Using Site Similarity to Generate New Matter Ideas

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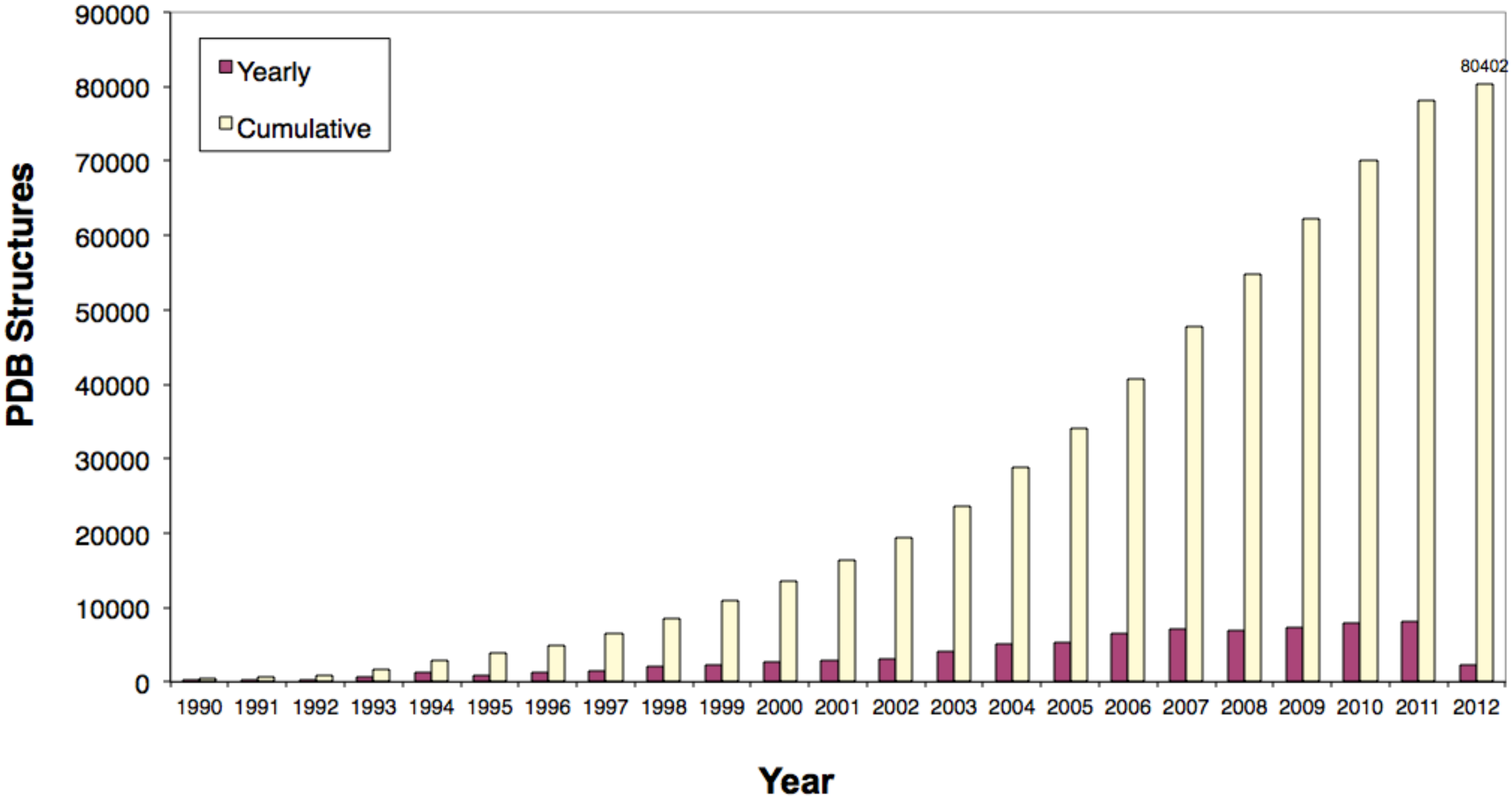
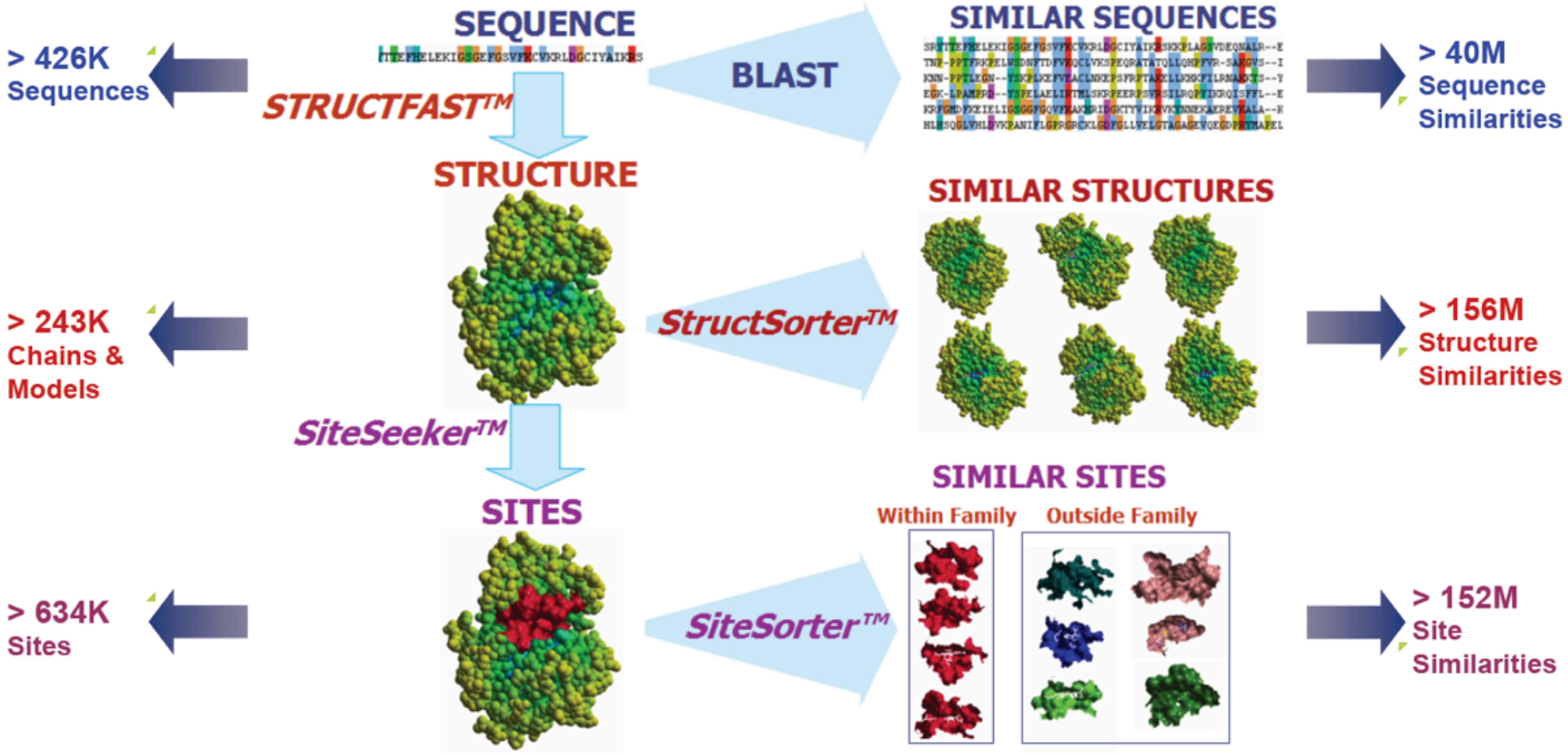
Protein Structure Growth Continues

> 80K structures/co-complexes “templates” (Apr-2012)
> 650 new templates/month (>150/week)



Target Informatics Platform (TIP)



SEQUENCE

STRUCTFAST™

STRUCTURE

SiteSeeker™

SITES

BLAST

StructSorter™

SiteSorter™

SIMILAR SEQUENCES

SIMILAR STRUCTURES

SIMILAR SITES

Within Family

Outside Family

> 426K Sequences

> 243K Chains & Models

> 634K Sites

> 40M Sequence Similarities

> 156M Structure Similarities

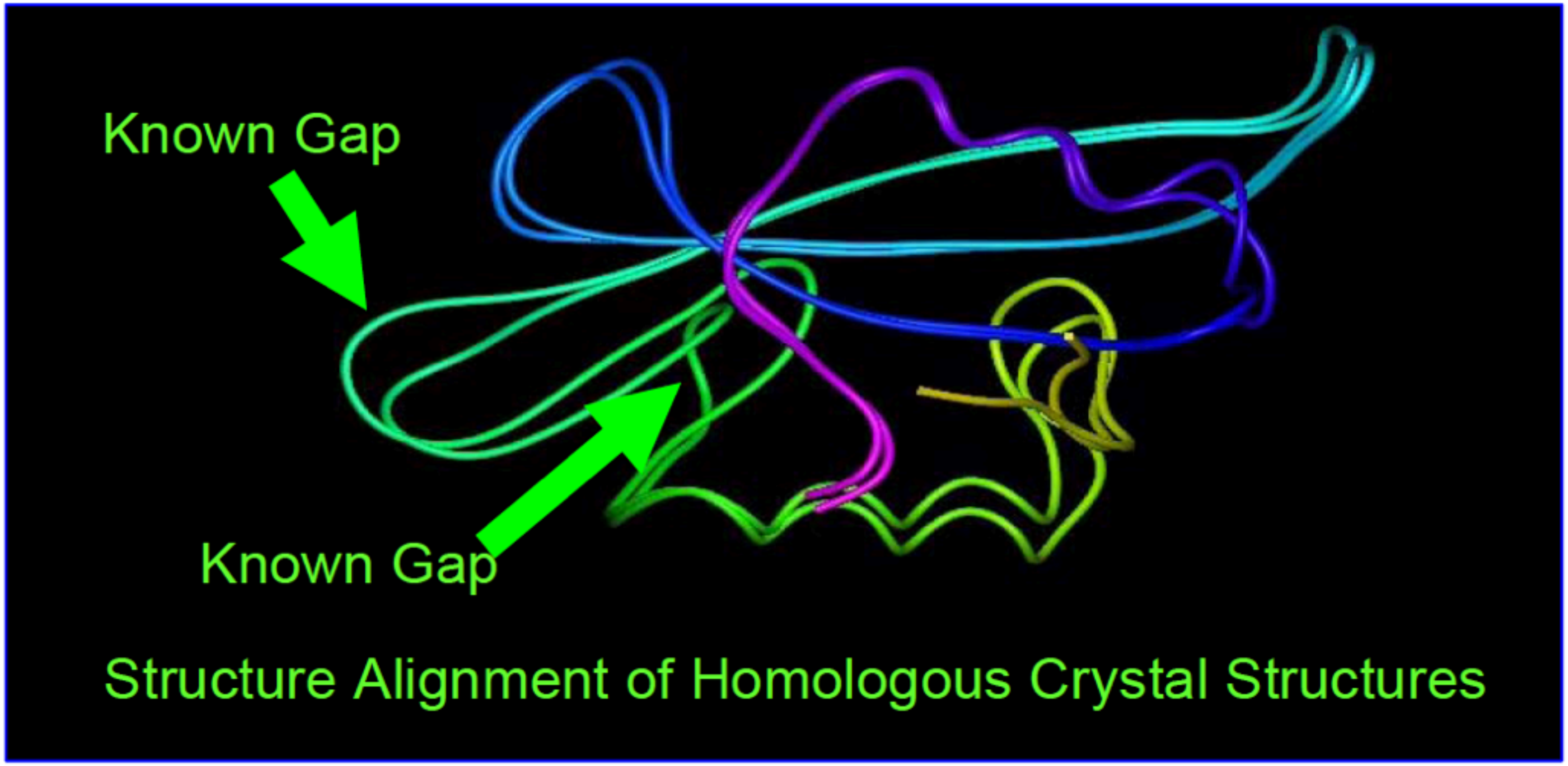
> 152M Site Similarities

- Interrogating the druggable genome with structural informatics **MolecularDiversity (2006)**
- STRUCTFAST: Protein Sequence Remote Homology Detection and Alignment Using Novel Dynamic Programming and Profile-Profile Scoring **Proteins. 2006 64:960-967**
- StructSorter: A Method for Continuously Updating a Comprehensive Protein Structure Alignment Database **J. Chem. Inf. Model. 2006, 46, 1871-1876**
- Convergent Island Statistics: A fast method for determining local alignment score significance. **Bioinformatics, 2005, 21, 2827-2831.**

STRUCTFAST

STructure Realization Utilizing Cogent Tips From Aligned Structural Templates

Basic Principle: Gaps known to exist should not be strongly penalized.



Structure Alignment of Homologous Crystal Structures

Leverages experimental structure and structural alignment data to create better alignments

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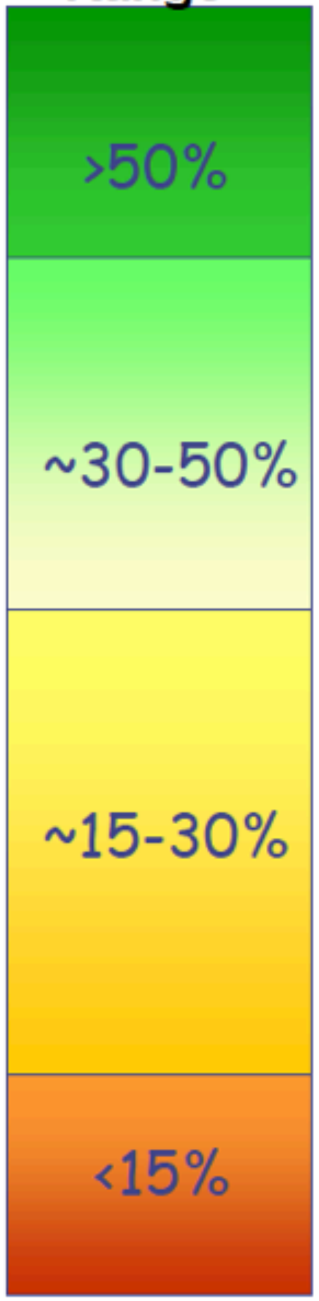
Modeling Algorithm Comparison

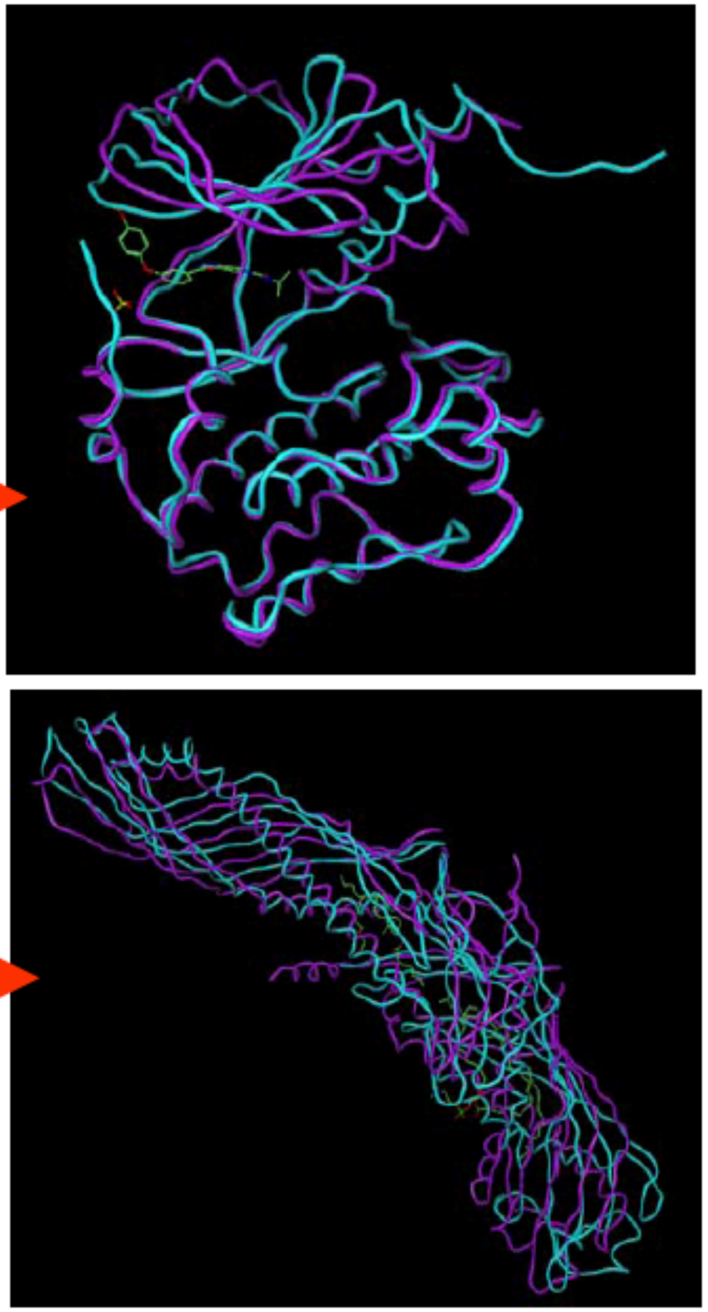
Alignment	Scoring Methods	Gap Treatment	Examples
Sequence-Sequence	BLOSUM PAM GONET	Length Proportional Affine	BLAST FASTA Smith-Waterman Needleman-Wunsch
Sequence-Profile	PSSM HMM	Affine Position-Specific	PSI-Blast HMMer
Sequence-Structure	Threading potential	Affine Position-Specific	Raptor GenThreader
Profile-Profile	Dot-product Log Average **Analytic Statistics**	Position-Specific **Structural Family-based**	3D-PSSM FFAS **STRUCTFAST**

