Curriculum Vita and List of Publications

Personal Details

Name: Ashraf Brik

Date and place of birth: June 29th, 1973, Israel
Date of immigration: NA
Regular military service: NA
Sex: Male
Nationality: Israeli
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Address and telephone number at work:
Department of Chemistry
Ben-Gurion University
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Education

B.Sc. 1993-1996: Ben-Gurion University of the Negev, Department of Chemistry, Beer Sheva, Israel.
M.Sc. 1996-1998: Technion-Israel Institute of Technology, Department of Chemistry, Haifa, Israel. Advisor: Dr. Nizar Haddad "Stereoselective Synthesis of Polysubstituted Fatty Acids Derivatives, Studies Towards Total Synthesis of Borrelidin."
Ph.D. 1998-2001: Technion-Israel Institute of Technology, Department of Chemistry, Haifa, Israel. Advisor: Professor Ehud Keinan, jointly with The Scripps Research Institute, La Jolla, California. Advisor: Professor Phillip Dawson. "Design and Synthesis of Novel Catalytic Proteins Based on Polypeptide Scaffolds."

Employment History

April 2011-Present: Associate Professor, Department of Chemistry, Ben-Gurion University of the
Negev.
February 2007-March 2011: Sr. Lecturer, Department of Chemistry, Ben-Gurion University of the
Negev.
2004-2006: Senior Research Associate, The Scripps Research Institute, La Jolla, California (With Professor Chi-Huey Wong).

2002-2004: Postdoctoral Associate, The Scripps Research Institute, La Jolla, California (With Professor Chi-Huey Wong).

Professional Activities

2001-present: Member of the American Chemical Society (ACS).
2006-2007: Consultant for Biomatrica, Inc "Stabilizing nucleic acids and prevent degradation without the need for cold storage."
2007-present: Member of the Israel Chemical Society (ICS).
2007-2008: Head of the library committee, Department of Chemistry, Ben-Gurion University of the Negev.
2008-present: Member of the graduate studies committee, Department of Chemistry, Ben-Gurion University of the Negev.
2009-present: Elected member of the executive board of the Israel Chemical Society.
2009-present: Member of the American Peptide Society (APS).

Educational Activities

Courses taught

1. Organic Chemistry II: Undergraduate students from the Pharmacy School and Chemical Engineering Department, Ben-Gurion University of the Negev.
2. Analytical and General Chemistry Laboratory: Undergraduate students from the Chemistry Department, Ben-Gurion University of the Negev.
3. Advanced Topics in Protein Chemistry: Graduate students, Ben-Gurion University of the Negev.
4. Organic Chemistry I Laboratory: Undergraduate students from the Chemistry Department, Ben-Gurion University of the Negev.

Research Students

Graduated:
2009: Marina Yamit Lutsky, MSc, Ben-Gurion University of the Negev
2010: Ziv Harpaz, MSc, Ben-Gurion University of the Negev
2010: Lesly Erlich, MSc, Ben-Gurion University of the Negev
2010: Rinat Roytman, MSc, Ben-Gurion University of the Negev
Currently Supervised:
Peter Siman (PhD)
Liat Spasser (PhD)
Mahmood Haj-Yahya (PhD)
Tal Moyal (MSc)
Shimrit Ohayon (MSc)
Najat El-tarteer (MSc)
Postdoc:
Dr. KS Ajish Kumar
Dr. Madhat Matar
Dr. Sudhir Bavikar
Dr. Karthikeya S.V.
Dr. Hemantha H. P.

Awards and Fellowship

1995-1996 B. Sc. Dean's Honors for Excellency (Ben-Gurion University).
1998-2001 Eshkol Scholarship for Graduate Students: Israel Ministry of Science.
2002-2004 Postdoctoral Fellowship: Israel Science Foundation (ISF).
2006-2009 Ma'of Fellowship: Established by the Kahanoff Foundation (The fellowship covers my university salary for the first three years of my position as a Sr. lecturer at Ben-Gurion University of the Negev, in addition to $30,000 for research).
2009 Dean's Honors for Excellent Researcher (Faculty of Natural Sciences Ben-Gurion University).
2011 JSP Fellowship, the 46th Bürgenstock Conference, May 1-6, 2011 at Brunnen.

