The use of effective fragment potentials in the design and synthesis of molecularly imprinted polymers for the group recognition of PCBs

Dougal Cleland and Adam McCluskey[1]*

[1]Chemistry, Centre for Chemical Biology, School of Environmental and Life Sciences, The University of Newcastle, University Drive, Callaghan NSW 2308, Australia. Phone: +612 4921 6486; Fax: +61 249 215472; E-mail: Adam.McCluskey@newcastle.edu.au

Corresponding Author Information: Adam McCluskey, Phone: +612 4921 6486; E-mail: Adam.McCluskey@newcastle.edu.au

Supplementary Figures

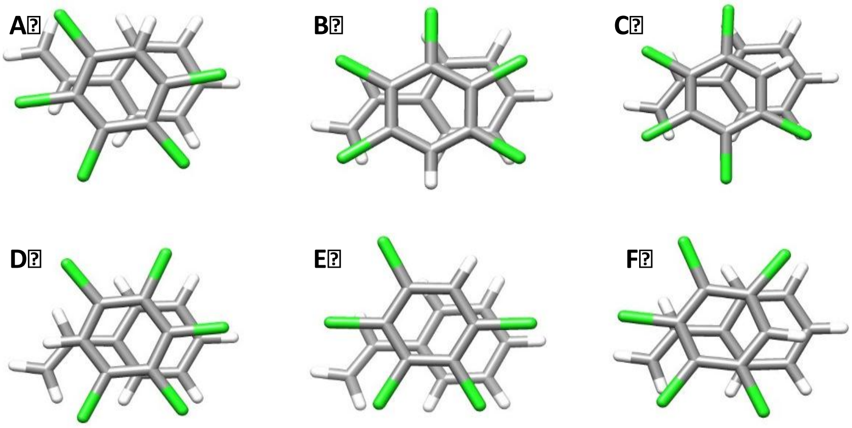

Figure S1: A selection of the gradient optimised configurations of STY with **4** in a π-π stacking interaction: (**A**) = 10.2 kcal mol^{-1}; (**B**) = 10.4 kcal mol^{-1}; (**C**) = 10.5 kcal mol^{-1}; (**D**) = 10.6 kcal mol^{-1}; (**E**) = 11.3 kcal mol^{-1}; and (**F**) = 11.7 kcal mol^{-1}. Atom colours: grey = carbon, white = hydrogen and dark green = chlorine.

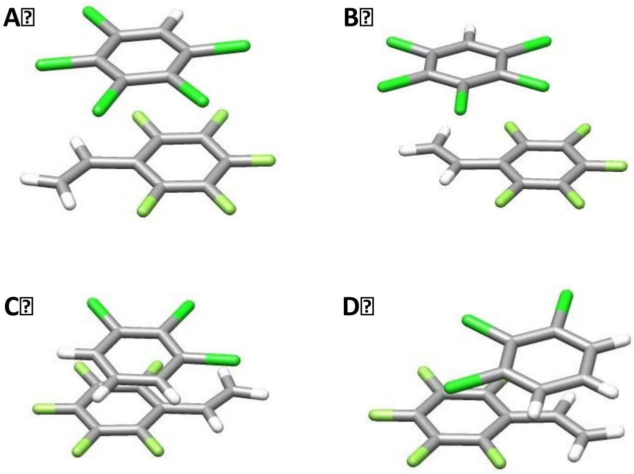

Figure S2: Gradient optimised configurations and intermolecular separation of PFS with (A) **4** in a π-π stacking interaction; (B) **4** in a vinyl-π interaction; (C) **5** in a π-π stacking interaction; (D) **5** in a vinyl-π interaction Atom colours: grey = carbon, white = hydrogen, dark green = chlorine and lime green = fluorine.

