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Temperature-controlled electrospray ionization tandem mass spectrometry study on protein/small molecule interaction

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ABSTRACT

Traditional electrospray ionization tandem mass spectrometry (ESI-MS^n) has been a powerful tool in diverse research areas, however, it faces great limitations in the study of protein-small molecule interactions. In this article, the state-of-the-art temperature-controlled electrospray ionization tandem mass spectrometry (TC-ESI-MS^n) is applied to investigate interactions between ubiquitin and two flavonol molecules, respectively. The combination of collision-induced dissociation (CID) and MS solution-melting experiments facilitates the understanding of flavonol-protein interactions in a new dimension across varying temperature ranges. While structural changes of proteins disturbed by small molecules are unseen in ESI-MS^n, TC-ESI-MS^n allows a simultaneous assessment of the stability of the complex in both gas and liquid phases under various temperature conditions, meanwhile investigating the impact on the protein's structure and tracking changes in thermodynamic data, and the characteristics of structural intermediates.

Protein-small ligand interactions are universal and of crucial importance in cellular processes [1–3]. Flavonoids serve as specialized plant metabolites and often act as effective pharmaceutical agents [4–9]. Previous studies on the interactions of flavonols and proteins, such as hemoglobin, bovine serum albumin, β-lactogolobulin, and others, reveal that flavonols form complexes with proteins, generating diverse structural and physicochemical characteristics [10–15]. However, limited information can be obtained regarding how the intrinsic property of protein is affected by flavonols.

Mass spectrometry (MS) has become an essential method for exploring non-covalent interactions [16–22]. It only requires minimal sample quantities, and can be easily conducted without the need for extensive purification or labeling [23–26]. By preserving either covalent or non-covalent interactions during the transition from solution to the gas phase, MS provides initial stoichiometric information on the composition of the complexes [27], their average charge states, and excels in extracting stability from protein mixtures [28]. The combination of MS with other techniques such as equilibrium dialysis, ion mobility (IM) and Förster resonance energy transfer (FRET) can also provide more information of the interactions [29–31]. However, the correlation between solution and gas-phase stabilities remains an unanswered question, despite extensive efforts from researchers to comprehend the behavior of proteins [32–34] in both gas and solution phases.

Employing MS detection to track changes in protein structures, temperature-controlled electrospray ionization (TC-ESI), also called variable-temperature electrospray ionization (vt-ESI), has been emergingly used as an equivalent to conventional solution-phase temperature annealing methods [35]. Measuring melting temperature (T_m) using calorimetry or spectroscopy is inherently slow, requires a substantial amount of purified material (0.1–2 mg), and poses challenges in terms of multiplexing. This creates a critical bottleneck for extracting stability measurements from protein mixtures [36–38]. However, TC-ESI is also useful to depict protein T_m values with MS-grade sensitivity and specificity, which proves to be in line with traditional technologies [39–48]. Moreover, its strength lies in its ability to unequivocally identify and quantify each of the multiple coexisting stoichiometries in a mixture. Structural information on ligand binding has also been primarily investigated by these methods [49–51].

Tandem MS (MS^n), usually using collision-induced dissociation (CID), has been widely employed for native protein complexes [52]. MS^n for protein-ligand complexes in the gas phase may offer valuable and quantitative insights into protein-ligand interactions [53,54]. Nevertheless, the degree of ion dissociation following the

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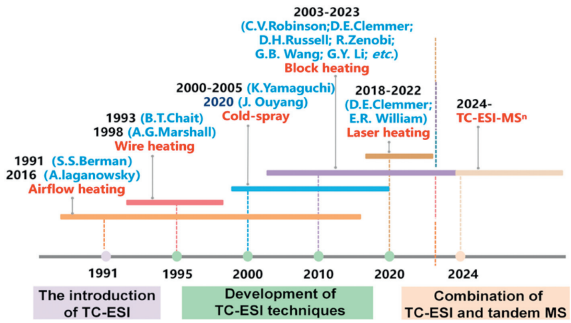


Scheme 1. Timeline of the development of TC-ESI-MSⁿ.

