

Mod 3 Day 1: Biomaterials Engineering

Growth of Phage Materials

11/12/2013

- 1. M13 bacteriophage – our nanomaterial
(spoiler alert)**
- 2. Phage titer assay**
- 3. React phage with SWNTs or Au**

Stuff!

- Two reflections are due today:
 - Journal Club & Mod 2 paper experiences
- ~~Quiz~~ next time! *Treat*
- Mod 2 papers will be returned on 11/19, revisions are due on 11/27 at 5pm

Module 3 motivation

- Biology interfaces with nano- and microscale materials!

- Engineered nanomaterials

Zicargus Cup
~ 375 AD
Gold/silver nanopart.

- In this context: bacteriophage is our nanomaterial!

– Natural, self-assembling properties

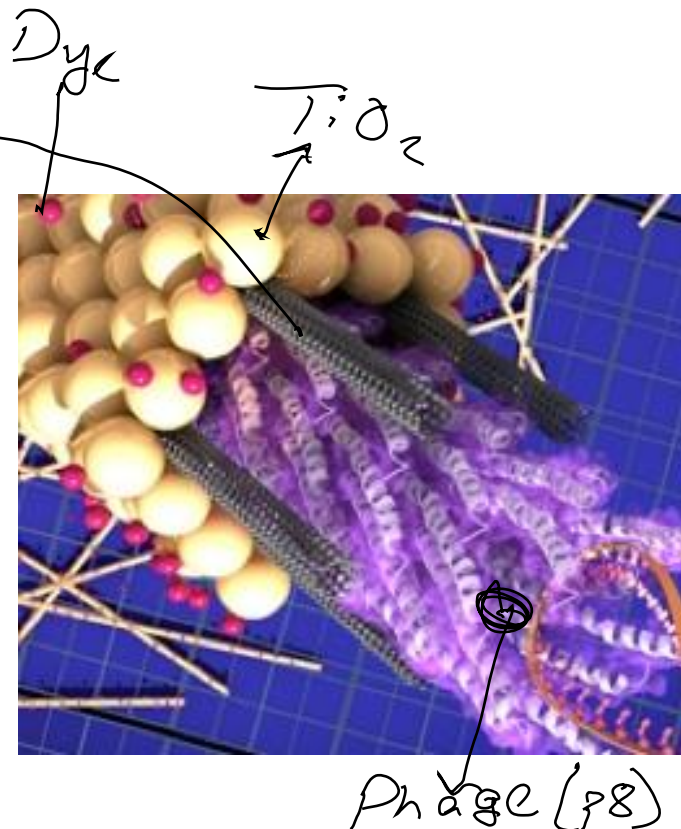
Carbon
Cementite
nano.

Damascus Saber
13-18th cent.

Overview

- Goal: Improve the photon-current efficiency of solar cells!

Ag & SWNT
Single Walled Carbon Nanotubes
↳ Efficient e^- paths.
Today
Phage to assemble SWNT/Ag

