Index

Page numbers in italics refer to illustrations; those in bold refer to tables

A769662 AMPK activator 211, 212
accuracy 58, 59
improvements 66–67
acid catalysis 4–6, 5, 10
α1 acid glycoprotein (AGP) 143
activator of thyroid and retinoic acid receptor (ACTR) 309, 310
CREB-binding protein interaction 309–316
hydrogen exchange kinetics 310–312, 311
residual helicity detection 312–316, 314, 315, 317
β2 adrenergic receptor 45, 213–214, 214, 287–290
affinity-column purification 249
aggregation hotspot identification, antibodies 334–336, 335
allostery studies 81–84, 83
Alzheimer’s disease
amyloid beta aggregation 203
tau protein 87
amide hydrogen exchange see hydrogen exchange
AMP-activated protein kinase (AMPK) 210–211, 212
amphitropic proteins 279, 281
AMPylator proteins 211–212
amyloid β peptide 156, 157
aggregation 203
Ana o 2 allergen 255, 256
angiotensin II 9
angiotensin III 167
Antarctic rock cod pepsins 99–100
anthrax toxin 174
antibody dynamics and stability 324
see also epitope mapping, monoclonal antibodies (mAbs)
apolipoprotein A-I 306–307
apolipoprotein E (ApoE)
Apo E4 142
oligomerization 254
asparagine deamidation, monoclonal antibody 328–329
aspartic acid isomerization, monoclonal antibody 328–329
Aspergillus saitoi protease 96
automation
case for 216
decoupled and real-time automation 216–218
back-exchange 3, 27–29, 64–65, 281, 282
correction for 110–111
epitope mapping 252
management of 65
see also quenching
backbone amide protection 154–158, 154, 157, 159
see also protection factors
base catalysis 4–6, 5
beta-lactamase TEM-1 82–83, 83
bevacizumab 331
bias 64–65
biogenerics 227
bioinformatics 296–298
biopharmaceuticals see protein biopharmaceuticals
biosimilars 227–228
biotin–streptavidin interaction 258
BmrA multidrug transporter 290–291, 290
bovine ADP/ATP mitochondrial carrier (bANC1p) 287, 288, 289
bovine carbonic anhydrase II (BCA II) 177–180, 178
bovine pancreatic trypsin inhibitor (BPTI) 8
buffer preparation 24
CalcDeut algorithm 50
calcineurin 296, 297, 308
activation by calmodulin 308–309, 309
calibration hierarchy 57, 58
calmodulin 157, 189, 193
calcineurin activation 308–309, 309
carboxypeptidase B active enzyme dynamics 86
carnivorous plant proteolytic fluid 100–104
carry-over 325
Carr–Purcell–Meiboom–Gill (CPMG) NMR 86

cashew allergen 255, 256
centroid of the isotopic envelope 57
calculation 29–33, 30, 31
centrifugation 73

cold denaturation 269
collision-induced dissociation (CID) 27
gas-phase fragmentation mechanism 129, 129
H/D scrambling 49, 128–130, 130
top-down HX-MS 151, 152

