Supporting Information

Site-Identification by Ligand Competitive Saturation (SILCS) Assisted Pharmacophore Modeling

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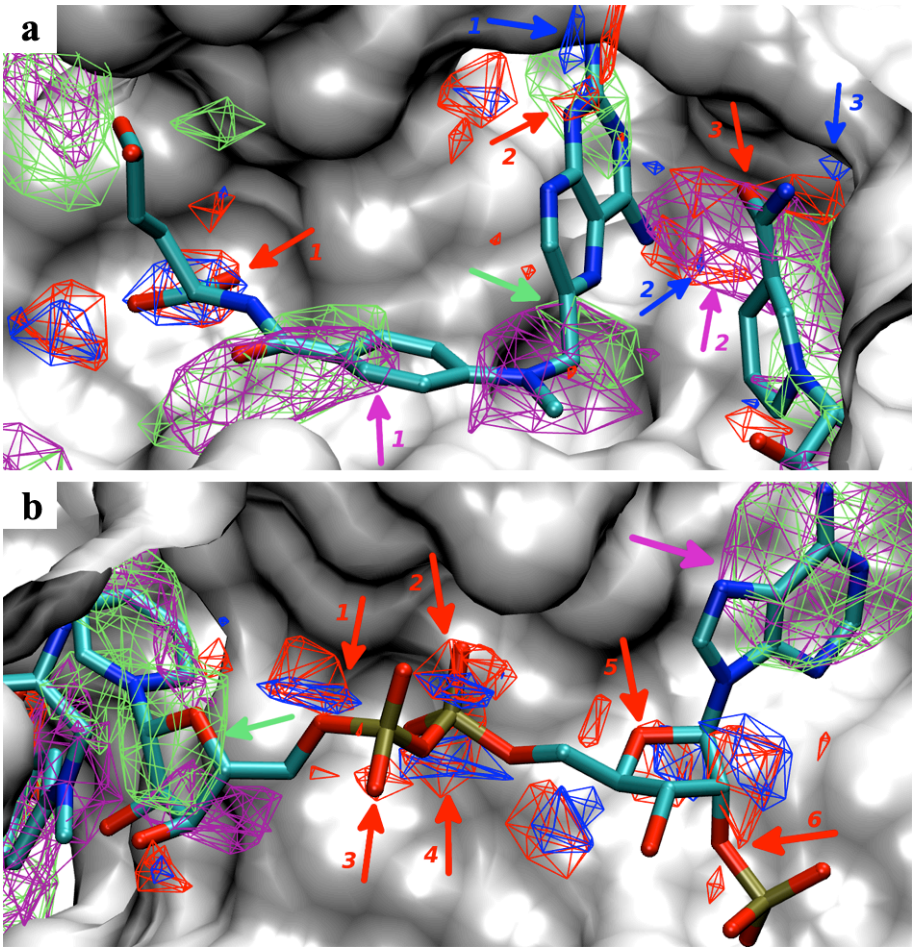

Figure S1. Surface of DHFR (PDB ID: 3DFR) overlaid with aromatic (purple), aliphatic (green), hydrogen-bond donor (blue) and acceptor (red) FragMaps and the crystal orientations of MTX (a) and NADPH (b). Regions of the protein occluding the view are removed from the visualization.

In the DHFR crystal structure, the ligand, MTX and nicotinamide adenine dinucleotide phosphate (NADPH) are present and both of them were removed prior to performing the SILCS simulation. As previously discussed,[1,2] four types of key interactions contribute to the binding of MTX. This includes interactions between the α-carboxylate group in the glutamate portion of MTX and the guanidinium group of Arg57; hydrophobic nonpolar interactions between the *p*-aminobenzoyl group of MTX and side chains of Leu27 and Phe30; nonpolar interactions between the pteridine ring and side chains of Leu4, Ala6, Leu19, Leu27, Phe30 and Ala97; and hydrogen-bond interactions between the hydrogens of the two amine groups in the pyrazine ring of MTX and Asp26 side chain and Leu4 backbone oxygens. These key interactions in the crystal binding mode of MTX are well reproduced by the corresponding

SILCS FragMaps as indicated by the overlaps of the MTX α-carboxylate group oxygens with hydrogen-bond acceptor FragMap (1st red arrow in Figure S1(a)); overlaps of the MTX *p*-aminobenzoyl group with aromatic FragMap (1st purple arrow in Figure S1(a)); overlaps of the MTX pteridine ring with aromatic FragMap (2nd purple arrow in Figure S1(a)); and overlaps of the likely hydrogen positions of the two amine groups in the pyrazine ring with hydrogen-bond donor FragMap (1st and 2nd blue arrows in Figure S1(a)).

FragMaps are also able to capture the crystal binding mode of the cofactor NADPH (Figure S1a).[2] Overlap of the oxygen and likely hydrogen position of the amide group in the nicotinamide portion of NADPH occur with the hydrogen-bond acceptor (3rd red arrow) and donor (3rd blue arrow) FragMaps, which reproduce the interactions between these chemical groups in NADPH with the Ala6 backbone amide hydrogen and carbonyl oxygen. In addition, the overlap of the nicotinamide ring with an aromatic FragMap (2nd purple arrow) captures the hydrophobic interactions between the nicotinamide ring and hydrophobic side chains of Ile13 and Leu19. It should be noted that the overlaps of the pteridine ring in MTX and the nicotinamide ring in NADPH with the aromatic FragMap are not ideal. This is due to the close contact between MTX pteridine and NADPH nicotinamide rings at the hydrophobic binding site in the crystal structure, such that the SILCS simulations yield a diffuse aromatic FragMap (2nd purple arrow in Figure S1(a)) between the crystal positions of two aromatic rings in MTX and NADPH. Good overlap of the adenine moiety with an aromatic FragMap (purple arrow in Figure S1(b)) is associated with the binding interactions between the adenine and residues Leu62, His64, Gln101 and Ile 102. In addition, good overlaps of the phosphate (red arrows 1-4) and sugar ring oxygens (red arrows 5 and 6) with hydrogen-bond acceptor FragMaps and of the sugar ring with aliphatic FragMaps (green arrow) are seen as shown in Figure S1(b).

