Integrative Proteomic Profiling of Protein Activity and Interactions Using Protein Arrays*

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Proteomic studies based on abundance, activity, or interactions have been used to investigate protein functions in normal and pathological processes, but their combinatorial approach has not been attempted. We present an integrative proteomic profiling method to measure protein activity and interaction using fluorescence-based protein arrays. We used an on-chip assay to simultaneously monitor the transamidating activity and binding affinity of transglutaminase 2 (TG2) for 16 TG2-related proteins. The results of this assay were compared with confidential scores provided by the STRING database to analyze the functional interactions of TG2 with these proteins. We further created a quantitative activity-interaction map of TG2 with these 16 proteins, categorizing them into seven groups based upon TG2 activity and interaction. This integrative proteomic profiling method can be applied to quantitative validation of previously known protein interactions, and in understanding the functions and regulation of target proteins in biological processes of interest. Molecular & Cellular Proteomics 11: 10.1074/mcpc1016964, 1167−1176, 2012.

Proteomics is the large-scale analysis of whole proteins and their role in biological systems. Abundance-based proteomics assigns protein functions in normal and pathological processes by quantification of global differences in protein expression levels (1). This classic approach identifies functional biomarkers by comparing samples from healthy individuals and patients. However, this abundance-based approach provides only indirect information about protein function (2). The abundance of a protein is not necessarily correlated with its activity because protein activities are predominantly regulated by a series of post-translational modifications (1, 2). Activity-based proteomics (activity-based protein profiling) is therefore considered an alternative approach to assigning protein functions in biological processes of interest (3). In this approach, specific activity-based probes using fluorescent, radioactive, and affinity tags are usually designed for detection of protein activity (2, 4–6). Activity-based proteomics identifies markers by comparative analyses of activity profiles between healthy and diseased cells and tissues (3, 7, 8). This approach is also used for profiling enzyme inhibitors, for developing therapeutic reagents, and for diagnosis (2, 5). Another functional proteomic approach using large-scale analysis is interactomics or interaction proteomics, which is a useful method for understanding the regulation of proteins in biological systems (9). To elucidate bioactive protein interactions with proteins or ligands, a number of technologies are currently used including the yeast two-hybrid system, affinity purification and mass spectrometry, the protein fragment complementation assay, the luminescence-based mammalian interactome, and protein arrays (9–13). Global differences in the dynamics of the interactome between healthy and diseased individuals provide new insights into causes of disease and can be used for biomarker identification and drug discovery (14–16). Thus, combinatorial analyses of abundance, activity, and interaction have great potential in revealing regulation mechanisms and functions of proteins, although such an integrated proteomic approach has not been widely used.

These proteomic methods have been coupled with various detection methods including one- or two-dimensional gel electrophoresis, one- or two-dimensional liquid chromatography and tandem mass spectrometry, surface plasmon resonance, and fluorometric assays for analyses of the proteome (9, 11). In combination with specific probes, colorimetric and fluorometric assays using multiwell plates have been extensively used for the determination of the abundance and activity of various proteins. Although often limited by the amount of sample, these methods nonetheless facilitate real-time measurement of changes in protein activity and high-throughput analyses of protein abundances and activities (17). Surface plasmon resonance, a method that does not necessitate labeling of proteins, has also been used for analysis of protein abundance, activity, and binding affinity (18–20). Using only very small amounts of sample, the microarray combined with fluorometric probes is a promising technology for the rapid analysis of a wide variety of biomolecular interactions, protein abundances, and activities. This approach has been used for serodiagnosis and identification of biomarkers by abundance-based protein profiling in human sera (21–25). It has also been
used for kinetic studies of carbohydrate-protein (17) and peptide-protein interactions (26, 27). In addition, this technology has been used for the rapid determination of enzyme activities and for the identification of enzyme substrates and inhibitors (24, 28–33). However, combinatorial profiling of protein activities and interactions based on array technology has yet to be reported.

