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## **NMR studies of protein-small molecule and protein-peptide interactions**

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**Author:** Guan, Jia-Ying

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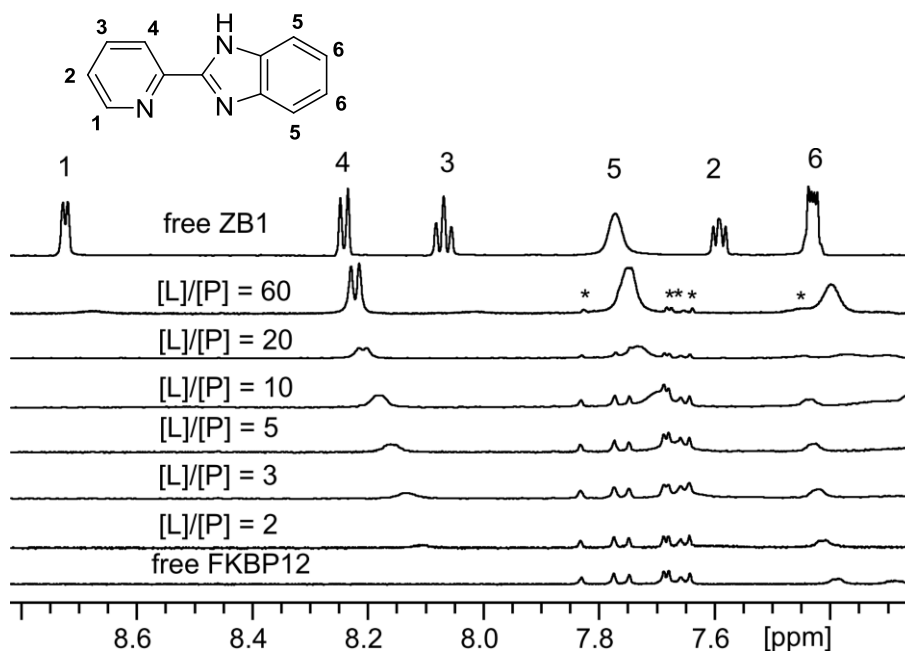
## Appendix A: 1D-<sup>1</sup>H NMR spectra of FKBP12 ligand titrations

**General description:** The proton assignments correspond to the numbers on the ligand structures shown in each figure. Signal intensities were scaled non-linearly for ease of viewing the shapes of the resonances. Asterisks indicate the protein/buffer/solvent resonances. Table A1 summarizes the details of the titration experiments shown in Figures A1-A8.

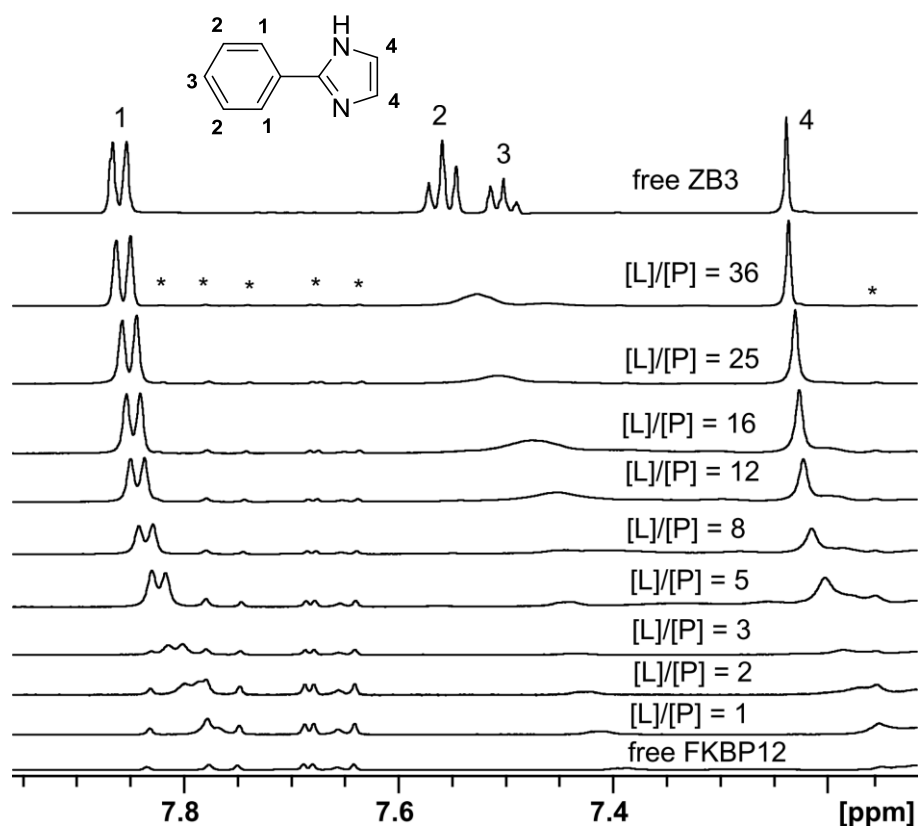
**Table A1:** Details of the titration experiments in Figures A1-A8.

| Figure | ZB code | Initial [P]<br>( $\mu$ M) | Final [L]<br>(mM) | Bound [L] at<br>end point (%) | T <sub>2</sub> filter<br>(ms) | K <sub>D</sub> |
|--------|---------|---------------------------|-------------------|-------------------------------|-------------------------------|----------------|
| A1     | 1       | 50                        | 3.0               | 1.6                           | 20                            | 83 $\mu$ M     |
| A2     | 3       | 50                        | 1.8               | 2.7                           | 20                            | 41 $\mu$ M     |
| A3     | 390     | 50                        | 2.5               | 0.8                           | 60                            | 3.6 mM         |
| A4     | 429     | 100                       | 1.0               | 2.9                           | 60                            | 2.4 mM         |
| A5     | 1051    | 100                       | 1.0               | n.d. <sup>a)</sup>            | 0.5                           | n.d.           |
| A6     | 1104    | 100                       | 1.0               | n.d.                          | 60                            | n.d.           |
| A7     | 1406    | 100                       | 1.0               | n.d.                          | 0.5                           | n.d.           |
| A8     | 1489    | 100                       | 1.0               | n.d.                          | 20                            | n.d.           |

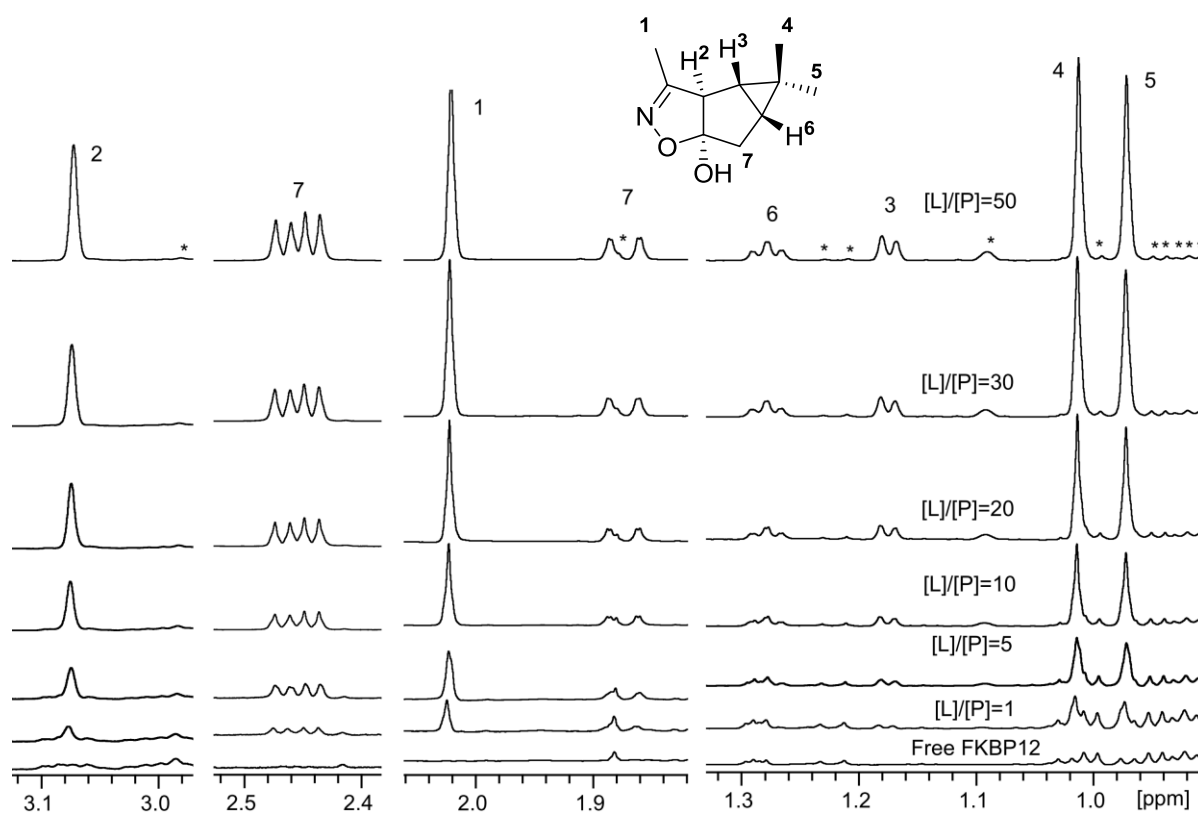
<sup>a)</sup> n.d.: not determined.



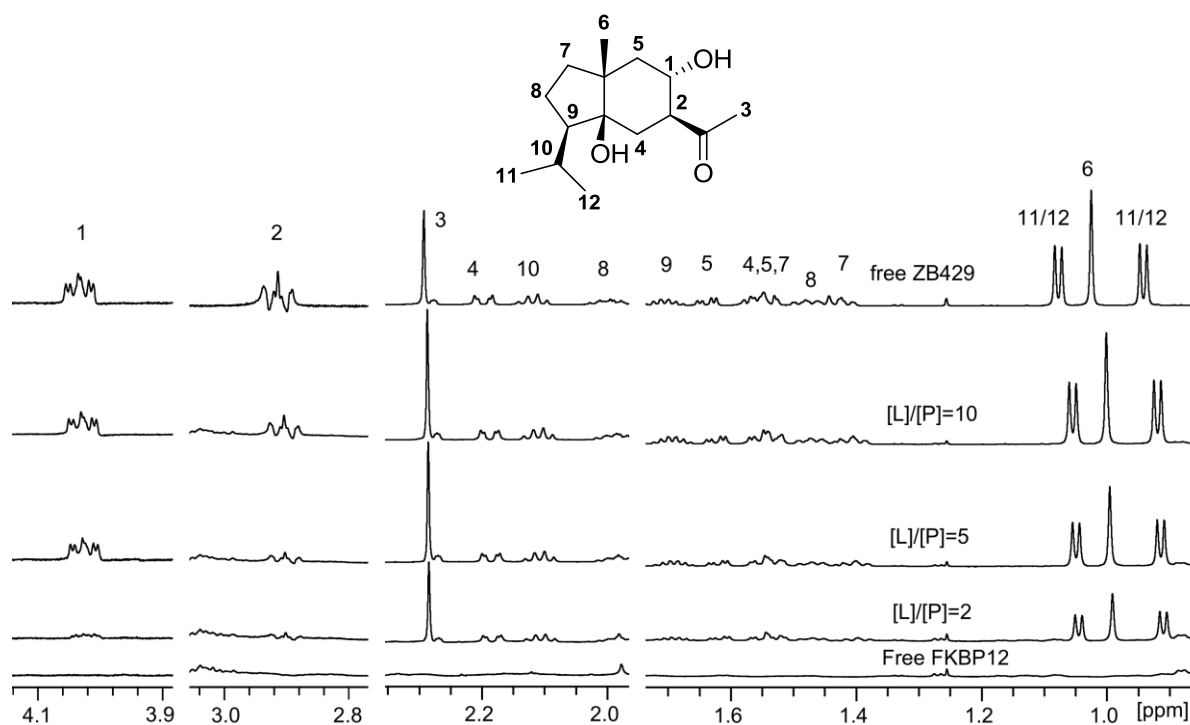
**Figure A1:** 1D-<sup>1</sup>H NMR spectra of ZB1 titrated into wt FKBP12.



