Supplementary Materials

**Off-pathway 3D-structure provides protection against spontaneous Asn/Asp isomerization:  
 shielding proteins Achilles heel**

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|  |  |  |  |
| --- | --- | --- | --- |
| A |  | | |
| B  pH 7.4 |  | C  pH 7.4 |  |
| D  pH 7.4 |  | E  pH 7.8 |  |

Supp.Figure 1: ***(A)*** *Isomerization followed by HPLC; Isomerization reaction and rate constants of Ac-N****G****AA–NH2 forming first the succinimide on-pathway intermediate (Ac-Suc-****G****AA–NH2) hydrolyzing subsequently to a product mixture of Ac-βD****G****AA–NH2 and Ac–αD****G****AA–NH2 with the schematic energy profile of the reaction along the reaction coordinate.* ***(B)*** *Decay of 1H-NMR-signal intensities as function of the time for selected resonances (e.g. acetyl protons: ~2 ppm), resulting in isomerization rate constant k1, k2, and k3 (T=310 K @ pH=7.4). Similar rate constants are obtained at* ***C)*** *T=319K @ pH=7.4,* ***D)*** *T=328K @ pH=7.4,* ***E)*** *T=319K @ pH=7.8,. Color-coding of the [Int.]~ f(time) functions* ***(B-E)*** *are the same as of the molecules depicted taking part of the isomerization reaction: panel* ***A****. Lines are drawn to guide the kinetic curves.*



***Supp.Scheme 1:*** *Positive charge catalyzed isomerization boosted. Backbone amide deprotonation stabilized by explicit positive charge (upper panel) [1939272] and positive charge as electrophile induced π-bond shift produces a lower probability of finding electrons at C creating a better electrophile (lower panel).*

***Supp.Table 1.*** *Selected examples of the in vivo Asn/Asp→βAsp+αAsp isomerization triggering proteopathy and risk in pathophysiology*

