Université de Montréal

Use of phospholipids in diagnostic applications

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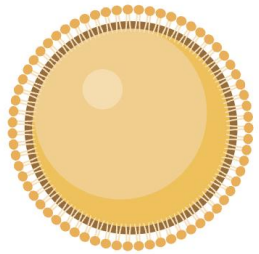
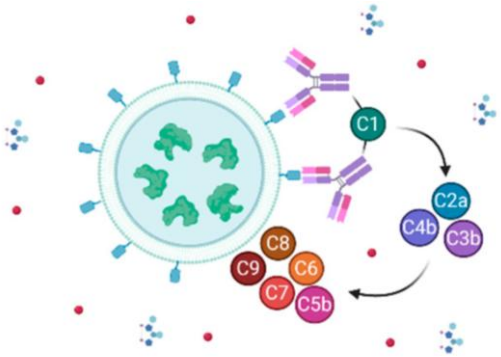
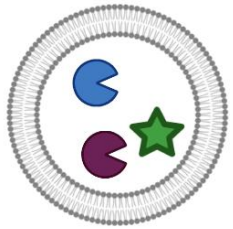
Use of phospholipids in diagnostics

- *In vivo* applications
  - *Microbubbles for ultrasound*
  - Liposomal delivery systems for diagnostics



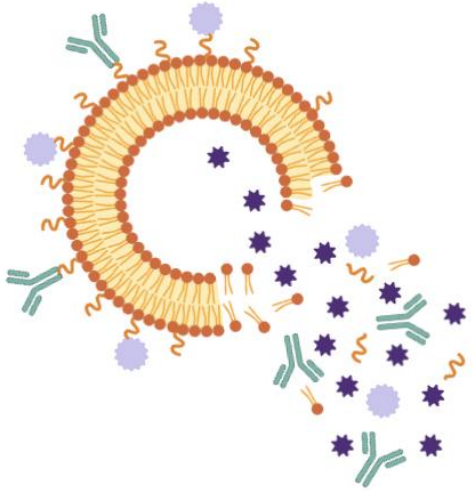
- *Ex vivo* applications
  - Lab assays *(the FDA-approved liposomes you have never heard of)*
    - *Complement assay*
    - *Anti-phospholipid autoantibody assays*
  - Point-of-care assays: liposomal microreactors
    - Blood assays
    - Wound assays





Repurposing liposomes for diagnostics: *in vivo* applications

- **Modulating biodistribution**: increasing accumulation at target
  - Prolonged circulation time and accumulation in target tissue
  - EPR effect, active targeting (cell-specific imaging)
  - Stimuli responsiveness: oxidative stress, pH



- **Combination products**
  - Multimodel imaging (CT, MRI, PET)
  - Theranostics

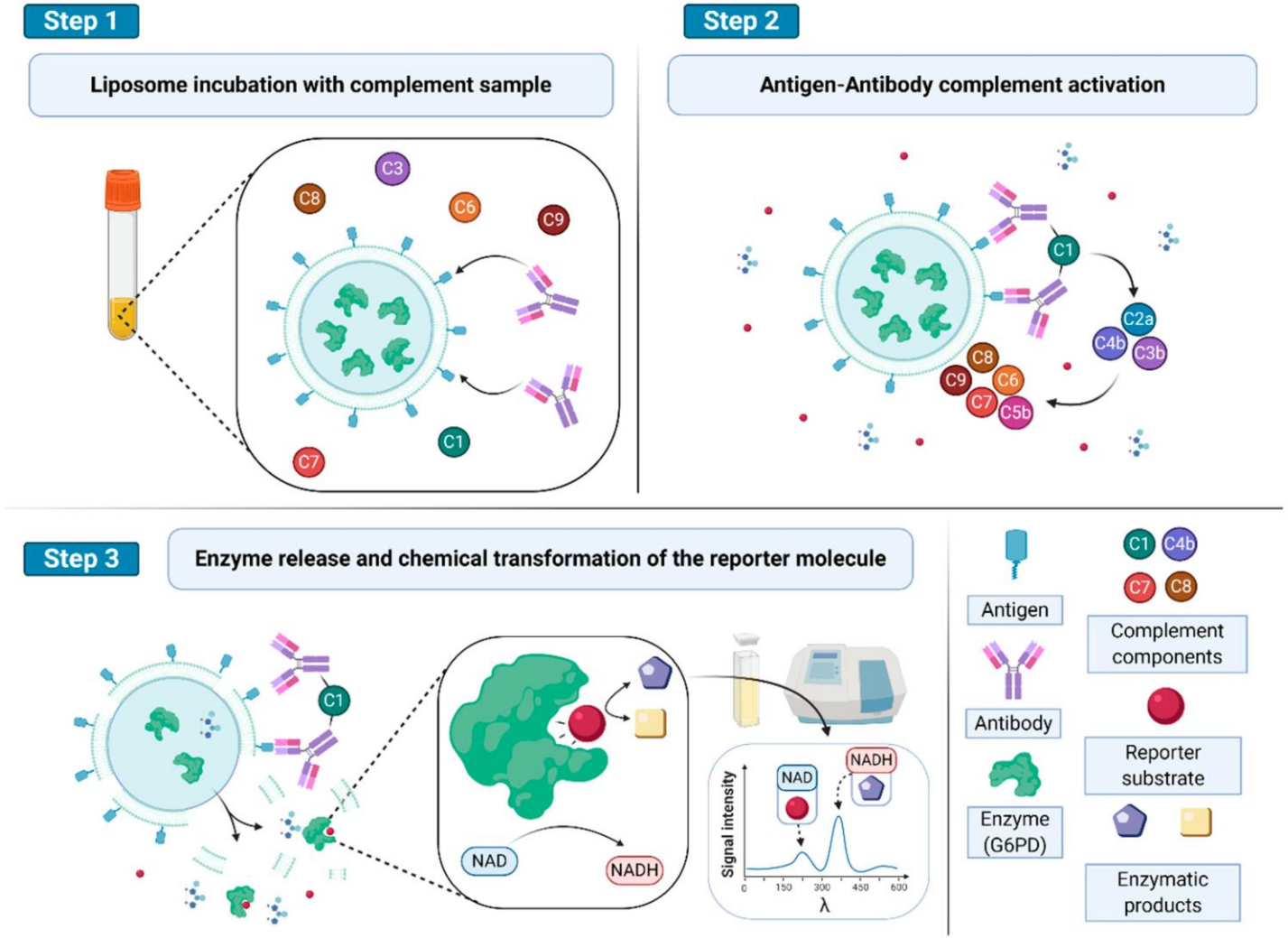
- Studying drug delivery systems
  - Imaging-based study of pharmacokinetics of drug delivery systemd

Use of phospholipids in lab assays

- Lab assays: phospholipids as
  - Analyte (standards): phospholipid quantification
  - Functional excipients
    - *Complement assay*
    - *Anti-phospholipid autoantibody assays*
      - *ELISA*
      - *Coagulation assays*

Liposomal assay for complement function

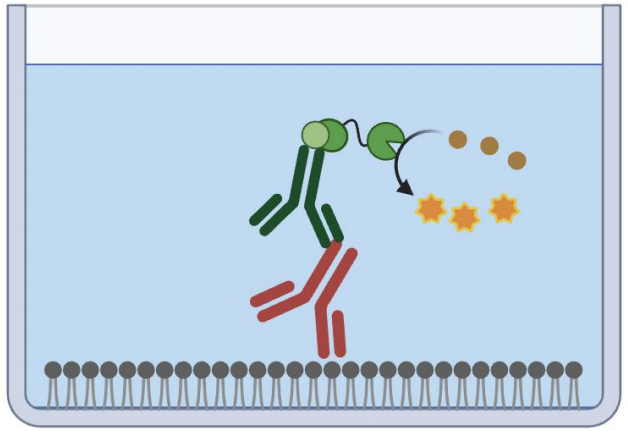
- Indication
  - First-tier screening test for **congenital complement deficiencies**
  - Detection of individuals with an **ongoing immune process**
- Method name: Automated Liposome Lysis Assay (Fujifilm Wako), serum
- **FDA-approved** and clinically used



