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Characterizing Biologics using *w*NMR

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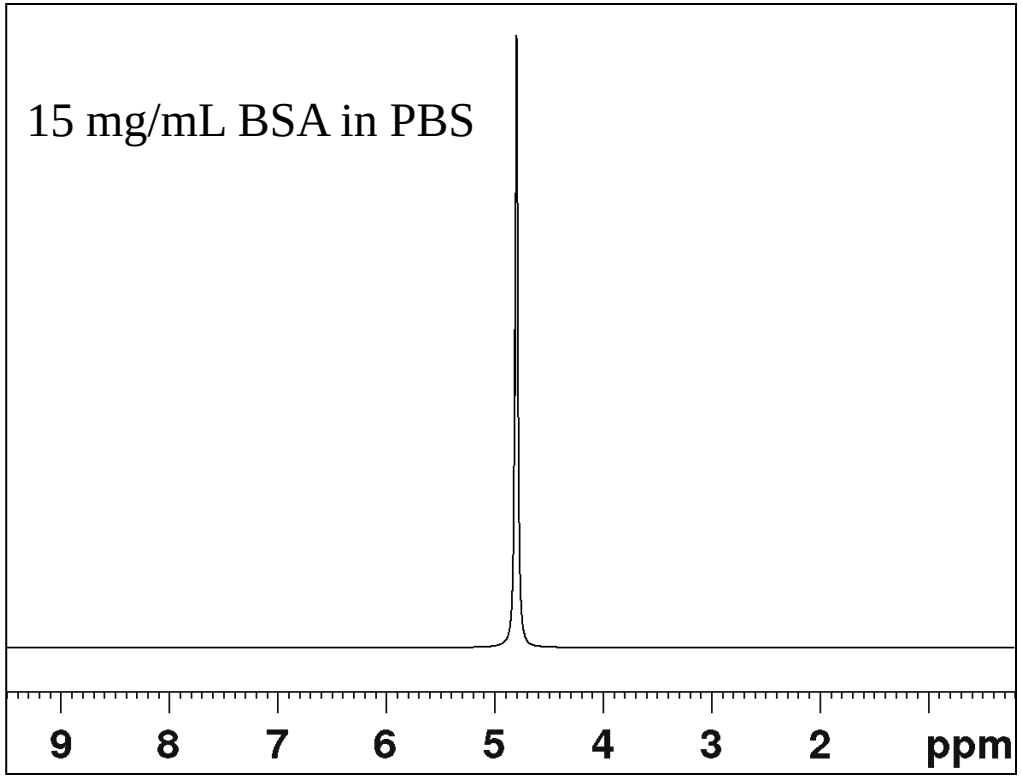
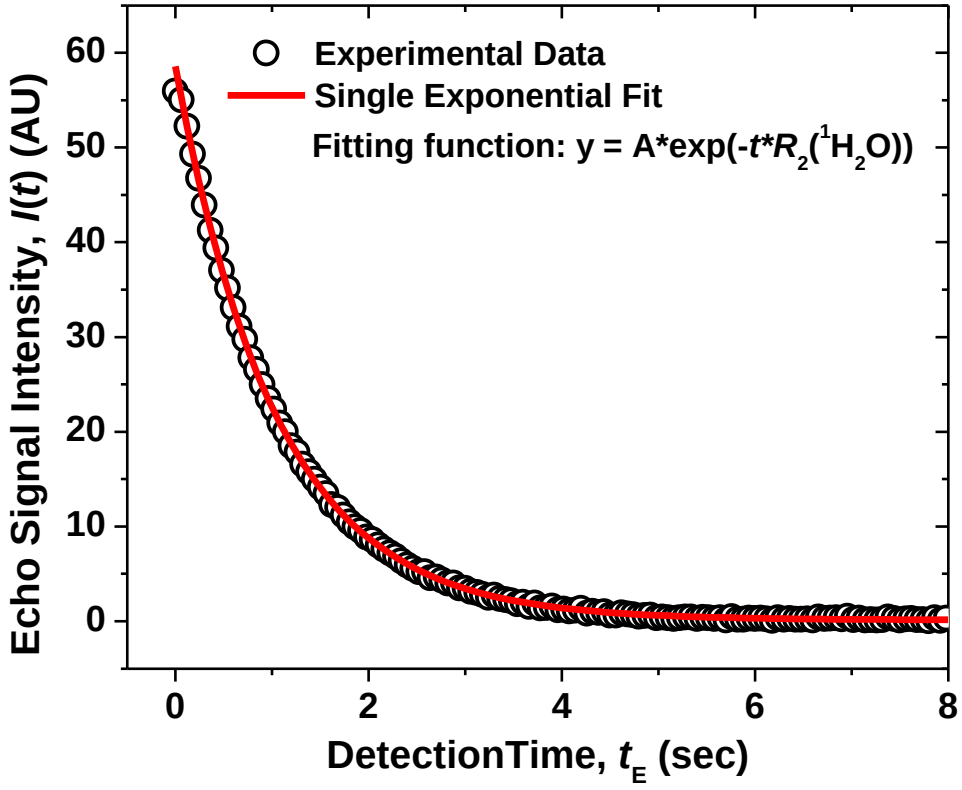
*w*NMR: Water as a Sensor and Amplifier in a Magnetic Field

frequency domain: single peak



time domain: single exponential decay



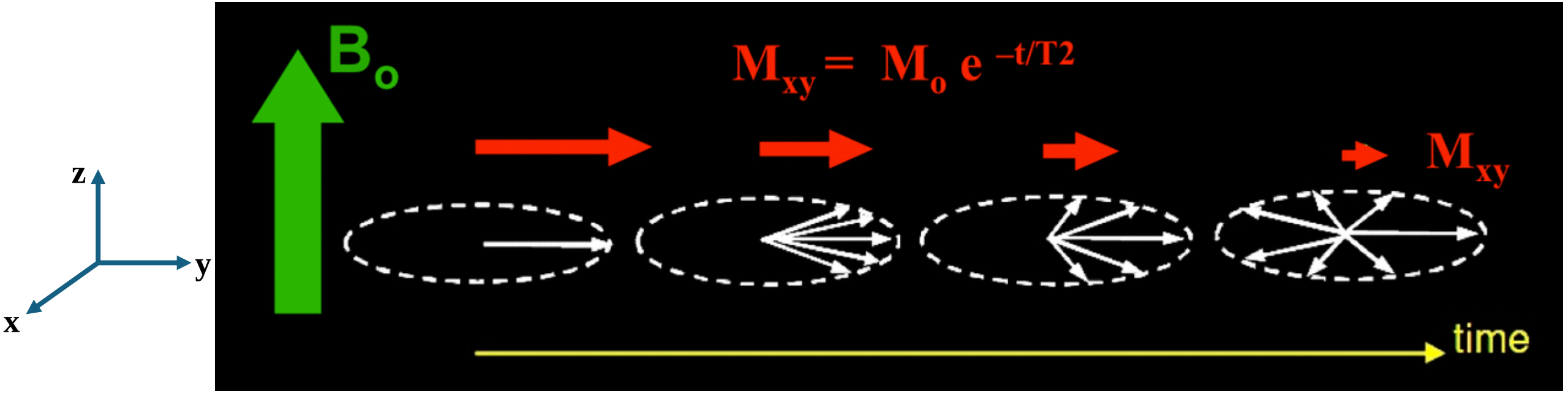
$^{1}H_2O$ molecules are tiny magnets

$^{1}H_2O$ interacts with solutes → sensor

$^{1}H_2O$ far outnumbers solutes → amplifier

Transverse Relaxation in NMR

– diminution of magnetic moment in the **xy** plane after a 90°-pulse



https://mriquestions.com/what-is-t2.html

$$R_2 = 1/T_2 \ (s^{-1})$$

(a dynamic property; does not depend the *absolute* NMR signal intensity)

Univariate vs. Multivariate *w*NMR

Univariate *w*NMR

Single $R_2(^1H_2O)$: timely decision making (product inspection, process monitoring, etc.).

