Predicting Virus Partitioning in Aqueous Two-Phase Systems

Motivation: Virus purification and concentration is critical for the production of vaccines and gene therapy vectors. Today, vaccines exist for 26 viral diseases[1], but low yields and high costs of production prevent access to many vaccines. Similarly, production limitations will reduce access to viral gene therapy, which may provide cures to single-mutation genetic diseases.

Although many technologies exist for virus particle purification, they each face unique roadblocks to developing rapid, high-yielding, and ultimately continuous processes. Chromatography is widely-used for virus purification, but suffers from low binding capacities, frequently inactivates viruses, and cannot operate continuously. Ultrafiltration is readily scalable and provides high throughput and recoveries, but fouling reduces flux and removal of contaminating proteins is often ineffective. Ultracentrifugation is difficult to scale up and has low yield, even though purities are high.

Aqueous two-phase systems (ATPS), most commonly constructed with two polymers or a polymer and a salt, are proposed as a replacement to simultaneously purify and concentrate virus. Biologically-gentle purification is achieved by choosing the identity and concentration (described by tie-line length, TLL) of phase-forming components, ionic strength, polymer molecular weight, and pH so that the desired bioparticle partitions to one phase and contaminants to the other. Many viral particles have been purified in ATPS, with yields as high as 79%[2], a significant result in an industry that often accepts overall downstream yields of 30%. Still, the vaccine industry is reluctant to adopt ATPS in part because each bioparticle requires a unique system for optimal recovery and predictive models are lacking to fill this critical gap.

So far, attempts to develop a predictive model for bioparticle partitioning in ATPS can be grouped into three approaches. A first approach, the Collander equation, uses known partitioning of bioparticles in ATPSs to predict partition coefficient (K) in new systems[3]. Because of its reliance on data, this model cannot predict K for new bioparticles. A more robust method combines density gradient theory with thermodynamic and association models. It successfully predicted the mass transfer of an amino acid in ATPSs[4], but cannot model partitioning of large bioparticles like viruses. A better solution by Chow, et al. uses surface and interfacial properties to predict partitioning[5]. Particle diameter, net charge, and contact angle with the ATPS components can predict K.

No one has yet successfully combined a theoretical model with measurements of ATPS and particle surface properties to predict bioparticle partitioning. Chow, et al. verified their model empirically by successfully comparing K to TLL and pH, but did not attempt predictions with surface measurements. I propose combining novel methods developed in my lab to measure virus surface characteristics with Chow's theoretical model to together predict virus partitioning in ATPS, a task which was not previously possible.

Hypothesis: Partitioning of virus particles in aqueous two-phase systems can be predicted using surface chemistry properties measured at the single-particle level.

Experimental Plan: I propose extending Chow, et al.'s models to predict partitioning of virus particles in polymer-salt ATPS. Chow's model requires phase properties of ATPS and surface properties of the viral particle to predict K. Methods for characterizing ATPS are rapid and well-known. Turbidity measurements will be used to determine the binodal points and tie lines. Characterization of viral particles is much more difficult. While virus diameters and isoelectric points (pI) (related to surface charge of viral particles) may be found in the literature, no method to measure the contact angle between ATPS and viral particles currently exists.

Historically, virus characterization has depended on bulk solution measurements or amino acid sequencing. Instead, my lab has developed a novel single-particle method to measure virus surface chemistry using chemical force microscopy (CFM), shown in Fig. 1. In a recently submitted article[6], we determined the pI of two model viruses using atomic force microscopy probes chemically functionalized to carry positive or negative charges. Adhesion to the viral particle was measured in varying solution pH and the pI determined[6], giving a direct characterization of the viral surface and avoiding error from contaminants. A similar single-particle characterization will be used to determine the parameters of Chow's partition model.

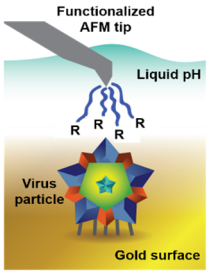

Fig. 1. CFM with virus particles

The first stage of this work will focus on how virus surface chemistry changes in the presence of ATPS components individually before combining them. First, virus-sized gold nanoparticles (AuNPs) coated in BSA or lysozyme will be immobilized on a gold surface. Then adhesion force between the AuNPs and probes modified with charged or hydrophobic ligands will be measured by CFM. These proteins are known to partition differently in ATPS, and since contact angle between these proteins and ATPS may also be determined by traditional sessile drop methods, comparison will confirm that CFM can be related to surface tension for well-defined systems before extending the method to viruses. Similar determinations of surface tension by CFM for non-biological surfaces have already been reported[7]. Then, two enveloped (surrounded by a lipid bilayer) and two non-enveloped viruses will be used to explore varying viral surface chemistry.

Once a relationship between CFM and contact angle is established, the second stage of this work will characterize multiple ATPS and adapt Chow's model to predict virus partitioning. To verify the model as a function of component concentration, three TLLs for three common polymer (PEG) and salt (citrate, phosphate, and sulfate) ATPS will be evaluated to show the model is robust for varying chemistries. Similarly, the pH of ATPS will be varied over the range of stability for each virus, typically $4.5<pH<7.5$. The model will be complete when it predicts the K of a virus as a function of TLL or pH.

Once this work establishes a reliable model for virus partitioning between the bulk phases in ATPS, partitioning to the interface, which frequently results in the irreversible aggregation and inactivation of virus particles, can be explored.

Intellectual Merit: Developing predictive models for virus partitioning in ATPS will add to the understanding of viral surfaces and the driving forces behind ATPS, reducing the experimental cost of developing ATPS to purify new bioparticles in the future.

Broader Impacts: By filling a critical gap in the literature, making ATPS research faster, and making ATPS more accessible to industry, this work will speed the development and production of vaccines and gene therapy, ultimately reducing outbreaks of viral and genetic disease and saving lives. In addition, I will mentor undergraduate researchers who will have the opportunity to develop contact angle measurements with protein-coated AuNPs.

References:

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