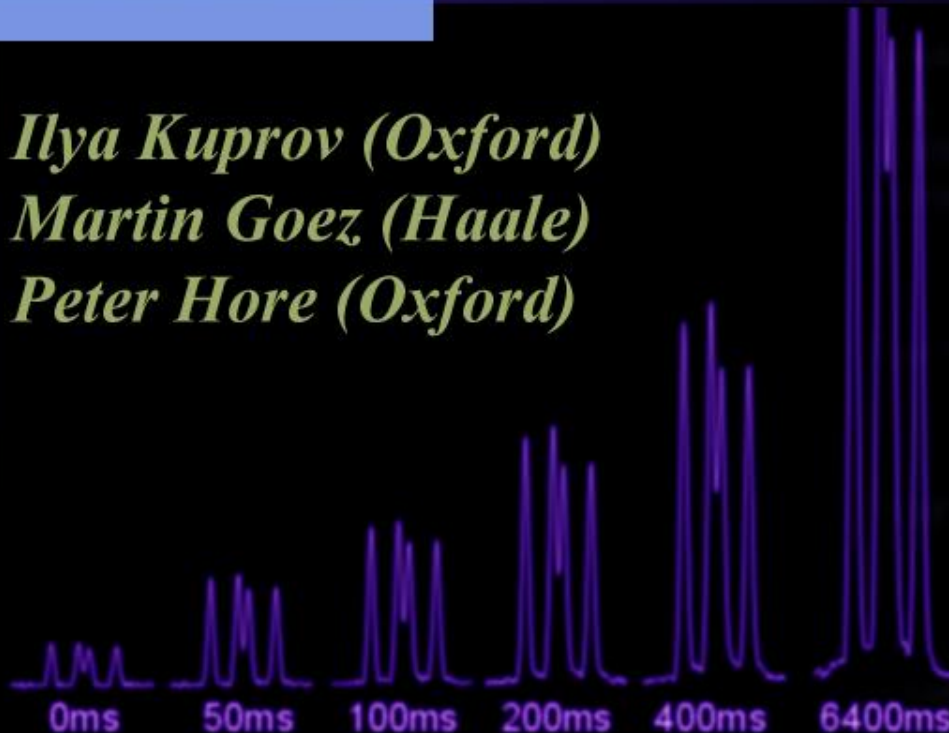




# High-field $^{19}\text{F}$ CIDNP: relaxation effects and applications

*Ilya Kuprov (Oxford)*  
*Martin Goez (Haale)*  
*Peter Hore (Oxford)*

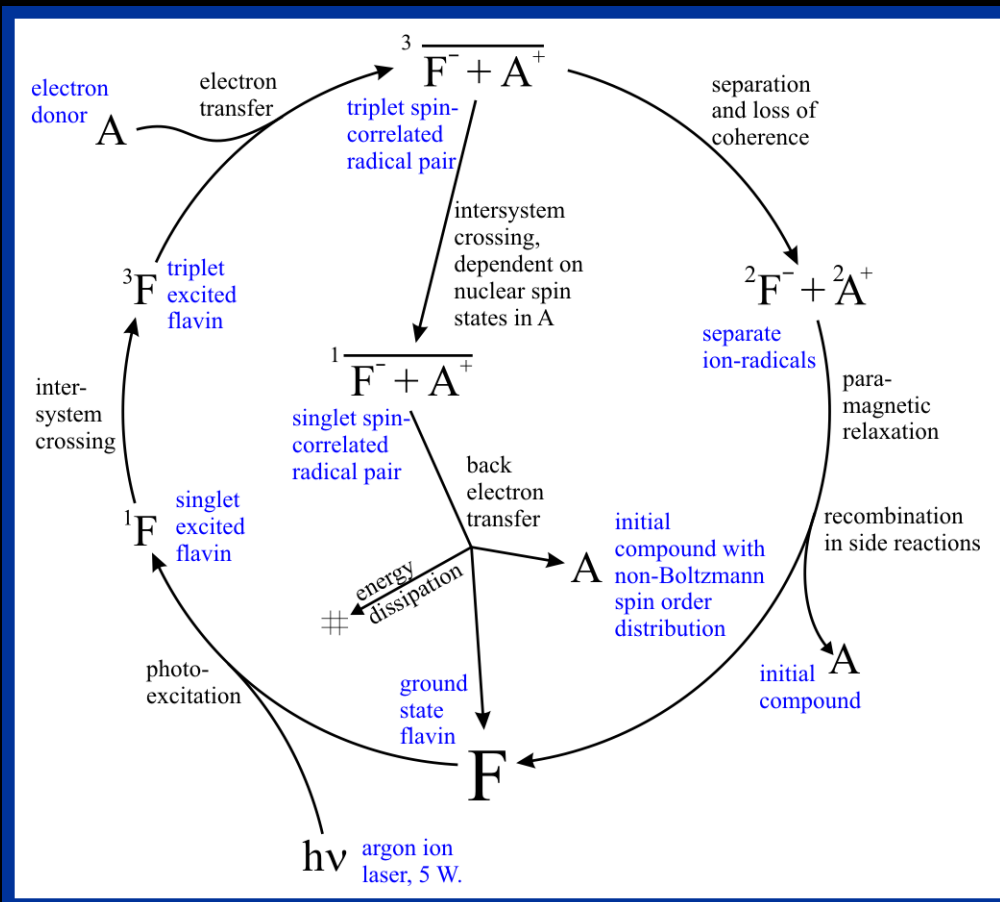


PHYSICAL CHEMISTRY



Oxford, 2005

# Chemically Induced Dynamic Nuclear Polarization (CIDNP)

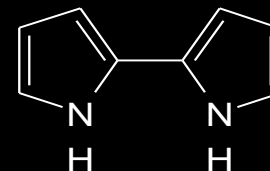


Most popular photosensitizers:

flavins (200-500 nm):



2,2'-bipyridyl (200-350 nm):

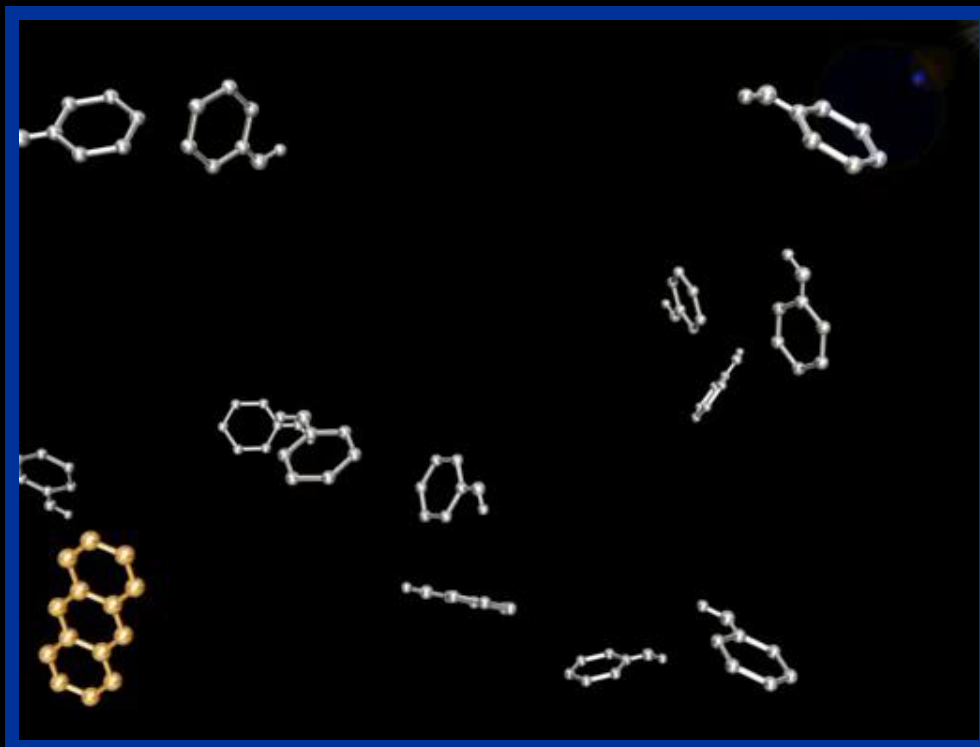


Target molecules:

aromatic amino acids, nucleotides, solvent-accessible aromatic side chains in proteins, solvent-accessible aromatic rings in DNA/RNA, phenols, thiols, etc.

CIDNP was discovered by Bargon and Fischer, and, independently, by Ward and Lowler in 1967. It has been recognized as a (rather exotic) sub-field of magnetic resonance ever since.

## CIDNP: origin and manifestation.



Origin: Different recombination probability of singlet and triplet spin-correlated radical pairs, and modulation of singlet-triplet interconversion by hyperfine interactions.

Manifestation: strongly enhanced ( $\times 10$  to  $\times 100$ ) NMR emission or absorption, sometimes with significant population of longitudinal multi-spin orders.

