Nanoparticle development for molecular imaging and drug delivery

Four specific aims were outlined for this two year project. Aim 1 sought to develop new ligands to bridge nanoparticles together for quantitative molecular imaging. Novel materials developed in this aim were targeted towards enhancing the properties of gold and silver nanoparticles to better facilitate their use in molecular imaging. The second aim was targeted towards understanding the behaviour of copper-binding proteins on the surface of neurons. Such proteins play an important role in signalling in the brain and have been implicated in neurological disorders. The third aim of this project was to use carbohydrates to coat the surface nanoparticles and to incorporate photo-cleavable linkers that could then release carbohydrates or other molecules on the surface of the nanoparticles in a controlled manner. The last aim was to determine if such carbohydrate-coated nanoparticles could interact with the surfaces or viral particles using carbohydrate-carbohydrate interactions.

Specific Aim 1: Develop new ligands that bridge nanoparticles together for quantitative molecular imaging. The initial synthesis of the linker ligands that were proposed was completed on time and analysis of clusters that resulted from binding to nanoparticles was performed. The initial results were inconclusive and as a result, conditions for the nanoparticle clustering were further optimized. New methodology for clustering nanoparticles was envisioned using small magnetic particles as opposed to bridging ligands. This approach should allow for easier separation of clusters from solution but result in larger aggregates of nanoparticles. Binding of silver particles to the surface of magnetic particles has been successful, however, control of aggregation has proved challenging and to date, aggregates that have formed have been too large for molecular imaging and their stability has been difficult ot control in an aqueous environment. Current efforts are now focussed on stabilizing the initial magnetic cores in solution as is appears that this is the primary challenge in synthesizing monodisperse rationally designed nanoparticle clusters for the newly designed targets of this aim.

Specific Aim 2: Use nanoparticles to probe the interactions between signalling proteins in the brain. Here a major modification to the project resulted in using fluorescent proteins instead of nanoparticles to study the effects of copper on protein localization; however, these studies have also had the greatest impact in scientific discovery of the four aims to date. Here we studied the effect of low doses of copper complexes on the localization of three proteins – the copper uptake protein hCTR1, amyloid precursor protein and prion protein. Cleavage of amyloid precursor protein results in beta-amyloid which is the primary component of plaques found in the brains of patients with Alzheimer's disease, while misfolding of the prion protein is responsible for Creutzfeldt–Jakob disease. Both proteins bind copper and misregulation of copper binding appears to be important the progression of both diseases. Our studies found that despite some reports which state that prion protein is internalized upon the addition of copper to the extracellular environment, that at physiologically relevant concentrations of copper, the levels of prion protein on the cell surface actually increase by up to an order of magnitude. This is an important finding as previous studies had used much larger doses of copper and forms of copper complexes that would not be present in the brain and thus observed trends that may not be consistent with those that actually occur in the a neurological environment. We attempted to reproduce conditions similar to what might be found in a normal brain and found very noticeable changes in protein numbers of localization at the surface. In addition to an increase in the number of proteins on the surface, it was also noted that while before adding copper, proteins were found in localized domains, after the addition of copper, proteins were found to be diffused across the entire cellular surface (Figure 1). This result suggest that the addition of copper may be disrupting interactions between prion protein and other components of the cell surface.

Specific Aim 3: Develop glycosylated nanoparticles with photo-cleavable linkers for drug delivery and proteomic studies. For Aim 3 completion of the initial photo-cleavable linker was achieved ahead of schedule, however, it was found to be incompatible with the functionalization chemistry to attach it to the nanoparticle surface. A new molecule was then designed and this molecule has been synthesized and is now being used to functionalize nanoparticles. Additionally, new particles that can upconvert light to shorter wavelengths have been synthesized and will be used in the development of the photochemical assays for drug release. The choice of drug candidate has been changed from cisplatin as originally proposed to a carbohydrate based on other work to develop novel vaccine candidates in the research group. The stabilization of these particles in aqueous media has proved challenging and is still an ongoing area of focus for this aim. Additionally carbohydrates have been used to

