Using Receptor-Site and Protein Structural Similarity to Generate New Matter Ideas

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Motivation

From: Individual biological target → “Selective” compounds
To: Target combinations → Multi-target compound (combinations)

➢ Opportunity Space:
  - Chemically tractable target combinations
  - Structural bioinformatics → first order assessment of likely selectivity and promiscuity with a protein family

~2000’s – Network pharmacology / systems chemical biology (polypharmacology, chemically tractable target combinations)

~80’s – Validated biological targets; selective modulators (molecular biology, HTS, combinatorial chemistry etc.)

~50’s – Synthetic compounds phenotypic effect (animal testing)

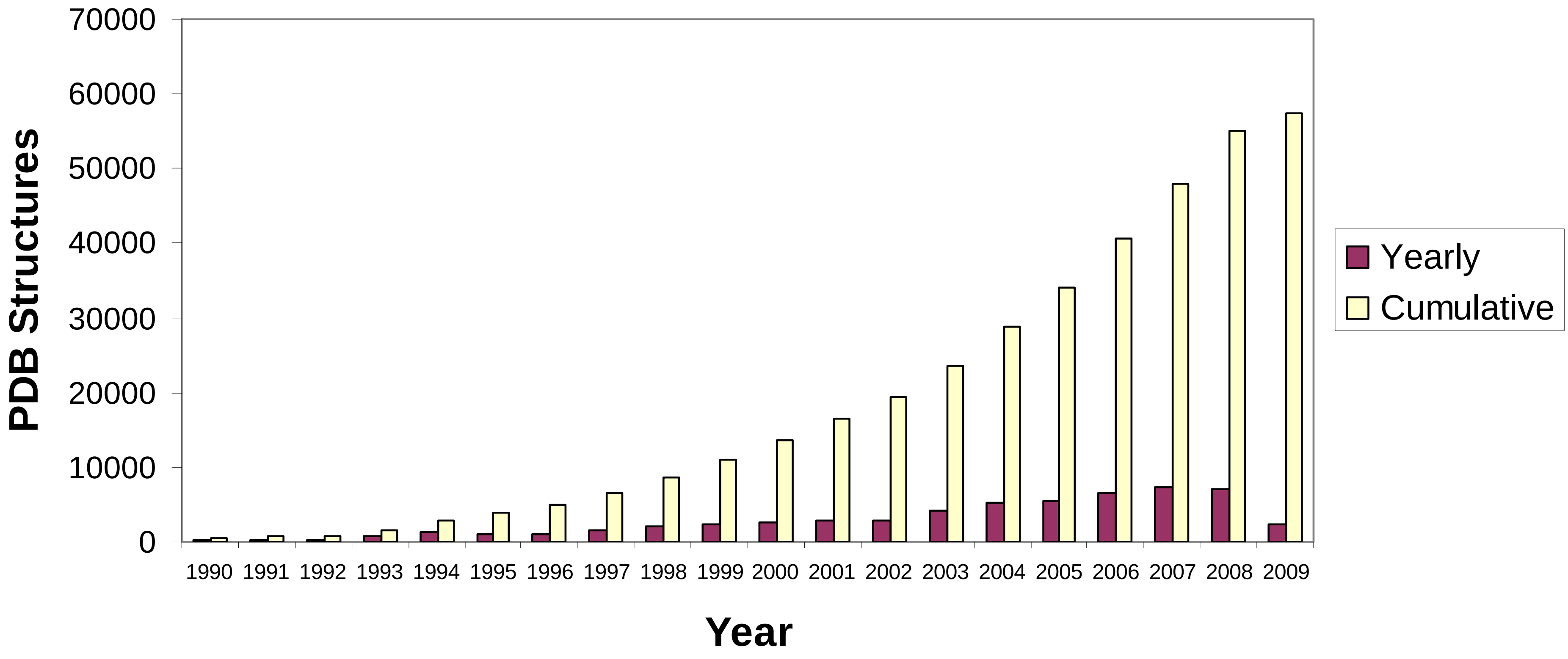
~1900’s – Identification (synthesis) of active natural components

~800BC – Natural extracts’ observed effect (traditional human experience)

Protein Structure Growth Continues

> 50K Structures/co-complexes (Apr-2008)
> 600 deposits per month → **>150/week!**

PDB Growth
source: rcsb.org

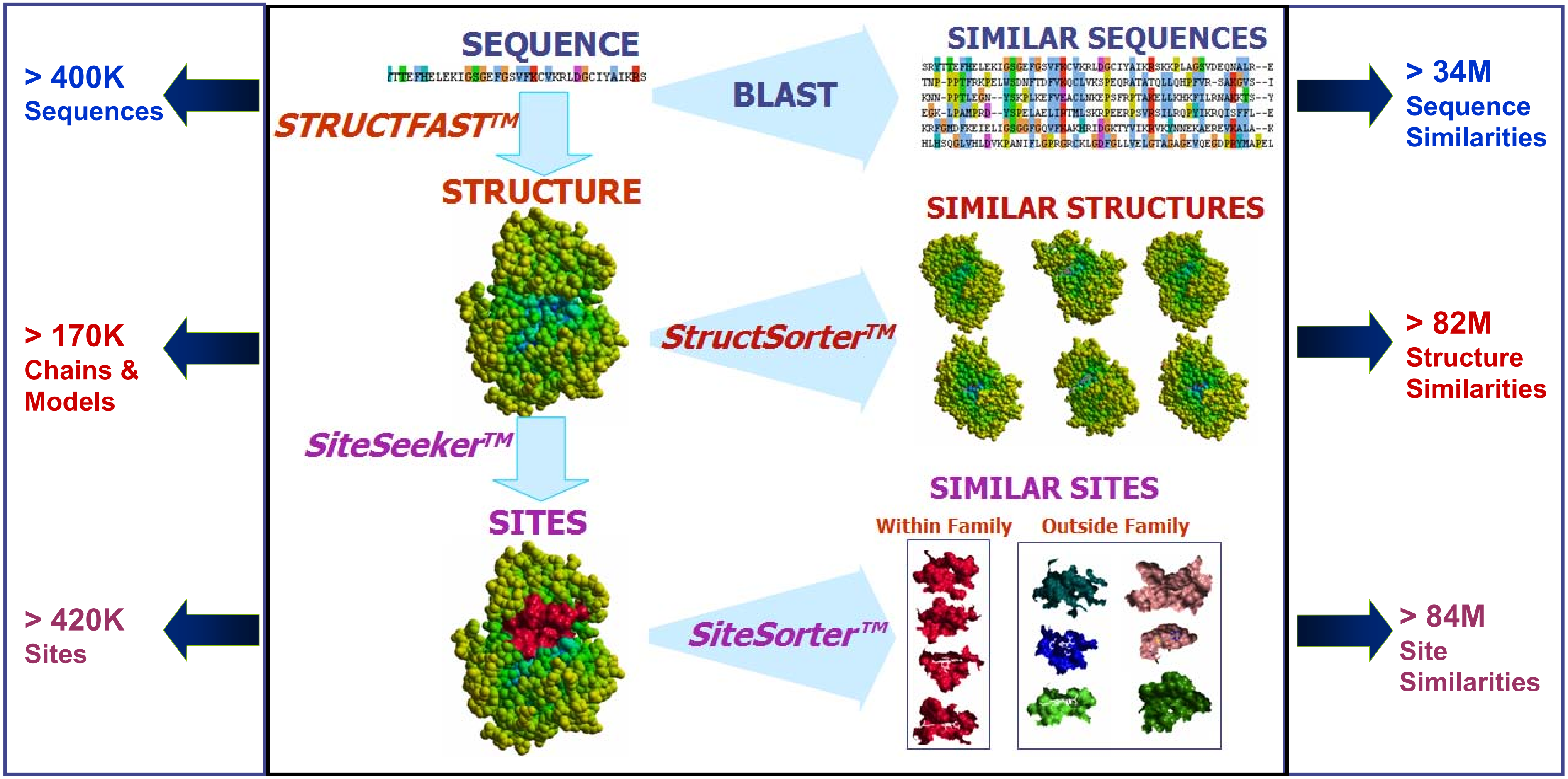


Drugs Developed using Structural Knowledge

Inhibitor/Drug	Disease	Company(s)	Protein targeted	Enzyme Family
STI-571/Gleevec	Chronic Myeloid Leukemia	Novartis	c-Abl kinase	Tyrosine kinase
Fluoroquinolone/Ciprofloxacin	Bacterial infection	Bayer	Gyrase	ATP Hydrolase
Saquinavir/Invirase, Ritonavir/Norvir, Indinavir/ Crixivan, Nelfinavir/Viracept, Amprenavir/Agenerase, Fosamprenavir/Lexiva,	AIDS	Roche, Abbott, Agouron, Merck, Vertex	HIV-1 Protease	Aspartylprotease
Trusopt	Glaucoma	Merck	Carbonic Anhydrase	Lyase
Thymitaq	Cancer	Agouron	Thymidylate synthase	Methyl transferase
Celecoxib/Celebrex, Rofecoxib/Vioxx	Inflammation, rheumatoid arthritis	Searle, Merck	Cox-2	Oxidoreductase
AG3340/Prinomastat	Cancer	Agouron	Matrix metalloprotease	Metalloprotease
Oseltamivir phosphate/Tamiflu, Zanamivir/Relenza	Influenza	Roche	Neuraminidase	Glycosidase

Source: http://www.active-sight.com/science/sbdd.html

TIP Content and Algorithm Engine



> 400K Sequences

> 170K Chains & Models

> 420K Sites

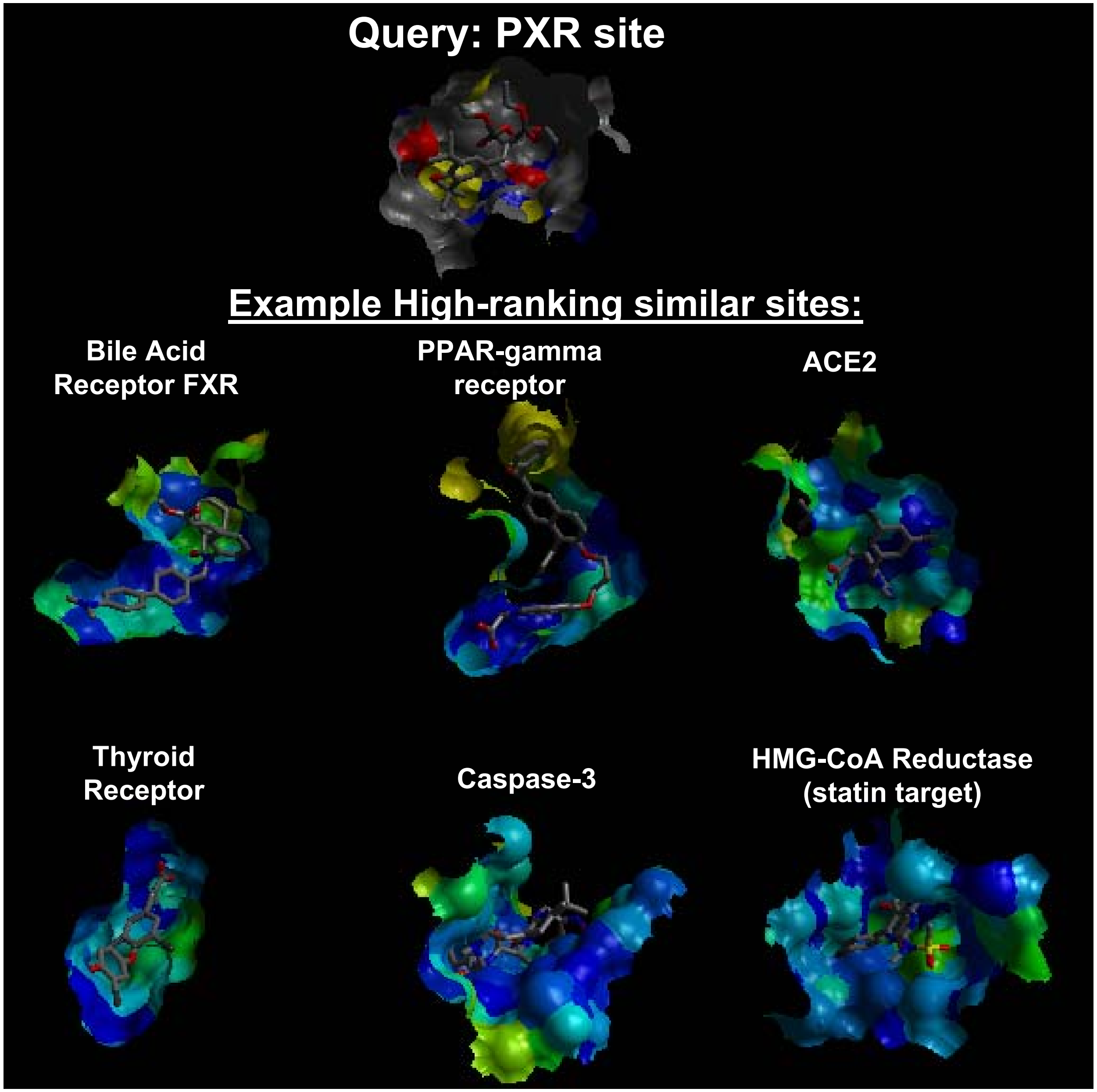
> 34M Sequence Similarities

> 82M Structure Similarities

> 84M 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.**

Nature Exploits Site Similarity...



Query: PXR site

Example High-ranking similar sites:

Bile Acid Receptor FXR

PPAR-gamma receptor

ACE2

Thyroid Receptor

Caspase-3

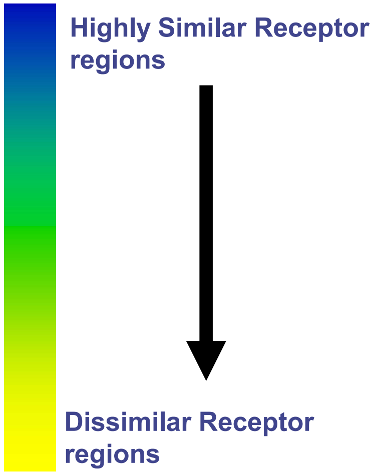
HMG-CoA Reductase (statin target)

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-ãantagonist, xenobiotics and endobiotics…

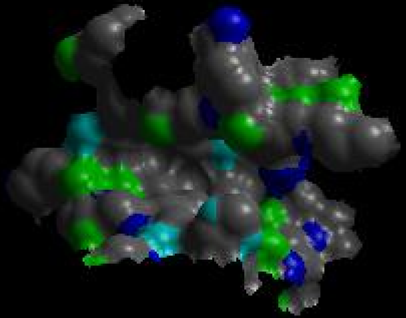
<u>Site Similarity Coloring</u>



Eidogen-Sertanty

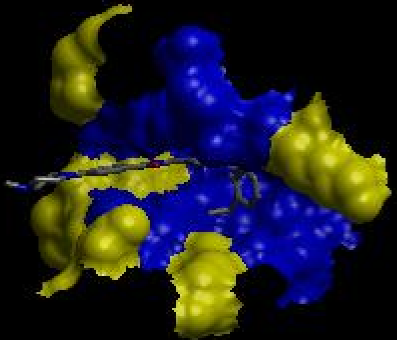
Borrowing Matter Ideas using Site Similarity

LIMK1



🟦 - H-bond donors
🟩 - H-bond acceptors
🟦 - H-bond donor/acceptors

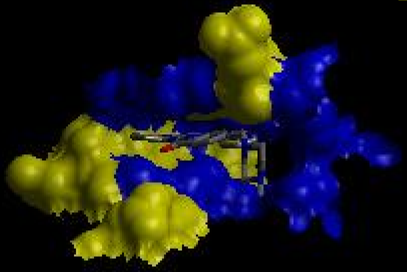
🟦 - Conserved with LIMK1
🟨 - Not conserved with LIMK1



