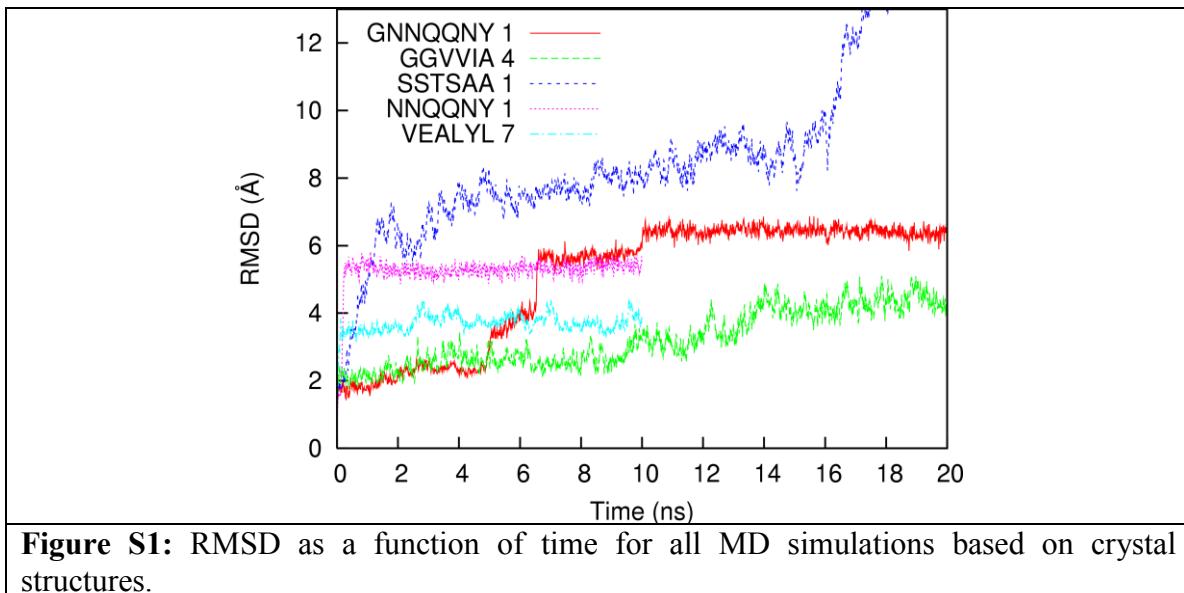


A Systematic Examination of Polymorphism in Amyloid Fibrils by Molecular Dynamics Simulation

Supplementary Information

Simulations performed from crystal structures: The root mean squared deviations of successive conformations from molecular dynamics (MD) started from crystallographic structures are shown below:



The RMSDs are larger than might be expected as the fibrils twist during the simulation, as previously reported (Esposito L., Pedone C. & Vitagliano L. *Proc. Natl. Acad. Sci. USA* (2006) 103, 11533; Berryman J., Radford S. E. & Harris S. A. *Biophys. J.* (2009) 97, 1). Nevertheless, the difference between the four stable ($OP < 0.07$) structures and the unstable SSTSAA class 1 is clear.

Control simulations: To demonstrate that the classification as ordered or disordered structures is not dependent on the forcefield chosen for the calculations, the simulations reported in Figure 1 were repeated using the AMBERFF03 forcefield, and simulations based on crystal structure data were also repeated using the CHARMM 22-CMAP forcefield. Figure S2 shows the final structures after the 10ns molecular dynamics simulations.

