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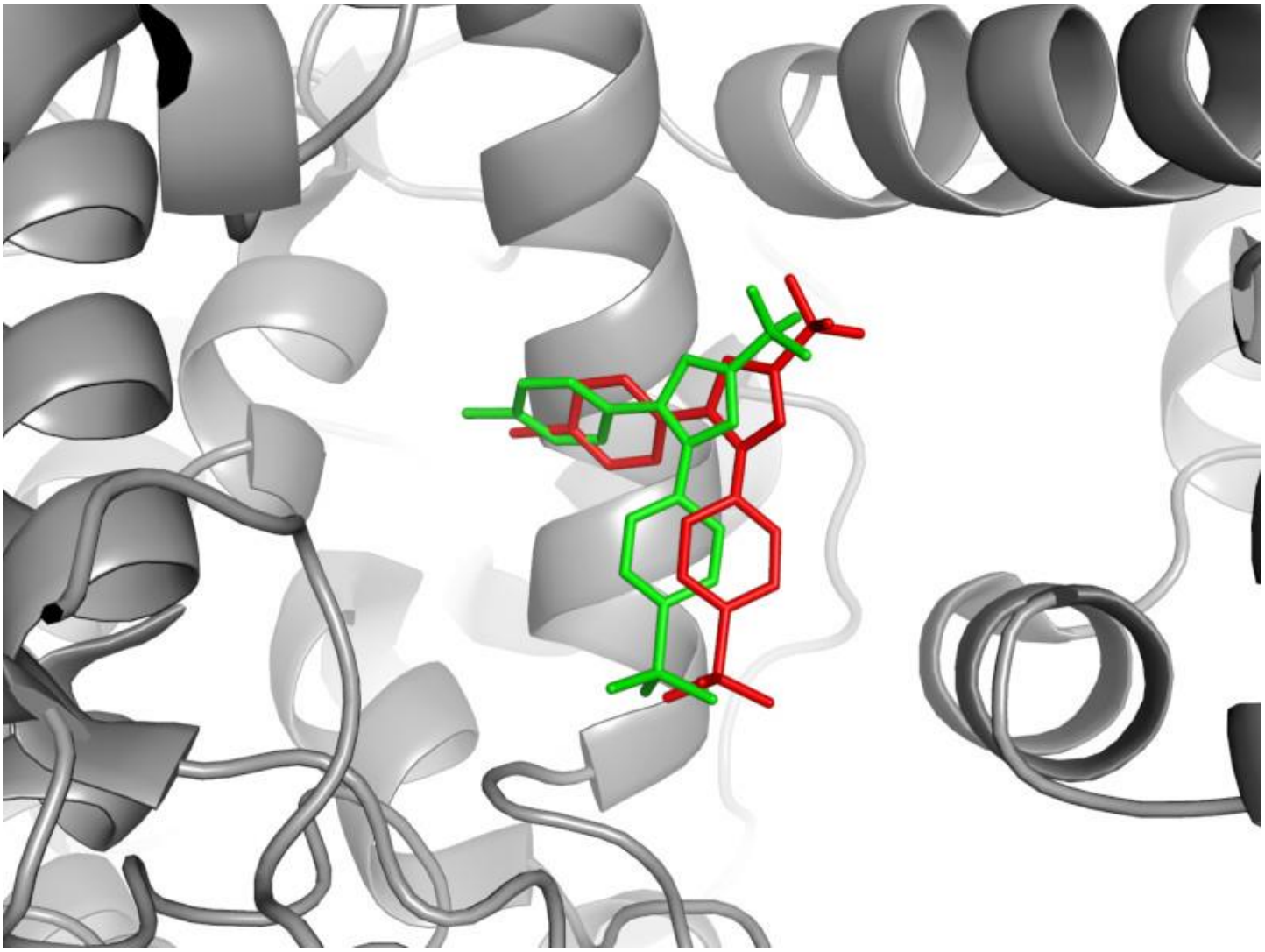
Supplementary information

