Thermokinetic Analysis of Protein Subunit Exchange by Variable-Temperature Native Mass Spectrometry

Senthil K. Thangaraj,† Salman James,† Juha Rouvinen and Janne Jänis†

†Department of Chemistry, University of Eastern Finland, PO BOX 111, FI-801101, Joensuu, Finland

Supporting Information Placeholder

ABSTRACT: Many protein complexes are assembled from a varying number of subunits, which are continuously exchanging with diverse timescales. This structural dynamics is considered important for many regulatory and sensory adaptation processes occurring *in vivo*. We have developed an accurate method to monitor protein subunit exchange by using native electrospray ionization mass spectrometry (ESI-MS), exemplified here for an extremely stable Rad50 zinc hook (Hk) dimer assembly, $Zn(Hk)_2$. The method has two steps: appropriate protein/peptide mutation and native ESI-MS analysis using a variable-temperature sample inlet. In this work, two Hk mutants were produced, mixed with the wild-type Hk and measured at three different temperatures. A thermokinetic analysis of the heterodimer formation allowed us to determine enthalpy, entropy and Gibbs free energy of activation for the subunit exchange, showing that the reaction is slow and associated with a high enthalpic barrier, consistent with exceptionally high stability of the $Zn(Hk)_2$ assembly.

Deciphering kinetics and assembly/disassembly pathways of protein complexes is a key to understand their function at a molecular level.[1,2] Biologically active signals are produced when proteins interact or reversibly bind to other proteins, ligands or metal ions, which is often critical for many cellular processes, e.g., DNA repair, replication, metabolism, and catalysis.[3] These interactions can be either "permanent" or transient".[4] Many protein complexes are assembled from two or more identical or non-identical subunits, which are continuously exchanging with diverse timescales.[5] This kind of structural dynamics is thought to be important in many regulatory and sensory adaptation processes, thus its quantification is an important part of protein structure-function studies.

A simplest situation, where subunit exchange is present, is protein homodimerization. Let's consider the dimerization of two proteins, a wild-type protein (W) and its mutant (M). Both proteins undergo homodimerization according to Equations 1 and 2:

$$W + W \leftrightarrows WW \quad (1)$$

$$M + M \leftrightarrows MM \quad (2)$$

Both homodimers WW and MM have characteristic equilibrium constant (K_{eq}) values, which are defined by the association and dissociation rate constants (k_1 and k_{-1}, respectively). Assuming that both K_{eq}(WW) and K_{eq}(MM) were large compared to the total protein concentrations, $[AA]_{tot}$ and $[BB]_{tot}$, the homodimers WW and MM dominate in solution. Upon mixing of the two protein samples, however, a heterodimer WM gradually forms from the free monomers W and M, according to equation 3:

$$2\ \{W + M \leftrightarrows WM\} \quad (3)$$

The statistical factor of 2 implies that for each mole of WW and MM, two moles of WM are produced. Let's consider a special case, in which $K_{eq}(WW) = K_{eq}(MM) \approx K_{eq}(WM)$, and $[WW]_{total} \approx [MM]_{total}$; in that case, the net dimer redistribution reaction can be described by Equation 4:

$$WW + MM \leftrightarrows 2WM \quad (4)$$

For both forward and backward reactions, the rate constant is k_{-1}, which is rate-limiting for the subunit exchange. Thus, at equilibrium, a statistical 1:2:1 distribution of the dimers WW, WM, and MM will be obtained, and the overall equilibrium constant for the reaction is $K_{eq} = k_{-1}/k_{-1} = 1$. While direct measurement of K_{eq} does not give information on subunit exchange, the quantification of k_{-1} at a varying temperature can be used to measure kinetics and thermodynamics of the process. This requires that time-dependent concentrations of WW, MM and WM can be measured.

There are a number of experimental techniques to study subunit exchange of protein complexes, including fluorescence-based methods,[6–9] surface plasmon resonance (SPR),[10] and small-angle neutron scattering (SANS).[11] In addition, electron paramagnetic resonance (EPR) and nuclear magnetic resonance (NMR) spectroscopies have been applied.[12] While fluorescence- and NMR-based methods have been widely used, protein labeling with radicals or stable isotopes is needed in order

to monitor the exchange reaction. These methods have also inability to provide binding stoichiometries or relative abundances of oligomeric species co-existing in solution.

