Supporting Information of

Design of Protein based Biosensors for Selective Detection of Benzene Group of Pollutants

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1. Supporting Results

Selective sensing of m-xylene isomer by MopR^{HY_YF}. The selectivity of the MopR^{HY_YF} sensor construct towards m-xylene was further explored by testing the response towards different isomers of xylene. As shown earlier, the shape of the pocket determines which isomer will be preferred. The design of the pocket can be altered appropriately via *in silico* docking to get leads as to how the pocket should be altered. The appropriate sensor- molecule combinations can then be tested via *in vitro* experiments. The selectivity analysis of the different isomers of xylene was performed and the *in silico* results show that MopR^{HY_YF} pocket is shaped to accommodate m-xylene best, in accordance with size and shape complementarily (Figure S5A). However, it also accommodates o-xylene; although the fit is not as appropriate as m-xylene as it encounters some steric crowding from F132 (Figure S5B). This is reflected in the slightly reduced ATPase activity (70%) (Figure S5D). On the other hand, p-xylene, owing to the para positioning of its methyl groups is unable to fit properly in the MopR^{HY_YF} pocket and adopts a flipped orientation (with respect to m-xylene) where it encounters a direct steric clash with F132 (Figure S5C). Consequently, it exhibits drastically reduced ATPase activity (Figure S5D). The results indicate that MopR$^{HY\text{-}YF}$ pocket mutation is adaptive to fit mainly *ortho* or *meta* oriented benzene derivatives.

2. Supporting Figures and Tables

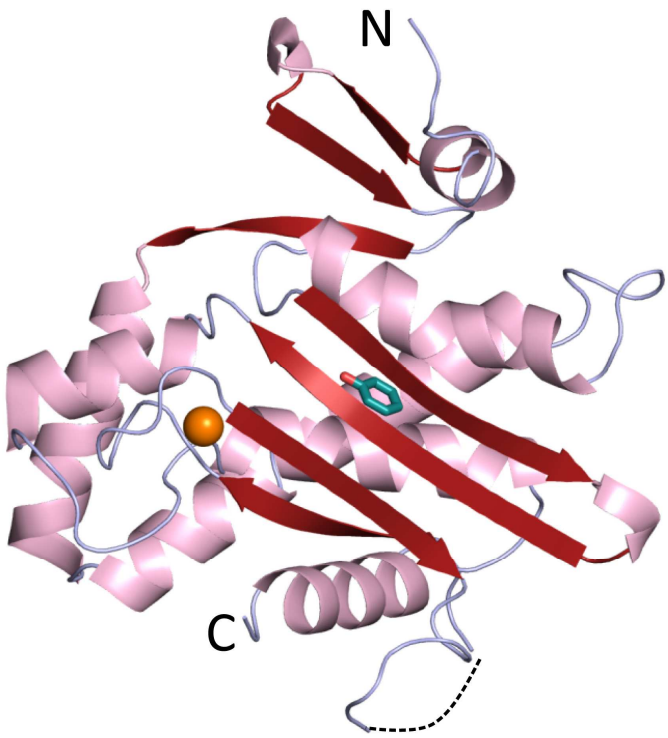

Figure S1. Crystal structure of the pollutant sensing domain of MopR nesting a zinc atom (in orange) and a bound phenol (in deepcyan). [Reprinted (in part) from 'Ray, S.; Gunzburg, M. J.; Wilce, M.; Panjikar, S.; Anand, R., Structural Basis of Selective Aromatic Pollutant Sensing by the Effector Binding Domain of MopR, an NtrC Family Transcriptional Regulator. ACS Chem. Biol. 2016, 11 (8), 2357–2365'. Copyright © 2016 American Chemical Society].

