Université de Montréal

Vesicular microreactors for diagnostic applications

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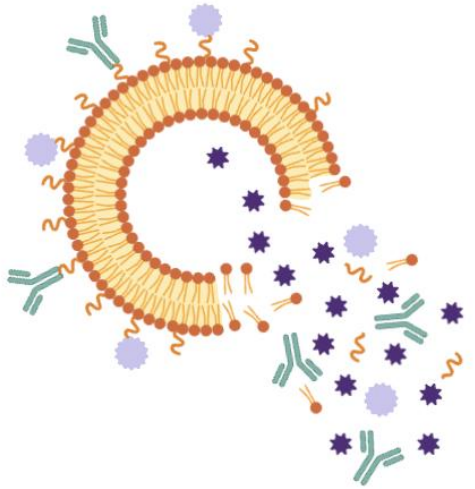
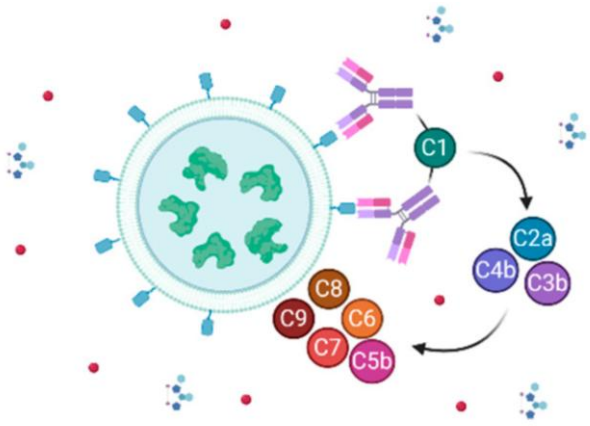
Leveraging vesicles in diagnostics

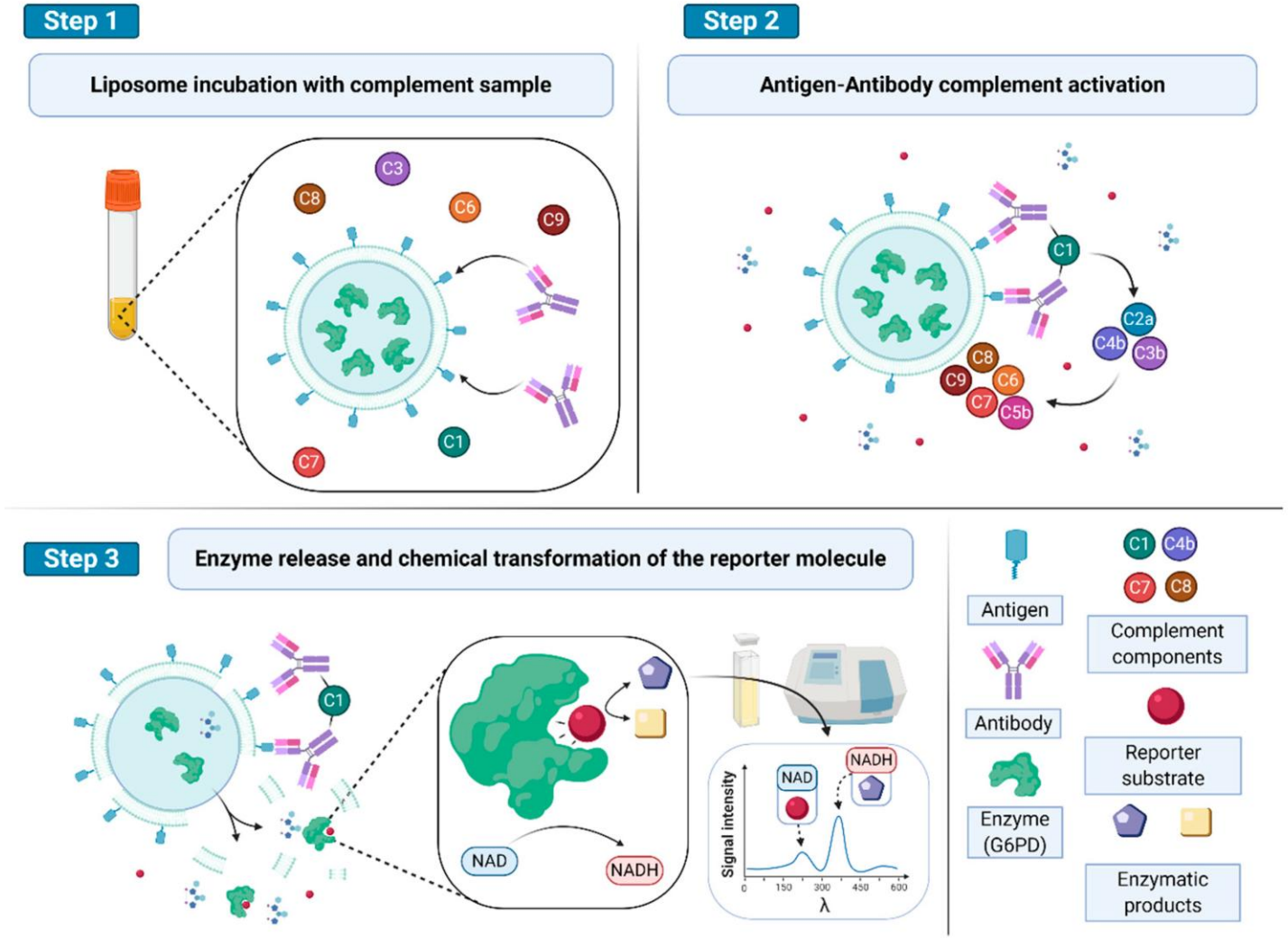
- *In vivo* applications
  - Vesicular delivery systems for imaging
    - Circulation time and biodistribution
    - Stimuli responsiveness
    - Combination treatment
- *Ex vivo* applications
  - Lab assays *(the FDA-approved liposomes you have never heard of)*
    - *Complement assay*
    - *Anti-phospholipid autoantibody assays*
  - Point-of-care assays: vesicular microreactors
    - Blood assays
    - Wound assays





Liposomal assay for complement function

- Indication
  - First-tier screening test for **congenital complement deficiencies**
  - Detection of individuals with an **ongoing immune process**
- Method name: Automated Liposome Lysis Assay (Fujifilm Wako), serum
- **FDA-approved** and clinically used

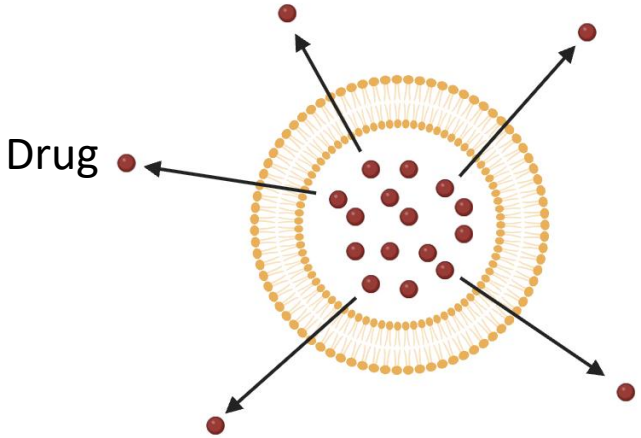


Mayo Labs
Sforzi+ *Biology* 2020

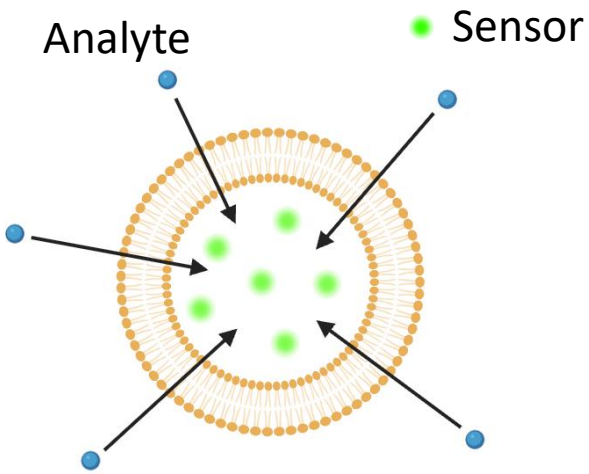
My contributions to the vesicular diagnostics

Repurposing vesicles for diagnostics

Liposome



Drug release



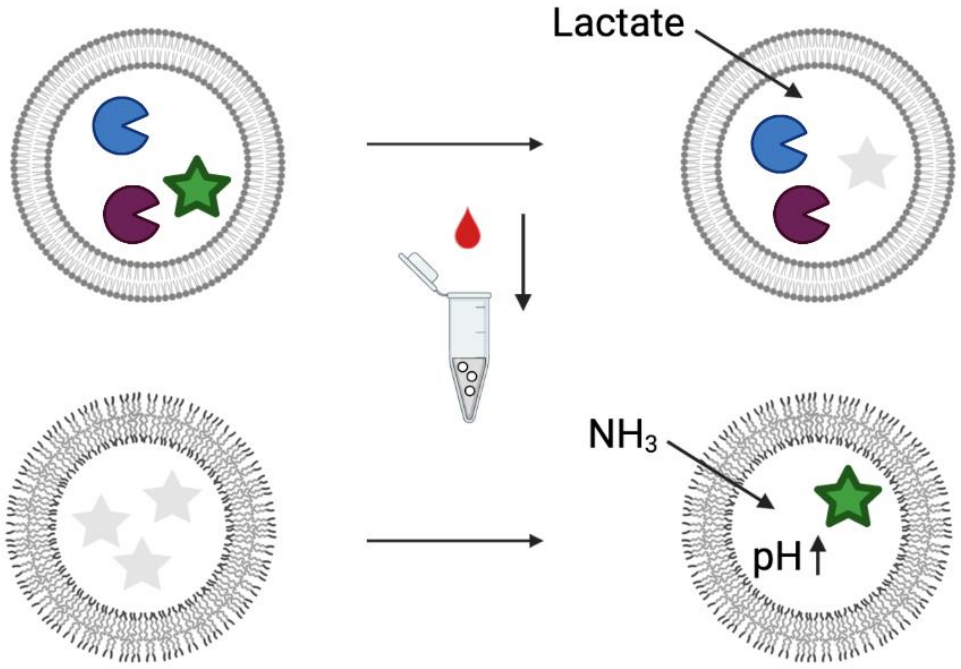
Vesicular microreactor for analyte sensing

