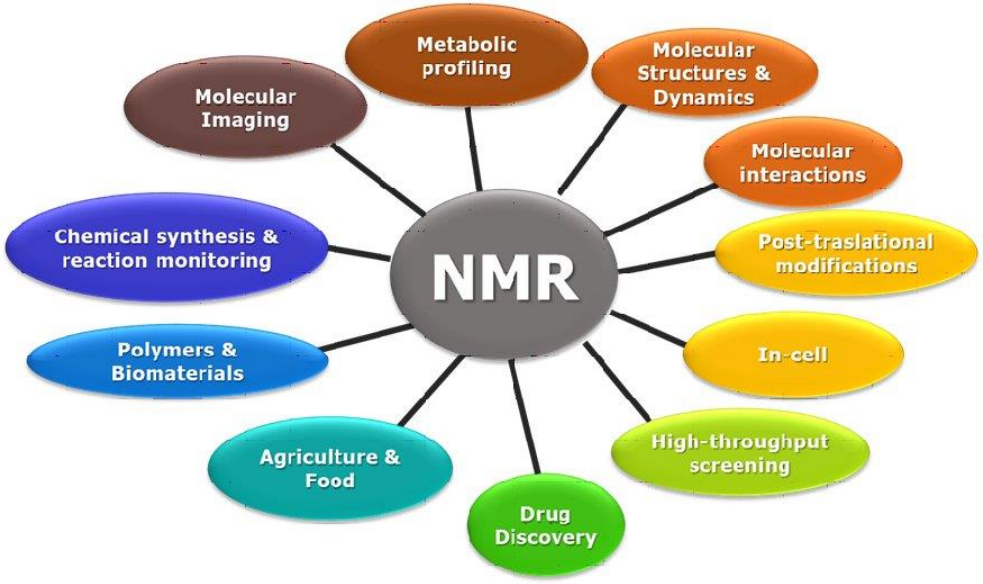
Applications



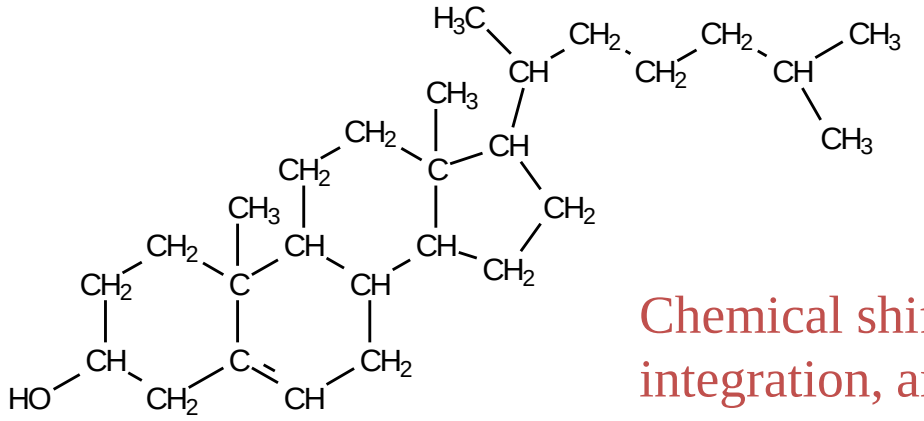
http://www.eurobionmr.eu/open-access-to-biological-nmr-in-europe/

Chemistry

Chemical structure

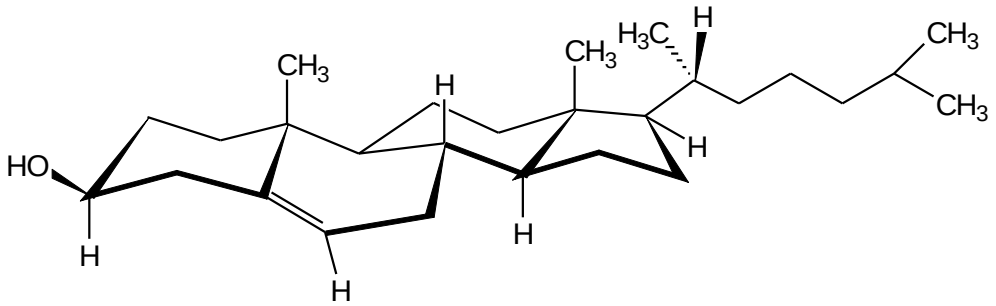
^{1}H: 46 hydrogen atoms

^{13}C: 27 carbon atoms.



Chemical shift δ, integration, and J correlations

Relative configuration



^{1}H-^{1}H splitting and NOE

Quality control/chemometrics – oil degradation

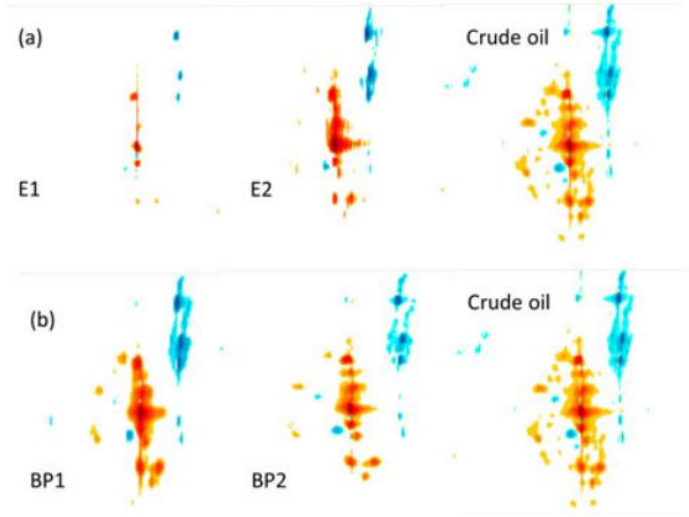


Figure 1. Fingerprint of the expanded HSQC-NMR spectra (δC/δH 10–45/0.5–3.0 ppm). (a) Crude oil and samples with natural attenuation (E1 and E2); (b) crude oil and biopiles (BP1 and BP2); f1: δC; f2: δH; $-CH_3$ and $-CH$ (blue); $-CH_2$ (orange)

Characterization of oil complex hydrocarbon mixtures by HSQC-NMR spectroscopy and PCA
https://doi.org/10.1002/poc.3233

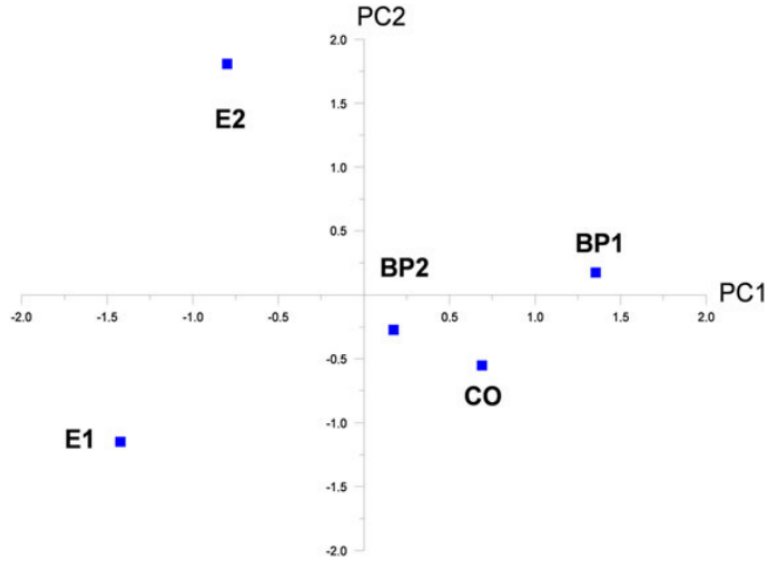
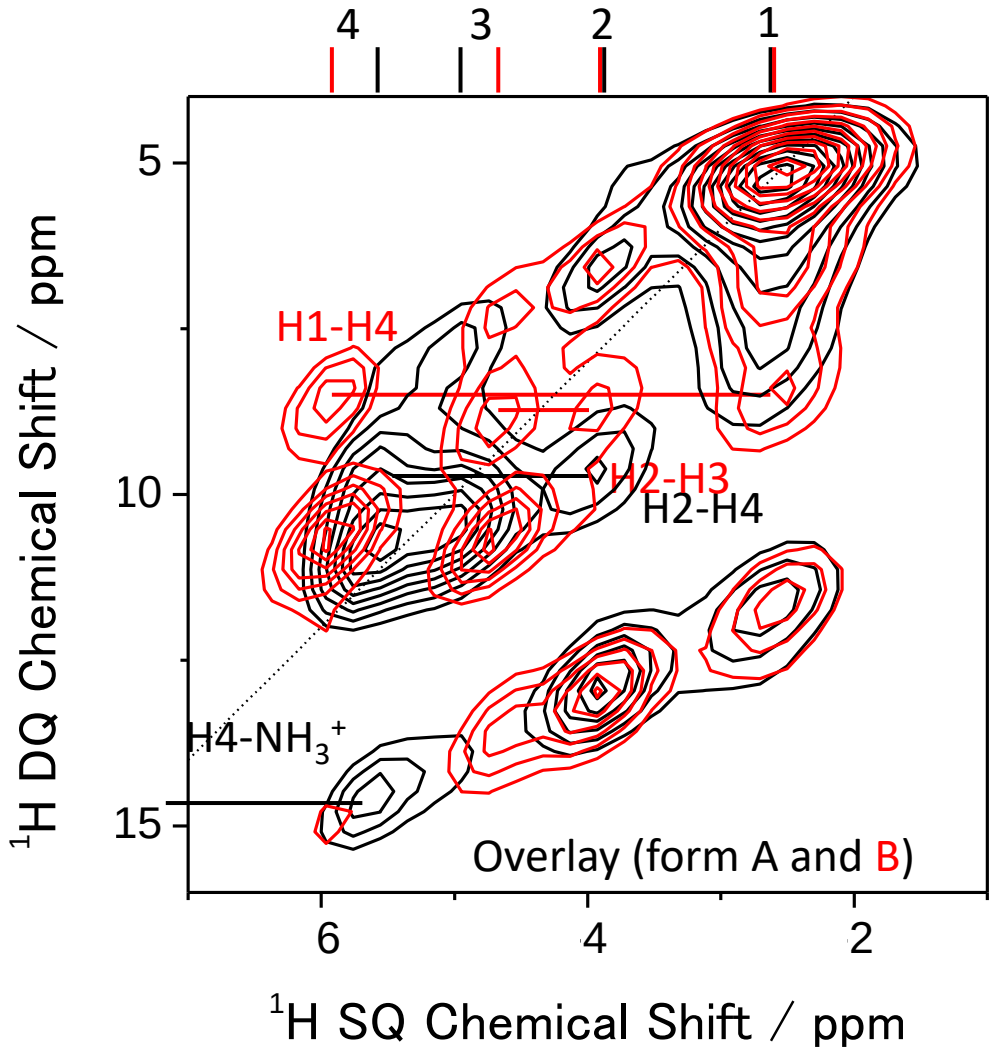
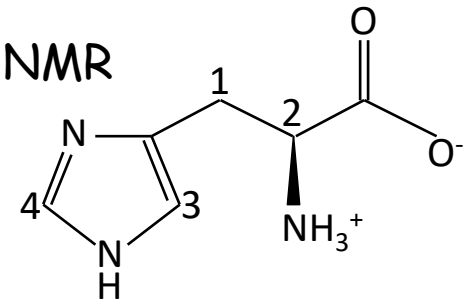


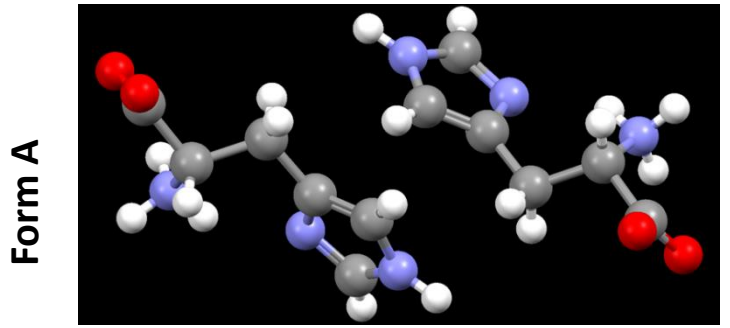
Figure 3. Score plot: crude oil (CO), residues with natural attenuation (E1 and E2), and biopiles (BP1 and BP2)

