How PEGylation enhances the stability and potency of insulin: a molecular dynamics simulation†

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TITLE RUNNING HEAD: Molecular dynamics simulation for PEG-insulin conjugates.

SUPPORTING INFORMATION

Molecular dynamics simulation using a simulated annealing procedure

The simulated annealing procedure for insulin-50 PEG units conjugate was performed by increasing the temperature from 300K to 400K linearly within 0.5 ns and equilibrating at 400K for 0.5 ns, followed by raising the temperature to 450K linearly in another 0.5 ns and equilibrating at 450K for 0.5 ns, and then decreasing the temperature symmetrically to 300K and equilibrating at each temperature for another 6.5 ns. For insulin-100 PEG units conjugate, the temperature was raised from 300K to 400K linearly within 0.5 ns and equilibrating at 400K for 1.5 ns, followed by raising the temperature to 450K linearly

in another 0.5 ns and equilibrating at 450K for 1.5 ns, and then decreasing the temperature symmetrically to 300K and equilibrating at each temperature for another 8.5ns. For insulin-200 PEG units conjugate, the temperature swing procedure was the same to that applied for insulin-50 PEG units.

Analysis tools for molecular simulation

The root mean square deviation (RMSD) reflects the derivation of a specific conformation with reference to its native counterpart. RMSD was computed by least-square fitting the structure at time $t=t_1$ to the reference structure a time $t=t_0$, according to equation (1).

$$RMSD(t_1,t_0)=\left[\frac{1}{M}\sum_{i=1}^{N}m_i\left\|\boldsymbol{r}_i(t_1)-\boldsymbol{r}_i(t_0)\right\|^2\right]^{\frac{1}{2}} \tag{1}$$

Where m_i represents the mass of atom i in insulin or PEG-insulin conjugate and $M=\sum_{i=1}^{N}m_i$ is the mass of the whole molecule, $\mathbf{r}_i(t)$ is the position of atom i at time t_1.

The radius of gyration (Rg) describes the structural compactness of the whole molecule, which was calculated according to equation (2).

$$Rg=\left(\frac{\sum_i\left\|\boldsymbol{r}_i\right\|^2 m_i}{\sum_i m_i}\right)^{\frac{1}{2}} \tag{2}$$

Where $\mathbf{r}_i$ is the position of atom i relative to the center of the mass of molecule.

