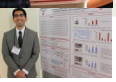

NIH Protein Biotechnology Training Program News & Reviews

2015 - 2016

News & Reviews

WASHINGTON STATE UNIVERSITY NIH BIOTECHNOLOGY TRAINING PROGRAM

Message from the Director

May 2016

Dr. Margaret E. Black

As we end the 27th year for the NIH Biotechnology Training Grant Program at WSU I'd like to reflect on the passed successful year. The Annual Protein Biotechnology Symposium was yet again a highlight of the Program, drawing students, faculty, and staff attendance from across campus. Throughout the year the students in the Protein Biotechnology Training Program work hard to plan, organize, and host this event and their efforts were very clear to see in the attendance and feedback I received about the symposium.

This year five speakers were invited to present at the all-day event on April 22, 2016 with an adjudicated graduate student poster session. The exciting theme this year was "Protein Evolution" and we were honored to have the following world-class scientists from academia, industry and private research institutes as our speakers: Dr. Sandy Merino (Novozymes), Dr. Phillipa Marrack (University of Colorado, Denver), Dr. Melvin Duvall (Northern Illinois University), Dr. Chris Amemiya (University of Washington), and Dr. Gerry Smith (Fred Hutchinson Cancer Research Center).

We have such talented, productive students and faculty associated with the Protein Biotechnology Training Program! The poster session was all-abuzz with active discussion of their great research activities. Thank you to all the poster judges (especially the invited speakers!) and to all the students who shared their hard work with the viewers. The scoring was very, very close and all the students should be very proud of their accomplishments. Congratulations to Darin Weed (first place), as well as Liz Zamora and Laura Ahlers (second place), awardees of the poster competition.

All the Officers (Darin Weed (President), Adan Medina (Vice President) and Chrystal Quisenberry (Secretary)) of the Protein Biotechnology Program and the numerous Symposium Committees who worked so diligently to put on such a wonderfully successful event deserve a huge cheer! A BIG thank you also to the other committees who work hard to engage and inform the trainees and trainers through your various important activities on behalf of the Biotech Program over this past year.

This past year we had a bumper crop of trainees successfully defended their dissertations and graduated from WSU with a Ph.D. including Kim Cotton (MPS, John Browse), Emily Davenport (CHEBE, Haluk Beyenal), David Favero (MPS, Michael Neff), Cory Gall (IID, Kelly Brayton), Tom Jacroux (CHEBE, Wenji Dong), Stacy (Hathcox) Martin (SMB, Margaret Black), Desiree Mendes (Chemistry, Cliff Berkman), Simon Newkirk (SMB, Wenfeng An), Jackie Stone (IID, Hector Aguilar-Carreno) and Leanne Whitmore (SMB, Ping Ye). Congratulations to each and every one of you and best wishes in your future endeavors.

Two students performed their industrial internships during the academic year (Ryan Christian (MPS), and Tom Jacroux (CHEBE)) and six students are performing internships this summer – Laura Ahlers (SMB), Jesse Bengtsson (MPS), Alexander Brown (SMB), Rachael DeTar (MPS), Seanna Hewitt (MPS), and Adan Medina (CHEBE). We all look forward to hearing about your internship adventures from this summer during the next year.

In faculty trainer news, Drs. Hector Aguilar-Carreno, Anthony Nicola, and Viveka Vadyvaloo (SGAH) were granted tenure and promoted to Associate Professor. Drs. David Gang and Mark Lange (MPS) were promoted to full Professor. Congratulations! In Biotech news, Drs. Guy Palmer (SGAH) and Mick Smerdon (SMB) have joined the elite ranks of emeritus faculty trainers! We thank them for their many years of dedication and enthusiastic support of the Biotech Program.

Continuing members of the Executive Steering Committee are Dr. Cliff Berkman, Associate Director (Chemistry), Dr. Nehal Abu-Lail (CHEBE), Dr. John Browse (MPS), and Dr. Doug Call (IID). In closing, I cannot thank Ms. Susan Cao enough for all she does for this program throughout the year. I encourage faculty trainers to attend the monthly Forum meetings next year. Have a wonderful summer!

Symposium Speaker Profiles

BIOTECHNOLOGY SYMPOSIUM 2016

The WSU NIH Biotechnology program held our 25th Annual Biotechnology Symposium on April 22nd 2016. Thank you all who added to its success this year!