Multivariate *w*NMR

$R_2(^1H_2O, x, y, \ldots)$: in-depth characterization (product fingerprinting, process understanding, etc.).

- Type I multivariate *w*NMR: $R_2(^1H_2O, \tau)$, τ is a pulse sequence parameter
- Type II multivariate *w*NMR: $R_2(^1H_2O, t)$, t is time
- Type III multivariate *w*NMR: $R_2(^1H_2O, \tau, t)$
- Other types possible

Time-domain multivariate NMR is analogous to frequency-domain multi-dimensional NMR

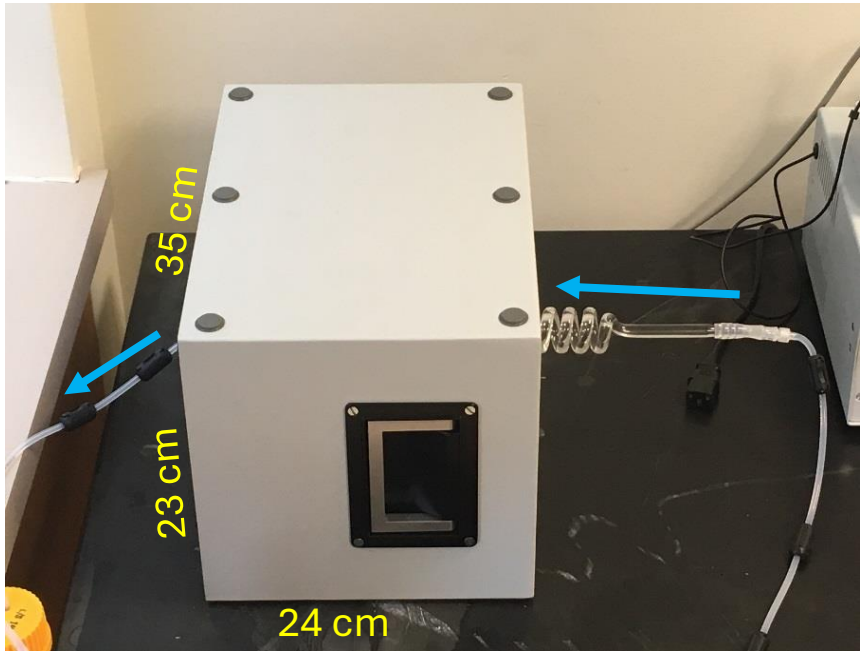
Why NMR but not IR or Raman?

	energy (Hz)	lifetime (s)
IR, Raman:	10^{14}	10^{-12}
NMR:	10^{8} (sensitive)	10^{0} (long memory)

Instrumentation for *w*NMR

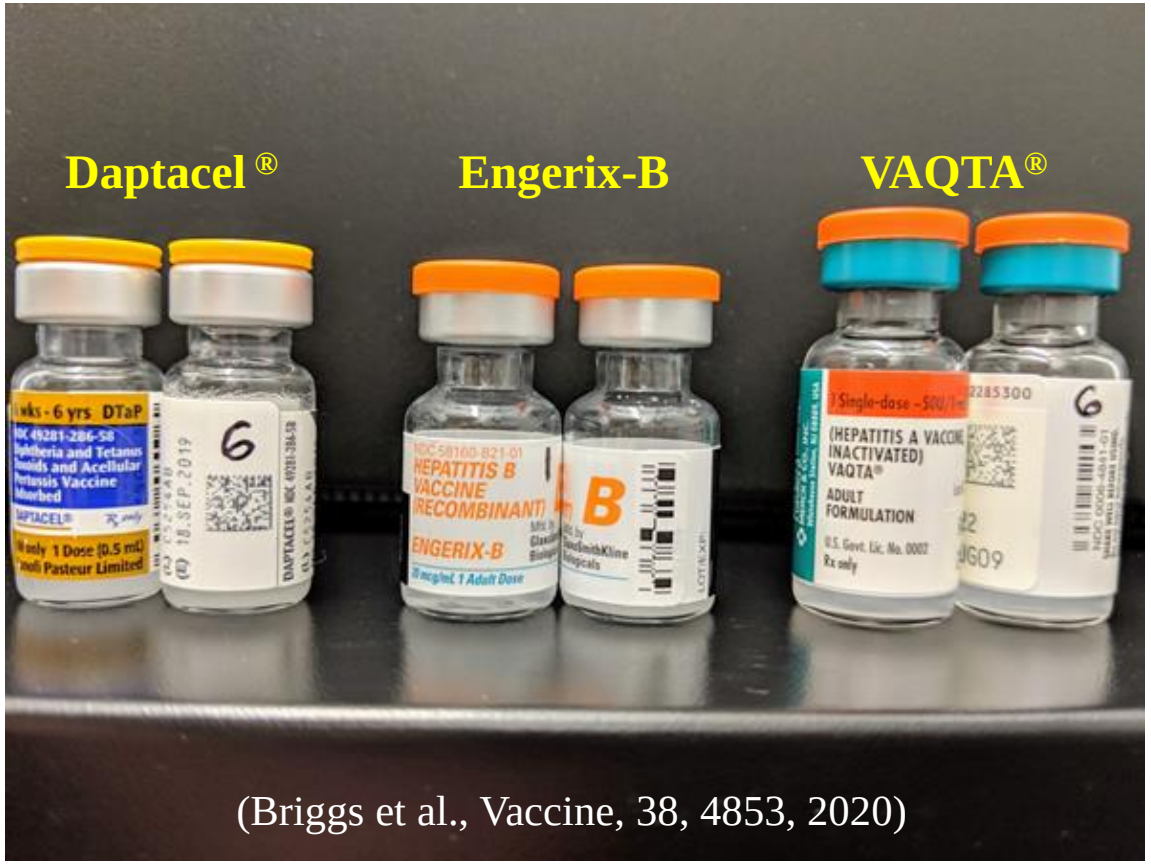
benchtop NMR instrument
(for product inspection)

benchtop flow NMR instrument
(for process monitoring)

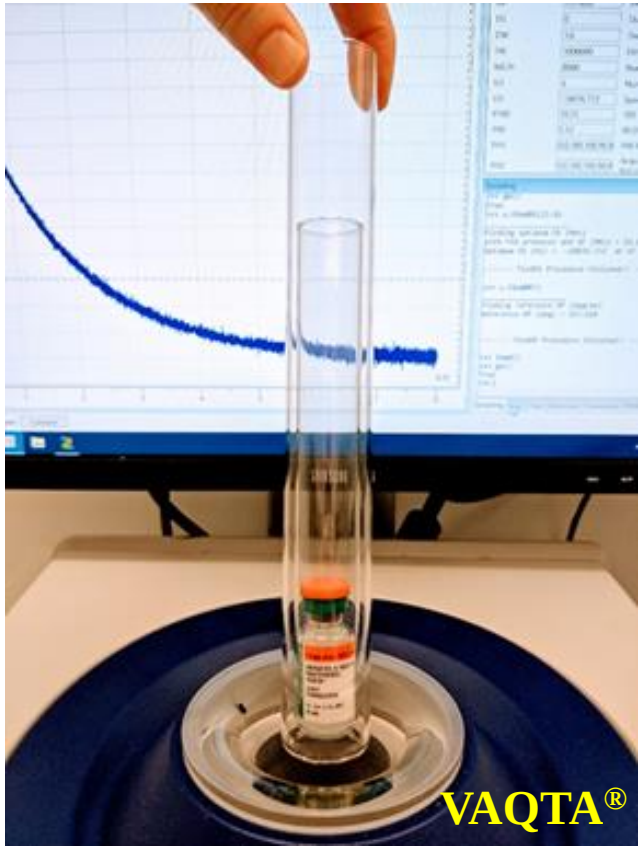


affordable, portable, robust, easy to operate

*w*NMR for Noninvasive Product Inspection



(Briggs et al., Vaccine, 38, 4853, 2020)



