Surveying ligand- and target-based similarities within the Kinome

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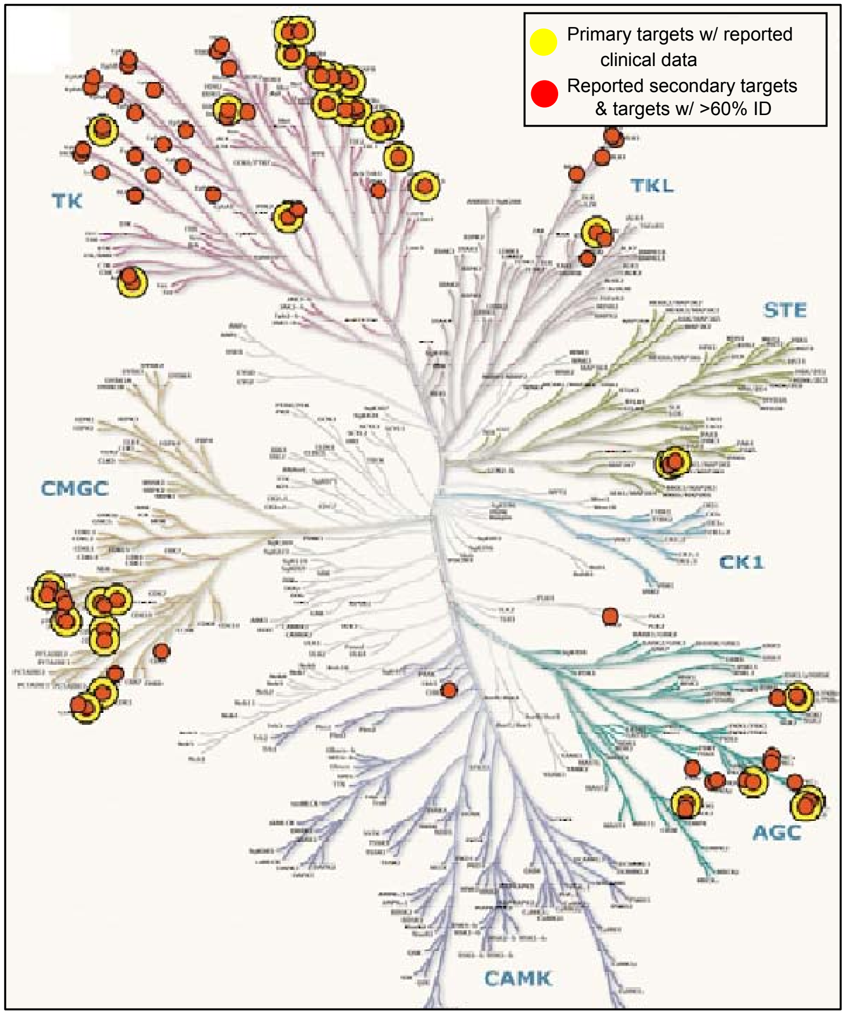
THE SCRIPPS RESEARCH INSTITUTE

Eidogen Sertanty

Kinase SAR Knowledgebase – Hot Targets

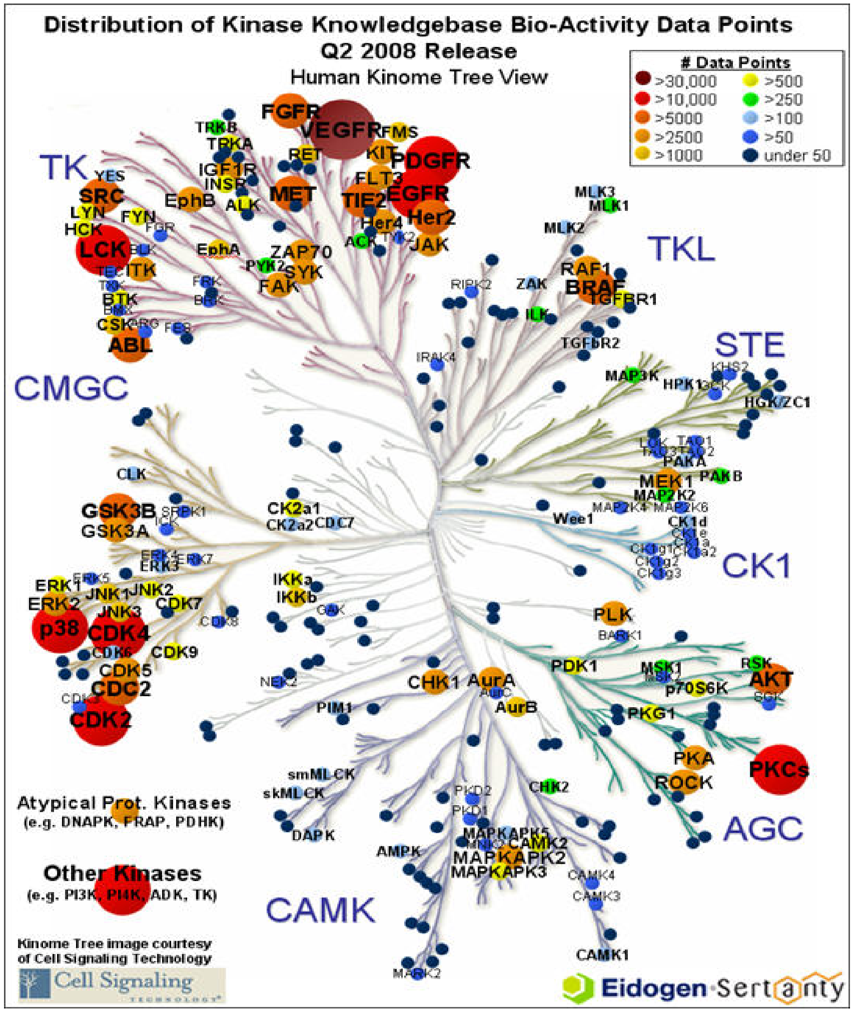
Kinase Targets of Clinical Interest

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



Eidogen-Sertanty KKB

SAR Data Point Distribution



>362,000 SAR data points curated from
>4,270 journal articles and patents
>130 Bayesian QSAR Models

Eidogen Sertanty

About Eidogen-Sertanty

- Knowledge-Driven Discovery Solutions Provider
  - Formed in March 2005 when Sertanty (Libraria→Sertanty 2003) acquired Eidogen (Bionomix 2000)
  - >$20M Invested in Technology Development
  - 12 FTE's
  - Worldwide Customerbase
  - Cash-Positive

- Chemogenomic Databases & Analysis Software

TIP™	- Structural Informatics Platform
KKB™	- Kinase SAR and Chemistry Knowledgebase
CHIP™	- Chemical Intelligence Platform

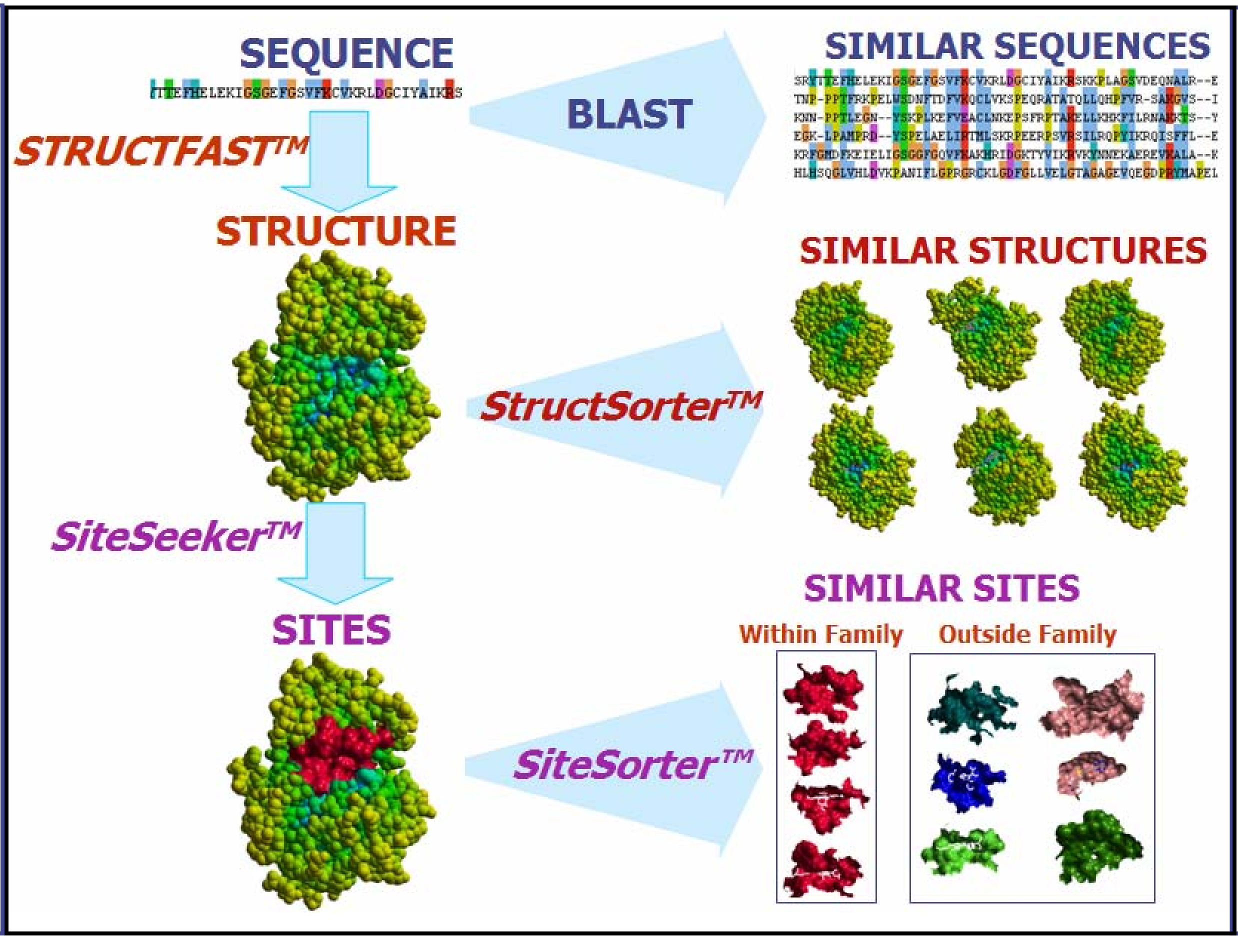
- DirectDesign™ Discovery Collaborations
  - In Silico Target Screening ("Target Fishing" and Repurposing)
  - Target and compound prioritization services
  - Fast Follower Design: Novel, Patentable Leads

