Fluorescence Detection

Supramolecular self-assembly for chem-bio sensors

Overview

Chem-bio-helices straddle the chemical-biological divide and play diverse roles in a variety of scientific disciplines including chemistry, biology and physics. Chem-bio helices illustrate an ordered, 3-dimensional geometry observed with several different molecular entities, both natural and synthetic. The role of biological helices such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) is well known. In addition, proteins (such as collagen) and carbohydrates (example, hyaluronic acid) with helical structures participate in key biological processes such as signal transduction, cartilage formation, joints lubrication and host-pathogen interactions. Helical structures are also prominent in the chemical arena, for example, the carbon nanotube.

Recently certain cyanine dyes were shown to form tight complexes with polymeric carbohydrates such as carboxymethylamylose (CMA), carboxymethyl cellulose (CMC) and hyaluronic acid (HA), resulting in intensely fluorescent *J*-aggregates as shown in Figure 1 with HA as the exemplar.

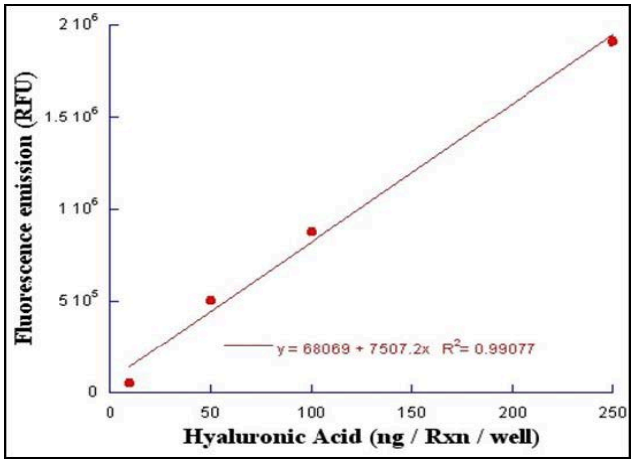

Figure 1. Bead 1.

Molecular aggregation occurred via cooperative self-assembly in which both the cyanine dye and the HA undergo conformational changes, wherein the aggregation of the cyanine dye drives a change in the chiral HA polymer to adopt a supramolecular helical structure. Reversible J-aggregation process (due to formation and disruption and destruction of the HA scaffold and the ensuing fluorescence changes were used to develop enzyme assays (Figure 2):

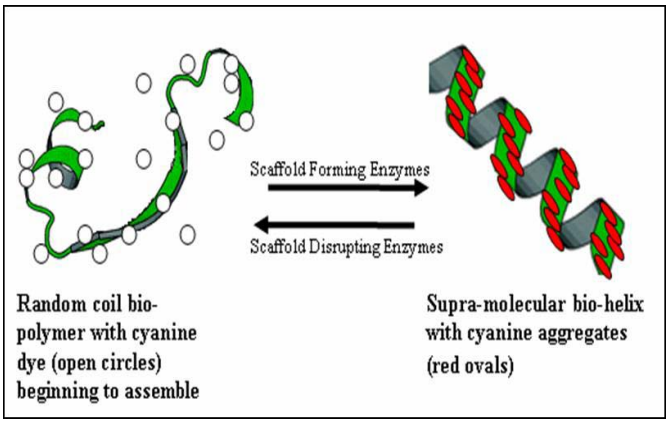

Figure 2. Supramolecular 1.

An example of the supramolecular self-assembly, reversible *J*-aggregation leading to useful assays is illustrated below with an activity assay for the enzyme Hyaluronidase.

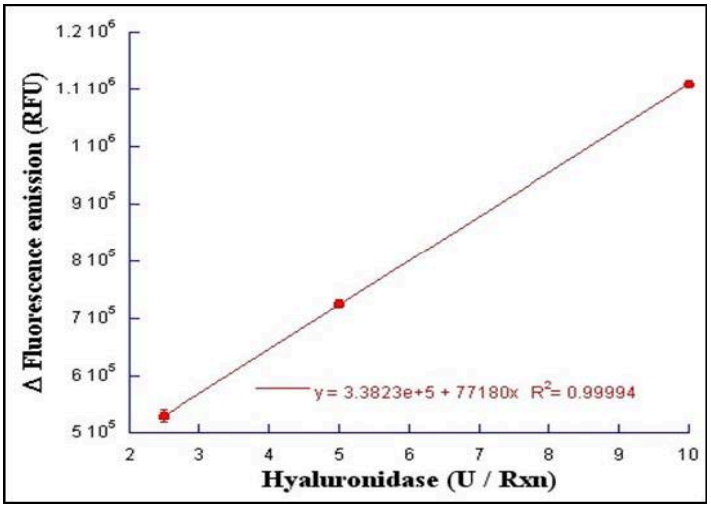

Figure 3. Activity assay for the enzyme Hyaluronidase.

These assays can be carried out in a *label-free* mode and thus do not involve chemically modifying either the substrate or the enzyme. Consequently, the enzymatic reactions can be screened in a high throughput screening (HTS) fashion, utilizing substrates in their natural conformational states, thus providing a greater degree of confidence regarding the validity of the data to mimic *in vivo* conditions. This novel fluorescence-based sensing technology is useful to detect, with high sensitivity, a variety of chem-bio-helices that have interesting geometries. The technology is capable of detecting fmol amounts of a specific analyte such as an infectious agent at its minimal infectivity dose, and pM concentrations of an enzyme inhibitor or a drug candidate during HTS applications.

An optical sensing system was thus developed that has potential applications for the high-throughput screening (HTS) of a broad range of biological molecules, whole cells, organisms and pathogens. The technology applications were illustrated by a HTS hyaluronidase enzyme activity assay. At the core of this technology, is the exciton concept that is relevant to molecular aggregation. J-aggregates of cyanine dyes have a narrower, red-shifted absorption band compared to monomer. Self-assembly may be driven by the helicogenic nature of the cyanine dye, converting the linear polymers of hyaluronic acid or carboxymethyl cellulose into supramolecular helical assemblies. This self-assembly is accompanied by an intense, sharp, red-shifted J-aggregate fluorescence. This property was utilized to develop an assay for the enzyme hyaluronidase, based upon the concept of "scaffold destruction," whereby the disruption/destruction of the hyaluronic acid polymer by hyaluronidase is accompanied by an attenuation of light emission from the J-aggregate. The extent of light attenuation provides an index of hyaluronidase activity. Other polymers of carbohydrates, proteins, nucleic acids and chemical polymers (such as the carbon nanotube) might provide a similar scaffold for helicogenic dyes upon which molecular aggregation can occur. A key feature of these assays is that they are label-free.

For additional information or questions, please email us at BioNano@sandia.gov.

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