Supporting Information

Dendrons and Dendrimers as Pseudochaperonins for Refolding of Proteins

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Materials

TLL (*Thermomyces lanuginosus lipase*), CAL B (*Candida antarctica* lipase B) and RML (*Rhizomucor miehei* lipase) were kind gifts from Novozymes (Denmark). BAA (α-amylase from *Bacillus amyloliquefaciens*) and BLA (α-amylase from *Bacillus licheniformis*) were generously supplied by Arun & Co. (Bombay, India) and NOVO Nordisk A/S (Bangalore, India), respectively. DTT (dithiothreitol), p-Nitrophenyl palmitate (PNPP) and SYPRO Orange™ were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade.

Chemical Synthesis

All amino acids used were of L-configuration. Unless otherwise stated, all reagents were used without further purification. All solvents employed in the reactions were distilled or dried from appropriate drying agent prior to use. Reactions were monitored wherever possible by thin layer chromatography (TLC). Silica gel G (Merck) was used for TLC and column chromatography was done on silica gel (100-200 mesh) columns, which were generally made from slurry in hexane, hexane/ethyl acetate or chloroform. Purification of peptide was done using HPLC (Schimdazu-LC-6AD) equipped with a phenomenex C-18 reversed phase column. Analytical HPLC was carried out using phenomenex C-18 column and acetonitrile/water as the solvent system (Figure S2 HPLC profiles of various dendrons and dendrimers). Detailed procedures have been described earlier.[1-4]

General procedure for the peptide coupling reaction

To an ice-cooled and well stirred solution of N-protected amino acid (1 mmoles) in dry dichloromethane, was added N-hydroxysuccinimide (1 mmol), dicyclohexylcarbodiimide (DCC) (1 mmol) and stirred for 10 min. To this mixture was added an amine component (1.1 mmol) in dichloromethane and triethylamine (1.1 mmol). The reaction mixture was stirred overnight, filtered and washed the filtrate with 0.2 N H_2SO_4, water and finally with saturated aqueous $NaHCO_3$ solution. The organic layer was separated dried over anhydrous Na_2SO_4, filtered and evaporated. Silica gel column chromatographic purification yielded the products in ~ 75-87 % yields.

General procedure for the Boc-Deprotection

To an ice-cooled solution of the Boc-protected compound (1mmol) was added 25 % solution of trifluroacetic acid (40 mmol) in dry dichloromethane and stirred at RT for 3h. The reaction mixture was subjected to vacuum, redissolved in ethylacetate and washed with sodium carbonate.

General procedure for the synthesis of diacetylenic dendrimer

To a solution of alkyne truncated dendron (0. 2 mmol) in dichloromethane was added *Hay* catalyst [prepared by stirring CuCl (10 mg, 0.10 mmol) and tetramethylethylenediamine (TMEDA) (0.033 mL, 0.22 mmol) in dichloromethane (4 mL) for 5 min] and stirred under air. After 3 hrs, more *Hay* catalyst (4 mL) was added and left stirred for 24 hours. The reaction mixture was diluted with distilled dichloromethane (20 mL) and washed sequentially with 0.5 N aq. H_2SO_4, water, saturated aqueous $NaHCO_3$ solution, aq. NH_4CI + NH_3 (9:1) solution, and water. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to yield the dendrimer 67-90 % in yields.

All the prepared dendrons and dendrimers were characterized by ^{1}H NMR, ^{13} C NMR, IR and HRMS.[1-4] Purity of the compounds was analyzed by analytical HPLC (Fig.S2)

(Schimdazu-LC-6AD) equipped with a phenomenex C-18 reversed phase column using acetonitrile/water as the solvent system.