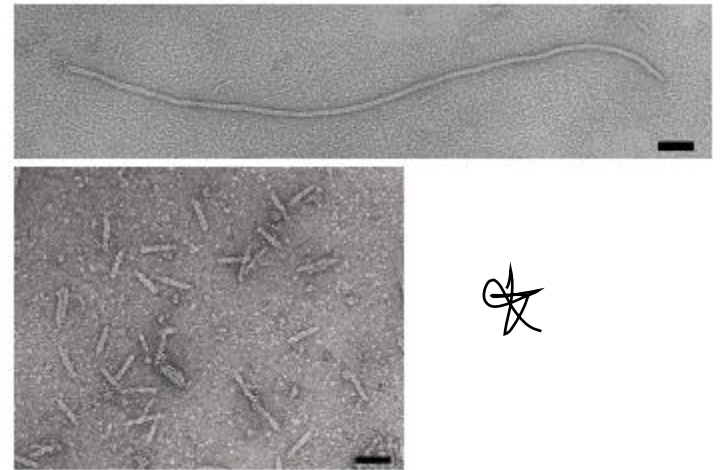


M13 Phage Design: Genome

- Genome encased: *ssDNA supercoiled circle*

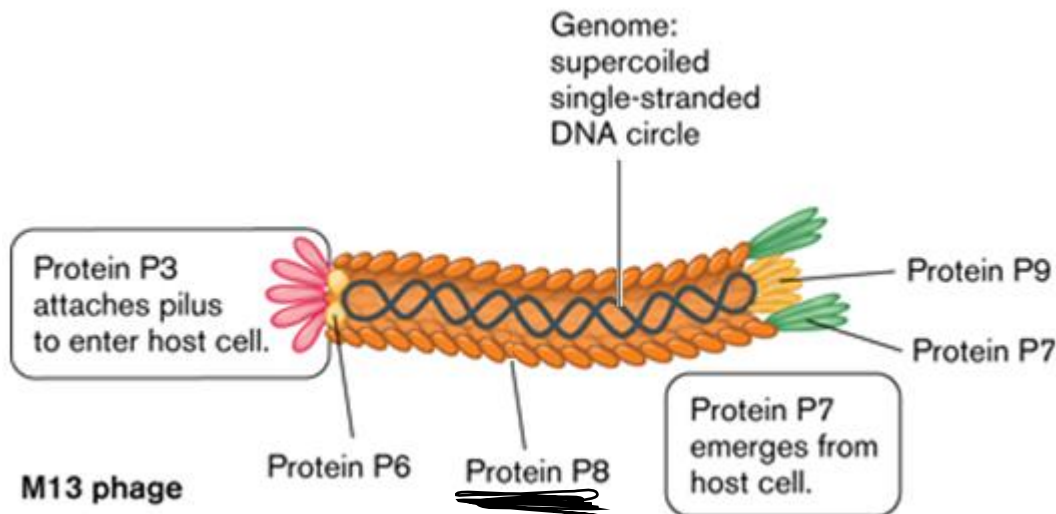
WT $\rightarrow \sim 6.4 \text{ kb}$

Encodes 11 proteins $\rightarrow$ 5 surface



✱

$\sim 880 \text{ nm}$ long
 $\sim 6-8 \text{ nm}$ diam



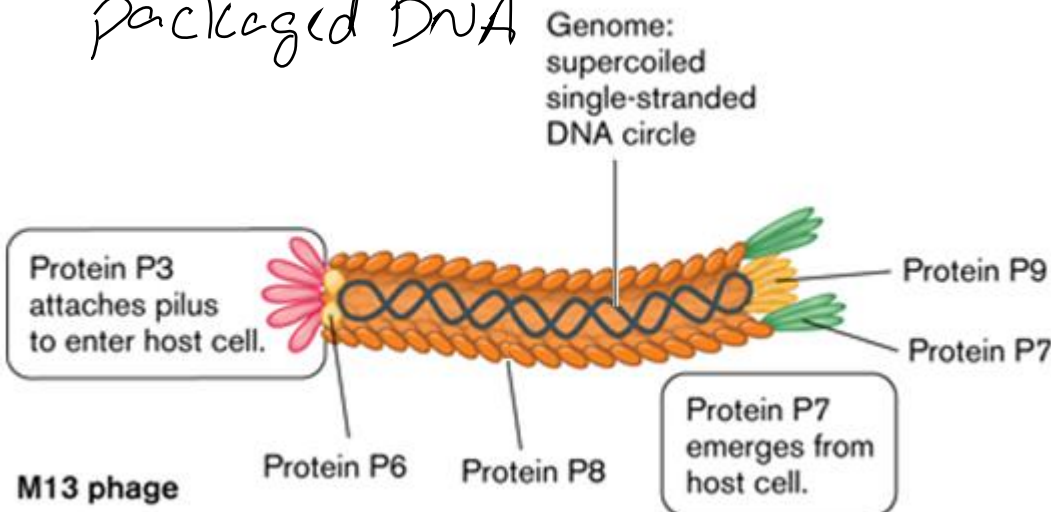
M13 Phage Design: p8

- Genome is wrapped in a protein coat – p8

50 aa protein

Gene 8

- Adjusts w/ length of packaged DNA



ENG12 substrate location

- Fuse peptide sequences to phage coat (p8)

Two M13 clones!

