# Prediction of protein assemblies, the next frontier: The CASP14-CAPRI experiment

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*Supplementary Material*



[1] CAPRI group "Czaplewski" corresponds to UNRES_template in the abstract text

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# Table S1 – Participation statistics

| CAPRI ID | CASP ID | Stoichiometry | #interfaces | Predictors | Uploaders | Scorers | CASP |
|---|---|---|---|---|---|---|---|
| T164 | T1032 | A2 | 1 | 28 | 18 | 23 | 21 |
| T165 | H1036 | A:HL | 1 | 27 | 12 | 22 | 25 |
| T166 | H1045 | AB | 1 | 24 | 16 | 19 | 26 |
| T168 | T1052 | A3 | 1 | 24 | 11 | 20 | 20 |
| T169 | T1054 | A2 | 1 | 27 | 16 | 20 | 22 |
| T170 | H1060 | A6B12C3D6 | 9 | 23 | 12 | 17 | 16 |
| T174 | T1070 | A3 | 1 | 24 | 13 | 19 | 21 |
| T176 | T1078 | A2 | 1 | 27 | 16 | 21 | 23 |
| T177 | H1081 | A20 | 3 | 24 | 12 | 18 | 23 |
| T178 | T1083 | A2 | 1 | 26 | 13 | 19 | 23 |
| T179 | T1087 | A2 | 1 | 25 | 13 | 19 | 22 |
| T180 | T1099 | A4 | 2 | 25 | 11 | 18 | 20 |

Target and participation statistics. Stoichiometry and #interfaces refer to the assessment entity. Predictors indicates the number of predictor groups uploading submissions. Whereas all predictor models enter the Scoring experiment, the number of Uploader groups indicates the number of predictor groups that submitted more than 10 models. The number of CASP groups excludes the CAPRI groups that also registered with CASP.

# Table S2 – Prediction results for Target/Interface T164.1

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Gray | 1 | 5/1** |
| Seok | 1 | 4/1** |
| Zou | 1 | 5 |
| Venclovas | 1 | 5 |
| Kihara | 1 | 5 |
| Chang | 1 | 5 |
| Bates | 1 | 5 |
| Shen | 1 | 4 |
| Pierce | 1 | 4 |
| Kozakov/Vajda | 0 | 4 |
| Huang | 1 | 4 |
| Nakamura | 1 | 3 |

| **CASP-only Predictors** | Top-1 | Top-5 |
|---|---|---|
| *CoDock* | 1 | 5 |
| *htjcadd* | 1 | 4 |
| *Baker* | 1 | 4 |
| *Takeda-Shitaka* | 0 | 2 |
| *DATE* | 1 | 2 |
| *Risoluto* | 1 | 1 |
| *Elofsson* | 1 | 1 |

| **Servers** | Top-1 | Top-5 |
|---|---|---|
| MDOCKPP | 1 | 4/1** |
| SWARMDOCK | 1 | 5 |
| HDOCK | 1 | 4 |
| HAWKDOCK | 1 | 4 |
| CLUSPRO | 0 | 4 |
| GALAXYPPDOCK | 1 | 3 |
| LZERD | 0 | 2 |

| **Scorers and Scoring Servers** | Top-1 | Top-5 |
|---|---|---|
| MDOCKPP | 1 | 5/1** |
| SWARMDOCK | 1** | 5/1** |
| Huang | 1** | 5/1** |
| Bates | 1** | 5/1** |
| HDOCK | 1 | 4/1** |
| Zou | 1 | 5 |
| Kihara | 1 | 5 |
| LZERD | 1 | 5 |
| Takeda-Shitaka | 1 | 5 |
| Bonvin | 1 | 5 |
| Shen | 1 | 5 |
| HAWKDOCK | 1 | 5 |
| Chang | 1 | 4 |
| Oliva | 1 | 4 |
| Grudinin | 0 | 3 |
| Fernandez-Recio | 0 | 2 |
| PYDOCKWEB | 0 | 2 |

# Table S2 – Prediction results for Target/Interface T165.1

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| *no acceptable models* | | |

| **CASP-only Predictors** | Top-1 | Top-5 |
|---|---|---|
| *no acceptable models* | | |

| **Servers** | Top-1 | Top-5 |
|---|---|---|
| *no acceptable models* | | |

| **Scorers and Scoring Servers** | Top-1 | Top-5 |
|---|---|---|
| *no acceptable models* | | |

# Table S2 – Prediction results for Target/Interface T166.1

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Vakser | 1** | 5** |
| Kozakov/Vajda | 1** | 5** |
| Huang | 1** | 5** |
| Chang | 1 | 5/3** |
| Venclovas | 0 | 1*** |
| Shen | 1** | 3** |
| Fernandez-Recio | 1** | 3** |
| Bates | 1** | 3** |
| Zou | 1** | 5/1** |
| Pierce | 1** | 3/2** |
| Kihara | 1** | 5/1** |
| Seok | 1 | 2/1** |
| Nakamura | 0 | 1** |

| **CASP-only Predictors** | Top-1 | Top-5 |
|---|---|---|
| *Takeda-Shitaka* | 1** | 5/1***/4** |
| *Baker* | 1** | 5/1***/4** |
| *AILON* | 1** | 5/4** |
| *CoDock* | 1 | 5/3** |
| *DellaCorte* | 1 | 4 |
| *Lamoureux* | 1** | 1** |
| *DATE* | 0 | 1** |

| **Servers** | Top-1 | Top-5 |
|---|---|---|
| MDOCKPP | 1 | 5 |
| SWARMDOCK | 0 | 2 |
| GALAXYPPDOCK | 1 | 1 |
| HDOCK | 0 | 1 |

| **Scorers and Scoring Servers** | Top-1 | Top-5 |
|---|---|---|
| Takeda-Shitaka | 1*** | 4/1***/3** |
| HDOCK | 1** | 5** |
| Huang | 1** | 5** |
| Kihara | 1*** | 3/1***/2** |
| HAWKDOCK | 1** | 5/4** |
| MDOCKPP | 1** | 4** |
| Zou | 1** | 4** |
| LZERD | 1*** | 2/1***/1** |
| Chang | 1** | 4/3** |
| Shen | 1** | 4/3** |
| Fernandez-Recio | 1 | 3/2** |
| PYDOCKWEB | 1 | 3/2** |
| Oliva | 1** | 2** |
| Grudinin | 0 | 1 |

# Table S2 – Prediction results for Target/Interface T168.1

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Zou | 1** | 5** |
| Venclovas | 1** | 5** |
| Vakser | 1** | 5** |
| Seok | 1** | 5** |
| Kozakov/Vajda | 1** | 5** |
| Kihara | 1** | 5** |
| Chang | 1** | 5** |
| Bates | 1** | 5** |
| Huang | 1** | 4/3** |
| Pierce | 1** | 5/2** |
| Fernandez-Recio | 0 | 3** |
| Liwo | 1 | 4 |
| Lubecka | 1 | 3 |
| Czaplewski | 1** | 1** |

| **CASP-only Predictors** | Top-1 | Top-5 |
|---|---|---|
| *CoDock* | 1** | 5** |
| *Baker* | 1** | 5/4** |
| *Lamoureux* | 1** | 3** |
| *UNRES* | 1 | 4 |
| *Takeda-Shitaka* | 0 | 1** |

| **Servers** | Top-1 | Top-5 |
|---|---|---|
| SWARMDOCK | 1** | 5** |
| LZERD | 1** | 5** |
| MDOCKPP | 1** | 5/3** |
| GALAXYPPDOCK | 1** | 2** |

| **Scorers and Scoring Servers** | Top-1 | Top-5 |
|---|---|---|
| Chang | 1** | 5** |
| Oliva | 1** | 5** |
| Bonvin | 1** | 5** |
| Venclovas | 1** | 5** |
| Kihara | 1** | 5** |
| Takeda-Shitaka | 1** | 5** |
| LZERD | 1** | 5** |
| Shen | 1** | 5** |
| Zou | 1** | 5/4** |
| MDOCKPP | 1** | 5/4** |
| HDOCK | 1** | 4** |
| Perthold | 1 | 5/3** |
| Huang | 1** | 4/3** |
| SWARMDOCK | 1** | 3** |
| Bates | 1** | 3** |
| Fernandez-Recio | 1** | 3** |
| PYDOCKWEB | 1** | 3** |

# Table S2 – Prediction results for Target/Interface T169.1

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| *no acceptable models* | | |

| **CASP-only Predictors** | Top-1 | Top-5 |
|---|---|---|
| *no acceptable models* | | |

| **Servers** | Top-1 | Top-5 |
|---|---|---|
| *no acceptable models* | | |

| **Scorers and Scoring Servers** | Top-1 | Top-5 |
|---|---|---|
| *no acceptable models* | | |

# Table S2 – Prediction results for Target/Interface T170.1

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Huang | 1** | 5** |
| Zou | 1 | 5/1** |
| Shen | 1 | 5/1** |
| Venclovas | 1 | 5 |
| Seok | 1 | 5 |
| Kozakov/Vajda | 1 | 5 |
| Fernandez-Recio | 1 | 5 |
| Chang | 1 | 5 |
| Kihara | 1 | 4 |
| Nakamura | 1 | 3 |

| **CASP-only Predictors** | Top-1 | Top-5 |
|---|---|---|
| *Takeda-Shitaka* | 1 | 4 |
| *DATE* | 1 | 2 |

| **Servers** | Top-1 | Top-5 |
|---|---|---|
| HDOCK | 1** | 5** |
| MDOCKPP | 1** | 5** |
| CLUSPRO | 0 | 2 |

| **Scorers and Scoring Servers** | Top-1 | Top-5 |
|---|---|---|
| HDOCK | 1** | 5/4** |
| Fernandez-Recio | 0 | 4/3** |
| PYDOCKWEB | 0 | 4/3** |
| Huang | 1** | 5/2** |
| SWARMDOCK | 0 | 4/2** |
| Bates | 0 | 4/2** |
| Zou | 1 | 5/1** |
| Shen | 1 | 5/1** |
| Takeda-Shitaka | 1 | 5/1** |
| MDOCKPP | 1 | 5 |
| Chang | 1 | 5 |
| Venclovas | 1 | 5 |
| LZERD | 1 | 5 |
| Kihara | 1 | 5 |
| Grudinin | 1 | 5 |
| Oliva | 0 | 3 |
| Bonvin | 1 | 2 |

## Table S2 – Prediction results for Target/Interface T170.2

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Fernandez-Recio | 1 | 5 |
| **CASP-only Predictors** | Top-1 | Top-5 |
| *no acceptable models* | | |
| **Servers** | Top-1 | Top-5 |
| *no acceptable models* | | |
| **Scorers and Scoring Servers** | Top-1 | Top-5 |
| *no acceptable models* | | |

## Table S2 – Prediction results for Target/Interface T170.3

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Venclovas | 1 | 5 |
| Seok | 1 | 5 |
| Zou | 1 | 3 |
| Shen | 0 | 3 |
| **CASP-only Predictors** | Top-1 | Top-5 |
| *no acceptable models* | | |
| **Servers** | Top-1 | Top-5 |
| MDOCKPP | 1 | 5 |
| **Scorers and Scoring Servers** | Top-1 | Top-5 |
| Venclovas | 1 | 4 |
| LZERD | 1 | 3 |
| Chang | 1 | 3 |
| Grudinin | 0 | 3 |
| Takeda-Shitaka | 1 | 3 |
| Shen | 0 | 3 |
| Zou | 0 | 2 |
| MDOCKPP | 0 | 2 |
| Oliva | 0 | 1 |
| Kihara | 0 | 1 |

# Table S2 – Prediction results for Target/Interface T170.4

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Huang | 1 | 5 |
| Venclovas | 0 | 4 |
| Chang | 1 | 2 |

| **CASP-only Predictors** | Top-1 | Top-5 |
|---|---|---|
| *no acceptable models* | | |

| **Servers** | Top-1 | Top-5 |
|---|---|---|
| HDOCK | 1 | 5 |

| **Scorers and Scoring Servers** | Top-1 | Top-5 |
|---|---|---|
| HDOCK | 1 | 4 |
| Fernandez-Recio | 0 | 3 |
| PYDOCKWEB | 0 | 3 |
| Venclovas | 1 | 3 |
| Huang | 1 | 2 |
| SWARMDOCK | 0 | 2 |
| Bates | 0 | 2 |
| Chang | 0 | 1 |
| Zou | 0 | 1 |

# Table S2 – Prediction results for Target/Interface T170.5

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Shen | 1** | 5** |
| Chang | 1** | 5** |
| Nakamura | 1** | 3** |
| Kozakov/Vajda | 1 | 5/2** |
| Venclovas | 1 | 5 |
| Seok | 1 | 5 |
| Kihara | 1 | 5 |
| Huang | 1 | 5 |
| Grudinin | 1 | 5 |
| Vakser | 1 | 2 |

| **CASP-only Predictors** | Top-1 | Top-5 |
|---|---|---|
| *DATE* | 1** | 2** |
| *Baker* | 1 | 5 |
| *VoroCNN-select* | 1 | 1 |

| **Servers** | Top-1 | Top-5 |
|---|---|---|
| CLUSPRO | 1 | 5/1** |
| HDOCK | 1 | 5 |

| **Scorers and Scoring Servers** | Top-1 | Top-5 |
|---|---|---|
| Shen | 1** | 5** |
| Takeda-Shitaka | 1** | 5** |
| Zou | 1 | 4/3** |
| MDOCKPP | 1 | 5/2** |
| Chang | 1 | 4/2** |
| Venclovas | 1 | 5/1** |
| Fernandez-Recio | 1 | 5/1** |
| PYDOCKWEB | 1 | 5/1** |
| Huang | 1 | 5/1** |
| Grudinin | 1 | 5 |
| HDOCK | 1 | 5 |
| Kihara | 1 | 5 |
| LZERD | 1 | 5 |
| Oliva | 0 | 3/1** |
| SWARMDOCK | 0 | 4 |
| Bates | 0 | 4 |

## Table S2 – Prediction results for Target/Interface T170.6

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Shen | 0 | 1 |
| **CASP-only Predictors** | Top-1 | Top-5 |
| *no acceptable models* | | |
| **Servers** | Top-1 | Top-5 |
| *no acceptable models* | | |
| **Scorers and Scoring Servers** | Top-1 | Top-5 |
| Shen | 0 | 1 |
| Takeda-Shitaka | 0 | 1 |

## Table S2 – Prediction results for Target/Interface T170.7

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Kihara | 1 | 2 |
| **CASP-only Predictors** | Top-1 | Top-5 |
| *no acceptable models* | | |
| **Servers** | Top-1 | Top-5 |
| *no acceptable models* | | |
| **Scorers and Scoring Servers** | Top-1 | Top-5 |
| Kihara | 1 | 2 |
| Oliva | 0 | 1 |

# Table S2 – Prediction results for Target/Interface T170.8

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Venclovas | 1** | 5** |
| Grudinin | 1 | 5 |
| Chang | 1 | 5 |
| Shen | 0 | 3 |
| Seok | 1 | 3 |
| Kihara | 0 | 2 |
| **CASP-only Predictors** | Top-1 | Top-5 |
| *Baker* | 1** | 5/3** |
| **Servers** | Top-1 | Top-5 |
| LZERD | 1 | 5 |
| **Scorers and Scoring Servers** | Top-1 | Top-5 |
| Venclovas | 1** | 5/4** |
| Chang | 1 | 4 |
| Oliva | 1 | 4 |
| Bonvin | 1 | 4 |
| LZERD | 1 | 3 |
| MDOCKPP | 0 | 3 |
| Grudinin | 0 | 3 |
| Zou | 0 | 3 |
| Shen | 0 | 3 |
| Fernandez-Recio | 1 | 2 |
| PYDOCKWEB | 1 | 2 |
| Kihara | 0 | 2 |
| Huang | 0 | 2 |

## Table S2 – Prediction results for Target/Interface T170.9

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Shen | 0 | 3 |
| Huang | 1 | 3 |
| Kihara | 0 | 2 |
| Chang | 1 | 2 |
| Seok | 1 | 1 |
| **CASP-only Predictors** | Top-1 | Top-5 |
| *Takeda-Shitaka* | 1 | 3 |
| **Servers** | Top-1 | Top-5 |
| HDOCK | 0 | 3 |
| **Scorers and Scoring Servers** | Top-1 | Top-5 |
| HDOCK | 1 | 4 |
| Huang | 1 | 3 |
| SWARMDOCK | 0 | 3 |
| Bates | 0 | 3 |
| Shen | 0 | 3 |
| MDOCKPP | 0 | 2 |
| Zou | 0 | 2 |
| Chang | 0 | 2 |
| Grudinin | 0 | 2 |
| LZERD | 0 | 2 |
| Fernandez-Recio | 1 | 1 |
| PYDOCKWEB | 1 | 1 |
| Kihara | 0 | 1 |

## Table S2 – Prediction results for Target/Interface T174.1

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| *no acceptable models* | | |
| **CASP-only Predictors** | Top-1 | Top-5 |
| *no acceptable models* | | |
| **Servers** | Top-1 | Top-5 |
| *no acceptable models* | | |
| **Scorers and Scoring Servers** | Top-1 | Top-5 |
| *no acceptable models* | | |

# Table S2 – Prediction results for Target/Interface T176.1

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Zou | 0 | 1 |
| Seok | 0 | 1 |
| **CASP-only Predictors** | Top-1 | Top-5 |
| *Elofsson* | 0 | 1 |
| **Servers** | Top-1 | Top-5 |
| MDOCKPP | 0 | 2 |
| **Scorers and Scoring Servers** | Top-1 | Top-5 |
| Venclovas | 0 | 1 |
| Zou | 0 | 1 |
| MDOCKPP | 0 | 1 |
| Takeda-Shitaka | 0 | 1 |
| SWARMDOCK | 0 | 1 |
| Chang | 0 | 1 |
| Perthold | 0 | 1 |
| Bates | 0 | 1 |
| HAWKDOCK | 0 | 1 |

# Table S2 – Prediction results for Target/Interface T177.1

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Zou | 1*** | 5*** |
| Vakser | 1*** | 5*** |
| Pierce | 1*** | 5*** |
| Kozakov/Vajda | 1*** | 5*** |
| Kihara | 1*** | 5*** |
| Huang | 1*** | 5*** |
| Fernandez-Recio | 1*** | 5*** |
| Chang | 1*** | 5*** |
| Bates | 1*** | 5*** |
| Nakamura | 1*** | 4*** |
| Grudinin | 1*** | 5/3***/2** |
| Venclovas | 1** | 5/2***/3** |
| Seok | 1** | 5** |
| Czaplewski | 1** | 5** |
| Liwo | 1 | 5 |

| **CASP-only Predictors** | Top-1 | Top-5 |
|---|---|---|
| *Baker* | 1*** | 5*** |
| *VoroCNN-select* | 1*** | 3*** |
| *Lamoureux* | 1*** | 2*** |
| *DellaCorte* | 1*** | 1*** |
| *Ornate-select* | 1** | 2** |
| *UNRES* | 1 | 5 |
| *SBROD* | 0 | 1** |
| *ricardo* | 1** | 1** |

| **Servers** | Top-1 | Top-5 |
|---|---|---|
| SWARMDOCK | 1*** | 5*** |
| HDOCK | 1*** | 5*** |
| LZERD | 1*** | 5*** |
| MDOCKPP | 1*** | 5*** |
| CLUSPRO | 1*** | 5*** |
| GALAXYPPDOCK | 1** | 5** |

| **Scorers and Scoring Servers** | Top-1 | Top-5 |
|---|---|---|
| SWARMDOCK | 1*** | 5*** |
| Oliva | 1*** | 5*** |
| Bonvin | 1*** | 5*** |
| Bates | 1*** | 5*** |
| HAWKDOCK | 1*** | 5*** |
| Takeda-Shitaka | 1*** | 5*** |
| MDOCKPP | 1*** | 5*** |
| Venclovas | 1*** | 5*** |
| Chang | 1*** | 5/4***/1** |
| Zou | 1*** | 5/4***/1** |
| HDOCK | 1*** | 5/4***/1** |
| Huang | 1*** | 5/4***/1** |
| PYDOCKWEB | 1 | 5/4*** |
| Shen | 1*** | 4*** |
| LZERD | 1*** | 5/3***/2** |
| Grudinin | 1 | 5/3*** |
| Fernandez-Recio | 1** | 5** |

