



WAVEGUIDE

**Breakthrough Portable NMR Platform for  
Quantitative Analysis & Screening Complementing  
Optical Methods and a Logical Alternative to HPLC  
Methods in BioPharma.**

**IFPAC SESSION JUNE 14, 2022**

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# Presentation Overview

## Introduction

## Nuclear Magnetic Resonance (NMR) Brief History

## WaveGuide $\mu$ NMR

## Applications

- Authentication of pharmaceutical and consumer products (i.e., mAbs, vaccines, hyaluronic acid)
  - Raw Materials
  - API
  - Final Product
- Stability studies, accelerated storage conditions for product degradation

# Executive Summary: WaveGuide Corporation

**Founded & funded:** 2015

**Location:** Waltham, MA. USA (ISO 13485:2016)

**Operating status:**

- Privately held, Delaware C corporation
- Angel investors through Series-A , \$30M raised

**Product & commercial status:**

- Beta ready platform for field trials
- NRE projects active with customers
- Commercial launch in 2020

**Patent & intellectual property portfolio:**

- Two assigned patents from Harvard University that encompass the core technology that enables small, low-cost, inhomogeneous magnets to produce a handheld chemical analyzer based on NMR. Fundamentally amplifying the NMR signal-to-noise by a factor of 10,000 times.
- Additional (6) patents filed by WaveGuide Corp bolstering the core technology.
- Company know-how, nanoparticle chemistry, algorithms, calibrations, and unit-unit repeatability.

**More WaveGuide online**

- Interview at NYSE: <https://vimeo.com/264443486>
- LinkedIn: <http://www.linkedin.com/company/portablenmr>



**HARVARD UNIVERSITY**  
Department of Physics



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# What is NMR?

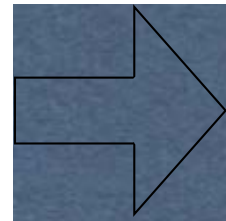
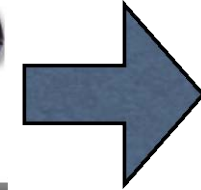
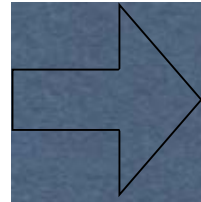
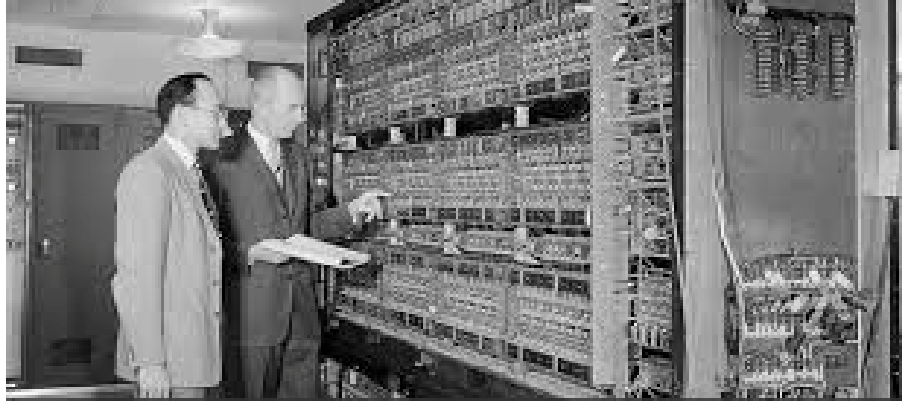
## Nuclear Magnetic Resonance

- NMR spectroscopy is a laboratory based analytical chemistry technique used in quality control and research for determining the content and purity of a sample as well as its molecular structure.
- The Purcell group at [Harvard University](#) and the Bloch group at Stanford University independently developed NMR spectroscopy in the late 1940s. Edward Purcell and Felix Bloch shared the 1952 Nobel Physics Prize for demonstrating NMR in condensed matter: water and paraffin (Isidor Isaac Rabi presented with the 1944 Nobel Prize for the discovery of the technique).



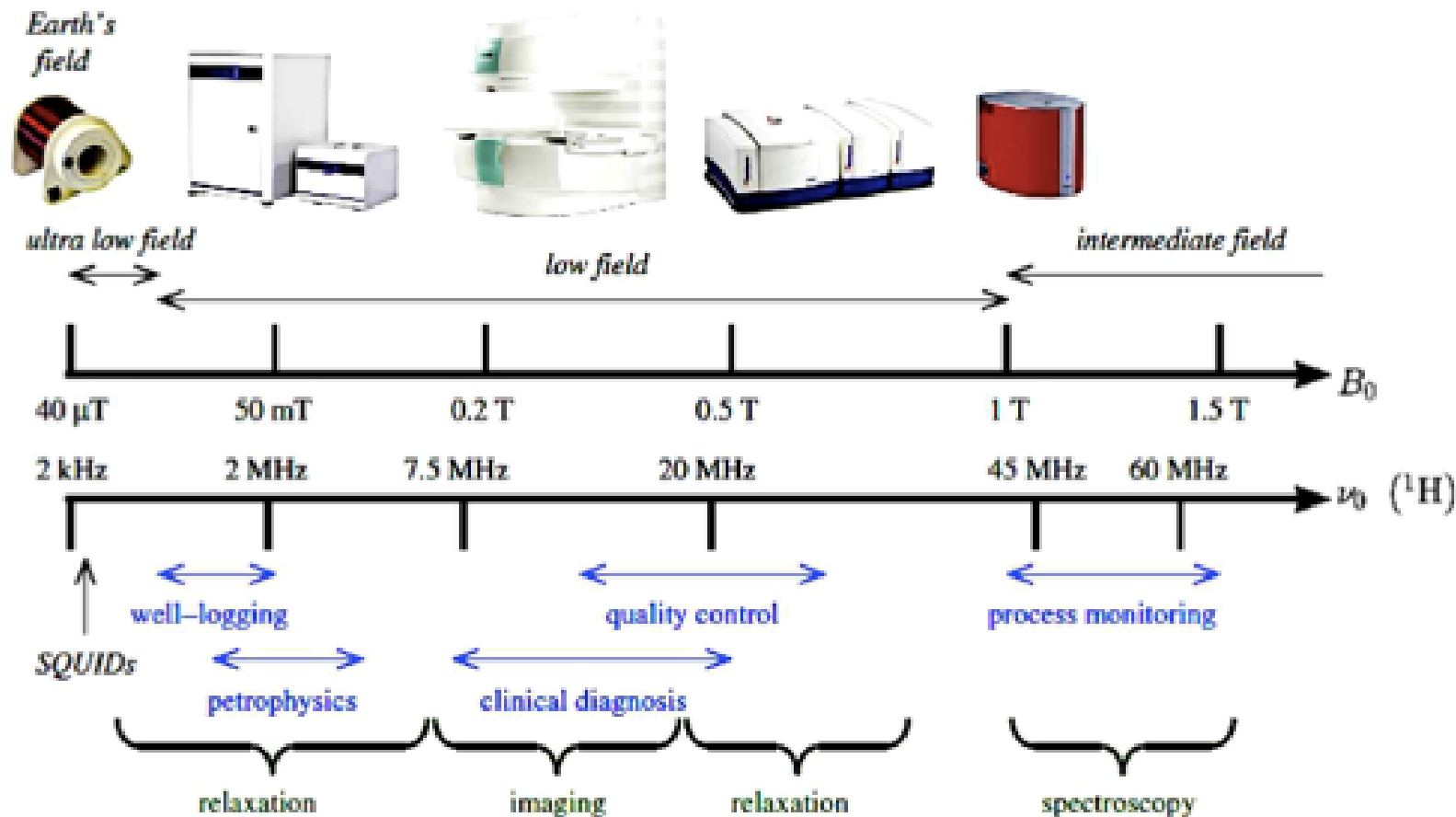
The First Commercial NMR, The HR-30, was sold in 1952 by Varian Associates to Humble Oil Company. This 30 MHz water-cooled electromagnet spectrometer weighed over two tons and had sufficient field homogeneity to resolve the chemical shifts of the three groups of protons in ethanol.

