THE UNIVERSITY OF CHICAGO

Diamond-based quantum sensor for molecular analytics

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B.S. in Chemical Biology – Xiamen University
Functional nanoparticles in biomedicine

Ph.D. in Chemistry – The Ohio State University
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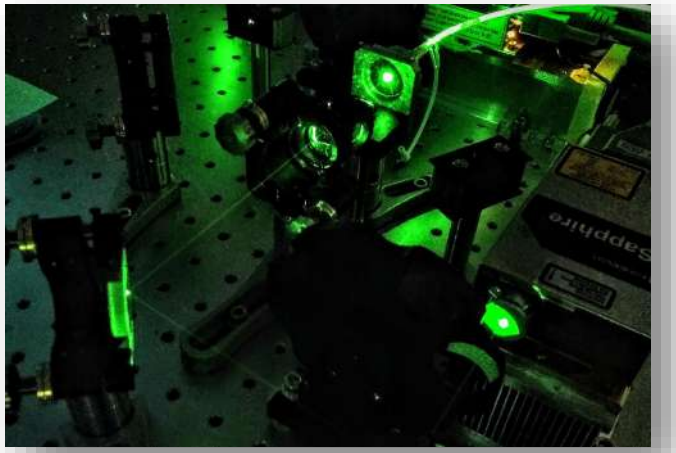
Protein dynamics and biophysics by NMR spectroscopy

Postdoc in Molecular Engineering – EPFL & UChicago
(Advisor: Peter Maurer)
Nanoscale NMR & quantum sensing

Conventional and optically detected NMR
to study biological system
at molecular level

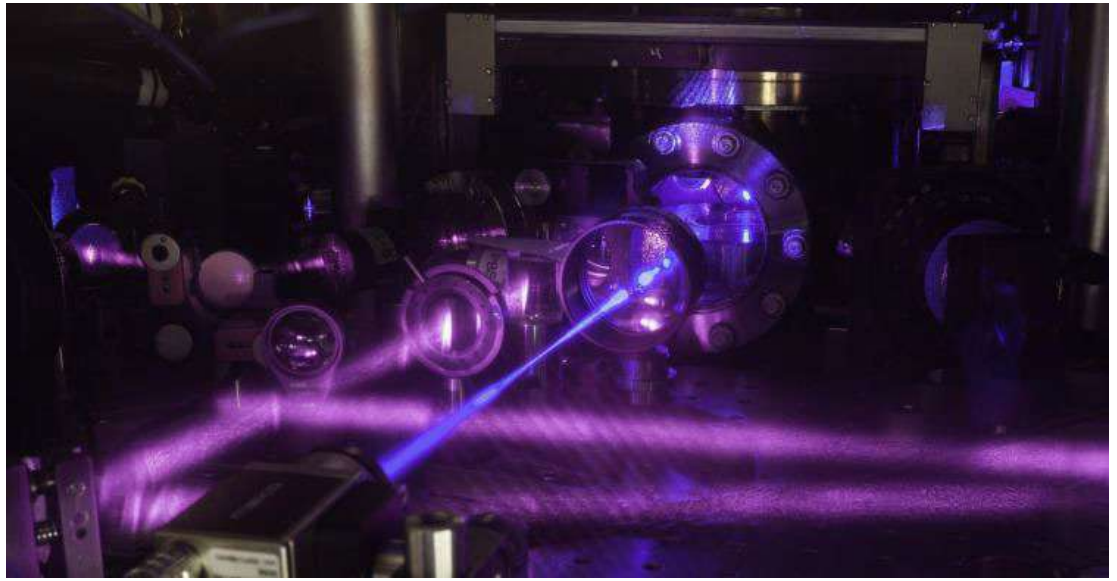
Conventional NMR spectroscopy


Quantum sensing based on
nitrogen-vacancy center in diamond

Quantum sensing permits some most precise measurements

Laser interferometer gravitational-wave observatory (LIGO)

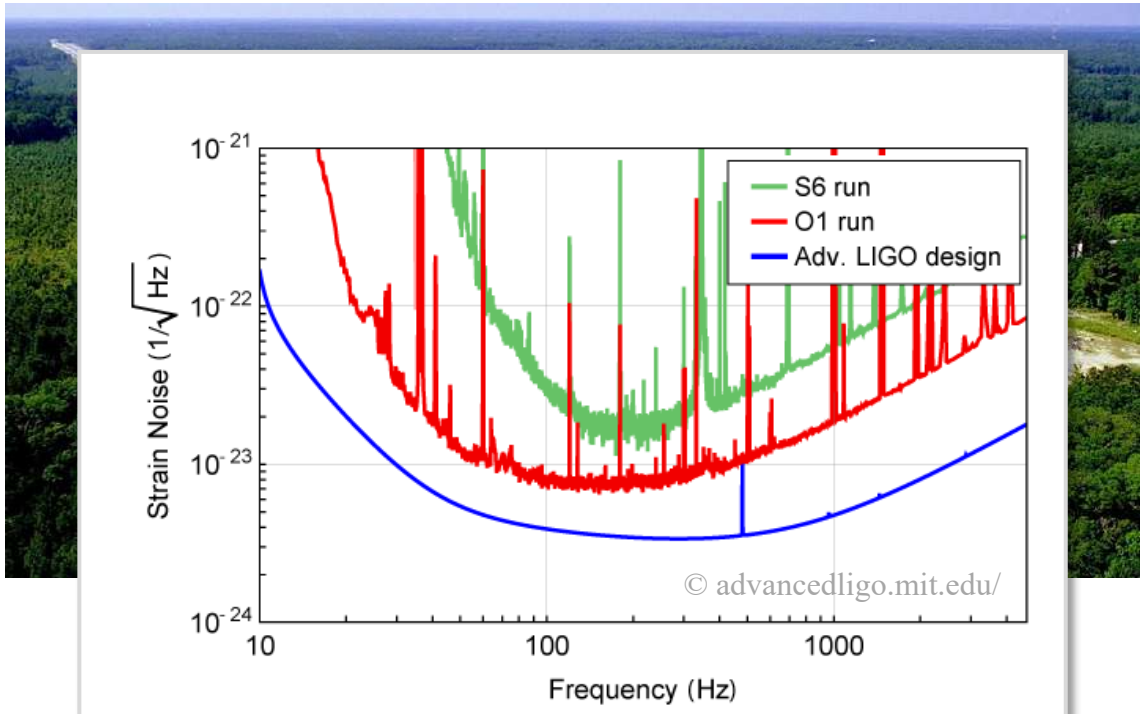
NIST's three-dimensional strontium quantum gas atomic clock



"Quantum sensing" describes the use of a quantum system, quantum properties, or quantum phenomena to perform a measurement of a physical quantity

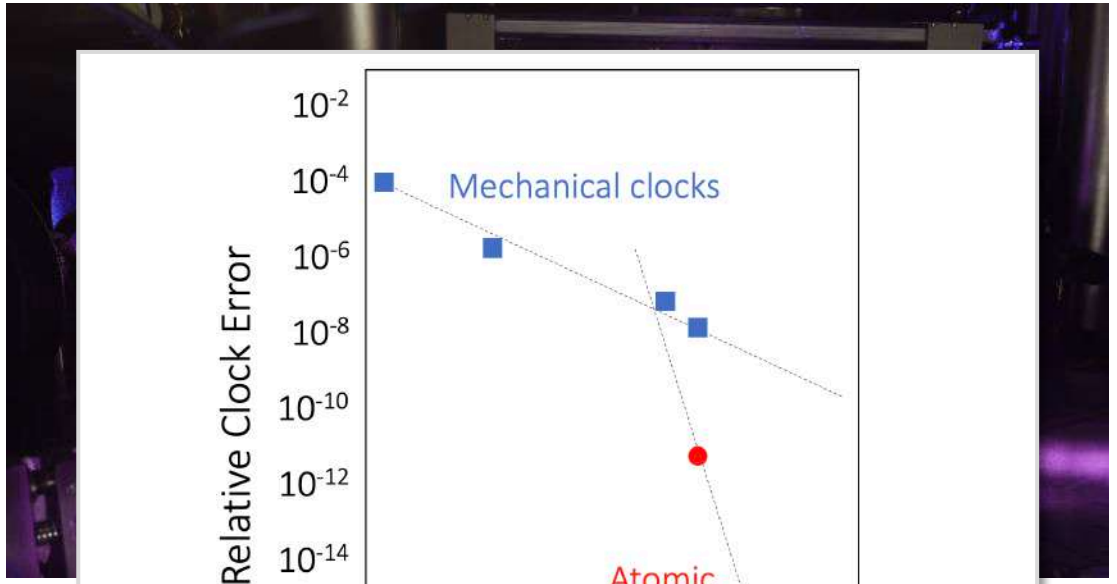
Quantum sensing permits some most precise measurements

Laser interferometer gravitational-wave observatory (LIGO)



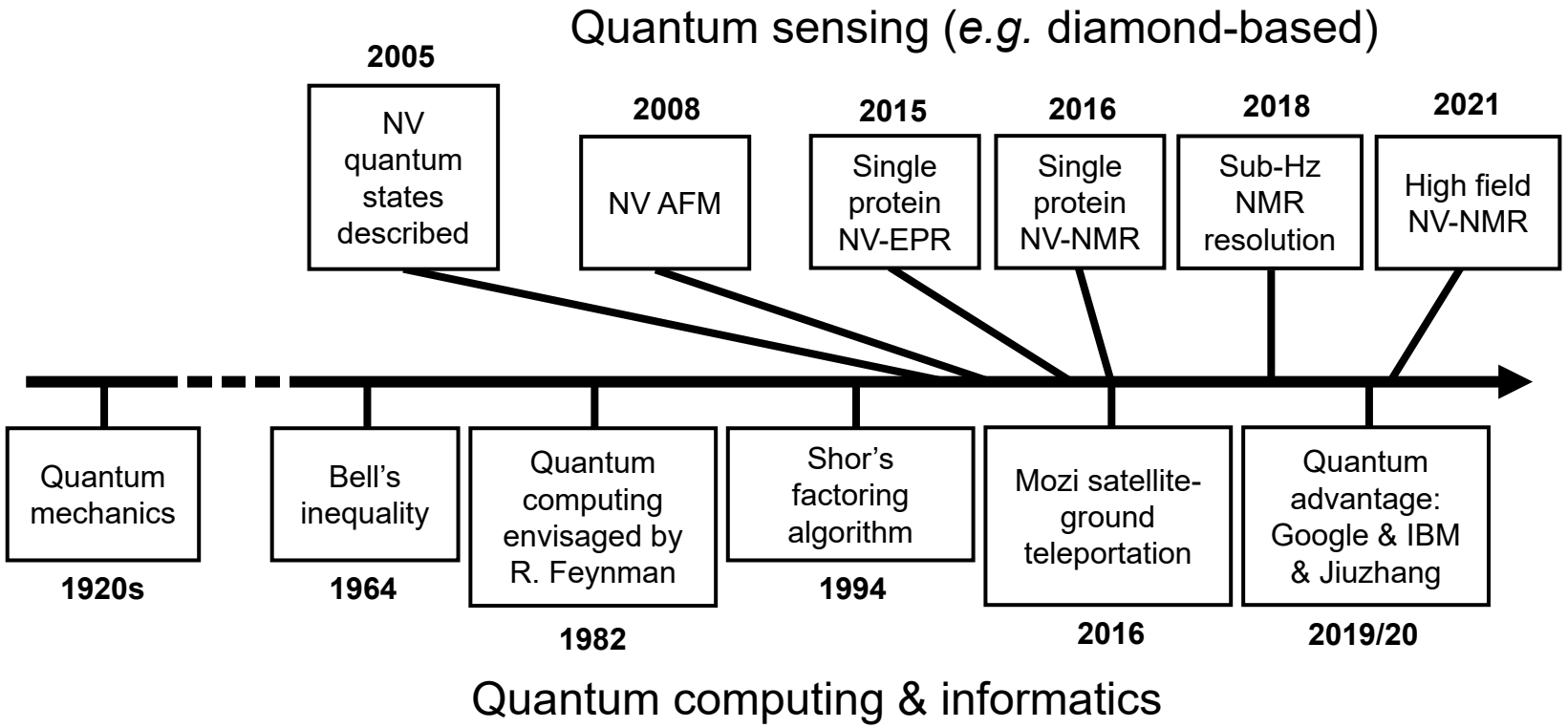
LIGO detects a change in distance between its mirrors **~1/10,000th the width of a proton**

NIST's three-dimensional strontium quantum gas atomic clock



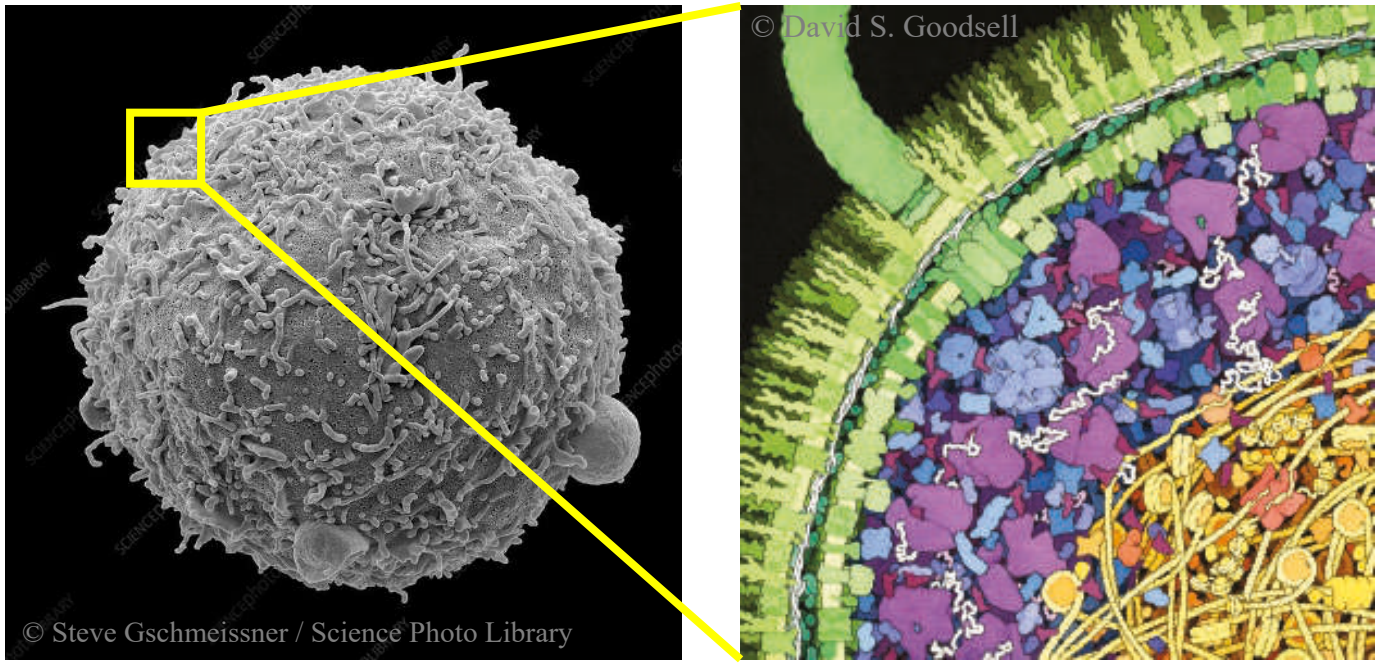
Atomic clock loses only **~0.1 s over the age of the universe**

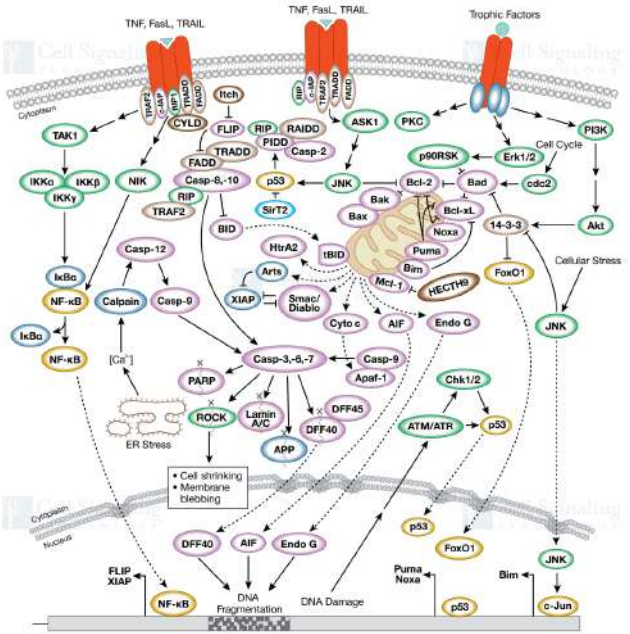
The quantum revolution



An era that we can **quantum engineer a state of matter** as advanced sensors to explore uncharted territories of knowledge

