SUPPORTING INFORMATION

"A molecular dynamics description of the conformational flexibility of the L-iduronate ring in glycosaminoglycans."

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Details of simulation protocols

Two models were considered with iduronate residues in the $^1C_4$ and ^2S_O conformations respectively. Starting structures were built taking as reference the coordinates of the dodecasaccharide fragments of natural heparin, from PDB databank (entry 1hpn) [1].

In all cases calculations were performed with AMBER force field (parameter set "parm91") [2], with the addition of explicit parameter modifications developed for carbohydrates (GLYCAM 93[3]) and sulfate groups [4]. Charges were taken from the CERMAV database at (http://www.cermav.cnrs.fr/bases/base_donnees_structure.html).[5]

In vacuo simulations were carried out using AMBER 5.0 as described in ref. 6.

Explicit solvent simulations were performed with periodic boundary conditions (PBC). The system was constructed by addition of 12 randomly placed Na^+ cations positioned far enough from the solute for electroneutrality and solvated by TIP3P water with sizes of: 2195 water molecules (box size $40 \times 45 \times 55$ Å^3) and 2474 water molecules (box size $55 \times 45 \times 43$ Å^3) in simulations with classic cutoff schemes; alternatively, 3893 water molecules (box size $54 \times 50 \times 48$ Å^3) and 3858 water molecules (box size $54 \times 50 \times 48$ Å^3) were used for the two models in PME simulations.

Explicit water simulations with classic cutoff scheme

Simulations were performed using the Sander module of AMBER 5.0[7] with SHAKE[8] on the hydrogen atoms with a tolerance of 0.0005 Å, and a 1 fs time step for the integration of Newton's equations. A 9 Å residue-based cutoff was applied to non-bonded interactions, and the nonbonded pair list was updated every 25 steps. A constant temperature of 298 K was kept using the Berendsen temperature coupling algorithm with a time constant of 0.2 ps. Equilibration was started by 100 cycles of minimization with steepest descent algorithm followed by full conjugate gradient minimization until the maximum derivative was <0.01 kcal/mol·Å, keeping the positions of the solute fixed. After this initial minimization 25 ps of constant pressure molecular dynamics were carried out once more with the positions of the solute fixed. Initial atomic velocities were assigned from a Maxwellian distribution at 298 K. This equilibration period was then followed by several nanoseconds of "productive" constant volume dynamics. Coordinates were stored every 0.5 ps.

### Explicit water simulations with Particle Mesh Ewald scheme

For these simulations, the Sander module of AMBER 6.0[9] was used with SHAKE on the hydrogen atoms with a tolerance of 0.0001 Å, and a 2 fs time step. A 9 Å atom-based cutoff was applied to Lennard-Jones interactions, and the nonbonded pair list was updated every 10 steps. A constant temperature of 300 K was maintained using the Berendsen temperature coupling algorithm with a time constant of 0.2 ps. All molecular dynamics simulations were carried out in the NPT ensemble at a pressure of 1 bar. To avoid the "flying ice cube" phenomena [10], the translational motion of the center of mass, and rotation about it, was periodically removed.

An 11-step protocol was used for the equilibration process[11]. It was started by 1000 cycles of minimization with steepest descent algorithm, and 25 ps of NPT molecular dynamics, keeping the positions of the solute and counterions fixed (restraint 500 Kcal/mol·Å^2). After this point, all

subsequent simulations were performed using the particle mesh Ewald method (PME) for the treatment of long-range electrostatic interactions. A charge grid spacing of approximately 1 Å with cubic B-spline interpolation and a direct sum tolerance of 1×10^{-6} were used. The initial velocities were assigned from a Maxwellian distribution at the starting temperature. Equilibration of the system was continued by 25 ps of NPT dynamics with the solute restrained by 500 Kcal/mol·$Å^2$. Afterwards, 1000 steps of minimization with the positions of the solute restrained by 25 Kcal/mol·$Å^2$ were performed, followed by 3 ps of molecular dynamics with the same restraint. This was then followed by five rounds of 600 step minimization, starting with a 20 Kcal/mol· $Å^2$ restraint on the solute and reducing it by 5 kcal/mol· $Å^2$ during each round. Finally, the system was slowly heated from 100 to 300 k and allowed to equilibrate at this temperature. Then, 2 ns of unrestrained molecular dynamics were performed, writing coordinates to the trajectory files after every 0.5 ps.

