Prediction of pKa from chemical structure using free and open-source tools

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pKa

What Is It ?

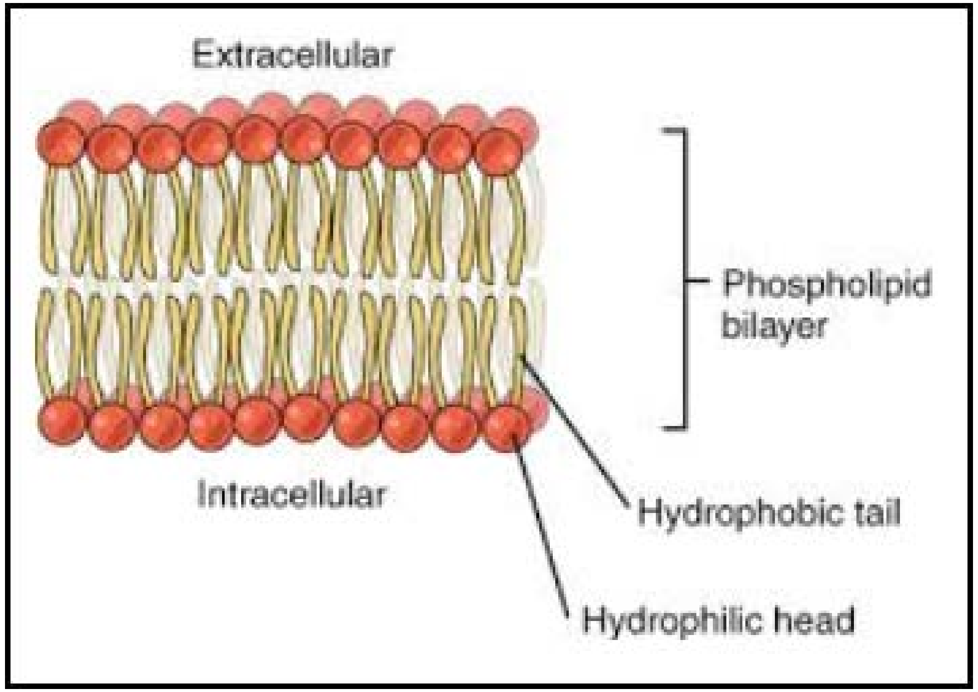
Why Is It Important ?

How Can We Use It ?

Absorption Of Chemicals Into Cells

Question: Which kind of chemicals will preferentially partition into the plasma membrane, charged or uncharged (ionized or non-ionized)?

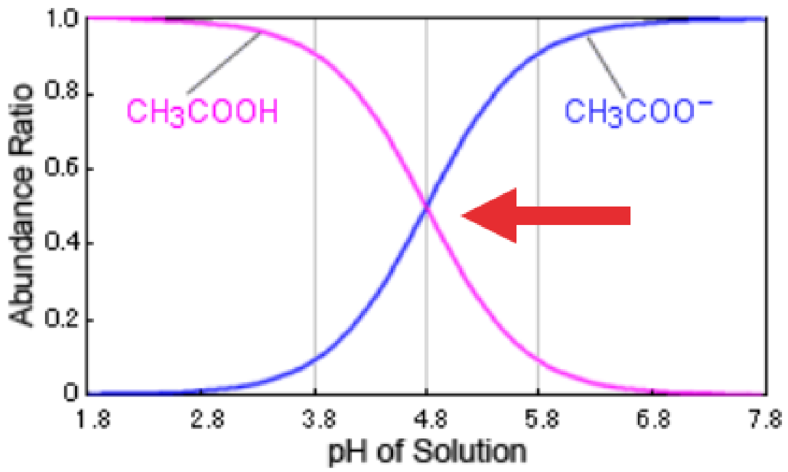
- In general, chemicals that make it into the plasma membrane (lipid bilayer) have a better chance of getting into the cell.
- The plasma membrane facing the extracellular space is hydrophobic and lipophilic so will charged or uncharged molecules cross the membrane best?



What Is pKa ?

- pKa is a property that tells us how acidic (or basic) a chemical is. The lower the pKa the stronger the acid.

- The pKa influences the protonation state (charged or uncharged) of the chemical in solution at a given pH value.



Relationship Between pH of Solution and Dissociation Status of Acetic Acid

The pKa of acetic acid is 4.75

At the pH of the pKa, the chemical is 50% ionized

Chemistry 101

- K_a is the acid dissociation constant which is a measure of the strength of an acid in solution.

- K_a is an equilibrium constant and pK_a is the -log10 value of K_a, therefore for acids

$$HA \leftrightarrow A^{-} + H^{+}$$

$$K_A = \frac{[A^{-}]\,[H^{+}]}{[HA]}$$

$$pK_a = -log10\ \frac{[A^{-}]\,[H^{+}]}{[HA]}$$

pKa Importance

- pKa values reflect the **ionization state** of a chemical

- Why is this important?
  - Ionization affects lipophilicity, solubility, protein binding and the ability of a chemical to cross the plasma membrane
    - This affects ADMET

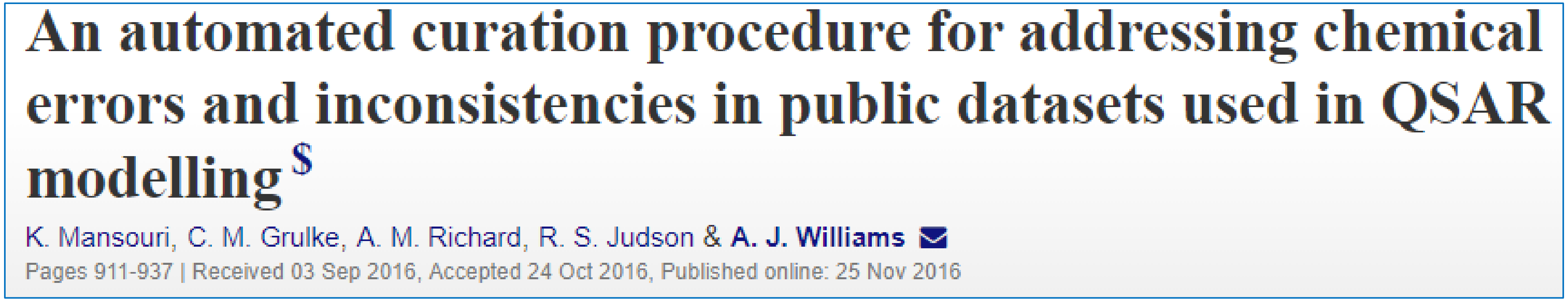
- pKa can be used, and is many times required, for
  - Physiologically Based Pharmacokinetic (PBPK) modeling
  - In Vitro To In Vivo Extrapolation (IVIVE)
  - Prediction of tissue:plasma partition coefficients

Using Open Source Software and Data to Build a pKa Prediction Algorithm:

Data Quality, Algorithm Development and Applications

Good Cheminformatics Data Is Hard To Obtain, Especially pKa

- Obtaining high-quality data sets is difficult
- Curation is generally VERY time-consuming without optimized workflows
- Many issues exist with available datasets



