Supplementary information

Label-free visualization of unfolding and crosslinking mediated protein aggregation in nonenzymatically glycated proteins

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1. Deep-UV-induced-Autofluorescence device (dUV-AF)

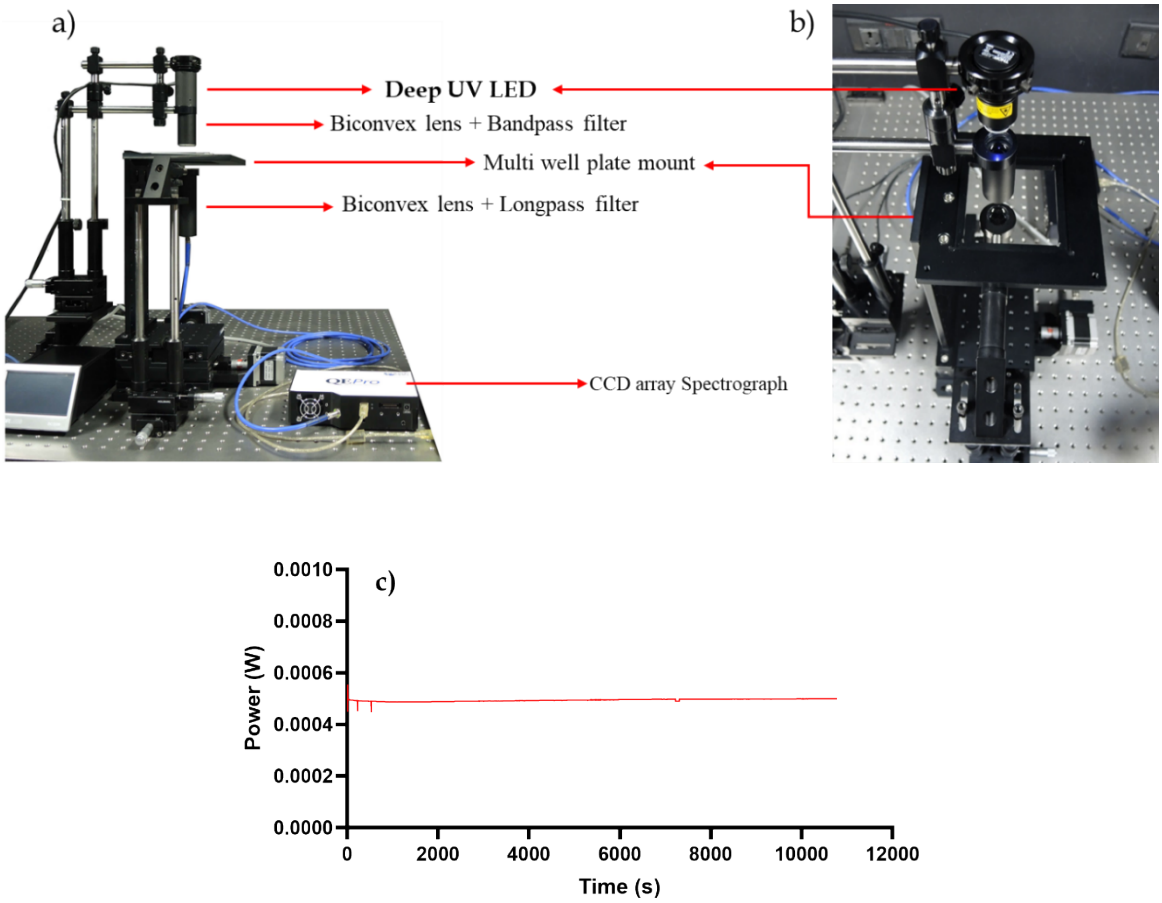

Figure S1. *Block diagram of the dUV-AF device with automated micro-controlled X-Y translational stage for mounting the quartz multi-well plate with protein solutions. Side view* ***(a);*** *Top view* ***(b).*** *Figure* ***c)*** *depicts the power stability of the deep-UV-LED used in the current study.*

2. UV-visible photometric absorbance

The Varioskan Flash multimode spectral reader (ThermoFisher Scientific, USA) was used to record the UV-visible absorption spectra **(Fig. S2)** of both the native and methylglyoxal-modified proteins across the 240–500 nm spectral range.

