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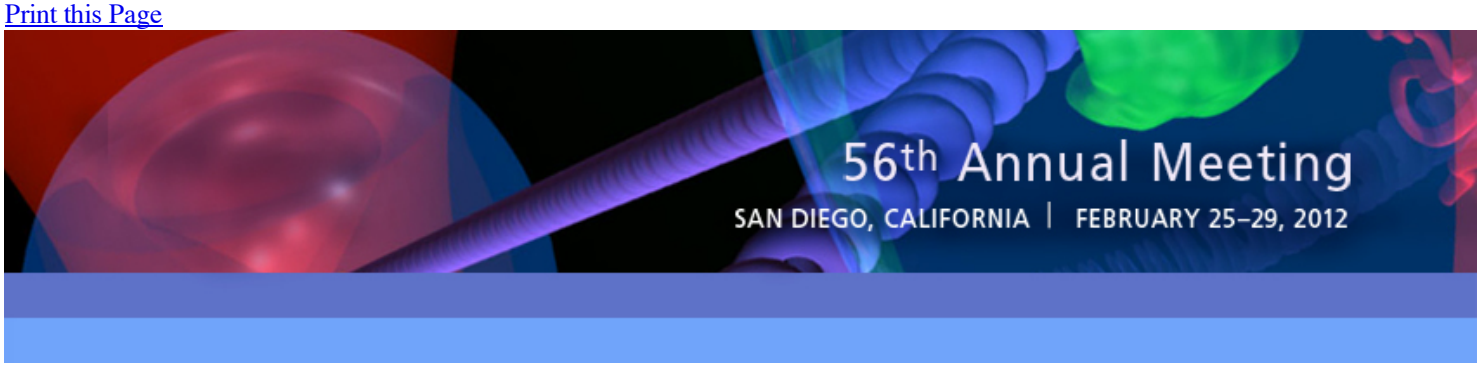

Key: ◆ Indicates Potential Conflict with another presentation.

	Session Start Time Presentation Time Location	Pres # & Type	Poster Board #	Authors & Institutions	Abstract Title Session # & Title
	Sat 2/25 9:00 AM 10:00 AM Room 29ABC	No Abstract Subgroup Symposia		**Benoit Roux**. BMB, University of Chicago, Chicago, IL, USA.	MOLECULAR DYNAMICS COMPUTATIONS OF STANDARD BINDING FREE ENERGIES 015.Subgroup: Molecular Biophysics
	Sat 2/25 1:30 PM 1:30 PM Room 28AB	No Abstract Subgroup Symposia		**Eduardo Perozo**. Dept of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL, USA.	A MOLECULAR MECHANISM OF ACTIVATION-DEACTIVATION GATING IN THE MG^{2+}-DEPENDENT MG^{2+} CHANNEL CORA 026.Subgroup: Permeation & Transport
	Sat 2/25 1:00 PM 1:35 PM Ballroom 20A	33-Subg Subgroup Symposia		**Emad Tajkhorshid, PhD**. Department of Biochemistry, and Beckman Institute, University of Illinois at Urbana Champaign, Urbana, IL, USA.	ACCELERATING MEMBRANE INSERTION OF PERIPHERAL PROTEINS WITH A NOVEL MEMBRANE MIMETIC MODEL 023.Subgroup: Membrane Structure & Assembly
	Sun 2/26 8:15 AM 9:45 AM - 10:00 AM Room 25ABC	71-Plat Platforms		**Katarina Ruscic**[1,2], Francesco Miceli[3], Carlos Villalba-Galea[4], Francisco Bezanilla[1], Steve A. N. Goldstein[1,2]. [1]University of Chicago, Chicago, IL, USA, [2]Brandeis University, Waltham, MA, USA, [3]IRCCS Bambino Gesù Children's Hospital, Rome, Italy, [4]Virginia Commonwealth University School of Medicine, Richmond, VA, USA.	KCNE1 SLOWS THE VOLTAGE SENSORS OF KCNQ1 042.Platform: Voltage-gated K Channels: Gating
	Sun 2/26 1:45 PM 1:45 PM - 3:45 PM Hall FGH	1028-Pos Posters	B814	**Chaitanya Sathe**, Xueqing Zou, Jean-Pierre Leburton, Klaus Schulten. UIUC, Urbana, IL, USA.	COMPUTATIONAL INVESTIGATION OF GRAPHENE NANO PORE DNA DETECTION 091.36.Micro & Nanotechnology: Nanopores I
	Sun 2/26 1:45 PM 1:45 PM - 3:45 PM Hall FGH	318-Pos Posters	B104	**Boon Chong Goh**[1], Xueqing Zou[2], Michael J. Rynkiewicz[3], Barbara A. Seaton[3], Klaus J. Schulten[1,2]. [1]University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Beckman Institute, Urbana, IL, USA, [3]Boston University, Boston, MA, USA.	MECHANISM OF INTERACTION BETWEEN LUNG SURFACTANT PROTEIN-D AND INFLUENZA A VIRUS HEMAGGLUTININ

					091.05.Protein-Ligand Interactions I
	Sun 2/26 1:45 PM 1:45 PM - 3:45 PM Hall FGH	654-Pos Posters	B440	**Angelica M. Lopez-Rodriguez**, Miguel Holmgren. NIH, Bethesda, MD, USA.	RESCUING PROPER TRAFFICKING OF CYSTEINE MUTANT PROTEINS 091.20.Cyclic Nucleotide-gated Channels
	Sun 2/26 1:45 PM 1:45 PM - 3:45 PM Hall FGH	570-Pos Posters	B356	**David Papke**, Claudio Grosman. University of Illinois, Urbana-Champaign, Urbana, IL, USA.	EXAMINING THE ROLE OF THE M1-M2 LOOP IN CYS-LOOP RECEPTOR DESENSITIZATION AND GATING 091.16.Ligand-gated Channels I
	Sun 2/26 4:00 PM 4:00 PM - 4:15 PM Room 29ABC	179-Plat Platforms		**Qufei Li**[1], Sherry Wanderling[1], Carlos A. Villalba-Galea[2], Eduardo Perozo[1]. [1]Institute for Biophysical Dynamics and Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL, USA, [2]Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Richmond, VA, USA.	THE RESTING AND ACTIVATED CONFORMATIONS OF THE VOLTAGE SENSOR OF CI-VSP FROM FUNCTIONAL AND SOLVENT ACCESSIBILITY DETERMINATIONS 081.Platform: Voltage-sensitive Proteins
	Sun 2/26 4:00 PM 5:30 PM - 5:45 PM Room 24ABC	169-Plat Platforms		**Martin L. Prieto**, Omer Oralkan, Butrus T. Khuri-Yakub, Merritt C. Maduke. Stanford University, Stanford, CA, USA.	ULTRASOUND-INDUCED CURRENTS IN PLANAR LIPID BLAYERS: ORIGINS AND POTENTIAL PHYSIOLOGICAL SIGNIFICANCE 079.Platform: Membrane Dynamics & Bilayer Probes
	Sun 2/26 7:30 PM 7:30 PM Ballroom 20BC	211-Wkshp Workshops		**Lewis Kay**. Medical Genetics & Microbiology, University of Toronto, Toronto, ON, Canada.	SEEING THE INVISIBLE BY SOLUTION NMR 090.Workshop: Measuring Excursions from the "Structure" (X-Ray Crystallography, NMR, Simulations)
	Mon 2/27 8:15 AM 8:30 AM - 8:45 AM Room 24ABC	1083-Plat Platforms		**Wei Han**, Emad Tajkhorshid. University of Illinois at Urbana-Champaign, Urbana, IL, USA.	TRANSIENT WATER CHAINS CONNECTING THE CYTOPLASMIC AND EXTRACELLULAR GLUTAMATE GATES IN CLC 101.Platform: Membrane Transporters & Exchangers I
	Mon 2/27 8:15 AM 10:00 AM - 10:15 AM Room 24ABC	1089-Plat Platforms		**Elena J. Levin**[1], Yu Cao[1], Giray Enkavi[2], Matthias Quick[1], Yaping Pan[1], Emad Tajkhorshid[2], Ming Zhou[1]. [1]Columbia University, New York, NY, USA, [2]University of Illinois at Urbana-Champaign, Urbana, IL, USA.	MECHANISM AND REGULATION OF UREA PERMEATION IN A MAMMALIAN UREA CHANNEL 101.Platform: Membrane Transporters & Exchangers I
	Mon 2/27 1:45 PM 1:45 PM - 3:45 AM	1314-Pos Posters	B84	**Melih Sener**, Johan Strumpfer, Klaus Schulten. UIUC, Urbana, IL, USA.	ATOMIC LEVEL SUPRAMOLECULAR ORGANIZATION AND

	Hall FGH				FUNCTION OF AN ENTIRE BIOENERGETIC ORGANELLE 144.04.Protein Assemblies
	Mon 2/27 1:45 PM 1:45 PM - 3:45 AM Hall FGH	1316-Pos Posters	B86	**Y Zenmei Ohkubo**, Emad Tajkhorshid. University of Illinois at Urbana-Champaign, Urbana, IL, USA.	FORMATION OF TERNARY COMPLEX OF FACTOR VIIA, TISSUE FACTOR, AND FACTOR X ON THE SURFACE OF ANIONIC MEMBRANE 144.04.Protein Assemblies
	Mon 2/27 1:45 PM 1:45 PM - 3:45 PM Hall FGH	1343-Pos Posters	B113	**Tomoya Kubota**, Jérôme J. Lacroix, Francisco Bezanilla, Ana M. Correa. The University of Chicago, Chicago, IL, USA.	PROBING S4 LENGTH CHANGES DURING GATING WITH LRET 144.05.Membrane Protein Structure I
	Mon 2/27 1:45 PM 1:45 PM - 3:45 PM Hall FGH	1832-Pos Posters	B602	**Johan A. Strumpfer**[1,2], Eleonore von Castelmur[3], Barbara Franke[4], Sonia Barbieri[4], Julijus Bogomolovas[5], Hiroshi Qadota[6], Petr Konarv[7], Dmitri Svergun[7], Siegfried Labeit[8], Klaus Schulten[1,2], Guy M. Benian[6], Olga Mayans[4]. [1]University of Illinois at Urbana Champaign, Urbana, IL, USA, [2]Beckman Institute, Urbana, IL, USA, [3]Institute of Integrative Biology, University of Liverpool, Liverpool, IL, USA, [4]Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom, [5]Universitätsmedizin Mannheim, Mannheim, Germany, [6]Department of Pathology, Emory University, Atlanta, GA, USA, [7]European Molecular Biology Laboratory, Hamburg, Germany, [8]Department for Integrative Pathophysiology, Universitätsmedizin Mannheim, Mannheim, Germany.	STRETCHING OF TWITCHIN KINASE 144.29.Muscle: Fiber & Molecular Mechanics & Structure II
	Mon 2/27 1:45 PM 1:45 PM - 3:45 PM Hall FGH	1439-Pos Posters	B209	**Tao Jiang**, Maria Spies, Emad Tajkhorshid. University of Illinois at Urbana-Champaign, Urbana, IL, USA.	CHARACTERIZING AN INTERMEDIATE STATE BETWEEN INACTIVE AND ACTIVE STATES OF RECA 144.10.DNA Replication, Recombination, and Repair
	Mon 2/27 1:45 PM 1:45 PM - 3:45 PM Hall FGH	1692-Pos Posters	B462	**Christopher N. Rowley**, Benoit Roux. University of Chicago, Chicago, IL, USA.	INTERPRETING THE BARIUM BLOCKADES OF POTASSIUM CHANNELS WITH THE MULTI-ION PERMEATION FREE ENERGY SURFACE 144.23.Biophysics of Ion Permeation
	Mon 2/27 1:45 PM 1:45 PM - 3:45 PM Hall FGH	2059-Pos Posters	B829	**Olivier Dalmas**, H. Clark Hyde, Eduardo Perozo. The University of Chicago, Chicago, IL, USA.	DISTANCE DETERMINATION IN MULTIMERIC MEMBRANE PROTEIN BY SYMMETRY CONSTRAINED ANALYSIS OF DOUBLE ELECTRON-ELECTRON RESONANCE SPECTROSCOPY. 144.41.EPR Spectroscopy
	Mon 2/27 1:45 PM 1:45 PM - 3:45 PM Hall FGH	1540-Pos Posters	B310	**Mark J. Arcario**, Emad Tajkhorshid. University of Illinois at Urbana-Champaign, Champaign, IL, USA.	CAPTURING SPONTANEOUS MEMBRANE INSERTION AND MEMBRANE-INDUCED CONFORMATIONAL CHANGES OF TALIN AT AN ATOMIC RESOLUTION 144.15.Lipids & Signaling on

					Membrane Surfaces
	Mon 2/27 1:45 PM 1:45 PM - 3:45 PM Hall FGH	1717-Pos Posters	B487	Pornthep Sompornpisut[1], **Olivier Dalmas**[2], Eduardo Perozo[2]. [1]Chulalongkorn University, Bangkok, Thailand, [2]The University of Chicago, Chicago, IL, USA.	THE OPEN CONFORMATION OF THE MG2+ CHANNEL CORA FROM SOLVENT ACCESSIBILITY AND DISTANCE CONSTRAINTS 144.24.Ligand-gated Channels II
	Mon 2/27 4:00 PM 5:15 PM - 5:30 PM Room 24ABC	1195-Plat Platforms		**Hang Yu**, Klaus Schulten, Ying Yin, Anton Arkhipov. University of Illinois, Urbana Champaign, Urbana, IL, USA.	COMPUTER SIMULATION OF MEMBRANE TUBULATION BY EFC F-BAR DOMAIN LATTICES 134.Platform: Interfacial Protein-Lipid Interactions I
	Tue 2/28 8:15 AM 8:45 AM - 9:15 AM Ballroom 20D	2071-Symp Symposia		**Merritt Maduke**. Stanford University, Stanford, CA, USA.	CLC TRANSPORTERS: THE SEARCH FOR CONFORMATIONAL CHANGE 152.Symposium: Transporter-Channel Interface
	Tue 2/28 8:15 AM 9:15 AM - 9:30 AM Room 29ABC	2101-Plat Platforms		**Taras V. Pogorelov**, Emad Tajkhorshid. University of Illinois, Urbana, IL, USA.	GLYCOPHORIN A HELIX INSERTION, POSITIONING, AND DIMERIZATION IN MODEL MEMBRANES 156.Platform: Membrane Structure II
	Tue 2/28 8:15 AM 9:30 AM - 9:45 AM Room 25ABC	2094-Plat Platforms		**Leigh D. Plant**[1], Leandro Zuniga[2], Dan Araki[2], Jeremy D. Marks[2], Steve AN Goldstein[1]. [1]Brandeis University, Waltham, MA, USA, [2]University of Chicago, Chicago, IL, USA.	K2P1 ASSEMBLES WITH K2P3 OR K2P9 TO FORM SUMO-REGULATED TASK BACKGROUND CHANNELS IN CEREBELLAR GRANULE NEURONS. 155.Platform: Channel Regulation & Modulation
	Tue 2/28 10:45 AM 11:25 AM - 11:45 AM Room 24ABC	2123-MiniSymp Minisymposia		Gisela D. Cymes, **Claudio Grosman**. University of Illinois at Urbana-Champaign, Urbana, IL, USA.	THE RING OF GLUTAMATES IN THE CHARGE-SELECTIVITY FILTER REGION OF THE NICOTINIC RECEPTOR FORMS A SYSTEM OF UNANTICIPATED COMPLEXITY 164.Minisymposium: Ligand-gated Channels
	Tue 2/28 1:45 PM 1:45 PM - 3:45 PM Hall FGH	2652-Pos Posters	B422	**Thomas A. Chew**[1,2], Sherwin J. Abraham[2], Shelley M. Elvington[2], Merritt C. Maduke[2]. [1]University of California, San Diego, La Jolla, CA, USA, [2]Stanford University, Stanford, CA, USA.	STRUCTURAL INVESTIGATIONS OF CLC-EC1, A LARGE INTEGRAL MEMBRANE PROTEIN, USING SOLUTION-STATE NMR AND NANODISC TECHNOLOGY 191.17.Membrane Transporters &

					Exchangers I
	Tue 2/28 1:45 PM 1:45 PM - 3:45 PM Hall FGH	2700-Pos Posters	B470	**Michael F. Priest**[1], Jérôme J. Lacroix[1], Carlos A. Villalba-Galea[2], Francisco Bezanilla[1]. [1]University of Chicago, Chicago, IL, USA, [2]Virginia Commonwealth University School of Medicine, Richmond, VA, USA.	REDUCING S3-S4 LINKER LENGTH IN SHAKER K+ CHANNELS STABILIZES THE RELAXED STATE 191.20.Voltage-gated K Channels: Gating II
	Tue 2/28 1:45 PM 1:45 PM - 3:45 PM Hall FGH	2327-Pos Posters	B97	**Christopher G. Mayne**, John A. Katzenellenbogen, Emad Tajkhorshid. University of Illinois at Urbana-Champaign, Urbana, IL, USA.	CHARACTERIZING STRUCTURAL AND DYNAMICAL IMPACTS OF AGONIST- AND ANTSGONIST-STABILIZED MUTANTS OF THE ESTROGEN RECEPTOR 191.04.Protein Folding & Stability II
	Tue 2/28 1:45 PM 1:45 PM - 3:45 PM Hall FGH	2653-Pos Posters	B423	**Ricky C.K. Cheng**, Shelley M. Elvington, Merritt C. Maduke. Stanford University, Stanford, CA, USA.	MONITORING SUBSTRATE-DRIVEN CONFORMATIONAL CHANGES OF CLC-EC1 BY [METHYL-13C] METHIONINE NMR 191.17.Membrane Transporters & Exchangers I
	Tue 2/28 1:45 PM 1:45 PM - 3:45 PM Hall FGH	2621-Pos Posters	B391	**Sayan Mondal**[1], George Khelashvili[1], Jennifer Khangulova (Johnston)[2], Hao Wang[2], Davide Provasi[2], Olaf S. Andersen[1], Marta Filizola[2], Harel Weinstein[1]. [1]Weill Medical College of Cornell University, New York, NY, USA, [2]Mount Sinai School of Medicine, New York, NY, USA.	INTERACTION WITH THE MEMBRANE UNCOVERS ESSENTIAL DIFFERENCES BETWEEN HIGHLY HOMOLOGOUS GPCRS 191.16.Membrane Receptors & Signal Transduction I
	Tue 2/28 1:45 PM 1:45 PM - 3:45 PM Hall FGH	2287-Pos Posters	B57	**Mert Gur**[1], Jeffry Madura[2], Ivet Bahar[1]. [1]Department of Computational & Systems Biology, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA, [2]Department of Chemistry and Biochemistry, Duquesne University, Pittsburgh, PA, USA.	TRANSITION PATHWAYS OF PROTEINS EXPLORED BY COMBINING MOLECULAR DYNAMICS SIMULATIONS AND MONTE CARLO SAMPLING OF COLLECTIVE MODES 191.03.Protein Dynamics II
	Tue 2/28 1:45 PM 1:45 PM - 3:45 PM Hall FGH	2702-Pos Posters	B472	**Jerome J. Lacroix**[1], Stephan A. Pless[2], Fabiana V. Campos[3], Luca Maragliano[1], Jason D. Galpin[2], Christopher A. Ahern[2], Benoit Roux[1], Francisco Bezanilla[1]. [1]University of Chicago, Chicago, IL, USA, [2]University of British Columbia, Vancouver, BC, Canada, [3]University of Heidelberg, Heidelberg, Germany.	POSITION OF THE SECOND GATING CHARGE ALONG S4 IN AN INTERMEDIATE CONFORMATION OF A K+ CHANNEL VOLTAGE SENSOR 191.20.Voltage-gated K Channels: Gating II
	Tue 2/28 1:45 PM 1:45 PM - 3:45 PM Hall FGH	2374-Pos Posters	B144	**Javier L. Baylon**, Emad Tajkhorshid. University of Illinois at Urbana-Champaign, Urbana, IL, USA.	CAPTURING INSERTION AND DYNAMICS OF MEMBRANE-BOUND CYTOCHROME P450 3A4 USING A NOVEL MEMBRANE MIMETIC MODEL 191.06.Heme Proteins
	Tue 2/28 1:45 PM 1:45 PM - 3:45 PM	2694-Pos Posters	B464	**Luis G. Cuello**[1], Doris M. Cortes[1], Eduardo Perozo[2]. [1]Texas Tech University Health Science Center,	ROLE OF STRUCTURAL WATER

