The effect of D-amino acids on the conformation of EntF

A MOLECULAR DYNAMICS STUDY

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EntF

enterobactin synthase component F
quorum sensing peptide produced by intestinal bacteria
plays a role in colorectal cancer metastasis

SNLVECVFSLFKKCN

EntF consists of L amino acids.
Each amino acid in the sequence is replaced
by a D amino acid.
These constructs were then analysed with
LC-ESI-TIMS-MS.

L-Amino Acid   D-Amino Acid

EntF Q76   10 nM
Q76 S1d   1 nM
Q76 N2d
Q76 L3d
Q76 V4d
Q76 E5d
Q76 C6d
Q76 V7d
Q76 S9d
Q76 L10d
Q76 F11d
Q76 K12d
Q76 K13d
Q76 C14d
Q76 N15d

420   430   440   450   460   470   480

CCS [Å²]   Voeten & Haselberg
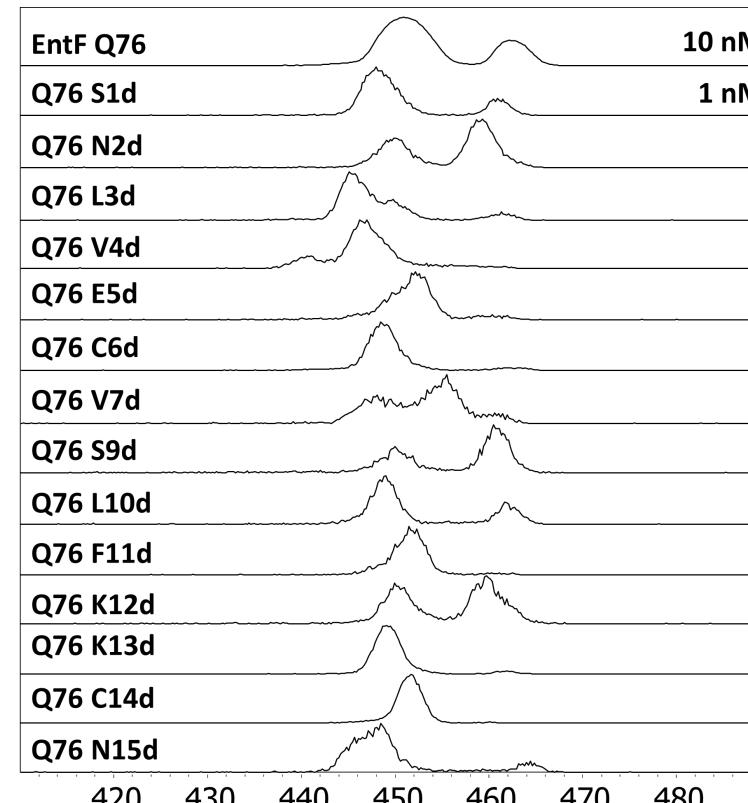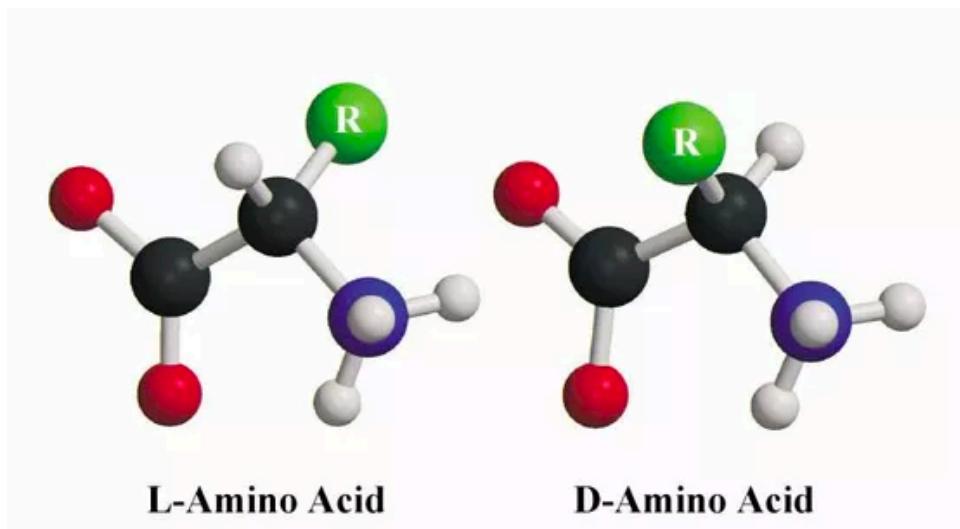
Research question

SNLVECVFSLFKKCN

Changing any L amino acid to its D form alters the conformation of EntF. How?

Structural information at atomistic resolution is required.

All-atom force field molecular dynamics provides required resolution

$$F = m\frac{d^2r}{dt^2} = ma$$

Given the potential, one can numerically integrate the trajectory of the whole system as a function of time.

$$F = -\frac{dV(r)}{dr}$$

The force F is given by the gradient of the potential *V(r)*. *V(r)* is also known as *force field*.

$$V(r) = \sum_{bonds} k_r(r - r_{eq})^2 + \sum_{angles} k_\theta(\theta - \theta_{eq})^2 + \sum_{dihedrals} \frac{1}{2}\nu_n(1 + cos(n\phi - \phi_0)) + \sum_{i<j}\left(4\epsilon\left(\left(\frac{\sigma}{r_{ij}}\right)^{12} - \left(\frac{\sigma}{r_{ij}}\right)^6\right) + \frac{q_i q_j}{\epsilon_d r_{ij}}\right)$$

bonds   bends   torsions   non-bonded

MOLECULAR POTENTIAL ENERGY

$$U = \sum \tfrac{1}{2} K_b (b-b_0)^2 + \sum \tfrac{1}{2} K_\theta (\theta-\theta_0)^2$$

All Bonds   All Angles

Hooke 1635
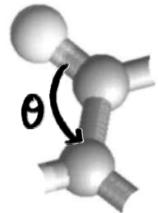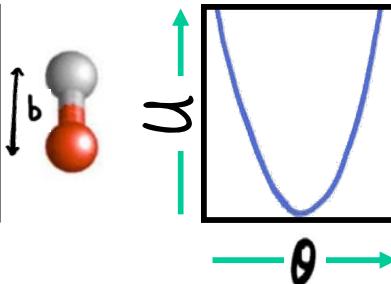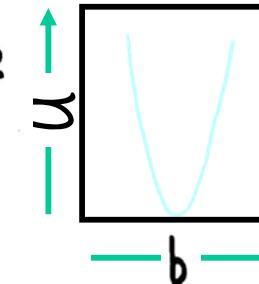
$$+ \sum K_\phi [1-\cos(n\phi+\delta)]$$