# Why Miniaturize NMR Instruments?



**Technology's Value Increases as it Migrates from Stationary to Mobile Formats**

# Commercial Low Field TD-NMR Instruments



- Contemporary Low Field TD-NMRs are \$80k to \$200k
- Permanently fixtured systems in a laboratory as these devices require significant temperature stabilization
- Expensive and sophisticated and requires a PhD/ Masters to use
- Wide range of applications in different fields of use

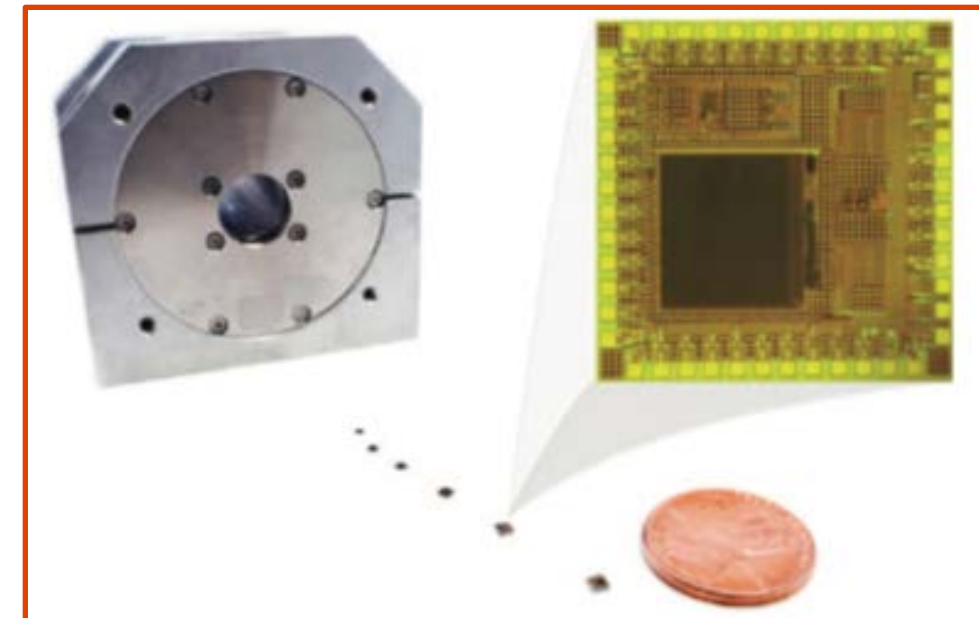
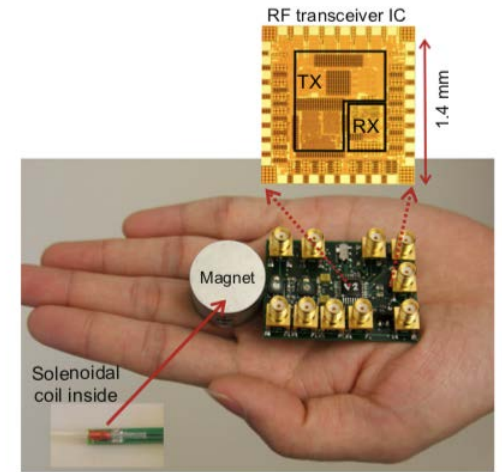
Can you take the PhD / Masters personnel out of the workflow??



# NMR Discovery at Harvard 1st CMOS

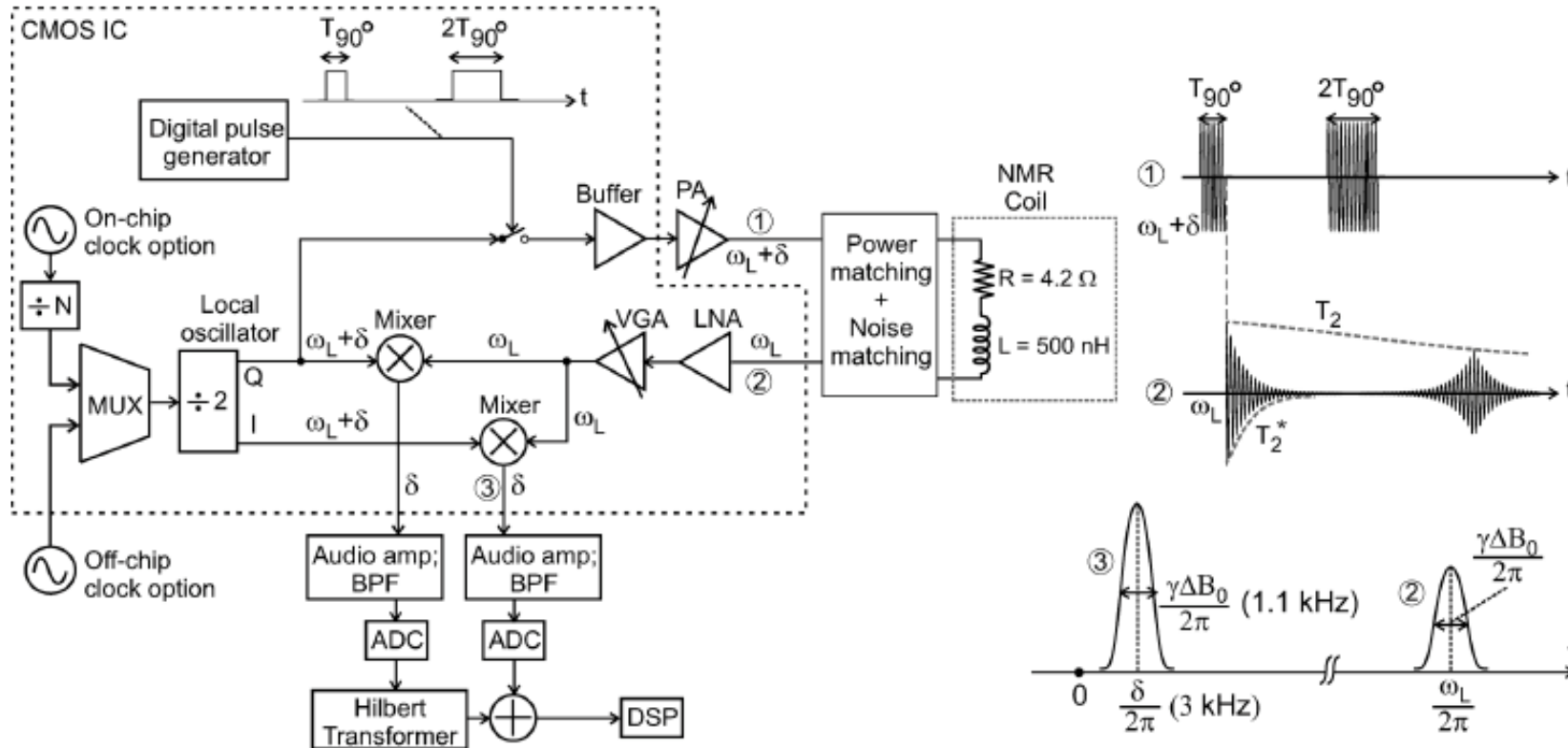
## Nuclear Magnetic Resonance

- Professor Donhee Ham and Graduate Student Nan Sun at Harvard University Physics Department discover a novel way to miniaturize NMR Spectroscopy and Time Domain NMR (Relaxometry).
- Upper right is the portable university grade miniaturized Time Domain NMR System and lower right is the portable university grade miniaturized NMR Spectroscopy System.
- Breakthrough technology is a novel network matching sequence between the low noise amplifier (LNA) stamped on a novel CMOS silicon chip and the probe.