AURKA

62% ID in ATP Site

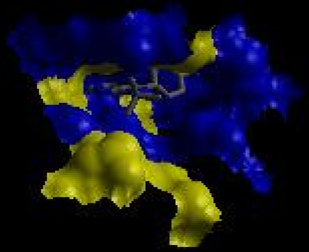
>3000 inhibitors in KKB



SRC

58% ID in ATP Site

>5100 inhibitors in KKB



LCK

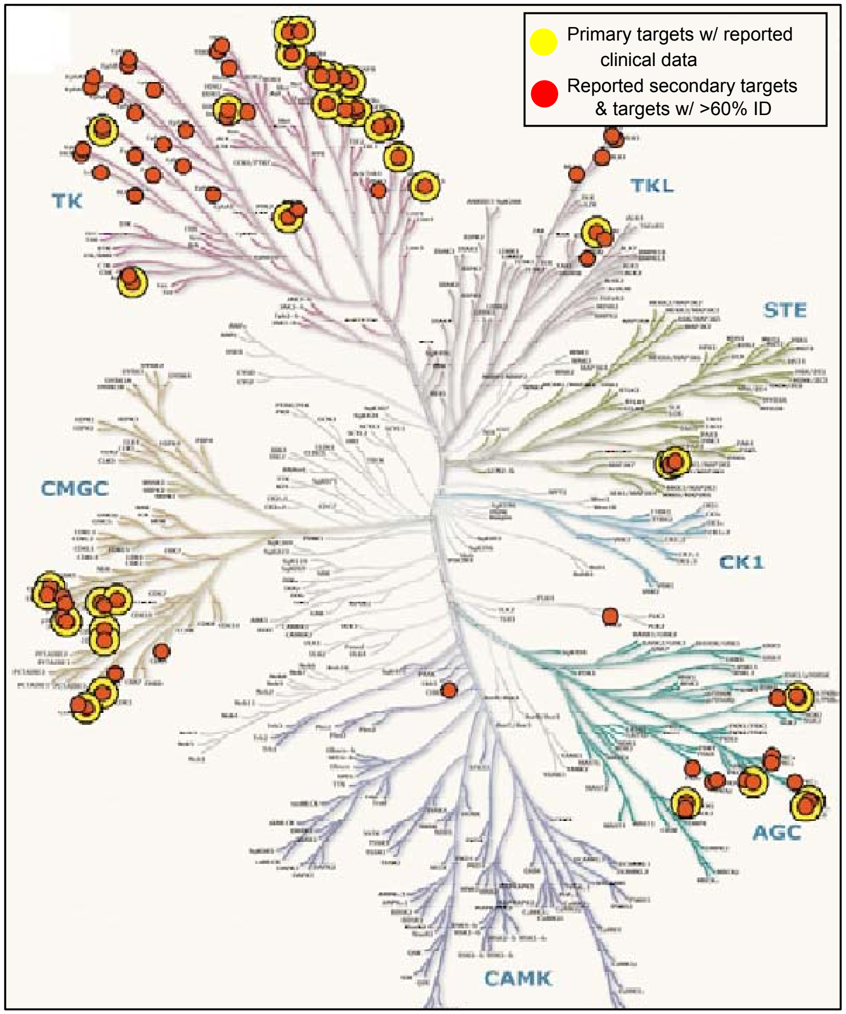
58% ID in ATP Site

>8200 inhibitors in KKB

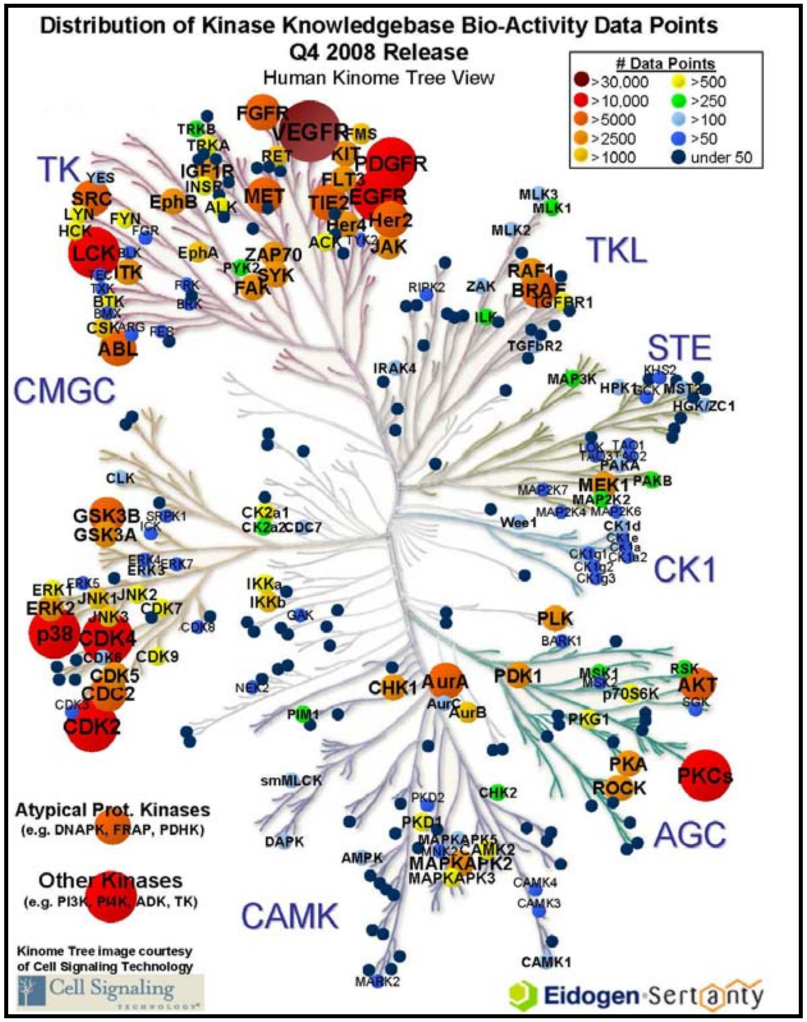
Kinase SAR Knowledgebase (KKB) – Hot Targets

Kinase Targets of Clinical Interest

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



Eidogen-Sertanty KKB
SAR Data Point Distribution



> 384,000 SAR data points curated from
> 5100 journal articles and patents

Kinase Knowledgebase (KKB)

Kinase inhibitor structures and SAR data mined from

- **> 4100 journal articles/patents**

Kinase Validation Set

Three sizable datasets freely available to the research community

http://www.eidogen-sertanty.com/kinasednld.php

Average **20-30K** unique structures added per quarter

Multi-Kinase Inhibitors

Nature Reviews | Drug Discovery   Vol 8 | February, 2009

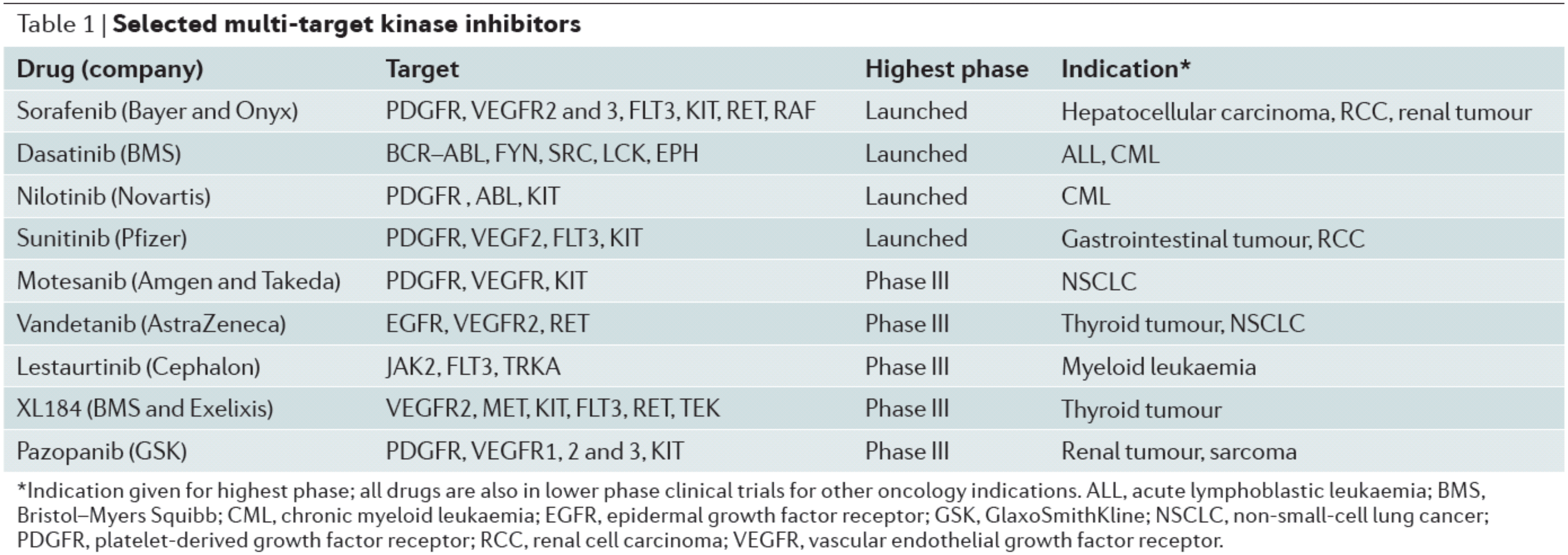


Table 1 | **Selected multi-target kinase inhibitors**

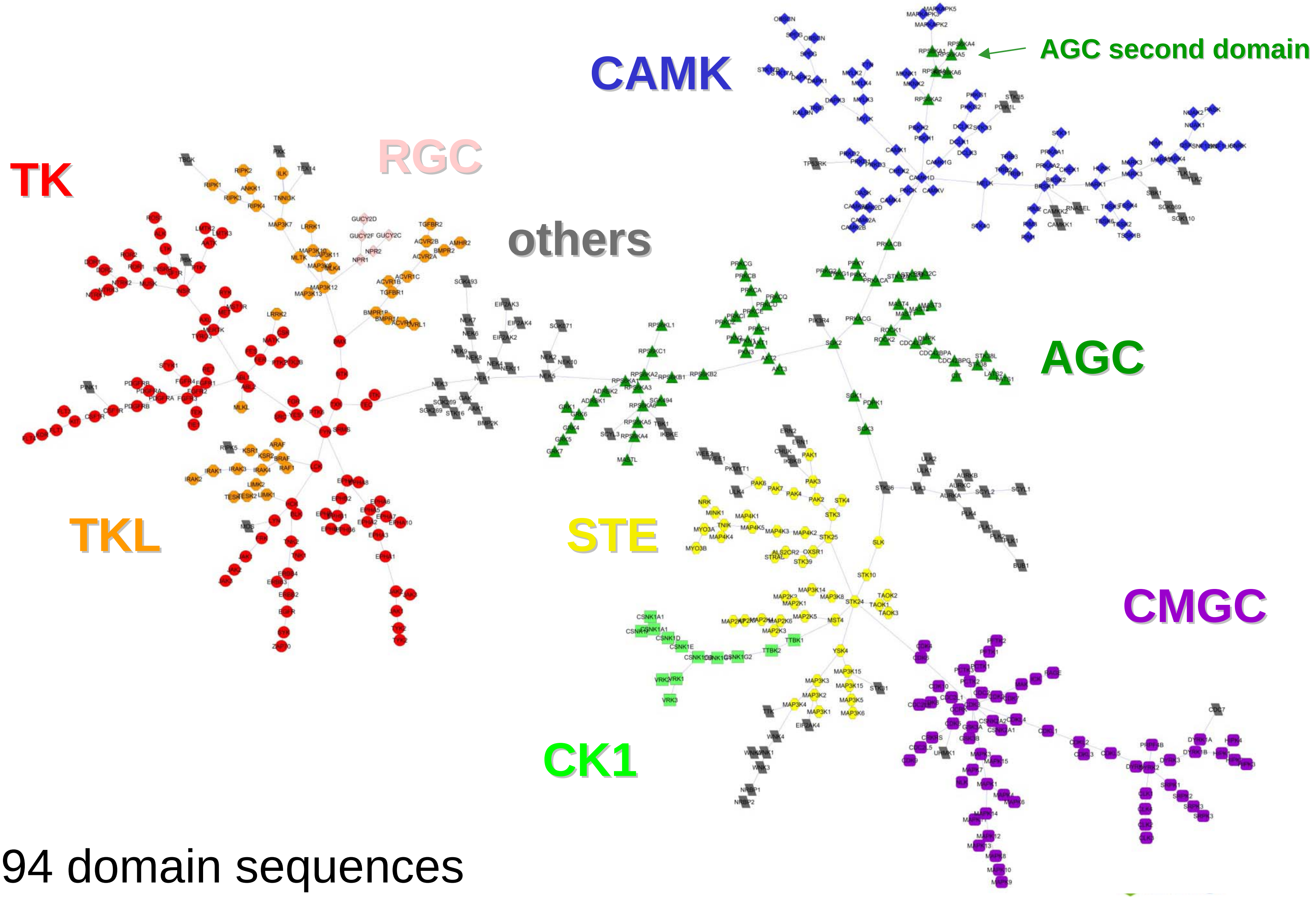
Drug (company)	Target	Highest phase	Indication*
Sorafenib (Bayer and Onyx)	PDGFR, VEGFR2 and 3, FLT3, KIT, RET, RAF	Launched	Hepatocellular carcinoma, RCC, renal tumour
Dasatinib (BMS)	BCR–ABL, FYN, SRC, LCK, EPH	Launched	ALL, CML
Nilotinib (Novartis)	PDGFR , ABL, KIT	Launched	CML
Sunitinib (Pfizer)	PDGFR, VEGF2, FLT3, KIT	Launched	Gastrointestinal tumour, RCC
Motesanib (Amgen and Takeda)	PDGFR, VEGFR, KIT	Phase III	NSCLC
Vandetanib (AstraZeneca)	EGFR, VEGFR2, RET	Phase III	Thyroid tumour, NSCLC
Lestaurtinib (Cephalon)	JAK2, FLT3, TRKA	Phase III	Myeloid leukaemia
XL184 (BMS and Exelixis)	VEGFR2, MET, KIT, FLT3, RET, TEK	Phase III	Thyroid tumour
Pazopanib (GSK)	PDGFR, VEGFR1, 2 and 3, KIT	Phase III	Renal tumour, sarcoma

*Indication given for highest phase; all drugs are also in lower phase clinical trials for other oncology indications. ALL, acute lymphoblastic leukaemia; BMS, Bristol–Myers Squibb; CML, chronic myeloid leukaemia; EGFR, epidermal growth factor receptor; GSK, GlaxoSmithKline; NSCLC, non-small-cell lung cancer; PDGFR, platelet-derived growth factor receptor; RCC, renal cell carcinoma; VEGFR, vascular endothelial growth factor receptor.

Imatinib (Gleevec: Novartis)	**ABL, PDGFR, KIT**	**CML, GIST**
Gefitinib (Iressa: Astra Zeneca)	**EGFR, (ERBB4,GAK,…)**	**NSCLC**

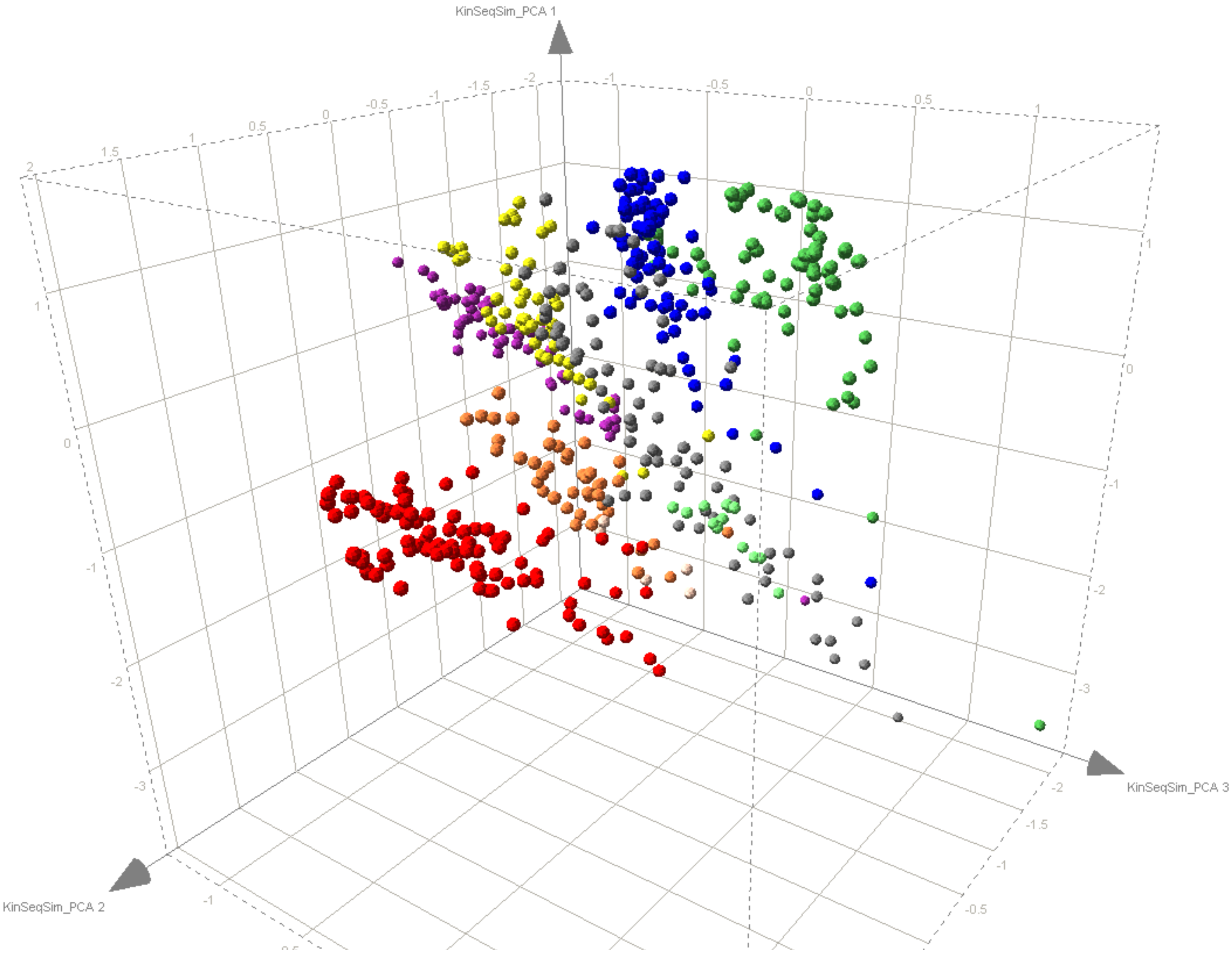
Kinome by Sequence Similarity

Kinase Domain Sequence Similarities - MST



494 domain sequences

PCA View – All Pairwise Similarities



494 domain sequences; 3 PCA dimensions preserve 61 % variability

Kinase Target Similarities by SAR

Extracting Kinase Data Sets

- Only enzymatic (homogeneous) assays with defined target
- Only high quality data (IC50, Ki, Kd)
- Standardizing chemical structures (salt forms, stereochemistry, E/Z geometry, tautomers, ionization)
- Kinase target Entrez Gene names and SwissProt accessions
- Aggregate data by structure first in an individual experiment and then globally by unique kinase and structure

- ➢ 189,119 unique (structure target) data points (366 kinases)
- ➢ 93,121 unique structures

Relating Kinase Targets by Compound Activity

- “ACTivity similarity” for compounds tested in common - which are active for one (or both) target(s)

$$ACTsim_{ij} = 1 - \frac{1}{N}\sum_{k=1}^{N>2}\frac{\left|pIC50_{ki} - pIC50_{kj}\right|}{\max pIC50_{diff}}$$

Vieth et.al. “Kinomics” Biochim Biophys Acta **2004** 243

- Activity cutoff pVal ≥ 6.5; minimum 20 actives per kinase pair
- Compute Minimum spanning tree (Kruskal)
  - ➢ Visualization as network tree (Cytoscape)

Side note: “Activity fingerprint” (for a comprehensive activity matrix)

Bamborough et.al. J Med Chem **2008**, 7898

Relating Kinase Targets by SARsim ‘Features’