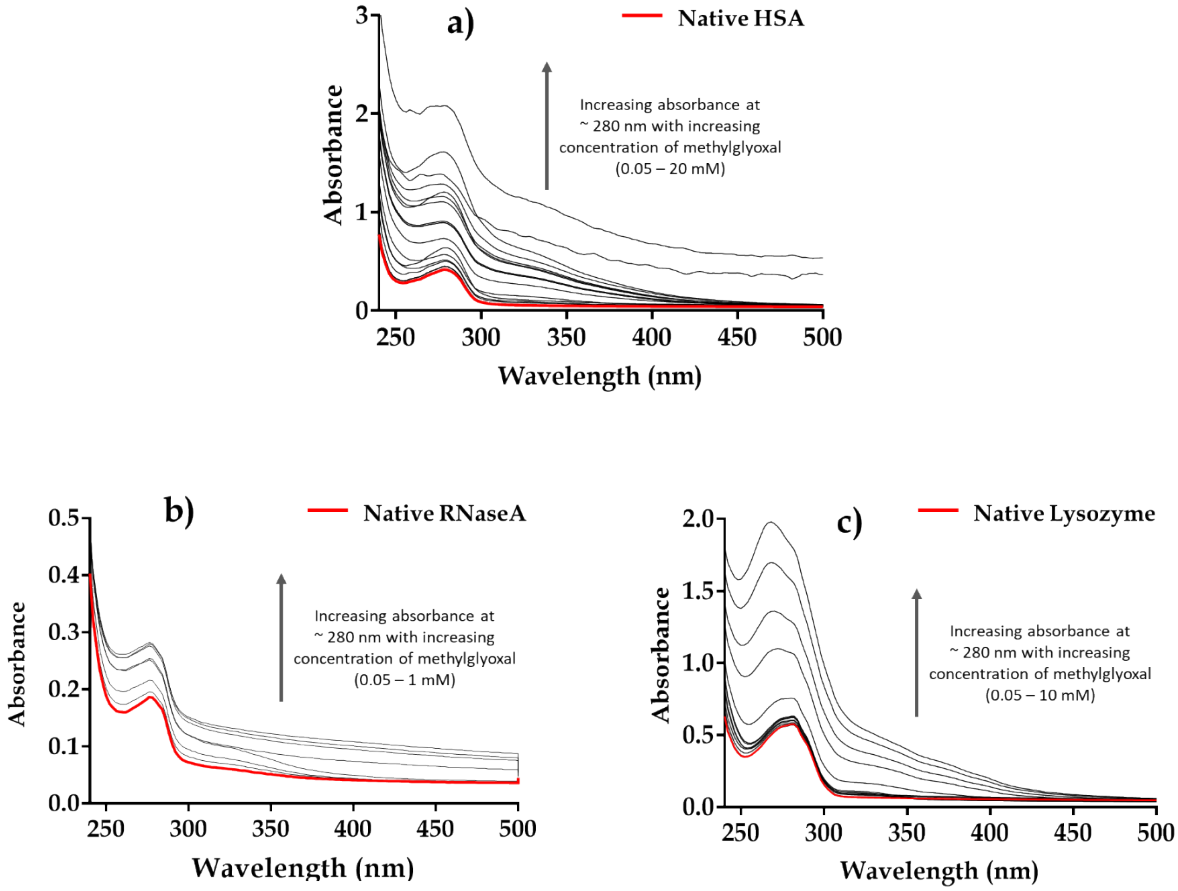

Figure S2. *The photometric absorbance spectra of methylglyoxal-modified proteins* ***a) HSA b) RNaseA c) Lysozyme***