Mayo Labs
Sforzi+ *Biology* 2020

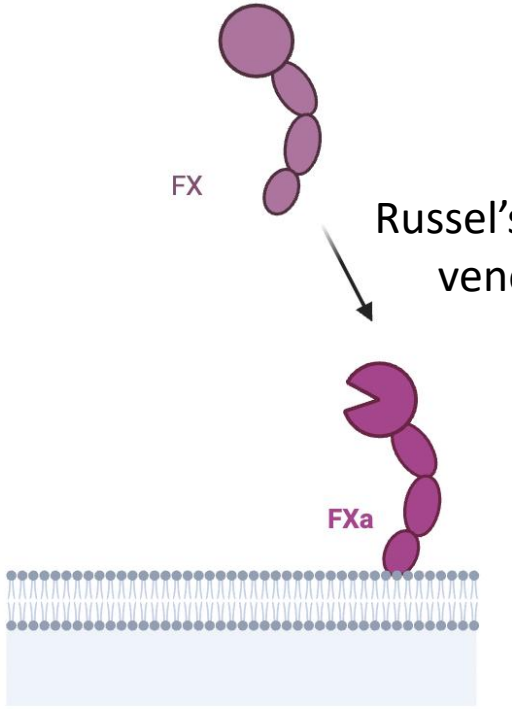
ELISA assay for anti-cardiolipin antibodies

- Indication
  - Diagnosis of **Antiphospholipid Syndrome**
    - Recurrent arterial or venous thrombosis (e.g., deep vein thrombosis, stroke)
    - Recurrent pregnancy loss
    - Often in association with systemic lupus erythematosus
- Method name: Phospholipid (Cardiolipin) Antibodies, IgG, IgA, IgM, Serum (Quanta)
- **FDA-approved** and clinically used

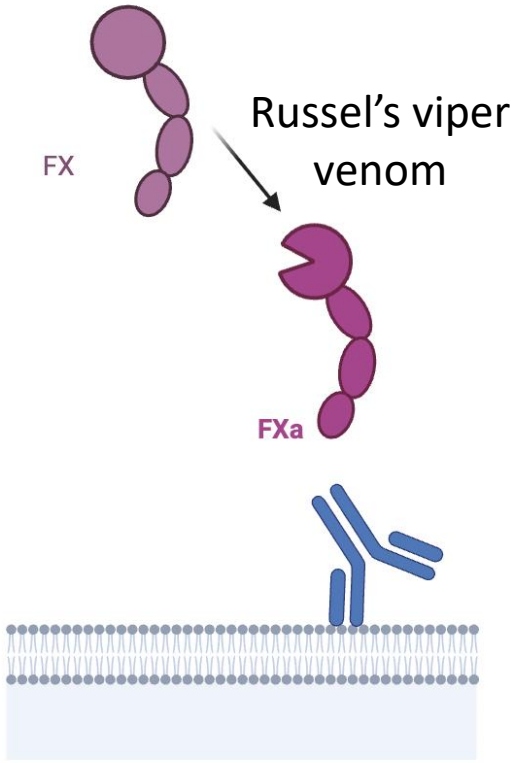


Coagulation assay for Lupus anticoagulants (1)

- Detection of **Lupus anticoagulants**
  - **IgG, IgM, IgA against negatively charged phospholipids** (such as phosphatidylserine or phosphatidylethanolamine)
  - Prolonged coagulation time
- Method name: Dilute Russell's Viper Venom Time (DRVVT), Plasma (Precision BioLogic)
- **FDA-approved** and clinically used



Activates coagulation cascade

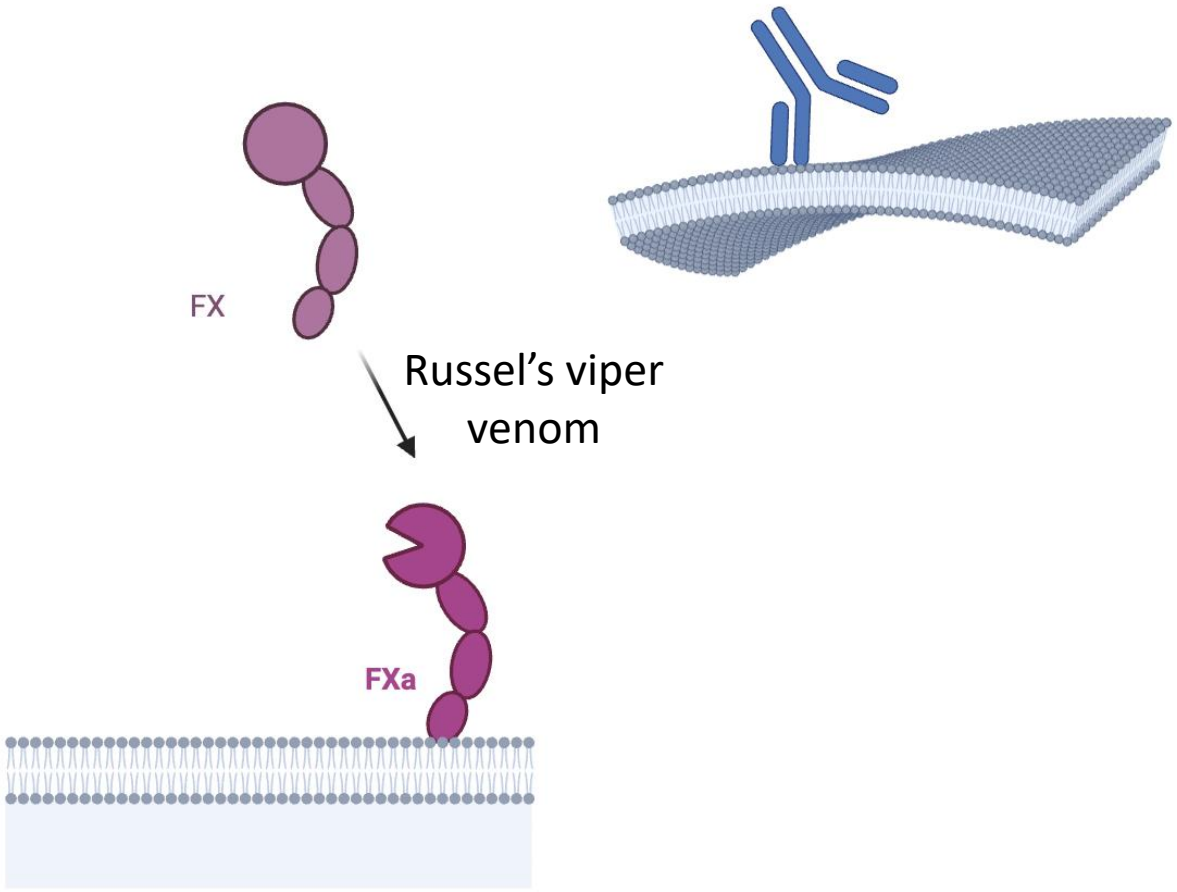


Activated Factor X cannot bind to plasma membrane
No activation of coagulation cascade

Mayo Labs

Coagulation assay for Lupus anticoagulants (2)

- Detection of Lupus anticoagulants
  - Control test
  - Anti-phospholipid antibody strongly binds to **hexagonal phase**
  - Coagulation occurs
- Method name: Hexagonal Phospholipid Neutralization, Plasma (Precision BioLogic)
- **FDA-approved** and clinically used



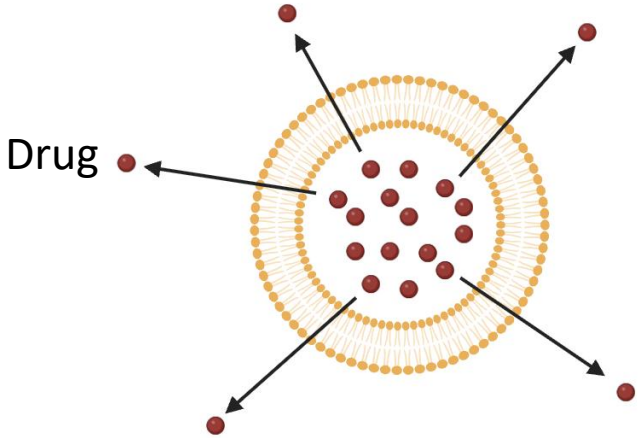
Antiphospholipid antibody binds to hexagonal phase
Activation of coagulation cascade

Mayo Labs

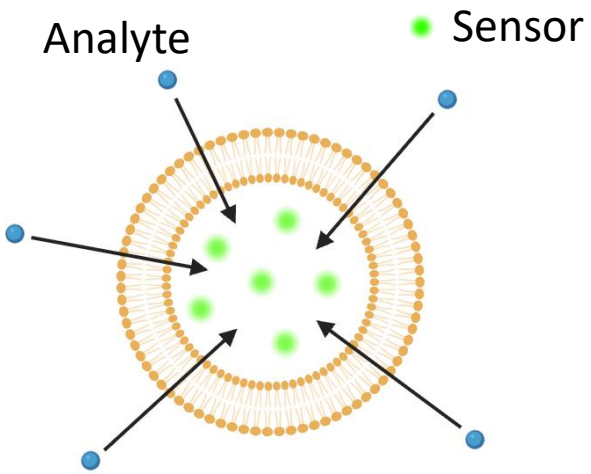
My contributions to the liposomal diagnostics