	Hall FGH			Lubbock, TX, USA, [2]The University of Chicago, Chicago, IL, USA.	IN THE STABILIZATION OF THE KCSA C-TYPE INACTIVATED SELECTIVITY FILTER: EVIDENCE FROM HIGH-RESOLUTION STRUCTURES 191.20.Voltage-gated K Channels: Gating II
	Tue 2/28 1:45 PM 1:45 PM - 3:45 PM Hall FGH	2267-Pos Posters	B37	**Wenxun Gan**, Mahmoud Moradi, Emad Tajkhorshid. University of Illinois at Urbana-Champaign, Urbana, IL, USA.	CHARACTERIZING TRANSITION PATHWAYS IN THE TRANSPORT CYCLE OF ABC TRANSPORTER MSBA 191.02.Protein Conformation II
	Tue 2/28 1:45 PM 1:45 PM - 3:45 PM Hall FGH	2511-Pos Posters	B281	**Joshua V. Vermaas**, Emad Tajkhorshid. University of Illinois at Urbana-Champaign, Urbana, IL, USA.	CONFORMATIONAL DYNAMICS OF MEMBRANE-BOUND α-SYNUCLEIN IN A HIGHLY MOBILE MEMBRANE MIMETIC 191.12.Interfacial Protein-Lipid Interactions II
	Tue 2/28 1:45 PM 1:45 PM - 3:45 PM Hall FGH	2645-Pos Posters	B415	**Sebastian Stolzenberg**, George Khelashvili, Harel Weinstein. Weill Cornell Medical College, Cornell University, New York, NY, USA.	STRUCTURAL INTERMEDIATES IN A MODEL OF THE SUBSTRATE TRANSLOCATION PATH IN THE BACTERIAL GLUTAMATE TRANSPORTER HOMOLOGUE GltPh 191.17.Membrane Transporters & Exchangers I
	Tue 2/28 1:45 PM 1:45 PM - 3:45 PM Hall FGH	2651-Pos Posters	B421	**Kimberly I. Matulef**[1], Andrew Howery[2], R. Lea Sanford[3], Sabrina Phillips[1], Sierra Simpson[2], Sherwin Abraham[2], Julian Whitelegge[4], Justin Du Bois[2], Olaf S. Andersen[3], Merritt Maduke[2]. [1]University of San Diego, San Diego, CA, USA, [2]Stanford University, Stanford, CA, USA, [3]Cornell University, New York, NY, USA, [4]UCLA, Los Angeles, CA, USA.	CHARACTERIZATION OF A NOVEL CLC-EC1 INHIBITOR 191.17.Membrane Transporters & Exchangers I
	Tue 2/28 1:45 PM 1:45 PM - 3:45 PM Hall FGH	2325-Pos Posters	B95	**Yanxin Liu**, Johan Strumpfer, Peter L. Freddolino, Martin Gruebele, Klaus Schulten. University of Illinois at Urbana-Champaign, Urbana, IL, USA.	FOLDING THERMODYNAMICS AND KINETICS OF LAMBDA-REPRESSOR FROM ALL-ATOM MOLECULAR DYNAMICS SIMULATIONS 191.04.Protein Folding & Stability II
	Tue 2/28 1:45 PM 1:45 PM - 3:45 PM Hall FGH	2381-Pos Posters	B151	**George Khelashvili**[1], Sayan Mondal[1], Martin Caffrey[2], Harel Weinstein[1]. [1]Weill Cornell Medical College, Cornell University, New York, NY, USA, [2]Membrane Structural and Functional Biology Group, Schools of Medicine and Biochemistry & Immunology, Trinity College, Dublin, Ireland.	QUANTITATIVE COMPARISON OF GPCR INTERACTIONS WITH THE LIPID BILAYER OF THE CUBIC AND LAMELLAR MESOPHASES 191.07.Membrane Protein Structure II
	Tue 2/28 1:45 PM 1:45 PM - 3:45 PM Hall FGH	2701-Pos Posters	B471	**Homer Clark Hyde**, Jeremy Treger, Francisco Bezanilla. The University of Chicago, Chicago, IL, USA.	VOLTAGE-CLAMPED SUPPORTED BILAYER SYSTEM TO RECORD ION CHANNEL ACTIVITY

					191.20.Voltage-gated K Channels: Gating II
	Tue 2/28 1:45 PM 1:45 PM - 3:45 PM Hall FGH	2695-Pos Posters	B465	**Amelia M. Randich**, Eduardo Perozo. University of Chicago, Chicago, IL, USA.	A RANDOM MUTAGENESIS APPROACH TO PROBING ELECTROMECHANICAL COUPLING IN THE HYPERPOLARIZATION-ACTIVATED CHANNEL MVP 191.20.Voltage-gated K Channels: Gating II
	Tue 2/28 4:00 PM 4:15 PM - 4:30 PM Room 29ABC	2200-Plat Platforms		**George Khelashvili**[1], Aurelio Galli[2], Harel Weinstein[1]. [1]Weill Cornell Medical College, Cornell University, New York, NY, USA, [2]Vanderbilt University School of Medicine, Nashville, TN, USA.	HOW PIP2 LIPIDS REGULATE THE POSITION AND PHOSPHORYLATION OF THE SYNTAXIN N-TERMINUS 184.Platform: Interfacial Protein-Lipid Interactions II
	Tue 2/28 4:00 PM 5:15 PM - 5:30 PM Room 24ABC	2188-Plat Platforms		**James C. Gumbart**[1], Benoit Roux[2], Klaus Schulten[3]. [1]Argonne National Lab, Argonne, IL, USA, [2]University of Chicago, Chicago, IL, USA, [3]University of Illinois, Urbana-Champaign, Urbana, IL, USA.	SPONTANEOUS MOVEMENT OF TRANSMEMBRANE SEGMENTS FROM SECY INTO THE MEMBRANE 182.Platform: Membrane Protein Function
	Wed 2/29 8:15 AM 8:30 AM - 8:45 AM Room 30ABC	3079-Plat Platforms		**Mahmoud Moradi**, Giray Enkavi, Emad Tajkhorshid. University of Illinois at Urbana-Champaign, Urbana, IL, USA.	CONFORMATIONAL TRANSITION PATHWAY OF GlpT TRANSPORTER, CHARACTERIZED BY NONEQUILIBRIUM MOLECULAR DYNAMICS SIMULATIONS 200.Platform: Membrane Transporters & Exchangers II
	Wed 2/29 8:15 AM 9:00 AM - 9:15 AM Room 30ABC	3081-Plat Platforms		**Kelli Kazmier**[1], Matthias Quick[2], Lei Shi[3], Harel Weinstein[3], Jonathan A. Javitch[2], Hassane S. Mchaourab[1]. [1]Vanderbilt University, Nashville, TN, USA, [2]Columbia University College of Physicians and Surgeons, New York, NY, USA, [3]Weill Medical College of Cornell University, New York, NY, USA.	STRUCTURE, DYNAMICS, AND MECHANISM OF THE LEUCINE TRANSPORTER STUDIED BY DOUBLE ELECTRON ELECTRON RESONANCE SPECTROSCOPY 200.Platform: Membrane Transporters & Exchangers II
	Wed 2/29 8:15 AM 9:15 AM - 9:30 AM Room 30ABC	3082-Plat Platforms		**Shruti Sharma**[1], Richard A. Stein[1], Matthias Quick[2], Sebastian Stolzenberg[3], Lei Shi[3], Harel Weinstein[3], Jonathan A. Javitch[2], Hassane S. Mchaourab[1]. [1]Vanderbilt University, Nashville, TN, USA, [2]Center for Molecular Recognition, Columbia University, New York, NY, USA, [3]Weill Cornell Medical College, New York, NY, USA.	CONFORMATIONAL DYNAMICS IN THE TRANSPORT CYCLE OF LEUCINE TRANSPORTER ON THE EXTRACELLULAR-SIDE 200.Platform: Membrane Transporters & Exchangers II
	Wed 2/29 8:15 AM 9:30 AM - 9:45 AM	3059-Plat Platforms		**Aaron Hoskins**[1], Larry Friedman[2], Ivan Correa[3], Ming-Qun Xu[3], Virginia W. Cornish[4], Jeff Gelles[2],	TURNING ON THE SPLICEOSOME

	Room 24ABC			Melissa J. Moore[5]. [1]U. Wisconsin-Madison, Madison, WI, USA, [2]Brandeis University, Waltham, MA, USA, [3]New England Biolabs, Ipswich, MA, USA, [4]Columbia University, New York City, NY, USA, [5]U. Massachusetts Medical School/HHMI, Worcester, MA, USA.	197.Platform: Protein-Nucleic Acid Interactions
	Wed 2/29 8:15 AM 9:45 AM - 10:00 AM Room 30ABC	3084-Plat Platforms		**Walter Sandtner**[1], Bernhard Egwolf[2], Fatemeh Khalili-Araghi[3], Jorge E. Sanchez-Rodríguez[3], Benoit Roux[3], Francisco Bezanilla[3], Miguel Holmgren[4]. [1]Medical University of Vienna, Vienna, Austria, [2]Max Planck Institute for Biophysical Chemistry, Goettingen, Germany, [3]University of Chicago, Chicago, IL, USA, [4]National Institutes of Health, Bethesda, MD, USA.	OUABAIN BINDING SITE IN A FUNCTIONING NA+/K+-ATPASE 200.Platform: Membrane Transporters & Exchangers II
	Wed 2/29 10:30 AM 10:30 AM - 12:30 PM Hall FGH	3615-Pos Posters	B476	**Paween Mahinthichaichan**, James Hemp, Robert B. Gennis, Emad Tajkhorshid. University of Illinois at Urbana-Champaign, Urbana, IL, USA.	PROBING GAS DIFFUSION PATHWAYS IN CYTOCHROME C OXIDASE WITH EXPLICIT AND IMPLICIT LIGAND SAMPLINGS 210.25.Membrane Transport
	Wed 2/29 10:30 AM 10:30 AM - 12:30 PM Hall FGH	3590-Pos Posters	B451	**Miguel Holmgren**[1], David C. Gadsby[2], Francisco Bezanilla[2], Robert F. Rakowski[2], Paul De Weer[2]. [1]NIH, Bethesda, MD, USA, [2]Marine Biological Laboratory, Woods Hole, MA, USA.	NA+ ACCESS KINETICS IN THE NA+/K+-ATPASE PUMP 210.24.Ion Motive ATPases
	Wed 2/29 10:30 AM 10:30 AM - 12:30 PM Hall FGH	Pos-L173 Posters	LB173	**Ernesto Vargas**, Francisco Bezanilla. University of Chicago, Chicago, IL, USA.	OBSERVING THE ROTATION OF A VOLTAGE-SENSING DOMAIN IN AN ION CHANNEL USING SPECTROSCOPIC METHODS 202.1.Late Poster Presentations
	Wed 2/29 10:30 AM 10:30 AM - 12:30 PM Hall FGH	3589-Pos Posters	B450	**Jorge E. Sanchez-Rodriguez**[1], Fatemeh Khalili-Araghi[1], Benoit Roux[1], Miguel Holmgren[2], Francisco Bezanilla[1]. [1]Department of Biochemistry and Molecular Biology, The University of Chicago, Gordon Center for Integrative Science, Chicago, IL, USA, [2]Molecular Neurophysiology Section, Porter Neuroscience Research Center, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA.	CHANGES OF INTRAMOLECULAR DISTANCES IN THE Na+/K+ ATPase UPON OUABAIN BINDING 210.24.Ion Motive ATPases
	Wed 2/29 10:30 AM 10:30 AM - 12:30 PM Hall FGH	3362-Pos Posters	B223	**Jing Li**, Emad Tajkhorshid. University of Illinois at Urbana-Champaign, Urbana, IL, USA.	MECHANISM OF ION COUPLED STATE TRANSITION IN LEUT-FOLD TRANSPORTERS 210.13.Membrane Transporters & Exchangers II
	Wed 2/29 10:30 AM 10:30 AM - 12:30 PM Hall FGH	3349-Pos Posters	B210	**Po-Chao Wen**, Emad Tajkhorshid. University of Illinois at Urbana-Champaign, Urbana, IL, USA.	FUNDAMENTAL DIFFERENCE OF TRANSPORT MECHANISMS BETWEEN ABC IMPORTERS AND EXPORTERS 210.13.Membrane Transporters & Exchangers II
	Wed 2/29 10:30 AM 10:30 AM - 12:30 PM Hall FGH	3253-Pos Posters	B114	**Yaroslav D. Bodnar**, Klaus J. Schulten. University of Illinois at Urbana-Champaign, Urbana, IL, USA.	MOLECULAR DYNAMICS FLEXIBLE FITTING OF POLIOVIRUS STRUCTURAL TRANSITIONS DURING INITIATION OF INFECTION AT ATOMIC RESOLUTION

					210.06.Virus Structure & Assembly
	Wed 2/29 10:30 AM 10:30 AM - 12:30 PM Hall FGH	3351-Pos Posters	B212	**Ping Zou**, Hassane Mchaourab. Vanderbilt University, Nashville, TN, USA.	STRUCTURE AND DYNAMICS OF THE MFS MULTIDRUG TRANSPORTER EMRD 210.13.Membrane Transporters & Exchangers II
	Wed 2/29 10:30 AM 10:30 AM - 12:30 PM Hall FGH	3605-Pos Posters	B466	Mizuki Sekiya[1], **Robert K. Nakamoto**[2], Mayumi Nakanishi-Matsui[1], Masamitsu Futai[1]. [1]Iwate Medical University, Yahaba, Japan, [2]University of Virginia, Charlottesville, VA, USA.	THE PHYTOPOLYPHENOL PICEATANNOL INHIBITS THE RATE LIMITING STEP OF ROTATIONAL CATALYSIS OF THE F1-ATPASE 210.24.Ion Motive ATPases
	Wed 2/29 10:30 AM 10:30 AM - 12:30 PM Hall FGH	3485-Pos Posters	B346	**Pablo Miranda**[1,2], Jorge E. Contreras[3], Diana Wesch[1,2], Fred J. Sigworth[4], Miguel Holmgren[5], Teresa Giraldez[1]. [1]Research Division, University Hospital NS Candelaria, Tenerife, Spain, [2]Department of Physiology, University of La Laguna, Tenerife, Spain, [3]University of Medicine and Dentistry of New Jersey (UMDNJ), Newark, NJ, USA, [4]Department of Cellular and Molecular Physiology Yale University School of Medicine, New Haven, CT, USA, [5]National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA.	STATE-DEPENDENT FRET REPORTS LARGE GATING-RING MOTIONS IN BK CHANNELS 210.20.Ca-activated Channels
	Wed 2/29 10:30 AM 10:30 AM - 12:30 PM Hall FGH	3591-Pos Posters	B452	**Juan P. Castillo**, Daniela De Giorgis, Daniel Basilio, David C. Gadsby, Joshua JC Rosenthal, Ramon Latorre, Miguel Holmgren, Francisco Bezanilla. Laboratorio de Fisiologia Celular, Montemar, Valparaiso, Chile.	ENERGETICS OF THE REACTIONS CONTROLLING THE DEEPLY OCCLUDED STATE OF THE NA+/K+ PUMP 210.24.Ion Motive ATPases
	Wed 2/29 10:30 AM 10:30 AM - 12:30 PM Hall FGH	3433-Pos Posters	B294	**David Medovoy**[1], Luis Cuello G. Cuello[2], Eduardo Perozo[1], Benoit Roux[1]. [1]University of Chicago, Chicago, IL, USA, [2]Texas Tech University Health Sciences Center, Lubbock, TX, USA.	A THREE-ION SELECTIVITY FILTER POTENTIAL ENERGY LANDSCAPE OF A PUTATIVE OPEN-CONDUCTIVE KCSA 210.17.Voltage-gated K Channels: Permeation
	Wed 2/29 10:30 AM 10:30 AM - 12:30 PM Hall FGH	3646-Pos Posters	B507	**Mikhail G. Shapiro**[1,2], Kazuaki Homma[3], Sebastian Villarreal[2], Claus-Peter Richter[3], Francisco Bezanilla[2]. [1]UC Berkeley, Berkeley, CA, USA, [2]University of Chicago, Chicago, IL, USA, [3]Northwestern University, Chicago, IL, USA.	INFRARED LIGHT EXCITES CELLS VIA TRANSIENT CHANGES IN MEMBRANE ELECTRICAL CAPACITANCE. 210.26.Biotechnology & Bioengineering II
	Wed 2/29 10:30 AM 10:30 AM - 12:30 PM Hall FGH	3359-Pos Posters	B220	**Giray Enkavi**, Emad Tajkhorshid. University of Illinois at Urbana-Champaign, Urbana, IL, USA.	TRANSPORT CYCLE OF MITOCHONDRIAL CARRIERS FROM INTERNAL SYMMETRIES 210.13.Membrane Transporters & Exchangers II
	Wed 2/29 10:30 AM 10:30 AM - 12:30 PM Hall FGH	3618-Pos Posters	B479	**Ryan Steed**, Kristin Trone, Hassane Mchaourab. Vanderbilt University, Nashville, TN, USA.	SUBSTRATE BINDING AND TRANSPORT BY A BACTERIAL MULTIDRUG MFS TRANSPORTER 210.25.Membrane Transport
	Wed 2/29 10:30	3475-Pos	B336	**H. Raghuraman**[1], Qufei Li[1], Sherry Wanderling[1], Carlos A. Villalba-Galea[2], Eduardo Perozo[1].	STRUCTURAL