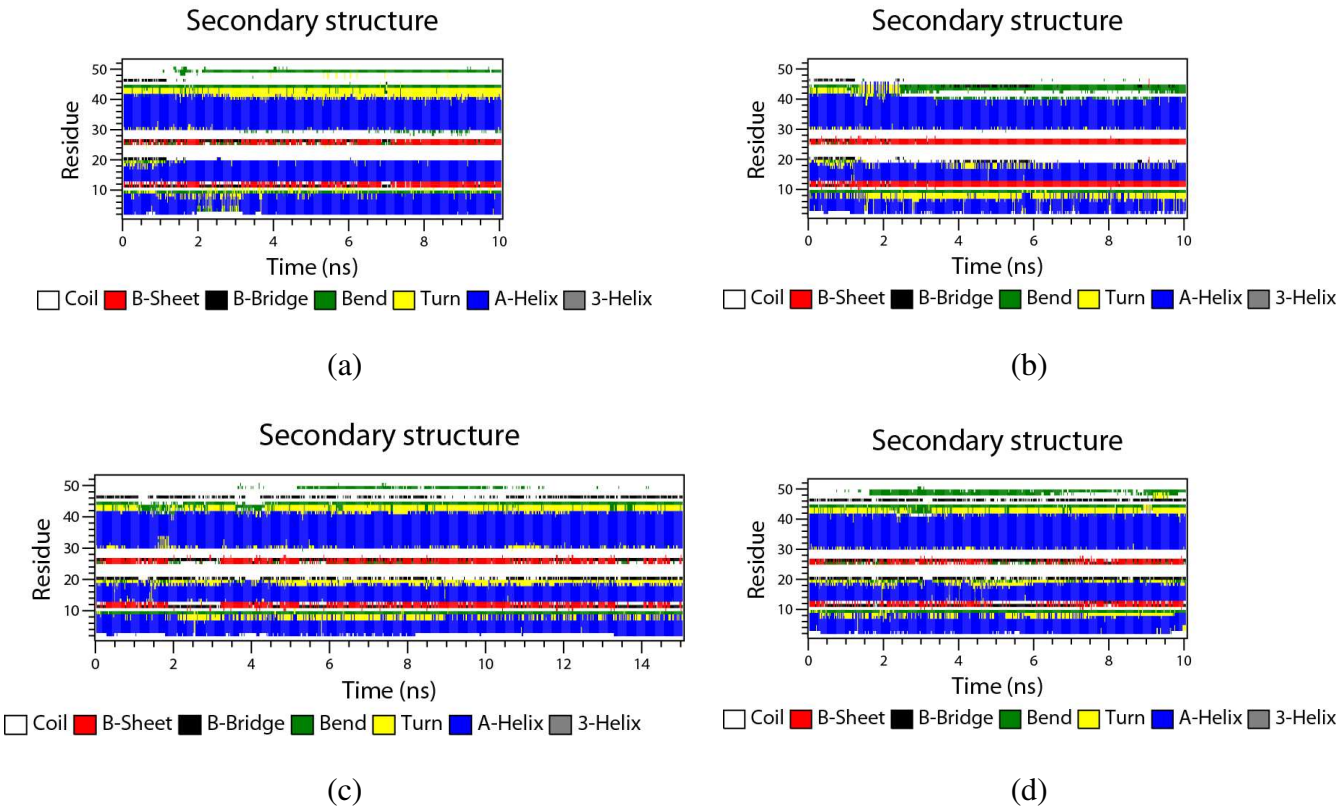
The radial distribution function (RDF) is used to illustrate the distribution of water around insulin or PEG-insulin conjugate, which was defined by equation (3)

$$RDF(r)=\frac{\langle\rho_W(r)\rangle}{\langle\rho_W\rangle_{local}}=\frac{1}{\langle\rho_W\rangle_{local}}\frac{1}{N_I}\sum_{i\in I}^{N_I}\sum_{j\in W}^{N_W}\frac{\delta(r_{ij}-r)}{4\pi r^2} \tag{3}$$

Where $\langle \rho_W(r) \rangle$ is the density of water at a distance r around insulin or PEG-insulin conjugate, $\langle \rho_W \rangle_{local}$ is the density of water averaged over all spheres around insulin or PEG-insulin conjugate with radius r_{max}, which is half of the box length.

