

# EPS: An Elusive Refuge for Bacteria in Water Systems

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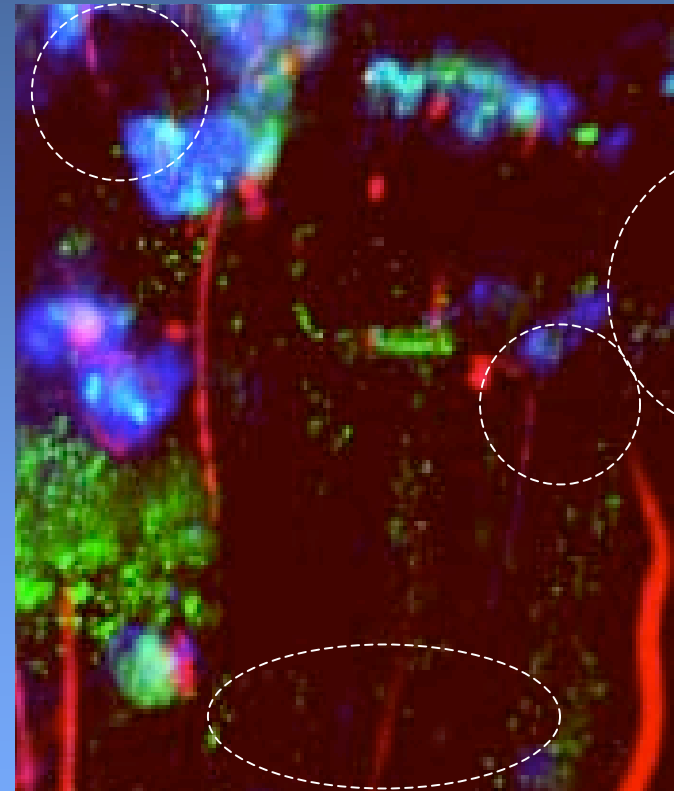
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**Also, an LSU  
Tiger!**  
(Ph.D. degree)



# Biofilm impacts:

- **RO Systems**  
(biofouling)
- **Sewage Trt**  
(activated sludge flocs & granule form.)
- **Trickling Filters**  
(resident flora)
- **Water Distrib. Systems**  
(recent *Salmonella* outbreak, Colorado)
- **Survival of bacteria in resistant 'flocs'** (in natural waters)

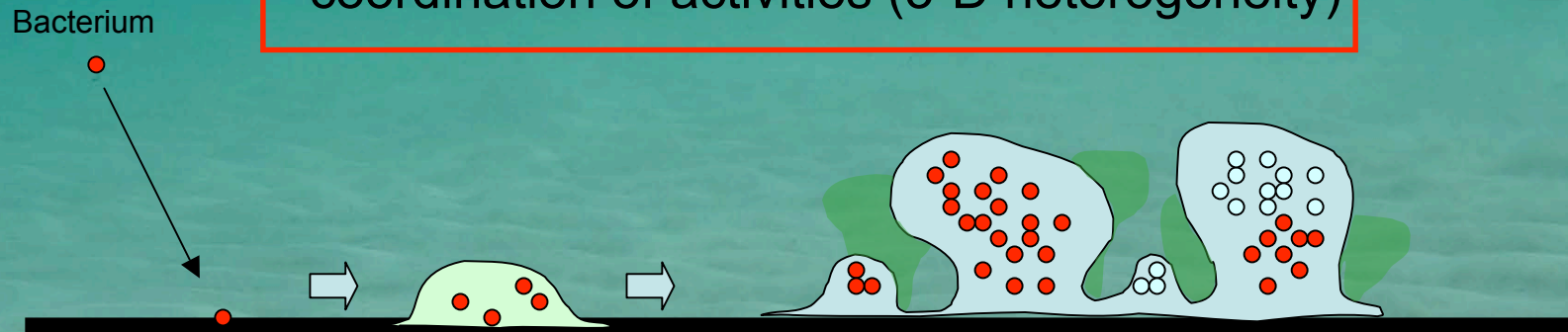


# What is EPS? (*Extracellular polymeric substances*)

-attached (or floc) bacteria



-secrete extracellular polymers (EPS)  
-communication (chemical signals)  
-coordination of activities (3-D heterogeneity)



- **EPS Enhances**: Attachment

- Sequester nutrients
- Defense vs. other microbes
- Avoid toxic compounds
- Coordinate virulence (escape immune response)
- microbial metal corrosion

- Produced by bacteria  
-Dissolved / Colloidal / Attached  
-Complex of Large to Small Molecules

# EPS: Sometimes more than a handful!

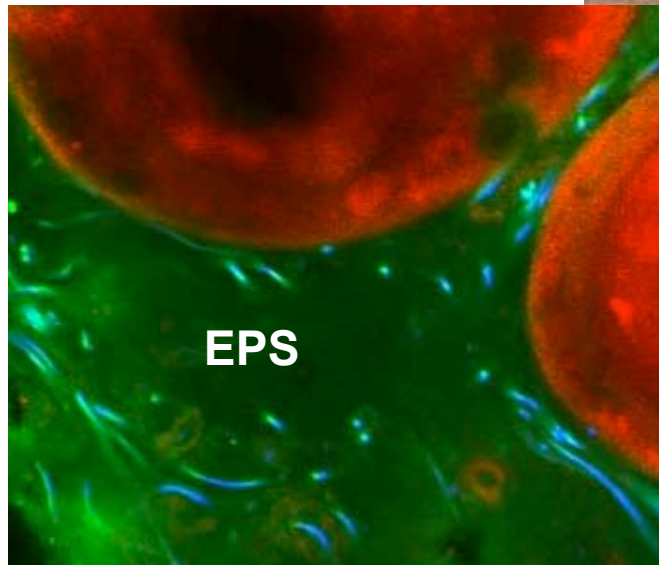
## COMPOSITION:

Uronic acids, sugars

Proteins, *peptides*,  
*dehydrins*, *GAGs*, etc

DNA → *viruses*, *EPS*?