All Torsion Angles

Fourier 1768
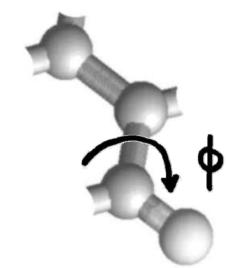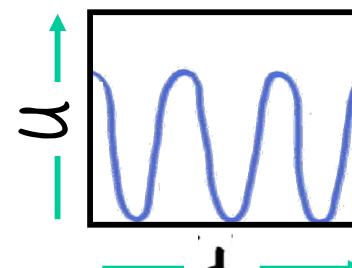
$$+ \sum \epsilon \left[\left(\tfrac{r_0}{r}\right)^{12} - 2\left(\tfrac{r_0}{r}\right)^{6} \right]$$

All Nonbonded pairs

Van der Waals 1837
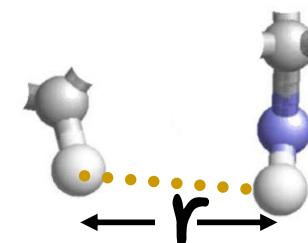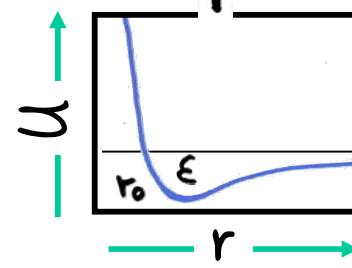
$$+ \sum 332 q_i q_j / r$$

All partial charges

Simple sum over many terms

Coulomb 1736
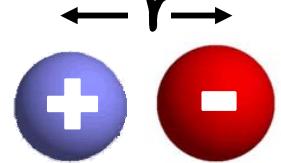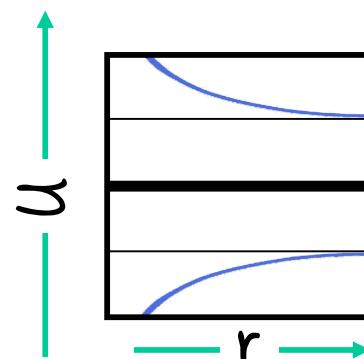
Numerical approach

Snapshots

From positions, get forces
From forces, get new positions

t $t + \Delta t$ $t + 2\Delta t$ $t + 3\Delta t$
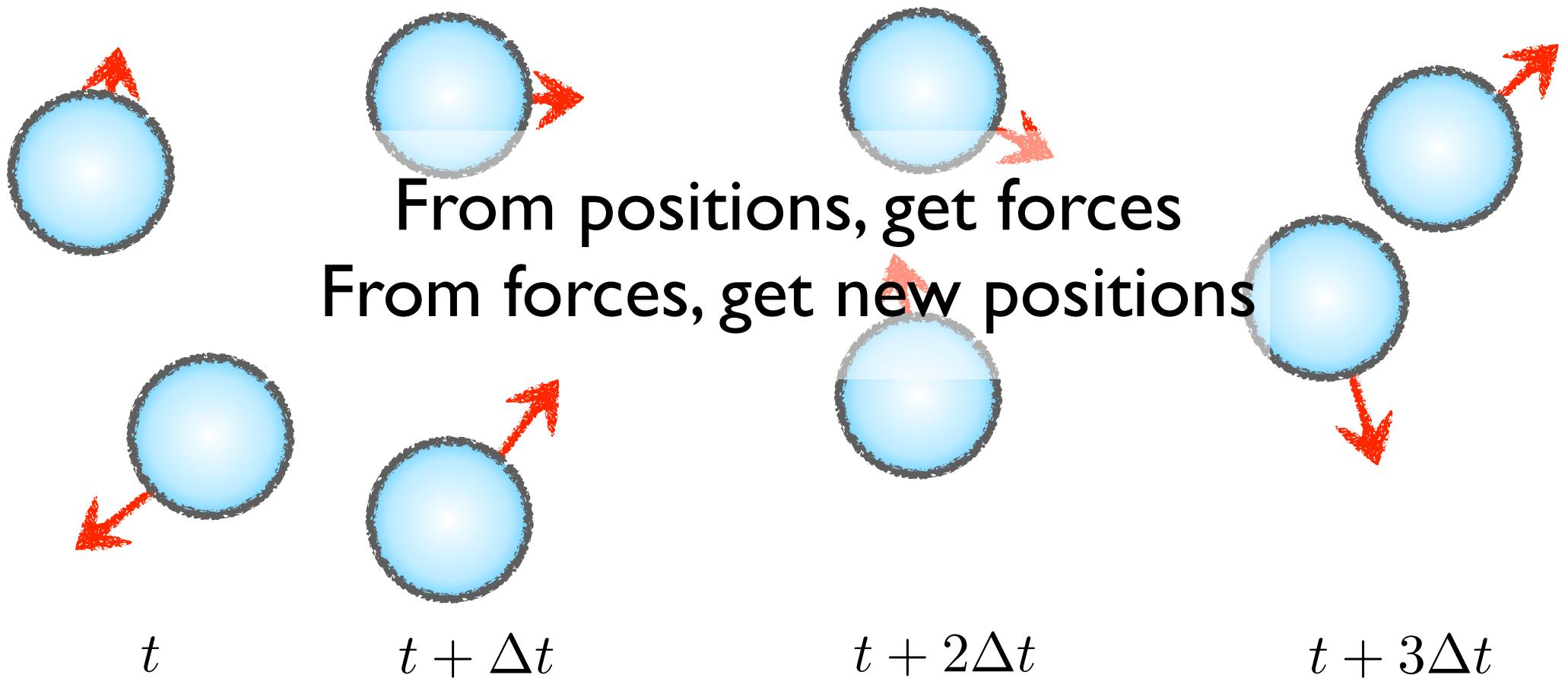
Choosing the time step

