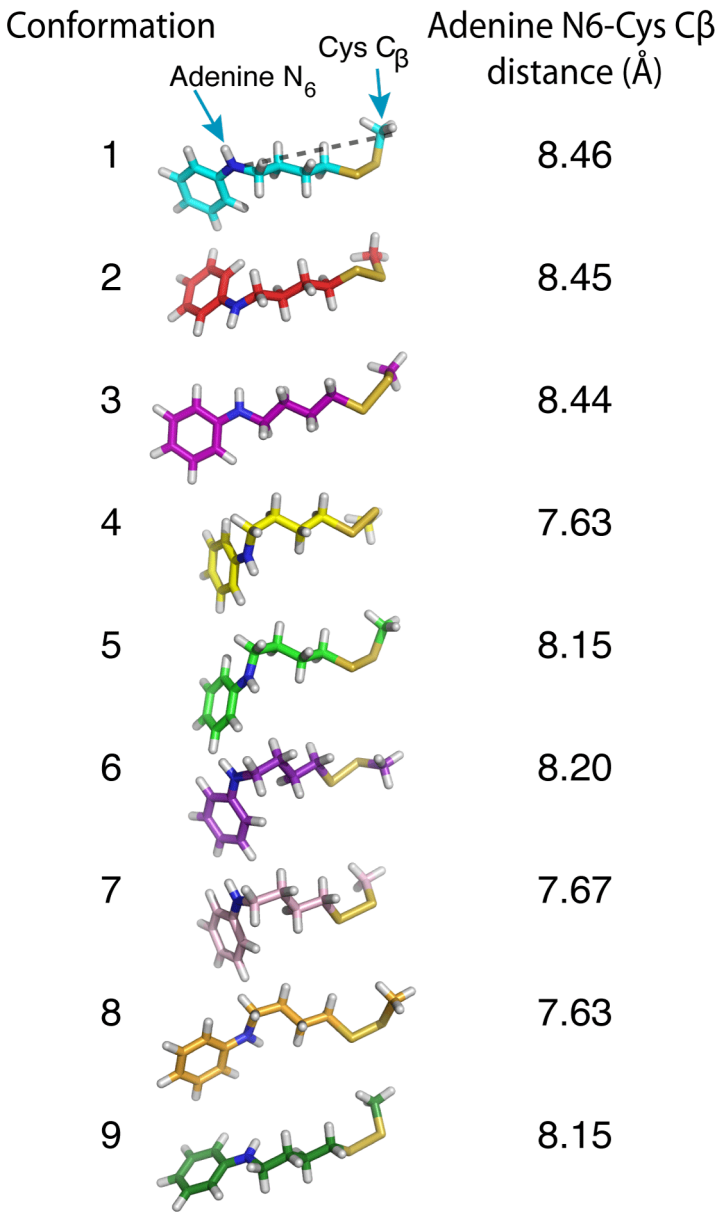

Supplemental Fig. 4. Conformations of the modeled crosslinker. The 9 lowest energy conformations of the modeled four-carbon linker are ranked in order of increasing energy with their corresponding N^6-Cβ distances. The lowest energy conformation (#1) is also depicted in figure 4B. Stick representations of models are colored by atom and were generated in Pymol (1). In order to produce these structures, a Monte Carlo (MC) conformational search was performed to locate all low energy conformations of the untethered crosslink. To generate the starting conformations for the MC conformational search, a crosslink was built using MacroModel's Maestro GUI (2). Since we were interested in the maximum distances accessible by the crosslink, we placed harmonic constraints (100 kJ/mol) on all X-CH_2-CH_2-X torsions, maintaining anti-conformation dihedrals of 180°. All other dihedrals were searched. Two

distinct 1000 step Monte Carlo conformational searches were run. For all calculations, energies were evaluated using the OPLS2005 force field, as implemented in Macromodel (2). For all minimizations the Polak-Ribiere Conjugate Gradient (PRCG) method was employed, and the convergence criterion for the minimization of gradient norm was set to <0.05 kJ/mol-Å. We employed the GB/SA solvation treatment (3) modeling the solvent as water. Bond dipole cutoffs were employed to truncate the electrostatic and GB terms. Non-bonded cutoffs were as follows: 8 Å in Van der Waals, 99999.0 Å in charge-charge (effectively infinite), $20^{3/2}$ Å (89.4 Å) in charge-dipole, and 20 Å in dipole-dipole. At each step of the Monte Carlo search, 2-4 crosslink dihedrals were randomly selected, and their values were adjusted by 0-180°. After each step, up to 500 steps of minimization were performed - if convergence was not achieved in fewer steps - and conformations within 50 kJ of the global minimum were saved. After the search, all remaining structures were fully minimized, and all conformations within 50 kJ of the global minimum were kept, while redundant structures (RMSD < 0.25 Å) were removed. No new structures were obtained after pooling the conformations obtained from the second run with those obtained from the first run, suggesting that conformational space had been fully explored.

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