

Robert A. Grassucci
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Education

B.S. in Biology, State University College at Oswego, 1984

AAS in Math/Science, Hudson Valley Community College, 1981

Awards

MSA 2011 Hildegard Crowley Outstanding Technologist in the Biological Sciences

Professional Experience

4/2008-Present Research Specialist 3 (HHMI/Columbia University)

8/2007-4/2008 Research Specialist 3 (HHMI/Wadsworth Center)

7/98-8/2007 Research Specialist 2 (HHMI/Wadsworth Center)

10/96-7/98 Research Scientist 1 (Health Research Inc.) Lab Management for 2 Principle Investigators, High Resolution Data collection, Equipment maintenance, Training of Students and Post Doctoral Fellows

4/91-10/96 Assistant Research Scientist (Health Research Inc.) Improved Cryo EM Protocols, Maintained Philips EM420 in Optimum Working Condition, High Resolution Data Collection

4/88-4/91 Sr. Laboratory Technician (Health Research Inc.) Developed Cryo Electron Microscopy Protocols

2/86-4/88 Laboratory Technician (Health Research Inc.) Prepared Specimens and Performed Transmission Electron Microscopy

8/85-2/86 Volunteer Laboratory Assistant: Prepared specimens for HVEM using plastic embedding and sectioning as well as culturing and critical point drying of whole cell mounts.

9/82-12/84 Laboratory Assistant (Oswego College Biology Dept.): Executed aseptic technique and tissue culture methods. Maintained cell biology lab.

Responsibilities 7/98-present

Lab management for Dr Joachim Frank

- Training: Graduate Students, postdocs, outside users and collaborators are trained in high resolution cryo-electron microscopy. This involved creation of a web based training syllabus and manuals as well as direct interaction with the trainees.
- Protocol Design: New techniques as well as constant improvements to preparation and data collection techniques to achieve highest resolution data were implemented.
- High resolution data for several investigators is generated. work independently from biological sample to finished 3D reconstruction
- Advise: Be a resource of knowledge for others because of my many years of experience in every aspect of this group's operation;
- Research: Independently conduct experiments and collect data in other labs throughout the world when necessary.
- Testing: Design performance acceptance tests and ensure these specifications are met for 2 state of the art Field Emission Transmission Electron Microscopes
- Equipment Management: Ensure Philips/FEI EM420, Tecnai F20 and Tecnai F30 Helium microscopes and Ancillary equipment are consistently operating at peak performance and with minimum down time using proper scheduling and testing.
- External Training: Travel to other labs to train their personnel in cryo EM preparation and data collection techniques.
- Networking: Communicate with personnel from other institutes as well as reading published articles to ensure that we are kept abreast of the latest developments in the field, suggest equipment and supplies to be ordered.

