Supporting Information

Backbone N-amination promotes the folding of β-hairpin peptides via a network of hydrogen bonds

Jožica Dolenc\textsuperscript{a}, Esme J. Haywood\textsuperscript{b}, Tingting Zhu\textsuperscript{b} and Lorna J. Smith\textsuperscript{b,*}

\textsuperscript{a} Chemistry | Biology | Pharmacy Information Center, ETH Zurich, Zurich, Switzerland

\textsuperscript{b} Department of Chemistry, University of Oxford, Inorganic Chemistry Laboratory, South Parks Road, Oxford OX1 3QR, United Kingdom
1. Molecular topology building blocks

1.1. ORNH Building Block

**Figure S1.** ORNH building block with atom numbering, GROMOS integer atom codes (IAC) and partial charges: $^{atom\ number}_{IC} \times ^{partial\ charge}_{IAC}$. Color boundaries indicate different charge groups.

**Table S1.** Atoms of the ORNH building block.

| Seq. | Name | IAC | Mass | Charge | Exclusions |
|------|------|-----|------|--------|------------|
| 1    | N    | 6   | 14   | -0.31000 | 2 3 4 11   |
| 2    | H    | 21  | 1    | 0.31000  | 3          |
| 3    | CA   | 14  | 3    | 0.00000  | 4 5 11 12 13 |
| 4    | CB   | 15  | 4    | 0.00000  | 5 6 11     |
| 5    | CG   | 15  | 4    | 0.00000  | 6 7        |
| 6    | CD   | 15  | 4    | 0.12700  | 7 8 9 10   |
| 7    | NZ   | 8   | 14   | 0.12900  | 8 9 10     |
| 8    | HZ1  | 21  | 1    | 0.24800  | 9 10       |
| 9    | HZ2  | 21  | 1    | 0.24800  | 10         |
| 10   | HZ3  | 21  | 1    | 0.24800  |            |
| 11   | C    | 12  | 12   | 0.45000  |            |
| 12   | O    | 1   | 16   | -0.45000 |            |
Table S2. Bond types of the ORNH building block.

| I  | J  | TYPE |
|----|----|------|
| 1  | 2  | 2    |
| 1  | 3  | 21   |
| 3  | 4  | 27   |
| 3  | 11 | 27   |
| 4  | 5  | 27   |
| 5  | 6  | 27   |
| 6  | 7  | 21   |
| 7  | 8  | 2    |
| 7  | 9  | 2    |
| 7  | 10 | 2    |
| 11 | 12 | 5    |
| 11 | 13 | 10   |

Table S3. Bond angle types of the ORNH building block.

| I  | J  | K  | Type |
|----|----|----|------|
| -1 | 1  | 2  | 32   |
| -1 | 1  | 3  | 31   |
| 2  | 1  | 3  | 18   |
| 1  | 3  | 4  | 13   |
| 1  | 3  | 11 | 13   |
| 4  | 3  | 11 | 13   |
| 3  | 4  | 5  | 15   |
| 4  | 5  | 6  | 15   |
| 8  | 7  | 9  | 10   |
| 8  | 7  | 10 | 10   |
| 9  | 7  | 10 | 10   |
| 3  | 11 | 12 | 30   |
| 3  | 11 | 13 | 19   |
| 12 | 11 | 13 | 33   |
| 5  | 6  | 7  | 15   |
| 6  | 7  | 8  | 11   |
| 6  | 7  | 9  | 11   |
| 6  | 7  | 10 | 11   |

Table S4. Dihedral angle types of the ORNH building block.

| I  | J  | K | L | Type |
|----|----|---|---|------|
| -2 | -1 | 1 | 3 | 14   |
Table S5. Improper dihedral angle types of the ORNH building block.

| I  | J | K | L | Type |
|----|---|---|---|------|
| 1  | -1| 3 | 2 | 1    |
| 3  | 1 | 11| 4 | 2    |
| 11 | 3 | 13| 12| 1    |

1.2. NLEU Building Block

Figure S2. NLEU building block with atom numbering, GROMOS integer atom codes (IAC) and partial charges: \(X^{\text{IAC}}\). Color boundaries indicate different charge groups.
Table S6. Atoms of the NLEU building block.

| Seq. | Name | IAC | Mass | Charge | Exclusions |
|------|------|-----|------|--------|------------|
| 1    | N    | 6   | 14   | 0.00000| 2 3 4 5 6 10 |
| 2    | NB   | 7   | 14   | -0.83000| 3 4 5     |
| 3    | HB1  | 21  | 1    | 0.41500| 4         |
| 4    | HB2  | 21  | 1    | 0.41500|           |
| 5    | CA   | 14  | 3    | 0.00000| 6 7 10 11 12 |
| 6    | CB   | 15  | 4    | 0.00000| 7 8 9 10 |
| 7    | CG   | 14  | 3    | 0.00000| 8 9       |
| 8    | CD1  | 16  | 5    | 0.00000| 9         |
| 9    | CD2  | 16  | 5    | 0.00000|           |
| 10   | C    | 12  | 12   | 0.45000|           |
| 11   | O    | 1   | 16   | -0.45000|          |

Table S7. Bond types of the NLEU building block.

| I   | J   | TYPE |
|-----|-----|------|
| 1   | 2   | 17   |
| 1   | 5   | 21   |
| 5   | 6   | 27   |
| 5   | 10  | 27   |
| 6   | 7   | 27   |
| 7   | 8   | 27   |
| 7   | 9   | 27   |
| 10  | 11  | 5    |
| 10  | 12  | 10   |
| 2   | 3   | 2    |
| 2   | 4   | 2    |

Table S8. Bond angle types of the NLEU building block.