3. dUV-AF spectra of BSA

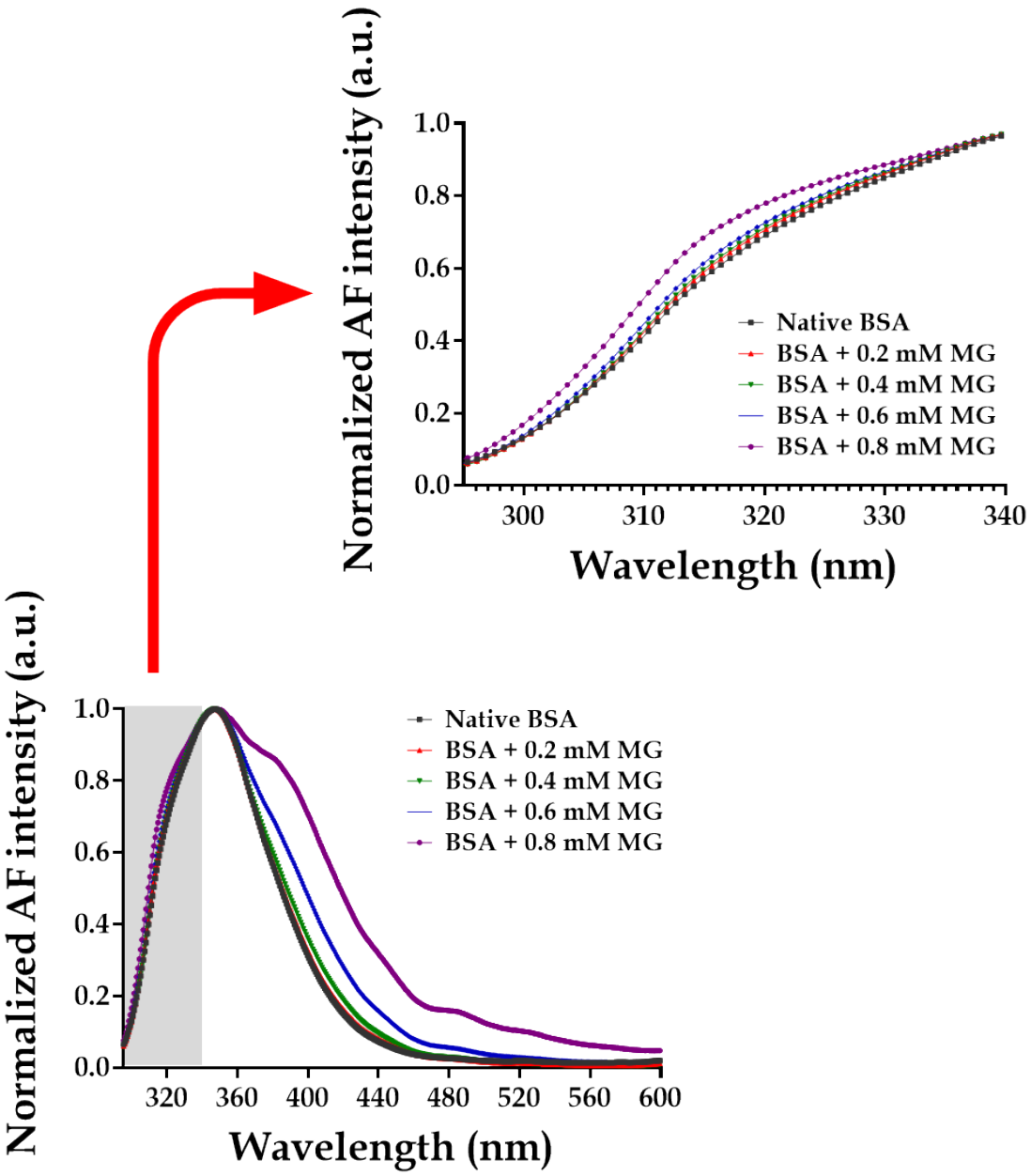

Figure S3. *The normalized dUV-AF spectra of methylglyoxal (0.2–0.8 mM) modified Bovine Serum Albumin (BSA) with the highlighted spectral region 290–340 nm.*

