

## Downstream Processing in Biomanufacturing: Multimodal Chromatography, Affinity Precipitation and Integrated Bioprocessing

Steven M. Cramer, RPI, Troy, NY

This talk will present recent developments in our lab in three areas of downstream bioprocessing; multimodal chromatography, affinity precipitation and integrated bioprocessing. Targeted experiments with different domains of mAbs with varying properties, biophysics, thermodynamics and molecular simulations will be used to shed light on the importance of protein surface cluster properties and multimodal ligand hydration and flexibility on creating selective separations. Hierarchical clustering is employed to examine preferred ligand conformations and the impact of water and dewetting phenomena in multimodal systems. NMR and molecular simulations will be used to examine the molecular basis for multimodal ligand binding to the FC region of mAbs both in solution and immobilized on SAM surfaces. In addition, pH and ligand chemistry will be employed to shift domain dominance in mAb multimodal chromatography from (Fab)<sub>2</sub> interactions to Fc binding. Affinity precipitation using smart biopolymers for the simultaneous recovery and purification of both mAb and non-mAb biologics will then be presented. The use of ELP-Z for mAb purification as well as the development of efficient processes based on this approach will be presented with industrial feed stocks. The extension of this approach using ELP-affinity peptides as well as the application to a new class of biologics will also be discussed. Finally, results will be given on a novel approach for the rapid development of integrated downstream biomanufacturing processes for biological products from *Pichia Pastoris*. This approach employs linear gradient screens in concert with high-throughput uplc analytics and custom software to identify sequences of orthogonal chromatography steps likely to recover and purify the product while also being amenable to integrated processing and a continuous flowpath. To illustrate the power of this novel approach, the rapid development of 3-step purification processes for three commercial biological products will be shown.

## CURRICULUM VITAE FOR STEVEN M. CRAMER

### Home Address

783 Trottingham Drive  
Schenectady, NY 12309  
(518) 377-9074

### Business Address

Department of Chemical Engineering  
Rensselaer Polytechnic Institute  
Troy, NY 12180-3590  
(518) 276-6198

Birthdate: 2/11/56

### Educational Background

1986 PhD in Chemical Engineering, Yale University, New Haven, Connecticut

1982 Master of Science in Chemical Engineering, Yale University, New Haven, Connecticut

1978 Bachelor of Science in Biomedical Engineering, Brown University, Providence, Rhode Island

### Professional Experience

**7/1/07-present: William Weightman Walker Professor**, Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, New York. The research in the Cramer laboratory involves state of the art experimental and theoretical investigations into novel bioseparations systems. Topics include: prediction of protein binding affinity and multiscale modeling of chromatographic systems, design of chemically selective displacers, development of efficient antibody separation systems, fundamental studies in multimodal chromatography, novel proteomics platform using carrier displacement chromatography, novel chromatographic and diode based electrophoretic lab on chip systems, protein unfolding in chromatographic systems, chemometrics for process analytical technology, multilevel automated peptide synthesis/screening system for design of affinity peptides, smart biopolymer affinity precipitation systems and hierarchical nanobio systems for bioprocessing. Recent work that combines protein libraries, NMR studies, molecular dynamics simulations and high throughput chromatographic investigations has shed significant insight into the design of a new generation of mixed mode chromatographic systems. Teaching courses in: material and energy balances, separation processes, and chromatographic separation processes.

**8/15/06-7/1/07: Professor of Chemical and Biological Engineering**, Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, New York.

**11/04-8/15/06: Acting Department Head and Professor of Chemical and Biological Engineering**, Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, New York.

**5/95-Present: Professor of Chemical and Biological Engineering**, Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, New York.

**7/93-5/95: Isermann Associate Professor of Chemical Engineering**, Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, New York.

**1/90-7/93: Associate Professor of Chemical Engineering**, Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, New York.

**9/86 - 1/90: Isermann Assistant Professor of Chemical Engineering**, Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, New York.

**9/78 - 8/81: Research Engineer**, Amicon Corporation, Lexington, Massachusetts.

### **Honors**

- 6/16 Elected Chair of the Recovery of Biological Products Board
- 3/16 ACS National Award in Separation Science and Technology
- 5/15 School of Engineering Outstanding Professor Award, RPI
- 4/15 Delivered the Horvath Distinguished Lecture at Yale
- 8/12 Elected a Fellow of American Chemical Society
- 7/11 Elected a Fellow of American Institute for Chemical Engineers
- 9/06 Alan S. Michaels Award for the Recovery of Biological Products (ACS Division of Biochemical Technology).
- 3/05 Presented the first honorary seminar in a new distinguished scientist lecture series at Centocore
- 12/04 Delivered a major keynote address at the joint Indian and US Chemical Engineering meeting in Mumbai India.
- 5/04 School of Engineering Research Excellence Award, RPI
- 4/04 Presented the first honorary seminar in a new distinguished scientist lecture series at Genzyme
- 2/03 Delivered a major keynote address at the International Society for the Separation of Peptides and Proteins in Aachen Germany.
- 10/03 Honorary speaker at the Central New England Chromatography Council
- 5/02 Delivered the major plenary address at the Taiwan-Japanese joint conference on Separations Technology.
- 6/01 Co-Chair of ACS Conference on Recovery of Biological Products
- 7/99 Chair of Gordon Conference on Reactive Polymers and Ion Exchange
- 12/96 Elected a Fellow of American Institute for Medical and Biological Engineering
- 3/96-present: Editorial Board, Separation and Purification Methods.
- 3/96-present Editor: Separation Science and Technology
- 5/95 Special Associate Editor: Biotechnology and Bioengineering
- 5/92 Honorary Focus Address at the Minnesota Chromatography Forum
- 5/90 Early Career Award, Rensselaer Polytechnic Institute.
- 3/89 Presidential Young Investigator, National Science Foundation.

3/89 Lilly Teaching Fellow Award, Rensselaer Polytechnic Institute.  
9/87 Dow Chemical Company Excellence in Teaching Award.  
9/86-1/96 Editorial Board, Isolation and Purification  
9/83-9/85 Awarded the Yale Exxon Graduate Fellowship

### **Professional Activities**

Membership in: American Chemical Society, American Institute of Chemical Engineers, American Association for the Advancement of Science, American Institute for Medical and Biological Engineering.

Chair of the Recovery of Biological Products Board, 2016-present  
Co-Chair Recovery of Biological Products Meeting, Bermuda, 2016  
Chair of 8<sup>th</sup> HIC/RPC Bioseparations Conference, Savannah, GA (2013)  
Chair of 6<sup>th</sup> HIC/RPC Bioseparations Conference, Nappa Valley, CA (2009)  
Co-Chair Recovery of Biological Products Meeting, Cancun MX 2001  
Chair of Gordon Conference on Reactive Polymers and Ion Exchange (1999)  
Co-Chair Biotechnology Secretariat Program for 1994 San Diego ACS Meeting.  
Executive Committee, Biotechnology Secretariat, American Chemical Society.  
Executive Committee, I & EC Division, American Chemical Society.  
Executive Committee, Separation Science and Technology Subdivision, American Chemical Society.

### **Reviewer for journals and agencies**

National Science Foundation	Trends in Analytical Chemistry
Journal of Chromatography	Chemical Engineering Communications
Biotechnology Journal	Biotechnology Progress
Reactive Polymers	AIChE Journal
Biotechnology and Bioengineering	University of Queensland (PhD Thesis)
I&EC Research	Yale University (PhD Thesis)
Preparative Chromatography	American Scientist
Chemical Engineering Science	Separations Technology
Proceedings of National Academy of Sciences	Chemistry of Materials
M.I.T. (Ph.D. Thesis)	Biotechnology Advances
Health Effects Institute	National Institute of Health
Nature	Computers in Chemical Engineering
	Journal of Colloid and Interface Science

### **Consulting**

Served as a consultant to Bio-Rad, Bristol Myers Squibb, Summit chemical Company, selective micro technologies, Millipore Corporation, H. R. Parrs Associates, Boehringer Labs, NASA, MARS Corporation, Allied Signal, National Institute of Health, Health Effects Institute, Merck, Protein Design Labs, Chiral Technologies, Genentech, IGEN,

DYAX, Regeneron, ISIS, Merck, GE Healthcare, SACHEM, Chiron, Biogen, Novo Nordisk, Antigenics, Cabot, Tanical Therapeutics.

