Electronic Supplementary Material (ESI) for RSC Advances

Naturally and Synthetically Linked Lys48 Diubiquitin: A QM/MM study

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Table S1 Results of the CHARMM minimized structure of the naturally linked system, with generated starting conformations (G_1 to G_4) and of minimizations based on the crystal structure. The values for the crystal structure and the gas-phase optimized linker length before insertion into the protein are also presented. Bold values are averaged across G_1 to G_4.

Conformation	Initial (G-P) linker length [Å]	Opt-Linker length [Å]	SD [Å]	Ile44 distance [Å]	SD [Å]
Crystal, M	10.17	10.70	0.23	11.51	0.13
G_1, M	4.38	8.99	0.09	11.29	0.15
G_2, M	4.71	7.59	0.36	11.81	0.25
G_3, M	4.72	7.31	0.22	10.80	0.21
G_4, M	7.31	10.97	0.18	11.75	0.28
	5.28	**8.72**	**0.21**	**11.41**	**0.22**

Table S2 Results of the SCC-DFTB/MM minimized structure of the naturally and synthetically linked system, with generated starting conformations (G_1 to G_4) and of minimizations based on the crystal structure. The values for the crystal structure and the gas-phase optimized linker length before insertion into the protein are also presented. Bold values are averaged across G_1 to G_4.

Conformation	Initial (G-P) linker length [Å]	Opt-Linker length [Å]	SD [Å]	Ile44 distance [Å]	SD [Å]
Crystal, Q	10.17	10.38	0.30	11.75	0.27
G_1, Q	4.38	10.16	0.40	11.79	0.21
G_2, Q	4.71	5.38	0.09	12.29	0.11
G_3, Q	4.72	6.28	0.41	11.44	0.21
G_4, Q	7.31	11.33	0.45	11.87	0.21
	5.28	**8.29**	**0.34**	**11.85**	**0.19**
G_1, Q	10.28	11.75	0.17	11.63	0.07
G_2, Q	5.37	11.28	0.15	11.11	0.17
G_3, Q	10.23	11.48	0.14	11.85	0.19
G_4, Q	7.55	11.04	0.14	11.84	0.09
	8.36	**11.39**	**0.15**	**11.61**	**0.13**

Table S3 Results of the DFT/CHARMM based optimization of the naturally and synthetically linked system with generated starting conformations (G_1 to G_4) and of optimitations based on the crystal structure. The values for the crystal structure and the gas-phase optimized linker length before insertion into the protein are also presented. Bold values are averaged across G_1 to G_4.

Conformation	Initial (G-P) linker length [Å]	Opt-Linker length [Å]	SD [Å]	Ile44 distance [Å]	SD [Å]
Crystal, Q	10.17	10.57	0.30	11.72	0.15
G_1, Q	4.38	10.17	0.43	11.80	0.28
G_2, Q	4.71	5.37	0.16	12.31	0.10
G_3, Q	4.72	6.21	0.35	11.46	0.26
G_4, Q	7.31	10.28	0.44	11.90	0.15
	5.28	**8.01**	**0.35**	**11.87**	**0.20**
G_1, Q	10.28	11.70	0.16	11.69	0.09
G_2, Q	5.37	11.28	0.18	11.21	0.18
G_3, Q	10.23	11.43	0.22	11.89	0.14
G_4, Q	7.55	10.99	0.12	12.01	0.16
	8.36	**11.35**	**0.17**	**11.70**	**0.14**

Table S4 Results of MD simulations using a pure force-field for the NL structure with generated starting conformations (G_1 to G_4) and of simulations based on the crystal structure. The values for the crystal structure and the gas-phase optimized linker length before insertion into the protein are also presented. Bold values are averaged across G_1 to G_4.