Scientific Publications

*Papers with * on my name indicates that I am a corresponding author and where my name is underlined indicates contribution from BGU.*

1. Nizar Haddad, **Ashraf Brik,** and Michael Grishko, Studies Towards Total Synthesis of Borrelidin, Regioselective Methylation of Bis-epoxides and Structure Determination, *Tetrahedron Letters* 1997, *38*, 6079-6082.
2. Nizar Haddad, Michael Grishko, and **Ashraf Brik**, Studies Towards Total Synthesis of Borrelidin, Stereoselective Synthesis of the Polysubstituted Macrolidic Part, *Tetrahedron Letters*, 1997, *38*, 6075-6078. (Primary Author).
3. **Ashraf Brik**, Ehud Keinan, and Philip E. Dawson, Protein Synthesis by Solid-Phase Chemical Ligation Using a Safety Catch Linker, *Journal of Organic Chemistry*, 2000, *65*, 3829-3835.
4. **Ashraf Brik**, Philip E. Dawson and Ehud Keinan, The Product of the Natural Reaction Catalyzed by 4-oxalocrotonate Tautomerase Becomes an Affinity Label of its Mutant, *Bioorganic & Medicinal Chemistry*, 2002, *10*, 3891-3897.
5. **Ashraf Brik,** Lawrence J. D'Souza, Ehud Keinan, Flavio Grynszpan and Philip E. Dawson, " Mutants of 4-Oxalocrotonate Tautomerase Catalyze the Decarboxylation of Oxaloacetate Through an Imine Mechanism" *ChemBioChem*, 2002**,** *3*, 845-851, (Highlighted on the cover page).
6. **Ashraf Brik**, Ying-Chuan Lin, John Elder and Chi-Huey Wong, A Quick Diversity-Oriented Amide-Forming Reaction to Optimize p-subsite Residues of HIV Protease Inhibitors, *Chemistry & Biology*, 2002, 9, 891-896.
7. **Ashraf Brik** and Chi-Huey Wong, HIV Protease: Mechanism and Drug Discovery" *Organic & Biomolecular Chemistry*, 2003, *1*, 5-14, Highlighted on the cover page, (Top 10 most downloaded OBC articles in 2006-2007 and 2009).
8. Chi Ching Mak, **Ashraf Brik**, Danica L. Lerner, John H. Elder, Garrett M. Morris, Arthur J. Olson, and Chi-Huey Wong, Design and Synthesis of Broad-Based Mono- and Bi-cyclic Inhibitors of FIV and HIV Proteases, *Bioorganic & Medicinal Chemistry*, 2003, *11*, 2025-2040.
9. **Ashraf Brik,** John Muldoon, Ying-Chuan Lin, John H. Elder, David S. Goodsell, Arthur J. Olson, Valery V. Fokin, K. Barry Sharpless and Chi-Huey Wong. Rapid Diversity-Oriented Synthesis and *In Situ* Screening of HIV Protease Inhibitors, *ChemBioChem*, 2003, *4*, 1246-1248.

10. Michael D. Best, **Ashraf Brik**, Lac V-Lee, Ali Chapman, Wei-Chieh Cheng and Chi-Huey Wong. Rapid Discovery of Potent Sulfotransferase Inhibitors by Diversity-Oriented Reaction in Microplates Followed by in Situ Screening, *ChemBioChem*, 2004, *5*, 811-819.
11. Norman Metanis, **Ashraf Brik**, Ehud Keinan, and Philip E. Dawson, Electrostatic interaction dominate the contribution of Arg39 in 44 Oxalocrotonate Tautomerase, *Journal of the American chemical society*, 2004, 126 (40), 12726-12727.
12. Ting-Jen Chen, **Ashraf Brik**, Chi-Huey Wong and Chen-Chen Kan, A model System for High-Throughput Screening of Novel Human Immunodeficiency Virus (HIV) Protease Inhibitors in *Escherichia Coli*, *Antimicrobial Agents and Chemotherapy*, 2004, *48(7)*, 2437-2447.
13. Chung-Yi Wu, Jia-Tsrong Jan, Shiou-Hwa Ma, Chih-Jung Kuo, Hsueh-Fen Juan, Yih-Shyun E Cheng, Hsuan Cheng Huang, Douglass Wu, **Ashraf Brik**, Fu-Sen Liang, Rai-Shung Liu, Jim-Min Fang, Shui-Tein Chen Po-Huang Liang, and Chi-Huey Wong, Small Molecules Targeting Severe Acute Respiratory Syndrome (SARS) Human Coronavirus, *Proceedings of the National Academy of Science*, USA, 2004, *101(27)*, 10012-10017.
14. **Ashraf Brik,** Chung-Yi Wu, Michael D. Best and Chi-Huey Wong, Tetrabutylammonium Fluride-Assisted Rapid N9-Alkylation on Purine Ring: Application to Combinatorial reaction in Microtiter plates for the Discovery of Potent Sulfotransferase Inhibitors In situ, *Bioorganic & Medicinal Chemistry*, 2005, 13, 4622-4626.
15. **Ashraf Brik**, Jerry Alexandratos, Ying-Chuan Lin, John H. Elder, Arthur J. Olson, David S. Goodsell, Alexander Wlodawer and Chi-Huey Wong, 1,2,3 Triazole as a Peptide Bond Surrogate in the Rapid Synthesis of HIV Protease Inhibitors, *ChemBiochem*, 2005, *6*, 1167-1169 (Highlighted on the cover page).
16. Chung-Yi Wu, **Ashraf Brik**, Sheng-Kai Wang, Yu-Hsien Chen, and Chi-Huey Wong, Tetrabutylammonium Fluoride-Mediated Rapid Alkylation Reaction in Microtiter Plates for Discovery of Enzyme Inhibitors In situ, *ChemBiochem*, 2005, 6, 2176-2180.
17. Fu-Sen Liang, **Ashraf Brik,** Ying-Chuan Lin, John H. Elder, and Chi-Huey Wong, Epoxide Opening in Water for Rapid Inhibitor Discovery in Microtiter Plate and In situ screening, *Bioorganic & Medicinal Chemistry*, 2006, 14, 1058-1062.
18. **Ashraf Brik**, Chung-Yi Wu and Chi-Huey Wong, Microtiter Plate Based Chemistry and In Situ Screening: A Useful Approach for Rapid Inhibitors Discovery, *Organic & Biomolecular Chemistry*, 2006, 4, 1446-1457.
19. **Ashraf Brik**, Yu-Ying Yang, Simon Ficht and Chi-Huey Wong, Sugar Assisted Glycopeptide Ligation, *Journal of The American Chemical Society*, 2006, 128, 5626-5627.
20. Ying-Chuan Lin, **Ashraf Brik**, Aymeric de Parseval, Karen Tam, Bruce Torbett, Chi-Huey Wong, and John H. Elder, Altered Gag-Pol Polyprotein Cleavage Specificity of FIV/HIV Mutant Proteases as Demonstrated in Cell-Based Expression System, *Journal of Virology*, 2006, 80, 7832-7843.
21. **Ashraf Brik**, Simon Ficht, and Chi-Huey Wong, Strategies for the Preparation of Homogenous Glycoproteins, *Current Opinion in Chemical Biology*, 2006, 10, 1-7.