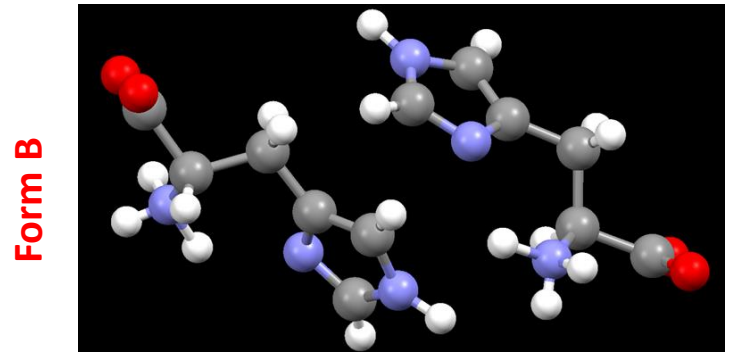
Intermolecular interactions

Determining molecular packing of L-histidide by solid-state NMR



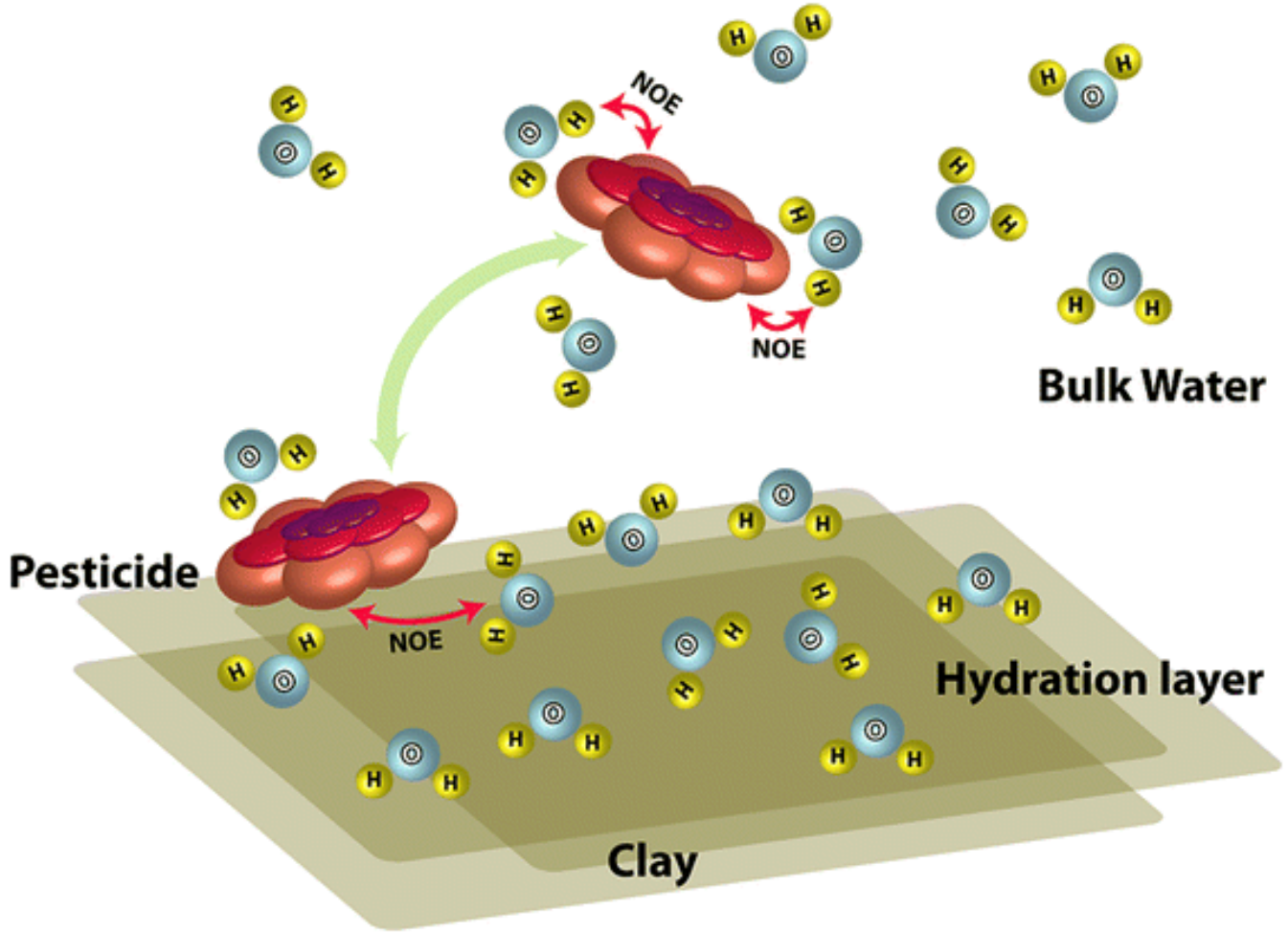






T. Oikawa, M. Okumura, T. Kimura, Y. Nishiyama, Acta Cryst. C73 (2017) 219–228.

Intermolecular interactions
pesticide – soils, drugs – proteins, etc.



WaterLOGSY using liquid-state probes

Soong, R., Botana, A. et al. Chemical and Biological Technologies in Agriculture (2017) 4:3

Antitrypanosomal 4-phenyl-6-(pyridin-3-yl)pyrimidines interaction with rhodesain

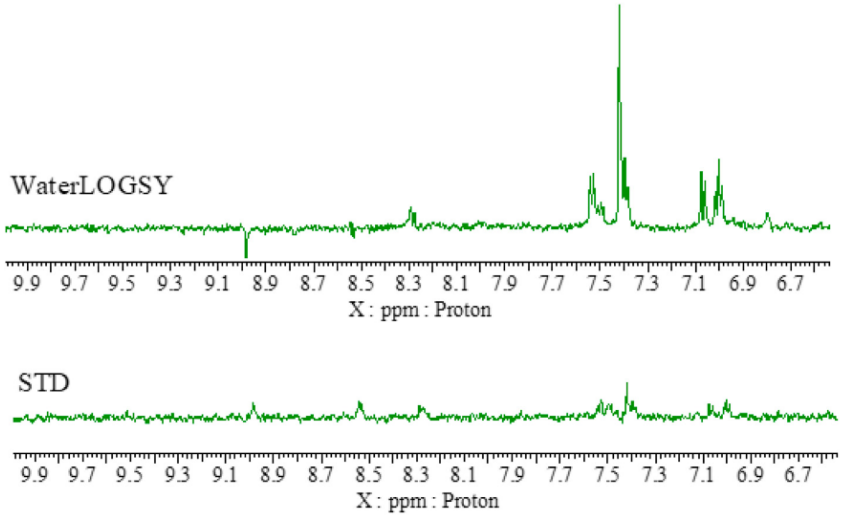
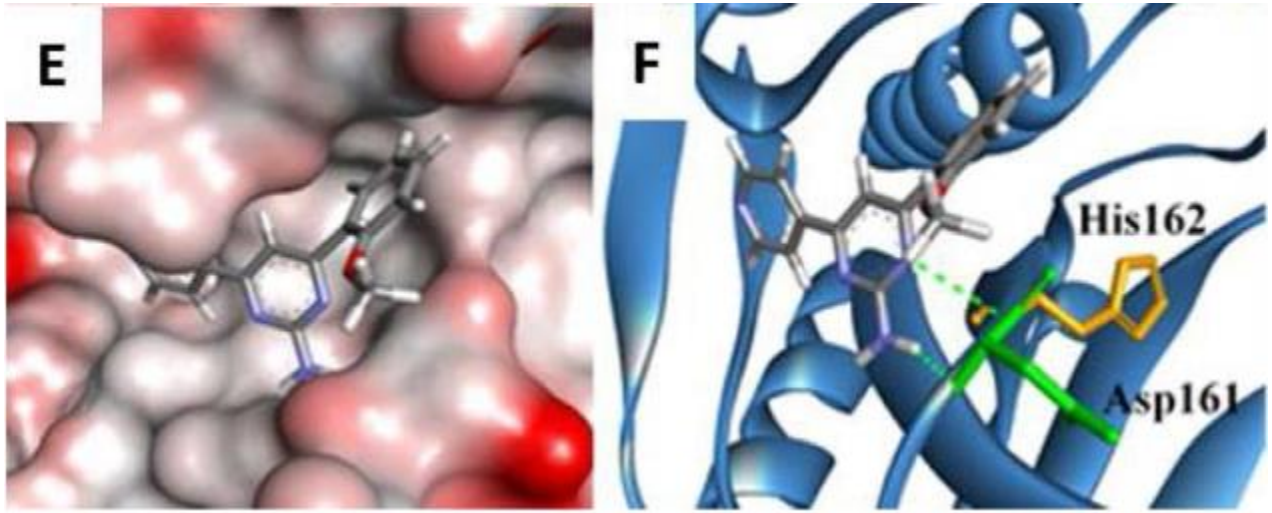


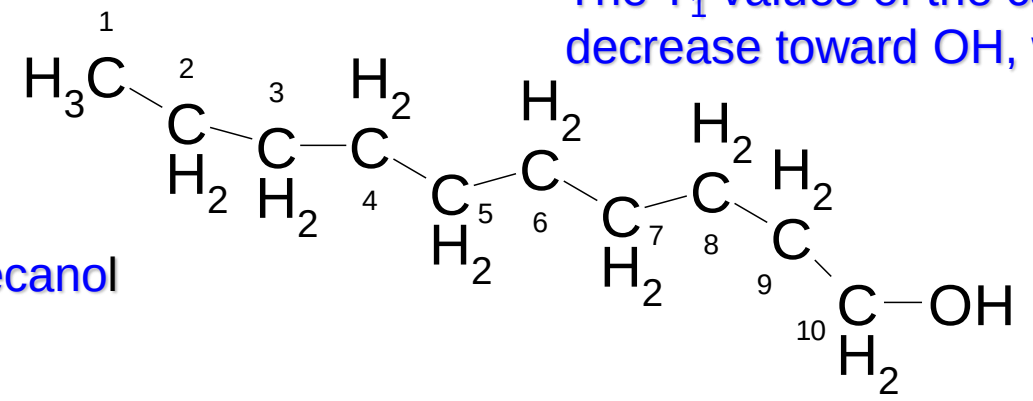
Fig. 6. STD and WaterLOGSY spectra of compound 13 in the presence of rhodesain.

https://doi.org/10.1016/j.ejmech.2020.112871



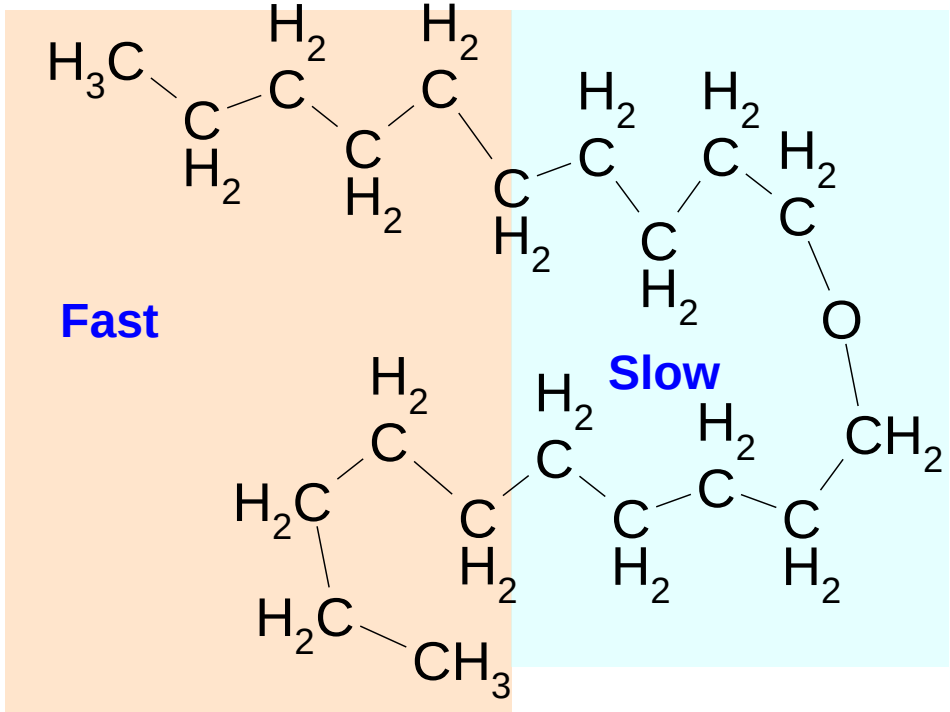
Information from T_1

The T_1 values of the carbons decrease toward OH, why?