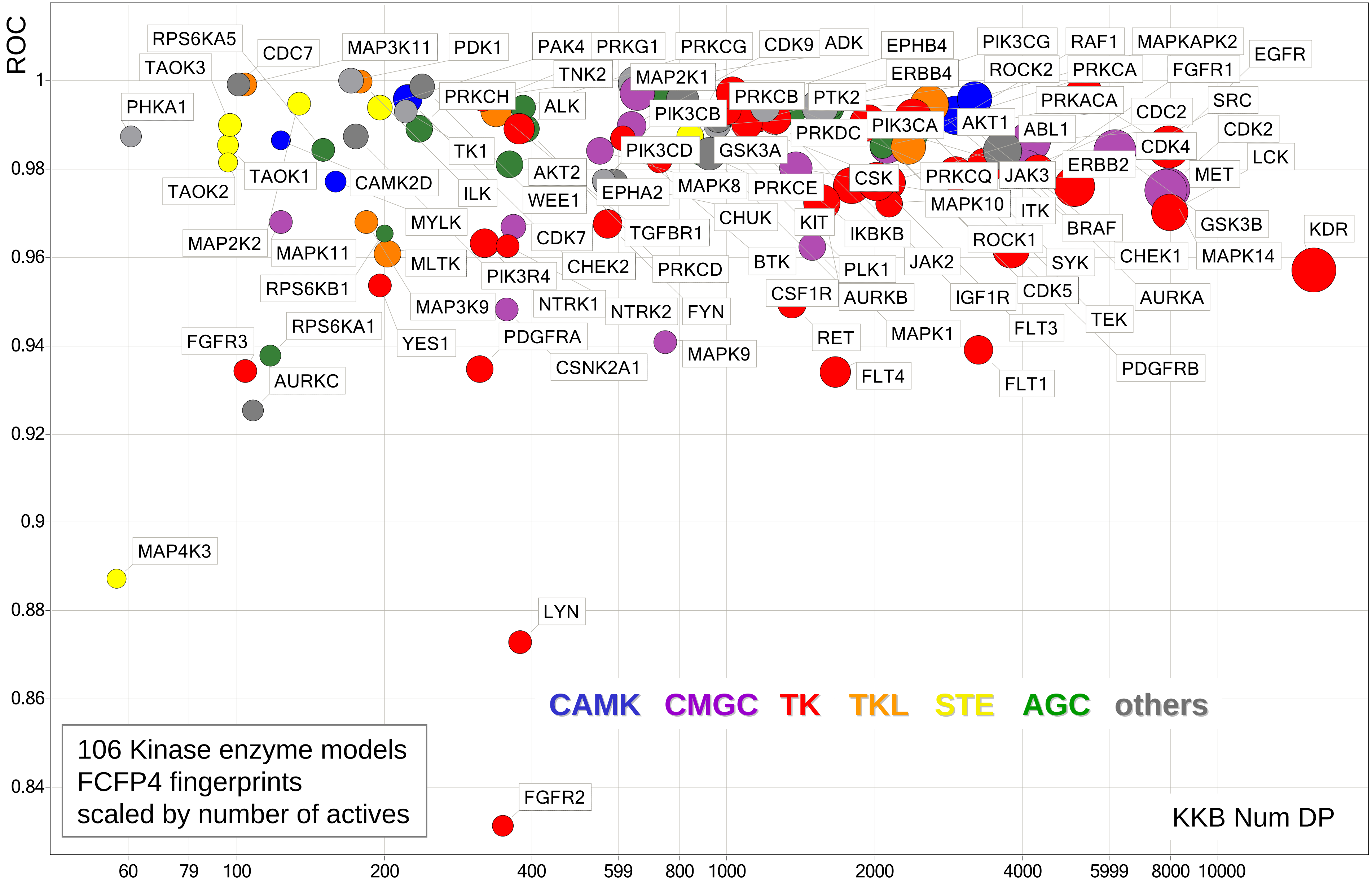
- Laplacien-modified Naïve Bayesian models using FCFP_4 fingerprints
  - Measure contribution of a bit in a fingerprint for a specific outcome
  - Assume all variables are independent
  - A compound is scored by summing the weights of its fingerprint bits

- Kinase models compared by the Pearson correlation coefficient of the vector of the probabilistic weights (log of Avidon weights) of all fingerprint bits

Adopted from Schuffenhauer *Org Biomol Chem* **2004** 3256

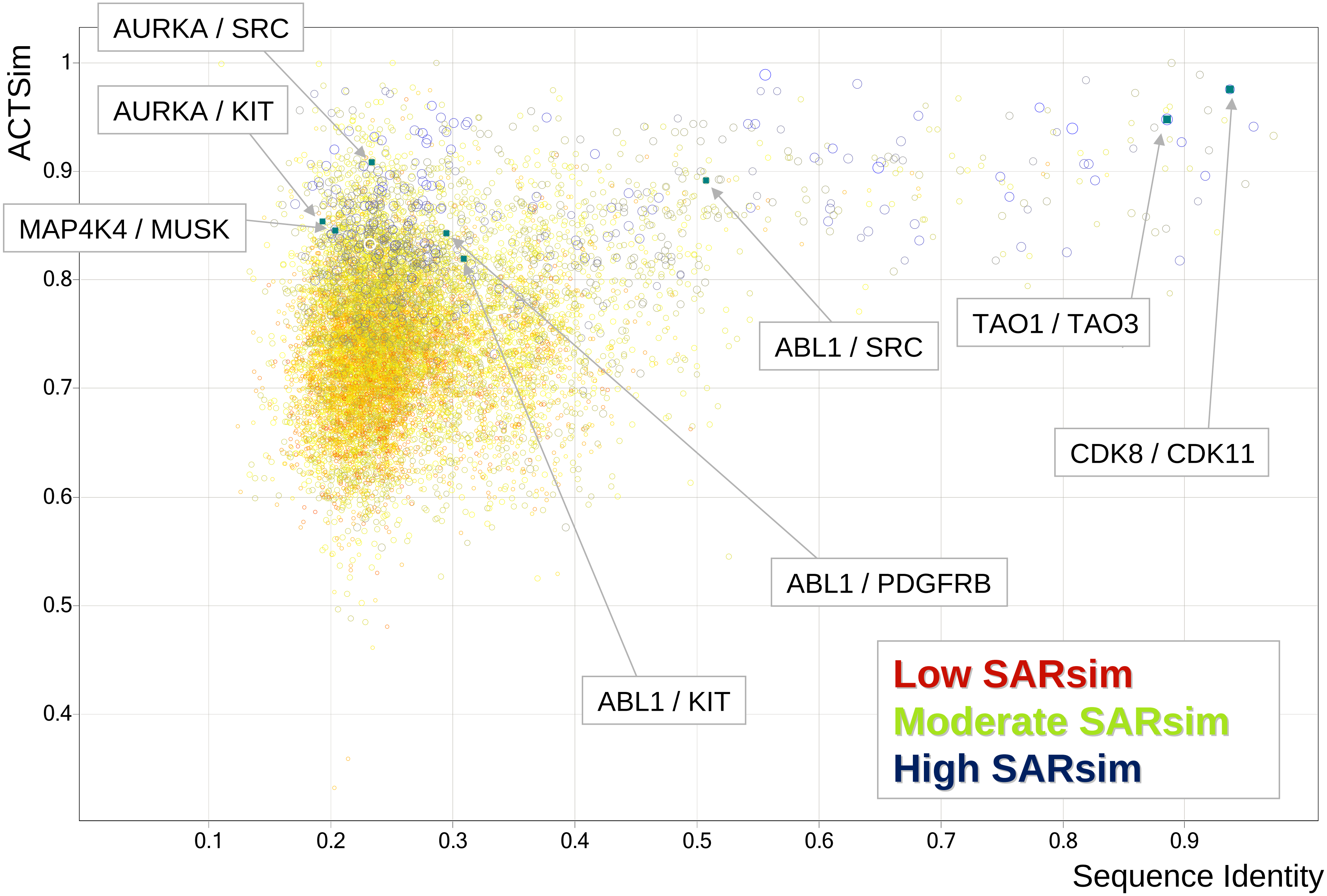
- Activity cutoff pIC50 > 6.5; all other compounds negative
- Select models with ROC > 0.8 and minimum 20 actives
- Compute the correlation matrix

Kinase SAR Naïve Bayse Models



106 Kinase enzyme models
FCFP4 fingerprints
scaled by number of actives

Kinase Target Similarity by ACTsim/SARsim



Kinase SAR-based Similarities – Summary

- Growing body of accessible kinase inhibition data facilitates a more comprehensive analysis of kinase polypharmacology
- Evolving picture, currently still a sparse kinase – inhibitor matrix
- SAR similarity analysis supports a global intuitive trend: the more similar a kinase the more likely to bind to the same compound
- Phylogenetic kinase tree breaks down in activity space; many examples of compounds that bind to “distant” kinases

- Bayesian models are robust and tolerant to noise and false positives
- Considering “features” maybe less sensitive to the gaps in the accessible data and has the potential to predict cross reactivity for novel compounds
- Fairly robust wrt activity cutoff and fingerprints used

- Be aware of limitations of descriptor-based statistical modeling
- No consideration of how a compounds binds (DFG-in/ -out)

- Small molecules can in many cases be optimized to differentiate between very similar (sequence) kinases in many cases

Kinome by Local Structural Binding Site Similarities (physicochemical)

Kinases Comparison by ATP Site Similarity

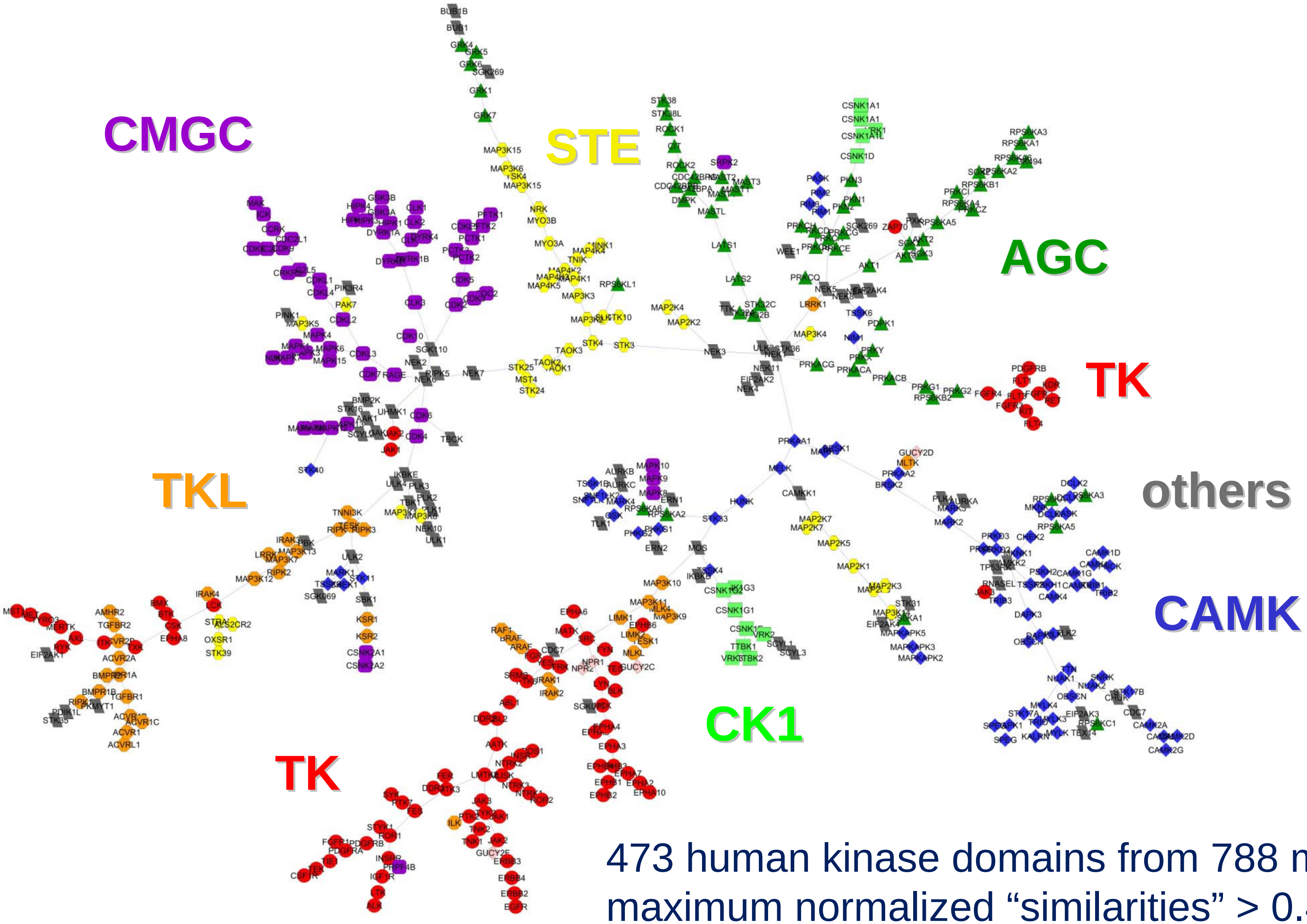
- Extract kinase domain sequences (Sugen, Swissprot, PFAM)
- Model almost the entire Kinome (501 sequences) using STRUCTFAST automated homology modeling (1,117 templates, > 5,000 models)

STRUCTFAST, Proteins **2006**, 960

- Define ATP binding sites for all models (homology and predicted)
- Compute binding site similarities
  - Define binding site amino acid features
  - Construct a graph: nodes are all corresponding features of the two sites; edges exist if the spatial distance of the a feature pair is similar between the two sites
  - Compute a complete sub-graph by clique detection (~100 solutions)
  - Overlay sites of the clique solution and sum up the corresponding surface areas
- Compute scores for all site pairs and each site for itself
- Normalize Tanimoto-like: AB_Norm := AB / (AA + BB - AB)
- Analyze and visualize (MST, PCA, hierarchical clustering)

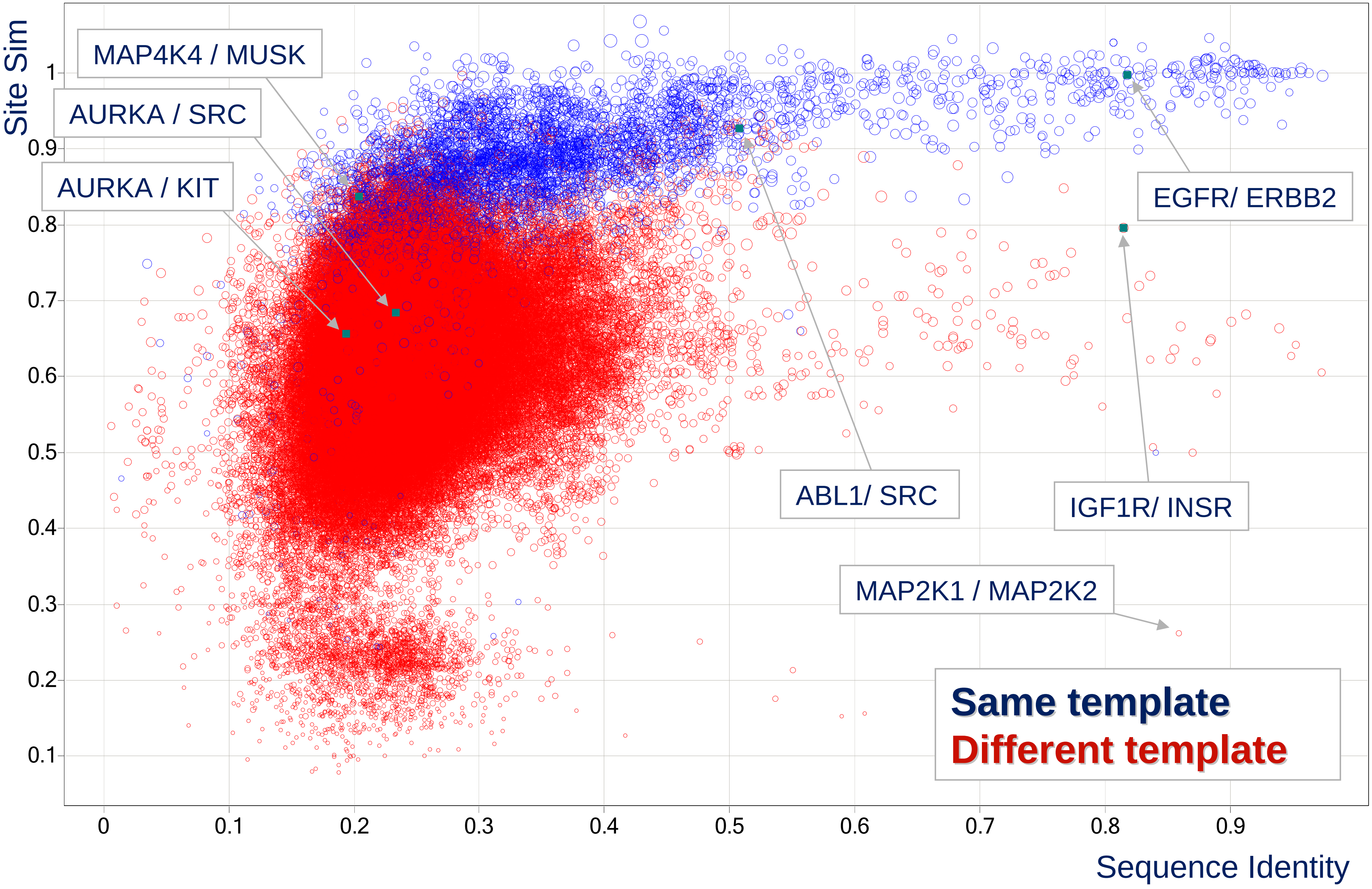
➢ Preliminary results reported (DFG-in only, homology sites only)

