External Forces in NAMD
Standard protocols are limited

SMD, moving (or rotating) constraints, constant force
F-ATP synthase

Rotary catalysis: Two protein motors coupled via common central stalk $\gamma \delta$

Solvent exposed $F_1$ unit ($\alpha_3 \beta_3 \gamma \delta \varepsilon$): central stalk rotation causes conformational changes in catalytic sites, driving ATP synthesis

Transmembrane $F_0$ unit ($ab_2c_{10}$): converts proton motive force into mechanical rotation of central stalk
Challenges

Atomic details matter: Displacing three atoms in F-ATP synthase can kill the cell

Multiple time and length scales make modeling difficult

Milliseconds: (rotation of the central stalk driving ATP synthesis)

Picoseconds: (proton hopping required to fuel the rotation)

$10^9$ gap
Structure of the Fo unit

How proton flux induces rotation of $c_{10}$?
Suggested Mechanism of Proton Translocation

(R.H. Fillingame, 2002)
Structural Model of *E. coli* $F_o$

$a_1c_{12}$ (Rastogi & Girvin, 1999, NMR) $+$ $c_{10}$ (Fillingame et al, 1999, NMR) = $a_1c_{10}$ (2001-2002, modeling)
Simulated Systems

$a_1 c_{10} + \text{POPE} + \text{water}$

$\sim 112,000 \text{ atoms}$

Water exposed parts of the $c_{10}$ oligomer alpha helices begun to unwind

![Graph showing RMSD over time](image)

- Water exposed parts of $C_{10}$
- Entire Protein
- Transmembrane Part

protein released
Forced Rotation of the $c_{10}$ Subunit

Forces were applied to all backbone atoms of $c_{10}$

Estimated friction coefficient $\zeta \sim 10^5$ kcal/(M sec)
With only one $\text{Asp}_{61}$ residue deprotonated, SMD rotation of $c_{10}$ breaks the structure apart.
To minimize steric hindrance (critical on nanosecond time scale), helix was forced to rotate in a reptation tube (local pivot points and directors).
Salt Bridge Can Be Transferred

The salt bridge can be transferred by the concerted rotation of the $c_{10}$ complex and the outer TMH of subunit $c$
Molecular mechanism for unidirectional rotation

cTMH-2 is tilted

4A

\[ H^+ \]
Stochastic model

Microscopic model

Reduced system

Principle degrees of freedom
Stochastic model

6 degrees of freedom:
\( \theta_0, \theta_1, \theta_2, \theta_3, \theta_4 \) are TMH rotation angles; \( \theta_A \) - position of the \( a \) subunit

Each Asp61 can be in either of two chemical states (protonated or deprotonated).

\[
\xi_i \frac{d\theta_i}{dt} = -\frac{d}{d\theta_i} \left[ U_{\text{group}} + U_{\text{hydroph.}} + U_{\text{internal}} - \tau \theta_A \right] + \eta_i(t)
\]
Stochastic Simulations of $F_o$ Operation

The c10 complex rotates in steps

Load torque 41pN nm
TclForces

Constant force to all atoms

\[ x = a \ t^2 / 2 \]

\[ F = ma \quad \rightarrow \quad a = F / m \]
NAMD config file

Exemplary script

```tcl
# Example NAMD config file

tclforces on
set linaccel "30 0 0"
tclforcescript push.tcl

set numatoms 1231
set atoms {}
for { set i 1 } { $i <- $numatoms } { incr i } {
    lappend atoms $i
}

foreach atom $atoms {
    addatom $atom
}

# Convert input to NAMD units: kcal/(mol*Ang*amu)
set linaccel_namd [vecscale [expr 1.0/418.68] $linaccel]

print "Linear acceleration applied: ($linaccel) Ang^2 ps^-2"

proc calcforces {} {
    global atoms numatoms linaccel_namd

    loadcoords coords
    loadmasses masses

    set comsum "0 0 0"
    set totalmass 0
    foreach atom $atoms {
        # Take force vector from NAMD config file
        set force [vecscale $masses($atom) $linaccel_namd]
        addforce $atom $force
        set comsum [veccadd $comsum [vecscale $masses($atom) $coords ($atom)]]
    }
    set totalmass [expr $totalmass + $masses($atom)]
    print "Center of mass - [vecscale [expr 1.0/$totalmass] $comsum]"
}
```

TclBC custom script
Rotation of the c-ring

\[ F = C \rho(z) [\mathbf{r} \times \mathbf{n}] \]

\[ F_{\text{rest}} = k (\mathbf{r} - \mathbf{r}_0) \]
Local directors and pivot points for rotation

\[ F_i = C \left[ r_i \times n_i \right] \]

\[ F^{(i)}_{\text{rest}} = k \left( r_i - r^{(i)}_0 \right) \]
Translocation of DNA through alpha-hemolysin

Experiment: Jerome Mathe and Amit Meller
Pushing a DNA strand through a pore is … like bringing a X-mass three through a door

Experiment:
J. Mathe et al
PNAS 2005
SMD simulation of DNA translocation through $\alpha$-hemolysin

3’-end-first translocation is about 10% faster

J. Mathe et al
PNAS 2005

3’-end first  5’-end first
SMD simulations of DNA hairpin permeation through α-hemolysin
Translocation of single-stranded DNA

MD simulation of single stranded DNA translocation through 1.5-nm-diameter pore

1.3 V / 10 nm;
Time: 17.5ns

The translocation halts b/c the DNA sticks to the wall!
140mV bias, 26 ns total simulation time

Hydrophobic adhesion of DNA bases to the pore wall slows down DNA translocation
Pruning DNA-nanopore interaction with phantom pores

Steric friction between DNA and the pore

Steric friction and screening of the DNA charge by counter ions

Mathematical surface confines DNA
Translocation of single-stranded DNA through a phantom pore
Screening by counter-ions dominates over steric friction in narrow (~1-nm-diameter) pores.
Making bubble with TclBC

Force is applied to all atoms inside the sphere.
Exemplary script

tclBC on
tclBCScript {
    set bubbleCenter   "0.0 0.0 0.0"
    set tclBCScript    < your working directory >/bubble.tcl
    source $tclBCScript
}
tclBCArgs {0. 15. 0.01 5.}

proc calcforces {step unique Rstart Rtarget Rrate K} {

    global bubbleCenter ;# defined in tclBCScript{ ... }

    # increase R, starting from $Rstart, by $Rrate at each step,
    # until it reaches $Rtarget; then keep it constant

    set R [expr $Rstart + $Rrate * $step]
    if { $R > $Rtarget } { set R $Rtarget }

    # let only the main processor print the output

    if { $unique } {

}
Condensing ions with **TcI BC**

Force is applied to all ions outside the sphere.
Simulating shear flow with TclBC
Summary

TclForces:
- Executed only on one (master) processor
- Many commands exists
- Can be made efficient if applied to a small number of atoms

TclIBC:
- Executed on each processor
- Few commands exists
- More efficient when applied to a large number of atoms
Pulling DNA with Constant Force

Constant force was applied to all heavy (non-hydrogen) atoms of the DNA molecule (about 100 pN per atom)