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The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling

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ABSTRACT

Motivation: Homology models of proteins are of great interest for planning and analysing biological experiments when no experimental three-dimensional structures are available. Building homology models requires specialized programs and up-to-date sequence and structural databases. Integrating all required tools, programs and databases into a single web-based workspace facilitates access to homology modelling from a computer with web connection without the need of downloading and installing large program packages and databases.

Results: SWISS-MODEL workspace is a web-based integrated service dedicated to protein structure homology modelling. It assists and guides the user in building protein homology models at different levels of complexity. A personal working environment is provided for each user where several modelling projects can be carried out in parallel. Protein sequence and structure databases necessary for modelling are accessible from the workspace and are updated in regular intervals. Tools for template selection, model building and structure quality evaluation can be invoked from within the workspace. Workflow and usage of the workspace are illustrated by modelling human Cyclin A1 and human Transmembrane Protease 3.

Availability: The SWISS-MODEL workspace can be accessed freely at http://swissmodel.expasy.org/workspace/

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 INTRODUCTION

Three-dimensional (3D) protein structures are of great interest for the rational design of many different types of biological experiments, such as site-directed mutagenesis or structure-based discovery of specific inhibitors. However, the number of structurally characterized proteins is small compared with the number of known protein sequences: as of November 2005, more than 33 000 experimentally determined protein structures were deposited in the Protein Data Bank (Westbrook *et al*., 2003), while the UniProt protein knowledge database (Bairoch *et al*., 2005) held more than 2.3 million sequences. Various computational methods for modelling 3D structures of proteins have been developed to overcome this limitation. Since the number of possible folds in nature appears to be limited (Chothia, 1992) and the 3D structure of proteins is better conserved than their sequences, it is often possible to identify a homologous protein with a known structure (template) for a given protein sequence (target). In these cases, homology modelling has proven to be the method of choice to generate a reliable 3D model of a protein from its amino acid sequence as impressively shown in several meetings of the bi-annual CASP experiment (Tramontano and Morea, 2003; Moult, 2005). Homology modelling is routinely used in many applications, such as virtual screening, or rationalizing the effects of sequence variations (Marti-Renom *et al*., 2000; Kopp and Schwede, 2004a; Hillisch *et al*., 2004). Building a homology model comprises four main steps: (1) identification of structural template(s), (2) alignment of target sequence and template structure(s), (3) model building and (4) model quality evaluation. These steps can be repeated until a satisfying modelling result is achieved. Each of the four steps requires specialized software as well as access to up-to-date protein sequence and structure databases.

Selection of the most suitable template structure and the alignment between target and template are still the predominant sources of errors in comparative models (Tramontano and Morea, 2003). In our own experience and that of others (Bates *et al*., 2001), manual intervention at different steps of model building can significantly improve the accuracy of the results. We have developed the SWISS-MODEL workspace to address this aspect by providing a range of tools that allow the user to validate and to modify the different modelling steps manually. The SWISS-MODEL workspace integrates programs and databases required for homology modelling in an easy-to-use web-based modelling workbench. It allows the user to construct comparative protein models from a computer with web connection without the need of downloading and installing large program packages and databases.

Depending on the difficulty of the individual modelling task, the workspace assists the user in building and evaluating protein homology models at different levels of complexity. For models that can be built based on sufficiently similar templates, we provide a highly automated modelling procedure with a minimum of user intervention. On the other hand, for more difficult modelling scenarios with less target–template sequence similarity, the user is given control over individual steps of model building: functional domains in multi-domain proteins can be detected, secondary structure and

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disordered regions can be predicted for the target sequence, suitable template structures identified by searching the SWISS-MODEL Template Library (SMTL), and target–template alignments can be manually adjusted. As quality evaluation is indispensable for a predictive method like homology modelling, every model is accompanied by several quality checks. All relevant data of a modelling project are presented in graphical synopsis.

