





# MANNOSE-BINDING LECTIN DETECTION ASSAY USING GOLD NANORODS

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#### Introduction

#### Importance of Mannose-Binding Lectin (MBL)

Role: MBL activates the complement system lectin pathway and the subsequent inflammatory mechanisms.

Blood levels: ~1 µg/mL in healthy adults.

The incidence and outcome of many human diseases are associated with and influenced by MBL activity and serum concentrations.

#### Why is it important to measure MBL?

- investigation of recurrent infections;
- monitoring patients at increased risk of developing diabetes complications and cardiovascular disease;
- > elucidation of suspected immune system deficiencies;
- > evaluation of medication therapies in immunosuppressed patients.

# How can we quantify MBL?

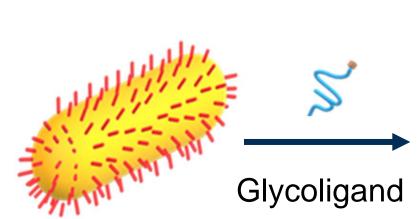
**ELISA** 

#### Problem:

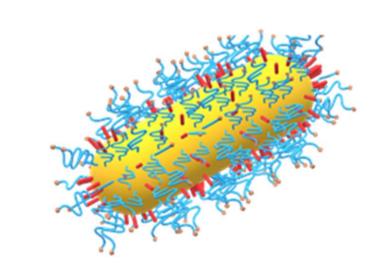
several incubation and washing steps; lengthy turnaround times.

#### Aim:

fast, non-invasive, one-step, wash-free nanoplasmonic-based assay for MBL detection with no dilutions required







Glycoligands coated GNRs

В.

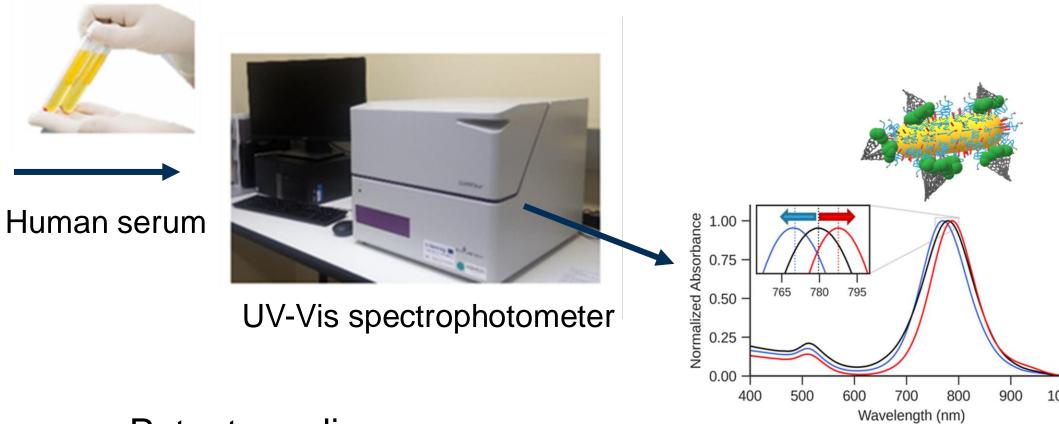
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LSPR peak shift (nm)

# Our Assay

### Assay specifications

- ✓ Label-free system based on the localised surface plasmon resonance (LSPR) of gold nanorods (GNRs)
- ✓ One-step reaction
- ✓ Performed directly in human serum without dilutions
- ✓ Read-out using simple microplate reader
- ✓ LoD ~ 160 ng/mL MBL
- ✓ Fast (15 min)
- √ Easy-to-use



#### Patent pending

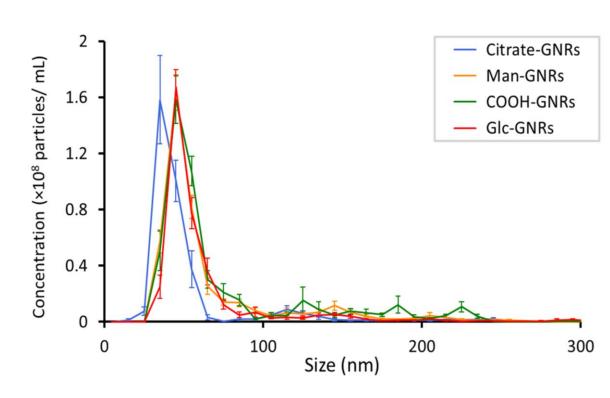
## Assay characteristics

#### Gold nanorods functionalization and coating density

# UV-Vis spectroscopy. Citrate-GNRs Man-GNRs COOH-GNRs Glc-GNRs Glc-GNRs 0.4 0.2 0.0 Wavelength (nm)

# Differential centrifugal sedimentation Citrate-GNRs Man-GNRs COOH-GNRs Glc-GNRs Glc-GNRs Particle diameter (µm)

# Nanoparticle tracking analysis



- ✓ High colloidal stability.
- ✓ Successful attachment of the glyco ligands to the gold particle surface.
- > Approx. 4 molecules of mannose-ligand × nm<sup>2</sup> of rods
- > 15 μg/mL of Man-GNRs is the best rods concentration to achieve the highest performance of MBL binding assay in human serum

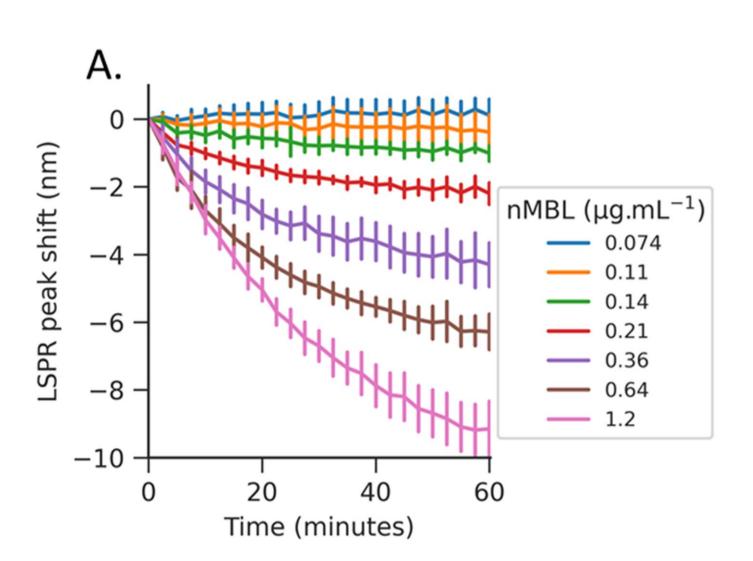
# Acknowledgements

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## MBL binding assay in human serum



- ✓ Quantitative analysis
   based on concentration dependent LSPR shift (A)
- ✓ Good sensitivity after15 minutes (B)
- ✓ LoD: 0.16 µg/mL
- ✓ Reproducible across four different coupling reactions from 2 batches of GNRs

### Quantification of MBL binding to Man-GNRs

✓ MBL strongly bound on the Man-GNR surface

8.0

1.0

0.6

nMBL ( $\mu$ g.mL<sup>-1</sup>)

0.4

√ 11 to 13 MBL molecules binding onto each functionalized nanorod

Man-GNRs (μg/mL)	MBL bound (ng/mL and %) by ELISA	MBL bound (ng/mL and %) by WB
15	210 ± 30 (18%)	227 ± 6 (19%)
30	430 ± 40 (36%)	370 ± 20 (31%)
60	810 ± 20 (68%)	740 ± 40 (61%)
80	955 ± 34 (80%)	890 ± 20 (74%)