Enzyme assays

The hydrolytic activity of lipase was monitored by following the rate of hydrolysis of p-nitro phenyl palmitate spectrophotometrically at 410 nm.[5] Amylase activity was assayed by the dinitrosalicylate (DNS) method of Miller.[6]

Estimation of protein concentration

The amount of proteins was estimated by the dye-binding method, using bovine serum albumin as a standard.[7]

Thermal shift assays

Thermal shift assays were performed on a Bio-Rad CFX 96 Touch Real Time PCR detection system. In a typical experiment, 10 µL of 0.5 mg mL^{-1} protein, 2.5 µL of 50X SYPRO Orange (Sigma-Aldrich, diluted from 5000X stock into buffer), 2.5 µL of compound, and 10 µL of 20 mM sodium phosphate buffer, pH 7.0, were mixed on ice in a 96-well white PCR plate (Bio-Rad), and the plate was heated from 20 °C to 95 °C in 0.5 °C steps. Fluorescence (AU, arbitrary units) was measured with excitation at 450–490 nm and emission at 560–580 nm. All measurements were carried out in triplicate. Data analysis and determination of T_m were performed using the Bio-Rad CFX Manager software supplied with the instrument.

Circular dichroism (CD) measurements

Far-UV CD spectra were recorded on a JASCO J-815 spectropolarimeter (Jasco Corporation, Tokyo, Japan) equipped with a Peltier-type temperature controller and a thermostatted cell holder, interfaced with a thermostatic bath at 25 °C using a cell with a path length of 0.1 cm. Typical spectral accumulation parameters were a scanning rate of 50 nm/min with a 2 nm bandwidth over the wavelength range 200 to 250 nm with six scans averaged for each far-UV spectrum using a protein concentration of 10-15 µM (calculated by using dye-binding method[7]) in 10 mM sodium phosphate buffer, pH 7.0. The CD data are presented in terms of mean residue ellipticity (MRE, expressed as deg cm^2 $dmol^{-1}$) as a function of wavelength, calculated as given below according to the procedure described earlier[8]:

$$[\theta]_{MRE} = MRW \times \theta_{obs}/10 \times d \times c$$

where $[\theta]_{MRE}$ is the calculated mean residue ellipticity (deg cm^2 $dmol^{-1}$), MRW is the mean residue weight for the peptide bond [MRW is calculated as, MRW = M/N-1, where M is the molecular mass of the polypeptide chain (in Da), and N is the number of amino acids in the chain], θ_{obs} is the observed ellipticity (expressed in degrees), d is the pathlength (cm), and c is the protein concentration (g mL^{-1}). All CD spectra were corrected for buffer contributions.

