Evaluation of Some Models in ADMET Predictor *(v 9.5)* used in Early Discovery Drug Metabolism and Pharmacokinetics Project Work

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Early Drug Discovery

- Predictions of basic compound properties (e.g. LogD, solubility, metabolic stability and permeability) from virtual structures can help prioritizing synthesis and testing and thus save cost and time for projects.

- To filter out compounds, the predictions need to be reliable.

- A few compounds with poor properties should be made and tested along with the best compounds to verify the predictions.

- **Note!** There is always a compromise to be made between phys chem, ADME, potency and toxicity.

Dataset characteristics (4794 compounds with measured HLM CL_{int} values)

Compounds from Medivir AB

Mostly protease and polymerase inhibitors
(i.e. many peptidomimetics and nucleoside/nucleotide analogs)

Purity >80%, vast majority >95%

Unstable or insoluble compounds were not included

2236	Zwitterions	
1888	Bases	(most weak, with pKa below 7. Only 385 had pKa >8)
623	Acids	(most weak, with pKa above 7. Only 52 had pKa <5)
44	Neutrals	
3	Mixed pKa	

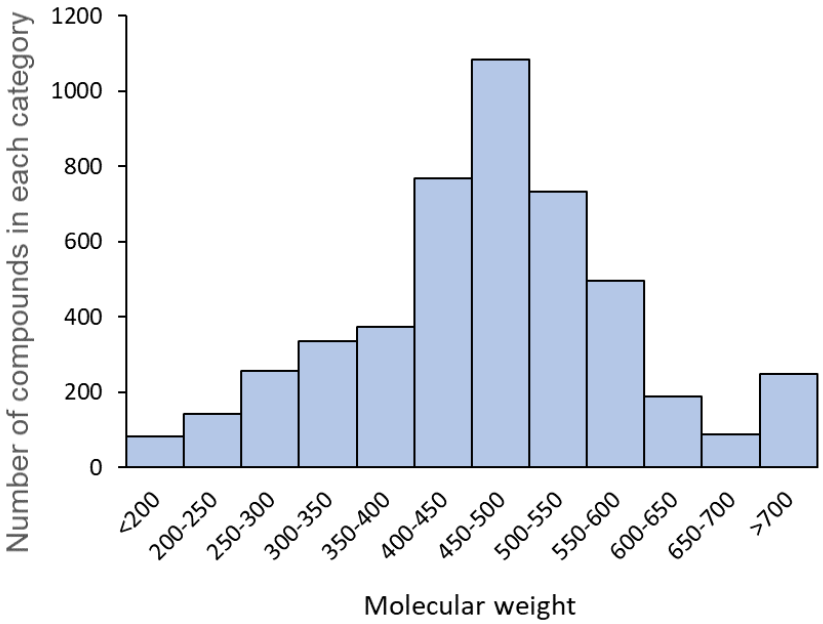
Measured quality data for		Predicted by global AP (v 9.5) model
• HLM CL_{int}	4794 compounds	CYP_HLM_CLint
• LogD	1198 compounds (from the 4794)	S+LogD
• Solubility	2778 compounds	S+Sw_pH (here pH 7.4)
• Caco-2 P_{app}	2586 compounds	S+P_{eff}

Molecular weight and LogD distribution

(4794 compounds with measured HLM CL_{int} values)