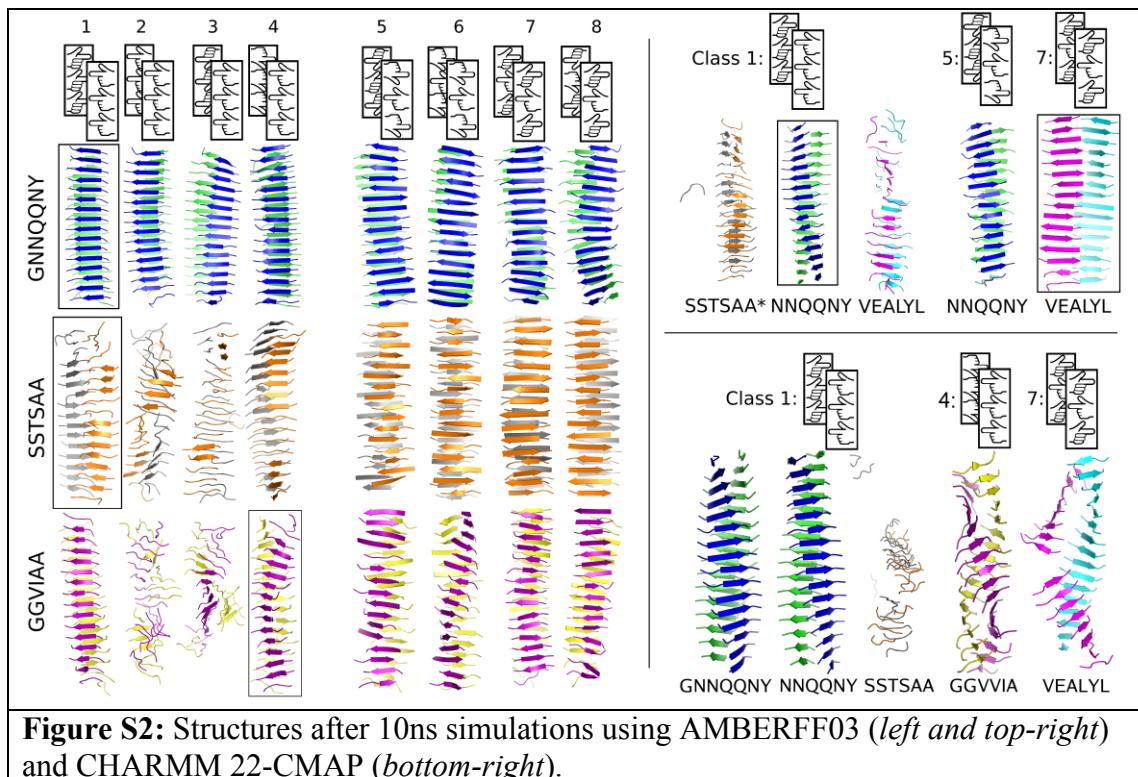
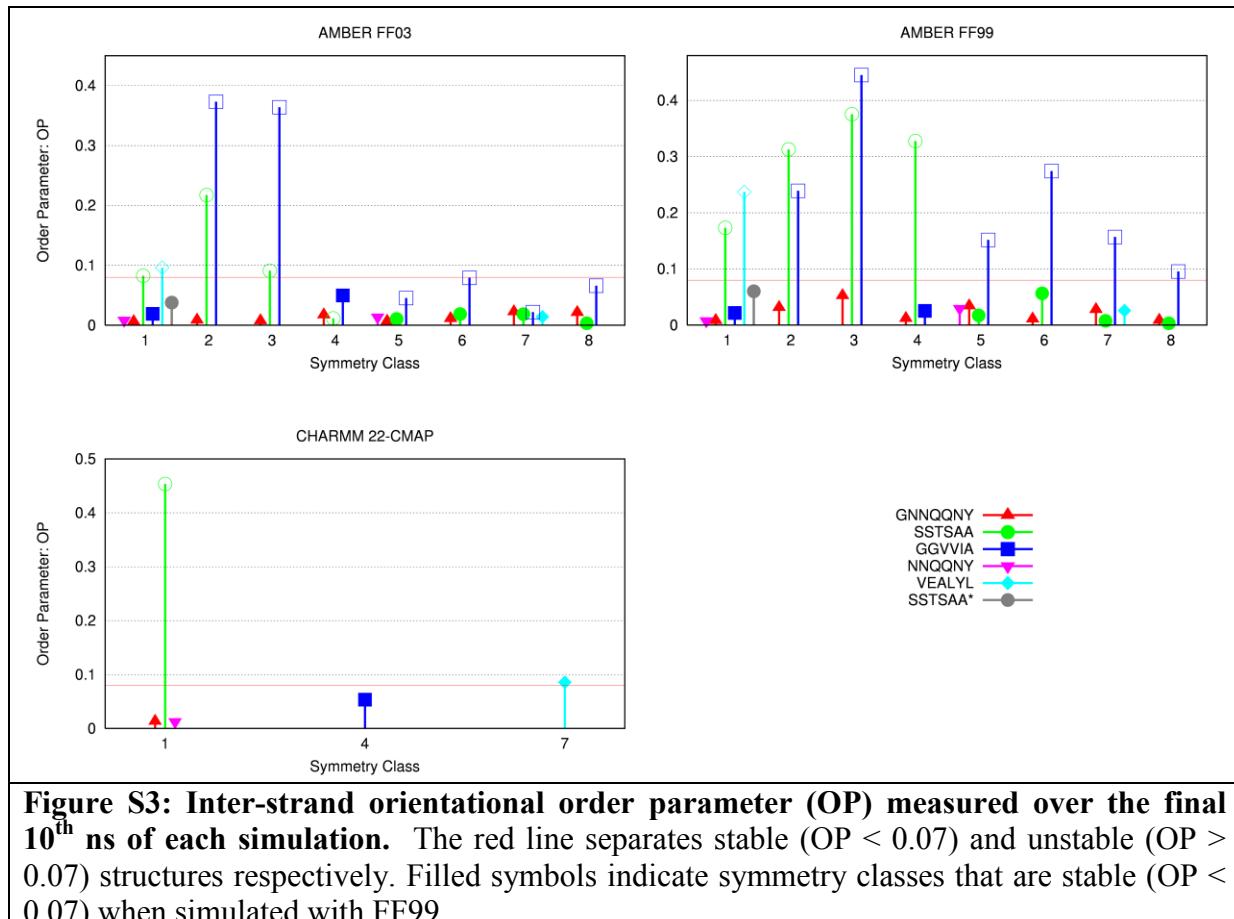
