

Supplement to: Protein Stability in Titan's Subsurface Water Ocean

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Sections 1-4 are mdp files used in Gromacs 5.1.2. Section 5 includes figures taken from simulation results for 1 bar and aqueous ammonia (a low pressure Titan-like environment), and 1 kbar in water (i.e., a high pressure Earth-like environment).

1. Em.mdp for Earth

```
define                = -DFLEXIBLE
integrator            = steep
nsteps                = 1000

nstlog                = 0
nstenergy             = 0
nstxout               = 0
nstxout-compressed    = 0
nstvout               = 0
nstfout               = 0

nstlist               = 20
pbc                   = xyz
rlist                 = 1.4
cutoff-scheme         = verlet

coulombtype           = Reaction-Field-zero
rcoulomb              = 1.2
vdwtype               = Cut-off
vdw-modifier          = Potential-shift-Verlet
rvdw                  = 1.2
```

2. Pr.mdp for Earth

```
define                = -DPOSRES
integrator            = md
dt                    = 0.002
nsteps                = 200000 ; total 1 000 ps

nstlog                = 0
nstenergy             = 0
nstxout               = 0
```

nstxout-compressed	= 0
nstvout	= 0
nstfout	= 0
nstcalcenergy	= 0
nstlist	= 20
pbcs	= xyz
rlist	= 1.4
cutoff-scheme	= verlet
coulombtype	= Reaction-Field-zero
rcoulomb	= 1.2
vdwtype	= Cut-off
vdw-modifier	= Potential-shift-Verlet
rvdw	= 1.2
constraints	= all-bonds
constraint_algorithm	= lincs
lincs_order	= 8
tcoupl	= v-rescale
tc_grps	= Protein Non-Protein
tau_t	= 0.1 0.1
ref_t	= 300 300
pcoupl	= Berendsen
tau_p	= 1.0
compressibility	= 4.5e-5
ref_p	= 1
refcoord_scaling	= all
annealing	= single single
annealing-npoints	= 3 3
annealing-time	= 0 500 1000 0 500 1000
annealing-temp	= 100 300 300 100 300 300

3. Md.mdp for Earth

integrator	= md
dt	= 0.002
nsteps	= 200000 ; total 1 000 ps

nstlog	= 2000
nstenergy	= 2000
nstxout	= 2000
nstxout-compressed	= 2000
nstvout	= 2000
nstfout	= 0
nstcalcenergy	= 0
nstlist	= 20
pbc	= xyz
rlist	= 1.4
cutoff-scheme	= verlet
coulombtype	= Reaction-Field-zero
rcoulomb	= 1.2
vdwtype	= Cut-off
vdw-modifier	= Potential-shift-Verlet
rvdw	= 1.2
constraints	= all-bonds
constraint_algorithm	= lincs
lincs_order	= 8
tcoupl	= v-rescale
tc_grps	= Protein Non-Protein
tau_t	= 0.1 0.1
ref_t	= 300 300
pcoupl	= Berendsen
tau_p	= 1.0
compressibility	= 4.5e-5
ref_p	= 1

4. Prod.mdp for Earth

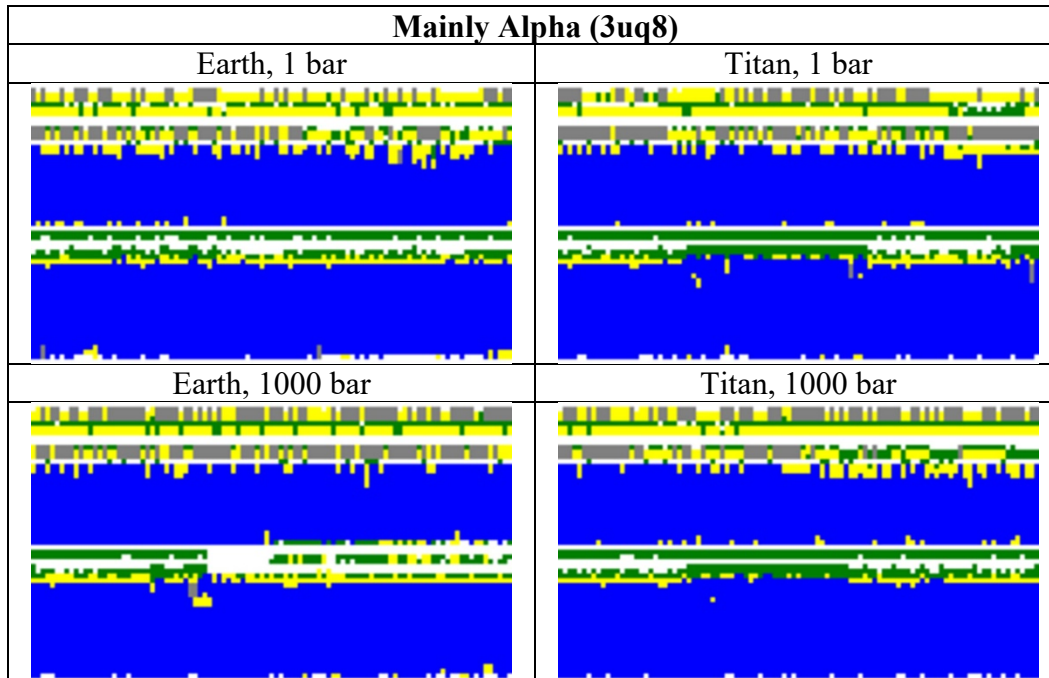
integrator	= md
dt	= 0.002
nsteps	= 50000000 ; total 100 000 ps
nstlog	= 2000
nstenergy	= 2000
nstxout	= 2000