Different products, same solvent (^1H_2O)

analytical chemistry

pubs.acs.org/ac

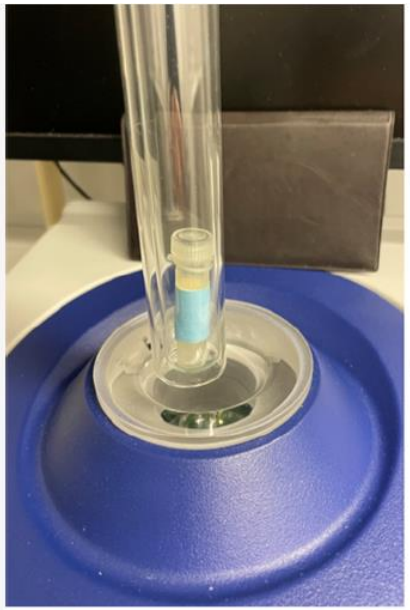
Letter

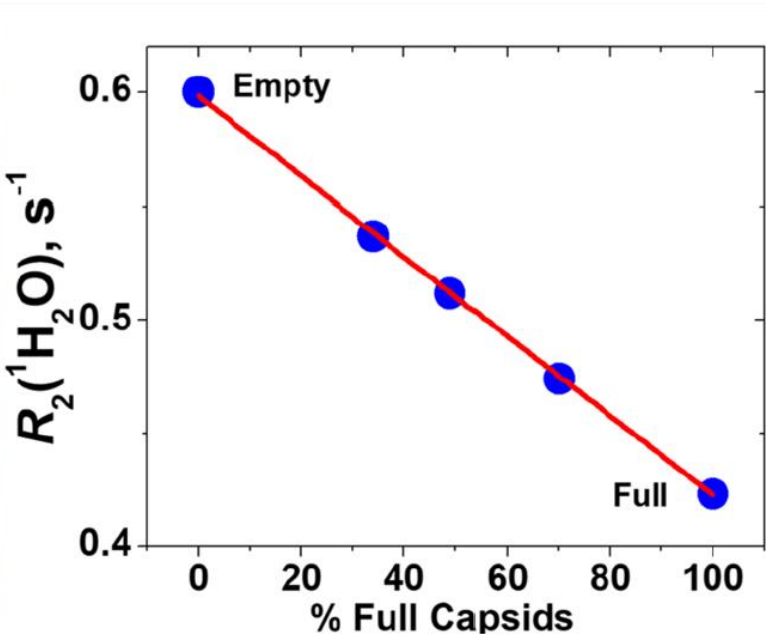
Rapid and Noninvasive Quantification of Capsid Gene Filling Level Using Water Proton Nuclear Magnetic Resonance

Marc B. Taraban, Michael T. Jones, and Yihua Bruce Yu*

Cite This: *Anal. Chem.* 2021, 93, 15816−15820

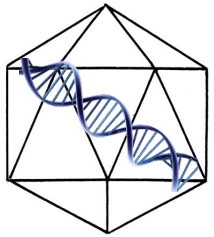
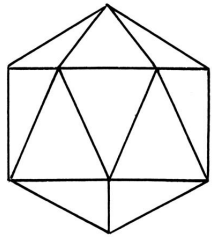
Read Online





- DNA inside protein capsids (☑)
- mRNA inside LNPs (ongoing)

Full vs. Empty Capsids in Gene Therapy Products

full capsid   empty capsid

- API of gene therapy products: DNA inside protein capsid
- Actual products: mixture of full and empty capsids
- Full% = *C*(full capsid)/[*C*(full capsid) + *C*(empty capsid)] × 100%
- Full% is a critical quality attribute (CQA) of gene therapy products

Invasive Methods for Full vs. Empty Capsids

- **AUC**, IEX-HPLC, Cryo-EM, qPCR/ELISA, CE…
- All invasive
- All involve some sample preparation/perturbation
- Most involve reagents
- Samples are consumed after measurement
- Time consuming (hours or more)

Noninvasive Characterization of Gene Therapy Capsids

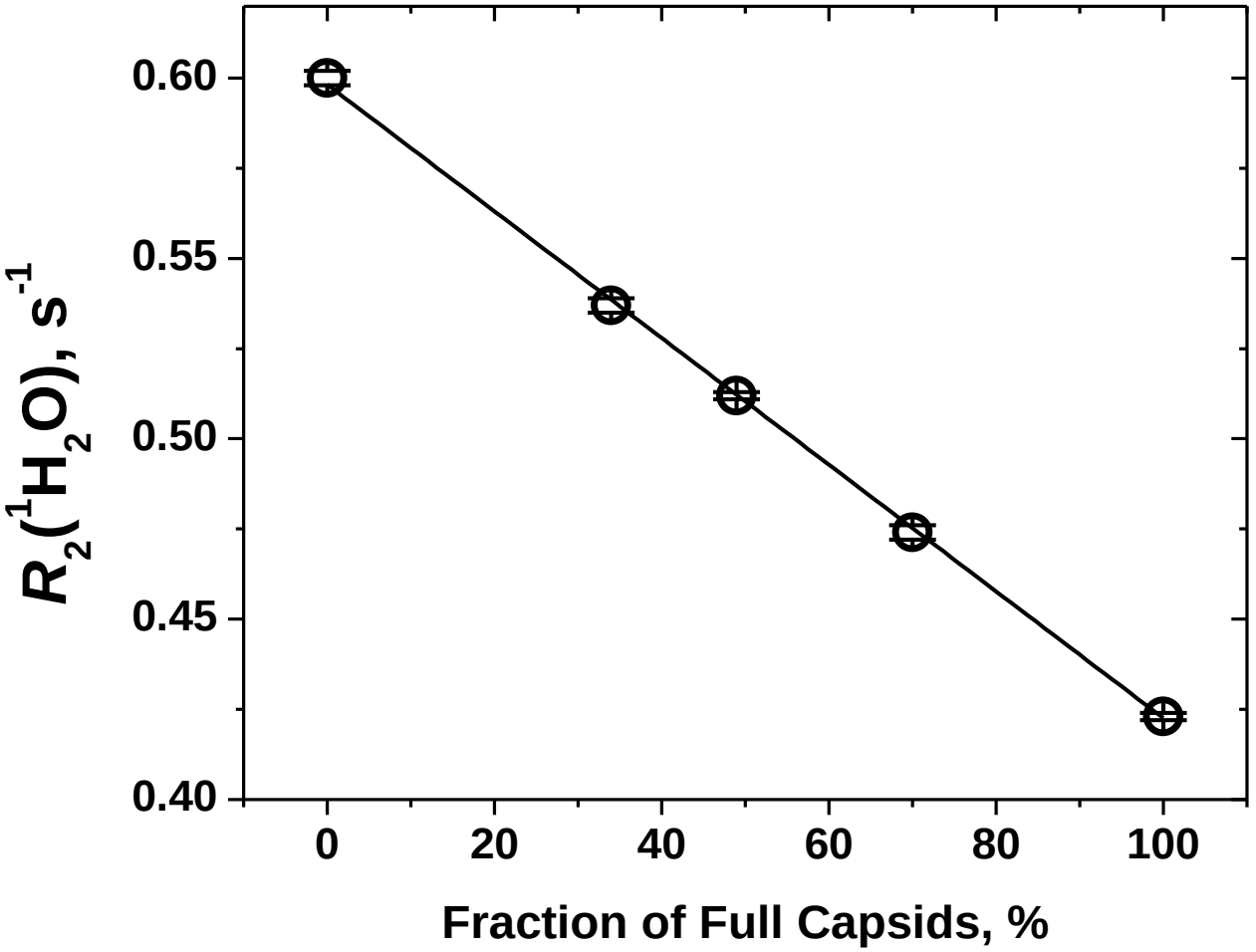
– full vs. empty capsids

Sample	Concentration (vp/mL)	$T_2(^1H_2O)$ (s)	$R_2(^1H_2O)$ (s^{-1})
Empty Capsid (AAV9-empty)	2.01×10^{13}	1.642 ± 0.008	0.609 ± 0.003
Full Capsid (AAV9-GFP, portion 1)	2.40×10^{13}	2.300 ± 0.009	0.435 ± 0.002
Full Capsid (AAV9-GFP, portion 2)	2.40×10^{13}	2.317 ± 0.008	0.432 ± 0.002