n-decanol

Carbon	T_1/sec
1	3.1
2	2.2
3	1.6
4	1.1
5	0.84
6	0.84
7	0.84
8	0.77
9	0.77
10	0.65



Those T_1 values prove the two molecular complex by hydrogen bound

Information from T_1 in solids

Table 2. ^{1}H T_1 relaxation times for α- and γ-CD, PCL-PEO-PCL tri-block copolymer, and inclusion complexes of the CDs and copoly-mer

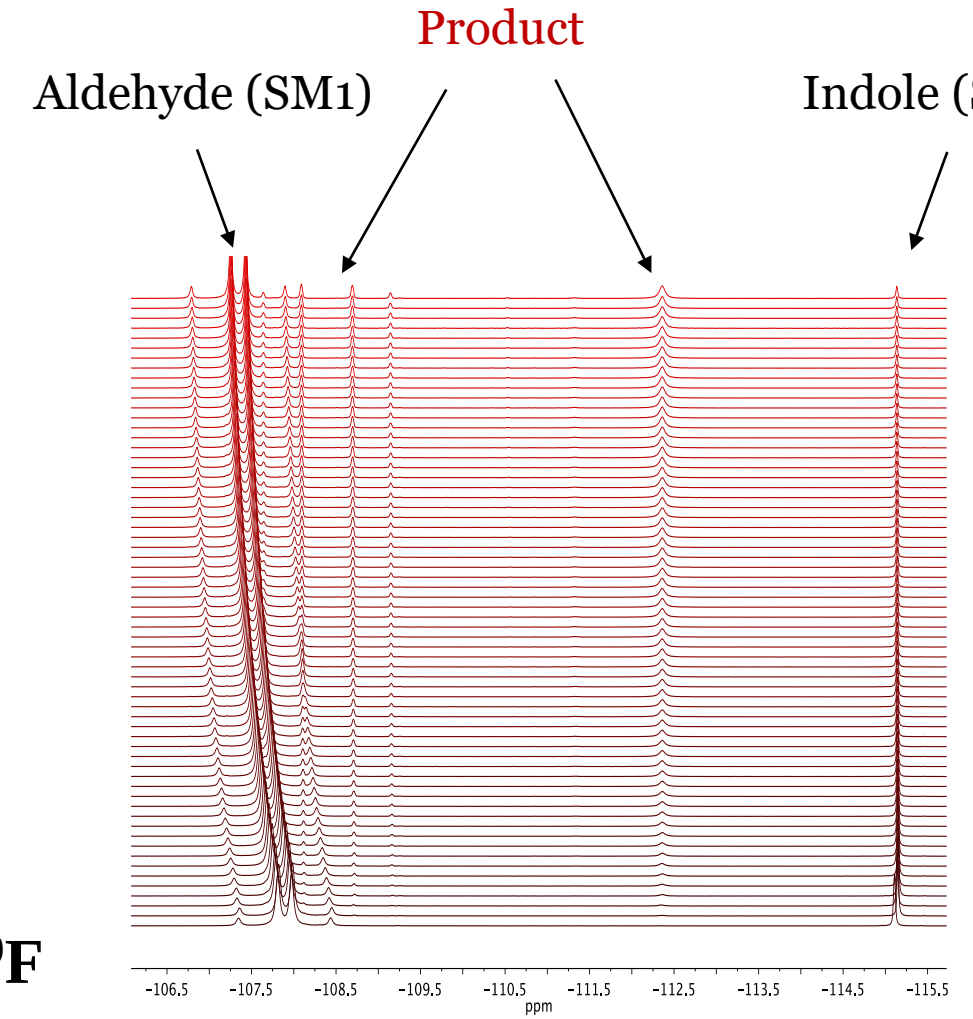
Sample	T_1 (s) PCL-PEO-PCL	T_1 (s) CD
α-CD	–	3.50
γ-CD	–	2.35
PCL-PEO-PCL	1.40	–
PCL-PEO-PCL-α-CD-IC	1.86	1.83
PCL-PEO-PCL-γ-CD-IC	1.07	1.02

The similarity of relaxation times for the CDs and the copolymer in the inclusion complexes demonstrates homogeneity of the com-plex. Adapted from [24].

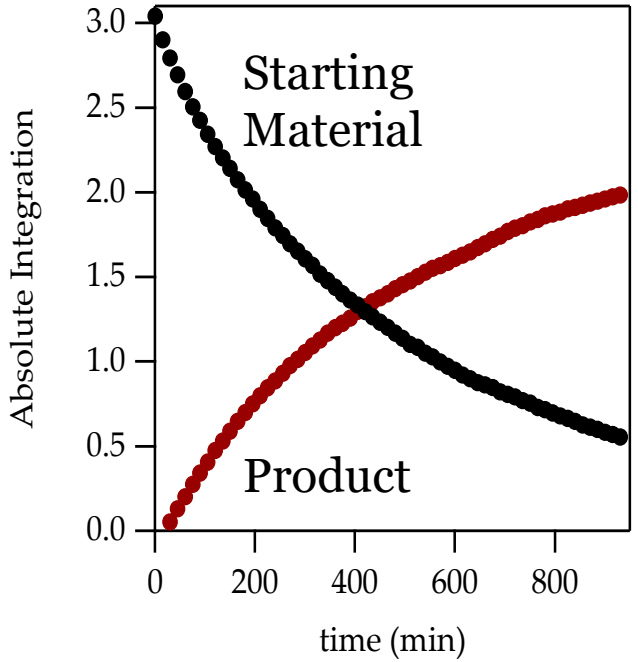
https://doi.org/10.1016/j.trac.2006.07.006

Reaction Monitoring

Genentech
A Member of the Roche Group

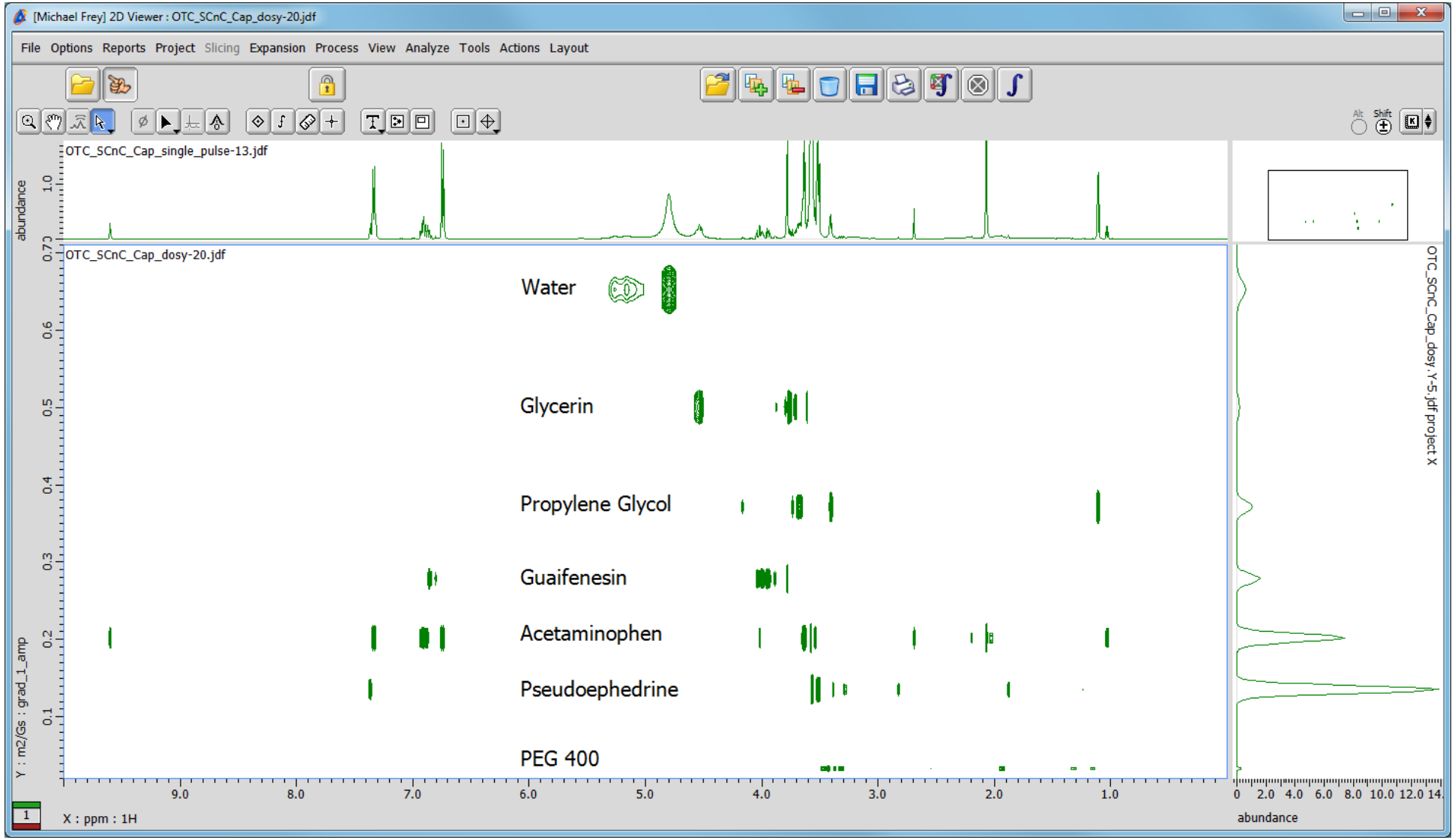


^{19}F



0.050 M aldehyde, 0.25 M indole, 0.25 M diphenyl phosphate, -80 ˚C

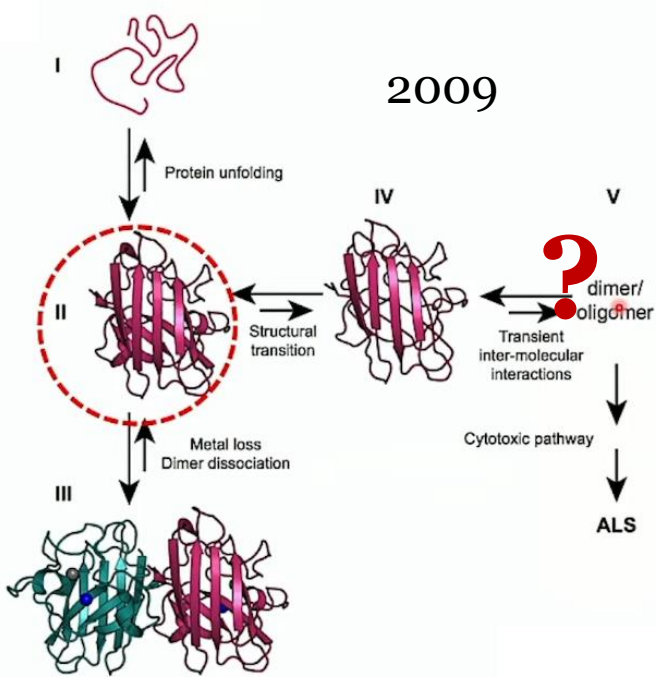
DOSY NMR of paracetamol tablet



Solvent Methanol-d_4

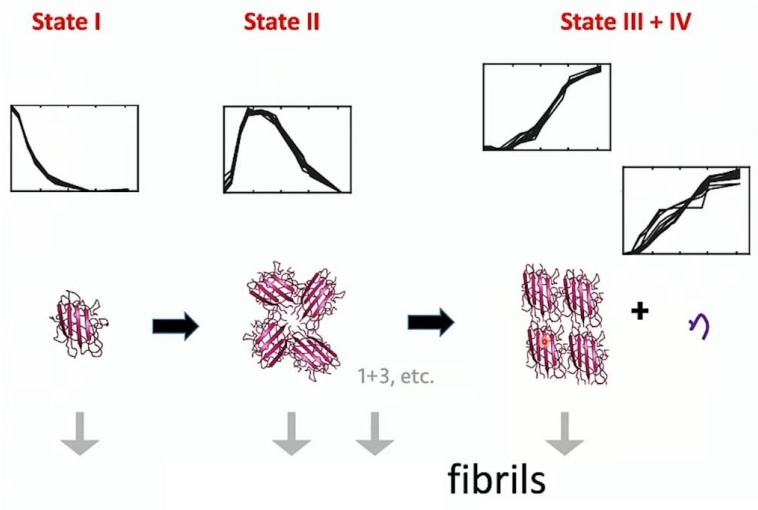
Insight into Amyotrophic Lateral Sclerosis via DOSY and reaction monitoring