Rapid bedside tests in blood

Vesicular reaction compartments

Enzymatic reaction compartment

Chemical reaction compartment

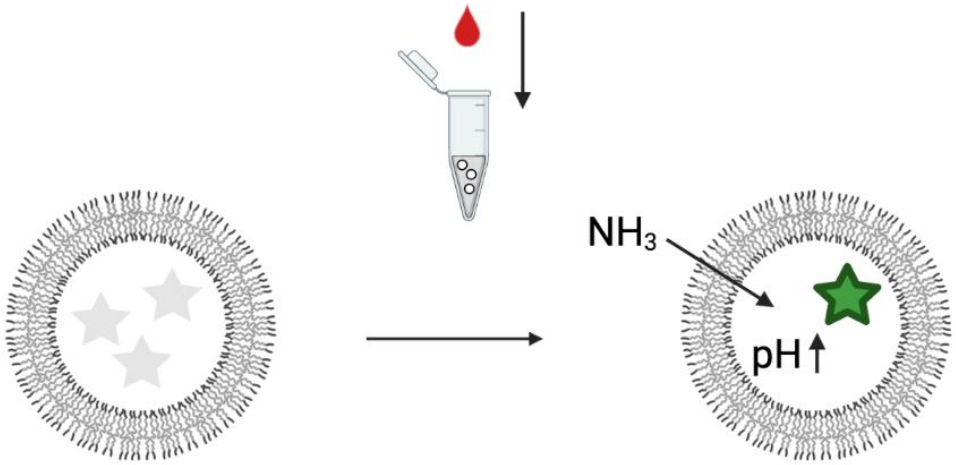


Leveraging the selectively permeable membrane to

- *retain sensor components*
- *exclude interfering substances*
- *… while allowing for rapid influx of analyte*

Matoori et al. *Small* 2019
Matoori et al. *Small* 2020
Matoori et al. *Biomaterials* 2022
Guirguis (…) Matoori* *ACS Pharmacol & Transl Sci* 2023
Al-Hawat (…) Matoori* *ACS Bio & Med Chem* 2024

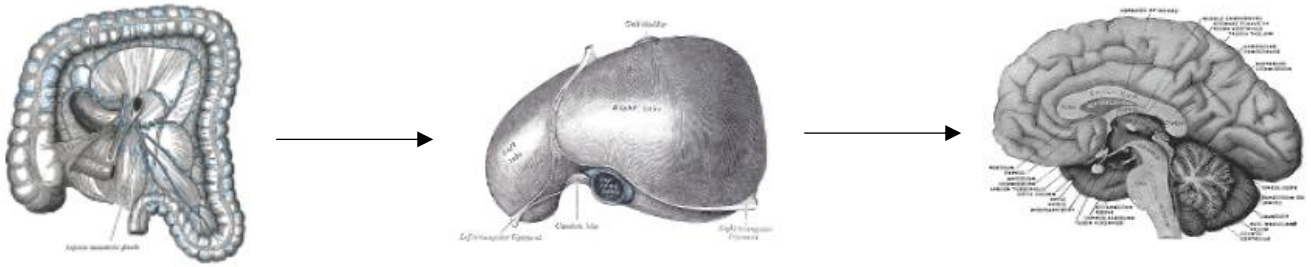
Chemical reaction compartment



Ammonia assay

Acute hyperammonemic crisis

Ammonia is a neurotoxic metabolite
Product of urease-producing bacteria in gut
Cleared by urea cycle in the liver



Liver function impairment leads to **ammonia buildup**

Gut-liver-brain axis

Acute hyperammonemia (e.g., acute-on-chronic liver failure)
Neuropsychiatric symptoms (somnolence, hepatic coma, risk of death)

Gines+ *Lancet* 2021

Current diagnostics
Gold standard: GLDH-based assay in plasma (hospital lab)
Time-to-result 3 h

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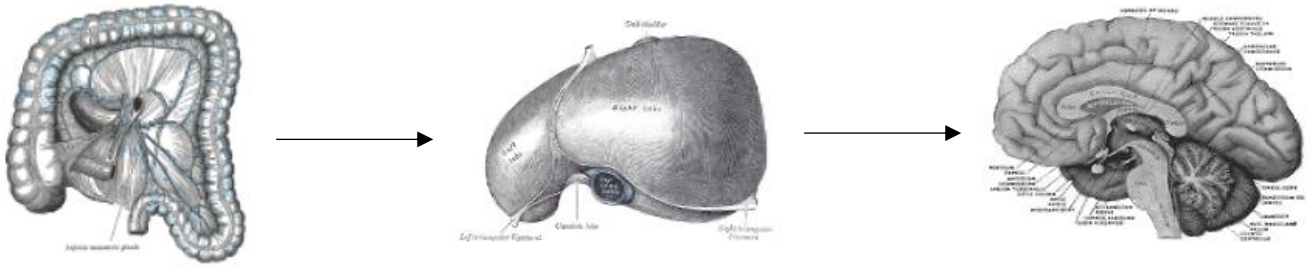

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Gines+ *Lancet* 2021

Current diagnostics
Gold standard: GLDH-based assay in plasma (hospital lab)
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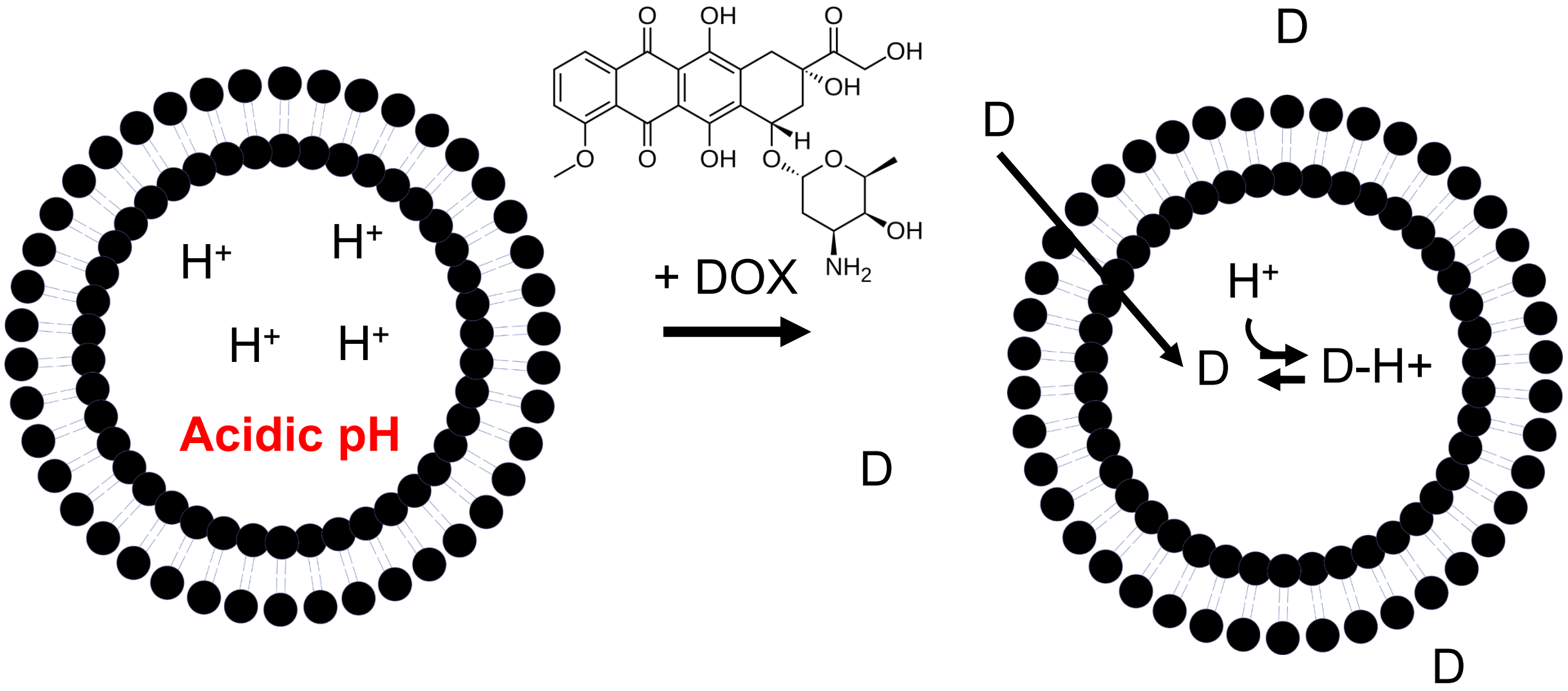
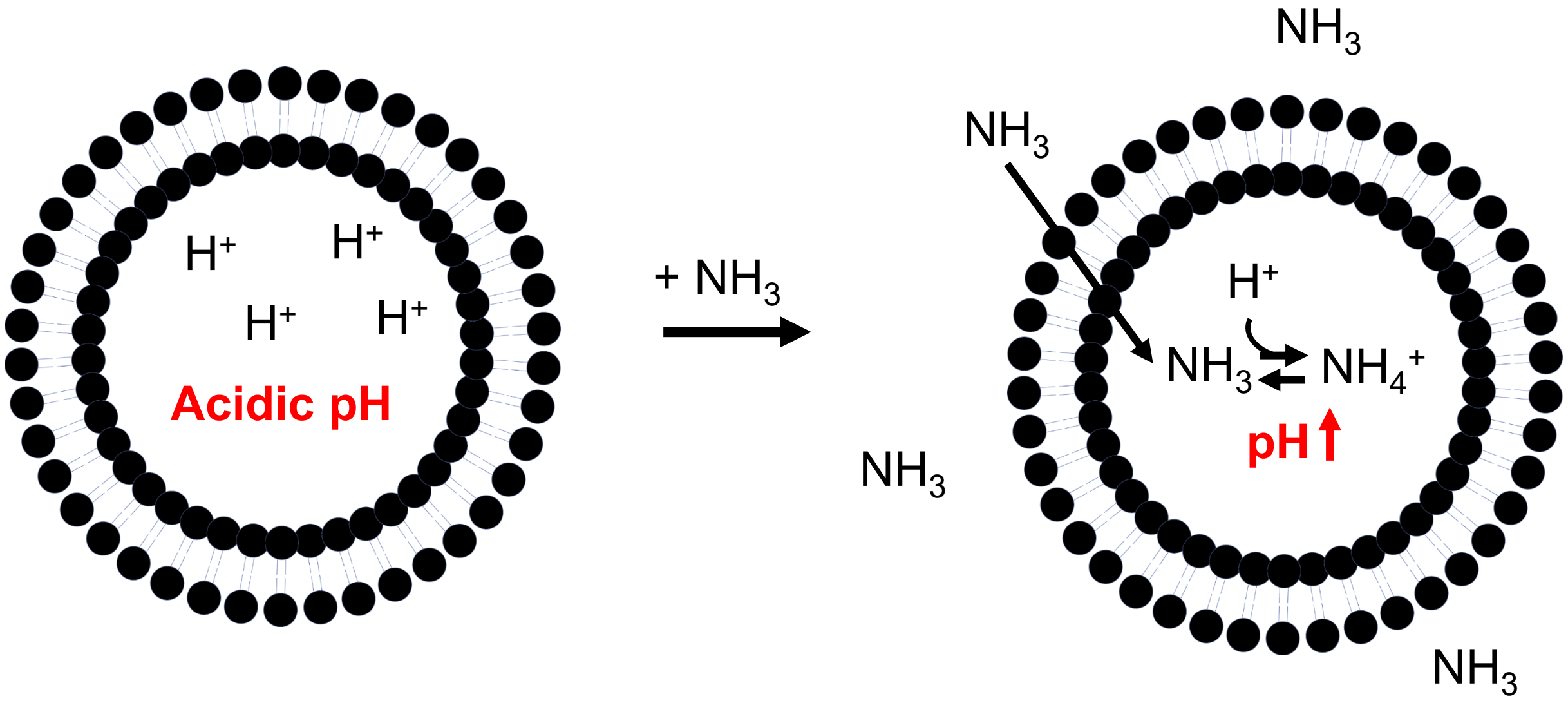
Clinical care gap: Rapid bedside blood ammonia assay