Molecular weight distribution

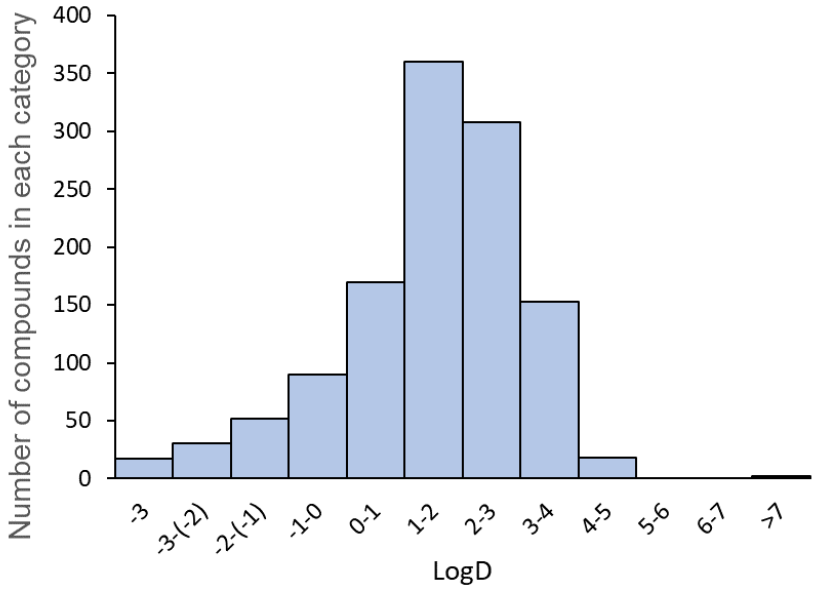
All 4794 compounds



Median: 467.7
Average: 468.6

Distribution of measured LogD

1198 out of the 4794 compounds



Median: 1.7
Average: 1.4

Measured data - Assays

- LogD — Compound (15 µM final, from 10 mM in DMSO) was vortexed with octanol/10mM phosphate buffer, pH 7.4, then allowed to settle and centrifuged.
- Solubility — Kinetic solubility, 100-fold dilution of a 10 mM compound (in DMSO stock) in 10 mM phosphate buffer, pH 7.4. Precipitate removed by vacuum filtration. Since 100 µM was the starting concentration in the assay, higher solubilities were reported as >100 µM.
- Permeability — Caco-2 cells from ATCC were used at passage 36, seeded in 96-well plates and cultured for 21 days. Permeability from A to B was measured during 120 min after adding 10 µM compound with 1% BSA in the basolateral buffer. Possible efflux was investigated for compounds with low permeability by blocking P-Gp (MDR1) and BCRP with 5 µM Elacridar GF120918). For some compounds a full ABBA assay was performed. Estimated +GF/-GF ratios of >1.5 in the A to B assay and efflux ratios >2 in the ABBA experiments were used as indications of efflux and compared with the P-gp substrate Yes/No predictions in AP.
- HLM total CL_{int} — 1 µM compound and 0.5 mg protein/mL in 100 mM phosphate buffer, pH 7.4. Ice-cold stop solution with losartan as internal standard was added. Protein precipitate was removed by centrifugation. A time curve 0-45 min was obtained and the disappearance of compound was fitted to a first-order elimination equation. As the f_u was not known, the AP predicted, unbound CL_{int} values were converted to total CL_{int} using the predicted $S+fu_{mic}$ for comparison.

Measured data

- Reference compounds were included in all experiments as quality controls and to check for reproducibility. Data from rejected experiments were not used.
- Data was characterized into 4 bins (A-D) for practical purposes so that A and D should be clearly separated and B and C were intermediate and gave less clear answers and were not used to predict actual values. The total number of compounds in categories B plus C was around 30%.

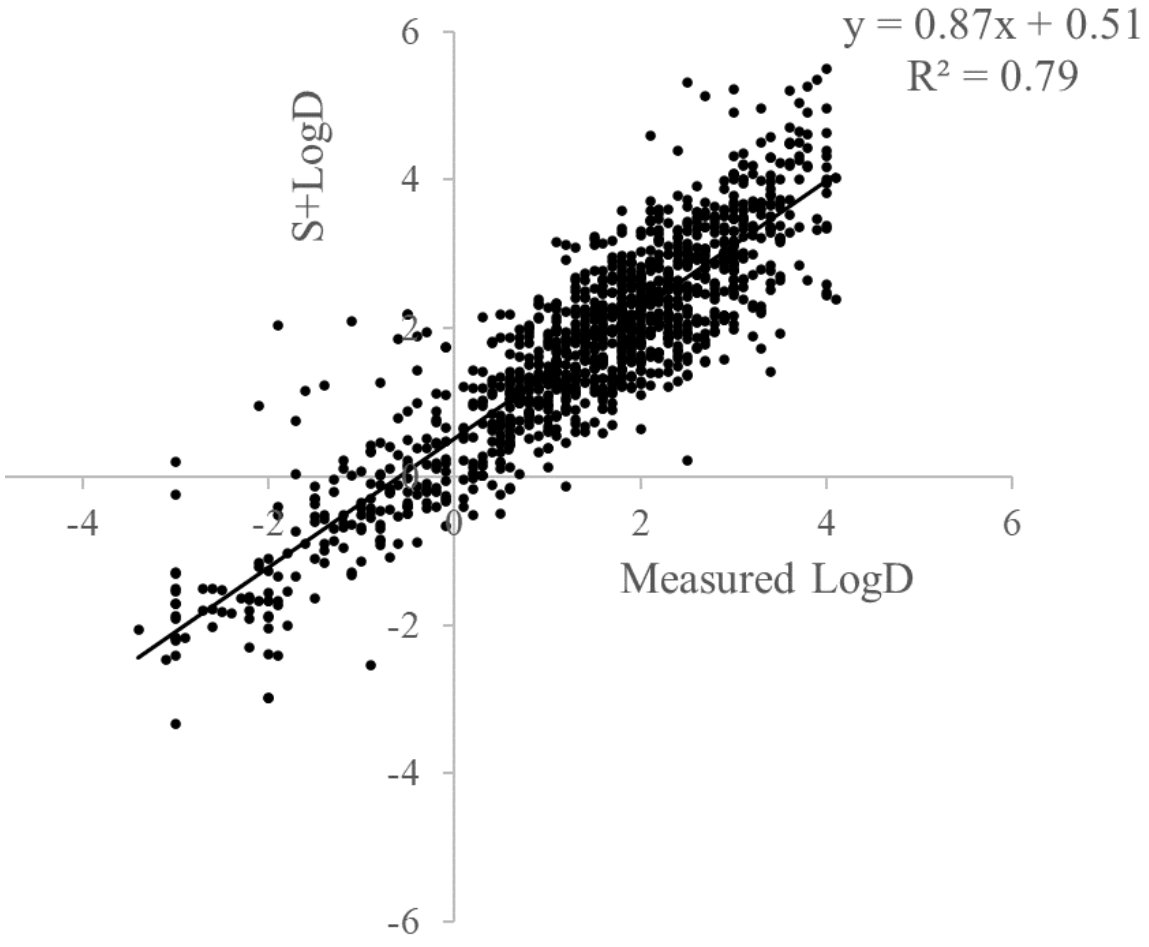
	Bin definitions			
Bin	**Solubility (µM)**	**Caco2 P_{app} (10^{-6} cm/s)**	**Predicted P_{eff} (10^{-4} cm/s)**	**HLM CL_{int} (µL/min/mg)**
A	<10	<2	<1	<15
B	10-50	2-5	1-2	15-30
C	50-90	5-10	2-3	30-80
D	>90	>10	>3	>80

