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APPENDICES

APPENDIX A

M. pneumoniae protein code of each gi accession number

Gi accession	code	Gi accession	code	Gi accession	code
13507749	MPN010	13508050	MPN311	13508225	MPN486
13507750	MPN011	13508051	MPN312	13508238	MPN499
13507751	MPN012	13508052	MPN313	13508239	MPN500
13507816	MPN077	13508053	MPN314	13508240	MPN501
13507827	MPN088	13508065	MPN326	13508241	MPN502
13507828	MPN089	13508077	MPN338	13508243	MPN504
13507830	MPN091	13508078	MPN339	13508244	MPN505
13507833	MPN094	13508082	MPN343	13508245	MPN506
13507837	MPN098	13508083	MPN344	13508263	MPN524
13507838	MPN099	13508088	MPN349	13508264	MPN525
13507839	MPN100	13508089	MPN350	13508265	MPN526
13507840	MPN101	13508097	MPN358	13508269	MPN530
13507842	MPN103	13508098	MPN359	13508298	MPN559
13507843	MPN104	13508103	MPN364	13508309	MPN570
13507846	MPN107	13508104	MPN365	13508326	MPN587
13507847	MPN108	13508107	MPN368	13508327	MPN588
13507849	MPN110	13508108	MPN369	13508328	MPN589
13507860	MPN121	13508109	MPN370	13508329	MPN590
13507867	MPN128	13508110	MPN371	13508330	MPN591
13507870	MPN131	13508115	MPN376	13508331	MPN592
13507871	MPN132	13508116	MPN377	13508332	MPN593
13507883	MPN144	13508139	MPN400	13508333	MPN594
13507888	MPN149	13508148	MPN409	13508372	MPN633
13507889	MPN150	13508149	MPN410	13508378	MPN639
13507902	MPN163	13508150	MPN411	13508379	MPN640
13507940	MPN201	13508187	MPN448	13508380	MPN641
13507941	MPN202	13508188	MPN449	13508381	MPN642
13507942	MPN203	13508193	MPN454	13508382	MPN643
13507944	MPN205	13508194	MPN455	13508386	MPN647
13508020	MPN281	13508195	MPN456	13508387	MPN648
13508021	MPN282	13508196	MPN457	13508388	MPN649
13508022	MPN283	13508198	MPN459	13508389	MPN650
13508023	MPN284	13508201	MPN462	13508401	MPN662
13508025	MPN286	13508202	MPN463	13508407	MPN668
13508026	MPN287	13508207	MPN468	13508419	MPN680
13508034	MPN295	13508208	MPN469	13508423	MPN684
13508048	MPN309				

UniProt web server has been cited on 19 June, 2006.

Sequence databases	
EMBL	U00089; AAB95884.1; -; Genomic_DNA. U43738; AAC43651.1; -; Genomic_DNA.
PIR	S73562; S73562.
3D structure databases	
HSSP	P08324; 1E9I.
Enzyme and pathway databases	
BioCyc	MetaCyc: MONOMER-551; -.
Family and domain databases	
HAMAP	MF_00318; -; 1.
InterPro	IPR000941; Enolase.
PANTHER	PTHR11902; Enolase; 1.
Pfam	PF00113; Enolase_C; 1. PF03952; Enolase_N; 1.
PRINTS	PR00148; ENOLASE.
ProDom	PD000902; Enolase; 1.
PROSITE	PS00164; ENOLASE; 1.
Genome annotation databases	
GenomeReviews	U00089_GR; MPN606.

Complete proteome; Glycolysis; Lyase; Magnesium; Metal-binding.

Key	From	To	Length	Description	FTId
CHAIN	1	456	456	Enolase.	PRO_0000133930
REGION	389	392	4	Substrate binding (By similarity).	
ACT_SITE	219	219		Proton donor (By similarity).	
ACT_SITE	362	362		Proton acceptor (By similarity).	
METAL	256	256		Magnesium (By similarity).	
METAL	310	310		Magnesium (By similarity).	
METAL	337	337		Magnesium (By similarity).	
BINDING	169	169		Substrate (By similarity).	
BINDING	178	178		Substrate (By similarity).	
BINDING	310	310		Substrate (By similarity).	
BINDING	337	337		Substrate (By similarity).	
BINDING	413	413		Substrate (By similarity).	
CONFLICT	309	311		IED -> HRS (in Ref. 2).	
CONFLICT	327	332		TLGQHI -> HWSTH (in Ref. 2).	