Biological systems are complex





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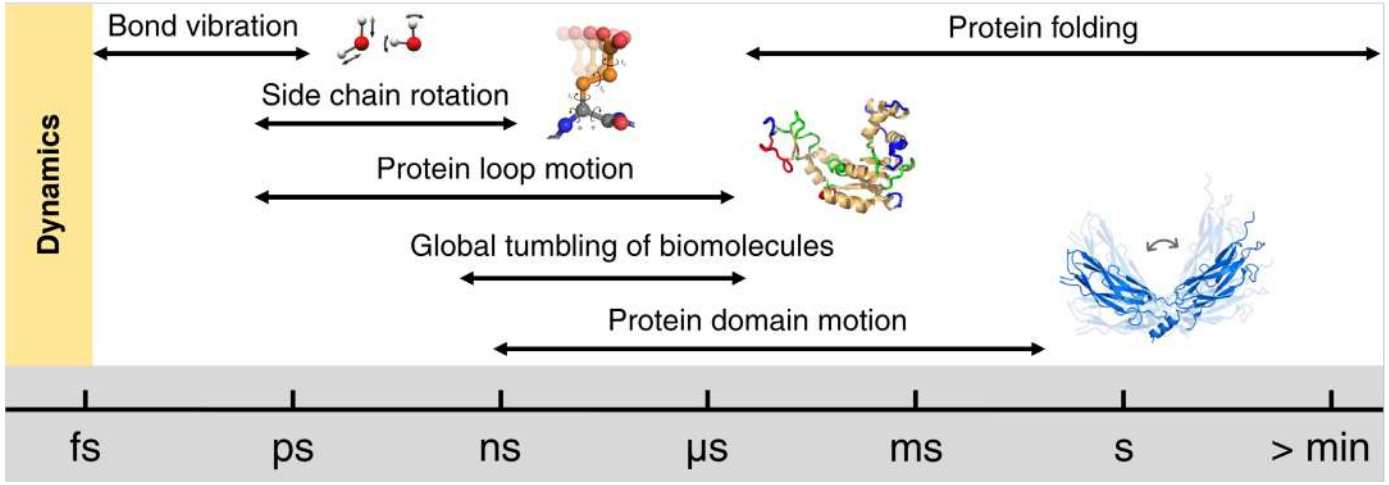
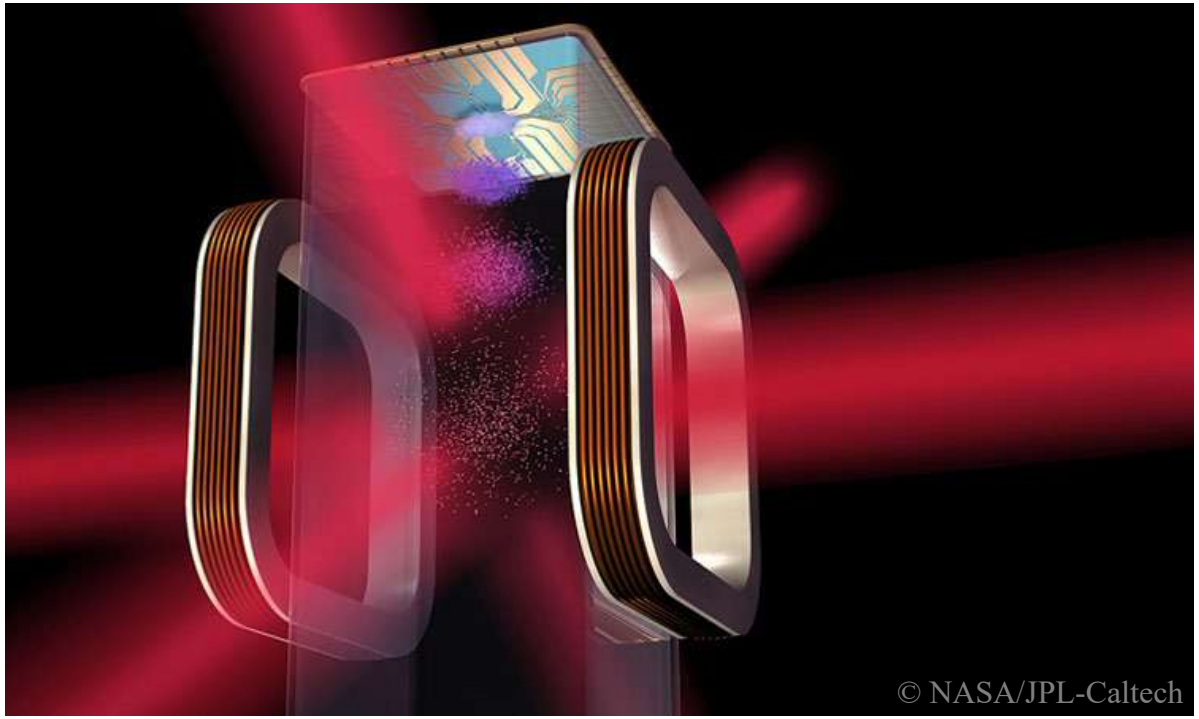


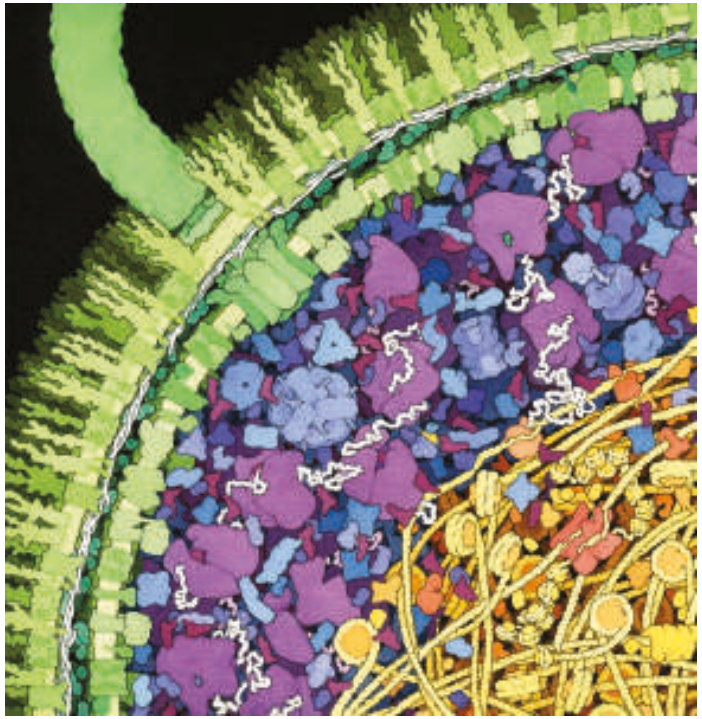
Mechanistic understand at

molecular level:

- Structure
- Interaction
- Dynamics

Vision: probing biological systems with quantum metrology





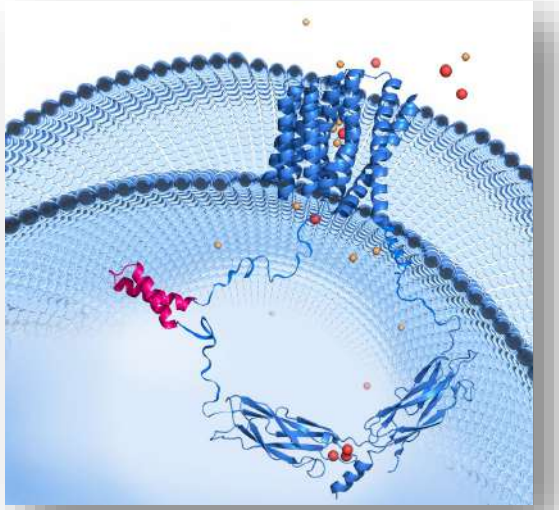
Nanoscale NMR enabled by diamond-based quantum sensor

- **Why NMR?**
- **Why nanoscale?**

Why NMR: a powerful molecular analytical technique

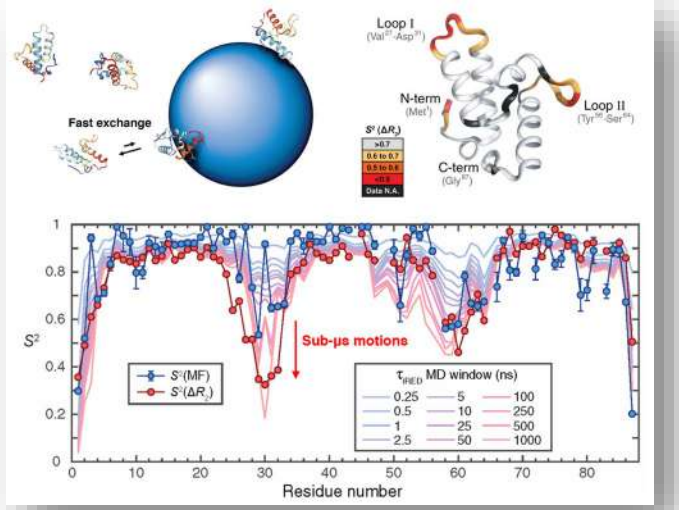
Ensemble molecules (~10^{16} spins)

- High spectral resolution
- No prerequisite of extrinsic labels
- Non-invasive
- Under (near-)physiological condition
- Probe dynamics on wide timescale



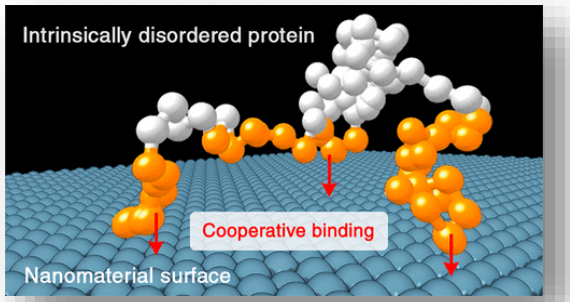
Structural determination

Yuan, Yuan, Xie *et al*. ***Biochemistry*** 2018, *57*, 5096



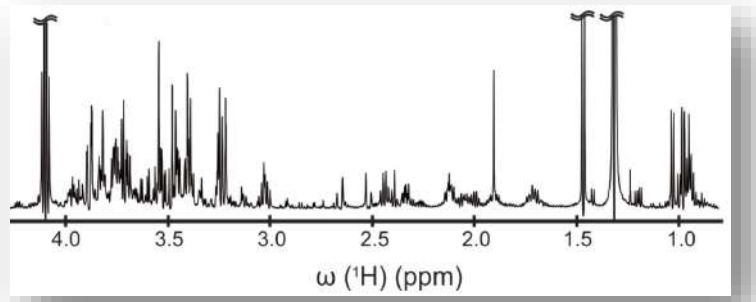
Protein dynamics

Xie & Yu *et al*. ***Sci. Adv***. 2019, *5*, eaax5560
Xie… *et al*. ***Angew. Chem.*** 2021, *133*, 150



Polymer physics

Li, Xie & Brüschweiler. ***JACS***. 2020, *142*, 10730
Xie *et al*. ***J. Phys. Chem. C.*** 2016, *120*, 24463

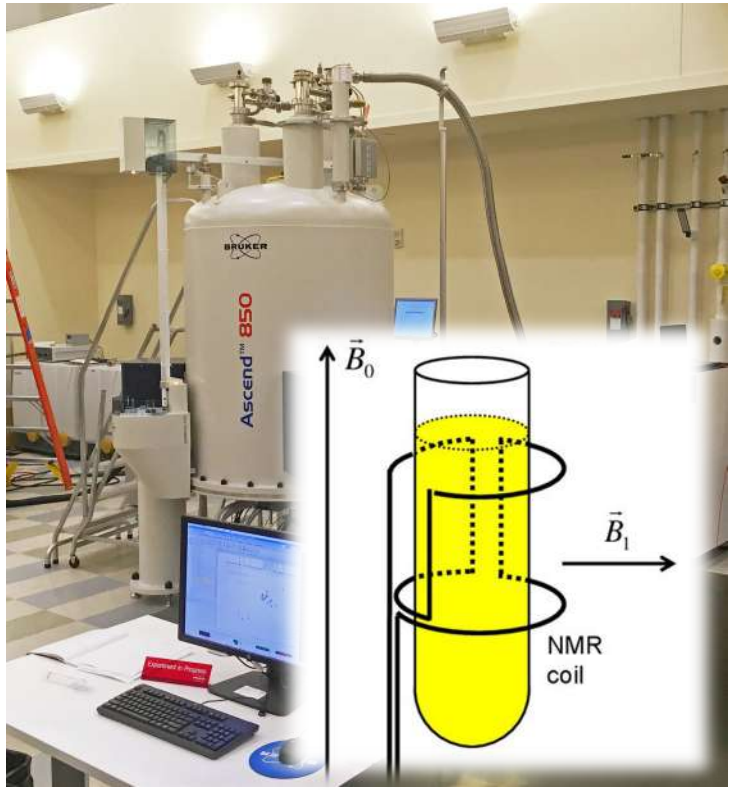


Metabolomics

Zhang, Xie *et al*. ***Anal. Chem***. 2016, *88,* 1003
Zhang, Xie *et al*. ***Metabolites***. 2018, *8*, 21

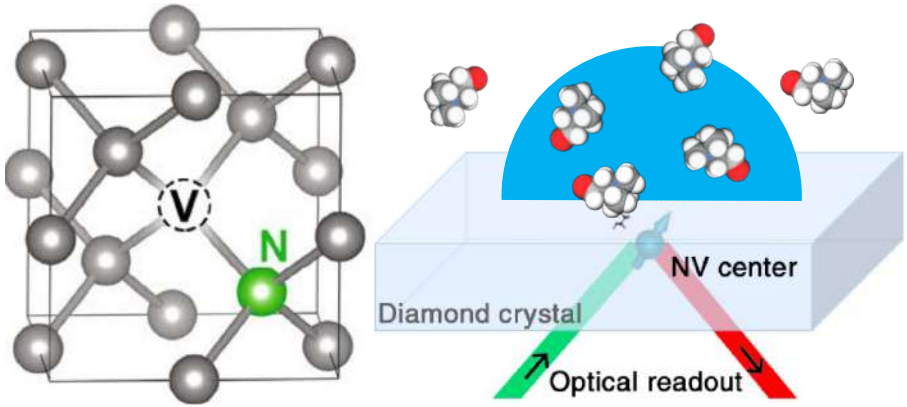
Why nanoscale: probe tiny systems that conventional NMR can't

Ensemble molecules (~10^{16} spins)



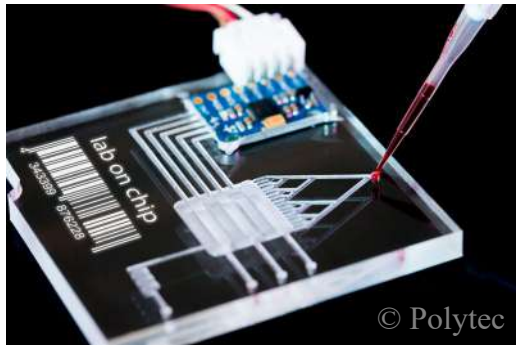
Coil-based detection:
macroscopic sample amount

Few molecules (<10^5 spins)

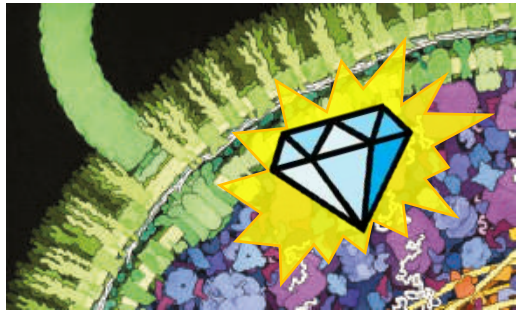


Nitrogen-vacancy (NV) center in diamond crystal can be used as a **quantum sensor** for **nanoscale NMR/EPR**

