Supplementary Information MMM: A toolbox for integrative structure modelling

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***Abstract*—This Supplementary Information provides protocols for reproducing the application examples in the main text. In MMM version 2017.2, functionality that depends on third-party programs is only accessible when you run Matlab on a Windows system.**

I. Localization by multilateration

A. Prerequisites

You need internet access for downloading the PDB file.

B. Localization

In the Matlab command window, change to the MMM subdirectory `demo\T4L_131` and start MMM. In the `File` menu use the `New from PDB/web` item to load the T4 Lysozyme structure 2LZM. Visualization will be more pleasing if you now type `color [2LZM] lightgrey` in the command line. In the `Display` menu, use the `Localization` item for loading the restraint file `T4L_localization_131_all.dat`. The result should look as in Fig. 1.

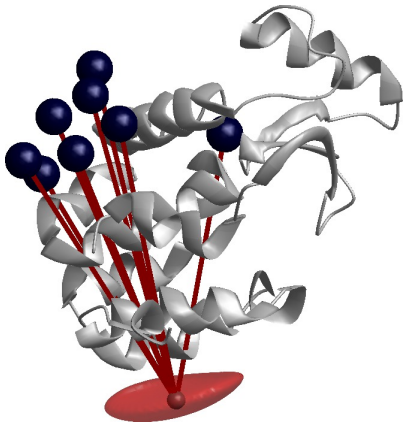

Fig. 1. Localization of an MTS label at residue 131 of T4 Lysozyme by eight distance distribution restraints. Experimental data from Ref.[1] and courtesy Christian Altenbach.

If you want to change color of the red semitransparent probability density isosurface, first click on it. The `Message board` will display its identifier (`131R1`), which was specified in the restraint file, as well as the coordinates of its center of gravity and of the point of highest probability. The latter point is visualized by the small grey sphere inside the semitransparent surface. If you now type `color $density:131R1 darkgreen` in the command line, the probability density isosurface will change its color.

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C. Rotamer library modelling of the site

In the command line, type `select 131` and use the `EPR` menu `Site scan/selected residues` item for *in silico* spin labelling. A window `Site scan setup` pops up, where you can just click the `OK` button. Now a window `Set labeling conditions for selected residues` pops up and, again, you can just click `OK`. Finally, a file save dialog pops up, where you can just click the `Save` button. After a short computation, a dialog window asks you whether you want to look a the result in a web browser. This is recommended, click `Yes`. A browser window opens and informs you that 114 rotamers could be attached with a partition function of 1.22533. This indicates a very well accessible site for spin labelling.[2] Go back to the MMM main window and in the `EPR` menu, use the `Attach precomputed rotamers` item to actually modify the protein model with the computed label. The `Set labeling conditions for selected residues` window pops up again (you could have computed rotamers of different labels for this site) and you click `OK`. Now unselect residue 131 by clicking on the `Unselect` toolbutton (this is the red cross toolbutton at the rightmost position in the toolbutton row). Finally visualize the spin centers for all rotamers by typing `show 131 label` in the Command line.

If you wish, you can search for a better viewing direction in the rotate mode. This mode is accessed by clicking on the `Rotate` toolbutton (the second one in the `View control` section of the toolbutton row). The result might look as shown in Fig. 2. If you wish to save the visualization in a common graphics format, you can do so either by using the `File` menu `Export visualization` item or by clicking on the `Camera` toolbutton in the `Graphics` section of the toolbutton row. The `Camera` toolbutton is available only after detaching the graphics panel with the leftmost toolbutton in the `3D panels mode` section of the toolbutton row. Clicking on the `Camera` copies a bitmap into the clipboard (Windows systems). Please close MMM after you are finished. It is not required, but good practice, to work with a fresh instance of MMM when you start a new task. If you have worked on one

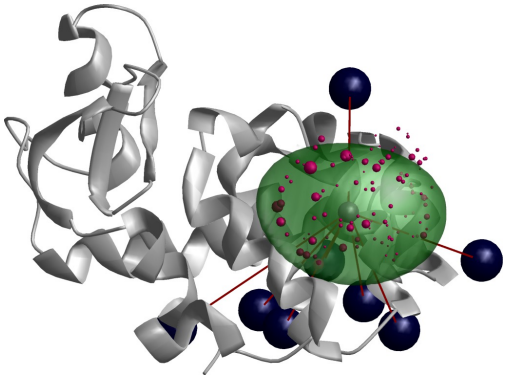

Fig. 2. Comparison of the rotamer prediction of the MTS label distribution at residue 131 of T4 Lysozyme with its experimental localization.

project with large files and for a long time, it may even be advisable to close and restart Matlab.

