
To Affinity… and Beyond!
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April 2021
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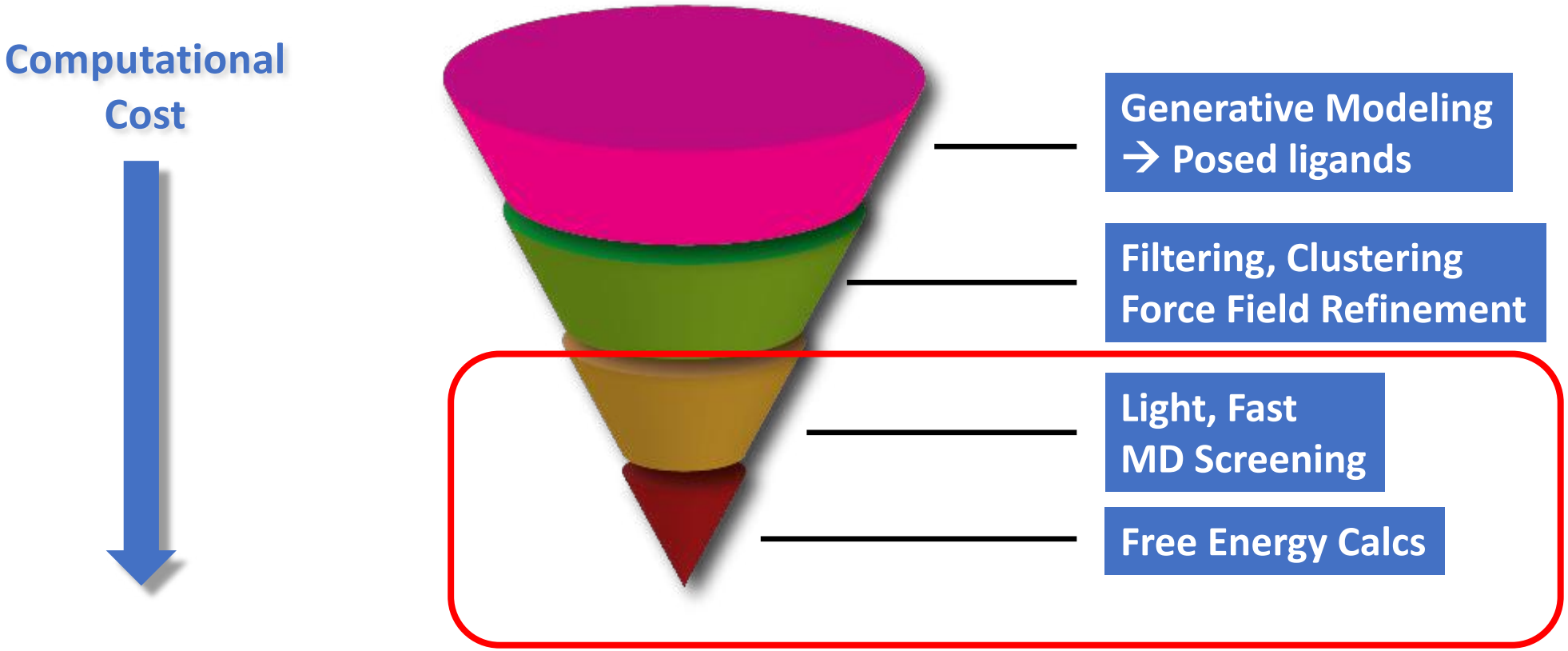
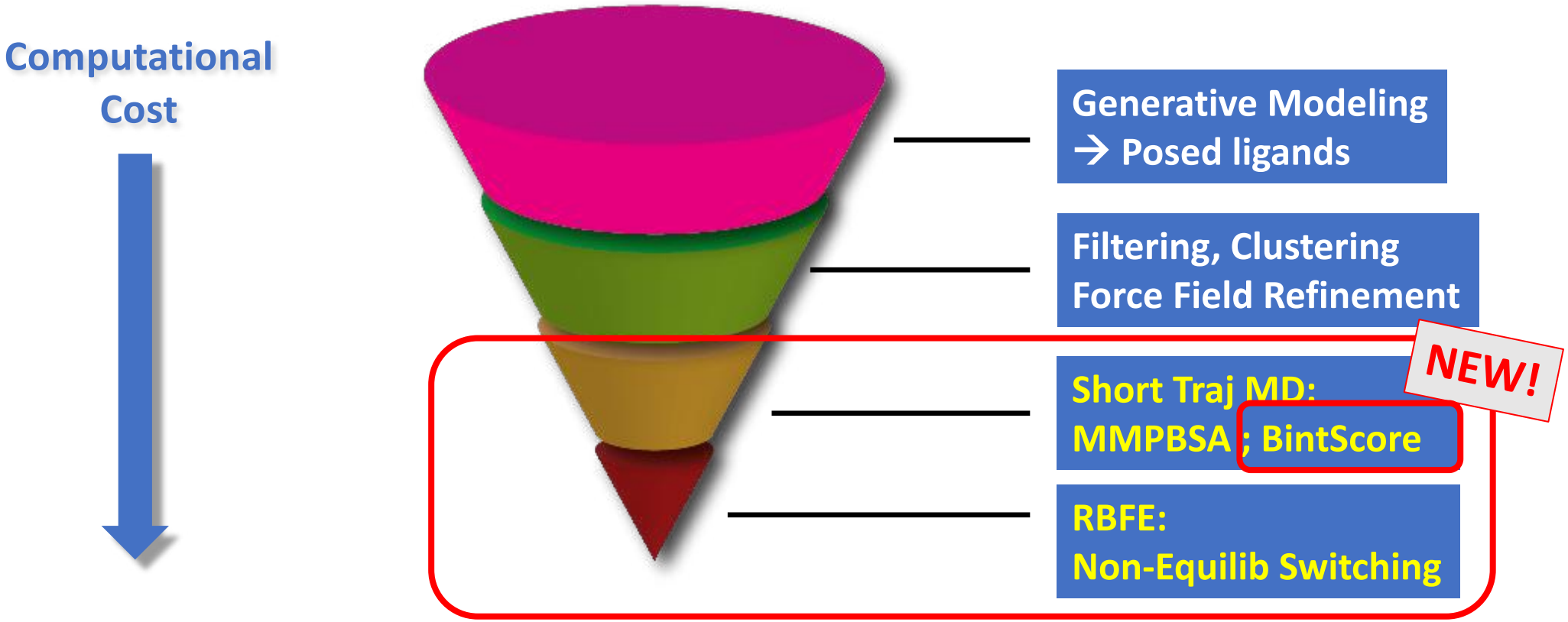
MD in Structure-Based Lead Optimization



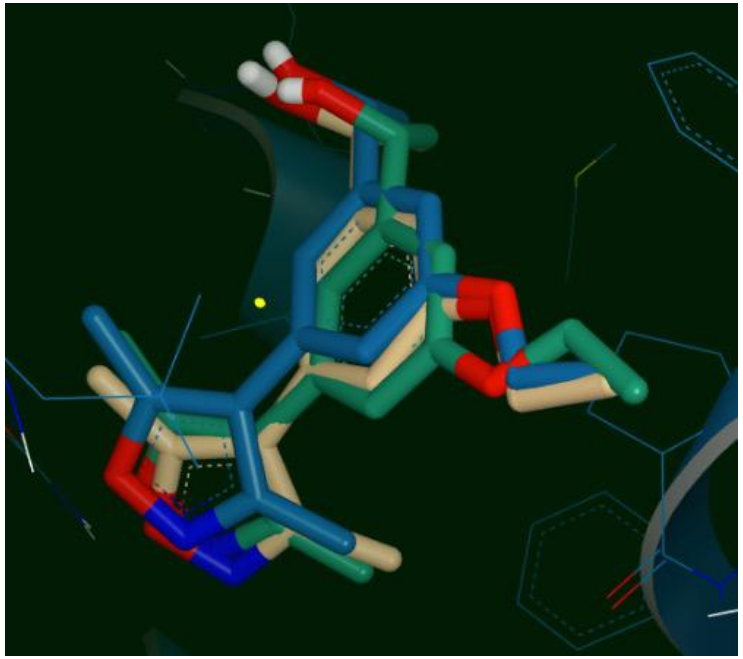
- Heavier MD methods staged to offer more value later in triaging

MD in Structure-Based Lead Optimization



- Heavier MD methods staged to offer more value later in triaging

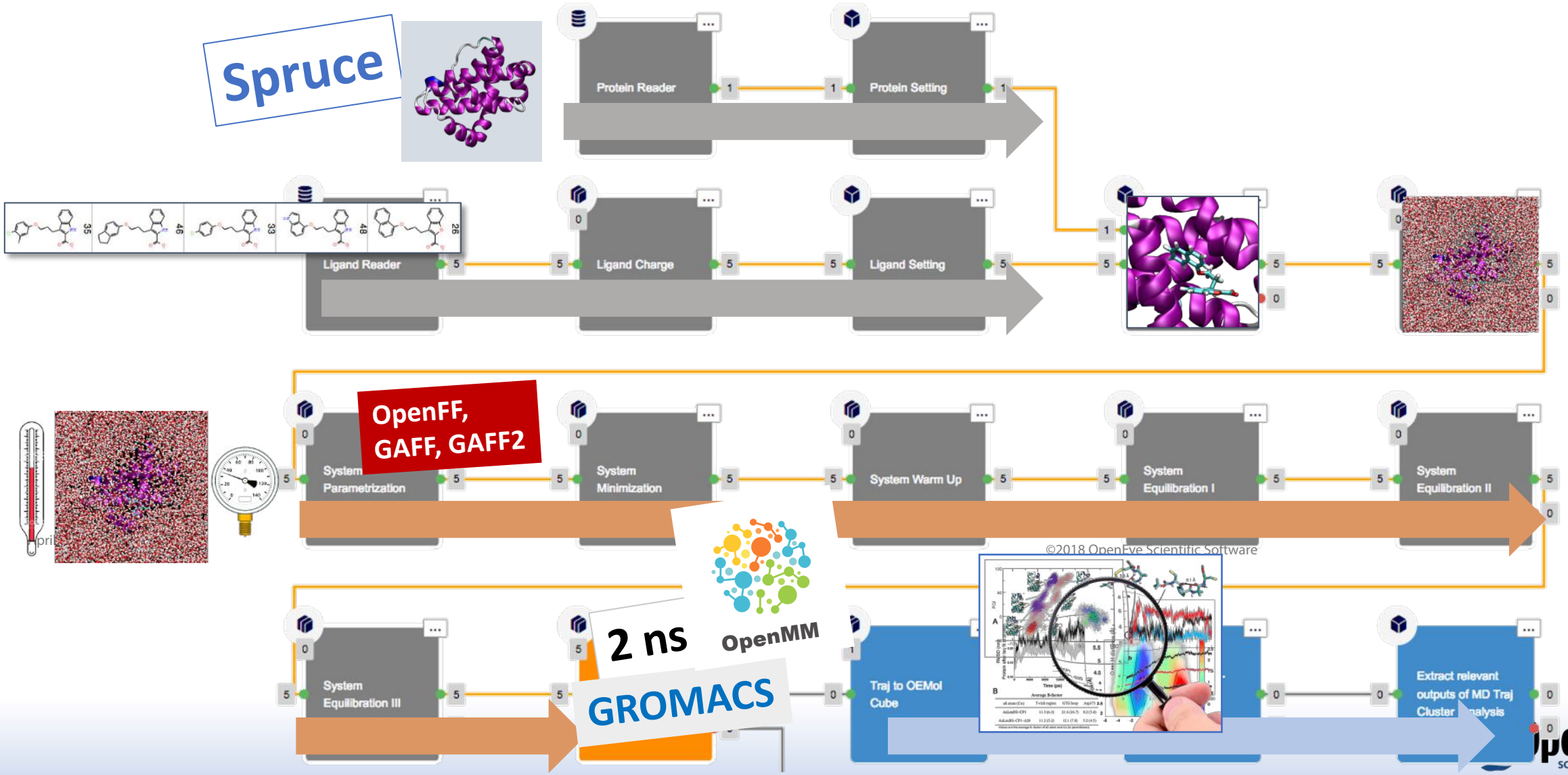
Short Trajectory MD: Pose Testing



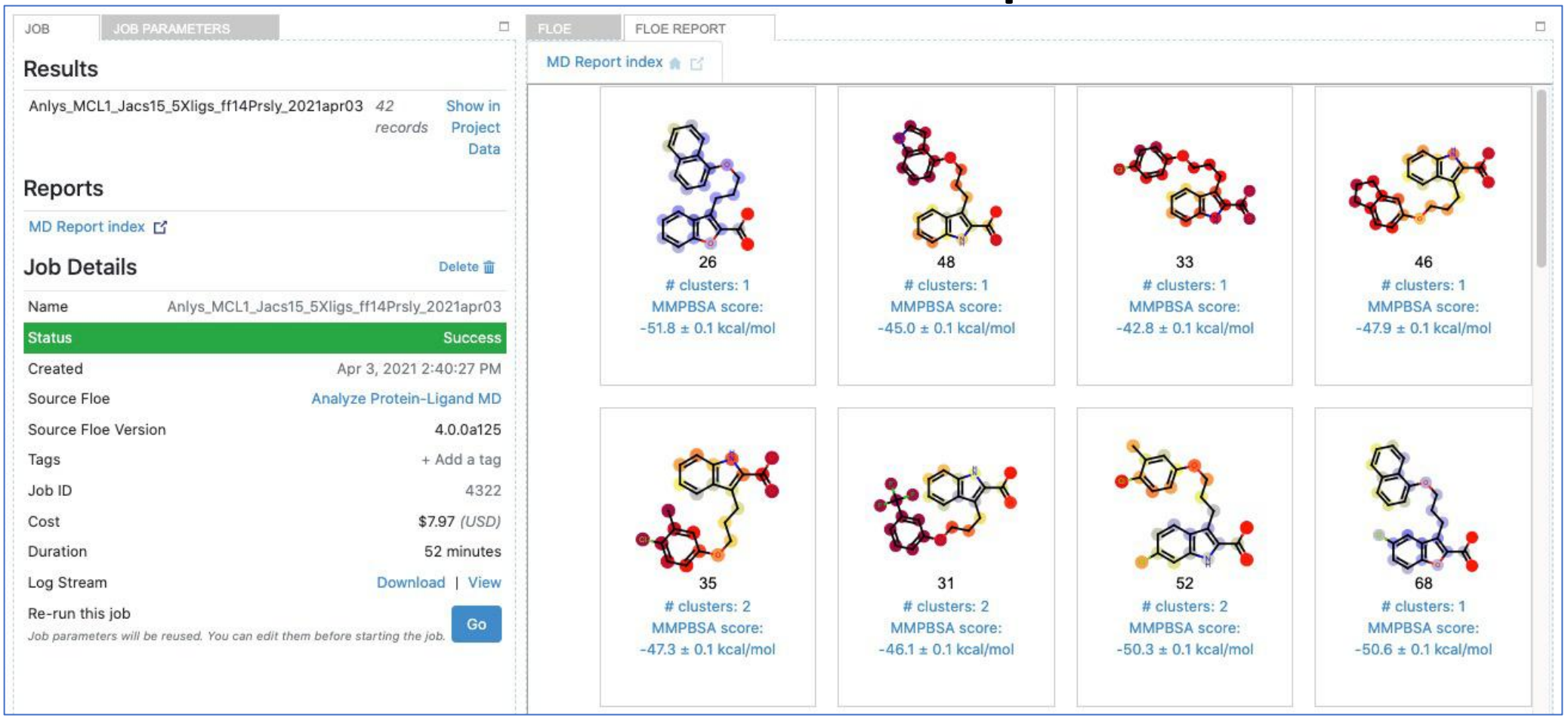
- Improved evaluation of a bound pose
  - Samples protein, ligand, and explicit water motion
  - Short Trajectory (2 ns)
    - Only the immediate region of the bound pose
  - Does the pose remain stable?
  - Do the protein-ligand interactions remain as designed?