*Optical excitation*



*Intersystem crossing to triplet electron spin state*



*Electron transfer from a donor molecule*



*Nuclear spin selective evolution of the radical pair*

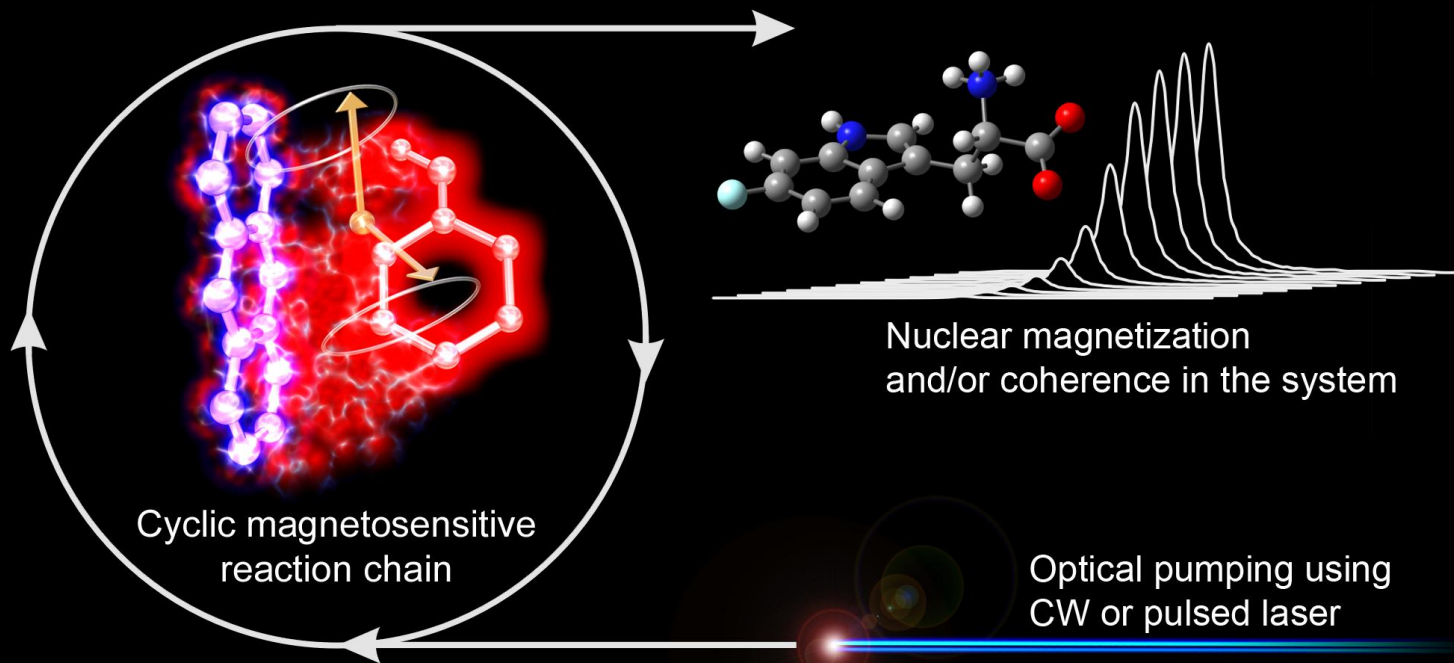


*Nuclear spin selective recombination*



*Initial compounds + nuclear magnetization*

# Chemical pumping of nuclear magnetization.

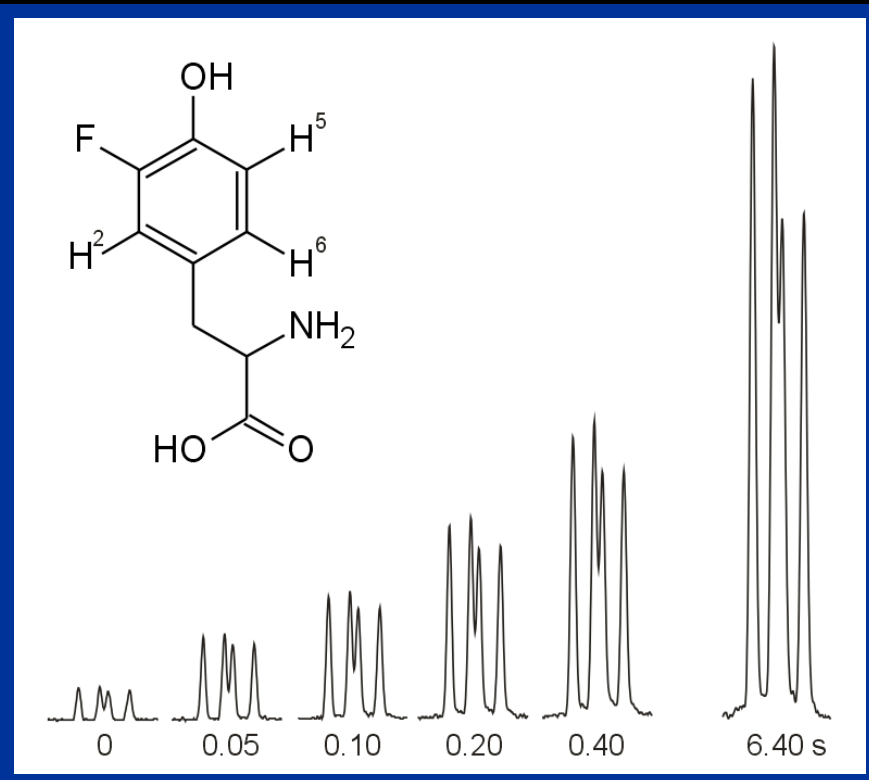


Origin: Radical recombination probability modulation by nuclear hyperfine interactions.

Applications:

- Protein folding and surface structure investigation.
- Chemically pumped nuclear magnetic resonance.
- Free radical structure and kinetics investigation.

# $^{19}\text{F}$ CIDNP and $^{19}\text{F}$ - $^1\text{H}$ DD-CSA cross-correlation.



$^{19}\text{F}$  photo-CIDNP effect in 3-fluorotyrosine and a number of fluorinated tryptophans: was intended for use in protein NMR spectroscopy. The observed photo-CIDNP enhancements are in the range of  $\times 10$ - $\times 50$  with consequently large NOEs and a possibility to map the solvent-accessible amino acids and analyze their motional regimes.

Need a model for magnetization evolution in a slowly and continuously pumped system.

$$\frac{\partial \hat{\rho}(t)}{\partial t} = -\frac{i}{\hbar} [\hat{H}(t), \hat{\rho}(t)] + \sum_i p_i \hat{K}_i \quad \leftarrow \text{"source" terms}$$

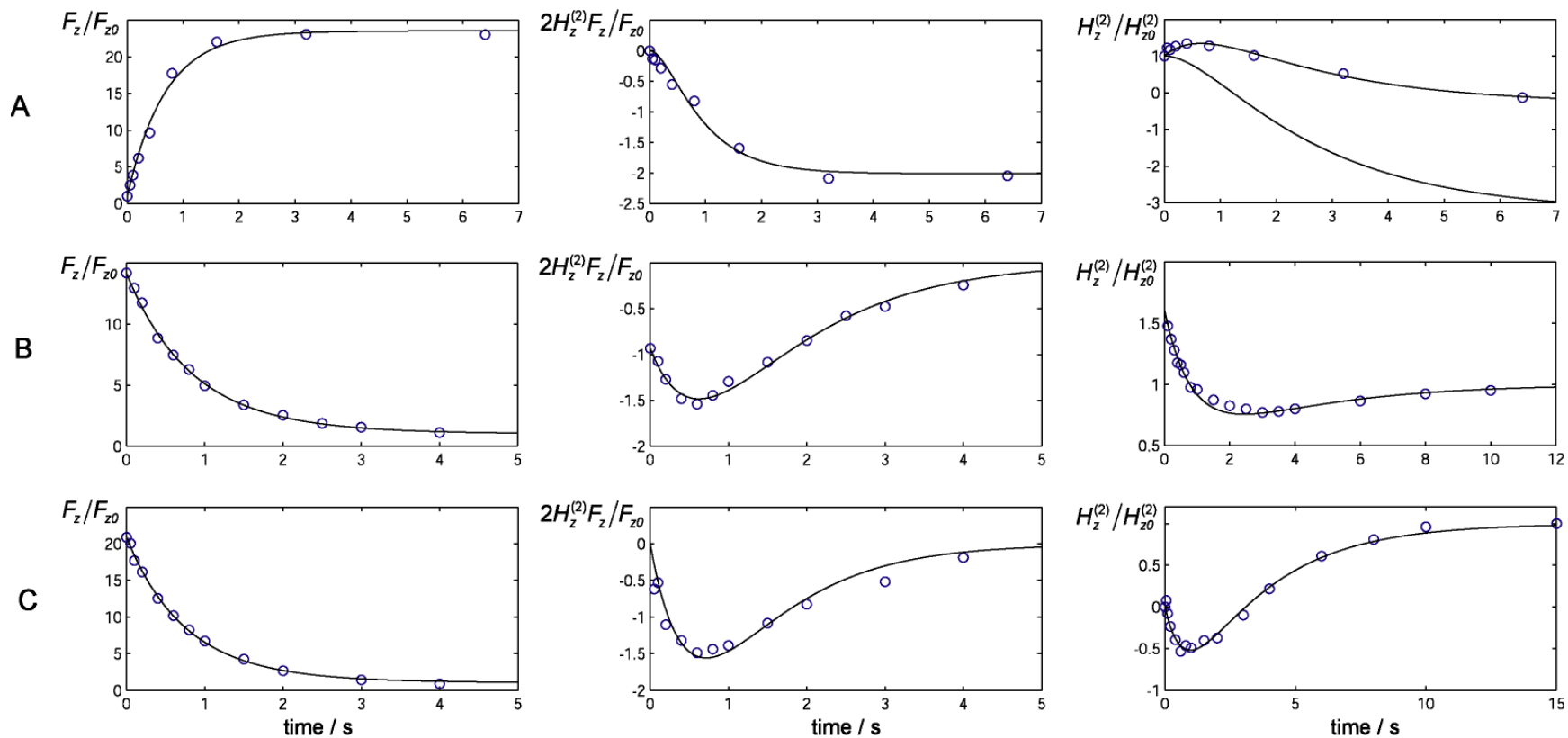
Relaxation treatment (including DD-CSA cross-correlation) yields:

$$\begin{cases} \frac{d}{dt} I_z = -\lambda_I (I_z - I_z^0) - \delta_{IS} 2I_z S_z - \sigma_{IS} (S_z - S_z^0) + p_I I_z \\ \frac{d}{dt} 2I_z S_z = -\lambda_{IS} 2I_z S_z - \delta_{IS} (I_z - I_z^0) + p_{IS} 2I_z S_z \\ \frac{d}{dt} S_z = -\lambda_S (S_z - S_z^0) - \sigma_{IS} (I_z - I_z^0) + p_S S_z \end{cases}$$

$\uparrow$  self-relaxation       $\uparrow$  cross-relaxation       $\uparrow$  chemical pumping

Will hopefully work with a slow enough pumping and low equilibrium radical concentration...

