RESUME

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EDUCATION:

INSTITUTION AND LOCATION	DEGREE	YEAR(S)	FIELD OF STUDY
Department of Pharmaceutical Technology and Biochemistry, Gdansk University of Technology, Poland	PhD	1997-2002	Biochemistry
Faculty of Chemistry, Gdansk University of Technology, Poland	M.Sc.	1991-1996	Biotechnology
Faculty of Chemistry, Gdansk University of Technology, Poland	Engineering	1991-1996	Biotechnology

RESEARCH AND PROFESSIONAL EXPERIENCE

2007 to present Assistant Molecular Biologist, Midwest Center for Structural Genomics, Argonne National Laboratory

2003 to 2007 Postdoctoral Fellow, MCL, Synchrotron Radiation Section, National Cancer Institute

2002 to 2003 Postdoctoral Fellow, Department of Molecular Medicine, University of Texas Health Science Center at San Antonio

PROFESSIONAL TRAINING

2002 Marie-Curie training fellowship, EMBL Hamburg

2001 University of Aberdeen, Scotland Department of Molecular and Cell Biology

PUBLICATIONS (CITATION INDEX IN PARENTHESES BASED ON GOOGLE SCHOLAR)

H Index = 9

1. Makowska-Grzyska M, Kim Y, Wu R, Wilton R, Gollapalli DR, Wang XK, Zhang R, **Jedrzejczak R**, Mack JC, Maltseva N, Mulligan R, Binkowski TA, Gornicki P, Kuhn ML, Anderson WF, Hedstrom L, Joachimiak A. *Bacillus anthracis* Inosine 5'-Monophosphate Dehydrogenase in Action: The First Bacterial Series of Structures of Phosphate Ion-, Substrate-, and Product-Bound Complexes. Biochemistry. 2012 Aug 7;51(31):6148-63.
2. **Jedrzejczak R**, Wojciechowski M, Andruszkiewicz R, Sowiński P, Kot-Wasik A, Milewski S. Inactivation of glucosamine-6-phosphate synthase by N3-oxoacyl derivatives of L-2,3-diaminopropanoic acid. Chembiochem. 2012 Jan 2;13(1):85-96.