TIP Algorithm Engine

> 400K
Sequences

> 158K
Chains & Models

> 388K
Sites



> 33M
Sequence Similarities

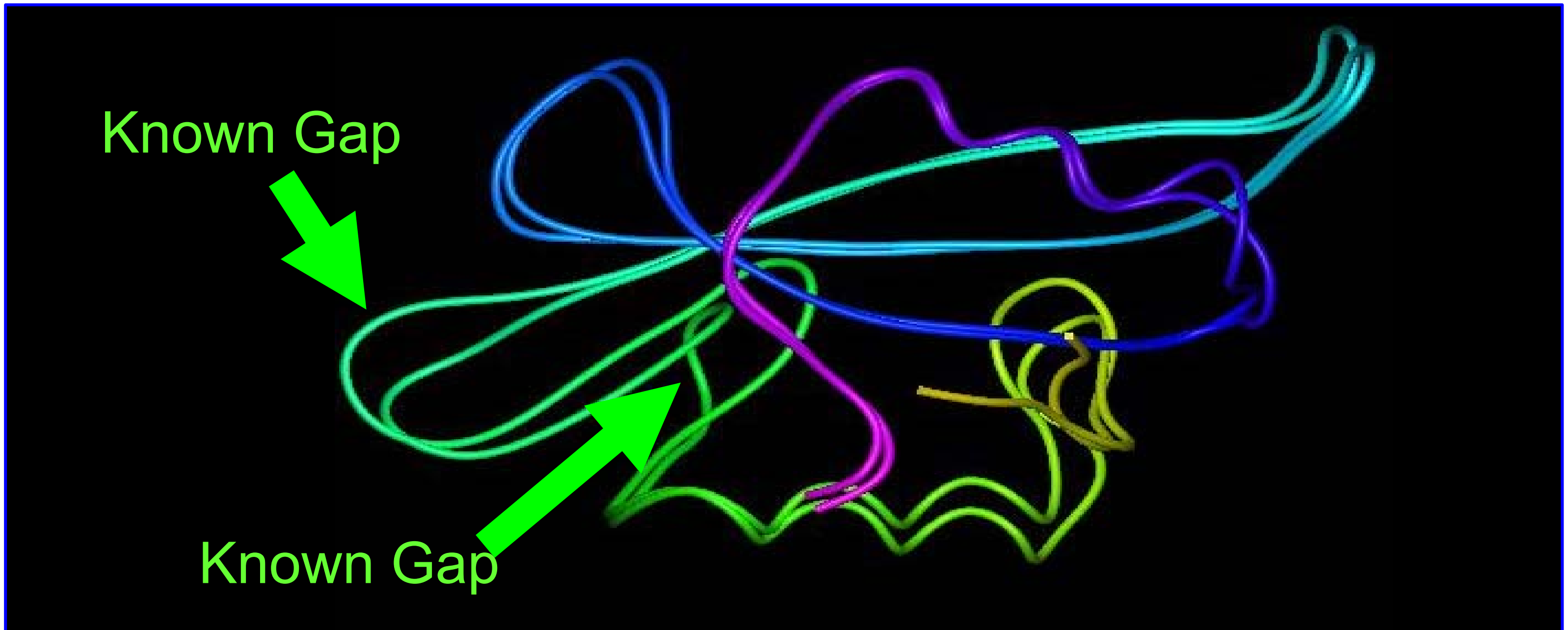
> 69M
Structure Similarities

> 62M
Site Similarities

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

1) Convergent Island Statistics: A fast method for determining local alignment score significance. Bioinformatics, 2005, 21, 2827-2831

2) STRUCTFAST: Protein Sequence Remote Homology Detection and Alignment Using Novel Dynamic Programming and Profile-Profile Scoring
Proteins. 2006 64:960-967

Eidogen·Sertanty

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

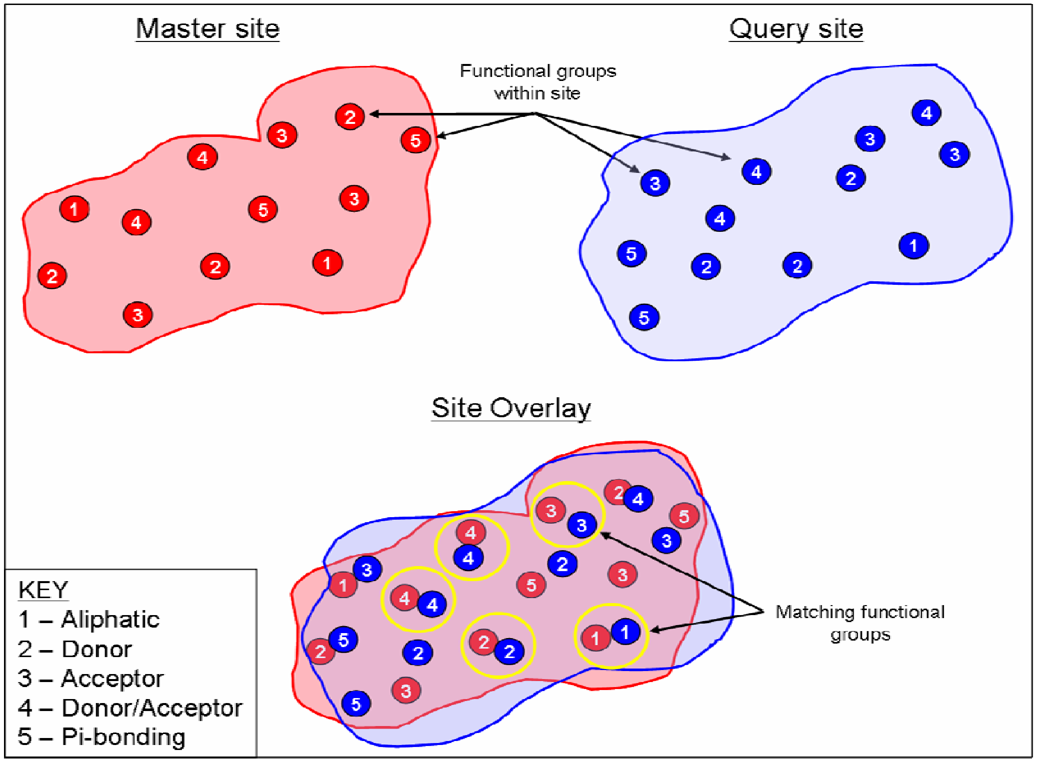
We use proteins with apo- & co-crystal structures in the PDB to test the accuracy & reliability of method