Publications

Research Papers

1. Wagenknecht, T., **R. Grassucci** and J. Frank. 1988. Electron microscopy of ice-embedded large ribosomal subunits from *Escherichia coli*. *J. Mol. Biol.*, 199:137-147.
2. Wagenknecht, T., **R.A. Grassucci**, J. Frank, A. Saito, M. Inui and S. Fleischer. 1989. Three-dimensional architecture of the calcium release channel from skeletal muscle. *Biophys. J.*, 55:14a.
3. Wagenknecht, T., **R. Grassucci**, J. Frank, A. Saito, M. Inui and S. Fleischer. 1989. Three-dimensional architecture of the calcium channel/foot structure of sarcoplasmic reticulum. *Nature*, 338:167-170.
4. Wagenknecht, T., **R. Grassucci** and D. Schaak. 1990. Cryoelectron microscopy of frozen-hydrated alpha-ketoacid dehydrogenase complexes from *Escherichia coli*. *J. Biol. Chem.*, 265:22402-22408.
5. Wagenknecht, T., **R. Grassucci**, G.A. Radke, and T.E. Roche. 1991. Cryoelectron microscopy of mammalian pyruvate dehydrogenase complex. *J. Biol. Chem.*, 266: 24650-24656.
6. Frank, J., P. Penczek, **R. Grassucci** and S. Srivastava. 1991. Three-dimensional reconstruction of the 70S *Escherichia coli* ribosome in ice: The distribution of ribosomal RNA. *J. Cell Biol.*, 115:579-605.
7. Radermacher, M., T. Wagenknecht, **R. Grassucci**, J. Frank, M. Inui, C. Chadwick and S. Fleischer. 1992. Cryo-EM of native structure of calcium release channel/ryanodine receptor from sarcoplasmic reticulum. *Biophys. J.*, 61:936-940.
8. Wagenknecht, T., **R. Grassucci**, J. Berkowitz and C. Forneris. 1992. Configuration of interdomain linkers in pyruvate dehydrogenase complex of *Escherichia coli* as determined by cryo electron microscopy. *J. Struct. Biol.*, 109:70-77.
9. Boisset, N., **R. Grassucci**, P. Penczek, E. Delain, F. Pochon, J. Frank and J. N. Lamy. 1992. Three-dimensional Reconstruction of a complex of human alpha2-macroglobulin with monomaleimido nanogold (Au 1.4nm) embedded in ice. *J. Struct. Biol.*, 109:39-45.
10. Boisset, N., M. Radermacher, **R. Grassucci**, J.-C. Taveau, W. Liu, J. Lamy, J. Frank and J.N. Lamy. 1993. Three-dimensional Immunoelectron Microscopy of Scorpion hemocyanin labeled with monoclonal fab fragment. *J. Struct. Biol.*, 109:234-244.
11. Penczek, P.A., **R.A. Grassucci** and J. Frank. 1994. The ribosome at improved resolution: new techniques for merging and orientation refinement in 3D cryo-electron microscopy of biological particles. *Ultramicroscopy*, 53:251-270.
12. Radermacher, M., V. Rao, **R. Grassucci**, J. Frank, A.P. Timmerman, S. Fleischer and T. Wagenknecht. 1994. Cryo-electron microscopy and three-dimensional reconstruction of the calcium release channel/ryanodine receptor from skeletal muscle. *J. Cell Biol.*, 127:411-423.
13. Wagenknecht, T., J. Berkowitz, **R. Grassucci**, A.P. Timmerman and S. Fleischer. 1994. Localization of the calmodulin binding sites on the ryanodine receptor from skeletal muscle by electron microscopy. *Biophys. J.*, 67:2286-2295.
14. Srivastava, S., A. Verschoor, M. Radermacher, **R. Grassucci** and J. Frank. 1995. Three-dimensional reconstruction of mammalian 40 S ribosomal subunit embedded in ice. *J. Mol. Biol.*, 245:461-466.
15. Frank, J., J. Zhu, P. Penczek, Y. Li, S. Srivastava, A. Verschoor, M. Radermacher, **R. Grassucci**, R.K. Lata and R.K. Agrawal. 1995. A model of protein synthesis based on cryo-electron microscopy of the *E. coli* ribosome. *Nature*, 376:441-444.
16. Frank, J., A. Verschoor, Y. Li, J. Zhu, R.K. Lata, M. Radermacher, P. Penczek, **R. Grassucci**, R.K. Agrawal and S. Srivastava. 1995. A model for the translational apparatus based on a three-dimensional reconstruction of the *Escherichia coli* ribosome. *Biochem. And Cell Biology*, 73:757-765.
17. Agrawal, R.K., P. Penczek, **R.A. Grassucci**, Y. Li, A. Leith, K.H. Nierhaus and J. Frank. 1996. Direct visualization of A-, P-, and E-site transfer RNA's in *E. coli* ribosome. *Science*, 271:1000-1002.
18. Wagenknecht, T., **R. Grassucci**, J. Berkowitz, G.J. Wiederrecht, H.-B. Xin and S. Fleischer. 1996. Cryoelectron microscopy resolves FK506-binding protein sites on the skeletal muscle ryanodine receptor. *Biophys. J.*, 70:1709-1715.
19. Lata, K.R., R.K. Agrawal, P. Penczek, **R. Grassucci**, J. Zhu and J. Frank. 1996. Three-dimensional reconstruction of *Escherichia coli* 30S ribosomal subunit in ice. *J. Mol. Biol.*, 262:43-52.
20. Verschoor, A., S. Srivastava, **R. Grassucci** and J. Frank. 1996. Native 3D structure of eukaryotic 80S ribosome: Morphological homology with the *E. coli* 70S ribosome. 133:495-505.
21. Beckmann, R., D. Bubeck, **R. Grassucci**, P. Penczek, A. Verschoor, G. Blobel and J. Frank. 1997. Alignment of conduits for the nascent polypeptide chain in the ribosome-sec61 complex. *Science*.