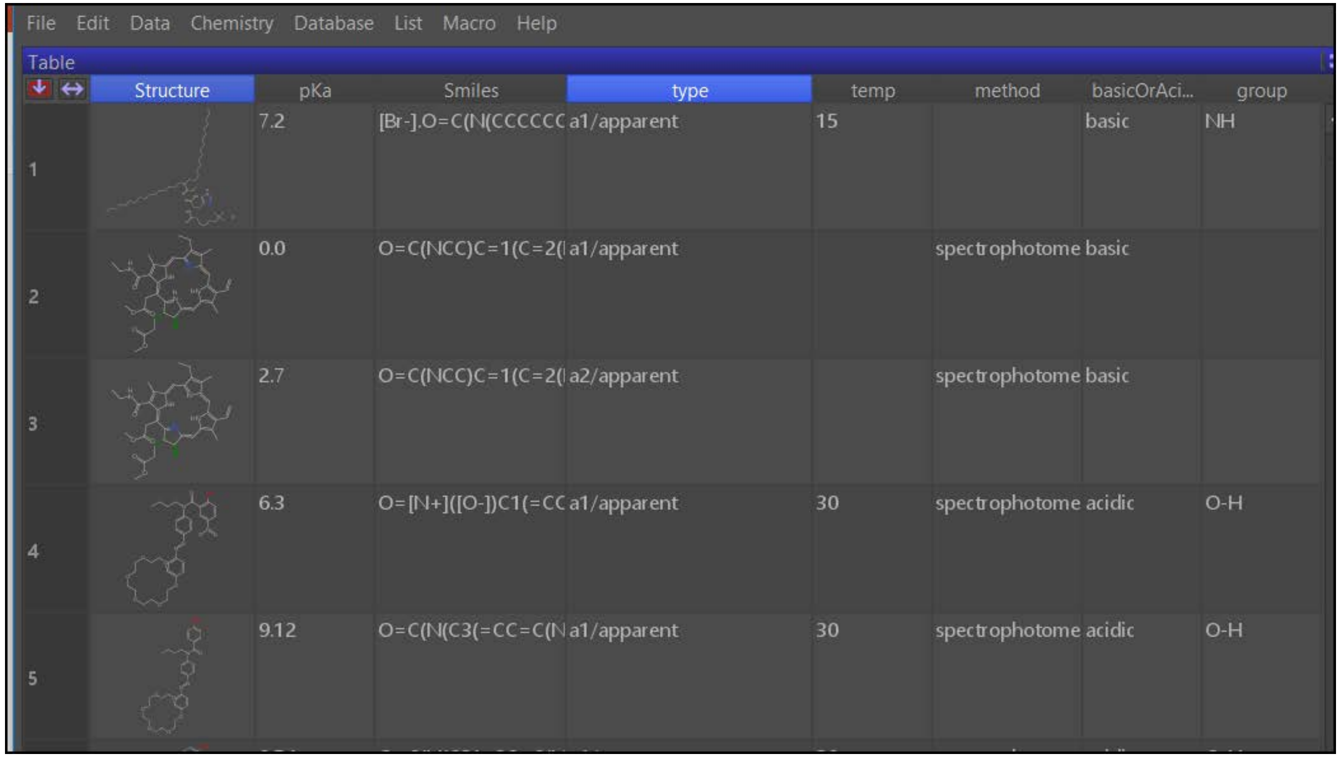
An automated curation procedure for addressing chemical errors and inconsistencies in public datasets used in QSAR modelling[$]

K. Mansouri, C. M. Grulke, A. M. Richard, R. S. Judson & **A. J. Williams**

Pages 911-937 | Received 03 Sep 2016, Accepted 24 Oct 2016, Published online: 25 Nov 2016

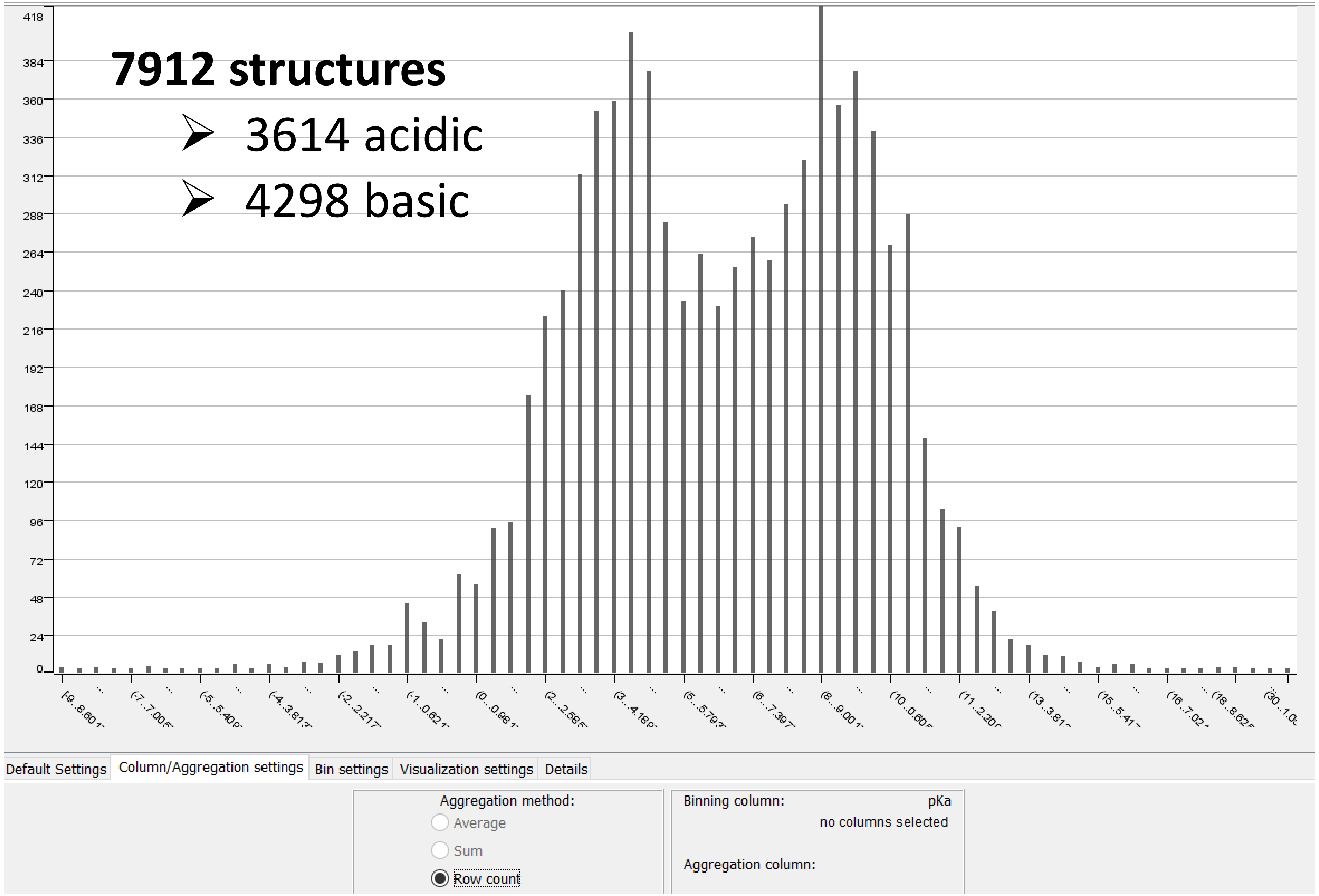
7912 Chemicals With pKa In Water Are Available From The Datawarrior Website

- This is not a widely-known dataset
- Datawarrior didn’t list the references for the data
- We checked ~60 DataWarrior chemicals against literature and the results were good (< 0.3 pKa units difference between DataWarrior and the literature)



