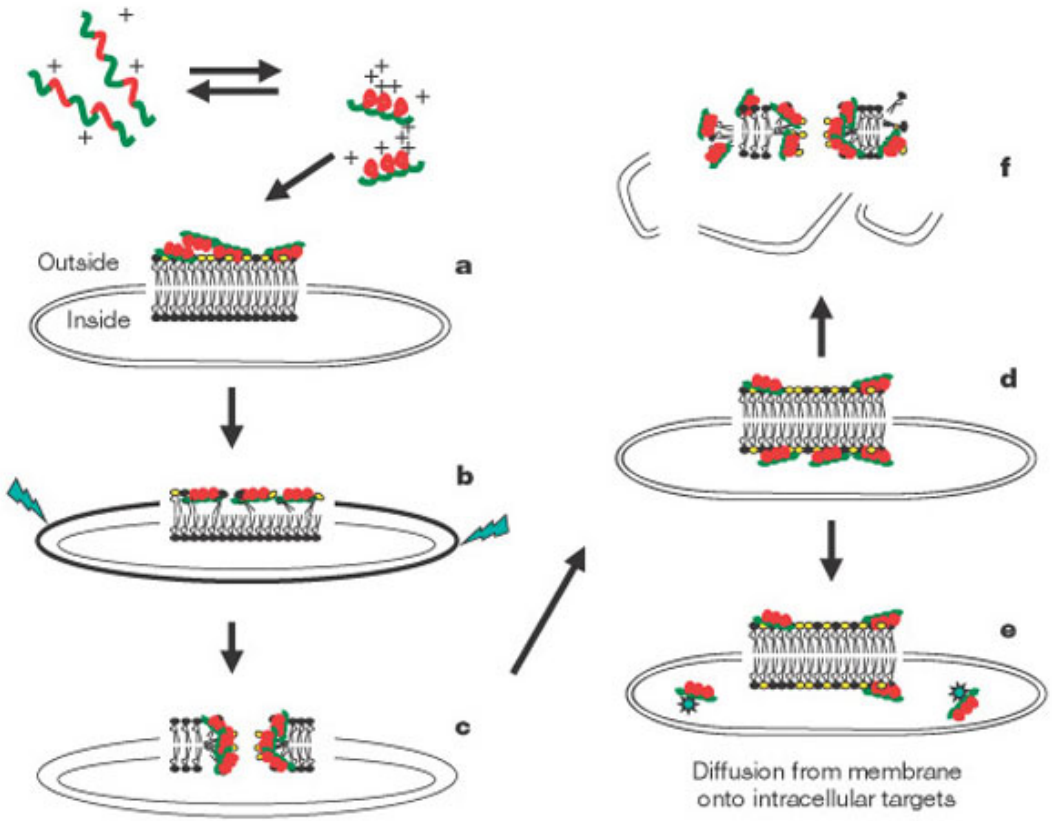
Protein-DNA AMBER Implicit Solvent Minimization