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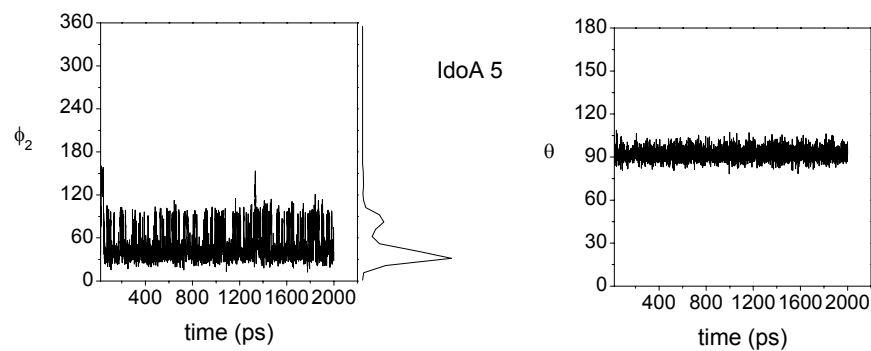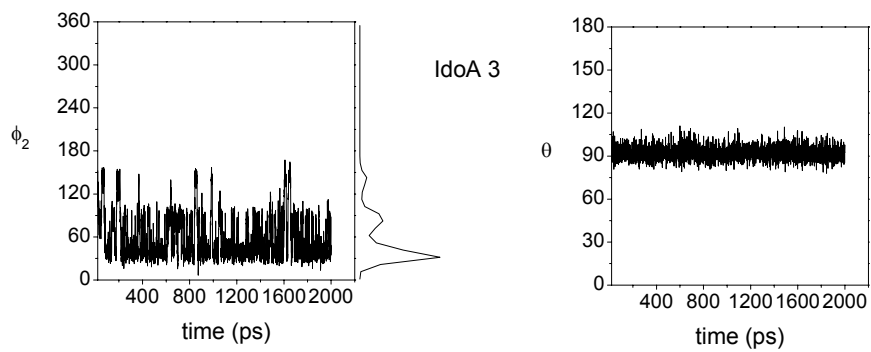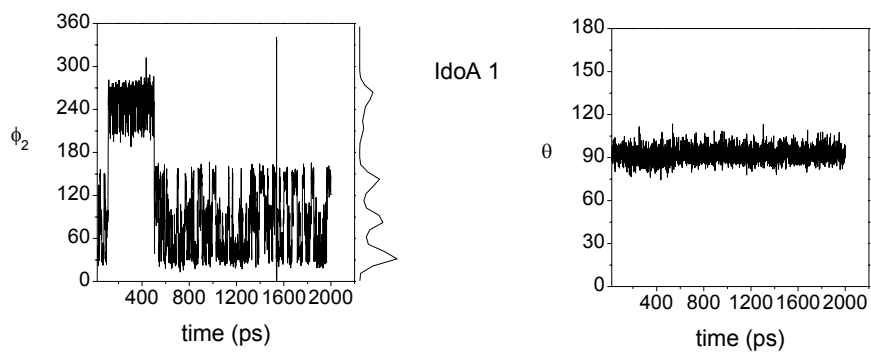
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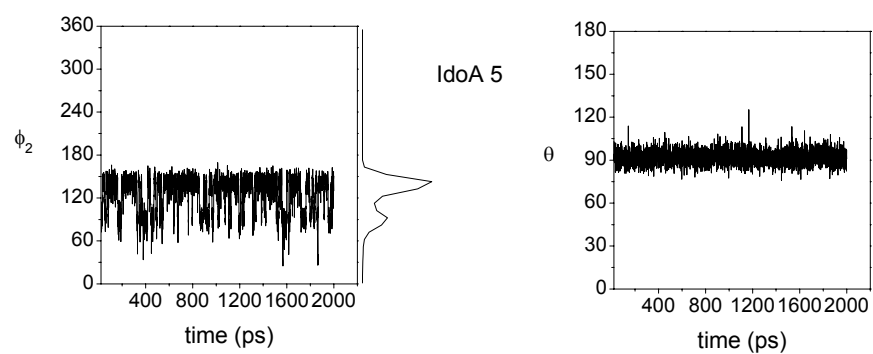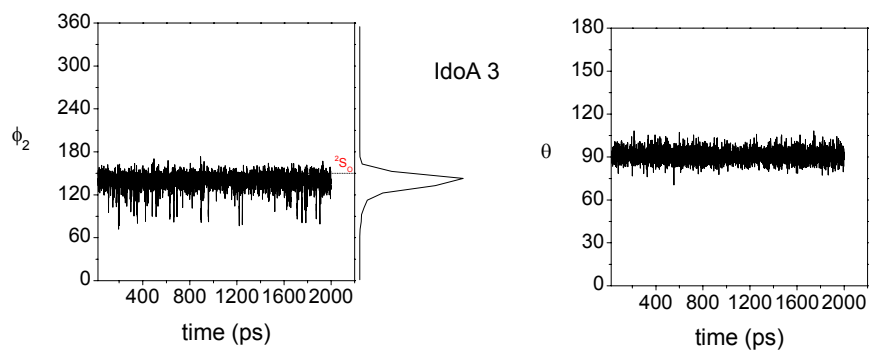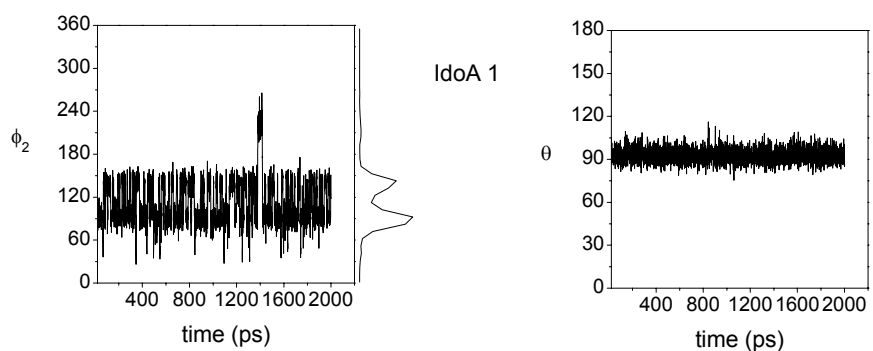
