficiency ε = -P(after)/P(before); high for remote protons)

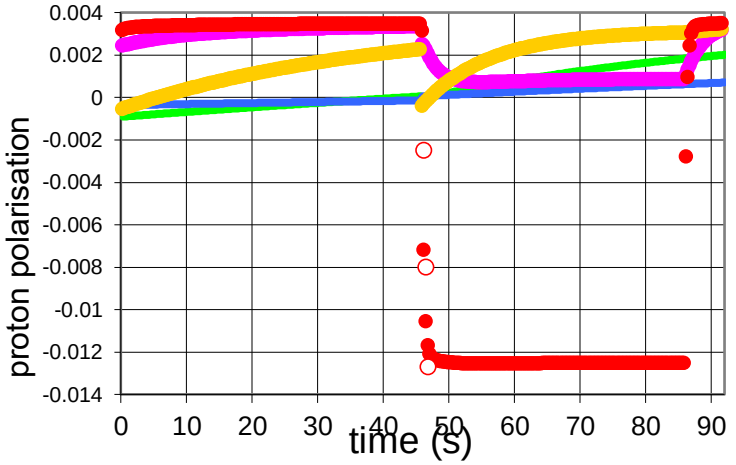
The influence of spin lattice relaxation is taken into account.

The model: five reservoirs (baths) coupled in sequence.

$$A(\boldsymbol{Q}, t) = A_0(\boldsymbol{Q}) + \sum_n P_n(t)\, A_n(\boldsymbol{Q})$$



A typical result obtained from the experimental data after drastic smoothing

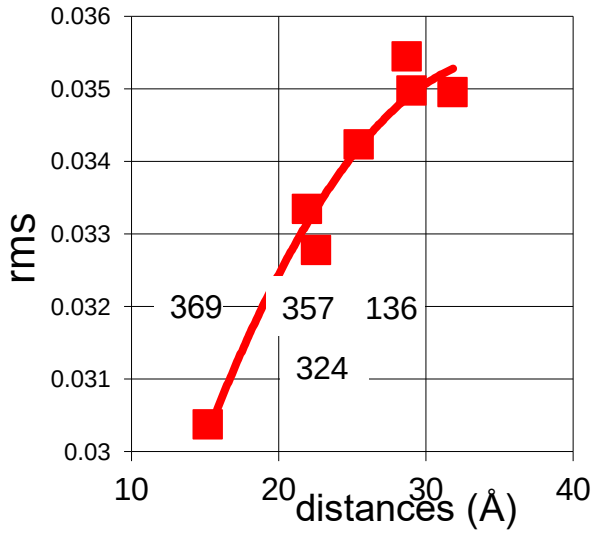


Note the exceptionally weak DNP at a microwave frequency of **97.50 GHz**. **The drift of proton polarisation towards thermal equilibrium polarisation prevails.**

Polarized neutron scattering from AFP modulated proton polarisation

and Conclusion

The selective inversion of nuclear spin polarisation by the method of Adiabatic Fast Passage (AFP) is a means to tailor the spatial distribution of dynamic polarised protons. As such, it adds a new dimension to the method using alternating directions of DNP, which focuses on the source of dynamic nuclear polarisation.



The radical tyrosyl-369 is confirmed.

AFP on the contrary develops its full power with nuclear spins far away from a radical site supporting DNP leaving protons close to the unpaired electron rather unaffected. Hence a radical shows up more clearly.

The joint use of AFP and DNP in a cleverly chosen sequence including relaxation is the method of choice. It presents optimal conditions for kinetic and structural studies on active centres supporting DNP and inactive ones like magnetic inhomogeneities in complex structures like radical proteins, for instance, by time-resolved polarised neutron scattering.

From Patrick Hautle, Oliver Zimmer, Hélène M. Jouve, Heinrich B. Stuhrmann, submitted

Acknowledgement

This work was performed by the French-Swiss-German collaboration on proton polarisation build-up by DNP, the team members of which are B. van den Brandt, H. Glättli, I. Grillo, P. Hautle, H. M. Jouve, J. Kohlbrecher, J.A. Konter, E. Leymarie, S. Mango, R.P. May, A. Michels, O. Zimmer and the author. We thank the Paul-Scherrer Institute (PSI) for the use of the facility for dynamic nuclear polarisation, the Institute Laue-Langevin for allocating beam time on the small-angle diffractometer D22, and last not least, J. Gaillard (CEN Grenoble) for the EPR measurements. We are grateful to Salvatore Mango with whom we conceived this project and to the late Ben van den Brandt for the successful continuation of the collaboration.

References: Heinrich B. Stuhrmann, Eur. Phys. J. E (2023) 46, 11 and citations therein