|  |  |  |  |
| --- | --- | --- | --- |
| change obs. a | –(N/D)X+1– site | Protein; UniProtID; PMID; PDB$ | physiological and pathological role; remarks |
| N→iD | EN29NV; NN30VL | p53; P04637; 227354551 | surveillance of DNA quality; regulation of cell cycle and apoptosis; several forms of cancer especially carcinoma |
| N→iD | TD121SH chicken | H2A; P02263; 187958042 | component of nucleosome, chromatin regulation |
| D→iD | KD25GK murine or rat; GD26KK canine or chicken | H2B; Q6ZWY9, P10853, P02279 demerged into P0C1H3-4-5; 159084253, 187958042 | DNA packing or chromatin remodeling |
| N→D/iD | AN321GE | HOX, Scr homeodomain; P09077; 264351414 | transcription factor of developmental genes |
| N→D/iD | AN32GD; YD45EA | RNAse U2; P00654; 206236665, 38278366, 208582087 | endoribonuclease |
| N→succ/D/iD | SD101GN | Hen egg white Lysozyme; P00698; 95710468, 116797269; PDB: 1at5 | bacterial cell wall carbohydrate degradation |
| N→succ/D/iD | LN25GR | Interferon *β*; P01574; 2941432510, 2734820911 | immunomodulatory activity; more active |
| N→l/d-D/iD | LD58SG; LD151AT; HN101ER | *α*A crystallin; P02489; 2665754412, 2187772313, 970218514, 954363215 | transparency of the eye lens; small heat shock protein; (chaperon) cataracts in the eye |
| N→D | SD36LF; FD62TG; VN78LD; VN146GP | *α*B crystallin; P02511; 2665754412, 2080248716; PDB: 2klr | transparency of the eye lens; small heat shock protein |
| D→l/d-iD | SD4HQ | *β*B2 crystallin; P43320; 2187772313 | transparency of the eye lens |
| N→l/d-D/iD | LN76DR | **S crystallin; P22914; 2253170417 | transparency of the eye lens |
| N→D/iD | SN27GP; GN132EE; GN140AG | SOD1; P00441; 2406678218, 24044898\*19, 2669460820; PDB: 1rk7 | superoxide dismutase; ALS (amyotrophic lateral syndrome) |
| D→iD or l/d-D | MD672AE; HD678SG; ED694VG | A*β*1-42; P05067, 1614152121, 818147822, 1103240923 | A*β*: neurite growth etc.; neurodegeneration |
| N→iD | GN45GR; EN51GE | GroeS; P0A6F9; 2150796124, 1642915425; PDB: 1pcq | chaperon; *E. coli* in GdnHCl |
| N→D/iD | PN25GA | ACTH; P01192, P01189; 434196226, 435057527 | decreased biological activity |
| N→D | VN27QH in B; CN110-- in A | insulin; P01308; 1457387528 | hormone; Type II diabetes |
| N→D/iD | GN203GY | calbindin D28k; P05937; 1574133529, 1679955930; PDB: 2f33 | Ca++-binding and subsequent signals |
| N→D/iD | MN16GH; NN45GN | *α*-lactalbumin; P00710; 2597703131 | regulatory subunit of lactose synthase; *Camelus* |
| N→iD | EN37GK; KN62GE | *β*2-microglobulin; P61769, 2312647632, 1184727233; PDB: 1jnj | component of MHC-I, antigen presentation; dialysis-related amyloidosis |
| N→l/d-D/iD | GN2A(A/P) | PKA; mammals; -; 952112334, 1115213835 | Ser/Thr kinase, tetramer of 2R and 2C, signal transduction |
| N→D/iD | IN52GN; VN66GA | Bcl-Xl; Q07817; 1237230036 | anti-apoptotic via blocking the apoptotic proteins to outflow from mitochondrion; programmed cell death |
| N→D/iD | DN28CN; CN30QN; QN32GA | Phe-4-hydroxylase; P00439; 1260332637 | metabolic enzyme (Phe → Tyr); increases catalytic activation, def. in phenylketonuria |
| N→D/iD | VN6GA; EN221GA | Ser-HO-CH3-transferase; P34896; 1060016438, 1517032339; PDB: 1rv3 | metabolic enzyme (Gly → Ser); activity increase |
| N→iD | MN53GR; TN109GA | Triose-phosphate isomerase; P60174, P00939; 2588463840; 857195741 | metabolic enzyme (DHAP → G3P); neurological disorder, hemolytic anemia; functional dimer changing the quaternary structure |
| N→D/iD | GN263GR | fibronectin; P02751; 1701545242, 830889243; PDB: 1fbr | cell adhesion, molecule binding; enhances cell-cell contact |
| N→D/iD | LN99NL; LN102NH | eIF4E-BP2; Q13542; 2042416344 | repressor of transcription initiation; autism-like symptoms |
| N→D | SN50GS Sardegna (H50N) | hemoglobin *α* SNPs P69905; 989533345 | oxygen transport; α-thalassemia |
| N→D | PN78GL Singapore (N78D & A79G) | hemoglobin *α* SNPs P69905; 1799438346 | oxygen transport; α-thalassemia |
| N→D | SK139YR, SN139TVKLEPR Wayne (frameshift) | hemoglobin *α* SNPs P69905; 106280147 | oxygen transport, decreased stability; α-thalassemia |
| N→D | DN80HK La-Roche-sur-Yon (L81H) | hemoglobin *β* SNPs P68871; 154065948 | oxygen transport, destabilization; *β*-thalassemia |
| N→D | LN82GT Providence (K82N) | hemoglobin *β* SNPs P68871; 1217249, 100269950 | oxygen transport. low oxygen affinity |
| N→D | LN92CD Redondo or Isehara (H92N) | hemoglobin *β* SNPs P68871; 195131851, 178709752 | oxygen transport; increased O2 affinity, macrocytic hemolytic disease |
| N→D | KN145H- Osler (Y145N), Nancy or Fort Gordon (Y145D) | hemoglobin *β* SNPs P68871; 910128053, 976618854, 116451055 | oxygen transport; *β*-thalassemia |
| N→succ | VD143GQ | endothiapepsin; P111838; 1208352756, 1287632357, 2252575258; PDB: 1gvt | aspartate protease in digestion |
| N→succ | AD75GA | Apo-CheY; P0AE67; 1227070359, 1141058460; PDB: 1jbe | chemosensor → motors |
| N→succ | TN290DE | banyan peroxidase; 2498020761 | peroxidase (metabolism) |
| N→succ | HD319CT | mandelate racemase (enolase superfamily); Q7D1T6; PDB: 4dx3, 4dxk | enolase |
| N→succ | CD38GA | thiamine pyrophosphokinase; Q8A545; PDB: 2omk | Thiamin pyrophosphokinase |
| N→succ | TD147HG | legumain; Q99538; 2563087762; PDB: 4n6n | hydrolysis of -N|X- bonds (endosome, lysosome) |
| N→succ | DD370GM | amylomaltase; O87172; 1742024563; PDB: 2owc | 4-*α*-glucanotransferase (starch metabolism) |
| N→succ | ED332GE | DLG4 or PSD-95; P78352; 2022750664 | disks large homolog 4, interact with the cytoplasmic region of NMDA receptor |

a depending on the available data provided: Asn → βAsp or N→iD for short, Asn → succ./Asp/βAsp or N→succ./D/iD for short, etc.

