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Insight to functional conformation and noncovalent interactions of protein-protein assembly using MALDI mass spectrometry

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Supplementary Table 11 Protein complex and conformation studies using MALDI-MS based approaches

Protein complex	Type of analysis	Biological function	Matrix	Mass analyzer	Polarity	Reference
Molecular assembly DNA-protein	Limited proteolysis	Transcription factor important for regulation of cell development and proliferation	CHCA[1] (saturated of 1:3:2 (v/v) formic acid: water:isopropanol)	TOF[8]	+	[92]
HIV-1_{sf2} P24 with anti-p24 antibody	Limited proteolysis (proteolytic footprinting)	Immune response to capsid protein p24 of HIV	CHCA[1] (saturated solution of 45:45:10 ethanol:water:formi c acid)	TOF[8]	+	[93]
p21 and Cdk2	Limited proteolysis	p21 inhibits the activity of cyclin-CDK2 which regulates cell cycle progression at G1 and S phase	CHCA[1] (saturated solution with 1:3:2 (v/v) formic acid: water:isopropanol)	TOF[8]	+	[94]
Viral capsids	Limited proteolysis	Protein shell enclosing genetic material	SA[2] (saturated solution of acetonitrile/water (50:50) 0.25% TFA)	TOF[8]	+	[96,141]

Protein complex	Type of analysis	Biological function	Matrix	Mass analyzer	Polarity	Reference
β-Arrestin 2 - V2R-pp	Limited proteolysis	Intracellular signal transduction via GPCR receptor V2R-pp	SA[2] (saturated solution of 45% acetonitrile 0.1% TFA[3])	TOF[8]	+	[98,99]
Conformation cytocrome c	HDX	Cell apoptosis	SA[2] (20 mg/mL in 100% acetone (precoating))	TOF[8]/ISD[9]	+	[114]
Kinase inhibitor and cyclic-AMP-dependent protein kinase	HDX	Regulation of glycogen, sugar, and lipid metabolism	CHCA[1] (5 mg/ml in 1:1:1 acetonitrile, ethanol, and 0.1% TFA - pH adjusted to 2.5 with 2% of TFA)	TOF[8]	+	[109,110]
Ubiquitin and insulin	HDX native	E3 ubiquitin ligases directly degrades the insulin receptor, promoting insulin resistance	SA[2] (5 mg/mL in acetonitrile with 0.1% 3-nitrobenzyl alcohol)	TOF[8]	+	[115]
Hemoglobin - O_2	HDX	O_2 transport in the blood from lungs or gills to the rest of the body	CHCA[1] (5 mg/mL in 2:8:1 acetonitrile/ethanol/0.1% aqueous TFA)	Triwave™ Technology	+	[111]
Factor XIII activation with calcium	HDX	Thrombin and Ca^{2+} activate Factor XII in the process of blood coagulation	CHCA[1] (10 mg/ml in 1:1:1 ethanol/acetonitrile/0.1%TFA at pH 2.2)	TOF[8]	+	[112]

Protein complex	Type of analysis	Biological function	Matrix	Mass analyzer	Polarity	Reference
Troponin C with Ca^{2+}	Limited proteolysis, HDX and LC-MS	Muscle contraction	CHCA[1] (saturated solution of in 50% acetonitrile/49.5% water/0.5% TFA)	TOF[8]	+	[113]
Integral membrane proteins complex: BtuCDF	Cross-linking	Uptake of vitamin B_{12} in *E. coli*	SA[2] (20 mg/mL in water/acetonitrile /TFA, 49.95/49.95/0.1, (v/v/v))	TOF[8]	+ / -	[120]
Nanobody•Membrane Protein Complexes	Cross-linking	Chaperones in crystallization and blockers or modifiers of protein activity	SA[2] (10 mg/mL in acetonitrile/water /TFA, 49.95/49.95/0.1, (v/v/v))	TOF[8]	+	[129]
Covalent 14-3-3ζWT Homodimeric Product	Cross-linking (bottom-up approach)	Phosphorylation process	CHCA[1] (5 mg/mL dissolved in 50% (v/v) aqueous acetonitrile containing 0.1% (v/v) TFA)	TOF[8]	+	[132]
calmodulin and peptide M13 complex	Cross-linking	Calmodulin regulates calcium levels in the cell. M13 resembles skeletal muscle myosin light chain kinase (skMLCK).	DHB[4] (saturated matrix solution of 30% acetonitrile, 69.9% (water, 0.1% TFA)	TOF[8]	+	[131]
Trimeric porin	Native MS	Transport of hydrophilic molecules	FA[5] (20-50 mg/mL in pure THF)	TOF[8]	+	[135]

Protein complex	Type of analysis	Biological function	Matrix	Mass analyzer	Polarity	Reference
Tetramer streptavidin	Native MS	Biotin binding protein. As antibody-streptavidin complex	SA[2] (in water with 20 mM TEAB at pH 8.5 or FA in pure THF)	TOF[8]	+	[137]
HU$\alpha\beta$ protein (heterodimer)	Native MS	Regulation of cell transcription	Ionic liquid matrices	TOF[8]	+/-	[138]

1. α-cyano-4-hydroxycinnamic acid; 2. Sinapinic acid; 3. Trifluoroacetic acid; 4. 2,5-dihydroxybenzoic acid; 5. Ferulic acid 6. Tetrahydrofuran; 7. 5-methoxysalicylic acid; 7. triethylammonium hydrogen carbonate; 8. Time-of-Flight; 9. In-source decay

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