2009

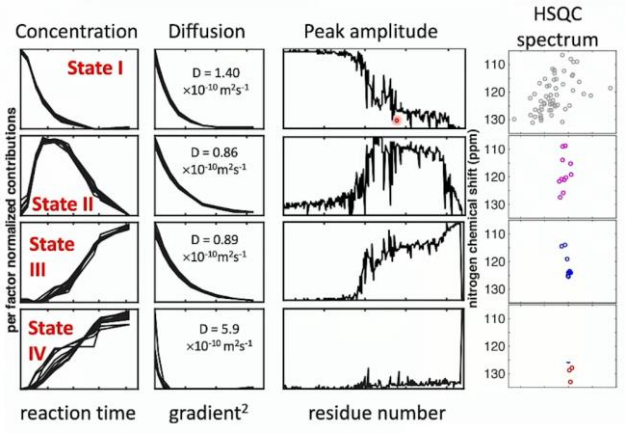


http://dx.doi.org/10.1073/pnas.0907387106

2019

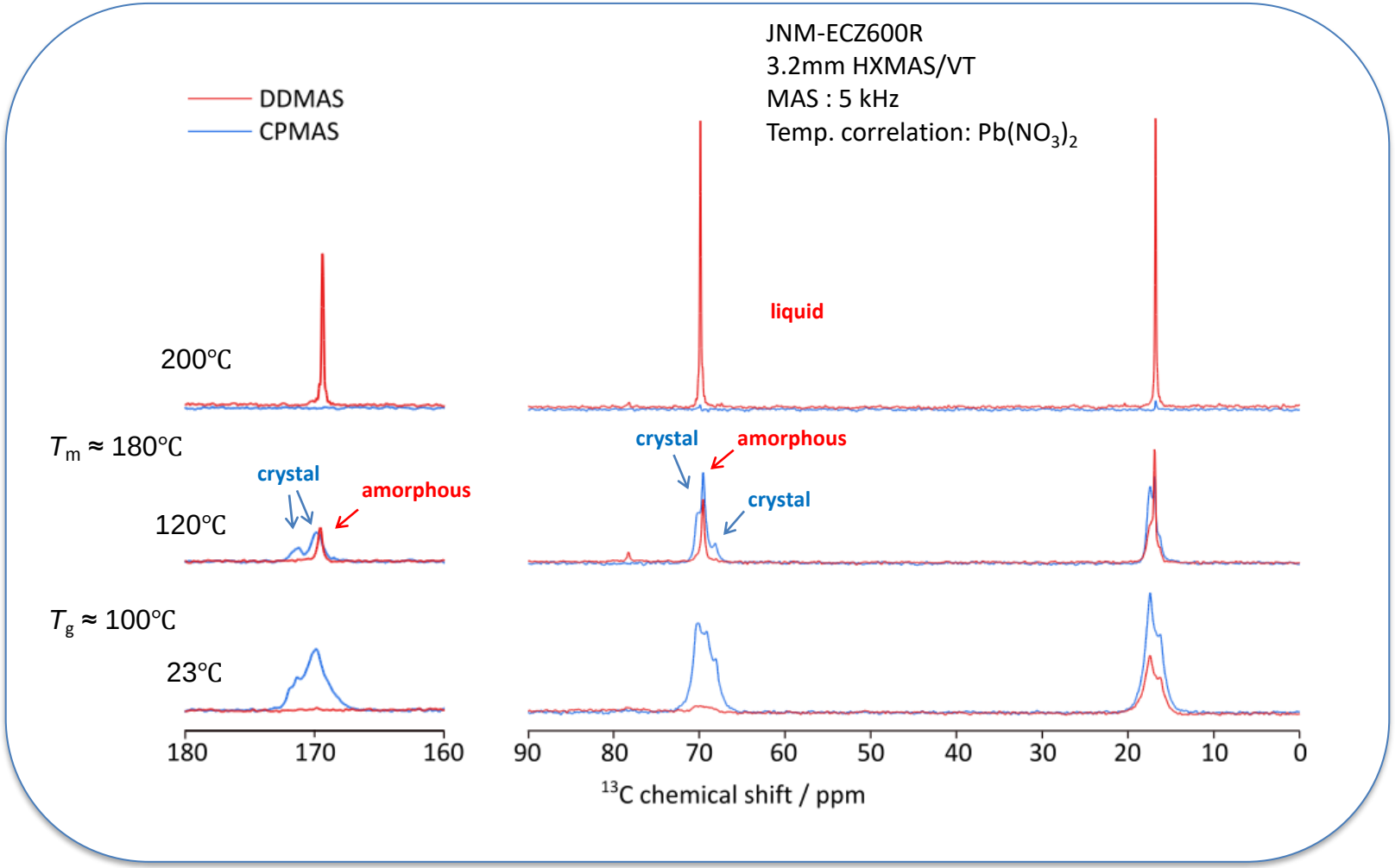


https://doi.org/10.1021/jacs.9b07952



Polymer analysis

poly(L-lactic acid) using extended VT solid-state probe



Sample – courtesy of Prof. Naoki Asakawa, Gunma University

Biological samples analysis

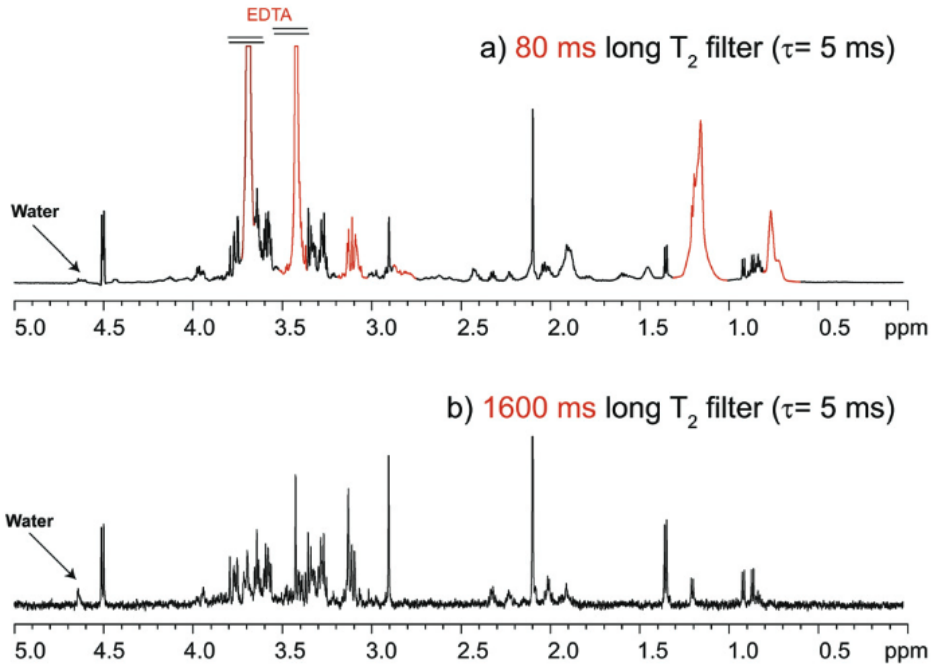


Fig. 5 Wasted pulse sequences allow the use of T_2 filters of a length impossible to use achieve with presat-CPMG. This allows the introduction of T_2 encoding to distinguish between different metabolite populations. Compare (b), in which a 1600 ms long filter has been made possible by Wasted-II, with (a) in which an 80 ms filter has been used (also using Wasted-II). Note that the suppression of the water signal is excellent even when using these long filters. The sample is human blood with non-deuterated EDTA.

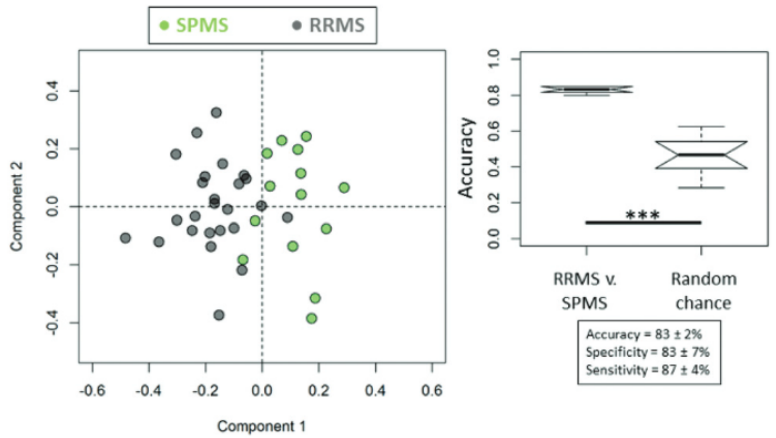
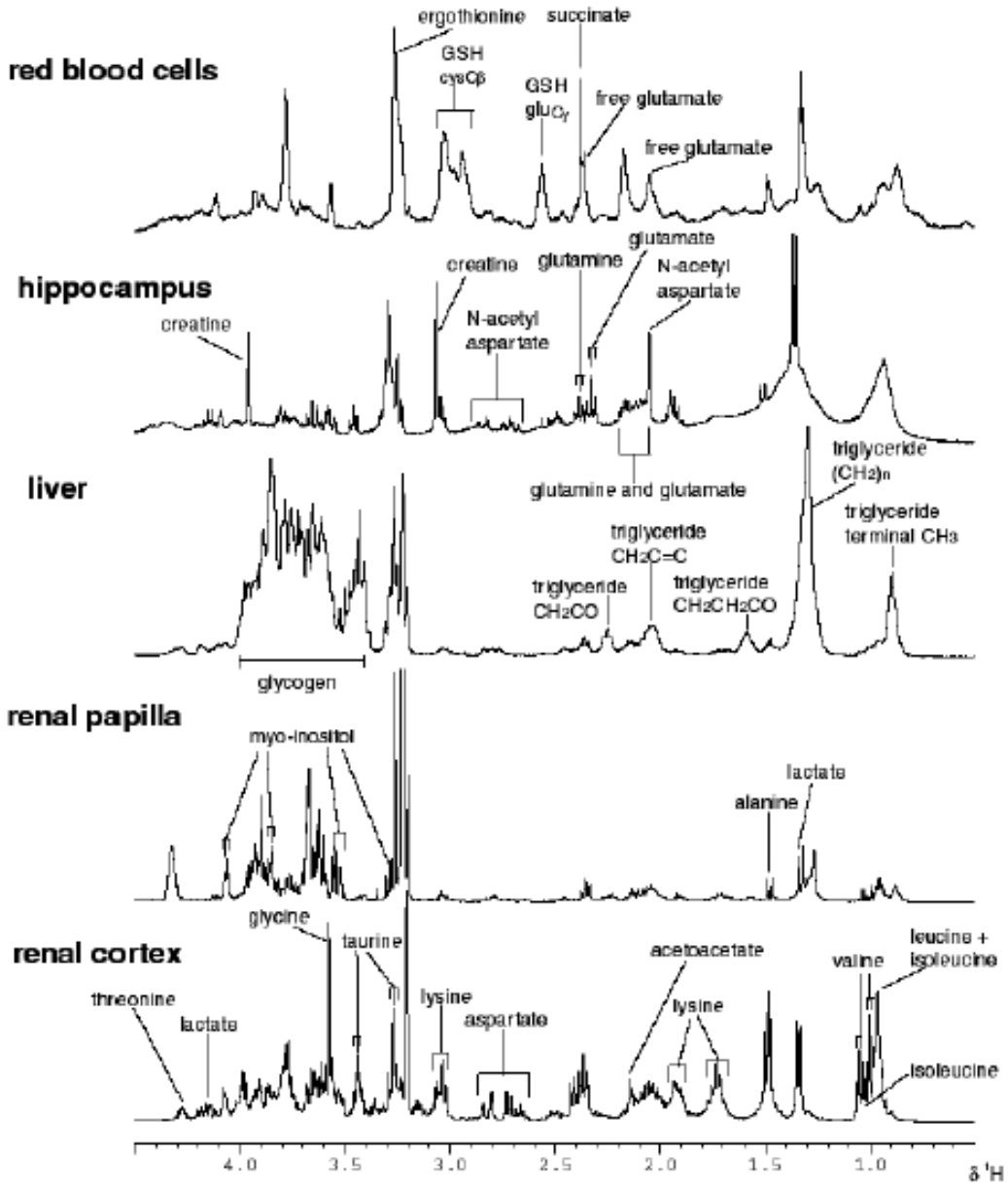
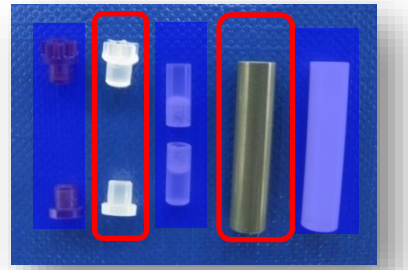


