BIOGRAPHICAL SKETCH

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NAME: Mario A. Bianchet

eRA COMMONS USER NAME: MBIANCH1

POSITION TITLE: Associate Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Universidad de Nacional de La Plata	Licenciado	1983	Physics
Universidad de Nacional de La Plata	Ph.D.	1988	Physics
Johns Hopkins University	Postdoctoral	1994	Biophysics

A. Personal Statement.

My research targets macromolecules of biomedical interest and focuses on understanding the relationship between their biological function/dysfunction and three-dimensional structure, with a disease-oriented emphasis. This research has utilized Biochemical, Molecular Biology, and Biophysical methods such as X-ray diffraction and small-angle scattering, cryo-EM, computer modeling and simulation and resulted in 75 publications in high impact journals and more than 60 structural entries in the protein database.

Of significant relevance to this proposal my participation in the structural and mechanistic studies of NAD[P]H:quinone oxidoreductases (NQOs) that are paradigms in the field. I have participated in the determination of the crystal structures of NQO, of its C-terminal truncated isoenzyme NQO2; of their complexes with substrates and small ligands that were essential to characterize the enzymatic mechanism; species enzymatic differences; specificity and active site plasticity; bio-activation of antitumor prodrugs; and NQO1 inhibition.

I am presently developing a research program in structural biology focusing on diseases associated with infectious microorganisms, seeking the determination and characterization in atomic detail of pharmacologically exploitable protein-protein interactions involved in these diseases' pathogenesis progression. Two ongoing collaborations are relevant to this proposal: with Dr. Nath's group (collaborator) we are working in several systems involved in neurodegenerative diseases related with viral infections, and with Dr. Mario Borgnia (collaborator) we are elucidating the single particle structure of MICAL1 using cryo-EM and beginning to work in the determination in situ of the structures of complexes related to assembly and budding of HIV, Tat toxicity, and peptidoglycan synthesis.

Unfortunately, an illness and its long convalescence, together with the COVID19 pandemic closures and limitations, severely affected my research output and negatively impacted the last three years. production of my laboratory. Nevertheless, I managed to produce three impactful contributions recently: [4,14,15].

Ongoing and recently completed projects that I would like to highlight

R21 NS10889
Bianchet (PI)
29/01/18-8/31/21
Characterization of the redox control of HIV-Tat proteostasis by cellular NQO1

Pamela Mars Discovery Award
Bianchet (PI)
8/1/19-7/31/22
Proteopathies and HAND: HIV-Tat modulation of amyloidogenesis

Key publications:

i. Rumbaugh JA, Bachani M, Li W, Butler TR, Smith KJ, **Bianchet MA**, Wang T, Prendergast MA, Sacktor N, Nath A. HIV immune complexes prevent excitotoxicity by interaction with NMDA receptors. Neurobiol Dis. 2013 Jan;49:169-76. doi: 10.1016/j.nbd.2012.08.013. Epub 2012 Aug 25. PMID: 22940423; PMCID: PMC3556339.

ii. Urzasci L., **Bianchet, M.A.**, Cotter R. J., Nath A. *Identification Of Nitrated Immunoglobulin Variable Regions In The HIV-Infected Human Brain: Implications In HIV Infection And Immune Response.* J Proteome Res. (2014) Mar 7;13(3):1614-23. PMID:24479669

iii. Hategan A., **Bianchet M.A.,** Steiner J, Karnaukhova E, Emilios, Dimitriadis E, Haughey, NJ., Nath *A. HIV-Tat protein complexes with amyloid β peptide to form multifibrillar structures and causes synergistic neurotoxicity.* Nat Struct Mol Biol. (2017) Feb 20. [Epub ahead of print] PMID:28218748

iv. Tyagi R, Li W, Parades D, **Bianchet M.A.**, Nath A. *Inhibition of human endogenous retrovirus-K by antiretroviral drugs.* Retrovirology. (2017) Mar 22;14(1):21. PMID:28330477

