Accurate *de novo* design of membrane-traversing macrocycles

Supplemental data file 1: Structure prediction plots for all designs. Related to Figures 1 and 3.

Figure D1.1: Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 6-amino acid macrocycles: Left panels show the torsion bin cluster and predicted conformational landscape for each design. * denotes N-methylated amino acids. Orange points denote an independently predicted structure with no cis-peptide bonds. Grey points represent predicted structures with at least one cis-peptide bond. Blue dots represent the local minimization of the designed macrocycle structure. For hydrogen bonding graphs, the orange boxes highlight the designed intramolecular hydrogen bonds. Amino acids without a backbone hydrogen bond donor (proline, D-proline, N-methylated amino acids) are marked by darker grey columns. Related to Figure 1.



Figure D1.2: Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 7-amino acid macrocycles: Left panels show the torsion bin cluster and predicted conformational landscape for each design. * denotes N-methylated amino acids. Orange points denote an independently predicted structure with no cis-peptide bonds. Grey points represent predicted structures with at least one cis-peptide bond. Blue dots represent the local minimization of the designed macrocycle structure. For hydrogen bonding graphs, the orange boxes highlight the designed intramolecular hydrogen bonds. Amino acids without a backbone hydrogen bond donor (proline, D-proline, N-methylated amino acids) are marked by darker grey columns. Related to Figure 1.



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Figure D1.3: Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 8-amino acid macrocycles: Left panels show the torsion bin cluster and predicted conformational landscape for each design. * denotes N-methylated amino acids. Orange points denote an independently predicted structure with no cis-peptide bonds. Grey points represent predicted structures with at least one cis-peptide bond. Blue dots represent the local minimization of the designed macrocycle structure. For hydrogen bonding graphs, the orange boxes highlight the designed intramolecular hydrogen bonds. Amino acids without a backbone hydrogen bond donor (proline, D-proline, N-methylated amino acids) are marked by darker grey columns. Related to Figure 1.



Figure D1.3 (cont.): Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 8-amino acid macrocycles *(full figure legend described earlier).* Related to Figure 1.



Figure D1.3 (cont.): Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 8-amino acid macrocycles. (full figure legend described earlier). Related to Figure 1.



1 2 RMSD to Design

RMSD to Design

Figure D1.3 (cont.): Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 8-amino acid macrocycles. *(full figure legend described earlier).* Related to Figure 1.



Figure D1.4: Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 9-amino acid macrocycles: Left panels show the torsion bin cluster and predicted conformational landscape for each design. * denotes N-methylated amino acids. Orange points denote an independently predicted structure with no cis-peptide bonds. Grey points represent predicted structures with at least one cis-peptide bond. Blue dots represent the local minimization of the designed macrocycle structure. For hydrogen bonding graphs, the orange boxes highlight the designed intramolecular hydrogen bonds. Amino acids without a backbone hydrogen bond donor (proline, D-proline, N-methylated amino acids) are marked by darker grey columns. Related to Figure 3.



Figure D1.4 (cont.): Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 9-amino acid macrocycles. *(full figure legend described earlier).* Related to Figure 3.



Figure D1.4 (cont.): Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 9-amino acid macrocycles. *(full figure legend described earlier).* Related to Figure 3.



-20

0

1 2 RMSD to Design

0 1 2 RMSD to Design

-20

10

Figure 1.4 (cont.): Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 9-amino acid macrocycles. *(full figure legend described earlier).* Related to Figure 3.



11

Figure 1.4 (cont.): Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 9-amino acid macrocycles. *(full figure legend described earlier).* Related to Figure 3.



Figure D1.5: Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 10-amino acid macrocycles: Left panels show the torsion bin cluster and predicted conformational landscape for each design. * denotes N-methylated amino acids. Orange points denote an independently predicted structure with no cis-peptide bonds. Grey points represent predicted structures with at least one cis-peptide bond. Blue dots represent the local minimization of the designed macrocycle structure. For hydrogen bonding graphs, the orange boxes highlight the designed intramolecular hydrogen bonds. Amino acids without a backbone hydrogen bond donor (proline, D-proline, N-methylated amino acids) are marked by darker grey columns. Related to Figure 3.