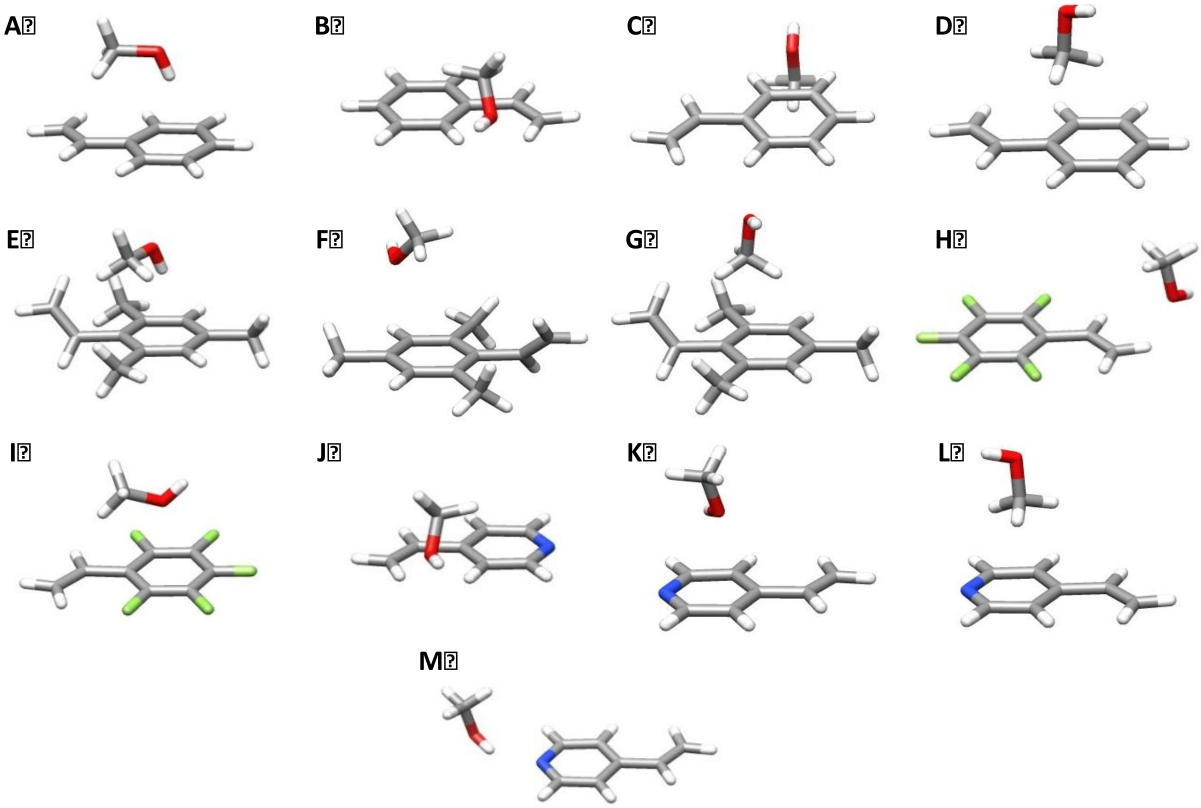

Figure S3: Gradient optimised configurations and intermolecular separation of STY with MeOH in (**A**) a H-π interaction; (**B**) a H-MeOH interaction with two hydrogens; (**C**) a H-MeOH interaction with a single hydrogen; and (**D**) a MeOH perpendicular interaction; TMS with MeOH in (**E**) an H-π interaction; (**F**) an H-MeOH interaction; and (**G**) a perpendicular interaction; PFS with MeOH in (**H**) an LP-π interaction; and (**I**) an H-MeOH interaction; 4VP with MeOH in (**J**) an H-MeOH interaction with two hydrogens; (**K**) an H-MeOH interaction with single hydrogen; (**L**) a perpendicular interaction; and (**M**) an H-N interaction. Atom colours: grey = carbon, white = hydrogen, blue = nitrogen, lime green = fluorine and red = oxygen.