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2021-	Associate Editor of Frontiers Cell and Developmental Biology in Bioinformatics and Artificial Intelligence for Molecular Medicine
2019-	Associate Professor, Department of Neurology The Johns Hopkins University, School of Medicine, Baltimore, MD
2019-	Associate Professor, Department of Biophysics and Biophysical Chemistry, The Johns Hopkins University, School of Medicine, Baltimore, MD
2018-	Adjunct Associate Professor Department of Microbiology and Immunology University of Maryland School of Medicine
2018-	Associate Editor of Frontiers Cell and Developmental Biology in Molecular Medicine
2011-2018	Assistant Professor, Department of Biophysics and Biophysical Chemistry, The Johns Hopkins University, School of Medicine, Baltimore, MD
2011-2018	Assistant Professor, Department of Neurology, The Johns Hopkins University, School of Medicine, Baltimore, MD
2007-2010	Adjunct Assistant Professor, Institute of Marine and Environmental Technology, Columbus Center, 701 East Pratt Street, Baltimore, MD 21202.
2004-2011	Instructor, JHUSOM
1999-2004	Associate Research Scientist, Department of Biophysics, Krieger School of Art and Sciences, Johns Hopkins University
1995-1999	Research Associate, Department of Biophysics and Biophysical Chemistry, JHUSOM
1990-1995	Postdoctoral Fellow, Department of Biophysics and Biophysical Chemistry, Johns Hopkins School of Medicine (JHUSOM)
1987-1988	Member of the Council of the Department of Physics, Facultad of Ciencias Exactas of The University of La Plata
1985-1987	Member of the Academic Council of the Facultad of Ciencias Exactas of The University of La Plata

Honors

2019	Pamela Mars Wright Discovery Award
2018	Michel Mirowski Discovery Award.
1984-1988	Graduated Fellowship of CICPBA. (Argentina)
1977	Undergraduate Fellowship of UNLP

C. Contributions to Science

During my career as a structural biologist and enzymologist, I have substantially contributed to the understanding of the relationship between structure and function of a number of macromolecules of biomedical relevance in different fields:

- Neurodegenerative diseases associated with viral infections (HIV-Tat, HERK protease).
- Oxidative stress, cytoprotection, and DNA repair, (NADPH Quinone oxidoreductases 1 and 2, and Uracil DNA Glycosylase, CaMKII).
- Infectious diseases (*Mycobacterium tuberculosis* L,D-transpeptidases)
- Nerve development: axon-guidance (MICAL-1).
- Bioenergetics (rat liver F1-ATPase).
- Glycobiology: carbohydrate-binding proteins (Galectin-1, F-lectins).

Neurodegenerative diseases associated with viral infections.- In this topic, I have contributed as a molecular modeler to seven publications on neurodegenerative diseases (ND) produced by a viral infection or its associated OS[1], and about Tat neurotoxic interactions with a neuronal receptor (NMDAR)], and amyloid β peptide germane to this application [2]. The endogenous HERV-K virus is reactivated in patients with amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease. The study looks for a mechanism of inhibiting this viral protease for treating this ND [3]. Galectins have been associated with HIV entry; this work models the interaction of galectins with elements of viral attachment and entry machinery [4].

1. Uzasci L., **Bianchet, M.A.**, Cotter R. J., Nath A. *Identification Of Nitrated Immunoglobulin Variable Regions In The HIV-Infected Human Brain: Implications In HIV Infection And Immune Response.* J Proteome Res. (2014) Mar 7;13(3):1614-23. PMID:24479669
2. Hategan A., **Bianchet M.A.,** Steiner J, Karnaukhova E, Emilios, Dimitriadis E, Haughey, NJ., Nath *A. HIV-Tat protein complexes with amyloid β peptide to form multifibrillar structures and causes synergistic neurotoxicity.* Nat Struct Mol Biol. (2017) Feb 20. [Epub ahead of print] PMID:28218748
3. Tyagi R, Li W, Parades D, **Bianchet M.A.**, Nath A. *Inhibition of human endogenous retrovirus-K by antiretroviral drugs.* Retrovirology. (2017) Mar 22;14(1):21. PMID:28330477
4. Ghosh A, Banerjee A, Amzel LM, Vasta GR, **Bianchet M.A**. Structure of the zebrafish galectin-1-L2 and model of its interaction with the infectious hematopoietic necrosis virus (IHNV) envelope glycoprotein. Glycobiology. (2019) May 1;29(5):419-430. doi: 10.1093/glycob/cwz015. PMID: 30834446.

