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Uniform amplification of phage display libraries in monodisperse emulsions

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ABSTRACT

In this paper, we describe a complete experimental setup for the uniform amplification of libraries of phage. Uniform amplification, which multiplies every phage clone by the same amount irrespective of the growth rate of the clone is essential for phage-display screening. Amplification of phage libraries in a common solution is often non-uniform: it favors fast-growing clones and eliminates those that grow slower. This competition leads to elimination of many useful binding clones, and it is a major barrier to identification of ligands for targets with multiple binding sites such as cells, tissues, or mixtures of proteins. Uniform amplification is achieved by encapsulating individual phage clones into isolated compartments (droplets) of identical volume. Each droplet contains culture medium and an excess of host (*Escherichia coli*). Here, we describe microfluidics devices that generate mono-disperse droplet-based compartments, and optimal conditions for amplification of libraries of different size. We also describe the detailed synthesis of a perfluoro surfactant, which gives droplets exceptional stability. Droplets stabilized by this compound do not coalesce after many hours in shaking culture. We identified a commercially available compound (Krytox), which destabilizes these droplets to recover the amplified libraries. Overall, uniform amplification is a sequence of three simple steps: (1) encapsulation of mixture of phage and bacteria in droplets using microfluidics; (2) incubation of droplets in a shaking culture; (3) destabilization of droplets to harvest the amplified phage. We anticipate that this procedure can be easily adapted in any academic or industrial laboratory that uses phage display.

1. Introduction

Phage display is an efficient method for the identification of ligands for any target from a library of billions of different peptides. Phage display involves panning of a phage library to enrich for target-binding clones followed by their amplification. Infection of bacteria by phage creates multiple copies of genetically identical phage. The sequence of the peptides displayed on phage is encoded in the phage DNA; these peptides, thus, are also amplified. The amplification process makes it possible to trace and identify peptides even if they are present as a single copy in a mixture of phage-displayed peptides.

Ideally, amplification should increase the amount of every clone uniformly. In practice, phage that present different peptide sequences have different growth rates. Each infection produces ca. 1000 copies of phage. Such rapid, exponential growth makes the amplification process sensitive to minute variations in growth rate. Both experimental results and modeling suggest that phage that amplify in bacteria a mere 10% faster can rapidly outcompete the phage that amplify more slowly [1,2]. Competition of phage clones during amplification leads to an undesired collapse of library diversity. This phenomenon has been first observed in libraries of peptides displayed on pVIII proteins [3–6]. For pIII-displayed libraries, Rodi and Makowski analyzed the sequences in phage libraries and demonstrated that amplification selects against sequences that interfere with the life cycle of phage [7]. Recently, we analyzed several hundred screens that used pIII-displayed libraries of peptides [2]. The results suggested that amplification is a major driving force in decreasing the diversity of libraries. We hypothesized that rounds of panning and amplification yield phage that (1) bind the target and (2) amplify faster than other target-binding clones [2]. This phenomenon does not present severe problems in selections for a single target: for these screens, identification of few binding ligands is usually sufficient. Nevertheless, enrichment of specific sequences in these screens has to be interpreted with caution, because the abundance of phage and binding capacity of phage-displayed peptides are unrelated [2]. It is not clear whether the strongest possible binders can be identified using this approach. On the other hand, loss of diversity is detrimental if the target has hundreds of binding sites (e.g. cells or mixtures of proteins). These screens rapidly converge on a few binding

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ligands [8]. It is, thus, difficult to find ligands for many binding sites in a single screen, because different binding clones compete with one another during amplification leading to a loss in diversity.

To eliminate amplification-induced loss of diversity, phage must be separated into different growth chambers. We believe that two conditions are necessary for uniform amplification: (1) growing individual phage separately to a saturating concentration (i.e., when bacteria reach stationary phase and all bacteria are infected); (2) combining equal volumes of phage solution after amplification. Clonal growth could be performed in separate test tubes or multiwell plates. However, this process is practical only for a small number of clones. Some research groups amplify phage clones as separate plaques in agar [9–12]. This process is laborious for large libraries. Also it is unclear if diversity is preserved in plaque based amplifications, because phage clones grow in plaques continuously and never reach saturation.

We have recently demonstrated that requirements (1) and (2) can be satisfied when each phage is encapsulated into a droplet of media suspended in oil (emulsion) [1]. Droplets act as isolated growth chambers for phage and bacteria. After a few hours of growth, destabilization of the emulsion combines the phage clones and yields a library that was uniformly amplified. As expected, the number of phage produced per droplet increases with droplet size. It is important, therefore, to amplify phage in emulsions in which droplets have identical size (monodisperse emulsions).

Microfluidics devices can be used to generate micrometer-sized droplets at high speed (100 Hz–10 kHz) [13,14]. These droplets can serve as compartments for growth of cells and bacteria [13,15,16]. Droplet size is the function of channel geometry and pressure in the channel; if pressure is constant, the droplets produced in the channel have nearly uniform size (polydispersity of 1.01) [17,18]. Microfluidics is one of the simplest approaches for rapid generation of large number of monodisperse growth compartments (e.g. 10^6). Other potentially interesting approaches for large-scale clonal isolation of cells are arrays of micro-wells [19], arrays of micropockets [20] or microbeads [21]. Polydisperse emulsions produced by simple mixing are useful for many biochemical applications [22–25]; however, they are not suitable for phage growth because amplification in polydisperse droplets is not uniform [1].

