Déjà Vu - Réjà Vu : on knowledge-based approaches linking ligand and target information to bioactivity

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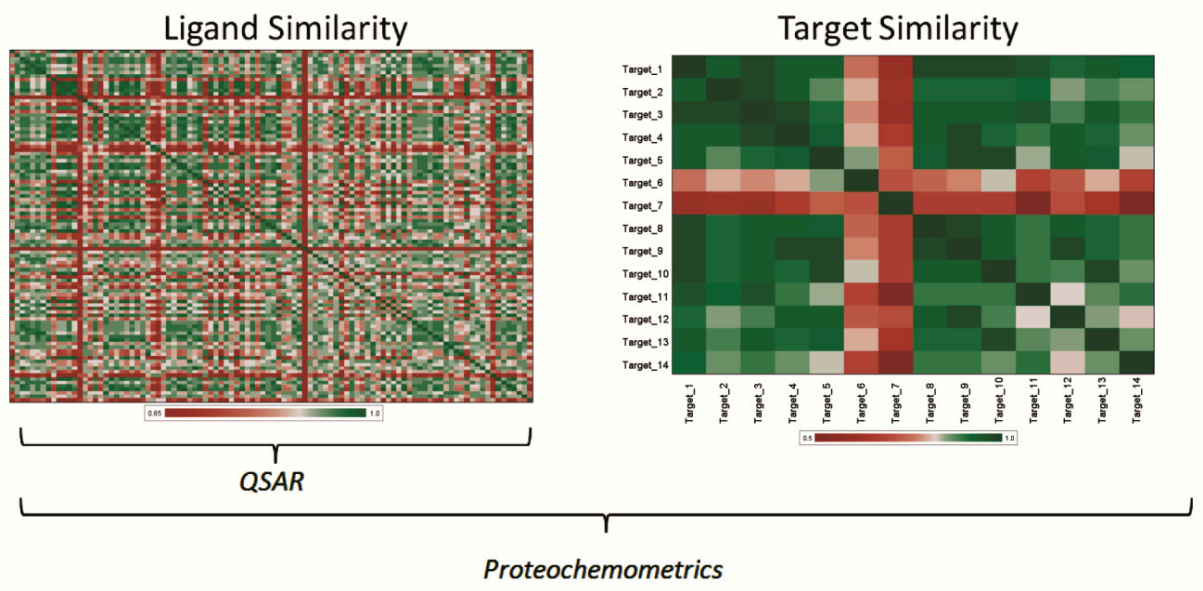
Chapter 2

Proteochemometric Modeling as a Tool to Design Selective Compounds and Extrapolate to Novel Targets



G.J.P. Van Westen, J.K. Wegner, A.P. IJzerman, H.W.T. Van Vlijmen, and A. Bender; Med. Chem. Commun.; 2011. ***2*** *(1): 16-30.*

Contents

2.1 Abstract

'Proteochemometric modeling' is a bioactivity modeling technique founded on the description of both small molecules (the ligands), and proteins (the targets). By combining those two elements of a ligand – target interaction, proteochemometric techniques model the interaction complex or the full ligand – target interaction space, and they are able to quantify the similarity between both ligands and targets simultaneously. Consequently, proteochemometric models or complex based models, can be considered and extension of QSAR models, which are ligand based. As proteochemometric models are able to incorporate target information they outperform conventional QSAR models when extrapolating from the activities of known ligands on known targets, to novel targets. Vice versa, proteochemometrics can be used to virtually screen for selective compounds that are solely active on a single member of a sub family of targets, as well as to select compounds with a desired bioactivity profile – a topic particularly relevant with concept such as 'ligand polypharmacology' in mind. Here we illustrate the concept of proteochemometrics and provide a review of relevant methodological publications in the field. We give an overview of the target families proteochemometric modeling has previously been applied to, and introduce some novel application areas of the modeling technique. We conclude that proteochemometrics is a promising technique in preclinical drug research that allows merging datasets that were previously considered separately, with the potential to extrapolate more reliably both in ligand as well as target space.

2.2 What is 'Proteochemometric Modeling' and what makes it useful for the design of bioactive compounds?

2.2.1 Structure-Activity Models. In 1962 Hansch *et al.* established the water/octanol partition coefficient (log P), discovered by Meyer and Overton,[1, 2] to quantitatively describe the relationship between the structure and biological activity of a substance using regression analysis;[3, 4] work that can be regarded as the first real Quantitative Structure- Activity Relationship (QSAR) study. Over the last decades this field has been greatly expanded, as computational methods can greatly reduce the number of experiments necessary to obtain a viable lead compound. They can do so by removing compounds from the set of candidates based on in silico experimentation before an actual 'wet lab' experiment needs to be performed.[5] The high expenses of innovative research and development have supported the case of computational research as it can be performed very cost effectively; i.e. it can help to reduce the costs of innovative drug research.[6] Furthermore the computational models obtained can be used to predict effects of untested substances, thus successfully finding their way into a virtual screening workflow.[7] Basic assumptions in structure activity studies are that (i) compounds sharing some chemical similarity should also share targets and (ii) targets sharing similar ligands should also share similar properties.[8-10] To summarize, conventional QSARs represent a very broad collection of computational tools that can model any output variable with input variables in the form of molecular descriptors using statistical approaches. However, QSARs have some limitations and drawbacks; these will be described in more detail below, followed by the extensions proteochemometric modeling makes to alleviate at least some of those limitations.

2.2.2 Why improve QSAR? A drawback of QSARs is that they consider the interaction of a group of compounds to only a single target, and often have a minimal ability to extrapolate (and sometimes even interpolate) into novel areas of chemical space.[11] This automatically requires that enough data is available on a specific target before a meaningful model can be constructed, which is rarely the case when searching for hits on a recently identified target. Furthermore, as conventional QSAR approaches consider only the ligands, the ability of QSAR approaches alone is very limited for identifying new classes of ligands or new binding modes of similar compounds outside the training set.

Although in practice there are usually multiple similar ligands that bind to a protein with varying affinities, these varying affinities are not caused only by the chemical structure, but also by the binding site. In fact, the concept of simultaneous considering ligand and binding site similarity has caused Kauvar to postulate that binding to any protein can be described by a linear combination of binding affinities to 'orthogonal' protein binding sites – a concept very much resembling current proteochemometric (and chemogenomics) thinking.[12]

The binding pocket is usually not a rigid pocket but has some flexibility present allowing an induced fit of the ligand molecule.[13, 14] As the pocket is not described by molecular descriptors in the case of QSAR, QSAR will naturally not always be able to describe all aspects of protein-ligand interaction.[15] Therefore, scientists should be cautious not to overstep the boundaries of the applicability domain for each QSAR (**Figure 2.1**). Overstepping this boundary might lead to the occurrence of 'activity cliffs' in the activity landscape present the modeler with situations where similar ligands do not always lead to similar activity.[13, 16-18] Possible causes of these cliffs include different binding modes, different binding sites and synergistic effects of chemical features of the ligand with features of the binding pocket; all of which cannot be covered in a QSAR model.

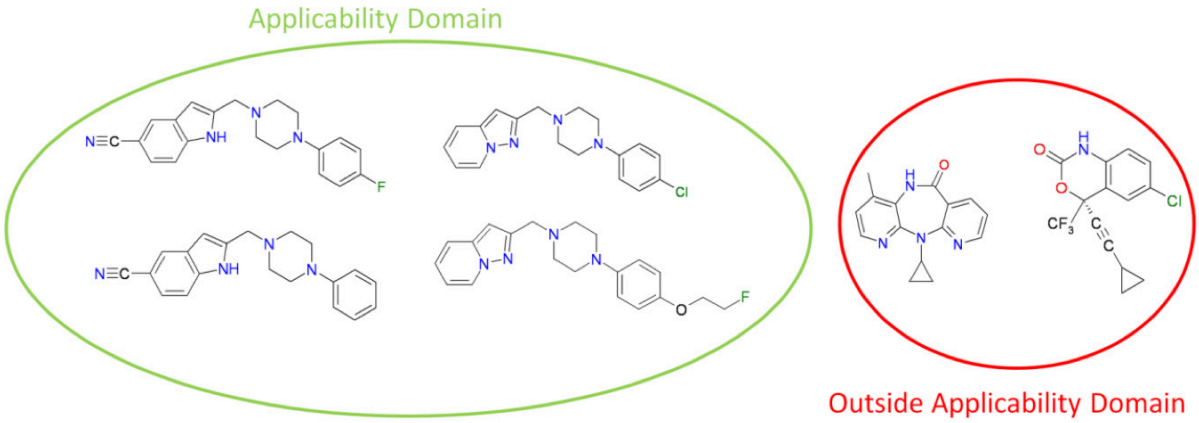


Figure 2.1: An example of the applicability domain concept. Depicted on the left side are known structures of Dopamine receptor binding compounds depicted. When a model is trained on these compounds it can only be expected to make reliable predictions for compounds that are chemically similar to this training set. On the right side two HIV reverse transcriptase inhibiting compounds are depicted, as these compounds are chemically very different from the training set the model cannot be expected to make reliable predictions of their possible affinity for the dopamine receptor.