Oxidative stress protection and DNA damage repair enzymes.- NAD[P]H:Quinone Oxidoreductase 1 and 2 (NQO1, NQO2). Ubiquitous antioxidant enzymes, NQO1 and NQO2, are highly expressed during the phase II cellular response to oxidative and xenobiotic stress; they carry out the obligatory two electrons reduction of toxic quinones to the more soluble and much easier excreted hydroquinones while performing the equally important function of gatekeeping the ubiquitin independent 20S proteasomal degradation pathway. NQOs dysfunction has been associated with multiple degenerative diseases, including Alzheimer's and Parkinson's diseases, cancers, and benzene toxicity. The frequent single nucleotide polymorphism (C609T) that inactivates the enzyme has been strongly associated with a predisposition to these diseases. I have highly significant contributions in the structural characterization of two members of this enzyme family on different species, elucidating their enzymatic mechanism and active-site plasticity, and the studies of their activity as bio-activator antitumor pro-drugs and enzyme inhibition [8,9]. Also, I significantly participated in characterizing and explain the observed species differences. I have performed the structural studies and modeling analysis of the Uracyl-DNA glycosylase-2 (UNG2) uracil-repair mechanism and several complexes of UNG2 with novel inhibitors. In the paper featured in Nature [10] using DNA containing modified thymine, we capture the structure of a reaction intermediate (flipping-out of the base to recognize mutated thymine) highly relevant to describe the repair mechanism of the enzyme.

5. Faig M., **Bianchet M. A.**, Winski S., Hargreaves R., Moody C. J., Hudnott A. R., Ross D., and Amzel L. M. *Structure-based development of anticancer compounds: Complexes of NAD(P)H:quinone*

oxidoreductase 1 with chemotherapeutic quinones. Structure (2001) **9**, 659-667. PMCID:PMC162127-1439.

6. Parker J. B, **Bianchet M. A**, Krosky D. J., Friedman J. I., Amzel L. M. and Stivers J. T. Enzymatic capture of an extrahelical thymine in the search for uracil in DNA. Nature (2007) Sep. 17; 449 (7161):433-7. PMCID: PMC2754044
7. Konstantinidis K, Bezzerides VJ, Lai L, Isbell HM, Wei AC, Wu Y, Viswanathan MC, Blum ID, Granger JM, Heims-Waldron D, Zhang D, Luczak ED, Murphy KR, Lu F, Gratz DH, Manta B, Wang Q, Wang Q, Kolodkin AL, Gladyshev VN, Hund TJ, Pu WT, Wu MN, Cammarato A, **Bianchet MA**, Shea MA, Levine RL, Anderson ME. MICAL1 constrains cardiac stress responses and protects against disease by oxidizing CaMKII. J. Clin Invest. (2020) Sep 1;130(9):4663-4678. doi: 10.1172/JCI133181. PMID: 32749237
8. Wang Q, Hernández-Ochoa EO, Viswanathan MC, Blum ID, Do DC, Granger JM, Murphy KR, Wei AC, Aja S, Liu N, Antonescu CM, Florea LD, Talbot CC Jr, Mohr D, Wagner KR, Regot S, Lovering RM, Gao P, **Bianchet MA**, Wu MN, Cammarato A, Schneider MF, Bever GS, Anderson ME. CaMKII oxidation is a critical performance/disease trade-off acquired at the dawn of vertebrate evolution. Nat Commun. 2021 May 26;12(1):3175. doi: 10.1038/s41467-021-23549-3. PMID: 34039988; PMCID: PMC8155201.

Infectious Diseases.- Interfering with late cell-wall biosynthesis is a successful strategy in antibiotics development. β-lactams inhibit the D,D-transpeptidases. These enzymes generate the (4,3) crosslink that makes the peptidoglycan mesh conferring to the bacteria mechanical stability. *However, mycobacterium* and a growing number of nosocomial pathogenic bacteria rely on a different crosslink, the (3,3). My group has determined the first structure of two of these enzymes (LdtMt2-5) in *Mycobacterium tuberculosis*, and it is presently performing structural studies of complexes of these enzymes with its carbapenem (a last resort antibiotic) inhibitors.

9. Erdemli SB, Gupta R, Bishai WR, Lamichhane G, Amzel LM, **Bianchet MA.** *Targeting the cell wall of Mycobacterium tuberculosis: structure and mechanism of L,D-transpeptidase 2.* Structure (London, England: 1993). 2012; 20(12):2103-15**.** PMID: 23103390**,** PMCID: PMC3573878
10. Brammer Basta LA, Ghosh A, Pan Y, Jean J, Lloyd EP, Townsend CA, Lamichhane G**, Bianchet MA**. *Loss of a functionally and structurally distinct L,D-transpeptidase, Ldt_{Mt5}, compromises cell wall integrity in Mycobacterium tuberculosis.* J Biol Chem. 2015 Aug 24. pii: jbc.M115.660753. [Epub ahead of print] PMID:26304120
11. **Bianchet M.A***, Pan Y, Brammer Basta L-A, Saavedra H., Lloyd E.P., Kumar P., Rohini Mattoo R., Townsend C.A., and Lamichhane G. *Structural insight into the inactivation of Mycobacterium tuberculosis non-classical transpeptidase LdtMt2 by biapenem and tebipenem* BMC Biochemistry (2017). *BMC Biochemistry*. PMID 28545389 (*corresponding author)

