Methods

SID Dataset

The dataset used for evaluation of the protein-protein docking poses using SID data consisted of a homodimer (triose phosphate isomerase, 8tim, 11+ charge state), a homotetramer (streptavidin, 1swb, 11+), a heterotetramer (hemoglobin, 1gzx, 11+), three homopentamers (cholera toxin B, 1fgb, 11+; C-reactive protein, 1gnh, 17+; serum amyloid P, 1sac, 19+), and a homohexamer (glutamate dehydrogenase, 3mvo, 25+). Medium- to high-resolution (< 3.5 Å resolution) crystal structures of the intact protein complexes existed for all members of the dataset and were used for evaluation purposes.

Trioce phosphate isomerase, hemoglobin, cholera toxin B, and glutamate dehydrogenase were purchased from Sigma-Aldrich (St. Louis, MO, USA). Serum Amyloid P and C-reactive protein were purchased from CalBioChem (EMD Biosciences, Inc., San Diego, CA, USA) and streptavidin was purchased from Thermo Scientific Pierce Biotechnology (Rockford, IL, USA). All samples were analyzed at approximately 10 µM complex concentration in 80 mM ammonium acetate (Sigma Aldrich, St. Louis, MO, USA) plus 20 mM triethyl ammonium acetate (TEAA) (Sigma Aldrich, St. Louis, MO, USA). We used TEAA to produce ‘charge reducing’ conditions, which are thought to keep the complex more compact and native-like.7-9 Residual salt impurities were removed by buffer exchanging using micro Spin 6 columns (Bio-Rad, Hercules, CA, USA), as required.

All SID experiments were performed on in-house modified Synapt G2 or G2S instruments (Waters, Milford, UK). The instruments were modified as previously described, however, in this case the SID device was placed between the Trap ion guide/collision cell and the IM cell and hence referred to as Trap-SID. All proteins were introduced into the mass spectrometer using nano-electrospray ionization in positive mode. Nano-electrospray tips were made in-house using thin-walled glass capillaries (i.d. 0.8 mm) using a Flaming/Brown micropipette puller (Sutter Instrument Company, Novato, CA, USA). The spray voltage (typically 1-1.4 kV) was applied using a thin (0.368 mm) platinum wire (Alfa Aesar, Ward Hill, MA, USA). The instrument was operated in ion mobility, sensitivity mode. For MS experiments, a Trap gas flow rate of 4 mL/min and a Trap DC bias of 45 V are applied and the SID device tuned to give a 1-5 V difference between the Trap exit and the entrance lens of the SID device and a 5-10 V difference between the exit lens of the SID device and the helium cell entrance. For SID experiments, the SID device is tuned to steer the ions for collision with the surface. The SID acceleration voltage is defined by...
the potential difference between the DC offset of the Trap and the surface and can be adjusted using the Trap bias setting. The collision energy is determined by multiplying the collision voltage by the charge state of the precursor. For SID experiments, the Trap gas flow rate was lowered to 2 mL/min, to limit any gas-collisions which would cause CID. For SID, typically the most intense, unique, mass-to-charge species in the charge-reducing conditions was chosen for study (see above for charge states used). Performing SID over a range of different collision energies and determining the relative abundance of precursor and products at each energy allows energy-resolved mass spectrometry plots to be produced and used for AE estimation. For each protein, ERMS plots were used to identify which interfaces fragmented during SID and to determine the experimental appearance energies, arbitrarily defined as the acceleration energy needed to reach 10% intensity with respect to the intensity of the native complex (to avoid the influence of hot/pre-fragmenting precursor ions). Appearance energies were subsequently normalized by the number of inter-subunit protein-protein contacts in order to properly account for the non-interface-dependent rigidity factor (RF, see main text).

Table S1: Complexes used for docking using ideal (computationally predicted from crystal structures) SID AE data. Percent helix and strand values were calculated from relaxed crystal structures using DSSP.\textsuperscript{11}