- Disease monitoring
- Selection of treatment
- Evaluation of treatment success

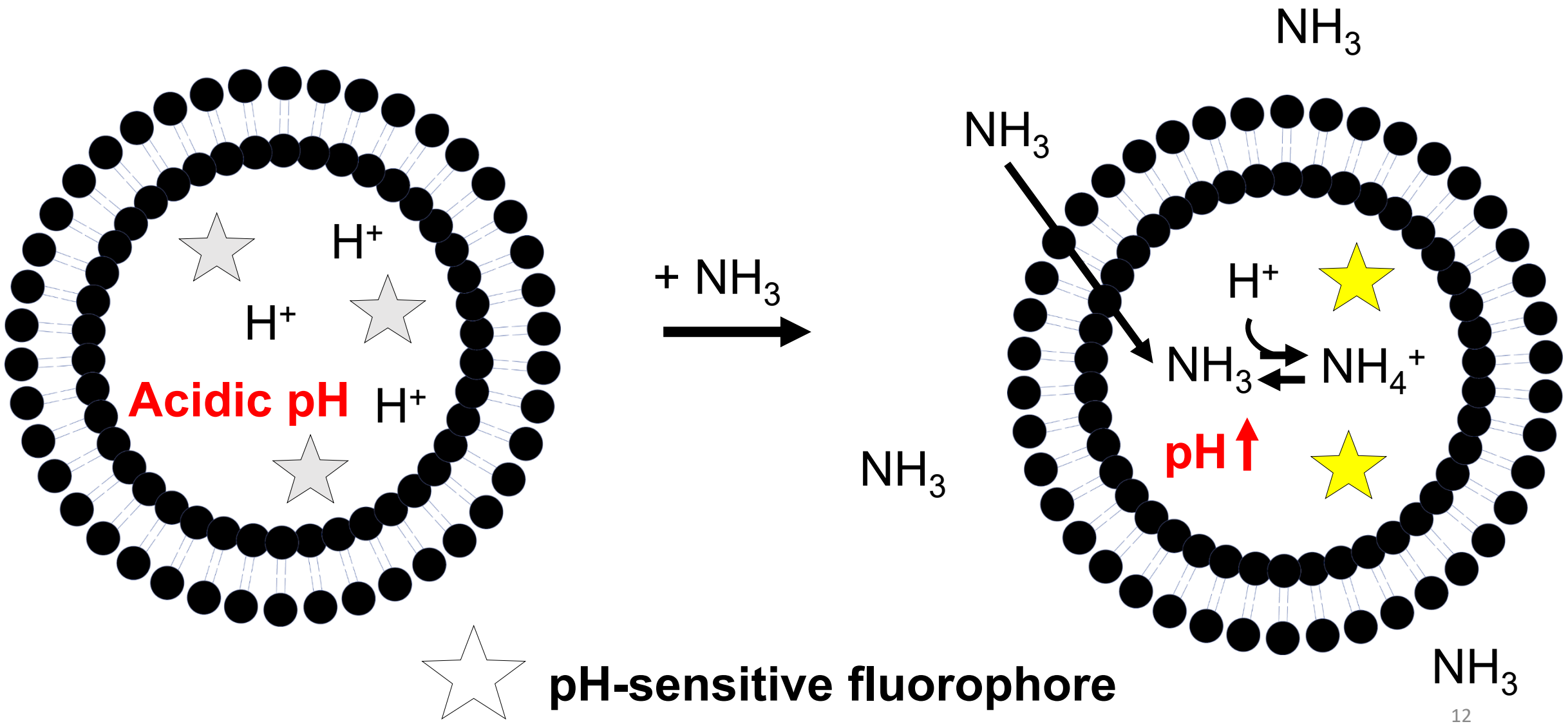
Concept from drug delivery: active loading of liposomes with doxorubicin



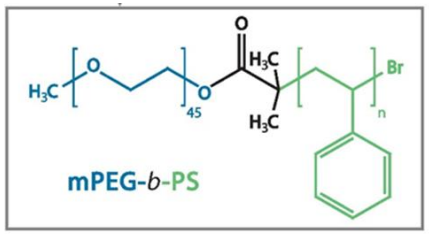
Chemical microreactor for ammonia sensing

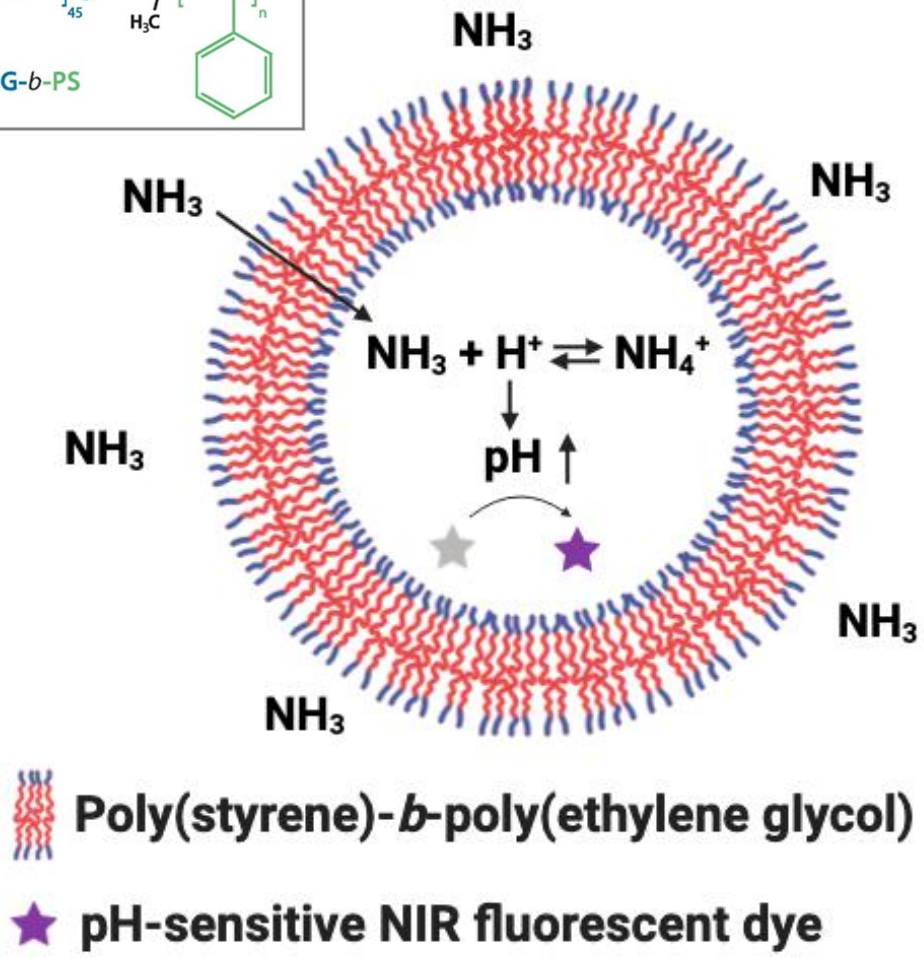


Chemical microreactor for ammonia sensing



Modulating membrane hydrophobility for selectivity towards ammonia



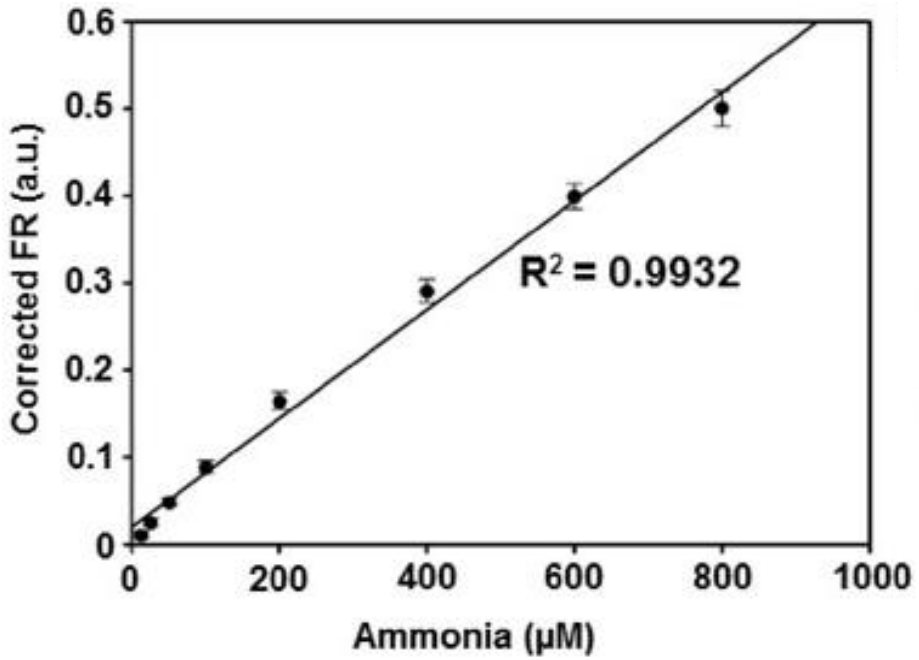


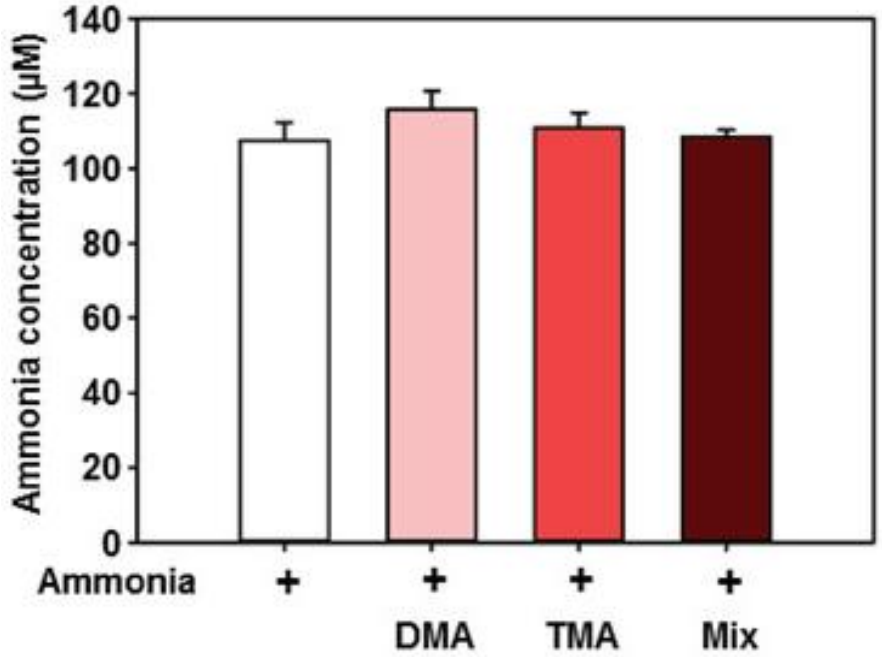
- *Hypothesis: highly hydrophobic membrane leads to low permeability for amine-containing weak bases*
  - *Permeable to ammonia (small, hydrophobic)*
- Poly(styrene)
  - High glass transition temperature (Tg) ≈73°C
  - Highly hydrophobic membrane
  - Rigid semi-crystalline state