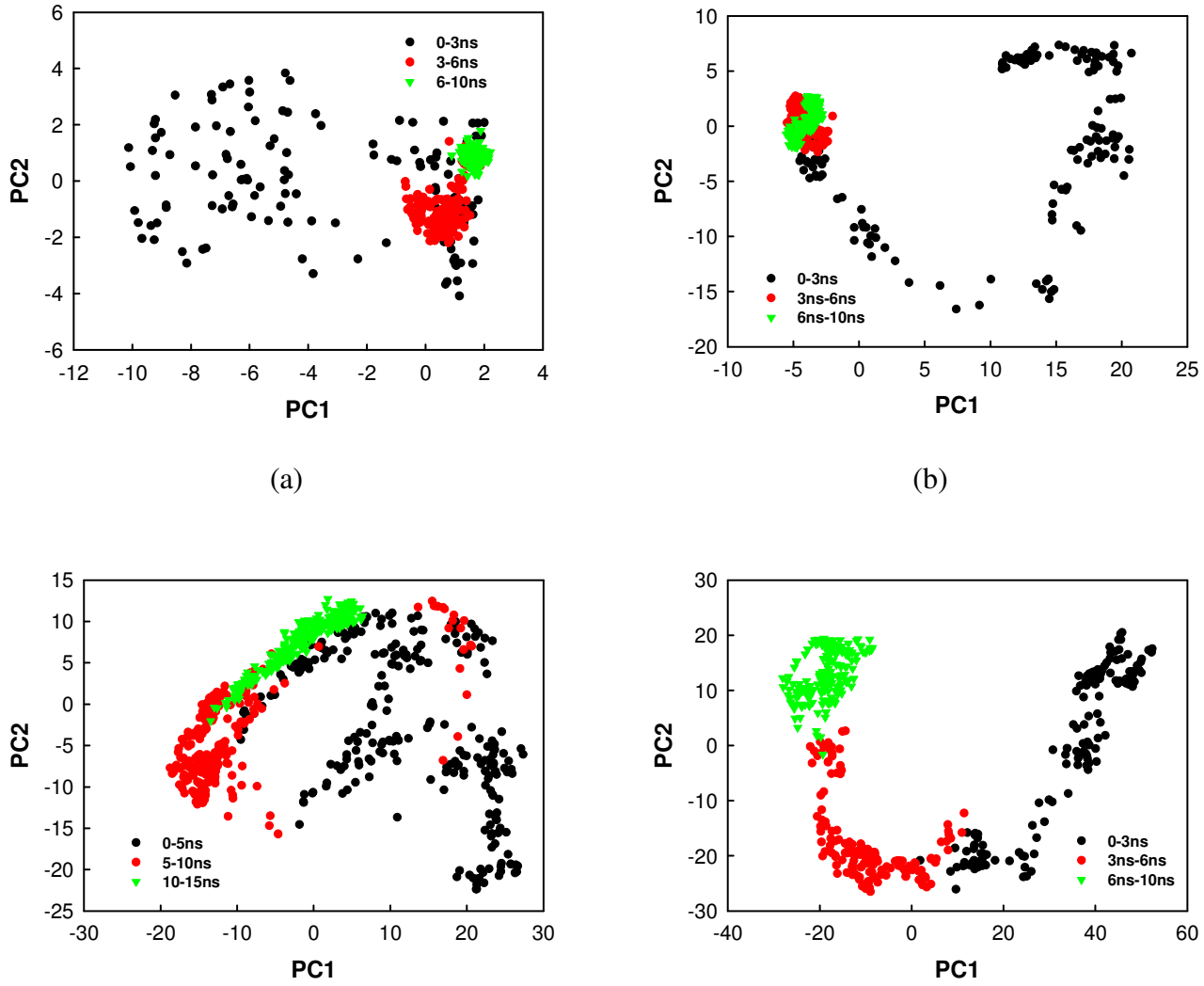
The solvent accessible surface area (SASA) was calculated by double cubic lattice method from the last 2ns of simulated trajectory, using probe with different sizes of 0.14nm, 0.35nm and 0.5nm. The number of hydrogen bond was recognized with geometrical criterion of Acceptor–Donor–Hydrogen angle (30°) and the Hydrogen–Accepter distance (0.35 nm). The Lennard-Jones (LJ) energy analysis was performed by a twin-range cutoff method with cutoff distance of 0.9nm.

Figure S1 Secondary structure analyses conjugated insulin using normal and simulated annealing procedure



(a) insulin-10 PEG units, (b) insulin-50 PEG units, (c) insulin-100 PEG units, (d) insulin-200 PEG units.

Figure S2 Principal compounent analysis of normal and simulated annealing MD simulation trajectory of insulin-PEG conjugate



(a) insulin-10 PEG units, (b) insulin-50 PEG units, (c) insulin-100 PEG units, (d) insulin-200 PEG units.

Figure S3 RMSD of insulin-10 PEG units conjugate with five different initial conformations under the same molecular dynamics simulation procedure. The RMSD at the last 4ns of trajectory indicates the system reaches equilibrium stage.

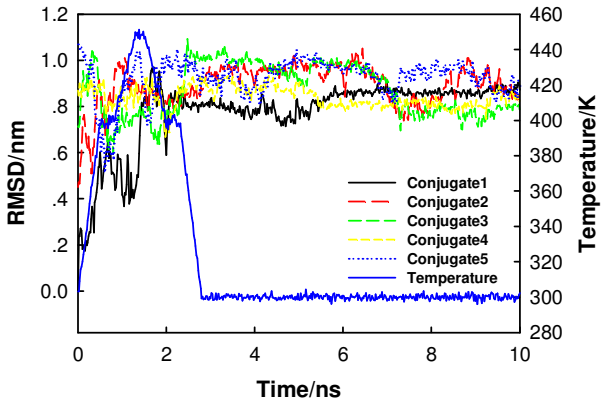


Figure S4 Radius of gyration of insulin-10 PEG units conjugate with five different initial conformations under the same molecular dynamics simulation procedure.