Repurposing liposomes for diagnostics

Liposome



Drug release



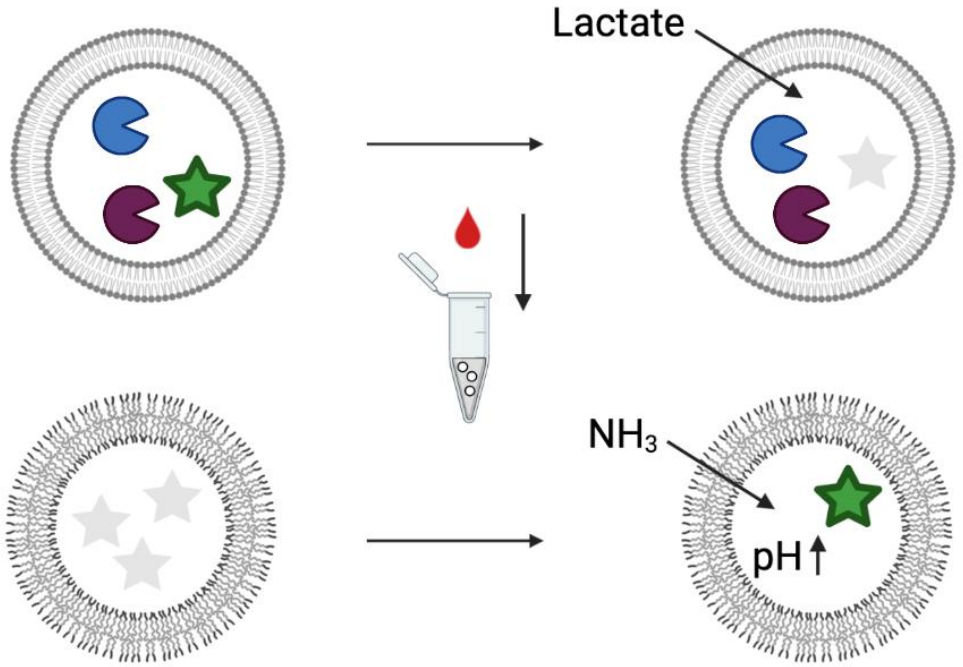
Liposomal microreactor for analyte sensing

Rapid bedside tests in blood

Vesicular reaction compartments

Enzymatic reaction compartment

Chemical reaction compartment



Encapsulation of diagnostic agents

Leveraging the selectively permeable membrane

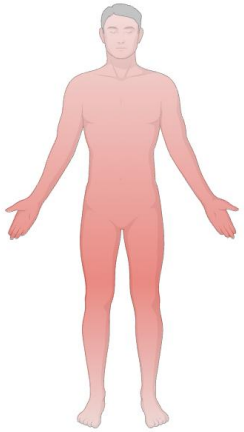
- *to exclude interfering substances*
- *… while allowing for diffusion of analyte*

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

Lactate assay

Lactate sensing in sepsis

- Sepsis
  - Highly prevalent complication of infection, mainly affects elderly
  - High mortality risk (one third of in-hospital deaths due to sepsis)
- Lactate
  - Key biomarker in sepsis (hypoxia)
    - Disease staging, treatment selection (volume replacement therapy)
    - Assessment of treatment success
  - Challenge: only measured in serum/plasma with large instruments (hospital lab)
    - Long time-to-result, delays clinical decision making
    - Need for a bedside test



Evans+ *Crit Care Med* 2021

Colorimetric lactate assay

I.) $$\text{**Lactate**} + O_2 \xrightarrow{\text{Lactate oxidase}} \text{Pyruvate} + \mathbf{H_2O_2}$$

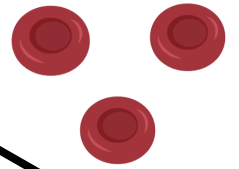
II.) $$4\text{-aminopyridine} + \mathbf{H_2O_2} \xrightarrow{\text{HRP}} 3\text{-(}\textit{N}\text{-ethyl-3-methylanilino)-2-hydroxypropanesulfonate}$$

Colorimetric lactate assay

Only for plasma

I.) Lactate oxidase

Lactate + O_2 ⇌ Pyruvate + $\mathbf{H_2O_2}$ —(Catalase)→ 2 H_2O + O_2

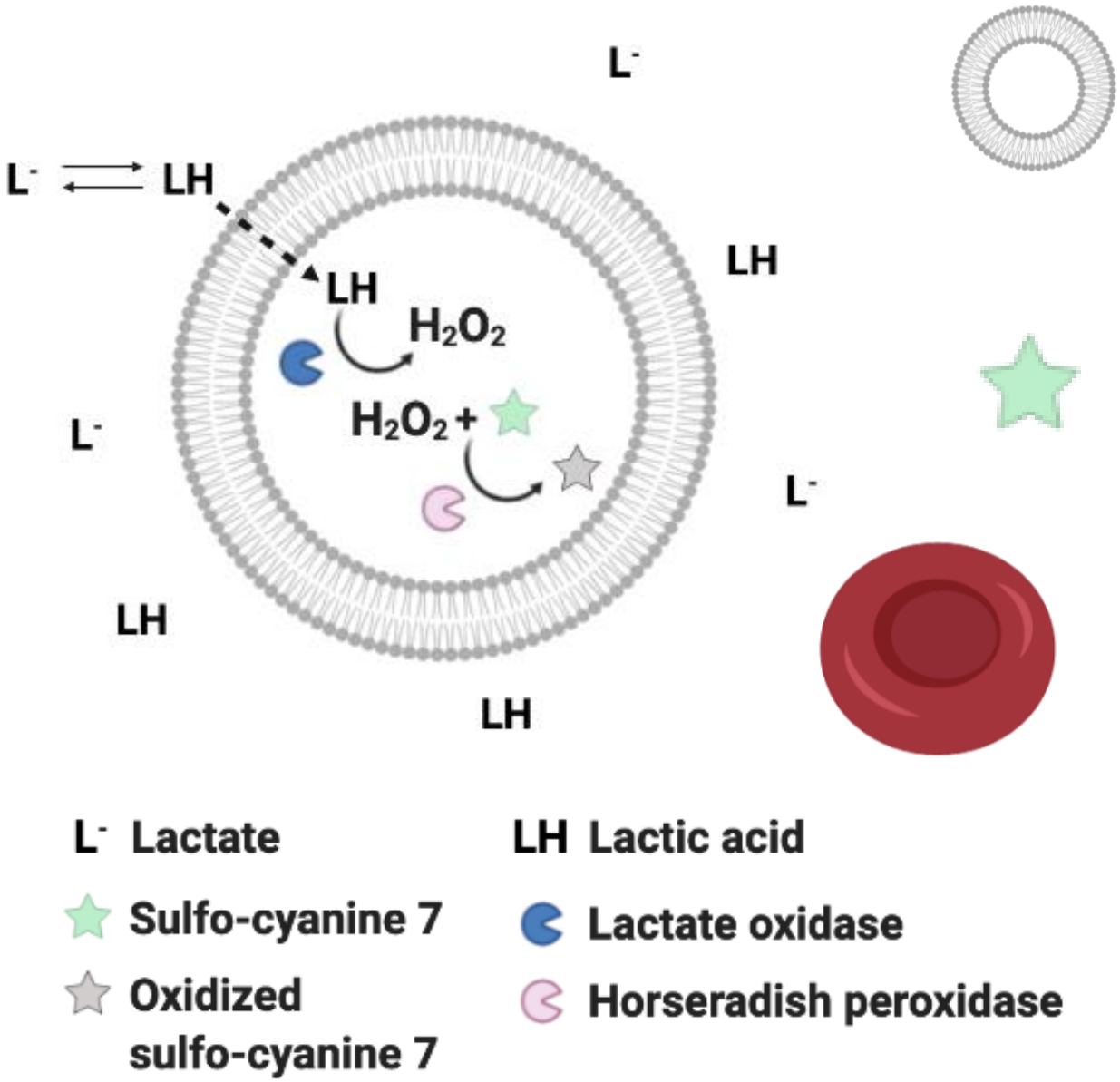


II.) HRP

4-aminopyridine + $\mathbf{H_2O_2}$ ⇌ 3-(*N*-ethyl-3-methylanilino)-2-hydroxypropanesulfonate

