Inactivating viruses with inert gas bubbles

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Bubbling and shaking inactivates viruses

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Inactivation of some Bacterial and Animal Viruses by Exposure to Liquid–air Interfaces

By T. TROUWBORST,* SJOUKJE KUYPER, J. C. DE JON AND A. D. PLANTINGA

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(*Accepted* 1 *March* 1974)

SUMMARY

Surface inactivation of the bacteriophages T_1, T_3, T_5, MS2, of EMC vir Semliki Forest virus was studied, exposing the viruses to a large air/water interface by aeration or by rotating the fluid in a spherical flask. EMC virus in 1 M-NaCl was not sensitive to this treatment, phage T_3 and T_5 were only little affected, but the phages T_1 and MS2 and Semliki Forest virus were rapidly inactivated by bubbling air or nitrogen gas through the suspension. In salt solutions at rest no inactivation of these viruses was observed. Inactivation by aeration was prevented by addition of peptone, by apolar carboxylic acids and by the surface active agent OED. If a large solution/glass interface is present, some loss of virus occurs by adsorption to the glass surface. Phenylalanine protected against adsorption to the glass surface, but protected less effectively against inactivation by aeration. The rate of surface inactivation was strongly dependent on the salt concentration in the medium. At low NaCl concentration (0·01 M) nearly no inactivation was found for phage T_1 and MS2 and phage T_3 was not sensitive to aeration in 1 M-NaCl but was rapidly inactivated in 2·6 M-NaCl. The rate of inactivation decreased with time of shaking and in the case of phage T_3 a nearly completely resistant

THE INACTIVATION OF EQUINE ENCEPHALITIS VIRUS BY MECHANICAL AGITATION

LT. WILLIAM F. McLIMANS, H-(S), USNR*

From the Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md.†

Received for publication February 11, 1947

A property of certain viruses is their marked susceptibility to oxidative destruction. Mueller (1) demonstrated that the rapid loss of infectivity of tumor filtrates (Rous Sarcoma) incubated at 37 C could be effectively inhibited by the addition of reducing agents such as hydrocyanic acid or cysteine monohydrochloride. Similar observations using herpes simplex had been made by Zinsser and Seastone (2) and confirmed by Perdrau (3). Mueller (4) states that eastern equine encephalitis virus rapidly becomes innocuous on incubation *in vitro* at 37 C.

Observations have also been made on the effect of H ion concentrations on the survival time of viruses *in vitro*. Thus, Howitt (5) showed that a California strain of equine encephalitis virus was rapidly destroyed below a pH of 5.5 but remained active up to a pH of 9.2. This marked sensitivity of the encephalitis virus to the H ion concentration distinguishes it from certain other viruses.

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SURFACE INACTIVATION OF BACTERIAL VIRUSES AND OF PROTEINS*

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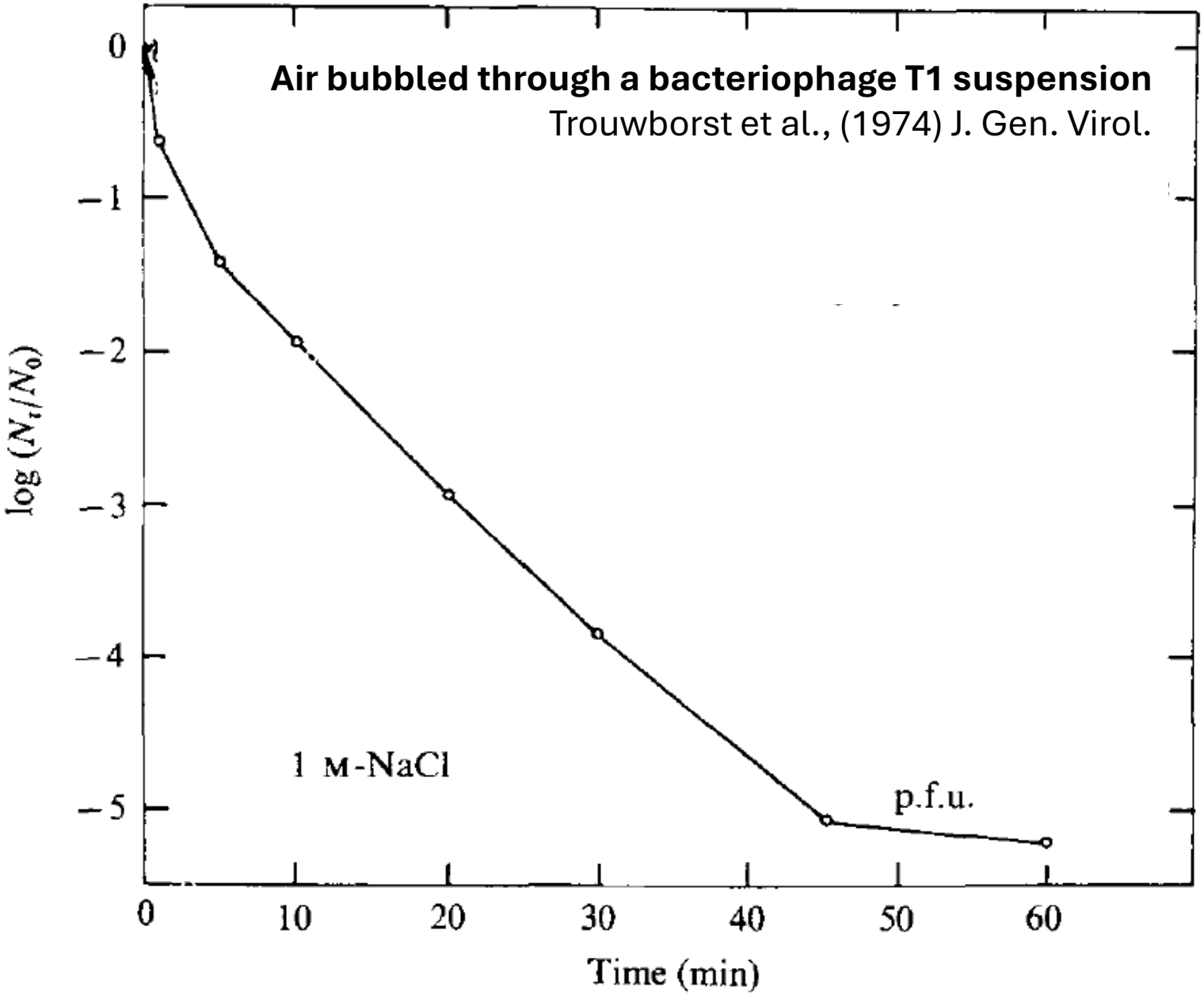
(*From the Department of Bacteriology, New York University College of Medicine, New York*)

(Received for publication, January 8, 1948)

It has been noticed previously that certain viruses can be rapidly inactivated by shaking or by bubbling gases through the virus suspensions. Campbell-Renton (1) studied the effect of violent mechanical shaking on bacteriophages and found them to be fairly rapidly inactivated, at rates which were characteristic for each phage. Grubb, Miesse, and Puetzer (2), while studying the effect of various vapors on influenza A virus, noted that bubbling air at the rate of 1 liter a minute through the virus suspension resulted in detectable reduction in infectivity in 10 minutes. In a somewhat more extensive study McLimans

Bubbling and shaking inactivates viruses



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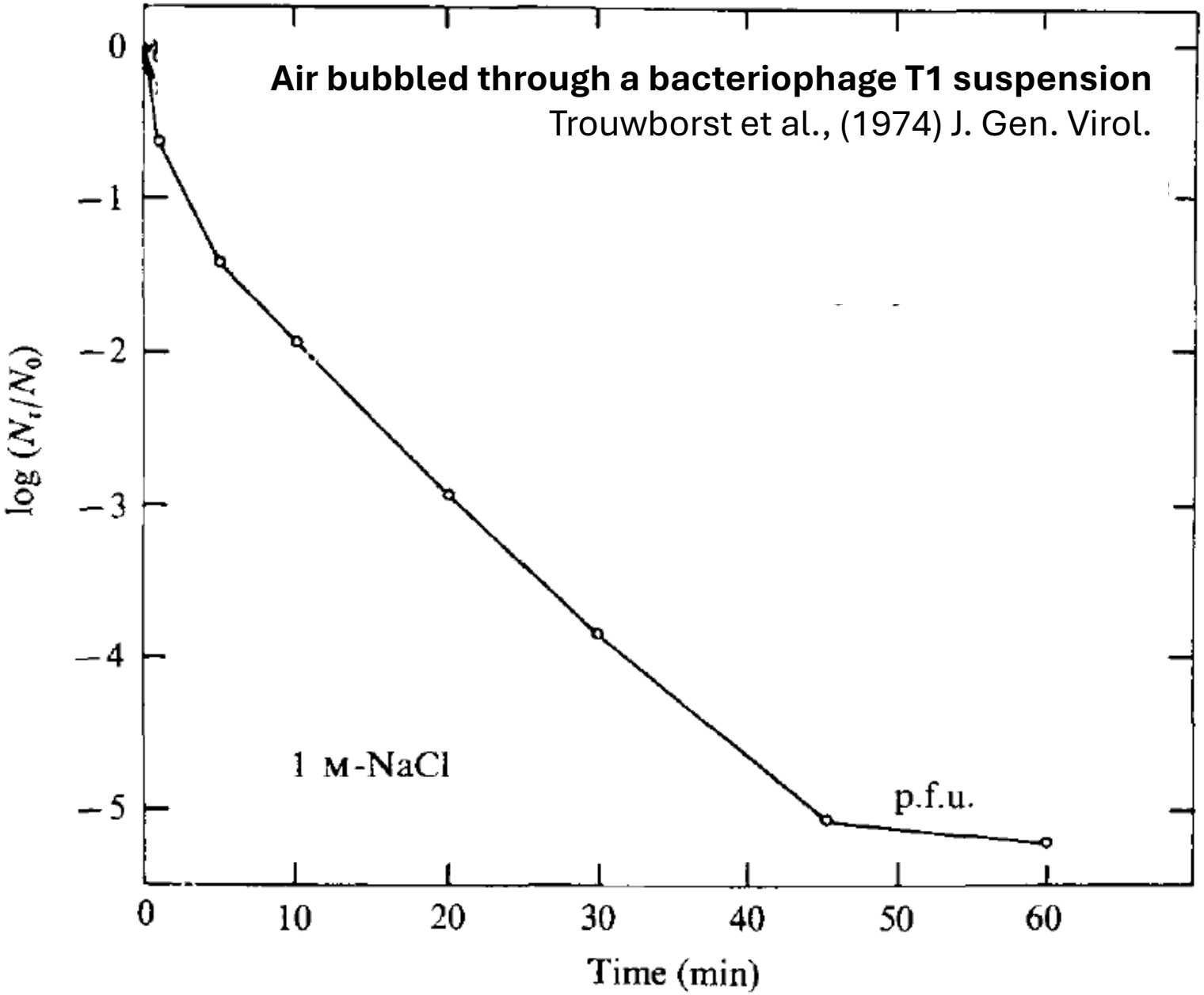


Main observations

Shaking and bubbling produce similar results

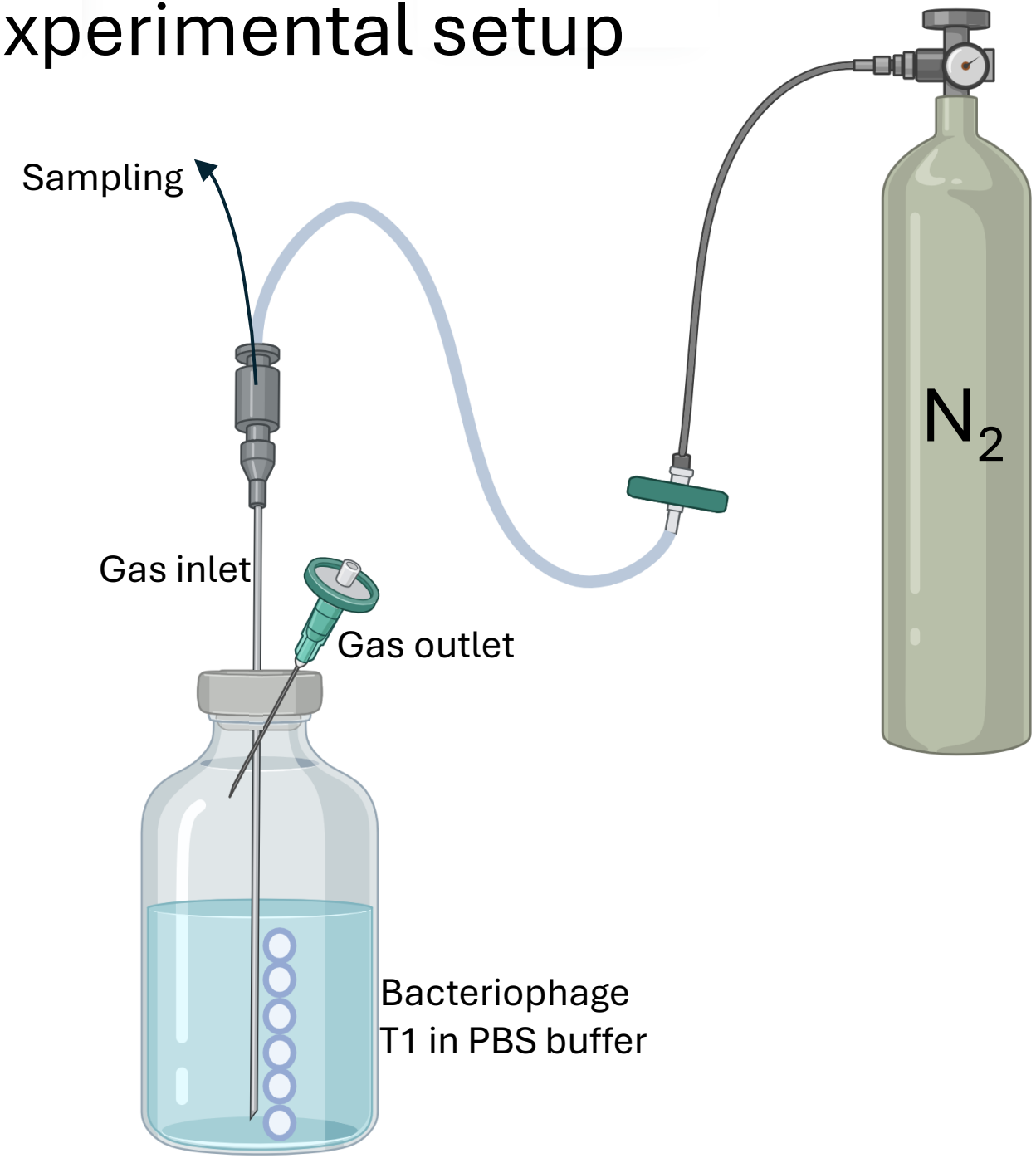
Sensitivity differs between viruses

High salt increases sensitivity

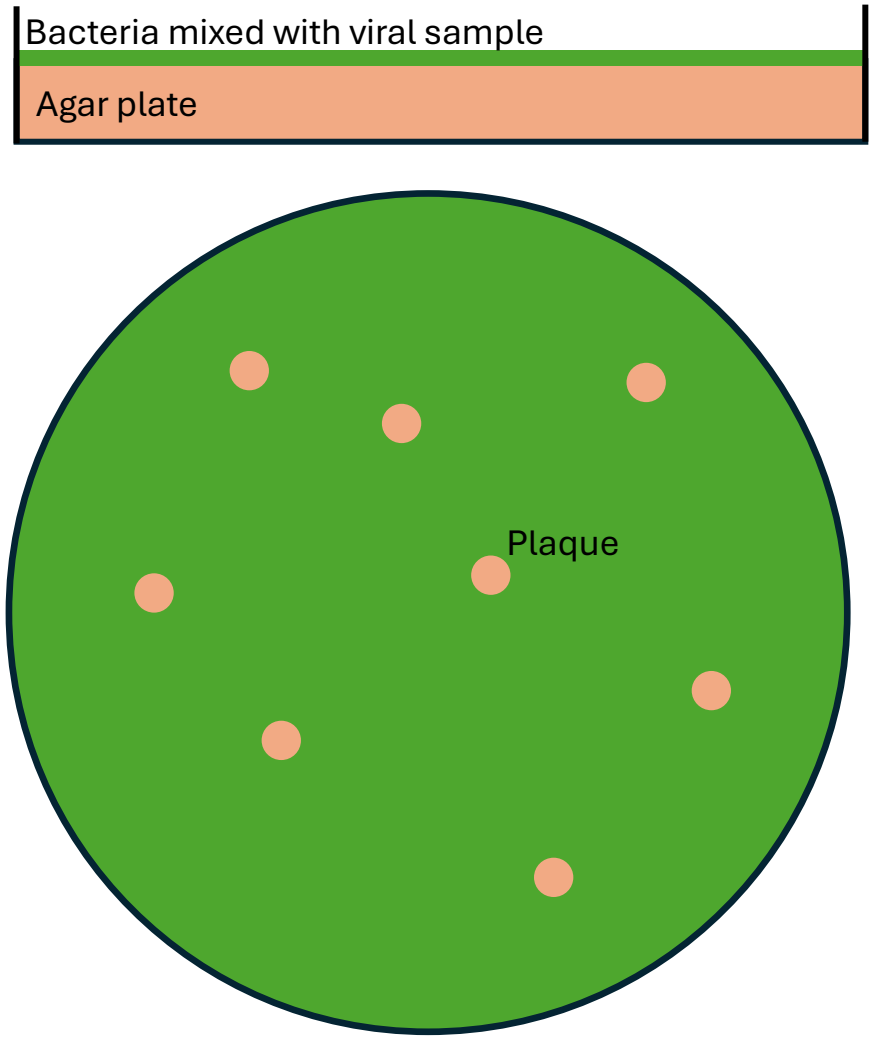
Resistance to inactivation is not heritable