50% (*dry wt EPS*) not identifiable



- *EPS* – nemesis of (cell) DNA Extraction
- *Diffusion-Slowing* properties
- 3D Matrix Effects: photolysis reactions
- Bind metals, ions, organics, immobilize water

# Composition of EPS:

The “*Dark Matter*” of *Bacteria* (*sensu T. Neu*)

“*Dark Matter in the Biofilm Universe*”!!! (*sensu Hans-Curt*)

**Carbohydrates:** Large AND small molecules

- **Proteins:** → -extracellular enzymes;  
structural proteins;  
-lectins; glycoproteins
- **Lipids:** Amphiphilic (‘cyclodextrins’)
- **eDNA:** -structural??; lysis products? plasmids?

***What is Role?***

## Post- Secretional Changes:

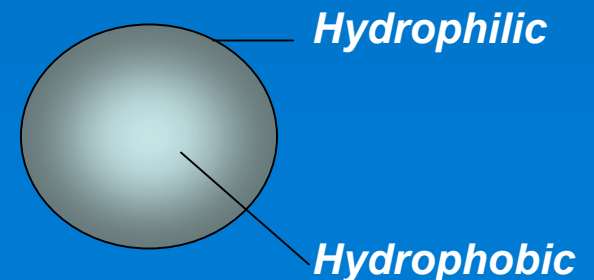
- **Protective Groups:** e.g. **O-Acetylation:**  
→ inhibit easy hydrolyses
- **Partial Degradation**
- **Steric Changes:** ‘Expose’ or ‘Hide’ functional groups  
→ change properties of molecules  
(50% non-ID’d)

