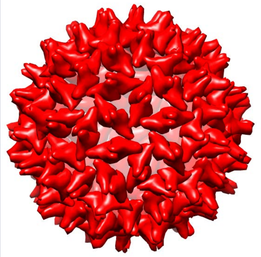

A Rapid Mixing Device to Detect Early Intermediates in Hepatitis B Virus Assembly by Charge Detection Mass Spectrometry

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HBV Capsid Assembly

Charge Detection Mass Spectrometry

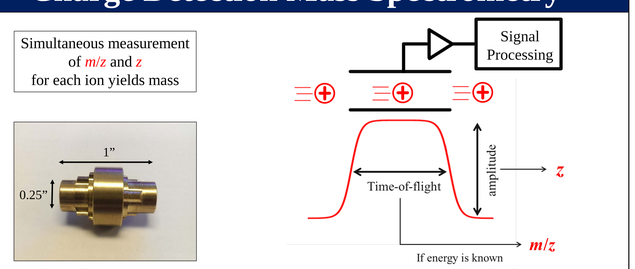


Figure 1. (Left) Photograph of the charge conducting cylinder used in CDMS. (Right) As an ion travels through the charge conducting cylinder, the ion induces a charge that is amplified. The time of flight is used to determine the mass-to-charge ratio (*m/z*) and the amplitude is proportional to the charge (z) of the ion.

Rapid Mixing Device

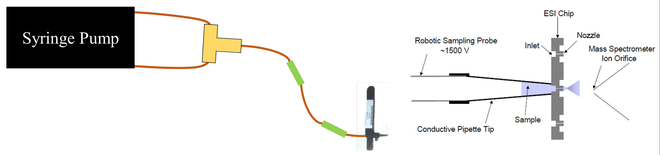


Figure 2. (Left) Schematic of the rapid mixing device that is interfaced with CDMS for these HBV assembly experiments. (Right) Schematic of conductive tip against ESI Chip.[1]

CDMS Instrument Schematic

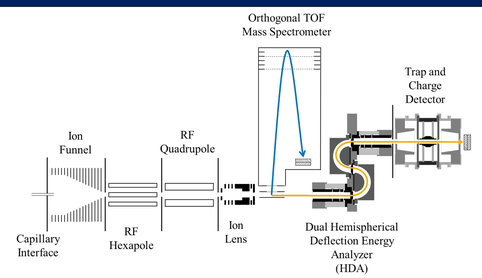


Figure 3. Schematic of the home-built charge detection mass spectrometer.