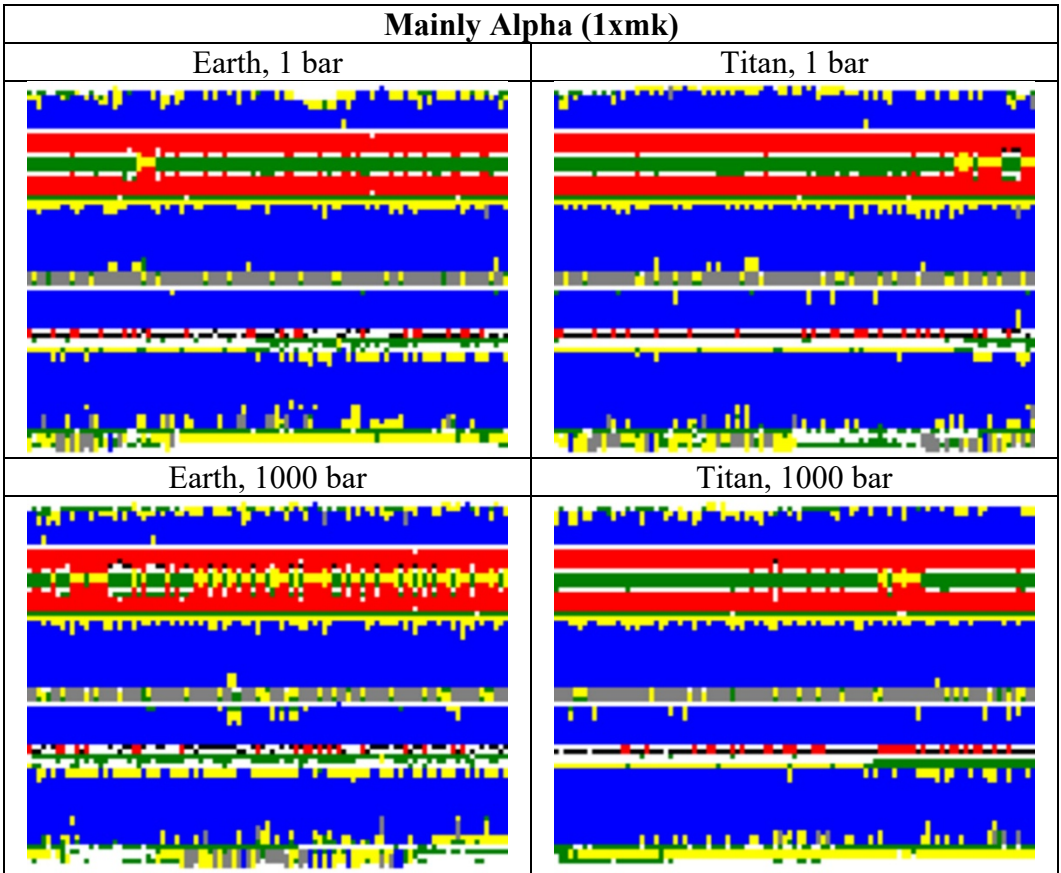
nstxout-compressed	= 2000
nstvout	= 2000
nstfout	= 2000
nstcalcenergy	= 2000
nstlist	= 20
pbc	= xyz
rlist	= 1.4
cutoff-scheme	= verlet
coulombtype	= Reaction-Field-zero
rcoulomb	= 1.2
vdwtype	= Cut-off
vdw-modifier	= Potential-shift-Verlet
rvdw	= 1.2
DispCorr	= EnerPres
constraints	= all-bonds
constraint_algorithm	= lincs
lincs_order	= 8
tcoupl	= v-rescale
tc_grps	= Protein Non-Protein
tau_t	= 0.1 0.1
ref_t	= 300 300
pcoupl	= Parrinello-Rahman
tau_p	= 1.6
compressibility	= 4.5e-5
ref_p	= 1

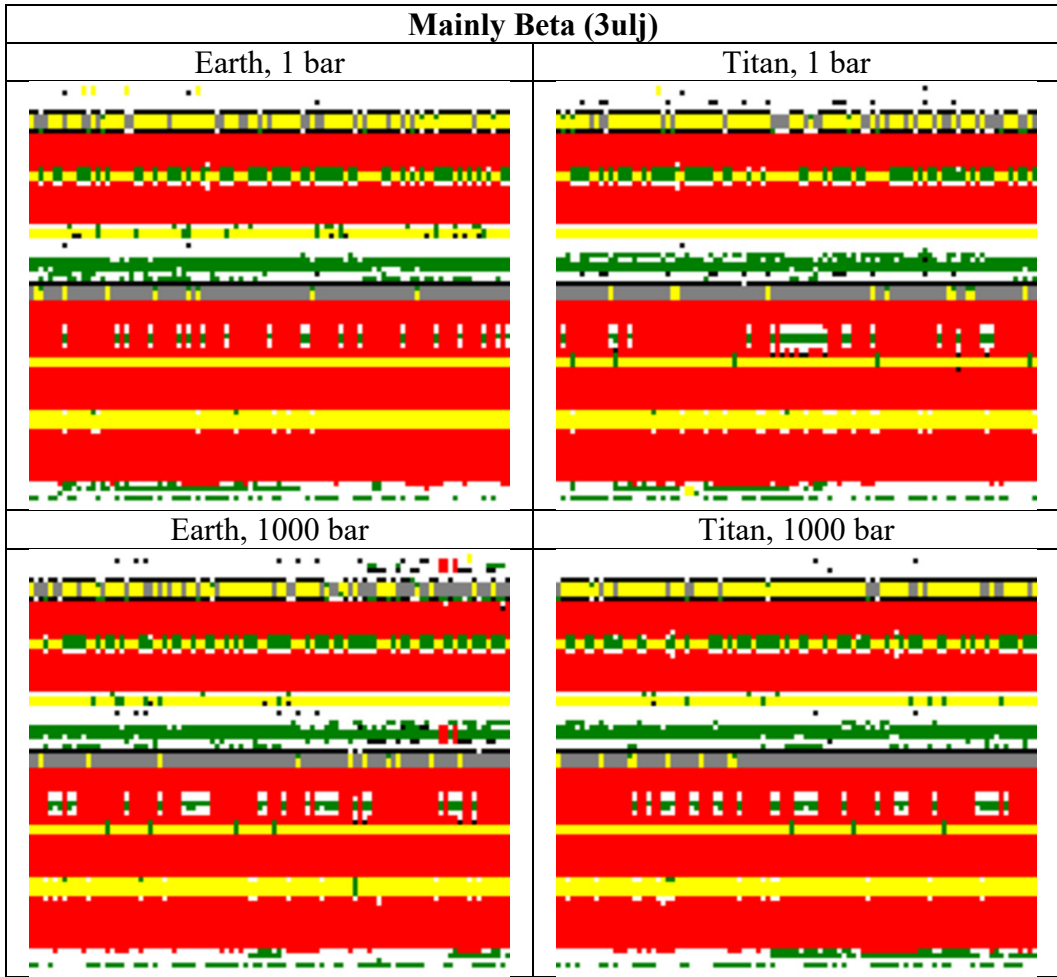
5. Simulations to examine effects of pressure and ammonia