- High throughput ; light calculation
  - Orion: 1 hr/ligand (~$1), highly parallel

Short Trajectory MD in Orion: The Floe



MCL1 Dataset* : STMD Floe Report



- 42 congeneric ligands

*Wang et al., *JACS* 2015, **137**, 2695

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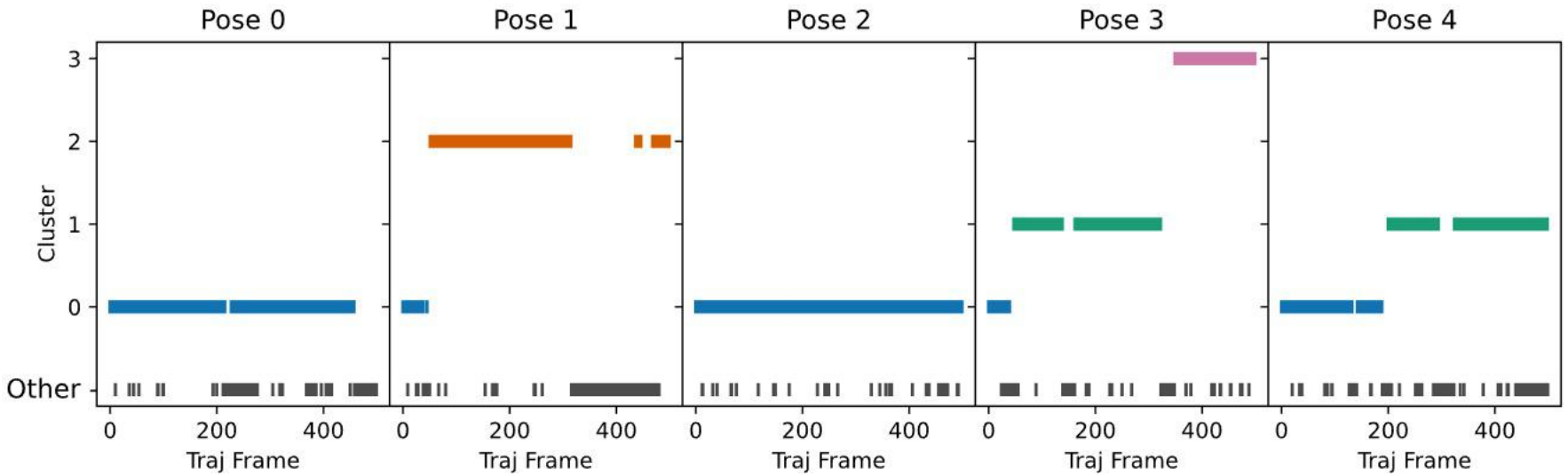
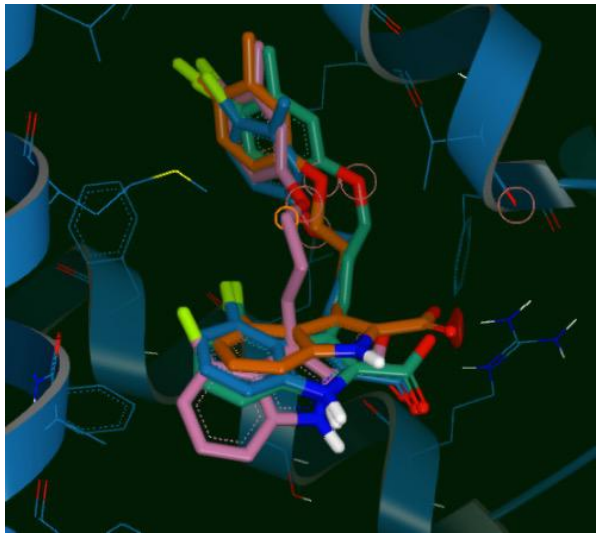
Aggregate Analysis of Ligand Trajectories





Cluster	Size	<MMPBSA>[a,b]	<BintScore>[a]
0	44.1%	-44.7 ± 0.2	-19.4 ± 0.3
1	18.6%	-44.5 ± 0.2	-15.6 ± 0.3
2	11.5%	-46.6 ± 0.3	-15.5 ± 0.4
3	5.6%	-41.5 ± 0.5	-13.7 ± 0.6
Other[c]	20.3%	-44.3 ± 0.4	-16.1 ± 0.5

- Aggregate Analysis for all poses for a ligand
- Reduces variance in Ensemble scoring : <MMPBSA>
- Consensus clustering yields a more global view of bound microstates

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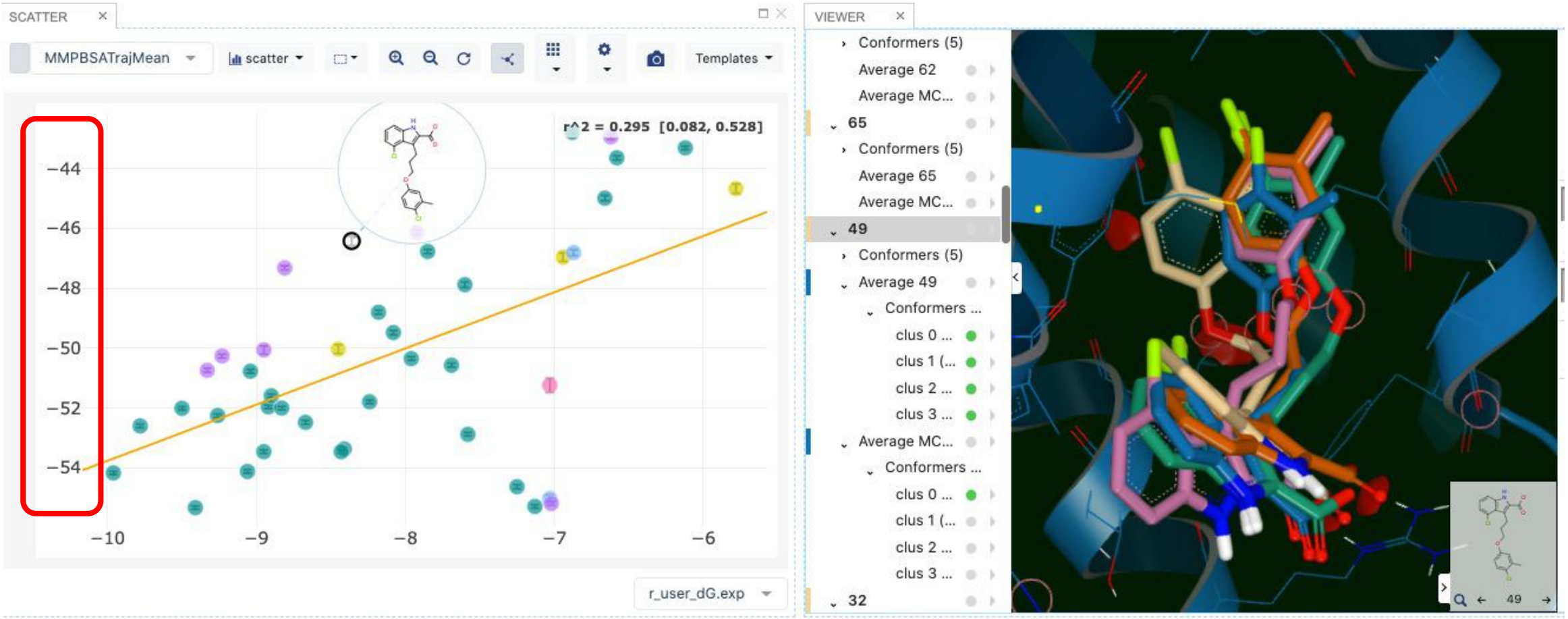
MCL1 Dataset : Orion Analysis page



- Ligands show varying degrees of pose stability
- Fitting a model is required: <MMPBSA> score itself is not useful

Wang et al., *JACS* 2015, **137**, 2695

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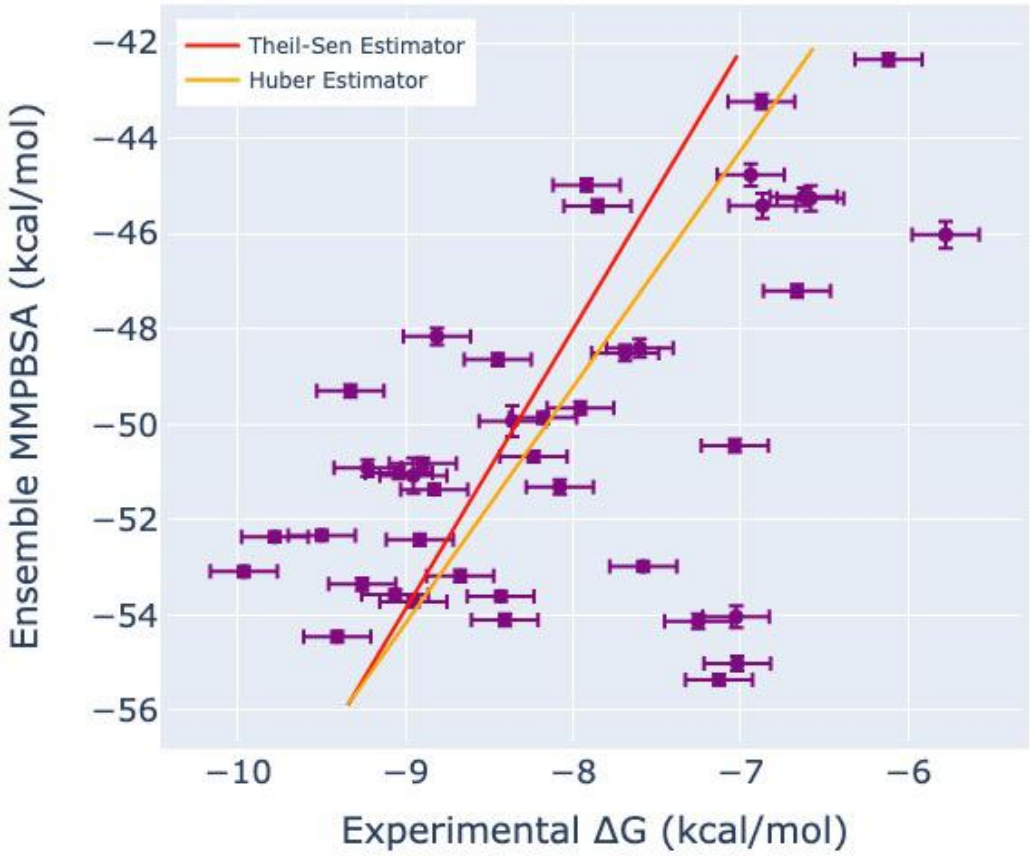
Modeling ΔG with <MMPBSA>: MCL1 Dataset



Correlations Between Experimental ΔG and Ensemble MMPBSA

Metric	Value	Median [5%,95%][a]	*p*-value
Pearson's r^2	0.295	0.301 [0.097, 0.526]	0.000
Kendall's τ [b]	0.339	0.341 [0.143, 0.517]	0.002

[a]Median with confidence intervals of 5% to 95% estimated using bootstrapping (2000 samples). [b]Kendall's τ is a recommended metric to assess rank ordering.

Robust Linear Models of Experimental ΔG

Metric	Theil-Sen	Huber
Slope	0.170	0.202
Intercept	0.186	1.930
MAE[a]	0.731	0.700
Relative MAE[b]	0.810	0.775

[a]Mean Absolute Error. [b]MAE divided by the Absolute Average Deviation of Experimental ΔG.

- Huber or Thiel-Sen estimators: robust linear models for ΔG

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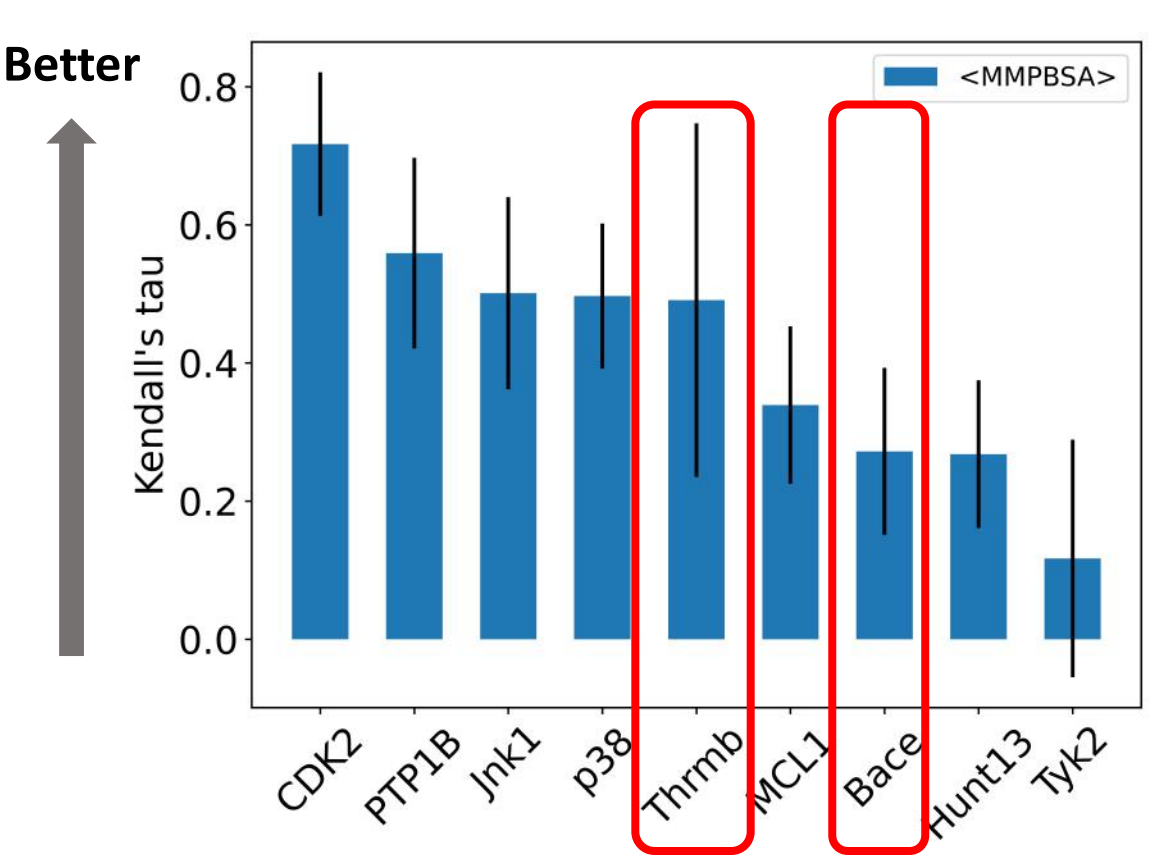
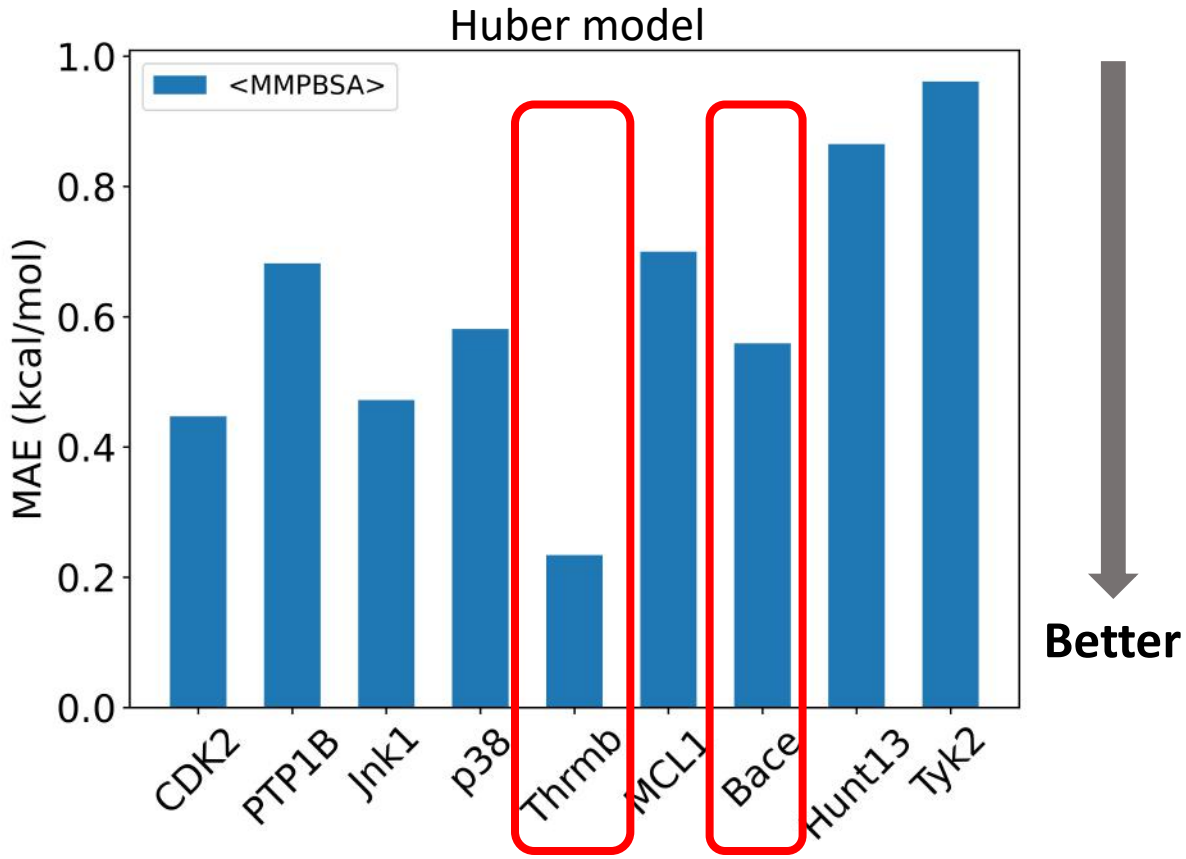
Huber Modeling of ΔG with <MMPBSA>: 9 Datasets





- Huber model gives better Mean Absolute Error (MAE) for datasets where Kendall's τ correlation is better