Template Homology Range



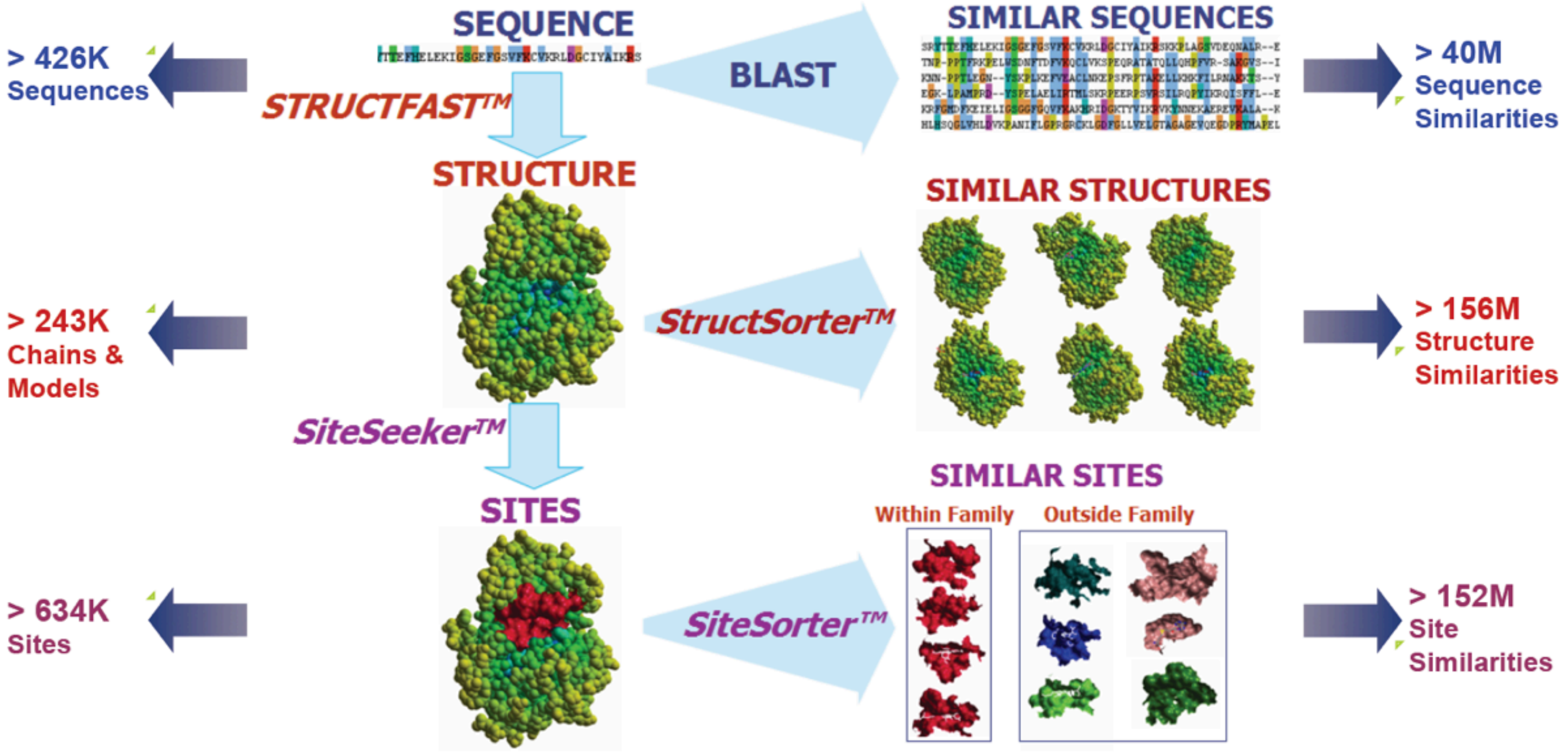
Comparison to a Client's Crystal Structures

Protein	# of residues	In house resolution	template resolution	PDB ID	% ID	# atoms fit	rmsd
Family 1	288	1.6 A	1.8 A		75%	976	0.88A
Family 2	347	1.7 A	2.3 A		87%	1148	1.23A
Family 3	285	2.5 A	2.4 A		34%	780	1.48A
Family 4	941	2.1 A	1.7 A		20%	1824	6.66A
Family 5	313	2.6 A	2.0 A		57%	1048	1.23 A
Family 6	442	2.05 A	2.1 A		95%	1376	0.87 A



Purple –client's in house
Cyan – homology model

Target Informatics Platform (TIP)



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SiteSeeker

Geometric Site-Finding Algorithms Find Many Pockets

But they don't know which pockets are important!

Evolutionary Trace Approach

Can't clearly define site boundary

Not all conserved residues are functionally relevant

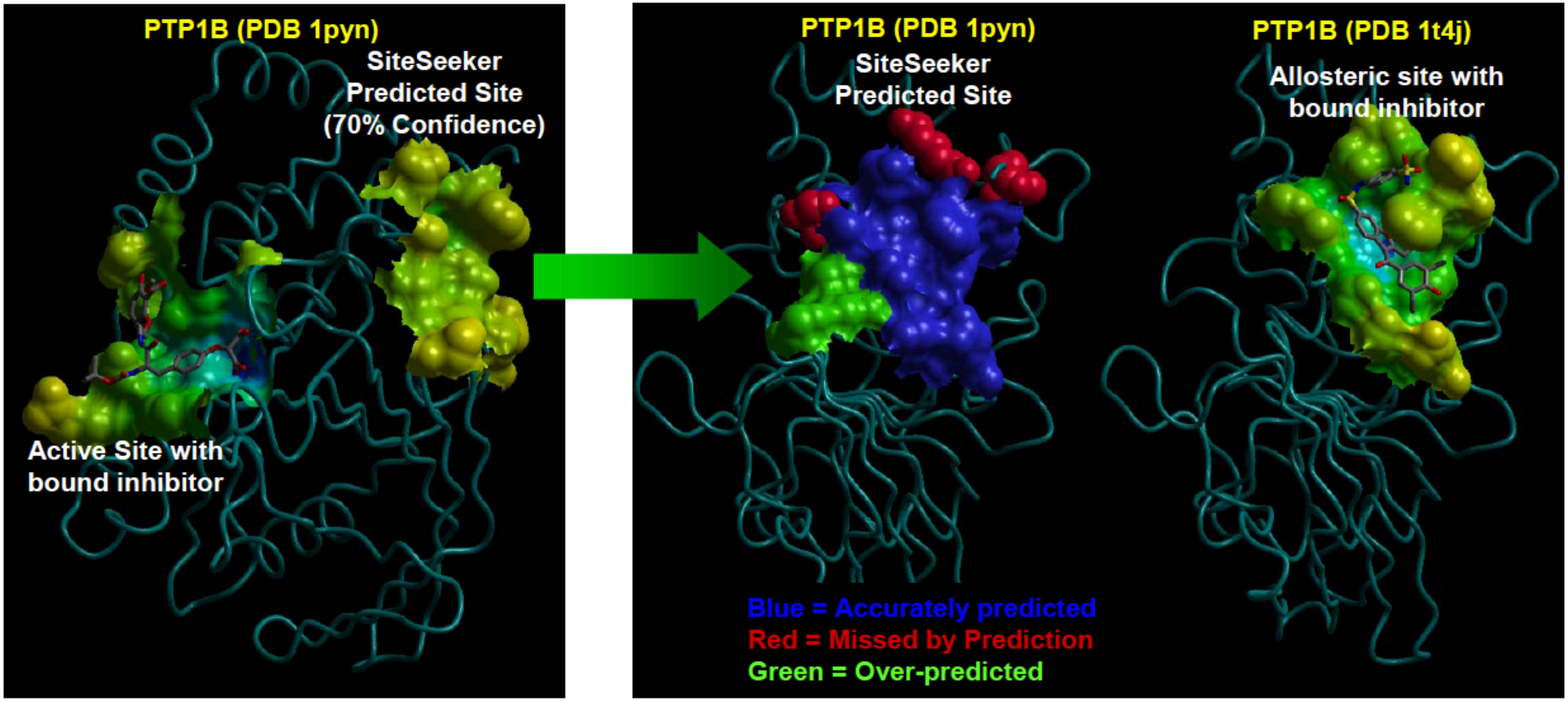
SiteSeeker combines *both* methods

Reliability & Confidence

We use proteins with apo- & co-crystal structures in the PDB to test the accuracy & reliability of method

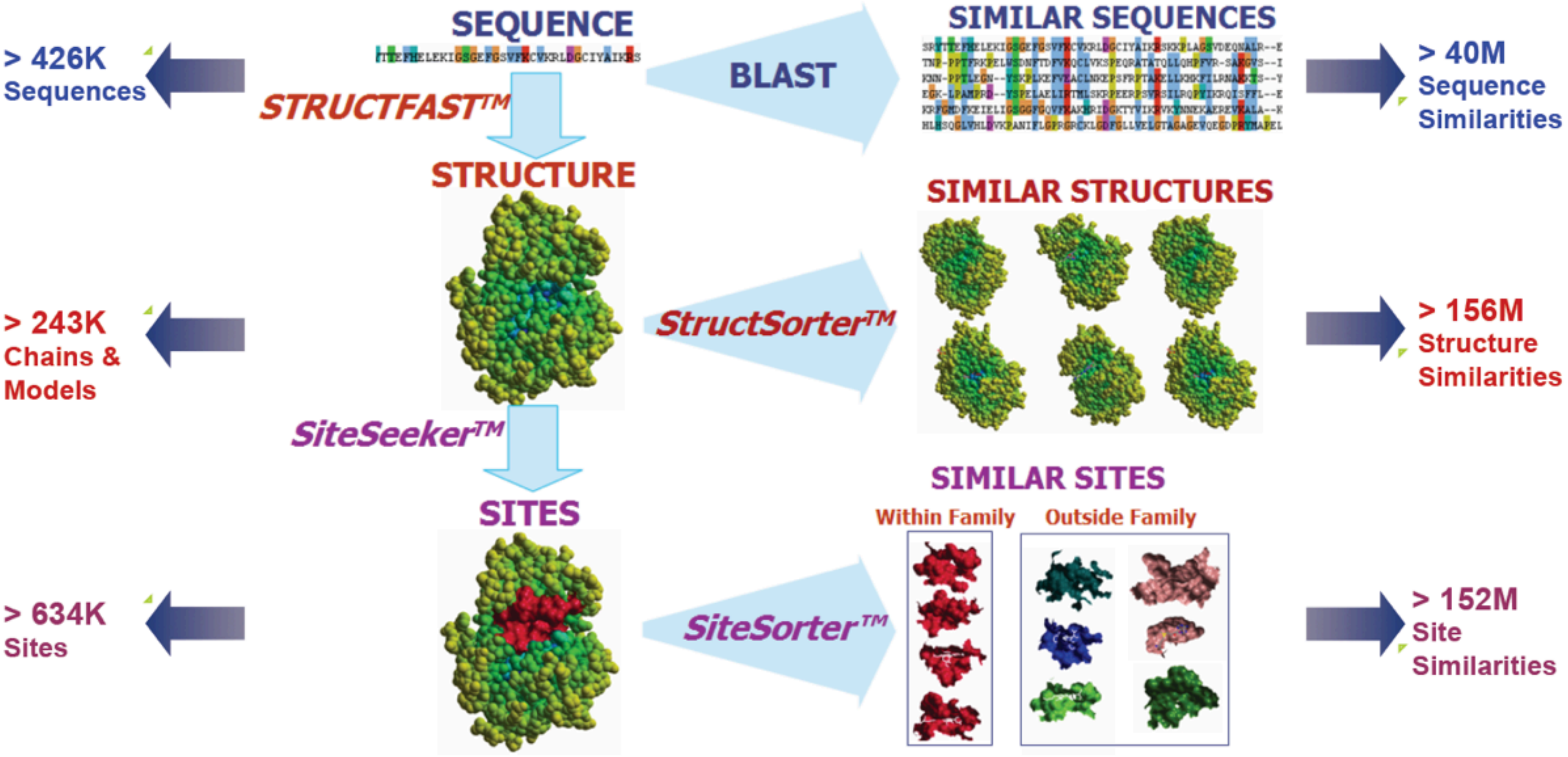
Allows us to map *SiteSeeker* score to predict confidence!
(e.g. At this *SiteSeeker* score, 80% are "real" co-crystal sites)
→ Sites with <60% confidence are not stored in TIP

SiteSeeker Example



All structures in TIP are annotated with known and predicted binding sites, along with **confidence** levels for each annotation

Target Informatics Platform (TIP)



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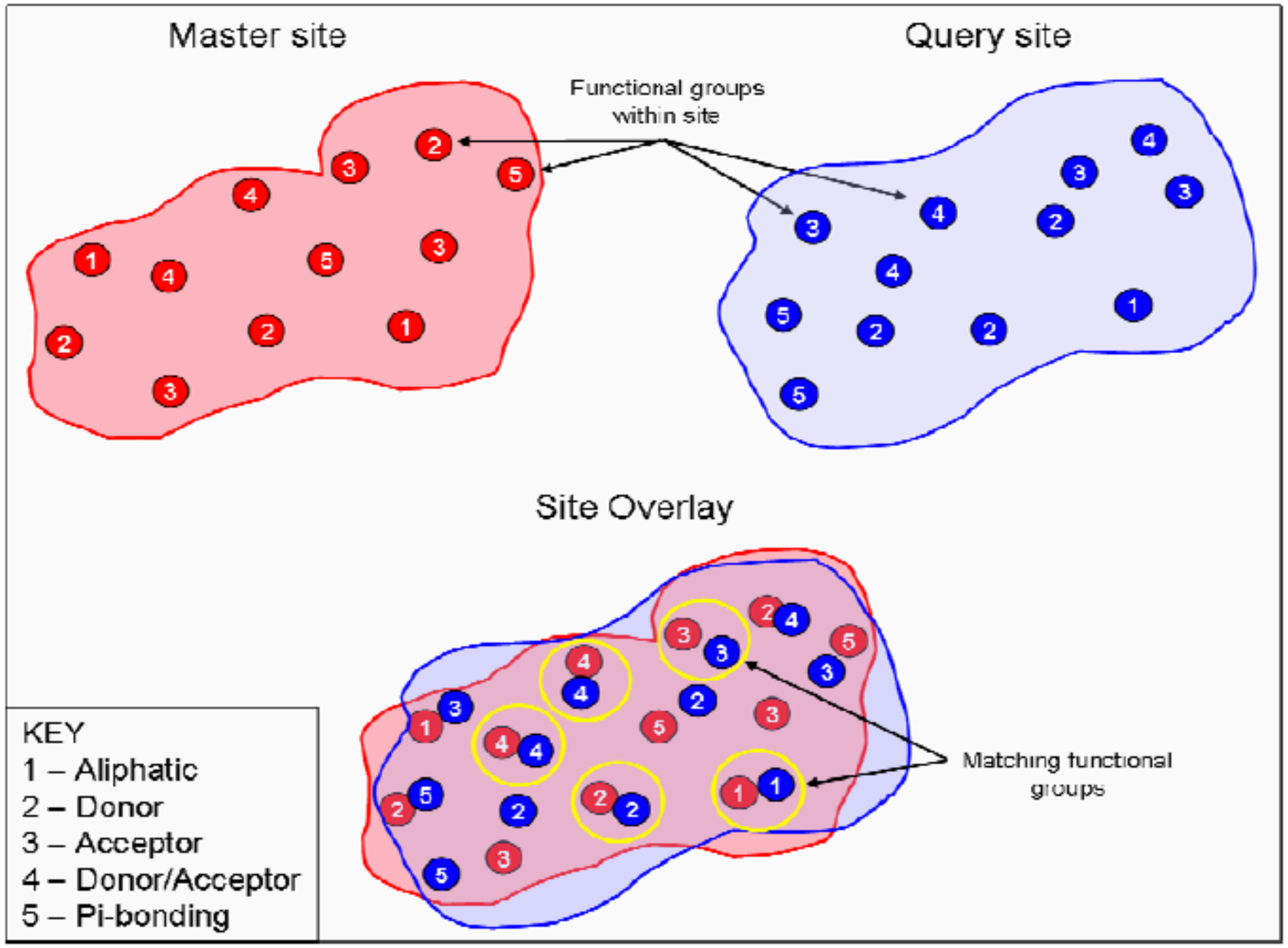
SiteSorter