(Taraban, Anal. Chem. 93, 15816, 2021)

Noninvasive Characterization of Gene Therapy Capsids

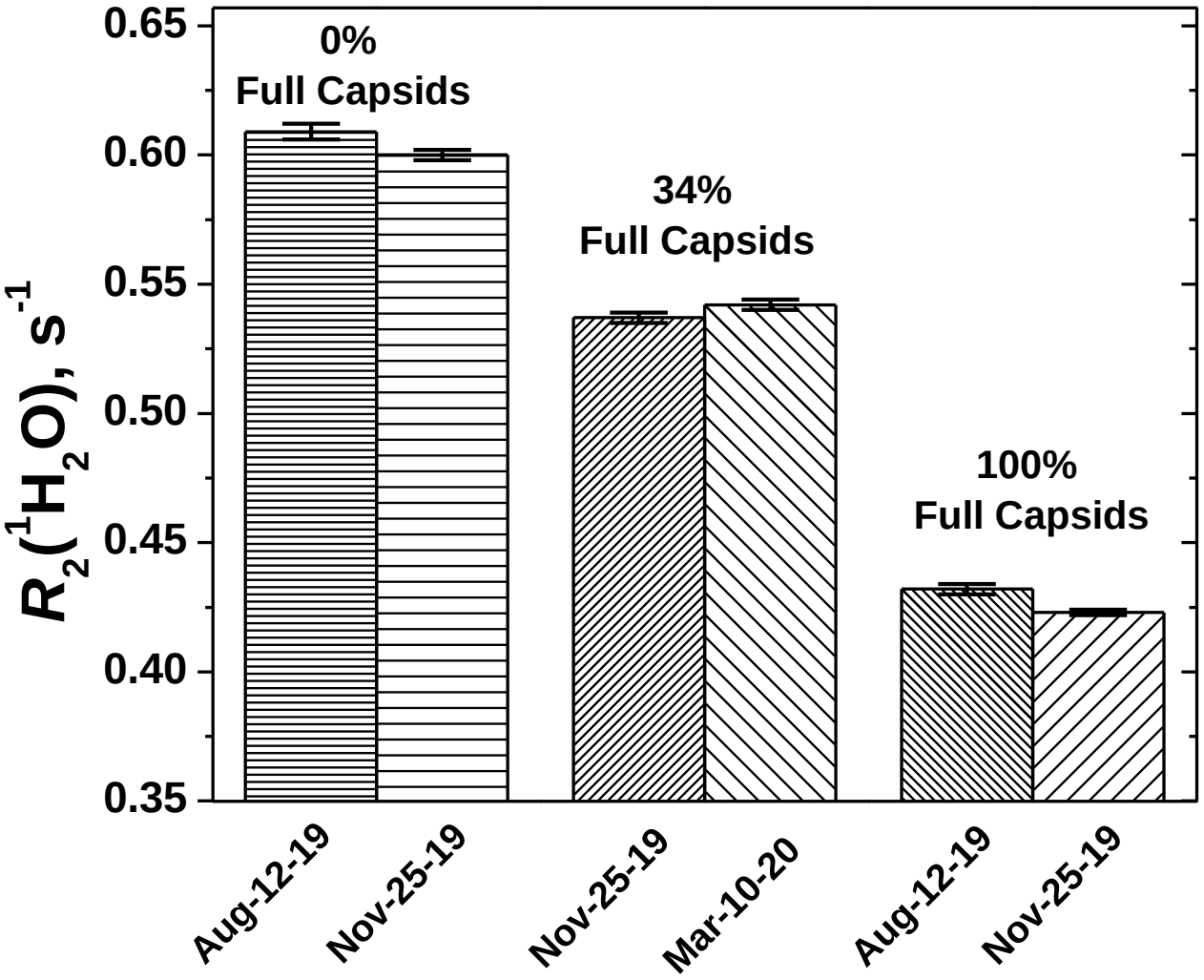
– Full% in a mixture



- Noninvasive (sealed vials)
- No sample preparation
- No reagents
- Sample NOT consumed
- Fast:1-2 min/sample

Noninvasive Characterization of Gene Therapy Capsids

– product stability over time

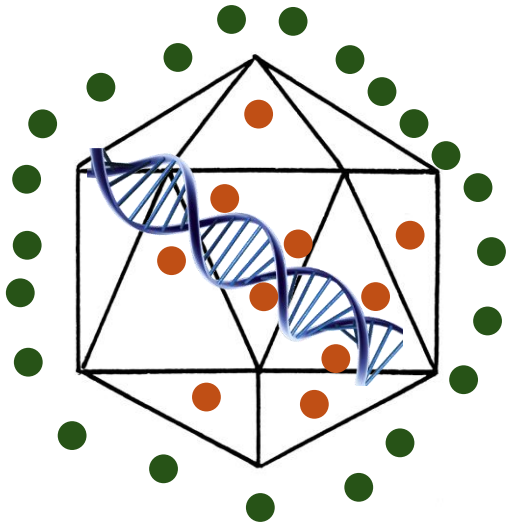


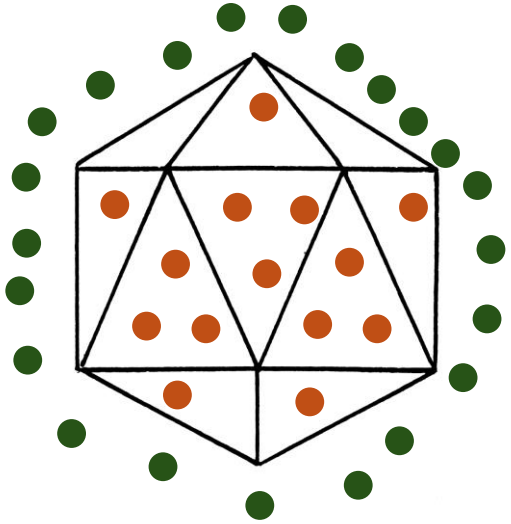
The same vial can be measured repeatedly over time (longitudinal stability monitoring)