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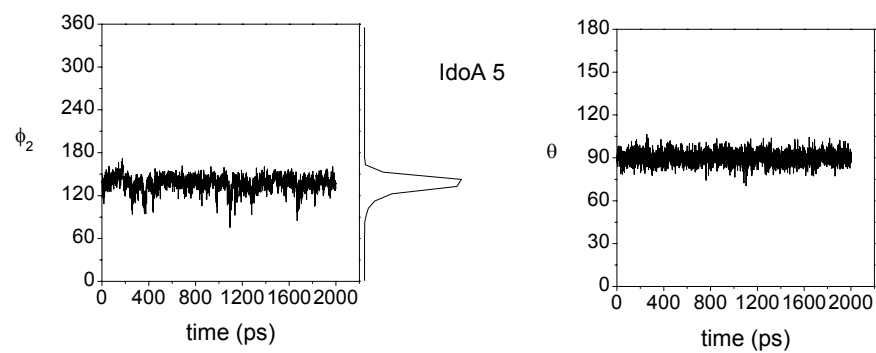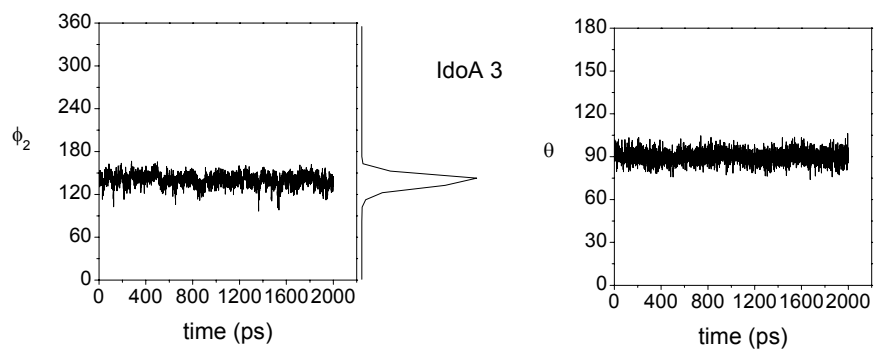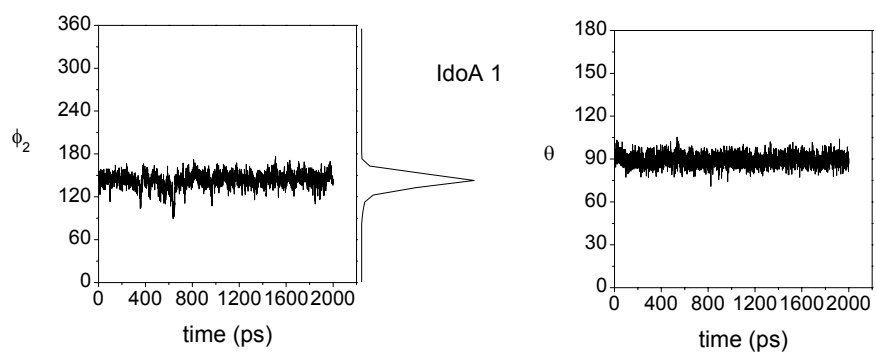
Trajectories of the Cremer-Pople puckering coordinates using PME electrostatic treatment