Absorbance at 450 nm

Liposomal lactate assay



Liposomal reaction compartment

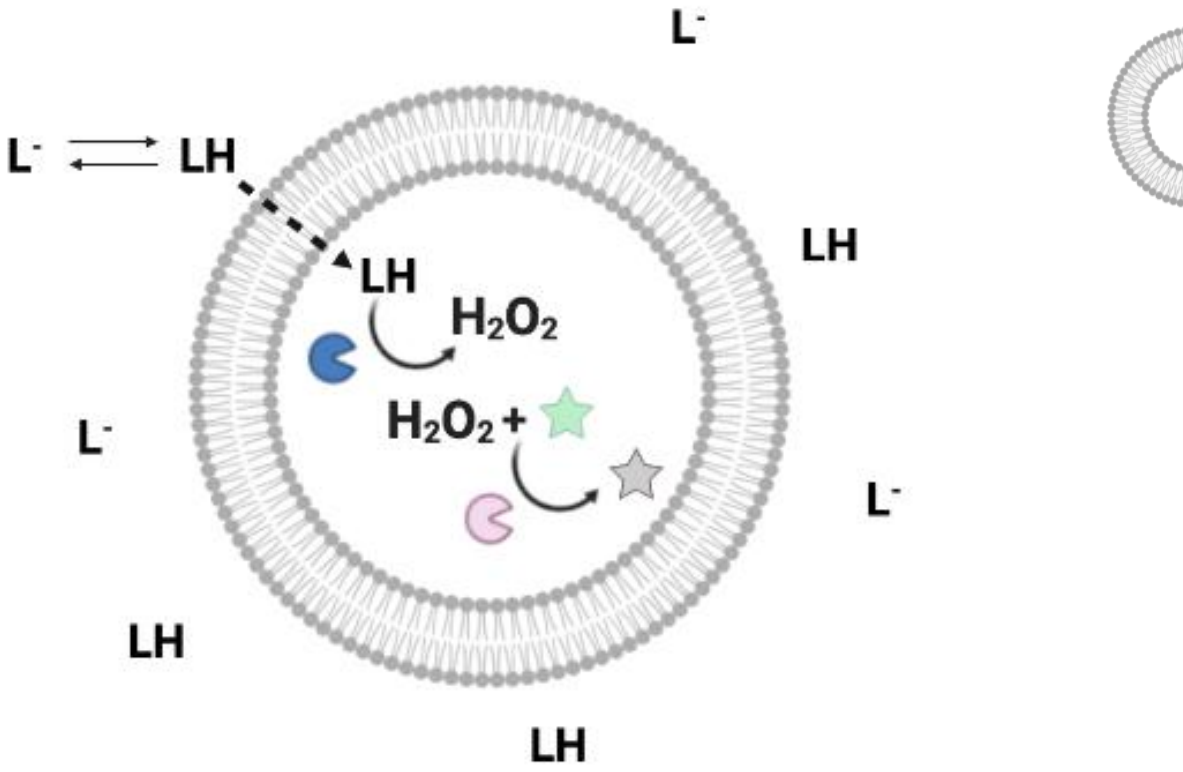
- Isolation of enzymatic cascade reaction
- Protection of H_2O_2 from degradation by catalase

NIR fluorescent HRP substrate Sulfocyanine 7

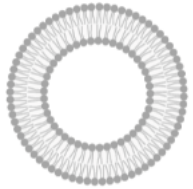
- No interference with hemoglobin fluorescence

Matoori & Mooney *Small* 2020
Matoori & Mooney *Biomaterials* 2022
Matoori & Mooney WO2021080951A1

Liposomal lactate assay



L⁻ Lactate
LH Lactic acid
Sulfo-cyanine 7
Lactate oxidase
Oxidized sulfo-cyanine 7
Horseradish peroxidase



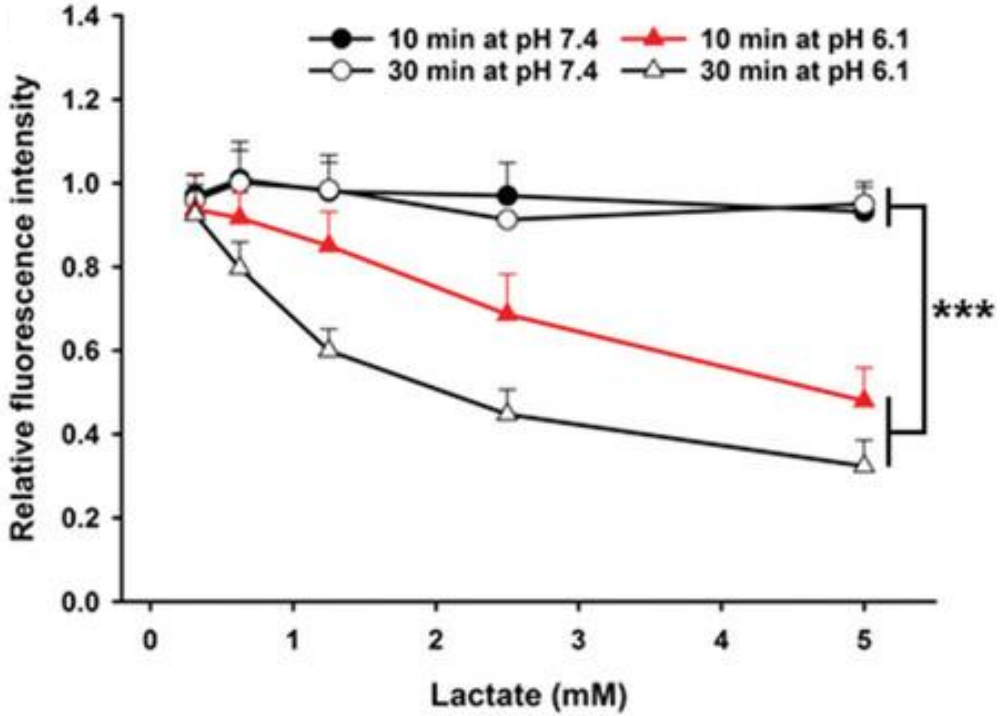
Formulation considerations

- High phase transition temperature phospholipids to ensure dye and transmembrane pH gradient is retained (stability)
- (low phase transition temp PL would enable faster diffusion)
- DPPC 45 mol%, Chol 54 mol%, DSPE-PEG 1 mol%

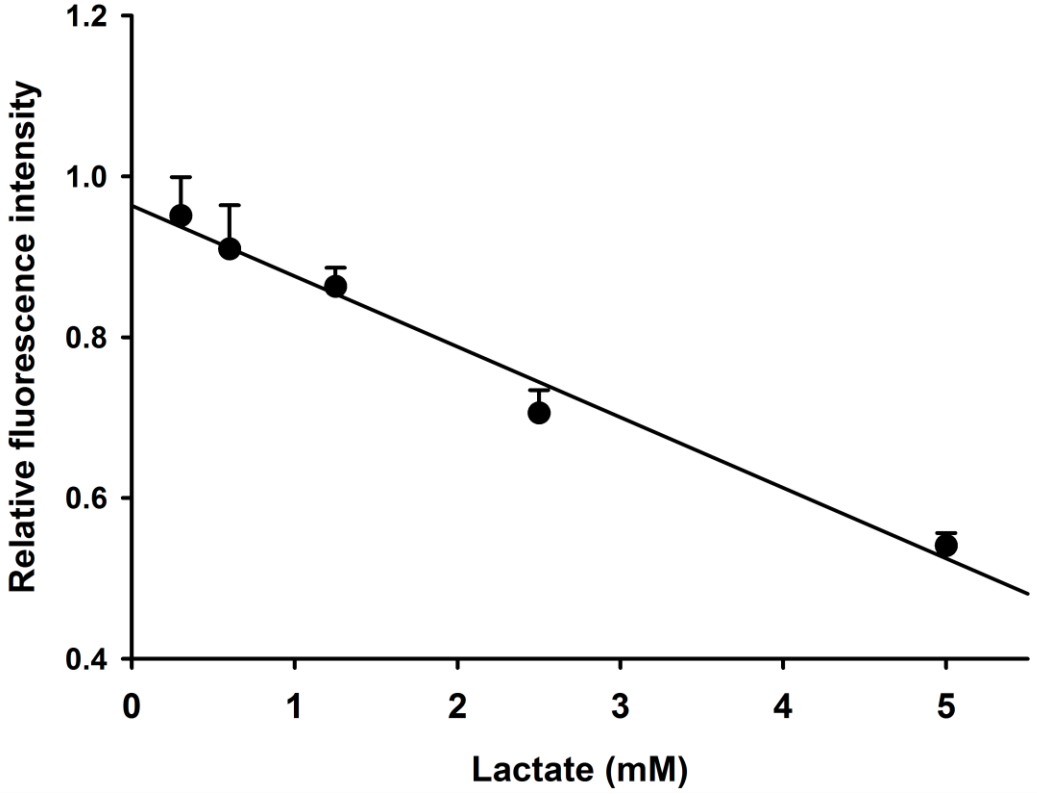
Matoori & Mooney *Small* 2020
Matoori & Mooney *Biomaterials* 2022
Matoori & Mooney WO2021080951A1