# Table S2 – Prediction results for Target/Interface T177.2

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Zou | 1*** | 5*** |
| Venclovas | 1*** | 5*** |
| Vakser | 1*** | 5*** |
| Seok | 1*** | 5*** |
| Pierce | 1*** | 5*** |
| Kozakov/Vajda | 1*** | 5*** |
| Kihara | 1*** | 5*** |
| Huang | 1*** | 5*** |
| Fernandez-Recio | 1*** | 5*** |
| DelCarpio | 1*** | 5*** |
| Chang | 1*** | 5*** |
| Bates | 1*** | 5*** |
| Nakamura | 1*** | 4*** |
| Czaplewski | 1*** | 5/2***/3** |
| Liwo | 1 | 5 |
| Shen | 1** | 2/1** |

| **CASP-only Predictors** | Top-1 | Top-5 |
|---|---|---|
| *Zhang-Assembly* | 1*** | 5*** |
| *DELCLAB* | 1*** | 5*** |
| *Baker* | 1*** | 5/4***/1** |
| *Lamoureux* | 1*** | 2*** |
| *Takeda-Shitaka* | 1*** | 4/1***/1** |
| *DellaCorte* | 1** | 2/1***/1** |
| *Risoluto* | 1*** | 1*** |
| *ricardo* | 1*** | 1*** |
| *UNRES* | 1 | 5 |
| *VoroCNN-select* | 0 | 1** |
| *SBROD* | 0 | 1** |
| *Ornate-select* | 0 | 1** |

| **Servers** | Top-1 | Top-5 |
|---|---|---|
| SWARMDOCK | 1*** | 5*** |
| HDOCK | 1*** | 5*** |
| GALAXYPPDOCK | 1*** | 5*** |
| LZERD | 1*** | 5*** |
| MDOCKPP | 1*** | 5*** |
| CLUSPRO | 1*** | 5*** |

| **Scorers and Scoring Servers** | Top-1 | Top-5 |
|---|---|---|
| SWARMDOCK | 1*** | 5*** |
| Chang | 1*** | 5*** |
| Oliva | 1*** | 5*** |
| HDOCK | 1*** | 5*** |
| Huang | 1*** | 5*** |
| Bonvin | 1*** | 5*** |
| Bates | 1*** | 5*** |
| LZERD | 1*** | 5*** |
| Zou | 1*** | 5*** |
| MDOCKPP | 1*** | 5*** |
| HAWKDOCK | 1*** | 5*** |
| Takeda-Shitaka | 1*** | 5*** |
| Venclovas | 1*** | 5*** |
| PYDOCKWEB | 1 | 5/4*** |
| Shen | 1*** | 4*** |
| Grudinin | 1 | 5/3*** |
| Fernandez-Recio | 1*** | 3/2*** |
| Kihara | 1** | 3** |

# Table S2 – Prediction results for Target/Interface T177.3

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Venclovas | 1** | 4** |
| Zou | 1** | 4/3** |
| Grudinin | 0 | 3** |
| Bates | 1 | 5 |
| Kozakov/Vajda | 0 | 2/1** |
| Pierce | 0 | 1** |
| Seok | 1 | 2 |
| Nakamura | 0 | 1 |
| Huang | 1 | 1 |
| DelCarpio | 1 | 1 |

| **CASP-only Predictors** | Top-1 | Top-5 |
|---|---|---|
| *VoroCNN-select* | 0 | 2** |
| *Ornate-select* | 1** | 1** |
| *Zhang-Assembly* | 0 | 2 |
| *DellaCorte* | 1 | 2 |
| *ricardo* | 1 | 1 |
| *DELCLAB* | 1 | 1 |
| *Baker* | 1 | 1 |

| **Servers** | Top-1 | Top-5 |
|---|---|---|
| MDOCKPP | 1** | 5/1***/4** |
| SWARMDOCK | 1 | 5 |
| CLUSPRO | 0 | 2/1** |
| HDOCK | 1 | 1 |

| **Scorers and Scoring Servers** | Top-1 | Top-5 |
|---|---|---|
| SWARMDOCK | 1** | 5/1***/2** |
| Bates | 1** | 5/1***/2** |
| HAWKDOCK | 1** | 3/1***/1** |
| Zou | 1** | 5/3** |
| MDOCKPP | 1** | 4/3** |
| LZERD | 1** | 3** |
| Bonvin | 1 | 3/1** |
| Fernandez-Recio | 1 | 3/1** |
| Chang | 0 | 2/1** |
| Takeda-Shitaka | 1** | 2/1** |
| Venclovas | 1 | 4 |
| Oliva | 1** | 1** |
| HDOCK | 1 | 2 |
| Huang | 1 | 2 |

# Table S2 – Prediction results for Target/Interface T178.1

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Venclovas | 1 | 4/1** |
| Seok | 1 | 4 |
| Lubecka | 1 | 4 |
| Pierce | 1 | 3 |
| Kihara | 0 | 2 |
| Chang | 1 | 2 |
| Zou | 0 | 1 |
| Liwo | 0 | 1 |
| Fernandez-Recio | 0 | 1 |
| Bates | 0 | 1 |

| **CASP-only Predictors** | Top-1 | Top-5 |
|---|---|---|
| *UNRES_contact* | 1 | 4 |
| *htjcadd* | 0 | 2 |
| *CoDock* | 1 | 2 |
| *Baker* | 1 | 2 |
| *UNRES* | 0 | 1 |
| *McGuffin* | 1 | 1 |

| **Servers** | Top-1 | Top-5 |
|---|---|---|
| LZERD | 1 | 2 |
| HAWKDOCK | 0 | 2 |
| MDOCKPP | 0 | 1 |

| **Scorers and Scoring Servers** | Top-1 | Top-5 |
|---|---|---|
| Takeda-Shitaka | 1 | 5/1** |
| Chang | 1 | 4/1** |
| LZERD | 1 | 4 |
| Kihara | 0 | 4 |
| HAWKDOCK | 0 | 2/1** |
| Venclovas | 1 | 3 |
| MDOCKPP | 0 | 3 |
| Oliva | 1 | 2 |
| Zou | 0 | 2 |
| Shen | 0 | 2 |

# Table S2 – Prediction results for Target/Interface T179.1

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Zou | 1 | 4 |
| Venclovas | 1 | 3 |
| Shen | 0 | 2 |
| Liwo | 1 | 2 |
| Chang | 0 | 2 |
| Vakser | 0 | 1 |
| Seok | 1 | 1 |
| Pierce | 0 | 1 |

| **CASP-only Predictors** | Top-1 | Top-5 |
|---|---|---|
| *Baker* | 1** | 1** |
| *UNRES* | 1 | 2 |
| *CoDock* | 0 | 2 |

| **Servers** | Top-1 | Top-5 |
|---|---|---|
| MDOCKPP | 0 | 1 |
| LZERD | 0 | 1 |

| **Scorers and Scoring Servers** | Top-1 | Top-5 |
|---|---|---|
| Venclovas | 1 | 3 |
| Fernandez-Recio | 1 | 3 |
| PYDOCKWEB | 1 | 3 |
| MDOCKPP | 0 | 2 |
| Takeda-Shitaka | 0 | 2 |
| Zou | 0 | 2 |
| LZERD | 1 | 2 |
| Kihara | 1 | 2 |
| Grudinin | 1 | 2 |
| Bonvin | 0 | 2 |
| Shen | 1 | 2 |
| Chang | 0 | 1 |
| HAWKDOCK | 0 | 1 |
| Oliva | 1 | 1 |
| SWARMDOCK | 0 | 1 |
| Huang | 0 | 1 |
| Bates | 0 | 1 |

# Table S2 – Prediction results for Target/Interface T180.1

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Seok | 0 | 1 |
| **CASP-only Predictors** | Top-1 | Top-5 |
| *no acceptable models* | | |
| **Servers** | Top-1 | Top-5 |
| *no acceptable models* | | |
| **Scorers and Scoring Servers** | Top-1 | Top-5 |
| PYDOCKWEB | 1 | 1 |
| Venclovas | 1 | 1 |
| Huang | 0 | 1 |
| Fernandez-Recio | 0 | 1 |

# Table S2 – Prediction results for Target/Interface T180.2

| **Predictors** | Top-1 | Top-5 |
|---|---|---|
| Venclovas | 1** | 5/2***/3** |
| Kihara | 1** | 5/1***/4** |
| Shen | 1** | 5/4** |
| Kozakov/Vajda | 1** | 5/4** |
| Chang | 1** | 5/3** |
| Fernandez-Recio | 1** | 4/2** |
| Zou | 1 | 5/1** |
| Seok | 1** | 5/1** |
| Pierce | 1** | 3/2** |
| Huang | 1** | 2** |
| Lubecka | 1 | 5 |
| Czaplewski | 1 | 5 |
| Liwo | 0 | 4 |
| Vakser | 1 | 1 |
| Grudinin | 0 | 1 |

| **CASP-only Predictors** | Top-1 | Top-5 |
|---|---|---|
| *Baker* | 1 | 5/4** |
| *CoDock* | 1** | 5/3** |
| *UNRES* | 1 | 5 |
| *UNRES_contact* | 1 | 4 |
| *Ornate-select* | 0 | 2 |
| *VoroCNN-select* | 0 | 1 |
| *SBROD* | 0 | 1 |
| *Lamoureux* | 1 | 1 |

| **Servers** | Top-1 | Top-5 |
|---|---|---|
| CLUSPRO | 1** | 5** |
| LZERD | 1** | 4** |
| GALAXYPPDOCK | 1** | 1** |
| MDOCKPP | 0 | 1 |

| **Scorers and Scoring Servers** | Top-1 | Top-5 |
|---|---|---|
| LZERD | 1** | 5** |
| Zou | 1** | 5** |
| MDOCKPP | 1** | 5** |
| Kihara | 1** | 5/4** |
| Huang | 1** | 5/4** |
| Chang | 1** | 5/3** |
| HDOCK | 1** | 4/2** |
| SWARMDOCK | 1** | 4/1** |
| Bates | 1** | 4/1** |
| Fernandez-Recio | 1 | 4/1** |
| Venclovas | 1 | 5 |
| Perthold | 1 | 5 |
| Grudinin | 0 | 4 |
| Shen | 0 | 2/1** |
| PYDOCKWEB | 1 | 3 |
| Oliva | 1 | 2 |
| Takeda-Shitaka | 0 | 2 |

# Table S3 – Assembly target performance for T170

| **Predictors** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | total |
|---|---|---|---|---|---|---|---|---|---|---|
| Shen | ** | 0 | * | 0 | ** | * | 0 | * | * | 6/2** |
| Venclovas | * | 0 | * | * | * | 0 | 0 | ** | 0 | 5/1** |
| Chang | * | 0 | 0 | * | ** | 0 | 0 | * | * | 5/1** |
| Seok | * | 0 | * | 0 | * | 0 | 0 | * | * | 5 |
| Kihara | * | 0 | 0 | 0 | * | 0 | * | * | * | 5 |
| Huang | ** | 0 | 0 | * | * | 0 | 0 | 0 | * | 4/1** |
| Zou | ** | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 2/1** |
| Nakamura | * | 0 | 0 | 0 | ** | 0 | 0 | 0 | 0 | 2/1** |
| Kozakov/Vajda | * | 0 | 0 | 0 | ** | 0 | 0 | 0 | 0 | 2/1** |
| Grudinin | 0 | 0 | 0 | 0 | * | 0 | 0 | * | 0 | 2 |
| Fernandez-Recio | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Vakser | 0 | 0 | 0 | 0 | * | 0 | 0 | 0 | 0 | 1 |
| **CASP-only Predictors** | | | | | | | | | | |
| *DATE* | * | 0 | 0 | 0 | ** | 0 | 0 | 0 | 0 | 2/1** |
| *Baker* | 0 | 0 | 0 | 0 | * | 0 | 0 | ** | 0 | 2/1** |
| *Takeda-Shitaka* | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | * | 2 |
| *VoroCNN-select* | 0 | 0 | 0 | 0 | * | 0 | 0 | 0 | 0 | 1 |
| *Kiharalab-assembly* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | * | 0 | 1 |
| **Docking Servers** | | | | | | | | | | |
| HDOCK | ** | 0 | 0 | * | * | 0 | 0 | 0 | * | 4/1** |
| MDOCKPP | ** | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 2/1** |
| CLUSPRO | * | 0 | 0 | 0 | ** | 0 | 0 | 0 | 0 | 2/1** |
| LZERD | 0 | 0 | 0 | 0 | 0 | 0 | 0 | * | 0 | 1 |
| **Scorers and Scoring Servers** | | | | | | | | | | |
| Zou | ** | 0 | * | * | ** | 0 | 0 | * | * | 6/2** |
| Shen | ** | 0 | * | 0 | ** | * | 0 | * | * | 6/2** |
| Venclovas | * | 0 | * | * | ** | 0 | 0 | ** | 0 | 5/2** |
| PYDOCKWEB | ** | 0 | 0 | * | ** | 0 | 0 | * | * | 5/2** |
| Huang | ** | 0 | 0 | * | ** | 0 | 0 | * | * | 5/2** |
| Fernandez-Recio | ** | 0 | 0 | * | ** | 0 | 0 | * | * | 5/2** |
| Chang | * | 0 | * | * | ** | 0 | 0 | * | * | 6/1** |
| Takeda-Shitaka | ** | 0 | * | 0 | ** | * | 0 | 0 | 0 | 4/2** |
| Oliva | * | 0 | * | 0 | ** | 0 | * | * | 0 | 5/1** |
| MDOCKPP | * | 0 | * | 0 | ** | 0 | 0 | * | * | 5/1** |
| Kihara | * | 0 | * | 0 | * | 0 | * | * | * | 6 |
| SWARMDOCK | ** | 0 | 0 | * | * | 0 | 0 | 0 | * | 4/1** |
| LZERD | * | 0 | * | 0 | * | 0 | 0 | * | * | 5 |
| HDOCK | ** | 0 | 0 | * | * | 0 | 0 | 0 | * | 4/1** |
| Grudinin | * | 0 | * | 0 | * | 0 | 0 | * | * | 5 |
| Bates | ** | 0 | 0 | * | * | 0 | 0 | 0 | * | 4/1** |
| Bonvin | * | 0 | 0 | 0 | 0 | 0 | 0 | * | 0 | 2 |

# Table S3 – Assembly target performance for T177

| **Predictors** | 1 | 2 | 3 | total |
|---|---|---|---|---|
| Zou | *** | *** | ** | 3/2***/1** |
| Venclovas | *** | *** | ** | 3/2***/1** |
| Pierce | *** | *** | ** | 3/2***/1** |
| Kozakov/Vajda | *** | *** | ** | 3/2***/1** |
| Nakamura | *** | *** | * | 3/2*** |
| Huang | *** | *** | * | 3/2*** |
| Bates | *** | *** | * | 3/2*** |
| Vakser | *** | *** | 0 | 2*** |
| Seok | ** | *** | * | 3/1***/1** |
| Kihara | *** | *** | 0 | 2*** |
| Fernandez-Recio | *** | *** | 0 | 2*** |
| Chang | *** | *** | 0 | 2*** |
| Grudinin | *** | 0 | ** | 2/1***/1** |
| Czaplewski | ** | *** | 0 | 2/1***/1** |
| DelCarpio | 0 | *** | * | 2/1*** |
| Shen | 0 | ** | 0 | 1/1** |
| Liwo | * | * | 0 | 2 |
| **CASP-only Predictors** | | | | |
| *VoroCNN-select* | *** | ** | ** | 3/1***/2** |
| *DellaCorte* | *** | *** | * | 3/2*** |
| *Baker* | *** | *** | * | 3/2*** |
| *ricardo* | ** | *** | * | 3/1***/1** |
| *Ornate-select* | ** | ** | ** | 3/3** |
| *Lamoureux* | *** | *** | 0 | 2*** |
| *UNRES* | ** | *** | 0 | 2/1***/1** |
| *Seok-assembly* | ** | *** | 0 | 2/1***/1** |
| *Zhang-Assembly* | 0 | *** | * | 2/1*** |
| *SBROD* | ** | ** | 0 | 2/2** |
| *DELCLAB* | 0 | *** | * | 2/1*** |
| *Takeda-Shitaka* | 0 | *** | 0 | 1*** |
| *Risoluto* | 0 | *** | 0 | 1*** |
| **Docking Servers** | | | | |
| MDOCKPP | *** | *** | *** | 3*** |
| CLUSPRO | *** | *** | ** | 3/2***/1** |
| SWARMDOCK | *** | *** | * | 3/2*** |
| HDOCK | *** | *** | * | 3/2*** |
| LZERD | *** | *** | 0 | 2*** |
| GALAXYPPDOCK | ** | *** | 0 | 2/1***/1** |
| **Scorers and Scoring Servers** | | | | |
| SWARMDOCK | *** | *** | *** | 3*** |
| HAWKDOCK | *** | *** | *** | 3*** |
| Bates | *** | *** | *** | 3*** |
| Zou | *** | *** | ** | 3/2***/1** |
| Takeda-Shitaka | *** | *** | ** | 3/2***/1** |
| Oliva | *** | *** | ** | 3/2***/1** |
| MDOCKPP | *** | *** | ** | 3/2***/1** |
| LZERD | *** | *** | ** | 3/2***/1** |
| Chang | *** | *** | ** | 3/2***/1** |
| Bonvin | *** | *** | ** | 3/2***/1** |
| Venclovas | *** | *** | * | 3/2*** |
| Huang | *** | *** | * | 3/2*** |
| HDOCK | *** | *** | * | 3/2*** |
| Fernandez-Recio | ** | *** | ** | 3/1***/2** |
| Shen | *** | *** | 0 | 2*** |
| PYDOCKWEB | *** | *** | 0 | 2*** |
| Grudinin | *** | *** | 0 | 2*** |
| Kihara | 0 | ** | 0 | 1/1** |

# Table S3 – Assembly target performance for T180

| **Predictors** | 1 | 2 | total |
|---|---|---|---|
| Venclovas | 0 | *** | 1*** |
| Seok | * | ** | 2/1** |
| Zou | 0 | ** | 1/1** |
| Shen | 0 | ** | 1/1** |
| Pierce | 0 | ** | 1/1** |
| Kozakov/Vajda | 0 | ** | 1/1** |
| Kihara | 0 | ** | 1/1** |
| Huang | 0 | ** | 1/1** |
| Chang | 0 | ** | 1/1** |
| Vakser | 0 | * | 1 |
| Lubecka | 0 | * | 1 |
| Liwo | 0 | * | 1 |
| Fernandez-Recio | 0 | * | 1 |
| **CASP-only Predictors** | | | |
| *Seok-assembly* | 0 | ** | 1/1** |
| *Kiharalab-assembly* | 0 | ** | 1/1** |
| *CoDock* | 0 | ** | 1/1** |
| *Baker* | 0 | ** | 1/1** |
| *UNRES_contact* | 0 | * | 1 |
| *UNRES* | 0 | * | 1 |
| *Seok-naive* | 0 | * | 1 |
| *Lamoureux* | 0 | * | 1 |
| **Docking Servers** | | | |
| LZERD | 0 | ** | 1/1** |
| GALAXYPPDOCK | 0 | ** | 1/1** |
| CLUSPRO | 0 | ** | 1/1** |
| **Scorers and Scoring Servers** | | | |
| Huang | * | ** | 2/1** |
| Zou | 0 | ** | 1/1** |
| Venclovas | * | * | 2 |
| SWARMDOCK | 0 | ** | 1/1** |
| Shen | 0 | ** | 1/1** |
| PYDOCKWEB | * | * | 2 |
| MDOCKPP | 0 | ** | 1/1** |
| LZERD | 0 | ** | 1/1** |
| Kihara | 0 | ** | 1/1** |
| HDOCK | 0 | ** | 1/1** |
| Fernandez-Recio | * | * | 2 |
| Chang | 0 | ** | 1/1** |
| Bates | 0 | ** | 1/1** |
| Takeda-Shitaka | 0 | * | 1 |
| Perthold | 0 | * | 1 |
| Oliva | 0 | * | 1 |
| Grudinin | 0 | * | 1 |