22. **Ashraf Brik**,***** Simon Ficht, Yu-Ying Yang, and Chi-Huey Wong***** "Sugar Assisted ligation for the Synthesis of *N*-linked Glycopeptide with Broad Sequence Tolerance at the Ligation Junction, *Journal of the American Chemical Society*, 2006, 128, 15026-15033, See highlight about this paper in News & Views, Nature, 2007, 445, 31-33.
23. Simon Ficht, Richard J. Payne, **Ashraf Brik** and Chi-Huey Wong, Second Generation Sugar-Assisted ligation: An effective New Method for the Synthesis of Cysteine Containing Glycopeptides, *Angewandte Chemie International Edition*, 2007, 46, 5975-5979.
24. **Ashraf Brik*** and Chi-Huey Wong*****, Sugar-Assisted Ligation for the Synthesis of Glycopeptides" *Chemistry-A European Journal*, 2007, 13, 5670-5675.
25. Yu-Ying, Yang, Simon Ficht, **Ashraf Brik*** and Chi-Huey Wong*****, Sugar-Assisted Glycoprotein Synthesis, *Journal of the American Chemical Society*, 2007, 129, 7690-7701.
26. Richard J. Payne, Simon Ficht, Sishi Tang, **Ashraf Brik**, Yu-Ying Yang, David A. Case and Chi-Huey Wong, Extended Sugar-Assisted Glycopeptide Ligations: Development, Scope and Applications, *Journal of the American Chemical Society*, **2007**, *129*, 13527-13536.
27. Marina-Yamit Lutsky, Natalia Nepomniashciy and **Ashraf Brik***, Peptide ligation via Side-Chain Auxiliary" *Chemical Communication*, 2008, 10, 1229-1231.
28. Michael J. Giffin, Holly Heaslet, **Ashraf Brik**, Ying-Chuan Lin, Gabrielle Cauvi1, Chi-Huey Wong, Duncan E. McRee, John H. Elder, C. David Stout and Bruce E. Torbett1, A Copper(I)-Catalyzed 1,2,3-Triazole Azide-Alkyne Click Compound Is a Potent Inhibitor of a Multidrug-Resistant HIV-1 Protease Variant, *J. Med. Chem.* 2008, 51, 6263-6267.
29. Clay S. Bennett, Richard J. Payne, Simon Ficht, Stephen M. Dean, **Ashraf Brik**, and Chi-Huey Wong, Sugar-Assisted Glycopeptide Ligation with Complex Oligosaccharides, *Journal of the American Chemical Society*, 2008, 130. 11945-11952.
30. **Ashraf Brik***, Metathesis in Peptides and Peptidomimetics, *Advanced Synthesis and Catalysis*, 2008, 350, 1661-1675.
31. Natalia Nepomniaschiy, Valerie Grimminger, Aviv Cohen, Saviana DiGiovanni, Hilal Lashuel,* and **Ashraf Brik*** "Switch Peptides via Staudinger Reaction, *Organic Letters*, 2008, 11, 5243-5246.
32. K. S. Ajish Kumar, Ziv Harpaz, Mahmood Haj-Yahya and **Ashraf Brik*,** Side-Chain Assisted Ligation in Protein Synthesis, *Bioorganic and Medicinal Chemistry letters*, 2009, 19, 3870-3874.
33. K. S Ajish Kumar, Mahmood Haj-Yahya, Diana Olschewski, Hilal A. Lashuel and **Ashraf Brik***, Highly Efficient and Chemoselective Peptide Ubiquityation, *Angewandte Chemie Int. Ed.*, 2009, 48, 8090-8094. (Highlighted in C&EN News, Vol 86, #46, 2009.
34. Lesly A. Erlich, K. S. Ajish Kumar, Mahmood Haj-Yahya, Philip E. Dawson and **Ashraf Brik***, N-Methyl Cysteine Mediated Total Chemical Synthesis of Ubiquitin Thioester, *Organic & Biomolecular Chemistry*, 2010, *8*, 2392-2396.
35. K. S. Ajish Kumar and **Ashraf Brik***, Accessing Posttranslationally Modified Proteins through Thiol Positioning, *Journal of Peptide Science*, 2010, 16, 524-529.