	Measured values		
Bin	**Solubility**	**Caco-2 P_{app}**	**HLM CL_{int}**
Total No	*2778*	*2586*	*4794*
% in A	21	37	28
% in B	11	12	12
% in C	12	15	20
% in D	55	36	40

In-house models built in the AP Modeler™ module

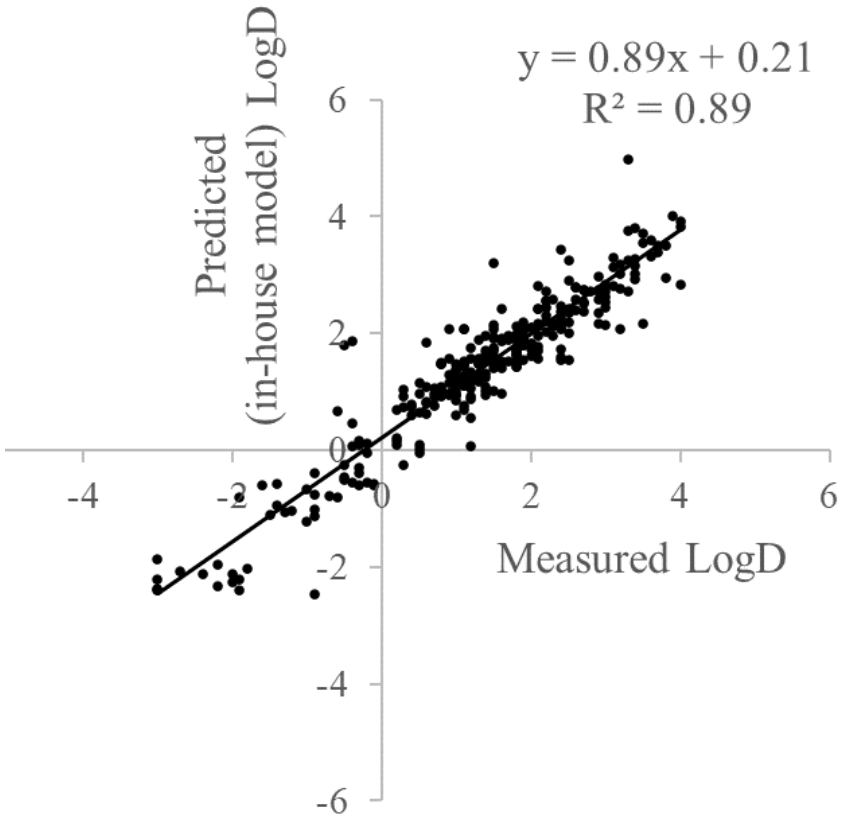
- Models for solubility, HLM CL_{int} and Caco-2 P_{app} were based on logarithmic data.
- Training sets used approximately 75-80% of the available measured data, with the remaining compounds used as test set.
- The test sets for the local model were chosen in the Modeler™, based on Kohonen mapping.
- The same test sets were also predicted with the global AP models for comparison.
- Each model was rebuilt at least 4 times and the deviations were around 10% or less.

