Interactions of viral particles and osmolytes for manufacturing, detection, and inactivation

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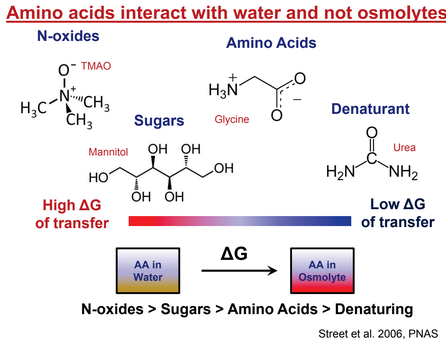
Background & Motivation

Abstract

In this age of modern medicine, viral diseases continue to take millions of lives. Our lab uses osmolytes to manipulate viral particle associations. Osmolytes are naturally occurring compounds that regulate osmotic pressure by controlling the structure of water molecules. Osmolytes can manipulate water molecules that surround viral particles. By understanding the interaction of water around large, hydrophobic viral particles, we can engineer methods to purify, detect and inactivate viral particles.

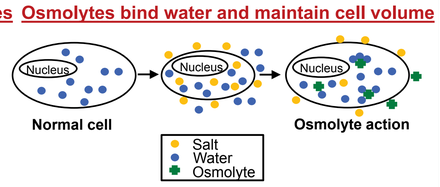
Osmolytes

Amino acids interact with water and not osmolytes



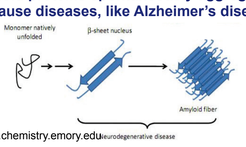
Street et al. 2006, PNAS

Osmolytes bind water and maintain cell volume



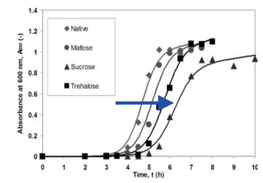
Osmolytes reduce protein aggregation

Amyloid proteins spontaneously aggregate and cause diseases, like Alzheimer's disease

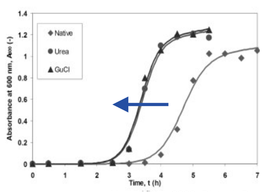


http://www.chemistry.emory.edu/neurodegenerative disease

Protecting osmolytes slow aggregation

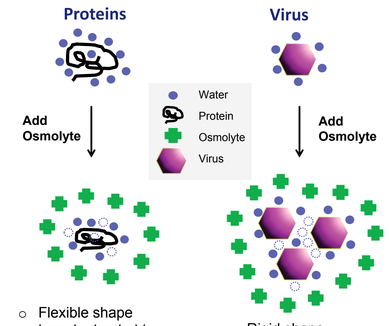


Denaturing osmolytes increase aggregation



Nayak et al. 2009, Biotech Prog

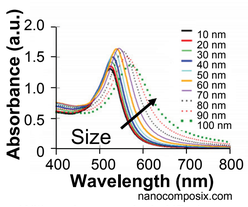
Virus interaction with osmolytes



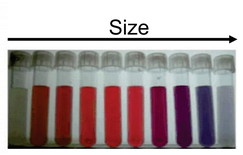
- Flexible shape
- Less hydrophobic
- Dehydration causes compaction and stabilization

- Rigid shape
- Highly hydrophobic
- Dehydration causes aggregation

Gold nanoparticles (AuNPs)

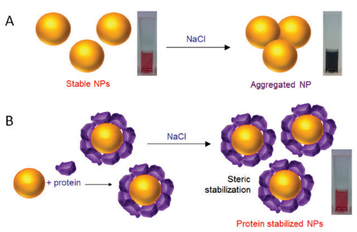


With an increase in AuNP size
- peak broadening
- red-shift in UV spectrum



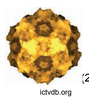
With an increase in AuNP size
- obvious color change
- also occurs with aggregation

Protein corona stabilizes AuNPs and stops aggregation in salt. (Ho et al. (2015) Analyst)



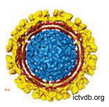
Model Virus Titration

	Porcine parvovirus	Sindbis virus
Abbreviation	PPV	SINV
Capsid	Non-enveloped	Enveloped
Nucleic Acid	ssDNA	ssRNA
Size (nm)	18-26	48-52
pI	~5.5	~4.2
Model for	B-19 human parvovirus, hepatitis A virus, and poliovirus	Eastern and western equine encephalitis viruses, hepatitis C



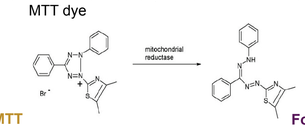
ictvdb.org

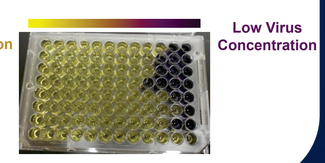
Dalrymple et al. (1976) Virology; Miesegaes et al. (2013) Biotechnol. Prog; Simpson et al. (2002) J. Mol. Biol; Stramer et al. (2009) Transfusion



ictvdb.org

Infectious virus detected with an MTT assay
- Set up the same as a $TCID_{50}$
- Put cells in a 96-well plate
- Put virus sample in the left wells
- Serially dilute virus across the plate
- After virus infection, detect viable cells with the MTT dye