II. Localization by distance geometry

A. Prerequisites

You need internet access for downloading the PDB file.

B. Localization of a spin-labelled lipid in soybean seed lipoxygenase

In the Matlab command window, change to the MMM subdirectory `demo\lipoxygenase` and start MMM. In the `File` menu use the `New from PDB/web` item to load the PDB structure 1YGE. You might want to type `color [1YGE] lightgrey` in the command line. In the `Display` menu, use the `Network (DMG)` item for loading the restraint file `network_Gaffney_explicit.dat`.[3] By rotating the model as described above, you can find the view as in Fig. 1B of the main text. In order to make the site polyhedron better visible, you can type `transparency [1YGE] 0.25` in the Command line.

III. Restraint-augmented homology modelling

A. Prerequistes

MODELLER [4] needs to be installed on your computer in a directory where you have write access (download at https://salilab.org/modeller/download_installation.html). The MODELLER directory and its subdirectories need to be included in the Matlab path. Copy all `.lib` files from the MMM subdirectory `\third_party\Modeller_stuff` to the `\modlib` subdirectory of your MODELLER directory. The program also needs to be registered with MMM preferences, as the name of the executable depends on the version number. For version 9.17 that we assume here, it is `mod9.17`. In the MMM `File` menu, select item `Preferences` and type the Modeller call for your version in the respective line. Click `OK`, close and restart MMM. This needs to be done only once after installing a new version of MODELLER.

If you want to remove the spin labels after modelling and you want to repack side chains, you also need SCWRL4[5], which you can obtain from http://dunbrack.fccc.edu/scwrl4/. The SCWRL4 directory needs to be on your Matlab path.

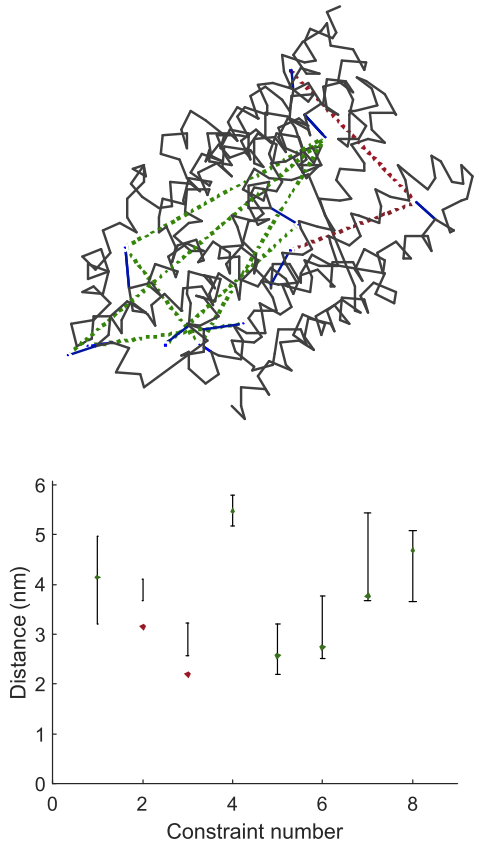

Fig. 3. Restraint matching for the Na^+/proline symporter PutP by the moderately homologous template structure of the Na^+/glucose symporter vSGLT. Experimental data taken from Ref. [6]

B. Modelling the eL4 loop of PutP

In the Matlab command window, change to the MMM subdirectory `demo\PutP_eL4` and start MMM. In the `File` menu use the `New from PDB/local` item to load the PDB file `2XQ2AG` that is stored in this subdirectory. The file has been prepared from PDB structure 2XQ2 by only keeping residues 9-500 of chain A. In the `Build` menu, for item `Fit from template` select the `via Modeller` subitem. A window `Model transition from template structure [2XQ2]` pops up. Use the `Alignment` pushbutton of this window to load the alignment file `Olkhova.ali`. The message line of this window informs you that sequence identity of the alignment is in the twilight zone and the Message board of the main window informs you that the target sequence of PutP has only 19.1% identity with the sequence of the template.