Δt too large
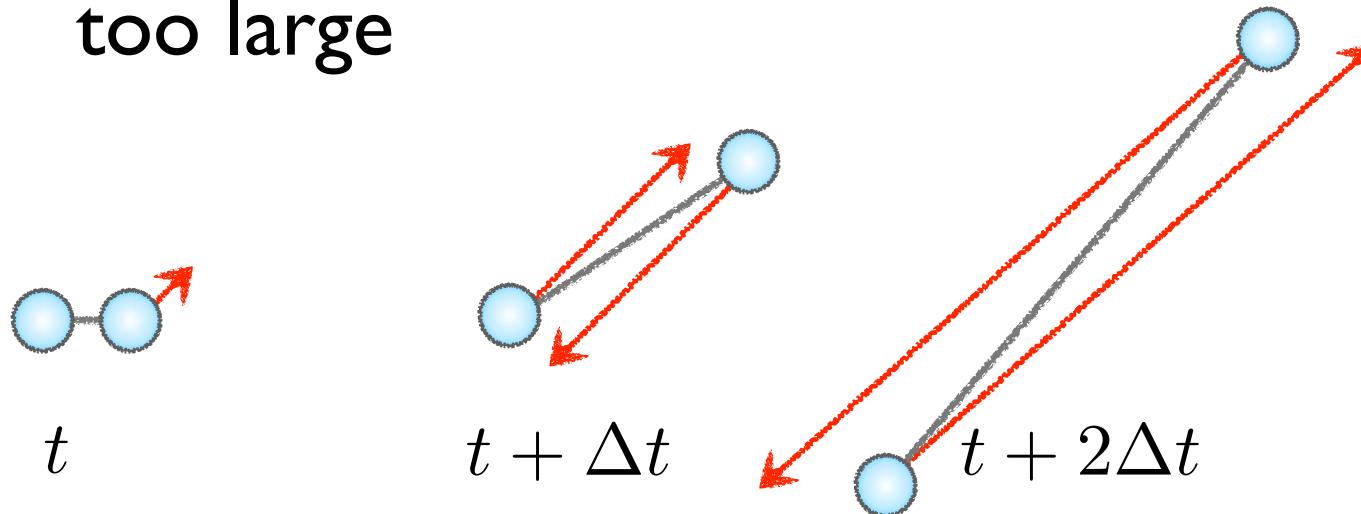
t $t + \Delta t$ $t + 2\Delta t$

Δt just fine (2 fs for force field MD)
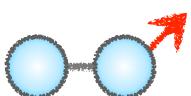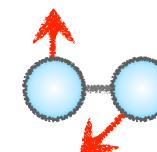
t $t + \Delta t$ $t + 2\Delta t$

Molecular dynamics

- assign positions and velocities to particles

- compute forces on all particles

- integrate equations of motion

- measure properties

- stop
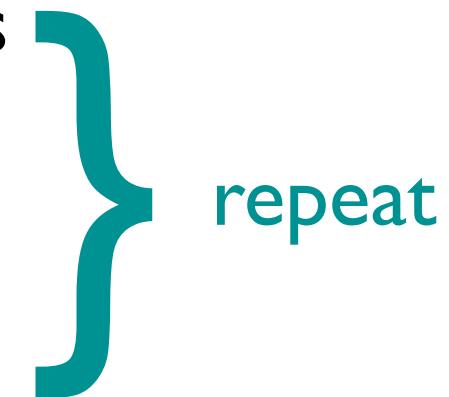
} repeat

Starting coordinates

No 3D structural information available for EntF

SNLVECVFSLFKKCN

pH = 3:
- glutamate E5 is protonated -COOH
- lysines K12 and K13 are protonated -NH$_3^+$
- N-terminus is protonated -NH$_3^+$
- C-terminus is deprotonated -COO$^-$

C6 and C14 form a disulphide bond.

generated with Avogadro
image created with pyMOL
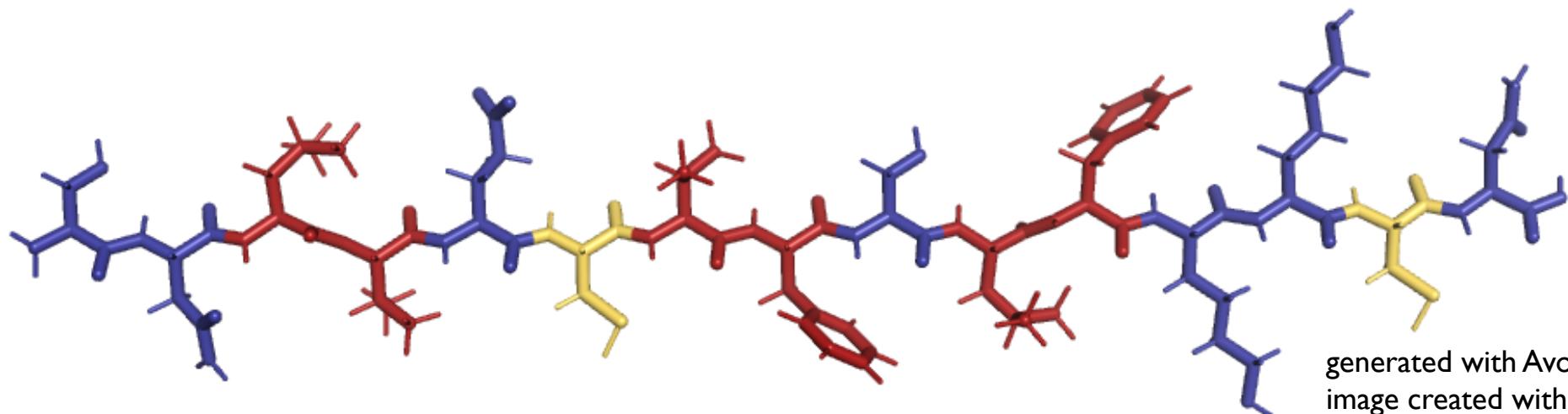
Generate EntF starting conformation

Solvate* linear peptide in periodic water box with
[NaCl] = 25 mM, after neutralizing the charge in the system

AMBER99SB-ILDN force field
TIP3P water

T = 298 K
p = 1 bar

Run for 200 ns.
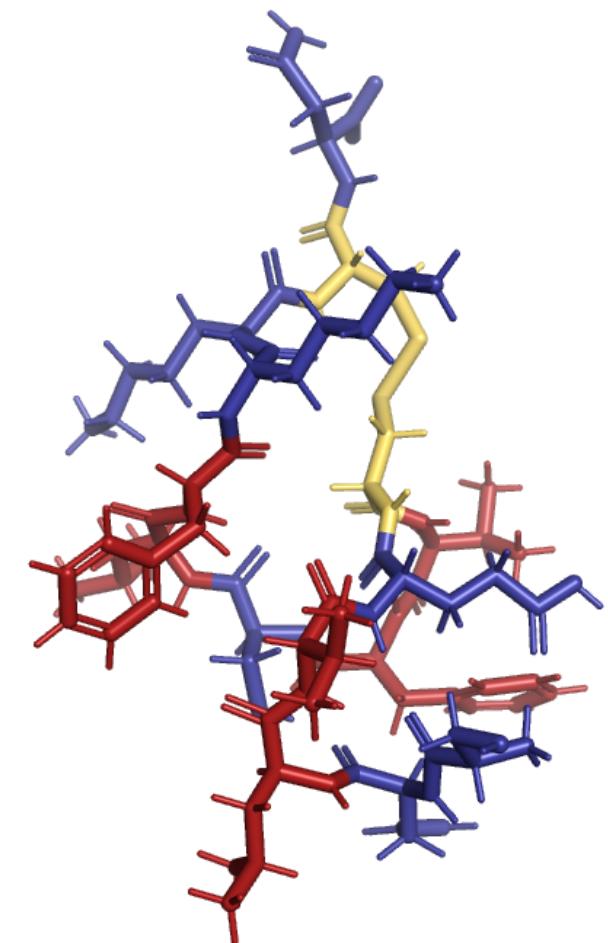
Take conformation with sulphur atoms close together and make disulphide bond.
Solvate* again.
Run for 1 microsecond. This takes about a week.