	Structure	pKa	Smiles	type	temp	method	basicOrAci...	group
1		7.2	[Br-].O=C(N(CCCCCC	a1/apparent	15		basic	NH
2		0.0	O=C(NCC)C=1(C=2(	a1/apparent		spectrophotome	basic	
3		2.7	O=C(NCC)C=1(C=2(	a2/apparent		spectrophotome	basic	
4		6.3	O=[N+]([O-])C1(=CC	a1/apparent	30	spectrophotome	acidic	O-H
5		9.12	O=C(N(C3(=CC=C(N	a1/apparent	30	spectrophotome	acidic	O-H

Dataset Has A Bimodal Distribution



7912 structures

- ➢ 3614 acidic
- ➢ 4298 basic

Default Settings | Column/Aggregation settings | Bin settings | Visualization settings | Details

Aggregation method:

○ Average

○ Sum

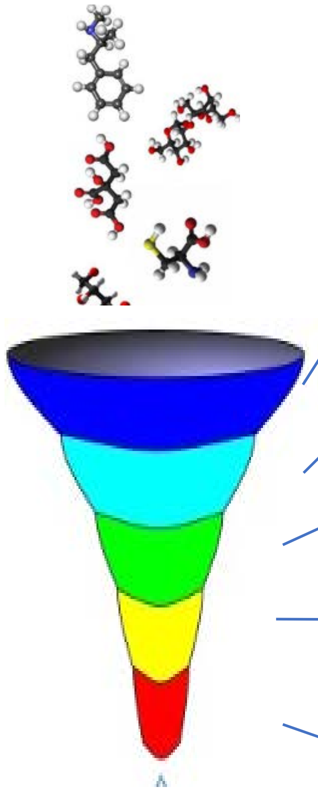
● Row count

Binning column: pKa

no columns selected

Aggregation column:

QSAR-ready Workflow



Remove inorganics and mixtures

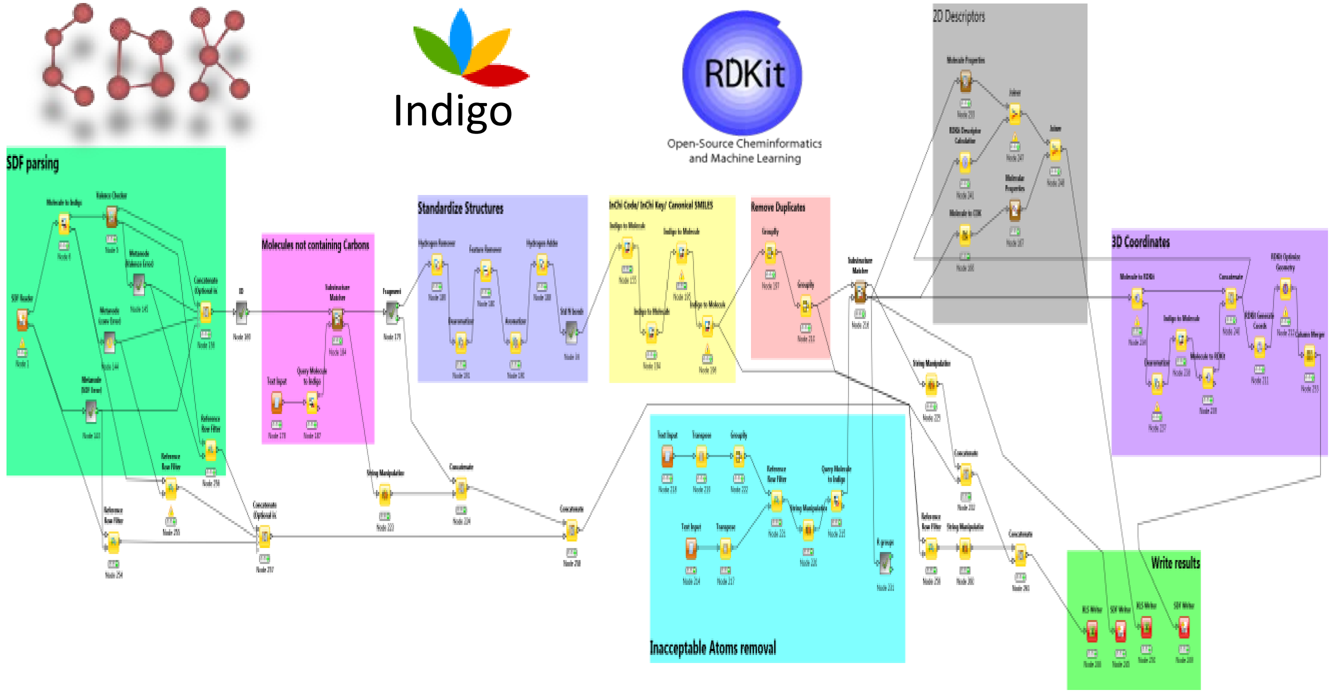
Clean salts and counterions

Normalize Nitros & tautomers

Remove of duplicates

Final inspection

QSAR-ready structures


Indigo
RDKit
Open-Source Cheminformatics and Machine Learning
SDF parsing
Molecules not containing Carbons
Standardize Structures
InChI Code/ InChI Key/ Canonical SMILES
Remove Duplicates
2D Descriptors
3D Coordinates
Inacceptable Atoms removal
Write results

KNIME workflow

QSAR-ready analysis

Full dataset:

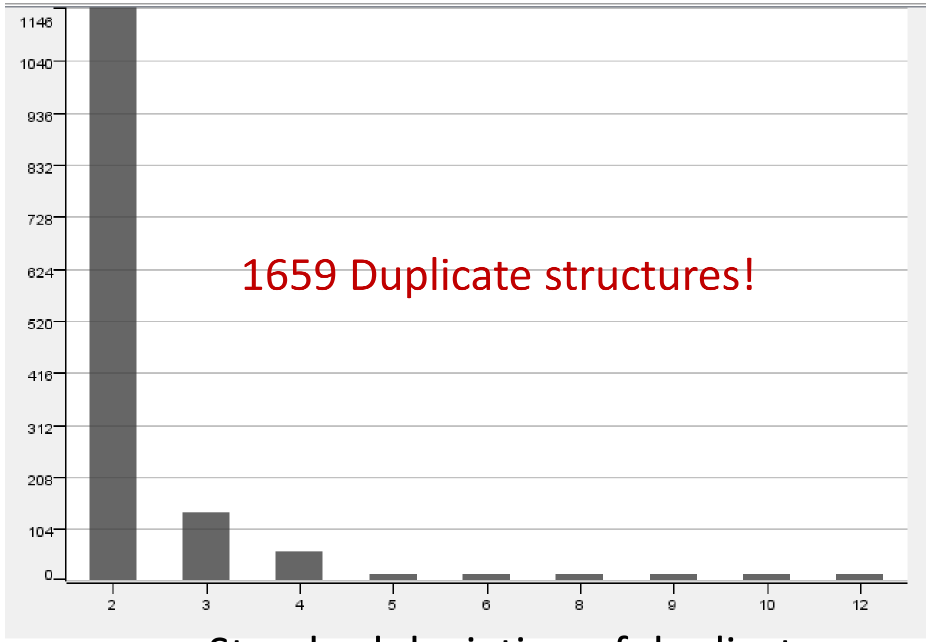
- 7904 QSAR-ready structures
- 6245 unique QSAR-ready structures