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***Supp.Table 2:*** *NMR relaxation data (1H-15N hetNOE and S2) retrieved from the BMRB2017 a of Asn/AspGly subunits of those proteins that have higher than average b flexible backbone motion for this site.*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Selection Criteria** | **Descriptor / BMRB ID** | ***S2*of selected Asn/AspGly-motifs** | | **hetNOEof selected Asn/AspGly-motifs** | |
| AsnGly | AspGly | AsnGly | AspGly |
| *normal* |  | 73/80 | 134/145 | 132/144 | 192/201 |
| *S*2 <0.7 for either Asn or Gly  or  hetNOE <0.25 for AsnGly or  hetNOE< 0.40 for AspGly | <*S*2>±*SD or*  *<hetNOE>*±*SD* | 0.594±0.261 (7)d | 0.632 ± 0.186 (11) d | -0.290 ± 0.434 (12 out of 9) | 0.052 ± 0.321 (9 out of 6) d |
| BMRB ID c | 6838, 18304, 18389, 18971, 19388, 26513, 26714 | 4267, 4689, 7219, 17306, 18305, 25852, 26710, 26712, 26713 | 15562, 16659, 18758, 19361, 19993, 26513, 26741, 26742, 26823 | 4689, 16069, 16876, 17513, 19993, 26515 |
| *S*2 < 0.7 for both Asn & Gly  or  hetNOE <0.25 for AsnGly or  hetNOE< 0.40 for AspGly | <*S*2>±*SD or*  *<hetNOE>*±*SD*  (position if required) | 0.408 & 0.183  0.408 & 0.183 | 0.560 & 0.608  0.539 & 0.680  0.379 & 0.165 | -0.338 & -0.445 (-N24G-)  -0.779 & -0.804 (-N24G-)  -0.532 & -0.412 (-N33G-)  -0.721 & -0.705 (-N33G-)  0.055 & -0.108  -0.700 & -0.454  -1.139 & -0.864  0.239 & 0.230  -0.071 & 0.040 | 0.321 & 0.177 (-D130G-)  0.322 & 0.590 (-D130G-)  0.437 & -0.140  0.201 & 0.235 (-D91G-)  0.023 & -0.034 (-D134G-)  0.023 & 0.018  -0.616 & -0.467  0.280 & 0.030 (-D117G-)  0.020 & -0.480 (-D121G-) |
| BMRB ID /(position | 19388 (-N161G-)  26513 (-N164G-) | 17306 (-D8G-)  26712 (-D121G-)  4689 (-D130G-) | 15562 (-N24G-) (-N33G-)  16659 (-N74G-)  18758 (-N12G-)  19993 (-N62G-)  26741 (-N8G-)  26823 (-N135G-) | 4689 (-D130G-)  16069 (-D89G-)  16876 (-D91G-) (-D134G-)  17513 (-D56G-)  19993 (-D12G-)  26515 (-D117G-) (-D121G-) |
| dynam. descriptor of  Asn/Asp < than that of Gly | values to compare  (position if required) | 0.672 < 0.914  0.282 < 0.798  0.582 < 0.952  0.640 < 0.759 | 0.557 < 0.796  0.516 < 0.702 | -0.532 < -0.412 (-N33G-)  -0.721 < -0.705 (-N33G-)  -0.700 < -0.454  -1.139 < -0.864  -0.071 < 0.040 | 0.322 < 0.590  0.201 < 0.235  -0.616 < -0.467 |
| BMRB ID /(position) | 18304 (-N103G-)  18971 (-N19G-)  26714 (-N43G-)  6838 (-N32G-) | 25852 (-D22G-)  7219 (-D53G-) | 15562 (-N33G-)  18758 (-N12G-)  19993 (-N62G-)  26823 (-N135G-) | 4689 (-D130G-)  16876 (-D91G-)  19993 (-D12G-) |
| dynam. descriptor of  Asn/Asp > than that of Gly | values to compare  (position if required) | 0.860 > 0.670 | 0.781 > 0.697  0.764 > 0.492  0.936 > 0.692  0.795 > 0.617  0.826 > 0.581  0.764 > 0.493  0.889 > 0.342 | -0.338 > -0.445 (-N24G-)  -0.779 > -0.804 (-N24G-)  0.055 > -0.108  0.239 > 0.230 | 0.321 > 0.177  0.437 > -0.140  0.023 > -0.034  0.023 > 0.018  0.280 > 0.030 (-D117G-)  0.020 > -0.480 (-D121G-) |
| BMRB ID /(position) | 18389 (-N95G-) | 18305 (-D66G-)  26713 (-D121G-)  25852 (-D24G-)  25852 (-D58G-)  26710 (-D134G-)  26713 (-D121G-)  4267 (-D178G-) | 15562 (-N24G-)  16659 (-N74G-)  26741 (-N8G-) | 4689 (-D130G-)  16069 (-D89G-)  16876 (-D134G-)  17513 (-D56G-)  26515 (-D117G-) (-D121G-) |
| Asn/AspGly the most mobile | values to compare | 0.282 & 0.798 | 0.379 & 0.165 | -1.139 ± -0.864 | -0.616 & -0.467 |
| BMRB ID /(position) | 18971 (-N19G-) | 4689 (-D130G-) | 19993 (-N62G-) | 19993 (-D12G-) |