Technology invented and developed at:
Stuttgart, Harvard, UCSB, IBM, USTC…
in the past two decades



Lab-on-a-chip analytics



In situ detection

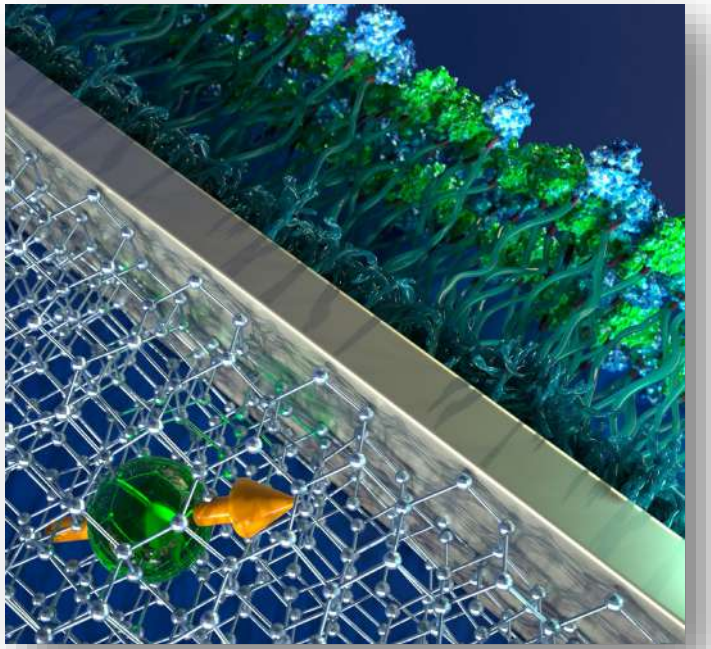
Portable device

Single-cell physiology

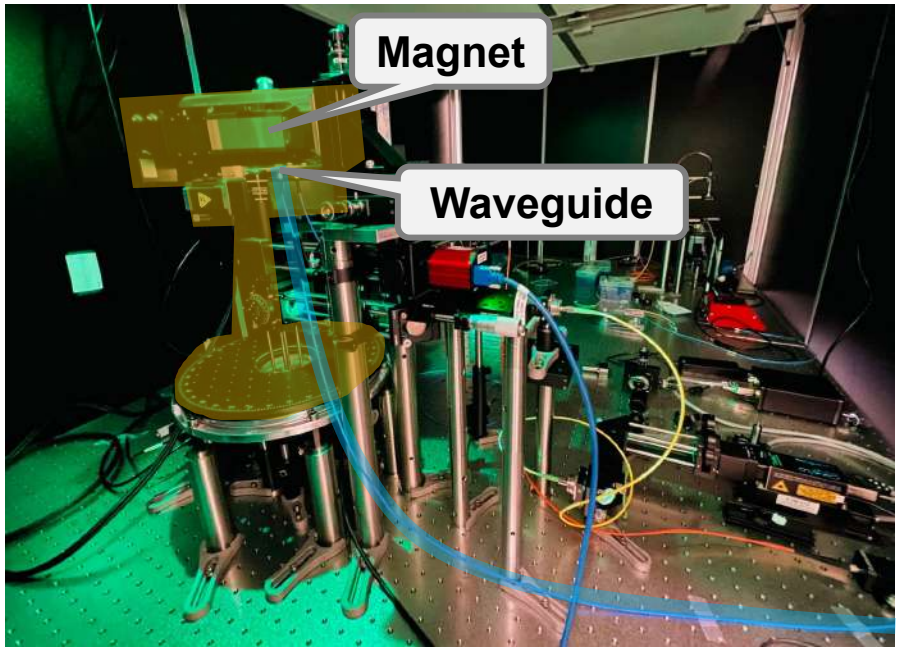
…

Outline

- NV-NMR sensor mechanism
- Biocompatible diamond surface functionalization
- Integrated sensing platform based on thin diamond membrane
- Conclusion & Outlook



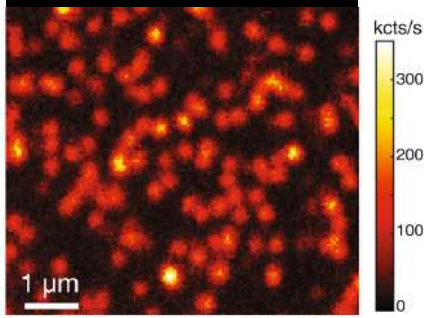
NV sensing mechanism

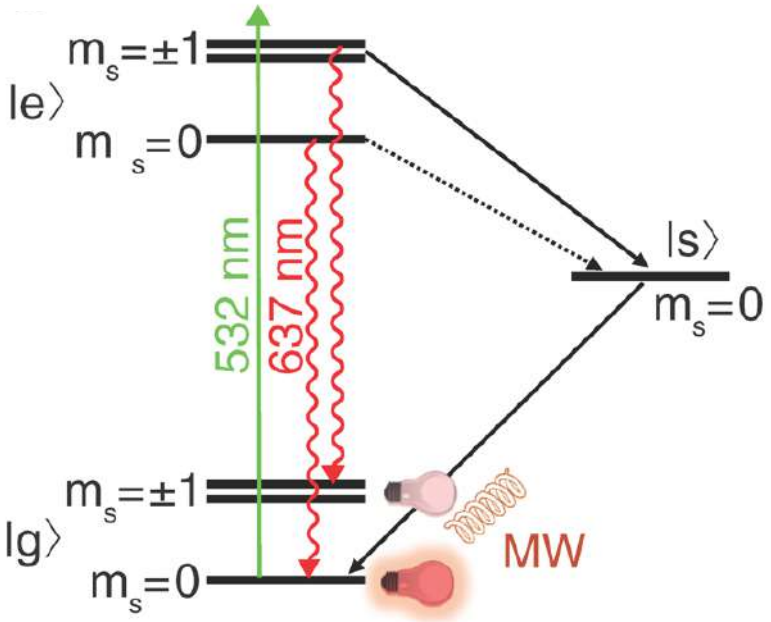


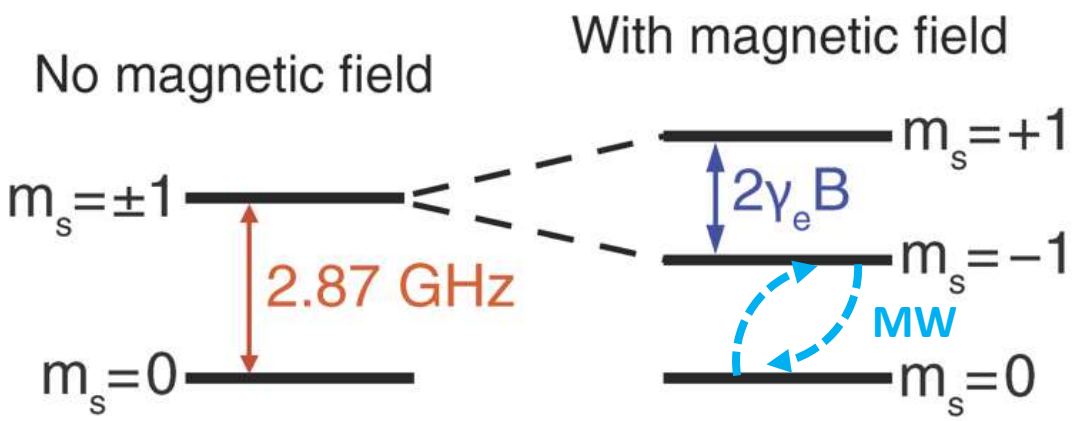
Contains shallow NVs

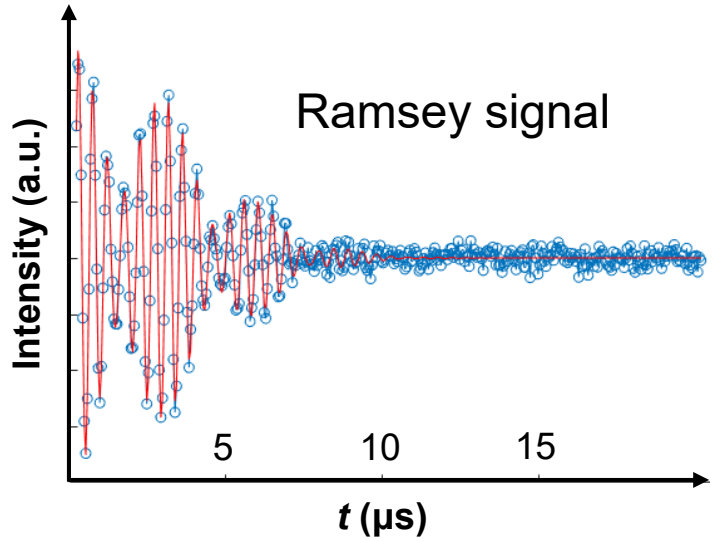
Diamond

Confocal scan



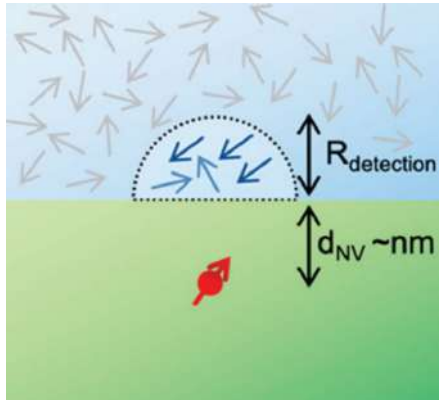






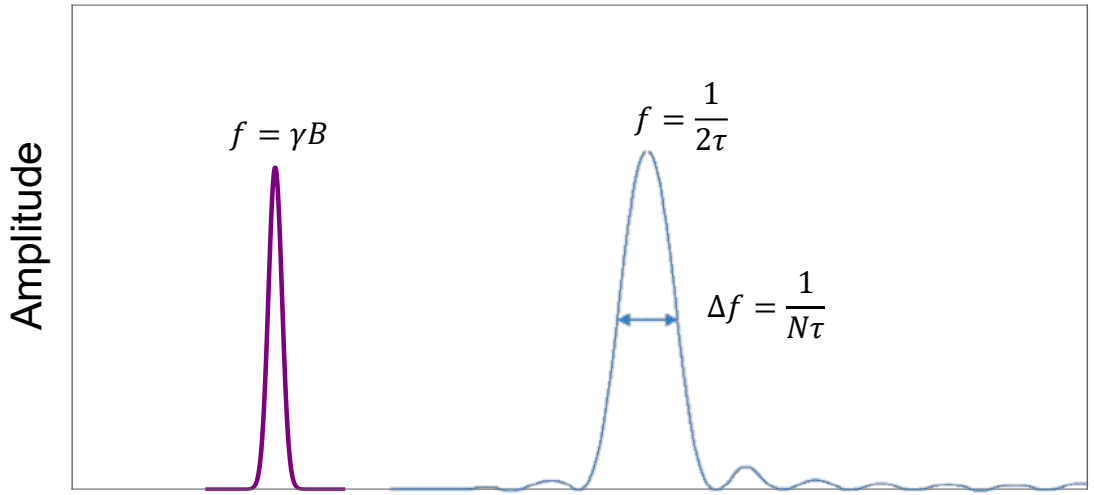
Room temperature

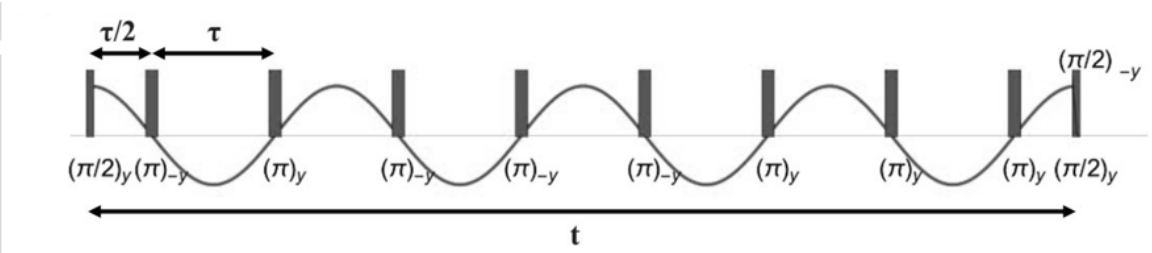
Detecting oscillating NMR signal through dynamical decoupling



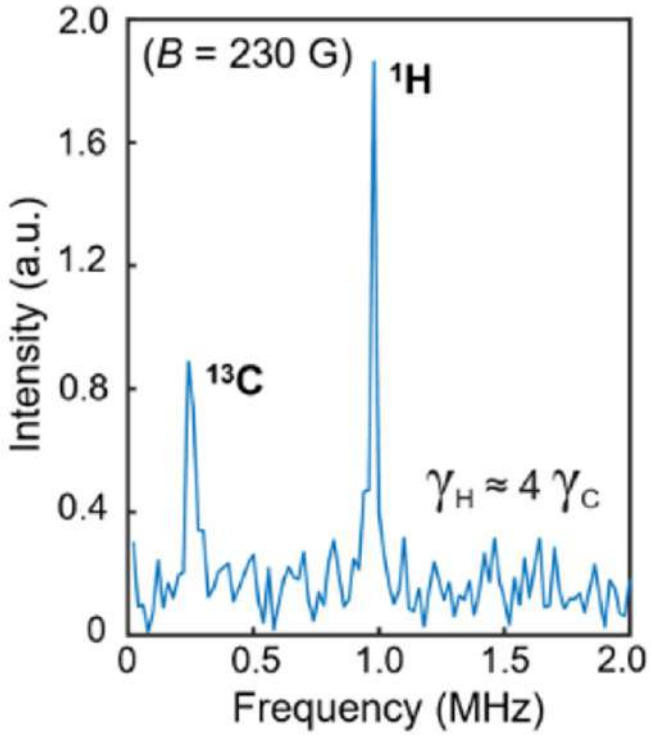
Sensitivity:
(10-1000) nT / $\sqrt{\text{Hz}}$

Frequency domain





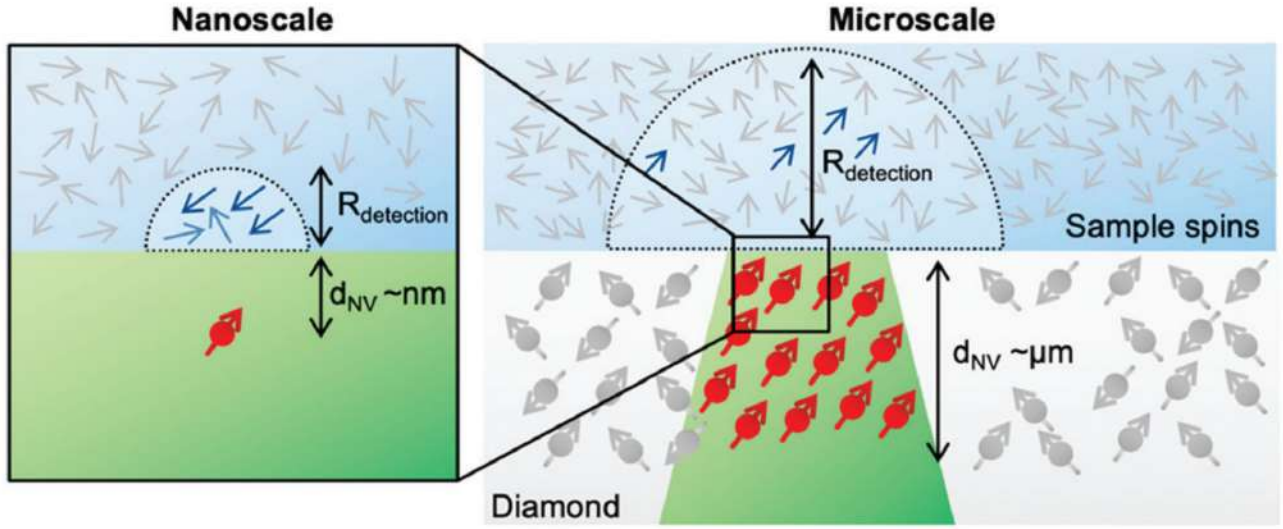
Dynamical decoupling on NV



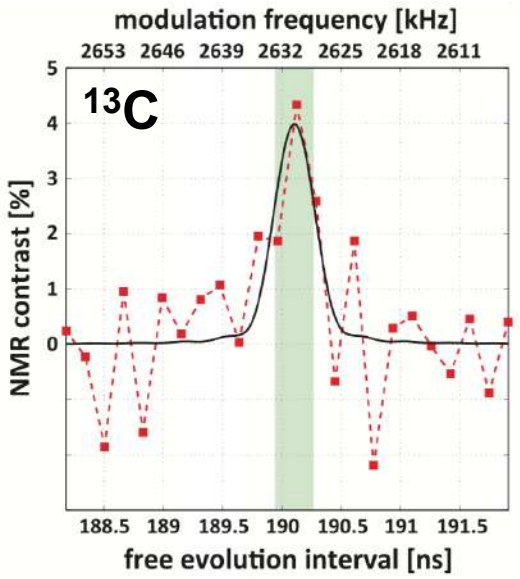
NV-NMR spectrum of objective oil (organic molecule)