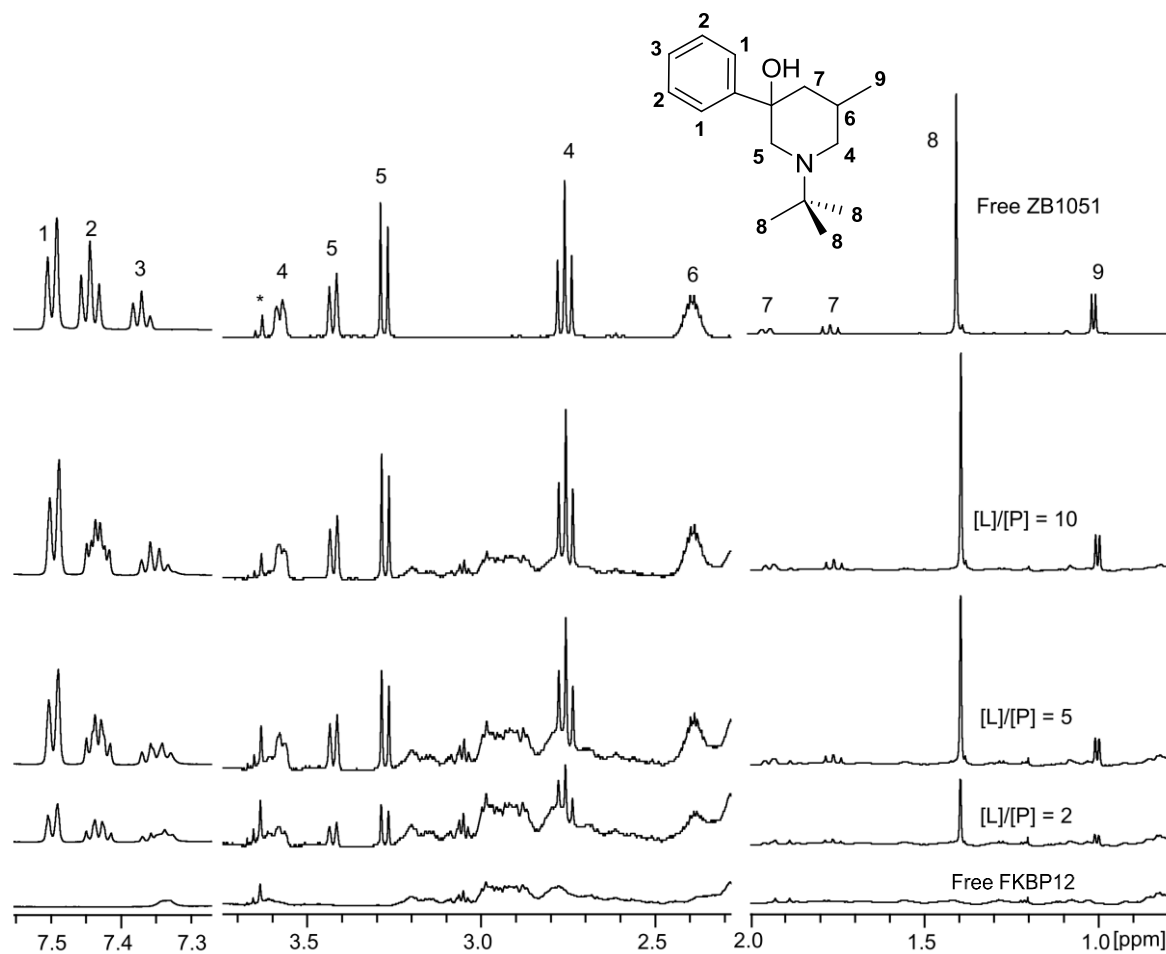
**Figure A2:** 1D-<sup>1</sup>H NMR spectra of ZB3 titrated into wt FKBP12.



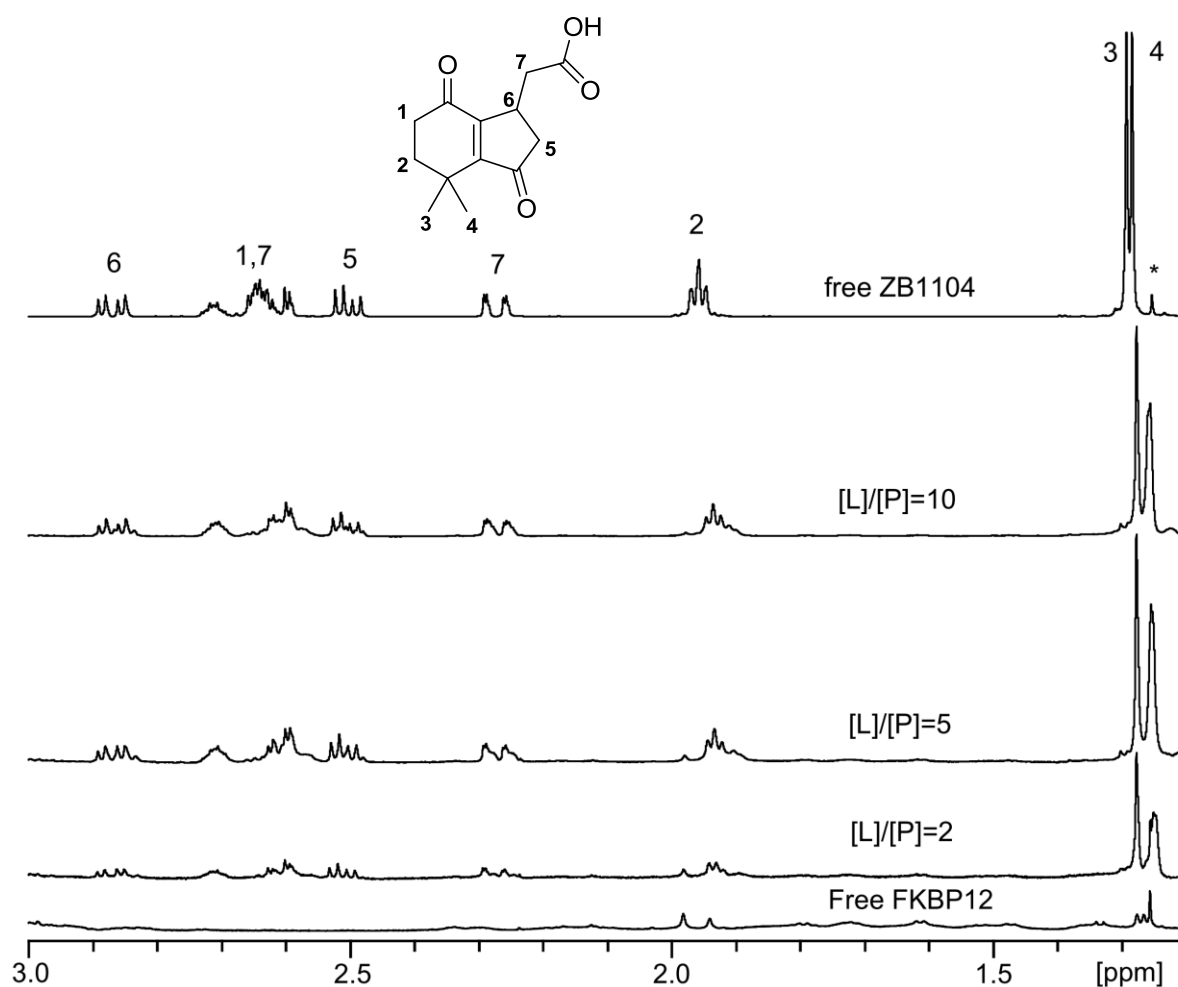
**Figure A3:** 1D-<sup>1</sup>H NMR spectra of ZB390 titrated into wt FKBP12.



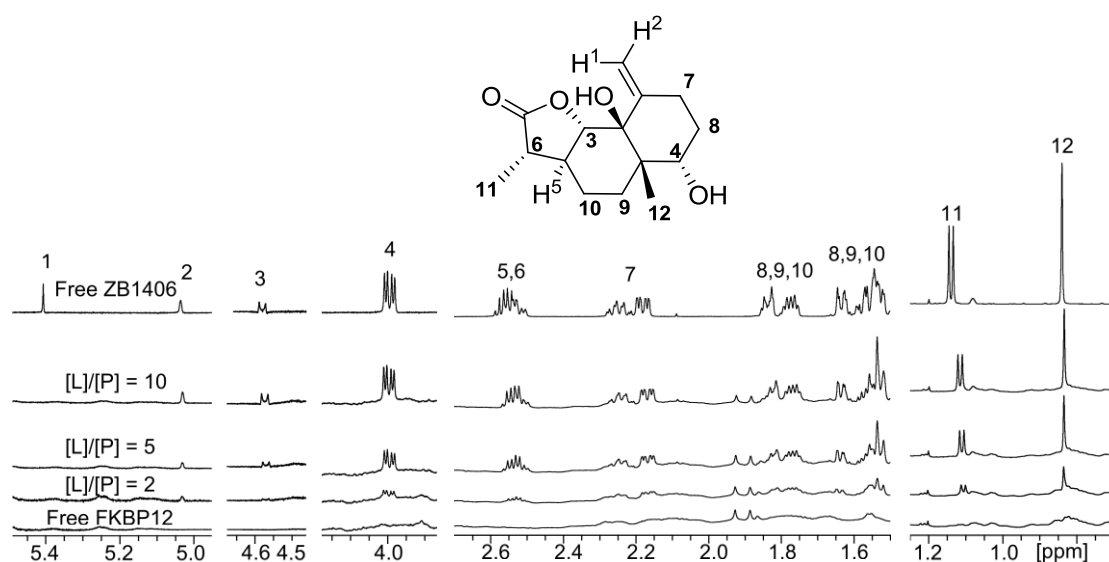
**Figure A4:**  $1D-^1H$  NMR spectra of ZB429 titrated into wt FKBP12.



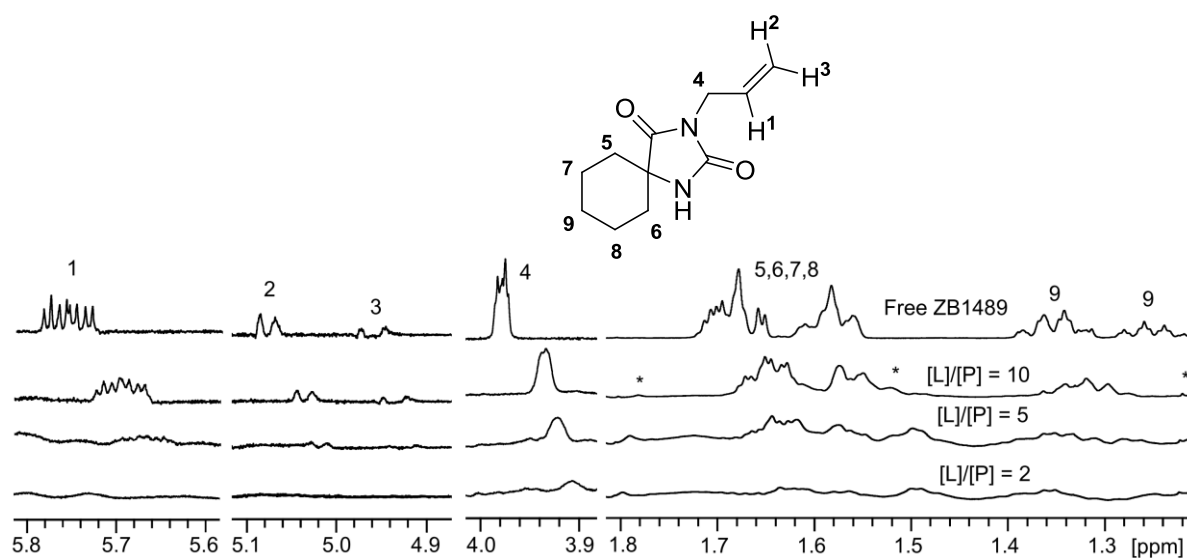
**Figure A5:**  $1D-^1H$  NMR spectra of ZB1051 titrated into wt FKBP12.



**Figure A6:** 1D- $^1\text{H}$  NMR spectra of ZB1104 titrated into wt FKBP12. The proton assignments were based on prediction.



**Figure A7:** 1D- $^1\text{H}$  NMR spectra of ZB1406 titrated into wt FKBP12. The proton assignments were based on prediction.