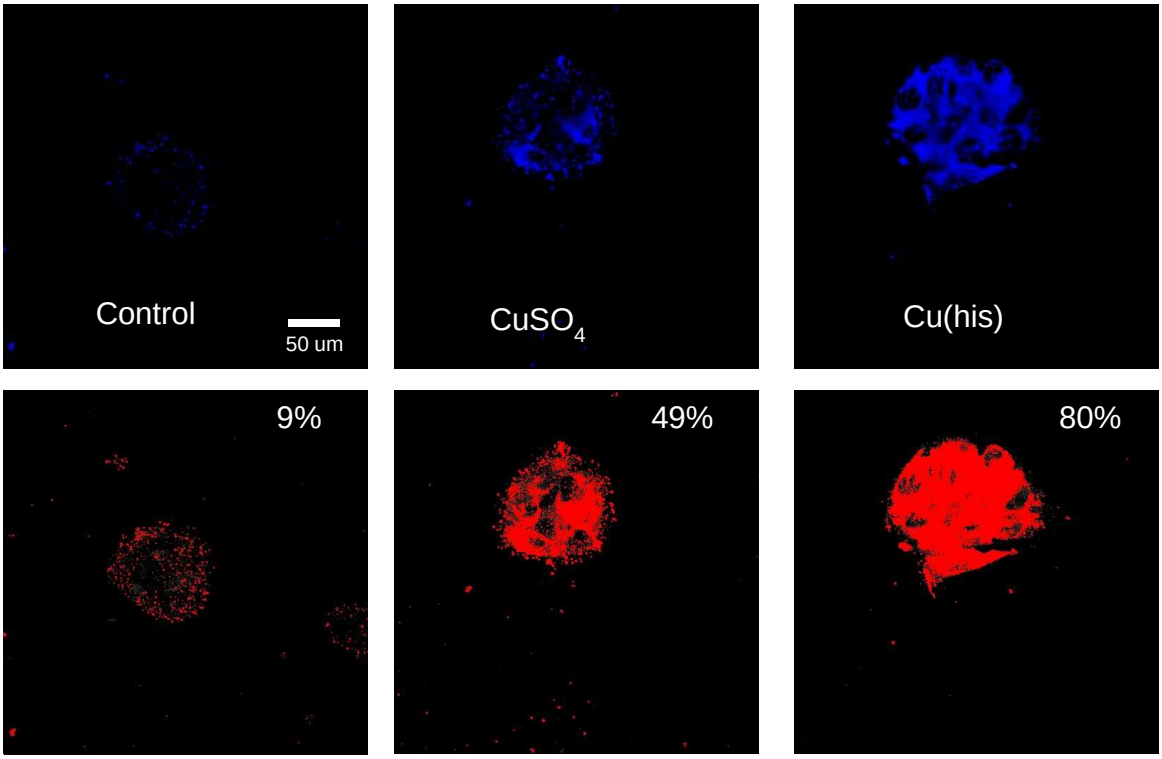

Figure 1. HepG2 liver cells treated with 25 uM copper for 1 h. Blue colour in top row shows fluorescence from antibody staining for prion protein using a FITC conjugated secondary antibody. The middle and right image show the difference in effect the form of copper has on the change in localization. Below, analysis using pixel counting in ImageJ quantifies the increase in fluorescence at the cell membrane. Percentages indicate the relative surface area of the cells exhibiting fluorescence from labelled prion protein.

functionalize both silver nanoparticles and CdSe/ZNS quantum dots. New procedures for producing stable particles with surface carbohydrates have been developed and the numbers of sugars on the surface have been quantified. Furthermore we have tested the toxicity of these particles against both neurons and liver cells and found that the identity of the sugar is critically important for particle stability, uptake and toxicity. We are currently examining whether this is a result of direct interactions with biomolecules on the surface of cells or whether simple properites such as differences in particle charge might be resulting in these observed biological differences.

Specific Aim 4: Use glycan functionalized nanoparticles to detect viral particles and inhibit viral entry. Aim 4 was started ahead of schedule. Glycan functionalized nanoparticles have been developed and characterized as described in specific aim 3. We have performed assays that show that sugar-binding lectins such as Concanavalin A can specifically bind glucose coated nanoparticles compared to galactose-coated particles. This work was carried out using an aggregation assay where glucose-coated particles aggregated in the presence of ConA while galactose-coated particles did not. This aggregation arises from the sugar-binding protein having multiple binding sites and thus bridging particles together resulting in large aggregates that precipitate from solution. This then results in a corresponding decrease in the absorbance of visible light in the solution because the concentration of particles in solution decreases. We have not yet found a suitable carbohydrate coating that can detect viral proteins, however, we are continuing to screen such ligands and can then use the absorbance assay to probe the effectiveness of different carbohydrates with similar structures to the initial candidate.