***Q. HOW DO EPS MOLECULES INTERACT?***

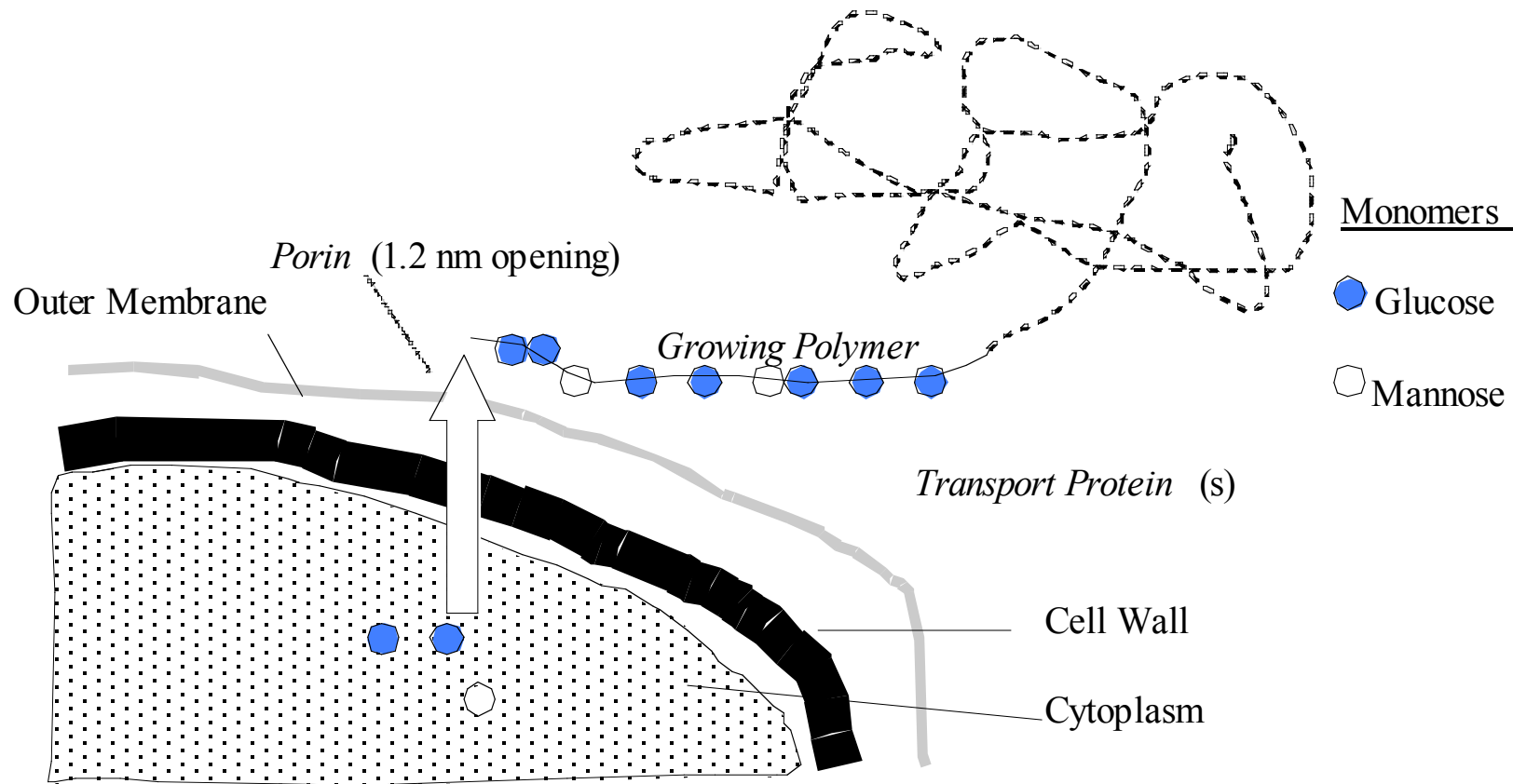
***→ POORLY-UNDERSTOOD***



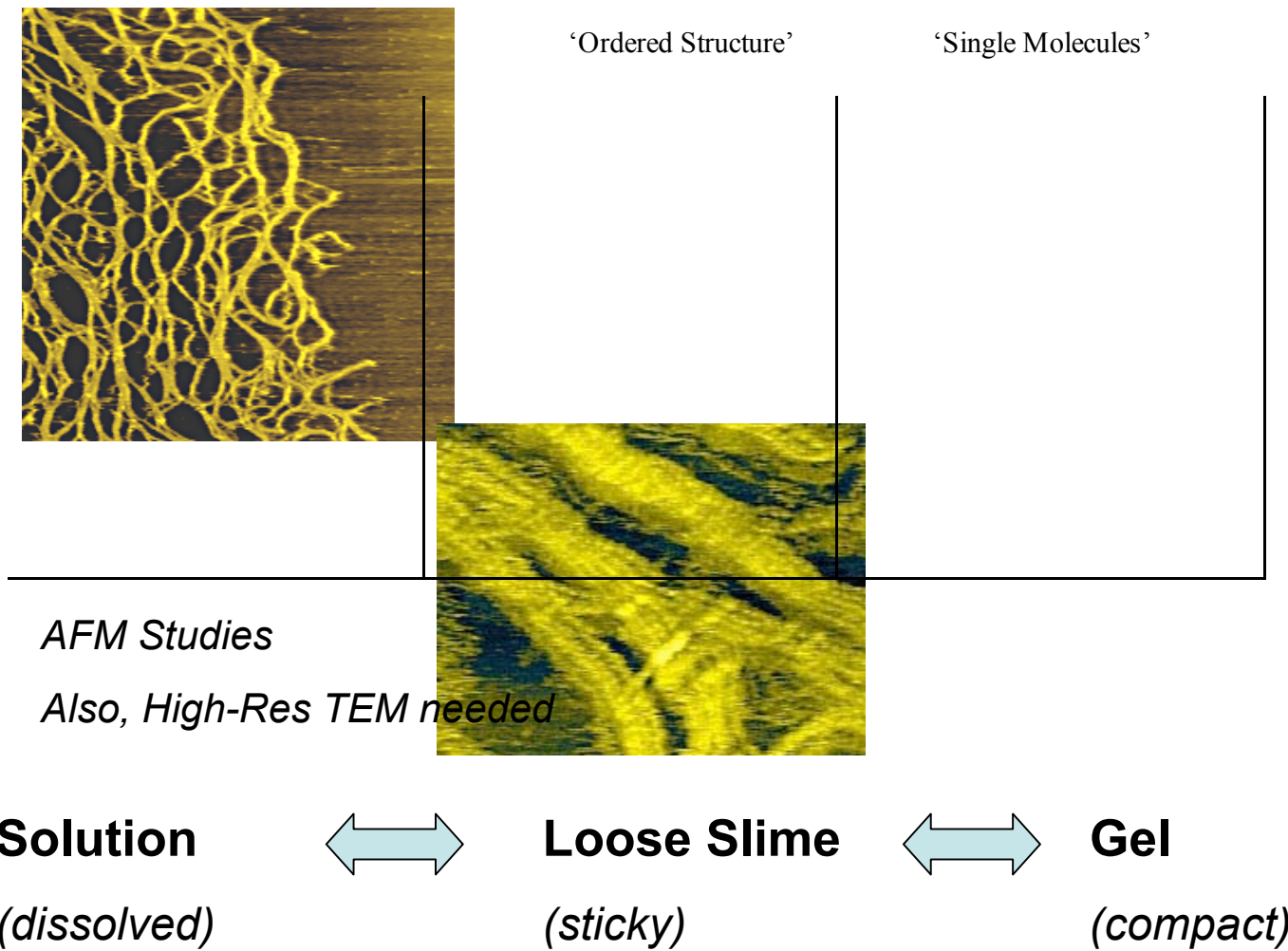
## ***Cyclodextrin Effect***



# Secretion of EPS by Cells

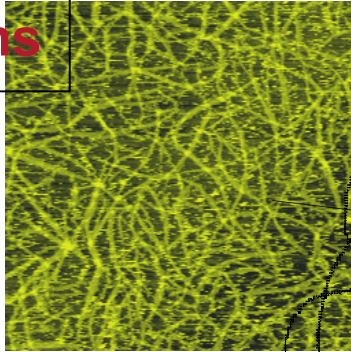


# Different Physical States of EPS

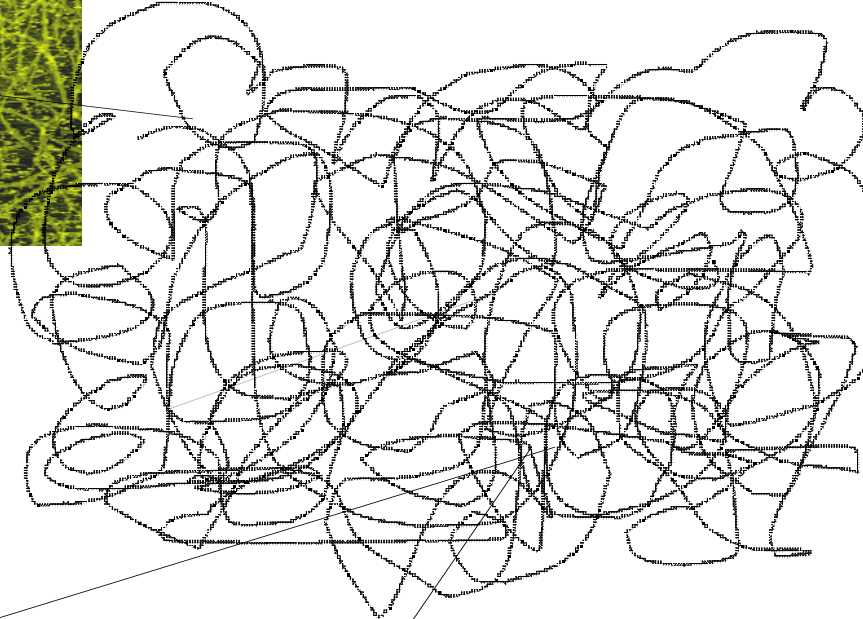


# What gives EPS Structural Stability??

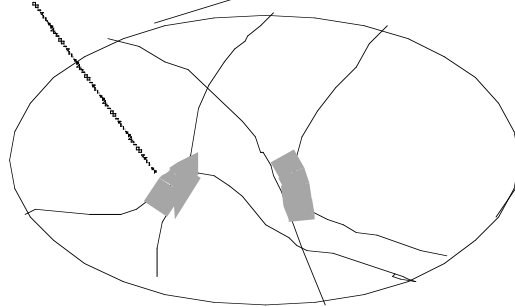
## 1. Proteins



” vs “ Tangled Networks ”  
Proteins in Structural Stability



Proteins



(*Sensu* Hans-Curt Flemming)

Extracellular proteins are typically small, but 'numerous', compared to larger polymers. Therefore, have the capability to form efficient bridges that link together polymers.