| I   | J   | K   | Type |
|-----|-----|-----|------|
| -1  | 1   | 2   | 32   |
| -1  | 1   | 5   | 31   |
| 2   | 1   | 5   | 23   |
| 1   | 2   | 3   | 11   |
| 1   | 2   | 4   | 11   |
| 3   | 2   | 4   | 10   |
| 1   | 5   | 6   | 13   |
| 1   | 5   | 10  | 13   |
| 6   | 5   | 10  | 13   |
| 5   | 6   | 7   | 15   |
| 6   | 7   | 8   | 15   |
| 6   | 7   | 9   | 15   |
Table S9. Dihedral angle types of the NLEU building block.

| I  | J  | K  | L  | Type |
|----|----|----|----|------|
| -2 | -1 |  1 |  5 |  14  |
| -1 |  1 |  5 | 10 |  44  |
| -1 |  1 |  5 | 10 |  43  |
|  1 |  5 |  6 |  7 |  34  |
|  1 |  5 | 10 | 12 |  45  |
|  1 |  5 | 10 | 12 |  42  |
|  5 |  6 |  7 |  8 |  34  |
|  5 |  1 |  2 |  3 |  14  |

Table S10. Improper dihedral angle types of the NLEU building block.

| I  | J  | K  | L  | Type |
|----|----|----|----|------|
|  1 | -1 |  5 |  2 |  1   |
|  5 |  1 | 10 |  6 |  2   |
|  6 |  8 |  9 |  7 |  2   |
| 10 |  5 | 12 | 11 |  1   |

GROMOS molecular topology building blocks for ORNH and for all the N-aminated amino acid residues used in this study have also been uploaded as an additional Supporting Information file.
2. NOE atom-atom distance upper bounds

Lists of NOE atom-atom distance upper bounds (in nm) derived from NMR experiments and \( r^6 \) averaged distances (in nm) calculated from the PepF MD simulations.

The NOE data are taken from Sarnowski et al. (Angew. Chem. Int. Ed. 2017, 56 2083-2086) unless otherwise stated. The upper distance bounds used for NOE's identified as strong, medium and weak were 0.28 nm, 0.35 nm and 0.5 nm respectively with the pseudoatom corrections of Wüthrich et al. (J. Mol. Biol. 1983 169,949-961) applied.

**Table S11. PepC simulation**

NOEs 1-19 from Sarnowski et al. Angew. Chem. Int. Ed. 2017, 56 2083-2086
NOEs 20-36 from Syud et al. J. Amer. Chem. Soc. 1999 121, 11577-11578

| NOE number | Residue and atom 1 | Residue and atom 2 | Upper bound in PepC simulation |
|------------|--------------------|--------------------|-------------------------------|
| 1          | ARG HD@            | 11 LEU HD@         | 0.890 0.957                   |
| 2          | TYR HA             | 11 LEU HA          | 0.350 0.271                   |
| 3          | TYR HD@            | 9 LYSH HB@         | 0.580 0.439                   |
| 4          | TYR HD@            | 9 LYSH HG@         | 0.580 0.514                   |
| 5          | TYR HD@            | 9 LYSH HD@         | 0.650 0.470                   |
| 6          | TYR HD@            | 10 ILE HA          | 0.550 0.564                   |
| 7          | TYR HD@            | 11 LEU HA          | 0.550 0.410                   |
| 8          | TYR HD@            | 11 LEU HD@         | 0.990 0.502                   |
| 9          | TYR HE@            | 9 LYSH HG@         | 0.580 0.646                   |
| 10         | TYR HE@            | 9 LYSH HD@         | 0.800 0.562                   |
| 11         | TYR HE@            | 10 ILE HA          | 0.700 0.594                   |
| 12         | TYR HE@            | 11 LEU HA          | 0.550 0.491                   |
| 13         | TYR HE@            | 11 LEU HD@         | 0.840 0.423                   |
| 14         | GLU HA             | 9 LYSH HA          | 0.280 0.261                   |
| 15         | VAL HA             | 6 DPRO HD@         | 0.380 0.216                   |
| 16         | VAL HG@            | 6 DPRO HA          | 0.380 0.216                   |
| 17         | VAL HG@            | 6 DPRO HD@         | 0.890 0.451                   |
| 18         | LEU HD@            | 14 GLY HA@         | 0.740 0.545                   |
| 19         | GLN HA             | 13 DPRO HD@        | 0.380 0.217                   |
| 20         | ARG HN             | 12 GLN HN          | 0.280 0.361                   |
| 21         | ARG HN             | 14 GLY HN          | 0.280 0.26                   |
| 22         | ARG HB@            | 14 GLY HN          | 0.600 0.502                   |
| 23         | TYR HD@            | 9 LYSH HA          | 0.700 0.585                   |
| 24         | TYR HD@            | 9 LYSH HE@         | 0.800 0.506                   |
| 25         | TYR HD@            | 11 LEU HB@         | 0.800 0.541                   |
| 26         | TYR HE@            | 11 LEU HB@         | 0.650 0.475                   |
| 27         | TYR HB@            | 9 LYSH HB@         | 0.550 0.389                   |
|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
| 28| 2 | TYR | HB@ | 9 | LYS | HG@ | 0.700 | 0.423 |
| 29| 2 | TYR | HB@ | 9 | LYS | HD@ | 0.700 | 0.376 |
| 30| 5 | VAL | HN  | 8 | ORNH| HN  | 0.280 | 0.390 |
| 31| 5 | VAL | HG@ | 6 | DPRO| HB@ | 0.890 | 0.718 |
| 32| 6 | DPRO| HA  | 8 | ORNH| HN  | 0.500 | 0.37  |
| 33| 6 | DPRO| HG@ | 7 | GLY | HN  | 0.600 | 0.472 |
| 34| 6 | DPRO| HD@ | 7 | GLY | HN  | 0.600 | 0.451 |
| 35| 7 | GLY | HN  | 8 | ORNH| HN  | 0.500 | 0.279 |
| 36| 12| GLN | HB@ | 13| DPRO| HB@ | 0.700 | 0.684 |
Table S12. PepF simulation

