Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Towards capturing cellular complexity: Combining encapsulation and macromolecular crowding in a reverse micelle

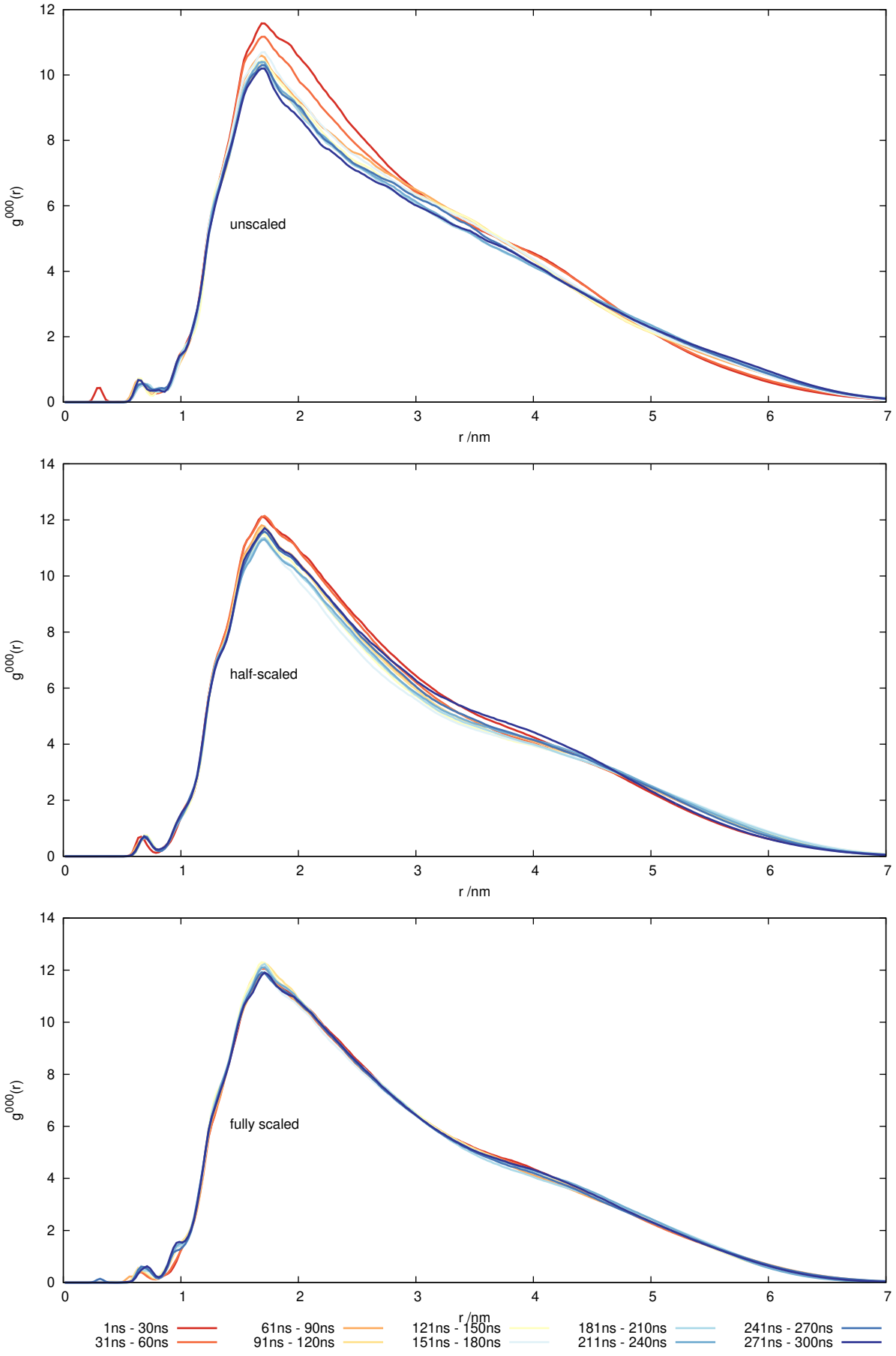
Supplementary Information

Philipp Honegger and Othmar Steinhauser

1 Convergence of the NVT simulation

The starting geometries of the simulation boxes were first energetically relaxed using the steepest-descent gradient method. Following this, the initial equilibrations of the systems were perfomed as NpT ensembles of 4 ns until the length of the cubic simulation box converged. This is necessary to know the equilibrium density of the simulation box.