36. Mahmood Haj-Yahya, K. S. Ajish Kumar, Lesly A. Erlich, and **Ashraf Brik***, Protecting Group Variations of δ-Mercaptolysine Useful in Chemical Ubiquitylation, *Biopolymers (Peptide Science)*, 2010, 94, 504-510.
37. Ziv Harpaz, K.S. Ajish Kumar, Peter Siman and **Ashraf Brik*,** Protein Synthesis Assisted by Native Chemical Ligation at Leucine, *ChemBioChem*, 2010, 11, 1232-1235.
38. Liat Spasser, K. S. Ajish Kumar, and **Ashraf Brik***, Scope and Limitation of Side Chain-Assisted Ligation, *Journal of Peptide Science*, 2011, 17, 252-255.
39. K. S. Ajish Kumar, Liat Spasser, Sudhir Bavikar, Lesly A. Erlich, and **Ashraf Brik***, Total Chemical Synthesis of di-Ubiquitin Chains, *Angewandte Chemie Int. Ed*, 2010, 49, 9126-9131. (Selected as the frontispieces of the communication section), (Highlighted in C&EN, October 11, 2010 and in *Angewandte Chemie Int. Ed.*, 2010, 49, 9042-9044, by L. Martin and R. T. Raines, Carpe di-ubiquitin and in C&EN, December 22, 2010, issue Chemical Year in Review).
40. Mirva Hajjouie[#], Mahmood Haj-Yahya[#], **Ashraf Brik*** and Hilal A. Lashuel*, Towards elucidating the role of ubiquitination in the Pathogenesis of Parkinson's disease using semisynthetic ubiquitinated α-Syn, *Angewandte Chemie International Edition*, 2011, 50, 405-409. Selected by the editors as a " Hot Paper" (# these authors contributed equally).
41. K. S. Ajish Kumar, Liat Spasser, Shimrit Ohayon, Lesly Erlich and **Ashraf Brik***, Expeditious Chemical Synthesis of Ubiquitinated Peptides Employing Orthogonal Protection and Native Chemical Ligation, *Bioconjugate Chemistry* (ACS publication), 2011, 22, 137-143.
42. Peter Siman, Ofrah Blatt, Tal Moyal, Tsafi Danieli, Mario Lebendiker, Hilal A. Lashuel, Assaf Friedler* and **Ashraf Brik***, Chemical Synthesis and Expression of the HIV-1 Rev Protein, *ChemBioChem*, 2011, 12, 1097-1104.
43. Rinat Roytman, Lihi Adler-Abramovich, K. S. Ajish Kumar, Ting-Chun Kuan, Chun-Cheng Lin, Ehud Gazit* and **Ashraf Brik*,** Exploring the Self-Assembly of Glycopeptides Using Diphenylalanine Scaffold, *Organic& Biomolecular Chemistry*, 2011, 9, 5755-5761.
44. K. S. Ajish Kumar#, Sudhir N. Bavikar#, Liat Spasser, Shimrit Ohayon, Tal Moyal and **Ashraf Brik*,** Total Synthesis of a 304 residue, K48-Linked Tetraubiquitin, *Angewandte Chemie International Edition*, 2011,50, 6137-6141. (# these authors contributed equally). Selected by the editors as a Hot Paper. See highlight on this paper in C&EN, May 30, 2011, Volume 89, number 22, p. 7.
45. Natalie Zeytuni, Ertan Ozyamak, Kfir Ben-Harush, Geula Davidov, Maxim Levin, Yair Gat, Tal Moyal, **Ashraf Brik**, Arash Komeili, Raz Zarivach*, Self-Recognition Mechanism of MamA, a Magnetosome-Associated TPR-Containing Protein, Promotes Complex Assembly; *Proceedings of the National Academy of Science*, USA, 2011, 108, E480-E487.
46. Carlos A. Castañeda, Liat Spasser, Sudhir N. Bavikar, **Ashraf Brik***, David Fushman*, Segmental Isotopic Labelling of Ubiquitin Chains to Unravel Monomer-Specific Molecular Behavior, *Angewandte Chemie International Edition*, Early View
47. K. S. Ajish Kumar, **Ashraf Brik***, δ-Mercaptolysine Assisted Ubiquitination, *Israel Journal of Chemistry*, In press. (Special Issue on Protein Synthesis, Edited by Ashraf Brik, Philip E. Dawson, Stephen BH Kent)