22. Wagenknecht, T., M. Radermacher, **R. Grassucci**, J. Berkowitz, H. B. Xin and S. Fleischer. 1997. Location of calmodulin and FK506-binding protein on the three-dimensional architecture of the skeletal muscle ryanodine receptor. *J. Biol. Chem.* 272:32463-32471.
23. Verschoor, A., J. R. Warner, S. Srivastava, **R. A. Grassucci** and J. Frank. 1998. Three dimensional structure of the yeast ribosome. *Nucl. Acids Res.* 26:655-661.
23. Agrawal, R.K., P. Penczek, **R.A Grassucci** and J. Frank. 1998. Visualization of elongation factor G on the *Escherichia coli* 70S ribosome: the mechanism of translocation. *PNAS*. 95:6134-6138.
24. Sharma, M. R., P. Penczek, **R. Grassucci**, HB. Xin, S. Fleischer and T. Wagenknecht. 1998. Cryoelectron microscopy and image analysis of the cardiac ryanodine receptor. *J. Biol. Chem.* 273:18429-18434.
25. Malhotra, A., P. Penczek, R.K. Agrawal, I.S. Gabashvili, **R.A. Grassucci**, R. Junemann, N. Burkhardt, K.H. Nierhaus and J. Frank. 1998. *Escherichia coli* 70S ribosome at 15 Å resolution by cryo electron microscopy: Localization of the fMET-tRNA and fitting of the L1 protein. *J. Mol. Biol.* 280:103-116.
26. Jeyakumar LH., JA. Copello, AM. O'Malley, GM. Wu, **R. Grassucci**, T. Wagenknecht and S. Fleischer. 1998 Purification and characterization of ryanodine receptor 3 from mammalian tissue. *Journal of Biological Chemistry*. 273:16011-20.
27. Ban N., B. Freeborn, P. Nissen, P. Penczek, **R.A. Grassucci**, R. Sweet, J. Frank, P.B. Moore and TA Steitz. 1998. A 9 Å resolution X-ray crystallographic map of the large ribosomal subunit. *Cell*. 93:1105-1115.
28. McCutcheon, JP., RK. Agrawal, SM. Philips, **RA. Grassucci**, SE. Gerchman, WM Jr. Clemons, V. Ramakrishnan and J. Frank. 1999. Location of translational initiation factor IF3 on the small ribosomal subunit. 96:4301-4306.
29. Agrawal RK., P. Penczek, **RA. Grassucci**, N. Burkhardt, KH. Nierhaus and J. Frank. 1999. Effect of buffer conditions on the position of tRNA on the 70 S ribosome as visualized by cryoelectron microscopy. *Journal of Biological Chemistry*. 274(13):8723-8729.
30. Gabashvili IS., RK. Agrawal, **R. Grassucci** and J. Frank. 1999. Structure and structural variations of the *Escherichia coli* 30 S ribosomal subunit as revealed by three-dimensional cryo-electron microscopy. *Journal of Molecular Biology*. 286:1285-1291.
31. Agrawal RK., AB. Heagle, P. Penczek, **RA. Grassucci** and J. Frank. 1999. EF-G-dependent GTP hydrolysis induces translocation accompanied by large conformational changes in the 70S ribosome. *Nature Structural Biology*. 6(7):643-647.
32. Gabashvili IS., RK. Agrawal, **R. Grassucci**, CL. Squires, AE. Dahlberg and J. Frank. 1999. Major rearrangements in the 70S ribosomal 3D structure caused by a conformational switch in 16S ribosomal RNA. *EMBO Journal*. 18:6501-6507.
33. Penczek P., N. Ban, **RA. Grassucci**, RK. Agrawal and J. Frank. 1999. Haloarcula marismortui 50S subunit-complementarily of electron microscopy and X-Ray crystallographic information. *Journal of Structural Biology*. 128:44-50.
34. Spahn, CM., **RA. Grassucci**, P. Penczek and J. Frank. 1999. Direct three-dimensional localization and positive identification of RNA helices within the ribosome by means of genetic tagging and cryo-electron microscopy. *Structure with Folding & Design*. 7:1567-73.
35. Gabashvili, IS., RK. Agrawal, CM. Spahn, **RA. Grassucci**, DI. Svergun, J. Frank and P. Penczek. 2000. Solution structure of the *E. coli* 70S ribosome at 11.5 Å resolution. *Cell*. 100:537-549.
36. Gomez-Lorenzo, MG., CM. Spahn, RK. Agrawal, **RA. Grassucci**, P. Penczek, K. Chakraborty, JP. Ballesta, JL. Lavandera, JF. Garcia-Bustos and J. Frank. 2000. Three-dimensional cryo-electron microscopy localization of EF2 in the *Saccharomyces cerevisiae* 80S ribosome at 17.5 Å resolution. *EMBO Journal*. 19(11):2710-8.
37. Frank J. P., Penczek, RK. Agrawal, **RA. Grassucci**, AB Heagle. 2000. Three-dimensional cryoelectron microscopy of ribosomes. *Methods in Enzymology*. 317:276-91.
38. Agrawal RK., CM. Spahn, P. Penczek, **RA. Grassucci**, KH. Nierhaus and J. Frank. 2000. Visualization of tRNA movements on the *Escherichia coli* 70S ribosome during the elongation cycle. *Journal of Cell Biology*. 150:447-60.

