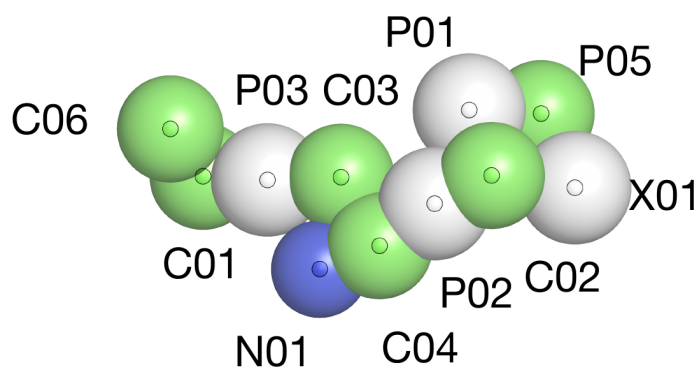
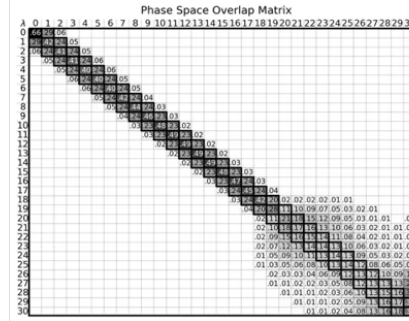




Supplementary Figure 1. MARTINI-3 coarse-grained representation of PAM_16A. The all-atom structure of PAM_16A mapped onto eleven coarse-grained beads using AutoMartini3. Beads are coloured by MARTINI bead type and labelled with the bead identifiers (P01–P03, C01–C06, N01, X01) that correspond to the [atoms] directive of Table S2, which lists the atom-to-bead assignments and the complete bonded topology.

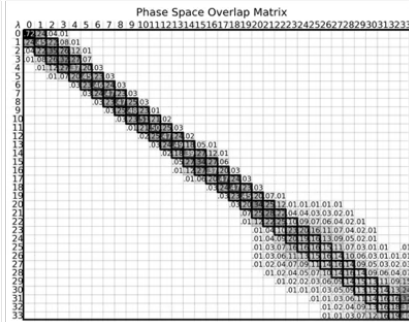
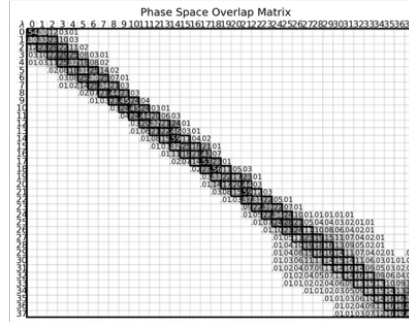
Free



Top site

Bottom site

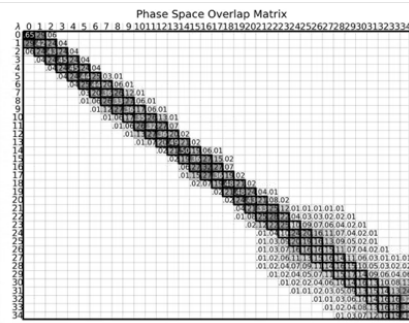
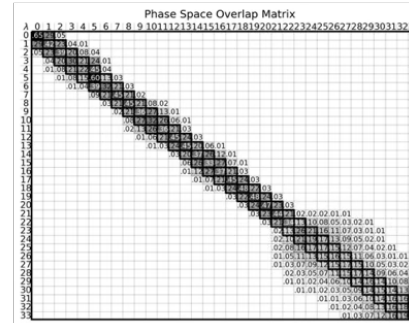
1Ca