Use the `Restraints` toolbutton of the MODELLER interface window to load the restraint file `PutP_eL4_Modeller_restraints.dat`. The plots show that some of the distance distribution restraints are already nicely matched by the template structure, but two of them are not (Figure 3). A `Report editor` window pops up that provides quantitative information on restraint matching. If you want to keep the initial restraint matching information, you might want to save this file to a directory of your choice. By default it is stored in the MMM `\tmp`

directory, which is cleared every 30 days. The Report editor window can be closed by clicking on the OK button.

If you have SCWRL4 installed, you might now want to activate the repacking checkbox in the Modeller control panel. By default, MMM will instruct Modeller to generate 20 models and will import up to 10 models with the highest DOPE score, but only models that have a GA341 score of at least 0.75, according to recommendations for MODELLER.[4]. If you need to change these values to obtain a sufficient number of models for your application, it is advisable to not compromise on the GA341 score, but rather to increase the number of initial models generated by MODELLER. Click the Run pushbutton. The Message board of the main window will display messages on start and, after some time, completion of the MODELLER run.

After completion of the MODELLER run, the Import button is enabled. After you have clicked it, MMM informs you how many models were rejected because of a too low GA341 score and imports the best models. After importing, a report editor window opens with the information on restraint matching of the homology models. This information is also visualized in the MODELLER interface window. In the case at hand, all restraints are well matched. If SCWRL4 is available and you had activated the repacking checkbox, MMM now uses SCWRL4 to repack the sidechains of all models. The Message board of the main window informs on progress of this task. After it is complete, you can close the report editor (OK) and MODELLER interface window.

The models are stored internally in MMM as structures targ, which is the original MODELLER result with spin-label side groups still attached, and targr, which is the ensemble with all native sidegroups and sidegroup repacking by SCWRL4. In order to save one or both of these ensembles, use the Display menu Hierarchy item to open the Hierarchy display window and the Structure dropdown menu in this window to select the wanted ensemble. You can save it with the File menu Save PDB as... menu item. After this, close the Hierarchy display window.

C. Visualization

The template structure 2XQ2 is already displayed as a ribbon model. Type color [2XQ2] peachpuff in the Command line to give it a less imposing appearance. Note that the Command line is available only in Selection mode. If it is inaccessible, you need to click the cursor arrow toolbutton in the View control section of the toolbutton row. To emphasize the residues of vSGLT that are aligned to the eL4 loop of PutP, type color [2XQ2]310-348 crimson in the Command line. Now type show [targr] ribbon. This displays all 10 homology models in MMM standard color. The visualization will be more clear after typing color [targr] lightgrey and color [targr]{:}294-324 darkgreen. The characters {:} in the address tell MMM that residues 294-324 should be colored darkgreen for all models in the ensemble. You can now rotate the model. The result may look slightly different from the one shown in Figure 2 of the main text, since homology modelling by MODELLER involves some degree of randomness.

IV. Rigid-body docking

A. Prerequisites

You need internet access for downloading one PDB file.

B. Making a single docking model

In the Matlab command window, change to the MMM subdirectory demo\integrin and start MMM. In the File menu use the New from PDB/local item to load the PDB file 4wtw_A that is stored in this subdirectory. It contains only chain A of the crystal structure of the FnIII-3 domain of integrin $\alpha 6\beta 4$. Now use the Add from PDB/web item to add the PDB file 4wtx as the second structure. It contains the crystal structure of the FnIII-4 domain. The Build menu Docking (rigid-body) item gives access to the MMMDock module. Using the Load restraints list button, load the restraint file integrin_docking_restraints.dat. Select the Docking method Grid search in the Initialize docking panel and activate the Automatically fit from the best grid point checkbox in the Grid search setup panel. Then click the Run docking pushbutton. After a few seconds, docking will be completed. PDB files with updated coordinates for the two individual domains are stored automatically.