Acidic dataset

- 3610 QSAR-ready total structures
- 3260 unique QSAR-ready structures

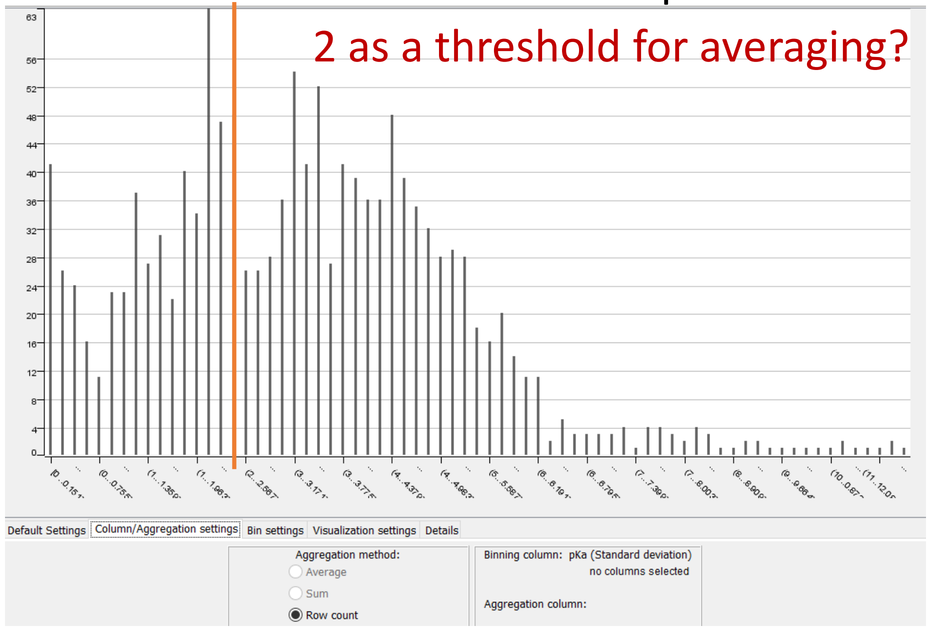
Basic dataset

- 4294 QSAR-ready total structures
- 3680 unique QSAR-ready structures



1659 Duplicate structures!

Standard deviation of duplicates



2 as a threshold for averaging?

Aggregation method:

○ Average

○ Sum

● Row count

Binning column: pKa (Standard deviation)

no columns selected

Aggregation column:

Modeling Options

To deal with complexity of multiple pKa's for a chemical, three datasets were produced and analyzed:

Option 1: Only structures with a unique pKa value were used.

- Pre-categorized Acidic dataset: 2960
- Pre-categorized Basic dataset: 3158
- Combined: 4897 (no amphoteric)

Option 2: A unique value/structure (average value if stdDev<2)

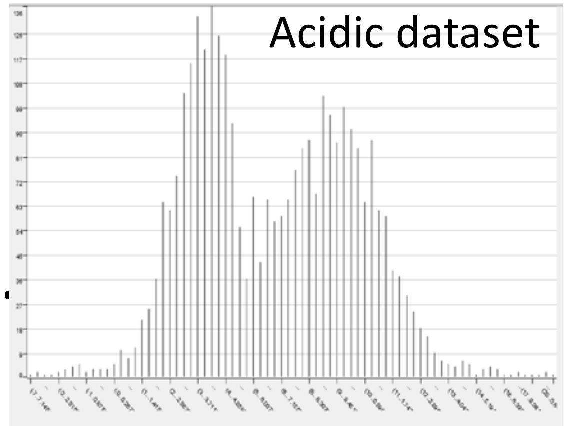
- Pre-categorized Acidic dataset: 3095
- Pre-categorized Basic dataset: 3370
- Combined: 5263 (no amphoteric)

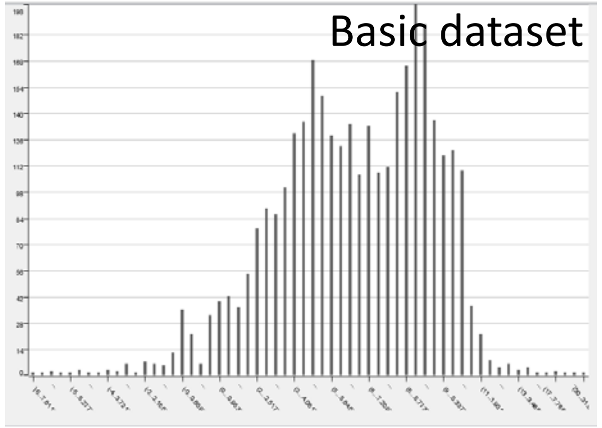
Option 3: The entire list of QSAR-ready chemicals was used with averaging for similar pKa values.

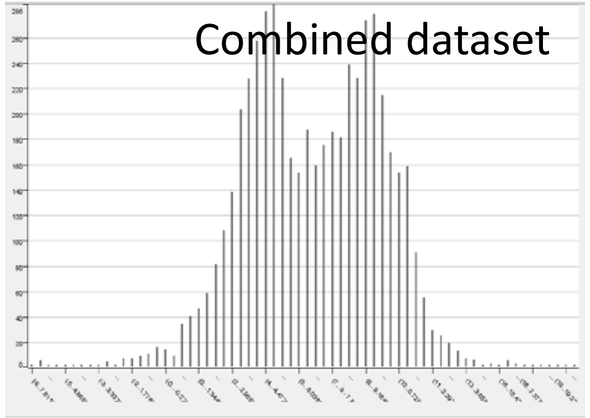
if stdDev =< 1: Average value;

if stdDev > 1: strongest pKa (min acidic/max basic)

- Acidic dataset: 3260 unique QSAR-ready structures
- Basic dataset: 3680 unique QSAR-ready structures





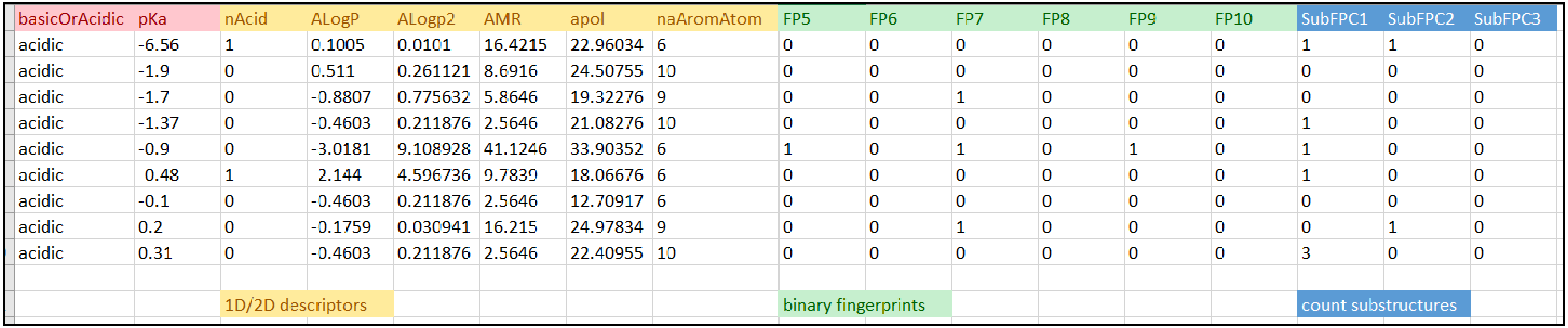


Machine Learning And Predicting pKa

- The term Machine Learning was coined in 1959

  Machine learning explores the study and construction of algorithms that can learn from and make predictions on data through building a model from sample inputs.