Electrospray ionization mass spectrometry (ESI-MS) is one of the most versatile analytical tools for structural elucidation of proteins.[13] When performed in nativelike solution conditions (a technique termed native MS),[14] which preserve proteins in their native, folded state, protein-ligand or protein-protein interactions, conformational changes or structural dynamics can be studied.[15–24] The biggest advantages of native MS are that protein labeling by chromophores or isotopes is not needed and stoichiometrical information is directly obtained. Moreover, kinetics and thermodynamics of interactions can be determined. Native ESI-MS has been previously employed for direct observation of protein subunit exchange as well.[23,25–28] However, many of these previous studies are concerning hetero-oligomeric protein complexes.

In this study, we developed a new experimental approach to investigate the kinetics of protein subunit exchange by using native ESI coupled with a high-resolution Fourier transform ion cyclotron resonance (FT-ICR) instrument. We first, developed a simple and effective hot-gun based sample inlet system to accurately alter the sample solution temperature prior to ESI. This system is based on the principle of performing a continuous flow of a reaction mixture at a constant temperature for ESI-MS analysis. To do this, first, a customized insulator box was made; second, a hot gun was placed inside the box to control an inner temperature where a syringe pump is located; finally, the temperatures of both the syringe and the atmospheric air inside the box were constantly monitored using a thermometer. By this way, the inner temperature could be accurately adjusted between the room temperature and 40 °C. A major advantage of this setup is to avoid a carryover, which is a typical problem during the off-line ESI-MS analysis. Furthermore, the results are highly consistent and reproducible with minimal sample consumption.

The use of the newly developed variable-temperature sample inlet for native MS was exemplified with an extremely stable, homodimeric zinc hook assembly, $Zn(Hk)_2$. This structural assembly, natively present in the Rad50 DNA-repair protein complex,[29,30] is based on two identical 14-residue Hk peptide motifs, which coordinate a single Zn^{2+} ion by four cysteine residues (two on each Hk peptide) in a tetrahedral fashion (**Figure 1**). This assembly has been studied previously and it has been shown to possess an extremely high thermodynamic stability ($-\log K_d \approx 19.19$), driven largely by entropy.[29,31] Since $Zn(Hk)_2$ is a homodimeric structural assembly made from the two identical Hk motifs, we produced two mutant peptides, whose masses differ from the wild-type peptide (Hk-wt), but retain the overall fold as well as the zinc binding affinity. In this way, native MS can be used to directly observe subunit exchange in $Zn(HK)_2$ in a time-dependent manner.

There were two mutants, namely T452A and E450D, used for the subunit exchange reactions. These mutants were designed and synthesized based on the previous reports by Kochańczyk *et al*.[29,31] The single-alanine mutant Hk-T452A was reported to have the same overall zinc affinity as Hk-wt ($-\log K_d \approx 19.17$) towards the Zn^{2+} ion.[31] In addition, we also designed another mutant with glutamic acid changed to aspartic acid (Hk-E450D), having a 14-Da mass difference to Hk-wt. Both mutated residues are solvent exposed in the β-hairpin forming $Zn(Hk)_2$ assembly. All peptides were terminated with an extra tryptophan residue for the UV detection (**Figure 1**)

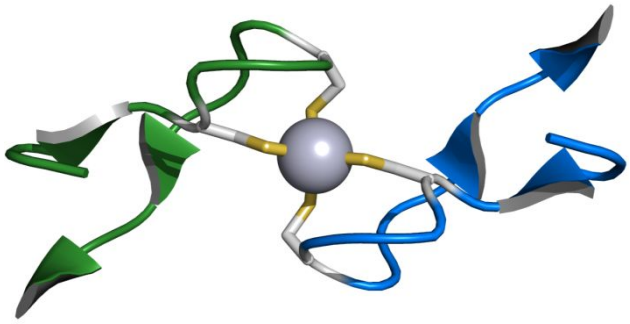

Figure 1. Structure of the zinc hook (Hk) dimer assembly of Rad50 (PDB: 1L8D; UNIPROT: P58301), composed of two identical Hk motifs (residues 440–453) and a tetrahedrally coordinated zinc ion (a grey sphere). Amino acid sequences are for the wild-type Hk peptide (Hk-wt) and the mutants T452A and E450D. The N-terminal W is a non-natural tryptophan residue, added to the construct for UV detection.