2.2.3 Proteochemometric modeling. Contrary to QSAR, proteochemometric (PCM) modeling is based on the similarity of a group of ligands and a group of targets, to the extent that PCM models the so-called ligand-target interaction space.[14, 19] Like in QSAR modeling, the PCM model is constructed based on chemical descriptors that describe the compound data set and it introduces an additional term, a descriptor of the protein or target (**Figure 2.2**). Therefore a PCM model is constructed on both ligand and target similarity and can be regarded as an extension of conventional QSAR modeling. Furthermore one more additional term can be introduced, which describes effects on both ligand and target and the specific interactions between a compound and a target, called the cross term.[19-22] Here the difference with chemogenomic approaches becomes clear; chemogenomics is founded mainly on ligand similarities rather than the combination of the two. Nonetheless, the two techniques are quite similar and even show overlap as reviewed recently.[23] In a direct comparison Lapinsh *et al.* showed PCM to outperform QSAR and these findings were corroborated by both Geppert *et al.* and Ning *et al.*[19, 24, 25]

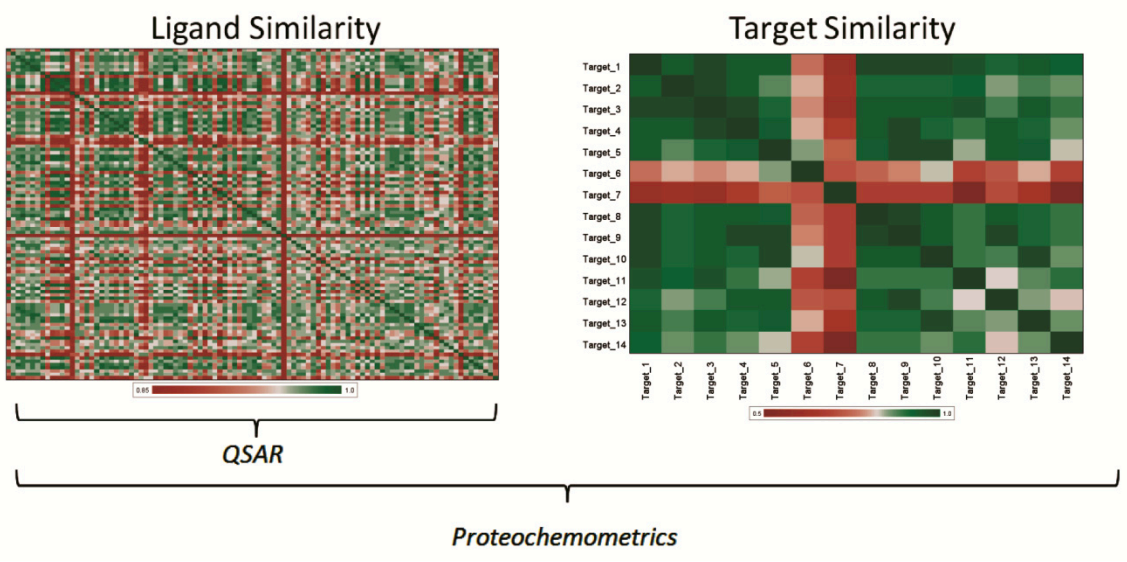


Figure 2.2: The difference between QSAR and PCM. The illustration shows two similarity matrices, one for a group of ligands and one for a group of related targets. In the heat maps green illustrates a high similarity while red illustrates a low similarity and white depicts an average similarity. PCM uses both ligand and target similarities for model generation, modeling the interaction complex. However, QSAR only uses ligand similarity, modeling only the left hand side of the ligand – target interaction space. Therefore, while QSAR and PCM are founded on similar principles, PCM can benefit from additional information in model training.

However, QSAR and PCM have also been shown to perform nearly identically on an identical training set by Lapinsh *et al.*,[11] although it should be noted that in that particular study a highly simplified form of protein description was used. The main advantage of PCM is that the model can describe different interactions of a series of compounds to a series of targets while still being able to describe specific interactions of individual compounds to individual targets in the data set. Effectively PCM can thereby connect neighboring QSAR datasets on the basis of the similarity between the targets contained in these data sets. Therefore, in order to create a true PCM model, it is necessary to have activity data of multiple compounds on multiple targets. When creating a PCM model on a single target, the fact that all targets are identical makes this PCM model in reality a QSAR model.[21, 26]

The fact that PCM can connect neighboring QSAR datasets makes it quite similar to inductive learning. Inductive learning is a QSAR based technique consisting of the learning of a property on a dataset and to use the extracted knowledge to better learn a related property (e.g. learn rat blood-brain barrier permeability based on a large rat dataset, then use the predicted rat BBB term as a descriptor in a human BBB model. Effectively only the difference between human and rat should then be learned, while letting the rat QSAR model account for the common issues that modulate BBB-permeation in both species). In the example given here, the predicted rat BBB descriptor can be compared with a protein descriptor in the case of PCM. The major difference is between the example and PCM is that the former requires two separate steps of model training where the latter requires one. Furthermore the interpretability of PCM will likely be higher as it relates to target (dis)similarity rather than a non-target related descriptor such as 'rat BBB penetration'

Since PCM contains a target descriptor, the major advantage is that PCM can create a single model predicting a single output variable of the interaction, e.g. affinity, between a very diverse series of compounds or targets and still provide a statistically solid model.[11, 20] It allows the modeler not only to extrapolate the activity of new compounds on known mutants or targets, but also to extrapolate the activity of known compounds on new mutants or targets (**Figure 2.3**). PCM can also be applied in situations where the 3D information of the targets is unavailable or when the 3D approach is unreliable. Typical examples are situations where no crystal structure is at hand or where only low quality homology models are available.

PCM, like QSAR, can implement a variety of machine learning techniques including both linear and non-linear methods to construct a model.[19, 27] Furthermore PCM has already been applied to a wide variety of relevant drug targets. To illustrate this versatility we will provide a short overview of the targets PCM has previously been applied to below. Subsequently we will provide an overview of previously used ligand descriptors, target descriptors and cross terms in PCM modeling. Thirdly we will outline some of the machine learning techniques compatible with PCM. We will end the review with some possible pitfalls and disadvantages and the final conclusions.

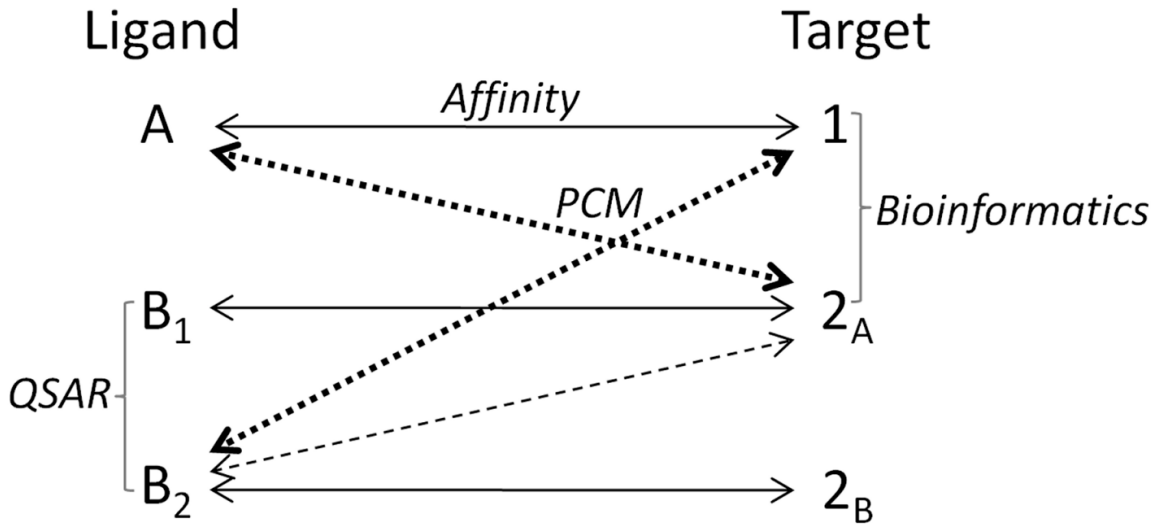


Figure 2.3: Possibilities of PCM in a hypothetical dataset where the affinity of three different compounds was measured on three different targets. QSAR is able to calculate an output variable based on the similarity of compounds (use the similarity between compound B_1 and B_2) and Bioinformatics can quantify the similarity between targets (similarity between target 1 and 2_A). However PCM can use this information to extrapolate the activity of compounds on targets (dashed double arrows). In this case a PCM prediction for the activity of B_2 on 2_A is likely more accurate than the prediction of the activity of B_2 on target 1.