Protein in solution reduces inactivation

Experimental setup



Plaque counting



Bubbling nitrogen through T1 virus

Buffer: Phosphate buffered saline
Flow rate: 0.6 mL/s
Needle inner diameter: 0.514 mm
Bubble volume fraction: 1%

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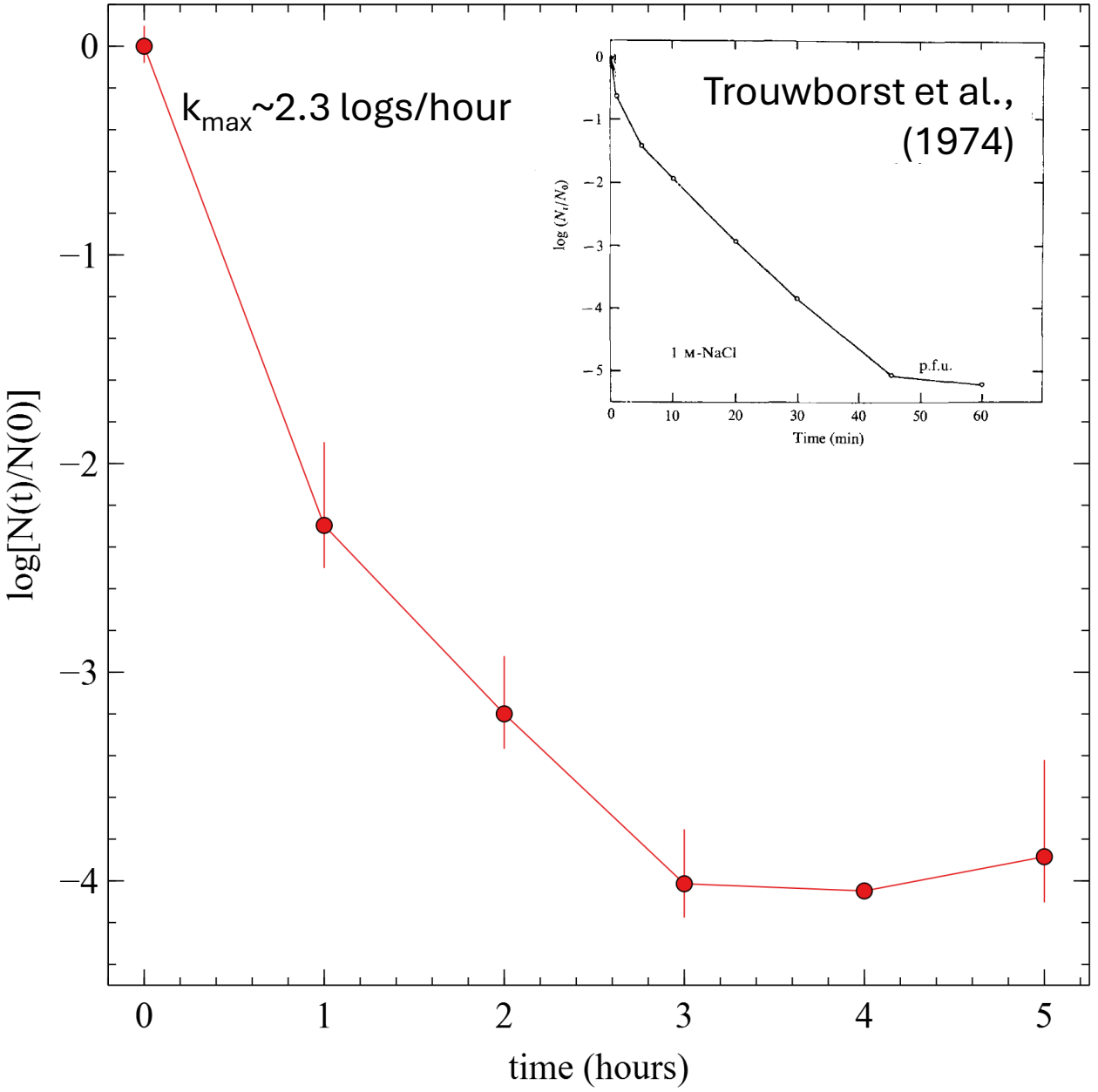
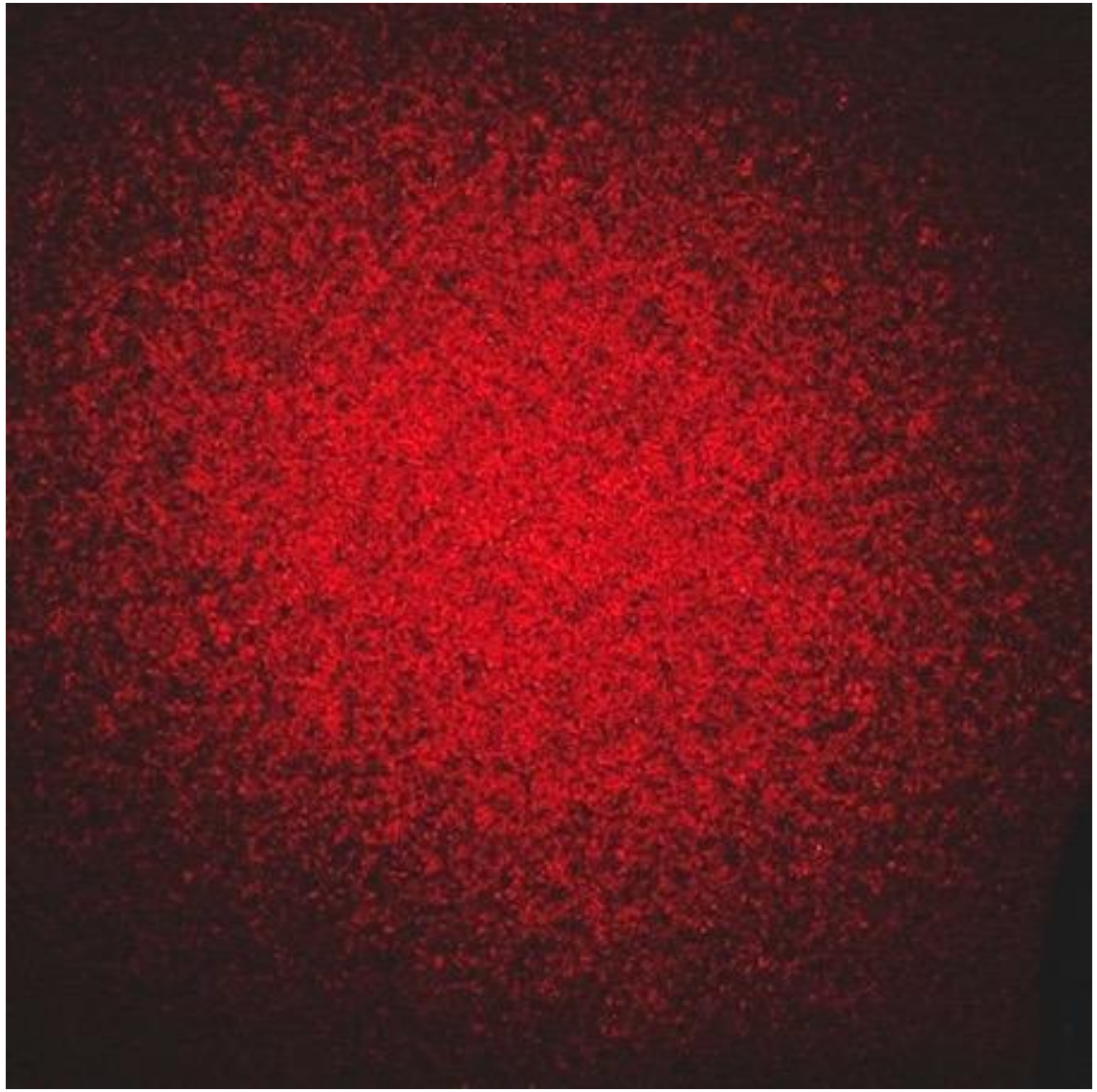