*a* for 11817 proteins deposited, in total, 76/151 *S*2, and 115/145 *hetNOE* data could have been retrieved

*b* average backbone dynamic data of all the AsnGly and AspGly-units: <*S*2>all.NGs=0.874±0.138, <*S*2>all.DGs=0.855±0.120, <*hetNOE*>all.NGs=0.597±0.401, <*hetNOE*>all.DGs=0.728±0.222, respectively

c BMRB ID: 19388, 26513, 19361: Protein Tyr phosphatase; 18304: HEWL; 18389: CtCBM11; 18971: Nedd4 WW (E3 ubiquitin protein ligase); 17306: Ankyrin; 4689: hGH; 25852: CaM; 7219: Human Ubiquitin-Conjugating Enzyme; 26712, 26713: MHC I B27; 4376: VAT ATPase from Thermoplasma;15562: PDCD5-like protein; 16659: IDP: CDK-inhibitor; 18758: E1; 19993: Lysyl tRNA synthetase; 26741, 26742: Prolyl hydroxylase domain 2; 26823: TAR DNA-binding protein 43; 16069: PilZ; 16876: IDP: ERD14; 17513: Immunity Protein 7; 26515: sf-ALR

d # of protein chains averaged

***Supp.Table 3:*** *Selected experimental conditions of the NMR measurements (unless mentioned otherwise 50 mM Na2HPO4 buffer was used set physiological conditions (pH=7.4 and also 7.8))*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **conditions: *pH*, *T*(K)** | **name** | **m (mg)** | **buffer (l)** | **D2O (l)** | **DSS and azid (l)** | **Mw (Da)** | **mg/l** | **concentration (mM)** |
| *pH=6.33, T=328* | *Ac*-NGAA-*NH2* | 0.8 | 500 | 40 | 5 | 372 | 0.8/545 | 3.95 |
| *pH=7.40, T=319* | *Ac*-NGAA-*NH2* | 1.0 | 500 | 50 | 5 | 372 | 1.0/555 | 4.93 |
| *pH=7.40, T=319* | *Ac*-NGRA-*NH2* | 0.8 | 500 | 45 | 5 | 458 | 0.8/545 | 3.15 |
| *pH=7.40, T=310* | *Ac*-NGAA-*NH2* | 0.9 | 600 | 50 | 5 | 372 | 0.9/655 | 3.69 |
| *pH=7.40, T=310* | *Ac*-NGRA-*NH2* | 0.8 | 600 | 50 | 5 | 458 | 0.8/655 | 3.28 |
| *pH=7.40, T=310* | *Ac*-NGAR-*NH2* | 1.8 | 550 | 45 | 6 | 458 | 1.8/601 | 6.54 |
| *pH=7.40, T=310* | *H*-NGAA-*NH2* | 1.1 | 600 | 50 | 5 | 331 | 1.1/655 | 5.07 |
| *pH=7.40, T=310* | *H*-NGRA-*NH2* | 1.1 | 600 | 50 | 5 | 417 | 1.1/655 | 4.03 |
| *pH=7.40, T=310* | cyclo(NGAA)1 | 0.7 | 540 | 40 | 5 | 313 | 0.7/585 | 3.82 |
| *pH=7.40, T=328* | cyclo(NGAA)1 | 1.0 | 510 | 45 | 5 | 313 | 1.0/560 | 5.71 |
| *pH=7.40, T=310* | cyclo(NGAA)2 | 0.9 | 600 | 50 | 5 | 626 | 0.8/655 | 1.95 |
| *pH=7.80, T=310* | *Ac*-NGAA-*NH2* | 1.1 | 500 | 45 | 5 | 372 | 1.1/550 | 5.38 |
| *pH=5.10, T=328* | *Ac*-NGAA-*NH2* | 1.1 | 600a | – | – | 372 | 1.1/600 | 4.93 |
| *pH=5.10, T=328* | *Ac*-NGRA-*NH2* | 1.0 | 640b | – | – | 458 | 1.0/640 | 3.41 |
| *pH=7.40, T=328* | *Ac*-NGAA-*NH2* | 1.0 | 600 | 50 | 5 | 372 | 1.0/655 | 4.10 |
| *pH=7.40, T=328* | *Ac*-NGRA-*NH2* | 1.2 | 600 | 50 | 6 | 458 | 1.2/656 | 3.99 |