Why Full Capsids have Lower $R_2(^1H_2O)$ than Empty Capsids

– proposed mechanism

full capsid

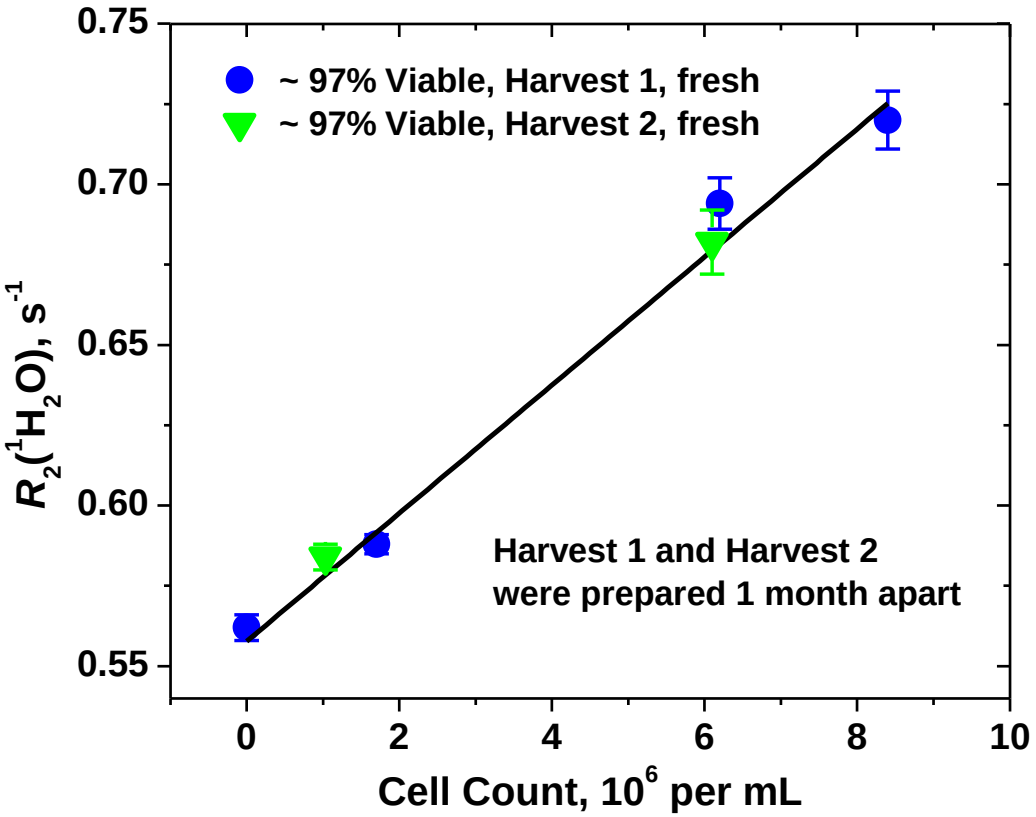


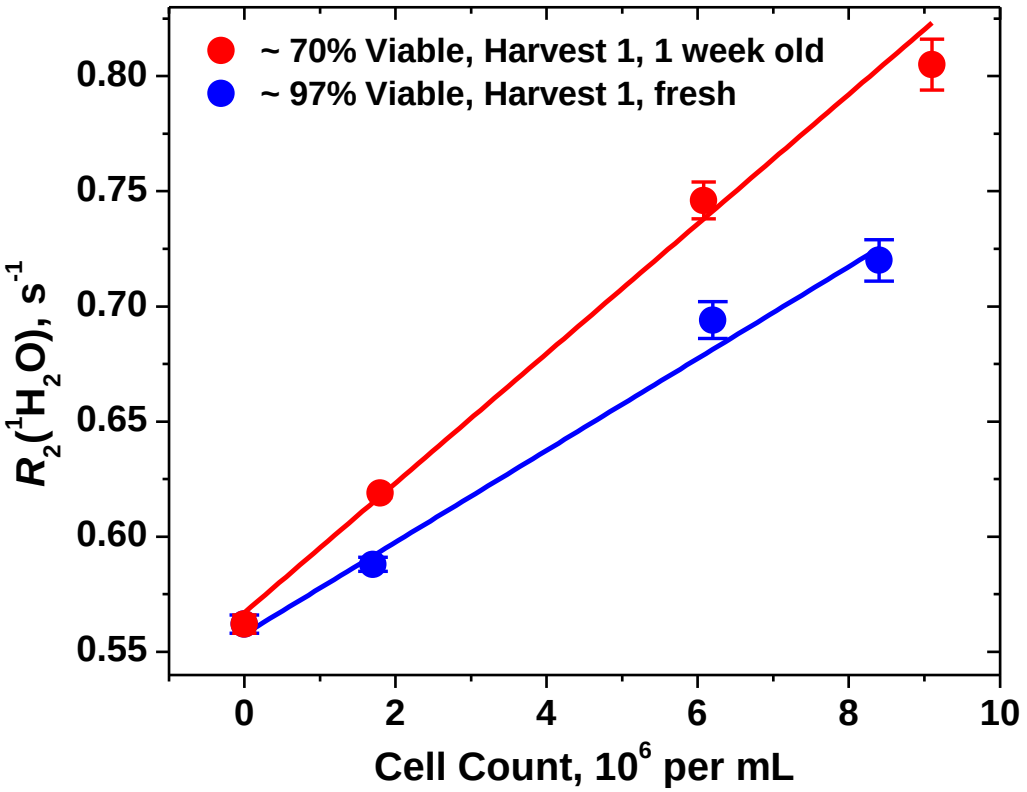


empty capsid

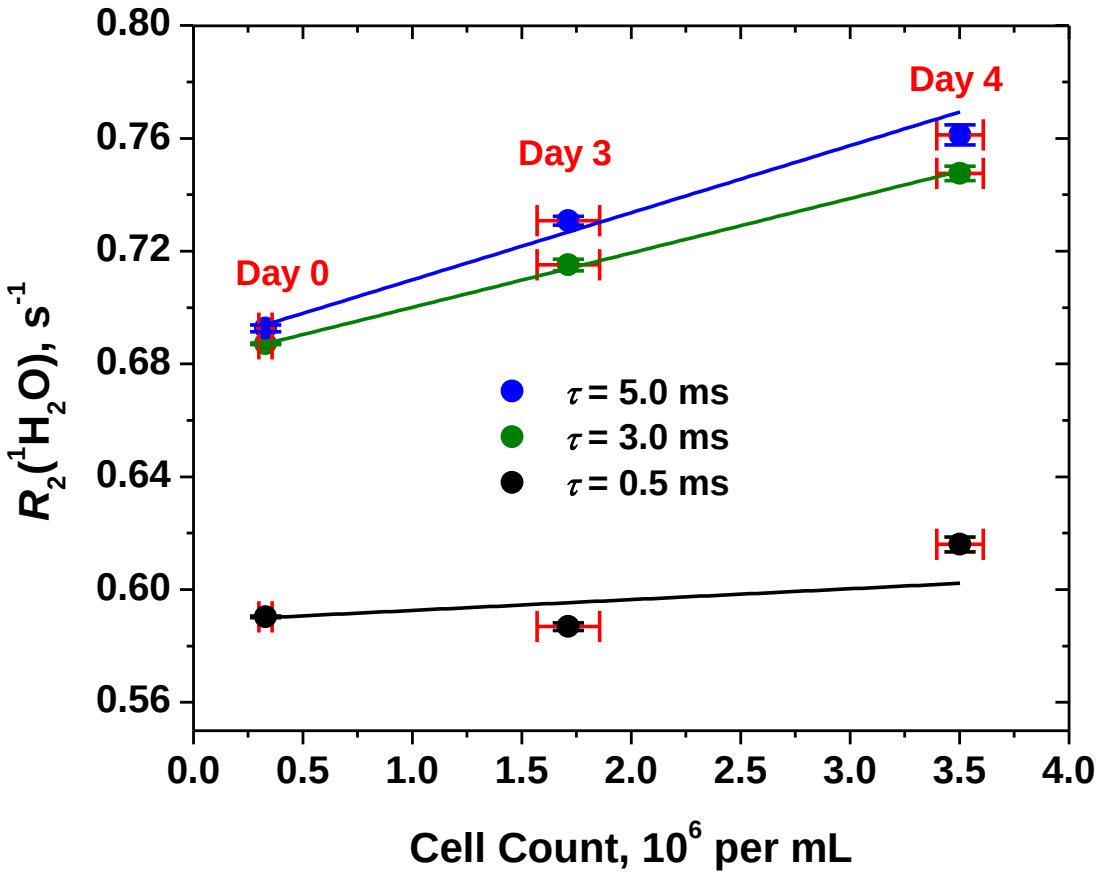
- External (●) and internal (●) water experience different magnetic environments
- Exchange between external and internal water elevates $R_2(^1H_2O)$
- Less water exchange in full capsids because: less water, and water is less mobile

Cell Count and Viability (NISTCHO cells)



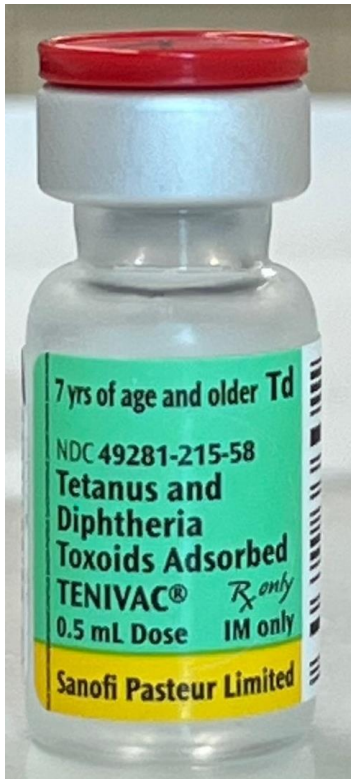


Monitoring Cell Growth (NISTCHO cells)