Ligand binding free-energy calculations with funnel metadynamics

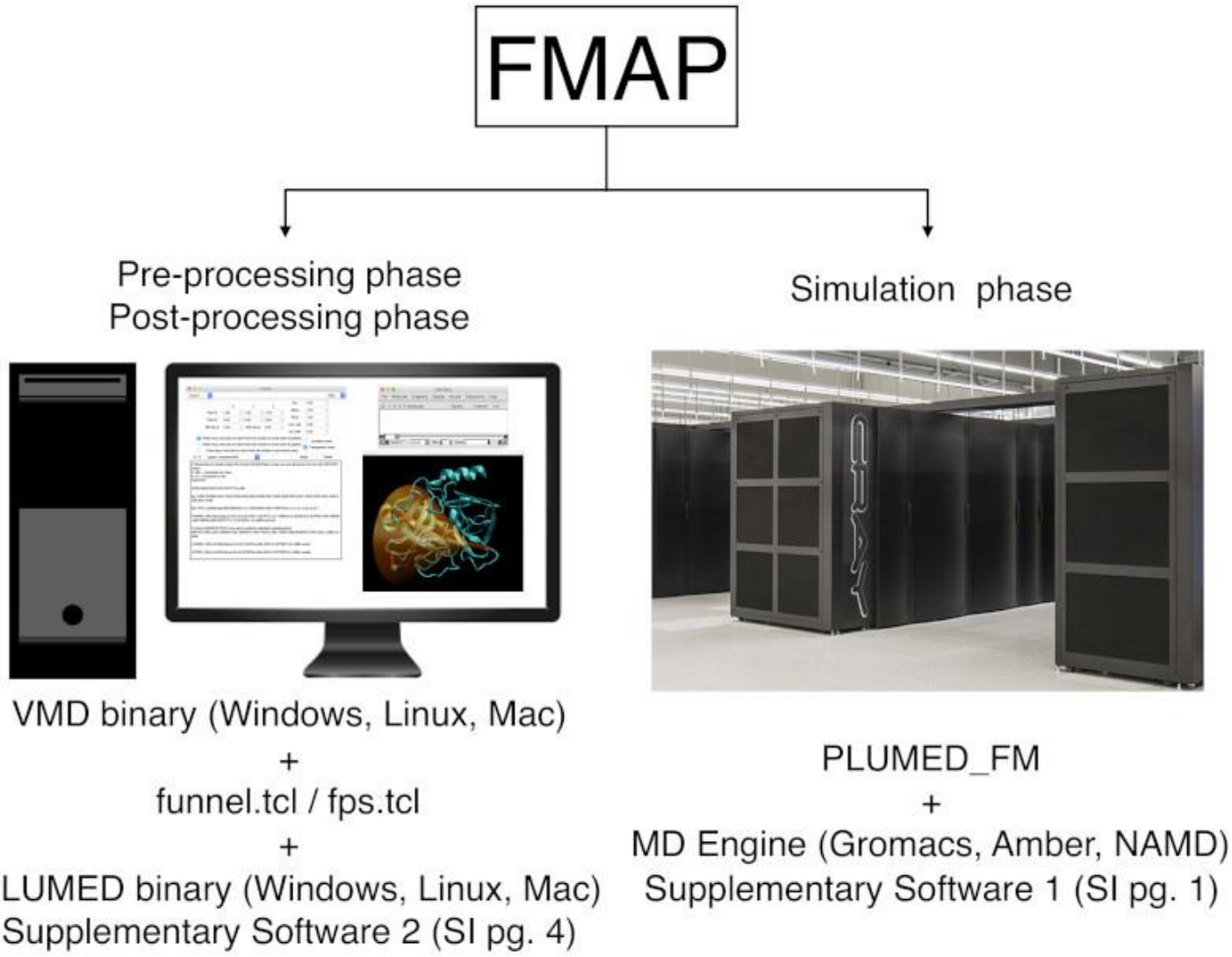
In the format provided by the authors and unedited



