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Tabhu: tools for antibody humanization

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ABSTRACT
Summary: Antibodies are rapidly becoming essential tools in the clinical practice, given their ability to recognize their cognate antigens with high specificity and affinity, and a high yield at reasonable costs in model animals. Unfortunately, when administered to human patients, xenogeneic antibodies can elicit unwanted and dangerous immunogenic responses. Antibody humanization methods are designed to produce molecules with a better safety profile still maintaining their ability to bind the antigen. This can be accomplished by grafting the non-human regions determining the antigen specificity into a suitable human template. Unfortunately, this procedure may result in a partial or complete loss of affinity of the grafted molecule that can be restored by back-mutating some of the residues of human origin to the corresponding murine ones. This trial-and-error procedure is hard and involves expensive and time-consuming experiments. Here we present tools for antibody humanization (Tabhu) a web server for antibody humanization. Tabhu includes tools for human template selection, grafting, back-mutation evaluation, antibody modelling and structural analysis, helping the user in all the critical steps of the humanization experiment protocol.
Availability: http://www.biocomputing.it/tabhu
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Supplementary information: Supplementary data are available at Bioinformatics online.

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1 INTRODUCTION
Monoclonal antibodies (mAbs) are an important class of therapeutic molecules. The high specificity and affinity towards their respective antigens, their modular structure that facilitates their engineering and the relative low costs for their production in model animals makes them excellent drug candidates against several diseases (Chames et al., 2009; Reichert, 2012).

However, together with all these desirable characteristics, xenogeneic mAbs have drawbacks that limit their therapeutic benefits and can ultimately endanger the patients’ health (Hansel et al., 2010; Hwang and Foote, 2005). To overcome these hurdles, different methods have been developed for increasing the mAbs ‘degree of humanness’ (Abhinandan and Martin, 2007) by replacing parts of the original non-human antibody with the corresponding human counterparts. This process is generally referred as ‘humanization’ and takes advantage of the particular architecture of the antibody molecule (Almagro and Fransson, 2008; Padlan, 1994). The molecules generated by such humanization procedures may partially or completely lose affinity for their intended antigen; this can be usually restored by re-introducing specific and case-dependent native residues in the humanized molecule through an experimental trial-and-error procedure going under the name of ‘back-mutation’ phase.

Taking advantage of our experience in antibody sequence and structure analysis (Chailyan et al., 2011; Ghiotto et al., 2011; Marcatili et al., 2013), we developed Tools for AntiBody Humanization (Tabhu), a comprehensive platform meant to help antibody humanization experiments. Tabhu integrates different methods to guide researchers through several steps of the humanization cycle, from the selection of a suitable human acceptor molecule to the evaluation of the back-mutations effect.

2 DESCRIPTION
The initial input page of Tabhu requires the sequence of the light and heavy chain variable domains (VL and VH, respectively; Padlan, 1994) of the xenogeneic antibody to be humanized (native Ab) and the antigen volume since the latter can be used to improve the prediction of the residues involved in antigen recognition (Olimpieri et al., 2013). Tabhu uses two alternative sources of human sequences to choose the framework donor with the highest sequence similarity to the xenogeneic V region: a database consisting of both light and heavy chain sequences retrieved from the Digit database (Chailyan et al., 2012) or human germline gene sequences compiled by IMGT (Giudicelli et al., 2005) from which the user can select the Variable and Joining genes, that are eventually assembled together with the mouse complementarity determining regions (CDRs) to form the initial acceptor molecule. Tabhu lists the possible templates and shows relevant information for each of them.

Once a receiving framework has been selected, the server starts an antibody humanization procedure that resembles what is usually done experimentally and involves four steps: (i) loop grafting, (ii) estimate of the binding mode similarity between the native and human antibody, (iii) back-mutations and (iv) re-evaluation of the binding mode similarity between input and humanized antibody (Supplementary Material, Supplementary Figure S1).

The first step consists of grafting the xenogeneic CDRs into the human framework. The evaluation of the expected similarity
of the binding mode is based on the proABC method that we have previously developed (Olimpieri et al., 2013), that predicts the probability that every single antibody residue is involved in antigen recognition taking into account the entire sequence of the variable domains. If the pattern of interaction is very different between the input and humanized sequence, it can be expected that the resulting binding mode, and most likely the affinity, will be different. More details on the formula used to evaluate individual back-mutation importance are reported as Supplementary Material.

Once the user selects which residues to back-mutate and submits them to the system, a new variant is generated and the process can be repeated. However, the introduction of mutations in the human antibody can lead to structural problems, such as the appearance of clashes or cavities in the modelled humanized antibody. Taking advantage of our antibody structure prediction tools (Chailyan et al., 2011; Marcatili et al., 2008), upon user request, Tabhu builds the three-dimensional models of the mouse and humanized antibodies, runs the procheck and EDTSurf tools (Laskowski et al., 1996; Xu and Zhang, 2009) and alerts the user if the introduction of a back-mutation generates clashes or cavities, that the user can ignore or use as a guide to remove or introduce additional back-mutations.

When the desired binding mode similarity between the xenogeneic and humanized antibody has been achieved the user can finalize the model and retrieve the three-dimensional model of the parental antibody, the amino acid sequence of the selected human template, the contact probabilities of the humanized antibody, the amino acid sequence of the final redesigned antibody and a back-translated nucleotide sequence optimized for being expressed in a number of organisms. Supplementary Material, Supplementary File S1 reports an example of antibody humanization with Tabhu.

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