NOEs 1-8 from Sarnowski Angew. Chem. Int. Ed. 2017, 56 2083-2086
NOEs 9-21 from Stanger and Gellman J. Amer. Chem. Soc. 1998 120, 4236-4237

| NOE number | Residue and atom 1 | Residue and atom 2 | Upper bound | Distance in PepF simulation |
|------------|-------------------|-------------------|-------------|----------------------------|
| 1          | 2 TYR HD@         | 9 LYSH HB@        | 0.800       | 0.431                      |
| 2          | 2 TYR HD@         | 9 LYSH HG@        | 0.800       | 0.490                      |
| 3          | 2 TYR HE@         | 11 LEU HA         | 0.550       | 0.427                      |
| 4          | 2 TYR HE@         | 11 LEU HA         | 0.550       | 0.515                      |
| 5          | 2 TYR HE@         | 11 LEU HG         | 0.480       | 0.429                      |
| 6          | 2 TYR HE@         | 11 LEU HD@        | 0.770       | 0.463                      |
| 7          | 5 VAL HA          | 6 DPRO HD@        | 0.380       | 0.216                      |
| 8          | 5 VAL HG@         | 6 DPRO HD@        | 0.670       | 0.450                      |
| 9          | 2 TYR HA          | 12 GLN HN         | 0.500       | 0.314                      |
| 10         | 2 TYR HA          | 11 LEU HA         | 0.500       | 0.279                      |
| 11         | 11 LEU HA         | 2 TYR HE@         | 0.700       | 0.515                      |
| 12         | 3 VAL HN          | 10 ILE HN         | 0.500       | 0.330                      |
| 13         | 4 GLU HA          | 9 LYSH HA         | 0.500       | 0.263                      |
| 14         | 5 VAL HN          | 8 ORNH HN         | 0.500       | 0.391                      |
| 15         | 7 GLY HN          | 8 ORNH HN         | 0.500       | 0.282                      |
| 16         | 2 TYR HD@         | 9 LYSH HD@        | 0.800       | 0.462                      |
| 17         | 2 TYR HD@         | 11 LEU HD@        | 0.990       | 0.532                      |
| 18         | 2 TYR HE@         | 11 LEU HB@        | 0.800       | 0.560                      |
| 19         | 2 TYR HD@         | 10 ILE HA         | 0.700       | 0.573                      |
| 20         | 10 ILE HD@        | 12 GLN HG@        | 0.750       | 0.544                      |
| 21         | 12 GLN HE21       | 10 ILE HG2@       | 0.650       | 0.717                      |

Table S13. PepF_N2 simulation

| NOE number | Residue and atom 1 | Residue and atom 2 | Upper bound | Distance in PepF_N2 simulation |
|------------|-------------------|-------------------|-------------|-------------------------------|
| 1          | 2 NTYR HA         | 11 LEU HA         | 0.280       | 0.291                         |
| 2          | 2 NTYR HD@        | 9 LYSH HB@        | 0.800       | 0.427                         |
| 3          | 2 NTYR HD@        | 9 LYSH HG@        | 0.800       | 0.499                         |
| 4          | 2 NTYR HD@        | 9 LYSH HD@        | 0.800       | 0.465                         |
| 5          | 2 NTYR HD@        | 10 ILE HA         | 0.480       | 0.563                         |
| 6          | 2 NTYR HD@        | 11 LEU HA         | 0.550       | 0.411                         |
| 7          | 2 NTYR HD@        | 11 LEU HD@        | 0.990       | 0.539                         |
| 8          | 2 NTYR HE@        | 9 LYSH HD@        | 0.580       | 0.531                         |
| 9          | 2 NTYR HE@        | 9 LYSH HE@        | 0.800       | 0.551                         |
| 10         | 2 NTYR HE@        | 11 LEU HA         | 0.700       | 0.487                         |
| 11         | 2 NTYR HE@        | 11 LEU HD@        | 0.840       | 0.448                         |
| NOE number | Residue and atom 1 | Residue and atom 2 | Upper bound | Distance in PepF_N9 simulation |
|------------|-------------------|-------------------|-------------|-----------------------------|
| 1          | 2 TYR HA          | 11 LEU HA        | 0.280       | 0.277                       |
| 2          | 2 TYR HD@         | 9 NKH HB@        | 0.650       | 0.434                       |
| 3          | 2 TYR HD@         | 9 NKH HG@        | 0.650       | 0.491                       |
| 4          | 2 TYR HD@         | 9 NKH HD@        | 0.800       | 0.474                       |
| 5          | 2 TYR HD@         | 10 ILE HA        | 0.700       | 0.577                       |
| 6          | 2 TYR HD@         | 11 LEU HA        | 0.480       | 0.427                       |
| 7          | 2 TYR HE@         | 9 NKH HD@        | 0.580       | 0.486                       |
| 8          | 2 TYR HE@         | 10 ILE HA        | 0.700       | 0.609                       |
| 9          | 2 TYR HE@         | 11 LEU HA        | 0.700       | 0.523                       |
| 10         | 2 TYR HE@         | 11 LEU HD@       | 0.840       | 0.476                       |
| 11         | 3 VAL HG@         | 12 GLN HG@       | 0.740       | 0.422                       |
| 12         | 5 VAL HA          | 6 DPRO HD@       | 0.380       | 0.216                       |
| 13         | 5 VAL HG@         | 6 DPRO HA        | 0.790       | 0.578                       |
| 14         | 5 VAL HG@         | 6 DPRO HD@       | 0.890       | 0.457                       |

