

MCB 4211; BASIC IMMUNOLOGY

FALL 2018

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Office: Biological Sciences/Physics (BSP) room 308

Office Hours: *No scheduled hours.* I am in my office by about 8:15AM, and usually stay until about 6. Catch me after class, drop by my office, phone, or email to make an appointment.

Class Meetings: Tuesday, Thursday room: BSP 131 (2-3:15 pm)

Teaching Assistants: Kristen Dostie (kristen.dostie@uconn.edu) and Amy Thees (amy.thees@uconn.edu) phone: 486-3648 Offices: BSP 317/318

Course Website: http://lynes.uconn.edu/Lynes_Lab/MCB4211.html

Goals of this course: This course draws together a multitude of different fundamental scientific fields that form the foundation for our understanding of how the immune system functions to distinguish "self" from "non-self". From this foundation, we will proceed to consider the variety of ways in which the immune system can fail to protect an organism, thus leading to a multitude of disease processes. We will also discuss the role the immune system can itself play in the initiation of disease and the range of autoimmune and neoplastic diseases that are influenced by human activities. Finally, we will discuss intentional manipulations of the immune system that can influence the direction of these diseases.

The course is specifically oriented to address how we know what we know about the immune system. Emphasis will be placed on specific experiments and assays that are important to our understanding of the immune system and how it works.

Recommended approach to studying course material

1. *Skim over each assigned chapter before class*, and look at figures in the assigned chapters.
2. *Take notes in class*; copy notes over that night after class to ensure you understand what you wrote. If you have trouble taking good notes, consider bringing a tape recorder to supplement your notes. If questions arise, ask them in class or set up an appointment with the TA or with Dr. Lynes.
3. Following each class, *read assigned chapter for comprehension* (don't fall behind!).
4. Be certain to *read primary literature assignments* before the assigned date! We discuss these papers in class, and this material will be included in the examinations.

Administrative notes

1. Please turn off your cell phone *before* class, do *not* bring cell phones, blackberry devices, pagers or other electronic devices to the examinations (you may leave your bookbags with these deactivated items in the front of the classroom during the exam if necessary).
2. Randomized seating will be assigned at the beginning of each examination.

Grades

Examinations will focus on material presented in class, and the supplemental assigned readings.

The final grade will be based on two **quizzes** (5% each), two **hour-long exams** (25 % each), and the **final examination** grade (40%). Exam coverage is listed in the class schedule below. While the material is cumulative, the quizzes and hour exams will emphasize material from the immediately preceding section of the course.

Statement on Academic Integrity

“A fundamental tenet of all educational institutions is academic honesty; academic work depends upon respect for and acknowledgement of the research and ideas of others. Misrepresenting someone else's work as ones own is a serious offense in any academic setting and it will not be condoned. Academic misconduct includes, but is not limited to, providing or receiving assistance in a manner not authorized by the instructor in the creation of work to be submitted for academic evaluation (e.g. papers, projects, and examinations); any attempt to influence improperly (e.g. bribery, threats) any member of the faculty, staff, or administration of the University in any matter pertaining to academics or research; presenting, as one's own, the ideas or words of another for academic evaluation; doing unauthorized academic work for which another person will receive credit or be evaluated; and presenting the same or substantially the same papers or projects in two or more courses without the explicit permission of the instructors involved. A student who knowingly assists another student in committing an act of academic misconduct shall be equally accountable for the violation, and shall be subject to the sanctions and other remedies described in The Student Code.” (taken from the UCONN student handbook.)

***** added at the request of the Office of Student Services and Advocacy *****

Students are required to be available for their exam during the stated time. If you have a conflict with this time you must visit the Office of Student Services and Advocacy to discuss the possibility of rescheduling this exam.

Please note that vacations, previously purchased tickets or reservations, graduations, social events, misreading the exam schedule and over-sleeping are not viable excuses for missing a final exam. If you think that your situation warrants permission to reschedule, please contact the Office of Student Services and Advocacy with any questions. Thank you in advance for your cooperation.