| *pH=7.40, T=328* | *Ac*-NGAR-*NH2* | 1.2 | 550 | 45 | 5 | 458 | 1.2/600 | 4.37 |
| *pH=7.40, T=328* | *H*-NGAA-*NH2* | 1.5 | 550 | 45 | 5 | 331 | 1.5/600 | 7.55 |
| *pH=7.40, T=328* | *H*-NGRA-*NH2* | 1.1 | 550 | 45 | 5 | 417 | 1.1/600 | 4.40 |
| *pH=7.40, T=328* | *Ac*-DGAA-*NH2* | 1.0 | 600 | 50 | 5 | 372 | 1.0/655 | 4.10 |
| *pH=7.40, T=328* | *Ac*-DGRA-*NH2* | 1.4 | 600 | 50 | 5 | 458 | 1.4/655 | 4.67 |
| *pH=7.40, T=310* | *Ac*-NSAA-*NH2* | 1.1 | 500 | 45 | 5 | 402 | 1.0/550 | 4.52 |
| *pH=7.40, T=328* | *Ac*-NSAA-*NH2* | 1.0 | 550 | 45 | 5 | 402 | 1.0/600 | 4.15 |
| *pH=7.40, T=310* | *Ac*-NSRA-*NH2* | 1.0 | 500 | 50 | 5 | 488 | 1.0/555 | 3.69 |
| *pH=7.40, T=328* | *Ac*-NSRA-*NH2* | 1.0 | 500 | 50 | 5 | 488 | 1.0/555 | 3.69 |
| *pH=7.40, T=310* | *Ac*-NAAA-*NH2* | 1.0 | 500 | 50 | 5 | 386 | 1.0/555 | 4.67 |
| *pH=7.40, T=328* | *Ac*-NAAA-*NH2* | 1.2 | 550 | 45 | 5 | 386 | 1.2/600 | 5.18 |
| *pH=7.40, T=310* | *Ac*-NARA-*NH2* | 1.2 | 500 | 45 | 5 | 472 | 1.2/550 | 4.62 |
| *pH=7.40, T=328* | *Ac*-NARA-*NH2* | 1.3 | 550 | 45 | 5 | 472 | 1.3/600 | 4.59 |
| *pH=7.40, T=328* | cyclo(NGAA)1 | 0.7 | 500 | 45 | 5 | 313 | 0.7/550 | 4.07 |
| *pH=7.40, T=328* | cyclo(NGAA)2 | 1.1 | 550 | 45 | 5 | 626 | 1.1/600 | 2.93 |
| *pH=7.40, T=328* | cyclo(NGAA)2 | 1.4 | 550 | 45 | 5 | 626 | 1.4/600 | 3.73 |
| *pH=7.40, T=328* | cyclo(NGAA)2 | 1.0 | 550 | 45 | 5 | 626 | 1.0/600 | 2.66 |
| *pH=7.40, T=328* | cyclo(NGRA)1 | 1.0 | 500 | 40 | 5 | 399 | 1.0/545 | 4.60 |
| *pH=7.80, T=319* | *Ac*-NGAA-*NH2* | 0.9 | 550 | 45 | 5 | 372 | 0.9/600 | 4.03 |
| *pH=7.80, T=328* | *Ac*-NGAA-*NH2* | 1.1 | 500 | 45 | 5 | 372 | 1.1/550 | 5.38 |
| *pH=7.40, T=310* | *Ac*-NGKA-*NH2* | 1.1 | 530 | 40 | 5 | 430 | 1.1/575 | 4.45 |
| *pH=7.40, T=328* | *Ac*-NGKA-*NH2* | 1.0 | 530 | 40 | 5 | 430 | 1.0/575 | 4.04 |
| *pH=7.40, T=310* | *Ac*-NGHA-*NH2* | ~1.0 | 530 | 40 | 5 | 438 | 1.0/575 | 3.97 |
| *pH=7.40, T=328* | *Ac*-NGHA-*NH2* | 1.0 | 530 | 40 | 5 | 438 | 1/575 | 3.97 |
| *pH=7.40, T=328* | *Ac*-NHAA-*NH2* | 1.1 | 530 | 40 | 5 | 452 | 1.1/575 | 4.23 |
| *pH=7.40, T=328* | *Ac*-ANGA-*NH2* | 0.9 | 530 | 50 | 5 | 372 | 0.9/585 | 4.14 |
| *pH=7.40, T=310* | *Ac*-ANGA-*NH2* | 1.0 | 530 | 50 | 5 | 372 | 1.0/585 | 4.60 |
| *pH=7.40, T=301* | *Ac*-NGAA-*NH2* | 1.3 | 500 | 45 | 5 | 372 | 1.3/550 | 6.35 |
| *pH=7.40, T=328* | *WVVW* | 1.5 | 500 | 45 | 5 | 1453 | 1.5/550 | 1.88 |
| *pH=7.40, T=328* | *TcN9* | ~1.0 | 550 | 45 | 5 | 2187 | 1.0/600 | 0.76 |
| *pH=7.40, T=328* | *TcD9* | ~1.0 | 550 | 45 | 5 | 2187 | 1.0/600 | 0.76 |

