
BIOGRAPHICAL SKETCH

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NAME: Bogdanov, Mikhail

eRA COMMONS USER NAME (credential, e.g., agency login): MVBOGDANOV

POSITION TITLE Professor of Biochemistry and Molecular Biology

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
Voronezh State University & Moscow State University, Russia	B.S., M.S. Summa Cum Laude	07/1980	Biochemistry
USSR Academy of Sciences, Pushchino, Moscow region, Russia	Ph.D.	09/1989	Biochemistry
The University of Texas Medical School Houston, Texas	Postdoc	01/1997	Biochemistry

A. Personal Statement

The goal of my first current project is to investigate the role of lipids in the membrane protein biogenesis, structure and function. Specifically, we are investigating the molecular mechanism of lipid-dependent membrane protein topogenesis by testing further a Charge Balance Rule for different proteins and lipid profiles *in vivo* and *in vitro* in order to establish the physiological significance of membrane protein topological heterogeneity and post-assembly dynamic changes in topological organization. The goal of my second project is to test a hypothesis that conformation of outer membrane β -barrel porins from pathogenic bacteria may adapt to *in vivo* changes in phospholipid composition in order to optimize or modulate their activity or folding and assembly pathway. The main hypothesis to be tested is that endogenous lipids with different molecular structure and thermotropic behavior act as molecular chaperones affecting conformational maturation of the model channel-forming oligomeric protein porin YOmpF from Gram-negative psychrotrophic bacteria *Y. pseudotuberculosis* resulting in optimization of functional properties of membrane proteins in a changing environment. The second goal of proposal is to investigate the role of glycerophospholipid asymmetry, externalization and remodeling within bacterial envelope in the development of bacterial resistance to antibiotics and ability to resist innate immunity of the host. We will address the question whether these adaptive changes are important not only for porin-mediated antibiotic permeability but also for the development of bacterial virulence and resistance to antibiotics and innate immune system of infected host and thus could be utilized to increase drug susceptibility of pathogenic microorganisms to known antibiotics and innate immune system. I have the expertise based on my position first as a Postdoctoral Fellow (beginning in 1991), Rinshoken Investigator (1997), co-investigator (since 1997) on NIH grant and Principal investigator of two Marie Skłodowska-Curie Research and Innovation Grant from European Commission (since 2015) and Project Director of NATO Science for Peace and Security Programme (since 2017) that provided the first evidence for lipid assisted folding and lipid-dependent topogenesis of membrane proteins in response to the changes in the lipid environment. My early interest in lipid-dependent membrane protein folding and topogenesis began from my M.Sc. project on involvement of phospholipids in the mechanism of protein translocation in bacteria headed by Professors Marina Nesmeyanova and Igor Kulaev at USSR Academy of Sciences. Since then I have been intrigued by the mechanism by which newly synthesized proteins are inserted into biological membranes. This led me to Professor William Dowhan's laboratory initially as a postdoctoral fellow where my journey with lipids along membrane protein folding pathways has continued. Over the years and since I was appointed to the Biochemistry Faculty, I invented and applied novel techniques

(coupled cell-free protein and phospholipid biosynthesis system, Eastern-Western blotting technique and novel genetic manipulation with *E. coli* "lipid" genes), which led to the discovery of novel functions for membrane phospholipids acting as molecular chaperones (lipochaperones) interacting transiently and reversibly with their membrane protein substrates. I further developed the Substituted Cysteine Accessibility Method (SCAM™) and brought this approach to its current state of development as evidenced by several invited published protocols in *Methods/Methods in Enzymology* (2005) and *Methods in Molecular Biology/Springer Protocols* (2010 and 2017). The application of this technique and using of *E. coli* "lipid" mutants established for the first time the membrane protein structure determining power of lipids by demonstrating that the topology of polytopic transmembrane proteins could be reversed bi-directionally simply by changing the lipid composition of the membrane. I always attempt to share my expertise in lipid metabolism, membrane proteins and lipid-protein interactions with my colleagues in Department, Medical School, University of Texas Health Science Center as well as worldwide. My fruitful collaboration with University of Texas MD Anderson Cancer Center resulted in the development of novel radiolabeled assay for PI(3,4,5)P₃ 3'phosphatase activity of PTEN tumor suppressor protein, discovery of novel interactions between transiently phosphorylated growth factor receptor-bound protein 2 (Grb2) and fibroblast growth factor receptor 2 (FGFR2) leading to activation of phospholipase C gamma-1 (PLC γ-1) and publication in *Nature Structural and Molecular Biology*. This activation/regulation cascade was successfully reconstituted *in vitro* in phospholipid micelle based system which contained all purified protein components (Grb2, FGFR, PLC γ-1) and radiolabeled PLC γ-1 substrate PtdIns (4,5) P₂. *In vitro* results perfectly matched metastatic outcome of this cascade *in situ* and *in vivo* (xenograft animal model) and recently been published in *Oncogene*. My current interests include also protein and lipid topogenesis and the origin and maintenance of transmembrane phospholipid asymmetry in plasma membrane of normal and cancer cells, apoptotic bodies and exosomes.