48. Sudhir N. Bavikar, Liat Spasser, Mahmood Haj-Yahya, Subramanian Vedhanarayanan Karthikeyan, Tal Moyal, K. S. Ajish Kumar and **Ashraf Brik*,** Chemical Synthesis of Ubiquitinated Peptides with Varying Lengths and Types of Ubiquitin Chains to Explore the Activity of Deubiquitinases, *Angewandte Chemie International Edition*, In press.
49. **Ashraf Brik*,** Fighting Hepatitis C Virus with Peptide Nanotubes, Chemistry and Biology, In press, (Invited Commentary).

Book Chapters

1. Ashraf Brik and Ehud Keinan, "Catalytic Antibodies in Natural Product Synthesis" *Catalytic Antibodies*. Ehud Keinan, ed. (WILEY-VCH), 2005, 132-152.
2. **Ashraf Brik*** "New Strategies for Glycopeptides, Neoglycopeptides and Glycoproteins Synthesis" *Comprehensive Natural Products II Chemistry and Biology*; Mander, L., Lui, H.-W., Eds.; Elsevier: Oxford, 2010; volume 6, pp.55–89.

Submitted

1. Gilad Fuchs, Efrat Shema, Rita Vesterman, Mahmood Haj-Yahya, **Ashraf Brik**, Eytan Domany and Moshe Oren,* RNF20 and USP44 regulate embryonic stem cell differentiation by modulating H2B monoubiquitylation, *Molecular Cell,* **Submitted**.

To be submitted shortly

1. Loay Awad, Nino Jejelava, **Ashraf Brik** and Hilal A. Lashuel*, A novel caged-glutamine derivative as a tool to control the assembly of glutamine-containing amyloidogenic peptides

2. Liat Spasser and **Ashraf Brik**, Chemical Biology with the Ubiquitin Signal, (Invited mini-review to Angew Chemie)

3. Peter Siman and **Ashraf Brik,*** Chemical and Semi-Synthesis of Posttranslationally Modified Proteins, (Invited review to Organic and Bimolecular Chemistry)

4. Shimrit Ohayon, Liat Spasser, Amir Aharoni*, and **Ashraf Brik***, Targeting Deubiquitinases Enabled by Chemical Synthesis of Proteins

Invited Lectures in Local and International Conferences:

1. **Ashraf Brik**, EMBO Conference (Ubiquitin and Ubiquitin Like Modifier, from Functional Modules to Systems Biology) Dubrovnik, September 21-25, 2011 "Advanced Chemical Tools to Study Ubiquitin Biology".
2. **Ashraf Brik**, EuCheMs Org Div Young Investigator Workshop-Crete, July 2011 "Advanced Chemical Tools to Study Ubiquitin Biology".
3. **Ashraf Brik**, The 22nd American Peptide Symposium, San-Diego, CA, USA, June 25-30, 2011 "Advanced Chemical Tools to Study Ubiquitin Biology".
4. **Ashraf Brik**, The 46th Bürgenstock Conference, May 1-6, 2011 at Brunnen "Advanced Chemical Tools to Study Ubiquitin Biology" (JSP fellowship).
5. **Ashraf Brik**, The ACS 241st meeting, Anaheim, March 27-31, 2011 "Advanced Chemical Tools to Study Ubiquitin Biology", to honor Prof. Stephen Kent for being the recipient of the A. Bader Award.

6. **Ashraf Brik**, The 7th Schulich symposium, Technion-Israel Institute of Technology, March 13 2011 "Advanced Chemical Tools to Study Ubiquitin Biology"
7. **Ashraf Brik**, The 5th International Peptide meeting, Kyoto, December 4-9 2010 "Advanced Chemical Tools to Study Ubiquitin Biology"
8. **Ashraf Brik**, The 31 EPS, Copenhagen, 4-9 September 2010 "Advanced Chemical Tools to Study Ubiquitin Biology"
9. **Ashraf Brik**, The 8th Congress of the Israel Association for Medicinal Chemistry, March 16th, 2010 "Advanced Chemical Tools to Study Ubiquitin Biology."
10. **Ashraf Brik**, The First Chemical Biology Symposium, 15 October 2009, Ben-Gurion University. "Accessing Posttranslationally Modified Proteins through Thiol Positioning."
11. **Ashraf Brik**, Probing Protein Function through Chemistry, 20-23 September, 2009, Ringberg Castel, Germany. "Accessing Posttranslationally Modified Proteins through Thiol Positioning."
12. **Ashraf Brik**, ACS National Meeting, Washington Dc, United States, Aug. 16-20, 2009. "Controlling Conformational Transition of Peptides-Protein via Staudinger Reaction."
13. **Ashraf Brik,** the 74 meeting of the Israel Chemical Society, 8-9 Feb, 2009 "Aminoacyl Transfer in Peptide-Protein Synthesis and Manipulation"
14. **Ashraf Brik,** 30th European Peptide Symposium, Helsinki, Finland 31-August - 5 September 2008. "Side-Chain Assisted ligation."
15. **Ashraf Brik,** the 73 meeting of the Israel Chemical Society, 4-5 Feb, 2008 " Peptide Ligation via side-chain auxiliary."
16. **Ashraf Brik**, 23 ACS National Meeting, San Francisco, CA, United States, Sept. 10-14, 2006. (Contributed)

