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Helix Handedness Inversion in Arylamide Foldamers: Elucidation and Free Energy Profile of a Hopping Mechanism

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Simulation setup. The general AMBER force field (GAFF),[1] with improved torsional parameters, was used in all simulations. The quantum mechanically derived torsional parameters ($V_n/2$, equation 1) for the X-C_Q-C_{am}-X and X-C_Q-N_{am}-X dihedral angles were 3.6 and 5.7 kcal/mol, respectively. All systems were simulated using the NAMD program.[2] Each simulation box measured ~50 Å along each side and contained one oligomer and up to ~5000 solvent molecules. The systems were initially equilibrated for 500 ps in an NPT ensemble at a constant temperature of 300 K and a pressure of 1 atm. The metadynamics production runs (300 to 350 ns for tetramer, 400 to 500 ns for pentamer and 500 ns for hexamer systems) were performed in an NVT ensemble with a 1 fs time step. In each metadynamics run, at least one pitch dihedral angle was used as a collective variable (CV). We carried out two sets of simulations, differing in the choice of the second CV. In *set 1* simulations, the end-to-end distance (between COMs of the terminal aromatic rings) was used as the second CV, to examine the extent of helical unfolding during handedness inversion. In *set 2* simulations, the second CV is another pitch dihedral angle, giving insight to local conformational information of intermediates along the inversion pathway. In all metadynamics simulations, the height of the biasing Gaussian potential ("hill") was 0.01 kcal/mol and the width was 0.313 Å for the end-to-end distance CV and 3.13 degrees for the pitch dihedral angle CV.

The torsional energy and parameters are defined by the following equation:

$$E_{torsion} = \frac{V_n}{2}(1 + cos(n\phi - f) \tag{1}$$

in which $n = 2$ and $f = 180°$ for torsions around the aryl-amide bonds.

Quantum mechanics studies of model compounds. Figure S1 shows the potential energy profiles with respect to the rotations around the aryl-C_{amide} and aryl-N_{amide} bonds in two model compounds. Each data point (filled circle) represents the energy of a partially optimized structure in which the dihedral angle of interest is fixed at the value shown on the X-axis and all other degrees of freedom are optimized at the B3LYP/6-311g(d,p) level of theory. As shown in Figure S1, a 180° rotation around the C_Q-N_{am} bond has a higher barrier than an analogous rotation around the C_Q-C_{am} bond.

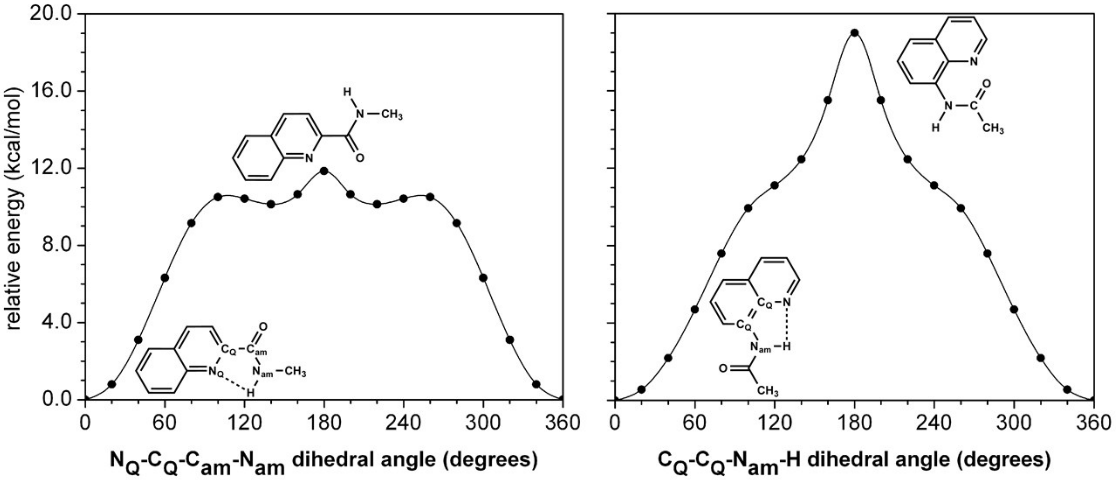

Figure S1. Quantum mechanics potential energy profiles with respect to the N_Q-C_Q-C_{am}-N_{am} and C_Q-C_Q-N_{am}-H dihedral angles for two arylamide model compounds. The structures of the minima (bottom) and maxima (top) are sketched in each figure and the dihedral angles of interest are labeled in the minimum structures.