### **Publications (Refereed Journal Articles)**

1. S Parimal, S Garde, SM Cramer , Effect of guanidine and arginine on protein-ligand interactions in multimodal cation-exchange chromatography, *Biotechnology Progress*, 12/1/2016
2. JR Robinson, HS Karkov, JA Woo, BO Krogh and S.M. Cramer, QSAR models for prediction of chromatographic behavior of homologous Fab variants, *Biotechnology and Bioengineering*, 12/1/2016.
3. W.R. Keller, S.T. Evans, G. Ferreira, D. Robbins and S.M. Cramer, “Use of MiniColumns for linear isotherm parameter estimation and prediction of benchtop column performance”, *J. Chromatogr. A* 1418, 94-102 (2015).
4. Karkov, H.S., Woo, J., Krogh, B.O., Ahmadian, H., Cramer, S.M., Evaluation of selectivity in homologous multimodal chromatographic systems using in silico designed antibody fragment libraries, *J. Chromatogr. A* 1426, 102-109, 2015
5. Woo, J.A., Chen, H., Snyder, M.A., Chai, Y., Frost, R.G. and Cramer, S.M. “Defining the property space for chromatographic ligands from a homologous series of mixed-mode ligands”, *J. Chromatogr. A.*, 1407 58-68 (2015).
6. Woo, J.A., Parimal, S., Brown, M.A., Heden, R., and Cramer, S.M. “The effect of geometrical presentation of multimodal cation-exchange ligands on selective recognition of hydrophobic regions on protein surfaces”, *J. Chromatogr. A.* (2015).
7. Parimal, S., Garde, S., and Cramer, S.M., “Interactions of Multimodal Ligands with Proteins: Insights into Selectivity Using Molecular Dynamics Simulations”, *Langmuir*, 27 7512-7523 (2015).
8. Karkov, H.S., Krogh, B.O., Woo, J., Parimal, S., Ahmadian, H. and Cramer, S.M., “Investigation of protein selectivity in multimodal chromatography using in silico designed Fab fragment variants, *Biotechnology and Bioengineering* (2015).
9. Srinivasan, K., Parimal, S., Lopez, M.M., McCallum, S.A. and Cramer, S.M. “Investigation into the Molecular and Thermodynamic Basis of Protein Interactions in Multimodal Chromatography Using Functionalized Nanoparticles” *Langmuir*, 30 (44), 13205-13216 , DOI: 0.1021/la502141q (2014).
10. Carvalho, R.J., Woo, J., Aires-Barros, M.R., Cramer, S.M., and Azevedo, A.M. “Phenylboronate chromatography selectively separates glycoproteins through the manipulation of electrostatic, charge transfer, and cis-diol interactions”, *Biotechnology Journal*, 2014 9 (10), 1250-1258. DOI: 10.1002/biot.201400170
11. Sejergaard, L., Karkov, H.S., Krarup, J.K., Hagel, A.B., and Cramer, S.M. “Model-Based Process Development for the Purification of a Modified Human Growth Hormone Using Multimodal Chromatography”, *Biotechnol Prog.* 2014 Sep;30(5):1057-64.
12. Sheth, RD., Jin, M., Bhut, BV., Li, ZJ., Chen, W., and Cramer, S.M. “Affinity Precipitation of a Monoclonal Antibody From an Industrial Harvest Feedstock Using an ELP-Z Stimuli Responsive Biopolymer” *Biotech. and Bioeng.* 111 (8) 1595-1603 (2014) DOI: 10.1002/bit.25230

13. Siddharth, P. Cramer, S.M., and Garde, S. “Application of a Spherical Harmonics Expansion Approach for Calculating Ligand Density Distributions around Proteins”, *J. Phys. Chem. B*, 2014, 118 (46), pp 13066–13076. (cover art)
14. Sheth, R.D., Bhut, B., Jin, M., Li, Z., Chen, W., and Cramer, S.M., “Development of an ELP-Z Based mAb Affinity Precipitation Process Using Scaled-Down Filtration Techniques”, *Journal of Biotechnology* 192 (2014) 11-19.
15. Buyel, J.F., Woo, J.A., Cramer, S.M., and Fischer, R. “The use of quantitative structure-activity relationship models to develop optimized processes for the removal of tobacco host cell proteins during biopharmaceutical production”, *J. Chromatogr. A.*, (1322), 18-28 (2013). DOI: 10.1016/j.chroma.2013.10.076
16. Levy, M., Plawsky, J., and Cramer, S.M., “Photopolymerized sol-gel monoliths for separations of glycosylated proteins and peptides in microfluidic chips”, *J. of Sep. Sci.* 36 (14) 2358-2365 (2013). DOI: 10.1002/jssc.201200990
17. Chandra, D., Morrison, C.J., Woo, J., Cramer, S.M. and Karande, P., “Design of peptide affinity ligands for S-protein: a comparison of combinatorial and de novo design strategies”, *Mol. Diversity* 17 (2) 357-369 (2013) DOI: 10.1007/s11030-013-9436-z
18. Madan, B., Chaudhary, G., Cramer, S.M., and Chen, W., “ELP-z and ELP-zz capturing scaffolds for the purification of immunoglobulins by affinity precipitation”, *J. of Biotechnology*, 163 (1) 10-16 (2013).
19. Holstein, M.A., Parimal, S., McCallum, S.A., and Cramer, S.M., “Effects of Urea on Selectivity and Protein-Ligand Interactions in Multimodal Cation Exchange Chromatography”, *Langmuir*, 29 (1), 158-167 (2013).
20. Karkov, H.S., Sejergaard, L., and Cramer, S.M. “Methods development in multimodal chromatography with mobile phase modifiers using the steric mass action model” . *Chromatogr. A*, 1318 149-155 (2013) DOI: 10.1016/j.chroma.2013.10.004
21. Sheth, R.D., Madan, B., Chen, W. and Cramer, S.M. “High-Throughput Screening for the Development of a Monoclonal Antibody Affinity Precipitation Step Using ELP-Z Stimuli Responsive Biopolymers”, *Biotech and Bioeng.* 110 (10) 2664-2676 (2013) DOI: 10.1002/bit.24945
22. Levy, M.H., Goswami, S., Plawsky, J. and Cramer, S.M. “Parameters Governing the Formation of Photopolymerized Silica Sol-Gel Monoliths in PDMS Microfluidic Chips”, *Chromatographia* 76 (15-16) 993-1002 (2013) DOI: 10.1007/s10337-013-2493-8
23. Holstein, M.A., Parimal, S, McCallum, S.A., and Cramer, S.M., “Mobile phase modifier effects in multimodal cation exchange chromatography”, *Biotech. & Bioeng.*, 109 (1) 176-186, (2012).
24. Holstein, M.A., Nikfetrat, A.M., Gage, M., Hirsh, A.G., and Cramer, S.M., “Improving selectivity in multimodal chromatography using controlled pH gradient elution”, *J. Chromatogr. A.*, 1233, 152-155, (2012).
25. Holstein, M.A., Chung, W.K., Parimal, S., Freed, A.S., Barquera, B., McCallum, S.A., and Cramer, S.M., “Probing multimodal ligand binding regions on ubiquitin using nuclear magnetic resonance, chromatography, and molecular dynamics simulations”, *J. Chromatogr. A.*, 1229, 113-120, (2012).

26. Sheth, R.D., Morrison, C.J. and Cramer, S.M. Selective displacement chromatography in multimodal cation exchange systems, *J. Chromatogr.* 1218 (51), 9250-9259, (2011).
27. Cramer, S.M. and Holstein, M. "Downstream bioprocessing: recent advances and future promise", *Current opinion in chemical engineering*, 1(1) pp 27-37 (2011).
28. Freed, A.S., Garde, S., and Cramer, S.M. "Molecular Simulations of Multimodal Ligand-Protein Binding: Elucidation of Binding Sites and Correlation with Experiments", *J. Phys. Chem. B* 115(45) 13320-13327, (2011).
29. Morrison, C.J., Gagnon, P. and Cramer, S.M. "Purification of Monomeric mAb From Associated Aggregates Using Selective Desorption Chromatography in Hydroxyapatite Systems", *Biotech. and Bioeng.* 108 (4) 813-821 (2011).
30. Nagrath, D., Xia F. and Cramer, S.M. "Characterization and modeling of nonlinear hydrophobic interaction chromatographic systems", *J. Chromatogr. A* 1218 (9) 1219-1226 (2011).
31. Hou, Y. and Cramer, S.M. "Evaluation of Selectivity in Multimodal Anion Exchange Systems: A Priori Prediction of protein retention and examination of mobile phase modifier effects", *J. Chromatogr. A* 1218(43) pp 7813-7820 (2011).
32. Freed, A. and Cramer, S.M., "Protein-Surface Interaction Maps for Ion-Exchange Chromatography", *Langmuir* 27 (7) 3561-3568 (2011).
33. Hou Y, Jiang C.P., Shukla A.A., and Cramer S.M., "Improved Process Analytical Technology for Protein A Chromatography Using Predictive Principal Component Analysis Tools", *Biotech. and Bioeng.* 108 (1) 59-68 (2011).
34. Evans, S.T., Holstein, M. and Cramer, S.M., "Detection of Trace Proteins in Multicomponent Mixtures using Displacement Chromatography", *Anal. Chem.* 83(11) 4184-4192 (2011).
35. Hou, Y., Morrison, C.J. and Cramer, S.M. "Classification of Protein Binding in Hydroxyapatite Chromatography: Synergistic Interactions on the Molecular Scale", *Anal. Chem.* 83(10) 3709-3716 (2011).
36. Morrison C.J., Gagnon P., and Cramer S.M., "Unique selectivity windows using selective displacers/eluent and mobile phase modifiers on hydroxyapatite", *J. Chromatogr.* 1217 (42), 6484-6495 (2010).
37. Chung W.K., Holstein M.A., Freed A.S., Evans S.T., Baer Z.C., and Cramer, S.M. "Ion Exchange Chromatographic Behavior of a Homologous Cytochrome C Variant Library Obtained by Controlled Succinylation", *Sep. Sci. and Tech.* 45 (15), 2144-2152 (2010).
38. Hou Y, Hansen T.B., Staby A., and Cramer S.M., "Effects of urea induced protein conformational changes on ion exchange chromatographic behavior", *J. Chromatogr.* 1217 (47), 7393-7400 (2010).
39. Morrison C.J., Moore J.A. and Cramer S.M., "Alkyl Based Selective Displacers for Protein Purification in Ion Exchange Chromatography", *Chromatographia*, 72 (11-12), 1025-1034 (2010).
40. Zhang S.J., Xia K., Chung W.K., Cramer S.M., and Colon W., "Identifying kinetically stable proteins with capillary electrophoresis", *Protein Sci.* 19 (4), 888-892 (2010).