Dr. Sandy Merino

Senior Scientist at Novozymes, Inc.

Dr. Merino earned her Ph.D. in the Cellular and Molecular Biology at the University of New Mexico studying sexual development in *Neurospora crassa* and *Neurospora testrasperma.* Afterwards, she completed a post doc position at Indiana University studying DNA repair and meiosis in the basidiomycete *Coprinus cinerius*. She then joined Novozymes as a senior scientist.

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Dr. Philippa Marrack

Distinguished Professor,
Department of Biomedical Research at National Jewish Health, UCDenver

Dr. Marrack was trained as a biochemist at Cambridge University. She came to the University of California, San Diego in 1971 to do postdoctoral work. Together with husband John Kappler, the couple worked at the University of Rochester and moved to National Jewish in Denver in 1979. She is now Chair of a newly created Department of Biomedical Science at that institution, a Distinguished Professor of the University of Colorado and an HHMI investigator.

In collaboration with John Kappler, Dr. Marrack studies the development, specificity and function of T cells, with side tracks to experiments on some kinds of B cells. Like many other immunologists, Drs. Marrack and Kappler have over the years shifted from a focus on basic biology to more applied studies on subjects such as the functions of vaccine adjuvants, the role of gender in autoimmunity and immune responses to self-antigens and metal ions in mice and humans.

University of Colorado Denver

Symposium Speaker Profiles

Dr. Melvin Duvall

Professor, Department of Biological Sciences, Northern Illinois University

Dr. Duvall first obtained a Master of Science degree in computer science from the University of Iowa before moving on to the University of Minnesota Twin Cities for a PhD in botanical systematics. He was an assistant and associate professor at South Dakota State University before moving to Northern Illinois University where he is a full professor. His research group studies focus on understanding both ancient and recent molecular evolution. Using genome sequencing and analysis, his group can compare mutation patterns and fill in biological history.

Northern Illinois University

Dr. Chris Amemiya

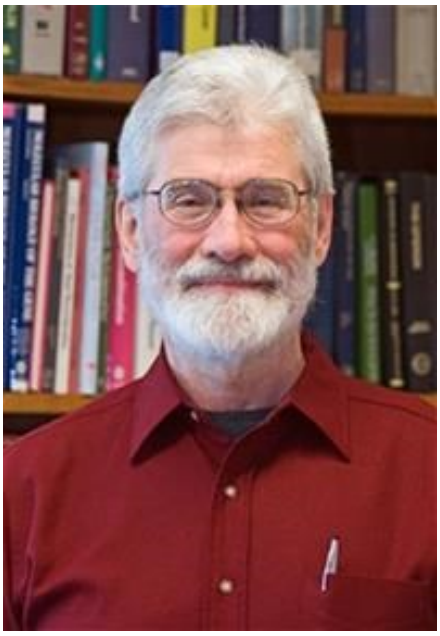
Affiliate Full Professor, Molecular Genetics, Director of Genome Resource Center, Benaroya Research Institute, University of Washington

Dr. Amemiya completed his undergraduate studies at Purdue University and received his PhD in genetics from Texas A&M. Completed postdoctoral studies with Dr. Gary Litman in comparative immunology (Tampa Bay Research Institute, Florida). He took a second postdoctoral fellowship where he worked on the Human Genome Project (Lawrence Livermore National Laboratory, California). Dr. Amemiya became a faculty member in the Center for Human Genetics at the Boston University School of Medicine, where he studied the genetics of X-linked immunodeficiency diseases. Dr. Amemiya moved to Benaroya Research Institute in 2001. He is also a full professor in the Biology Department at the University of Washington.

Dr. Amemiya is interested in the origins of novelty and innovation in vertebrates, with special emphasis on the adaptive immune system and vertebrate bauplan. His laboratory wishes to understand the mode by which diversity is generated at the genomic and developmental levels, and how the mechanism emerged in the first place.

