BIOGRAPHICAL SKETCH

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NAME: **Chapman, Michael S.**

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POSITION TITLE: Wurdack Prof. Biochemical Sci. & Chair, Dept. Biochemistry, Univ. Missouri - Columbia

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of London, Kings College, England	B.Sc. / AKC	08/1982	Cell & Molecular Biol.
University of London, Birkbeck College, England	M.Sc.	08/1983	Crystallography
University of California, Los Angeles	Ph.D.	12/1987	Biochemistry
Purdue University	(Post-Doc)	08/1993	Structural Virology

A. Personal Statement

Michael Chapman is a biophysicist who develops and applies multi-disciplinary approaches to viral-host interactions and enzyme mechanism/dynamics. A PhD on enzyme structure with David Eisenberg was followed by post-doctoral research on virus structure with Michael Rossmann. As an independent investigator, he has led a research group combining structural techniques: x-ray diffraction, electron microscopy (EM) and NMR with biochemical kinetics, molecular virology and computer modeling to understand the functional workings of large and dynamic complexes. He has pushed the boundaries through holistic integration of techniques, developing computer algorithms to optimize structure against diverse experimental data and to incorporate additional *a priori* stereochemical principles. These multi-disciplinary approaches have advanced our understanding of enzyme turnover kinetics, and of the virus-host interactions key to improving vectors for human gene therapy.

He has served in multiple research / administrative capacities. Starting mid-career at Florida State University, he led an initiative to add a Center of Excellence in Computational Biology. At Oregon Health & Science University, he oversaw the interdepartmental graduate curriculum, established training and internship programs in Quantitative Bioscience and served as Interim Chair for four years. He currently serves as Chair of Biochemistry at the University of Missouri, a department with missions in undergraduate teaching (400 majors), graduate research (26 faculty groups) and pre-clinical medical education. The pandemic provided leadership challenges in establishing on-line education, safely reopening research laboratories, and managing revenue losses (State, tuition & medical center) that threatened faculty/staff payroll. Of broader impact, he was founding Associate Director of one of the NIH National Centers for Cryo-EM. In Missouri, he helped develop, and now chairs the oversight committee for an EM Center of Excellence, a collaboration with Thermo Fisher Inc., for multi-scale EM imaging in the life and materials sciences at the NextGen Precision Health Institute.

Research products:

American Society of Gene & Cell Therapy 19th Annual Meeting, **Presidential Symposium** (2016): An Essential and Ubiquitous Protein Receptor for AAV; Glycans as Attachment Receptors.

US Patent 10633662 Methods and compositions for modulating AAV infection (2020); Pillay, S., Carette, J, Chapman, M.S., Meyer, N., Pushnik, A. & Davulcu, O.

Meyer, N.L. & Chapman, M.S. (2021): Adeno-associated virus (AAV) cell entry: structural insights. Trends in Microbiology, **Trends in Microbiology** 30: 432-451 (2022) doi: 10.1016/j.tim.2021.09.005.

Stagg, S.L., Yoshioka, C., Davulcu, O. & Chapman, M.S. *Cryo-Electron Microscopy of Adeno-Associated Virus*. In *Cryo-EM* in *Biology and Materials Research*, Danino, D. & Subramaniam, S. Eds., **Chemical Reviews**, *in press* (2022) doi: 10.1021/acs.chemrev.1c00936.

Ongoing & completed research projects:

NIH R35 GM122564-05 Chapman (PI) — 8/1/17–7/31/22
Adeno-Associated Virus Gene Therapy Vectors: Molecular Interactions on Cell Entry.

NIH U24GM129547 PIs: E. Gouaux, M. Chapman (5/18-1/20), J. Evans & S. Reichow (1/20-) — 5/1/18–4/30/24
Pacific Northwest (National) Center for Cryo-EM. Role: Assoc. Director (though 1/20):
Role: Center planning, recruitment, oversight of training programs, application review & biosafety; Since 1/20, executive committee & advisory board chair.

