Мега-грант «Многочастотный электронный парамагнитный резонанс (ЭПР) для биохимических исследований» Руководитель-проф. Майкл Боуман

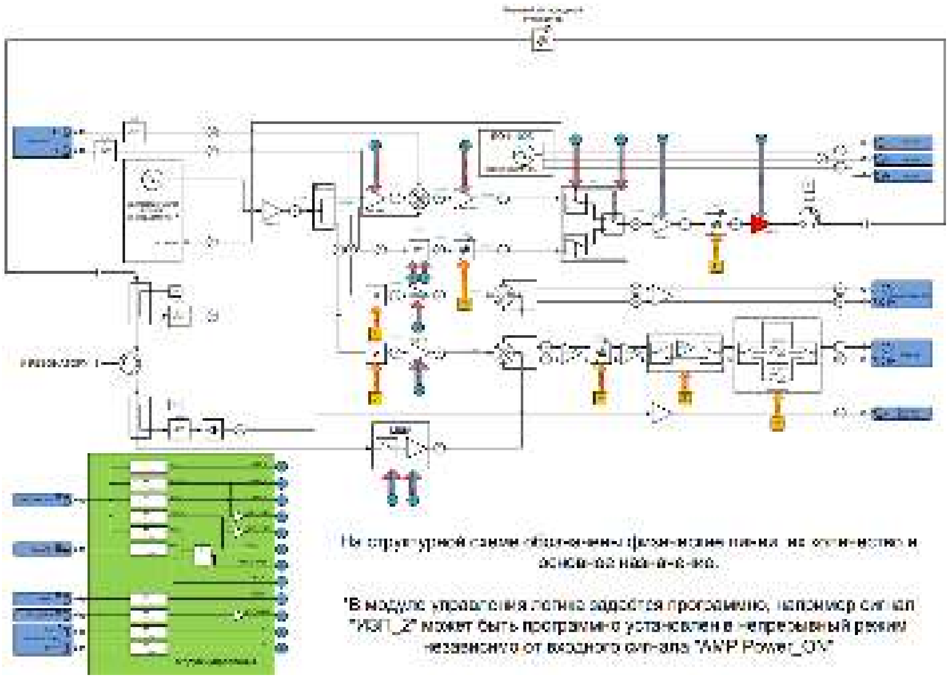
Наименование проекта: **Многочастотный электронный парамагнитный резонанс (ЭПР) для биохимических исследований**

Федеральное государственное бюджетное учреждение науки Новосибирский институт органической химии им. Н.Н. Ворожцова Сибирского отделения Российской академии наук

Цель проекта: Целью проекта является развитие и применение передовых методов спектроскопии электронного парамагнитного резонанса (ЭПР) к актуальным задачам в области биохимии и биомедицины, имеющие принципиальную значимость для развития методов диагностики и разработки стратегий лечения ряда социально важных заболеваний, таких как болезни сердечно-сосудистой системы, болезнь Альцгеймера, онкологические заболевания и др.

Задача проекта: Основной задачей данного проекта является создание высокотехнологичной ЭПР лаборатории мирового уровня, имеющей в своем распоряжении самый современный приборный парк с максимальной функциональностью, и укомплектованной высококвалифицированными кадрами.

Разработка импульсного ЭПР спектрометра (10гГц, 34 гГц)



1. **Применен цифровой синтезатор сигнала СВЧ-диапазона, рабочий диапазон СВЧ/точность: 5.0 – 15.0 ГГЦ ± 1 Гц.**
2. **Применен твердотельный усилитель мощности 350 Ватт, обеспечивает высокую стабильность мощности и фазы импульсов.**

- **Развертка по полю/точность: 50 – 6500 ± 0.01 Гс**
- **Длительности импульсов: от 12нс**
- **Шаг установки импульсов и задержек: 2 нс**
- **Частота повторений импульсов: до 10 кГц**
- **Рабочая температура/точность криостата: от 3.5 ± 0.1 °К**

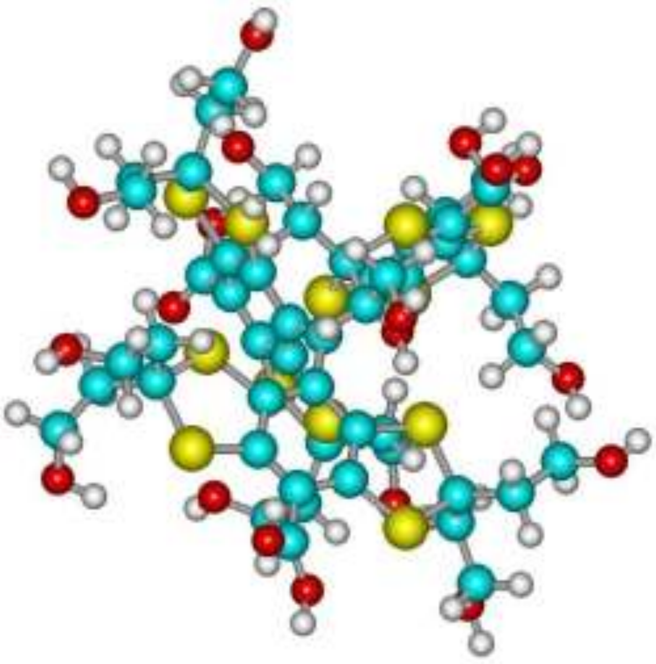
Triarymethyl radical and its applications

Elena Bagryanskaya, Victor Tormyshev

N.N.Vorozhtsov Novosibirsk Institute of Organic Chemistry SB RAS

Physics and Chemistry of Elementary Chemical Processes

September 5-9, 2022,
Akademgorodok,
Novosibirsk, Russia



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OUTLINE

- History of trityl radicals
- Synthesis: general concept, capabilities & limitations
- Trityls vs nitroxides: advantages and disadvantages
- Trityl properties – EPR and stability
- Trityl application in EPR tomography as OXYGEN sensors
- Trityls as spin labels for PELDOR/DEER
- Trityl- nitroxide biradical – polarizing agents for DNP
- Trityl- 19F for ENDOR distance measurements

Triarylmethyl radicals (TAM, trityls): what they are ?

AN INSTANCE OF TRIVALENT CARBON: TRIPHENYL-METHYL.

By M. Gomberg.

Received October 4, 1900.

[PRELIMINARY PAPER.]

SOME time ago[1] I published a method of preparing tetra-phenylmethane. The yield was rather small and I was obliged to study the solubilities, composition, molecular weight, and the nitro derivative on about 0.5 gram of the hydrocarbon. The stereochemical interest attached to this compound has induced me to take up the subject once more, in the hope of obtaining larger yields. I have, therefore, gone over most of the methods which have been tried by others for the preparation of tetraphenylmethane. My results, while differing in detail from those published by others, agree in the main,—the hydrocarbon could not be obtained by the usual reactions. One of the main proofs advanced by me for the constitution of tetraphenylmethane was that it furnished a tetranitro derivative which gave no colored salts with alcoholic potash, while most of the less phenylated methanes do respond to this test. To prove whether

1 Hemilian: Ber. d. chem. Ges., 7, 1207.
2 Ber. d. chem. Ges., 30, 2043; This Journal, 20, 773.

24-22

The University of Michigan - Ann Arbor, (1900-1905)

Moses Gomberg (Моисей Гомберг)
the FOUNDER of RADICAL CHEMISTRY

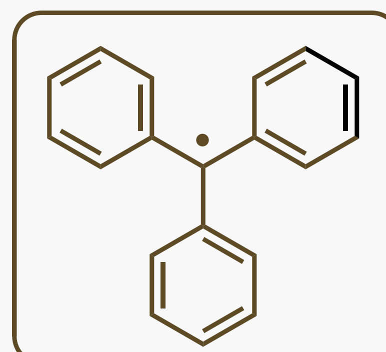
Elisavetgrad (1866) – Ann Arbor (1947)

at 92° C.

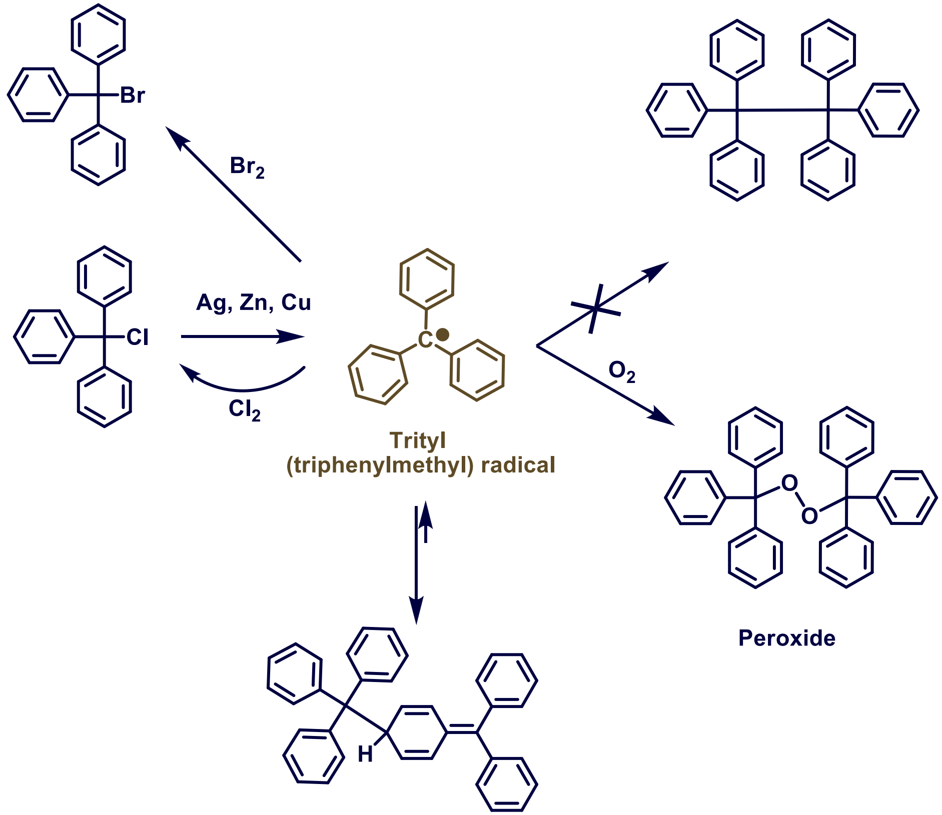
V. TRIPHENYLMETHYL,

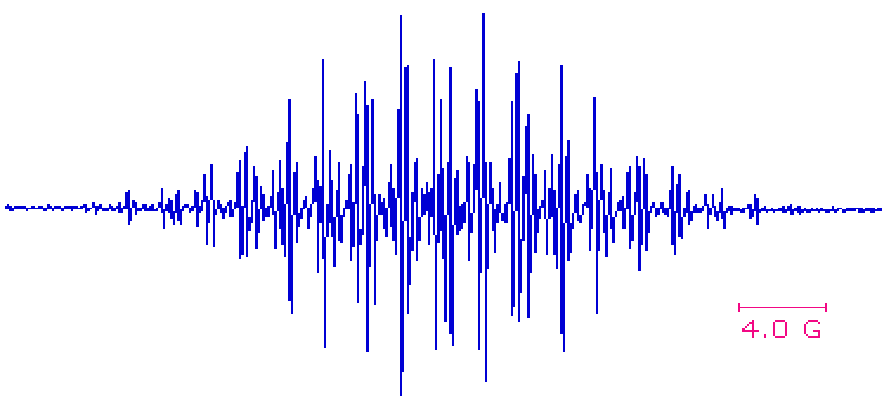
$(C_6H_5)_3\equiv C.$

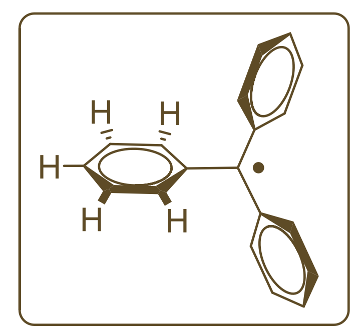
experimental evidence presented above forces



Triarylmethyl radicals (TAM, trityls): what they are ?

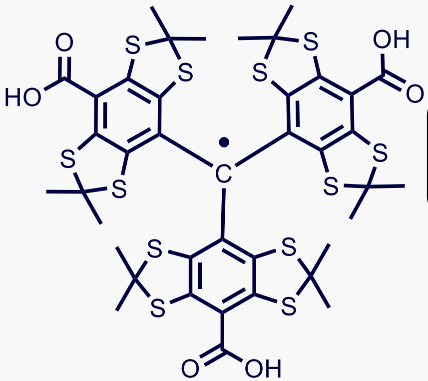






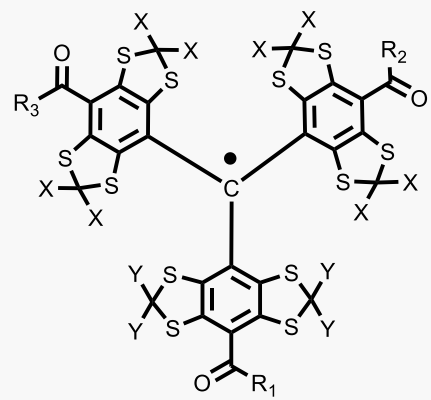
Chiral propeller: *J. Sciebura et al, Angew. Chem. Int. Ed., 2009, 48, 7069*

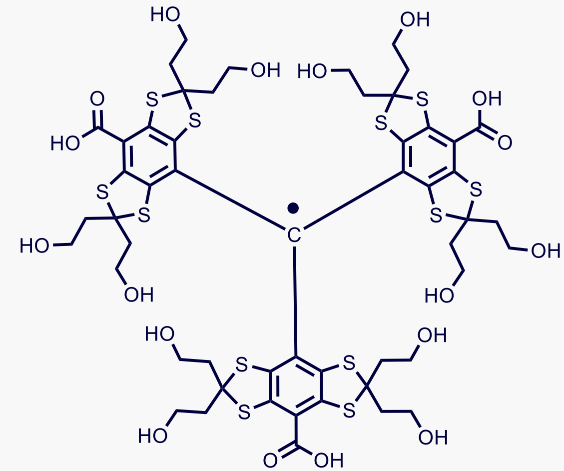
Sterically hindered tris-(tetrathiaryl)methyls – a new generation of trityls (NYCOMED INNOVATIONS, 1990-1998)



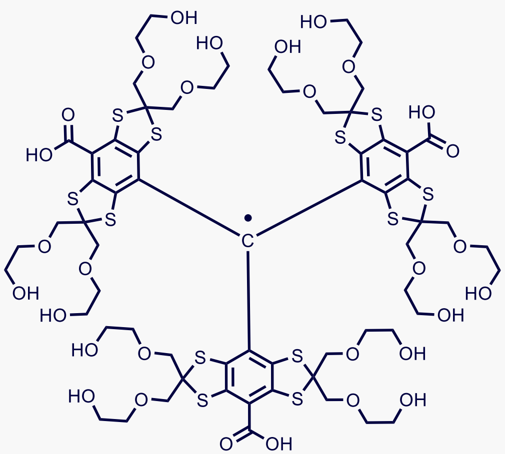
Finland trityl

Tris(8-carboxy-2,2,6,6-tetramethylbenzo [1,2-d;4,5-d′] bis[1,3]dithiol-4-yl)methyl

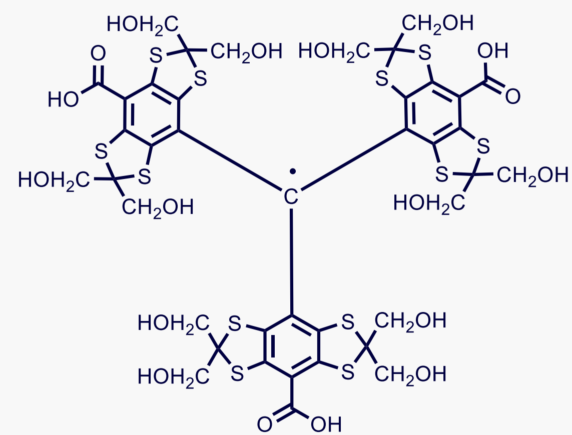




OX 063



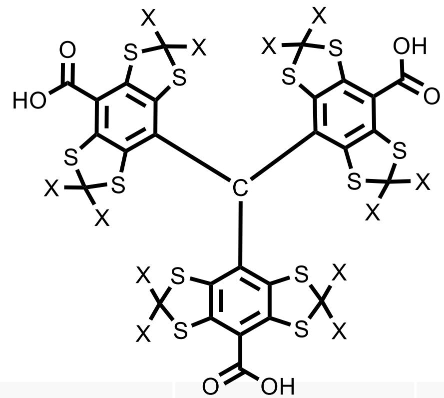
OX 031



OX 021

The key objective of studies:
new efficient agents for Overhauser-enhanced MRI

Trityl radicals: lipophilicity/hydrophilicity, and stability in blood



Side substituent	Trityl	logP (pH=2.0)	logP (pH=7.0)	$t_{1/2}$ (blood, mice)
CH_3	**Finland**	**> 5**	**-2.43**	**> 1.5 h**
CH_2CH_2OH	**OX063**	**-1.84**	**< -5.2**	**> 1.5 h**
$CH_2CH_2OCH_2CH_2OH$	**OX031**	**-2.62**	**< -4.8**	30 min
CH_2OH	OX021	-1.65	-3.40	< 5 min

Stability and toxicity of trityls

Trityl	Solid state Destruction in the presence of air at RT after storage for 1 year	Water solution, pH 7.0 Destruction in the presence of air at 0^{o}C after 2 months	Water solution, pH 7.0 Destruction in the absence of air at 0^{o}C after 1 year	Toxicity LD_{50}
Finland	0-1%	<1%	< 1%	0.24-0.48 g/kg
OX063	0-1%	2.5%	< 1%	**11.8 g/kg**

Trityl radicals *vs* Nitroxide radicals: spectroscopy, physical and chemical features

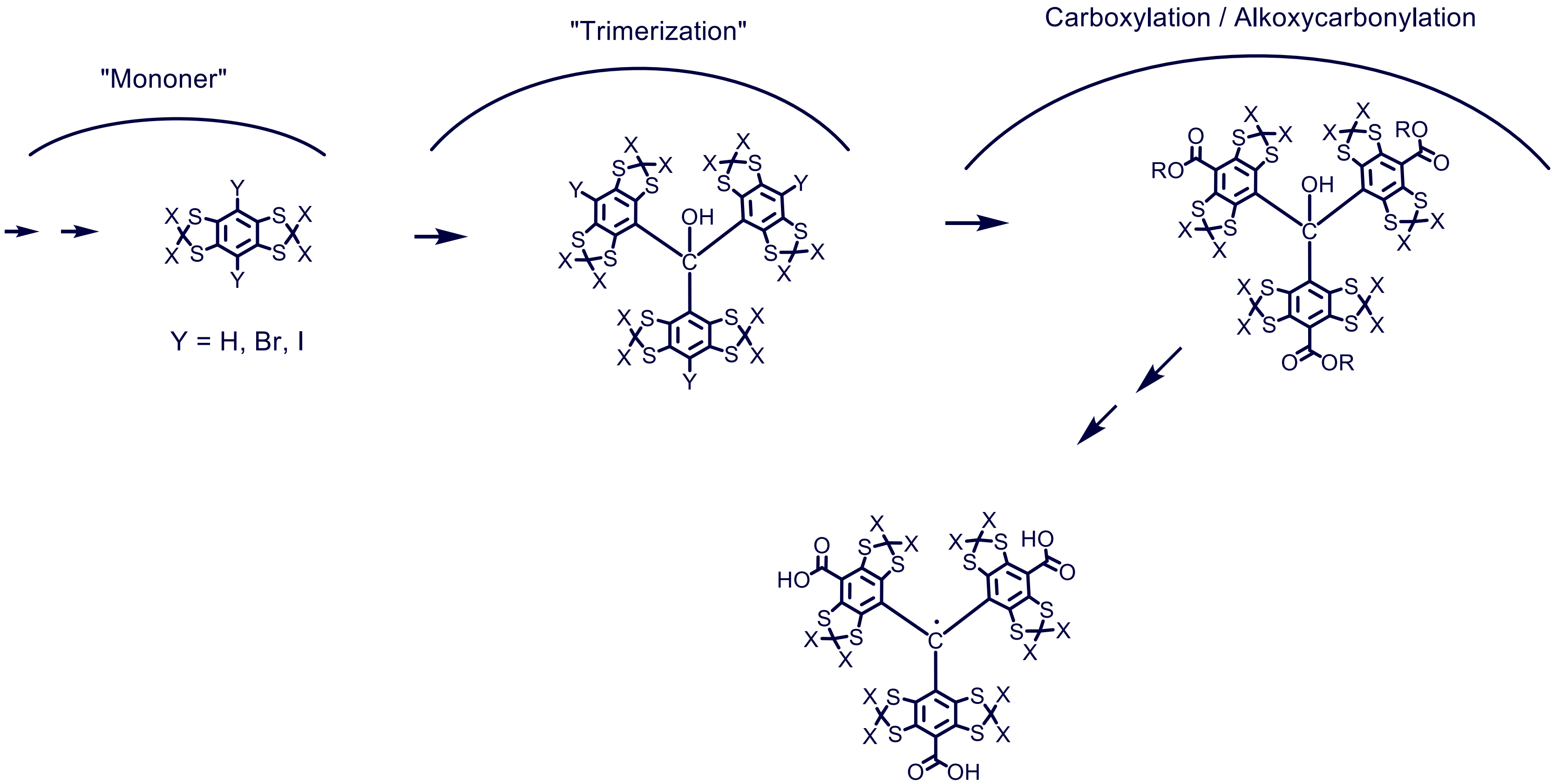
Nitroxides

- EPR signal: a rather broad EPR triplet
- EPR resolution: relatively low
- Sensitivity in registering oxygen: relatively low
- Stability in biological media: easily reduced, but stability permits to be altered, and thus may be notably increased
- Main uses: measurements of redox status and pH, ROS probes, spin traps, SDSL techniques, antioxidants, Nitroxide-Mediated radical Polymerization (NMP)