- Each chemical with a pKa produces ~16.5K data points in 12 datasets
- We need to find the best combination of variables (columns) for pKa prediction



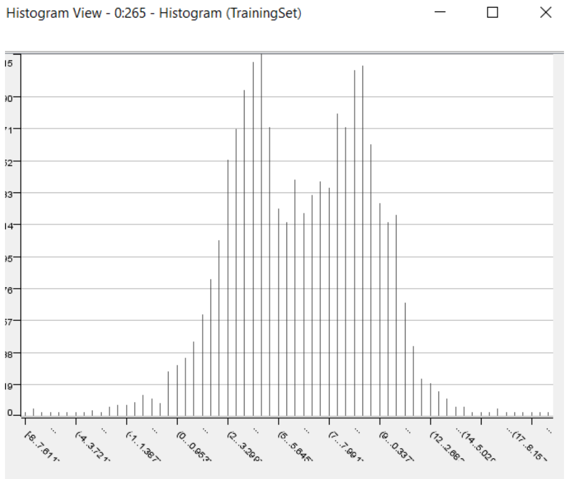
basicOrAcidic	pKa	nAcid	ALogP	ALogp2	AMR	apol	naAromAtom	FP5	FP6	FP7	FP8	FP9	FP10	SubFPC1	SubFPC2	SubFPC3
acidic	-6.56	1	0.1005	0.0101	16.4215	22.96034	6	0	0	0	0	0	0	1	1	0
acidic	-1.9	0	0.511	0.261121	8.6916	24.50755	10	0	0	0	0	0	0	0	0	0
acidic	-1.7	0	-0.8807	0.775632	5.8646	19.32276	9	0	0	1	0	0	0	0	0	0
acidic	-1.37	0	-0.4603	0.211876	2.5646	21.08276	10	0	0	0	0	0	0	1	0	0
acidic	-0.9	0	-3.0181	9.108928	41.1246	33.90352	6	1	0	1	0	1	0	1	0	0
acidic	-0.48	1	-2.144	4.596736	9.7839	18.06676	6	0	0	0	0	0	0	1	0	0
acidic	-0.1	0	-0.4603	0.211876	2.5646	12.70917	6	0	0	0	0	0	0	0	0	0
acidic	0.2	0	-0.1759	0.030941	16.215	24.97834	9	0	0	1	0	0	0	0	1	0
acidic	0.31	0	-0.4603	0.211876	2.5646	22.40955	10	0	0	0	0	0	0	3	0	0
		1D/2D descriptors						binary fingerprints						count substructures		

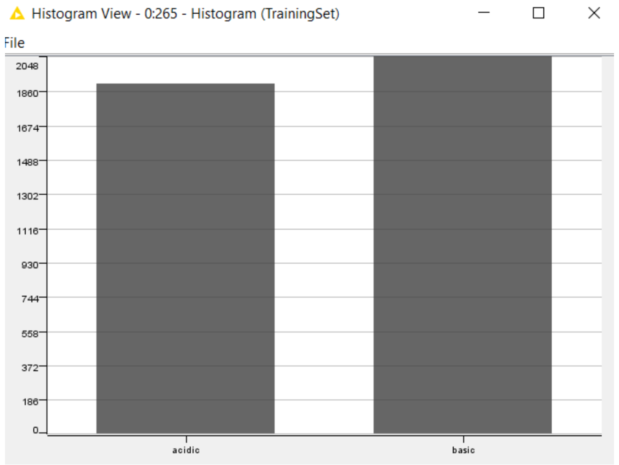
Train And Test Sets For Modeling

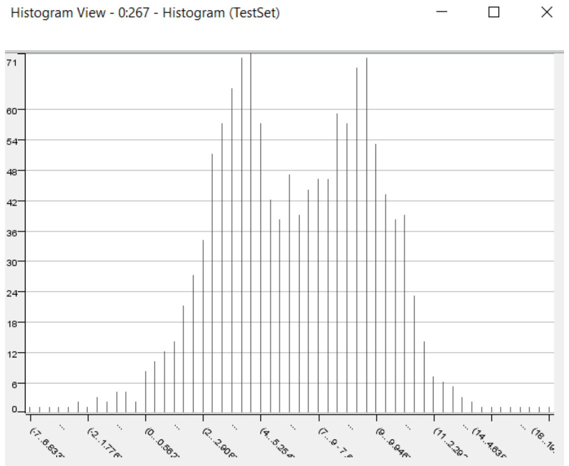
For each one of the data options:

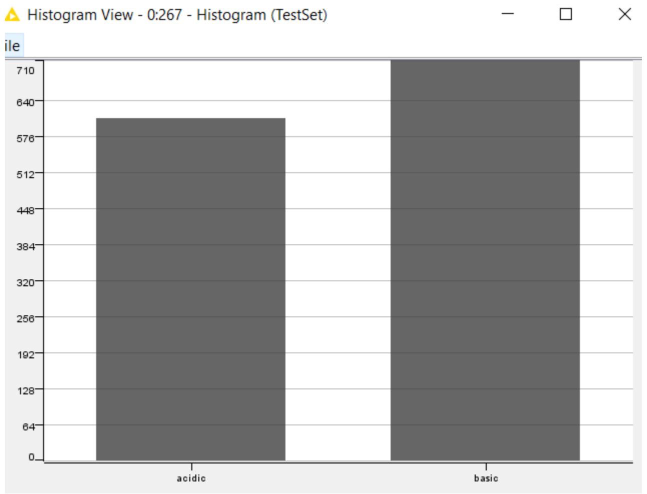
- Split into training (75%) and test (25%)
- Keep similar distribution of pKa values
- Keep similar distribution of acidic and basic pKas for combined datasets
- Descriptors (and fingerprints) are generated for all QSAR-ready structures and can be matched by the generic ID (integers)

A classification model to determine if a molecule will have an **acidic pKa**, **basic pKa** or **both** is trained too.









Training Models

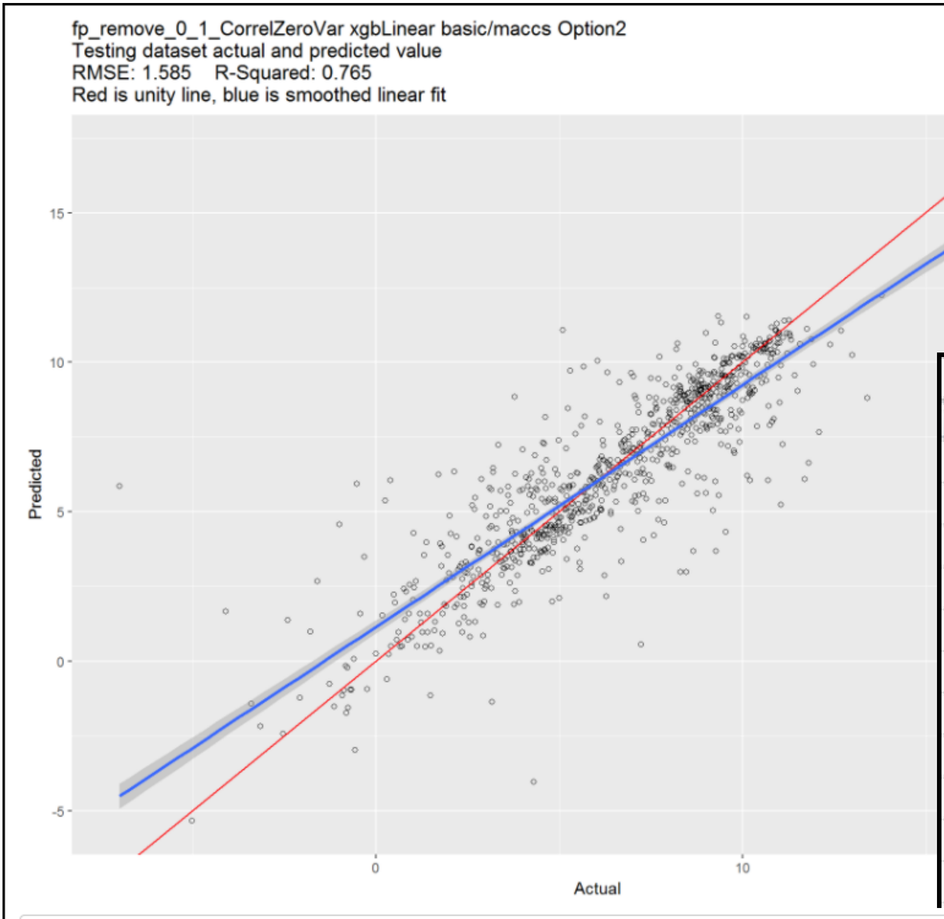
- Create model and estimate performance using only the training dataset
- 5-fold cross-validation was used for training, model performance evaluation and tuning
- Root mean squared error (RMSE) was used as a performance metric for training optimization.
- Choice of machine learning methods:
  - Extreme Gradient Boosting (XGBoost), the advanced **traditional (shallow) machine learning** (SML) method.
  - Deep Neural Network (DNN), a **deep machine learning method** (DML).
  - Support Vector Machines (SVM): defines a decision boundary that optimally separates two classes by maximizing the distance between them.