Predicted LogD versus measured LogD using the global S+LogD model (ADMET Predictor, v 9.5) for all 1198 compounds with measured values

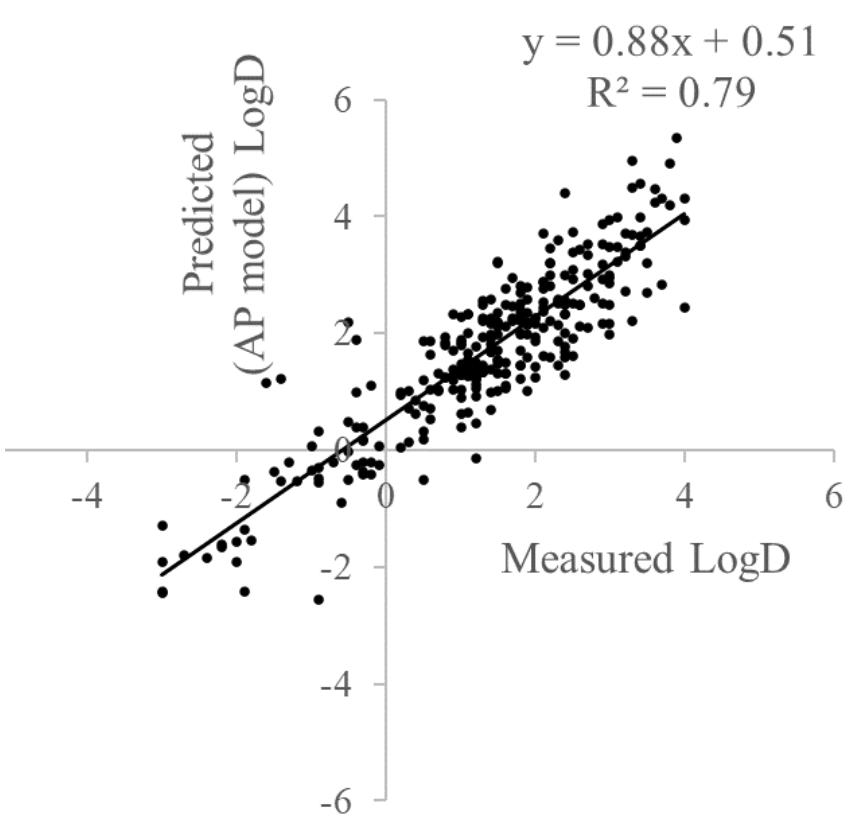


Predicted LogD vs measured LogD using (A) the in-house (local) model for the test set (287 cpds) and (B) the S+LogD (AP) global model for the same test set.

A)