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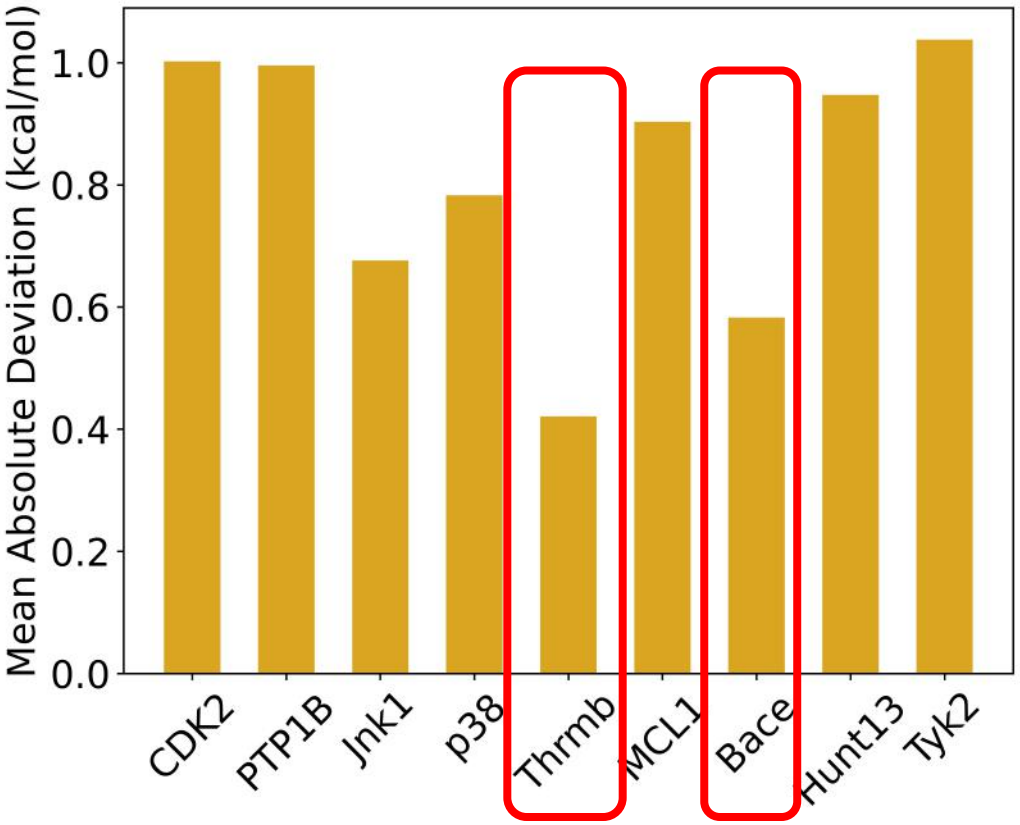
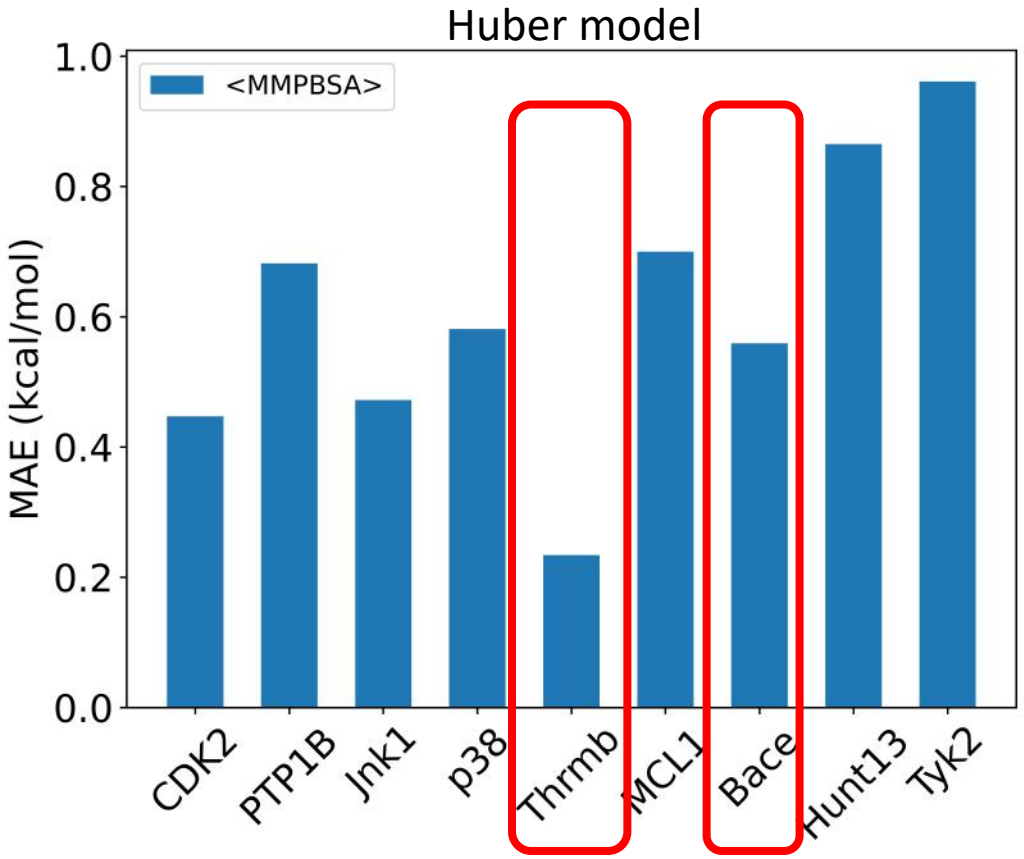
MAE is Sensitive to Mean Absolute Deviation





- Mean Absolute Deviation (MAD): property of the data (Exptl ΔG)
- Narrower affinity range (**poorer** dataset) → **lower** MAEs !

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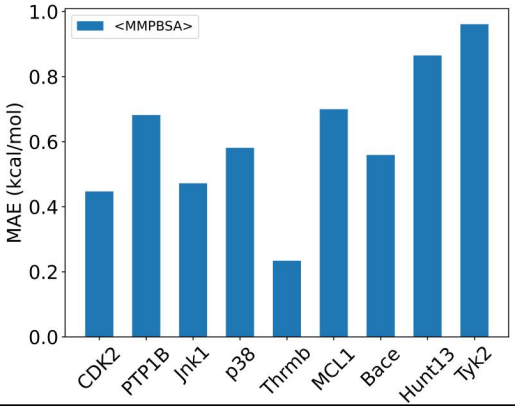
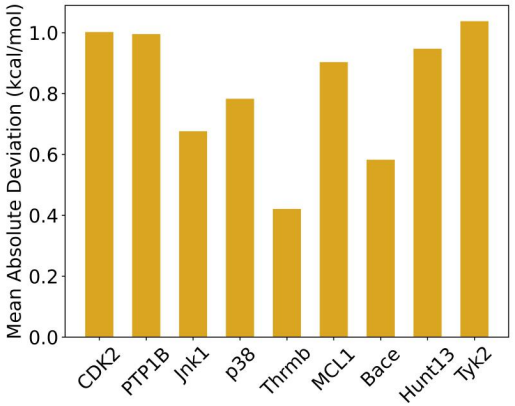
Use Relative MAE instead of MAE

MAE



MAD



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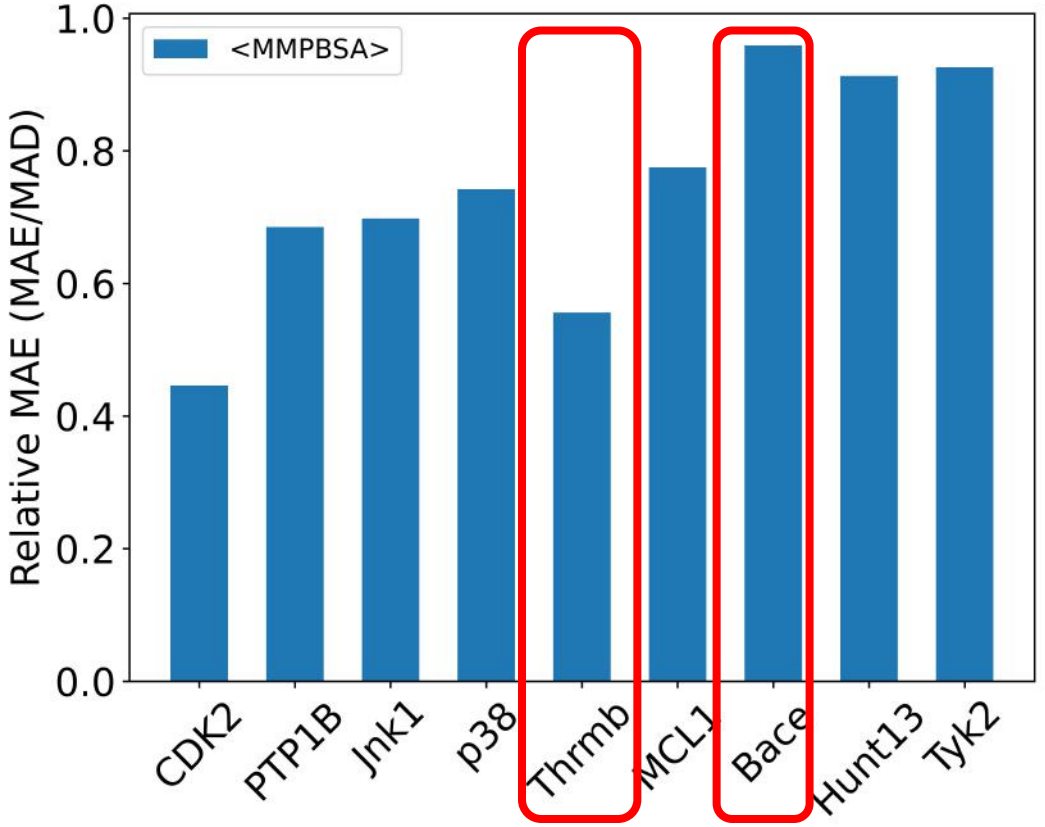

- Relative Mean Absolute Error: **RMAE** = **MAE/MAD**
- Normalizes MAE between datasets (and dimensionless!)

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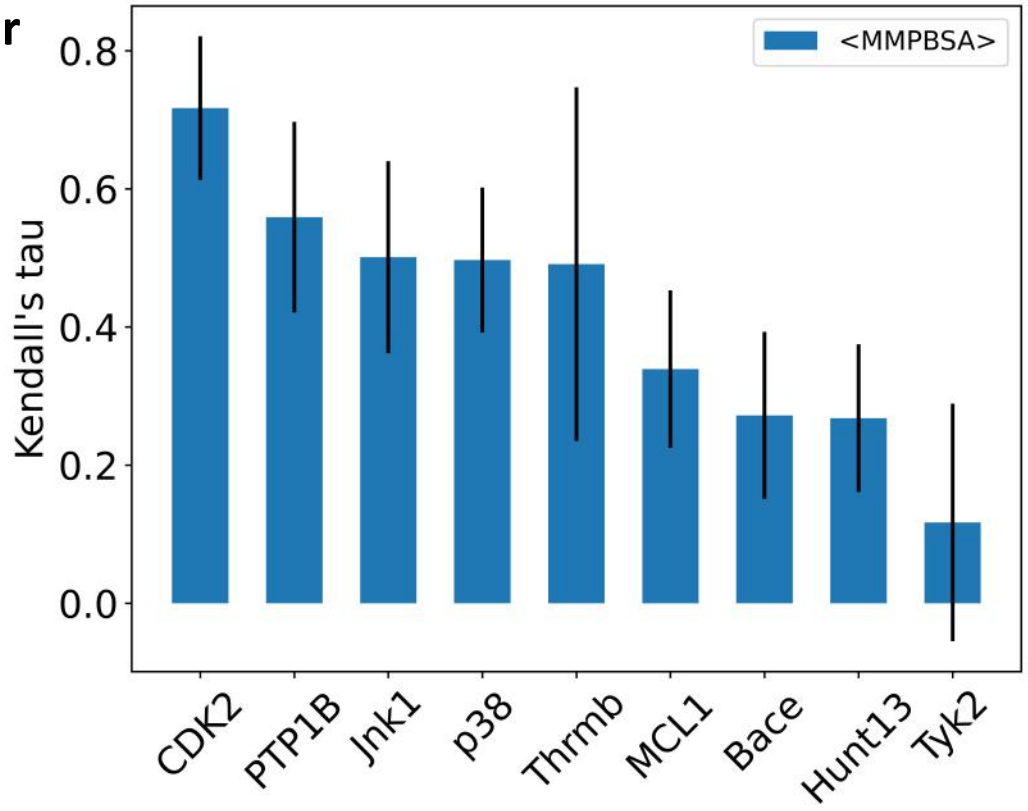
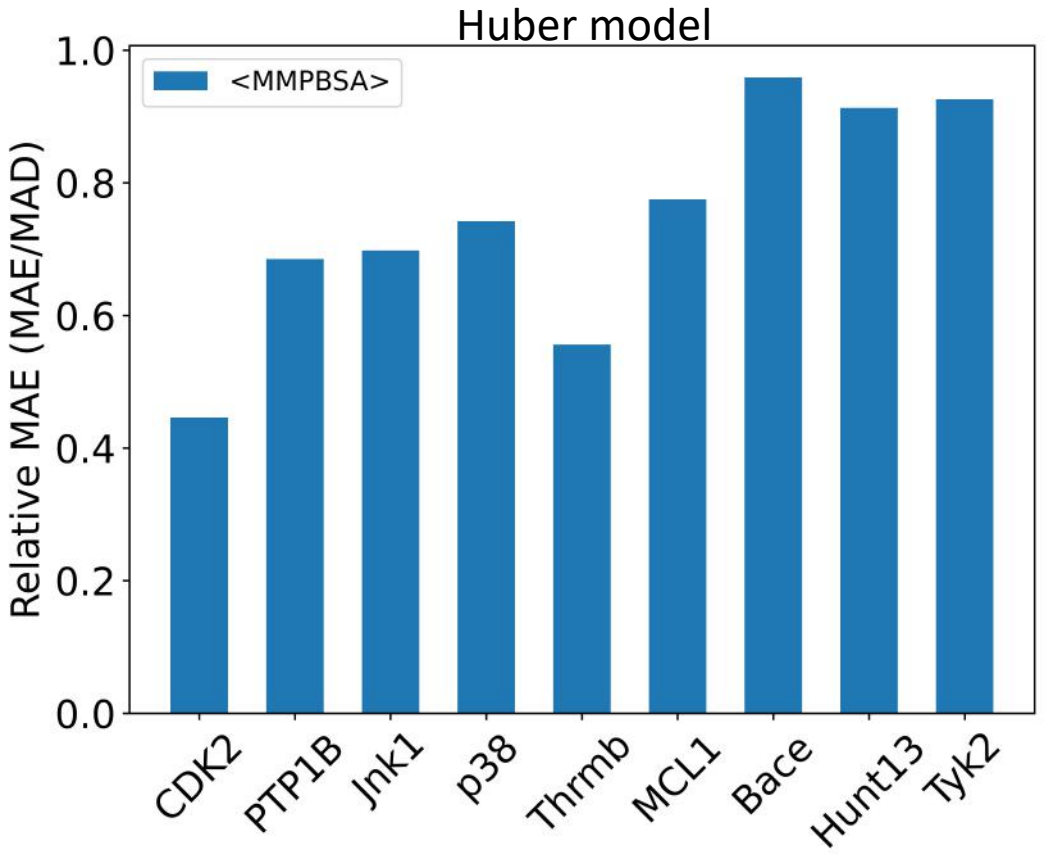
Modeling ΔG with <MMPBSA>: 9 Datasets

Better





Better

- Huber model tends to give better Relative Mean Absolute Error (RMAE) for datasets where Kendall's τ correlation is better

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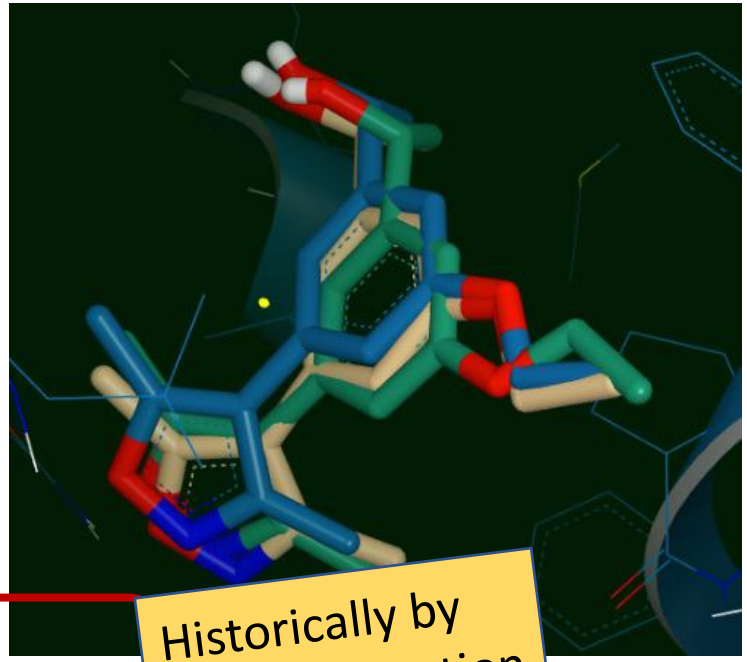
Short Trajectory MD: Pose Testing

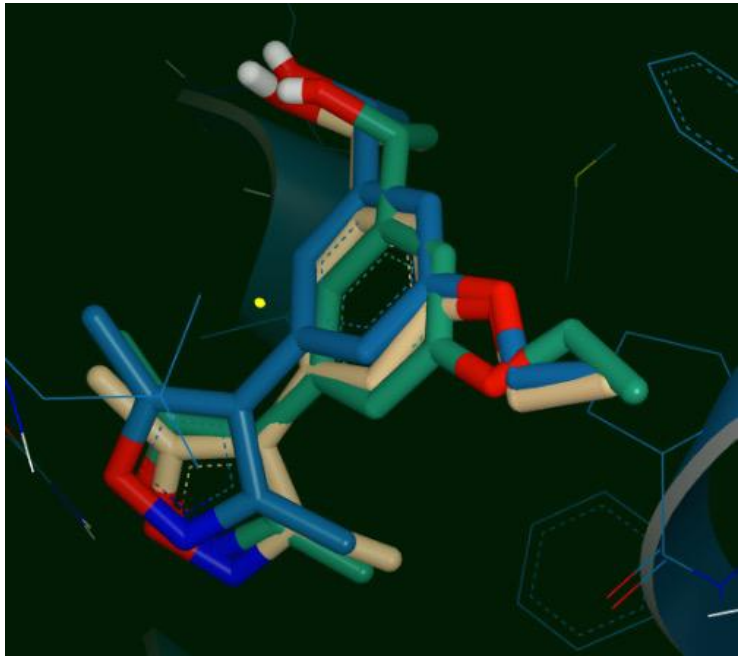


- Improved evaluation of a bound pose
  - Samples protein, ligand, and explicit water motion
  - Short Trajectory (2 ns)
    - Only the immediate region of the bound pose
  - Does the pose remain stable?
  - Do the protein-ligand interactions remain as designed?