Conformation	Initial (G-P) linker length [Å]	Opt-Linker length [Å]	SD [Å]	Ile44 distance [Å]	SD [Å]
Crystal, M	10.17	10.48 ± 0.07	0.35 ± 0.06	11.65 ± 0.15	0.29 ± 0.02
G_1, M	4.38	9.12 ± 0.07	0.20 ± 0.01	11.76 ± 0.06	0.33 ± 0.04
G_2, M	4.71	7.05 ± 0.94	0.38 ± 0.02	12.06 ± 0.17	0.39 ± 0.06
G_3, M	4.72	7.74 ± 1.24	0.59 ± 0.05	12.34 ± 0.82	0.48 ± 0.06
G_4, M	7.31	10.85 ± 0.03	0.36 ± 0.15	11.86 ± 0.22	0.34 ± 0.02
	5.28	$\mathbf{8.69 \pm 0.57}$	$\mathbf{0.38 \pm 0.06}$	$\mathbf{12.01 \pm 0.32}$	$\mathbf{0.39 \pm 0.05}$

Table S5 Measured distances between and to atoms of the amino acids of the hydrophibic patches. Shown are the averages and standard deviations over QM/MM MD simulations of the naturally linked system based on the crystal structure and all trajectories of the synthetically linked system. The differences between both systems are shown in bold.

Distance between		Native diUb	Artificially linked diUb	**Difference**
Ub_{prox}Ile44-C$_\alpha$	Ub_{dist}Ile44-C$_\alpha$	11.70 ± 0.06	11.77 ± 0.05	**-0.07**
Ub_{prox}Ile44-C$_\beta$	Ub_{dist}Ile44-C$_\beta$	8.99 ± 0.06	9.05 ± 0.05	**-0.06**
Ub_{prox}Val70-C$_\alpha$	Ub_{dist}Val70-C$_\alpha$	10.12 ± 0.10	10.00 ± 0.07	**0.13**
Ub_{prox}Val70-C$_\beta$	Ub_{dist}Val70-C$_\beta$	7.43 ± 0.06	7.30 ± 0.09	**0.14**
Ub_{prox}Leu8-C$_\alpha$	Ub_{dist}Leu8-C$_\alpha$	9.82 ± 0.20	10.38 ± 0.13	**-0.56**
Ub_{prox}Leu8-C$_\beta$	Ub_{dist}Leu8-C$_\beta$	8.32 ± 0.13	8.70 ± 0.09	**-0.38**
Ub_{prox}Ile44-C$_\alpha$	Ub_{dist}Val70-C$_\alpha$	7.74 ± 0.07	7.67 ± 0.03	**0.07**
Ub_{prox}Ile44-C$_\alpha$	Ub_{dist}Leu8-C$_\alpha$	7.79 ± 0.07	7.59 ± 0.11	**0.20**
Ub_{prox}Val70-C$_\alpha$	Ub_{dist}Leu8-C$_\alpha$	10.77 ± 0.11	10.82 ± 0.12	**-0.06**
Residue 48-C$_\alpha$	Ub_{dist}Val70-C$_\alpha$	5.68 ± 0.07	6.09 ± 0.11	**-0.41**
Residue 48-C$_\alpha$	Ub_{dist}Leu8-C$_\alpha$	7.72 ± 0.19	7.62 ± 0.08	**0.10**
Residue 48-C$_\alpha$	Ub_{dist}Ile44-C$_\alpha$	11.64 ± 0.06	12.10 ± 0.09	**-0.46**
Residue 48-C$_\alpha$	Ub_{prox}Val70-C$_\alpha$	11.91 ± 0.03	12.02 ± 0.06	**-0.11**
Residue 48-C$_\alpha$	Ub_{prox}Leu8-C$_\alpha$	13.54 ± 0.10	13.82 ± 0.09	**-0.28**
Residue 48-C$_\alpha$	Ub_{prox}Ile44-C$_\alpha$	5.51 ± 0.04	5.59 ± 0.04	**-0.08**

Figure S1 The RMSD between the original crystal strucutre (1IDQ) and various models during the MD trajectory after equilibration. Only backbone atoms are considered. The residues within the linking region are excluded.