# A CIDNP-pumped two-spin system – relaxation analysis.



The equation fitted:

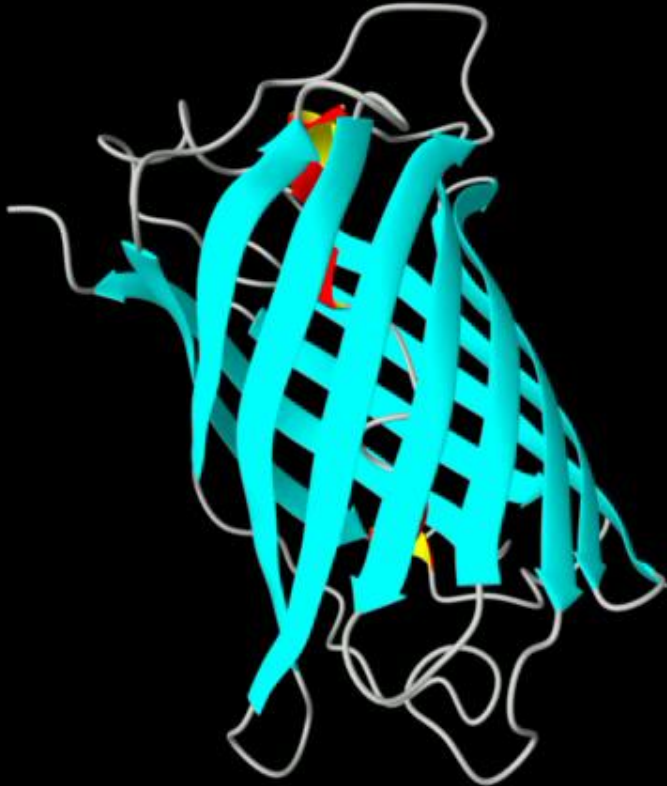
$$\frac{d}{dt} \begin{bmatrix} 1 \\ H_z \\ F_z \\ 2H_z F_z \end{bmatrix} = - \begin{bmatrix} 0 & 0 & 0 & 0 \\ -\rho_H & \rho_{HH} & \sigma_{HF} & 0 \\ -\rho_F & \sigma_{HF} & \rho_{FF} & \delta_{F,HF} \\ 0 & 0 & \delta_{F,HF} & \rho_{HFHF} \end{bmatrix} \begin{bmatrix} 1 \\ \Delta H_z \\ \Delta F_z \\ 2H_z F_z \end{bmatrix}$$

$$\sigma_{HF} = \frac{1}{10} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{\gamma_H^2 \gamma_F^2 \hbar^2 \tau_c}{r_{HF}^6} \left( \frac{6}{1 + (\omega_F + \omega_H)^2 \tau_c^2} - \frac{1}{1 + (\omega_F - \omega_H)^2 \tau_c^2} \right)$$

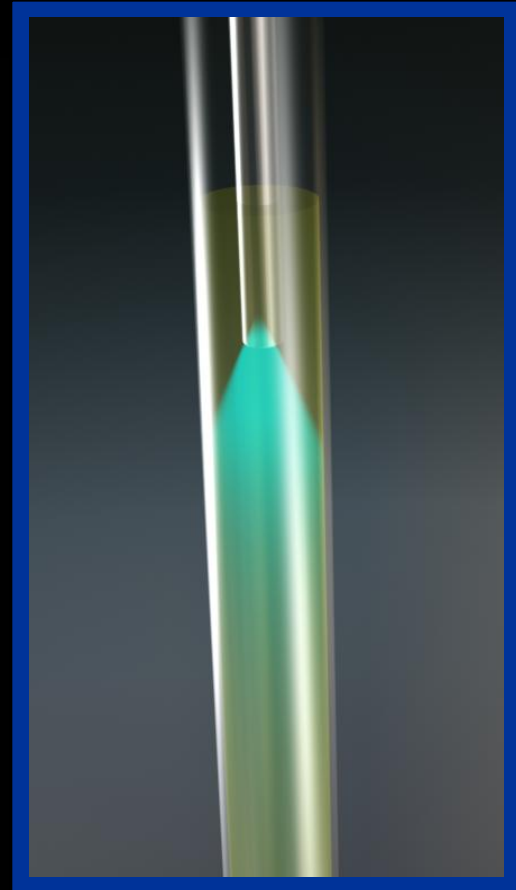
$$\delta_{F,HF} = \frac{2}{5} \frac{\mu_0}{4\pi} \frac{\gamma_F^2 \gamma_H \hbar B_0}{r_{HF}^3} \frac{\tau_c}{1 + \omega_F^2 \tau_c^2} \Delta \sigma_F^g$$

(I. Kuprov, P.J. Hore, *J. Magn. Res.* 168 (2004) 1-7)

## Green fluorescent protein – an application?



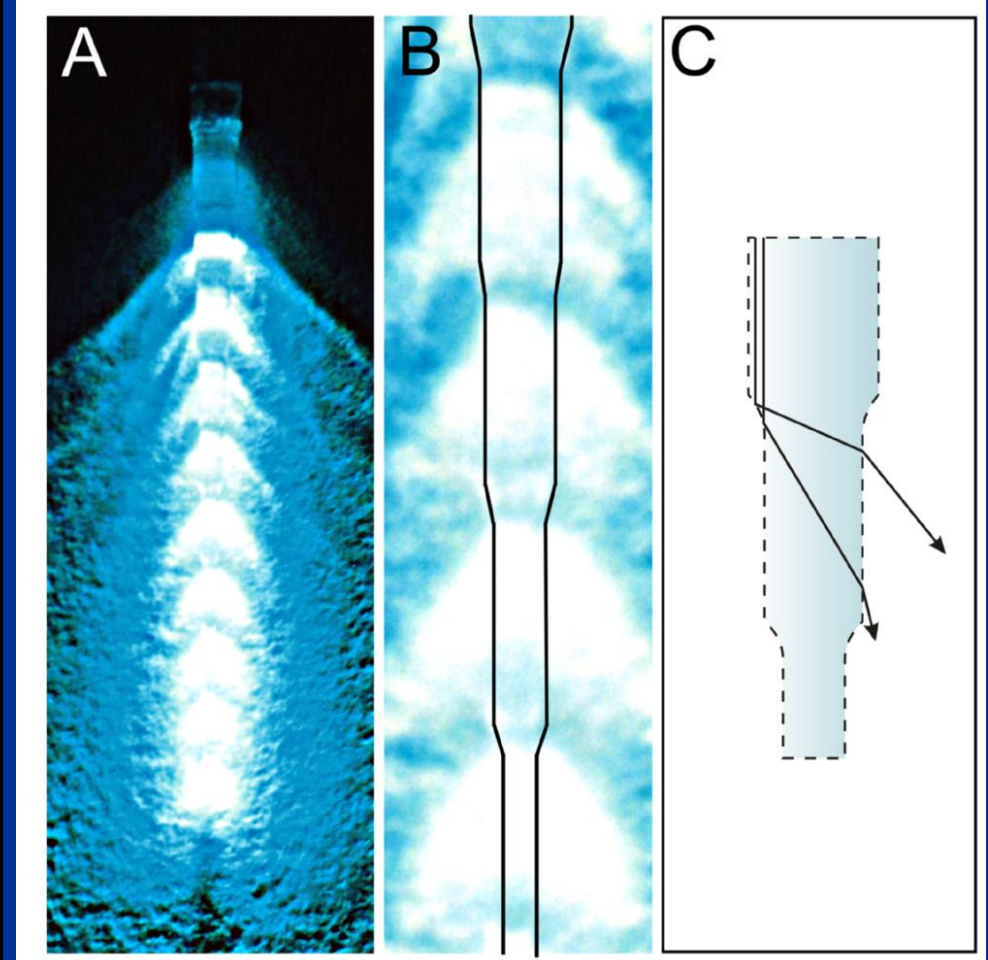
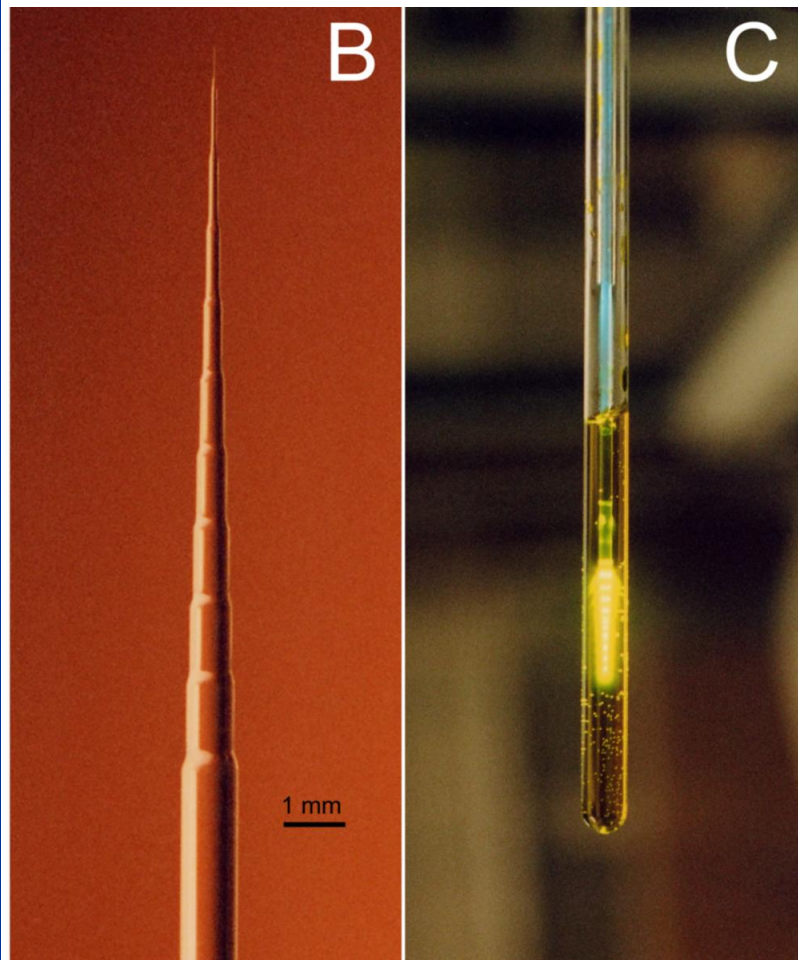
Originally found in fluorescent medusas, used primarily as a coexpressing protein to identify microorganisms with successfully embedded plasmids.



