

FRET-Aptamer Assays for Bone Marker Assessment, C-Telopeptide, Creatinine, and Vitamin D

Applications include assessment of osteoporosis, and aptamer assays for veterinary analytes, infectious disease, food- and water-borne pathogens, and chemical/biological threats.

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Astronauts lose 1.0 to 1.5% of their bone mass per month on long-duration spaceflights. NASA wishes to monitor the bone loss onboard spacecraft to develop nutritional and exercise countermeasures, and make adjustments during long space missions. On Earth, the same technology could be used to monitor osteoporosis and its therapy.

Aptamers bind to targets against which they are developed, much like antibodies. However, aptamers do not require animal hosts or cell culture and are therefore easier, faster, and less expensive to produce. In addition, aptamers sometimes exhibit greater affinity and specificity vs. comparable antibodies. In this work, fluorescent dyes and quenchers were added to the aptamers to enable pushbutton, one-step, bind-and-detect fluorescence resonance energy transfer (FRET) assays or tests that can be freeze-dried, rehydrated with body fluids, and used to quantitate bone loss of vitamin D levels with a handheld fluorometer in the spacecraft environment.

This work generated specific, rapid, one-step FRET assays for the bone loss marker C-telopeptide (CTx) when ex-

tracted from urine, creatinine from urine, and vitamin D congeners in diluted serum. The assays were quantified in nanograms/mL using a handheld fluorometer connected to a laptop computer to convert the raw fluorescence values into concentrations of each analyte according to linear standard curves.

DNA aptamers were selected and amplified for several rounds against a 26-amino acid form of CTx, creatinine, and vitamin D. The commonalities between loop structures were studied, and several common loop structures were converted into aptamer beacons with a fluorophore and quencher on each end. In theory, when the aptamer beacon binds its cognate target (CTx bone peptide, creatinine, or vitamin D), it is forced open and no longer quenched, so it gives off fluorescent light (when excited) in proportion to the amount of target present in a sample. This proportional increase in fluorescence is called a "lights on" FRET response. The vitamin D aptamer beacon gives a "lights off" or inversely proportional fluorescence response to the amount of vitamin D present in diluted serum.

These FRET-aptamer assays are rapid (<30 minutes), sensitive (low ng/mL detection limits), and quite easy to carry out (add sample, mix, and detect in the handheld reader). Benefits include the speed of the assays as well as the small amount of space taken up by the handheld reader and cuvette assays.

The aptamer DNA sequences represent novel additional features of the existing (patent-pending) FRET-aptamer assay platform.

This work was done by John G. Bruno of Operational Technologies Corporation for Johnson Space Center. For further information, contact the JSC Innovation Partnerships Office at (281) 483-3809.

In accordance with Public Law 96-517, the contractor has elected to retain title to this invention. Inquiries concerning rights for its commercial use should be addressed to:

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