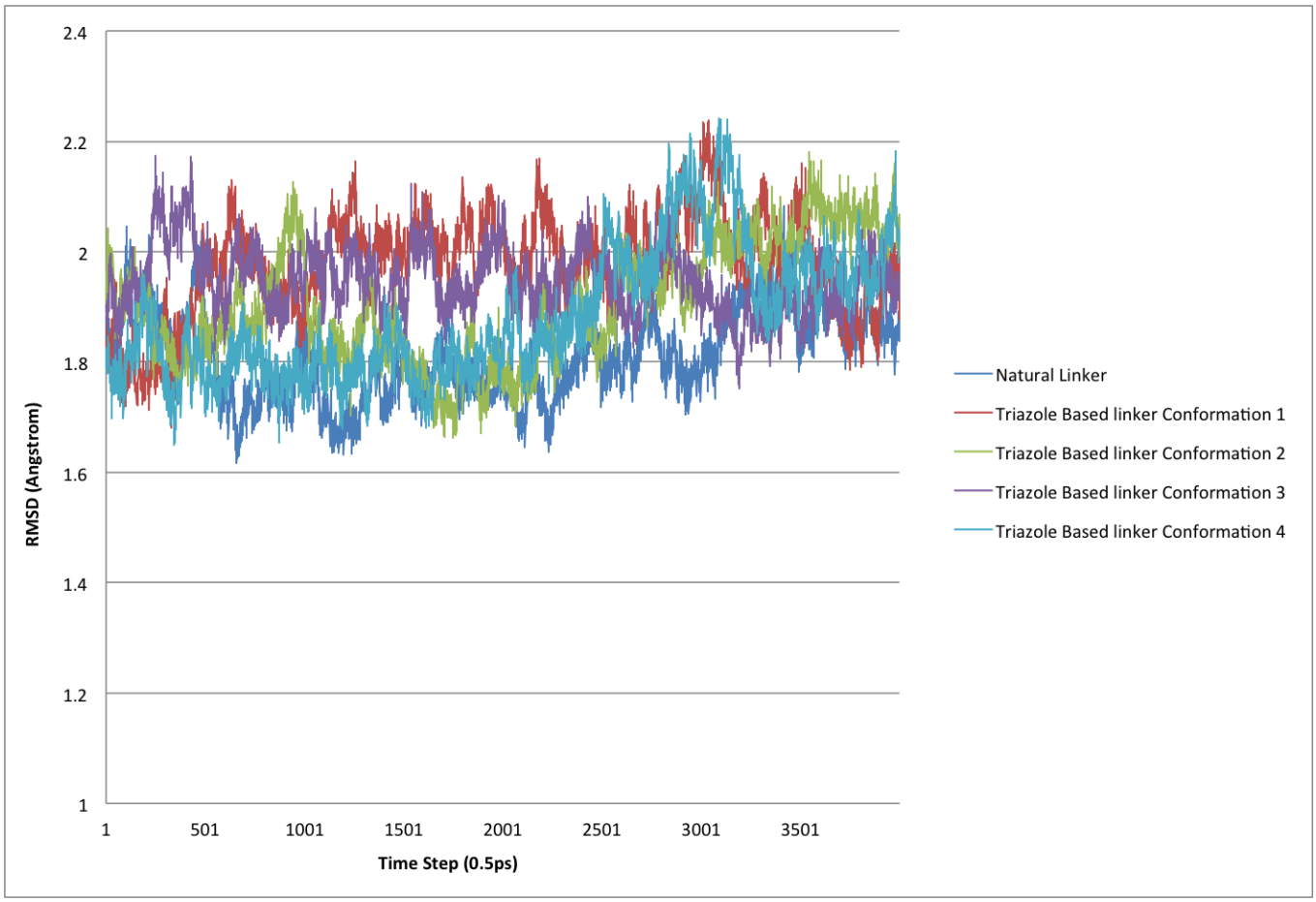
