Electronic Supplementary Material (ESI) for RSC Advances.

Experimental and computational evidence on conformational fluctuations as a source of catalytic defects in genetic diseases.

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Supplementary Information

Figure S1. 2D-RMSD plots showing 34-45 loop movements. 200 snapshots have been periodically extracted from the simulations and pair-wise RMSD values (0-8 Å) are shown as a heatmap. Data for both loops of all dimeric systems are presented. Major conformational rearrangements are observed for apo systems of WT GALE (A, B), p.N34S (C, D), p.G90E (E, F) and p.V94M (G, H). In contrast, NAD^+-bound systems show drastically reduced loop flexibility for all four systems: WT GALE (I, J), p.N34S (K, L), p.G90E (M, N) and p.V94M (O, P).

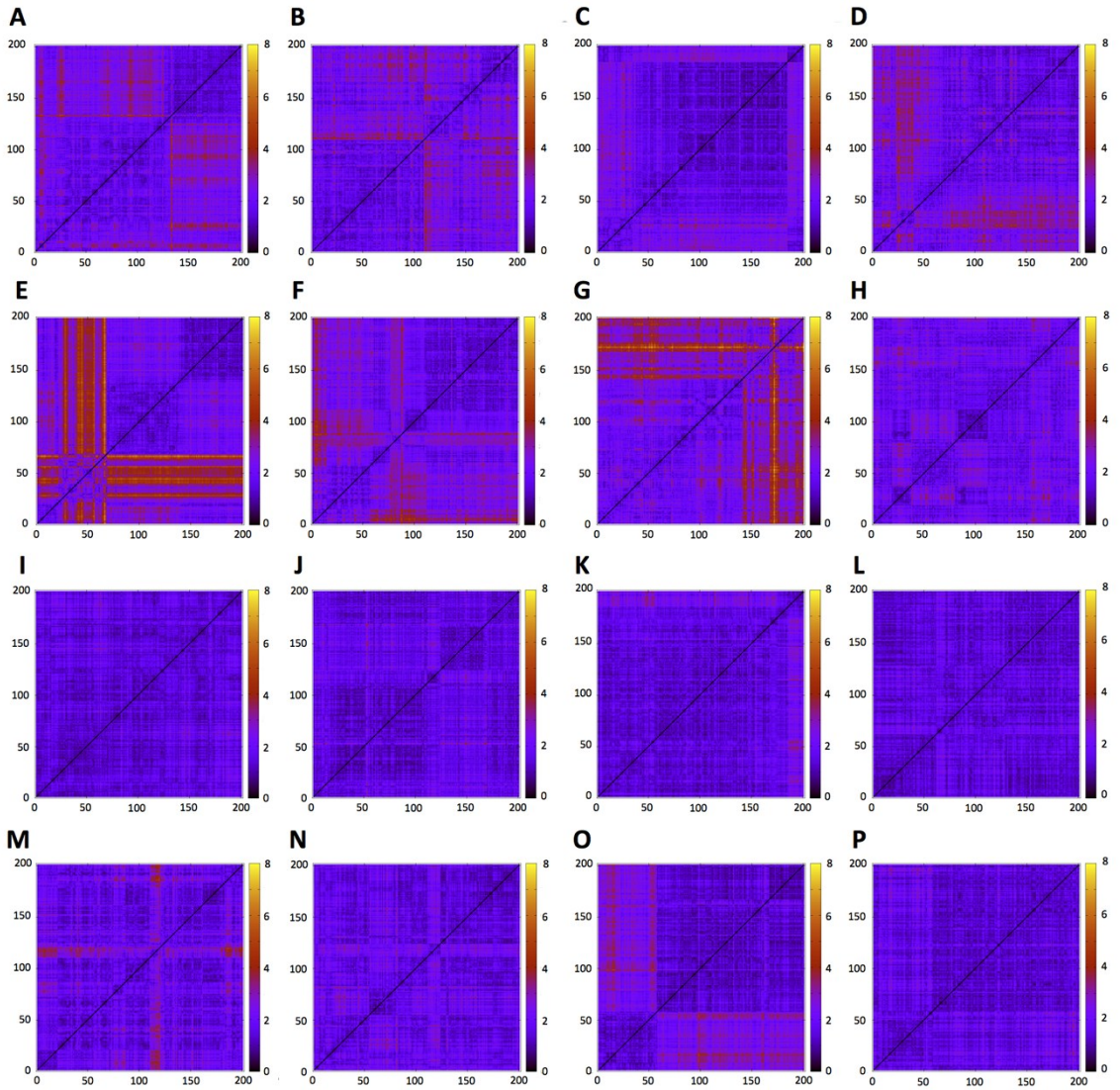

Figure S2. Urea denaturation of GALE enzymes monitored by Far-UV CD spectroscopy.

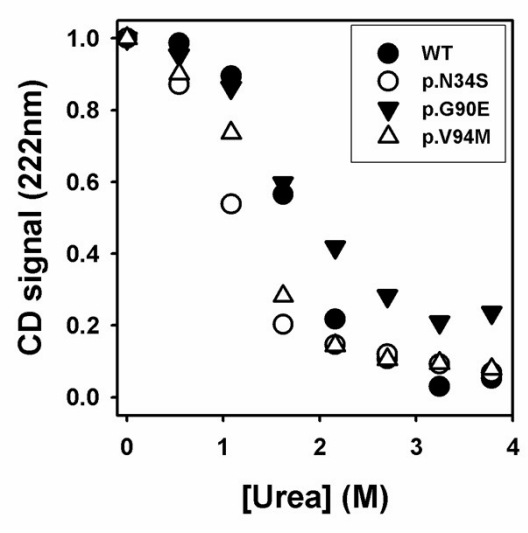

Figure S3. Proteolysis kinetics by thermolysin in the presence of urea. A and B) representative SDS-PAGE gels for thermolysin degradation kinetics of WT (A) and p.N34S (B) in the absence or presence of 0.6 M urea. C-F) proteolysis kinetics in the absence (black symbols) or the presence of 0.6 M urea (grey symbols), and in the absence (circles) or presence of NAD^+ 80 μM NAD (triangles). Data are the mean from two independent experiments, and fits to a single exponential function.