Set 1 metadynamics simulations. Figure S2 shows the free energy profiles (FEPs) of pentamer and hexamer in chloroform, obtained by using the end-to-end distance and one pitch dihedral angle as CV. The FEPs show that the largest end-to-end distance and the highest energy along the inversion pathways are ~14 Å and ~14-16 kcal/mol, respectively. The fact that the largest end-to-end distances of pentamer and hexamer are comparable to the **E1** of tetramer suggested that their handedness inversion also goes through partially extended intermediates. To decipher their inversion pathways, we ran *set 2* simulations, with two pitch dihedral angles used as CVs.

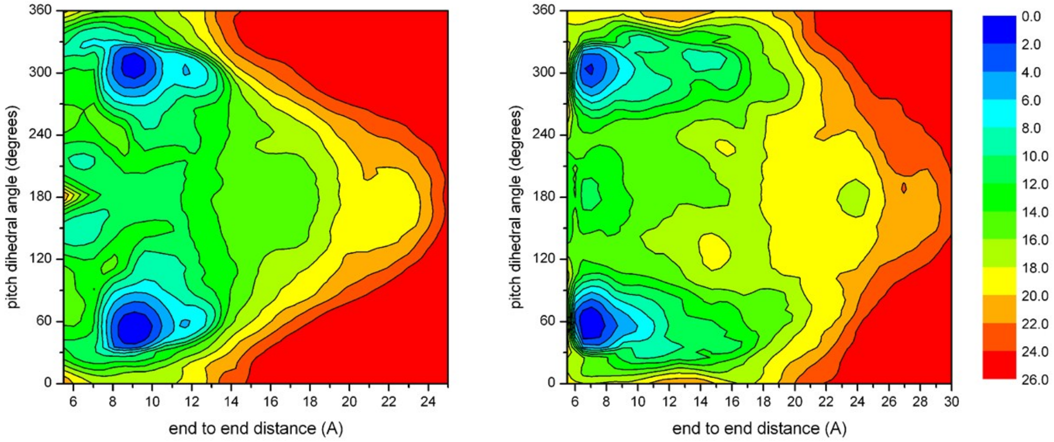

Figure S2. Folding-unfolding free energy profiles (energy in kcal/mol) with respect to end-to-end distance and the first pitch dihedral angle (Q1-Q2-Q3-Q4) for pentamer (left) and the second pitch dihedral angle (Q2-Q3-Q4-Q5) for hexamer (right).

Influence of solvents. Figures S3, S4 and S5 show free energy profiles in water for handedness inversion of tetramer, pentamer and hexamer, respectively. Qi *et al.*'s experimental study[3] has shown that polar protic solvents (such as methanol/water mixtures) significantly enhance the stability of an analogue quinoline based helical octamer. The half-life of the helical octamer increases by about two to three orders of magnitude when going from chloroform to methanol/water mixtures, which corresponds to ~3 to 4 kcal/mol difference in activation barrier of inversion. Our results (Figures S3 to S5) show a similar trend, i.e. in water the free energy of both the partially folded intermediates and transition states with respect to the folded global minimum increases as compared to that in chloroform. The highest barrier along the inversion path increases by ~1-3 kcal/mol, in line with experimental results considering the differences in structure (experimentally studied octamer with side chains vs simulated pentamer/hexamer without side chains).

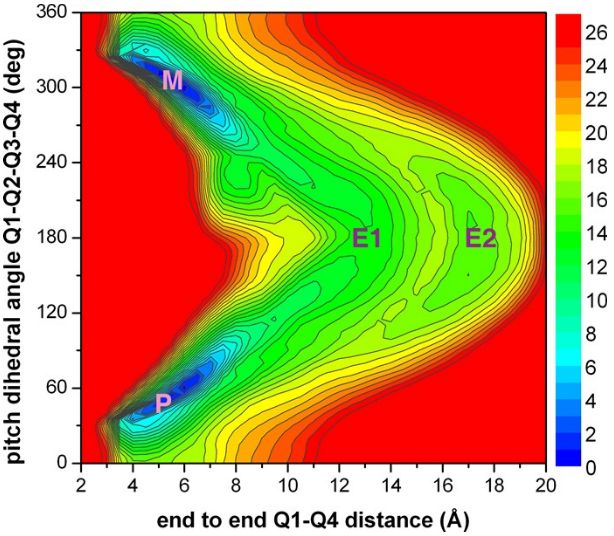

Figure S3. Tetramer free energy profile (energy in kcal/mol) with respect to end-to-end (Q1-Q4) distance and pitch dihedral angle Q1-Q2-Q3-Q4, in water.