Taking advantage of conventional NMR techniques

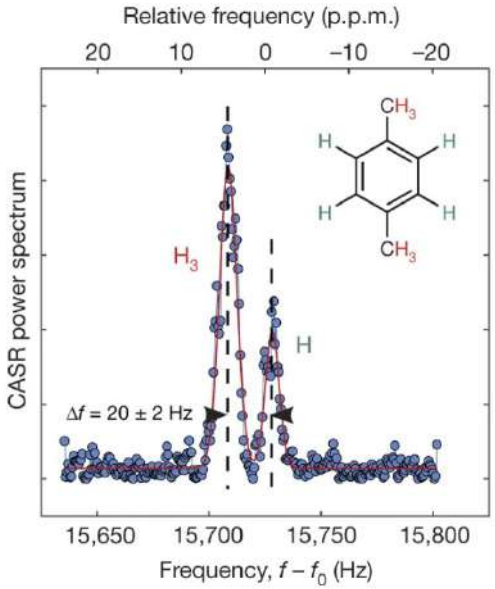
Domains of NV-NMR detection



Allert *et al.*, *Chem. Commun*. 2022, *58*, 8165



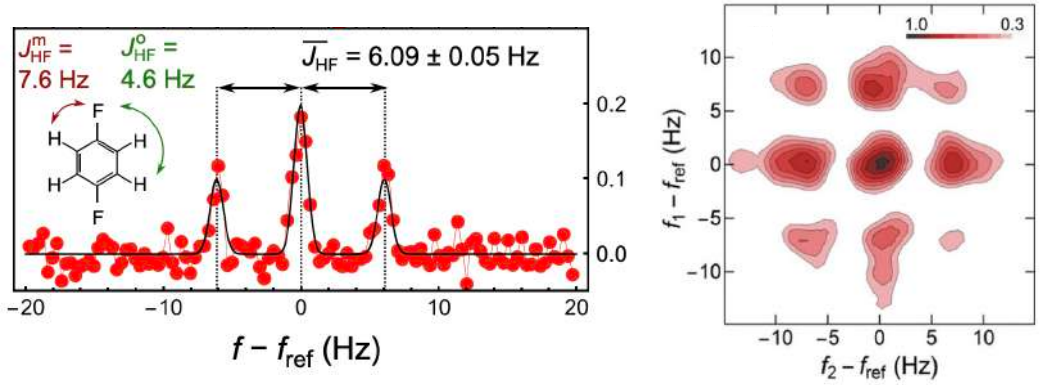
Lovchinsky *et al.*,
Science 2016, *351*, 836



Glenn & Bucher *et al.*,
Nature 2018, *555*, 351

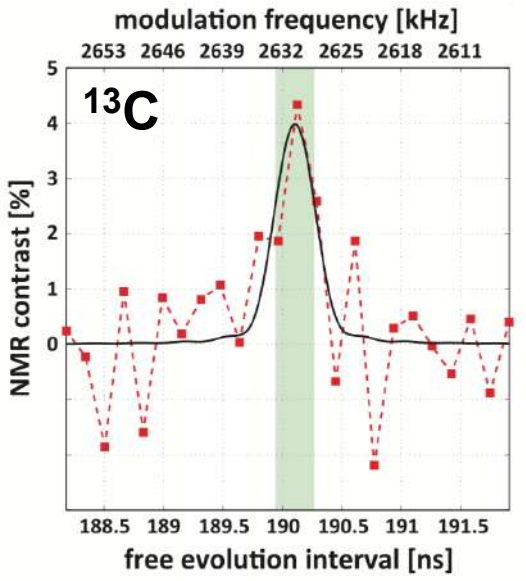
- Individual protein sensitivity
- Low spectral resolution

- High spectral resolution
- Low sensitivity (pL volume)



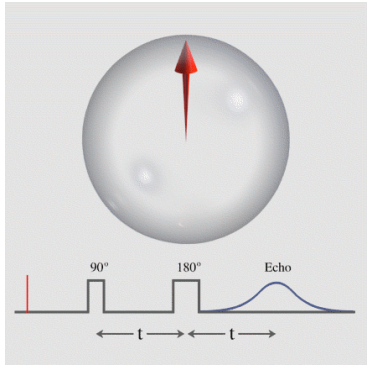
Smits *et al.*, *Sci. Adv*. 2019, *5*, aaw7895

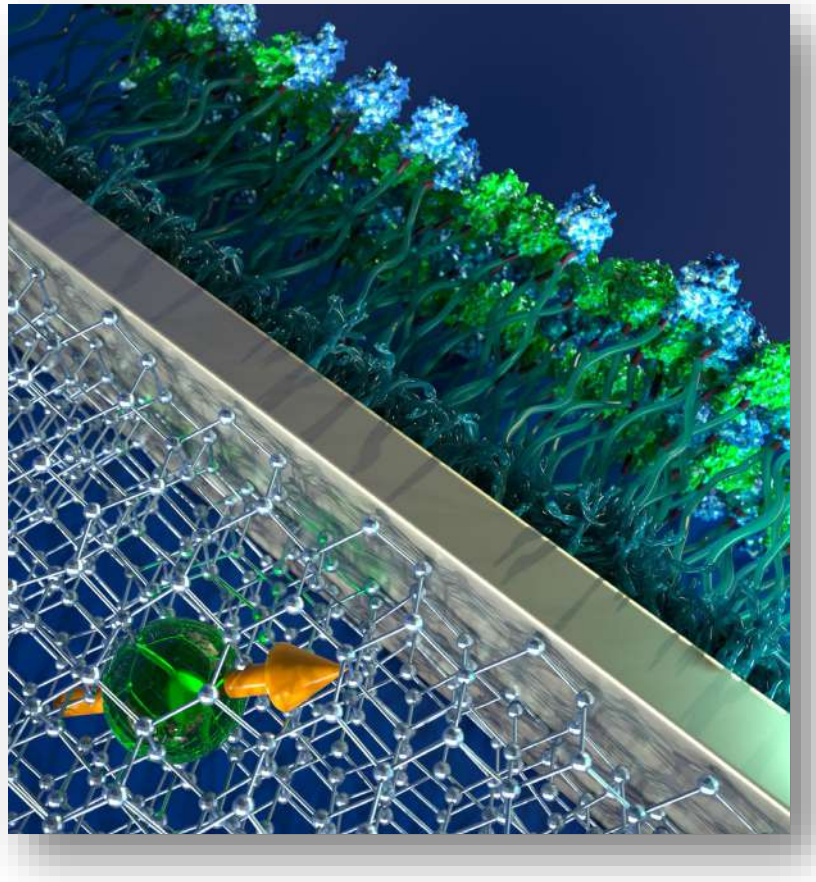
Missing piece: biocompatible interfacing methods



Lovchinsky *et al.*,
Science 2016, *351*, 836

Surface-dried ubiquitin sample soaked in objective oil

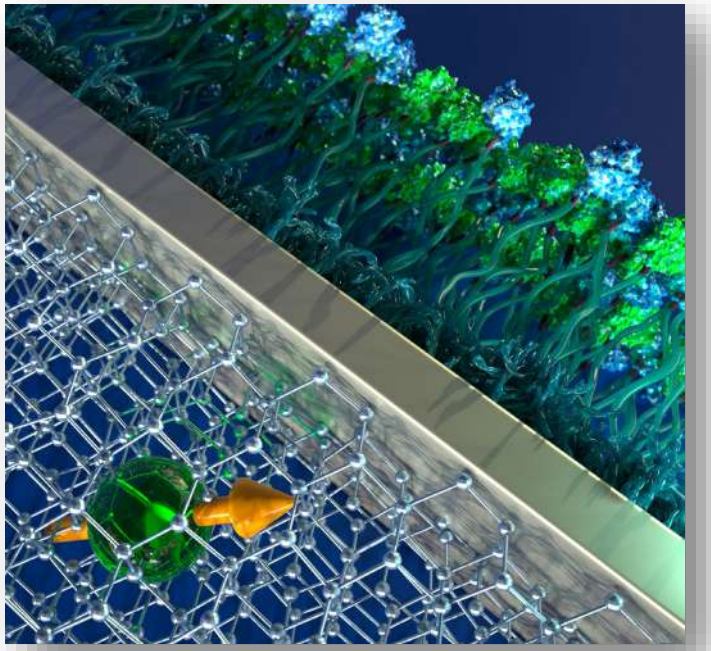




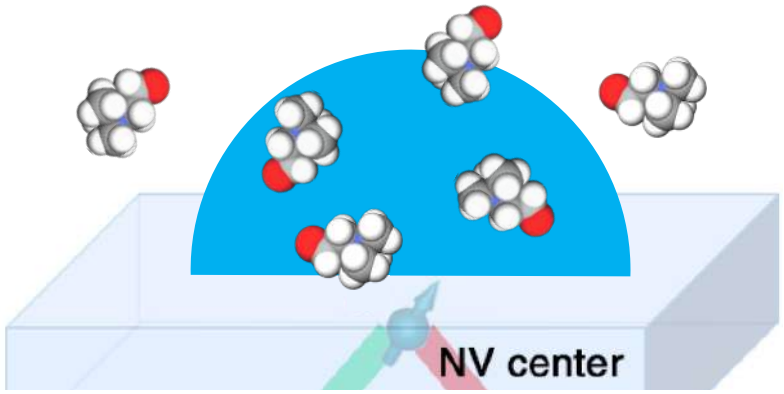
We need a **biocompatible surface functionalization** method to interface target molecules with diamond quantum sensor

Outline

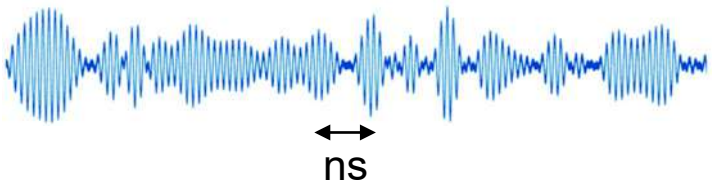
- NV-NMR sensor mechanism
- Biocompatible diamond surface functionalization
- Integrated sensing platform based on thin diamond membrane
- Conclusion & Outlook

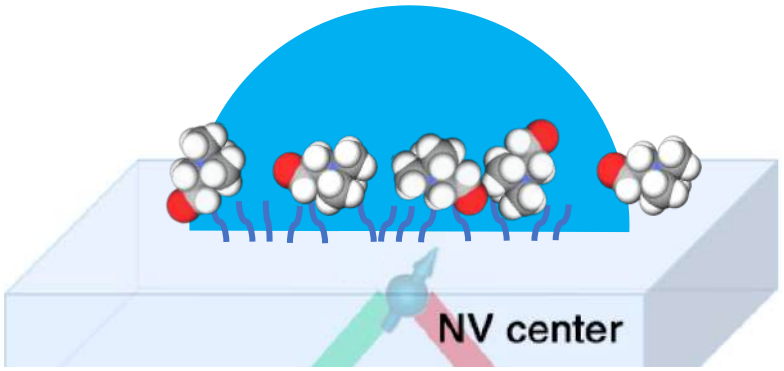


Motivation: why immobilize biomolecules on diamond surface?