8aa + p8

1) "DSPH" – SWNT

2) "p8#9" – Au

M13 Phage design

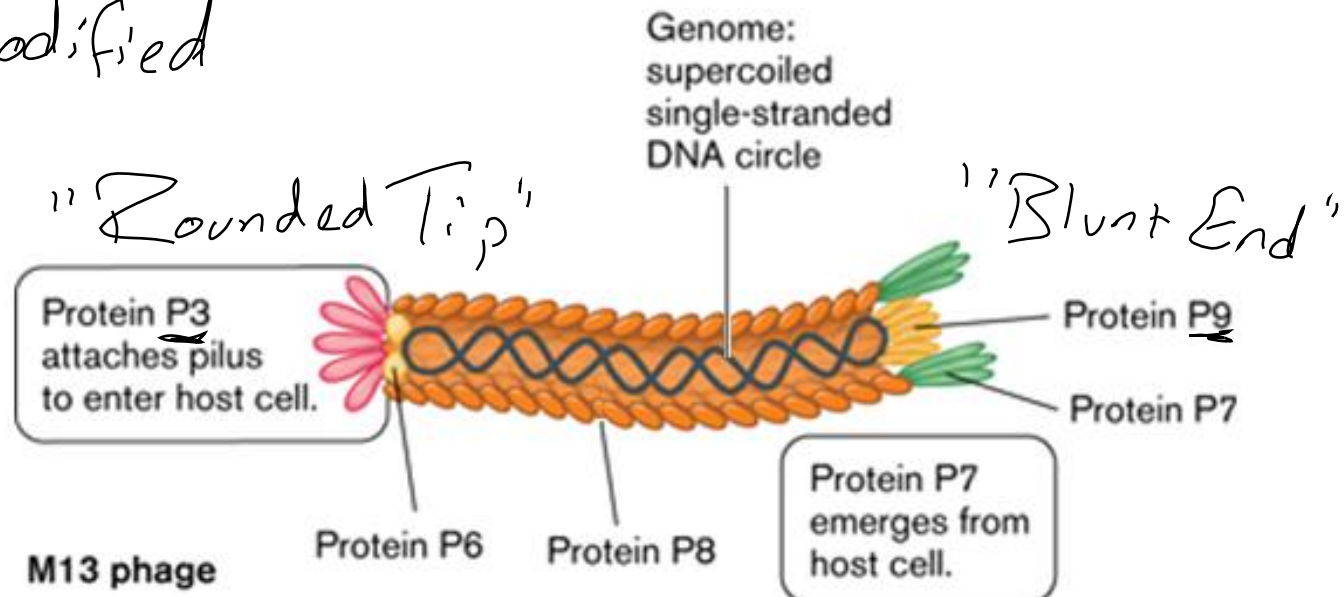
R1

- p3 minor coat protein & p6 accessory protein
- First point of contact w/ targeted surface
- Last POC as secreted
- Often modified

Blunt End

- p9 minor coat protein & p7 accessory protein

- small → no modification
- Phage assembly starts here



M13 Phage life cycle *E. coli*

1. Infection: $p3 \rightarrow F$ pilus

Infection to Secretion in 10 minutes!
1000 new phage per cell in first hour.

2. Amplification!

Translocates genome to *E. coli* cytoplasm

Infective Form to Replicative (IRF)
Form: ssDNA $\rightarrow$ dsDNA (Template)

$p2 \rightarrow$ nicks dsDNA

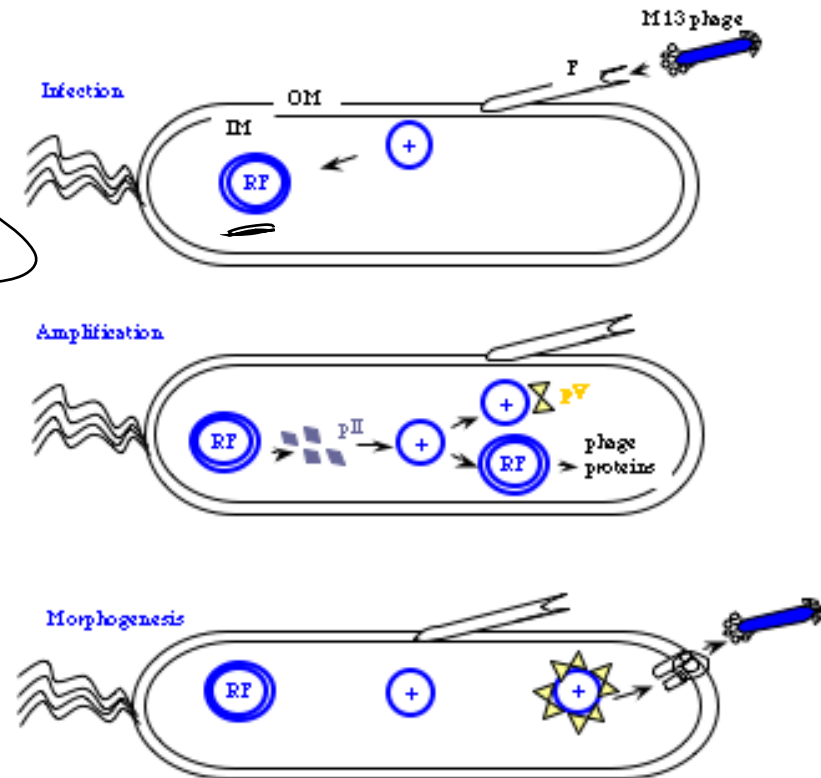
Packaging prepared:

$p5 \rightarrow$ complexes w/ + DNA

Morphogenesis/Secretion:

$p1/p1/4 \rightarrow$ secretion channels

$p8 \rightarrow p5$ replace!
"shrink wrap"



Cells don't lyse!
 $\frac{1}{2} - \frac{3}{4}$ normal metab.