**Figure A8:** 1D- $^1\text{H}$  NMR spectra of ZB1489 titrated into wt FKBP12. This compound showed complicated scalar coupling due to the aliphatic ring. The proton assignments were based on prediction.

## Appendix B: XPLOR-NIH script for NOE- and PCS-based structure calculations

```

display $DATE $TIME
! dock n ligand copies in FKBP12 using NOE restraints (pdb:1FKR14)
! metal positions and tensor directions placed on PDB of protein
! mutant 2 = 34-35 Cys
! mutant 3 = 44-47 Cys
! mutant 5 = 61-65 Cys, 22 Ala

!-----DEFINE USER VARIABLES-----
eval ($fbound2 = 0.015)      ! fraction ligand bound to mutant 2
eval ($fbound3 = 0.009)      ! fraction ligand bound to mutant 3
eval ($fbound5 = 0.019)      ! fraction ligand bound to mutant 5
eval ($field = 600.13)       ! magnetic field in MHz
eval ($MUT2="Y")            ! data for mutant 2 Y/N
eval ($MUT3="Y")            ! data for mutant 3 Y/N
eval ($MUT5="Y")            ! data for mutant 5 Y/N
eval ($metal2="Yb")          ! Ln in mutant 2: Yb or Tm
eval ($metal3="Yb")          ! Ln in mutant 3: Yb or Tm
eval ($metal5="Yb")          ! Ln in mutant 5: Yb or Tm
eval ($chaxYbm2=8.7e-32)     ! chi axial in m^3 CLaNP-5 Yb mutant 2
eval ($chrhoYbm2=3.4e-32)    ! chi rhombic in m^3 CLaNP-5 Yb mutant 2
eval ($chaxYbm3=8.9e-32)     ! chi axial in m^3 CLaNP-5 Yb mutant 3
eval ($chrhoYbm3=3.3e-32)    ! chi rhombic in m^3 CLaNP-5 Yb mutant 3
eval ($chaxYbm5=7.8e-32)     ! chi axial in m^3 CLaNP-5 Yb mutant 5
eval ($chrhoYbm5=2.7e-32)    ! chi rhombic in m^3 CLaNP-5 Yb mutant 5
eval ($fres= 2)              ! first residue to be used
eval ($lres= 107)            ! last residue to be used
eval ($PRF= (1e30*1e6)/(12*3.14159)) ! factor in PCS equation
eval ($f01=6.0)              ! force constant xpcs
eval ($f02=6.0)              ! force constant xpcs
eval ($f03=6.0)              ! force constant xpcs
eval ($stdev1=0.04)          ! stdev for PCS
eval ($stdev2=0.04)          ! stdev for PCS
eval ($stdev3=0.04)          ! stdev for PCS
eval ($a20=50)               ! random distance max + 0 A
eval ($a20a=360)             ! random rotation max +/-0.5*
eval ($a05= 40000)           ! nr cycle
eval ($a53= 300)             ! nr of steps
eval ($a59= 10)              ! .pdb writing threshold
eval ($a55= 300.0)           ! TBATH (temperatuur)
eval ($rot_5ring="Y")        ! rotate ligand oxazole ring
eval ($intac="N")            ! interactions to BB (Y) or BB + side chain (N)
eval ($rigid="N")            ! dynamics mode = rigid (Y) or rigid+langevin (N)
eval ($pcs_r="Y")            ! docking with ligand PCS restraints
eval ($noe_r="N")            ! docking with NOE restraints
eval ($vdw_r="Y")            ! switch on/off vdw
eval ($flex_noe="Y")         ! also docking with NOE from flexible loop
eval ($lig_noe="Y")          ! include ligand intramolecular (transferred) NOE
eval ($attraction_noe = "Y") ! switch on/off attraction noe
eval ($pcs_calc="Y")         ! back-calc PCS at end
eval ($noe_calc="Y")         ! back-calc NOE distance at end
eval ($opttens="N")          ! optimize tensor orientations iteratively
eval ($kick=100)             ! # cycles per approach !100
eval ($vdwmax=0.2)           ! starting rcon value, scaling of vdW energy
eval ($lignr = 1)            ! number of ligand copies to be used
eval ($genscale = 0.1)       ! general scale for noe
eval ($noescale = 0.5)       ! scale real noes
eval ($g01 = 20)             ! nr of cycles to get to max vdw in rigid docking
(should be 2<$g01< $kick) !20
!-----
!-----OTHER VARIABLES; DO NOT CHANGE-----
!-----
eval ($vleckaxYbm2=$chaxYbm2/3.77E-35) !chi axial in van Vleck units
eval ($vleckrhYbm2=$chrhoYbm2/3.77E-35) !chi rhombic in van Vleck units
eval ($vleckaxYbm3=$chaxYbm3/3.77E-35) !chi axial in van Vleck units
eval ($vleckrhYbm3=$chrhoYbm3/3.77E-35) !chi rhombic in van Vleck units
eval ($vleckaxYbm5=$chaxYbm5/3.77E-35) !chi axial in van Vleck units
eval ($vleckrhYbm5=$chrhoYbm5/3.77E-35) !chi rhombic in van Vleck units
eval ($a01=1)                 !cycle counter
eval ($a02=$kick)             !counts cycles after last kick
eval ($a02a=1) !counts approach step in which min ener was reached
eval ($a02b=1)                !counts cycle number in current approach
eval ($c01=0)                 !counts number of saved structures

```



```

eval ($a58=30.0)          !fbeta, frictinal constant 30.0
eval ($a56=1.5)          !velocity factor! 1.5
eval ($a48=$cpu*1e4)      !($cpu*1e4)
set seed=$a48 end        !generate random number
eval ($a54 = 0.01)        !timestep in ps
eval ($f1 = 0)           ! van der waals scaling factor
eval ($kk2 = $kick/2)
!-----
!----- READ STRUCTURE -----
!-----
set echo=off end
set mess=off end
structure @1FKR14.opt_lig.h.psf end
param @1FKR14.opt_lig.h.par end
coord @1FKR14.opt_lig.h.pdb
set echo=on end
set mess=on end

parameter
  nbonds
    inhi=0.75
    ctofnb=5.0
    ctonnb=4.0
    repel=0.8
    rexp=2
    irex=2
    rcon=$f1
  end
end

parameters
  BOND (segid "drg") (segid "drg") 400. TOKEN
  ANGLE (segid "drg") (segid "drg") (segid "drg") 60. TOKEN
  IMPR (segid "drg") (segid "drg") (segid "drg") (segid "drg") 50. TOKEN TOKEN
end

para
  nonb (name OO) 0.12 9.00 0.12 9.00
  nonb (name X) 0.0498 2.2272 0.0498 2.2272
  nonb (name Y) 0.0498 2.2272 0.0498 2.2272
  nonb (name Z) 0.0498 2.2272 0.0498 2.2272
  IMPR OO X Z Y 500.0 0 -54.7356
end
!-----rotate ligand oxazole ring-----
if ($rot 5ring="Y") then
  vector show (x) (resn DRG and name CAC)
  eval ($ix=$RESULT)
  vector show (y) (resn DRG and name CAC)
  eval ($iy=$RESULT)
  vector show (z) (resn DRG and name CAC)
  eval ($iz=$RESULT)
  vector show (x) (resn DRG and name CAD)
  eval ($jx=$RESULT)
  vector show (y) (resn DRG and name CAD)
  eval ($jy=$RESULT)
  vector show (z) (resn DRG and name CAD)
  eval ($jz=$RESULT)

  eval ($kx=$jx-$ix)
  eval ($ky=$jy-$iy)
  eval ($kz=$jz-$iz)

  coord rota SELE=(resn DRG and (name CAD or name OAE or name CAF or name HAG
    or name HAF or name CAK or name NAJ or name CAO or name CAL or name
    HAL or name HAM or name HAN or name HAR or name HAS or name HAQ))
    CENT=($jx $jy $jz) axis=($kx $ky $kz) -90.0 end
end if
!-----
!----- DEFINE RESTRAINTS -----
!-----
!-----DEFINE PCS-----
! ---- mutant 2, pcs in Hz, as observed ---
vector do (store1=-2.5) (segid="drg" and name CAL)
vector do (store1=-3.5) (segid="drg" and name CAO)
vector do (store1=-2.5) (segid="drg" and name HAF)
vector do (store1=-2.5) (segid="drg" and name HAG)
vector do (store1=-3.1) (segid="drg" and name HAB)

```

```

vector do (store1=-6.9) (segid="drg" and name HAA)
vector do (store1=-9.4) (segid="drg" and name HAH)
vector do (store1=-5.7) (segid="drg" and name HAI)
! ---- mutant 3, pcs in Hz, as observed ---
vector do (store2=2.2) (segid="drg" and name CAL)
vector do (store2=2.2) (segid="drg" and name CAO)
vector do (store2=1.3) (segid="drg" and name HAF)
vector do (store2=1.3) (segid="drg" and name HAG)
vector do (store2=0.9) (segid="drg" and name HAB)
vector do (store2=0.4) (segid="drg" and name HAA)
vector do (store2=0.9) (segid="drg" and name HAH)
vector do (store2=2.3) (segid="drg" and name HAI)
! ---- mutant 5, pcs in Hz, as observed ---
vector do (store3=3.5) (segid="drg" and name CAL)
vector do (store3=2.9) (segid="drg" and name CAO)
vector do (store3=3.7) (segid="drg" and name HAF)
vector do (store3=3.7) (segid="drg" and name HAG)
vector do (store3=1.6) (segid="drg" and name HAB)
vector do (store3=1.5) (segid="drg" and name HAA)
vector do (store3=1.5) (segid="drg" and name HAH)
vector do (store3=1.3) (segid="drg" and name HAI)

if ($pcs_r="Y") then
!-----DEFINE CONDITIONS MUTANT 2-----
xpcs reset end
xpcs nres 500 end

if ($MUT2="Y") then
xpcs
class 2
force $f01
if ($metal2="Yb") then
coeff $vleckaxYbm2 $vleckrhYbm2
elseif ($metal2="Tm") then
coeff $vleckaxTm $vleckrhTm
end if
end

for $id in ID (store1)
loop E
vector show elem (store1) (ID $id)
eval ($obs=$RESULT/($fbound2*$field))
xpcs
assign (atom "tens" 1 00)
(atom "tens" 1 Z)
(atom "tens" 1 X)
(atom "tens" 1 Y)
(ID $id) $obs $stdev1
end
end loop E
end if
!-----DEFINE CONDITIONS MUTANT 3-----

if ($MUT3="Y") then
xpcs
class 3 !B
force $f02
if ($metal3="Yb") then
coeff $vleckaxYbm3 $vleckrhYbm3
elseif ($metal3="Tm") then
coeff $vleckaxTm $vleckrhTm
end if
end

for $id in ID (store2)
loop A
vector show elem (store2) (ID $id)
eval ($obs=$RESULT/($fbound3*$field))
xpcs
assign (atom "tens" 2 00)
(atom "tens" 2 Z)
(atom "tens" 2 X)
(atom "tens" 2 Y)
(ID $id) $obs $stdev2
end
end loop A
end if

```

```

!-----DEFINE CONDITIONS MUTANT 5-----

if ($MUT5="Y") then
  xpcs
  class 5 !B
  force $f03
  if ($metal5="Yb") then
    coeff $vleckaxYbm5 $vleckrhYbm5
  elseif ($metal5="Tm") then
    coeff $vleckaxTm $vleckrhTm
  end if
end

for $id in ID (store3)
loop G
  vector show elem (store3) (ID $id)
  eval ($obs=$RESULT/($frbound5*$field))
  xpcs
  assign      (atom "tens" 3 00)
              (atom "tens" 3 Z)
              (atom "tens" 3 X)
              (atom "tens" 3 Y)
              (ID $id) $obs $stdev3
  end
end loop G
end if
end if
!-----DEFINE REAL NOEs-----
set echo=off end
set mess=off end
if ($noe_r="Y") then
@noe.xpl
end if
set echo=on end
set mess=on end
!-----DEFINE ATTRACTION NOE-----
if ($attraction_noe = "Y") then
noe
  class      attraction
  scale      attraction 0.5      !0.3 0.1
  sqconstant  attraction $genscale
  sqexponent  attraction 2
end

eval ($l1 = 1)
while ($l1 LE $lignr) loop G1
  noe
    assign (segid = "drg" and resi $l1 and name CAD) (name C2 and resn CM2) 13.0
  13.0 5.0
    assign (segid = "drg" and resi $l1 and name HAT) (segid = "drg" and resi $l1
and name NAJ) 2.0 1.0 0.1 end
  eval ($l1 = $l1 +1)
end loop G1

end if

!-----
!-----DYNAMICS RUN & OUTPUT-----
!-----

vector show (x) (name C2)
eval ($xc2 = $RESULT)
vector show (y) (name C2)
eval ($yc2 = $RESULT)
vector show (z) (name C2)
eval ($zc2 = $RESULT)

while ($a01 LE $a05) loop calc
  eval ($a02 = $a02 + 1)
  if ($a02 > $kick) then ! -----put ligand randomly away-----
    coor init end ! always use the same starting position of ligands
    coor @01FKR14.opt_lig.h.pdb
    if ($rot 5ring="Y") then
      coor rota SELE=(resn DRG and (name CAD or name OAE or name CAF or name HAG
or name HAF or name CAK or name NAJ or name CAO or name CAL or name