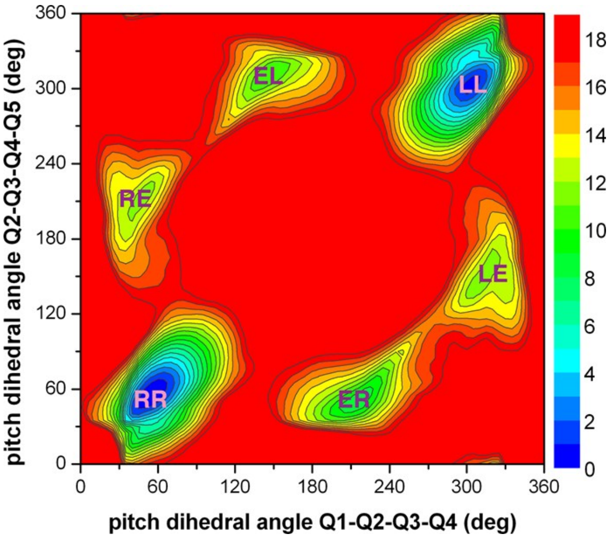

Figure S4. Pentamer free energy profile (energy in kcal/mol) with respect to pitch dihedral angles Q1-Q2-Q3-Q4 and Q2-Q3-Q4-Q5, in water. Note that **LL** and **RR** correspond to **M** and **P**, respectively.

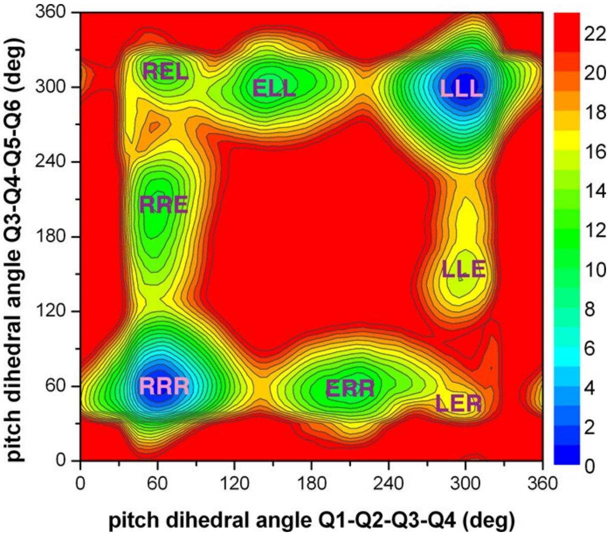

Figure S5. Hexamer free energy profile (energy in kcal/mol) with respect to pitch dihedral angles Q1-Q2-Q3-Q4 and Q3-Q4-Q5-Q6, in water. Note that **LLL** and **RRR** correspond to **M** and **P**, respectively.

Error analysis. In the metadynamics simulation, at certain time, *t*, accumulation of the biasing "hills" fills the energy wells in the conformational space of interest and the underlying free energy landscape becomes "flat" for the molecule to move freely. The additional simulation time adds hills over the entire conformational space evenly; these hills should not affect the relative energy and positions of minima and intermediates. Therefore, the standard deviation of the differences (Figure S6) between the FEPs at *t (i.e. 300 ns)* and *t' (i.e. 400 ns)* reflects error. The errors estimated for the tetramer and pentamer FEPs are ~1.1 and ~0.55 kcal/mol, respectively.

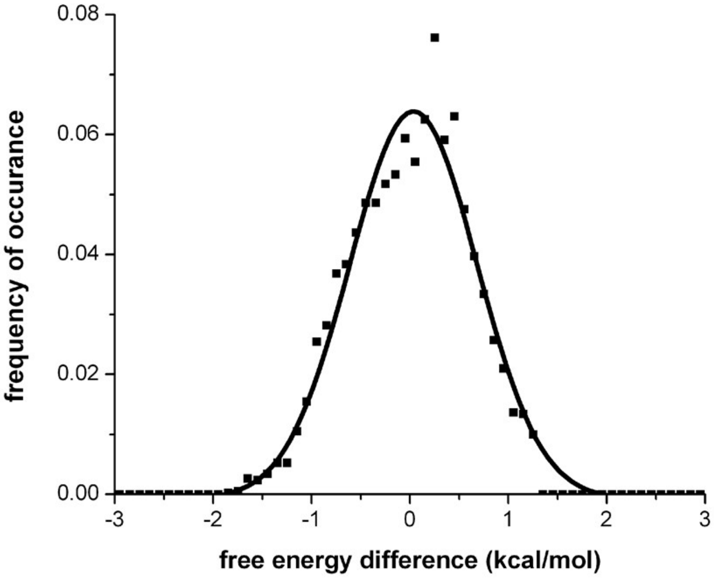

Figure S6. Distribution of the free energy differences between the 300 ns and 400 ns FEPs for the pentamer. The scatter plot (squares) is the actual data and the solid line is its Gaussian fit.

References:

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2. J. C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R. D. Skeel, L. Kalé, K. Schulten, *J. Comput. Chem.* **2005**, *26*, 1781–802.
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