The mass spectrum of Hk-wt was initially measured in denaturing solution conditions (CH_3CN:H_2O:HOAc, 49.5:49.5:1 *v/v*), to verify the amino acid sequence and purity of the sample **(Figure S1a)**. The experimental monoisotopic mass of Hk-wt (1661.8086 Da) was perfectly matching with the mass calculated from the sequence (1661.8018 Da). No signs of zinc ion binding were observed. This indicates that in these conditions, Hk-wt existed as a fully unfolded monomer in solution. Free cysteines in small peptides (two in the Hk construct) are prone to quickly oxidize in solution, producing both intra- and intermolecular disulfide bonds. To ensure that the peptides remained fully reduced, a reducing agent (1 mM dithiothreitol; DTT) was added to the samples.[32]

To examine the zinc ion binding and dimerizartion of Hk-wt, we attempted to use pure water or 20 mM ammonium acetate (pH 6.9) as a solvent for the ESI-MS analysis. However, re-oxidation occurred very rapidly in the absence of Zn^{2+} and even in the presence of DTT. After many trials of solvents, we found CH_3CN:20 mM

NH_4OAc (50:50, *v/v*) mixture as an optimal solvent for this. In these conditions, the oxidation rate was much reduced. The mass spectra measured in native-like solution conditions, in the absence and presence of zinc, are presented in **Figures S1b and S1c**. The experimental and theoretical masses are reported in **Table S1**. In the absence of zinc, two signals were observed in the mass spectrum, one representing a free monomeric peptide and the other representing a complex with a single Zn^{2+} ion (~1:2 abundance ratio). The Zn^{2+} ion binding of the zinc hook peptide can be described by the two consequtive reactions 5 and 6:

$$Zn^{2+} + Hk \leftrightarrows Zn(Hk) \quad (5)$$

$$Zn(Hk) + Hk \leftrightarrows Zn(Hk)_2 \quad (6)$$

Thus, at substoichiometric zinc concentrations, free Hk and a monomeric Zn(Hk) complex dominate in solution. Traces of zinc can always be found in the sample solutions; therefore the monomeric zinc complex Zn(Hk) is observable even without addition of zinc to the samples. The zinc ion binding is also highly dependent on pH, due to protonation of the coordinating cysteine residues; thus at low pH, the free Hk dominates.[29,31] In contrast, the mass spectrum measured for Hk-wt in the presence of zinc (5-fold molar excess) showed the dimeric $Zn(Hk)_2$ complex as a major species in solution (**Figure S1c**). The experimental monoisotopic mass (3385.4554 Da) agreed well to the theoretical mass calculated for $Zn(Hk)_2$ complex (3385.5171 Da).

Additionally, we evaluated whether a few other metals (Co^{2+}, Mn^{2+}, or Au^{3+}) could efficiently coordinate to the zinc hook peptide and induce a dimeric complex formation. Both Co^{2+} and Mn^{2+} have been previously used in many spectroscopic studies of zinc finger (ZnF) proteins due to their ability to coordinate to zinc binding sites. In addition, Au^{3+} ions can also coordinate to ZnFs in a bidentate fashion. Among the metals tested, Hk-wt was able to bind Co^{2+} which partially replaced Zn^{2+} ion in the dimeric $Zn(Hk)_2$ complex, although the binding affinity was low (**Figure S2**). The replacement of zinc ion by other metals in ZnF proteins may cause structural defects such as incomplete metal ion coordination or oxidation of cysteine residues.[3,33,34] Neither of the other two metals had any measurable affinity with Hk-wt.