TEXTBOOK and other READINGS

The newest version of "Janeway's Immunobiology" by Kenneth Murphy, **9th edition** has a publication date of March 2016, and is published by Taylor and Francis/Garland Scientific. The ISBN for the paperback version of the text is 9780815345053. There is a looseleaf, unbound version that is about half the price of the paperback. There are also electronic versions of the

textbook available for both computers and e-readers. These can be purchased at reduced cost with either unlimited access, or access for 1 year or 1 semester. The e-book version will be available for purchase or rental <https://www.crcpress.com/Janeways-Immunobiology/Murphy-Weaver/p/book/9780815345053>. Amazon and other vendors also sell this book in used condition.

Statement on textbook use: The text is a highly recommended component of the course, and specific readings are noted in the semester schedule below. Students are strongly encouraged to follow the recommended approach to studying the class material. This includes reading the relevant sections of the text. Much like learning a new language, the study of immunology requires a familiarity with the technical language of the immune system, and with the ways in which concepts interrelate. The text is organized to emphasize these relationships.

Primary literature readings: There are several readings from the primary scientific literature that are required during the semester. You will see them noted on the days that they will be discussed. Read these papers in advance of the class. Note, for some journals, you need to set up a vpn link if you want to access to the library copy of the journals from an off-campus location. You can do that at https://vpn.uconn.edu/dana-na/auth/url_default/welcome.cgi.

“Other links”: In some places in the syllabus, there are additional interesting readings that are related to a specific lecture topic. These other readings or videos are optional, but are both informative and possibly entertaining. The links can be found at the class website.

Semester Schedule

Date Topic and Readings

8/28 Lecture: Introduction, overview of course, course expectations

- A. Organization of the course
 - 1. content: lecture with associated primary literature readings (references are noted in outline)
 - 2. additional resource materials: assigned text readings/note additional textbook features, websites
 - 3. course format: lectures and discussions during class: *feel free to ask questions in class; you may tape the lectures if you wish.*
 - 4. examination format and content; remarks regarding practice exam availability
- B. Historical roots of the study of Immunology
 - 1. Immunology is a relatively young science as compared to botany, zoology, physics, etc.
 - a. 1798 Jenner: cowpox immunization
 - b. 1891 Koch: DTH vs tuberculin Ag
 - c. 1895 Bordet: C' + Ab + bacteria = lysis
 - d. 1901 Landsteiner: ABO blood groups
 - e. 1914 Little: genetic theory of tumor transplantation
 - f. 1936 Gorer: identification of MHC antigens
 - g. 1939 Kabat and Tselius: Antibodies as gamma globulins

- C. At its most basic: simple concept of immunological system activation
 1. signal molecule interaction with receptor molecule leading to information processing that produces either tolerance or some response
 2. signal molecule = antigen; smallest unit that is recognized as non-self
 3. Characteristics of the immune response that may be present (but not always)
 - a. specificity
 - b. memory
- D. Current trends in immunological research
 1. manipulation of the response to disease for therapeutic benefit
 - a. AIDS: small molecule therapeutics, vaccines
 - b. cancer: pdx mice/ avatar transplants for drug susceptibility determinations, biologic therapies, CAR-T therapy
 - c. autoimmunity, biologic therapies
 - d. tissue transplantation; stem cell biology
 2. antibodies as enzymes (“abzymes”)
 3. antibodies as probes of biochemical/cellular structure and as probes of biomarker signatures
 4. psychoneuroimmunology: learned/conditioned immune responses
 5. cytokine immunotherapies
 6. xenogeneic transplants of humanized tissues /stem cell transplants of autologous tissues,
 7. diseases with newly recognized immunological components
 8. therapeutic manipulations of disease with novel antibody constructs (single chain, minibodies, etc.)
 9. viral infection of cancer cells to make them selectively susceptible to CTL attack
- E. Lynes laboratory research interests (<http://web.uconn.edu/lyneslab/>)
 1. mechanisms of immunotoxicity & role of metallothionein in stress-mediated immunomodulation
 - a. MT and immune regulation
 - b. MT and inflammatory bowel disease, diabetes, inflammatory hepatitis and the treatment of these diseases
 - d. the role of bacterial MT (PmtA) and pathogenesis
 2. automated measurements of chemotaxis
 3. Grating-coupled surface plasmon resonance imaging (GCSPRI) and grating coupled surface plasmon coupled emission (GCSPCE) cell and protein microarray platform applications
 - a. use of the surface plasmon resonance (SPR) microarray platform for immune characterization of autoimmune biomarker signatures
 - b. SPR based pathogen-, toxin-, and toxicant- biosensors
 - c. Functional phenotyping in a microarray format; Cytometer on a chip,
 - e. effects of toxicants on stem cell differentiation