Sensing in human whole blood: kinetics

Arterial blood



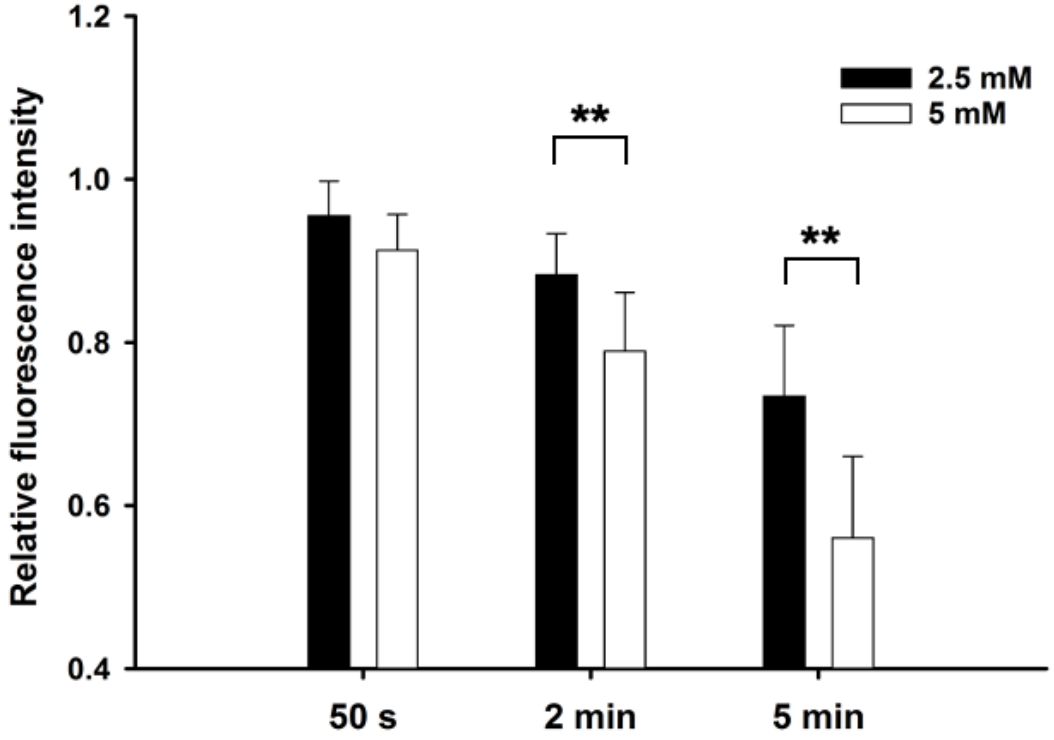
Outer phase pH 6.1
Luminal pH 7.4
Optimized LO and HRP concentrations
Under one minute



Matoori & Mooney *Small* 2020
Matoori & Mooney *Biomaterials* 2022
Guirguis (…) Matoori* *ACS PTS* 2023

Sensing in human whole blood: fresh capillary blood

IRB-approved study in healthy volunteers
Capillary blood

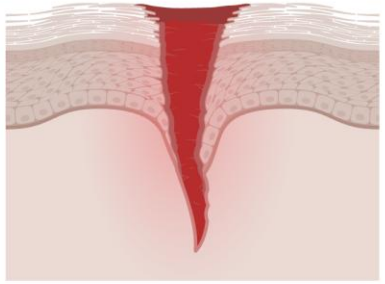


Matoori & Mooney *Biomaterials* 2022

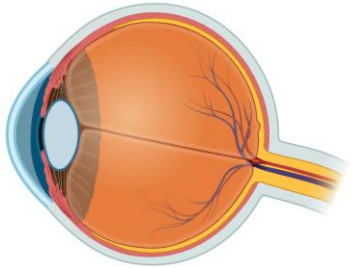
The next frontier: molecular monitoring at the disease site

Blood, plasma/serum, urine

External (surface)