Supplementary Figure 1

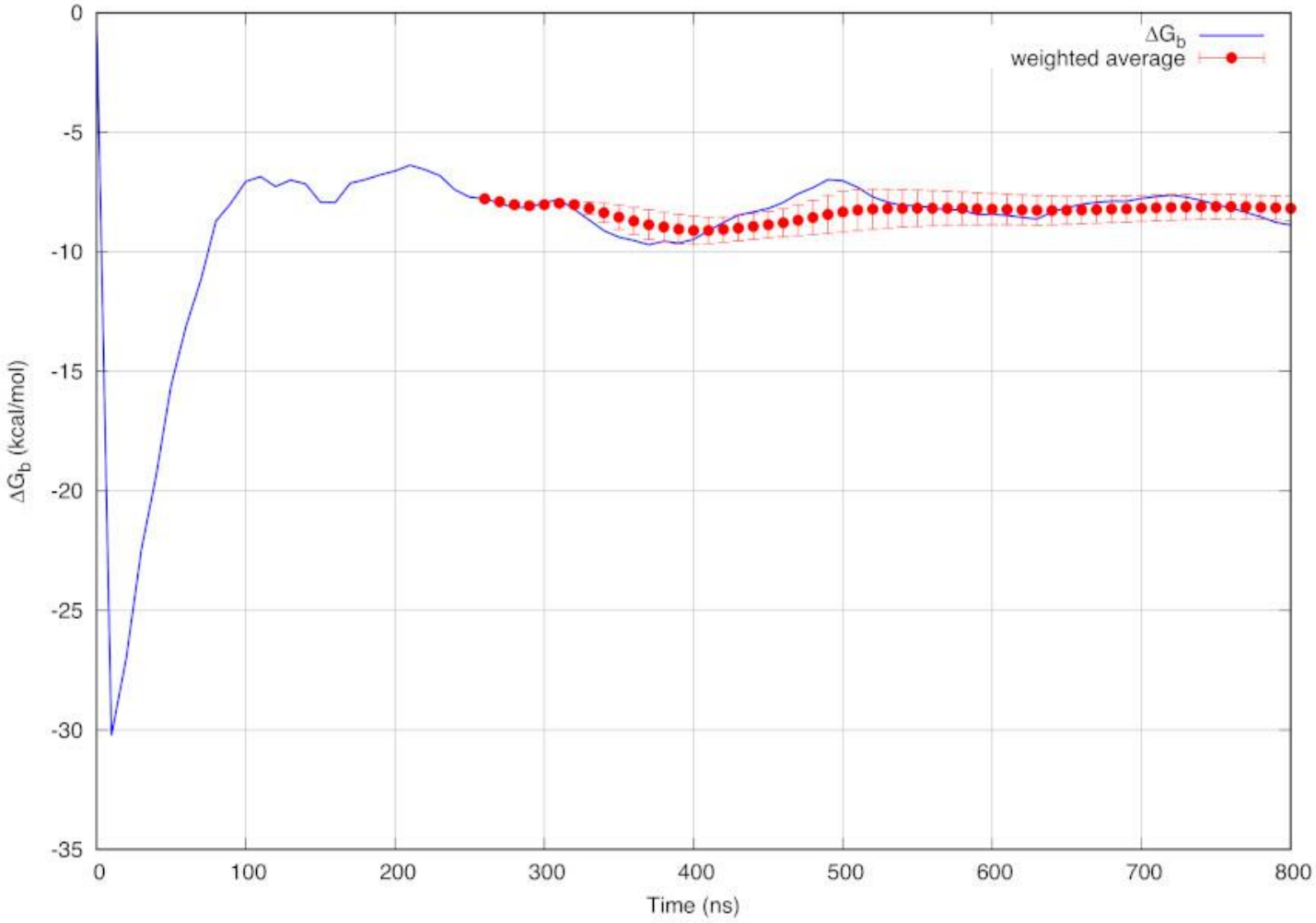
Prediction of ligand binding mode.

Comparison of the binding mode of the coxib drug SC-558 (red) to cyclooxygenase 1 (grey) predicted by metadynamics with the binding mode of the SC-558 analogue celecoxib (green) to the same target (pdb code 3kk6) determined by X-ray crystallography. The root mean square deviation computed for the ligand heavy atoms is 1.46 Å. Image taken from Limongelli et al. Proc. Natl. Acad. Soc. USA 2013.



Supplementary Figure 2

Software requirement for FMAP



Supplementary Figure 3

Convergence of the ligand binding free energy

Plot of the absolute trypsin-benzamidine binding free energy as a function of the simulation time (blue). Its weighted average with the standard deviation (see Supplementary Methods for details) is reported in red and leads to the final value of -8.2 ± 0.5 kcal/mol.

Supplementary Information

Ligand binding free-energy calculations with Funnel-Metadynamics

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Table contents

Supplementary Software 1
FMAP GUI version 1

The FMAP GUI version 1 is available in the "funnel_gui" folder included in the github repository here. This folder contains the two tcl files, "funnel.tcl" and "ffs.tcl", to equip VMD with the pre-processing and post-processing GUI, respectively.

Supplementary Software 2
Funnel-Metadynamics code

The new code of Funnel-Metadynamics is implemented in the Plumed-master, therefore it is available as a module of Plumed. It can be installed as reported in the module section of Plumed by specifying in the configuration command of Plumed the flag:

```
--enable-modules=funnel
```

The source code is available on the github repository in the "funnel_code" folder (https://github.com/limresgrp/FMAP_v1.git).