**Table S14.** PepF_N9 simulation
### Table S15. PepF_N11 simulation

| NOE number | Residue and atom 1 | Residue and atom 2 | Upper bound | Distance in PepF_N11 simulation |
|------------|-------------------|-------------------|-------------|-------------------------------|
| 1          | TYR HA            | NLEU HA           | 0.280       | 0.285                         |
| 2          | TYR HD@           | LYSH HB@          | 0.650       | 0.450                         |
| 3          | TYR HD@           | LYSH HG@          | 0.650       | 0.507                         |
| 4          | TYR HE@           | LYSH HB@          | 0.580       | 0.463                         |
| 5          | TYR HE@           | NLEU HD@          | 0.770       | 0.474                         |
| 6          | VAL HG@           | GLN HG@           | 0.890       | 0.462                         |
| 7          | VAL HA            | DPRO HD@          | 0.380       | 0.215                         |
| 8          | VAL HG@           | DPRO HA           | 0.640       | 0.568                         |
| 9          | VAL HG@           | DPRO HD@          | 0.740       | 0.447                         |
| 10         | GLU HA            | LYSH HA           | 0.280       | 0.272                         |
| 11         | GLU HG@           | LYSH HA           | 0.450       | 0.385                         |

### Table S16. PepF_N9_N11 simulation

| NOE number | Residue and atom 1 | Residue and atom 2 | Upper bound | Distance in PepF_N9_N11 simulation |
|------------|-------------------|-------------------|-------------|-----------------------------------|
| 1          | TYR HA            | NLEU HA           | 0.280       | 0.281                             |
| 2          | TYR HA            | NLEU HD@          | 0.790       | 0.487                             |
| 3          | TYR HD@           | NKH HD@           | 0.650       | 0.477                             |
| 4          | TYR HD@           | NKH HG@           | 0.800       | 0.514                             |
| 5          | TYR HD@           | NLEU HA           | 0.550       | 0.432                             |
| 6          | TYR HE@           | NKH HD@           | 0.650       | 0.457                             |
| 7          | TYR HE@           | NKH HG@           | 0.800       | 0.522                             |
| 8          | TYR HE@           | NLEU HA           | 0.550       | 0.528                             |
| 9          | VAL HG@           | GLN HA            | 0.640       | 0.600                             |
| 10         | VAL HG@           | GLN HG@           | 0.670       | 0.472                             |
| 11         | VAL HA            | DPRO HD@          | 0.380       | 0.217                             |
| 12         | VAL HG@           | DPRO HA           | 0.790       | 0.577                             |
| 13         | VAL HG@           | DPRO HD@          | 0.670       | 0.457                             |
3 Conformational clustering analysis

Table S17. Clustering of the PepF trajectory. Populations of clusters and of the respective hydrogen bonds (%).

The clustering was performed with a cut-off RMSD of 0.1 nm for the backbone atoms of residues 2-11.

The hydrogen bonds were identified using the definition for medium-strong hydrogen bonds: H-acceptor distance < 0.25 nm and donor-H-acceptor angle > 135°. Populations < 5% are excluded.

| Hydrogen bond     | Cluster 1 | Cluster 2 | Cluster 3 |
|-------------------|-----------|-----------|-----------|
| 1 NH1 – 12 O      | 12.0      | 15.9      |           |
| 1 NH2 – 12 O      | 11.8      | 6.1       |           |
| 1 NH3 – 12 O      | 11.9      | 9.1       |           |
| 1 HH11 – 4 OE1    | 5.5       | 7.6       | 6.3       |
| 1 HH21 – 12 OE1   | 7.6       | 6.8       |           |
| 1 HH11 – 12 OE1   | 5.3       |           |           |
| 1 HE – 12 OE1     | 11.8      | 9.1       |           |
| 3 NH – 10 O       | 95.6      | 94.7      | 9.4       |
| 3 NH – 12 OE1     |           |           | 60.9      |
| 4 NH – 4 OE1      | 6.7       | 21.2      |           |
| 4 NH – 4OE2       | 7.3       | 25.0      |           |
| 5 NH – 3 O        | 9.3       | 19.7      |           |
| 5 NH – 4 OE1      | 5.3       |           |           |
| 5 NH – 4 OE2      | 5.3       |           |           |
| 5 NH – 8 O        | 74.7      | 11.4      | 93.8      |
| 8 NH – 5 O        | 36.0      | 7.6       | 34.4      |
| 8 NH – 6 O        | 16.1      |           | 10.9      |
| 9 NH – 7 O        |           |           | 37.9      |
| 9 HNZ* – 6 O      |           |           | 7.6       |
| 10 NH – 3 O       | 81.5      | 25.8      | 87.5      |
| 10 NH – 8 O       |           |           | 14.4      |
| 12 NH – 1 O       | 76.0      | 84.1      | 6.2       |
| 12 NH – 10 O      |           |           | 25.0      |
| 12 HE21 – 1 O     |           |           | 15.6      |
| 12 HE21 – 4 OE1   |           |           | 9.9       |
| 12 HE21 – 4 OE2   |           |           | 6.1       |

* The highest hydrogen bond population from the donor NZ-HZ1, NZ-HZ2 or NZ-HZ3 is listed
Table S18. Clustering of the PepE trajectory. Populations of clusters and of the respective hydrogen bonds (%).

The clustering was performed with a cut-off RMSD of 0.1 nm for the backbone atoms of residues 2-11.