41. Chung, W.K., Freed, A.S., Holstein, M.A., McCallum, S.A., and Cramer, S.M. "Evaluation of protein adsorption and preferred binding regions in multimodal chromatography using NMR", *PNAS* 2010 107 (39) 16811-16816;
42. Evans, S.T., Huang, X., and Cramer, S.M. "Using Aspen to Teach Chromatographic Bioprocessing: A Case Study in Weak Partitioning Chromatography for Biotechnology Applications", *Chemical Eng. Education*, 2010, 44(3), p 198.
43. Evans, S.T., Morrison, C.J., Freed, A.S., and Cramer, S.M. "The effect of feed composition on the behavior of chemically selective displacement systems", *J. Chromatogr.* 1217 (8). 1249-1254 (2010).
44. Chung, W.K., Hou, Y., Holstein, M., Freed, A., Makhatadze, G.I., and Cramer, S.M. "Investigation of protein binding affinity in multimodal chromatographic systems using a homologous protein library" *J. Chromatogr.* 1217 (2) 191-198 (2010).
45. Chung, W.K., Evans, S.T., Freed, A.S., Keba, J.J., Baer, Z.C., Rege, K., and Cramer, S.M., "Utilization of Lysozyme Charge Ladders to Examine the Effects of Protein Surface Charge Distribution on Binding Affinity in Ion Exchange Systems", *Langmuir* 2010, 26(2), 759–768
46. Morrison CJ, Breneman CM, Moore JA, and Cramer SM, "Evaluation of Chemically Selective Displacer Analogues for Protein Purification" *Anal. Chem.* 81(15) 6186-6194 (2009).
47. Chung, W.K.; Hou, Y.; Freed, A; Holstein, M.; Makhatadze, G., and Cramer, S.M. "Investigation of protein binding affinity and preferred orientations in ion exchange systems using a homologous protein library", *Biotech. Bioeng.* Volume 102, Issue 3 (p 869-881) (2009). (cover art featured on journal cover).
48. Evans, S. and Cramer, S.M. "Displacer concentration effects in displacement chromatography. Implications for trace solute detection", *J. Chromatogr.* 1216 (1) 79-85 (2009).
49. Goswami S, Bajwa N, Asuri P, Ci LJ, Ajayan PM, and Cramer SM "Aligned Carbon Nanotube Stationary Phases for Electrochromatographic Chip Separations" *CHROMATOGRAPHIA* Volume: 69 (5-6) 473-480 (2009)
50. Morrison, C.J. and Cramer, S.M. "Characterization and Design of Chemically Selective Cationic Displacers Using a Robotic High Throughput Screen," *Biotech. Progress.* 25 (3) 825-833 (2009).
51. Morrison, C.J.; Godawat, R.; McCallum, S.A.; Garde, S.; Cramer, S.M. "Mechanistic Studies of Displacer-Protein Binding in Chemically Selective Displacement Systems using NMR and MD Simulations", *Biotech. Bioeng.* 102(5) 1428-1437 (2009). (featured article)
52. Barua S, Joshi A, Banerjee A, Matthews D, Sharfstein ST, Cramer SM, Kane RS, and Rege K. "Parallel Synthesis and Screening of Polymers for Nonviral Gene Delivery", *MOLECULAR PHARMACEUTICS* Volume: 6 (1) 86-97 (2009).
53. Morrison, C.J.; Park, S.K., Simocko, C., McCallum, S.A., Cramer, S.M., Moore, J.A "Synthesis and Characterization of Fluorescent Displacers for Online Monitoring of Displacement Chromatography" *J. Am. Chem. Soc* 130 (50), 17029–17037 (2008).



54. Liu J, Hilton Z, Cramer S.M., “Chemically selective displacers for high-resolution protein separations in ion-exchange systems: Effect of displacer-protein interactions” *Anal. Chem.* 80 (9) 3357-3364 (2008)
55. Vutukuru S, Kate SD, McCallum SA, Cramer, S.M., Kane, R., “An affinity-based strategy for the design of selective displacers for the chromatographic separation of proteins” *LANGMUIR* 24 (13), 6768-6773 (2008).
56. Jindal R, Plawsky J.L., and Cramer S.M. “Synthesis and characterization of particle loaded sol-gel composite material for microfluidic chip system”, *J. of Sol-Gel Sci. and Tech.* 45 (2), 133-141 (2008).
57. Chen J., Yang T. and Cramer S.M. “Prediction of Protein Retention Times in Gradient Hydrophobic Interaction Chromatographic Systems” *J. Chromatogr. A*, 1177 (2008) 207–214
58. Yang T., Breneman C., and Cramer S.M., “Investigation of multi-modal high-salt binding ion-exchange chromatography using quantitative structure–property relationship modeling” *Journal of Chromatography A*, Volume 1175, Issue 1, Pages 96-105 (2007).
59. Chen J., Yang T., Luo ., Breneman C., and Cramer S.M. “Investigation of protein retention in hydrophobic interaction chromatographic (HIC) systems using the preferential interaction theory and quantitative structure property relationship models” *Reactive & Functional Polymers* 67: 1561–1569 (2007).
60. Yang T, Sundling M, Freed A, Breneman C. and Cramer, S.M. “Prediction of pH-Dependent Chromatographic Behavior in Ion-Exchange Systems”, *Anal. Chem.*; 79(23); 8927-8939. (2007).
61. Chen J and Cramer S.M. “Protein adsorption isotherm behavior in hydrophobic interaction chromatography”, *Journal of Chromatography A* 1165 (1-2): 67-77 (2007).
62. Yang, T.; Malmquist, G.; Cramer, S.M., “Evaluation of multi-modal high salt binding ion exchange materials,” *Journal of Chromatography A*, 1157(1-2): 171-177 2007
63. Ghose S, Hubbard B, Cramer SM, “Binding capacity differences for antibodies and Fc-fusion proteins on protein A chromatographic materials “ *Biotech. & Bioeng.* 96 (4): 768-779 (2007)
64. Chen J, Luo Q, Breneman CM, and Cramer, S.M., “Classification of protein adsorption and recovery at low salt conditions in hydrophobic interaction chromatographic systems, *Journal of Chromatography A* 1139 (2): 236-246 (2007).
65. Liu J, Park SK, Moore JA, Cramer SM, “Effect of displacer chemistry on displacer efficacy for a sugar-based anion exchange displacer library”, *Ind. & Eng. Chem. Res.* 45 (26): 9107-9114 (2006).
66. Liu J, Yang T, Ladiwala A, Cramer SM, Breneman CM , “High throughput determination and QSER modeling of displacer DC-50 values for ion exchange systems”, *Sep. Sci. Tech.* 41 (14): 3079-3107 2006
67. Teske CA, von Lieres E, Schroder M, Ladiwala A, Cramer SM, Hubbuch JJ, “Competitive adsorption of labeled and native protein in confocal laser scanning microscopy”, *Biotech. and Bioeng.* 95 (1): 58-66 SEP 5 2006

68. Ghose, S., Hubbard, B., Cramer S.M., "Evaluation and comparison of alternatives to Protein A chromatography Mimetic and hydrophobic charge induction chromatographic stationary phases" *J. Chromatogr. A*, 1122 (2006) 144–152
69. Ladiwala, A., Xia, F., Luo, Q., Breneman, C.N., Cramer, S.M., "Investigation of protein retention and selectivity in HIC systems using quantitative structure retention relationship models", *Biotech. and Bioeng.* 93(5), 836-850 (2006).
70. Rege K, Ladiwala A, Hu S, Breneman CM, Dordick JS, Cramer SM "Investigation of DNA-binding properties of an aminoglycoside-polyamine library using quantitative structure-activity relationship (QSAR) models, *J. of Chem. Information and Modeling.* 45 (6): 1854-1863 (2005).
71. Ghose S, Allen M, Hubbard B, Brooks C, Cramer SM, "Antibody variable region interactions with Protein A: Implications for the development of generic purification processes" *Biotech. And Bioeng.* 92 (6): 665-673 (2005).
72. Ladiwala A, Rege K, Breneman CM, Cramer SM, "A priori prediction of adsorption isotherm parameters and chromatographic behavior in ion-exchange systems", *PNAS* 102 (33): 11710-11715 (2005)
73. Tugcu N, Cramer SM, "The effect of multi-component adsorption on selectivity in ion exchange displacement systems", *J. Chromatogr. A* 1063 (1-2): 15-23 (2005).
74. Rege K, Ladiwala A, Cramer SM, "Multidimensional high-throughput screening of displacers", *Anal. Chem.* 77 (21): 6818-6827 (2005).
75. Xia F, Nagrath D, Cramer SM, "Effect of pH changes on water release values in hydrophobic interaction chromatographic systems", *J. Chromatogr. A* 1079 (1-2): 229-235 (2005).
76. Nagrath D, Bequette BW, Cramer SM, Messac A, "Multiobjective optimization strategies for linear gradient chromatography", *AIChE J.* 51 (2): 511-525 (2005).
77. Ghose S, Hubbard B, Cramer SM, "Protein interactions in hydrophobic charge induction chromatography (HCIC)", *Biotech. Progress.* 21 (2): 498-508 (2005).
78. Jindal R, Plawsky JL, Cramer SM, "Selective filling for patterning in microfluidic channels", *LANGMUIR* 21 (10): 4458-4463 (2005).
79. Jindal R., and Cramer S.M., "On-Chip Electrochromatography Using Sol-Gel Immobilized Stationary Phase With UV Absorbance Detection" *J. Chromatogr.* 1044 (1-2): 277-285 (2004).
80. Rege K., Hu S., Moore J., Dordick S., and Cramer S.M., "Chemoenzymatic Synthesis and High-Throughput Screening of an Aminoglycoside-Polyamine Library: Identification of High- Affinity Displacers and DNA-Binding Ligands" *JACS.* 126 (39): 12306-12315 (2004).
81. Nagrath D, Messac A, Bequette BW, and Cramer, S.M., "A hybrid model framework for the optimization of preparative chromatographic processes", *Biotech. Prog.*, 20 (1): 162-178 (2004).
82. Rege K, Ladiwala A, Tugcu N, Breneman, C. and Cramer, S.M., "Parallel screening of selective and high-affinity displacers for proteins in ion-exchange systems", *J. Chromatogr. A*, 1033 (1): 19-28 (2004).