Fig. 6 OPLS-DA results from blood plasma Wasted-II ^{1}H NMR data discriminating SP from RR multiple sclerosis patients. Left, a representative scores plots illustrating separation between SPMS and RRMS plasma spectra in the multivariate models, and right, the accuracy of the cross-validated ensemble of OPLS-DA models is significantly better than random chance. Kolmogorov–Smirnov test *p*-values <0.001 are represented by ***.

https://doi.org/10.1039/C9AN01005J

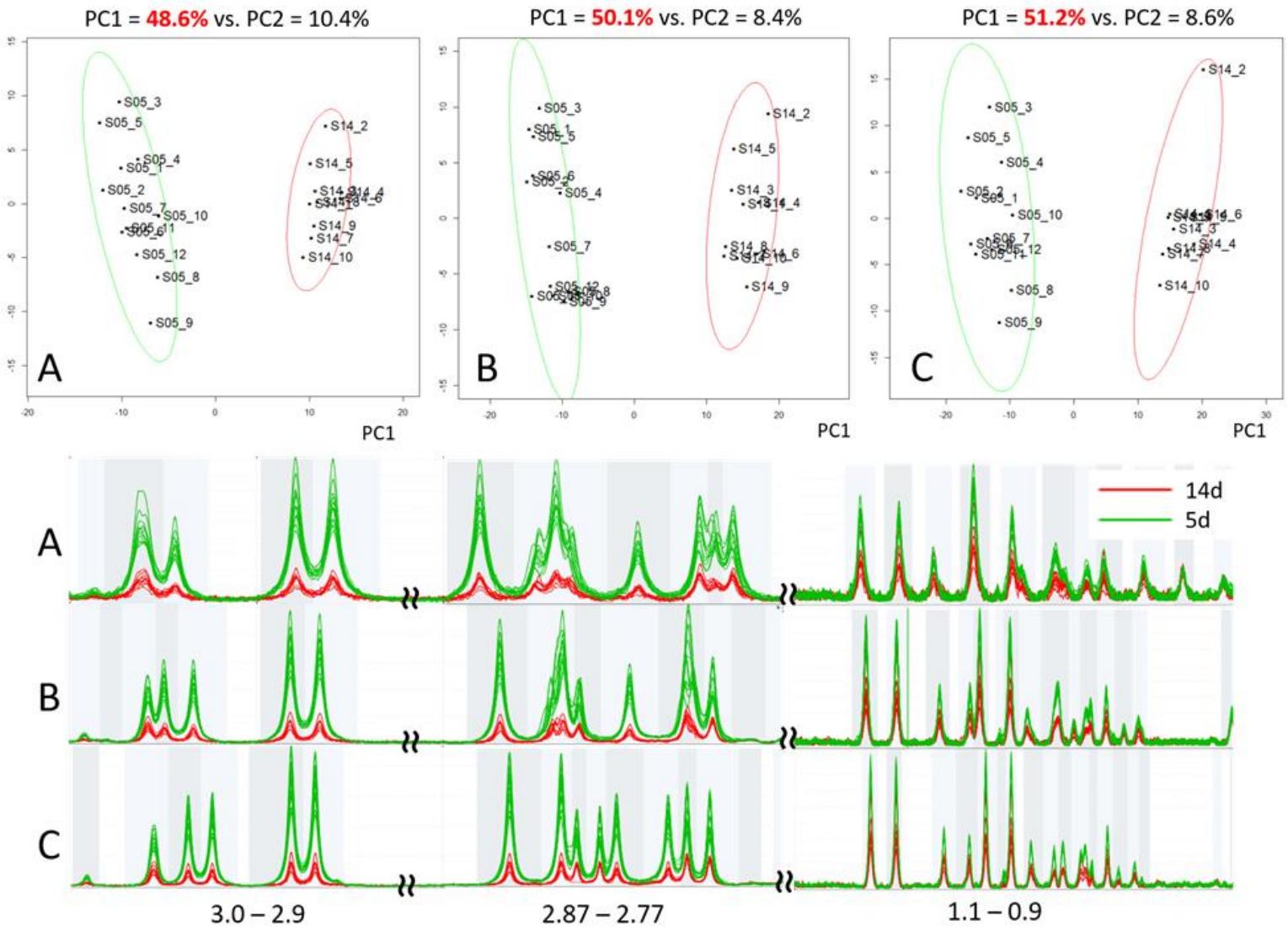
Semi-solids analysis in biology





Study of tissues using FGMAS probe reveals clear differences between them

Metabolomics analysis



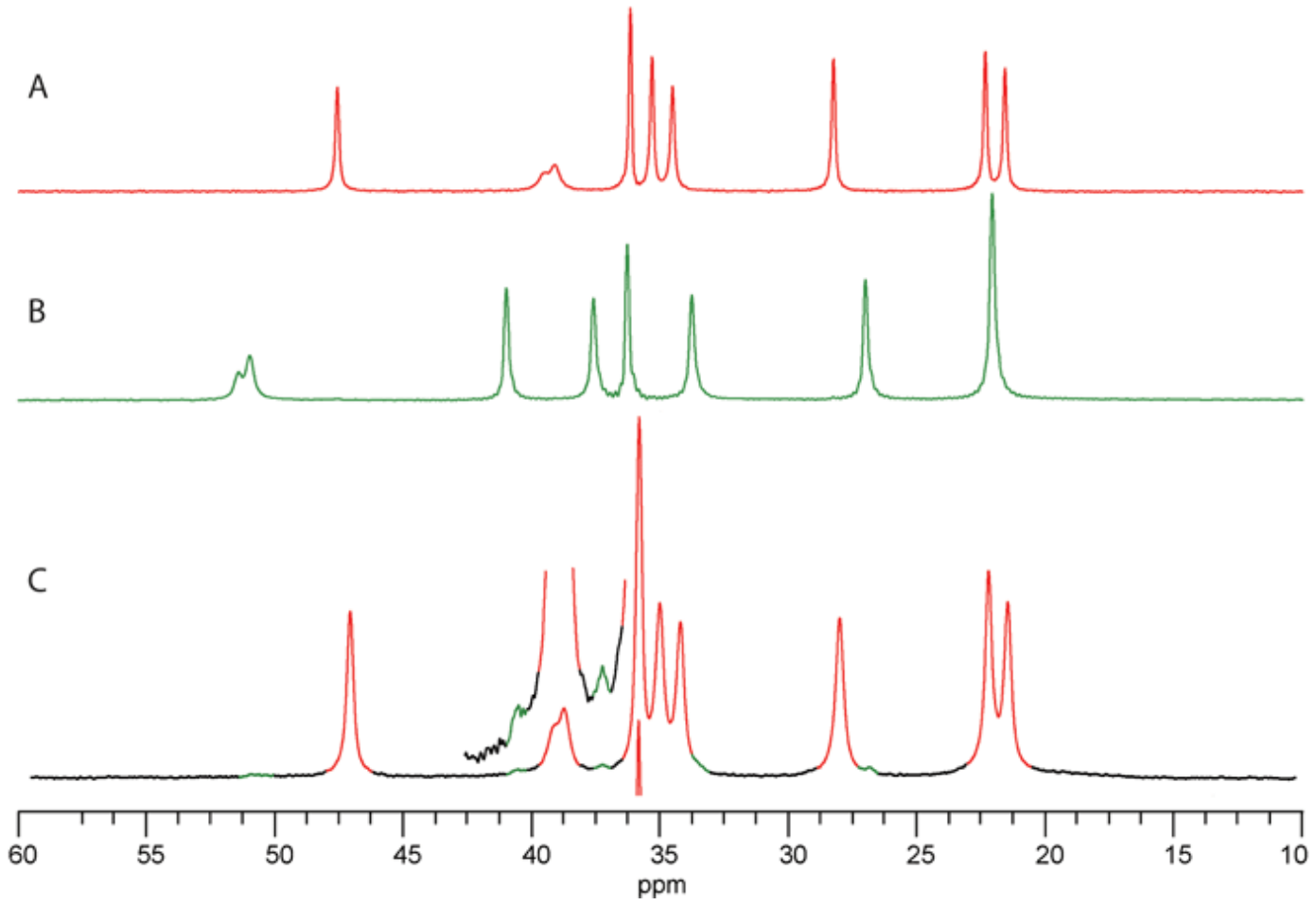
A: JEOL 400
B: Bruker 500
C: Bruker 600

Green: 5 days after flowering

Red: 14 days after flowering

Deborde, C., Fontaine, JX., Jacob, D. Botana, A. et al. Metabolomics (2019) 15: 28

Quantitation of gabapentin polymorphs



Form II

Form III

Form II mixed with 6.5% hydroxyl-propylcellulose (excipient) and milled for 45 min

https://www.europeanpharmaceuticalreview.com/article/17182/applications-of-solid-state-nmr-spectroscopy-to-pharmaceuticals/
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3581669/

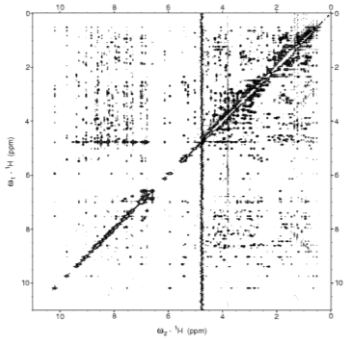
Protein structure determination

GSDIIDEFGTLDDSATICRVCQKPG
DLVMCNQCEFCFHLDCHLPALQD
VPGEEWSCSLCHVLPDLKEEDVDL
QACKLN

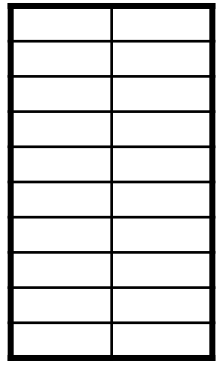
Protein sequence

Express and purify protein (or isolate from natural source)

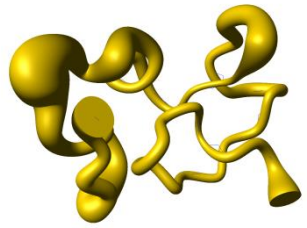
Initial characterisation
- **Identity, composition**
- **Concentration**
- **Stability (buffers, salt, pH, temperature)**



Acquire NMR spectra



Evaluation:
Sequential Assignment
Extraction of distance restraints and other structural data