comparability studies 225–226
biopharmaceuticals 226–227
challenges 229
HX-MS role 242–244
internal versus external comparability 227–228
difference criteria 239–241
difference interpretation 238–241, 240–241
structure–function comparability 242
conformational dynamics
Linderstrøm-Lang structural unfolding model 10–11, 10
mapping perturbation effects 20
measurement 19
continuous labeling 25, 122
continuous-flow millisecond HX 74
correlated exchange see EX1 kinetics
coupled binding and folding 215, 298–299, 308
calcineurin activation by calmodulin 308–309
conformational selection model 298–299
CREB-binding protein and activator of thyroid retinoic acid receptor 309–316, 310
induced folding model 299
creatine kinase (CK) unfolding 98
CREB-binding protein (CBP) 309, 310
activator of thyroid and retinoic acid receptor (ACTR) interaction 309–316
hydrogen exchange kinetics 310–312, 311
hydrogen exchange in molten globular CBP 312, 313
CTC HTS PAL HDX systems 218
cytochrome c
epitope mapping 255–257
loop dynamics 85
data processing and analysis 37–38
deuterium uptake 38–41, 108, 109
average deuteration calculation 39–40, 108
distribution analysis 40–41
millisecond HX 76–79
monoclonal antibody studies 326, 327
PLIMSTEX data 190–193
protein dynamics hidden in the isotope distributions 117–122, 117
deconvolution of natural isotope distributions 118, 119
local unfolding dynamics 118–122, 120
rate constant extraction 110–113
nonlinear curve fitting 111, 112
numerical inverse Laplace transform 112–113, 113
semilogarithm plot 111–112, 113
software packages 46–50, 47, 107
solid state HX-MS study 272–274
workflow 41–46
data validation 43
feature processing 43
file import and project creation 42–43
integration 46
statistical analysis 43–44
visualization 44–46, 45
deuterium exchange reactions 25–26
continuous labeling 25, 122
detection of 42
off-exchange reactions 25–26
on-exchange reactions 25–26
pulsed labeling 25, 122
rate constants 33, 109
extraction 110–113
see also hydrogen exchange
average deuteration calculation 39–40, 108
distribution analysis 40–41
difference plot 236, 237–238
differential scanning calorimetry (DSC) 331
dihydrofolate reductase (DHFR) 170–171, 173–174, 173
dilution PLIMSTEX (dPLIMSTEX) 197–198, 197, 258, 259
diphtheria toxin–vesicle interaction 284–285, 285, 286
disordered proteins 295
characterization methods 299
coupled binding and folding 298–299, 308–316
disorder prediction 296–298, 297
HX-MS application 299–306
hydrogen exchange kinetics 299–304, 303
identifying disordered regions 306–308
millisecond hydrogen exchange 304–305
see also intrinsically disordered proteins (IDPs)
disulfide bonds
monoclonal antibodies 325
top-down HX-MS 160–161
dodecyl maltoside (DDM) 289, 290–291
drug discovery 209–221
automation requirement 216–218
challenges and future directions 219–221
HX-MS applications 210–216
binding site identification 210–212
structure–activity relationships 212–215
Index

targeting intrinsically disordered proteins 215–216
statistical analysis need 218–219, 220
see also protein biopharmaceuticals
DynamX software package 46–47
electron capture dissociation (ECD) 26, 49, 128
ammonia loss 135
disulfide-containing protein ions 160
gas-phase fragmentation mechanism 131, 131, 134–135
H/D scrambling 130–131, 133–135
top-down HX-MS 152–156
protein–ligand interaction studies 188
electron transfer dissociation (ETD) 26, 49, 128
ammonia loss 135
disulfide-containing protein ions 160
gas-phase fragmentation mechanism 131, 131
H/D scrambling 130–131, 133–135
top-down HX-MS 152–154
HX-ETD applications 141–143
integration into HX-MS workflow 135–141
ion transmission efficiency 138–139
peptide charge state 139
peptide selection 141
spectral overlap 139
supplemental activation 139
targeted HX-MS/MS acquisition 139–141, 140, 142
protein–ligand interaction studies 188
electrospray ionization-MS (ESI-MS) 168–170, 174
pKₐ analyses 170–171, 171, 174
protein–ligand interaction studies 187
enzyme efficiency 60
enzyme-linked immunosorbent assay (ELISA) 248
sandwich ELISA 249
epitope mapping 248
case studies 254–258
protein–peptide interactions 258
protein–protein interactions 255–258
HX-MS methodology 251–254, 252
complementary strategies 253–254
data interpretation 252–253, 253
experimental design 251–252
methods 248–251, 249
see also monoclonal antibodies (mAbs)
ethylene glycol 65
EX1 kinetics 11, 13, 21, 22, 40, 41, 109, 120–122, 121
accuracy improvements 66–67
detection 13–14
disordered proteins 300–302, 303
histidine hydrogen exchange 177
isotope patterns produced 117, 117
millisecond HX 76–78, 77
EX2 kinetics 11, 13, 14, 21, 22, 40, 41, 109, 122
accuracy improvements 66–67
detection 13–14
disordered proteins 300–302, 303
histidine hydrogen exchange 177
isotope patterns produced 117, 117
millisecond HX 76–78, 77
ExMS software package 50
extracted ion chromatograms (EICs) 42, 43
EXX (mixed) kinetics 14, 40, 41
disordered proteins 300–302, 303
factor VIIa 2
fast photochemical oxidation of proteins (FPOP) 250–251
FAST-HSQC 73
Finke–Watzky modeling 203
formic acid 24, 27
Fourier transform ion cyclotron resonance (FTICR) mass spectrometer 159, 255, 267
fragmentation methodology see protein fragmentation
freeze-drying 270
see also proteins in lyophilized solids
freezing 269
freeze-thaw cycles 24–25, 269
impact on monoclonal antibody local dynamics 331
see also proteins in frozen solutions
fuzzy complexes 299
FVIIa conformational changes 141–142, 142
G protein-coupled receptors (GPCR) 213, 219–220
activation by modulators 213–214, 214
β₂ adrenergic receptor 45, 213–214, 214, 287–290
rhodopsin (Rho) 169, 171, 174
gas-phase fragmentation 3, 128
combination with proteolytic fragmentation 158–159
continuous-flow millisecond HX 74
fast fragmentation MS/MS techniques 130–133
future directions 143
H/D scrambling 128–135
avoidance of 128–135, 134
quantitating 133–135
integration into bottom-up HX-MS workflow 135–141
applications 141–143
ion transmission efficiency 138–139
peptide charge state 139
peptide selection 141
spectral overlap 139
suitable mass spectrometers 138
supplemental activation 139
targeted HX-MS/MS acquisition 139–141, 140, 142
slow fragmentation MS/MS techniques 128–130
glucocorticoid receptor ligand-binding domain (GR LBD) unfolding 221
γ-glutamyl carboxylase (GGCX) 291
GroEL protein 334

