Supporting Information

Selenoprotein P and Selenoprotein M block Zn^{2+}-mediated $A\beta_{42}$ aggregation and toxicity

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Mass Spectrometry

Purified SelP-H and SelM′ were analyzed by SDS-PAGE (13%, tris-glycine running buffer) and visualized with Coomassie Blue R-250. Bands corresponding to SelP-H and SelM′ were digested with trypsin (Sigma) at 37 ^{o}C overnight. Peptide fragments were then analyzed with matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-MS) and compared with theoretically digested peptides calculated by MS-digest software (http://prospector.ucsf.edu/).

MALDI-TOF mass spectra of SelP-H and SelM′ digested with trypsin confirmed the identities of these proteins, as shown in Fig. s1. Spectrum of SelP-H showed peaks at m/z 837.45, 1083.52 and 1099.52, corresponding to the peptides: QGHPENR (calc, 837.40) and QGHPENRDM (calc, 1083.46 and 1099.46 with oxidation), respectively. Spectrum of SelM′ revealed peaks at m/z 946.50, 994.52, 1423.70 and 1486.86, corresponding to the peptides IPLSEMTR (calc, 946.50), RYEELER (calc, 994.50), GTSTAYRPDWNR (calc, 1423.67) and HLPGADPELVLLGR (calc, 1486.84), respectively.

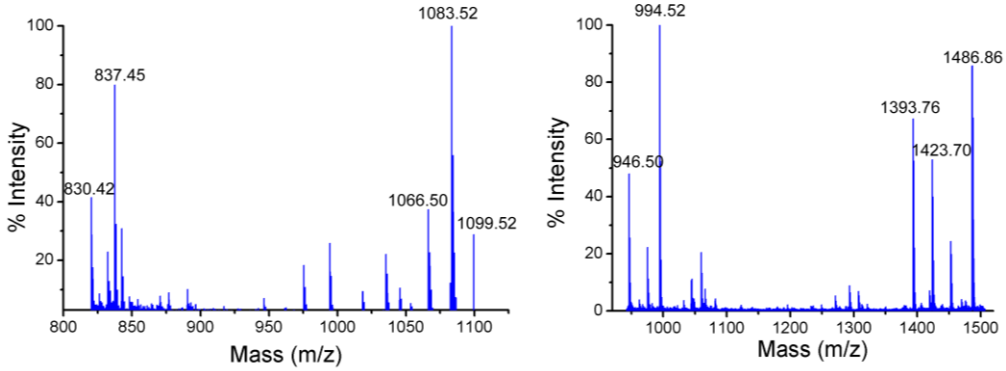

Fig.s1 MALDI-TOF-MS analysis of SelP-H (left) and SelM′ (right) digested with trypsin.

One set of sites binding model

The equilibrium constant and mass balance expression for the one set sites (n identical sites) model are shown as:

$$K = \frac{\theta}{(1-\theta)[L]} \quad (1)$$

Where K is the binding constant, Θ is fraction of sites occupied by ligand and [L] is free concentration of ligand.

Total heat evolved (or absorbed) during the binding process at the end of the i^{th} injection, Q(i), is given by Equation (2) [1]:

$$Q_{(i)} = \frac{nP_t\Delta HV\left\{1+\frac{L_t}{nP_t}+\frac{1}{nKP_t}-\left[\left(1+\frac{L_t}{nP_t}+\frac{1}{nKP_t}\right)^2-\frac{4L_t}{nP_t}\right]^{\frac{1}{2}}\right\}}{2} \quad (2)$$

where n is the number of binding sites, P_t is the total protein concentration, L_t is the total ligand concentration, V is the cell volume, K is the binding constant and ΔH is the binding enthalpy. The heat corresponding to the i^{th} injection only, $\Delta Q(i)$, is equal to the difference between $Q(i)$ and $Q(i-1)$ and is given by Equation (3):

$$\Delta Q_{(i)} = Q_{(i)} + \frac{dV_i}{V_0}\left[\frac{Q_{(i)}+Q_{(i-1)}}{2}\right] - Q_{(i-1)} \quad (3)$$

$\Delta Q(i)$ value for each injection was measured and then fitted to Equations (2) and (3) by a nonlinear least squares method. The fit process involves initial guess of n, K and ΔH which allows calculation of $\Delta Q(i)$ values as mentioned above for all injections and comparing them with the corresponding experimentally determined values. Based on this comparison the initial guess of n, K and ΔH is improved and the process is repeated till no further significant improvement in the fit can be obtained.

1.T. Wiseman, S. Williston, J. F. Brandts, L. N. Lin, Rapid measurement of binding constants and heats of binding using a new titration calorimeter. *Analytical biochemistry* 1989, *179*. 131-7.