```

```

    HAL or name HAM or name HAN or name HAR or name HAS or name HAQ))
    CENT=($jx $jy $jz) axis=($kx $ky $kz) -90.0 end
end if

eval ($l1 = 1)
while ($l1 LE $lignr) loop F2

    vector show ave (x) (segid="drg" and resi $l1)
    eval ($x1 = $RESULT)
    vector show ave (y) (segid="drg" and resi $l1)
    eval ($y1 = $RESULT)
    vector show ave (z) (segid="drg" and resi $l1)
    eval ($z1 = $RESULT)
    eval ($xr1 = $xc2 - $x1)
    eval ($yr1 = $yc2 - $y1) ! move ligands to centre of protein
    eval ($zr1 = $zc2 - $z1)
    coor trans SELE=(segid="drg" and resi $l1) VECT=($xr1 $yr1 $zr1) end

    eval ($ang1=(0.5-RANDOM())*$a20a)
    eval ($vec1=(0.5-RANDOM())*3)
    eval ($vec2=(0.5-RANDOM())*3)
    eval ($vec3=(0.5-RANDOM())*3) ! move and rotate ligands randomly away
    coor trans SELE=(segid="drg" and resi $l1) VECT=($vec1 $vec2 $vec3) DIST=50.0
end

    vector show ave (x) (segid="drg" and resi $l1)
    eval ($x1 = $RESULT)
    vector show ave (y) (segid="drg" and resi $l1)
    eval ($y1 = $RESULT)
    vector show ave (z) (segid="drg" and resi $l1)
    eval ($z1 = $RESULT)
    coor rota SELE=(segid="drg" and resi $l1)
        cent=($x1 $y1 $z1) axis ($vec3 $vec1 $vec2) $ang1 end
    eval ($l1 = $l1 + 1)
end loop F2

vector do (fbeta=$a58) (segid="drg")
vector do (vx=$a56) (segid="drg")
vector do (vy=$a56) (segid="drg")
vector do (vz=$a56) (segid="drg")

eval ($a02=1)
eval ($miner=999999)
!write coor FROM=MAIN SELE=(all) OUTPUT= temp2.pdb end
end if

! -----dynamics run-----
if ($intac="Y") then
    constraints interactions
        (segid="drg")
        (segid= "tens" or (segid "fkbp" and (name CA or name N or name C or name CB or
name HN or name O))) end
    else
        constraints interactions (segid="drg") (segid "tens" or segid "fkbp") end
    end if

    constraints FIX=(not segid "drg") end

if ($pcs_r="Y") then
    if ($vdw_r="Y") then
        if ($attraction_noe="Y") then
            flag exclude * include noe vdw xpcs end
        else
            flag exclude * include vdw xpcs end
        end if
    else
        flag exclude * include noe xpcs end
    end if
else
    if ($vdw_r="Y") then
        flag exclude * include noe vdw end
    else
        flag exclude * include noe end
    end if
end if

para nbond rcon = $f1 end end
if ($f1 < $vdwmax) then

```

```

    eval ($f1 = $f1 + $vdwmax/($g01-1)) !valid for next round
end if
display QQQQQ $f1

display cycle $a01
dynamics rigid
  dt=$a54
  group=(segid="drg" and resi 1)
  dynmode=TCOU !TCOU FREE LANG
  tbath=$a55
  nprint=50
  nstep=$a53
  NTRFRQ=0 !new for XPLOR vs 3.8
end

if ($rigid="N") then
  if ($a02b > $g01) then
    !-----local langevin dynamics-----
    constraints
      interactions ((segid "fkbp" and not (name Ca or name C or name O or name N or
name
  HN or name C2) and segid "drg" around 8.0) ) (segid "fkbp")
      interactions (segid "drg") (all)
    end

    constraints !fix protein Bb + sc outside 8A from ligand + tens; ligand free
    FIX (not ((segid "fkbp" and not (name Ca or name C or name O or name N or name
    HN or name C2) and segid "drg" around 8.0) or (segid="drg") ))
    end

    display cycle $a01

    if ($pcs r="Y") then
      if ($vdw_r="Y") then
        if ($attraction_noe="Y") then
          flag exclude * include noe vdw bond angle dihedral impro xpcs end
        else
          flag exclude * include vdw bond angle dihedral impro xpcs end
        end if
      else
        flag exclude * include noe bond angle dihedral impro xpcs end
      end if
    else
      if ($vdw_r="Y") then
        flag exclude * include noe vdw bond angle dihedral impro end
      else
        flag exclude * include noe bond angle dihedral impro end
      end if
    end if
    energy end

    vector do ( fbeta = 6.657235 ) ((segid "fkbp" and not (name Ca or name C or name
O or name N or name HN or name C2) and segid "drg" around 8.0) or segid "drg" )

    dynamics langevin
      timestep = 0.001
      nstep = 2000
      ilbfrq = 2000
      nprint = 200
      tbath = 300.
      iasvel = maxwell
      rbuf = 0.0
      origin = ( 0. 0. 0. )
    end

    minimize powell
      drop=1
      nprint=10
      nstep=50
    end

  end if
end if !-----local langevin dynamics finished-----

!-----CALCULATE ENERGIES AFTER EACH CYCLE-----
constraints

```

```

interactions (segid "drg") (segid "fkbp")
end
if ($pcs_r="Y") then!!MAKE SURE THIS MATCHES THE FLAG DURING THE RUN
  if ($vdw_r="Y") then
    if ($attraction_noe="Y") then
      flag exclude * include noe vdw xpcs end
    else
      flag exclude * include vdw xpcs end
    end if
  else
    flag exclude * include noe xpcs end
  end if
else
  if ($vdw_r="Y") then
    flag exclude * include noe vdw end !xpcs
  else
    flag exclude * include noe end !xpcs
  end if
end if
energy end

set disp=ener.dat end          !write energy values
if ($a01=1) then
  display file: ener.dat      $DATE $TIME
  if ($pcs_r="Y") then
    if ($vdw_r="Y") then
      if ($attraction_noe="Y") then
        display cycle   appr.step Etot Evdw Enoe Epcs
      else
        display cycle   appr.step Etot Evdw Epcs
      end if
    else
      display cycle   appr.step Etot Enoe Epcs
    end if
  else
    if ($vdw_r="Y") then
      display cycle   appr.step Etot Evdw Enoe
    else
      display cycle   appr.step Etot Enoe
    end if
  end if
end if

if ($pcs_r="Y") then
  if ($vdw_r="Y") then
    if ($attraction_noe="Y") then
      display $a01      $a02a$a02 $ENER$VDW $NOE $XPCS
    else
      display $a01      $a02a$a02 $ENER$VDW $XPCS
    end if
  else
    display $a01      $a02a$a02 $ENER$NOE $XPCS
  end if
else
  if ($vdw_r="Y") then
    display $a01      $a02a$a02 $ENER$VDW $NOE
  else
    display $a01      $a02a$a02 $ENER$NOE
  end if
end if
set disp=OUTPUT end

if ($miner>$ENER) then
if ($a02b > $g01) then
coor copy end
eval ($miner = $ENER)
eval ($mincyc = $a01)
eval ($minstep = $a02)
if ($vdw_r="Y") then
  eval ($minvdw = $VDW)
end if
if ($noe_r="Y") then
  eval ($minnoe = $NOE)
end if
if ($pcs_r="Y") then
  eval ($minpcs = $XPCS)
  if ($attraction_noe="Y") then

```

```

        eval ($minnoe = $NOE)
    end if
end if
end if
end if
!-----END OF APPROACH REACHED -----
if ($a02 = $kick) then
    if ($miner LE $a59) then
        !---write structure---
        eval ($c01 = $c01 + 1)
        eval ($a52="cycle_"+encode($c01)+".pdb")
    coor swap end
    write coord output=$a52 end
    !---write information about this cycle---
    set disp=coor.dat end!write details of step
    if ($c01=1) then
        display file: coor.dat    $DATE $TIME
        if ($pcs_r="Y") then
            if ($vdw_r="Y") then
                if ($attraction noe="Y") then
                    display file    cycleapproach    app. step    Etot    Evwd    Enoe    Epcs
                else
                    display file    cycleapproach    app. step    Etot    Evwd    Epcs
                end if
            else
                display file    cycleapproach    app. step    Etot    Enoe    Epcs
            end if
        else
            if ($vdw_r="Y") then
                display file cycleapproach    app. step    Etot    Evwd    Enoe
            else
                display file cycleapproach    app. step    Etot    Enoe
            end if
        end if
    end if
    if ($pcs_r="Y") then
        if ($vdw_r="Y") then
            if ($attraction noe="Y") then
                display $c01 $mincyc $a02a $minstep $miner $minvdw $minnoe $minpcs
            else
                display $c01 $mincyc $a02a $minstep $miner $minvdw $minpcs
            end if
        else
            display $c01 $mincyc $a02a $minstep $miner $minnoe $minpcs
        end if
    else
        if ($vdw_r="Y") then
            display $c01 $mincyc    $a02a $minstep    $miner    $minvdw    $minnoe
        else
            display $c01 $mincyc    $a02a $minstep    $miner    $minnoe
        end if
    end if
    set disp=OUTPUT end
    !---write file with PCS violations for this cycle---
    if ($pcs_calc="Y") then
        @pcs_calc.xpl
    end if
    !---write file with distances of atoms with NOEs---
    if ($noe_calc="Y") then
        @noe_calc.xpl
    end if
    set echo=on end
    set mess=on end
end if
eval ($a02a = $a02a + 1 )    !approach counter
eval ($f1 = 0)    !set vdw back to zero
eval ($a02b = 0)    !cycle per approach
end if

eval ($a02b = $a02b + 1)    !cycle per approach
eval ($a01 = $a01 + 1)    !cycle counter
end loop calc
display $DATE $TIME
!-----
!-----FINISH-----
!-----
Stop