Sara Cheng

Ren Lab Meeting

February 20, 2015

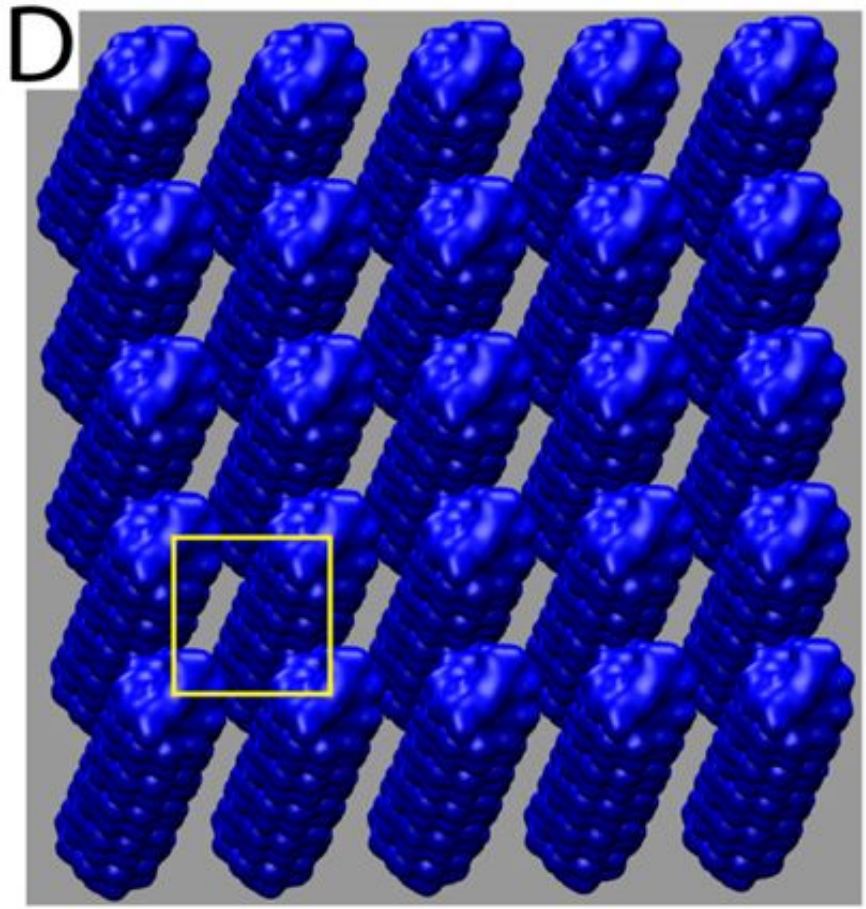
Antimicrobial Properties of LL-37



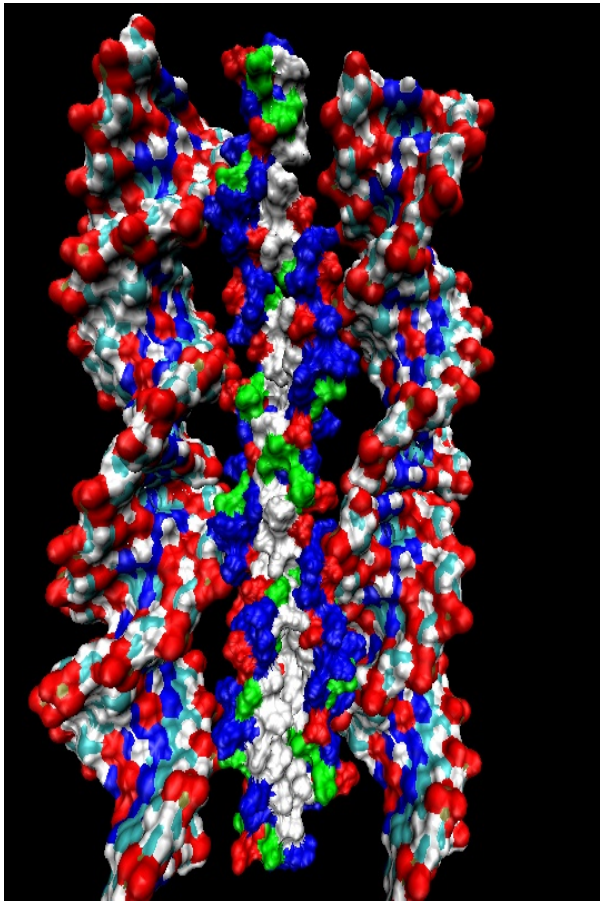
- Multi-drug resistant bacteria is a growing global health concern
- LL-37 is a human cathelicidin
- **LL-37 antimicrobial behavior has been shown to be inhibited by DNA** which is released from lysed cells

Figure 3 from M. Zasloff. Antimicrobial Peptides of Mutlicellular Organisms (2002). Nature

Organization of DNA into an ‘open’ columnar complex can bind to TLR-9 receptors in dendritic cells



DNA square lattice (only DNA shown); LL37 superhelix (16 x LL37 ‘monomers’ in 7 full helical turns) at 4-fold interstititial sites, with inter-DNA separation of 3.51nm

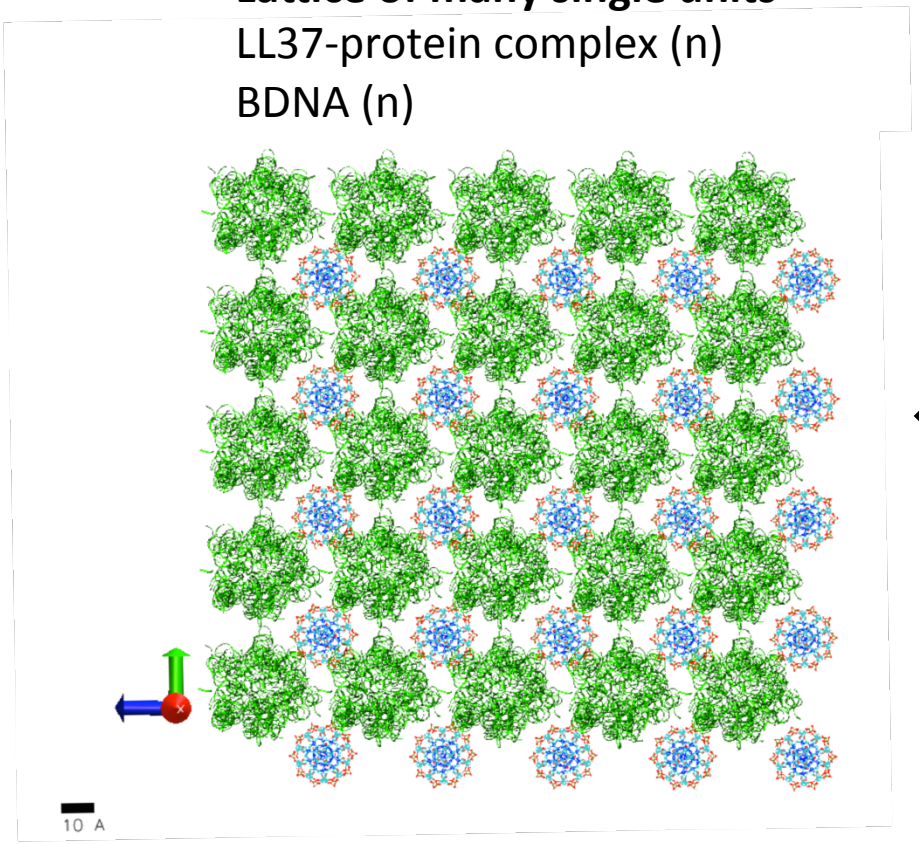


DNA LL37 superhelix DNA

Slide Provided by Dr. G Wong UCLA

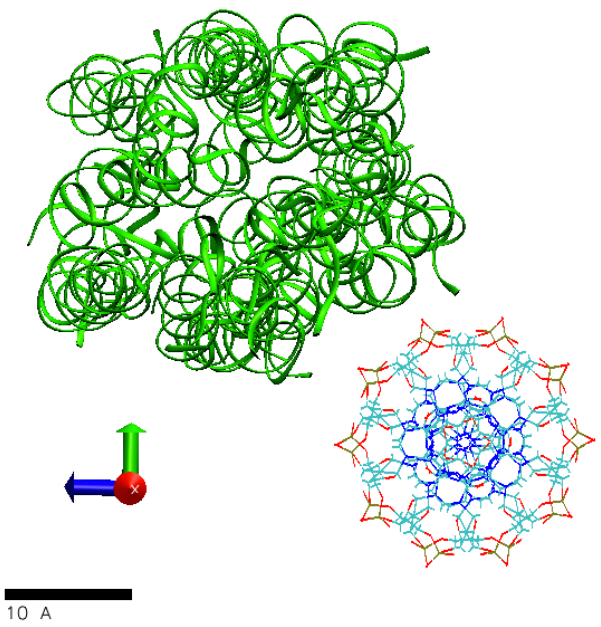
Lattice to Single Unit

Lattice of many single units
LL37-protein complex (n)
BDNA (n)



PBC

Single Unit
LL37-protein complex (1)
BDNA (1)