XGBoost training method

- XGBoost is an implementation of gradient boosted decision trees designed for speed and performance.
- Coding was done using R. The caret and xgboost packages were used for all analysis.
- RMSE was a metric to be minimized. 5-fold cross validation was used to train the model using the training dataset
- Highly correlated variables were removed using caret::findCorrelation with a cutoff of 0.90
- Low variance variables were removed using caret::nearZeroVar with a cutoff of 95/5
- The following data subsets were modeled using all binary fingerprints
    - Remove variables that are all 0's (many) and all 1's (few)
    - As above with removal of highly correlated variables
    - As above with removal of near zero variance variables removed

XGBoost training results

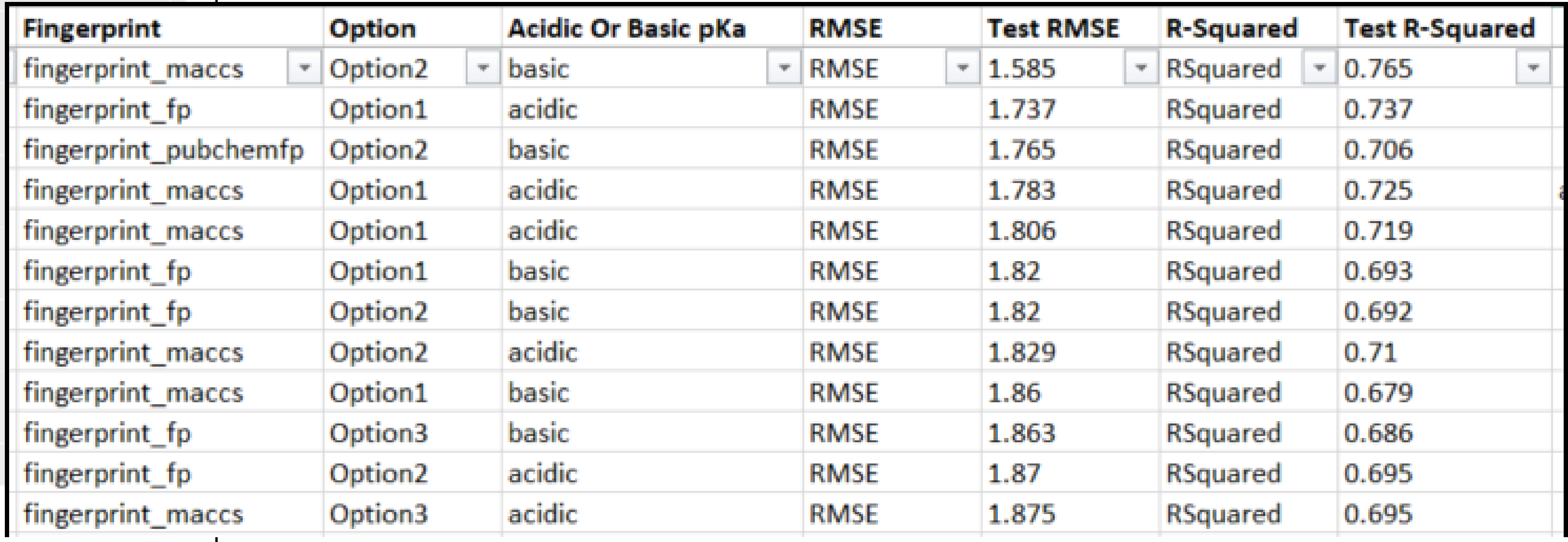
- Performance using the basic dataset was substantially better than the acidic dataset
- MACCS and FP (Morgan's, 1024 bins) binary fingerprints generally gave the best performance



fp_remove_0_1_CorrelZeroVar xgbLinear basic/maccs Option2
Testing dataset actual and predicted value
RMSE: 1.585   R-Squared: 0.765
Red is unity line, blue is smoothed linear fit

Best **RMSE** and **R-Squared** are:

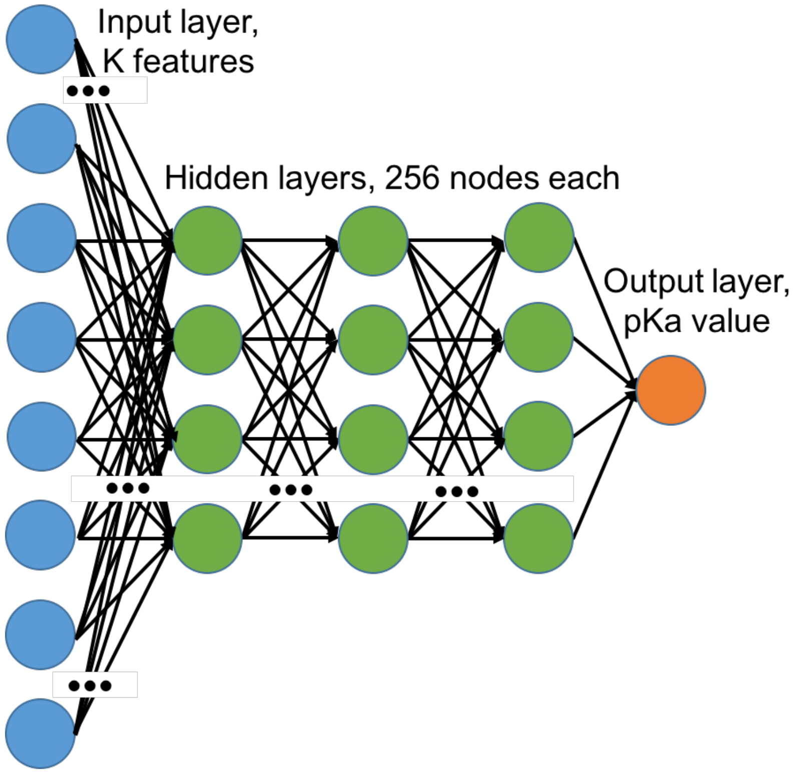
- **Basic** pKa: **1.585** and **0.765**
- **Acidic** pKa: **1.737** and **0.737**