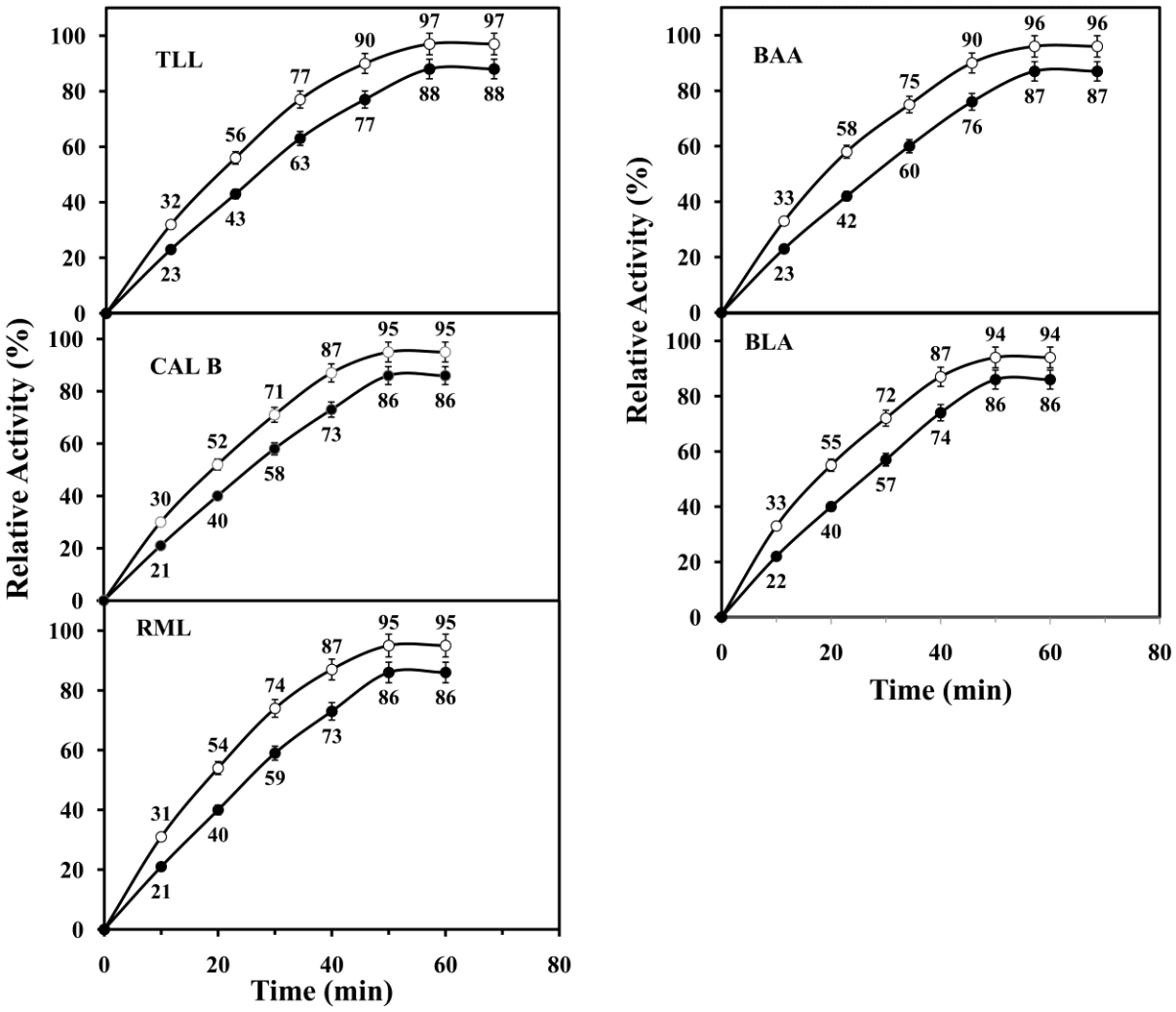

Fig. S1 Enzymatic activity of different proteins refolded by K7 dendron (black circles) and K7 dendrimer (open circles) with different time intervals. The relative activity (%) was calculated by taking the activity of starting protein to be 100% and calculating activity at each step relative to it.