| PDB ID | Complex type | Number of residues (per subunit) | Percent Helix | Percent Strand |
|--------|--------------|---------------------------------|---------------|---------------|
| 1eym   | Homodimer    | 107                             | 10.6          | 39.8          |
| 1f37   | Homodimer    | 110                             | 29.4          | 20.8          |
| 1ix9   | Homodimer    | 205                             | 57.3          | 11.7          |
| 1qlw   | Homodimer    | 328                             | 33.6          | 19.3          |
| 1x8j   | Homodimer    | 351                             | 50.1          | 9.4           |
| 2ear   | Homodimer    | 196                             | 36.5          | 24.7          |
| 2qqq   | Homodimer    | 110                             | 17.1          | 41.5          |
| 2vha   | Homodimer    | 287                             | 38.2          | 24.4          |
| 2voc   | Homodimer    | 112                             | 31.0          | 24.4          |
| 2xdi   | Homodimer    | 107                             | 59.9          | 0.0           |
| 3cby   | Homodimer    | 108                             | 18.1          | 35.8          |
| 3cdy   | Homodimer    | 109                             | 2.8           | 49.5          |
| 3e18   | Homodimer    | 359                             | 35.5          | 23.0          |
| 3f11   | Homodimer    | 252                             | 45.2          | 14.1          |
| 3gmx   | Homodimer    | 154                             | 19.0          | 32.9          |
| 3hg5   | Homodimer    | 398                             | 28.7          | 23.5          |
| 3o1n   | Homodimer    | 276                             | 45.5          | 20.0          |
| 3vm9   | Homodimer    | 153                             | 79.2          | 0.0           |
| 4amb   | Homodimer    | 400                             | 42.6          | 14.8          |
| 4iwh   | Homodimer    | 363                             | 43.1          | 21.3          |
| 4r8d   | Homodimer    | 394                             | 42.4          | 15.1          |
| 4u13   | Homodimer    | 109                             | 31.8          | 44.5          |
| 4unu   | Homodimer    | 111                             | 2.7           | 48.6          |
| 5fi3   | Homodimer    | 357                             | 30.4          | 29.1          |
| 5idb   | Homodimer    | 142                             | 0.0           | 65.0          |
| Protein | Average RMSD of top 100 for Rosetta (Å) | Average RMSD of top 100 for Rosetta with SID (Å) |
|---------|----------------------------------------|-----------------------------------------------|
| 1fgb    | 15.44                                  | 14.74                                         |
| 1gnh    | 21.01                                  | 20.94                                         |
| 1gpx    | 21.61                                  | 18.20                                         |
| 1sac    | 23.20                                  | 21.04                                         |
| 1swb    | 17.91                                  | 15.72                                         |

**Table S2:** Average RMSD’s of top 100 scoring models with Rosetta and Rosetta with SID. Results show improvement in 8/9 cases.
Table S3: $P_{\text{near}}$ values for Rosetta and Rosetta with SID to quantify funneling of score vs. RMSD plots. Three cases (1gzx, 1sac, 1gzx_dimers) show a drastic improvement (>3-fold).

| Protein         | $P_{\text{near}}$ Rosetta | $P_{\text{near}}$ Rosetta with SID | X-fold increase |
|------------------|---------------------------|------------------------------------|-----------------|
| 1fgb             | 0.757                     | 0.562                              | 0.742           |
| 1gnh             | 0.00504                   | 0.00522                            | 1.04            |
| 1gzx             | 0.0115                    | 0.485                              | 42.2            |
| 1sac             | 0.0145                    | 0.0534                             | 3.68            |
| 1swb             | 0.945                     | 0.964                              | 1.02            |
| 3mvo             | 1.77e-14                  | 3.70e-30                           | 2.09e-16        |
| 8tim             | 0.000106                  | 2.82e-12                           | 2.70e-8         |
| 1gzx_dimers      | 3.66e-5                   | 0.00155                            | 42.4            |
| 1swb_dimers      | 0.0171                    | 0.0171                             | 1.00            |

Docking Tutorial
To use RosettaDock to rescore structures with SID AE, two main stages need to be performed in Rosetta:

1. Generate docked structures using RosettaDock.
2. Rescore the poses using Rosetta SID_rescore application.

In this tutorial, variables that need to be specified by the user are shown in brackets (< >).

Dockings and analysis from this paper were performed using talaris2014 scoring function. To use the scoring function, include the flag `--restore_talaris_behavior` in all Rosetta command lines. Without this flag, REF15 will be used by default.

Step 1: RosettaDock

a. Prepare a pdb file containing both partners in a predicted starting position.

b. Prepак the chains by running the following command:

```
~/Rosetta/main/source/bin/docking_prepack_protocol.default.<os><compiler>release -in:file:s <pdb> -partners <chains>
```

- `<os>` operating system (macos, linux).
- `<compiler>` compiler used (gcc, clang, etc.).
- `<pdb>` name of coordinate file in pdb format.
- `<chains>` chains of docking partners, separated by underscore. ex: A_B where A is the static chain, B is the mobile chain.

Example: If gcc was used to compile on linux to dock chains A and C of complex.pdb:

```
~/Rosetta/main/source/bin/docking_prepack_protocol.default.linuxgccrelease -in:file:s complex.pdb -partners A_C
```

c. To dock the chains, use the following command:

```
~/Rosetta/main/source/bin/docking_protocol.default.<os><compiler>release -in:file:s <prepacked_pdb> -partners <chains> -nstruct <n structs>
```
- `<prepacked_pdb>` output pdb from prepack step.
- `<n_structs>` number of structures to generate (>10,000 recommended)
- Additionally, a randomization flag (-randomize1, -randomize2, or -spin) can be given to search more conformational space.