B. Positions, Scientific Appointments and Honors

Employment

1988-1993	Post-doctoral Assoc., Dept. of Biological Science, Purdue Univ. (with Michael Rossmann)
1993-2006	Asst. Prof., Assoc. Prof. (1998), Prof. (2003), Dept. Chem. & Biochem., Florida State Univ.
1998-2001	Associate Director, Institute of Molecular Biophysics, Florida State Univ.
2000-2006	Director, Center of Excellence in Biomolecular Computer Modeling & Simulation, FSU.
2006-2018	Jones Prof. Structural Biology, Dept. Biochem. & Mol. Biol., Oregon Health & Science Univ.
2014-2018	Interim Chair, Dept. Biochem. & Mol. Biol.; Dir. Training, Quant. Biosci. & Biomed. Engin.
2018- curr.	Wurdack Prof. Biochem. Sci. & Chair, Dept. Biochemistry, Univ. Missouri.

Professional Service

1999-2019	Executive Board SERCAT APS x-ray beam line (1999-06); MBC ALS beam line (2006-).
2002	Chair, Gordon Research Conference – Diffraction Methods in Structural Biology
2018-2019	Founding Assoc. Director, Pacific NW Ctr. *Cryo*-EM; 2020– curr.: Chair, Ext. Advisory Board
2020- curr.	NIH National Resource for Advanced NMR Technology - Ext. Advisory Board

Federal Committees

1999-2013	Proposal reviewer: NSF MCB; Dept. Defense; UK Med. Res. Council; Biotech. & Biol. Res. Council, Welcome Trust, French Agence Natl. de Researches; IHFSP.
2005-2008	*NIH* panel member: Macromolecular Structure & Function C
2000-2019	*NIH* Special Panels (22, chair of 8): P41 centers, P01, K99, ZRG1 CB-N MIRA, *etc*..
2004-2020	*NIH* panels (8), temp. member: Exp. Virol., Virology, Macromol. Struct./Function B, C, D.

Recent Honors

2005- curr.	Fellow, American Association for the Advancement of Science

C. Contributions to Science

The five contributions fall in three areas. Growing from PhD research were interests in: (a) mechanisms of enzyme catalysis, then (b) protein dynamics in enzyme turnover. A second theme, extending from post-doctoral research, involves contributions to: (c) virus structure and (d) host interactions of the viral gene therapy vector, AAV. This 4th topic is currently our highest priority and the focus of this proposal. A final (5th) continuing endeavor is development of computer algorithms for refining structural models *vs*. biophysical data.

Structural Enzymology

Graduate research with David Eisenberg focused on structure determination of RuBisCO, the enzyme responsible for photosynthetic carbon fixation in plants. Later, the Chapman group developed arginine kinase (AK) as a model system for two-substrate reactions. AK, like homolog creatine kinase, buffers cellular ATP levels. The structure of a transition state analog (TSA) complex at 1.2 Å resolution indicated precise alignment of reactants, begging two questions: (1) is substrate alignment part of the catalytic effect? (2) how is precise alignment achieved in an enzyme undergoing large conformational changes on substrate-binding? With help from Jeff Evanseck, the Chapman group used quantum mechanics (QM) to dissect catalytic contributions. High level *ab initio* calculations led to a fundamental discovery. Stereoelectronic destabilization of the scissile bond in phosphoarginine, ATP, and other organic phosphates, was achieved by transfer of electron density from a oxygen lone pair into a proximal anti-bonding orbital. Experimental free energies of hydrolysis were correlated with the computed stereoelectronic effect, superseding textbook rationalizations of “high energy” bonds. The interaction can be modulated by neighboring solvent and polar side chains. A survey of kinase structures showed active site configurations where the stereoelectronic interaction could be enhanced for catalytic effect.