Historically by visual inspection (Merck Frosst)

- High throughput ; light calculation
  - Orion: 1 hr/ligand (~$1), highly parallel

Short Trajectory MD: Pose Testing



- Improved evaluation of a bound pose
  - S… explicit water motion
  - S…
    - Only the im…ediate region of the bound pose
  - Does the pose remain stable?
  - Do the protein-ligand interactions remain as designed?
- Hig…ion
  - C… aral

"MD Trajectory" property: Ensemble Average

Qualitative assessment of protein-ligand interactions

Based on the "goodness" of the ligand's Initial Pose

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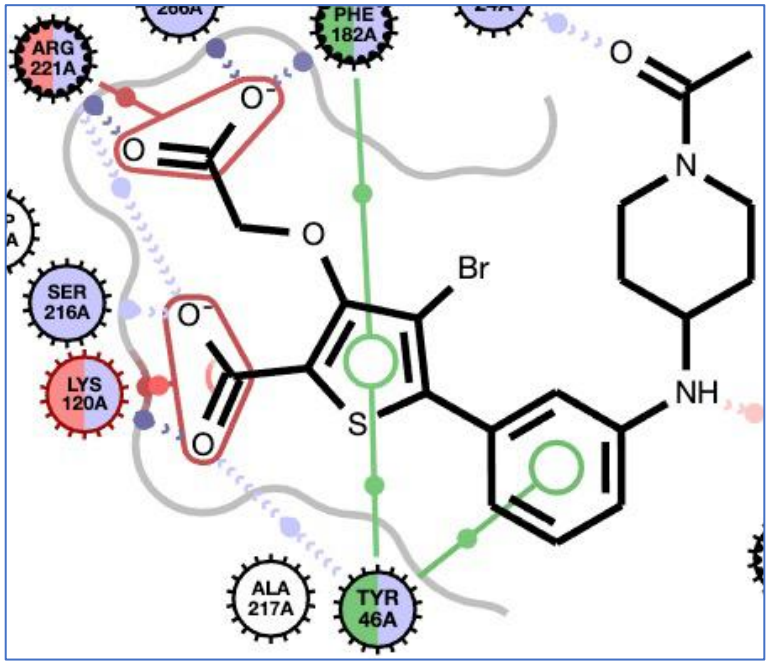
“Do the **Protein-Ligand interactions** …

OEInteractions:

…
OEInteraction Type: contact
<ligand: 0 O protein:3561 C>

OEInteraction Type: hbond:protein2ligand
<ligand: 2 O protein:4300 N>

OEInteraction Type: hbond: non-ideal-protein2ligand
<ligand: 0 O protein:3555 N>
…



- OEInteractions: Qualitative protein-ligand binding interactions
  - Knowledge-based approach
  - OEChem TK → Grapheme TK

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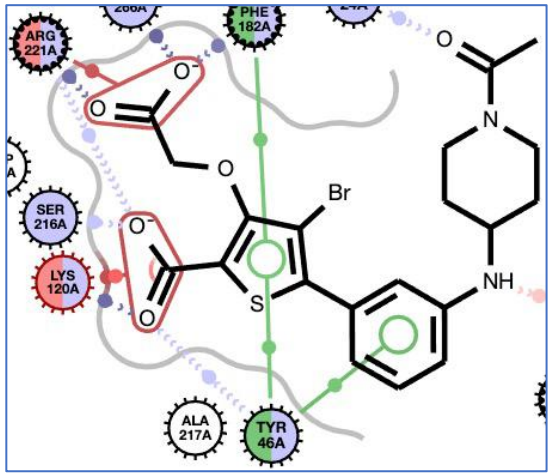
“Do the **Protein-Ligand interactions** …

- Use OEInteractions of the initial ligand Pose
  - Principle: The more **good** OEInteractions there are, the better
  - Prerequisite to relate to affinity

- Scoring the Initial Pose:
  - With **good** OEInteraction Types ***j*** having $weight_j$
  - For ***i*** OEInteractions of Type ***j***:

BintScore = sum_j (***i*** * $weight_j$)



Binding
interactions
—
Bint

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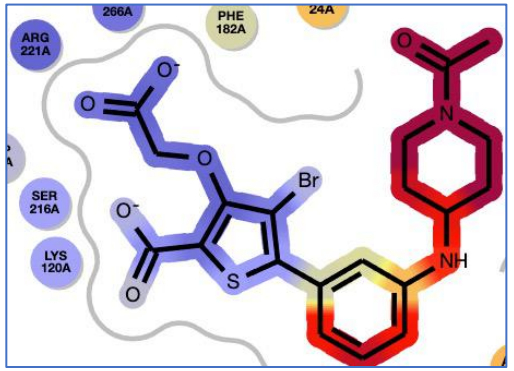
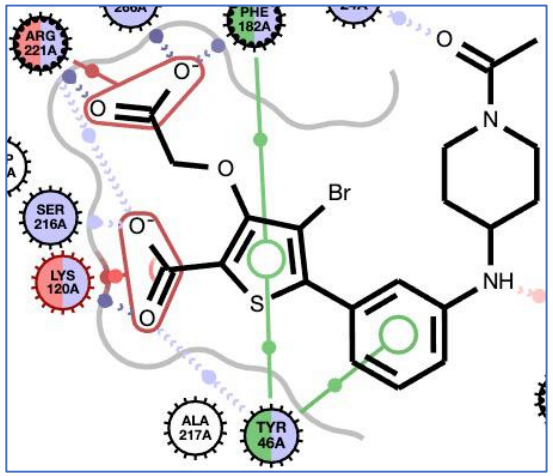
"… remain as designed?"

- Initial Pose BintScore: **Reference** OEInteractions
  - Initial Pose = "the design"
  - Initial Pose BintScore = "goodness" of the design



- Trajectory BintScore = **<BintScore>**
  - Per-frame occupancy of **Reference** OEInteractions
  - Simple ensemble average



- A **change** in binding interactions, **even if good**, is rejected
  → "Does the pose remain stable?": **No**

- **<BintScore>** strongly depends on the Initial Ligand Pose

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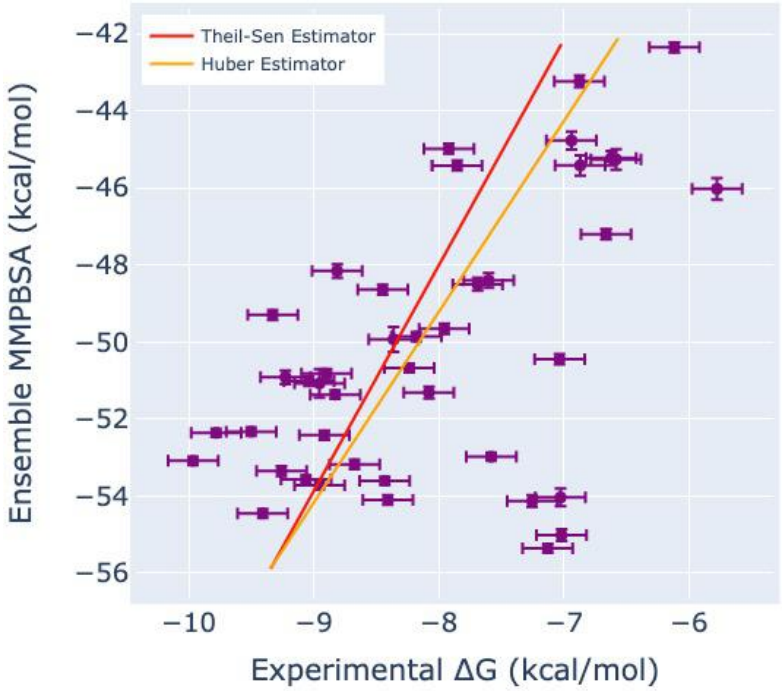
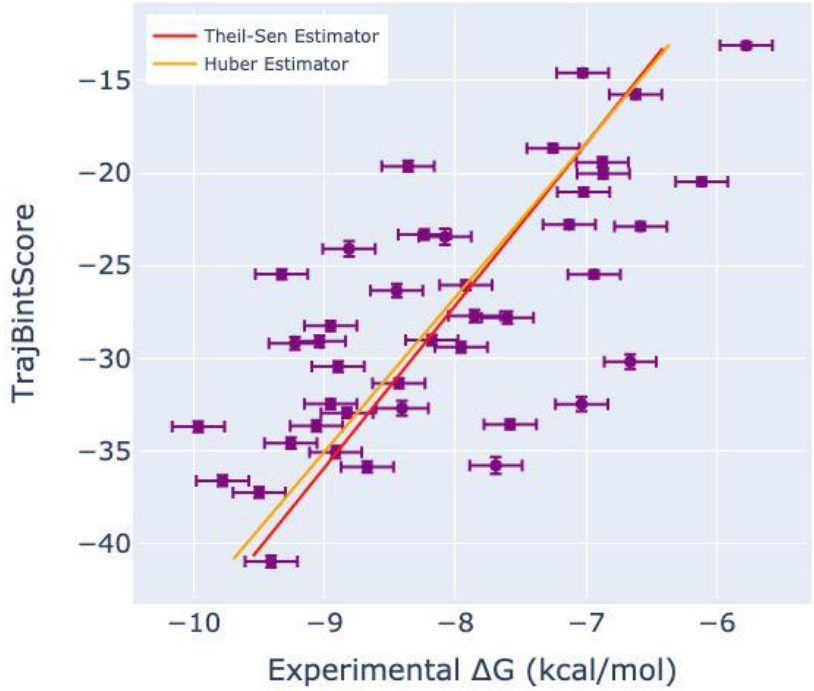
<MMPBSA>, <BintScore>, and InitBintScore: MCL1 Dataset

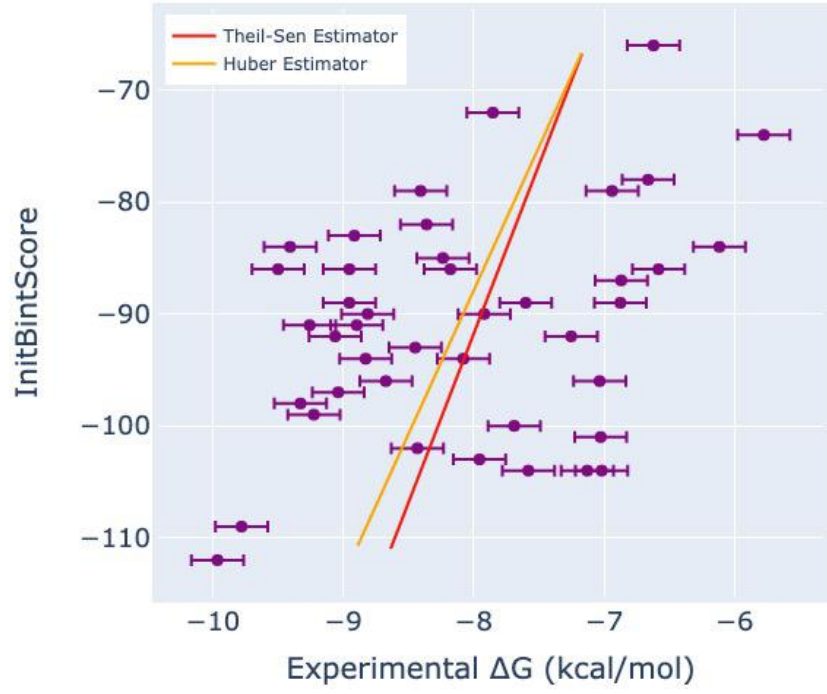
<MMPBSA>



<BintScore>



InitBintScore



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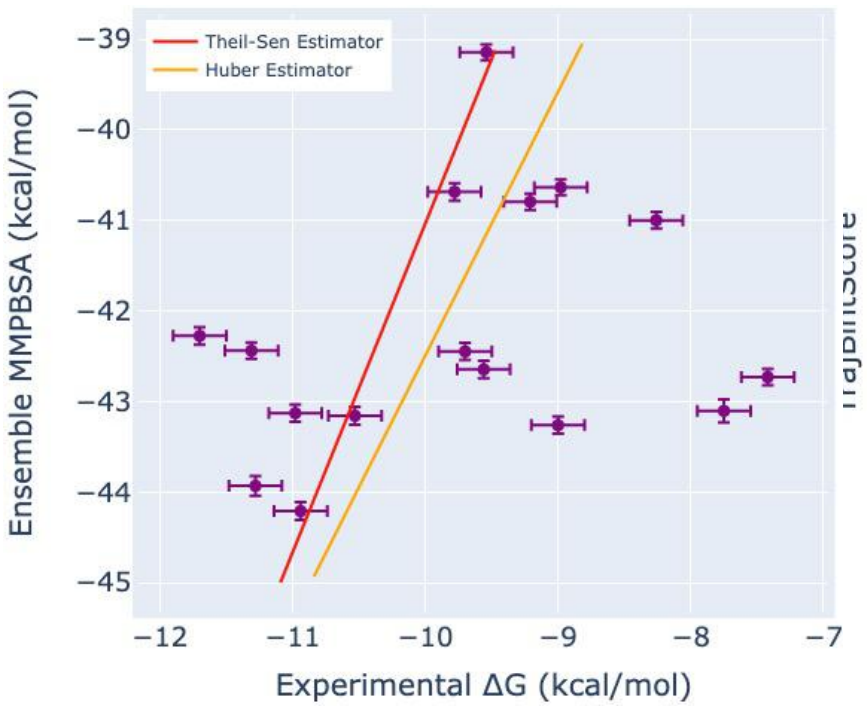
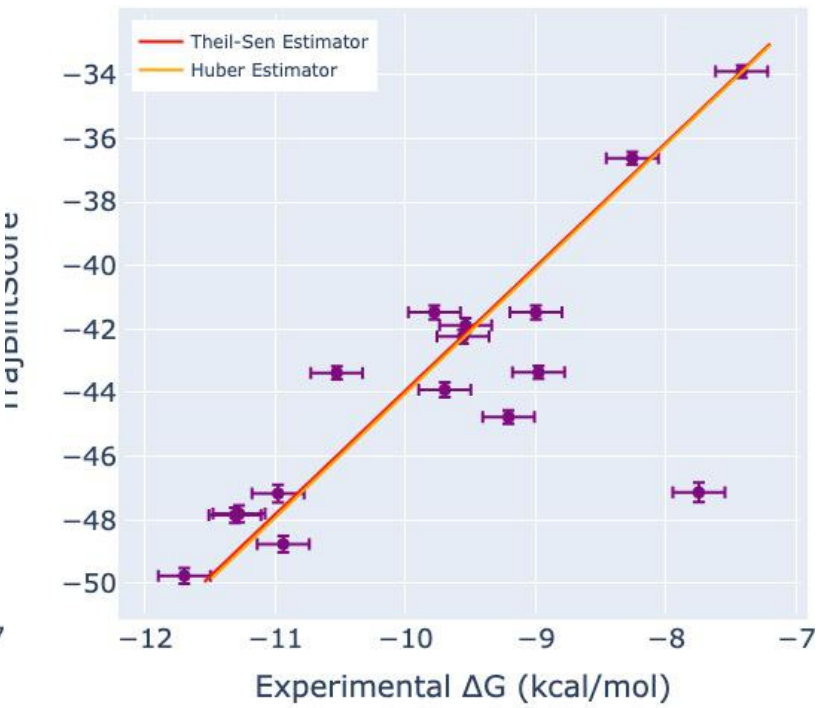
<MMPBSA>, <BintScore>, and InitBintScore: Tyk2 Dataset

