Lilliputian Particles: Scattering and Spectroscopy Applied to New Large Molecule Delivery Vehicles

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Problem Solving: particlesellc.com

Kevin Dahl, PhD - 20 years of particle and spectroscopic experience in pharma

- Technology Consulting
  - Instrumentation, laboratory
- Data Consulting
  - Reanalysis, interpretation, CMC support
- Document Consulting
  - Drafting and Review
- Method Consulting
  - Development, Optimization, Qualification, Validation
- Experimental Consulting and Services
  - Study design, execution, analysis, interpretation
- Training Services
  - Selected Instruments

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“Vision is the art of seeing things invisible.”

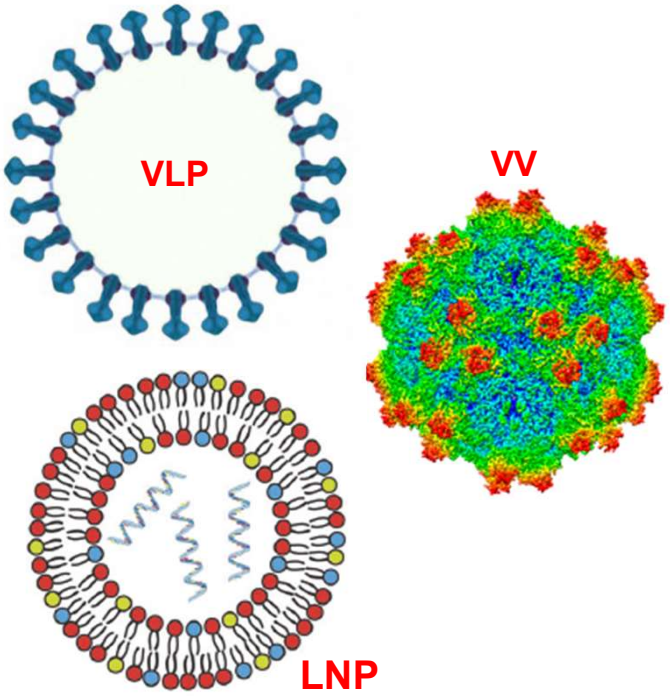
- Jonathan Swift

The Lilliputians: Discovered

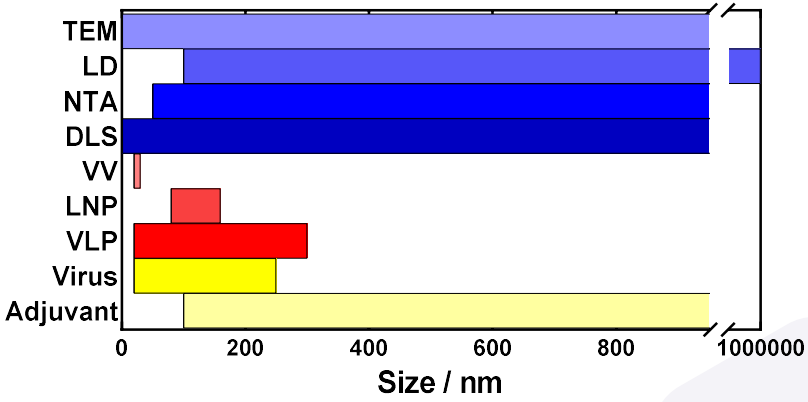
- New therapeutics require new delivery methods
  - Molecular and *in vivo* DP stability are critical
  - Target the cell interior
- Traditional delivery doesn't suffice
  - Solid dosage forms
  - Buffered
- Structural targeting
  - Delivery direct to specific cell type
- Nano-scale structures can address these challenges

Illustration by Louis John Rhead from: http://www.childrensbooksonline.org/Gullivers_Travels/index.htm

The Lilliputians: Nano-scale Structures



- Nanoscale delivery vehicles include:
  - Virus-like Particles
  - Viral Vectors
  - Lipid Nanoparticles
- Size scales differ significantly



VLP Illustration from: Nooraei, et al., *Journal of Nanobiotechnology*, 19 (2021).
VV Illustration from: Mietzsch, et al., *Viruses*, 13 (2021).
LNP Illustration from: Blakney, et al., *Gene Therapy*, 26 (2019).

Nano-scale Delivery Analytical Challenges

I ROARED SO LOUD THAT THEY ALL RAN BACK IN A FRIGHT

- Formulation, Characterization
  - Stability, Storage, etc.
- Critical Quality Attributes
  - Count (Concentration)
  - Size Distribution
  - ‘Chemistry’
- How have nano-scale structures been studied?
  - DLS
  - UV-Vis spectroscopy
  - Cryo-TEM
  - Analytical Ultracentrifugation