Fig. 4. Single docking model for the FnIII-3,4 domains of integrin $\alpha 6\beta 4$.

In order to save the docked model to a single PDB file, type select [trs3,trs4] in the Command line. The model displayed in the main window should now be highlighted yellow, since [trs3] and [trs4] are the MMM structure addresses of the docked domains. Select the File menu Save selection as PDB... item and use INTG as a pseudo PDB identifier (must have 4 characters, but should not start with a digit). You should obtain the result that is stored as INTG_docked.pdb in the same subdirectory and displayed in Fig. 4.

C. Testing further solutions of the grid search

To inspect more results of the grid search, use the `Edit` menu item `Reports` and load the docking session report of your previous fit (the file name contains date and time). Towards the end of the file, you will find the 20 grid points that gave the lowest RMSD. The model shown in Fig.4 resulted from local optimization of the first of these points (best-fitting grid point).

In the `MMMDock` window, you can now switch to the `fit only` mode in the `Initialize docking` panel. Copy the second best grid point (Alpha = 135, Beta = 51.43, Gamma = 315, x = 18.75, y = -18.75, z = 0) into the edit fields of the `Fit setup` panel and click the `Run docking` pushbutton. The solution that you obtain will slightly differ from the one obtained by automatic local optimization from the best grid point (compare the result reported in the `MMMDock` window to the one in the report file that you still have open in the Report Editor).

The second solution is stored in MMM structures [trs5] and [trs6]. You can save it as described above (`INTG_docked_2.pdb`) and, if you wish, you can explore solutions that you get from further grid points. In the following we assume that a fit was also performed from the third best grid point. The third solution is then stored in MMM structures [trs7] and [trs8] and we assume that it was saved in `INTG_docked_3.pdb`.

Fig. 5. Three docking models for the FnIII-3,4 domains of integrin $\alpha 6\beta 4$.

D. Generation of an ensemble and visualization

Close all windows except for the MMM main window. With the `File` menu `New from PDB/local` item load the PDB file `INTG_docked` and with the `Add from PDB/local` item first add `INTG_docked_2` and then `INTG_docked_3`. The three models will now have MMM addresses [INTG], [INTG_1], and [INTG_2] (if you have saved all of them with pseudo-PDB identifier INTG). Now rotate the model to a convenient view, for instance the one shown in Fig. 5. If you want to also display a rotation by 90°, as in Fig. 3 of the main text, you best define this view as a standard view along the x axis by using the `Build` menu item `Transform to viewing frame` subitem `x along viewing vector`. The superposition will look somewhat better after you type `transparency [INTG,INTG_1,INTG_2] 0.5` in the Command line (Fig. 5). If you now want to see the model rotated by 90°, you can select `y` in the dropdown menu of the `View` panel of the main window. Note that you can also use the `M` pushbutton right beside this dropdown menu to store any current view and can later return to it by selecting `memory` in the dropdown menu.

V. Conformational change by elastic network modelling

A. Prerequisites

You need internet access for downloading the PDB file.

B. Modelling the apo form of the HCN ion channel

In the Matlab command window, change to the MMM subdirectory `demo\HCN_change` and start MMM. In the `File` menu use the `New from PDB/local` item to load the PDB file `3etq_A` that is stored in this subdirectory. This file contains only chain A of the cAMP-bound form of HCN. In the `Build` menu, item `Fit from template` select the `by Elastic network model` subitem. A window `Fit transition from template structure [3ETQ]` pops up. In this window, use the `Load restraints` pushbutton to load restraint file `Puljung_et_al_PNAS_restraints.dat`. Click the `Fit` button. Progress in terms of an improvement of restraint fitting is reported in a separate plot window that is continuously updated and after completion will look approximately as in Fig. 6. The results may vary somewhat, since the algorithm uses random numbers for relative phases of the normal modes. This feature can be used to generate more than one model and to get some estimate of the uncertainty.