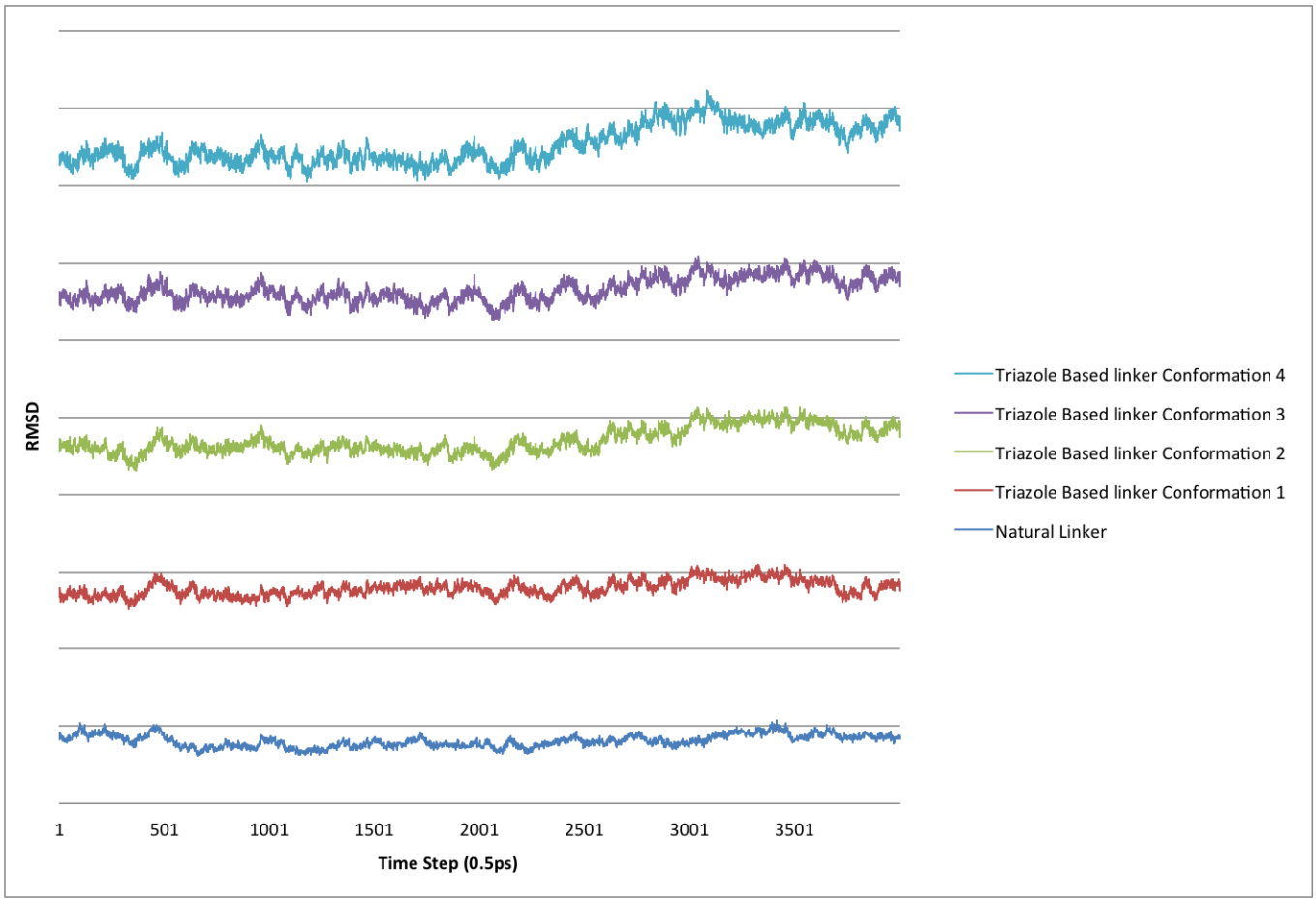