*Actually: solvate, energy minimise, equilibrate positions of water and ions, all using GROMACS v2018.4

1 μs molecular dynamics of L-EntF

Extract relevant information from the 1 μs MD simulation

Experiment:
What happens is real.
Macroscopic properties

Simulation:
Molecular understanding
Positions and velocities of particles

Data from experiment:
Collisional Cross Section CCS

Size and shape of peptide

Data from simulations:
Radius of gyration R_{gyr}

$$\text{CCS} = \pi R_{\text{gyr}}^2$$
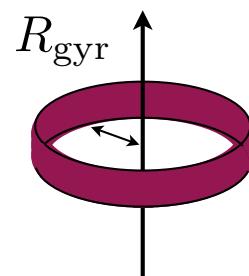
$$R_{\text{gyr}} = \sqrt{\frac{\sum_i m_i r_i^2}{\sum_i m_i}}$$

particle index - i
mass - m
distance to center of mass - r

Collisional Cross Section
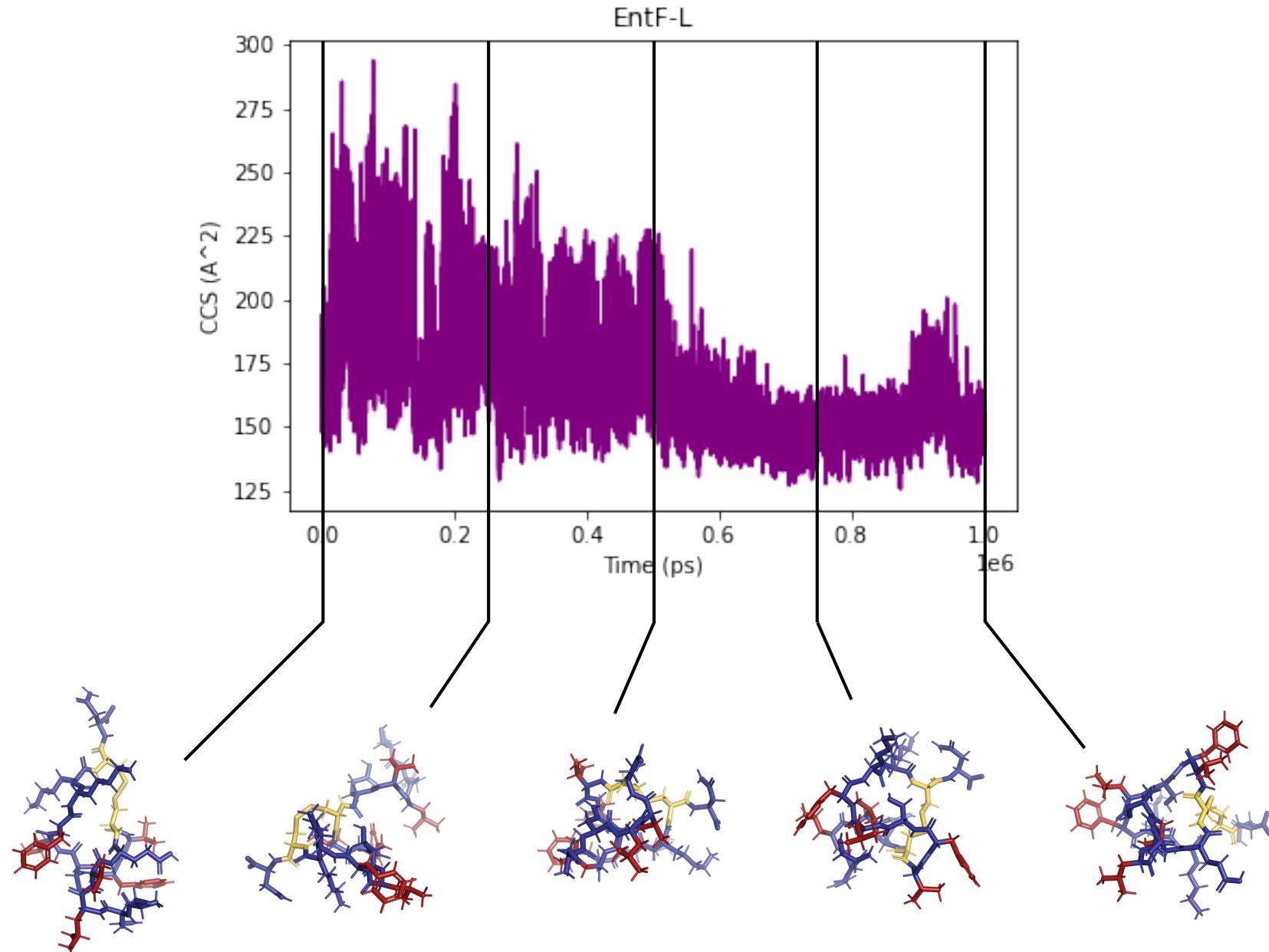
Collisional Cross Section
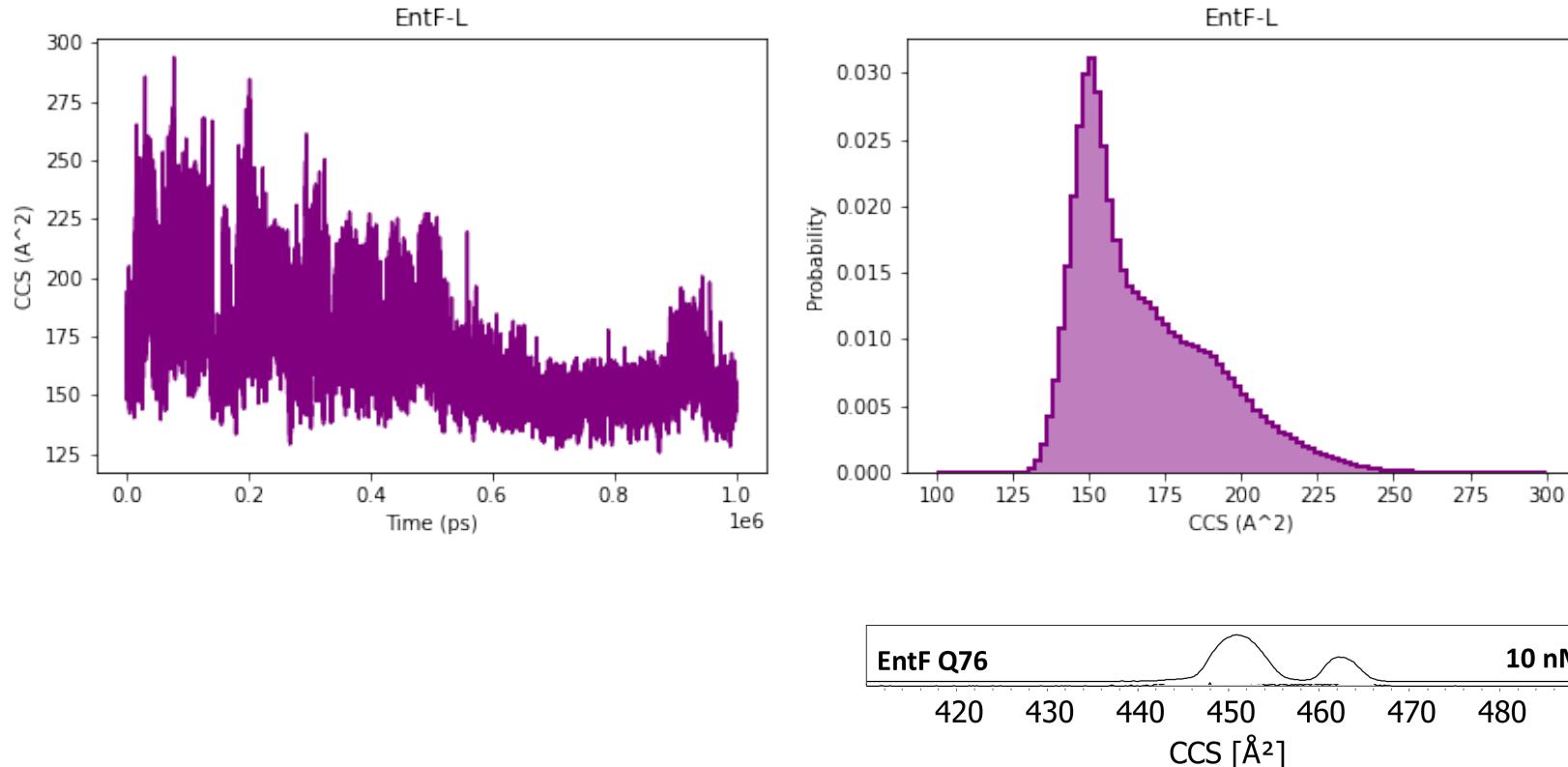