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

SiteSorter™

Weighted Clique Detection Algorithm

Importance of Points Related To Conservation In Multiple Sequence Alignment



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

TIP Content

>75,000 Human Sequences

>33M Sequence Similarities

>116,000 Total PDB chains (~50K PDBs)
> 42,000 Homology Models

>69M Structural Similarities

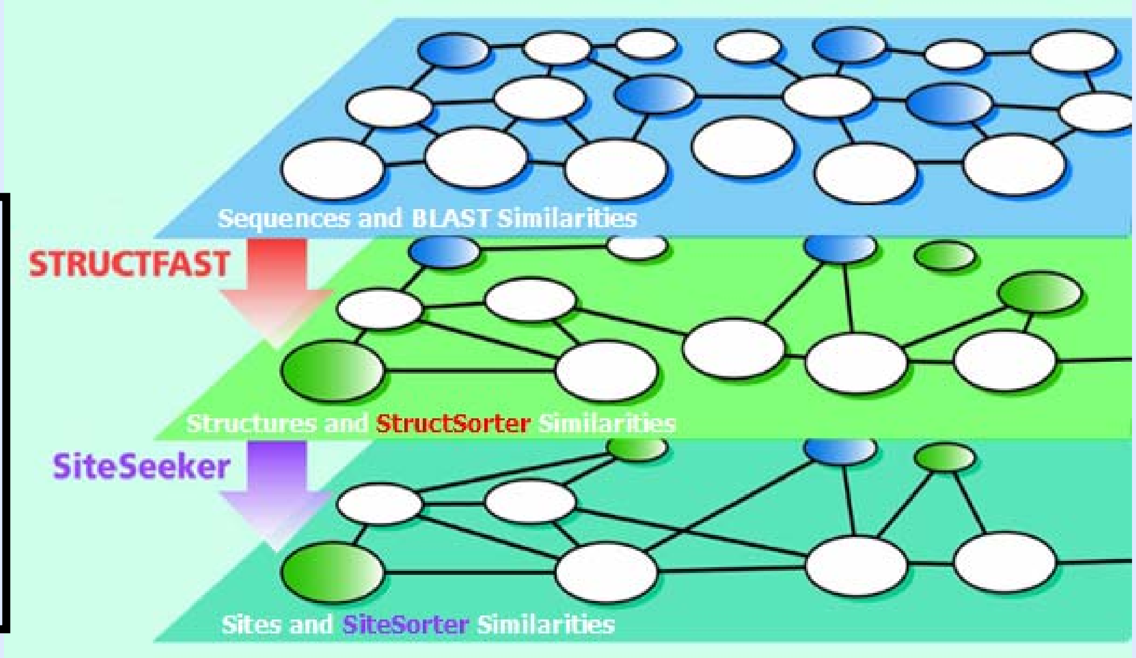
>194,000 PDB co-crystal sites
>190,000 Predicted Sites (on PDBs & Models)

>62M Site Similarities

Updated monthly with new PDBs and models:

e.g. March 2006:
→ 661 new PDBs added
→ 447 new models built
- 153 had no previous structure in TIP
- 294 had "better" models built

e.g. July 2008:
→ 576 new PDBs added
→ 1045 new models built



Automatically updated with new models as the PDB grows

Eidogen-Sertanty

Kinase Knowledgebase (KKB)

Kinase inhibitor structures and SAR data mined from

> 4278 journal articles/patents

- **KKB Content Summary (Q2-2008):**

  \# of kinase targets: **>390**
  \# of SAR Data points: **> 362,000**
  \# of **unique** kinase molecules with SAR data: **>120,000**
  \# of annotated assay protocols: **>16,000**
  \# of annotated chemical reactions: **>2,300**
  \# of unique kinase inhibitors: **>465,000** **(~340K enumerated from patent chemistries)**

- **KKB Growth Rate:**
  - Average **15-20K** SAR data points added per quarter
  - Average **20-30K** unique structures added per quarter

Kinase Knowledgebase (KKB)

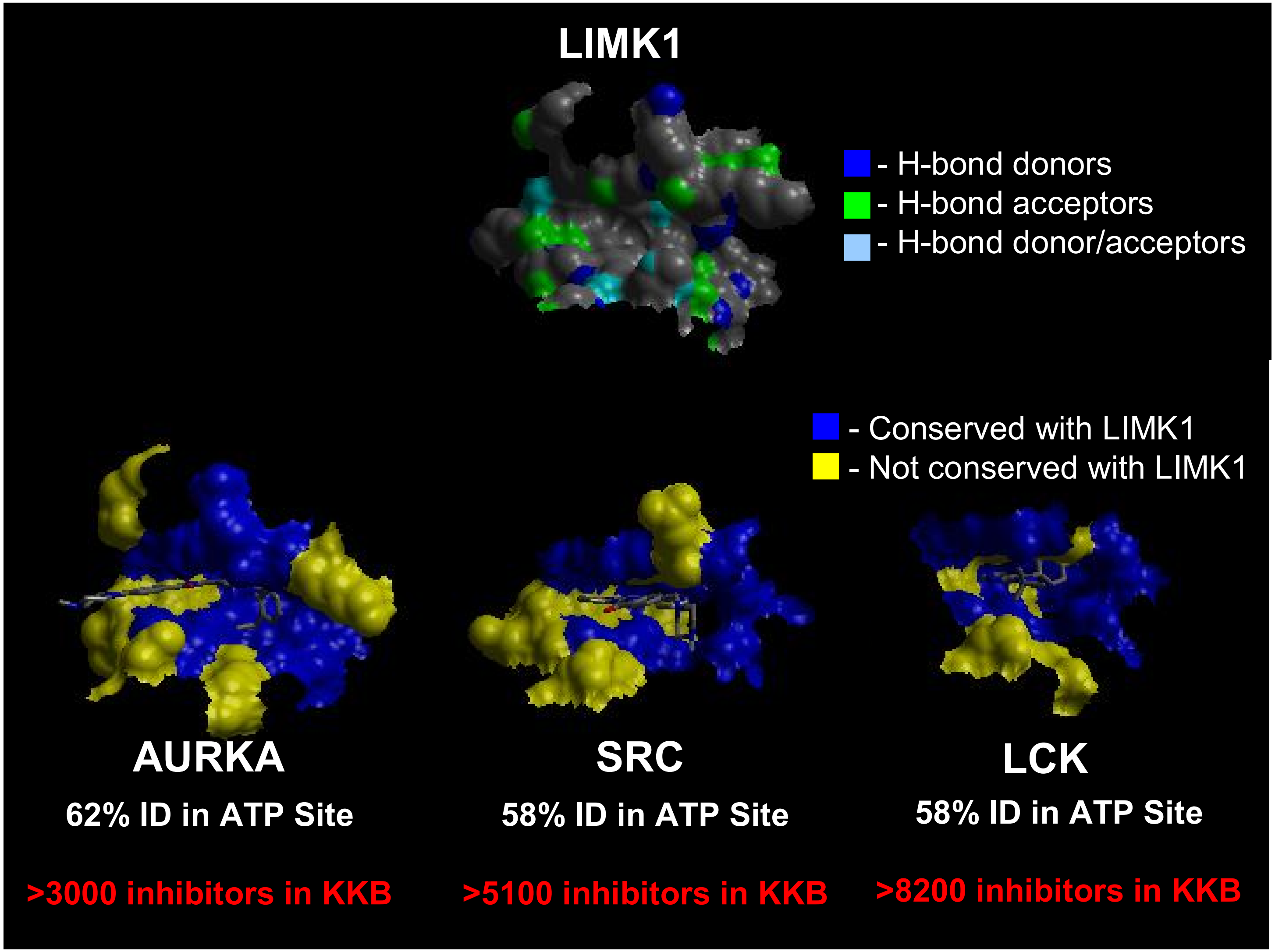
Kinase inhibitor structures and SAR data mined from