B. Positions and Employment

1980-1981 Junior Research Scientist, Department of Plant Physiology and Biochemistry, Voronezh State University, Voronezh.
1981-1991 Junior Research Scientist and Research Scientist, Laboratory of the Regulation of Biochemical Processes, Group of Biochemistry of Protein Secretion in Bacteria. Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences. Pushchino, Moscow Region, Russia.
1991-1997 Research Fellow I, II, III, Department of Biochemistry and Molecular Biology, University of Texas Medical School, Houston.
1997-2002 Assistant Professor at Department of Biochemistry and Molecular Biology, University of Texas Medical School, Houston
2002-2017 Associate Professor at Department of Biochemistry and Molecular Biology, University of Texas Medical School, Houston
2017 - present Professor at Department of Biochemistry and Molecular Biology

Other Experience and Professional Memberships

1994-present American Society for Biochemistry and Molecular Biology, Member
1994-present American Society for Microbiology, Member
1998 Visiting Professor at Department of Molecular Biodynamics (Director M.Umeda), The Tokyo Metropolitan Institute, Japan
2001-2002 Co-Chair and organizer of Annual Texas Protein Folders Meeting
2017-present Lipid Research Division of ASBMB, Member

Membership on Editorial Boards:

Journal of Biological Chemistry (since 2020)
Biomolecules, Senior Editorial Board (since 2019)
International Journal of Biochemistry and Molecular Biology, Senior Editorial Board (since 2011)
Journal of Molecular Microbiology and Biotechnology Co-editor (with Milton Saier) of Volume on "Prokaryotic Membrane-bounded Organelles: Intracellular and Extracellular prokaryotic membranes" (2012)
Member of Review panel the Pegasus-The Research Foundation – Flanders (Belgium) (2016-present)

Honors and Awards

1998 Rinshoken Research Award (Japan) on "Invention of Eastern-Western, a novel membrane protein refolding blotting technique" and "A study on the molecular chaperone function of phospholipids"
2000 Jump Start Program Award from The University of Texas - Houston Medical School
2014 Open Lecture at Stockholm University, Science for Life Laboratory, Sweden
2020 Life Sciences Faculties Tenure Track Appointments Committee, Weizmann Institute of Science, Israel

C. Contribution to Science

1. Traditionally, molecular chaperones have been a class of proteins that bind transiently to substrate proteins to promote their proper folding by interacting non-covalently with non-native folding intermediates and not with either the native or totally unfolded protein. When folding is complete, molecular chaperones are not required to maintain proper conformation. To test an idea that specific lipids can facilitate membrane protein folding through transient interaction i.e. act in the way molecular chaperones of protein origin an Eastern-Western blotting was invented by me as a first technique which allows one to identify the transient effect of lipids on membrane protein conformation. This technique makes possible the detection of proper membrane protein refolding (with the aid of specific phospholipid) followed by transfer of proteins onto the same nitrocellulose membrane preblotted with selected phospholipids (TLC-blotting, Eastern or Fare-Eastern). By using this and other techniques I have demonstrated that one of the most abundant bacterial lipid, phosphatidylethanolamine (PE), is required either during refolding of partially denatured protein in vitro or during the assembly in vivo but is not required once “native structure” has been attained. Thus novel function of lipids acting as molecular chaperones was discovered. Discovery of the first molecular chaperone of non-protein origin led to introduction of concepts of lipid-assisted membrane protein folding and membrane “protein conformational memory” which led to discovery of new lipochaperones by many labs since 1996.

