



Single-Cell, Single-Molecule Analysis of TNF- α Signaling with Quantum Dots

Prerana Jaikumar¹, Phuong Le², Andrew M. Smith²

¹Case Western Reserve University

²Department of Bioengineering, University of Illinois at Urbana-Champaign



Introduction

Traditional molecular biology assays are performed in bulk; individual cells are ground up, and the combined components are quantified. Single-cell imaging, however, allows for detection of heterogeneity between cells through quantitative imaging. The number of proteins or nucleic acids per cell can be measured quantitatively on a single-cell basis.

This study focuses on single-cell quantification of the cytokine TNF- α using quantum dots (QDs). TNF- α is responsible for many inflammatory diseases, including rheumatoid arthritis, Crohn's disease, and psoriasis. TNF- α binds to its cell surface receptor and stimulates an intracellular signaling cascade that leads to translocation of the transcription factor NF- κ B from the cytoplasm to the nucleus. NF- κ B is responsible for the expression of over 500 genes and is frequently deregulated in disease states.

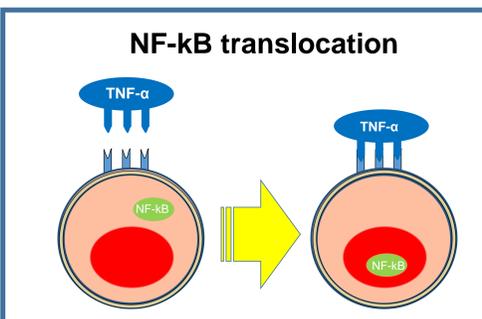
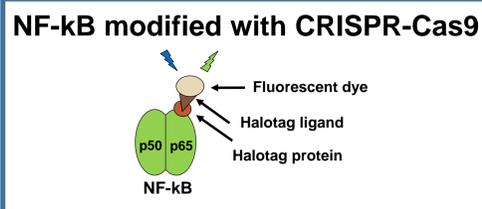
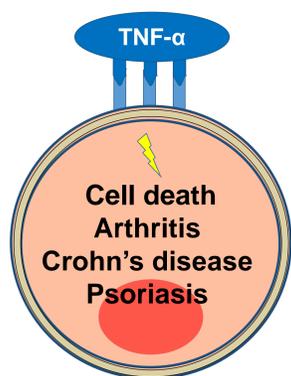
The goal of this study is to quantify the amount of TNF- α required to induce NF- κ B translocation from the cytoplasm to the nucleus at the single-cell level, with single-molecule sensitivity. We first optimized the conjugation of QDs to TNF- α through streptavidin-biotin linkers. We then analyzed the specific and nonspecific binding of these probes and quantified the average number of QDs per cell. We also tested the functionality of the probes without TNF- α to ensure that they would not induce NF- κ B translocation. We found that while there is specific binding of QD-TNF- α conjugates, the correlation between the number of QDs per cell and the amount of p65 translocation remains unclear.

Aims

Aim 1. Conjugate QDs to TNF- α through streptavidin-biotin linkers.

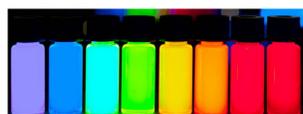
Aim 2. Quantify functionality of conjugates through phenotype assays.

TNF- α and NF- κ B Signaling



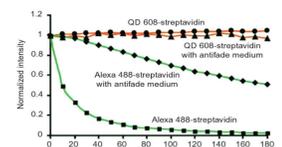
Quantum Dots

- Semiconductor core
- No photobleaching
- Size tunable color
- Narrow emission bands



Increasing Diameter

Right: QD conjugated with streptavidin (SA), which is then conjugated to TNF- α through a biotin linker.



Kumar, C. S. R. *Semiconductor Nanomaterials*. (2010).

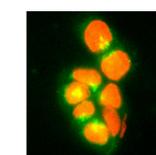
Above: Comparison between QD intensity and organic fluorophore intensity over time.

Left: Size-tunable QDs.

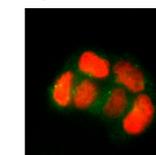


QD-SA Does Not Affect NF- κ B Pathway

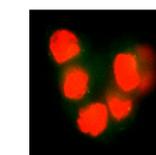
- HCT 116 cell line
- CRISPR-Cas9 modified NF- κ B p65 subunit with HaloTag® (labeled with chloroalkane-dye conjugate shown in green)
- Hoechst stain for nucleus (shown in red)



