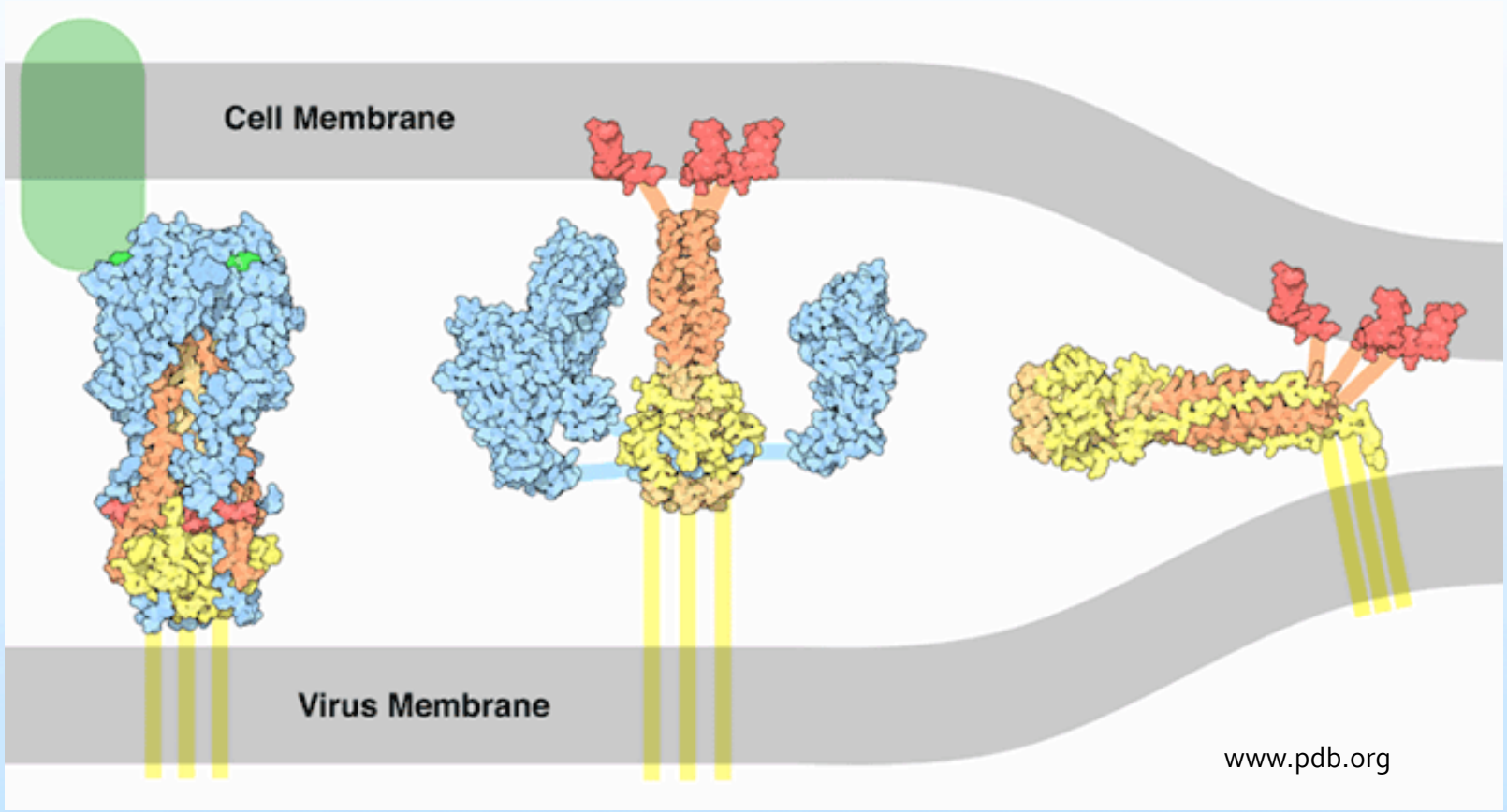
Monitoring fusion of vesicles containing hemagglutinin to endosomes using Förster Resonance Energy Transfer (FRET)