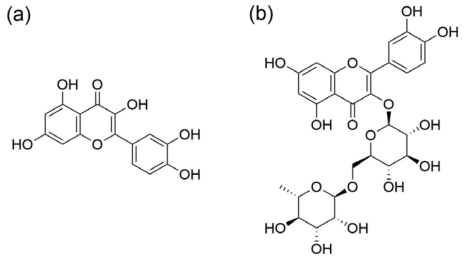
implementation of CID is contingent on the dissociation rate constant for the gaseous ion [55]. Therefore, the gas-phase stability of protein-ligand complexes relies on the dissociation thermochemistry and kinetics of the complex in the gas phase. This is not anticipated to accurately mirror solution-phase binding affinities [55–57].

The development of TC-ESI primarily focused on the temperature control methods (Scheme 1) [58], but it was seldom combined with other MS techniques. The integration of TC-ESI with MSⁿ greatly expands the application field of this ion source, enabling more effective exploration of interactions between biomacromolecules and ligands. Furthermore, due to the capability of block heating to effectively control parameters such as heating time, flow rate, and temperature, it is highly suitable for compensating MSⁿ method to study subtle changes in protein-small molecule interactions. Combining TC-ESI with MSⁿ allows for a rapid and simultaneous assessment of the stability of the complex in both gas and liquid phases under various temperature conditions. Additionally, it facilitates the examination of how these conditions affect protein structure, highlighting structural alterations in proteins affected by small molecules that often go undetected in traditional ESI-MSⁿ. Temperature-controlled ESI-MS in combination with tandem mass spectrometry (TC-ESI-MSⁿ) experiments allow for the observation of differences in dissociation patterns and affinities of complexes across various temperature dimensions in the gas phase. By integrating with TC-ESI experiments, it can facilitate a more comprehensive comparison of flavonol-protein interactions between gas and solution phases. Furthermore, this combined strategy will provide new insights into proteins and enable careful tracking of changes in thermodynamic data, and the characteristics of structural intermediates. Compared with traditional ESI-MS, this method can offer unprecedented insights and an additional layer of detail into protein-small molecule interactions. A schematic drawing of the experimental setup is shown in Fig. S1 in Supporting information.

In this work, a prototype protein ubiquitin (U), one of the most investigated proteins with +5 to +8 charge states under native MS conditions, is firstly selected [59]. Then, TC-ESI-MSⁿ is used to study the interaction of ubiquitin with two typical flavonols (Scheme 2), quercetin (Q) and rutin (R), respectively.

At room temperature, the spray of ubiquitin and quercetin mixture in methanol/water solution produces ions in U, U-Q_1 to U-Q_3 at +5 and +6 charge states (Fig. 1a). The 1:1 U-Q_1 complex is to date the most abundant in the spectrum while the 1:2 U-Q_2 complex is of lower abundance. Similarly, rutin and ubiquitin complex solution sprays to forms U, U-R_1 to U-R_2 at +5 and +6 charge states (Fig. 1b).

Weighted average charge state of ubiquitin (Fig. 2) and mass spectra (inset of Fig. 2) *versus* solution temperature of ubiquitin obtained in methanol/water mixture are in line with temperature-dependent unfolding transitions of ubiquitin observed by El-Baba



Scheme 2. Structures of (a) quercetin (Q, left) and (b) rutin (R, right).

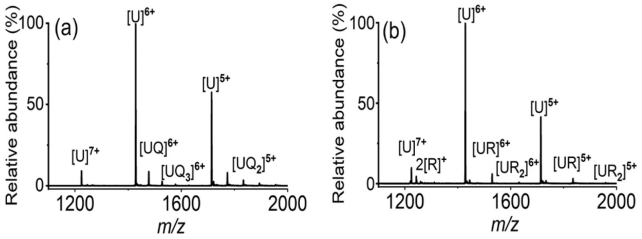


Fig. 1. Mass spectra of ubiquitin with different flavonols.