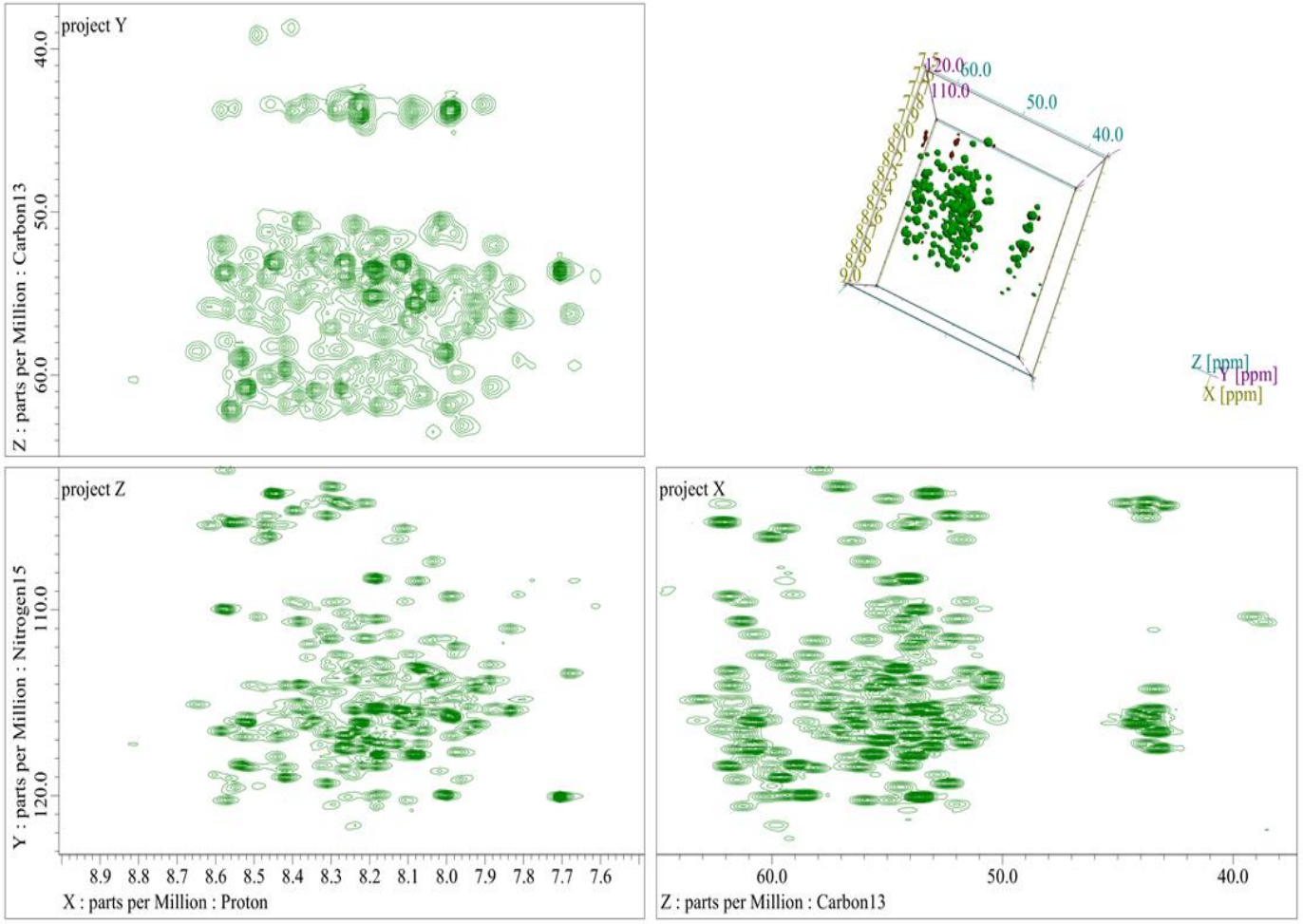
3D structure

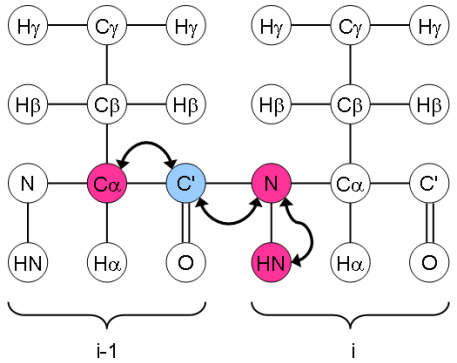
Protein spectra using UltraCOOL HCN 800

3D HNCOCA-TROSY, 2H decoupled, 20 hours

~0.2 mM Stt3p* (~30kDa) in lipid micelles at 55C

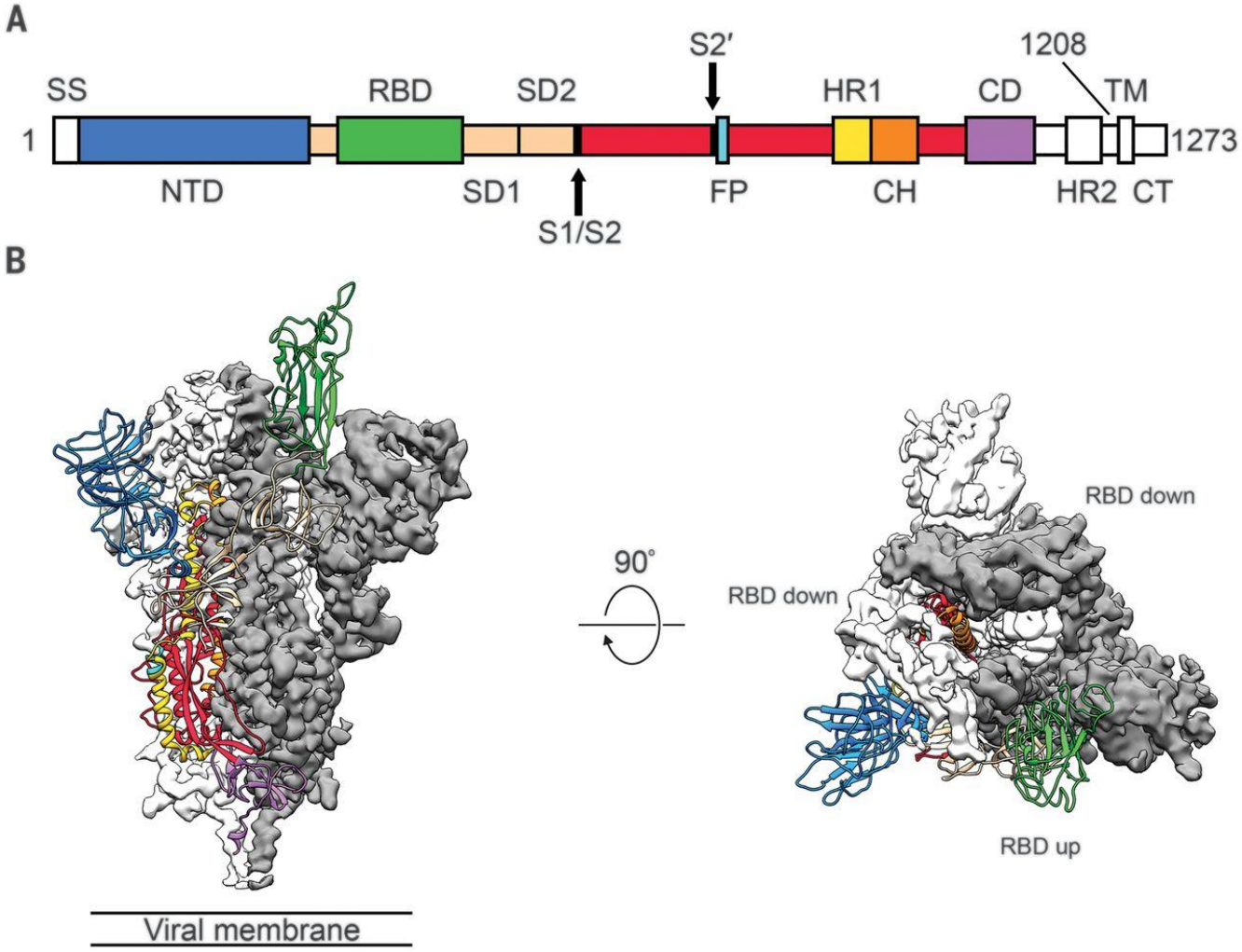
*Protein sample was kindly provided by Professor Smita Mohanty, Oklahoma State University.





https://www.protein-nmr.org.uk/solution-nmr/spectrum-descriptions/hncoca/

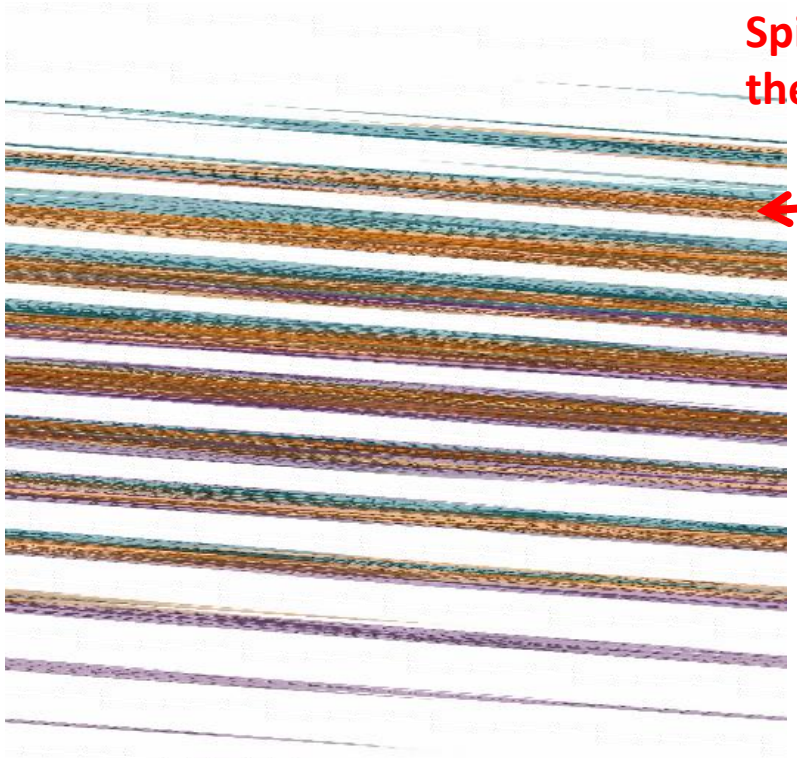
But… March 2020, in Science



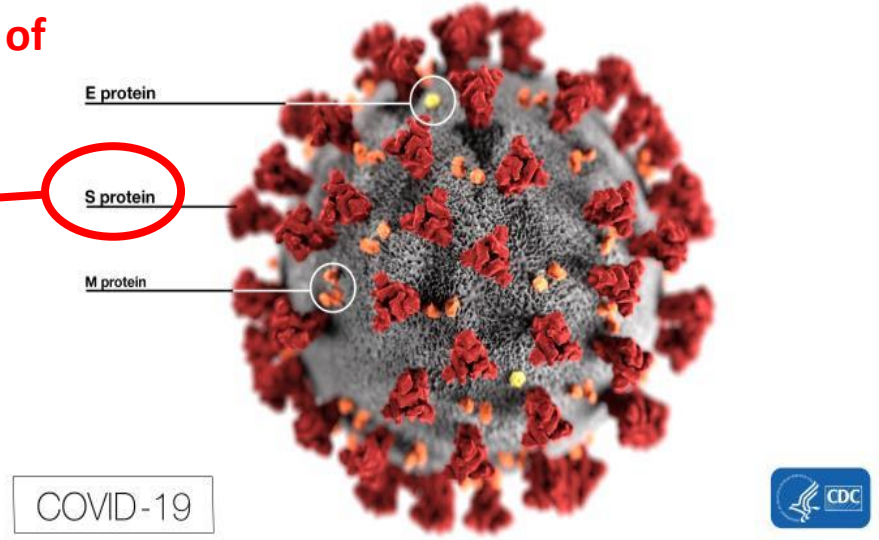
Cryo-EM enabled quick structure identification of spike protein of COVID-19
DOI: 10.1126/science.abb2507

X-Ray and cryo-EM are preferred for determining protein structures
NMR still main method to determine structure in disordered proteins

But… Molecular dynamics
(simulation with cryo-EM and SAXS data & Folding@Home)



Spike protein of the COVID-19



https://blogs.plos.org/dnascience/files/2020/02/ESM-proteins.jpg

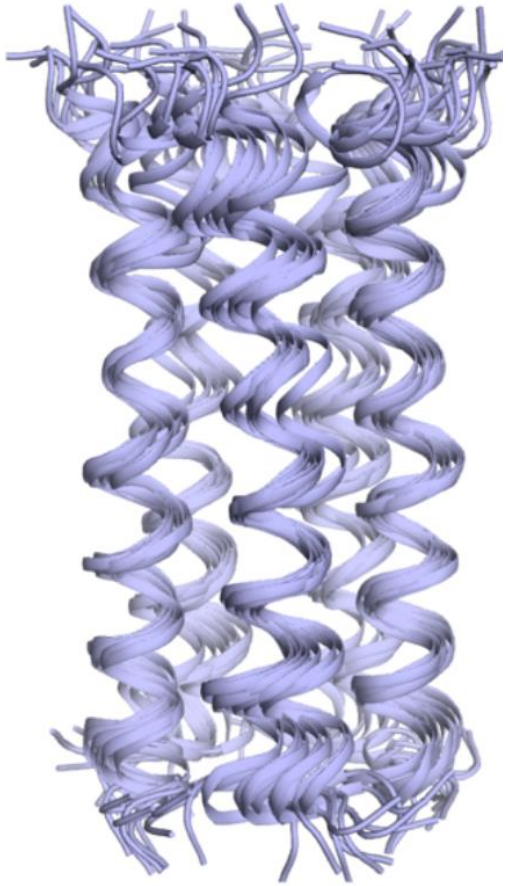
Greg Bowman @drGregBowman · Apr 3
Replying to @drGregBowman
The three colors are the three proteins that come together to form the spike. Each is made of a linear chain of chemicals called amino acids. The ribbons trace out each chain. The transparent surface is the surface of the spike.
2 9 92

Greg Bowman @drGregBowman · Apr 3
The three proteins that make up the #Demogorgon/spike must spread apart to reveal the ACE2 binding site, which initiates infection by attaching to a protein called ACE2 on the surface of human cells. This movie captures part of that opening motion.