# NMR – New Technology 60 Years After The Invention

## Core Technology: Harvard's Discovery



***A 50  $\Omega$  matching with abundantly available 50  $\Omega$  off-the-shelf LNAs has been the dominant choice for the LNA-coil connection in all NMR's commercialized***

***Harvard switched to power matching and noise matching at the Larmor frequency and integrated on a CMOS***



# NMR – WaveGuide $\mu$ NMR TD-NMR

## Commercial TD-NMR

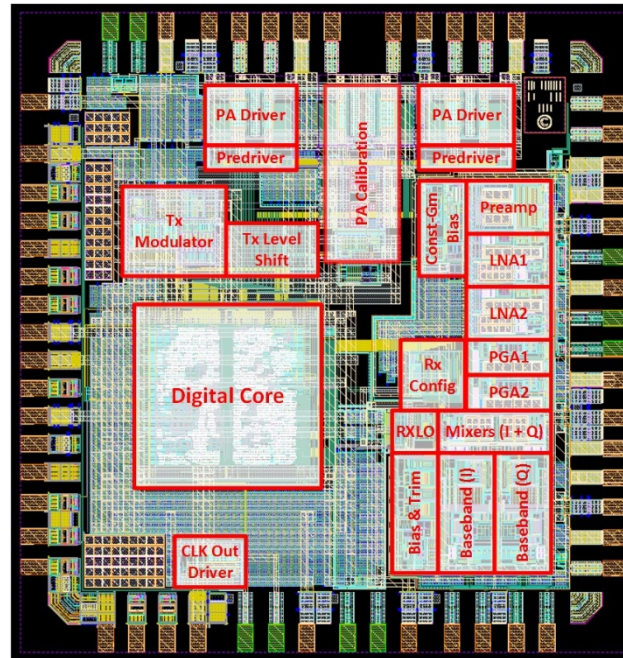
- Expensive and sophisticated
- Typical user PhD
- Permanently fixtured systems in a laboratory
- **Large sample volume required (>2 mL)**
- Discreet electronics average S/N ratio
- No data aggregation collection



Commercial TD-NMR

## WaveGuide $\mu$ NMR TD-NMR

- Inexpensive and sophisticated
- Typical user non-PhD
- Portable / battery operated field use
- **Small sample 15  $\mu$ L**
- CMOS / RF Transceiver excellent S/N ratio
- Data aggregation collection

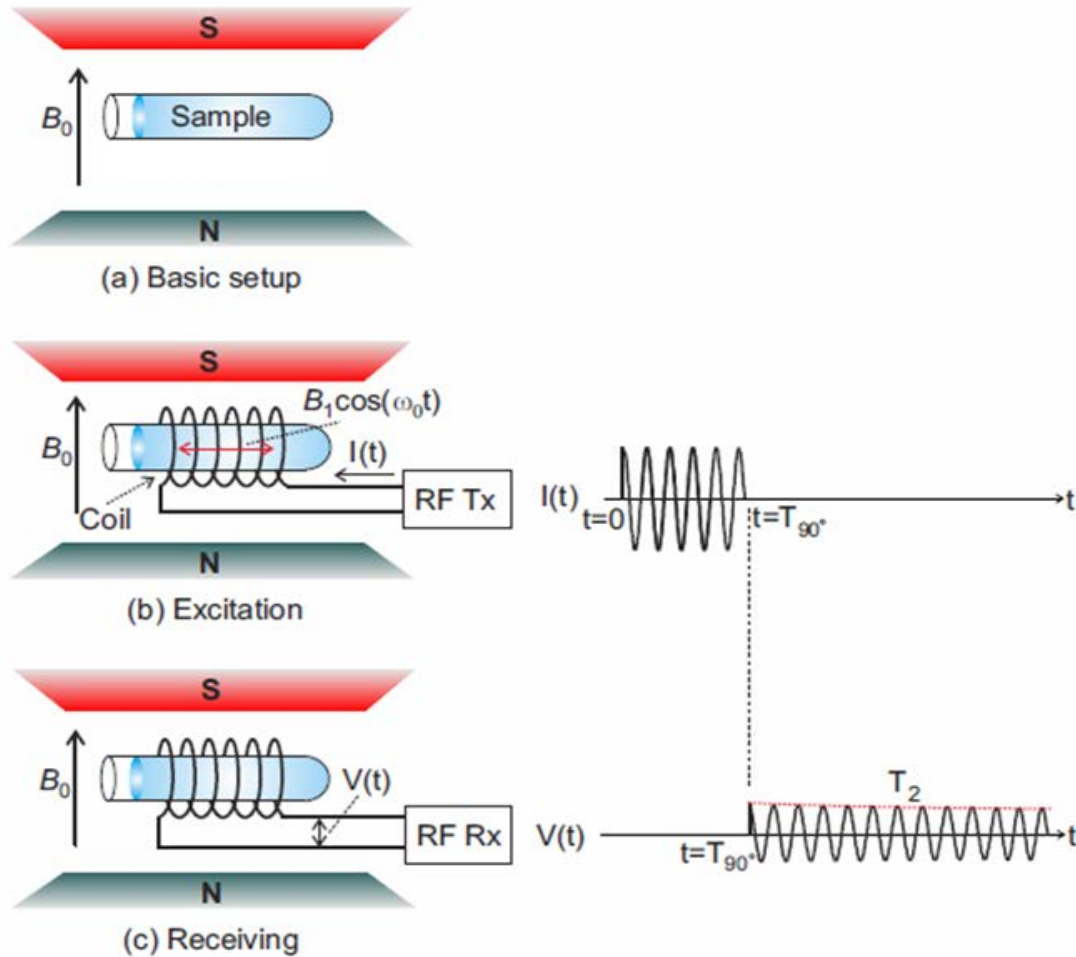


TSMC-0.18u Mixed-Signal RF  
CMOS Transceiver



WaveGuide  $\mu$ NMR TD-NMR

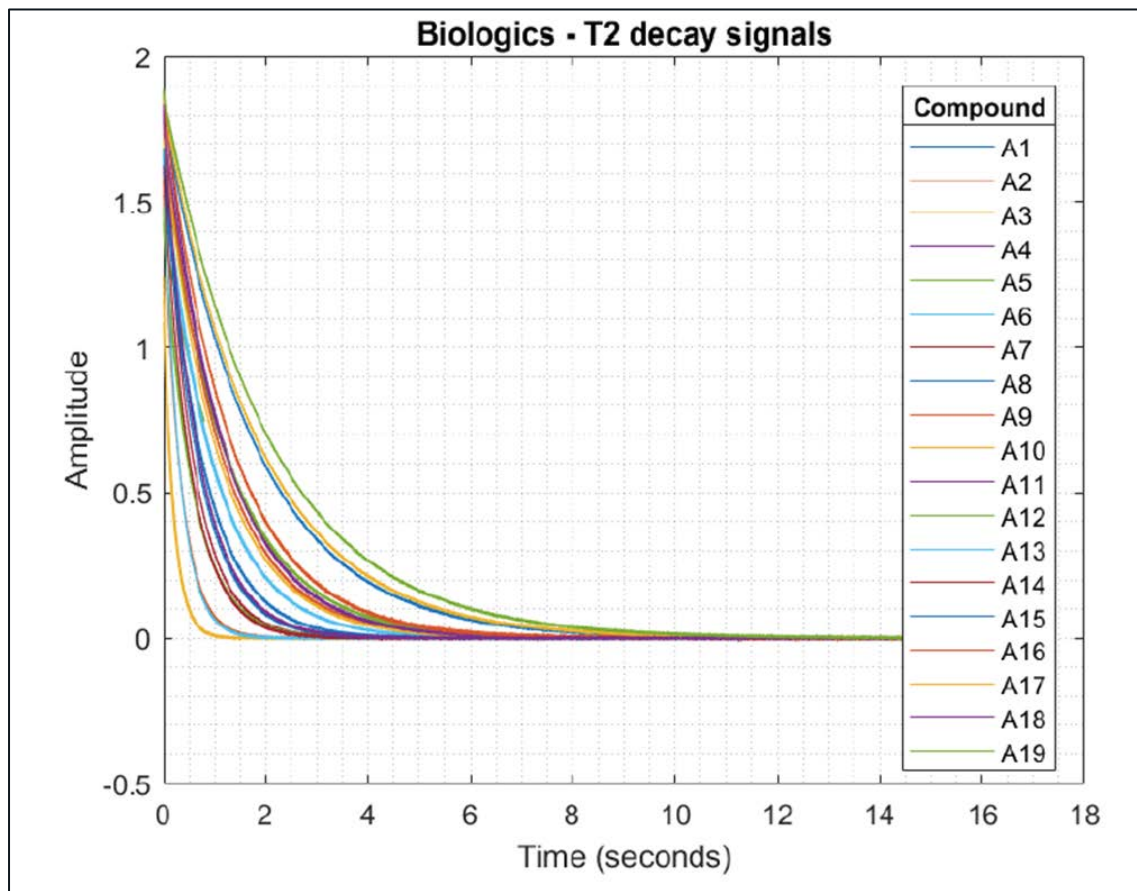
# Basic Setup of TD-NMR System for Relaxation Experiments