8/30 Lecture: Macromolecules, cells, and tissues of the immune response

Reading: Immunobiology textbook Chapter 1: “Basic concepts in Immunology”

- A. Cells of the immune response
 - 1. hematopoiesis
 - a. lymphoid lineage
 - b. other cells (erythroid and myeloid lineages)
 - c. how can these cells be identified, separated and functionally characterized?
 - 2. structure/function of cells in these lineages
 - 3. organization of cells into tissues and organs
 - 4. lymphocyte trafficking
- B. Major soluble components of the immune system
 - 1. antibodies
 - 2. complement
 - 3. cytokines

9/4 Lecture: Experimental systems used to study immune mechanisms

Optional extra reading: “Mouse Genetics; Concepts and Applications” by Lee M. Silver

<http://www.informatics.jax.org/silver/>

- A. Experimental model systems
 - 1. phylogenetic studies: immune mechanisms and their evolution/ appearance in different organisms
 - 2. commonly used mammalian systems
 - a. mouse: Mendelian inheritance and breeding manipulations
 - b. other experimental mammalian animal systems
 - c. human
 - a. ethical limitations vs experimental opportunities
- B. Artificial *in vivo* and *in vitro* systems
 - 1. cell and tissue culture systems: *in vitro*
 - 2. immunologically compromised animals
 - a. immunological mutants
 - b. radiation-induced immunodeficiency
 - 3. transgenic animals, targeted gene disruptions, chimeric animals
 - 4. "humanized" mice/avatar mice

9/6 Lecture: Antigenicity and immunogenicity

Reading: Appendix I pages 791-810 (a listing of CD antigens)

- A. Antigenicity vs. immunogenicity
- B. Fundamental characteristics of antigens
- C. Prototypical antigens
 - 1. mitogens as polyclonal activators
 - 2. T-dependent and T-independent antigens
 - 3. the MHC as an antigenic system
 - 4. CD (cluster of differentiation) antigens
- D. Adjuvants and their mechanisms of action

9/11 Lecture: Antibodies: structure and function

Reading: Immunobiology textbook Chapter 4: “Antigen recognition by B cell and T cell receptors”

- A. Immunoglobulin structure
 - 1. subunit structure
 - 2. heavy and light chains
 - 3. Immunoglobulin fragments and their uses: Fc, Fab, and F(ab')₂
- B. Antigenic determinants of immunoglobulins
 - 1. isotypes and subclasses
 - 2. idiotypes
 - 3. allotypes

9/13 Lecture: Antibodies: detecting antibody/antigen interactions

Reading: Primary Literature Reference #1

Reading: Immunobiology textbook (Appendix I: p 748-790) “The immunologist’s toolbox”

- A. Antibody-antigen binding: Law of mass action and calculation of affinity constants
 - 1. parameters of binding
 - 2. structural contributions to binding
- B. Assays of antibody binding
 - 1. precipitation
 - 2. agglutination
 - 3. radioimmunoassay (RIA)
 - 4. ELISA
 - 5. Fluorescent immunoassay/ Flow cytometry/Fluorescence activated cell sorting (FACS)
 - 6. Western immunoblot analysis
 - 7. immunohistochemistry/immunoelectronmicroscopy
- C. Monoclonal antibodies (MAb)
 - 1. how they are made, isolated, and "humanized"
 - 2. functional differences between polyclonal antisera, monospecific sera and MAb

****** sample questions that can be used to prepare for the quiz and first examination will be posted online at the course website ***** answers to the questions will be posted after an interval*

9/18 Quiz # 1 10 questions, 15 minutes; will focus on the material presented to date.