This equilibration was followed up with the trajectory production as NVT ensemble, altogether covering 300ns $\times$ 5 replica $\times$ 3 scaling levels = 4.5 microseconds. However, large and slow molecules such as ubiquitin and complicated systems such as RMs are known to be plagued by long equilibration processes, where the system needs to dissipate surplus energy to reach its equilibrium. For instance, the protein seeks its optimal conformation and its preferred location within the reverse micelle which takes dozens of nanoseconds.[1]. Since all subsequent analyses of the trajectory require equilibrium conditions, the system was controlled for convergence on the basis of a series of properties, *e.g.* radial distribution functions (RDFs) shown below. The water-protein curves explain why we decided to discard the first 200 ns of each replicum and use the last 100 ns only.


unscaled
half-scaled
fully scaled
g000(r)
r /nm
1ns - 30ns
31ns - 60ns
61ns - 90ns
91ns - 120ns
121ns - 150ns
151ns - 180ns
181ns - 210ns
211ns - 240ns
241ns - 270ns
271ns - 300ns

2 λ-scaling

Bests and Mittals[2] λ-scaling approach consists of modifying the Lennard-Jones potential U_{ij} between two atoms i and j

$$U_{ij}(r_{ij}) = 4\epsilon_{ij}\left\{\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right\} \tag{1}$$

with r_{ij} as the interatomic distance, σ_{ij} as the repulsive/attractive switching distance and ϵ_{ij} as the potential well depth. In λ-scaling, the potential well depth ϵ_{ij} is then adjusted by multiplication:

$$\epsilon_{ij}^{\text{scaled}} = \lambda\epsilon_{ij} \tag{2}$$

To date, there is no extensive screening study optimizing λ, but Best and Mittal found that $\lambda = 1.1$ considerably improves protein-protein behaviour, hence we used this value in this study as well.

Technically, CHARMM does not store pair Lennard Jones parameters σ_{ij} and ϵ_{ij}, but single-particle parameters σ_i and ϵ_i designated for each atom type individually. Upon force field construction, pair-wise Lennard Jones parameters σ_{ij} and ϵ_{ij} are obtained via the Lorenz-Berthelot relations forming the arithmetic and the geometric means respectively:

$$\sigma_{ij} = \frac{1}{2}(\sigma_i + \sigma_j) \tag{3}$$

$$\epsilon_{ij} = (\epsilon_i \cdot \epsilon_j)^{\frac{1}{2}} \tag{4}$$

The parameter ϵ_{ij} is then simply modified *post festum* using the NBFIX command to overwrite the nonbonded parameters evaluated by CHARMM based on the CHARMM36 force field.[3–6] The two tables below list the NBFIX commands employed for the half-scaled ($\lambda_{\text{UBQ}} = 1.1$, $\lambda_{\text{surfactant}} = 1.0$) and the fully scaled ($\lambda_{\text{UBQ}} = 1.1$, $\lambda_{\text{surfactant}} = 1.1$) system, respectively.

NBFIX commands for the half-scaled system

atom type 1	**atom type 2**	ϵ	σ
OT	C	-0.142283	3.777
OT	CA	-0.113503	3.7694
OT	CC	-0.113503	3.777
OT	CD	-0.113503	3.777
OT	CE1	-0.11187	3.867
OT	CE2	-0.108529	3.857
OT	CP1	-0.06067	4.052
OT	CP2	-0.100609	3.952
OT	CP3	-0.100609	3.952
OT	CPH1	-0.095927	3.577
OT	CPH2	-0.095927	3.577
OT	CS	-0.142283	3.977
OT	CPT	-0.134982	3.637
OT	CY	-0.115909	3.767
OT	CAI	-0.115909	3.767
OT	CT	-0.06067	4.052
OT	CT1	-0.076742	3.777
OT	CT2	-0.10152	3.787
OT	CT2A	-0.10152	3.787
OT	CT3	-0.119813	3.817
OT	H	-0.09201	2.0015
OT	HA	-0.063631	3.097
OT	HB1	-0.063631	3.097
OT	HB2	-0.071785	3.117
OT	HE1	-0.075533	3.027