GFP is coloured and absorbs strongly at and above 488 nm.

:(

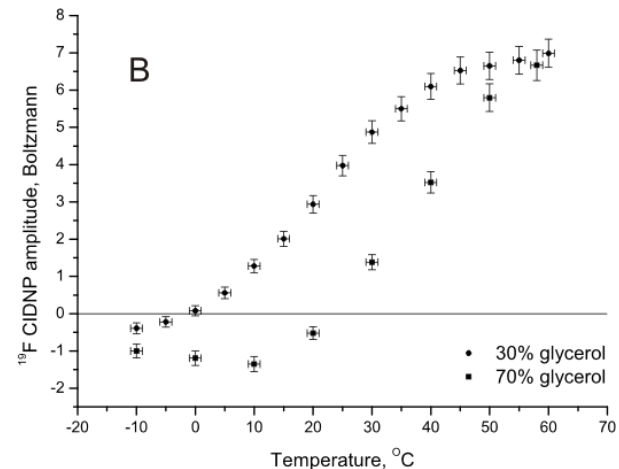
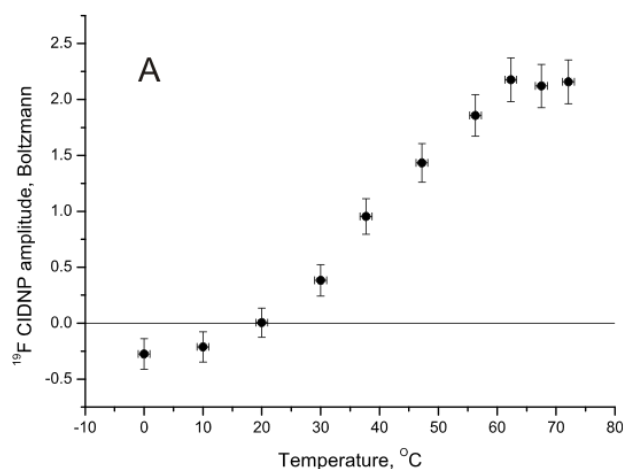
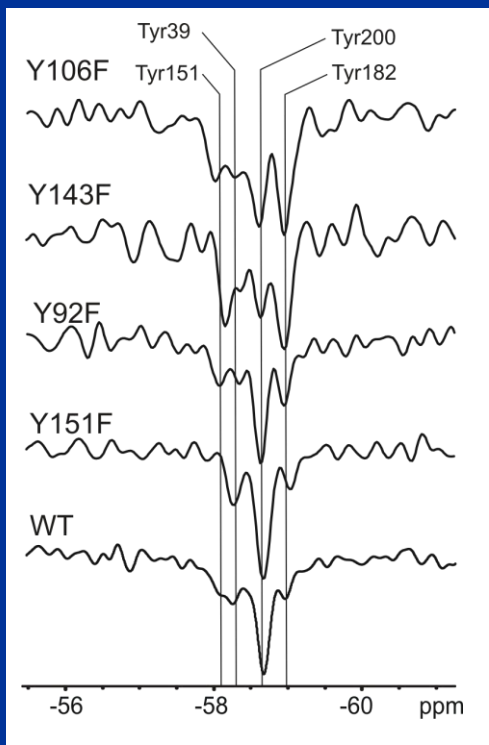
# Optically dense sample illumination setup



Based on HF-etched fused silica optical fibre, uniformly illuminates the sample “from within”, doesn’t lead to noticeable resolution or filling factor loss.

*I. Kuprov, P.J. Hore, J. Magn. Res. 171 (2004) 171-175.*

# GFP and Trp-cage proteins – $^{19}\text{F}$ CIDNP experiments.

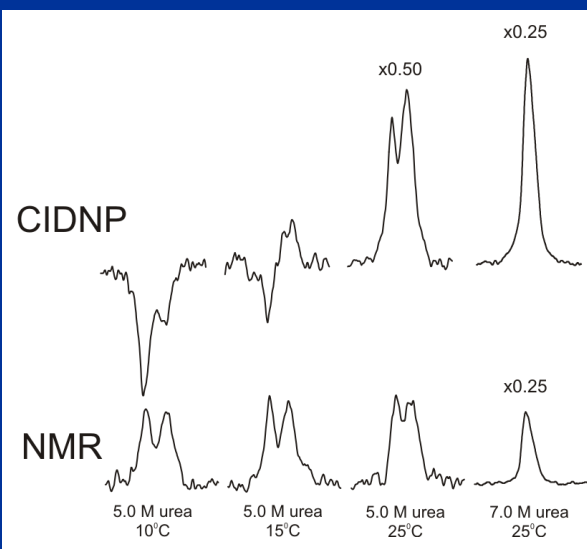


The viscosity-dependent sign change only happens on fluorine (protons unchanged) ruling out overall kinetic effects and pure electron-related dynamics effects. Suggests relaxation.

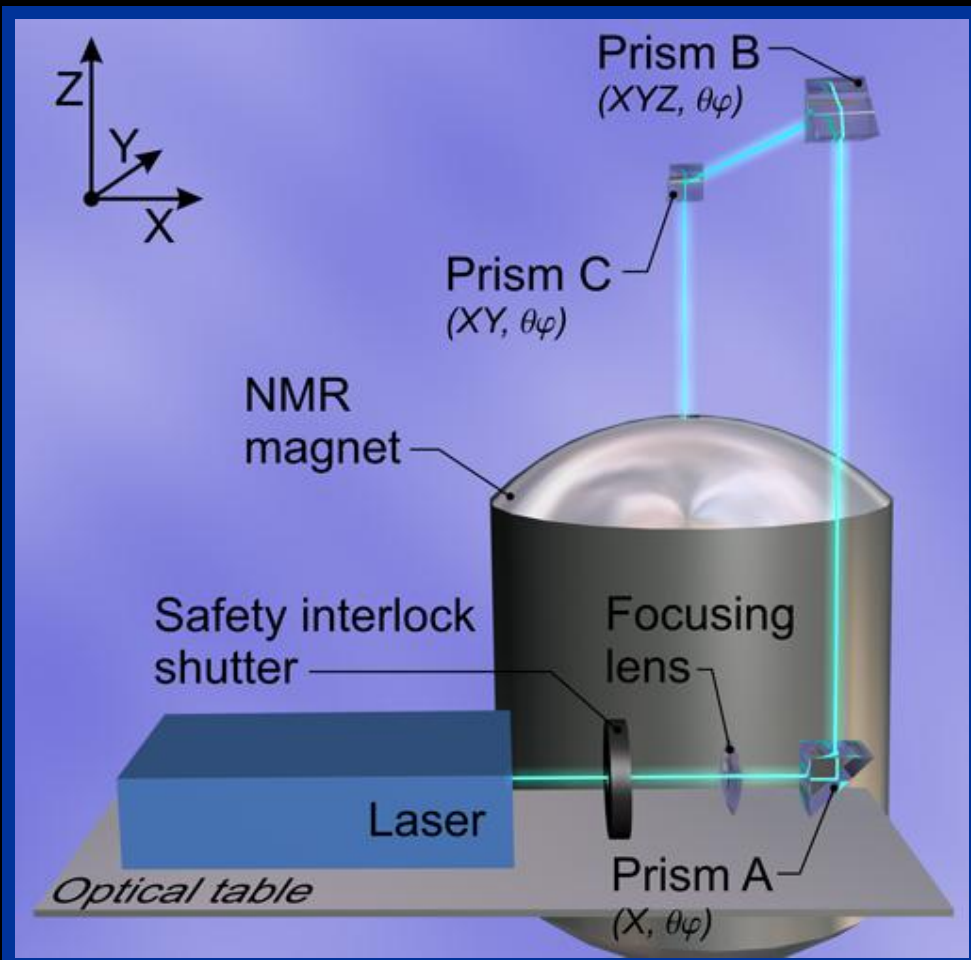
F. Khan, I. Kuprov, T.D. Craggs, P.J. Hore, S.E. Jackson, *J. Am. Chem. Soc.*, submitted.