4. dUV-AF spectra of RNaseA

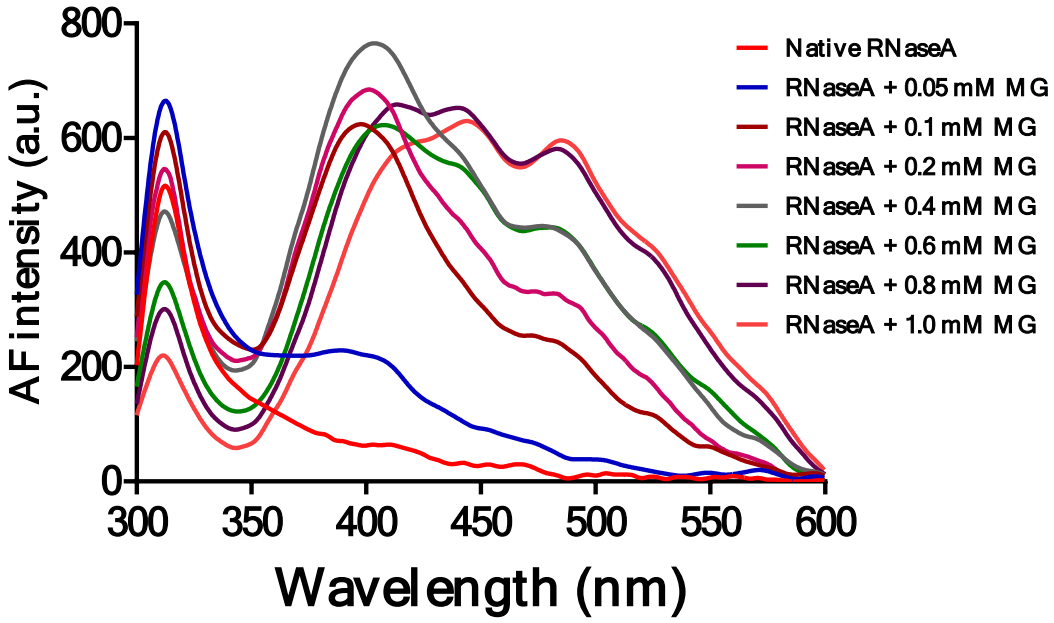

Figure S4. *dUV-AF spectra of methylglyoxal (0.05 – 1.0 mM) modified RNaseA*

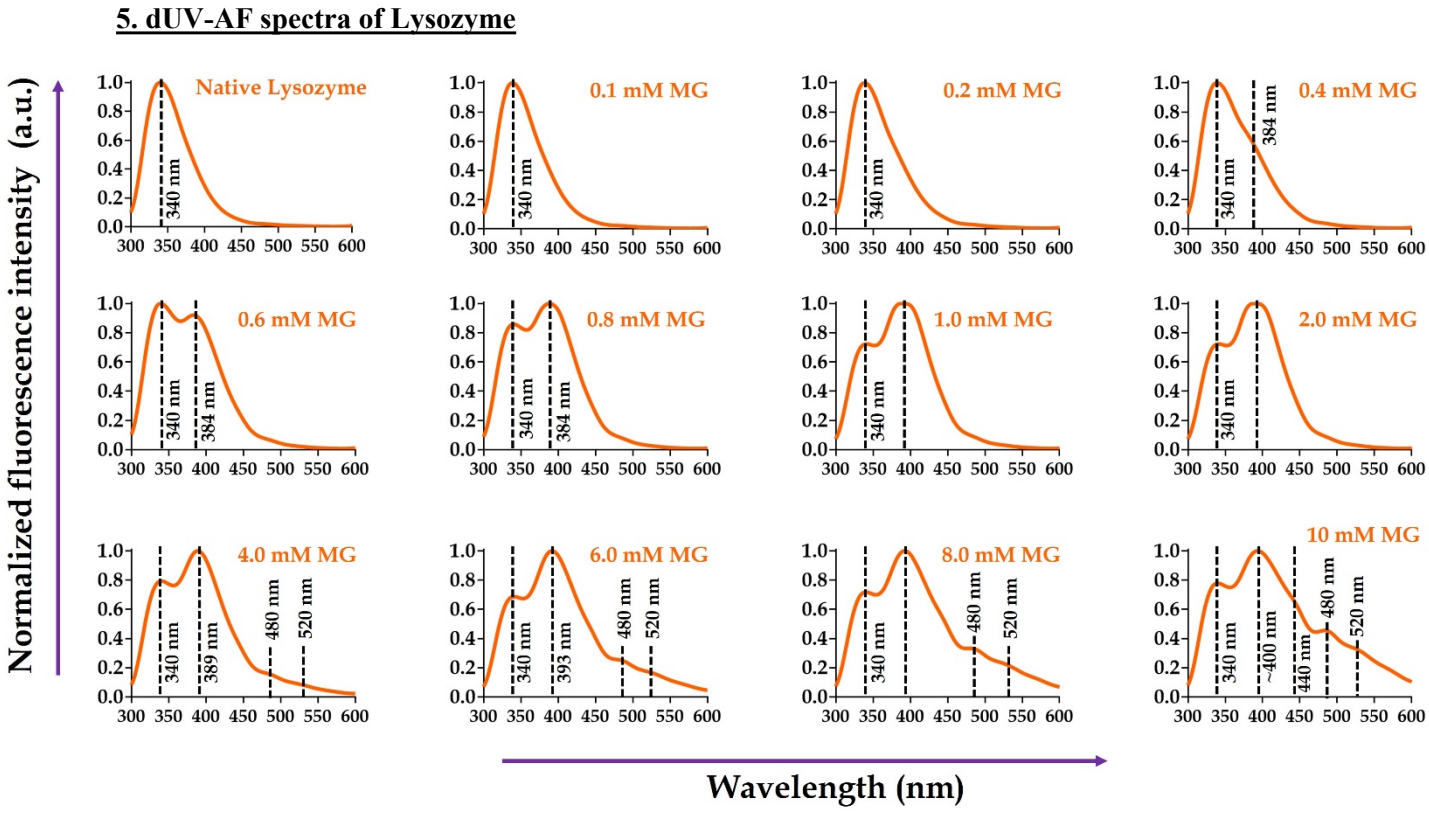

Figure S5. *dUV-AF spectra of methylglyoxal (0.1 – 10.0 mM) modified Lysozyme with AGEs and Trp-specific peaks highlighted with black dotted lines.*