Diffusing





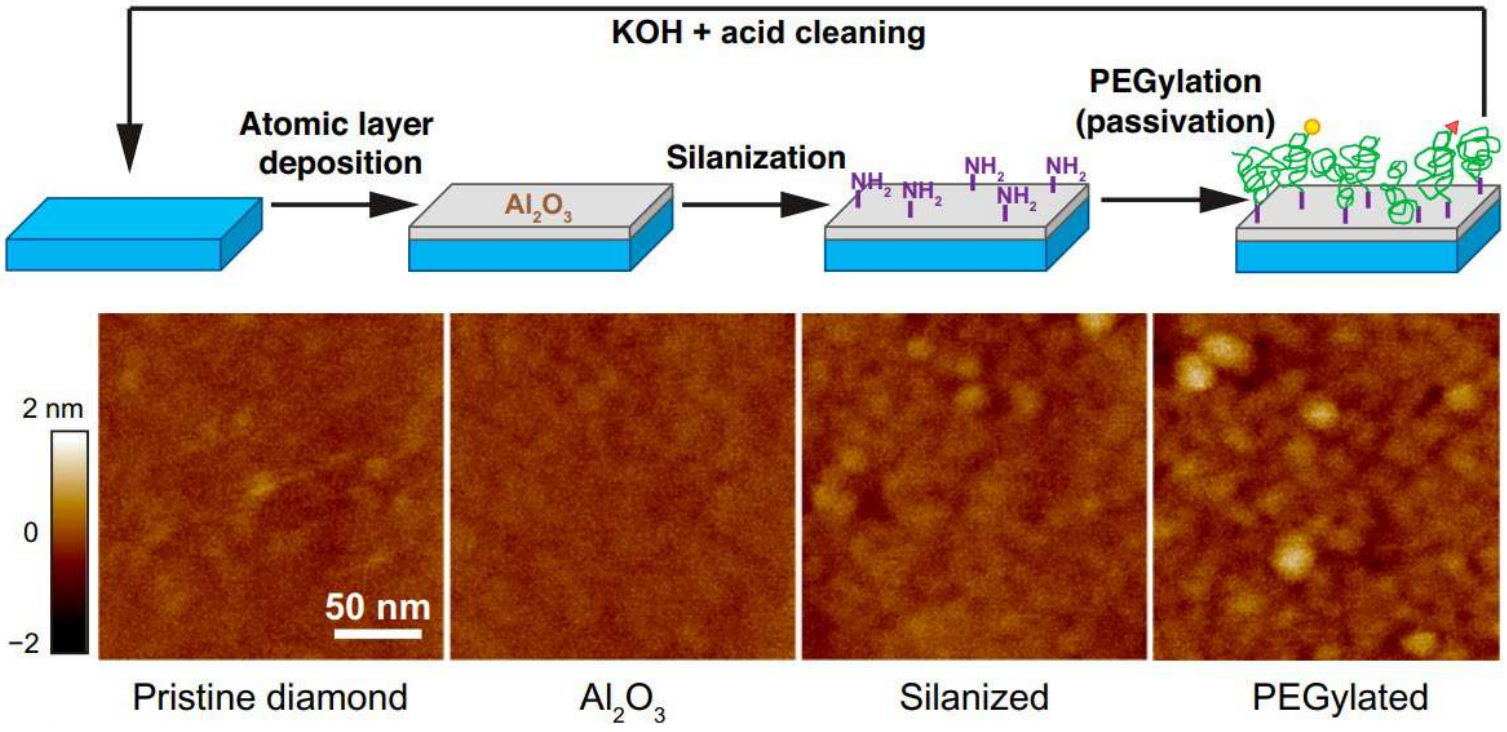
Immobilized

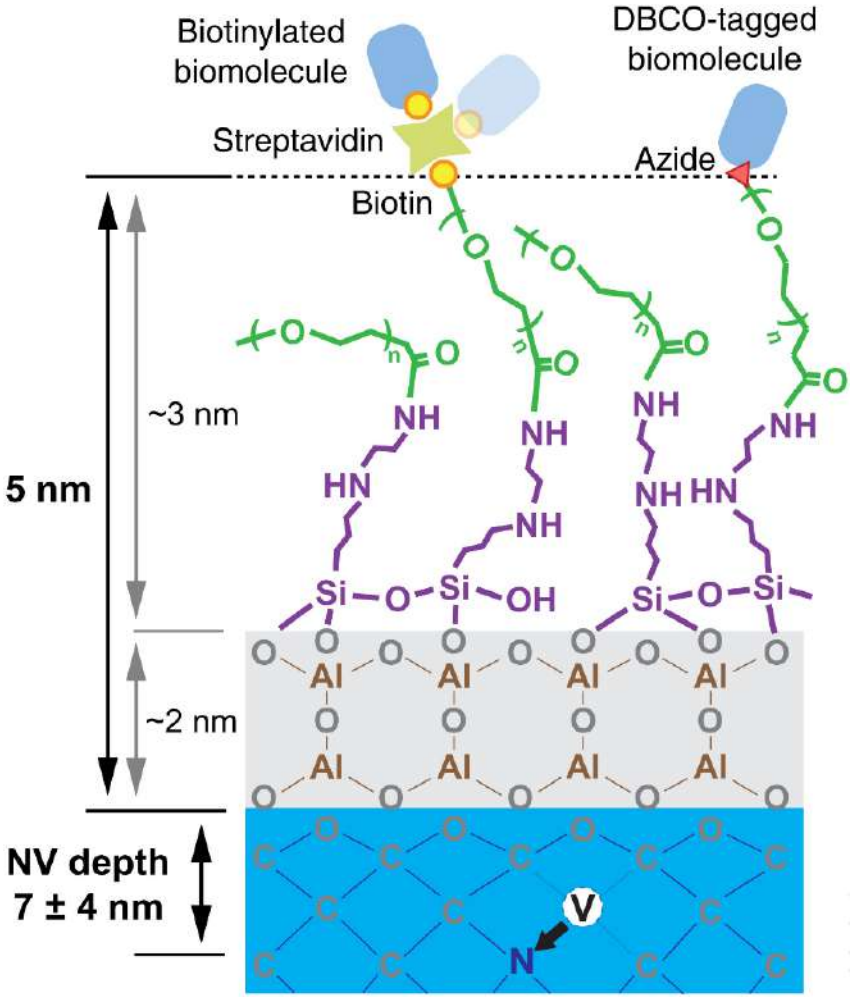
- Increase the effective concentration
- Reduce diffusion noise
- Investigating the same molecules over a long time

Criteria to satisfy

- Retain sensor coherence
- Thin (tunable thickness)
- Biocompatible
- Specific conjugation
- Stable
- Recyclable

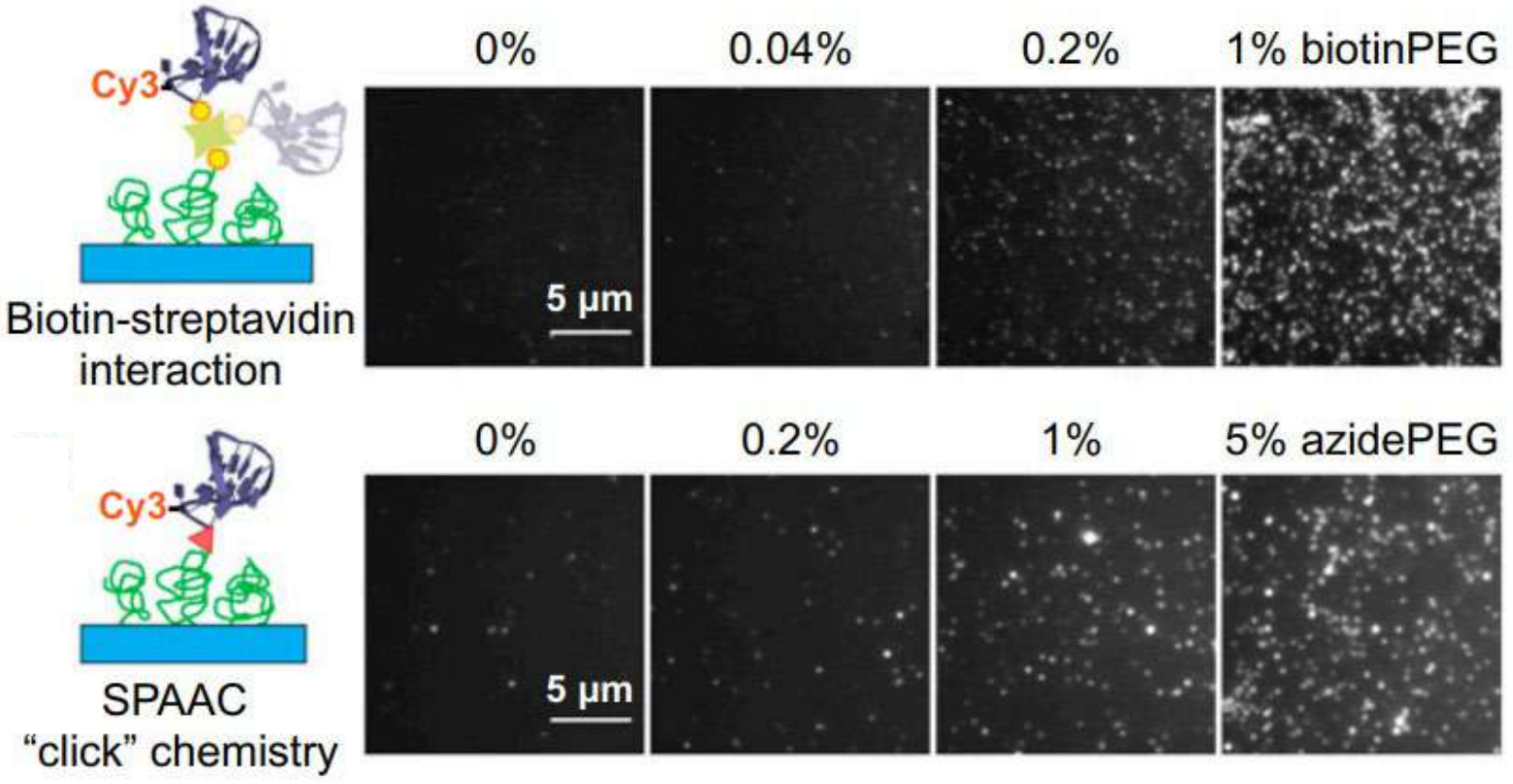
Biocompatible diamond surface functionalization architecture

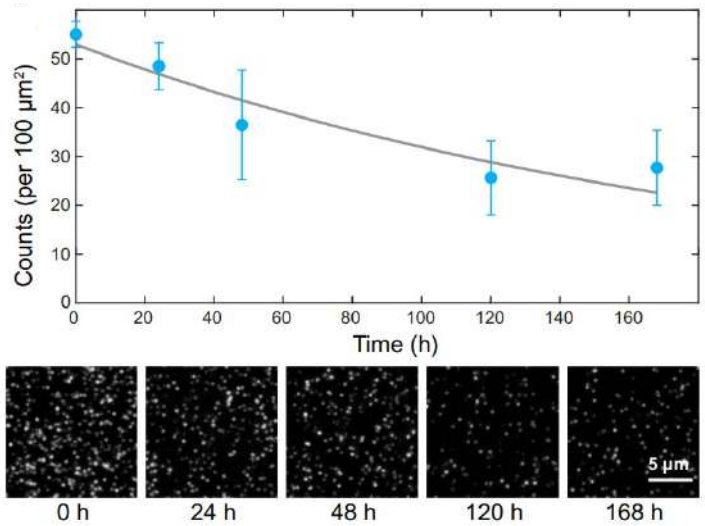




Xie & Yu *et al*. ***PNAS*** 2022, *119*, e2114186119

Immobilizing ssDNA via two conjugation methods



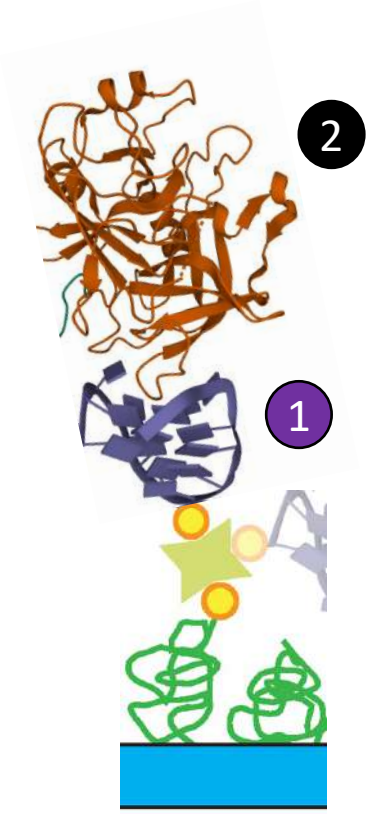


Stable for days
(phosphate buffer pH 7.4)

Single-molecule fluorescence microscopy

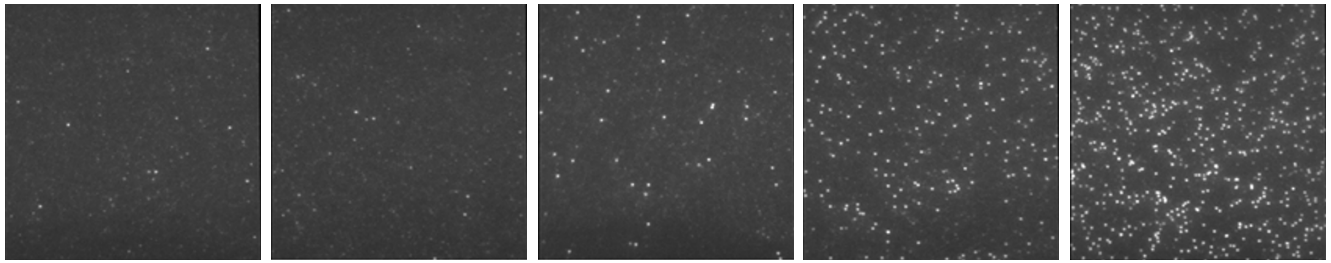
Xie & Yu *et al*. ***PNAS*** 2022, *119*, e2114186119

Molecular “pull-down” by ssDNA aptamer

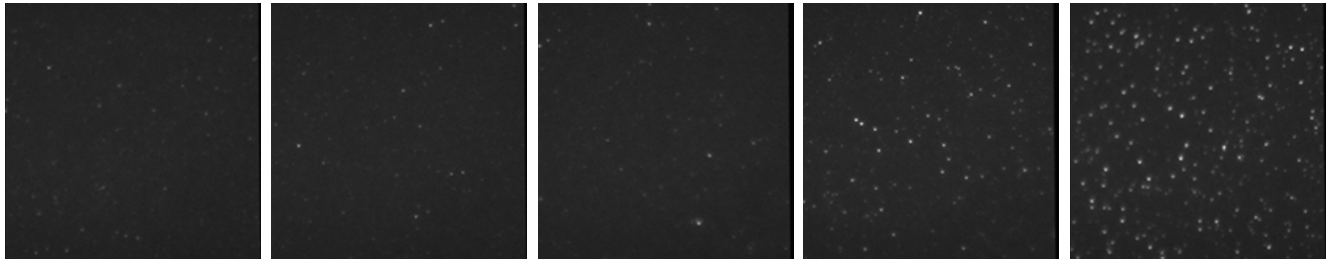


K_D ~5 nM

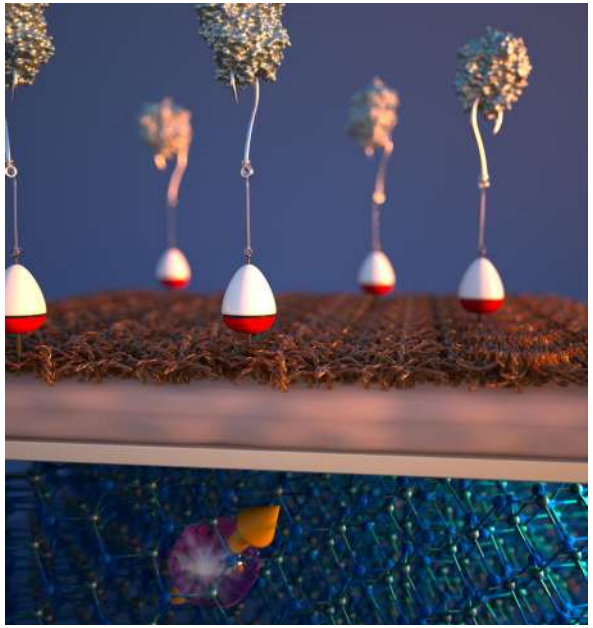
1 Only label **aptamer** (HD22)



