Light-triggered β-hairpin folding and unfolding

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**SI Materials and Methods**

Here we describe the details of the computational methods applied to (i) simulate the photoinduced refolding of the model peptide in deuterated methanol by molecular dynamics (MD) using a molecular mechanics (MM) force field and (ii) calculate the corresponding amide I IR bands of the peptide backbone for the cis and trans isomeric states of the integrated AMPP chromophore using a hybrid force field (1), which combines density functional theory (DFT) for the peptide backbone with MM models of the amino acid side-chains and of the solvent molecules.

**Simulation System and MM–MD Methods.** A periodic orthorhombic dodecahedron (inner radius: 25 Å) was filled with 1232 rigid MM methanol–d₄ models (2). The system was equilibrated for 1 ns by MD in the NpT ensemble, i.e., at constant atom number N, pressure p and temperature T, using the program EGO–MMII (3) and controlling the temperature (T = 300 K) and the pressure (p = 1 bar) by Berendsen thermostats and barostats (4), respectively [coupling constants, 0.5 ps (T) and 5 ps (p); isothermal compressibility, 12·10⁻⁵ bar⁻¹ (2)]. Here, the equations of motion were integrated by a multiple time–step algorithm (5) with a basic time step of 1 fs. The long–range electrostatics was treated by fast hierarchical multipole expansions (6) combined with a moving boundary reaction field approach (3), where a dielectric continuum [ε = 32.6 (2)] was assumed at distances beyond 25 Å. Lennard–Jones interactions were cut off at 10 Å and a long–range correction to the Lennard–Jones energy was applied (7).

Subsequently, the 10 best refined NMR structures (8) of the cis–AMPP hairpin were solvated by removing all overlapping methanol models. For the peptide the CHARMM22 force field (9) was chosen and supplemented by DFT derived parameters for the AMPP dye and for its linkage to the peptide (10). The peptide–solvent systems were carefully equilibrated by 250–ps NpT simulations keeping all covalent bonds involving hydrogen atoms rigid by M–SHAKE (relative tolerance, 10⁻⁶) (11). The resulting ensemble of solvated cis–hairpin structures served as a starting point for MM–MD simulations of the light–induced cis–trans isomerization and for computing the IR spectra of the cis–hairpin in methanol by DFT/MM.

In addition, the 300 K cis– and trans–hairpin equilibrium ensembles were estimated by two REST MD simulations (12) of 5 ns length, in which eight replicas covered the temperature range from 300 K up to 500 K (300 K, 322 K, 346 K, 372 K, 401 K, 432 K, 465 K, 500 K). Here, the starting structures were the eight best refined NMR structures which were additionally equilibrated for 100 ps to adopt the designated temperatures. After equilibration, exchanges between neighboring replicas were attempted every 2 ps as described in Ref. (13) with an average acceptance ratio of ≈20%. Inter–β–strand H–bond statistics were derived from the 300 K results.

**MM–MD Simulations of the Light–Induced cis–trans Relaxation.** The MM–MD simulations of the light–induced cis–trans photoisomerization of the AMPP chromophore and of the subsequent peptide relaxation from the cis into the trans conformational equilibrium
ensemble were enabled by a model potential (10, 14) driving an inversion reaction at one of the atoms within the central N=N bond of AMPP. That model potential drives an ultrafast (∼250 fs) cis–trans isomerization of AMPP and deposits an energy into the molecule, which equals that of the absorbed photon (70.7 kcal/mol). Each of the equilibrated cis–hairpin structures was simulated for 1 ps in the NVE ensemble using now the Verlet algorithm (15) for integration until the photoisomerization was triggered by switching on the quoted model potential. The NVE ensemble was maintained for the following 10 ps. Then the solvent molecules were coupled to a Berendsen thermostat (\(T = 300\) K) and NVT simulations were continued for another 3,000 ps applying the multiple time–step integrator (5). The coordinates, kinetic and potential energies of the peptide were recorded every 5 fs during the first 10 ps, every 50 fs during the next 90 ps, and every 500 fs during the remainder of the simulation for analysis of energy dissipation and structural relaxation dynamics. Intermediate structures obtained 100 ps after photoisomerization were selected as representatives for the model peptide on its way to the equilibrium ensemble of trans–conformations.

**DFT/MM Calculations of Amide I Bands.** The IR bands of the cis and trans peptide in the amide I spectral range were derived from DFT/MM calculations (1) by the protocol for an “instantaneous normal mode analysis” (INMA) described in refs. 16 and 17. INMA requires snapshots of the peptide solvent system. For the cis–case we selected three snapshots with a maximal number of inter–β-strand H–bonds and for the trans–case three snapshots without such H–bonds from ensembles of equilibrated NMR structures. For the INMA calculations the solvent–solute snapshots were partitioned into DFT and MM fragments by assigning in the cis–case the complete peptide backbone and in the trans–case the backbone of one of the two isolated branches (residues 1–5 or 6–10, treated independently) to the DFT fragment, resulting in 78 (cis) and ≈40 (trans) atoms to be treated by DFT. To enable the application of the plane–wave DFT code CPMD (18) the DFT fragments were enclosed by rectangular boxes such that each DFT atom was located at least 3 Å away from the next box surface (e.g., resulting in a size of \(24 \times 14 \times 14\) Å\(^3\) for cis). A plane wave cut–off of 70 Ry, the gradient–corrected exchange functional of Becke (19), the correlation functional of Perdew (20), and the norm–conserving pseudopotentials of Troullier and Martins (21) were applied. The covalent linkages between the peptide backbone and MM fragment were treated as described in ref. 1.

In the INMA computation of the amide I bands the solvent cages of the structural snapshots were kept rigid and the DFT/MM energies of the systems were minimized by relaxing the coordinates of the azopeptide until the maximal gradient fell below 1.0 kcal/(mol·Å). At the optimized geometries the Hessians were calculated for all DFT atoms by symmetric finite differences (\(\Delta x = 0.01\) Å) of analytical DFT/MM forces. The amide groups of the peptide backbone were chosen to be deuterated as in the experimental setting. The INMA procedure yielded then for each of the snapshots the vibrational frequencies and IR intensities of all backbone normal modes. IR spectra of the backbone C=O stretching modes in the cis and trans conformations of the peptide were constructed by superposing the obtained line spectra and convolving them with a Gaussian of 13 cm\(^{-1}\) width.
Time–Dependent Absorption Changes at Distinct Probing Wavenumbers. The time
dependence of the absorption changes allows to obtain insight into the time scales of
specific processes that occur during the unfolding reaction (see SI Fig. 6). It reflects
transients on different time scales: Directly after the photoisomerization of the AMPP optical
switch, which is finished within a few picoseconds, the absorption is changed over the
complete recorded spectral range. Subsequent strong transient absorption changes occur
with a time constant of ≈4 ps. They lead to a reduced absorption on the far low frequency
side of the amide I band (see curve A, 1,628 cm$^{-1}$). The strong initial absorption decrease
observed at higher frequencies recovers partially with the same time constant (trace B).
Subsequently there is a weak transient on the 20 ps and 70 ps time scale (traces A, C, and
D). A pronounced absorption increase is found at 1,655 cm$^{-1}$ (trace C) which occurs with a
time constant of ≈700 ps. The absorption changes reached at the end of the observation
period fit well to those of the stationary difference spectrum (Fig. 1B, dashed curve). A fit
performed with the presented set of time constants describes well the recorded absorption
data.

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