DMAN: a Java tool for analysis of multi-well differential scanning fluorimetry experiments

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ABSTRACT

Summary: Differential scanning fluorimetry (DSF) is a rapid technique that can be used in structural biology to study protein–ligand interactions. We have developed DMAN, a novel tool to analyse multi-well plate data obtained in DSF experiments. DMAN is easy to install and provides a user-friendly interface. Multi-well plate layouts can be designed by the user and experimental data can be annotated and analysed by DMAN according to the specified plate layout. Statistical tests for significance are performed automatically, and graphical tools are also provided to assist in data analysis. The modular concept of this software will allow easy development of other multi-well plate analysis applications in the future.

Availability and implementation: DMAN is implemented in Java to provide a cross-platform compatibility. It is freely available to academic users at http://www.structuralchemistry.org/pcsb/. To download DMAN, users will be asked for their name, institution and email address. A manual can also be downloaded from this site.

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1 INTRODUCTION

Assays in multi-well plate formats have become very popular and are frequently employed in laboratories in different disciplines of the life sciences. Due to their wide applicability, UV/Vis absorbance and fluorescence emission measurements are the most common readouts in such assays.

In the area of structural biology, differential scanning fluorimetry (DSF) has established itself as a tool to assess protein stability in the context of structure–function studies, but most importantly to rapidly identify low molecular weight ligands that bind to the protein of interest (Lo *et al.*, 2004; Niesen *et al.*, 2007; Pantoliano *et al.*, 2001; Poklar *et al.*, 1997). Due to the relatively low amounts of sample required, and the parallel measurement of many conditions on one multi-well plate, DSF also lends itself as a first-line screening method for potential small molecule ligands in structure-based drug discovery, including fragment-based drug design.

DSF experiments are typically conducted with a real-time Polymerase Chain Reaction (PCR) instrument, where the protein sample is mixed with an additive to be screened and the fluorescence dye SYPRO Orange that shows enhanced fluorescence emission in hydrophobic environment (Vedadi *et al.*, 2006). The dye binds to the exposed hydrophobic residues of proteins in their unfolded states, and thus serves as a reporter to monitor thermally induced protein unfolding.

Analysis software for any instrument-acquired data is provided by the manufacturers and shows varying degrees of convenience and user-friendliness. In case of DSF, analysis of data from some instruments requires subsequent application of several different software programs, therefore making the entire process rather cumbersome.

We set out to design a simple-to-use and portable Java application, DMAN, which enables analysis of DSF data on multi-well plates with any user-chosen layout of replica. Typically, biochemical measurements are done in triplicate. In order to prevent instrument-biased systematic errors due to non-uniform temperature distribution across the PCR block, we prefer to measure replica data on one plate, with the individual replica mixtures spread over the plate. The Java application reported here can analyse these repetitions, given the particular plate layout, and perform standard statistical tests.

2 METHODS

DMAN is a Java application that builds on and extends fundamental Java classes developed within the programme collection for structural biology and biophysical chemistry (PCSB) (Hofmann and Wlodawer, 2002). We have also implemented a method to perform Welch's modification of Student's *t*-test (Welch, 1947). Typically, individual protein thermal unfolding curves adopt a sigmoid shape as a function of temperature. The melting temperature T_m is then determined by finding the temperature that corresponds to the maximum of the first derivative of the melting curve around the estimated point of inflection.

3 RESULTS

The graphical user interface of the stand-alone Java application DMAN possesses four tabbed panels (*Input parameters*, *Raw data*, *Results*, *Plate*). On the first panel, *Input parameters*, the user needs to specify the data file and its origin (i.e. instrument type), the plate layout (provided as an ASCII text file), the output file, a title and description of the assay, the temperature range of the experiment and the *slope threshold*. The user can also specify the ΔT_m *threshold* and the *Outlier cutoff*, which are used during analysis to evaluate the