The accuracy, stability and reliability of the automated SWISS-MODEL server pipeline (Peitsch, 1995; Guex and Peitsch, 1997; Schwede *et al*., 2003) has been continuously validated by the EVA-CM evaluation project (Koh *et al*., 2003). The workspace extends the web-based SWISS-MODEL system by assisting manual user intervention in model building and evaluation. Here, we will illustrate the application of SWISS-MODEL workspace in two modelling projects of different complexity: human Cyclin A1 and Transmembrane Protease 3.

2 SYSTEMS AND METHODS

2.1 Modelling modes in SWISS-MODEL workspace

Depending on the difficulty of the modelling task, three different types of modelling modes are provided, which differ in the amount of user intervention: automated mode, alignment mode and project mode. Modelling requests are directly computed by the SWISS-MODEL server homology modelling pipeline (Schwede *et al*., 2003). The 'automated mode' has been developed for cases where the target–template similarity is sufficiently high to allow for fully automated modelling. As a rule of thumb, automated sequence alignments are sufficiently reliable when target and template share >50% identical residues (Rost, 1999). 'Automated mode' submissions require only the amino acid sequence or the UniProt accession code of the target protein as input data. The modelling pipeline automatically selects suitable templates based on a Blast *E*-value limit, which can be adjusted upon submission. The automated template selection will favour high-resolution template structures with reasonable stereochemical properties as assessed by ANOLEA mean force potential (Melo and Feytmans, 1998) and Gromos96 force field energy (van Gunsteren *et al*., 1996).

Multiple sequence alignments are a common tool in many molecular biology projects, and are often the result of extensive theoretical and experimental exploration of a certain family of proteins. If a 3D structure for at least one of the members is known, a given alignment can be used as starting point for comparative modelling using the 'alignment mode'. In order to facilitate the use of alignments, the submission is implemented as a three-step procedure: The input alignment, which can be provided in several different formats (FASTA, MSF, ClustalW, PFAM, SELEX), is converted into a standard ClustalW format in the first step. The user indicates which sequence in the alignment corresponds to the target protein, and which corresponds to a protein with an experimentally determined structure deposited in the PDB database (Westbrook *et al*., 2003). Commonly, protein sequences used for multiple sequence alignments are not identical to their corresponding PDB entries, e.g. due to missing atom coordinates, mutations or sequence tags introduced for easier purification and crystallization. Therefore, in the subsequent step the submitted alignment is matched against the sequence of the template structure coordinates extracted from the SWISS-MODEL template library. Since most modelling programs depend on a perfect match between the template sequence in the input alignment and the residues present in the coordinate file, this step relieves the user from the obligation to deliver 'perfect' alignments to PDB entries. Instead one can actually work with sequences directly retrieved from sequence databases. The server pipeline will build the model based on this alignment. During the modelling process, implemented as rigid fragment assembly in the SWISS-MODEL pipeline, the modelling engine might introduce minor heuristic modifications to improve the placement of insertions and deletions based on the structural context (Schwede *et al*., 2003).

In difficult modelling projects, where the correct alignment between target and template cannot be clearly determined by sequence-based methods, visual inspection and manual manipulation of the alignment can significantly improve the quality of the resulting model (Bates *et al*., 2001). The program DeepView—Swiss-PdbViewer (Guex and Peitsch, 1997)—can be used to generate, display, analyse and manipulate modelling project files for SWISS-MODEL workspace. Project files contain the superposed template structures and the alignment between the target and the template. In this mode the user has full control over essential modelling parameters, i.e. the choice of template structures, the correct alignment of residues, and the placement of insertions and deletions in the context of the 3D structure. Project files can also be generated by the workspace template selection tools and are the default output format of the modelling pipeline. This allows analysing and iteratively improving the output of 'automated mode' and 'alignment mode' modelling projects. The program DeepView can be downloaded freely from the ExPASy web site (http://www.expasy.org/spdbv/).

2.2 SWISS-MODEL template library

The template structure database used by SWISS-MODEL (SMTL) is derived from the Protein Data Bank (Westbrook *et al*., 2003). In order to allow sequence-based template searches, each PDB entry is split into individual chains. The separated template chains are annotated with information about experimental method, resolution (if applicable), ANOLEA mean force potential, Gromos96 energy and PQS (Henrick and Thornton, 1998) quaternary state assignment to allow for rapid retrieval of the relevant structural information during template selection. Theoretical models, structures only consisting of C_α atoms and incorrectly formatted database entries are removed.