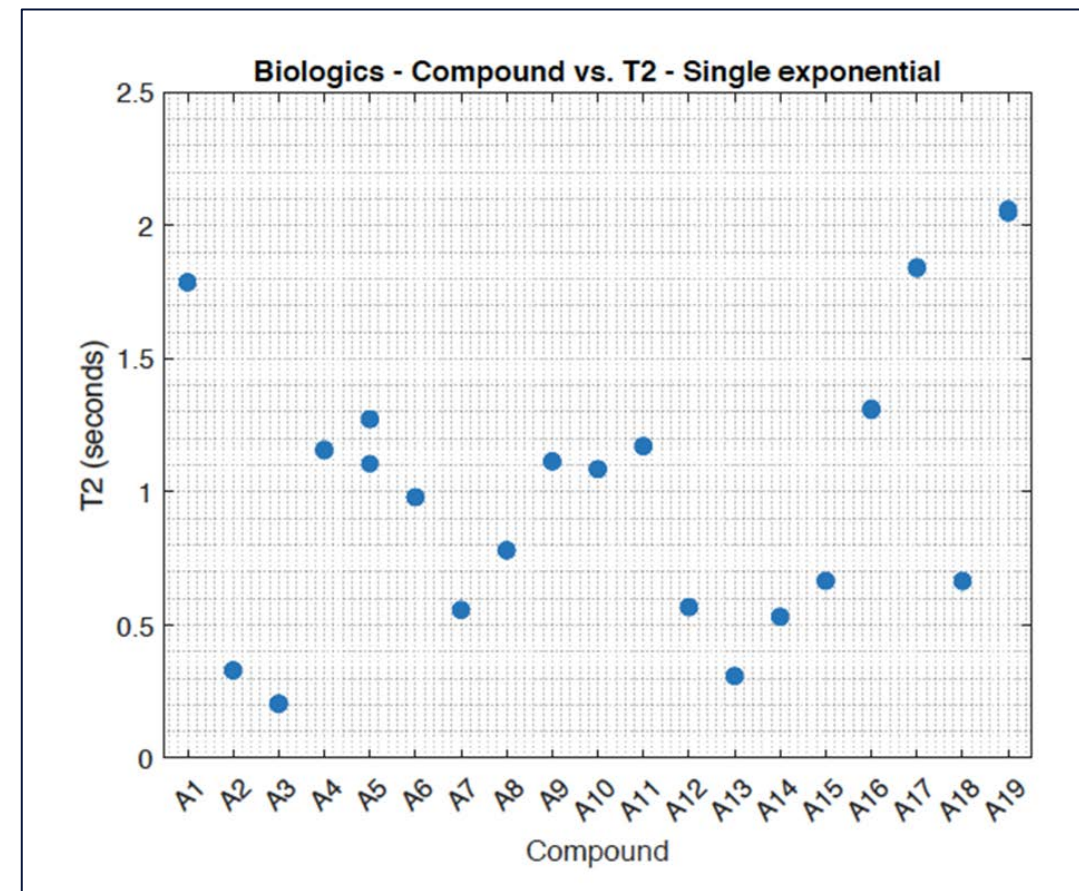
- The permanent magnetic field causes the sample nuclei (protons) to align and causes an energy gap
- RF energy is transmitted into the sample through the coil perturbs the equilibrium
- The time required for the nuclei (protons) to return to equilibrium after excitation is the “relaxation time”
- The “relaxation time constants” are unique to the sample chemistry and physical properties and are sensitive, e.g., to molecular mass, concentration, conformation and viscosity of a sample
- As a non-optical analysis technique, the color or texture of the material does not interfere with the TD-NMR measurement

# Raw Material Identification: 20 Unique Biologics Identified

## Proof of Concept study: Blind analysis of 20 Biological Drugs



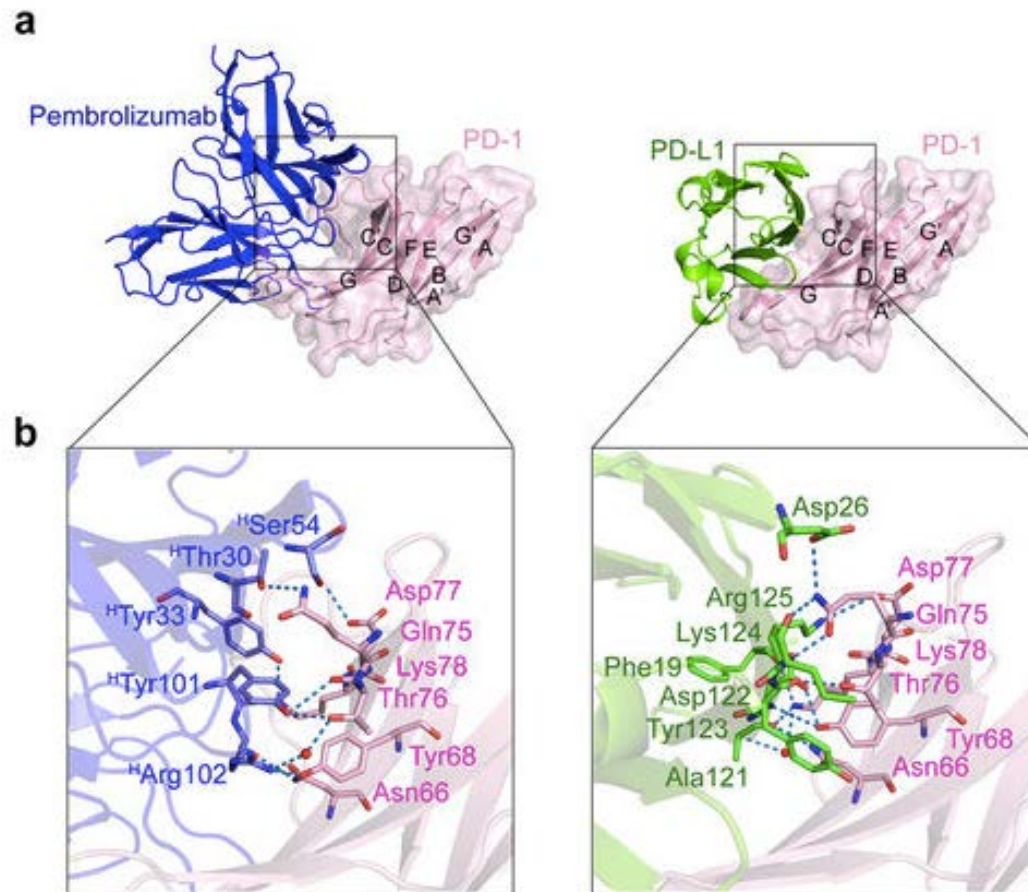
T2 decay relaxation times from all the 20 unique samples



T2 value of the single exponential component had a range of 0.256 (sec) to 2.014 (sec). All compounds displayed a unique single component exponential fit