# Table S4 – Complete participant performance

| Rank | **Predictors** | Participation | Top-1 | Top-5 | Score |
|---|---|---|---|---|---|
| 1 | Seok | 14 | 8/2** | 9/4** | 13 |
| | Venclovas | 14 | 7/2** | 8/1***/3** | 13 |
| 3 | Chang | 14 | 7/2** | 8/3** | 11 |
| | Zou | 14 | 5/3** | 8/3** | 11 |
| 5 | Kihara | 14 | 5/3** | 7/3** | 10 |
| | Pierce | 13 | 6/3** | 7/3** | 10 |
| 7 | Huang | 14 | 5/3** | 5/3** | 8 |
| | Bates, Kozakov/Vajda | 14 | 4/3** | 5/3** | 8 |
| | Fernandez-Recio | 14 | 3/2** | 5/3** | 8 |
| 11 | Shen | 14 | 3/1** | 6/1** | 7 |
| | Vakser | 14 | 3** | 4/3** | 7 |
| 13 | Nakamura | 11 | 2/1** | 3/2** | 5 |
| 14 | Liwo | 12 | 2 | 3 | 3 |
| | Czaplewski | 13 | 2/1** | 2/1** | 3 |
| 16 | Lubecka | 8 | 2 | 2 | 2 |
| | Gray | 1 | 1 | 1** | 2 |
| 18 | Del Carpio | 9 | 1 | 1 | 1 |
| | Grudinin | 13 | 1 | 1 | 1 |
| | **CASP-only Predictors** | Participation | Top-1 | Top-5 | Score |
| | *Baker* | 14 | 7/4** | 8/1***/3** | 13 |
| | *CoDock* | 10 | 5/1** | 6/2** | 8 |
| | *Takeda-Shitaka* | 14 | 2/1** | 4/1***/1** | 7 |
| | *Seok-assembly* | 14 | 5/1** | 5/1** | 6 |
| | *Kiharalab-assembly* | 13 | 3/1** | 5/1** | 6 |
| | *Lamoureux* | 11 | 3** | 3** | 6 |
| | *UNRES* | 13 | 2 | 3 | 3 |
| | *DellaCorte* | 6 | 2/1** | 2/1** | 3 |
| | *DATE* | 11 | 1 | 2/1** | 3 |
| | *Risoluto* | 14 | 2 | 2 | 2 |
| | *Elofsson* | 13 | 1 | 2 | 2 |
| | *htjcadd* | 6 | 1 | 2 | 2 |
| | *AILON* | 6 | 1** | 1** | 2 |
| | *ricardo* | 3 | 1** | 1** | 2 |
| | *VoroCNN-select* | 13 | 1 | 1** | 2 |
| | *Ornate-select* | 10 | 1 | 1** | 2 |
| | *Seok-naive* | 6 | 0 | 1** | 2 |
| | *SBROD* | 11 | 0 | 1 | 1 |
| | *DELCLAB* | 9 | 1 | 1 | 1 |
| | *McGuffin*, *UNRES_contact* | 7 | 1 | 1 | 1 |
| | *Zhang-Assembly* | 5 | 1 | 1 | 1 |
| Rank | **Servers** | Participation | Top-1 | Top-5 | Score |
| 1 | MDOCKPP | 14 | 4/2** | 7/1***/2** | 11 |
| 2 | LZERD | 14 | 4/2** | 6/2** | 8 |
| 3 | GALAXYPPDOCK | 14 | 5/1** | 5/1** | 6 |
| | SWARMDOCK | 14 | 3/2** | 4/2** | 6 |
| 5 | HDOCK, CLUSPRO | 14 | 2/1** | 3/1** | 4 |
| 7 | HAWKDOCK | 6 | 1 | 2 | 2 |
| Rank | **Scorers and Scoring Servers** | Participation | Top-1 | Top-5 | Score |
| 1 | Zou | 14 | 5/3** | 10/3** | 13 |
| | Chang | 14 | 6/3** | 9/4** | 13 |
| | MDOCKPP | 14 | 5/3** | 9/4** | 13 |
| | Takeda-Shitaka | 14 | 5/1***/2** | 8/1***/3** | 13 |
| 5 | Shen | 14 | 5/3** | 9/3** | 12 |
| | LZERD | 14 | 7/1***/2** | 8/1***/2** | 12 |
| 7 | Huang | 14 | 5/4** | 7/4** | 11 |
| 8 | Oliva | 14 | 6/3** | 7/3** | 10 |
| | Fernandez-Recio | 14 | 5/2** | 7/3** | 10 |
| | PYDOCKWEB | 14 | 5/1** | 7/3** | 10 |
| | Kihara | 14 | 5/1***/1** | 7/1***/1** | 10 |
| | Bates, SWARMDOCK | 14 | 4/3** | 6/1***/2** | 10 |
| | HAWKDOCK | 10 | 3/2** | 6/1***/2** | 10 |
| 15 | Venclovas | 13 | 6/2** | 7/2** | 9 |
| | HDOCK | 14 | 5/3** | 5/4** | 9 |
| 17 | Grudinin | 14 | 1 | 5/1** | 6 |
| | Bonvin | 14 | 3/2** | 4/2** | 6 |
| 19 | Perthold | 9 | 1 | 2/1** | 3 |

# Table S5 – Interface prediction accuracy

| Rank | Name | #correct | #total | #interface | #target | fraction of correct interfaces | Recall $\mu$ | Recall $\sigma$ | Precision $\mu$ | Precision $\sigma$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **Predictors** | | | | | | | | | | |
| 1 - | Huang | 30 | 111 | 23 | 12 | 0.27 | 48.4% | 30% | 52.0% | 31% |
| 2 - | Liwo | 27 | 100 | 20 | 10 | 0.27 | 39.1% | 31% | 32.6% | 21% |
| 3 - | Czaplewski | 28 | 105 | 21 | 11 | 0.27 | 31.1% | 30% | 38.3% | 27% |
| 4 - | Venclovas | 28 | 115 | 23 | 12 | 0.24 | 55.2% | 29% | 59.8% | 27% |
| 5 - | Kozakov/Vajda | 27 | 115 | 23 | 12 | 0.23 | 42.9% | 29% | 54.1% | 27% |
| 6 - | Shen | 24 | 109 | 23 | 12 | 0.22 | 52.5% | 27% | 52.4% | 24% |
| 7 - | Zou | 25 | 115 | 23 | 12 | 0.22 | 41.2% | 31% | 56.2% | 32% |
| 8 - | Bates | 24 | 115 | 23 | 12 | 0.21 | 38.3% | 32% | 45.5% | 30% |
| 9 - | Grudinin | 23 | 110 | 22 | 11 | 0.21 | 33.5% | 23% | 51.4% | 30% |
| 10 - | Vakser | 18 | 94 | 23 | 12 | 0.19 | 45.4% | 31% | 51.9% | 29% |
| 11 - | Kihara | 21 | 115 | 23 | 12 | 0.18 | 54.9% | 27% | 50.0% | 25% |
| 12 - | Pierce | 20 | 110 | 22 | 11 | 0.18 | 42.9% | 28% | 53.9% | 26% |
| 13 - | Chang | 19 | 115 | 23 | 12 | 0.17 | 56.1% | 27% | 58.0% | 26% |
| 14 - | Seok | 18 | 115 | 23 | 12 | 0.16 | 54.0% | 28% | 49.2% | 26% |
| 15 - | DelCarpio | 9 | 56 | 12 | 9 | 0.16 | 33.0% | 29% | 31.9% | 26% |
| 16 - | Nakamura | 10 | 73 | 20 | 9 | 0.14 | 50.9% | 29% | 54.6% | 25% |
| 17 - | Lubecka | 6 | 45 | 9 | 8 | 0.13 | 42.1% | 23% | 36.0% | 17% |
| 18 - | Fernandez-Recio | 12 | 115 | 23 | 12 | 0.10 | 35.8% | 28% | 52.8% | 27% |
| **CASP-only Predictors** | | | | | | | | | | |
| 1 - | Risoluto | 15 | 54 | 23 | 12 | 0.28 | 34.4% | 26% | 37.2% | 26% |
| 2 - | Elofsson | 8 | 30 | 22 | 11 | 0.27 | 40.9% | 28% | 40.1% | 24% |
| 3 - | Seok-assembly | 25 | 95 | 22 | 12 | 0.26 | 31.4% | 27% | 30.2% | 23% |
| 4 - | UNRES | 27 | 105 | 21 | 11 | 0.26 | 38.7% | 30% | 31.6% | 21% |
| 5 - | Kiharalab-assembly | 22 | 100 | 20 | 11 | 0.22 | 36.9% | 27% | 39.0% | 28% |
| 6 - | Ornate-select | 14 | 65 | 13 | 10 | 0.22 | 36.4% | 24% | 48.3% | 28% |
| 7 - | Lamoureux | 10 | 51 | 14 | 11 | 0.20 | 50.8% | 29% | 46.5% | 26% |
| 8 - | DATE | 10 | 54 | 18 | 9 | 0.19 | 47.6% | 27% | 48.9% | 23% |
| 9 - | VoroCNN-select | 13 | 77 | 21 | 12 | 0.17 | 31.3% | 23% | 45.0% | 30% |
| 10 - | bioinsilico_sbi | 5 | 30 | 6 | 6 | 0.17 | 21.4% | 13% | 43.8% | 24% |
| 11 - | MULTICOM-AI | 7 | 45 | 9 | 7 | 0.16 | 33.1% | 21% | 31.6% | 21% |
| 12 - | CoDock | 8 | 55 | 11 | 10 | 0.15 | 52.8% | 26% | 51.1% | 23% |
| 13 - | UNRES_contact | 6 | 40 | 8 | 7 | 0.15 | 39.0% | 22% | 34.2% | 17% |
| 14 - | Takeda-Shitaka | 14 | 106 | 23 | 12 | 0.13 | 46.6% | 27% | 56.2% | 26% |
| 15 - | DELCLAB | 7 | 55 | 11 | 9 | 0.13 | 33.9% | 28% | 35.9% | 26% |
| 16 - | htjcadd | 4 | 30 | 6 | 6 | 0.13 | 33.1% | 22% | 43.4% | 20% |
| 17 - | Baker | 13 | 115 | 23 | 12 | 0.11 | 46.1% | 29% | 58.1% | 27% |
| 18 - | SBROD | 8 | 70 | 14 | 11 | 0.11 | 32.3% | 22% | 47.7% | 30% |
| 19 - | Seok-naive | 3 | 29 | 7 | 6 | 0.10 | 33.9% | 24% | 42.8% | 21% |
| **Servers** | | | | | | | | | | |
| 1 - | MULTICOM-CLUSTER | 23 | 90 | 18 | 12 | 0.26 | 24.0% | 24% | 22.8% | 22% |
| 2 - | HDOCK | 27 | 109 | 23 | 12 | 0.25 | 48.2% | 27% | 49.2% | 30% |
| 3 - | GALAXYPPDOCK | 25 | 104 | 22 | 12 | 0.24 | 34.8% | 30% | 33.6% | 26% |
| 4 - | LZERD | 27 | 115 | 23 | 12 | 0.23 | 40.2% | 30% | 42.5% | 30% |
| 5 - | CLUSPRO | 20 | 109 | 23 | 12 | 0.18 | 45.2% | 27% | 53.0% | 25% |
| 6 - | SWARMDOCK | 16 | 100 | 20 | 12 | 0.16 | 32.7% | 31% | 42.4% | 31% |
| 7 - | MDOCKPP | 17 | 114 | 23 | 12 | 0.15 | 38.1% | 31% | 53.3% | 34% |
| 8 - | HAWKDOCK | 4 | 30 | 6 | 6 | 0.13 | 33.1% | 22% | 43.4% | 20% |
| **Scorers** | | | | | | | | | | |
| 1 - | Bonvin | 35 | 115 | 23 | 12 | 0.30 | 44.3% | 29% | 43.7% | 29% |
| 2 - | Perthold | 15 | 50 | 10 | 9 | 0.30 | 50.0% | 27% | 39.8% | 22% |
| 3 - | Zou | 29 | 115 | 23 | 12 | 0.25 | 52.8% | 29% | 53.0% | 26% |
| 4 - | Takeda-Shitaka | 24 | 100 | 23 | 12 | 0.24 | 56.0% | 27% | 57.4% | 26% |
| 5 - | Huang | 27 | 115 | 23 | 12 | 0.23 | 57.8% | 26% | 50.3% | 26% |
| 6 - | Venclovas | 25 | 110 | 22 | 11 | 0.23 | 51.3% | 30% | 56.5% | 29% |
| 7 - | Chang | 25 | 115 | 23 | 12 | 0.22 | 55.8% | 28% | 54.7% | 26% |
| 8 - | Shen | 25 | 115 | 23 | 12 | 0.22 | 47.9% | 29% | 53.0% | 26% |
| 9 - | Grudinin | 22 | 115 | 23 | 12 | 0.19 | 45.3% | 28% | 46.6% | 27% |
| 10 - | Fernandez-Recio | 21 | 114 | 23 | 12 | 0.18 | 51.3% | 29% | 52.2% | 29% |
| 11 - | Oliva | 19 | 115 | 23 | 12 | 0.17 | 47.1% | 28% | 50.6% | 28% |
| 12 - | Kihara | 18 | 115 | 23 | 12 | 0.16 | 52.7% | 26% | 49.3% | 26% |
| 13 - | Bates | 17 | 113 | 23 | 12 | 0.15 | 58.4% | 28% | 48.3% | 26% |
| **Scoring Servers** | | | | | | | | | | |
| 1 - | MDOCKPP | 29 | 115 | 23 | 12 | 0.25 | 57.9% | 26% | 54.0% | 24% |
| 2 - | HDOCK | 25 | 112 | 23 | 12 | 0.22 | 53.2% | 28% | 51.9% | 28% |
| 3 - | PYDOCKWEB | 21 | 114 | 23 | 12 | 0.18 | 51.8% | 30% | 50.8% | 28% |
| 4 - | SWARMDOCK | 17 | 113 | 23 | 12 | 0.15 | 58.4% | 28% | 48.3% | 26% |
| 5 - | LZERD | 15 | 114 | 23 | 12 | 0.13 | 53.8% | 29% | 50.8% | 27% |
| 6 - | HAWKDOCK | 4 | 60 | 12 | 10 | 0.07 | 68.4% | 24% | 56.0% | 24% |

**Table S5 – Interface prediction accuracy.** Interface rank based on the fraction of correctly predicted interfaces. Interfaces are defined as correct when both recall and precision values exceed 50%. Recall and precision values are averaged over ligand and receptor entities. Columns list the number of correct interfaces (#correct), the total number of interfaces (#total), and the target (#target) and interface (#interface) participation. $\mu$ and $\sigma$ values are average and standard deviation of recall and precision values over all predicted interfaces.

# Extended Abstracts of individual participants

*The responsibility for the accuracy of the individual Extended Abstracts lies with the authors of each Abstract.*

## I. Protein complex assembly by employing particle swarm optimization

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The construction, optimization and docking of protein models remains challenging. All require extensive sampling of the high dimensional conformational space, which is intractable with methods based on exhaustive enumeration of all possible solutions. Moreover, the exact contributions of the two recognized mechanisms for protein-protein complex formation, 'conformational selection' and 'induced fit', are not known for any specific interaction. In order to address these problems, we have developed a series of heuristic methods based on Particle Swarm Optimization (PSO).

### Methods

Our general methodology for protein fold construction and docking can be described as follows:

*i) Fold construction using our automatic server 3D-Jigsaw-SL*

The protocol first searches for homologous sequences to the query sequence using HHBlits[1] against a sequence profile database of known structures clustered at 70% sequence identity. A linear *ab initio* polypeptide corresponding to the query sequence is constructed, taking into account the bond lengths, angles and torsion angles accordingly to identified homologous fragments. All the coil regions that are not matched with a structural template are automatically adjusted in torsion angle space. The central core of the algorithm is a constricted PSO[2], which searches for a minimal Dfire[3] statistical pair potential energy. When distance information was available, either from PSICOV[4] or from discontinuous templates, a Hookean force was applied as a distance restraint mechanism. Two strategies were applied for folding the structures, the first one adjusts all the torsion angles between all the fragments at once, whereas the second one adjusts the torsion of each linker region (i.e., regions between fragments from templates) one at a time, starting from the N-terminal. The latter technique is computationally more expensive; however, it achieves to generate structures with a smaller radius of gyration (i.e., the structures are more globular). This property allows to generate better, i.e., biophysically sound, models. Finally, the top 10 ranking models from 100 replicates of the algorithm at 10000 iterations (according to Dfire) are then minimized with CHARMM[5] (version 22) and the top structure, identified as having the best CHARMM energy after minimization, is selected for subsequent submission to our protein docking server, SwarmDock. For each section of a protein model different templates might have been chosen; therefore, relating models to single templates is not always possible with this methodology.

*ii) Docking using SwarmDock*

For the modelling of all protein complexes, we used a modification to our binary protein-docking algorithm SwarmDock[6]. Our method uses the principles of PSO to search the parameter docking space. The innovations added to our automated binary server is, for homo-oligomers, to treat each particle within the swarm as an instance of a packed homo-oligomer, constrained by the appropriate symmetry operators. The objective is to optimize the particle space in order to find the most energetically favourable homo-oligomer. Particles move through a multi-parameter space by the optimization of two sets of parameters: orientations and translations of each monomeric unit relative to the imposed symmetry and linear combinations of normal modes that adjust the conformation of each monomer, in the presence of the other monomers, in this simultaneous docking process. For hetero-oligomeric structures we employed our standard SwarmDock (https://bmm.crick.ac.uk/~svc-bmm-swarmdock) protocol[6]. This docking methodology isn't template based. Moreover, additional information, such as potential sequence conservation at the protein-protein interface, was not considered. The ranking of docked poses was obtained using our 'democratic' scoring system, as previously described[7]. To an extent, we considered both the principle of 'conformational selection' and 'induced fit' in our docking procedure. Conformational selection, by using a variety of starting protein conformations[8], obtained either by our own protein modelling server, *3D-Jigsaw-SL,* or protein models taken from the CASP14 server tar file. Induced fit, is considered too since small adjustments are made in both the backbones and sidechains of the interacting proteins upon docking via the employment of our PSO procedure.

## Results

For this round of CASP-CAPRI our results show substantial room for improvement. Only for targets classified as easy (T164, T166, T177), and on one target classified as Difficult (T178, manual model), did the above method show some utility. Interestingly, our manual submissions ranked slightly better than for our server, SwarmDock. This we attribute to having more accurately built models to feed into the docking procedure. For the automated server runs, we didn't have the time to consider alternative input model protein folds to those produced by our fold construction software, *3D-Jigsaw-SL,* a software package in the early stages of development. For the manual submission phase, we were able to test other input model constructs from the CASP14 server tar file. Perhaps not surprisingly, this seems to have made a noticeable difference; the accuracy of the output depends upon the accuracy of the input.

## Conclusion

It is not formally a requirement that docking servers in CASP-CAPRI should both build protein folds as well as dock them. However, since success in docking, at least in our hands, requires high quality input models, and high quality protein fold modelling servers around the world tend to have long queues and waiting times, to keep a cutting edge in developing our docking server, our own input models, generated from primary amino acid sequences of the unbound component parts, must be developed to a higher standard; therefore, to facilitate and end-to-end docking methodology, as much time will need to be invested in producing accurate input models as to developing further the actual docking modules.

## Availability

Our automated binary protein-protein docking server, SwarmDock, can be located at:

https://bmm.crick.ac.uk/~svc-bmm-swarmdock/

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## II. Summary, Chang group, CAPRI Round 50 (CASP14-CAPRI)


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In CASP14-CAPRI, our group combined template-based modeling and *ab-initio* docking protocol as hybrid docking strategy called CoDock [1, 2] for the docking and scoring experiments. For each target, we queried Protein Data Bank (PDB) for structures of protein homologs that can be used as template for modeling the homo and hetero protein complexes. The templates for each target are listed in Table 1. If no proper template was available, we used the *ab-initio* docking protocol to perform the global searching, such as T172 and T173. For T170, we partly modeled the hetero 15-mer complex according to the template 6J0N and applied *ab-initio* docking to obtain the final structure of the hetero 27-mer complex. Then, two different binding modes are submitted by our group, as shown in Figure 1.

A knowledge-based scoring function was trained based on the statistical mechanics-based iterative method [3] and used in CoDock program. This knowledge-based scoring function included much more information of near-native structures in the observed pair distribution function, enabling it more robust for conformational changes. Since the scorer results for all targets in Round 50 are evaluated by CAPRI, we summarized our performance in Table 1. For T165, T169 and T174, no group obtained acceptable models. For T167, T175 and T181, there are no evaluated data provided by CAPRI. The 3 targets of T171-173 are canceled by CASP. Only 9 targets have available data for analysis. For 8 targets of T164, T166, T168, T170, T176, T177, T178, and T179, our docking protocol achieved acceptable quality or better models in the scoring competitions. Our group failed mainly in the target of T180. The minimum number of subunits of T180 may not be chosen correctly in our submissions.

Table 1. List of templates and scoring performance of our group (S3) for each target

| CAPRI ID (CASP ID) | Template | Stocihiometry | Scoring performance of Top-5 [a] |
|---|---|---|---|
| **T164 (T1032)** [b] | 1GXL, 6OIS | A2 | * |
| *T165 (H1036)* [c] | 5ZS0, 4OT1, 5C6T | A3B3C3 | -- |
| **T166 (H1045)** | 2Y9M, 4BWF, 5NKZ | A1B1 | ** |
| T167 (T1050) | 4A2L, 3V9F, 4A2M, 3OTT, 3VA6 | A2 | No data |
| **T168 (T1052)** | 6F7D, 6F7K | A3 | ** |
| *T169 (T1054)* | 4QO6, 2OBV, 4ODJ | A2 | -- |
| **T170 (H1060)** | 6J0N | A6B3C12D6 | * |
| T171( T1063) | 2MVW, 6QAJ | A4 | Canceled |
| T172 (H1066) | --[d] | A1B1 | Canceled |
| T173 (H1069) | -- | A1B1 | Canceled |
| *T174 (T1070)* | 1S2E, 5IV5, 5IV7, 1QEX | A3 | -- |
| T175 (T1073) | 4G6Q | A4 | No data |
| **T176 (T1078)** | 3V0R | A2 | * |
| **T177(H1081)** | 2VYC, 5XX1 | A20 | ** |
| **T178 (T1083)** | 1U4Q, 3GWK, 5MTO, 5ME8 | A2 | ** |
| **T179 (T1087)** | 3GWK, 5LOS | A2 | * |
| T180(T1099) | 3J2V, 6HTX | The minimum number of subunits | -- |
| T181 (H1103) | 6XDC | A1B1 | No data |
| Total | | | **8/4**** |

[a] '**' and '*' indicate medium and acceptable quality models achieved by our group, respectively. '--' represents no acceptable models obtained by our group. 'No data' represents no evaluated data provided by CAPRI. 'Canceled' represents the target canceled by CASP.
[b] In these 8 targets, our group achieved acceptable or better models in scoring experiments.
[c] No groups obtained acceptable models in T165, T169 and T174.
[d] No proper template was available for T172 and T173.