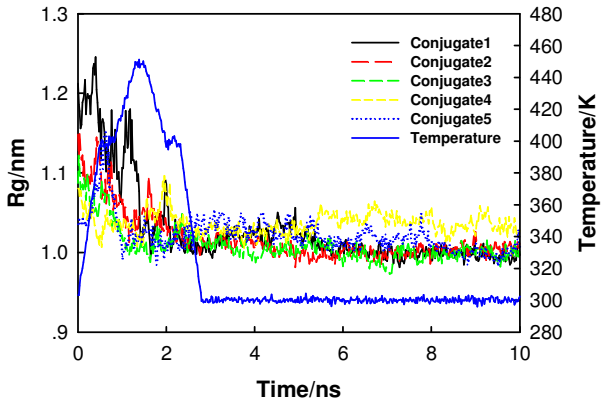
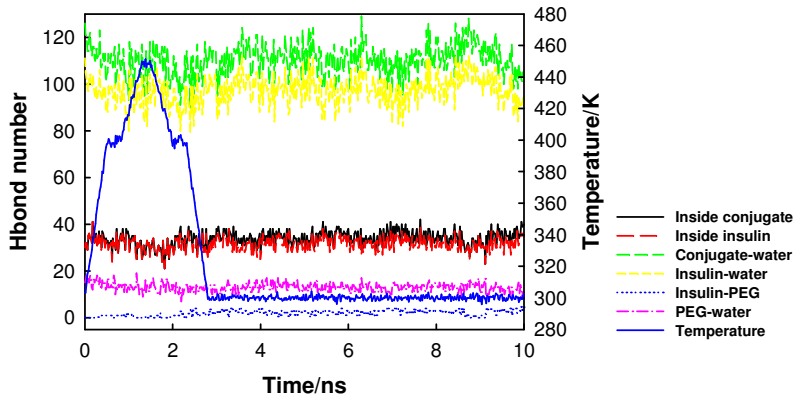
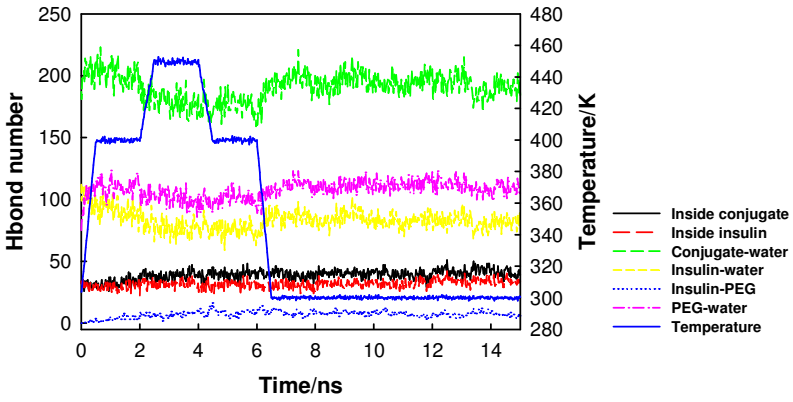


