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Homogeneous and duplexed immunoassay of EGFR receptors based on terbium complex to quantum dot FRET

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Förster resonance energy transfer (FRET) has attracted much research interest in bioanalytical application, because its transfer distances (1-20 nm) are in the range of biomolecular interactions. Combining terbium complexes (Tbs) and semiconductor quantum dots (QDs) for FRET biosensors has many advantages. Tbs provide multiple narrow photoluminescence (PL) emission lines in a broad wavelength range and exceptionally long PL excited-state lifetimes (ms), which enable time-gated detection void of autofluorescence background. QDs add very strong absorption and narrow and symmetric PL bands, whose colors can be tuned by the composition and the size of the QDs. The combination in Tb-to-QD FRET offers homogeneous (no washing and separation steps) and multiplexed biosensors. Here we exploit the nanosurface features of the QD by applying small single domain antibodies (V_HH nanobodies) as biological recognition molecules. Nanobodies do not only offer high surface coating density but also a possibly reduced FRET distance compared to large IgG antibodies. We present a systematic investigation of random vs. oriented nanobody-QD conjugation for FRET-based immunoassays. The homogeneous assays provide sub-nanomolar (few ng/mL) detection limits of the two epidermal growth factor receptors EGFR and Her2 in 50 µL buffer or serum samples. These very low EGFR and Her2 concentrations measured on a KRYPTOR diagnostic plate reader system under "real-life" sample conditions demonstrate the direct applicability of our nanobody-based Tb-to-QD FRET immunoassays for fast and sensitive biomarker detection in both point-of-care and high throughput *in-vitro* diagnostics.

Biography

Xue Qiu received her MS degree from Fudan University (P. R. China) in 2012 and now she is a PhD student under the supervision of Prof. Hildebrandt at Université Paris-Sud (France). Her research interests include development of assays and sensors for biomarkers (proteins and DNA/RNAs) based on time-resolved Forster resonance energy transfer.

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