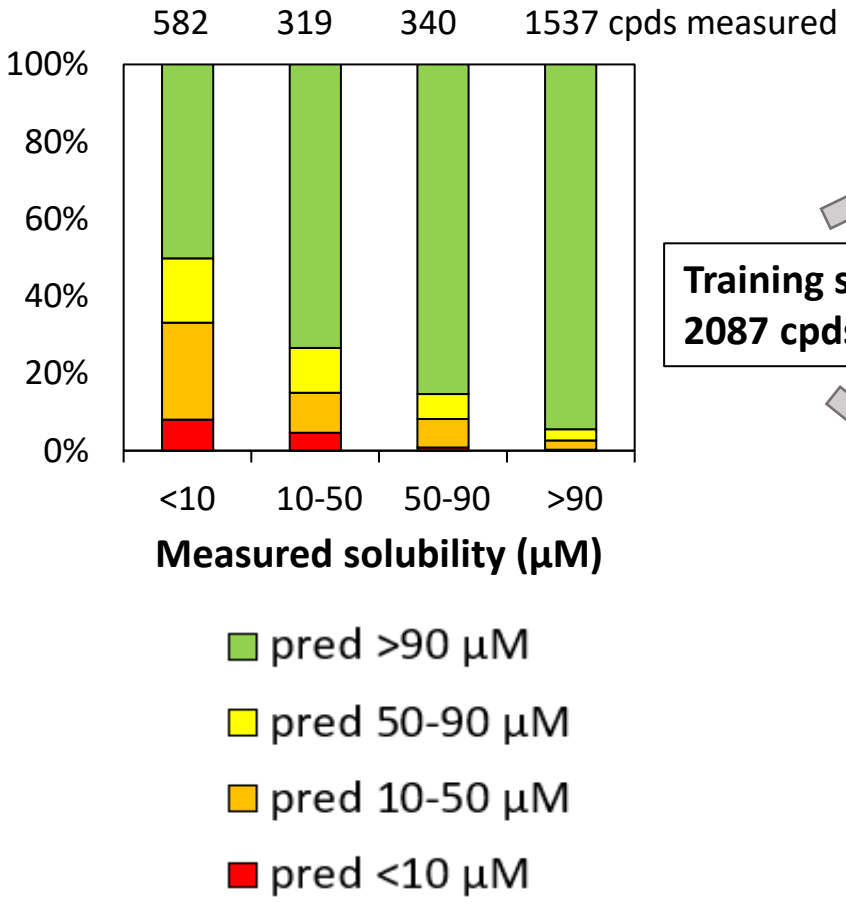


B)



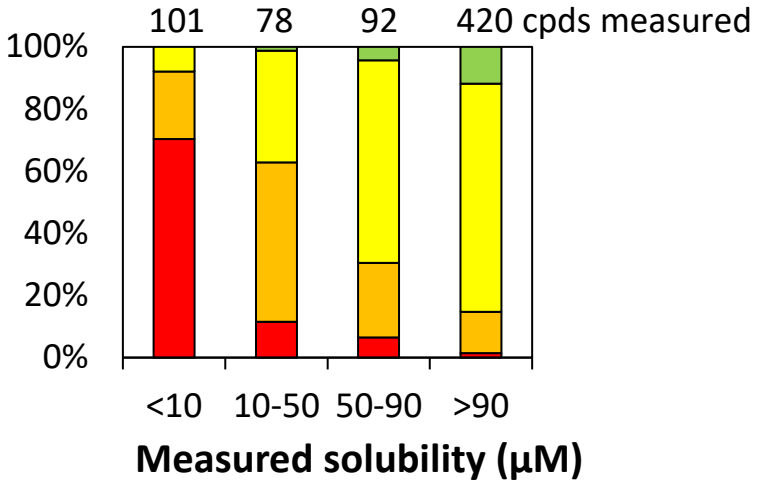
Predicted versus measured solubility (pH 7.4) for in-house compounds

Predictions in % of measured for each bin of measured values total: 2778 compounds (AP global model)