Maximum local site similarity – MST



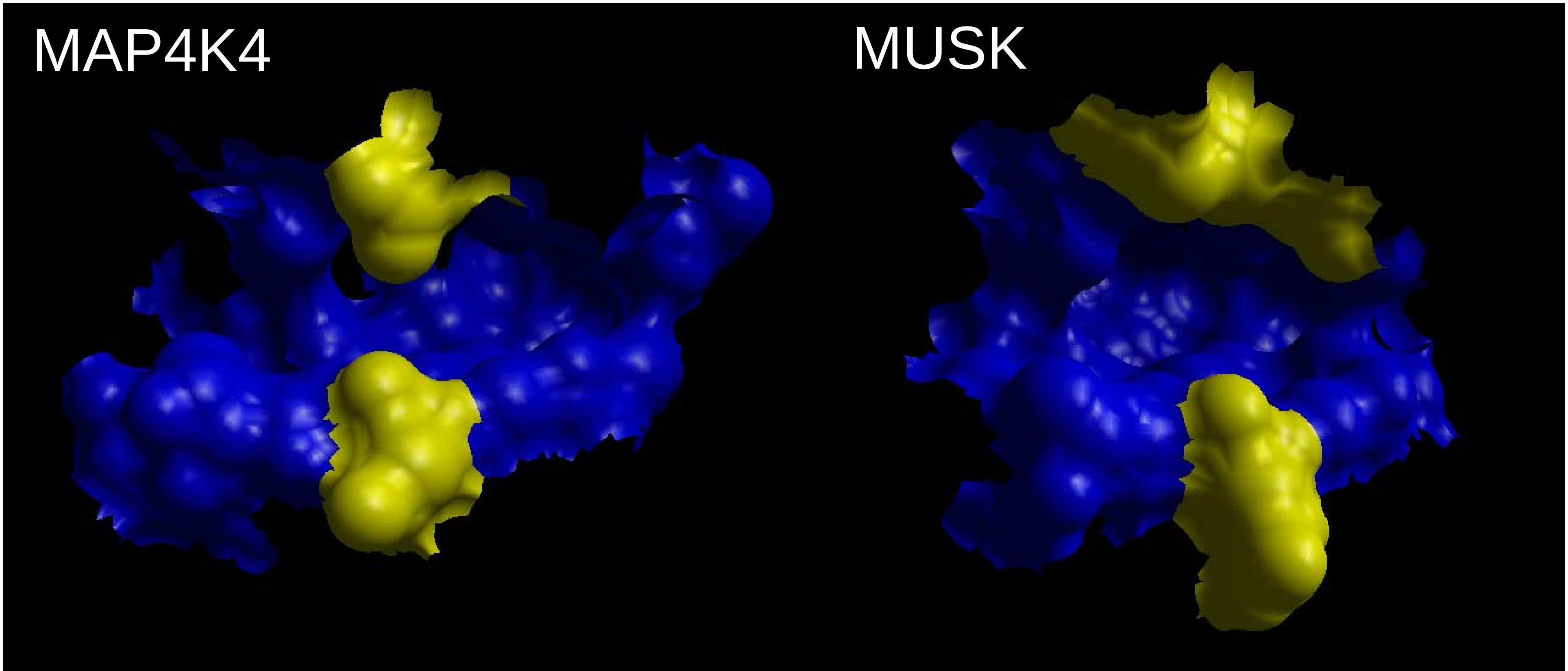
473 human kinase domains from 788 models
maximum normalized “similarities” > 0.45

PhysChem SiteSim vs. Domain Sequence Identity



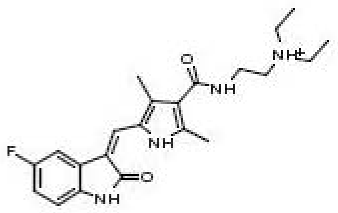
Example: PhysChem SiteSim vs. Domain Seq ID

- STE_STE2_HGK (MAP4K4): template 1u5rA
- TK_Musk_MUSK (MUSK) : template 1ir3A
- Full Sequence identity: 0.22 Site Sequence identity: 0.55
- Normalized (physicochemical) site similarity: 0.84

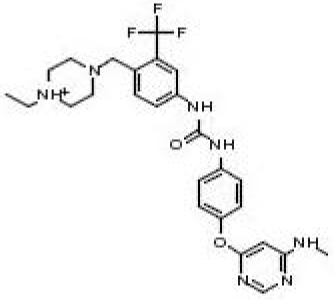


```
MAP4K4 .VGNGTY.V.A.K.M.E.A.MEFC.AGS.D.D.QN.L.D
  MUSK .IGEGAF V A K - E V FEYM -GD - N -N L D
```

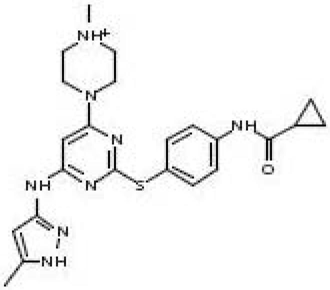
MAP4K4 and MUSK Small Molecule Inhibitors



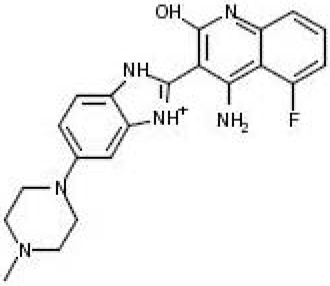
MAP4K4 / 6.9
MUSK / 6.3



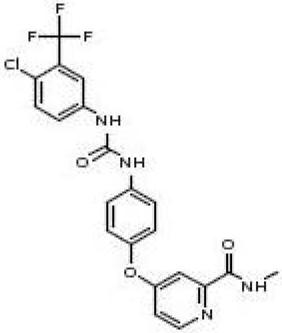
MAP4K4 / 7.8
MUSK / 8.5



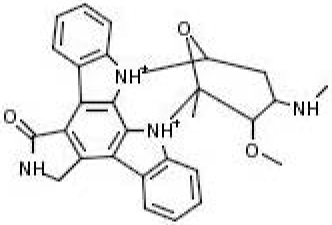
MAP4K4 / 5.6
MUSK / 6.5



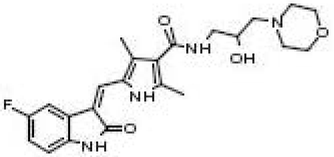
MAP4K4 / 6.8
MUSK / 6.1



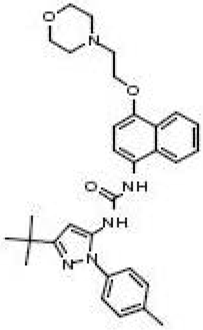
MAP4K4 / 5.3
MUSK / 6.9



MAP4K4 / 7.6
MUSK / 7.1

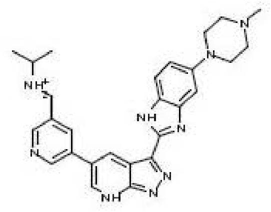


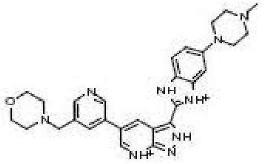
MAP4K4 / 6.6
MUSK / 7.0

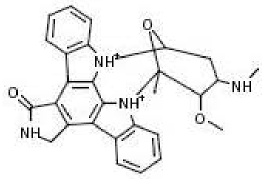


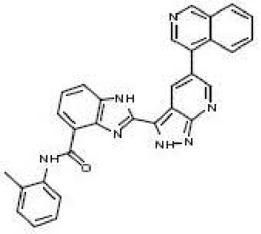
MAP4K4 / 7.0
MUSK / 5.7

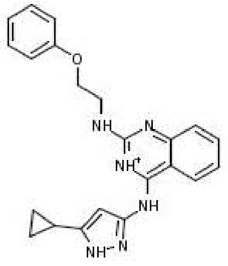
AURKA and SRC Kinase Dual Inhibitors

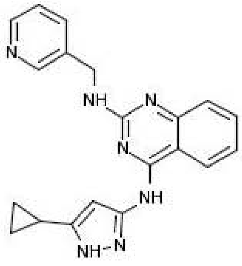

AURKA / 6.6
SRC / 8.2

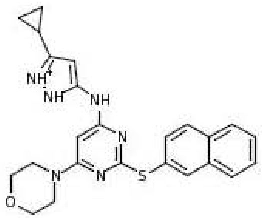

AURKA / 7.1
SRC / 8.3

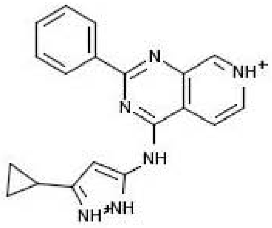

AURKA / 7.0
SRC / 7.8

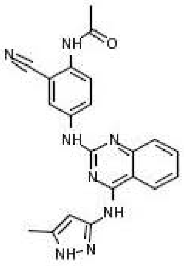

AURKA / 6.6
SRC / 8.9

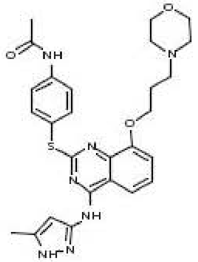

AURKA / 7.0 (min)
SRC / 7.0 (min)


AURKA / 7.0 (min)
SRC / 7.0 (min)


AURKA / 7.0 (min)
SRC / 7.0 (min)


AURKA / 7.0 (min)
SRC / 7.0 (min)


AURKA / 7.0 (min)
SRC / 6.5 (est)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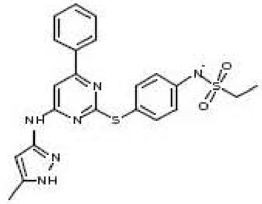
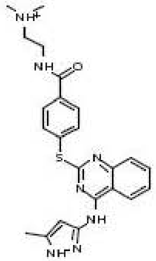
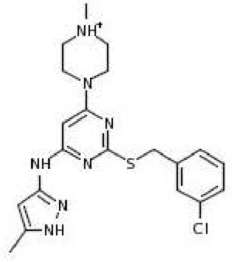
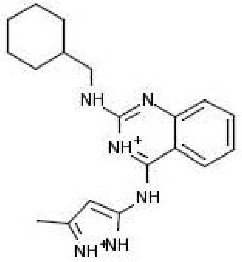
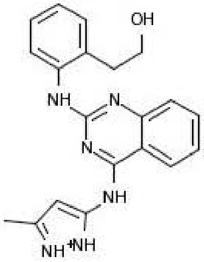
SRC / 6.5 (est)

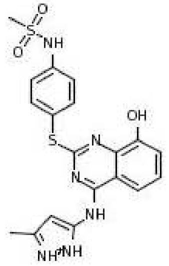

AURKA / 7.0 (min)
SRC / 6.5 (est)

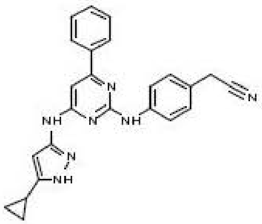

AURKA / 6.5 (est)
SRC / 6.5 (est)

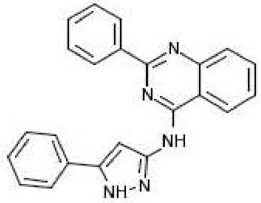

AURKA / 7.0 (min)
SRC / 7.0 (min)


AURKA / 7.0 (min)
SRC / 7.0 (min)


AURKA / 6.5 (est)
SRC / 6.5 (est)


AURKA / 7.0 (min)
SRC / 6.5 (est)


AURKA / 7.0 (min)
SRC / 7.0 (min)


AURKA / 6.5 (est)
SRC / 6.5 (est)

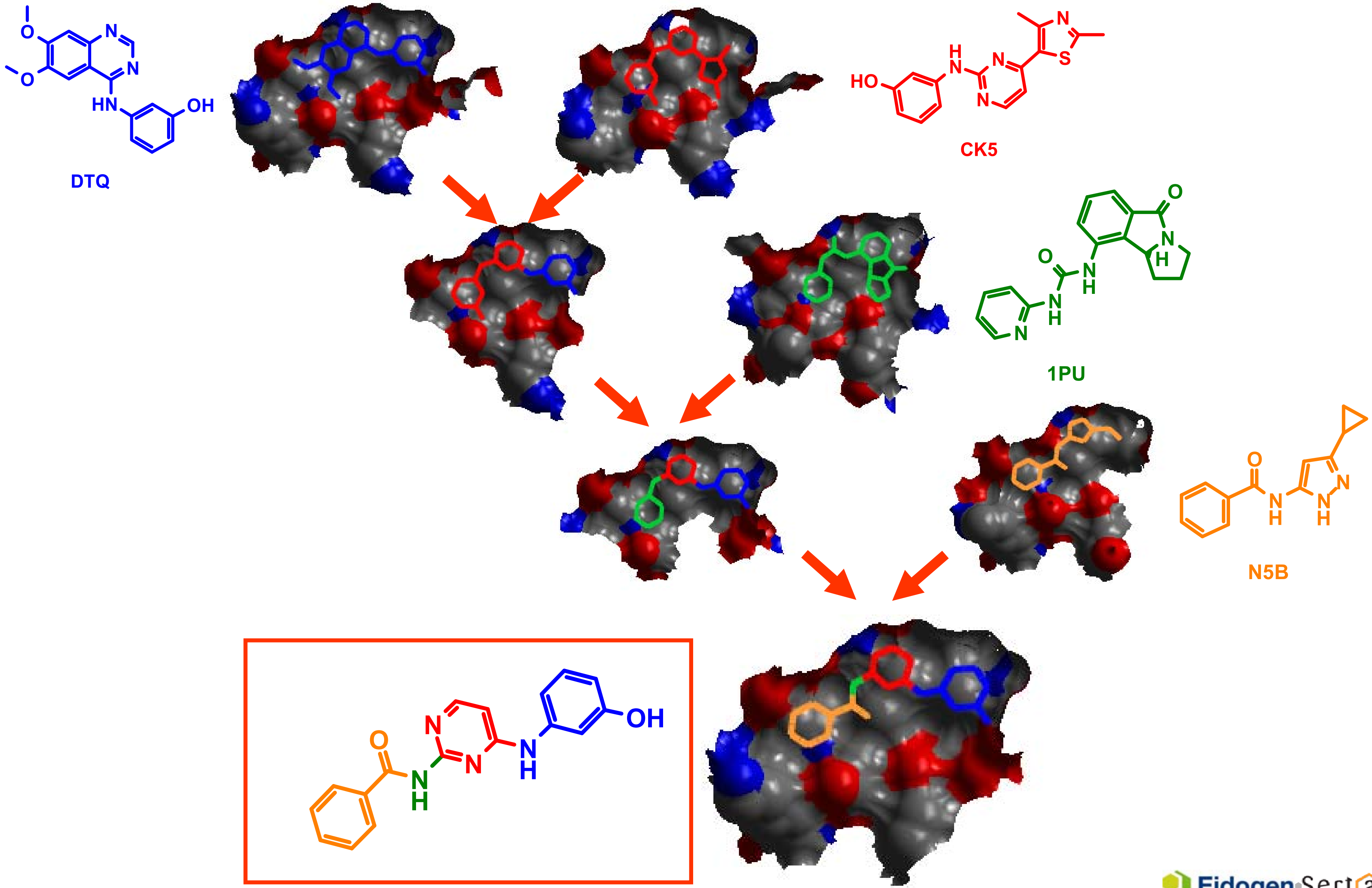
diverse subset

Kinome Site Similarities – Summary

- Relating kinases by local binding site similarity may be meaningful for development of selective inhibitors or compounds with desired profiles
- Many experimental examples confirm the validity of this approach
- Results suggest an expected global trend that similar sequence results in structural- and physicochemical- similar binding sites
- Dissimilar sequences do not always result in different binding sites
- There are subtle differences in the kinase site relationships among groups and sub-types
- Strong template effect
  - only homology sites (from co-crystal templates) are used in the present analysis (similarities using entire solvent accessible ATP sites)
  - for many kinases no experimental structures exist, but they can be modeled
- Although almost all kinases are modelable; experimental coverage and quality of structures will likely influence results
- Growing body of structural information will optimize this picture (in particular co-crystal structures)

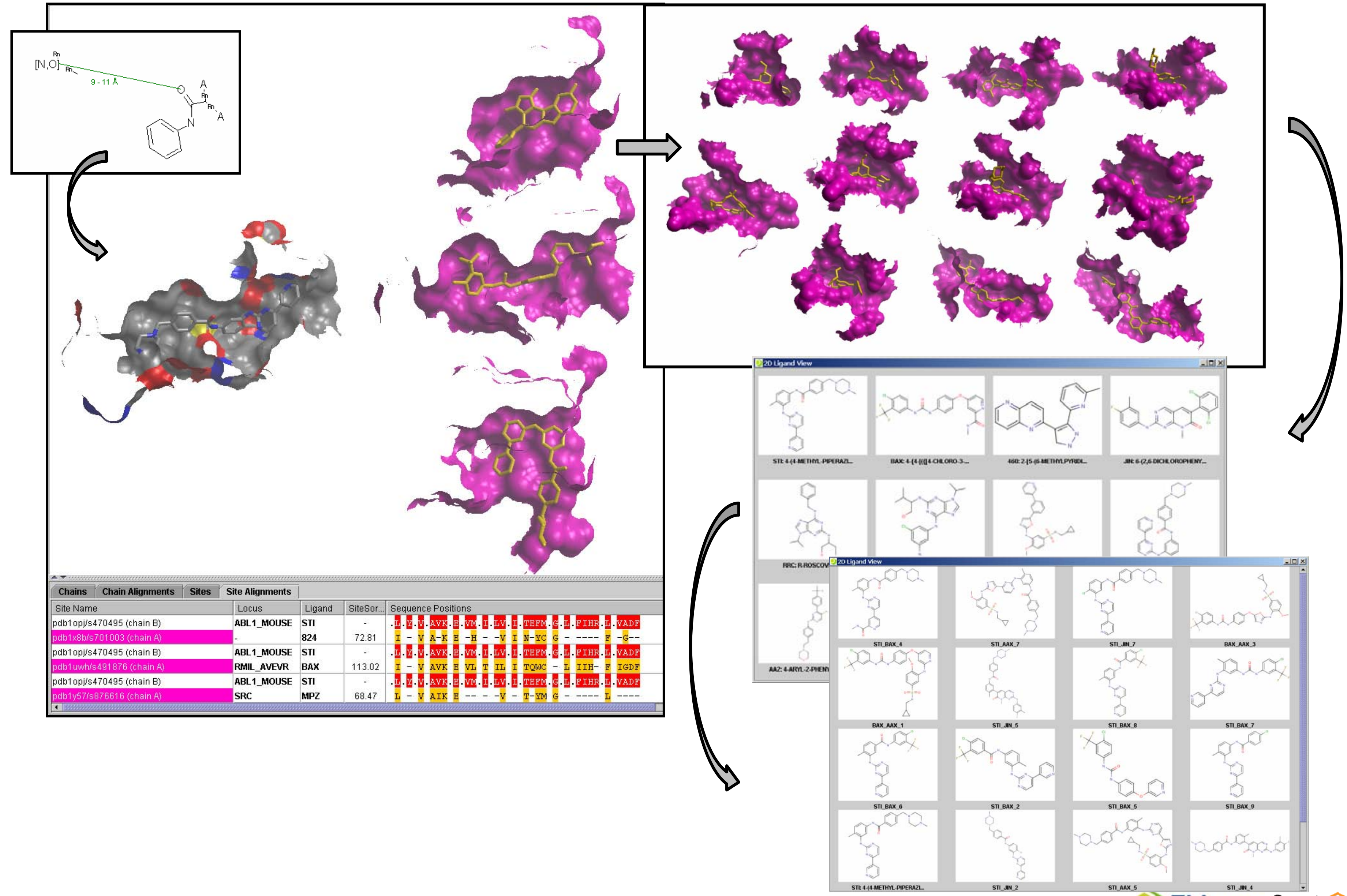
LigandCross: Shuffling Ligand Functionality