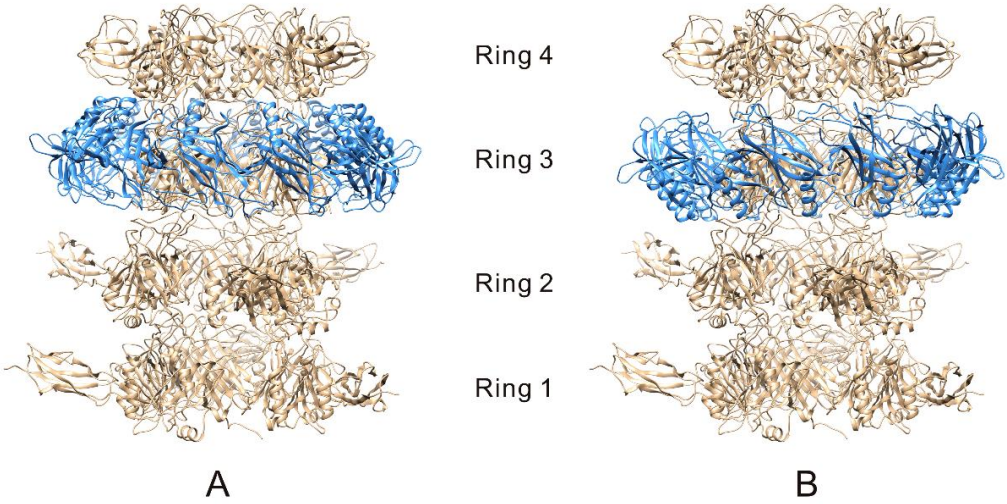


Figure 1. Two different binding modes of T170 are predicted by the *ab-initio* docking of CoDock. The hetero 15-mer is colored brown and the homo 12-mer is colored blue.

References:
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## III. Reconstructing Protein Complex Structure from Predicted Inter-Chain Contacts in CAPRI-Round50

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Our MULTICOM-AI protein complex structure predictor uses ab initio deep learning-based interchain contact prediction tool (DNCON2_Inter[1]) as well as a template-based prediction method **(**TBP**)** to predict interchain contacts for complex targets in CASP14 and CAPRI50. The predicted inter-chain contacts are then used to create the quaternary structure of the target by a custom distance-geometry protocol based on Crystallography & NMR System (CNS)[2].
**Availability:** https://github.com/jianlin-cheng/DNCON2_Inter

**Methods**
The individual sequences and predicted ("known") tertiary structures of the subunits of a target protein complex are given to the MULTICOM-AI system as input. Its prediction workflow is illustrated in **Figure 1.**

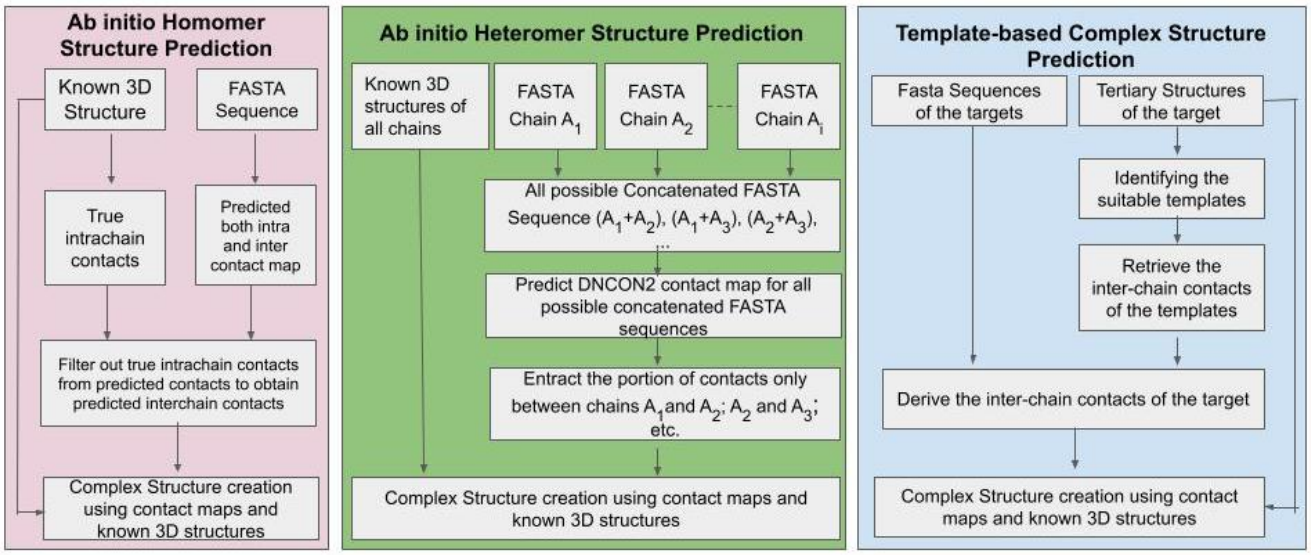


**Figure 1**: The workflow of the MULTICOM-AI complex structure prediction system. The box on the left illustrates the deep learning prediction of inter-chain contacts for homodimers, the box in the middle the deep learning prediction of inter-chain contacts for heterodimers, and the box on the right the template-based prediction of inter-chain contacts. The predicted contacts are used to generate the quaternary structure of a dimer. Interchain residue pairs are considered interchain contacts if the Euclidean distance between the two closest heavy atoms of the two residues is $\leq 6.0$ Å[1,4].

*Interchain Contact Prediction*. The pairs of chains in a complex are treated as dimers - homodimer if two chains are identical and heterodimer otherwise. Since the multiple sequence alignment (MSA) of a monomer in a homodimer is essentially the same as that of the homodimer itself, the deep learning-based contact predictor (DNCON2_Inter) using the MSA of a monomer in a homodimer as input predicts both interchain and intrachain contacts[3]. The intra-chain contacts are then filtered out according to the "known" tertiary structure of the monomer to keep interchain contacts only (pink box in Figure 1). The tertiary structure is predicted by MULTICOM[4]. The process for heterodimer contact prediction is slightly different (green box in Figure 1). We concatenate the individual sequences of the two chains and generate MSA for heterodimers by searching the combined sequence against a custom database of concatenated sequences of interacting dimers derived from the Protein Data Bank (PDB). It is then fed to our deep learning predictor to generate the interchain contact map. For TBP (blue box in Figure 1), we search the tertiary structures of the protein chains against the custom dimer structure database to find complex templates and then extract the interchain contacts from them. The tertiary structures of individual chains and the predicted interchain contact maps are then fed to the complex structure construction system based on CNS to generate the final structures of the complex as follows.

*Inter-Chain Contact-Guided Complex Structure Generation.* The method is implemented on top of the distance geometry protocol of CNS, which uses a stochastic simulated annealing method to build the complex structure of protein dimers, leveraging predicted interchain protein contacts and tertiary structures of individual protein chains. It can build complex structures consisting of two or more protein chains, keeping the individual protein chains unchanged and trying to satisfy inter-chain contacts as well as possible. It generates 100 models, which are then sorted in the ascending order of the distance-restrain energy. The top 5 models with minimum energy are selected.

**Results**

Table 1 shows the precision of the inter-chain contact prediction for a good example - Target T164. It is a homodimer, where each chain contains 284 residues. Figures 2 (A) and (B) depict the contact map predicted by DNCON2_Inter and TBP in comparison with the true contact map, respectively.

**Table 1**: The precision of top k contact predictions by DNCON2_Inter and TBP for T164, where k = {5, 10, L/10, …, 2L} and L: length of protein sequence.

| Precision (%) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Method | Top 5 | Top 10 | Top L/10 | Top L/5 | Top L/2 | Top L | Top 2L |
| Dncon2_Inter | 100 | 100 | 100 | 100 | 52.8 | 26.4 | 13.2 |
| TBP | 100 | 100 | 100 | 100 | 100 | 54.6 | 27.3 |

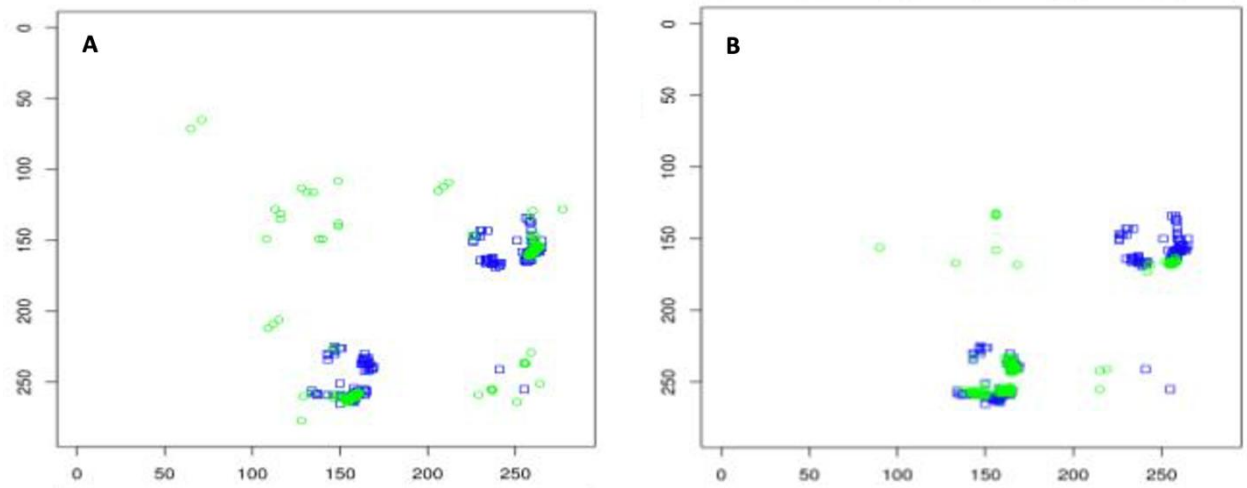


**Figure 2**: **(A)** Inter-chain contacts predicted by DNCON2_Inter (green) versus true contacts (blue); **(B)** Inter-chain contacts predicted by TBP (green) versus true contacts (blue).

**Acknowledgements**

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## IV. Extended Abstract: UNRES group

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We participated in CAPRI Round 50 as three groups: UNRES (group 55; 22 predictions), UNRES-contact (group 53; 12 predictions), and UNRES-template (group 04; 22 predictions), respectively, all of which used the latest version of the coarse-grained UNRES force field [1]. The UNRES group used plain UNRES force field with only weak restraints on secondary structure [2] obtained from PSIPRED, the UNRES-contact group used contact prediction accomplished by using the DNCON2 method [3] or extracted from server models, while the UNRES-template group used distance- and local-structure restraints extracted from server models, as described previously [4]. The restraints were imposed only on monomers. The UNRES software was modified to handle large oligomeric targets.

**The protocol for oligomer-structure prediction.** Initial multimeric models were built from monomer structures. The monomers were, in turn, modeled, within the CASP14 experiment, by using our hierarchical protocol, in which restrained MREMD (Multiplexed Replica Exchange Molecular Dynamics) [5] simulations with the coarse-grained UNRES force field [1] were carried out. Simulations of monomers were started from multiple server models.

Targets 177 (CASP14 target H1081) and 180 (CASP14 target T1099) were special cases. The initial 20-mer of target 177 was obtained as two stacked rings of the experimental decamer (PDB: 3n75), rotated with respect to each other structure to avoid overlaps. The initial structure of target 180 was built using symmetry constraints from the i-TASSER model of monomer selected to avoid overlaps, for which purpose the C-terminal part of each oligomer had to be regenerated, subject to symmetry constraints. Whole structure of this target was simulated, while the minimal asymmetric unit was submitted.

When good oligomer templates were available (as found by HHpred [6]), the initial structures of oligomers were modeled on template scaffold, while in other cases the monomers were assembled into the initial oligomer structures by using the random-oligomer-positioning algorithm of UNRES-dock [7]. For each target, multiple oligomer structures were constructed. The starting structures were subjected to restrained MREMD simulations with the UNRES force field [1], as described previously [2,4]. Subsequently, simulation results were processed by using the Weighted Histogram Analysis Method (WHAM) [8] and cluster analysis to extract 10 families of conformations [2,4]. The families are ranked based on their free energies (each computed over the entire conformational sub-ensemble constituting a family [1]). Coarse-grained structures which were closest to the cluster centers were subsequently converted to all-atom representation by using the PULCHRA [9] and SCWRL [10] knowledge-based algorithms, and subjected to final refinement at the all-atom level with the AMBER ff14SB force field [11].

**Results**

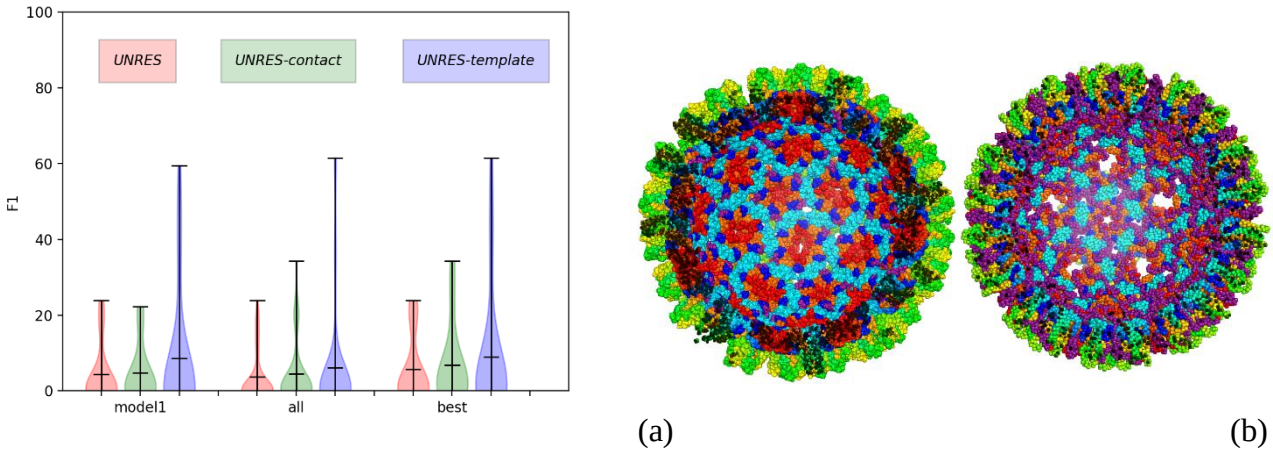


**Figure 1.** (a) Violin plots of the distributions of the F1 measure of fitting the modeled structures to the experimental structures of the targets treated by the three UNRES-based groups. (b) The experimental 2vgh structure of the duck hepatitis virus capsid (target 180) (left) and the structure modeled by UNRES (right).

As shown from Figure 1a, including knowledge-based restraints on monomers results in much better quality of the best models; however its influence on the average F1 value is less significant.

In our experience [4], round 50 was more difficult compared to the previous rounds of CAPRI because of lesser availability of good templates for the oligomers and because of significantly greater size of the targets.

## Acknowledgments

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# V. INCORPORATING FLEXIBILITY BY SOFT DOCKING IN MIAX

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***Introduction:***

Incorporating flexibility in protein docking algorithms has become a key issue in computational methodologies oriented to solve the protein-protein interaction (PPI) prediction problem in molecular biology. Backbone rearrangement and, to a larger extent, amino acid side chain rearrangement shape the interacting subunits at the interface of the complex, post-docking re-modeling of the interfaces being required when rigid docking algorithms are applied to solve the PPI problem for unbound subunits. The difficulty arises in predicting and scoring high the best decoys (closest to the native structure), the interfaces of which may undergo extensive rearrangement at interaction. Here we propose a genuine methodology to undertake this problem that consists in an a-priori treatment of the molecules to dock that combines a softening technique of the surface of the molecules, and the utilization of graph morphological operations to deal with protein flexibility resulting in a new concept of "soft docking" that mainly targets the docking of unbound protein molecules (reported at the isolated state).

The methodology is embeded as an independent module into our system for macromolecular interaction assessment system MIAX (Macromolecular Interaction Assessment Computer System)[1,2,3], that has been in continuous development for the last decade. Results of applying the newly proposed soft docking technique to the set of benchmark protein complexes reported in the literature illustrate its ability to generate close-to-native decoys even before re-modeling the interaction interfaces.

***Methodology:***

The key strategy is the mapping of a characteristic shape from the unbound conformation for the protein by applying a three dimensional filter to the function representing the surface. The high flexibility of the amino acids on the molecular surface can be expressed introducing in this way "softness" in the rigid docking algorithm, allowing some unrealistic penetrations among the interacting proteins that may lead to better prediction of the complex configuration. Moreover, the elimination of the atomistic detail of the molecular surface by replacing it with a smoother function enables the treatment of the protein as a three dimensional graph, to which morphological operations can be applied in order to accentuate or smooth protuberances or groofs on the surface that may play the main roles in the interaction process. This allows the manipulation of flexible docking in a way mimicking induced fit in PPI.

***Results and Discussion:***

If only the geometrical features of the interacting monomers are used to dock the structures, the system performs well for a set of difficult docking problems. We show, thus, that a priori knowledge on the binding sites or the automatic identification of binding regions on the surfaces of the interacting macromolecules can substantially improve the ranking of the decoys output by the rigid body docking of the unbound proteins. Moreover, a refinement of the structures can be performed to deal with the most flexible chains in the interacting interface of the best decoys. The optimization process would be confined to a relatively small section of the conformational space as we have shown in previous reports about flexible docking; the algorithm constitutes therefore a powerful tool to predict those starting conformations. Nonetheless, since more and more structures resulting from large scale genome sequencing projects may have to be modeled rather than determined by experimental methods, containing therefore several significant structural errors, methodologies like the one presented here may probe of great assistance in structure-based functional studies requiring

computational techniques with the capacity of docking large number of protein models within acceptable limits of accuracy as our methodology does before the refinement process by flexible docking. Indeed, the methodology proposed here was validated with monomers that in several cases are not 100% similar in amino acid sequence, the effectiveness of the methodology having been proven and the results are described in a paper under publication.

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## VI. Rosetta docking strategies in the CASP14-CAPRI experiment

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### Introduction

Performance of docking methods in prior CAPRI challenges have revealed that the intrinsic flexibility of proteins still hampers the accuracy of docking predictions[1]. In this edition of CASP14-CAPRI, we expanded and evaluated our docking methods in Rosetta to tackle the binding-induced conformational flexibility in protein-protein docking. To model the targets in this round, we employed our recent progress in RosettaDock[2] and SymDock[3]; along with our upcoming replica-exchange method, ReplicaDock 2.0 (built upon work from Zhang, Schindler, Lange and Zacharias[4,5]), that utilizes induced-fit with docking.

### Model Curation

From the given amino acid sequence, we used the BLAST program to identify homologous proteins. Based on the availability of a suitable template, we determined the three-dimensional structure of a target or the monomer with MODELLER[6]. If no template was available, we built models using the Robetta web server. For antibody targets such as T165, modeling the complementarity-determining region is challenging as it is less conserved. In order to improve our predictions with antibody targets, we employed RosettaAntibody[7] and DeepH3[8] developed by our group.

### Docking Methods

*Rigid-docking for identification of binding sites:* To obtain putative binding sites, we performed ab-initio docking using rigid-body Fast Fourier Transform (FFT) based docking using the ClusPro[9] web server. We also performed rigid-body global sampling using our ReplicaDock protocol and clustered the lowest energy decoys. As both of these tools generate multiple bound structures, we compared the ligand root mean square deviations (L_rmsd) and chose those poses that had the ligand in same relative neighborhood for local docking. By using this pipeline, we were able to narrow down the global search into a local search, where it was feasible to sample conformational changes near putative binding regions.

*RosettaDock 4.0:* Our recent work on protein docking uses a conformer-selection based Monte Carlo Minimization (MCM) approach. Starting from a putative binding region obtained

post global search, we dock the targets using our RosettaDock 4.0 protocol that adaptively swaps receptor and ligand conformers from a pre-generated ensemble of structures in the coarse-grained stage followed by an all-atom refinement in the high-resolution stage. To diversify the backbone conformations in the ensemble, we generate structures using Rosetta Relax, backbone flexing with Backrub[10] and normal-modes. Further, we use an updated coarse-grained energy function i.e. Motif Dock Score (MDS)[2] obtained from 6-dimensional residue pair data to efficiently discriminate non-native decoys from native ones.