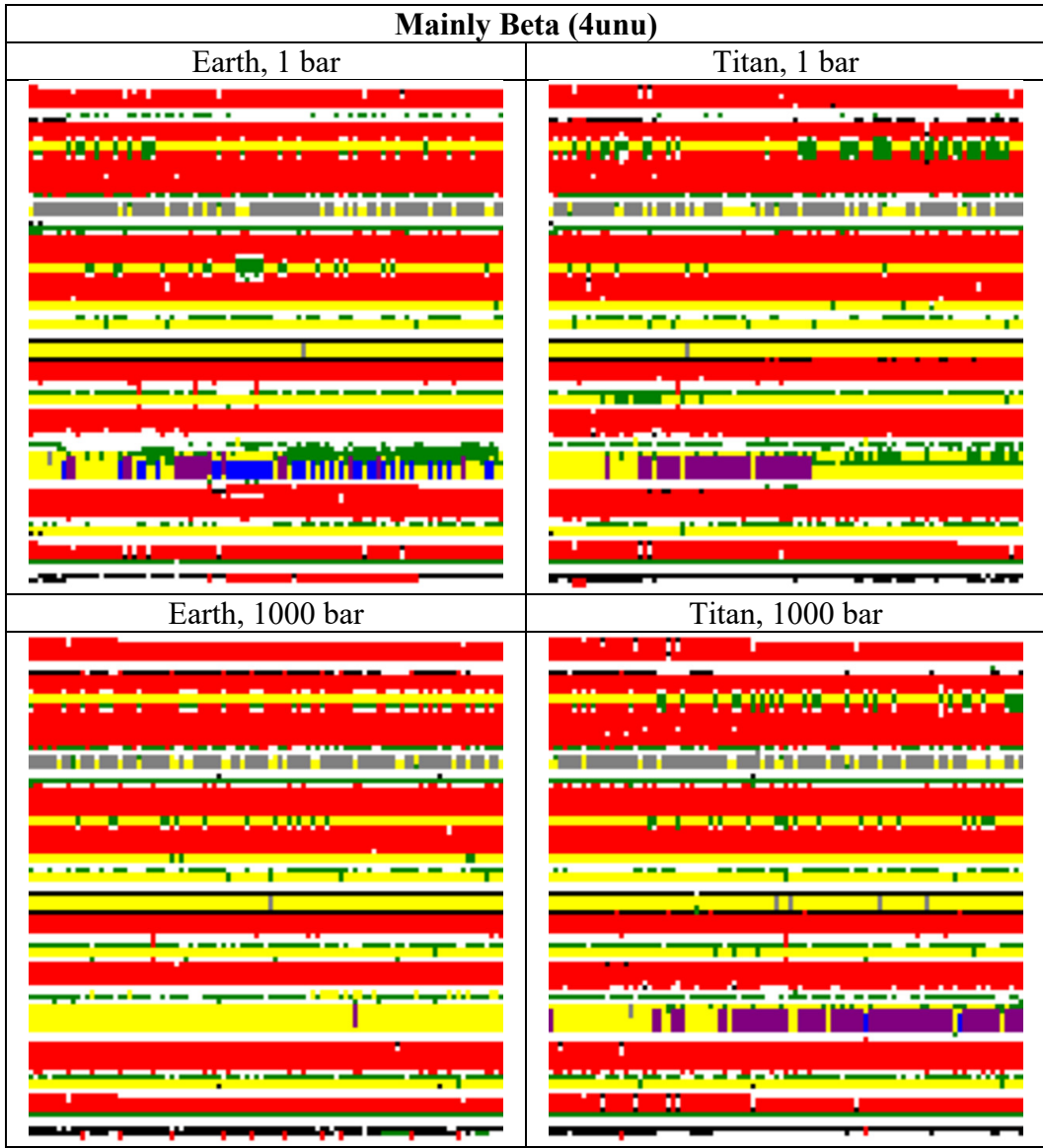
The following results are for simulations of proteins in a low pressure Titan environment (1 bar, aqueous ammonia) and in a high pressure Earth environment (1000 bar, water).