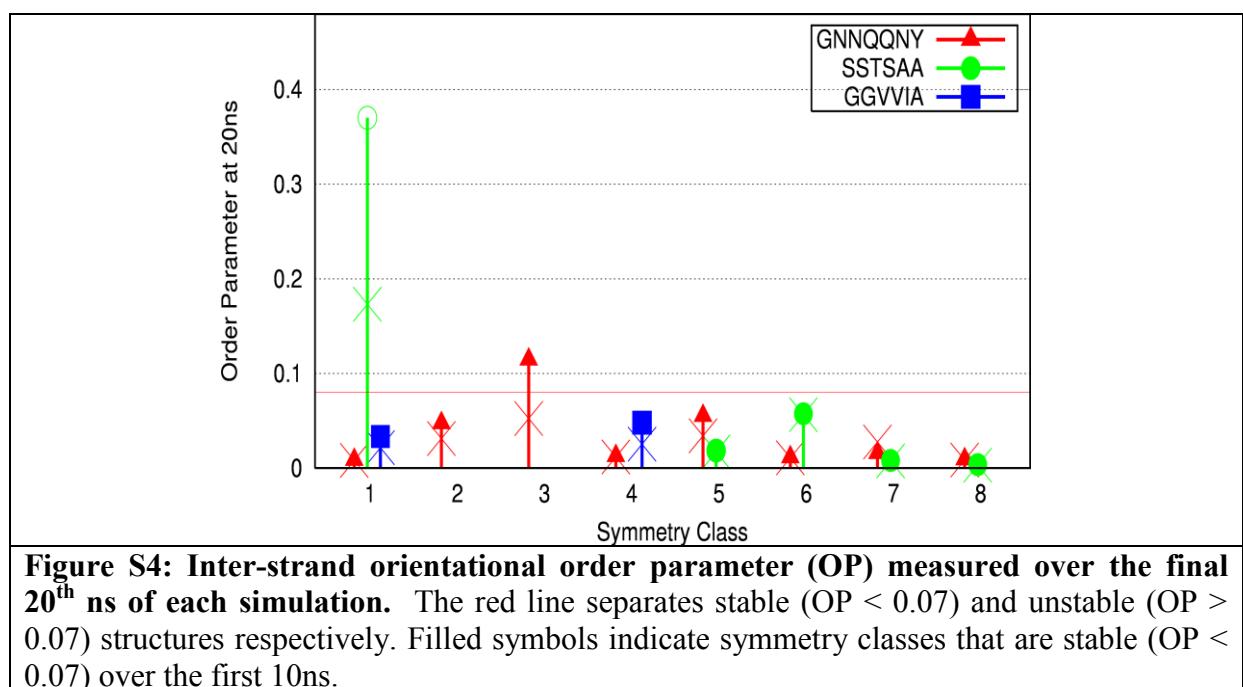