*ReplicaDock 2.0:* We recently developed a new, aggressive conformational sampling methodology incorporating temperature and Hamiltonian-replica exchange Monte Carlo (T-REMC[4] and H-REMC[5]) techniques coupled with the induced-fit (IF) mechanism of protein binding. By capturing backbone motions of putative interface residues on-the-fly, we mimic the partner-specific moves of IF approaches within our docking protocol. This approach communicates between multiple replicas with different energy functions and temperatures to better explore the backbone conformational space. For this CASP14-CAPRI round, we first performed a global docking with the replica exchange protocol restricting motion to only rigid body moves to sample the protein energy landscape. Upon narrowing down putative local binding sites, we performed replica exchange docking with IF-backbone motion. We initiated 8 trajectories of the docking simulation, each trajectory spanning over 3 temperature replicas, run for 2.5 x $10^5$ MC steps. With inverse temperatures set to $\beta$, of $1.5^{-1}$ $kcal^{-1}$.mol, $3^{-1}$ $kcal^{-1}$.mol and $5^{-1}$ $kcal^{-1}$.mol, replica exchange swaps are attempted every 1000 MC steps generating 6,000 decoys at a local binding site.

*SymDock 2.0:* To navigate the challenges pertaining to targets with point symmetries, we utilize the symmetry framework in our docking protocol SymDock2[3]. We dock monomer chains related by a symmetry-axis. The use of a new scoring function (MDS) and an all-atom backbone flexibility in the high-resolution stage allows us to obtain tighter interface packing for symmetric targets, and in turn improve the quality of our predictions. This is reflected in our medium quality targets obtained for target T164, a symmetric homo-dimer with C2 symmetry.

**Successes and Failures**

We participated in 2 targets of the CASP14-CAPRI blind prediction experiment. For target T164 (Fig. 1), we were one of the only two groups that generated medium-quality predictions (and multiple acceptable-quality predictions). This was particularly interesting as we fused the SymDock2 ensembles with monomer chains obtained from the ReplicaDock2 IF-based docking approach. The method of induced-fit outperformed other methods, perhaps by capturing putative native-like conformations in the presence of the partner. For target T165 (Fig. 2) which involved a glycoprotein-antibody complex with C3 symmetry, like all other groups we were unable to obtain acceptable predictions. The failure in modeling target T165 stems from the limitations in our ability to accurately model the H3 loop of the antibody.

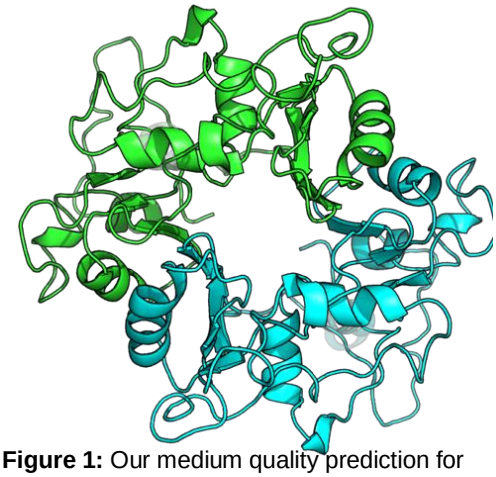

**Figure 1:** Our medium quality prediction for T164 (human predictor group). This model had an i-RMSD under 2 Å. Only two CAPRI human participants achieved medium quality predictions.

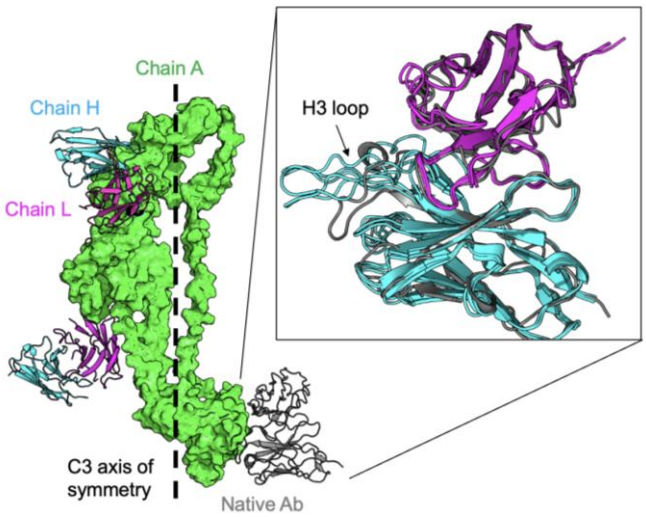


**Figure 2:** Our top models for Target T165, superimposed over the native. In box, the H-L chains of the Ab superimposed over the Ab crystal structure (grey), showing the challenge of modeling H3 loops.

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# VII. HADDOCK scoring of CAPRI Round50 models.

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**Scoring methodology**

The HADDOCK team only participated as scorers in this CASP/CAPRI round. The scoring was purely based on energetics considerations, not making use of any experimental and/or bioinformatics information such as conservation or co-evolution. In this section, we describe the followed scoring pipeline, consisting of the following steps:

**1) *Pre-processing of models*.** This initial step consists of identifying possible problematic models (e.g., cases with missing chains, missing atoms, etc) and correcting for amino-acid nomenclature (e.g., renaming HSD/HSE, AMBER nomenclature, to HIS).

**2) *Short energy minimisation*.** Each model is subjected to a short energy minimisation using HADDOCK2.4[1] (https://www.bonvinlab.org/software/haddock2.4). For each model, any missing atoms (but not missing segments) are built, the protonation state of histidines is automatically set by comparing the electrostatic energies of the various histidine states (charged or neutral with the proton attached to either the N or E nitrogen atom on the ring) and disulphide bridges are automatically detected based on a distance cut-off. Once all missing atoms have been built, the model is subjected to 50 steps of energy minimisation with the OPLS forcefield[2] with a non-bonded cutoff of 8.5Å.

**3) *Scoring***. The energy minimized models generated by the last step are scored with the simple HADDOCK scoring function (HS)[3], which is a linear weighted sum of energetic and structural terms:

$$HS_{it} = 1.0\, E_{vd}^{w} + 0.2\, E_{ele}^{w} + 1.0\, E_{deso}^{w}$$

where $E_{vd}^{w}$, $E_{ele}^{w}$ and $E_{deso}^{w}$ stand for van der Waals, Coulomb electrostatics and desolvation energies, respectively. The non-bonded components of the score ($E_{vd}^{c}$, $E_{ele}^{c}$) were calculated with the OPLS forcefield[2]. The desolvation energy is a solvent accessible surface area-dependent empirical term[4], which estimates the energetic gain or penalty of burying specific sidechains upon complex formation.

**4) *Clustering*.** Despite the possible heterogeneity in model numbering, we perform a clustering of all models based on the fraction of common contacts [5] using a 0.65 cutoff. Two different clusterings are performed requiring a minimum of 4 and 2 models per cluster, respectively.

**5) *Cluster-based scoring*.** For each cluster we calculate an average score based on the top 2 or top 4 models. Clusters are then ranked based on their average HADDOCK score.

**6) *Model selection*.** Depending on the degree of clustering, the final selection of models is based either on cluster statistics obtained with a minimum of 2 or 4 models per cluster. A short visual inspection of the cluster is done to exclude "suspect models". Those are often models scoring much better than any other models, which upon visual inspection appear to be unrealistic models with highly intertwined chains and optimized energetics. Although the origin of the models is unknown to us, we venture to speculate that those most likely originate from fold and dock approaches. The final submission consists of 1 model per cluster for the top5 re-scored set, and depending on the scoring statistic, more models from the top cluster might be included in positions 6 to 10, or models from new clusters are included.

**Scoring performance**

Table 1 summarises our scoring results for the 12 targets that were assessed, for a total of 23 different interfaces (with T170, T177 and T180 assessed as nine, three and two different interfaces respectively). Overall, we got acceptable or better models for 11 of the 23 interfaces (48%) in the top 10 predictions, which drops to 8 (35%) and 7 (30%) for the top5 and top1, respectively. Note that in our scoring procedures all interfaces are considered simultaneously, and we do not score per interface.

We generally observe that our short minimisation strategy is unable to refine strongly clashing models which end up with very high positive scores. Such models often originate from rigid body docking approaches and might well contain good predictions when it comes to the backbone orientations. Due to their potential high clash content at the interfaces, those models are however severely penalized by our energy-based scoring procedure. Further, comparing our results with others, while we do identify acceptable models for close to 50% of the interfaces in the top10 (the best groups reach ~64% in this round), there is room for improvement since our top5 and top1 performance is dropping. A more aggressive refinement might be needed to further remove clashes and improve the scoring of models together potentially with a re-optimisation of our scoring function.

**Table1** – HADDOCK scoring performance per interface. The stars indicate the quality of the selected models and the number before those the number of models of a given quality (*: acceptable; **: medium; ***: high) based on CAPRI criteria.

| TARGET/INTERFACE | TOP1 | TOP5 | TOP10 |
|---|---|---|---|
| **T164** | **1*** | **5*** | **9*** |
| **T165** | **-** | **-** | **-** |
| **T166** | **-** | **-** | **1**** |
| **T168** | **1**** | **5**** | **10**** |

| | | | |
|---|---|---|---|
| **T169** | **-** | **-** | **-** |
| **T170/1** | **1*** | **2*** | **5*** |
| **T170/2-7** | **-** | **-** | **-** |
| **T170/8** | **1*** | **4*** | **7*** |
| **T170/9** | **-** | **-** | **1*** |
| **T174** | **-** | **-** | **-** |
| **T176** | **-** | **-** | **-** |
| **T177/1** | **1***** | **5***** | **10***** |
| **T177/2** | **1***** | **5***** | **10***** |
| **T177/3** | **1*** | **2*/1**** | **2*/2**** |
| **T178** | **-** | **-** | **2*** |
| **T179** | **-** | **2*** | **3*** |
| **T180/1** | **-** | **-** | **-** |
| **T180/2** | **-** | **-** | **-** |
| **OVERALL** | **4*/1**/2***** | **4*/3**/2***** | **6*/3**/2***** |

**Funding**

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**Conflict of interest**

Dr. A.M.J.J. Bonvin is a member of the CAPRI management committee but has no access to target information.

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# VIII. Hybrid ClusPro approach in 2020 CASP-CAPRI round: template-assisted docking and docking of protein models

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In the latest joint CASP-CAPRI assembly round our group used two modeling methods, one of them being a template-based method recently updated and implemented as a fully automated public server ClusPro TBM[1,2] (tbm.cluspro.org) and the other being the original ClusPro[3–6] server (cluspro.bu.edu) that performs rigid-body docking. Here we briefly describe the basic features of these protocols and present some of the applications and performance highlights.

## Methods

### Model Preparation

Given the sequences and expected stoichiometry of the target protein complex, we search for available templates in the pdb100 database using HHsearch[7] and identify those that contain homologs of the interacting biological unit to be predicted (it is also possible to use manually pre-selected templates as input). If no template of the complex is found, we perform free docking using ClusPro. Since free docking requires three-dimensional structures as input, we use the HHpred[8,9] top template to build a homology model of the subunits using Modeller[10,11]. Unaligned regions of the target sequence are removed to avoid the addition of unstructured loops and tails into the model, while aligned portions of the target are built with fixed backbone atoms. In difficult cases, we build an “ab initio” model of the subunit using TrRosetta[12].

### Template-based docking

Once potential templates containing homologs for all subunits have been identified, the method performs a stoichiometry check, testing whether the template can accommodate the required number of copies of each subunit type. This includes some nontrivial cases like the use of homomeric templates for heteromeric targets or the use of single-chain templates for multimers. If a template of the biological complex satisfying the required stoichiometry is found then we choose the best template for each unique subunit of the complex, align multiple copies of this subunit template to the complex template and then model the whole complex of full-sequence chains using Modeller. In order to diversify the pool of models, we also used the CASP server models of the subunits by aligning those onto our initial template-based models and minimizing the resulting structures. To remove structural redundancy in the final set of resulting assembly models we calculate Cα RMSD

between all pairs of models taking into account complex stoichiometry and symmetry (there might be several ways to align the chains of one model onto the other, the minimal RMSD is calculated) and perform greedy clustering at 10 Å radius. Finally, we report the centers of up to 10 largest clusters ranked by the cluster size.

**Free Docking**

Our free docking protocol starts from running PIPER[13] - a global rigid-body docking program that performs a systematic search of protein complex conformations on a grid using the fast Fourier transform (FFT). It represents the energy score as a sum of correlation terms that includes vdW interaction energy, electrostatic energy, and desolvation energy contributions calculated by a DARS[14] structure-based statistical pairwise potential. The docking run can be additionally informed by XL-MS cross-links[15] and SAXS data[16,17] that helps to guide and restrain the search. The top 1000 lowest energy docked complex conformations generated by PIPER are used to compute a matrix of pairwise ligand interface Cα RMSD values. Namely, for each docked conformation, we select ligand residues with at least one atom within 10 Å of receptor and calculate Cα RMSD for these residues against the remaining 999 ligand poses. Using this not necessarily symmetric matrix as a distance measure we perform greedy clustering of the poses with a clustering radius of 9 Å. After removing potential side chain clashes by fixed backbone minimization of CHARMM[18] vdW energy, we report the centers of the 10 largest clusters as predictions. They are ranked by the cluster size.

**Results**

**Performance highlights**

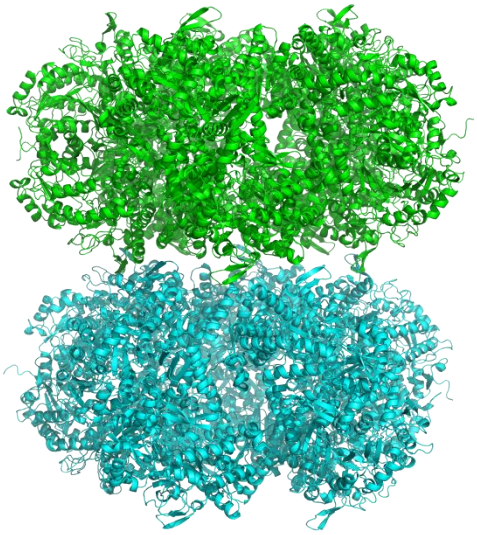

**Figure 1.** A model of CASP-CAPRI target H1081/T177 generated by a combined TBM/free docking approach. The two identical partial assemblies of A10 stoichiometry modeled with ClusProTBM (shown in green and cyan) were docked to each other using free docking ClusPro capabilities to produce a near-native model.

As mentioned above, in CAPRI/CASP14 we used both template-based and free modeling approaches. A particularly interesting case in which the two methodologies needed to be combined was CASP-CAPRI target H1081/T177, representing a homomultimer of A20 stoichiometry. Here, we found no reasonable template for the whole assembly, and thus a

straightforward TBM approach was not a way forward. However, templates for partial (A10) assembly were abundantly available.

We, therefore, modeled an A10 subcomponent of the target using ClusproTBM based on available templates and then docked two copies of this partial model using the free docking functionality of the ClusPro server. This approach thus represented a synthesis of the two methodologies and resulted in a near-native model of the full target assembly as a result (see Fig. 1).

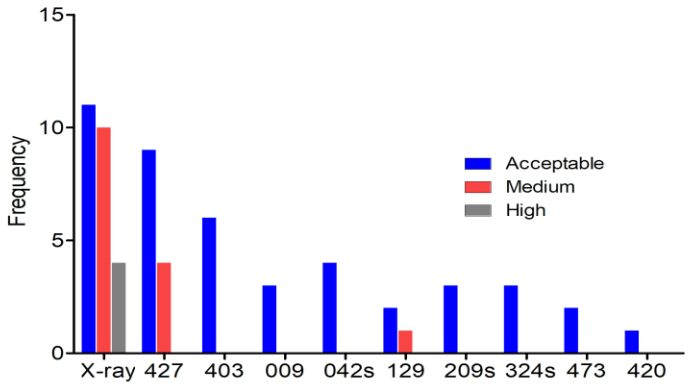


**Figure 2.** Comparison of free docking results for a set of CASP14 multimeric cases using models generated by top CASP14 predictors as inputs. For each group, the number of acceptable or better predictions in top-5 is given. Docking results obtained by re-docking the subunits taken from the X-ray structure of the complex are provided as a baseline.

Another observation we make in CASP/CASP14 is how the improvement in the quality of folding models leads to a dramatic improvement in the quality of free docking results. When using subunit models produced by the top-performing CASP14 groups as inputs to free docking, the number of modeled interfaces with acceptable or better quality (as estimated by DockQ[19]) is comparable to those obtained by re-docking subunits taken from the X-ray structures. Fig. 2 provides the summary of docking results for a set of CASP14 multimeric targets.

## Encountered difficulties

Despite the progress in the development of scoring functions, template-based modeling of protein assemblies generally outperforms free docking when good templates are available. However, working with some of the CASP-CAPRI targets demonstrated that even in presence of good but remotely homologous templates, modeling quality might be significantly affected by the quality of sequence alignment, especially in key regions such as complex interfaces.

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# IX. Fernandez-Recio's group performance in CASP14 / CAPRI round 50

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We have participated as human predictors, human scorers, and server scorers, in all the 12 evaluated targets, comprising a total of 14 assessed interfaces. We applied a similar strategy to that in the CASP13-CAPRI experiment, combining *ab initio* docking, template-based modeling, and energy-based scoring [1].

## Methods

The models of the individual subunits were taken from the best predictions of ZHANG, RaptorX, and QUARK CASP-hosted servers, except for target T170, which had available structures for two of the subunits (see Results).

We applied our pyDock [2] docking pipeline to the models of the individual subunits, in order to build the binary interactions in each assembly. In homo-oligomers, only docking poses satisfying the expected symmetry (e.g. cyclic $C_2$ symmetry for homo-dimers; $C_3$ for homo-trimers) were selected. For that, rotation angles and translation distances along the

symmetry axis between two docked subunits were calculated by ICM-Browser (www.molsoft.com) as previously described [3]. A given docking pair was defined as $C_2$ or $C_3$ symmetry when rotation angle was $180^o \pm 5^o$ or $120^o \pm 1^o$, respectively, and translation along symmetry axis < 5 Å.

Additionally, we searched for available templates for the assembly interfaces, using BLAST as well as the top five released predictions from the ZHANG, QUARK, RaptorX, MULTICOM-CONSTRUCT and ROSETTA CASP-hosted servers. The models for the individual subunits were superimposed on each template (template-based docking) and minimized with AMBER 12. In targets T165 and T177, some interfaces were modelled with MODELLERv9.19 (template-based modeling) because there were not available models at the CASP-hosted servers (see Results).

Finally, all the (*ab initio* or template-based) modelled interfaces were scored with pyDock. The number of available templates and their reliability determined the proportion of template-based complex models included in the set of submitted models. We eliminated redundant predictions and minimized the top ten submitted models.

In the scorers experiment, we first removed models with more than 250 clashes (i.e., intermolecular pairs of atoms closer than 3 Å). Then, we scored the models with pyDock (or pyDockWEB [4] as servers) and applied the same additional criteria as in predictors (i.e. in case of reliable templates we favored models similar to such templates, we checked for symmetry, we applied *ad-hoc* distance restraints for specific targets, etc., more details in the Results section). As human scorers we introduced more manual intervention than as server scorers, i.e., removing loops with non-realistic conformations, and re-scoring some of these models afterwards.

**Results**

In targets for which we could not find available templates (T169, T174, T178, T179), we applied our automatic pyDock docking and scoring pipeline as predictors, and the built-in scoring function of pyDock or pyDockWEB as human or server scorers, respectively, selecting only symmetric orientations ($C_2$ in T169, T178 and T179; $C_3$ in T174). We got acceptable results in two of these difficult targets, T178 (as predictors) and T179 (both as human and as server scorers). For the two failing targets, no other participant group was successful.

For the rest of targets, we could find potentially suitable templates for all or some of the predicted interfaces. In most of these cases, we generated models by *ab initio* docking and by template-based modeling independently, and the final proportion of models derived from these two approaches was determined by pyDock scoring and/or by the reliability of the available templates. Thus, *ab initio* docking was favored in target T176, with unsuccessful results (indeed, this was a difficult case, with few successful participants). In target T164, we also favoured *ab initio* docking, since we incorrectly focused on the potential dimerization of the helices. As a consequence, we had unsuccessful results as predictors, but got acceptable models as human and server scores. On the other side, template-based modeling was favored in targets T166, and T168, for which we got medium-quality models as predictors, human scorers and server scorers. Finally, only template-based modeling was applied in target T180, consisting in the assembly of a virus capsid with icosahedral symmetry. We modelled a homo-tetramer, as the minimal number of subunits necessary to define the unique interfaces, and got acceptable results (averaged over the two evaluated interfaces) as predictors, human scorers and server scorers (together with Venclovas, our server scorers were the only acceptable rank 1 submissions of all participants for this target).

In the most challenging multi-molecular targets, in order to build the full assembly we combined template-based docking for some interfaces and *ab initio* docking for the other ones. This is the case of the homo-20mer T177, in which the homo-decamer was modelled with MODELLERv9.19 based on available templates, followed by pyDock scoring, and the final assembly was built by docking two decamers. For this target, we obtained medium-quality results (averaged over the 3 interfaces) as predictors, human scorers and server scorers. In the hetero-nonameric target T165, the homo-trimeric glycoprotein was modelled by template-based docking (i.e. superimposing the subunit models from the CASP-hosted servers on the available templates), and the hetero-dimeric antibody was modelled with MODELLERv9.19 because there were not available models at the CASP-hosted servers. Then, they were docked to form the evaluated hetero-trimeric interface. However, we got unsuccessful results, as the rest of participants.