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



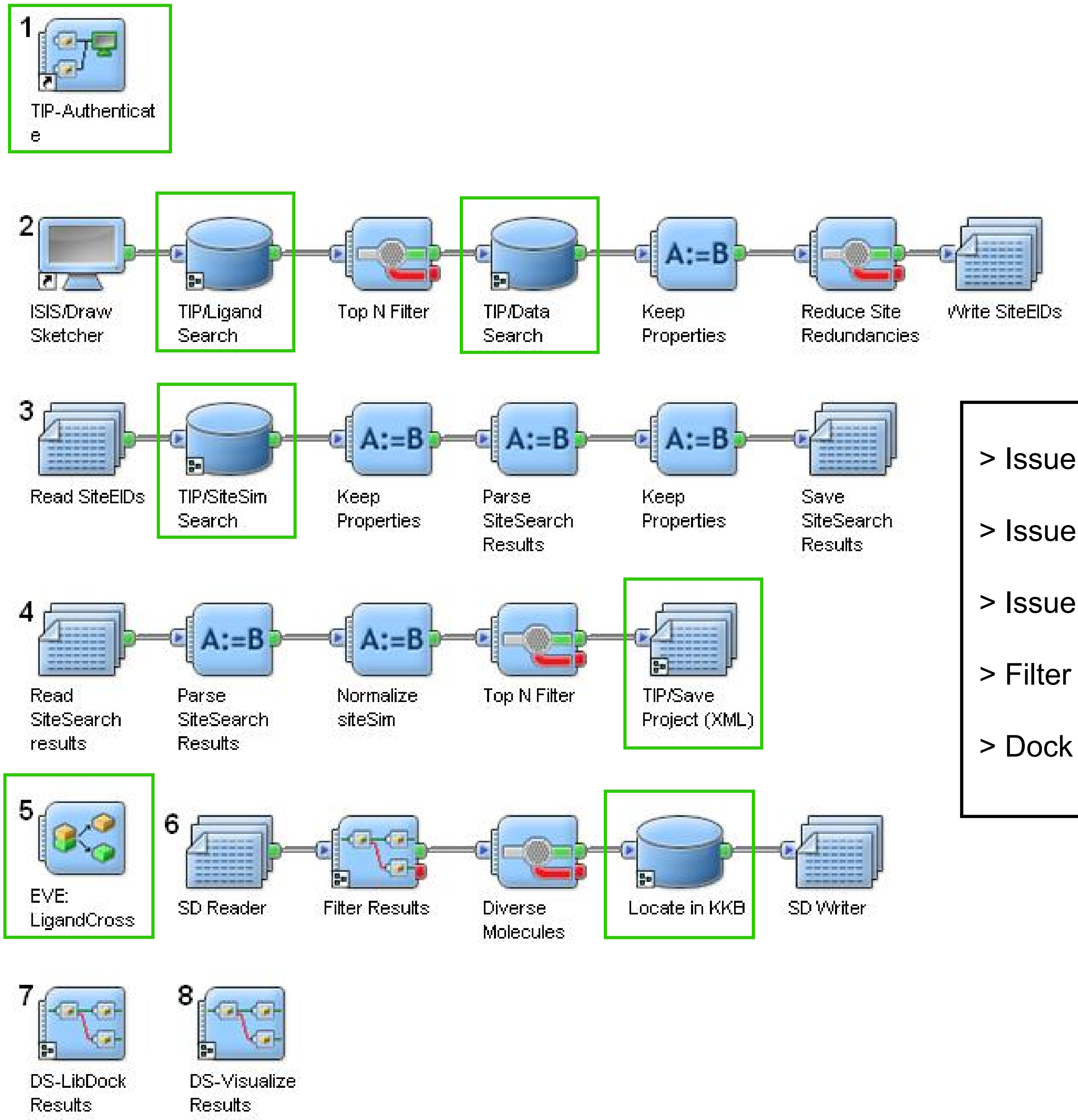
Eidogen-Sertanty

From Ligand Query to Sites to New Ligand Ideas



Site Name	Locus	Ligand	SiteSor...	Sequence Positions
pdb1opj/s470495 (chain B)	ABL1_MOUSE	STI	-	.L.Y.V.AVK.E.VM.I.LV.I.TEFM.G.L.FIHR.L.VADF
pdb1x8b/s701003 (chain A)	-	824	72.81	I - V A-K E -H - -V I N-YC G - ---- F -G--
pdb1opj/s470495 (chain B)	ABL1_MOUSE	STI	-	.L.Y.V.AVK.E.VM.I.LV.I.TEFM.G.L.FIHR.L.VADF
pdb1uwh/s491876 (chain A)	RMIL_AVEVR	BAX	113.02	I - V AVK E VL T IL I TQWC - L IIH- F IGDF
pdb1opj/s470495 (chain B)	ABL1_MOUSE	STI	-	.L.Y.V.AVK.E.VM.I.LV.I.TEFM.G.L.FIHR.L.VADF
pdb1y57/s876616 (chain A)	SRC	MPZ	68.47	L - V AIK E -- - -V - T-YM G - ---- L ----

Eidogen·Sertanty



> Issue TIP/LigandSearch

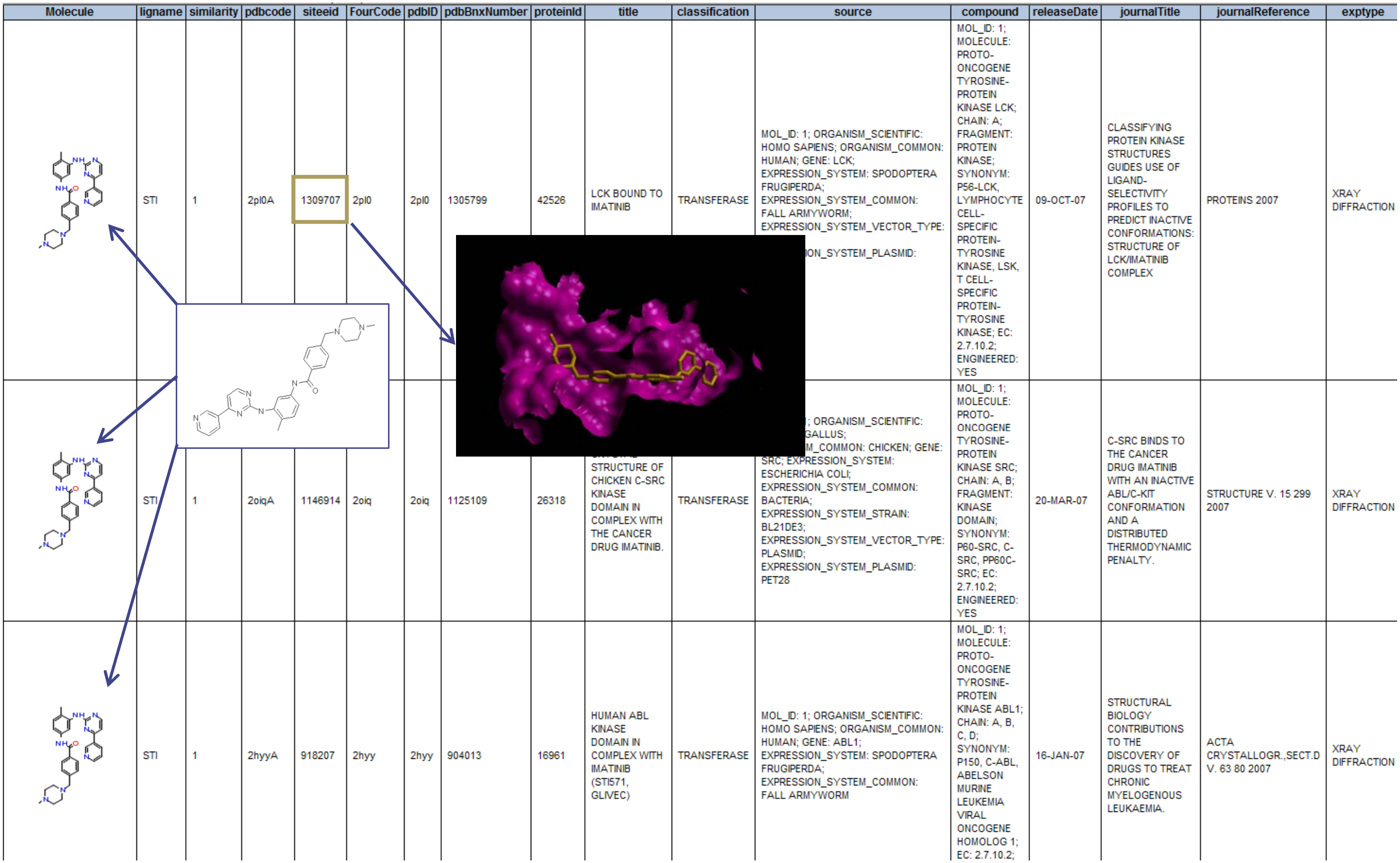
> Issue TIP/SiteSimSearch

> Issue LigandCross

> Filter and locate results in KKB

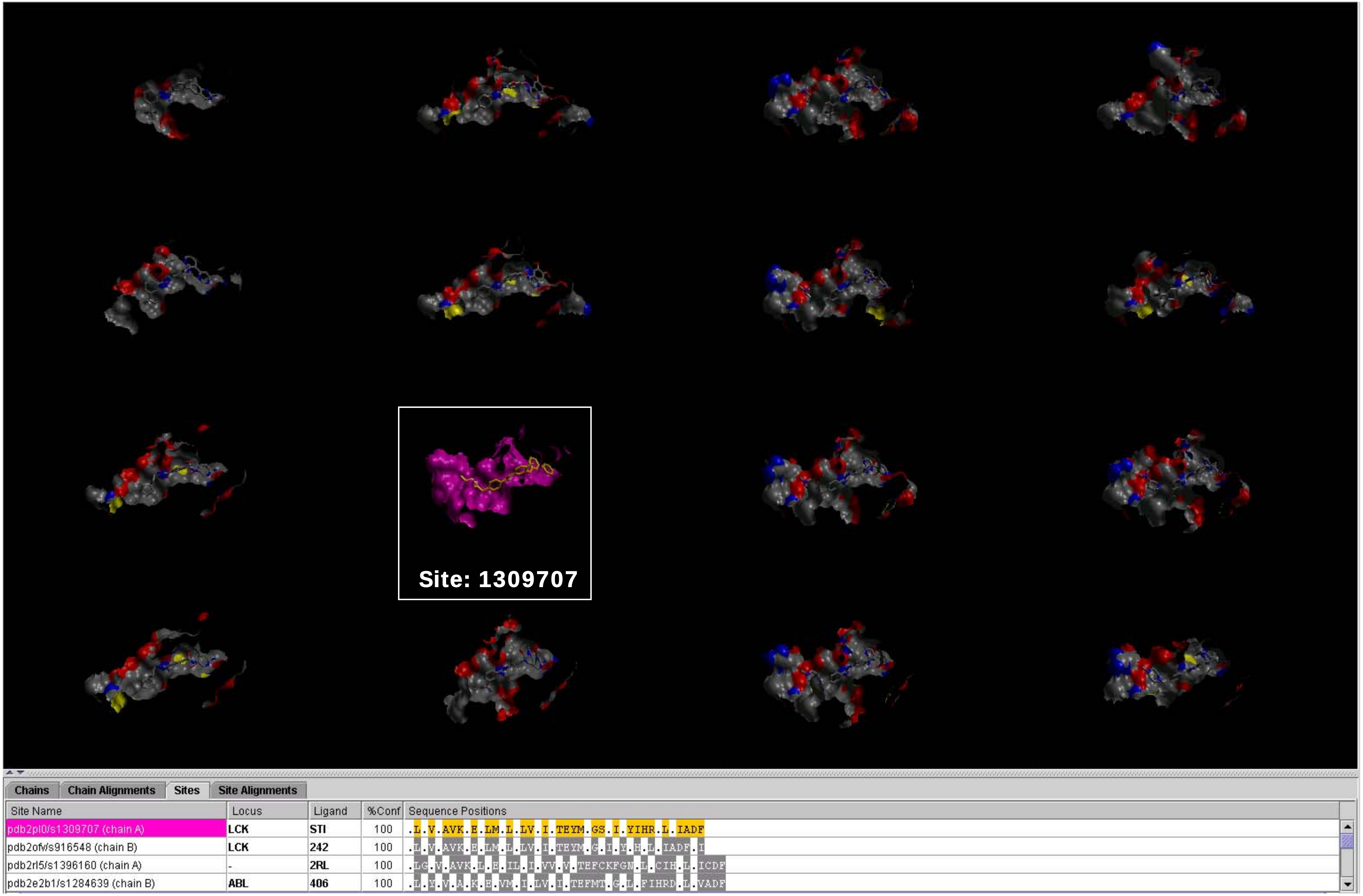
> Dock and visualize results

Step 1: Find Co-complexes and Sites from Ligand-Structure-Search



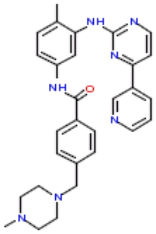
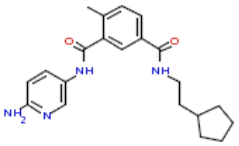
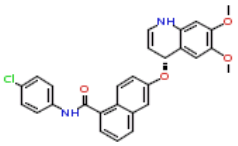
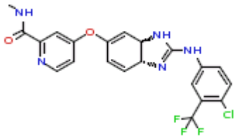
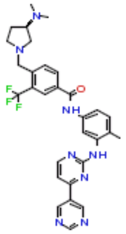
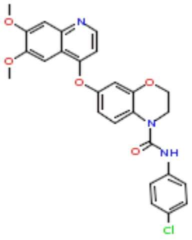
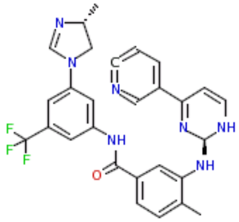
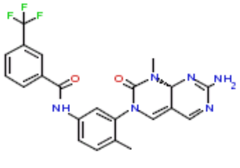
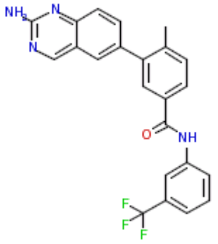
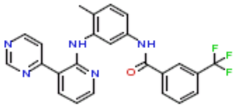
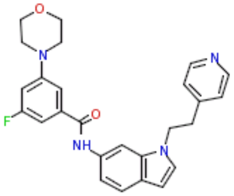
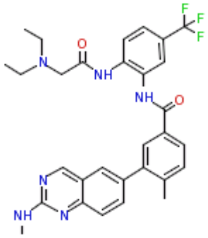
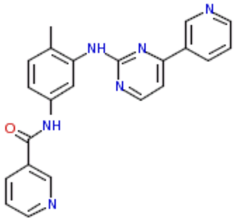
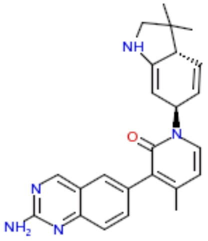
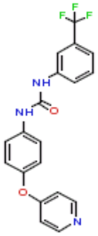
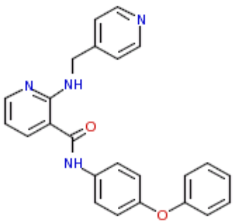
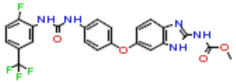
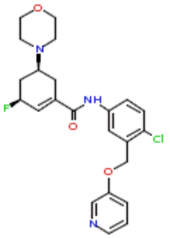
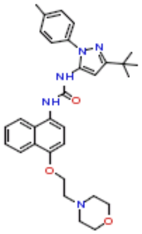
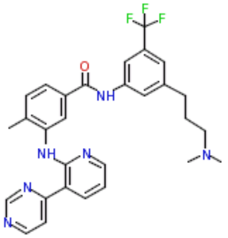
Molecule	ligname	similarity	pdbcode	siteeid	FourCode	pdbID	pdbBnxNumber	proteinId	title	classification	source	compound	releaseDate	journalTitle	journalReference	exptype
	STI	1	2pl0A	1309707	2pl0	2pl0	1305799	42526	LCK BOUND TO IMATINIB	TRANSFERASE	MOL_ID: 1; ORGANISM_SCIENTIFIC: HOMO SAPIENS; ORGANISM_COMMON: HUMAN; GENE: LCK; EXPRESSION_SYSTEM: SPODOPTERA FRUGIPERDA; EXPRESSION_SYSTEM_COMMON: FALL ARMYWORM; EXPRESSION_SYSTEM_VECTOR_TYPE: ION_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 Receptor Sites from <u>Site-Similarity</u> Search