```

## Appendix C: XPLOR-NIH script for PRE-based ensemble docking

```

! Energy minimization of TOAC pseudoatom position using PRE restraints
! Using Plastocyanin for docking TOAC on tetra-Lys peptide
! M. Ubbink 15.2.2013
! file: red_run.inp

set mess=off end
set echo=off end

!-----
!-----VARIABLES-----
!-----
eval ($idi="plas")          ! SEGID of plastocyanin
eval ($d1= 0.1)             ! lower bound CL3, real PRE
eval ($d2= 0.1)             ! upper bound CL3, real PRE
! eval ($d3= 10.0)          ! target distance CL1
eval ($d4= 7)               ! lower bound CL1, disappeared
eval ($d5= 0)               ! upper bound CL1, disappeared
eval ($d6= 15)              ! target distance CL2, unaffected
eval ($d7= 0)               ! lower bound CL2, unaffected
eval ($d8= 100.0)           ! upper bound CL2, unaffected
eval ($d9= 0.5)             ! general scaling
eval ($d10= 0.1)            ! scaling CL1 (disappeared) 1
eval ($d11= 0.1)            ! scaling CL2 (not affected) 0.1
eval ($d12= 0.1)            ! scaling CL3 (real PREs) 1
eval ($d13= 100)            ! nr steps Powell energy minimization
eval ($d15= 0.01)           ! scaling NOE to pull all the Toac to the protein
eval ($tau_c= 5.54E-9)! tau-c in s
eval ($fbeta=0.06)          !TC friction coefficient in psec-1
eval ($run_nr= 1)           !cycle counter, $a01 in fkbp script
eval ($kick=20)              ! # cycles per approach
eval ($total_cycles= 11000) ! nr runs EM (nr cycles), $a05 in fkbp script
eval ($a02=1)                !counts cycles after last kick
eval ($min_app=1)            !counts approach step in which min ener
                                ! was reached, $a02a in fkbp script
eval ($min_cycle=1)          !counts cycle number in current approach, $a01 in fkbp script
eval ($a20=40)                ! random distance max + 0 A
eval ($a20a=30)               ! random rotation max +/-0.5*
eval ($c01=0)                 !counts number of saved structures, $c01 in fkbp script
eval ($threshold= 4.00944*1.25) ! .pdb writing threshold, $a59 in fkbp script

eval ($frb=1)                 ! fraction in which the peptide is bound to PoPc
eval ($frl=1)                 ! fraction of spin labelled TOAC peptide
eval ($sens=6)                ! nr of TOAC molecules in ensemble
eval ($t3=1)                  ! first residue number of TOAC
eval ($t4=$t3+$sens-1)
eval ($a48=$cpu*1e4)          !($cpu*1e4)
set seed=$a48 end             !generate random number
eval ($Crd="Y")               ! Control loop Crd (Y/N)to kick TOAC after each approach
eval ($ener_check="Y")        ! switch on energy barrier (Y/N)
eval ($checkener=2.1*$threshold) ! energy barrier to prevent coordinate
explosion
eval ($attraction_noe="Y")    ! switch on attraction noe (Y/N)
eval ($fix_CL2="Y")           ! fix experimental distance for CL2 (unaffected)
!-----
!-----PARAMETERS & STRUCTURE -----
!-----

parameter
    @PoPc.cu.h.par
end

parameter
    NONBonded TC      0.1200  3.3      0.1200  3.3
end

structure
    @PoPc.cu.h.psf
end

coordinates
    @PoPc.cu.h.pdb

!-----define TOAC -----

```



```

topology
  residue TOAC
    atom TC type=C charge=0.000 mass 12.00 end
  end
end

segment
  molecule
    name=TOAC
    number=$ens
  end
end

para
  nbon
    repe = 0.65
    rcon = 60
  end
end

vector do (X=10.0) (resn TOAC and name TC)!
vector do (Y=10.0) (resn TOAC and name TC)!define initial TC coordinates
vector do (Z=10.0) (resn TOAC and name TC)!

!-----
!-----RESTRRAINTS -----
!-----

!DEFINE NMR DERIVED DISTANCES FOR TC-NH
@restraints_PoPc_KKKKX_Kd93uM.xpl

if ($attraction_noe="Y") then !
NOE
  class CL4
    averaging CL4 cent
    potential CL4 square
    sqconstant CL4 0.01
    sqoffset CL4 0.0
    scale CL4 $d15
    sqexponent CL4 2
    ceil=10.0 !20
  end

eval ($t1 = $t3)
while ($t1 LE $t4) loop D ! set random coordinates for TOAC atoms
  noe
    assign (name TC and resi $t1) (name C2 and resn CM2) 20.0 10.0 10.0
  end
eval ($t1 = $t1 + 1)
end loop D
end if ! if ($attraction_noe="Y") then

!-----
!-----RUN -----
!-----

set echo=off end
set mess=off end

constraints fix (not name TC) end
constraints interactions (name TC) (not name TC and not name C2) end
flag exclude * include noe vdw end

while ($run_nr LE $total_cycles) loop RUNS
  eval ($t1 = $t3)
  !coor orient sele=(segid "plas") end
  if ($Crd="Y") then
    if ($a02 = 1) then
      while ($t1 LE $t4) loop Crd ! set random coordinates for TOAC atoms
        !-----put TC at origin-----
        vector do (fbeta=0.025) (name TC and resi $t1)
        vector do (vx=0.1) (name TC and resi $t1)
        vector do (vy=0.1) (name TC and resi $t1)
        vector do (vz=0.1) (name TC and resi $t1)
        vector show ave (x) (name TC and resi $t1)
      end loop Crd
    end if
  end if
end loop RUNS

```

```

eval ($x2= $RESULT)
vector show ave (y) (name TC and resi $t1)
eval ($y2= $RESULT)
vector show ave (z) (name TC and resi $t1)
eval ($z2= $RESULT)
eval ($dist=sqrt($x2**2+$y2**2+$z2**2))
coor trans SELE=(name TC and resi $t1) VECT=(-$x2 -$y2 -$z2) DIST=$dist end

!----- move TOAC randomly away-----

eval ($vec1=0.5-RANDOM())
eval ($vec2=0.5-RANDOM())
eval ($vec3=0.5-RANDOM())
coor trans SELE=(name TC and resi $t1) VECT=($vec1 $vec2 $vec3) DIST=$a20 end
eval ($t1 = $t1 +1)
end loop Crd

minimize powell
drop=10
nprint=50
nstep=$d13
tolgradient=0.0001
end

end if ! if ($a02 = 1) then
end if ! if ($Crd="Y") then

dynamics internal
nstep = 500
depred = 1
etol = 1
maxenergy = 100
timestep = 0.002
friction = $fbeta
nsavc = 20
fix = (segid $idi)
group = (name TC and resi 1)
group = (name TC and resi 2)
group = (name TC and resi 3)
group = (name TC and resi 4)
group = (name TC and resi 5)
group = (name TC and resi 6)
tbath = 300
nprint = 50
NTRFRQ=0 !new for XPLOR vs 3.8
end

energy end

!-----write all energy info-----

set disp=ener.dat end !write energy values
if ($run_nr=1) then
display ener.dat $DATE $TIME
display cycle appr step Etot Enoe Evdw
end if
display $run_nr $min_app $a02 $ENER $NOE $VDW
set disp=OUTPUT end

!-----check energy-----
if ($ener_check="Y") then
if ( $a02 = 1 ) then
if ($ENER>$checkener) then
display -----ENERGY TOO HIGH, GO TO NEXT CYCLE -----
-----
eval ($a02=0)
eval ($min_app = $min_app + 1 ) !approach counter
end if ! if ($ENER>$checkener) then
end if !if ( $a02 = 1 ) then

if ( $a02 = 2 ) then
if ($ENER>$miner) then
display -----ENERGY TOO HIGH, GO TO NEXT CYCLE -----
-----
eval ($a02=0)
eval ($min_app = $min_app + 1 ) !approach counter
end if ! if ($ENER>$miner) then

```

```

        end if ! if ( $a02 = 2 ) then
        end if !if ($ener_check="Y") then
!-----save energy info for currently lowest energy structure-----

        if ($a02 = 1) then
            eval ($miner=999999)
        end if

        if ($miner>$ENER) then
            coor copy end
            eval ($miner = $ENER)
            eval ($mincyc = $run_nr)
            eval ($minstep = $a02)
            eval ($minvdw = $VDW)
            eval ($minnoe = $NOE)
        end if

!-----END OF APPROACH REACHED -----

!-----ANALYSIS-----
!-----ANALYSIS-----
!-----ANALYSIS-----

!-----write the coordinates for lowest energy structure-----

        if ($a02 = $kick) then

            if ($miner LE $threshold) then
                eval ($c01 = $c01 + 1)
                eval ($a52="cycle_"+encode($c01)+".pdb")
                coor swap end
                write coord sele=(name TC) OUTPUT=$a52 end

                !-----save the PRE info for lowest energy structure-----
                @pre_calc.xpl

                !-----write the energy info for lowest energy structure-----

                set disp=coor.dat end !write details of step
                if ($c01=1) then
                    display file: coor.dat    $DATE $TIME
                    display file    cycleapproach  app.step    Etot  Enoe  Evwd  violation
                end if
                display $c01 $mincyc    $min_app    $minstep    $miner    $minnoe    $minvdw
                $viol
                set disp=OUTPUT end

            end if ! if ($miner LE $threshold) then

                eval ($min_app = $min_app + 1 )    !approach counter
                eval ($min_cycle = 0) !cycle per approach
                eval ($a02 = 0)
            end if ! if ($a02 = $kick) then
            display -----END OF RUN $run_nr-----

!-----go to the next run-----
            eval ($run_nr = $run_nr +1) ! run counter
            eval ($a02 = $a02 + 1)
            eval ($min_cycle = $min_cycle + 1) !cycle per approach

        end loop RUNS
        display $DATE $TIME
        set echo=off end
        set mess=on end
!-----FINISH-----
!-----FINISH-----
!-----FINISH-----
Stop

```

**Appendix D: Backbone and side chain assignments of Zn-substituted PoPc**

| Residue | C $\alpha$ | C $\beta$ | H     | N     | Residue | C $\alpha$ | C $\beta$ | H     | N     |
|---------|------------|-----------|-------|-------|---------|------------|-----------|-------|-------|
| I1      | 59.73      | 40.89     | -     | -     | H37     | 53.92      | 36.59     | 7.852 | 110.2 |
| I1      | 59.76      | 38.60     | -     | -     | N38     | 52.66      | 39.93     | 9.196 | 120.3 |
| D2      | 52.86      | 42.05     | 7.793 | 126.2 | I39     | 61.92      | 40.27     | 6.082 | 112.6 |
| D2      | 53.10      | 41.58     | 8.310 | 111.2 | V40     | 61.15      | 34.73     | 8.605 | 126.4 |
| V3      | 60.62      | 34.86     | 8.810 | 124.1 | F41     | 57.89      | 39.58     | 8.529 | 123.0 |
| V3      | 60.64      | 35.08     | 8.773 | 124.0 | D42     | 53.75      | 42.31     | 8.385 | 121.3 |
| L4      | 54.68      | 42.58     | 8.963 | 126.0 | E43     | 59.06      | 29.61     | 8.748 | 125.7 |
| L5      | 53.18      | 41.42     | 8.626 | 119.4 | D44     | 55.37      | 41.47     | 8.314 | 117.6 |
| G6      | 44.37      | 0.00      | 7.816 | 116.2 | S45     | 56.75      | 64.33     | 8.125 | 116.5 |
| A7      | 50.76      | 20.87     | 7.995 | 125.1 | I46     | 58.83      | 39.45     | 7.060 | 114.9 |
| D8      | 57.51      | 39.98     | 8.935 | 119.9 | P47     | 59.77      | 31.97     | -     | -     |
| D9      | 53.42      | 39.59     | 7.627 | 115.3 | S48     | 59.76      | 63.32     | 8.235 | 115.5 |
| G10     | 44.71      | 0.00      | 8.189 | 108.7 | G49     | 45.11      | 0.00      | 8.729 | 112.8 |
| S11     | 60.35      | 63.09     | 8.046 | 115.8 | V50     | 62.26      | 32.42     | 7.372 | 121.1 |
| L12     | 53.16      | 39.11     | 8.682 | 125.9 | D51     | 52.25      | 41.03     | 8.251 | 126.7 |
| A13     | 50.06      | 22.39     | 7.351 | 124.6 | A52     | 54.95      | 18.47     | 9.348 | 130.0 |
| F14     | 58.35      | 40.45     | 9.077 | 121.2 | S53     | 61.47      | 63.13     | 8.617 | 114.2 |
| V15     | 59.31      | 34.20     | 8.823 | 121.5 | K54     | 56.76      | 32.96     | 7.314 | 118.8 |
| P16     | 63.63      | 35.03     | -     | -     | I55     | 61.15      | 38.30     | 6.984 | 109.6 |
| S17     | 59.21      | 64.62     | 8.160 | 107.7 | S56     | 58.17      | 67.13     | 6.649 | 110.2 |
| E18     | 55.26      | 32.27     | 7.500 | 119.9 | M57     | 56.75      | 33.54     | 8.213 | 122.5 |
| F19     | 56.00      | 39.07     | 7.632 | 119.0 | M57     | 56.53      | 33.12     | 8.247 | 122.6 |
| S20     | 56.74      | 66.30     | 8.592 | 115.3 | S58     | 58.50      | 63.50     | 8.548 | 115.8 |
| S20     | 56.67      | 66.27     | 8.626 | 115.3 | E59     | 59.72      | 29.48     | 8.597 | 122.9 |
| I21     | 59.62      | 42.71     | 8.716 | 116.5 | E60     | 56.38      | 29.80     | 8.047 | 113.4 |
| I21     | 59.70      | 43.03     | 8.716 | 116.5 | D61     | 54.05      | 42.14     | 7.601 | 122.5 |
| S22     | 56.78      | 63.29     | 8.228 | 116.8 | L62     | 53.52      | 46.62     | 7.723 | 118.3 |
| S22     | 56.99      | 63.17     | 8.406 | 117.4 | L63     | 54.07      | 40.34     | 8.882 | 119.7 |
| P23     | 64.51      | 31.46     | -     | -     | N64     | 54.79      | 42.10     | 8.467 | 124.7 |
| P23     | 64.39      | 31.48     | -     | -     | A65     | 50.11      | 21.55     | 8.349 | 121.2 |
| G24     | 44.97      | 0.00      | 9.216 | 113.3 | K66     | 58.67      | 32.49     | 8.538 | 125.0 |
| G24     | 44.95      | 0.00      | 9.095 | 113.9 | G67     | 45.13      | 0.00      | 8.806 | 113.6 |
| E25     | 56.99      | 31.06     | 7.655 | 121.7 | E68     | 58.18      | 32.33     | 7.183 | 119.9 |
| E25     | 56.60      | 30.65     | 7.801 | 123.1 | T69     | 59.38      | 73.68     | 8.373 | 110.6 |
| K26     | 56.16      | 33.67     | 8.040 | 122.3 | T69     | 59.48      | 73.63     | 8.382 | 110.8 |
| K26     | 56.19      | 33.98     | 7.981 | 120.4 | F70     | 57.87      | 43.78     | 8.661 | 120.7 |
| I27     | 60.34      | 39.64     | 8.867 | 125.6 | F70     | 57.88      | 43.72     | 8.632 | 120.8 |
| I27     | 60.65      | 39.11     | 8.858 | 125.0 | E71     | 54.00      | 32.74     | 7.789 | 126.2 |
| V28     | 61.64      | 31.39     | 8.468 | 126.3 | E71     | 54.01      | 32.75     | 7.826 | 126.2 |
| V28     | 61.63      | 31.14     | 8.437 | 126.8 | V72     | 60.21      | 35.52     | 8.568 | 120.1 |
| F29     | 57.18      | 39.29     | 9.042 | 126.5 | V72     | 60.24      | 35.50     | 8.509 | 119.9 |
| K30     | 54.83      | 35.19     | 9.173 | 122.8 | A73     | 50.39      | 20.61     | 8.235 | 128.9 |
| K30     | 54.88      | 35.11     | 9.138 | 122.6 | L74     | 53.46      | 43.46     | 7.595 | 120.3 |
| N31     | 54.46      | 38.45     | 9.229 | 126.8 | L74     | 53.35      | 43.48     | 7.556 | 120.3 |
| N32     | 56.50      | 42.50     | 9.016 | 129.2 | S75     | 59.70      | 64.97     | 8.685 | 115.2 |
| A33     | 52.28      | 22.05     | 8.647 | 119.8 | N76     | 53.95      | 37.54     | 7.901 | 120.0 |
| G34     | 47.51      | 0.00      | 7.750 | 116.5 | K77     | 58.09      | 33.51     | 8.726 | 124.5 |
| F35     | 52.80      | 36.98     | 5.720 | 114.5 | G78     | 43.70      | 0.00      | 8.683 | 108.2 |
| P36     | 62.47      | 36.82     | -     | -     | G78     | 43.69      | 0.00      | 8.745 | 108.2 |

