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Quantifying protein loading in individual mesoporous silica nanoparticles

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Abstract

Mesoporous silica nanoparticles have gained popularity over the past decade as vehicles for the delivery of therapeutic proteins. However accurate quantification of protein loading on a per particle basis remains lacking, hindering full understanding of delivery efficiency. In an effort to develop more robust quantification approaches we are developing a microscopy based analytical method for assessing loading of a fluorescently labeled protein into mesoporous silica nanoparticles cores.

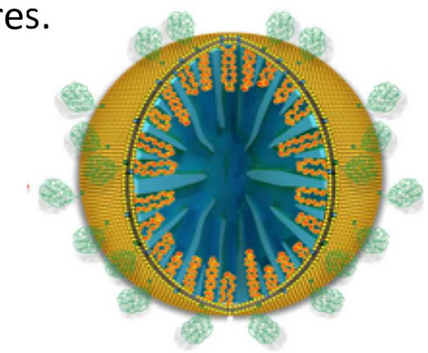


Image 1: A graphical representation of a mesoporous silica nanoparticle

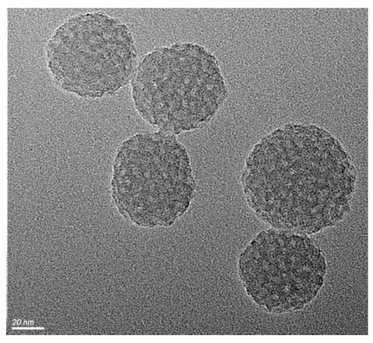


Image 2; A TEM image of mesoporous silica nanoparticles, specifically AK2

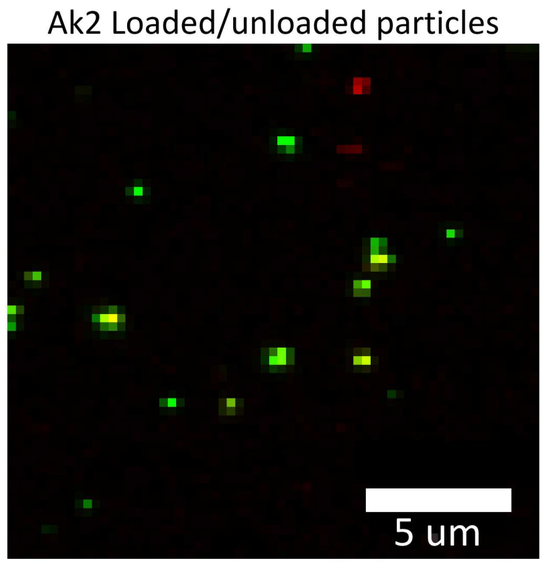
Experiment

- Investigating two MSN core types, Ak2 and PD43
- These two cores have been fluorescently labeled with Cy3
- Cy-5 labeled ACR protein is loaded in cores, which are then coated with lipid bi-layer
- Different loading ratios and mixtures of particles are studied for method development

Results

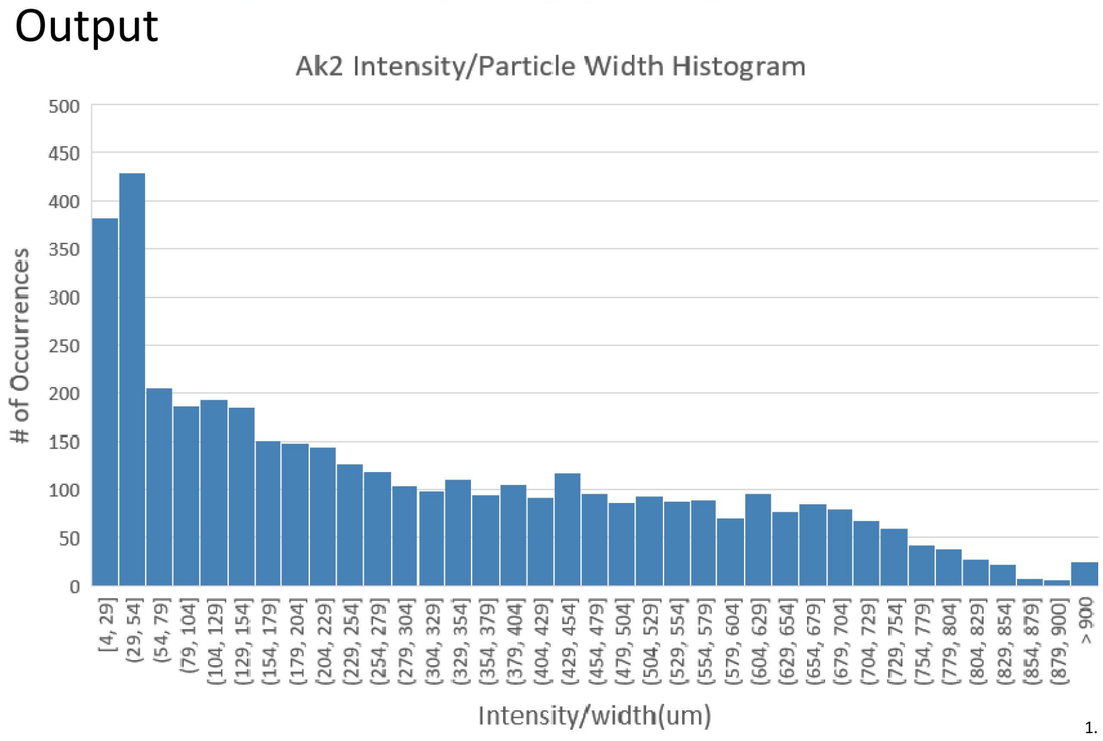
Ak2 Loaded/unloaded particles

Input



Output

Image 3 : Unloaded Ak2 particles are green, loaded particles are yellow, and unloaded ACR is red



Background

- Mesoporous silica nanoparticles are nanoparticles that are made from silica and contain pores with diameters of 2-50 nm.
- Mesoporous silica nanoparticles are promising nanoparticle delivery vehicles, due to their high stability, easy synthesis, and simplistic surface functionalization
- Current methods for assessing protein loading rely on bulk measurements of particle populations, hampering full pharmacokinetic characterization of protein delivery
- We are utilizing microscopy and single particle image analysis to compute particle loading on a per particle basis

Methods

Particle Synthesis
Incubate protein with MSN cores, add lipid, sonicate and wash[1]

Slide preparation
Mix loaded particles with Prolong Gold mounting reagent, add coverslip and dry overnight

Microscopy
Acquired confocal image stacks of 30 different areas on the slide

Image Preprocessing
- Select 3 most in focus slices from each image
- Verify files are in pairs – one for each channel

Particle Segmentation
- Perform background subtraction
- Select particles based on Core Channel image
- Project those positions to the ACR image
- Calculate ACR intensities for that particle

Single Particle Statistics
- X and Y positions
- Core diameter
- Core intensity
- Total intensity (5x5 pixel area around particle)

Discussion

- Accurately and reliably quantifying protein loading for individual nanoparticles will improve pharmacokinetic characterization for protein delivery
- We have developed a quantitative method that uses microscopy and image analysis to extract a mathematical relationship between the intensity of a fluorescently labeled protein and the amount of protein present
- Future work will use images from pure fluorescently labeled protein to create a calibration curve that relates the intensity per pixel of the protein to the number of molecules of protein. With this calibration we can determine the amount of protein present inside an individual nanoparticle

References

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