The hydrogen bonds were identified using the definition for medium-strong hydrogen bonds: H-acceptor distance < 0.25 nm and donor-H-acceptor angle > 135°. Populations < 5% are excluded.

| Population of clusters | Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 |
|------------------------|-----------|-----------|-----------|-----------|
| 54.6                   | 6.2       | 4.8       | 3.7       |

| Hydrogen bond          | Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 |
|------------------------|-----------|-----------|-----------|-----------|
| 1 HNE – 3 O            |           |           |           | 6.3       |
| 1 HNE – 6 O            |           |           | 6.8       |           |
| 1 HNE – 10 O           |           |           | 5.8       |           |
| 1 HNE – 12 O           |           |           | 24.7      |           |
| 1 HH11 – 4 O           |           |           | 7.7       |           |
| 1 HH21 – 6 O           |           |           | 32.6      |           |
| 1 HH21 – 9 O           |           |           |           | 11.4      |
| 1 HH 11 – 12 O         |           |           |           | 10.3      |
| 2 NH – 10 O            |           |           | 19.1      |           |
| 2 NH – 11 O            |           |           | 30.5      |           |
| 2 NH – 12 O            |           |           | 62.0      |           |
| 2 NH – 12 OE1          |           |           |           | 61.0      |
| 3 NH – 11 O            |           |           | 41.7      |           |
| 3 NH – 12 OE1          |           |           | 12.0      |           |
| 3 NH – 12 O            |           |           | 5.3       | 74.4      |
| 4 NH – 7 O             |           |           |           | 80.4      |
| 4 HN – 4 OE1           | 35.4      | 25.1      |           |           |
| 4 HN – 4 OE2           | 32.8      | 30.5      |           |           |
| 5 NH – 9 O             | 89.1      |           |           |           |
| 5 NH – 4 OE1           | 25.4      | 15.7      | 44.7      |           |
| 5 NH – 4 OE2           | 23.1      | 13.7      | 17.4      |           |
| 7 NH – 12 OE1          |           |           | 25.3      |           |
| 8 NH – 4 OE1           | 12.2      |           |           |           |
| 8 NH – 4 OE2           | 12.1      |           |           |           |
| 8 NH – 6 O             | 11.0      |           |           |           |
| 8 NH – 12 OE1          | 13.1      | 7.7       |           |           |
| 9 NH – 2 O             |           |           |           | 95.6      |
| 9 HN – 7 O             | 63.4      | 22.3      |           |           |
| 9 NH – 12 OE1          |           | 66.8      |           |           |
| 11 NH – 3 O            | 90.8      |           |           |           |
| 12 NH – 3 O            | 5.9       | 72.2      |           |           |
| 12 NH – 9 O            | 64.2      | 18.5      |           |           |
| 12 NH – 10 O           | 11.0      | 21.7      |           |           |
| 12 NH – 12 OE1         | 12.6      |           |           |           |
|                |     |     |
|----------------|-----|-----|
| 12 HE22 – 2 OH | 6.0 |     |
| 12 NH2* – 5 O  |     | 61.0|
| 12 NH2* - 9 O  |     | 10.7|

* C-terminal NH$_2$ group
Table S19. Combined clustering of the PepF and PepE trajectories. Populations of clusters and of the respective hydrogen bonds (%).

The clustering was performed with a cut-off RMSD of 0.1 nm for the backbone atoms of residues 2-11.

The hydrogen bonds were identified using the definition for medium-strong hydrogen bonds: H-acceptor distance < 0.25 nm and donor-H-Acceptor angle > 135°. Populations < 5% are excluded.

| Population of clusters by PepF | Cluster 1 | Cluster 2 | Cluster 3 |
|--------------------------------|-----------|-----------|-----------|
| 98.3                           | 0         | 0         |
| Population of clusters by PepE | 0         | 54.6      | 6.1       |

| Hydrogen bond       | Cluster 1 | Cluster 2 | Cluster 3 |
|---------------------|-----------|-----------|-----------|
| 1 NH1 – 12 O        | 12.0      |           |           |
| 1 NH2 – 12 O        | 12.0      |           |           |
| 1 NH3 – 12 O        | 11.8      |           |           |
| 1 HNE – 10 O        |           |           | 6.4       |
| 1 HNE – 12 OE1      | 5.6       |           |           |
| 1 HH21 – 12 OE1     |           |           | 7.4       |
| 2 NH – 11 O         |           |           | 29.7      |
| 2 HN – 12 O         |           |           | 50.0      |
| 3 NH – 10 O         | 96.0      |           |           |
| 3 NH – 11 O         |           | 41.3      |           |
| 3 NH – 12 O         |           | 5.5       | 93.6      |
| 3 NH – 12 OE1       |           |           | 12.3      |
| 4 HN – 4 OE1        | 6.6       |           | 31.2      |
| 4 HN – 4 OE2        | 7.4       |           | 34.8      |
| 5 NH – 3 O          | 9.5       |           |           |
| 5 NH – 4 OE1        |           |           | 25.7      |
| 5 NH – 4 OE2        |           |           | 23.1      |
| 5 NH – 8 O          | 74.6      |           |           |
| 5 NH – 9 O          |           | 89.1      |           |
| 8 NH – 4 OE1        |           | 12.3      |           |
| 8 NH – 4 OE2        |           | 12.7      |           |
| 8 NH – 5 O          | 36.3      |           |           |
| 8 NH – 6 O          | 15.8      | 10.7      |           |
| 8 NH – 12 OE1       |           |           | 13.9      |
| 9 NH – 7 O          |           | 64.2      |           |
| 9 NH – 5 O          |           | 10.7      |           |
| 9 NH – 12 OE1       |           |           | 54.9      |
| 10 NH – 3 O         |           | 81.5      |           |
| 10 NH – 8 O         |           | 7.1       |           |
| 11 NH – 3 O         |           | 98.4      |           |
| 12 NH – 1 O         |           | 76.5      |           |
| 12 NH – 9 O         |           |           | 40.9      |
| 12 NH – 12 OE1      |           |           | 12.6      |
| Formula            | Value |
|--------------------|-------|
| 12 HE22 – 2 OH     | 5.1   |
| 12 NH2* – 1 O      | 8.2   |
| 12 NH2* - 3 O      | 6.3   |
| 12 NH2* – 9 O      | 55.9  |

* C-terminal NH₂ group
Table S20. Clustering of the PepF_N9_N11 trajectory. Populations of clusters and of the respective hydrogen bonds (%).

The clustering was performed with a cut-off RMSD of 0.1 nm for the backbone atoms of residues 2-11.

