BIOGRAPHICAL SKETCH

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NAME: Christian Baron

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POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Ludwig-Maximilians-University, Munich, Germany	Diploma	04/1989	Microbiology
Ludwig-Maximilians-University, Munich, Germany	Ph.D	04/1993	Microbiology
Ludwig-Maximilians-University, Munich, Germany	Post-doc	02/1994	Microbiology
UC Berkeley, Berkeley, CA, USA	Post-doc	02/1997	Microbiology/Plant Biology

A. Personal Statement

The project proposed here continues the exploration of the structure and mechanism of type IV secretion systems (T4SS), a subject we have pursued since I became leader of an independent research group in 1997. T4SS are macromolecular transporters in many pathogenic bacterial that translocate virulence factors across the cell envelope. They typically comprise 12 membrane proteins (VirB1-VirB11 and VirD4) that assemble the translocation machinery, the extracellular pilus that contacts recipient cells and that mediate the translocation process. T4SS also mediate the transfer of plasmids between bacteria and thereby contribute to the spread of antimicrobial resistance genes, causing increased morbidity and mortality in the health system.

We primarily use the *Agrobacterium* system for our basic research on the mechanism of type IV secretion and this work has inspired research the human pathogen (*Brucella and Helicobacter*) systems. We were the first ones to study T4SS assembly using a biochemical approach with purified components and we have systematically added *in vivo* (bacterial two-hybrid system), standard biochemical (gel filtration, pulldown assays and cross-linking) and quantitative *in vitro* approaches (AUC, ITC and SPR) to study the protein-protein interactions required for complex assembly. In collaboration with G. Waksman (London, UK), James Omichinski and Jurgen Sygusch (Université de Montréal) we have gained detailed molecular insights into protein structures (using NMR spectroscopy and X-ray crystallography) and have conducted structure-function analyses based on this information. Using structural information we have designed *in vivo* VirB8 interaction and VirB11 ATPase assays, we have isolated inhibitors of T4SS functions, determined their binding sites to the targets and we have optimized inhibitor action by medicinal chemistry.

Independently, we have pursued the high resolution structural analysis of T4SS components from bacterial conjugation systems using negative-staining and cryo-electron microscopy in collaboration with Huy Bui, Natalie Zeytuni and Joaquin Ortega (McGill University). We present exciting preliminary data on the structures of the TraE/VirB8 and the TraD/VirB6 protein that were collected on a 120 kV screening microscope at Université de Montréal and on the Titan Krios 300 kV microscope at McGill University. We here propose to collect data to determine high-resolution structures on of these proteins alone, in a TraE-TraD complex as well as in complexes with DNA. The capacity limits of the instrument at McGill University are a constant impediment to the progress of our research activities. The beamtime at allocation would enable us to advance our project in a much more rapid fashion leading to scientific publications beginning in the winter of 2023.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

1993	Postdoctoral Fellow, Ludwig-Maximilians-University, Microbiology Department, Munich, Germany
1994-1996	Postdoctoral Fellow, University of California at Berkeley, Department of Plant & Microbial Biology, Berkeley, USA,
1997-2002	Assistant Professor/University Assistant, Ludwig-Maximilians-University, Microbiology Department, Munich, Germany
2002-2008	Associate Professor, McMaster University, Biology Department, Ontario, Canada
2008-2015	Professor & Chair, Université de Montréal, Department of Biochemistry, Montréal, Canada
2015-2020	Vice-Dean of Research and Development, Université de Montréal, Faculty of Medicine, Montréal, Canada
2008-now	Professor, Université de Montréal, Department of Biochemistry, Montréal, Canada
2022-now	Vice-President of Research – Programs, Canadian Institutes of Health Research (CIHR), Government of Canada, Ottawa, Canada

