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

Julian E. Fuchs, Ines G. Muñoz, David J. Timson and Angel L. Pey

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.

<table>
<thead>
<tr>
<th>Data collection parameters</th>
<th>Instrument</th>
<th>Diamond Light Source (Oxfordshire, UK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (Å)</td>
<td></td>
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<tr>
<td>s-range (Å⁻¹)</td>
<td></td>
<td>0.01–0.6</td>
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<tr>
<td>Exposure time (s)</td>
<td></td>
<td>30×10</td>
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<td>Temperature (K)</td>
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<td>277</td>
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<table>
<thead>
<tr>
<th>Structural parameters</th>
<th>GALE WT</th>
<th>GALE p.G90E</th>
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</thead>
<tbody>
<tr>
<td>Concentration (mgml⁻¹)</td>
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<td>3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
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<tr>
<td>R_g (Å) (from Guinier)</td>
<td>29±3</td>
<td>30±3</td>
</tr>
<tr>
<td></td>
<td>31±3</td>
<td>32±3</td>
</tr>
<tr>
<td></td>
<td>33±3</td>
<td>34±3</td>
</tr>
<tr>
<td>R_g (Å) (from P(r))</td>
<td>29±3</td>
<td>30±3</td>
</tr>
<tr>
<td></td>
<td>31±3</td>
<td>33±3</td>
</tr>
<tr>
<td></td>
<td>34±3</td>
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<tr>
<td>D_max (Å)</td>
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<td>112±11</td>
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<tr>
<td></td>
<td>116±11</td>
<td>112±11</td>
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<td></td>
<td>118±12</td>
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Molecular mass determination

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<th>58±6</th>
<th>62±6</th>
<th>62±6</th>
<th>66±7</th>
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<tr>
<td>Calculated MM (kDa) from sequence (Monomer / Dimer)</td>
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Software employed

<table>
<thead>
<tr>
<th>Software employed</th>
<th>Data processing</th>
<th>Ab initio analysis</th>
<th>Validation and averaging</th>
<th>Computation of model intensities</th>
<th>3D graphics representations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRIMUS, GNOM</td>
<td>DAMMIF, DAMMIN</td>
<td>SUPCOMB, DAMAVER</td>
<td>CRYSOL</td>
<td>PYMOL</td>
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