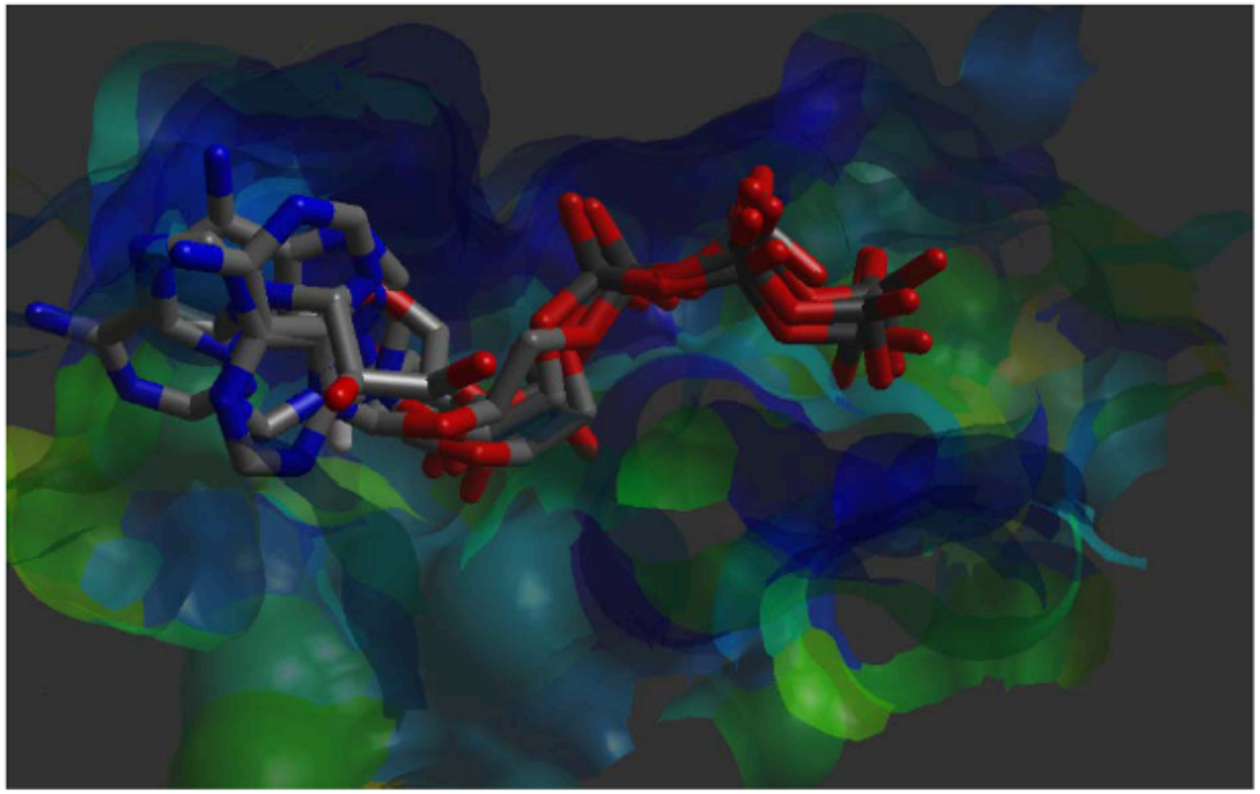
Weighted Clique Detection Algorithm

Importance of Points (Weights) Related to their Similarity

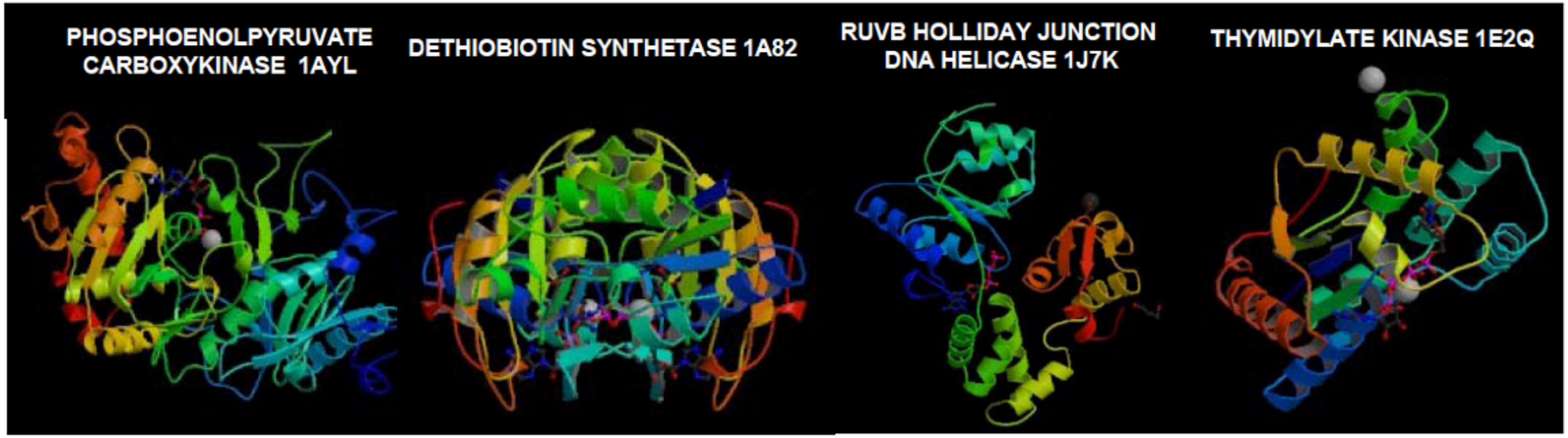


Surface Atoms Assigned One of 5 Different Chemical Characters
Matching points increase the *SiteSorter* similarity score

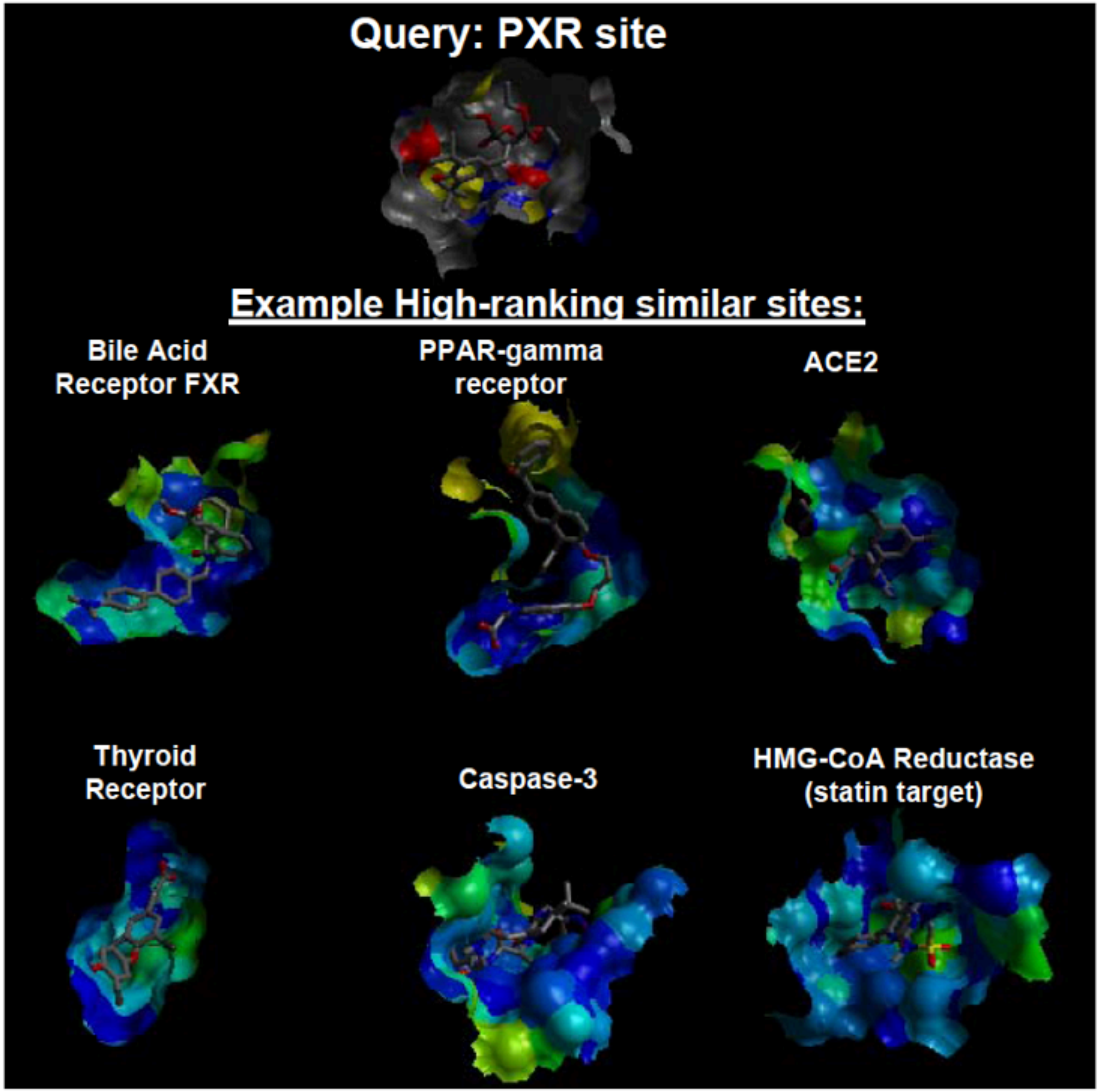
SiteSorter Example



Overlay of ATP binding sites from completely different folds

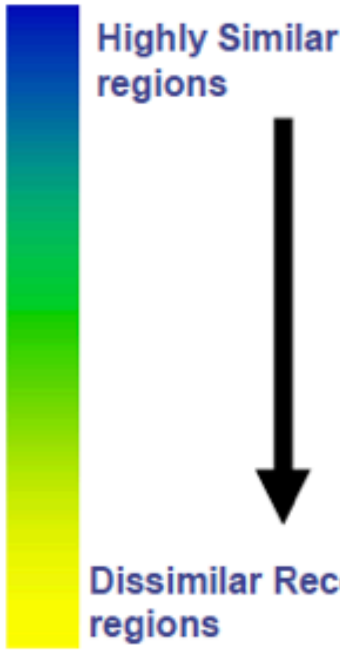


Nature Exploits Site-Similarity



Pregnane X-receptor – PXR ("sensor)" →CYP3A4 ("executioner")
<u>PXR Binds > 50% drugs</u>
Including some bile acids, statins, herbal components, a selection of HIV protease inhibitors, calcium channel modulators, numerous steroids, plasticizers and monomers, organochlorine pesticides, a peroxisome proliferator-activated receptor-äantagonist, xenobiotics and endobiotics…