- NIR fluorescent dye for sensing in blood (low interference with hemoglobin)

Matoori (…) Leroux *Small* 2019
Al Hawat, Caron, Djebbar, Matoori* *ACS Bio & Med Chem* 2024

Polymersome ammonia assay

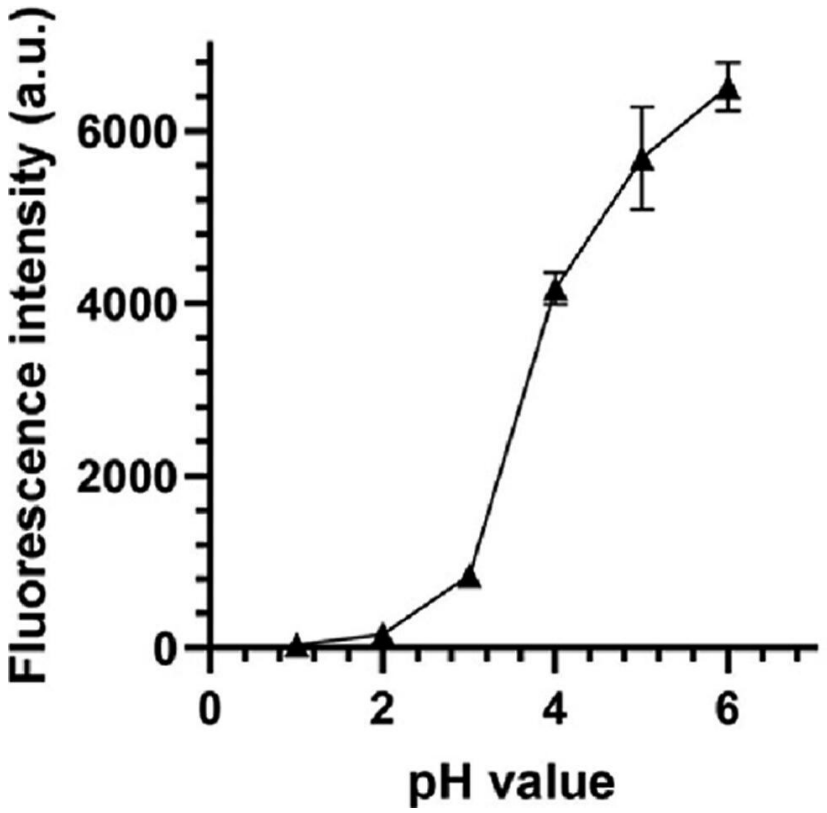




- Rapid and linear sensing in buffer
- High selectivity towards ammonia
- Proof-of-concept in plasma of cirrhotic rats

Matoori (…) Leroux *Small* 2019

pH sensitive NIR fluorescent dye



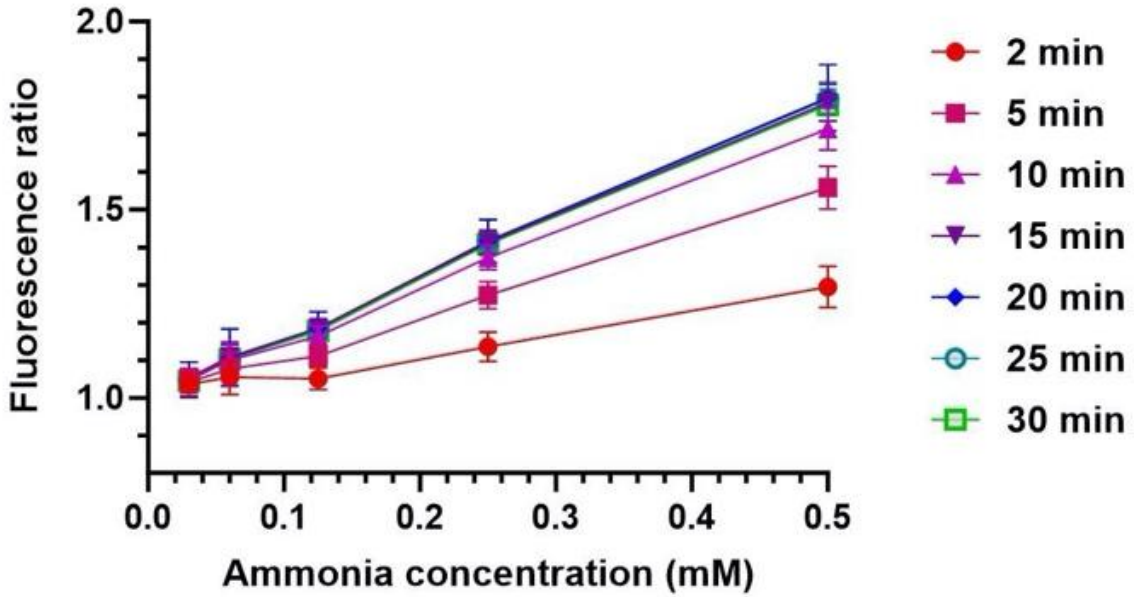
Marie-Lynn Al-Hawat

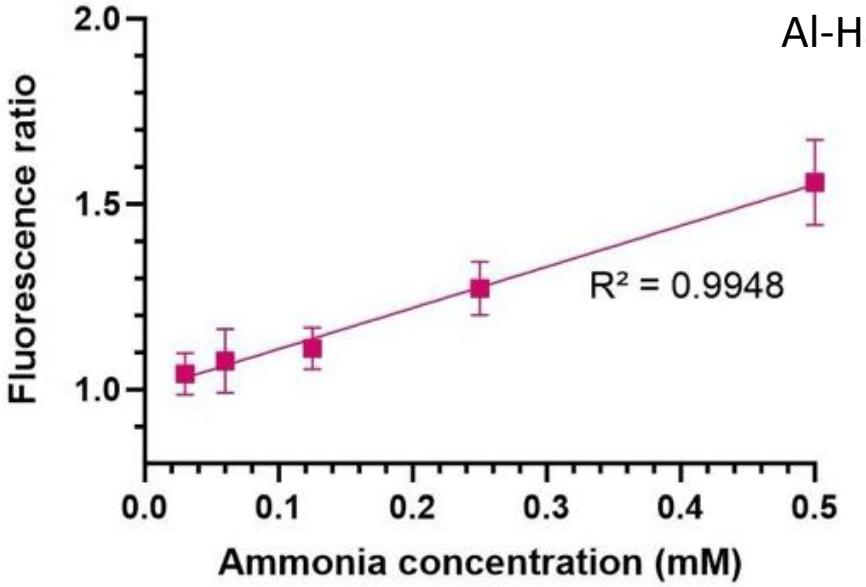
- Identified a novel pH sensitive NIR fluorescent dye with pKa of approx 4.0

Al Hawat, Caron, Djebbar, Matoori* *ACS Bio & Med Chem* 2024

Blood ammonia assay

Marie-Lynn Al-Hawat





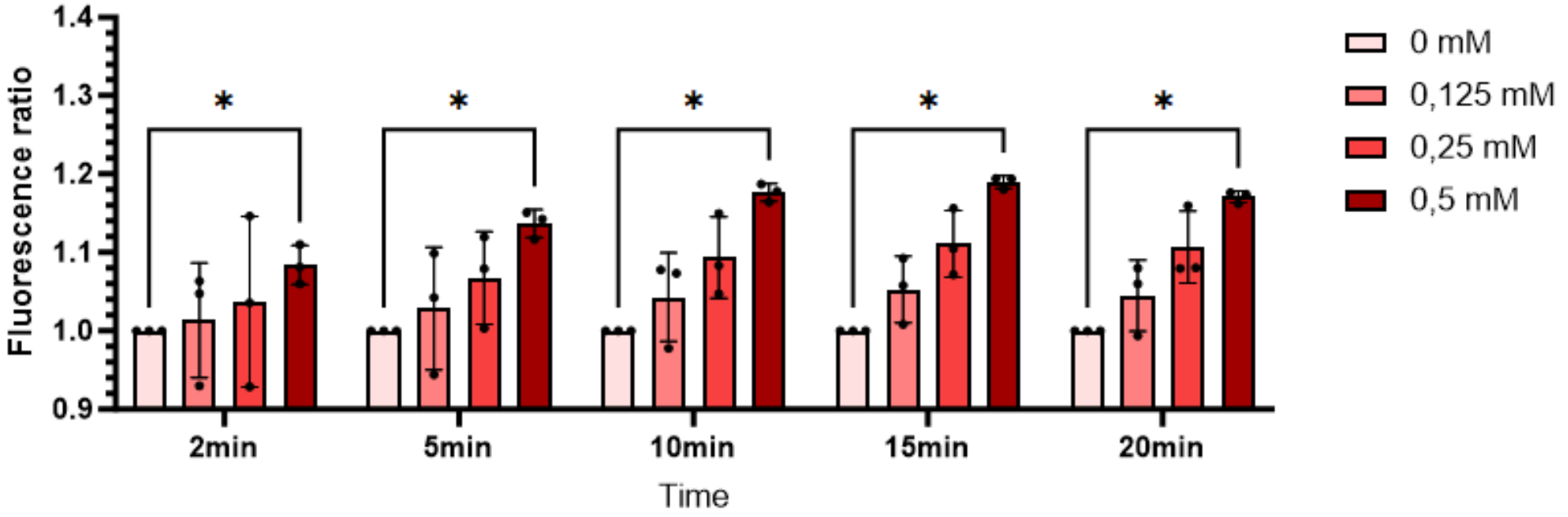
- Rapid and linear sensing in fresh whole blood