Kinase Validation Set

Three sizable datasets freely available to the research community

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

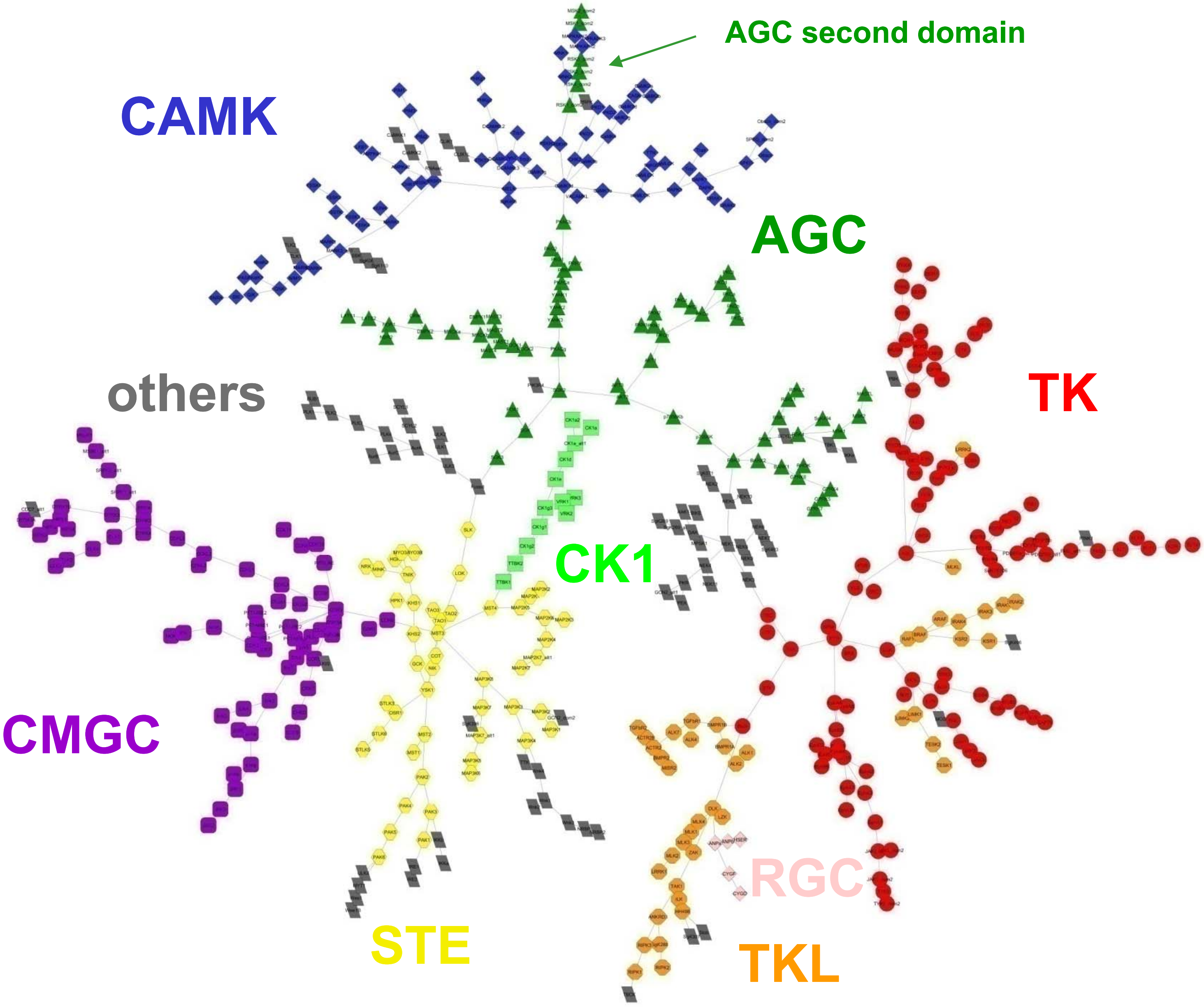
LIMK1 – ATP binding site comparison



The ATP site of LIMK1 shares a high level of homology with several well-studied kinases

Kinome by Sequence

Kinase domain sequence similarities - MST

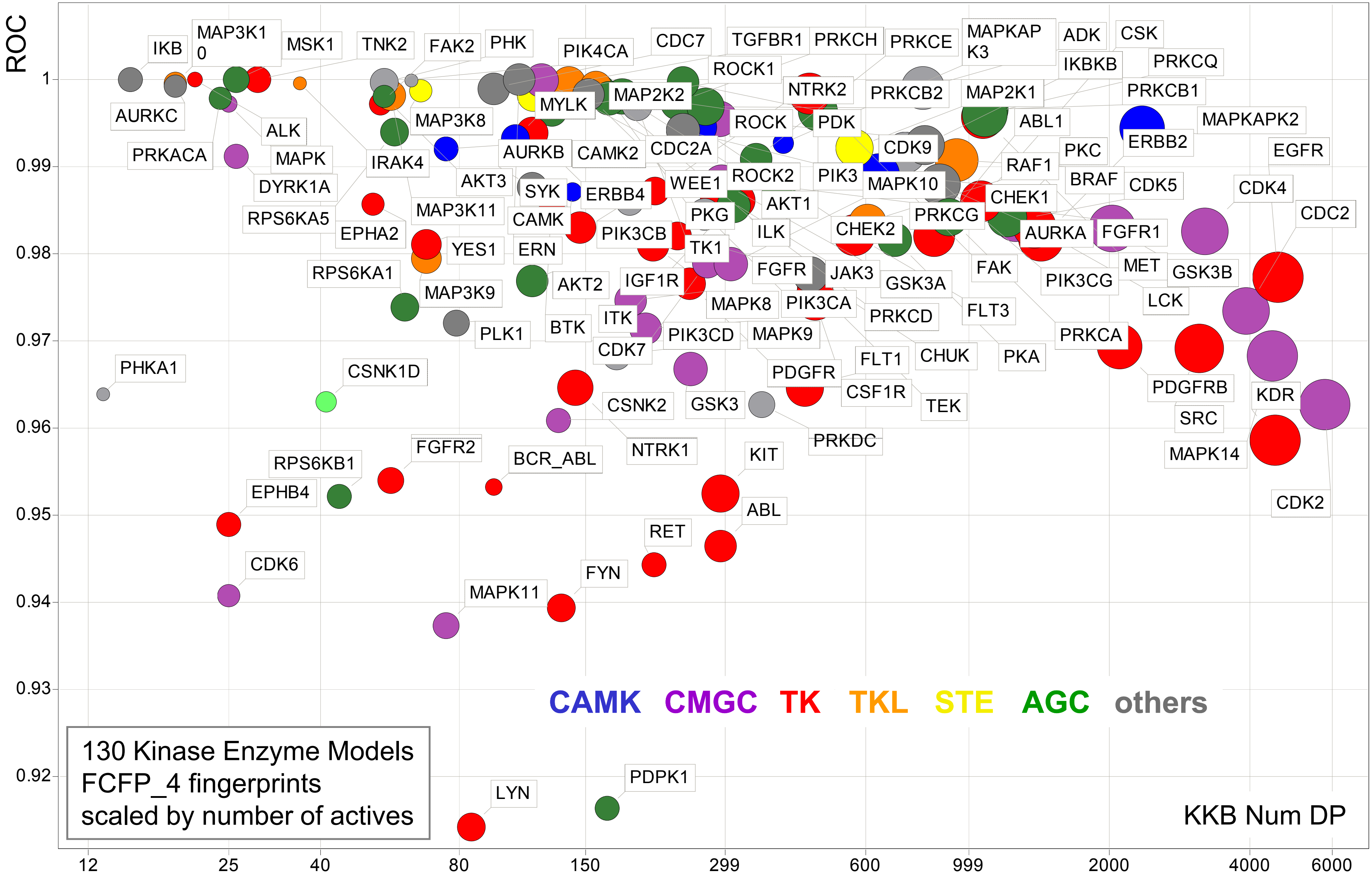


Kinome by SAR

Relating kinase targets by SAR

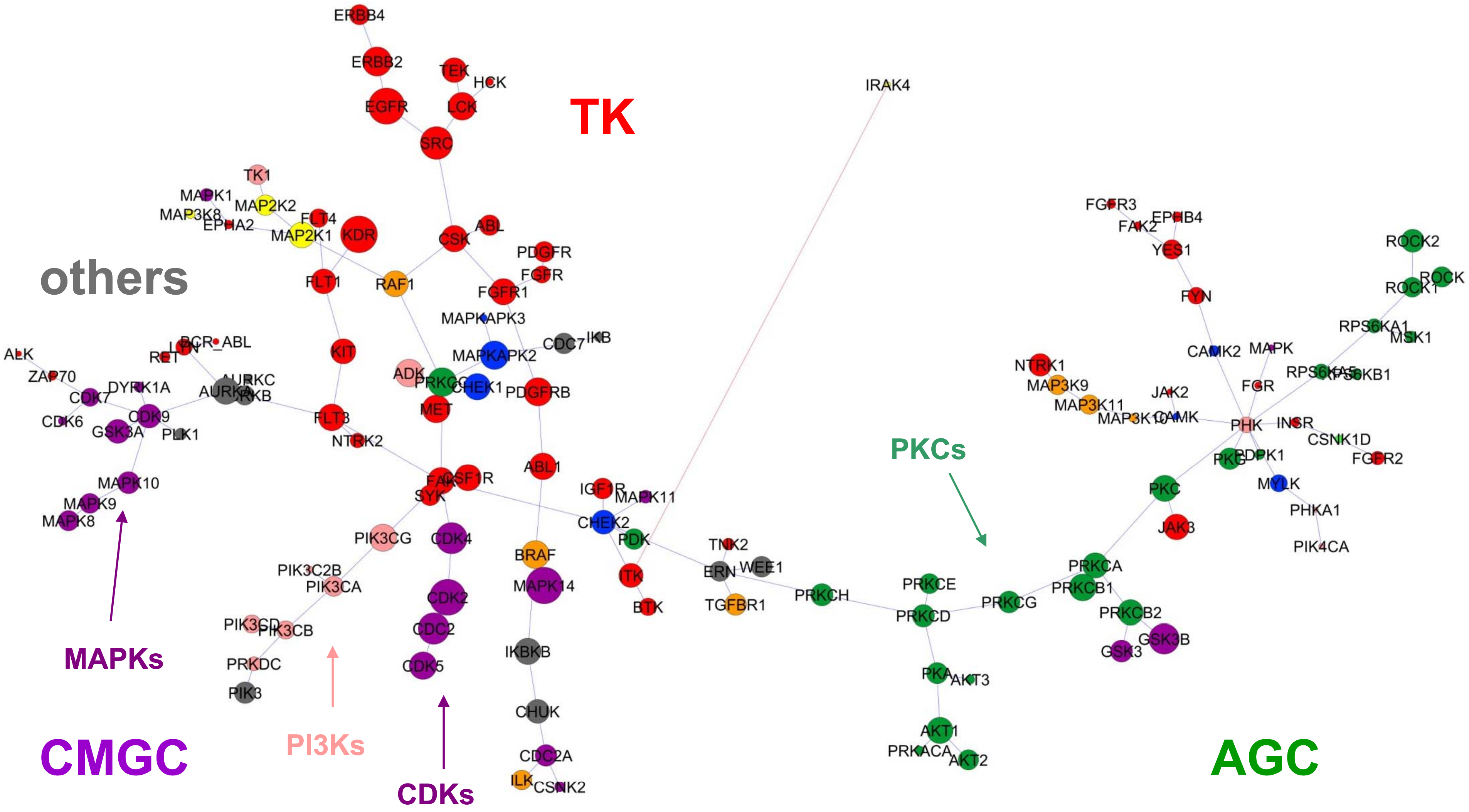
- Relationships derived from Bayesian categorization models
    - Adopted from Schuffenhauer *Org Biomol Chem* **2004** 3256
- Bayesian categorization models built within PipelinePilot:
    - Kinase enzyme assay data, activity cutoff pIC50 > 6.5; all other compounds “negative”
    - Functional group connectivity fingerprints length 4
    - ROC > 0.7
- Bayesian feature weights (~10,000 features) extracted for each model
- Correlation matrix determined between Bayesian vectors
- Visualization via minimum spanning trees (Kruskal algorithm)