The hydrogen bonds were identified using the definition for medium-strong hydrogen bonds: H-acceptor distance < 0.25 nm and donor-H-Acceptor angle > 135°. Populations < 5% are excluded.

| Population of clusters | Cluster 1 | Cluster 2 | Cluster 3 |
|------------------------|-----------|-----------|-----------|
| 99.0                   | 0.5       | 0.3       |

| Hydrogen bond          | Cluster 1 | Cluster 2 | Cluster 3 |
|------------------------|-----------|-----------|-----------|
| 1 NH2 – 11 O           | 5.0       |           |           |
| 1 NH1 – 12 O           | 13.7      | 18.0      | 5.7       |
| 1 NH2 – 12 O           | 13.6      | 9.0       | 10.0      |
| 1 NH3 – 12 O           | 13.4      | 12.0      | 5.7       |
| 1 NH1 – 12 OE1         |           |           | 8.6       |
| 1 NH2 – 12 OE1         |           |           | 11.4      |
| 1 NH3 – 12 OE1         |           |           | 8.6       |
| 1 HH21 – 12 OE1        |           |           | 6.4       |
| 2 OH – 4 OE1           |           | 23.0      |           |
| 2 OH – 4 OE2           |           | 16.0      |           |
| 3 NH – 1 O             |           |           | 18.6      |
| 3 NH – 10 O            | 93.0      | 95.0      | 42.9      |
| 4 NH – 4 OE1           |           |           | 8.0       |
| 4 NH – 4 OE2           |           |           | 11.0      |
| 5 NH – 3 O             |           |           | 17.2      |
| 5 NH – 4 OE1           |           |           | 8.0       |
| 5 NH – 4 OE2           |           |           | 14.0      |
| 5 NH – 8 O             | 81.8      | 55.0      | 61.4      |
| 5 NH – 12 OE1          |           |           |           |
| 7 NH – 4 O             |           |           | 10.0      |
| 8 NH – 5 O             | 48.6      | 40.0      | 22.9      |
| 8 NH – 6 O             | 10.0      |           | 45.7      |
| 9 HNB1 – 7 O           | 6.6       |           | 42.0      |
| 9 HNB2 – 9 O           |           | 8.3       | 8.0       |
| 9 HZ1 – 6 O            |           | 8.0       |           |
| 9 HZ2 – 6 O            |           | 6.0       |           |
| 10 NH – 3 O            | 85.4      | 68.0      | 61.4      |
| 10 NH – 8 O            |           | 5.0       |           |
| 11 HNB1 – 9 O          |           |           | 15.0      |
| 12 NH – 1 O            | 61.8      | 51.0      | 32.9      |
| 12 NH – 10 O           |           |           | 11.4      |
Table S21. Clustering of the PepE_N9_N11 trajectory. Populations of clusters and of the respective hydrogen bonds (%).

The clustering was performed with a cut-off RMSD of 0.1 nm for the backbone atoms of residues 2-11.

The hydrogen bonds were identified using the definition for medium-strong hydrogen bonds: H-acceptor distance < 0.25 nm and donor-H-acceptor angle > 135°. Populations < 5% are excluded.

| Hydrogen bond       | Cluster 1 | Cluster 2 | Cluster 3 |
|---------------------|-----------|-----------|-----------|
| 1 NH1 – 10 O        | 19.9      |           |           |
| 1 NH2 – 10 O        | 20.5      |           |           |
| 1 NH3 – 10 O        | 20.2      |           |           |
| 1 NH1 – 12 O        |           | 5.3       |           |
| 1 NH3 – 12 O        |           | 5.7       |           |
| 1 NH1 – 12 OE1      | 6.6       |           |           |
| 1 NH2 – 12 OE1      | 6.7       |           |           |
| 1 NH3 – 12 OE1      | 6.1       |           |           |
| 1 HNE – 12 O        |           |           | 10.9      |
| 1 HH11 – 12 O       |           |           | 7.0       |
| 1 HH21 – 12 O       |           |           | 15.0      |
| 2 NH – 11 O         |           | 9.1       | 59.5      |
| 2 NH – 12 OE1       |           |           | 5.2       |
| 2 OH – 4 OE1        |           | 11.9      |           |
| 2 OH – 4 OE2        |           | 11.7      |           |
| 3 NH – 8 O          | 96.1      |           |           |
| 3 NH – 10 O         |           |           | 78.1      |
| 4 NH – 4 OE1        | 32.6      |           | 14.1      |
| 4 NH – 4 OE2        | 31.5      |           | 13.8      |
| 5 NH – 3 O          |           |           | 27.7      |
| 5 NH – 4 OE1        |           | 11.0      |           |
| 5 NH – 4 OE2        |           | 10.1      |           |
| 5 NH – 8 O          |           |           | 85.7      |
| 5 NH – 12 OE1       |           |           | 14.7      |
| 7 NH – 4 O          |           | 74.9      |           |
| 8 NH – 3 O          |           | 67.0      |           |
| 8 NH – 5 O          | 8.2       |           | 50.4      |
| 9 HNB1 – 7 O        |           | 8.9       | 24.6      |
| 9 HNB2 – 9 O        | 7.9       | 9.6       | 15.3      |
| 9 HNB2 – 12 O       |           |           | 17.2      |
| 9 HNB1 – 12 OE1     |           |           | 12.5      |
| 9 HNB2 – 12 OE1     |           |           | 10.8      |
| 10 NH – 1 O         |           | 61.2      |           |
| 10 NH – 3 O         |           |           | 87.6      |
| Bond                  | Distance |
|-----------------------|----------|
| 11 HNB1 – 9 O         | 14.2     |
| 12 NH – 2 O           | 69.9     |
| 12 NH – 9 O           | 30.9     |
| 12 NH – 10 O          | 7.1      |
| 12 HE21 – 7 O         | 15.9     |
| 12 HE21 – 9 O         | 9.5      |
| 12 HE22 – 3 O         | 8.6      |
| 12 NH – 12 OE1        | 6.1      |
| 12 NH2* – 3 O         | 5.6      |
| 12 NH2* – 4 OE1       | 5.7      |
| 12 NH2* – 4 OE2       |          |

* C-terminal NH₂ group
Table S22. Combined clustering of the PepF_N9_N11 and PepE_N9_N11 trajectories. Populations of clusters and of the respective hydrogen bonds (%).