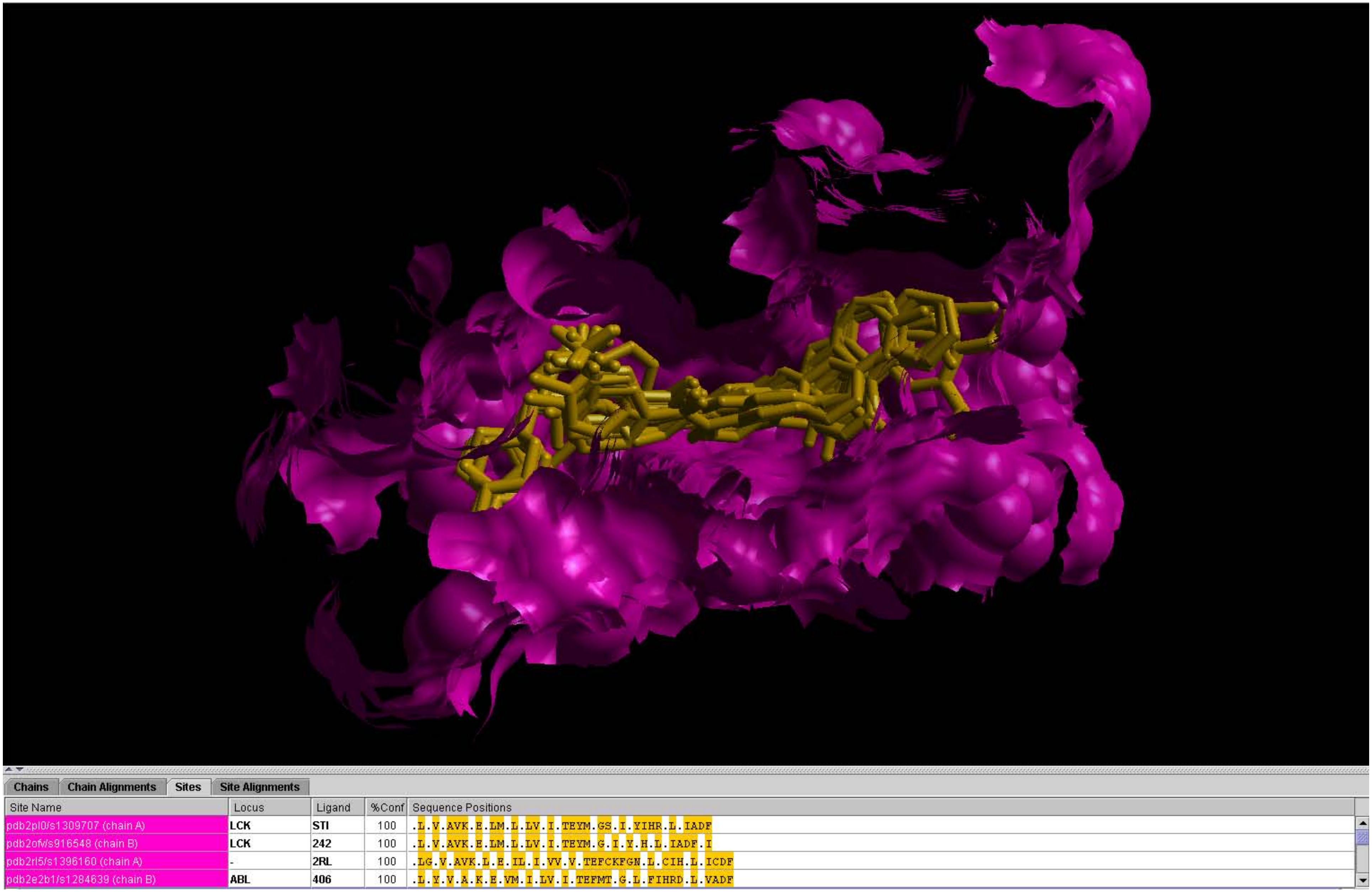


Site Name	Locus	Ligand	%Conf	Sequence Positions
pdb2pl0/s1309707 (chain A)	LCK	STI	100	.L.V.AVK.E.LM.L.LV.I.TEYM.GS.I.YIHR.L.IADF
pdb2ofv/s916548 (chain B)	LCK	242	100	.L.V.AVK.E.LM.L.LV.I.TEYM.G.I.Y.H.L.IADF.I
pdb2rl5/s1396160 (chain A)	-	2RL	100	.LG.V.AVK.L.E.IL.I.VV.V.TEFCKFGN.L.CIH.L.ICDF
pdb2e2b1/s1284639 (chain B)	ABL	406	100	.L.Y.V.A.K.E.VM.I.LV.I.TEFMT.G.L.FIHRD.L.VADF

Example Ligands Extracted from Similar Sites

 STI	 C92	 900	 276	 406
 2RL	 NIL	 7MP	 242	 MUH
 WBT	 1N8	 PRC	 C19	 KIN
 608	 GIG	 L11	 B96	 RAJ

Step 3: LigandCross – Mixing Ligand Features from Aligned Sites



Site Name	Locus	Ligand	%Conf	Sequence Positions
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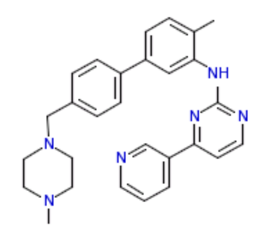
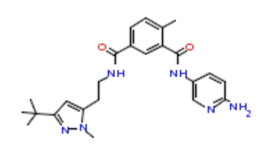
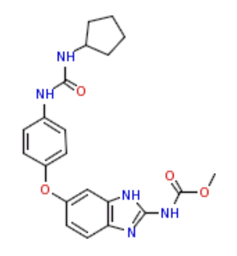
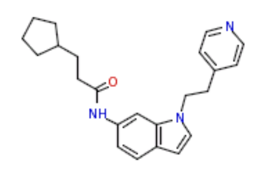
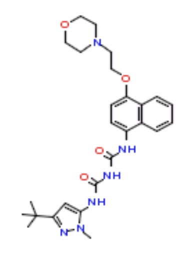
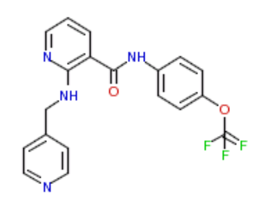
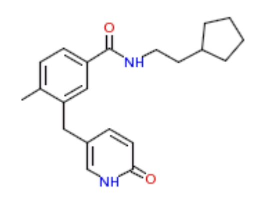
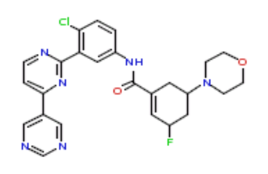
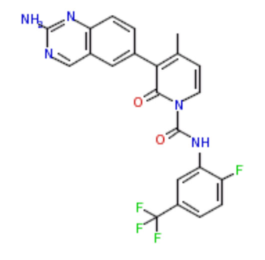
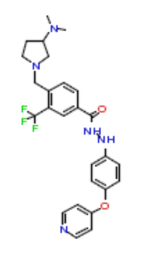
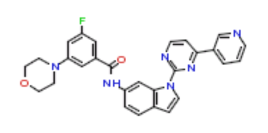
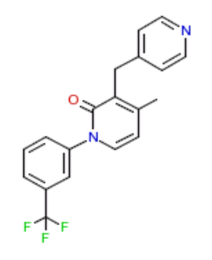
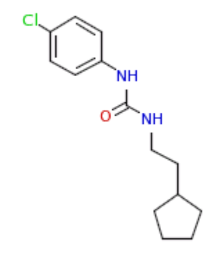
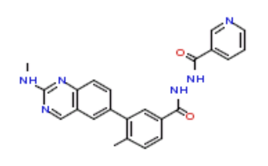
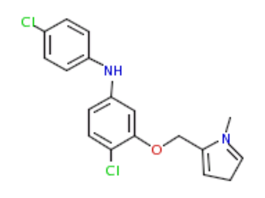
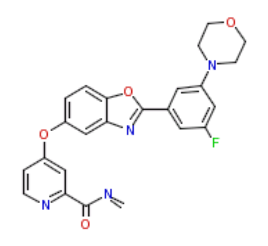
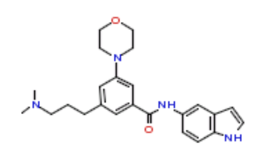
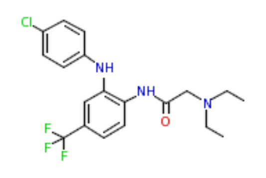
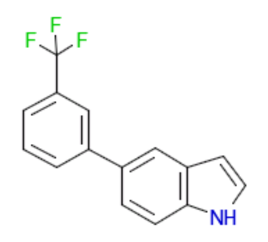
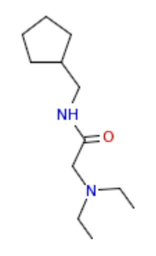
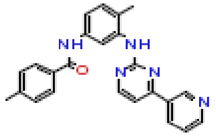
pdb2ofv/s916548 (chain B)	LCK	242	100	.L.V.AVK.E.LM.L.LV.I.TEYM.G.I.Y.H.L.IADF.I
pdb2rl5/s1396160 (chain A)	-	2RL	100	.LG.V.AVK.L.E.IL.I.VV.V.TEFCKFGN.L.CIH.L.ICDF
pdb2e2b1/s1284639 (chain B)	ABL	406	100	.L.Y.V.A.K.E.VM.I.LV.I.TEFMT.G.L.FIHRD.L.VADF

Example LigandCross Results

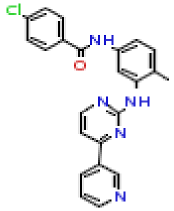
 STI_PRC_2 0.667	 C92_BMU_5 0.635	 C92_GIG_3 0.633	 C92_WBT_1 0.625	 B96_BMU_2 0.623
 608_276_3 0.608	 C92_GIN_7 0.608	 406_L11_6 0.577	 GIG_C52_1 0.574	 406_KIN_2 0.545
 NIL_WBT_6 0.538	 608_C52_2 0.529	 C92_BMU_1 0.520	 1N8_PRC_3 0.491	 857_BMU_4 0.480
 857_WBT_2 0.472	 RAJ_LI3_1 0.462	 1N8_BMU_2 0.449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

Step 4: LigandCross Ligands with Reported Biological Activity

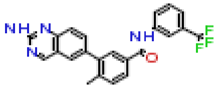
	Kinase Knowledgebase (pIC50)										Bayesian Model Predictions (PP)									
LC-ID	ABL	PDGFR	PDGFRB	JAK3	KDR	LCK	MAPK14	TEK	KIT	RAF1	ABL	PDGFR	PDGFRB	JAK3	KDR	LCK	MAPK14	TEK	KIT	RAF1
G2G_STI_12	6.7	8	8								0.40	0.90	0.76	0.81	0.59	0.15	0.89	0.45	0.70	0.37
900_STI_1	6.1	8	8								0.38	0.91	0.76	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



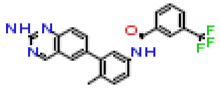
1: G2G_STI_12



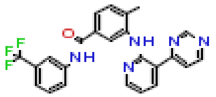
2: 900_STI_1



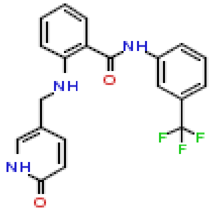
3: 7MP_1N8_4



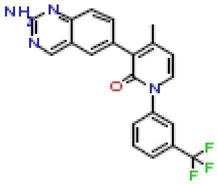
4: 7MP_1N8_2



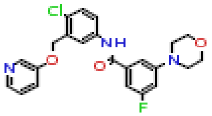
5: 7MP_RAJ_3



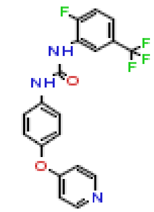
6: 7MP_GIN_4



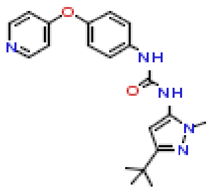
7: 242_C52_2



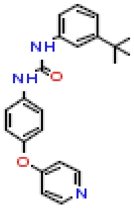
8: LI3_L11_1



9: 608_GIG_7



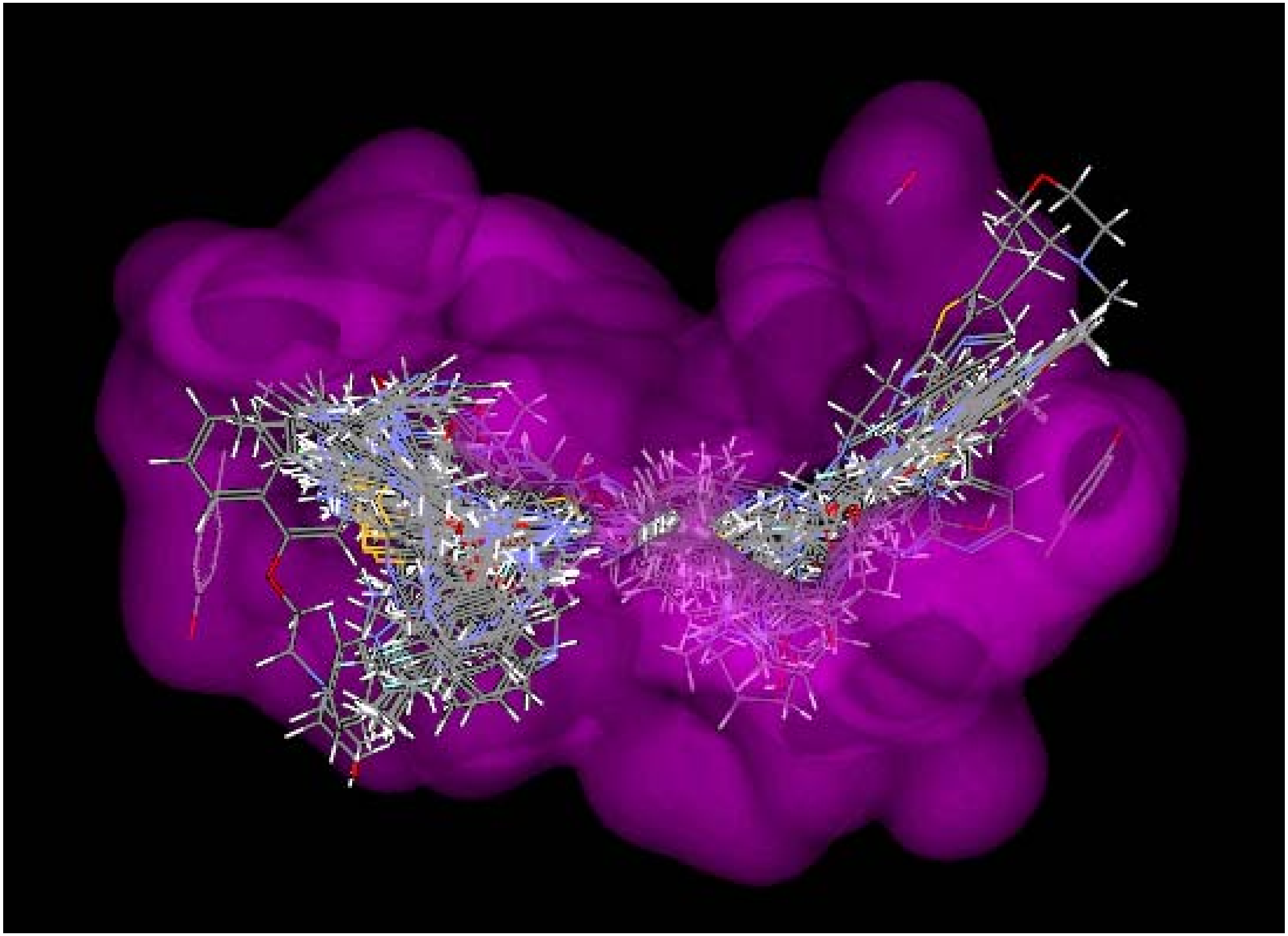
10: KIN_BMU_4



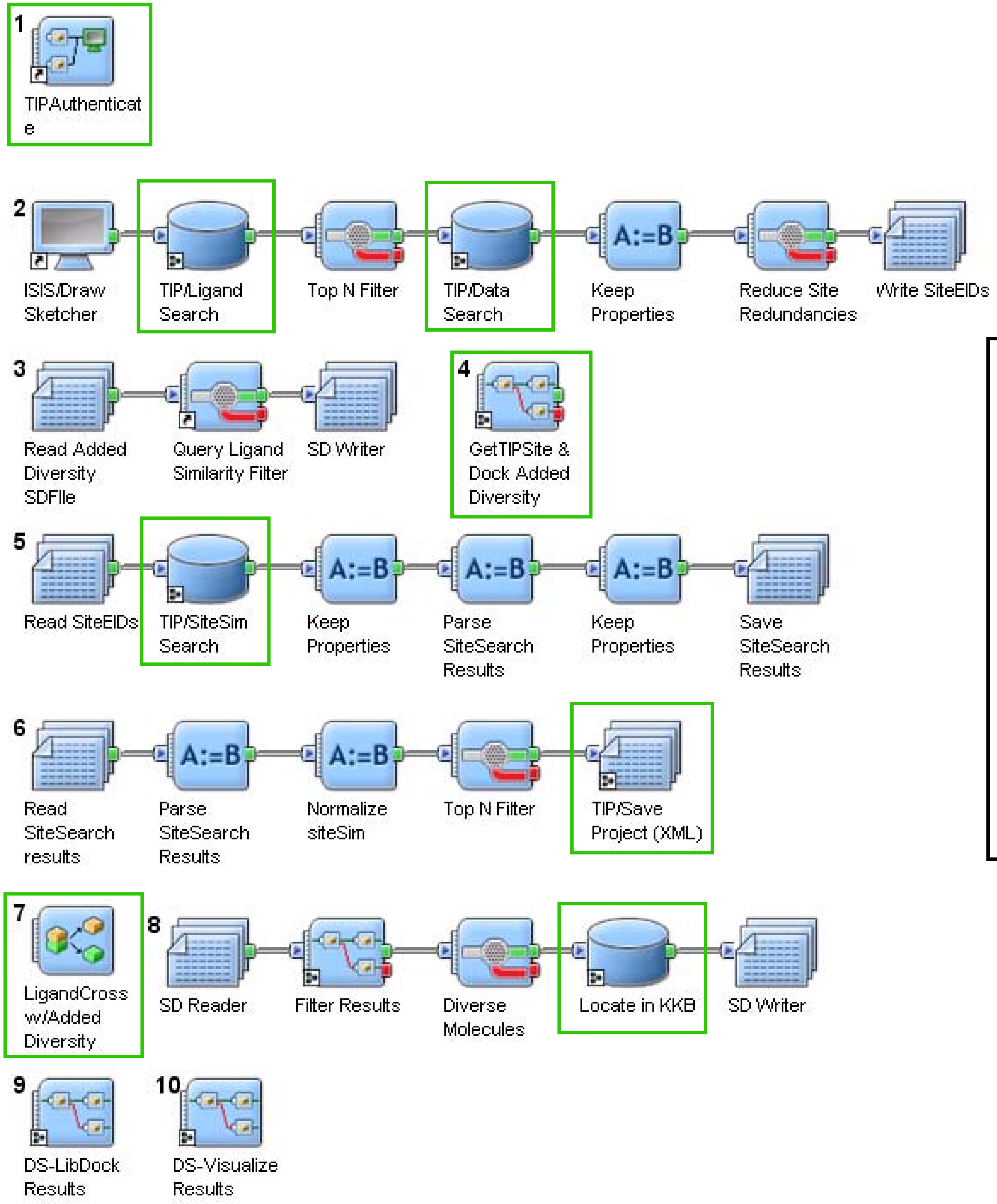
11: G2G_KIN_3

Eidogen-Sertanty

Step 5: LigandCross Ligands reDocked into s1309707



Eidogen-Sertanty



> Issue TIP/LigandSearch

> Identify/Dock "AddedDiversity"