2.3 Biochemical applications of PCM techniques

2.3.1 G Protein-Coupled Receptors. Although PCM has been introduced fairly recently the technique has been tested on a wide variety of relevant drug targets. For a comprehensive overview of targets to which PCM has been applied see **Table 2.1**. Overall PCM has mainly been applied to datasets of G protein-coupled receptors (GPCRs), in particular the rhodopsin-like class A receptors. Dopamine, histamine, adrenergic and melanocortin receptors have been described in various ways ranging from binary to local descriptors of protein structures.[28] Interestingly, Lapinsh *et al.* showed that PCM was able to create a viable model from a dataset containing multiple related class A receptors based on their transmembrane alpha-helical regions with a pKi error of approximately 0.55 log units, which is a rather well-performing model.[14] It should be noted though that this dataset contained limited chemical diversity as it was based on only 22 ligands. The targets side contained 31 GPCRs, being described by 159 TM domain amino acids. In related work, Weil and Rognan also create a model including multiple class A GPCRs. Their model was based on a custom fingerprint encoding both ligand and target features in one fixed length array of bits.[29] Using several classification models, they showed that it is in fact possible to create one global GPCR PCM model. Furthermore, they demonstrated that their models are able to retrieve the natural receptor ligands from a decoy-spiked dataset of 200,000 ligand – target pairs.

Likewise, Bock *et al.* modeled multiple GPCR receptor families in a single model, a PCM based approach, which they applied to orphan GPCRs.[30] Using a Support Vector Machines (SVM) learning approach they were able to separate a small group (2%) of highly active ligand – compound pairs from the bulk of their 1.9 million data point dataset. As an extension of this work, Jacob *et al.* applied a PCM approach to the GLIDA GPCR database to predict the ligands of orphan GPCRs.[31] They achieved a prediction accuracy of approximately 90 %.

Table 2.1: List of applications of PCM modeling (near-comprehensive to the knowledge of the

Year	Modeling Technique	Targets	Validated
2001	PLS	Melanocortin Receptors	No
2001	PLS	1a, 1b and 1d Alfa-Adrenergic Receptors	No
2002	PLS	Serotonin, Dopamine, Histamine, and Adrenergic receptors	No
2002	SVM	CLiBE Selection	No
2003	PLS	Melanocortin Receptors	No
2005	PLS	Melanocortin Receptors	No
2005	PLS	Set I Serotonin, Dopamine, Histamine, Adrenergic receptors Set II 1a, 1b and 1d Alfa- Adrenergic Receptors	No
2005	PLS	Serotonin, Dopamine, Histamine, Muscarinic Acetylcholine and Adrenergic receptors	No
2005	SVM	Orphan GPCRs	No
2006	PLS	Melanocortin Receptors	Yes
2006	RS	Set I Melanocortin Receptors Set II Melanocortin Receptors Set III Adrenergic Receptors	No
2006	RS and PLS	Hydrolases, Lysases, Neuramidases, Anhydrases	No
2006	PLS	PDBind subset	No
2007	PLS	Melanocortin Receptors	No
2007	PLS	Antigen recognizing Antibodies	No
2007	PLS	Melanocortin Receptors	No
2008	PLS	(point mutated) HIV Proteases	No
2008	PLS	Cytochrome P450 enzymes	No
2008	Linear and NN	Matrix Metalloproteinases	No
2008	PLS	Dengue Virus NS3 Proteases	No
2008	SVM	Large Crystal Structure data set	No
2008	SVM	GLIDA subset	No
2009	SVM	Proteases	No
2009	PLS	(point mutated) HIV Proteases	No
2009	PLS	(point mutated) HIV Proteases	Yes
2009	SVM, RF, NB	MDL Drug Data Report subset	No
2009	SVM	117 Pubchem Targets	No
2009	SVM	DrugBank	Yes
2010	PLS	Major Histocompatibility Complex Proteins	No
2010	SVM, DT, NB	Multi target BindingDB dataset	No
2010	DT, NN, SVM, PLS	Kinase Inhibitors	No
2010	SVM	Kinase inhibitors (ProLINT database)	No

Explanation of abbreviations: Protein Database (PDB), Computed Ligand Binding Energy (CLiBE), GPCR LIgand Database (GLIDA), Random Forest (RF), Naïve Bayesian (NB), Decision Tree (DT), Grid Independent Descriptors (GRINDs),

authors, though publications using a different name for the technology might have been missed):

Ligand Descriptors	Target Descriptors	Cross Terms	Ref.
Binary	Binary	Multiplication	28
Binary	Sequential (Z-scales)	Multiplication	19
GRINDs	Sequential (Z-scales)	Multiplication	62
1D projection of Physicochemical properties	Sequential Physicochemical Properties	-	42
GRINDs	TM Identity	Multiplication	11
Physicochemical	Binary	Multiplication	20
GRINDs	Sequential (Z-scales)	Multiplication	85
GRINDs	Sequential (Z-scales)	Multiplication	14
Physicochemical and 2D	Sequential Physicochemical Properties	-	30
Binary	Sequential (Z-scales)	Multiplication	21
ASCII String	ASCII String	-	27
Physicochemical, 1D, 2D, 3D	Local Descriptors of Protein Structure	Multiplication	35
2D and 3D	3D Structural	Protein-Ligand Interaction	67
Binary	Binary / Sequential (Z-scales)	Multiplication	65
Sequential (Z-scales)	Sequential (Z-scales)	Multiplication	36
Sequential (Z-scales)	Sequential (Z-scales)	Multiplication	26
GRINDs	Sequential (Z-scales)	Multiplication	32
GRINDs	Binary and Sequential	Multiplication	41
2D autocorrelation vectors	Amino Acid Sequence Autocorrelation vectors	-	93
Physicochemical	Binary	Multiplication	34
Physicochemical, 1D, 2D, 3D	Local Descriptors of Protein Structure	-	70
2D and 3D kernels	Multitask, Hierachy and Binding pocket kernel	-	31
2D Fingerprints	Sequence Similarity	-	24
Physicochemical	Sequential (Z-scales)	Multiplication	33
Sequential (Z-scales)	Sequential (Z-scales)	Multiplication	112
2D, Shannon Entropy, Pharmacophoric	Pysicochemical property based phylogenetic tree	Merger of ligand and target descriptors	29
Topological Graph Based	Sequence Similarity	-	25
2D graph vector	Physicochemical property vector	-	113
Sequential (Z-scales)	Sequential (Z-scales)	Multiplication	40
Physicochemical	Sequence Similarity (multiple descriptors)	-	46
Physicochemical, Geometrical, Molecular	Sequential (Z-scales) + Sequence Similarity (multiple descriptors)	Multiplication	39
2D autocorrelation vectors	Amino Acid Sequence Autocorrelation vectors	-	38

one dimensional (1D), two dimensional (2D), three dimensional (3D), Human Immunodeficiency Virus (HIV), Partial Least Squares (PLS), Rough Set (RS), Neural Net (NN), Support Vector Machines (SVM), Reference (Ref.).

2.3.2 Viral Targets. While GPCRs are quite amenable to PCM modeling due to their relatedness, the same is true for mutants of enzymes. Hence, another target type that has been adequately covered in PCM modeling are viral proteins, such as HIV Protease,[32, 33] Dengue virus NS3 Protease,[34] and Influenza virus A and B Neuraminidase.[35] The average similarity between these targets is very high when compared to the average similarity between multiple GPCR families. This is especially true in the case of HIV proteases, where the differences between targets may be a single amino acid.

2.3.3 Other macromolecules. In addition to these larger target groups, PCM has also been applied to antigen recognizing antibodies,[36] matrix metalloproteinases,[37] kinases,[38, 39] Major Histocompatibility Complex (MHC) proteins,[40] and Cytochrome P450 enzymes.[41] The ligands PCM has been applied to include both small molecules and peptides. Furthermore, the output variables modeled by PCM models include classification,[29, 31] binding affinity,[20] and even equilibrium binding free energy.[42] The nature of the datasets PCM has been applied to confirm the potential of PCM as a versatile technique suitable for any dataset. Whether the targets are highly related or more dissimilar, a functional model that provides better extrapolation capabilities than QSAR can be created. However, it should be noted that the type of target description must be tuned to the nature of the dataset.