# API: Analysis of Water Exchangeable OH and NH Protons in the PD1 PD-L1 Family of Proteins



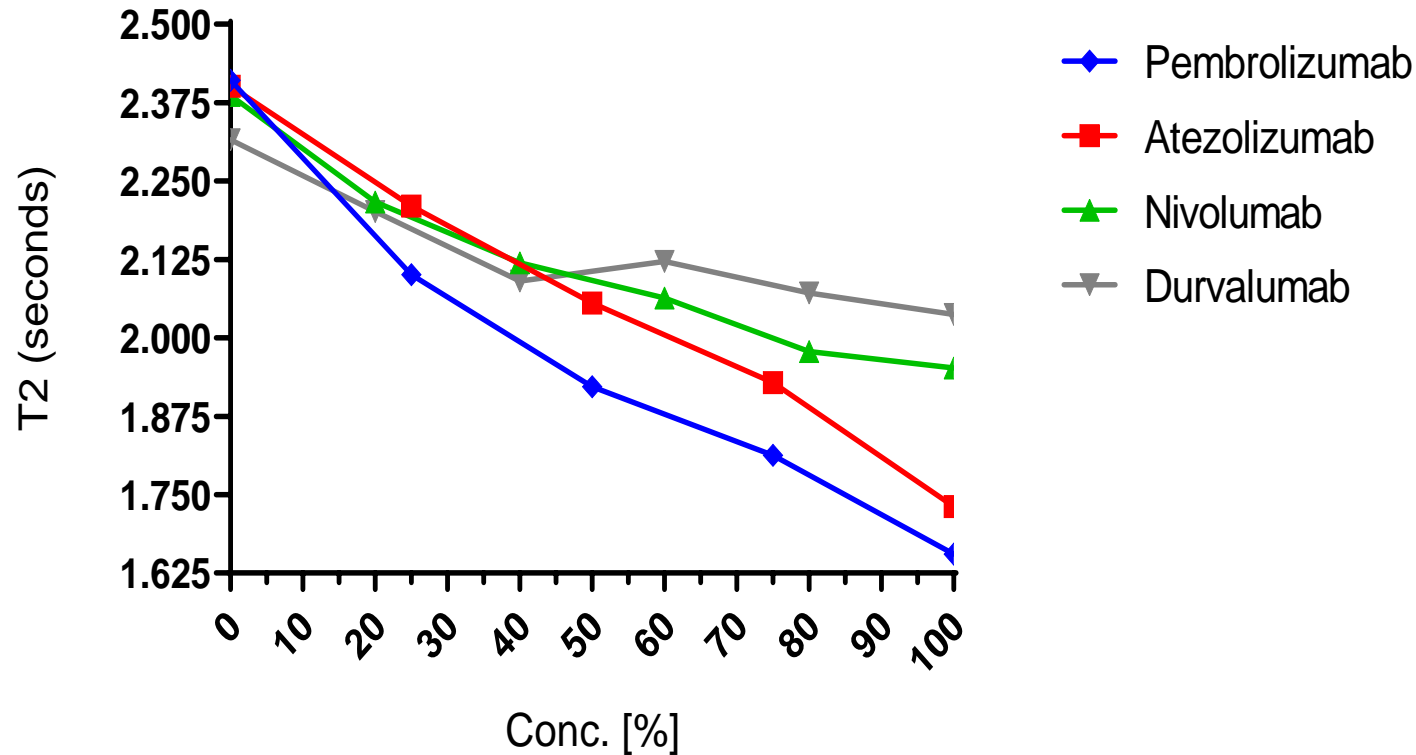
**a. Structure of the pembrolizumab/PD-1 complex and comparison with the PD-L1/PD-1 complex.**

**b. Residues involved in hydrogen bonds (blue dashes) are shown with water molecules in red**

**WaveGuide Proof of Concept Study:  
Analysis of native PD-1 and PD-L1 family  
of inhibitors to examine the effect of  
exchangeable OH and NH protons on  
relaxation in water**

**Each structure predicted to have unique  
T2 relaxation profile**

# API Proof of Concept Analysis of PD-1 and PD-L1 Inhibitors



- The plot on the left shows measured T2 relaxation values of four different monoclonal antibodies used in cancer immunotherapy using a single exponential data fit
- Each of the APIs have a unique T2 value even at the same effective protein concentration (100% far right data points)
- Each API was diluted from original concentration (100%) in either 20% or 25% decrement
- Quantitative analysis method
- These results suggested that this novel TD-NMR technique can distinguish biosimilars in the PD-1 and PD-L1 family



# API Proof of Concept Analysis of PD-1 and PD-L1 Inhibitors

**Atezolizumab** Each 20 mL vial contains 1200 mg of atezolizumab and is formulated in glacial acetic acid (16.5 mg), L-histidine (62 mg), polysorbate 20 (8 mg), and sucrose (821.6 mg), with a pH of 5.8

**Pembrolizumab** Each 1 mL of solution contains 25 mg of pembrolizumab and is formulated in: L-histidine (1.55 mg), polysorbate 80 (0.2 mg), sucrose (70 mg), and Water for Injection, USP

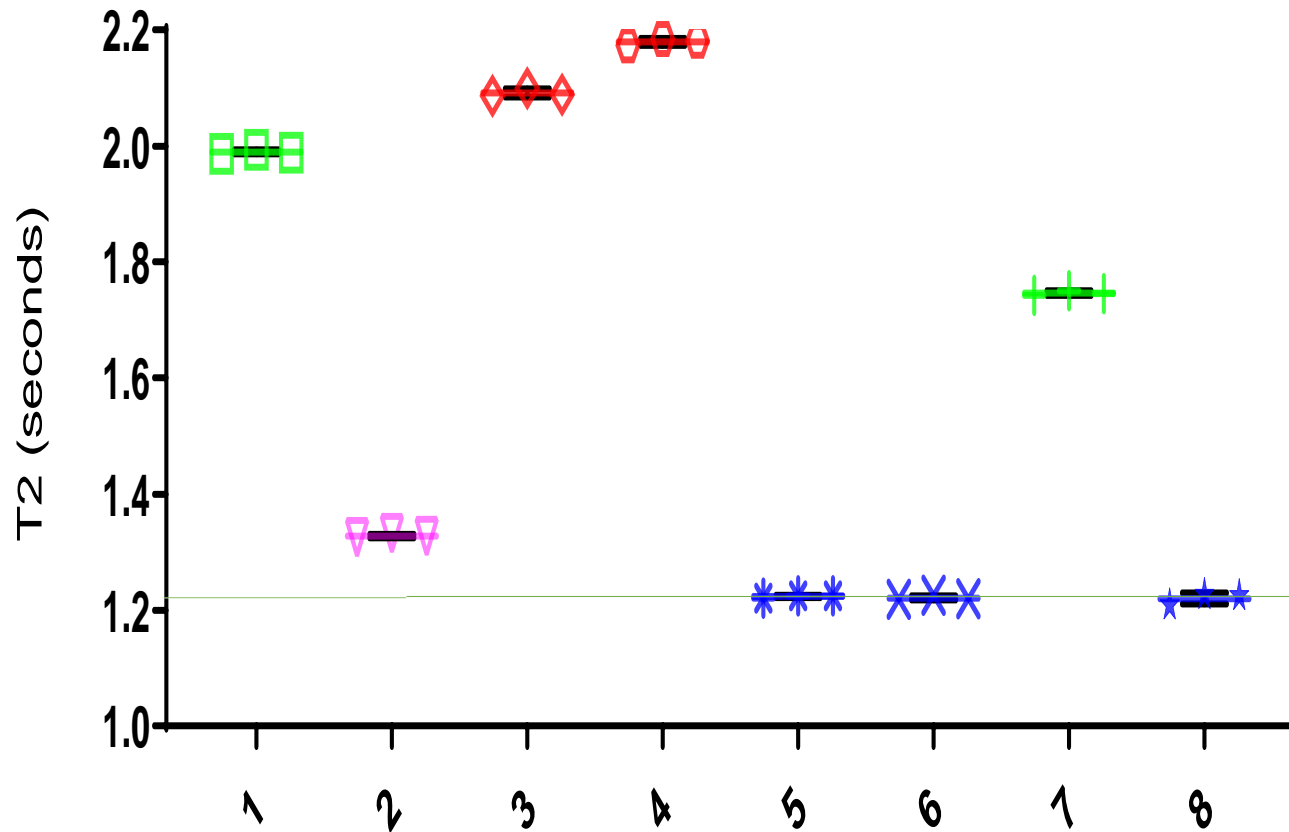
**Nivolumab** Each mL of solution contains nivolumab 10 mg, mannitol (30 mg), pentetic acid (0.008 mg), polysorbate 80 (0.2 mg), sodium chloride (2.92 mg), sodium citrate dihydrate (5.88 mg), and Water for Injection, USP. May contain hydrochloric acid and/or sodium hydroxide to adjust pH to 6

**Durvalumab** Each mL contains durvalumab 500 mg, 50 mg, L-histidine (2 mg), L-histidine hydrochloride monohydrate (2.7 mg),  $\alpha,\alpha$ -trehalose dihydrate (104 mg), Polysorbate 80 (0.2 mg), and Water for Injection, USP.