Dynamic Light Scattering Reveals Aggregation



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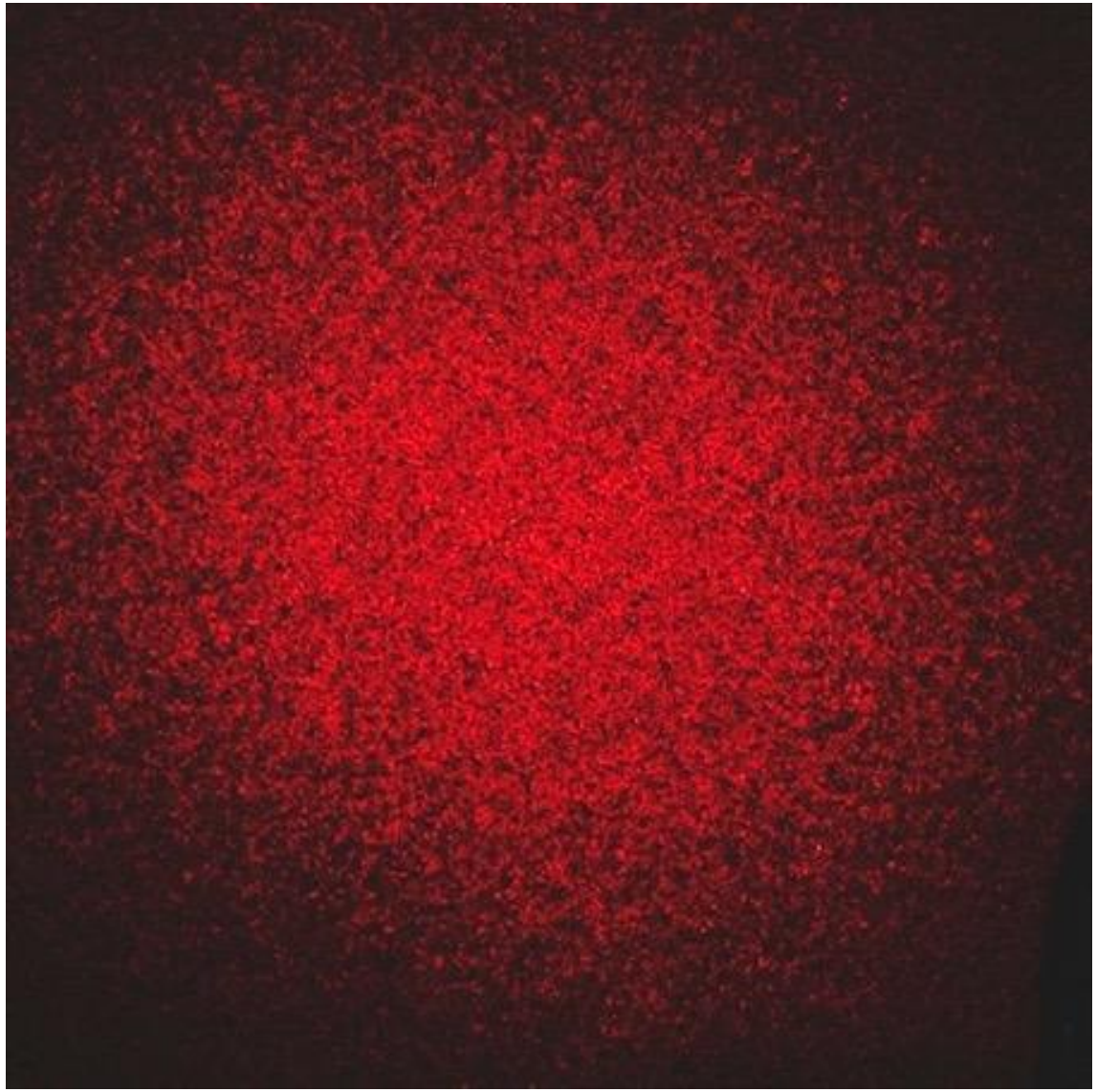
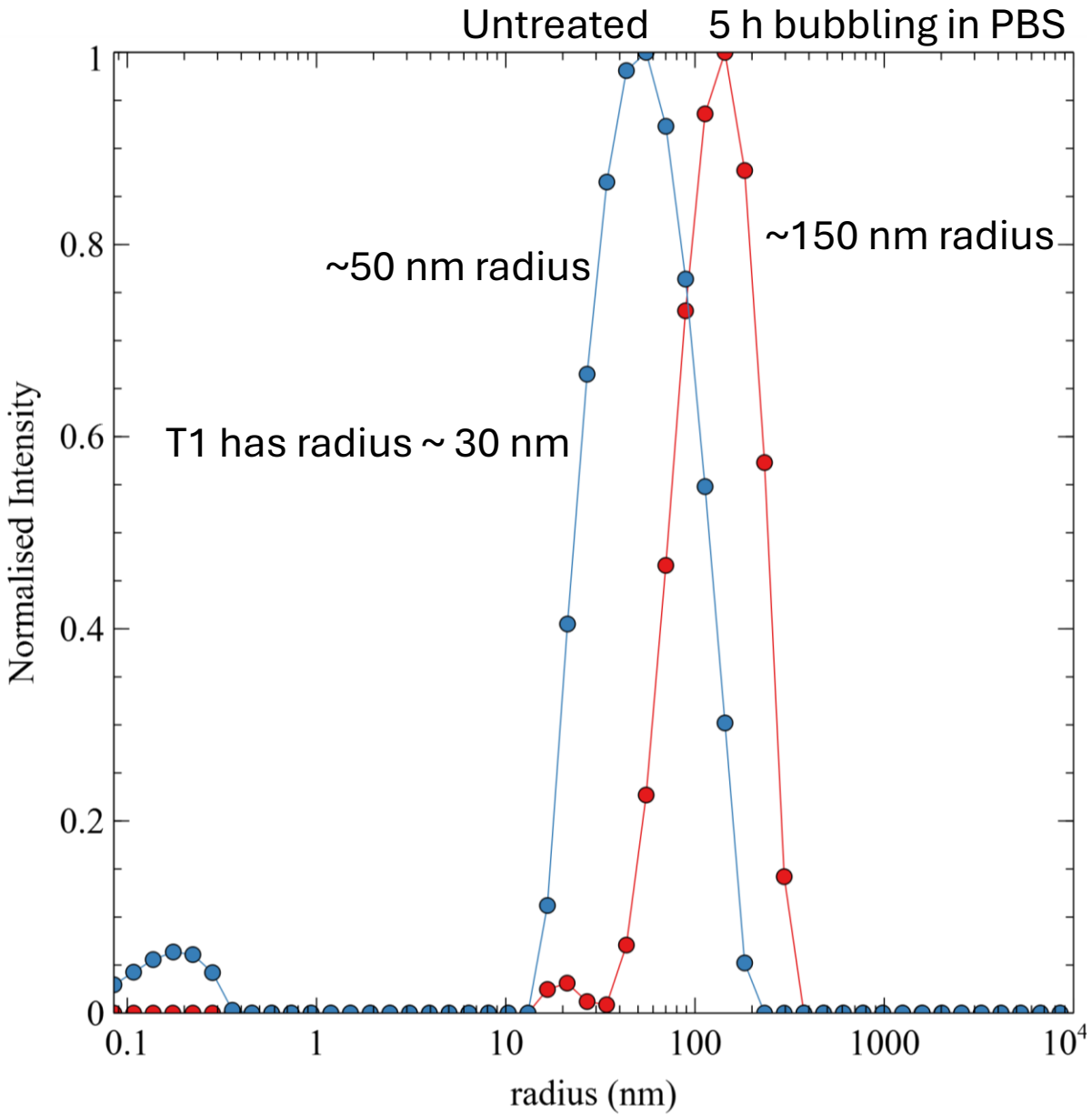
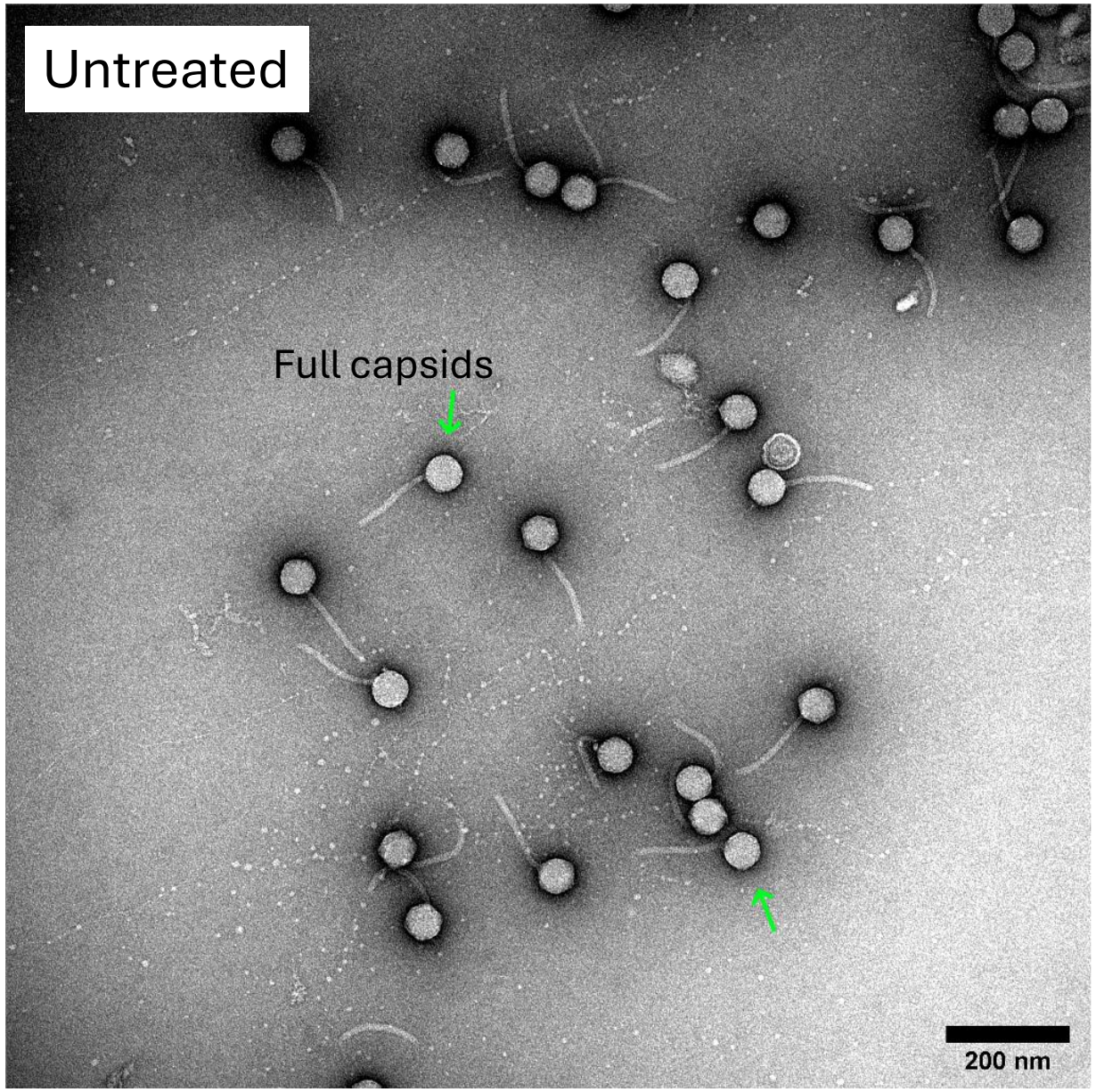




Electron Microscopy Reveals Nature of Aggregates



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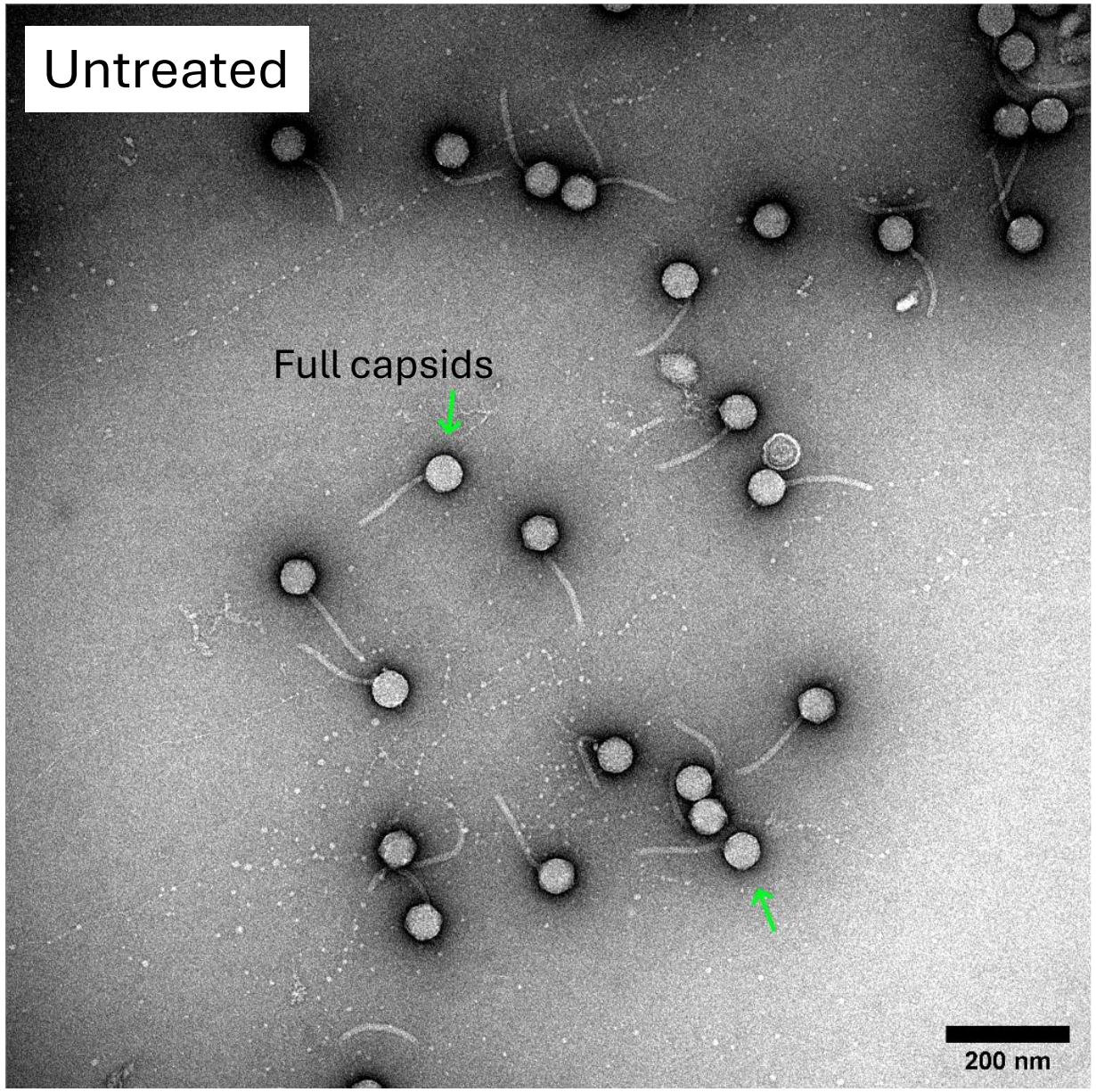