6. Chapman, M. S., Suh, S. W., Curmi, P. M. G., Cascio, D., Smith, W. W. & Eisenberg, D. S. (1988). *Tertiary Structure of Plant RuBisCO: Domains and their Contacts*. ***Science*** 241, 71-74. PMID: 3133767; https://doi.org/10.1126/science.3133767.
38. Zhou, G., Somasundaram, T., Blanc, E., Parthsarathy, G., Ellington, W. R. & Chapman, M. S. (1998). *Transition state structure of arginine kinase: Implications for catalysis of bimolecular reactions*. **Proc.**

Natl. Acad. Sci., USA 95, 8449-54. https://doi.org/10.1073/pnas.95.15.8449; PMCID PMC21096; NIH R01 GM55837, NSF IBN 96–31907 & BIR 94-18741.

78. Ruben, E., Chapman, M.S. & Evanseck, J. (2008) *Anomeric effect in "high energy" phosphate bonds – selective destabilization of the scissile bond and modulation of the exothermicity of hydrolysis.* **J Am Chem Soc**, 130: 3349-58. PMID: 18302368; https://doi.org/10.1021/ja073652x; NIH R01GM077643.
106. Summerton, J.C., Martin, G.M., Evanseck, J.D. and Chapman, M.S. (2014). *Common Hydrogen Bond Interactions in Diverse Phosphoryl Transfer Active Sites*. **PLoS-One**, 9: e108310. PMCID PMC4169622; https://doi.org/10.1371/journal.pone.0108310; NIH R01GM077643.

Enzyme Dynamics

Structures of substrate-bound and –free AK revealed a wide repertoire of conformational changes from loop closures to domain rotations. Despite its size (42 kDa), AK yields exceptional NMR data, providing dynamics information to complement crystal structures. In collaboration with Jack Skalicky and Rafael Brűschweiler, intrinsic dynamics were characterized with relaxation dispersion (RDA) and residual dipolar coupling (RDC). Enzyme turnover corresponded to rates of loop closure and domain rotation even in the absence of substrates. The temperature dependence of the NMR exchange constants and catalytic turnover indicated a common activation barrier, implying that intrinsic concerted millisecond protein motions limited turnover. We showed that these slow large-amplitude motions are correlated to (and perhaps built from) faster low-barrier modes of flexibility, and that substrate-associated changes could arise by selection from conformers sampled in the intrinsic motions. By comparing X-ray anisotropic atomic displacement parameters with rigid-domain models fit to solution NMR RDCs, the slow / large transitions (observed by NMR) are correlated to small and fast (ns/ps) oscillations reflected in crystallographic B-factors. Furthermore, hinges, implied by these crystallographic indicators of thermal motion, have NMR-measurable relaxation exchange, likely because of intrinsic flexing in the ms to μs regime. All is consistent with a hierarchy of large motions built from component modes of fast oscillations. Surprisingly, the protein dynamics of the transition state analog complex are more widespread and somewhat faster than substrate-free. With stabilization of transition states as a means of catalysis, this might seem counter-intuitive, but disorder in the surrounding protein is lowering entropic barriers. Few enzymes are amenable to the array of approaches needed to characterize turnover dynamics robustly. AK retains unique advantages as a model, the first of a size in which is displayed the full repertoire of loop and domain motions typical of metabolic enzymes.

In our computer analyses, conservative low-parameter models gave greatest consistency between NMR and X-ray datasets, perhaps reflecting a parsimony in nature. It inspires development (below) of a parsimony restraint to counter overfitting of atomic models to sparse or low resolution biophysical datasets.

92. Davulcu, O, Skalicky, J. & Chapman, M (2011) *Rate-Limiting Domain & Loop Motions in Arginine Kinase*. **Biochem.** 50:4011-8. PMCID PMC3091953; https://doi.org/10.1021/bi101664u; NIH R01 GM77643.
107. Chapman, B.K., Davulcu, O., Skalicky, J., Brűschweiler, R. & Chapman, M. (2015). *Parsimony in Protein Conformational Change*, **Structure**, 23: 1190-98; https://doi.org/10.1016/j.str.2015.05.011; PMCID: PMC4497923; NIH R01 GM77643.
113. Peng,Y., Hansen, A.L., Bruschweiler-Li, L., Davulcu, O., Skalicky, J.J., Chapman, M.S. and Brüschweiler, R.P. (2017). *The Michaelis Complex of Arginine Kinase Samples the Transition State at a Frequency that Matches the Catalytic Rate*. **J. Am. Chem. Soc.**, 139: 4846-4853; PMCID PMC5663447; https://doi.org/10.1021/jacs.7b00236; NSF MCB-1360966 (RPB) & NIH GM-077643 (MSC).
115. Davulcu O, Peng Y, Brüschweiler R, Skalicky JJ, Chapman MS. *Elevated μs-ms timescale backbone dynamics in the transition state analog form of arginine kinase*. (2017) **J. Struct. Biol**., 200: 256-66. https://doi.org/10.1016/j.jsb.2017.05.002; PMCID PMC5677571; NIH R01GM077643.