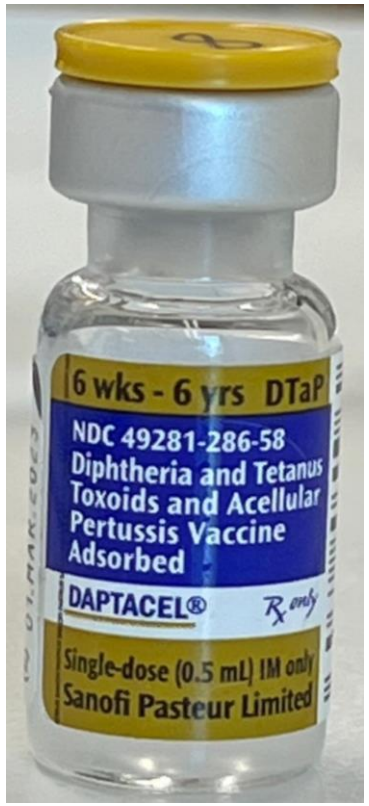


Sensitivity (slope) can be increased by adjusting τ (NMR pulse sequence parameter)

Vaccines: Tenivac® vs. Daptacel®

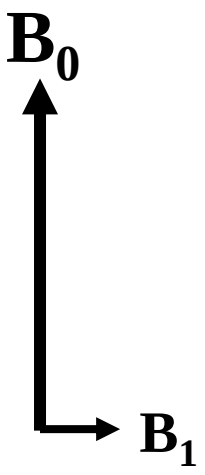


- Both made by Sanofi
- Both contain aluminum phosphate as adjuvant (0.66 mg/mL of Al(III))
- Both single-dose vial of 0.5 mL
- Tenivac® against tetanus, diphtheria
  Dapacel® against tetanus, diphtheria, pertussis

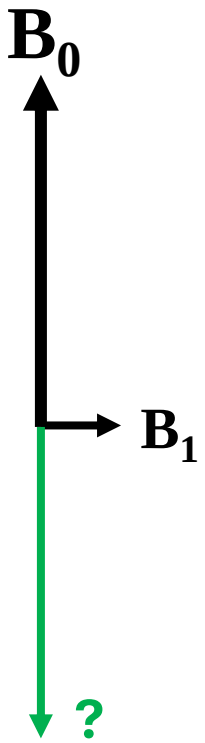


Can *w*NMR distinguish them without opening the vials?

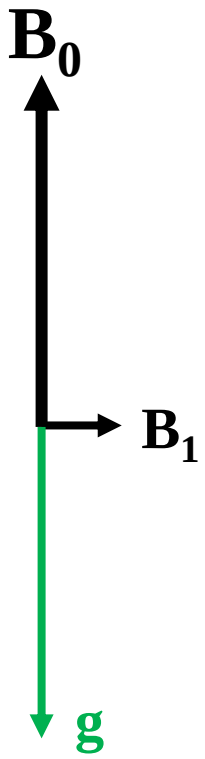
Back to NMR basics and Physics



Conventional NMR involves two magnetic fields, $\mathbf{B_0}$ and $\mathbf{B_1}$.
They are vector fields.
Vector fields induce polarization.



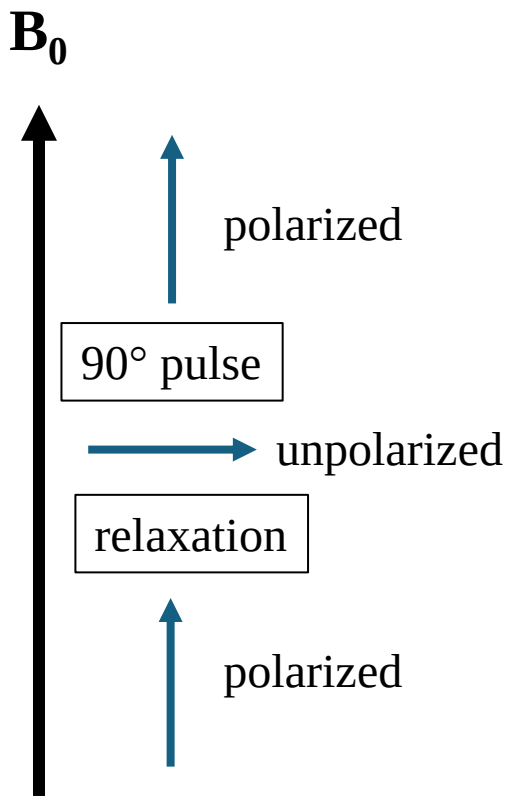
Add another vector field to induce additional polarization.
But what field?



The gravitational field!