Honors and Committee membership

1987-1990	Undergraduate fellowship, Studienstiftung des Deutschen Volkes (German foundation for the support of excellent students)
1991	EMBO short term graduate fellowship for research exchange visits (3 months)
1994-1995	DFG Research Fellowship (2 years)
2001-2003	Euresco/European Union conference series chairperson: Two meetings on type IV Secretion Systems (2001 and 2003)
2002	Habilitation/Privatdozent, Ludwig-Maximilians University, Munich, Germany
2004-2007	Natural Sciences and Engineering Research Council of Canada (NSERC), member of grant selection committee 32/Cell Biology, co-chair 2007
2009-2015	Canadian Institutes of Health Research (CIHR), Biochemistry and Molecular Biology-A (BMA) or Microbiology and Infectious Diseases (MID), grant selection panel, member

C. Contributions to Science

C.1. Biochemistry of bacterial type IV secretion systems. We have studied the *Agrobacterium tumefaciens* T4SS since I was a postdoctoral fellow at UC Berkeley in Patricia Zambryski's lab. We made several key contributions to the understanding of the biochemistry (protein-protein interactions) of this macromolecular translocation system and of its extracellular pilus. This work contributes to the body of knowledge on the mechanism of this process that is of basic and of applied biomedical importance.

1.) **Mary C, Fouillen A**, **Bessette B,** Nanci A, **Baron C.** (2018). Interaction via the N-terminus of the type IV secretion system (T4SS) protein VirB6 with VirB10 is required for VirB2 and VirB5 incorporation into T-pili and for T4SS function, *Journal of Biological Chemistry*. 115: 5950-5955 *(*CIHR)

2.) **Villamil-Giraldo, A.-M., Mary, C., Sivanesan, D.,** and **Baron, C.** (2015) VirB6 and VirB10 from the *Brucella* type IV secretion system interact via the N-terminal periplasmic domain of VirB6. *FEBS Letts*, 589: 1883-1889 (CIHR)

3.) **Villamil-Giraldo, A.M., Sivanesan, D.**, **Carle, A., Paschos, A., Smith, M.A.,** Plesa, M., Coulton, J**.** and **Baron, C.** (2012) Type IV secretion system core component VirB8 from *Brucella* binds to the globular domain of VirB5 and to a periplasmic domain of VirB6. *Biochemistry*, 51: 3391-3390 (CIHR)

4.) **Sivanesan, D.**, and **Baron, C.** (2011) Dimerization of VirB8 is important for stabilization of the type IV secretion system and for association of VirB2 with the core complex. *J. Bacteriol.,* 193:2097-106. (CIHR)

5.) **Aly, K. A.** and **Baron, C.** (2007) The VirB5 protein localizes to the T-pilus tips in *Agrobacterium tumefaciens*. *Microbiology* 153 : 3766-3775

6.) **Paschos, A.,** Patey, G., **Sivanesan, D., Gao, C.,** Bayliss, R., Waksman, G., O'Callaghan, D. and **Baron, C.** (2006) Dimerization and interactions of *Brucella suis* VirB8 with VirB4 and VirB10 are required for its biological activity. *Proc. Natl. Acad. Sci. USA* 103: 7257-7257

7.) **Yuan, Q., Carle, A., Gao, C., Sivanesan, D., Aly, K., Höppner, C., Krall, L. Domke, N.,** and **Baron, C.** (2005) Identification of the VirB4-VirB8-VirB5-VirB2 pilus assembly sequence of type IV secretion systems. *J. Biol. Chem.* 280: 26349-26359

8.) **Krall, L., Wiedemann, U., Unsin, G., Weiss, S., Domke, N.** and **Baron, C.** (2002) Detergent extraction identifies different VirB protein subassemblies of the type IV secretion machinery in the membranes of *Agrobacterium tumefaciens. Proc. Natl. Acad. Sci. USA* 99 : 11405-11410

C.2. Inhibitors of bacterial type IV secretion systems. Since my initial appointment at McMaster University in 2002 we have established innovative screening systems to identify inhibitors of T4SS in *Brucella*, *Helicobacter* and in bacterial conjugation systems. These molecules have potential for the development into anti-virulence drugs for the treatment of bacterial infections and as inhibitors of bacterial conjugation and antimicrobial resistance gene spread.