3. **Jedrzejczak R**, Wang J, Dauter M, Szczesny RJ, Stepien PP, Dauter Z. Human Suv3 protein reveals unique features among SF2 helicases. Acta Crystallogr D Biol Crystallogr. 2011 Nov;67(Pt 11):988-96.
4. Kim Y, Tesar C, Mire J, **Jedrzejczak R**, Binkowski A, Babnigg G, Sacchettini J, Joachimiak A. Structure of apo- and monometalated forms of NDM-1--a highly potent carbapenem-hydrolyzing metallo-β-lactamase. PLoS One. 2011;6(9). (**Cited 6 times**).
5. Kim Y, Babnigg G, **Jedrzejczak R**, Eschenfeldt WH, Li H, Maltseva N, Hatzos-Skintges C, Gu M, Makowska-Grzyska M, Wu R, An H, Chhor G, Joachimiak A. High-throughput protein purification and quality assessment for crystallization. Methods. 2011 Sep;55(1):12-28. (**Cited 2 times**)
6. Nocek B, Stein AJ, **Jedrzejczak R**, Cuff ME, Li H, Volkart L, Joachimiak A. Structural studies of ROK fructokinase YdhR from *Bacillus subtilis*: insights into substrate binding and fructose specificity. J Mol Biol. 2011 Feb 18;406(2):325-42. (**Cited 3 times**)
7. Malecki M, **Jedrzejczak R**, Puchta O, Stepien PP, Golik P. In vivo and in vitro approaches for studying the yeast mitochondrial RNA degradosome complex. Methods Enzymol. 2008;447:463-88. (**Cited 4 times**)
8. Pereira M, Mason P, Szczesny RJ, Maddukuri L, Dziwura S, **Jedrzejczak R**, Paul E, Wojcik A, Dybczynska L, Tudek B, Bartnik E, Klysik J, Bohr VA, Stepien PP. Interaction of human SUV3 RNA/DNA helicase with BLM helicase; loss of the SUV3 gene results in mouse embryonic lethality. Mech Ageing Dev. 2007 Nov-Dec;128(11-12):609-17. (**Cited 14 times**)
9. Malecki M, **Jedrzejczak R**, Stepien PP, Golik P. In vitro Reconstitution and Characterization of the Yeast Mitochondrial Degradosome Complex Unravels Tight Functional Interdependence. J Mol Biol. 2007 Jul 3. (**Cited 26 times**)
10. Szczesny RJ, Obriot H, Paczkowska A, **Jedrzejczak R**, Dmochowska A, Bartnik E, Formstecher P, Polakowska R, Stepien PP. Down-regulation of human RNA/DNA helicase SUV3 induces apoptosis by a caspase- and AIF-dependent pathway. Biol Cell. 2007 Jun;99(6):323-32. (**Cited 20 times**)
11. **Jedrzejczak R**, Dauter M, Dauter Z, Olszewski M, Dlugolecka A, Kur J. Structure of the single-stranded DNA-binding protein SSB from *Thermus aquaticus*.Acta Crystallogr D Biol Crystallogr. 2006 Nov;62(Pt 11):1407-12. (**Cited 12 times**)
12. **Jedrzejczak R**, Dauter Z, Dauter M, Piatek R, Zalewska B, Mroz M, Bury K, Nowicki B, Kur J. Structure of DraD invasin from uropathogenic *Escherichia coli*: a dimer with swapped beta-tails. Acta Crystallogr D Biol Crystallogr. 2006 Feb; 62(Pt 2): 157-64. (**Cited 21 times**)
13. Olchowy J, **Jedrzejczak R**, Milewski S, Rypniewski W Crystallization and preliminary X-ray analysis of the isomerase domain of glucosamine-6-phosphate synthase from *Candida albicans*. Acta Crystallograph Sect F Struct Biol Cryst Commun. 2005 Nov 1;61(Pt 11):994-6. (**Cited 5 times**)
14. Stanislawska-Sachadyn A, Sachadyn P, **Jedrzejczak R**, Kur J. Construction and purification of his(6)-*Thermus thermophilus* MutS protein. Protein Expr Purif, 2003, 28(1):69-77. (**Cited 11 times**)
15. Zgodka D, **Jedrzejczak R**, Milewski S, Borowski E. Amide and ester derivatives of N3-(4-methoxyfumaroyl)-(S)-2,3-diaminopropanoic acid: the selective inhibitor of glucosamine-6-phosphate synthase. Bioorg Med Chem, 2001, 9(4):931-8.
16. Andruszkiewicz R, **Jedrzejczak R**, Zieniawa T, Wojciechowski M, Borowski E. N3-oxoacyl derivatives of L-2,3-diaminopropanoic acid and their peptides; novel inhibitors of

glucosamine-6-phosphate synthase. J Enzyme Inhib, 2000; 15(5):429-41. (**Cited 10 times**)

17. Sachadyn P, **Jedrzejczak R**, Milewski S, Kur J, Borowski E. Purification to homogeneity of *Candida albicans* glucosamine-6-phosphate synthase over expressed in Escherichia coli. Protein Expr Purif, 2000, 19(3):343-9. (**Cited 19 times**)
18. Milewski S, Kuszczak D, **Jedrzejczak R**, Smith RJ, Brown AJ, Gooday GW. Oligomeric structure and regulation of *Candida albicans* glucosamine-6-phosphate synthase. J Biol Chem. 1999, 274(7):4000-8. (**Cited 36 times**)

PDB publications (70 total)

4G2P, 4EW7, 4EXK, 2FXQ, 3S9X, 3DO8, 3CAN, 4FB7, 3FVV, 3T9Y, 4FXS, 4ERU, 4EVX, 3UH9, 4ERH, 4H0D, 3HTR, 3SBL, 4E5U, 4ER9, 4ESH, 4F79, 4GYQ, 4GYU, 3RC3, 3D0K, 3ED5, 3F5B, 3GYG, 3SOY, 3SOZ, 3FDI, 3GKU, 3H1Q, 3HDT, 3IVP, 3ONP, 3T68, 3T6M, 4DQ1, 2AXW, 3DNP, 3K1U, 3SQN, 3H36, 3IV3, 3RC8, 3FRW, 4E2G, 3LOQ, 3SRX, 3US6, 4GMD, 3FH3, 3RKJ, 3RKK, 3RPF, 3TY7, 3EDH, 3FGG, 3IC3, 3D3S, 3L1W, 3SFP, 3RRL, 4G6X, 4XF5, 4F4I, 4EAQ, 4DWJ, 4EW7

