Calculation of free energy differences is of central importance in the simulation of biochemical systems. It is particularly difficult to calculate between pairs of macromolecular conformations as well as a computationally expensive task with existing methods. In this work, confinement approach is used to calculate absolute free energies of biomolecular systems. This method provides two main advantages: it does not require a reaction coordinate or transition path and it is fast to compute. Free energy calculated can be decomposed into a per residue contribution in an approximate way. Per residue free energy allows us to identify the reason behind conformational preferences in biomolecules. Through out the article we show its use in different challenging modeling problems. In particular, we show its use in predicting the conformational preference of chameleon sequences (sequences with high sequence identity and different folds). This sequence dependent conformational preferences and per residue free energy decomposition set the stage for the use of this method in protein design.

To better understand the mechanism behind these chameleon proteins, we

decomposed the calculated free energy into per-residue contributions in

an approximate way28. We decompose each confinement step (\_GA;A\_ , \_GB;B\_ ) into per-residue contributions. We can also decompose the remaining

enthalpy in the confined ensemble into per-residue contributions. However,

the method is approximate because we do not include the residual

conformational entropy from the normal mode or quasiharmonic analysis

steps. Computing per-residue contributions helps us to identify important

residues that stabilize a particular conformation. The per residue free

energy, \_\_G((\_) 􀀀 (\_)) is shown in Figure 4.

Although the overall free energy difference between the two structures is

small (within 3–5 kcal/mol), individual residues can show marked preferences

for being in either \_ or \_ conformation. Such differences can be

understood by looking in detail at the local environment for those residues.

For example, the region around residue 7 forms a random coil in the \_ structure,

whereas it is forming a beta sheet structure in the \_ structure. These

residues strongly favour the locally well packed and hydrogen bonded

environment found in the \_ sheet. Overall, favoring \_ or \_ structure is a

delicate balance, were the relative global free energy difference is small

and the contributions of different per residue tendencies balance out. It is

therefore very likely that by changing key residue preferences the global

fold preferences can be changed.

Three residues are mutated between GA95 and GB95, at positions 20, 30,

and 45. In GA95, these residues (L20, I30 and L45) stabilize the \_ structure

(compare the upper and lower panels on Figure 4). In GB95, two of these

residues (F30 and Y45) favor the \_ structure, because they have large

solvent exposed surface areas in the \_ structure, but are more buried in

the \_ structure. Additionally Y45 forms a hydrogen bond with D47 in the

\_ structure. On the other hand, residue A20 from GB95 still favors the \_

structure, although less strongly than L20 in GA95.

The \_ and \_ sequences are nearly identical, so there are some common

features observed for all sequences. The experimental observations classified

the protein into two parts: Amino acids 9–51 are fully structured in

both folds, whereas residues 1–8 and 52–56 are unstructured in the \_ fold,

but form \_-strands in the \_ fold. Most of the amino acid residues in the

region 1–9 have negative per-residue free energies, which means that these

residues favor the \_ structure. Roles of some other important residues in

stabilizing either the \_ or \_ conformation are summarized in Figure S1.

In addition to the direct effects of the mutation, there are also indirect

effects due to small perturbations in the environment around the mutations.

For example, the L20A mutation causes a slight repacking around residue

20. This causes large changes in the per-residue free energies of nearby

residues T25 and A26. However, the changes for these two residues have

opposite signs and nearly cancel.

These per-residue free energy decompositions provide a great deal of insight

into the driving forces behind protein folding and conformational

change. We believe that such calculations may also be useful for protein

design—designing proteins with specific structures and functions.

**2.4 The confinement method is a useful tool for structure**

**prediction**

We have tested the ability of the confinement method to act as a “metapredictor”

for structure prediction. Here, the task is to correctly identify

the most accurate models out of a set of “decoys” generated by different

methods during the CASP experiment. CASP is a blind test in which

different groups apply methods to predict the 3-dimensional structure

of proteins from their sequences. Each group is allowed to submit five

possible structures, which they are supposed to rank from best to worst.