UNIVERSITY of WASHINGTON

Symposium Speaker Profiles



Dr. Gerry Smith

Lead Scientist, Fred Hutchinson Cancer Research Center, Basic Sciences Division, University of Washington

Dr. Gerry Smith earned his Bachelor's of Science Degree from Cornell University emphasizing in microbiology. He then went on to Massachusetts Institute of Technology (MIT) to earn a Ph.D. in biology. He currently runs a lab at Fred Hutch in the Basic Sciences Division. His research interests include recombination and DNA repair regulation using yeast *Schizosaccharomyces pombe* and the bacterium *Escherichia coli* as model systems.

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Internship Reports

Kim Chiok

Internship: I obtained a spot at Illumina's iAspire summer program. Illumina is a private biotech company that specializes in sequencing technologies, which we currently use in my research project. The program lasted 12 weeks and took place at their headquarters office in San Diego, CA. My specific activities were focused on surveying current scientific literature and writing a literature review that will be published and distributed worldwide as a booklet in the next few months. I also participated in the activities fostered by the program that aim to provide tools for professional development in private industry.

Nathan Havko

Internship: I worked as a research intern at Matrix Genetics, Seattle WA. I was part of a team working to improve the photochemical efficiency of cyanobacteria. I used strains of cyanobacteria that contained recombinant promoter-gene fusions to alter expression of genes involved in light harvesting and photosynthetic electron transport to uncover effects of altering photosynthetic electron flow on cell heath and growth rates. I combined my previous experience with basic molecular biology with techniques I learned related to the study of photosynthesis to contribute to the analysis of two different strains of cyanobacteria.

Internship Reports

Kelcey Dinkel

Internship: I began and completed my internship at VMRD in Pullman, WA. I was immediately immersed in the company. I was taught various items related to every day projects and tasks that occurred in all departments, including production and marketing. These included standard operating procedures, transitioning assays from R&D to production, and the mass production of kits. Within the R&D department, I familiarized myself with current and everyday projects through shadowing and assisting my fellow research scientists/technicians. I was also given my research project that focused on the immune response to Porcine Respiratory and Reproductive Syndrome virus (PRRSV). Specifically, I would design an assay to measure responses to PRRSV infection in porcine cell cultures. I spent the remaining time of my internship focused on designing and optimizing this assay. During the final month, I expanded my work from my core project to additional areas within the company. My work continued on assay optimization, as well as the design of a new assay. In addition, I began helping a co-worker in the design of a new ELISA kit. With this design, I learned the complicated task of creating an ELISA from the ground up. My work focused on producing a reliable antigen, which was the first step in the design. My experience working in the R&D department at VMRD was enjoyable and rewarding. From my internship, I gained knowledge regarding different scientific techniques, unfamiliar areas within the field of immunology and infectious diseases, and the biotechnology industry as a whole.

vmrd Veterinary Medical Research & Development

Kevin Gray

Internship: I interned at Seattle Genetics, located in Seattle, WA. The goal of my project was to develop a toolbox of media supplements that could be used to modulate the PQAs of Seattle Genetics Antibodies which are recombinantly expressed in CHO cells.

Potential media supplements were screened using a new statistical technique called definitive screening design. This technique allows for greater data mining from small data sets. The set of 15 supplements screened in my work were done with only 33 experiments instead of 3350 experiments to test the same number of components at the same ranges independently and in combination. The impact of these supplements were checked for various process attributes including cell growth, culture yield, and PQAs. JMP11, a powerful statistical program, was used to develop models that describe the concentration dependent impact of each of the components independently and in combination. JMP11 allowed us to develop predictions of optimal media supplements. At the completion of my internship, the group invited me to return and present a poster based on my work and I was thrilled to see the predicted media supplements were being tested and yielding encouraging results.

It was a great pleasure to work with the process development group. I learned about suspension culture of CHO cells and JMP11 statistical software. More fundamentally, I got to experience the differences between industrial and academic science which helped me refine my career objectives.

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Internship Reports

Benjamin Kilian

Internship: As an intern at Phytelligence, I was given the opportunity to work very much independently on various projects. Some of the smaller projects involved optimizing light conditions for containers that have variable air exchange capabilities. Another project I worked on was a genome re-sequencing project with the goal of demonstrating genetic differences and distinguishing among closely related tree fruit varieties.