6. SDS-PAGE of Lysozyme

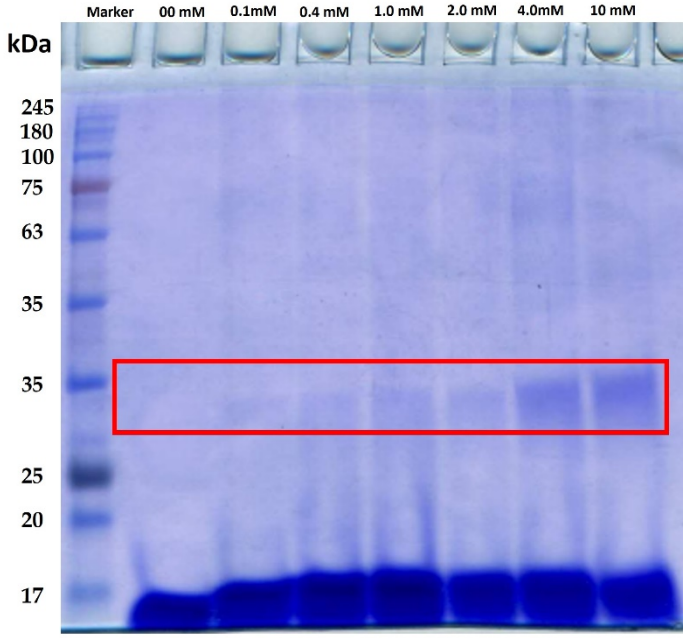

Figure S6. *Cross-linking of Lysozyme under varied concentrations of methylglyoxal. each lane indicates the different concentrations of methylglyoxal. The red box highlights the protein dimer bands corresponding to ~30 kDa.*