Site Similarity Coloring



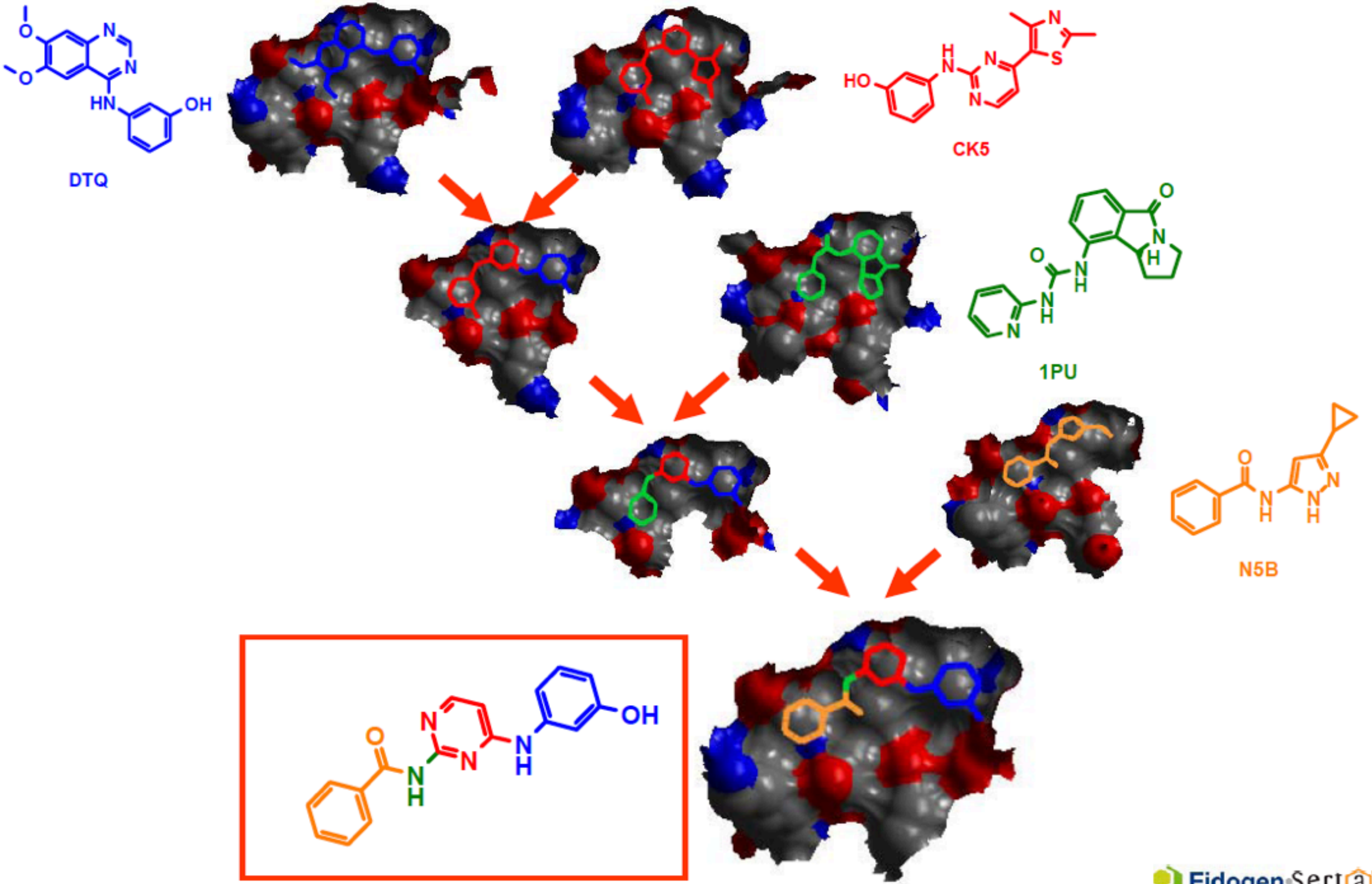
Highly Similar Receptor regions

Dissimilar Receptor regions

Eidogen-Sertanty

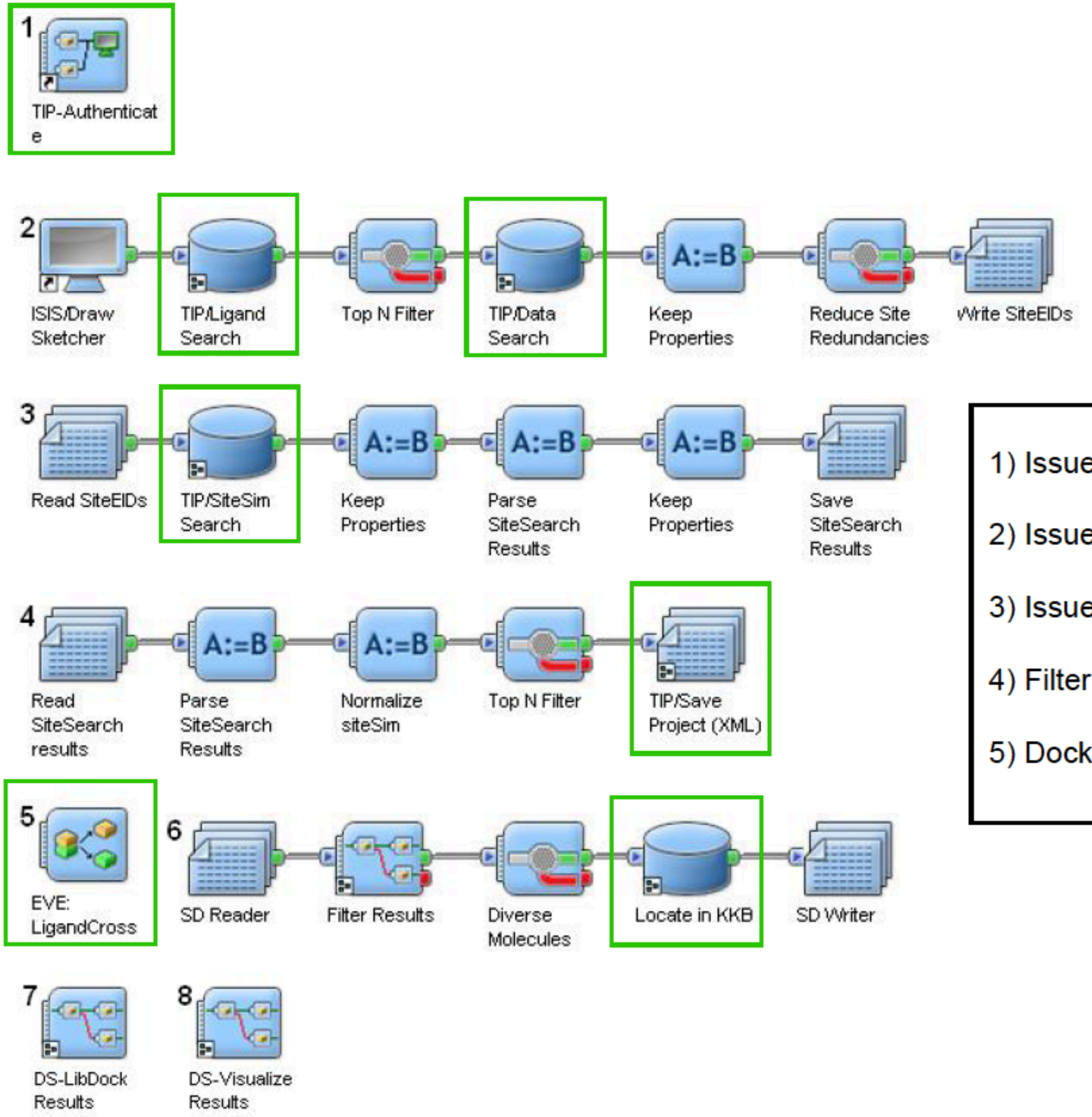
LigandCross: Shuffling Ligand Functionality

Similar to Vertex's BREED: J. Med. Chem. **47**, 2768 (2004)



Eidogen-Sertanty

LigandCross via PipelinePilot



1) Issue TIP/LigandSearch

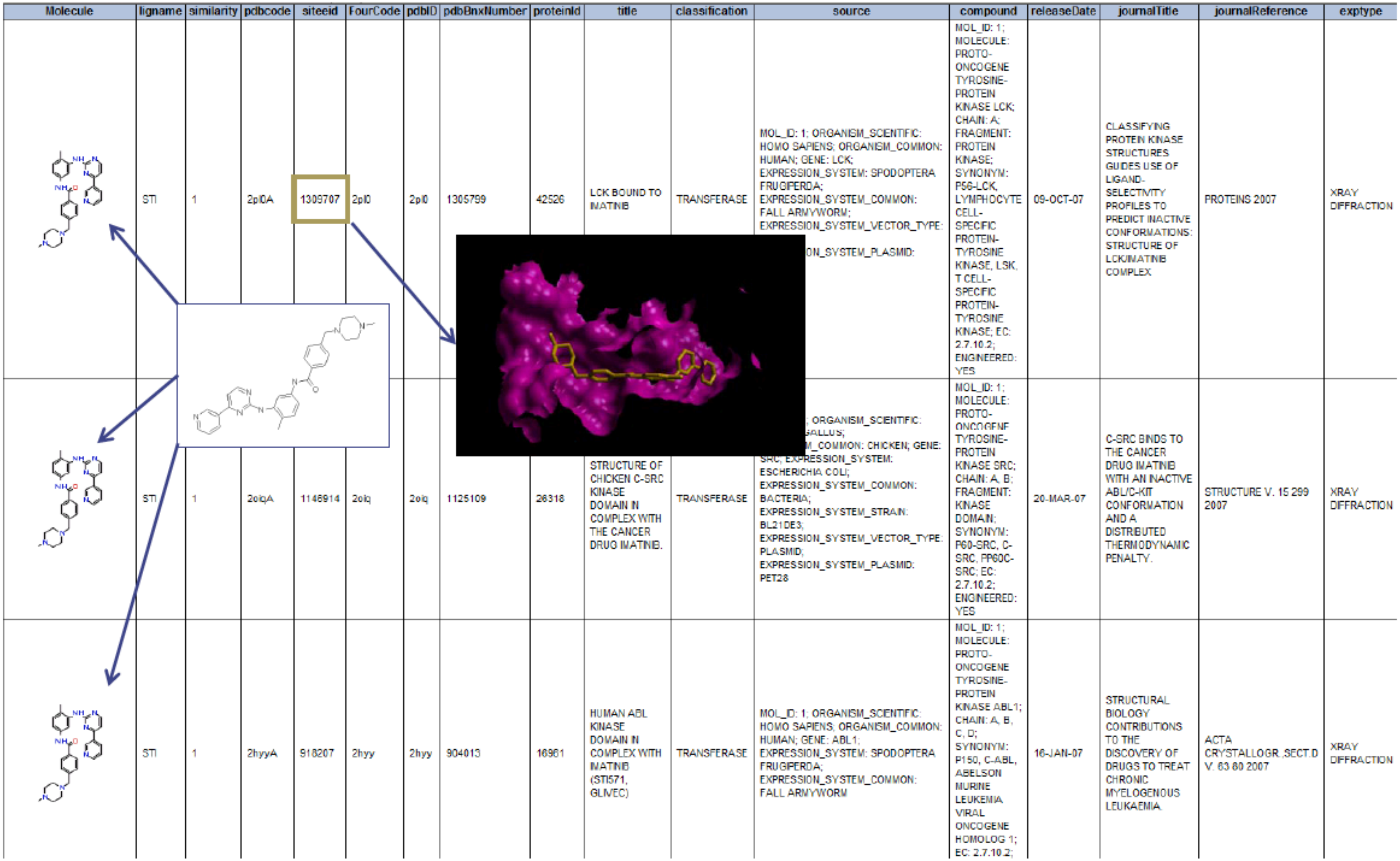
2) Issue TIP/SiteSimSearch

3) Issue LigandCross

4) Filter and locate results in KKB

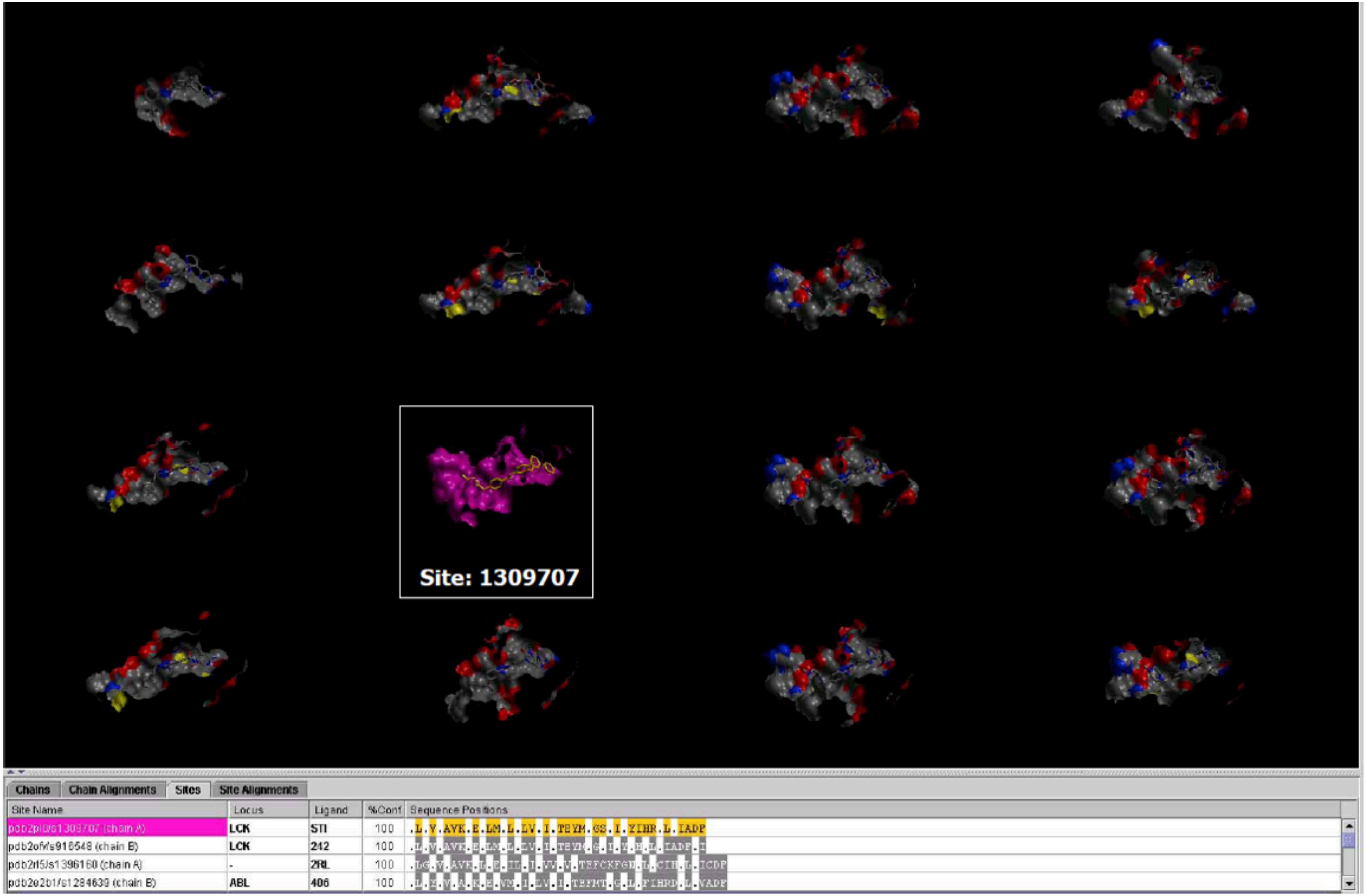
5) Dock and visualize results

Step 1: Find Co-complexes and Sites



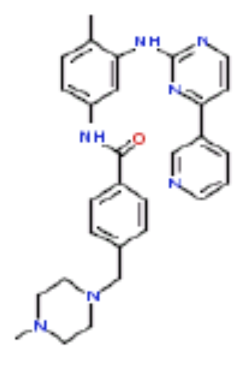
Molecule	ligname	similarity	pdbcode	siteid	FourCode	pdbID	pdbBnxNumber	proteinId	title	classification	source	compound	releaseDate	journalTitle	journalReference	exptype
	STI	1	2pl0A	1309707	2pl0	2pl0	1305799	42526	LCK BOUND TO IMATINIB	TRANSFERASE	MOL_ID: 1; ORGANISM_SCIENTIFIC: HOMO SAPIENS; ORGANISM_COMMON: HUMAN; GENE: LCK; EXPRESSION_SYSTEM: SPODOPTERA FRUGIPERDA; EXPRESSION_SYSTEM_COMMON: FALL ARMYWORM; EXPRESSION_SYSTEM_VECTOR_TYPE: ON_SYSTEM_PLASMID:	MOL_ID: 1; MOLECULE: PROTO-ONCOGENE TYROSINE-PROTEIN KINASE LCK; CHAIN: A; FRAGMENT: PROTEIN KINASE; SYNONYM: P56-LCK, LYMPHOCYTE CELL-SPECIFIC PROTEIN-TYROSINE KINASE, LSK, T CELL-SPECIFIC PROTEIN-TYROSINE KINASE; EC: 2.7.10.2; ENGINEERED: YES	09-OCT-07	CLASSIFYING PROTEIN KINASE STRUCTURES GUIDES USE OF LIGAND-SELECTIVITY PROFILES TO PREDICT INACTIVE CONFORMATIONS: STRUCTURE OF LCK/IMATINIB COMPLEX	PROTEINS 2007	XRAY DIFFRACTION
	STI	1	2oiqA	1146914	2oiq	2oiq	1125109	26318	STRUCTURE OF CHICKEN C-SRC KINASE DOMAIN IN COMPLEX WITH THE CANCER DRUG IMATINIB.	TRANSFERASE	; ORGANISM_SCIENTIFIC: GALLUS; M_COMMON: CHICKEN; GENE: SRC; EXPRESSION_SYSTEM: ESCHERICHIA COLI; EXPRESSION_SYSTEM_COMMON: BACTERIA; EXPRESSION_SYSTEM_STRAIN: BL21DE3; EXPRESSION_SYSTEM_VECTOR_TYPE: PLASMID; EXPRESSION_SYSTEM_PLASMID: PET28	MOL_ID: 1; MOLECULE: PROTO-ONCOGENE TYROSINE-PROTEIN KINASE SRC; CHAIN: A, B; FRAGMENT: KINASE DOMAIN; SYNONYM: P60-SRC, C-SRC, PP60C-SRC; EC: 2.7.10.2; ENGINEERED: YES	20-MAR-07	C-SRC BINDS TO THE CANCER DRUG IMATINIB WITH AN INACTIVE ABL/C-KIT CONFORMATION AND A DISTRIBUTED THERMODYNAMIC PENALTY.	STRUCTURE V. 15 299 2007	XRAY DIFFRACTION
	STI	1	2hyyA	918207	2hyy	2hyy	904013	16961	HUMAN ABL KINASE DOMAIN IN COMPLEX WITH IMATINIB (STI571, GLIVEC)	TRANSFERASE	MOL_ID: 1; ORGANISM_SCIENTIFIC: HOMO SAPIENS; ORGANISM_COMMON: HUMAN; GENE: ABL1; EXPRESSION_SYSTEM: SPODOPTERA FRUGIPERDA; EXPRESSION_SYSTEM_COMMON: FALL ARMYWORM	MOL_ID: 1; MOLECULE: PROTO-ONCOGENE TYROSINE-PROTEIN KINASE ABL1; CHAIN: A, B, C, D; SYNONYM: P150, C-ABL, ABELSON MURINE LEUKEMIA VIRAL ONCOGENE HOMOLOG 1; EC: 2.7.10.2;	16-JAN-07	STRUCTURAL BIOLOGY CONTRIBUTIONS TO THE DISCOVERY OF DRUGS TO TREAT CHRONIC MYELOGENOUS LEUKAEMIA	ACTA CRYSTALLOGR.,SECT.D V. 63 80 2007	XRAY DIFFRACTION

