

Cell-free Detection of Small-Molecules Using Nanobody Conjugates

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ABSTRACT: Assay formats such as ELISAs have effectively employed antibodies for detection of small molecules, but often require numerous steps, complex protocols, and lack modularity making them useful, but ultimately unfieldable technologies for the warfighter. Nanobodies are small, single chain forms of antibodies and hold several advantages for use as biological sensors, including their compact size (~12-15 kDa), superior folding stability, and simple single peptide chain that make them amenable to rapid evolution towards desired targets. In this work, we have developed sensors incorporating nanobodies that bind to insecticides fipronil and cyantriliprole that when fused to nanobody FRET pair proteins and brought together with a dimerizer produce an effective sensor for the insecticides. To reduce the complexity of the optics needed for measuring the sensor signal, the FRET pair was replaced with a split luciferase and the system was further tested in one-pot, lyophilized cell-free reactions containing the DNA for the fusion proteins, the organic dimerizer, and one of the small molecule targets. We found that both liquid and lyophilized cell-free reactions produced a visible signal presence of either of the nanobody targets. We hypothesize that the continued development of nanobody-based biological sensors will enable high-throughput production of fieldable detection tools for a variety of chemical and biological threats.

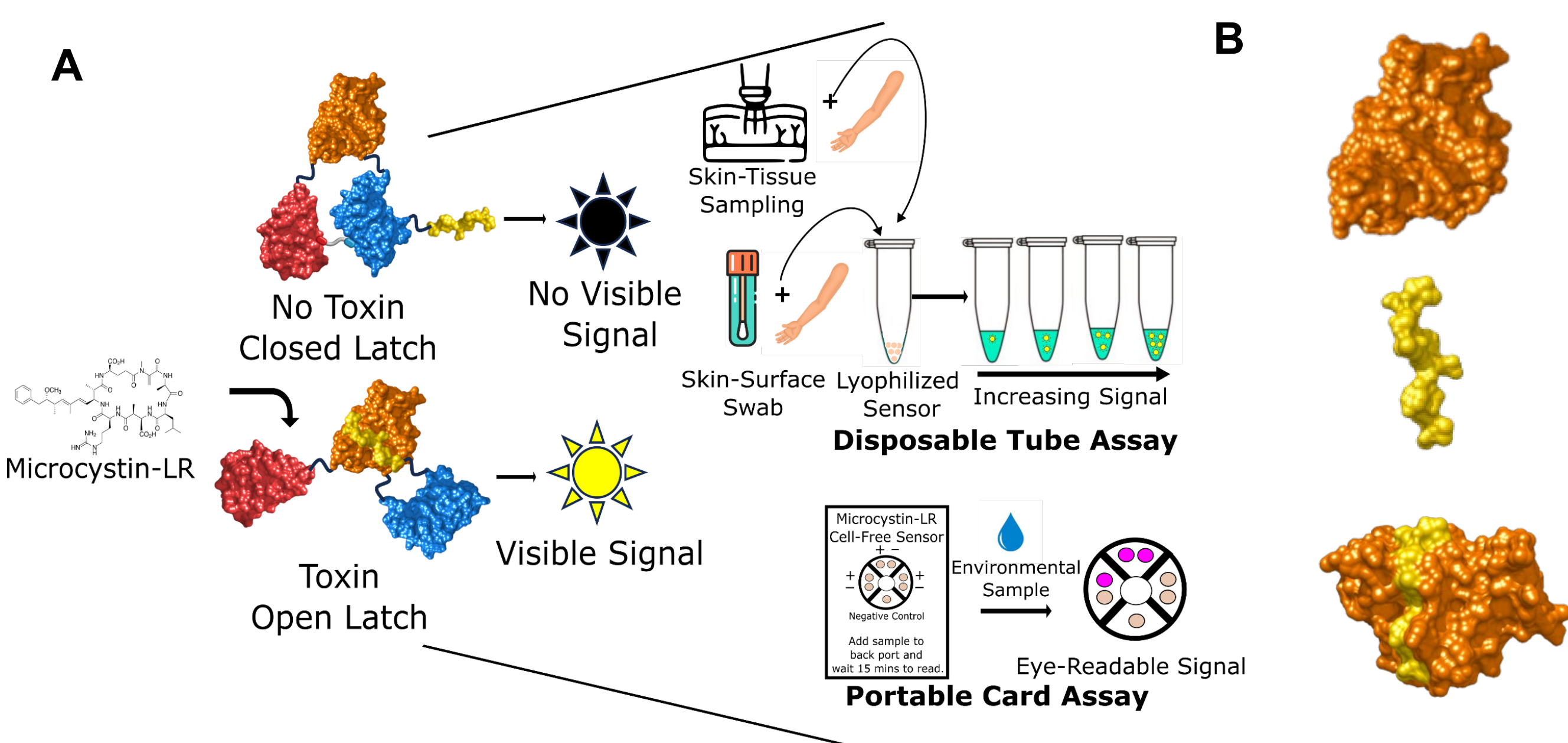


Figure 1. A) Protein-based luciferase "on" sensor. Fusion protein containing two nanobodies and each component of split-NanoLuciferase or split-LacZ fused with cognate antigen "latch" and activated by the presence of the antigen. B) Split version of nanoluciferase. Combined protein produces a visible luminescent signal.

