The Impact of Bioconjugation on Antibody Immunoreactivity

Eric C. Silberman | June – August, 2018

Laboratory of Jason S. Lewis

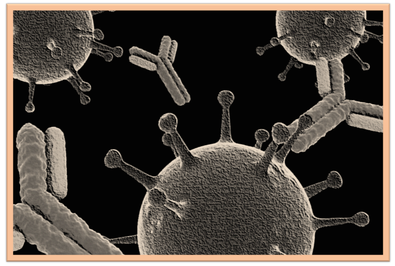
Department of Radiology, Memorial Sloan Kettering Cancer Center, New York

Weill Cornell Medicine Graduate School
A partnership with the Sloan Kettering Institute
High School Science Immersion Program

Memorial Sloan Kettering Cancer Center

Background

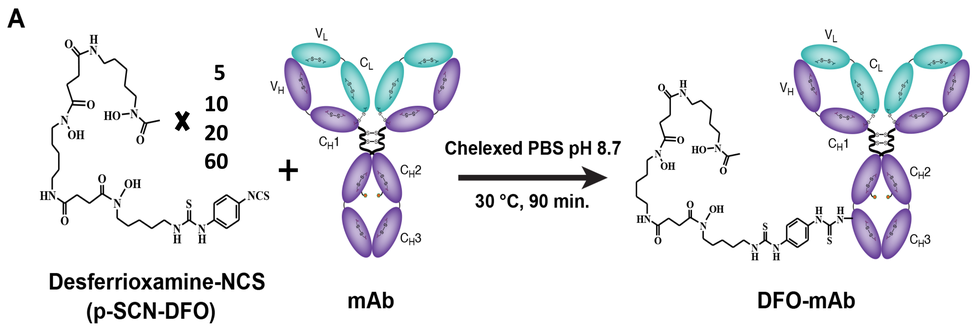
The exquisite property of antibodies to bind to specific molecular targets has made them an important class of drugs used for the molecular imaging and therapy of cancer. Since the direct labeling of antibodies with radiometals is not possible, it necessitates the conjugation of macrocyclic chelators such as desferrioxamine (DFO), that encage ^{89}Zr via coordination chemistry to produce a radioimmunoconjugate.

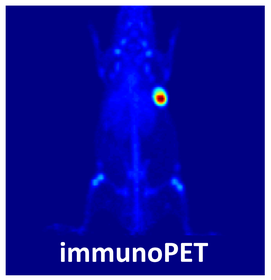


Purpose

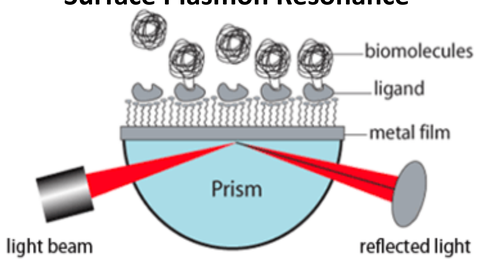
- Conjugation of chelators may impact the ability of the antibody to bind to its target antigen (immunoreactivity).
- Studying the impact of bioconjugation on the final synthesis of an antibody-based radiotracer used for immunoPET is critical.

Radioimmunoconjugate Synthesis



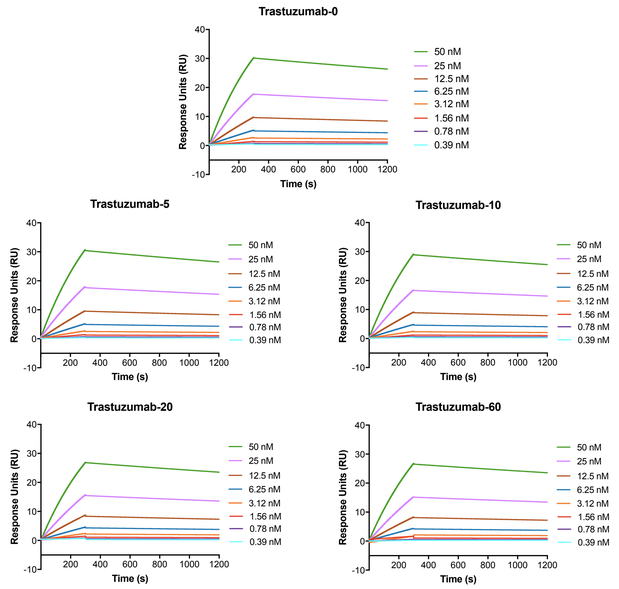


Surface Plasmon Resonance



Results

mAb: DFO Ratio	DFOs per mAb
1:5	1.4 ± *0.5*
1:10	2.6 ± *0.6*
1:20	4.0 ± *1.0*
1:60	10.9 ± *0.7*



Constructs	K_a (M^{-1} s^{-1})	K_d (s^{-1})	K_D(M)
T-0	4.74 x 10^4	1.45 x 10^{-4}	3.15 x 10^{-9}
T-5	4.39 x 10^4	1.54 x 10^{-4}	3.52 x 10^{-9}
T-10	4.03 x 10^4	1.38 x 10^{-4}	3.44 x 10^{-9}
T-20	4.16 x 10^4	1.46 x 10^{-4}	3.65 x 10^{-9}
T-60	3.84 x 10^4	1.32 x 10^{-4}	3.43 x 10^{-9}

Method

- Trastuzumab was conjugated separately with 5-fold, 10-fold, 20-fold, and 60-fold molar excess amounts of isothiocynato-desferrioxamine (*p*-SCN-DFO) to synthesize immunoconjugates.
- The immunoconjugate was purified from excess DFO via size exclusion chromatography using standard PD-10 desalting columns.
- The number of DFOs per antibody in each of the trastuzumab immunoconjugates (T-5, T-10, T-20 and T-60) was determined using matrix-assisted laser desorption ionization-time of flight (MALDI-ToF).
- The binding affinity of the various trastuzumab-DFO immunoconjugates was assessed via surface plasmon resonance (SPR) technology on a Biacore T200 instrument.

Conclusion

- While the immunoreactivity (K_D values) of the antibody does not seem compromised despite conjugation with 60-fold molar excess of DFO, there was a trend for decreased association rate (k_a) between the unconjugated trastuzumab versus the T-60 immunoconjugate.
- This trend in depreciating k_a is indicative of a change to the binding kinetics of the antibody for its cognate antigen – Her2.

References

- Brinkley M (1992) Bioconjug Chem 3(1); 2-13
- Deri MA (2013) Nucl Med Biol (1); 3-14
- Kalia J (2010) Curr Org Chem (2); 138-147

Acknowledgements

Thanks to Dr. Sai Kiran Sharma for invaluable mentorship; Dr. Jason Lewis for having me in his lab.