In the same line, target T170 was a challenging hetero-27mer, in which we applied an *ad-hoc* modeling procedure, also combining *ab initio* docking and template-based modeling. This assembly was formed by three rings with different composition and stoichiometry. The first ring was a homo-hexamer arranged as a dimer of trimers (A3:A3) and was modelled by manually fitting (Figure 1) the monomeric x-ray structure (PDB 5NGJ, chain A) into the available cryo-EM map of bacteriophage T5 tail (EMDB ID: 3689) [5] (trimers built by *ab initio* docking did not fit well into the cryo-EM map). In the final hexameric ring, residues 3-5 and 216-220 were removed due to steric clashes between rings. Interestingly, in the evaluated interface #2 (A3:A3) we submitted the only acceptable model among all participants, as predictors. The second ring was formed by three subunits of one protein and twelve subunits of a second protein (B3:C12) and was modelled by a combination of *ab initio* docking, symmetry restraints, and template-based docking. The third homo-hexameric ring (D6) was modelled by superimposing the x-ray structure of the monomer (PDB 4JMQ) on available templates (PDB 4DIV and 2X8K), followed by minimization and pyDock scoring (Figure 1). The final assembly of the modelled rings was done by *ab initio* docking, selecting only models in which the symmetry axes of the rings were aligned. The same criteria was used in the scorers experiment. The different interfaces in this target were evaluated in three separate groups. In the average evaluation over interfaces #1 (A3), #2 (A3:A3), #3 (B3) and #4 (A3:B3), we got unsuccessful results (despite having acceptable models for interfaces #1 and #2 as predictors). In the averaged evaluation over interfaces #5 (C12), #6 (B3:C12) and #7 (B3:C12), we also got unsuccessful results. However, in the averaged evaluation for interfaces 8 (D6) and 9 (B3:D6), we obtained acceptable results as human and server scorers (together with Venclovas, these were the only acceptable rank 1 submissions of all participants).

In summary, our results for the modeling of multi-meric assemblies in CASP14 were in line with those in the past CASP13-CAPRI edition. Interestingly, our performance as human predictors, human scorers, and human servers has been quite consistent, and in the majority of cases our successful predictions were achieved with our rank 1 submissions, which is an improvement with respect to past editions. However, in the interfaces without suitable template or experimental information we had significantly worse predictions, which shows that *ab initio* docking of multi-meric assemblies is far from being solved.

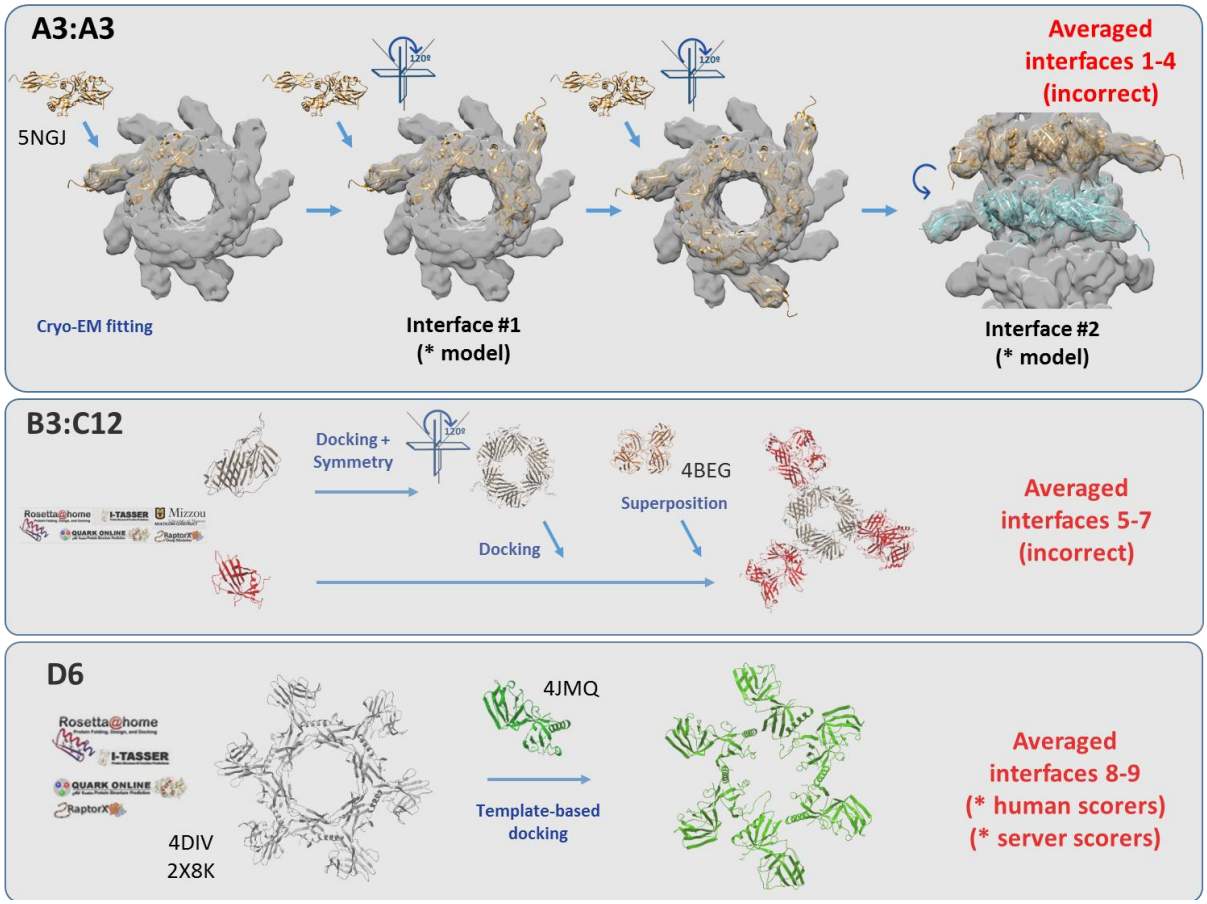


**Figure 1.** Modeling strategies for the three major rings in target T170.

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## X. Quaternary structure prediction using a combination of physics-based approaches with machine learning

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In the CAPRI round 50, we combined rigid-body free docking with several quality assessment (QA) methods developed in our lab. We extensively used the symmetry assembler SAM[4] and the binary docking method Hex[5]. Some targets also motivated us to develop novel methods. For example, we extended SAM[4] symmetry assembler for helical symmetries, we introduced new options into the symmetry analyzer AnAnaS[10,11], we developed a novel rigid-body replica-exchange Markov-chain Monte Carlo simulation technique, we introduced symmetry constraints into the interactive docking engine[12], and more.

**Methods:**

**Prediction round:**

Firstly, we selected best-scored CASP14 stage-2 server predictions according to QA methods developed in our lab: VoroCNN[1], VoroCNN-sh[8], Ornate[2], and SBROD[3]. Next, we used them as initial monomeric models for molecular docking. For each target, we used around 40 initial monomeric models. For the homo-oligomeric targets, we ran SAM[4] on each model, specifying the desired symmetry. For the hetero-dimers, we performed cross-docking using the Hex program. We generated from 5,000 to 20,000 docking poses, depending on the size of the target, and then we scored them using our QA methods. Finally, we submitted the best assembly predictions based on the competition rank. More precisely, for each of the models, we computed *weighted interface scores* as $S_{interface} = (S_{AB}*N_{AB} - S_A*N_A - S_B*N_B) / N_{AB}$, where $S_{AB}$ is a QA score of the multimeric model, $N_{AB}$ is the number of residues in the multimeric model, $S_A$ and $S_B$ are the individual QA scores of the assembly components A and B, and $N_A$ and $N_B$ are the corresponding numbers of residues. We then ranked all the interfaces by the standard competition ranking. We repeated this procedure for each QA method. The 100 assembly models with the highest number of summed up ranking points were submitted to CAPRI as our predictions.

**Scoring round:**

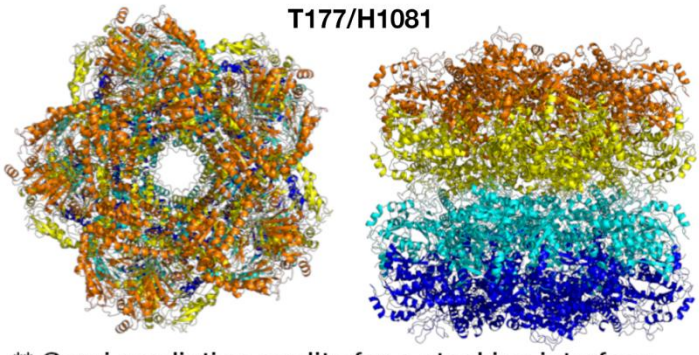
For the scoring round, we calculated scores using our QA methods for each provided complex model and its subunits. Then, we computed the interface score for each model and rank them according to the standard competition ranking described above.

**Performance:**

**Predictions:** We participated in 13 prediction targets (all except T168). According to results presented at the CASP14 meeting, our method correctly predicted structures for targets: T177 (Interface 3, **) and T170 (Interfaces: Z:Z and D:D both *). Final models for both targets were selected based on either Hex or KSENIA[9] scores, which were specifically developed for scoring oligomeric structures.

**Scoring:** We participated in all 14 scoring targets and our general performance is as follows: 1* hit per Top-1 model, 5*/1** hits per Top-5 models, and 5*/1** hits per Top-10 models. To score the models, we combined our 4 QA methods. All of them were initially designed for scoring monomeric models only. Therefore, the interface scores in the assemblies can be incorrect and noisy.

**Difficulties encountered:**



** Capri prediction quality for a stacking interface

For several targets, we did not follow our standard protocol because of their specificity. For the T165/H1036 target with the stoichiometry $(ABC)_3$, firstly we ran our trimeric docking algorithm DockTrina (a protein docking method for modeling the 3D structures of nonsymmetrical triangular trimers)[13] for the trimer ABC. Initial models for subunit A were taken from the CASP's T1036s1 stage-2 server predictions. Subunits B and C were modeled with iTASSER[6] v.5.1. Then, we applied SAM using the C3 symmetry on top of the obtained DockTrina predictions to generate the required stoichiometry.

The T190/H1099 target was modeled using an exhaustive scan of all 150 CASP14 stage-2 server submissions using SAM for the 2-fold and 3-fold symmetry axes in the asymmetric subunit. Then, the predictions were supplemented with 60 icosahedral symmetry operators between the asymmetric subunits, and a local optimization with the KSENIA potential[9], sidechain repacking, and an interactive in-house docking application applied[12]. We ranked the predictions according to the KSENIA scores.

We modeled the T170/H1060 and T177/H1081 targets starting from the general protocol for the homo-oligomers. To generate a monomeric subunit of H1081, we used Swiss-Model[7] with 2VYC as a template. We used experimental structures for subunits in rings A and D. We applied D5 symmetry to H1081, C3 symmetry to the A and B subunits of H1060, C12 symmetry to the C subunit of T170/H1060, and C6 symmetry to the D subunit of T170/H1060. We stacked the two D5 dimers of T177/H1081 and the rings A and B of T170/H1060 using SAM extended to helical symmetries. The other rings in T170/H1060 were stacked along the symmetry axis using the Hex docking engine with only 2 degrees of freedom active, the translation between the subunits, and the twist angle between them. We ranked the T170/H1060 models based on the Hex docking scores. The T177/H1081 models were additionally optimized and ranked using KSENIA[9].

## Availability

More information about our methods can be found at https://team.inria.fr/nano-d/software.

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# XI. Integrating residue-residue contact prediction and hybrid scoring function into hybrid docking in CASP14-CAPRI

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## Methods

During the CASP14-CAPRI experiment, we integrated template-based docking and ab-initio docking into a hybrid docking protocol, which is similar to that used in our HDOCK webserver [1, 2] and that used by our group in CASP 13-CAPRI [3], to predict the complex structures for the given targets. Specially, for a given protein sequence, the HHblits [4] program was first used to search against the PDB database for the monomer templates. Then the searched templates were filtered by 20% cutoff of sequence identity and 80% cutoff of sequence coverage. If no templates were found through the filter, the cutoff of the sequence identity was relaxed to 15%-20%. For the hetero-oligomer target, the complex template was selected from the common PDB templates of receptor and ligand according to the sequence identity, sequence coverage and the resolution of the template structure. For the homo-oligomer target, the homo-oligomeric template with the same stoichiometry given by CAPRI organizers was selected. If a complex template was found, MODELLER[5] was subsequently adopted to construct the monomer structure(s) of the target using the corresponding component(s) as monomer template(s). Otherwise, the best monomer template(s) was/were used for homology modeling. Then template-based docking was performed by superimposing the modeled monomer structure(s) onto the complex template if found. The *ab-initio* docking was performed using our HDOCK-lite [6] for hetero-complex target or our HSYMDOCK-lite [7] for homo-complex. A new hybrid scoring function was used to rank the sampled binding modes of *ab-initio* docking. The hybrid scoring function is a linear combination of our distance-based iterative scoring function ITScorePP [8] and the contact-based scoring function IFACE previously used in ZDOCK [9]. At last, the template-based model of the complex and the ranked *ab-initio* docking models were combined together and clustered with an $L_{rmsd}$ cutoff of 5 Å. The selected models were further refined and then submitted. For homo-oligomer targets, a deep learning model, named as DeepHomo [10], was used to predict the inter-protein residue-residue contacts, by integrating evolutionary coupling, sequence conservation, distance map, docking pattern, and physic-chemical information of monomers. Then the top prediction was used to filter the *ab-initio* docking models in the last step. The overall workflow of our hybrid docking protocol is shown in **Figure 1**.

## Performance

The hybrid scoring function has been tested in protein-protein docking benchmark 4.0 and outperformed the previous scoring function ITScorePP. When separately tested on the benchmark, IFACE and ITScorePP have shown a complementary effect in terms of characterizing protein-protein interactions. Therefore, the combination of two scoring functions improved the top 1 success rate from 7.4% to 12.5% in unbound docking. DeepHomo model has been tested on a test set of 300 targets with experimental monomer structures and 28 CASP-CAPRI targets with predicted monomer structures. It has shown a much better performance than direct-coupling analysis (DCA) and machine learning (ML)-based approaches, and obtained the precisions of top 1 prediction more than 60%. Integrating the predicted contacts into *ab-initio* docking also significantly improved the top 5 success rate from 42.9% to 64.3% when tested on the 28 realistic CASP-CAPRI targets.

## Difficulties

The most difficult thing we encountered in this round was how to integrate the residue-residue contact predictions into the docking protocol efficiently. As now, we only use the top prediction to filter the sampled binding modes. Another challenge was the refinement of the complex models especially the template-based complex model.

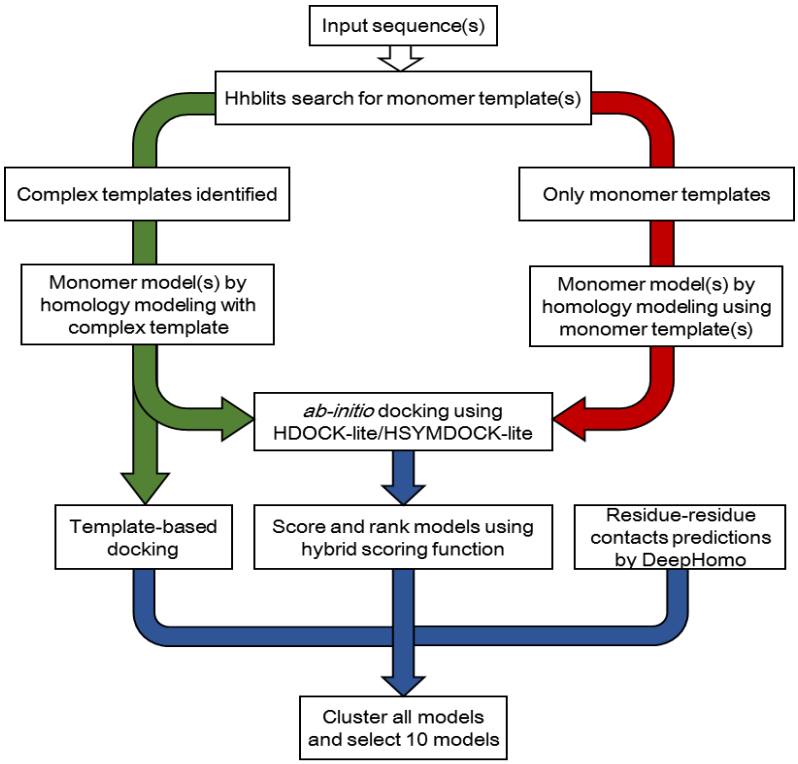


**Figure 1:** Workflow of our hybrid docking


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## XII. Kihara human team and LZerD server performance in CAPRI 50 / CASP 14

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We report the protein docking prediction pipeline of our group and the results for CAPRI50/CASP14. The pipeline integrates programs developed in our group as well as other existing scoring functions and modeling tools. HHpred[1] and PSI-BLAST[2] with default settings were used to search for template structures for the complex structure. If one or more templates of the complex are found, models were generated using MODELLER[3]. When subunit structures were otherwise unavailable, structures generated by our CASP14 free modeling (de novo) pipeline were used[4].
If templates of the full complex were not found in the PDB, we generated ab initio docking models using the LZerD protein-protein docking algorithm developed in our group[5]. In cases of complexes with more than two subunits, a restricted and modified version of the multiple-chain docking protocol Multi-LZerD[6], developed in our group, was used on top of LZerD. Partial complex templates and symmetry information were integrated. In the case of human group prediction, we surveyed the literature to find information to guide the modeling, such as protein-protein interface information. We also integrated available SAXS data, but the applicable target was canceled. In addition to any literature information, generated docking decoys were selected by a combination of scoring functions, including DFIRE[7], GOAP[8], and ITScorePro[9]. These scores were combined by the simple rank aggregation scheme of adding their numerical rank values, called ranksum[10]. The top ten decoys were relaxed by a short molecular dynamics simulation before submission to remove atom clashes and improve side-chain conformations.

**Performance Summary**
In CAPRI50/CASP14, our groups ranked near the top of the preliminary assessment released in December 2020. For prediction, our human and server groups produced models of at least acceptable quality for 7 and 6 targets respectively, as well as models of at least medium quality for 3 and 2. Broken down by modeling category, our human and server groups achieved at least acceptable quality for 9 and 3 target-interfaces respectively for targets where ab initio docking was used. Of the 3 targets where de novo subunit structure prediction was relied on exclusively, we were able to achieve acceptable complex models for 2 of them. Templates used are listed in Table 1.

**Sucess**
Our docking pipeline achieved acceptable model quality for T179, a bacterial prototoxin, although no templates for either the complex or for the subunit were found. Our T179 models were generated by modeling the subunit de novo and then docking de novo models using LZerD in C2 symmetry mode (see Figure 1). LZerD was one of only two servers to acceptably model this target.

**Failure/Challenges**

T170 was a particularly challenging target. This phage tail complex with A6B3C12D6 stoichiometry would have likely been impossible as a completely ab initio target. Of the 9 interfaces in this target, we were able to acceptably model 5. Particularly, 2 of the interfaces that we modeled acceptably were classified as difficult by the organizers; only one other group was able to model 2 difficult interfaces, and no groups were able to model all the difficult interfaces. These difficult interfaces were within the 12-chain cyclic ring, between that cyclic ring and the concentric 3-chain cyclic ring, and within that 3-chain ring. We were the only group to successfully model one of the specific interfaces between the two rings. However, the other such interface was missed. Furthermore, we missed 3 of the 4 easy interfaces among the two stacked 3-chain cyclic identical-sequence rings, despite the fact that most groups which succeeded on any interface of this target were able to model at least 2 of those interfaces.

**Figure 1. T179 acceptable LZerD server model.**

LZerD server model #4 is shown in green and cyan. At 78% sequence recovery, this model is of acceptable quality with an $f_{na}$ of 0.30 and an L-RMSD of 9.1 Å. At time of writing, the native structure was not publicly available and so is omitted here.

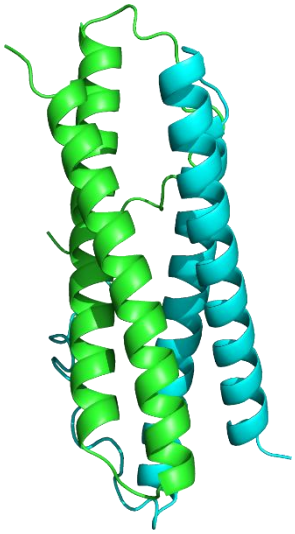

**Table 1. Templates used for all evaluated CAPRI 50 submissions.**

Targets where no template was used for subunit modeling as well as for the assembly are marked “n/a”. A template being listed does not indicate that de novo models were not also used for that target.

| Target | List of templates |
|---|---|
| T164 | 4RSJ |
| T165 | 2GUM, 4PFE, 5JYM, 5C6T, 3FVC, 3NWA, 5V2S, 6ESC |
| T166 | 4BWF, 5NKZ |
| T168 | 3DC7, 6F7D |
| T169 | n/a |
| T170 | 3UH8, 5NGJ, 6F2M, 5IV5 |
| T174 | 1S2E |
| T176 | 3V0R |
| T177 | 2VYC |
| T178 | n/a |
| T179 | n/a |

| | |
|---|---|
| T180 | 3J2V |

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## XIII. Template-based Structure Prediction and Inter-residue Distances and Orientations Prediction-based Structure Prediction

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In CAPRI 50 (CASP 14), we used a multiple sequence alignment (MSA) generated by our method[1] as a seed input for HHblits[2] to a perform profile−profile sequence search, and we also used the template profile database created in a similar method. To construct 3D-models, we used template-based structure prediction by MODELLER[3], interresidue distances and orientations prediction-based structure prediction by trRosetta[4], and combined them in some targets. In addition, we predicted quaternary structures that replicate experimental evidence based on a literature search.