- a. **Bogdanov, M.**, Sun, J., Kaback, H.R. and Dowhan, W.: A phospholipid acts as a chaperone in assembly of membrane transport protein. *J. Biol. Chem.* **271**: 11615-11618, 1996. (considered as *JBC Classics*)
- b. **Bogdanov M.** and Dowhan W.: Phospholipid-assisted protein folding: phosphatidylethanolamine is required at a late step of the conformational maturation of the polytopic membrane protein lactose permease. *EMBO J.* 1998 **17**: 5255. . PMID 1170853
- c. **Bogdanov, M.** and Dowhan, W.: Lipid-assisted protein folding. *J. Biol. Chem.* **274**: 36827-36830, 1999.
- d. ***Bogdanov, M.**, Heacock, P., Guan, Z., Dowhan, W. Plasticity of lipid-protein interactions in the function and topogenesis of the membrane protein lactose permease from *Escherichia coli*. *Proc Natl Acad Sci U S A.* **107**:15057-62, 2010. PMID 2930521

2. Since translational gene fusion approaches which were developed in 90s could not faithfully assign the predicted transmembrane topology for many polytopic membrane proteins, I have developed further the Substituted Cysteine Accessibility Method as applied to TMs (SCAM™) and brought this approach to its current state of development. SCAM™ is based on the controlled membrane permeability of the thiol-specific reagent MPB (maleimide attached to biotin), In this approach cysteine replacements in otherwise cysteine-less protein are expressed and reactivity with MPB in intact cells (extracellular exposure) or only after cell disruption by sonication (cytoplasmic exposure) was used to establish TM orientation. SCAM™ was also further developed to map a dual, mixed or unusual membrane protein topology in either intact cells, isolated membranes vesicles or liposomes by using a two-step labeling protocol. Thus this strategy can be adapted to any membrane system.

- a. **Bogdanov, M.**, Zhang, W., Xie, J., Dowhan, W.: “Transmembrane protein topology mapping by the substituted cysteine accessibility method (SCAM™): application to lipid-specific membrane protein topogenesis”. *Methods.* **36**:148-171. 2005.
- b. ***Bogdanov, M.**, Heacock, P., Dowhan, W.: Study of polytopic membrane protein topological organization as a function of membrane lipid composition. *Methods in Molecular Biology* **619**:79-101, 2010. PMID 3099133.
- c. Dowhan, W., ***Bogdanov, M.** Molecular genetic and biochemical approaches for defining lipid-dependent membrane protein folding. *Biochim. Biophys. Acta.* **1818**(4):1097-107, 2012. PMID 3253330
- d. ***Bogdanov, M.** Mapping of Membrane Protein Topology by Substituted Cysteine Accessibility Method (SCAM™) *Methods in Molecular Biology.* **1615**:105-128, 2017. PMID: 28667607

3. The application of the advanced SCAM™ technique to membrane proteins expressed in mutants of *E. coli* in which membrane phospholipid composition can be changed either before or after membrane protein synthesis and assembly helped us to (i) to establish for the first time the topology determining power of lipids by demonstrating that the topology of a polytopic transmembrane protein could be reversed simply by changing the lipid composition of membranes (ii) to discover the mechanism by which membrane proteins can exhibit structural and functional duality in the same membrane or different membranes by demonstrating a lipid-dependent generation of dual topology for a membrane protein in vivo and in vitro. These results clearly demonstrated that

the lipid composition is a determinant of transmembrane domain orientation and challenges the dogma that once transmembrane domain orientation is established during assembly it is static and not subject to change.