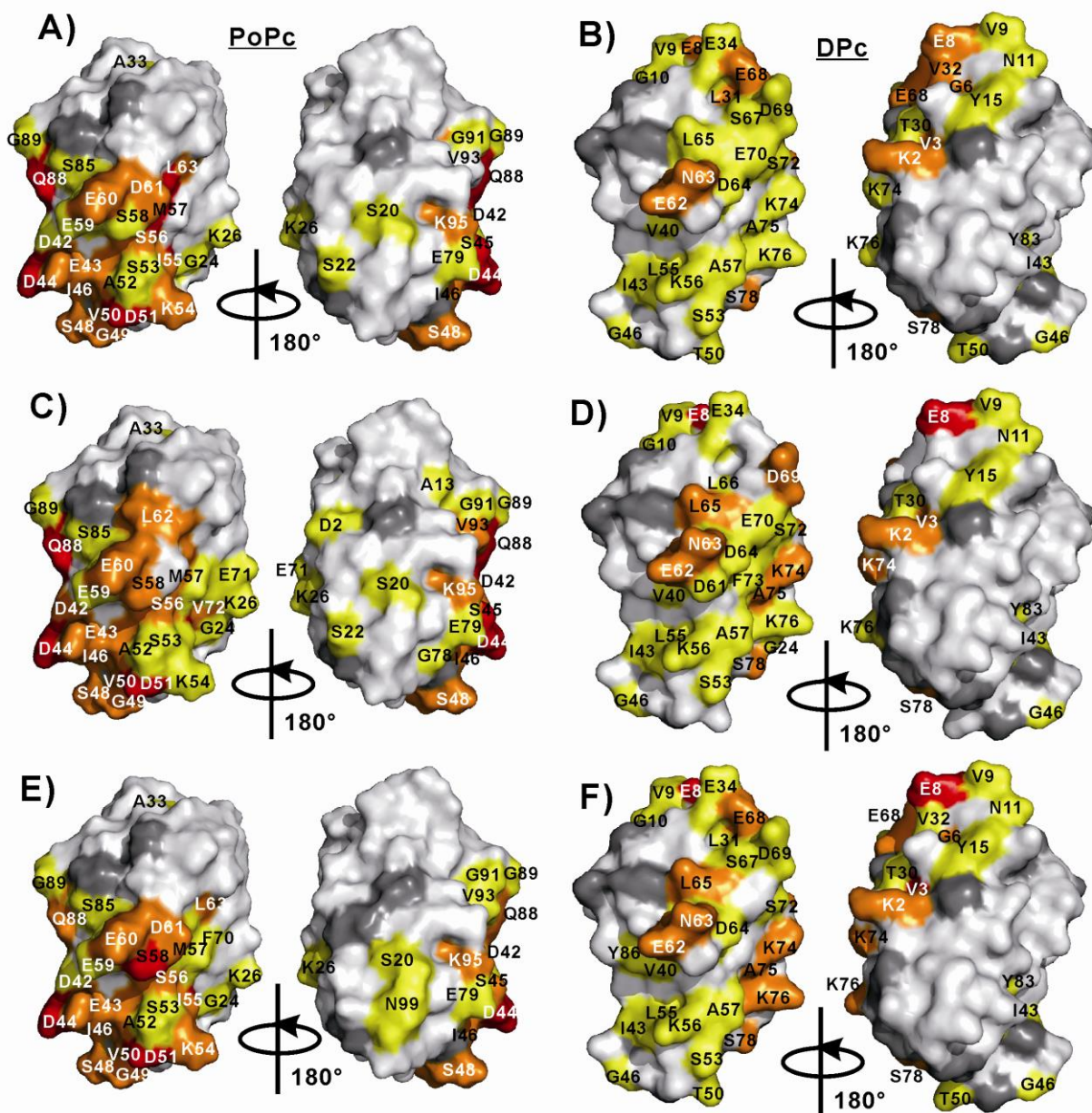
| Residue    | C $\alpha$ | C $\beta$ | H      | N     |
|------------|------------|-----------|--------|-------|
| <b>E79</b> | 56.04      | 31.99     | 8.224  | 119.2 |
| <b>Y80</b> | 56.54      | 39.77     | 9.513  | 122.6 |
| <b>S81</b> | 58.36      | 64.76     | 8.268  | 118.7 |
| <b>F82</b> | 55.10      | 41.15     | 7.978  | 119.8 |
| <b>Y83</b> | 56.12      | 41.21     | 9.183  | 115.4 |
| <b>C84</b> | 56.22      | 29.46     | 7.804  | 121.2 |
| <b>S85</b> | 64.80      | 61.34     | 10.230 | 126.5 |
| <b>P86</b> | 65.14      | 30.36     | -      | -     |
| <b>H87</b> | 54.11      | 34.88     | 7.189  | 112.5 |
| <b>Q88</b> | 60.88      | 28.55     | 7.982  | 115.1 |
| <b>G89</b> | 46.30      | 0.00      | 8.964  | 108.5 |
| <b>A90</b> | 51.44      | 19.11     | 7.296  | 121.3 |
| <b>G91</b> | 45.35      | 0.00      | 7.829  | 110.4 |
| <b>M92</b> | 56.89      | 30.37     | 7.758  | 121.5 |
| <b>V93</b> | 58.74      | 35.98     | 8.016  | 121.5 |
| <b>G94</b> | 45.22      | 0.00      | 8.258  | 112.2 |
| <b>K95</b> | 55.78      | 36.67     | 8.394  | 119.7 |
| <b>V96</b> | 56.90      | 33.50     | 9.022  | 122.2 |
| <b>T97</b> | 61.77      | 70.28     | 8.244  | 124.4 |
| <b>V98</b> | 60.94      | 31.57     | 9.236  | 128.7 |
| <b>N99</b> | 55.42      | 42.04     | 8.648  | 132.1 |

| Residue    | H(sc1) | H(sc2) | N(sc) |
|------------|--------|--------|-------|
| <b>N38</b> | 7.043  | 6.242  | 107.2 |
| <b>N64</b> | 7.631  | 6.865  | 113.5 |
| <b>Q88</b> | 6.731  | 6.189  | 110.8 |
| <b>N99</b> | 7.418  | 6.683  | 112.2 |

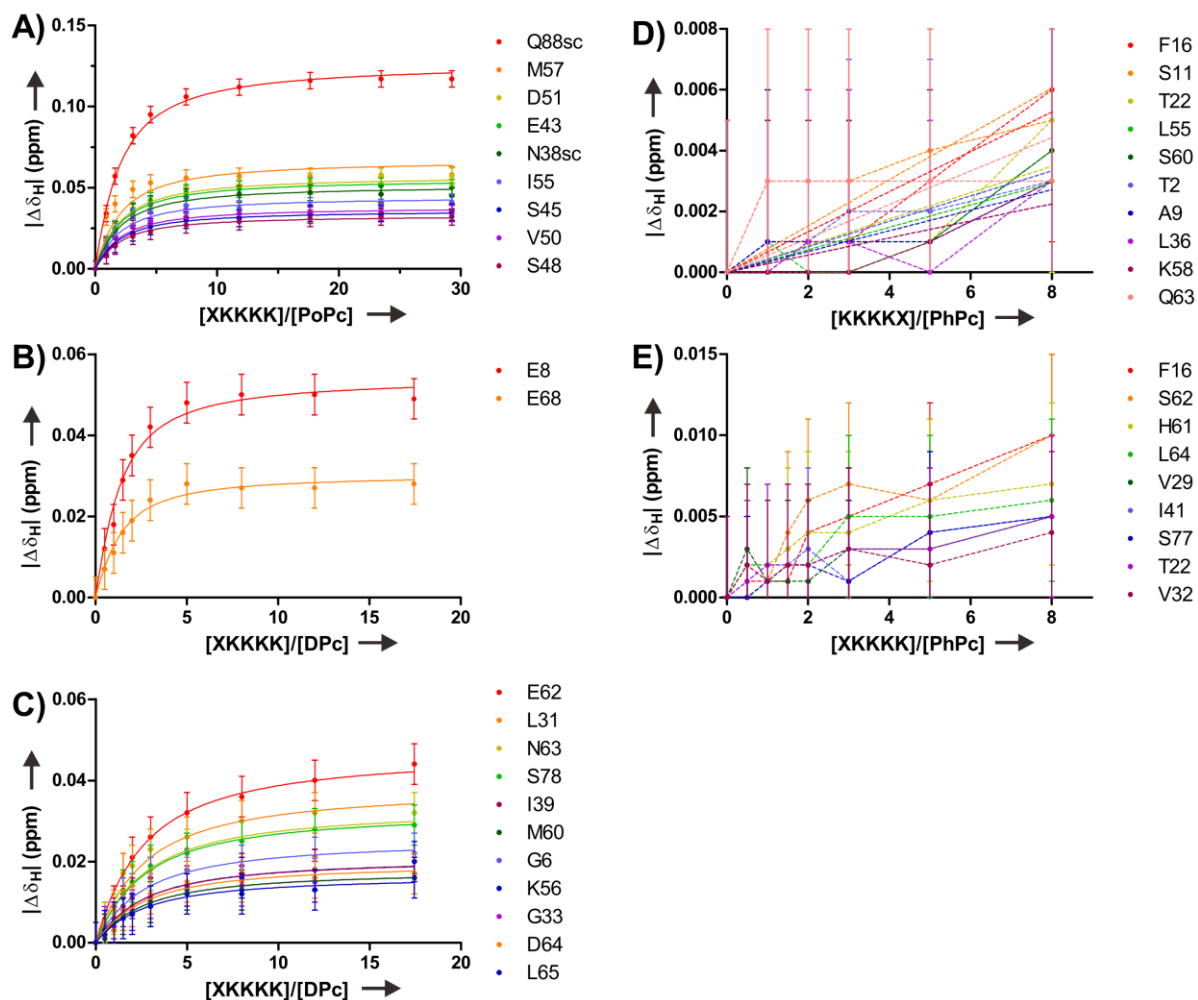
## Appendix E: Backbone assignments of Zn-substituted DPc