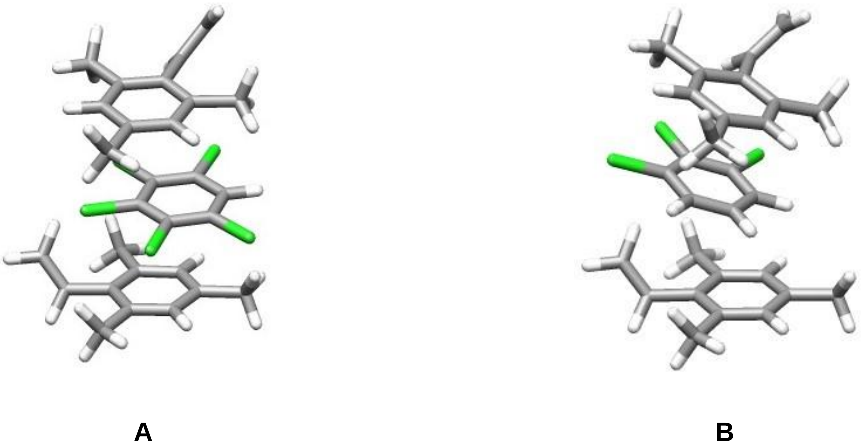

Figure S4: Energy optimised configurations of (**A**) a TMS cluster with **4** and (**B**) a TMS cluster with **5**. Atom colours: grey = carbon, white = hydrogen, dark green = chlorine.

Supplementary Table

Table S1: The BET determined surface areas of MIPs and NIPs.

Polymer Name	Surface Area (m^2g^{-1})
$\text{MIP}_{\textbf{4-TMS}}$	328
$\text{NIP}_{\textbf{4-TMS}}$	255
$\text{MIP}_{\textbf{5-PFS}}$	305
$\text{NIP}_{\textbf{5-PFS}}$	330

Materials and Methods

1. Reagents

Solvents, dichloromethane (DCM), acetonitrile (MeCN), methanol (MeOH), toluene (Tol) and acetone (Ac) were of bulk grade and re-distilled from glass. 1,2,3,4,5-Pentachlorobenzene (**4**) and 1,2,3-trichlorobenzene (**5**) (Sigma Aldrich) were recrystallised from methanol-water and dried under vacuum at 40 °C. 1,3,5-Trichlorobenzene (**11**), 2,4,6-trichloroaniline (**13**), 2,6-dimethylaniline (**12**), 2,2',3,5'-tetrachlorobiphenyl (**15**), 2,3,3',4,4'-pentachlorobiphenyl (**16**), and 2,2',3,3',4,5,6'-heptachlorobiphenyl (**17**) (Sigma Aldrich) were used as supplied. 4-Vinylpyridine (4VP), styrene (**STY**), 2,3,4,5,6-pentafluorostyrene (**PFS**), 2,4,6-trimethylstyrene (**TMS**), and ethyleneglycol dimethacrylate (**EGDMA**) (Sigma Aldrich) were distilled under reduced pressure. Azobisisobutyronitrile (AIBN) (Sigma Aldrich) was recrystallised from methanol and dried under vacuum at 40 °C.

2 Synthesis of MIPs

2.1 1,2,3,4,5-Pentachlorobenzene (4) MIPs

The pre-polymerisation mixtures were prepared by adding appropriate quantities of 4, TMS and EGDMA to 1 mL of solvent; the quantities and solvent type for Synthesis 1-5 are shown in Table S2. The order of addition for Synthesis 1, 2 and 3 was 4 followed by TMS and EGDMA. The order of addition for Synthesis 4 and 5 was EGDMA followed by 4 and TMS. AIBN (10 mg) was then added and the pre-polymerisation mixtures were sonicated for 5 minutes to ensure the complete dissolution of 4 and AIBN. The solution was then purged with nitrogen to remove dissolved oxygen. Synthesis 1 and 2 were placed in an oven where thermal polymerisation was conducted at 60 °C for 24 hours. Synthesis 3, 4 and 5 were placed in an ice-water bath where they were allowed 30 minutes to equilibrate with the surrounding temperature. Photochemical polymerisation was then initiated by ultra-violet (UV) radiation emitted from a 450 watt quartz mercury vapour lamp (Ace Glass) over a period of 24 hours. NIPs were prepared in an identical manner as the MIPs, excepting for the addition of 4.