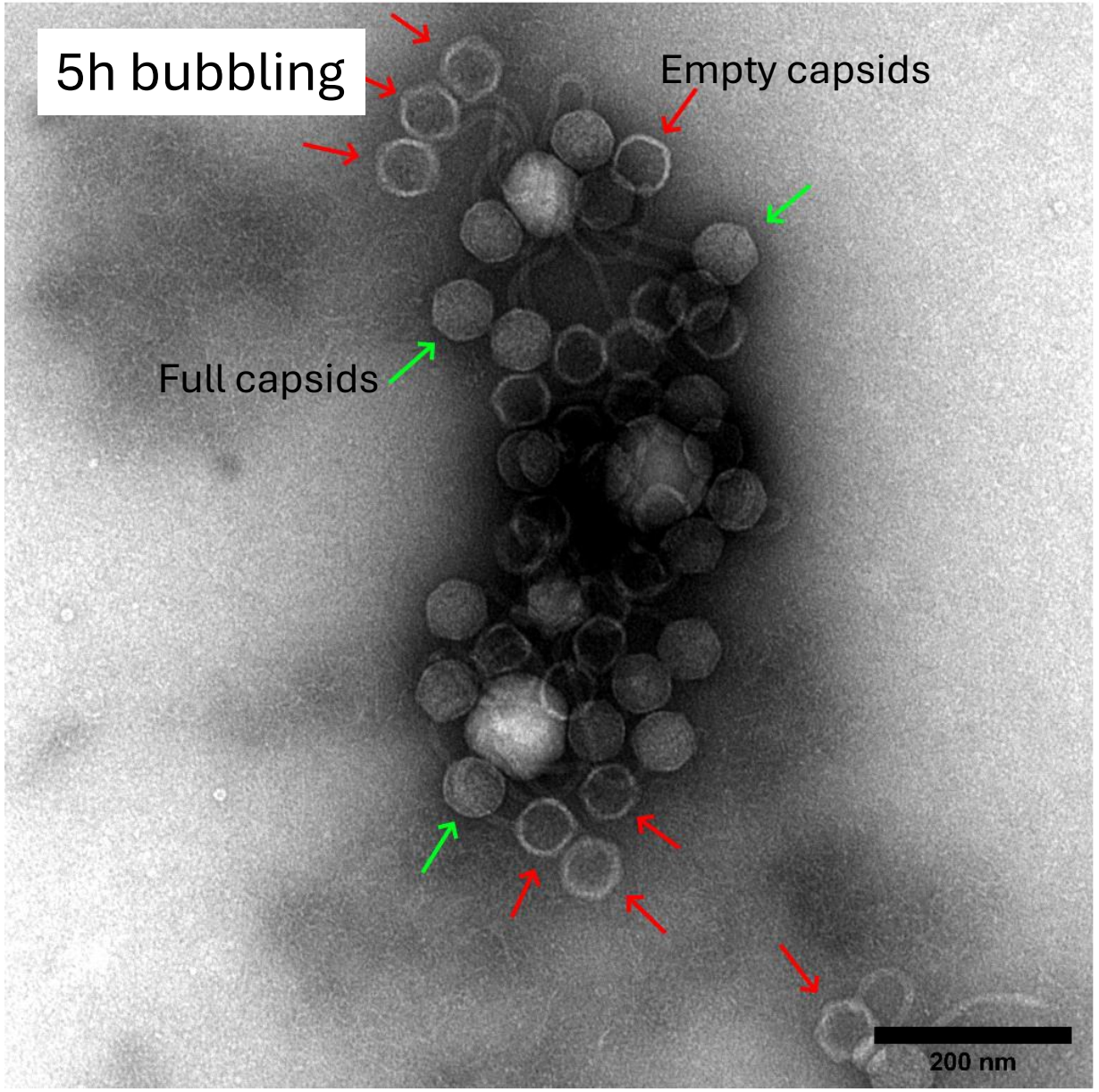

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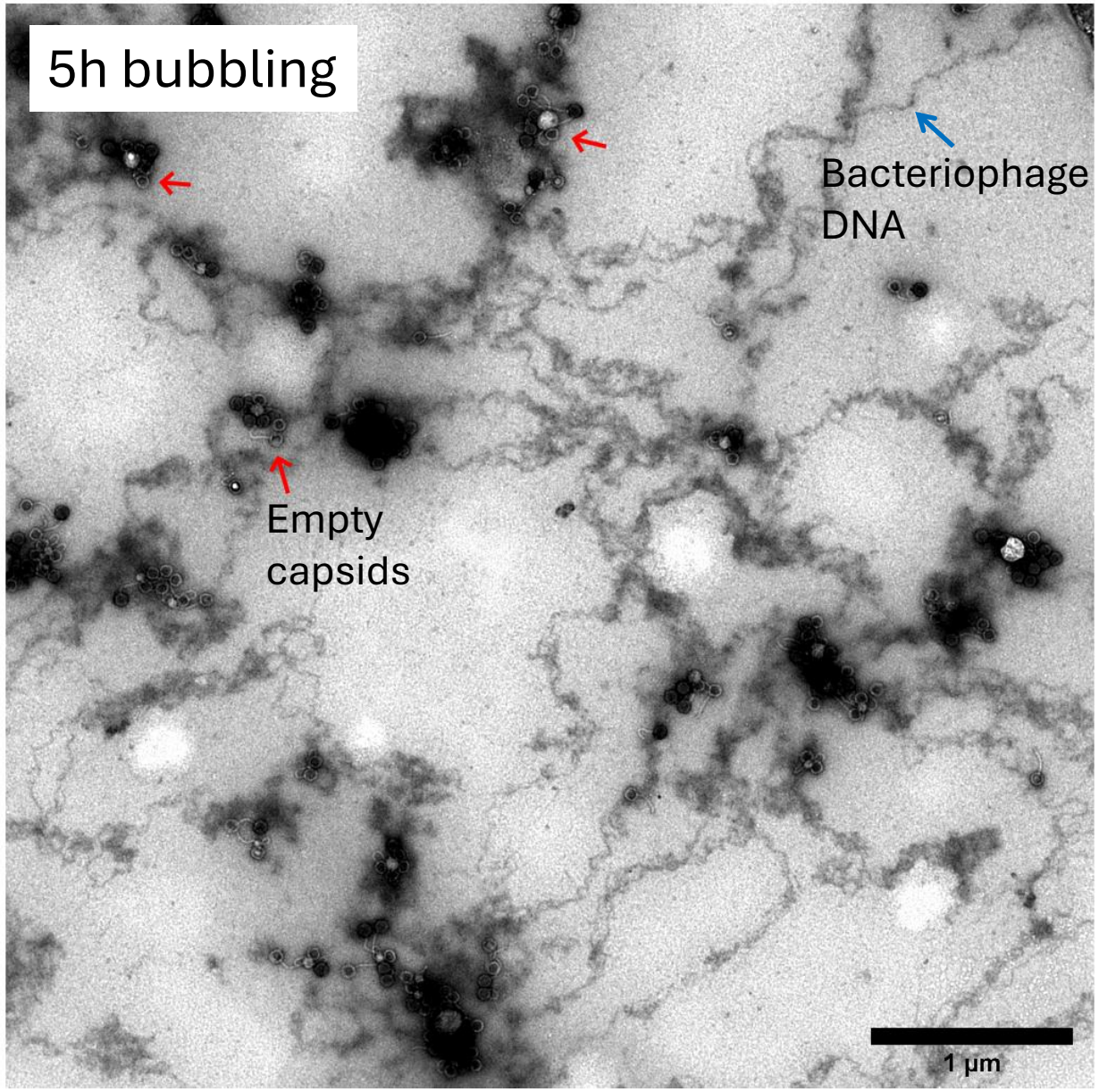
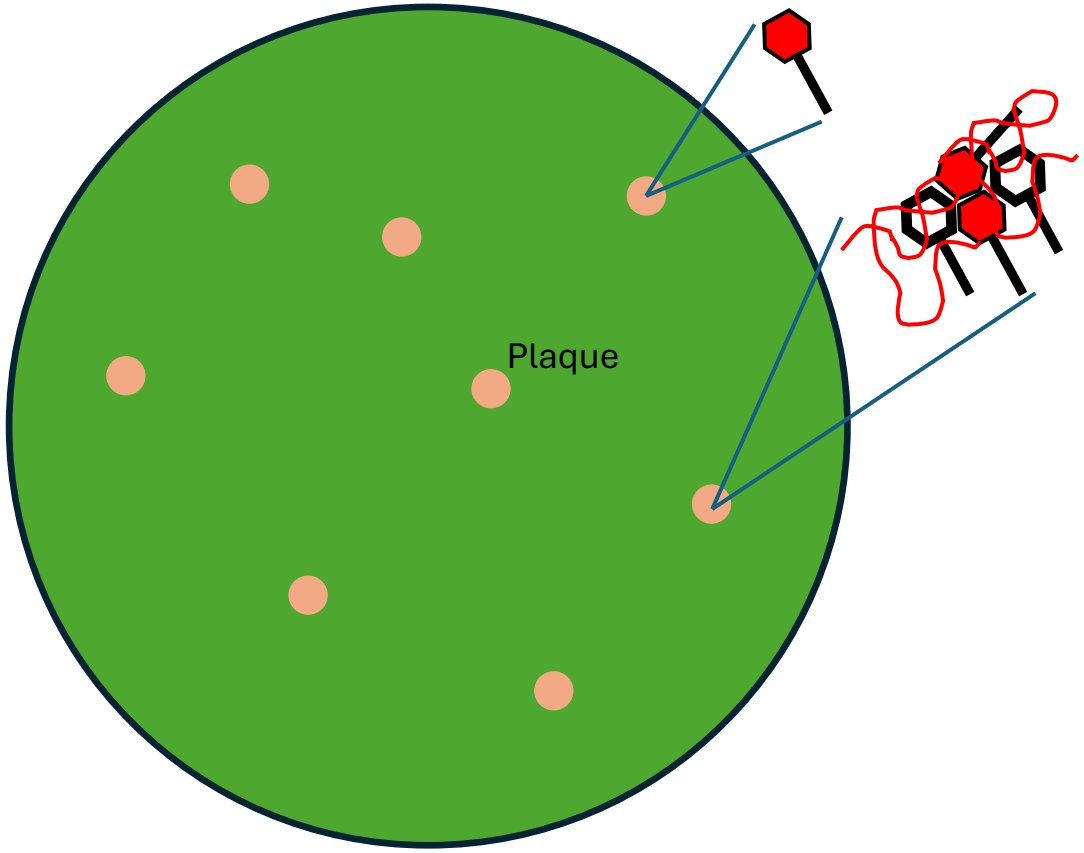
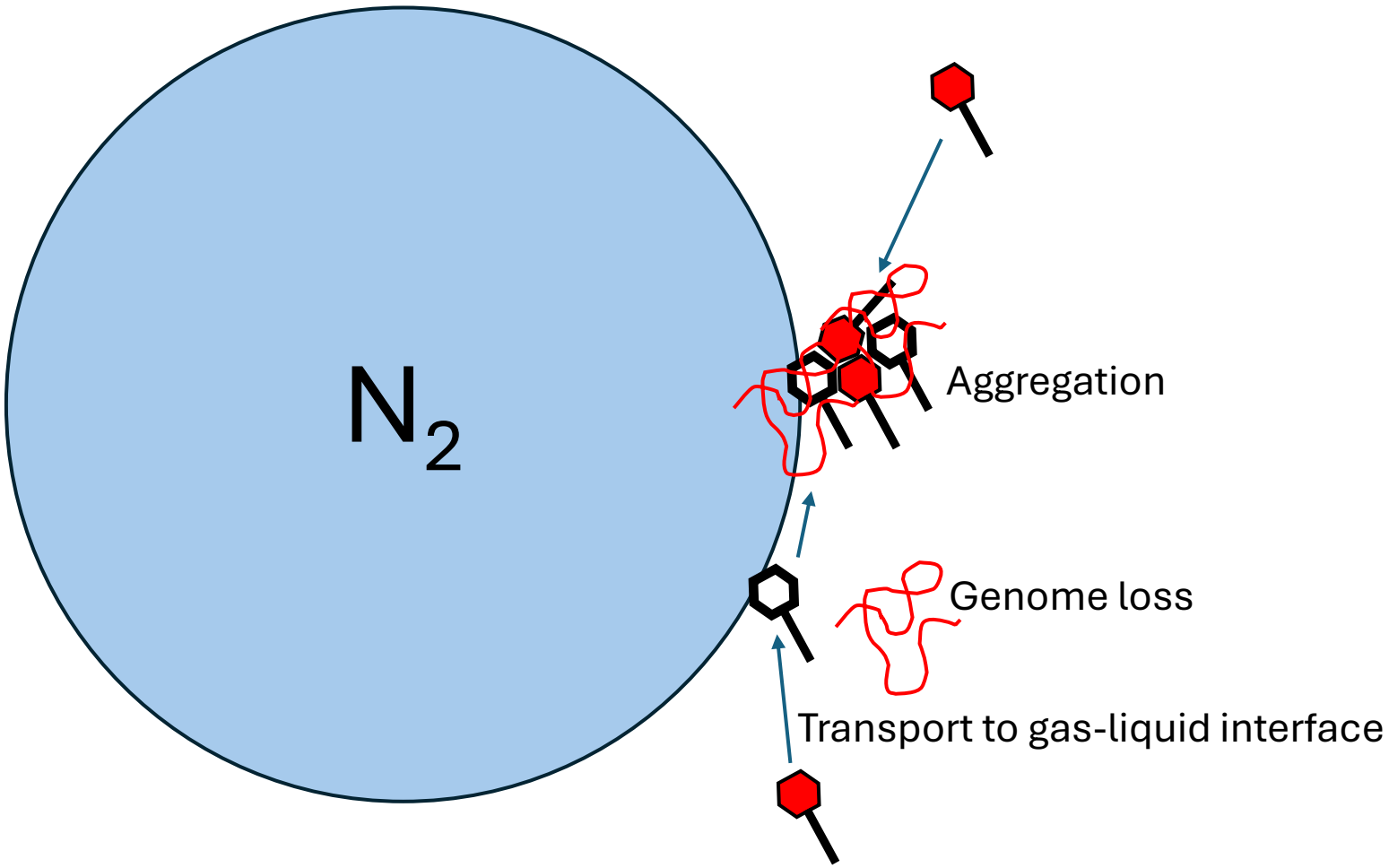




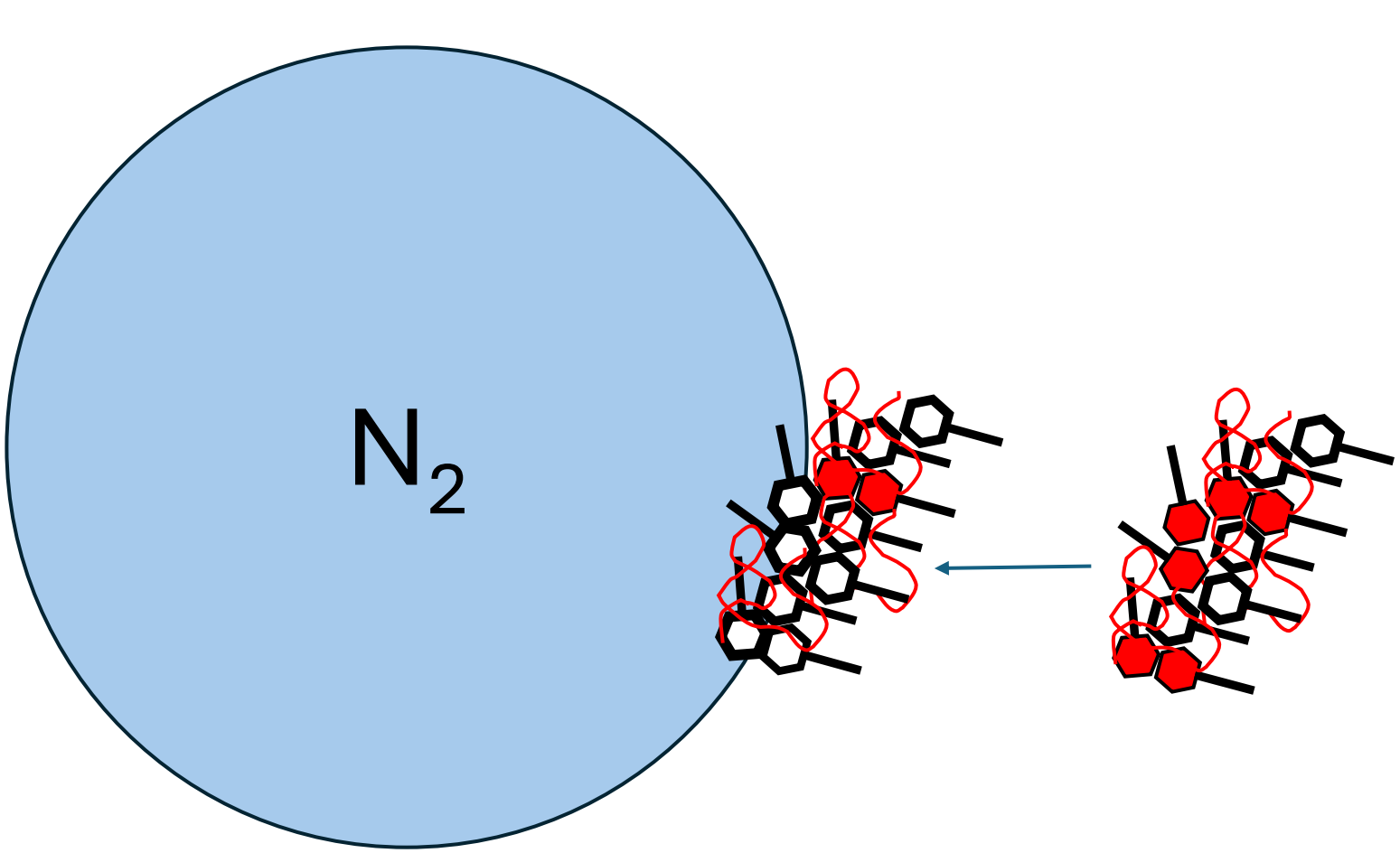
1 plaque= 1 phage OR 1 aggregate

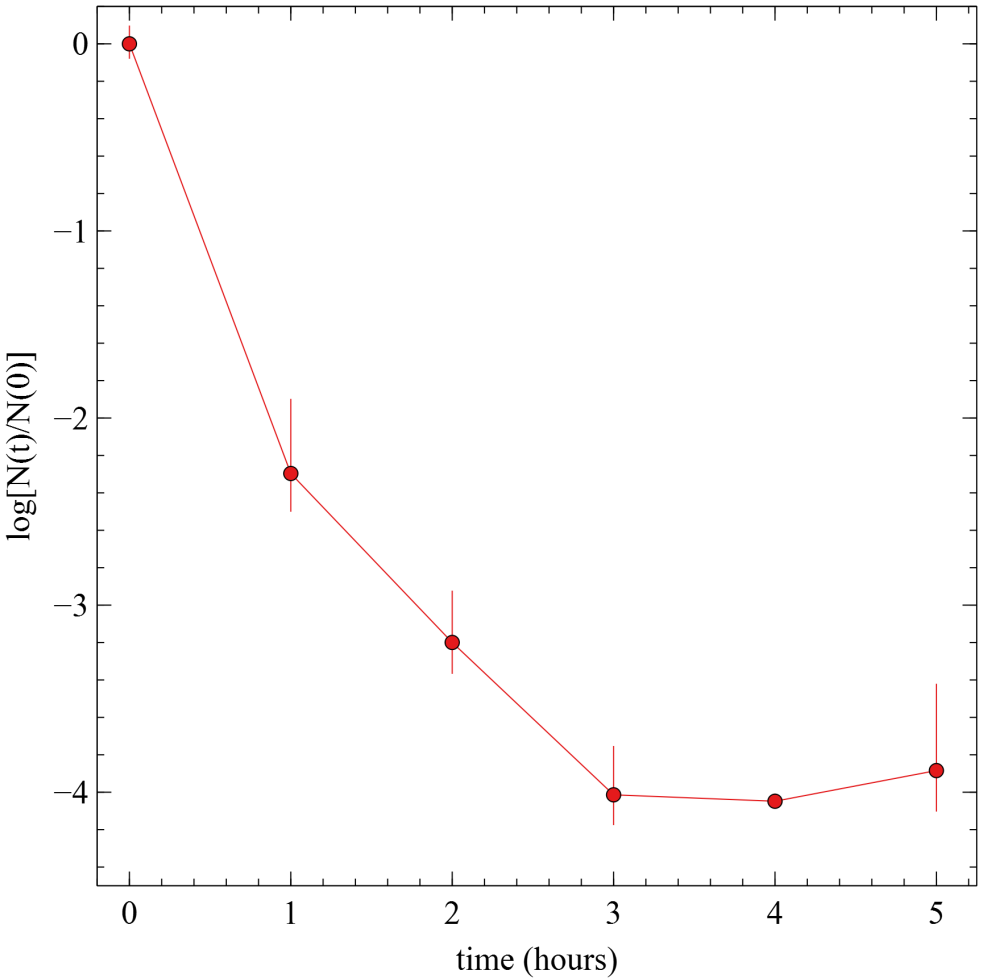
Inactivation by genome loss and aggregation



