EXAMINING THE BINDING SITES OF BOVINE SERUM ALBUMIN AND INDOCYANINE GREEN DYE

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Introduction:
When radiated, indocyanine green (ICG) dye fluoresces (Figure 1). This fluorescence allows for infrared imaging detection within the human body. By using photoluminescence scanning to measure the fluorescence of ICG when bound to bovine serum albumin (BSA) and differential scanning calorimetry and isothermal titration calorimetry to study the thermodynamic parameters of interactions of BSA with ICG in solution, a model representing the same effects in the human body can be created.

Materials and Methods:
Bovine serum albumin is an albumin derived from cows that is the most abundant plasma protein in mammals. It acts as a carrier and transport protein by binding dyes\textsuperscript{2}. Indocyanine green dye (ICG) is a dye used for medical imaging. When bound to BSA, ICG becomes a fluorophore that can re-emit light upon excitation. The fluorescence of a solution of BSA (8.4E-5 M) and ICG (varying concentrations from 1.0E-7 to 1.6E-6 M) is measured with photoluminescence detectors. The thermodynamic parameters of a solution of BSA (.5 mM) and titrations of ICG (.05 mM) are measured through isothermal titration calorimetry\textsuperscript{3} and differential scanning calorimetry\textsuperscript{4}.

Results and Discussion:
The relative fluorescence as a function of ICG dye concentration excited at 279 nm in a BSA solution (8.4E-5 M) produces a slope trend line that corresponds to the binding constant of BSA and ICG. That binding constant is 3.25E-5 M\textsuperscript{-1} which validates previous studies\textsuperscript{5}. The fluorophores exhibited quenching of the albumin emission. This is shown in Figure 2. The thermodynamic parameters of BSA (.5 mM) titrated into an ICG solution (.05 mM) are shown in Figure 3. The curve of the peak areas as a function of the total volume injected shows the heat released from the binding of BSA with ICG. The plateau of the curve shows that the heat reactions from the binding of ICG to BSA have ceased.

The fluorescent experiments yielded favorable results regarding the binding constant of the order of magnitude
10^5. The binding constant of 10^{-7} represented in the isothermal titration calorimetry experiment between BSA and ICG is still preliminary and will need further experiments. Future studies in isothermal titration may lead to varying concentrations of ICG and BSA to find a binding constant corresponding to that of the fluorescence results.

**Conclusions:**
The fluorescence results are conclusive that the binding constant of ICG and BSA is on the order of 10^5 magnitude. The titration results of BSA into ICG and its ligand binding are still preliminary. However, the results will be considered for further experiments. These preliminary experiments testing the binding sites of BSA and ICG have great value in the realm of cancer research. Every small experiment is one step closer to a better and more effective treatment to cancer.

**Acknowledgements:**
The authors would like to acknowledge their funding from the National Science Foundation (DMR 1062873).

**References:**