**2. DNA???**; **3. Ions (Ca<sup>++</sup>)??;** **4. numerous H-bonds**

# Post-Secretional Changes

- 1) *Partial or Selective Degradation*
- 2) *Binding of Proteins, DNA, Ions (Ca<sup>++</sup>)*
- 3) Amphiphilic
- 4) Steric Hindrance
- 5) Often Resist Drying –Dehydration- Glass Formation

# 'Glass' Formation by EPS

- Protect Bacteria against Drying (e.g. during WWT dewatering process)

Trehalose  
Water-Replacement Hypothesis  
Vitrification Hypothesis:

- Glycerol (freeze-dry bacteria)

- **Ordered Glass Formation:**

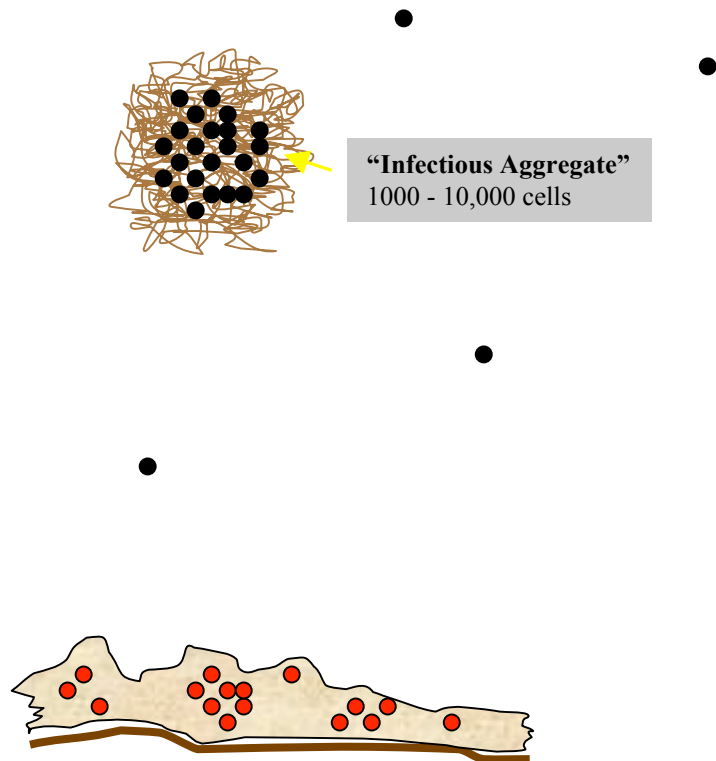
Glass  
(breaks)

T<sub>g</sub>  
/

Amorphous Polymer  
(pliant)

- Later, Re-wet bacteria → wake up
- Implications of Glass Formation
- Drying of 2<sup>nd</sup> Sludge? → fertilizer
- **Q:** Can this add dried pathogens (or their genes) to plant crops?

# Biofilms/Aggregates as a “*Pathogen Refugia*” for Bacteria in Natural and Artificial Waters



**Biofilm => Protective refugia for survival and propagation**

**EPS gel: BUFFER against STRESSORS** salinity shock, digestion, etc.

- **Aggregates** -> Transport and Infection vehicle
- **Interactions: Metals**  
w/**EPS** may enhance protective effect vs. salinity, chlorination, digestion, UV,

***Ingestion of single ‘microscopic’ aggregate → Infective Dose!***

# Survival of Pathogenic Bacteria in Natural Waters:

1. Sewage: flocs and granules
2. Natural: “feces” of animals, leaky septic tanks, etc

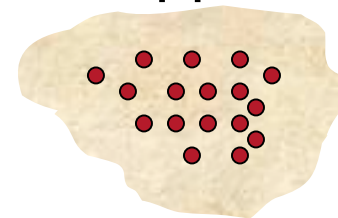
## Relative Survival/Persistence:

Free-Cells



vs.

“EPS-Wrapped Cells”

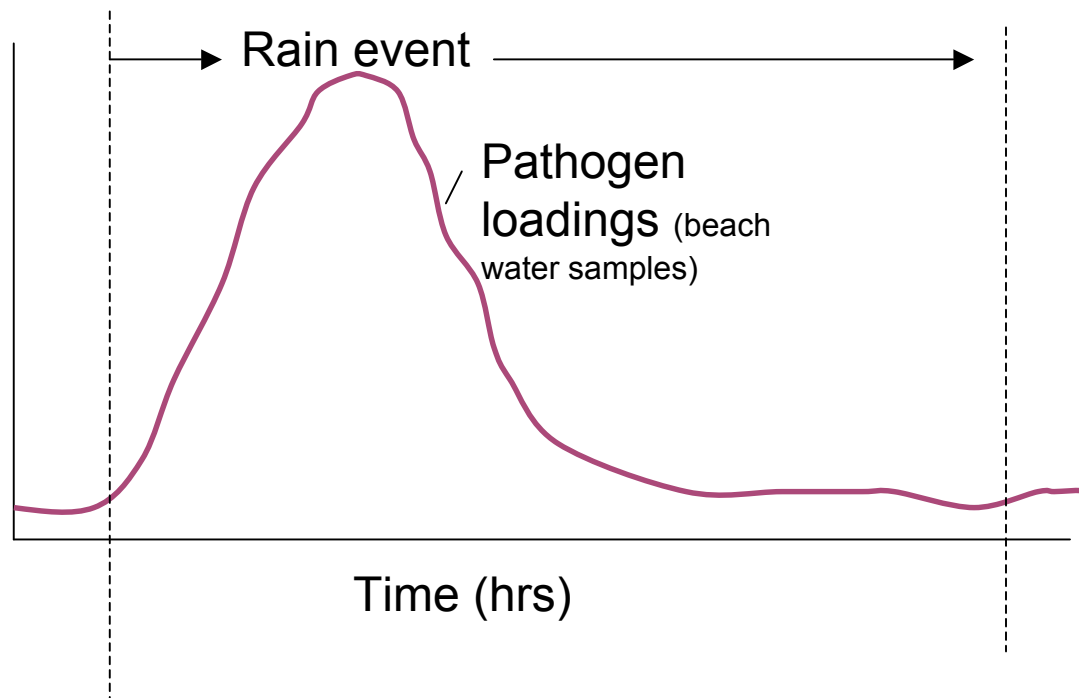


## Important Pathogen Adaptations:

- *survive changing environments; desiccation;*
- *turn virulence on/off; swap genes*
- **EPS** affords protection & more stable environment

# Rain Events

- ‘Pulse’ of pathogens occurs shortly after Rain Event begins



# The Aggregate 'Microenvironment': *A Pathogen's Perspective*

- EPS
- FW to Marine systems
  - Terrestrial Environ.