- **Complex formulations of final drug substance**
- **Optical analysis techniques will have issues with the color and excipients contained in the final formulation**
- **Proof of concept study conducted to analyze final formulation comparing manufacturing lots and counterfeits**

\*The small sample set was purchased from Selleckchem (see <https://www.selleckchem.com/>)

# Finished Product Authentication: Oncology Treatment



| Blind samples key                      |  |
|--|--|
| <span style="color: green;">□</span>   | #1: authentic, diluted (20%)                     |
| <span style="color: magenta;">▽</span> | #2: biosimilar from Europe                       |
| <span style="color: red;">◇</span>     | #3: biosimilar from Mexico (i)                   |
| <span style="color: red;">◻</span>     | #4: biosimilar form Mexico (ii)                  |
| <span style="color: blue;">✱</span>    | #5: <u>known</u> authentic (25 mg/mL)            |
| <span style="color: blue;">✕</span>    | #6: blind authentic (25 mg/mL)                   |
| <span style="color: green;">+</span>   | #7: authentic, diluted (40%)                     |
| <span style="color: blue;">★</span>    | #8: <u>Known</u> WG Control authentic (25 mg/mL) |

- Final drug lots of product #8 analyzed to set the T2 relaxation decay time baseline
- Blinded Samples #5 and #6 were known as Authentic to the company: WG Result Consistent with Authentic Product (#8)
- Blinded Samples #2 #3 and #4 were known as counterfeit biosimilars to the company: WG Result Not Consistent with Authentic Product (#8)

# Proof of Concept Analysis of Final Formulation Multivalent Vaccines

## Examples of Commercially Available Multivalent Vaccines

**TWINRIX** 1-mL dose of vaccine contains 720 ELISA Units of inactivated hepatitis A virus and 20 mcg of recombinant HBsAg protein. One dose of vaccine also contains 0.45 mg of aluminum in the form of aluminum phosphate and aluminum hydroxide as adjuvants, amino acids, sodium chloride, phosphate buffer, polysorbate 20, and Water for Injection.

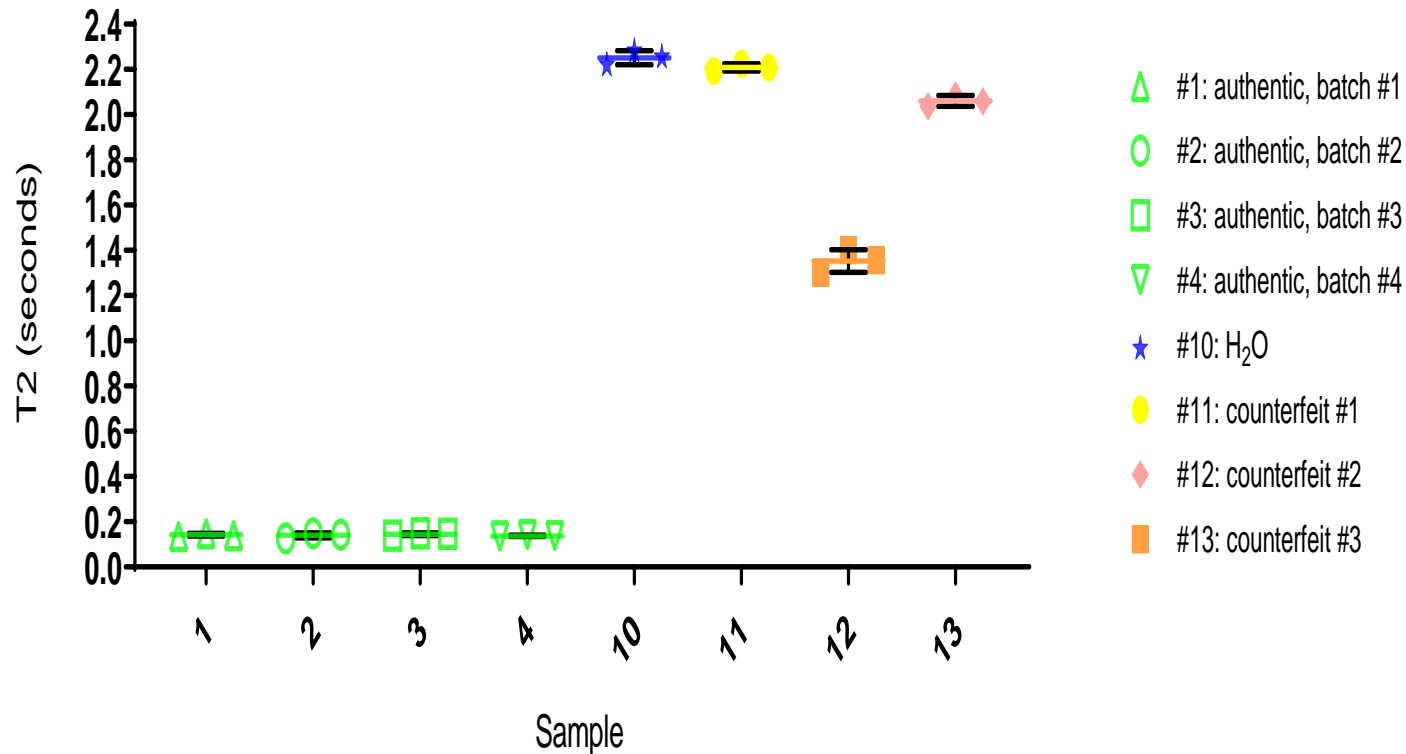
**Flublok**, Influenza Quadrivalent Vaccine A/Tasmania/503/2020 (an A/Cambodia/e0826360/2020-like virus) (H3N2), B/Washington/02/2019 and B/Phuket/3073/2013. A single 0.5 mL dose of Flublok Quadrivalent contains sodium chloride (4.4 mg), monobasic sodium phosphate (0.2 mg), dibasic sodium phosphate (0.5 mg), and polysorbate 20 (Tween®20) (27.5 mcg). Each 0.5 mL dose of Flublok Quadrivalent may also contain residual amounts of baculovirus and Spodoptera frugiperda cell proteins ( $\leq 19$  mcg), baculovirus and cellular DNA ( $\leq 10$  ng), and Triton X-100 ( $\leq 100$  mcg).

**PedvaxHIB®** Each 0.5 mL dose of Liquid PedvaxHIB is a sterile product formulated to contain: 7.5 mcg of Haemophilus b PRP, 125 mcg of Neisseria meningitidis OMPC and 225mcg of aluminum as amorphous aluminum hydroxyphosphate sulfate in 0.9% sodium chloride and is a slightly opaque white suspension.

- Complex formulations of final vaccines as Aluminum adjuvant suspensions
- Must re-suspend the vaccine before delivery to patient into uniform hazy white suspension
- Extremely low concentration of virus / proteins in the vaccine “soup”
- Optical analysis techniques will have issues with the cloudy Aluminum adjuvant suspension
- Proof of concept study conducted to analyze final formulation of vaccines comparing manufacturing lots and counterfeits