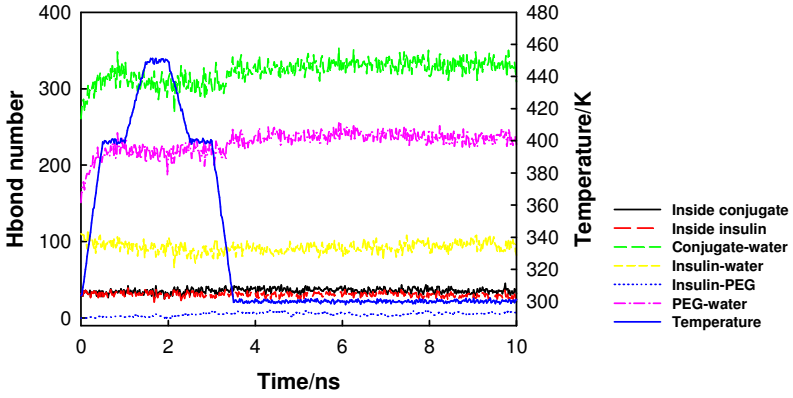
Figure S5 Analysis of the number of hydrogen bonds involving the insulin-PEG conjugate



(a)



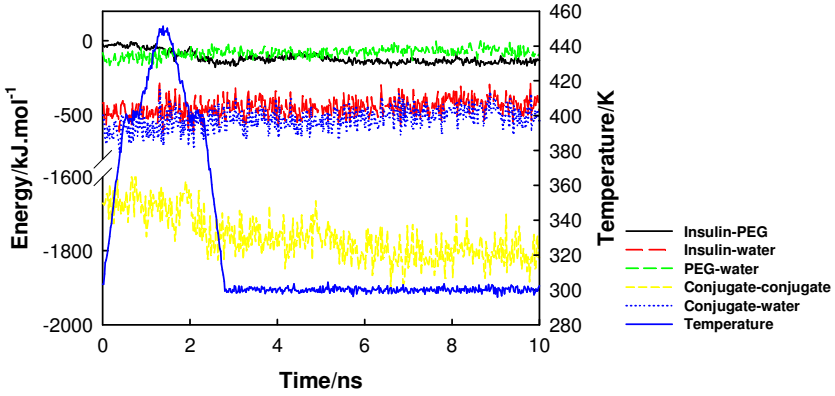
(b)



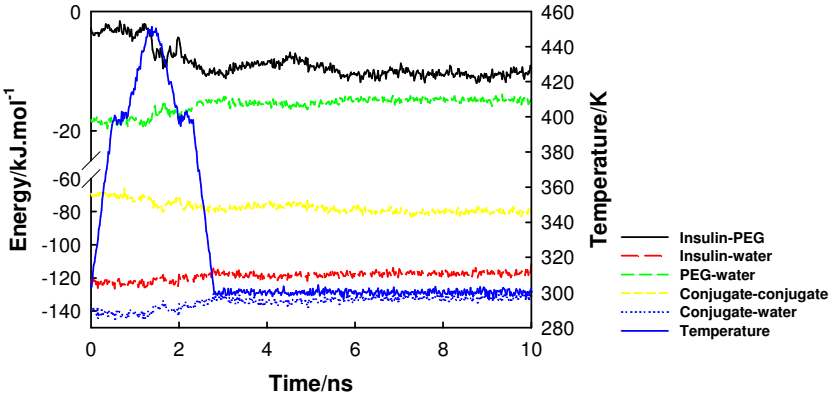
(c)

(a) insulin-10 PEG units, (b) insulin-100 PEG units, (c) insulin-200 PEG units.

Figure S6 Lennard-Jones (LJ) energy analysis of the insulin-10 PEG units conjugate



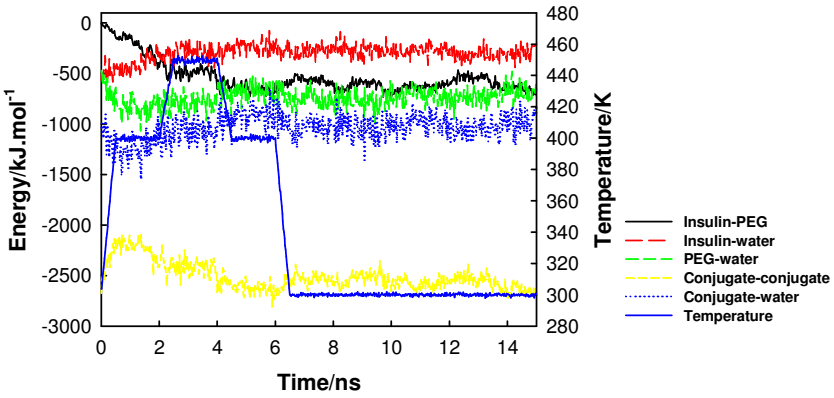
(a)