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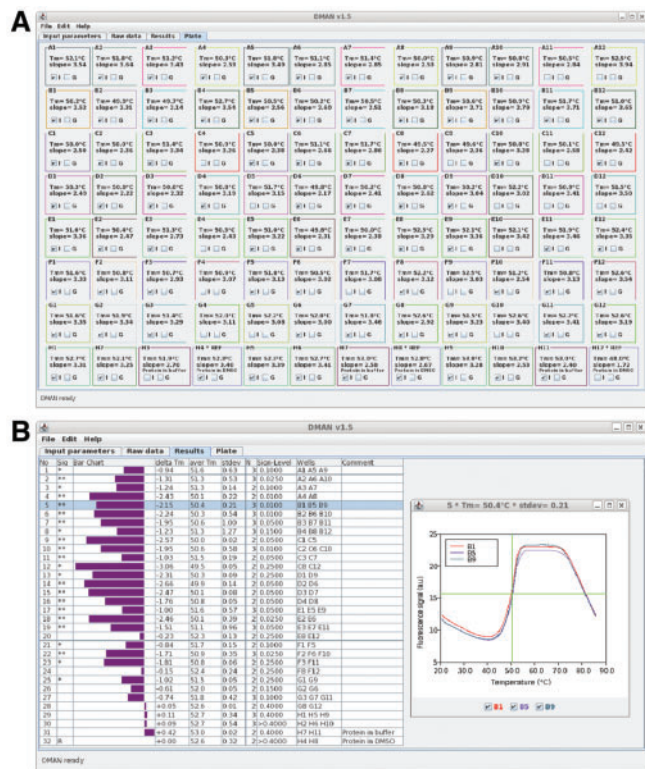
Fig. 1. Screenshot of the Plate (**A**) and Results (**B**) panel after parsing raw data. A window showing the melt curves (inset of B) can be opened from both panels.

significance of the results. The raw data file is parsed and analysed by clicking the *Read Data* button.

3.1 Data processing

After the data have been parsed, the *Raw data* panel becomes active and displays a table of the extracted melt curve parameters per well (T_m, slope, signal at T_{start}, lowest signal and highest signal). This information is visualized in the *Plate* panel, where the individual values for T_m and slope are mapped onto a representation of the actual plate (Fig. 1A). The boxes are labelled and coloured to help identify the replica. A checkbox labelled *I* allows the user to include or exclude a particular well into the subsequent analysis; a well is automatically excluded if the slope of the curve around the point of inflection is less than the specified *slope threshold*. A checkbox labelled *G* opens a new window that shows the melt curves for the current well and its replica on the plate.

For each dataset, a *z*-test is performed, and individual wells that show a T_m value differing by more than *Outlier cutoff* × estimated SD are automatically excluded from the analysis. The user can revise these suggestions by a simple mouse click.

3.2 Data analysis

As shown in Figure 1B, the *Results* panel lists the analysis results for each set of replica (dataset). The user can also review the melt

curves for each dataset from this panel. The average T_m values and the SD as well as ΔT_m as compared to the reference dataset (annotated with the symbol 'R') are calculated and shown. If ΔT_m is larger than the user-specified threshold, the dataset is labelled with a '*'. If the significance level from a *t*-test is better than 0.05, a second '*' is added to that dataset. An embedded bar chart provides a convenient means for visualizing significant datasets.

3.3 Plate layout

The type of plate used, as well as the location of datasets on the multi-well plate, along with comments for each dataset is coded in an ASCII file that can by read by DMAN. This file can easily be written or modified by the user with a text editor to implement any plate layout. Alternatively, the plate layout can be designed with the graphical user interface accessible from the *Edit* menu. In this case, replica wells labelled with the same number or character are recognized as one dataset; the location of the reference dataset can also be defined.

A description for each dataset can also be entered, either as part of the plate layout design, or only for the plate currently being analysed.

3.4 Future development

Currently, we have implemented parsers for data obtained with Roche LC480 real-time PCR instrument. Development of additional raw data parsers for other real-time PCR instruments are planned. Feedback from potential users in that context is highly welcome.

The modular design of DMAN will also enable easy development of further multi-well plate analysis applications, such as UV/Vis absorbance and fluorescence emission measurements.

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