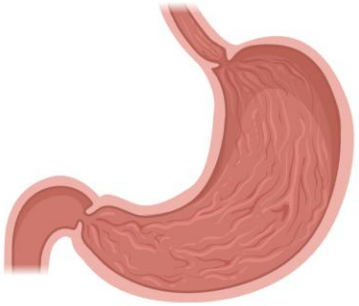
Wound



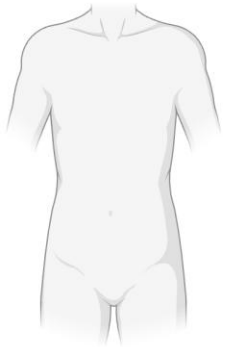
Eye, tears

Buccal

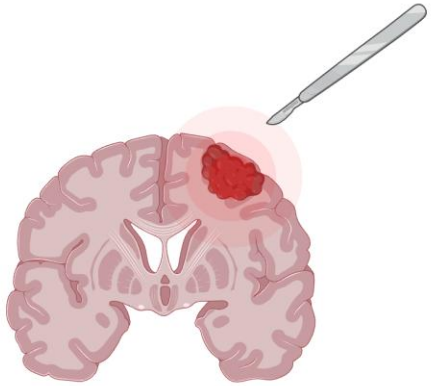
Internal



Gastrointestinal tract



Genito-urinary tract

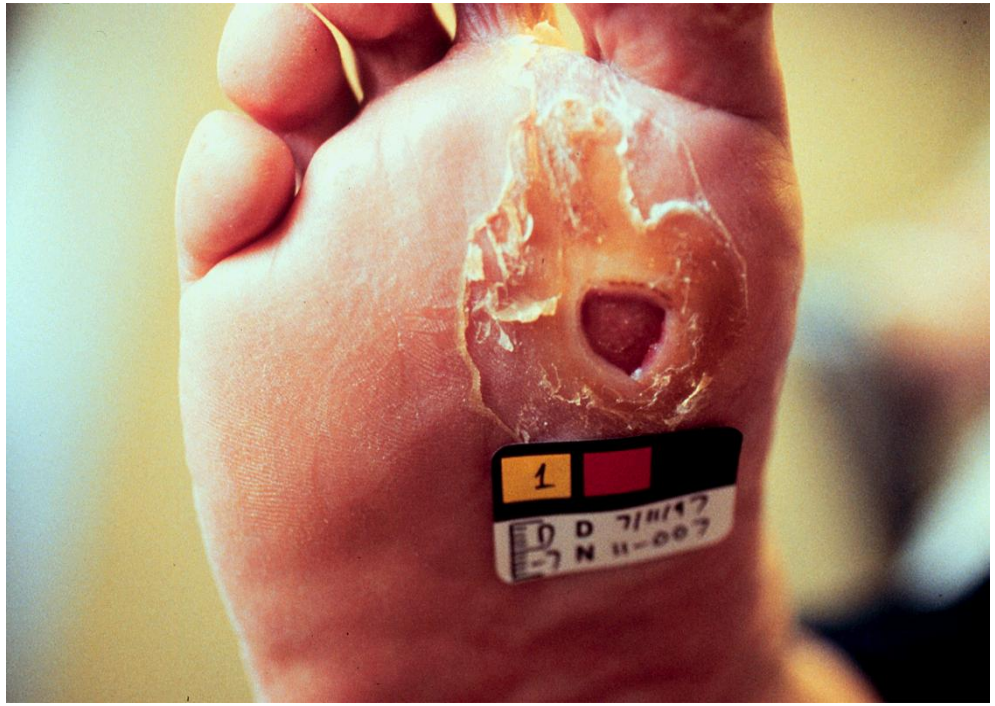


Post-tumor resection

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**

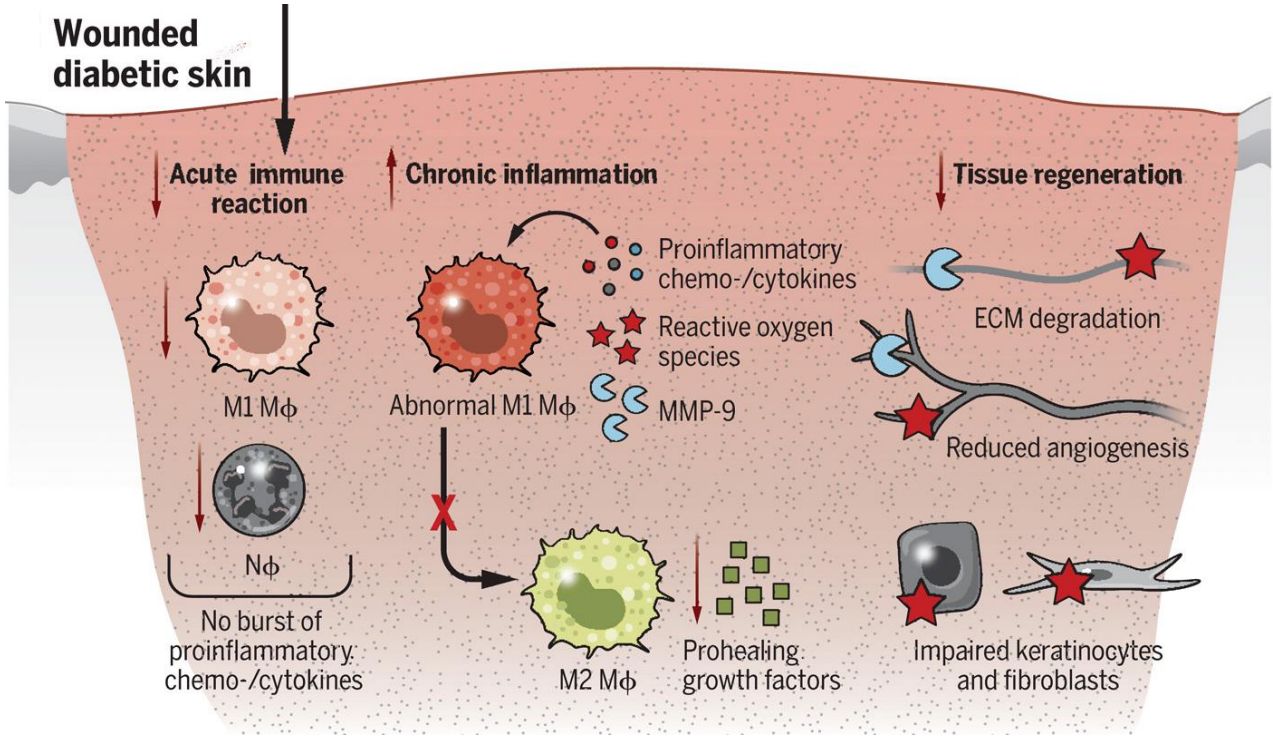
Matoori, Veves, Mooney *Science Translational Medicine* 2021
Freedman*, Hwang, Talbot, Higler, Matoori*, Mooney* *Science Advances* 2023
Mahmoudi (…) Matoori, Mahmoudi* *Nature Reviews Bioengineering* 2024

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

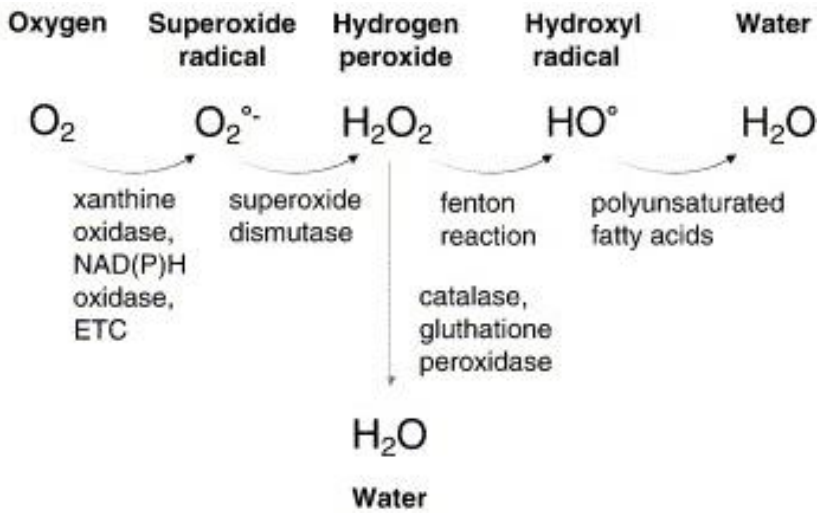


Fu (…) Matoori* *ACS Measurement Sci* 2022
Matoori, Veves, Mooney *Sci Transl Med* 2021

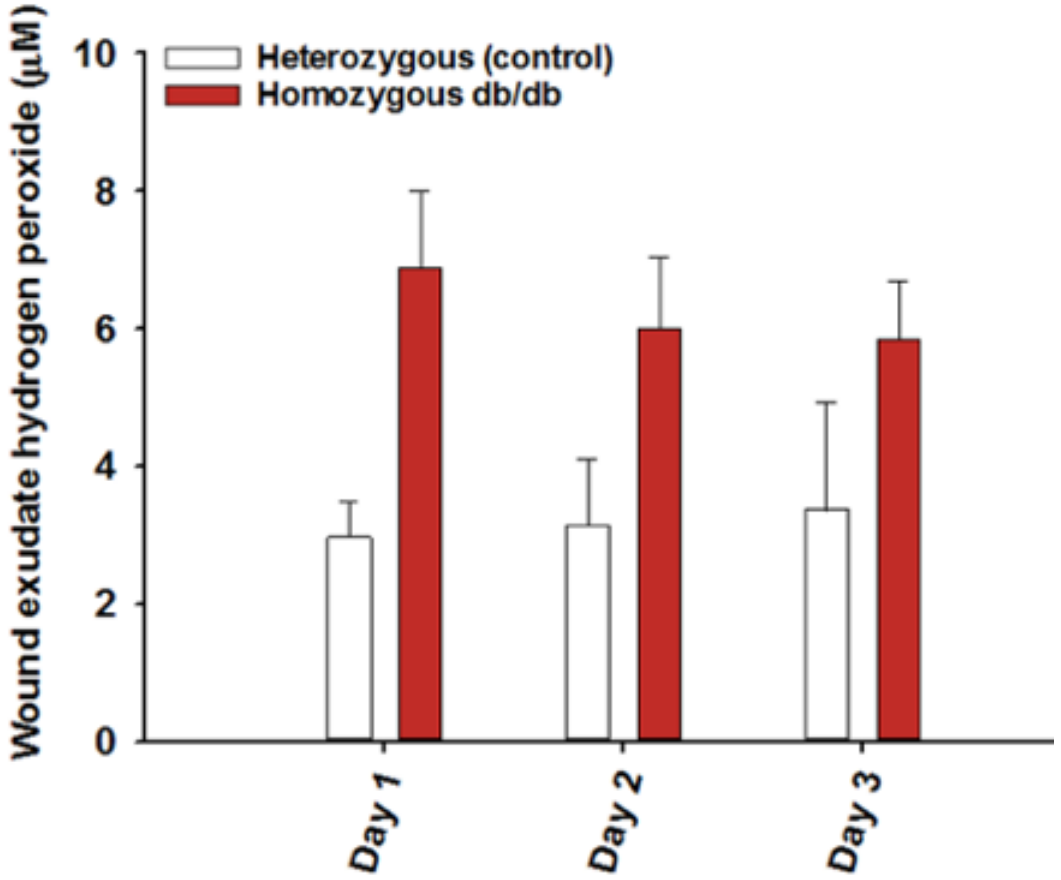
Hydrogen peroxide is a key ROS

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

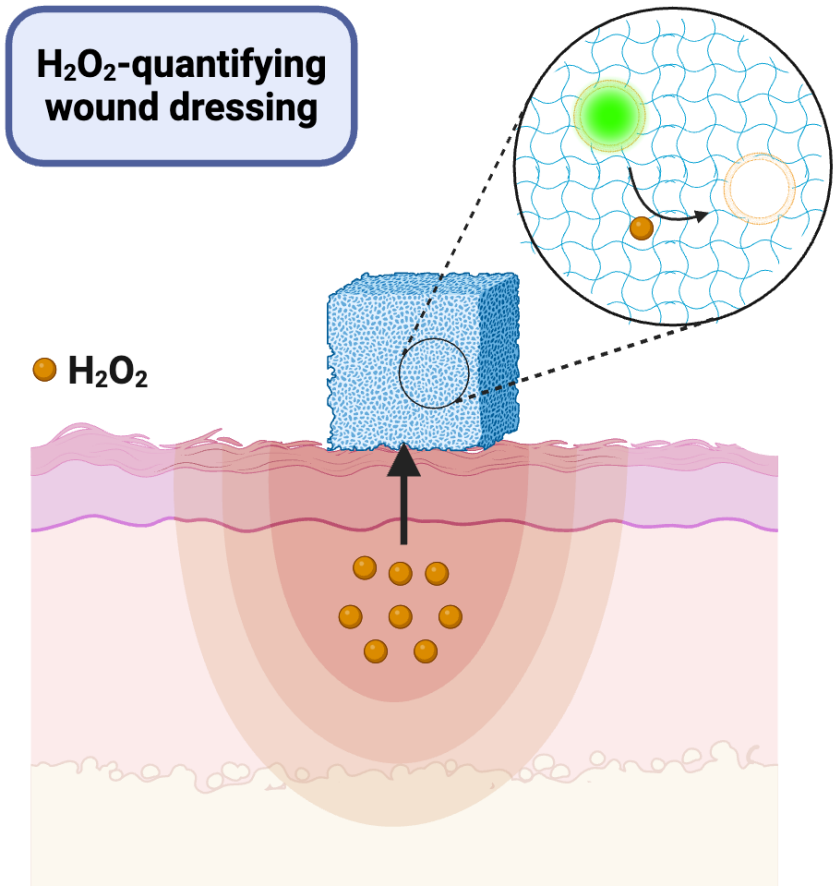
Wang+ *Journal of Controlled Release* 2015
Dhall+ J. *Diabetes Res*. 2014
Fadini+ *Diabetes* 2010