The clustering was performed with a cut-off RMSD of 0.1 nm for the backbone atoms of residues 2-11.

The hydrogen bonds were identified using the definition for medium-strong hydrogen bonds: H-acceptor distance < 0.25 nm and donor-H-acceptor angle > 135°. Populations < 5% are excluded.

| Hydrogen bond       | Cluster 1 | Cluster 2 | Cluster 3 |
|---------------------|-----------|-----------|-----------|
| 1 NH1 – 10 O        | 19.9      |           |           |
| 1 NH2 – 10 O        | 20.5      |           |           |
| 1 NH3 – 10 O        | 19.6      |           |           |
| 1 NH1 – 12 O        | 12.5      |           |           |
| 1 NH2 – 12 O        | 12.5      |           |           |
| 1 NH3 – 12 O        | 12.4      |           |           |
| 1 NH1 – 12 OE1      |           | 6.3       |           |
| 1 NH2 – 12 OE1      |           | 6.7       |           |
| 1 NH3 – 12 OE1      |           | 5.7       |           |
| 1 HNE – 12 O        |           |           | 11.3      |
| 1 HH11 – 12 O       |           |           | 7.1       |
| 1 HH21 – 12 O       |           |           | 16.0      |
| 1 HH21 – 12 OE1     |           |           | 5.5       |
| 2 NH – 11 O         |           |           | 63.7      |
| 3 NH – 8 O          |           |           | 96.0      |
| 3 NH – 10 O         |           |           | 91.6      |
| 4 NH – 4 OE1        |           | 31.8      | 13.6      |
| 4 NH – 4 OE2        |           | 31.4      | 13.0      |
| 5 NH – 3 O          |           |           | 30.5      |
| 5 NH – 4 OE1        |           |           | 11.6      |
| 5 NH – 4 OE2        |           |           | 10.7      |
| 5 NH – 8 O          |           |           | 82.5      |
| 5 NH – 12 OE1       |           |           | 13.2      |
| 7 NH – 4 O          |           |           | 75.0      |
| 8 NH – 3 O          |           |           | 68.1      |
| 8 NH – 5 O          |           | 49.6      | 7.2       |
| 8 NH – 6 O          |           | 9.1       |           |
| 9 HNB1 – 7 O        |           | 7.1       | 23.4      |
| 9 HNB2 – 9 O        |           | 8.6       | 7.9       |
| 9 HNB2 – 12 O       |           |           | 16.0      |
|                |          |      |
|----------------|----------|------|
| 9 HNB1 – 12 OE1|          | 13.7 |
| 9 HNB2 – 12 OE1|          | 10.3 |
| 9 HZ3 – 5 O    |          | 5.1  |
| 10 NH – 1 O    |          | 60.3 |
| 10 NH – 3 O    |          | 86.0 |
| 11 HNB1 – 9 O  |          | 14.7 |
| 12 NH – 1 O    |          | 52.9 |
| 12 NH – 2 O    |          | 66.3 |
| 12 NH – 9 O    |          | 28.4 |
| 12 NH – 10 O   |          | 7.3  |
| 12 HE21 – 7 O  |          | 14.9 |
| 12 HE21 – 9 O  |          | 8.5  |
| 12 HE22 – 3 O  |          | 9.5  |
| 12 NH – 12 OE1 |          | 6.0  |
| 12 NH2* – 3 O  |          | 5.8  |
| 12 NH2* – 4 OE1|          | 7.1  |
| 12 NH2* – 4 OE2|          | 8.4  |

* C-terminal NH$_2$ group
Table S23. Clustering of the PepF_N2_N4_N9_N11 trajectory. Populations of clusters and of the respective hydrogen bonds (%).

The clustering was performed with a cut-off RMSD of 0.1 nm for the backbone atoms of residues 2-11.

The hydrogen bonds were identified using the definition for medium-strong hydrogen bonds: H-acceptor distance < 0.25 nm and donor-H-Acceptor angle > 135°. Populations < 5% are excluded.

| Hydrogen bond                | Cluster 1 | Cluster 2 | Cluster 3 |
|------------------------------|-----------|-----------|-----------|
| 1 NH1 – 12 O                 | 20.9      | 10.6      | 7.3       |
| 1 NH2 – 12 O                 | 18.3      | 16.0      | 18.2      |
| 1 NH3 – 12 O                 | 19.8      | 12.0      | 18.2      |
| 1 NH2 – 12 OE1               |           |           | 5.9       |
| 1 HH21 – 12 OE1              | 10.2      | 5.2       | 12.7      |
| 1 HE – 12 OE1                |           | 10.3      | 10.9      |
| 2 OH – 4 OE1                 | 8.6       | 14.3      |           |
| 2 OH – 4 OE2                 | 7.5       | 9.1       |           |
| 3 NH – 10 O                  | 94.7      | 88.7      | 89.1      |
| 4 HNB1 – 2 O                 | 9.2       | 19.9      |           |
| 5 NH – 3 O                   |           |           | 14.6      |
| 5 NH – 8 O                   | 75.5      | 20.2      | 63.6      |
| 7 NH – 4 O                   |           |           | 5.5       |
| 7 NH – 4 OE1                 |           |           | 5.5       |
| 8 NH – 5 O                   | 57.2      | 26.0      | 36.4      |
| 8 NH – 6 O                   | 5.7       |           | 43.6      |
| 9 HNB1 – 7 O                 | 13.8      | 52.1      |           |
| 9 HNB2 – 9 O                 | 8.5       | 10.1      |           |
| 9 HNZ* – 4 O                 |           | 12.8      |           |
| 9 HNZ* – 6 O                 |           | 7.7       |           |
| 9 HNZ* – 7 O                 |           |           | 5.5       |
| 10 NH – 3 O                  | 86.4      | 69.8      | 81.8      |
| 11 HNB1 – 9 O                | 8.3       | 15.0      |           |
| 12 NH – 1 O                  | 67.9      | 40.3      | 70.9      |

* The highest hydrogen bond population from the donor NZ-HZ1, NZ-HZ2 or NZ-HZS3 is listed
Table S24. Clustering of the PepE_N2_N4_N9_N11 trajectory. Populations of clusters and of the respective hydrogen bonds (%).