Using protein arrays, we propose as a model system an integrative proteomic approach for simultaneous profiling of the transamidating activity and interactions of transglutaminase 2 (TG2) with TG2-related proteins. TG2, known as tissue transglutaminase, is a member of the calcium-dependent transglutaminase family. Its activity and interactions are associated with a wide variety of diseases and cellular events (34). TG2 is implicated in the pathogenesis of a wide variety of diseases including inflammatory diseases such as celiac sprue, neurodegenerative disorders such as Huntington’s, Alzheimer’s, and Parkinson’s disease, as well as cancers, cardiovascular diseases, and diabetes (34–36). TG2 is also involved in various cellular events including cell growth, cell differentiation, cell adhesion, extracellular matrix crosslinking, and apoptosis (34, 37, 38). In the present study, the transamidating activity and binding affinity of TG2 for 16 proteins were simultaneously monitored using Cy5-conjugated TG2 and protein arrays (Fig. 1). Using this large-scale analysis, we constructed a quantitative activity-interaction (AI)1 map to describe the quantitative interaction of TG2 with its related proteins. Thus, this integrative proteomic approach can be used to characterize functions and regulation mechanisms of a target protein in many biological processes of interest.

EXPERIMENTAL PROCEDURES

Chemicals and Reagents—3-Aminopropyltrimethoxysilane, dithiothreitol, thrombin, Cy3-conjugated streptavidin, human serum albumin (HSA), fibrinogen and fibronectin (from human plasma), α-synuclein (human recombinant), retinoblastoma (human recombinant), E-cadherin (human recombinant), and G-actin were obtained from Sigma. 5-(biotinamido)pentylamine (BAPA) was obtained from Pierce (Rockford, IL). TG2 from guinea pig liver was purchased from Innovative Research (Novi, Michigan). Human recombinant aldolase A, grancalcin, S100 calcium binding protein A7 (S100A7), osteopontin, B-cell lymphoma 2 (Bcl-2), Sma- and Mad-related protein 2 (SMAD2), and annexin A1 were purchased from ATGen (Seongnam, Korea). Cy5 NHS ester and Bio-spin columns were purchased from Amersham Biosciences (Piscataway, NJ) and Bio-Rad (Hercules, CA), respectively. Human recombinant GST and Rac1 (GST-free) were prepared as previously reported (22).

Preparation of Amine Arrays—Amine-modified glass slides were fabricated according to the procedures of Jung et al. (22). Briefly, glass slides were cleaned with a solution of NH2OH·H2O·H2O (1:1:5, v/v/v) at 70 °C for 10 min. The slides were immersed in 1.5% 3-aminopropyltrimethoxysilane solution (v/v) in 95% ethanol for 2 h, dried under air, and baked at 110 °C. Teflon tape containing arrayed holes of 1.5 mm diameter were attached to the modified glass slides to prepare well-type amine arrays.

Conjugation of TG2 and Its Related Proteins with Cy5 NHS Ester and Characterization of the Cy5-conjugates—TG2 was labeled with Cy5 NHS ester as previously reported (22). TG2 (100 μl, 100 μg/ml) in 100 mM sodium bicarbonate buffer (pH 8.3) was mixed with 2 μl of 1 mg/ml Cy5 mono NHS-ester in 10% dimethyl sulfoxide and incubated for 2 h on ice. To quench the reactions, 5 μl of 1 M Tris-HCl (pH 8.0) was added to the reaction solution. Reaction mixtures were loaded onto 1.5 ml Sephadex G-25 columns, and Cy5-conjugated TG2 was eluted by centrifugation for 3 min at 1050 × g. To calculate the molar extinctions of Cy5 and TG2 in Cy5-conjugated TG2, molar extinction coefficients of pure TG2 at 280 nm and Cy5 at 280 and 650 nm were determined using the Nanodrop® ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE). TG2 concentrations of Cy5-conjugates were determined using equation (1).

\[
[TG2] = \frac{A_{280} - (a/b \times A_{650})}{c}
\]  

(Eq. 1)

where \(A_{280}\) is the absorbance of Cy5-conjugates at 280 nm, a is the molar extinction coefficient of Cy5 at 280 nm, b is the molar extinction coefficient of Cy5 at 650 nm, and c is the molar extinction coefficient of TG2 at 280 nm.

TG2-related proteins were also labeled with Cy5 NHS ester and absorbance of Cy5-conjugates was determined using the Nanodrop® ND-1000 UV-Vis spectrophotometer. Protein concentrations of Cy5-conjugates were determined using equation (1), where c is the molar extinction coefficient of the proteins at 280 nm.