Figure D1.5 (cont.): Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 10-amino acid macrocycles. (full figure legend described earlier). Related to Figure 3.

20

10

0

-10

-20

-30

0

Energy (kcal/mol)



D10.15: PRO DPRO DPHE *DLEU *DPHE DLEU ILE PRO *DLEU LEU



D10.17: *LEU *PHE LEU DPRO *LEU DILE PRO DPRO ALA DVAL



D10.19: DALA LEU VAL DPRO *LEU VAL PRO *DLEU VAL *ILE



D10.21: *LEU *DLEU PRO *DLEU DALA *LEU PRO *DLEU LEU PHE



D10.14: *DPHE VAL DLEU PRO *DLEU DLEU *DPHE *LEU *DALA PRO

>

H-bond acceptor *L *f | *| P |

æ *

Bins: [*AXBY*BB*B*X*BY]

RMSD to Design

v

.

H-bond donor P *| | *f *L *a P





D10.20: PRO *DLEU ALA PRO *DLEU DPHE LEU VAL *LEU *LEU



D10.22: PRO LEU *LEU *LEU PRO *DLEU DLEU *DPHE *GLY DPRO



Figure D1.5 (cont.): Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 10-amino acid macrocycles. (full figure legend described earlier). Related to Figure 3.



-30 0 RMSD to Design

۳ *

-10

-20

А *|

H-bond donor *A p p p P v *L *I A

D10.30: PRO *DLEU PHE DILE PRO LEU DLEU DPRO *DALA DVAL





15

Figure D1.5 (cont.): Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 10-amino acid macrocycles. (full figure legend described earlier). Related to Figure 3.



0 1 2 RMSD to Design

-30



Figure D1.5 (cont.): Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 10-amino acid macrocycles. *(full figure legend described earlier).* Related to Figure 3.



D10.46: PRO DVAL DILE PRO *DALA *LEU DPRO DLEU DLEU DILE



D10.49: DALA PRO *DPHE DPRO PHE DPRO PRO ALA LEU LEU



D10.51: ILE *DPHE *PHE DVAL *PHE ALA PRO DPRO LEU DLEU



D10.53: ALA DPRO PHE PRO VAL *DLEU DVAL ALA PRO PRO



D10.45: DVAL DALA DPHE ILE *DLEU *DALA PRO DPRO PRO MET H-bond donor Bins: [AAAY*B*BYBXX] n 20 10 rgy (kcal/mol) 0 æ -10 Ē Ŧ -20 < -30 2 3 RMSD to Design



D10.50: LEU VAL DPHE *DLEU DPRO *PHE ILE PRO LEU DPRO



D10.52: ILE DVAL *LEU *PHE *DPHE PRO ALA *DPHE ALA PRO



D10.54: DVAL DLEU DPRO *PHE ALA PRO DPRO PRO DPHE LEU



Figure D1.5 (cont.): Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 10-amino acid macrocycles. *(full figure legend described earlier).* Related to Figure 3.



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Figure 1.6: Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 11-amino acid macrocycles: Left panels show the torsion bin cluster and predicted conformational landscape for each design. * denotes N-methylated amino acids. Orange points denote an independently predicted structure with no cis-peptide bonds. Grey points represent predicted structures with at least one cis-peptide bond. Blue dots represent the local minimization of the designed macrocycle structure. For hydrogen bonding graphs, the orange boxes highlight the designed intramolecular hydrogen bonds. Amino acids without a backbone hydrogen bond donor (proline, D-proline, N-methylated amino acids) are marked by darker grey columns. Related to Figure 3.