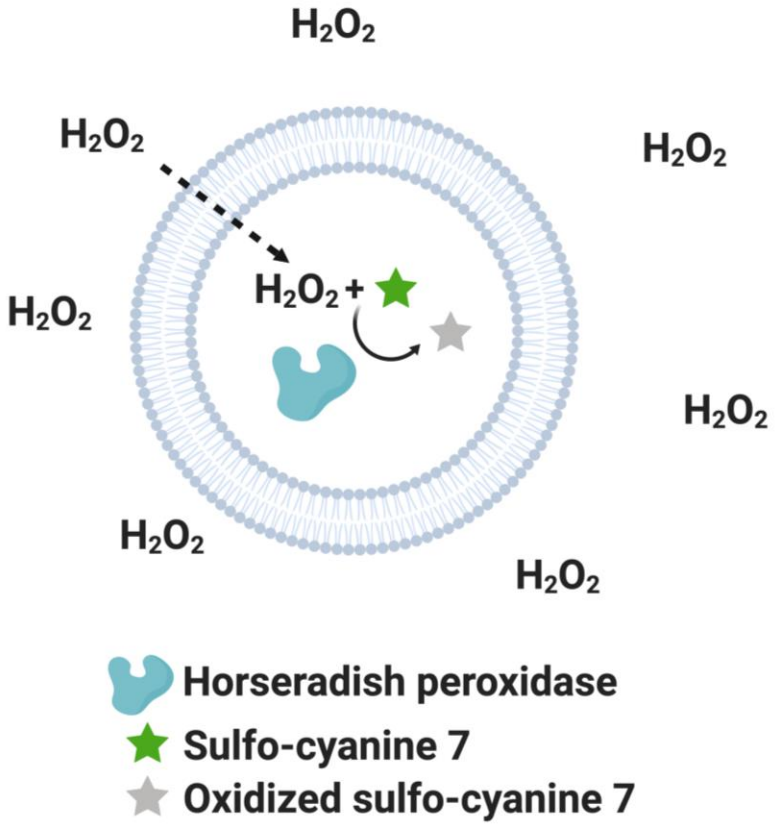


In vivo study performed by us (Matoori/Veves)