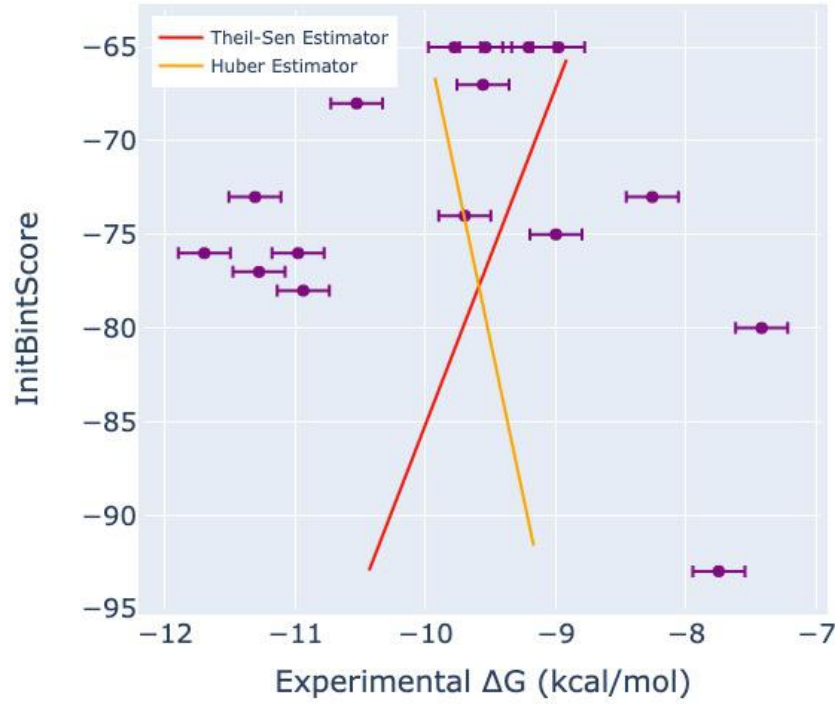
<MMPBSA>



<BintScore>



InitBintScore



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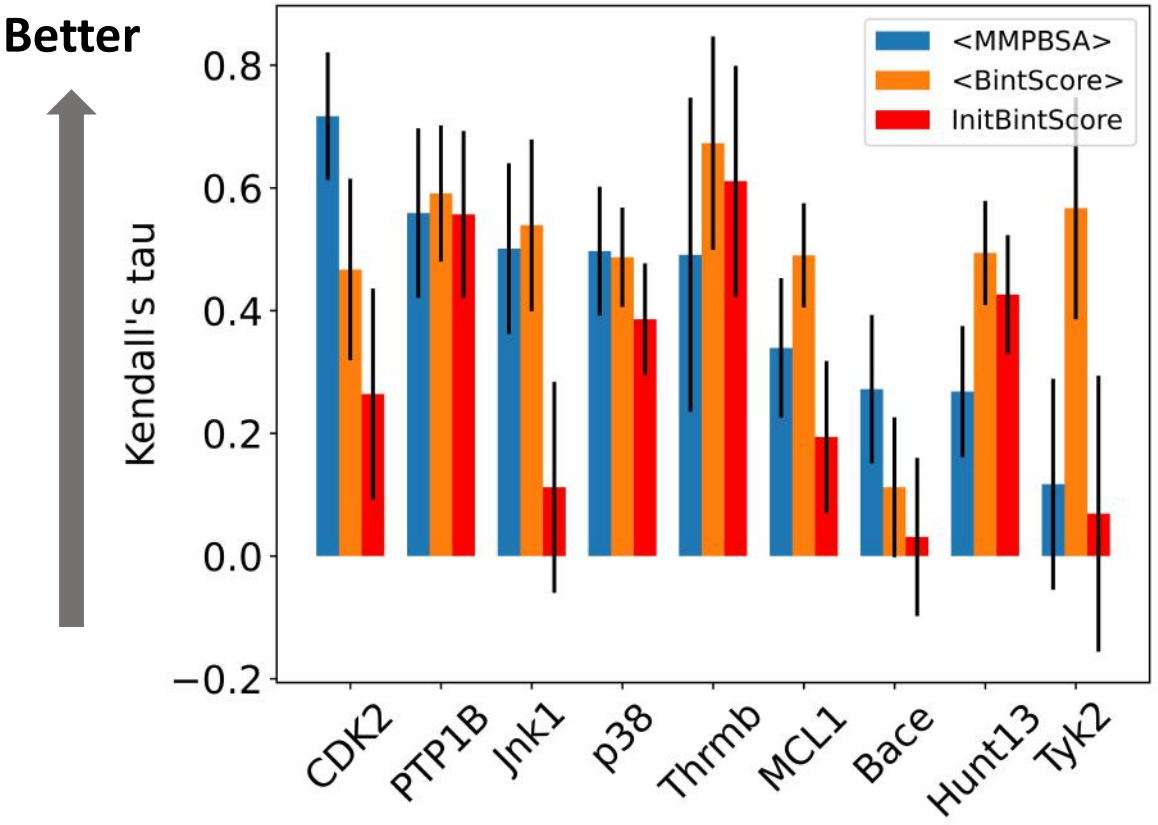
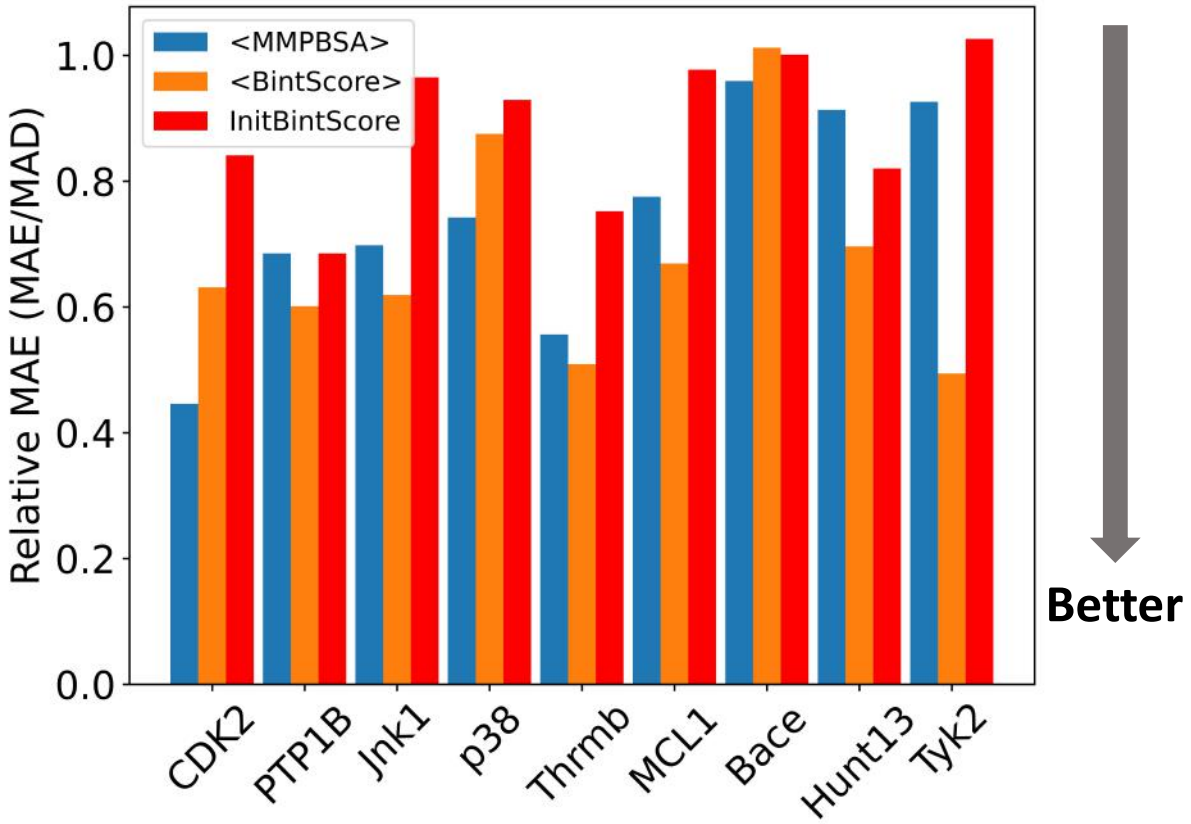
Huber Models of ΔG : 9 Datasets





- <MMPBSA> and <BintScore> have similar **aggregate** performance
  - Complimentarity on specific targets
- InitBintScore performs less well → pose stability matters

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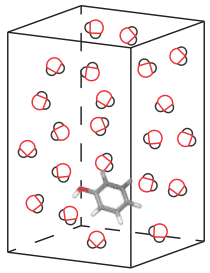
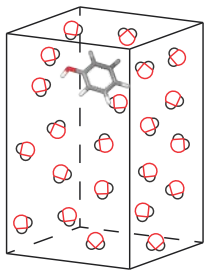
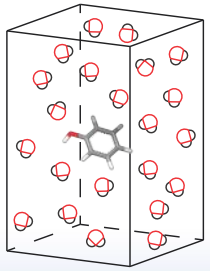
RBFE with Non-Equilibrium Switching (NES)



Equilibrium





$W_{A \to B}$

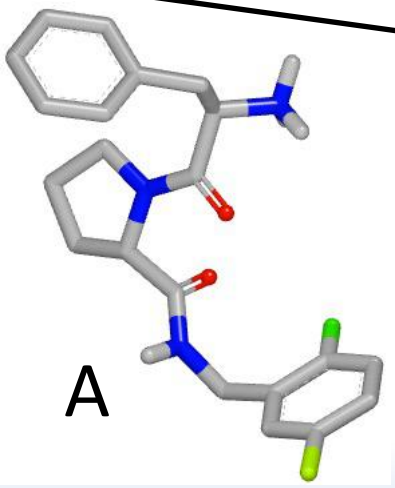
$W_{A \to B}$

$W_{A \to B}$

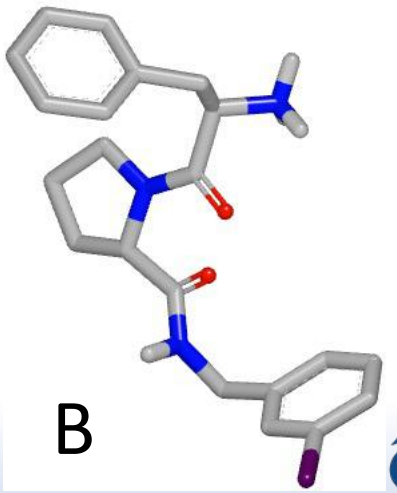
Non-Equilibrium $A \to B$

Non-Equilibrium $A \to B$

Non-Equilibrium $A \to B$



A



B

Bert L. De Groot

Vytautas Gapsys

Gapsys et al., *Chem. Sci.*, 2020, **11**, 1140-1152

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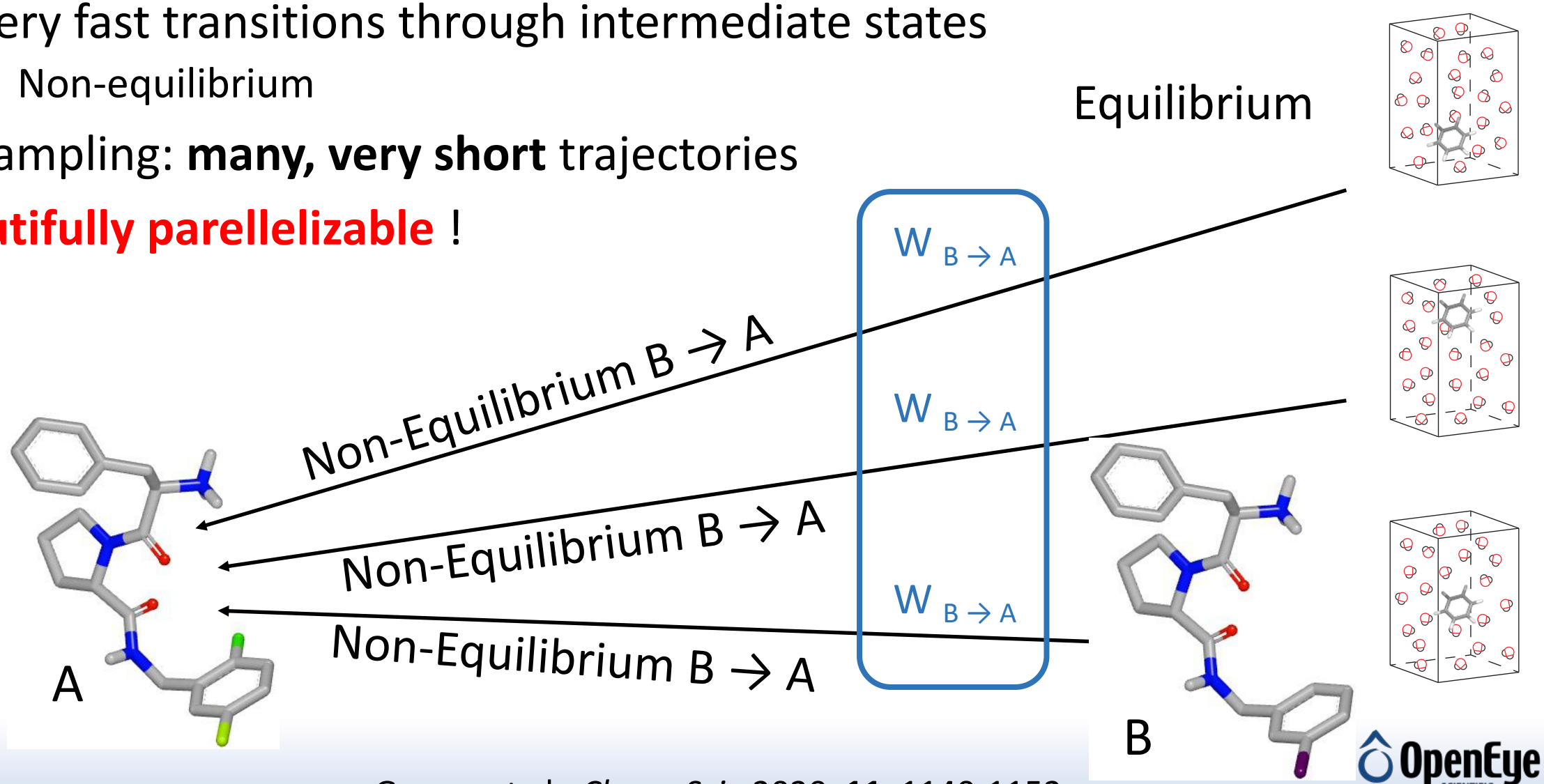
RBFE with Non-Equilibrium Switching (NES)

- Very fast transitions through intermediate states
  - Non-equilibrium
- Sampling: **many, very short** trajectories

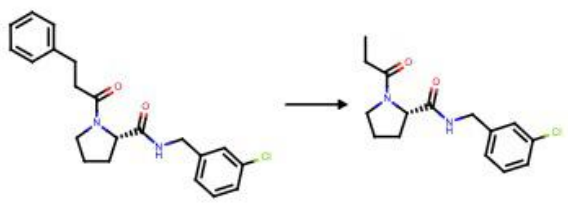
Beautifully parellelizable !



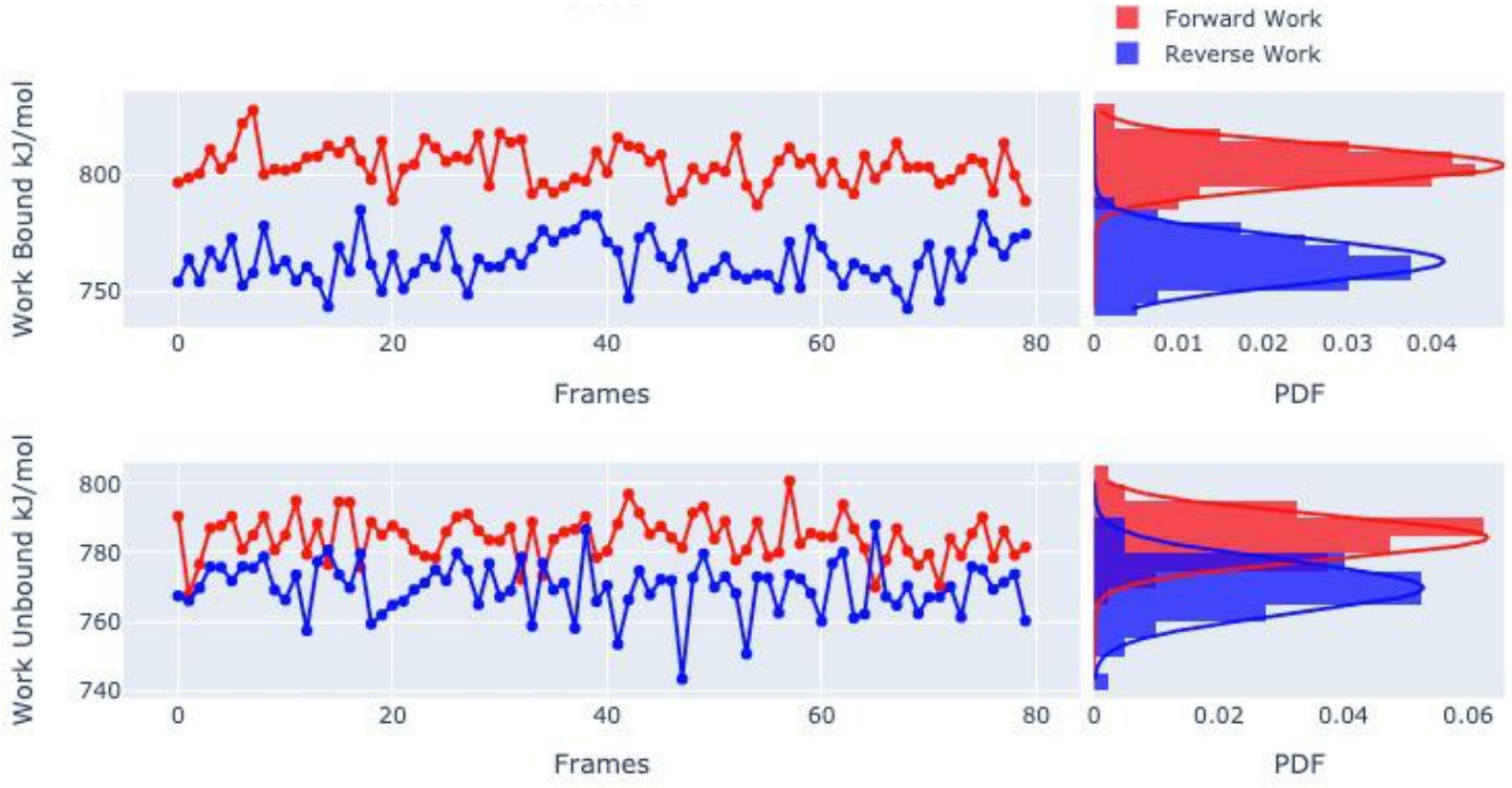
Gapsys et al., *Chem. Sci.*, 2020, **11**, 1140-1152

RBFE with Non-Equilibrium Switching (NES)

3K to 3B



BAR score:
8.30 ± 1.02 kJ/mol



BAR:

$$\sum_{i=1}^{n_f} \frac{1}{1+\exp\left(\ln\frac{n_f}{n_r}+\beta(w_i-\Delta G)\right)} = \sum_{j=1}^{n_r} \frac{1}{1+\exp\left(\ln\frac{n_r}{n_f}+\beta(w_j-\Delta G)\right)}$$