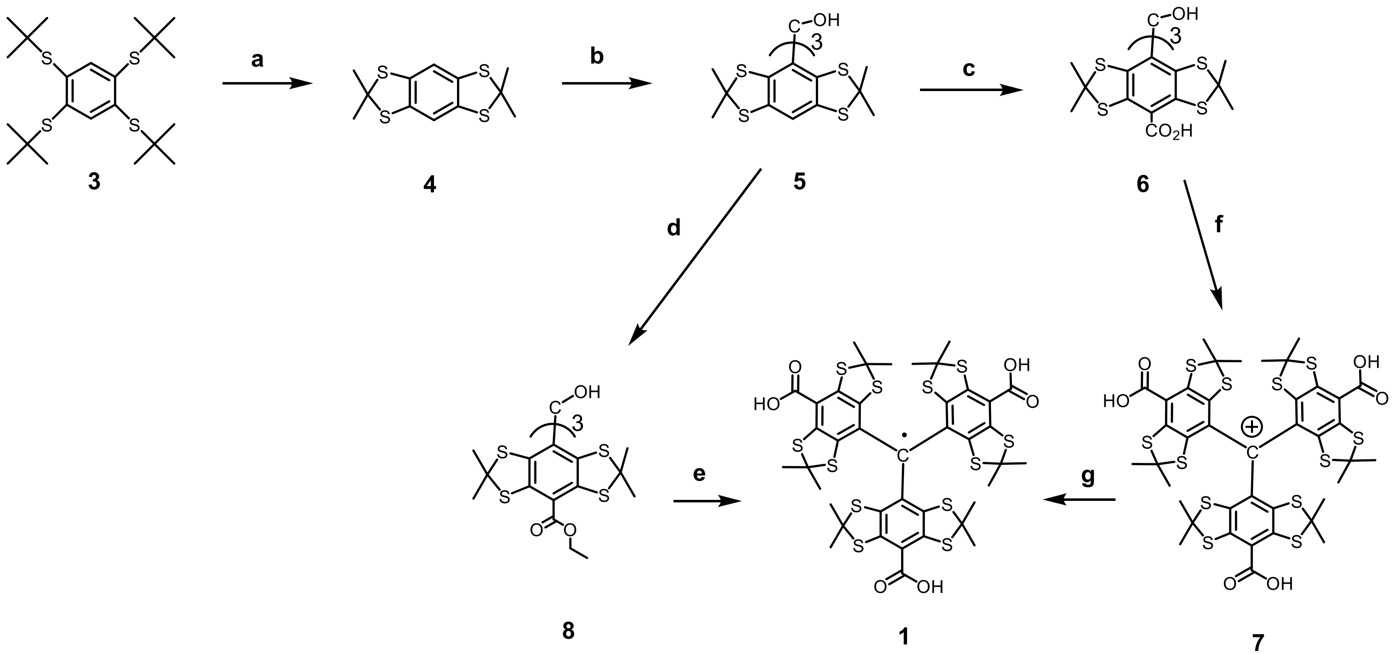
Trityls

- EPR signal: narrow, sharp
- EPR resolution: high, LW < 100 mG
- Sensitivity in registering oxygen: high
- Stability in biological media : stable for hours or longer
- Main uses: EPR, EPR oximetry, Overhauser-enhanced MRI, spin labels, materials for EPR microscopy

Trityl synthesis: general concept



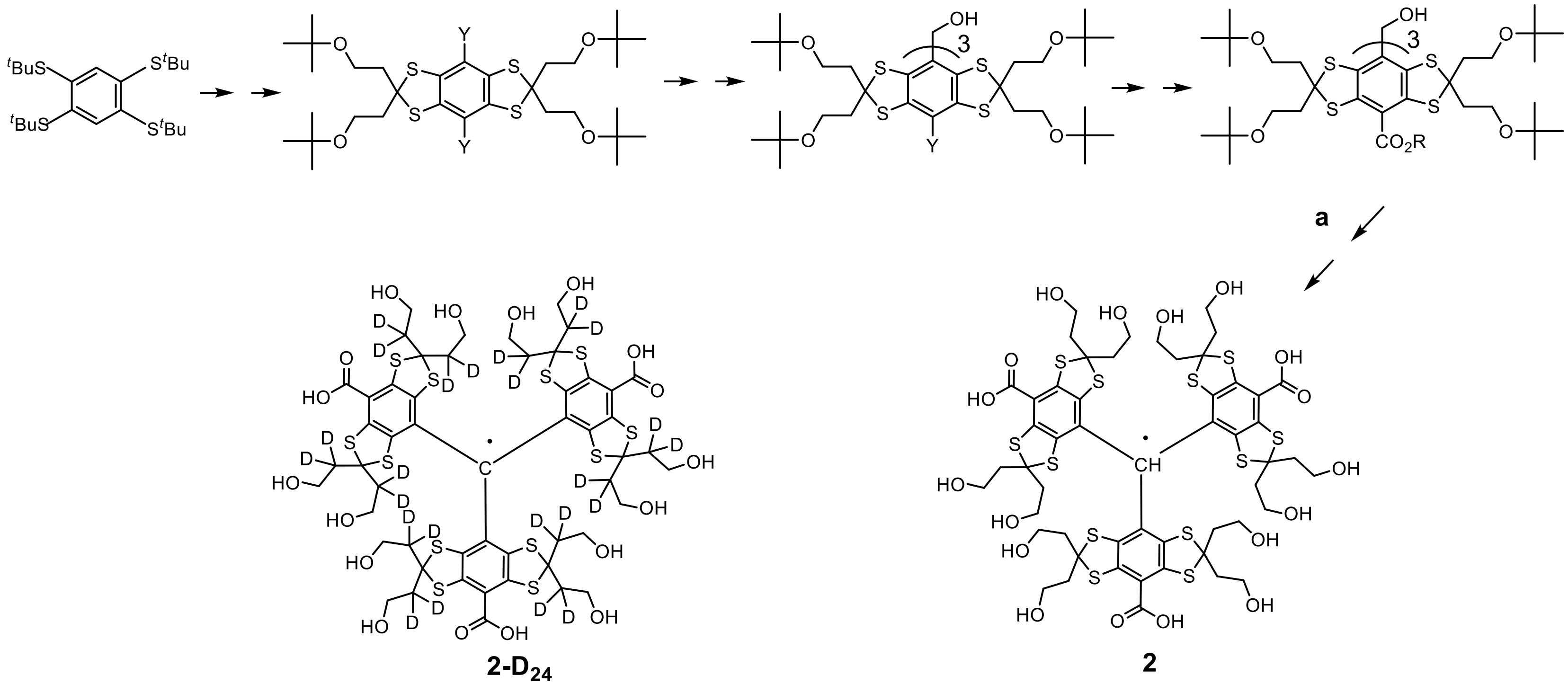
Synthesis of Finland TAM



(a) ацетон, BF_3, D-10-камфорсульфоновая кислота, $CHCl_3$, 93%;
(b) 2.5 M раствор *н*-BuLi (1.1 экв) в *н*-гексане, диэтиловый эфир, диэтилкарбонат (0.32 экв), 72%;
(c) 2.5 M *н*-BuLi в *н*-гексане (10 экв), *н*-гексан/TMEDA, CO_2 (тв), 67%;
(d) 2.5 M *н*-BuLi в *н*-гексане (10 экв), *н*-гексан/TMEDA, диэтилкарбонат (40 экв), 32%;
(e) CF_3SO_3H (15 экв) в дихлорметане, $SnCl_2$ (1 equiv), гидролиз водным раствором KOH (10 экв), HCl, 92 %;
(f) TFA; (g) $SnCl_2$ (0.5 equiv.), 96 %

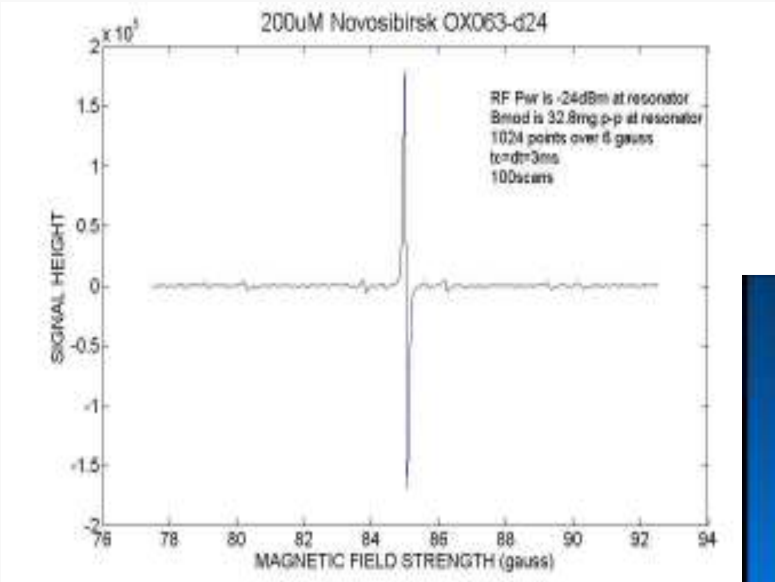
S. Andersson, F. Radner, A. Rydbeck, R. Servin, L.-G. Wistrand. U.S. Patent 5530140, 1996.
Yu. Rogozhnikova, V.G. Vasiliev, T.I. Troitskaya, D.V. Trukhin, T.V. Mikhalina, H.J. Halpern, V.M. Tormyshev, *Eur. J. Org. Chem.*, 2013, **2013**, 3447.

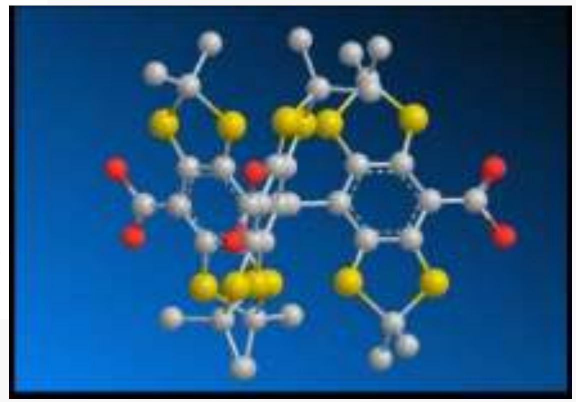
Synthesis of OXO63

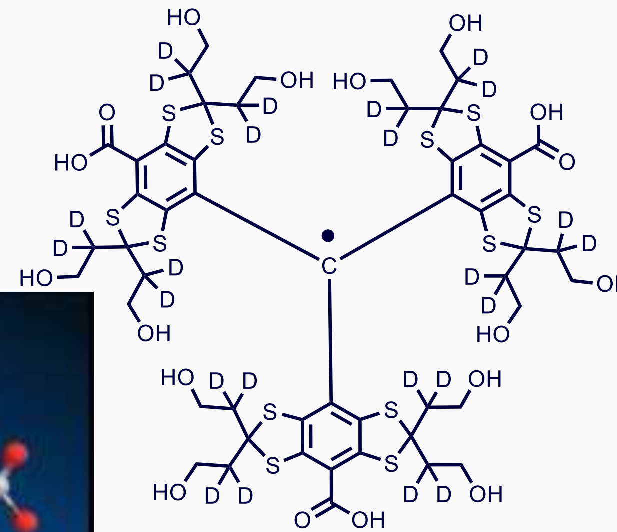


(a) (R = H), муравьиная кислота, 32 ч, 40 ^oC; раствор CF_3SO_3H (5 экв) в дихлорметане; $SnCl_2$ (1 экв); водный раствор KOH (5 экв), HCl, 92 %

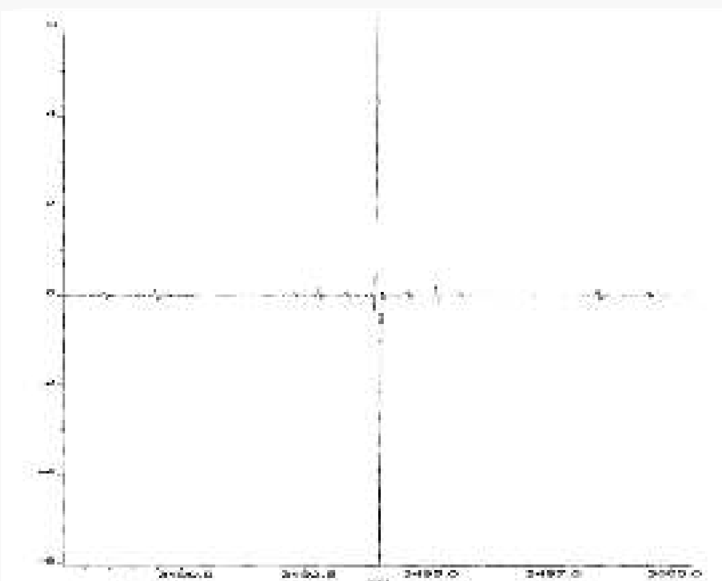
Typical persistent narrow-line triarylmethyls (TAMs) with numerous applications in spectroscopy, biology and material science

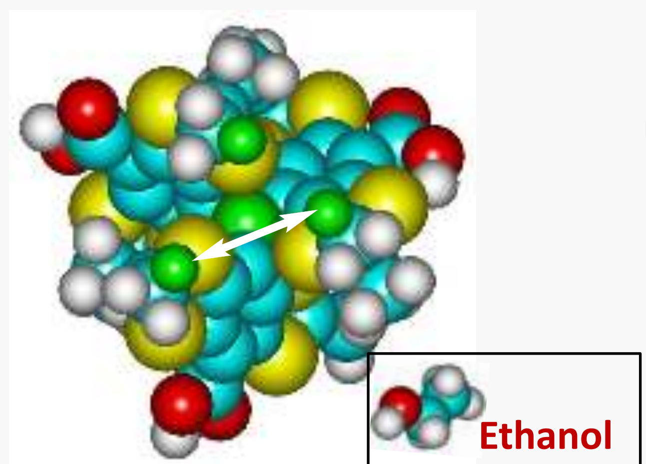




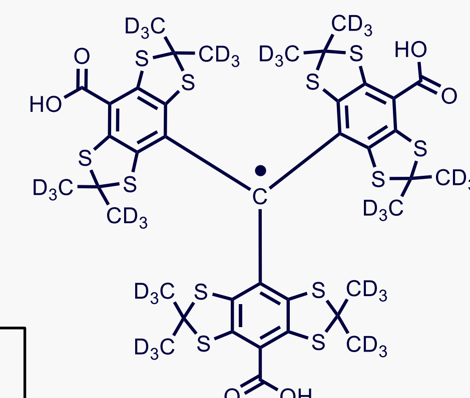


OX 063-D_{24}



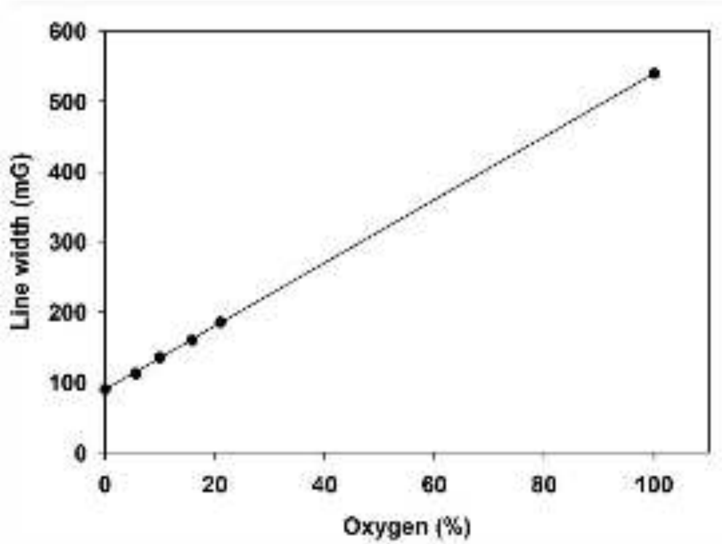


CH_3-CH_3 distance 0.49 nm



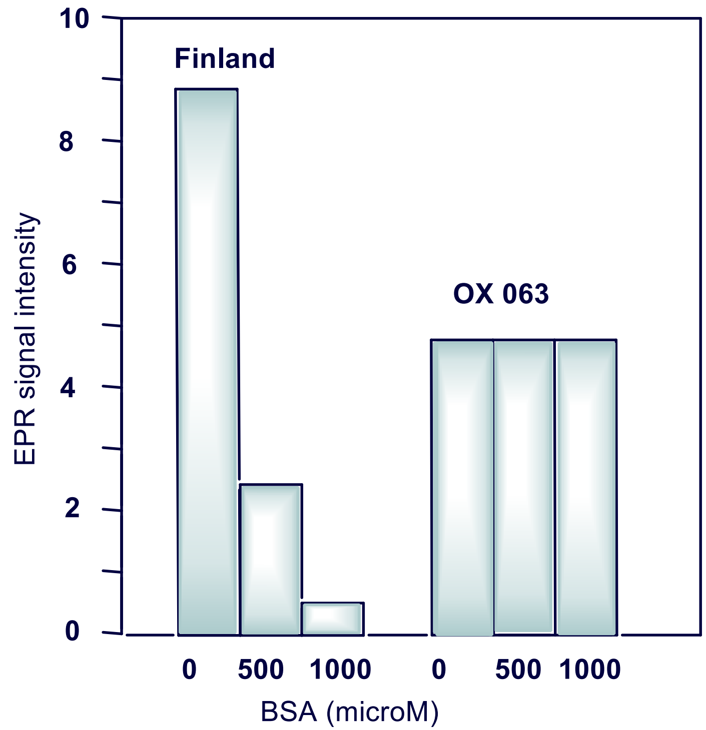
Finland-D_{36} trityl

Trityl application in EPR tomography as OXYGEN sensors



The dependency of the EPR line-width Finland TAM on the fraction of oxygen in gas phase.

I. Dhimitruka et al. *Bioorg. Med. Chem. Lett.*, 17, 2007, 6801–6805

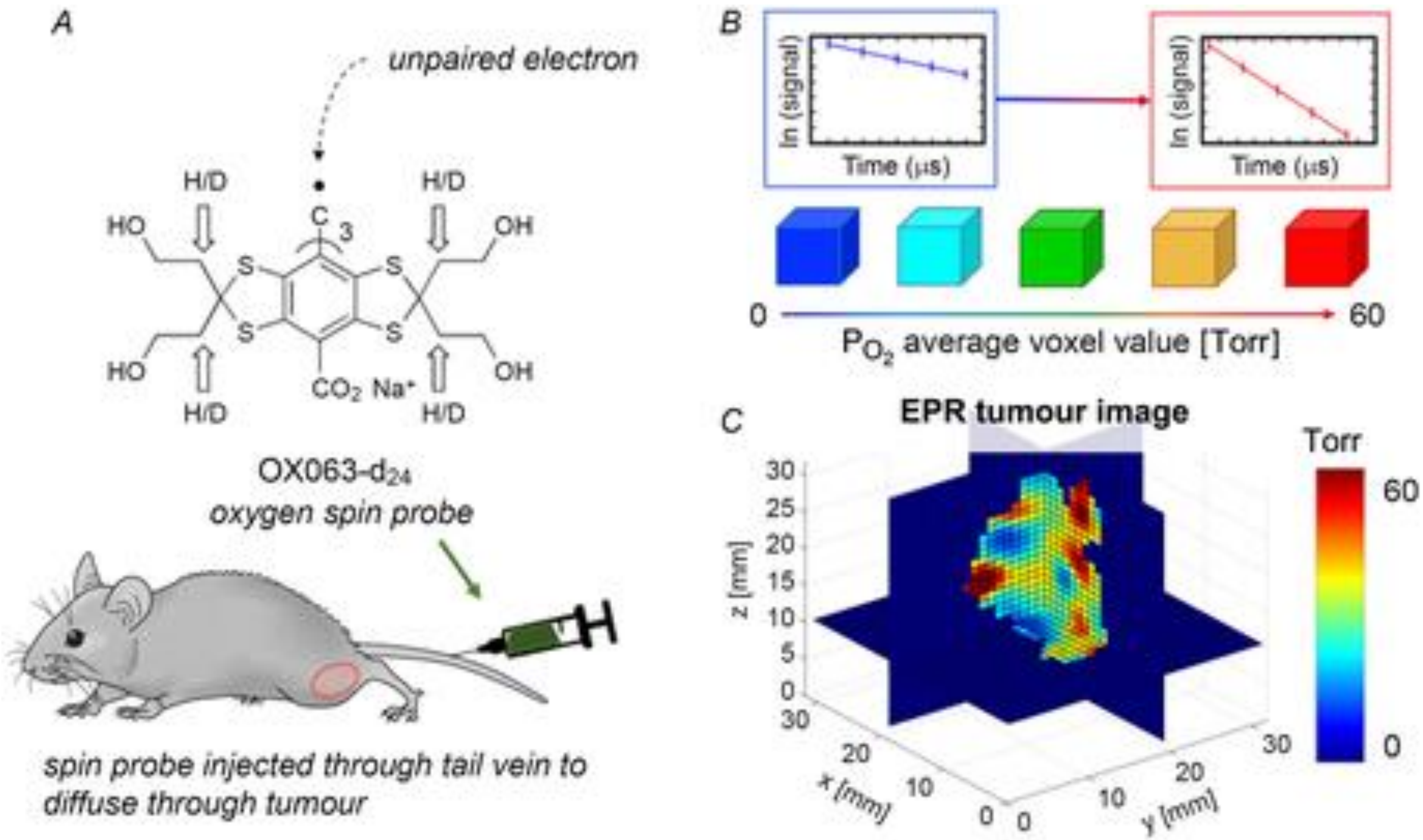


Relative EPR signal intensity of TAM radicals (20 µM) in PBS (20 mM, pH 7.4) in the presence of bovine serum albumin (0, 500 and 1000 µM).

Y. Qu et al. *Chem. Eur. J.*, 25, 2019, 7888
H. Chen,A.G.Maryasov,O.Y.Rogozhnikova, D. V. Trukhin, V. M. Tormyshev,M.K.Bowman,Phys.Chem.Chem.Phys.2016,18,24954.