APPENDIX B

Parameter for model building

Script for generating *.PIR format sequence

```
# Color the first template structure according to gaps in alignment
env = environ()
code = 'enol'
aln = alignment (env)
mdl = model (env)
mdl.read (file = code, model_segment = ('FIRST:@','LAST:@'))
aln.append_model(mdl=mdl,align_codes=code,atom_files=code)
aln.write (file=code + '.seq')
```

Script for align2D

```
env = environ()
aln = alignment(env)
# file = 'name of template pdb file'
mdl = model(env, file='1E9I', model_segment=('FIRST:A','LAST:A'))
aln.append_model(mdl, align_codes='1e9iA', atom_files='1E9I')
# align_code command must the same as title of first line in sequenced which saved
as below
aln.append(file='enolase.seq', align_codes='Enolase')
aln.align2d()
aln.write(file='enolase-1e9iA.ali', alignment_format='PIR')
aln.write(file='enolase-1e9iA.pap', alignment_format='PAP')
```

The align2D in *.PIR format

```
>P1;1e9iA
structureX:1E9I:   1 :A: 430 :A:undefined:undefined:-1.00:-1.00
---------SKIVKIIGREIIDSRGNPTVEAEVHLEGGFVGMAAAPSGASTGSREALELRDGDKSRFLGKGVTKA
VAAVNGPIAQALIGKDAKDQAGIDKIMIDLDGTENKSKFGANAILAVSLANAKAAAAAKGMPLYEHIAELNG--T
PGKYSMPVPMMNIINGGEHADNNVDIQEFMIQPVGAKTVKEAIRMGSEVFHHLAKVLKAKGMNTAVGDEGGYAPN
LGSNAEALAVIAEAVKAAGYELGKDITLAMDCAASEFYKD----------GKYVLAGEGNKAFTSEEFTHFLEEL
TKQYPIVSIEDGLDESDWDGFAYQTKVLGDKIQLVGDDLFVTNTKILKEGIEKGIANSILIKFNQIGSLTETLAA
IKMAKDAGYTAVISHRSGETEDATIADLAVGTAAGQIKTGSMSRSDRVAKYNQLIRIEEALGEKAPYNGRKEIK-
----GQ*

>P1;Enolase
sequence:Enol:     : :     : ::: 0.00: 0.00
MSAQTGTDLFKIADLFAYQVFDSRGFPTVACVVKLASGHTGEAMVPSGASTGEKEAIELRDGDPKAYFGKGVSQA
VQNVNQTIAPKLIGLNATDQAAIDALMIQLDGTPNKAKLGANAILAVSLAVAKAAASAQKTSLFKYLANQVMGLN
KTEFILTVPMLNVINGGAHADNNIDFQEFMIMPLGANSMHQALKMASETFHALQKLLKQRGLNTNKGDEGGFAPN
LKLAEEALDLMVEAIKAAGYQPGSDIAIALDVAASEFYDDTTKRYVFKKGIKAKILDEKEWSLTTAQMIAYLKKL
TEQYPIISIEDGLSEHDWEGMETLTKTLGQHIQIVGDDLYCTNPAIAEKGVAHKATNSILIKLNQIGTLTETIKA
INIAKDANWSQVISHRSGETEDTTIADLAVAACTGQIKTGSMSRSERIAKYNRLLQIELELGNNAKYLGWNTFKN
IKPQKA*
```

Model building

```
# Very fast homology modelling by the automodel class
from modeller.automodel import *
# Load the automodel class
log.verbose()
env = environ()
# directories for input atom files
env.io.atom_files_directory = './:../atom_files'
a = automodel(env, alnfile='enol-1e9iA.ali', knowns ='1e9iA', sequence ='enol',
assess_methods=assess.DOPE)
# request DOPE model assessment
a.very_fast()
# prepare for extremely fast optimization
a.starting_model = 1
a.ending_model = 2000
a.final_malign3d = True
a.make()
# make the homology model
```

Model loop script

```
# Homology modelling by the automodel class
from modeller.automodel import *
# Load the automodel class
log.verbose()
env = environ()
# directories for input atom files
env.io.atom_files_directory = './:../atom_files'
a = loopmodel(env, alnfile = 'enol-1e9iA.ali', knowns = '1e9iA', sequence = 'enol')
# index of the first model
a.starting_model= 1
# index of the last model
```

```
a.ending_model = 1
# (determines how many models to calculate)
# No refinement of model
a.md_level = None
# First loop model
a.loop.starting_model = 1
# Last loop model
a.loop.ending_model = 2000
# Loop model refinement level
a.loop.md_level = refine.fast
# do homology modelling
a.make()
```