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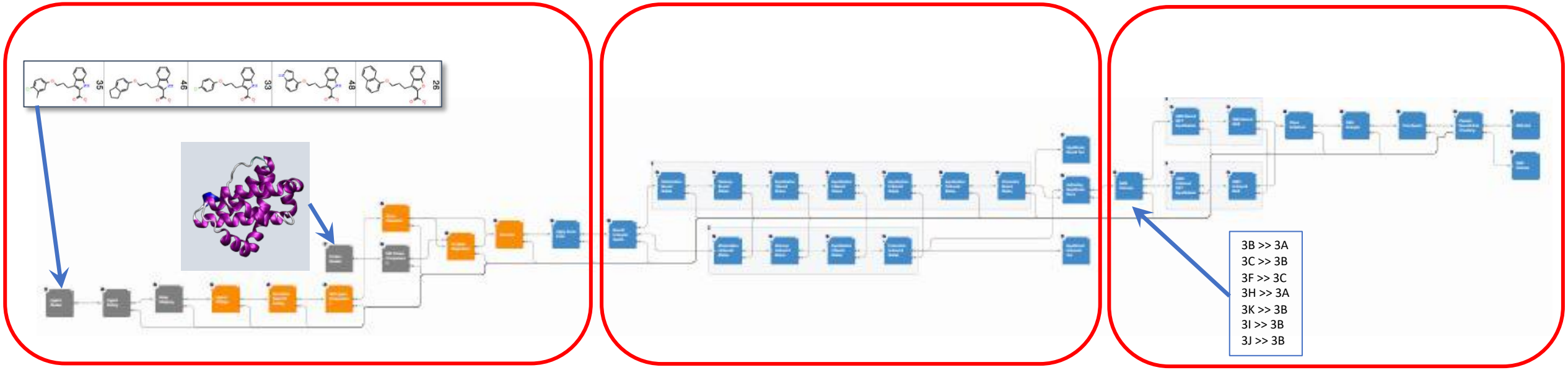
RBFE-NES Floe: Bird's eye view



Stage 1: System Setup

Stage 2: End-point Equilibration

Stage 3: NES

- Almost entirely based on existing OE Orion infrastructure for MD
- User input Edge mapping will come from RBFE mapper (LOMAP)
- Stages 1 and 2 can be from a prior MD run

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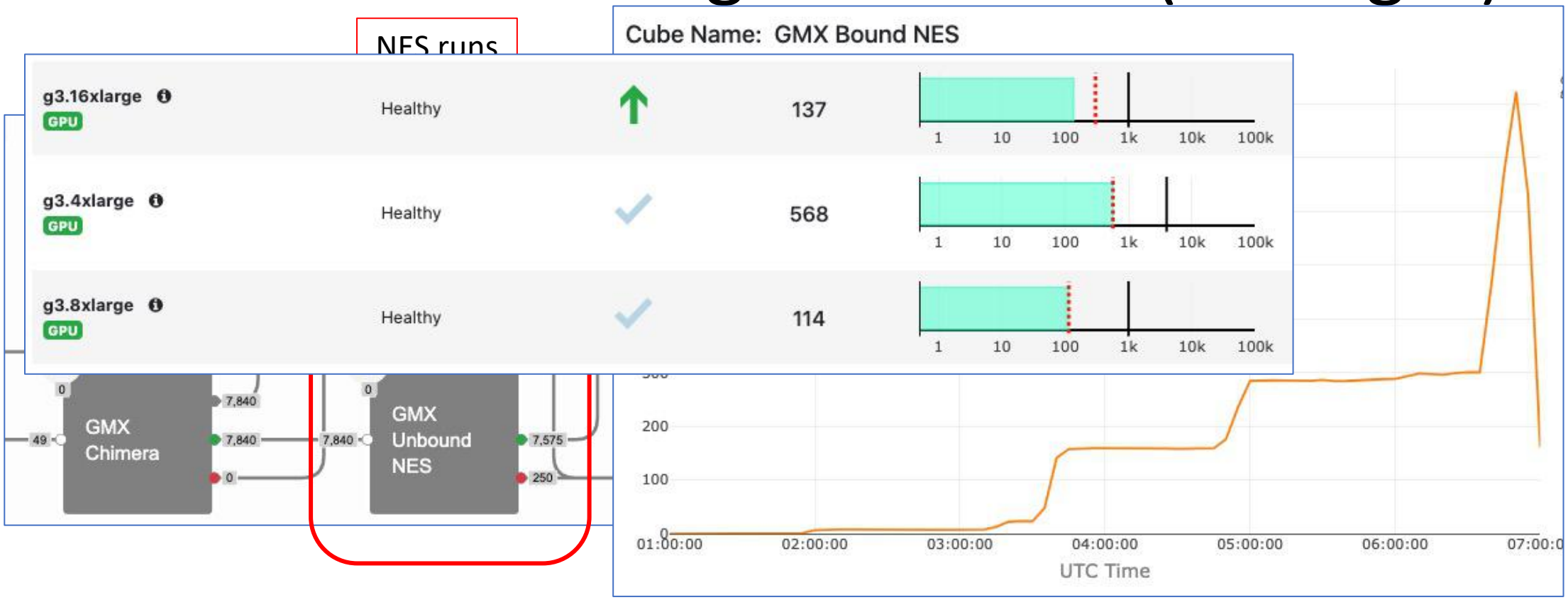
RBFE-NES Floe: NES Stage for PTP1B (49 edges)



- Orion scheduler dynamically allocates spot instances (availability)
  - Not a concern for the user: everything finishes!
  - Runtime may vary based on availability

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NES Protocol mainly followed Gapsys et al.

- Gromacs 2020
- OpenFF 1.3 (Parsley) with Amber ff14
- Equilibrium runs done separately
  - Bound and unbound ligand
  - 1X 6 ns, no clustering
  - No NES knowledge embedded
- NES runs: 80 frames with 50ps switching per frame
  - OpenEye alchemical chimeric A/B ligands
  - $\Delta\Delta G$ correlations symmetrized around A→B | B→A
- Schrodinger JACS ‘15 datasets: 8 targets
- Hunt ‘13 Bace dataset

*Gapsys et al., *Chem. Sci.,* 2020, **11**, 1140-1152

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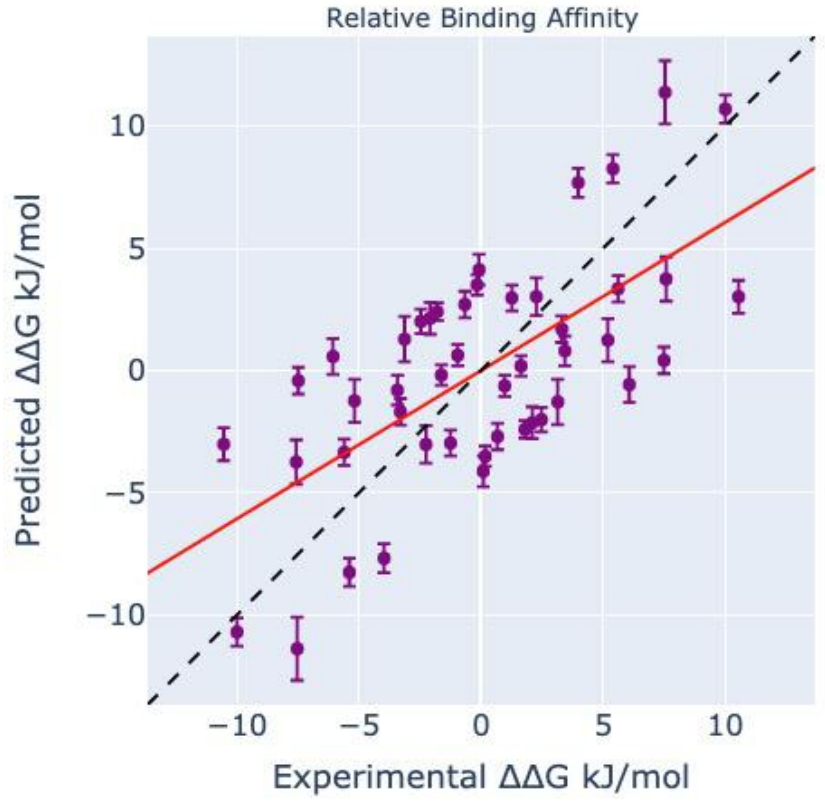
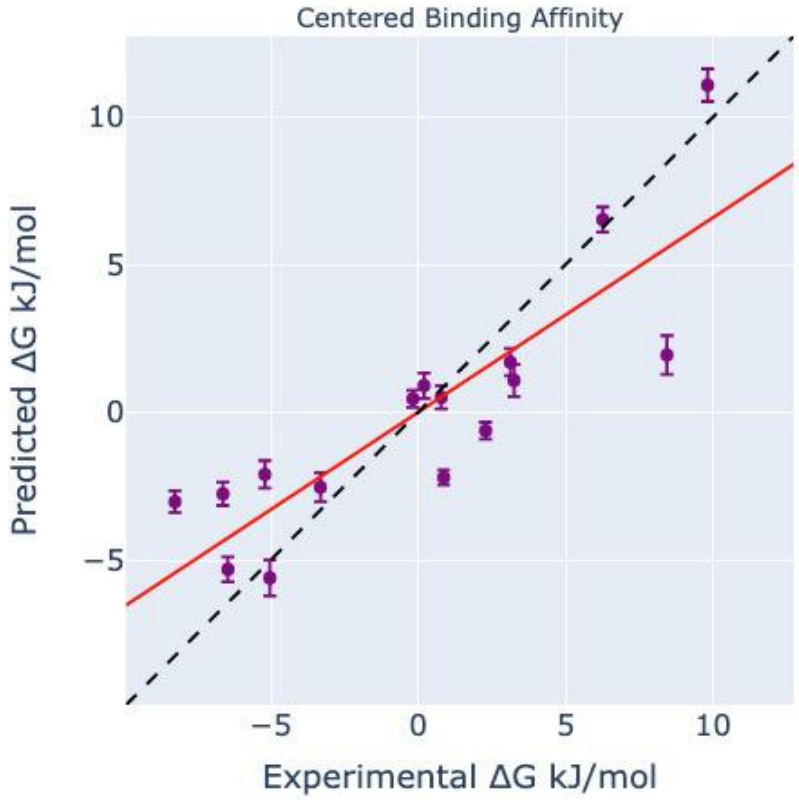
Tyk2

- 16 ligands
- 24 edges
- Equil: $15
- NES: $232, 6h
- NES failures
  - Bound 8%
  - Unbound 7%





Method	ΔΔG kJ/mol
MAE	3.51 [3.86 5.13]
Relative MAE	0.87 [0.66 0.96]
RMSE	3.95 [4.84 6.34]
Relative RMSE	0.79 [0.66 0.94]
Pearson's R^2	0.44 [0.26 0.62]
Spearman's ρ	0.66 [0.51 0.78]
Kendall's τ	0.45 [0.32 0.57]

Method	ΔG kJ/mol
MAE	2.12 [1.38 3.04]
Relative MAE	0.48 [0.30 0.74]
RMSE	2.78 [1.72 3.70]
Relative RMSE	0.52 [0.35 0.72]
Pearson's R^2	0.73 [0.60 0.90]
Spearman's ρ	0.86 [0.77 0.94]
Kendall's τ	0.72 [0.50 0.89]

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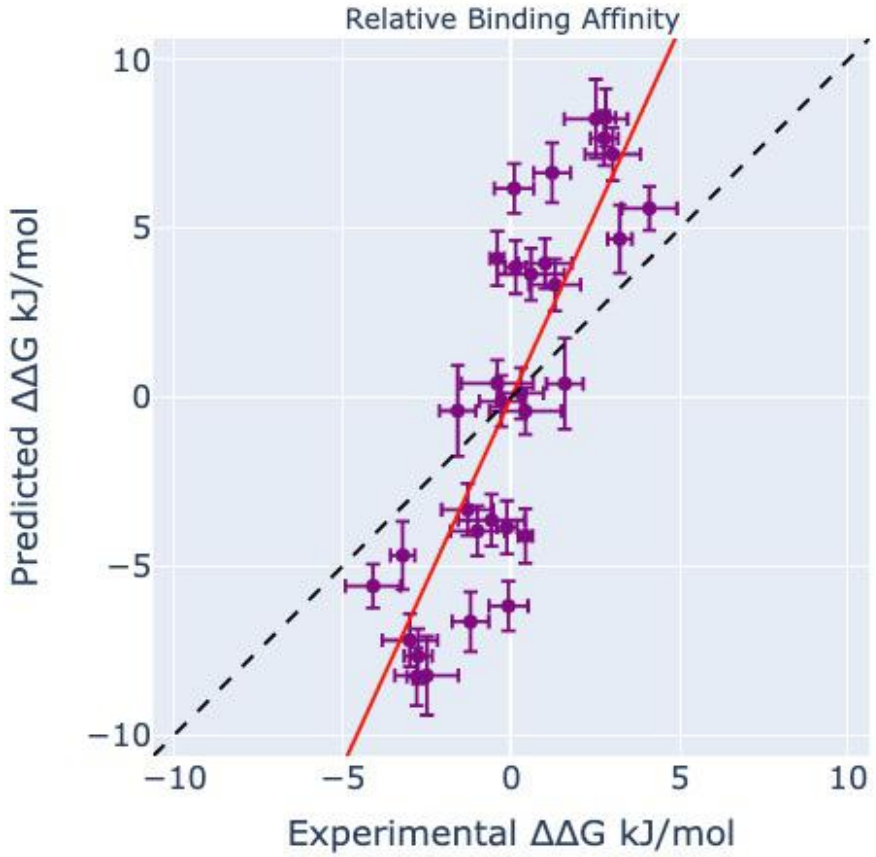
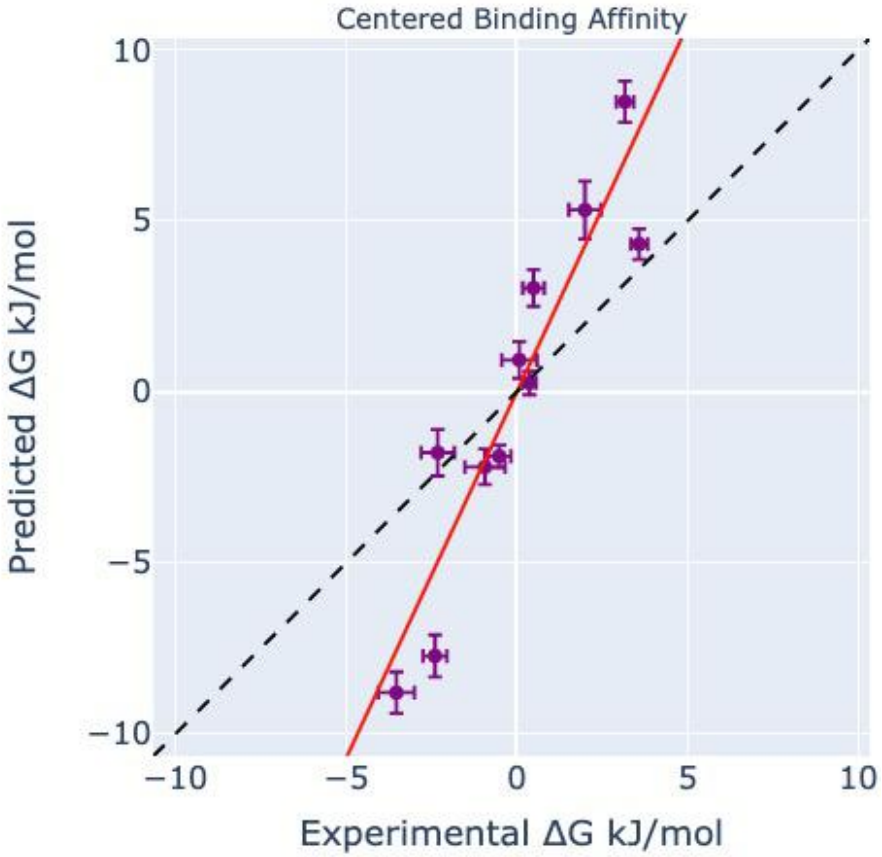
Thrombin

- 11 ligands
- 16 edges
- Equil: $9
- NES: $144, 2.7h
- NES failures
  - None





Method	ΔΔG kJ/mol
MAE	3.33 [3.66 5.09]
Relative MAE	2.10 [1.43 2.60]
RMSE	3.83 [4.48 6.18]
Relative RMSE	1.89 [1.47 2.50]
Pearson's R^2	0.69 [0.51 0.82]
Spearman's ρ	0.83 [0.72 0.90]
Kendall's τ	0.60 [0.52 0.72]

Method	ΔG kJ/mol
MAE	2.42 [1.35 3.59]
Relative MAE	1.38 [0.80 2.17]
RMSE	3.12 [1.88 4.11]
Relative RMSE	1.44 [0.93 2.29]
Pearson's R^2	0.86 [0.71 0.99]
Spearman's ρ	0.93 [0.85 0.99]
Kendall's τ	0.82 [0.53 1.00]