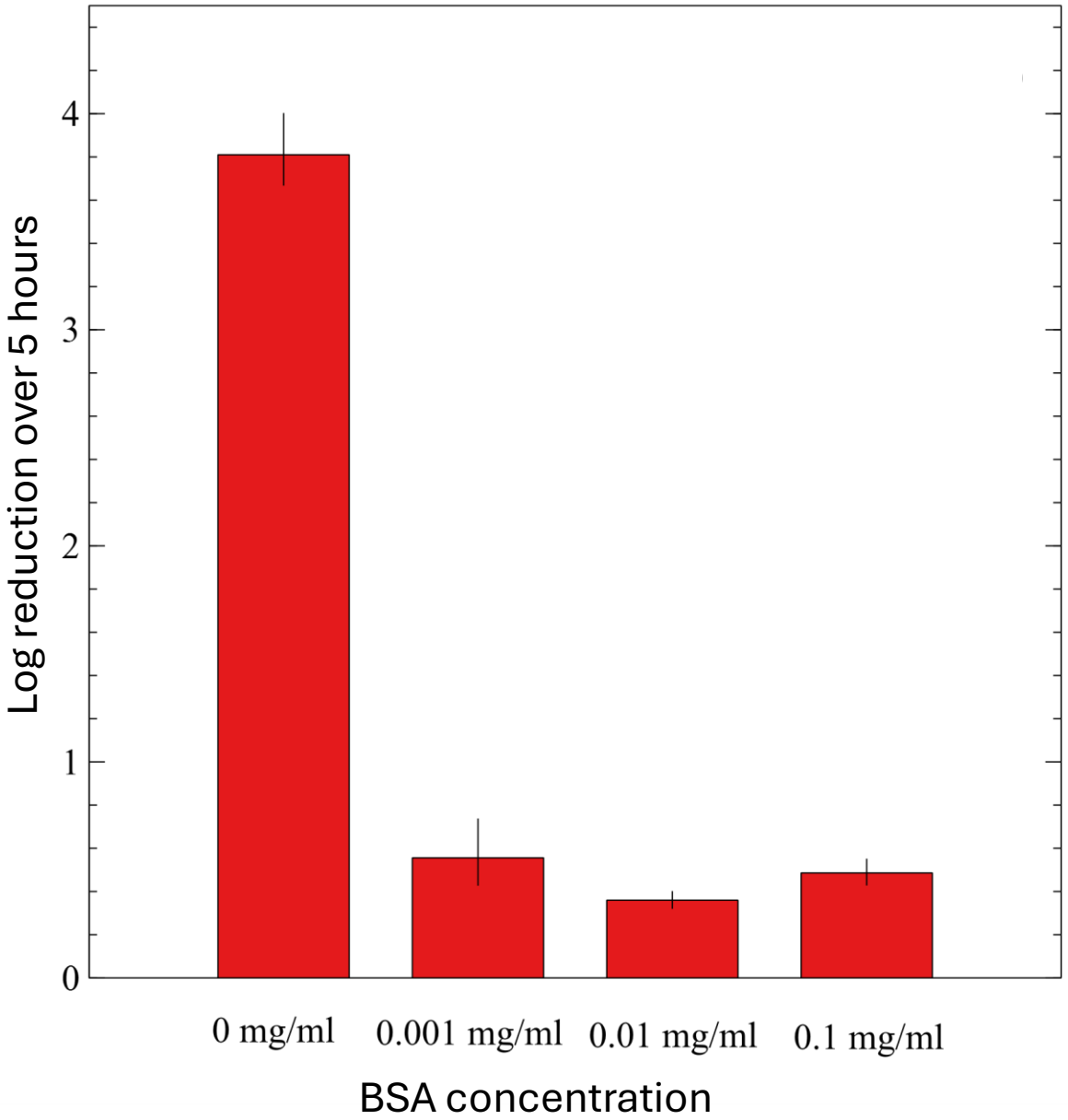
Mechanisms for destabilization… at interfaces
Brackley et al., (2021) *Nature Communications*

The long-time plateau is probably due to aggregation

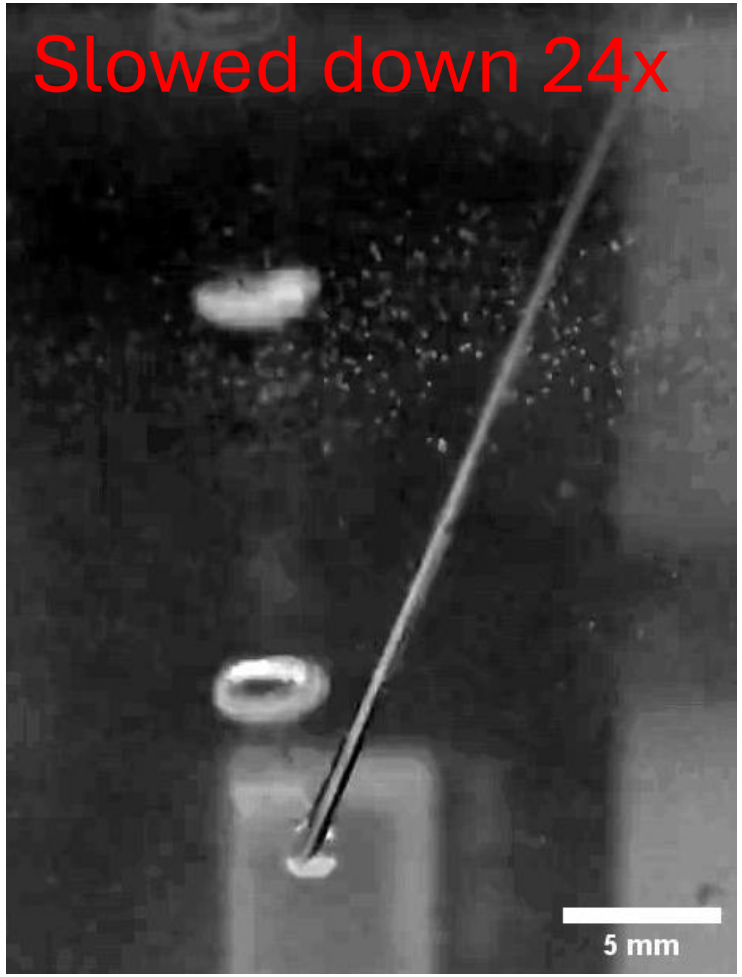




The impact of a protein layer: bovine serum albumin



Shape, size and speed of bubbles depend on the media

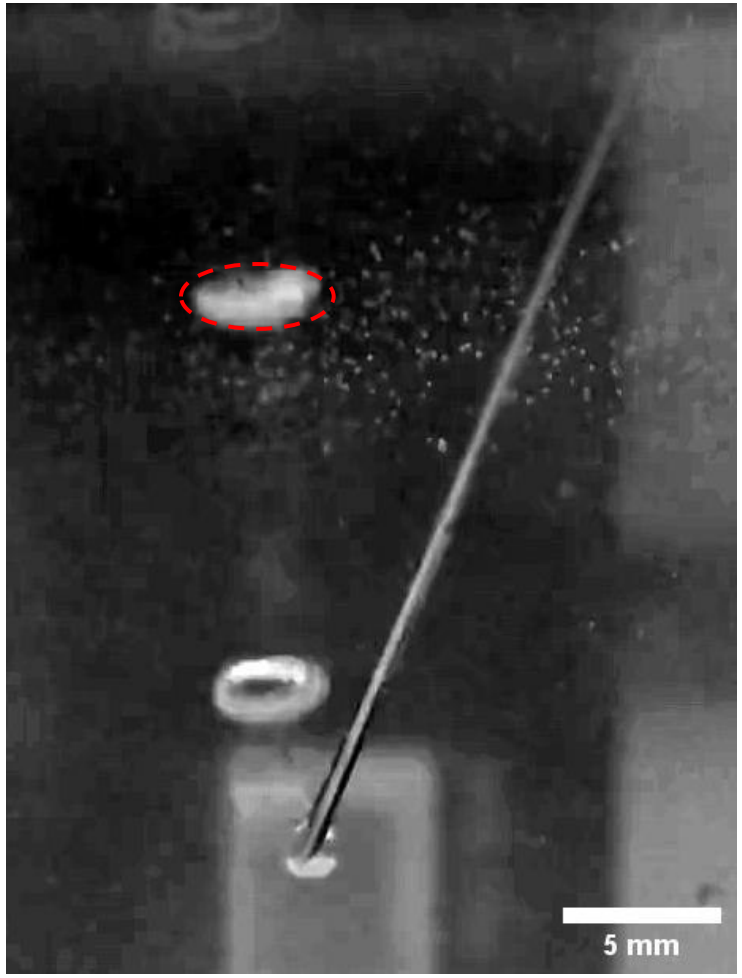


Water

PBS

...with 0.01 mg/mL BSA

Shape, size and speed of bubbles depend on the media



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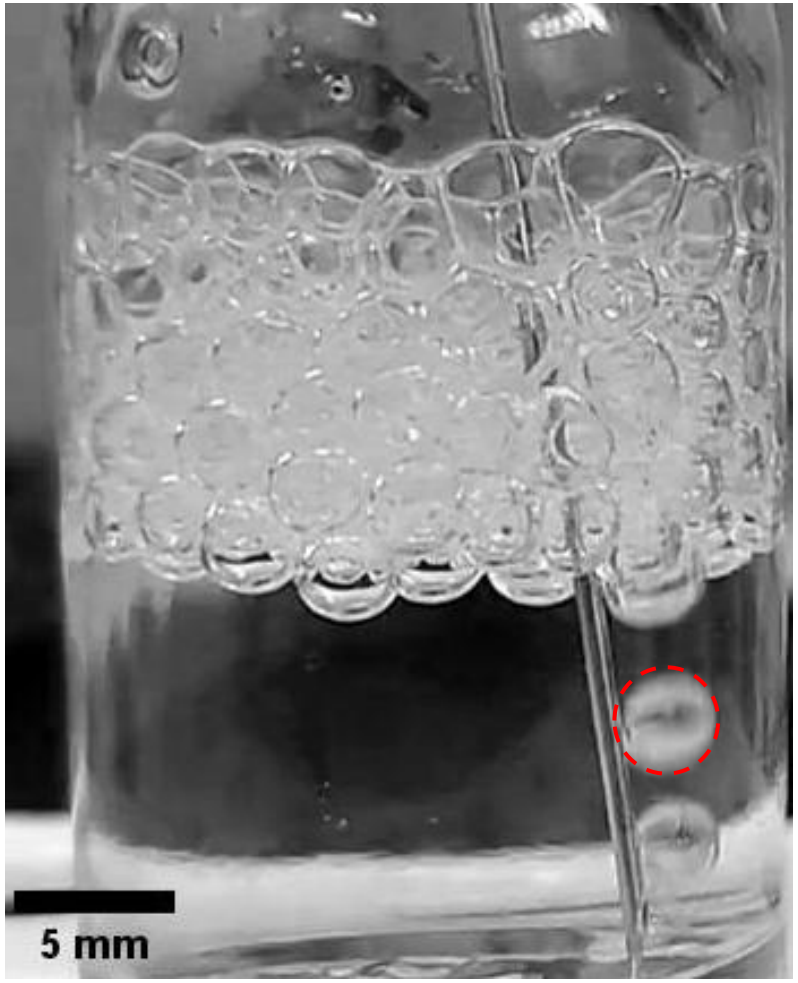
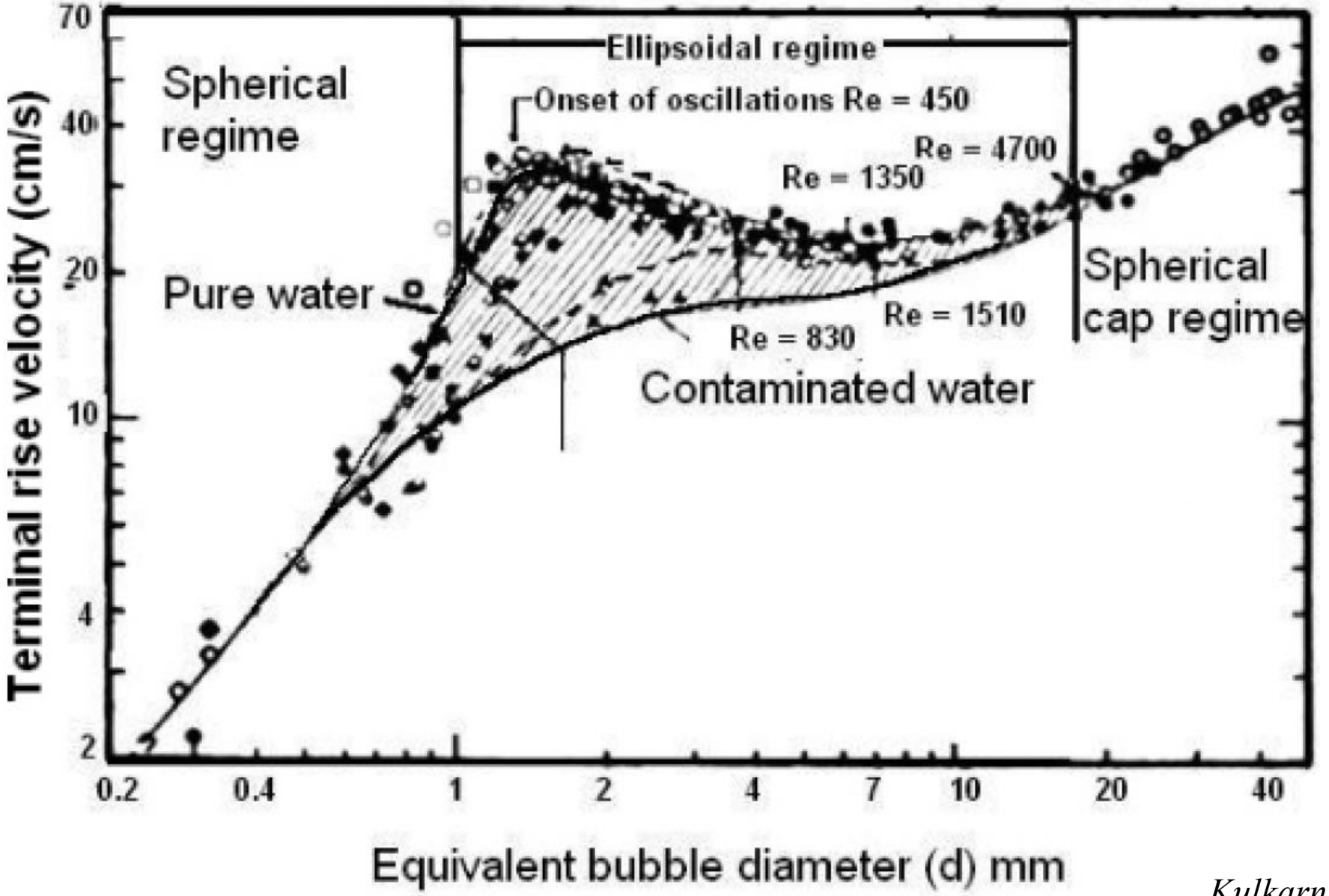