Finland trityl and OX063: reversible broadening of linewidth & aggregation

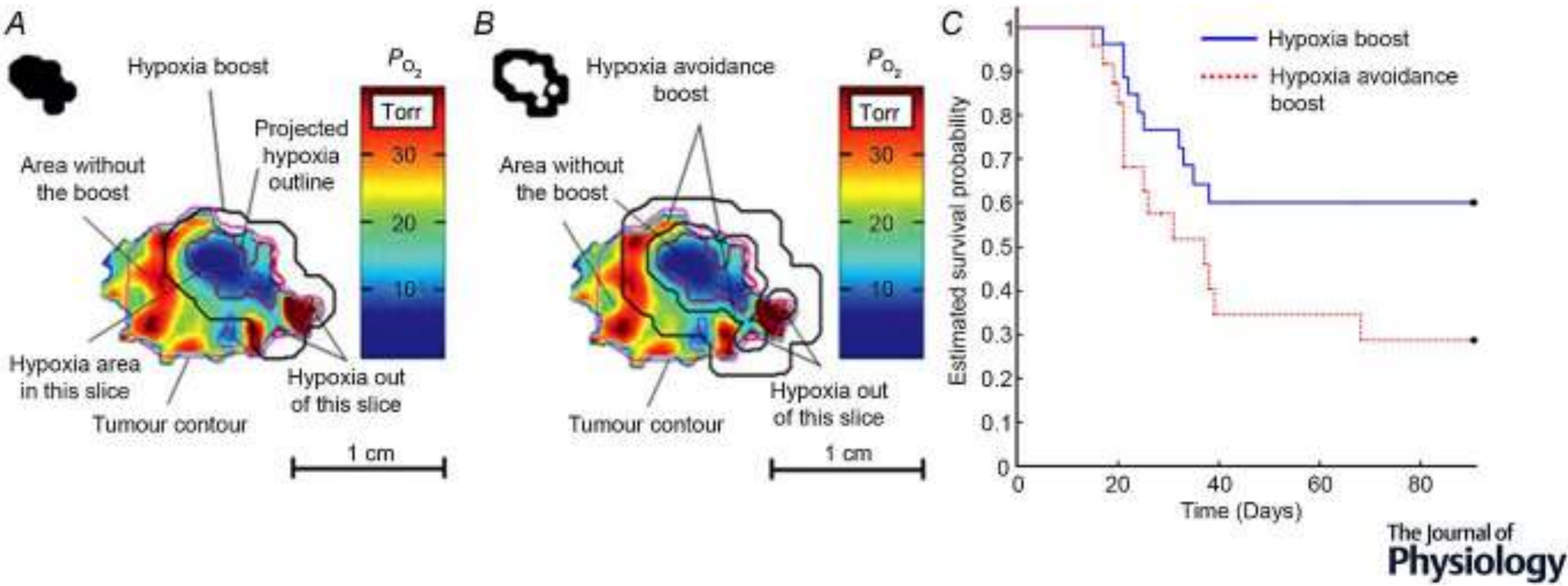
Quantitative PO_2 imaging with spin lattice relaxation EPR



The Journal of Physiology

I.Gertsenshteyn, M. Giurcanu,P. Vaupel and H.Halpern, J Physiol 599.6 (2021) pp 1759–1767

Biological validation of electron paramagnetic resonance (EPR) image oxygen thresholds in tissue

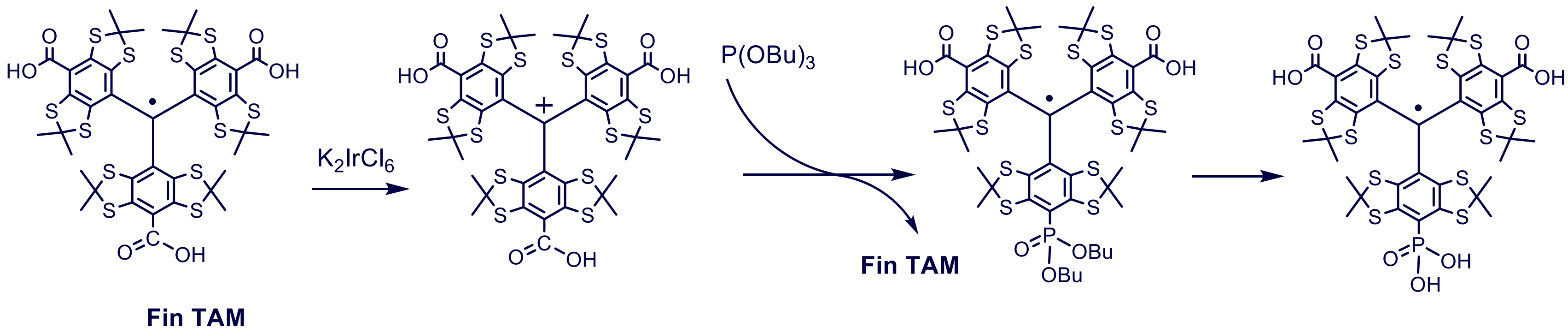


B. Epel et al. *Int J Radiation Oncol Biol Phys*, 103, 2019 pp. 977

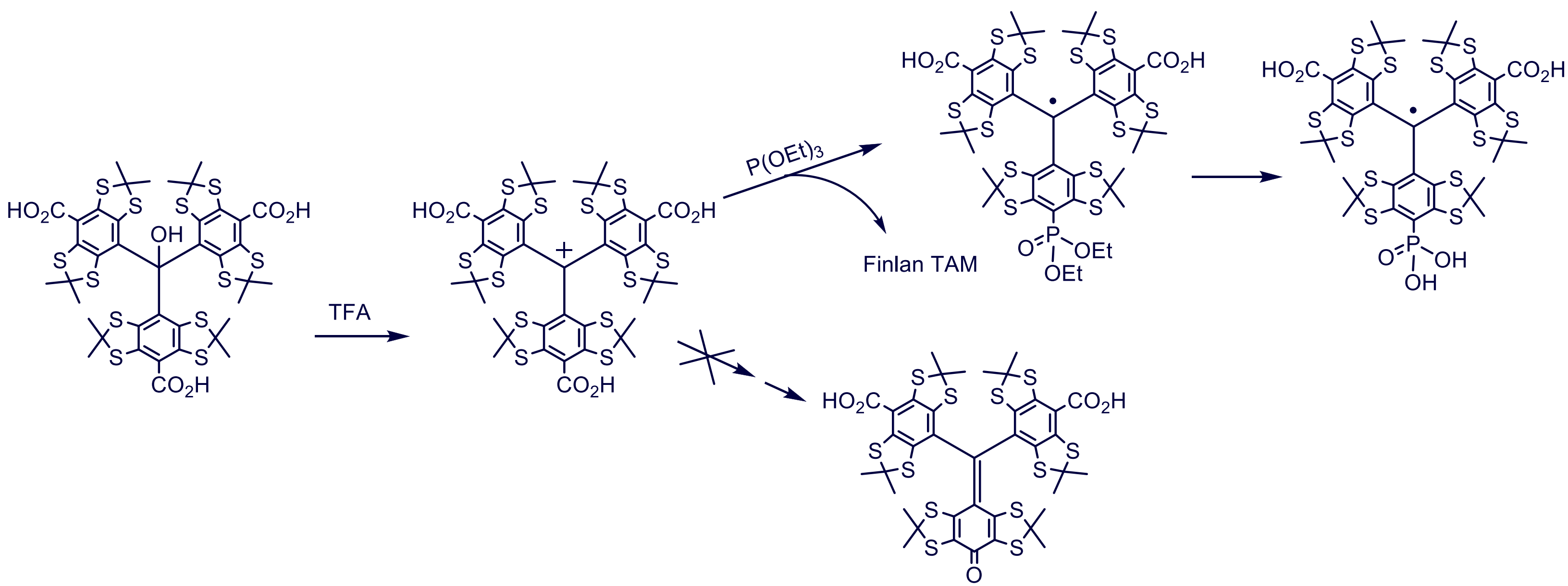
B. Epel, G. Redler, V. Tormyshev, H.J. Halpern *Advances in Experimental Medicine and Biology*, 876, 2016, pp 363

B. Epel, G. Redler, C. Pelizzari, V.M. Tormyshev, H.J. Halpern *Advances in Experimental Medicine and Biology*, 876, 2016, 185

Dual-function water-soluble phosphonate spin probes

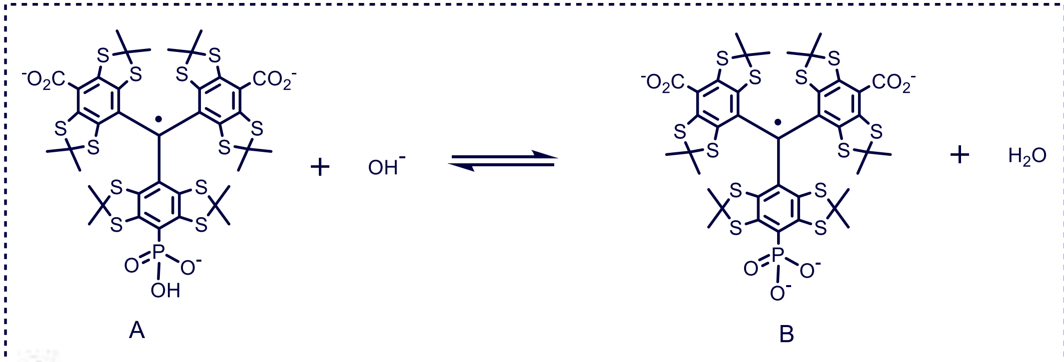


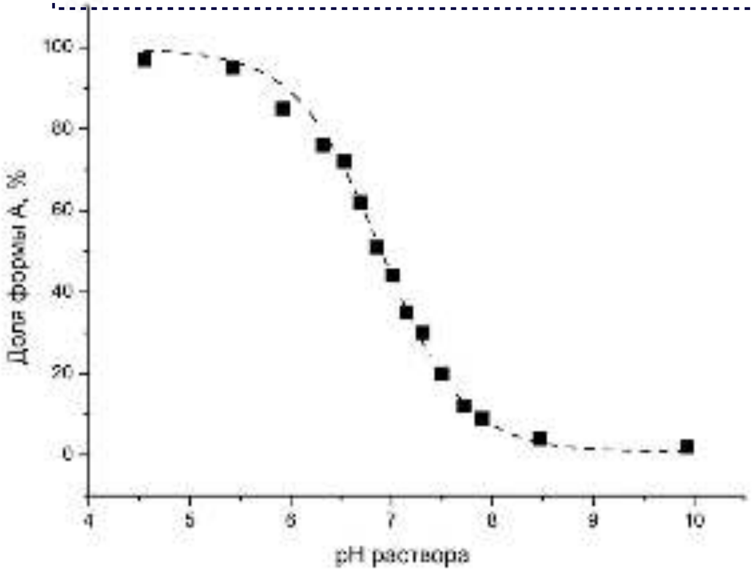
I. Dhimitruka, A.Bobko, T.Eubank, D.Komarov, V.Khramtsov, *J. Am. Chem.Soc.*, 2013, 135, 5904

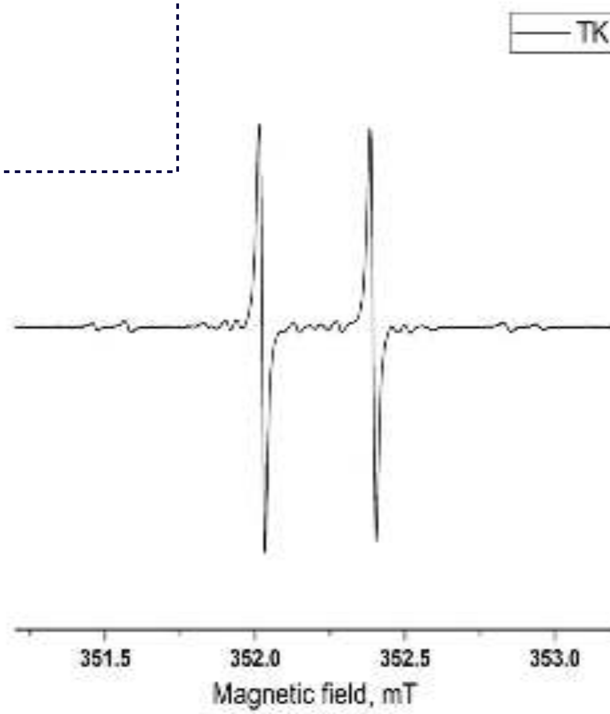


Д.В. Трухин, О.Ю. Рогожникова, Т.И. Троицкая, С.С. Овчеренко, Е.В. Амосов, В.М. Тормышев Журнал органической химии. 2020. Т. 56. № 11. С. 1693-1699

pH –sensitve water-soluble TAM-phosphonate



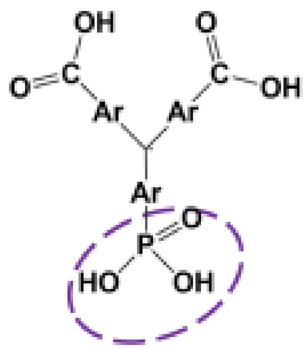




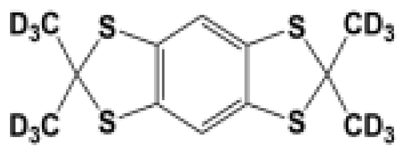
Зависимость доли кислотной формы А ТАМ **1** от pH среды (представлены квадратами). Пунктиром представлена кривая титрования, соответствующая значению pKa= 6.90 для монокислоты А

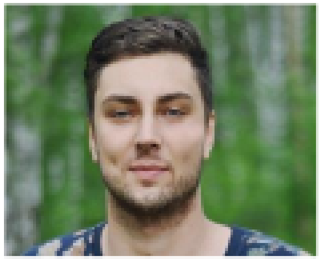
Multiparametric OMRI: spin probe concentration, pO_2 , pH and Pi imaging of mouse tumor

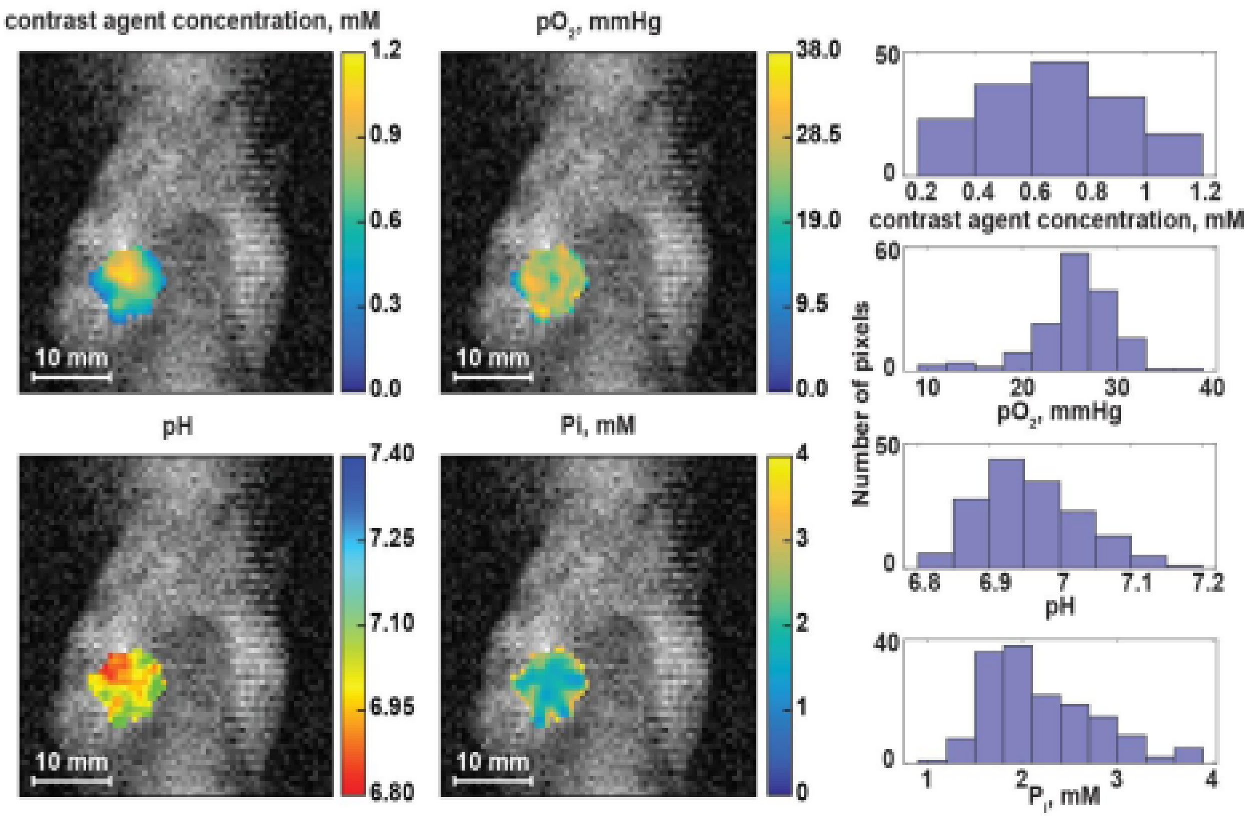
phosphonated trityl probe



pH and Pi sensitive center

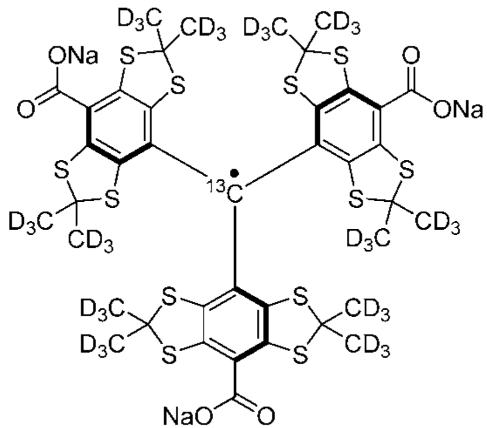
Ar = 

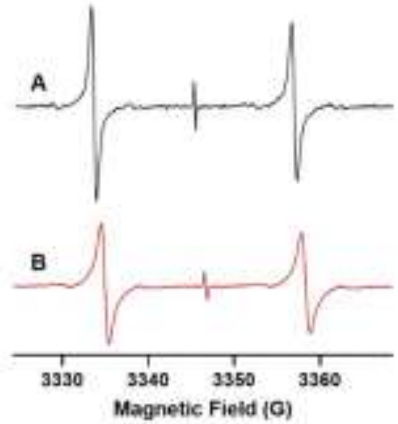


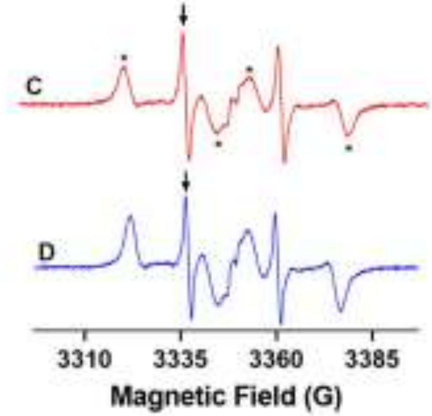


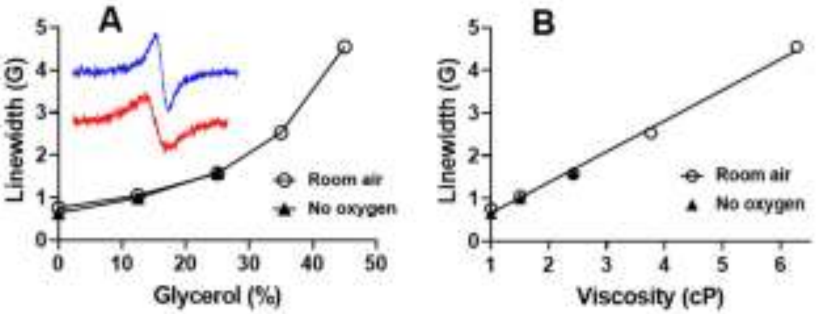
A.A. Gorodetskii, T.D. Eubank, B. Driesschaert, M. Poncelet, E. Ellis, V.V. Khramtsov, A.A. Bobko *Scientific Reports*, 2019, V. 9 (1), Art.number 12093

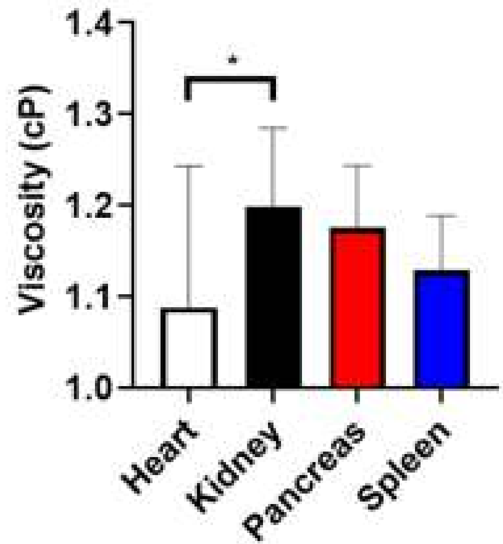
Biological Applications of Electron Paramagnetic Resonance Viscometry Using a ^{13}C-Labeled Trityl Spin Probe





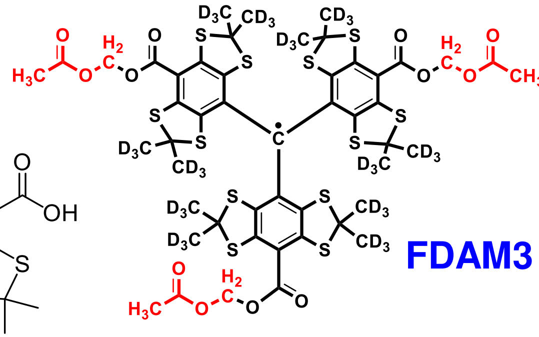




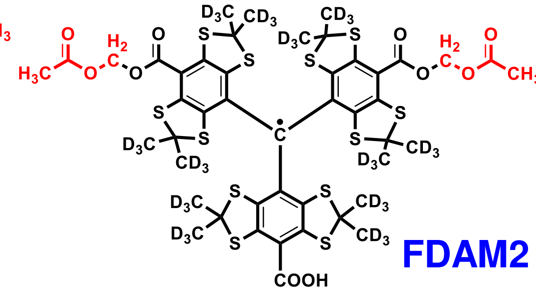


Poncelet, M.; Driesschaert, B. A Angew. Chem. Int. Ed. Engl. 2020, 59, 16451–16454.
M.Velayutham, M.Poncelet, T.D. Eubank, B.Driesschaert, Valery V. Khramtsov, Molecules 2021, 26, 2781

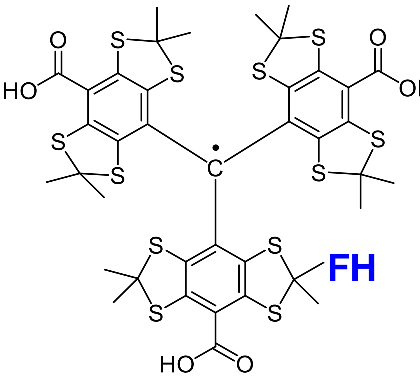
Variety of Trityls synthesized in NIOCH SB RAS