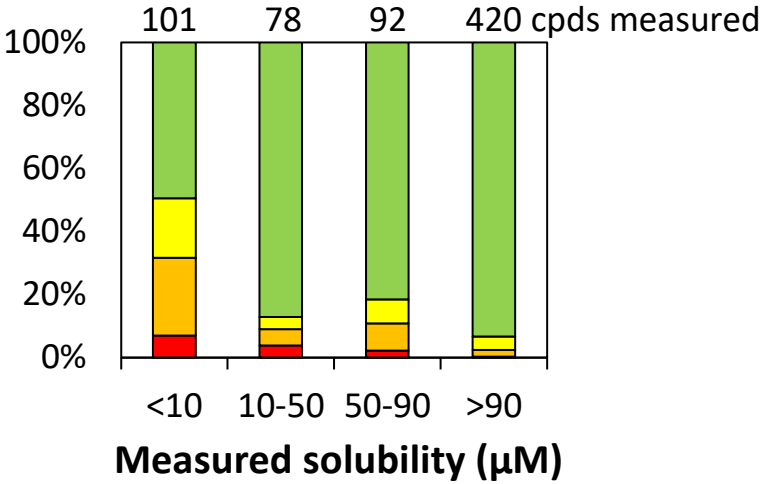


Training set: 2087 cpds

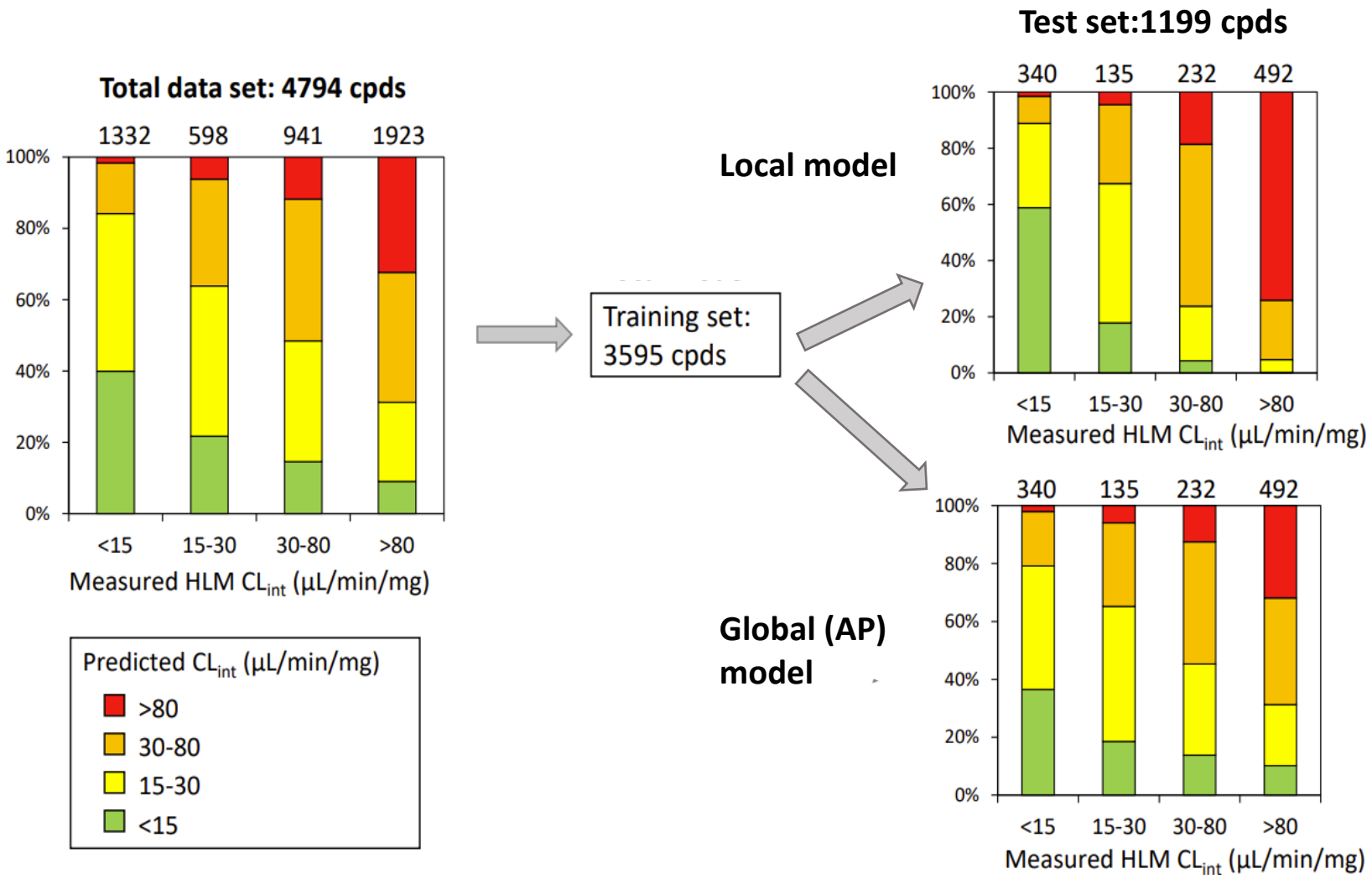
Local model: test set (691 cpds)



Global AP model: test set (691 cpds)



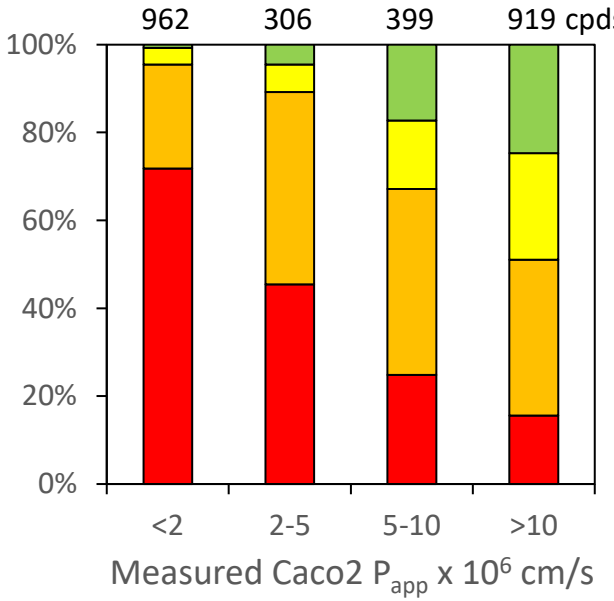
Predicted versus measured HLM CL_{int} for in-house compounds