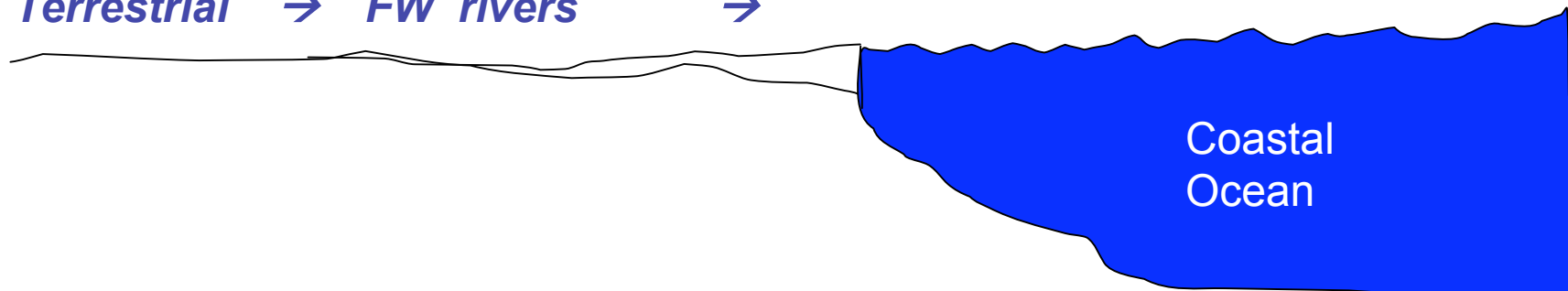
Animal (**Nutrient-rich; temp-controlled, hydrated Environ!**)

→ Feces → Runoff → Oceans

– Entry to Water Systems as Runoff

– Stressors: [*Hydration/dehydration; Metals, Organics, UV; Nutrients*  
*Salinity (ionic) shock/changes*]

*Terrestrial* → *FW rivers* →



# Small EPS-Flocs → Entering coastal Oceans

*Do aggregates 'enhance' survival of pathogens changing environments?*

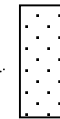
# Predicting ‘Pathogen loadings’ in Water: *Aggregates Mislead Estimates?*

- Water sampling: Is it accurate and precise?

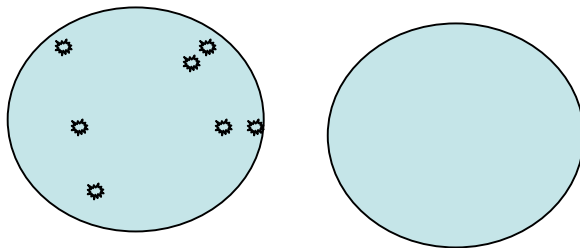
- 100 ml sample:



Single Aggregate w/  
500 – 2000  
pathogenic cells



- Plate counts (viable cells?)