Poster presentations (The first author is a primary presenter)

1. B. Nocek, C. Hatzos-Skintges, **R. Jedrzejczak**, G. Babnigg, A. Joachimiak. A tale of two redesigned proteins, Diffraction Methods in Structural Biology, Gordon Conference, Lewiston, ME, 15-20 July 2012.
2. **R. Jedrzejczak**, G. Babnigg, W. Eschenfeldt, S. Clancy, J. Bearden, A. Joachimiak. Delayed expression of TVMV protease eliminates the co-purification of a solubility enhancer. High-Throughput Structural Biology, Breckenridge, CO, 2012.
3. C. Chang, N. Marshall, L. Bigelow, B. Feldmann, G. Chhor, Y. Kim, **R. Jedrzejczak**, A. Joachimiak. Crystal structure of GroEL chaperonin from *Chlorobium tepidum*. American Crystallographic Association, Annual meeting, New Orleans, LA, 2011.
4. **R. Jedrzejczak**, G. Babnigg, N. Marshall, A. Sather, A. Joachimiak. High-throughput Expression of Protein/Protein Complexes. Expanding the Horizons of Structural Biology, Keystone Symposia, Breckenridge, CO, 8-13 January 2010.
5. G. Babnigg, **R. Jedrzejczak**, B. Nocek, A. Stein, W. Eschenfeldt, N. Marshall, A. Weger, L. Stols, K. Buck, A. Joachimiak. Comparison of high-throughput techniques for the expression of protein complexes. Structural Genomics: Expanding the Horizons of Structural Biology, Keystone Symposia, Breckenridge, CO, 8-13 January 2010.
6. **R. Jedrzejczak**, G. Babnigg, N. Marshall, A. Sather, A. Joachimiak. High-throughput Expression of Protein/Protein Complexes. Structural Genomics: Expanding the Horizons of Structural Biology, Keystone Symposia, Breckenridge, CO, 8-13 January 2010.
7. **R. Jedrzejczak**, K. Buck, L. Keigher, J. Bearden, A. Joachimiak. Strategy for optimizing protein expression in *E. coli*. 2009 NIGMS Workshop: Enabling Technologies for Structural Biology, Bethesda, MD, 2009.
8. N. Marshall, A. Stein, B. Nocek, **R. Jedrzejczak**, G. Babnigg, A. Joachimiak. High-throughput Structure Determination of Molybdopterin Converting Factor from *Helicobacter pylori*. NIGMS Workshop, Enabling Technologies in Structure and Function, Bethesda, MD, 19-21April 2010.
9. G. Babnigg, **R. Jedrzejczak**, B. Nocek, A. Stein, W. Eschenfeldt, N. Marshall, A. Wagner, R. Wu, L. Stols, K. Buck, A. Joachimiak. A high-throughput pipeline for the

production of protein-protein complexes. NIGMS workshop, Enabling Technologies in Structure and Function, Bethesda, MD, 19-21 April 2010.

10. B. Nocek, D-H Chen, A. Stein, R. Mulligan, J. Abdullah, **R. Jedrzejczak**, W. Chiu, A. Joachimiak. Crystallographic and Cryo-EM studies of the HK97-like bacteriophage DNA packaging portal. American Crystallographic Association, Annual meeting, Chicago, IL, 24-29 July 2010.
11. G. Babnigg, **R. Jedrzejczak**, B. Nocek, A. Stein, W. Eschenfeld, L. Bigelow, C.-S. Chang, G. Chhor, M. Cuff, Y. Fan, G. Joachimiak, Y.-C. Kim, H. Li, J. Osipiuk, E. Rakowski, K. Tan, C. Tesar, A. Weger, R. Wu, A. Joachimiak. The Production of Protein-Protein Complexes for Structural Characterization. American Crystallographic Association, Annual meeting, Chicago, IL, 24-29 July 2010.