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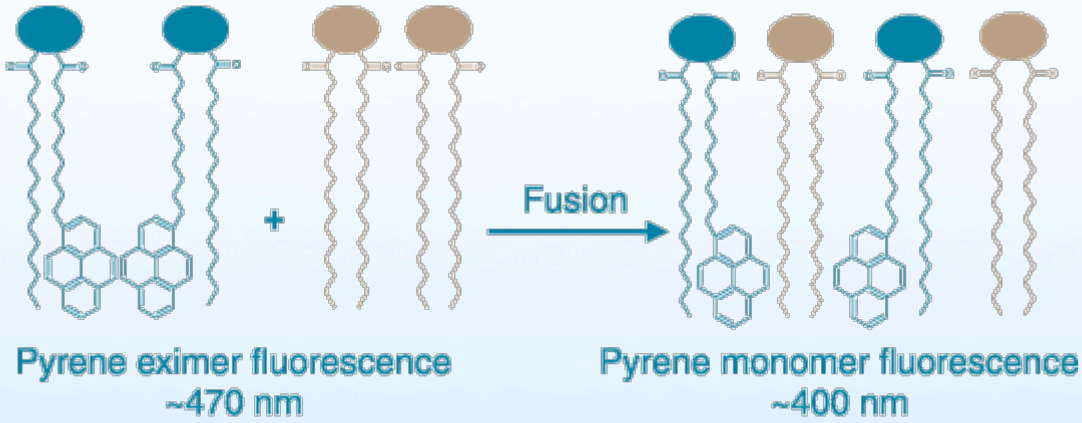
CHE 345 Presentation

Motivation: Efficient Drug Delivery using Hemagglutinin Vesicles

- Difficult to effectively deliver therapeutic drugs to tumor cells
- Cargo-loaded vesicles are endocytosed by endosome
  - Drug needs to get to cytosol.
  - Vesicle must fuse with endosomal membrane to release contents to cytosol.
  - Can get enveloped by lysosome instead, which degrades the vesicle and cargo inside
- Viral fusion protein hemagglutinin (HA) is known to cause the fusion of viral membrane with endosome due to external pH drop, releasing viral load into the cell cytosol.

Using FRET to Determine Vesicle-Endosome Fusion



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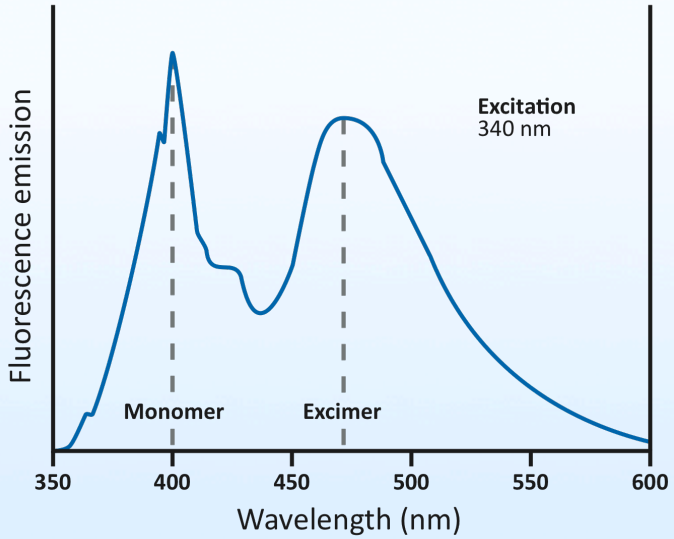
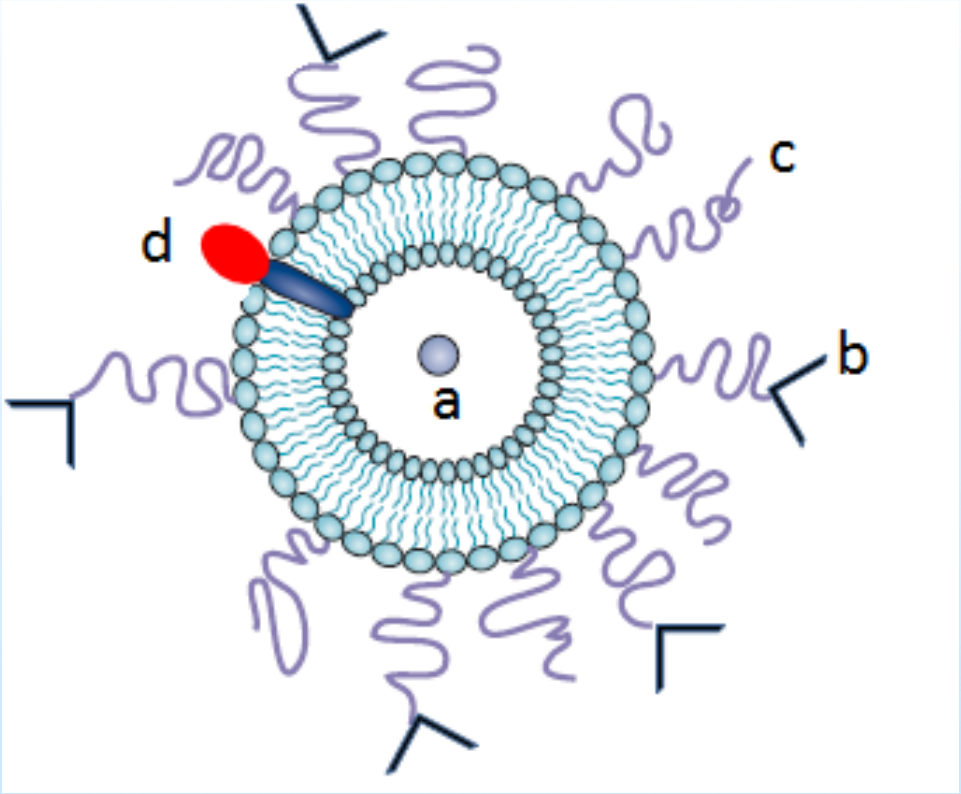