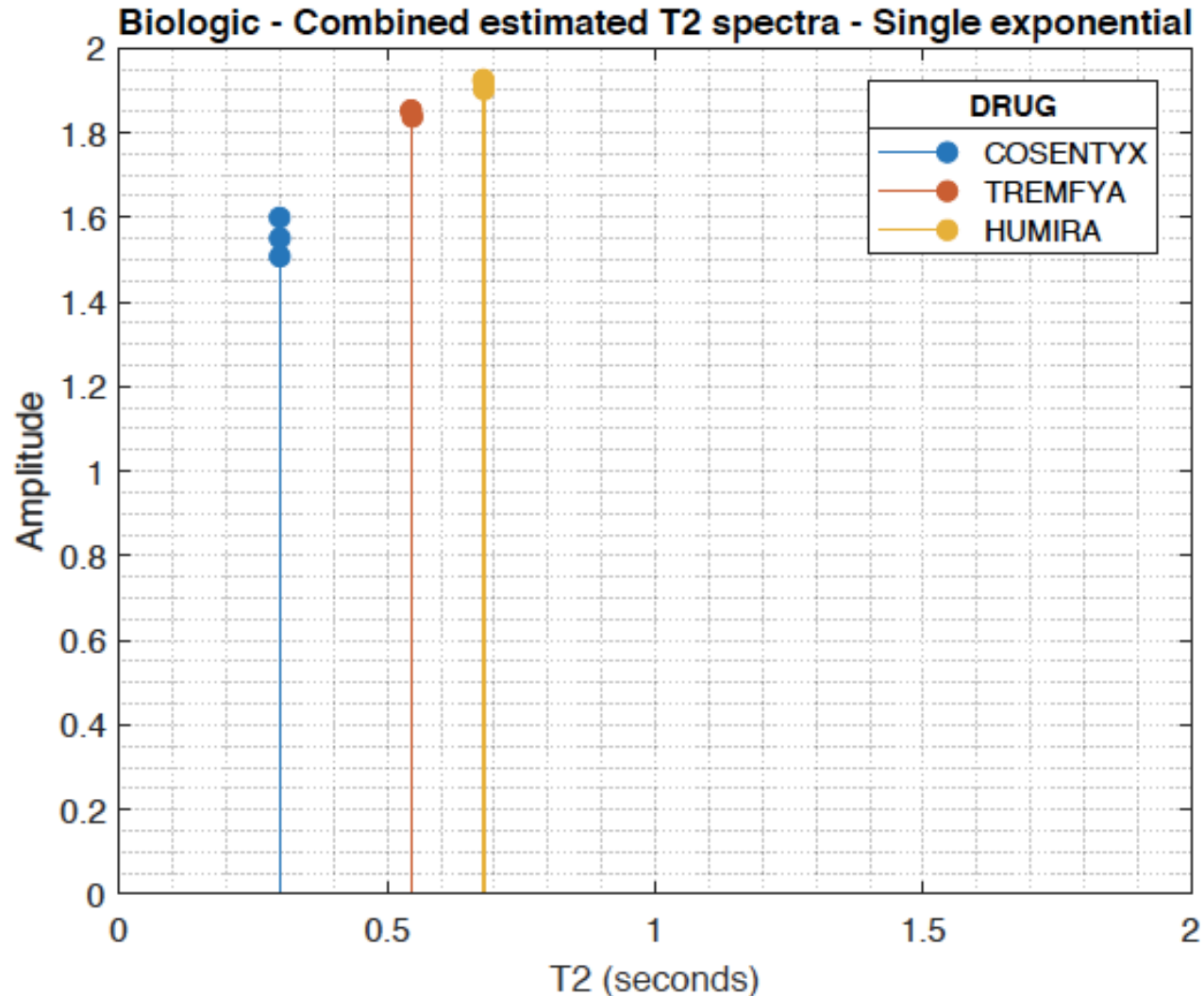
# Finished Product Authentication: Multivalent Vaccine

Method development for the authentication of multivalent vaccine



- An authentic multivalent vaccine was analyzed against a similar vaccine product and counterfeit samples
- The plot on the left shows the measured T2 values for the authentic vaccine, a molecular biology grade water sample, and three counterfeit products from the foreign market
- The authentic multivalent vaccine was tested across four different batches and the results were very consistent
- The authentic multivalent vaccine was clearly distinguishable from diluted, biosimilar, or counterfeit products

# WaveGuide TD- $\mu$ NMR Analysis: Stability Baseline

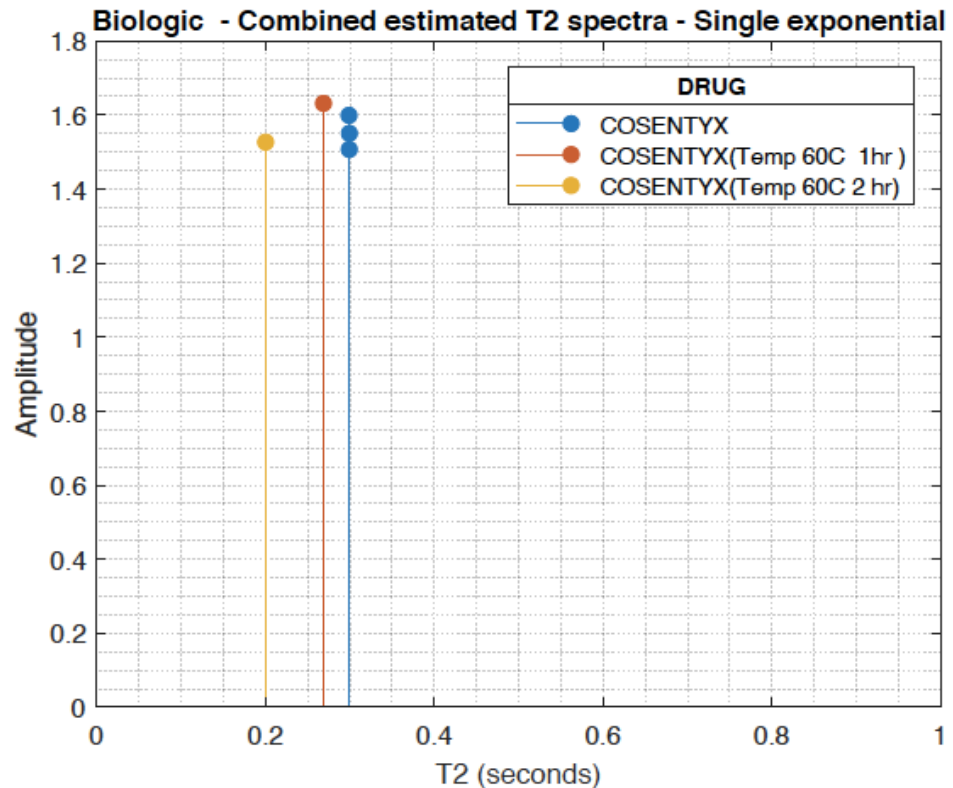


- The T2 relaxation data were analyzed using a single component exponential fit
- COSENTYX® single exponential value of 0.299 (sec)
- TREMFYA® single exponential value of 0.546 (sec)
- HUMIRA® single exponential value of 0.679 (sec)
- Early results suggest that this novel TD-NMR technique can readily distinguish different pharmaceutical medicines

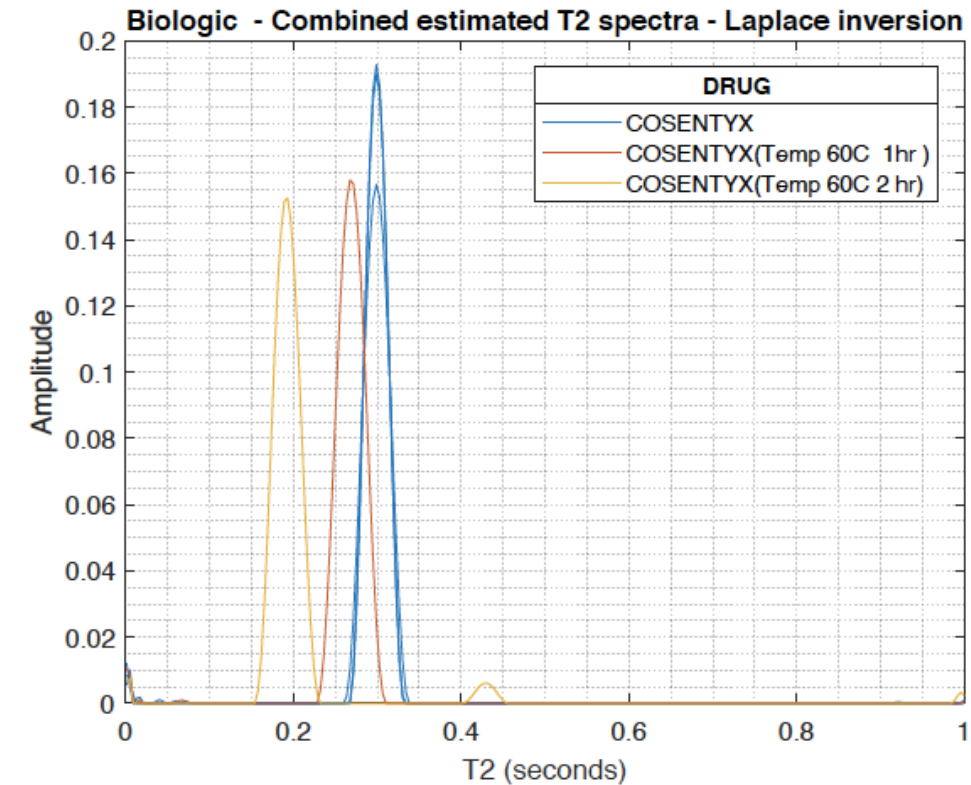
\*The small sample set was purchased from Evidentic (see <https://evidentic.com>)