Top site

Bottom site

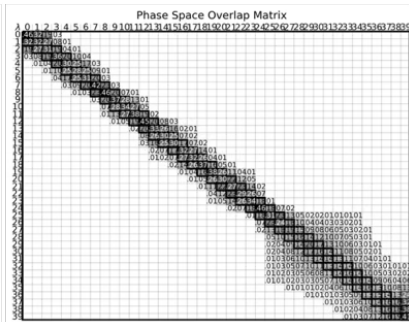
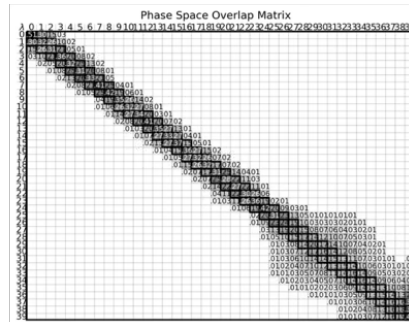
2Ca



1Ca

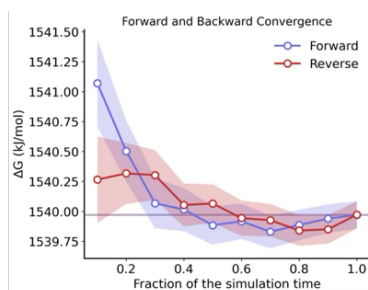
2Ca

**Bound +
PAM_16A**



Supplementary Figure 2. Phase-space overlap matrix between neighbouring λ windows from the MBAR analysis; near-diagonal density confirms sufficient overlap for reliable estimates. Data shown are representative of $n = 3$ independent replicates.

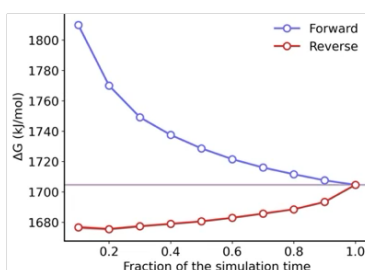
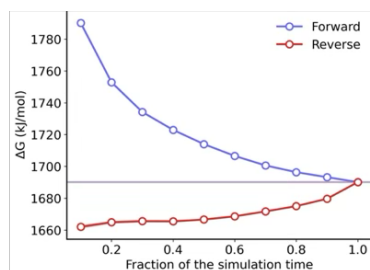
Free



Top site

Bottom site

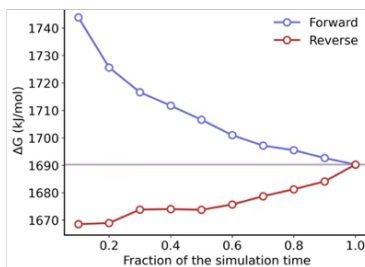
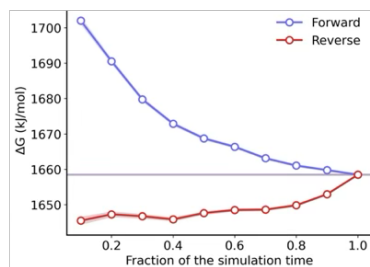
1Ca



Top site

Bottom site

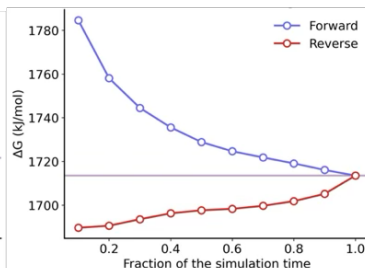
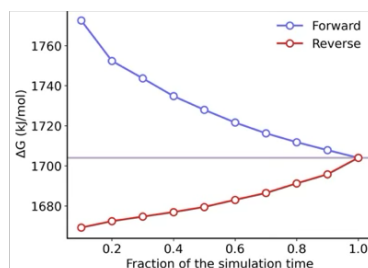
2Ca