83. Ghose S, Nagrath D, Hubbard B, and Cramer, S.M., "Use and optimization of a dual-flowrate loading strategy to maximize throughput in protein-A affinity chromatography *Biotech. Prog.* 20 (3): 830-840 (2004).
84. Xia F, Nagrath D, Garde S, Cramer, S.M. "Evaluation of selectivity changes in HIC systems using a preferential interaction based analysis", *Biotech. And Bioeng.* 87 (3): 354-363 (2004).
85. Ladiwala, A., Rege, K., Breneman, C. and Cramer, S.M., "Mobile Phase Salt Type Effects on Protein Retention and Selectivity in Cation Exchange Systems". *Langmuir* 19(20): 8443-8454 (2003).
86. Tugcu, N., Ladiwala, A., Breneman, C. and Cramer, S.M., "Identification of Chemically Selective Displacers using Parallel Batch Screening experiments and quantitative structure efficacy relationship models". *Anal. Chem.* 21: 5806-5816 (2003).
87. Tugcu, N., Song, M., Breneman, C. and Cramer, S.M., Prediction of the effect of mobile phase salt type on protein retention and selectivity in anion exchange systems, *Anal. Chem.* 75(14): 3563-3572 (2003).
88. Rege, K., Tugcu, N., Cramer, S.M., "Predicting column performance in displacement chromatography from batch screening experiments", *Sep. Sci. and Tech.* 38 (7): 1499-1517 (2003).
89. Sunasara K., Xia F., Gronke R.S. and Cramer S.M. "Application of Hydrophobic Interaction Displacement Chromatography for an Industrial Protein Purification", *Biotech and Bioeng.* 82 (3): 330-339 (2003).
90. Xia F, Nagrath D, Cramer SM. "Modeling of adsorption in hydrophobic interaction chromatography systems using a preferential interaction quadratic isotherm", *J. Chromatogr.* 989 (1): 47-54 (2003).
91. Tugcu N, Deshmukh RR, Sanghvi YS, et al. " Displacement chromatography of anti-sense oligonucleotide and proteins using saccharin as a non-toxic displacer". *React. Funct. Polym.* 54 (1-3): 37-47 2003
92. Nagrath D., Bequette B.W. and Cramer S.M. "Evolutionary Operation and Control of Chromatographic Processes" *AIChE.* 49(1): 82-95 (2003).
93. Tugcu N, Park SK, Moore JA, Cramer SM , Synthesis and characterization of high-affinity, low-molecular-mass displacers for anion-exchange chromatography *Ind. & Eng. Chem. Research.* 41 (25): 6482-6492 (2002).
94. Song MH, Breneman CM, Bi JB, Sukumar N, Bennett KP, Cramer S, Tugcu N, Prediction of protein retention times in anion-exchange chromatography systems using support vector regression *J. of Chem. Inf. And Comp. Sci.* 42 (6): 1347-1357 (2002).
95. Natarajan, V; Ghose, S; Cramer, SM, "Comparison of linear gradient and displacement separations in ion-exchange systems", *Biotechnol. Bioeng.*, 78 (4): 365-375 (2002).
96. Tugcu, N.; Mazza, C.; Breneman, C.; Sanghvi, Y.; Moore, J. and Cramer, S. M., High throughput screening and quantitative structure efficacy relationship models for designing displacers for anti-sense oligonucleotide purification in anion-exchange systems, *Sep. Sci. Technol*, 37 (7): 1667-1681, 2002.

97. Tugcu, N.; Bae, S.; Moore, J. A. and Cramer, S. M., The role of the stationary phase on the dynamic affinity of various low-molecular-mass displacers, *J. Chromatogr.* 954: pp 127-135, 2002.
98. Mazza C.B., Rege K., Breneman C., Sukumar N., Dordick J.S. and Cramer S.M. High Throughput Screening and Quantitative Structure-Efficacy Relationship Models of Potential Displacer Molecules for Ion Exchange Systems. *Biotech. And Bioeng.*, 80 (1): 60-72 (2002).
99. Mazza, C.B., Whitehead, C.E., Breneman, C.M. and Cramer, S.M., Predictive quantitative structure retention relationship models for ion-exchange chromatography, *Chromatographia*, 56 (3-4): 147-152 (2002).
100. Mazza, C. B.; Sukumar, N. and Breneman, C. M., et al. Prediction of protein retention in ion-exchange systems using molecular descriptors obtained from crystal structure, *Anal. Chem.*, 73 (22): 5457-5461, ( 2001).
101. Sunasara, K. M.; Rupp, R. G. and Cramer, S. M., Purification of recombinant brain derived neurotrophic factor using reversed phase displacement chromatography, *Biotechnol. Progr.*, 17 (5): 897-906, Sept. - Oct., 2001
102. Ghose, S. and Cramer, S. M., Characterization and modeling of monolithic stationary phases: application to preparative chromatography, *J Chromatogr. A*, 928 (1): 13-23 (2001).
103. Tugcu, N.; Deshmukh, R. R. and Sanghvi, Y. S., et al., Purification of an oligonucleotide at high column loading by high affinity, low-molecular-mass displacers, *J Chromatogr. A*, 923 (1-2): 65-73 July 20, 2001
104. Tugcu, N.; Moore, J. A. and Cramer, S. M., Synthesis and characterization of high-affinity, low molecular mass displacers for anion-exchange chromatography, submitted to *Industrial and Engineering Chemistry*.
105. Kalra, A.; Tugcu, N.; Cramer, S. M.; and Garde, S., Salting-in and salting-out of hydrophobic solutes in aqueous salt solutions, *J. Phys. Chem. B*, 105 (27), 6380 - 6386, 2001.
106. Shukla, A., Deshmukh, R., Moore, J. and Cramer, S.M., "Purification of oligonucleotides by high affinity, low molecular weight displacers", *Biotechnol. Prog.*, 16 (6), 1064-1070 Nov.-Dec., 2000
107. Shukla, A., Sunasara, K. M.; Rupp, R. G. and Cramer, S. M., Hydrophobic displacement chromatography of proteins", *Biotech and Bioeng*, 68 (6) 672-680, 2000.
108. Natarajan, V.; Bequette, B.W. and Cramer, S. M., Optimization of ion exchange displacement separations. I. Validation of an iterative scheme and its use as a methods development tool", *J Chromatogr. A*, 876 (1-2) 51-62 April 21, 2000
109. Natarajan, V. and Cramer, S. M., Optimization of ion exchange displacement separations. II. Comparison of displacement separation on various ion exchange resins, *J Chromatogr. A*, 876 (1-2) 63-73 April 21, 2000
110. Natarajan, V. and Cramer, S. M., A methodology for the characterization of ion-exchange resins, *Separ. Sci. Technol.*, 35 (11) 1719-1742, 2000.
111. Shukla, A. and Cramer, S. M., Bioseparations by displacement chromatography, in the *Handbook of Bioseparations*, Academic Press, 2000.