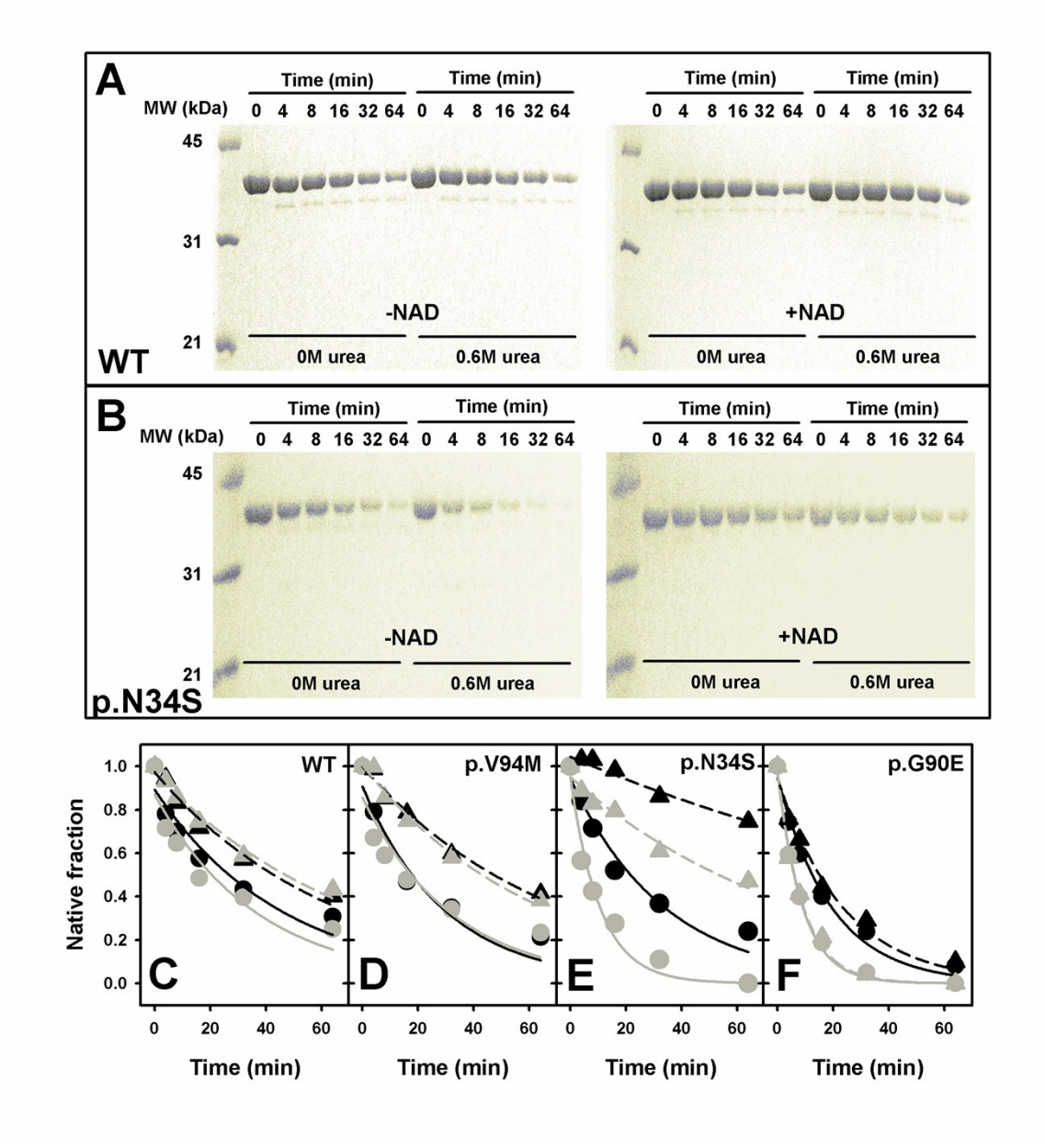

Table S1. SAXS Data Collection and derived parameters.

Data collection parameters					
Instrument	Diamond Light Source (Oxfordshire, UK)				
Wavelength (Å)	1				
s-range ($Å^{-1}$)	0.01–0.6				
Exposure time (s)	30×10				
Temperature (K)	277				
Structural parameters	GALE WT		GALE p.G90E		
Concentration ($mgml^{-1}$)	1	3	2	6	8
R_g (Å) (from Guinier)	29±3	30±3	31±3	32±3	33±3
R_g (Å) (from P(r))	29±3	30±3	31±3	33±3	34±3
D_{max} (Å)	97±10	112±11	116±11	112±11	118±12
Molecular mass determination					
MM (kDa) from Porod volume	59±6	58±6	62±6	62±6	66±7
Calculated MM (kDa) from sequence (Monomer / Dimer)	39 / 78				
Software employed					
Data processing	PRIMUS, GNOM				
Ab initio analysis	DAMMIF, DAMMIN				
Validation and averaging	SUPCOMB, DAMAVER				
Computation of model intensities	CRYSOL				
3D graphics representations	PYMOL				