Lectures at Academic Institutes:

1. School of Chemistry, Tel-Aviv University, April 10, 2011 "Advanced Chemical Tools to Study Ubiquitin Biology"
2. Department of Chemistry and Biochemistry, University of California, San-Diego, 30 March, 2011 "Advanced Chemical Tools to Study Ubiquitin Biology"
3. Department of Chemical Engineering, Ben-Gurion University, 8 March, 2010 "Advanced Chemical Tools to Study Ubiquitin Biology"
4. Department of Life Sciences, Ben-Gurion University, 15 October, 2010 "Advanced Chemical Tools to Study Ubiquitin Biology"
5. Academia Sinica, Taiwan, 19 April, 2010, "Adventure in the Synthesis of Posttranslationally Modified Proteins"
6. Department of Chemistry, National Taiwan University, Taiwan 21 April, 2010, "Adventure in the Synthesis of Posttranslationally Modified Proteins"
7. Department of Chemistry, National Tsing Hua University, Taiwan, 22 April, 2010, "Adventure in the Synthesis of Posttranslationally Modified Proteins"
8. Department of Chemistry, Ben-Gurion University; 13 April, 2010 "Advanced Chemical Tools to Study Ubiquitin Biology"
9. Department of Chemistry, Weizmann Institute of Science; 3 March, 2010 "Adventure in the Synthesis of Posttranslationally Modified Proteins"

10. Department Chemistry, Technion-Israel Institute of Technology; 14 Dec, 2009 "Adventure in the Synthesis of Posttranslationally Modified Proteins"
11. Department Chemistry, The Hebrew University of Jerusalem; 26 May, 2009 "Aminoacyl Transfer in Peptide-Protein Synthesis and Manipulation"
12. Pharmacy School, Ben-Gurion University; 1 Feb, 2009 "Aminoacyl Transfer in Peptide-Protein Synthesis and Manipulation"
13. Department Chemistry; Bar-Ilan University; 15 May, 2008 "New Synthetic approaches for Protein Synthesis and Manipulation"
14. Department of Clinical Biochemistry; Ben-Gurion University; 11 March, 2008 "Evolving New Strategies for Protein Manipulation and Synthesis"
15. Department of Biotechnology Engineering; Ben-Gurion University; 27 Jan, 2008 "New Strategies for Protein Manipulation and Synthesis"
16. NIBN; Ben-Gurion University; 6 Jan, 2008 "New Strategies for Protein Manipulation and Synthesis"
17. School of Pharmacy; The Hebrew University of Jerusalem 18 Nov, 2007 " New Synthetic Approaches for Protein Synthesis and Manipulation"
18. Department of Chemistry, Tel-Aviv University; 4 Nov, 2007 " New Synthetic Strategies for Protein Synthesis and Manipulation"
19. Department of Chemistry, Ben-Gurion University; 26 March, 2007 " Sugar-Assisted Ligation in Glycopeptides and Glycoproteins Synthesis"

Patents

1. Wong, C.-H.; **Brik, A.;** Yang, Y.-Y.; Ficht, S.; Payne, R. "Method of Preparing Glycopeptides" PCT Int. Appl. (2007), CODEN: PIXXD2 WO 2007111952 A2 20071004 AN 2007:1116927.
2. **Brik, A.**; H.-Yahya, M.; Kumar, A.; Erlich L.; Spasser, L. "Chemical Synthesis of Ubiquitin Thioester and Modification thereof" From PCT Int. Appl. (2011), WO 2011098999 A2 20110818.
3. **Brik, A.**; Haj-Yahya, M.; Kumar, A.; Erlich, L.; Spasser, L. "Chemical Preparation of Polyubiquitin Chains" From PCT Int. Appl. (2011), WO 2011098998 A2 20110818

Received Competitive Grants

2010-2014: Personal grant, Israel Science Foundation NIS 936,000. Advanced Chemical Tools to Study Ubiquitin Chains.

2010-2014: US-Israel Binational Science Foundation, (Jointly with Professor Peter Schultz, TSRI). $188,000, Genetically Encoded Ubiquitination.