Guide to the Expression of Uncertainty in Measurement (GUM) 57

H/D scrambling 128
  avoidance in gas-phase MS/MS 128–135, 134
    fast fragmentation MS/MS techniques 130–133
    slow fragmentation MS/MS techniques 128–130
  collision-induced dissociation (CID) 49, 128–130, 130
  influences 132
  quantitating 133–135
  top-down HX-MS 150, 151–156
  determinants of 151

HDExaminer 65
HDX Workbench software package 47–48
HDX-Analyzer software package 50
HDXFinder software package 50
  heat map 19, 33, 45, 233
  heat shock protein 85, 221
  hemoglobin–haptoglobin interaction 180, 181
Hexicon software package 50
  Hexicon2 50

high-throughput screening (HTS) 200
histidine hydrogen exchange 165–182
  denaturant-dependent experiments 175–182, 175
    advantages and disadvantages 181–182
    protein folding 177–180
    protein–ligand binding analysis 180–181
    workflow 176–177
  historical context 167–168
  imidazole side chain C-2 proton 165–167, 166, 175
  kinetics 167, 167
    mechanism 166–167, 166
    pH dependence of 166, 167
  pH-dependent experiments 168–175
    advantages and disadvantages 174
    pK_a analyses 170–171, 171, 174
    solvent accessibility 171–174, 172, 173
    workflow 168, 169

HTS Twin PAL auto-sampler 218
human mitotic kinesin Eg5 254
human telomeric repeat binding factor 2 (hTRF2) 193
HX-Express software package 50
hydrogen exchange
  acid catalysis 4–6, 5, 10
  base catalysis 4–6, 5
  folded polypeptides 9–15
    conformational unfolding model 10–11, 10
  histidine  see histidine hydrogen exchange
  ionic strength effect 8
  kinetics 21, 22, 109

  see also EX1 kinetics; EX2 kinetics; EXX (mixed) kinetics
  mechanisms 4
  pH dependence 6–7, 6, 20
  pressure effect 9
  rate constants 33, 109
  extraction 110–113
  sequence effect 8
  solvent effect 8–9
  temperature dependence 7–8, 7, 20–21
  unstructured polypeptides 3–9, 4
  see also back-exchange; deuterium exchange reactions; millisecond HX
hydrogen exchange mass spectrometry (HX-MS) 1
  centroid calculation 29–33, 30, 31
  future challenges 50–51
  HX-NMR comparisons 67–68, 68, 127
  process control 39
  resolution 95–96
  results presentation 33, 44–46, 45
  workflow 2, 3, 22–29, 136
  automated 217
  back-exchange consideration 27–29
  buffer preparation 24
  deuterium exchange reactions 25–26
  gas-phase fragmentation integration 135–141
  LC separation 27, 28
  proteolytic digest fragment identification 27
  proteolytic digestion 26–27
  quench solution preparation 24–25
  sample preparation 22–23
  see also data processing and analysis; millisecond HX; top-down HX-MS
hydrogen exchange NMR spectroscopy (HX-NMR) 1–2
  HX-MS comparisons 67–68, 68, 127
  hydrophobic resins, protein adsorption 267–269
  hydroxyl radical oxidative modification 250–251
IgG1 121, 122, 122
  chemical modification impact 328–329
  environmental impacts 331
  formulation additive impacts 332–334
  see also monoclonal antibodies (mAbs)
IgG2 112, 113, 114, 115, 116
  immunoaffinity purification 249
  inhibitor of nuclear factor κB 307
  insulin self-association 202
  interferon (IFN) 238
  IFN-β-1a 45, 62
  intermediate measurement precision (IMP) 60–62, 61, 63
  intestinal fatty acid binding protein (I-FABP) 193
  intrinsically disordered proteins (IDPs) 295–299
  coupled binding and folding 215, 298–299, 308–316
  disorder prediction 296–298, 297
  HX-MS application 299–306
identifying disordered regions 306–308
millisecond hydrogen exchange 304–305
hydrogen exchange kinetics 299–304, 303
peptide mapping 305–306
residual structure 87
detection of 312–316, 314, 315, 317
targeting in drug discovery 215–216
isotopic exchange monitoring 1–2