Phage titer assay

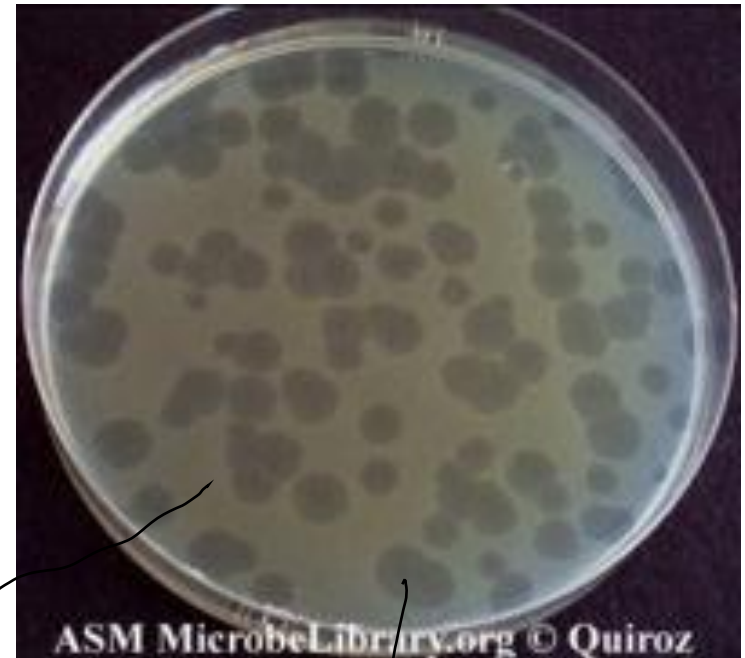
- Count phage particles!

Phage molar extinction
 ϵ DNA base size

$$\# \text{ phage particles per mL} = \frac{(6 \times 10^{16})(A_{269} - A_{320})}{\# \text{ DNA Bases in the phage genome}} \quad \frac{DSPH}{\sim 7220}$$

269 $\rightarrow$ DNA content
 260 $\rightarrow$ NA 280 $\rightarrow$ proteins

320 $\rightarrow$ Background solvent



Uninfected
bacteria
lawn

Infected bacteria
Less dense "plaques"

Complex phage with SWNTs or Au

Group (T/R)	Material	Ratio (material:phage)
Red	SWNT	1:1 (SWNT:phage)
Orange	SWNT	2.5:1 (SWNT:phage)
Yellow	SWNT	5:1 (SWNT:phage)
Green	SWNT	5:1 (SWNT:phage)
Blue	AuNP	1:1 (AuNP:phage)
Pink	AuNP	5:1 (AuNP:phage)
Purple	AuNP	5:1 (AuNP:phage)
Gray	AuNP	10:1 (AuNP:phage)
White	AuNP	10:1 (AuNP:phage)

Calculate volume needed for
 4×10^{13} phage/mL

- SWNT

- Calculate SWNT needed from stock of $20 \mu\text{g/mL}$
- Mix in dialysis tubes
- Dialyze against NaCl pH 5.3 then 10

- Au

- Calculate volume Au needed from stock 5×10^{13} nanoparticles/mL
- Mix in glass scintillation vial
- Store in fridge

Today

- **Goals:**
- Prepare phage using PEG/NaCl precipitation
 - Phage are in the supernatant!!!
 - Pellet is..... BACTERIA
- Viral titer
 - Clean quartz cuvettes after use
- Complex phage with SWNT/Au

Mod 3 Assignments

- **Research Proposal – Dec 10th**
 - Identify a topic of interest, gather relevant background information, understand what you read
 - NEW research question! [not your UROP work]
 - 12 minute talk describing background, research problem and goals, proposed methods, expected results, resources needed, and societal impact.
- **Mini-Report – Due Dec 5th (5pm)**
 - Not as bad as Mod 2!
 - Background & Specific Approach, Results/Data Interpretation, Future Work
 - Schematic, appropriate data figures from each step