Figure S2 The RMSD between the original crystal strucutre (1IDQ) and various models during the MD trajectory after equilibration, plotted as a stack chart to more clearly display the trend of the RMSD over time. Only backbone atoms are considered. The residues within the linking region are excluded.



The CHARMM parameters and additional topology file entries for the synthetic linker:

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MASS 223    HX   1.008    H
MASS 224    CX   12.001   C
MASS 225    NX   14.001   N
MASS 226    OX   16.001   O
MASS 227    SX   32.060   S
MASS 228    QQH  1.00800  H ! Link Atom
RESI RLN
GROUP
ATOM  N1   NX     -0.88
ATOM  C2   CX     -0.23
ATOM  H22  HX      0.19
ATOM  H23  HX      0.33
ATOM  H24  HX      0.38
ATOM  H44  HX      0.21
GROUP
ATOM  C3   C       0.77
ATOM  O4   O      -0.66
ATOM  N5   N      -0.82
ATOM  C6   CX     -0.21
ATOM  H25  HX      0.23
ATOM  H26  HX      0.21
ATOM  H27  HX      0.48
GROUP
ATOM  C7   CX      0.76
ATOM  O8   OX     -0.64
ATOM  N9   NX     -0.85
ATOM  C10  CX     -0.18
ATOM  H28  HX      0.20
ATOM  H29  HX      0.22
ATOM  H30  HX      0.49
GROUP
ATOM  C11  CX      0.74
ATOM  O12  OX     -0.62
ATOM  C20  CX     -0.12
ATOM  N21  NX     -0.81
ATOM  H40  HX      0.20
ATOM  H41  HX      0.17
ATOM  H45  HX      0.44
GROUP
ATOM  C19  CX     -0.32
ATOM  H38  HX      0.16
ATOM  H39  HX      0.16
GROUP
ATOM  C18  CT2    -0.35
ATOM  H36  HX      0.20
ATOM  H37  HX      0.15
GROUP
ATOM  C17  CT2    -0.36
ATOM  H34  HA      0.17
ATOM  H35  HA      0.19
GROUP
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ATOM  C14  CX     -0.08
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ATOM  H32  HX      0.37
ATOM  H33  HX      0.22
GROUP
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ATOM  O16  OX     -0.61
ATOM  O42  OX     -0.70
ATOM  H43  HX      0.47
BOND     C2     N1     C3     C2     O4     C3     N5     C3     C6     N5
BOND     C7     C6     O8     C7     N9     C7     C10    N9     C11    C10
BOND     O12    C11    N13    O12    C14    N13    C15    C14    O16    C15
BOND     C17    C14    C18    C17    C19    C18    C20    C19    N21    C11
BOND     H22    C2     H23    N1     H24    N1     H25    C6     H26    C6
BOND     H27    N5     H28    C10    H29    C10    H30    N9     H31    N13
BOND     H32    N13    H33    C14    H34    C17    H35    C17    H36    C18
BOND     H37    C18    H38    C19    H39    C19    H40    C20    H41    C20
BOND     O42    C15    H43    O42    H44    C2     H45    N21
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IC  C3    C2     N1     -99    1.52910645  113.08862617    0.00000000    0.00000000    0.00000000
IC  O4    C3     C2     N1     1.22799333  123.32148130   57.29743866  113.08862617    1.47784817
IC  N5    C3     C2     N1     1.39769977  115.42793327 -124.65708355  123.32148130    1.52910645
IC  C6    N5     C3     C2     1.46950994  121.69692806 -176.03982532  123.32148130    1.22799333
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IC  C11   C10    N9     C7     1.54208889  112.22631326   63.40252909  115.11107993    1.22569728
IC  O12   C11    C10    N9     1.22179796  121.56232099   96.03893024  112.22631326    1.47242068
IC  N13   O12    C11    C10    3.03384388   97.91542514  -76.49325205  121.56232099    1.54208889
IC  C14   N13    O12    C11    1.48397035  106.37069608  -87.06355271   97.91542514    1.22179796
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IC  O16   C15    C14    N13    1.21447959  131.73615995  -64.79118650  106.18157120    1.48397035
IC  C17   C14    N13    O12    1.54019119  115.20626878   40.23917216  131.73615995    1.53055317
IC  C18   C17    C14    N13    1.53360862  110.54835992  -86.41643953  106.18157120    1.21447959