2.4 Novel applications of PCM

2.4.1 Hit identification for orphan targets. The main advantage of the PCM extension of conventional QSAR modeling is that it allows the merging of datasets that describe the affinity to highly similar but not identical targets; hence it allows for the extrapolation of affinity modeling between these datasets. Thereby PCM can fulfill a need in hit identification for newly identified targets – including applications such as the prediction of polypharmacology or the deorphanization of receptors. PCM not only models similar datasets simultaneously, it also quantifies the distance between the different targets. Therefore it allows the scientist to estimate the reliability of any model prediction depending on the distance of both the ligand and target to the training set, through determining the applicability domain of the model.

2.4.2 Simultaneous modeling of orthosteric and allosteric ligands. PCM includes a target description in addition to the ligand descriptors; hence it is able to quantify the similarity between different binding sites. As a result it could be used for the simultaneous modeling of a series of orthosteric and allosteric ligands of a single target even though they act through a different binding site.[43-45] In this case not two (or more) proteins are used for modeling the target side, but rather two binding sites of ligands that are in different parts of the protein which are able to accommodate completely different ligand chemistry. Previously it has already been shown that targets sharing a low similarity and accommodating a completely different chemistry can be modeled successfully with PCM.[35, 46]

Capturing the chemical information of different types of ligands that act on different binding sites on a single target can provide advantages as the increase in information that serves as model input could lead to better extrapolation capabilities of a single target model. The single target model can in this case be seen as a two targets model. (**Figure 2.4**)

A second possible application is the simultaneous modeling of a drug regimen incorporating both nucleoside reverse transcriptase inhibitors (orthosteric) and non-nucleoside reverse transcriptase inhibitors (allosteric) for a single dominant HIV mutant in a patient (**Figure 2.4**).

Finally, combining allosteric and orthosteric information in one model can provide advantages in preclinical research when looking for novel allosteric inhibitors or enhancers of proteins. Allosteric drugs have been shown to provide advantages in treatment by better resembling physiological signaling.[47] Furthermore these models could come in use, when research is targeting a protein for which the orthosteric (natural) ligand is known and part of an essential physiological process. As this process cannot be completely disrupted (orthosteric inhibition) or continuously activated (orthosteric activation or agonism) the possibility of allosteric modulation is very promising here. The addition of the orthosteric inhibitors to this model potentially could potentially detect cross reactivity with the orthosteric binding site at an early stage.

While to our knowledge this research has not been performed yet, it illustrates the versatility of PCM modeling approaches. PCM can cover a much larger bioactivity space than conventional QSAR models alone as well as, introduced here, multiple modes of action.

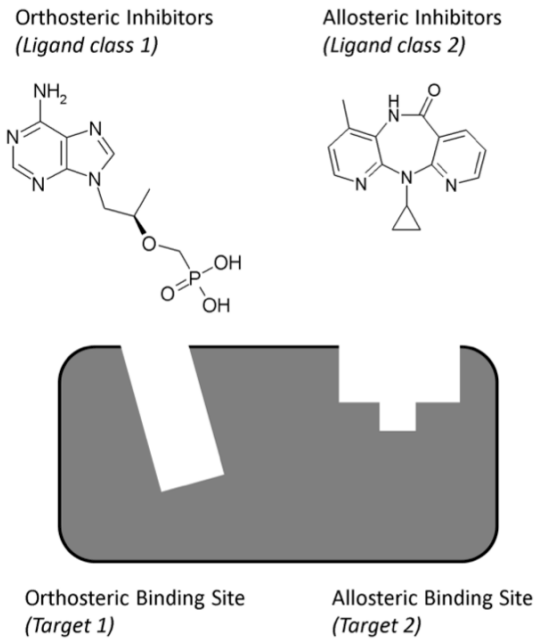


Figure 2.4: A single PCM could also potentially be used to model both allosteric and orthosteric binders to a given target, as an extension of current PCM models. Hereby a single target model is effectively turned into a multi target model, with the additional variable being introduced the binding site a given ligand binds to, this being particularly relevant in cases where a single chemical class of molecules can bind to each of the receptor subsites. Shown here are Tenofovir (an orthosteric HIV Reverse Transcriptase inhibitor) and Nevirapine (an allosteric HIV Reverse Transcriptase inhibitor). While modeling of this type has not been performed yet and its precise performance remains to be validated in the future, it still illustrates the ability of PCM models to incorporate not only ligand and target variables, but also additional variable types such as those of subpockets of a given protein target.

2.5. Ligand descriptors

Ligand descriptors are merely numerical methods to describe either properties of or differences between the compounds to be modeled, therefore a large number of different descriptor methods for ligands are available (For reviews see [8, 10, 13, 48, 49]). In this review article we will restrict our focus on ligand descriptors previously used in PCM. There is no optimal descriptor for all datasets and it is therefore wise to sample several different descriptors to identify the optimal descriptor for each setting.[13] In the following we will start our discussion with the simplest descriptors used in PCM, namely binary descriptors.

2.5.1 Binary compound descriptors. Binary descriptors are descriptors based on one or more binary flags set to 1 or 0. These descriptors can be based on the differences between functional groups on one common scaffold. Lapinsh *et al.* implemented them in PCM by creating a table listing all possible combinations of three functional groups and assigning unique binary descriptors to each of those combinations.[19] These descriptors are relatively simple and therefore computationally very fast; however results from the model have to be translated back to the functional group combination before model results can be interpreted.

Furthermore predicting values outside the range of descriptors is not self-evident, so there is little possibility of numerical inter- and extrapolation.[23, 50] Therefore, the authors recommend not to use binary descriptors in PCM. Additionally, binary descriptors function on the assumption that it is already known which properties are relevant in the QSAR. Furthermore they are very sensitive to the creation of 'ties',[51] namely the creation of equidistant similarity distances for non equal compound pairs. Binary descriptors are also more affected by possible overfitting of the data, since they do not allow a fuzzy interpolation of relevant groups.[52] One-Dimensional (1D) numerical (real-valued) descriptors resolve most of these problems and are better interpretable.

2.5.2 One dimensional and physicochemical compound descriptors. The main advantage of one-dimensional descriptors is that they are quickly calculated and modeled. Physicochemical descriptors are a subtype of these 1D descriptors, such as molecular weight, polar surface area or polarizability. When compared to binary descriptors, the usage of physicochemical descriptors increases the interpretability of the model and makes a translation step obsolete. The downside of this subtype of descriptors is the high number of possible descriptors that can be calculated for every compound. Therefore it is common to make a selection of descriptors that are important in each interaction model while also removing possible covariance in the descriptors. This goal can be achieved by preprocessing of the descriptors before a reliable model can be constructed, leading to the risk that only those descriptors are selected that are applicable to the training set on which the model is constructed. 1D descriptors have previously been applied successfully in PCM allowing the creation of a predictive and highly interpretable model (reaching an R^2 of 0.92).[20]

2.5.3 Two dimensional topological compound descriptors. Topological descriptors are two dimensional representations of compounds, visualizing bond properties, atomic properties and the inner atomic distances between the functional groups. These descriptors can be transformed into molecular graphs, a method widely used in substructure searching and clustering. In graph descriptors, the atoms form the nodes of the molecular graph and the bonds the edges.[10] Advantages of this type of descriptors are that they are relatively quickly computed and easily interpreted, but a downside can be that they lack three-dimensional (3D) information (for a recent review see Van der Horst *et al.* [53]). To the knowledge of the authors these descriptors have only been used in a PCM-like approach by Ning *et al.*[25] Their particular graph-based descriptor performs quite well and has previously been shown to perform comparably to 2D circular fingerprints, which are mentioned below.[50]

2.5.4 Two dimensional circular fingerprints. Descriptors based on 2D fingerprints convert the two dimensional information of the compound structure into a linear binary string. This class of descriptors is overall similar to 2D graph-based approaches, however it tends to be computationally much faster.[10] These 2D fingerprints are constructed from several substructures present in the compounds while the substructures are limited by a radius defined by a certain number of covalent bonds.[54, 55]

Circular fingerprints have previously been found to capture a large amount of information and to provide a high retrieval rate while at the same time being chemically interpretable as individual (localized) features.[54, 56] In recent studies the authors have been applying circular fingerprints in PCM models of HIV Reverse Transcriptase producing models with an average prediction error of 0.5 log units.[57, 58]

2.5.5 Alignment based 3D compound descriptors. 3D descriptors preserve more information of the compounds by adding information concerning the conformation of the compound.[59] However, most 3D descriptors require superposition of the compounds in their active conformation in 3D space. Only then useful information can be obtained, making the calculation more complex. The process of compound superposition is error prone and can introduce more noise than functional information,[60] a step which can be avoided by using internal distances between atoms or surface points of a compound. This translation preserves all possible pharmacophore triplets and quadruplets, features of the compound and inter-feature distances but simplifies the calculation step.[10] The increase of information in these descriptors also increases their size and consequently the calculation times of the models. To the knowledge of the authors, 3D descriptors have as yet not been used in PCM and the previously mentioned disadvantages might support the usage of GRINDs instead (see next section).