2009-2012: Tashtiot grant, NIS 1,800,000 (Shared with Prof Ehud Keinan and Ohad Medalia) "Nano Reactors"

2009-2012: HFSP grant, $750,000 (Shared with Prof H. A. Lashuel, EPFL), "Developing novel chemical approaches to control protein folding and self-assembly in health and disease"

2007-2011: Personal grant, Israel Science Foundation $270,000 "New synthetic approaches for the incorporation of peptidomimetic structures into proteins"

2008-2011: Safra Foundation $1,870,000, Principal investigator,

(Shared with another five groups), "Establishment of center of biopolymers"

2007-2010: Wolfson Foundation €307,000, Principal investigator, (Shared with another three PIs) "Protein Design and Engineering Laboratory at Ben-Gurion University of the Negev"

2007-2010: Rich Foundation, $250,000, "Protein synthesis for biological studies"

2007-2009: European Commission FP6 - Marie Curie International Reintegration Grant, €80,000, "HTLV Protease: Synthesis and Inhibition"

2006-2009: Ma'of fellowship: Established by the Kahanoff Foundation (the fellowship covers my university salary for the first three years of my position as a Sr. lecturer at Ben-Gurion University of the Negev, in addition to $30,000 for research).

2007-2008: Equipment grant, Israel Science Foundation $140,000, "Analytical and Preparative System for Synthetic Peptides, Peptidomimetics, and Proteins"

Synopsis of Research

For the past three decades, scientists have obtained proteins to conduct their studies by recombinant DNA-based expression in genetically engineered cells. This mature technology allows the production of large amounts of proteins of well-defined molecular compositions for studies at the molecular level. Unarguably, DNA-based expression has dramatically influenced our knowledge and understanding of the molecular basis of protein function and will remain the core for the study of biological systems. Yet, the method has some limitations, such as difficulties in expressing large multidomain proteins and over expression of proteins that are toxic to the cells (proteases). Moreover, studies in most cases are inherently limited to the 20 natural encoded amino acids, despite some successes of using cell-free synthesis to expand the repertoire of ribosomal synthesis to incorporate unnatural amino acids in proteins.

Chemists have offered an attractive and complementary approach to biological systems for protein production. Chemical synthesis of proteins allows for variations of the covalent protein structure, virtually in an unlimited manner. Although Solid-Phase Peptide Synthesis (SPPS) is reliable up to ~50 amino acids, protein synthesis is complicated due to the accumulation of side products during chain assembly and deprotection. Consequently, protein synthesis is dominated by approaches that depend on the assembly of smaller peptide fragments. Most notably, the Native Chemical Ligation (NCL) utilizes a highly chemoselective coupling of two unprotected peptides, one of which bears an N-terminal cysteine residue, while the other contains a C-terminal thioester group. In continuation to these efforts, our lab is exploring new ligation methods to construct large polypeptides that correspond to folded proteins (*ChemBioChem*, 2010, 11, 1232-1235; *Chemical Communications*, 2008, 10, 1229-1231). This work was supported by the ISF grant and European Commission FP6 - Marie Curie International Reintegration Grant). The above-described strategies are used in the following areas.

A) *Manipulating Proteins with Chemistry (ISF grant):* Manipulating proteins with chemistry to study their biological function and to increase their stability has attracted synthetic chemists to contribute to these studies in ways that are difficult to achieve by using traditional biochemical approaches. The increasing momentum of the field is supported by the emerging chemical and biochemical approaches that allow the incorporation of unnatural functionalities into the protein of interest. In addition, there are an increasing number of organic reactions that can be carried out in aqueous media, which permit their use to modify proteins in physiological conditions. The introduction of unnatural entities into proteins at a specific position allows a subsequent chemistry to be carried out in a selective manner, contrary to previous methods where selectivity is sacrificed. We are investigating several chemistries that are compatible to physiological conditions in order to introduce the peptidomimetic motifs into protein scaffold (*Advanced Synthesis and Catalysis*, 2008, 350, 1661-1675). The effect of such modifications on the protein structure and activity will also be examined.

B) *Chemical Synthesis of posttranslationally modified proteins*: Posttranslational modifications play an important role in regulating protein structure and function in health and disease. Ubiquitylation is one example for such a modification wherein both the extent (polyubiquitylation vs mono-ubiquitylation) and the sequence position of this modification dictates the function and fate of the ubiquitylated protein. In the ubiquitylation process three distinct enzymes, known as the E1-E3 system, collaborate to achieve a site-specific tagging of the lysine residue(s) in target protein. This condensation step generates an isopeptide linkage between the $-NH_2$ of the lysine residue and the activated C-terminal glycine of ubiquitin (Ub). Chemical synthesis of proteins offers exceptional opportunities to prepare homogeneous posttranslationally modified protein with high purity and large quantities for functional and structural analysis. We have recently reported a new method for peptide ubiquitylation employing mercaptolysine residue to mediate thioesterification followed by amino acyl transfer to form the isopeptide linkage between ubiquitin and specific lysine residue of the tagged protein (*Angewandte Chemie Int. Ed.*, 2009, 48, 8090-8094). Various derivatives of this amino acid were prepared so it could be used in various chemistries (*Biopolymers, 2010, 54, 504-510*). We are currently working to apply this approach to study the effect of ubiquitylation on the function of a wide-range of proteins. (This work is supported by Edmond J. Safra foundation).