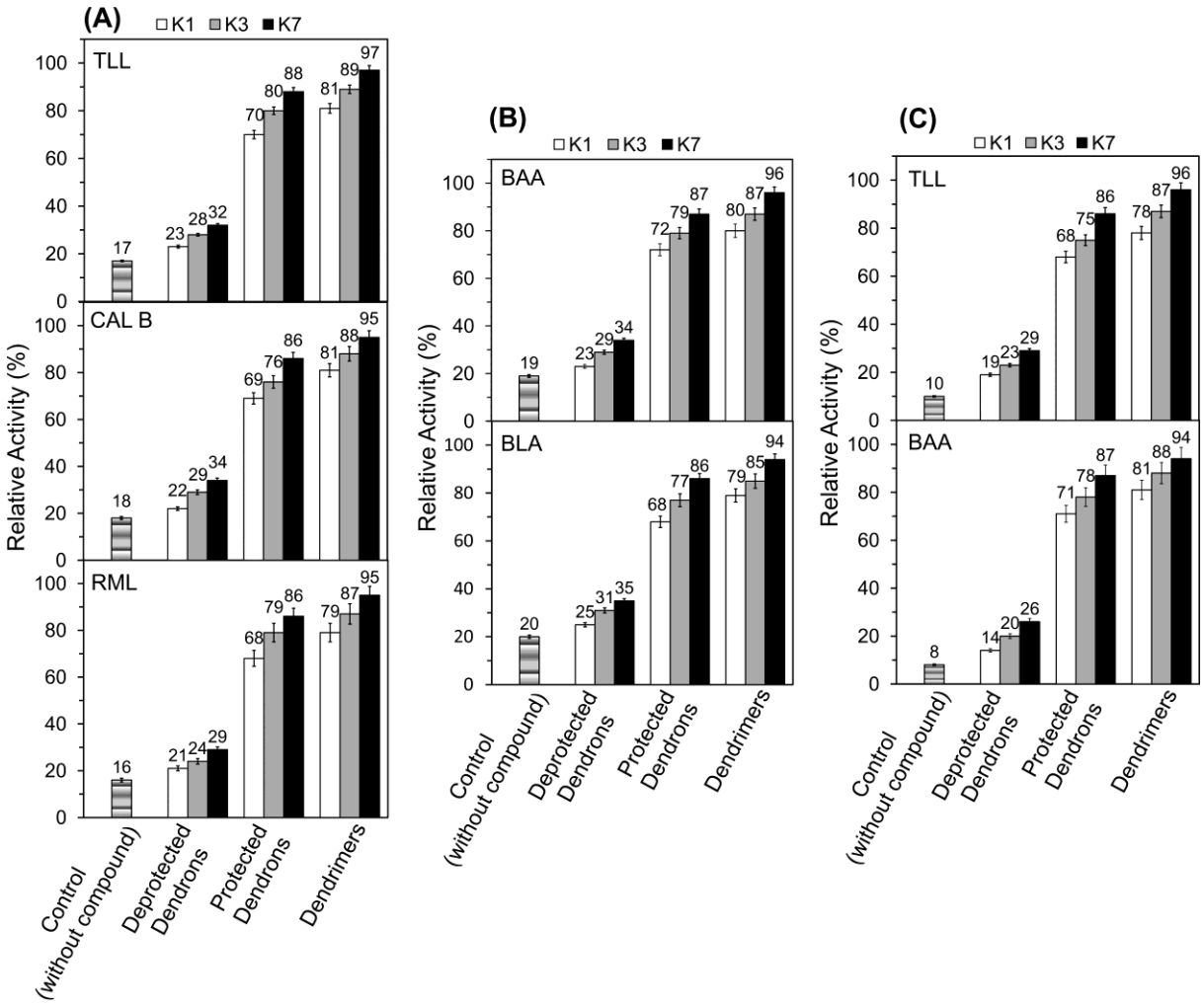

Fig. S2 Enzymatic activity of different proteins refolded by dendrons and dendrimers: (A) thermally denatured lipases, (B) thermally denatured α-amylases and (C) urea denatured TLL and BAA. The relative activity (%) was calculated by taking the activity of starting protein to be 100% and calculating activity at each step relative to it.