2.5.6 Grid independent descriptors. Grid Independent Descriptors (GRINDs) are descriptors that are obtained starting from a set of molecular interaction fields using different probes. This procedure involves a first step, simplifying the fields, and a second step, encoding the fields into alignment-independent variables using an autocorrelation transform.[61] The obtained descriptors can also be used to provide graphical diagrams and the original descriptors can be regenerated from the transformation in order to visualize the results of the analysis graphically in 3D. As one of few 3D descriptors GRINDs have previously been used in PCM with a prediction error of around 0.5 log units on datasets of GPCRs.[11, 62] This confirms the compatibility of PCM with GRINDs on this dataset. In this case, the GRIND descriptors were preprocessed using principal component analysis (PCA), a step to handle the high dimensionality common to many descriptor spaces.[11]

2.6 Protein descriptors

The main difference between ligand and protein descriptors is that the protein is, in general, a larger structure to describe and hence in most cases a selection of a subset of the residues (such as those lining the binding site) would be recommended. This selection can be made on the basis of a crystal structure if available, on data from mutational experiments or from an information-based bioinformatics analysis, e.g. a two-entropy analysis.[63]

At the level of the residues, a distinction can be made between descriptors that describe a (sub)structure or general property of the protein and those that stand for properties of individual amino acids on a sequential basis. Protein descriptors have been reviewed extensively and only methods that have been previously used in PCM will be highlighted here.[10, 64]

2.6.1 Binary protein descriptors. Similar to binary ligand descriptors, binary description of proteins can be performed based on several binary flags corresponding to different substructures of the protein. Although the make-up of the data set dictates the binary method used for the description and hence the length of the descriptors, binary descriptors are generally fast from a computational point of view.

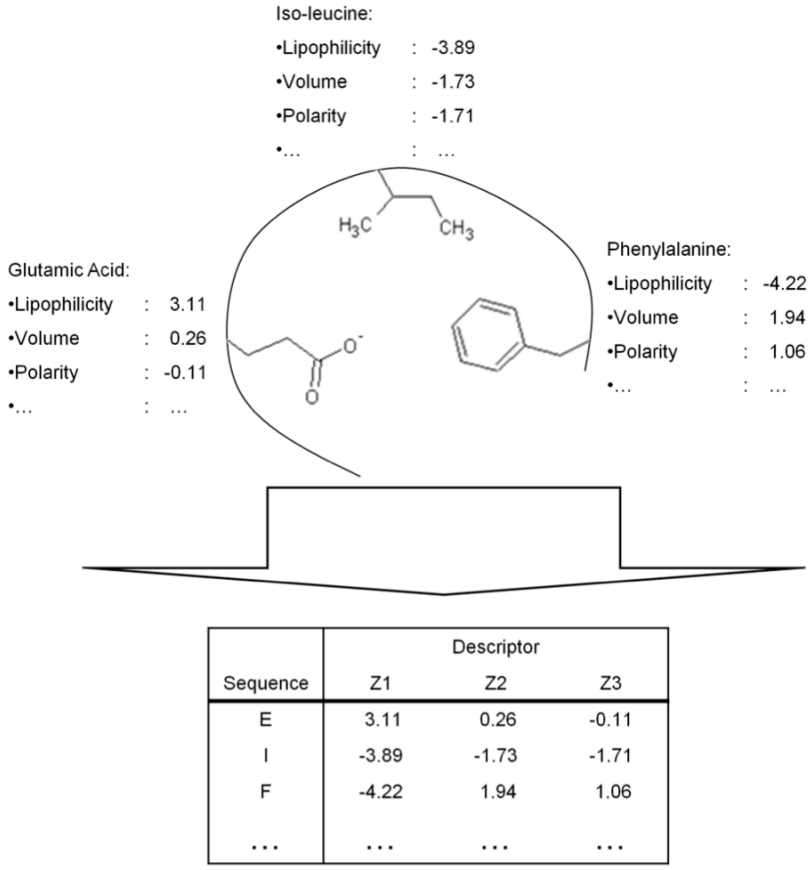
An example of a binary descriptor in PCM is given by Kontijevskis *et al.* who created several chimeric proteins divided into five segments based on building blocks obtained from four different receptors.[65] Thereby every segment was described by four binary descriptors and every protein by five segments, leading to each protein being described by 20 binary descriptors and to a unique descriptor for every protein. When directly compared with a sequential protein descriptor the binary descriptors (Root Mean Squared Error (RMSE) 0.61) outperformed the sequential descriptors (RMSE 0.76). However, in this form it is far less interpretable than sequential descriptors and limited in extrapolation capabilities.

A related subtype of binary protein descriptors is a feature-based semi-binary protein descriptor. In this descriptor each individual amino acid gets assigned a unique identifier resulting in a unique descriptor for each sequence.[57, 58] The final model is then trained on the collection of unique identifiers per sequence. The authors found this type of descriptors to outperform sequential descriptors, as is the case with the previously mentioned binary descriptors. The main advantage of these descriptors is that they are better interpretable than the binary descriptors as important residues can be individually identified rather than identifying an entire important subsection of a protein.

2.6.2 Three dimensional protein descriptors. While the descriptors used above encode only 2D properties of protein sequences, the targets are three-dimensional entities and this information can also be used in PCM modeling. Considering multiple mutants of HIV Reverse Transcriptase in parallel, Van Westen *et al.* used protein energy fields as descriptors for the investigation of complexes between mutant forms of HIV-RT and different ligands.[66] This appeared very useful in understanding the molecular mechanism and in suggesting novel chemistry ideas for anti-resistant inhibitor design. A 3D protein descriptor taking into account Cα coordinates and ϕ/ψ angles is introduced by Lindström *et al.*[67] Alignment problems and the large number of variables emerging from these descriptors were circumvented by preprocessing the data by PCA and covariance transformations. Lindström et al. show that this form of descriptor contains sufficient information to generate both global and sub-class specific PCM models.[67]

Contrary to full protein 3D descriptors, an example of a (local) 3D protein descriptor was introduced by Hvidsten and Kryshtafovych.[68, 69] Here 3D substructures are a collection of continuous short backbone fragments centered on a single residue within a predefined radius. These descriptors provide a highly generalized descriptor of the protein binding pocket and can therefore be used between completely different classes of proteins without the need for a multiple sequence alignment. They have been applied to PCM modeling of a very diverse target library of PDB structures by Strombersson et al.[35, 70] Performance was limited (cross validation prediction error of 1.7 log units), although it should be taken into account that this was a very diverse data set. We conclude that these descriptors can be especially useful when building a PCM model of a diverse dataset for which 3D structures are available.

2.6.3 Sequential protein descriptors. As for nearly all possible drug targets (human proteins) the amino acid sequence is available, one can use information derived from this sequence as a protein descriptor. Jacob *et al* employed this information to create several similarity measures.[31] In this case a hierarchical tree of the sequences guided a division in classes and through these classes a similarity measure of the binding pockets is obtained. They showed that using sequence as a protein descriptor enables the creation of a PCM model, obtaining a prediction accuracy of 90 %.[31] This performance can most likely be improved by converting a purely sequence based protein descriptor to a sequential descriptor of physicochemical properties of the amino acids, as described below.



	Descriptor		
Sequence	Z1	Z2	Z3
E	3.11	0.26	-0.11
I	-3.89	-1.73	-1.71
F	-4.22	1.94	1.06
…	…	…	…

Figure 2.5: Conversion of physicochemical properties of amino acids in the binding site into a protein descriptor via sequential protein descriptors using the Z-scales as an example. Based on the physicochemical properties of the amino acids side chains a protein descriptor is constructed. A trained PCM model can subsequently compare the binding sites of the proteins based on the differences in the physicochemical properties of the side chain.