Suspensions in Magnetic and Gravitational Fields

— interplay between two dynamic processes



magnetic field

$B_0 \leftrightarrow g$

polarized ↔ settled

90° pulse ↔ shaking

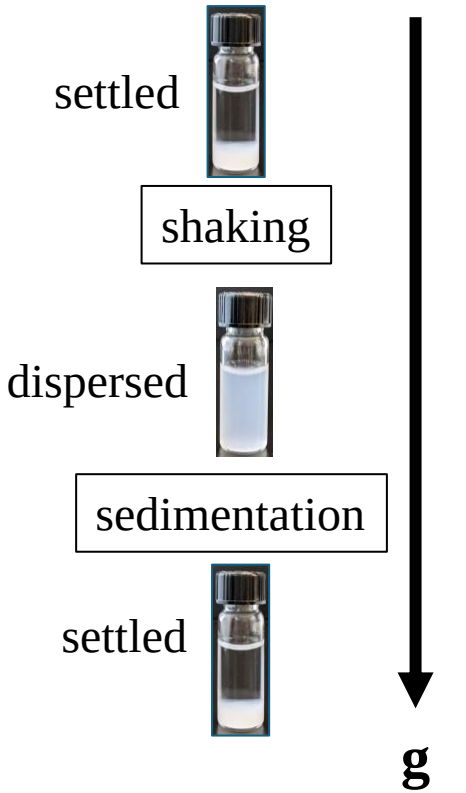
unpolarized ↔ dispersed

relaxation ↔ sedimentation

Interplay of two dynamic processes

^1H_2O relaxation time scale: ms to s

Particle sedimentation time scale: hr to day



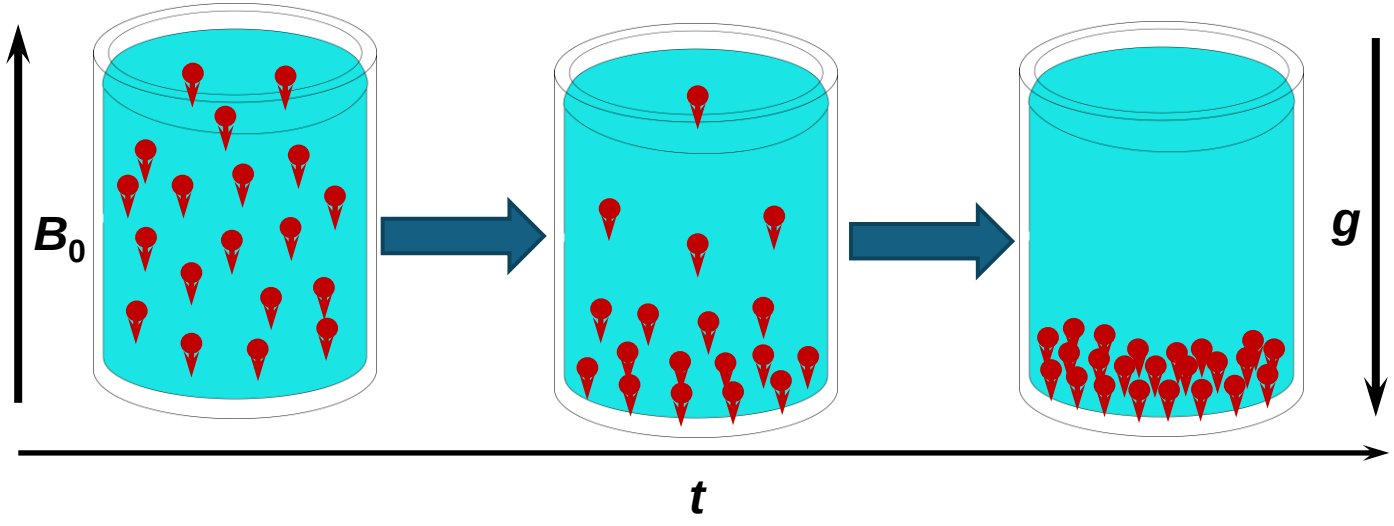
gravitational field

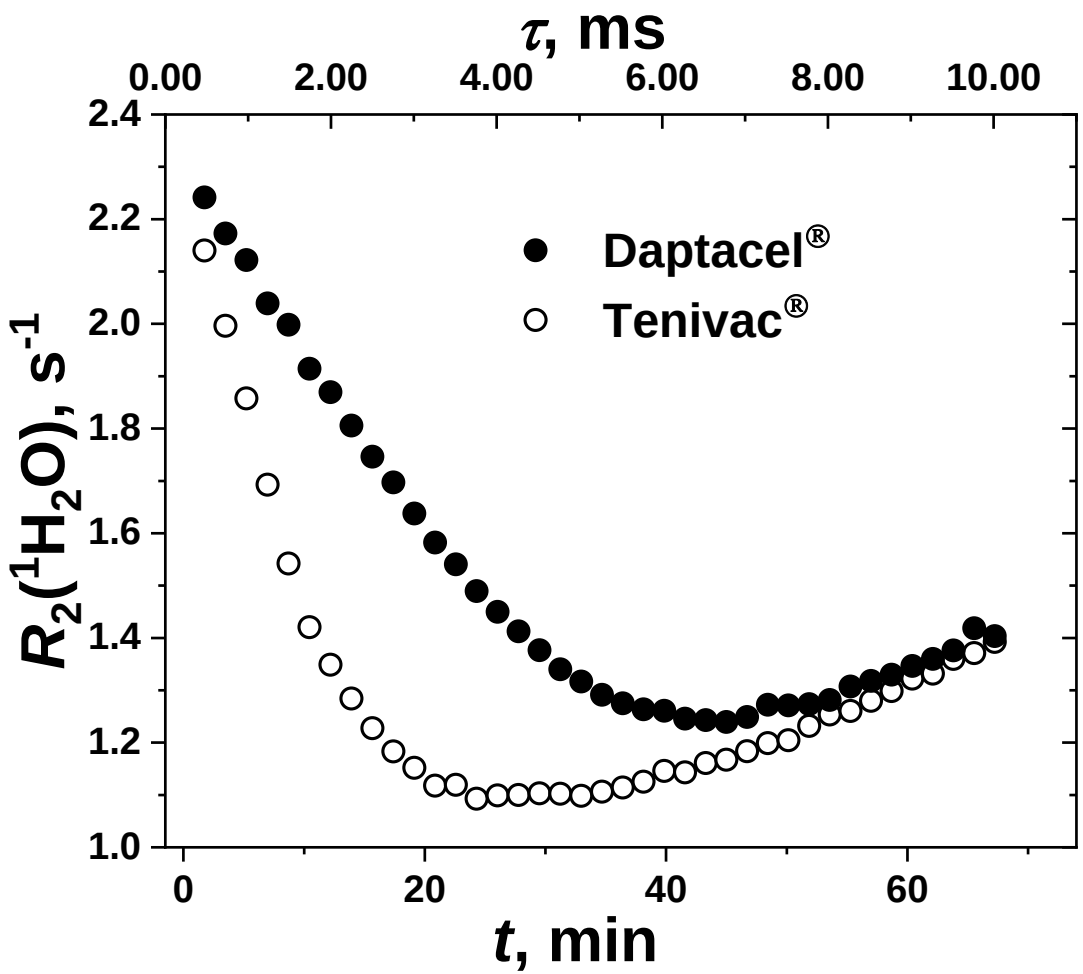
Water is already there, gravity is always there

Differentiating Tenivac® vs. Daptacel® by *w*NMR

– exploiting *dia*magnetic susceptibility contrast and gravity

An antigen-adjuvant microparticle in water is a micromagnetic moment that settles under gravity. Different antigen-adjuvant pairs settle differently, forming the basis for differentiation by *w*NMR.

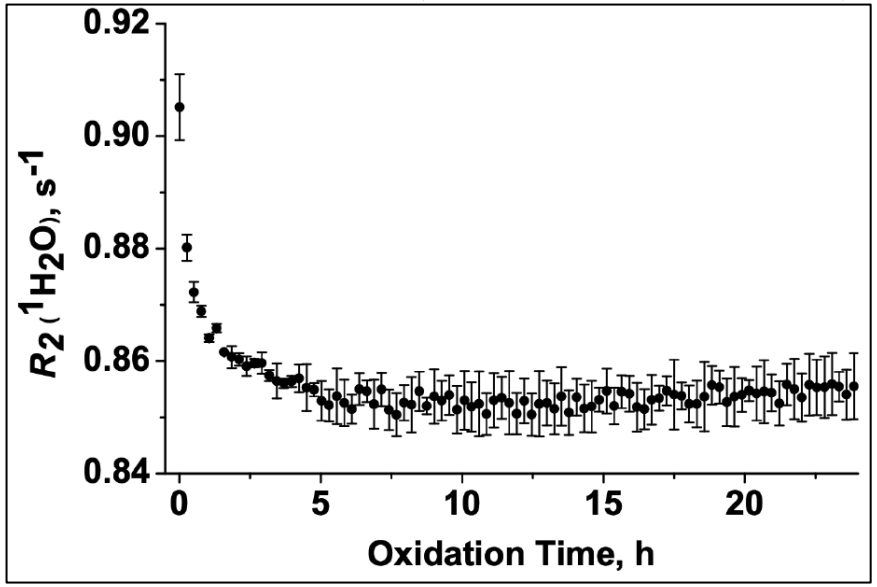




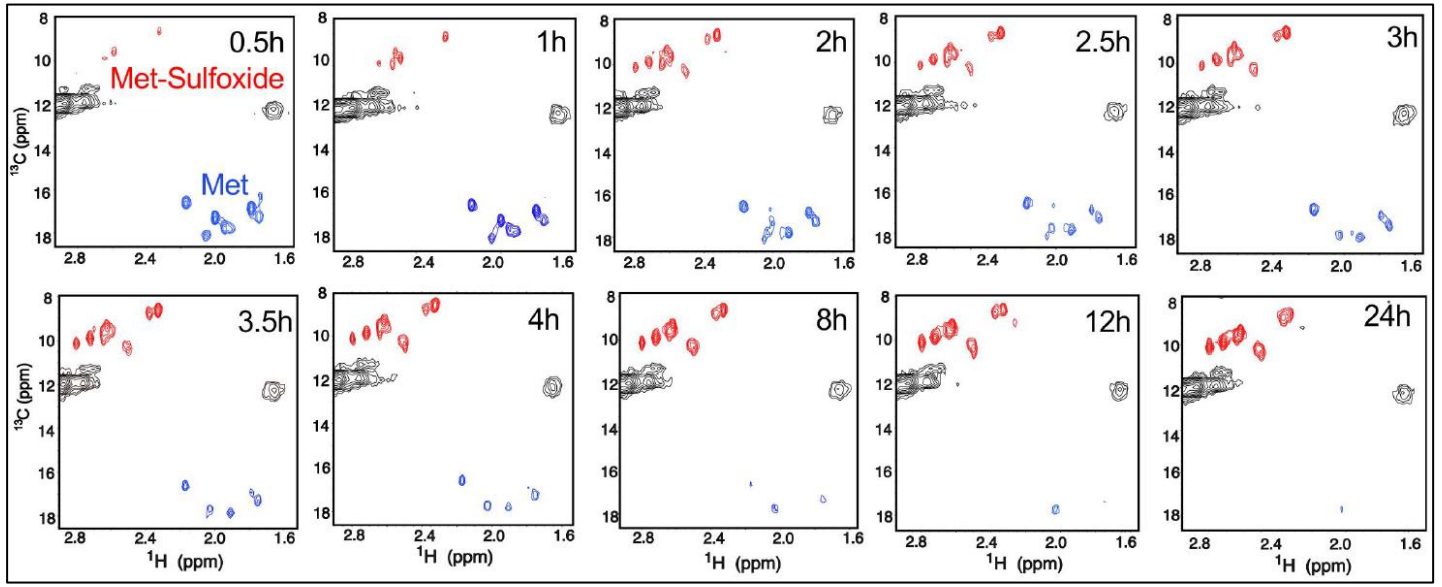
Potential application: noninvasive vaccine fingerprinting