	AM 10:30 AM - 12:30 PM Hall FGH	Posters		[1]Institute for Biophysical Dynamics, The University of Chicago, Chicago, IL, USA, [2]Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Richmond, VA, USA.	DYNAMICS IN THE RESTING AND ACTIVATED STATES OF THE VOLTAGE SENSOR OF Ci-VSP FROM DIPOLAR DISTANCE MEASUREMENTS 210.19.Voltage-gated K Channels: Gating III
	Wed 2/29 10:30 AM 10:30 AM - 12:30 PM Hall FGH	Pos-L54 Posters	LB54	**Hassane S. Mchaourab**[1], Johanna Baldus-Becker[2], Andrea Lakatos[2], Shruti Sharma[1], Clemens Glaubitz[2]. [1]Vanderbilt University, Nashville, TN, USA, [2]Goethe University, Frankfurt, Germany.	DYNAMIC NUCLEAR POLARISATION ENHANCED MAS-NMR ON ^{13}C-LEUCINE BOUND TO THE SECONDARY TRANSPORTER LEUT 202.1.Late Poster Presentations
	Wed 2/29 10:30 AM 10:30 AM - 12:30 PM Hall FGH	3350-Pos Posters	B211	**Matthieu Masureel**[1], Smriti Smriti[2], Chloé Martens[1], Ping Zou[2], Jean-Marie Ruysschaert[1], Hassane S. Mchaourab[2], Cédric Govaerts[1]. [1]Université Libre de Bruxelles, Brussels, Belgium, [2]Vanderbilt University Medical Center, Nashville, TN, USA.	STUDYING THE CONFORMATIONAL CYCLE OF THE SECONDARY MULTIDRUG TRANSPORTER LMRP BY EPR SPECTROSCOPY 210.13.Membrane Transporters & Exchangers II
	Wed 2/29 1:00 PM 1:30 PM - 2:00 PM Ballroom 20D	3099-Symp Symposia		Aaron A. Hoskins[1], Larry J. Friedman[2], Daniel J. Crawford[3], Eric J. Anderson[3], Inna Shcherbakova[3], Virginia W. Cornish[4], Jeff Gelles[2], **Melissa J. Moore**[5]. [1]Univ. Wisconsin, Madison, WI, USA, [2]Brandeis University, Waltham, MA, USA, [3]UMass Medical School/HHMI, Worcester, MA, USA, [4]Columbia University, New York, NY, USA, [5]Department of Biochemistry and Molecular Pharmacology, UMass Medical School/HHMI, Worcester, MA, USA.	INTO THE COSMOS: SINGLE MOLECULE ANALYSIS OF SPLICEOSOME ASSEMBLY AND ACTIVATION 204.Symposium: Dynamics and Localization of RNAs
	Wed 2/29 1:00 PM 2:00 PM - 2:15 PM Ballroom 20BC	3112-Plat Platforms		**Hector P. Salazar Garcia**[1], Albert Y. Lau[2], Lydia Blachowicz[3], Andrew J. Plested[1], Benoît Roux[3]. [1]Leibniz-Institut für Molekulare Pharmakologie & NeuroCure Initiative, Charité Universitätsmedizin, Berlin, Germany, [2]Johns Hopkins University School of Medicine, Dept. of Biophysics and Biophysical Chemistry, Baltimore, MD, USA, [3]The University of Chicago, Dept. of Biochemistry and Molecular Biology, Chicago, IL, USA.	INTERDIMER CONTACTS PAINT A NEW PICTURE OF GLUTAMATE RECEPTOR ACTIVATION. 206.Platform: Ligand-gated Channels

33-Subg. ACCELERATING MEMBRANE INSERTION OF PERIPHERAL PROTEINS WITH A NOVEL MEMBRANE MIMETIC MODEL

Emad Tajkhorshid, PhD.
Department of Biochemistry, and Beckman Institute, University of Illinois at Urbana Champaign, Urbana, IL, USA.

Membrane binding and insertion of peripheral proteins constitutes a key step in their function, both through localizing them into specific regions of the membrane and often also regulating their activity. Characterizing membrane-bound forms of peripheral protiens, however, has proven challenging both experimentally and in simulations, with the latter being significantly hindered by the slow dynamics of membrane reorganization. To expedite lateral diffusion of lipid molecules without sacrificing the atomic details of such interactions, we have developed a novel membrane representation, termed HMMM (Highly Mobile Membrane Mimetic) to study binding and insertion of various molecular species into the membrane. The model is based on the novel concept of molecular fragmentation, i.e., representing the lipid molecules by smaller fragments that faithfully preserve the interactions in a full membrane. The model takes advantage of an organic solvent layer to represent the hydrophobic core of the membrane and short-tailed phospholipids for the headgroup region. The model describes spontaneous, rapid bilayer formation regardless of the initial lipid position and orientation, a phenomenon reported for the first time at an atomistic level. In the HMMM membrane, lipid molecules

exhibit 1-2 orders of magnitude enhancement in lateral diffusion, while the atomic density profile is essentially identical to full-membrane models. Most importantly, the model is extremely efficeint in capturing spontaneous binding and insertion of various membrane-anchoring domains and proteins/peptides, e.g., coagulation proteins, talin, alha-synuclein, and viral fusion peptide, consistently in multiple unbiased simulations. The model and the applications will be presented and discussed in this talk.

71-Plat. KCNE1 SLOWS THE VOLTAGE SENSORS OF KCNQ1

Katarina Ruscic[1,2], Francesco Miceli[3], Carlos Villalba-Galea[4], Francisco Bezanilla[1], Steve A. N. Goldstein[1,2].
[1]University of Chicago, Chicago, IL, USA, [2]Brandeis University, Waltham, MA, USA, [3]IRCCS Bambino Gesù Children's Hospital, Rome, Italy, [4]Virginia Commonwealth University School of Medicine, Richmond, VA, USA.

KCNQ1 α-subunits assemble with KCNE1 forming the slow cardiac potassium channel I_{Ks}. KCNE1 alters currents of α-subunits by slowing activation and deactivation kinetics, suppressing inactivation, and increasing single-channel conductance. Attempts to elucidate the mechanism of KCNE1-induced slowing of kinetics have come to discrepant conclusions. Two studies using chemical modifiers and cys-substitution in the KCNQ1 S4 supported one model where KCNE1 acts on voltage-sensors alone and another where only the activation-gate is affected. A fluorimetric study argued for effects on sensors and the gate (Osteen et al, PNAS 2010). To address the controversy, we used cut-open oocyte vaseline-gap clamp to record gating currents, ionic currents and perform site-directed, voltage-clamp fluorimetry (VCF).

Gating currents show that voltage-sensor movement precedes pore opening in channels with only KCNQ1 α-subunits. VCF confirms this result. While ionic currents have a lag before activation, gating and fluorescence changes do not; this indicates a final concerted step after voltage sensors move before pore opening. In contrast, channels with KCNE1 show voltage-sensor movements with no lag that are ~20-fold slower and mirror slowing of ionic current activation. These findings are unlike those made by Osteen et al. We used a smaller dye inserted closer to S4 and measured ionic current and fluorescence simultaneously in the same cells. We observe a matching and expected shift in the I_{Ks} fluorescence-voltage and ionic current-voltage relationships of +50 mV with KCNE1 and do not see voltage-sensor movements at hyperpolarized voltages as they report.

Both KCNQ1 and I_{Ks} channels have voltage-sensor kinetics almost as slow as ionic activation. Our simultaneous kinetic recordings of fluorescence and ionic currents support a model in which I_{Ks} channels are slowed purely by an effect of KCNE1 on the KCNQ1 voltage sensors. Support: HL105949 and GM030376.

1028-Pos/B814. COMPUTATIONAL INVESTIGATION OF GRAPHENE NANO PORE DNA DETECTION

Chaitanya Sathe, Xueqing Zou, Jean-Pierre Leburton, Klaus Schulten.
UIUC, Urbana, IL, USA.

Recently, voltage-induced transport of dsDNA molecules
through nano pores in graphene
membranes has been demonstrated experimentally. Graphene, due
to its sub-nanometer thickness,
shows great potential to realize DNA sequencing at single-base
resolution. The kinetics of electrophoretically
driven DNA translocation through graphene nanopores was studied using molecular
dynamics simulations (Sathe et al., ACS Nano, in press).
The simulations provide guidance in the design of
graphene-based DNA sequencing
devices and single molecule sensors. We
report the effects of applied voltage,
DNA conformation, pore charge as well as sequence on the translocation
characteristics of DNA revealed in the simulations. Simulations
yield also, consistent with recent measurements, the characteristics of ion currents passing pores alongside with DNA.
The simulations
demonstrate, furthermore, that under suitable voltage bias
conditions A-T and G-C base pairs can be discriminated using graphene
nanopores.

318-Pos/B104. MECHANISM OF INTERACTION BETWEEN LUNG SURFACTANT PROTEIN-D AND INFLUENZA A VIRUS HEMAGGLUTININ

Boon Chong Goh[1], Xueqing Zou[2], Michael J. Rynkiewicz[3], Barbara A. Seaton[3], Klaus J. Schulten[1,2].
[1]University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Beckman Institute, Urbana, IL, USA, [3]Boston University, Boston, MA, USA.

Lung collectin surfactant proteins are pulmonary host defense proteins that contribute to innate, front-line defense against influenza A virus (IAV) and other inhaled pathogens. Collectins recognize viral glycans on the globular head of hemagglutinin (HA) on the IAV surface and initiate events leading to pathogen neutralization. Thus, effective pulmonary host defense requires fast recognition of IAV HA by collectins. In order to assist development of new approaches to collectin-based antiviral therapeutics, we investigated the mechanism underlying SP-D recognition of IAV HA using molecular dynamics simulations. Comparing the binding affinities of SP-Ds of human and swine on different IAV HA proteins, we showed that swine's SP-D has a higher binding affinity towards the glycans of HA proteins. In addition, starting from crystalized protein structures from X-ray diffraction experiments, our simulations identified the most stable docking configuration of the SP-D-HA complex, revealing how composition and location of glycans affect binding of SP-D to HA.

654-Pos/B440. RESCUING PROPER TRAFFICKING OF CYSTEINE MUTANT PROTEINS

Angelica M. Lopez-Rodriguez, Miguel Holmgren.
NIH, Bethesda, MD, USA.

Cysteine substitution has been widely used in structure and function studies, but sometimes mutant proteins get retained during trafficking and the cell is unable to deliver full-length membrane proteins to the cell surface. Similarly, in many inheritable genetic diseases cysteine mutant proteins encounter the same fate. In the visual system, for example, some cysteine mutants of cyclic nucleotide-gated channel (CNG channels) are retained in the ER, leading to achromatopsia (color blindness). We reasoned that it should be possible to modify the chemical structure of the mutation in order to mimic the side chain of the wild type amino acid and recover proper trafficking. As proof of principle, we have studied two naturally occurring cysteine mutants (Y181C and R277C in CNGA3). These mutations are responsible for hereditary cone photoreceptor disorders. We introduced both achromatopsia-related cysteines in a cysteine-less CNG channel, and used them as targets for specific chemical modification with hydroxybenzyl- (MTSHB) and aminoethyl-methanethiosulfonate (MTSEA). These reagents readily attach to the side chain of cysteines and mimic the chemistry of tyrosine and arginine, respectively. Cell surface expression was assayed in *Xenopus* oocytes using fluorescence microscopy and electrophysiology. We successfully restored trafficking and normal function to CNG mutant channels R→C and Y→C, as well as three more cysteine mutants within the S4 transmembrane segment that are known to be retained in the ER. This chemical method provides a unique opportunity to functionally characterize previously inaccessible proteins, as well as it can be readily implemented to assess the chemical nature of misfolding problems, conformational dynamics of folding processes or to study protein conformational changes at the site of the cysteine mutation.

570-Pos/B356. EXAMINING THE ROLE OF THE M1-M2 LOOP IN CYS-LOOP RECEPTOR DESENSITIZATION AND GATING

David Papke, Claudio Grosman.
University of Illinois, Urbana-Champaign, Urbana, IL, USA.

The intracellular M1-M2 loop of Cys-loop receptors connects the pore-lining M2 α-helix with the adjacent M1 α-helix, and it is located near the narrowest constriction of the open pore. In ganglionic acetylcholine receptors (AChRs), oxidation of the -4' cysteine in this linker greatly affects the kinetics of recovery from desensitization; in glycine receptors (GlyRs), a naturally occurring mutation significantly alters the biophysical properties of these receptors, leading to hyperekplexia in humans. However, despite these findings, the M1-M2 loop's role in gating and desensitization of Cys-loop receptors has yet to receive thorough investigation. To this end, we engineered a series of mutations to the M1-M2 loops of the human α3β4 AChR, adult muscle AChR, and α1 GlyR. Mutations to the -4' cysteine of α3β4 AChRs had only small effects on deactivation and responses to low-frequency repetitive stimulation in the whole-cell configuration. In the GlyR, alanine and threonine scans of the M1-M2 loop did not consistently change receptor kinetics, as measured in fast-perfused outside-out patches; however, the large patch-to-patch variability in GlyR behavior complicated our comparison across mutants. In the muscle AChR, alanine, glycine, proline, and valine scans were performed on the M1-M2 loop of the α1 subunit, and their effects were measured in fast-perfused outside-out patches. Remarkably, just as mutations to M2 consistently lead to a prolongation of the rate of deactivation, so too mutations to the M1-M2 loop invariably led to an increase of the rate of entry into desensitization; thus, just as residues in M2 very likely change microenvironments during channel gating, so too do residues in the M1-M2 loop during desensitization. This result suggests that the desensitization gate of the AChR is located near the narrowest constriction of the open channel.

179-Plat. THE RESTING AND ACTIVATED CONFORMATIONS OF THE VOLTAGE SENSOR OF CI-VSP FROM FUNCTIONAL AND SOLVENT ACCESSIBILITY DETERMINATIONS

Qufei Li[1], Sherry Wanderling[1], Carlos A. Villalba-Galea[2], Eduardo Perozo[1].
[1]Institute for Biophysical Dynamics and Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL, USA, [2]Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Richmond, VA, USA.

The voltage sensor domain (VSD) is responsible for electromechanical transduction in voltage-gated ion channels and enzymes. In all known VSDs, both architecture and voltage-sensing mechanism are conserved: the positive charged residues (R/K) on the fourth

transmembrane segment S4 respond to the voltage change across the membrane, which trigger its own conformation change leading to the response of downstream domain. A wealth of biophysical information on voltage sensors in the last two decades has revealed one of the major functional states - “up” or activated state. However, the structure and functional properties of the “down” or resting state remains controversial. Here, we show electrophysiological and structural studies of the voltage sensor from *Ciona intestinalis* voltage sensitive phosphatase (Ci-VSP), that point to conformational transitions between the resting and activated conformations of the sensor. The voltage dependence of Ci-VSP mutants, analyzed by gating charge measurement in oocytes, show significant shift in their Q-V relationships along the voltage axis (R217E -60 mV, R217Q -20 mV, WT +60 mV, D136N +130 mV). At 0 mV, these mutants populate different functional states under biochemical conditions: WT and D136N mostly in the “down” state while R217E is mostly in the “up” state. A Ci-VSD biochemical preparation was developed for each of the four mutants and studied by site-directed spin labeling EPR (SDSL-EPR) methods in proteoliposomes. Mobility and accessibility information revealed the secondary structure of transmembrane segments and their positions relative to membrane and each other, suggesting the extend and direction of the motion of S4 between “up” and “down” states. These results are consistent with the down movement of S4 under hyperpolarization and render critical structural information, that allow us to propose a gating mechanism for Ci-VSD.

169-Plat. ULTRASOUND-INDUCED CURRENTS IN PLANAR LIPID BLAYERS: ORIGINS AND POTENTIAL PHYSIOLOGICAL SIGNIFICANCE

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Low-intensity focused ultrasound shows great promise for non-invasive, spatially resolved modulation of neural activity *in vivo*. To determine the mechanisms involved in ultrasonic modulation of neural activity and guide the development of this technology, we have been investigating the effects of ultrasound on protein-free planar lipid bilayers. Previously, we reported that ultrasound causes decaying current oscillations in planar bilayers at the onset and offset of the stimulus. These on and off responses are of opposite polarity but otherwise identical. Here, we report that if the rise time of the ultrasound pulse is prolonged, the on response is resolved into two distinct components: a sigmoidal component during the rise time and a damped oscillating component once the pulse reaches its final value. This result suggests that changes in ultrasound intensity during the rise time of the pulse may be important in determining the response to ultrasound *in vivo*, and is consistent with the observation that pulsed ultrasound is more effective than continuous ultrasound in modulating neural activity. To investigate further the origins of the on/off behavior, we used an optical interferometer to measure the velocity of the ultrasound-induced movement (acoustic streaming) in the solution surrounding the bilayer. We find that the time course of the ultrasound-induced current matches the time course of the streaming velocity, with a ratio of 162 pA/(mm/s). This acoustic streaming is probably due to the action of ultrasonic radiation force. To explore the potential physiological relevance of these effects, and to obtain further mechanistic insight, we are investigating the response of planar bilayers to ultrasound under current-clamp. In preliminary experiments, we find that ultrasound pulses with intensity comparable to those used *in vivo* produce voltage changes that would be sufficient to initiate an action potential.