Kinase SAR Bayesian models



130 Kinase Enzyme Models
FCFP_4 fingerprints
scaled by number of actives

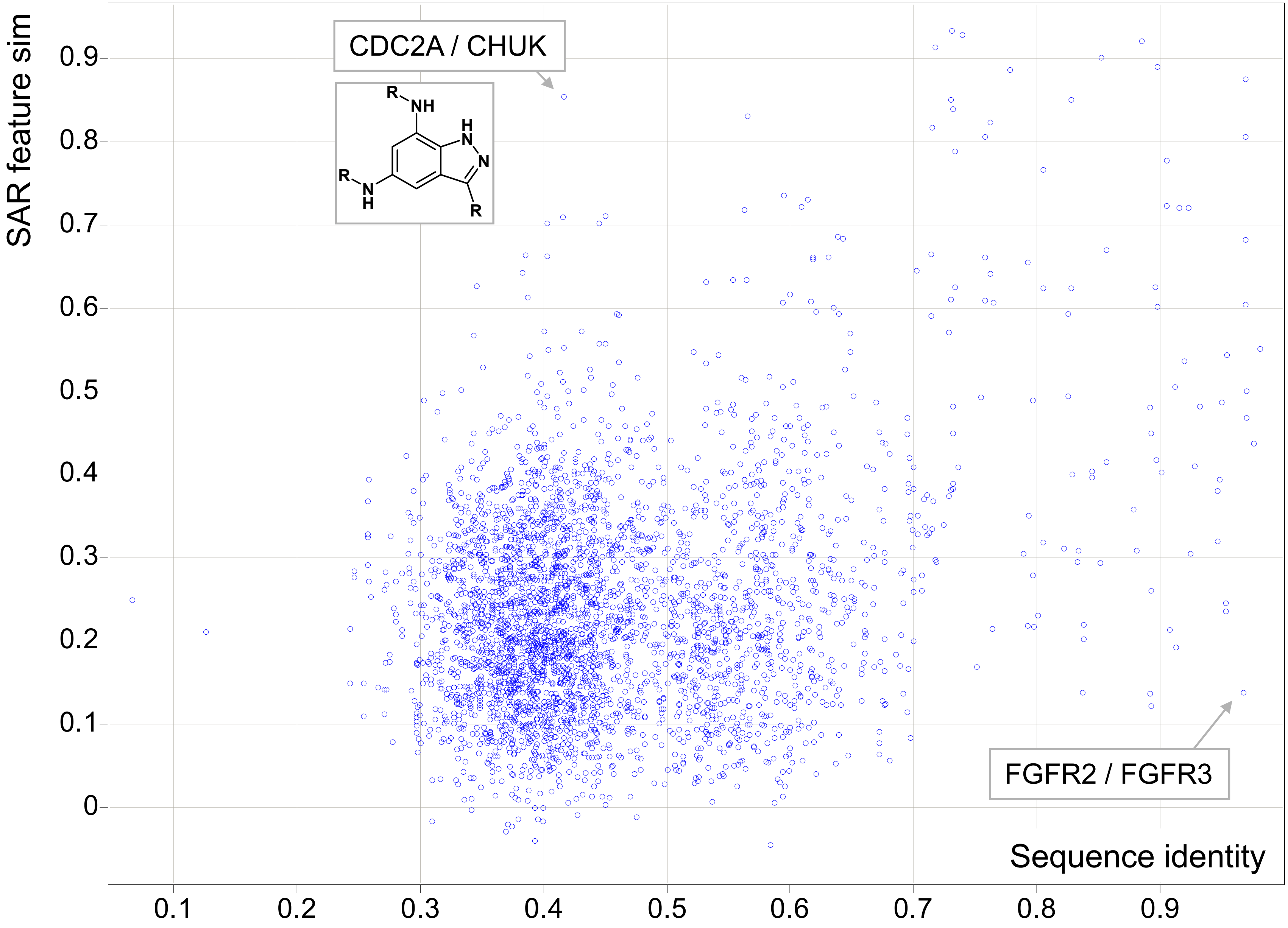
Kinase target relationships by SAR – MST



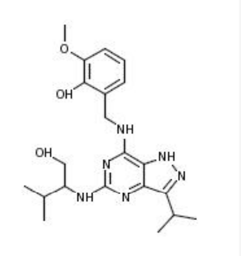
130 kinase models

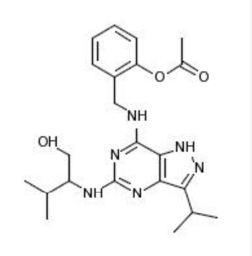
MST – all “similarities” > 0.27

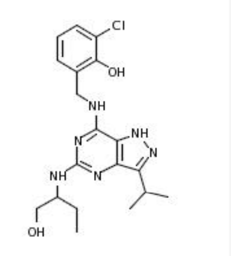
SAR-based similarity vs. Sequence identity



CDC2A and CHUK: > 90 ligands with activity against both targets


CDC2A / 8.2
CHUK / 7.8


CDC2A / 8.2
CHUK / 7.9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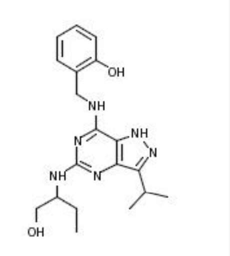
CHUK / 7.9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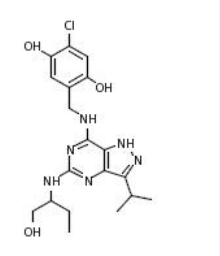
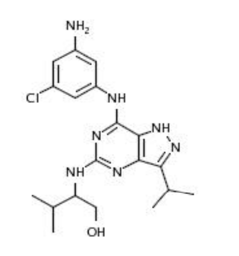
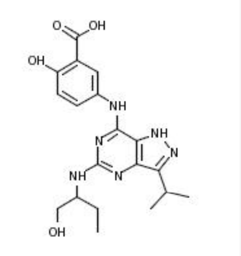
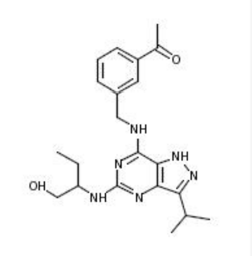
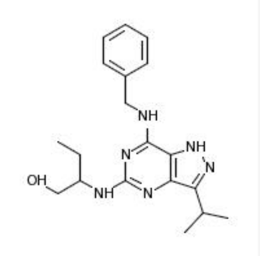
CHUK / 7.9