- ***Which Pathogens are Detected?***

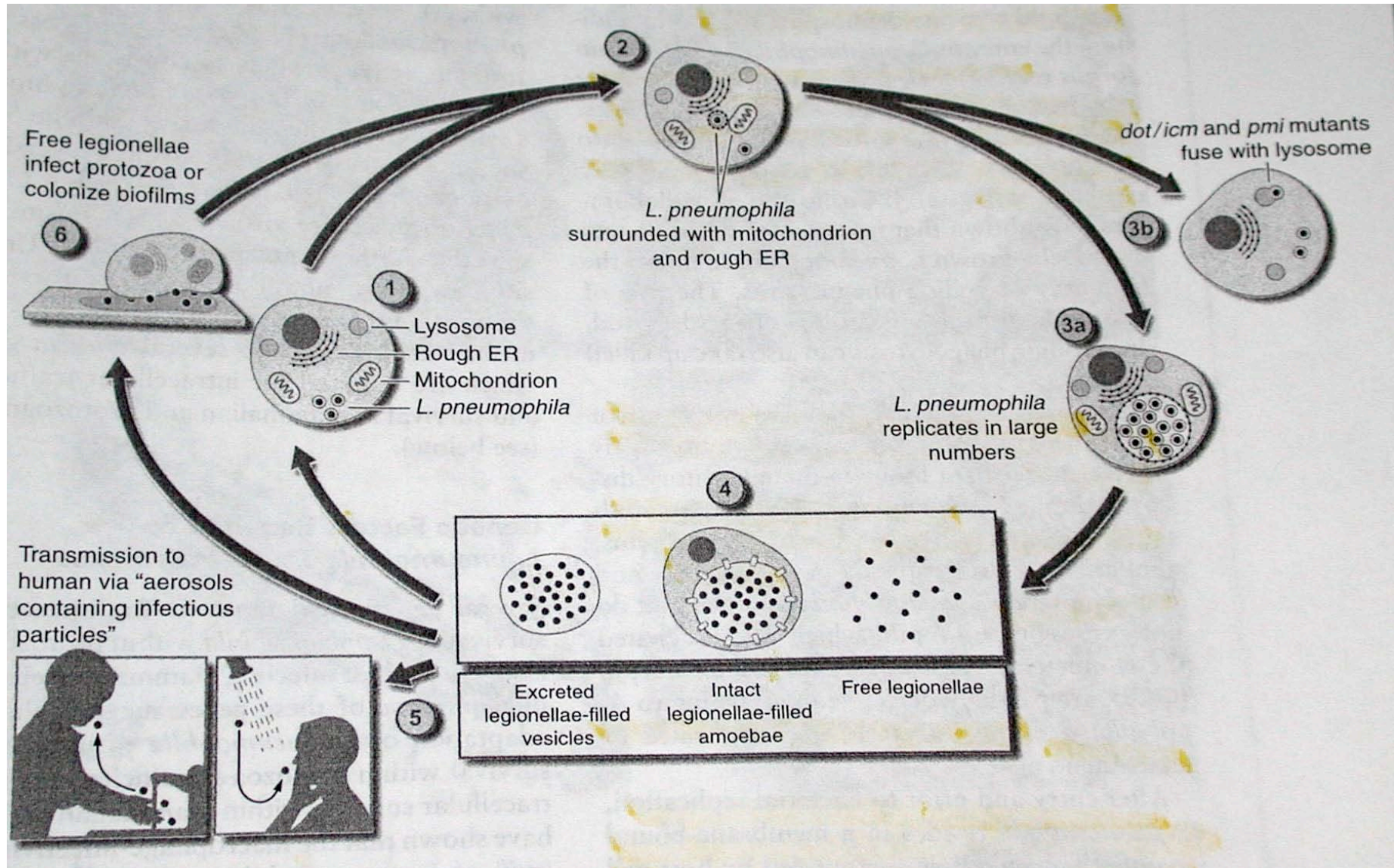
E. coli, Enterococcus, Vibrio

*Can you  
miss  
aggregates  
during  
sampling?*

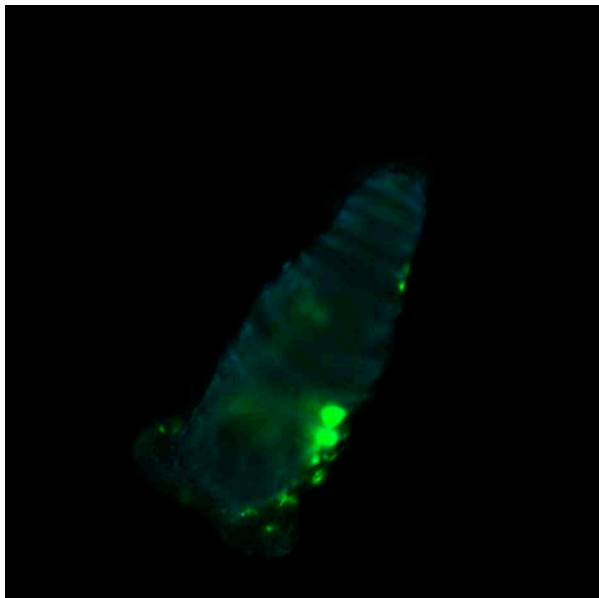
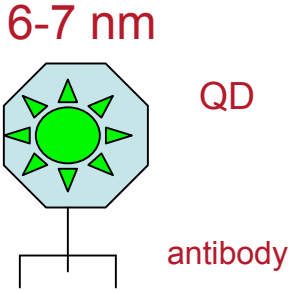
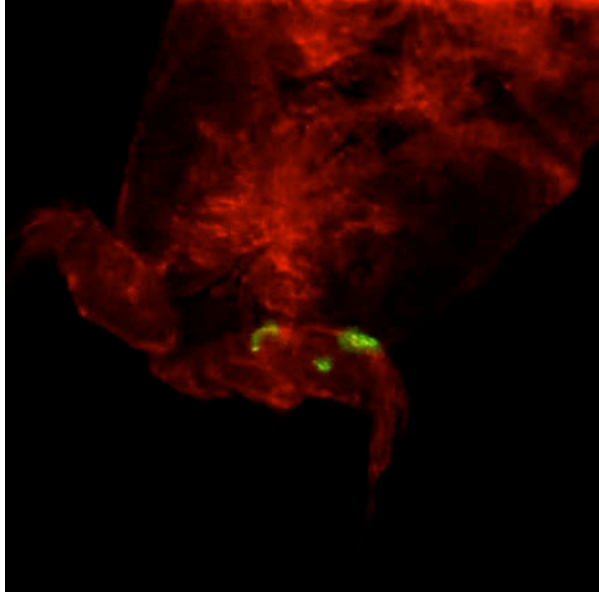
**VBNC (*Viable but Non-culturable Cells*) ??**

# Legionella pneumophila - in Water Systems

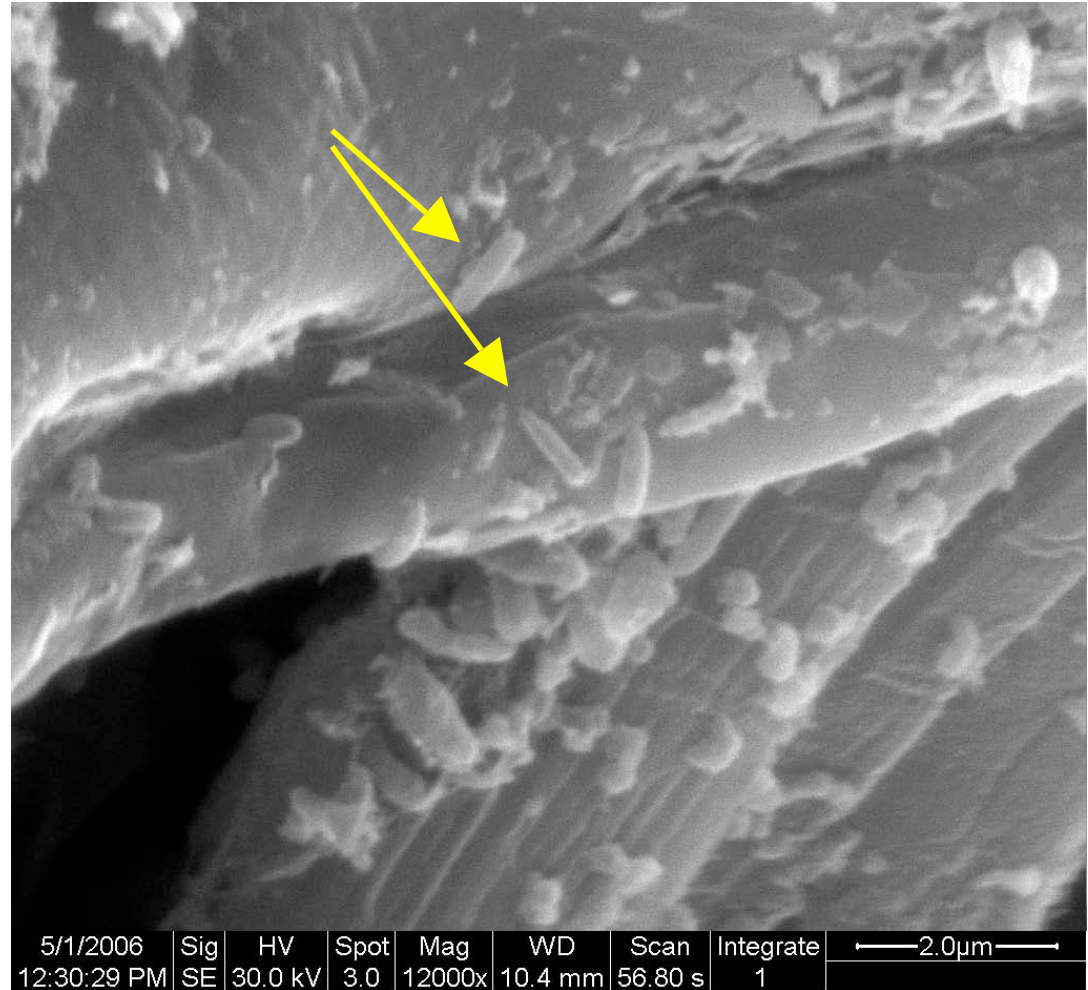
→ amoeba vs human host → showers, aerosol exposure



