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Carolyn Bertozzi's Lecture Part 2:
Imaging the Glycome

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1. Review Questions

1. What is the most commonly used imaging technique to visualize changes in living systems in basic research laboratories?

    A. Magnetic Resonance Imaging (MRI)

    B. Fluorescence/optical imaging

    C. Positron Emission Tomography (PET)

    D. Electron microscopy

2. Why can't glycans be labeled with genetically-encoded reporters?

3. A "bioorthogonal" reaction is a reaction that…

    A. doesn't interact with biology

B. is mutually and selectively reactive

C. is not harmful to the biological system

D. All of the above

4. What monosaccharide is used as the starting point for sialic acid biosynthesis?

    A. N-acetylmannosamine

    B. Xylose

    C. Fucose

    D. N-acetylgalactosamine

5. What are the names of two reactions used as bioorthogonal reactions?
6. Why do bioorthogonal reactions need to be very fast reactions for imaging in live animals?
7. Why is a strained alkyne so much more reactive with an azide than a linear alkyne?
8. Why are zebrafish a good model organism for optical imaging?

2. Answers to Review Questions

1. b. Fluorescence/optical imaging
2. Glycans are the product of metabolism and are not encoded in the genome.
3. d. All of the above
4. a. N-acetylmannosamine
5. The Staudinger Ligation and the Huisgen 1,3-dipolar cycloaddition between azides and alkynes.
6. The reaction needs to proceed before the reporter and/or probe is cleared by the animals' metabolism.
7. The linear alkyne has a higher activation energy barrier than a strained alkyne.

8. The embryos of zebrafish are transparent, enabling structures labeled with fluorophores inside zebrafish to be readily observed under a light microscope.

3. Discussion Questions

1. What is metabolic labeling?
2. What is a “fluorogenic”, or smart probe? Why are smart probes desirable? What are the three components necessary to make a smart fluorogenic probe for use in the bioorthogonal chemical reporter approach?
3. What is a control experiment? Why is a control experiment necessary?
4. Why is the development of technology to optically image glycans important in the understanding of human disease? How might you imagine this technology to be used clinically?

4. Answers to Discussion Questions

1. Metabolic labeling is the incorporation of an unnatural metabolite into a target biomolecule via a biosynthetic pathway. This is often done to allow researchers to follow a specific biomolecule. For example, this approach can be used to label biomolecules with radioisotopes or chemical reporters such as azide, by using appropriately labeled biosynthetic precursors.
2. A smart probe is a chemical probe that is only fluorescent after it has chemically reacted with the chemical reporter. The smart probes are desirable because they can only be seen after reaction with the chemical reporter giving little or no background fluorescence. The three components necessary to make a smart probe are a fluorophore, a chemically reactive probe, and a quencher.
3. A control experiment is an experiment where the outcome is known and fixed. In the case of the Bertozzi group research, azide-containing sugars are used for the experiment and natural sugars are used as the control. The control experiments show that the chemical probes are reacting selectively with the azide and not with anything else in the biological system.

4. The development of technology to image glycans is important to understand how the glycosylation patterns change in the development of diseases. In addition, understanding these changes could help us develop ways to discover and diagnose disease non-invasively at an earlier time point. Technology to image glycans could be used clinically in many different ways. One way, would be a non-invasive method to either detect or diagnose cancer at very early stages.

5. Explain or Teach These Concepts to a Friend

1. Explain why the Bertozzi group chose sialic acid to label fluorescently.
2. Explain why the azide was chosen as the chemical reporter and why it is considered safe in the context of a larger molecule in biological systems when the chemical sodium azide is not safe.
3. Explain how glycans can be imaged with different colors to compare the trafficking of old glycans versus new glycans.

6. Research the Literature on Your Own

Into what other molecules are scientists incorporating azides, for metabolic labeling? (search azide metabolic labeling)

7. Papers for Journal Club

1. Sletten, E.M.; Bertozzi, C.R.; Bioorthogonal chemistry: fishing for selectivity in a sea of functionality. *Angew Chem Int Ed Engl.* 2009, *48,* 6974-98.
2. Chang, P.V.; Prescher, J.A.; Hangauer, M.J.; Bertozzi, C.R.; Imaging cell surface glycans with bioorthogonal chemical reporters. *J Am Chem Soc.* 2007, *129*, 8400-1.
3. Baskin, J.M.; Prescher, J.A., Laughlin, S.T.; Agard, N.J.; Chang, P.V.; Miller, I.A.; Lo, A.; Codelli, J.A.; Bertozzi, C.R.; Copper-free click chemistry for dynamic *in vivo* imaging. *Proc Natl Acad Sci USA.* 2007, *104*, 16793-7.