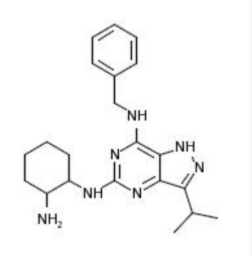

CDC2A / 8.1
CHUK / 7.8

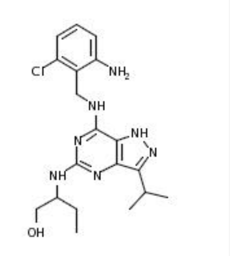

CDC2A / 8.1
CHUK / 7.8

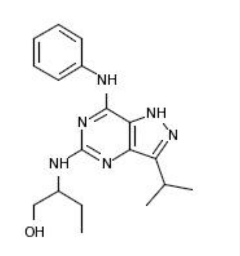

CDC2A / 8.0
CHUK / 7.5

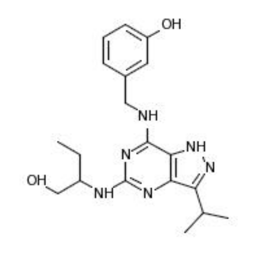

CDC2A / 8.0
CHUK / 7.4

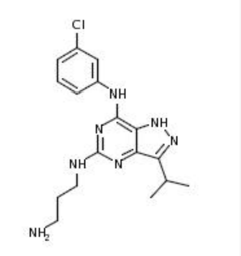

CDC2A / 8.0
CHUK / 7.5

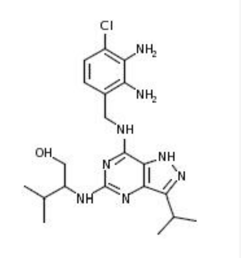

CDC2A / 8.0
CHUK / 7.7

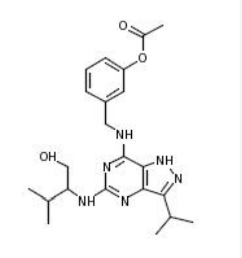

CDC2A / 7.9
CHUK / 8.5

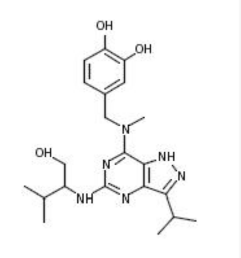

CDC2A / 7.7
CHUK / 7.0


CDC2A / 7.7
CHUK / 7.4


CDC2A / 7.7
CHUK / 7.1


CDC2A / 7.7
CHUK / 7.4


CDC2A / 7.7
CHUK / 7.2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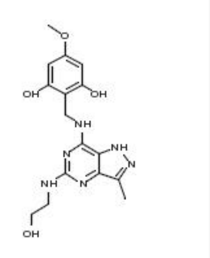
CHUK / 7.0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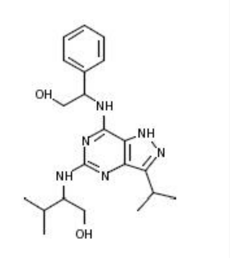
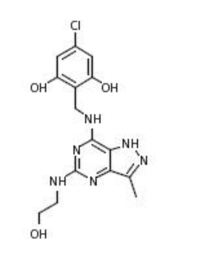
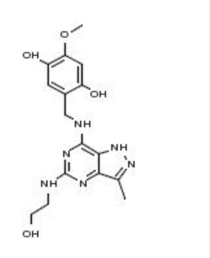
CHUK / 8.0


CDC2A / 7.2
CHUK / 6.8


CDC2A / 7.2
CHUK / 7.9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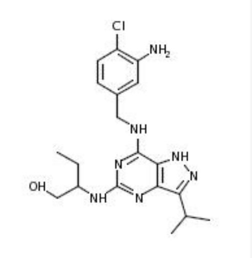
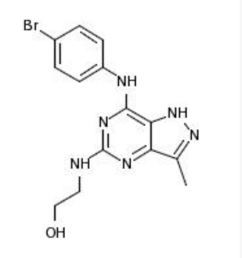
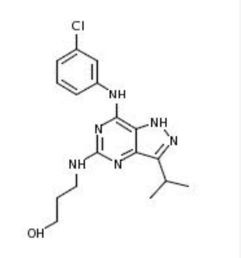
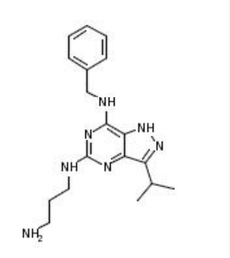
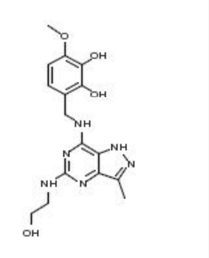
CHUK / 7.8


CDC2A / 7.1
CHUK / 7.6


CDC2A / 7.1
CHUK / 7.7


CDC2A / 7.1
CHUK / 6.7


CDC2A / 7.1
CHUK / 7.0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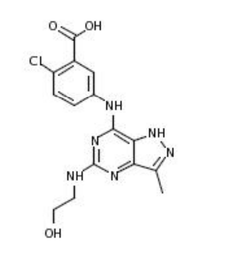
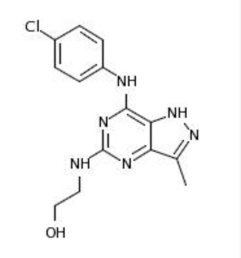
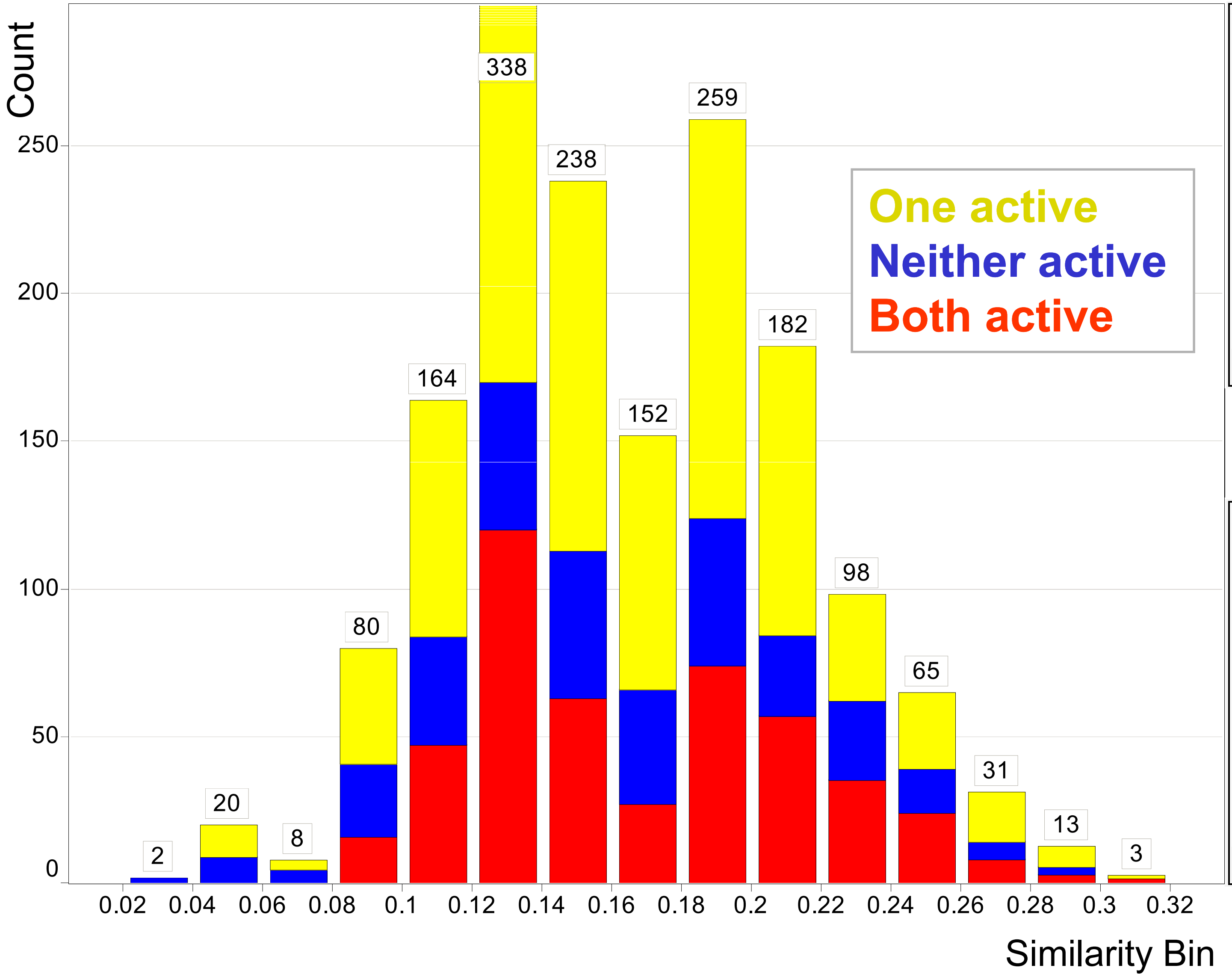