Ensemble Averaged Iduronate H-H Coupling Constants (Hz) Observed During Molecular Dynamics Simulation

(a1) Dynamics of IdoA-1 of **1** in Vacuo ($\varepsilon = 80$)		
Coupling	$^1C_4$	«Pseudorotational ensemble»
H1-H2	1.8 ± 0.5	5.8 ± 2.0
H2-H3	2.5 ± 0.9	3.7 ± 3.5
H3-H4	2.6 ± 0.9	4.0 ± 2.4
H4-H5	1.4 ± 0.8	3.0 ± 2.2

Least-squares analysis

$f(^1C_4) = 0.52$ $\qquad\qquad$ $f(^xS_y\ \text{ensemble}) = 0.48$

Σerrors squared = 0.59 *

Mean weighted calculated couplings

$J_{12} = 3.7$ $\quad$ $J_{23} =\ $-- $\quad$ $J_{34} = 3.3$ $\quad$ $J_{45} = 2.2$

Experimental Coupling Constants

$J_{12} = 3.4$ $\quad$ $J_{23} =\ $-- $\quad$ $J_{34} = 3.4$ $\quad$ $J_{45} = 2.9$

(a2) Dynamics of IdoA-3 of **1** in Vacuo ($\varepsilon = 80$)		
Coupling	$^1C_4$	«Pseudorotational ensemble»
H1-H2	1.8 ± 0.5	6.7 ± 1.0
H2-H3	2.7 ± 1.0	2.4 ± 2.7
H3-H4	2.7 ± 0.9	3.3 ± 1.6
H4-H5	1.5 ± 0.8	2.6 ± 1.7

Least-squares analysis

$f(^1C_4) = 0.74$ $\qquad\qquad$ $f(^xS_y\ \text{ensemble}) = 0.26$

Σerrors squared = 5.41

Mean weighted calculated couplings

$J_{12} = 3.1$ $\quad$ $J_{23} = 2.6$ $\quad$ $J_{34} = 2.9$ $\quad$ $J_{45} = 1.8$

Experimental Coupling Constants

$J_{12} = 2.9$ $\quad$ $J_{23} = 5.6$ $\quad$ $J_{34} = 3.9$ $\quad$ $J_{45} = 2.7$

(a3) Dynamics of IdoA-5 of **1** in Vacuo ($\varepsilon = 80$)		
Coupling	$^1C_4$	«Pseudorotational ensemble»
H1-H2	1.8 ± 0.5	6.7 ± 0.9
H2-H3	2.7 ± 1.0	1.7 ± 2.0
H3-H4	2.8 ± 0.9	3.3 ± 1.5
H4-H5	1.5 ± 0.8	2.7 ± 1.7

Least-squares analysis

$f(^1C_4) = 0.87$ $\qquad\qquad$ $f(^xS_y\ \text{ensemble}) = 0.13$

Σerrors squared = 5.78

Mean weighted calculated couplings

$J_{12} = 2.4$ $\quad$ $J_{23} = 2.6$ $\quad$ $J_{34} = 2.9$ $\quad$ $J_{45} = 1.7$

Experimental Coupling Constants

$J_{12} = 2.7$ $\quad$ $J_{23} = 5.7$ $\quad$ $J_{34} = 3.5$ $\quad$ $J_{45} = 2.8$

(b1) Dynamics of IdoA-1 of **1** in Vacuo ($\varepsilon = 5r$)		
Coupling	$^1C_4$	«Pseudorotational ensemble»
H1-H2	1.9 ± 0.5	7.0 ± 1.3
H2-H3	2.7 ± 1.0	6.9 ± 2.6
H3-H4	2.8 ± 1.0	2.6 ± 2.3
H4-H5	1.3 ± 0.8	1.7 ± 1.7

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Least-squares analysis

$f(^1C_4) = 0.69$ $f(^xS_y$ ensemble$) = 0.31$

Σerrors squared $= 2.62$ *

Mean weighted calculated couplings

$J_{12} = 3.5$ $J_{23} = --$ $J_{34} = 2.7$ $J_{45} = 1.4$

Experimental Coupling Constants

$J_{12} = 3.4$ $J_{23} = --$ $J_{34} = 3.4$ $J_{45} = 2.9$

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(b2) Dynamics of IdoA-3 of **1** in Vacuo ($\varepsilon = 5r$)		
Coupling	$^1C_4$	«Pseudorotational ensemble»
H1-H2	1.9 ± 0.5	6.3 ± 1.0
H2-H3	2.7 ± 0.9	9.5 ± 1.1
H3-H4	2.8 ± 0.9	4.7 ± 1.9
H4-H5	1.4 ± 0.8	3.2 ± 1.4