Amino acids can also be described according to their physicochemical properties, like descriptors of small molecules. Several descriptor scales are available from the field of peptide drug modeling.[71-74] In PCM, the 'z-scales' introduced by Sandberg et al. that describe the properties of the amino acids, have been shown to perform well in many cases (**Figure 2.5**).[19, 67] The z-scales are based on a PCA used to compress a large input matrix that describes a broad selection of physicochemical properties into 5 principal components (PCs). In a plot of PC1 versus PC2, similar amino acids are located close together and dissimilar amino acids are spread further apart (**Figure 2.6**). PC1 can be interpreted as a lipophilicity scale and PC2 as volume/polarizability scale.[73] PC3 mainly describes polarity while PC4 and PC5 are more difficult to interpret. It has been shown that these sequential descriptors can predict contributions to selectivity without 3D information available 20 or predict the site of origin of indirect effects on ligand recognition by proteins.[21]

One possible problem with the z-scales or similar descriptors is the slightly limited interpretability since these descriptors are obtained through a data reduction step performed on an initially large matrix. Furthermore this initial data matrix contains far more amino acids than the 20 natural amino acids needed for the creation of PCMs, and therefore the descriptors might not perform optimal within the 20 natural amino acid space needed for description of protein targets. The authors are currently working on a novel protein descriptor that eliminates some of the issues described above.

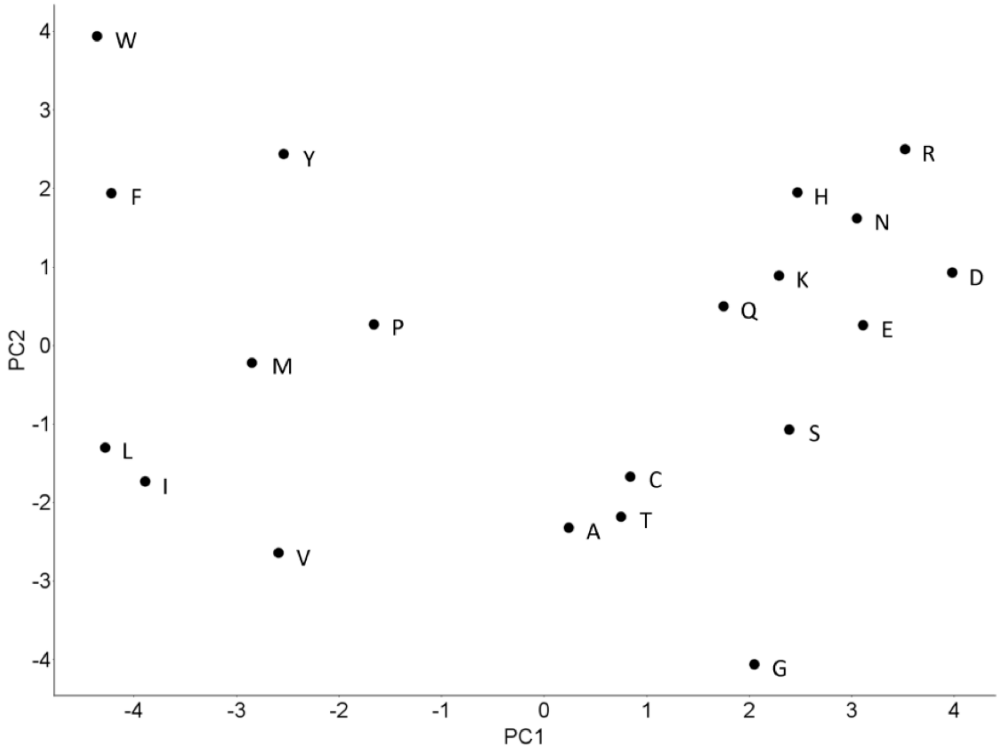


Figure 2.6: Principal components 1 and 2 of the PCA analysis which resulted in the Z-scales. In this plot similar amino acids are located closely together and dissimilar amino acids are spread further apart. PC1 can be interpreted as a lipophilicity scale and PC2 as volume/polarizability scale. While overall distances between amino acids agree with chemical intuition, this is not always the case (such as lysine being located closer to negatively charged amino acids than to arginine, which is reasonable when thinking about locations of the amino acids in the outside of a protein, but less so when relating to ligand binding properties).

2.7 Cross terms

2.7.1 Non-linear term. Descriptor cross terms are used to allow linear machine learning methods like Partial Least Squares (PLS) to model the non-linear interactions that guide ligand-target interactions,[75, 76] which seem to be present in at least half of all structure-activity datasets.[77] Cross terms are influenced by both the ligand and the target part of the dataset and they are intended to model particular interactions between the ligand and the target, such as charge interaction sets.[19, 22] However, when using non-linear machine learning methods in our work in practice, we found that cross terms may in fact deteriorate model performance, likely due to the introduction of a large number of additional parameters, so it is often beneficial to leave them out completely.[57] In their simplest forms, cross terms describe the similarity between different compound – target pairs; therefore they can be constructed as any similarity measure.

2.7.2 Drawbacks. One problem with cross-terms is that their functional form is undefined, and they can be any function of ligand and target properties. In practice often properties of the ligand and target are multiplied.[19, 65] However, this step follows more the intuition of the user than any thorough theoretical derivation. (For a comprehensive overview of previously used cross terms see **Table 2.1**.) Variable selection, mentioned under data pre-processing, can be applied to descriptors prior to cross term calculation when the calculation of all possible cross terms is too time consuming or computationally infeasible.[14, 35]

2.7.3 Alternative approaches. A second and completely different approach to cross term calculation is provided by Lindstrom *et al.*[67] They applied PCM to model a dataset that includes structural protein information, while using the target – ligand interactions as a cross term. The interactions were limited to steric and electrostatic complementarity, the ligand strain energy, logP (octanol/water partition coefficient), steric fit, complementary surface area interactions and the number of rotatable bonds in the ligand as described by Head *et al.*[78] Although their final global model had a prediction error of approximately 1.7 log units, the authors showed that any property dependent on both target and ligand features can be used as a cross term.

Weil and Rognan extend this work by compressing the ligand and target descriptors to a single fixed length bit-string.[29] They described the ligands and targets by a combination of different descriptors and subsequently they merged both the ligand and target bit-strings into one single protein-ligand fingerprint (PLFP). Thereby they used a single descriptor consisting of a ligand and target part to describe the dataset, effectively constructing a model on only the cross term. The authors obtain predictive models with average ROC values of around 0.80 with these descriptors and a variety of machine learning techniques.[29]

In conclusion a cross term can be any descriptor that depends on properties from the compound and properties from the target. As long as this condition is met there is no difference if the cross term is obtained by mathematically combining the compound and target descriptors (e.g. by multiplication, addition or exponentially) or if the cross term is obtained by introduction of an independent descriptor. As far as the authors know, the different mathematical operators to obtain cross terms have not been thoroughly researched, therefore this provides some interesting research opportunities.

2.8 Data pre-processing

Before a viable PCM model can be constructed, the data should usually be pre-processed allowing it to be compatible with the machine learning technique of choice and to obtain optimal model performance. Depending on the data and machine learning technique this processing can be very extensive or relatively simple. Here we will provide an overview of data preprocessing steps, with applications in PCM modeling in mind.

2.8.1 Scaling and mean centering. When a PCM model is created using multiple descriptors (descriptor blocks), it should be prevented that a biased model is created, wherein a subset of descriptors mask the influence of the other descriptors. Scaling and mean centering the different descriptors prevents this bias, makes them compatible and is especially necessary when using PLS modeling.[79] In block scaling one block of descriptors is scaled according to:

$$1/(Nb)1/2 \quad (1)$$

Here, Nb is the number of descriptors in block b. Block scaling prevents larger blocks to mask small ones and it is often incorporated in modern modeling tools.[26, 65] In the case of PCM modeling mean centering is always advised.

2.8.2 Covariance removal. To prevent overfitting of the model, which is likely when PLS is used,[80] it is important to remove covariance within the descriptor blocks before the final model is built. Covariance can lead to misinterpretations of the final model and poor extrapolation capabilities, and it increases the dimensionality of the model with no apparent benefit. Several methods are available for removal of covariance and determination of the descriptors that best describe the data. Two of the covariance removal methods previously used in PCM will be highlighted, namely variable extraction (e.g. PCA) and variable selection. For a general review about the underlying principles and methods see Wegner *et al.*[81]

2.8.3 Variable extraction. An example of variable extraction is PCA, a method to find the underlying latent variables present in a data set, e.g. in a set of descriptors. The original multidimensional space defined by the descriptors is summarized by a smaller number of descriptive dimensions which describe the main variation in the data; these are called the principal components.[82] PLS is the regression extension of PCA and provides the ability to construct a model based on the extracted principal components. PCA can be used when modeling of the original dataset is infeasible due to reasons such as a high dimensionality of the dataset.[14, 65] However, at the same time the interpretability of the model is usually decreased.