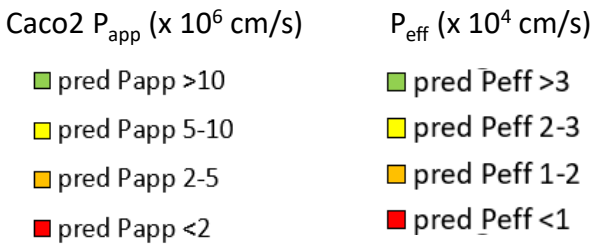


Predicted versus measured permeability for in-house compounds

No Caco2 model available in AP v9.5

Predicted P_{eff} (AP model) in % of measured Caco2 P_{app} for each bin: all 2586 compounds



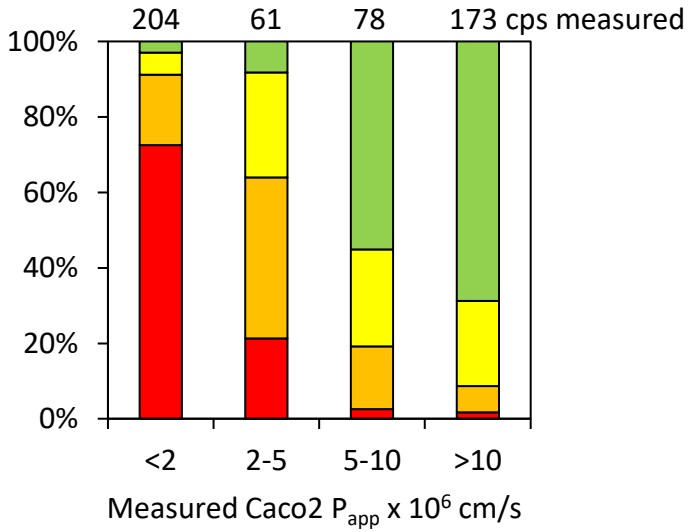


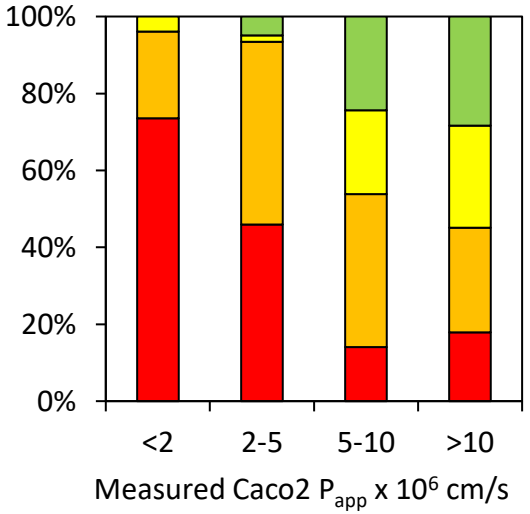
Local model
Predicted vs Measured
Caco2 P_{app}

Training set: 2070 cpds

Global model
Predicted P_{eff} vs Measured Caco2 P_{app}

Test set: 516 cpds





The AP model for prediction of P-gp substrate (Yes/No) evaluated against measured in-house data

387 in-house compounds were tested in the A to B +/- P-gp inhibitor assay.
A total of 264 compounds (68% of tested) had a +/- inhibitor ratio >1.5.

103 in-house compounds were tested in the ABBA assay.
A total number of 94 compounds (91% of tested) had an ABBA ratio >2.

Compounds with measured values above these ratios were considered to be true P-gp substrates.

A confusion table demonstrating the AP performance in predicting the tested compounds was constructed and resulted in an accuracy of 0.75-0.86

Caco2 assay setup	Precision	Sensitivity	Specificity	Accuracy
+/- P-gp inhibitor >1.5	0.75	0.96	0.30	0.75
ABBA >2.0	0.93	0.93	0.22	0.86