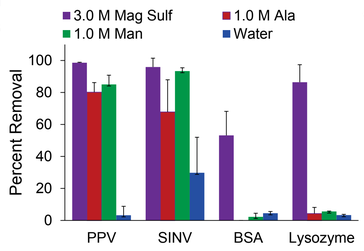


Results & Discussion

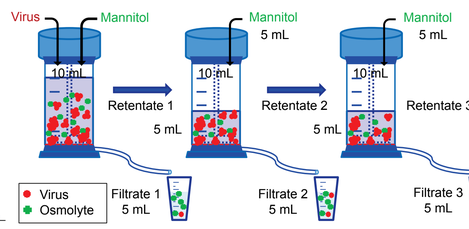
Manufacturing

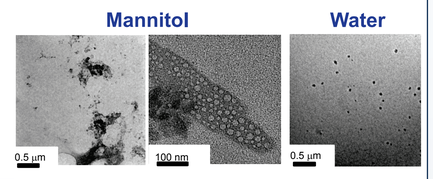
For vaccine manufacturing, osmolytes can be used as virus flocculants by inducing hydrophobic interactions between virus particles that do not occur in most proteins. This allows for the separation of virus from contaminating proteins using a large pore-size membrane. Osmolyte flocculation can purify an enveloped and non-enveloped leading to a cost-effective purification method for a variety of viral products.

Batch virus flocculation

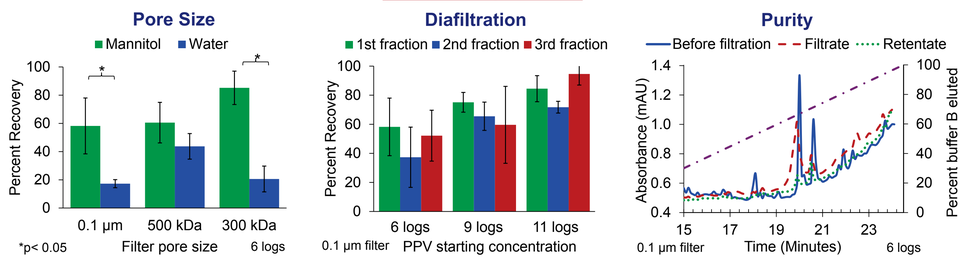


Semi-continuous virus flocculation

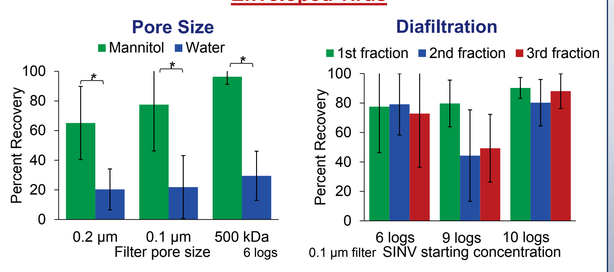




Non-enveloped virus



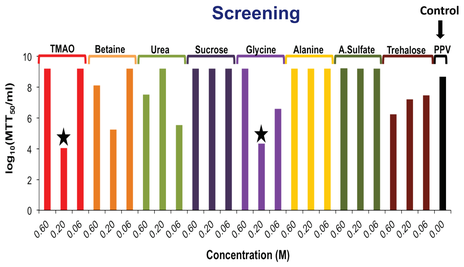
Enveloped virus



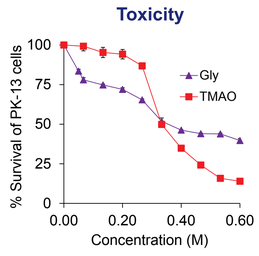
Inactivation

Osmolytes were explored as a method to inactivate virus. Glycine and TMAO were able to stop cell lysis and the release of infectious virus particles. It was determined that capsid proteins are still produced in an infected cell, but no infectious virus particles can be found. It was concluded that osmolytes, which stabilize proteins, stabilize the individual capsid proteins and interfere with the capsid assembly process. This process could be a new method to inactivate virus particles post-infection.