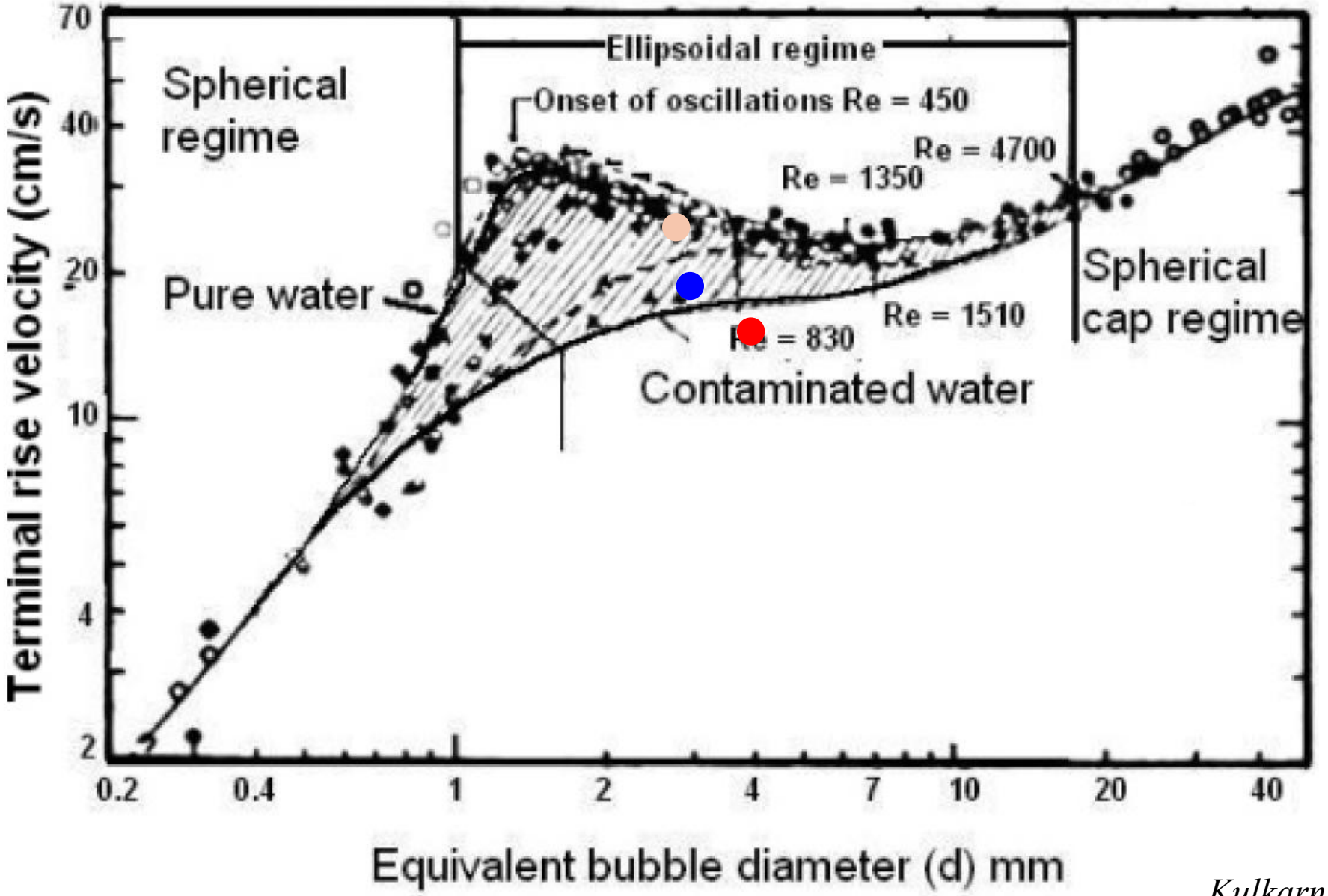
...with 0.01 mg/mL BSA

Terminal bubble rise speed



Kulkarni & Joshi, (2005) Ind. Eng. Chem. Res.
Clift, Grace, Weber (1978) Bubbles, Drops, and Particles

Terminal bubble rise speed



- Water
- PBS
- PBS + BSA (protein)

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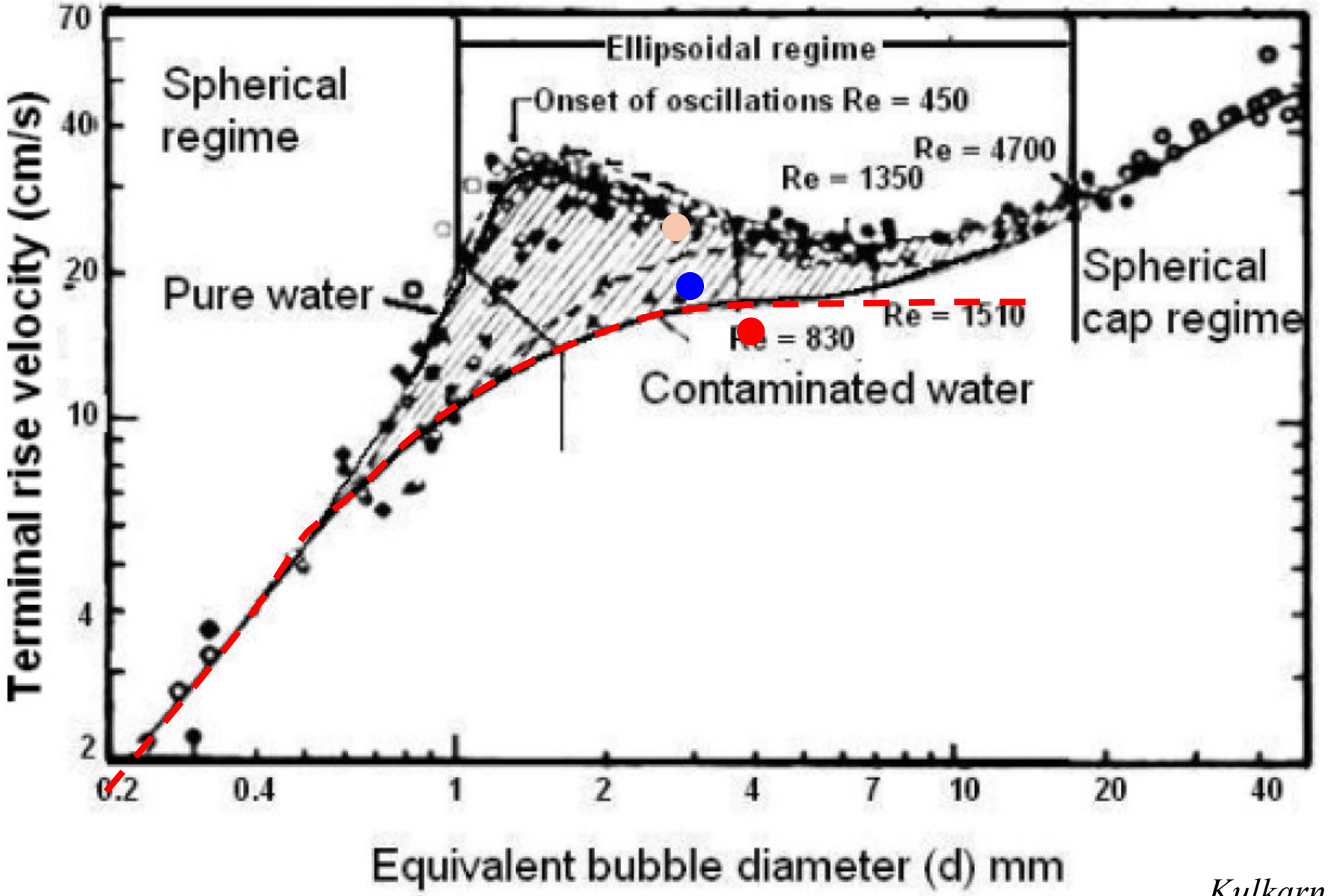
Terminal bubble rise speed



Water

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PBS + BSA (protein)

Empirical form used here

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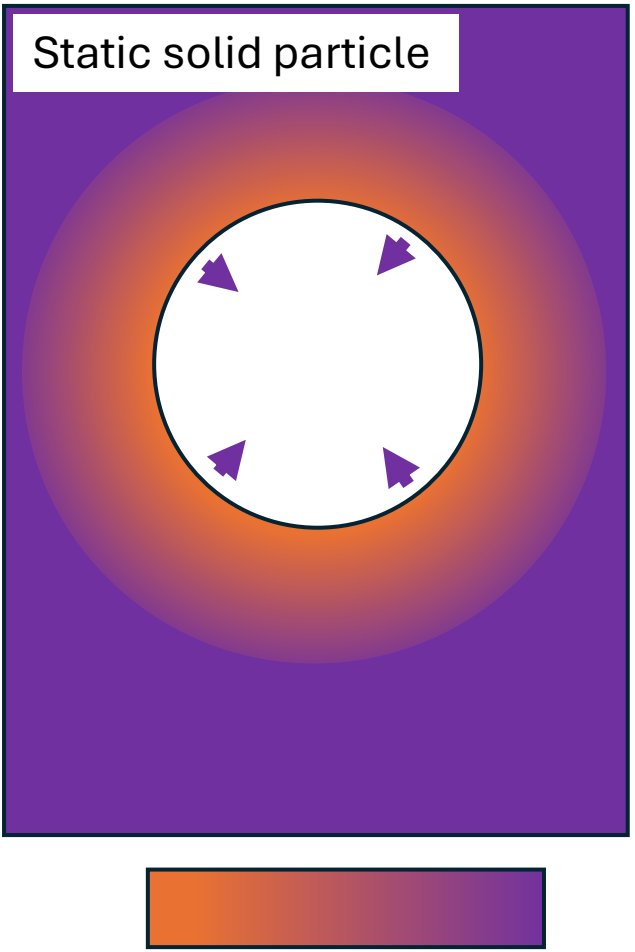
Factors controlling viral adsorption rate



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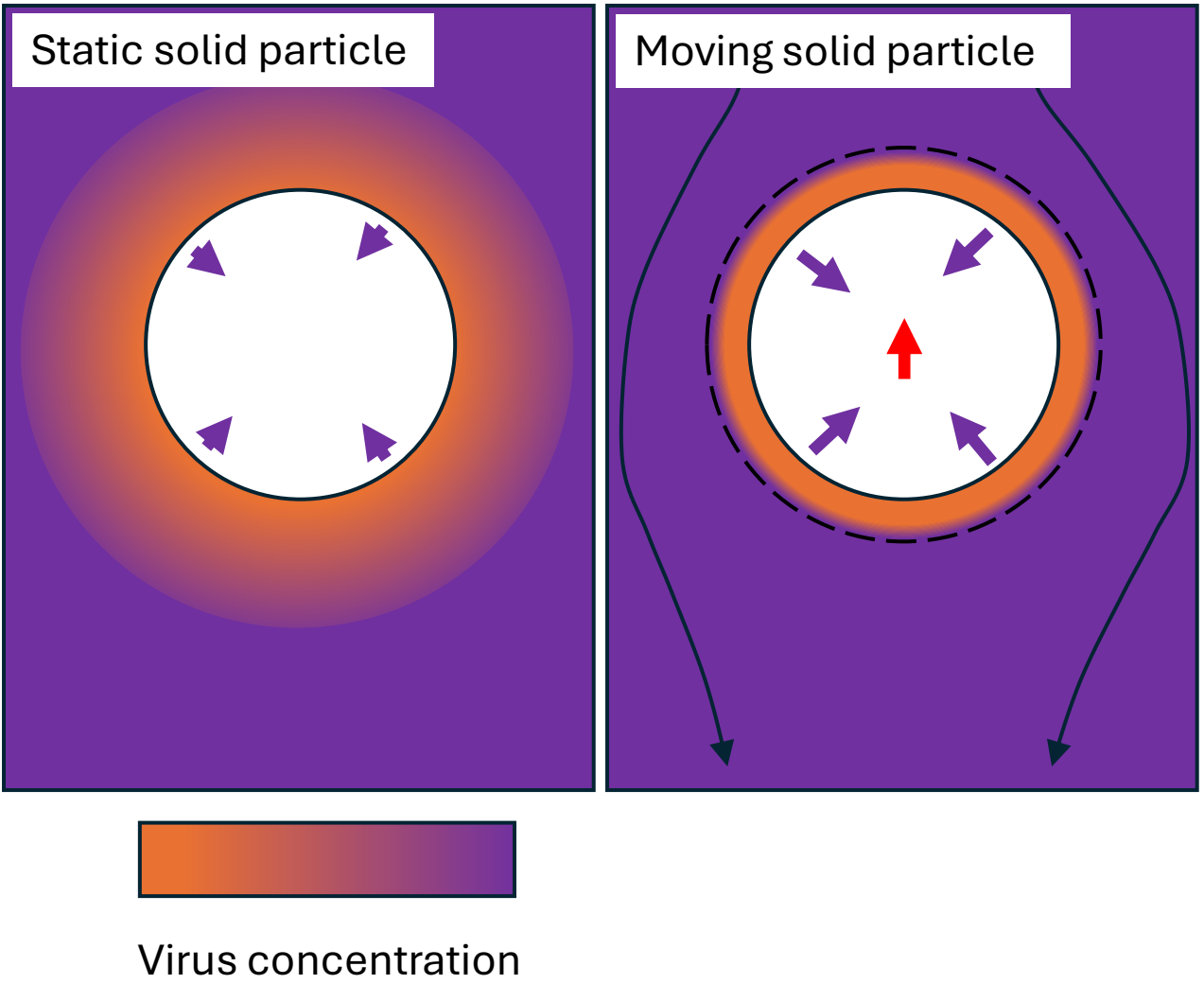

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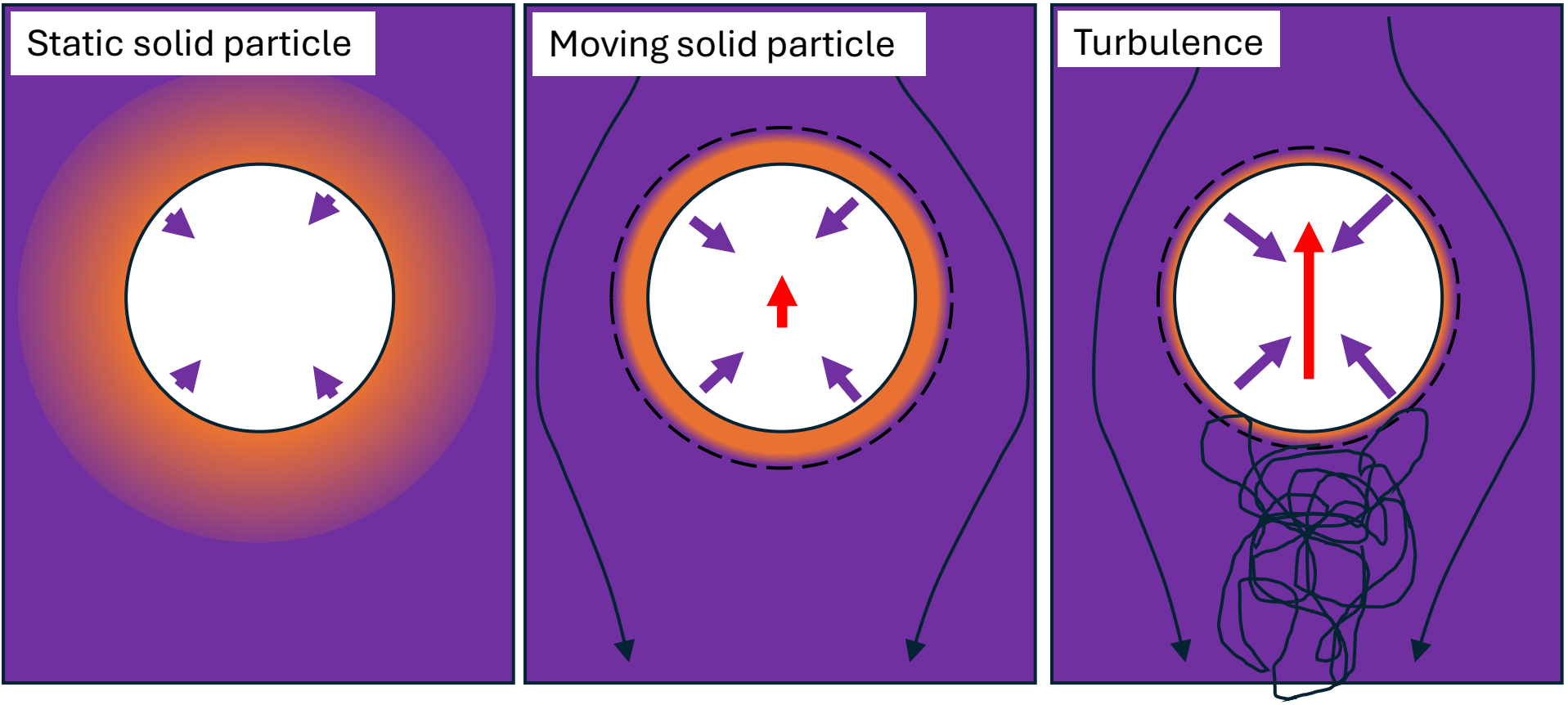