Determination of Protein Concentration Bound to the Array Surface—We used dry-off measurements to determine the concentrations of proteins bound to the surface of well-type amine arrays, as previously described (39). Briefly, various concentrations of Cy5-conjugated proteins in 1 μl of 9.3 μM phosphate buffer (pH 7.4) were applied in duplicate sets to amine arrays for 1 h at 37 °C. One set of array spots (washed set) was washed with 0.1% Tween 20 in PBS and with milli-Q-purified water whereas another set of array spots (dried set) was dried under ambient conditions. The resulting arrays were scanned with a fluorescence scanner (ScanArray Express, Perkin-Elmer Life and Analytical Sciences). Protein concentrations bound to the array surface were calculated from fluorescence intensities obtained from washed sets using standard curves generated from dried sets of array spots.

Simultaneous Transamidating Activity and Binding Assay of TG2 Against TG2-related Proteins—To simultaneously determine transamidating activity and interaction of TG2 with TG2-related proteins, we selected 16 commercially available proteins (fibrinogen, fibronectin, α-synuclein, retinoblastoma, aldolase A, grancalcin, S100A7, osteopontin, Bcl-2, SMAD2, annexin A1, Rac1, GST, E-cadherin, G-actin, and HSA) from the list of predicted functional partners on the STRING database, one of the widely used resources for prediction of functional interactions between two proteins, and from the literature (29, 40, 41). For each protein pair, STRING provides a probabilistic confidence score, representing the probability of a functional linkage between two proteins (42). The scores of TG2 with TG2-related proteins are shown in Table I. Sixteen proteins with the indicated concentrations were immobilized on the surface of well-type amine arrays for 1 h at 37 °C and blocked with 2 μl BSA containing 0.1% (v/v) Tween 20 in phosphate buffered saline (PBS) (8.1 mM Na2HPO4, 1.2 mM KH2PO4, 2.7 mM KCl, and 138 mM NaCl, pH 7.4) for 30 min at 37 °C. The resulting protein arrays were washed with 0.1% Tween 20 in PBS and with milli-Q-purified water. Reaction mixtures were prepared in 30 μl of solution containing 40 μM Tris-HCl (pH 7.5), 140 mM NaCl, 2 mM CaCl2, 50 mM dithiothreitol, 5 mM BAPA, 0.01% (v/v)
Trition X-100, and 10 μg/ml Cy5-conjugated TG2. Then, 0.7 μl of reaction mixture was applied to array wells and incubated at 37 °C for 30 min. After washing with 0.1% (v/v) Tween-20 in PBS and with milli-Q-purified water, BAPA incorporation catalyzed by TG2 was probed with 10 μg/ml Cy3-conjugated streptavidin at 37 °C for 30 min. Following washing with 0.1% (v/v) Tween-20 in PBS and with milli-Q-purified water, the arrays were dried under compressed air and scanned with a fluorescence scanner using 543 nm and 633 nm lasers (PerkinElmer Life and Analytical Sciences). Fluorescence intensities (FI) of array spots were analyzed using the ScanArray Express program (PerkinElmer Life and Analytical Sciences). Fluorescence intensities at Cy3 and Cy5 channels represent TG2 activities and interactions, respectively, with TG2-related proteins.

**Determination of Dissociation Constants (Kd) Between TG2 and TG2-related Proteins**—We quantitatively analyzed binding affinities of TG2 with 16 TG2-related proteins by determining the dissociation constants (Kd). Kd values were calculated by following the modified Langmuir isotherm using GraphPad PRISM (GraphPad Software; San Diego, CA) as follows:

\[
F_{\text{obs}} = \frac{F_{\text{max}} \times [\text{protein}]}{K_d + [\text{protein}]} + \text{background}
\]

where \(F_{\text{obs}}\) is the fluorescence intensity of triplicate spots, \(F_{\text{max}}\) is the maximum fluorescence at saturation, [protein] is the protein concentration on the surface, and \(K_d\) is the apparent equilibrium dissociation constant.

**Data Analysis—**ScanArray Express software was used for quantitation of fluorescence intensities and extraction of data. TG2 activities were normalized by the median-centering.

**RESULTS**

**Characterization of Cy5-conjugated TG2**—To better characterize the regulation and functions of proteins in biological systems, we developed an integrative proteomic profiling method to simultaneously monitor the activities and interactions of target proteins with their related proteins in a high-throughput assay. We used TG2 and 16 related proteins as a model system for investigating this proteomic profiling. TG2 was conjugated with the Cy5 NHS ester to visualize TG2 interaction with the proteins, and BAPA and Cy3-conjugated streptavidin were used for probing TG2 activity for the proteins, as illustrated in Fig. 1.