| Residue | C $\alpha$ | C $\beta$ | H     | N     | Residue | C $\alpha$ | C $\beta$ | H     | N     |
|---------|------------|-----------|-------|-------|---------|------------|-----------|-------|-------|
| A1      | 51.87      | 20.70     | -     | -     | A52     | 56.14      | 18.11     | 8.234 | 122.0 |
| K2      | 55.70      | 35.28     | 8.579 | 123.2 | S53     | 61.86      | 63.17     | 8.185 | 111.9 |
| V3      | 61.23      | 35.98     | 8.568 | 125.4 | E54     | 59.61      | 30.32     | 7.695 | 124.9 |
| E4      | 55.79      | 30.83     | 9.265 | 126.7 | L55     | 57.47      | 41.49     | 8.406 | 119.8 |
| V5      | 61.75      | 31.83     | 9.060 | 125.4 | K56     | 59.66      | 32.20     | 8.160 | 122.0 |
| G6      | 43.85      | -         | 8.015 | 112.6 | A57     | 54.16      | 17.97     | 7.772 | 120.9 |
| D7      | 52.58      | 43.17     | 6.938 | 109.9 | A58     | 51.60      | 19.40     | 7.223 | 121.2 |
| E8      | 59.14      | 29.44     | 8.915 | 116.9 | S59     | 58.12      | 66.67     | 7.159 | 108.3 |
| V9      | 62.03      | 31.73     | 7.415 | 113.5 | M60     | 56.24      | 34.29     | 8.489 | 123.1 |
| G10     | 45.51      | -         | 7.841 | 108.9 | D61     | 55.08      | 42.98     | 8.567 | 120.2 |
| N11     | 54.45      | 39.01     | 9.108 | 119.1 | E62     | 59.42      | 29.74     | 8.460 | 123.5 |
| F12     | 51.90      | 34.75     | 8.662 | 125.6 | N63     | 53.28      | 39.44     | 8.655 | 115.4 |
| K13     | 54.33      | 38.05     | 6.459 | 117.4 | D64     | 53.26      | 43.33     | 7.593 | 121.7 |
| F14     | 57.37      | 41.32     | 8.332 | 119.6 | L65     | 54.30      | 47.17     | 8.001 | 117.4 |
| Y15     | 54.80      | 41.22     | 9.328 | 118.4 | L66     | 55.37      | 44.15     | 8.308 | 121.3 |
| P16     | 63.69      | 35.15     | -     | -     | S67     | 56.81      | 67.73     | 8.003 | 115.1 |
| D17     | 53.10      | 40.29     | 7.859 | 111.0 | E68     | 59.52      | 29.62     | 8.688 | 118.2 |
| S18     | 56.93      | 64.35     | 7.184 | 114.5 | D69     | 55.86      | 41.60     | 7.757 | 115.1 |
| I19     | 59.99      | 42.59     | 8.649 | 125.1 | E70     | 53.79      | 30.18     | 7.251 | 120.2 |
| T20     | 61.43      | 70.56     | 8.469 | 124.6 | P71     | 65.22      | 33.45     | -     | -     |
| V21     | 58.40      | 35.73     | 9.124 | 119.6 | S72     | 56.90      | 66.60     | 7.904 | 114.3 |
| S22     | 58.03      | 64.49     | 8.138 | 116.4 | F73     | 57.84      | 44.44     | 9.029 | 125.5 |
| A23     | 54.41      | 18.25     | 8.672 | 125.7 | K74     | 54.81      | 34.76     | 7.569 | 127.5 |
| G24     | 45.16      | -         | 8.738 | 113.1 | A75     | 50.40      | 23.60     | 8.753 | 125.6 |
| E25     | 55.98      | 30.80     | 8.118 | 123.4 | K76     | 55.85      | 34.50     | 8.355 | 122.0 |
| A26     | 51.72      | 19.64     | 8.497 | 127.6 | V77     | 62.23      | 33.58     | 8.862 | 128.0 |
| V27     | 61.44      | 32.63     | 8.924 | 122.4 | S78     | 59.58      | 64.54     | 9.329 | 123.0 |
| E28     | 54.95      | 30.76     | 8.439 | 126.7 | T79     | 61.79      | 68.79     | 8.145 | 123.5 |
| F29     | 57.07      | 40.30     | 9.443 | 127.5 | P80     | 63.84      | 32.46     | -     | -     |
| T30     | 60.83      | 71.85     | 8.771 | 118.2 | G81     | 44.78      | -         | 8.537 | 110.4 |
| L31     | 56.23      | 43.71     | 9.455 | 130.6 | T82     | 62.25      | 71.00     | 7.628 | 115.2 |
| V32     | 62.51      | 33.33     | 8.743 | 131.5 | Y83     | 56.60      | 42.36     | 9.569 | 128.0 |
| G33     | 43.85      | -         | 8.279 | 112.5 | T84     | 61.67      | 71.15     | 9.610 | 119.2 |
| E34     | 57.38      | 31.12     | 8.006 | 114.3 | F85     | 54.32      | 40.36     | 8.089 | 121.4 |
| T35     | 63.05      | 69.04     | 7.865 | 117.7 | Y86     | 56.42      | 41.47     | 8.616 | 115.1 |
| G36     | 46.38      | -         | 8.556 | 112.7 | C87     | 57.18      | 29.85     | 7.105 | 120.1 |
| H37     | 55.24      | 36.53     | 6.585 | 116.3 | T88     | 69.99      | 67.19     | 9.995 | 126.6 |
| N38     | 51.16      | 40.36     | 9.503 | 123.8 | P89     | 65.03      | 31.09     | -     | -     |
| I39     | 61.51      | 40.08     | 5.990 | 113.0 | H90     | 57.96      | 32.67     | 7.459 | 114.7 |
| V40     | 61.34      | 35.83     | 8.706 | 125.3 | K91     | 61.58      | 29.21     | 7.903 | 128.0 |
| F41     | 58.75      | 40.72     | 8.264 | 124.0 | S92     | 60.28      | 62.36     | -     | -     |
| D42     | 54.35      | 42.60     | 8.822 | 122.9 | A93     | 51.33      | 18.54     | 7.202 | 123.7 |
| I43     | 58.10      | 37.75     | 8.469 | 125.2 | N94     | 54.62      | 36.96     | 8.195 | 114.0 |
| P44     | 62.77      | 32.41     | -     | -     | M95     | 56.66      | 31.08     | 7.492 | 119.7 |
| A45     | 53.68      | 18.05     | 8.606 | 126.7 | K96     | 54.22      | 37.51     | 7.369 | 125.5 |
| G46     | 45.31      | -         | 8.774 | 110.3 | G97     | 44.21      | -         | 8.212 | 112.7 |
| A47     | 50.79      | 17.41     | 7.482 | 123.5 | T98     | 61.09      | 71.56     | 8.074 | 112.9 |
| P48     | 62.40      | 32.67     | -     | -     | L99     | 53.01      | 46.81     | 9.640 | 128.8 |
| G49     | 47.82      | -         | 8.887 | 110.9 | T100    | 62.59      | 69.82     | 9.384 | 126.3 |
| T50     | 64.51      | 67.95     | 8.040 | 112.2 | V101    | 60.99      | 33.30     | 9.301 | 128.9 |
| V51     | 65.49      | 32.34     | 6.966 | 124.0 | K102    | 57.42      | 34.75     | 8.780 | 133.5 |

## Appendix F: Supplementary figures for Chapter 5

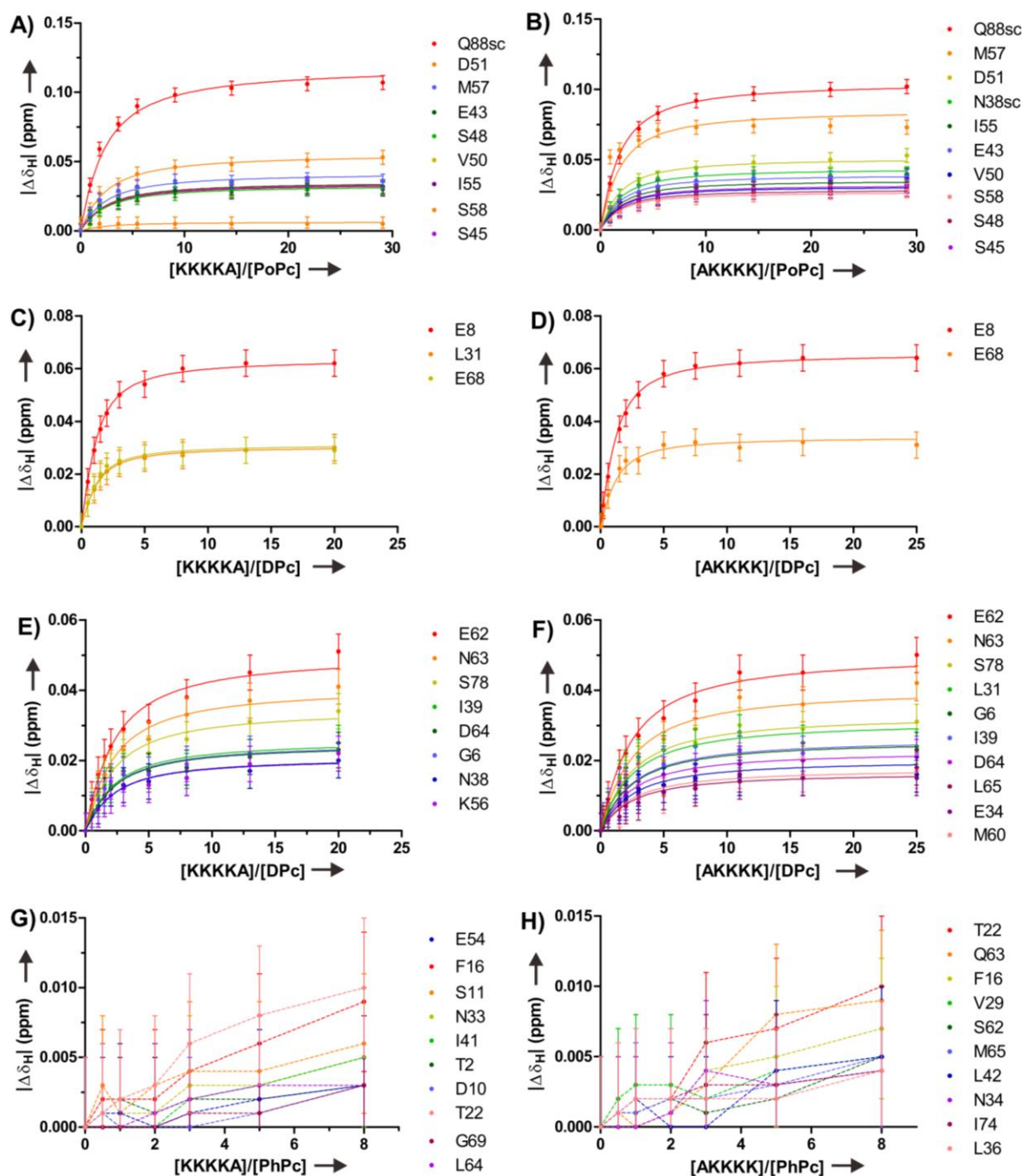


**Figure F1:** CSPs (extrapolated to 100% bound) mapped onto the protein surfaces from the binding of KKKKX (panels A and B), KKKKA (panels C and D) and AKKKK (panels E and F) to PoPc (left, PDB entry 1TKW<sup>47</sup>) and DPc (right, PDB entry 1KDI<sup>43</sup>). Color representations: red,  $\Delta\delta_{ave} \geq 0.04$  ppm; orange,  $0.04 > \Delta\delta_{ave} \geq 0.02$  ppm; yellow,  $0.02 > \Delta\delta_{ave} \geq 0.01$  ppm; white,  $\Delta\delta_{ave} < 0.01$  ppm; grey, no data or overlapping resonances.