2 Only label **thrombin**



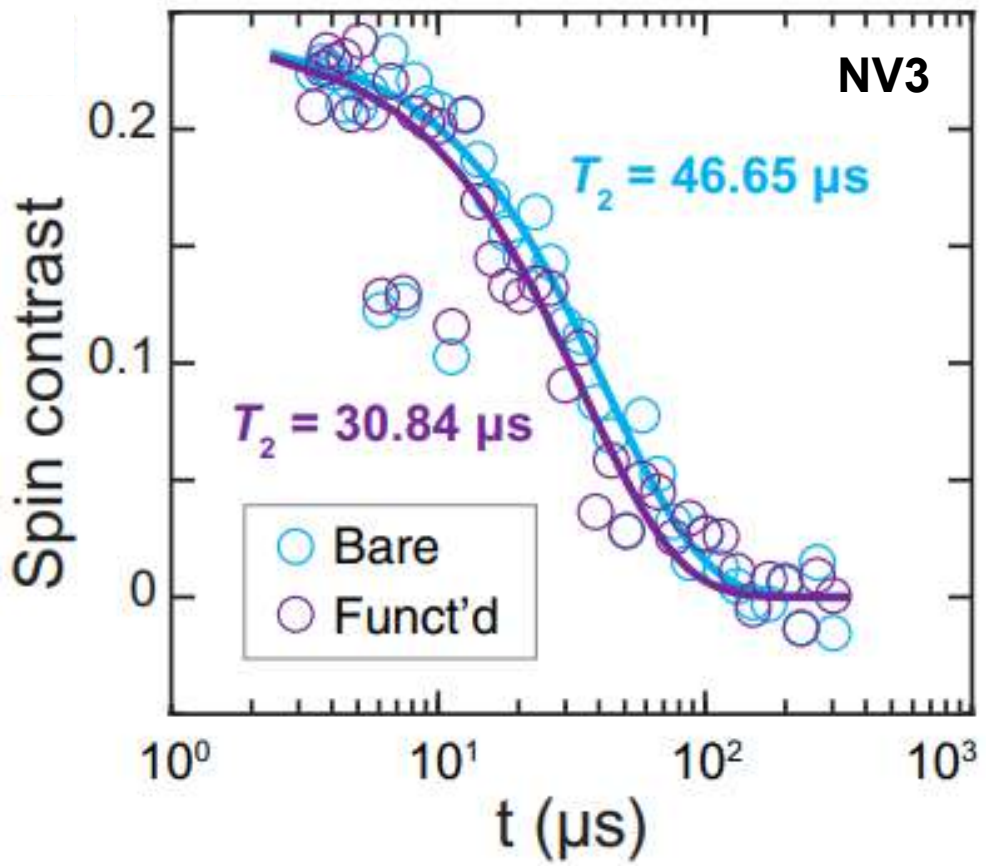
Increasing biotinPEG percentage

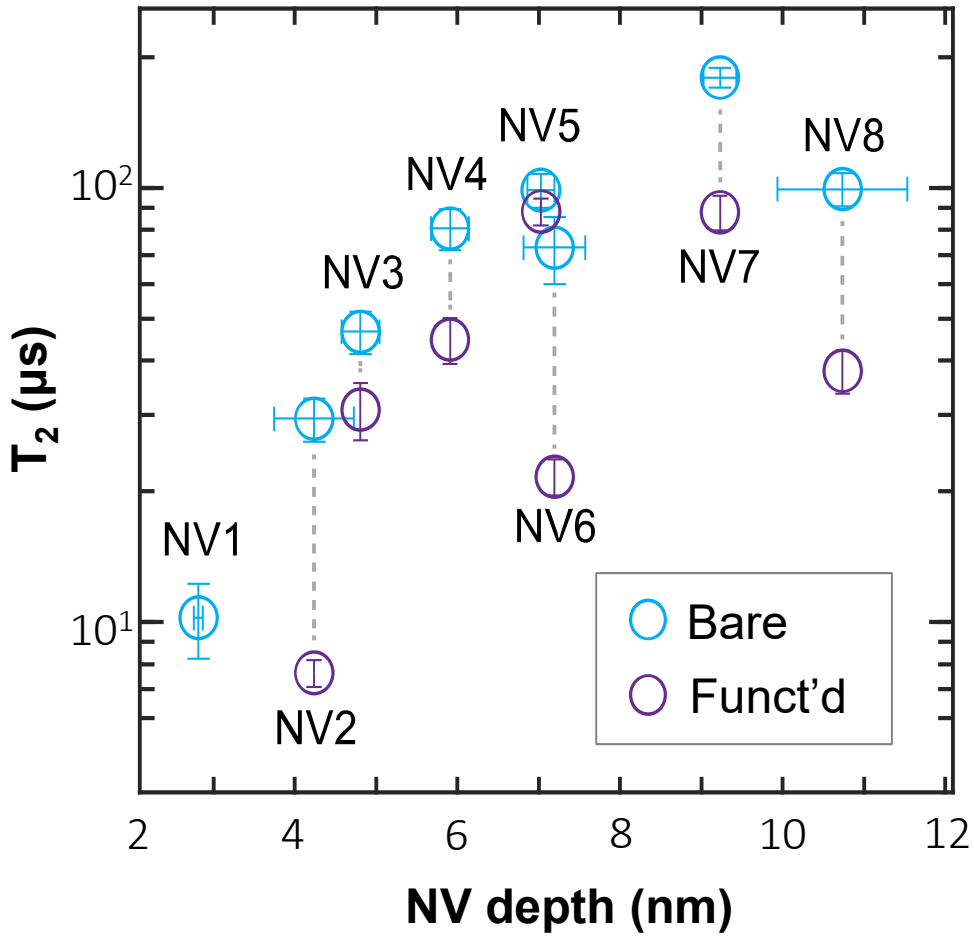


Preliminary data acquired on glass coverslips
PDB: 4DIH

Impact on NV coherence: minimal

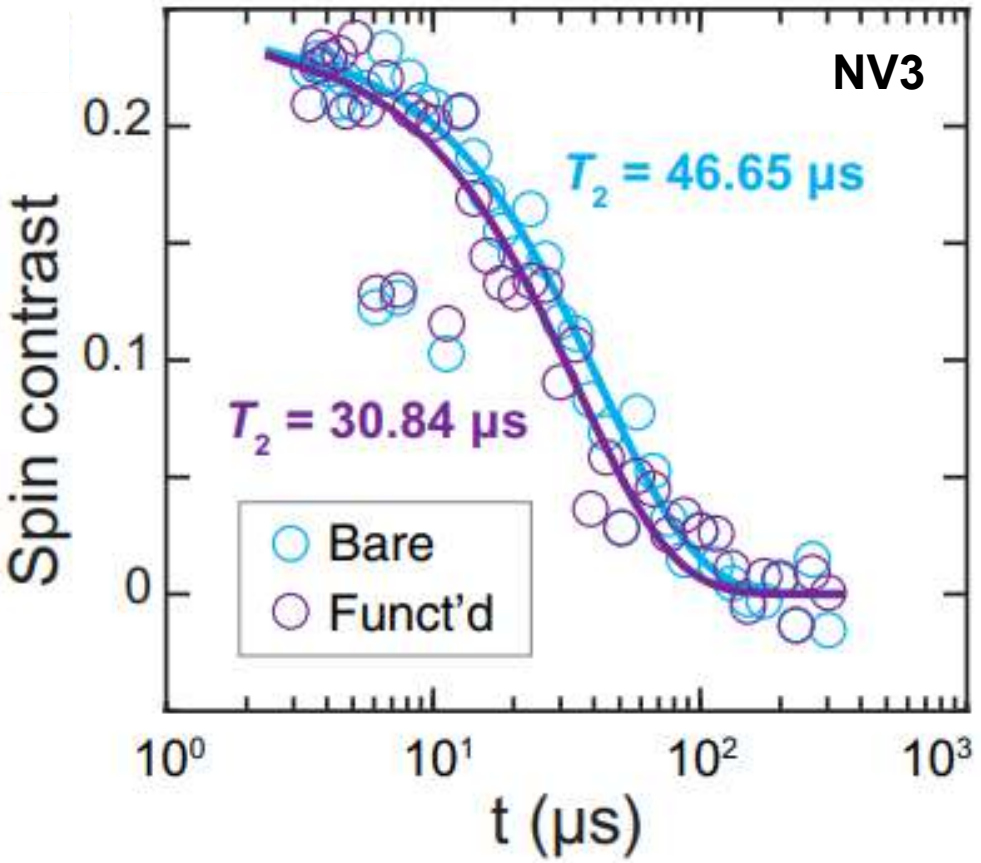
15 ± 18% reduction in T_2 by spin-echo sequence
49 ± 22% reduction in T_2 by $(YY\text{-}8)_{N=8}$



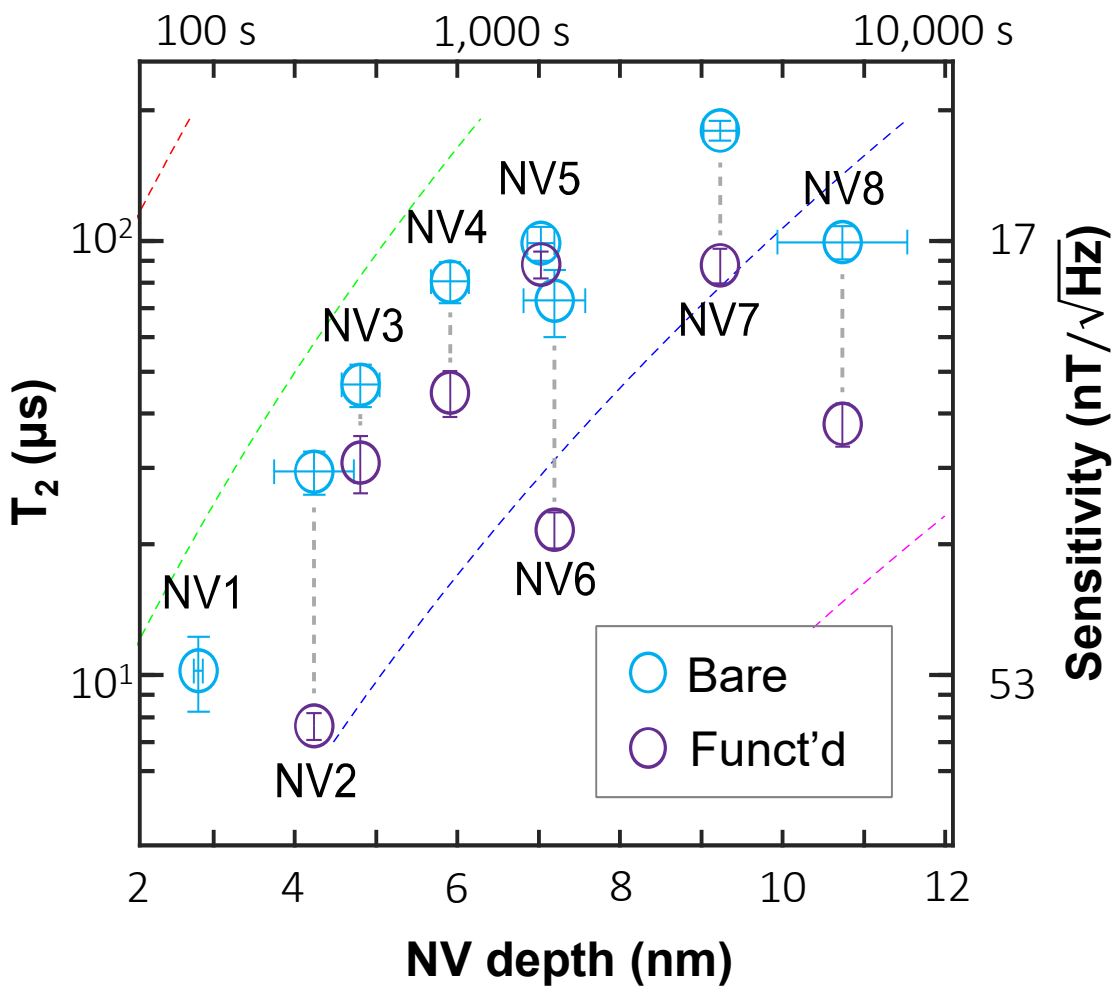


Xie & Yu *et al*. ***PNAS*** 2022, *119*, e2114186119

Impact on NV coherence: minimal



Integration time required for detecting a single ^{1}H spin with SNR = 1 and 5 nm thick functionalization layer



Xie & Yu *et al*. ***PNAS*** 2022, *119*, e2114186119

Quick notes

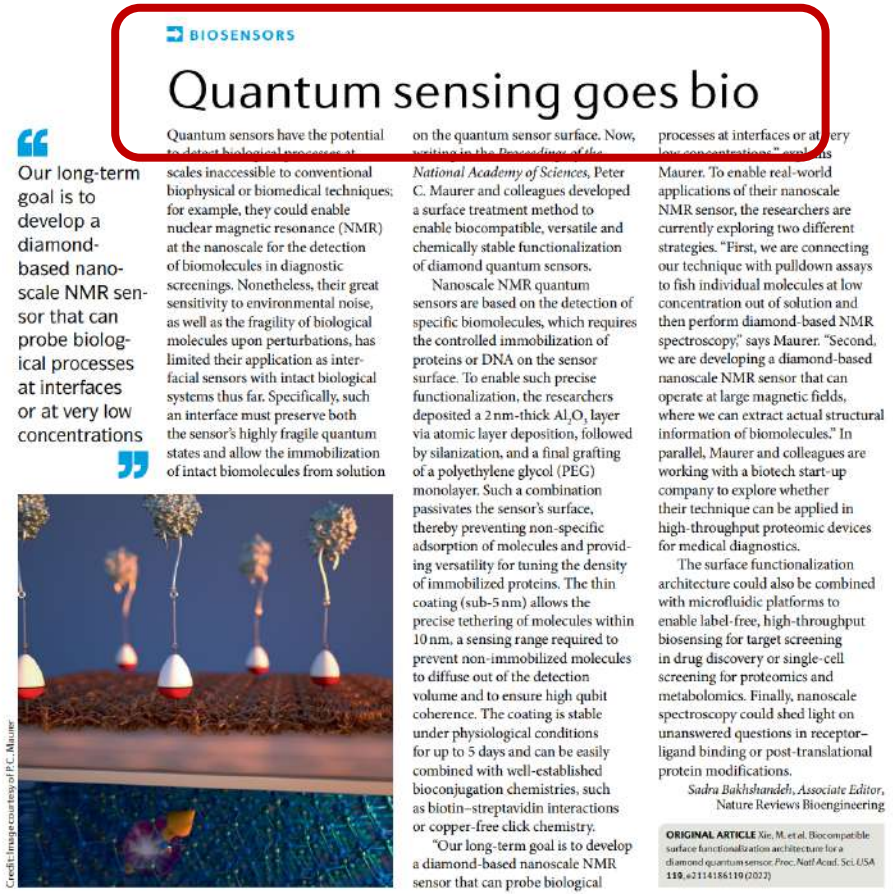
- We developed a **biocompatible**, **versatile**, **stable**, and **recyclable** functionalization method on the surface of diamond quantum sensor with long spin coherence

Commonly used labeling techniques

Aptamer-mediated "pull-down"

Specifically attach molecules of interest for NV sensing under physiological conditions

A step closer to real-world applications



BIOSENSORS

Quantum sensing goes bio

"Our long-term goal is to develop a diamond-based nanoscale NMR sensor that can probe biological processes at interfaces or at very low concentrations"

Quantum sensors have the potential to detect biological processes at scales inaccessible to conventional biophysical or biomedical techniques; for example, they could enable nuclear magnetic resonance (NMR) at the nanoscale for the detection of biomolecules in diagnostic screenings. Nonetheless, their great sensitivity to environmental noise, as well as the fragility of biological molecules upon perturbations, has limited their application as interfacial sensors with intact biological systems thus far. Specifically, such an interface must preserve both the sensor's highly fragile quantum states and allow the immobilization of intact biomolecules from solution on the quantum sensor surface. Now, writing in the *Proceedings of the National Academy of Sciences*, Peter C. Maurer and colleagues developed a surface treatment method to enable biocompatible, versatile and chemically stable functionalization of diamond quantum sensors.

Nanoscale NMR quantum sensors are based on the detection of specific biomolecules, which requires the controlled immobilization of proteins or DNA on the sensor surface. To enable such precise functionalization, the researchers deposited a 2 nm-thick Al_2O_3 layer via atomic layer deposition, followed by silanization, and a final grafting of a polyethylene glycol (PEG) monolayer. Such a combination passivates the sensor's surface, thereby preventing non-specific adsorption of molecules and providing versatility for tuning the density of immobilized proteins. The thin coating (sub-5 nm) allows the precise tethering of molecules within 10 nm, a sensing range required to prevent non-immobilized molecules to diffuse out of the detection volume and to ensure high qubit coherence. The coating is stable under physiological conditions for up to 5 days and can be easily combined with well-established bioconjugation chemistries, such as biotin–streptavidin interactions or copper-free click chemistry.

"Our long-term goal is to develop a diamond-based nanoscale NMR sensor that can probe biological processes at interfaces or at very low concentrations," explains Maurer. To enable real-world applications of their nanoscale NMR sensor, the researchers are currently exploring two different strategies. "First, we are connecting our technique with pulldown assays to fish individual molecules at low concentration out of solution and then perform diamond-based NMR spectroscopy," says Maurer. "Second, we are developing a diamond-based nanoscale NMR sensor that can operate at large magnetic fields, where we can extract actual structural information of biomolecules." In parallel, Maurer and colleagues are working with a biotech start-up company to explore whether their technique can be applied in high-throughput proteomic devices for medical diagnostics.

The surface functionalization architecture could also be combined with microfluidic platforms to enable label-free, high-throughput biosensing for target screening in drug discovery or single-cell screening for proteomics and metabolomics. Finally, nanoscale spectroscopy could shed light on unanswered questions in receptor–ligand binding or post-translational protein modifications.