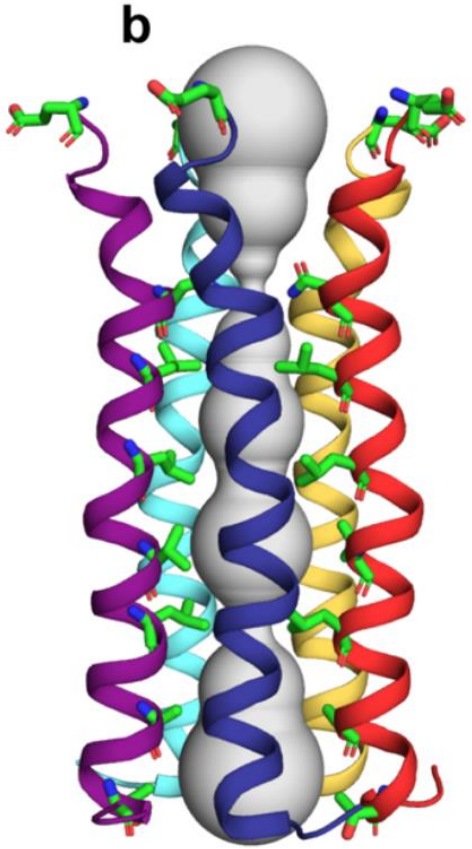
Greg Bowman @drGregBowman · Apr 3
The jitteriness of the movie isn't an artifact, its representative of the stochastic motions on this size scale (i.e. random motions with different probabilities)

https://twitter.com/drGregBowman/status/1246106316864708608

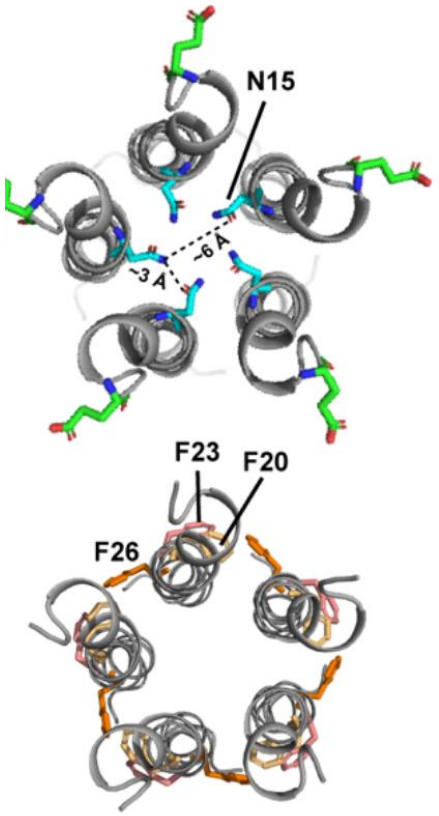
And… structure of the SARS-CoV-2 envelope protein TM domain via solid-state NMR (3.2mm and 1.9mm MAS)

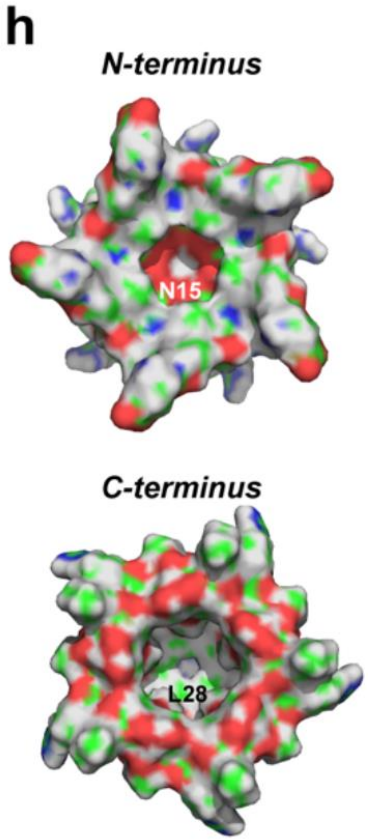


Ensemble of the ten lowest-energy structures



Sideview of the lowest-energy structure along with pore water(gray)

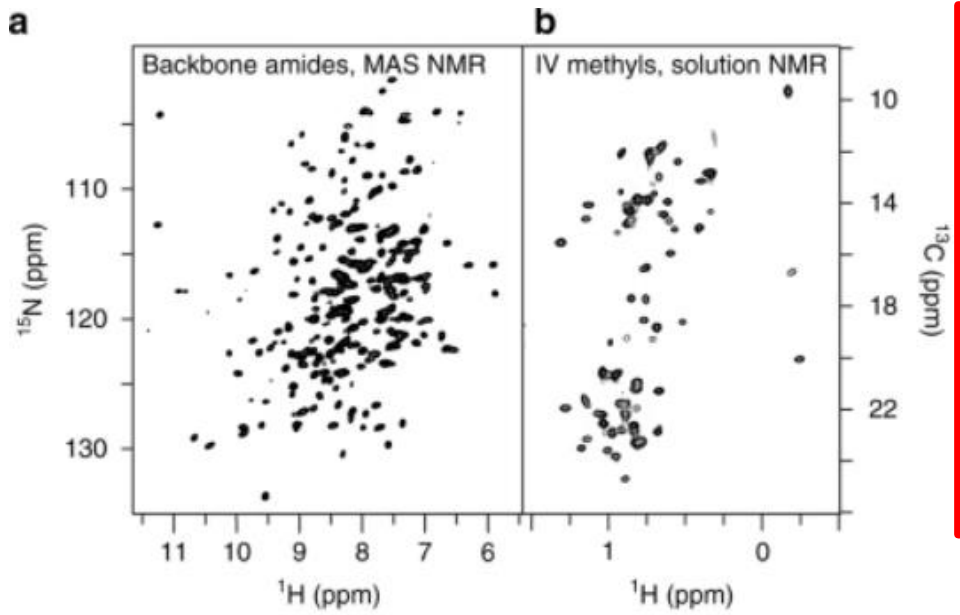


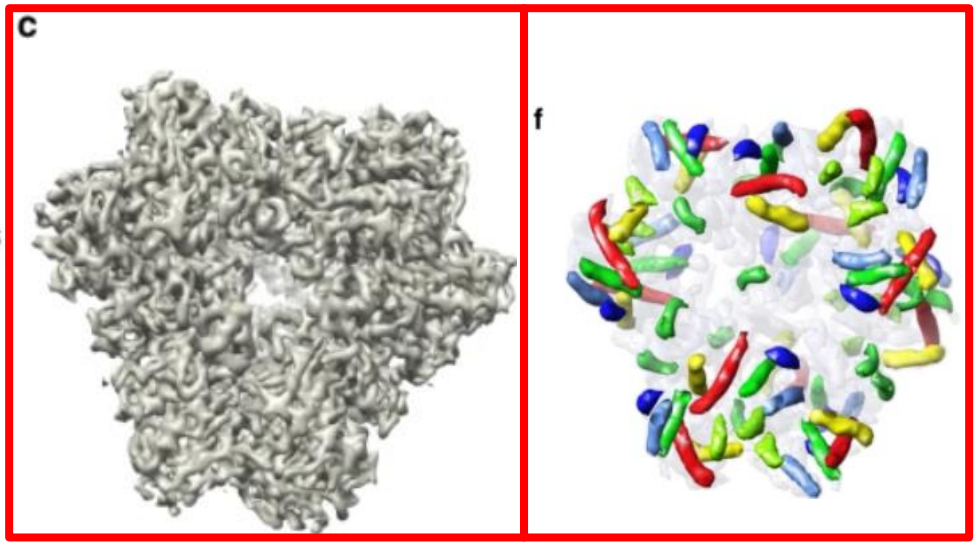


Top views of the **disordered** N-terminal E8and surface plots

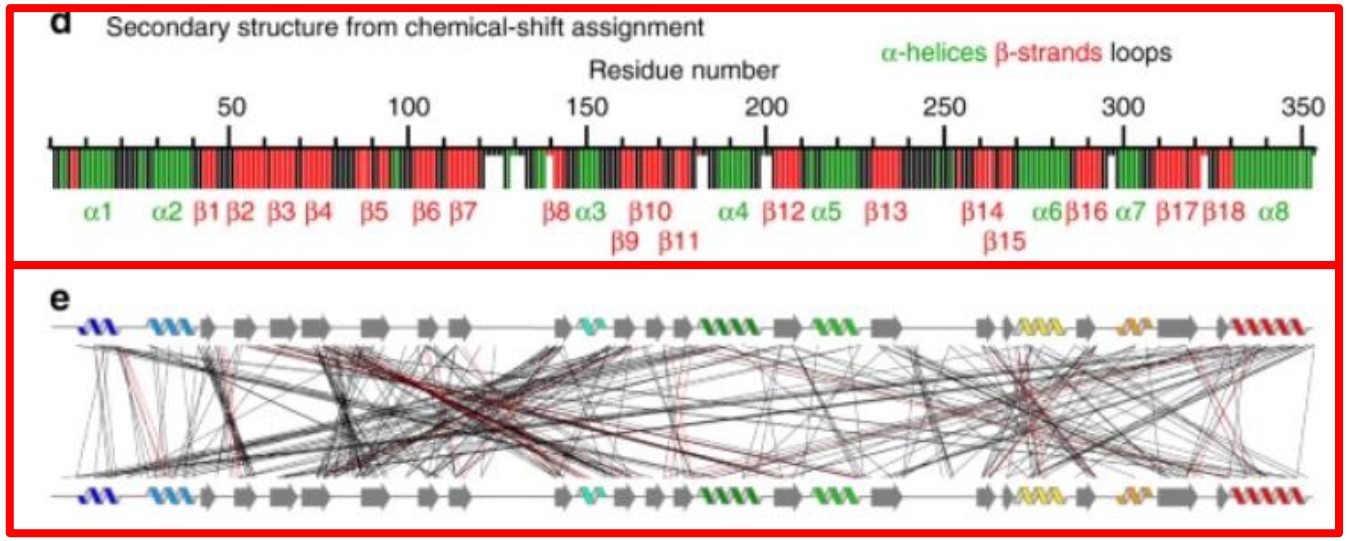
https://doi.org/10.21203/rs.3.rs-77124/v1