- a. **Bogdanov, M.**, Heacock, P. and Dowhan, W.: A polytopic membrane protein displays a reversible topology dependent on membrane lipid composition. *EMBO J.* 21: 2107-2116, 2002. PMID 125992
- b. **Bogdanov, M.**, Jun Xie, J., Heacock, P., Dowhan, W. To flip or not to flip: protein–lipid charge interactions are a determinant of final membrane protein topology *J.Cell Biology.* 182: 925-935, 2008. PMID 252857.
- c. ***Bogdanov, M.** and Dowhan, W.: Lipid-dependent generation of a dual topology for a membrane protein. *J. Biol. Chem.* 287: 37939-37948 (2012). PMID 348806
- d. Vitrac, H., Dowhan, W., ***Bogdanov, M.** Effects of mixed proximal and distal topogenic signals on the topological sensitivity of a membrane protein to the lipid environment. *Biochim. Biophys. Acta.* 1859(7):1291-1300, 2017. PMID: 28432030

4. SCAMTM experiments with membrane protein expressed in various "lipid mutants" or reconstituted in liposomes allowed us to (i) establish the physiological basis for the Positive Inside Rule of polytopic membrane protein assembly; (ii) propose a thermodynamically based model for how changes in lipid composition can result in changes in the ratio of topologically distinct conformers of proteins and; (iii) understand how lipid-protein interactions govern reversible flipping in both directions. Such lipid-dependent post-insertional and post-reconstitution reversibility of transmembrane domain orientation indicates a thermodynamically driven process that can occur at any time and in any cell membrane driven by changes in the lipid composition. According to the new Charge Balance Rule, lipid-protein interactions affect the potency of charged residues as topological signals. An increase or decrease of PE level simply tips the topological equilibrium of LacY toward a correct or an inverted TM topology demonstrating that membrane protein topogenesis is probabilistic by nature. This proof of principle observation has important implication for membrane proteins in eukaryotic cells and can provide important information for understanding the unresolved mechanism of self-propagating conformational diseases. The results provide a thermodynamic basis for lipid-dependent interconversions between populations of native and non-native conformers separated by a high activation energy that prevents equilibration unless lipid composition is changed.

- a. ***Bogdanov, M.**, Jun Xie, J., Dowhan,: Lipid-protein interactions drive membrane protein topogenesis in accordance with the positive-inside rule *J.Biol.Chem.* 284: 9637-9641, 2009. PMID 2665083.
- b. Dowhan, W, and ***Bogdanov, M.**: Lipid-dependent topogenesis. *Annu. Rev. Biochem.* 78:515-540, 2009. PMID: 3033430.
- c. Vitrac, H., ***Bogdanov, M.** and Dowhan,: W. *In vitro* reconstitution of lipid-dependent dual topology and post-assembly topological switching of a membrane protein. *Proc. Nat'l. Acad. Sci., U.S.A* 110: 9332-9337 (2013). PMID 3677496.
- d. ***Bogdanov, M.**, Dowhan, W., Vitrac, H.: Lipids and Topological Rules Governing Membrane Protein Assembly. *Biochim. Biophys. Acta* 1843:1475-1488, 2014. PMID 4057987

5. By mimicking *Yersinia pseudotuberculosis* transition from temperature conditions of saprophytic growth in soil (< 8°C) to those in warm-blooded infected mammals (37°C) we demonstrated at first time the unique ability of this bacteria to reciprocally regulate the level of LPE and ratio of anionic PG and net neutral PE within envelope of **aerobically grown cells. We extended this finding in this proposal and analyzed distribution of phospholipids in *Y. pseudotuberculosis* grown at anaerobic conditions in the presence of glucose e.g. conditions closely imitating parasitic stage of growth.** Differential scanning calorimetry and intrinsic protein fluorescence demonstrated that the increase of LPE content and the corresponding increase in the phase transition temperature of bacterial lipids was accompanied by enhanced OM trimeric YOmpF protein thermostability. Deconvolution of fluorescence spectra have shown that thermostabilising effect of LPE was due to its effect on the tertiary structure of porin. Unsaturated LPE increases the protein stability due to more dense packing of monomers in porin and preserves its trimeric form at elevated temperature, while saturated LPE weakens the contact between monomers and promotes dissociation of the protein monomers. It was suggested that these rearrangements in conformation of YOmpF might specifically regulate the porin channel permeability at stress conditions for bacteria and during switches of life cycles. It was demonstrated that adaptive accumulation of LPE **in aerobically glucose grown *Y. pseudotuberculosis*** coincides with increase of minimal inhibitory concentration (MIC) for ampicillin. It was proposed that adaptive changes in lipids composition can be directly related to the development of their resistance to antibiotics.