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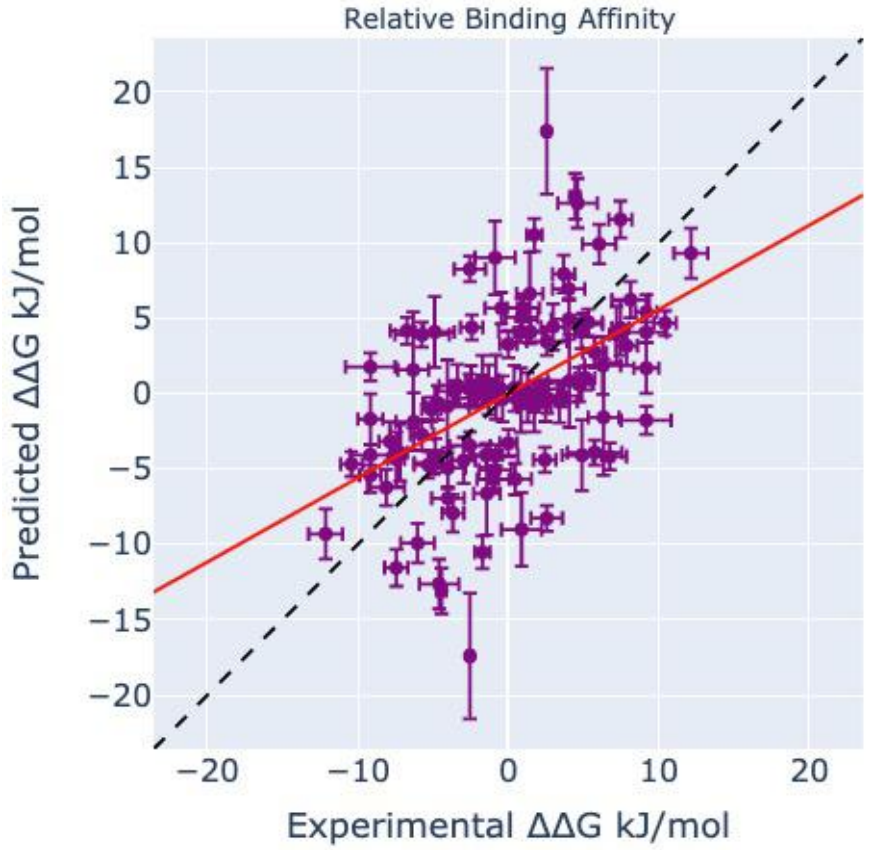
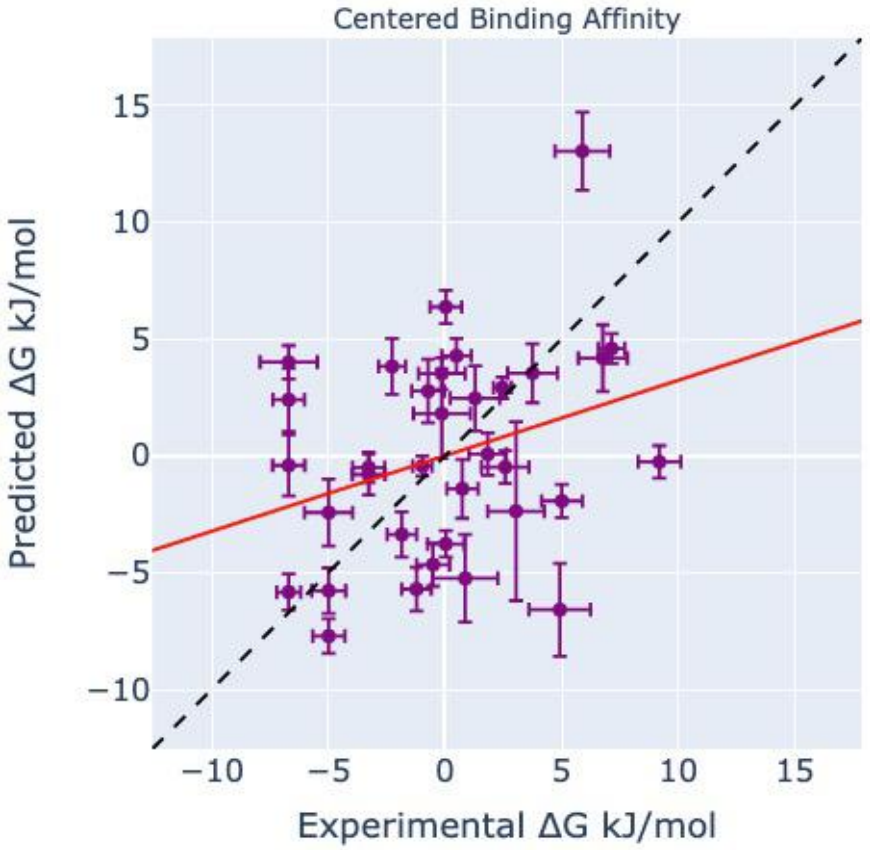
JACS15 p38

- 34 ligands
- 56 edges
- Equil: $39
- NES: $660 6.1h
- NES failures:
  - Bound 3%
  - Unbound 3%

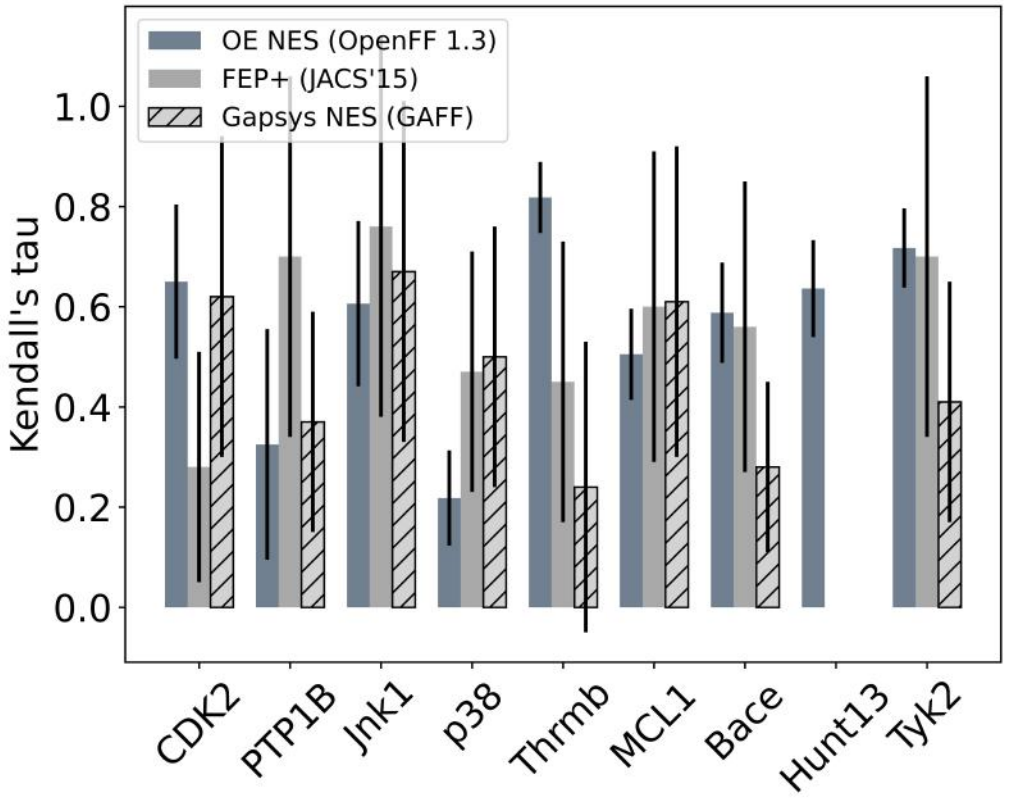


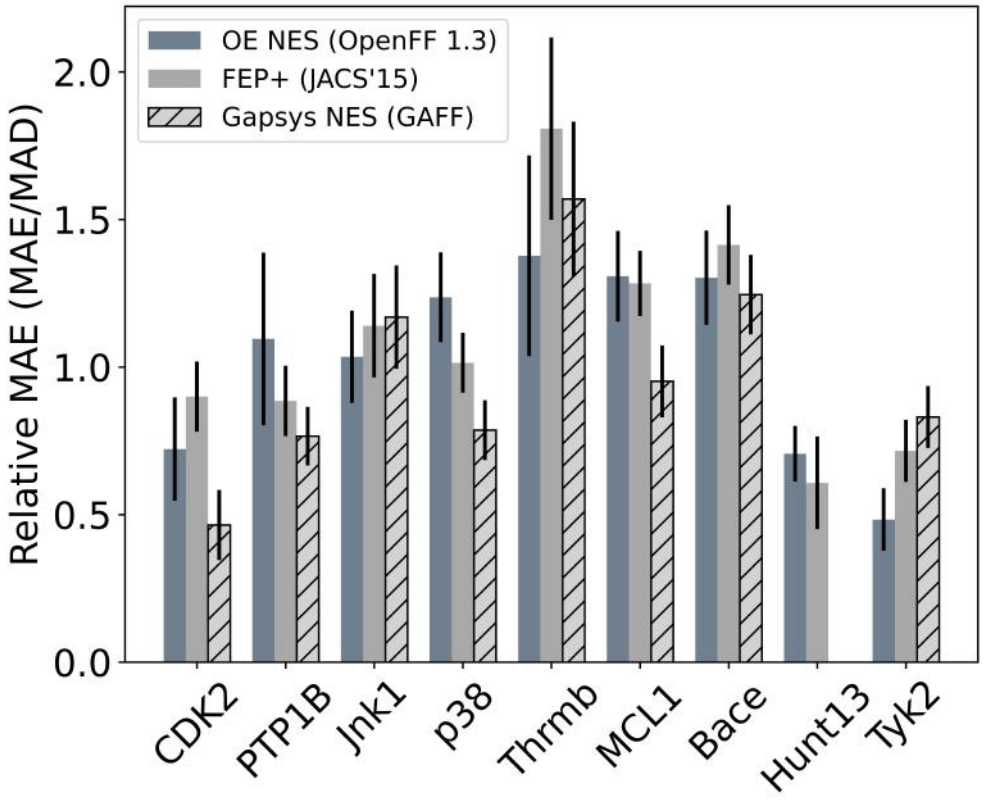


Method	ΔΔG kJ/mol		Method	ΔG kJ/mol
MAE	4.56 [5.55 7.15]		MAE	4.08 [3.11 5.10]
Relative MAE	1.06 [0.90 1.27]		Relative MAE	1.24 [0.94 1.64]
RMSE	5.59 [6.89 8.95]		RMSE	5.04 [3.84 6.06]
Relative RMSE	1.08 [0.91 1.26]		Relative RMSE	1.20 [0.98 1.52]
Pearson's R^2	0.24 [0.14 0.37]		Pearson's R^2	0.10 [0.00 0.35]
Spearman's ρ	0.49 [0.37 0.60]		Spearman's ρ	0.31 [-0.03 0.57]
Kendall's τ	0.36 [0.25 0.44]		Kendall's τ	0.22 [-0.02 0.46]

Direct Predictions of ΔG : 9 Datasets

Better





Better

- OE NES has comparable accuracy to literature benchmarks

Wang et al., *JACS* 2015, **137**, 2695

Gapsys et al., *Chem. Sci.*, 2020, **11**, 1140-1152

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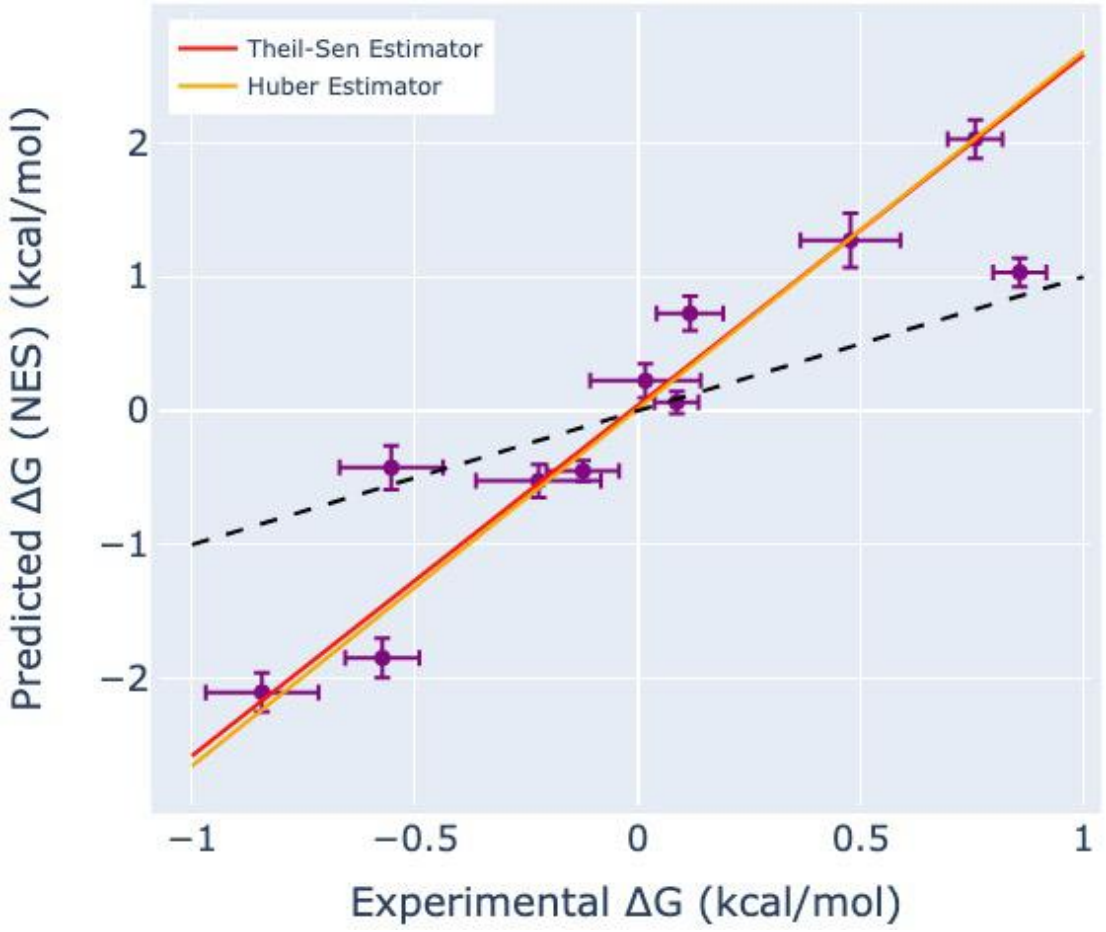
Modeling ΔG with ΔG_NES: Thrombin Dataset



Correlations Between Experimental ΔG and Predicted ΔG (NES)

Metric	Value	StdErr;[5%,95%][a]	*p*-value
MAE[b]	0.580	0.143 [0.358, 0.826]	n/a
RMAE[c]	1.464	0.360 [0.930, 2.075]	n/a
Pearson's r^2	0.859	0.071 [0.741, 0.978]	4.051e-05
Kendall's τ	0.818	0.117 [0.600, 1.000]	1.323e-04

Robust Linear Models of Experimental ΔG

Metric	Theil-Sen	Huber
Slope	0.382	0.375
Intercept	-0.015	-0.006
MAE[a]	0.126	0.127
Relative MAE[b]	0.299	0.302

- Huber or Thiel-Sen estimators: robust linear models for ΔG

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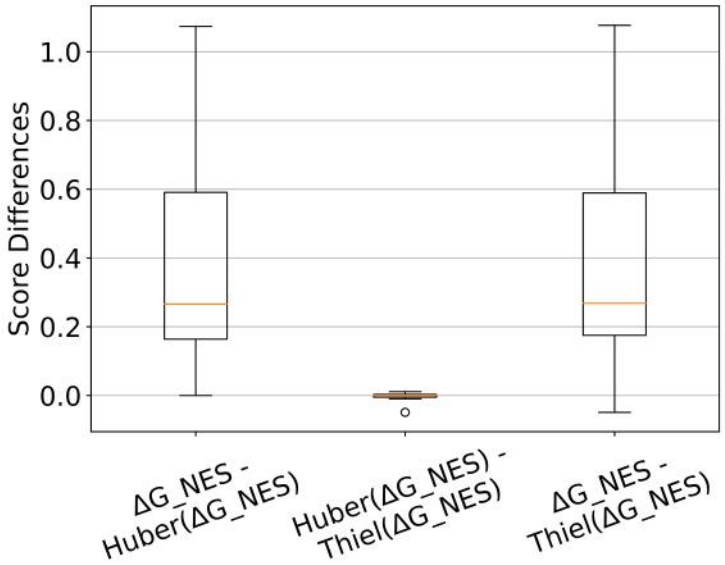
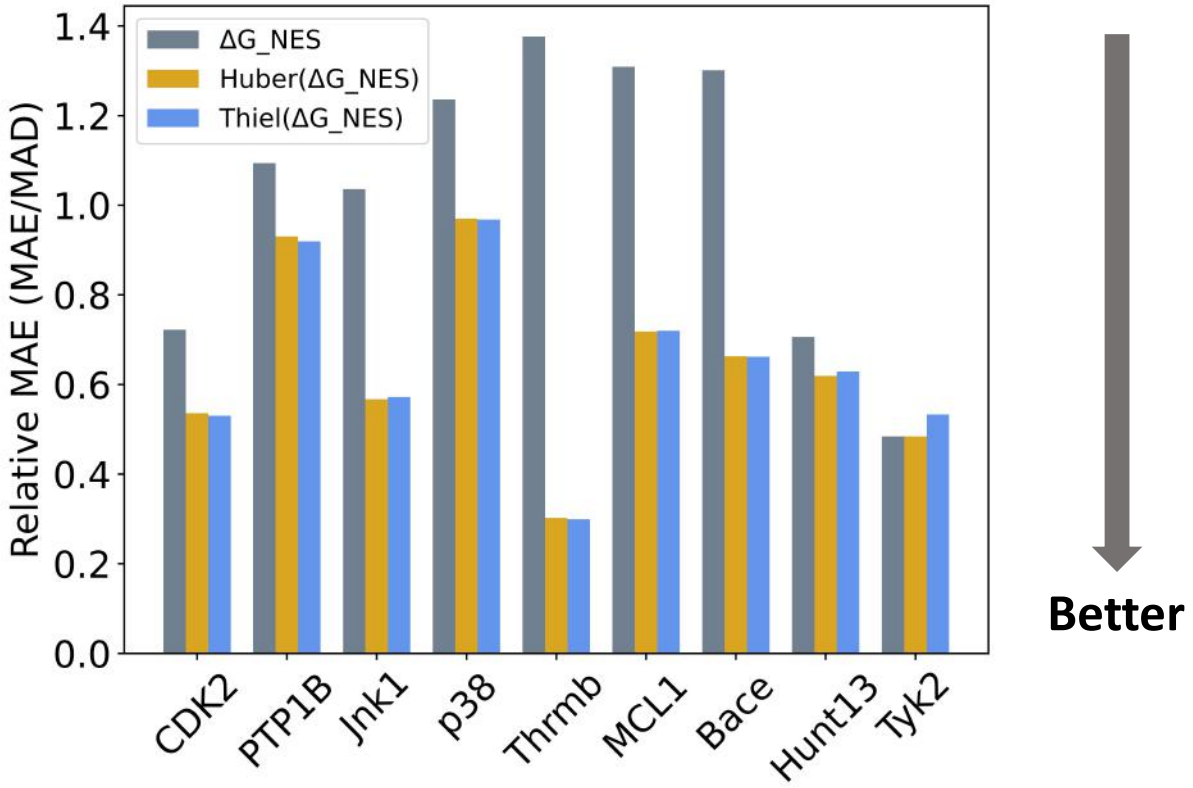
Modeling ΔG with ΔG_NES: 9 Datasets