Ultimately, we sought to analyze the subunit exchange reactions of Hk-wt with its mutants. The preliminary ESI FT-ICR MS measurements with both mutants in denaturing and near-native conditions indicated that the experimental monoisotopic masses perfectly matched with the theoretical ones **(Figure S3 and Table S2)**. Further, to monitor the kinetics of the subunit exchange, an equimolar mixture of Hk-wt and T452A (both 5 μM) in the presence of 25 μM Zn(II) and 1 mM DTT in CH_3CN: 20 mM NH_4OAc (50:50, *v/v*; pH 6.8) was measured at three different temperatures (25, 30, and 40 °C) and the progress of the subunit exchange was monitored (see, Supporting Information for technical details).

Figure 2 is a representative ESI FT-ICR mass spectrum of the subunit exchange reaction of Hk-wt and Hk-T452A at 25 °C measured 5, 60, and 160 after mixing the two peptides. The mass spectra were measured at 5 min intervals until the equilibrium was reached. The represengtative mass spectra measured at 30 and 40 °C are presented in **Figure S4**.

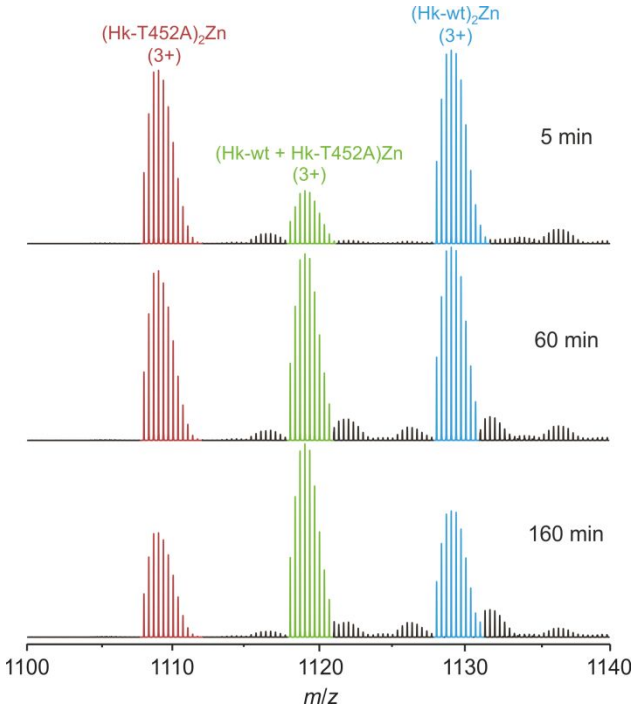

Figure 2. Native ESI FT-ICR mass spectra of the dimeric zinc-bound complexes (3+ charge state) of an equimolar mixture of the zinc hook peptides Hk-wt and Hk-T452A (1 μM); 5, 60, and 160 min after mixing.

After 5 min of mixing at 25°C, the zinc-bound homodimers of Hk-wt (blue trace) and Hk-T452A (red trace) were observed predominantly along with a minor signal of the heterodimer, Zn(Hk-wt + Hk-T452A) (green trace). With respect to the increasing reaction time, the abundance of both homodimers slowly decayed and the heterodimer started to dominate ca. 1 h after mixing. After 160 min of reaction time, the signals of the three dimers approximated an abundance ratio of 1:2:1. At even longer time points, this distribution of signals remained the same, indicating that the equilibrium was reached. Such the same scenario was noticed at the other two temperatures as well, with the rate getting faster with an increasing temperature. Similar subunit exchange reactions were performed with Hk-wt and Hk-E450D at 25, 30, and 40 °C. The mass spectra are presented in **Figure S5**.

To determine rate constants (k) for the exchange reactions, we determined the homodimer-to-heterodimer

peak (R_{dimer}) ratios from the mass spectra, and used a single-phase exponential decay function 7,

$$R_{dimer} = A\, e^{-kt_{react}} + B \quad (7)$$

to follow the kinetics of subunit exchange process by plotting R_{dimer} as a function of reaction time (t_{react}), measured at the indicated reaction temperatures.[36] The scaling factors $A = B = 0.5$, since, at equilibrium, a statistical distribution of 1:2:1 is observed, and the zinc hook peptides were mixed at the 1:1 molar ratio in the beginning. The data were fitted to this equation with non-linear regression to obtain the values of k (**Figure 3a**). The rate constants ($k = k_{-1}$) for subunit exchange of Hk-wt with Hk-T452A at 25, 30, and 40 °C were determined to be 1.31×10^{-2}, 1.92×10^{-2} and 3.46×10^{-2}, respectively.