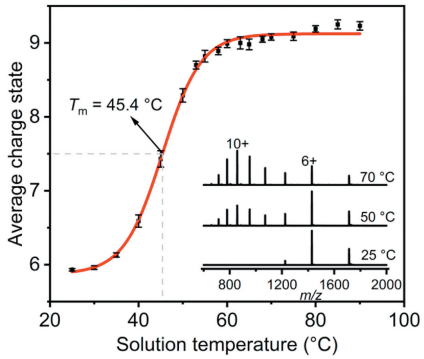


Fig. 2. Weighted average charge state of ubiquitin *versus* solution temperature with midpoint melting temperature T_m = 45.4 °C. Inset shows mass spectra of ubiquitin in 20% (v/v) methanol in water in an acidified aqueous solution collected at 25, 50, and 70 °C at flow rate of 5 μL/min. Weighted average charge state is calculated using Eq. S1 in Supporting information. The values are presented as mean ± standard deviation (n = 3).

et al. [42]. Notably, the melting temperature derived in Fig. 2 is 45 °C, which is different from previous results, such as in aqueous solution with pH 3 [60]. This result is consistent with earlier studies and is primarily attributed to changes in solvent conditions within the solution (Figs. S2 and S3 in Supporting information) [61,62].

As shown in Fig. 3a, investigation on temperature-dependent effects of flavonols binding to ubiquitin has been conducted. As temperature grows, binding of quercetin keeps the same binding stoichiometries. A zoomed mass spectrum for ubiquitin-quercetin (Fig. 3b) shows the formation of the noncovalent U-Q_1 to U-Q_3 complex during thermal denaturation. However, the peaks for +5 and +6 ions decrease against increasing temperature, making it challenging to intuitively compare the binding affinities between ligands and ubiquitin. Similarly, thermal denaturation of ubiquitin-rutin complex (Figs. 3c and d) has similar trend mass spectra during the entire heating process and no obvious new binding peaks of U-R complex are observed.

To better understand the complex mechanism, Eq. 1 has been utilized to assess the relative binding affinities of flavonols [63], where I(M) and I(1:n) represent the relative abundance of each charge state of the free ubiquitin and various binding stoichiometries of ubiquitin-flavonol complexes. It is assumed that the rela-

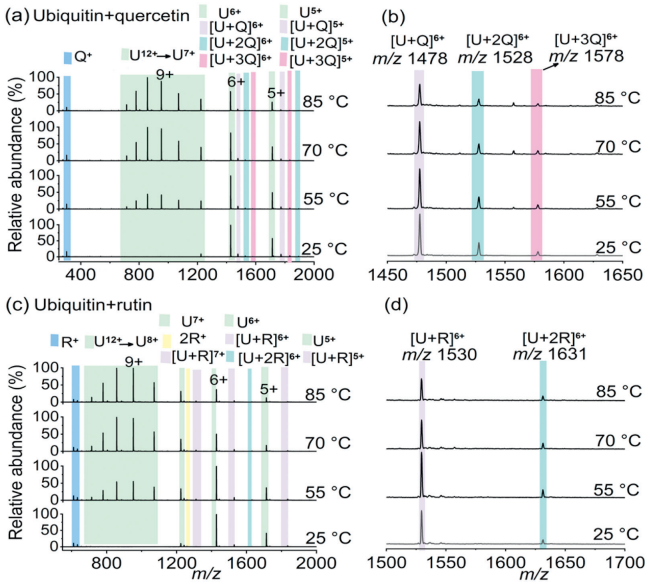


Fig. 3. (a) Mass spectra of the ubiquitin-quercetin complex. (b) Zoomed-in mass spectra (m/z 1450–1650) of the $[U\text{-}Q_{1\text{-}3}]^{6+}$ ions. (c) Mass spectra of the ubiquitin-rutin complex and (d) zoomed-in mass spectra (m/z 1500–1700) of the $[U\text{-}R_{1\text{-}2}]^{6+}$ ions, all at different temperatures, respectively.