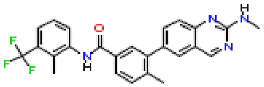
> Issue TIP/SiteSimSearch

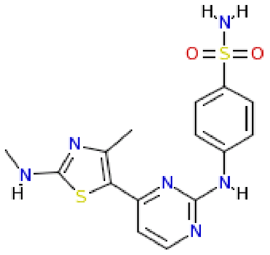
> LigandCross w/AddedDiversity

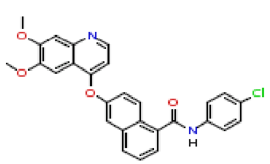
> Filter and locate results in KKB

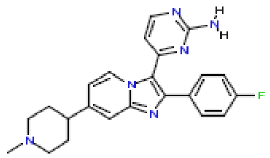
> Dock and visualize results

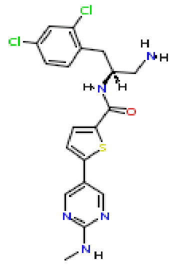
Example Potent Kinase Inhibitors ("Added Diversity")


4336533 LCK pval: 11.00

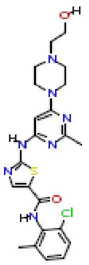

4302493 CDK9 pval: 10.54

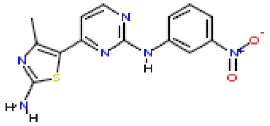

4332561 KDR pval: 10.52

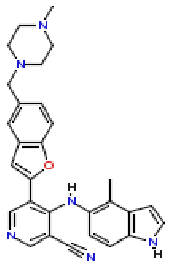

4318145 PKG pval: 10.40

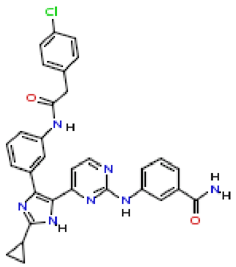


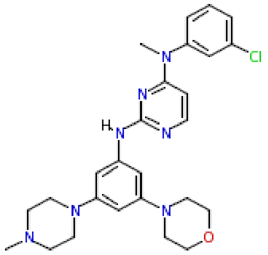
4336686 PKA pval: 10.00

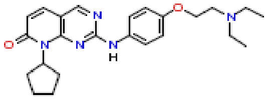

4272835 ABL1 pval: 10.00

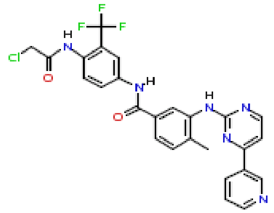

894611 CDK2 pval: 9.70

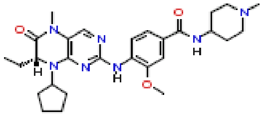

4358565 PRKCQ pval: 9.70


4363734 RAF1 pval: 9.30

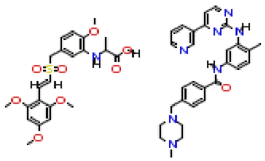

4369892 EPHB4 pval: 9.24

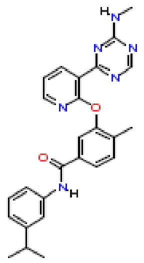

809 CDK4 pval: 9.15

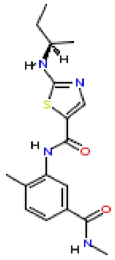

4374385 PDGFRA pval: 9.14



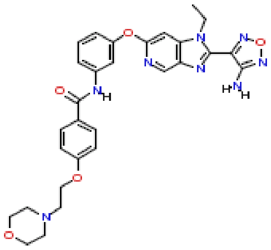
4366691 PLK1 pval: 9.10

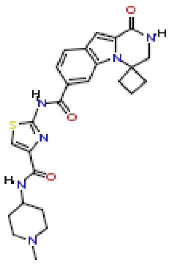

4301886 BCR_ABL pval: 9.08

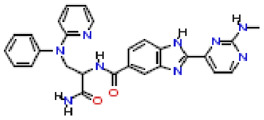

4307551 TEK pval: 9.00

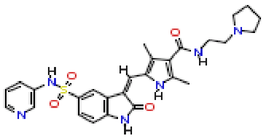


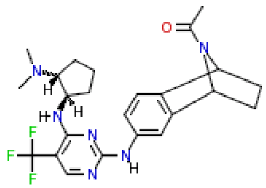
4363016 MAPK11 pval: 8.82


4343448 ROCK1 pval: 8.74

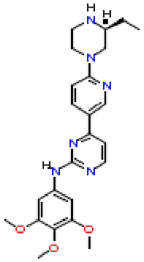

4363247 MAPKAPK2 pval: 8.70


4291996 IKB pval: 8.70

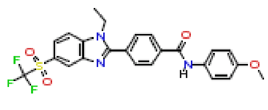

4208857 FAK2 pval: 8.22

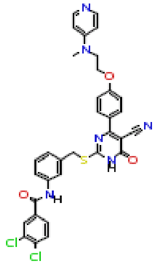


4373725 PTK2B pval: 8.22

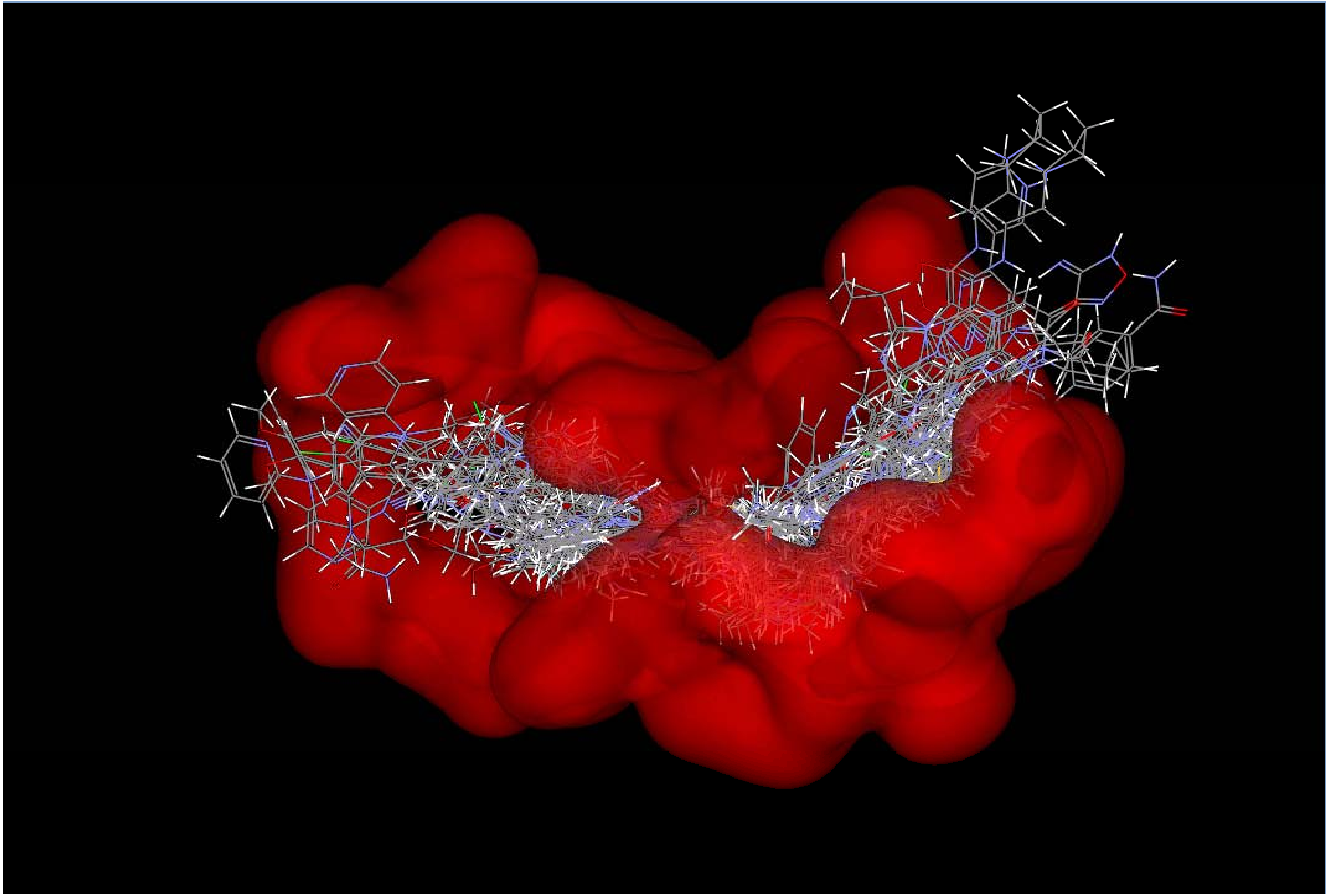


1788 ZAP70 pval: 8.10

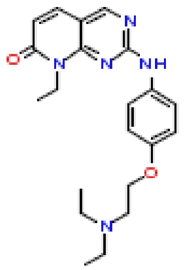

2425813 PTPN9 pval: 5.96

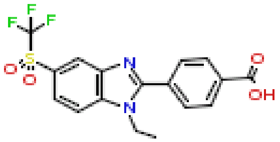

4303129 MAP3K2 pval: 4.70

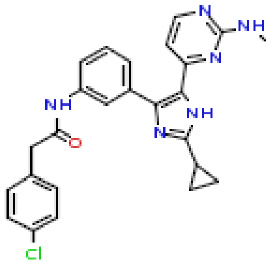
Potent Kinase Inhibitors Docked (s1309707)

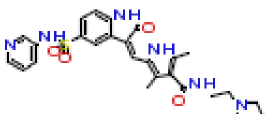


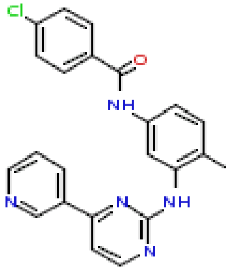
LigandCross Examples using "Added Diversity"

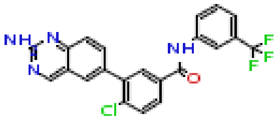

4343448_809_27

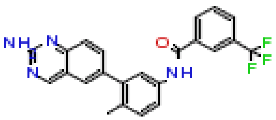

4272835_2425813_23

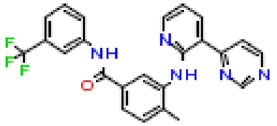

4363734_4291996_2

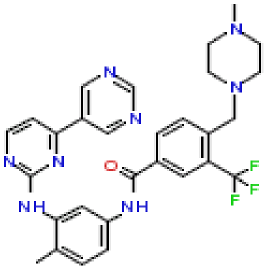

4208857_4208857_1


900_STI_1


242_A96_5


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

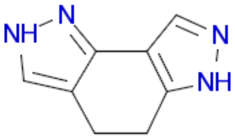
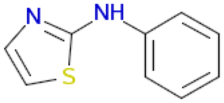
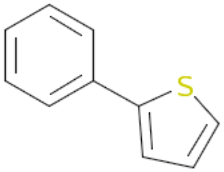
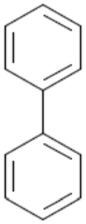
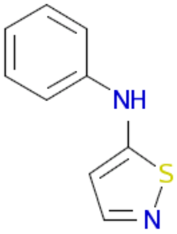
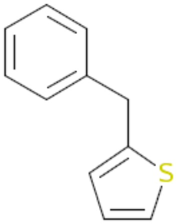
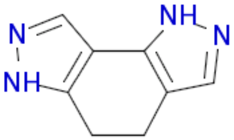
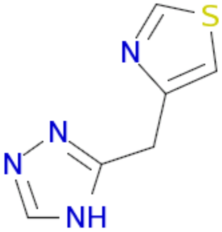
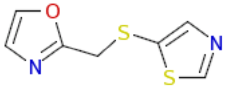
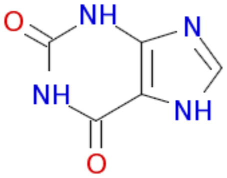
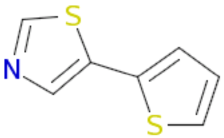
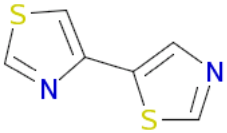
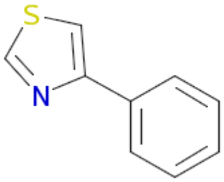
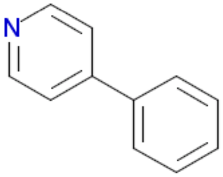
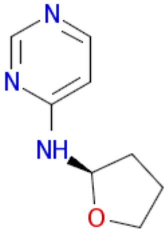
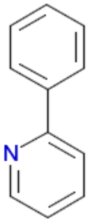
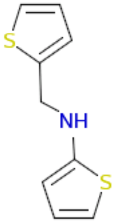
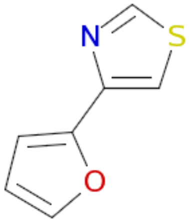
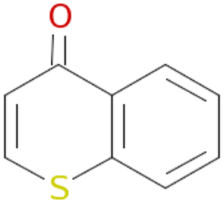
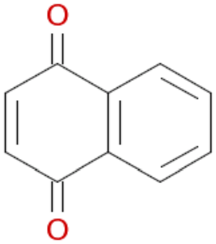
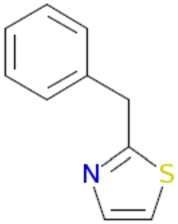
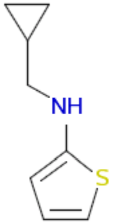
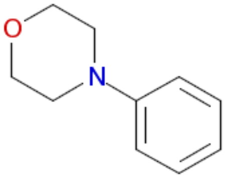
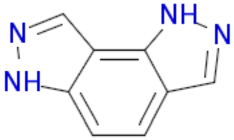
242_MUH_1:
LCK: 9.40 TEK: 7.68 KDR: 8.22 MAPK14: 9.00 JAK3: 6.81

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

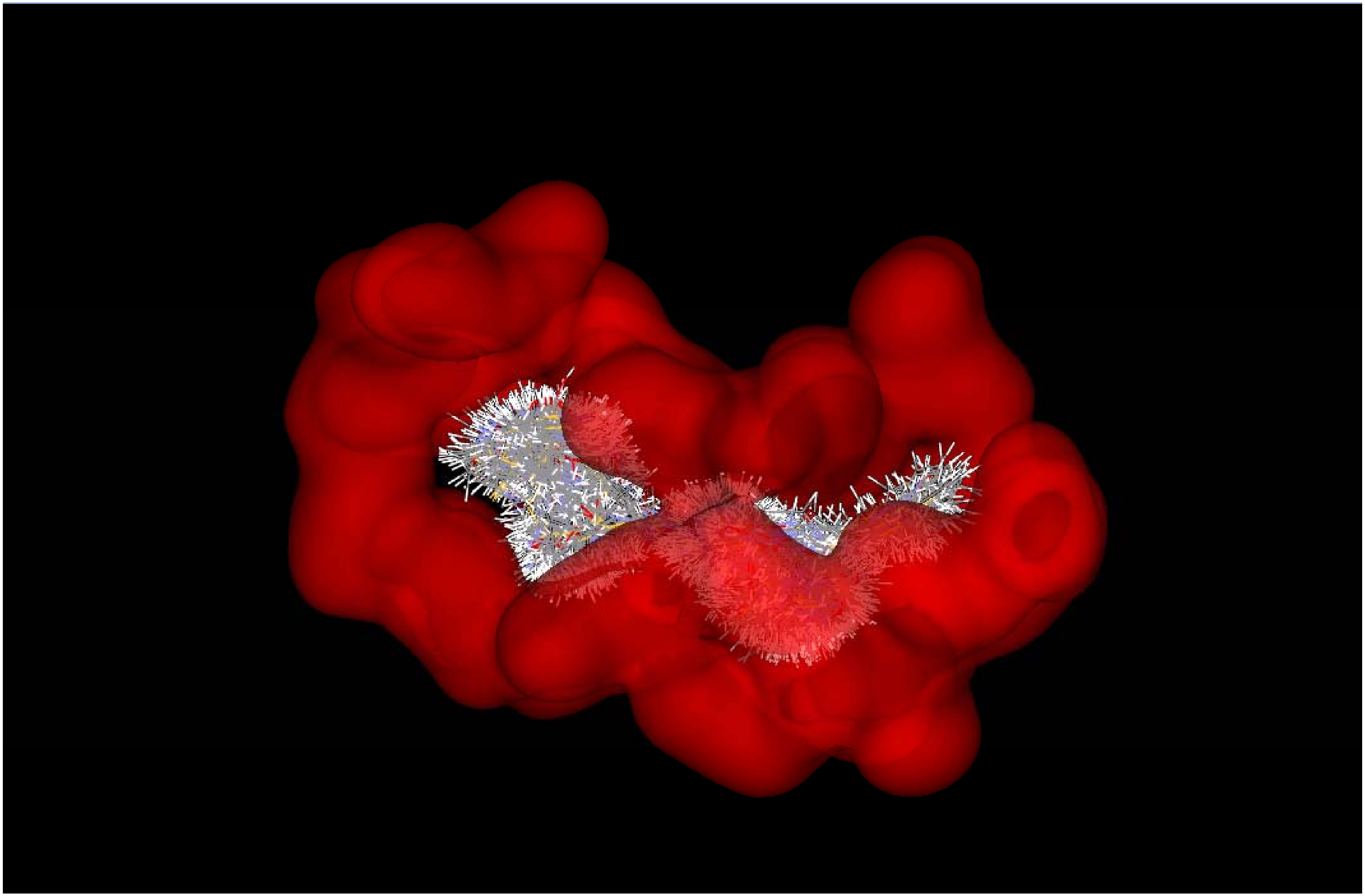
Eidogen·Sertanty

Murcko Assemblies Found in Kinase Inhibitors

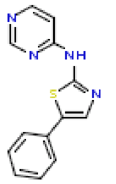
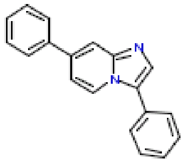
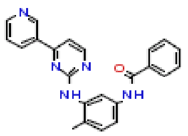
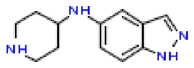
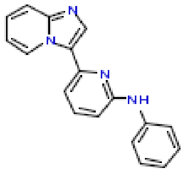
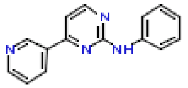
 4271715	 4361736	 4299112	Chiral  13176	 4325163	 4295206
 4271959	 4342495	 813719	 2425137	 4367928	 4344540
 4184755	 3432838	Chiral  13702	 4330411	 4295114	 2426312
 1634982	 4278310	 4278150	 4295148	 4271026	 4290503