Relaxation contribution to the  $^{19}\text{F}$  CIDNP effect at high viscosities?

A time-resolved investigation is needed to separate geminate and secondary effects.



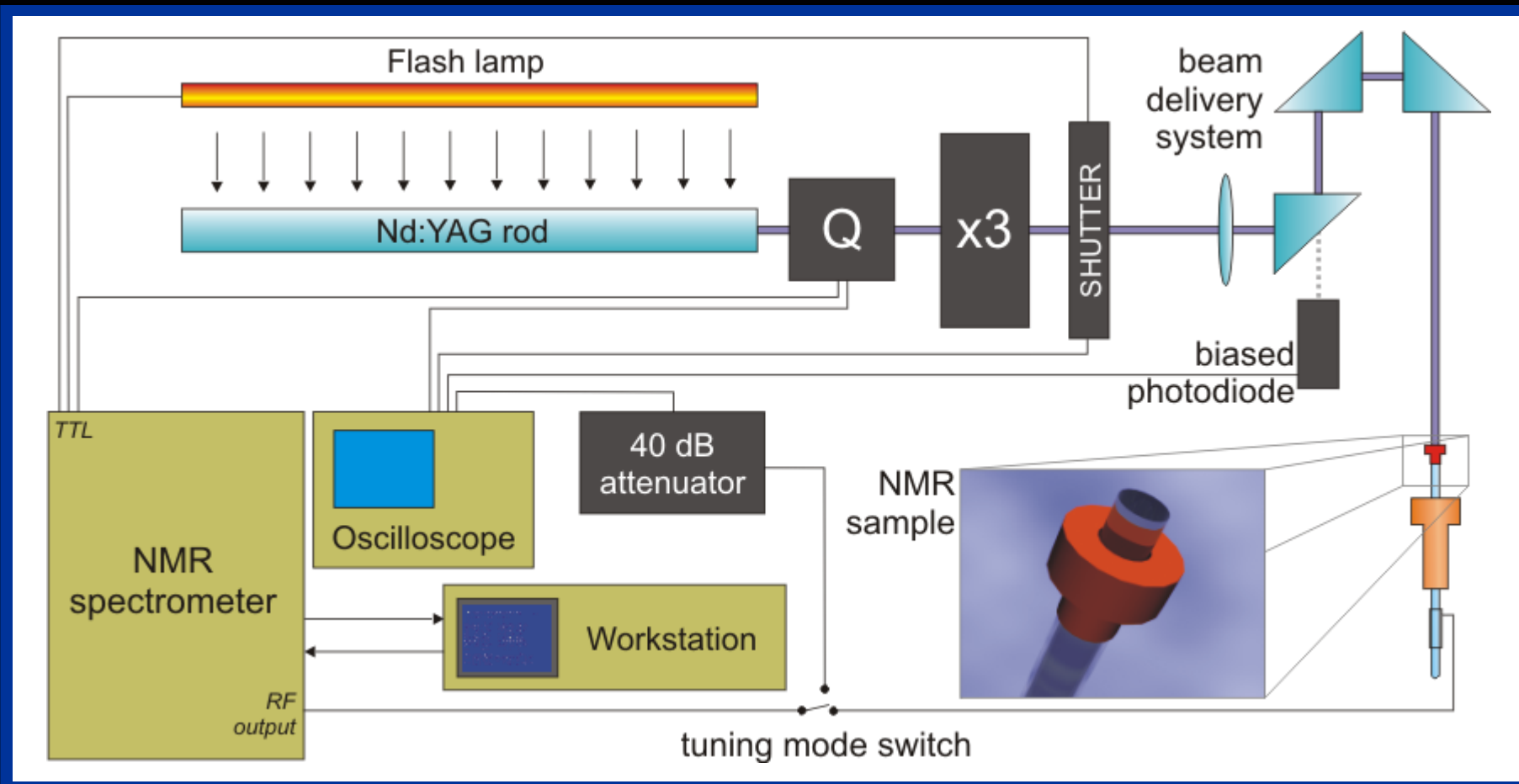
# Microsecond time-resolved photo-CIDNP installation



Basic principle: it looks like the only way left is from the top. The only viable way to bring the pulsed light in appears to be through-space routing by a series of prisms (mirrors in the latest incarnation).

100 mJ in 8 ns  
equals  
12.5 megawatts

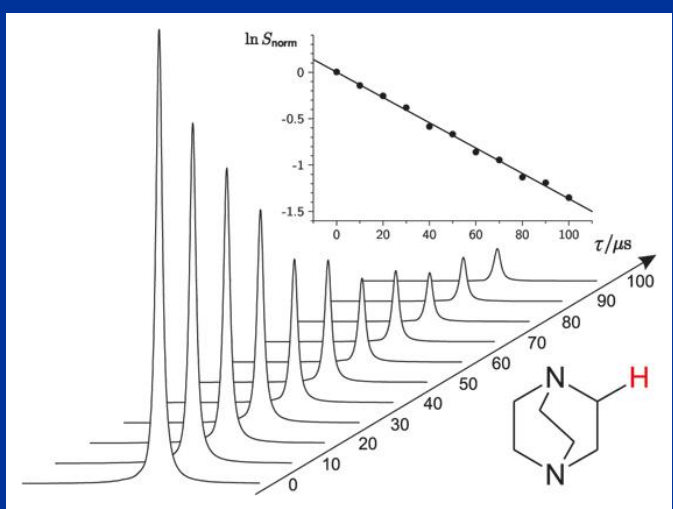
# Microsecond time-resolved photo-CIDNP installation



*Probe modification is not required. Easily installable on any spectrometer, including ones equipped with cryoprobes.*

*I. Kuprov, M. Goetz, P.A. Abbott, P.J. Hore, Rev. Sci. Instr. 76 (2005) 084103.  
M. Goetz, I. Kuprov, P.J. Hore, J. Magn. Reson. 177 (2005) 146-152.*

# Microsecond time-resolved $^{19}\text{F}$ photo-CIDNP experiments



The escape  $^1\text{H}$  photo-CIDNP dynamics of DABCO/FMN is dominated by the degenerate electron exchange at high concentrations. So the kinetics should be exponential.

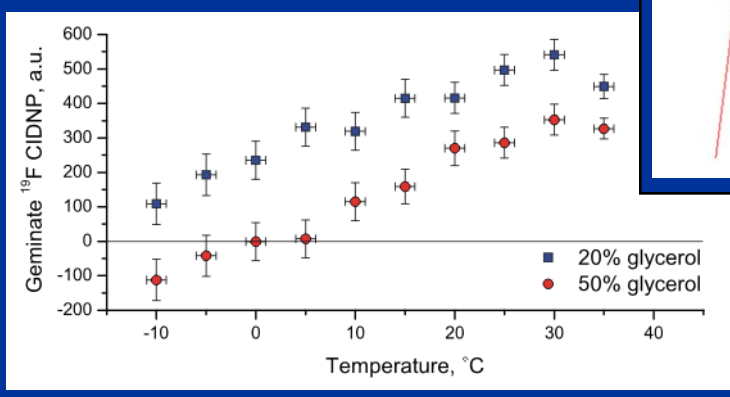
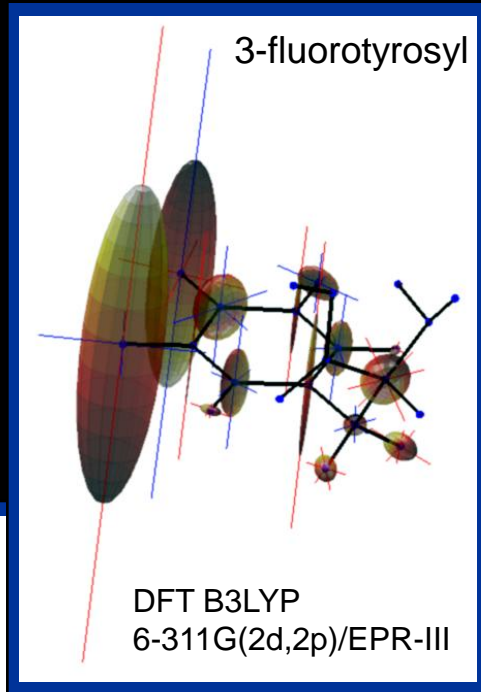
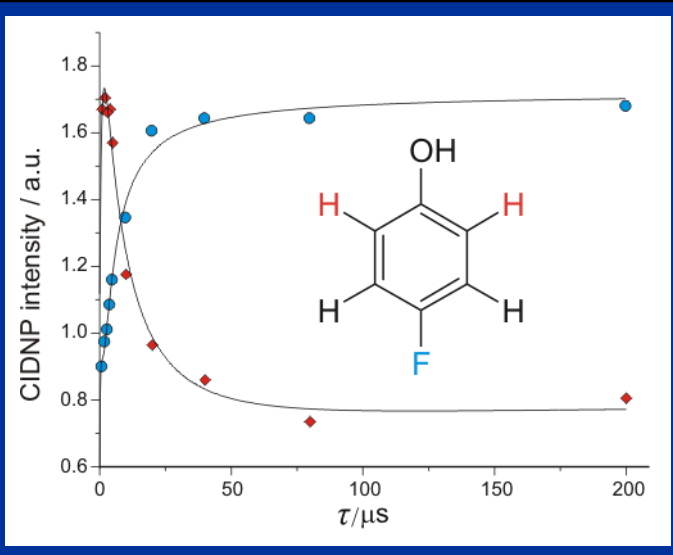
The experiment shows that it *is* exponential. Cool.