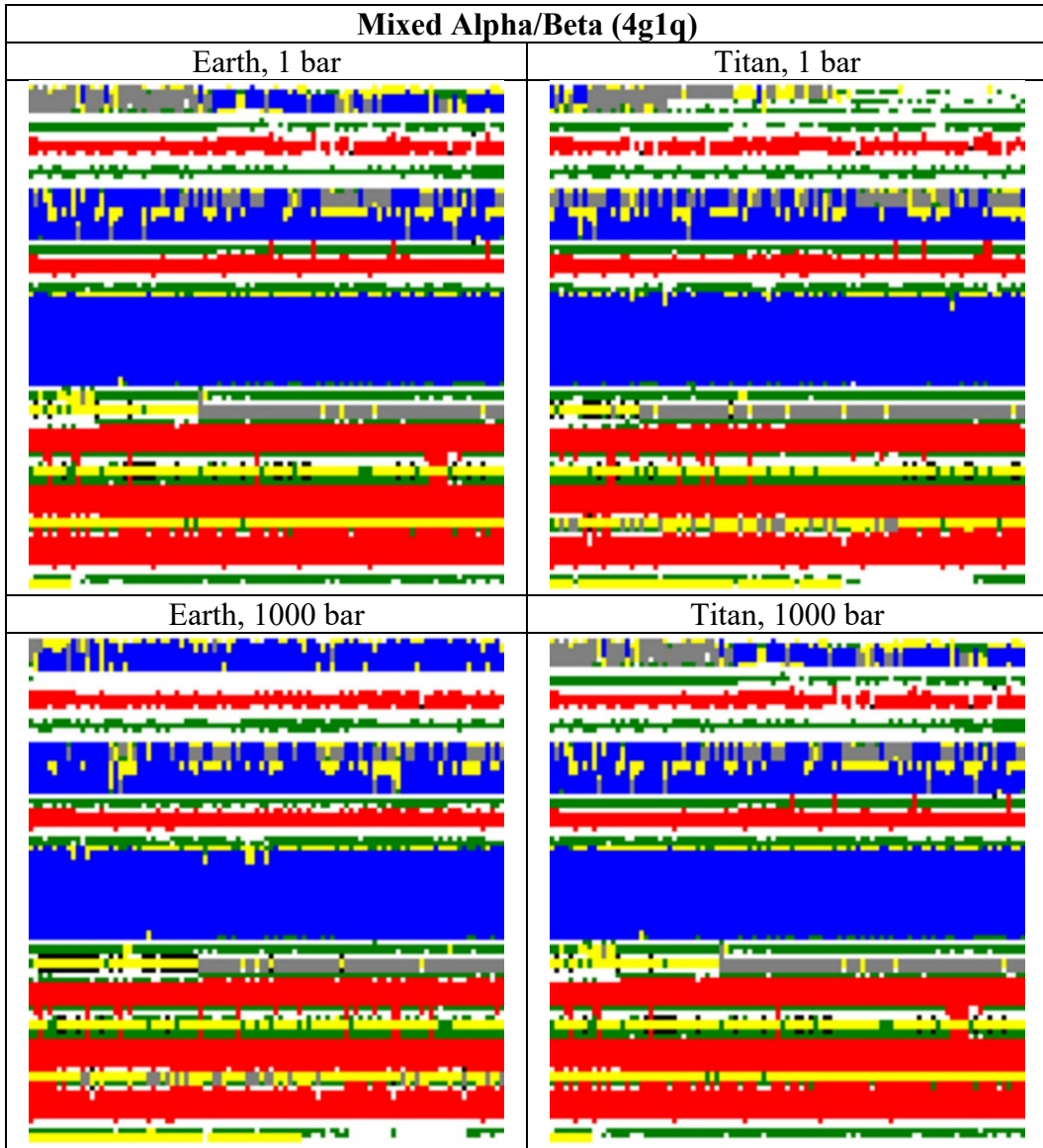
SECONDARY STRUCTURE

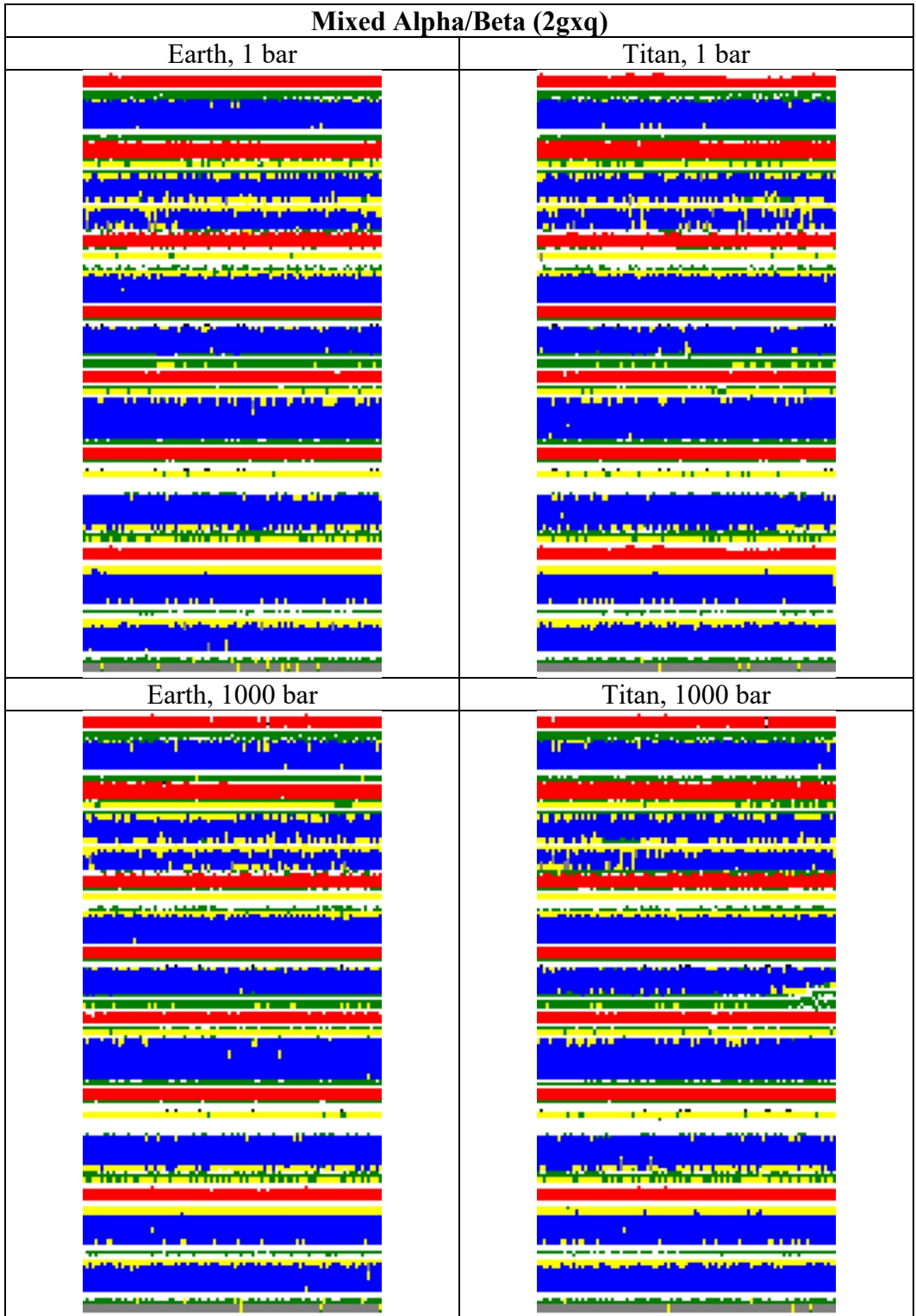




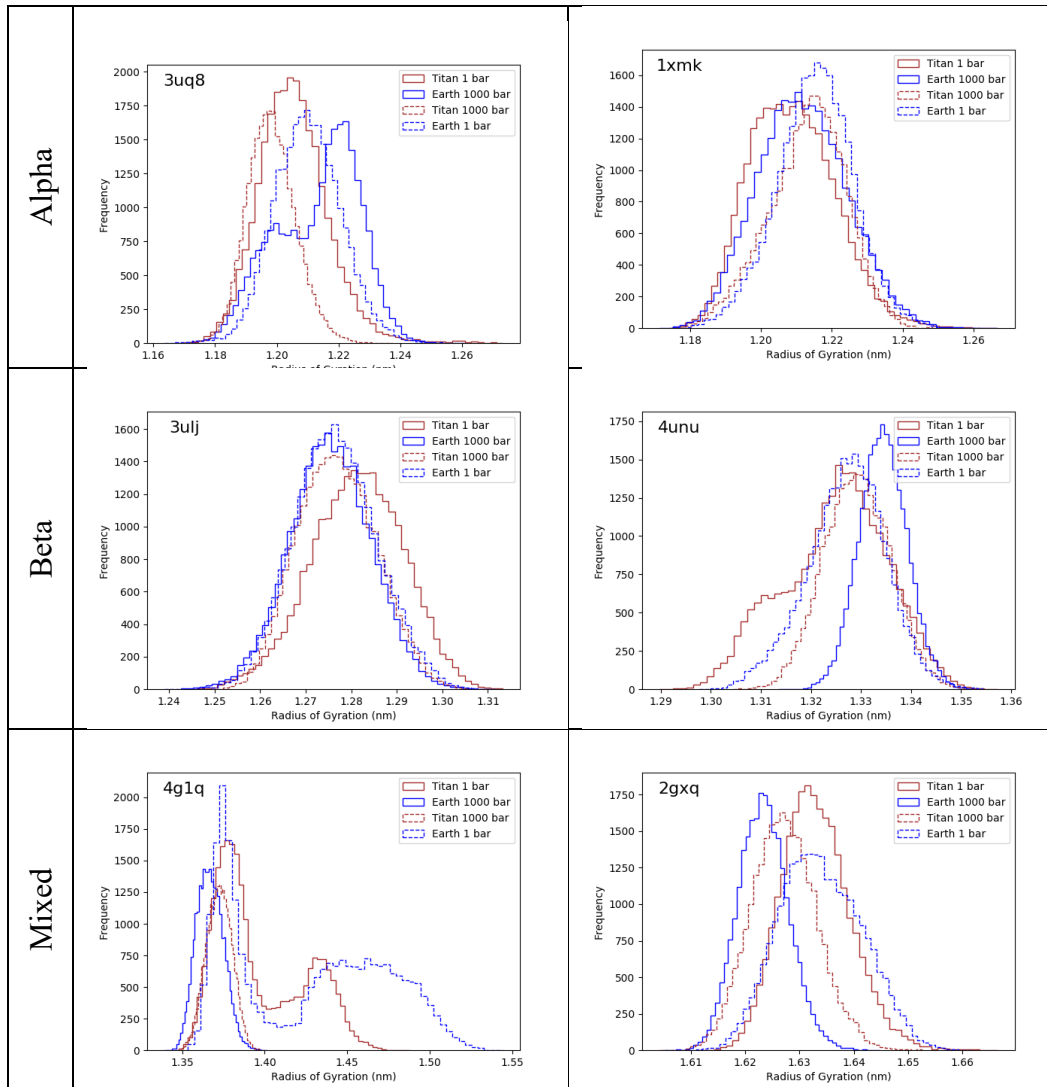




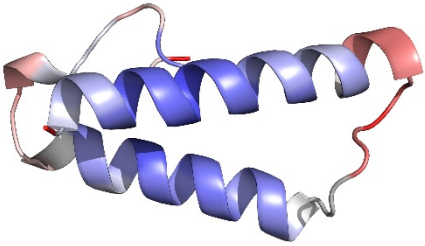
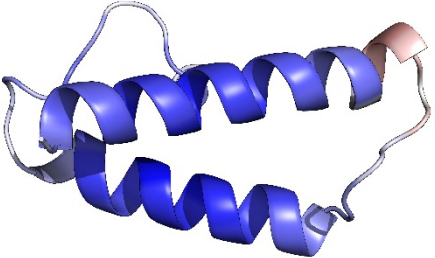