Solvate parameter script with VMD program

```
>VMD

package require psfgen

topology top_all27_prot_lipid.inp

pdbalias residue HIS HSE

pdbalias atom ILE CD1 CD

segment u {pdb model.pdb}

coordpdb umoldel.pdb u

guesscoord

writepdb protein1.pdb

writepsf protein1.psf

exit
```

Molecule minmax measurement script

>VMD

VMD> set protein [atomselect top protein]

VMD> measure minmax $protein

VMD> measure center $protein

Solvation script

>VMD

VMD> package require solvate

VMD> solvate protein.psf protein.pdb –minmax{{-X –Y –Z} {X Y Z}}

Or

VMD> package require solvate

VMD> solvate protein.psf protein.pdb –o solvate –rotate –t 10, (10 Å water box size)

PSF file generating script

>VMD

package require psfgen

topology top_all27_prot_lipid.inp

pdbalias residue HIS HSE

pdbalias atom ILE CD1 CD

segment u {pdb protein1.pdb}

coordpdb uprotien1.pdb u

segment w {pdb protein2.pdb}

coordpdb wprotien2.pdb w

guesscoord

writepdb protein1.pdb

writepdb protein2.pdb

writepsf protein1.psf

writepsf protein2.psf

exit

Molecule minmax measurement script

>VMD

VMD> set protein [atomselect top protein]

VMD> measure minmax $protein

VMD> measure center $protein

Solvation script

>VMD

VMD> package require solvate

VMD> solvate protein.psf protein.pdb –minmax{{-X –Y –Z} {X Y Z}}

Or

VMD> package require solvate

VMD> solvate protein.psf protein.pdb –o solvate –rotate –t 20 # (20 Å water box size)

APPENDIX C

Parameter for Energy minimization by NAMD

```
# input topology and initial structure
structure          solvate.psf
coordinates        solvate.pdb
paratypecharmm          on
parameters      par_all27_prot_lipid.inp
exclude            scaled1-4
1-4scaling              1.0
dielectric              1.0
switching                on
switchdist              8.0
cutoff                 12.0
pairlistdist           13.5
margin                  0.0
stepspercycle           20
rigidBonds             all
rigidTolerance      0.00001
rigidIterations         100
PME                      on
PMETolerance        0.000001
PMEGridSizeX            32
PMEGridSizeY            32
PMEGridSizeZ            32
minimization             on
outputenergies         1000
```

```
outputtiming            1000
binaryoutput              no
outputname      output/nameem
restartname output/nameem_re
restartfreq            10000
binaryrestart             no
DCDfile    output/nameem.dcd
dcdfreq                 1000
numsteps                2000
cellBasisVector1    X 0.0 0.0
cellBasisVector2    0.0 Y 0.0
cellBasisVector3    0.0 0.0 Z
cellOrigin          X' Y' Z'
wrapWater                 on
```

Remarks

$X = X_{max}-X_{min}$, $X' = (X_{max}-X_{min})/2$

$Y = Y_{max}-Y_{min}$, $Y' = (Y_{max}-Y_{min})/2$

$Z = Z_{max}-Z_{min}$, $Z' = (Z_{max}-Z_{min})/2$

Parameter for Heating by NAMD

```
# input topology and initial structure
structure          solvate.psf
coordinates   output/nameem.coor
paratypecharmm              on
parameters    par_all27_prot_lipid.inp
exclude             scaled1-4
1-4scaling                1.0
dielectric                1.0
switching                  on
switchdist                8.0
cutoff                   12.0
pairlistdist             13.5
margin                    0.0
stepspercycle              20
rigidBonds                all
rigidTolerance          0.00001
rigidIterations            100
PME                        on
PMETolerance          0.000001
PMEGridSizeX               32
PMEGridSizeY               32
PMEGridSizeZ               32
timestep                  1.0
fullElectFrequency          4
outputenergies           1000
```

```
outputtiming              1000

binaryoutput                no

outputname      output/nameheat

restartname output/nameheat_re

restartfreq               10000

binaryrestart               yes

DCDfile     output/nameheat.dcd

dcdfreq                   1000

seed                      1010

numsteps                300000

reassignIncr             0.001

reassignHold               300

cellBasisVector1   X 0.0 0.0

cellBasisVector2   0.0 Y 0.0

cellBasisVector3   0.0 0.0 Z

cellOrigin          X' Y' Z'

wrapWater                  on
```

