OMB No. 0925-0001 and 0925-0002 (Rev. 11/16 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.  
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NAME: Konigsberg, William

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

POSITION TITLE: Professor of Molecular Biophysics & Biochemistry

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 |
| --- | --- | --- | --- |
| Rensselaer Polytechnic Inst., NY  Columbia University, NY | B. Sci  Ph.D, | 1952  1956 | Chemistry Organic  Chemistry |
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**A. Personal Statement**

My lab has been focused almost exclusively on studying the structure and function of DNA polymerases and their associated replication partner proteins. We cloned, sequenced and produced some of the key proteins required for T4 phage replication including its DNA polymerase, clamp, clamp loader and its single strand binding protein. We collaborated in determining the 3D structure of the DNA polymerase from phage RB69 a close relative of phage T4. This was the first B family DNApol whose structure was determined at atomic resolution. It has served as a model and a reference for all B family DNA pols that followed. We then showed which amino residues were important for catalysis and for base selectivity. We have used X-ray crystallography, pre-steady state kinetics and fluorescence, both on the ensemble and single molecule level, to relate structure to function for this class of enzymes which are essential for maintaining the genetic integrity of an organism from one generation to the next. Our current studies are aimed at understanding the structural and dynamic features that determine replication fidelity at an atomic level. For these reasons, I believe that I have the necessary leadership and research experience to successfully carry out the research project proposed in this application. I have been supported by NIH (GM) for the last 50 years. I have also been supported for ten years by a program project grant from HL that resulted in the identification and cloning of the gene for tissue factor, an essential component of the extrinsic pathway of blood coagulation. This new project on the biophysical properties of a tumor suppressor protein (PSF) demands expertise in biophysical and molecular biological methods such as cloning, protein expression, fluorescence spectroscopy and X-ray crystallography. These are areas which are included in publications that have appeared in prominent journals where I am the senior author.. The current proposal is a joint project with Dr. Alan Garen, who pioneered many of the biological studies on PSF and was the first to demonstrate that it acted as a tumor suppressor protein, showing that transcription repression can be reversed by lncRNAs such as VL 30-I in the mouse and MALAT-I in humans. We have assembled a competent team: Aristidis Sachpatzidis, an expert X-ray crystallographer, and Stephen Chin-Bow, who has performed many of the cloning, mutagenesis and protein production experiments described in the preliminary results of this application.

1. Luo, G., Wang, M., Konigsberg, W.H. and Xie, S. (2007). Conformational Changes in a T7 DNA Polymerase-Primer/Template Complex During Catalysis Observed at a Single-Molecule Level, PNAS 104:12610-5
2. Song Y., Wang, B., Bromberg, M., Hu, Z., Konigsberg, W. and Garen, A. (2002). Retroviral-mediated transmission of a mouse VL30 RNA to human melanoma cells promotes metastasis in a SCID mouse model. Proc. Nat. Acad. Sci (USA) 99:6269-6273.
3. Xia, S., Wang, M., Blaha, G., Koniigsberg, W., Wang, J. (2011). Structural Insights into Complete Metal Ion Coordination from Ternary Complexes of B Family RB69 DNA Polymerase. Biochemistry 50:9114-9124.
4. Xia, S., Konigsberg, W.H. (2014). RB69 DNA Polymerase Structure, Kinetics, and Fidelity. Biochemistry 53: 2752-67 PMCID: PMC4018061.

**B. Positions and Honors**

**Positions and Employment**

1956 - 1957 N.S.F. Fellow, The Rockefeller Institute

1957 - 1959 Research Associate, The Rockefeller Institute

1959 - 1964 Assistant Professor, Rockefeller University

1964 - 1976 Associate Professor of Biochemistry, Yale University

1976 - 1984 Professor of Molecular Biophysics and Biochemistry and genetics, Yale University

1984 - 1987 Chairman, Department of Molecular Biophysics and Biochemistry, Yale University

1987 - present Professor, Molecular Biophysics and Biochemistry, Yale University

**Other Experience and Professional Memberships**

1968 - 1972 Editorial Board: Archives of Biochemistry

1969 - 1973 Editorial Board: Biochem. Biophys. Acta.

1969 - 1970 American Society of Biological Chemistry (Membership Committee)

1970 - 1974 National Institutes of Health, Biochemistry Study Section

1970 - 1974 National Institutes of Health, Physiological Chemistry Study Section

1974 - 1984 U.S. Israel Binational Science Foundation

1976 - 1984 Minority Career Opportunity Section – Advisory Council, National Institutes of Health Ad Hoc

Consultant: National Science Foundation; American Cancer Society; Heart and Lung Institute

1976 - 1977 Chairman: Gordon Conference of Proteins

1980 - 1985 National Science Foundation Study Section

1984 - present Federation American Society for Experimental Biology

1986 - 1992 Editorial Board: Proteins: Structure, Function and Genetics

2000 - present Reviewer for Journal of Biological Chemistry, NAR, PNAS, Biochemistry, EMBO.J.,