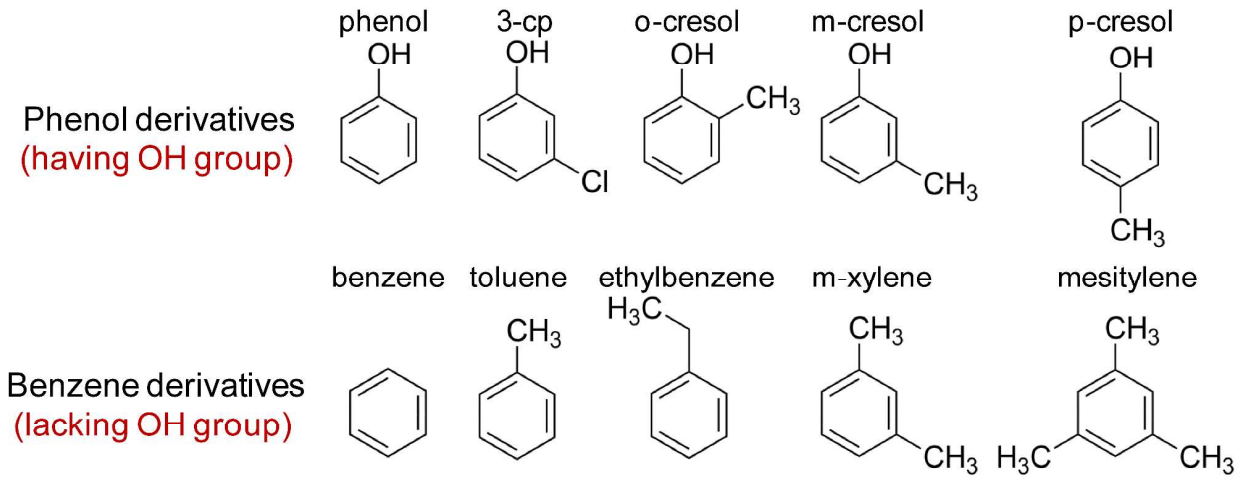

Figure S2. Structures of aromatic compounds. Structures of pollutant in the first row constitute of phenol and its derivatives that are sensed by native MopR. The second row depicts structures of benzene and its derivatives for which mutant MopR sensors were engineered.