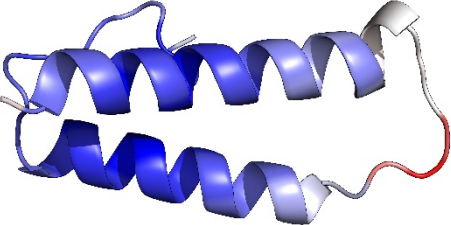
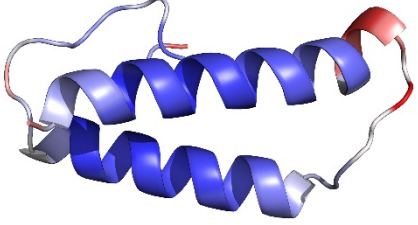


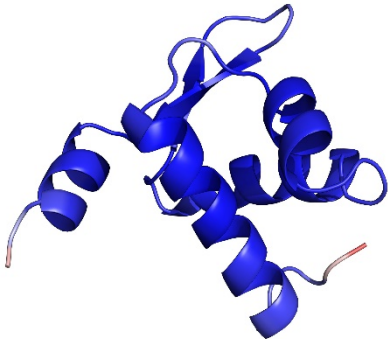
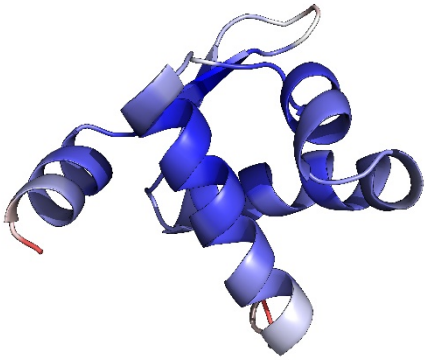
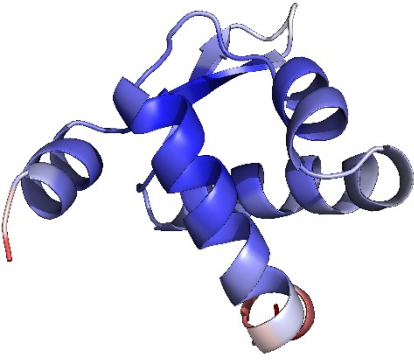
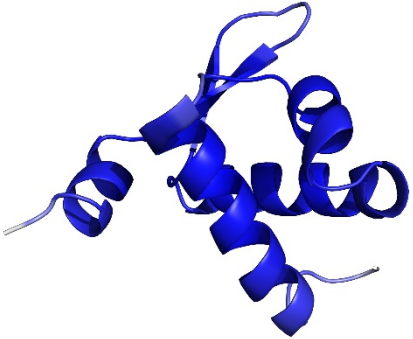