The bulk polymers were ground in MeOH by mortar and pestle and wet sieved (< 45 μM). Smaller particles were removed by repeated mixing in Ac and, after 5 minutes, decanting the supernatant containing suspended fines. T removal was achieved by exhaustive soxhlet extraction in the same solvent as used for polymerisation.

Table S2: Factors of the synthetic procedures for **4** MIPs

	4 (mmol)	TMS (mmol)	EGDMA (mmol)	Solvent Selection	Initiation Method
Synthesis 1	63	146	991	DCM	thermal
Synthesis 2	63	146	991	MeCN	thermal
Synthesis 3	63	146	991	MeCN	UV
Synthesis 4	63	146	991	MeOH	UV
Synthesis 5	125	146	991	MeOH	UV

2.2 1,2,3-trichlorobenzene (5) MIPs

MIPs were prepared by adding the appropriate FM, **5** and EGDMA to 0.25 mL of MeOH; the quantities for **5**, STY MIPs are shown in Table S3 and the quantities for **5**, 4VP, TMS, and PFS MIPs are shown in Table S4. EGDMA was added first followed by **5** and FM. AIBN (5 mg) was then added and the mixtures were sonicated for 5 minutes to ensure the complete dissolution of **5** and AIBN. The solution was then purged with nitrogen to remove dissolved oxygen. Reaction vials were placed in an ice-water bath where they were allowed 30 minutes to equilibrate with the surrounding temperature. Photochemical polymerisation was then initiated by ultra-violet (UV) radiation emitted from a 450 watt quartz mercury vapour lamp (Ace Glass) over a period of 24 hours. NIPs were prepared in an identical manner as the MIPs, excepting for the addition of **5**.

The bulk polymers were ground in MeOH by mortar and pestle and wet sieved (< 45 μM). Smaller particles were removed by repeated mixing in Ac and, after 5 minutes, decanting the supernatant containing suspended fines. T removal was achieved by exhaustive soxhlet extraction in MeOH.

Table S3: Quantities of T, FM and XL added to **5**, STY MIPs.

Polymer Identification	TriCB	FM	EGDMA
	Quantity (mmol)		
A	0.25	0.17	1.25
B	0.10	0.58	1.25
C	0.19	0.48	1.25
D	0.07	0.15	1.25
E	0.08	0.33	1.25
F	0.27	0.27	1.25
G	0.29	0.38	1.25
H	0.16	0.16	1.25
I	0.50	0.75	1.25
J	0.63	0.21	1.25
K	0.94	0.94	1.25
L	1.00	0.25	1.25
M	1.25	0.63	1.25
N	0.42	0.42	1.25
O	0.75	0.50	1.25
P	0.86	0.04	1.25

Table S4: Quantities of T, FM and XL added to **5**, 4VP, TMS and PFS MIPs.

Polymer Identification	TriCB	FM	EGDMA
	Quantity (mmol)		
A	0.86	0.04	1.25
B	1.04	0.31	1.25
C	1.32	0.72	1.25
D	0.65	0.26	1.25
E	0.78	0.57	1.25
F	0.43	0.47	1.25

3 Rebinding Assays

3.1 Batch Rebinding Assays

A measured quantity (20 mg) of the polymers was suspended in 1 mL of 0.1mM **4** solution with shaking at 180-200 rpm for 24 h and then centrifuged at 10 000 rpm for 5 min. An aliquot of the supernatant was then removed for analysis. To insure reliability, all rebinding assays were conducted in triplicate involving the preparation of 3 samples of MIPs and 3 samples of NIPs.

3.2 Solid Phase Extraction Rebinding Assays

A measured quantity (20 mg) of the polymers was packed into 1 mL solid phase extraction cartridges between porous polyethylene frits. Rebinding solutions with a concentration of 0.1 mM were then prepared by adding the appropriate target to a mixture of MeOH and water (7: 3). The cartridges were first conditioned by eluting 1 mL of MeOH and water (7: 3) under manual pressure applied by a 3 mL syringe. 1 mL of rebinding solution was then eluted under manual pressure (20 seconds) and collected in a 1.7 mL chromatography vial for analysis. All rebinding assays were conducted in triplicate involving preparation of 3 samples of MIPs and 3 samples of NIPs.