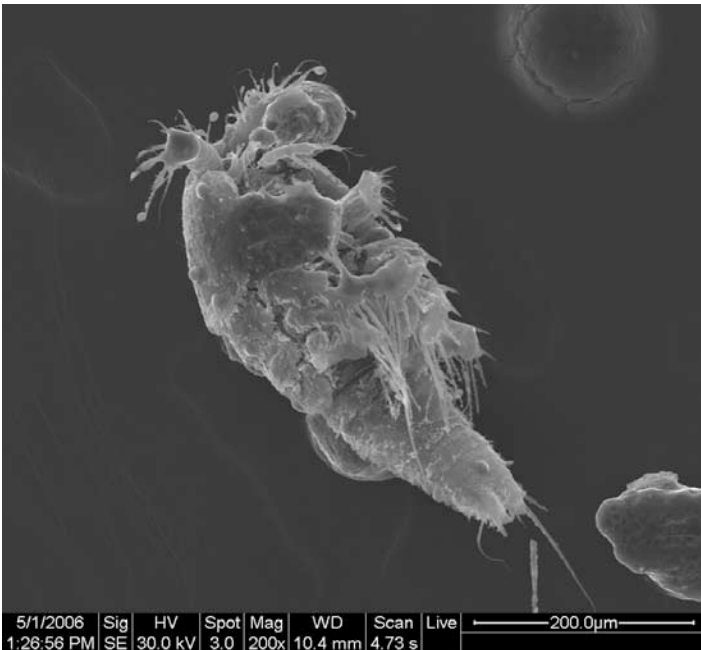
# Probing for Pathogens: Quantum Dot Technology (Fluorescent nanoprobess) with specific antibody linkage



## *Alteromonas* sp. on Copepod exuvia



5/1/2006	Sig	HV	Spot	Mag	WD	Scan	Integrate	2.0µm
12:30:29 PM	SE	30.0 kV	3.0	12000x	10.4 mm	56.80 s	1	



5/1/2006	Sig	HV	Spot	Mag	WD	Scan	Live	200.0µm
1:26:56 PM	SE	30.0 kV	3.0	200x	10.4 mm	4.73 s		

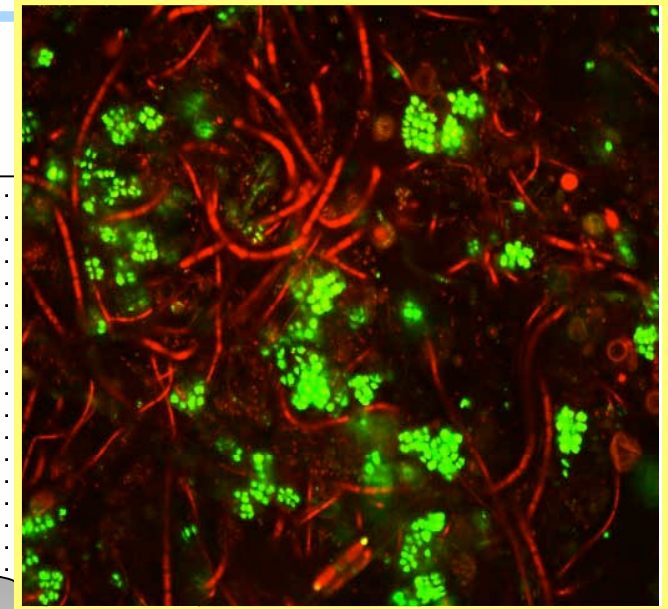
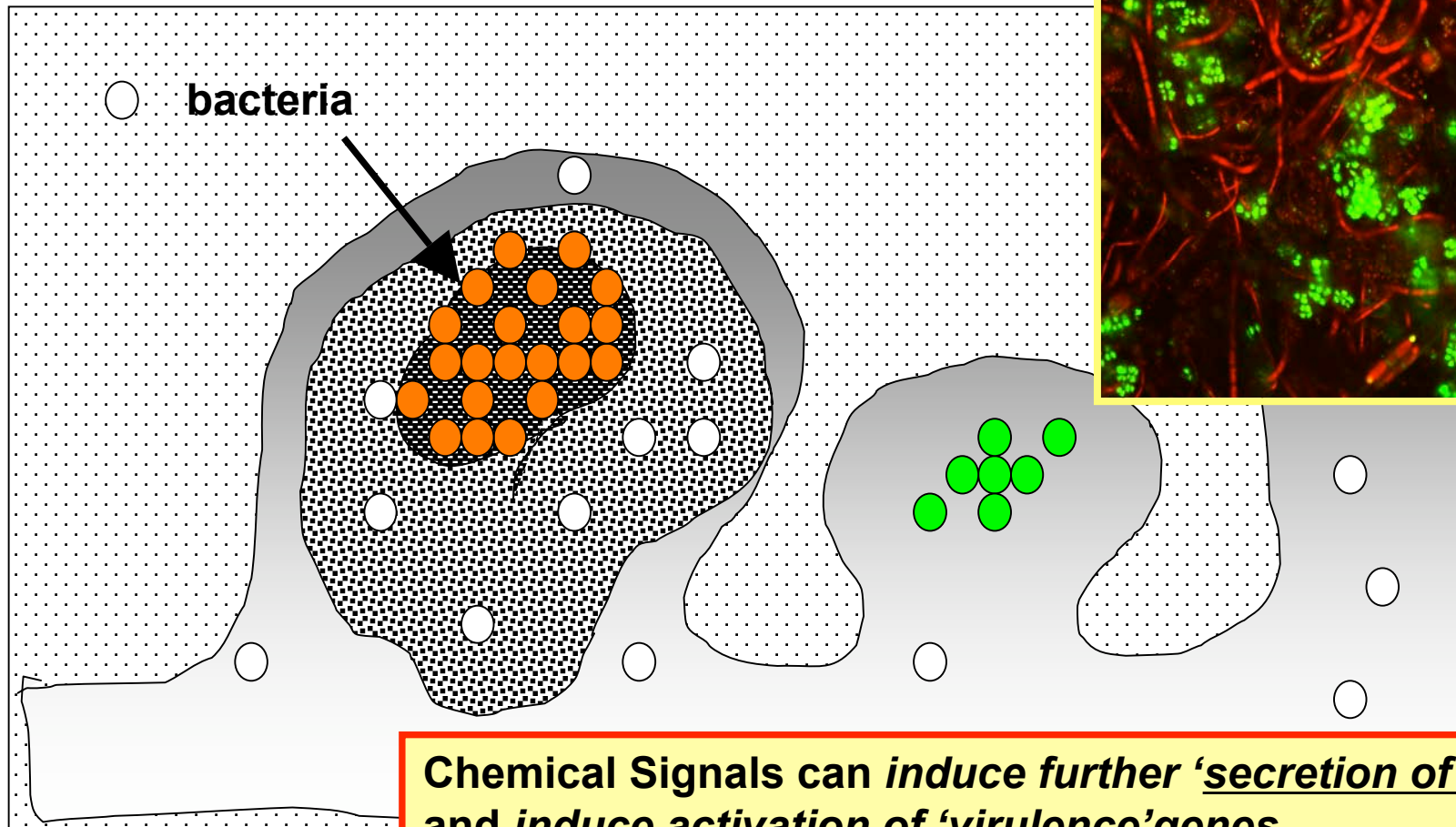
***Targeting Specific 'Strains' of Pathogens using QDs***