\( \alpha \)-lactalbumin 11, 12
Laplace transform 113
large unilamellar vesicles (LUVs) see unilamellar vesicle interaction studies
laulimalide binding to microtubule 210
limits of quantitation (LOQ) 229
lipid nanodiscs 291
liquid chromatography (LC) 27, 28
proteolytic digest fragment separation 27
liquid chromatography–mass spectrometry (LC-MS) 65
lyophilized solids see proteins in lyophilized solids
lysozyme 9
adsorption kinetics 267
folding processes 267
m-nitrobenzyl alcohol (m-NBA) 154
MALDI see matrix-assisted laser desorption ionization (MALDI)
maltose-binding proteins 200
mannitol 273
mass difference plot 236, 237–238
Mass Spec Studio software package 48–49
matrix-assisted laser desorption ionization (MALDI) 281
in-source decay (ISD) 130
measurement errors 64
measurement reproducibility 60, 62–64
membrane proteins 23, 279–292
integrated membrane proteins 285–291
\( \beta \)-adrenergic G-protein-coupled receptor (\( \beta \)-AR) 287–290
bovine ADP/ATP mitochondrial carrier (bANC1p) 287, 288, 289
membrane proteins in organello 291–292
proteins inserted in lipid nanodiscs 291
unilamellar vesicle interaction 280–285
diphtheria toxin–vesicle interaction 284–285, 285, 286
myoglobin–vesicle interaction 98, 281, 282, 283
peptide–vesicle interactions 280, 280
phospholipase–vesicle interaction 281–284, 283
membrane scaffold proteins (MSP) 291
metalloproteins 181
methionine oxidation, monoclonal antibody 328–329
method quantization limit 60
method robustness 60
method validation 58–68
accurate improvements 66–67
bias 64–65
general conditions 58–60
precision 60–64
methyl CpG-binding protein 2 307
metrological terminology 58
metrological traceability 56–57
microbial antigens 255
microfluidic device 75, 75, 85, 87
\( \beta \)-microglobulin 136, 137, 160, 162
millisecond HX 73–74, 75
conformational dynamics in weakly structured protein regions 84–85, 84, 85
data analysis 76–79
agreement with crystal structure 78–79, 79
millisecond HX kinetics 76–78
disordered protein studies 304–305
enzyme dynamics 85–86
instrumentation 74–75
outlook 87–88
pulse labeling for protein folding 80–81, 80, 82, 83
pulse labeling for studying allostery 81–84, 83
residual structure in intrinsically disordered proteins 87
mitochondrial membrane protein study in organello 291–292
mitotic centromere-associated kinesin (MCAK) 44
molten globular protein 296, 302
CREB-binding protein (CBP) 312, 313
monitoring program 69
monoclonal antibodies (mAbs) 247, 323–339
aggregation hotspot identification 334–336, 335
global dynamics 328–329, 330
disulfide cross-links 325
environmental stress impact 329–331
formulation additive impacts 331–334, 333
formulation development challenges 337–338
see also epitope mapping
Monopterus albus 100
MS/MS techniques see tandem mass spectrometry (MS/MS)
myoglobin 98–99, 99
adsorption onto solid surface 267
backbone amide protection maps 154, 154
folding behavior 177–179, 179
folding intermediate 81, 83
overlapping peptides 96
partially denatured, conformational dynamics 84–85, 84
solid-state study 272–273, 273
vesicle interaction study 98, 281, 282, 283
N-protonation 6
nanodiscs 291
natural isotope deconvolution 118, 119
Neisseria meningitides factor H binding
protein 255, 256
nepenthesins 100–104, 101
neutralizing antibody 255
nuclear magnetic resonance (NMR) spectroscopy 1
CPMG NMR 86
solid-state (ssNMR) 271
see also hydrogen exchange NMR spectroscopy (HX-NMR)