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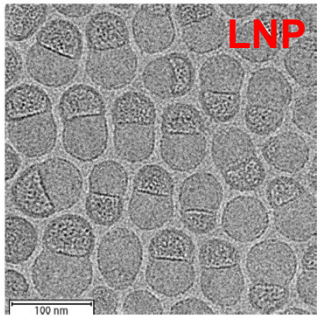
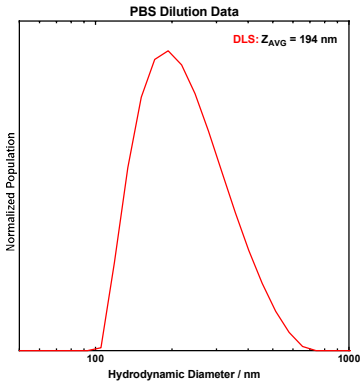
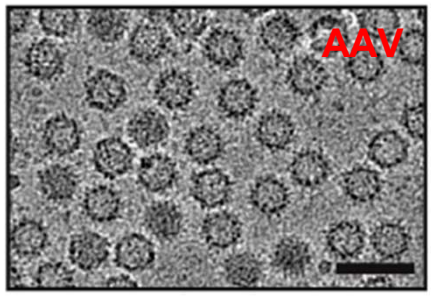
Analytical: Too Much or Too Little



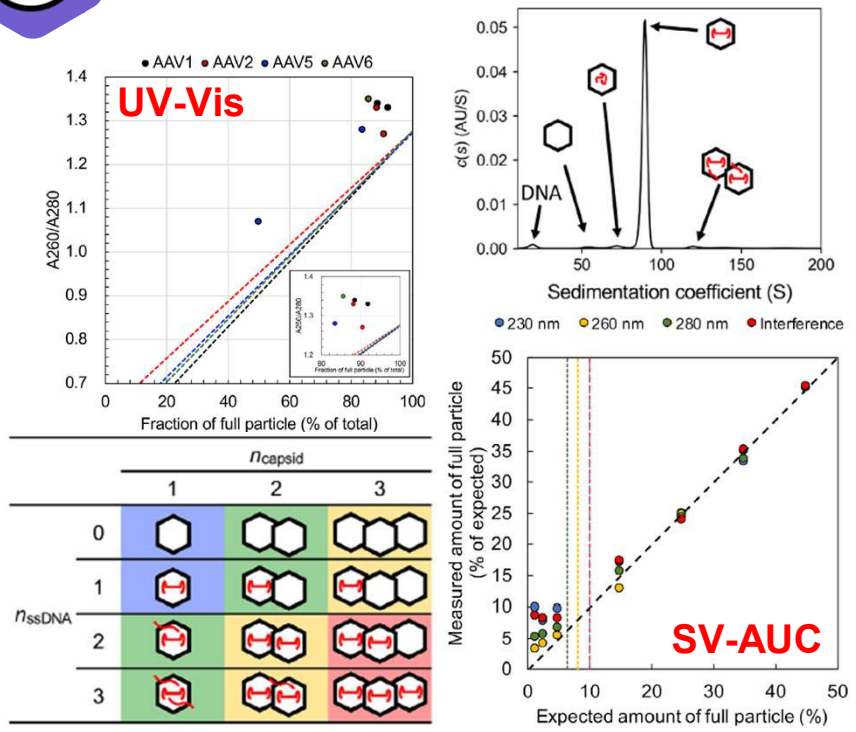




- DLS
  - Size and Polydispersity
  - Cheap, rapid, no expertise
  - Poor resolution
  - Difficult interpretability
- Cryo-TEM
  - Morphology, count, ‘chemistry’
  - Extreme resolution, full/empty ratio
  - Statistically weak
  - Expertise required
  - Expensive
  - Slow

LNP TEM Image from: Schoenmaker, et al., *International Journal of Pharmaceutics*, 601 (2021).
AAV TEM Image from: Mietzsch, et al., *Viruses*, 13 (2021).
DLS Plot from: Nguyen and Dahl, Webinar (2022).