3.3 Rebinding Analysis

The concentration of target remaining in a solution after a rebinding experiment was analysed by high performance liquid chromatography (Shimadzu LC-20AD) conducted using a 5μm C18 column (Grace Econosphere). The mobile phase for the different targets consisted of 70% MeCN in water. A 20 μL injection volume was used with a run time of 15 minutes and a flow rate of 1.0 mL / minute. Detection was conducted by a photodiode array detector and analysed at 230 nm using Shimadzu LC Solution software. The response of the detector to the target concentration was calibrated using a series of target solutions over a concentration range of 1 to 100 nM.

After the analysis of the rebinding solution, the amount of target bound to a polymer (T_b) was calculated from the difference between the amount of target in the bulk rebinding solution (C_i) and the amount of target remaining in solution after a rebinding experiment (C_f) using Equation 1.

$$T_b = C_i - C_f \qquad \text{(Equation 1)}$$

The imprinting factor (IF) was then calculated from the amount of target bound to the MIP (T_bMIP) and NIP (T_bNIP) using Equation 2.

$$IF = \frac{T_bMIP}{T_bNIP} \qquad \text{(Equation 2)}$$

4 Surface Area

The surface area of the polymers was analysed by CO_2 absorption measurements made by a Micromimetics ASAP 3030 surface area analyser. The data was then analysed by Brunauer-Emmet-Teller (BET) theory.

5 Effective Fragment Potential Study

The Effective Fragment Potential (EFP)[1] method is a feature of GAMESS (General Atomic and Molecular Electronic Structure System)[2] computational chemistry software package. GAMESS version 09 was used for all calculations involved in the EFP study, including Monte Carlo simulations and gradient optimisations of the molecular structures and inter-molecular configurations.

5.1 Interaction Energies of the S22 Test Set

The S1, S2 and S3 set of parameters were calculated from the published geometries of the S22 test set.[3] The S1 parameters were calculated by the standard EFP method and the 6-311++G (3df, 2p) basis set. This is the systems default and recommended basis set for calculation of EFP parameters and includes diffuse and polarisable functions. The S2 and S3 parameters were composed of the S1 parameters with the exception of electrostatic parameters for aromatic molecules. For the S2 parameters, the electrostatic parameters for aromatic molecules were calculated using a numerical DMA and the 6-311++G (3df, 2p) basis set. For the S3 parameters, the electrostatic parameters for aromatic molecules were calculated using an analytic DMA and the 6-311G basis set, which does not include diffuse or polarisable functions.

Calculations of the interaction energies began with the molecules in the optimised coordinates, as supplied by the S22 test set. Gradient optimisations were then performed by applying each set of parameters, in turn, to determine the EFP optimised coordinates. A visual inspection of the molecules in the EFP optimised coordinates was then conducted using MacMolPlt software[4] to confirm that the interaction had maintained a similar configuration as the interaction in the S22 test set. During calculation of the interaction energies, high order screening was selected to account for charge penetration effects on electrostatic energy calculations.

The results of calculations were evaluated by their errors, which were determined by subtracting the EFP calculated interaction energies ($\hat{E}_i$) from the benchmark CCSD(T) calculated interaction energies (E_i). The root mean square deviation (RMSD) of errors for each set of interactions was then calculated by Equation 3 where n is the number of interactions in a set.

$$RMSD = \sqrt{\frac{\sum_{i=1}^{n}(E_i - \hat{E}_i)^2}{n}} \qquad \text{(Equation 3)}$$

5.2 Functional Monomer Interaction Energies

Energy optimised geometries for the Ts, FMs and MeOH were obtained by gradient optimisations using the 6-311++G(3df, 2p) basis set. The EFP parameters were then calculated by the default settings in the GAMESS program using the 6-311G basis set for electrostatic parameters for aromatic molecules and the 6-311++G(3df,2p) basis set for all other parameters.