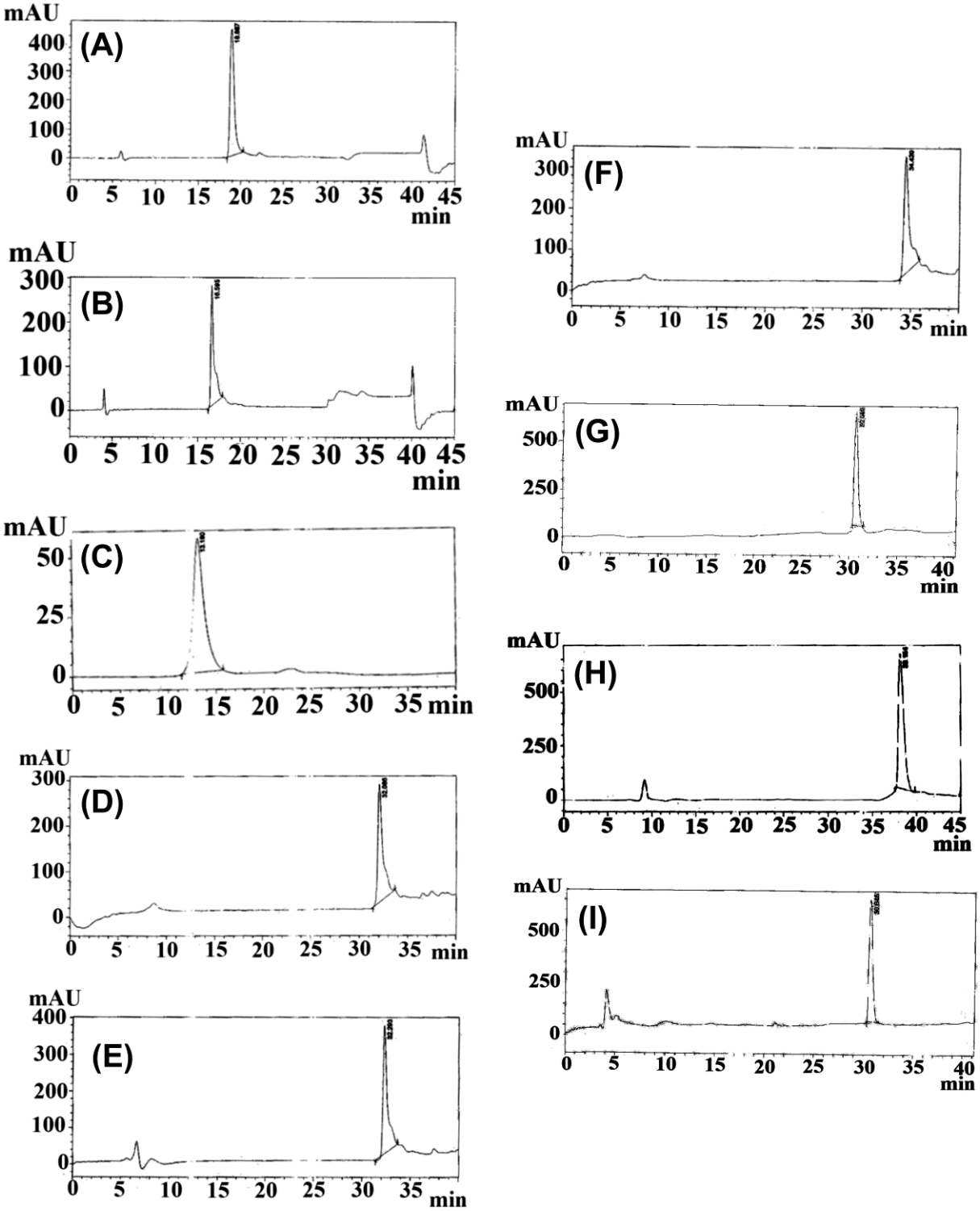

Fig. S3 HPLC profiles for dendrons and dendrimers in a binarygradient system (Acetonitile/ Water) using UV detector at 220 nm: (A) Protected K1 Dendron, (B) Protected K3 Dendron, (C) Protected K7 Dendron, (D) Deprotected K1 Dendron, (E) Deprotected K3 Dendron, (F) Deprotected K7 Dendron, (G) K1 Dendrimer, (H) K3 Dendrimer, and (I) K7 Dendrimer.