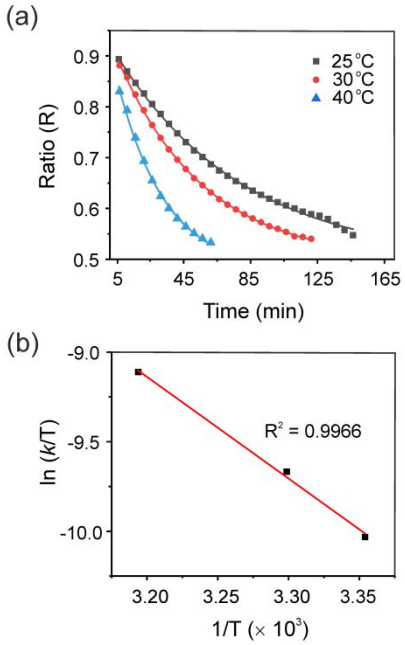

Figure 3. Subunit exchange of the zinc-bound dimer of Hk-wt: and Hk-T452A. (a) The primary kinetic plots (R_{dimer} vs t_{react}) using a first-order exponential decay equation at 25, 30, and 40 °C. (b) The secondary plot ($\ln k/T$ *vs* $1/T$) to determine Gibbs free energy ($\Delta G^{\ddagger}$), entropy ($\Delta S^{\ddagger}$) and enthalpy ($\Delta H^{\ddagger}$) of activation for subunit exchange using Eyring-Polanyi equation.

The values entropy ($\Delta S^{\ddagger}$) and enthalpy ($\Delta H^{\ddagger}$) of activation for subunit exchange were then obtained by plotting the $\ln(k/T)$ versus $1/T$ of absolute temperature and the data were fitted using an Eyring–Polanyi equation (8):

$$k = \frac{k_B T}{h} e^{-\Delta H^{\ddagger}/RT} e^{-\Delta S^{\ddagger}/R} \quad (8)$$

The linear form of Eyring-Polanyi equation is,

$$ln\frac{k}{T} = \frac{-\Delta H^{\ddagger} 1}{R \quad T} + ln\frac{\kappa\, k_B}{h} + \frac{\Delta S^{\ddagger}}{R} \quad (9)$$

where, k is the subunit rate constant (s^{-1}), T is the absolute temperature (K), $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are the standard enthalpy and entropy of activation, R is the universal gas constant, k_B is the Boltzmann constant, κ is a transmission coefficient (usually one) and h is the Planck's constant. The slope and the intercept of the plot can be used to determine the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ (see, Supporting Information for details)

The Gibbs free energy (at 298 K) was then obtained through a standard Gibbs equation, $\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$.

Table 1. Thermokinetic parameters of subunit echange of zinc-bound dimers of the wild-type zinc hook peptide Hk-wt with Hk-T452A or Hk-E450D.

Mutant	$\Delta G^{\ddagger}$ (kcal/mol) at 298 K	$\Delta H^{\ddagger}$ (kcal/mol)	$\Delta S^{\ddagger}$ (cal/mol K)
T452A	20.01	11.3 ± 0.7	–29.2 ± 3.5
E450D	20.29	15.8 ± 1.2	–14.9 ± 1.7