Structural Virology: Picornaviruses, Parvoviruses & Adeno-associated Virus (AAV)

Contributions as a Rossmann post-doc included the first algorithm for *ab initio* crystallographic phase determination using viral symmetry (avoiding heavy atom derivatives), and crystal structures of rhinoviruses and canine parvovirus (CPV). It was then a surprise that DNA and RNA viruses shared the same capsid protein fold. By developing algorithms for mapping sequence diversity to 3D structure, and comparing very different viruses, I revealed evidence of competing evolutionary pressures. Cell receptor sites were conserved, but elsewhere, surfaces had evolved greater diversity, presumably in escaping recognition by neutralizing antibodies.

As an independent investigator, I turned to AAV, emerging as a candidate gene therapy delivery vector for genetic diseases. Its potential importance attracted the attention of both young and well-established structural virologists. For 4 years, our AAV-2 was the only structure, because we, alone, invested in development of

large-scale preparative virology to support wide crystallization screens. Our structure provided a road map for site-directed mutagenesis and mapping of functions to the viral surface. AAV-2 was followed by structures of other serotypes by our group and others, supporting comparative analyses of host preferences and immune neutralization. Our work provided a foundation for the selection, by directed evolution, of new cell tropisms using targeted insertion of peptides of random sequence.

In the late 2000s, analysis of complexes with cellular attachment factors and neutralizing antibodies led us to cryo-electron microscopy (EM). This started at nanometer resolution, requiring development of computer methods for optimal modeling of hybrid structures (see below). Our work has since pushed the limits through the "cryo-EM resolution revolution". AAV-DJ has, at times, been the highest resolution cryo-EM structure, and now, at 1.56 Å resolution, is 2nd only to apoferritin. Hydrogens are observable, motivating further development of refinement methods, so that cryo-EM can be used for analysis of hydrogen-bond networks. AAV-DJ is a chimeric vector selected, in directed evolution, for liver tropism and immune-escape. Differences from the parental strains are biggest at the epitope where we had previously visualized the binding of neutralizing monoclonal antibody A20 to AAV-2. This explained the resistance of AAV-DJ to neutralization by polyclonal serum and suggested that the epitope might also be a receptor interaction site affecting tropism.

13. Tsao, J., Chapman, M. S., Agbandje, M., Keller, W., Smith, K., Wu, H., Luo, M., Smith, T. J., Rossmann, M. G., Compans, R. & Parrish, C. (1991). The Three-Dimensional Structure of Canine Parvovirus and its Functional Implications. ***Science*** 251:1456-64. PMID: 2006420. https://doi.org/10.1126/science.2006420.
14. Chapman, M. S., Tsao, J. & Rossmann, M. G. (1992). *Ab initio* Phase Determination for Spherical Viruses: Parameter Determination for Spherical Shell Models. ***Acta Crystallogr***. A48, 301-12. PMID: 1605933. https://doi.org/10.1107/S0108767391013211.
51. Xie, Q., Bu, W., Bhatia, S., Hare, J., Somasundaram, T., Azzi, A., and Chapman, M.S. 2002. The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy. ***Proc Natl Acad Sci USA*** 99: 10405-10410. PMCID: PMC124927; https://doi.org/10.1073/pnas.162250899. ACS RPG-99-356-01-GMC.
119. Xie, Q., Yoshioka, C. K. & Chapman, M. S. Adeno-Associated Virus (AAV-DJ)-Cryo-EM Structure at 1.56 A Resolution. **Viruses** 12: 1194 (2020); https://doi.org/10.3390/v12101194; PMCID PMC7589773. NIH R35GM122564 & U24GM129437.