Sadra Bakhshandeh, Associate Editor, Nature Reviews Bioengineering

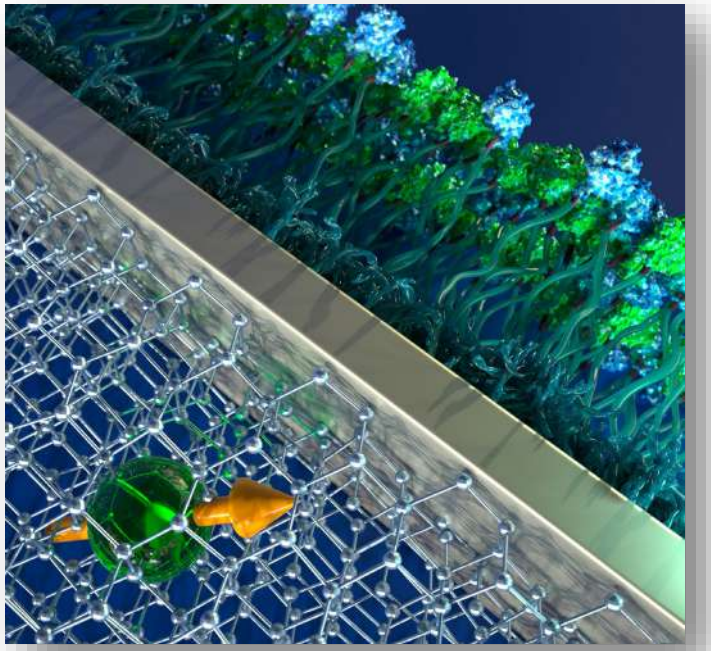
ORIGINAL ARTICLE Xie, M. et al. Biocompatible surface functionalization architecture for a diamond quantum sensor. *Proc. Natl Acad. Sci. USA* **119**, e2114186119 (2022)

Credit: Image courtesy of P.C. Maurer

Research highlight in *Nat. Rev. Mater.*

Outline

- NV-NMR sensor mechanism
- Biocompatible diamond surface functionalization
- Integrated sensing platform based on thin diamond membrane
- Conclusion & Outlook

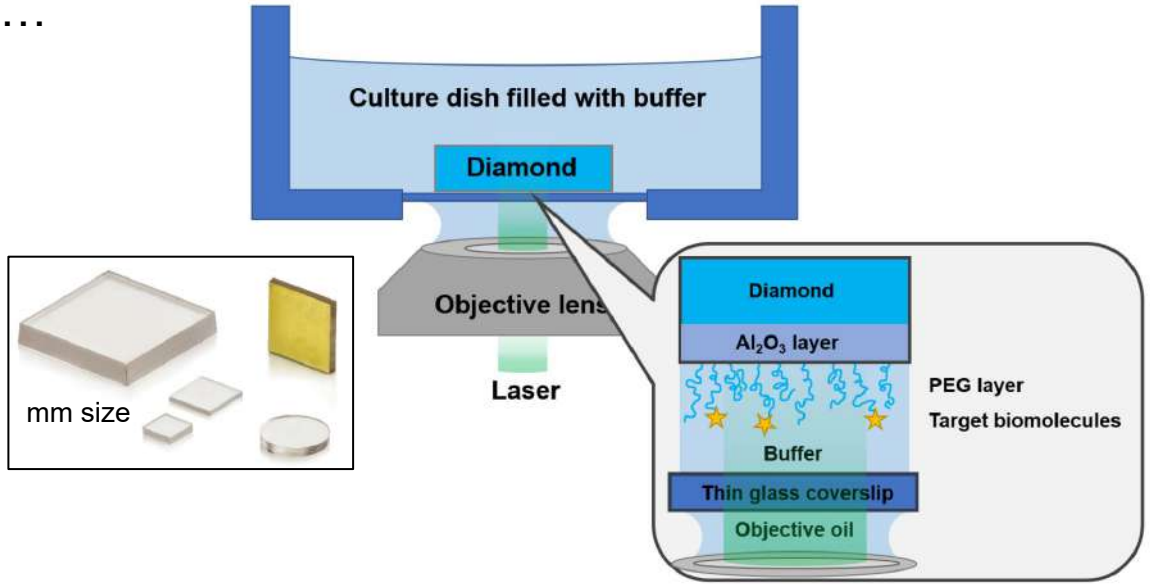


Motivation: novel platform based on diamond-membrane

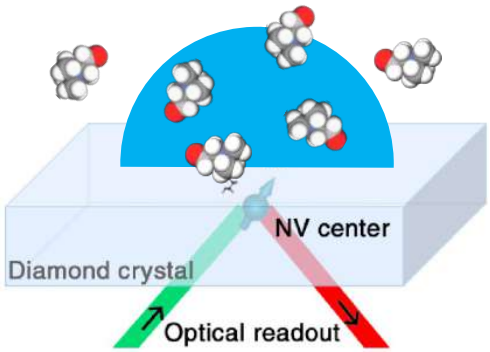
When study a biological system, it is common to:

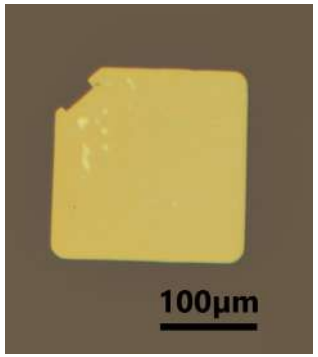
- Attach/detach samples repeatedly
- Add or remove substrate, binding partner, co-factor etc.
- Change conditions such as pH or salt concentration
- Apply chemogradient

…



Difficult to rapidly apply environmental change

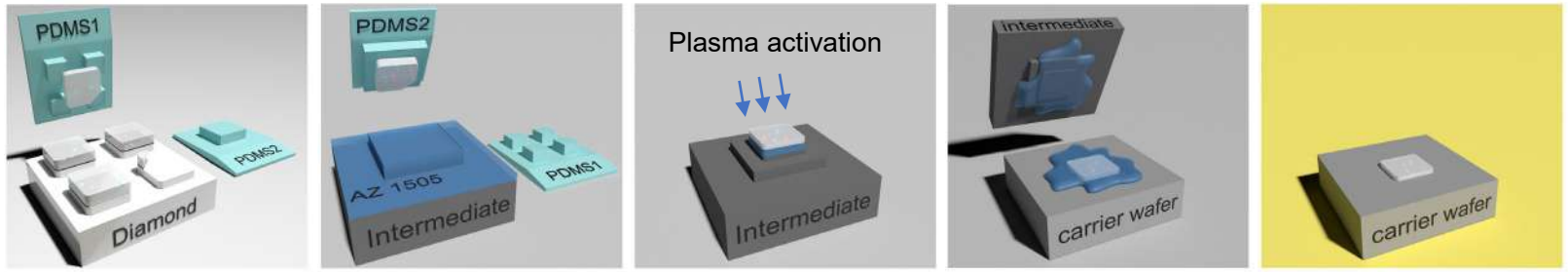


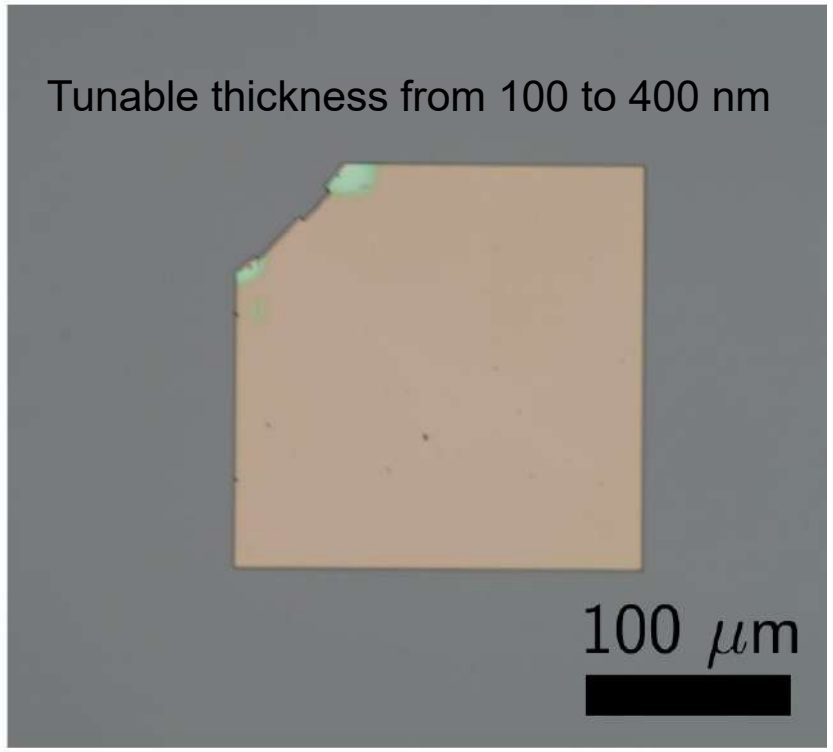


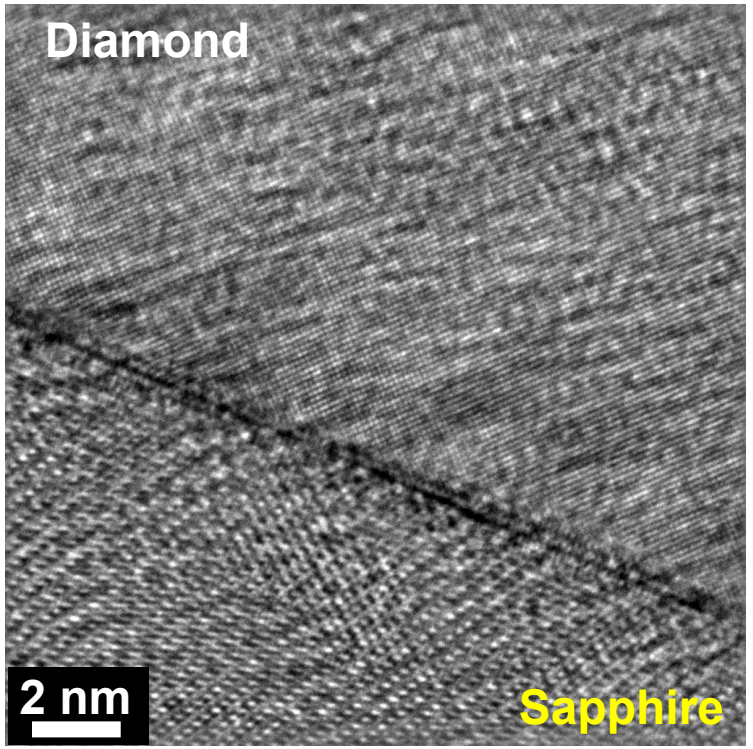
Diamond membrane?

Nanofabrication of diamond membrane-based heterostructure

“smart-cut”







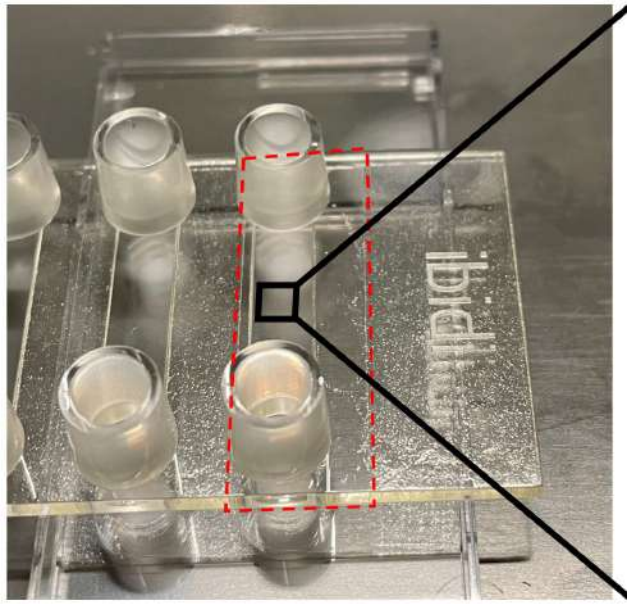
THE UNIVERSITY OF CHICAGO

Prof. Alex High

Xinghan Guo

Guo & Xie *et al*. Manuscript in preparation

Sensing platform based on diamond membrane heterostructure



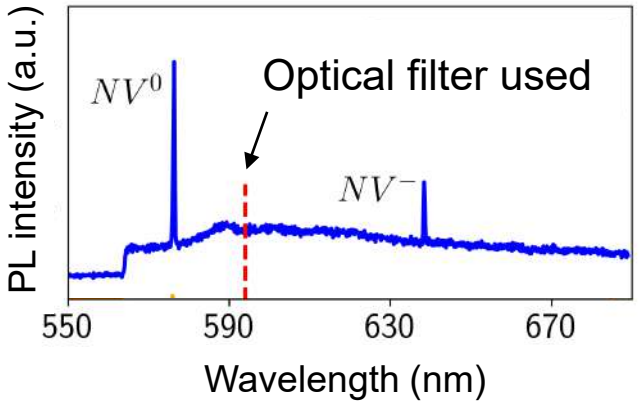
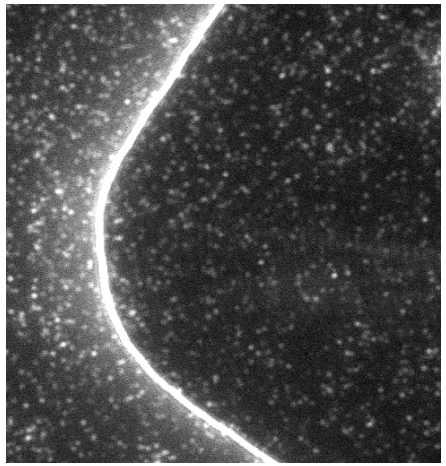


