



## Contents

### Foreword

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#### Foreword

John R. Engen, Thomas J.D. Jørgensen

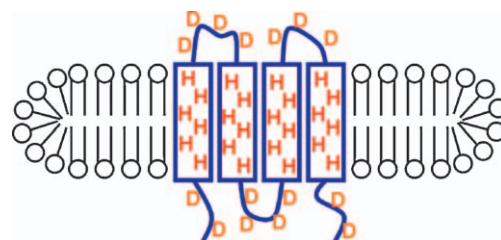
### Regular articles

3–11

#### Hydrogen/deuterium exchange mass spectrometry and optical spectroscopy as complementary tools for studying the structure and dynamics of a membrane protein

Yan Pan, Leonid Brown, Lars Konermann

Hydrogen/deuterium exchange mass spectrometry provides insights into structural and dynamic aspects of the membrane protein bacteriorhodopsin under various biophysical conditions.

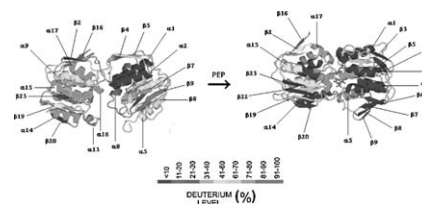


12–18

#### Hydrogen/deuterium exchange mass spectrometry for characterizing phosphoenolpyruvate-induced structural transitions in *Mycobacterium tuberculosis* 5-enolpyruvylshikimate-3-phosphate synthase (EC 2.5.1.1)

Alessandra Vaso, Diógenes S. dos Santos, Luis Augusto Basso, Mario S. Palma

H/D exchange monitored by ESI-MS and MS/MS was used to investigate PEP-induced conformational changes in *Me*EPSPS.

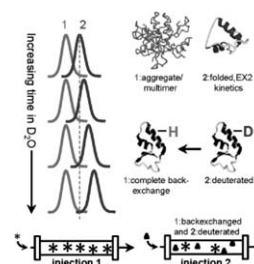


## 19–25

**False EX1 signatures caused by sample carryover during HX MS analyses**

Jing Fang, Kasper D. Rand, Penny J. Beuning, John R. Engen

Isotope patterns resembling real EX1 signatures in hydrogen exchange mass spectra may be confused with false EX1 arising from experimental conditions.

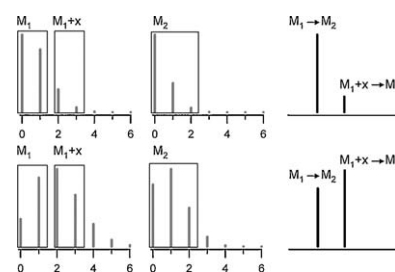


## 26–35

**MRM methods for high precision shift measurements in H/DX-MS**

Andrew J. Percy, David C. Schriemer

Multiple reaction monitoring provides a sensitive and targeted means of monitoring mass shifts in H/D exchange experiments.

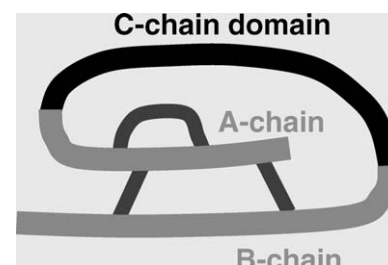


## 36–43

**Conformational transmission in proinsulin and its derivatives: A study using H/D exchange**

Hooria Younas, Qurra-tul-Ann Afza Gardner, Naeem Rashid, J. Neville Wright, Muhammad Akhtar

The length of the C-peptide (black) can subtly influence the conformation within the insulin core (red) of proinsulin.

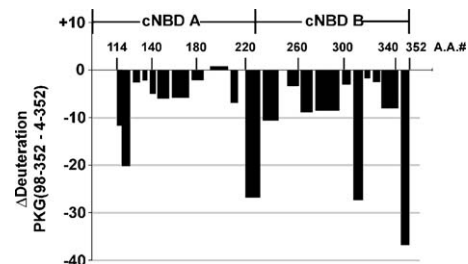


## 44–52

**The amino terminus of cGMP-dependent protein kinase Iβ increases the dynamics of the protein's cGMP-binding pockets**

Jun H. Lee, Sheng Li, Tong Liu, Simon Hsu, Choel Kim, Virgil L. Woods Jr., Darren E. Casteel

► The N-terminus of PKG Iβ modulates cGMP-affinity in its cyclic nucleotide binding pockets. ► The N-terminus of PKG Iβ increases the dynamics of the nucleotide binding pockets. ► The increased dynamics may account for the increased cGMP-affinity.