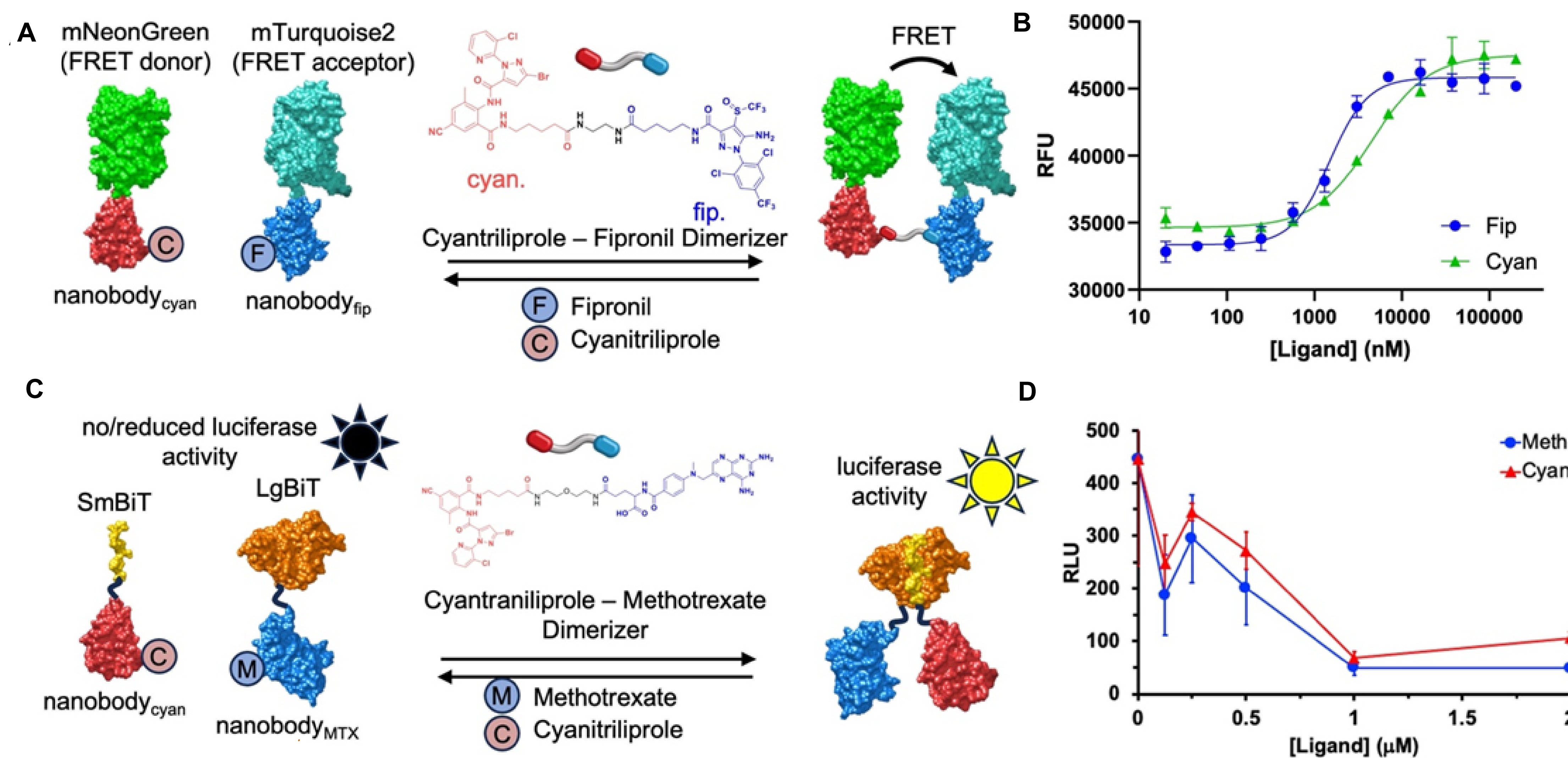


Figure 2. Protein-based sensors for small molecules. A) Nanobodies fused to FRET pair proteins are brought together by an organic "dimerizer" terminated with the target compounds of the nanobodies, resulting in an increased FRET signal. Introduction of either of the target small molecules disrupts dimerization, decreasing FRET. B) Donor fluorescence increases (indicating less FRET) with introduction of target pesticides fipronil or cyantriliprole. RFU: relative fluorescence units. C) Nanobodies fused to split luciferase (SmBiT and LgBiT) are brought together with a dimerizer, resulting in light generation. D) Addition of methotrexate or cyantriliprole reduces light production. RLU: relative light units.