Example: Dock 10,000 structures of chains A and C of complex_0001.pdb

```
~/Rosetta/main/source/bin/docking_protocol.default.linuxgccrelease -in:file:s complex_0001.pdb -partners A_C -nstruct 10000
```

Step 1 will result in `<n_structs>` docked structures. Step 2 will use SID data to rescore and rank the generated structures. A more detailed description for generating docked structures can be found here: [https://www.rosettacommons.org/demos/latest/tutorials/Protein-Protein-Docking/Protein-Protein-Docking](https://www.rosettacommons.org/demos/latest/tutorials/Protein-Protein-Docking/Protein-Protein-Docking)

**Step 2: SID_rescore**

a. First create a text file containing the names of the docked structures (pdb files).

Example:

```
complex_0001_00001.pdb
complex_0001_00002.pdb
complex_0001_00003.pdb
complex_0001_00004.pdb
complex_0001_00005.pdb
... complex_0001_10000.pdb
```

b. To rescore the poses, run the SID_rescore application using the following command:

```
~/Rosetta/main/source/bin/SID_rescore.default.<os><compiler>release -in:file:l <file_with_docked_poses> -AE <AE_from_SID> -interface <chains> -n_ints <n_ints> -out:file:o <output_file> -native <native_pdb>
```

- `<file_with_docked_poses>` file created in Step 2a.
- `<AE_from_SID>` appearance energy from SID experiment (eV).
- `<n_ints>` number of intra-chain contacts in docking.
- `<output_file>` *(optional)* name of output file from this command. (SID_rescore_default.out by default)
- `<native_pdb>` *(optional)* native pdb. Will calculate RMSD if given.

Example: Rescoring list_docked_pdbs.txt (list of pdb files). Experimental AE of 100.0 eV, one intra-chain contact, output file named complex_docking_scores.out, and calculate RMSD to native.pdb.

```
~/Rosetta/main/source/bin/SID_rescore.default.linuxgccrelease -in:file:l list_docked_pdbs.txt -AE 100.0 -interface A_C -n_ints 1 -out:file:o complex_docking_scores.out -native native.pdb
```

Step 2 will result in an output file containing the predicted AE, Rosetta_score, SID_score, Rosetta_SID_score, and RMSD (if native specified) for each of the docked structures. Use the Rosetta_SID_score value to sort the poses and thus select optimal predicted structures.

Example output file:

| Pose_number | AE_pred | Rosetta_score | SID_score | Rosetta_SID_score | RMSD |
|-------------|---------|---------------|-----------|-------------------|------|
| 1           | 7.651   | -87.837       | 0.00703   | -87.795           | 18.34|
| 2           | 44.428  | -91.943       | 0.0       | -91.943           | 2.053|
| ...         |         |               |           |                   |      |
**Figure S1:** Docked complexes of five subcomplexes for which including SID restraints (from ideal AE data) improved the RMSD by more than 14 Å (3vm9, 3gmx, 3jcf, 4ix2, and 4hy3). Green structures are the natives, blue are the models predicted without SID data, and red are models predicted with the Bayesian Rosetta SID rescore. For each dimer, the stationary subunit (left) was aligned to show the discrepancy or lack thereof for the mobile (docked) subunit (right).
Figure S2: Raw SID Score vs. RMSD plots for 1gzx, 1sac, 1swb, and 1gzx dimers. SID score generally scored low RMSD models well while penalizing most high-RMSD structures.
**Figure S3**: Comparison of funneling metrics with the use of ideal AE (predicted from crystal structures): $P_{near}$ (A) and score difference between high RMSD models and minimum score (B). $P_{near}$ improved for 56/57 cases when SID ideal AE was used and average high RMSD separation improved for all cases when ideal SID was used.
Figure S4: Score vs. RMSD plots of each complex for which $P_{\text{near}}$ (quantification of “goodness of funnelling”) decreased by more than half (absolute values in Table S2) when SID data was used. 8tim: 2.70e-8-fold increase, 3mvo: 2.09e-16-fold increase.
**Figure S5**: Function used to evaluate SID likelihood score. Structures with small deviation from the measured experimental data (low $\Delta$) have lower scores and thus higher probability while structures with large deviation from the measured experimental data (high $\Delta$) have higher scores and thus lower probability. This function contains two cutoffs, a lower cutoff ($E_{\text{low}}$, below which the score is minimum) and a higher cutoff ($E_{\text{high}}$, above which the score is maximum). We hypothesize that the inclusion of the $E_{\text{low}}$ helps account for experimental error.

\[
\text{Score} = \begin{cases} 
0 & \Delta < E_{\text{low}} \\
100(2b^3 - 3b^2 + 1) & E_{\text{low}} < \Delta < E_{\text{high}} \\
100 & \Delta > E_{\text{high}}
\end{cases}
\]

where $b = - (\Delta - E_{\text{high}})/(E_{\text{high}} - E_{\text{low}})$

$E_{\text{low}} = 100$ eV

$E_{\text{high}} = 1750$ eV
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