D11.5: ALA PRO *DLEU VAL DLEU DILE PRO *DLEU *ALA DPRO *LEU



D11.7: PHE PRO *DLEU *ALA PRO DPRO PRO ALA VAL LEU LEU



D11.9: VAL *DLEU *LEU DPRO DLEU DALA DPRO PRO ALA DPHE DLEU









RMSD to Design



D11.8: PRO DPRO VAL DVAL *VAL VAL DPRO ILE DPRO *DLEU ALA



D11.10: DPRO ILE *ALA DILE DPRO PRO DLEU ILE *ALA DVAL *LEU



Figure D1.6 (cont.): Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 11-amino acid macrocycles. *(full figure legend described earlier).* Related to Figure 3.



D11.13: PRO DPRO VAL DVAL *DLEU DLEU DPRO PRO ILE *ALA ILE



D11.15: LEU DPRO DPRO *LEU LEU *DALA DLEU DPRO *DLEU DALA *DLEU



D11.17: PRO *DLEU DVAL LEU DPRO PRO DPRO DALA PHE LEU ILE



D11.19: DILE DPRO *DLEU DALA DPRO PRO DPRO DLEU DALA DLEU DLEU



D11.12: DVAL DPRO PRO *DLEU DALA PHE DPRO PRO DVAL LEU *DALA Bins: [AY*BBBY*BBYB] v p * to the temperature of te



H-bond donor Bins: [AAAAXB*YYYYY] L РМ p 20 ما 10 H-bond acceptor . *a f l l f a (kcal/mol) 0 -10 Energ -20 ۵. -30 ⋝ RMSD to Design

D11.14: PRO DPRO DALA DPHE DLEU ILE DPHE *DALA LEU PRO MET

D11.16: LEU DPHE *LEU *DALA DPRO DPRO *DLEU DVAL PRO DPRO DALA



D11.18: DALA DILE DPHE LEU DLEU *DALA *DLEU PRO PRO DPHE DLEU



D11.20: PRO *DALA LEU PRO DPRO *DALA *DLEU PHE *DLEU VAL ILE



Figure 1.6 (cont.): Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 11-amino acid macrocycles. *(full figure legend described earlier).* Related to Figure 3.

20

10

0

-10

-20

-30

0

1

Energy (kcal/mol)



D11.23: DVAL DPRO *DVAL DLEU *ILE ILE PRO *DLEU DILE ILE PRO



D11.25: DLEU DPRO *DPHE DALA DPRO PRO DPRO DALA DALA DLEU DLEU

D11.22: PHE *DLEU LEU *DLEU *DLEU DILE LEU PRO DPRO PRO LEU

*I L

F

H-bond acceptor p P L i *I *I L

Bins: [*A*AAYYBXXY*AY]

2 3 RMSD to Design H-bond donor *I *I i L P p P L



Figure S1.7: Structure-energy landscapes and hydrogen-bonding patterns for designed and selected 12-amino acid macrocycles: Left panels show the torsion bin cluster and predicted conformational landscape for each design. * denotes N-methylated amino acids. Orange points denote an independently predicted structure with no cis-peptide bonds. Grey points represent predicted structures with at least one cis-peptide bond. Blue dots represent the local minimization of the designed macrocycle structure. For hydrogen bonding graphs, the orange boxes highlight the designed intramolecular hydrogen bonds. Amino acids without a backbone hydrogen bond donor (proline, D-proline, N-methylated amino acids) are marked by darker grey columns. Related to Figure 3.











D12.7: DPHE DPRO *PHE DILE *DALA DILE DPRO PRO DVAL LEU *DLEU DILE







D12.6: DPRO *PHE ILE DPRO LEU DLEU LEU DPRO PRO DALA *DALA ILE



D12.8: PHE ALA DPRO PRO LEU DVAL PRO LEU ALA ILE DPRO DLEU



D12.10: *LEU DPRO DLEU LEU *DALA DILE DPRO PRO DVAL VAL *DLEU DVAL