We have performed two experiments centered on CASP. In the first experiment,

we tried to rank-order predictions for several targets. For each target,

the predictions were either produced by a single group—presumably using

the same method for each prediction, or were produced by several different

groups—using different methods. The goal of this experiment is to determine

if the physics-based confinement method can correctly identify more native-like structures as having lower free energies. Our second experiment

was to see if the confinement method can identify structures that are

missed by other meta-prediction servers. Most successful meta-prediction

servers are based on the idea of consensus: if many different prediction

methods produce similar results, then that is probably a correct prediction

[ref]. This is often a powerful heuristic, but it can miss cases where there is

a very good result that is only predicted by one method. We chose several

cases where such structures were missed by the best meta-predictors in

CASP and assessed if the confinement method can correctly identify these

accurate models.

As is common in the CASP experiment, we assess our results in terms of

Global Distance Test Total Score (GDT-TS)45, which is a C\_ based measure

of structural accuracy. It can be understood roughly as the percentage of

residues that are correctly positioned in the model (range 0 to 100, higher

is better). We did have enough computer model to analyze every model,

so the initial models for the test were chosen from a selection of different

server groups that have done well in past CASP events.

Overall, the confinement method performs well on our CASP tests. Figure 4

shows that in almost every case, the native structure has the lowest free

energy and the best model has the next lowest free energy. The confinement

method appears to be a useful tool for ranking structure predictions.

**The confinement method can correctly rank-order structure predictions**

First, we examined the ability of the confinement method to rank different

models for a target that have been generated by a single group using the

same methodology for all predictions. We examined two targets: T0559

and T0560 (see Table ?).

[Arijit: Let’s move the protein names, organisms, pdb ids, etc to a separate

table and just use the CASP ids.]

The first test case is target T0559. The best predictor group for this 69

amino acid target was “BAKER-ROSETTASERVER”. To save computer time, we excluded two models that were very similar to other models that

we did include. For this target, the difference free energy computed by the

confinement method can be used to accurately rank-order all of the models

and the native structure (Figure 4).

We performed a similar calculation for target T0560 with two models form

the group “Splicer”. The remaining three models were discarded as they

were too similar to the rest of the models. Again, we can correctly identify

the native sate and our calculated free energy based ranking matches well

with GDT-TS (Figures S?).

Next, we tested the ability of the confinement method to rank models for

target T0540 that were produced by different prediction groups. The top

models from groups “LTB” (Model 1) and “Mufold” (Model 2) were chosen

for analysis. Once again, the free energy based ranking correlates well with

the GDT-TS based score (Figure 7).

**The per-residue free energy is sensitive to small changes in protein**

**conformation**

In section 2.3 we discussed how the per-residue free energy can reveal

mechanistic detail behind the conformational preference of a chameleon

sequence. In this section, our aim is to apply the same method and try

to understand if the per-residue free energy can help us identify residues

which stabilize or destabilize a particular region of a protein. In this case,

the two conformations have similar folds with only small changes in localized

regions. We chose CASP target T0569 and compared the experimental

NMR structure with the best predicted model (GDT-TS=78; predicted by

the “Mufold” group). The confinement method predicts that the native

structure is more stable by 20 kcal/mol (Figure S2).

The confinement method clearly identifies two hydrophobic residues V59

and I61, which destabilize the predicted model with respect to the crystallographic

structure (Figure 8 and Figure S3). The sidechains of these

hydrophobic residues are oriented towards the protein hydrophobic core in

the native NMR structure but oriented towards the exterior of the protein

and are solvent exposed in the model. These residues also form part of

a beta-sheet in the experimental structure, but do not form inter-strand

hydrogen bonds in the predicted model. There is also a large difference

around K76, which forms a salt-bridge with D11 in the predicted model,

but not in the experimental structure. This suggests that salt-bridge interactions

are too favorable for the combination of force field and implicit

solvent model we use, which has been a problem noted in the past [ref

carlos].

**The confinement method occasionally produces incorrect results**

Arijit: let’s move Figure 9 to the supporting info and move Figure S4 into

the main text.

Despite success for most of the studied systems, there are few failures, specially

when the GDT-TS scores of the compared structures are very close.

One example is Target T0538, where we compared the crystal structure with

three models (Model 1: “PconsR”—GDT-ts=96; Model 2: “Shell”—GDTTS=

90; Model 3: “FOLDIT”—GDT-TS=86). Contrary to our expectation, the

confinement method predicts that Model 1 is more stable than the crystal

structure (Figure ?). Per-residue free energy calculations (not shown) show