Al Hawat, Caron, Djebbar, Matoori* *ACS Bio & Med Chem* 2024

Portable fluorometer

Deniro, Detect Diagnostics, Groningen, NL

Polymersome ammonia assay



- Portable meter: rapid ammonia sensing in fresh whole blood

Al Hawat, Caron, Djebbar, Matoori* *ACS Bio & Med Chem* 2024

Platform technology

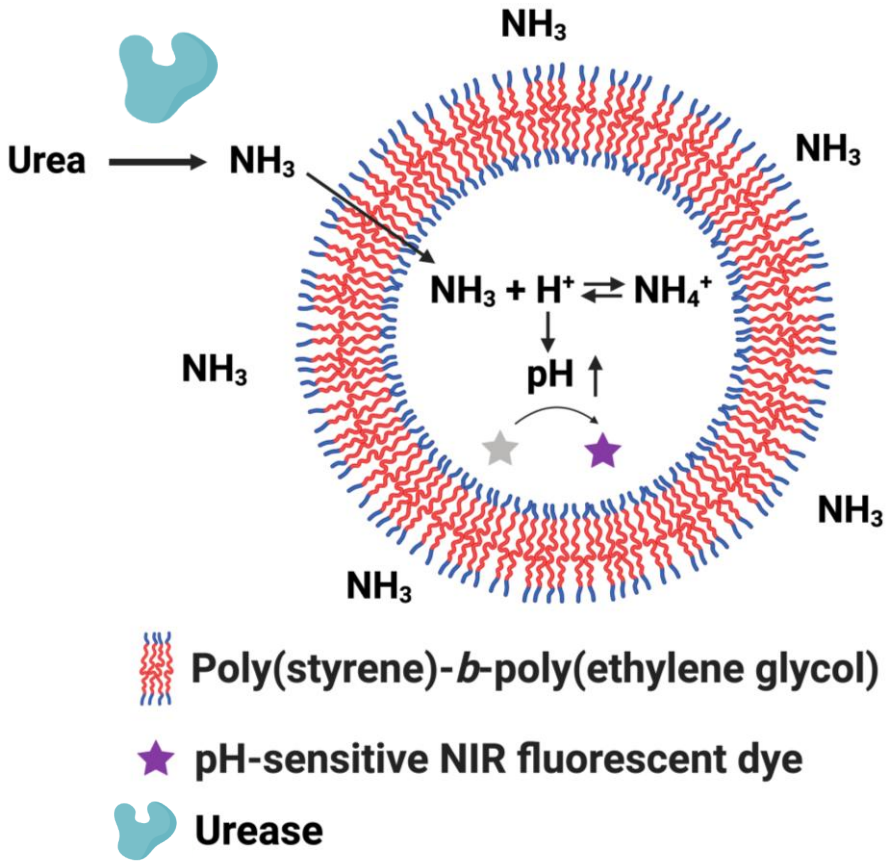
Platform technology

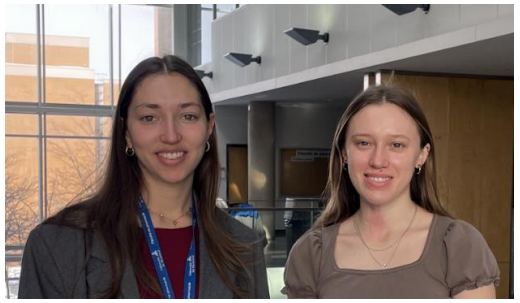
Combination with **ammonia-generating enzymes**
Expansion of analyte space

Urease: degrades urea to ammonia and CO_2

Urea
- Test to evaluate optimal dialysis duration
  - To prevent under- and overdialysis
- Performed once per month
- Currently limited to plasma assay (hospital lab)

Bedside test would enable optimal dialysis duration at each session (lower risk of electrolyte imbalances, potential for cost savings)

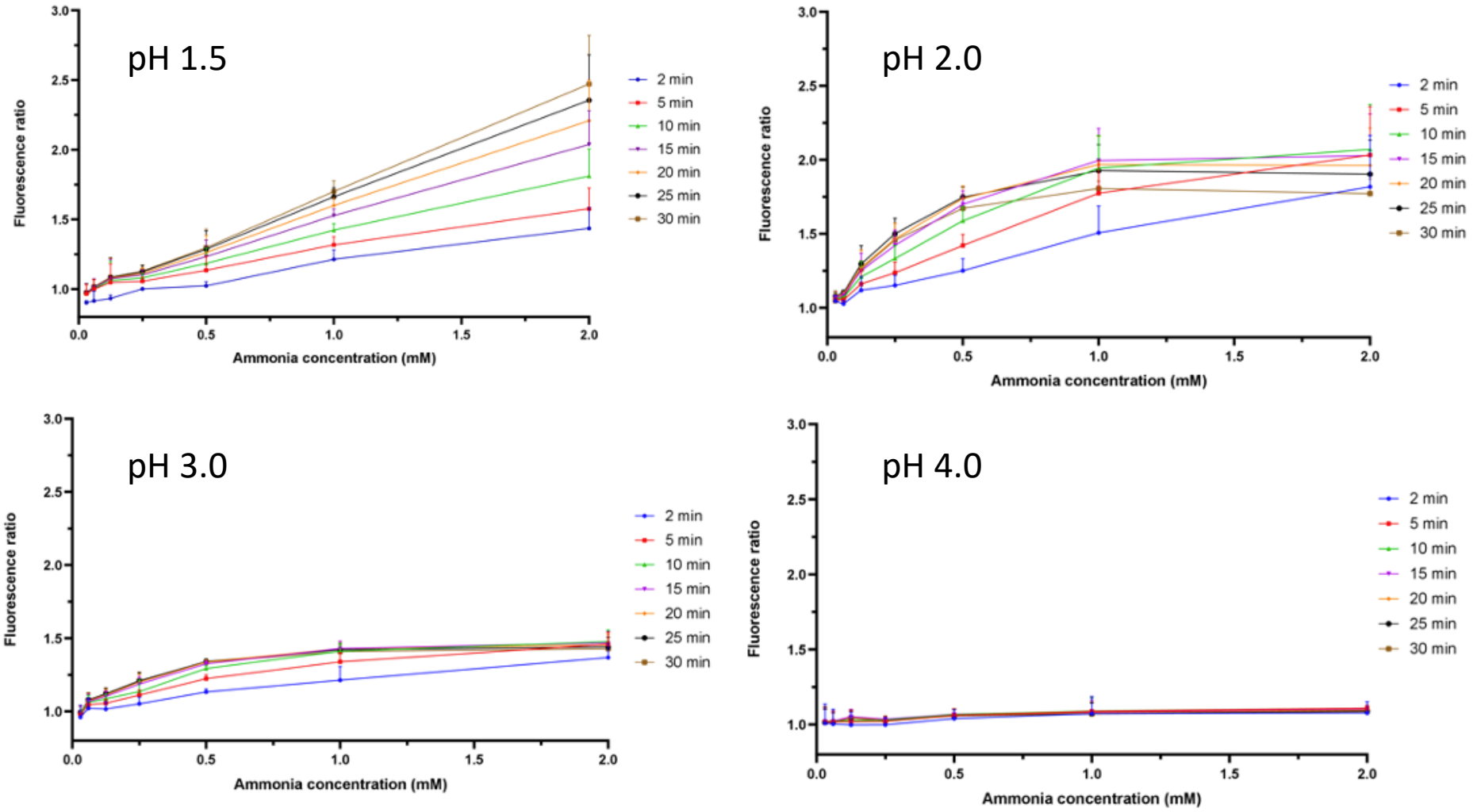




Emma Lenglet et Cléa Belin

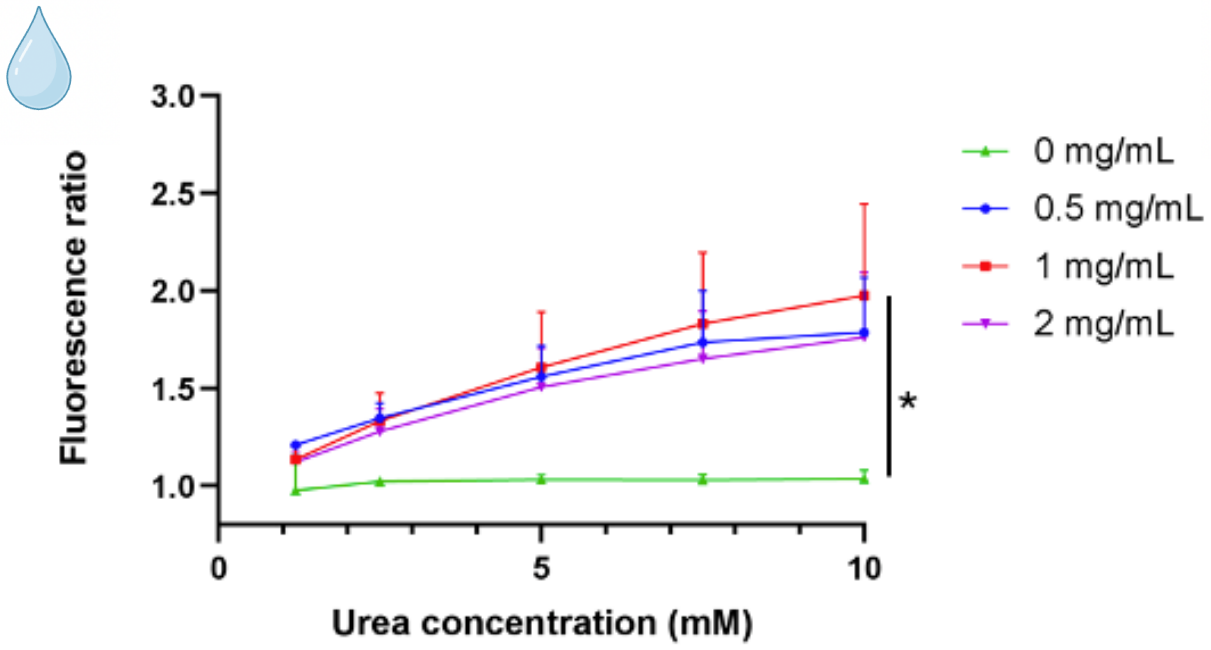
Belin, Lenglet, Caron, Al-Hawat, Matoori*, *under review*