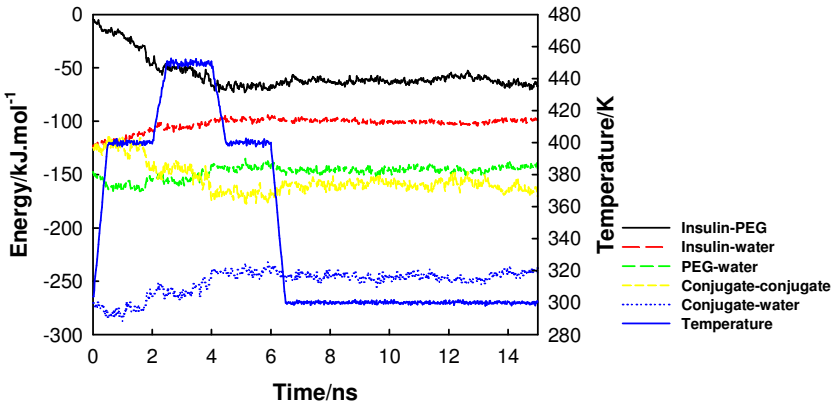
(b)

(a) LJ short-range energy, (b) LJ long-range energy.

Figure S7 Lennard-Jones (LJ) energy analysis of the insulin-100 PEG units conjugate



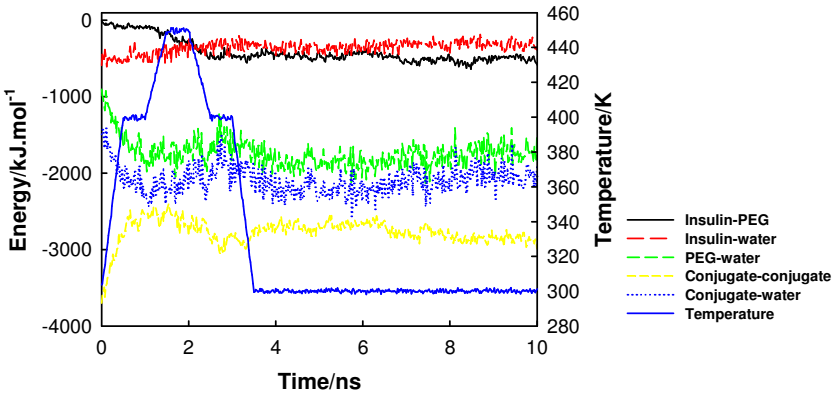
(a)



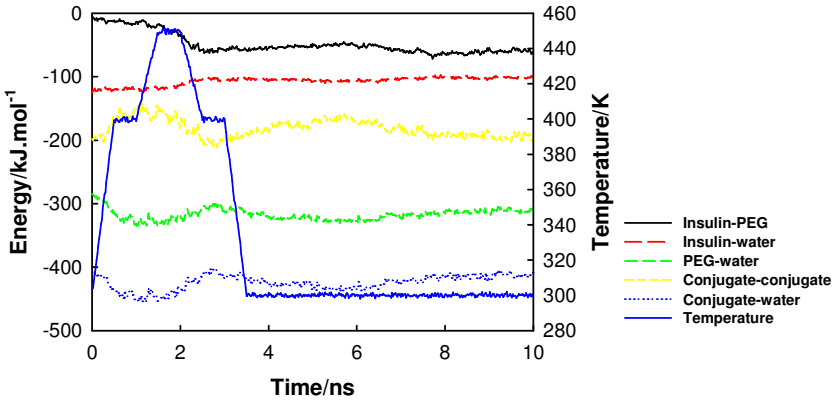
(b)

(a) LJ short-range energy, (b) LJ long-range energy.

Figure S8 Lennard-Jones (LJ) energy analysis of the insulin-200 PEG units conjugate



(a)



(b)

(a) LJ short-range energy, (b) LJ long-range energy.