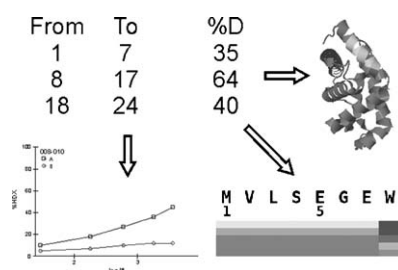


## 53–58

**MSTools—Web based application for visualization and presentation of HXMS data**

Daniel Kavan, Petr Man

► MSTools are a web based platform for visualization of HXMS data. ► Several different modules aid in creation of various graphical outputs. ► Figures showing peptide map, plots for deuterium uptake over time, known 3D structure colored according to HXMS, or a heat map can be easily created using a simple text file.

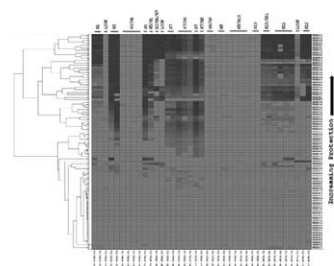


## 59–68

**Methods for the analysis of high precision differential hydrogen–deuterium exchange data**

Michael J. Chalmers, Bruce D. Pascal, Scooter Willis, Jun Zhang, Stephen J. Iturria, Jeffery A. Dodge, Patrick R. Griffin

► Evaluation of a workflow for the cross comparison of differential HDX datasets. ► Tukey multiple comparison of HDX data. ► Evaluation of the precision of an automated HDX platform across 127 datasets.

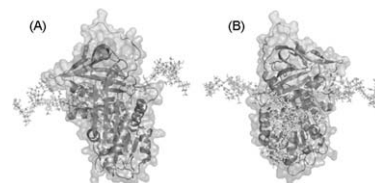


## 69–75

**Effects of glycosylation on the stability and flexibility of a metastable protein: The human serpin  $\alpha_1$ -antitrypsin**

Anindya Sarkar, Patrick L. Wintrode

► First study of the dynamics of a glycosylated serpin. ► Glycosylation stabilizes the compact denatured state but not the native state. ► Glycans perturb local dynamics but flexibility in important areas is retained.

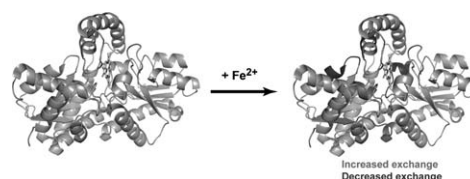


## 76–84

**Analysis of human ferrochelatase iron binding via amide hydrogen/deuterium exchange mass spectrometry**

Awuri P. Asuru, Laura S. Busenlehner

► Structural response of ferrochelatase to iron gives insight into heme synthesis. ► Iron-responsive channel from surface of enzyme towards the active site identified. ► Iron does not induce structural changes around the key catalytic residue His263.

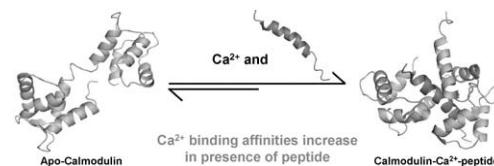


## 85–92

### Hydrophobic peptides affect binding of calmodulin and $\text{Ca}^{2+}$ as explored by H/D amide exchange and mass spectrometry

Justin B. Sperry, Richard Y-C. Huang, Mei M. Zhu, Don L. Rempel, Michael L. Gross

► Capabilities of MS-based PLIMSTEX illustrated for calmodulin-peptide binding. ► Digestion of calmodulin following HDX reveals binding at component peptide level. ► The presence of peptide ligands increases  $\text{Ca}^{2+}$  binding affinity to calmodulin. ► Similar HDX patterns for binding of three peptides suggest common structures.