211-Wkshp. SEEING THE INVISIBLE BY SOLUTION NMR

Lewis Kay.
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Seeing the Invisible by Solution NMR Spectroscopy
Lewis E. Kay
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Many biochemical processes proceed through the formation of functionally important intermediates. For example, ligand binding, enzyme catalysis and protein folding may all involve the formation of one or more intermediates along the reaction coordinate connecting the initial and final protein states. A complete understanding of each process, requires, therefore, characterization of these intermediates in detail. While methods exist for studying the endpoints of these processes at atomic resolution in many cases, similar studies of the intermediates remain elusive. NMR methods for seeing such ‘invisible’ states will be described, along with a number of applications to protein folding illustrating the power of the methodology.

1083-Plat. TRANSIENT WATER CHAINS CONNECTING THE CYTOPLASMIC AND EXTRACELLULAR GLUTAMATE GATES IN CLC

Wei Han, Emad Tajkhorshid.
University of Illinois at Urbana-Champaign, Urbana, IL, USA.

CLC-ec1, a bacterial member of the CLC family, exchanges chloride ions and protons across the cellular membrane. A key step of the transport cycle of CLC- ec1 is the transfer of protons between the extracellular and cytoplasmic gates, E148 and E203, respectively. These residues are 15 Å apart, and in dearth of any intermediate titratable groups needed for proton shuttling. Hence, it is an open question as to how protons shuttle between the two gates. Proton hopping through water chains provides a possible mechanism in this regard, having been investigated in numerous computational studies, given the lack of experimental structural data on water. Two possible chains, either involving Y445 or side-chain rotation of E203, have been proposed based on searching

algorithms and short molecular dynamics (MD) simulations. We herein propose another water chain characterized through extended (0.42 μs) MD simulations of CLC-ec1 dimer. The water chain forms frequently (once every 50-100 ns) but transiently (lasting for <1-2 ns). Neither Y445 nor the side-chain rotation of E203 is needed for the water chain. The presence of the water chain, however, coincides with significant side-chain conformational changes of F199 and F357 around the chloride-binding site and F208 and F219 at the dimer interface remote from the ion permeation pathway. We further performed a 0.25 μs simulation of monomeric CLC-ec1, which has been shown to be structurally identical to the dimer, but with a halved activity. We show that water chains don't form as readily in the monomeric simulation as what was observed in the dimer simulation; side-chain conformations of F199, F357, F208 and F219 are also different from the dimer. Our study supports the idea that both local and long-range factors could be important for the CLC-ec1 activity.

1089-Plat. MECHANISM AND REGULATION OF UREA PERMEATION IN A MAMMALIAN UREA CHANNEL

Elena J. Levin[1], Yu Cao[1], Giray Enkavi[2], Matthias Quick[1], Yaping Pan[1], Emad Tajkhorshid[2], Ming Zhou[1].
[1]Columbia University, New York, NY, USA, [2]University of Illinois at Urbana-Champaign, Urbana, IL, USA.

To maintain constant fluid volume and osmolarity in the face of infrequent access to water, terrestrial animals accumulate high concentrations of urea in the kidney interstitium to allow the reabsorption of water. This mechanism is dependent on the facilitated diffusion of urea through members of a family of integral membrane proteins known as urea transporters (UTs). While the structure of a bacterial homolog was solved previously, the bacterial UT transports urea much slower than the mammalian ones and its similarity to the mammalian proteins remained unclear. In addition, little was known of the mechanism of permeation. To answer these questions, we have solved the first structure of a mammalian urea transporter at 2.4 Å, and probed the energetics of urea permeation with a combination of molecular dynamics simulations, functional characterization of mutants and co-crystallization with urea analogs. Similarities with the bacterial UT suggest that many features of the structure are broadly conserved across the UT family, including a trimeric assembly, and the presence of a channel-like, continuous permeation pathway through each monomer. The functional and computational studies revealed a large energy barrier at the center of the permeation pathway, whose presence may hint towards a possible gating mechanism.

1314-Pos/B84. ATOMIC LEVEL SUPRAMOLECULAR ORGANIZATION AND FUNCTION OF AN ENTIRE BIOENERGETIC ORGANELLE

Melih Sener, Johan Strumpfer, Klaus Schulten.
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An atomic level structural model of a bioenergetic pseudo-organelle, the chromatophore, is presented along with a stochastic spatiotemporal description of its function. The chromatophore is an intracytoplasmic membrane vesicle of 50-70 nm size found in purple bacteria, comprising of over a hundred proteins that cooperate to produce ATP by converting light energy. The primary function of the chromatophore is performed through the subprocesses of photon absorption, excitation energy transfer, charge separation, diffusion of electron carriers, and the generation of a proton-motive force, culminating, finally, in ATP synthesis. These subprocesses bridge quantum mechanical and classical domains over timescales ranging from picoseconds to milliseconds. We present an atomic detail structural model of an entire chromatophore vesicle obtained by combining atomic-force microscopy, cryo-electron microscopy, crystallography, spectroscopy, and proteomics data. The chromatophore model thus constructed features 99 LH2 complexes, 15 LH1-RC dimer complexes, 7 bc1 complexes, and 1 ATP synthase, as well as over 3000 bacteriochlorophyll pigments. Based on the resulting supramolecular organization, a stochastic description of the aforementioned subprocesses are presented, revealing the principles of efficiency and robustness in the light harvesting function of the chromatophore.

1316-Pos/B86. FORMATION OF TERNARY COMPLEX OF FACTOR VIIA, TISSUE FACTOR, AND FACTOR X ON THE SURFACE OF ANIONIC MEMBRANE

Y Zenmei Ohkubo, Emad Tajkhorshid.
University of Illinois at Urbana-Champaign, Urbana, IL, USA.

Most steps in the clotting cascade involve formation of protein complexes between an enzyme, a cofactor, and a substrate, highly catalyzed by membrane binding of individual proteins. Upon binding to anionic regions of the cellular membrane, the rate of enzymatic activity of these proteins is enhanced by several orders of magnitude. Despite its established key role, the mechanism by which the membrane mediates this activation process has remained largely unknown. The complex of factor VIIa (FVIIa), tissue factor (TF), and factor X (FX), namely the TF:FVIIa:FX ternary complex, is the first such complex in the clotting cascade. We report atomistic models of the TF:FVIIa:FX complex, revealing specific interactions between individual proteins in the ternary complex and the structural transitions resulted from such interactions.

The ternary complex was constructed in two steps, combining docking and molecular dynamics (MD) methodologies, in conjunction with a highly mobile membrane mimetic (HMMM) model for enhanced lipid mobility. Starting with the known structures of the TF:FVIIa binary complex and a modeled FX, first several candidates of the complex of the enzyme (TF:FVIIa) and its substrate (FX) were generated by docking hundreds of structural snapshots of individual components collected from their respective MD

simulations. The best-scored candidates were then subjected to MD simulations on the surface of the HMMM membrane, which can efficiently describe binding/insertion of proteins to the membrane due to the enhanced lipid mobility. During the simulations, weak distance restraints were imposed between residues that have been experimentally reported to be involved in protein-protein interaction. The complexes at the end of the equilibrium process resulted in a well-converged ternary structure characterizing FX residues that interact with the TF exosite.

1343-Pos/B113. PROBING S4 LENGTH CHANGES DURING GATING WITH LRET

Tomoya Kubota, Jérôme J. Lacroix, Francisco Bezanilla, Ana M. Correa.
The University of Chicago, Chicago, IL, USA.

Voltage-activated proteins containing a Voltage Sensor Domain (VSD) respond to changes in the membrane potential by transferring across the electric field several positively charged residues (gating charges) located in the fourth transmembrane segment (S4). Even though the mechanistic details of gating charge translocation and S4 re-arrangement are presently unclear, a prevailing hypothesis suggests that the S4 segment adopts a 3_{10} helix conformation during gating, thus aligning the gating charges and facilitating their transport across the membrane electric field. If a whole typical S4 segment were to change its conformation from an α-helix to a 3_{10} helix, its length would stretch by about 8 Å. Here we tested the existence of such transition by measuring the length of the S4 segment during gating using the LRET technique. We used the VSD domain of the *Ciona intestinalis* Voltage-Sensitive Phosphatase (C_iVSP), truncated from its phosphatase and phospholipid-binding domains, to genetically encode a lanthanide (Tb^{3+})-binding-tag at the extracellular end of S4 and the red fluorescent protein mCherry at its intracellular end. We expressed the protein in *Xenopus* oocytes from which we recorded gating currents using the cut-open and two-electrode voltage-clamp techniques. The distance-dependent efficiency of energy transfer between the two probes was measured at steady-state voltages and also during voltage pulse protocols of varying amplitudes and durations. Our technique could only detect distance changes larger than 2.5 Å. We found that in our C_iVSP construct, the results are not consistent with the expected length change if the whole S4 were converted (and remained converted) from a 3_{10} helix to an α-helix (or vice-versa) when the membrane potential is changed from -100 to +80 mV. Supported by NIH GM68044-07 and GM30376-30S1.

1832-Pos/B602. STRETCHING OF TWITCHIN KINASE

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The giant proteins from the titin family, that form cytoskeletal filaments, have emerged as key mechanotransducers in the sarcomere. These proteins contain a conserved kinase region, which is auto-inhibited by a C-terminal tail domain. The inhibitory tail domain occludes the active sites of the kinases, thus preventing ATP from binding. It was proposed that through application of a force, such as that arising during muscle contraction, the inhibitory tail becomes detached, lifting inhibition. The force-sensing ability of titin kinase was demonstrated in AFM experiments and simulations [Puchner, et al., 2008, PNAS:105, 13385], which showed indeed that mechanical forces can remove the autoinhibitory tail of titin kinase. We report here steered molecular dynamics simulations (SMD) of the very recently resolved crystal structure of twitchin kinase, containing the kinase region and flanking fibronectin and immuniglobulin domains, that show a variant mechanism. Despite the significant structural and sequence similarity to titin kinase, the autoinhibitory tail of twitchin kinase remains in place upon stretching, while the N-terminal lobe of the kinase unfolds. The SMD simulations also show that the detachment and stretching of the linker between fibronectin and kinase regions, and the partial extension of the autoinhibitory tail, are the primary force-response. We postulate that this stretched state, where all structural elements are still intact, may represent the physiologically active state.

1439-Pos/B209. CHARACTERIZING AN INTERMEDIATE STATE BETWEEN INACTIVE AND ACTIVE STATES OF RECA

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RecA is an essential enzyme for the repair of DNA double-strand breaks by homologous recombination. The repair requires the formation of a RecA nucleoprotein filament, in which ATP and DNA are known to promote each other's binding to RecA. The RecA filament exists in two distinct conformations: a wide and compressed "inactive" conformation in the absence of DNA, and a narrow and extended "active" conformation in the presence of ATP and DNA. The inactive conformations share similar crystal

structures, although biochemically only the ATP-bound state has a high DNA binding affinity. Our molecular dynamics (MD) simulations on inactive RecA filament reveal that upon ATP binding, only parts of the RecA filament adopt active-like conformations. During the simulations, the DNA binding loops assemble into an arrangement optimal structure for binding of extended ssDNA, i.e., similar to the active ssDNA-RecA state. However, the overall structure remains in its inactive form. This suggests that the ATP-bound RecA might be an intermediate state between the inactive apo-RecA and active ssDNA-RecA states. This intermediate shows overall compressed inactive conformation, with key loops in extended active-like conformation. In order to study the cooperativity between DNA and ATP, we have also performed MD simulations on nucleoprotein filament, showing that the ATP-bound state is able to maintain the organized DNA binding loops both with and without ssDNA. However, in the absence of ATP, the DNA binding loops tend to lose their organized arrangement rapidly once the DNA is removed, a process which is followed by the overall conformational changes of the protein.

1692-Pos/B462. INTERPRETING THE BARIUM BLOCKADES OF POTASSIUM CHANNELS WITH THE MULTI-ION PERMEATION FREE ENERGY SURFACE

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Barium blockade experiments on the BK channel provided an early quantitative determination of the ion binding selectivity of a potassium channel (Neyton, Miller, *J. Gen. Physiol.* 1988, 569). Recently, these experiments were repeated for KcsA by Piasta and Miller, providing the first site selectivity electrophysiological measurements for a channel of known atomic structure (Piasta, Theobald, Miller, *J. Gen. Physiol.* 2011). Site selectivity can be determined from these blockade experiments because the binding of an external alkali cation in the so-called lock-in site impedes the translocation of Ba^{2+} toward the external side, thus increasing the length of blockade. As K^+ and Na^+ impede the rate of Ba^{2+} translocation to vastly different degrees, their relative binding affinities to the lock-in site can be determined quantitatively. Here, we have used molecular dynamics simulations of KcsA to model the permeation process of Ba^{2+} by computing the ion permeation potential of mean force (PMF) with umbrella sampling enhanced by Hamiltonian exchange. Although a Ba^{2+}-bound crystallographic structure (Lockless, Zhou, MacKinnon, *PLoS Biology*, 2007, 5, e121) showed Ba^{2+} binding in the S4 and S2 sites, we find that Ba^{2+} can bind in all five internal sites, with site binding affinities ranked in the order: S2 > S1≈S3 > S0≈S4. Permeation of Ba^{2+} in the absence of external K^+ proceeds through the familiar knock-on mechanism, where the Ba^{2+} moves in concert with an internal K^+ ion. The barriers for the transition between binding sites are high (>15 kcal/mol), consistent with the observation of long-lived channel blockades and slow permeation of Ba^{2+} relative to K^+. The K^+ lock-in effect is examined by a multi-ion PMF of Ba^{2+} permeation in the presence of an external K^+ ion that impedes the permeation of Ba^{2+}.

2059-Pos/B829. DISTANCE DETERMINATION IN MULTIMERIC MEMBRANE PROTEIN BY SYMMETRY CONSTRAINED ANALYSIS OF DOUBLE ELECTRON-ELECTRON RESONANCE SPECTROSCOPY.

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Distance determinations are often a core strategy to decipher the molecular mechanism by which membrane proteins execute their biological function. EPR spectroscopy and pulsed Double Electron-Electron Resonance (DEER) methods are unparalleled tools for the measurement of probe-based long-range distances in protein. However, distance determination from echo intensity modulation in pulsed DEER experiments is a moderately ill-posed problem. Here, we show that by using protein symmetry-based geometrical constraints in homo-oligomeric membrane proteins, we are able to greatly facilitate the fitting solution. We modeled the distance distribution with 2 Gaussians, with each mean distance identified by its Gaussian peak. During the fit process, the mean distance ratio was constrained to be within a specified tolerance of the theoretical distance ratio determined by the symmetry of the oligomeric assembly (1:1.4 for tetramers; 1:1.6 for pentamers). We compared this approach against the classical Tikhonov regularization, concluding that our method stabilizes a solution that is much easier to interpret in molecular modeling terms. Importantly, we also show that when the qualities of the dipolar evolution signal increases (more observed periods and higher SNR), there is little or no difference in the distance distribution obtained by our method vs. Tikhonov regularization. This suggests that the geometrically constrained fit does not artificially distort the distance distribution. Our approach was validated on 2 different ion channels of different oligomeric states: CorA, a homopentameric Mg2+ channel and KcsA, a homotetrameric K+ channel. In all cases, the distances obtained by DEER are in excellent agreement with respective crystal structures.

1540-Pos/B310. CAPTURING SPONTANEOUS MEMBRANE INSERTION AND MEMBRANE-INDUCED CONFORMATIONAL CHANGES OF TALIN AT AN ATOMIC RESOLUTION

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Integrins are a diverse set of proteins that play a central role in complex biological processes, such as tumor metastasis and thrombus formation. The integrin heterodimer is often expressed in a low-affinity, inactive state, relying on specific cytoplasmic or extracellular signals for its activation. The cytoskeletal-associated protein talin constitutes one of the major activation pathways of integrin through a membrane-mediated mechanism. While the involvement of activated, membrane-bound talin in this process is well established, atomic details of membrane binding of talin and of talin-dependent integrin activation have been lacking. Using our novel, highly mobile membrane mimetic simulation system, we have successfully captured complete insertion of the talin head domain (THD) in a phosphatidylserine membrane in three independent unbiased simulations, revealing key molecular events involved in the process. The THD is initially recruited to the membrane via the documented membrane orientation patch (MOP), consisting of a large number of positively charged residues. Electrostatic potential calculations revealed THD to be highly polarized, providing a potential mechanism explaining how the protein is aligns for optimal encounter with the membrane. We also observe a large, membrane-induced interdomain conformational change (>2.5 nm), which brings the F3 subdomain into contact with the anionic membrane via residues K325, N326, and K327. This result explains how F2 and F3 subdomains can simultaneously bind the membrane, a biochemically established aspect that could not be explained by the crystal structure. Moreover, we characterize a phenylalanine-rich region as the hydrophobic membrane anchor, consisting mainly of F261 and F283, which is released through the snorkeling motion of a few critical lysine residues within the membrane. Although such an anchor has been hypothesized to exist, none had been identified prior to this study.

1717-Pos/B487. THE OPEN CONFORMATION OF THE MG2+ CHANNEL CORA FROM SOLVENT ACCESSIBILITY AND DISTANCE CONSTRAINTS

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Using a combination of electrophysiology and spectroscopic approaches we have recently shown that CorA acts as a Mg2+-gated Mg2+-selective channel. NiEddA/O2 accessibility together with intersubunit distances were measured in two conditions: 1) Saturating Mg2+, which stabilizes CorA in the closed conformation and 2) The nominal absence of divalent ions, which stabilizes the open state. Here, we use a computational approach for incorporating both solvent accessibility data and distance constraints through restrained molecular dynamics (MD) simulations using CorA in a closed conformation as the starting structure. The accessibility restraints were enforced through interactions between a pseudoatom representation of the spin-label and environmental probe-surrounding particles. Intersubunit Cβ-Cβ distances estimated from DEER experiments, together with the accessibility data were used to generate hundreds of models by varying the upper and lower bound of distance constraints and increasing the number of MD refining cycles. The top 25 models show structural convergence especially around the stalk and inner helices, where the EPR restraints were imposed. After a second round of refinement, the stability of the top ranked structure was evaluated by an all-atom MD simulation in a fully hydrated phospholipid bilayer. After 2ns of the simulation, the RMSD of the stalk and inner helices is stable around 4-5Å. Based on the pore-radius profile, the permeation pathway has dilated enough to allow a hydrated Mg2+, as expected if this conformation is conductive. A linear interpolation between the closed and open conformations suggests a gating mechanism for CorA, where the tips of the stalk helix come together like the ribs of an umbrella. After a kink, this motion translates into an expansion of the cavity and the mouth of the pore opens up with a motion reminiscent of an iris of a camera.