Lecture: Assays of Antibody/antigen Interactions (continued from 9/13)

9/20 Lecture: Sources of antibody diversity

Reading: Immunobiology textbook Chapter 5: “The generation of lymphocyte antigen receptors”

- A. Genetics of antibody synthesis (the B cell receptor)
- B. Generation of antibody diversity (p179-186)
 - 1. germline vs. somatic mutation
 - associational, junctional and combinatorial diversity
- C. Class (isotype) switching

9/25 Lecture: Major Histocompatibility Complex (MHC)

Reading: Immunobiology textbook Chapter 6: “Antigen presentation to T lymphocytes”

- A. Discovery as a transplantation antigen, and genetics
- B. Cellular expression/tissue distribution
- C. Contribution to cellular recognition (genetic restriction)
- D. Structure/function

9/27 FIRST EXAM will cover material through 9/25

10/2 Lecture: Cell biology of the MHC/protein biology of the MHC; antigen processing and presentation (guest lecture: Dr. Larry Silbart)

Reading: Immunobiology textbook Chapter 6 continued

- A. Mechanisms of antigen association with MHC molecules
 - 1. endocytic pathway (exogenous antigen)
 - 2. cytosolic pathway (endogenous antigen)
- B. Interactions of antigen/MHC with the T cell antigen receptor

10/4 Lecture: T cell/antigen interactions and consequences of TcR engagement

Reading: Immunobiology textbook Chapter 7: “Lymphocyte receptor signaling

- A. Structure of the TcR
- B. Genetics of TcR
- C. Other molecular components of the TcR
- D. Signal transduction following TcR engagement
- E. T cell populations in the thymus and periphery
- F. T cell maturation
- G. Mechanisms of T cell activation
- H. Products of T cell activation (see soluble mediators)
- I. Small molecules and biologic inhibitors of receptor mediated signaling

10/9 Lecture: Cell mediated immunity (CMI)

Reading: Immunobiology textbook Chapter 8, 9: “The development of B and T lymphocytes” and “T cell mediated immunity”

- A. Cytotoxicity
- B. Delayed type hypersensitivity
- C. Immunological protection conferred by CMI

10/11 Lecture: Soluble mediators of immunity

**Reading: Immunobiology textbook Chapter 2: “Innate Immunity: the first line of defense”
Primary literature reading #2, and Appendix III “Cytokines and their receptors”**

- A. Cytokines and lymphokines: structure and function
- B. Pattern recognition receptors and lymphokine receptors
- C. Complement
- D. Anti-idiotypic Immunoglobulins
- E. Selective migration
- F. Neuroendocrine regulation

10/16 Lecture: Immune responses to infectious agents: innate vs adaptive immunity

Reading: Immunobiology textbook Chapter 3,10,11,12: “The induced response to innate immunity”, “The humoral response”, “Integrated dynamics of Innate and adaptive immunity”, and “The mucosal immune system”

- A. The players in an innate immune response
- B. Innate immunity and inflammation
- C. Responses to pathogens
 - 1. viral
 - 2. bacterial
 - 3. protozoan
 - 4. invertebrate parasites; optional reading #1
 - 5. the hygiene hypothesis

10/18 Quiz #2: 10 questions, 15 minutes; covers all material from 10/4 through 10/16 *** sample questions that can be used to prepare for the quiz and second examination will be posted online at the course website ***** answers to the questions will be posted after an interval**

Lecture: Immune responses to infectious agents (continued from 10/16)

10/23 Lecture: Manipulation of the immune response

**Reading: Immunobiology textbook Chapter 16: “Manipulation of the immune response”
Optional reading #2 and 4**

- A. Vaccines (live/attenuated/killed; protein;DNA)
- B. Drugs
- C. Irradiation

10/25 SECOND EXAM will focus on material from 10/2 through 10/23, and will depend on material from the first section of the course. Note that you are responsible for material in the required primary literature. These readings that available on the MCB 4211 homepage and from the e-journal site at the UConn library website.

