Progress toward Barium Tagging in High Pressure Xenon Gas with Single Molecule Fluorescence Imaging

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Abstract. We present an update on the development of techniques to adapt Single Molecule Fluorescent Imaging for the tagging of individual barium ions in high pressure xenon gas detectors, with the goal of realizing a background-free neutrinoless double beta decay technology. Previously reported progress is reviewed, including the recent demonstration of single barium dication sensitivity using SMFI. We then describe two important advances: 1) the development of a new class of custom barium sensing fluorescent dyes, which exhibit a significantly stronger response to barium than commercial calcium sensing compounds in aqueous solution; 2) the first demonstration of a dry-phase chemosensor for barium ions. This proceeding documents work presented at the 9th Symposium on Large TPCs for Rare Event Detection in Paris, France.

1. Barium Tagging in High Pressure Xenon Gas with SMFI
A convincing detection of neutrinoless double beta decay (0νββ) would demonstrate that the neutrino is its own antiparticle. Such a discovery would prove that the standard model is a low energy effective theory; demonstrate a new mechanism of mass generation that explains the disparity of charged and neutral lepton mass scales; prove that lepton number is not a symmetry of nature; and lend weight to leptogenesis as a viable explanation for the matter-antimatter asymmetry of the Universe. The search for 0νββ is accordingly considered to be a top scientific priority in nuclear and particle physics worldwide.

To realize half-life sensitivities nearing 10^{-28} yr, an ideal experiment would be a fully active, ultra-low background, ton-scale or larger detector with strong positive signal criteria capable of providing an unambiguous discovery claim. For detectors using the isotope 136Xe, it has long been recognized that efficient and selective detection of the daughter nucleus 136Ba, in coincidence with electron energy measurements of precision better than \sim 2% FWHM to reject the two-neutrino double beta decay background, would represent such a technology.

R&D on techniques for “barium tagging” has been pursued actively for both liquid and gaseous xenon experiments for at least 17 years [1]. Several single-atom or single-ion-sensitive detection methods exist, emerging from various disciplines in physics and chemistry. The
Figure 1. Left: TIRFM image showing individual Ba$^{++}$ ions near the sensor surface; Right: The time profile of a single spot, showing the discrete photo-bleaching process that is the hallmark of single molecule fluorescence. Reproduced from [14].

question facing the field is: can any of these techniques be implemented with high efficiency, in a liquid or gaseous xenon environment?

An important consideration when assessing barium tagging approaches concerns the charge state of the daughter nucleus. Barium from double beta decay is born in a highly ionized state Ba$^{N+}$ that quickly captures electrons from neutral Xe until further capture is energetically disfavored, stopping at Ba$^{++}$. In liquid xenon, recombination with locally thermalized electrons then produces further neutralization, producing an ensemble of ionic and atomic species including Ba and Ba$^+$. The lack of recombination in gaseous xenon, on the other hand, implies that Ba$^{++}$ will be the dominant outcome [3]. Distinct technologies thus appear optimal for these two cases.

Barium tagging approaches for liquid xenon [4, 5] have traditionally focused on cycling fluorescence transitions of the outer electron in Ba$^+$. Ba$^{++}$, on the other hand, has a noble-like electron configuration without low-lying fluorescence transitions. To detect Ba$^{++}$ using fluorescence techniques it is necessary to add such transitions artificially. A method for achieving this was proposed in [6] and further developed in [7], using the technique of Single Molecule Fluorescence Imaging (SMFI). This method is widely used in biochemical microscopy for sensing Ca$^{++}$ ions in biological media [8–13]. In SMFI, a specially designed molecule is employed that is non-fluorescent in isolation but becomes fluorescent upon chelation of a target ion. The fluorescence enabled upon binding can be observed by probing with an excitation laser and collecting the longer wavelength fluorescence emission using highly sensitive EM-CCD cameras.

The NEXT collaboration has been developing SMFI-based barium tagging since 2015. In early exploratory work, we demonstrated that commercially available molecules FLUO-3 and FLUO-4, designed for Ca$^{++}$ detection, are sensitive probes for Ba$^{++}$, thus making them promising barium tagging agents [7]. Using total internal fluorescence microscopy [15] (TIRFM) with FLUO-3, the first single barium dication fluorescence detection was then demonstrated [14]. Individual ions were spatially resolved with 2 nm super-resolution and identified with 13 $\sigma$ statistical significance over background via their sharp photo-bleaching transitions (Fig. 1).

We have also studied the drift and survival properties of the dication state in high pressure
Figure 2. Wet and dry response of various fluors that may be used in SMFI molecules.