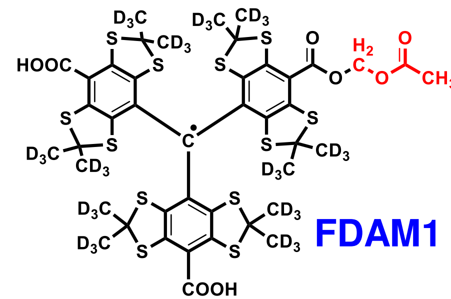
FDAM3



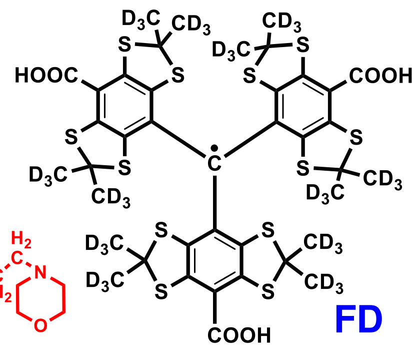
FDAM2



FH



FDAM1



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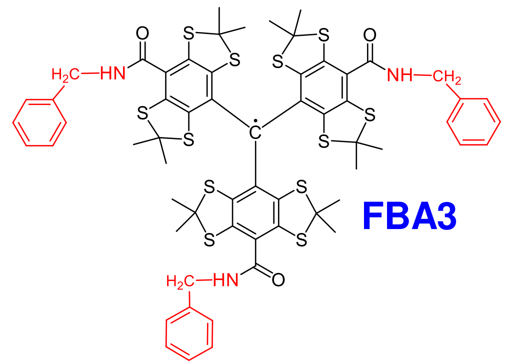
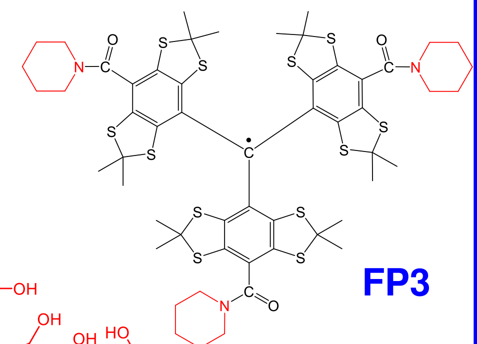
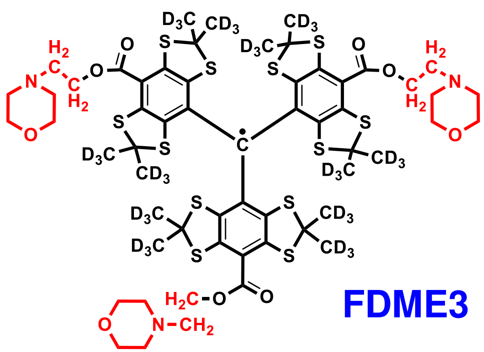


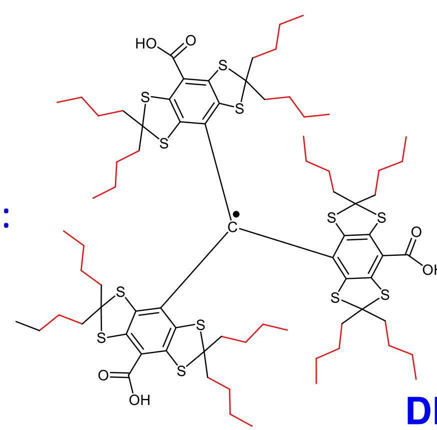
FBA3



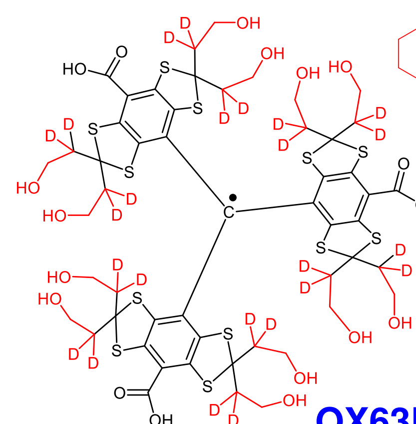
FP3



FDME3



DBT



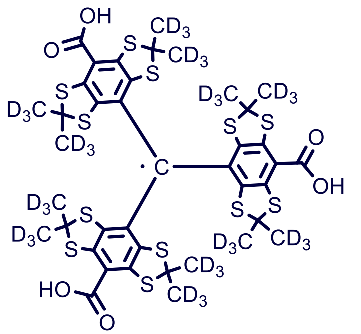
OX63D

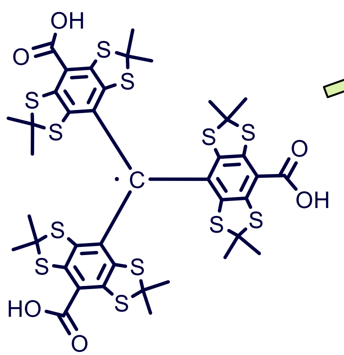
Phase memory time depending on:

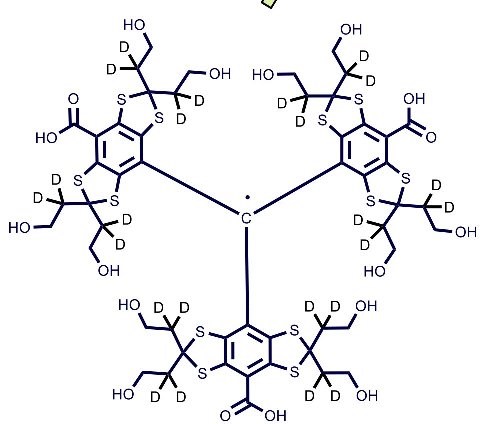
- solvent viscosity
- magnetic field
- deuteration of radical/solvent
- structure of radical

A.A. Kuzhelev, D.V. Trukhin, O.A. Krumkacheva, R.K. Strizhakov, O.Yu. Rogozhnikova, T.I. Troitskaya, M.V. Fedin, V.M. Tormyshev, E. G. Bagryanskaya *J. Phys. Chem. B,* 2015, 119 (43), pp 13630-13640

T_2 (μs) and T_1 (μs) in H_2O, D_2O and methanol at 300 K at X- and Q-bands







TAM	Solvent	X-band		Q-band	
		T_2	T_1	T_2	T_1
FTD_{36}	MeOH	9.2	15.6	3.8	15.6
	H_2O	10.8	17.1	4.5	16.2
	D_2O	13.6	17.7	4.5	17.0
FTH_{36}	MeOH	6.3	16.0	2.0	15.3
	H_2O	9.2	15.0	4.6	14.4
	D_2O	10.4	18.0	4.0	16.8
OX $063D_{24}$	MeOH	5.8	16.5	1.8	15.6
	H_2O	7.3	16.0	2.2	15.3
	D_2O	7.6	16.1	2.0	16.1

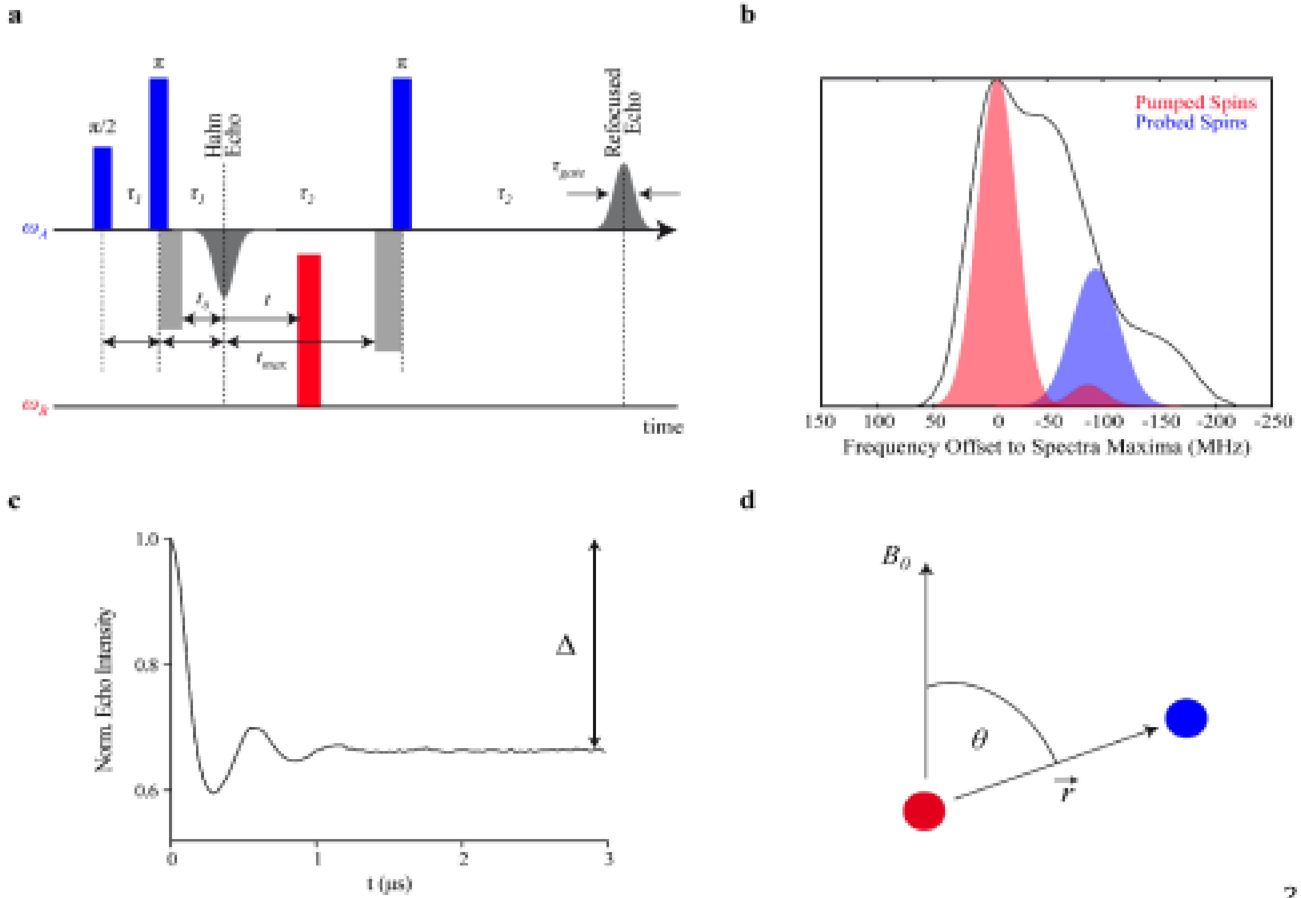
Phase memory time depending on:

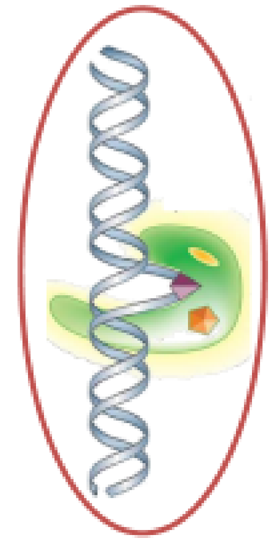
- solvent viscosity
- magnetic field
- deuteration of radical/solvent
- structure of radical

A.A. Kuzhelev, D.V. Trukhin, O.A. Krumkacheva, R.K. Strizhakov, O.Yu. Rogozhnikova, T.I. Troitskaya, M.V. Fedin, V.M. Tormyshev, E. G. Bagryanskaya *J. Phys. Chem. B,* 2015, 119 (43), pp 13630-13640

Trityls as spin labels for PELDOR/DEER

Pulse Dipole EPR Spectroscopy





$$\omega_{\mathrm{AB}}(\theta, r) = -\frac{\mu_0 g_{\mathrm{A}} g_B \mu_{\mathrm{B}}^2}{4\pi\hbar} \cdot \frac{1}{r_{\mathrm{AB}}^3}\left(3\cos^2\theta - 1\right)$$

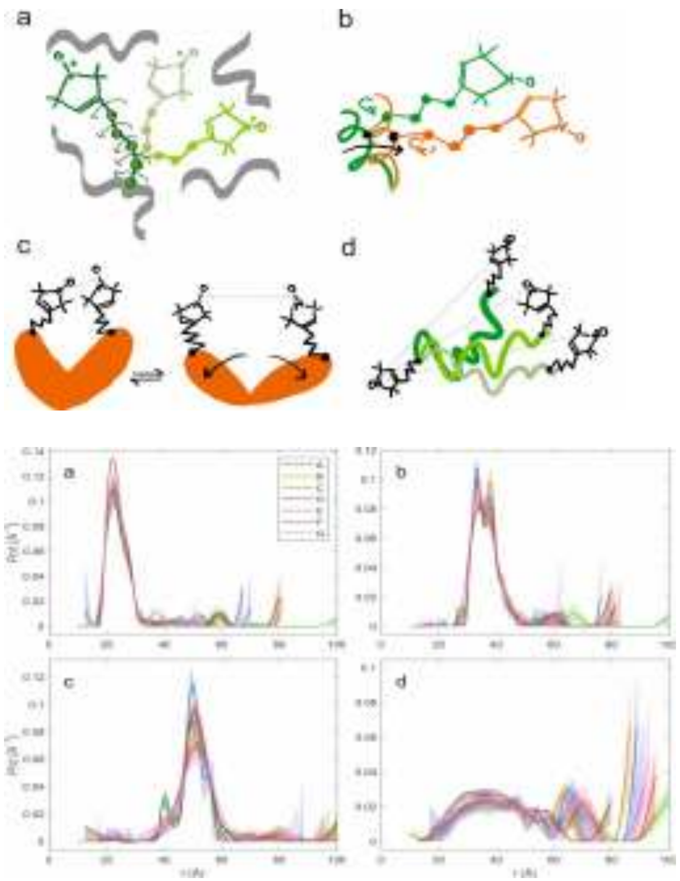
A.D.Milov , K.M.Salikhov, et.al Fiz. Tverd.Tela., 1981, 23,9751984,

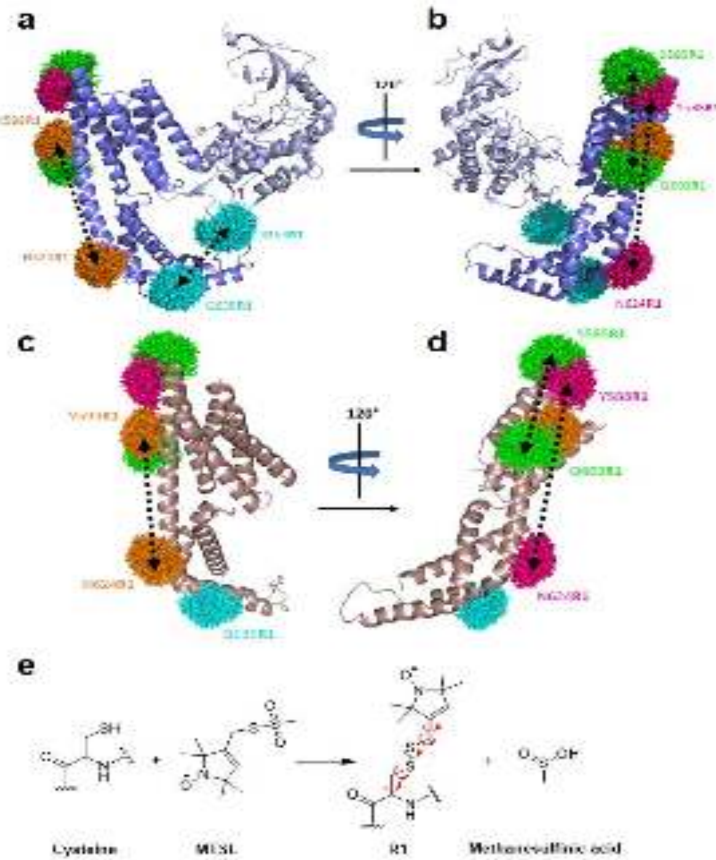
A.D.Milov, Y.D.Tsvetkov, Zhur.Struc.Khim., 1984, 25, 5.

Jeschke, Phys. Chem. Chem. Phys., 2007, 9, 1895–1910

Pannier *et.al*, J. Magn. Res., (2000) 142: p. 331-40

Benchmark test and guidelines for DEER/PELDOR experiments on nitroxide-labeled biomolecules

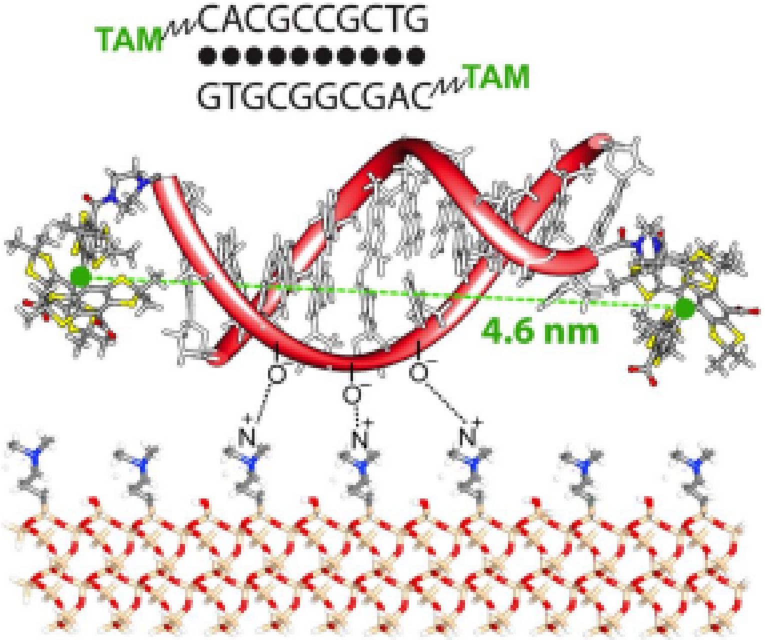


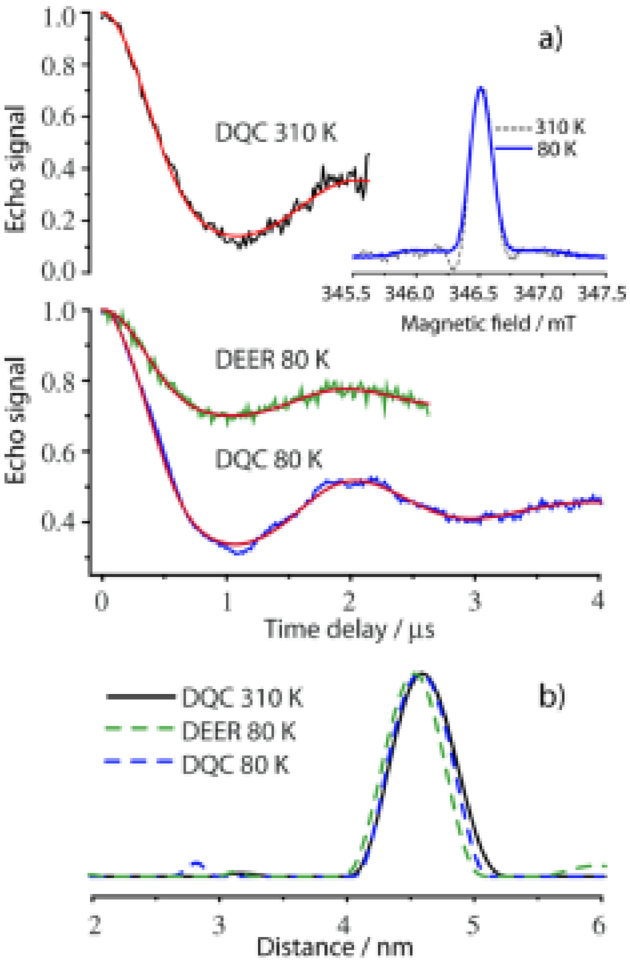


O. Schieman, C.A. Heubach, D. Abdullin, K. Ackermann, M. Azarkh, E.G. Bagryanskaya, Malte Drescher, Burkhard Endeward, Jack H. Freed, Laura Galazzo, Daniella Goldfarb, Tobias Hett, Laura Esteban Hofer, Luis Fábregas Ibáñez, Eric J. Hustedt, Svetlana Kucher, Ilya Kuprov, Janet Eleanor Lovett, Andreas Meyer, Sharon Ruthstein, Sunil Saxena, Stefan Stoll, Christiane R. Timmel, Marilena Di Valentin, Hassane S. Mchaourab, Thomas F. Prisner, Bela Ernest Bode, Enrica Bordignon, Marina Bennati, and Gunnar Jeschke.

JACS, 2021, 143, 43, 17875-17890

Physiological-Temperature Distance Measurement in Nucleic Acid using Triarylmethyl-Based Spin Labels and Pulsed Dipolar EPR Spectroscopy





Shevelev G.Y., Krumkacheva O.A., Lomzov A.A., Kuzhelev A.A., Bagryanskaya E.G. // J. Am. Chem. Soc. – **2014**.– V. 136.– № 28.– P. 9874–9877.

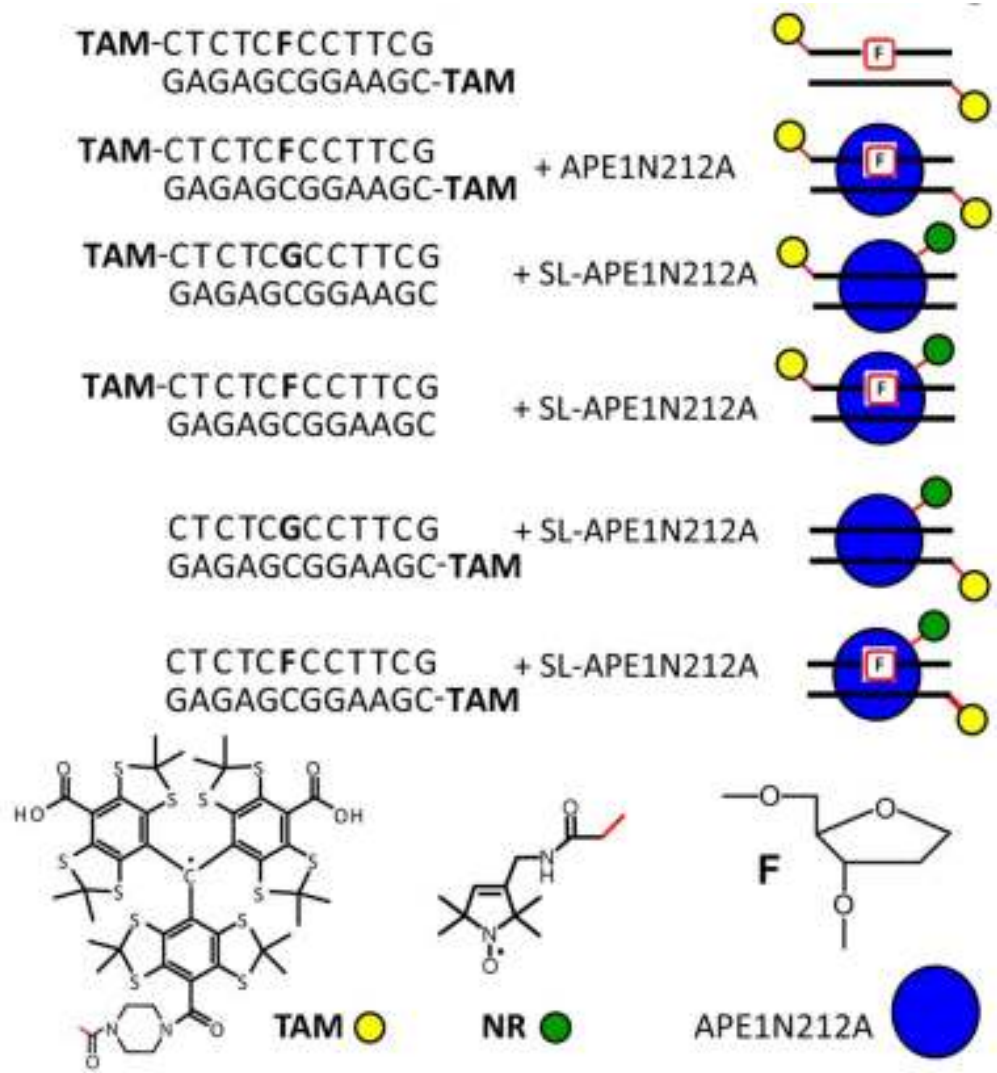
DNA complexes with human apurinic/*apyrimidinic* endonuclease 1: structural insights revealed by pulsed dipolar EPR with orthogonal spin labeling

A number of endogenous and exogenous damaging factors continuously affect DNA, leading to a variety of DNA lesions, the most common of which are apurinic/apyrimidinic sites (abasic or AP sites).