Structural determination of 468 kDa TET2 with NMR and cryo-EM



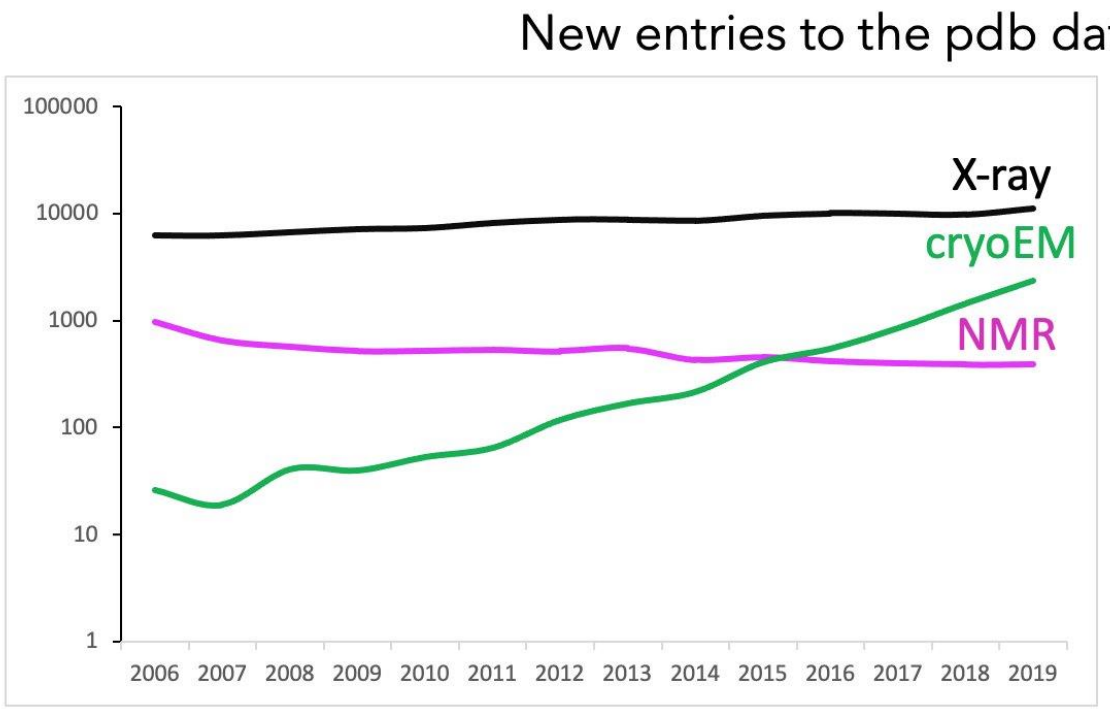


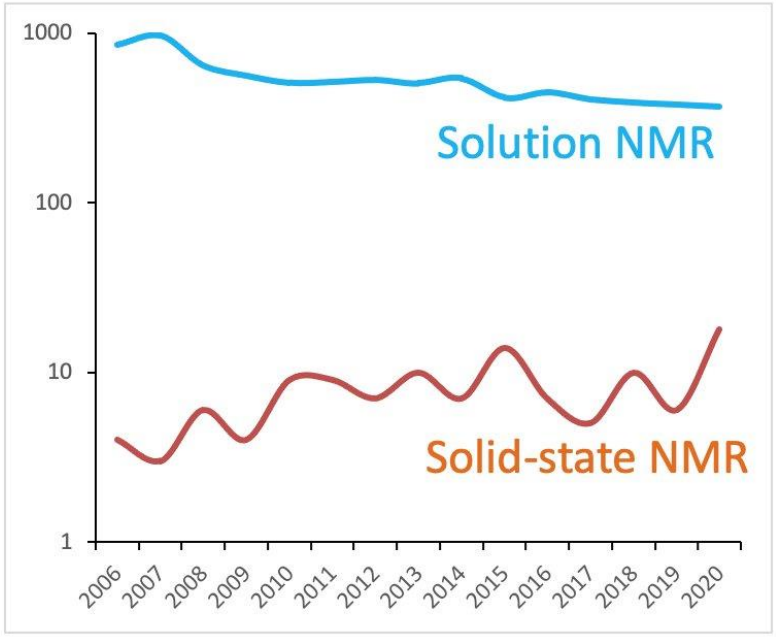
https://doi.org/10.1038/s41467-019-10490-9

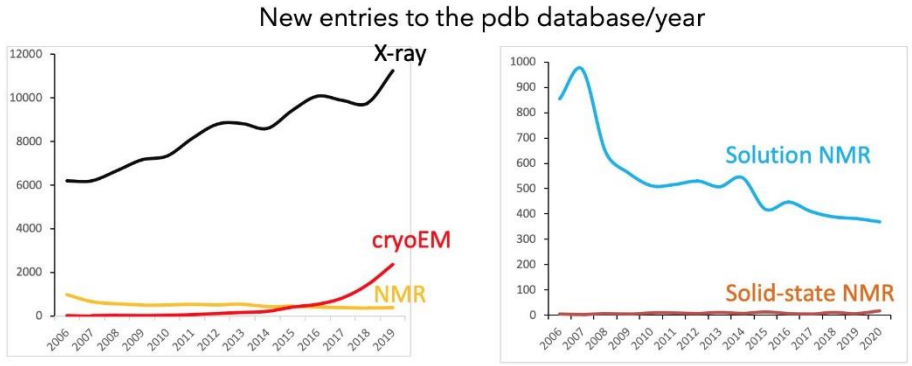


Tertiary structures can now also be predicted from primary structure https://deepmind.com/blog/article/alphafold-a-solution-to-a-50-year-old-grand-challenge-in-biology

Protein structures submitted to pdb







Linear scale

https://twitter.com/MarkusWeingarth/status/1443299113026654215

In-cell NMR

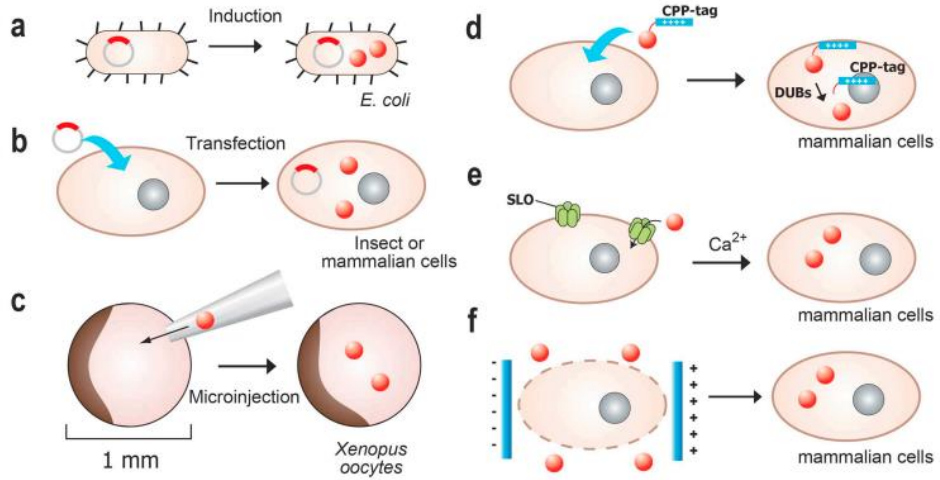
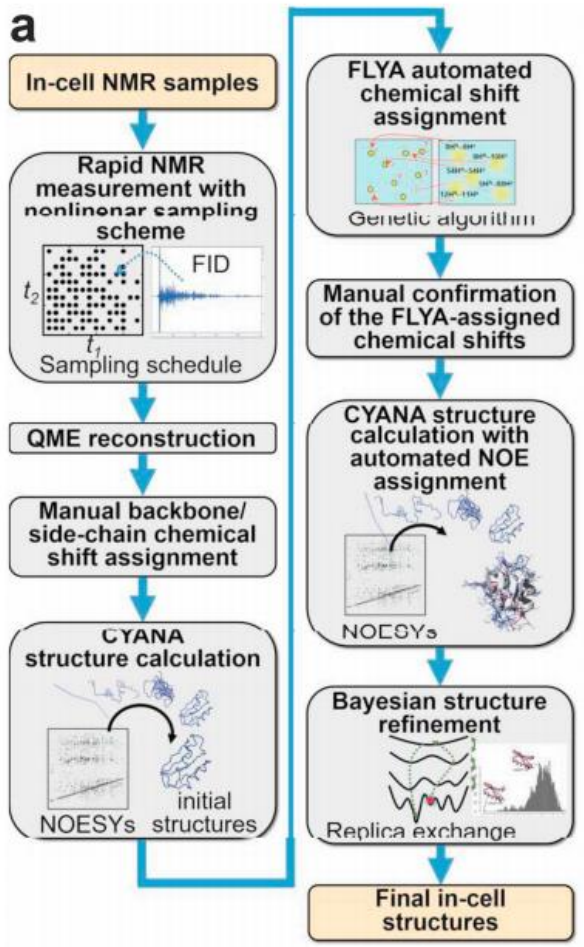


Fig. 1. Overview of the methods to prepare the cells enriched with isotope-labeled proteins. (a–b) Overexpression of target proteins within the cells for (a) bacterial cells and (b) eukaryotic cells, such as insect cells or mammalian cells. Cells were cultured in the medium containing the isotope enriched medium after IPTG induction or transfections of expression vectors. (c) The target proteins or nucleic acids were injected into *X. laevis* oocytes by microinjection. (d) The arginine-rich cell-penetrating peptide (CPP) guide the translocation of the target protein (Ub) across the plasma membrane. The CPP tag, which contains a positive charge cluster, tends to stick to a negatively charged surface, such as a plasma membrane. Therefore, the release of the target protein from the CPP tag by endogenous DUBs is necessary for NMR observation. (e) The target proteins are introduced into the mammalian cells through the pores formed by SLO. (f) The cells are permeabilized by electroporation to transduce the target proteins.

https://doi.org/10.1039/C3MT00224A



Chemical shift perturbation

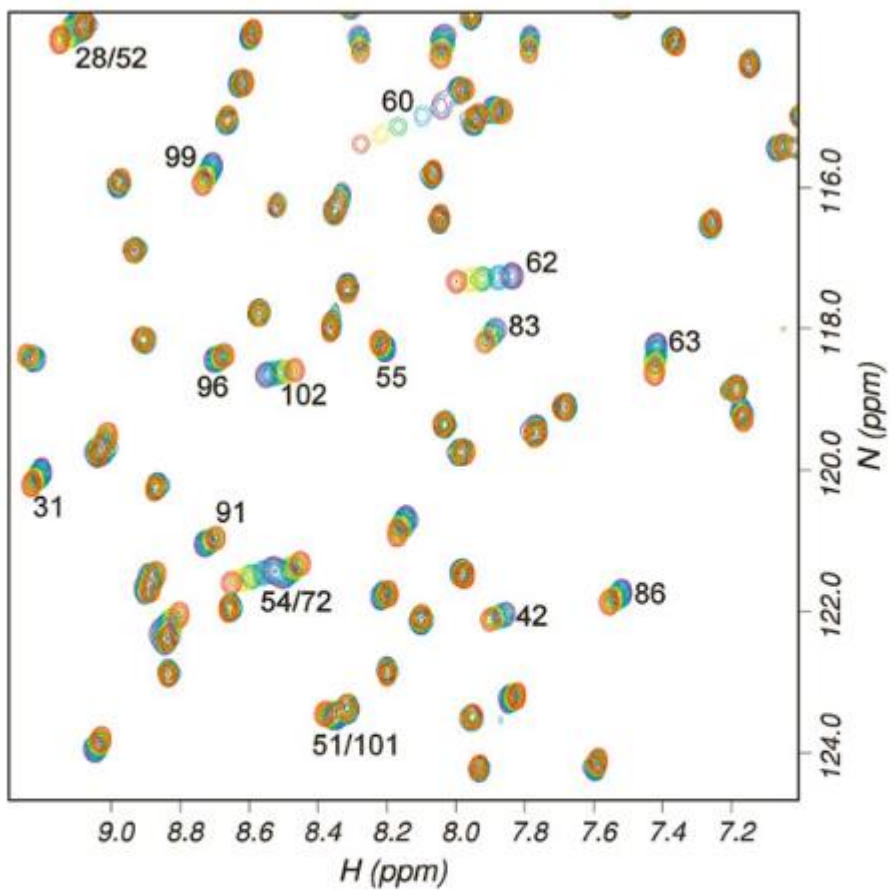


Fig. 1 Part of a ^{1}H, ^{15}N HSQC spectrum of the ribonuclease barnase. Each peak is the ^{1}H, ^{15}N correlation for a different amino acid residue. The figure shows a superposition of HSQC spectra with addition of increasing amounts of the deoxynucleotide ligand d(GC), going from *red* (no addition) to *blue*. Protein concentration is 200 μM, and ligand concentration goes from zero to a 4.5-fold excess. Peaks that shift are numbered by the amino acid (From Ref. [29], with permission from Springer Science + Business)

https://doi.org/10.1007/978-3-319-28388-3_76