CDC2A / 7.1
CHUK / 8.0

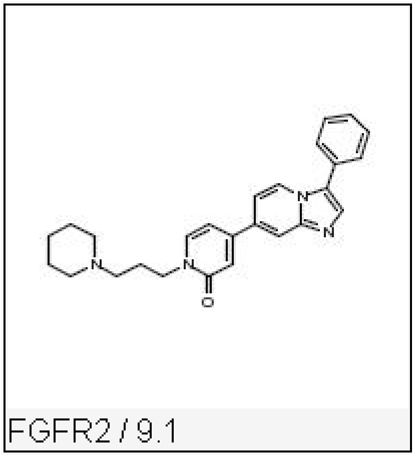

CDC2A / 7.0
CHUK / 7.7

FGFR2 / FGFR3: no similar ligands

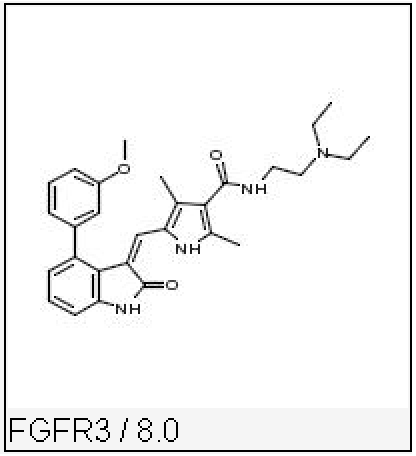
FCFP4 Tanimoto (all pairs)



Top active FGFR2:



Top active FGFR3:

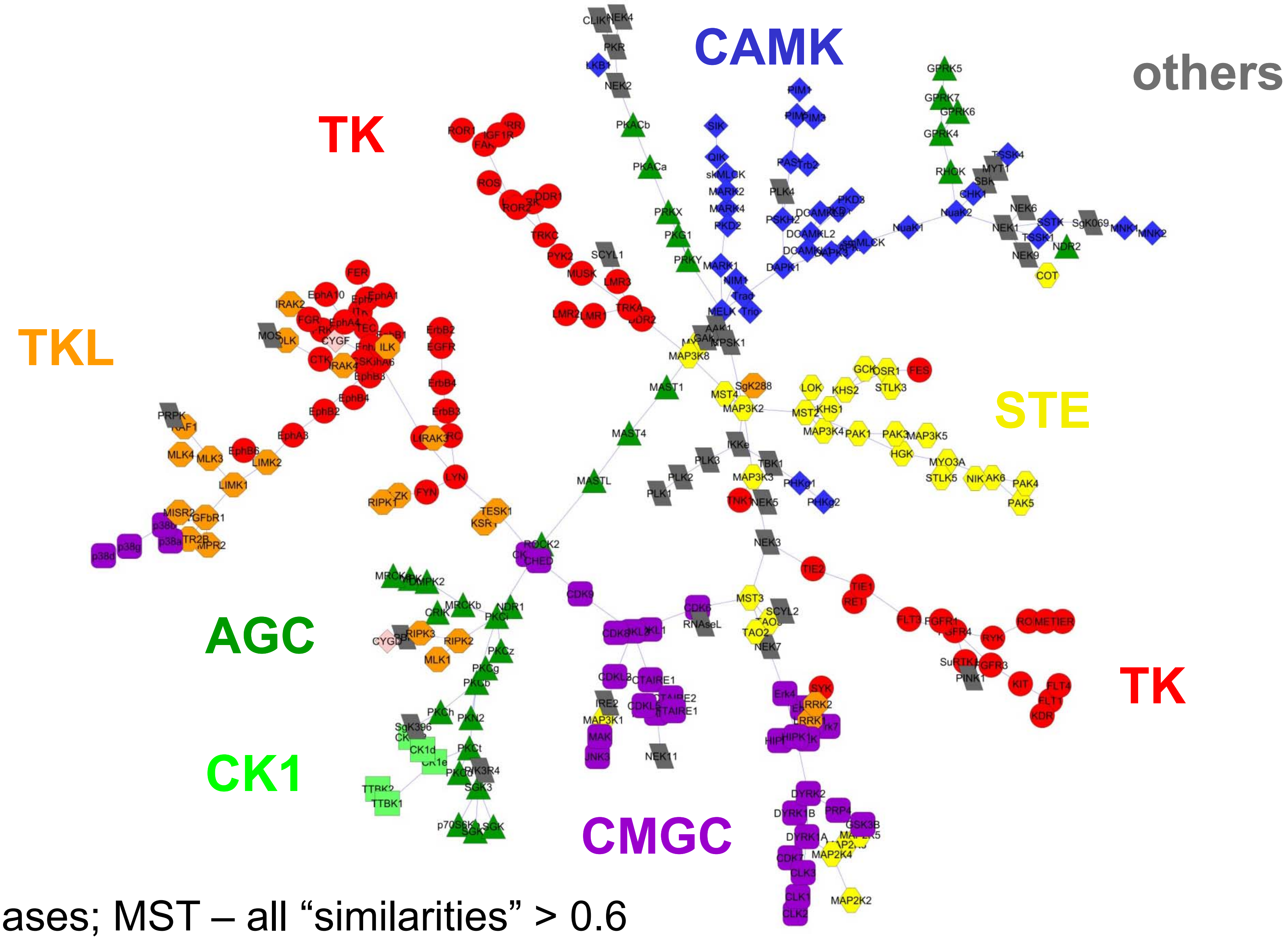


Kinome by structure binding site similarities

Relating kinases by ATP binding-site similarity

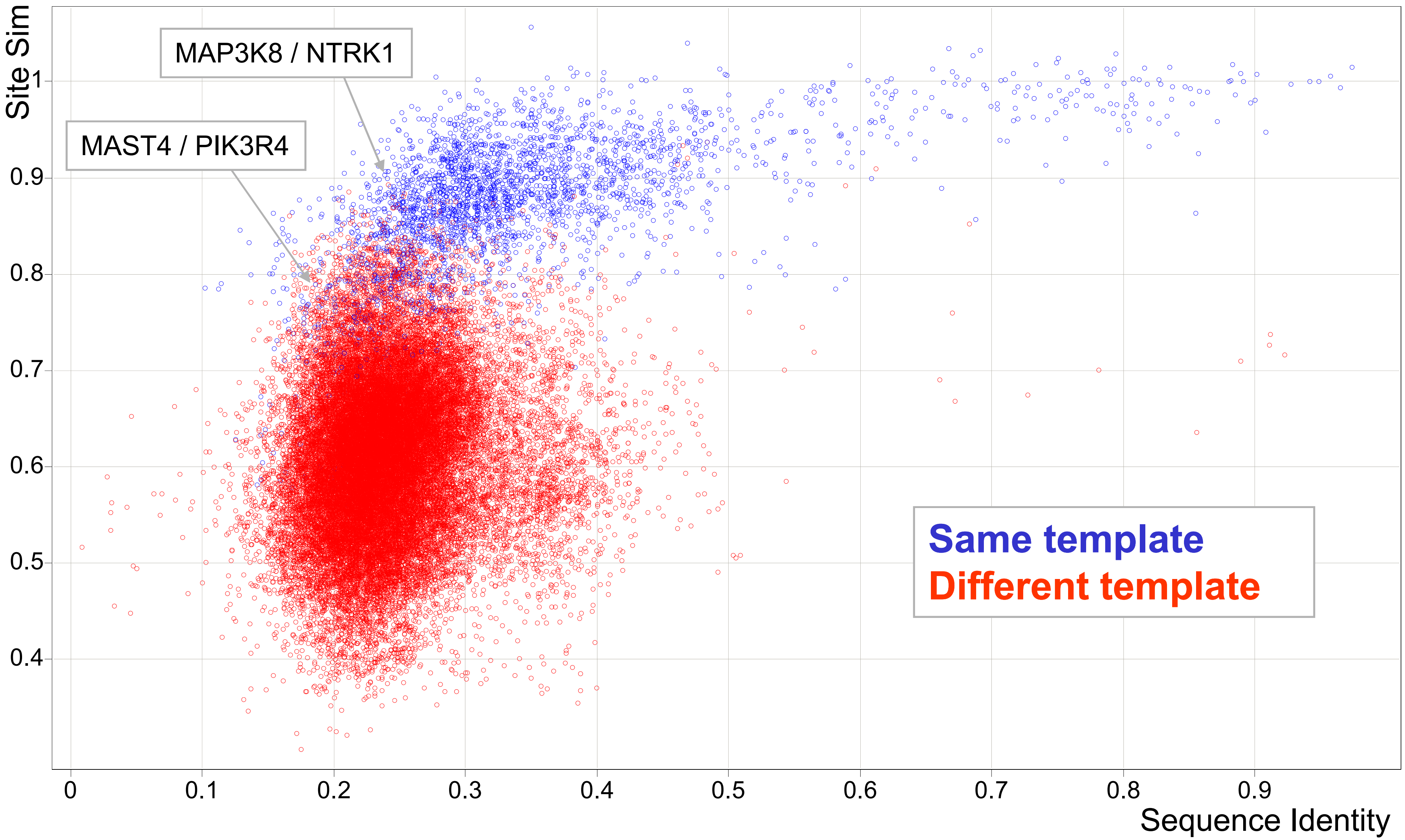
- Human Kinase domain sequences extracted (Sugen, Swissprot, PFAM)
- Human Kinome (500 sequences) modeled using STRUCTFAST
  - Multiple models per sequence (subset of 263 presented here)
- Binding sites for all models computed (SiteSeeker)
- Binding site similarity scores computed (SiteSorter)
- Similarity scores normalized: AB_Norm := AB / (AA + BB - AB)
  - AB – Site Similarity between sites A & B
  - AA / BB – “Self Site” Similarity Scores
- Analysis and visualization with MST