FM interactions with the Ts and MeOH were evaluated by EFP calculation of the interaction energy in the energy optimised configuration. The energy optimised configurations were identified by Monte Carlo simulations using the Metropolis algorithm. During calculation of the interaction energies, high order screening was selected to account for charge penetration effects on electrostatic energy calculations.

The Monte Carlo simulations began in randomly generated configurations and included 1000 translational and rotational steps performed at 500 K. During the simulations, gradient optimisations were conducted every 10 steps and resulted in 100 gradient optimised configurations for each simulation. A 3D visual inspection of all gradient optimised configurations was conducted using MacMolPlt software to correlate the range of FM interactions with the Ts and MeOH with the corresponding interaction energies.

6 Molecular Dynamics Study

Molecular dynamics simulations were performed using the AMBER version 8.0 suite of programs (UCSF, San Francisco, CA).[5]

Preparation of a simulation began by entering the chemical structures for each type of pre-polymerisation component into the Amber antechamber program. The antechamber program then assigned the most appropriate set of molecular mechanics parameters to each atom of the pre-polymerisation components selected from the Amber99[6] and GAFF[7] force fields. Coulomb energy parameters required calculation of the partial charge of each atom, which was conducted by the AM1-BCC method.[8] Once parameters had been assigned, the systems were built by randomly placing the appropriate numbers of each pre-polymerisation component within an 80 x 80 x 80 Å box with periodic boundary conditions. Energy minimisations were then performed by 5000 steepest-descent and 5000 conjugate gradient steps to remove unfavourable contacts. The pre-polymerisation mixtures were then heated at constant volume from 0 to 273 K. A pressure of 1 bar was then applied to the box which was allowed to expand and contract in response to internal pressure until the pre-polymerisations mixtures had achieved a constant density. After confirming that the density of the pre-polymerisation mixture had stabilised, the dimensions of the box were fixed. A 5 ns production phase simulation then commenced with coordinates of the atoms recorded to a trajectory file every 2 ps.

Temperature and pressure were kept constant during the simulation by Langevin dynamics with a collision frequency of 1 ps. A 9.0 Å cut-off was selected for the calculation of non-bonded interactions. The motion of hydrogen atoms were constrained using the SHAKE algorithm permitting a time step 0.002 ps.

After the completion of a simulation, interactions were analysed by calculation of the radial distribution function (RDF) between pre-polymerisation components with a 0.05 Å bin size.

References

1 M. S. Gordon, L. Slipchenko, H. Li and J. H. Jensen. *Ann. Rep. Comp. Chem.* 2007, **3**, 177-193.

2 M. W. Schmidt, K. K. Baldridge, J. A. Boatz, S. T. Elbert, M. S. Gordon, J. H. Jensen, S. Koseki, N. Matsunaga, K. A. Nguyen, S. J. Su, T. L. Windus, M. Dupuis and J. A. Montgomery. *J. Comput. Chem*. 1993, **14**, 1347-1363.

3 P. Jurečka, J. Šponer, J. Černỳ and P. Hobza. *Phys. Chem. Chem. Phys.* 2006, **8**, 1985-1993.

4 B. M. Bode and M. S. Gordon. *J. Mol. Graphics Mod.* 1998, *16*, 133-138.

5 D. A. Case, T. E. Cheatham, T. Darden, H. Gohlke, R. Luo, K. M. Merz, A. Onufriev, C. Simmerling, B. Wang and R. J. Woods. *J. Comput. Chem.* 2005, **26**, 1668-1688.

6 J. Wang, P. Cieplak and P. A. Kollman. *J. Comput. Chem.* 2000, **21**, 1049-1074.

7 J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman and D. A. Case. *J. Comput. Chem.* 2004, **25**, 1157-1174.

8 A. Jakalian, D. B. Jack and C. I. Bayly. *J. Comput. Chem.* 2002, **23**, 1623-1641.