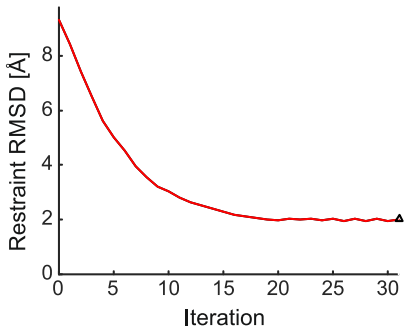

Fig. 6. Convergence of restraint RMSD during elastic network modeling of the apo form of the HCN ion channel.

The final model is internally stored in structure [tr2]. After closing the Fit window, select it by typing `select [tr2]` and use the `File` menu `Save selection as PDB...` item and `HCNA` as a pseudo PDB identifier.

C. Visualization

Click on the unselect toolbutton (red cross). Hide the model of the cAMP-bound form by typing `hide [3ETQ]` in the Command line. Show the motion model by typing `motion [tr2] [3ETQ]`. Color the coil model of the fitted apo structure by typing `color [tr2] goldenrod`. The cones that visualize the motion are a graphics object with identifier `motion:tr2_to_3ETQ`. You can change their color by the command `color $motion:tr2_to_3ETQ darkgreen`. By rotating the model, you can obtain the visualization shown in Fig. 4A of the main text.

To obtain the visualization in Fig. 4B, hide the motion cones by the command `hide $motion:tr2_to_3ETQ`. Type `show [3ETQ] coil` and then `color [3ETQ] darkgreen` to display a coil model of the experimental cAMP-bound conformation. Now click on the `Depth cueing` pushbutton in `View` panel. You can change the depth cueing by playing with the front and back plane setting in the `Depth cueing control` window, but note that this may hide parts of the model. Usually the default setting is close to optimum. Once you close the `Depth cueing control` window, display will change back to the normal appearance.

VI. Ensemble model of a flexible peptide section

A. Prerequisites

You need internet access for downloading the PDB file. Generation of flexible peptide sections requires SCWRL4 [5], which you can obtain from http://dunbrack.fccc.edu/scwrl4/. The SCWRL4 directory needs to be on your Matlab path. For adding the bilayer model, you need MSMS [7], which can be downloaded from http://mgl.scripps.edu/people/sanner/html/msms_home.html.

B. Transforming the crystal structure to the symmetry frame

In the Matlab command window, change to the MMM subdirectory `demo\flex_LHCII` and start MMM. In the `File` menu use the `New from PDB/web` item to load the PDB file `2BHW`. This is a trimer, but C_3 symmetry is not perfect in this crystal structure. By selecting one residue in each protomer, you can define symmetry-related sites that allow for determining the C_3 axis to a good approximation. Type `select (A,B,C)60` in the command line and use the `Edit` menu `Symmetry frame` item to transform coordinates to a frame where the C_3 axis is the z axis. The structure will be displayed with the viewing direction along z.

C. Removing residues 10-13 and water from the structure

Click the unselect toolbutton (red cross). Select all residues in all chains by the `select (A,B,C):` command (the : character selects all objects on some hierarchy level). Unselect residues 10-13 in all chains by the `unselect (A,B,C)10-13` command. Unselect water by the `unselect (A,B,C)"HOH"` command. Using the `File` menu `Save selection as PDB` item, save this edited structure to a file `2BHW_sym_no_10_13_no_water`. You can still use the PDB identifier 2BHW for this.

In order to work with the edited structure in the following, you need to reload it using the `File` menu `New from PDB/local` item. Don't use the `Add from PDB/local` item, because this would load the edited structure with MMM name [2BHW_1] instead of [2BHW] as assumed in the restraint file.

In the `View` panel select `z` from the dropdown menu as the viewing direction. Type `color [2BHW] lightgrey` in the command line.

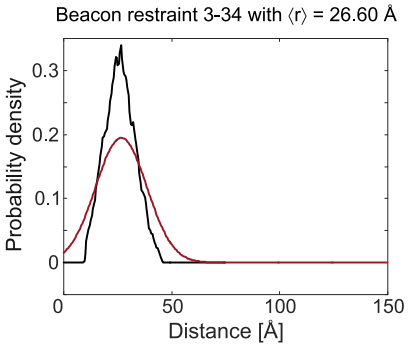

Fig. 7. Matching of the beacon restraint between residue 3 in the modelled flexible N-terminal section and residue 34 in the ordered section of the N-terminal domain of major plant light harvesting complex LHCII. The Gaussian restraint is shown in red and the simulated distance distribution for all 25 models in the ensemble in black.