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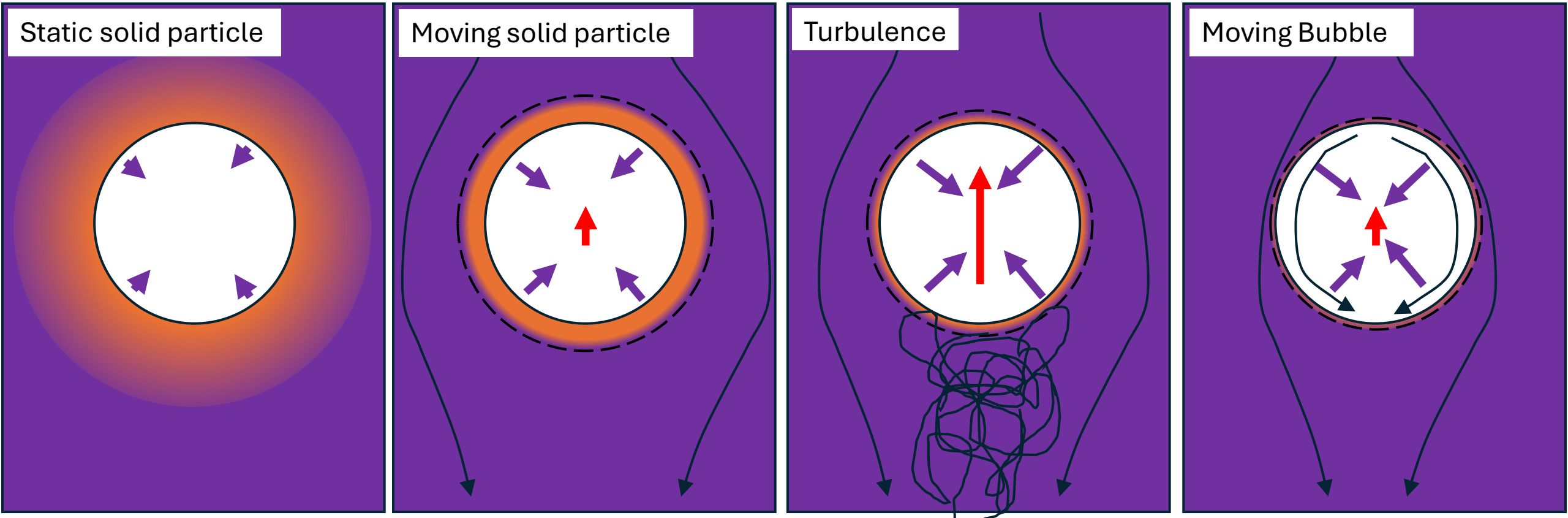
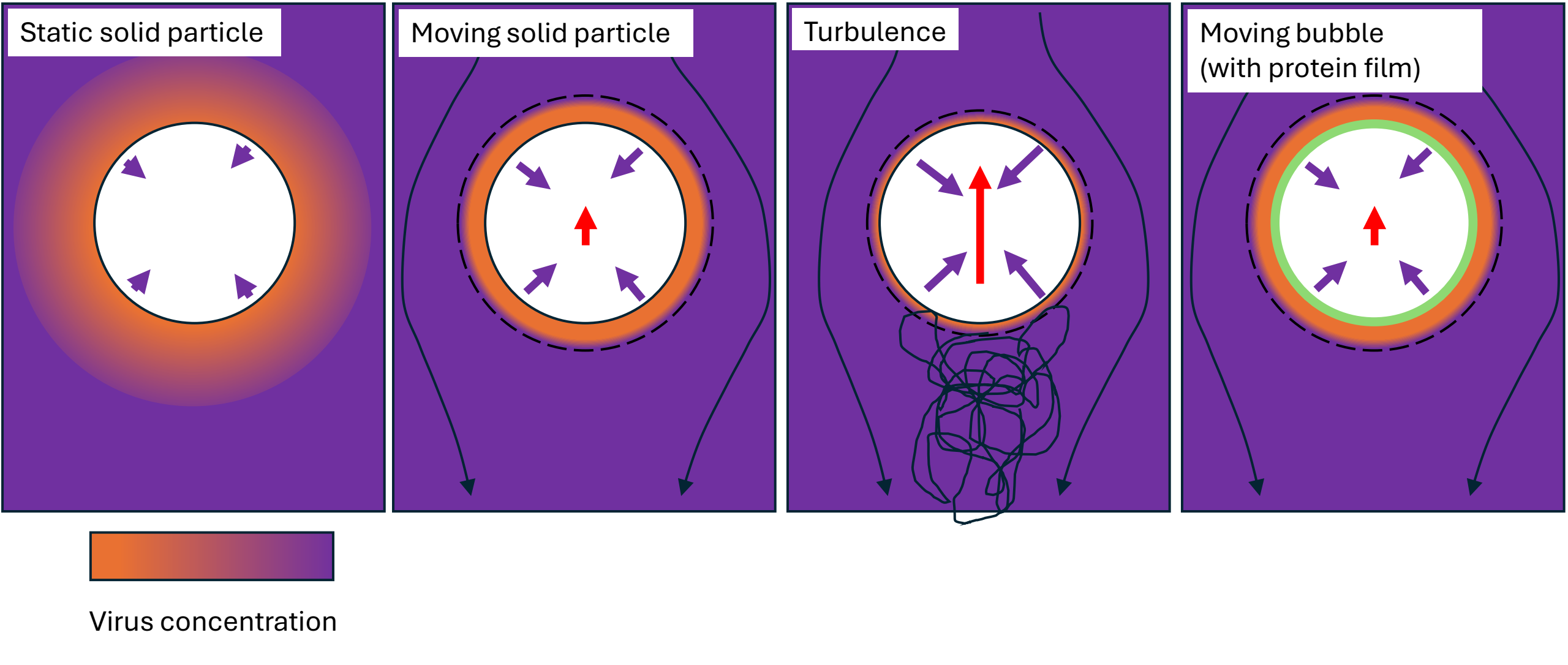
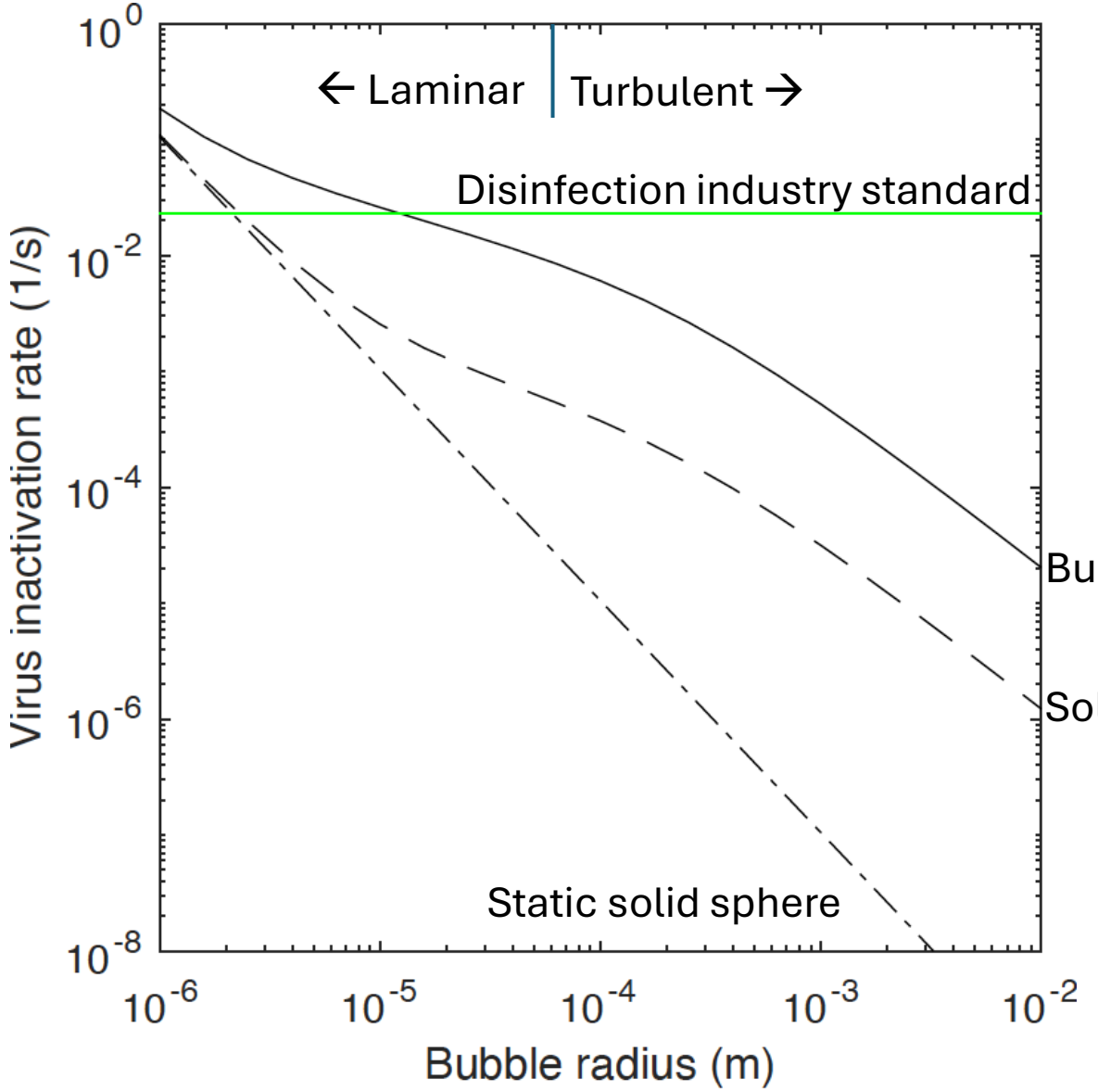


Virus concentration

Factors controlling viral adsorption rate



Theoretical inactivation rate at 1% bubble volume fraction



Bubble (simulation + theory)

Solid sphere (theory + exp.)

Landau & Lifshitz, *Fluid Mechanics*
Kumar Forsoiya et al., *(2023) JFM*
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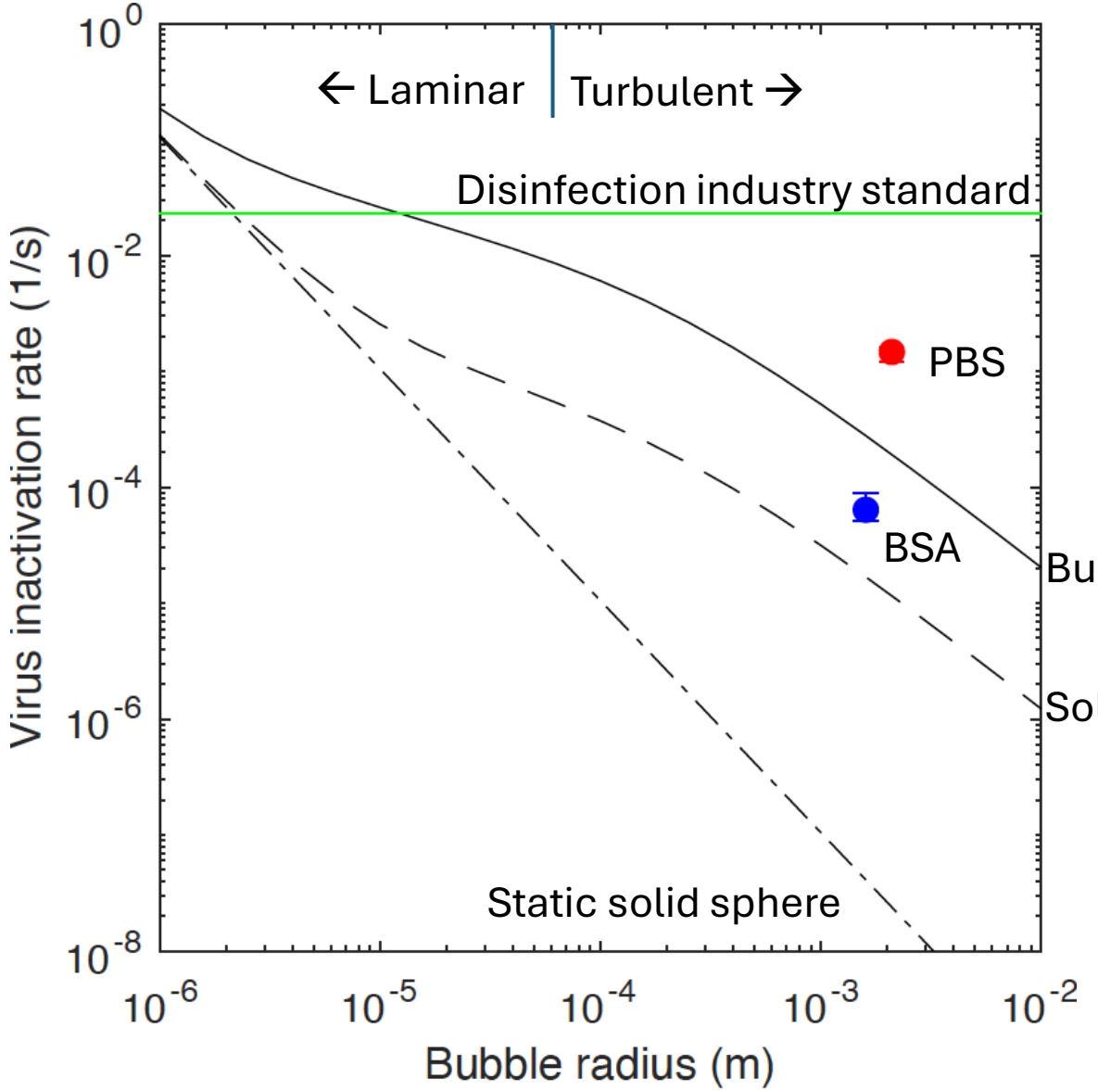