CCS values from experiment and simulation do not match.

Conformations with lower CCS dominant in both experiment and simulation.

Extract relevant information from the 1 μs MD simulation

Experiment:
What happens is real.
Macroscopic properties

Simulation:
Molecular understanding
Positions and velocities of particles

Data from experiment:
Retention time
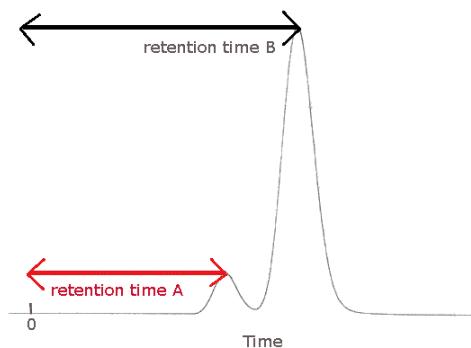
hydrophilicity
hydrophobicity
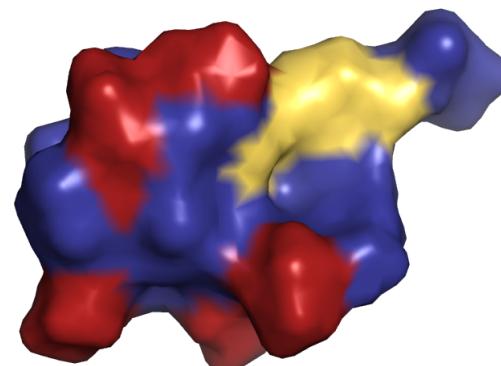
Data from simulations:
Solvent accessible surface area
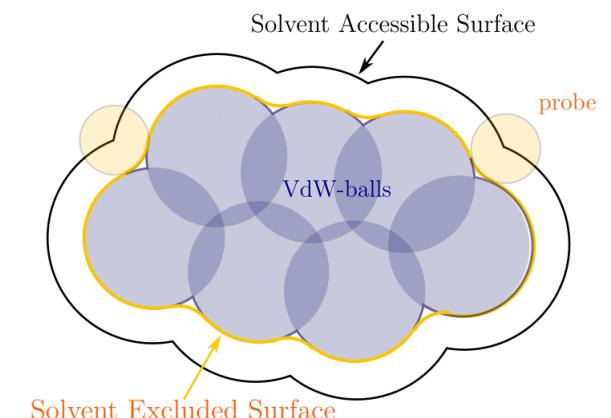
Hydrophobicity/hydrophilicity
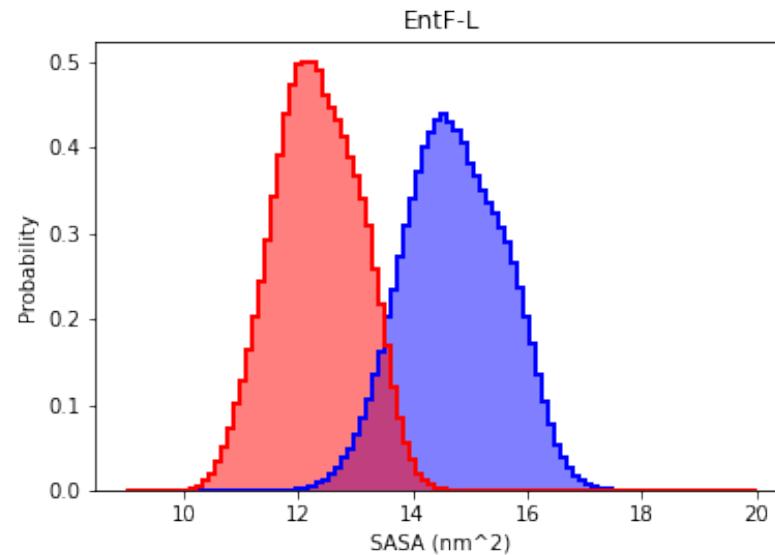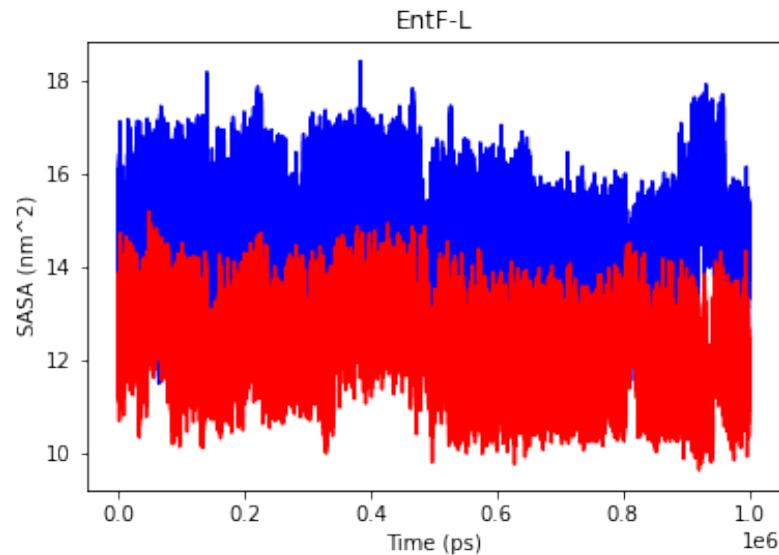
hydrophilic SASA: backbone and side chains of SNEK
hydrophobic SASA: side chains of LVF