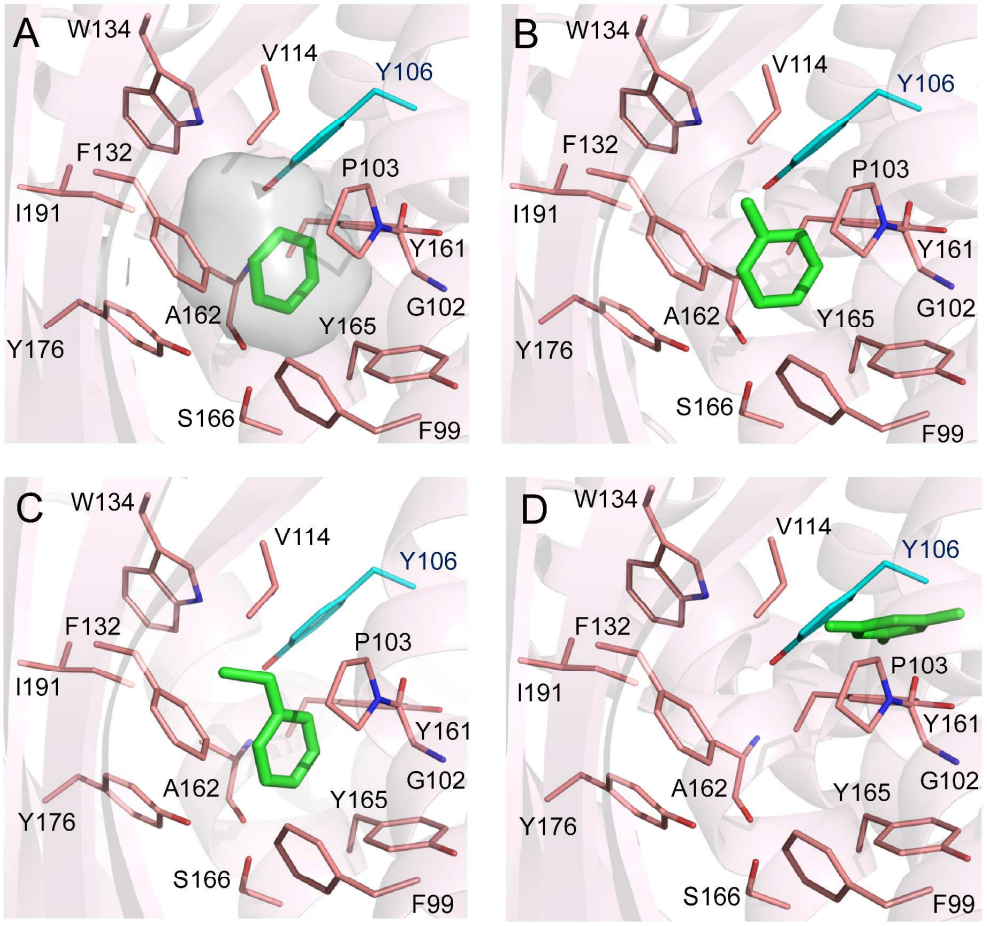

Figure S3. Selective sensing by MopR^{HY}. The panels represent the binding pocket of the MopR^{HY} mutant docked with the following benzene derivatives - (**A**) benzene, **(B)** toluene, **(C)** ethylbenzene and **(D)** mesitylene. Carbon atoms of the ligands are coloured green, pocket residues are in salmon and mutated residues in cyan. Oxygen and nitrogen atoms are in red and blue respectively. Docking shows that MopR^{HY} pocket is tuned to accommodate the smaller hydrocarbons (benzene and toluene) with the best energetics.

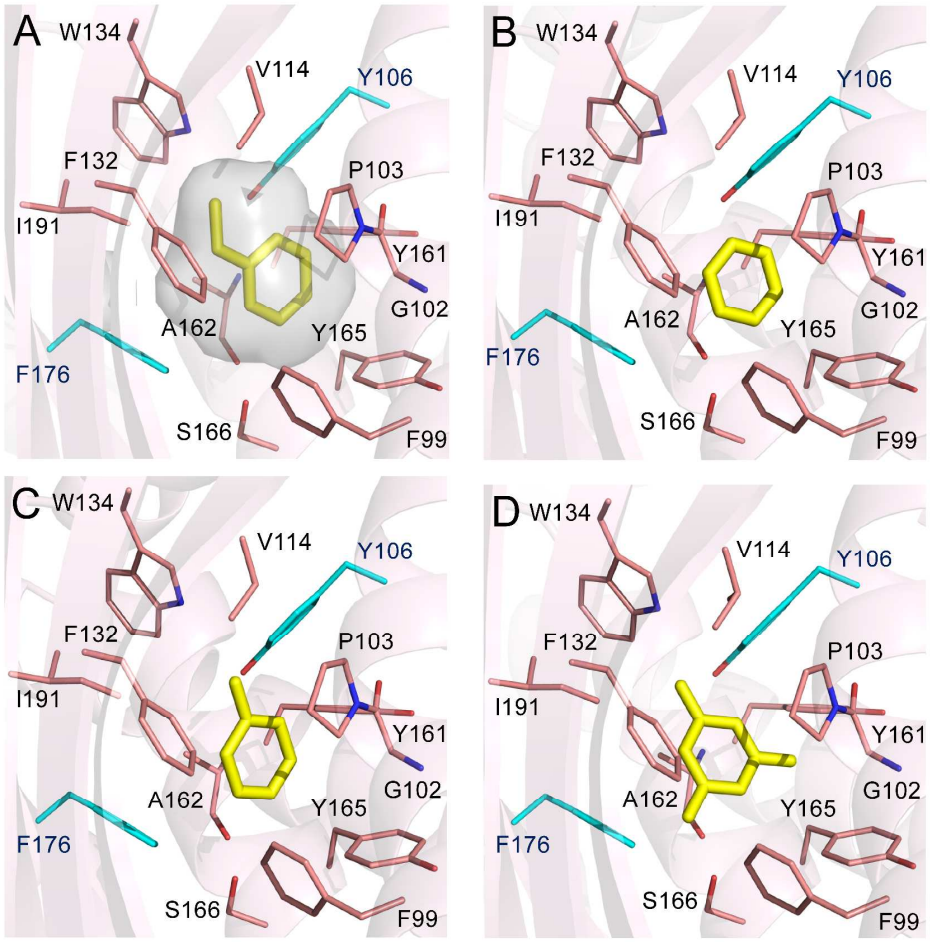

Figure S4. Selective sensing by MopR^{HY_YF}. The panels represent the binding pocket of the MopR^{HY_YF} mutant docked with the following benzene derivatives - (**A**) ethylbenzene, **(B)** benzene, **(C)** toluene and **(D)** mesitylene. Carbon atoms of the ligands are coloured yellow, pocket residues are in salmon and mutated residues in cyan. Oxygen and nitrogen atoms are in red and blue respectively. Docking shows that MopR^{HY_YF} pocket is tuned to accommodate the moderate sized hydrocarbons (ethylbenzene and m-xylene) with the best energetics.