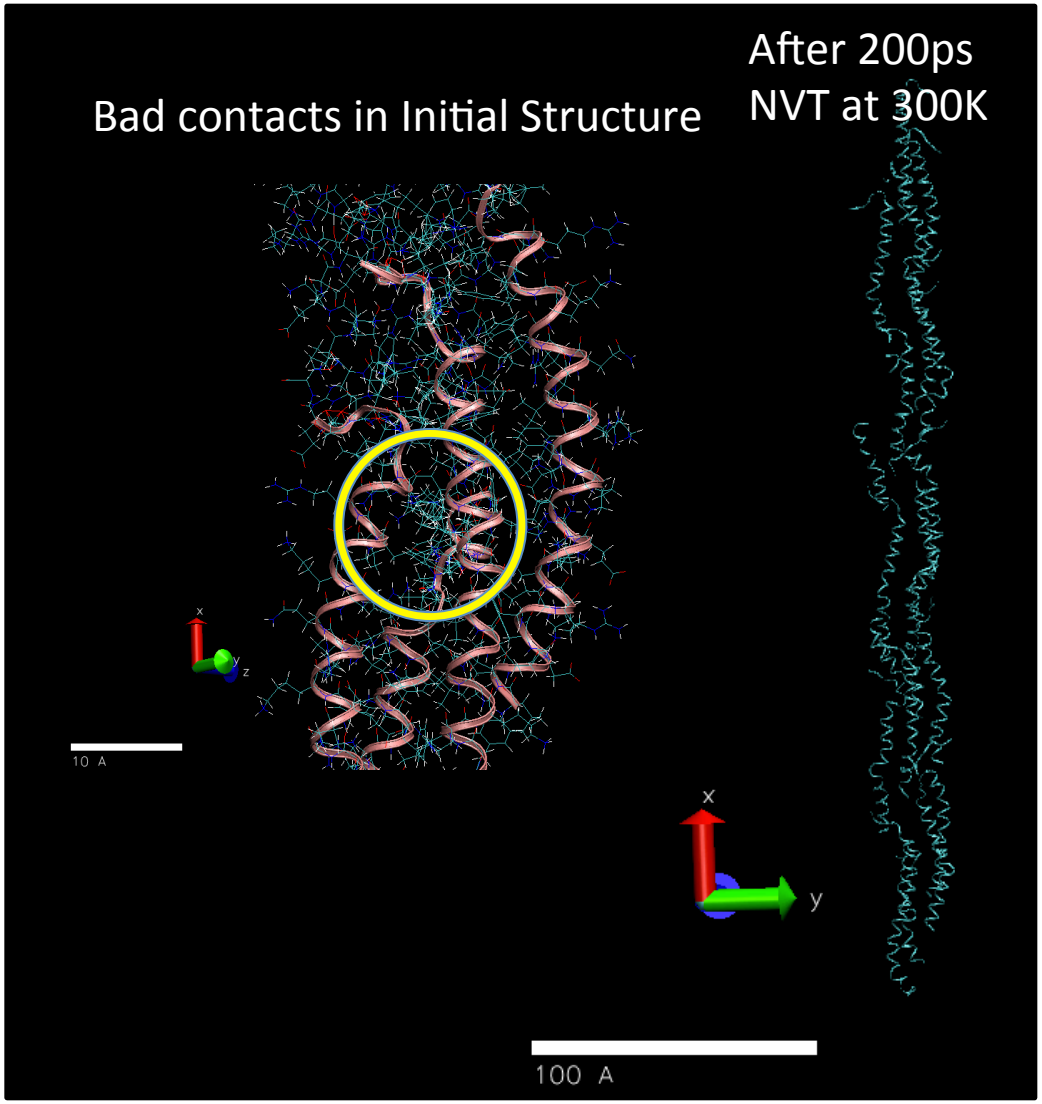


PBC: Periodic Boundary Conditions

MD and Software Used

- GROMACS 4.5.3 MD Package
- Visual Molecular Dynamics (VMD)
- AMBER99SB force field
- AMBER 12 MD Package
- Generalized Born Implicit Solvent

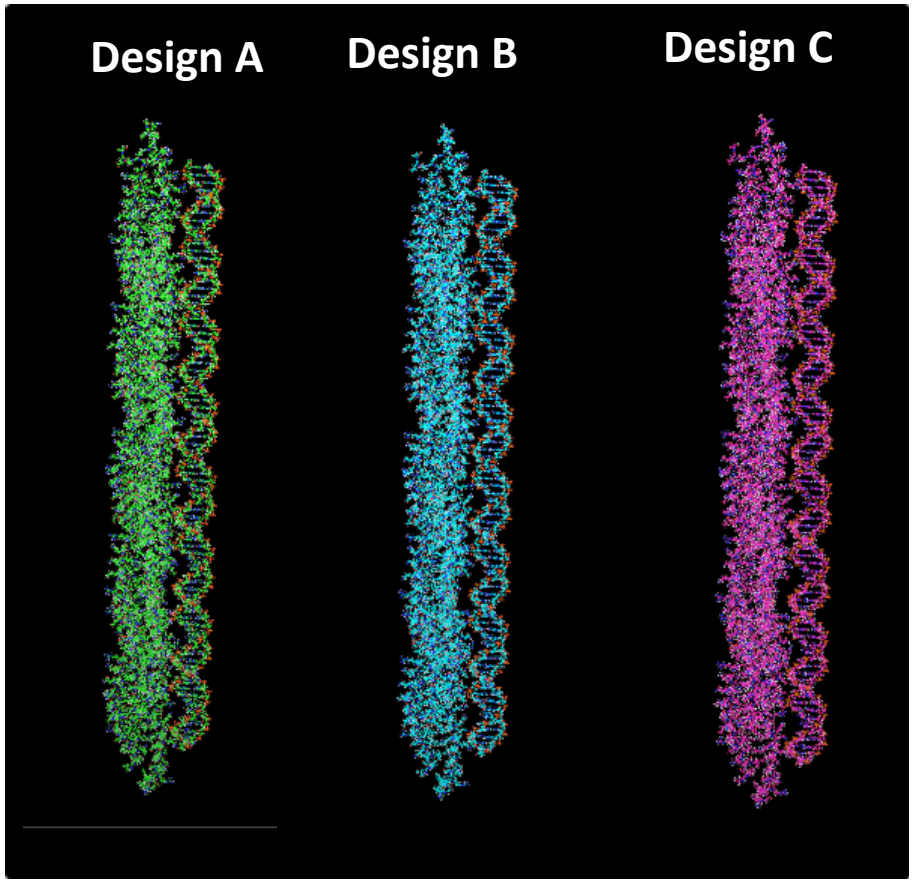
Equilibration of Superhelix



Equilibration Procedure

1. Design Initial Structure (box-size, counter ions, explicit water)
1. Minimization Steep Integrator (2000 steps)
2. Restrain backbone of BDNA and Protein: Equilibration 5ps NVT simulations at 50K, 100K,150K, 200K, 250K, 300K
3. No restraints, Equilibration for 50ps NVT simulations at 300K
4. No restraints, Equilibration for 200ps NPT simulations at 300K