Through this internship I learned a great deal about how biotech startup companies actually operate. I was offered the opportunity to have a unique perspective in several areas of business that I had not encountered previously. For example, I was allowed to sit in on several employee interviews and had input in the development of an inventory tracking program that was coming online. During my time at Phytelligence I learned the importance of fostering relationships in a business context. These include industry partners, investors and even employee/employer relationships. It was helpful to see how a company has to balance these varied relationships. I had a great experience at Phytelligence and think it was a very valuable addition to my educational experiences at WSU.

Corey Knadler

Internship: As part of my internship at Phytelligence, I learned nearly every aspect of propagation of plants through tissue culture, and helped to improve this process and solve various problems that their rapid growth has posed to their operation. I developed a method of tracking plants and what actions are taken with them using a system of barcoding that greatly reduces the amount of work required to maintain the database and improves the quality of the data that is available. As the system that I invented reaches full implementation they will be able to track the precise lineage of all their plants of who propagated them when, and what specific plants they were propagated from and when. Additionally, Phytelligence genetic testing services are relatively new, and I assisted in designing and implementing some of their methods. I learned a lot about evaluating high throughput sequencing data, which I had never done before and using SSRs to detect plant varieties. To try to determine how related a pair of trees were, I learned how to use a number of programs for analyzing high throughput sequencing data and I became much more proficient with perl programming. During my internship I developed a perl program that searches sets of high throughput sequencing data for repeated sequences, and I designed PCR based methods of performing genetic tests.

On the whole, I feel that it was a valuable experience because I initially had no idea what starting a biotech company would be like. Now I have a much more realistic view of what to look for in future employers, or what to think about if I decide to start my own company. Along the way I learned a lot and gained skill in analysis of high throughput sequencing data and computer programming.

Internship Reports

Chrystal Quisenberry

Internship: I worked at TransTissue Technologies GmbH in Berlin, Germany. During this internship, I learned many useful techniques in tissue engineering research that I do not currently use in my research at WSU. At TransTissue Technologies GmbH, I cultured primary human chondrocytes and learned how to seed them into PGA scaffolds. From these scaffolds, we used portions of the cultured scaffolds for mRNA expression of chondrogenic markers, histological staining, and live-dead staining. From these I learned how to fluorescently stain scaffolds with PI and FDA to see the morphology, location and quantity of viable and dead cells. I learned how to prepare and stain samples for histology, cut them with the microtome and place samples on slides for staining. For the proliferation assays, I cultured the samples for 9 days with three time points, collected the cells, digested the cells and quantified the DNA content. In addition to these experiments, I was able to work with my supervisor on his project which included a chemotaxis assay to see the migration of mesenchymal progenitor cells in response to different concentrations of platelet rich plasma (PRP) and take images of the migrated cells to quantify PRP's effect on migration. This internship was a great learning experience because it taught me techniques that I do not currently use in my research but would greatly enhance the quality of my research at WSU. Working at TransTissue Technologies GmbH for this short period in the summer confirmed my impression that I would like to work at a small biotech company after I have completed my graduate studies.

Leanne Whitmore

Internship: I performed my internship at the Center for Infectious Disease (CID) Research in Seattle, WA in the laboratory of Dr. Peter Myler. This lab studies the protozoan parasite Leshmainia which causes the disease Leshmaniasis resulting in skin ulcers, fever, enlarged spleen and kidney. My project was focused on annotating transposons in the Crithidia Fasciculata genome, which a closely related protozoan species to Leshmania. Using genome alignment tools and software, and phylogenetic analyses, we were able to annotate within the Crithidia genome four types of retrotransposons. Additionally, each transposons' location (i.e. iTSS, tTSS, SSRs, synteny breaks, and telomeric) in the genome were classified showing that each type of transposon appears to have a distinctive role in the evolution of the C. fasciculate genome. Specifically, TATEs appear to have expanded the telomeric regions of both L. brazilienesis and C. fasciculate while VIPERs are located in iTSS sites for polycistronic regions suggesting part of their sequence enhances initiation. CREs and CIREs based on their location may facilitate joining segments of the genome together. Furthermore, each of the four transposons discovered in C. fasciculate were classified into retrotransposon groups such as non-LTR retrotransposons, copia/ty1, bel, tyrosine recombinases and gypsy/ty3. CIREs and CREs clustered with non LTR-retrotransposons while VIPERs and TATEs we showed to cluster with tyrosine recombinase LTR- retrotransposons. Further studies are necessary in order to annotate and classify transposons in other protozoan species to better understand their role in the evolution of these species.