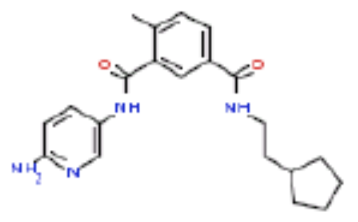
Step 2: Find Other Sites via SiteSimilarity

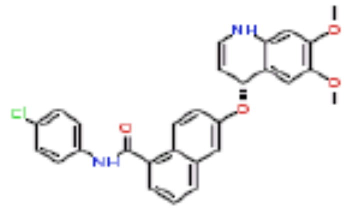


Site Name	Locus	Ligand	%Conf	Sequence Positions
pdb2pl0/s1309707 (chain A)	LCK	STI	100	.L.V.AVK.E.LM.L.LV.I.TEYM.GS.I.YIHR.L.IADP
pdb2ofv/s916548 (chain B)	LCK	242	100	.L.V.AVK.E.LM.L.LV.I.TEYM.G.I.Y.H.L.IADF.I
pdb2rl5/s1396160 (chain A)	-	2RL	100	.LG.V.AVK.L.E.IL.I.VV.V.TEFCKFGN.L.CIH.L.ICDF
pdb2e2b/s1284639 (chain B)	ABL	406	100	.L.Y.V.A.K.E.VM.I.LV.I.TEFMT.G.L.FIHRD.L.VADF

Example Ligands from Similar Sites


STI


C92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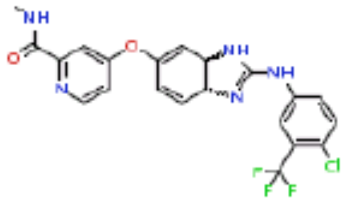
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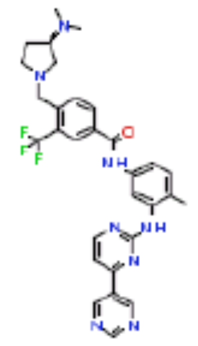
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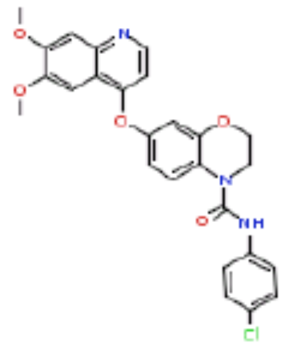
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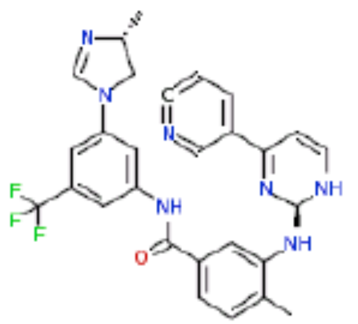
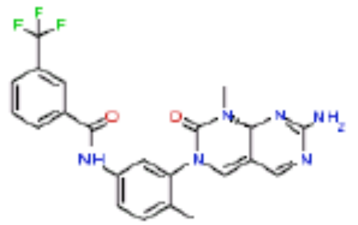

NIL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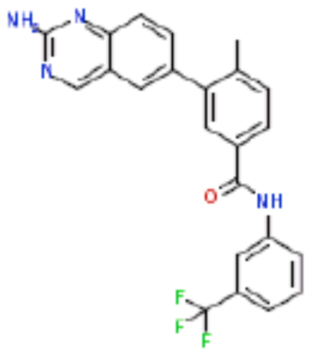
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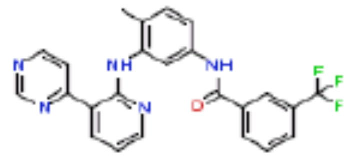
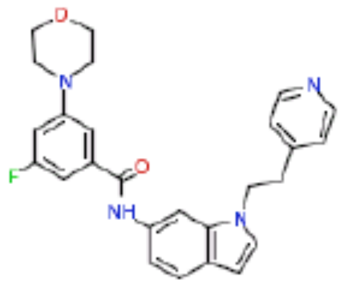

MUH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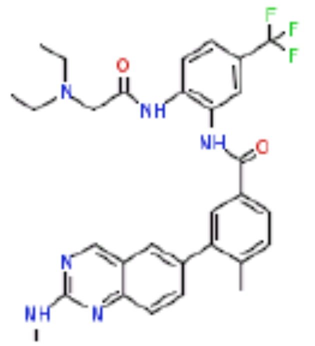
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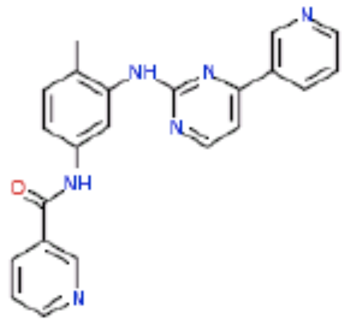
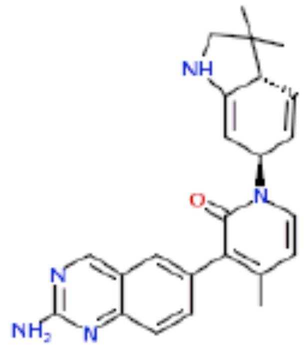

PRC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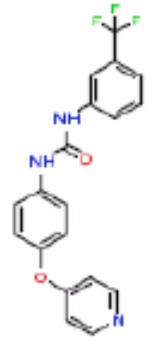
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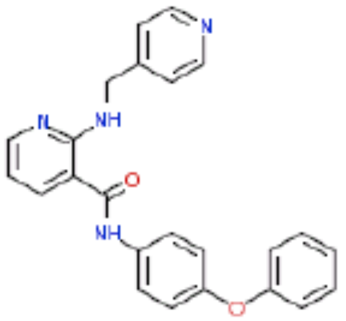
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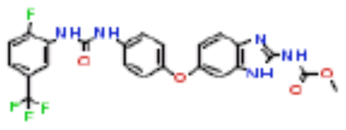
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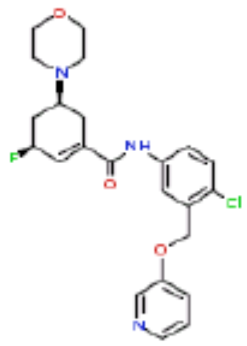
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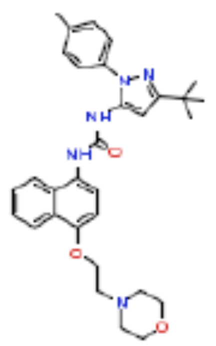
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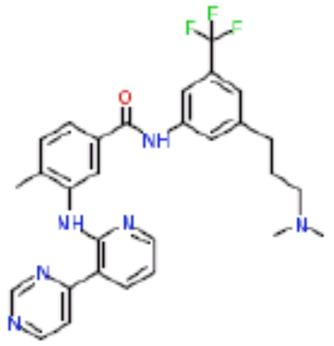
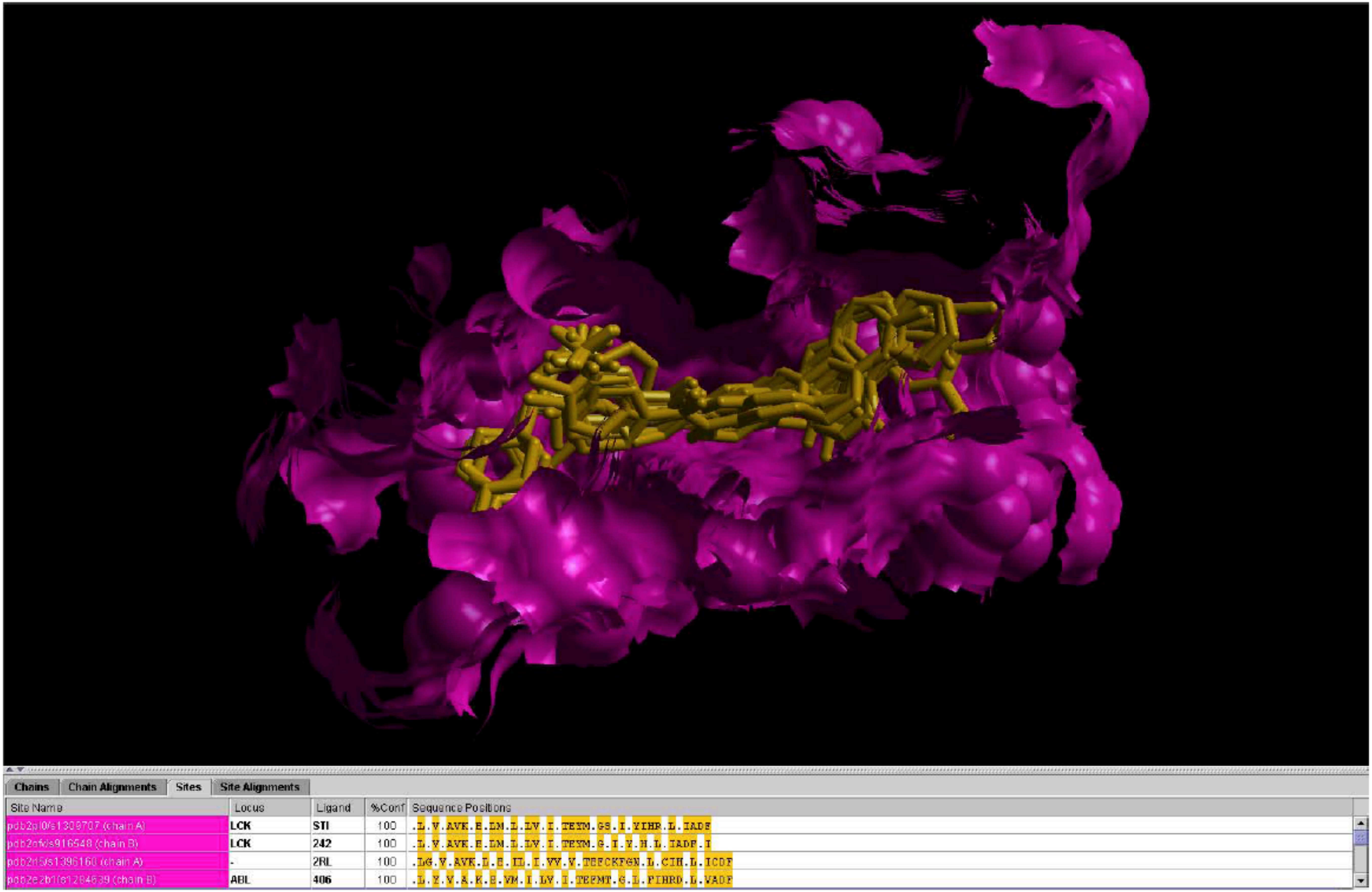
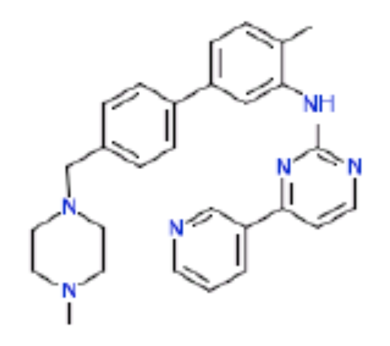
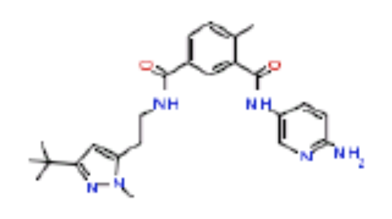
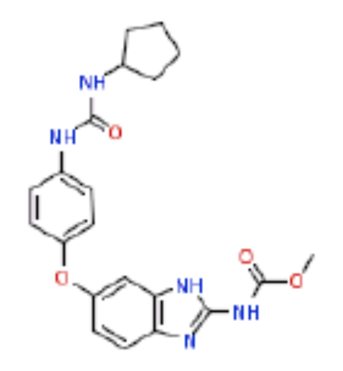
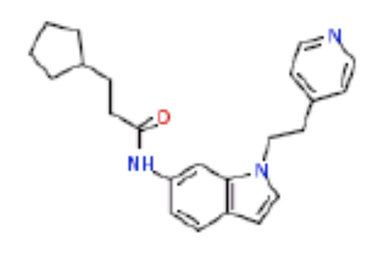
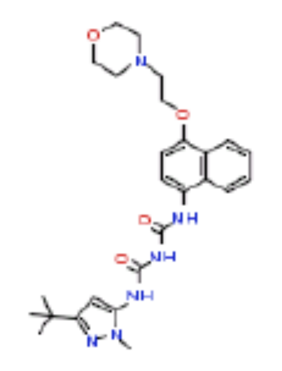
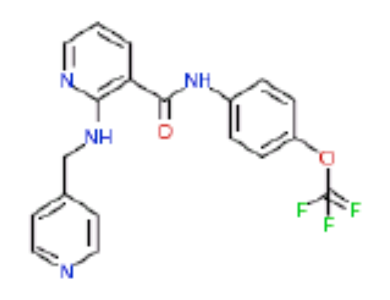
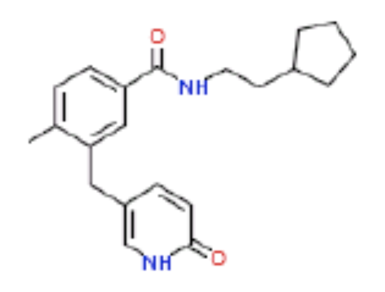
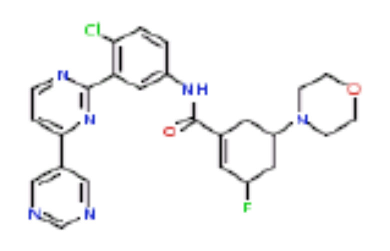
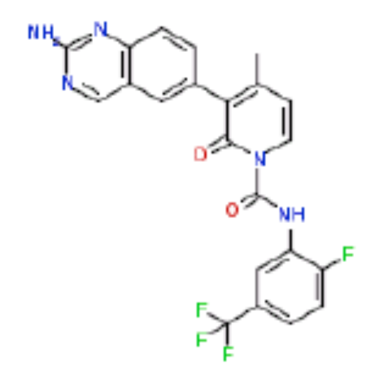
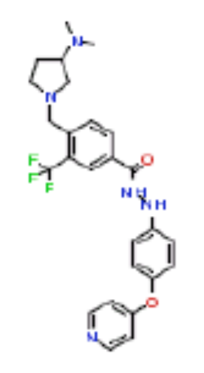
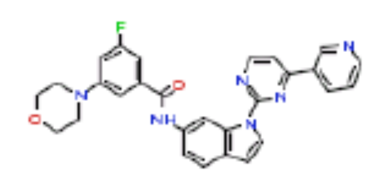
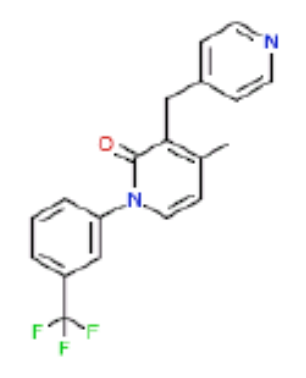
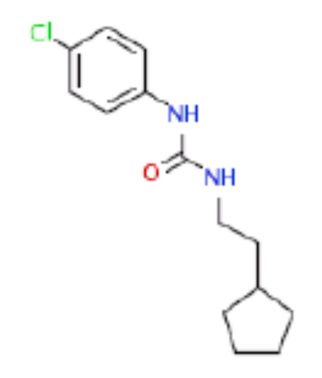
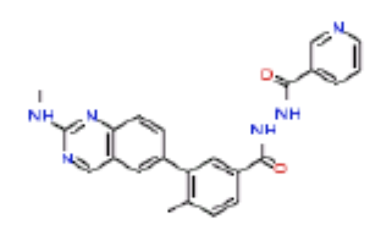
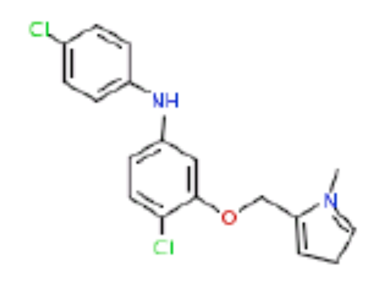
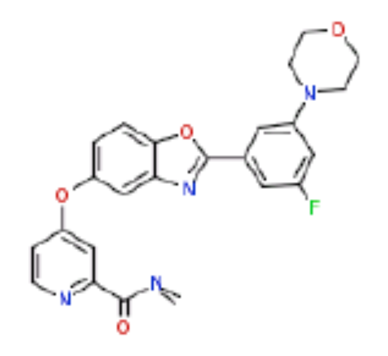
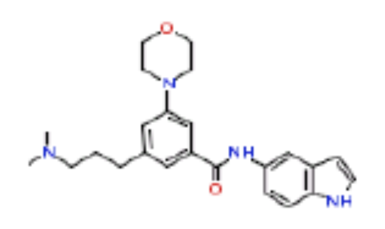
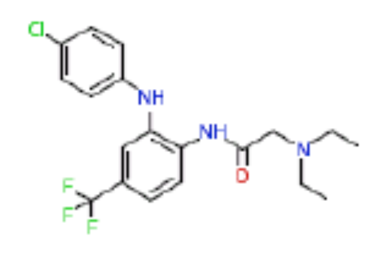
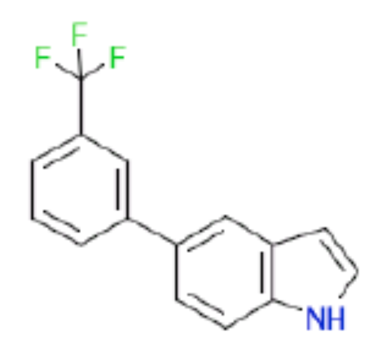
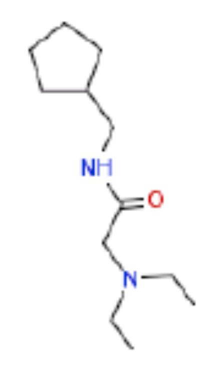