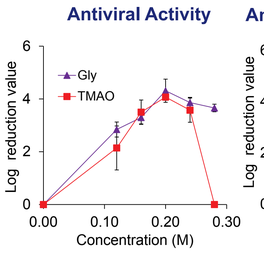
Screening



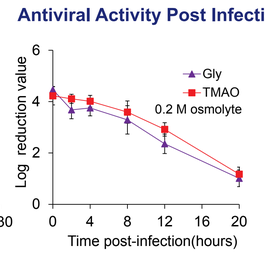
Toxicity



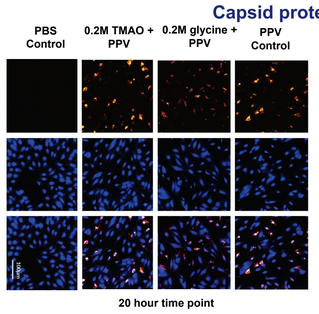
Antiviral Activity

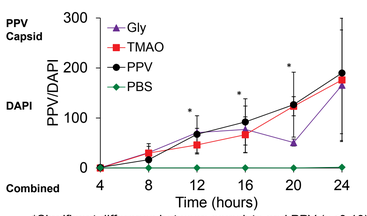


Antiviral Activity Post Infection



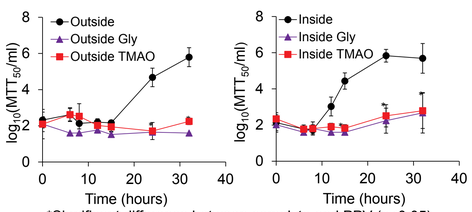
Capsid protein detection in cells





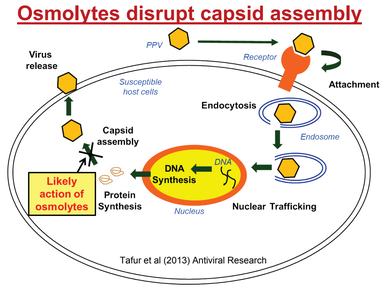
*Significant difference between osmolyte and PPV (p<0.10)

Viable virus production inside and outside cells



*Significant difference between osmolyte and PPV (p<0.05)

Osmolytes disrupt capsid assembly

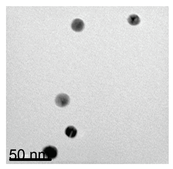


Tafur et al (2013) Antiviral Research

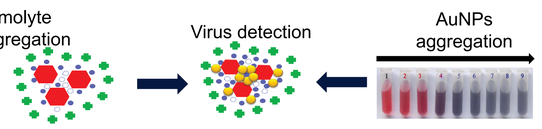
Detection

Osmolytes aggregation of virus is being used as a virus detection method. The aggregation of virus-coated AuNPs can be detected by the UV spectrum shifts found by the aggregation of AuNPs. We are able to measure a difference between the aggregation of virus-coated and proteins-coated AuNP by comparing the aggregation in salt and osmolyte solutions.

TEM image of synthesized AuNPs

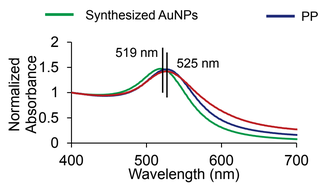


To detect virus using AuNPs aggregation induced by osmolytes

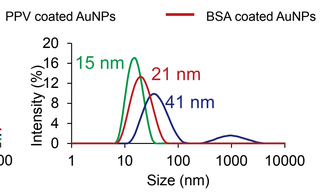


Advantages:
- Quick
- Sensitive
- Inexpensive
- Portable
- Detect virus non-specifically

UV-Vis spectra of AuNPs



Size of synthesized AuNPs



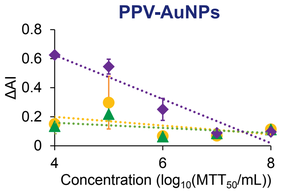
$$AI = \frac{\lambda_{max+60nm}}{\lambda_{max}}$$

$$\Delta AI = AI_{Before\ treatment} - AI_{After\ treament}$$

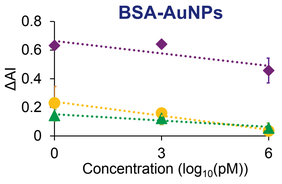
$$\Delta\Delta AI = \Delta AI_{Salt} - \Delta AI_{Osmolyte}$$

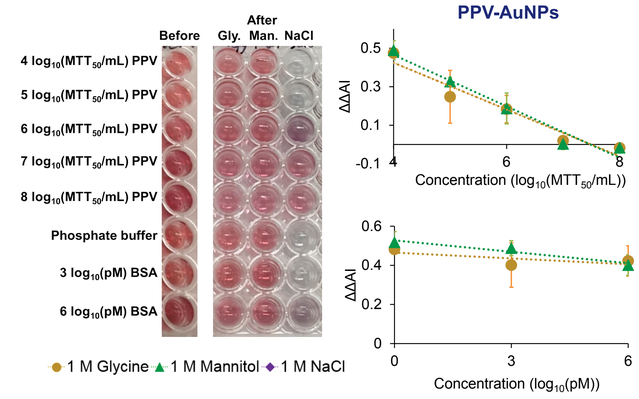
Limit of detection

PPV-AuNPs



BSA-AuNPs





PPV-AuNPs

Acknowledgements

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