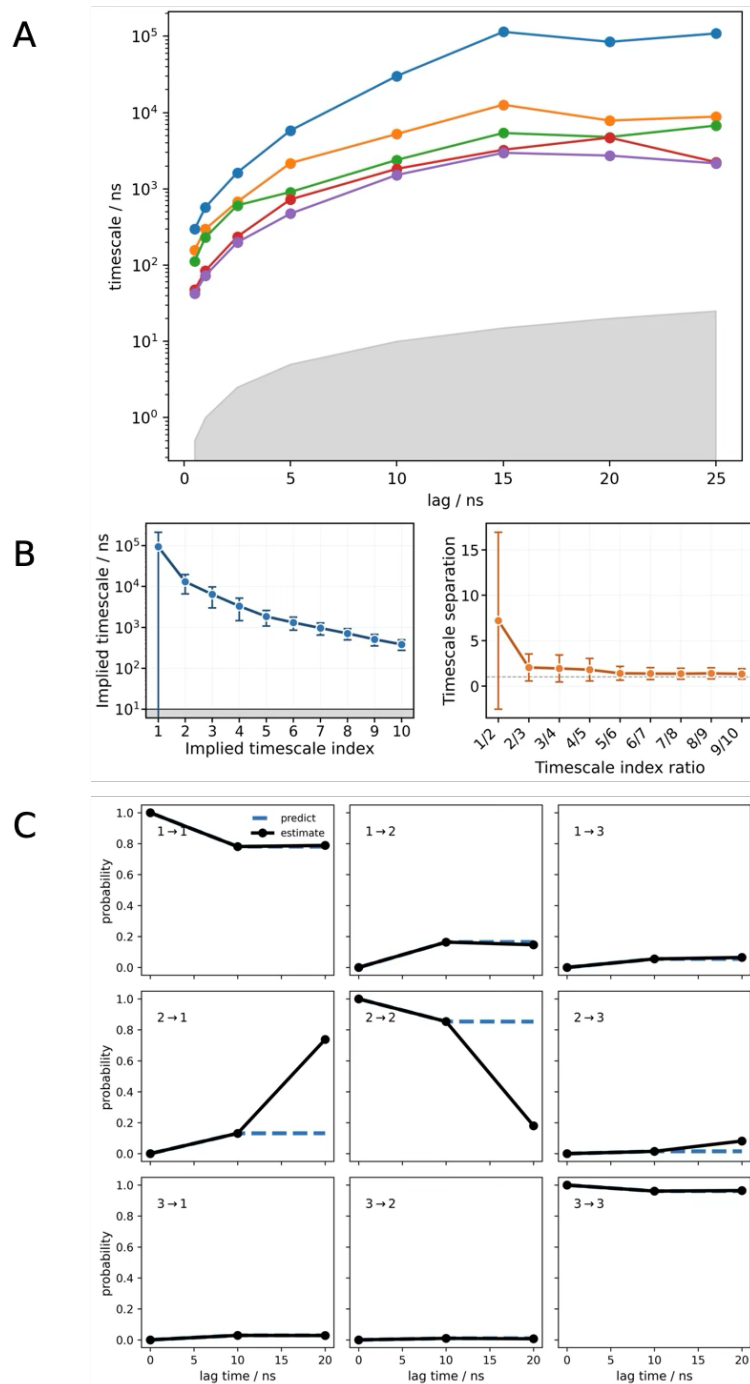
1Ca

2Ca

Bound +
PAM_16A

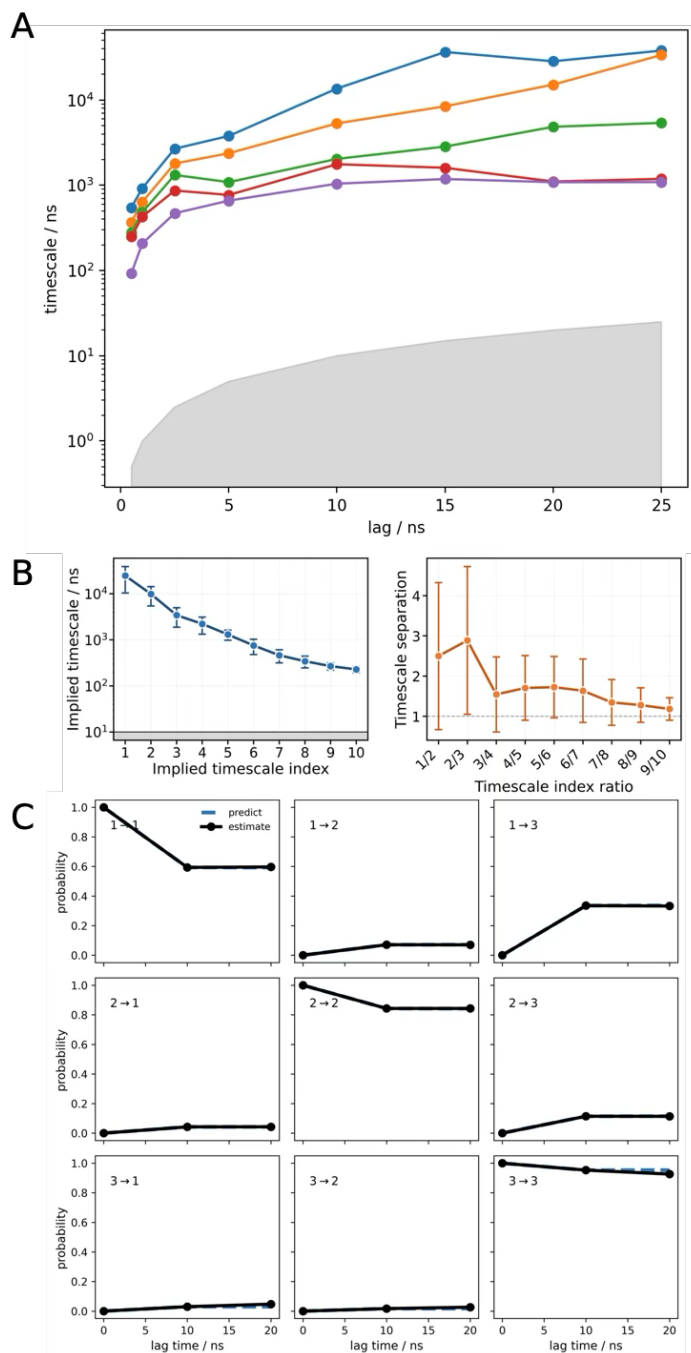


Supplementary Figure 3. Forward and reverse convergence of the calcium free energy perturbation (FEP) calculations. For each transformation, the free energy estimate (ΔG , MBAR) is plotted against the fraction of simulation time analysed from the start of the trajectories (Forward, blue) and from the end (Reverse, red); shaded bands denote the standard deviation and the horizontal line marks the estimate. Panels show the decoupling of Ca^{2+} in bulk water (Free) and in the channel without drug (Bound + Apo) and with PAM_16A (Bound + PAM_16A), for one or two bound calcium ions (1Ca, 2Ca) perturbed at the top or bottom site; one representative replicate is shown per condition.

