Poster

THE USE OF QUANTUM DOTS IN NOVEL TWO PHOTON EXCITATION FLUORESCENCE CROSS CORRELATION SPECTROSCOPY LIGAND BINDING ASSAYS

Jody L. Swift[1], Romey Heuff[1], Tanya E.S. Dahms[2] & David T. Cramb[1]
[1]Department of Chemistry, University of Calgary, Alberta Canada
[2]Department of Chemistry and Biochemistry, University of Regina, Saskatchewan Canada
jlswift@ucalgary.ca, dcramb@ucalgary.ca

A significant goal in the application of nanoscience to medicine is to develop novel biomimetic systems that can be used to model biological responses at the molecular level. It is only through understanding disease at this level that rational molecular therapeutic advances can be made. Since a large fraction of a cell's response to its environment begins with the binding of a ligand to a membrane protein receptor, an objective of this research is to develop technology essential in examining model systems at the nanometer scale. This is especially true for G-protein coupled receptors (GPCRs). The challenge of such assays is establishing a fast and reliable technology that can monitor the desired binding events potentially in a sea of background biochemistry. For traditional assays, large backgrounds arising from high levels of autofluorescence from the sample result in high instances of false positive tests. Additionally, many fluorescence assays report on secondary effects of ligand receptor interaction, such as increases in intracellular signalling molecules, rather than direct ligand binding.

Quantum dot (QD) based fluorescence cross-correlation spectroscopy (XCS) can be utilized to address the limitations of current fluorescence based high context screening, allowing for direct detection of ligand-receptor interactions. In XCS interacting species are labelled with fluorophores of different and non-overlapping spectra. Two–photon excitation (TPE) enables the simultaneous excitation of both fluorophores using a single wavelength. Emission from the sample is spectrally separated and collected simultaneously in two separate detection channels. A cross–correlation signal is obtained only when the two distinctly labelled species are physically linked, and the uncorrelated signal (autofluorescence) will time average to zero. The XCS signal will be diminished if the two molecules are dissociated or removed from the field of detection by any process. Thus, XCS is perfectly suited for tracking the dynamics of dimerized (and/or oligomerized) species in a sea of monomers. The advantages offered by this state-of-the-art technique are essential for our studies of intermolecular interaction dynamics.

In order for TPE – XCS to be used for ligand binding assays it is desirable to ensure that relevant kinetic information can be extracted from the experimental data. There are a growing number of examples of correlation spectroscopy used in the examination of ligand binding. These studies have largely examined binding indirectly through quantifying the loss of free fluorescently labeled ligand in the presence of the receptor both in solution and in living cells. However, these assays infer ligand binding by monitoring drastic changes in ligand diffusion behavior. In fact, it is only possible to discern binding if the relative molecular weight of the bound ligand is 4 – 8 times greater than the free ligand. We present a simple alternative approach which employs an extension to the Hill – Langmuir theorem allows us to quantify ligand – receptor interactions using standard pharmacological parameters including percent occupancy and equilibrium constants (K_d) which can be easily obtained from a combination of FCS and XCS data.

One obstacle which limits our ability to accurately examine ligand binding interactions is the limited amount of information which can be obtained at low ligand concentrations. Due to the small excitation volume used in TPE – XCS it is theoretically possible to work at the single molecule level and thus obtain very accurate binding information in these traditionally unattainable regions. However, many commercially available organic dyes have poor two photon cross sections and quantum yields. As a result in order to fully actualize the potential of TPE – XCS for sensitive, accurate ligand binding assays we have employed the use of conjugated quantum dots as alternatives to conventional fluorophores labels.

Luminescent semiconductor nanocrystals, or quantum dots have many potential advantages in the development of TPE-XCS-based ligand-receptor binding assays. QDs are beginning to be examined as possible fluorescent label for multicolour imaging in biological samples [13AG]. The major advantages of QDs for imaging are: broad excitation spectra; narrow Gaussian emission spectra; low photobleaching yield; and large brightness. Additionally, colloidal quantum dots (nanocrystals) have several features motivating their use as fluorescence labels for multiphoton imaging in biological systems. Firstly, the particles themselves have gigantic two-photon absorption cross sections – vastly greater than exiting labels [9AG]. Secondly, the structural organization of individual colloidal particles enables them to be made compatible with a variety of environments and biofunctionalized for nanosensor and fluorescence tagging applications [14-22AG]. The key to these applications is that the surface of the nanocrystal can be modified to make it water soluble, then silica-coated and functionalised such that biotin can be bound to the surface. The resulting nanobioconjugates can be coupled to, for example, biotin or streptavidin.

We have successfully shown that biotinylated and Streptavidin conjugated quantum dots could be used as a simple ligand binding system. Using our modified FCS/XCS approach to conventional Hill – Langmuir theory we were able to extract relevant binding information and equilibrium constants for this simple system. Recent developments will be described illustrating the suitability of TPE–XCS for direct detection of ligand – receptor interactions, and the essential role that quantum dots play in increasing the sensitivity of this detection method. We will show that it is also possible to characterize and quantify ligand – receptor interactions for more complex systems including GPCRs.

Extensions of the binding assay are applied to the human mu opioid receptor (hMOR). This GPCR is of great interest in the pharmaceutical industry as a target for pain control. This class of receptors are often difficult to purify in high yields and because of their transmembrane structure require a lipid matrix to maintain structure and function. The necessary lipid matrix associated with GPCRs may result in high non – specific binding as most hydrophobic organic dyes used to label the potential ligands will partition into the membrane bilayer. We have shown this to be large problem with the commercial dye fluorescein. We propose that it is possible to utilize quantum dots to label ligands for the human mu opioid receptor. Specifically we will show that it is possible to attach a biotinylated enkephalin (endogenous ligand) to a streptavidin coated quantum dot. This method will both increase the sensitivity of the assay by allowing us to examine binding which occurs at concentrations as low as 0.5 nM, and reduce the problems of non – specific binding.

References:

1. K.G. Heinze et al, *PNAS* 2000, 97, 10377.
2. I. S. Zuhorn and D. Hoekstra. *J Membr Biol.* 2002, 189, 167.
3. P. Mitchell, *Nature Biotech.* 2003, 21, 233
4. C. Wingren et. al., *Nature Biotech.* 2003, 21, 247.
5. C. Taylor et. al., *Nature Biotech.* 2003, 21, 223
6. C.C. Wu and J.R. Yates III, *Nature Biotech.* 2003, 21, 262.
7. Z. Zhang et. al, *Anal. Chem.* 2000, 72, 318.
8. K.M. Berland et al, *Biophys. J.* 1995, 68, 694.