2.8.4 Variable selection. Variable selection is the selection and exclusion of variables with negligible importance for the data to be modeled. It is also known as Variable importance projection (VIP) or variable subset selection (VSS). When building a PCM model using PLS, the reliability is heavily influenced by the variable selection before model training. In the case of PLS it can therefore be seen as both a data preprocessing and model tuning procedure.[83] Variable selection is an iterative process aimed at selecting a subset of variables from the full set that optimally describes the variance of the dataset. Two possible forms of variable selection exist.[84] Backward selection consists of iterative elimination starting from the full set, and forward selection consists of iterative addition starting from a single variable. To our knowledge in PCM only backwards selection has been previously used. The iterative process proceeds as follows, firstly a model is constructed on the full data set, subsequently descriptors with negligible importance are removed and a new model on the improved descriptor set is built.[19, 82, 83] This procedure can be repeated until variable selection leads to model deterioration. In models where many variables receive low weights, the variable selection can significantly improve the model.[85]

2.9 Modeling techniques in PCM

Apart from statistical methods, both linear machine learning techniques and non-linear machine learning techniques can be and have been used in PCM. We will describe several approaches, starting with modeling learning techniques already used in PCM and subsequently including new suggestions. We will highlight positive and negative consequences of the different techniques, a short summary of which is given in **Table 2.2**.

Table 2.2: Modeling techniques previously used, and which can potentially be used, in proteochemometric modeling with their main advantages and disadvantages

Technique	Linear ?	Previously used in PCM?	Advantage	Disadvantage
PLS	Linear	Yes	Highly Interpretable	Requires Cross-Terms
RS	Non-linear	Yes	Highly Interpretable	Classification
NN	Non-linear	Yes	Performs well on complex data	High Dimensionality
SVM	Non-linear	Yes	Very Robust on complex data	Poorly Interpretable
NB	Linear	Yes	Performs well on complex data	Requires Cross-Terms
RF	Non-linear	Yes	Low risk of Overfitting	-
DT	Non-linear	Yes	Highly Interpretable	Variable performance
GP	Non-linear	No	Confidence Estimate	Long training time

Explanation of abbreviations: Partial Least Squares (PLS), Rough Set (RS), Neural Net (NN), Support Vector Machines (SVM), Naïve Bayesian (NB), Random Forest (RF), Decision Tree (DT).

2.9.1 Partial least squares. By far the most commonly used modeling technique in PCM is PLS.[80] PLS is the regression extension of PCA and specifies the relationship between an output variable Y and a set of predictor variables Xi. The final model is able to display the role of the individual predictor variables in the model. Advantages of PLS are that it is highly interpretable and requires low computational expense. A large number of PCM models have been created founded on PLS with a prediction error usually ranging between 0.4 and 0.8 log units. Targets for which PLS-PCM models were constructed include melanocortin receptors, HIV Proteases and dengue virus NS3 proteases.[20, 33, 34] However, as PLS is linear it requires cross terms to be calculated in order to obtain an optimal model as opposed to Rough Set modeling which alleviates this restriction.[21, 22, 26, 62, 65, 85, 86]

2.9.2 Rough set modeling. Non-linear Rough Set (RS) modeling has been introduced to PCM by Strömbergsson *et al.* as they proposed that RS might be capable of modeling data to a higher level than PLS because of its non-linearity.[29, 36] RS constitutes a mathematical framework that induces basic IF-THEN decision rules to classify a compound – ligand pair as active or inactive,[27] therefore these models are highly interpretable. However, as RS is a classification tool, RS is unable to perform regression and provide a numerical value, e.g. an affinity (pKi) value. RS modeling has been applied to melanocortin and adrenergic receptor datasets as well as a dataset containing a very broad target collection obtained from the PDB.[27, 35] RS modeling performed very well with an area under the retrieval curve (ROC) usually above 0.9, reliably distinguishing between actives and inactives on a particular target.

However, while the non-linear RS cannot be used in order to model a numerical output variable rather than a class, SVM as described in the following section is a non-linear machine learning technique that is capable of both classification and regression.

2.9.3 Support vector machines. SVM is a non-linear modeling technique also applied multiple times in PCM.[24, 25, 57, 58, 70, 87, 88] The main advantage of this machine learning technique is that it has been proven to be very robust and very capable of modeling QSAR datasets, especially in the case of many dimensions.[89, 90] The disadvantage of SVMs at the moment is the degree to which the models can be interpreted. However, a recent paper by Carlsson *et al.* introduced an approach to improve the interpretation capabilities.[91] When these interpretation methods improve they might lead to SVM models being the machine learning technique of choice in PCM models. Three publications are available that describe PCM modeling based on SVMs. The first one is by Strömbergsson *et al.* who model the entire Enzyme-Ligand interaction space.[70] Although the absolute performance of the PCM model is not very accurate with a prediction error of 1.5 log units, the authors were able to model a very diverse dataset. The second publication by Geppert *et al.* showed recovery rates of active compounds from a database of around 60-70 %,[24] leading to the conclusion that SVM can successfully extrapolate from a combination of ligand and target information to retrieve new active compounds on a related target. This conclusion is supported by Ning *et al.*,[25] who also used multiple assay based models to gain improved performance compared to single assay models.

We share a similar view, having used SVM based PCM models to model an adenosine receptor dataset and an HIV Reverse Transcriptase inhibitor dataset.[58] The SVM models we generated were able to model the data with a prediction error of around 0.5 log units. In conclusion SVM is a robust machine learning technique capable of modeling the complicated PCM data. However, it should be noted that SVM specific parameters like 'gamma' and 'cost' must always be optimized through a proper model selection, for instance by cross validation.[92]

2.9.4 Neural net modeling. The final previously used machine learning technique is neural network (NN) modeling. Fernandez *et al.* have applied NN to PCM modeling using a Bayesian Regularized form of NN.[93] NNs are known for their ability to handle complex input-output relationships and provide robust models of non-linear data. For an extensive review on NN please see Grossberg *et al.*[94] In PCM NNs would not be an optimal machine learning technique since NNs often possess too many parameters for this purpose (as an often mentioned very approximate rule of thumb, when training NNs one should have at least three times more datapoints than variables). In PCM modeling the number of variables is much larger than in QSAR as PCM requires two variables per input dimension. Fernandez *et al.* used a simplified correlation matrix as ultimate input descriptor, keeping the number of input variables low and using Bayesian Regularization to diminish the inherent complexity of the NN. Therefore their model output requires a translation to the original descriptors before the results can be interpreted. Furthermore, NNs are not easily interpretable by themselves. However, Browne *et al.* have described ways of improving the interpretability.[95] A much better interpretable modeling technique known from QSAR models is a Naïve Bayesian classifier. The possibility of using this classifier in PCM models will be described below.

2.9.5 Naïve Bayesian classifier. Naïve Bayesian (NB) classification models have been shown to be able to model datasets with a high number of variables and relate bioactivity predictions from multiple activity classes with each other.[96] These two qualities make Bayesian classification another suitable machine learning technique for PCM. However, as Bayesian classification is linear, cross terms might be required to allow confident modeling. Two publications on PCM with a Naïve Bayesian (NB) classifier have appeared, one by Weil and Rognan and one by Strömbergsson *et al.*[29, 46] However, in the former the model is created merely on cross terms as descriptors and in the second publication no cross terms are used. We speculate that an NB model will reach a better performance and interpretability when separate ligand, protein and cross term descriptors would be used.

2.9.6 Decision trees algorithm. The decision trees (DT) algorithm has been known from the creation of QSAR models. The decision based output makes a decision tree highly interpretable. The DT algorithm has been applied to PCM by Lapins *et al.* and by Strömbergsson *et al.* [39, 46] In the former publication their performance is somewhat disappointing with a squared correlation coefficient of 0.45, which puts it slightly below PLS. The authors contribute this performance to the high non-linearity of the dataset. In the latter publication by Strömbergsson *et al.* the performance of DT, as a classifier, is superior to both SVM and NB based classification models, with an ROC score of 0.84 and an accuracy of 82 %. It can therefore be concluded that the performance of DT can vary with the dataset and that further research is required.

2.9.7 Random forest. Random forest modeling techniques, which can be used for both classification and regression, have previously been shown to perform well on QSAR datasets.[97-99] Weil and Rognan have also applied this technique to PCM data.[29] In their paper RF performs very well on a number of datasets with a recall larger than 0.5 and precision value higher than 0.7 on average, on average RF performs roughly equal to SVM. However, since RF can provide the following additional features: built-in performance assessment, a measure of relative importance of descriptors, and a measure of compound similarity that is weighted by the relative importance of descriptors. RF might very well be a better choice for usage in PCM models than SVM with its low interpretability.