# WaveGuide TD- $\mu$ NMR Analysis: Stability and Forced Degradation

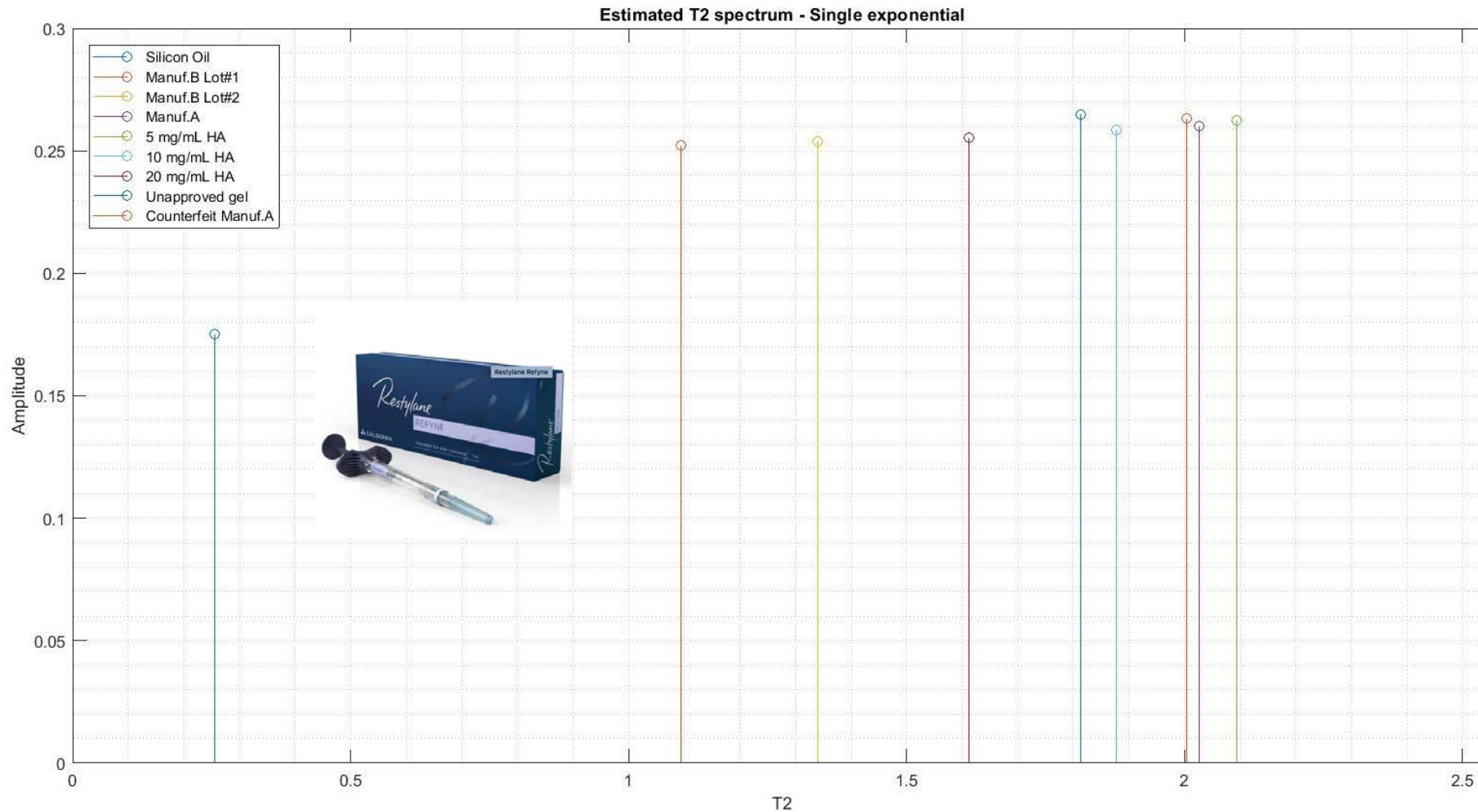


- Fresh COSENTYX®  
T2 = 0.299 (s)
- 1 hr. after heating at 60 °C  
T2 = 0.269 (s)
- 2 hrs. after heating at 60 °C  
T2 = 0.191 (s)



- The T2 relaxation data were analyzed using a Laplace inversion and a single exponential fit
- A forced temperature aggregation study was conducted to mimic poor storage conditions of the final formulated drug
- The WaveGuide TD- $\mu$ NMR clearly detected a change in the T2 relaxation of the different aggregation indicating a change in the product state during the study

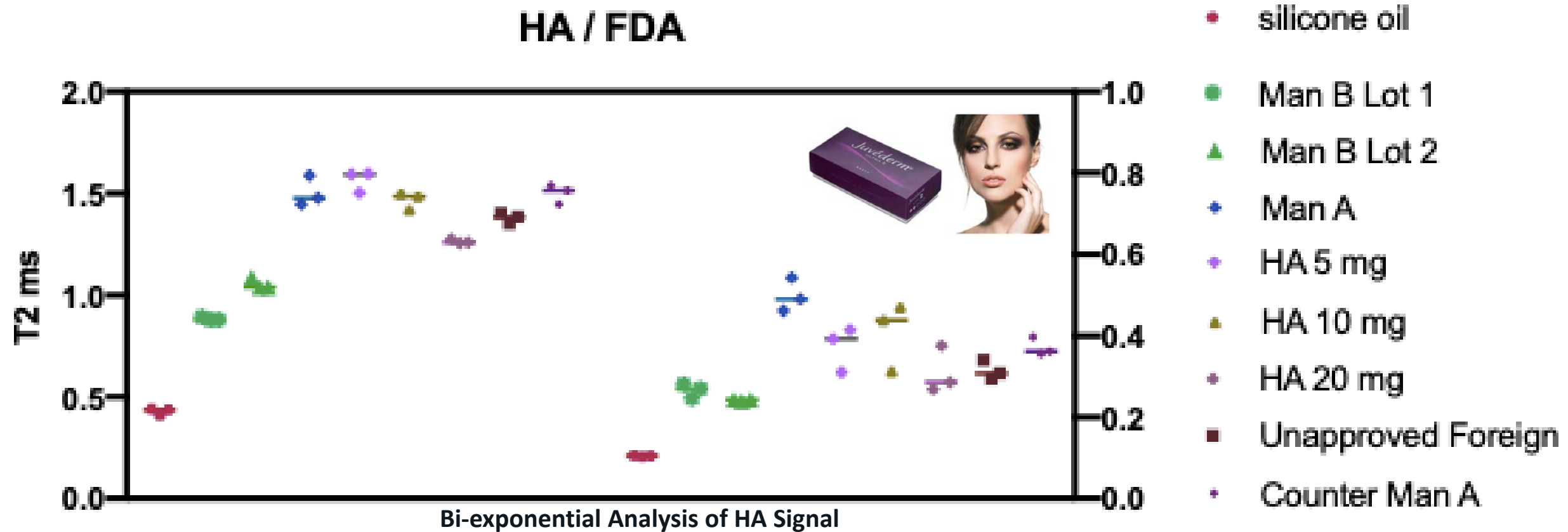
# Consumer Product Authentication: Hyaluronic Acid Injectables



## WaveGuide PoC Study: showed .....

- Hyaluronic acid (HA) is currently the most widely used filler & most adulterated dermal filler
- HA has a longevity of ~ 6 months, depending on the molecular size, method of cross-linking, and the region of injection

# WaveGuide $\mu$ NMR Forensic Analysis of Consumer Products



- In 2017, the FDA issued an official warning urging consumers to “Never buy dermal fillers on the internet: They may be fake, contaminated, or harmful.”

# Summary

- **TD-NMR can be utilized to screen a diverse range of biological pharmaceuticals from raw materials to final formulation.**
- **TD-NMR has shown the ability to aid in a manufacturing environment from incoming raw material inspection to in process phase testing to final product release.**
- **The portability of the WaveGuide TD-NMR allows for testing in the field for authentication of different classes of products.**
- **Minimal sample size 15µl required**



# WaveGuide self-contained $\mu$ NMR instrument





# Thank You

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