RAJ

Step 3: LigandCross: Shuffle Ligand Features from Aligned Sites



Site Name	Locus	Ligand	%Conf	Sequence Positions
pdb2pl0/s1309707 (chain A)	**LCK**	**STI**	100	.L.V.AVK.E.LM.L.LV.I.TEYM.GS.I.YIHR.L.IADF
pdb2ofv/s916548 (chain B)	**LCK**	**242**	100	.L.V.AVK.E.LM.L.LV.I.TEYM.G.I.Y.H.L.IADF.I
pdb2il5/s1396160 (chain A)	-	2RL	100	.LG.V.AVK.L.E.IL.I.VV.V.TEFCKFGN.L.CIH.L.ICDF
pdb2e2b1/s1284639 (chain B)	ABL	**406**	100	.L.Y.V.A.K.E.VM.I.LV.I.TEFMT.G.L.FIHRD.L.VADF

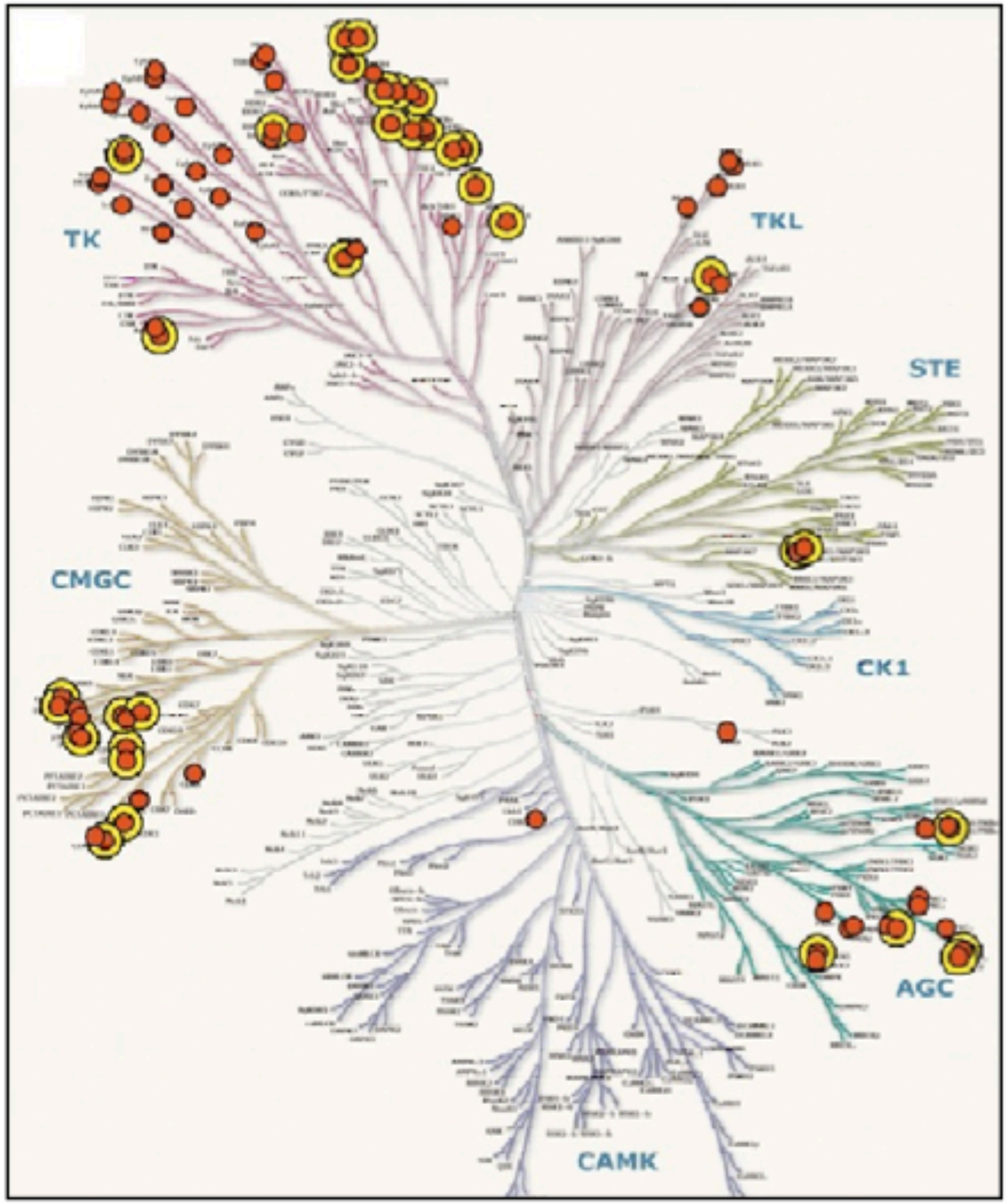
Example LigandCross Results

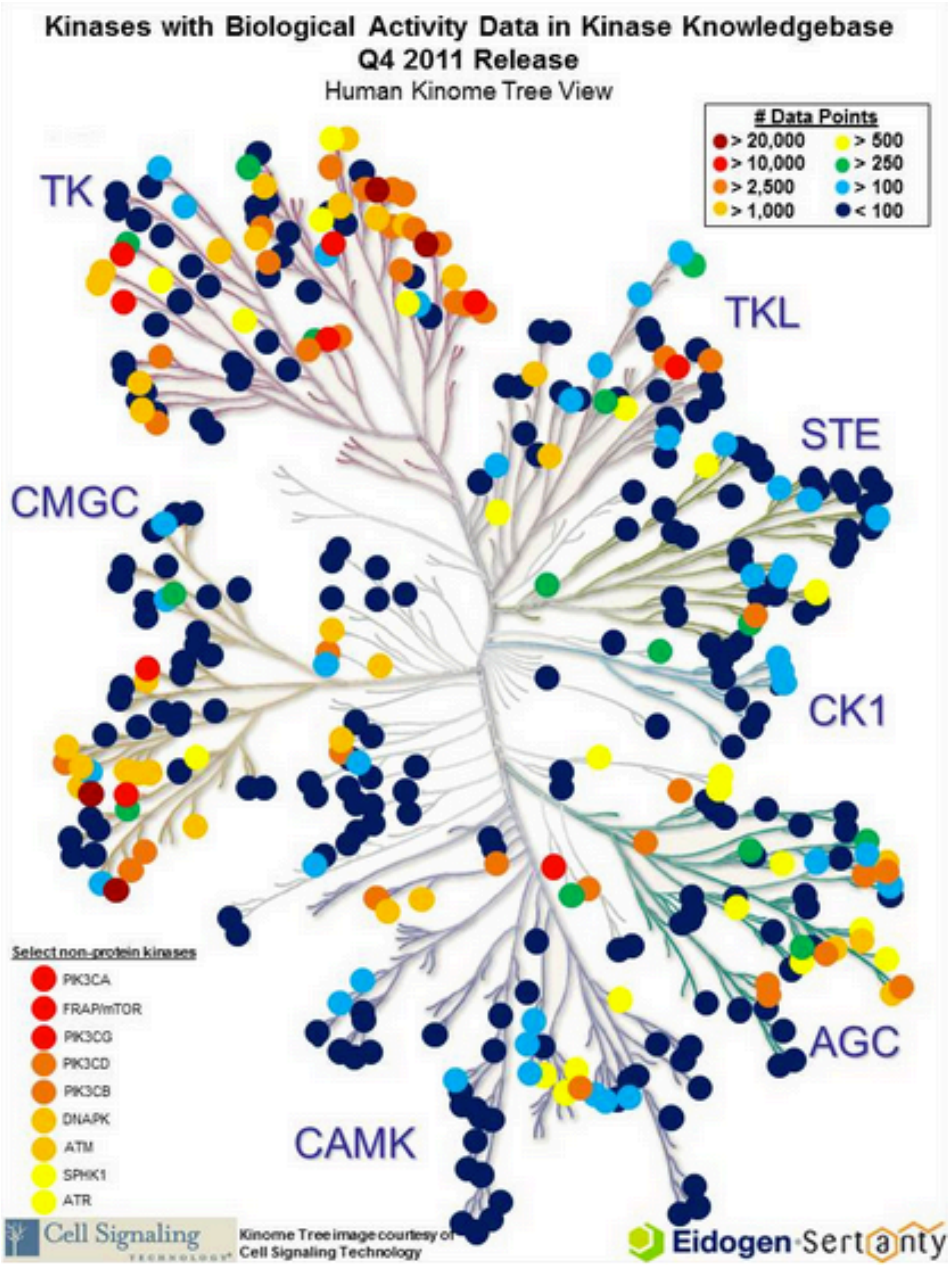
 STI_PRC_2 0.667	 C92_BMU_5 0.635	 C92_GIG_3 0.633	 C92_WBT_1 0.625	 B96_BMU_2 0.623
 608_276_3 0.608	 C92_GIN_7 0.608	 406_L11_6 0.577	 GIG_C52_1 0.574	 406_KIN_2 0.545
 NIL_WBT_6 0.538	 608_C52_2 0.529	 C92_BMU_1 0.520	 1N8_PRC_3 0.491	 857_BMU_4 0.480
 857_WBT_2 0.472	 RAJ_LI3_1 0.462	 1N8_BMU_2 0.449	 LI3_C52_2 0.385	 C92_1N8_1 0.375

Kinase Knowledgebase (KKB)

Kinase Targets of Clinical Interest

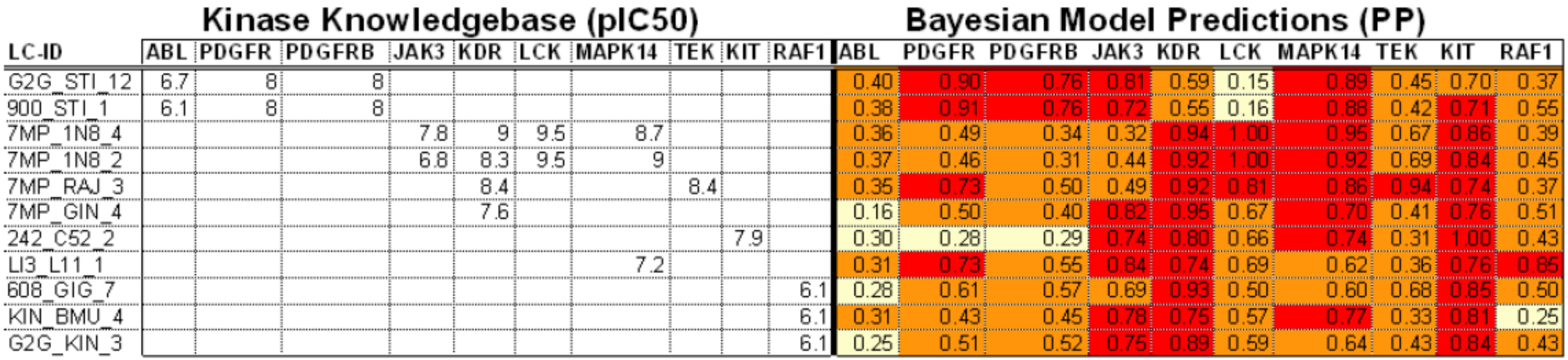
from Vieth *et al.* *Drug Disc. Today* **10**, 839 (2005).



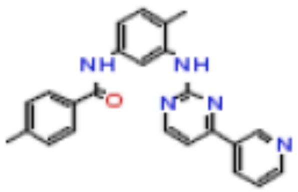


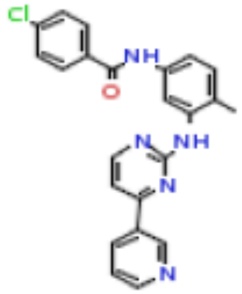
> 583K SAR data points curated from
> 7700 articles and patents

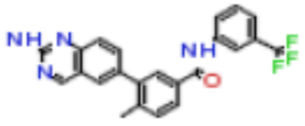
LigandCross Validation

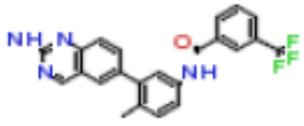


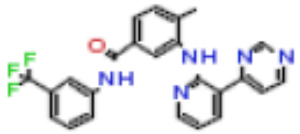
	Kinase Knowledgebase (pIC50)										Bayesian Model Predictions (PP)									
LC-ID	ABL	PDGFR	PDGFRB	JAK3	KDR	LCK	MAPK14	TEK	KIT	RAF1	ABL	PDGFR	PDGFRB	JAK3	KDR	LCK	MAPK14	TEK	KIT	RAF1
G2G_STI_12	6.7	8	8								0.40	0.90	0.76	0.81	0.59	0.15	0.89	0.45	0.70	0.37
900_STI_1	6.1	8	8								0.38	0.91	0.76	0.72	0.55	0.16	0.88	0.42	0.71	0.55
7MP_1N8_4				7.8	9	9.5	8.7				0.36	0.49	0.34	0.32	0.94	1.00	0.95	0.67	0.86	0.39
7MP_1N8_2				6.8	8.3	9.5	9				0.37	0.46	0.31	0.44	0.92	1.00	0.92	0.69	0.84	0.45
7MP_RAJ_3					8.4			8.4			0.35	0.73	0.50	0.49	0.92	0.81	0.86	0.94	0.74	0.37
7MP_GIN_4					7.6						0.16	0.50	0.40	0.82	0.95	0.67	0.70	0.41	0.76	0.51
242_C52_2									7.9		0.30	0.28	0.29	0.74	0.80	0.66	0.74	0.31	1.00	0.43
LI3_L11_1							7.2				0.31	0.73	0.55	0.84	0.74	0.69	0.62	0.36	0.76	0.85
608_GIG_7										6.1	0.28	0.61	0.57	0.69	0.93	0.50	0.60	0.68	0.85	0.50
KIN_BMU_4										6.1	0.31	0.43	0.45	0.78	0.75	0.57	0.77	0.33	0.81	0.25
G2G_KIN_3										6.1	0.25	0.51	0.52	0.75	0.89	0.59	0.64	0.43	0.84	0.43