In vivo, AP sites are thought to be repaired via the DNA base excision repair pathway (BER). In humans, BER initially involves apurinic/apyrimidinic endonuclease 1 (APE1).

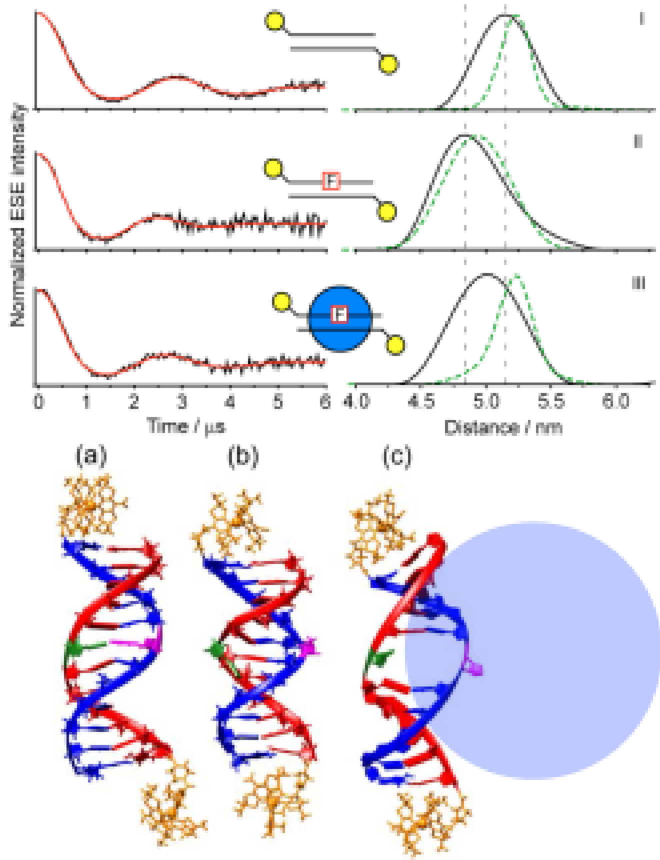
We applied PD ESR spectroscopy in combination with molecular dynamics simulations to investigate in-depth conformational changes in DNA containing an AP site and in a complex of this DNA with AP endonuclease 1 (APE1).

For this purpose TAM-based spin labels were attached to the 5'-ends of an oligonucleotide duplex, and nitroxide spin labels were introduced into APE1.



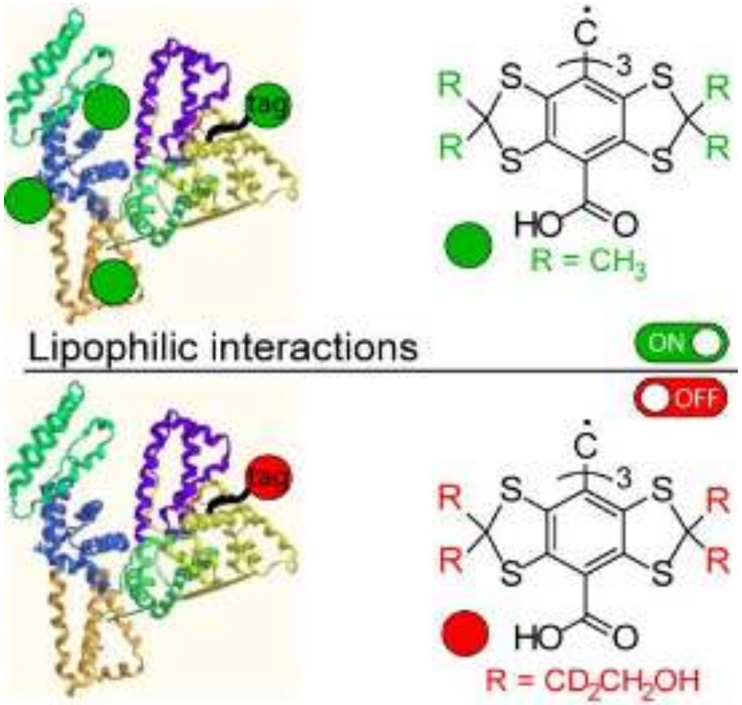
O.A. Krumkacheva, G.Yu. Shevelev, A.A. Lomzov, N.S. Dyrkheeva, A.A. Kuzhelev, V.V. Koval, V.M. Tormyshev, Yu.F. Polienko, M.V. Fedin, D.V. Pyshnyi, O.I. Lavrik, E.G. Bagryanskaya, Nucleic Acids Research, 2019, 47(15)7767-7780

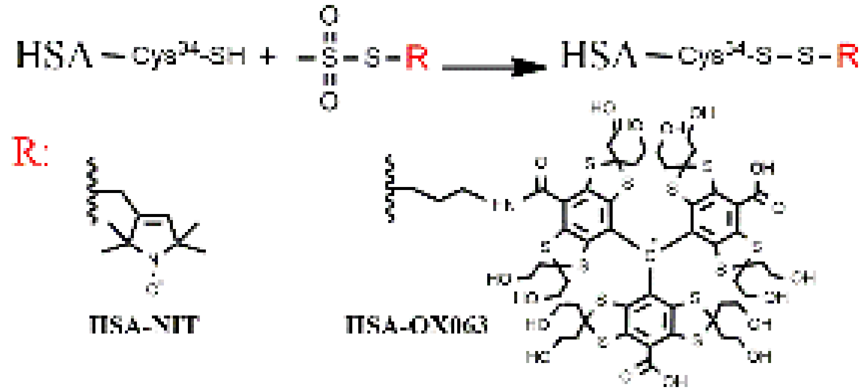
DNA complexes with human apurinic/*apyrimidinic* endonuclease 1: structural insights revealed by pulsed dipolar EPR with orthogonal spin labeling

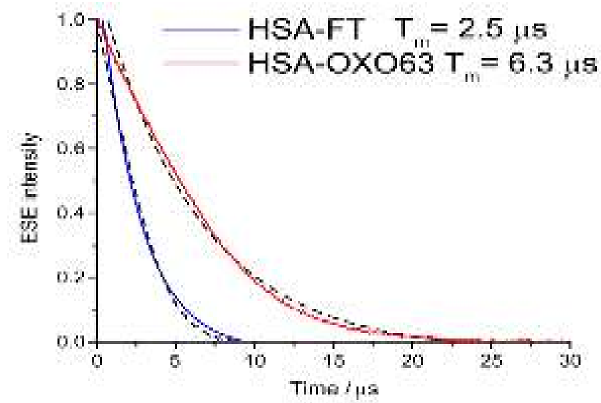


O.A. Krumkacheva, G.Yu. Shevelev, A.A. Lomzov, N.S. Dyrkheeva, A.A. Kuzhelev, V.V. Koval, V.M. Tormyshev, Yu.F. Polienko, M.V. Fedin, D.V. Pyshnyi, O.I. Lavrik, E.G. Bagryanskaya, Nucleic Acids Research, 2019, 47(15)7767-7780

Methanethiosulfonate Derivative of OX063 Trityl: A Promising and Efficient Reagent for Side-Directed Spin Labeling of Proteins



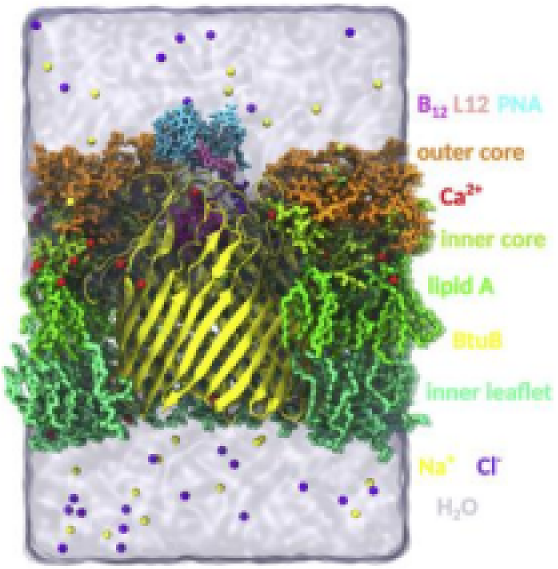


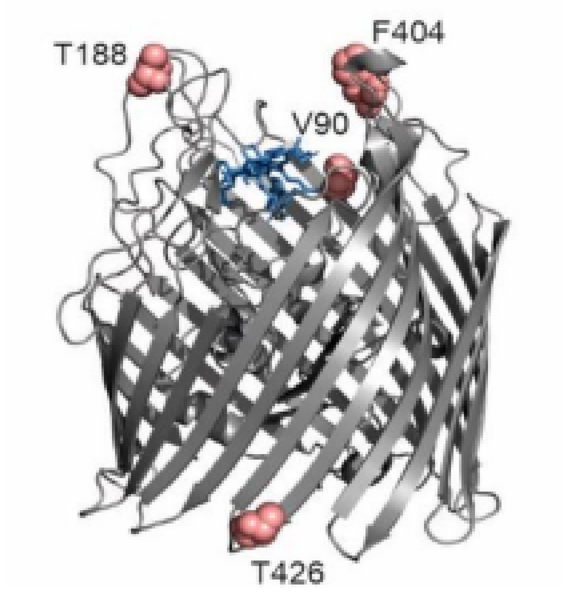


The very hydrophilic spin labels based on OX063 with very-low toxicity and little tendency for aggregation was developed.
New spin labels have the longest electron spin relaxation time among any TAM-based spin labels.

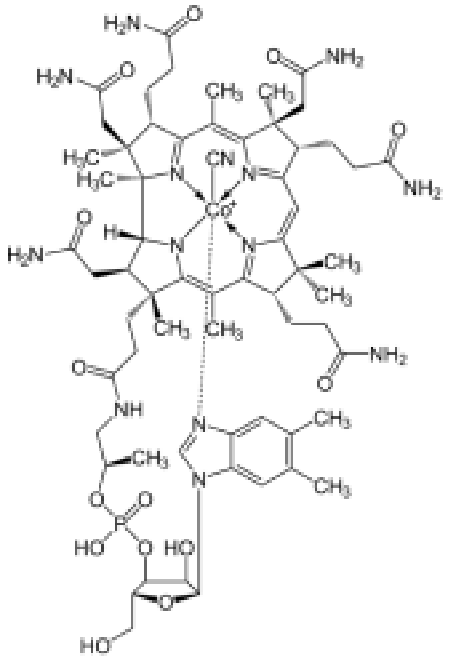
Tormyshev, V.M.; Chubarov, A.S.; Krumkacheva, O.A.; Trukhin, D. V; Rogozhnikova, O.Y.; Spitsyna, A.S.; Kuzhelev, A.A.; Koval, V. V; Fedin, M. V; Godovikova, T.S.; M. Bowman, Bagryanskaya E. G. *Chem. – A Eur. J.* **2020**, *26*, 2705–2712.

Distance measurements on outer membrane proteins (OMPs) of Gram-negative bacteria in isolated outer membranes and intact cells





Cobalamin transporter BtuB

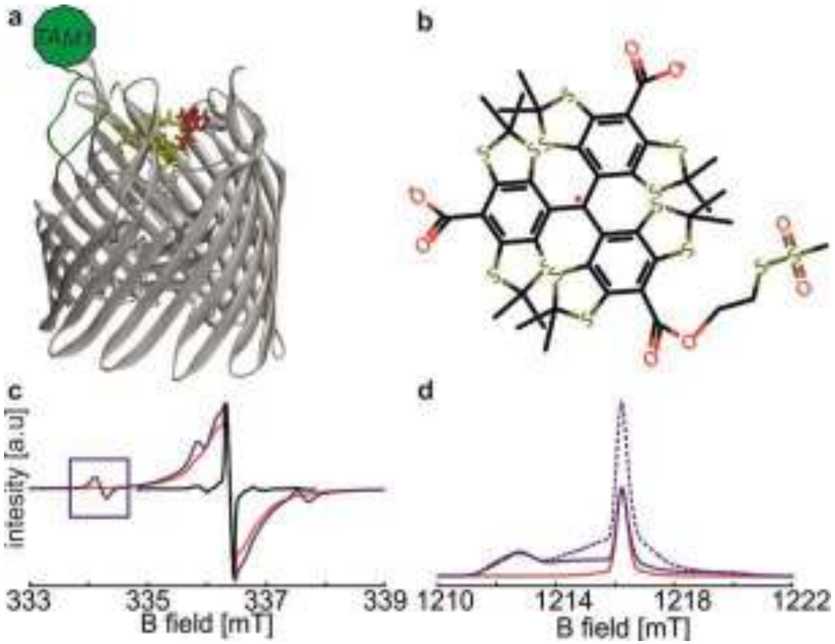


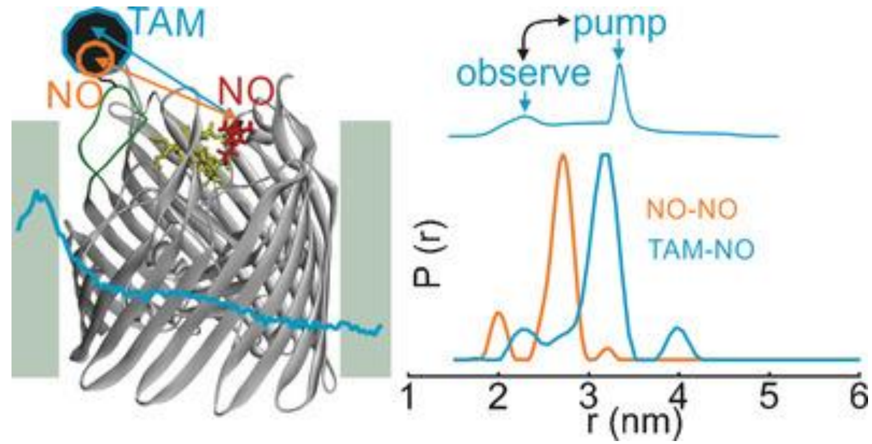
Colabamin B12

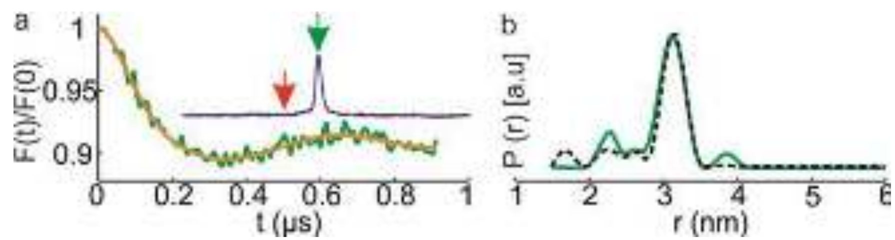
A.Kugele, S. Ketter, B. Silkenath, V. Wittmann, B. Joseph, M. Drescher, *Chem Commun (Camb)* **2021**, *57*, 12980.

B. Joseph, A. Sikora, E. Bordignon, G. Jeschke, D. S. Cafiso, T. F. Prisner, *Angew Chem Int Ed Engl* **2015**, *54*, 6196.

Selective Detection of Protein-Ligand Interaction in Native Membranes Using Trityl - Nitroxide Distance Measurement







T=150 K NO(pump)-TAM1 (observer)

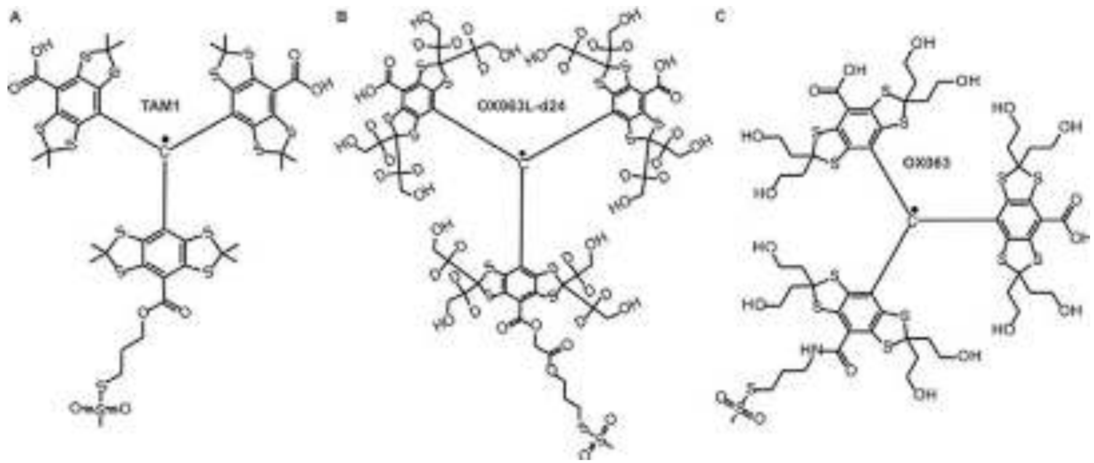
Aggregation of TAM in the membrane environment.

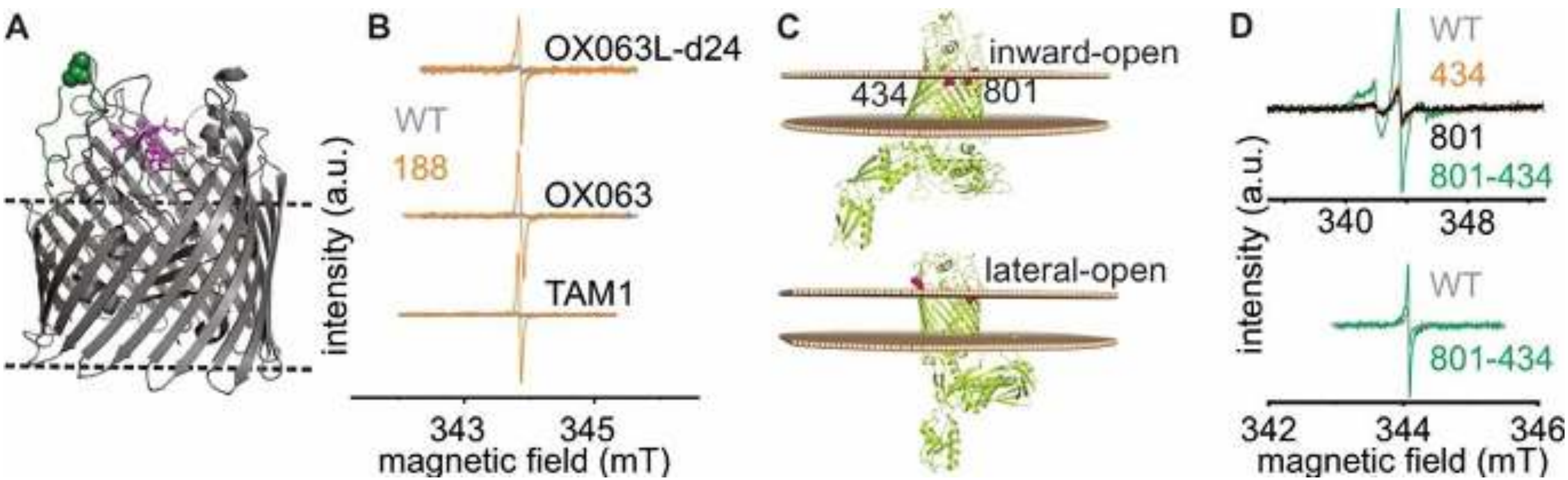
The selective and sensitive detection of protein-protein or protein-ligand interactions in the complex native membranes.

Temperature measurements - 150 K

B. Joseph, V. M. Tormyshev, O. Y. Rogozhnikova, D. Akhmetzyanov, E. G. Bagryanskaya, T. F. Prisner, *Angewandte Chemie International Edition* **2016**, *55*, 11538.