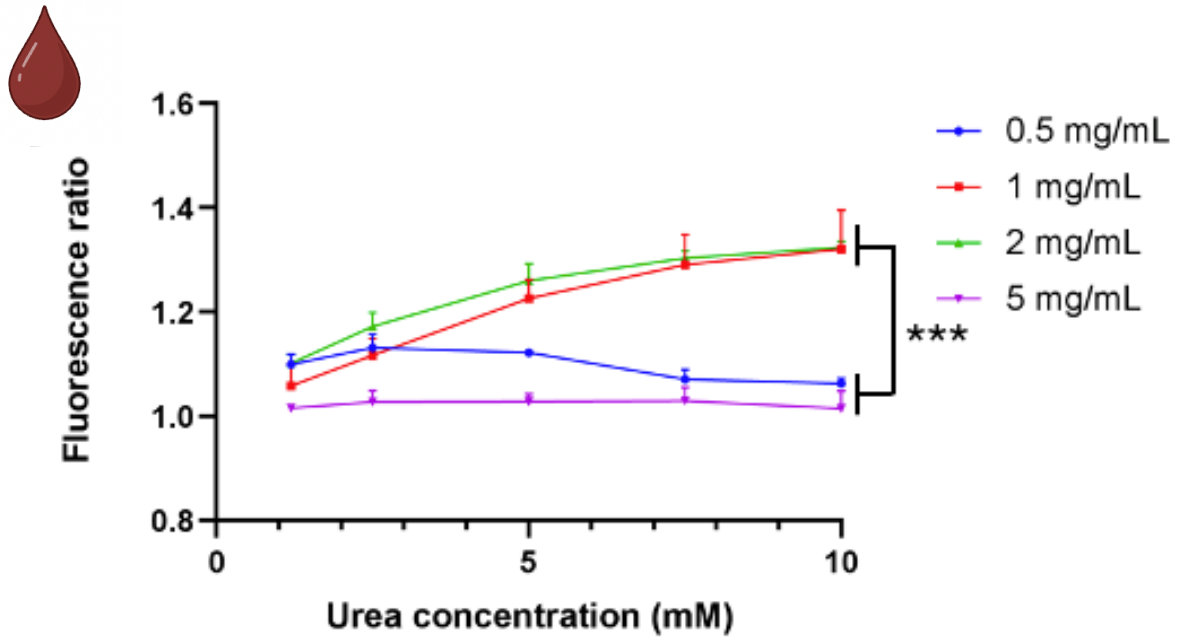
Optimizing luminal pH of ammonia sensor



- Luminal pH strongly influences ammonia sensor response and linearity

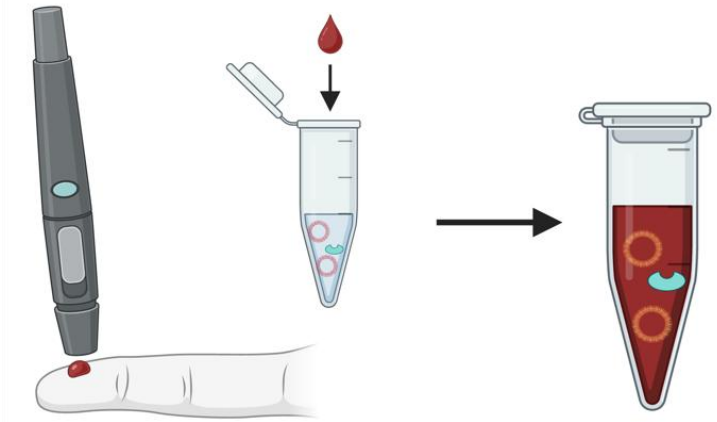
Urea sensing

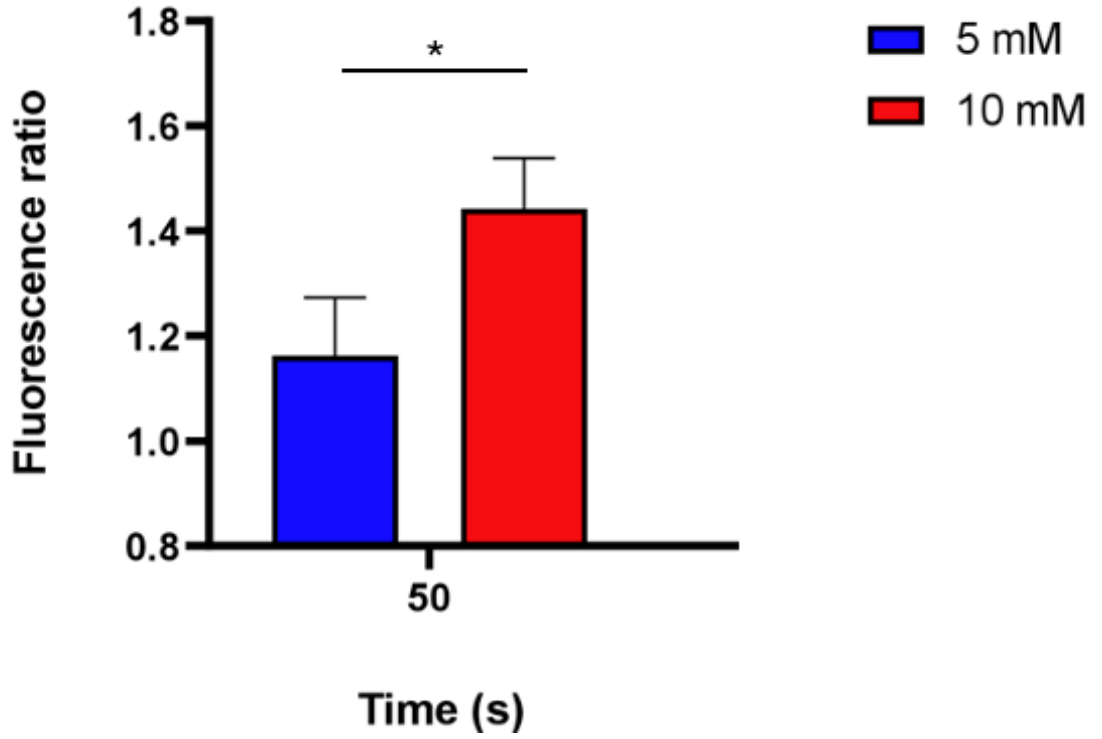




- Urease concentration-dependent sensing
- Stronger response in buffer than in spiked fresh mouse blood
  - Likely due to natural urea in mouse blood (saturation)

IRB-approved study in human volunteers



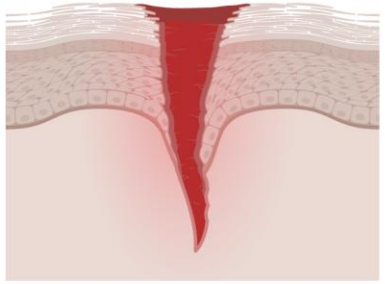


- IRB-approved study in urea-spiked fresh capillary blood from human volunteers (n=4)
- Test discriminates two clinically relevant urea concentrations

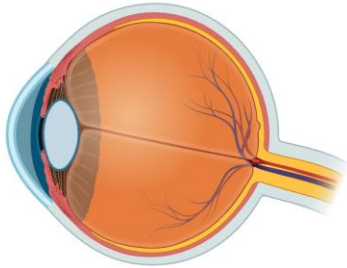
The next frontier: molecular monitoring at the disease site

Blood, plasma/serum, urine

External (surface)



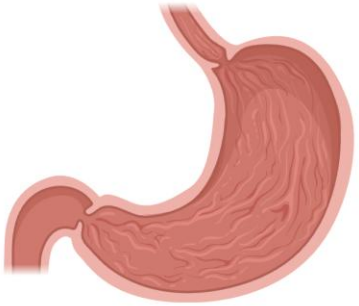
Wound



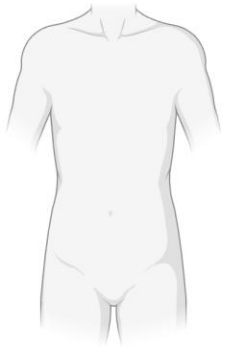
Eye, tears

Buccal

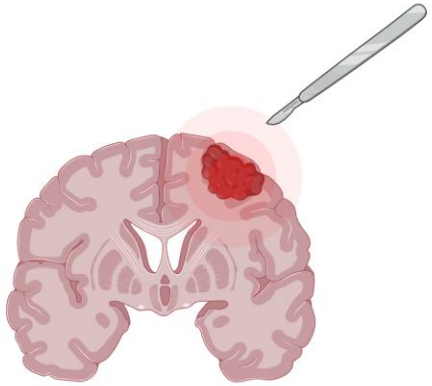
Internal



Gastrointestinal tract



Genito-urinary tract

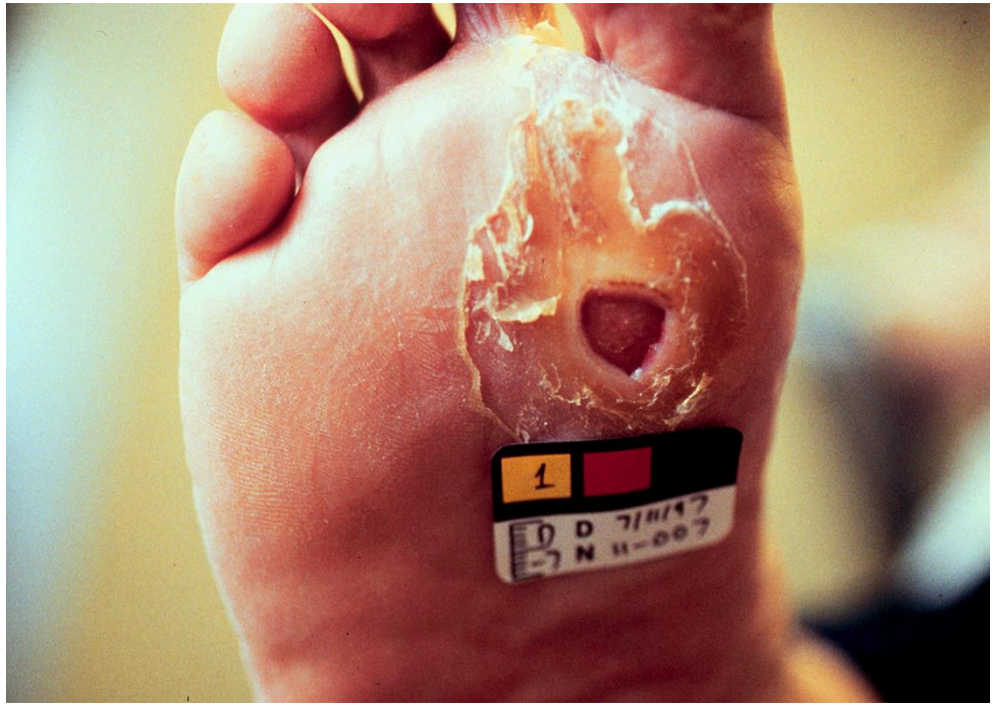


Post-tumor resection

Phospholipid Research Center

Wound hydrogen peroxide assay

Diabetic foot ulcers



- **Diabetic foot ulcer**
  - Major complication in diabetes
  - Life time incidence in patients with diabetes: 15-25%
  - Most common cause for amputations in North America

- **Limited diagnostic options**
  - **Ulcer depth**
  - **Unspecific infection markers (redness, swelling, temperature)**
  - **No molecular diagnostics**

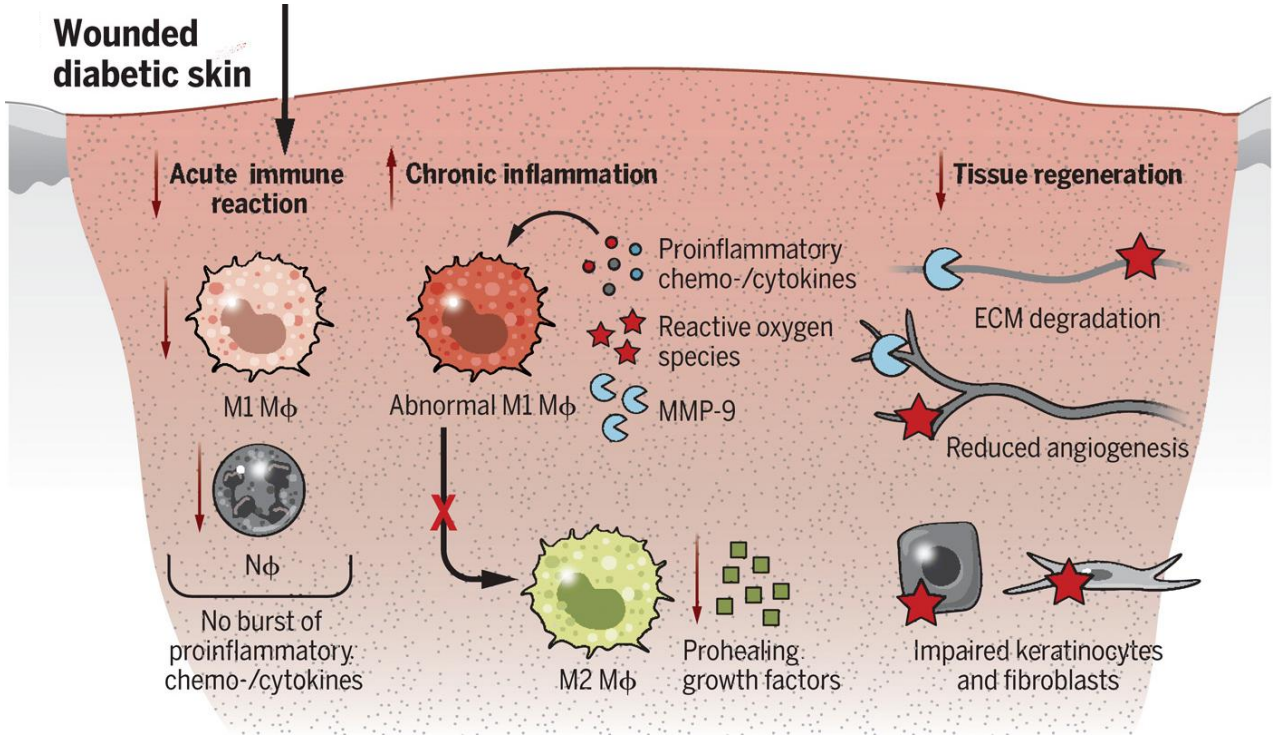
Matoori, Veves, Mooney *Science Translational Medicine* 2021
Freedman*, Hwang, Talbot, Higler, Matoori*, Mooney* *Science Advances* 2023
Mahmoudi (…) Matoori, Mahmoudi* *Nature Reviews Bioengineering* 2024