Parameter for Equilibrium by NAMD2

```
# input topology and initial structure
structure       solvate.psf
coordinates output/nameheat.coor
paratypecharmm          on
parameters  par_all27_prot_lipid.inp
exclude            scaled1-4
1-4scaling              1.0
dielectric               1.0
switching                 on
switchdist               8.0
cutoff                  12.0
pairlistdist            13.5
margin                   0.0
stepspercycle             20
rigidBonds               all
rigidTolerance       0.00001
rigidIterations          100
PMETolerance        0.000001
PMEGridSizeX              32
PMEGridSizeY              32
PMEGridSizeZ              32
outputenergies          1000
outputtiming            1000
binaryoutput              no
outputname     output/nameequi
```

```
restartname output/nameequi_re

restartfreq              10000

binaryrestart              yes

DCDfile     output/nameequi.dcd

dcdfreq                  1000

seed                     2010

numsteps                50000

temperature               300

rescaleFreq                 1

rescaleTemp               300

cellBasisVector1    X 0.0 0.0

cellBasisVector2    0.0 Y 0.0

cellBasisVector3    0.0 0.0 Z

cellOrigin           X' Y' Z'

wrapWater                  on
```

Parameter for Quench by NAMD

```
# input topology and initial structure
structure          solvate.psf
coordinates output/nameequi.coor
bincoordinates  output/nameequi_re.coor
#binvelocities  output/nameequi.coor.vel
paratypecharmm              on
parameters   par_all27_prot_lipid.inp
exclude             scaled1-4
1-4scaling                1.0
dielectric                1.0
switching                  on
switchdist                8.0
cutoff                   12.0
pairlistdist             13.5
margin                    0.0
stepspercycle              20
rigidBonds                all
rigidTolerance          0.00001
rigidIterations            100
PME                        on
PMETolerance          0.000001
PMEGridSizeX               32
PMEGridSizeY               32
PMEGridSizeZ               32
timestep                  1.0
```

```
fullElectFrequency          4
outputenergies           1000
outputtiming             1000
binaryoutput               no
outputname     output/namequench
restartname    output/namequench_re
restartfreq             10000
binaryrestart             yes
DCDfile    output/namequench.dcd
dcdfreq                  1000
seed                     3010
numsteps              4000000
temperature               300
cellBasisVector1      X 0.0 0.0
cellBasisVector2      0.0 Y 0.0
cellBasisVector3      0.0 0.0 Z
cellOrigin             X' Y' Z'
wrapWater                 on
```

APPENDIX D

Script to analyze DCD file to compute the RMSD between each frame in a DCD file and a reference pdb file

```
Bigdcd.tcl (package require bigdcd)
proc bigdcd { script args } {
global bigdcd_frame bigdcd_proc bigdcd_firstframe vmd_frame
set bigdcd_frame 0
set bigdcd_firstframe [molinfo top get numframes]
set bigdcd_proc $script
uplevel #0 trace variable vmd_frame w bigdcd_callback
foreach dcd $args {
animate read dcd $dcd waitfor 0
 }
}
proc bigdcd_callback { name1 name2 op } {
global bigdcd_frame bigdcd_proc bigdcd_firstframe vmd_frame
# If we're out of frames, we're also done
set thisframe $vmd_frame($name2)
if { $thisframe < $bigdcd_firstframe } {
bigdcd_done
return
 }
incr bigdcd_frame
if { [catch {uplevel #0 $bigdcd_proc $bigdcd_frame} msg] } {
puts stderr "bigdcd aborting at frame $bigdcd_frame\n$msg"
bigdcd_done
return
}
animate delete beg $thisframe end $thisframe
return $msg
}
proc bigdcd_done { } {
```

```
puts "bigdcd_done"
after idle uplevel #0 trace vdelete vmd_frame w bigdcd_callback
}
```

Script to extract RMSD from trajectory file to EXCELL plot

```
set outfile [open rmsd.csv w]
proc myrmsd { frame } {
global ref sel all
$all move [measure fit $sel $ref]
puts $outfile "$frame,[measure rmsd $sel $ref]"
puts "$frame is finished"
 }
mol load psf solvate.psf
set all [atomselect top all]
set ref [atomselect top "name CA" frame 0]
set sel [atomselect top "name CA"]
animate read pdb protein.pdb
source bigdcd.tcl
bigdcd myrmsd quench.dcd
close $outfile
```

VITA

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