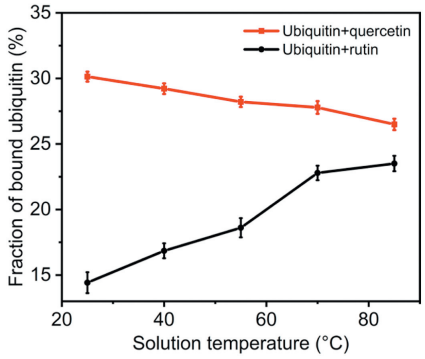


Fig. 4. Binding affinities based on TC-ESI-MS measurements of flavonol-ubiquitin complexes at different temperatures. The values are presented as mean ± standard deviation ($n = 3$).

tive intensities of the proteins or complexes in the mass spectra are directly proportional to their relative abundances in the solution [63,64]. The higher fraction value of ubiquitin in the TC-ESI-MS experiment indicates greater binding affinities.

$$\text{Fraction} = \frac{I(1{:}1) + I(1{:}2) + \cdots}{I(\text{M}) + I(1{:}1) + I(1{:}2) + \cdots} \quad (1)$$

As shown in Fig. 4, the relative ligand affinities of flavonol-ubiquitin complexes at different temperatures can be calculated from their extractive mass spectra, respectively. It illustrates two distinctly different trends. The binding affinity between quercetin and ubiquitin consistently decreases as temperature rises. In contrast, the binding affinity between rutin and ubiquitin increases with temperature elevation.

The discrepancy in the interaction of proteins with quercetin and rutin is proposed to be attributed to the structural differences between the flavonoid ligands [65,66]. The U/Q complex becomes less stable at elevated temperatures, indicating an exothermic nature of their interaction as in soy protein [67]. In contrast, the binding affinity of U/R complexes exhibits notable increments as the temperature rises, indicating that these complexes are formed through endothermic processes. These results align with those observed in the interactions between rutin and soybean protein isolates [68]. Hydrogen bonds and hydrophobic interactions are reported to have key roles in the non-covalent binding of flavonoids and proteins [69]. The strengthening of the hydrophobic interactions in the U/R complexes at higher temperatures could potentially enhance their stability [70], while the binding between ubiquitin and quercetin may primarily be mediated by hydrogen bonds.

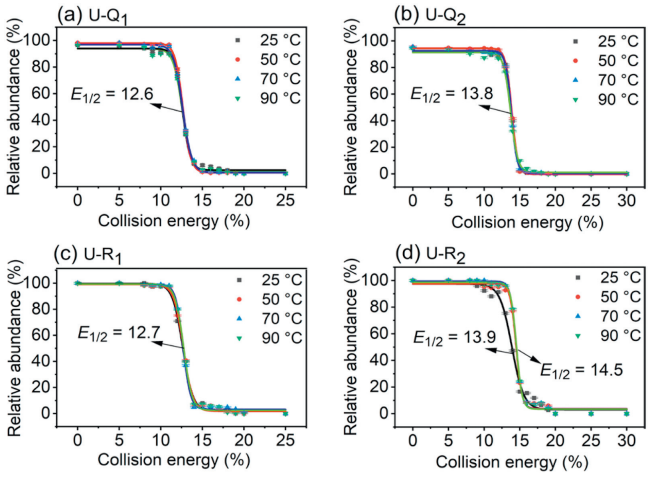


Fig. 5. Dissociation profiles of (a) U-Q_1, (b) U-Q_2, (c) U-R_1 and (d) U-R_2 at different temperatures. The values are presented as mean ± standard deviation ($n = 3$).

In order to further explore the interactions between the complexes in gas phase, the gas phase stability of the two ligand complexes by TC-ESI-MS^n has been introduced. What deserves expecting is that combination of CID to quantify the stability of the protein-ligand complexes in the gas phase can offer meaningful and quantitative insights into the interactions.