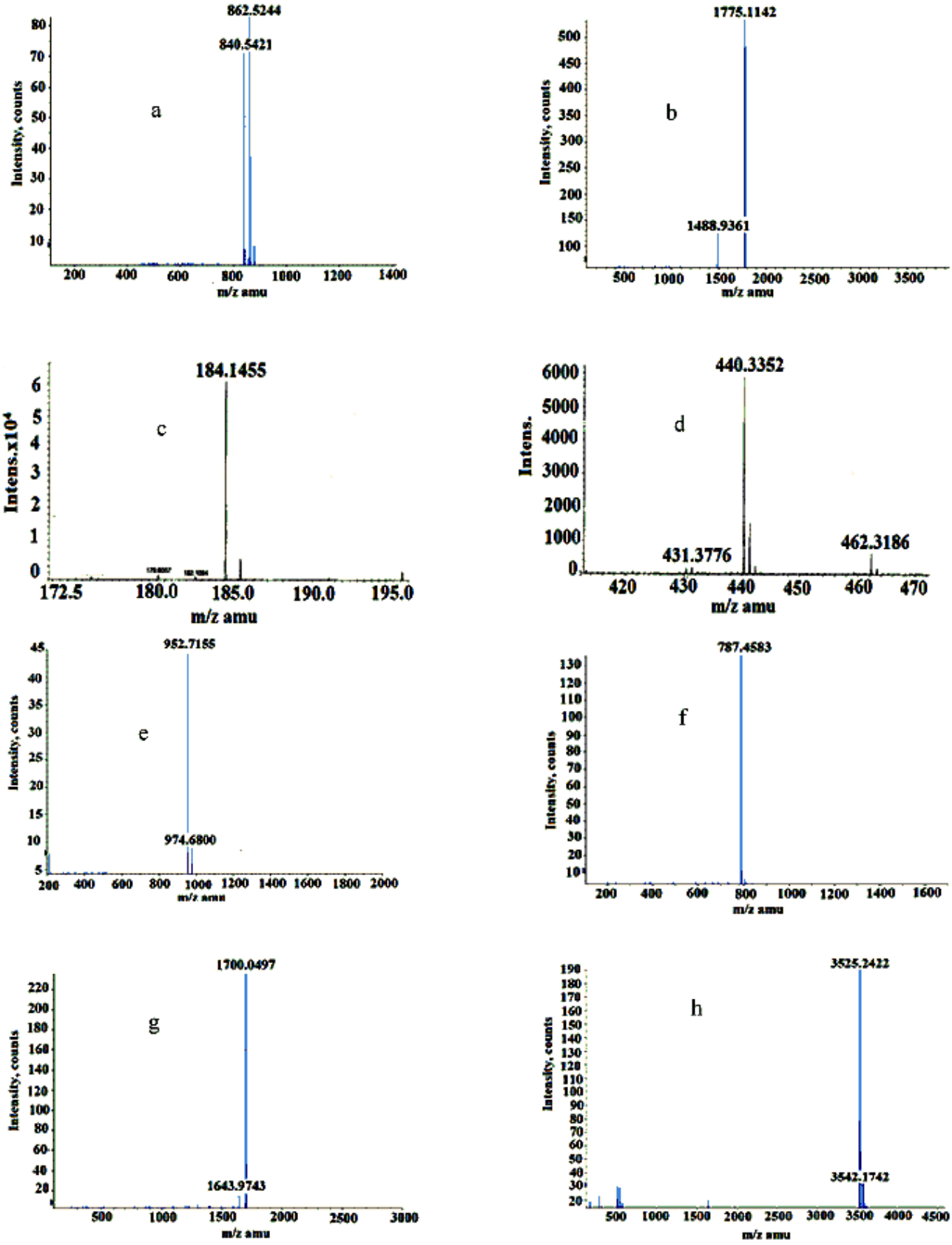

Fig. S4. HRMS of (a) Protected K3 Dendron, (b) Protected K7 Dendron, (c) Deprotected K1 Dendron, (d) Deprotected K3 Dendron, (e) Deprotected K7 Dendron, (f) K1 Dendrimer, (g) K3 Dendrimer, and (h) K7 Dendrimer.

Table S1 T_m (°C) and shift in the T_m of the proteins calculated from thermal shift assay for ligand binding to proteins from Fig. 2. The thermal shift assays were carried out as described in materials and methods.

Compound with protein	**TLL**		**BAA**	
	T_m (°C)	**ΔT_m (°C)**	**T_m (°C)**	**ΔT_m (°C)**
No compound (protein in 20 mM sodium phosphate buffer, pH 7.0)	65.0	0.0	81.0	0.0
Deprotected K1 Dendron	66.5	1.5	82.0	1.0
Deprotected K3 Dendron	67.0	2.0	82.5	1.5
Deprotected K7 Dendron	67.0	2.0	83.0	2.0
Protected K1 Dendron	69.0	4.0	86.0	5.0
Protected K3 Dendron	69.5	4.5	87.0	6.0
Protected K7 Dendron	73.0	8.0	89.0	8.0
K1 Dendrimer	71.0	6.0	88.0	7.0
K3 Dendrimer	72.0	7.0	89.0	8.0
K7 Dendrimer	74.0	9.0	90.0	9.0

Table S2 Size distribution analysis by DLS of TLL and BAA after denaturation in 8 M urea/100 mM DTT with and without compounds (K7 Dendron and K7 Dendrimer) and then diluted 4 times with 20 mM sodium phosphate buffer, pH 7.0 (final urea concentration in all the cases was 2 M except native protein). Final protein concentration in all the cases was 1 mg mL^{-1}. The average diameter of 2 M urea was 482.0 nm without any compound, 368.0 nm with K7 dendron and 361.0 nm with K7 dendrimer.

Protein	**Compound**	**Average diameter (nm)**	**Average diameter after dialysis (nm)**
Native TLL	No compound	5.5	5.5
TLL	No compound (Dilution control)	1105.0	1207.0
TLL	Protected K7 Dendron	361.0	5.7
TLL	K7 Dendrimer	356.0	5.6
Native BAA	No compound	6.0	6.0
BAA	No compound (Dilution control)	908.0	1082.0
BAA	Protected K7 Dendron	350.0	6.5
BAA	K7 Dendrimer	342.0	6.5

References for Supporting Information

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