In order to speed up the sequence database search step of the template identification algorithms and to provide a clear and concise overview of the results, templates sharing 100% sequence identity are grouped into an SMTL100 library using the program CD-HIT, a fast clustering method for sequences at high identity thresholds (Li *et al*., 2001, 2002). Clusters of sequences having 90, 70 and 50% sequence identity are derived from the RCSB non-redundant PDB lists. Information about the members of the cluster is presented in the detailed output of the different template search programs. For each template, the SWISS-MODEL workspace provides a summary showing a small ribbon representation, experimental details, information about bound molecules, as well as links to PDB (Westbrook *et al*., 2003), SCOP (Andreeva *et al*., 2004), CATH (Pearl *et al*., 2005), PDBsum (Laskowski *et al*., 2005) and MSD (Velankar *et al*., 2005).

2.3 Domain assignment, secondary structure and disorder prediction

Many proteins are modular and made up of several structurally distinct domains, which often reflect evolutionary relationships and may correspond to units of molecular function (Andreeva *et al*., 2004; Bateman *et al*., 2004; Pearl *et al*., 2005). The member databases of InterPro (Mulder *et al*., 2005) allow for both the identification of protein domains and the assignment of protein function. Using IprScan (Zdobnov and Apweiler, 2001), a PERL-based InterProScan implementation, protein domains and functional sites can be assigned to regions of a target sequence. Splitting multi-domain proteins into separated domains often improves the sensitivity and performance of profile-based template search methods (see below). Secondary structure prediction methods are provided, which are especially useful when combined with other types of analyses: e.g. in cases where only templates with very low sequence homology can be detected by sequence-based search methods, predicted secondary structure may help to decide if a putative template shares structural features of the target protein. The secondary structure prediction program PsiPred (Jones, 1999) is accessible from the tools section of the workspace.

Most soluble proteins adopt well-defined 3D structures. However, some proteins have regions that are natively disordered, unstructured or have flexible regions without permanent regular secondary structure. It has been suggested that disordered regions may possess biological functions, and could be involved in signalling and regulation processes (Dunker and Obradovic, 2001; Iakoucheva *et al*., 2002). The protein disorder prediction program Disopred (Jones and Ward, 2003) estimates the propensity of protein sequences to be disordered. The result of secondary structure, disorder and domain boundary prediction (Fig. 1) can aid the selection of modelling templates for specific regions of the target protein.

2.4 Template identification

The degree of difficulty in identifying a suitable template for a target sequence can range from 'trivial' for well-characterized protein families to 'impossible' for proteins with a so far unknown fold. Consequently, the SWISS-MODEL workspace provides access to a set of increasingly complex and computationally demanding methods to search for templates.

Blast. Templates which are close homologues of the target can be identified using a gapped BLAST (Altschul *et al*., 1997) query against the SWISS-MODEL template library extracted from PDB. When no suitable templates are identified, or only parts of the target sequence are covered, two additional approaches for more sensitive detection of distant relationships among protein families are provided: an iterative profile Blast search and a Hidden Markov Model-based template search.

Iterative profile blast. In the iterative profile Blast approach (Altschul *et al*., 1997) a profile for the query sequence is built from homologue sequences found by iterative searches of the NR database (Wheeler *et al*., 2005). Subsequently, this profile is used to search for homologous structures in the template library. This method has been initially introduced as PDB-Blast by Godzik and coworkers.

HMM-based template library search. A library of Hidden Markov Models for a non-redundant set of the sequences in the template library was generated. Each model of the library was created from a multiple sequence alignment generated by an iterative search of the NR databases using SAM-T2K (Hughey and Krogh, 1996). Model building, calibration and library searches were performed using the SAM (v 3.4) software package (Karplus *et al*., 1998). Although computationally intensive, model calibration against a large, non-redundant database was calculated in order to obtain more accurate *E*-values during the scoring step. To select suitable template structures, the target sequence is scored against the template HMM library for statistically significant matches.

