Title: Calculating the free energy of antimicrobial peptide (HHC-36) dimerization in bulk

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Abstract: The increasing demand for antibiotics has contributed to the investigation of possible novel antibiotics by many researchers. For this purpose experimental and theoretical studies have been carried out to draw scientists' attention to antimicrobial peptides and their interaction with the surface of bacterial membranes. Their ability to disrupt the functioning of bacterial membranes has been probed from different perspectives. The most desirable antimicrobial peptides are those which do not harm plant or animals' membranes but which disrupt bacterial membranes. It has been found that some cationic antimicrobial peptides (CAPs) satisfy these requirements. CAPs interacting with the outer membrane of gram-negative bacteria and the membrane of gram-positive bacteria have been studied recently. We conduct a Molecular Dynamics simulation study of peptide-peptide interactions in physiological solutions and investigate the mechanism of CAPs aggregation since aggregation of the peptides could precede their interaction with the membrane. Different algorithms are applied to calculate the potential mean force of the aggregation process of peptides to select the most efficient one. Also we have run CD spectroscopy and calorimetry experiments to predict the structure of the peptide and measure the peptide-peptide binding enthalpy and compared these results with our simulation data. The particular CAP studied is HHC-36 a peptide selected by high throughput screening (A. Cherkasov et al ACS Chem. Biol. 2009 4 (1) pp 6574) (M. Kazemzadeh-Narbat et al Biomed. Mater. Res. Part B 2012 5 pp1344-1352) (M. Ma et al Biomed. Mater. Res. Part A 2012 2 pp278-185) which has nine amino acid residues and charge +5.
CALCULATING THE FREE ENERGY OF ANTIMICROBIAL PEPTIDE (HHC-36) DIMERIZATION IN BULK

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Outline

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**HHC-36**

- **Sequence:** KRWWKWWRR (Cherkasov, 2008)
- Active against multi-drug resistant and Superbugs and pathogens
- Adding C to R can function as antibacterial coat for implants (Kazemzadeh-Narbat, 2012)

[Image of the molecule structure]

[Table with amino acids]

Experiments
CD Spectroscopy

\[ T = 25 \, ^\circ \text{C} \]

\[ T = 37 \, ^\circ \text{C} \]

Isothermal Titration Calorimetry

The truncated virial series used here is:

\[ \frac{\Delta H}{N_A} = A_A^{(H)} + B_{AA}^{(H)} c \]

- \( \Delta H = H(\text{solution}) - H(\text{solvent}) \) as a function of peptide concentration \( c \). The enthalpy 2nd virial coefficient \( B \) is equal to the slope and here has the value \( B = -1.17 \times 10^{-3} \) cm³/mol.
Simulations
Dynamic/Static Restraint Method

Oscillating Forward-Reverse (Nategholeslam, 2011)
\[ x = xA + vt + Asin(\omega t) \]
\[ \Delta F \cong \frac{\langle W_F \rangle - \langle W_R \rangle}{2} \quad (\text{Forward-Reverse method, Kosztin et al. 2006}) \]
\[ \langle W_d \rangle = \langle W_{dF} \rangle = \langle W_{dR} \rangle \cong \frac{\langle W_F \rangle + \langle W_R \rangle}{2} \]

Assumptions: Brownian Dynamics and stiff spring approximation

Advantages: need only one oscillatory pull and keep track of \[ \Delta W_F \] and \[ \Delta W_R \] across each bin \[ \Delta x \]

(Analysis by Holland, Vafaei, Tomberli, J. Comp. Physics 2012)
Methodology

- Softwares: NAMD and VMD
- Water model: TIP3P
- Forcefield: CHARMM36
- Timestep: 2 fs
- Ensemble: NPT, NVT
- Temperature: different for each case
HHC-36 Dimerization, Dynamic Restraint Method, OFR, v=0.5 Å/ns, k=500, n=200, T=310 K, TIP3P
Theory
Virial Coefficients

- Explains the behavior of the solution
- Can be rigorously calculated from statistical mechanics
- Pressure or thermodynamic potentials can be expanded as an infinite series (Taylor series) in the density

\[ \Delta F(N_A, V, T) = A_A^F(V, T) N_A + B_{AA}^F(V, T) \frac{N_A^2}{V} + \ldots \]

\( A_A^F(V, T) \): chemical potential \((\mu_A)\) of inserting the solute

\[ B_{AA}^{(F)} = -2 \pi \int_0^\infty \left( e^{-\frac{w(r)}{kT}} - 1 \right) r^2 dr \]

\( B_{AA}^{(F)} > 0 \) repulsive interaction dominant

\( B_{AA}^{(F)} < 0 \) attractive interaction dominant
Statistical Mechanics

\[
\langle e^{-\beta W} \rangle_{\text{quasi-static}} = e^{-\beta \Delta F} = \frac{Q_{A+B}}{Q_B}
\]

\[
\frac{Q_{A+B}}{Q_B} = \frac{\text{Partition Function of the solution} (A + B)}{\text{Partition Function of the solvent} (B)}
\]

Helmholtz free energy \(\Delta F(N_A, V, T) = -k_B T \ln \frac{Q_{A+B}}{Q_B}\)
Thermodynamic Potentials

- $\Delta F = \Delta U - \Delta(TS)$, \hspace{1cm} $\Delta H = \Delta U + \Delta(PV)$
- $\Delta H = \Delta F + \Delta(TS) + \Delta(PV)$

@ Constant T and V:

$$\Delta H = \Delta F + T \Delta S + V \Delta P$$

$$\Delta H(N_A, V, T) = \Delta F(N_A, V, T) + T \Delta S + V \Delta P$$

- Enthalpy of Mixing = $\Delta H(P, T) = \Delta H(N_A, V, T) + \text{corrections}$
- $\Delta H(P, T) =$

- $\Delta H(P, T) = A_A^H(P, T)N_A + B_A^H(A, P, T) \frac{N_A^2}{V} + ...$

$$A_A^H(P, T) = A_A^F(V, T) - T A_A^F(V, T) - V A_A^F(V, T) + \text{corrections}$$

$$B_A^H(A, P, T) = B_A^F(V, T) - T B_A^F(V, T) - V B_A^F(V, T) + B_A^F(V, T) + \text{corrections}$$
Comparison of Theory and Experiment

Benzene-Benzene in water PMF

<table>
<thead>
<tr>
<th>Reference</th>
<th>Osmotic virial coeff. (\text{Å}^3)</th>
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<th>Temp. (K)</th>
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<tr>
<td>Tucker and Christian 1979</td>
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<td>Experiment</td>
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