Figure S3 shows the order parameters (OP) calculated over the final 10th ns using AMBERFF99, AMBERFF03 and CHARMM 22-CMAP. Only three (SSTSAA class 4, GGVVIA classes 5 and 7) of the 29 simulations have a different stability classification with FF99 and FF03 (note that class 8 GGVVIA has a very similar order parameter with both forcefields although strictly the classification is different). Interestingly, fibrils simulated with FF03 have the lowest order parameter in all three cases.



All of the polymorphs of GNNQQNY, SSTSAA and GGVVIA classified as stable ($OP < 0.07$) after 10ns were extended to 20ns to demonstrate that they remain ordered over this longer timescale. Figure S4 shows the order parameter calculated over the final 1ns for the simulations which were extended from 10ns to 20ns. Crosses indicate the order parameter after 10ns, symbols show the value after 20ns. The red line indicates $OP = 0.07$. All structures apart from SSTSAA were classified as ordered ($OP < 0.07$) at 10ns. After 20ns, all of these classifications remain valid, apart from GNNQQNY class 3, which has $OP > 0.07$ after 20ns. On inspection of the molecular structures for this sequence, this reduction in ordering with time is due to melting from the ends of the fibril. Note that the order parameter which changes the most on extending the simulations from 10ns to 20ns is for SSTSAA class 1, which was in a disordered aggregated conformation ($OP > 0.07$) after 10ns. As this symmetry has already disintegrated into a disordered aggregate after 10ns, the order parameter continues to increase as the simulation is extended to 20ns and the systems moves further away from an ordered aggregated state.



Backbone/side-chain hydrogen bonding interactions in polymorphs of GNNQQNY and SSTSAA: Hydrogen bonding interactions are crucial in determining the thermodynamic stability of a given polymorph. For the sequence SSTSAA, the number of hydrogen bonds per donor varied from around 0.3 to 0.5 depending on the symmetry class, however, the sequences GNNQQNY and NNQQNY satisfied > 0.5 of their hydrogen bonding potential over all polymorphic symmetries. The numbers of backbone and side-chain hydrogen bonds per residue for the polar sequences GNNQQNY and SSTSAA are compared for each of the 8 symmetries in Fig. S5 (left and right respectively). When GNNQQNY switches from anti-parallel (fibril classes 5 to 8) to parallel β -sheets (fibril classes 1 to 4), the loss of backbone hydrogen bond interactions is compensated by an increase in the number of side-chain/side-chain hydrogen bonds, as was previously suggested based on an analysis of this peptide in symmetry classes 1 and 5 (Berryman J., Radford S. E. & Harris S. A. *Biophys. J.* (2009) 97, 1). Although the total number of hydrogen bonds per residue in the anti-parallel polymorphs is comparable for both sequences (1.2 for GNNQQNY compared to 0.9 for SSTSAA), SSTSAA is unable to achieve an equivalent side-chain/backbone hydrogen bond compensation on switching from anti-parallel to parallel β -sheets. So although GNNQQNY has, on average a total of 1.4 hydrogen bonds per residue in the parallel polymorphs, SSTSAA has only 0.6. The serine and threonine alcohol side-chains are too short to span the 4.8 \AA distance between successive peptide strands; in contrast with the glutamine and asparagine amide side-chains in GNNQQNY which are exactly the right size to achieve this span. Therefore, the continuous Q/N ladders observed in ordered β -sheets of GNNQQNY (Fig S6 left) cannot be formed by SSTSAA, rather the ladder is forced to “zig-zag” between pairs of β -strands (Fig S6 right). Consequently, parallel (classes 1-4) polymorphs of SSTSAA are predominantly unstable ($OP > 0.07$) in the simulations. Figure S6 (right) shows that the SSTSAA 1* polymorph looses a monomer from the end of the fibril. End effects are common in simulations of amyloid, so the ends are removed prior to analysis.