# Antagonistic Interactions

(between/among bacteria)

- *e.g. Alteromonas sp. \* Vibrio cholera*
- **Small Animals as “*Transport Vehicles*”**  
for pathogens
- Do *Alteromonas* exclude *Vibrios* on copepods?

# Microspatial Clustering of Bacteria within Biofilms and Chemical Communication

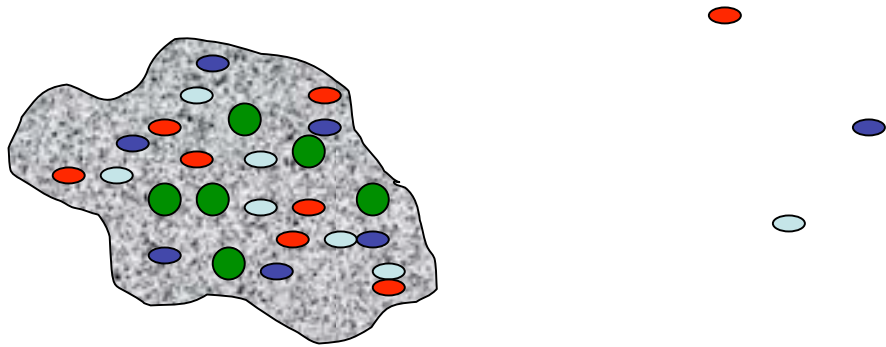


**Chemical Signals can induce further 'secretion of EPS' and induce activation of 'virulence' genes**

# Polymicrobial Infections

## *Do Pathogens travel in Groups?*

- e.g. *Enterococcus* sp. complex (many 'redundant' species)
- Cooperative associations of different bacteria
- Strength in Numbers but also Strength in Diversity!

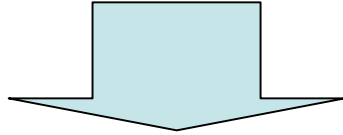


*Formation of 'flocs' can facilitate this!*

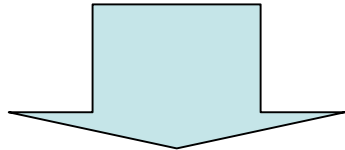
- Aerosolization of Pathogens by wave actions. As wave energy is dispersed on a sandy beach, seawater is aerosolized as “sea spray”. The microscopic droplets may contain pathogenic cells and viruses, and result in potential exposure to individuals (e.g. child) in proximity to splash zone. Potential exposure to pathogen-containing aerosols will vary depending on a number of factors, including distance from splash zone. This is called a “*Sand-Castle* effect because children playing in sand close to the water’s edge will potentially receive greatest potential exposure to aerosol mists.

# Antibiotic Resist. & Gene Exchange in flocs

- Enhanced gene exchange in aggregates (sewage trt)



- Transfer Antibiotic-Resistance Genes (to non-pathogens)

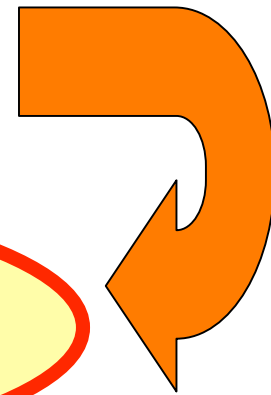


- Non-Pathogens exit (as free cells with genes)



- Transport to Environment

**Idea: Do we Need to ‘chase Genes’ not ‘Bugs’?**



## Summary: Hiding Places for Pathogens in Water Systems:

- Biofilms: A “*Resistant Refugia*” for Pathogens

-*Any Surfaces*

-Sediments

-Plant roots

-Small Animals

-Aggregates

→ *Why? Not Typically Sampled*