2.5 Continuous evaluation of modelling accuracy

Evaluation of model quality is a crucial step in homology modelling. In our view, benchmarking the individual steps and components of the comparative modelling workflow separately is not sufficient to assess the overall performance of a modelling pipeline, because the observed differences may not reflect critical steps in practical application and are therefore often inconclusive in judging the accuracy of an entire modelling workflow. The reliability of different protein modelling methods can be assessed by evaluating the results of blind predictions after the corresponding protein structures have been determined experimentally. During the biannual 'Community Wide Experiment on the Critical Assessment of Techniques for Protein Structure Prediction' CASP (Moult, 2005), new algorithmic developments are evaluated based on a number of test cases, e.g. in the 2004 CASP6 experiment 43 homology modelling targets were assessed (Tress *et al*., 2005). The continuous and automated assessment of modelling servers by the LiveBench (Rychlewski and Fischer, 2005) or EVA (Koh *et al*., 2003) projects is based on a large number of blind predictions. SWISS-MODEL was the first comparative modelling server to join the EVA project in May 2000, and has been continuously monitored since then. EVA-CM has performed a comparative evaluation of the following servers: 3D-Jigsaw (Bates *et al*., 2001), ESyPred3D (Lambert *et al*., 2002), CPHModels (Lund *et al*., 1997), SDSC1 (Shindyalov and Bourne, 2000) and SWISS-MODEL (Guex and Peitsch, 1997; Schwede *et al*., 2003). The evaluation has been based on 261 weekly releases of the PDB database consisting of 48 098 protein models for 19 698 protein target chains. Figure 2a (supplementary material) gives an estimation of the overall accuracy of the different modelling servers displaying the C_α atom RMSD after global superposition of the model and the experimental target structures versus percentage of sequence identity between target and best template. As expected, model RMSD is increasing with decreasing alignment accuracy as defined by the percentage of equivalent C_α positions (within 3.5 Å) between the optimally superimposed target and model structures (Figure 2b, supplementary material). For target–template pairs sharing sequence identities of >40%, only small differences between the prediction servers are observed.

The cumulative evaluation of global C_α atom RMSD over all models in the test set shows that the models built by SWISS-MODEL exhibit on average the lowest overall deviation (2.0 Å versus 2.7 Å for 3DJIGSAW) from the experimental control structures (Figure 2c, supplementary material). However, this is linked to a lower coverage of SWISS-MODEL for low homology templates. In the EVA-CM comparison, the models built by SWISS-MODEL appear to be on average more accurate, but shorter than the models of the other servers in the evaluation.

In general, the modelling accuracy for different target proteins is much more variable than the differences observed between different