The numbers of backbone and side-chain hydrogen bonding interactions per residue for the polar sequences GNNQQNY (left) and SSTSAA (right) is shown are Figure S5:

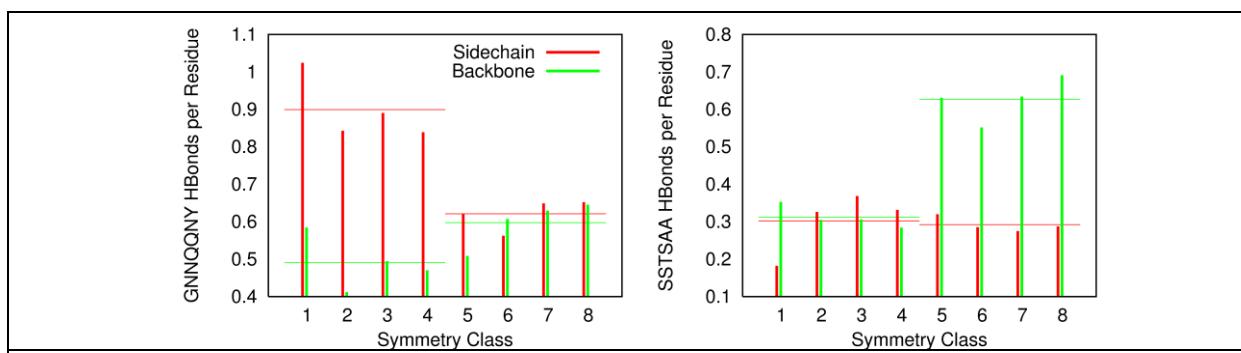
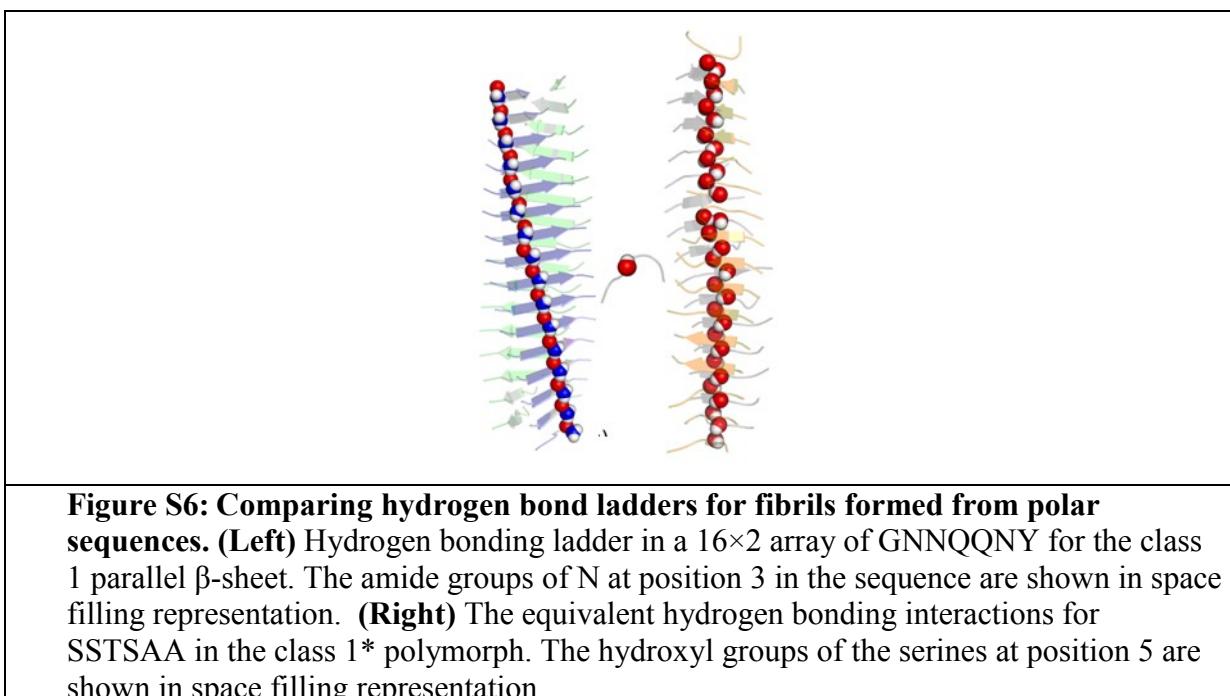
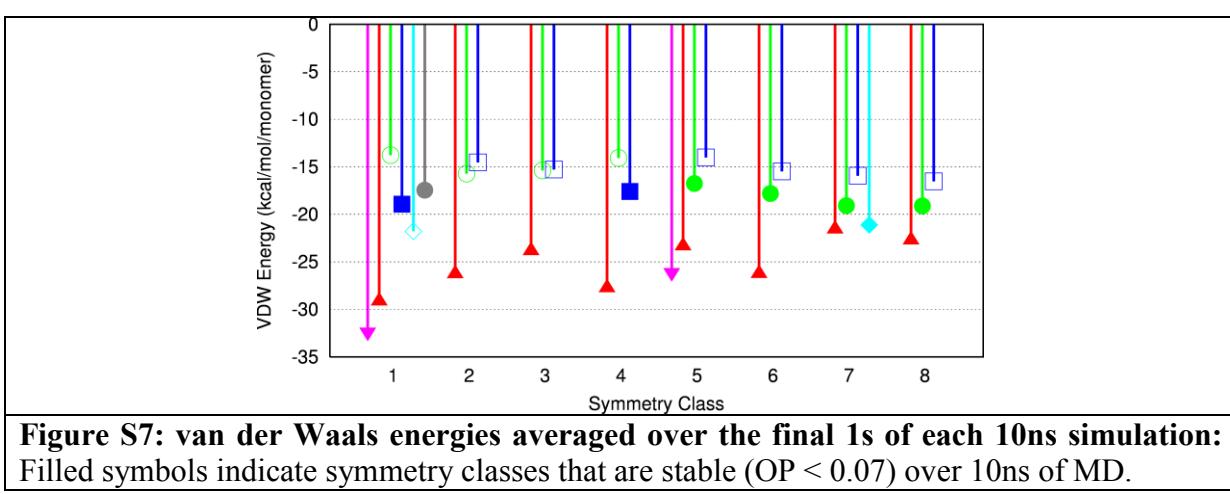