D. Modelling residues 3-13 of trimeric LHCII

Select in the `Build` menu the item `Domain ensemble model` to open the window `Monte Carlo ensemble at structure [2BHW].` With the `Restraints` toolbutton, load the restraint file `LHCII_Nterminus_restraints.dat`. Click the `Run` button. Whenever a model is created, it will be immediately displayed. You will find that the N-terminal loop is on the backside.

After completion, the `Restraint fulfillment by the ensemble` panel of the fit window shows a plot similar to the one in Fig. 7. Using the > and < button in this panel you can scan through all distance distribution restraints. After you are done, you can close the fit window. Change the viewing direction to `-z`.

E. Adding a lipid bilayer model

Select in the `Build` menu the `Bilayer` item. By using MSMS, MMM computes the solvent-accessible surface of the protein. As we know that the C_3 symmetry axis is the bilayer normal and as we already did transform the structure to a frame where the C_3 axis is the z axis, we don't need to fit the membrane normal. Since LHCII is a helical bundle, the default settings in the `Build bilayer` window can be kept. Just click on the `Fit` button and then on `OK`. This will update the graphics, which may take a while for the whole ensemble. Type `show [2BHW](A){:}3.CA space-filling`, then `color (A){:}3-14 palegreen`, and finally `transparency (A){:}3-14 0.5` in the Command line.

After rotation, the model should look similar to Fig. 5B in the main text. Details may differ, since ensemble generation features some randomness.

VII. RigiFlex modelling

A. Prerequisites

Generation of flexible peptide sections requires SCWRL4[5], which you can obtain from http://dunbrack.fccc.edu/scwrl4/. The SCWRL4 directory needs to be on your Matlab path. Fitting of the SAXS curve requires ATSAS [8], which you can obtain from https://www.embl-hamburg.de/biosaxs/software.html. ATSAS must be on your Matlab path. The MMM interface is tested up to ATSAS version 2.8.1. The SAXS curve needs to be downloaded from https://www.sasbdb.org/data/SASDAT6/ and saved to the subdirectory `\integrin` by the name `SASDAT6.dat`. MMM should be started from this directory.

B. Running Rigi for the FnIII-3,4 domains of integrin $\alpha6\beta4$

In the Matlab command window, change to the MMM subdirectory `demo\integrin` and start MMM. In the `File` menu use the `New from PDB/local` item to load the PDB file `INTG_rigid_bodies`. This PDB file was constructed from crystal structures 4WTW for the FnIII-3 domain and 4WTX for the FnIII-4 domain [9] by making sure that the two rigid bodies are well separated in space and by removing crystal water. Select in the `Build` menu the item `RigiFlex` to open the window `RigiFlex structure model based on rigid bodies [INT0]`. With the `Restraints` button, load the file `integrin_rigiflex_restraints.dat`. In the `Display mode` panel, activate the radio button `SAXS fit`. That way the SAXS fits for valid rigid-body arrangements will be displayed during the run of the Rigi module. Now click the `Run Rigi` button and provide the file name `INTG.pdb`. Rigi should generate the requested 15 models within a few minutes. After all models are generated, the module requires some more time to clean them up, save them to the PDB file, and diagnose restraint fulfillment for the whole ensemble.