Internship Reports

Thu Ly

Internship: I was offered a genetics internship at Matrix Genetics in Seattle, WA thanks to my experiences in microbiology and biochemistry. The project that I was involved in was to modulate the expression of two important genes encoding for β-carotene ketolase and hydroxylase that are responsible for converting β-carotene to astaxanthin in transgenic cyanobacteria Synechococcus elongatus PCC 7942 in order to optimize astaxanthin production. Astaxanthin is a commercially important keto-carotenoid that is responsible for the reddish color of salmonids and crustaceans. It is an extremely efficient antioxidant and has great beneficial effects on human health, thus it can be consumed as a food supplement. The goal of my internship was to analyze the impacts of different genetic backgrounds and operon designs on astaxanthin productivity. For this I performed PCR, molecular cloning, DNA sequencing and plasmid purification on a daily basis. In addition to creating new cyanobacterial strains, I designed weekly experiments to test cell growth and astaxanthin productivity of these new strains. By the end of the internship I had created more than twenty new strains, half of which I successfully tested and reported the results. I was able to incorporate an optimal operon design into the strain with the best genetic background. The internship gave me an incredible opportunity to enhance my molecular cloning skills which will greatly benefit my future graduate research.

Trainee Awards, Achievements, and Publications

2016 Symposium Poster Winners

First Place

Darin Weed

(Nicola – VMP)

Second Place—TIE!

Laura Ahlers (Goodman – SMB) And Liz Zamora (Aguilar—IID)

Honorable Mentions

Mia Kiamco

(Beyenal – CHEBE), Gunner Jonston (Aguilar – IID), and Adan Medina (Beyenal – CHEBE)

Trainee Awards

Laura Ahlers

Dr. Bruce Gibbins Summer 2015 Travel Award

American Society for Virology Student Travel Grant

Kevin Grey

Anne & Russ Fuller Interdisciplinary Research Fellowship

Desiree Mendes

E.L. Wagner Memorial Scholarship – Academic Year 2014-2015

William J. Shelton Fellowship – Academic Year 2015-2016

Natalie Peer

Richard R. & Constance M. Albrecht Scholarship

Chrystal Quisenberry

Hariet B. Rigas Award from Association of Faculty Women, April 2016

Trainee Awards, Achievements, and Publications

Graduations

Name (Advisor - Area)	Thesis Title	Degree	Current Position
Cotton, Kimberly (Browse - MPS)	Genetic and Biochemical Analysis of Essential Enzymes in Triacylglycerol Synthesis in Arabidopsis	Ph.D. Dec. 2015	Genetic Analysis Scientist. Phytelligence, Pullman WA
Hopkins, Mandy. (Meier - Pharm. Sci.)	Characterizing the effects of free fatty acid receptor 4 (FFA4) agonists in human prostate cancer cells	Ph.D. Dec. 2015	Postdoctoral Fellow. University of Colorado-Denver, Anschutz Medical Campus
Martin (Hathcox), Stacy. (Black - SMB)	Development of Suicide Enzyme-Based Therapeutics for Cancer Therapy	Ph.D. Dec. 2015	Looking for employment
Stone, Jackie. (Aguilar - IID)	Studying entry mechanisms of Nupah Virus, an emerging zoonotic virus of the para-myxoviridae family	Ph.D. Dec. 2015	Medical Writer. Scimentum, Hamilton NJ
Whitmore, Leanne. (Ye - SMB)	Discovering the source of retinoic acid (RA), required for meiotic initiation, in mouse spermatogenesis	Ph.D. Dec. 2015	Postdoctoral Fellow, Sandia National Laboratories, Albuquerque NM
Desiree Mendes (Chemistry, Berkman)	Development of phosphoramidate inhibitors for cell surface proteases in metastatic cancers	Ph.D. Spring 2016	Looking for employment in industry.
Emily Davenport (CHEBE, Beyenal)	Differential defensive mechanisms against antibiotics present in biofilm extracellular Polymeric Substance	Ph.D. Spring, 2016	Process Development Engineer. Illumina, Inc, San Diego CA.
David Favero (MPS, Neff)	Transcriptional control of Arabidopsis development of AHLs	Ph.D. Spring, 2016	Postdoctoral Fellow. Riken Yokohama Institute. October 2016
Cory Gall (IID, Brayton)	Functional Characterization of the bacterial microbiome of the *Dermacentor andersoni* tick exhibits interactions with pathogen acquisition	Ph.D. Spring, 2016	Postdoctoral Fellow. University of Pretoria, South Africa
Tom Jacroux (CHEBE, Dong)	Development of microchip isotachophoresis for the detection of novel cardiac troponin I biomarkers.	Ph.D. Spring, 2016	Genentech, South San Francisco, CA.