To calculate molar concentrations of Cy5 and TG2 in Cy5-conjugated TG2, we determined molar extinction coefficients of TG2 at 280 nm and Cy5 at 280 nm and 650 nm using the Nanodrop® spectrophotometer. The molar extinction coefficient of Cy5 at 650 nm was 219,500 M⁻¹·cm⁻¹, whereas that of TG2 at 280 nm was 109,600 M⁻¹·cm⁻¹. However, the molar extinction coefficient of Cy5 at 280 nm was very low (3600 M⁻¹·cm⁻¹, ~2% of that of Cy5 at 650 nm) (Fig. 2A). We then reacted 1.27 pmole of TG2 with 1-, 5-, 10-, 20-, and 40-fold molar excesses of Cy5 NHS ester and determined Cy5 and TG2 concentrations in Cy5-conjugated TG2 using the extinction coefficient of Cy5 and equation (1), respectively. The concentration of Cy5 in the Cy5-conjugates increased in proportion to the molar excess of the Cy5 NHS ester, and the molar ratio of Cy5 to TG2 increased from 0.65 to 7.59 (Fig. 2B). However, the TG2 concentration in Cy-5 conjugates was in a similar range from 0.78 μM to 0.84 μM, demonstrating that up to a 40-fold molar excess, the efficiency of TG2 conjugation with Cy5 NHS ester was not significantly affected.

Because covalent conjugation of an enzyme with a fluorophore may affect the activity of the enzyme, we determined the optimal molar ratio of Cy5 NHS ester to TG2 for TG2 conjugation by measuring transamidating activity and interaction of Cy5-conjugated TG2 using fibronectin arrays (Fig. 2C). The transamidating activity of Cy5-conjugated TG2 was dependent on the molar ratio of Cy5 NHS ester to TG2. The transamidating activity of Cy5-conjugated TG2 at the ratio of 1:1 (Cy5 NHS ester:TG2) decreased to about 61% of unconjugated TG2, and continuously decreased in proportion to an increase in the concentration of Cy5 NHS ester, with a minimal activity at a ratio of 40:1. We then investigated the effect of Cy5-conjugation on TG2 interaction using fibronectin arrays. Interaction of Cy5-conjugated TG2 with fibronectin increased with increased molar ratio of Cy5 NHS ester and TG2 (Fig. 2C), which was opposite to the effect of Cy5-conjugation on the transamidating activity of the conjugated TG2. Thus, for conjugation of TG2 with the Cy5 NHS ester, we chose the molar ratio of 10:1 to minimize the loss of transamidating activity and to maximize the interaction of Cy5-conjugated TG2 with fibronectin.

**Determination of the Transamidating Activity of TG2 with TG2-related Proteins**—For quantitative analysis of the transamidating activity and interaction of TG2 for TG2-related proteins, we fabricated protein arrays by immobilizing 16 proteins, with indicated concentrations , to the amine surface of well-type arrays, then incubated with reaction mixtures containing 10 μg/ml of Cy5-conjugated TG2 (Fig. 3A). The concentrations of the proteins bound to the array surface were determined by dry-off measurements using solid-phase standard curves. Fluorescence intensities obtained with Cy3 and Cy5 channels represent the transamidating activity and interaction of TG2 with its related proteins, respectively.

For the analysis of transamidating activity, fluorescence intensities at the Cy3 channel were plotted against the concentrations of 16 TG2-related proteins. Most of the proteins, except Rac1, showed a concentration-dependent increase of fluorescence with saturation at 1000 nM (Fig. 3B). TG2 activity was not detectable with Rac1, indicating that there was no nonspecific binding of BAPA to Rac1. Consistently, fluorescence was not detectable when reaction mixtures without TG2 were applied to arrays of fibrinogen and G-actin, with concentrations ranging from 20 nM to 2 μM (applied concentrations; data not shown), demonstrating that the transamidating activity was mostly contributed by TG2-mediated bio-tylation of proteins on arrays. To analyze suitability of the proteins as substrates for TG2, we monitored maximal fluorescence intensities for 15 proteins (Table I). The resulting transamidating activities were normalized using the median centering, then used to construct an activity-interaction (AI) map (Fig. 4). The 16 proteins were assigned to three groups.
FIG. 1. Schematic diagram for the simultaneous analysis of transamidating activity and interaction of TG2 with TG2-related proteins. BAPA, 5-(biotinamido)pentylamine; Pr, protein; SA, streptavidin; TG2, transglutaminase 2.
based upon the mean value (1.830) of the normalized transamidating activities: high activity (greater than the mean, $A_H$), low activity (lower than the mean, $A_L$), and nondetectable activity ($A_N$) (Table I). Six proteins (fibronectin, G-actin, E-cadherin, retinoblastoma, osteopontin, and fibrinogen) were grouped into the $A_H$ group. Nine proteins—annexin A1, grancalcin, S100 calcium binding protein A7 (S100A7), aldolase A, α-synuclein, GST, Bcl-2, SMAD2, and HSA—belonged to the $A_L$ group. Rac1 was a member of the $A_N$ group. Thus, by determining transamidating activity using protein arrays, we successfully applied the integrative proteomic profiling approach to ascertain the suitability of the 16 proteins as TG2 substrates.