PLOS, Science, Nature

2009 Invited seminar speaker Mount Sinai Hospital

2009 - 2011 Special NIH study section for large program projects

2012 Invited seminar speaker Penn State

2012 Invited seminar speaker University of Rochester

2013 Invited seminar speaker New York University

2014 Invited seminar speaker Indiana University

2015 Invited speaker Enzyme Mechanism Conference, Galveston, Texas

2015 Invited speaker for the annual conference on enzymology, Galveston, Texas

2015 Invited seminar speaker NIEHS

1. **Contributions to Science**

1. **Cloning and expression of the Human Tissue Factor (hTF) cDNA.**

There is a long history filled with controversy about the relative roles of the intrinsic and extrinsic pathways regarding the initiation of blood coagulation. Although it was known for many years that tissue factor (TF) had a role in blood clotting, its importance relative to the role of the well-studied intrinsic system was unclear and controversial... Together with the lab of Yale Nemerson the details of the entire pathway, which included kinetics of the extrinsic system were worked out and shown to be the major initiator of blood coagulation. The cloning and expression of hTF cDNA enabled *in vitro* testing of hTF which established the central role of the extrinsic pathway in blood coagulation. Recombinant human TF is used in every diagnostic lab for determining patients’ clotting times. I was the PI on the project directed at purifying and cloning the TF gene and the expression of human TF in E.coli.

1. Konigsberg, W.H. and Nemerson, Y. Molecular cloning of the cDNA for human tissue factor. *Cell* 52, 639 (1988). PMCID: PMC 3345565
2. Banner, D.W., D’Arcy, A., Chene, C., Winkler, F.K., Guha, A., Konigsberg, W.H., Nemerson, Y. and Kirchhofer, D. The crystal structure of the complex of blood coagulation factor VIIa with soluble tissue factor. *Nature* 380, 41-46 (1996). PMID 8598903
3. Konigsberg, W., Kirchhofer, D, Riederer, M. and Nemerson, Y. (2001) The TF:VIIa Complex: Clinical Significance, Structure-Function Relationships and Its Role in Signaling Metastasis, *Thromb. Haemost*. 86: 757-71. PMID 11583305
4. Kirchhofer, D., Guha, A., Nemerson, Y., Konigsberg, W.H., Vilbois, F., Chene, C., Banner, D. W. & D’Arcy, A. (1995) Activation of blood coagulation factor VIIa with cleaved tissue factor extracellular domain and crystallization of the active complex. Protein: Struc., Fun. And Genet. 22, 419-425 PMID 7479715
5. **Structure / Function Relationships In RB69 Phage DNA polymerase, a close analog to human DNA polymerase alpha and delta.**

When this project was started it was not clear how fidelity of DNA replication was achieved. The structure of phage RB69 DNA polymerase was the first complete structure of a replicative DNA polymerase that had sequence hormology with human DNA polymerases. We were able to produce the recombinant DNA polymerase in E. coli. By introducing site-specific mutations, we could relate structure to function and understand the role of particular amino acid residues in maintaining fidelity. This has been important not only for understanding how the integrity of the genome is preserved but also has been crucial in improving methods for DNA sequencing. I was the P.I. on the project of cloning, and expressing RB69 pol in E.coli and in helping to determine its structure and those of mutant RB69 complexes with DNA.

* 1. Wang, J., Sattar, A.K.M., Wang, C.C., Karam, J.D., Konigsberg, W.H. and Steitz, T.A. (1997) Crystal structure of a pol a family replication DNA polymerase from backteriophage RB69. Cell 89, 1087-1099. PMID 9215631
  2. Xia, S., Eom, S., Wang, J., Konigsberg, W.H. (2012) Structural basis for different insertion kinetics of dNMPs opposite a difluorotoluene nucleotide residue. Biochemistry 51: 1476-85 PMCID: PMC3292180
  3. Xia, S., Konigsberg, W.H. (2014) RB69 DNA Polymerase Structure, Kinetics, and Fideity. Biochemistry 53: 2752-67 PMCID: PMC4018061
  4. Wang M, Lee HR, Konigsberg W. (2009) Effect of A and B Metal Ion Site Occupancy on Conformational Changes in an RB69 DNA Polymerase Ternary Complex. *Biochemistry* 48(10):2075-86.

1. **The development of protein-induced fluorescent enhancement (PIFE) as a method for monitoring conformational changes in protein DNA complexes and protein RNA** **complexes**.

These was a paucity of methods, especially at the single-molecule level to detect conformational changes in protein DNA complexes undergoing enzymatic reactions. We were the first to develop the PIFE method for measuring conformational changes in protein complexes at the single-molecule level. This was based on the observation that the intensity of a fluorescent dye attached to DNA or RNA is enhanced when the labeled nucleic acid is bound to a protein. This method of protein-induced fluorescence enhancement can be employed at the ensemble or single molecule level to study protein-nucleic acid interactions. This method has been used in conjunction with smFRET to measure distances between labeled bases in DNA and for RNA when complexed with proteins that are undergoing processes involving conformational changes. We plan to use PIFE in the projects that are outlined in this grant application. I was a collaborator with Dr. Luo in Sunny Xie’s lab in the development of this method which we plan to use in our current grant application.

1. Luo, G., Wang, M., Konigsberg, W.H. and Xie, S. (2007) Single-molecule and Ensemble fluorescence assays for a functionally important conformational Changes in a T7 DNA Polymerase-Primer/Template Complex During Catalysis Observed at a Single-Molecule Level, *PNAS* 104:12610-5. PMCID: PMC1937514

Complete list of my published work can be found at the following URL:

<https://www.ncbi.nlm.nih.gov/pubmed/?term=konigsberg+w>

1. **Research Support**

**Ongoing Research Support**

None

**Completed Research Support**

None