1.) **Arya T., Oudouhou F., Casu B., Bessette B.,** Sygusch J. and **Baron C.** (2019). Fragment-based screening identifies inhibitors of the ATPase activity and of hexamer formation of Cagα from the *Helicobacter pylor*i type IV secretion system. *Scientific reports*. 9 : 6474 (CRS)

2.) **Casu, B., Arya, T., Bessette, B.,** and **Baron, C.** (2017) Fragment-based screening identifies novel targets for inhibitors of conjugative transfer of antimicrobial resistance by plasmid pKM101, *Sci. Rep. (*CIHR) 7: 14907 doi: 10.1038/s41598-017-14953-1

3.) **Smith, M.A., Coinçon, M., Paschos, A.,** Jolicoeur, B., Lavallée, P., Sygusch, J. and **Baron, C.** (2012) Identification of the binding site of *Brucella* VirB8 interaction inhibitors. *Chem. & Biol.* 19 : 1041-8 (CIHR)

4.) **Paschos, A.,** den Hartigh, A., **Smith, M. A.,** Atluri, V.L., **Sivanesan, D.,** Tsolis, R.M. and **Baron, C**. (2011) An *in vivo* high-throughput screening approach targeting the type IV secretion system component VirB8 identified inhibitors of *Brucella abortus* 2308 proliferation. *Infect. & Immun. 79 :* 1033–1043. (CIHR)

C.3. Structural biology and mechanism of bacterial type IV secretion systems. In collaborations with specialists in X-ray crystallography, NMR spectroscopy and cryo-EM we have made significant contributions to the understanding of the molecular mechanism of the T4SS. This work is of basic as well as of applied importance since high-resolution structures can guide the development of T4SS inhibitors into drugs.

1.) **Amro A**, Black C, **Jemouai Z, Rooney N**, Daneault C, Zeytuni N, Ruiz M, Bui KH, and **Baron C.** (2022). Cryo-EM structure of the *Agrobacterium tumefaciens* T-pilus reveals the importance of positive charges in the lumen, submitted to *Structure*, https://www.biorxiv.org/content/10.1101/2022.04.28.489814v1

2.) **Casu B, Mary C,** Sverzhinsky A, **Fouillen A**, Nanci A, **Baron C**. (2018). The VirB8 homolog TraE from the plasmid pKM101 type IV secretion system interacts with TraD and forms a hexameric ring-like pore structure, *Proceedings of the National Academy of Sciences of the USA,* 115: 5950-5955 *(*CIHR)

3.) **Sharifahmadian, M., Nlend, I.U.,** Lecoq, L., Omichinski, J.G. and **Baron, C.** (2017) The type IV secretion system core component VirB8 interacts via the β1-stand with VirB10. *FEBS Letts.,* 591: 2491-2500 (CIHR)

4.) **Sharifahmadian, M., Arya, T., Bessette, B.,** Lecoq, L., Ruediger, E., Omichinski, J.G. and **Baron, C.** (2017) Monomer-to-dimer transition of *Brucella suis* type IV secretion system component VirB8 induces conformational changes, *FEBS J.,* 284 : 1218-1232 (CIHR)

5.) **Smart, J., Fouillen, A.**, Casu, B., Nanci, A. and **Baron, C.** (2017) Cag-delta protein from the Helicobacter pylori 26695 cag type IV secretion system forms ring-like supramolecular assemblies. *FEMS Microbiol. Letts* 364, 1-7 (CIHR)

6.) **Casu, B., Smart, J.,** Hancock, M.A., **Smith, M.,** Sygusch, J. and **Baron, C.** (2016) Structural analysis and inhibition of TraE from the pKM101 type IV secretion system. *J. Biol. Chem.* 291 : 23817-23829 *(*CIHR)

7.) Yeo, H.-J., **Yuan, Q.,** Beck, M. R., **Baron, C.**, Waksman, G. (2003) Structural and functional characterization of the VirB5 protein from the type IV secretion system encoded by the conjugative plasmid pKM101. *Proc.Natl. Acad. Sci. USA* 100 : 15947-15962

8.) Terradot, L., Bayliss, R., Oomen, C., Leonard, G., **Baron, C.,** Waksman, G. (2005) Structures of two core subunits of the bacterial type IV secretion system, VirB8 from *Brucella suis* and ComB10 from *Helicobacter pylori*. *Proc. Natl. Acad. Sci. USA* 102: 4596-4601