Fingerprint	Option	Acidic Or Basic pKa	RMSE	Test RMSE	R-Squared	Test R-Squared
fingerprint_maccs	Option2	basic	RMSE	1.585	RSquared	0.765
fingerprint_fp	Option1	acidic	RMSE	1.737	RSquared	0.737
fingerprint_pubchemfp	Option2	basic	RMSE	1.765	RSquared	0.706
fingerprint_maccs	Option1	acidic	RMSE	1.783	RSquared	0.725
fingerprint_maccs	Option1	acidic	RMSE	1.806	RSquared	0.719
fingerprint_fp	Option1	basic	RMSE	1.82	RSquared	0.693
fingerprint_fp	Option2	basic	RMSE	1.82	RSquared	0.692
fingerprint_maccs	Option2	acidic	RMSE	1.829	RSquared	0.71
fingerprint_maccs	Option1	basic	RMSE	1.86	RSquared	0.679
fingerprint_fp	Option3	basic	RMSE	1.863	RSquared	0.686
fingerprint_fp	Option2	acidic	RMSE	1.87	RSquared	0.695
fingerprint_maccs	Option3	acidic	RMSE	1.875	RSquared	0.695

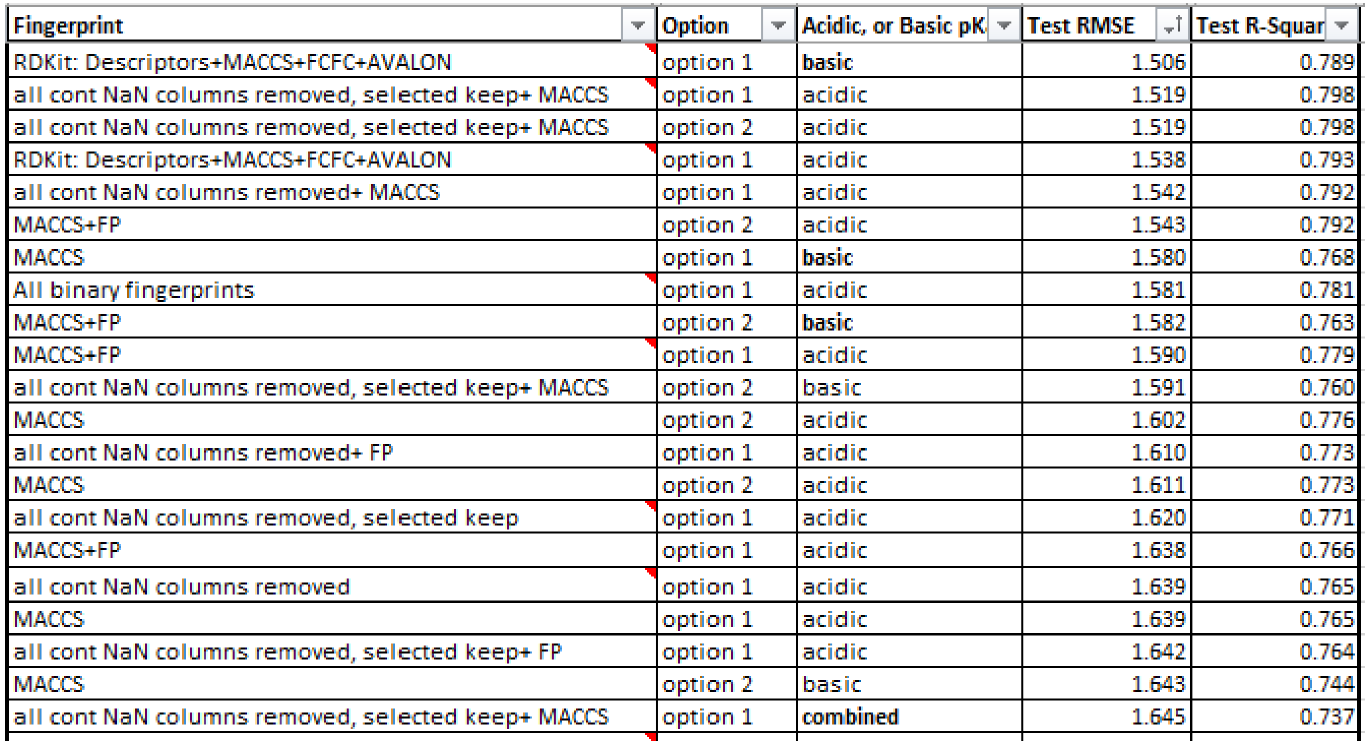
DNN training method

- The following Deep Neural Network parameters were optimized: optimization algorithm, weight initialization, hidden layers activation function, L2 regularization, dropout regularization, number of hidden layers and nodes in the hidden layers, and learning rate. Keras (https://keras.io/) and Tensorflow (www.tensorflow.org) were used for deep learning models training.
- The final DNN: 3 hidden layers of 256 nodes each followed by a batch normalization and a drop out layer to generalize trained models.
- 5-fold cross validation on training data using mean square error as a loss function with earlier training stopping base on validation loss, thus further improving of the models' generalization.



DNN training results

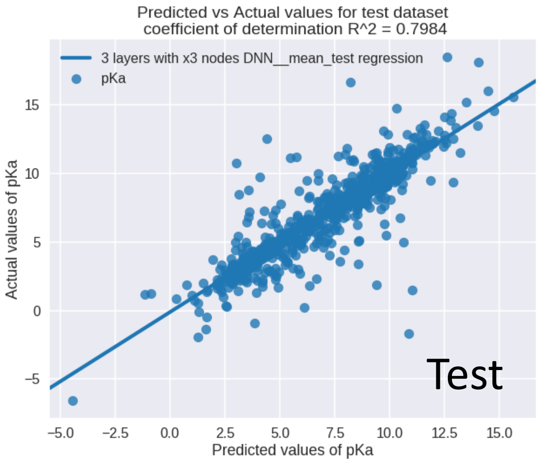
- Performance using the acidic dataset was substantially better than the basic dataset, and slightly outperforming XGBoost models
- Combination of RDKit Descriptors+MACCS+FCFC(512 bins, radius 3)+Avalon(512 bins), PADEL continues descriptors+MACCS, and MACCS or MACCS+FP (Morgan's, 1024 bins) gave the best DNN models performance

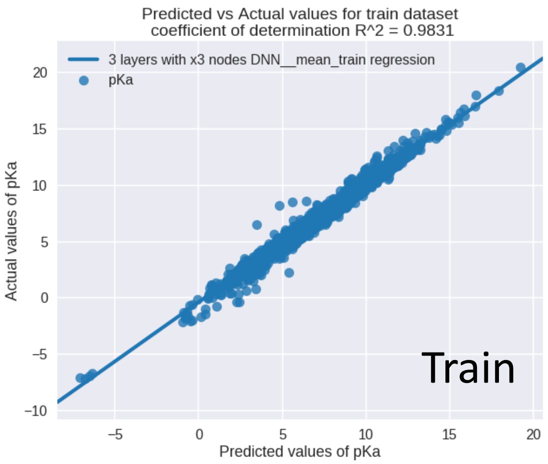


Fingerprint	Option	Acidic, or Basic pK	Test RMSE	Test R-Squar
RDKit: Descriptors+MACCS+FCFC+AVALON	option 1	**basic**	1.506	0.789
all cont NaN columns removed, selected keep+ MACCS	option 1	acidic	1.519	0.798
all cont NaN columns removed, selected keep+ MACCS	option 2	acidic	1.519	0.798
RDKit: Descriptors+MACCS+FCFC+AVALON	option 1	acidic	1.538	0.793
all cont NaN columns removed+ MACCS	option 1	acidic	1.542	0.792
MACCS+FP	option 2	acidic	1.543	0.792
MACCS	option 1	**basic**	1.580	0.768
All binary fingerprints	option 1	acidic	1.581	0.781
MACCS+FP	option 2	**basic**	1.582	0.763
MACCS+FP	option 1	acidic	1.590	0.779
all cont NaN columns removed, selected keep+ MACCS	option 2	basic	1.591	0.760
MACCS	option 2	acidic	1.602	0.776
all cont NaN columns removed+ FP	option 1	acidic	1.610	0.773
MACCS	option 2	acidic	1.611	0.773
all cont NaN columns removed, selected keep	option 1	acidic	1.620	0.771
MACCS+FP	option 1	acidic	1.638	0.766
all cont NaN columns removed	option 1	acidic	1.639	0.765
MACCS	option 1	acidic	1.639	0.765
all cont NaN columns removed, selected keep+ FP	option 1	acidic	1.642	0.764
MACCS	option 2	basic	1.643	0.744
all cont NaN columns removed, selected keep+ MACCS	option 1	**combined**	1.645	0.737

