

# Pathogen Capture Using Floating Films

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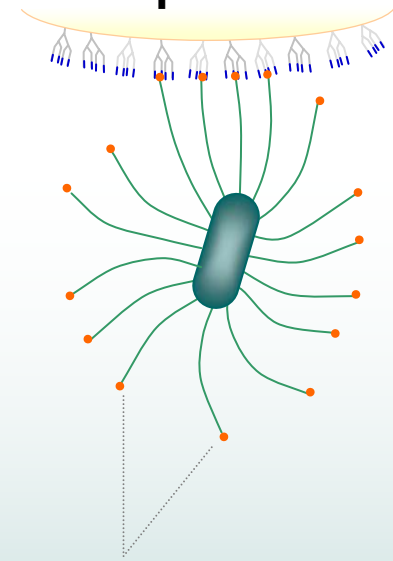
# Problem

- **Monitoring water for the introduction of infectious disease agents is expensive**
- **Collecting samples of pathogens and biotoxins present in low concentration is cumbersome and time consuming**
- **Can we quickly isolate human pathogens from a mixture of microbes and other particulate matter using inexpensive biocapture films?**

# Background

- In order to cause disease, pathogens and their toxins must bind to host tissues
- Most pathogens produce adhesive proteins that attach to specific sugar sequences on host cell surfaces
- Carbohydrates found on human cells are sometimes present on inexpensive glycoproteins of other organisms
- Biocapture films are produced at the interface between oil and a solution of glycoprotein

**Floating biocapture film bears receptor sugar sequences**



**Proteins (adhesins) produced by pathogenic bacteria bind to sugar sequences on film surface**

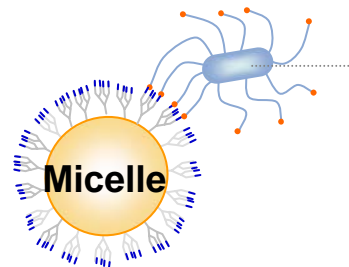
# Objective

- **Determine whether glycoprotein films can selectively capture human pathogens present in low concentration and lift them to the surface of water containing a mixture of microbes and particulate matter**
- **Define mixing time and other requirements for capturing specific pathogens on floating glycoprotein films**

# Activities

- Testing biocapture in low concentrations of human pathogens on glycoprotein films presenting different carbohydrate targets
- Developing quality-control methods for determining whether a batch of biocapture micelles\* bears intact carbohydrate receptors
- Refining methods for mass-producing glycoprotein micelles within an optimal size range

\* A biocapture micelle is a bubble of glycoprotein film filled with oil that causes it to float to the water surface



Pathogen captured by sugars coating outer surface of micelle

# Highlight

**Biocapture\* following mixture for 10 minutes of 6 milliliters (ml) pathogen suspension with 1 ml micelles laced with the sugar, mannose**

Strain of bacteria	Micelle size**	Ratio of captured to residual cells*	
		Trial 1	Trial 2
<i>E. coli</i> (CFT073) expressing mannose-binding adhesins	Large	1.86	2.96
	Medium	5.96	5.53
	Small	10.14	8.15
Control: CFT073 mutant unable to express mannose-binding adhesins	Large	0.46	0.98
	Medium	0.38	0.56
	Small	0.50	0.25

\* Measured in colonies that formed from 100 microliters ( $\mu\text{m}$ ) of biocapture micelles (or remaining suspension) following overnight incubation at 37 degrees centigrade.  
Original pathogen concentration: 1000 colony-forming units per ml.

\*\*Operational definitions of micelle diameter:

“Large”	> 300 $\mu\text{m}$
“Medium”	100 to 300 $\mu\text{m}$
“Small”	10 to 100 $\mu\text{m}$

# Highlight



**SugarBindDB**  
Pathogen Sugar-Binding Database

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## Search Results

Pathogen or Toxin	Carbohydrate or Ligand
Rotavirus	NeuGc(a2-3)Gal(b1-4)Glc(b1-1)
Salmonella	Man
Sendai virus	NeuAc
Sendai virus	NeuAc(a2-3)[NeuAc(a2-3)Gal(b1-4)] Gal(b1-4)Glc(b1-1)
Serratia marcescens	Man
Shiga toxin	Gal(a1-4)Gal(b1-1)
Shigella dysenteriae	Gal(a1-4)Gal(b1-4)Glc(b1-1)Cer
Staphylococcus saprophyticus	Gal(b1-4)GalNAc
Streptococcus aureus	GalNAc(b1-4)Gal

MITRE offers a free database listing primary research on carbohydrate receptors of human pathogens

<http://sugarbinddb.mitre.org/>

MITRE will integrate SugarBindDB with other Web-based databases to provide a comprehensive bioinformatics resource for scientists studying the glycobiology of disease

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# Impacts

- **Floating glycoprotein micelles offer an affordable means of lifting pathogens and biotoxins from contaminated water to the surface where they can be collected for analysis**
- **Biocapture on glycoprotein micelles will facilitate the identification of pathogens in surface water and other infected fluids**

# Future Plans

- **Collaborate with potential users of the technology to test biocapture in field samples**
- **Develop efficient collection and sample preparation protocols**
- **Challenge the technology in fluids containing a low pathogen concentration and high concentrations of other microbes and particulate matter**