SNLVECVFSLFKKCN
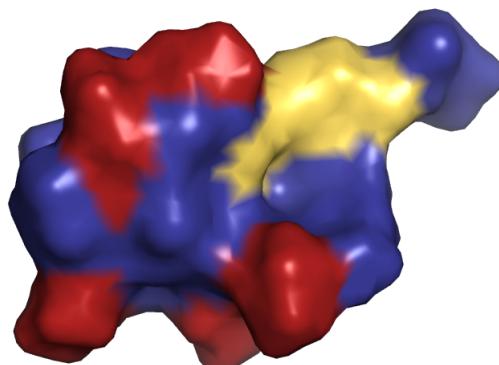
Hydrophilic SASA shows more detail than hydrophobic SASA.

Combine CCS and SASA
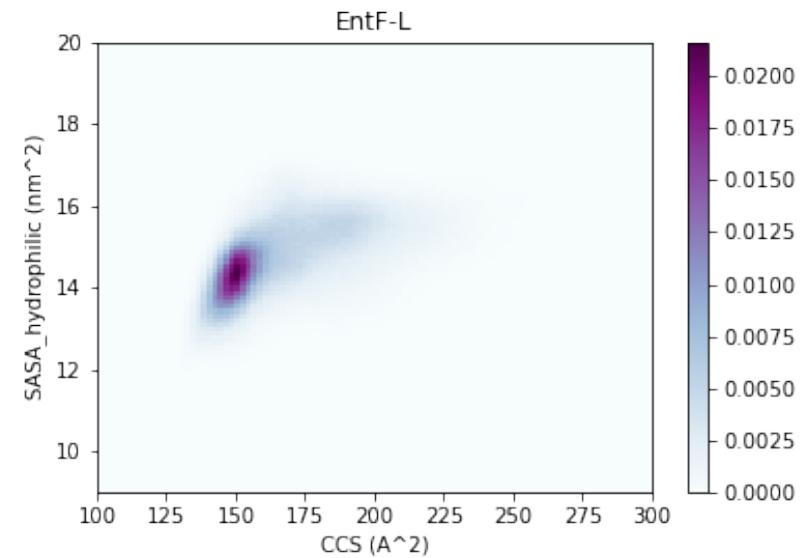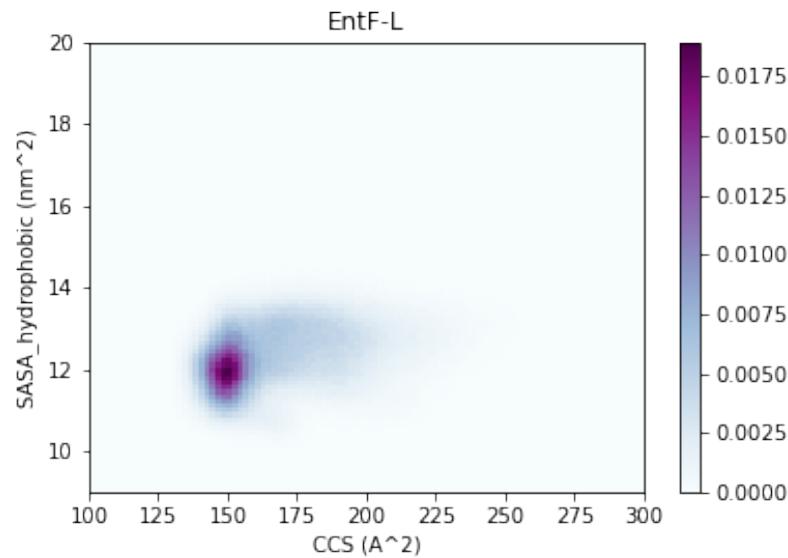
Increase in CCS correlates with increase in hydrophilic SASA.

Setting up the D amino acid series

SNLVECVFSLFKKCN
sNLVECVFSLFKKCN
SnLVECVFSLFKKCN
SNlVECVFSLFKKCN
SNLvECVFSLFKKCN
SNLVeCVFSLFKKCN
SNLVEcVFSLFKKCN
SNLVECvFSLFKKCN
SNLVECVfSLFKKCN
SNLVECVFsLFKKCN
SNLVECVFSlFKKCN
SNLVECVFSLfKKCN
SNLVECVFSLFkKCN
SNLVECVFSLFKkCN
SNLVECVFSLFKkcN
SNLVECVFSLFKkcn

Replace L amino acid with D amino acid
Solvate* peptide in periodic water box with
[NaCl] = 25 mM, after neutralizing the charge in the system

AMBER99SB-ILDN force field
TIP3P water
T = 298 K
p = 1 bar

Run for 1 microsecond.

Peptides with D-cysteine:
First run linear peptide for 200 ns. Then take conformation
with sulphur atoms close together and make disulphide bond.
Solvate* again.
Run for 1 microsecond.

*Actually: solvate, energy minimise, equilibrate positions of water and ions, all using GROMACS v2018.4

Results

SASA$_{hydrophilic}$ (nm^2)