Xe gas theoretically [16] and this is the subject of an ongoing experimental program. Also ongoing are the development of ion concentration methodologies at the cathode and creation of dry-compatible fluorophores for ion imaging in Xe gas.

2. Requirements for a Dry-Phase SMFI Agent

Fluorophores FLUO-3 and FLUO-4 comprise of a BAPTA-like receptor bonded onto a fluorescein-like fluor, shown in Fig 3, left. The on-off fluorescence response in the FLUO family is understood to arise from quenching of the fluorescein group, presumably by photoinduced electron transfer from the lone pair of electrons on nitrogen. When unbound to an ion, these electrons may move freely into the fluorescein group to inhibit fluorescence. In the presence of a Ba\(^{++}\), however, the BAPTA-like receptor binds the ion in a cage-like structure, with its electrostatic force pulling the nitrogen electrons into a bonding-type configuration described as Lewis acid-base complexation. This inhibits the movement of nitrogen’s lone pair of electrons into the fluorescein group, and prevents the fluorescent response from being quenched.

The FLUO family of chemosensors is typically deployed in aqueous solutions. For example, our previous work [14] used FLUO-3 suspended in a PVA matrix with liquid pockets to resolve Ba\(^{++}\) ions from barium perchlorate solution. Two unfortunate features appear to render this family of molecules inappropriate for use in a dry environment: 1) the binding to Ba\(^{++}\) requires deprotonation of the four carboxylic acid groups in the BAPTA-like receptor, which is not expected to occur in a dry state. 2) While fluorescein is a bright fluor in solution, our data (Fig. 2, first panel) show that fluorescence is suppressed when dried.

To address these issues, we have designed and synthesized custom SMFI molecules based off known alkaline earth metal binding crown ethers that avoid these problems. First, we have identified binding groups based on aza-crown ethers and aza-cryptands as good candidates for dry Ba\(^{++}\) capture, since they can bind the ion strongly but without deprotonation; Second, other fluors such as pyrene exhibit less sensitivity to the solvent and microenvironment (Fig. 2, third panel). Our hypothesis is that connection of these two building blocks via a nitrogen “switch” similar to that used in FLUO-3 and FLUO-4 should yield molecules with a strong on-off response to Ba\(^{++}\) in dry environments.

3. Preliminary Results with custom-made aza-crown derivatives

Based on the considerations above, we have embarked upon systematic study of aza-crown and aza-cryptand-based binders coupled to a variety of fluorophores, starting with pyrene and subsequently investigating similarly promising fluorophores naphthalimides, boron-
Figure 3. Molecules described in this proceeding. FLUO-3 and FLUO-4 are commercially available calcium sensors and NEXT-1 and NEXT-2 are our own custom-made molecules.

Dipyrromethenes and anthracene. Here we present early results using mono-aza-crown ethers (15-crown-5 and 18-crown-6) linked to methylpyrenes, 15-crown-5-pyrene and 18-crown-6-pyrene. Also under development are 21-crown-7 receptors, though we do not present results from those here.

Synthesis of 15-crown-5 ether (15c5) and 18-crown-6 ether (18c6) binding domains is achieved in parallel, via ether synthesis under phase transfer conditions [17] with N-benzylbisethanolamine acting as the linchpin that reacts at two ends of various length polyethers. Hydrogenolysis of the N-benzyl protecting group yields unmodified aza-crown ethers ready for the installation of various fluorophores. The late installation of fluorophores via S_N2 substitution allows for a divergent synthetic pathway to a wide variety of potentially successful fluorescent barium tags without significantly altering the synthesis [13].

Fig. 4 shows the Ba^{2+} response of the most promising of our molecules to date, based on 15c5 and pyrene, which we have named NEXT-2 and show in Fig. 3, right. Initially, buffered aqueous solutions of fluorophores were prepared by serial dilution and fluorescent response was measured using a Cary Agilent Eclipse fluorescence spectrometer. The molecule is excited at its lowest energy \( \lambda_{max} \) (357 nm), near the absorption peak of pyrene, and emits a broad, two-peaked spectrum at 375 and 400 nm when bound to Ba^{2+}.

