To first map the theoretically accessible space of USP14 interdomain positions, an initial set of 13 000 USP14 interdomain conformations were generated by sampling different conformations of the loop connecting the two domains in USP14. No experimental restraints were used in this stage. A starting model of USP14 was modelled using AlphaFold2, and the model was energy minimized in Rosetta’s force field using the relax application with the option *relax:constrain\_relax\_to\_start\_coords* enabled. Starting from the relaxed AlphaFold model of USP14 (*AF.rlx.pdb* in Rosetta command line below)*,* the relative positioning of the two USP14 domains was explored by sampling the backbone degrees of freedom of the loop residues (76-102 residue position) connecting the two domains using the FloppyTail application in Rosetta. The backbone degrees of freedom were sampled with 3-mer backbone fragments and smaller changes to individual backbone psi/phi dihedrals. The backbone fragments were taken from known structures with local sequences similarity and secondary structure preference. In total, 13,000 conformations were generated using Monte Carlo energy minimization by first sampling the backbone orientations in low-resolution followed by all-atom refinement. The Rosetta command line used to generate the 13000 conformations is shown below:

FloppyTail.mpi.linuxgccrelease

-in:file:s *AF.rlx.pdb*

-nstruct 13000

-out:file:silent silent.out

-packing:repack\_only

-in:file:movemap *movemap.dat*

-FloppyTail::perturb\_cycles 10000

-FloppyTail::refine\_cycles 1000

-in:file:frag3 frags.200.3mers

-FloppyTail:C\_root

The *movemap.dat* file instructs the protocol to sample the backbone and side-chains degrees of freedom for residue 76-102:

RESIDUE \* NO

JUMP \* NO

RESIDUE 76 102 BBCHI

The SAXS-guided molecular USP14 solution structural ensemble was constructed from the initial 13,000-membered structural space using the iterative Bayesian/Maximum Entropy approach (iBME) as described in Pesce F, Lindorff-Larsen K (2021) Refining conformational ensembles of flexible proteins against small-angle x-ray scattering data. Biophys J 120:5124–5135. In short, SAXS profiles were calculated from each structure in the initial 13 000-membered ensemble, and iBME was then used to obtain normalized weights (wi) representing their contribution to a c2- optimised iBME fit of an ensemble of SAXS profiles to the experimental USP14 SAXS data. The final USP14 solution structural ensemble was defined as the structures corresponding to the SAXS profiles that contributed 0.5% or more (wi>0.005) to the iBME fit, resulting in an ensemble of 30 structures.