Figure 3b shows the Eyring plot constructed from ln *(k/t)* vs *1/T*, which exhibits a linear behavior with R^2 value of 0.9966. The primary and secondary kinetics plots of Hk-wt with Hk-E452D are shown in **Figure S6**. The corresponding thermokinetic parameters for subunit exchange of Hk-wt with Hk-T452A are presented in **Table 1**, which revealed that the subunit exchange reaction of zinc hook dimer assembly the reaction is slow and associated with a high enthalpic barrier, consistent with exceptionally high stability of the $Zn(Hk)_2$ assembly. Moreover, the negative value for entropy of activation suggests that the subunit exchange of $Zn(Hk)_2$ occurs via associative rather than dissociative reaction pathway. However, structural basis for this remains elusive at this point. The obtained thermokinetic values between the two Hk mutants, Hk-452A and Hk-E450D were almost indistinguishable regarding the obtained $\Delta G^{\ddagger}$ values, suggesting that both mutants can be used to study the subunit exchange in the $Zn(Hk)_2$ assembly. However, the values of entropy and enthalpy of activation varied quite substantially, suggesting that even subtle changes in the amino acid residues located in the solvent exposed surface of a protein can affect the overall structure and its dynamics.

In conclusion, the current study provides a detailed thermokinetic analysis of subunit exchange of an extremely stable zinc hook assembly. A precise control over the sample solution temperature and high reproducibility of the ESI FT-ICR MS analysis provides accurate estimates for thermokinetic parameters. The same ESI-MS based approach can also be extended to study subunit exchange of any oligomeric protein complexes provided that 1) appropriate mutation(s) can

be found and 2) time-scale of the process is reachable by on-line ESI-MS analysis. One possibility is to use isotopic labeling instead of direct amino acid mutations; however this is prone to signal broadening due to partial heavy atom incorporation as well as high cost for the isotopically labeled reagents.

ACCESSION ID FOR THE PROTEIN

UNIPROT: P58301

ASSOCIATED CONTENT

Supporting Information

The supporting information is available free of charge on the ACS Publication website at DOI:
Experimental details (sample preparation, subunit exchange procedure, instrumentation), supplementary mass spectra and tables, and kinetic plots. (PDF)

AUTHOR INFORMATION

Corresponding Author

*Email: janne.janis@uef.fi

Address

Department of Chemistry, University of Eastern Finland, PO BOX 111, FI-801101, Joensuu, Finland.

ORCID

Janne Jänis: 0000-0002-8446-4704

ACKNOWLEDGMENTS

The financial support from the Biocenter Kuopio, the European Regional Development Fund (Grant A70135), and the EU's Horizon 2020 Research and Innovation Programme (EU FT-ICR MS project; grant agreement 731077) is gratefully acknowledged.

REFERENCES

(1) Marsh, J. A.; Teichmann, S. A. Structure, Dynamics, Assembly, and Evolution of Protein Complexes. Annu. Rev. Biochem. 2015, 84 (1), 551–575.

(2) Marsh, J. A.; Rees, H. A.; Ahnert, S. E.; Teichmann, S. A. Structural and Evolutionary Versatility in Protein Complexes with Uneven Stoichiometry. Nat. Commun. 2015, 6, 6394.

(3) Witkiewicz-Kucharczyk, A.; Bal, W. Damage of Zinc Fingers in DNA Repair Proteins, a Novel Molecular Mechanism in Carcinogenesis. Toxicol. Lett. 2006, 162 (1), 29–42.

(4) Perkins, J. R.; Diboun, I.; Dessailly, B. H.; Lees, J. G.; Orengo, C. Transient Protein-Protein Interactions: Structural, Functional, and Network Properties. Structure 2010, 18 (10), 1233–1243.

(5) Tusk, S. E.; Delalez, N. J.; Berry, R. M. Subunit Exchange in Protein Complexes. J. Mol. Biol. 2018, 430 (22), 4557–4579.

(6) Bova, M. P.; Huang, Q.; Ding, L.; Horwitz, J. Subunit Exchange, Conformational Stability, and Chaperone-like Function of the Small Heat Shock Protein 16.5 from Methanococcus Jannaschii. J. Biol. Chem. 2002, 277 (41), 38468–38475.

(7) Chen, Z.; Chang, K.; Capraro, B. R.; Zhu, C.; Hsu, C. J.; Baumgart, T. Intradimer/Intermolecular Interactions Suggest Autoinhibition Mechanism in Endophilin A1. J. Am. Chem. Soc. 2014, 136 (12), 4557–4564.