CCS (Å^2)
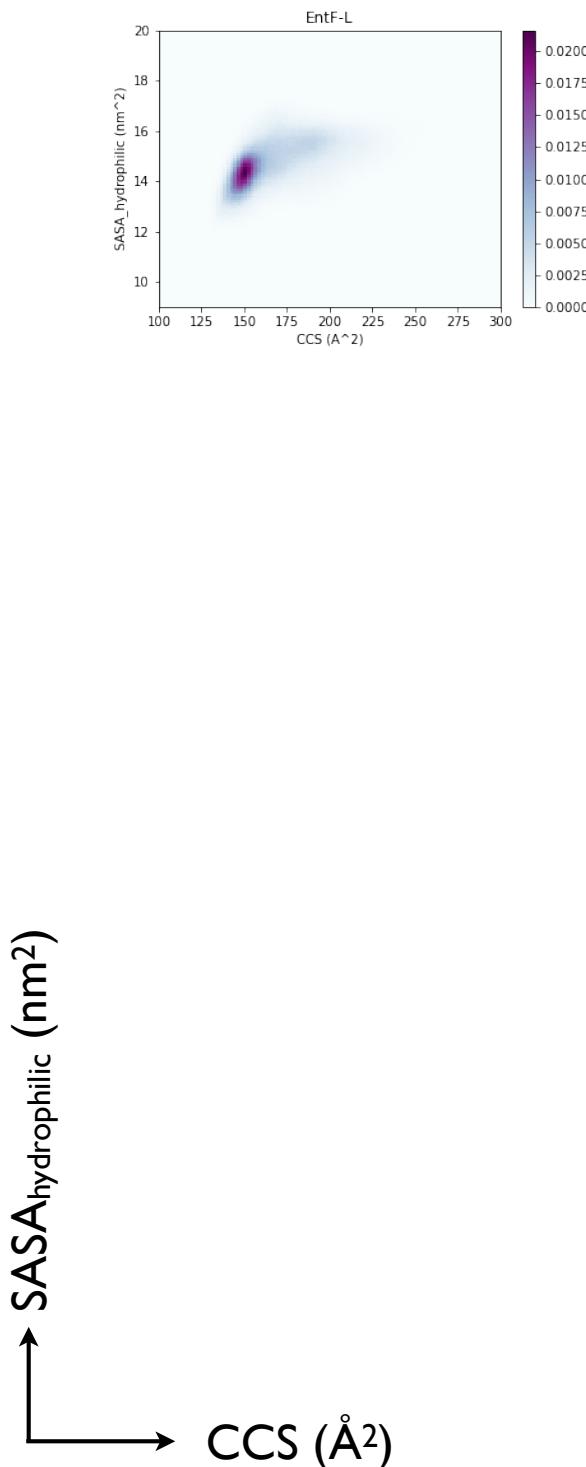
Results

Comparison to experiment I

EntF Q76   10 nM
Q76 S1d   1 nM
Q76 N2d
Q76 L3d
Q76 V4d
Q76 E5d
Q76 C6d
Q76 V7d
Q76 S9d
Q76 L10d
Q76 F11d
Q76 K12d
Q76 K13d
Q76 C14d
Q76 N15d

CCS (A^2)