10/30 Lecture: Inappropriate immune reactions

Reading: Immunobiology textbook Chapters 14, 15 “Allergy and allergic disease” and “Autoimmunity and Transplantation” and Primary Literature Reference #3

- A. Hypersensitivities
- B. Immune tolerance and Autoimmune diseases
- C. Animal models

11/1 Lecture: Inappropriate immune reactions (autoimmunity, continued from 10/30)

- C. Human diseases and treatment
- D. Genome Wide Associational Studies (GWAS) and autoimmune disease
- E. Biomarkers of disease (autoantibodies as biomarkers of autism spectrum disorder)

11/6 Lecture: Immunodeficiencies

Reading: Immunobiology textbook Chapter 13 “Failures of Host defense mechanisms”

- A. Animal models for congenital syndromes
 - 1. nude (T cell defect)
 - 2. SCID (T and B cell Defect)
 - 3. beige (NK cell defect)
- B. Examples of Human congenital immunodeficiencies

11/8 Lecture: Immunodeficiencies (continued from 11/6)

Optional reading #7

- C. Acquired causes of immunodeficiencies
 - 1. Environmental
 - 2. Drug induced
 - 3. Viral
- D. AIDS Acquired Immunodeficiency Syndrome
 - 1. Etiology/viral replication cycle
 - 2. symptoms/immune effects
 - 3. epidemiology
 - 4. current and future therapies

11/13 no class; catch up if you are behind in your reading!

11/15 Lecture: HIV and AIDS; current and future therapies (continued from 11/13)

Optional reading #3 and 5

- 5. pharmacological therapeutics
- 6. immune stem cell replacement therapies

11/19-11/23 No class; Fall recess!

11/27 Lecture: The immune response to cancer

Reading: Immunobiology textbook Chapter 16 “Manipulation of the immune response”

Primary Literature Reference #4

- A. Mechanisms of carcinogenesis
- B. Natural immune responses to neoplasia
- C. Immunological diagnosis of neoplastic disease

11/29 Lecture: Cancer immunology (continued).

- A. Tumor antigens
- B. Immunotherapies for neoplasia
- C. “Magic bullets”: immune conjugates
- D. Antibody/drug conjugates
- E. Antibody/radionuclide conjugates
- F. Anti-idiotypic antibodies
- G. Irradiation and transplantation to reconstitute immunity
- H. TILs, LAKs
- I. Gene therapies
- J. CAR-T therapies; Chimeric antigen receptors or artificial T cell receptors

12/4 Lecture: Transplantation biology

Reading: Immunobiology textbook Chapter 15 “Autoimmunity and transplantation”

Primary Literature Reference #5; *Optional reading* #6.

- A. Immunological mechanisms of histo-incompatibility
- B. Manipulation of histo-incompatibility
- C. Clinical value of transplantation
- D. Value of MHC polymorphism in population responses to infection; consequences to transplant success

12/6 LAST MCB 4211 class--Summary, overview, and final exam comments

12/7 LAST DAY OF CLASSES

****** sample questions that can be used to prepare for the final examination will be posted online at the course website ***** answers to the questions will be posted after an interval*

12/10-12/16 FINAL EXAM PERIOD; You can find the entire preliminary exam schedule at <http://www.registrar.uconn.edu/exams.htm>

..... The final exam will emphasize material from 10/30 through 12/6, but will cover material from the entire course. Remember that you are responsible for material in the required primary literature readings as well as the material covered in class **.....**

Required primary literature

Primary literature references from the scientific literature and their web links

1. Kohler, G. and Milstein, C., Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*, 1975. 256: p. 495-7.
<http://www.nature.com/nature/journal/v256/n5517/pdf/256495a0.pdf>
2. Rennard, B.O., et al., Chicken soup inhibits neutrophil chemotaxis in vitro. *Chest*, 2000. 118(4): p. 1150-7. <http://www.sciencedirect.com/science/article/pii/S0012369215377217>
3. Youn, J., et al., Metallothionein suppresses collagen-induced arthritis via induction of TGF-beta and down-regulation of proinflammatory mediators. *Clin Exp Immunol*, 2002. 129(2): p. 232-9.
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1906452/>
4. Dolk, E., M. van der Vaart, et al. (2005). "Isolation of llama antibody fragments for prevention of dandruff by phage display in shampoo." *Appl Environ Microbiol* 71(1): 442-50. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC544197/pdf/0258-04.pdf>
5. Siddle, H. V., A. Kreiss, M. D. Eldridge, E. Noonan, C. J. Clarke, S. Pyecroft, G. M. Woods, and K. Belov. 2007. Transmission of a fatal clonal tumor by biting occurs due to depleted MHC diversity in a threatened carnivorous marsupial. *Proc Natl Acad Sci U S A* 104:16221.
<http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=1999395&blobtype=pdf>