O-protonation 6, 10
on-exchange experiments 255–257, 257
on/off-exchange experiments 255–258, 257
overlapping peptides 96, 127–128

Parkinson’s disease 203
penicillin binding protein (PBP-2X) 96, 97
PEPSSCAN method 250
pepsin fragmentation 2, 26, 29, 74, 93–96
carry-over during digestion 325
immobilization 95
proteolysis mechanisms 94
reproducibility 95
resolution 95–96
specificity 94
tandem MS 94–95
peptide map 60
PeptideCutter tool 94
performance monitoring 69
peroxidase proliferator-activated receptor γ (PPARγ) 87, 142–143, 210
activation by small molecules 215
coactivator-1 alpha (PGC-1α) 216
disordered region identification 307
selective PPAR modulators (SPPARMs) 215
PGC-1α 87
pH dependence 6–7, 6, 20
histidine hydrogen exchange 166, 167
millisecond hydrogen exchange 304–305
phosphodiesterase, RegA 32
phospholipase–vesicle interaction 281–284, 283
pioglitazone 215
Plasmodium falciparum plasmepsins 100
PLIMSTEX 188–197, 258
advantages 193–194
data processing 190–193
dilution PLIMSTEX (dPLIMSTEX) 197–198, 197, 258, 259
disadvantages 194–197
examples 193
sharp-break curve 194, 194
workflow 189
polypeptide hydrogen exchange see hydrogen exchange
POROS AL-20 95
precision 58, 59
intermediate measurement precision (IMP) 60–62, 61, 63
prostacyclin synthase (PCS), tyrosine
nitration 258, 259
proteases 96–104, 103
Antarctic rock cod pepsins 99–100
nepenthesins 100–104, 101
Plasmodium falciparum plasmepsins 100
rhizopuspepsin (Rp) 99
rice field eel protease 100
type XIII 96–98, 99
type XVIII 96–98
see also pepsin fragmentation

protein biopharmaceuticals 225–244
biosimilars/biogenerics 227–228
comparability studies 226–227
challenges 229
difference criteria 239–241
difference interpretation 238–241, 240–241
HX-MS role 242–244
internal versus external comparability 227–228
structure–function comparability 242
data handling challenges and approaches 232–238, 233
difference plot 236, 237–238
example 241–242
relative fractional exchange comparability plot 235–237, 236
development path 226
higher-order structure and HX-MS 229–232, 230, 231
posttranslational modification 229
protein conformational dynamics see conformational dynamics
protein folding
conformational unfolding model 10–11, 10
coupled binding and folding 215, 298–299, 308–316
local unfolding dynamics 118–122, 120
millisecond HX applications 80–81, 80, 82, 83
protein footprinting 250
protein fragmentation 2, 26–27
fragment identification 27
offline digestion 26–27
online digestion 26–27
see also pepsin fragmentation; proteases
protein quartet model 295, 296
protein sample preparation 22–23
ProteinLynx Global Server (PLGS) 46
proteins adsorbed to solid surfaces 266–269
Index