112. Mazza, C. and Cramer, S. M., Evaluation of lot-to-lot consistency in ion exchange chromatography, *J Liq. Chromatogr. Relat. Techno.*, 22 (11) 1733-1758, 1999
113. Natarajan, V. and Cramer, S. M., Modeling Shock Layers in Ion Exchange Displacement Chromatography, *AIChE J.* 45, 27-37, 1999.
114. Sane, S., Cramer, S. M. and Przybycien, T. M., "A holistic approach to protein secondary structure characterization using amide I B and raman spectroscopy, *Analytical Biochemistry* 269, 255-272, 1999.
115. Sane, S., Cramer, S. M. and Przybycien, T. M., "Protein structure perturbations on chromatographic surfaces", *Journal of Chromatography A* 849,149-159, 1999.
116. Cramer, S. M. and Natarajan, V., Chromatography, ion exchange, in the *Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Bioseparation*, 612-627, 1999.
117. Barnthouse, K. A.; Trompeter, W.; Jones, R. and Cramer, S. M., Ion-exchange displacement chromatography: Scale-up and displacer clearance for recombinant human brain-derived neurotrophic factor (rHuBDNF)", *BioPharm* 35-44, 1999.
118. Sunasara, K. M.; Cramer, S. M.; Hauer, C. R.; Rupp, R. G. and Shoup, V. A., Characterization of recombinant human brain-derived neurotrophic factor variants, *Archives of Biochemistry and Biophysics*, 372 ( 2), 248-260, 1999.
119. Natarajan, V. and Cramer, S. M., Modeling shock layers in ion exchange displacement chromatography, *AIChE J.* 45, 27-37, 1999.
120. Barnthouse, K. A; Trompeter, W; Jones, R.; Rupp, R. and Cramer, S. M., Ion-exchange displacement chromatography: Scale-up and displacer clearance for recombinant human brain-derived neurotrophic factor LC GC-mag, *Separation Sci.*, 17 (11) 1028-, Nov., 1999
121. Vunnum, S., Natarajan, V. and Cramer, S. M., Non-linear multicomponent gradient chromatography in IMAC systems, *Sep. Sci. and Tech.* 33 (16) 2465-2489, 1998.
122. Shukla, A. A; Bae, S. S. and Moore, J. A., et al., Synthesis and characterization of high-affinity, low molecular weight displacers for cation-exchange chromatography, *Ind. Eng. Chem. Res.*, 37 (10) 4090-4098, 1998.
123. Vunnum, S.; Natarajan, V. and Cramer, S. M., IMAC: Self-sharpening of protein-modulator interfaces in frontal chromatography, *J. Chromatogr.* 818 (1) 31-41, 1998.
124. Barnthouse, K. A.; Trompeter, W.; Jones, R.; Inampudi, P.; Rupp, R. and Cramer, S. M., Purification of recombinant human brain-derived neurotrophic factor (rHuBDNF) by cation-exchange displacement chromatography, *J. of Biotech.* 66 (2-3) 125-136, 1998.
125. Shukla, A. A.; Barnthouse, K. A.; Bae, S. S.; Moore, J. A. and Cramer, S. M., Structural characteristics of low molecular weight displacers for cation-exchange chromatography, *J. Chromatogr.* 814 (1-2), 1998.
126. Shukla, A. A; Bae, S. S.; Moore, J. A., and Cramer, S. M., Structural characteristics of low-molecular-mass displacers for cation-exchange

- chromatography - II. Role of the stationary phase”, *J. Chromatogr.*, 827 (2) 295-310, 1998.
127. Mazza, C.; Kundu, A. and Cramer, S. M., Preparative ion-exchange chromatography of challenging protein separations, *Biotech. Techniques*, 12 ( 2) 137-141, 1998.
  128. Goel, M.; Agrawal, V.; Kulkarni, A. K.; Cramer, S. M. and Gill, W. N., Stability and transport characteristics of reverse osmosis membranes using cyanide rinse waters, *Journal of Membrane Science* 141, 245-254, 1998.
  129. Kundu, A.; Vunnum, S. and Cramer, S. M., Displacement chromatography of proteins using low molecular weight anionic displacers, *Adsorption* 4 (3), 1998.
  130. Kundu, A.; Barnthouse, K. and Cramer, S. M., Selective displacement chromatography of proteins, *Biotech. and Bioeng.*, 56 (2), 119-129, 1997.
  131. Vunnum, S. and Cramer, S. M., IMAC: Non-linear elution chromatography of proteins, *Biotech. and Bioeng.*, 54 (4), 373-390, 1997.
  132. Kundu, A. Shukla, A., Barnthouse, K. Moore, J. and Cramer S. M., Displacement chromatography of proteins using sucrose octasulfate, *BioPharm* 10 (5), 64, 1997.
  133. Kundu, A. and Cramer, S. M., Low molecular weight displacers for high resolution protein separations, *Anal. Biochem.*, 248, 111-116, 1997.
  134. Gallant, S. and Cramer, S. M., Productivity and operating regimes in protein chromatography using low molecular weight displacers, *J. Chromatogr.*, 771, 9-22, 1997.
  135. Shukla, A., Hopfer R., Chakravarti, D., Bortell E. and Cramer, S.M. "Purification of an Antigenic Vaccine Protein by Selective Displacement Chromatography", *Biotech. Prog.* 14 (1998) 92-101.
  136. Gallant, S.; Vunnum, S. and Cramer, S.M., Modeling gradient elution of proteins in ion exchange chromatography, *AIChE J.*, 42, 2511-2520, 1996.
  137. Brooks, C. and Cramer, S.M., Solute affinity in ion exchange displacement chromatography, *Chem. Eng. Sci.*, 51, 3847-3860, 1996.
  138. Vunnum, S.; Gallant, S. and Cramer, S. M., Immobilized metal displacement chromatography: displacement characteristics of traditional mobile phase modifiers, *Biotechnology Progress*, 12, 87-91, 1996.
  139. Gallant, S.; Vunnum, S. and Cramer, S. M., Optimization of preparative ion-exchange chromatography of proteins-linear gradient separations, *Journal of Chromatography*, 725, 295-314, 1996.
  140. Buragohain, P.; Gill, W. N. and Cramer, S. M., Novel resin based ultrapurification system for reprocessing IPA in the semiconductor industry, *IEC Res.*, 35, 3149-3154, 1996.
  141. Kundu, A.; Vunnum, S.; Jayaraman, G. and Cramer, S. M., Protected amino acids as novel low-molecular-weight displacers in cation-exchange displacement chromatography, *Biotechnology and Bioengineering*, 48, 452-460, 1995.
  142. Kundu, A.; Vunnum, S. and Cramer, S. M., Antibiotics as low molecular weight displacers in ion exchange displacement chromatography, *Journal of Chromatography*, 707, 57-67, 1995.

143. Gallant, S.; Kundu, A. and Cramer, S. M., Optimization of step gradient separations - consideration of nonlinear adsorption, *Biotechnology and Bioengineering*, 47, 355-372, 1995.
144. Gallant, S.; Kundu, A. and Cramer, S. M., Modeling nonlinear elution of proteins in ion-exchange chromatography, *Journal of Chromatography*, 702, 125-142, 1995.
145. Vunnum, S.; Gallant, S.; Kim, Y. and Cramer, S. M., Immobilized metal affinity chromatography - modeling of nonlinear multicomponent equilibrium, *Chemical Engineering Science*, 50, 1785-1803, 1995.
146. Patrickios, C.; Gadam, S.; Hatton, T. A. and Cramer, S. M., Block methacrylic polyampholtes as protein displacers in ion-exchange chromatography, *Biotechnology Progress*, 11, 33-38, 1995.
147. Brooks, C. and Cramer, S. M., Investigation of displacer equilibrium properties and mobile phase operating conditions in ion exchange displacement chromatography, *Journal of Chromatography*, 693, 187-196, 1995.
148. Jayaraman, G.; Li, Y.; Moore, J. A. and Cramer, S. M., Ion-exchange displacement chromatography of proteins: dendritic polymers as novel displacers, *Journal of Chromatography*, 702, 143-155, 1995.
149. Gadam, S.; Gallant, S. and Cramer, S. M., Transient profiles in ion-exchange displacement chromatography, *AIChE Journal*, 41, 1676-1686, 1995.
150. Gerstner, J. A.; Bell, J. and Cramer, S. M., Gibbs free energy of adsorption of biomolecules in ion-exchange systems, *Biophysical Chemistry* 52, 97-106, 1994.
151. Kim, Y. and Cramer, S. M., Experimental studies in metal affinity displacement chromatography of proteins, *J. Chromatogr.* 686 ( 2), 193-203, 1994.
152. Gadam, S. and Cramer, S. M., Salt effects in anion exchange displacement chromatography - comparison of pentosan polysulfate and dextran sulfate displacers, *Chromatographia*, 39, (7/8), 409-418, 1994.
153. Mhatre, R.; Qian, R.; Krull, I. S., Gadam, S. and Cramer, S. M., Characterization of proteins separated by displacement chromatography using low angle laser light scattering photometry *Chromatographia*, 38, ( 5/6), 349-354, 1994.
154. Cramer, S. M. and Jayaraman, G., Preparative chromatography in biotechnology, *Current Opinions in Biotechnology* 4, 217-225, 1993.
155. Wu, D.; Cramer, S. M. and Belfort, G., Enzymatic resolution of a racemic mixture using a multiphase membrane bioreactor: experiments and model verification, *Biotech. Bioeng.* 41, 979-990, 1993.
156. Gallant, S. R.; Fraleigh, S. P. and Cramer, S. M., CMAC neural network applications in chromatography, *Chemometrics* 18, 41-57, 1993.
157. Jayaraman, G., Gadam, S. and Cramer, S. M., High affinity dextran based displacers for ion exchange displacement chromatography of proteins, *J. Chromatogr.* 630, 53-68, 1993.
158. Gadam, S.; Jayaraman, G. and Cramer, S. M., Characterization of high affinity dextran based displacers, *J. Chromatogr.* 630, 37-52, 1993.
159. Brooks, C.; Rosano, T. and Cramer, S. M., Preparative chromatographic purification of cyclosporine metabolites, *Clinical Chemistry* 39, 457-466, 1993.