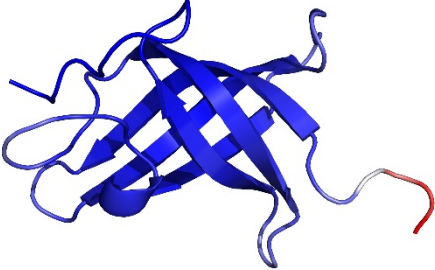
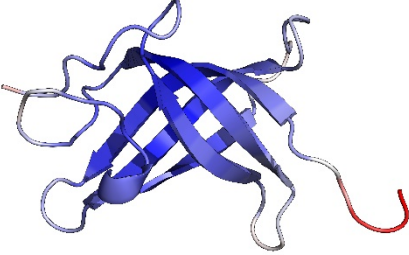
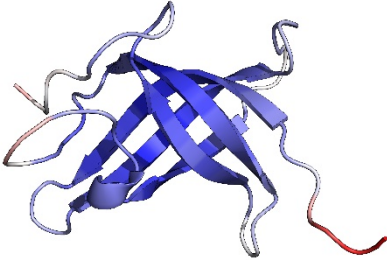
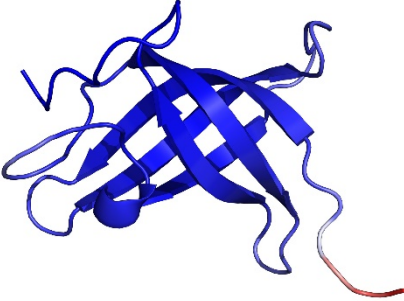
RADIUS OF GYRATION

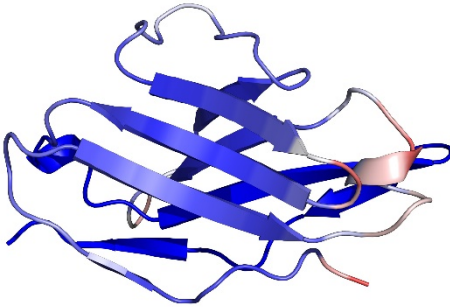
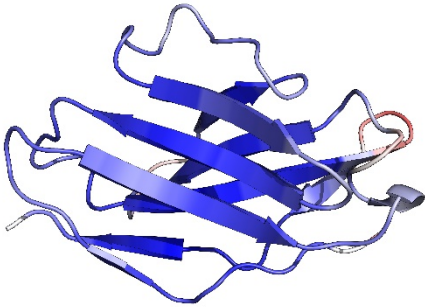
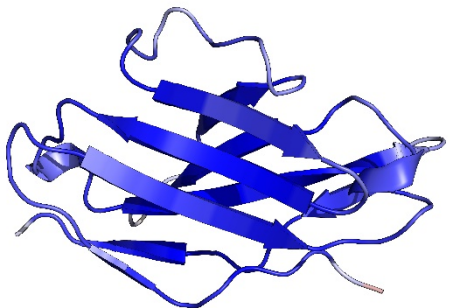
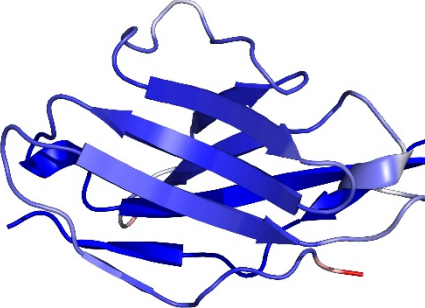


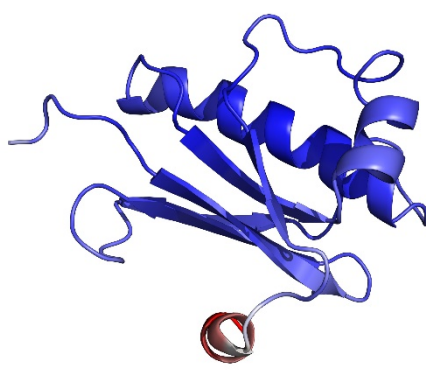
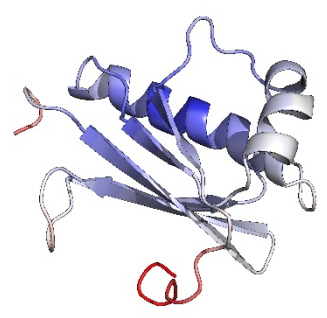
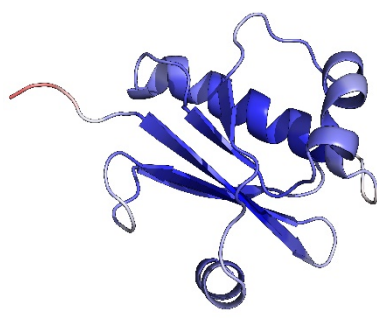
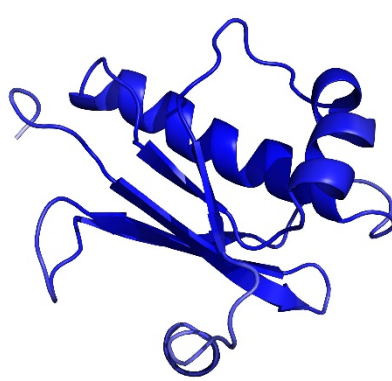
RMSF

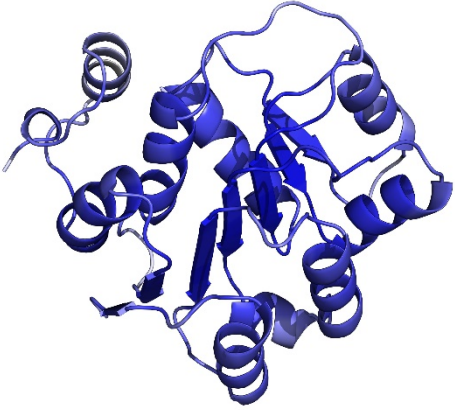
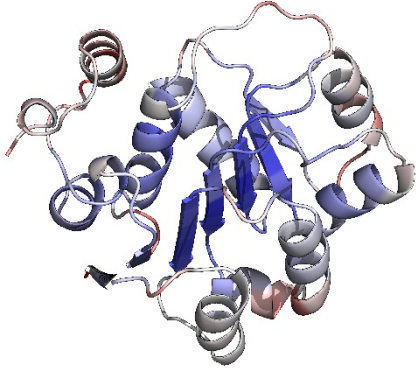
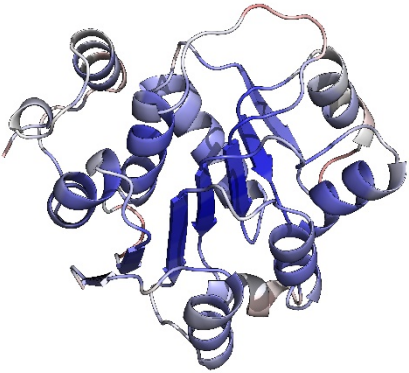
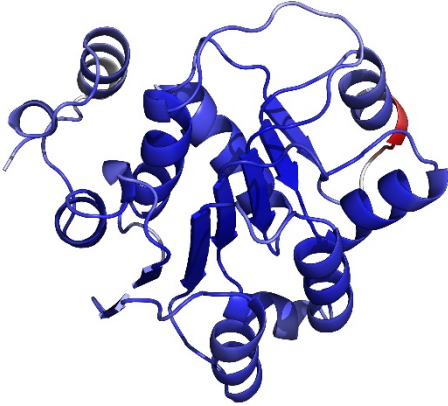
Mainly Alpha (3uq8)	
Earth, 1 bar	Titan, 1 bar
	