**Determination of TG2 Affinity with TG2-related Proteins**—For quantitative analysis of the interaction of TG2 with its related proteins, fluorescence intensities obtained at the Cy5 channel from the simultaneous activity and binding assay of TG2 (Fig. 3A), were plotted against the concentrations of 16 TG2-related proteins and fitted using equation (2) (Fig. 3C). With the exception of osteopontin, SMAD2, E-cadherin, and HSA, binding of TG2 increased in proportion to the concentration of protein. The binding affinity ($K_d$) was determined using equation (2) and ranged from 1.15 nM to 469.50 nM (Table I), but was not detectable for the four proteins. The $K_d$ values, together with transamidating activities, were used to construct a quantitative AI map (Fig. 4).

The 16 proteins were assigned to three groups using the log $K_d$ values (logKd); strong (logKd > 1, IS), weak (logKd < 1, IW), and nondetectable (IN) interaction groups (Table I). Six proteins (fibronectin, annexin A1, grancalcin, retinoblastoma, S100A7, and fibrinogen) showed a strong interaction with TG2 (logKd > 1). The log Kd values of another six proteins (G-actin, aldolase A, α-synuclein, GST, Bcl-2, and Rac1) were higher than 1 (logKd < 1), suggesting a weak interaction with TG2. The $K_d$ values for E-cadherin, osteopontin, SMAD2, and HSA were undetectable, indicating that these proteins did not interact with TG2. Thus, we could simultaneously and quantitatively analyze the transamidating activity and binding affinity of TG2 for 16 TG2-related proteins by the use of integrative proteomic profiling in conjunction with protein arrays.

**Integrative Proteomic Networks of Quantitative Transamidating Activity and Interaction of TG2 with TG2-related Proteins**—Visualization of protein interaction networks is the best method for presenting known and predicted protein interactions, using experimental and computational information from databases. Web-based databases, such as STRING, provide extensive information on interactomes. However, these databases provide predictive rather than quantitative information of protein activity and dissociation constant ($K_d$). Using data derived from large-scale analyses, we circumvented this limitation by constructing a quantitative AI map of TG2 with 16 TG2-related proteins (Fig. 4). In this map, the node size and distance between TG2 and various nodes represent the transamidating activity and binding affinity, respectively, of
Fig. 3. Simultaneous transamidating activity and binding assays of TG2 with TG2-related proteins. A, Protein arrays were fabricated by immobilizing 16 TG2-related proteins, with indicated concentrations, onto well-type amine arrays. B, C, Reaction mixtures (1 μl) containing 10 μg/ml Cy5-conjugated TG2 were applied to protein arrays for 30 min. Transamidating (TG) activities and interactions of TG2 with 16 proteins were simultaneously determined as described in Methods. The results are expressed as means of fluorescence intensities (FI) ± S.D. from three separate experiments.
### Table I

Comparison of predicted protein association scores with experimental values of TG2 activity (normalized FI) and interaction ($K_d$)

Confidence scores were obtained from the STRING database. TG2 activities of the TG2-related proteins were normalized by median-centering. N.D., nondetectable; AI grade, activity-interaction grade: A, activity; H, high; L, low; I, interaction; S, strong; W, weak; N, non-detectable.

