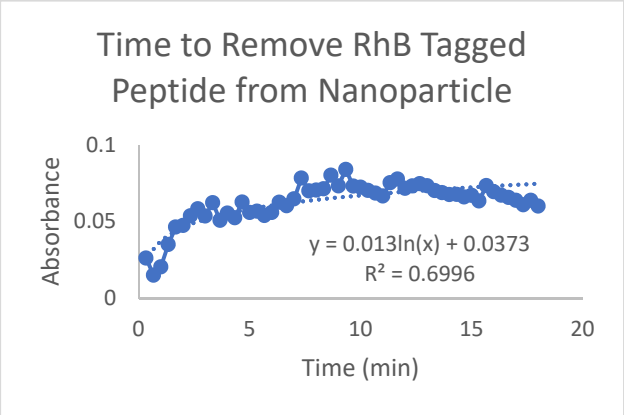
A diagnostic platform for the early detection of multiple types of cancer is being developed. This platform will use a nanobiosensor to measure enzyme activity in biological samples. The biosensor consists of a peptide substrate that a specific enzyme will cleave or phosphorylate attached to a magnetic nanoparticle though a disulfide bond. The nanosesor can be added to a biological sample and later removed using the magnetic nanoparticle handle. The substrate reporter can then be removed from the magnetic nanoparticle through reduction of the disulfide bond. The ratio of the peptide in its original state versus the cleaved/phosphorylated state can then be used to determine enzymatic activity in the sample. This specific project focused on studying the efficiency of the disulfide reduction. The rate and completeness of the reduction was measured using a rhodamine tagged peptide attached to iron/iron oxide nanoparticles. Tris-hydroxypropyl phosphine (THPP) was used to reduce the disulfide bond in a Tris-HCl pH 8.8 buffer.

In one milliliter of reaction solution This reduction experiment was carried out in a cuvette and measured using a spectrophotometer. The nanoparticles were held on the side of the cuvette using rare earth magnets. Mixing was achieved with a pipet which would be removed every thirty seconds so the spectrophotometer could record the absorbance of the solution at 532 nm. As the rhodamine labeled peptide was released from the nanoparticle through the deduction of the disulfide bond the absorbance increased.




With constant flow of solution over the magnetic nanoparticles approximately 80% of the peptide was removed from the nanoparticle in 2.3 minutes. THPP was shown to be a novel, quick acting reducing reagent that can be used to irreversibly reduce disulfide bonds.