Earth, 1000 bar	Titan, 1000 bar
	

Mainly Alpha (1xmk)	
Earth, 1 bar	Titan, 1 bar
 A ribbon diagram of a protein structure on Earth at 1 bar. The protein is primarily composed of blue alpha-helices and loops, with a few light blue and orange segments. The structure is compact and well-defined.	 A ribbon diagram of a protein structure on Titan at 1 bar. The protein is primarily composed of blue alpha-helices and loops, with a few light blue and orange segments. The structure is more relaxed and less compact than the Earth version.
Earth, 1000 bar	Titan, 1000 bar
 A ribbon diagram of a protein structure on Earth at 1000 bar. The protein is primarily composed of blue alpha-helices and loops, with a few light blue and orange segments. The structure is more relaxed and less compact than the Earth version at 1 bar.	 A ribbon diagram of a protein structure on Titan at 1000 bar. The protein is primarily composed of blue alpha-helices and loops, with a few light blue and orange segments. The structure is more relaxed and less compact than the Titan version at 1 bar.

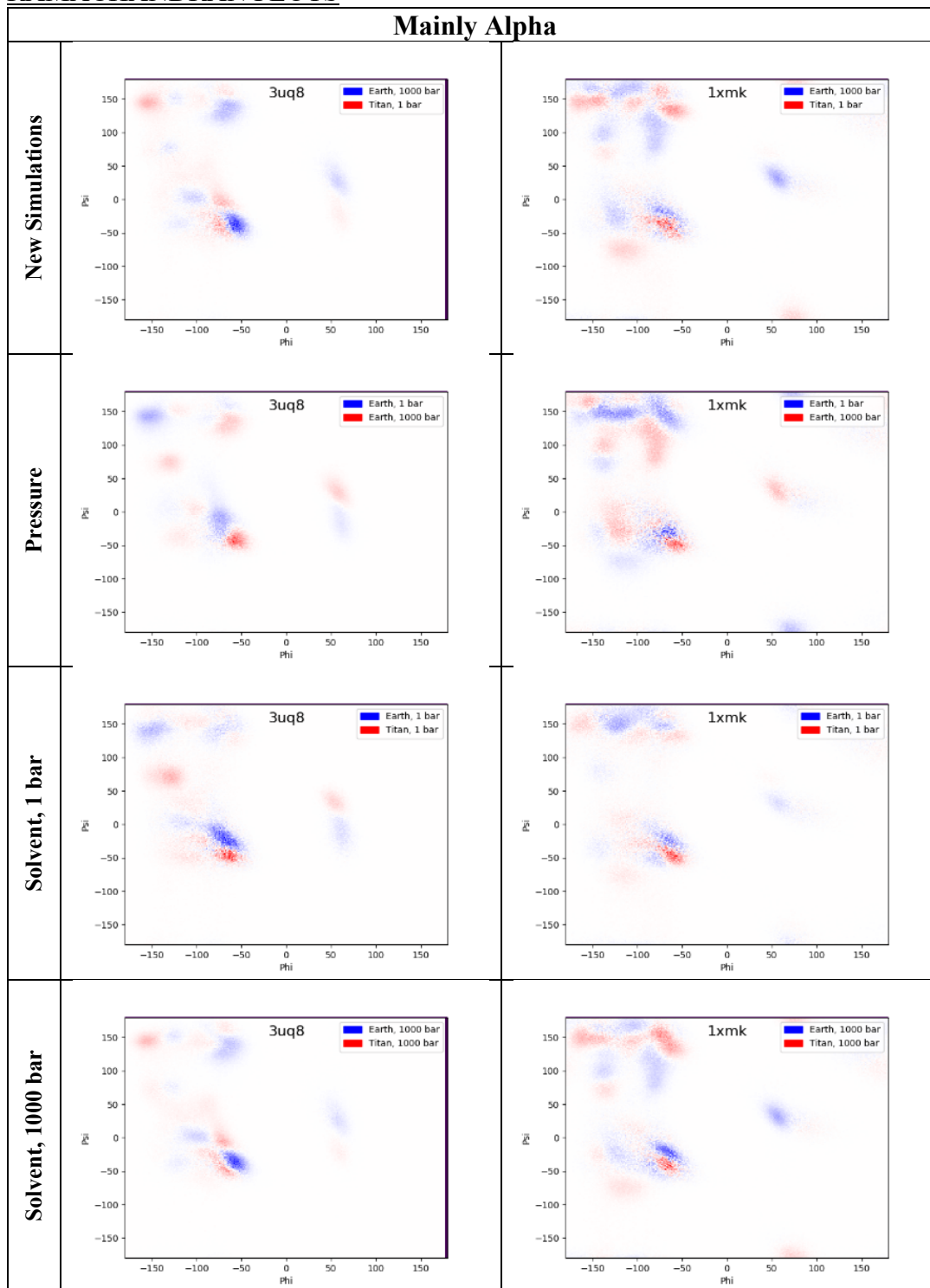
Mainly Beta (3ulj)	
Earth, 1 bar	Titan, 1 bar
 <p>A ribbon diagram of a protein structure on Earth at 1 bar. The protein is primarily composed of blue beta-strands forming a sheet, with several loops and a red C-terminal tail extending to the right.</p>	 <p>A ribbon diagram of a protein structure on Titan at 1 bar. The protein is primarily composed of blue beta-strands forming a sheet, with several loops and a red C-terminal tail extending to the right. The structure appears slightly more compact than the Earth 1 bar version.</p>
Earth, 1000 bar	Titan, 1000 bar
 <p>A ribbon diagram of a protein structure on Earth at 1000 bar. The protein is primarily composed of blue beta-strands forming a sheet, with several loops and a red C-terminal tail extending to the right. The structure appears significantly more compact and rigid than the Earth 1 bar version.</p>	 <p>A ribbon diagram of a protein structure on Titan at 1000 bar. The protein is primarily composed of blue beta-strands forming a sheet, with several loops and a red C-terminal tail extending to the right. The structure appears significantly more compact and rigid than the Titan 1 bar version.</p>