(8) Merickel, S. K.; Sanders, E. R.; Vázquez-Ibar, J. L.; Johnson, R. C. Subunit Exchange and the Role of Dimer Flexibility in DNA Binding by the Fis Protein. Biochemistry 2002, 41 (18), 5788–5798.

(9) Liang, J. J.; Liu, B.-F. Fluorescence Resonance Energy Transfer Study of Subunit Exchange in Human Lens Crystallins and Congenital Cataract Crystallin Mutants. Protein Sci. 2006, 15 (7), 1619–1627.

(10) Pan, C. Measuring Dissociation Rate Constants of Protein Complexes through Subunit Exchange: Experimental Design and Theoretical Modeling. PLoS One 2011, 6 (12), e28827.

(11) Fukunaga, T.; Yagi, H.; Kurimoto, E.; Kato, K.; Zaccai, G.; Mori, K.; Sugiyama, M.; Hirai, M. Kinetic Asymmetry of Subunit Exchange of Homooligomeric Protein as Revealed by Deuteration-Assisted Small-Angle Neutron Scattering. Biophys. J. 2011, 101 (8), 2037–2042.

(12) Yang, Y.; Ramelot, T. A.; Ni, S.; McCarrick, R. M.; Kennedy, M. A. Measurement of Rate Constants for Homodimer Subunit Exchange Using Double Electron-Electron Resonance and Paramagnetic Relaxation Enhancements. J. Biomol. NMR 2014, 55 (1), 47–58.

(13) Nordhoff, E.; Kirpekar, F.; Roepstorff, P. Mass Spectrometry of Nucleic Acids. Mass Spectrom. Rev. 1996, 15 (2), 67–138.

(14) Heck, A. J. R. Native Mass Spectrometry: A Bridge between Interactomics and Structural Biology. Nat. Methods 2008, 5 (11), 927–933.

(15) Sharon, M.; Robinson, C. . The Role of Mass Spectrometry in Structure Elucidation of Dynamic Protein Complexes. Annu. Rev. Biochem. 2007, 76, 167–193.

(16) Eckart, K.; Spiess, J. Electrospray Ionization Mass Spectrometry of Biotin Binding to Streptavidin. J. Am. Soc. Mass Spectrom. 1995, 6 (10), 912–919.

(17) Liu, L.; Bagal, D.; Kitova, E. Hydrophobic Protein− Ligand Interactions Preserved in the Gas Phase. J. Am. Chem. Soc. 2009, 82, 15980–15981.

(18) Sinz, A. Isotope-Labeled Photoaffinity Reagents and Mass Spectrometry to Identify Protein-Ligand Interactions. Angew. Chemie - Int. Ed. 2007, 46 (5), 660–662.

(19) Daniel, J. M.; McCombie, G.; Wendt, S.; Zenobi, R. Mass Spectrometric Determination of Association Constants of Adenylate Kinase with Two Noncovalent Inhibitors. J. Am. Soc. Mass Spectrom. 2003, 14 (5), 442–448.

(20) Pacholarz, K. J.; Garlish, R. a.; Taylor, R. J.; Barran, P. E. Mass Spectrometry Based Tools to Investigate Protein-Ligand Interactions for Drug Discovery. Chem. Soc. Rev. 2012, 41 (11), 4335.

(21) Loo, J. A. Electrospray Ionization Mass Spectrometry: A Technology for Studying Noncovalent Macromolecular Complexes. Int. J. Mass Spectrom. 2000, 200 (1–3), 175–186.

(22) Beck, J. L.; Lo, A. T. Y.; Wang, Y.; Dixon, N. E.; Mason, C. E.; Jergic, S. Escherichia Coli Single-Stranded DNA-Binding Protein: NanoESI-MS Studies of Salt-Modulated Subunit Exchange and DNA Binding Transactions. J. Am. Soc. Mass Spectrom. 2013, 24 (2), 274–285.

(23) Painter, A. J.; Jaya, N.; Basha, E.; Vierling, E.; Robinson, C. V.; Benesch, J. L. P. Real-Time Monitoring of Protein Complexes Reveals Their Quaternary Organization and Dynamics. Chem. Biol. 2008, 15 (3), 246–253.