Figure S5: Backbone/side-chain hydrogen bonding compensation for fibrils with polar sequences. Bars show the mean hydrogen bonding over the final 1 ns of the simulations in each symmetry class; horizontal lines show averages over parallel (classes 1-4) and anti-parallel (classes 5-8) β -sheets. Note that the vertical axis has a different scale for the two sequences.

Figure S6 shows that SSTSAA is unable to achieve equivalent side-chain/backbone hydrogen bond compensation on switched from anti-parallel to parallel β -sheets due to the shorter length of the serine and threonine sidechains compared to glutamine and asparagine.



The van der Waals energies: Figure S7 shows the van der Waals interaction energies extracted from the MD forcefield averaged over the last 1ns of each of the 29 10ns simulations performed with AMBER FF99. The interaction energy was obtained by subtracting the energy of the individual peptide strands from the van der Waals energies of the aggregates.



Equilibrium population of polymorphs estimated from the relative enthalpies: Figure S8 shows the relative thermodynamic populations of stable polymorphs of GNNQQNY, SSTSAA and GGVVIA calculated from the enthalpies of the defect free GB/SA simulations (symbols) and from the enthalpies of the last 1ns of the 10ns explicitly solvated MD simulations (horizontal lines). The difference between these two approaches provides an honest estimate of the error in the relative energies of the polymorphs, given that two contrasting methods of *in silico* design have been employed.