Wound ROS sensing

- Chronic inflammation: high oxidative stress is common in diabetic wounds

- Oxidative stress is an emerging **biomarker**
  - Correlated with histolopathological markers of disease
  - ➢ Molecular wound characterization
  - ➢ **Prognosis**
  - ➢ **Treatment selection**
  - ➢ Assessment of **treatment effects**

- Oxidative stress is a key therapeutic target
  - Companion diagnostics with antioxidant treatment

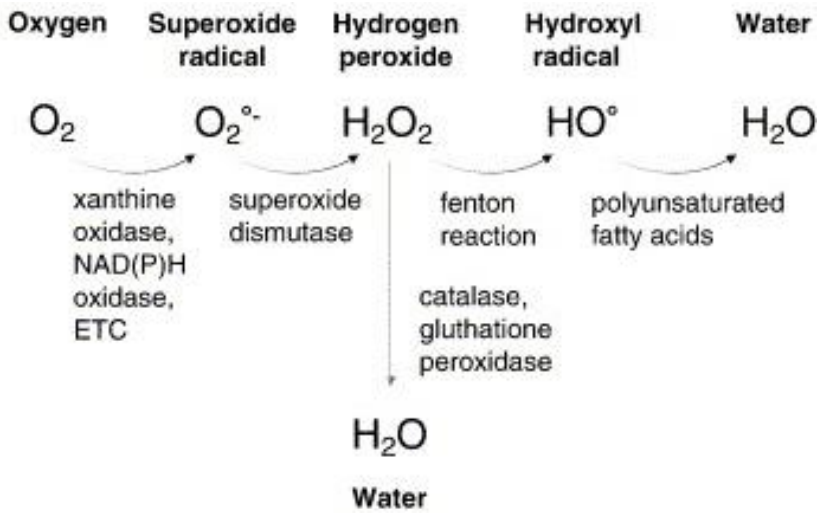


Fu (…) Matoori* *ACS Measurement Sci* 2022
Matoori, Veves, Mooney *Sci Transl Med* 2021

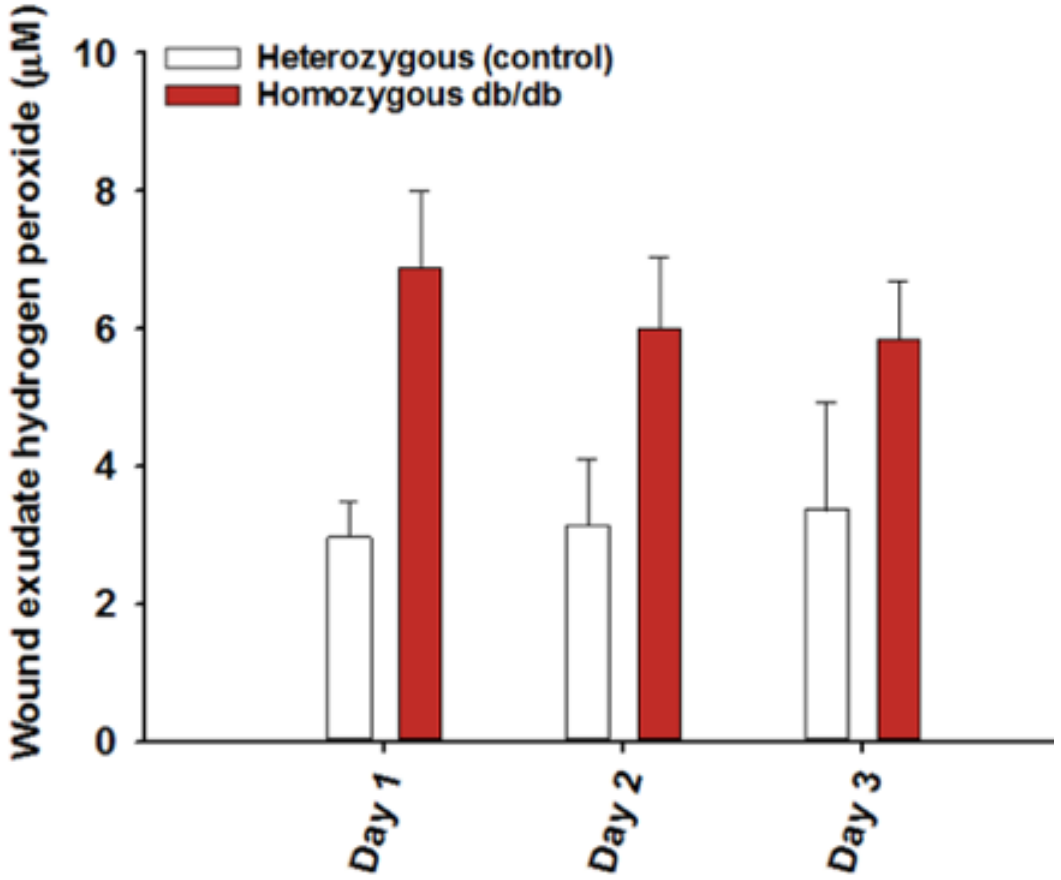
Hydrogen peroxide is a key ROS

- Hydrogen peroxide is upregulated in diabetic wounds
- Directly contributes to **oxidative damage**
- Key molecule in ROS metabolism

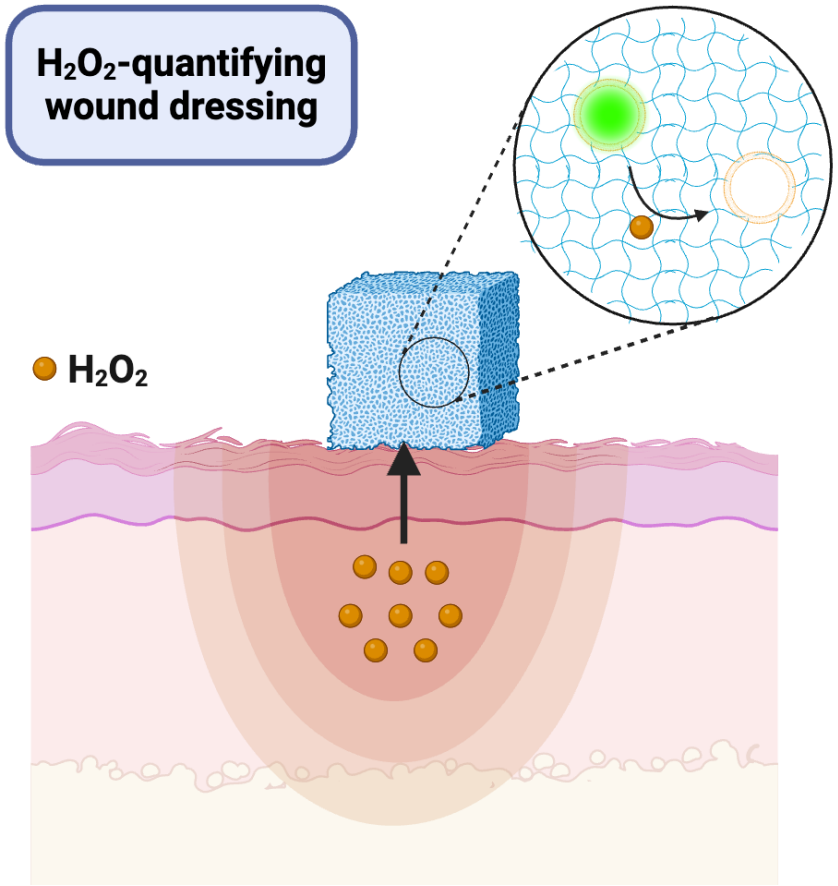
Wang+ *Journal of Controlled Release* 2015
Dhall+ J. *Diabetes Res*. 2014
Fadini+ *Diabetes* 2010



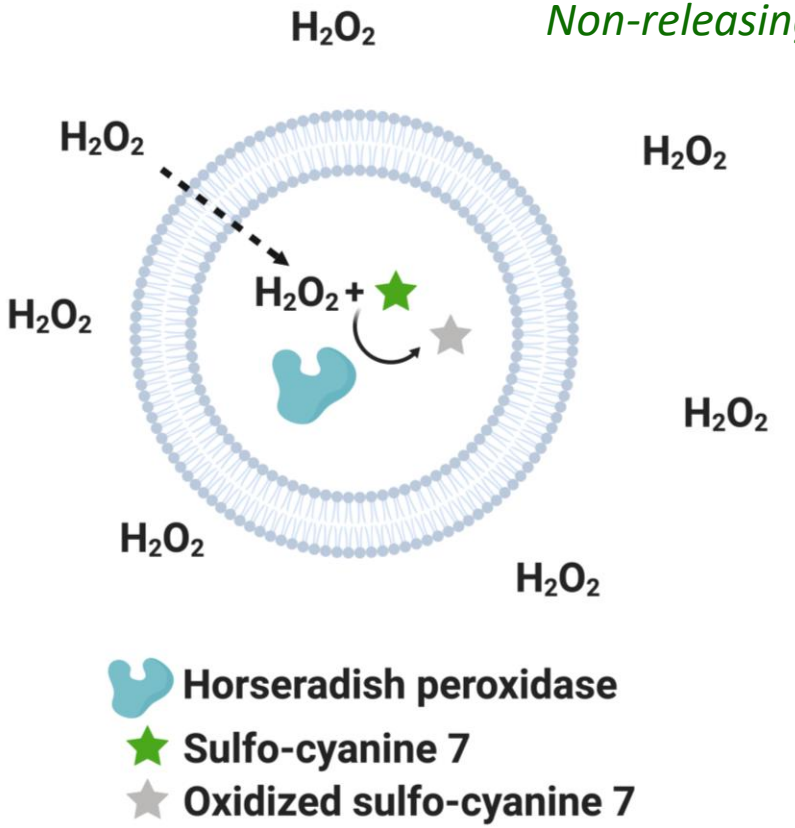
In vivo study performed by us (Matoori/Veves)