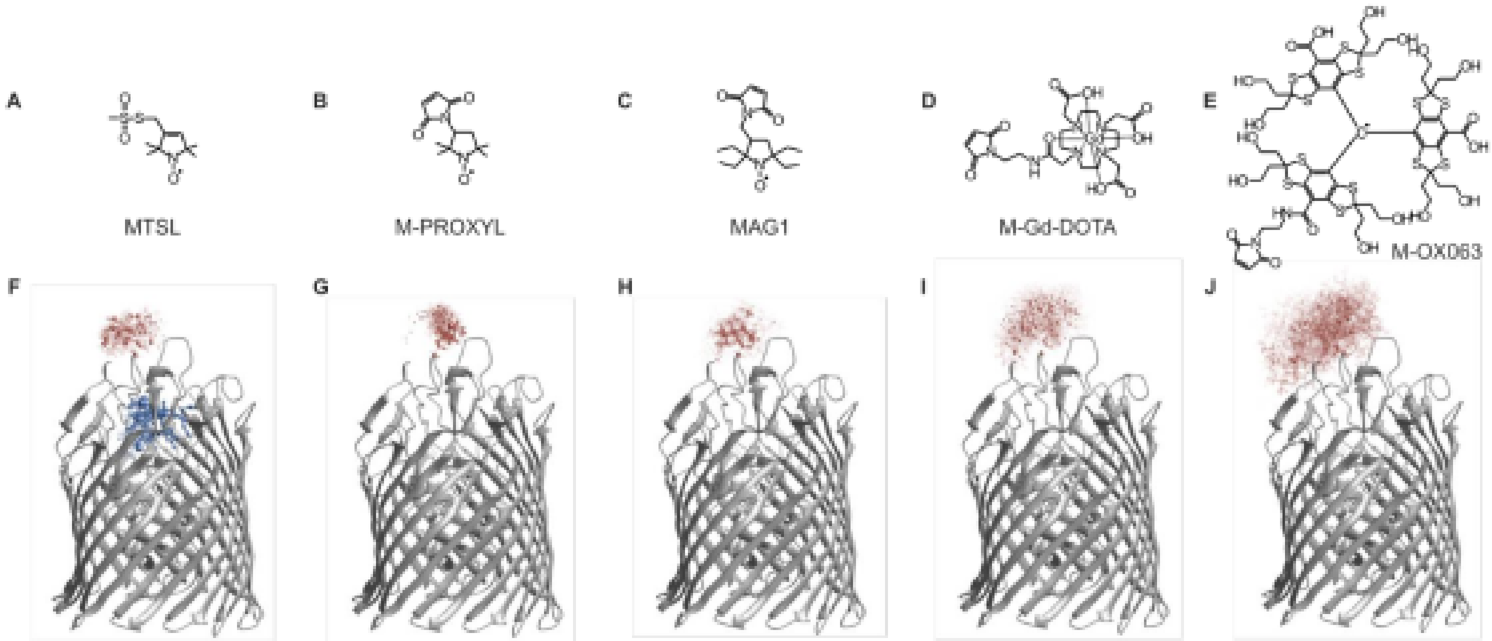
In Situ Labeling and Distance Measurements of Membrane Proteins in E.coli Using Finland and OX063 Trityl Labels



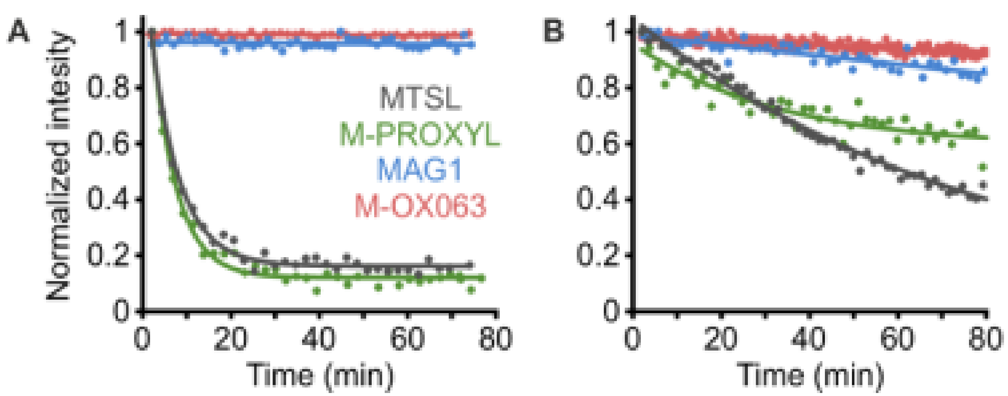


S.Ketter, A.Gopinath, O.Rogozhnikova, D.Trukhin, V. Tormyshev, E.Bagryanskaya, B.Joseph, Chem.Eur.J. 2021,27,2299 –2304

Comparison of different spin labels stability



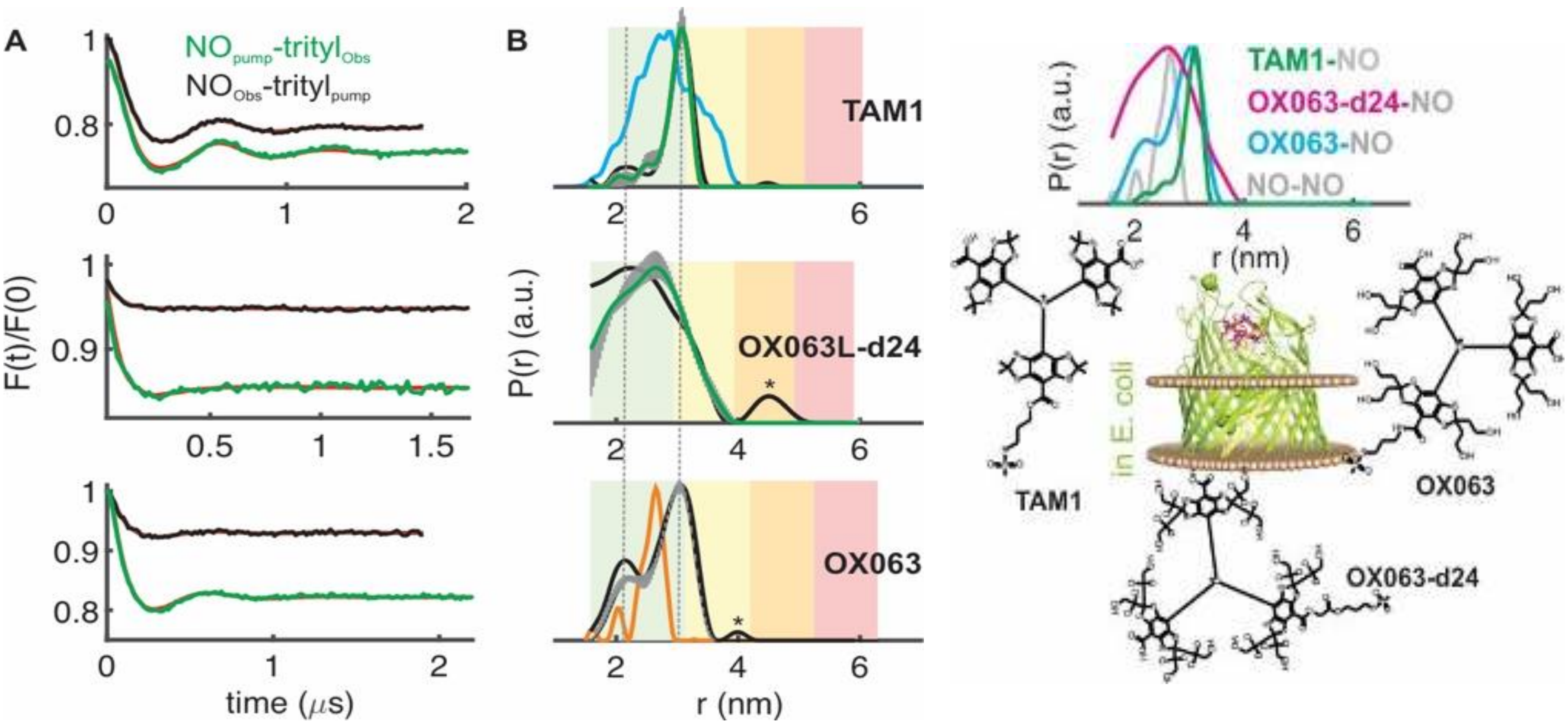
Reduction of 200 µM spin labels with 5 mM ascorbic acid pH 7.5 buffer



Stability of 200 µM spin label in *E. coli* cell suspension in the same buffer.

The MAG1 and M-OX063 labels exhibited superior stability in both *E. coli* suspension and 5 mM ascorbate solution

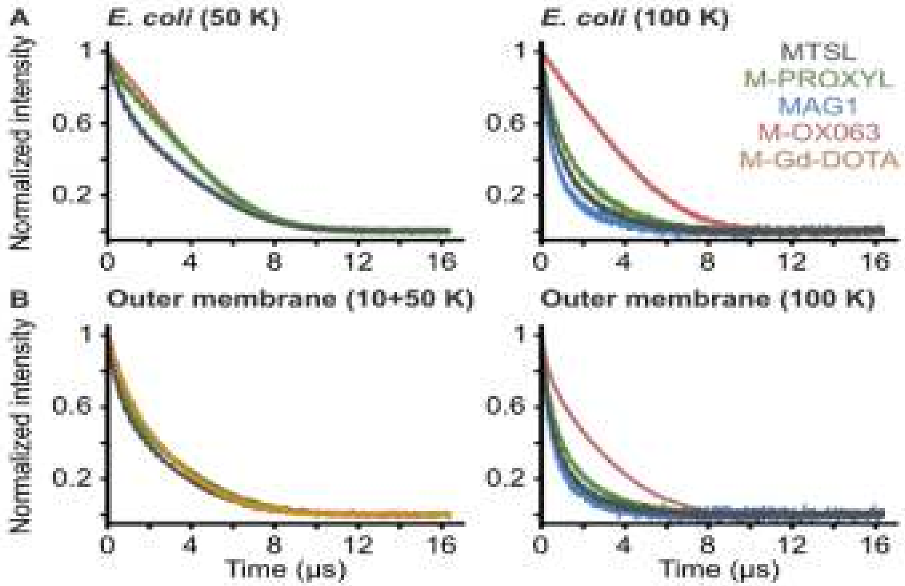
In Situ Labeling and Distance Measurements of Membrane Proteins in *E. coli* Using Finland and OX063 Trityl Labels



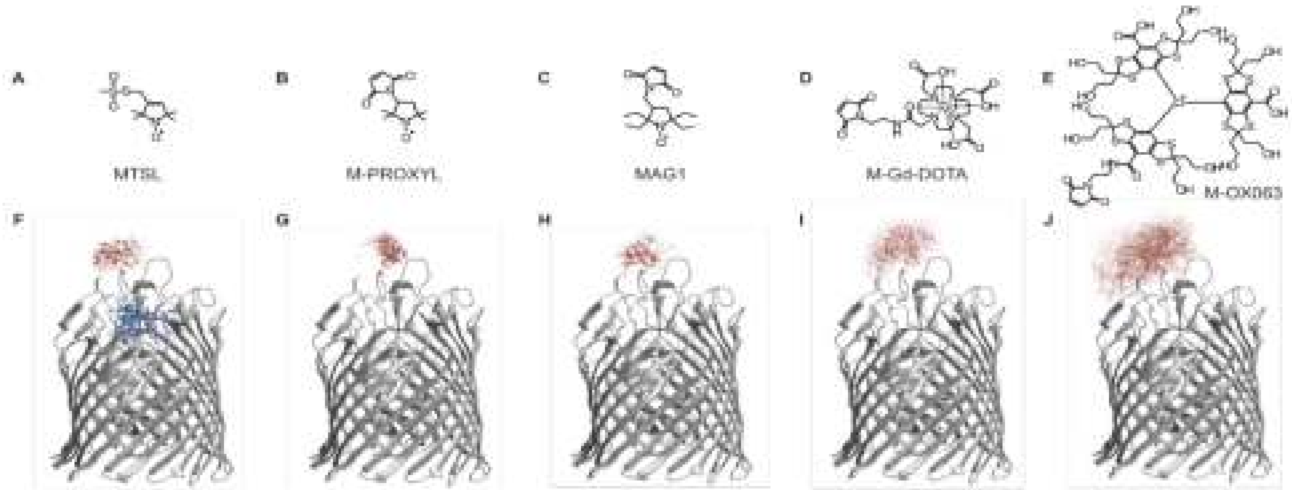
Benesh Joseph, Sophie Ketter, Aathira Gopinath, Olga Rogozhnikova, Dmitrii Trukhin*, Victor M. Tormyshev, Elena Bagryanskaya,* Chemistry - A European Journal, 2021, 20 N7 2299-2304

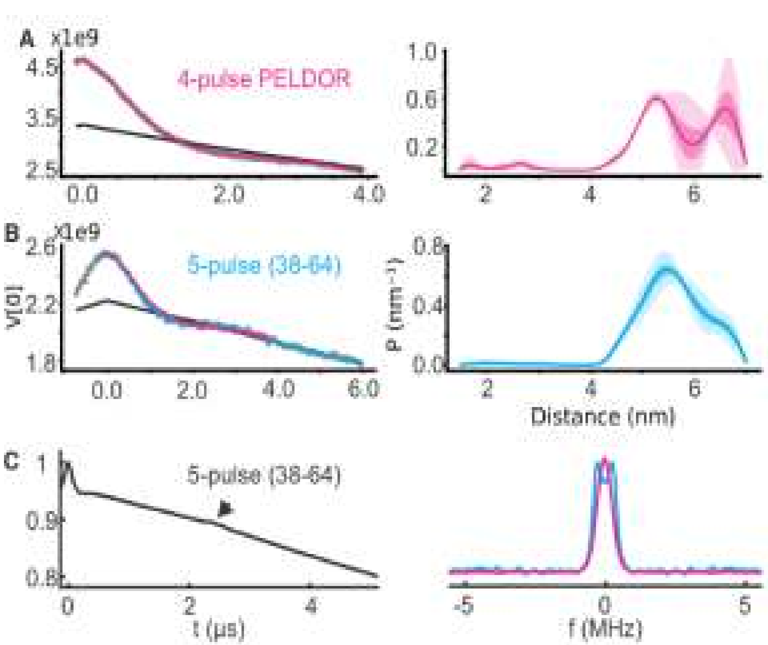
Phase memory time (T_M) and stretch factor κ determined from fitting the 2-pulse decay curves in *E. coli* cells and native outer membranes

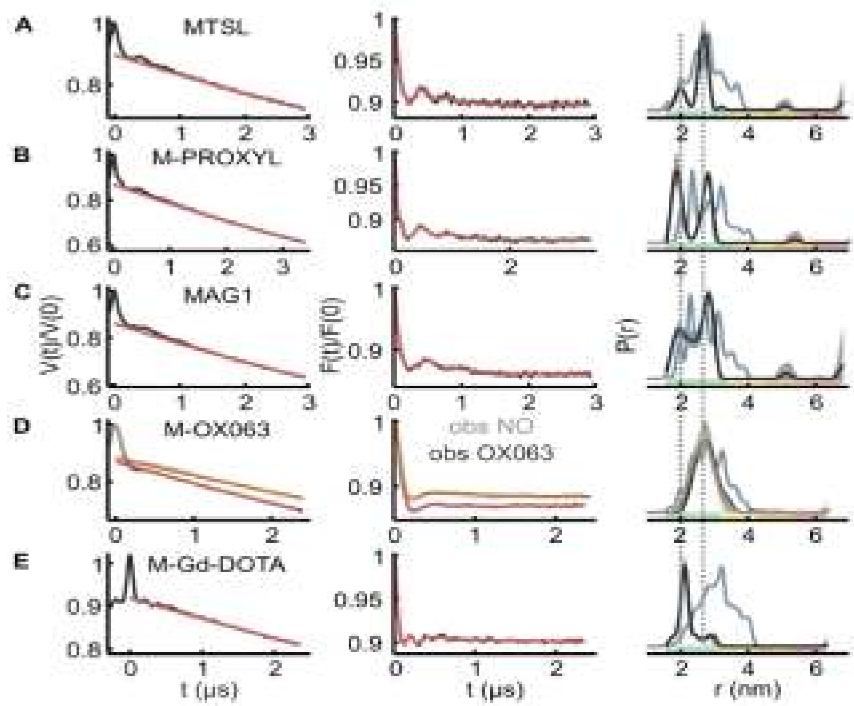
Spin label	T_M Outer membrane (µs)			κ Outer membrane			T_M E. coli (µs)		κ E. coli	
	10 K	50 K	100 K	10 K	50 K	100 K	50 K	100 K	50 K	100 K
MTSL	-	2.0	0.8	-	0.8	0.7	3.0	1.2	1.0	0.8
M-PROXYL	-	2.4	1.1	-	0.9	0.8	4.1	1.6	1.3	0.8
MAG1	-	2.3	0.7	-	0.8	0.7	3.0	0.8	1.0	0.7
M-OX063	-	2.5	2.5	-	1.0	1.0	4.2	4.1	1.5	1.4
M-Gd-DOTA	2.6	-	-	1.0	-	-	-	-	-	-



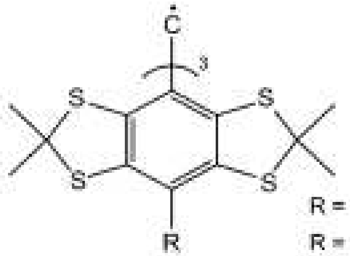
Comparison of PELDOR data for nitroxides and M-OX063 labels attached to BtuB T188C in whole *E. coli* cells.



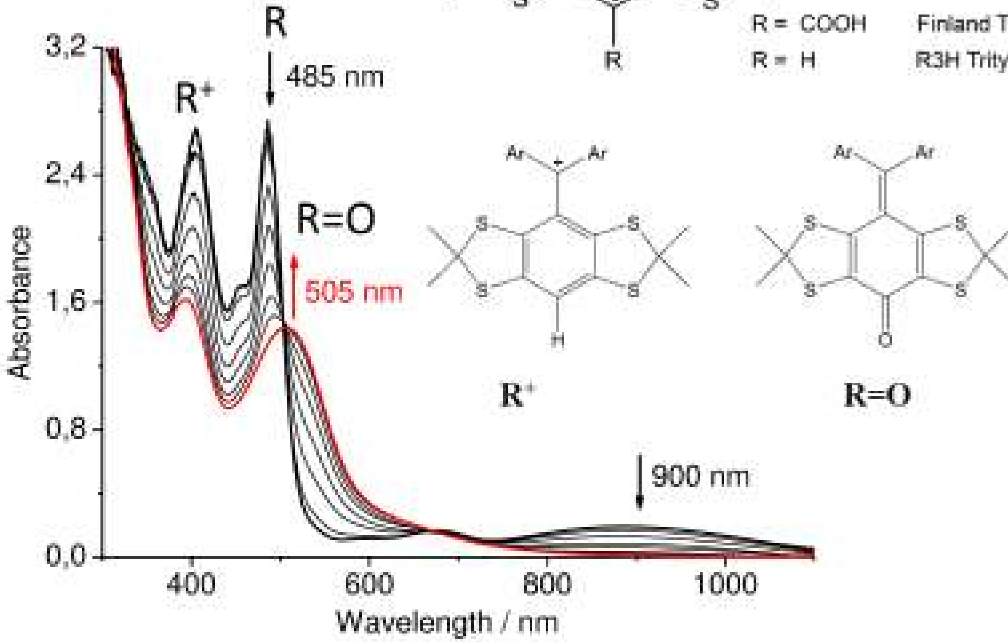




TAM stability against photoirradiation (308 nm)

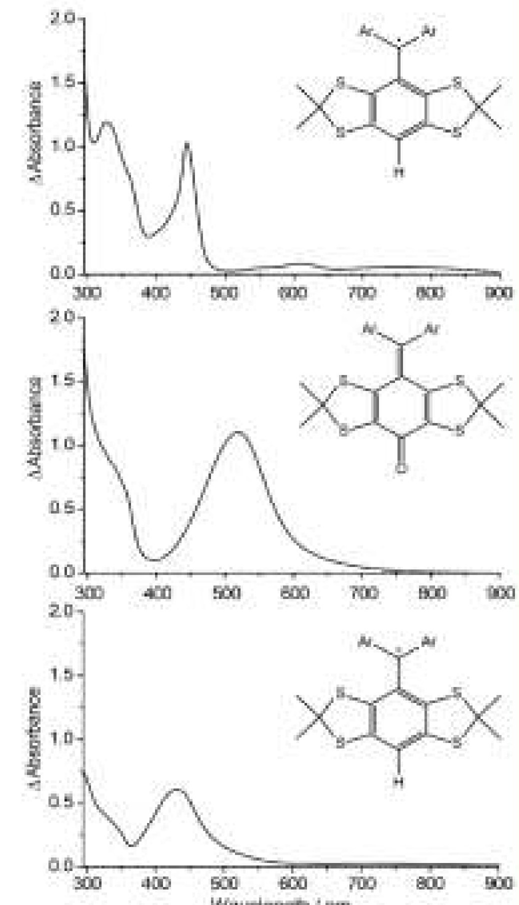


R = COOH Finland Trityl
R = H R3H Trityl



R^+ R=O

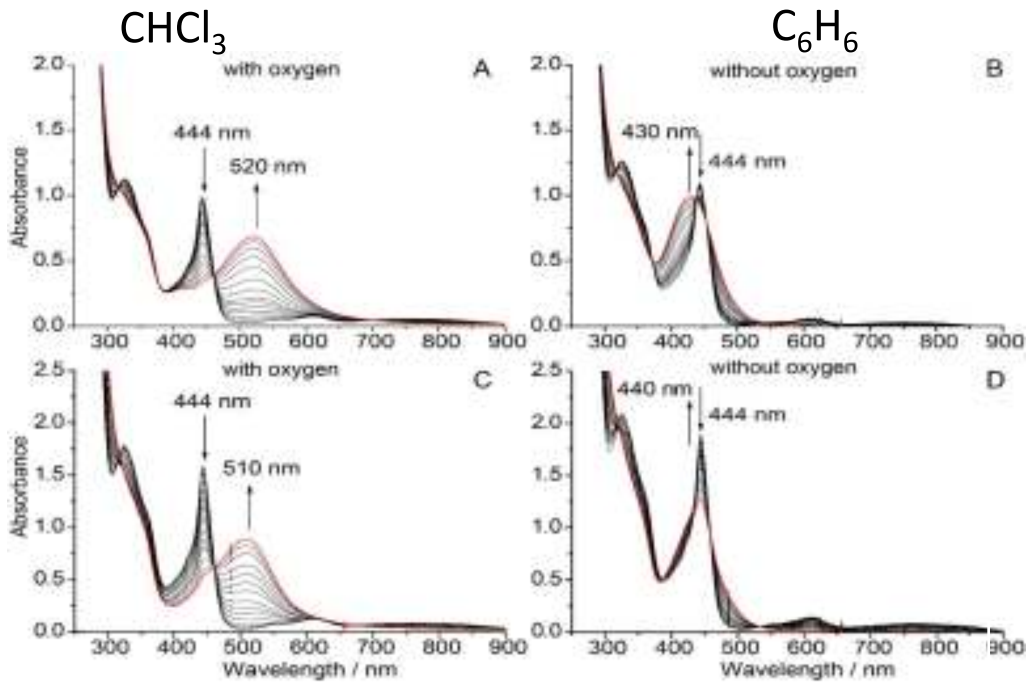
Andrey Kuzhelev



$$R \xrightarrow{h\nu} R^+ + e^- \longrightarrow R{=}O$$

Can we use TAM for as spin label in pair with photoinduced triplet spin label?