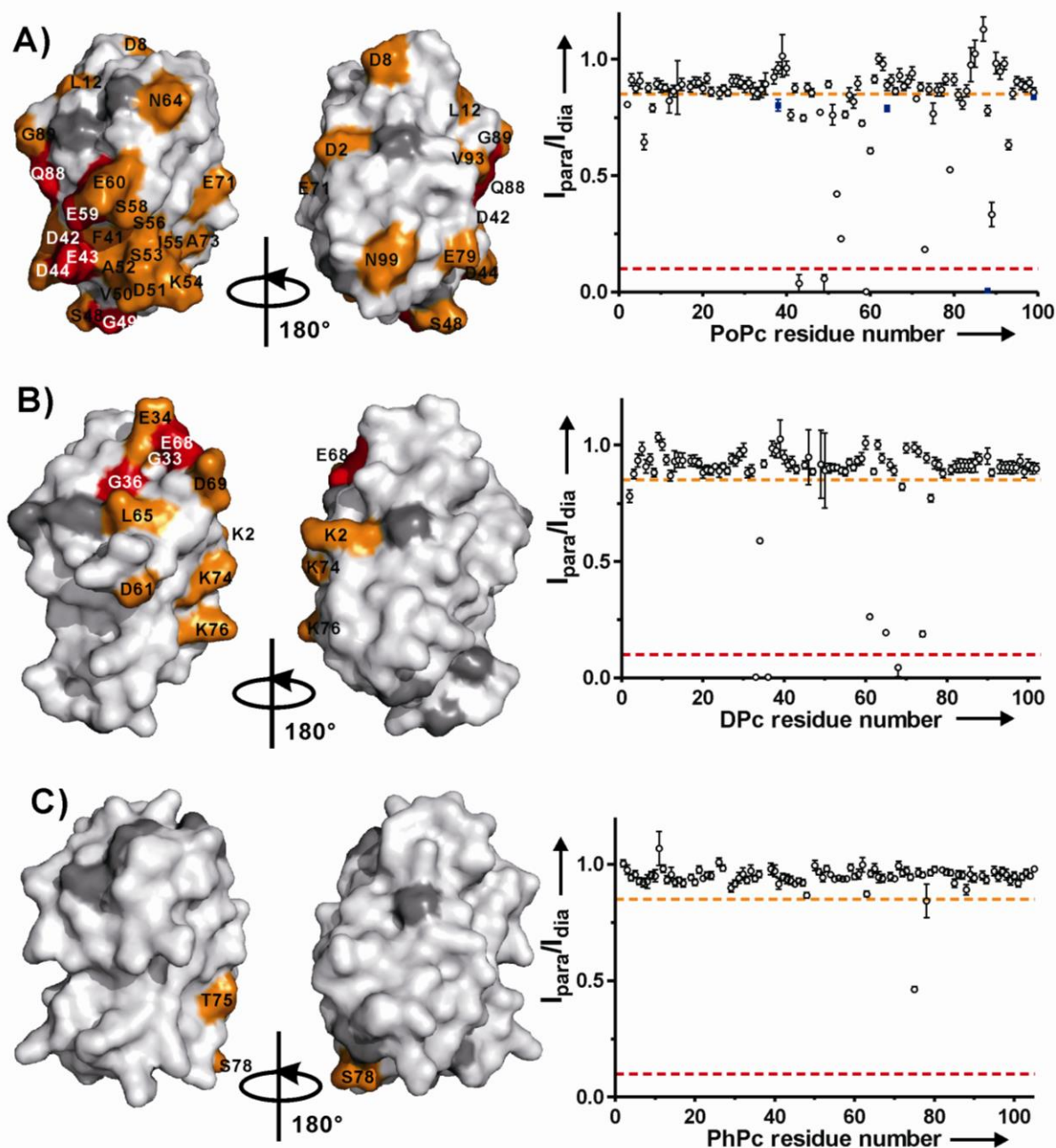


**Figure F2:** Chemical shift changes of Pcs resonances as a function of increasing  $[peptide]/[Pc]$  for peptides XKKKK and KKKKX. The dissociation constants of the corresponding peptides (Table 5.1) were obtained by simultaneous fitting to a 1:1 binding model for PoPc (solid lines) and by simulation for 2-site binding for DPc. (A) XKKKK with PoPc; (B) XKKKK with DPc, strong-binding residues; (C) XKKKK with DPc, weak-binding residues; (D) KKKKX with PhPc; (E) XKKKK with PhPc. The titration points for each residue in (D) and (E) are connected with dashed lines. Error bars represent  $\pm 0.005$  ppm.

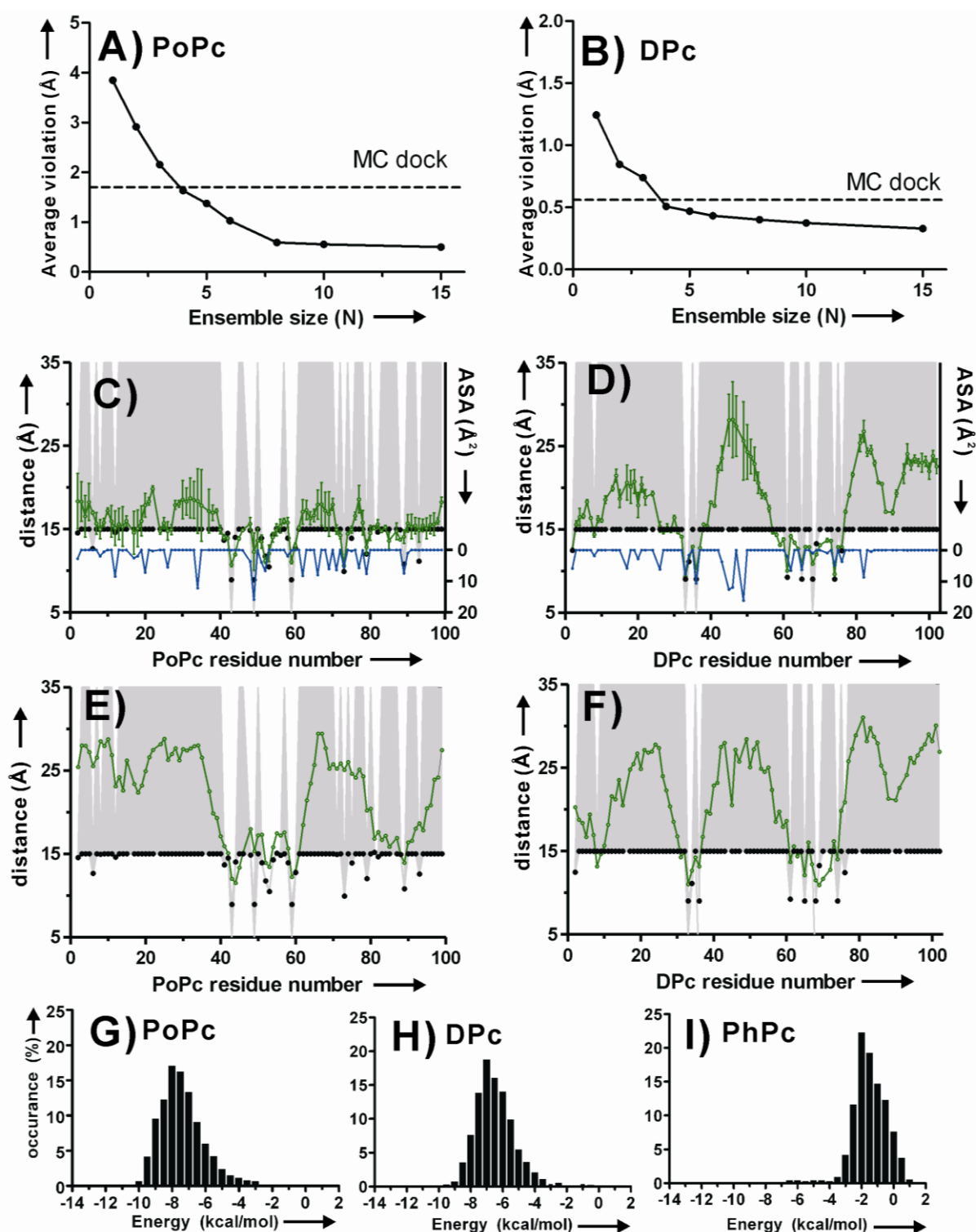




**Figure F3:** Chemical shift changes of Pcs resonances as a function of increasing  $[\text{peptide}]/[\text{Pc}]$  for peptides KKKKA and AKKKK. The residues which showed largest perturbations are shown. The dissociation constants of the corresponding peptides (Table 5.1) were obtained by simultaneous fitting to a 1:1 binding model for PoPc (solid lines) and by simulation for 2-site binding for DPc. (A) KKKKA with PoPc; (B) AKKKK with PoPc; (C) KKKKA with DPc, strong-binding residues; (D) AKKKK with DPc, strong-binding residues; (E) KKKKA with DPc, weak-binding residues; (F) AKKKK with DPc, weak-binding residues; (G) KKKKA with PhPc; (H) AKKKK with PhPc. The titration points for each residue in (G) and (H) are connected with dashed lines. Error bars represent  $\pm 0.005$  ppm.



**Figure F4:** PRE effects in Pc-XK KKK complexes. Left: PRE maps of PoPc (A, PDB entry 1TKW<sup>47</sup>), DPc (B, PDB entry 1KDI<sup>43</sup>) and PhPc (C, PDB entry 2Q5B) bound to XK KKKK peptide, color-coded on surface models of Pc: red,  $I_{\text{para}}/I_{\text{dia}} < 0.1$ ; orange,  $0.1 \leq I_{\text{para}}/I_{\text{dia}} < 0.85$ ; white,  $I_{\text{para}}/I_{\text{dia}} \geq 0.85$ ; grey, prolines, unassigned, and overlapping resonances. Right: relative  $[^1\text{H}, ^{15}\text{N}]$ -HSQC intensities of amides PoPc (A), DPc(B) and PhPc(C) in the complex with TOAC-containing peptides. For PoPc, the side chains are also included (blue squares). The dashed horizontal lines indicate  $I_{\text{para}}/I_{\text{dia}} = 0.85$  (orange lines) and 0.1 (red lines). The error bars denote  $2 \times$  standard deviations, derived from spectral noise levels using standard error propagation procedures.



**Figure F5:** (A-B) Averaged distance violations against number of XKKKK peptides ( $N=1-6,8,10,15$ ) in the ensemble docking for PoPc (A) and DPc (B). (C-D) Correlation of experimental distances (black dots) and back-calculated average distances (green circles with connecting lines) from the ensemble docking ( $N=6$ ) of XKKKK bound to PoPc (C) and DPc (D). The average distances from the 20 lowest-energy solutions of the PRE driven ensemble docking are shown as black circles connected by black lines with error bars representing the standard deviation. Right y axes show the accessible surface area (ASA) of each amide. Grey areas indicate the error margins of the

experimental distances. (E-F) Comparison of experimental distances (black dots) and back-calculated average distances (green dots with connecting lines) between Pc amides and the 2000 ensembles of peptide paramagnetic oxygen atoms from MC simulations for PoPc (E) and DPc (F). Grey areas indicate the error margins of the experimental distances. (G-I) Histograms showing the energy distribution of 2000 ensembles from MC simulations: (G) PoPc-XK KKKK, (H) DPc-XK KKKK, (I) PhPc-XK KKKK.