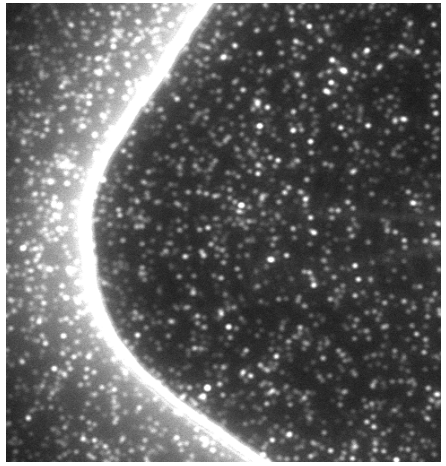
Figure removed due to confidentiality

NV centers under wide-field fluorescence microscope

Quantum properties as good as bulk diamond



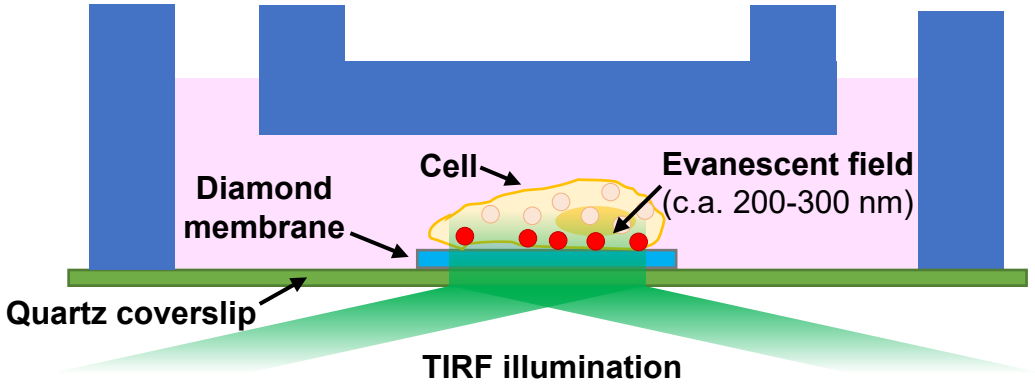
Alexa488-labeled streptavidin

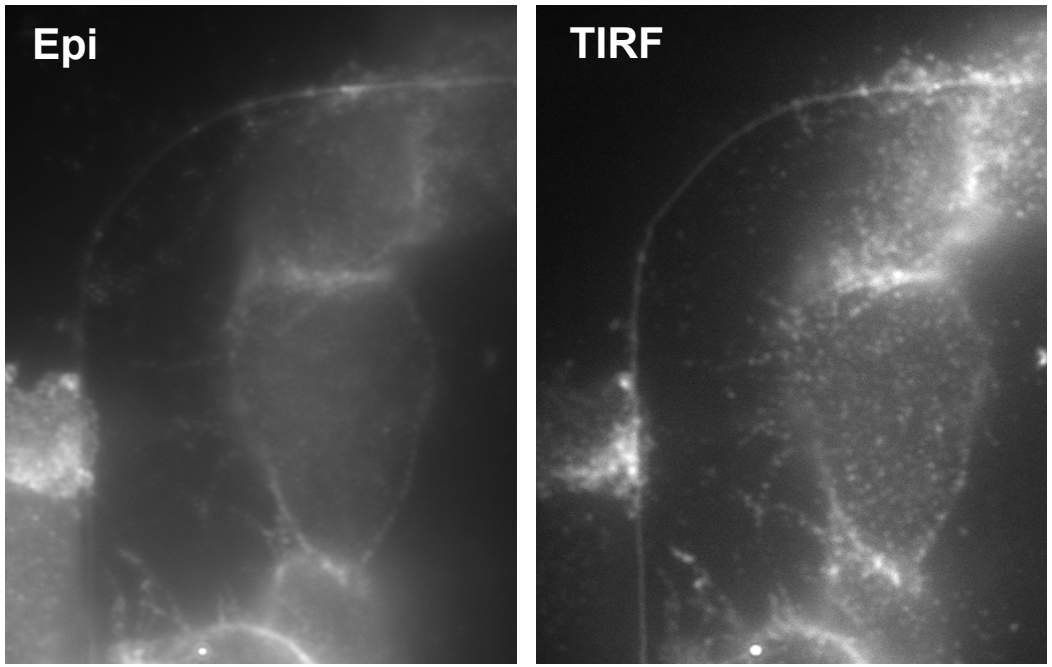


Streptavidin-conjugated QDot-525

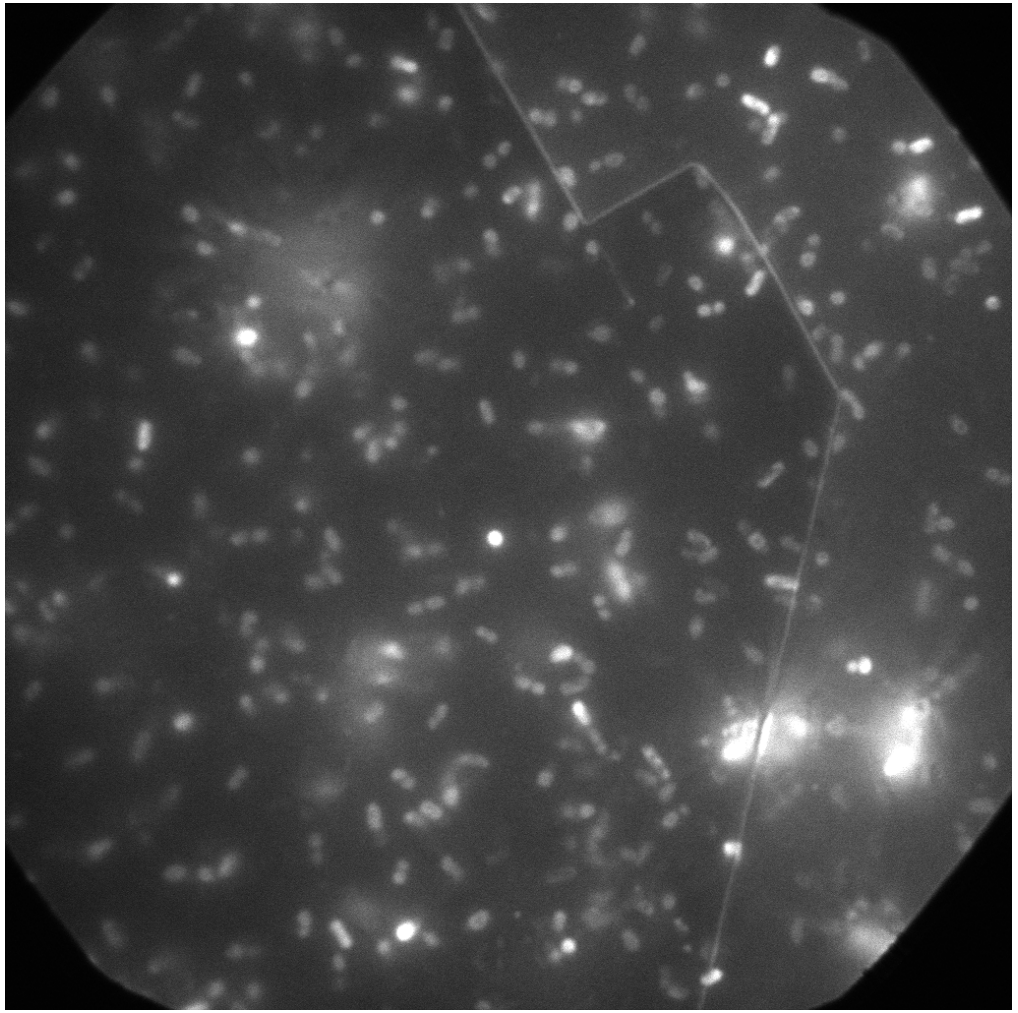
Guo & Xie *et al*. Manuscript in preparation

Sensing platform based on diamond membrane heterostructure





Toll-like receptors (dye-labeled) on RAW cell membrane



Living *E.coli* bacteria (that over-expressing GFP protein)

Guo & Xie *et al*. Manuscript in preparation

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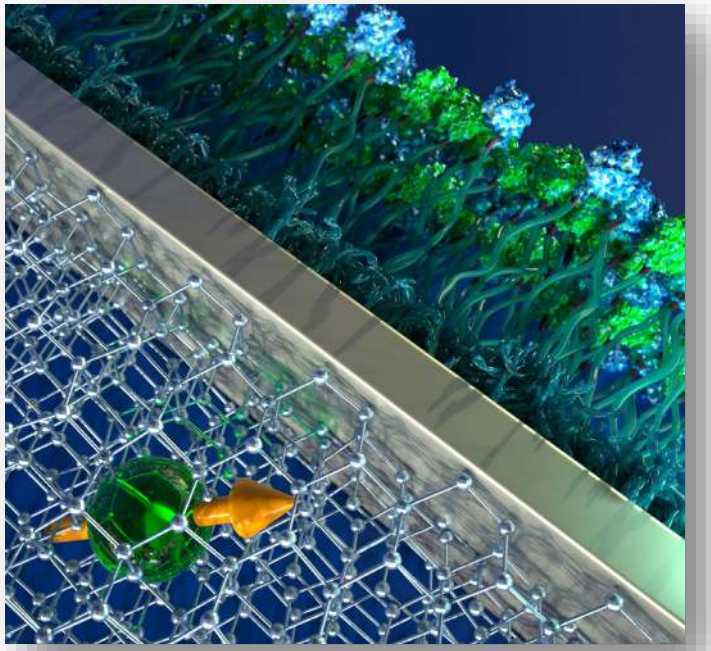
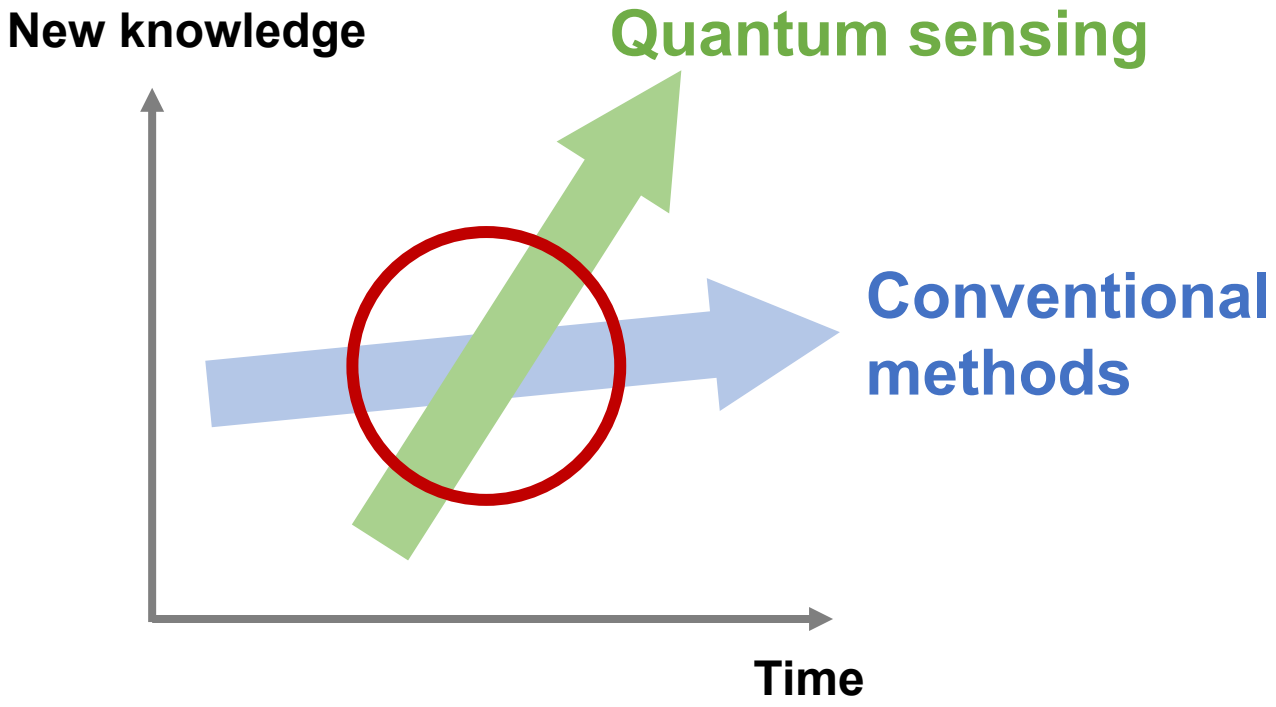


Conclusion

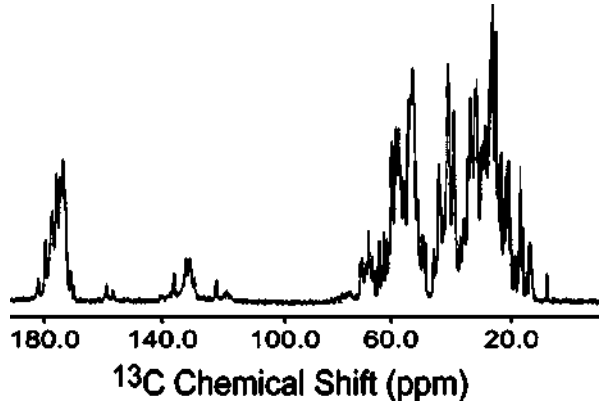
- NV-NMR (NV-EPR) is an emerging molecular sensing technology that has unprecedented spatial resolution (single molecule)

- Biocompatible surface functionalization and diamond-membrane based sensing modality pave the road for the future applications of NV quantum sensing technology to better understand biological systems

Outlook

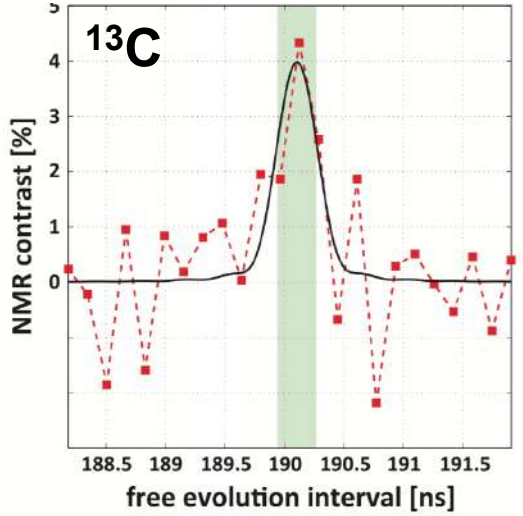


Ubiquitin by conventional NMR



Igumenova *et al.*, *JACS* 2004, *126*, 6720

Ubiquitin by NV-NMR



Lovchinsky *et al.*, *Science* 2016, *351*, 836

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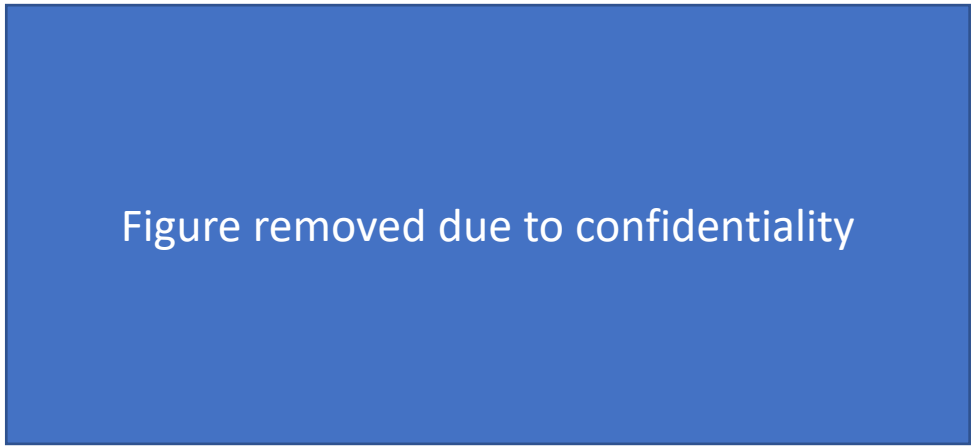
I look forward to starting my independent research lab this fall!

working at the interface of *Physics* and *Biology*



- Technological development on quantum biosensing platforms
- NV-NMR (NV-EPR) on single-molecule biophysics
- Multi-disciplinary approach to decipher molecular mechanisms of biological processes

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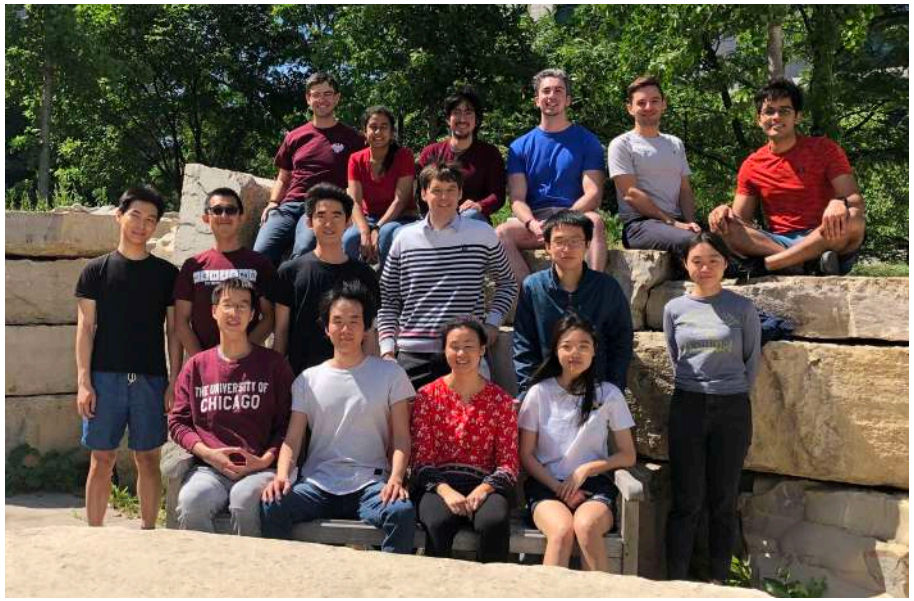
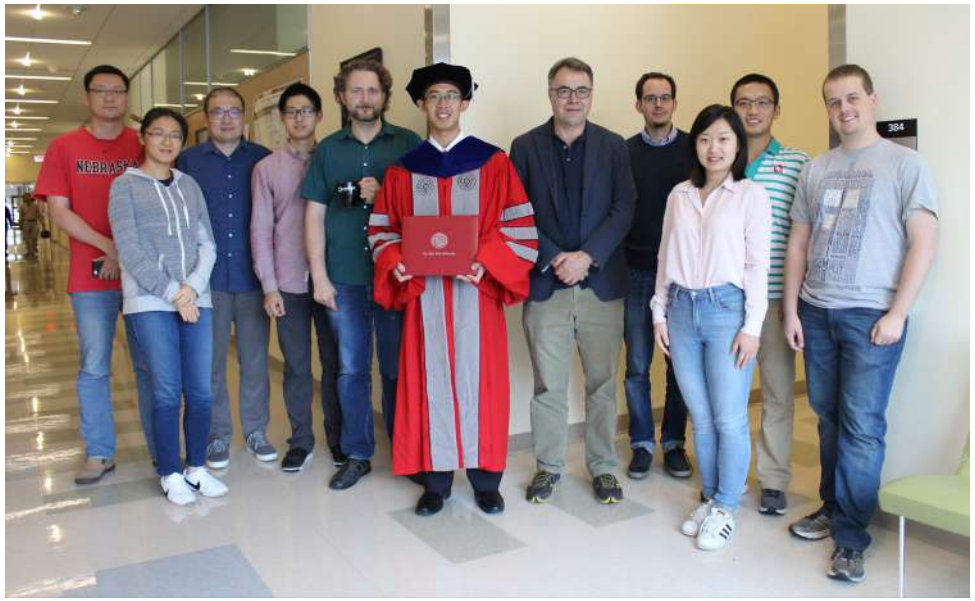
Thank you for your attention and hospitality!

The Peter Maurer lab @ UChicago



The Rafael Brüschweiler lab @ Ohio State



Collaboration

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