The  $^{19}\text{F}$  nucleus in 3-fluorotyrosyl sports an enormous hyperfine anisotropy of 400 MHz.

Experimental  $T_1$ :  $0.3 \mu\text{s} \pm 0.1$

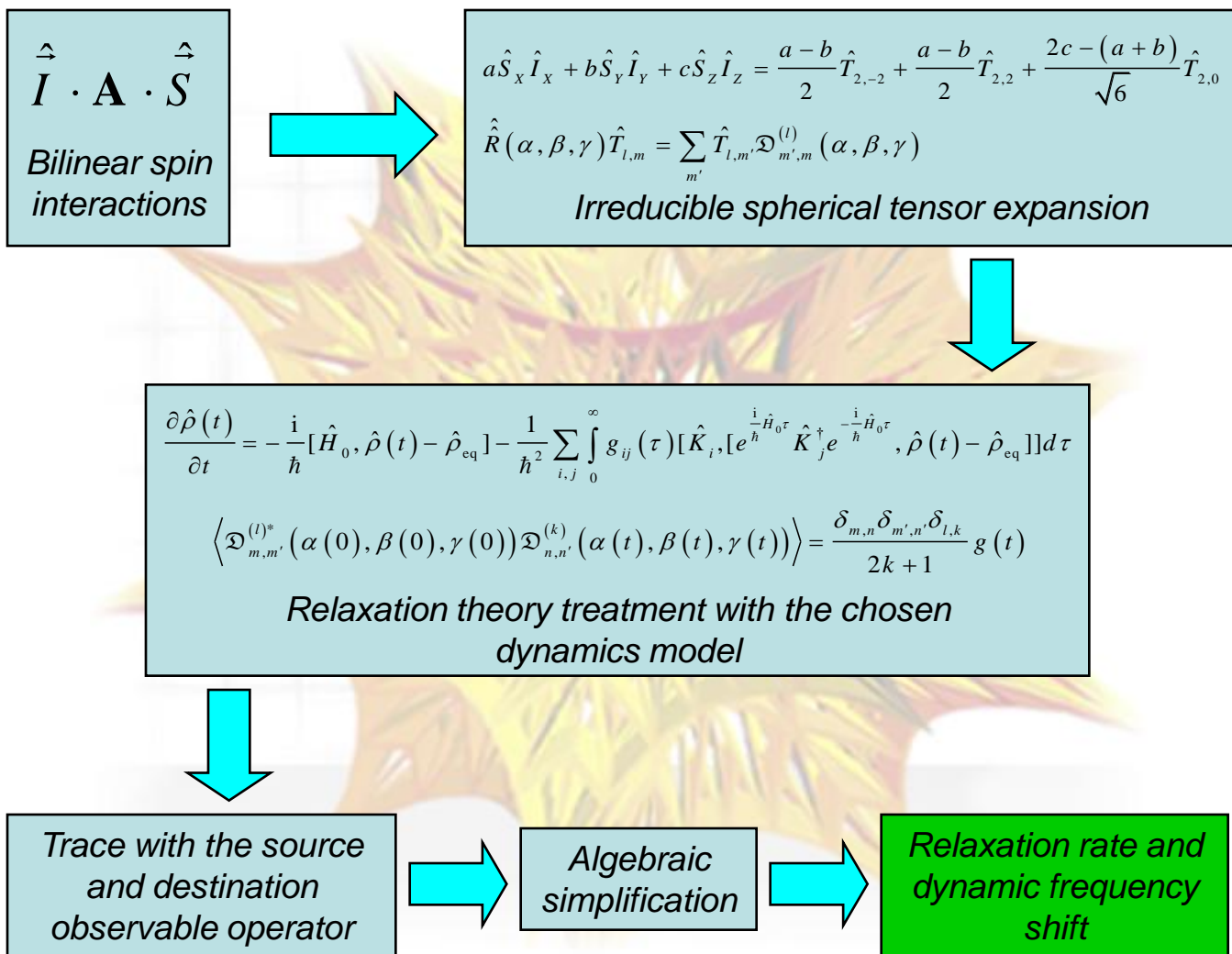
*Ab initio*  $T_1$  ( $\tau_c=100$  ps):  $0.18 \mu\text{s}$

The recombination cancellation is suppressed on  $^{19}\text{F}$  due to very fast paramagnetic relaxation.



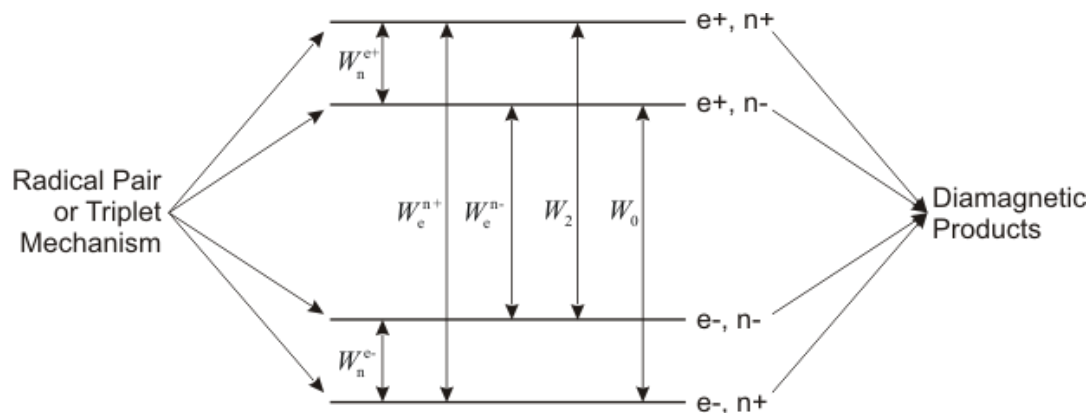
Some kind of extreme relaxation behavior is clearly present...

# Analytical “Redfield processor” in *Mathematica* 5.2



All relaxation, cross-relaxation and cross-correlation rates in a 3-spin system (HFC anisotropy,  $g$ -tensor anisotropy,  $g$ -HFC cross-correlation) in 40 seconds.

# Electron-nucleus dipolar cross-relaxation – too slow at 14.1 Tesla



$$W_n^{e+} = W_n^{e-} = \frac{\Delta A^2}{240} \frac{\tau_c}{1 + \tau_c^2 \omega_n^2}$$

$$W_e^{n+} = \frac{\Delta A^2 \tau_c}{240} \left( \frac{1}{1 + \tau_c^2 \omega_e^2} \right) + \frac{\Delta g^2 \tau_c}{60} \left( \frac{1}{1 + \tau_c^2 \omega_e^2} \right) + \frac{\Delta A \Delta g \tau_c}{240} \left( \frac{1 + 3 \cos(2\theta)}{1 + \tau_c^2 \omega_e^2} \right)$$

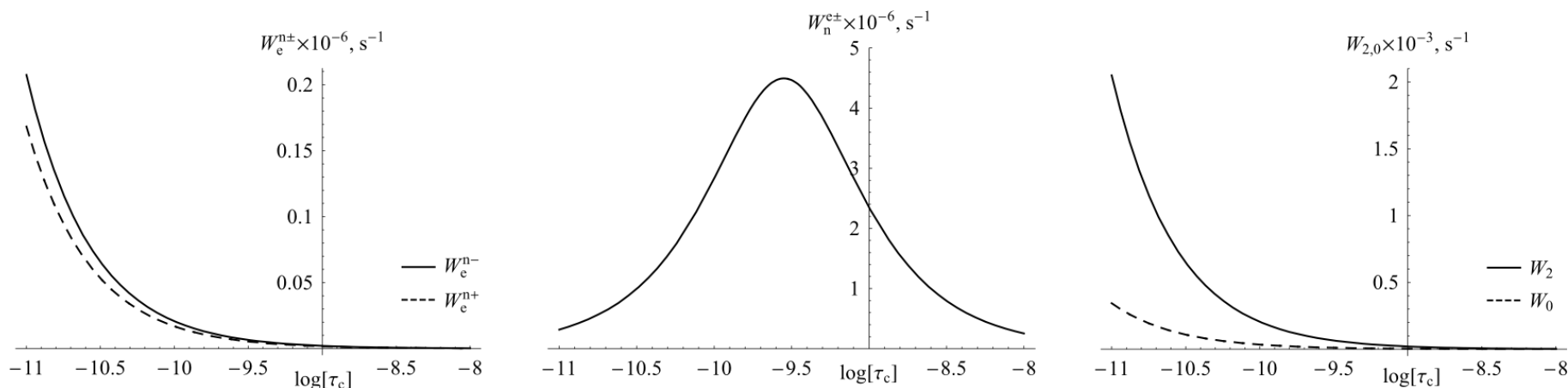
$$W_e^{n-} = \frac{\Delta A^2 \tau_c}{240} \left( \frac{1}{1 + \tau_c^2 \omega_e^2} \right) + \frac{\Delta g^2 \tau_c}{60} \left( \frac{1}{1 + \tau_c^2 \omega_e^2} \right) - \frac{\Delta A \Delta g \tau_c}{240} \left( \frac{1 + 3 \cos(2\theta)}{1 + \tau_c^2 \omega_e^2} \right)$$

$$W_2 = \frac{\Delta A^2 \tau_c}{60} \left( \frac{1}{1 + \tau_c^2 (\omega_e + \omega_n)^2} \right)$$

$$W_0 = \frac{\Delta A^2 \tau_c}{360} \left( \frac{1}{1 + \tau_c^2 (\omega_e - \omega_n)^2} \right)$$