- Measures the proximity of a donor and acceptor fluorophore
- Sample is probed at donor excitation wavelength.
  - If the fluorophores are more than 10 nm apart, only donor emission is detected.
  - If they are close, then donor transfers energy to acceptor, and acceptor emission is detected.
- Pyrene bound to a lipid is both donor and acceptor.
  - When pyrenes are close (within the vesicle), excimer is formed, emitting at 470 nm.
  - When pyrene diffuses after vesicle-endosome fusion, monomers are primarily emitting at 400 nm.

Hemagglutinin Vesicle Composition



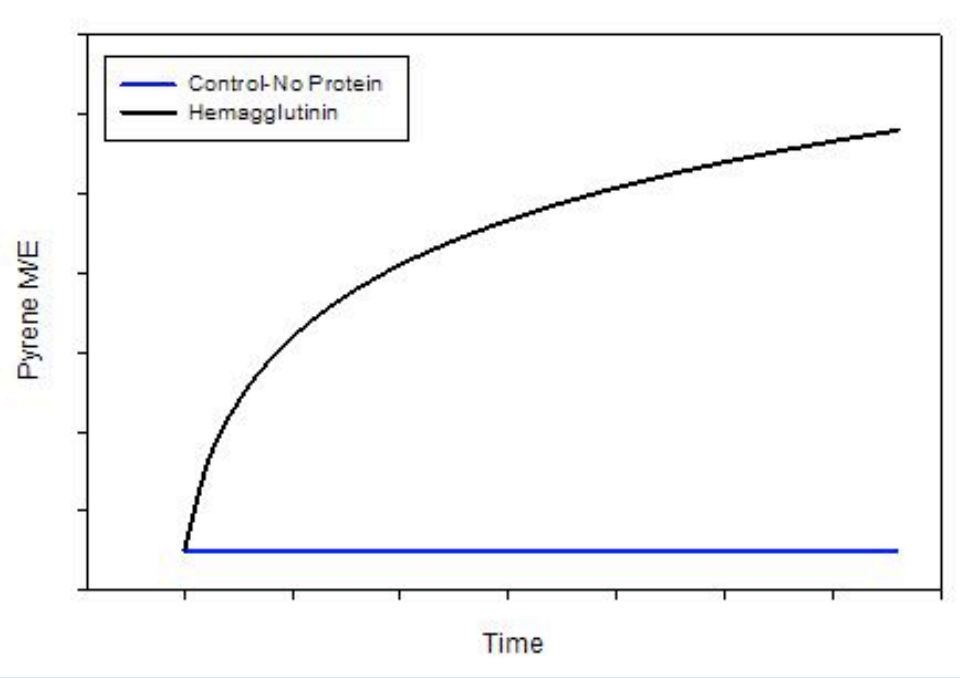
- PEGylated (c) liposome with neutral internal pH
- Lipids are labeled with rhodamine and pyrene.
- Interior contains pH-sensitive fluorophore pyranine (a).
- Hemagglutinin (d) (HA) is incorporated into liposome membrane.
  - Conformation of HA changes with acidity.
  - When inside endosome , exterior pH drops, changing HA conformation.

Experimental Outline

- Using rhodamine and successive centrifugation and washes, determine % vesicles bound to cell surface and % vesicles internalized over time.
- Using pyranine, determine spectra over time for non HA control vesicles being endocytosed by lysosomes and HA-vesicle fusion with endosomes.
- Using pyrene and FRET, measure the ratio of pyrene monomer to excimer (M/E) over time to determine extent of endosomal fusion with HA-vesicles.
  - Also measure the pH of vesicles to ensure delivery to cytosol and not to lysosomes.

Anticipated Results

- M/E Ratio should increase over time, indicating fusion of HA-vesicle with endosome.
- These experiments will allow us to determine if HA is a viable candidate for increased drug delivery efficiency
- Future studies:
  - Compare efficiency of standard liposomes with HA-vesicles using luciferase assay
  - Add functionalized groups to outer surface to increase specificity to cancer cells
  - *In vivo* mouse studies for tumor treatment



Questions?