Nerve development (axon guidance).- Axon guidance is a process essential in nerve development associated with steering the axon growth across different tissues up to the point of innervation. A multidomain enzyme, MICAL-1, mediates the dramatic cytoskeleton reorganization of the axon growth-cone required to steer the axon away/towards repulsive/attractive signals. I have participated in the structural determination of two constructs: one including only the monooxygenase domain of MICAL-1 and another that also includes MICAL's calponin homology domain that interacts with F-actin. Research on the interactions of full-length MICAL-1 and other proteins of the guidance pathway is in an advanced state of preparation.

12. Nadella M., **Bianchet M. A.**, Gabelli S. B, Barrila J., Amzel L. M. *Structure and activity of the axon guidance protein MICAL.* Proc. Natl. Acad. Sci. U S A. (2005) Nov 15; 102(46): 16830-5. PMCID:PMC1277968
13. Alqassim SS, Urquiza M, Borgnia E, Nagib M, Amzel L. M and **Bianchet M.A.** *Modulation of MICAL Monooxygenase Activity by its Calponin Homology Domain: Structural and Mechanistic Insights. Nature* Scientific Reports. (2016) Mar 3;6:22176. doi: 10.1038/srep22176

Bioenergetics.- F1F0-ATP-Synthase is a large protein complex in the mitochondria that carry out the synthesis of ATP using the proton gradient across the mitochondrial membrane. I have performed the structural

determination of the soluble portion of the complex F1-ATPase from the rat liver. The observed symmetrical structure represents a critical intermediate in ATP hydrolysis and synthesis.

14. **Bianchet M. A.**, Ysern X., Hullihen J., Pedersen P. L. and Amzel L. M. *Mitochondrial ATP Synthase: Quaternary Structure of F1 Moiety at 3.6 Å Determined by X-Ray Diffraction Analysis.* J. Biol. Chem.(1991) **266**, 21197-21201.
15. **Bianchet M. A.**, Hullihen J., Pedersen P. L., and Amzel L. M. The 2.8 Å *structure of rat liver F1-ATPase: Configuration of a critical intermediate in ATP synthesis/hydrolysis.* Proc. National Acad. of Science (1998) **95**,11065-70.
16. **Bianchet M. A.**, Pedersen PL. and Amzel LM. *Notes on the Mechanism of ATP Synthesis.* Journal of Bioenergetics and Biomembranes (2000) **32** (5) 517-521. PMID:15254387.
17. **Bianchet M. A.**, Amzel L. M. *Making the right moves.* Structure. (2007) Aug;15(8):885-6. PMID:17697991

Glycobiology.- Carbohydrate binding proteins. I have determined the structure and performed modeling studies of several animal lectins involved in innate immune responses. My work has characterized a novel carbohydrate binding fold (F-lectins).

18. **Bianchet MA.**, Ahmed H., Vasta G. R. and Amzel L. M.. *Soluble beta-galactosyl-binding lectin (galectin) from toad ovary: crystallographic studies of two protein-sugar complexes.* PROTEINS (2000) **40** (3) 378-388.
19. **Bianchet M. A.***, Odom E., Vasta G. R. and Amzel L. M. *Novel Fucose Carbohydrate Recognition Domain involved in Innate Immunity.* Nature Structural Biology (2002) **9**, (8) 628-634. (*corresponding author)
20. **Bianchet, M. A.***, Odom, E. W., Vasta G. R. and Amzel L. M. *Structure and Specificity of a Binary Tandem Domain F-Lectin from Striped Bass (Morone saxatilis)*, J. Mol. Biol. (2010), Aug 13;401(2):239-52.doi:10.1016/j.jmb.2010.06.018. PMCID:PMC2935320 (*corresponding author)
21. Vasta G.R., Ahmed H., **Bianchet M. A**., Hernandez-Robledo J. A., and Amzel L.M. *Diversity in recognition of glycan by type F-lectins and Galectins: molecular, structural, and Biophysical aspects*. Ann. N. Y. Acad. of Sci., issue Glycomics of Immune System. (2012) Apr;1253:E14-26. PMCID:PMC3683447

Complete List of Published Work in NCBI MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/mario.bianchet.1/bibliography/public/