## 93–100

### Conformational studies of the robust 2-Cys peroxiredoxin *Salmonella typhimurium* AhpC by solution phase hydrogen/deuterium (H/D) exchange monitored by electrospray ionization mass spectrometry

Sasidhar Nirudodhi, Derek Parsonage, P. Andrew Karplus, Leslie B. Poole, Claudia S. Maier

► First HX-MS studies of the robust peroxiredoxin, *StAhpC*, and two mutants in which the Thr-77 was substituted by isoleucine, a decamer-disruptive mutation, or valine, a decamer-promoting mutation. ► Global HX-MS studies indicate that disulfide reduction causes a reduction in overall conformational stability. ► HX-MS at the peptide level demonstrate enhanced conformational mobility in the peroxidatic active site of loop as a consequence of disulfide formation. ► HX-MS studies reveal allosteric interaction between the mutations in the dimer-dimer interface and the active site loop.

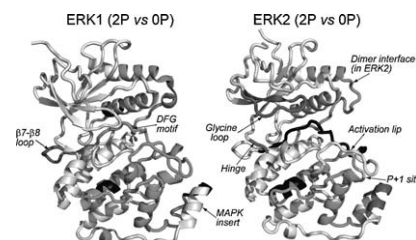


## 101–109

### Distinct patterns of activation-dependent changes in conformational mobility between ERK1 and ERK2

Adam Y. Ring, Kevin M. Sours, Thomas Lee, Natalie G. Ahn

► Hydrogen-exchange mass spectrometry (HX-MS) measures protein conformational mobility. ► Inactive vs active forms of the MAP kinase, ERK1, are compared by HX-MS. ► Kinase activation affects conformational mobility differently in related MAP kinases. ► Interdomain closure is constrained prior to activation of ERK2, but not ERK1. ► MAP kinases have distinct mechanisms for activation via control of protein motions.

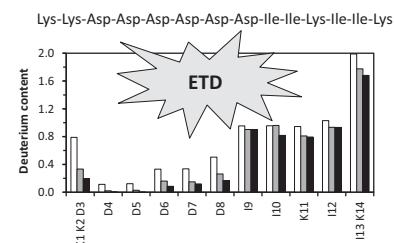


## 110–115

### Investigation of amide hydrogen back-exchange in Asp and His repeats measured by hydrogen ( $^1\text{H}/^2\text{H}$ ) exchange mass spectrometry

Kasper D. Rand, Frederik W. Lund, Sabine Amon, Thomas J.D. Jørgensen

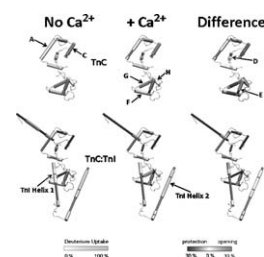
► 100% back-exchange occurs in His repeats ( $\text{His}_6$ ) at HDX-MS quench conditions. ► Asp repeats ( $\text{Asp}_6$ ) retain deuterons for several minutes at HDX-MS quench conditions. ► Acetic acid accelerates the back-exchange rate at HDX-MS quench conditions. ► Residue-specific deuterium levels were obtained using a LTQ-Orbitrap with ETD.



**116–124****Complexation and calcium-induced conformational changes in the cardiac troponin complex monitored by hydrogen/deuterium exchange and FT-ICR mass spectrometry**

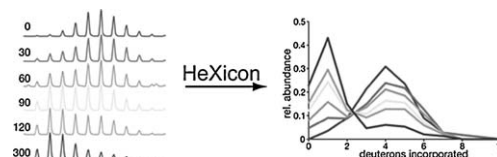
George M. Bou-Assaf, Jean E. Chamoun, Mark R. Emmett, Piotr G. Fajer, Alan G. Marshall

► The structural dynamics and the Ca-induced conformational changes of the cardiac isoform of troponin are revealed by comparing H/D exchange rate constants for TnC alone, the binary TnC:TnI complex, and the ternary TnC:TnI:TnT complex for Ca-free and Ca-saturated states. ► TnC:TnI and TnC:TnI:TnT complexes possess both highly flexible and very rigid domains. ► The N-terminal extension of TnI (specific to the cardiac isoform), the DE linker in TnC alone, and the mobile domain of TnI exhibit fast H/D exchange rates. ► The present results corroborate prior X-ray crystallography and NMR interpretations and also illuminate domains that were absent or not resolved in those experiments.