In conclusion, all machine learning methods that have currently been applied to PCM have their advantages and disadvantages, and none can be considered a universal optimal approach. It might therefore be interesting to apply established machine learning methods that have previously not been used in PCM. One suggestions will be discussed below.

2.9.8 Possible new machine learning techniques to be applied in PCM. One of the most promising machine learning techniques not yet applied in PCM are Gaussian Processes (GP).[100] The non-linear GP not only provide the scientist with a prediction of the output variable but also a measure of reliability for this prediction in the form of variance estimation for each prediction. This quality makes it invaluable in PCM as it directly links the application domain to model predictions and allows the selection of the most reliable predictions for decision making. Previously GPs have been shown to perform very well in the modeling of ADME and physicochemical properties.[101, 102] Although the training time required surpasses that of the linear PLS technique, the superior performance combined with the prediction confidence parameter tips the scales in favor of GP.

2.10 Validation of a PCM model

One of the key differences between PCM and QSAR is that PCM significantly increases the number of variables to be modeled as the descriptor space is increased. Therefore there is an increased risk of both chance correlations and model overfitting.[85] The modeler should consequently take care to rule out the possibility of a model built on chance correlations. Validation in PCM is based on established validation techniques normally applied in QSAR modeling. The key goal is to get a reliable estimate of the model quality and applicability. This goal can be achieved by calculation of the correlation coefficient, coefficient of determination and RMSE. For an overview of validation techniques please see a set of recent comprehensive publications.[103-106]

2.10.1 Y-scrambling. Response permutation testing, or Y-scrambling is an approach to estimate the risk of chance correlations.[103, 107] Y-scrambling consists of keeping the X-variables, or the descriptor space, fixed and to randomly shuffle the output or Y- variable and subsequently retraining the model. A typical approach is to create 100 random models and to assess their performance using standard validation parameters and to compare this performance to the actual model, where the performance of the proper model should be significantly higher (for details see above references). Due to the highly increased variable space that a PCM is founded on, this validation technique is very relevant and should always be applied to validate the final model.

2.10.2 Internal validation. Cross validation or internal validation is used to estimate the ability of the model to reliably predict the activity of the data points used in the training set. There are three forms of cross validation, namely Leave-One-Out (LOO)) cross validation, n-fold cross validation and double loop cross validation.[85] Double loop cross validation was introduced by Freyhult *et al.* to improve the quality of cross validation performance estimates and prevent overoptimistic assumptions.

Two independent loops are used to tune model parameters in the inner loop and provide a performance estimate P2 in the outer loop. Here we will only consider n-fold cross validation as it is currently the state of the art.[108] In n-fold cross validation the total training set is divided into 'n' equal subsets. Subsequently a model is trained on n-1 of the subsets and used to predict the activity of the data points in the remaining subset. This process is repeated until all subsets have been left out of the training and the plots of these iterations are used to calculate q^2 and cross-validated RMSE.[109] Cross validation, while useful, cannot always reliably be used to estimate the performance of the final model on unknown data points as it has been shown that there is no direct correlation between q^2 and R^2.[59, 109] However, cross-validation provides a very useful framework for tuning modeling parameters like the number of components in PLS and the values for 'gamma', 'epsilon' and 'cost' in SVM.[65, 89, 92]

2.10.3 External validation. In external validation a trained model is used to predict the output variable for a set of data points for which an observed activity value is available. These points have been separated from the original dataset prior to model training (as well as selection!) or are assembled only after the model has been constructed, and hence they and are completely unknown to the model. Separation of this so called test-set from the training set can be performed using a wide extent of different parameters. (For a full review see Tropsha *et al.*[103]) After model training, the performance of the model on the test set is estimated using conventional validation parameters. The main goal of this exercise is to provide a more reliable performance estimate than internal validation to assess the quality of model predictions on a dataset of unknown compounds. This performance estimation becomes even more critical when using any sort of model selection, e.g. feature selection.[110] Otherwise there is a considerable risk that models will become overfitted as we reported earlier,[111] and is explained by Wegner et al.[81]

2.10.4 Prospective validation. The only true validation for any computational model is prospective validation. Prospective validation assesses model performance by experimentally determining compound activity subsequent to model predictions. Unfortunately in the field of PCM not much work has been published containing a prospective validation. Currently, to the knowledge of the authors, only three publications contain prospective validation of modeling results.

The first prospectively validated PCM model was constructed on a dataset consisting of melanocortin wild-type and chimeric receptors and their ligand α-MSH (and synthetic analogues).[21] In this publication a small scale prospective validation was performed in which PCM predicted a correct response of the binding affinity to point mutations in 80 % of the datapoints. The second published prospective validated PCM model was constructed on a HIV protease dataset (containing point mutations in the protein that change binding affinity) and a selection of octapeptides as ligands.[112] Here PCM was able to prospectively predict the affinity of 10 peptides on 4 different protease mutants with an R of 0.63 (R^2 of 0.40). Lastly, a prospectively validated PCM modeling study was published by Nagamine et al.[113] They used an SVM based model to find androgen receptor ligands from a pool of 19 million compounds while iteratively prospectively validating model predictions. With their model they obtained an area under the ROC curve of 0.717 compared to 0.558 for QSAR. These results firmly establish PCM as a reliable modeling technique with applications in preclinical research.

2.11 Pitfalls and disadvantages

In any form of statistical modeling there are a large number of possible pitfalls which are caused by the modeling technique, data set preparation or can be the result of a simple bias. An extensive review on risks in statistical modeling has been published by Gedeck *et al.*;[13] here we will focus on specific dangers that can arise when performing PCM.

The main risk present in PCM has also been discussed under validation, and is the fact that the large increase in descriptor variables increases the risk of chance correlation models. This should at all times be kept in mind and an extensive validation (including cross-validation, Y-scrambling and prospective testing) is indicated for all PCM models.

A second pitfall comes from the fact that the data to be modeled by PCM is inherently non-linear, whereas many machine learning techniques in QSAR rely on (multiple) linear regression. Therefore the data should be modified in many cases by the introduction of cross-terms. The risk of cross-terms is that they will account for most of the described variance, as cross terms describe variance of the ligands and the targets simultaneously. If this is the case, cross terms can mask the contributions of the pure compound or protein descriptors.

Furthermore, since they rely on both compound and protein contributions, cross terms are not always readily interpretable;[27] however, this depends to a significant extent on the precise way in which cross terms are constructed. When using cross terms one needs to ensure that both compound and protein descriptors are compatible; which is not always the case. A workaround that circumvents the use of cross terms, can be the use of non-linear machine learning techniques; however their low interpretability might lead to highly accurate but non-interpretable black box models. Currently research efforts are underway to alleviate this problem,[91, 95] but for now a decision needs to be made in many cases between better interpretable models or more accurate models. As mentioned above, it also needs to be ensured that cross terms indeed improve model performance, which was not always the case in the experience of the authors.

A disadvantage of PCM is that the calculation time of the models increases compared to QSAR when protein descriptors are added. Depending on the machine learning technique this increase in calculation time can be small, as in PLS, or exponential, as in radial basis function based SVMs.[114]

2.12 Conclusions

PCM is a relatively new technique that, by including target descriptors in addition to ligand descriptors, enables modeling of datasets that could previously only be modeled separately using conventional QSAR based techniques. PCM has been applied successfully to a variety of targets, among which are GPCRs, Viral Proteins and Cytochrome P450 enzymes. However, relatively few of those studies have included prospective validations – with the notable exceptions of the studies by Prusis *et al.*, Kontijevskis *et al.*, and Nagamine *et al*.[21, 112, 113] Hence, while limited data exists, the authors are of the opinion that PCM modeling should indeed, in many cases, be able to make better use of bioactivity data of molecules than previous QSAR models.

In conclusion, by more comprehensively exploiting the information contained in datasets that were previously considered separate, PCM models allow for improved extrapolation both on the ligand side (by taking more 'chemistry' into account) as well as on the target side (by incorporating the relationship between targets into the model). This enables applications in areas such as the deorphanization of compounds or the selection of compounds that are selective, or show a desired bioactivity profile. Until the current stage few prospective PCM studies are available in the literature. However PCM is one of the areas where other research groups are currently active, and where a large-scale validation will be published also by the authors very shortly.

2.13 Acknowledgements

The authors would like to thank Olaf O. van den Hoven for his supporting work and discussions. GJPvW thanks Tibotec BVBA for funding his PhD project.

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