Fig. 5 shows the dissociation profiles of the TC-ESI-MS^n experiment performed on the +6 charge state of the ubiquitin-flavonols complex, with molar ratios of 1:1 and 1:2 at various temperatures. As will be shown, structural changes of proteins disturbed by small drug molecules are unseen in traditional ESI-MS^n, but the introduction of temperature dimension in this study can reveal subtle interactions of flavonols on ubiquitin.

The half-wave collision energy ($E_{1/2}$) is defined as the collision energy at which the relative abundance of the complex precursor ion, reaches 0.5 [71]. $E_{1/2}$ corresponds to T_m in a ubiquitin melting curve. The larger the $E_{1/2}$ value, the more stable the complex in the gas phase. As depicted in Figs. 5a and b, quercetin gives similar $E_{1/2}$ values for its complexes with ubiquitin at different temperatures. The similarity in the dissociation profiles suggests that the noncovalent complexes adopt very similar gas-phase conformations even when they transition into an unfolded state. Meanwhile, as the molar ratio of the complex increases from 1:1 to 1:2, the $E_{1/2}$ value increase significantly. The observed greater stability of U-Q_2 complex may be due to the stronger and more opportunities of intramolecular interactions between the two ligands. This pattern is consistent with the data shown in Figs. 5c and d. Rutin exhibits comparable $E_{1/2}$ values for its interactions with ubiquitin across various temperatures.

It is also worth noting that when ubiquitin with one ligand is activated, it dissociates to give back the ubiquitin. When ubiquitin with two ligands is activated, it initially loses one ligand before reverting to ubiquitin itself (Figs. S4 and S5 in Supporting information). The CID results of corresponding ubiquitin-flavonol complexes fail to reflect the solution behavior. This can be attributed to the destruction of the specific interaction between the flavonols and the ubiquitin in the protonated complex. A possible explana-

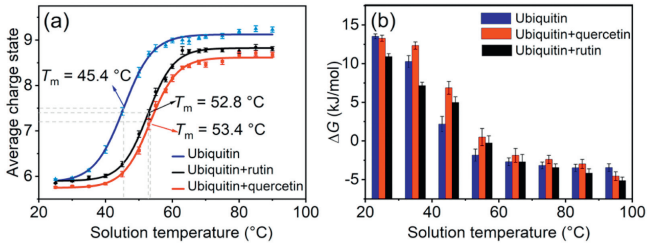


Fig. 6. (a) Weighted average charge state *versus* solution temperature for ubiquitin (blue triangles), ubiquitin + rutin (red circles), ubiquitin + quercetin (black squares). The midpoint melting temperatures (T_m) determined from sigmoidal curve are shown with colored arrows. (b) ΔG values for ubiquitin (blue rectangle), ubiquitin + rutin (red rectangle), and ubiquitin + quercetin (black rectangle) are calculated at various temperatures. The values are presented as mean ± standard deviation ($n = 3$).

tion is that the charge state of U-Q or U-R depends on the aquas structure. Thus, U-Q or U-R with the same charge state but produced from different temperatures have the same or similar conformations, resulting in unchanged gas phase stability. Therefore, the addition of small molecule ligands under different temperature conditions does not alter the gas-phase stability of the complex, in contrast to the alterations observed in the liquid phase. However, it should be noted that the comparison between gas phase with solution stabilities does not provide insights into the stability changes of ubiquitin itself under the influence of quercetin and rutin.

To even further understand the thermal denaturation of ubiquitin in the presence of quercetin and rutin, thermal stability is investigated [72]. The average charge states of ubiquitin in the mixed solutions are then calculated from the intensities, and plotted as a function of temperature (Fig. 6a).

In the presence of quercetin or rutin, the melting temperatures of ubiquitin are both increased from 45.4 °C to 53.4 °C or 52.8 °C, respectively. The results indicate that the binding of quercetin and rutin significantly enhances the stability of ubiquitin. Yet the shape of the melting curve appears to be similar to ubiquitin individually, suggesting no big changes happen during the ubiquitin unfolding process. One common approach to assess ligand binding affinities is by calculating the ΔT_m values obtained from experiments conducted with and without the ligand. Based on MS-melting studies, the two flavonols obtain similar ΔT_m values and show their significant stabilization.