(24) Schermann, S. M.; Simmons, D. a; Konermann, L. Mass Spectrometry-Based Approaches to Protein – Ligand Interactions. Expert Rev. Proteomics 2005, 2 (4), 475–485.

(25) Fändrich, M.; Tito, M. A.; Leroux, M. R.; Rostom, A. A.; Hartl, F. U.; Dobson, C. M.; Robinson, C. V. Observation of the Noncovalent Assembly and Disassembly Pathways of the Chaperone Complex MtGimC by Mass Spectrometry. Proc. Natl. Acad. Sci. U. S. A. 2000, 97 (26), 14151–14155.

(26) Sobott, F.; Benesch, J. L. P.; Vierling, E.; Robinson, C. V. Subunit Exchange of Multimeric Protein Complexes. J. Biol. Chem. 2002, 277 (41), 38921–38929.

(27) Aquilina, J. A.; Benesch, J. L. P.; Ding, L. L.; Yaron, O.; Horwitz, J.; Robinson, C. V. Subunit Exchange of Polydisperse Proteins. J. Biol. Chem. 2005, 280 (15), 14485–14491.

(28) Keetch, C. A.; Bromley, E. H. C.; McCammon, M. G.; Wang, N.; Christodoulou, J.; Robinson, C. V. L55P Transthyretin Accelerates Subunit Exchange and Leads to Rapid Formation of Hybrid Tetramers. J. Biol. Chem. 2005, 280 (50), 41667–41674.

(29) Kochanczyk, T.; Nowakowski, M.; Wojewska, D.; Kocyla, A.; Ejchart, A.; Kozminski, W.; Krezel, A. Metal-Coupled Folding as the Driving Force for the Extreme Stability of Rad50 Zinc Hook Dimer Assembly. Sci. Rep. 2016, 6 (October), 2–13.

(30) Hopfner, K. P.; Craig, L.; Moncalian, G.; Zinkel, R. A.; Usui, T.; Owen, B. A. L.; Karcher, A.; Henderson, B.; Bodmer, J. L.; McMurray, C. T.; Carney, J. P.; Petrini, J. H. J.; Tainer, J. A. The Rad50 Zinc-Hook Is a Structure Joining Mre11 Complexes in DNA Recombination and Repair. Nature 2002, 418 (6897), 562–566.

(31) Kochańczyk, T.; Jakimowicz, P.; Krężel, A. Femtomolar Zn(II) Affinity of Minimal Zinc Hook Peptides-a Promising Small Tag for Protein Engineering. Chem. Commun. 2013, 49 (13), 1312–1314.

(32) Scigelova, M.; Green, P.; Giannakopulos, A.; Rodger, A.; Crout, D.; Derrick, P. A Practical Protocol for the Reduction of Disulfide Bonds in Proteins Prior to Analysis by Mass Spectrometry. Eur. J. Mass Spectrom. 2001, 7 (1), 29–34.

(33) Quintal, S. M.; Depaula, Q. A.; Farrell, N. P. Zinc Finger Proteins as Templates for Metal Ion Exchange and Ligand Reactivity. Chemical and Biological Consequences. Metallomics 2011, 3, 121–139.

(34) Shi, Y.; Beger, R. D.; Berg, J. M. Metal Binding Properties of Single Amino Acid Deletion Mutants of Zinc Finger Peptides: Studies Using Cobalt(II) as a Spectroscopic Probe. Biophys. J. 1993, 64 (3), 749–753.

(35) Jänis, J.; Pulkkinen, P.; Rouvinen, J.; Vainiotalo, P. Determination of Steady-State Kinetic Parameters for a Xylanase-Catalyzed Hydrolysis of Neutral Underivatized Xylooligosaccharides by Mass Spectrometry. Anal. Biochem. 2007, 365 (2), 165–173.

(36) Yang, Y.; Ramelot, T. A.; Ni, S.; McCarrick, R. M.; Michael Kennedy, A. Measurement of Rate Constants for Homodimer Exchange Using Double Electron-Electron Paramagnetic Relaxation Enhancements. J Biomol NMR. 2013, 55 (1), 47–58.