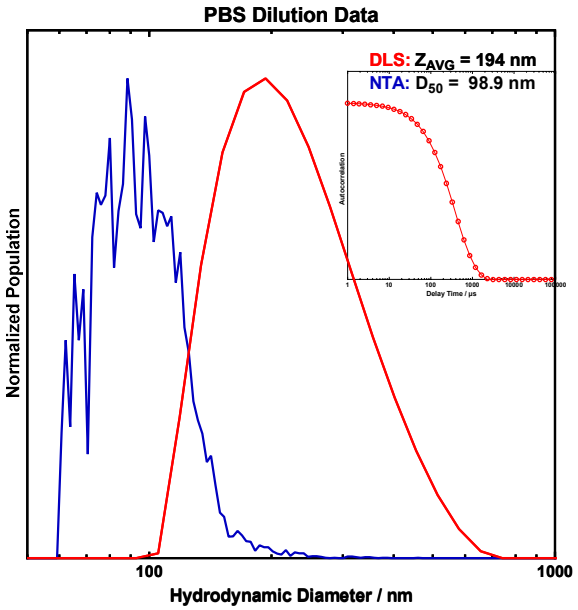
Analytical: More Too Much or Too Little



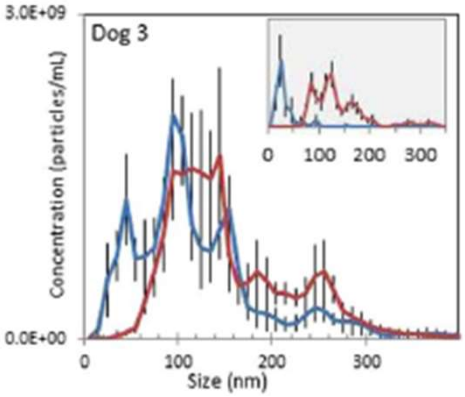
- UV-Vis (A_{260}/A_{280})
  - Concentration, Full/Empty capsid ratio
  - Cheap, rapid, no expertise
  - Poor performance (unresolved bands)
  - Excipient interference
  - 2-state assumption (full or empty)
- Analytical Ultracentrifugation
  - Gold standard
  - HUGE information density
  - Multi-state possible
  - EXPENSIVE
  - High expertise requirements

Figure from: Maruno, et al., *Journal of Pharmaceutical Sciences*, 110 (2021).

Analytical: The Middle

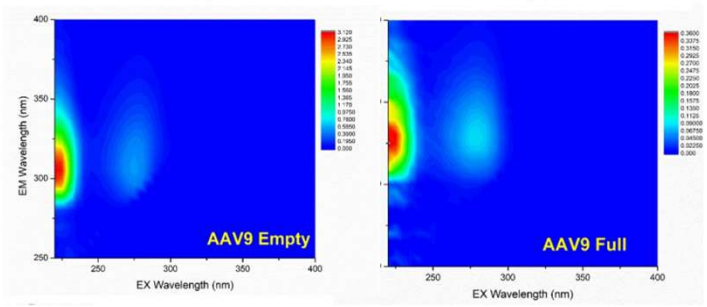


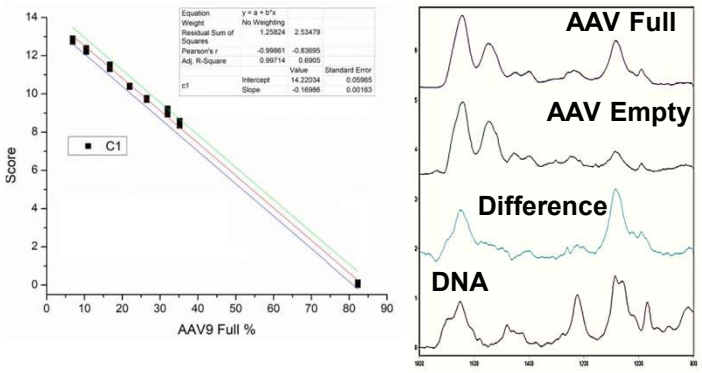
- NTA (number) results show a single LNP mode at 100 nm
  - Counts (conc.) and size down to viral sizes
  - Fluorescence capable for segregation
  - Blue laser systems can go to <50 nm
- NTA struggles with polydispersity
  - Multicolor systems mitigate



DLS/NTA Figure from: Nguyen and Dahl, Webinar (2022).
FL NTA Figure from: Thane, et al., *Scientific Reports*, 9 (2019).

Analytical: The Middle

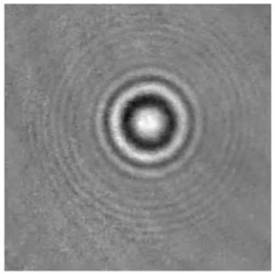


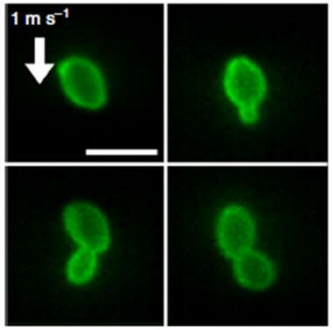


- Multi-dimensional electronic spectroscopy
  - UV-Vis on steroids
  - Excitation-emission shows promise
  - Combined with multivariate method(s)
- Vibrational Spectroscopy
  - Ubiquitous in biotech labs
  - Multivariate method(s)
  - See Pyatski @1530

A-TEEM Figures from: Kidder, *A-TEEM for Vaccines Webinar (*2022).
FTIR Figures from: Pyatski, *Webinar* (2022).

Analytical: What Could Be?





- Interference Techniques
  - Bridge sub-visible and sub-micron
  - Provide count, size, and ‘chemistry’
- Imaging flow cytometry
  - Analogue of Flow Microscopy
  - Chemical sensitivity (label specificity)
  - Provide count, size, and ‘chemistry’

Figure from: Mikami, et al., *Nature Communications*, 11 (2020).

Summary

I SET THEM A-GRAZING IN A BOWLING-GREEN AT GREENWICH

- Nano-particulate delivery systems are a challenge for analytical professionals
  - Tools are evolving
- Information density doesn’t have to be all or nothing
  - Characterization ‘in the middle’
- Keep your eyes on new technologies