Gibbs free energy (ΔG) derived from the MS-melting experiment is used to simulate the direction of unfolding process of proteins during heating. As the solution temperature increases, the folded ubiquitin transforms into the unfolded form, and the unfolding process can be described and calculated (Eqs. S2 and S3 in Supporting information). As shown in Fig. 6b, in the presence of quercetin and rutin, ΔG increases significantly as the temperature increases from 25 °C to 55 °C, demonstrating that ubiquitin necessitates higher energy input for denaturation. However, ΔG does not change significantly at above 55 °C, implying that ubiquitin has the similar tendency to unfold. Notably, at 55 °C, ΔG values of isolated ubiquitin are negative, but in the presence of quercetin and rutin, ΔG values remain positive. This demonstrates that in a solution containing only ubiquitin, a spontaneous unfolding process occurs at this temperature.

In summary, TC-ESI-MS^n is used to study the interactions of ubiquitin with two typical flavonols: quercetin and rutin in both solution and gas phases. The interactions at different temperature conditions can be obtained and determined from the MS data. The capability to distinguish and quantify distinct binding stoichiometries, binding affinities and stabilities in both solution and gas phases at various temperatures offers detailed insights into the complexes formed by ubiquitin and flavonols.

These results further demonstrate that TC-ESI-MS^n also has the capacity to investigate protein T_m and ΔG that aligns with conventional technologies. In the presence of quercetin and rutin, the T_m and ΔG of ubiquitin are significantly higher than ubiquitin alone, indicating an enhanced stability of ubiquitin. As compared to conventional ESI-MS^n experiment, TC-ESI-MS^n has yielded more valuable insights into the protein-small molecule complexes. It simultaneously and rapidly compares the stability of complexes in both the gas and condensed phases, and further reveals the intrinsic property of proteins. Since there are quite few studies on ubiquitin-flavonol complexes using TC-ESI-MS^n previously, this combined strategy will provide new insights into proteins and inspire further studies on a wider-range of protein-small molecule interactions.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Wen Su: Writing – review & editing, Writing – original draft, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Siying Liu:** Writing – review & editing, Software, Methodology, Formal analysis, Data curation. **Qingfu Zhang:** Methodology, Investigation. **Zhongyan Zhou:** Software. **Na Wang:** Writing – review & editing, Funding acquisition, Conceptualization. **Lei Yue:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2024.110237.

References

[1] F. Chen, B. Gülbakan, S. Weidmann, et al., Mass Spectrom. Rev. 35 (2016) 48–70.
[2] J.A. Loo, Mass Spectrom. Rev. 16 (1997) 1–23.
[3] J.L. Bennett, G.T. Nguyen, W.A. Donald, Chem. Rev. 122 (2022) 7327–7385.
[4] C.F. Lourenço, B. Gago, R.M. Barbosa, et al., J. Agric. Food Chem. 56 (2008) 3798–3804.
[5] J.J. Peterson, J.T. Dwyer, P.F. Jacques, et al., Nutr. Rev. 70 (2012) 491–508.
[6] P. Xin, S. Han, J. Huang, et al., Chin. Chem. Lett. 34 (2023) 108125.
[7] D. Treutter, Environ. Chem. Lett. 4 (2006) 147–157.
[8] J. Laoué, C. Fernandez, E. Ormeño, Plants 11 (2022) 172.
[9] X. Zhang, J. Li, K.Z. Lu, et al., Chin. Chem. Lett. 35 (2024) 109456.
[10] C.D. Kanakis, I. Hasni, P. Bourassa, et al., Food Chem. 127 (2011) 1046–1055.
[11] Y. Yan, J. Hu, P. Yao, Langmuir 25 (2009) 397–402.
[12] V. de Freitas, N. Mateus, Curr. Org. Chem. 16 (2012) 724–746.
[13] A. Papadopoulou, R.A. Frazier, Trends Food Sci. Technol. 15 (2004) 186–190.
[14] J. Xiao, G. Kai, Crit. Rev. Food Sci. 52 (2012) 85–101.
[15] J. Xi, R. Guo, Int. J. Biol. Macromol. 40 (2007) 305–311.
[16] J.D. Eschweiler, R. Kerr, J. Rabuck-Gibbons, et al., Annu. Rev. Anal. Chem. 10 (2017) 25–44.
[17] A.J. Heck, Nat. Methods 5 (2008) 927–933.