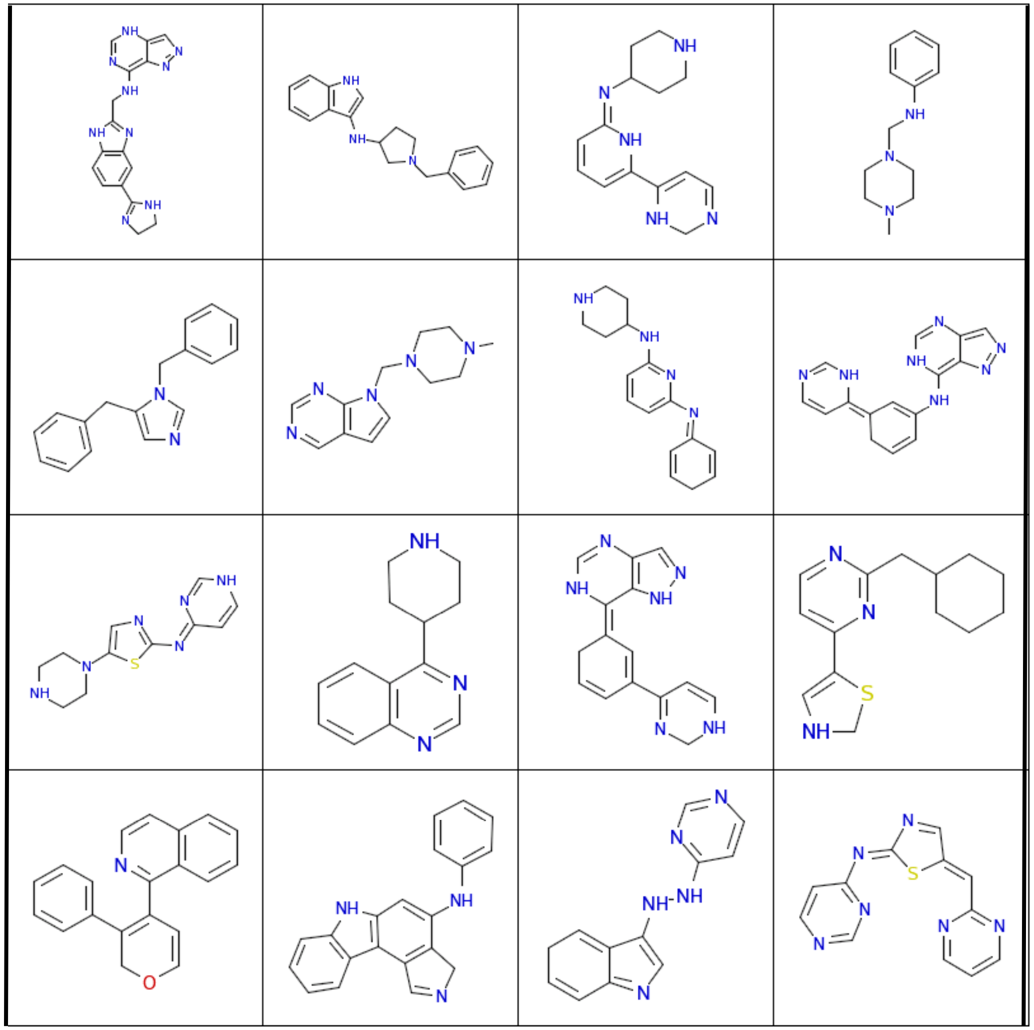
Murcko Assemblies: Contiguous ring systems plus chains that link two or more rings
"The Properties of Known Drugs. 1. Molecular Frameworks", Guy W. Bemis and Mark A. Murcko, *J. Med. Chem.* 1996, 39, 2887-2893.

Positional Murcko Assemblies (parent inhibitors docked into s1309707)



LigandCross Results: Positional Murcko Assemblies
from docked Kinase inhibitors (s1309707)

	KDR KDR	Enzyme Assay Enzyme Assay	7.4437 7.4437
	KDR	Enzyme Assay	7.0088
	PDGFR PRKCA PRKCA ABL EGFR PDGFR PDGFR PDGFRB ABL PDGFRB PDGFRB PDGFRA ABL PDGFRB	Enzyme Assay Enzyme Assay Enzyme Assay Enzyme Assay Enzyme Assay Enzyme Assay Enzyme Assay Cell-Based Assay Enzyme Assay Cell-Based Assay Enzyme Assay Enzyme Assay Enzyme Assay Cell-Based Assay	7 4.1427 4.1427 6.3979 4.1871 7 7 7.1871 6.3979 6.2218 7 5.2218 6.3979 6.7696
	ROCK ROCK1	Enzyme Assay Enzyme Assay	6.5421 6.5229
	IRAK4	Enzyme Assay	5.9370
	PRKCA PRKCD ABL EGFR	Enzyme Assay Enzyme Assay Enzyme Assay Enzyme Assay	4.9788 4.4089 5.7447 4



Kinase Activity ????

Eidogen-Sertanty

Conclusions

- Systematic modeling and analysis of both small molecule activity data and protein structure site similarities can reveal pharmacologically relevant insights and predict possible cross reactivity within (and across) target families
- Systematic analysis of protein site similarities is in many cases consistent with existing experimental SAR
- The structurally resolved and modelable proteome is a very rich source for new matter ideas
- LigandCross can be an effective strategy to generate novel, bioactive molecules from co-complex information
- There is synergy between protein structure information and small molecule SAR data

Acknowledgements

- Stephan Schürer
- Kevin Hambly
- Joe Danzer
- Brian Palmer
- Derek Debe
- Aleksandar Poleksic

- Accelrys/Scitegic - Shikha Varma-O'Brien/Ton van Daelen

Add’l slides

Conclusions

- Significant receptor-site similarities exist within and across target families

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

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

About Eidogen-Sertanty

- Knowledge-Driven Solutions Provider
  - Sertanty established in 2003, acquired Libraria assets
  - Sertanty acquired Eidogen/Bionomix in 2005→ Eidogen-Sertanty
  - $20M invested: Libraria ($6M), Eidogen/Bionomix ($12M), Sertanty/ES ($2M)
  - 14 distributed FTE's (4 US and 10 India)
  - Worldwide (bio)pharmaceutical customer base
  - Cash-positive since 2006

- Databases & Software – Annual Subscriptions
  - *TIP™* - Protein Structural Informatics Platform
  - *KKB™* - Kinase SAR and Chemistry Knowledgebase
  - CHIP™ - Chemical Intelligence Platform

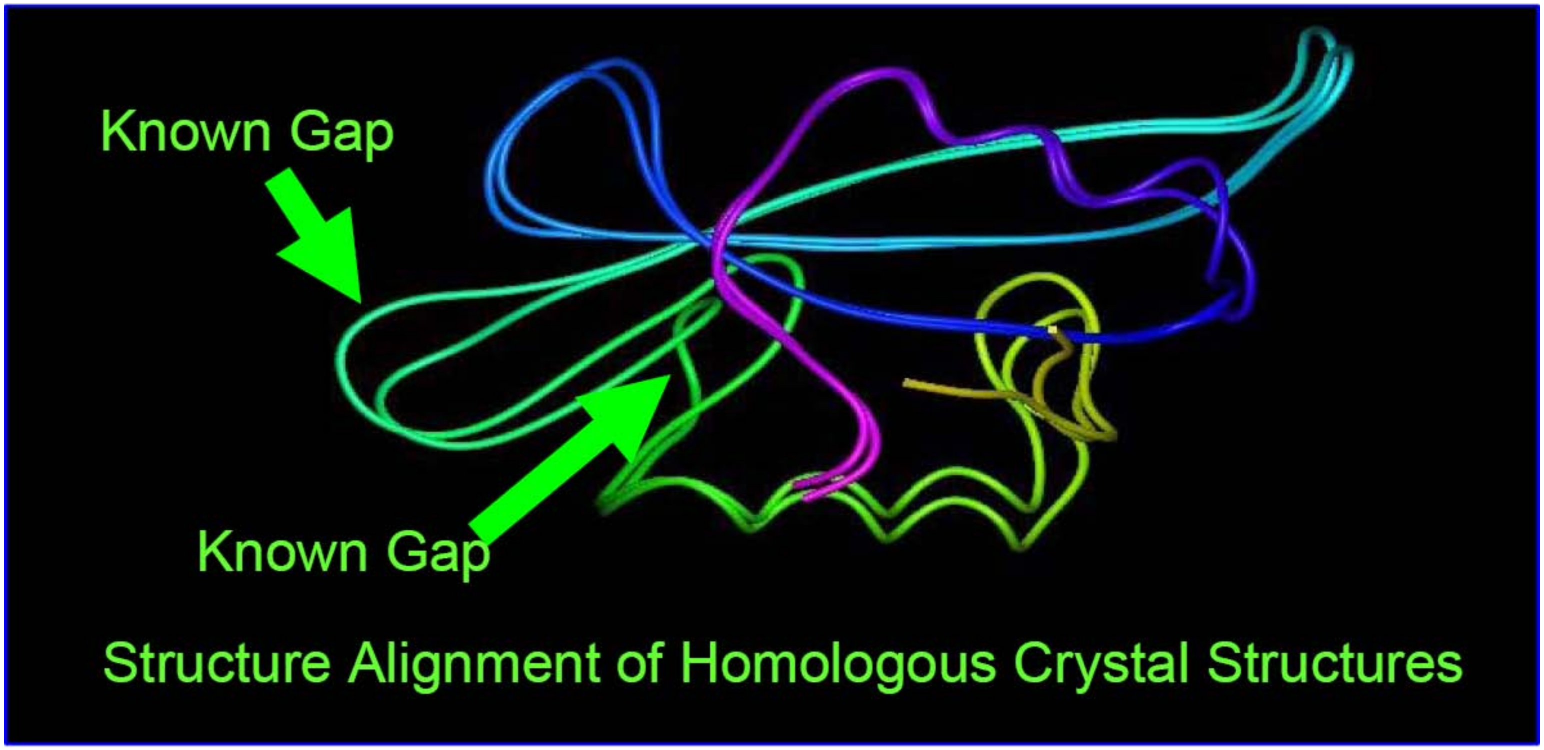
- DirectDesign™ Fee-For-Service
  - In Silico Target Screening ("Target Fishing" and Repurposing)
  - Target and compound prioritization services
  - Fast Follower Design: Novel, Patentable Leads

Eidogen·Sertanty

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

TIP (Eidogen-Sertanty) / Debe et. al. Proteins 2006, 960

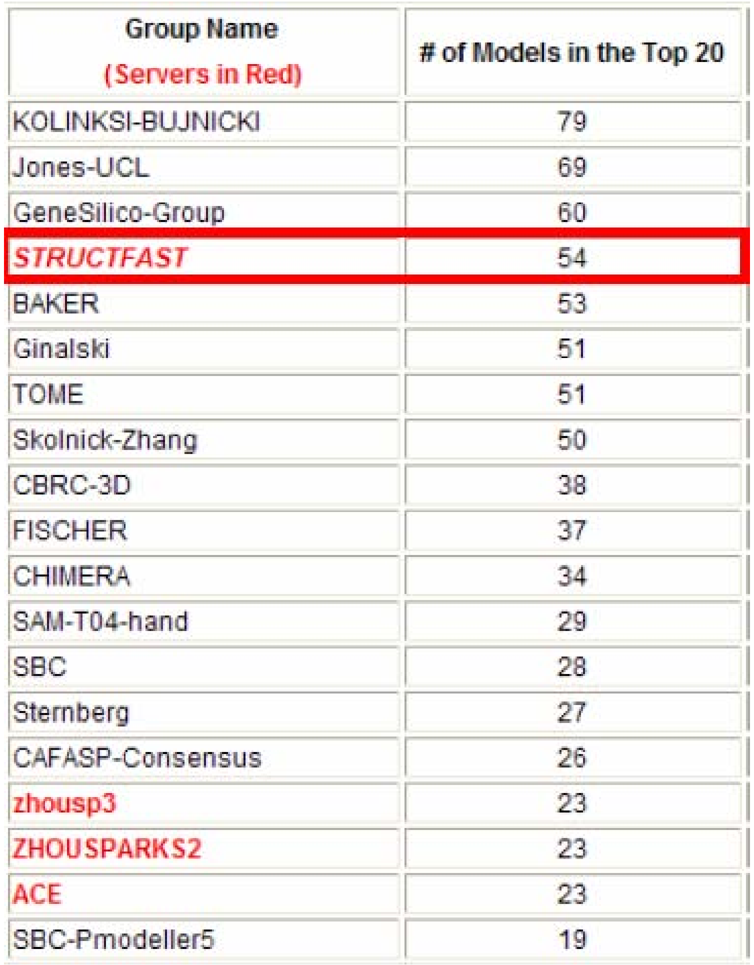
STRUCTFAST™ Algorithm Comparison

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**

STRUCTFAST^{TM} CASP6 Results

December 2004 CASP6 Total Comparative Modeling Results

of models placed in the top 20 according to the number of correctly aligned residues



Group Name (Servers in Red)	# of Models in the Top 20
KOLINKSI-BUJNICKI	79
Jones-UCL	69
GeneSilico-Group	60
STRUCTFAST	54
BAKER	53
Ginalski	51
TOME	51
Skolnick-Zhang	50
CBRC-3D	38
FISCHER	37
CHIMERA	34
SAM-T04-hand	29
SBC	28
Sternberg	27
CAFASP-Consensus	26
zhousp3	23
ZHOUSPARKS2	23
ACE	23
SBC-Pmodeller5	19

Other Notables:

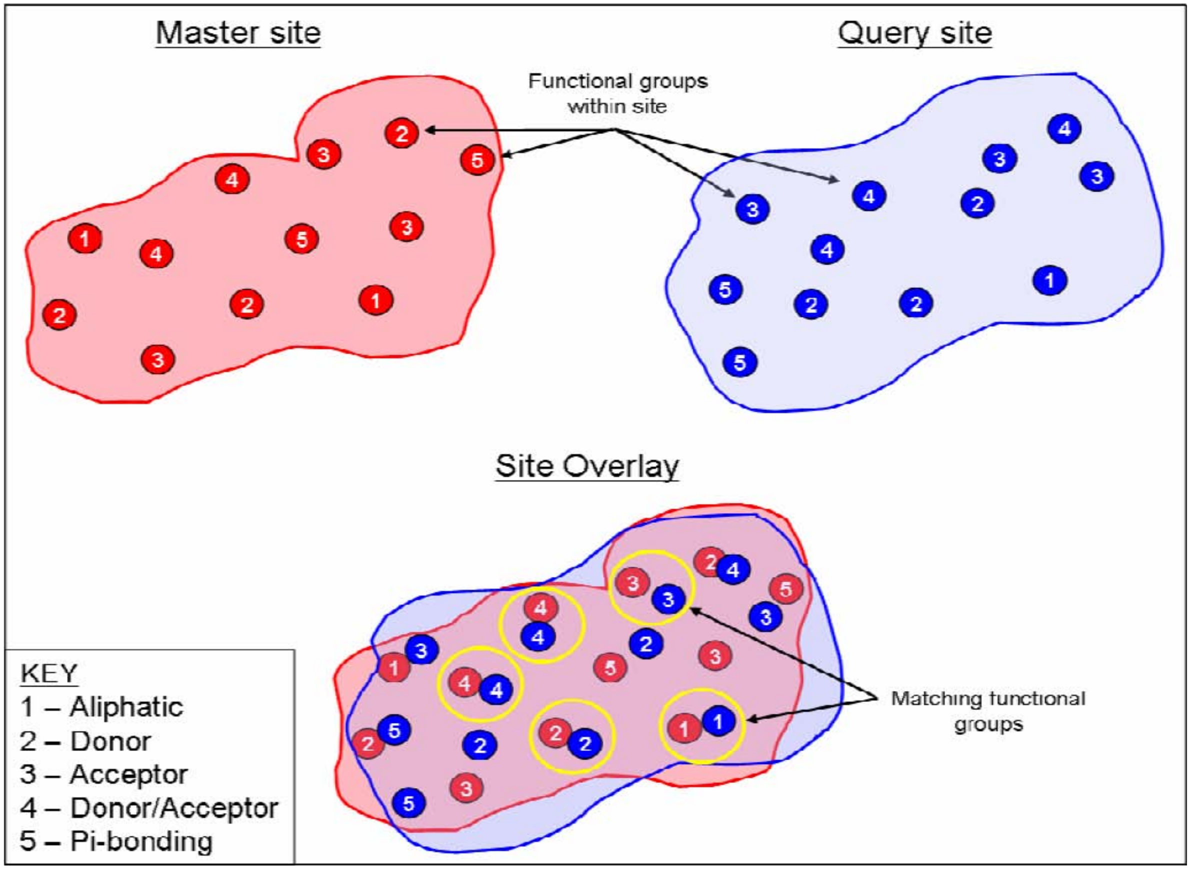
FAMS	15
Accelrys	4

STRUCTFAST had more than twice as many models in the top 20 compared to the second best automated server.

Only 3 of 124 hand modeling teams produced better alignments than ***STRUCTFAST***.

SiteSorter^{TM} binding site comparison

Weighted Clique Detection Algorithm (importance of points related to conservation in multiple sequence alignment)



Surface atoms assigned one of 5 different chemical characters (pseudocenters); matching points increase the site similarity score

TIP (Eidogen-Sertanty) / Klebe et. al. J Med Chem 2002