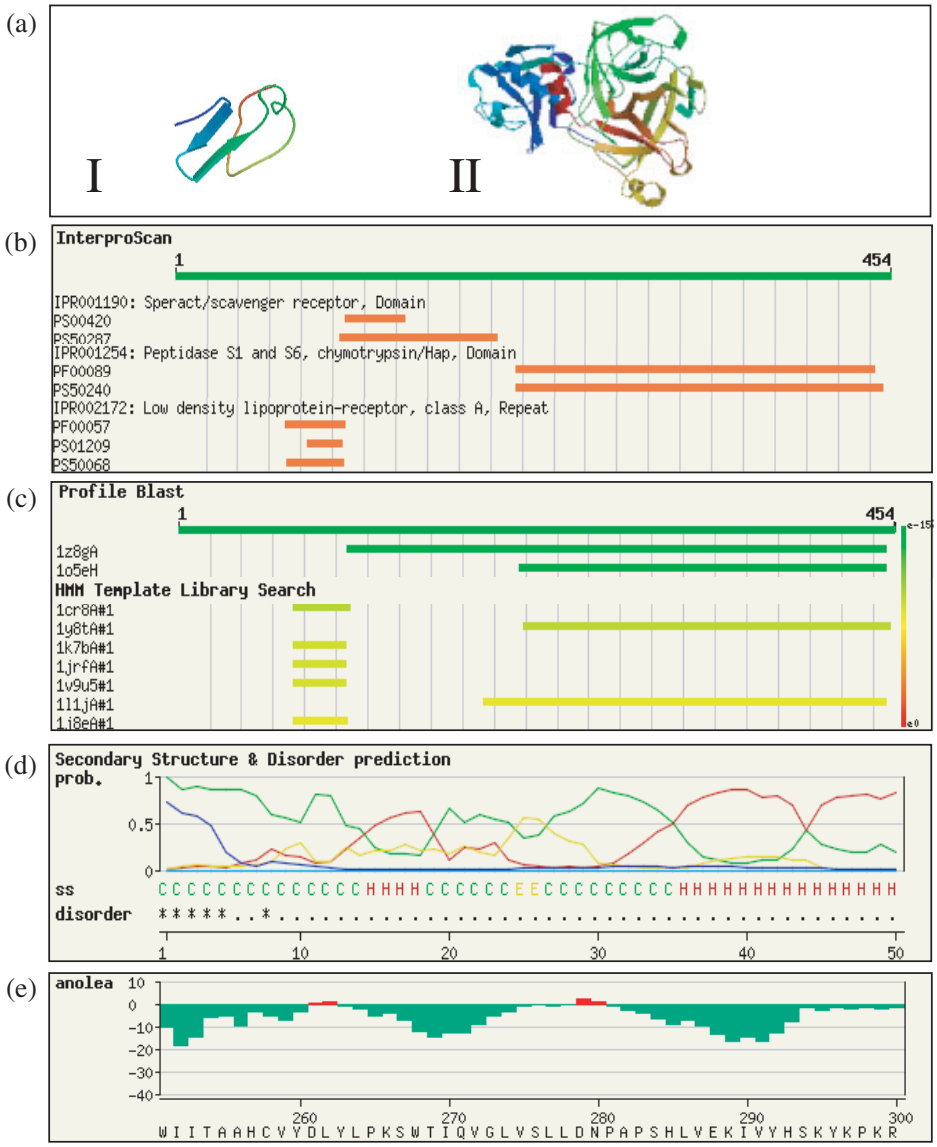

Fig. 1. Graphical output of SWISS-MODEL workspace representing typical steps of a modelling experiment. (**a**) Ribbon representation of three domains modelled for the target protein human transmembrane protease 3 (TMPRSS3): I, LDL receptor domain and II, Scavenger receptor in complex with the protease domain. (**b**) IprScan of the target sequence detected three domains in the target protein. (**c**) Sequence-based searches of the template library identified two segments with suitable template structures. (**d**) Secondary structure and disorder prediction of the target protein. (**e**) Anolea mean force potential plot allows for quality assessment of the final models.

modelling methods for the same target. The EVA-CM database is sufficiently large to allow the prediction of the expected accuracy of the participating servers for a given protein sequence based on previous data of similar modelling scenarios.

2.6 Tools for protein structure and model assessment

The quality of individual models can vary significantly from the average accuracy expected for a given target–template similarity or modelling method. Therefore, individual assessment of each model is essential. SWISS-MODEL workspace provides graphical plots of Anolea mean force potential (Melo and Feytmans, 1998), GROMOS empirical force field energy (van Gunsteren *et al*., 1996), Verify3D profile evaluation (Eisenberg *et al*., 1997), Whatcheck (Hooft *et al*., 1996) and Procheck (Laskowski *et al*., 1993) reports are generated to enable the user to estimate the quality of protein models and template structures. To facilitate the description of template and model structures, DSSP (Kabsch and Sander, 1983) and Promotif (Hutchinson and Thornton, 1996) can be invoked to classify structural features.