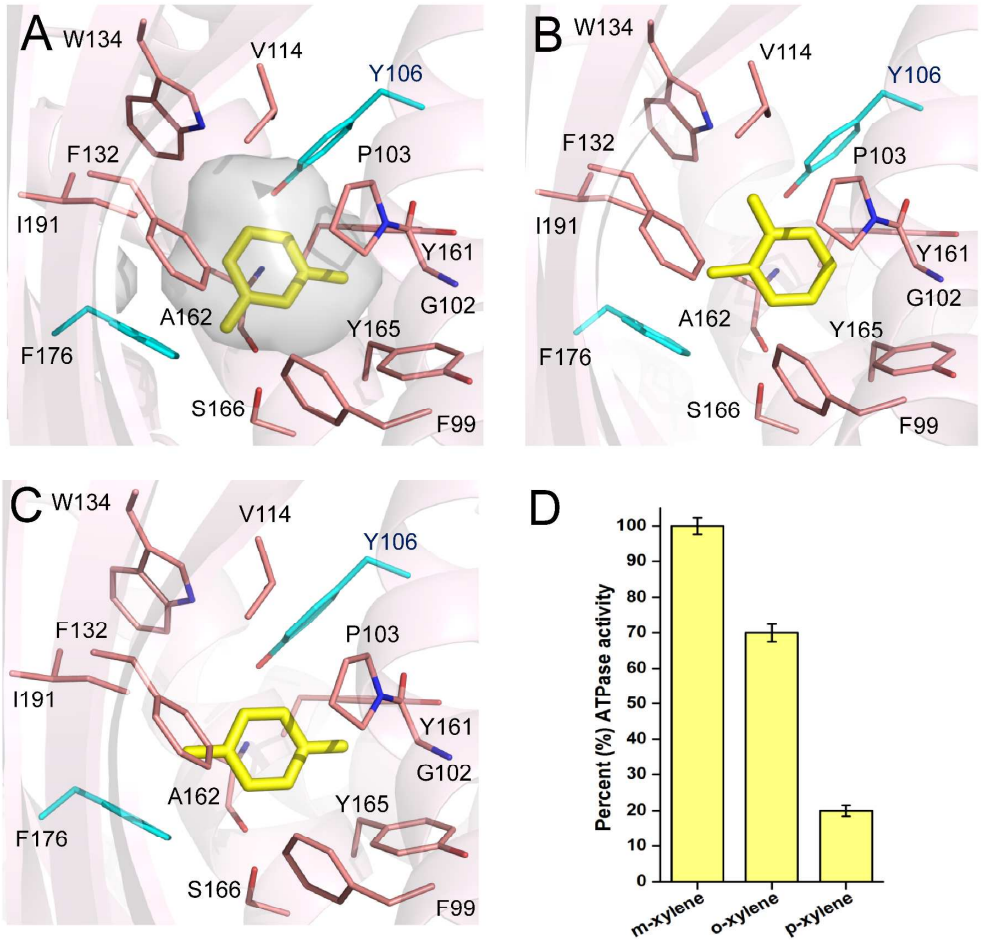

Figure S5. Sensing response of MopR^{HY_YF} towards different isomers of xylene. Panels **(A-C)** represent docked m-xylene **(A)**, o-xylene **(B)** and p-xylene **(C)** in the MopR^{HY_YF} pocket. **(D)** represents percent ATPase activity of MopR^{HY_YF} sensor in response to xylene derivatives. The concentrations of all the compounds used in the assay is 10µM.