160. Gerstner, J. and S. M. Cramer, Heparin as a non-toxic displacer for anion exchange displacement chromatography of proteins, *BioPharm* 5 (9), 42, 1992.
161. Brooks, C. and Cramer, S. M., Steric mass action ion exchange displacement profiles and Induced salt gradients, *AIChE Journal* 38 (12), 1969-1978, 1992.
162. Gerstner, J. and Cramer, S. M., Cation exchange displacement chromatography of proteins with protamine displacers: effect of induced salt gradients, *Biotech. Progress* 8, 540-545, 1992.
163. Gerstner, J.; Hamilton, R. and Cramer, S. M., Chromatographic membrane systems for high throughput gradient separations of biomolecules, *J. Chromatogr.* 596, 173-180, 1992.
164. Kim, Y. and Cramer, S. M., Metal chelate displacement chromatography of proteins, *J. Chromatogr.* 549, 89-99, 1991.
165. Cramer, S. M., Displacement chromatography, *Nature* 351, 251-252, 1991.
166. Freed, B. M.; Stevens, C.; Brooks, C.; Cramer, S. M.; Lampert, N. and Rosano, T. G., Assessment of the biological activity of cyclosporine metabolites using the human TURKAT cell line, *Transplant Proc.* 23, 980-981, 1991.
167. Cramer, S. M. and Subramanian, G., Recent advances in the theory and practice of displacement chromatography, *Sep. Purif. Methods*, 19 (1), 31-91, 1990.
168. Rosano, T. G.; Brooks, C. A.; Dybus, M. T.; Cramer, S. M.; Stevens, C. and Freed, B. M., Selection of an optimal assay method for monitoring cyclosporine therapy, *Transplant Proc.* 22 (3) 1125-1128, 1990.
169. Wu, D.; Belfort, G. and Cramer, S. M., Enzymatic resolution with a multiphase membrane bioreactor: a theoretical analysis, *I & EC Research*, 29, 1612-1621, 1990.
170. Subramanian, G.; Phillips, M. W.; Jayaraman, G. and Cramer, S. M., Displacement chromatography of biomolecules with large particle diameter systems, *J. Chromatogr.*, 484, 225, 1989.
171. Subramanian, G. and Cramer, S. M., Displacement chromatography of proteins under elevated flow rate and crossing isotherm conditions, *Biotech. Progress* 5 (3), 1989.
172. Cramer, S. M. and Horvath, Cs., Peptide synthesis and deamidation with chemically modified immobilized carboxypeptidase Y., *Enzyme Microb. Technol.* 11 (2), 74, 1989.
173. Cramer, S. M. and Horvath, Cs., Peptide synthesis with immobilized carboxypeptidase Y, *Biotechnol. Bioeng.* 33, 344, 1989.
174. Phillips, M. W., Subramanian, G. and Cramer, S. M., A theoretical optimization of operating parameters in non-ideal displacement chromatography, *J. Chromatogr.*, 454, 1, 1988.
175. Subramanian, G., Phillips, M. W. and Cramer, S. M., Displacement chromatography of biomolecules, *J. Chromatogr.*, 439, 341, 1988.
176. Cramer, S. M. and Horvath, Cs., Displacement chromatography in peptide purification, *Prep. chromatogr.*, 1, 29, 1988.
177. Cramer, S. M.; Rassi, Z. and Horvath, Cs., Tandem use of carboxypeptidase Y reactor and displacement chromatograph for peptide synthesis, *J. Chromatogr.*, 394, 305, 1987.



178. Cramer, S. M., Nathanael, B. and Horvath, Cs., High-performance liquid chromatography of deferoxamine and ferrioxamine: interference by iron present in the chromatographic system, *J. Chromatogr.*, 285, 404, 1984.
179. Cramer, S. M.; Schornagel, J. H.; Kalghatgi, K.; Bertino, J. R. and Horvath, Cs., Occurrence and significance of D-methotrexate as a contaminant of commercial methotrexate, *Cancer Res.*, 44, 1843, 1984.
180. Pitt, A. M.; Cramer, S. M.; Czernicki, A. B., Kalghatgi, K.; Horvath, Cs. and Solomon, B. A. Anisotropic membranes with carboxypeptidase G1, *Appl. Biochem. Biotech.*, 8, 55, 1983.

## **Patents**

US Patent # 5,478,924 “Displacement Chromatography of Proteins using Low Molecular Weight Displacers”, S. M. Cramer, J. A. Moore, A. Kundu, Y. Li, G. Jayaraman. (1995).

US Patent # 5,606,033 “Displacement Chromatography of Proteins using Low Molecular Weight Anionic Displacers”, S. M. Cramer, J. A. Moore, A. Kundu, Y. Li, G. Jayaraman. (1997).

US Patent # 6,239,262 “Low Molecular Weight Displacers for Protein Purification in Hydrophobic Interaction and Reversed Phase Chromatographic Systems”, S. M. Cramer, A. Shukla, and K. Sunasara. (2001).

US Patent #6,573,373 “High Affinity, Low Molecular Weight Displacers for Oligonucleotide Purification”, S. M. Cramer, A. Shukal, R. Deshmukh, J. Moore. (2003).

US Patent #6,828,436 “Method for selecting a high affinity, low molecular weight displacer for oligonucleotide purification”, A. Shukla, R. Deshmukh, S. M. Cramer, and J. Moore. (2004).

US Patent #6,881,540 B2 “High Throughput Screening of Potential Displacer Molecules”, S. M. Cramer, K. Rege, J. Dordick (2005).

US Patent #6,929,747 “High-affinity, low-molecular-mass displacers for ion-exchange chromatography”, J. Moore, S.M. Cramer, N. Tugcu, S. K. Park (2005).

US Patent #7,189,324 “High-affinity, low-molecular-mass displacers for ion-exchange chromatography”, S.M. Cramer, J. Moore, S. K. Park, N. Tugcu, (2007).

US Patent #7,439,343 "Aminoglycoside-polyamine displacers and methods of use in displacement chromatography" S.M. Cramer K. Rege, J. Dordick,etc.. (2008).

US Patent #20,150,258,539 “Mixed Mode Ligands” Steven Cramer, James Woo, Hong Chen, Jiali Liao, Russell Frost (2015).

### **Books (Article)**

Shukla, A., and Cramer, S. M., "Bioseparations by Displacement Chromatography", in "Handbook of Bioseparations", Vol.1, S. Ahuja (ed.), Academic Press (2000).

Cramer, S.M. and Natarajan, V., "Chromatography, Ion Exchange", in Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Bioseparations", M" Flickinger and S. Drew (eds.), Wiley (1999).

S. M. Cramer and C. A. Brooks, "Ion Exchange Displacement Chromatography of Proteins: Theoretical and Experimental Studies", in "Chromatography in Biotechnology", C. Horvath and L. S. Ettre (eds.), ACS Symposium Series, 529 (1993).

C. Patrickios, T. A. Hatton, W. R. Hertler and S. M. Cramer, "Chromatographic Characterization of Arcylic Polyampholytes", Polymer preprints, Vol. 34 (1) p. 1074 (1993).

S. M. Cramer, "Displacement Chromatography of Proteins: Practical Considerations", in Methods in Molecular Biology, Vol. II: "Practical Protein Chromatography", A. Kenney, ed., Human Press Inc. (1992)

S. M. Cramer and G. Subramanian, "Preparative Liquid Chromatography of Biomolecules - New Directions", in "New Directions in Sorption Technology", G. Keller and R. Yang, eds., Butterworth (1989).

S. M. Cramer, G. Subramanian and M. W. Phillips, "Chromatographic Separation Processes", in Chemical Engineering Education in a Changing Environment", S. Sandler and B. Finlayson, eds., United Engineering Trustees, Inc. (1988).

### **International Proceedings**

S. M. Cramer, "Recent Advances in the Theory and Practice of Displacement Chromatography", Journal of Cellular Biochemistry, Supplement 14D, 1990, Abstract K 201, 19th Annual UCLA Symposia.

S. M. Cramer, "Displacement Chromatography of Biopolymers", Journal of Cellular Biochemistry, Supplement 14D, 1990, Abstract K 015, 19th Annual UCLA Symposia.

S. M. Cramer and Cs. Horvath, "Peptide Synthesis with Immobilized Carboxypeptidase Y", Proceedings 4th European Congress on Biotechnology, Volume 2, Neijssel, A. M., Van Der Meer, R. R., Luyben, K.Ch.A.M., eds., 1987.

### **Keynote or Plenary Addresses:**

- 2013 Keynote at the 2nd European Congress of Applied Biotechnology World Forum  
The Hague, The Netherlands
- 2012 Keynote at PepTalk 2012  
Keynote at MSS 2012 Monolith Conference in Slovenia.  
Keynote at SPICA meeting in Brussels, Belgium.  
3 Invited Keynotes in Japan
- 2011 Keynote address at the High Throughput Process Development meeting in Krakow.
- 2008 Featured speaker at the Connecticut Chromatography Council  
Keynote Presentation at the International Society for Peptide and Protein Purification
- 2007 Plenary lecture at the Cell Culture Meeting  
Keynote at the International Ion Exchange Conference  
Michaels Award Address at the ACS Meeting
- 2005 First honorary seminar in a new distinguished scientist lecture series at Centocore
- 2004 Keynote address at the joint Indian and US Chemical Engineering meeting in Mumbai  
India.  
First honorary seminar in a new distinguished scientist lecture series at Genzyme
- 2003 Keynote address at the International Society for the Separation of Peptides and  
Proteins in Aachen Germany.  
Honorary speaker at the Central New England Chromatography Council
- 2002 Plenary address at the Taiwan-Japanese joint conference on Separations Technology.
- 1992 Honorary Focus Address at the Minnesota Chromatography Forum

## **RESEARCH GRANTS**

GOALI: Collaborative Research: Industrial Implementation of Smart Biopolymers for Purification of Biolog, \$300,000 (NSF), 9/14-9/17

Integrated and Scalable Cyto-Technologies (InSCyT), DARPA, \$1,000,000 (RPI part, note this is part of major MIT lead grant), 6/25/13-6/25/15

Purification of HCPs from mAbs using multimodal chromatography, \$200,000 (MedIMMune), 9/13-9/15

Investigation of Protein Binding and Selectivity in Novel Bio-Rad Multimodal Systems, Biorad, \$200,000, 9/13-9/15

Affinity Reagents for Purification of Therapeutic Enzymes, Genzyme, CO-PIs Steve Cramer and Pankaj Karande, \$150,000, 4/13-4/14

Proposal for Group Travel Grant for 8th Eastern Mediterranean Chemical Engineering Conference for Collaborative Research, NSF, \$49,000, 1/13-1/15.