Initial LL37-BDNA Designs

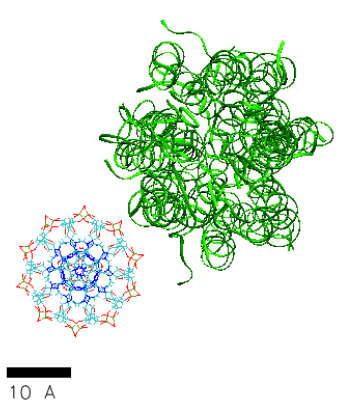


- Goal: **Evaluate binding energy of a unit-cell of LL-37 BDNA square lattice**
- Design A: Shift BDNA
  0.5 BDNA-turn relative to LL37-complex
- Design B: Shift BDNA
  0.25 BDNA-turn relative to LL37-complex
- Design C: Shift BDNA
  0.125 BDNA-turn relative to LL-37-complex

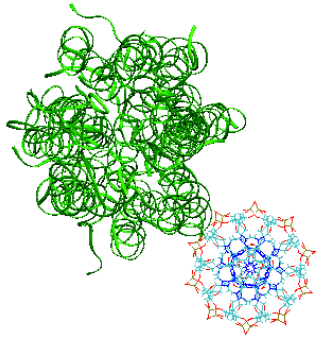
LL-37 PDB structure Ref: G. Wang, et al. (2008)
Initial square lattice design provided by G. Wong UCLA