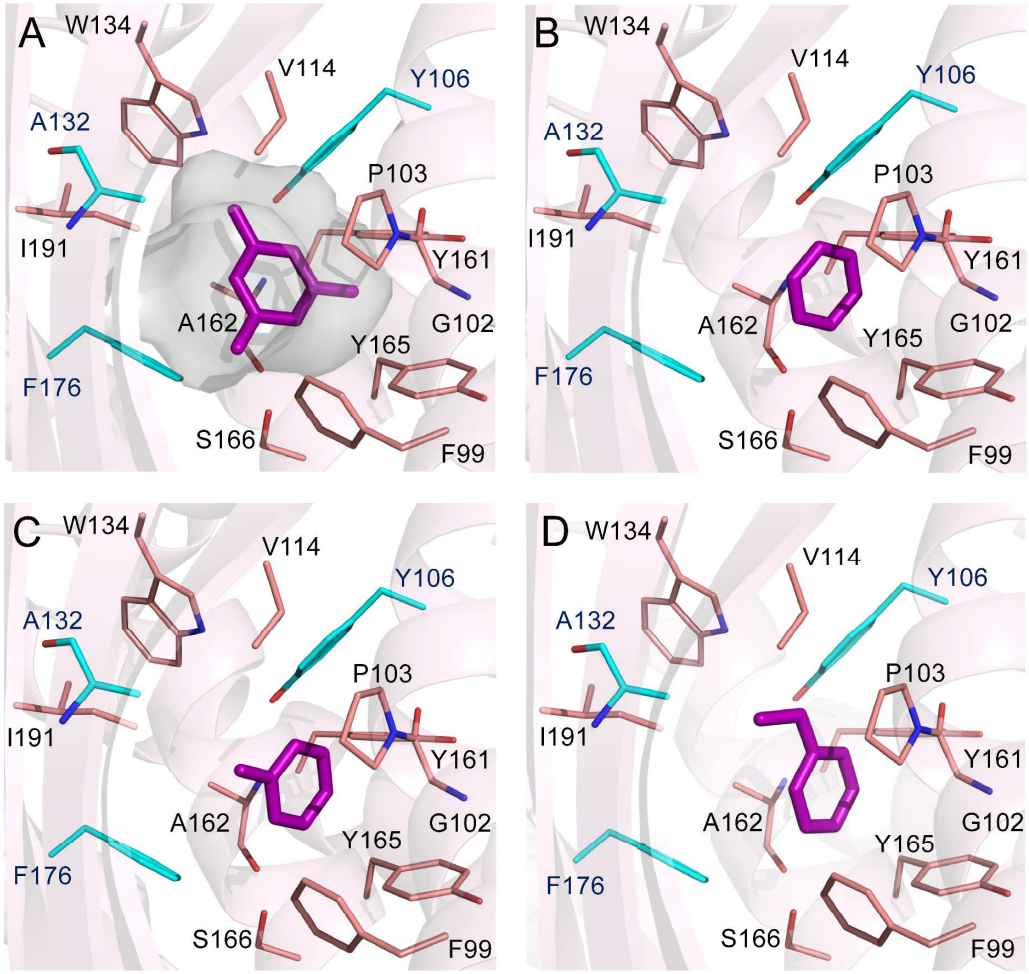

Figure S6. Selective sensing by MopR^{HY_YF_FA}. The panels represent the binding pocket of the MopR^{HY_YF_FA} mutant docked with the following benzene derivatives - (**A**) mesitylene, **(B)** benzene, **(C)** toluene and **(D)** ethylbenzene. Carbon atoms of the ligands are colored purple, pocket residues are in salmon and mutated residues in cyan. Oxygen and nitrogen atoms are in red and blue respectively. Docking shows that MopR^{HY_YF_FA} pocket is tuned to accommodate the bulkier hydrocarbons like mesitylene with the best energetics.

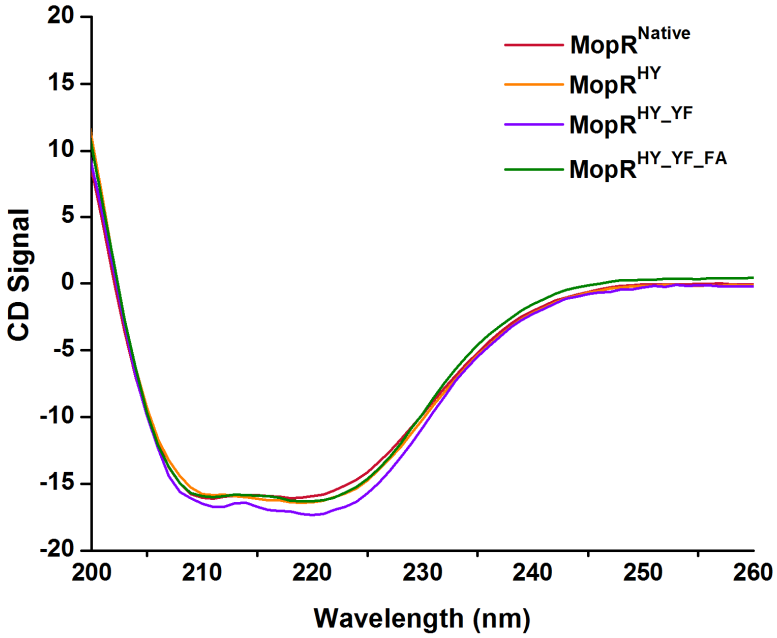

Figure S7. Comparative CD between native and mutated MopR proteins. The concentration of each protein variant used for the CD experiment has been kept constant at 0.2mg/ml.