| Protein          | Confidence score | Integrative proteomic profiling |
|------------------|------------------|---------------------------------|
|                  |                  | TG2 activity (normalized)       | TG2 interaction $K_d$ (nM) | AI grade |
| Fibronectin      | 0.996            | 4.199                           | 6.44                         | A_HiS    |
| Annexin A1       | 0.747            | 0.396                           | 6.99                         | A_LiS    |
| Grancalcin       | 0.708            | 0.453                           | 3.82                         | A_LiS    |
| G-actin*         | 0.688            | 4.012                           | 25.42                        | A_LiW    |
| E-cadherin       | 0.659            | 5.653                           | N.D.                         | A_LiN    |
| Retinoblastoma   | 0.649            | 2.881                           | 7.33                         | A_LiS    |
| S100A7           | 0.638            | 0.565                           | 2.82                         | A_LiS    |
| Aldolase A       | 0.619            | 1.074                           | 43.21                        | A_LiW    |
| α-synuclein      | 0.619            | 0.926                           | 44.93                        | A_LiW    |
| Osteopontin      | 0.593            | 3.230                           | N.D.                         | A_LiN    |
| GST*             | 0.530            | 0.415                           | 469.5                        | A_LiW    |
| Bcl-2            | 0.372            | 0.708                           | 26.83                        | A_LiW    |
| Rac1             | 0.218            | N.D.                            | 15.41                        | A_LiW    |
| SMAD2            | 0.196            | 0.232                           | N.D.                         | A_LiN    |
| Fibrinogen       | No information   | 3.175                           | 1.15                         | A_LiS    |
| HSA              | No information   | 1.356                           | N.D.                         | A_LiN    |

*β-actin and GSTP1 were used to determine the confidence scores of G-actin and GST from the STRING database.

Fig. 4. **Quantitative AI map of TG2 with its related proteins.** Normalized transamidating activities and dissociation constants ($K_d$) of TG2 with 16 proteins were used to construct an integrative proteomic map. In this map, the node volume represents the transamidating activity and the distance between the node and TG2 indicates the $K_d$ value. ALDOA, aldolase A; ANXA, annexin A1; Bcl2, B-cell lymphoma 2; E-cad, E-cadherin; FI, fibrinogen; FN, fibronectin; GCA, grancalcin; GST, glutathione S-transferase; HSA, human serum albumin; OPN, osteopontin; RB1, retinoblastoma; S100A7, S100 calcium binding protein A7; SMAD2, Smad-related protein 2; SNCA, α-synuclein.
TG2 for different proteins. Four proteins (E-cadherin, osteopontin, SMAD2, and HSA) are located outside the $K_d$ of 1,000, because $K_d$ values were not detectable for these proteins. The AI map can therefore provide a systematic view of protein interactions based both on activity and binding affinity.

The 16 proteins were assigned to seven groups by an AI grading system: high activity and strong interaction (AIHIS), high activity and weak interaction (AIHIW), high activity and non-detectable interaction (AIHW), low activity and strong interaction (AILIS), low activity and weak interaction (AILIW), low activity and non-detectable interaction (AILN), and low activity and non-detectable interaction (AILN). No proteins were assigned to the group for AHWN. Thus, this integrative proteomic profiling based on the quantitative AI map and AI grading system is a potential approach to construct real functional networks of the quantitative activities and interactions of target proteins with various proteomes to understand the functions and regulation of the target proteins.

**DISCUSSION**

In this report, we present an integrative proteomic profiling method for the simultaneous analysis of activities and interactions of target proteins, in order to provide an overview of the functions and regulation mechanisms of target proteins in biological processes of interest. TG2 was used as a model system because this protein is involved in the pathogenesis of various diseases including celiac disease, and in a variety of cellular events (34, 36). TG2 is a member of the transglutaminase family and catalyzes the formation of an e-(γ-glutamyl)-lysine bond between glutamine and lysine in a Ca$^{2+}$-dependent manner (29, 34). In the Ca$^{2+}$-dependent transamidation reaction, Ca$^{2+}$ activates transamidating activity of TG2 by inducing a conformational change. The side chain of glutamine forms a thioester with the active site cysteine of TG2 by induction a conformational change. The side chain of glutamine forms a thioester with the active site cysteine of TG2 by activating transamidating activity of TG2 by inducing a conformational change. The side chain of glutamine forms a thioester with the active site cysteine of TG2 by activating transamidating activity of TG2 by inducing a conformational change. The side chain of glutamine forms a thioester with the active site cysteine of TG2 by activating transamidating activity of TG2 by inducing a conformational change.
Array-based Profiling of Protein Activity and Interactions

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