Rotation of BDNA

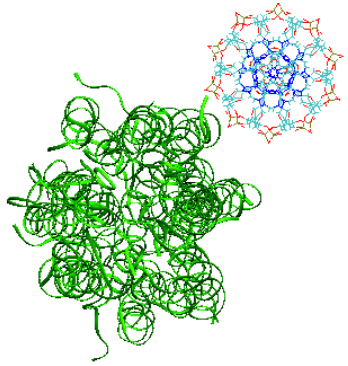
0° rotation



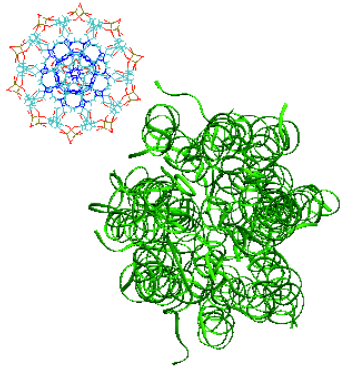
90° rotation



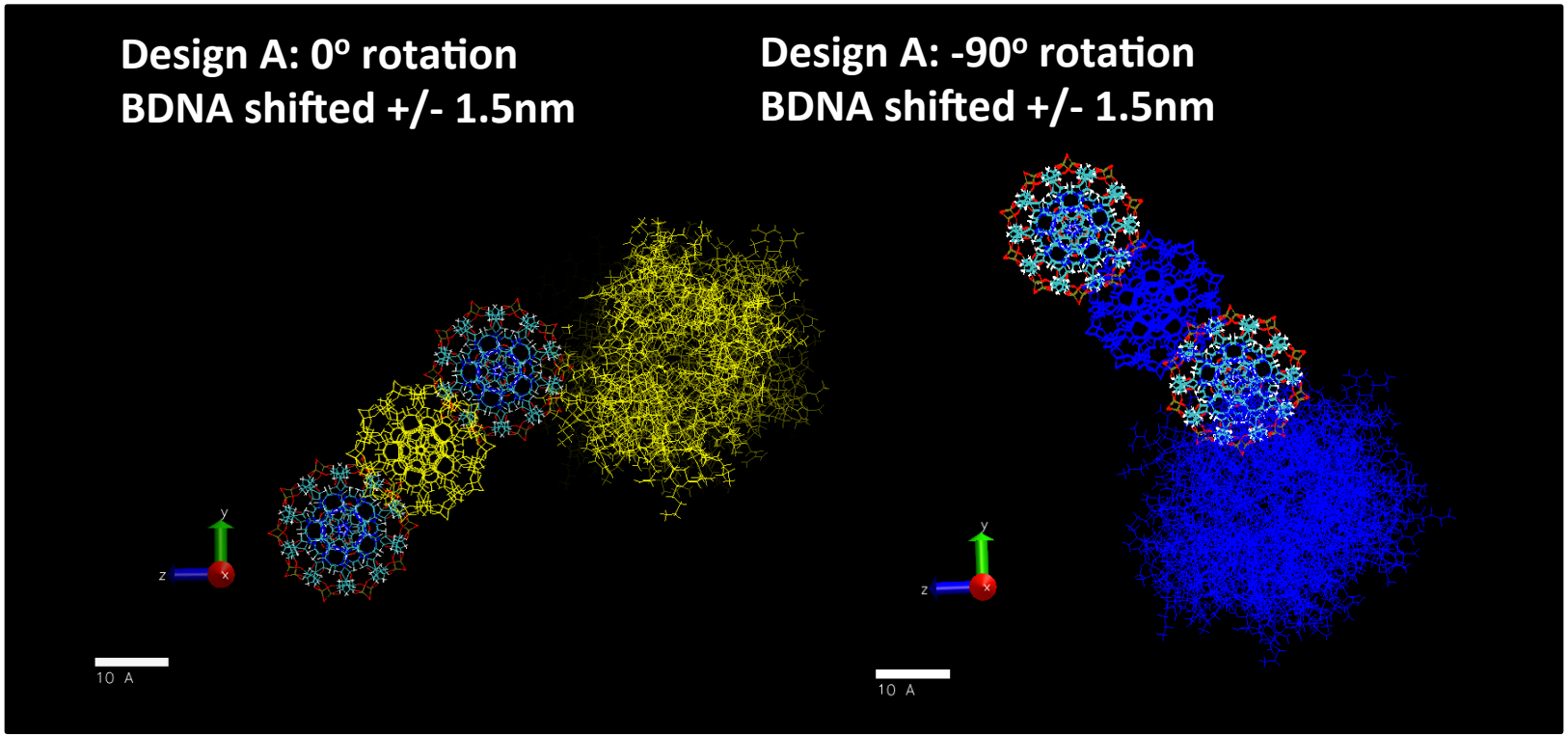
180° rotation



270° rotation

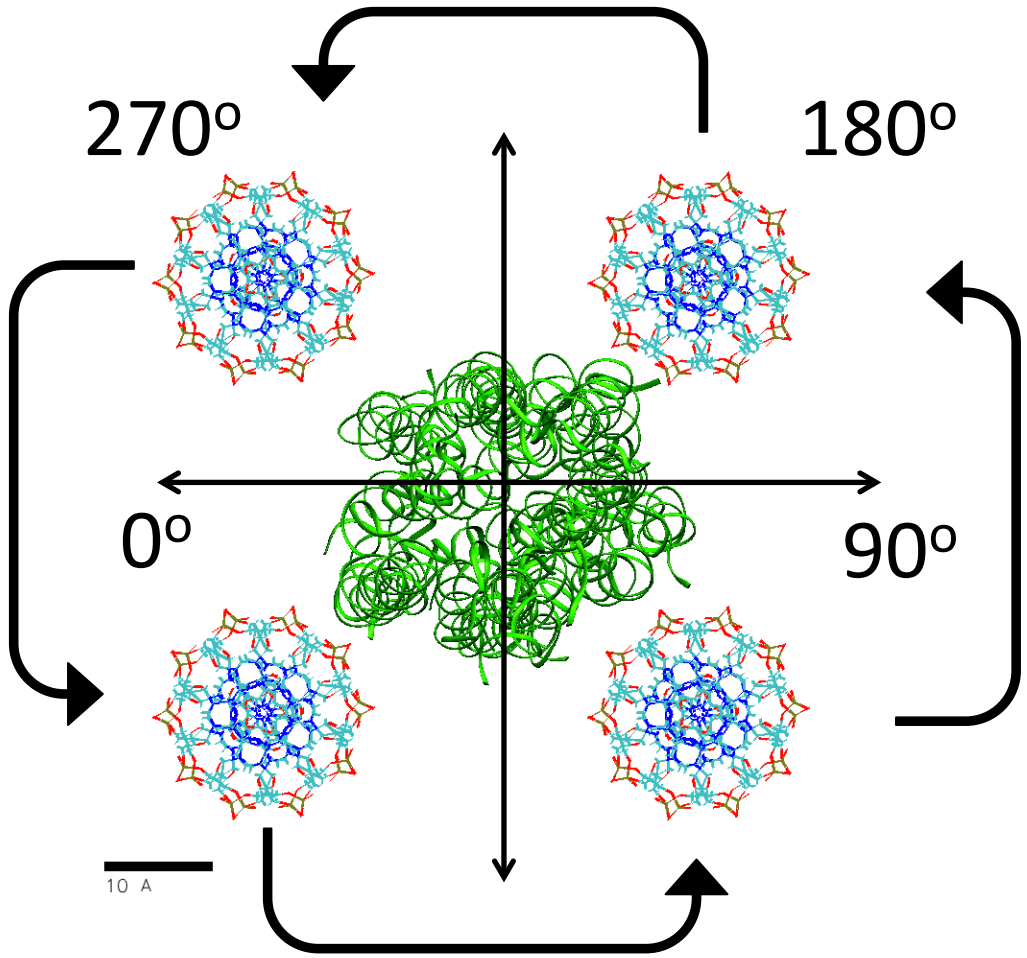


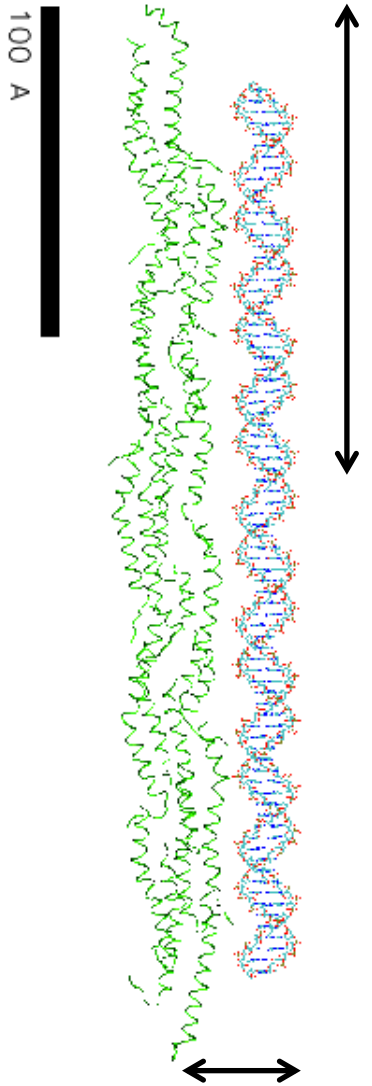
BDNA Translation