OT	HE2	-0.069174	3.037
OT	HB	-0.063631	3.097
OT	HC	-0.09201	2.0015
OT	HP	-0.074305	3.1352
OT	HR1	-0.09201	2.677
OT	HR2	-0.09201	2.477
OT	HR3	-0.037888	3.245
OT	HS	-0.135662	2.227
OT	HA1	-0.091005	3.117
OT	HA2	-0.079104	3.117
OT	HA3	-0.06646	3.117
OT	N	-0.191855	3.627
OT	NC2	-0.191855	3.627
OT	NH1	-0.191855	3.627
OT	NH2	-0.191855	3.627
OT	NH3	-0.191855	3.627
OT	NP	-0.191855	3.627
OT	NR1	-0.191855	3.627
OT	NR2	-0.191855	3.627
OT	NR3	-0.191855	3.627
OT	NY	-0.191855	3.627
OT	O	-0.14861	3.477
OT	OB	-0.14861	3.477
OT	OC	-0.14861	3.477
OT	OH1	-0.16731	3.547
OT	OS	-0.16731	3.547
OT	S	-0.287782	3.777
OT	SM	-0.264453	3.752
OT	SS	-0.294108	3.97

NBFIX commands for the fully scaled system

atom type 1	**atom type 2**	ϵ	σ
OT	C	-0.142283	3.777
OT	CA	-0.113503	3.7694
OT	CC	-0.113503	3.777
OT	CD	-0.113503	3.777
OT	CE1	-0.11187	3.867
OT	CE2	-0.108529	3.857
OT	CP1	-0.06067	4.052
OT	CP2	-0.100609	3.952
OT	CP3	-0.100609	3.952
OT	CPH1	-0.095927	3.577
OT	CPH2	-0.095927	3.577
OT	CS	-0.142283	3.977
OT	CPT	-0.134982	3.637
OT	CY	-0.115909	3.767
OT	CAI	-0.115909	3.767
OT	CT	-0.06067	4.052
OT	CT1	-0.076742	3.777
OT	CT2	-0.10152	3.787
OT	CT2A	-0.10152	3.787
OT	CT3	-0.119813	3.817
OT	H	-0.09201	2.0015
OT	HA	-0.063631	3.097
OT	HB1	-0.063631	3.097
OT	HB2	-0.071785	3.117

OT	HE1	-0.075533	3.027
OT	HE2	-0.069174	3.037
OT	HB	-0.063631	3.097
OT	HC	-0.09201	2.0015
OT	HP	-0.074305	3.1352
OT	HR1	-0.09201	2.677
OT	HR2	-0.09201	2.477
OT	HR3	-0.037888	3.245
OT	HS	-0.135662	2.227
OT	HA1	-0.091005	3.117
OT	HA2	-0.079104	3.117
OT	HA3	-0.06646	3.117
OT	N	-0.191855	3.627
OT	NC2	-0.191855	3.627
OT	NH1	-0.191855	3.627
OT	NH2	-0.191855	3.627
OT	NH3	-0.191855	3.627
OT	NP	-0.191855	3.627
OT	NR1	-0.191855	3.627
OT	NR2	-0.191855	3.627
OT	NR3	-0.191855	3.627
OT	NY	-0.191855	3.627
OT	O	-0.14861	3.477
OT	OB	-0.14861	3.477
OT	OC	-0.14861	3.477
OT	OH1	-0.16731	3.547
OT	OS	-0.16731	3.547
OT	S	-0.287782	3.777
OT	SM	-0.264453	3.752
OT	SS	-0.294108	3.97
OT	CG2O2	-0.134298	3.477
OT	CG311	-0.076742	3.777
OT	CG321	-0.10152	3.787
OT	CG334	-0.119043	3.992
OT	CH2	-0.134374	4.0612
OT	CH3	-0.19531	3.8805
OT	HGA1	-0.091005	3.117
OT	HGA2	-0.080259	3.117
OT	HGP1	-0.09201	2.0015
OT	HGP5	-0.09201	2.477
OT	NG3P0	-0.191855	3.627
OT	OG2D1	-0.14861	3.477
OT	OG302	-0.135662	3.427
OT	OG311	-0.188027	3.542
OT	OG312	-0.14861	3.527

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