Kinase Site Similarity Relationships – MST



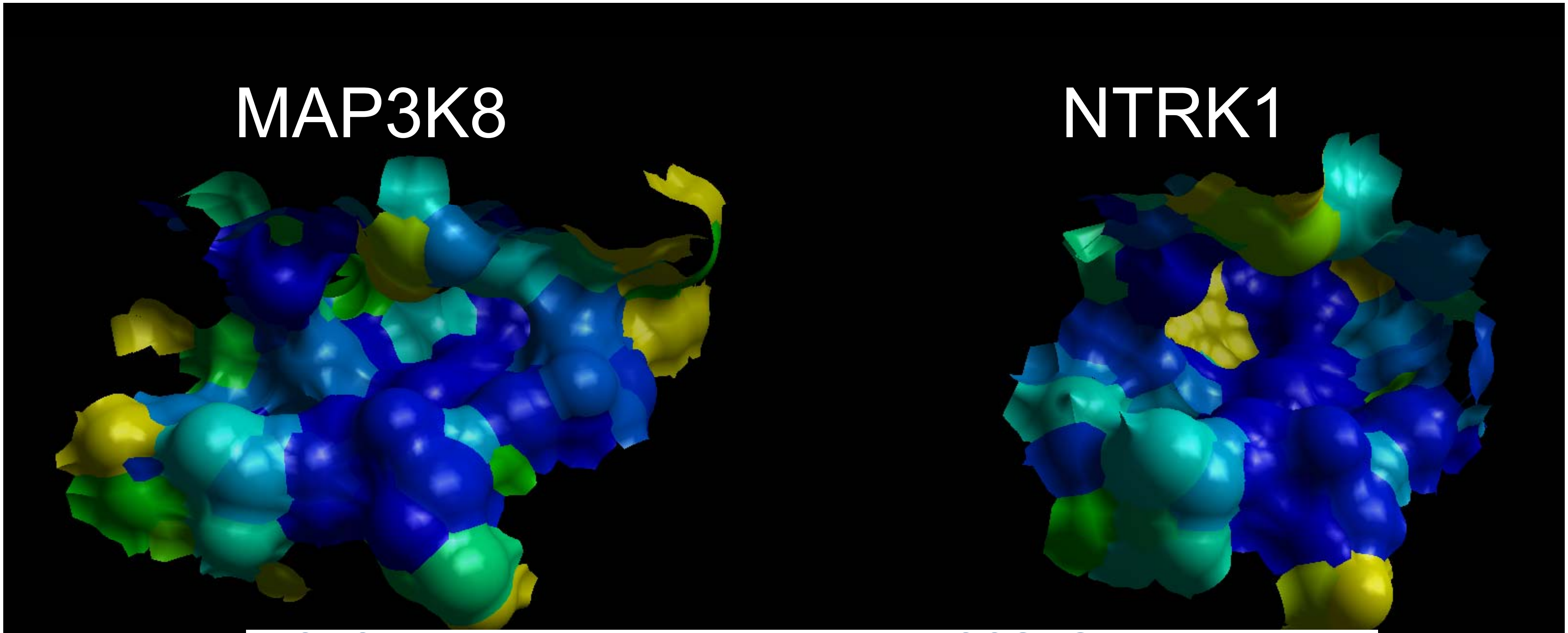
263 kinases; MST – all “similarities” > 0.6

Sequence vs. Site Similarity



Similar sites – different sequences

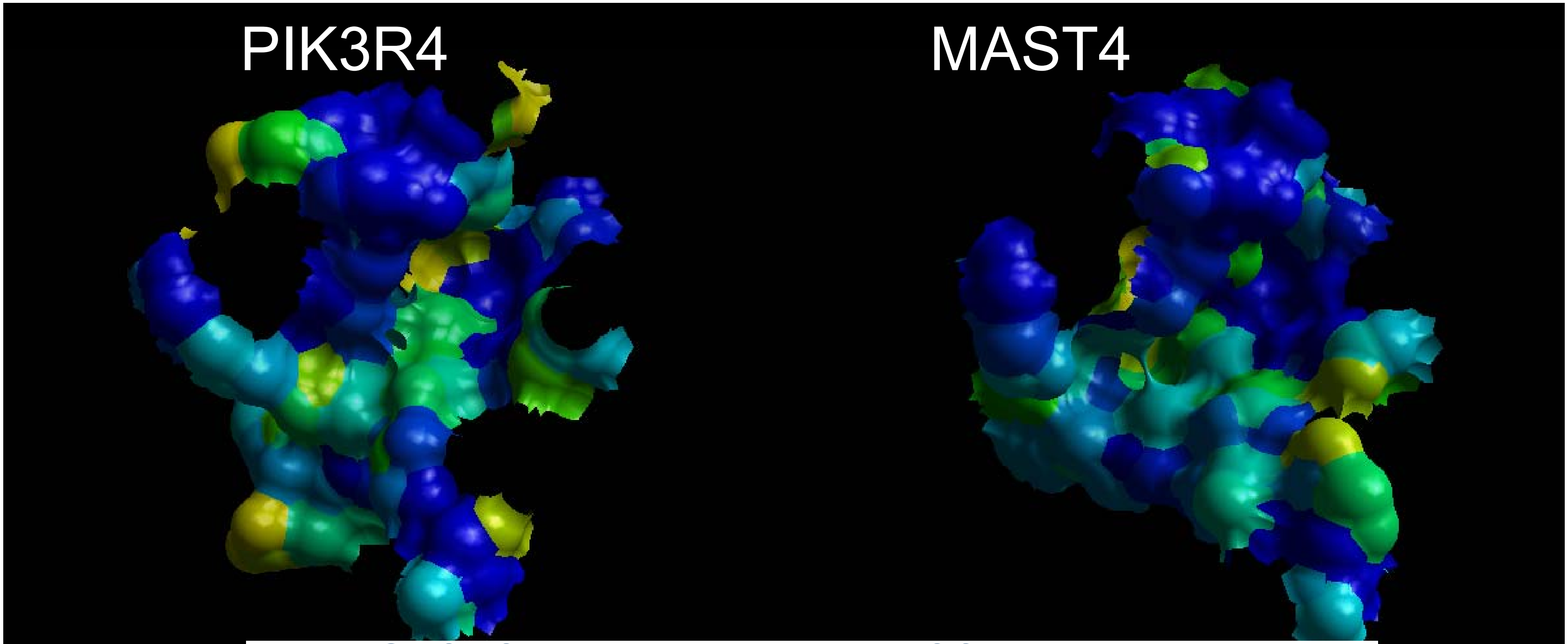
- STE_STE11_MAP3K8: template 1u5rA
- TK_Trk_TRKA (NTRK1): template 1ir3A



```
MAP3K8 LGKGAY.V.A.K.V.E.V.MEFV.GGS.S.D.NN.M.D
 NTRK1 LGEGAF V A K - E V FE-M -GD - D -N L D
```

Similar sites – different site AA composition

- AGC_MAST_MAST4: template 1z5mA
- Other_VPS15_PIK3R4: template 1z5mA
- Site sequence similarity: 0.2
- Normalized (physicochemical) site similarity: 0.78



```
MAST4   .K.ISNG.GAV.A.K.V.MEYVEGGD.T.K.DN.L.TD
PIK3R4  .K.LGST.FKV.K.F.P.FRQYVRDN.D.S.EN.M.TD
```

What did we learn?

- Expected global trend:Similar sequence results in physicochemical- and fold-similar binding sites
- Dissimilar sequences do not always result in different binding sites
- Binding site similarities group in “patches” by domain sequence similarity
  - Subtle differences in site relationships among groups and sub-types
- Modeling templates influence results:
  - For many kinases no experimental structures exist, but can be modeled
  - Growing body of structural information will optimize the picture
- Body of selective Kinase compounds continues to grow
- In principle, small molecules can be optimized to differentiate between very similar (sequence) kinases

Conclusions and Next steps

- Quantifying similarity relationships within the Kinome can provide insight in early Kinase drug development
- Similarity within the Kinome should consider SAR-based <u>and</u> structure-based binding site similarity (v. domain sequence-based similarity)
- Next steps include
  - Analyze trends with respect to DFG-In/DFG-out
  - Quantify template effects
  - Investigate effects of site size and predicted vs. templated sites

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- Aleksandar Poleksic

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