Pure TNF- α

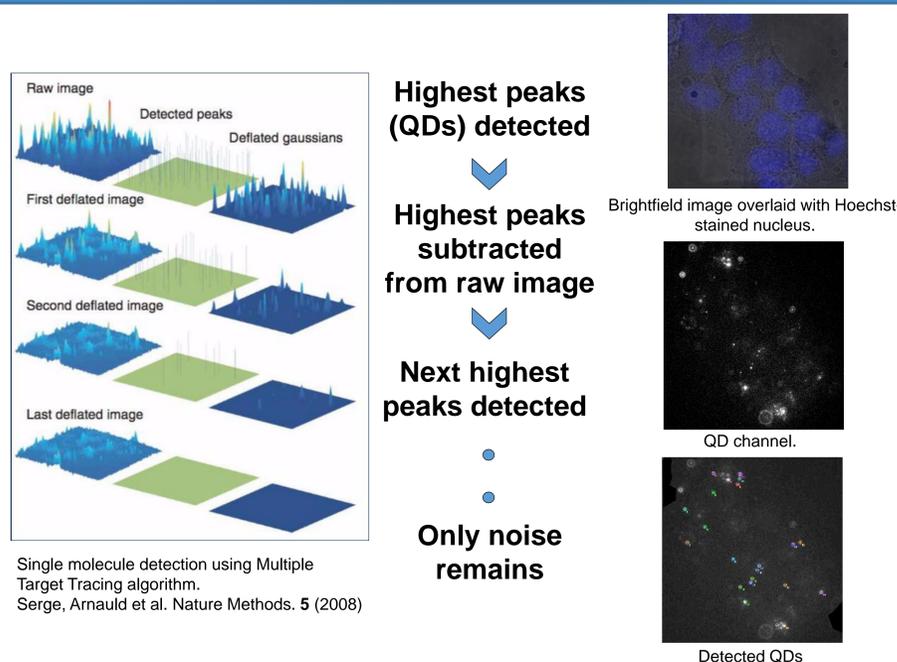


No treatment

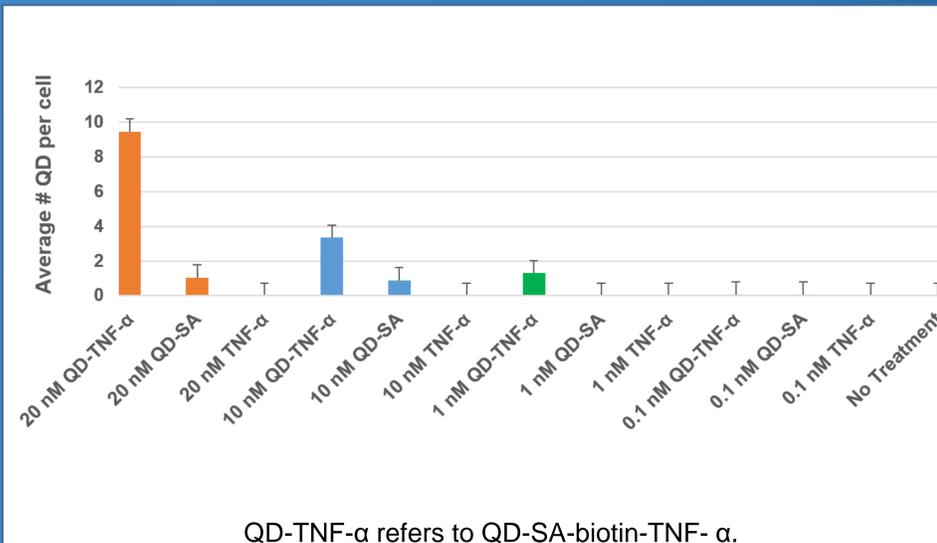


QD-SA

Detecting Single Molecules



Quantification of QDs Per Cell



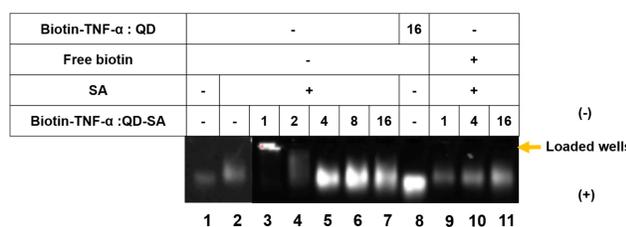
Conclusions and Future Directions

- Aggregation occurred at low ratios of biotin-TNF- α to QD-SA.
- Saturation of QD-SA binding sites at high ratios of biotin-TNF- α to QD-SA yielded homogeneous conjugates. The optimal conjugate ratio is 4:1.
- QD-SA has low nonspecific binding across a wide range of concentrations and QD-SA alone does not induce NF- κ B translocation.
- No clear trends NF- κ B translocation was observed for number of QD-TNF- α conjugates bound per cell.
- **Future:** Use longer incubation times for QD-TNF- α in cells.

Acknowledgments

The Discoveries in Bioimaging REU is funded by the National Science Foundation (EEC 14-61038). I would like to thank Phuong Le, Dr. Andrew Smith, and the rest of Smith lab for their support. I would also like to thank the Summer Opportunities Program (SROP) at UIUC, Dr. Marina Marjanovic and Joanne Li.

Conjugation of QDs to TNF- α



- Row 1: QD alone migrates furthest due to its small size.
- Row 2: QD-SA migrates a shorter distance due to an increased size.
- Row 3,4: Aggregation of biotin-TNF- α and QD-SA conjugates occurs when there is a small number of biotin-TNF- α per QD-SA.
- Row 5,6,7: Monodisperse QD-TNF- α conjugates occur for larger numbers of biotin-TNF- α per QD-SA.
- Row 9,10,11: Free biotin blocks QD-SA binding to biotin-TNF- α .