TAM stability against photoirradiation (308 nm)



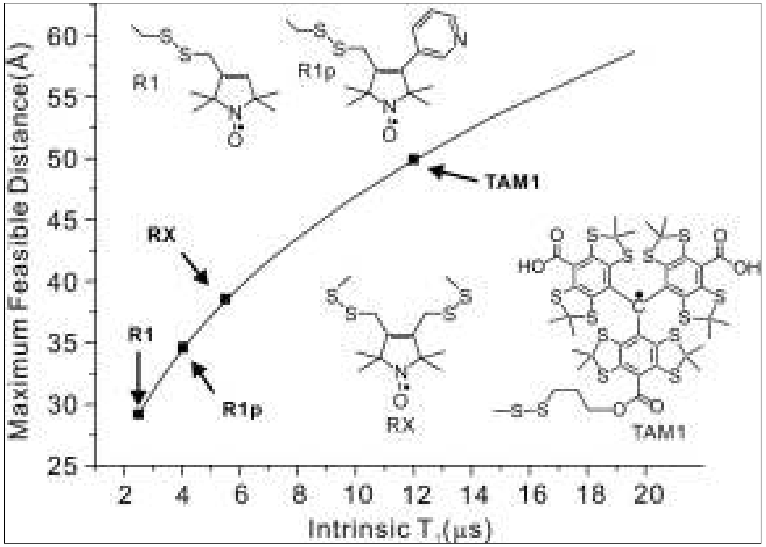
Main product of photolysis – quinone

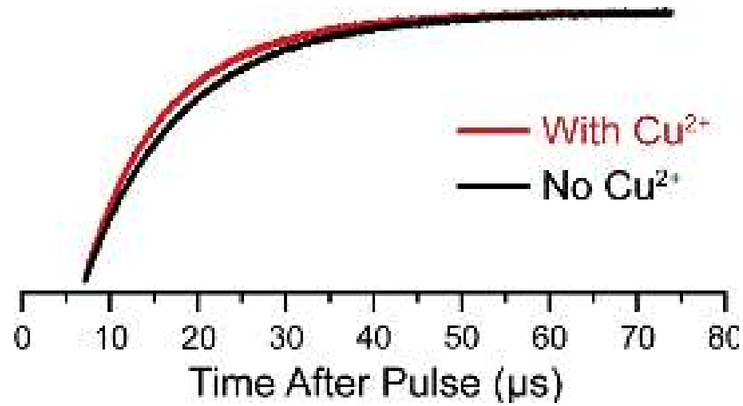
Quantum yield for Finland TAM <<0.1

Solvent	Concentration / mM	Quantum yield / %
Ethanol	0.1	2.2
Acetonitrile	0.1	3.1
Benzene	0.1	4.4
Chloroform	0.1	7.9
Chloroform	(0.01	7.3
Chloroform	(0.0013	6.8

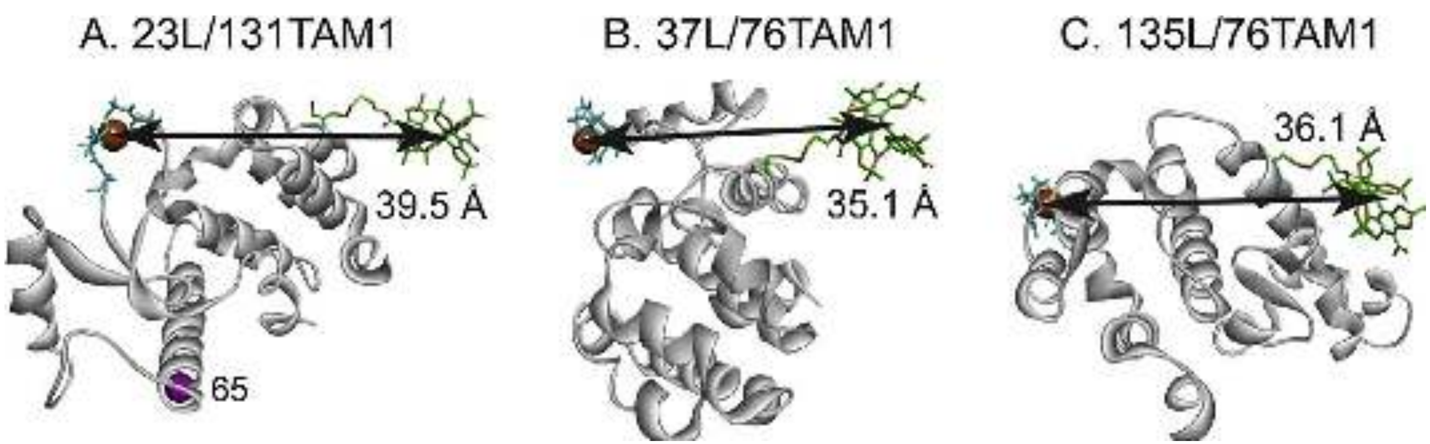
A.A. Kuzhelev, V.M. Tormyshev, V.F. Plyusnin, O.Yu. Rogozhnikova, M.V. Edeleva, S.L. Veber, E.G. Bagryanskaya
Phys. Chem. Chem. Phys., 2020, V. 22, N 3, Pp 1019-1026

A Triarylmethyl Spin Label for Long-Range Distance Measurement at Physiological Temperatures Using T_1 Relaxation Enhancement



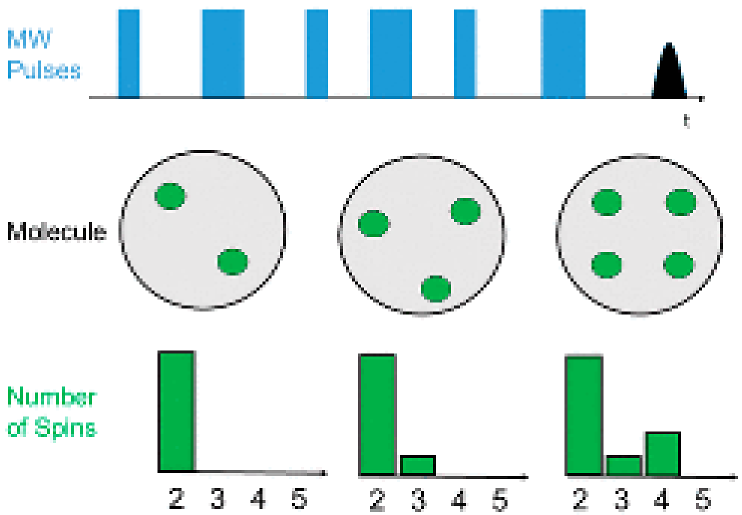


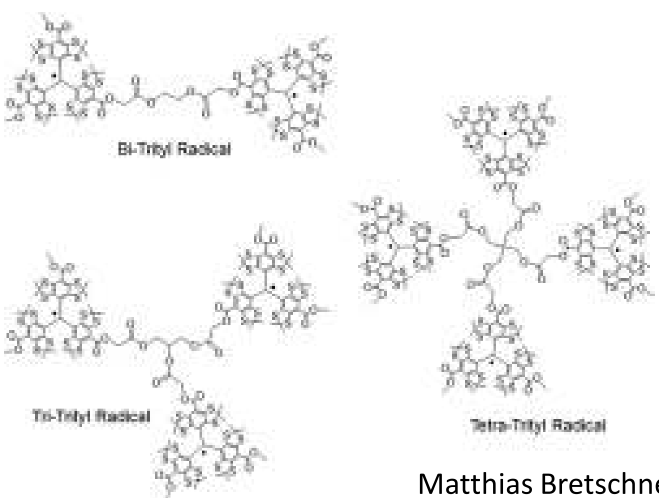
Representative relaxation enhancement data. The saturation recovery traces obtained for the 23L/131TAM1 sample in the absence (black) and presence (red) of Cu^{2+} at a microwave observe power of 50 µW

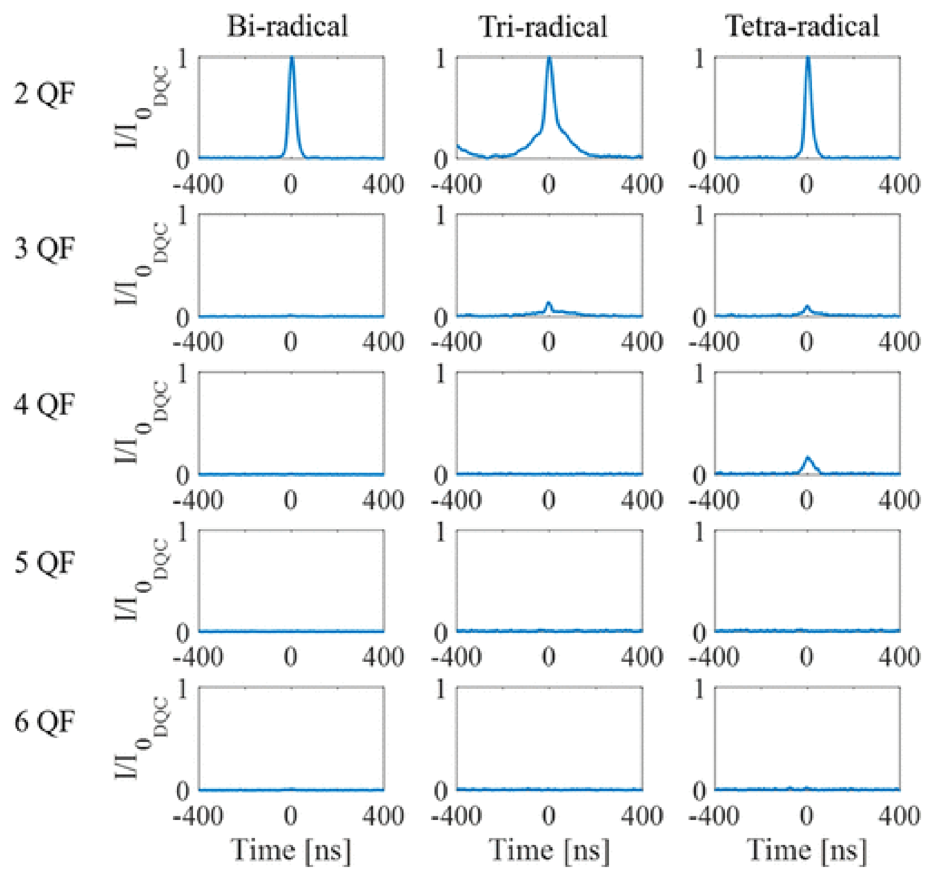


Z. Yang, M.D. Bridges, C.J. Lopez, O.Yu. Rogozhnikova, D.V. Trukhin, E.K. Brooks, V. Tormyshev, H.J. Halpern, W.L. Hubbell, Journal of Magnetic Resonance, **269**, 2016, 50–54

Multi-quantum counting/filtering of spins



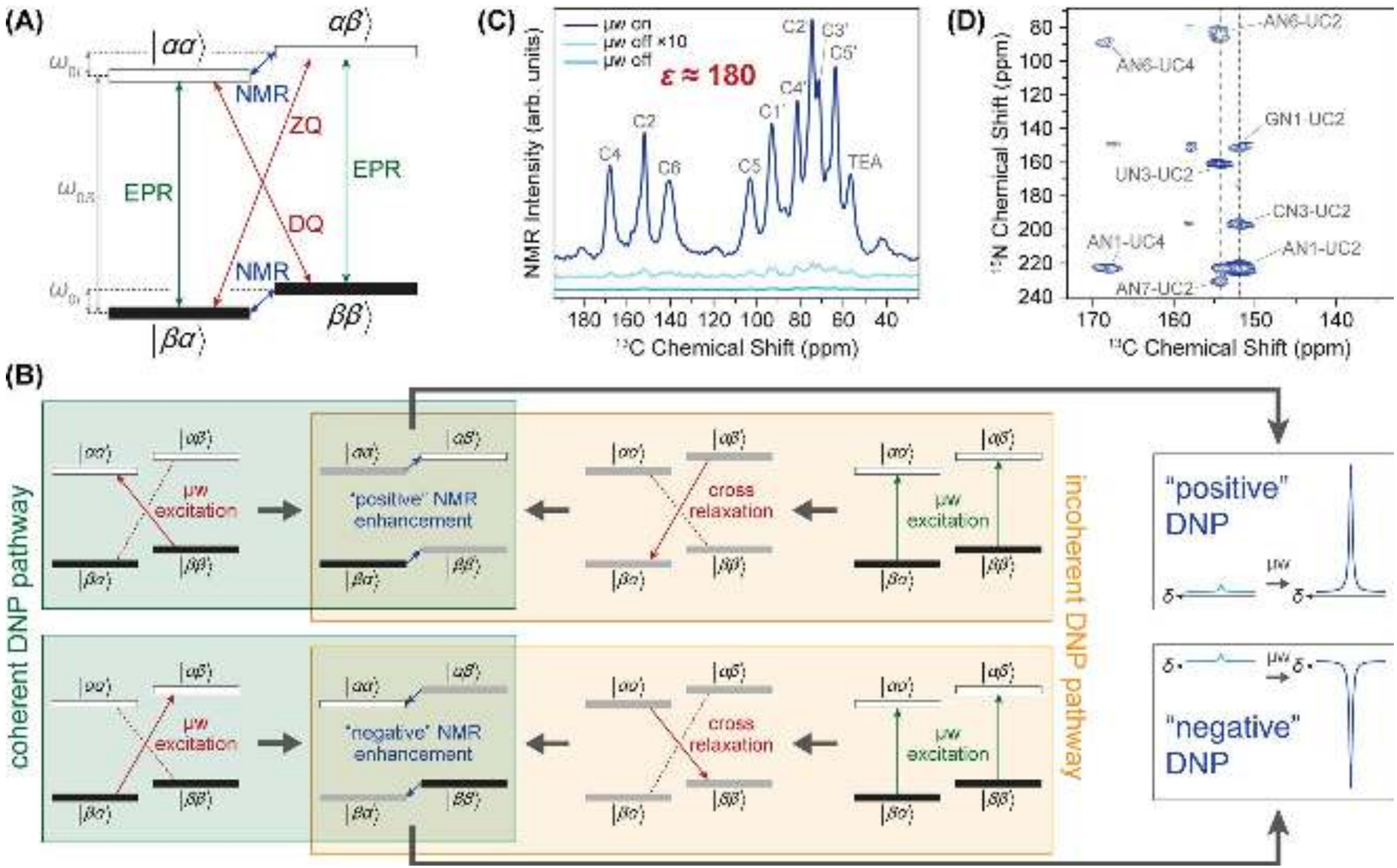




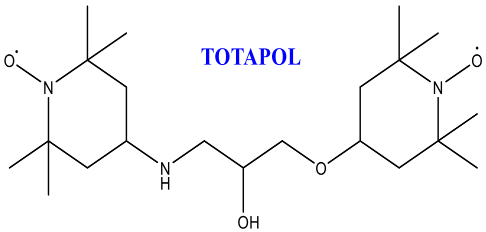
Matthias Bretschneider, Phillip E. Spindler, Olga Yu. Rogozhnikova, Dmitry V. Trukhin, Burkhard Endeward, Andrey A. Kuzhelev, Elena Bagryanskaya, Victor M. Tormyshev, and Thomas F. PrisnerJ. Phys. Chem. Lett. 2020, 11, 15, 6286-6290

Dynamic Nuclear Polarization for Sensitivity Enhancement in Biomolecular Solid-State NMR

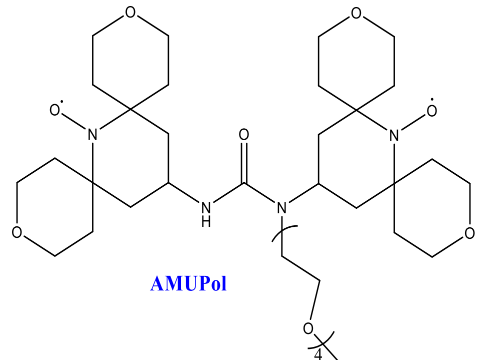
Thomas Biedenbänder, Victoria Aladin, Siavash Saeidpour, and Björn Corzilius Chem. Rev. 2022, 122, 9738–9794



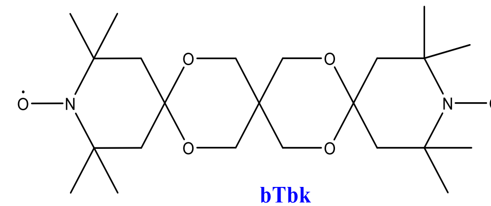
Biradicals as polarizing agents for DNP



TOTAPOL



AMUPol



bTbk

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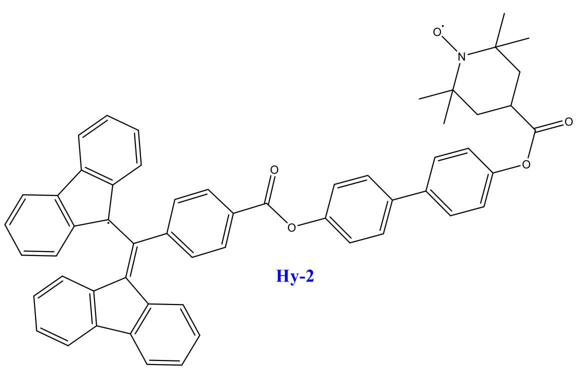
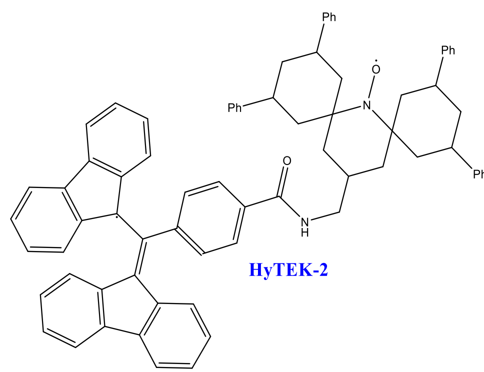
C. Sauvée et al., Angew. Chem. 52, 10858-10861 (2013)

A. Zagdoun et al., JACS 135,
B. 12790-12797 (2013)



Hy-2



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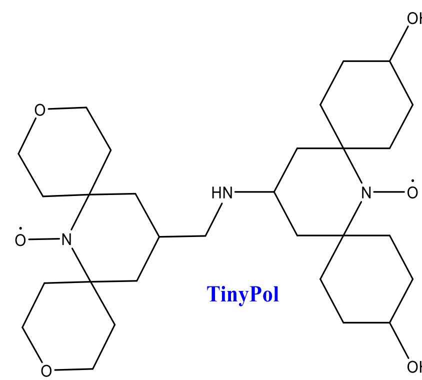
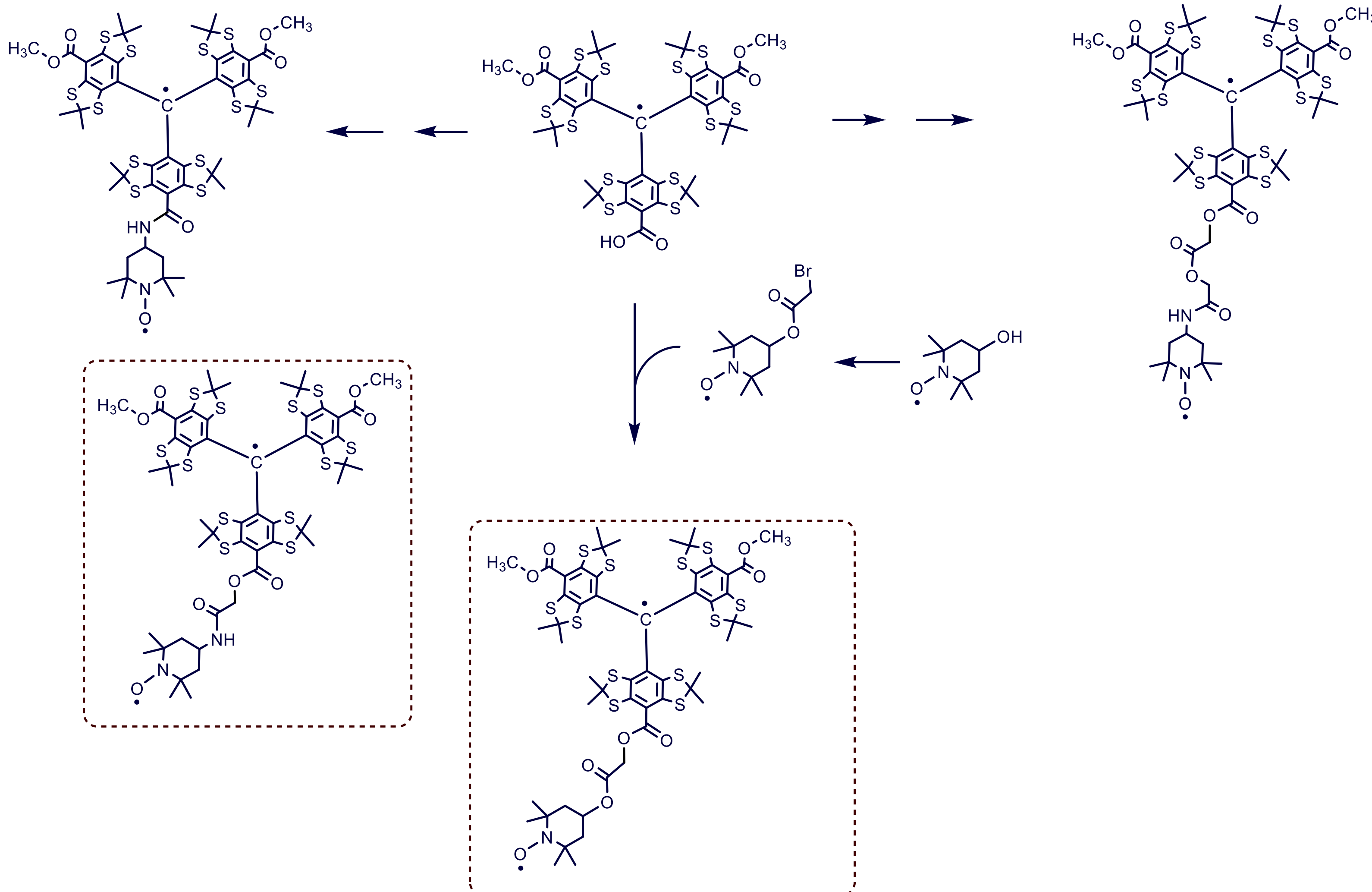


TinyPol

D. Wisser et al., JACS 140 (41), 13340-13349 (2018)

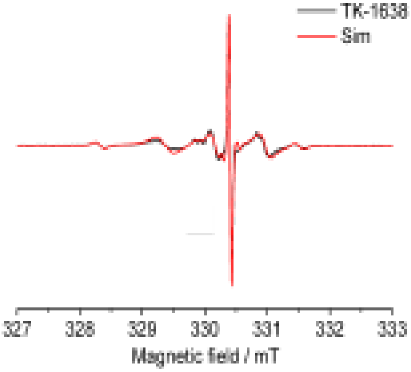
A. Lund et al., Chem. Sci. 11, 2810-2818 (2020)

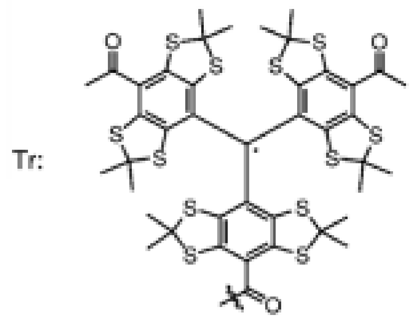
TAM-Nitroxide biradicals for direct excitation high field Dynamic Nuclear Polarization

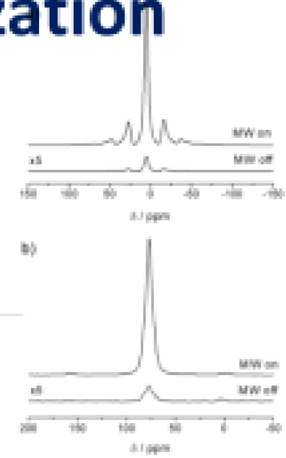


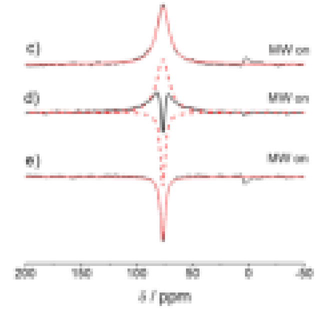
S. Bothe, J. Nowag, V. Klimavicius, M. Hoffmann, T.I. Troitskaya, E.V. Amosov, V.M. Tormyshev, I. Kirilyuk, A. Taratayko, A.A. Kuzhelev, D. Parkhomenko, E.G. Bagryanskaya, T. Gutmann, G. Buntkowsky, *J. Phys. Chem. C.,* 2018, **122**, 11422.