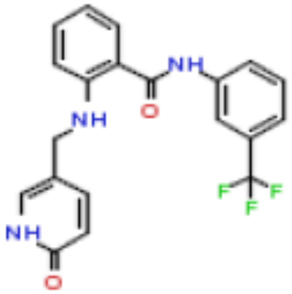

1: G2G_STI_12

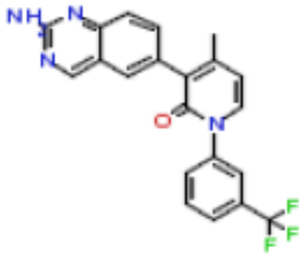

2: 900_STI_1

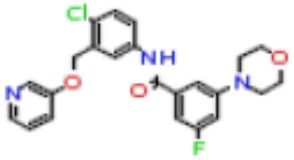

3: 7MP_1N8_4

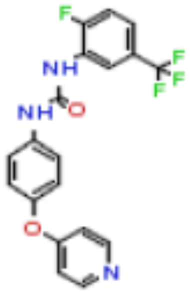

4: 7MP_1N8_2

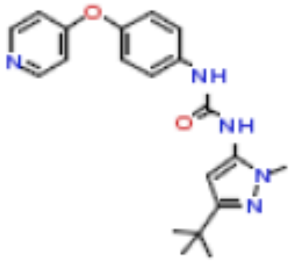

5: 7MP_RAJ_3

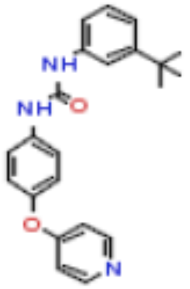

6: 7MP_GIN_4


7: 242_C52_2


8: LI3_L11_1

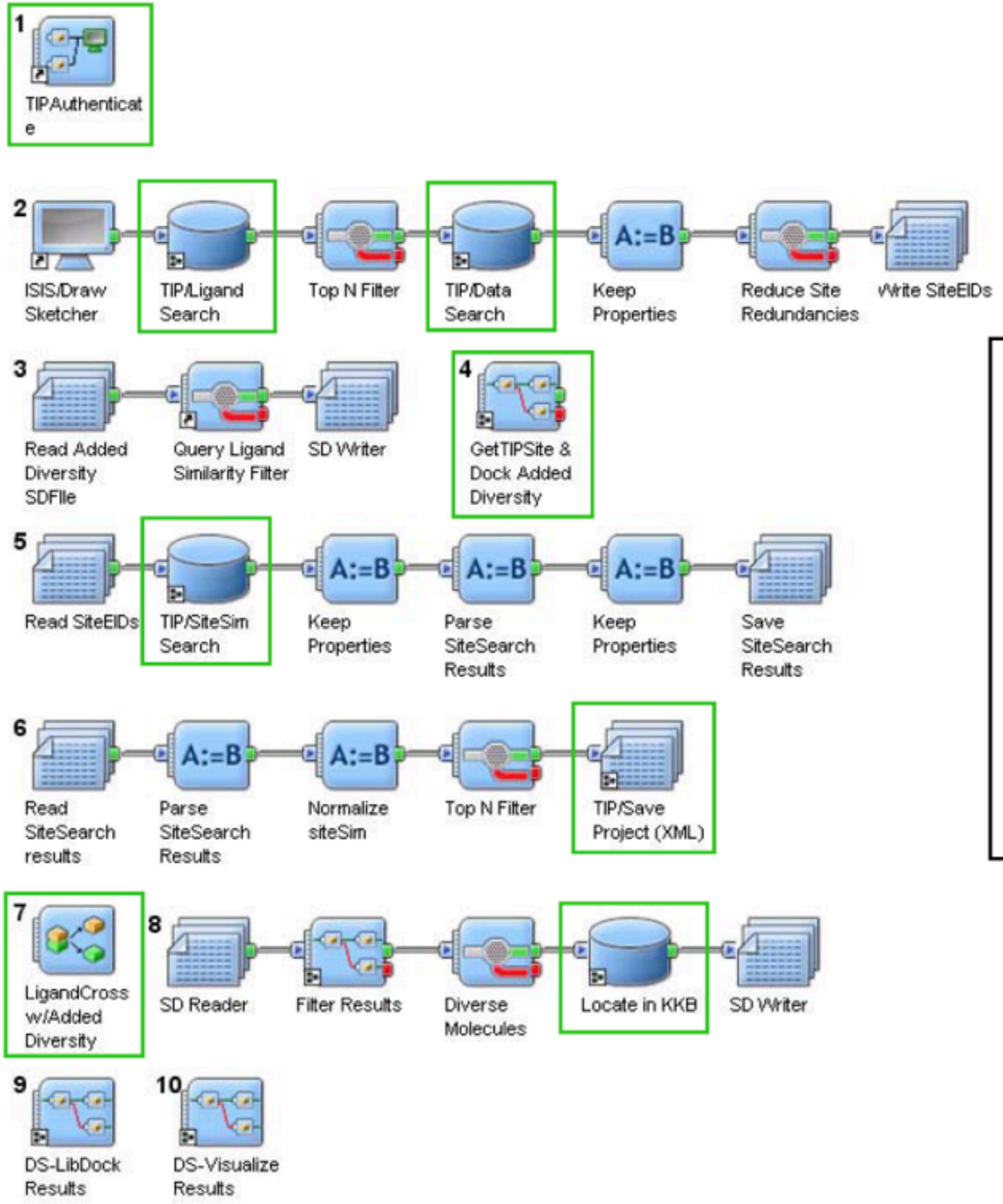

9: 608_GIG_7


10: KIN_BMU_4


11: G2G_KIN_3

Eidogen-Sertanty

Enhance LigandCross with Added Diversity



> Issue TIP/LigandSearch

> Identify/Dock “AddedDiversity”

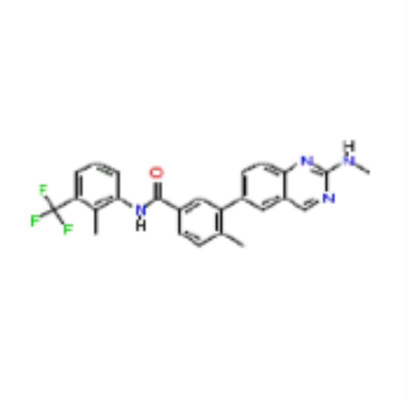
> Issue TIP/SiteSimSearch

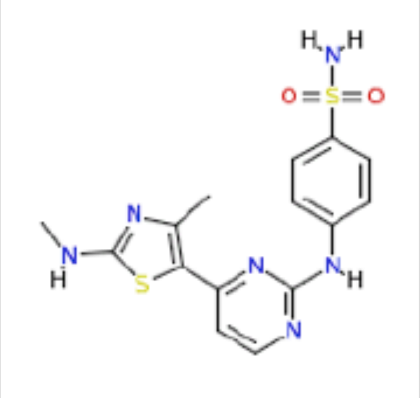
> LigandCross w/AddedDiversity

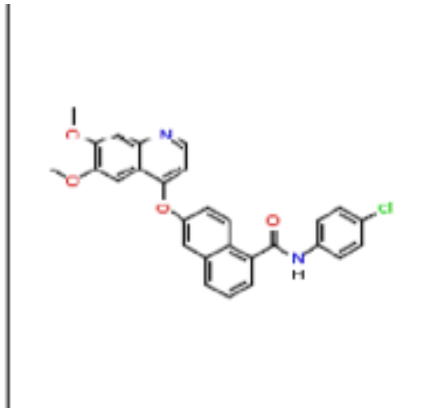
> Filter and locate results in KKB

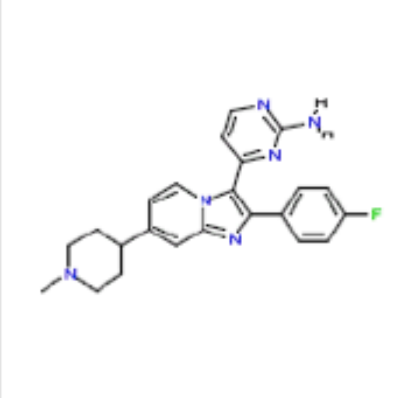
> Dock and visualize results

Example Potent Kinase Inhibitors (From KKB)


4336533 LCK pval: 11.00


4302493 CDK9 pval: 10.54


4332561 KDR pval: 10.52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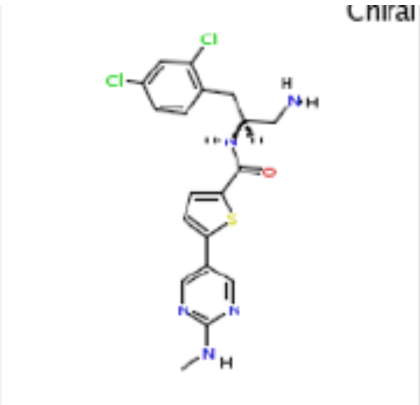
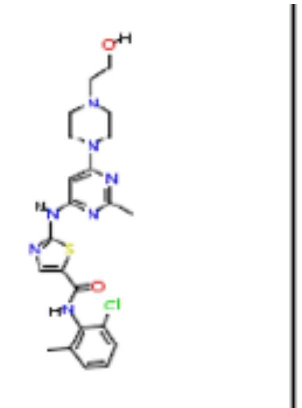
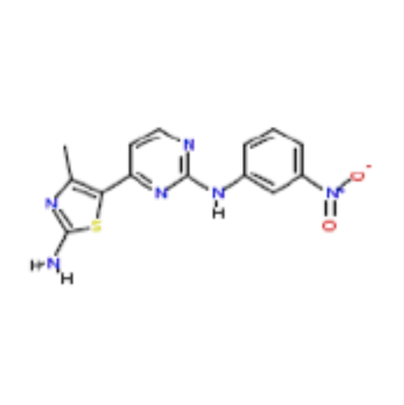
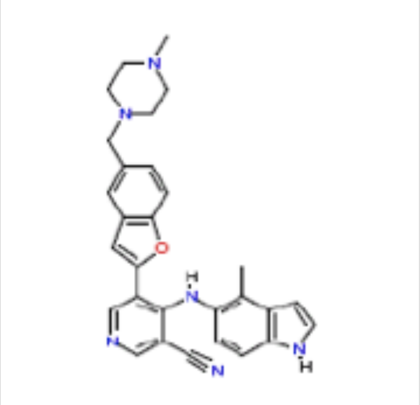
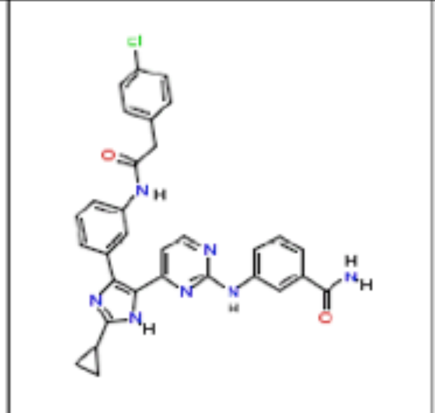