Host interactions of the Gene Therapy Vector, AAV

Cellular attachment and entry are current *foci* of our work with AAV. Glycans were previously considered to be "primary receptors", but SPR binding analyses, and our cryo-EM structures of glycan-analog complexes, revealed low specificity, flexible side chains accommodating diverse glycan types. This was the start of a now-accepted paradigm change that glycans serve as AAV attachment factors not as classical entry receptors. Our structures refuted the belief that glycan-binding triggered conformational changes for viral uncoating.

Turning next to reported co-receptors, we found no biophysical evidence of direct interactions, or that siRNA interference ablated viral transduction. Preparing for a genome-wide re-appraisal of cellular receptors, we developed an approach for FACS-based selection of AAV-resistant cells. We initiated a collaboration with Jan Carette, applying FACS-selection to his gene trap knockout (KO) library in a human haploid cell line. Deep sequencing of resistant cells identified a transmembrane protein (AAVR) used by most AAV serotypes, as validated by knock-outs in multiple cell lines and a mouse. AAV takes advantage of AAVR's retrograde trafficking from the cell surface, hitching a ride to the perinuclear *trans* Golgi network. Our Nature paper over-came a 20-year impasse, prompting new analyses of the entry pathway, now that the receptor was identified correctly. We have since elucidated the pertinent binding domains and expressed appropriate constructs. Cryo-electron tomography (with Scott Stagg) and cross-linking mass spectrometry provided an overview of flexible domain configuration. We integrated this holistically with details of the binding interfaces coming from our cryo-EM structures at 2.5 Å resolution of AAVs complexed with domain fragments of the receptor. AAVs are falling into three groups. The majority interact most closely with AAVR's PKD2 domain, but, surprisingly, the AAV5-like clade binds PKD1 at a distinct site, and the AAV-4 clade is AAVR-independent. Regarding immune neutralization, mechanisms were thought to be predominantly post-entry, but we are now finding multiple examples of neutralizing antibodies competing at AAVR-binding sites. Our results are not only correcting a fundamental mis-conception but will provide a key foundation in designing immune-evading vectors that preserve cell entry.

109. Pillay, S[1]., Meyer, N.L.[1], Puschnik, A., Davulcu, O., Diep, J., Ishikawa, Y., Jae, L., Wosen, J., Nagamine, C., Chapman, M.S.[2], and Carette, J.E.[2] (2016). *An essential receptor for adeno-associated virus infection*. **Nature** 530, 108-112. [1]Co-1st / [2]corresp. auth. https://doi.org/10.1038/nature16465; PMCID PMC4962915;

NIH R01 GM066875 (MSC), DP2 AI104557 (JEC) & U19 AI109662 (JEC).

115. Pillay, S., Zou, W., Cheng, F., Puschnik, A. S., Meyer, N. L., Ganaie, S. S., Deng, X., Wosen, J. E., Davulcu, O., Yan, Z., Engelhardt, J. F., Brown, K. E., Chapman, M. S., Qiu, J. & Carette, J. E. *Adeno-associated Virus (AAV) Serotypes Have Distinctive Interactions with Domains of the Cellular AAV Receptor*. **J Virol** 91, e00391-00317 (2017); https://doi.org/doi:10.1128/JVI.00391-17; PMCID PMC5571256; R01 GM066875 & R35 GM122564 (MSC), DP2 AI104557 & U19 AI109662 (JEC), R01 AI070723 & R21 AI112803 (JQ) and P01 HL051670 (JFE).
117. Meyer, N. L., Hu, G., Davulcu, O., Xie, Q., Noble, A. J., Yoshioka, C., Gingerich, D. S., Trzynka, A., David, L., Stagg, S. M. & Chapman, M. S. *Structure of the gene therapy vector, adeno-associated virus with its cell receptor, AAVR*. eLife 8, e44707 (2019); https://doi.org/10.7554/eLife.44707; PMCID PMC6561701; NIH R35-GM122564 & R01-GM066875.
120. Silveria, M. A., Large, E. E, Zane, G. M., White, T. A. & Chapman, M. S. *The Structure of an AAV5-AAVR Complex at 2.5 A Resolution: Implications for Cellular Entry and Immune Neutralization of AAV Gene Therapy Vectors*. **Viruses** 12:1326 (2020); https://doi.org/10.3390/v12111326. PMCID PMC7698955; NIH R35 GM122564.