1195-Plat. COMPUTER SIMULATION OF MEMBRANE TUBULATION BY EFC F-BAR DOMAIN LATTICES

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Cells are dynamically sculpted into many types of compartments by cellular membranes, in some cases with the help of BAR domain proteins. BAR domain proteins act under in vitro conditions are found to induce formation of tubules. We have seen in coarse-grained molecular dynamics simulation stretching over 100 microseconds how a flat membrane is curved into a tube when F-BAR domain proteins are arranged on the membrane surface as a regular lattice of parallel rows. The simulations could also characterize the membrane bending properties of F-BAR domains in different lattice arrangements, showing membrane curvatures with radii ranging from 25 to 100 nm.

Lastly, the simulations reveal two key structural features of F-BAR domain that facilitate efficient binding to membranes and membrane curving: (1) Curving is promoted by close contact between phosphoserine lipid head groups and clusters of cationic residues along the membrane facing surface of F-BAR domains, namely lysine and arginine residues 30, 33, 110, 113, 114, and 139, 140, 146, 150, respectively. (2) Within the 100 ns of contact, the F-BAR domain hinge region, through a 20 degree rotation of the helix moment of inertia, establishes a close contact between protein and membrane. (1) and (2) result in membrane bending on a microsecond-to-millisecond time scale.

2071-Symp. CLC TRANSPORTERS: THE SEARCH FOR CONFORMATIONAL CHANGE

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The conventional alternating-access mechanism for transporter function requires outward-facing, inward-facing, and occluded conformational states. The CLC mechanism has been suggested to deviate from the conventional transporter mechanism and involve almost no global protein movement. However, because this idea is based chiefly on our inability thus far to observe additional conformations crystallographically, alternative strategies for investigating conformational change are essential.

2101-Plat. GLYCOPHORIN A HELIX INSERTION, POSITIONING, AND DIMERIZATION IN MODEL MEMBRANES

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Membrane proteins play vital and diverse roles in the cell, e.g., in membrane transport, signal transduction and molecular recognition, and inter-cellular communication in higher organisms. The membrane's lipid composition affects membrane protein helix insertion, positioning and oligomerization, but molecular mechanisms of these effects are largely unknown. We employ our novel highly mobile membrane mimetic (HMMM) model combined with molecular dynamics simulations to investigate structural and dynamic properties determining these lipid-protein interactions during insertion, hydrophobically driven positioning and dimer formation of transmembrane helices of glycophorin A (GpA). Extended simulations of GpA transmembrane helix monomers and dimers with HMMM membranes including zwitterionic large head groups of phosphatydilcholine (POPC) and small head groups of phosphatidylethanolamine (POPE), provide a detailed view of structural changes in membrane during spontaneous insertion of the GpA helix. Simulations revealed dependence of helix insertion on the lipid head group content. In particular, the bulky head groups of POPC, in contrast to POPE, impede GpA insertion by obstructing accessibility of the phosphate group charges. Interestingly, we also observed that the lipid content influences dimer formation and stability, which correlates well with recently reported experimental studies of GpA dimers in native and model membranes. Prior to the development of the HMMM method these inquiries were out of reach of atomistic simulations.

2094-Plat. K2P1 ASSEMBLES WITH K2P3 OR K2P9 TO FORM SUMO-REGULATED TASK BACKGROUND CHANNELS IN CEREBELLAR GRANULE NEURONS.

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Cell-surface K2P1 channels are most often electrically quiet despite their widespread expression in excitable tissues. Previously, we studied human K2P1 expressed in Chinese hamster ovary cells and found one small ubiquitin-like modifier protein (SUMO) conjugated to one K2P1 subunit to be sufficient to silence the dimeric channels (Plant et al., 2010, PNAS 107). Here, we assess K2P1 sumoylation in cultured rat cerebellar granule neurons (CGN). K2P channels have been posited to produce IKso (the standing outward potassium current responsive to changes in pH and volatile anesthetics) in CGN. First, we developed a fluorescent method to count single mRNA transcripts and showed that those for KCNK1, KCNK3 and KCNK9 (encoding K2P1, K2P3 and K2P9, respectively) were present together in individual CGNs at a ratio of 2:8:1. Next, using antibody-mediated FRET, we observed native K2P1, K2P3 and K2P9 to interact with SUMO1 at the neuronal surface whereas native K2P2 (TREK1) did not. Unlike channels with K2P1, the function of channels with K2P3, K2P9 or K2P2 were found to be insensitive to SUMO1. We reconciled the apparently discrepant findings by showing that K2P1 subunits are incorporated into mixed assemblies with K2P3 or K2P9 (but not K2P2) to form novel two P domain, acid-sensitive (TASK) channels. Channels with just one K2P1 subunit were held in silent reserve at the CGN surface by sumoylation. Accordingly, intracellular application of the desumoylating enzyme SENP1 to CGN doubled the magnitude of IKso (decreasing excitability via expected shifts in resting membrane potential and resistance) and tripled the response to halothane. Because K2P1, K2P3 and K2P9 are expressed together throughout the body and the SUMO pathway is ubiquitous this mechanism of regulation is expected be common.

2123-MiniSymp. THE RING OF GLUTAMATES IN THE CHARGE-SELECTIVITY FILTER REGION OF THE NICOTINIC RECEPTOR FORMS A SYSTEM OF UNANTICIPATED COMPLEXITY

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Of all the rings of ionizable side chains that decorate the permeation pathway of nicotinic-type receptors (AChRs), the ring of glutamates at the intracellular end of M2 (the "intermediate ring") is, by far, the one that lowers the energetic cost of cation permeation the most. Although these glutamates have received much attention, several properties of this ring (such as the pH insensitivity) have remained unexplained, especially when compared to the properties of similar rings in CNG, Nav and Cav channels. To gain a more detailed understanding of this "catalytic" site, we performed a thorough mutational analysis of the intermediate ring of the muscle AChR (which consists of four glutamates and one glutamine) using single-channel electrophysiology. Our results indicate that the commonly held idea that each glutamate represents a negative charge and that each additional charge makes an incremental contribution to the single-channel conductance is incorrect: for example, we found that (in the wild-type channel) only two, not all four, of the glutamates contribute to the single-channel conductance. And, in mutant muscle AChRs bearing five glutamates in the ring (and thus mimicking the non-muscle type), only three glutamates (not five) contribute to the conductance. Our data point to a

model of unanticipated complexity in which the conformational dynamics of the glutamate side chains (rather than their acid-base properties) in the ambivalent environment afforded by the membrane-water interface play a fundamental role in determining the amplitude of unitary currents.

2652-Pos/B422. STRUCTURAL INVESTIGATIONS OF CLC-EC1, A LARGE INTEGRAL MEMBRANE PROTEIN, USING SOLUTION-STATE NMR AND NANODISC TECHNOLOGY

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CLC antiporters mediate Cl^-/H^+ exchange across cell membranes in organisms ranging from bacteria to mammals. This exchange is accomplished through elegant coupling of protein conformational changes to ion binding, unbinding and translocation events. To understand this mechanism, molecular details of the different conformations and the dynamics of their interchange must be known. While X-ray crystallographic structures have provided essential molecular pictures, the details of protein conformational change have remained elusive. To address this issue, we are using solution-state NMR to probe conformational change in ClC-ec1, a well-characterized CLC homolog of known structure. Selective ^{13}C labeling of lysine residues and the N-terminus is achieved by post-translational reductive methylation. ^1H-^{13}C HSQC spectra of these samples reveal reversible, substrate-dependent spectral changes that may reflect protein conformational changes (studies in progress). A chronic concern with the use of detergent-solubilized protein is the possible effects of the non-native detergent environment. To address this concern we are also implementing nanodisc technology. Nanodiscs present a promising alternative for studying membrane proteins such as CLCs, offering both a native bilayer environment and a size small enough for solution-state NMR studies. Each disc consists of two amphipathic helices wrapped around a bilayer patch of lipids surrounding the membrane-embedded protein. The protocol for the incorporation of the monomeric, ^{13}C-methylated mutant form of ClC-ec1 was optimized and the samples tested for homogeneity and monodispersity. The ^1H-^{13}C HSQC spectra of methylated, monomeric ClC-ec1 in nanodiscs are being investigated, with preliminary results indicating substrate-dependent spectral changes in this system. While further experiments will be needed to determine if these spectral changes represent functionally-relevant conformational changes, these studies demonstrate the potential for using solution-state NMR and nanodisc technology to study ClC-ec1 structure in a native lipid environment.

2700-Pos/B470. REDUCING S3-S4 LINKER LENGTH IN SHAKER K+ CHANNELS STABILIZES THE RELAXED STATE

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The voltage sensing domain (VSD) of voltage-gated channels contains four transmembrane segments (S1 through S4), where the S4 segment is the main voltage sensor. It has been shown that upon prolonged depolarization the VSD enters a third conformational state, the relaxed state, resulting in a dramatic slowing of gating current kinetics upon a repolarization. The length of the linker between the third and fourth transmembrane domains (the S3-S4 linker) is highly variable between different voltage-gated potassium channels. Here we investigated whether the S3-S4 linker length affects the relaxation transition. We generated mutant clones of the Shaker K^+ (ShK) IR-H4, W434F background (wild type) with S3-S4 linkers progressively shortened by, typically, three residues. We found that upon prolonged depolarization, the kinetics of repolarizing gating currents going from the relaxed state to the resting state slow down with a linear correlation with the length of the S3-S4 linker (R^2>0.9). In addition, the entry to the relaxed state for short linker constructs was generally faster than that for wild type. These results show that shortening the S3-S4 linker favors the relaxed conformation. In addition, we recorded gating currents from oocytes expressing two fragments of ShK, one from the N terminus through part of the S3-S4 linker, and the other comprised of the remainder of the S3-S4 linker to the C terminus. This “split linker” construct and our short linker constructs suggest that shorter S3-S4 linkers impose greater constraints on the voltage sensor, stabilizing the relaxed state. Supported by NIH-GM030376.

2327-Pos/B97. CHARACTERIZING STRUCTURAL AND DYNAMICAL IMPACTS OF AGONIST- AND ANTSGONIST-STABILIZED MUTANTS OF THE ESTROGEN RECEPTOR

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The estrogen receptor (ER) is a ligand-regulated transcription factor belonging to the nuclear receptor superfamily and is involved in numerous physiological and disease states, most notably breast cancer. Extensive mutational studies have identified several point mutations within the binding domain that stabilize agonist and antagonist functional states and allow for structural characterization of the ER in these states. Characterizing differential dynamics of these mutants and wildtype ER, therefore, can be used as a way to understand the nature of conformational changes induced by binding of each class of ligands and to characterize the impact of specific residues on the overall functional state of the receptor.

Equilibrium molecular dynamics (MD) simulations of the agonist-stabilized Y537S structure have revealed the formation of a new hydrogen bond (S537-D351), but challenged the role of solvent exposure for hydrophobic residues in the enhanced stability of the mutant structures, as has been suggested based on x-ray structures. Furthermore, we have observed the formation of transient hydrogen bond networks surrounding the base of critical helix 12 (H12) that were not observable from static structures. Steered MD (SMD) simulations have also shown different unbinding modes of H12 upon applying a force perpendicular to the length of the helix. Analysis of the antagonist-stabilized L536S structure indicated that the mutation resulted in a 157-degree rotation of the mutated residue, repositioning the preceding flexible loop into an ordered conformation against the protein surface. Attempts to replicate this effect *in silico* by mutating the WT structure of ER-bound hydroxytamoxifen and simulating for 20 ns resulted in only a partial rotation of the loop. Applying a harmonic force to slowly complete the rotation of the S536 psi angle revealed a significant energy barrier to obtain the final mutant conformation.

2653-Pos/B423. MONITORING SUBSTRATE-DRIVEN CONFORMATIONAL CHANGES OF CLC-EC1 BY [METHYL-13C] METHIONINE NMR

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ClC-ec1, a bacterial Cl^-/H^+ antiporter of the CLC family, has been crystallized under many conditions. Yet, only one major conformation of the protein has been observed, leading to the view that the antiport cycle only involves local conformational changes near the chloride binding sites. This view has been challenged in a recent study (Elvington et al, 2009), in which a residue up to 25 Å away from the chloride binding sites was shown to undergo substrate-induced, antiport-dependent conformational changes. To gain further understanding of this conformational change, we are using specific ^{13}C-methyl labeling to facilitate the study of the ClC-ec1 structure and dynamics by NMR. Use of a monomeric ClC-ec1 (Robertson et al, 2010) greatly improves spectral quality of this 50-kD (per subunit) membrane protein. Heteronuclear single quantum correlation (HSQC) spectra reveal substrate-dependent spectral changes that may correspond to functionally relevant conformational change. Resonance assignment is in progress.

2621-Pos/B391. INTERACTION WITH THE MEMBRANE UNCOVERS ESSENTIAL DIFFERENCES BETWEEN HIGHLY HOMOLOGOUS GPCRS

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The lipid membrane environment has been shown to play a significant role in the function and organization of G-protein coupled receptors (GPCRs) and other transmembrane proteins. We now show quantitatively how small sequence differences between otherwise highly homologous GPCRs can result in strikingly different membrane interaction characteristics. This is evidenced by comparing the membrane interactions of two pairs of functionally related family A GPCRs - (1) the beta1 and beta2 adrenergic receptors; and, (2) the kappa- and delta- opioid receptors, embedded in a lipid bilayer composed of a 16:0-18:1 PC (POPC) /10% Cholesterol mixture. We used the recently described 3D Continuum-Molecular dynamics (3D-CTMD) approach (Mondal et al., BJ (in press)) to quantify the membrane deformation profile and corresponding energy costs due to the protein/membrane hydrophobic mismatch. The novel computational method accounts for the irregular hydrophobic surface of the protein and the hydrophobic mismatch at particular TMs that is not alleviated by membrane deformations. A description of the irregular membrane-protein interface from MD simulations of protomeric receptors with the coarse-grained Martini force field provided the information on the membrane-protein boundary needed to quantify with 3D-CTMD the energetics of membrane deformation for each system. The specific residues involved in unfavorable polar-to-hydrophobic interactions not alleviated by membrane deformations at each TM were identified from solvent accessibilities in the MD trajectories. We found strikingly different energy costs of hydrophobic mismatch at TMs 4,5 between the beta1 and beta2 adrenergic receptors. In contrast, both kappa and delta opioid receptors exhibited a similar pattern of (small) energy cost around the protein with slightly more pronounced residual mismatch at TM4. These distinct patterns of energy differences indicate how small sequence differences in otherwise homologous GPCRs can affect the mechanisms driving their organization in the cell membrane.

2287-Pos/B57. TRANSITION PATHWAYS OF PROTEINS EXPLORED BY COMBINING MOLECULAR DYNAMICS SIMULATIONS AND MONTE CARLO SAMPLING OF COLLECTIVE MODES

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The conformational transition of proteins is explored using a molecular dynamics (MD) simulation protocol which is guided by the normal modes derived from coarse-grained anisotropic network model (ANM). The methodology applies to the cases where the passage from one substate to another (e.g., the open and closed forms of an enzyme, or outward-facing and inward-facing states of a

transporter) within a global energy minimum (native state) involves relatively low energy barriers, based on the assumption that low energy barriers may be surmounted/overlooked by adopting a coarse-grained description of the structure and energetics, which smoothes out the energy landscape. The basic approach is to deform the structure along ANM modes, similar to the adaptive ANM (*a*ANM) procedure adopted in our previous work,(Yang et al., 2009) but with the major improvement that the intermediate structures are selected from the complete pool of all accessible ANM modes of motion using a Monte Carlo/Metrolopis algorithm. Application to two proteins with different functional mechanisms, *Escherichia coli* adenylate kinase (AK) and dopamine transporter, shows that the transition between the two alternative forms does not necessarily obey the same pathway(s). For example, in the case of AK, the open-to-closed (O->C) and closed-to-open (C->O) transitions of AK proceed via distinct intermediate conformers: the ATP-binding (LID) domain closing (opening) takes place faster than the nucleotide monophosphate-binding (NMP) domain closing (opening) in the O->C (C->O) transition.

Yang,Z., Majek,P., and Bahar,J. (2009). Allosteric transitions of supramolecular systems explored by network models: application to chaperonin GroEL. PLoS Comput. Biol. *5*, e1000360.

2702-Pos/B472. POSITION OF THE SECOND GATING CHARGE ALONG S4 IN AN INTERMEDIATE CONFORMATION OF A K+ CHANNEL VOLTAGE SENSOR

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Voltage-sensing in ion channels and some phosphatases relies on the movement of positively-charged residues (gating charges) carried by the fourth (S4) helix of the voltage-sensor domain (VSD). Structures of Kv channels revealed the position of the gating charges in the open/inactivated conformation of the VSD yet the pathway they follow during gating remains unclear. To this aim, we previously showed that at hyperpolarized potentials, the first gating charge of the Shaker K+ channel (R362) resides within atomic proximity of I241, located in the middle of the S1 segment. Here, we show that I241 mutations shift the voltage dependence of channels activation toward positive potentials and this effect is most pronounced for the I241W mutation. This mutation isolates two equal components of the charge movement during activation, thereby stabilizing the S4 in an intermediate position between resting and active states. Using non-natural amino acid incorporation, electrophysiology recordings and molecular dynamic simulations, we show that this S4-immobilization involves a specific interaction between I241W and R365, the second gating charge. We found that this interaction requires the presence of the nitrogen atom of the indole ring and is not affected by a dispersion of its Pi electrons. This interaction is present regardless of whether the S4 moves from its resting, active or relaxed state but the charge component that is being isolated during deactivation only represents 30% compared to 50% during activation. Taken together, our results indicate that each of the gating charges move in slightly different pathways, the overall S4 motion is similar during activation and deactivation, and the electric field is shaped differently between activation and deactivation.