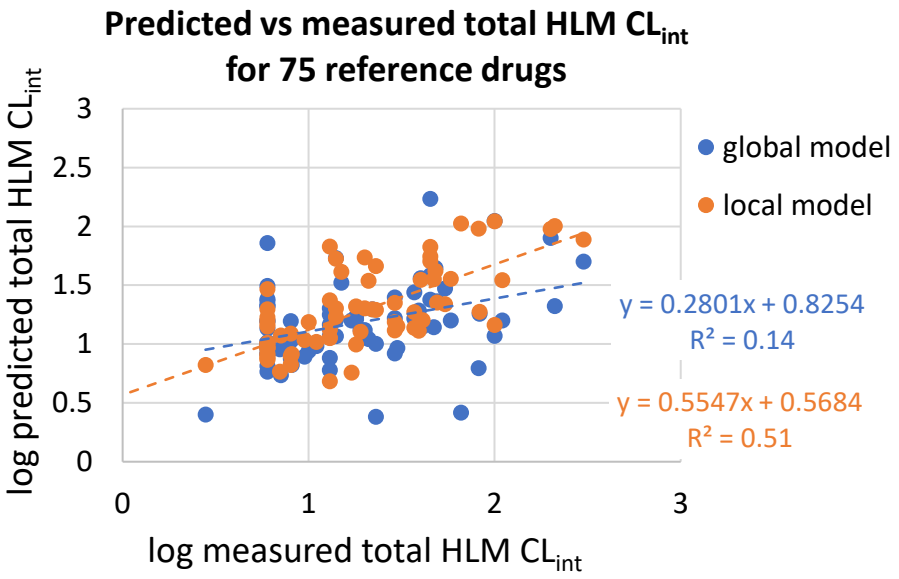
Precision: True predicted Yes/All predicted Yes
Sensitivity: True predicted Yes/All measured Yes
Specificity: True predicted No/All measured No
Accuracy: True total predictions (predicted Yes and No/Total measured)

The predictions picked up almost all P-gp substrates but did not pick up all negatives. The overall prediction accuracy was good but the ABBA assay had very few negatives (only 9% of compounds tested). As the +/- inhibitor assay dataset is unbalanced, the confusion table may “overestimate” precision and accuracy.

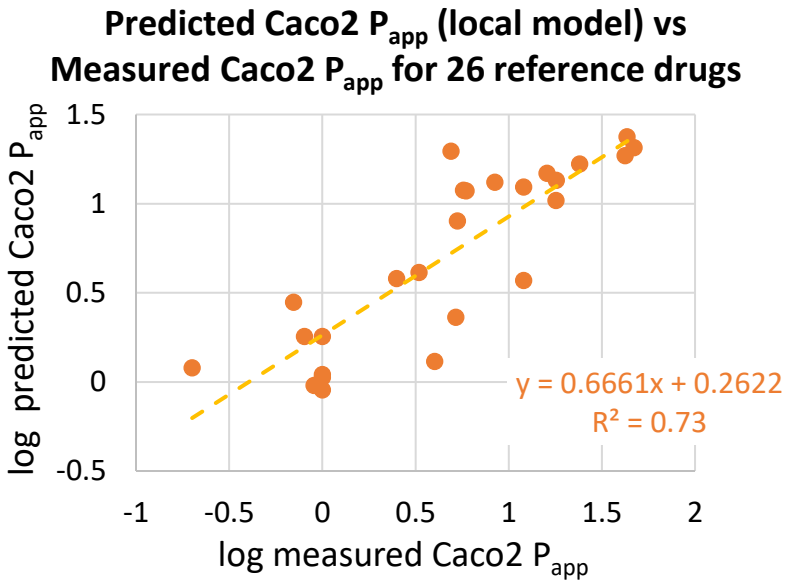
Predicted HLM CL_{int} and Caco2 P_{app} for reference drugs

Measured data are the mean of at least 3 independent experiments

A)



B)



Summary

Prediction outcome for the ADMET Predictor (v 9.5) models (global models) and the in-house models (local models) built with the AP Modeler™ module.

Assay	Total number of compounds	Number of compounds in Training set	Number of compounds in Test set	R^2		
				Global AP		Local model
				All compounds	Test set	Test set
LogD	1198	911	287	0.79	0.79	0.89
Solubility*	2778	2087	691	0.26	0.2	0.59
HLM CL_{int}*	4794	3595	1199	0.53	0.5	0.72
Caco2 P_{app}*	2586	2070	516	NA	NA	0.61

* Model based on logarithmic data

Take-home message

NOTE! The dataset used here comprises mostly protease inhibitors and polymerase inhibitors, while global models are normally built on a chemically more diverse set of compounds.

Predictions can almost always be improved by building local models on good quality in-house data (such as a chemical series from a specific project). The separate AP Modeler™ module can be used by non-modelers to build useful local models.

However, global models can also be useful, especially when there is insufficient in-house data, i.e. when starting new projects. AP is especially useful for companies that do not have dedicated in-house modeling groups. AP can save time and money by helping to prioritize virtual compounds for synthesis and/or testing.

Reasons for improved predictions with models built with in-house data:

- Better representation of in-house structures.
- All data based on the same assay conditions.

  Biological assay systems can vary quite a lot (e.g. here: the same batch of pooled HLM)

However, when using local model predictions to guide synthesis of better compounds, structures move away from the chemical space used in the model building. This often makes it necessary to rebuild models, including the new structures in the training set.

Thanks to

- The former chemists and DMPK staff at Medivir
- Medivir for allowing us to use the dataset