In the following sections, we describe the steps for generation, handling, and destabilization of monodisperse emulsions. We describe the steps for efficient separation of phage into individual droplets, and identify conditions in which this separation is possible. The manuscript also provides a detailed description of perfluorinated surfactants that stabilize or destabilize these emulsions. Unlike previous reports [1,13,26], we generate these surfactants from commercially available materials.

2. Description of materials

2.1. Synthesis of bis(tetrachlorophthalimido)-polyethylene glycol (1)

Reagents: Polyethylene glycol-Mn 600 (PEG_{600}) (Sigma 202401), Triphenylphosphine (Sigma T84409), 3,4,5,6-Tetrachlorophthalimide (TCI T0918), Diisopropyl azodicarboxylate (DIAD) (Sigma 225541).

Reaction solvent: Anhydrous tetrahydrofuran (THF) (Sigma 196562).

Workup solvents: (technical grade): Hexane, Ethyl acetate, Methanol, Dichloromethane.

Materials: 2× Round bottom flasks (1 × 250 mL and 1 × 100 mL), Funnel (8 cm O.D.), Column (50 mL), Filter paper.

Characterization: The ^{1}H-NMR of **1** was recorded on Varian i400 and ^{13}C-NMR was recorded on Varian u500 in Chloroform-d ($CDCl_3$) (Sigma 151823).

2.2. Synthesis of diaminopolyethylene glycol (NH_2–PEG–NH_2) (2)

Reagents: Hydrazine monohydrate (Sigma 207942).

Reaction solvent: Anhydrous ethanol, Dichloromethane (ACS Grade) (VWR BDH1113),

Workup reagents: Celite 545 (Sigma 22140).

Workup solvents: Anhydrous ethanol, Distilled water, Diethyl ether (Fisher E134-1), Toluene (Sigma 179418).

Materials: 4× Round bottom flasks (1 × 50 mL and 3 × 100 mL), 1 mL Syringe (Fisher 14-817-25), Needle 20G (Fisher 14-826-5C), Pump arm sinter funnel (30 mL), Separatory funnel (125 mL).

Characterization: The ^{1}H-NMR and ^{13}C-NMR of **2** was recorded on Varian u500 in Deuterium oxide (D_2O) (Sigma 521965).

2.3. Synthesis of (perfluoropolyether)–acid chloride (PFPE–COCl) (3)

Reagents: Krytox (Miller-Stephenson Chemical Company 157FSH), Thionyl chloride (Sigma 88952).

Reaction solvent: HFE-7100 (VWR 98-0211-8941-4).

Materials: 200 mL Round bottom flask, Condenser, Heating mantle, Tubing, 1 mL Syringe (Fisher 14-817-25), Needle 20G (Fisher 14-826-5C).

2.4. Synthesis of bis(perfluoropolyether)–polyethylene glycol (PFPE–PEG–PFPE) (4)

Reagents: 4-(Dimethylamino)pyridine, polymer (PolyDMAP) (Sigma 359882).

Reaction solvent: HFE-7100 (VWR 98-0211-8941-4), Anhydrous tetrahydrofuran (THF) (Sigma 196562).

Workup reagent: Celite 545 (Sigma 22140).

Materials: 2× Round bottom flask (200 and 100 mL), Condenser, Heating mantle, Tubing.

Charaterization: The ^{1}H-NMR of **4** was recorded on Varian i400 in 1,1,1,3,3,3-hexafluoroisopropanol-d2 (Cambridge Isotopes Labs Inc. DLM-143-5 × 1).

2.5. Fabrication of master

Reagents: SU8-50 (Microchem), Trichloro(1H,1H,2H,2H-perfluorooctyl)silane.

Materials: File to make photo-mask (available for download at Derda Lab Website: http://www.chem.ualberta.ca/~derda/), Si-wafer (Silicon Sense Inc.).

2.6. Fabrication of channel

Reagents: PDMS elastomer base and curing agent (Dow Chemicals 184 Sylgard Elastomer Kit), Aquapel (Aquapel Canada).

Materials: 1.2 mm Biopsy puncher (Ted Pella Inc. 15074), Plasma cleaner.

2.7. Amplification of phage library in droplets

Reagents: Phage libraries: Ph.D-12™ (NEB E8110S, lot 0101002), F+ *Escherichia coli* K12 ER2738 (NEB E41045 lot 0141011), bacto-tryptone (Fisher DF0123-17-3), bacto-yeast extract (Fisher DF0127-179), sodium chloride (NaCl) (Fisher S271-1), agar (Fisher DF0140-01-0), isopropyl-β-D-thiogalatopyranoside (IPTG) (Fisher BP1755-10), 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) (Fisher BP1615-1).

Material: 14 mL Petri dishes (Fisher 08-757-12), 3 mL Petri dishes (Fisher 08-757-11YZ), BD 10 mL Syringe (VWR CABD309604), BD 3 mL Syringe (VWR 97048-656), BD needle 21G1½ (VWR CABD305167), Syringe filter 0.2 μm (VWR 28145-