- a. Sanina, N., Davydova, L., Bakholdina, S., Novikova, O., Pornyagina, O., Solov'eva, T., Shnyrov, V. and ***Bogdanov, M.** Effect of phenol-induced changes in lipid composition on conformation of OmpF-like porin of *Yersinia pseudotuberculosis*. *FEBS Lett.* 587:2260-5, 2013. PMID: 23742936.
- b. Davydova, L., Sanina, N., Novikova, O., Portniagina, O., S Bakholdina, S., Khomenko, V., Velansky, P., Vorobyeva, N., Shnyrov, V., and ***Bogdanov, M.** "Opposite Effects of Lysophosphatidylethanolamines on Conformation of OmpF-like Porin from *Yersinia pseudotuberculosis*. *Protein & Peptide Letters.* 2015 22(12):1060-5. PMID: 26349609.
- c. Davydova, L., Bakholdina, S., Barkina, M., Velansky, P. **Bogdanov, M.** and Sanina, N. Effect of elevated growth temperature and heat shock on lipid composition of inner and outer membranes of *Yersinia pseudotuberculosis*. *Biochimie*, 2016 123:103-9. PMID:26853818
- d. Sanina, N., Pomazenkova, L., Bakholdina, S., Chopenko, N., Zabolotnaya, A., Reutov, V., Stenkova, A., Bystritskaya, E., and ***Bogdanov, M.** Relationship between adaptive changing of lysophosphatidylethanolamine content in bacterial envelope and ampicillin sensitivity of *Yersinia pseudotuberculosis*. *Journal of Molecular Microbiology and Biotechnology*, 2019 28(5):236-239 PMID: 30844797

* indicated shared correspondence or corresponding author. These are the major directions of my current research program. My complete list of published work (*h index* = 32) includes 85 original research manuscripts and 9 book chapters. 70 of these publications are available through PubMed.

Complete List of Published Work in MyBibliography:

<https://www.ncbi.nlm.nih.gov/myncbi/mikhail.bogdanov.1/bibliography/public/?page=1>

D. Additional Information: Research Support

Ongoing Research Support

SPS 985291, NATO Science for Peace and Security Programme Bogdanov (PI) 05/2017- 07/2020
"A novel method for the detection of biohazards" The goal of this just awarded project is to develop a new technology for quick screening for various bacterial biohazards. This technology is based on the changes in lipid composition that are correlated with pathogenic potential of common pathogenic bacteria (such as *Helicobacter pylori*, *Yersinia pestis* and *Vibrio cholera*) and on spectroscopic sensing of the membrane lipids.
 \$ 432,151 Role: Project Director/Principal Investigator

H2020-MSCA-RISE EU Bogdanov (PI) 08/2016-08/2020
"The influence of the cell membrane asymmetry and curvature on the functioning of membrane proteins". The goal of this study is to develop convergent methodology of studying asymmetric lipid membranes, lipid-protein and drug-membrane interactions by the combination of experimental techniques and computer simulations. To quantify energetic cost of envelope remodeling that underlie the lipid-dependent protein rearrangement and interaction of envelope components with antibiotics and their adjuvants.
 \$ 571,530 Role: Principal Investigator

R01 GM121493

National Institutes of Health General Medical Science Bogdanov (Co-PI) 07/2017-06/21
"Protein sequence determinants and properties of the lipid bilayer that govern membrane protein dynamic organization"

The long-term goal of this project is to investigate the role of lipids in the synthesis and assembly of the cell membrane particularly in membrane protein topogenesis of polytopic membrane proteins. A broad range of recombinant bacteria with varied lipid composition and novel techniques will be utilized to craft a Charge Balance Rule for membrane protein topogenesis which supports the synergistic relationship between interfacial charge of extramembrane domains and the negative charge density of the membrane surface in maintaining native topological organization of membrane protein.

Annual direct funding of \$312,000. Role: Co-Investigator