2374-Pos/B144. CAPTURING INSERTION AND DYNAMICS OF MEMBRANE-BOUND CYTOCHROME P450 3A4 USING A NOVEL MEMBRANE MIMETIC MODEL

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Cytochrome P450 (CYP) enzymes constitute a large family of enzymes present in a wide variety of organisms that are involved in the metabolism of xenobiotics. In humans, CYP3A4 is the most abundant isoform in the liver and is responsible for the metabolism of a large variety of drugs. CYP enzymes are anchored in the cellular membrane by a transmembrane alpha helix and by the insertion of an unknown hydrophobic region from their globular domain into the lipid bilayer. Despite its high relevance to drug entry and binding, an experimental membrane-bound structure of CYP3A4 has not been reported to this date, and only soluble structures are currently available. Molecular dynamics (MD) simulations of other soluble CYP structures have suggested that the presence of the lipid bilayer might initiate important conformational changes in CYPs, but due to the limited lipid motion in such studies, the nature of these changes are still largely uncharacterized. In order to study the interaction of CYP3A4 with a membrane, we performed MD simulations employing a highly mobile membrane mimetic (HMMM) model developed by our group. The HMMM model allows the unbiased association of CYP3A4 with a phosphatidylcholine (PC) bilayer, providing an all-atom description of this process for the first time. The enhanced lipid mobility achieved by the HMMM model allows for a detailed description of the dynamics of CYP3A4, reveling the mechanism of opening and closing of the tunnels from the active site upon membrane binding. In particular, it is observed that the presence of the PC bilayer induces the closing of the access tunnel going through the BC loop of the globular domain. The resulting membrane-bound model exhibits an orientation that is in close agreement with experimental data.

2694-Pos/B464. ROLE OF STRUCTURAL WATER IN THE STABILIZATION OF THE KCSA C-TYPE INACTIVATED

SELECTIVITY FILTER: EVIDENCE FROM HIGH-RESOLUTION STRUCTURES

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A first glimpse into the structural changes underlying the interconversion between conductive and non-conductive C-type inactivated forms of the K+ channel selectivity filter (SF) at modest resolution was obtained from crystal structure of open-inactivated KcsA (O/I) [1]. On the basis of these structures, we developed a set of double cysteine crosslinking pairs that sharply increase the resolution of KcsA crystals with its gate trapped in the open configuration. Here, we present the crystal structures of KcsA locked-open (O) together with mutations that stabilize either the C-type inactivated filter, Y82A (O/I); or the fully conductive filter, E71A (O/O) @ 2.3 and 2.1 Å, respectively. Comparison of these structures helps identify a novel water network (H2O_1, H2O_2 and H2O_3) behind the channel SF that stabilizes the O/I conformation of the channel. H2O_1 interacts with the backbone carbonyl groups of V76 and E71 from a neighboring subunit and with H2O_2+H2O_3 within the same subunit. In KcsA, the presence of the E71 residue on the channel pore helix is essential to drive the channel to the O/I state while providing a high-dielectric media suitable for the formation of this water-molecule network behind the SF. The intersubunit nature of this interaction, which is present only in the O/I state, provides a cogent structural explanation for the cooperative character of the C-type inactivation process in K+ channels. Additionally, we propose that the formation of a similar water-molecules mediated H-Bond network behind the SF of other K+ channels is essential to stabilize the O/I state.

1. Cuello, L.G., et al. Nature, 2010. 466(7303): p. 203-8.

2267-Pos/B37. CHARACTERIZING TRANSITION PATHWAYS IN THE TRANSPORT CYCLE OF ABC TRANSPORTER MSBA

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MsbA is a member of the ABC (ATP-binding cassette) family transporters that uses energy from ATP hydrolysis to transport various substrates across cellular membrane. The transport cycle of MsbA has been described by a simple "Alternating Access" mechanism in which the transporter changes its conformation between an outward-facing (OF) and an inward-facing (IF) state while coupling the translocation of substrates. Here, to investigate the detailed transport cycle of MsbA at atomistic resolution, we have performed molecular dynamics (MD) simulations using a combination of several computational methods. Starting from the recently solved X-ray crystal structure of MsbA in OF state, we first generate high resolution all-atom models for the two IF states (IF open and IF closed) by using targeted MD simulations. We then define two collective variables to describe the relative motion of the two transmembrane domains (TMDs) and nucleotide binding domains (NBDs), respectively. Steered MD simulations along the collective variables have been performed to induce the conformational changes between the three states (OF, IF open and IF closed) of MsbA. Assessing the energetics associated with the induced transitions, approximated by calculating the non-equilibrium work involved in going from one state to another suggest that the OF-to-IF conformational transition follows two steps: the two TMDs close first, and then the two NBDs open, as opposed to the pathway observed from initial targeted MD simulations. Taken together, these results not only provide a better understanding of the functionality of ABC transporters, but also help define a general mechanism for membrane transport process.

2511-Pos/B281. CONFORMATIONAL DYNAMICS OF MEMBRANE-BOUND α-SYNUCLEIN IN A HIGHLY MOBILE MEMBRANE MIMETIC

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α-Synuclein is a widely studied unstructured protein that plays an important role in pathophysiology of Parkinson's and Alzheimer's diseases through its aggregation, a process closely connected to its binding to and restructuring of the cellular membrane. Thus the dynamics of the membrane-bound form of the protein is highly relevant to unravel the molecular mechanism of its involvement in these diseases. In order to characterize the dynamic range of conformations accessible to membrane-bound α-Synuclein, molecular dynamics offers a potentially powerful method, owing to its detailed atomistic and dynamic description of the semi-liquid environment of biological membranes. Nonetheless, the applicability of the method is hampered by the slow diffusion of lipid molecules when described atomisticly relative to simulation accessible timescales. We have developed and employed a novel membrane representation (termed HMMM, highly mobile membrane mimetic) with enhanced lipid dynamics and without compromising atomic details, which was used to perform 10 independent simulations of binding of α-Synuclein to bilayers composed of a mixture of PS and PC lipids. In all simulated systems, α-Synuclein spontaneously binds to the membrane without the application of an external force. Rather than converging to a single structure, these simulations capture an ensemble of diverse, highly dynamic structures of α-Synuclein in the presence of the membrane. While the observed conformational diversity can be attributed primarily to two highly flexible regions, namely near the turn and the region bracketed by critical Gly residues. Not only do the resulting ensemble of membrane-bound structures reflect the horseshoe-shape of the original structure, but also show similarities to a proposed linear configuration of the protein. The observed structural diversity suggests that in its membrane-bound form, α-Synuclein exists in an equilibrium between the horseshoe and linear conformations that have been also postulated experimentally.

2645-Pos/B415. STRUCTURAL INTERMEDIATES IN A MODEL OF THE SUBSTRATE TRANSLOCATION PATH IN THE BACTERIAL GLUTAMATE TRANSPORTER HOMOLOGUE GltPh

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Excitatory Amino Acid Transporters (EAATs) are membrane proteins responsible for reuptake of glutamate from the synaptic cleft to terminate neurotransmission and help prevent neurotoxically high extracellular glutamate concentrations. Important structural information about these proteins emerged from crystal structures of GltPh, a bacterial homologue of EAATs. The extra- and intra-cellular facing conformations of GltPh are structurally different and considered to be the end points of the substrate translocation path (STP), which suggests that the transport mechanism involves major conformational rearrangements that remain uncharted. To investigate possible steps in the structural transitions of the STP between the two end point conformations we applied a combination of computational methods (Motion Planning, Molecular Dynamics Simulations, and mixed Elastic Network Models). We found conformational changes involving a repositioning of the transport and trimerization domain in opposite directions along the membrane normal, and a tilt of the transport domain by ~20° with respect to this axis. In addition, the TM3-4 loop undergoes a flexible, "restraining bar"-like conformational change with respect to the transport domain. Together, these changes increase the lipid-protein interface area by 11% in the intermediates compared to the end states, and decrease the area of the transport and trimerization domain interface (TTDI) by as much as 16%, even though the TTDI areas in the end states differ from each other by only 4%. With these changes, water penetrates the TTDI in the modeled intermediates, but not in the end point structures - a finding that agrees with experimental results of residue accessibility studies and suggests new testable hypotheses about the STP. Monomer intermediates replicated to construct a complete trimeric model adopt different conformations in some of the MD simulations, leading to asymmetry in the trimer.

2651-Pos/B421. CHARACTERIZATION OF A NOVEL CLC-EC1 INHIBITOR

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CLC chloride channels and transporters are vital to many physiological processes. Defects in these proteins can lead to diseases that affect muscle, cardiovascular system, kidneys, and bones. Historically, high affinity inhibitors have played crucial roles in the characterization of ion transporter structure and function and in the treatment of disease. However, there is a dearth of known CLC inhibitors. To search for improved inhibitors, we synthesized derivatives of DIDS (4,4'-diisothio-cyanostilbene-2,2'-disulfonic acid), a small molecule that inhibits several CLC proteins with low affinity and specificity. We found that linking DIDS and octanoic acid generated a CLC-ec1 inhibitor (OADS) that inhibits CLC-ec1 with low micromolar affinity. We are characterizing the mechanism of inhibition using flux assays, electrophysiology, isothermal titration calorimetry, photoaffinity labeling, and mutagenesis. Given that OADS and OADS derivatives that inhibit ClC-ec1 are rather hydrophobic, we also explored their potential to alter lipid bilayer properties using a gramicidin based fluorescence assay.

2325-Pos/B95. FOLDING THERMODYNAMICS AND KINETICS OF LAMBDA-REPRESSOR FROM ALL-ATOM MOLECULAR DYNAMICS SIMULATIONS

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The five-helix bundle lambda repressor fragment ($\lambda_{6\text{-}85}$) is a fast-folding protein. A length of 80 residues puts it on the large end among all known fast folders, and hence its size poses a computational challenge for molecular dynamics (MD) studies. We simulated the folding of $\lambda_{6\text{-}85}$ in explicit solvent using an all-atom model. By means of a single copy tempering method, adaptive tempering sampling, we observed folding, unfolding, and refolding of $\lambda_{6\text{-}85}$ in a 6-microsecond long trajectory. The best root mean square deviation (RMSD) relative to the crystal structure native state arising in the trajectory was 1.8 Å. Thermodynamic quantities, such as heat capacity and transition temperature of the protein, were calculated from the simulation and compared with experiment. The folding kinetics was investigated through a set of equilibrium MD simulations at different temperatures that together covered more than 100 microseconds. The protein was seen to fold into the native-like topology with RMSD relative to the crystallographic structure of less than 4 Å. Folding pathway and several folding intermediates were identified through cluster analysis. The simulations suggest a new fast-folding 4-helix truncation waiting to be tested in experiment.

2381-Pos/B151. QUANTITATIVE COMPARISON OF GPCR INTERACTIONS WITH THE LIPID BILAYER OF THE CUBIC AND LAMELLAR MESOPHASES

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Recent successes in the crystallographic determination of structures of transmembrane (TM) proteins in the G protein-coupled receptor (GPCR) family have established the lipidic cubic phase (LCP or "in meso") environment as useful for growing diffraction quality crystals. The mechanism underlying in meso crystallogenesis is currently at a descriptive level. To begin developing a quantitative, energy-based nucleation and crystallization mechanism we are conducting molecular dynamics studies of the GPCR, rhodopsin, reconstituted into the LCP using the coarse-grained representations of the Martini force-field. The first aim is to quantify differences in the hydrophobic/hydrophilic exposure of the GPCR to lipids in the cubic and lamellar phases. Simulations of a single rhodopsin molecule in these monoolein-based mesophases showed more energetically unfavorable hydrophobic-hydrophilic interactions between the protein and lipid in planar bilayers of the lamellar phase. The reduced level of hydrophobic mismatch in the LCP, by contrast, is attributable to the highly curved geometry of the cubic phase that provides for more efficient shielding of the protein from unfavorable hydrophobic exposure. Since hydrophobic mismatch can drive oligomerization (Mondal et al., BJ 2011 - in press), these differences suggest that compared to the LCP, lamellar structures provide a more favorable setting in which GPCRs can oligomerize as a prelude to nucleation and crystal growth. These new findings lay the foundation for future studies of in meso crystallization mechanisms and for a rational approach to the generation of structure-quality crystals of membrane proteins.

2701-Pos/B471. VOLTAGE-CLAMPED SUPPORTED BILAYER SYSTEM TO RECORD ION CHANNEL ACTIVITY
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Structure-function studies of voltage-dependent ion channels require controlled electrical stimuli to drive channel activity. Under voltage control, site-directed fluorescence spectroscopy is often used to probe structural rearrangements that underlie channel function. Many voltage-dependent ion channels, such as the KvAP K+ channel, must be purified from bacteria and reconstituted in a lipid membrane. The ensuing (conventional) experiment in a black lipid membrane has several problems: 1) too few channels can be measured to observe gating currents; 2) the bilayer stability is generally poor and inversely related to protein density; 3) fluorescence analysis is difficult due to channel diffusion. To circumvent these problems, we previously developed a system to voltage clamp a supported bilayer with simultaneous fluorescence imaging (Hyde et al, 2010 BPS Meeting). This system employed a 5 mm diameter supported bilayer grown atop a transparent electrode-coated coverslip. We could measure gating currents of small charged molecules and voltage-dependent responses from fluorescent membrane probes; however, we have since found that direct contact with the supporting electrode significantly hinders recordings of functional channels. We have thus introduced a self-assembled monolayer cushion grown atop a gold-coated coverslip as the support electrode. Importantly, the gold-coated coverslip is also designed to enable simultaneous fluorescence imaging of ion channels in the supported bilayer using surface plasmon-assisted microscopy. Preliminary results indicate that in response to an applied transmembrane potential, we can observe voltage-dependent fluorescence changes from S4-labeled KvAP channels consistent with presumptive gating behavior. Support: NIH GM030376; Medical Scientist National Research Service Award 5 T32 GM007281.

2695-Pos/B465. A RANDOM MUTAGENESIS APPROACH TO PROBING ELECTROMECHANICAL COUPLING IN THE HYPERPOLARIZATION-ACTIVATED CHANNEL MVP
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MVP, the hyperpolarization-activated potassium channel from the archaeon M. jannaschii, poses an interesting case study for understanding how the voltage-sensing domain (VSD) couples to the pore domain in Kv channels. Although the VSD of MVP adopts the same orientation in the membrane and senses changes in the polarity of the membrane potential in the same manner as canonical Kv channels, the pore of MVP opens in response to hyperpolarization; thus, like the eukaryotic hyperpolarization-activated cyclic-nucleotide-gated (HCN) channels, MVP opens when the S4 helix moves inwards and closes when the S4 moves outwards. Because hyperpolarization-activated channels use the same VSD for inverse gating, they offer a unique opportunity for understanding the molecular basis of coupling the VSD and pore domain. Since structural evidence in canonical Kv channels suggests that the movement of the S4 is transmitted to the pore domain via the S4-S5 linker, we have set out to identify key residues of MVP necessary for electromechanical coupling. We probed the S4-S5 linker and S6 helix with a random mutagenesis screen using the potassium uptake deficient strain LB2003 in conjunction with a MVP library consisting of single amino acid substitutions along the linker with full codon coverage. By screening this library under different potassium concentrations, we have identified mutations in the linker and S6 that abolish WT complementation on potassium-depleted media (Loss of Function). This approach has allowed high-throughput screening of all possible amino acid identities along the linker that are incompatible with channel function. Here we present preliminary assessments of the expression profile, stability and functional properties of these mutants.

2200-Plat. HOW PIP2 LIPIDS REGULATE THE POSITION AND PHOSPHORYLATION OF THE SYNTAXIN N-

TERMINUS

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Syntaxin, a member of the family of soluble N-ethylmaleimide-sensitive factor attachment protein receptor proteins can bind and regulate plasma membrane ion channels and neurotransmitter transporters (NSS). Studies of such mechanisms for the dopamine transporter (DAT) have established the N-terminal segment of Syntaxin as the site of direct interactions, and have shown the critical role of highly charged PIP2 lipids in regulating Syntaxin-DAT interactions. We used a computational approach that combines mesoscale continuum modeling of protein-membrane interactions with all-atom molecular dynamics (MD) simulations to compare conformational states of Syntaxin in complex with PIP2-enriched and PIP2-depleted membranes. Our mesoscale approach is based on non-linear Poisson-Boltzmann theory of electrostatics and diffusion-like Cahn-Hilliard dynamics that makes possible the quantitative tracking of lipid-type demixing in the membrane due to the interaction with the protein. The calculations with this method identified strong electrostatic interactions of specific sites of Syntaxin with PIP2 lipids that diffused to their vicinity. MD simulations of the resulting system established that as many as five PIP2 lipid molecules can simultaneously bind Syntaxin. The attending segregation of PIP2 lipids appears to have a dramatic effect on the positioning of the Syntaxin N-terminal segment with respect to the membrane/water interface. These results are discussed in the context of the suggested role of PIP2 lipids in regulating Syntaxin-DAT interactions by modulating phosphorylation of Syntaxin at its N-terminus.

2188-Plat. SPONTANEOUS MOVEMENT OF TRANSMEMBRANE SEGMENTS FROM SECY INTO THE MEMBRANE

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At an early stage of synthesis, nearly all membrane proteins are targeted to a protein-conducting channel in the membrane, the SecY/Sec61 complex. Access of the nascent protein to the membrane occurs via a lateral gate, located at the interface of the two halves of the channel. Recently, we structurally resolved a membrane-protein insertion intermediate state of SecY bound to a translating ribosome with a transmembrane (TM) segment at the lateral gate, between channel and membrane. Beginning from that state, we have now carried out multi-microsecond simulations of different putative TM segments at SecY's lateral gate using Anton, a special-purpose molecular dynamics computer from DE Shaw Research. The simulations reveal spontaneous motion of the TM segment, either toward membrane or toward the channel interior, depending on its sequence. Diffusion of the TM segment into the membrane is generated through interactions with individual lipid molecules, which rapidly envelop a hydrophobic TM segment but reject a hydrophilic one. Subsequent free-energy calculations quantify and support the generality of the spontaneous motions observed in individual simulations. Thus, we suggest that the discrimination step between membrane-inserted and secreted states of a nascent protein occurs primarily in the SecY channel.