7. AGEs-based autofluorescence imaging

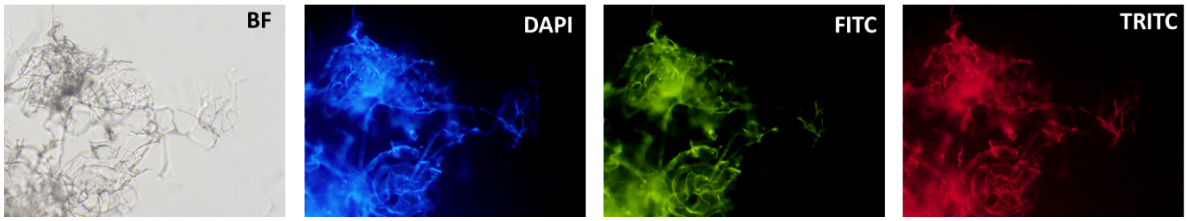

Figure S7. *The autofluorescence imaging of methylglyoxal-induced Lysozyme aggregates at 0.8 mM methylglyoxal treatment.*

8. AGEs specific fluorescence

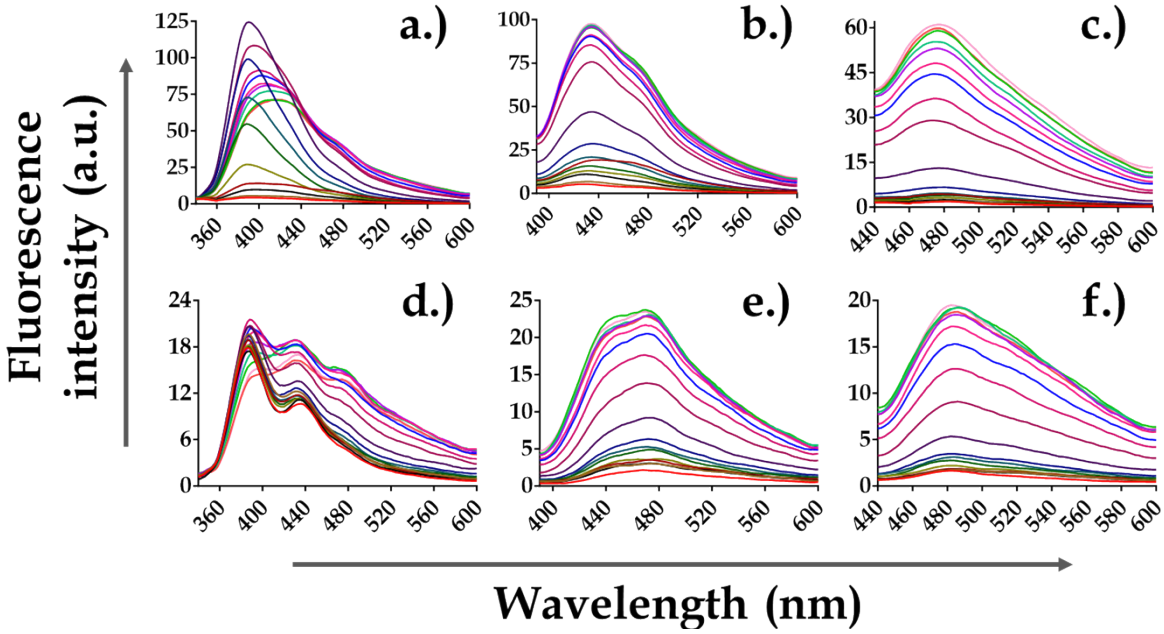

Figure S8. *AGEs specific fluorescence spectra of HSA at excitations, (a)320 nm, (b)370 nm, (c) 420 nm and Hemoglobin at excitations (d) 320 nm, (e)370 nm, and (f) 420 nm at their varying concentrations (0.05 mM – 20 mM).*