Trainee Publications in 2015-2016

Grandi FC, Rosser JM, Newkirk SJ, Yin J, Jiang X, Xing Z, **Whitmore L**, Bashir S, Ivics Z, Izsvák Z, Ye P, Yu YE, An W. Retrotransposition creates sloping shores: a graded influence of hypomethylated CpG islands on flanking CpG sites. Genome Res. 2015 Aug;25(8):1135-46. PubMed PMID: 25995269; PubMed Central PMCID: PMC4509998.

Harrington TD, Tran VN, Mohamed A, Renslow R, Biria S, Orfe L, Call DR, Beyenal H. The mechanism of neutral red-mediated microbial electrosynthesis in Escherichia coli: menaquinone reduction. Bioresour Technol. 2015 Sep;192:689-95. PubMed PMID: 26094195; PubMed Central PMCID: PMC4516386.

Mendes, D.E.; Wong-on-Wing, A.; Berkman, C.E. "Phosphoramidate Peptidomimetic Inhibitors of Matrix Metalloproteinase 1." Journal of Enzyme Inhibition and Medicinal Chemistry. March 27, 2015, 1-5 published online ahead of print.

Nazempour A**., Quisenberry C.R**., Van Wie B.J., and Abu-Lail N.I. Nanomechanics of engineered cartilage tissues from human adipose stem cells: Synergistic influences of transforming growth factor-β3 and oscillating pressure. Journal of Nanoscience and Nanotechnology, 16(3), 3136-3145, 2016.

O'Loughlin JL, Samuelson DR, Braundmeier-Fleming AG, White BA, Haldorson GJ, **Stone JB**, Lessmann JJ, Eucker TP, Konkel ME. The Intestinal Microbiota Influences Campylobacter jejuni Colonization and Extraintestinal Dissemination in Mice. Appl Environ Microbiol. 2015 Jul;81(14):4642-50. PubMed PMID: 25934624; PubMed Central PMCID: PMC4551207.

Quisenberry C.R., Nazempour A., Van Wie B.J., and Abu-Lail, N.I. β1-integrin expression on chondrogenically differentiating human adipose-derived stem cells using atomic force microscopy. Biointerphases, Accepted, 2016.

Quisenberry C.R., Nazempour A., Van Wie B.J., and Abu-Lail, N.I. Expression of N-cadherin on chondrogenically differentiating human adipose-derived stem cells using single-molecule force spectroscopy. Journal of Nanomedicine Research, 3(1):00045, 2016. 3.

Rodriguez Y, Hinz JM, Smerdon MJ. Accessing DNA damage in chromatin: Preparing the chromatin l andscape for base excision repair. DNA Repair (Amst). 2015 Aug;32:113- 9. PubMed PMID: 25957487; PubMed Central PMCID: PMC4522338.

Stone JA, Nicola AV, Baum LG, Aguilar HC. Multiple Novel Functions of Henipavirus O-glycans: The First O-glycan Functions Identified in the Paramyxovirus Family. PLoS pathogens. 2016;12 (2):e1005445

Shockey J, Regmi A, **Cotton K**, Adhikari N, Browse J, Bates PD. Identification of Arabidopsis GPAT9 (At5g60620) as an Essential Gene Involved in Triacylglycerol Biosynthesis. Plant Physiology, Nov 2015.

Zhao Z, Eberhart LJ, Orfe LH, **Lu SY**, Besser TE, Call DR. Genome-Wide Screening Identifies Six Genes That Are Associated with Susceptibility to Escherichia coli Microcin PDI. Appl Environ Microbiol. 2015 Oct 15;81(20):6953-63. PubMed PMID: 26209678; PubMed Central PMCID: PMC4579430.