Optional (added value) Readings

1. Hookworm mediated immune suppression and allergy/asthma treatment.
http://www.nytimes.com/2008/07/01/health/research/01prof.html?_r=3&pagewanted=1&sq&st=nyt&scp=2
2. Lack of connection between vaccination and autism, and recent connections of autism to specific non-coding RNAs
<http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0003140>
<http://the-scientist.com/2012/04/04/multiple-strikes-against-autism/>
3. autologous stem cells are rejected
http://www.nytimes.com/2011/05/14/science/14stem.html?_r=2
<http://www.nature.com/nature/journal/vnfv/ncurrent/pdf/nature10135.pdf>
4. Social/Legal issues in Genetics and Immunology
Vaccination programs and international policy
<http://www.ibtimes.com/articles/363531/20120716/pakistan-taliban-polio-vaccinations.htm>
Vaccination in the US
<http://www.latimes.com/science/sciencenow/la-sci-sn-disneyland-measles-under-vaccination-20150316-story.html>
5. HIV prevention pill "Truvada"
<http://www.npr.org/blogs/health/2012/07/17/156868446/deciding-on-truvada-who-should-take-new-hiv-prevention-pill>
6. CAR-T cancer immunotherapies.
Ruella, M., and June, C. (2018) Predicting Dangerous Rides in CAR T Cells: Bridging the Gap

between Mice and Humans. Mol. Ther. 26(6) 1401-1403.

<https://doi.org/10.1016/j.ymt.2018.05.005>

<https://www.mdanderson.org/publications/cancerwise/2018/02/car-t-cell-therapy--9-things-to-know.html>

7. Environmental toxicants and immune disease

Kreitinger JM, Beamer CA, Shepherd DM. Environmental Immunology: Lessons learned from exposure to a select panel of immunotoxicants. Journal of immunology (Baltimore, Md : 1950). 2016;196(8):3217-3225. doi:10.4049/jimmunol.1502149.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4824550/>

Some website addresses of interest to immunologists: Immunology techniques biological materials, and instrumentation websites

1. <http://flowcyt.cyto.purdue.edu> PUCL is the leading cytometry site since 1993
2. <http://www.perkinelmer.com/lab-solutions> {a manufacturers guide to immunoassays}
3. <http://www.bdbiosciences.com/us/home/> {a home page for a flow cytometer manufacturer}
4. <http://www.atcc.org/> {American Type Culture Collection; a source of cell lines and genetic resources}
5. <http://www.jax.org> {a resource of inbred mouse strains, transgenic and knockout mice}

Immunology databases

1. <http://www.ncbi.nlm.nih.gov/protein> {a site that shows structural features of CD molecules}
2. <http://rarediseases.info.nih.gov/> {the office of rare diseases at NIH; for autoimmune diseases like Lupus, arthritis}

Genome databases

1. <http://www.informatics.jax.org> {mouse genome information}
2. <http://www.ncbi.nlm.nih.gov/omim> {online mendelian inheritance in man}

Scientific journal websites

1. <http://www.cell.com/> {the journal Cell}
2. <http://journals.lww.com/jaids/pages/default.aspx> {Journal of Acquired Immune Deficiency Syndromes}
3. <http://www.jimmunol.org/> {The Journal of Immunology}
4. <http://flowcyt.cyto.purdue.edu/flowcyt/websites/cytsites/sitesed.htm> {listing of other scientific journal websites}

Scientific funding websites

1. <http://www.projectreporter.nih.gov/reporter.cfm> {a site from which to search for currently funded NIH grants}
2. <http://www.nsf.gov/awardsearch/> {NSF awards data base}

Scientific document search engines

1. <http://www.ncbi.nlm.nih.gov/pubmed/> {for searching scientific journal citations “PubMed/Medline”}
2. <http://toxnet.nlm.nih.gov/> {the National Library of Medicine’s Toxline database of toxic chemicals}

Scientific reagent and instrumentation companies:

1. <http://www.bdbiosciences.com/reagents/#research> {a supplier of monoclonal antibodies to human, mouse antigens and a flow cytometry company}
2. http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_2421.pdf {BioRad fluorescent probe spectra}