Supplementary Methods
Scripts for the ligand binding free-energy calculation (for interested reader)

Here below we provide the algorithm used to calculate the absolute protein-ligand binding free energy from BFES files as implemented in FMAP (Step 29).

```
#!/bin/bash

zmin=$1      # in nm
zmax=$2      # in nm
zbulk=$3     # in nm
rcyl=$4      # in nm
```

```
nfes=$6      # amount of BFESs

C0=0.6020
kbt=2.479

 binsize=$(awk '{if(NR==16) bin0=$1;if(NR==17) bin1=$1}END{print (bin1-bin0)}' <name of the FES>)

 Funbound=$(awk -v zbulk=$zbulk -v binsize=$binsize '{if($1!="#!" && $1>=zbulk && $1<zbulk+binsize &&
$2!="Infinity") Funbound=$2}END{print Funbound}' <name of the FES>)

 KB=$(awk -v zmin=$zmin -v zmax=$zmax -v pi=$pi -v rcyl=$rcyl -v dr=$binsize -v Funbound=$Funbound -v
kbt=$kbt 'BEGIN{tot=0.0}{if($1!="#!" && $1>=zmin && $1<=zmax && $1!="Infinity")tot+=exp(-($2-
Funbound)/kbt)*dr}END{print pi*rcyl*rcyl*tot}' <name of the FES>)

 DG=$(echo $KB $C0 | awk -v kbt=$kbt '{print -kbt*log($1*$2)}')

 echo $i $KB $DG
```

The obtained values are stored in a file and can be plotted as in Supplementary Fig. 3. The blue line shows the values obtained using the above algorithm with the BFESs generated for the benzamidine/trypsin system.
Finally, the following script computes the average value in kJ/mol of the absolute protein-ligand binding free energy with its standard deviation:

```
#!/bin/bash

# input
# $1 = reject time
# $2 = column number where DeltaG values are reported in the DeltaG file
# $3 = DeltaG file name
# $4 = output file name

awk 'BEGIN{tot1=0.0;tot2=0.0}{if($1>rej) {tot2+=($1-rej);tot1+=($1-rej)*$i;mean=tot1/tot2; tot3+=($1-
rej)*($i-mean)^2; print $1-rej, mean,sqrt(tot3/tot2)}}' rej=$1 i=$2 $3 > $4.meantime

mean=`tail -n 1 $4.meantime | awk '{print $2}'`
```

Considering that well-tempered metadynamics converges to the right value[1], the computed value is the time-weighted average value. The last output value of this script is the value reported in the tk_console clicking “Convergence!” in the GUI.

Supplementary Data 1
Data for the FMAP demo

The data for the FMAP demo are available in the Supplementary_data_2 folder included in a compressed file in Zenodo. This folder contains all the input and output files necessary to replicate using FMAP the analysis and the results presented in the protocol manuscript. A detailed list is following:

- start.pdb – pdb file of the starting structure of the benzamidine/trypsin complex for the FM simulation. It is used to set up the funnel-shape potential.
- trj.trr – strided trajectory coming from the FM simulation of the benzamidine/trypsin complex. It is used to enable all functionalities of the post-processing FMAP GUI (“ffs.tcl”).
- HILLS_800ns - file containing the Gaussian functions added in the FM simulation on the benzamidine/trypsin system. It is used to produce the BFES using both a small and large bin number with the post-processing FMAP GUI (“ffs.tcl”).

- COLVAR – file containing statistics of the FM simulation on the benzamidine/trypsin system. It has been generated using the post-processing FMAP GUI ("ffs.tcl") and it has the same number of frames of the simulation trajectory "trj.trr". It is used in all the Steps requiring a "COLVAR" file.
- 2D-distance.dat – 2D BFES of the benzamidine/trypsin complex as a function of a ligand-protein CV and a ligand-protein torsion CV. The latter has been computed using a reweight algorithm[2]. It is used in the post-processing FMAP GUI ("ffs.tcl").
- fes_$$.dat – 80 1D BFESs, from 1 to 80, representing the BFES evolution during the 800 ns of FM simulation on the benzamidine/trypsin system. It is used to compute the average estimate of the absolute protein-ligand binding free energy.

Supplementary Data 2
Input examples for MW-FM

The following are examples of Plumed-2 input files to run MW-FM.