Hydrogen peroxide-sensing wound dressing



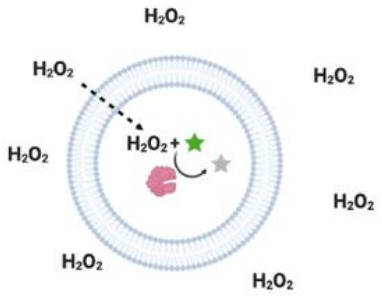
Non-releasing release system

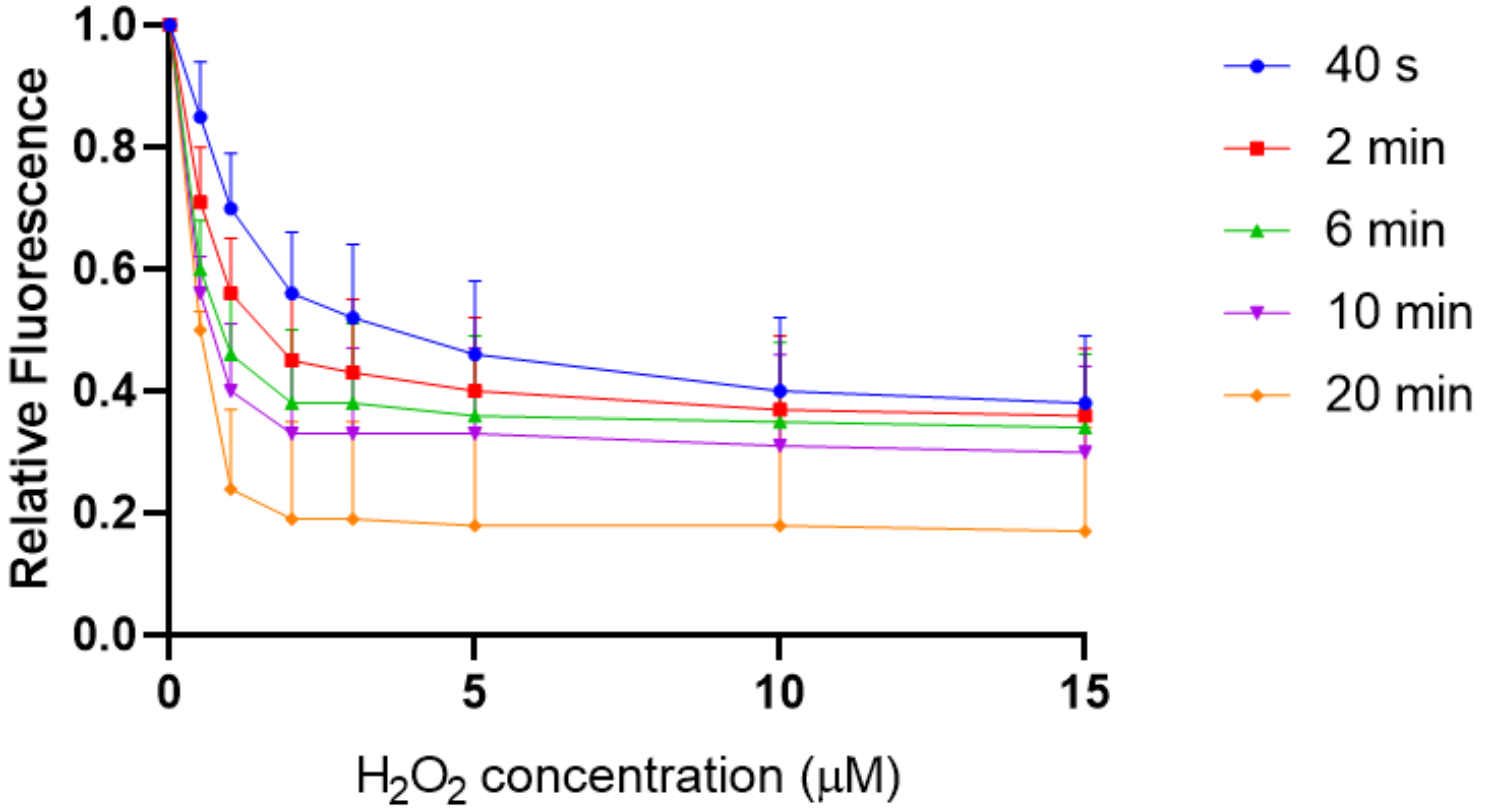


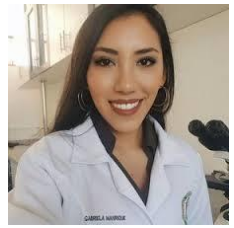
Reaction HRP and S7

Manrique, Gottraux, Matoori* *ACS Measurement Sci* 2025

Liposomal hydrogen peroxide sensing





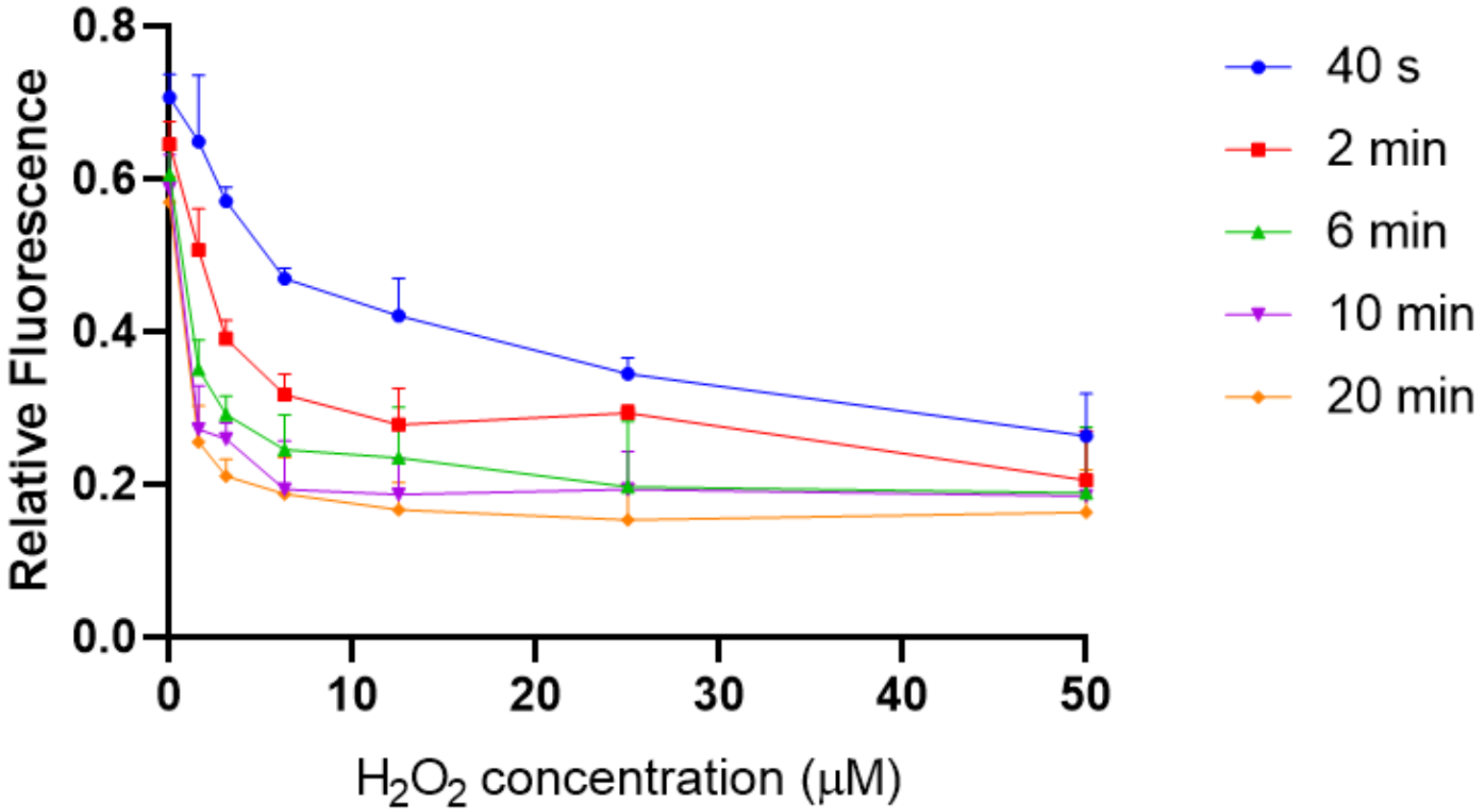


Gabriela Manrique

- HRP/S7-loaded liposomes are responsive to preclinically relevant hydrogen peroxide concentrations
- Similar response in presence of FBS

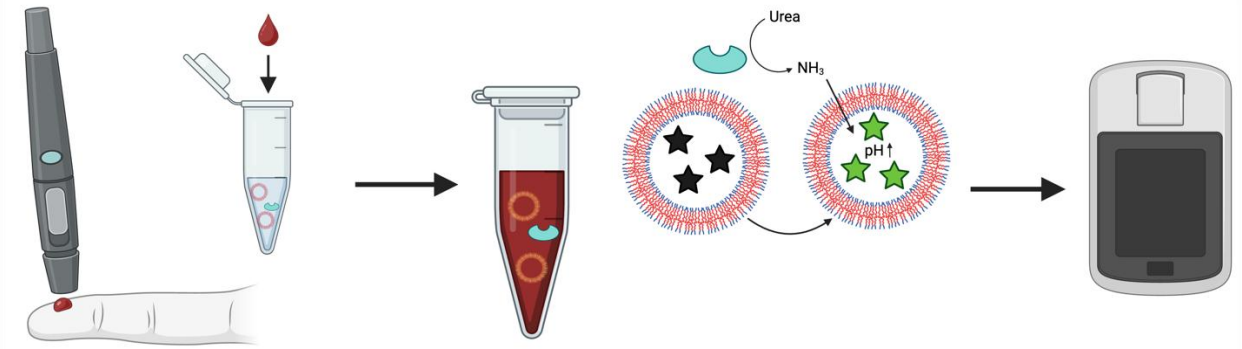
Liposomal hydrogel

Encapsulation of liposomes in calcium crosslinked alginate hydrogel



Leveraging vesicles in diagnostics

- *Ex vivo* applications
  - Lab assays *(the FDA-approved liposomes you have never heard of)*
    - *Complement assay*
  - Point-of-care assays: vesicular microreactors
    - Blood assays
      - Ammonia, urea
    - Wound assays
      - ROS



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Collaborators

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Prof. Christopher Rose (UdeM)
Prof. Parisa Mehrkovandi (U British Columbia)
Prof. Ben Freedman (Harvard Medical School)
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Prof. Rivka Stone (University of Miami)
Prof. Morteza Mahmoudi (Michigan State U)

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Phospholipid Research Center

NSERC CRSNG

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Fonds de recherche Nature et technologies Québec

RQRM — RÉSEAU QUÉBÉCOIS DE RECHERCHE SUR LES MÉDICAMENTS

CQMF QCAM

LES ALLIÉS DE LA FACULTÉ DE PHARMACIE

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