**Methods**

To execute a sequence search of a target, we used SSearch[5] with MIQS[6] against the latest NCBI nr database. Then we made an MSA by using MPI-parallelized MAFFT[7,8] with

homologous sequences. With the MSA as input, we used HHblits to execute an iterative profile−profile sequence search against the UniClust30[9] and BFD[10] databases.

To execute a template search and acquire profile−profile alignment between target and templates, we used HHsearch[2] against the latest PDB70 and an in-house profile database that was made by three iterations of HHblits with MSAs as input. These MSAs were made with PDB98 against NCBI nr in a similar manner for target sequences. However, we made the MSAs partly by stacking pairwise sequence alignments by SSearch instead of using MPI-parallelized MAFFT.

In our 3D-model construction step, we used MODELLER with the result of the profile−profile alignment against PDBs and trRosetta with the result of the sequence search. We intervened in the processes of trRosetta by partly substituting the input with the distances and orientations of 3D-models made by MODELLER in some targets that had good templates and made 3D-models well.

In our model selection step, we used VoroMQA[11] mainly, dDFire[12], ProQ4[13], and the rate of fit with the servers' distance predictions.

For multimeric targets, the stoichiometry of the template protein was considered to select a model. Also, experimental evidence (e.g., the number of disulfide bonds by mass spectrometry and interacting regions by pull-down assay) based on a literature search was heavily considered and we tried to replicate the evidence in 3D-models by adding restraints manually. If we needed to perform free-docking, we used Haddock[14] and ZDOCK[15]. If we considered that the target must be coiled-coil but it was hard to construct a model, we used ISAMBARD[16].

**Performance**

In CAPRI 50, our group was able to submit models for 9 out of the 12 targets (if including cancelled and no structure targets, 15 out of the 18 targets). In the preliminary assessment, our models achieved medium quality for 2 targets (T166, T170K:L(Z:Z)) and acceptable quality for 3 targets (T164, T170A:B(A:A), T177). Regarding T166, we superimposed a good server model for PEX4 and a model which we produced for PEX22 to the interface structure of 2y9m. Regarding T170K:L, we superimposed a good server model to 6v8i. Regarding T170A:B, we used Fit in Map of UCSF Chimera with EMD-3691 and 5ngj.

**Failure**

In the case of T168, although we produced the multimeric structure of domain 1 (1-539) based on 6f7k, we misplaced the other domains and it caused too many clashes (presumably more than the disqualification threshold).

**Acknowledgments**

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## XIV. CAPRI Round 50 Extended Abstract, Pierce Team

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Our team submitted models for 17 out of 18 targets, and 11 of our submitted targets were evaluated out of the 12 evaluated targets. Our basic modeling strategy for this CAPRI round entailed these main steps: 1) Select monomer structure(s) from the CASP server model sets, 2) Utilize one or more of the following docking protocols: RosettaDock[1], ZDOCK 3.0.2[2], SymDock[3] and M-ZDOCK[4] (the latter two for $C_n$ symmetric docking), 3) Refinement and model selection, including one or more of the following protocols: clustering using FCC[5], model refinement using Rosetta FastRelax[6] or local RosettaDock, and re-scoring with ZRANK2[7] and Rosetta REF15[8] scoring functions. For ZDOCK and M-ZDOCK docking input, certain residues or regions were blocked to avoid their participation in modeled interfaces, when information from known structures or the literature was identified by our team.

Table 1 summarizes the methods and results for all evaluated targets from our team. Our multi-stage strategy, which utilized a range of available tools, achieved Acceptable or

better models for 7 targets, including Medium or higher quality models for four targets (T166, T168, T177, T180). Also, for two homodimeric targets classified as Difficult, we generated Acceptable quality models (T178, T179). Among 47 CASP teams that participated in CASP-CAPRI targets, our team was ranked 7$^{th}$ based on average z-score (> 0.0). While for most targets we used major docking and refinement tools, for the large ring-shaped assemblies of T170 and T177 we performed template-based homomultimer modeling with SWISS-MODEL[9], in conjunction with PyMOL (Schrodinger, Inc.) to position the rings in the respective assemblies. Lack of successful predictions for four Difficult targets (T169, T170, T174, T176) highlights the need for improved docking protocols in scenarios with conformational changes in unbound or modeled input structures.

**Table 1.** Prediction methods and results for evaluated targets.

| Target | Assembly | Quality[1] | Server Model(s)[2] | Methods[3] | Difficulty |
|---|---|---|---|---|---|
| T164 | Homodimer | Acceptable | Zhang-Server<br>BAKER-ROSETTASERVER<br>MULTICOM-DEEP<br>RaptorX | SymDock, M-ZDOCK, FastRelax | Easy |
| T166 | Heterodimer | Medium | Zhang-Server<br>ZhangS | RosettaDock<br>ZDOCK<br>FastRelax | Easy |
| T168 | Homotrimer | Medium | ZhangS<br>MultiAI | FastRelax | Easy |
| T169 | Homodimer | Incorrect | RaptorX,<br>Zhang_Ab_Initio | M-ZDOCK, FastRelax, ZRANK2, FCC | Difficult |
| T170.1-9 | Homultimer/Heteromultimer rings | Incorrect (Interface 1-9) | SWISS-MODEL<br>Templates: 5NGJ | MZDOCK, PyMOL | Difficult |
| T174 | Homotrimer | Incorrect | Zhang-Server, Zhang-CEthreader, Quark | Assembly templates: 5HX2, 1QEX<br>FastRelax, ZRANK2 | Difficult |
| T176 | Homodimer | Incorrect | BAKER_ROSETTASERVER | M-ZDOCK, SymDock, FastRelax, ZRANK2 | Difficult |
| T177.1<br>T177.2<br>T177.3 | Two stacked decamers | High (Interface 1)<br>High (Interface 2)<br>Medium (Interface 3) | SWISS-MODEL<br>Template: 2VYC | PyMOL | Easy |
| T178 | Homodimer | Acceptable | Zhang-Server<br>MUFOLD2 | M-ZDOCK, SymDock | Difficult |
| T179 | Homodimer | Acceptable | BAKER-ROSETTASERVER | M-ZDOCK, FastRelax, SymDock, FloppyTail, ZRANK2 | Difficult |
| T180.1<br>T180.2 | Capsid | Incorrect (Interface 1)<br>Medium (Interface 2) | BAKER-ROSETTASERVER<br>Zhang-Server<br>Yang-Server<br>MULTICOM-DIST | Assembly templates: 6HTX, 1QGT<br>SymDock, FastRelax, ZRANK2 | Easy |

[1]Best CAPRI model rating in top 5 submitted structures.
[2]CASP server model used as docking/modeling input. In most cases, the top model from the specified server ("TS1") was used in modeling.
[3]Methods used for docking and/or refinement: RosettaDock[1], ZDOCK[2], SymDock[3], M-ZDOCK[4] , FCC[5], Rosetta FastRelax[6], ZRANK2[7], PyMOL (Schrodinger, Inc.), SWISS-MODEL[9]. PDB codes for multimeric structures used to guide or fit assemblies are also noted.

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## XV. Oliva team performance, CASP14-CAPRI, Round 50


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**Scoring function/scheme used**

We submitted scoring predictions for all the 14 targets assessed in the scoring experiment, using an approach similar to the one we first applied in the CASP13/CAPRI round. In order to assign a tentative easy/difficult classification to each target, as a preliminary step, we used HHPRED [1] to search for possible templates for the modeling of the single proteins and of the assembly. All the targets (with the partial exception of T170 and T177, see below) were scored with our tools CONSRANK (CONSensus RANKing) and Clust-CONSRANK. CONSRANK is a pure consensus method for the ranking of docking decoys [2-3]. It calculates the frequency of inter-molecular contacts in a decoys ensemble and then ranks each decoy based on its ability to match the most frequent contacts. Clust-CONSRANK is a CONSRANK implementation introducing a contact-based clustering of the models as a preliminary step of the scoring process [4]. For the clustering, a threshold on the number of clusters was set as 1/10 of the total number of models per target.

For each target, the ≈20-30 models with the highest CONSRANK score and the 3-4 top ranked models for the 15 most populated clusters from the Clust-CONSRANK output were further analyzed with CoCoMaps [5], a web tool we developed for the analysis of the

interface in macromolecular complexes. Models showing a large number of clashes were removed from the selection. The weaker was the overall consensus highlighted by CONSRANK, the higher was the number of models we selected from the Clust-CONSRANK output. Ranking of the selected models from the top-1 to the top-10 position was guided by results of the interface analysis, especially in terms of extension of the contacts network and of the interface area.

Models for the hetero 27-mer T170 and the homo 20-mer T177 target were analysed by CONSRANK focusing on single interfaces and also subjected to short *in vacuo* molecular dynamics (MD) simulations, carried out with GROMACS in a microcanonical (NVE) ensemble [6]. The MD simulations were followed by a clustering step to single out representatives of the different clusters. Submitted models for these two targets were partially selected from the CONSRANK results, partially from the MD approach.

**Short description of successes and failures**

Our performance was especially effective in the top-1 ranking, where we were second, with at least one correct solution for 6 targets, of which 3 of medium quality (see Figure 1), on a par with Takeda-Shitaka, and second after LZERD, which exceeded our performance for having one target with a top-1 high quality model instead of a medium-quality one.

Our performance for the top-5 ranking coincides with that for the top-1 ranking. As for the top-10 ranking, we submitted correct models for 7 targets, for 1 of them of high quality- and for 2 of them of medium-quality. This compares with the 9 targets with at least one correct model, of which 4 of medium quality, of the best performing scorers in this CAPRI Round.

Our successful targets (both in the top-1 and top-5 rankings) correspond to T164, T166, T168, T177, T178 and T179, which include different types of homo- and hetero-assemblies. The targets for which we could not submit any correct solutions (at the top-10 positions), while at least another scorer could, were instead T176 and T180. These targets, a homodimer and a 240-mer assembly, respectively, were particularly difficult ones, for which most of the scorer groups could not identify any correct solution. These are most probably targets whose scoring sets included only very few correct models and remain the critical cases for us.

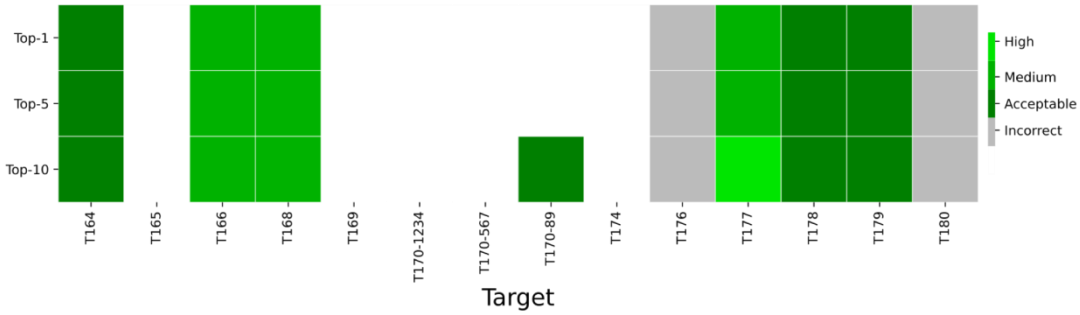


**Figure 1.** Our performance (Oliva's group) as scorers for the 14 assessed targets of the CASP14/CAPRI50 joint experiment. The presence of high, medium quality, acceptable or incorrect models in each ranking is reported in a green-to-gray color code. Targets for which no acceptable solutions were available are colored white.

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## XVI. Performance of the Shen team in CASP14-CAPRI Round 50

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### Methods

We first adopted monomer structures from CASP14 tar ball (stage 2 webserver predictions, specifically “Zhang-Server”, “BAKER-ROSETTASERVER”, and “RaptorX”) and performed rigid docking using the web server ClusPro. Multimer docking, antibody mode, and attractions were used in ClusPro whenever applicable. Homology models are built if oligomer templates are identified (we used HHpred for monomer sequences).

We then refined oligomer models using our BAL (Bayesian Active Learning) [1] and rescored the refined models using our EGCN (Energy-based Graph Convolutional Networks) [2].

Our innovations include:
1. *Conformational flexibility: basis directions and ranges*. We used encounter complex-based normal mode analysis, incorporating both effects of conformational selection and induced fit, to predict the directions and the ranges of conformational change (first proposed in [3][4] and then upgraded in BAL [1]).
2. *Conformational search with uncertainty awareness*. By directly modeling the posterior distribution of the unknown global optimum (the native structure), we guide conformational search and improve model scoring with uncertainty estimation [1].
3. *Absolute and relative quality estimation*. In BAL [1], we had developed a funnel-like energy function using random forest of MM-PBSA features, which is used for both absolute quality estimation (iRMSD estimation) and relative quality estimation (ranking/scoring of structure models). For this round of CASP/CAPRI, we have adopted the newly-developed EGCN [2] for both absolute and relative quality estimation, which is the first graph neural networks for the purpose. Compared to our earlier shallow models (random forest in [1]), the new deep-learning model [2] directly learns energy values from 3D structures represented as graphs rather than indirectly doing so from structure-based semi-empirical energy calculations. EGCN was used for both re-scoring our models in the docking experiment and re-scoring community submissions in the scoring experiment.

### Performances

In the CASP multimeric evaluation on CAPRI targets only, our submissions for 10 targets led to a sum of Z score ($\geq 0.0$) being 5.94, placing us 14$^{th}$ out of 47 CASP+CAPRI groups. In particular, our CASP ranking on three hard (FM) targets were 8$^{th}$/34.

In the CAPRI docking evaluation, our submissions for 12 targets (not counting 2 canceled targets and 4 targets without structures determined) led to at least acceptable prediction for 6 of the 12 targets, including medium-accuracy prediction for 1 target. The docking performance (a sum score of 7) placed us tied 15$^{th}$ out of 28 teams, being 1-point shy of the top 10.

Target-based break downs of our docking performances are as follows. First, for 2 easy dimers, we had one acceptable (T164/T1032) and one medium-accuracy (T166/H1045) prediction. Second, for 4 difficult dimers (T169/T1054, T176/T1078, T178/T1083, T179/T1087), we had acceptable prediction for T169 and T179 whereas the community had at least acceptable prediction for 3 of the 4 (the one where we didn't succeed was T178). Third, for 3 homo-trimers (T165/H1036, T168/T1052, and T174/T1070), we had no acceptable prediction and the community had only such for T168; however, T168 turned out to be easy for the community (19 teams had acceptable prediction, including 2 teams with high accuracy and 9 with medium accuracy). Last, for the 3 remaining targets of cryo-EM hetero-oligomer assemblies including T177/H1081 (the decarboxylase), T180/T1099 (viral capsid), and T170/H1060 (T5 phage tail distal complex), we had at least acceptable prediction for T180 and T170, but we missed T177 which turned out to be easy for the community (16 teams had at least acceptable predictions including 8 teams with medium accuracy and 1 with high accuracy). Taken together, we did well for difficult dimers and the 240-mer T170. In particular, we had at least acceptable prediction for 6 of the 9 interfaces of T170, including 2 interfaces with medium-accuracy prediction, which was the community best. Our models for the difficult T170 interfaces of C3/C5 and min unit also had the highest TM scores among all CASP-assessed submissions. However, our performance was poor for the easy cases of T168 and T177, for which the reasons remain to be validated and may include interfacial clashes in the models.

In the CAPRI scoring evaluation, our submissions for 12 targets (14 interfaces) had identified (among top-5 models) at least acceptable prediction for 7 targets/interfaces, including medium-accuracy prediction for 3 of the 7. The scoring performance placed us tied 7$^{th}$ out of 19 teams. We note that our scoring results included medium-accuracy top-5 models for both T168 and T177.

**Challenge**

Regardless of the performances, the cryo-EM hetero-assemblies present a major challenge (and potential opportunity) to our pipeline that has mainly been developed for dimers.

**Acknowledgement**

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## XVII. Predicting protein complex structures using GALAXY in CAPRI round 50

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We participated in CAPRI round 50 as a server group GalaxyPPDock and a human group Seok. GalaxyPPDock submitted server predictions generated by GALAXY programs with minimal human intervention. Human intervention was made only when the stoichiometry of the target did not correspond to $A_n$ or $A_1B_1$ or when additional information was provided. Human predictions submitted by Seok were also generated by GALAXY, but human intuitions were utilized in selecting monomer models, using information obtained from the literature search, scoring complex models, etc.

**Methods**

The newly developed GalaxyHomomer2 and GalaxyHeteromer were used to predict the structures of homo-oligomers and hetero-oligomers, respectively, from sequences. These oligomer structure prediction pipelines were run following the monomer modeling pipeline of CASP14 Seok-server. Monomer models, selected among those predicted by template-based modeling GalaxyTBM and by an in-house distance-based modeling method that employs distance prediction from sequence coevolution, were further subject to quaternary structure prediction.

GalaxyHomomer2 performed homo-oligomer structure prediction by template-based and *ab initio* docking depending on template availability, just like GalaxyHomomer. In GalaxyHomomer2, the two template-based modeling methods, sequence-based template detection followed by restraint-based modeling-building and structure-based template detection followed by superimposition-based model building, were re-balanced, considering improved monomer structure prediction owing to improved distance prediction.

GalaxyHeteromer performed automatic hetero-dimer structure prediction also by template-based and *ab initio* docking depending on the availability of hetero-dimer templates. Templates to be used for hetero-dimer modeling were selected from HHsearch high-rankers including monomers and homo-oligomers as well as from a hetero-dimer structure database based on structure similarity between the monomer models and the templates. The hetero-dimer structure database consisted of non-redundant hetero-dimer structures prepared by compiling all hetero-dimers with atomic contacts in PDB and sequence- and structure-based clustering. Hetero-dimer models generated by superimposing the monomer models on the templates were ranked by monomer structure similarity, with additional consideration of the number of clashes, the number of contacting residue pairs, and interface area. If template-based modeling produces less than five models, *ab initio* asymmetric docking method GalaxyTongDock_A was used to generate more models to get a total of five models.

**Results and Discussion**

We confirmed that there remains a large gap between performances of human and server predictions. In most cases, manual prediction showed higher performance than server prediction, as shown in Figure 1. In the case of T174, incorrect complex structure prediction originated from incorrect monomer structure prediction by the server. However, human prediction started with manual domain splitting of the monomer into four domains, and the trimer structure of each domain was predicted first before full domain docking. The human predictions resulted in a trimer interface of the 2nd domain (Fnat=0.667) and the 4th domain (Fnat=0.417) with acceptable quality. In the case of T180, incorporation of key interactions

extracted from mutagenesis studies of capsid protein assembly resulted in an acceptable model, which turned out to be the only acceptable model submitted by all.

As a post-analysis, monomer structures of AlphaFold2 (AF2) were subject to *ab initio* docking with TongDock for eight targets of stoichiometry $A_n$ or $A_1B_1$ to assess the effect of improved monomer structure quality. In one case (T179), docking of the AF2 monomer model resulted in a medium quality model (Fnat=0.81) compared to an acceptable quality model (Fnat=0.18) obtained previously. In the remaining seven cases, AF2 monomer structures had incorrect interface structures, and resulted in incorrect complex structure prediction by docking. In six of the seven cases, superposition of AF2 monomers to the crystal complex structures caused severe steric clashes. So, the current AF2 monomer models do not seem to be enough to be accurately docked by rigid-body docking.