2.7 Implementation

We have implemented the SWISS-MODEL workspace using standard web browsers as graphical user interface to the dynamic server front-end based on Apache/PHP. Protein sequence and structure databases, modelling software and user data are located on a back-end system. Front-end server and the compute back-end

communicate via XML-RPC calls, separating the graphical display from the computationally demanding tasks. Batch jobs are executed on a high-performance Linux cluster via Sun GRID engine to ensure load balancing and optimal response times. Databases at the back-end are automatically updated in regular intervals. The SWISS-MODEL workspace provides a personal working area for each registered user, which is not visible for other users. Email notification is sent to indicate when the calculation of a work unit has been completed. Users can access the workspace system anonymously without providing an email address; however, it is then necessary to bookmark the individual work units in the web browser to be able to retrieve the results once the browser has been closed. The SWISS-MODEL workspace is accessible at http://swissmodel.expasy.org/workspace/

3 RESULTS AND DISCUSSION

In the following chapter, we describe how the different modelling modes and tools available from the SWISS-MODEL workspace were applied to identify suitable templates and to build homology models for two biologically relevant human proteins: Cyclin A1 (CCNA1) and Transmembrane Protease 3 (TMPRSS3).

3.1 Modelling of human Cyclin A1

Cyclins are eukaryotic proteins that play an active role in controlling nuclear cell division cycles and in regulating cyclin-dependent kinases (CDKs). Inactive CDK apoenzymes are partially activated by complex formation with regulatory cyclin subunits and the complexes are further activated by phosphorylation of a Thr residue in CDKs. There are four classes of cyclins: A, B, D and E, which bind to CDKs at different stages of the cell cycle. The mammalian A-type cyclin family consists of two members, Cyclin A1 and Cyclin A2. While Cyclin A2 is widely expressed in different tissues (Liu *et al*., 1998), Cyclin A1 is limited to male germ cells. Cyclin A1 is essential for spermatocyte passage into the first meiotic division in male mice (Liu *et al*., 1998). Therefore its role in human male infertility is explored and it is investigated whether it could be a novel target for new approaches for male contraception (Wolgemuth *et al*., 2004).

To date no experimentally determined 3D structure of Cyclin A1 is available. Blast searches to identify suitable templates for the modelling of Cyclin A1 lead to several highly significant matches. These matches span the second half of the protein (starting at about residue 210), which correspond to the Cyclin domain, as assigned by scanning the InterPro database of protein families and domains. All detected templates belong to the Cyclin family; in particular the Cyclin A2 proteins share the highest sequence similarity with Cyclin A1. For Cyclin A2 several experimental structures are known, which contain two subdomains of similar all-alpha fold forming the Cyclin domain of the protein.

The Blast alignment between the sequences of the Cyclin domain of Cyclin A1 and Cyclin A2 is unambiguous: the two sequences share ∼60% identity and contain only one gap. Based on these observations, the Cyclin A1 regulatory domain can be easily modelled using the automated mode of the SWISS-MODEL pipeline. The modelling pipeline selects the structure of the human Cyclin A2 in complex with CDK2 and an inhibitor [PDB: 1h1rB (Davies *et al*., 2002)] as a template. Although other structures of the same protein have been deposited in the PDB, 1h1rB is selected by the modelling pipeline because it has the highest resolution among the suitable templates. Alternatively, other Cyclin A2 apo-protein structures or complexes with other molecules could be used to model the Cyclin A1 C-terminal domain. Exhaustive information about the individual templates is available directly from the template selection output as link to the SWISS-MODEL template library and external resources: MSD (Velankar *et al*., 2005), PDB (Westbrook *et al*., 2003), PDBsum (Laskowski *et al*., 2005), SCOP (Andreeva *et al*., 2004) and CATH (Pearl *et al*., 2005). For instance, in order to model the Cyclin-CDK activated complex bound to ATP, the bovin template 1finB (Jeffrey *et al*., 1995) could be used instead of 1h1rB. In the automated mode of the modelling pipeline, it is sufficient to specify the SMTL code for the template to be used.

As expected from the high target–template similarity the quality of both models (based on structures 1h1rB and 1finB) is very good. Overall ANOLEA, GROMOS and Verify3D quality assessments do not detect major problems in the models. Visual inspection of the model and the template structure using DeepView shows good sequence conservation at the interface between CDK2 and the cyclin molecules.