3079-Plat. CONFORMATIONAL TRANSITION PATHWAY OF GlpT TRANSPORTER, CHARACTERIZED BY NONEQUILIBRIUM MOLECULAR DYNAMICS SIMULATIONS

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Glycerol-3-phosphate transporter (GlpT) is a member of major facilitator superfamily (MFS), the largest family of secondary transporters. Similar to other transporters, GlpT is believed to function using an “alternating-access” mechanism during which it undergoes large conformational changes. The only available structure of GlpT is in the *apo* inward-facing (IF) state. In our earlier work using equilibrium molecular dynamics (MD) simulations, the binding site of GlpT was characterized and the initial substrate-induced closing on the cytoplasmic side was observed. However, an atomic model of the outward-facing (OF) state of GlpT and the IF-to-OF transition pathway remain unknown. Here we have used a novel approach based on nonequilibrium MD simulations to drive the transition of GlpT from its available IF state to its unknown OF state in an explicit membrane environment. Based on the interhelical symmetries, a set of non-conventional collective variables were defined for the transmembrane helices to specifically reflect the conformational changes involved during the IF-to-OF transition. The system was then steered along these collective variables in a nonequilibrium scheme. The obtained IF-to-OF transition pathway was further optimized adaptively by minimizing the nonequilibrium work, which is a good way to produce more realistic transition pathways. Thus, the protocol of inducing the transition was optimized after each simulation based on the resulted trajectory. Nonequilibrium work relations were used to analyze the trajectories and extract information about the OF state structure, the IF-to-OF transition mechanism, the intermediate/occluded states, and the characteristics of the OF-state binding site, providing insight about the general mechanism of MFS transporters and more generally introducing a novel approach to study the IF-to-OF transitions in membrane transporters.

3081-Plat. STRUCTURE, DYNAMICS, AND MECHANISM OF THE LEUCINE TRANSPORTER STUDIED BY DOUBLE ELECTRON ELECTRON RESONANCE SPECTROSCOPY

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The neurotransmitter/sodium symporter (NSS) family of transporters includes the human dopamine, serotonin, and norepinephrine transporters. These transporters harness sodium and chloride gradients to facilitate reuptake of neurotransmitters from the synapse thus terminating neurotransmission. Due to their critical regulatory role, NSSs are the targets of numerous psychiatric therapeutics and drugs of abuse. Progress in understanding and treating diverse conditions including depression and anxiety will require a detailed description of the structure and function of this class of proteins. Structural investigations of NSS have focused on a bacterial homolog of these transporters, LeuT. This work will describe a systematic investigation of the intracellular dynamics of LeuT as well as detail progress in defining the LeuT transport mechanism and open-inward structure. Using double electron-electron resonance (DEER) spectroscopy, a pulsed EPR technique, over 40 double mutants were analyzed for their distance distributions and relative dynamics. Mechanistic descriptions were inferred by tracking shifts in equilibria between multi-component distance distributions and relating these shifts to biochemical conditions across all mutants. Closed-inward distances were largely consistent with the LeuT crystal structures, while primary gating motions were identified at helices 1 and 6 with additional reorientations of helices 5 and 7, resulting in the open-inward structure. Distance data are being implemented into restrained computational modeling to generate preliminary models of the open-inward conformation of LeuT. These results will be compared to the current models of LeuT open-in structure and transport mechanism derived using alternative approaches.

3082-Plat. CONFORMATIONAL DYNAMICS IN THE TRANSPORT CYCLE OF LEUCINE TRANSPORTER ON THE EXTRACELLULAR-SIDE

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LeuT is a bacterial homolog of the Neurotransporter:Sodium Symporters (NSS) which mediates the sodium dependent re-uptake of neurotransmitters from the synaptic cleft thereby terminating neuronal transmission. LeuT has been crystallized bound with various amino acids (leucine, glycine, methionine, tyrosine and tryptophan), tricyclic antidepressants (TCA), and selective serotonin re-uptake inhibitors (SSRIs). Although these structures reveal molecular details of ion/substrate binding and inhibition, the conformational dynamics underlying a complete transport cycle have yet to be elucidated. Computational modeling studies have led to a proposal of a rocking bundle mechanism that is based on the inherent inverted pseudo-symmetry between TM1-5 and TM6-10. According to this model, TM1, 2, 6, and 7 form a bundle that tilts with respect to other surrounding helices and results in closing and opening of cytoplasmic and extracellular molecular gates. We are investigating the conformational changes of transmembrane helices predicted to move at different stages of the transport cycle by measuring distances between spin labels. Monitoring these dynamics should provide insights into molecular interactions that govern the extracellular gating of the transporter during the transport process. Our results show that the bundle does not behave as a single rigid body structural unit as previously proposed. Some TM helices show a distinct increase in conformational flexibility as observed from the width of distance distributions, whereas others show a change in the equilibrium between different conformational intermediates in response to ion and or substrate binding. We are also analyzing these conformational changes with respect to the crystal structure through geometric optimization in order to consolidate distance changes into a model of the sodium bound intermediate.

3059-Plat. TURNING ON THE SPLICEOSOME

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The spliceosome is the complex macromolecular machine responsible for removing introns from pre-mRNAs. The processes of spliceosome assembly and activation rely on the coordinated interactions of many dozens of spliceosome components to identify splice sites in a pre-mRNA, build a spliceosome, and activate the spliceosome for catalysis by formation of an active site prior to transesterification. The activation step itself likely involves many intermediates. It results in loss of the U1 and U4 snRNPs from the spliceosome, removal of SF3 from the branchsite, and several conformational rearrangements of the snRNAs and pre-mRNA prior to lariat formation. These assembly and activation events are best studied using endogenous spliceosome components found in whole or nuclear cell extracts. We recently demonstrated that a single molecule technique (CoSMoS: Co-localization Single Molecule Spectroscopy) in combination with yeast genetic engineering and chemical biology provides a powerful method for studying spliceosome assembly in S. cerevisiae whole cell lysate (Hoskins et al., Science, v331, pg. 1289-95 (2011)). That study provided significant novel insight into the kinetics of the assembly reaction. We are now extending these results to spliceosome activation. By monitoring the relative association and dissociation kinetics of the U1, U4, U5, NTC, and SF3b spliceosome components on single pre-mRNAs, we are able to definitively order the snRNP association and dissociation events involved in spliceosome activation.

3084-Plat. OUABAIN BINDING SITE IN A FUNCTIONING NA+/K+-ATPASE

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The Na+/K+ ATPase is an almost ubiquitous integral membrane protein within the animal kingdom. It is also the selective target for cardiotonic derivatives, widely prescribed inhibitors for patients with heart failure. Functional studies revealed that ouabain-sensitive residues were distributed widely throughout the primary sequence of the α subunit, which spans ten times across the cell membrane and contains all the necessary components for ion transport: the ion permeation pathway, the phosphorylation site and the ATP binding domain protein. Recently, structural work has revealed that ouabain binds at the external end of the ion permeation pathway. To elucidate the ouabain binding site in a functioning Na+/K+ ATPase we use a spectroscopic approach that estimates distances between a fluorescent ouabain and a lanthanide binding tag (LBT). We introduced LBTs at five different positions in the Na+/K+ ATPase sequence. These five normally functional LBT-Na+/K+ ATPase constructs were expressed in the cell membrane of Xenopus laevis oocytes, operating under physiological internal and external ion conditions. The spectroscopic data suggest two mutually exclusive distances between the LBT and the fluorescent ouabain. From the estimated distances and using homology models of the LBT-Na+/K+ ATPase constructs, approximate ouabain positions could be determined. Our results suggest that ouabain binds at two sites along the ion permeation pathway of the Na+/K+ ATPase. The external site (low apparent affinity) occupies the same region as previous structural findings. The high apparent affinity site is, however, slightly deeper towards the intracellular end of the protein. Interestingly, in both cases the lactone ring faces outward. We propose a sequential ouabain binding mechanism that is consistent with all functional and structural studies. This work was supported by NIH grants R01-GM062342, R01-GM030376 and U54-GM087519, and the Intramural Research Program of the National Institutes of Health, NINDS.

3615-Pos/B476. PROBING GAS DIFFUSION PATHWAYS IN CYTOCHROME C OXIDASE WITH EXPLICIT AND IMPLICIT LIGAND SAMPLINGS

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Cytochrome C oxidase (CCO) couples reduction of O_2 to water with proton pumping across the membrane, thereby generating a driving force for ATP synthesis. The x-ray structures of A-, B- and C-type CCOs suggest that an elongated hydrophobic cavity connecting the membrane core to the protein's active site might serve as an O_2 access point to the catalytic active site of the enzyme. The structure of this cavity is, however, varied in different CCOs. While B- and C-type CCOs exhibit two entrances into this cavity, A-type CCOs appear to only have one entrance. To investigate the involvement of the hydrophobic cavity in O_2 diffusion and to identify (potential) additional O_2 entry pathways, we employed two complementary approaches using molecular dynamics simulations, performed on membrane-bound models of various CCO isoforms. In one approach, using a large ensemble of equilibrated protein conformations collected in the absence of O_2, free-energy of O_2 insertion over a grid covering the entire protein matrix is calculated using the “implicit ligand sampling” method. In the other approach, we included O_2 molecules explicitly in the simulations and monitored their diffusion through the system. We observed favorable O_2 binding and rapid O_2 diffusion primarily from the membrane core, characterizing the hydrophobic cavity as a major O_2 delivery pathway. Moreover, through simulations performed on a mutant enzyme, we identify a site that may contribute to the experimentally observed diffusion-controlled O_2 binding kinetics in B-type CCO from *Thermus thermophilus*.

3590-Pos/B451. NA^+ ACCESS KINETICS IN THE NA^+/K^+-ATPASE PUMP

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In exchanging three Na^+ for two K^+, Na^+/K^+ pumps move net charge through the membrane’s electric field. Most charge moves during transitions that release Na^+ to the exterior, as Na^+ traverse a fraction of the membrane field along an access channel connecting their binding sites deep inside the pump with the extracellular space. We separate the resulting electrical signals from others in the pump cycle by withholding K^+ and intracellular ADP, thereby constraining pumps to steps that release and rebind external Na^+. Under these conditions, at a given external Na^+ concentration ($[Na^+]_o$) and membrane potential, the pumps distribute among conformations with zero, one, two, or three bound Na^+. After a sudden jump to a new potential, as pumps redistribute to a new steady-state arrangement, transient currents are generated upon Na^+-ion release or binding. By rapidly changing the squid giant axon

membrane potential, we previously identified three distinct components in these transient current relaxations: fast (comparable to the voltage-jump time course), medium-speed (τ_m ~0.2-0.5 ms), and slow (τ_s ~1-10 ms). Technical advances now allow us to simultaneously follow the charge amounts in all three phases, with unprecedented temporal resolution. We find that, over a broad range of $[Na^+]_o$ and potential, the charge amount contained in the fast component is dictated by how far the medium-speed component has progressed towards equilibrium at that time, which is itself determined by how far the slow component has progressed. Thus, under all conditions examined, as Na^+-bound and -unbound pump populations redistribute, the amounts and time courses of the three charge movements are closely correlated. These strict correlations reveal the dynamics of the conformational changes by which Na^+ are released from (or sequestered into) their binding sites one at a time, in an obligatorily sequential manner. HL36783, U54-GM087519.

Pos-L173/LB173. OBSERVING THE ROTATION OF A VOLTAGE-SENSING DOMAIN IN AN ION CHANNEL USING SPECTROSCOPIC METHODS

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In potassium voltage-gated ion channels, the voltage sensor undergoes a conformational change upon membrane depolarization. Experimental evidence indicates that this conformational change involves translation and rotation of its fourth transmembrane helix (S4). Previous studies have inferred a rotation of the S4 helix indirectly from distance measurements between subunits. A direct measurement of the rotation of the S4 helix would generate significant progress towards understanding a historically contentious mechanism. Here we apply the method of polarized fluorescence recovery after photobleaching (pFRAP) to directly observe the rotation of fluorescently-labeled ion channels. This method combines spectroscopic techniques with voltage clamp control of the membrane to provide a model-independent measurement of the rotation of the S4 helix. Our preliminary experiments using pFRAP in conjunction with electrophysiological recordings have allowed us to directly observe a rotation of the Shaker S4 during membrane depolarization.

3589-Pos/B450. CHANGES OF INTRAMOLECULAR DISTANCES IN THE Na^+/K^+ ATPase UPON OUABAIN BINDING

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The Na^+/K^+ ATPase is an integral membrane protein formed mainly by two subunits; α and β. Recently, we showed that ouabain (a cardiotonic steroid used in patients with heart failure) binds at two mutually exclusive sites along the ion permeation pathway of the Na^+/K^+ ATPase, indicating a sequential ouabain binding mechanism. Here, we used a spectroscopic approach (LRET) to measure distances between a genetically encoded lanthanide binding tag (LBT that binds Tb^{+3} with high affinity) and a cysteine-reactive fluorescent compound (TMR). We created energy transfer pairs by encoding the LBT's in several of the external loops of the α subunit and cysteines substituted in different positions of the β subunit. These normally functional LBT-Na^+/K^+ ATPase constructs were expressed in *Xenopus laevis* oocytes that were voltage clamped with two microelectrodes to obtain simultaneously electrical and LRET recordings under physiological ionic conditions. By measuring donor-only decays and sensitized emission decays, we estimated the distance between donor and acceptor. Distances from several LBT-(Tb^{3+})-TMR pairs were estimated, one at time, in the absence and then in the presence of ouabain. Interestingly, in the presence of ouabain, the distances increased. Ours results suggests that the Na^+/K^+ ATPase might transit to a different conformation (not yet detected by crystallography) when ouabain binds to the ion permeation pathway. This new conformation could be a separation between the α and β subunits or a rearrangement of the transmembrane segments to accommodate ouabain deep into the permeation pathway. Further experiments are required to discern between these possibilities. Supported by U54GM087519 and GM030376.

3362-Pos/B223. MECHANISM OF ION COUPLED STATE TRANSITION IN LEUT-FOLD TRANSPORTERS

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Several highly conserved features, including similarities in the overall architecture and in substrate and ion binding sites, suggest that secondary transporters sharing the LeuT-fold topology might form an emerging superfamily with a similar transport mechanism. Similar to other transporters, LeuT-fold transporters operate via the alternating access mechanism, in which during the transport cycle the transporter protein undergoes conformational transitions between two major states, an inward-facing (IF) and an outward-facing (OF) one. However, despite the availability of a wealth of structural data, a clear and consistent understanding for the transition mechanism and how it might be coupled to ion binding/unbinding events has not been achieved yet.

Using microsecond-long equilibrium molecular dynamics (MD) and special-protocol target MD simulations and comparative structural analysis, we demonstrate that the conformational changes of the conserved Na2 site are closely coupled with the global transition between the OF and IF states in the LeuT-fold transporters. In our TMD simulations, we show that inducing structural transitions in only two transmembrane (TM) helices that are directly involved in Na+ binding site in Mhp1, is sufficient to drive the transition between the IF and OF states in the entire protein. Additional data from equilibrium simulations along with structural analyses suggest that the state transitions captured in the simulations are highly relevant to the natural pathway during the transport cycle. Furthermore, the results clearly show a high correlation between Na+-binding TMs and extracellular and cytoplasmic gates, and how the movement of specific TMs switches the states of the transporter. Meanwhile, our extended equilibrium simulations performed in different transport states, and following free energy calculations suggest the effect of Na+-binding in LeuT-fold is to reshape the free energy landscape, and thereby to change the distribution of the states involved the transport cycle.

3349-Pos/B210. FUNDAMENTAL DIFFERENCE OF TRANSPORT MECHANISMS BETWEEN ABC IMPORTERS AND EXPORTERS

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ABC transporters use the energy of ATP to drive the active transport of various substrates across the membrane. All members of this superfamily share a common machinery of two nucleotide binding domains (NBDs) at their cytoplasmic side, which close and open in response to ATP binding and hydrolysis. Despite highly homologous NBDs, substrates can be either imported or exported in different subfamilies. Although crystal structures of ABC transporters have been resolved in different conformational/functional states, it is still puzzling how a common set of motor domains can drive transport in opposite directions. We use comparative molecular dynamics simulations of an ABC-importer (maltose transporter) and an ABC-exporter (P-glycoprotein), to investigate functionally relevant dynamical differences. The two transporters exhibit very different structural flexibility and fluctuations in their inward-facing states, especially with regard to the separation of the NBDs. Comparing the dynamics of NBDs, it becomes evident that ABC importers fluctuate significantly lower than ABC exporters, suggesting that the conformational ensembel obtained for the former represents a deeper energy well. In contrast, the NBDs of P-glycoprotein are able to reach near dimerized conformations even in the absence of the nucleotide due to the overall higher structural flexibility of the protein architecture. The dynamical difference between the two ABC transporters appears to present a fundamental mechanistic difference between the subfamilies they belong to, which can be described as different energy landscapes along their pathways for NBD dimerization. The difference in energy landscapes is evidenced by their transport activities in that the NBDs of the maltose transporter dimerize only in the presence of its substrate, whereas P-glycoprotein possesses a remarkable substrate-independent ATPase activity.

3253-Pos/B114. MOLECULAR DYNAMICS FLEXIBLE FITTING OF POLIOVIRUS STRUCTURAL TRANSITIONS DURING INITIATION OF INFECTION AT ATOMIC RESOLUTION

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Poliovirus attaches to the surface of a host cell by binding to the poliovirus receptor (PVR). Interaction of poliovirus with PVR triggers a conformational change that converts it from the 160S to the 135S state. The 135S poliovirus particle unbinds from PVR and directly associates with the membrane to initiate cell entry. Structures of the poliovirus-receptor complex and the 135S particle are only available as cryo-electron microscopy density maps that do not resolve atomic details. However, such maps permit atomic level structural assignment when x-ray structures are flexibly matched to them, for example through molecular dynamics simulation. The respective method is called Molecular Dynamics Flexible Fitting (MDFF). A complete atomic model of the poliovirus 160S particle bound to PVR has been determined by MDFF with explicit solvent and symmetry restraints based on a 2.2 Å x-ray structure of poliovirus, 3.5 Å x-ray structure of the D1-D2 domains of PVR, and an 8.0 Å cryoEM structure of the 160S-PVR complex. PVR is found to form extensive interactions with the core beta strands, GH loop, and C-terminus of the VP1 capsid protein. A complete atomic structure of the poliovirus 135S cell entry intermediate was also determined by MDFF based on a 9.6 Å cryo-EM density map. Flexible rearrangements of the VP1 GH and VP2 EF loops are observed. Molecular dynamics simulations based on the 160S-PVR and 135S atomic structures determined by MDFF provide an opportunity to identify the pathway of the 160S-to-135S structural transition and visualize the infection process of a non-enveloped virus in full atomic detail.