Best test **RMSE** and **R-Squared** are:

- **Basic** pKa: **1.506** and **0.789**
- **Acidic** pKa: **1.519** and **0.798**





SVM training method

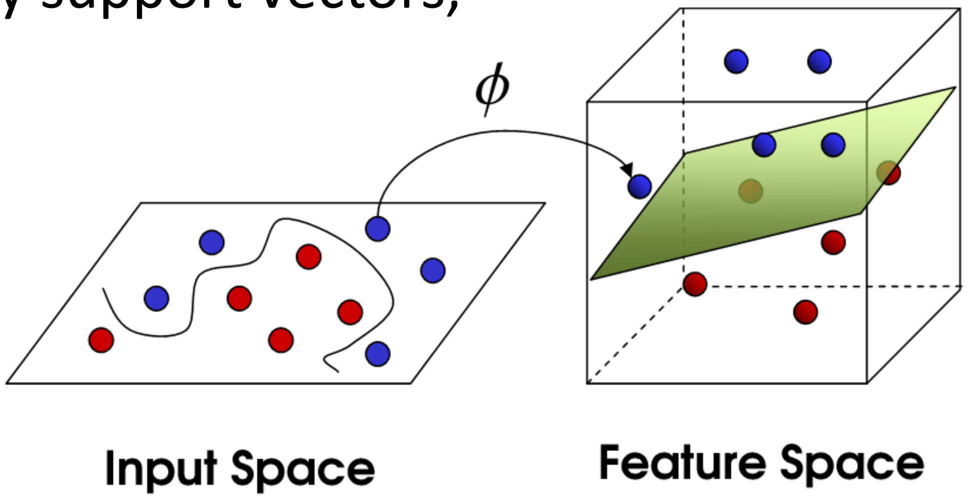
- Used the free and open source package LibSVM3.1 (Chang and Lin 2001).
- Originally designed to solve classification problems then generalized to fit continuous models as well.
- Its algorithm defines a decision boundary that optimally separates two classes by maximizing the distance between them.
- The decision boundary can be described as an hyperplane that is expressed in terms of a linear combination of functions parametrized by support vectors,

which consist in a subset of training molecules.

- SVM algorithms search for the support vectors

that give the best separating hyperplane using

a kernel function



SVM kernel function maximizing the margin between the classes.

SVM training results

Results of Option 1

		Train		5f CV		Test	
	variables	R2	RMSE	Q2	RMSE	R2	RMSE
Acidic							
Continuous	870	0.96	0.65	0.58	2.18	0.68	1.91
Fingerprint	1548	0.91	1	0.64	2.02	0.71	1.81
Fingerprint Count	556	0.9	1.1	0.6	2.16	0.65	2.01
Fingerprint – FP Count	2104	0.94	0.8	0.64	2.02	0.72	1.8
Fingerprint - Continuous	2418	0.99	0.11	0.67	1.92	0.76	1.65
Basic							
Continuous	876	0.96	0.64	0.65	1.94	0.65	1.93
Fingerprint	1535	0.91	0.99	0.69	1.84	0.69	1.83
Fingerprint Count	544	0.9	1.05	0.68	1.88	0.69	1.83
Fingerprint – FP Count	2079	0.93	0.87	0.72	1.73	0.7	1.8

Results of Option 2

		Train		5f CV		Test	
	variables	R2	RMSE	Q2	RMSE	R2	RMSE
Acidic							
Continuous	913	0.98	0.49	0.61	2.1	0.69	1.89
Fingerprint	1552	0.9	1.05	0.63	2.04	0.69	1.87
Fingerprint Count	589	0.9	1.09	0.59	2.17	0.65	1.98
Fingerprint – FP Count	2141	0.94	0.85	0.63	2.05	0.71	1.81
Basic							
Continuous	913	0.97	0.52	0.67	1.88	0.66	1.88
Fingerprint	1534	0.9	1.02	0.68	1.83	0.75	1.63
Fingerprint Count	551	0.9	1.02	0.67	1.87	0.73	1.69
Fingerprint – FP Count	2085	0.93	0.88	0.71	1.76	0.78	1.53

Results of Option 3

		Train		5f CV		Test	
	variables	R2	RMSE	Q2	RMSE	R2	RMSE
Acidic							
Continuous	510	0.96	0.66	0.59	2.17	0.57	2.2
Fingerprint	1580	0.91	1	0.64	2.01	0.68	1.91
Fingerprint Count	815	0.88	1.19	0.6	2.14	0.61	2.11
Fingerprint – FP Count	2395	0.93	0.86	0.65	1.99	0.69	1.87
Basic							
Continuous	510	0.95	0.75	0.61	2.01	0.6	2.09
Fingerprint	1543	0.91	0.94	0.72	1.72	0.67	1.9
Fingerprint Count	815	0.89	1.06	0.69	1.79	0.69	1.84
Fingerprint – FP Count	2358	0.93	0.84	0.73	1.67	0.71	1.79

kNN and SVM classification models

		Train		5f CV		Test
kNN	variables	BA		BA		BA
Continuous	15	0.8		0.8		0.77
SVM						
Continuous	15	0.92		0.8		0.73
Continuous	511	0.98		0.79		0.72
Fingerprints	1565	0.98		0.8		0.74
Fingerprint Count	815	0.96		0.8		0.73

These models are used to decide if a test chemical has an acidic pKa, basic pKa, or both (amphoteric)

Future Work

- Predict pKa values for all ionizable chemicals in the EPA CompTox Chemistry Dashboard (https://comptox.epa.gov)
- Develop web service for pKa prediction – used for calculation on the fly when registering new chemicals
- Integrate web service into online systems: e.g. the CompTox Chemistry Dashboard to allow for real time prediction of pKa values (https://comptox.epa.gov/dashboard/predictions/index)

Summary

- 7912 Chemicals With pKa In Water were scrapped from from the public Datawarrior Website: http://www.openmolecules.org/datawarrior/
- Automated QSAR data preparation workflow was developed. Three different options of automated split into Acidic, Basic, and Combined sub-sets was developed and tested.
- A classification model to determine if a molecule will have an acidic pKa, basic pKa or both was trained. Will be used for prediction workflow in a dashboard.
- XGBoost models for pKa predictions were trained. MACCS and FP (Morgan’s, 1024 bins) binary fingerprints gave the best performance with the following best RMSE and R-Squared are: basic pKa: 1.585 and 0.765; acidic pKa: 1.737 and 0.737.
- The DNN exhibited very good performance and generalization characteristics. The best performance with the following best RMSE and R-Squared are: basic pKa: 1.506 and 0.789; acidic pKa: 1.519 and 0.798.
- For SVM: the results for the acidic dataset reached an R2 test of 0.76 and for the basic dataset, an R2 test of 0.78.