**125–131****Automated detection and analysis of bimodal isotope peak distributions in H/D exchange mass spectrometry using HeXicon**

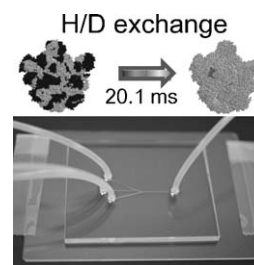
Anna Kreshuk, Marta Stankiewicz, Xinghua Lou, Marc Kirchner, Fred A. Hamprecht, Matthias P. Mayer

► HeXicon was modified to specifically analyze bimodal isotope distributions in amide hydrogen exchange mass spectrometry datasets. ► In a dataset of a pulse-labeling HX-MS experiment the modified HeXicon was able to find all previously known peptides with bimodal isotope distribution. ► The modified HeXicon is able to identify previously unknown peptides with bimodal isotope distribution.

**132–138****Application of micro-reactor chip technique for millisecond quenching of deuterium incorporation into 70S ribosomal protein complex**

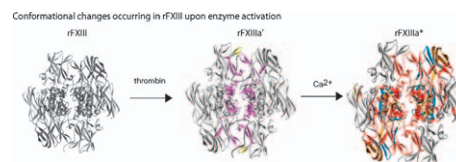
Tatsuya Yamamoto, Yoshihiro Shimizu, Takuya Ueda, Yoshitsugu Shiro, Makoto Suematsu

► Designed a micro-reactor chip for rapid quenching of H/D exchange. ► Observation the movement of 70S ribosome at the functional time scale. ► The structure-function relationship of 70S ribosome.

**139–148****Structural characterization of both the non-proteolytic and proteolytic activation pathways of coagulation Factor XIII studied by hydrogen–deuterium exchange mass spectrometry**

Mette Dahl Andersen, Johan Henrik Faber

► FXIII can be activated both via a proteolytic pathway and a non-proteolytic pathway. ► Weakened dimer interaction is observed in rFXIIIa', rFXIIIa\* and rFXIIIa°. ► Extensive conformational changes occur upon full activation to either rFXIIIa\* or rFXIIIa°. ► Both activation pathways of rFXIII produce similar activated conformations.

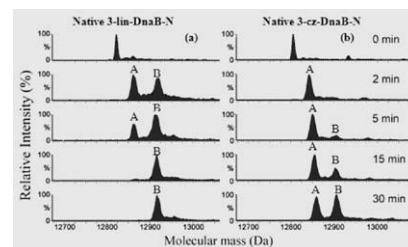


## 149–156

**EX1 hydrogen–deuterium exchange in an all-helical protein and its cyclized derivative at neutral pH**

Thitima Urathamakul, Neal K. Williams, Nicholas E. Dixon, Jennifer L. Beck

► The all-helical protein, DnaBN, exhibited EX1 type hydrogen exchange at pH 7.2. ► The protein was cyclized by joining the N- and C-termini through peptide linkers that were three, four, five or nine amino acids long. ► The rate of exchange for the slowly exchanging protons decreased for both the linear and cyclized proteins as linker length increased, correlating with predictions that the C-terminal helix of the protein would be extended by addition of these extra amino acids and stabilizing the protein.

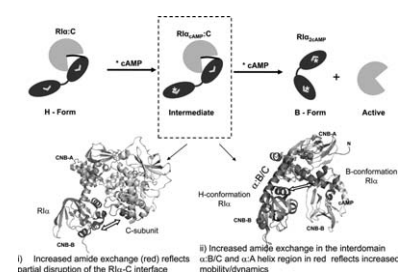


## 157–166

**Cooperativity and allostery in cAMP-dependent activation of Protein Kinase A: Monitoring conformations of intermediates by amide hydrogen/deuterium exchange**

Balakrishnan Shenbaga Moorthy, Suguna Badireddy, Ganesh S. Anand

► Amide H/D exchange to monitor conformations of intermediates in the cAMP-dependent activation of Protein Kinase A. ► Binding of a single molecule of cAMP to the CNB-B domain of the regulatory subunit partially disrupts R–Cintersubunit interface of PKA without leading to dissociation. ► cAMP binding to CNB-B increases deuterium exchange in the interdomain helices representing enhanced dynamics/conformational changes. ► Increased dynamics facilitates recruitment of a second molecule of cAMP binding to CNB-A enabling positive cooperativity.



## 167–173

**Hydrogen exchange mass spectrometry as an analytical tool for the analysis of amyloid fibrillogenesis**

Carsten Scavenius, Shirin Ghodke, Daniel E. Otzen, Jan J. Enghild

► MALDI based hydrogen exchange mass spectrometry analyses for amyloid fibril formation. ► Quantitative direct analysis in combination with high sensitivity. ► Glucagon fibrillation is a two-component system.