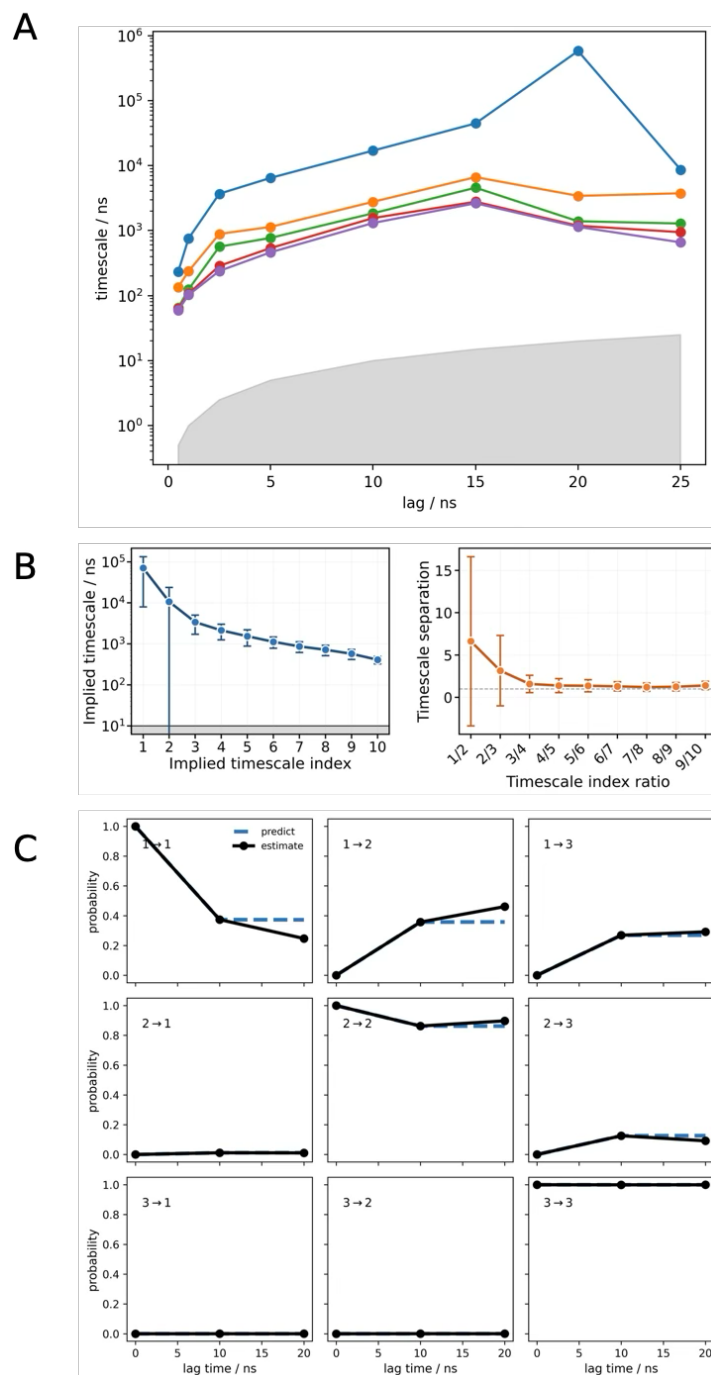


Supplementary Figure 4. The convergence analysis of the simulation with calcium, without PAM_{16A} (A) Implied timescale (ITS) convergence: the five slowest implied timescales as a function of MSM lag time. Each line is one timescale, ordered from slowest to fastest — timescale 1 (blue), 2 (orange), 3 (green), 4 (red), 5 (purple); the slowest process (blue) is uppermost. The timescales become approximately lag-independent from ~10 ns onward and lie above the grey