Slow Reaction Kinetics

1 second

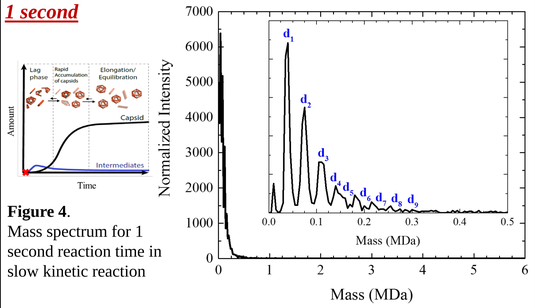


Figure 4. Mass spectrum for 1 second reaction time in slow kinetic reaction

1-90 seconds

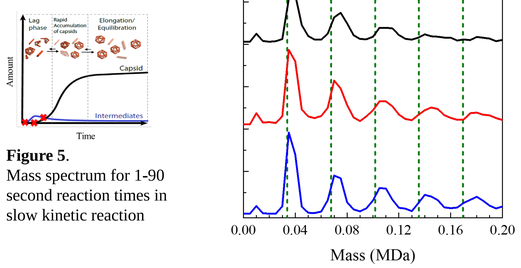


Figure 5. Mass spectrum for 1-90 second reaction times in slow kinetic reaction

120 seconds

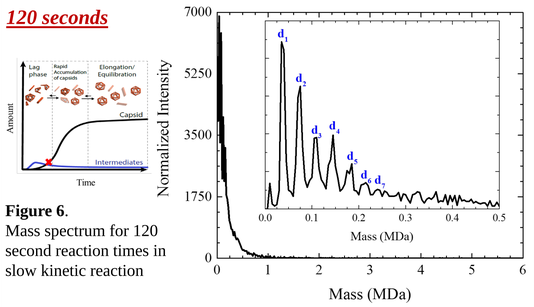


Figure 6. Mass spectrum for 120 second reaction times in slow kinetic reaction

180 seconds

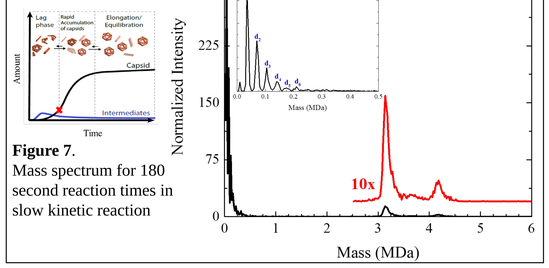


Figure 7. Mass spectrum for 180 second reaction times in slow kinetic reaction

Fast Reaction Kinetics

25 seconds

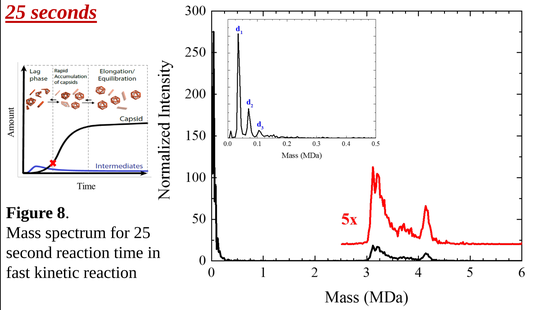


Figure 8. Mass spectrum for 25 second reaction time in fast kinetic reaction

60 seconds

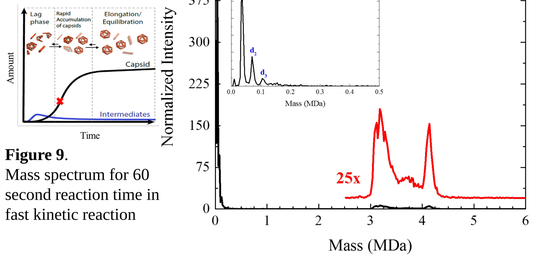


Figure 9. Mass spectrum for 60 second reaction time in fast kinetic reaction

120 seconds

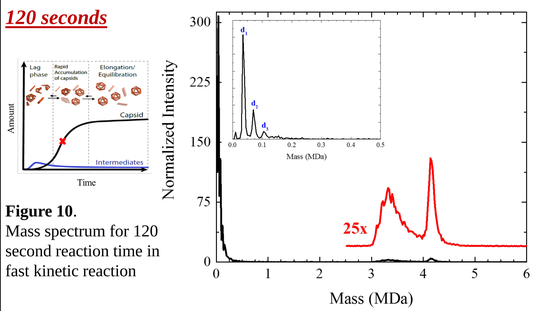


Figure 10. Mass spectrum for 120 second reaction time in fast kinetic reaction

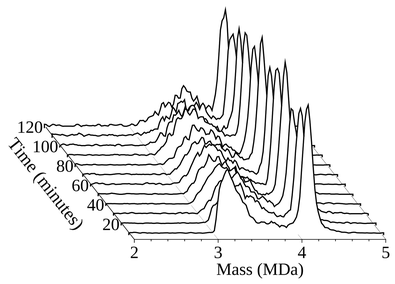
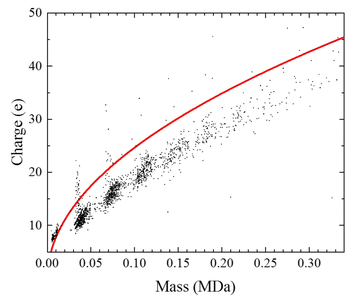


Figure 11. Long term monitoring of HBV assembly. Multiple reactions were averaged and binned into 10 minute intervals (Figure Credit to Corinne Lutomski).

Dual Dimer Conformations



Charge Residue Model

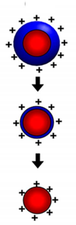


Figure 12. Charge versus mass plot for the 1 second reaction time in slow kinetic reaction. The red trace is the Rayleigh limit. The ions are assumed to charge according to the charge residue model.[2]

HBV Assembly Summary

- HBV assembly kinetics on the sub-second to second timescale were able to be monitored
- One of the few techniques to be able to monitor the lag phase of assembly
- There are consistent trends in the assembly reaction that dictate when capsid will be the majority species
- We can adjust settings (e.g. protein concentration, salt concentration and reaction time) to monitor assembly

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IU Electronic Instrument Services

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1: Triversa Nanomate User Manual
2: Konermann, L.; Ahadi, E.; Rodriguez, A.D.; Vahidi, S. *Anal. Chem.* **2013**, *85*, 2-9.
Kinetics Scheme produced by Dr. Lisa Selzer