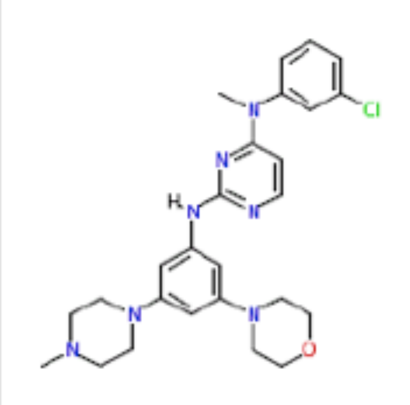
4336686 PKA pval: 10.00

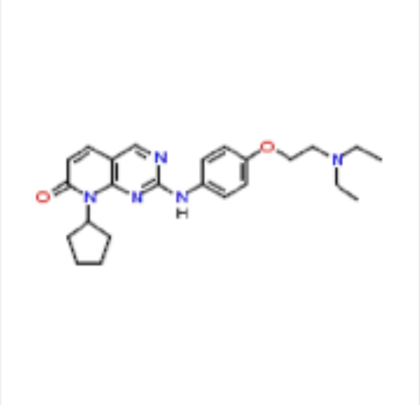

4272835 ABL1 pval: 10.00

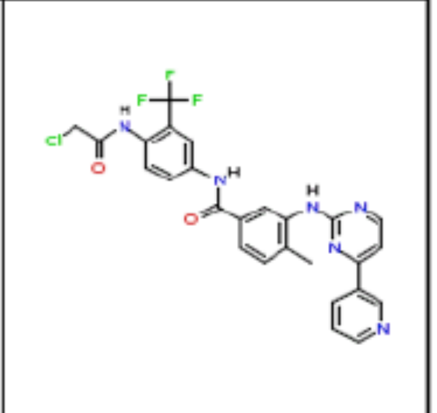

894611 CDK2 pval: 9.70

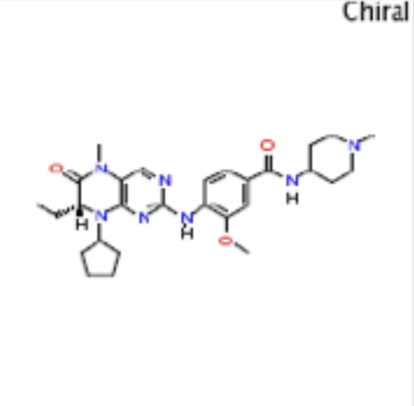

4358565 PRKCG pval: 9.70


4363734 RAF1 pval: 9.30

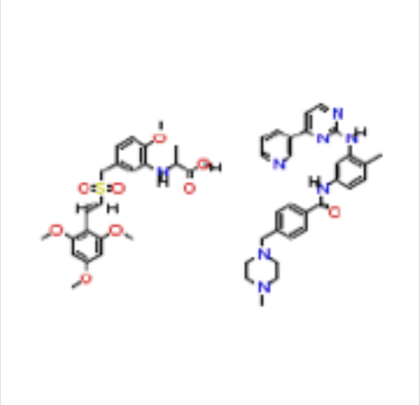

4369892 EPHB4 pval: 9.24

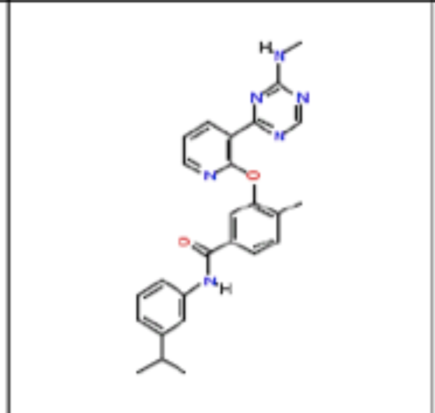

809 CDK4 pval: 9.15

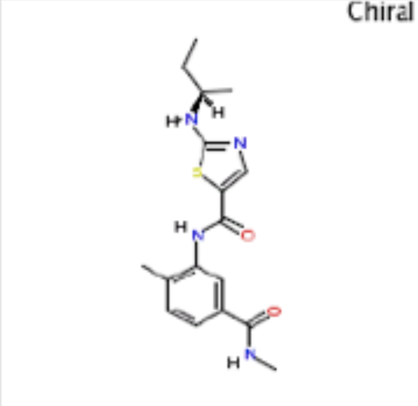

4374385 PDGFRA pval: 9.14



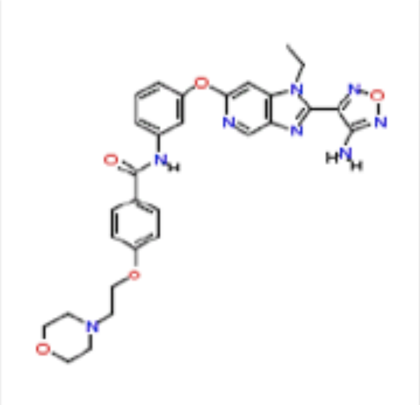
4366691 PLK1 pval: 9.10

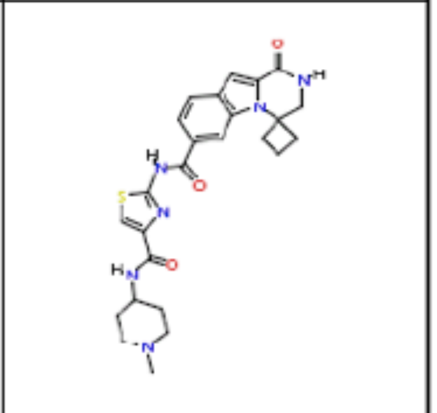

4301886 BCR_ABL pval: 9.08

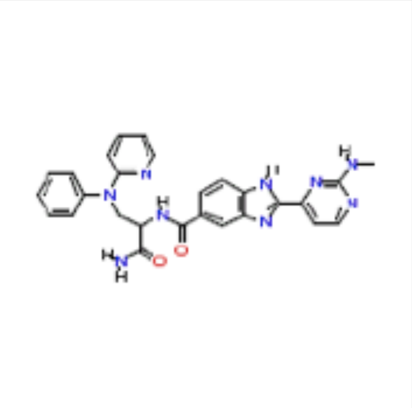

4307551 TEK pval: 9.00

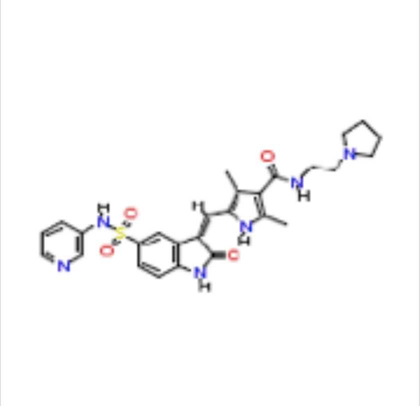


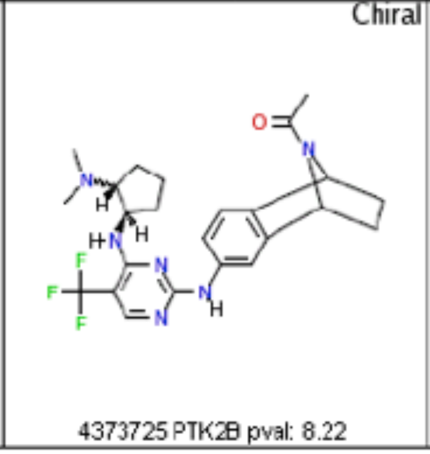
4363016 MAPK11 pval: 8.82


4343448 ROCK1 pval: 8.74

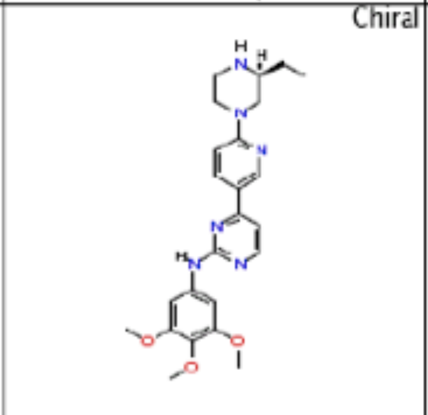

4363247 MAPKAPK2 pval: 8.70


4291996 IKB pval: 8.70

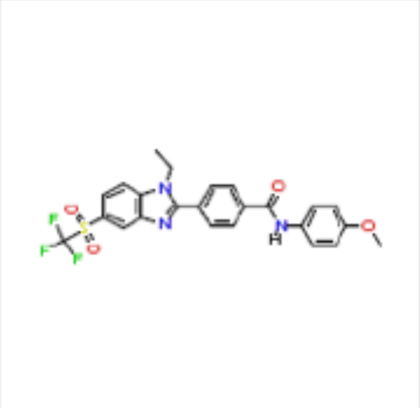

4208857 FAK2 pval: 8.22

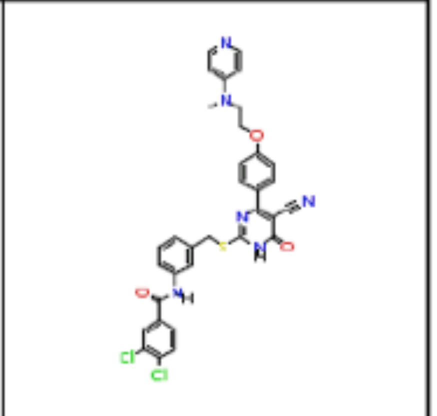


4373725 PTK2B pval: 8.22

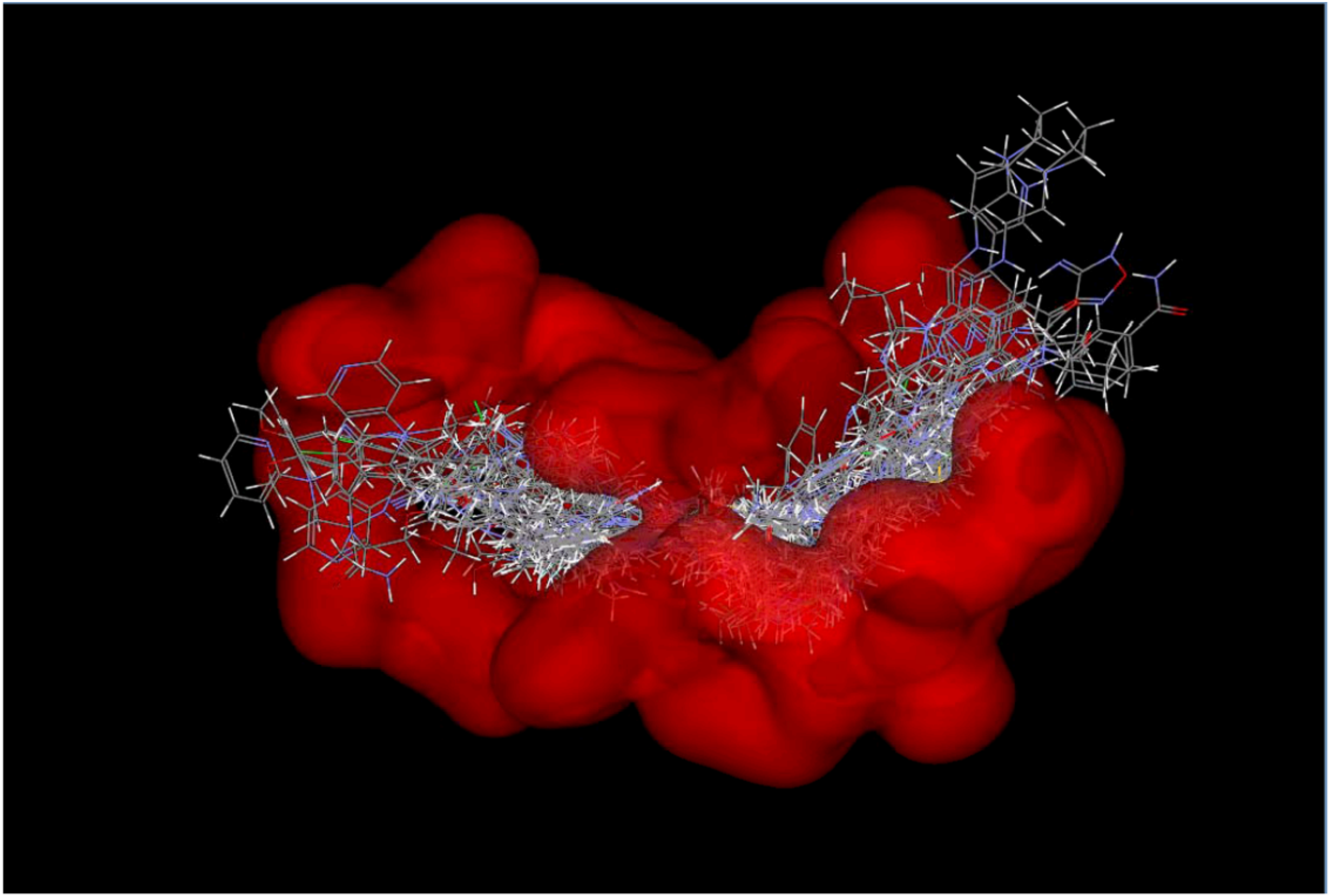


1788 ZAP70 pval: 8.10

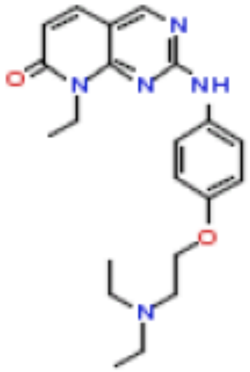

2425813 PTPN9 pval: 5.96


4303129 MAP3K2 pval: 4.70

Potent Kinase Inhibitors Docked (s1309707)



Added Diversity LigandCross Validation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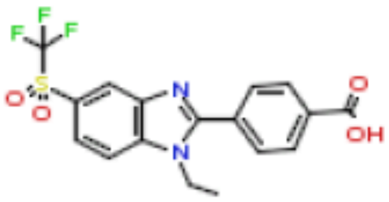
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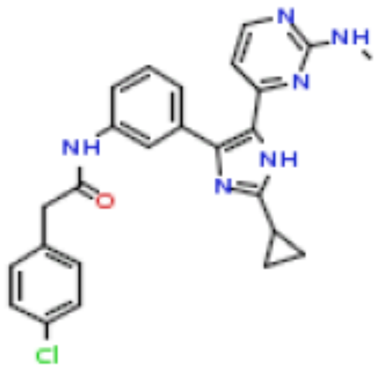
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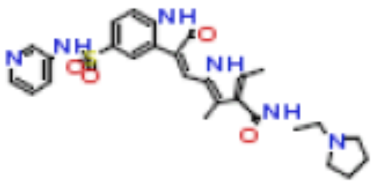
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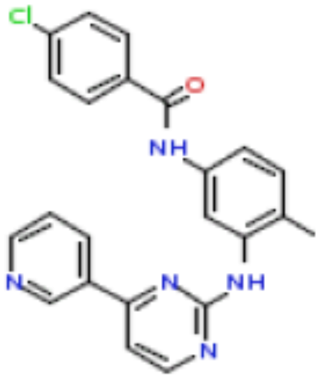
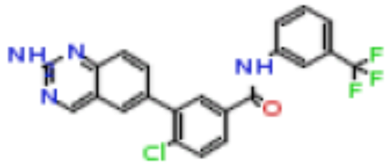

900_STI_1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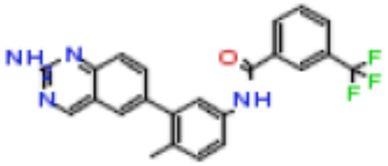
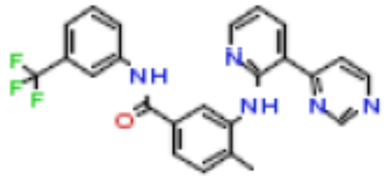

242_MUH_1


242_MUH_2


406_STI_1

4343448_809_27:
CDK4: 6.80 CDK2: 5.63 CDK2: 6.12 CDC2: 5.58 CSK: 5.99 CDK5: 6.81 CDK4: 6.80 CDK2: 5.63 CDK2: 6.12 CDC2: 5.58 CDK4: 6.80

4272835_2425813_23:
PTPN1: 4.24 PTPRA: 4.21

4363734_4291996_2:
RAF1: 9.00 MAPK1: 5.29 BRAF: 8.05 BRAF: 8.52

4208857_4208857_1:
FAK2: 8.22 KDR: 5.86 PDGFRB: 4.90 EGFR: 4.17 ERBB2: 5.23

900_STI_1:
PDGFR: 8.00 PDGFR: 8.00 ABL: 6.10 PDGFRB: 8.00 PDGFR: 8.00 ABL: 6.10

242_A96_5:
LCK: 9.40

242_MUH_1:
LCK: 9.40 TEK: 7.68 KDR: 8.22 MAPK14: 9.00 JAK3: 6.81

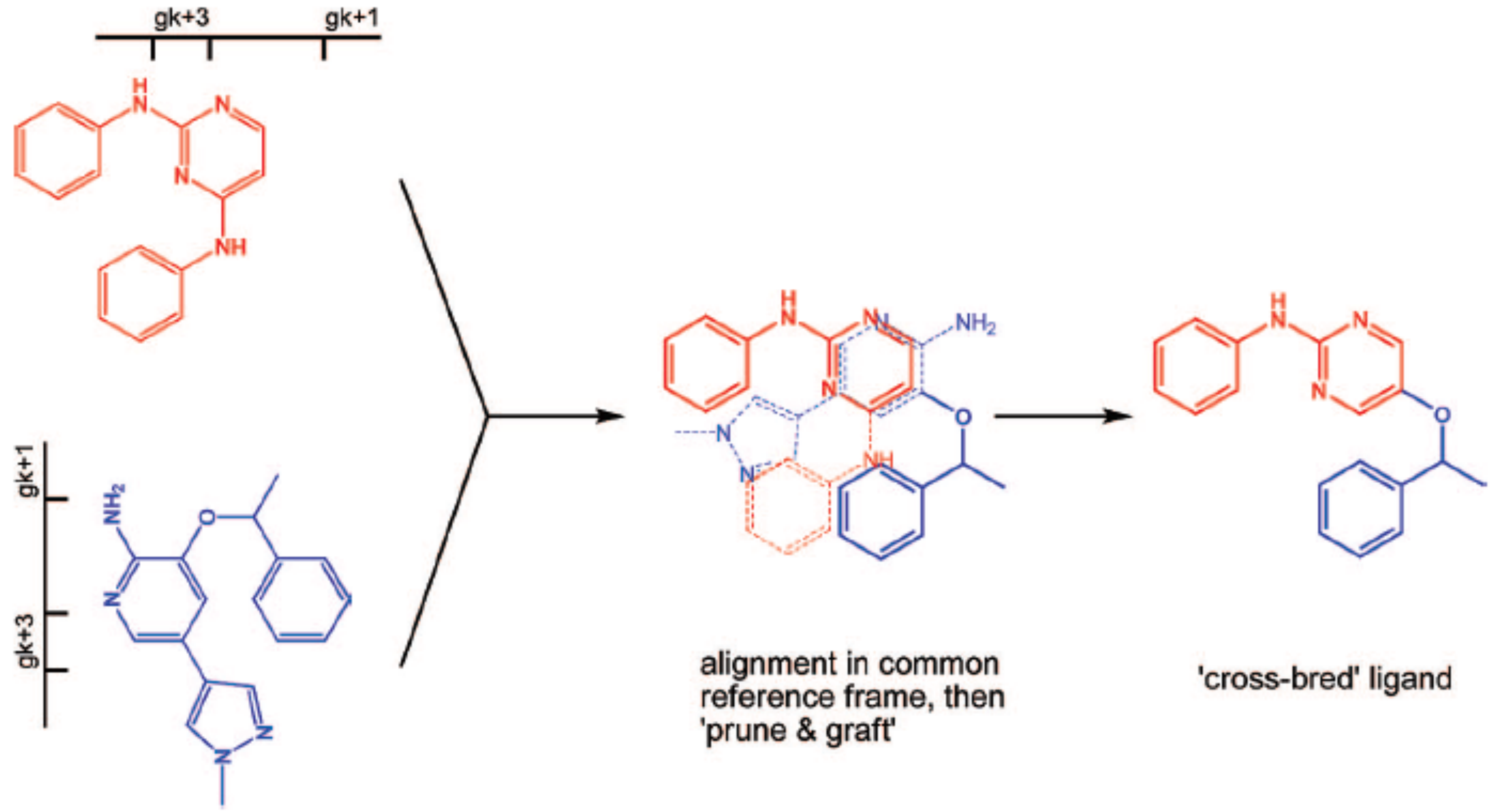
242_MUH_2:
KDR: 8.40 TEK: 8.40 TEK: 8.40 KDR: 8.40 TEK: 8.40 KDR: 8.40

406_STI_1:
BCR_ABL: 8.40 BCR_ABL: 5.30 LYN: 8.06 ABL1: 8.07 ABL1: 8.40

Generation of Cross-Bred Kinase Ligands

Arup K. Ghose *et al. J. Med. Chem*. 2008, 51, 5149-5171

1. Align all relevant PDB kinases on a reference kinase structure.
2. Optional step: ‘Refine’ the binding poses of the ligands for the target kinase using any docking pose refinement program (e.g. Schrodinger: Glide/Refine).
3. Apply Ligand-Cross to generate first generation ligands.



alignment in common reference frame, then 'prune & graft'

'cross-bred' ligand

Before alignment

A Few Useful Comments on Using LigandCross

- A few steps of steepest descent followed by conjugate gradient of the ligand in the protein binding site, often fixes distorted geometry problems.

- Use both Type-I and Type-II ligands.

- Use aligned DFG-in kinase structure/model during minimization and binding mode evaluation for generating Type-I ligands.

- Use aligned DFG-out kinase structure/model during minimization and binding mode evaluation for generating Type-II ligands.

- The method was successfully used to design novel Type-I and Type-II ligands for ALK, JAK2, FAK and TAM/RTK kinases.

- For further information contact Arup K. Ghose (akghose@msn.com)

Conclusions

- The structurally resolved and modelable proteome is a very rich source for new matter ideas

- Receptor-site similarity can rapidly identify “feed-stock” functionality for favorable ligand decoration

- LigandCross can be an effective approach to generate novel, bioactive matter using co-complexes, known inhibitors, and/or fragment-based information.

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iKinasePro

iProtein

Mobile Reagents

Reaction101

Yield101

SPRESImobile

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