[18] N.P. Barrera, S.C. Isaacson, M. Zhou, et al., Nat. Methods 6 (2009) 585–587.
[19] J. Marcoux, C.V. Robinson, Structure 21 (2013) 1541–1550.
[20] N.P. Barrera, C.V. Robinson, Annu. Rev. Biochem. 80 (2011) 247–271.
[21] L. Ma, S. Liu, N.S. Xu, et al., Chin. Chem. Lett. 25 (2014) 1179–1184.
[22] Z. Guo, Y. Liu, N. He, et al., Chin. Chem. Lett. 32 (2021) 40–47.
[23] E.B. Erba, R. Zenobi, Annu. Rep. Prog. Chem. Sect. C 107 (2011) 199–228.
[24] D. Rathore, A. Faustino, J. Schiel, et al., Expert Rev. Proteomics 15 (2018) 431–449.
[25] C. Guo, X. Zhang, X. Hong, et al., Chin. Chem. Lett. 35 (2024) 108867.
[26] X. Chang, N. Wang, D. Jiang, et al., Chin. Chem. Lett. 34 (2023) 107522.
[27] H. Hernández, C.V. Robinson, Nat. Protoc. 2 (2007) 715–726.
[28] D.D. Vallejo, C. Rojas Ramiŕez, K.F. Parson, et al., Chem. Rev. 122 (2022) 7690–7719.
[29] R. Wu, J.B. Metternich, A.S. Kamenik, et al., Nat. Commun. 14 (2023) 2913.
[30] J.F. Van Dyck, J.R. Burns, K.I.P. Le Huray, et al., Nat. Commun. 13 (2022) 3610.
[31] K.G. Hicks, A.A. Cluntun, H.L. Schubert, et al., Science 379 (2023) 996–1003.
[32] Y. Zhong, L. Han, B.T. Ruotolo, Angew. Chem. Int. Ed. 126 (2014) 9363–9366.
[33] A. Al-jabiry, M. Palmer, J. Langridge, et al., Chemistry 27 (2021) 13783–13792.
[34] L. McAlary, J.A. Harrison, J.A. Aquilina, et al., Anal. Chem. 92 (2020) 1702–1711.
[35] S.K. Chowdhury, V. Katta, B.T. Chait, J. Am. Chem. Soc. 112 (1990) 9012–9013.
[36] B. Ibarra-Molero, A.N. Naganathan, J.M. Sanchez-Ruiz, et al., Modern analysis of protein folding by differential scanning calorimetry, in: A. Feig (Ed.), Methods in Enzymology, Academic Press, New York, 2016, pp. 281–318.
[37] F.E. Torres, M.I. Recht, J.E. Coyle, et al., Curr. Opin. Struct. Biol. 20 (2010) 598–605.
[38] R. Mahran, N. Vello, A. Komulainen, et al., Sci. Rep. 13 (2023) 20066.
[39] G. Wang, R.R. Abzalimov, I.A. Kaltashov, Anal. Chem. 83 (2011) 2870–2876.
[40] A. Pruska, A. Marchand, R. Zenobi, Angew. Chem. Int. Ed. 60 (2021) 15518–15526.
[41] G. Li, S. Zheng, Y. Chen, et al., Anal. Chem. 90 (2018) 7997–8001.
[42] T.J. El-Baba, D.W. Woodall, S.A. Raab, et al., J. Am. Chem. Soc. 139 (2017) 6306–6309.
[43] S.A. Raab, T.J. El-Baba, D.W. Woodall, et al., J. Am. Chem. Soc. 142 (2020) 17372–17383.