39. Gabashvili, IS., S.T. Gregory, M. Valle, **R. Grassucci**, M. Worbs, M.C. Wahl, AE. Dahlberg, J. Frank. 2001. The polypeptide tunnel system in the ribosome and its gating in erythromycin resistance mutants of L4 and L22. *Molecular Cell* . 8(1):181-8.
40. Valle, M., J. Sengupta, N. Swami, **R.A.Grassucci**, N. Burkhardt, K.H Nierhaus.,R.K.Agrawal and J. Frank. (2002). Cryo-EM structure of the ribosome-bound EF-Tu ternary complex: an active role for the aminoacyl-tRNA in the accommodation process. *EMBO J.* 21(13):3557-67.
41. Gao, H., CM. Spahn, **R.A.Grassucci** and J.Frank, (2002). An assay for local quality in cryo-electron micrographs of single particles. *Ultramicroscopy.* 93(2):169-78.
42. Rawat, UB, AV. Zavialov, J. Sengupata, M.Valle, **R.A. Grassucci**, J.Linde, B.Vestergarrd, M. Ehrenberg, and J. Frank.(2003). A cryo-electron microscopic study of ribosome-bound termination factor RF2. *Nature.* 421(6918):87-90.
43. Spahn CM, Gomez-Lorenzo MG, **Grassucci RA**, Jorgensen R, Andersen GR, Beckmann R, Penczek PA, Ballesta JP, Frank J. (2004) Domain movements of elongation factor eEF2 and the eukaryotic 80S ribosome facilitate tRNA translocation. *EMBO J.* 2004 Mar 10;23(5):1008-1019. Epub 2004 Feb 19.
44. Halic M, Becker T, Pool MR, Spahn CM, **Grassucci RA**, Frank J, Beckmann R. (2004) Structure of the signal recognition particle interacting with the elongation-arrested ribosome. *Nature.* 2004 Feb 26;427(6977):808-14.
45. Agrawal RK, Sharma MR, Kiel MC, Hirokawa G, Booth TM, Spahn CM, **Grassucci RA**, Kaji A, Frank J.(2004) Visualization of ribosome-recycling factor on the Escherichia coli 70S ribosome: functional implications. *Proc Natl Acad Sci U S A.* 2004 Jun 15;101(24):8900-5. Epub 2004 Jun 3.
46. Spahn CM, Jan E, Mulder A, **Grassucci RA**, Sarnow P, Frank J. (2004) Cryo-EM visualization of a viral internal ribosome entry site bound to human ribosomes: the IRES functions as an RNA-based translation factor. *Cell.* 2004 Aug 20;118(4):465-75.
47. **Grassucci RA**, Taylor DT and Frank J. (2007) Preparation of Macromolecular complexes for cryo-electron microscopy. *Nature Protocol.*2007;2(12):3239-3246.
48. **Grassucci RA**, Taylor DT and Frank J. (2008) Visualization of macromolecular complexes using cryo-electron microscopy with FEI Tecnai transmission electron microscopes. *Nature Protocol.*2008; 3(2): 330-339.
49. Jamie LeBarron, **Robert A. Grassucci**, Tanvir R. Shaikh, William T. Baxter, Jayati Sengupta, Joachim Frank (2008) Exploration of parameters in cryo-EM leading to an improved density map of the E. coli ribosome. *Journal of Structural Biology* 164:1 October 2008: 24-32.
50. Elizabeth Villa , Jayati Sengupta, Leonardo G. Trabuco, Jamie LeBarron, William T. Baxter, Tanvir R. Shaikh, **Robert A. Grassucci**, Poul Nissen, Måns Ehrenberg, Klaus Schulten, and Joachim Frank (2009) Ribosome-induced changes in elongation factor Tu conformation control GTP hydrolysis. *PNAS* 106:4 January 27 2009 1063-1068.
51. William T. Baxter, **Robert A. Grassucci**, Haixiao Gao and Joachim Frank (2009) Determination of signal-to-noise ratios and spectral SNRs in cryo-EM low-dose imaging of molecules *Journal of Structural Biology* 166: 126-132.
52. Tao Huang, Tanvir Shaikh, Kushol Gupta, Lydia Contreras-Martinez, , **Robert Grassucci**, Gregory Van Duyne, Joachim Frank, Marlene Belfort. (2010) The group II intron ribonucleoprotein precursor is a large, loosely packed structure. *Nucleic Acids Research.* Dec 3,2010.