Structure Optimization for Cryo-EM and Hybrid Methods

Mechanistic conclusions rarely come directly from the biophysical data, but from derived atomic models, which must be fit robustly to the data. We have previously contributed supplementary restraints, such as directional hydrogen-bonding, and target functions to use new datatypes, such as solid-state NMR, but increasingly focus on cryo-EM. The popular program, Phenix, maximizes map values at atom centers, subject to stereochemical restraints. Unique to our approach are rigorously calculated 3D atomic profiles whose summed contributions are fit to map values at all grid point within the molecular envelope, allowing refinement of effective resolution, atomic B-factors and improving coordinates through better map-model consistency. It has been applied in many resolution regimes to structures of ribosomes, viruses, acto-myosin complexes and ion channels. Our approach was adopted by of Schröder, Levitt & Brűnger, but other programs retain unnecessary approximations for expediency. A goal is a drop-in module to upgrade popular 3rd party refinement packages.

We work throughout cryo-EM regimes. Despite advances, the average resolution of deposited structures is 6.5Å, where tests show nothing but overfitting with commonly applied all-atom flexible fitting (#100 below). We are working on reduced model parameterizations and parsimony restraints (developed for our analyses of sparse NMR data) as low-resolution alternatives to all-atom molecular dynamics. At high resolution, only unsharpened apoferritin maps were amenable to Refmac-refinement, so it is our RSRef refinement of AAV-DJ that has been breaking new ground with inclusion of explicit hydrogens and optimization of atomic interaction networks (#119 above). Improving instrumentation will provide increasing opportunities for EM analyses in hitherto inaccessible detail, enabled by our software algorithms.

55. Gao, H., Sengupta, J., Valle, M., Korostelev, A., Eswar, N., Stagg, S., VanRoey, P., Agrawal, R., Harvey, S., Sali, A., Chapman, M., & Frank, J. *Study of the Structural Dynamics of the E. coli 70S Ribosome Using Real Space Refinement.* **Cell**, 2003. 113: 789-801. PMID: 12809609; https://doi.org/10.1016/S0092-8674(03)00427-6; NIH R37 GM 29169, R01 GM 55440, P01 GM 64676, R01 GM 53827, R01 GM 61576, R01 GM 54762, P50 GM62529, NSF DBI-98-08098.
58. Bertram, R., Asbury, T., Fabiola, F., Quine, J., Cross, T. & Chapman, M. (2003). *Atomic Refinement with Correlated Solid-State NMR Restraints*. **J. Mag. Res.**, 2003. 163: 300-9. PMID: 12914845; https://doi.org/10.1016/s1090-7807(03)00147-2; NIH P01 GM64676, NSF DMS 99-81822 & 99-86036 and DBI 98-08098.
65. Fabiola, F. and Chapman, M.S. (2005) *Fitting of High Resolution Structures into Electron Microscopy Reconstruction Images*, **Structure**, 13: 389-400. https://doi.org/10.1016/j.str.2005.01.007; PMID: 15766540; NIH P01 GM64676.
100. Chapman, M.S., A. Trzynka, and B.K. Chapman (2013), *Atomic Modeling of cryo-Electron Microscopy Reconstructions - Joint refinement of Model and Imaging Parameters*. **J. Struct. Biol.** 182:10-21. PMCID: PMC3662558; https://doi.org/10.1016/j.jsb.2013.01.003; NIH R01GM66875 & R01GM78538

Complete publication list: https://www.ncbi.nlm.nih.gov/myncbi/michael.chapman.1/bibliography/public/