Table S1. Parameters used to develop SILCS pharmacophore models for the three tested targets.

Targets	Aromatic		Aliphatic		H-bond Donor		H-bond Acceptor	
	GFE_{cutoff} (kcal/mol) [a]	d (Å) [b]	GFE_{cutoff} (kcal/mol) [a]	d (Å) [b]	GFE_{cutoff} (kcal/mol) [a]	d (Å) [b]	GFE_{cutoff} (kcal/mol) [a]	d (Å) [b]
HIVPR	-1.0	2.8	-1.0	2.6	-1.0	1.0	-1.0	1.0
FXa	-1.5	2.8	-1.5	2.6	-0.6	1.0	-0.6	1.0
DHFR	-1.2	2.8	-1.2	2.6	-0.4	1.0	-0.9	1.0

[a] Any voxels in the binding pocket with GFE lower than the GFE_{cutoff} are used to develop pharmacophore features. [b] Clustering distance parameter d used to put all voxels whose distance between each other are within d into one cluster.

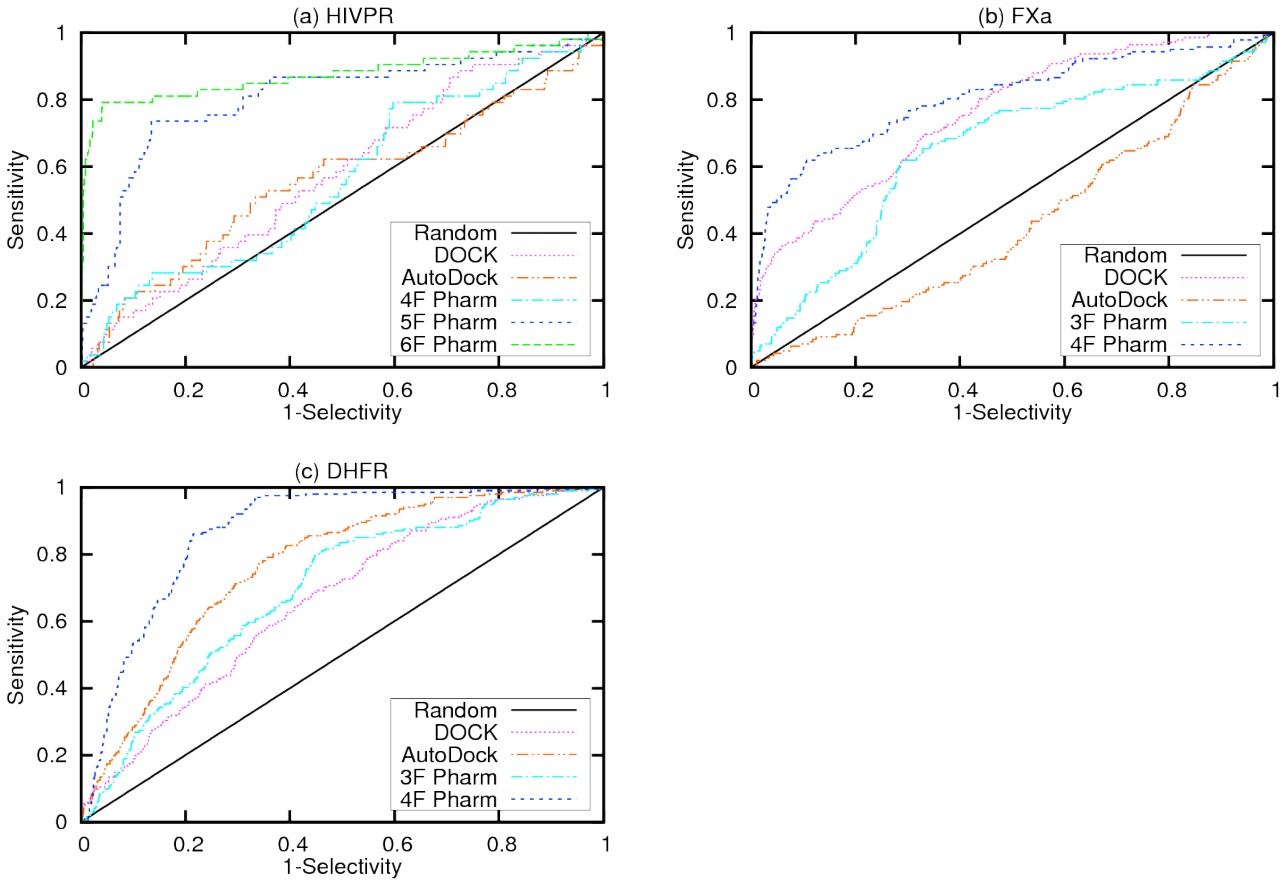

Figure S2. ROC plots of VS results using DOCK, AutoDock and SILCS pharmacophore modeling against DUD data sets for the three protein targets (a) HIVPR, (b) FXa and (c) DHFR. The black line indicates random selection of compounds from the database.

References:

1. Bolin, J. T.; Filman, D. J.; Matthews, D. A.; Hamlin, R. C.; Kraut, J. *J. Biol. Chem.* **1982**, *257*, 13650-13662.
2. Blaney, J. M.; Hansch, C.; Silipo, C.; Vittoria, A. *Chem. Rev.* **1984**, *84*, 333-407.