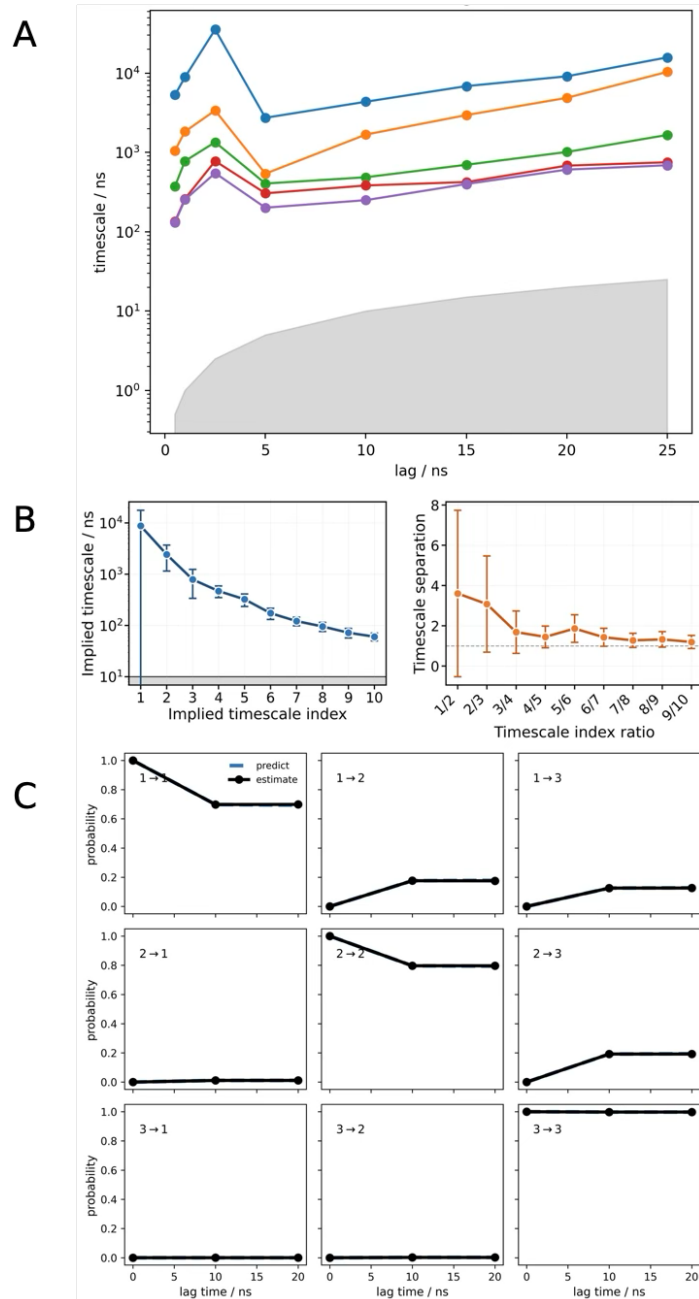
region (timescales faster than the lag time, which cannot be resolved). A 10 ns lag time was used for the production MSM.



Supplementary Figure 5. The convergence analysis of the simulation with calcium, with PAM_16A (A) Implied timescale (ITS) convergence: the five slowest implied timescales as a function of MSM lag time. Each line is one timescale, ordered from slowest to fastest — timescale 1 (blue), 2 (orange), 3 (green), 4 (red), 5 (purple); the slowest process (blue) is uppermost. The timescales become approximately lag-independent from ~10 ns onward and lie above the grey region (timescales faster than the lag time, which cannot be resolved). A 10 ns lag time was used for the production MSM.



Supplementary Figure 6. The convergence analysis of the simulation without calcium, without PAM_16A (A) Implied timescale (ITS) convergence: the five slowest implied timescales as a function of MSM lag time. Each line is one timescale, ordered from slowest to fastest — timescale 1 (blue), 2 (orange), 3 (green), 4 (red), 5 (purple); the slowest process (blue) is uppermost. The timescales become approximately lag-independent from ~10 ns onward and lie above the grey region (timescales faster than the lag time, which cannot be resolved). A 10 ns lag time was used for the production MSM.



Supplementary Figure 7. The convergence analysis of the simulation without calcium, with PAM_16A (A) Implied timescale (ITS) convergence: the five slowest implied timescales as a function of MSM lag time. Each line is one timescale, ordered from slowest to fastest — timescale 1 (blue), 2 (orange), 3 (green), 4 (red), 5 (purple); the slowest process (blue) is uppermost. The timescales become approximately lag-independent from ~10 ns onward and lie above the grey region (timescales faster than the lag time, which cannot be resolved). A 10 ns lag time was used for the production MSM.