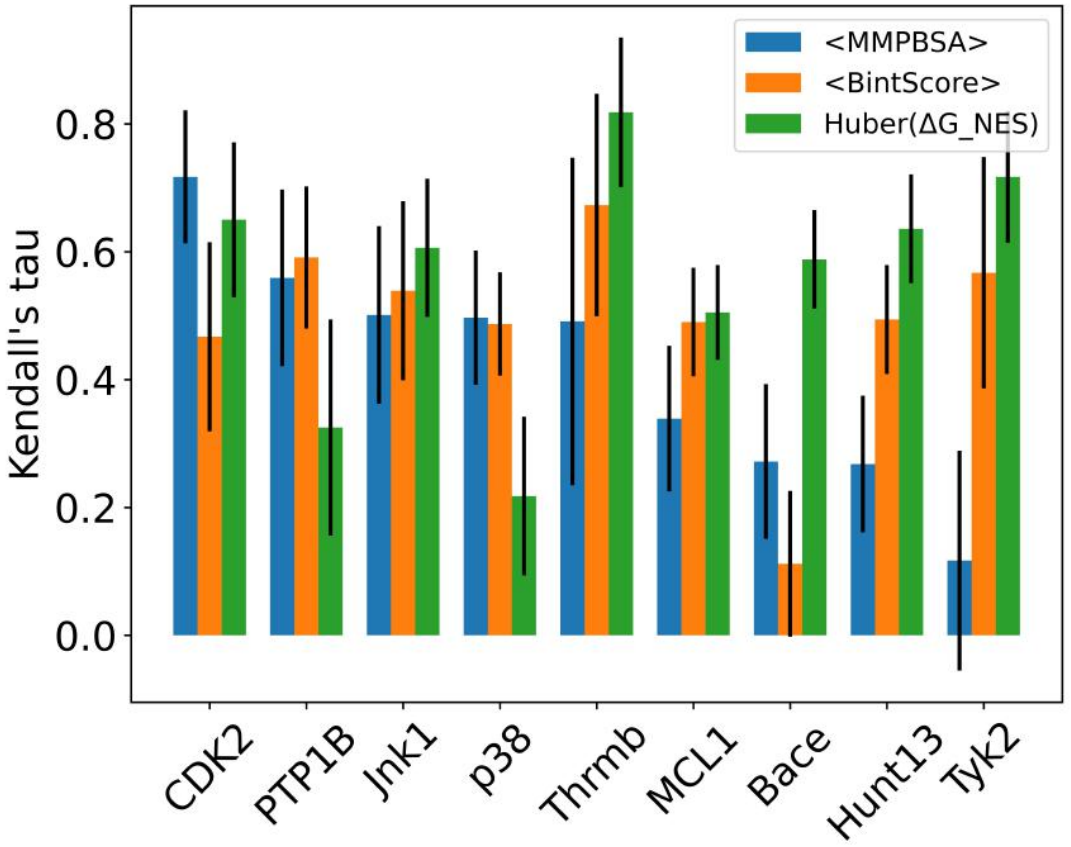


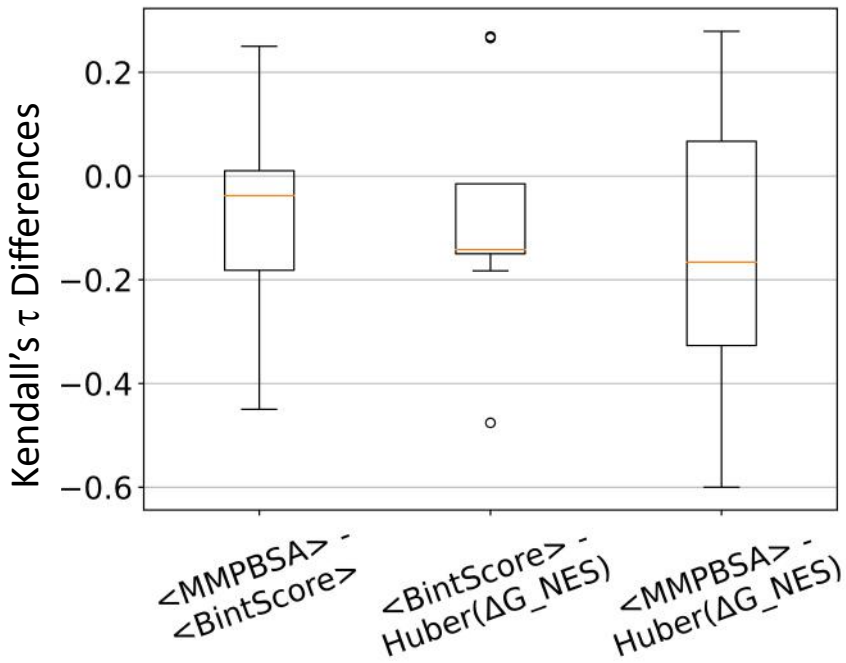


- Robust Linear (Huber) Modeling of Modeling ΔG with ΔG_NES substantially improves RMAE compared to direct prediction

Kendall's τ for <MMPBSA>, <BintScore>, and ΔG_NES

Better





- Kendall's τ correlation between <MMPBSA> ,<BintScore>, and ΔG_NES show no significant differences in **aggregate** performance.
  - Sometimes large differences on individual targets.

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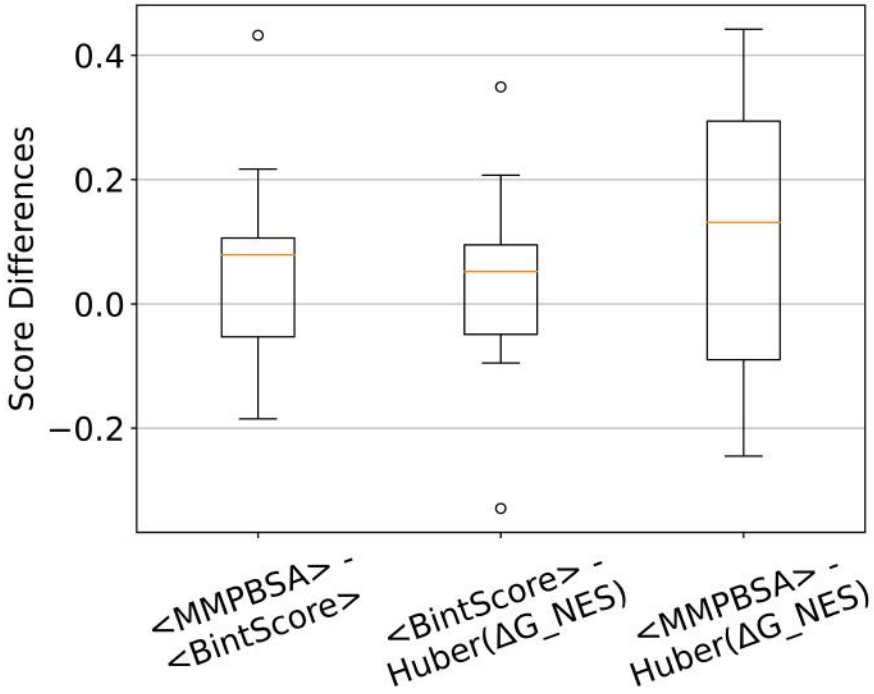
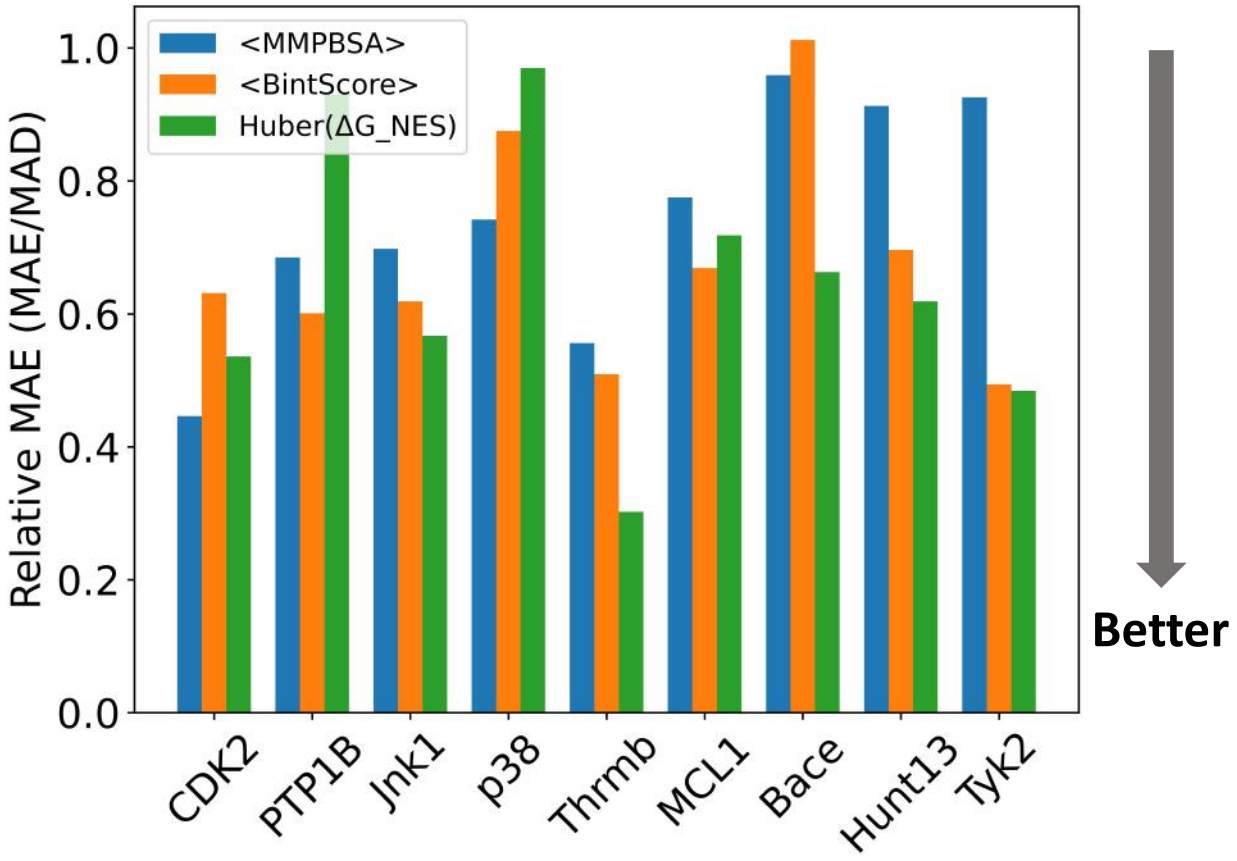
Modeling ΔG with <MMPBSA>, <BintScore>, and ΔG_NES





- Huber Models of <MMPBSA> ,<BintScore>, and ΔG_NES show no significant differences in **aggregate** performance.
  - Sometimes large differences on individual targets.

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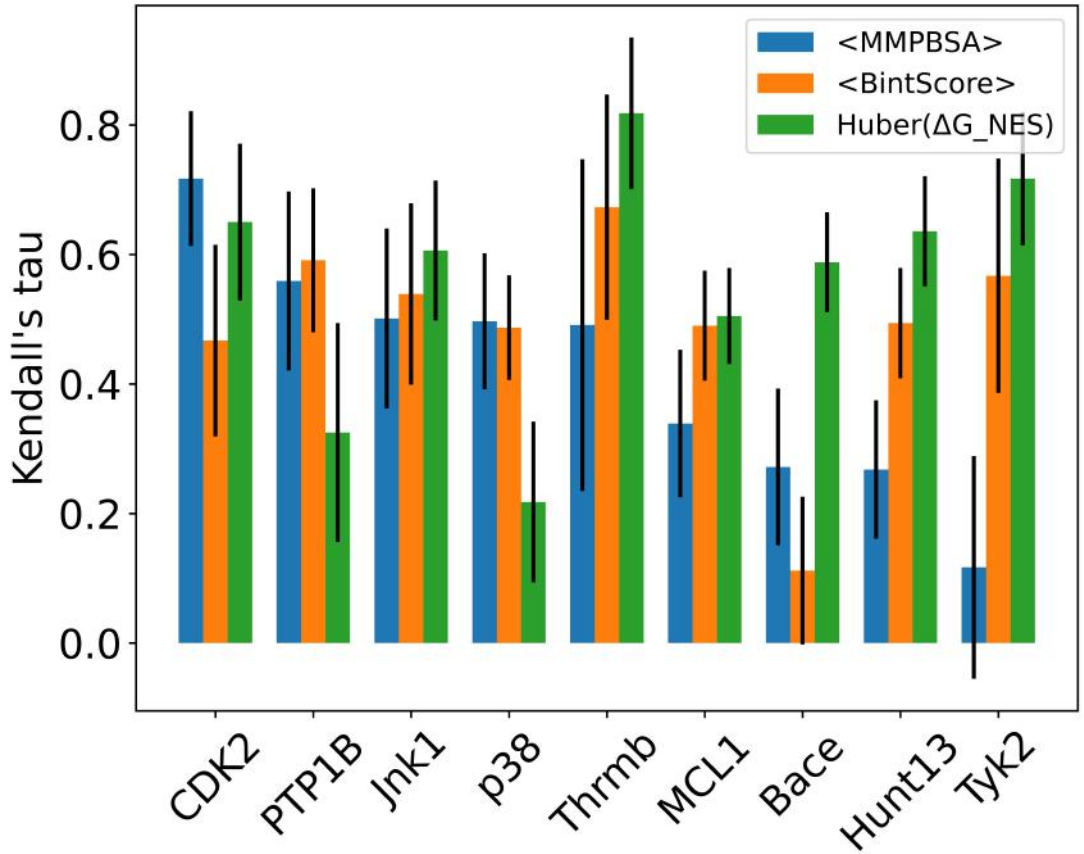
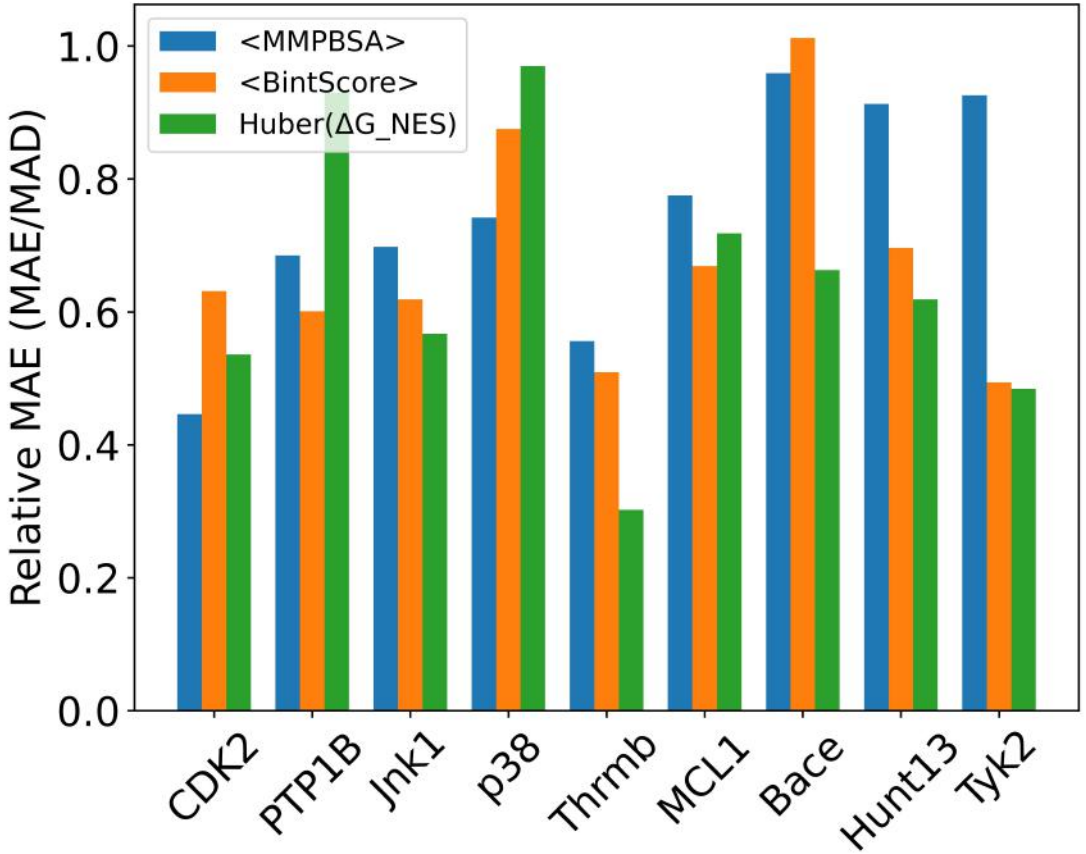
Modeling ΔG with <MMPBSA>, <BintScore>, and ΔG_NES

Better





Better

- Huber Models of <MMPBSA> ,<BintScore>, and ΔG_NES show no significant differences in **aggregate** performance.
  - Sometimes large differences on individual targets.

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Conclusions

- OpenEye will have a massively parallel Relative Binding Free Energy (RBFE) floe available in the upcoming Orion release
  - Non-Equilibrium Switching (NES)
  - Comparable accuracy to literature RBFE benchmarks (limited testing)
- Pose Evaluation using Short Trajectory MD will be improved by the addition of Ensemble BintScore.
  - New knowledge-based evaluation
- ΔG_NES, Ensemble BintScore, and Ensemble MMPBSA can all be effectively used in Robust Linear Modeling of Affinity
  - Aggregate performance is similar
  - Specific targets may (strongly) favor one model

Acknowledgements

- **Gaetano Calabro**
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