3.2 Modelling of TMPRSS3

While the automated approach yields satisfying results for closely related proteins, modelling of proteins based on templates with remote homology requires more user intervention as presented in the case of TMRSS3. Human Transmembrane Protease 3 (TMPRSS3) belongs to a family of transmembrane serine proteases expressed in several tissues. Mutations in the protein have been reported to cause neurosensory deafness (Masmoudi *et al*., 2001; Scott *et al*., 2001; Wattenhofer *et al*., 2002; Lee *et al*., 2003). *In silico* predictions indicate that the protein contains four domains: a transmembrane (TM) domain, a low-density lipoprotein receptor A (LDLRA), a scavenger receptor cystein-rich (SRCR) domain and a protease domain. Pathogenic mutations have been described in all three non-membrane domains. Currently, no experimentally determined protein structure of human TMPRSS3 is available. Therefore, homology models of the protein are valuable resources to rationalize the functional impact of these mutations.

The boundaries of the individual domains of TMPRSS3 were identified using IprScan. The sensitivity and performance of template detection can often be improved when the template search is performed on individual domains rather than the whole target sequence. As previously reported (Masmoudi *et al*., 2001; Scott *et al*., 2001; Wattenhofer *et al*., 2002; Lee *et al*., 2003), IprScan reports the occurrence of LDLRA, SRCR and protease domains in the TMPRSS3 protein. Blast, Iterative Profile Blast and HMM-based template identification methods display several matches to structures corresponding to the protease domain and some templates spanning both SRCR and protease domain. For the LDLRA domain only Iterative Profile Blast and HMM-based template identification methods are sensitive enough to detect potential structural templates with significant scores (Fig. 1). Each template of the hit list is linked to the SMTL and from there to other external resources, to allow for verification and a plausibility check. The biological annotations (e.g. domain classifications) of the different potential templates can be compared with the annotations of the target sequence to avoid the selection of non-homologous templates. This is especially relevant when trying to increase the coverage of the model by including low homology templates. The templates covering both SRCR and protease domain correspond to the structure of human Hepsin which

belongs to the family of serine proteases. Crystal structures of a protein–substrate complex [PDB: 1z8g (Herter *et al*., 2005)] or the apo-protein [PDB: 1P57 (Somoza *et al*., 2003)] could be used to build a model for the TMSSR3 protease and SRCR receptor domains. The alignment between target sequence and template 1z8g contains several gaps and therefore should be checked carefully. Alignments with possible template structures can be directly loaded into DeepView, where the placement of insertion and deletions is examined in the structural context. Modified alignments can be saved as project files and submitted to the Project mode of the modelling workspace. The protein structure deposited as PDB entry 1z8g contains the cleaved activated form of the protease. To build a model of the activated form, the two fragments of the template were treated as individual chains within DeepView. Several alternative alignments were submitted and the quality of the resulting models was inspected based on the output of protein model assessment tools, which accompany the resulting models. Geometric correctness of the models e.g. Ramachandran plots were analysed with Procheck (Laskowski *et al*., 1993). The alignment, model building and evaluation steps were iterated until a satisfactory result was obtained.

The same procedure was applied to the modelling of the LDLRA domain using the crystal structure 1jja of the human low-density lipoprotein receptor. The LDL-receptor class A domain contains 6 disulphide-bound cysteins (Bieri *et al*., 1995). Particular care was therefore taken in correctly aligning the cysteins. A highly conserved cluster of negatively charged amino acids forms a Ca^{2+} coordination site (Daly *et al*., 1995). The LDLRA repeat has been shown to consist of a beta-hairpin structure followed by a series of beta turns. Based on the pattern of cystein residues, and on the homology detected by template identification tools, the TMPRSS3 sequence appears to contain only one occurrence of the repeat. The quality assessment for the final models is satisfactory and essential structural features such as the disulfide bridge pattern and the Ca^{2+} coordination site of the LDLRA domain appear to be correctly retained. Regions with slightly positive energy value reported for ANOLEA and GROMOS correspond to residues where the alignment is less conserved and gaps in the alignment require *ab initio* loop re-modelling.

The presented examples demonstrate the usefulness and the capabilities of the integrated web-based modelling infrastructure SWISS-MODEL workspace. In combination with DeepView (Swiss-PdbViewer), it complements the server pipeline and repository (Kopp and Schwede, 2004b) as powerful and easy to use web-based resources for comparative protein modelling.

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