More recently, we developed a novel method for the expeditious synthesis of ubiquitinated peptides relying only on solid phase peptide synthesis (SPPS) coupled with native chemical ligation (NCL). This should enable the rapid assembly of a variety of ubiquitinated peptides for various studies related to the biology of ubiquitin (Bioconjugate Chemistry 2011).

The conjugation of Ub molecule can be as a monomer so called monoubiqutination or as a chain of various length and linkage types, known as polyubiquitination. As a result, these modifications lead to a variety of molecular signals e.g. regulation of endocytosis and DNA repair wherein the outcome depends on the ubiquitination nature (polyubiquitination vs monoubiquitination). Polyubiquitination is a complex and diverse modification where the outcome of the molecular signal depends on which one of the seven lysines in ubiquitin (K63, K48, K33, K29, K27, K11, K6) is linked to consecutive ubiquitin molecules. Most studies up until today were done on

chains through K48 (Nobel prize in chemistry 2004) and through K63. Besides recent molecular modeling studies for exploring the linkage dependence of di-ubiquitin conformations of the chains through K33, K29, K27, K11, K6, no structural data is available regarding these chains. Moreover, despite the importance of these chains in vivo their isolation in enough quantity for the in vitro biochemical studies and providing structural information have been very challenging. This is mainly being hindered by the unavailability of the E2/E3 enzymes to reconstitute the desired chain in vitro, as is in the case with K48- and K63-Ub chains.

To allow for the preparation of ubiquitin chains for biological and structural analysis in which the enzymatic machinery has not yet been identified, we developed a chemical approach for the preparation of ubiquitin thioester a key intermediate in the ubiquitination process (*Organic& Bimolecular Chemistry 2010*). Together these tools were used to chemically prepare all seven analogues of di-ubiquitin chains which allowed examining their secondary structure as well as their activity with the IsoT, a deubiquitinating enzyme that hydrolyze the ubiquitin chain in vivo (Angew Chemie, 2010). We have also accomplished the total chemical synthesis of tetra- ubiquitin chain, which represent the largest protein made to date using synthesis (Angew Chemie 2011).

These studies should assist in unraveling several aspects of these chains with ubiquitin binding proteins and should enable a comprehensive structural analysis study to shed more light on how the diversity of Ub signaling is achieved.

2-Developing novel chemical approaches to control protein self-assembly in health and disease

Protein misfolding and aggregation, more specifically amyloid formation, play a central role in the pathogenesis of several incurable systemic and neurodegenerative diseases that affect the population today. Therefore, detailed mechanistic understanding of these processes in and outside the cell is of critical importance to elucidating the fundamental roles governing protein folding, understanding disease mechanisms and developing therapeutic strategies to prevent, treat and/or reverse these devastating diseases. This joint interdisciplinary collaborative project (with Prof. Hilal A. Lashuel) aims to bring together expertise in organic chemistry, proteins biochemistry, biophysics and molecular/cellular biology to develop innovative chemical approaches and novel tools to control and characterize protein misfolding and self-assembly of peptides and proteins in and outside of living cells. Our efforts will focus on developing new chemical switch elements to facilitate mechanistic studies aimed at elucidating the mechanisms of protein misfolding and aggregation and their role in the pathogenesis of amyloid diseases. More specifically, we will seek to develop new chemical tools to enhance the solubility and allow controlled disruption of folding and self-assembly in amyloid formation. We will then apply these tools to elucidate the molecular mechanisms underlying the misfolding, self-assembly and amyloid formation of various systems of increasing complexity. Our work represents the first attempt to extend the concept of molecular switches based on secondary structure disruption elements and acyl migration to control the folding, self-assembly, and aggregation properties of proteins. The work will result in the development of new chemical tools that allow greater spatial and temporal control

over protein structure and function without altering the native sequence of the protein. These tools will contribute significantly to addressing many of the technical and experimental limitations to study protein folding and self-assembly. The knowledge to be gained from the proposed studies will have significant impact on our understanding of the molecular basis of amyloid diseases, including Alzheimer's and Parkinson's, Huntington's disease and Type II diabetes, and contribute to advances in biotechnology, where protein aggregation remains a major challenge in the development of protein therapeutics. (This work is supported by the HFSP grant).

Towards these goals, we have recently shown a new transformation based on the Staudinger reaction and demonstrate its application in the design of a novel switch element to control the folding of the NPY peptide from random coil to α-helix conformation (*Organic Letters* 2008). The azido functionality in the depsipeptide unit is activated rapidly in water using TCEP via Staudinger reaction. This work expands the repertoire of uses of the Staudinger reaction in chemical biology and the number of available triggers for use in switch peptides.