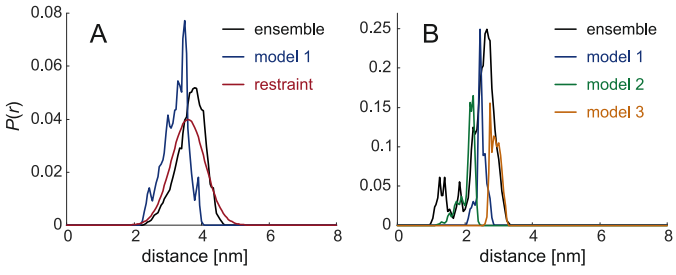

Fig. 8. Matching of the restraints by the rigid-body arrangements of the FnIII-3,4 domains of integrin $\alpha6\beta4$. (A) Restraint between residues 1472 in the FnIII-3 domain and 1626 in the FnIII-4 domain. Distance distributions of all models overlap quite well with each other and with the specified restraint. (B) Potential restraint between reference sites 1472 in the FnIII-3 domain and 1598 in the FnIII-4 domain. This restraint had not been obtained experimentally and the distance distributions vary more strongly among the models in the ensemble.

To see how well the restraints are fulfilled, activate the `DEER` radiobutton in the `Display mode` panel. With the < and > buttons for `Core restraints` you can navigate to the desired restraint. If the restraint was specified, you obtain a plot similar to the one in Fig. 8A. With the < and > buttons for `Model` you can display the predicted distance distribution for any of the rigid-body arrangements.

C. Running Flex for the FnIII-3,4 domains of integrin $\alpha6\beta4$

Click on the `Run Flex` button. For this particular problem, the Flex module requires long computation times since for some of the otherwise valid rigid-body arrangements most of the initially generated loop models clash with the protein. Therefore, you might want to run the Flex module over night. The restraint file specifies 15 rigid-body arrangements and a maximum time of 1 h for generating up to 5 flexible linker models for each of them, i.e., Flex will run at most for 15 h. Since for some of the rigid-body arrangements flexible linkers can be constructed more easily, runtime is shorter in practice. The RigiFlex window displays progress of the computation.

D. Running Assembler for the FnIII-3,4 domains of integrin $\alpha6\beta4$

Click on the `Run Assembler` button. For this particular problem, Assembler spends most of its run time in calls of the `crysol` program of the ATSAS package [8] for fitting the SAXS curve for all valid combinations of rigid-body arrangements with flexible section models. After the Assembler run is completed, you may want to open the file `INTG_assembler_report.txt` with the report editor (`Edit` menu item `Reports`). The report summarizes how many models for the flexible section were found for each rigid-body arrangement and gives the χ^2 values for the SAXS curve fits of all valid combinations. Near the end of the file, the best-scoring models are sorted by χ^2. The requested number of models in the ensemble (20 in the case at hand) is automatically written to the PDB file `INTG_ensemble.pdb`. You can now close the RigiFlex and Report editor windows.

E. Visualization

Using the `File` menu item `New from PDB/local`, load the ensemble `INTG_ensemble.pdb`. All models are superimposed on the FnIII-3 domain, as it was specified in the restraint file `integrin_rigiflex_restraints.dat`. In the Command line, type `color {:}1454-1548 darkgreen` to color this domain for all 20 models, then `color {:}1549-1571 crimson` to color the flexible section, and then `color {:}1572-1666 grey` to color the FnIII-4 domain. Rotate the structure to a suitable view, for instance, the one in the left panel of Fig. 6 of the main text, and use the `Build` menu item `Transform to viewing frame` with subitem `viewing vector along x` to transform coordinates to this frame. The command `transparency {:}1549-1666 0.25` improves visualization of the sections with distributed relative conformation. By selecting `y` in the `View control` panel of the

main window of MMM, you obtain a view similar to the one in the right panel of Fig. 6 of the main text. If you wish, you can save the ensemble with coordinates in this viewing frame using the `File` menu `Save PDB as...` item.

In order to visualize a distance restraint, you can use the `plot` command. For instance, `plot 1463.CA 1608.CA 3 darkblue` draws a darkblue line with width 3 between the Cα atoms of residues 1463 in the FnIII-3 domain and 1608 in the FnIII-4 domain, corresponding to the first restraint. You could now repeat this for all restraints, but it is more convenient to use MMM scripting for this visualization. A script file `plot_restraints.mmm` is contained in the MMM subdirectory `demo\integrin`. After clicking on the `Run script from file` toolbutton, which you find above the right corner of the command line, you need to navigate to this directory. When you load the script file, all restraints are displayed as in Figure 6 of the main text.

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