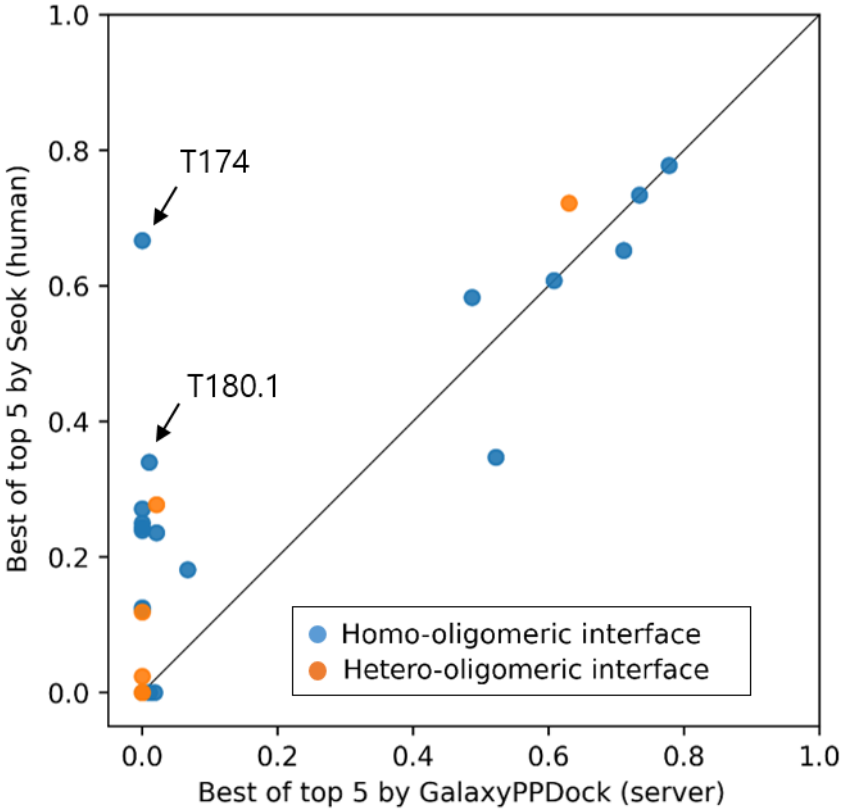


**Figure 1**. Performance comparison of the server and human predictions in terms of the fraction of native contacts (Fnat) for the best of top 5 models. Targets mentioned in the text, T174 and T180.1 (interface 1), are marked with their target numbers.

## XVIII. Performance of Takeda-Shitaka Team in CASP14-CAPRI Round 50

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**Introduction**

We participated in the assembly category of CASP14-CAPRI50. We predicted both homo- and hetero-oligomeric protein structures according to the oligomeric state in the CASP14-CAPRI50 target list. Our modeling procedure was based on template-based docking method.

**Methods (Human Predictor)**

**Monomer model selection**

We basically used CASP14 server models (Stage 2) as monomer models. We selected high quality monomer models using combined score of ProQ3[1], ProQ3D[2] and VoroMQA[3]. The score was adjusted to pick up high quality models. When we could not obtain high quality server models, we constructed the monomer models by MODELLER[4] based on alignments from HHalign[5].

**Oligomeric template search**

To find reliable oligomeric templates, we carried out two-step template search. Firstly, the oligomeric templates were searched by HHblits[5] against PDB70 database. Secondly, to search oligomeric templates more widely, we ran PSI-BLAST[6] on PDBaa using HHblits hits as inputs. According to the results of two-step template search and information of biological unit, oligomeric templates were selected.

**Oligomeric model construction**

To construct oligomeric models, we performed template-based docking. We superposed the monomer models onto the oligomeric templates using TM-align[7] or CAB-align[8]. When we could not obtain the oligomeric templates, we used DOCKGROUND[9] database as templates. For some targets, we used SymDock2[10] to construct the oligomeric models.

**Quality assessment and refinement of oligomeric models**

The quality of oligomeric models were assessed by combined score of VoroMQA and SOAP-PP[11]. The selected 5 models were refined using MODELLER to remove steric clashes.

**Methods (Scorer)**

When oligomeric templates were available, oligomeric models were superposed onto oligomeric templates using MM-align[12]. Models with insufficient TMscore or many clashes in the interface were removed before scoring. The same scoring method for human prediction was used for scoring models. Based on the results, 10 models were selected.

**Successes and Failures**

For T166, our model was assessed as high (***) by the CAPRI measure. We could find a good oligomeric template (PDB ID: 2Y9M) and select good monomer models from the CASP14 server models (step2) for both chains using our method described above.

T170 was a challenging target because it was 27-mer and had many interfaces. This hetero-complex target had ring A (A3), ring B (A3), ring C (B3/C12) and ring D (D6). We prepared ring-shaped templates for each ring (5NGJ for ring A and B, 6F2M for ring C, and 4JMQ for ring D). After constructing these rings, we assembled whole structure based on two templates (6V8I and 4V96). It was difficult to remove steric clashes. As a result, two out of nine interfaces of our model were assessed as acceptable (*) by the CAPRI measure.

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## XIX. Modeling CAPRI and Oligomeric CASP Targets by Template-Based and Free Docking

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We performed the initial alignment of the CAPRI and CASP targets by HHpred.[1] If the alignment had > 90% probability and covered > 80% of the target sequence, we utilized NEST[2] to build the protein models for the docking. Otherwise, we used CASP stage 2 server models of the individual proteins, except those with loose packing. For the template-based docking, the structure alignment by TM-align[3] was scored by a combination of structure similarity metrics, normalized AACE18[4] values for the interface, fraction of shared target/template contacts, target/template interface sequence identity, interface solvation score, and the extent of clashes in the unrefined predictions.[5] The free docking by GRAMM was performed at lower resolution (3.5 Å grid step) to accommodate structural inaccuracies of the modeled proteins. The predicted matches were scored by the AACE18 potential. The text-mining procedure[6] was used to identify the binding site residues, which served as additional docking constraints. All final predictions were minimized by TINKER.[7] The results are summarized in Table 1.

*Table 1. Docking of CASP14-CAPRI targets*

| CAPRI target | CASP target | Proteins | Organism | Assembly | Experimental method | Number of residues | Number of HHpred templates | Rank of the best model by CASP |
|---|---|---|---|---|---|---|---|---|
| T164 | T1032 | smchD1 | Human | A2 | X-ray | 284 | 107 | 95 |
| T165 | H1036 | Glycoprotein gB/antibody 93k | Varicella-zoster virus/human | A3B3C3 | EM | 622/128/106 | 9/250/250 | N/A |
| T166 | H1045 | PEX4/PEX22 | *Arabidopsis Thaliana* | A1B1 | X-ray | 157/173 | 22 | 19 |
| T167 | T1050 | ATPase | *Bacteroides Ovatus* | A2 | X-ray | 779 | 250 | N/A |
| T168 | T1052 | Tail spike protein | Salmonella phage epsilon15 | A3 | X-ray | 832 | 250 | 11 |
| T169 | T1054 | Outer-membrane lipoprotein | *Acinetobacter baumannii* | A2 | X-ray | 190 | 18 | 105 |
| T170 | H1060 | tail subcomplex | T5 phage | A6B3C12D6 | EM | 464/298/140/204 | 11/1/4/6 | 9 |
| T171 | T1063 | CCNB1IP1 | Human | A4 | X-ray | 196 | 208 | N/A |
| T172 | H1066 | CCPol/MP-2 | - | A1B1 | X-ray | 366/123 | 11/0 | |
| T173 | H1069 | CCPol/MP-1 | - | A1B1 | X-ray | 369/122 | 11/0 | |
| T174 | T1070 | Tail spike protein | Escherichia virus CBA120 | A3 | X-ray | 335 | 2 | 11 |
| T175 | T1073 | DUF4423 | *Bdellovibrio bacteriovorus* | A4 | X-ray | 255 | 250 | 9 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| T176 | T1078 | Tsp1 | *Trichoderma virens* | A2 | X-ray | 138 | 1 | 24 |
| T177 | H1081 | Arginine decarboxylase | *Providencia stuartii* | A20 | EM | 758 | 250 | N/A |
| T178 | T1083 | Nitro | *Nitrosococcus ocear* | A2 | X-ray | 98 | 0 | 48 |
| T179 | T1087 | Tuna | *Methylobacter tundripaludum* | A2 | X-ray | 93 | 1 | 18 |
| T180 | T1099 | Capsid protein | Duck hepatitis B virus | A? | EM | 262 | 4 | 17 |
| T181 | H1103 | Orf3a-HMOX1 | SARS2-Human | A1B1 | X-ray | 275/288 | 1/37 | N/A |

*Table 1. Continued*

| CAPRI target | CASP target | Proteins | Organism | Assembly | Experimental method | Number of residues | Number of HHpred templates | Rank of the best model by CASP |
|---|---|---|---|---|---|---|---|---|
| | | | *CASP only oligomeric targets* | | | | | |
| | T1034 | BIL2 | *Tetrahymena thermophila* | A4 | X-ray | 156 | 0 | 41 |
| | T1038 | TSWV glycoprotein | Tomato spotted wilt virus | A2 | X-ray | 199 | 0 | 13 |
| | H1047 | FlgH-FlgI | *Shigella sonnei* | A1B1? | EM | 232/365 | 1/0 | 7 |
| | T1048 | HD_1495 | *Haemophilus ducreyi* | A4 | X-ray | 109 | 0 | 23 |
| | T1061 | tail subcomplex | T5 phage | A3 | EM | 949 | 192 | 36 |
| | T1062 | tail subcomplex | T5 phage | A3 | EM | 35 | 0 | N/A |
| | H1065 | Cytosine Methyltransferase | *Serratia marcescens* | A1B1 | X-ray | 127/98 | 0/0 | 8 |
| | H1072 | SYCE2/TEX12 | Human | A2B2 | X-ray | 101/69 | 0/2 | 17 |
| | T1080 | Bd3182 | *Bdellovibrio bacteriovorus* | A3 | X-ray | 922 | 129 | 14 |
| | T1084 | Meio | *Meiothermus silvanus* | A2 | X-ray | 73 | 0 | 48 |
| | H1097 | AR9 | *Bacillus phage PBS1* | ABCDE | EM | 426/631/496/665/464 | 14/16/0/12/0 | 44 |

For *n*-homomeric targets, we performed spatial rearrangement of the target protein to match the monomers in the experimentally determined complexes either from the full-structure template library[8] or from an *ad hoc* library generated from PDB for a particular target. The *ad hoc* library contained structures which (a) were identified by the HHpred as likely templates (> 90% probability) and (b) had oligomeric state in the biounit corresponding to that of the target. For target T177/H1081, due to anticipated conformational changes and large size of the putative interface, the free docking of the two 10-mers was performed with $C^{\alpha}$ atoms only. For *n*-heteromeric targets, we looked for common HHpred templates, when the templates for the target monomers were identified either as interacting chains in a PDB entry, or non-overlapping parts of the same chain. If no reliable templates were found, we performed free docking, including cross-docking of all selected CASP stage 2 server models.

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## XX. Modeling of protein complexes in CAPRI Round 50

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### Methods

In CASP14-CAPRI round 50 we used the same general modeling workflow as in CASP13[1] with several improvements. For every target we initially attempted to identify multimeric templates. If sequence-based searches using PPI3D[2] and HHpred[3] failed, we employed structure-based searches against PDB by submitting CASP server models to the DALI server[4]. If templates were identified, structural models were generated using MODELLER plugin AltMod[5, 6]. Otherwise, free docking of five selected monomeric CASP server models was done using Hex[7] for hetero-complexes and Sam[8] for homomultimers. For some larger target protein complexes templates were available only for some of the subunits or domains. In such cases a hybrid strategy was used, where part of the complex was generated by homology modeling, whereas remaining subunits were docked either by simply using TM-align[9] or by free docking. If the reliability of identified templates was questionable, we used a different hybrid strategy, performing both template-based modeling and free docking. In those cases we submitted best models generated by both approaches.

For model selection we utilized VoroMQA[10] taking into account both global scores and interface scores as described previously[1] with some modifications. More specifically, we used an improved version of VoroMQA (VoroMQA-dark) for global structure evaluation and an improved tournament-based ranking algorithm. Standard automated procedure was used to select the best template-based models and 10 best models in the CAPRI scoring challenge. In the case of free docking the top 100-500 selected models were subsequently relaxed by a very short molecular dynamics simulation using OpenMM[11] and then re-ranked. Constraints obtained from literature searches or CASP contact prediction servers, if available, were also used in selection of free-docking models. All models, resulting from both template-based modeling and free docking, were visually inspected before submission and, if necessary, their ranking was adjusted manually.

### Results

Our CAPRI results are summarized in Table 1 and two highlight cases are provided in Figure 1. We used template-based modeling for five, free docking for one and a hybrid approach for six targets. Template-based modeling usually resulted in models of acceptable or higher quality according to the CAPRI criteria[12] except for two cases. Of these two, the first one featured large insertions in the sequence-structure alignment corresponding to the interface region (interface No. 1 in T180, CASP T1099v1). The second case represents the failure to

identify the correct epitope in the antibody-antigen interaction (T165, CASP H1036v0). The success of free docking was mixed: sometimes models were completely incorrect (T169, CASP T1054), whereas sometimes docking outperformed the template-based approach (T178, CASP T1083; see Fig. 1). In the case of a large target (T170, CASP H1060), higher quality models were obtained for the interfaces that could be modeled using structural templates.

**Table 1.** Summary of the results obtained by the "Venclovas" group in modeling CAPRI targets

| CASP Target | CAPRI Target | Template search methods | Notes | CAPRI evaluation of our best model | Maximum $f_{nat}$ |
|---|---|---|---|---|---|
| **Template-based modeling:** | | | | | |
| T1032 | T164 | PPI3D, HHpred | | acceptable | 0.45 |
| H1036v0 | T165 | PPI3D, HHpred | Antibody-antigen interface which is not conserved | incorrect | 0 |
| H1045 | T166 | HHpred | | high | 0.81 |
| T1052 | T168 | PPI3D | | medium | 0.75 |
| T1099 | T180 | PPI3D | Interface 1 had large insertions | incorrect/ medium | 0.01-0.51 |
| **Hybrid modeling:** | | | | | |
| H1060 | T170 | DALI | | incorrect/ acceptable/ medium | 0-0.61 |
| T1070 | T174 | HHpred, PPI3D | Low resolution template and low quality alignment, reliable templates only for domains | incorrect | 0.11 |
| T1078 | T176 | DALI | A mixture of template-based and free docking models, the best model from docking | incorrect | 0.36 |
| H1081 | T177 | PPI3D | Free docking of two template-based 10mer models | medium/ high | 0.50-0.84 |
| T1083 | T178 | DALI | A mixture of template-based and free docking models, the best model from docking | medium | 0.68 |
| T1084 | T179 | DALI | A mixture of template-based and free docking models | acceptable | 0.51 |
| **Free docking:** | | | | | |
| T1054 | T169 | | | incorrect | 0.02 |

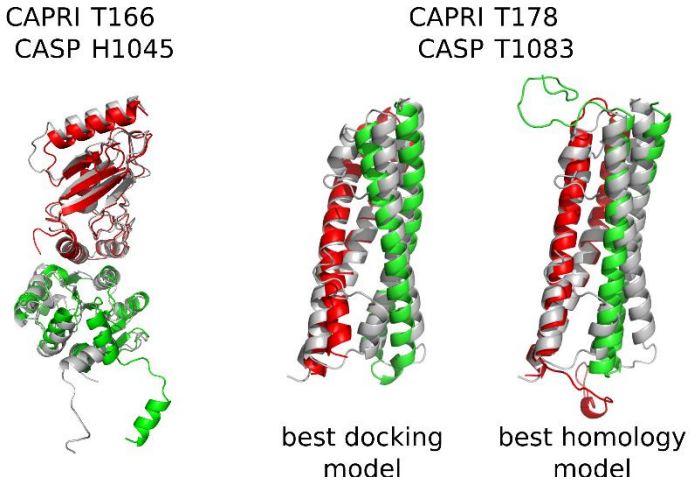

**Figure 1.** Top CAPRI results obtained by the "Venclovas" group: a template-based high quality model for T166 (CASP H1045) and the best models for T178 (CASP T1083). Model chains are colored green and red, and the experimental target structure is shown in grey.

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## XXI. Summary of the Zou Group and the MDOCKPP server

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We participated in the joint CASP14-CAPRI experiment as both predictor and scorer in all the announced targets. Different from our approaches used in the previous CASP13-CAPRI experiment[1], in-house developed GPU version of MDockPP server[2,3] and deep-learning-based DLScorePP scoring function were applied to the prediction of the binding modes in the current competition. In addition, a novel version of the MULTICOM-CLUSTER server[4] was used to generate monomeric structures.

In the prediction challenge, templated-based and/or docking-based methods were applied depending on the features of the targets. Specifically, for a given target, the BLAST program[5] was utilized to search for appropriate templates in the Protein Data Bank. If all the chains were found in a single PDB entry, the Modeller program[6] was applied to build the structure of the target using the complex structure in this PDB entry as a template. On the other hand, if templates for different chains of a target were found in different PDB entries, the Modeller program was utilized to construct the monomeric structure of each chain. If no template was found, the structures of the monomers were generated using the CASP14 MULTICOM-CLUSTER server. Moreover, the 150 best monomeric structures for each target provided by CASP groups were also evaluated using both ITScorePro[7] and DeepRank[8–10]. About 10 monomeric structures were selected using the consensus of these two scoring functions and biological information, which were then used as the input for docking with our MDockPP server.

Our in-house developed GPU version of the MDockPP server uses a fast Fourier transform (FFT)-based rigid docking algorithm[11] to generate putative binding modes. The biological information searched by an in-house Rebipp server[3] were also used as an input for the MDockPP server. The generated binding poses were optimized and ranked with our ITScorePP scoring function[12], which is an atomic-level, statistical potential-based scoring function for protein-protein interactions. If a target is dimeric, the top ranked binding modes by ITScorePP were further ranked with our recently developed deep learning model called DLScorePP. Next, the ranked binding modes were clustered according to their RMSDs. The best model from each of the top 10-ranked clusters were selected and submitted to CAPRI as the MDOCKPP server prediction. For human prediction, up to 100 binding modes were manually inspected, and ten models were selected for CAPRI submission.

In the scoring experiment, the same protocol was used for both the server and human scoring challenge, except that now the putative binding modes were collected from the groups participating in the docking experiment and redistributed by CAPRI.

In CASP14-CAPRI, our human prediction ranked at the #4 position in the prediction category and MDockPP ranked at the #1 position in the server prediction category, according to the preliminary assessment released by CAPRI in December 2020. Specifically, our human group (server) produced models of at least acceptable quality for 8 (7) out of 14 targets/interfaces whose structures are solved. Our server achieved high-accuracy prediction for T177. The performance of the MDockPP server is approaching to the performance of our human prediction. In the scoring category, our human and server (MDOCKPP) scoring prediction both ranked at the first positions when the Top-5 models are considered; our human (server) groups produced models of at least acceptable quality for 10 (9) out of 14 targets/interfaces with solved structures (including the three sub-targets of T170). The performance of our group for each target is summarized in Table 1. Similar with the previous CASP13-CAPRI experiment, the major difficulties are 1) how to further improve the acceptable accuracy models to medium or high accuracy models, and 2) how to make the rigid docking algorithm less sensitive to the conformations of monomeric structures.

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Table 1. List of templates and target performance of our group.

| Target | Possible templates* | Stoichiometry | Best prediction of our Top-5 models | | | |
|---|---|---|---|---|---|---|
| | | | Prediction | | Scoring | |
| | | | Server | Human | Server | Human |
| **T164** | 1gxl | A2 | Medium | Acceptable | Medium | Acceptable |
| **T165** | 5ys6, 5avg | A:HL | Incorrect | Incorrect | Incorrect | Incorrect |
| **T166** | 2oxq | AB | Acceptable | Medium | Medium | Medium |
| **T167** | - | Cancelled | - | - | - | - |
| **T168** | 6f7k | A3 | Medium | Medium | Medium | Medium |
| **T169** | 1fg9 | A2 | Incorrect | Incorrect | Incorrect | Incorrect |
| **T170.1** | | A6B12C3D6 | Medium | Medium | Acceptable | Medium |
| **T170.2** | | | Incorrect | Incorrect | Incorrect | Incorrect |
| **T170.3** | | | Acceptable | Acceptable | Acceptable | Acceptable |
| **T170.4** | | | Incorrect | Incorrect | Incorrect | Acceptable |
| **T170.5** | | | Incorrect | Incorrect | Medium | Medium |
| **T170.6** | | | Incorrect | Incorrect | Incorrect | Incorrect |
| **T170.7** | | | Incorrect | Incorrect | Incorrect | Incorrect |
| **T170.8** | | | Incorrect | Incorrect | Acceptable | Acceptable |
| **T170.9** | | | Incorrect | Incorrect | Acceptable | Acceptable |
| **T171** | - | No structure | - | - | - | - |
| **T172** | - | No structure | - | - | - | - |
| **T173** | - | No structure | - | - | - | - |
| **T174** | | A3 | Incorrect | Incorrect | Incorrect | Incorrect |
| **T175** | - | Cancelled | - | - | - | - |
| **T176** | | A2 | Acceptable | Acceptable | Acceptable | Acceptable |
| **T177.1** | | A10:A10 | High | High | High | High |
| **T177.2** | | | High | High | High | High |
| **T177.3** | | | High | Medium | Medium | Medium |
| **T178** | | A2 | Acceptable | Acceptable | Acceptable | Acceptable |
| **T179** | | A2 | Acceptable | Acceptable | Acceptable | Acceptable |
| **T180.1** | | A2:A2 | Incorrect | Incorrect | Incorrect | Incorrect |
| **T180.2** | | | Acceptable | Medium | Medium | Medium |
| **T181** | 3czy, 1ni6 | No structure | - | - | - | - |

*Only the templates used for homology modeling with the Modeller program are listed.