SI Tables

Rank	Predicted site	Aggregate score	ipTM	pTM
1	Calcium-binding site (TM6)	0.294	0.167	0.800
2	Steric gate (TM4–TM6)	0.293	0.166	0.804

Supplementary Table 1. Chai-1 predicted binding poses for PAM_16A on a TMEM16A monomer. The top-ranked clusters fall into two intracellular regions — the steric gate (between TM4 and TM6) and the calcium-binding site (on TM6). The confidence scores for the two sites are comparable.

```
[moleculetype]
; molname      nrexcl
PAM            2
```

```
[atoms]
; id  type  resnr residue atom  cgnr  charge  mass ; smiles  ; atom_num
1    TP2d   1    PAM    P01    1      0    36 ; CO      ; atoms: O2, C18,
2    SP3d   1    PAM    P02    2      0    54 ; NC=O    ; atoms: O3, N5, C16,
3    TN5a   1    PAM    N01    3      0    36 ; CN      ; atoms: N6, C22,
4    SC3    1    PAM    C01    4      0    54 ; CCC     ; atoms: C7, C8, C9,
5    SP3d   1    PAM    P03    5      0    54 ; NC=O    ; atoms: O1, N4, C11,
6    TC4    1    PAM    C02    6      0    36 ; CC      ; atoms: C10, C12,
7    TC5    1    PAM    C03    7      0    36 ; C=C     ; atoms: C13, C15,
8    TC5    1    PAM    C04    8      0    36 ; C=C     ; atoms: C14, C20,
9    TC5    1    PAM    C05    9      0    36 ; C=C     ; atoms: C21, C24,
10   SX3    1    PAM    X01   10      0    54 ; C=CCl   ; atoms: Cl0, C19, C23,
11   TC6    1    PAM    C06   11      0    36 ; C#C     ; atoms: C17, C25,
```

```
[bonds]
; i j  funct  length  force.c.
1  6    1      0.29   25000.00
1  9    1      0.28   25000.00
2  6    1      0.22  100000.00
2  8    1      0.23  100000.00
3  7    1      0.30   25000.00
3  8    1      0.22   25000.00
4  5    1      0.27   25000.00
4 11    1      0.33  10000.00
5  7    1      0.26   25000.00
6 10    1      0.33  10000.00
7  8    1      0.27   25000.00
9 10    1      0.30   5000.00
```

```
[constraints]
; i j  funct  length
6  9    1      0.43
1 10    1      0.42
```

```
[angles]
; i j k  funct  angle  force.c.
1  2  6    1     58.9   30.0
1  2  8    1    132.6   30.0
1  3  7    1     30.9  100.0
1  3  8    1     31.8  100.0
1  4  5    1     23.8  100.0
1  4 11    1     82.3   30.0
1  5  7    1     20.2   30.0
1  6  3    1     83.8  100.0
1  6  4    1     57.1  250.0
1  6  5    1     59.7  250.0
1  6  7    1     59.4  100.0
1  6  8    1     87.4  100.0
1  6  9    1     39.8  100.0
1  6 10    1     83.6  250.0
1  6 11    1     60.0  100.0
1  7  8    1     85.7  100.0
1  9  2    1     36.2  250.0
1  9  3    1     44.0  100.0
1  9  4    1     46.7  250.0
1  9  5    1     39.1  250.0
1  9  7    1     32.1  100.0
```

1	9	8	1	41.4	100.0
1	9	10	1	92.6	250.0
1	9	11	1	31.4	100.0
1	10	2	1	44.5	100.0
1	10	3	1	54.1	30.0
1	10	4	1	38.3	100.0
1	10	5	1	36.4	100.0
1	10	7	1	34.1	30.0
1	10	8	1	52.9	30.0
1	10	11	1	28.4	30.0
2	3	7	1	51.9	100.0
2	4	5	1	9.3	25.0
2	8	4	1	124.5	100.0
2	8	5	1	116.9	100.0
2	8	11	1	115.2	100.0
3	4	5	1	28.6	100.0
3	7	4	1	81.5	250.0
3	7	11	1	92.9	100.0
4	5	11	1	60.4	45.0

Supplementary Table 2. MARTINI-3 coarse-grained topology of PAM_16A generated with AutoMartini3. Bead types and atom-to-bead assignments ([atoms]), together with the bonded parameters ([bonds], [constraints], [angles]) used in the coarse-grained flooding simulations.