The performance of SMFI agents is often characterized by fluorescence ratio, \( f = (F_{max} - F_{min}) / F_{min} \), where \( F_{max} \) is the fluorescence intensity when saturated with ions and \( F_{min} \) is the minimum observable intensity when uncontaminated. Integrating the spectral region above 375 nm, NEXT-2 exhibit a fluorescence ratio exceeding 200 for barium. This is to be compared to the commercially available dyes FLUO-3 and FLUO-4, where our previous studies found \( f = 17 \) and \( f = 85 \), respectively. Remarkably, this custom-made molecule exhibits a much stronger transition between the on- and off-states than the the commercial molecules we tested for Ba^{2+} sensitivity in [7]. The closely related molecule based on 18c6 and pyrene (which we have named NEXT-1, and show in Fig. 3, second from right), also exhibits barium sensitivity although less strongly, with \( f = 6 \).

The demonstration of strong barium response from custom made SMFI agents in our laboratory is an important step in this R&D program, opening a path to systematic exploration of a wide variety of related molecular structures for SMFI-based barium tagging in xenon gas.

4. Preliminary Dry Fluorescence Response
The primary goal of custom SMFI fluorophore development is not brightness optimization, but performance in a dry environment. No commercially available calcium sensors that we have
tested have satisfied this requirement. To assess the fluorescence of our custom molecules in a dry environment, we used the microscopy setup described in [14] with dried samples of fluorophore, with and without added Ba$^{++}$. Due to the non-availability of ultraviolet wavelength dichroic mirrors and filters, excitation is delivered at 400 nm with a dichroic separation of excitation and emission at 409 nm. This is far from ideal for these molecules, which would respond much more strongly with a shorter wavelength excitation and fluorescence collection integrating above 375 nm. Nevertheless, we established that with sufficiently long microscope exposures, fluorescence from raw pyrene can be observed in this configuration, both in bulk solution and at the single molecule level. This demonstrates that even with significantly off-peak excitation, the system is sufficiently sensitive for these tests.

To test for dry fluorescence, samples of barium-spiked and barium-un-spiked NEXT-1 and NEXT-2 dyes were dried onto microscope slides. These slides were then scanned for fluorescence, with emission collected by Hamamatsu ImagEM X2 EM-CCD camera. Example images are shown in Fig. 5, left, with the corresponding pixel intensity distributions shown in Fig. 5, right.

The un-spiked samples are optically quiet, showing a background of small isolated spots of low-level fluorescence on a smooth background of scattered light. Barium-spiked samples, on the other hand, exhibit very bright fluorescent regions. These are understood to be macroscopic clusters of fluorescent material rather than single molecules, based on their lack of photo-bleaching and high fluorescence intensity. Similar behaviour was observed for both NEXT-1 and NEXT-2. A full account of these data and their analyses will be forthcoming in a future publication.

These data demonstrate a strong fluorescent response in the presence of chelated Ba$^{++}$ ions in the dry phase. Single molecule detection was not achieved in this test because of the imperfect

**Figure 4.** Response of NEXT-2 dye with steadily increasing Ba$^{++}$ concentrations. The light grey shaded area represents the response in the “off” state, primarily from scattered excitation light.
preparation of the SMFI layer, leading to crystals. In the next stage of this program we will deposit modified fluorophores in a well-controlled monolayer using molecular self-assembly via siloxane surface tethers. This will allow individual fluorescent molecules to be resolved, rather than large clusters.

5. Conclusion and Outlook
In this talk and proceeding we have summarized previous work on barium tagging using SMFI for the NEXT experiment. We presented two important new advances: 1) the first demonstration of fluorescence response from custom-made barium sensing molecules from our laboratories that exceeds the barium-induced response from commercial calcium sensors; 2) the first demonstration of a barium chemosensor that maintains responsiveness in the dry phase. A fully systematic exploration of the fluorescent response of $^{15}c5$, $^{18}c6$ and $^{21}c7$ derivatives with a variety of quenchable fluors in wet and dry environments is presently under way.

In addition, work is commencing to produce a well-controlled monolayer via self-assembly of SMFI molecules onto microscope slides, with the goal of producing a high quality, robust, uniform, single-ion-resolving sensing surface. This builds upon our previous demonstration of single ion identification in aqueous suspensions, translating the approach to thin layer of immobilized molecules on a sensitized glass or quartz surface. We will proceed to test the response of this layer under bombardment of barium ions in a gaseous xenon environment, to demonstrate their viability as SMFI agents within the unfamiliar environment of a xenon gas detector.
Following successful demonstration of single barium dication resolution with SMFI and creation of a dry-compatible barium chemosensor, the identification of barium ions in high pressure xenon gas may be close at hand. If realized, this could yield a new technology to enable large, effectively background-free $0\nu\beta\beta$ experiments, that would represent a powerful tool for discovery of this elusive process.

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