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Least-squares analysis

$f(^1C_4) = 0.61$ $f(^xS_y$ ensemble$) = 0.39$

Σerrors squared $= 0.53$

Mean weighted calculated couplings

$J_{12} = 3.6$ $J_{23} = 5.4$ $J_{34} = 3.5$ $J_{45} = 2.1$

Experimental Coupling Constants

$J_{12} = 2.9$ $J_{23} = 5.6$ $J_{34} = 3.9$ $J_{45} = 2.7$

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(b3) Dynamics of IdoA-5 of **1** in Vacuo ($\varepsilon = 5r$)		
Coupling	$^1C_4$	«Pseudorotational ensemble»
H1-H2	1.9 ± 0.5	6.7 ± 1.1
H2-H3	2.7 ± 0.9	8.3 ± 2.2
H3-H4	2.9 ± 0.9	3.6 ± 2.3
H4-H5	1.3 ± 0.8	2.4 ± 1.7

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Least-squares analysis

$f(^1C_4) = 0.60$ $f(^xS_y$ ensemble$) = 0.40$

Σerrors squared $= 1.53$

Mean weighted calculated couplings

$J_{12} = 3.8$ $J_{23} = 5.0$ $J_{34} = 3.2$ $J_{45} = 1.7$

Experimental Coupling Constants

$J_{12} = 2.7$ $J_{23} = 5.7$ $J_{34} = 3.5$ $J_{45} = 2.8$

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(c1) Dynamics of IdoA-1 of **1** with Particle Mesh Ewald		
Coupling	$^1C_4$	«Pseudorotational ensemble»
H1-H2	1.9 ± 0.6	6.5 ± 1.0
H2-H3	2.4 ± 1.0	10.0 ± 0.6
H3-H4	2.1 ± 0.9	5.6 ± 1.7
H4-H5	1.6 ± 0.9	3.8 ± 1.4
	Least-squares analysis	
	$f(^1C_4) = 0.63$	$f(^xS_y\ \mathrm{ensemble}) = 0.37$
	Σerrors squared = 0.28*	
	Mean weighted calculated couplings	
$J_{12} = 3.6$ $J_{23} = --$	$J_{34} = 3.4$ $J_{45} = 2.4$	
	Experimental Coupling Constants	
$J_{12} = 3.4$ $J_{23} = --$	$J_{34} = 3.4$ $J_{45} = 2.9$	

(c2) Dynamics of IdoA-3 of **1** with Particle Mesh Ewald		
Coupling	$^1C_4{}^\dagger$	«Pseudorotational ensemble»
H1-H2	1.7 ± 0.5	6.4 ± 0.9
H2-H3	2.3 ± 1.0	9.8 ± 0.7
H3-H4	2.0 ± 0.8	5.2 ± 1.6
H4-H5	1.6 ± 0.8	3.2 ± 1.3
	Least-squares analysis	
	$f(^1C_4) = 0.58$	$f(^xS_y\ \mathrm{ensemble}) = 0.42$
	Σerrors squared = 0.56	
	Mean weighted calculated couplings	
$J_{12} = 3.7$ $J_{23} = 5.4$	$J_{34} = 3.3$ $J_{45} = 2.3$	
	Experimental Coupling Constants	
$J_{12} = 2.9$ $J_{23} = 5.6$	$J_{34} = 3.9$ $J_{45} = 2.7$	

(c3) Dynamics of IdoA-5 of **1** with Particle Mesh Ewald		
Coupling	$^1C_4$	«Pseudorotational ensemble»
H1-H2	1.9 ± 0.5	6.5 ± 0.9
H2-H3	2.6 ± 0.9	9.7 ± 0.9
H3-H4	2.4 ± 0.9	4.7 ± 1.8
H4-H5	1.5 ± 0.8	2.9 ± 1.3
	Least-squares analysis	
	$f(^1C_4) = 0.62$	$f(^xS_y\ \mathrm{ensemble}) = 0.38$
	Σerrors squared = 0.84	
	Mean weighted calculated couplings	
$J_{12} = 3.7$ $J_{23} = 5.3$	$J_{34} = 3.3$ $J_{45} = 2.0$	
	Experimental Coupling Constants	
$J_{12} = 2.7$ $J_{23} = 5.7$	$J_{34} = 3.5$ $J_{45} = 2.8$	