The clustering was performed with a cut-off RMSD of 0.1 nm for the backbone atoms of residues 2-11.

The hydrogen bonds were identified using the definition for medium-strong hydrogen bonds: H-acceptor distance < 0.25 nm and donor-H-Acceptor angle > 135°. Populations < 5% are excluded.

| Hydrogen bond          | Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 |
|------------------------|-----------|-----------|-----------|-----------|
| Population of clusters |           |           |           |           |
| 1 NH1 – 12 O           | 15.2      | 20.0      |           |           |
| 1 NH2 – 12 O           | 14.2      | 12.7      |           |           |
| 1 NH3 – 12 O           | 15.0      | 14.2      |           |           |
| 1 HH21 – 12 O          |           | 5.5       |           |           |
| 1 HH21 – 12 OE1        | 6.8       |           |           |           |
| 2 HNB2 – 9 O           |           | 43.6      |           |           |
| 2 HNB2 – 11 O          | 5.3       | 4.6       |           |           |
| 2 OH – 4 OE1           | 7.3       | 36.5      | 10.9      |           |
| 2 OH – 4 OE2           | 16.8      | 37.8      | 10.5      | 7.4       |
| 3 NH – 10 O            | 92.5      |           | 92.4      |           |
| 4 HNB1 – 2 O           | 9.6       | 67.6      | 18.8      | 22.4      |
| 4 HNB2 – 8 O           |           |           | 94.4      |           |
| 5 NH – 8 O             | 76.1      |           | 12.0      |           |
| 5 NH – 4 OE1           |           |           |           | 41.2      |
| 5 NH – 4 OE2           |           |           |           | 28.9      |
| 7 NH – 4 O             |           |           |           | 60.5      |
| 8 NH – 4 OE1           |           |           |           | 43.2      |
| 8 NH – 5 O             | 56.7      |           | 24.7      |           |
| 8 HNZ* – 2 O           |           |           |           | 15.5      |
| 9 HNB1 – 7 O           | 15.7      | 6.1       | 39.9      |           |
| 9 HNB2 – 9 O           | 8.6       |           | 8.6       |           |
| 9 HNZ* – 4 O           |           |           |           | 9.4       |
| 9 HNZ* – 6 O           |           |           |           | 5.9       |
| 10 NH – 3 O            | 84.1      |           | 65.9      |           |
| 11 HNB1 – 9 O          | 6.4       | 14.2      | 5.3       |           |
| 12 NH – 1 O            | 47.9      |           | 32.6      |           |

* The highest hydrogen bond population from the donor NZ-HZ1, NZ-HZ2 or NZ-HZS3 is listed.
Table S25. Combined clustering of the PepF_N2_N4_N9_N11 and PepE_N2_N4_N9_N11 trajectories. Populations of clusters and of the respective hydrogen bonds (%).

The clustering was performed with a cut-off RMSD of 0.1 nm for the backbone atoms of residues 2-11.

The hydrogen bonds were identified using the definition for medium-strong hydrogen bonds: H-acceptor distance < 0.25 nm and donor-H-acceptor angle > 135°. Populations < 5% are excluded.

| Hydrogen bond | Cluster 1 | Cluster 2 | Cluster 3 |
|---------------|-----------|-----------|-----------|
| 1 NH1 – 12 O  | 19.6      |           | 16.3      |
| 1 NH2 – 12 O  | 18.6      |           | 13.8      |
| 1 NH3 – 12 O  | 17.3      |           | 14.1      |
| 1 HH21 – 12 OE1 | 9.3      |           |           |
| 2 OH – 4 OE1  | 8.3       | 36.5      | 12.1      |
| 2 OH – 4 OE2  | 9.8       | 37.5      | 10.2      |
| 2 HNB2 – 9 O  |           |           | 47.4      |
| 3 NH – 10 O   | 94.3      |           | 92.3      |
| 4 HNB1 – 2 O  | 9.4       | 67.6      | 18.8      |
| 4 HNB2 – 8 O  |           |           | 95.4      |
| 5 NH – 8 O    | 76.3      |           | 14.6      |
| 7 NH – 4 O    |           |           | 61.0      |
| 8 NH – 4 OE1  |           |           | 43.8      |
| 8 NH – 4 OE2  |           |           | 42.8      |
| 8 NH – 5 O    | 57.3      |           | 26.1      |
| 8 NH – 6 O    | 5.4       |           |           |
| 9 HNB1 – 7 O  | 14.7      | 6.1       | 44.2      |
| 9 HNB2 – 9 O  | 8.4       |           | 9.4       |
| 9 HNZ* – 4 O  |           |           | 9.8       |
| 9 HNZ* – 6 O  |           |           | 5.8       |
| 10 NH – 3 O   | 85.7      |           | 68.6      |
| 11 HNB1 – 9 O | 7.7       |           | 13.4      |
| 12 NH – 1 O   | 62.6      |           | 36.4      |
| 12 NH – 12 OE1|           |           | 5.1       |

* The highest hydrogen bond population from the donor NZ-HZ1, NZ-HZ2 or NZ-HZS3 is listed.