Molecular Engineering of Multiple Weak Interactions for High Selectivity Bioseparations, NSF, PI Steve Cramer, \$388,000, 10/12-10/15.

Purification of FC fusion proteins by multimodal chromatography, Pfizer, PI Steve Cramer, \$50,000, 1/12-1/13.

Two dimensional microfluidic platform for rapid DNA separation by fragment length, NIH, PI Linda McGown, S. Cramer co-PI, \$562,000, 8/12- 7/15.

High Selectivity and Capacity Bioseparations using Hybrid Displacement-Multimodal Chromatography, NSF, PI Steve Cramer, Co-PI Shekhar Garde, \$300,000, 9/11-9/14.

Effect of mobile phase modifiers on product quality in multimodal systems, NovoNordisk PI Steven Cramer, \$250,000. 8/11-8/15

Use of high throughput screening for quality by design, Meddimune.  
PI Steven Cramer, \$200,000. 5/11-5/13

GOALI: Industrial Implementation of Smart Biopolymers for Antibody Purification, NSF, PI Wilfred Chen, Co-PI Steve Cramer \$209,222, 9/09- 9/12.

Improving the Understanding and Application of Multi-Modal Chromatography, NSF, PI Steve Cramer, Co-PI Shekhar Garde and Curt Breneman, \$300,000, 9/09-9/12.

The Use of Multimodal Chromatography for the Purification of Fusion Proteins, Glaxo Smith Kline, PI Steven Cramer, 100 % responsibility, \$200,000. 6/09-6/13.

Predicting Protein Chromatographic Behavior in HA Systems, Biorad, \$190,000, PI Steve Cramer, 100 % responsibility, funding period 10/08-10/13.

New Generation of Lab on Chip Separators based on Independent Fluid and Analyte Control, NSF PI Dimitar Petsev, co-PIs Orlin Velez and Steve Cramer, 15% responsibility, 7/08-7/12, \$337,000.

High Selectivity and Capacity Bioseparations using Hybrid Displacement-Multimodal Chromatography, NSF, PI Steve Cramer, Co-PI Shekhar Garde, \$300,000, 9/11-9/14.

Effect of mobile phase modifiers on product quality in multimodal systems, NovoNordisk PI Steven Cramer, \$250,000. 8/11-8/15

Use of high throughput screening for quality by design, Meddimune.  
PI Steven Cramer, \$200,000. 5/11-5/13

The Use of Multimodal Chromatography for the Purification of Fusion Proteins, Glaxo Smith Kline, PI Steven Cramer, \$200,000. 6/09-6/11.

GOALI: Industrial Implementation of Smart Biopolymers for Antibody Purification, NSF, PI Wilfred Chen, Co-PI Steve Cramer \$418,444, 9/09- 9/12.

Improving the Understanding and Application of Multi-Modal Chromatography, NSF, PI Steve Cramer, Co-PI Shekhar Garde and Curt Breneman, \$300,000, 9/09-9/12.

Predicting Protein Chromatographic Behavior in HA Systems, Biorad, \$50,000, PI Steve Cramer, funding period 10/08-10/09.

Protein Unfolding in Chromatographic Systems, Novo Nordisk, \$100, 000, PI Steve Cramer, funding period 9/07-9/09

Chemically Selective Displacers for Protein Purification, NSF, PI Steve Cramer, co-PI Jim Moore, funding period 9/07-9/10, \$270,000.

Travel Grant for Fifth Mediterranean Chemical Engineering Conference for Collaborative Research (\$68,000), 10/01/07-9/30/09.

Selective Filling of Nanostructured Packings for Chromatographic Chip System, NSF, PI Steve Cramer, co-PI Joel Plawsky, funding period 8/05-8/08, \$327,000.

Travel Grant for Fourth Mediterranean Chemical Engineering Conference for Collaborative Research (\$68.995), 10/01/05-9/30/07.

Prediction and Understanding of Protein Affinity, NSF, PI Steve Cramer, Co-PI's C. Breneman, S. Garde, and R. Kane, funding period 9/04-9/08, \$1,600,000.

NIRT: Miniaturized Chemical Sensors Featuring Electrical Breakdown near Carbon Nanotube Tips, NSF, PI N. Koratkar, Co-PI's P Ajayan, T. Borca-Tasciuc, S. Cramer and S. Nayak, funding period 9/04-9/08, \$1,300,000.

Low Molecular Weight Displacers for Protein Purification, National Institute of Health, P.I., S. Cramer; Co-PI's J. Moore, C. Breneman, and J. Dordick, funding period 5/1/03-5/1/07 \$1,264,000 direct costs.

Protein Bioprocessing with Hydrophobic Separations Media, NSF, PI Todd Przybycien, Co-PI's, Steven Cramer, Curt. Breneman, Erik Fernandez, John O'Connell. Funding period 9/02-9/07, \$2,000,000.

Quantitative Combinatorial Design of Displacers and Affinity Ligands, NSF, PI Steven Cramer; Co-PI's Jon Dordick, and Curt. Breneman, funding period 12/00-1/04, \$703,000.

Hydrophobic Displacement Chromatography of Proteins, NSF, PI Dr. Steven Cramer, funding period 1/99-7/02, grant award \$240,000.

Low Molecular Weight Displacers for Protein and Oligonucleotide Purification, National Institute of Health, P.I. Steven Cramer, Co-PI James Moore, funding period 12/1/1999 -1/03, \$641,934.

Optimization of Preparative Ion Exchange Chromatography, NSF, PI Dr. Steven Cramer and Dr. Wayne Bequette, funding period 10/1/98-10/1/00, grant award \$160,000.

Hazardous Source and Waste Reduction in Metals Finishing Industry by Hybrid Membrane Separation Systems, NYSERDA, Co-P.I.'s, S. Cramer and W. Gill, funding period 1/95 - 1/99, grant award \$269,000.

Purification of Complex Biological Mixtures by Displacement Chromatography, Lederle Praxis., grant award \$30,000.

"Purification of Complex Biological Mixtures by Displacement Chromatography", Regeneron Pharmaceuticals, funding period 5/96-6/99, grant award \$100,000.

Low Molecular Weight Displacers for Protein Purification, National Institute of Health, Co-P.I.'s Dr. Steven Cramer and Dr. James Moore, funding period 9/1/95 - 9/1/98, grant award \$557,898.

Reversed Phase Purification of Antibiotics, Pfizer, funding period 1/98-12/99, grant award \$24,251

Purification of Oligonucleotides, ISIS Pharmaceuticals, \$40,000.

Hydrophobic Displacement Chromatography, Biogen, \$81,375.

Optimal Design of Stationary Phase Materials, Pharmacia, \$30,000.

"Purification of Complex Biological Mixtures by Displacement Chromatography", Regeneron Pharmaceuticals, funding period 5/96-6/97, grant award \$50,000.

"Reproducibility of Preparative Ion Exchange Materials", Pharmacia, funding period 5/96-6/97, grant award \$15,000.

Optimization of Preparative Ion Exchange Chromatography, PI Dr. Steven Cramer (consultant Dr. Wayne Bequette), funding period 8/15/95-8/15/98, grant award \$189,473.

Engineering Research Equipment: An Infrared Spectrometer for the Analysis of Protein - Surface Interactions, Co PI's T. M. Przybycien, G. Belfort, and S. M. Cramer, grant award \$32,000.

Hazardous Source and Waste Reduction in Metals Finishing Industry by Hybrid Membrane Separation Systems, NYSERDA, Co-P.I.'s, S. Cramer and W. Gill, funding period 1/95 - 1/97, grant award \$219,000.

Low Molecular Weight Displacers for Protein Purification, National Science Foundation, CO-P.I.'s Dr. Steven Cramer and Dr. James Moore, funding period 10/1/94 - 10/1/95, grant award \$124,650.

"Novel Supports for Displacement Chromatography", Pharmacia AS, P.I., Dr. Steven Cramer, funding period, 4/93 - 8/95, grant award \$54,350.

"Chromatographic Purification of Isopropyl Alcohol", NYSERDA, Co-P.I.'s, S. Cramer and W. Gill, funding period 2/1/94 - 6/1/95, grant award \$100,000.

Presidential Young Investigator, National Science Foundation, funding period 10/1/89 - 10/1/94, total grant award \$312,500.

"Optimization and Scale-Up of Displacement Chromatography", Millipore Corporation, P.I., Dr. Steven M. Cramer, funding period 9/1/89 - 9/1/94, grant award \$120,000.