- Translated BDNA +/- 1.5nm, +/- 1.0nm, +/- 0.5 nm from LL-37 DNA

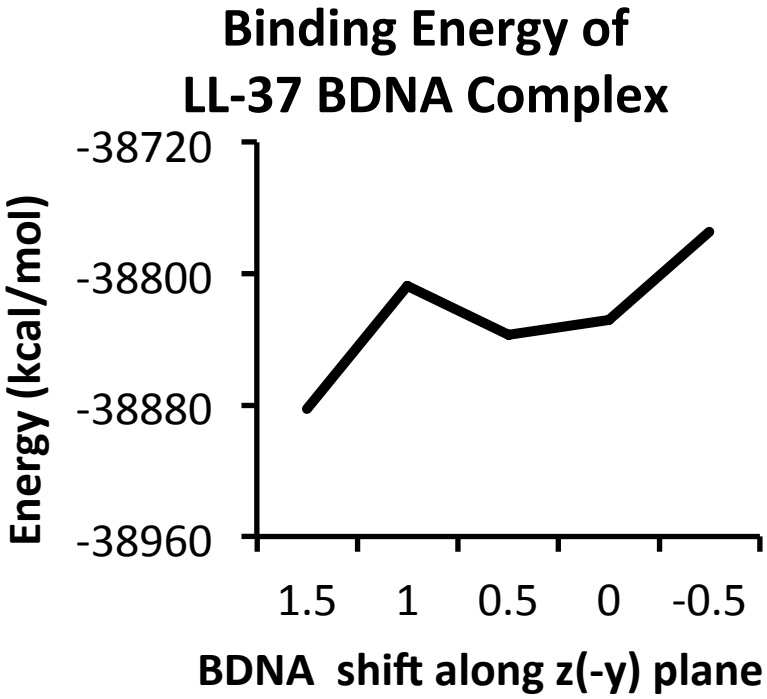
Designs: Rotation and Translation



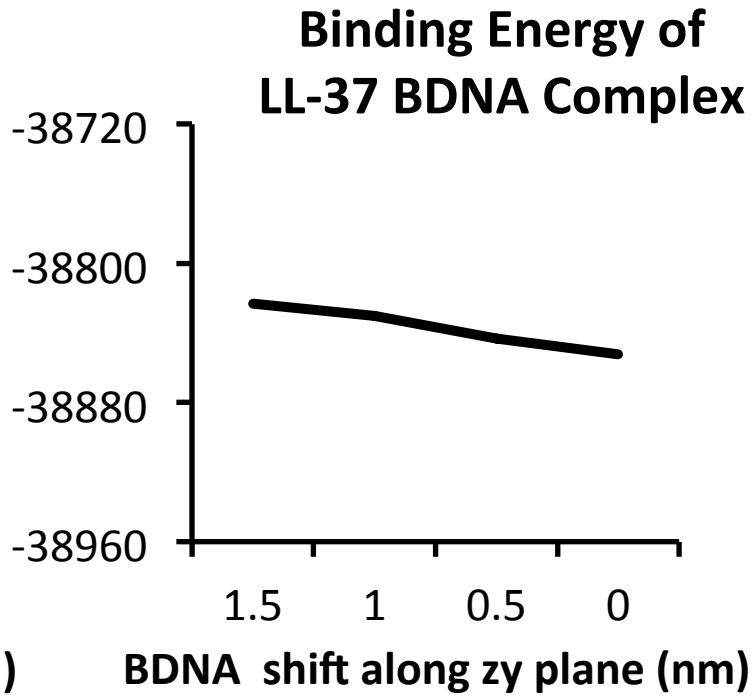


Structural Evaluation

Design A rot BDNA 0°



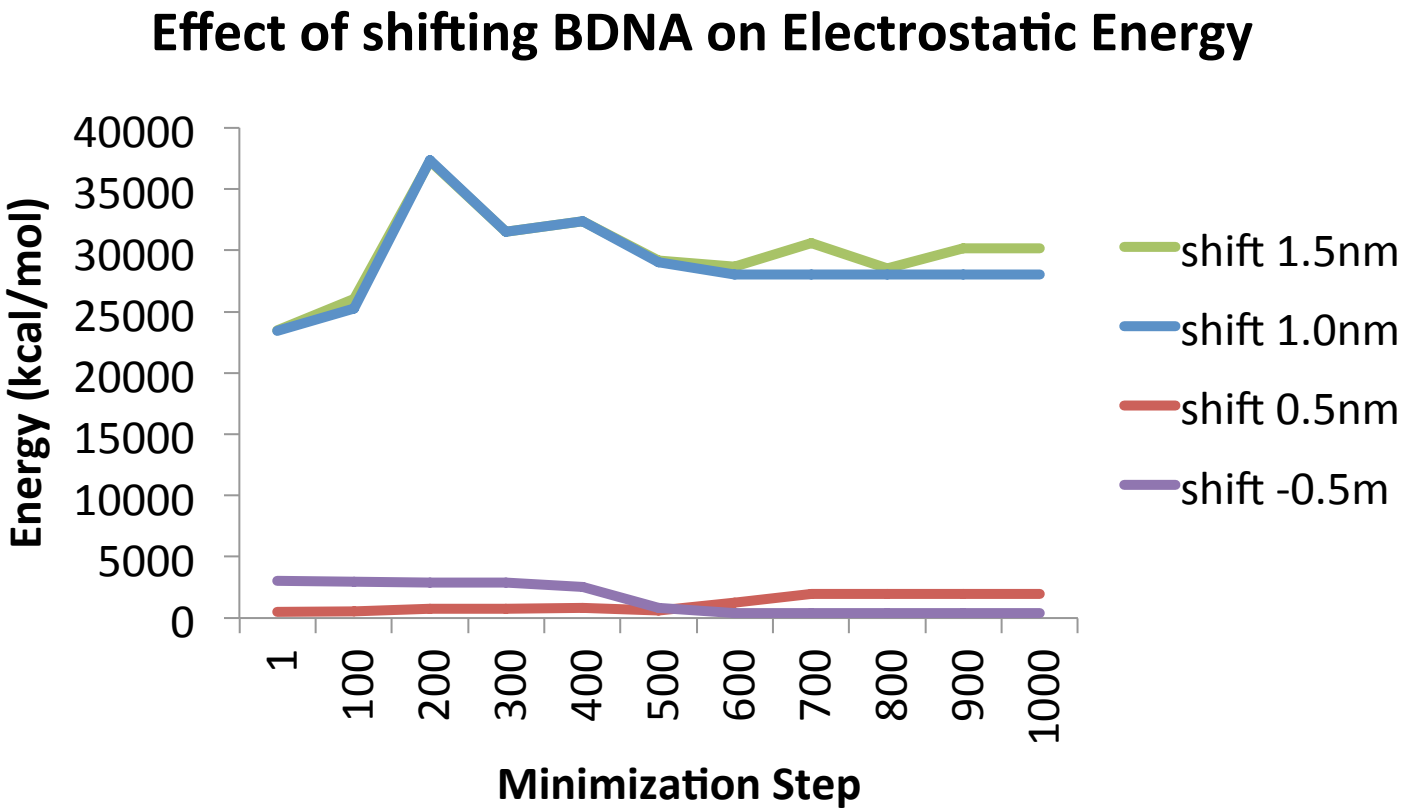
Design A rot BDNA -90°



Binding Energy = Energy(complex) – Energy (BDNA) – Energy (superhelix)