Gromacs:

```
d1: DISTANCE ATOMS=2472,3224
lig: COM ATOMS=3221,3224,3225,3228,3229,3231,3233,3235,3237
fps: FUNNEL_PS LIGAND=lig REFERENCE=start.pdb ANCHOR=2472
POINTS=4.724,5.369,4.069,4.597,5.721,4.343
rmsd: RMSD REFERENCE=start.pdb TYPE=OPTIMAL

FUNNEL ARG=fps.lp,fps.ld ZCC=1.8 ALPHA=0.55 RCYL=0.1 MINS=-0.5 MAXS=3.7 KAPPA=35100
NBINS=500 NBINZ=500 SAFETY=1.0 SLOPE=1.0 FILE=BIAS LABEL=funnel WALKERS_MPI

# remove BIASFACTOR if you want to perform standard metadynamics
METAD ARG=d1 SIGMA=0.01 HEIGHT=2.0 PACE=500 TEMP=300 BIASFACTOR=20 LABEL=metad
GRID_MIN=0.0 GRID_MAX=4.0 GRID_WFILE=grid_w.dat GRID_RFILE=grid_r.dat
GRID_WSTRIDE=250000 REWEIGHTING_NGRID=2000 REWEIGHTING_NHILLS=1 WALKERS_MPI

LOWER_WALLS ARG=fps.lp AT=-0.3 KAPPA=500000 EXP=2 OFFSET=0 LABEL=lwall
UPPER_WALLS ARG=rmsd AT=0.09 KAPPA=500000 EXP=2 OFFSET=0 LABEL=uwall-rmsd
UPPER_WALLS ARG=fps.lp AT=3.4 KAPPA=500000 EXP=2 OFFSET=0 LABEL=uwall
UPPER_WALLS ARG=d1 AT=3.8 KAPPA=500000 EXP=2 OFFSET=0 LABEL=distwall

PRINT STRIDE=500 ARG=* FILE=COLVAR
```

NAMD or Amber:

```
d1: DISTANCE ATOMS=2472,3224
lig: COM ATOMS=3221,3224,3225,3228,3229,3231,3233,3235,3237
fps: FUNNEL_PS LIGAND=lig REFERENCE=start.pdb ANCHOR=2472
POINTS=4.3502,4.6759,4.042,4.2205,5.0289,4.3134
rmsd: RMSD REFERENCE=start.pdb TYPE=OPTIMAL

FUNNEL ARG=fps.lp,fps.ld ZCC=1.8 ALPHA=0.55 RCYL=0.1 MINS=-0.5 MAXS=3.7 KAPPA=35100
NBINS=500 NBINZ=500 SAFETY=1.0 SLOPE=1.0 FILE=BIAS LABEL=funnel

# remove BIASFACTOR if you want to perform standard metadynamics
METAD ARG=d1 SIGMA=0.01 HEIGHT=2.0 PACE=500 TEMP=300 BIASFACTOR=20 LABEL=metad
GRID_MIN=0.0 GRID_MAX=5.0 GRID_WFILE=grid_w.dat GRID_WSTRIDE=250000
REWEIGHTING_NGRID=2000 REWEIGHTING_NHILLS=1 WALKERS_ID=0 WALKERS_N=10
WALKERS_DIR=.. FILE=HILLS

LOWER_WALLS ARG=fps.lp AT=-0.3 KAPPA=500000 EXP=2 OFFSET=0 LABEL=lwall
UPPER_WALLS ARG=rmsd AT=0.09 KAPPA=500000 EXP=2 OFFSET=0 LABEL=uwall-rmsd
UPPER_WALLS ARG=fps.lp AT=3.4 KAPPA=500000 EXP=2 OFFSET=0 LABEL=uwall

PRINT STRIDE=500 ARG=* FILE=COLVAR
```

Supplementary References

1. Dama, J.F., Rotskoff, G., Parrinello, M., Voth, G.A. Transition-tempered metadynamics: robust, convergent metadynamics via on-the-fly transition barrier estimation. J. *Chem. Theory Comput*. **10**, 3626-3633, (2014).
2. Bonomi, M., Barducci, A., Parrinello, M. Reconstructing the equilibrium Boltzmann distribution from well-tempered metadynamics. *J. Comput. Chem*. **30**, 1615-1621 (2009).
3. Limongelli, V., Bonomi, M., Parrinello, M. Funnel-metadynamics as accurate binding free-energy method. *Proc. Natl. Acad. Soc. USA*. **110**, 6358-6363 (2013).