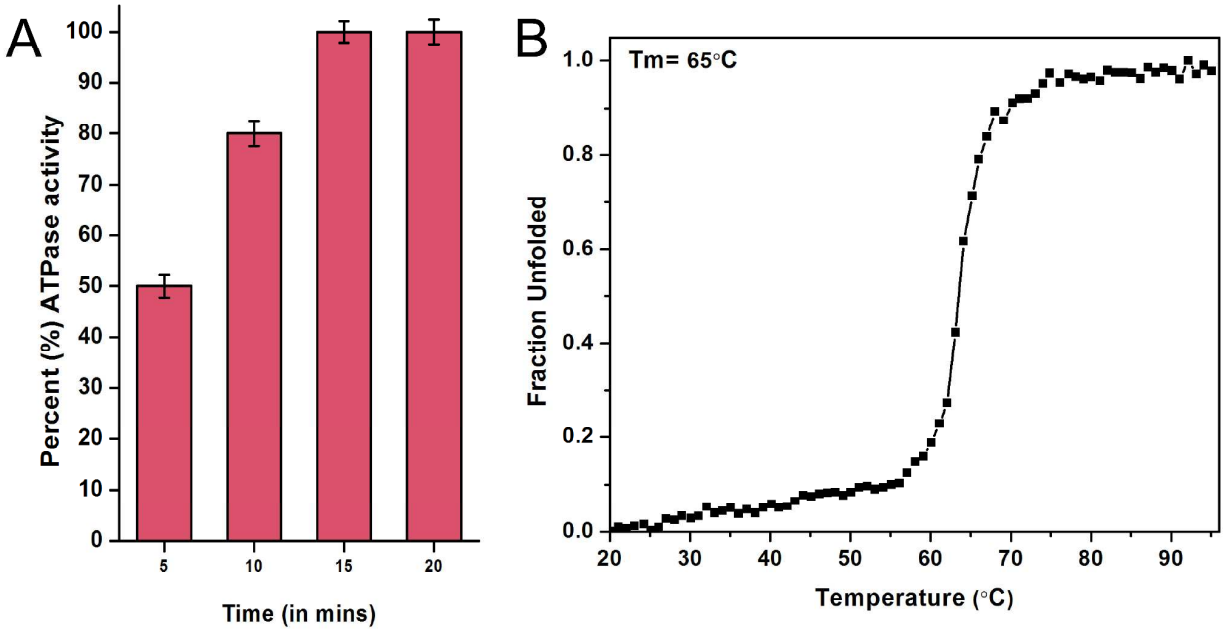

Figure S8. Stability and response time of MopR biosensor. (A) Time dependent ATPase activity of MopR in response to pollutants. **(B)** CD-based melt curve of MopR protein showing a T_m of 65ºC.

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Table S1. Docking of MopR^{AB} mutants with different aromatic pollutants

MopR mutant	Aromatic Ligand	No. of clusters	Population in the chosen cluster	ΔG (kcal/mol) of the top ranked ligand orientation in the chosen cluster
MopR^{HY}	phenol	2	248	-4.55 ± 0.34
MopR^{HY}	benzene	1	250	-6.18 ± 0.23
MopR^{HY}	toluene	2	245	-6.01 ± 0.41
MopR^{HY}	ethylbenzene	3	191	-4.99 ± 0.19
MopR^{HY}	m-xylene	4	207	-4.90 ± 0.14
MopR^{HY}	mesitylene	6	104	-4.85 ± 0.91
MopR^{HY_YF}	benzene	3	225	-4.28 ± 0.12
MopR^{HY_YF}	toluene	1	250	-4.67 ± 0.09
MopR^{HY_YF}	ethylbenzene	3	236	-6.05 ± 0.44
MopR^{HY_YF}	m-xylene	2	240	-6.37 ± 0.57
MopR^{HY_YF}	mesitylene	6	196	-4.09 ± 0.63
MopR^{HY_YF_FA}	benzene	2	230	-4.65 ± 0.13
MopR^{HY_YF_FA}	toluene	2	203	-4.76 ± 0.54
MopR^{HY_YF_FA}	ethylbenzene	4	183	-4.88 ± 0.71
MopR^{HY_YF_FA}	m-xylene	3	217	-4.97 ± 0.45
MopR^{HY_YF_FA}	mesitylene	2	243	-6.24 ± 0.15