CCS [Å²]   Voeten & Haselberg
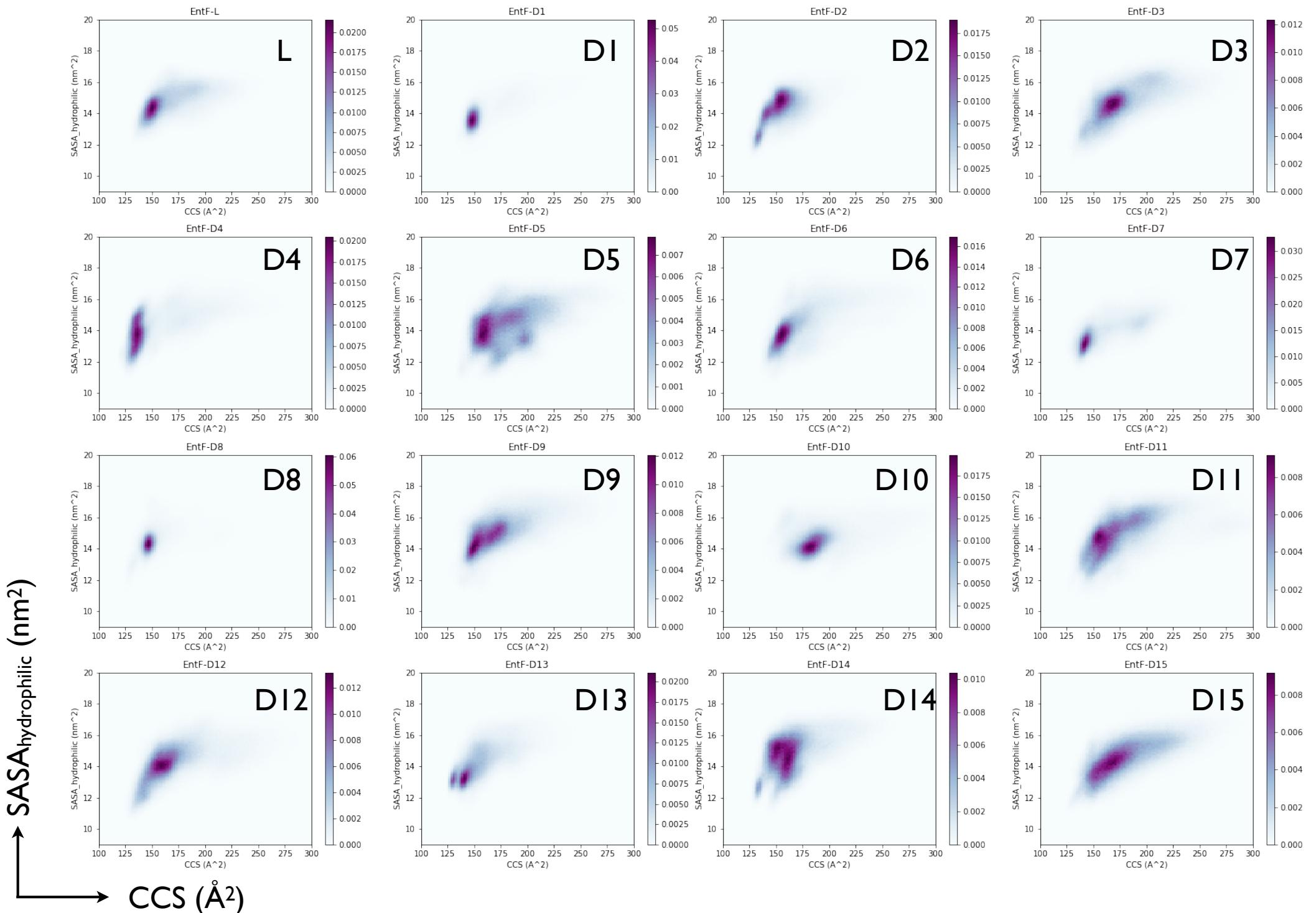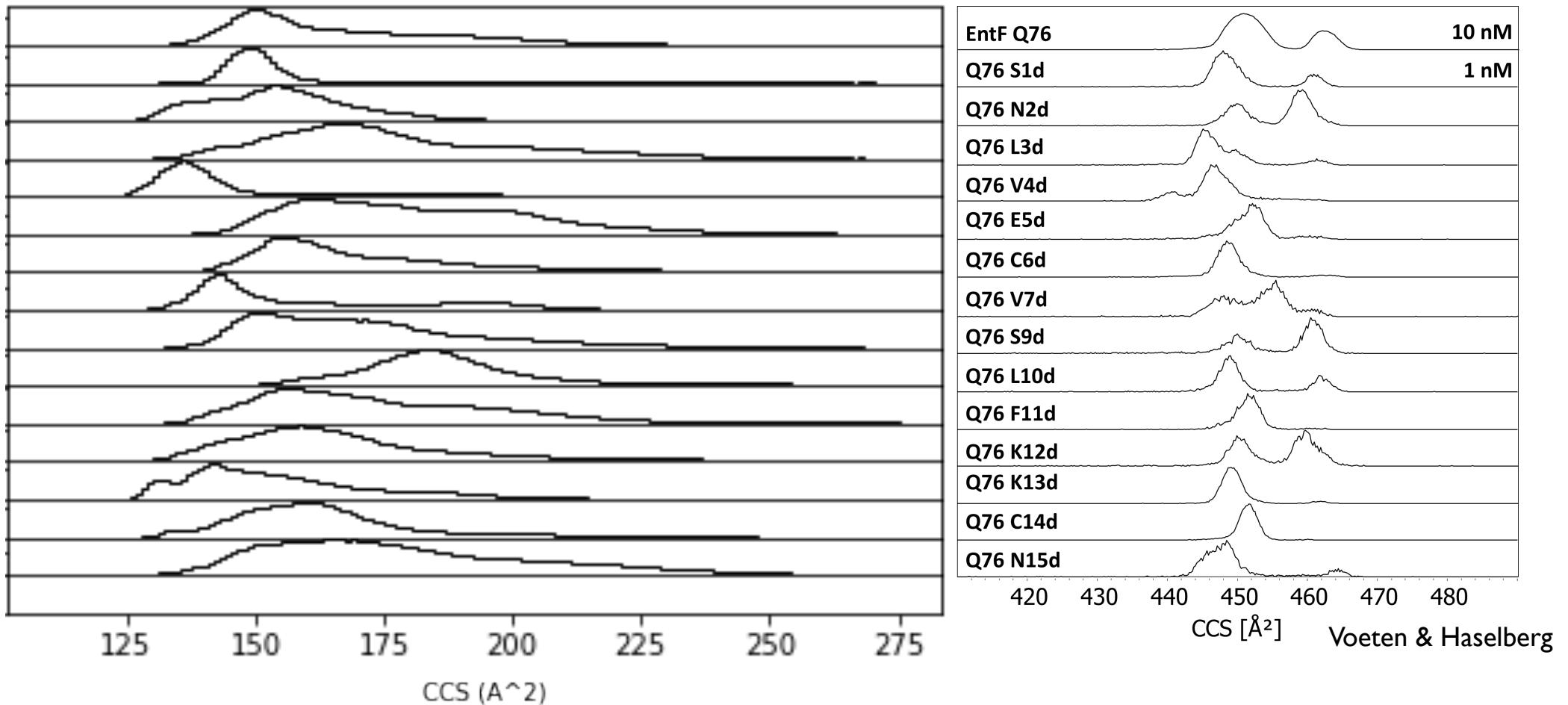
Comparison to experiment II
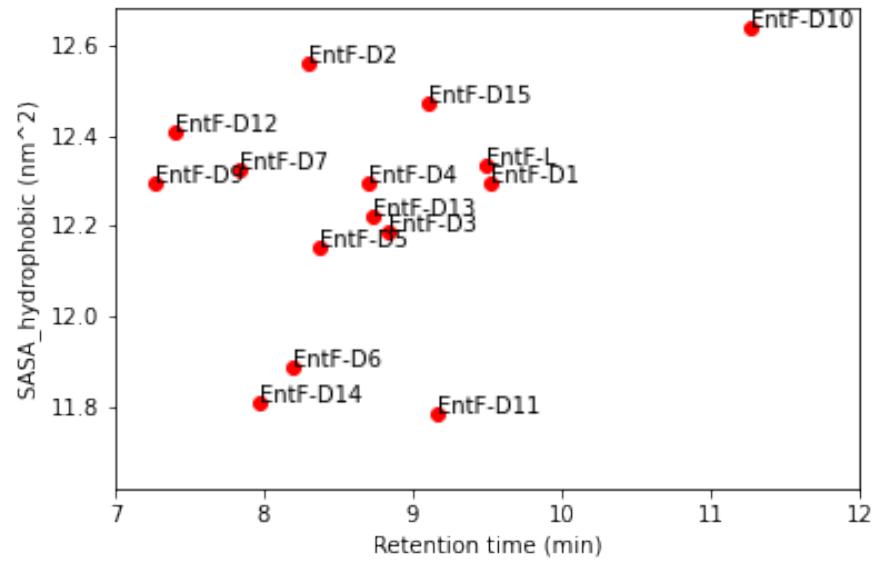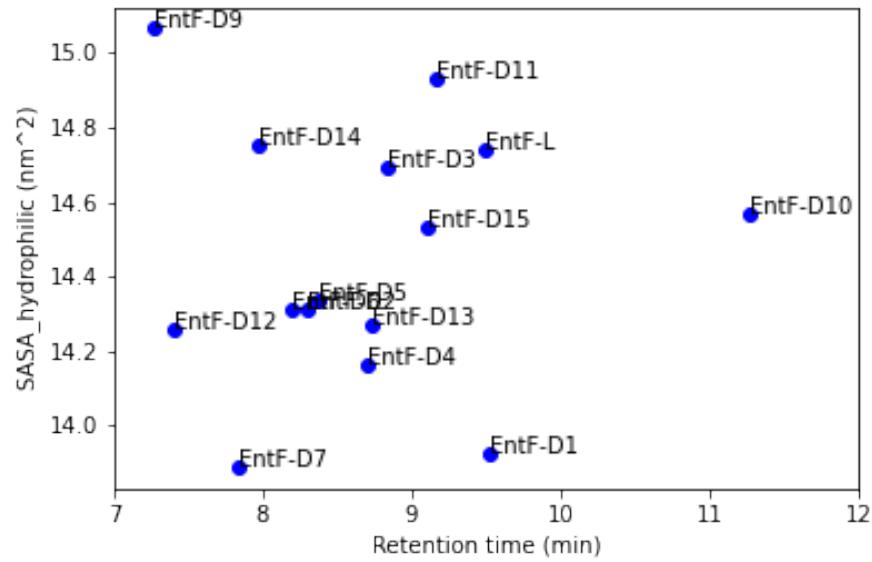
Conclusions

- Including a D amino acid in EntF has an effect on the conformation of the peptide.

- Simulation data can be analysed further, focusing on specific interactions.

- Connection between simulations and experimental results is not entirely clear yet.

EntF

ACKNOWLEDGEMENTS

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Performing simulations   Cindy van der Schaaff & Isa Vos

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