3351-Pos/B212. STRUCTURE AND DYNAMICS OF THE MFS MULTIDRUG TRANSPORTER EMRD

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Major Facilitator Superfamily (MFS) transporters harness the free energy stored in ion or solute gradients for active transport of a variety of substrates. Although most of the 58 distinct families of MFS transporters are substrate-specific, six families contain multidrug transporters (MDR). EmrD is a MFS-MDR from E.coli whose crystal structure has been determined. Similar to the lac permease, the topology consists of 12 transmembrane helices arranged in two bundles of six helices. However, EmrD has a more compact conformation that occludes access to a hydrophobic cavity/chamber from both sides of the membranes. To define the

conformational motion involved in substrate transport, we are using spin labeling and electron paramagnetic resonance (EPR) spectroscopy. Spin labels were introduced at selected sites along transmembrane helices 5, 6 and 8. EPR analysis of mobilities and accessibilities reveal distinct changes in spin label dynamics and exposure to NiEDDA upon protonotation of the transporter. The sites of these changes cluster at the periplasmic side of EmrD suggesting that this end of the transporter undergoes conformational rearrangements at low pH. Double electron electron resonance analysis is in progress to determine the nature and amplitude of the protein conformational motion.

3605-Pos/B466. THE PHYTOPOLYPHENOL PICEATANNOL INHIBITS THE RATE LIMITING STEP OF ROTATIONAL CATALYSIS OF THE F1-ATPASE

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Single molecule observations of the F1 sector of the E. coli ATP synthase were made in Vmax conditions with minimal load. The enzyme rotates through continuous cycles of catalytic dwells (pauses lasting ~0.2 ms) and 120° rotation steps (~0.6 ms in duration). We previously established that the rate limiting transition state step occurs during the catalytic dwell just prior to the initiation of the 120° rotation. Here we use the phytopolyphenol stilbenoid inhibitor, piceatannol, which binds to a pocket formed by contributions from α and β stator subunits and the carboxyl terminal region of the rotor γ subunit. The inhibitor did not interfere with the movement through the 120° rotation step, but caused increased duration of the catalytic dwell. Because all of the beads rotate at a lower rate in the presence of saturating piceatannol and the dissociation rate of the inhibitor is relatively slow, it appears that inhibitor stays bound throughout the rotational catalytic mechanism. Furthermore, piceatannol does not cause a bias in the behavior of the three catalytic dwell positions suggesting that the inhibitor rotates with the γ subunit against the α/β subunits. Arrhenius analysis of the duration of the catalytic dwell shows significantly increased activation energy of the rate-limiting step that triggers the 120° rotation. The activation energy was further increased by combination of piceatannol and the γM23K mutation indicating that the inhibitor and the β-γ interface mutation affect the same transition step, even though they perturb physically separated rotor-stator interactions. Our data indicate that both rotor-stator interaction sites contribute to formation of the rate limiting transition state. These studies were supported by a grant from Ministry of Science and Culture of Japan.

3485-Pos/B346. STATE-DEPENDENT FRET REPORTS LARGE GATING-RING MOTIONS IN BK CHANNELS

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Large conductance voltage- and calcium-dependent potassium channels (BK channels) are key regulators of many important physiological processes and a key feature to their physiological role is that the channel's open probability is regulated both by changes in transmembrane voltage and by intracellular calcium concentration. The voltage sensor resides within the transmembrane region of the channel, while Ca2+ binding is sensed by a large C-terminal intracellular region, where eight Regulator of Conductance for K+ (RCK) domains form a "gating ring". Calcium binding to this region reduces the energy required to open the channel, but the exact mechanism underlying this process is still uncertain. Structural studies using isolated gating rings from prokaryotic channels and a biochemical study of the isolated gating ring from the human channel suggest that Ca2+ binding expands the gating ring. The large movement of the gating ring would physically pull and open the gate located at the pore domain. In the present study we investigate the calcium and voltage-dependence of conformational changes in the intact human BK channel by patch-clamp recordings and simultaneous measurements of fluorescence energy transfer between CFP and YFP variants of the green fluorescent protein, inserted into three sites in the BK gating ring. Depending of the site studied, different movements are detected that differ in their Ca- and V-dependence. Here we show that Ca2+ binding produces surprisingly large structural changes that, contrary to current theories, are not obligatorily coupled to the opening of the pore and are not strictly cooperative. Instead, a mechanism such as the "flip" transitions that have been identified in pentameric neurotransmitter receptor-channels is operative.

3591-Pos/B452. ENERGETICS OF THE REACTIONS CONTROLLING THE DEEPLY OCCLUDED STATE OF THE NA^+/K^+
PUMP

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The Na^+/K^+ pump is a nearly ubiquitous membrane protein in animal cells that uses the free energy of ATP hydrolysis to alternately export $3Na^+$ from the cell and import $2K^+$ per cycle. This exchange of ions produces a steady outward current, proportional in

magnitude to the cycle turnover rate. When the absence of K^+ prevents cycling, a sudden voltage jump causes the Na^+/K^+ pump to generate temporally distinct transient currents that represent the kinetics of extracellular Na^+ binding/release and Na^+ occlusion/deocclusion transitions. For many years, these electrical signals have escaped a proper thermodynamic treatment due to the relatively small size of the currents. Here, taking advantage of the large diameter of the axons from the Humboldt squid *Dosidicus gigas*, we have separated the kinetic components of the transient currents over an extended temperature range and thus characterized the energetic landscape, both of the Na^+/K^+ pump cycle and of those transitions associated with extracellular release of the first Na^+ from the deeply occluded state. A global fit of the energetic parameters of a model that included the occlusion/deocclusion transition followed by binding/unbinding reactions was made using 150 measurements spanning a voltage range between -120 and +60 mV and a temperature range between 17 and 32°C. The occlusion/deocclusion transition involves large changes in enthalpy (Q_{10}=3.3) and entropy that largely compensate each other as the ion is deoccluded for release to the external milieu. The subsequent binding/unbinding reaction is substantially less costly (Q_{10}=2), though still larger than predicted for the energetic cost of an ion diffusing through a permeation pathway, suggesting that ion binding/unbinding must involve amino acid side chain rearrangements at the ion coordination site. Supported by FIRCA grant R03 TW008351 and U54GM087519, GM030376, NS64259, HL36783, Fondecyt-1110430.

3433-Pos/B294. A THREE-ION SELECTIVITY FILTER POTENTIAL ENERGY LANDSCAPE OF A PUTATIVE OPEN-CONDUCTIVE KCSA

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Potassium channels are tetrameric integral membrane proteins that passively allow rapid potassium conduction through the cell membrane while being strongly selective over other monovalent cations. A narrow region known as the selectivity filter, conserved in both structure and sequence, presents a series of backbone carbonyls towards the permeating ion. Here, we have performed a full three-ion permeation free energy landscape calculation for the selectivity filter region using all-atom umbrella sampling molecular dynamics simulations of the KcsA channel in a membrane with explicit lipids and solvent. The cases of pure potassium conduction and of a single sodium chaperoned by potassium ions were examined. The Potential of Mean Force (PMF) dependent on the Z-coordinates of three ions was calculated on the basis of a new unpublished high-resolution crystal structure of KcsA in a conformation which includes both an open gate and a conductive filter. The effect of flipping of Valine 76 was examined as well as the permeation and selectivity in a model with Glycine 77 replaced by a D-Alanine.

3646-Pos/B507. INFRARED LIGHT EXCITES CELLS VIA TRANSIENT CHANGES IN MEMBRANE ELECTRICAL CAPACITANCE.

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Control of excitable cells using optical technologies such as optogenetics has enabled important advances in neuroscience and the development of clinical applications. Most existing methods of optical control require the use of genetic or chemical sensitizers that enable light to alter the ionic conductance of cell membranes. By contrast, infrared (IR) light of wavelengths > 1.5μm has been shown in vivo to excite neural and muscle tissue without any pre-treatment. Unfortunately, the mechanism of IR stimulation is unknown. Here, we describe how IR light excites cells by transiently altering their membrane electrical capacitance. Our data from voltage clamped Xenopus laevis oocytes, mammalian cells and artificial lipid bilayers shows that IR energy absorbed by water produces a rapid local increase in temperature at the cell membrane, transiently increasing its electrical capacitance, and generating depolarizing currents. Correspondingly, under current clamp conditions, IR pulses produce rapid changes in membrane potential. This unexpected mechanism is fully reversible and requires only the most basic properties of cell membranes. Changes in capacitance were verified by direct measurement in mammalian cells and artificial bilayers, and are consistent with a classical theoretical description of cell membranes as coupled double-layer capacitors. In shedding light on the mechanism of IR stimulation, our findings point to this technology's unique generality as a means to control excitable cells, and raise questions about other thermal phenomena that may meaningfully affect membrane electrostatics. Supported by the NIH: GM030376 and DC011481-01A1.

3359-Pos/B220. TRANSPORT CYCLE OF MITOCHONDRIAL CARRIERS FROM INTERNAL SYMMETRIES

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Mitochondrial carriers (MC) are nuclear-encoded transporters which exchange charged molecules across the inner membrane of mitochondria. The common transport cycle of structurally and functionally similar MCs involves transitions between two major

cytoplasmic-open (c-) and matrix-open (m-) states. The only available structure of an MC is that of ADP/ATP carrier (AAC) in c-state. Previous computational and experimental work have characterized the binding site and functional conformational changes of AAC, but fallen short of explaining the full transport cycle. MCs are consisted of three structurally similar repeats each composed of two helices. Based on sequence alignments of individual helices across all MCs, a model for conformational changes resulting in m-state is suggested. The model implies that the helices kink or straighten around certain key residues involving prolines and glycines, so that helices in each repeat exchange conformations among each other. In order to get a detailed realistic view of the transport cycle and to obtain an atomic resolution m-state model of AAC, we combined homology modeling with non-equilibrium driven MD simulations. Initially, we generated crude models of the m-state AAC by modeling each helix in each repeat based on its partner helix. We used the crude m-state models as targets in targeted molecular dynamics (TMD) simulations. Besides, we applied time dependent harmonic restraints on certain collective variables reflecting the conformational change between the c-state and the model m-state. The collective variables include dihedrals of the key prolines and glycines along with rotational orientations of the pieces of helices with respect to the binding site region. Here, we present plausible m-state structures of AAC and transition pathways along with several intermediates identified from the common features of the non-equilibrium simulations requiring the lowest work.

3618-Pos/B479. SUBSTRATE BINDING AND TRANSPORT BY A BACTERIAL MULTIDRUG MFS TRANSPORTER

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Bacterial multidrug efflux pumps confer resistance to a broad range of antibiotics, thus reducing the efficacy of clinical treatment of bacterial infection. EmrD is the only structurally-characterized representative of a drug:H^+ antiporter of the major facilitator superfamily (MFS). However, the determinants of substrate binding and transport by EmrD remain unclear. Unlike other MFS transporters, EmrD contains no membrane-embedded acidic residues thought to be necessary for coupling drug:H^+ antiport. In an effort to characterize the determinants of substrate binding and mechanism of transport by EmrD we have tested the binding and transport of several fluorescent substrates by anisotropy and quenching, respectively. EmrD bound Doxorubicin and Hoechst33342 with micromolar affinity whereas substrates with constitutive positive charges bound with low affinity, suggesting a preference for neutral species. Additionally, EmrD carried out ΔpH-driven transport of Hoechst in inside-out membrane vesicles. Interestingly, we found that no single acidic residue, including the conserved Asp68 in the MFS signature motif, is essential for Hoechst transport.

3475-Pos/B336. STRUCTURAL DYNAMICS IN THE RESTING AND ACTIVATED STATES OF THE VOLTAGE SENSOR OF Ci-VSP FROM DIPOLAR DISTANCE MEASUREMENTS

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The mechanism of electromechanical transduction in voltage sensing domains remains controversial. Here, we have probed the conformation of the voltage sensor of Ci-VSP in different functional states by means of EPR-based distance measurements. Ci-VSP is a voltage-sensing phosphatase from *Ciona intestinalis*. Although it is coupled to a cytoplasmic phosphatase, its voltage-sensing domain (VSD) is homologous to voltage sensors found in voltage-gated ion channels. It therefore serves as an excellent model to study voltage sensor movement independent of the interaction with pore domain. On the basis of voltage dependence of Ci-VSP sensing currents (Q-V curves), it is agreed that, at 0 mV, the S4 of wild-type Ci-VSP is in the resting conformation (down state). The arginine at position 217, located in the extracellular end of S4, has a strong effect on the voltage dependence of Ci-VSP sensing currents. Mutations at arginine 217 with a neutral or negative residue (R217Q or R217E), lead to a large leftward shifts in the Q-V curve so that, at 0 mV, the sensor is in the activated conformation (up state). This provides a unique opportunity to monitor the conformational differences in the VSD between resting and activated states in the absence of membrane potential. We expressed and purified a series of double cysteine mutants in the isolated voltage sensor (S1 to S4) of Ci-VSP in wild-type and R217E backgrounds, and measured distances using CW-based dipolar broadenings (for short distances, 8 to 20 Å) and double electron-electron resonance (DEER) spectroscopy (for longer distances, 20 to 50 Å). Our preliminary analysis of the distance measurements suggest defined conformational differences between resting and activated states of the VSD.

Pos-L54/LB54. DYNAMIC NUCLEAR POLARISATION ENHANCED MAS-NMR ON ^{13}C-LEUCINE BOUND TO THE SECONDARY TRANSPORTER LEUT

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Neurotransmitter: Sodium symporters (NSS) terminate synaptic signaling by re-uptake of neurotransmitters from the synaptic cleft. The bacterial homolog LeuT from *Aquifex aeolicus* is the structural paradigm for this family of transporters. Here, we describe the first cwDNP enhanced MAS-NMR experiments on LeuT reconstituted into lipid bilayers. The work was carried out using a non-commercial DNP setup consisting of a high power 258 GHz gyrotron connected via corrugated waveguides to a specially modified

Bruker 3.2mm cryo-MAS probehead operating at 110 K at a 393 MHz Bruker Avance II spectrometer. Reconstituted LeuT sample was doped using 20mM of the biradical TOTAPOL yielding a 15 - 20 fold signal enhancement compared to conventional solid-state NMR. This enabled us to detect ^{13}C-Leu within the high affinity binding site using double quantum filtering techniques. The resulting spectra are well resolved and enable lineshape and chemical shift analysis. We have also carried out first ^{13}C-^{15}N TEDOR experiments to probe direct ligand-protein contacts. Our data highlight the potential of cwDNP-MAS NMR to probe high affinity ligand binding to LeuT. Using this technique, we are conducting experiments to shed light on the controversy surrounding the presence and role of two leucine high affinity sites.

3350-Pos/B211. STUDYING THE CONFORMATIONAL CYCLE OF THE SECONDARY MULTIDRUG TRANSPORTER LMRP BY EPR SPECTROSCOPY

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LmrP, a Major Facilitator Superfamily (MFS) multidrug transporter from Lactococcus lactis, acts a secondary antiporter, catalysing the extrusion of a large spectrum of hydrophobic drugs by dissipating a proton gradient. This implies that it has evolved to bind and export a series of structurally diverse cytotoxic substances from within the membrane, in contrast to the majority of MFS transporters that are specialized in the transport of a single soluble substrate, captured from the aqueous medium. Its transport mechanism could thus potentially differ from the alternating access model of the lactose permease that currently stands as a paradigm for all MFS transporters. Using Electron Paramagnetic Resonance spectroscopy on a library of cysteine mutants, labeled with either one or two spin probes, we aim at mapping the conformational dynamics of LmrP during its transport cycle. We are using accessibility patterns and intramolecular distance changes to understand the conformational rearrangements involved in the energization and subsequent substrate recognition and extrusion mechanisms of the transporter. Preliminary measurements on a first set of mutants show profound structural changes upon ligand binding or protonation of key acidic residues. In particular, we observe a significant conformational rearrangement of helix VIII upon substrate binding, suggesting that the interface between the N-and C-terminal halves could be key in the controlled access of drugs to the substrate binding pocket. These findings could shed light on the mechanistical divergence with MFS transporters recognizing soluble substrates from the extracellular medium. Based on these first results, we present a blueprint of the catalytic cycle of LmrP.

3099-Symp. INTO THE COSMOS: SINGLE MOLECULE ANALYSIS OF SPLICEOSOME ASSEMBLY AND ACTIVATION

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Excision of introns from pre-mRNAs is mediated by the spliceosome, a large, dynamic complex consisting of five small ribonucleoprotein particles (snRNPs) and scores of associated proteins. Current understanding of spliceosome assembly is based largely on the procession of stable complexes that can be resolved from in vitro splicing reactions. Such ensemble experiments have suggested a highly ordered, linear assembly pathway in which initial binding of U1 snRNP to the 5' splice site is followed by stable U2 association with the branch site and subsequent U4/U5/U6 tri-snRNP and Nineteen Complex (NTC) addition to form the fully assembled spliceosome. Previously unknown, however, were the detailed forward and reverse kinetics of each assembly step, the extent to which branched and/or dead-end assembly pathways exist, and whether or not different introns utilize the same or alternate assembly pathway(s). We are now addressing these questions by combining yeast genetic engineering, chemical biology, and multi-wavelengthfluoresence microscopy to follow assembly of single spliceosomes in real time. Because no protein purification or reconstitution is required for such Colocalization Single Molecule Spectroscopy (CoSMoS), this experimental strategy should prove widely useful for mechanistic analysis of many other macromolecular machines in environments approaching the complexity of living cells.

3112-Plat. INTERDIMER CONTACTS PAINT A NEW PICTURE OF GLUTAMATE RECEPTOR ACTIVATION.

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The ionotropic glutamate receptors (iGluR) mediate the majority of the rapid signalling at excitatory synapses in the brain. The

binding of glutamate and other agonist molecules to the ligand-binding domains (LBDs) of the iGluR provides the free energy for driving intra-LBD conformational transitions that open the gate of the ion channel. However, much less information is available about inter-LBD motions. We recently showed that disulfide crosslinks between kainate receptor LBD dimers inhibit receptor activation (Das et al, 2010, PNAS). Here, we used a combination of structural studies and electrophysiology to map the conformational transitions of the LBD dimers between different states of the GluA2 receptor. Interdimer disulfide trapping with exquisite functional sensitivity shows that the two subunit dimers must translate relative to each other during activation, with the center of the dimers moving towards the overall axis of the channel. The crosslink captures an intermediate state between resting and fully activated and has geometry (including reduced linker separation) that provides new insight to glutamate receptor activation.

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