Major accomplishments

1. **Protein–protein complexes**

I have optimized a high-throughput (HT) cloning method for the engineering of artificial polycistronic genes via rPCR. At the moment, the method is efficient for the co-expression of two different proteins. Additional enhancements to increase the number of co-expressing proteins and maintain system compatibility with the MCSG pipeline include the modification of an arabinose promoter-based vector, pGro7, replacement of the chloramphenicol-resistant gene with a “user friendly” kanamycin gene cassette, and modification of the cloning site region to improve system flexibility. A unique design advantage of the two different vectors is the ability to cross-test expressed proteins and identify strong interactions of partner proteins. This approach has been validated in the MCSG histone-chaperone project (8 of two partner histones versus 11 chaperones). In addition, the presence of the arabinose promoter allows the user to independently control timing of expression one of the complex partners which is critical for observation of some protein complexes.

2. **pMCSG73**

The majority of structures in the MCSG program were derived from proteins expressed in a simple but very efficient vector, pMCSG7, and its derivatives. However, a limitation of pMCSG7 vector in the inability to improve the solubility of expressed protein due to a reliance on the *E. coli* folding machinery. As a consequence, t\The average fraction of soluble clones expressed in pMCSG7 is only about 20% suggesting considerable room for improvement. I developed a design strategy for a new vector based on expression of the target protein in fusion with a solubility enhancer coupled with the option of separating the fusion with TVMV protease prior to the first chromatography purification step (IMAC-I). Initially, I have tested four solubility enhancers: SUMO, Halo, MBP and NusA; NusA to identify protein fusion partners with the best potential to improve solubility. Several *in vivo* approaches for controlled but efficient TVMV expression were evaluated but rejected due to inefficient cleavage. The outcome of the evaluation process was the construction of two vectors, one expressing NusA in fusion with the target protein (pMCSG73) and a second one for the strong expression of TVMV in a separate batch of cells. The protocol involves simultaneous growth with TVMV expressing cells added to target cells prior to sonication in a ratio of 1:16. The process is optimized so there is no visible NusA after IMAC-I. Currently cloning is performed in two vectors: pMCSG68 (a pMCSG7 derivative) and pMCSG73. Only clones that are not soluble in pMCSG68 are purified out of pMCSG73. The impact of this project is illustrated by the observation that 30% of targets in purification and 25% of structures in refinement have come from vector pMCSG73 since its first appearance in February of 2012.

3. Eliminating “empty clones”

When I joined the MCSG, the major problem for the purification and cloning groups were reoccurring epidemics of “empty clones.” “Empty clone” in MCSG jargon refers to a protein target that failed to yield any protein after the first purification step (IMAC-I). Every “empty clone” costs about one thousand dollars in chemicals and labor, and the number was reaching 40% of targets in milligram scale purification. The main cause of “empty clones” was an unreliable method used for solubility evaluation. It was based on analyzing the supernatant fraction of lysed cells grown in 1 ml of culture. I have introduced an original semi-automated purification procedure based on Ni affinity in a 96-well format. The method combines: 1) cell growth in 4 ml of culture, 2) sonication in a 96-well format (eliminates the need for any supplementary enzyme, such as lysozyme or Benzonase), 3) centrifugation, 4) automated mixing of the supernatant with Ni resin and loading onto a 96-well filter, 5) wash followed by elution with a high imidazole buffer containing TEV protease. Since the application of the method, the overall number of “empty clones” has dropped to about 5%. The introduced method also gives information about TEV cleavage efficiency for a target. It allows for early direction of a clone with poor TEV digestion into a salvage pathway of one of our C-terminal vectors.

4. Mutagenesis and LIC ready vectors

In the past five years at the MCSG, I have also simplified the mutagenesis procedure by combining Polymerase Incomplete Primer Extension (PIPE) cloning with a T4 polymerase treatment. The method decreased the price per mutagenesis reaction from $37 (Agilent) to the cost of primers (about $4) and PCR reaction ($2.50). The procedure was successfully used for multipoint mutagenesis, insertion and deletions. None of the above would be possible without the addition of betaine to the standard PCR protocol used at the MCSG; for unknown reasons, pMCSG vectors were very difficult to amplify via PCR. Amplification of up to 10 kbp templates simplified the vector preparation for Ligation Independent Cloning (LIC). Instead of labor and time consuming methods based on vector digest with restriction enzymes followed by gel extraction, PCR is used for vector amplification. This methodology speeds up modification to existing vectors which, in consequence, allows for faster verification of new cloning approaches.