HX-MS studies 267–269
  workflow 268
  properties at the solid–liquid interface 266, 266
  study methods 266–267
proteins in frozen solutions 269–270
  HX-MS studies 270
  protein structure and dynamics 269
  study methods 269
proteins in lyophilized solids 270
  data analysis and interpretation 272–274
  solid state HX-MS studies 271–272
  stability 270–271
  study methods 271
protein–DNA interaction 193
protein–ligand interactions 12–13, 185
  affinity measurements 185–186
  conventional methods for characterization 186–187
  direct mass spectrometry method 187
  HX-MS studies 187–188
  binding affinity 188
  binding order 201, 202
  binding regions 188
PLIMSTEX studies 188–197, 258
  advantages 193–194
  data processing 190–193
dilution PLIMSTEX 197–198, 197, 258, 259
  disadvantages 194–197
  examples 193
SUPREX studies 198–201
  advantages 200
dilution PLIMSTEX 197–198, 197, 258, 259
  disadvantages 194–197
dilution PLIMSTEX 197–198, 197, 258, 259
  examples 193
protein–protein interactions 12–13, 201–203
  antibody aggregation hotspot identification 334–336, 335
  epitope mapping 255–258
  hemoglobin–haptoglobin interaction 180–181
  pulsed HX studies 203, 204
  self-association 201–203
  SIMSTEX studies 201–203
see also protein–ligand interactions
proteolytic digestion see protein fragmentation
proton transfer 4
  see also hydrogen exchange
  pulsed labeling 25, 122
  purification procedures 23
quenching 24
  quench solution preparation 24–25
Ras-GDP 193
  rate constants 33, 109
  extraction 110–113
  nonlinear curve fitting 111, 112
  numerical inverse Laplace transform 112–113, 113
semi-logarithm plot 111–112, 113
  histidine hydrogen exchange 167, 167
  recombinant factor IX (rFIX) 232, 234, 236, 241, 243
  reference measurement (RM) 56–58, 68–69
  relative fractional exchange comparability plot 235–237, 236
  repeatability 60–62, 61, 63
  reproducibility 60, 62–64
  pepsin digestion 95
  residual structure, intrinsically disordered proteins 87
  detection of 312–316, 314, 315, 317
  retinoic acid-related orphan receptor gamma t (RORγt) 210
  retinoid X receptor–vitamin D receptor (RXR–VDR) complex 210
Rhizopus protease 96
  rhizopuspepsin (Rp) 99
  rhodopsin (Rho) 169, 171, 174
  ribonuclease (RNase) 8, 9
  RNase A 170, 171, 177–179
  rice field eel protease 100
  rosiglitazone 215
  sample preparation 22–23
  flash-frozen samples 24–25
  sandwich ELISA 249
  selective estrogen receptor modulators (SERMs) 210
  selective PPAR modulators (SPPARMs) 215
  functional (FSPPARMs) 215
  SIMSTEX studies 201–203
  site-directed mutagenesis, epitope mapping 250, 254
  small-angle X-ray scattering (SAXS) 220–221, 331
SOFAST 73
  software packages 46–50, 47, 107
  solid surface interactions see proteins adsorbed to solid surfaces
  solid-state HX-MS 271–272
  data analysis and interpretation 272–274
  solvent accessibility studies 171–174, 172, 173
  spectral envelope 29–32, 31
  standards 56, 69
  reference measurement 68–69
  standard operating procedures (SOPs) 55
  staphylococcal nuclease (SNase) 67–68, 68
  statistical analysis 43–44
  drug discovery 218–219, 220
  see also data processing and analysis
  subzero temperature reversed-phase chromatography 65
  supercharging protein ions 154–155
  superoxide dismutase 1 (SOD-1) 180–181
SUPREX studies 181, 198–201
- advantages 200
- disadvantages 200–201
- examples 200
- workflow 198, 199

surface plasmon resonance spectroscopy (SPR)
- epitope mapping 249–250
- protein–ligand binding analysis 180

α-synuclein 203
- disordered region identification 307–308

surface plasmon resonance spectroscopy (SPR)
- epitope mapping 249–250
- protein–ligand binding analysis 180

α-synuclein 203
- disordered region identification 307–308

tandem mass spectrometry (MS/MS) 27, 94–95
- fast fragmentation MS/MS techniques 130–133
- H/D scrambling 128–135
- slow fragmentation MS/MS techniques 128–130
- tau protein 87
- TEM-1 beta-lactamase 82–83, 83
- temperature dependence 7–8, 7, 20–21
- therapeutic antibodies see monoclonal antibodies (mAbs)
- thiazolidinedione (TZD) antidiabetic drugs 215
- top-down HX-MS 149–162, 150
- appeal of 149–151
- challenges 160–161
- conformation-specific characterization of nonnative states 156–158
- convergence with classical schemes 158–159
- future directions 160–161
- hydrogen scrambling problem 150, 151–156
- determinants of 151
- electron-based fragmentation techniques and 152–156
- small proteins 151–156
- traceability, metrological 56–57
- trehalose 270
- Trematomus bernacchii 99
- trifluoroacetic acid 24, 27
- tris(2-carboxyethyl)phosphine (TCEP) 160, 325
- troponin C (TnC) binding order 201, 202
- trueness 58, 59
- ubiquitin 154, 157–158, 158
- folding intermediate 81, 82
- uncorrelated exchange see EX2 kinetics
- unilamellar vesicle interaction studies 280–285
- myoglobin–vesicle interaction 98, 281, 282, 283
- peptide–vesicle interactions 280, 280
- phospholipase–vesicle interaction 281–284, 283
- urokinase-type plasminogen activator receptor (uPAR) 133
- validation see method validation
- vesicle interactions see unilamellar vesicle interaction studies
- VopS AMPylator protein 211–212
- workflow see data processing and analysis; histidine hydrogen exchange; hydrogen exchange mass spectrometry (HX-MS)
- yeast display 248