"Displacement Chromatography of Proteins: Development of Pentaerythritol-Based Displacers", National Science Foundation, CO-P.I.'s Dr. Steven Cramer and Dr. James Moore, funding period 9/91 - 9/93, grant award \$160,000.

"Purification of IgG's from Whey", Immucell Corp., P.I., Dr. Steven Cramer, funding period 1/93 - 1/94, grant award \$8,000.

"Preparative-Scale Separation of Biopolymers by Displacement Chromatography", National Science Foundation, P.I., Dr. Steven M. Cramer, funding period, 6/1/87 - 10/30/89, grant award \$70,000.

"Lilly Teaching Fellowship", funding period 7/1/89 - 7/1/90, grant award, \$6,500.

"Dow Chemical Company Excellence in Teaching Award", grant award, \$10,000.

"Multicomponent Adsorption Behavior of Biopolymers on Chromatographic Surfaces", The Petroleum Research Fund, American Chemical Society, P.I., Dr. Steven M. Cramer, funding period 1/88 - 1/90, grant award \$18,000.

"Identification and Isolation of Cyclosporine Metabolites Associated with Nephrotoxicity and Immunosuppression", with Albany Medical College, National Institute of Health, funding period 7/88 - 7/91, total grant award \$390,000. Dr. Cramer's award, \$100,000.

"Purification of Complex Biological Mixtures by Displacement Chromatography: Extension to Group Specific Affinity Supports", National Science Foundation, P.I. Dr. Steven M. Cramer, funding period, 2/1/90 - 7/31/92, grant award \$60,000.

"Purification of Proteins", Eastman Kodak, P.I., Dr. Steven M. Cramer, funding period 9/1/89 - 9/1/92, grant award \$30,000.

"Downstream Bioprocessing with Novel Membrane Systems", Hoechst Celanese, P.I. Dr. Steven M. Cramer, funding period 1/90 - 9/92, grant award \$20,000.

"Purification of Therapeutic Proteins using Group Specific Affinity Displacement Chromatography", Abott Biotech, Inc., P.I. Dr. Steven Cramer, funding period 9/91 - 9/92, grant award \$28,000.

### EQUIPMENT GRANTS

Biocad automated Chromatography System- Isis  
Akta Automated Chromatography System- Amersham Pharmacia  
Diode Array Detector, Protein Design Labs  
Autoprep 500 Preparative Chromatographic System, Millipore Corporation.  
Deltaprep Preparative Chromatographic System, Millipore Corporation.  
FPLC Preparative Chromatographic System, Pharmacia-LKB Corporation.  
Automated Electrophoresis System, Pharmacia - LKB Corporation.

### **Thesis supervision**

#### **Masters Thesis**

Stuart Gallant: "Neural Network Applications in Chromatography", May 1991.

Regina Mesquita: "Purification of Natural Products by Displacement Chromatography", completed 1992

Partha Burogohain: "Ultrapurification of Isopropyl alcohol", completed 1995.

Khurram Sunasara: "High Temperature Reversed Phase Displacement Chromatography of BDNF", completed 1997.

Manish Goel: "Hybrid Membrane Processes for Metal Plating Waste Recycling". conferred September 1995.

Vikash Agrawal: "Hybrid Membrane Processes for Metal Plating Waste Recycling". Completed in 1998.



Sanchayita Ghoses: "Modeling of Novel Chromatographic Media", completed 2001.

A. Kapur: "High Throughput Affinity Separations for Identification of Transcription Factors", completed 2002.

Pinar Tabur: "Multicomponent Adsorption Studies in Protein and Displacer Affinity", completed 2003.

J. Keba: "Rapid Methods Development using AKTA Express", completed 2006.

### **Doctoral Theses**

Guhan Subramanian: "Displacement Chromatography of Biomolecules", degree conferred May 1990.

Michael Phillips: "Optimization and Scale-Up of Displacement Chromatography", degree conferred May 1991.

Dauh-Rung Wu: "Multiphase Enzyme Reactors for Organic Synthesis", conferred December 1991.

Clayton Brooks, III: "On the Characterization of Equilibrium in Ion Exchange Chromatography: Steric Mass Action Ion Exchange", conferred August 1993.

Guhan Jayaraman: "Ion-Exchange Displacement Chromatography of Proteins: Heuristic Approaches to Displacer Design", conferred August 1993.

Joseph Gerstner: "Membrane Chromatography", conferred May 1993.

Young Kim: "Metal Affinity Displacement Chromatography of Protein", conferred May 1993.

Shishir Gadam: "Characterization of Non-Linear Adsorption Properties of Biopolymers", conferred May 1994.

Stuart Gallant: "Control of Optimization of Displacement Systems", conferred, 1995.

Amitava Kundu: "Low Molecular Weight Displacers for Protein Purification in Ion Exchange Systems", conferred July 1996.

Suresh Vunnum, "Non-Linear Protein Separations by Immobilized Metal Affinity Chromatography", conferred, December 1996.

Kristopher Barnthouse, "Purification of Complex Biological Mixtures by High Throughput Chromatography", conferred 1997.

Abhinav Shukla "Novel Low Molecular Weight Displacers", conferred 1999.

Venkatesh Nataragan "Optimization of Preparative Ion Exchange Chromatography", conferred 1999.

Samir Sanes "RAMAN and FTIR for evaluation of protein conformation on chromatographic surfaces", conferred 1999.

Cecilia Mazza " Quantitative-structure based predictions of displacer and protein affinities in chromatographic systems", conferred 2001.

Khurram Sunasara "Hydrophobic Displacement Chromatography", conferred 2002.

Nihal Tugcu "Design of high affinity low molecular weight displacers and investigation of the selectivity of displacers and proteins in ion exchange systems", conferred 2003.

Kaushal Rege "Chemoenzymatic synthesis, high-throughput screening, and structure-property relationships of novel bioseparation and DNA-binding agents", conferred 2004.

Fang Xia "Hydrophobic interaction chromatography: Displacement modeling, process development, selectivity investigation, and displacer design", conferred 2002.

Deepak Nagrath: "Evolutionary design, multiobjective optimization and control of chromatographic processes", conferred 2003.

A. Ladiwala: "A multiscale investigation of protein affinity and displacer efficacy in chromatographic systems using quantitative structure-property relationship modeling", conferred 2005.

Rohit Jindal "Selective filling for patterning in microfluidic channels and integration of chromatography in 'lab-on-a-chip' devices using sol-gel technology" conferred 2005.

Sanchayita Ghose: "Purification of monoclonal antibodies and Fc-fusion proteins: Protein A and beyond" conferred 2005.

J. Liu: "High affinity and selective displacers for high resolution protein separations in ion-exchange chromatography: displacer ranking, mechanism elucidation, and displacer design", conferred 2006.

T. Yang: "Protein Binding Affinity in Ion Exchange Chromatography and Quantitative Structure Property Relationship Modeling for Chromatographic Systems and Proteomics" conferred 2007.

Jie Chen: “Investigation of protein adsorption in hydrophobic interaction chromatographic (HIC) systems”, conferred 2007.

S. Gowami: “Chip electrochromatographic systems: novel vertically aligned carbon nanotube and silica monoliths based separations”, conferred 2009.

W. Chung: “An investigation into the effects of protein surface modifications on protein binding affinity in ion exchange and multimodal chromatography”, conferred 2009.

S. Evans: “Displacement chromatography for proteomic applications”, conferred 2009.

A. Freed: “Multiscale modeling of chromatographic systems”, conferred 2010.

C. Morrison: “Automated high throughput screening of selective displacers”, conferred 2010.

M. Levy: “Selective filling for creating novel chromatographic chip systems”, conferred 2010.

Y. Hou: “QSPR Models of homologous protein libraries”, conferred 2011.

M. Holstein: “Variant libraries for evaluation of multimodal chromatography”, conferred 2012.

R. Sheth: “Smart biopolymers for affinity precipitation of antibodies”, conferred 2014.

S. Parimal: “Molecular simulations of protein surface interactions”, conferred 2014.

J. Woo: “Affinity peptides for enzyme therapeutic separations”, conferred 2015.

K. Srinivasan: “Hybrid displacement multimodal separations”, conferred 2015.

B. Keller: “Quality by design considerations in scale up and scale down of bioprocessing”, conferred 2016.

S. Karkov: “Purification of fusion proteins using optimal multimodal systems”, conferred 2016.

S. Timmick: “Biomanufacturing in a box”, in process.

S. Banerjee: “Molecular simulations of multimodal chromatographic systems”.

C. Goodwin: “Novel affinity peptides for protein purification”, in process.,

Swarnim Ranjan: “Biophysical investigation of HCP interactions with MM Systems”, in process.

Julie Robinson: “Novel Multimodal Ligands for Bioprocessing”

Nickolas Vecchiarello, “New Classes of Bioprocesses for next generation biologics”

Akshat Mullerpatan: “Smart biopolymer peptide systems for affinity precipitation capture of proteins”.

Ronak Gudhka: Biophysics of Protein Interactions

Camille Bilodeau: Molecular simulations of protein interaction systems

Siddharth Parasnavis: Multimodal Separations of Bispecific Antibodies

Ronit Ghosh: Affinity Sensors for Evaluation of Bioproduct Quality

### **ACTIVITIES**

Accomplished jazz and classical pianist.