[44] A. Laganowsky, D.E. Clemmer, D.H. Russell, Annu. Rev. Biophys. 51 (2022) 63–77.
[45] J.L.P. Benesch, F. Sobott, C.V. Robinson, Anal. Chem. 75 (2003) 2208–2214.
[46] J.S. Jordan, E.R. Williams, Anal. Chem. 94 (2022) 16894–16900.
[47] A. Marchand, M.F. Czar, E.N. Eggel, et al., Nat. Commun. 11 (2020) 566.
[48] A. Pruška, J.A. Harrison, A. Granzhan, et al., Anal. Chem. 95 (2023) 14384–14391.
[49] A. Marchand, F. Rosu, R. Zenobi, et al., J. Am. Chem. Soc. 140 (2018) 12553–12565.
[50] T.E. Walker, M. Shirzadeh, H.M. Sun, et al., J. Am. Chem. Soc. 144 (2022) 2667–2678.
[51] X. Cong, Y. Liu, W. Liu, et al., J. Am. Chem. Soc. 138 (2016) 4346–4349.
[52] K.J. Light-Wahl, B.L. Schwartz, R.D. Smith, J. Am. Chem. Soc. 116 (1994) 5271–5278.
[53] K.J. Light-Wahl, B.E. Winger, R.D. Smith, J. Am. Chem. Soc. 115 (1993) 5869–5870.
[54] L. Dai, N. Guo, Y. Liu, et al., Chin. Chem. Lett. 30 (2019) 103–106.
[55] L. Deng, E.N. Kitova, J.S. Klassen, J. Am. Soc. Mass Spectrom. 24 (2013) 988–996.
[56] M.C. Jecklin, D. Touboul, R. Jain, et al., Anal. Chem. 81 (2009) 408–419.
[57] S. Yin, Y. Xie, J.A. Loo, J. Am. Soc. Mass Spectrom. 19 (2011) 1199–1208.
[58] J.A. Harrison, A. Pruška, I. Oganesyan, et al., Chem. Eur. J. 27 (2021) 18015–18028.
[59] T. Wyttenbach, M.T. Bowers, J. Phys. Chem. B 115 (2011) 12266–12275.
[60] T.J. El-Baba, D.R. Fuller, D.W. Woodall, et al., Chem. Commun. 54 (2018) 3270–3273.
[61] K.R. Babu, A. Moradian, D. Douglas, J. Am. Soc. Mass Spectrom. 12 (2001) 317–328.
[62] M. Jourdan, M.S. Searle, Biochemistry 39 (2000) 12355–12364.
[63] C. Wan, M. Cui, F. Song, et al., Int. J. Mass Spectrom. 283 (2009) 48–55.
[64] C.L. Mazzitelli, Y. Chu, J.J. Reczek, et al., J. Am. Soc. Mass Spectrom. 18 (2007) 311–321.
[65] Z. Jiang, T. Li, L. Ma, et al., Food Res. Int. 131 (2020) 109006.
[66] Y. Jia, X. Yan, Y. Huang, et al., Food Chem. 396 (2022) 133685.
[67] C. Ren, W. Xiong, J. Li, et al., Food Hydrocolloid 92 (2019) 155–162.
[68] J. Ye, L. Deng, Y. Wang, et al., Food Chem. 362 (2021) 130238.
[69] J. Li, R. Tian, G. Liang, et al., Food Chem. 355 (2021) 129617.
[70] I.J. Joye, G. Davidov-Pardo, R.D. Ludescher, et al., Food Chem. 185 (2015) 261–267.
[71] K.X. Wan, M.L. Gross, T. Shibue, J. Am. Soc. Mass Spectrom. 11 (2000) 450–457.
[72] A. Jarzab, N. Kurzawa, T. Hopf, et al., Nat. Methods 17 (2020) 495–503.