Energy Component Analysis

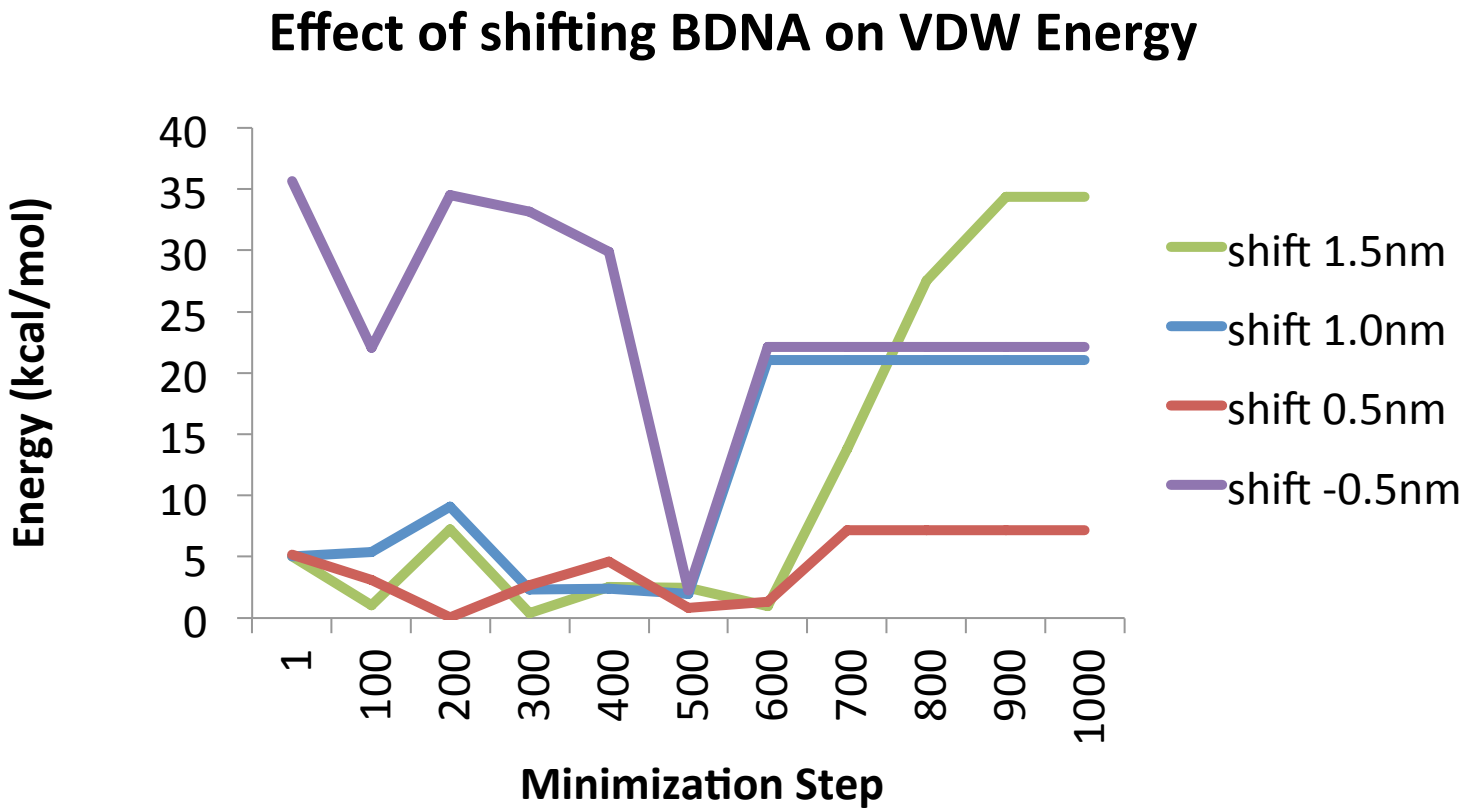
Design A rot BDNA 0°



Relative Energy Difference as compared with 0nm shift of BDNA

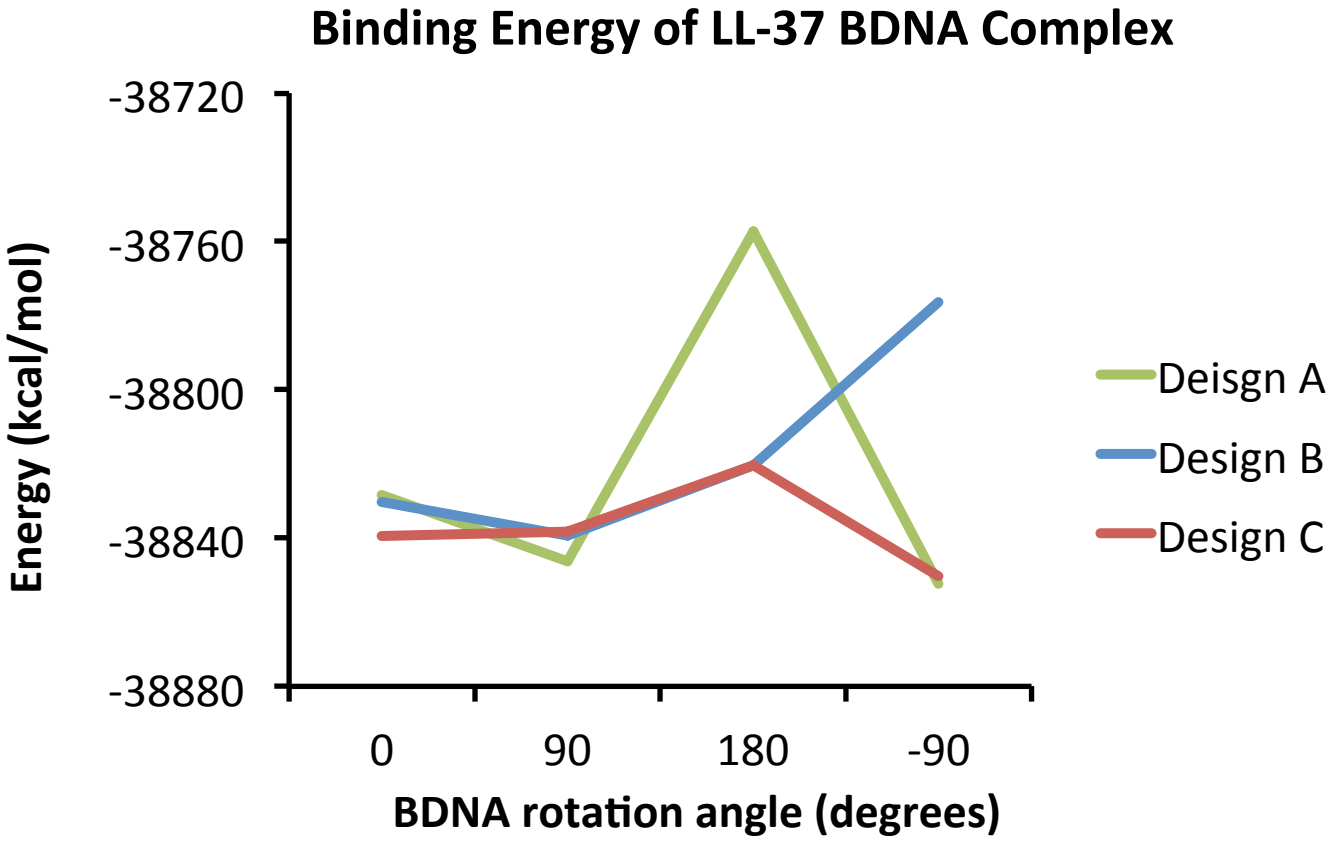
Energy Component Analysis

Design A rot BDNA 0°



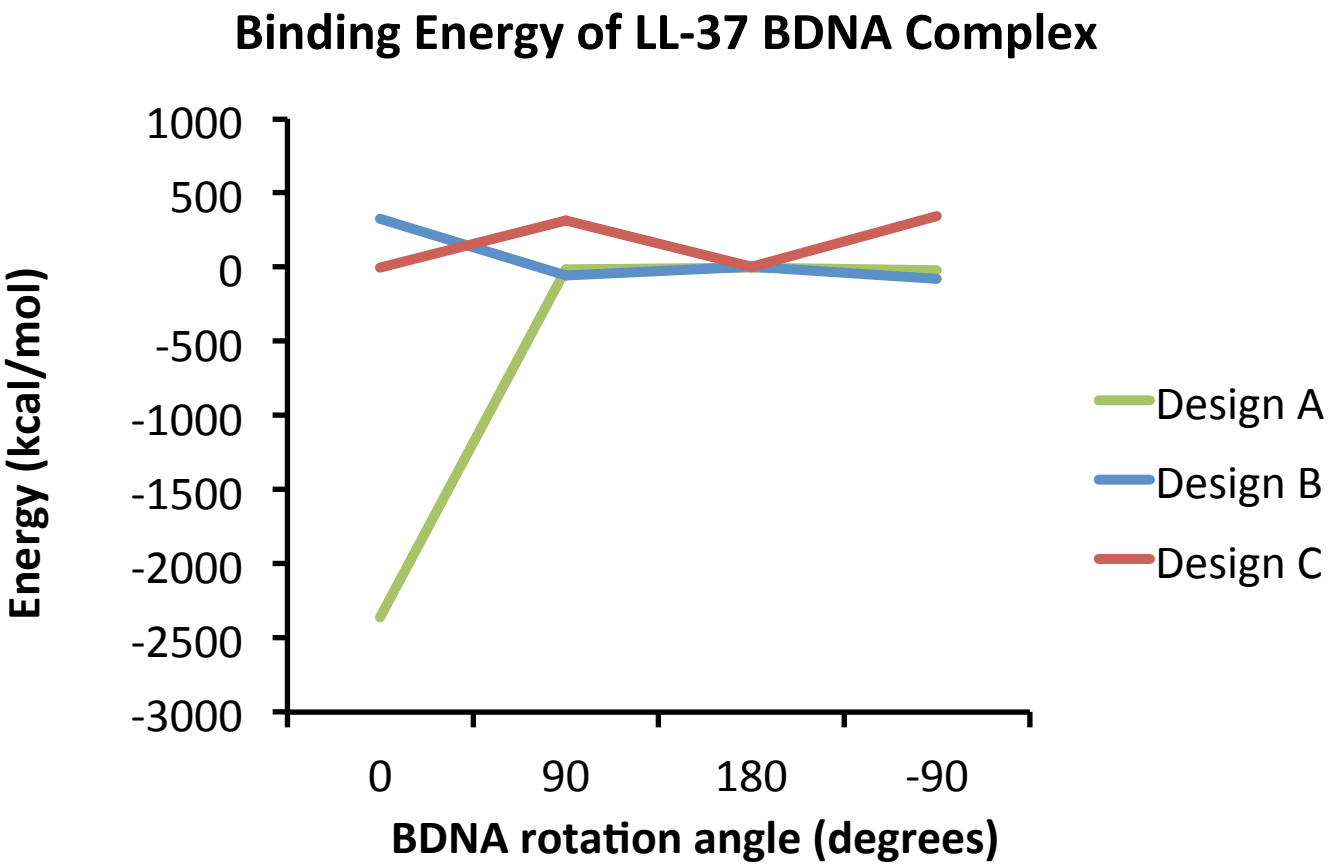
Absolute Relative Energy Difference between Design A, BDNA rotation of 0° with no shift of BDNA

Minimization Results



- Binding Energy = Energy(complex) – Energy (BDNA) – Energy (superhelix)
- Amber 99SB force field, Amber 12 MD Package
- 0.1M salt concentration
- 1000 cycles of minimization with steepest descent algorithm

Importance of Counter ions



- Binding Energy = Energy(complex) – Energy (BDNA) – Energy (superhelix)
- Amber 99SB force field, Amber 12 MD Package
- No counter ions
- 1000 cycles of minimization with steepest descent algorithm

Summary

- Implicit Solvent calculations can provide preliminary structural information about protein-DNA systems
- All-atom MD simulations is needed to equilibrate lowest energy configurations of LL-37 and BDNA

Future Goals

- Generate a Binding Energy Surface
- Identify minimum/minima and perform all-atom MD simulations on these systems

References

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