National Institute of Environmental Health Sciences

Uniform Amplification of Phage Display Libraries Using Microfluidic Technology

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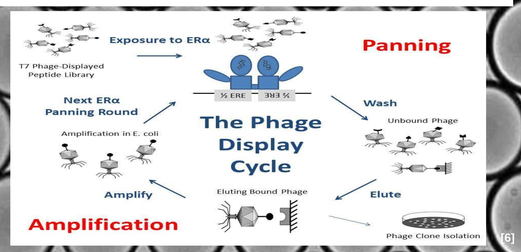
Abstract

Phage display is a widely-used research and drug discovery technique for creating and screening highly diverse peptide libraries to identify ligands for any target. Affinity selection involves panning of a phage library to enrich for target-binding clones followed by their amplification. Uniform amplification of all members of a library is of utmost importance for all selection experiments. If uniform amplification is not achieved, bias is introduced into the selection that favors faster growing clones regardless of the selection pressure applied. This results in the potential elimination of many biologically relevant binding clones from the screen. Microfluidic flow-focusing technology is used to generate monodisperse droplet-based compartments to encapsulate individual phage clones, resulting in the elimination of competition between phage clones with different growth rates. The elimination of growth-based competition ensures that selection of binding clones is driven only by the binding strength of each clone. The knowledge of these target interactions is an integral part of investigating the mechanistic interactions in cell signaling and is a novel approach for library screening.

200 µm

Introduction

Selection from phage display libraries is driven by two **independent** selection processes: (1) the <u>panning</u> step enriches clones that bind to the desired target or any other physical moieties present during the panning step (2) the <u>amplification</u> step (infection of bacteria by a single phage particle to create multiple copies of genetically identical phage) enriches clones that have a growth advantage/amplify faster during any of the amplification steps. As a result, fast growing weak or nonspecific binders may overgrow and ultimately be 'selected' over more desirable slowly growing strong binders. Amplification induced loss of diversity severely hinders the identification of ligands from any display system. To eliminate this bias, individual phage must be separated into different growth chambers so they cannot compete for bacterial hosts.

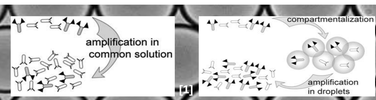


Objective

Uniform amplification cannot be achieved when multiple phage having different growth rates compete with each other in a common solution.

→ **Use Microfluidic Technology to generate monodisperse droplet-based compartments to encapsulate individual phage clones from a mixture of clones possessing different growth characteristics.**

The ratio between clones can be preserved if, and only if, the clones are isolated from each other and all compartments are the same size.



200 µm

Materials and Methods

Materials:
Microfluidic Flow-Focusing Device: Droplet Junction Chip (Dolomite DJC 3000301) + Fluorophilic Coating
Single Syringe Pump: Harvard Apparatus Pump 11 Plus (PY270-2208)
Double Syringe Pump: Harvard Apparatus Pump 11 Plus (PY270-2209)
PFPE-PEG-PFPE (RAN Biotechnologies)
HFE-7500 (3M 98-0212-2928-5)
FC-40 (Sigma F9755)
Perfluoro phase: 2% (w/v) PFPE-PEG-PFPE in HFE-7500
BD 10 mL syringe + 21G$1_{1/2}$ needle, syringe filter 0.2 µm

Generation of and Culture in Droplets:
Fill channel with perfluoro phase: add 3 mL LB + 150 µL log phase bacteria (syringe 1) + 10 µL 10^{10} pfu/mL T7 phage in 3 mL 1x PBST (syringe 2) = 1 phage/droplet and ~5 bacteria/droplet.
240 ul/min oil / 60 ul/min aqueous: R1=95 um, R2=195 um, R3=95 um
Collection in 3 cm petri dish with 500 µL HFE-7500 inside 14 cm petri dish with water (provides humid environment for droplets and minimizes accumulation of static electricity on dry plastic dishes). Place PE tubing from outlet of channel into petri dish on top of perfluorocarbon layer.
Amplification of phage (4-5 hr), harvest phage from droplets via destabilizing solution:0.5% (w/v) Krytox in HFE-7100

Imaging of Droplets:
ANDOR high speed camera (500 Hz) with Image J ROI

200 µm

Results

- True uniform amplification of phage libraries can be achieved in monodisperse droplets formed in a microfluidics channel
  - ◊ **Droplets isolate phage clones from one another**
    Impermeability: bacteria & phage cannot jump b/w droplets
  - ◊ **Supply each clone with an equal number of bacteria**
    Monodispersity: each droplet is identical in size
  - ◊ **Allow amplification to go to completion**
    Droplet Stability: allowing all bacteria to be infected by phage
- Droplet amplification of phage libraries eliminates the competition between phage clones that have different growth characteristics
- The elimination of growth-based competition ensures that selection of binding clones is driven only by the binding strength of each clone.
- Elimination of undesired competition between different phage clones enable:
  * Selection of wider repertoire of target-binding phage independent of their relative rates of replication
  * Identification of rare ligands
  * Prediction of target affinity based on abundance of clones

The Problem:

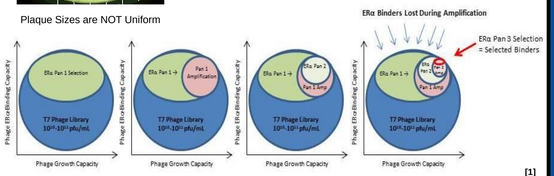
Panning vs. Rate of Amplification

Individual Phage Have Different Growth Rates

Plaque Sizes are NOT Uniform

Selection from Phage Display Libraries is driven by 2 **INDEPENDENT** Processes:

1. Panning
   Enriches clones that bind ERα
2. Amplification
   Enriches clones that have a growth advantage



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Microfluidic Flow-Focusing Device

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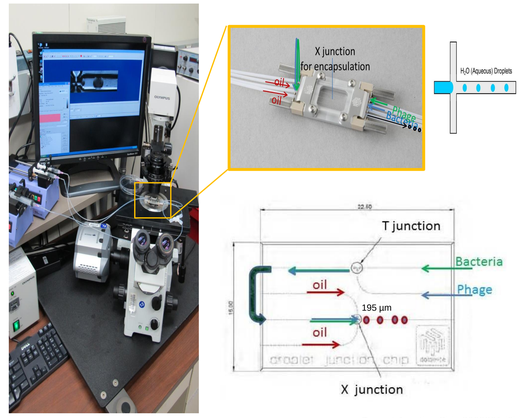
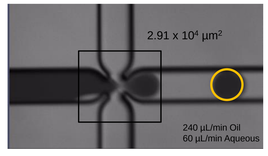
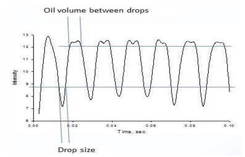


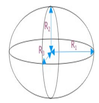
Droplet Junction Chip 3000301 [9]

Droplet Monodispersity

Encapsulate Nanoliter Volumes of Aqueous Solutions



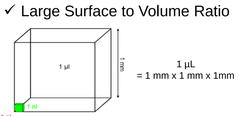




R_1= 95 µm
R_2= 195 µm (channel width)
R_3= 95 µm

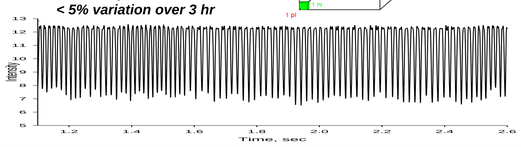
Droplet Volume = 7.37 nL

✓ Small Volume & Size
✓ Large Surface to Volume Ratio



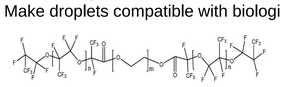
1 µL
= 1 mm x 1 mm x 1mm

Stable Droplet Production:
< 5% variation over 3 hr



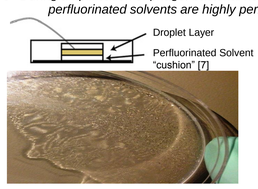
Droplet Stability

Water-In-Oil Emulsion:
- Stabilized by non-ionic Fluorosurfactant PFPE-PEG-PFPE
- Make droplets compatible with biological molecules and cells

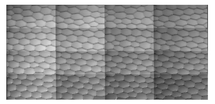


[9]

- Phage must remain in separate droplets with bacteria hosts for the duration of amplification (4-5 hr). *No coalescence of droplets during this incubation period*
- Each droplet must contain a clonal population of phage. *Dilution*
- During amplification of phage, bacterial host require oxygen and nutrients *perfluorinated solvents are highly permeable to O2*



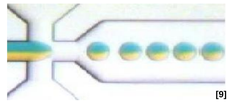
Droplet Incubation



Droplets remain stable/monodisperse and do not coalesce for 72+ hrs

Bacteria & Phage Encapsulation

T Junction



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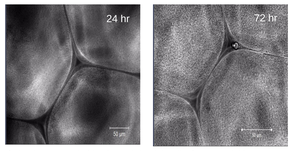
No Mixing Prior To Encapsulation
High Reynolds Constant

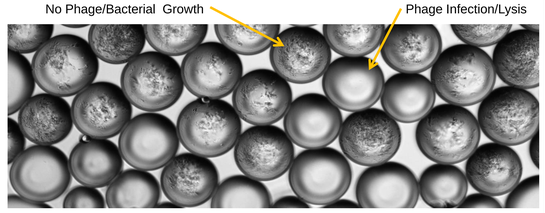
Bacterial Growth In Droplets
Encapsulation = 5 bacteria / droplet



Lateral movement of bacteria at droplet wall = Impermeability

Isolation of Phage In Separate Droplets Containing Bacteria Eliminates Competition During Amplification

No Phage/Bacterial Growth → Phage Infection/Lysis



Phage Do Not Hop Between Droplets

The Solution:

Uniform Amplification Between ERα Pans

Ratios between Phage Clones are Preserved

Applications of Droplet-Based Microfluidics

Benefits:
- Creates discrete monodisperse isolated environments
- Sample volume significantly reduced
  lower costs
- Manipulation at kilohertz speeds (10,000 droplets/sec)
  shorter time to assay large libraries
- Compartmentalization
  higher sensitivity
- Monodispersity/Size Consistency
  accurate & repeatable experiments
- Droplet Stability/No Cross Contamination
  monitor biological process over extended period of time

- ➢ **Uniform Amplification of Display System**
  Phage, Ribosome, RNA, DNA
- ➢ **Ultrahigh-Throughput Biological Screens**
- ➢ **Compartmentalized Chemistry**
  Mini Chemical Reactors
- ➢ **DNA Pyrosequencing/PCR Amplification/ RT-PCR**

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