Hydrogen peroxide-sensing wound dressing

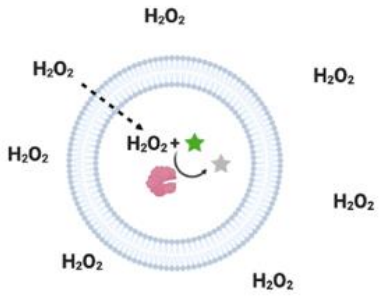


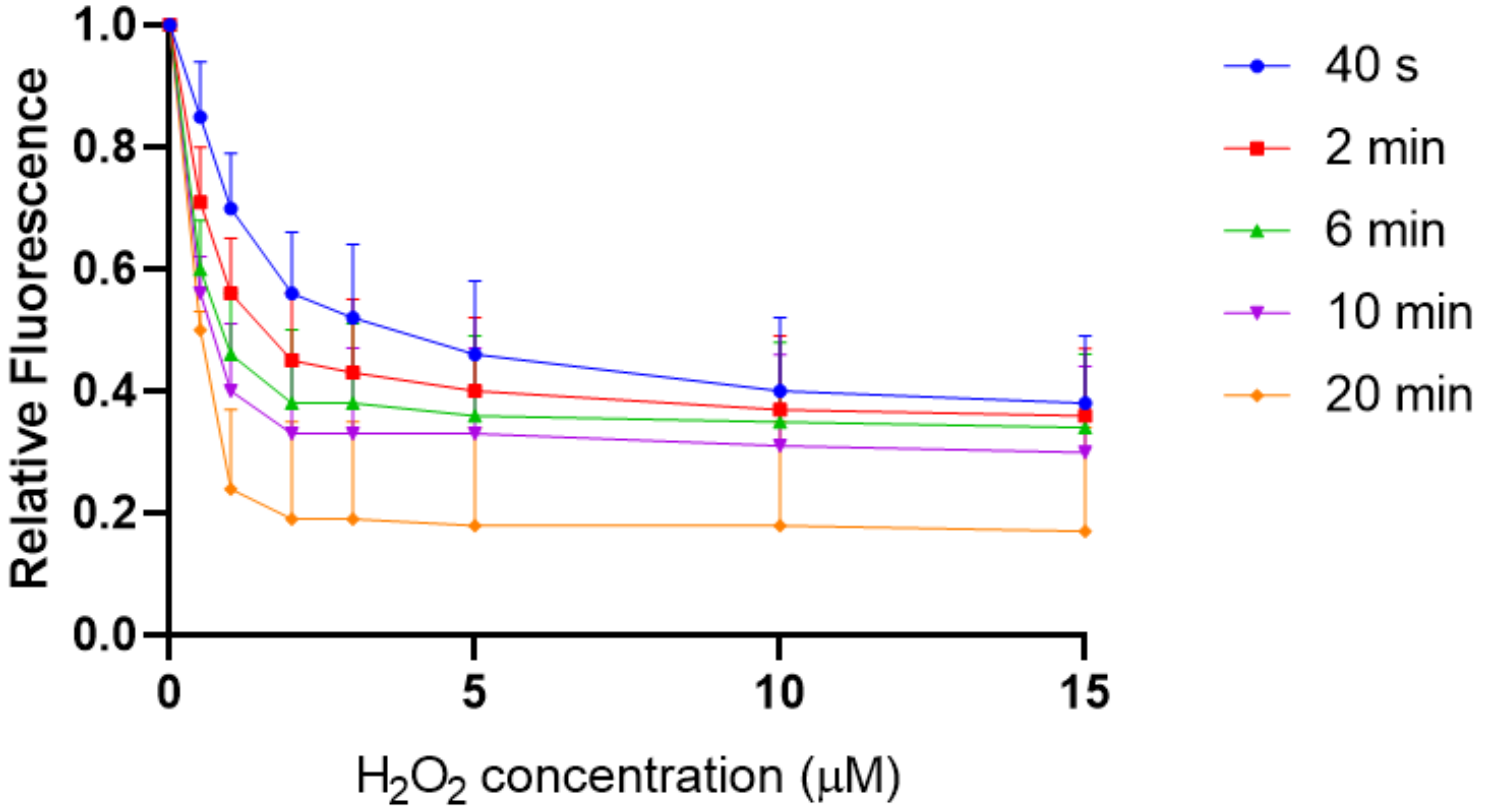


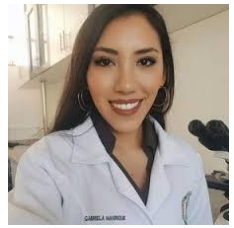
Reaction HRP and S7

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

Liposomal hydrogen peroxide sensing



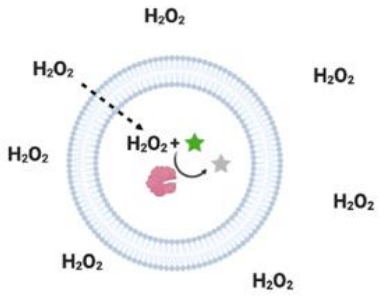


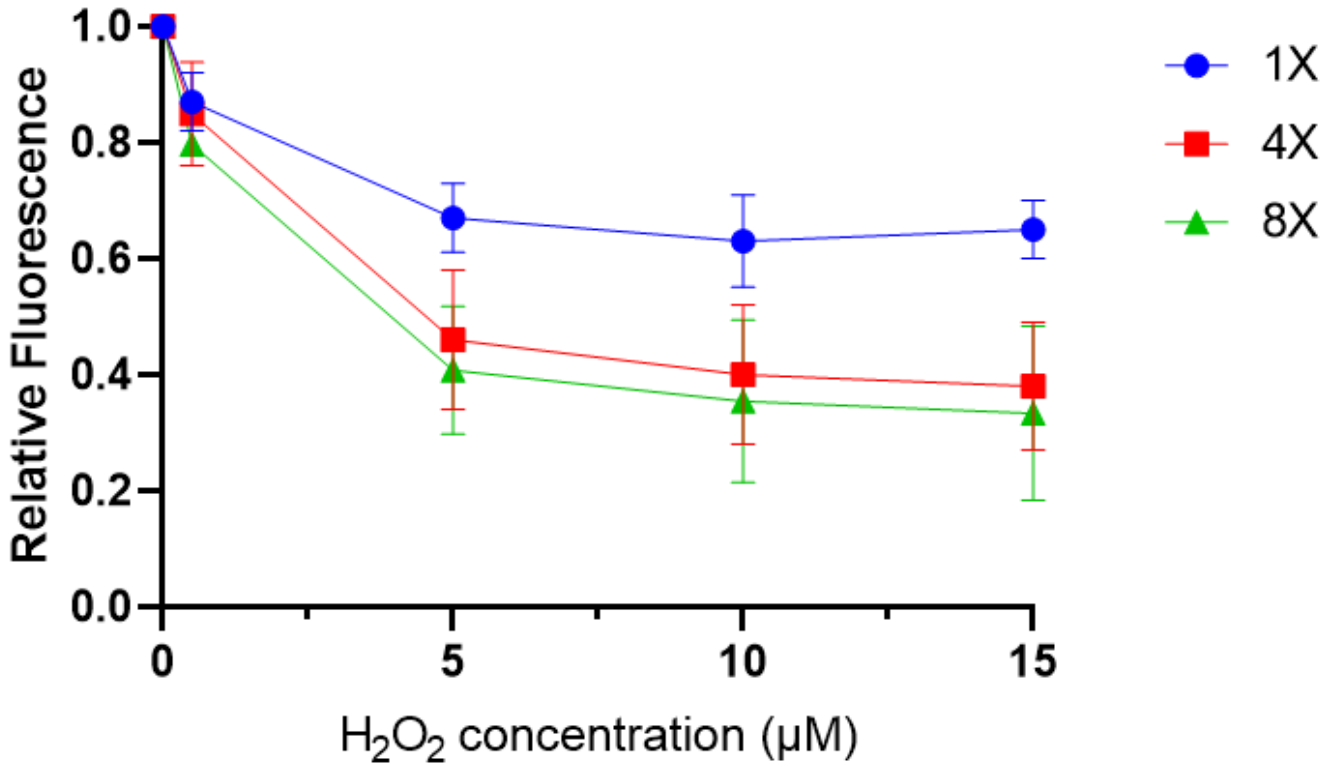


Gabriela Manrique

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

Liposomal hydrogen peroxide sensing

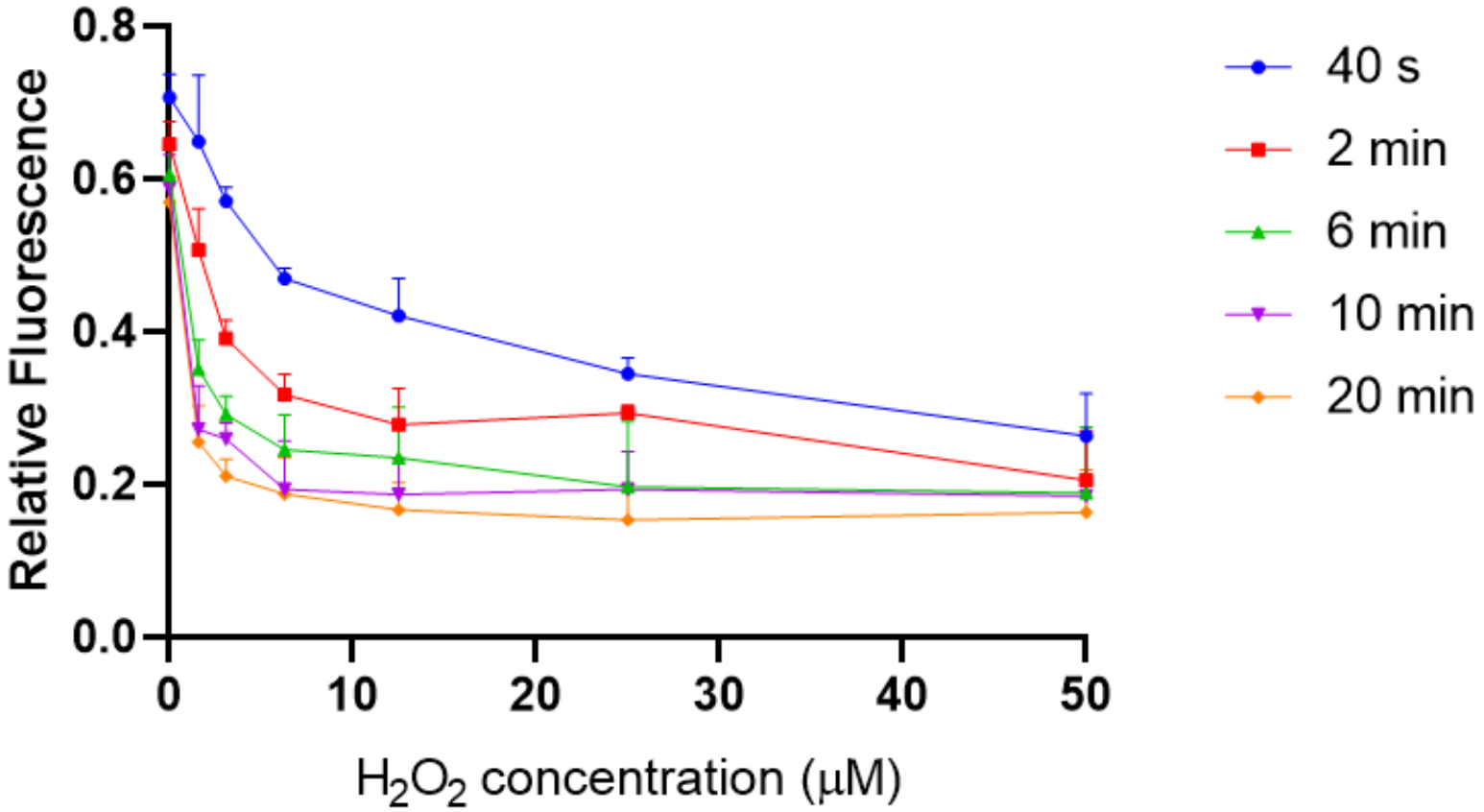




- Increasing the HRP concentration acclerates the response

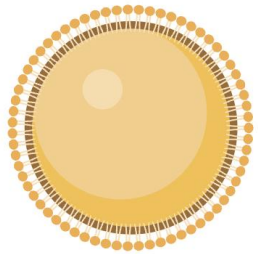
Liposomal hydrogel

Encapsulation of liposomes in calcium crosslinked alginate hydrogel

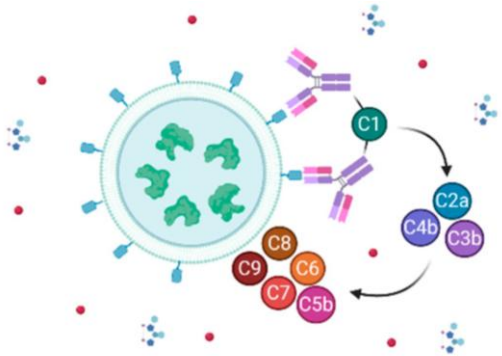


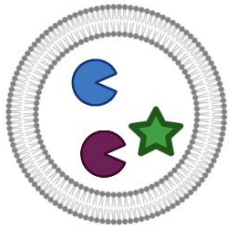
Phospholipids in diagnostics

- Liposomal *in vivo* diagnostics
  - *Microbubbles for ultrasound*
  - MRI/CT/PET: Liposomal delivery systems for contrast agents



- Lab assays *(the FDA-approved liposomes you have never heard of)*
  - *Complement assay*
  - *Anti-phospholipid autoantibody assays*
  - Point-of-care assays: liposomal microreactors
    - Blood assays
    - Wound assays





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Prof. Ben Freedman (Harvard Medical School)
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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

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