IC  C19   C18    C17    C14    1.53462000  112.50331559  160.14148075  131.73615995    1.53055317
IC  C20   C19    C18    C17    1.53681702  112.36360562 -108.39766999  112.50331559    1.53360862
IC  N21   C11    C10    N9     1.39283584  113.94269164  -90.20790577  112.36360562    1.53462000
IC  H22   C2     N1     C3     1.11644581  109.45246695  120.62153134  121.56232099    1.53681702
IC  H23   N1     C2     C3     1.01835694  110.43344378  -61.69020956  113.08862617    1.22179796
IC  H24   N1     C2     C3     1.02173095  110.07845823   56.29583478  123.32148130    1.52910645
IC  H25   C6     N5     C3     1.11830604  108.90533561  162.19998949  123.32148130    1.47784817
IC  H26   C6     N5     C3     1.11939831  109.87513513   45.91038173  114.15627544    1.47784817
IC  H27   N5     C3     C2     1.03389846  119.31517172   25.50072765  114.15627544    1.53616198
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IC  H29   C10    N9     C7     1.11790917  108.82836049 -177.52315735  112.22631326    1.46950994
IC  H30   N9     C7     C6     1.04252234  119.46442049    5.69098034  112.22631326    1.54208889
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IC  H33   C14   N13   O12     1.12984375  108.71253920  -83.56334297  106.37069608    1.48397035
IC  H34   C17   C14   N13     1.10793338  110.86515431  151.80588612  106.18157120    1.48397035
IC  H35   C17   C14   N13     1.11173760  110.48580660   36.14103952  131.73615995    1.53055317
IC  H36   C18   C17   C14     1.11462712  109.87347658   38.08876396  131.73615995    1.53360862
IC  H37   C18   C17   C14     1.10683296  109.63330595  -77.28755495  112.50331559    1.53360862
IC  H38   C19   C18   C17     1.10860581  110.91268903   15.33759871  112.50331559    1.53462000
IC  H39   C19   C18   C17     1.10817230  109.60077250  130.80534298  112.36360562    1.53462000
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IC  H41   C20   C19   C18     1.11433211  110.04171277 -167.46211838  113.94269164    1.53681702
IC  O42   C15   C14   N13     1.36635700  109.03672107  117.53349212  113.94269164    1.39283584
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PRES XLN1
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ATOM 1HN  H       0.31
ATOM 1CA  CT1     0.07
ATOM 1HA  HB      0.09
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DELETE ATOM 1O
DELETE ATOM 2N1
DELETE ATOM 2C2
DELETE ATOM 2H22
DELETE ATOM 2H23
DELETE ATOM 2H24
DELETE ATOM 2H44
BOND 1CA  2C3
PRES XLN2
GROUP
ATOM 1N   NH1    -0.47
ATOM 1HN  H       0.31
ATOM 1CA  CT1     0.07
ATOM 1HA  HB      0.09
GROUP
ATOM 2C17 CT2    -0.36
ATOM 2H34 HA      0.17
ATOM 2H35 HA      0.19
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DELETE ATOM 1HB1
DELETE ATOM 1HB2
DELETE ATOM 1CG
DELETE ATOM 1HG1
DELETE ATOM 1HG2
DELETE ATOM 1CD
DELETE ATOM 1HD1
DELETE ATOM 1HD2
DELETE ATOM 1CE
DELETE ATOM 1HE1
DELETE ATOM 1HE2
DELETE ATOM 1NZ
DELETE ATOM 1HZ1
DELETE ATOM 1HZ2
DELETE ATOM 1HZ3
DELETE ATOM 2N13
DELETE ATOM 2C14
DELETE ATOM 2C15
DELETE ATOM 2O16
DELETE ATOM 2H31
DELETE ATOM 2H32
DELETE ATOM 2H33
DELETE ATOM 2O42
DELETE ATOM 2H43
BOND 1CA  2C17
PRES LNGL         0.00 ! Linkage between Glycine and Lysine
GROUP                  !
ATOM 2NZ  NH1    -0.42 ! (HA1)  O   HE1
ATOM 2HZ1 H       0.31 !   |   //   |
ATOM 2CE  CT2     0.21 ! -(CA)-C   CE
ATOM 2HE1 HA      0.05 !   |    \  / \
ATOM 2HE2 HA      0.05 ! (HA2)   NZ  HE2
ATOM 1C   C       0.31 !         |
ATOM 1O   O      -0.51 !        HZ1
DELETE ATOM 2HZ2
DELETE ATOM 2HZ3
BOND 1C 2NZ
!ANGLE 1C 2NZ 2HZ1
!ANGLE 1O 1C 2NZ
!DIHED 1O 1C 2NZ 2HZ1
IMPR 1C 1CA 2NZ 1O
IMPR 2NZ 1C 2CE 2HZ1
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