a 600 l from a stock of 1000 l H2O + 125 l buffer + 125 l D2O + 18 l DSS/azid

b l\* from a stock of 1000 l H2O + 125 l buffer + 125 l D2O + 18 l DSS/azid

Buffers used: 20 mM CH3COONH4 (pH=5.1); 50 mM Na2HPO4/50 mM NaH2PO4 (pH=6.3)

**WVVW:=** (*H+*)-SWTV**E(-)NGK(+)**VTWK-*NH2,* **TcN9:=** NLYIQWLK(+)**NG**GPSSGR(+)PPPS, **TcD9:=** NLYIQWLK**D**(**–**)**G**GPSSGR(+)PPPS

***Supp.Table 4:*** *Selected structural parameters of molecular scaffolds of different internal mobility and their isomerization half-life*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **molecular scaffold** | **isomerization half-life @ 55°C** | **most populated fold** | | **mean fold** | | **the on-pathway fold a** | |
|  | *τ*±SD(h) | ***θ***pop(º) | ***d***pop (Å) | ***θ***mean±SD (º) | ***d***mean±SD (Å) | ***θ***opt(º) | ***d***opt(Å) |
| ***Ac*-NGAA-*NH2*** | ~4.1±0.6 | 89 | 3.4 | 95.3±25.4 | 3.9±0.5 | 110 | 3.0 |
| **TcN9** | ~5.4±0.6 | 102.5 | 4.4 | 106.5±20.1 | 4.4±0.4 | 110 | 3.5 |
| **cyclo(NGAA-NGAA)** | ~10.1±2.2 | 86 | 4.2 | 95.3±22.6 | 4.0±0.5 | 107 | 3.3 |
| **cyclo(NGAA)** | ~21.6±5.9 | 68 | 4.1 | 85.6±21.8 | 3.9±0.3 | 110 | 3.2 |

**a** the optimum molecular structure for isomerization found during 300 ns MD simulation

***Supp.Table 5:*** *Measurement details of Co2+ shift reagents used for  and  peptide identification*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **conditions / pH** | **peptide** | **m / mg** | **buffer / l** | **concentration / mM** | **peptide / mol** | **Co2+ / mol**  **(1 : 0.023)** | **Mw / g/mol** | **CoCl2 ∙ 6 H2O / g** | **given Co2+ solution / ml** |
| *pH=7.40* | *Ac*-NGRA-*NH2* | 1.2 | 660 | 4.23 | 2.79 | 0.064 | 238 | 15.3 | 10.0 |
| *pH=7.40* | *Ac*-NGAA-*NH2* | 1.0 | 660 | 4.07 | 2.69 | 0.062 | 238 | 14.7 | 9.6 |
| *pH=7.40* | *H-WVVW-NH2* | 1.5 | 550 | 1.90 | 1.05 | 0.024 | 238 | 5.7 | 10 |