Abstracts

1. Hammill, T.M., S.W. Beattie and **R.A. Grassucci**. 1985. Scanning electron microscopy of zygosporogenesis in *Mucor mucedo*. *Mycological Society of America Newsletter.* 36(1):26.
2. Hammill, T.W., S.W. Beattie, **R.A. Grassucci**, D.A. Sussman and C.T. Ware. 1985. Transmission electron microscopy of zygosporogenesis in *Mucor mucedo*. I. Early stages. *Mycological Society of America Newsletter.* 36(1):26.
3. Turner, J.N., W.G. Shain, Jr., V. Madelain, **R.A. Grassucci** and D.L. Foreman. 1986. High Voltage electron microscopy of astroglial cell whole mounts. In: *Proc. 44th EMSA Meeting.* ed. G.W. Bailey. 316.

4. McEwen, B.F., C.L. Rieder, M. Radermacher, **R.A. Grassucci**, J.N. Turner and J. Frank. 1987. The application of three-dimensional tomographic reconstruction methods to high-voltage electron microscopy. In: Proc. 45th EMSA Meeting. Ed. G.W. Bailey. 570.
5. **Grassucci, R.A.**, T. Wagenknecht, G.A. Radke and T.E. Roche. 1990. Cryoelectron microscopy of frozen-hydrated pyruvate dehydrogenase complexes. In: Proc. XIIth International Congress for Electron Microscopy. Ed. L.D. Peachey and D.B. Williams, 260-261.
6. Wagenknecht, T., **R. Grassucci**, J. Berkowitz, A.P. Timmerman and S. Fleischer. 1993. Cryo-electron microscopy of frozen-hydrated isolated triads and terminal cisternae from sarcoplasmic reticulum. *Biophys. J.*, 64:A153.
7. Wagenknecht, T., J. Berkowitz, **R. Grassucci**, A.P. Timmerman and S. Fleischer. 1994. Localization of calmodulin binding sites on the skeletal muscle ryanodine receptor by electron microscopy. *Biophys. J.*, 66:A19.
8. Radermacher, M., V. Rao, T. Wagenknecht, **R. Grassucci**, J. Frank, A.P. Timmerman and S. Fleischer. 1994. Three-dimensional cryo-electron microscopy of the calcium release channel/ryanodine receptor (Ryr) from skeletal muscle. *Biophys. J.*, 66:A19.
9. **Grassucci, R.A.** and J. Frank .2000. Cryo-electron microscopy training at the Wadsworth Center. In: Proc. Microsc. And Microanalysis 2000. Ed. G.W. Bailey. 278-279.
10. **Grassucci, R.A.**, Z. Liu, T. Wagenknecht and J. Frank. 2002. HHMI Tecnai F30 Helium Microscope: Initial Results and Observations. In: Proc. Microscopy and Microanalysis 2002. 8 Suppl.2, 854CD.
11. **Grassucci, R.A.**, W.T. Baxter, E.S. Barnard and J. Frank 2005. Efficient Strategy for Processing Single Particle Data for a Cryo-EM Exposure Series. In: Proc. Microscopy and Microanalysis 2005. 11Suppl 2 286CD.