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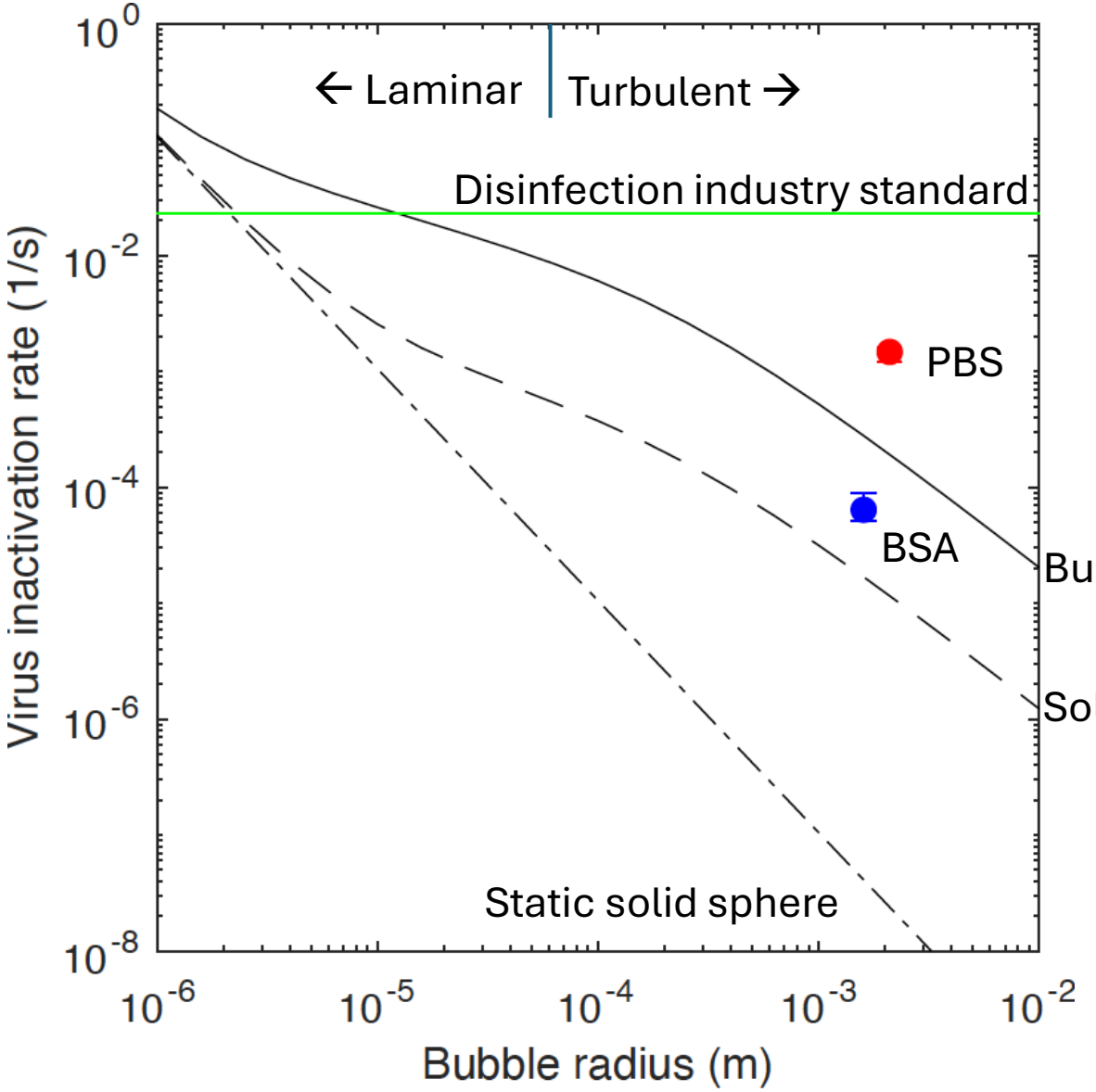

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Conclusions

1. Inactivation of T1 virus is due to a combination of capsid rupture, resulting in genome loss, and aggregation

2. A small fraction of viruses are not inactivated. This may be due to aggregation.

3. Bubble motion and turbulence contribute significantly to the predicted inactivation rate.

4. The rate of inactivation in PBS is 5x larger than the maximum predicted rate.

5. Adding protein reduces the inactivation rate. This could be due to solidification of the interface.

Questions

1. To what extent are these conclusions reproduced in other viruses?

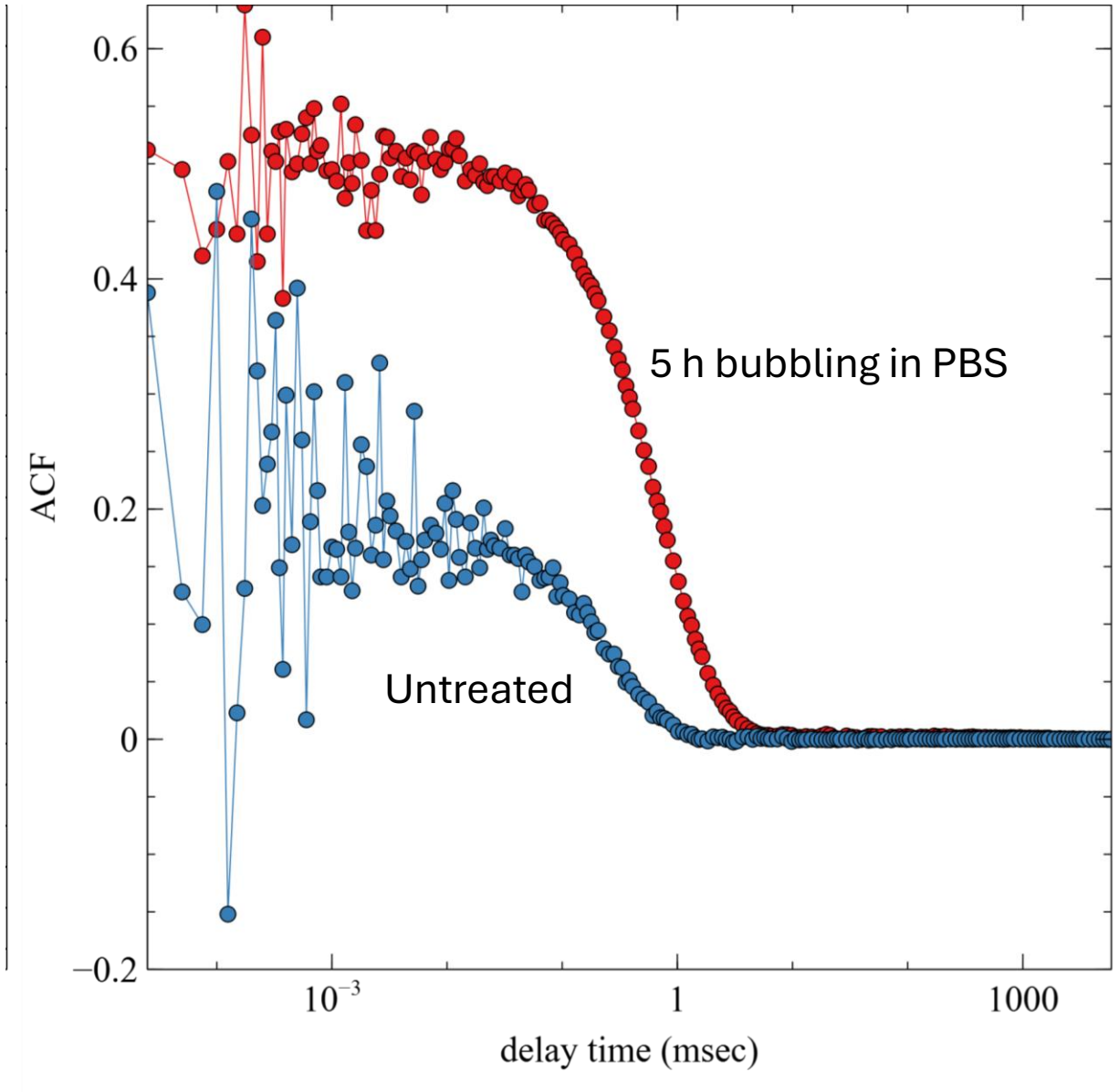
2. Why are some viruses more stable than others?

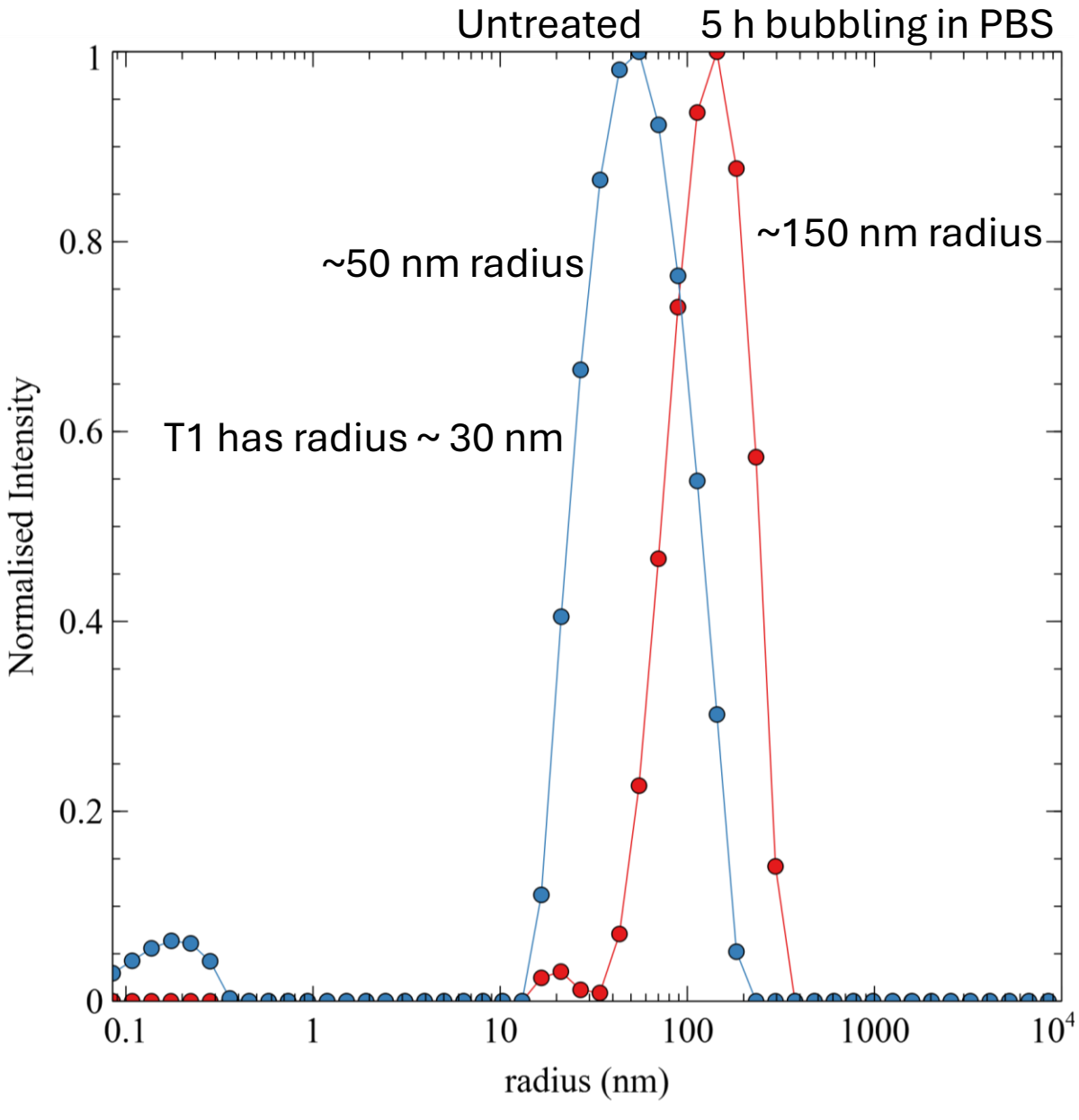
3. Why is the rate of inactivation faster than the predicted rate of adsorption?

4. What is the role of charge, salt concentration etc.?

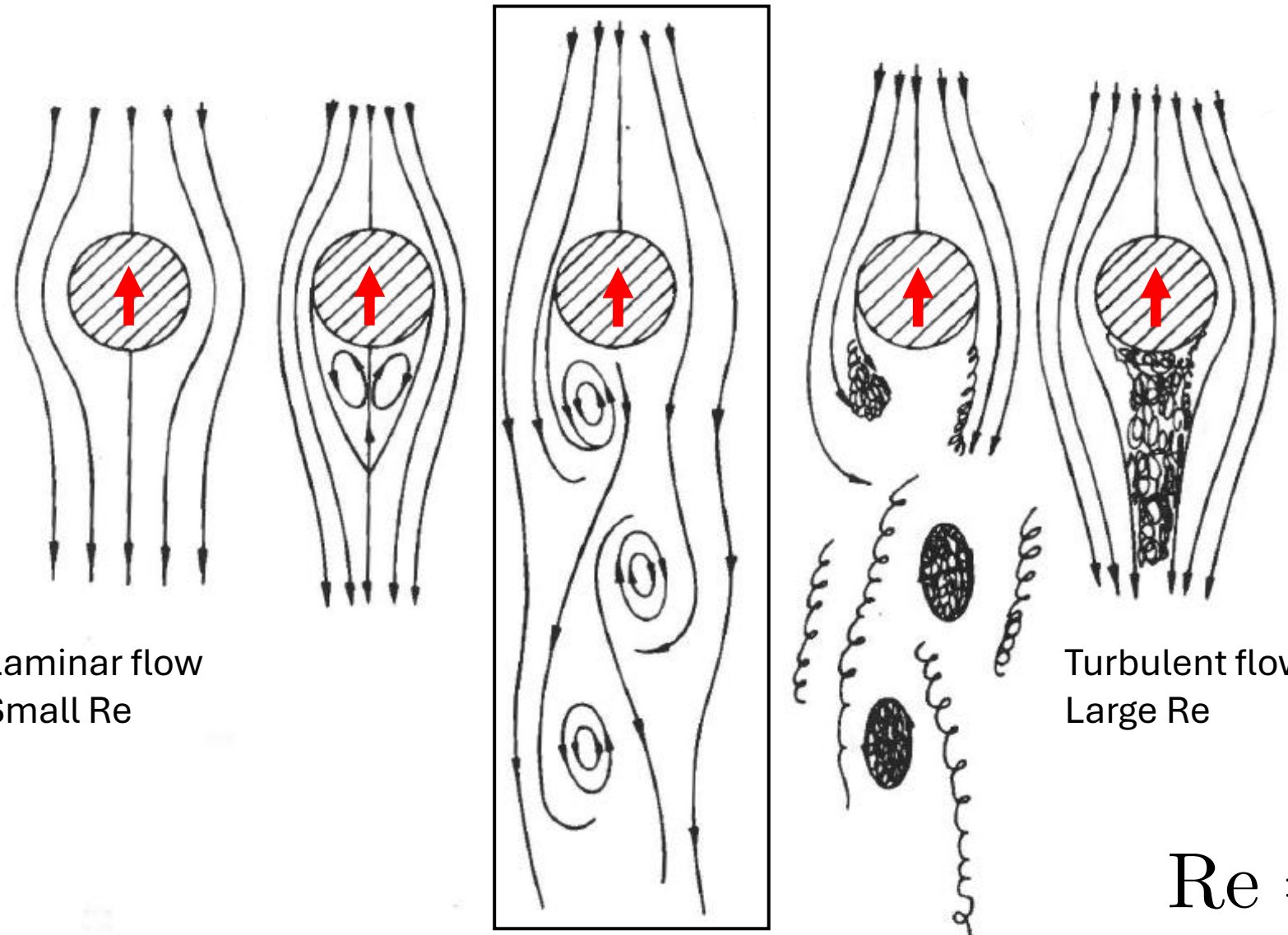
Review: Poon et al., (2020) *Soft Matter*
Soft Matter Physics & the Covid-19 Pandemic

Dynamic Light Scattering Reveals Aggregation





The Reynolds number



Laminar flow
Small Re

Experiments

Turbulent flow
Large Re

$$\mathrm{Re} = \frac{U a \rho}{\eta}$$

Feynman Lectures on Physics