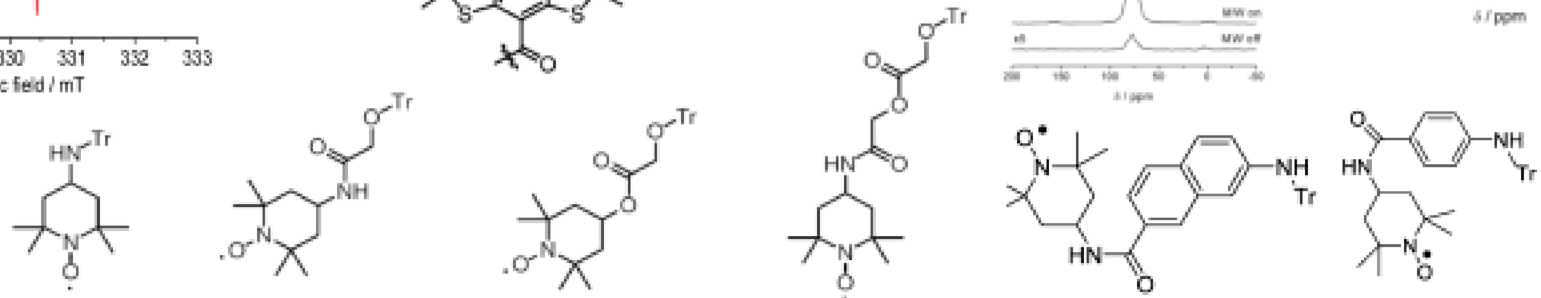
Biradicals TAM-nitroxides – polarizing agents for dynamic nuclear polarization











Radical	1	2	3	4	5	6
^{1}H MAS	2.8±0.1	40.0±0.1	49.8±0.1	14.3±0.1	30.1 ± 0.1	19.8 ± 0.1
$^{1}H \rightarrow ^{13}C$ CP MAS	2.8±0.1	43.2±1.8	52.2±2.1	13.7±0.7	28.7 ± 0.6	2.7±0.1

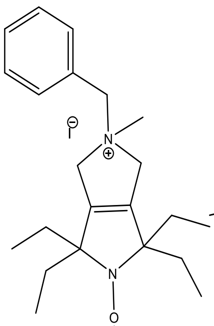
The dominant effect is the cross effect.

The maximum DNP is observed for a flexible linker and interradical distances of ~14 Å.

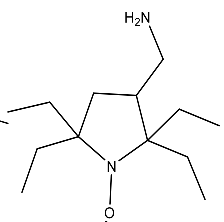
S. Bothe, J. Nowag, V. Klimavicius, M. Hoffmann, T.I. Troitskaya, E.V. Amosov, V.M. Tormyshev, I. Kirilyuk, A. Taratayko, A.A. Kuzhelev, D. Parkhomenko, E.G. Bagryanskaya, T. Gutmann, G. Buntkowsky, *J. Phys. Chem. C.*, 2018, **122**, 11422.

EPR study of highly stable biradicals perspective for DNP in cell

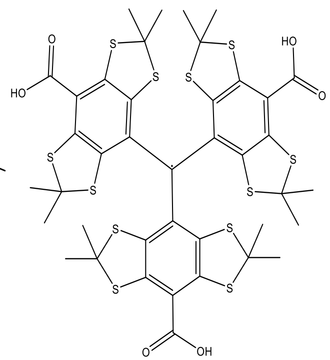
Monoradicals – highly stable tetraethyl substituted nitroxides and triarylmethyl radical



N1



N2

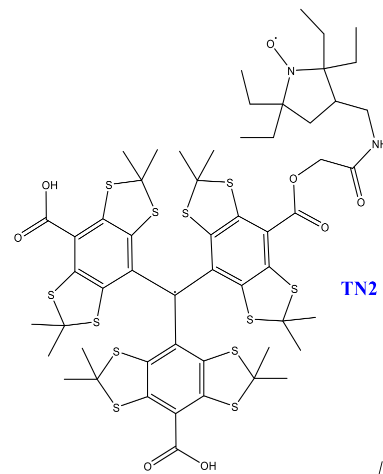


TAM

For biomolecular applications:

Water-soluble
Inert to biological reductunts

Nitroxyl-nitroxyl and Trityl-nitroxyl biradicals



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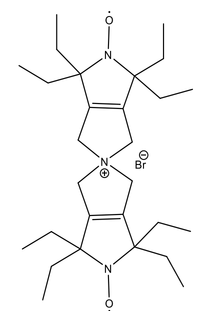
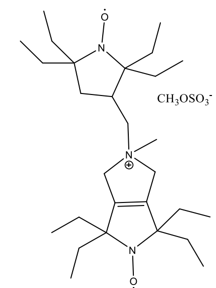


NN1



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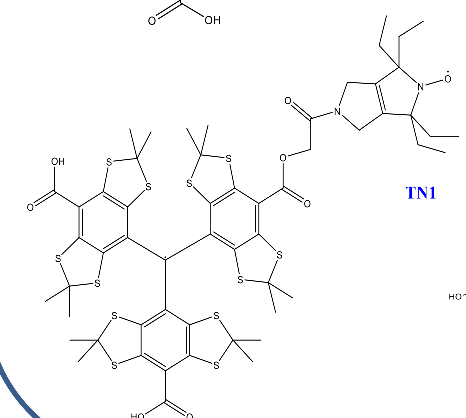
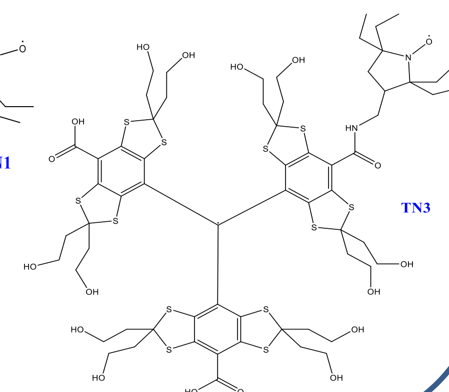


TN1



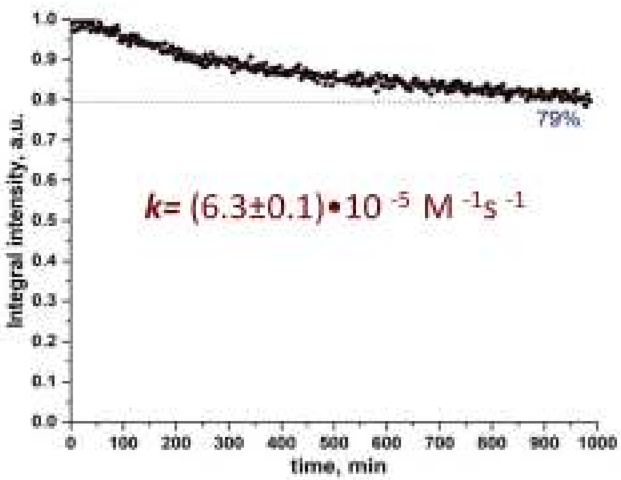
TN3

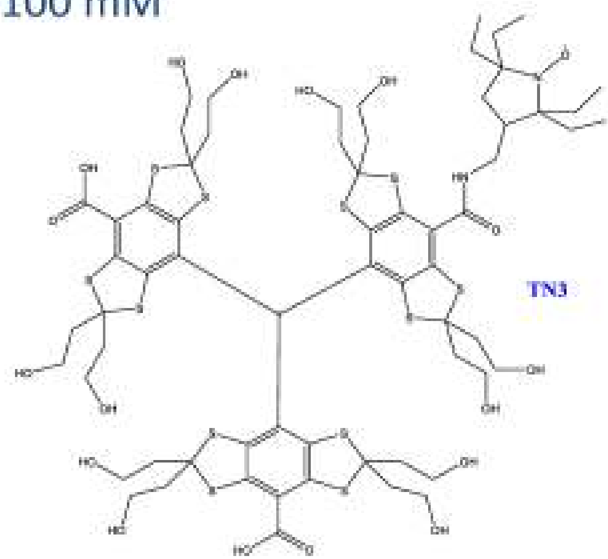
Trityl-Nitroxide Highly Stable Biradicals for DNP in Cells

a_N = 1.46 mT
J = 392 MHz

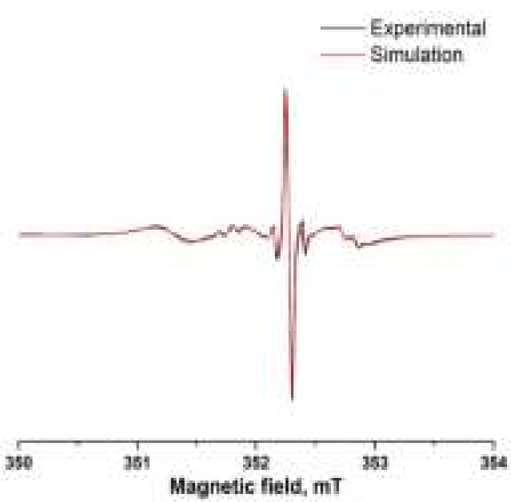
Concentrations:

Radical – 0.3 mM   Ascorbate – 100 mM

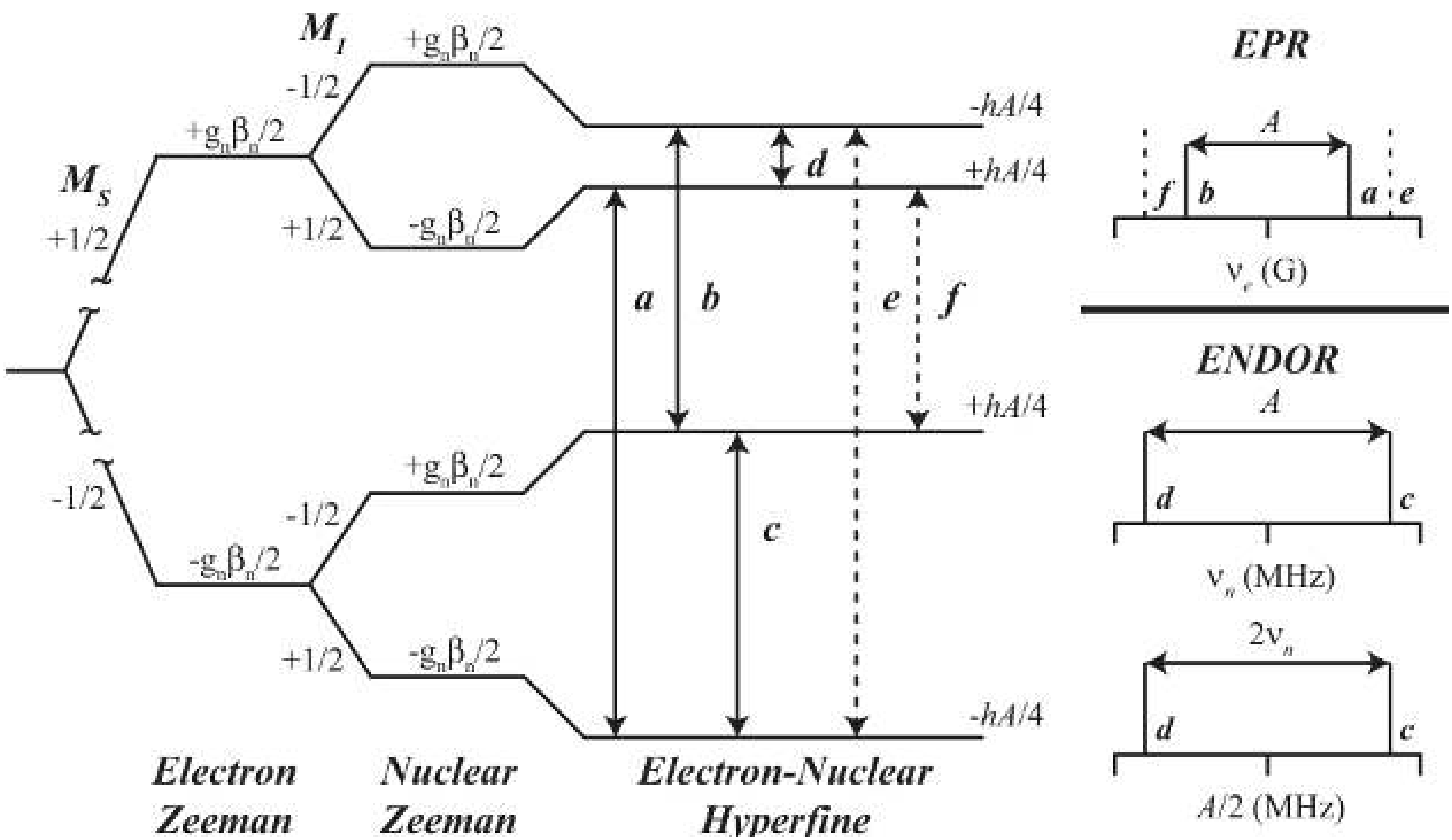




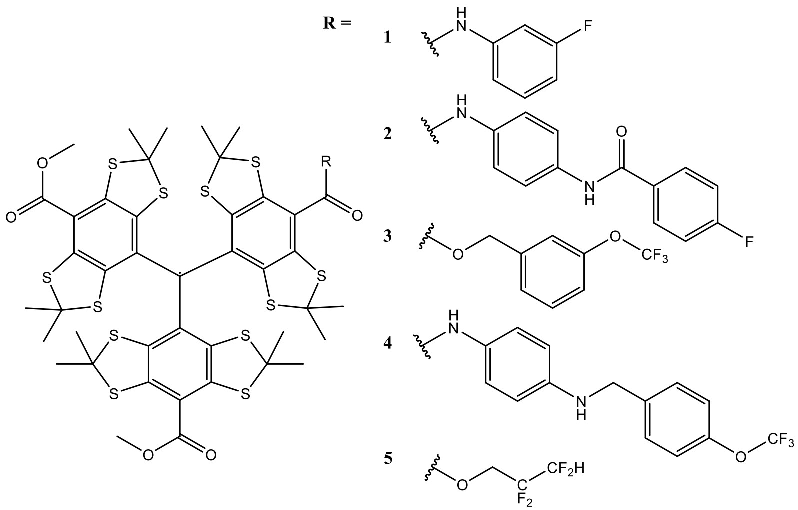
TN3

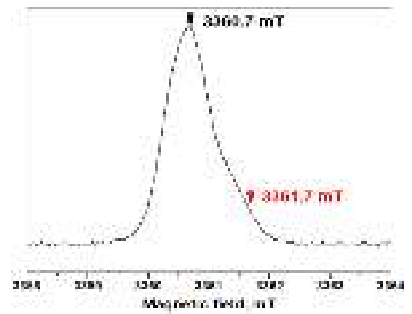


Electron Nuclear DOuble Resonance

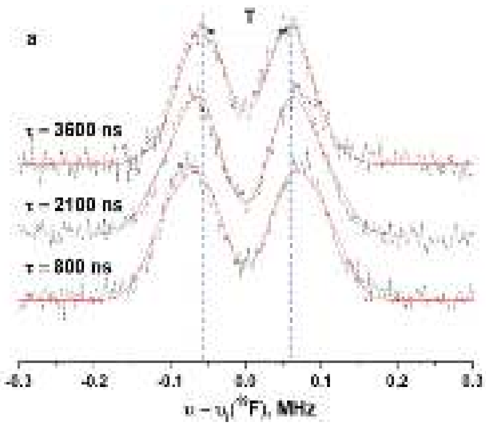


Application of W-band ^{19}F ENDOR spectroscopy for distance measurements (0.5-1 nm)

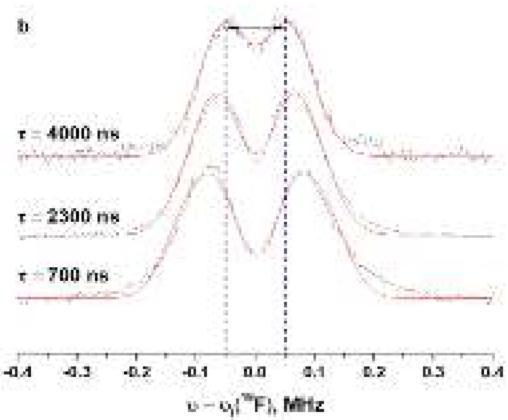




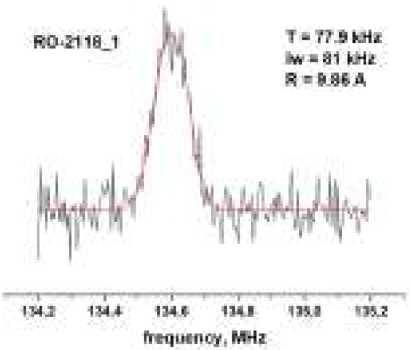
А.Суханов, КФТИ



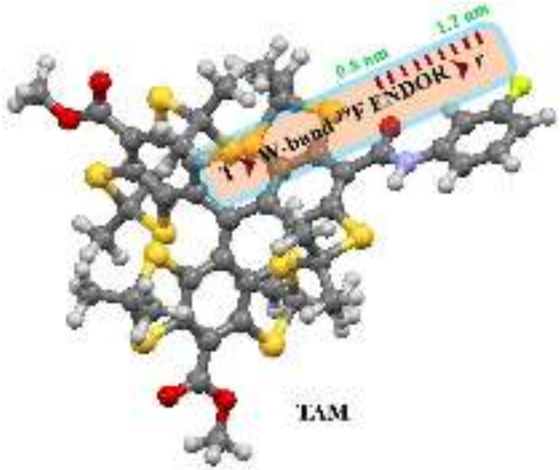
94 GHz ^{19}F Mims ENDOR spectra of **5** in $[d_6]$DMSO/$[d_4]$MeOD (2:3) were recorded at different values of τ. R= 7.0 ± 0.2 Å

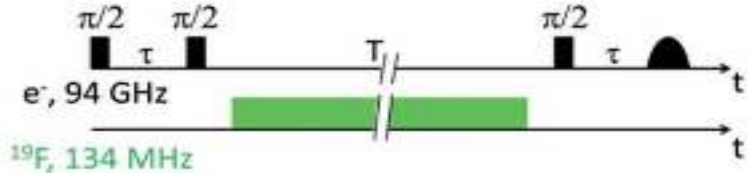


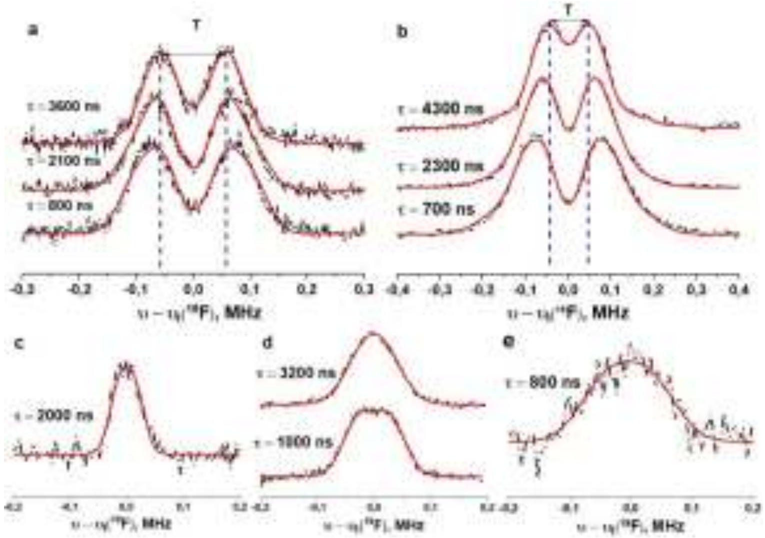
94 GHz ^{19}F Mims ENDOR spectra of **1** in $[d_6]$DMSO/$[d_4]$MeOD (2:3) at EPR resonances 3360.7 mT (black) and 3361.7 mT (red) at 80 K. R= 8.2 ± 0.3 Å



Application of W-band ^{19}F electron nuclear double resonance (ENDOR) spectroscopy to distance measurement using a trityl spin probe and a fluorine







✓*Использование триарилметильных меток для избежания фактора ориентационной селективности*

✓*Использование фторных меток и высокочастотной ENDOR спектроскопии позволяет хорошо разделить сигналы от разных ядер*

Измерение расстояний в диапазоне 8 -12 Å!

N.B. Asanbaeva, A.A. Sukhanov, A.A. Diveikina, O. Yu. Rogozhnikova, D.V. Trukhin, V.M. Tormyshev, A.S. Chubarov, A.G. Maryasov, A.M. Genaev, A.V. Shernyukov, G.E. Salnikov, A. A. Lomzov, D.V. Pyshnyi, E.G. Bagryanskaya, Phys. Chem. Chem. Phys., 2022, 24 (10), 5982-600

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Thomas Prisner


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A. Kuzhelev

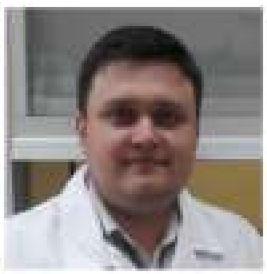

Gerd Buntkowky


Torsten Gutmann

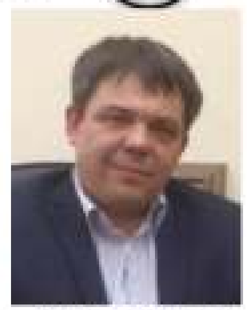
ICB FM SB RAS


Georgiy Shevelev


Alexander Lomzov,


Alexey Chubarov,


Tatyana Godovikova


Dmitrii Pyshnui


Benesh Joseph


Andrey Sukhanov


Olesya Krumkacheva


Matvey Fedin


Ivan Timofeev

ITC SB RAS

Министерство науки и высшего образования Российской Федерации

RSF Russian Science Foundation

RFBR RUSSIAN FOUNDATION FOR BASIC RESEARCH

Thank you very much!