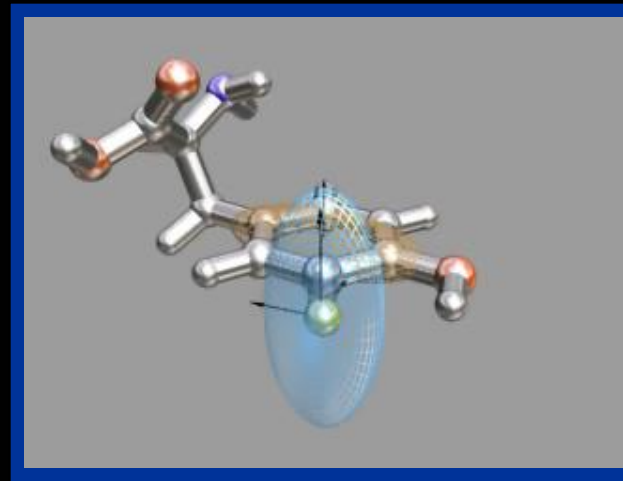
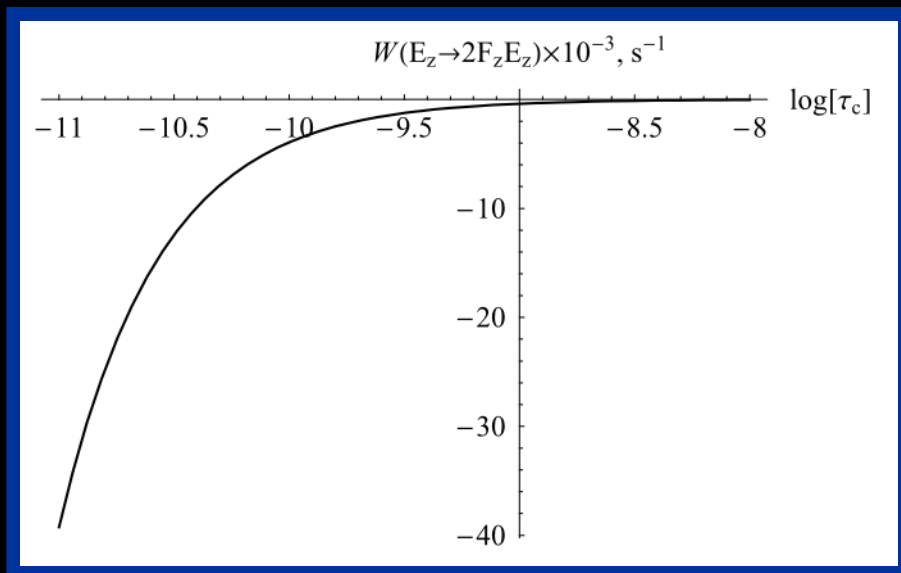
Early dipolar relaxation model suggested by Adrian et al.

Our coefficients.



No Overhauser CIDNP at 14.1 Tesla – too slow, at least for the geminate dynamics.

# Longitudinal $\Delta g$ -HFC cross-correlation – still too slow



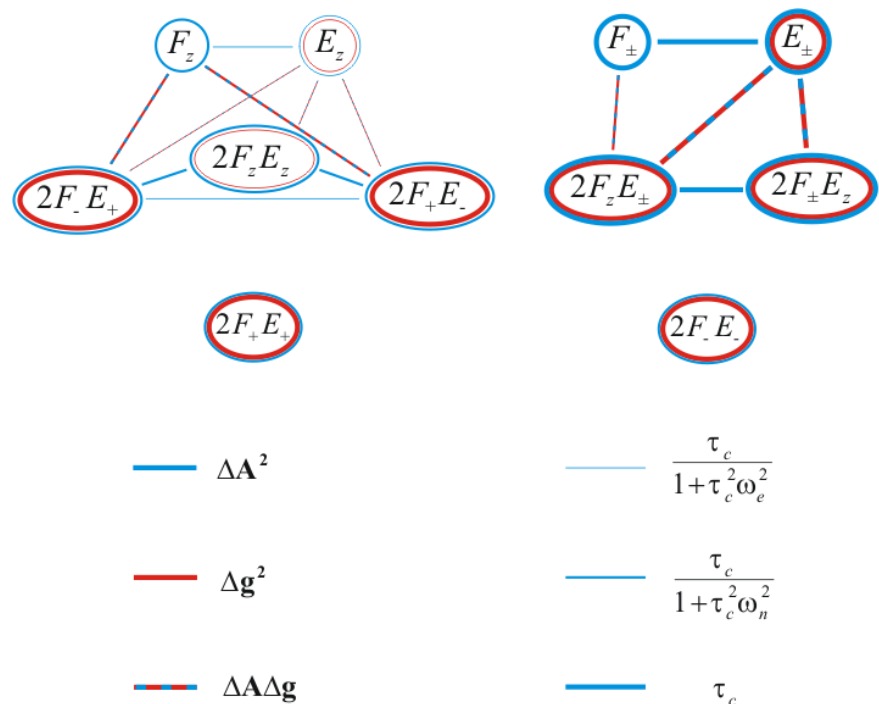
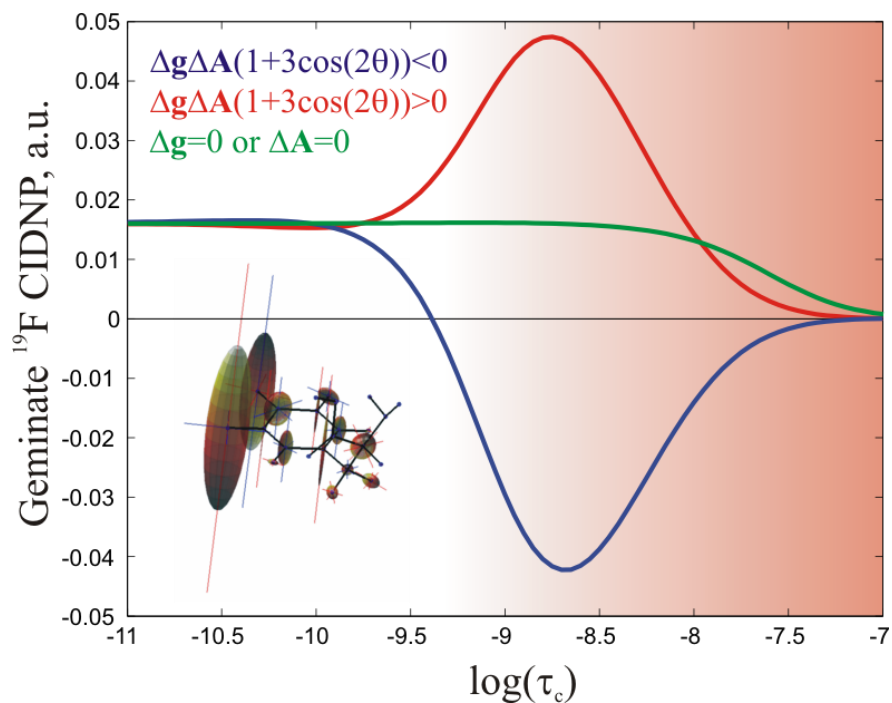
The square of the electron frequency in the denominator kills all longitudinal cross-relaxation processes at 14.1 Tesla.

Are there any processes that do not have that bug?

Yes.

The transverse relaxation processes.