Mainly Beta (4unu)	
Earth, 1 bar	Titan, 1 bar
 <p>A ribbon diagram of a protein structure, primarily composed of blue beta-strands. A specific region is highlighted in red and orange, indicating a flexible or disordered loop. The structure is shown in a compact, folded state.</p>	 <p>A ribbon diagram of a protein structure, primarily composed of blue beta-strands. A specific region is highlighted in red and orange, indicating a flexible or disordered loop. The structure is shown in a compact, folded state, similar to the Earth, 1 bar condition.</p>
Earth, 1000 bar	Titan, 1000 bar
 <p>A ribbon diagram of a protein structure, primarily composed of blue beta-strands. A specific region is highlighted in red and orange, indicating a flexible or disordered loop. The structure is shown in a compact, folded state, similar to the Earth, 1 bar condition.</p>	 <p>A ribbon diagram of a protein structure, primarily composed of blue beta-strands. A specific region is highlighted in red and orange, indicating a flexible or disordered loop. The structure is shown in a compact, folded state, similar to the Earth, 1 bar condition.</p>

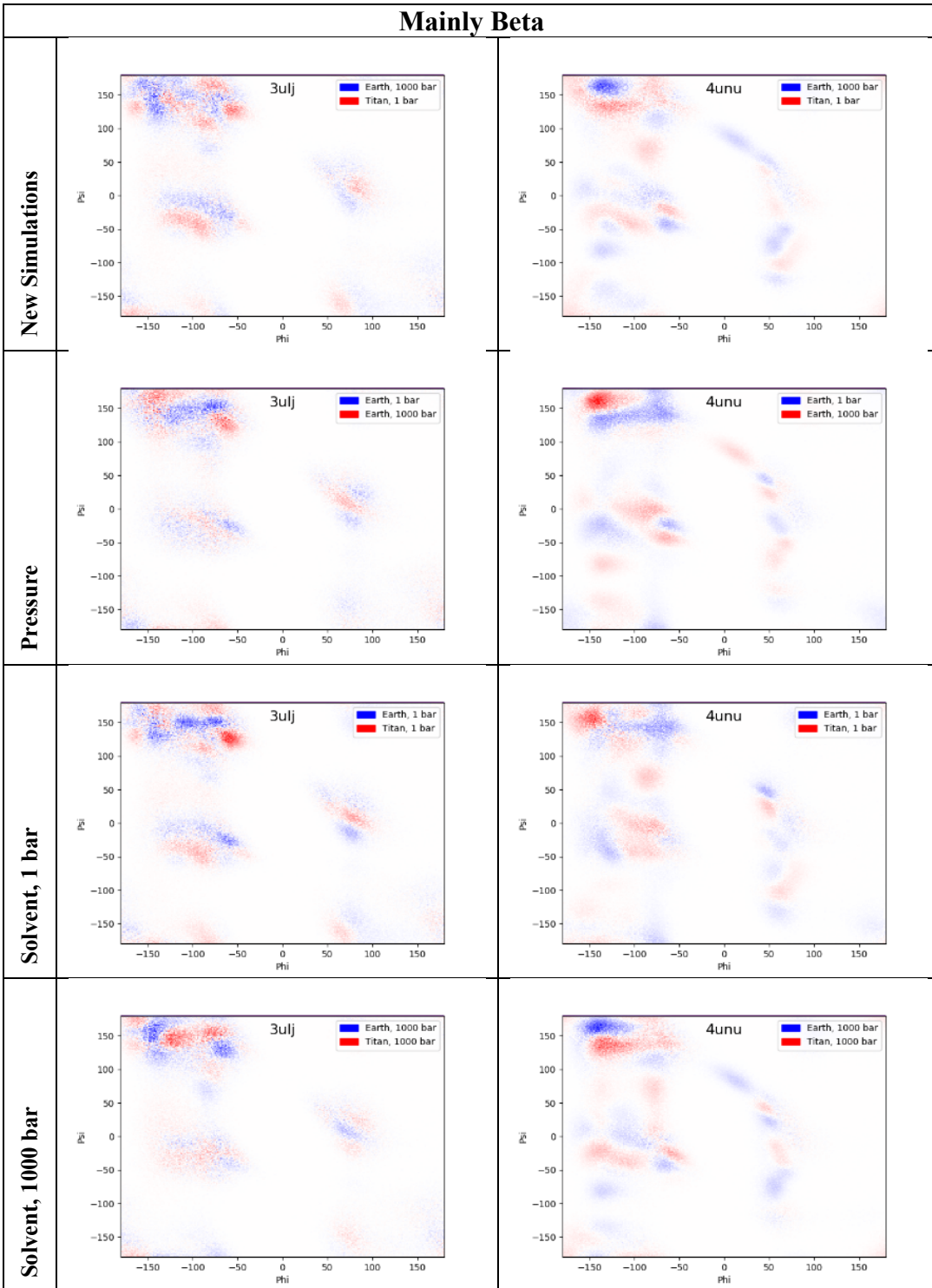
Mixed Alpha/Beta (4g1q)	
Earth, 1 bar	Titan, 1 bar
	
Earth, 1000 bar	Titan, 1000 bar
	

Mixed Alpha/Beta (2gxq)	
Earth, 1 bar	Titan, 1 bar
 <p>A ribbon diagram of a protein structure (Mixed Alpha/Beta, 2gxq) at Earth, 1 bar. The structure is shown in a blue color, indicating a stable, well-defined conformation.</p>	 <p>A ribbon diagram of a protein structure (Mixed Alpha/Beta, 2gxq) at Titan, 1 bar. The structure is shown in a multi-colored (blue, grey, red) representation, indicating a partially denatured or flexible state.</p>
Earth, 1000 bar	Titan, 1000 bar
 <p>A ribbon diagram of a protein structure (Mixed Alpha/Beta, 2gxq) at Earth, 1000 bar. The structure is shown in a multi-colored (blue, grey, red) representation, indicating a partially denatured or flexible state.</p>	 <p>A ribbon diagram of a protein structure (Mixed Alpha/Beta, 2gxq) at Titan, 1000 bar. The structure is shown in a blue color, indicating a stable, well-defined conformation.</p>

RAMACHANDRAN PLOTS



Mainly Beta



Mixed Alpha/Beta