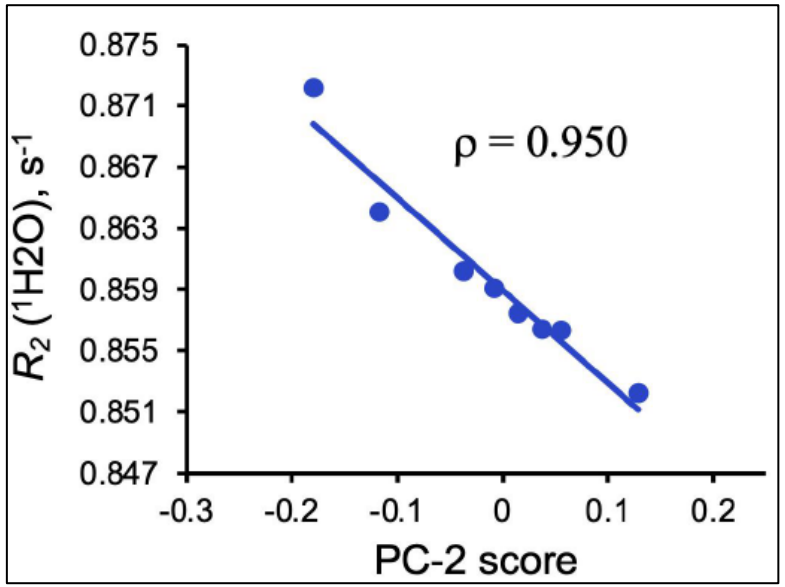
Correlation between *w*NMR and HOS: an example (NISTmAb oxidation)

*w*NMR at 25°C (0.56 T, 23.8 MHz)



High-field NMR at 50°C (14.1 T, 600 MHz)





Correlation between *w*NMR and HOS

(PC-2 score: principal component analysis of high-field NMR data)

(Solomon et al., mAbs, 15, 2160227, 2023)

More to come!

Evolution of NMR: turning liabilities into assets

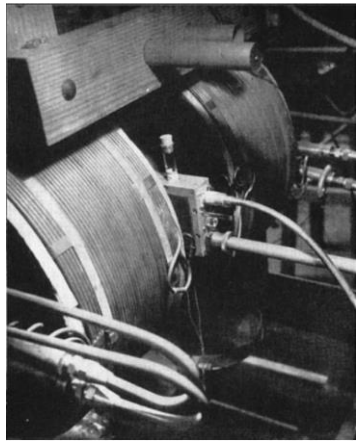
Physics: atomic nuclei N

$\omega_N = -\gamma_N \cdot B_0$

↓

$\mu_N = \gamma_N \cdot S_N$

Liability: $(1 - \sigma_{MN}) \cdot B_0$



Chemistry: molecules M

$\omega_N = -\gamma_N \cdot (1 - \sigma_{MN}) \cdot B_0$

↓

structure of M

Liability: $B_0 + \Delta B$; ^1H_2O

Quality control: products

ingredients ↔ ^1H_2O in B_0

↓

product quality

process monitoring

*w*NMR

Medicine: human body

^1H_2O in B_0 + $B(x, y, z)$

↓

images of body

MRI

Different NMR, different information

Information from Different Types of NMR

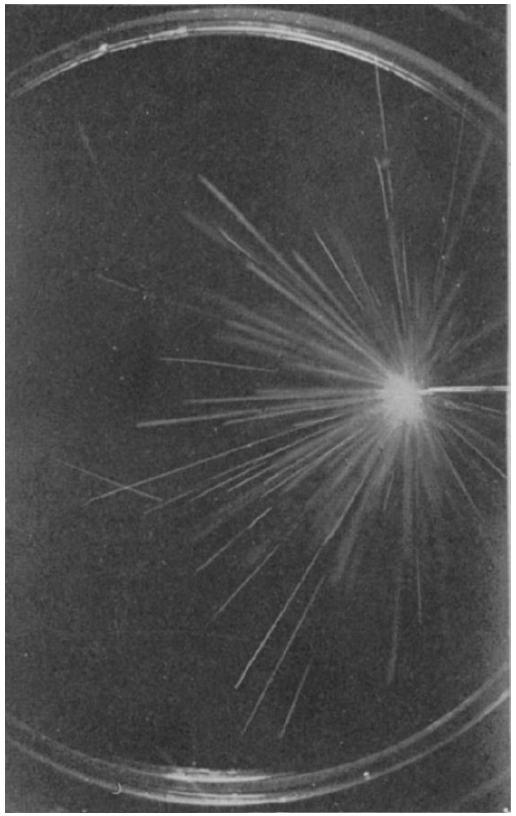
		Information on…			
		Nuclear moments	Molecular structure	Human body	Intact Drug Products
NMR Type	Physics	yes	no	no	no
	Chemistry	no	yes	no	no
	MRI	no	no	yes	no
	*w*NMR	no	no	no	yes

No one technique can do all; no one application requires all.

Another Example of Water as a Sensor and Amplifier in a Magnetic Field

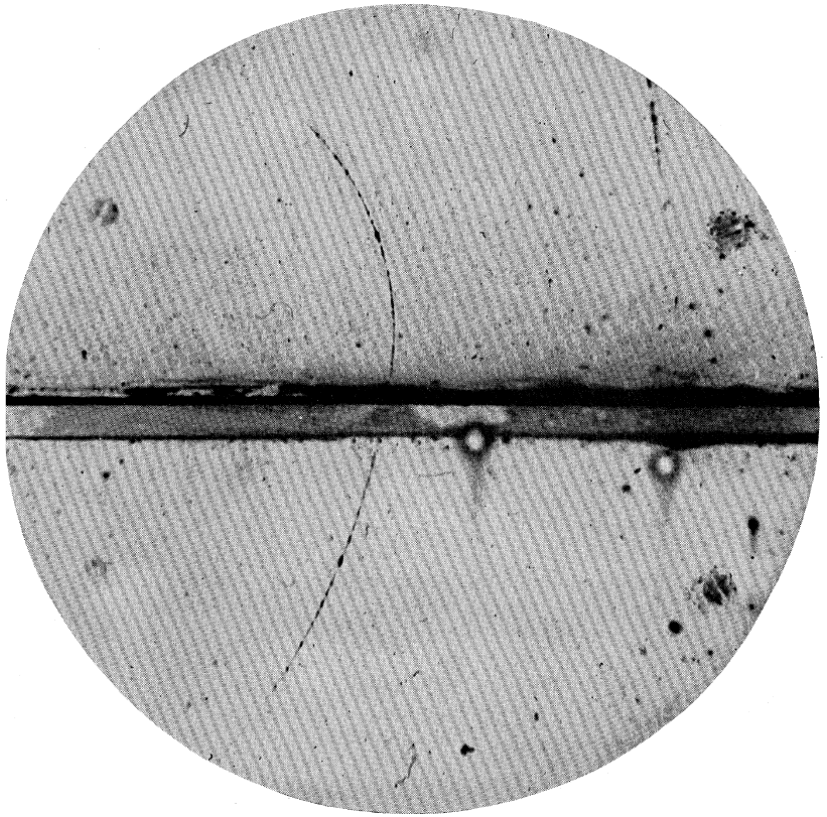
— the Wilson cloud chamber in particle physics

particle tracks



(Wilson, Proc. R. Soc. Lond. A 87, 277, 1912)

discovery of positron



(Anderson, Phys. Rev. 43, 491, 1933)

PET scanner

Healthline

The long arc of measurement science

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