# Full brute force relaxation analysis – works!

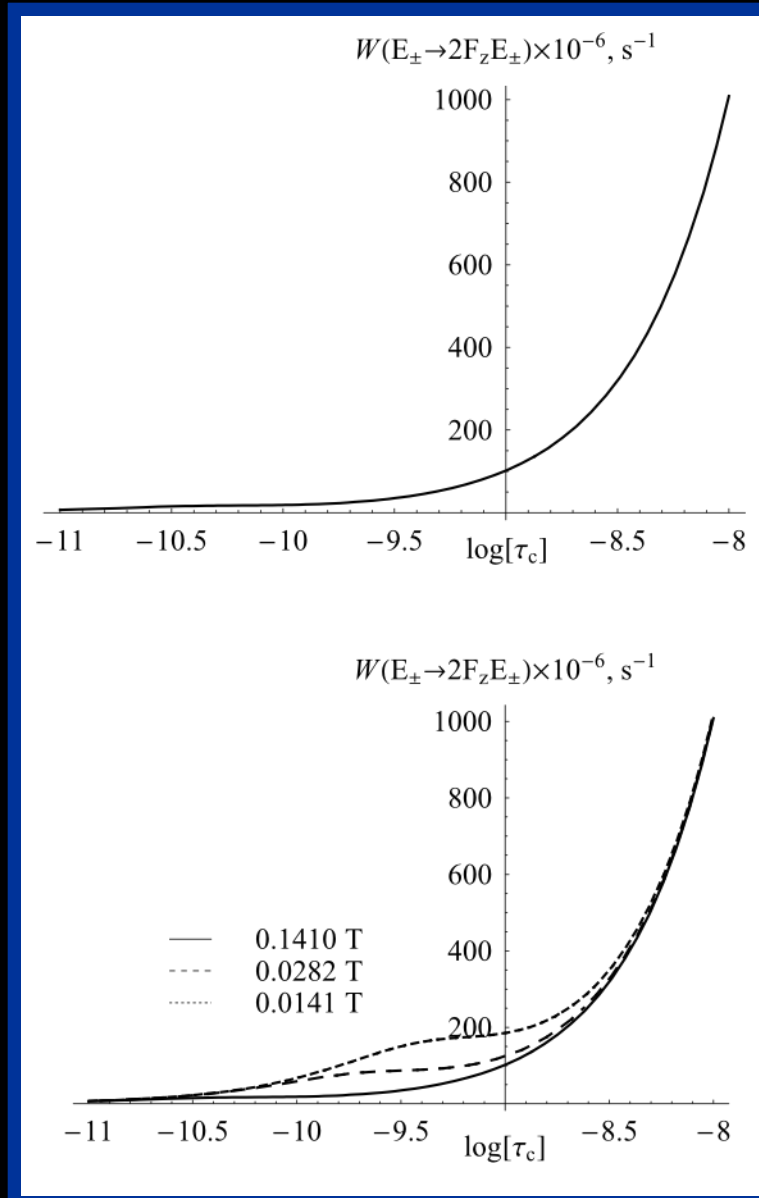
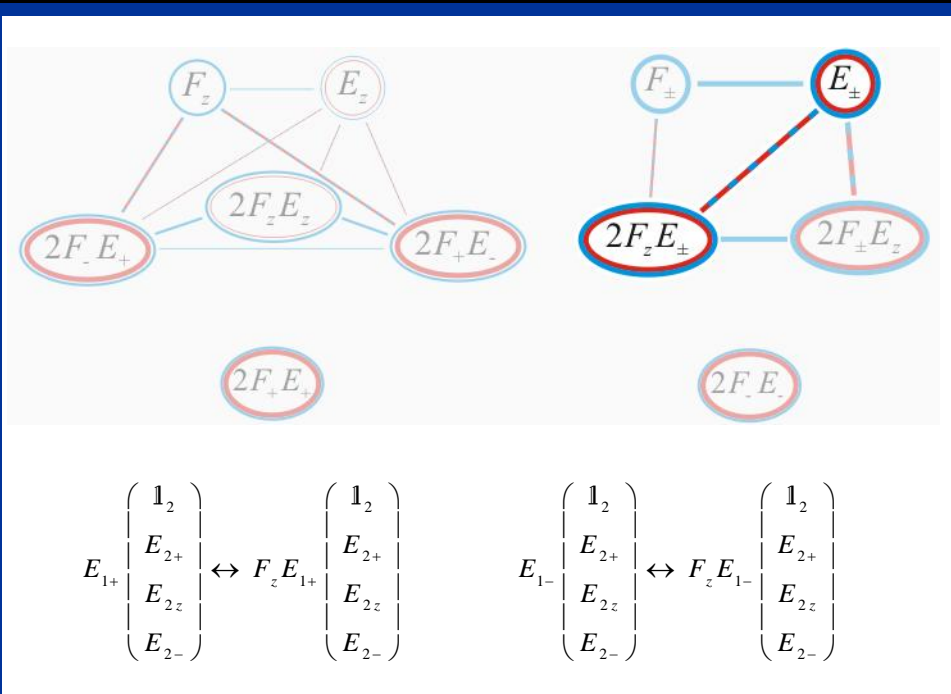


## Simulation details:

- Exponential propagator solution with the full relaxation matrix in a complete Liouville space.
- Hyperfine anisotropy, g-tensor anisotropy and g-HFC cross-correlation included.
- Noyes' re-encounter statistics.

The brute-force simulation does predict the experimentally observed geminate  $^{19}\text{F}$  photo-CIDNP sign change at long correlation times. Cool. Now need to hunt down the pathway responsible...

# Transverse $\Delta g$ -HFC cross-correlated relaxation



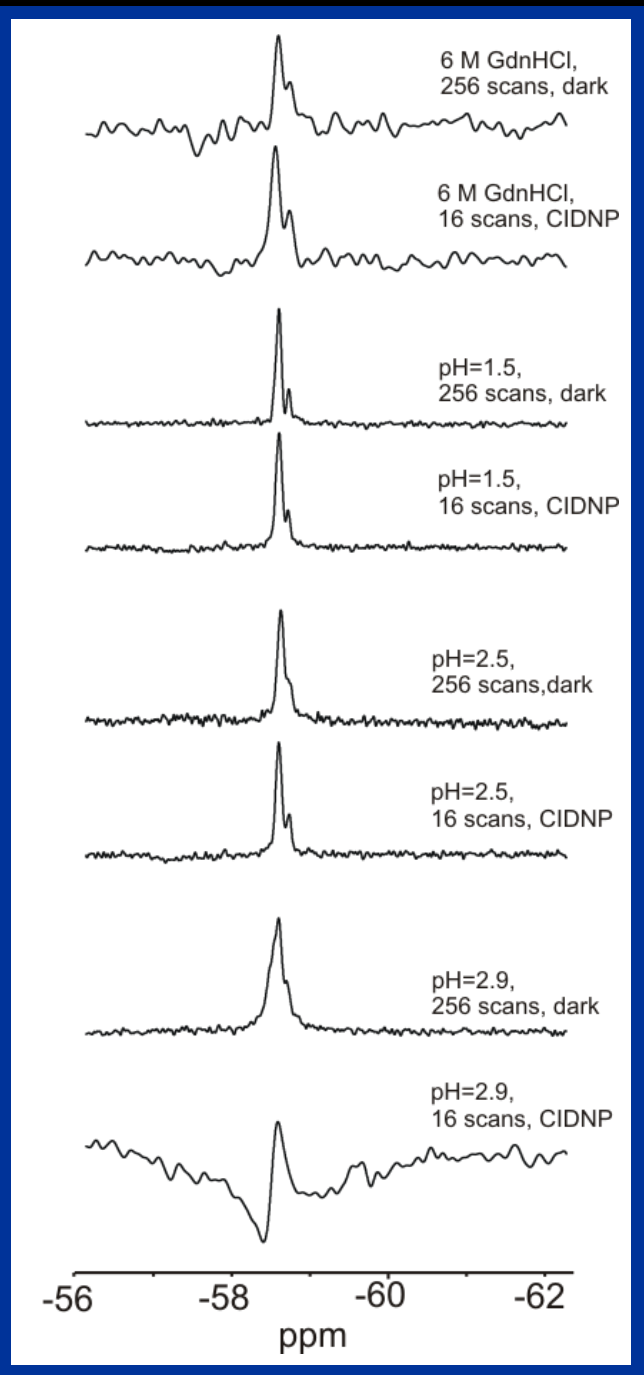
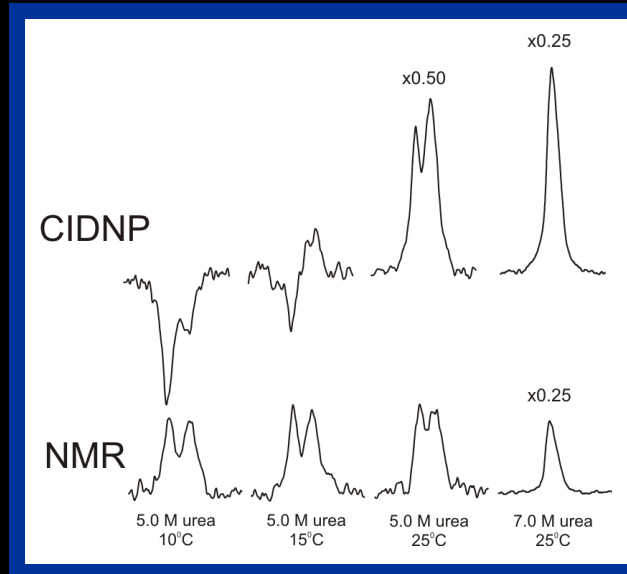
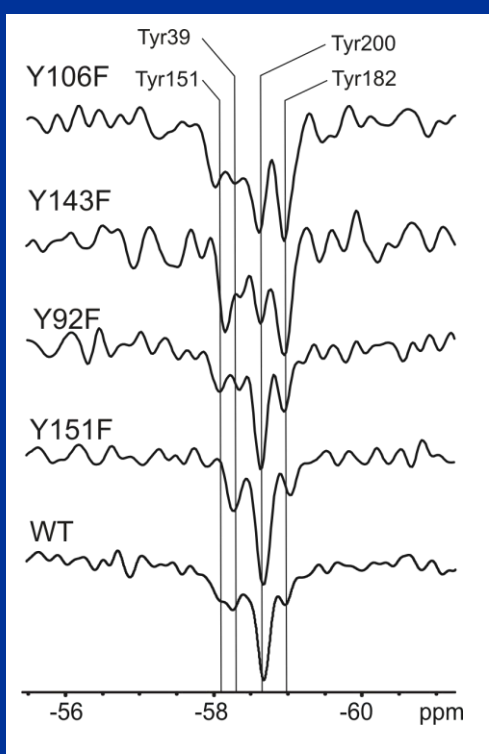
Electron singlet or triplet



Electron singlet or triplet conditional upon the nuclear spin state

Conceptually similar to the common CIDNP generation mechanism, but driven by transverse cross-correlated relaxation.

# Applications.



## Applications:

- Traditional – solvent accessibilities.
- Unique – real-time measure of side chain mobility.
- Fundamental – a testing ground for relaxation theories of CIDNP and CIDEP.

Can now do brute-force analytical RT – new opportunities.

# Acknowledgements

## Collaborators:

Peter J. Hore (Oxford, UK)

Martin Goez (Halle, Germany)

Sophie Jackson (Cambridge, UK)

Tim Craggs (Cambridge, UK)

## Financial support:

Hill Foundation

Scatcherd European Foundation