SCIENCE CHINA
Information Sciences

• Supplementary File •

OPUS-Design: a 3D convolutional approach for protein sequence design targeting complex spatial microenvironments

Gang Xu[1], Qinghua Wang[2] & Jianpeng Ma[1,3*]

[1]*Multiscale Research Institute of Complex Systems, Fudan University, Shanghai, 200433, China*
[2]*Center for Biomolecular Innovation, Harcam Biomedicines, Shanghai, 200131, China*
[3]*School of Physics and Electronics, Hunan Normal University, Changsha, 410082, China*

Appendix A Supplementary results

Table A1 Comparison of structural representations and prediction strategies in inverse-folding methods.

Method	Structural representation	How residue predictions are integrated
ProDCoNN	Local backbone voxel grid and 3DCNN	Residue types are predicted from individual local environments.
DenseCPD	Local backbone-atom density grid and 3D DenseNet	Residue types are predicted from individual local environments.
PiFold	Backbone-derived residue graph with geometric and virtual-atom features	Graph features are aggregated before one-shot sequence prediction.
ProteinMPNN	Backbone-geometric residue graph	Message passing is followed by autoregressive sequence decoding.
LigandMPNN	Protein residue graph and ligand/context-atom graphs	Protein-ligand message passing is followed by autoregressive sequence decoding.
CARBonAra	Atomic point cloud of the protein and molecular context, encoded by a geometric transformer	Atom-level features are pooled to residues; sequence information can be incorporated through imprint-conditioned prediction.
ESM-IF	Invariant backbone-geometric features	A structure-conditioned sequence-to-sequence transformer decodes the sequence.
ABACUS-T	Protein backbone, side-chain, and ligand representations	Iterative sequence and side-chain denoising incorporates embeddings from a pretrained ESM protein language model.
OPUS-Design	Local atom-level voxel grid and 3D U-Net, followed by sequence and pairwise features	Predicted side-chain density and residue probabilities enter protein-wide feature aggregation, followed by sequential context updates and ESM-2 refinement.

* Corresponding author (email: jpma@fudan.edu.cn)

Table A2 Performance comparison of different methods on the CAMEO and CASP15 datasets, measured by core residues.

Method	Sequence recovery (%) ↑	Charge MAE ↓	Hydropathy MAE ↓	AA JSD ↓	BLOSUM62 ↑	ESM-C PLL ↑
CAMEO						
PiFold	61.58%	0.12	1.10	0.09	3.06	-2.02
ProteinMPNN	56.74%	0.12	1.18	0.09	2.86	-1.75
LigandMPNN	61.06%	0.12	1.08	0.09	3.08	-1.77
CARBonAra	62.92%	0.11	0.99	0.08	3.23	-1.96
ESM-IF	68.46%	0.10	0.82	0.07	3.62	-1.56
ABACUS-T	69.12%	0.09	0.85	0.08	3.52	-1.36
OPUS-Design	73.15%	0.08	0.77	0.07	3.69	-1.52
CASP15						
PiFold	56.53%	0.14	1.41	0.15	2.56	-2.11
ProteinMPNN	51.36%	0.14	1.50	0.17	2.32	-1.83
LigandMPNN	56.81%	0.14	1.37	0.16	2.55	-1.92
CARBonAra	60.10%	0.13	1.06	0.16	2.85	-2.12
ESM-IF	64.22%	0.12	1.22	0.16	2.86	-1.74
ABACUS-T	60.74%	0.13	1.17	0.15	2.74	-1.64
OPUS-Design	65.18%	0.11	1.00	0.13	3.13	-1.71

Table A3 Sequence recovery of 3DCNN-based methods on the CAMEO dataset. All OPUS-Design variants in this table use a single 3DCNN prediction pass, without feature aggregation or ESM-2 refinement.

Method	All residues	Core residues
ProDCoNN	37.91%	42.59%
DenseCPD	43.85%	52.00%
OPUS-Design (3DCNN-only)	54.42%	65.95%
OPUS-Design (3DCNN w/o side-chain density)	50.83%	61.81%
OPUS-Design (3DCNN w/o side-chain density and voxel occupancy)	49.50%	59.67%

Table A4 OmegaFold-based evaluation of different methods on the CAMEO-TM90 dataset. Sequence recovery is calculated as the percentage of correctly predicted residues among all residues.

	Sequence recovery (%)	sc-TM	OmegaFold confidence
PiFold	53.36%	0.86	85.04
ProteinMPNN	48.37%	0.89	89.62
LigandMPNN	52.46%	0.89	89.15
CARBonAra	54.10%	0.88	86.00
ESM-IF	60.67%	0.90	88.89
ABACUS-T	59.28%	0.92	90.57
OPUS-Design	63.58%	0.90	88.32
OPUS-Design-rand18	63.00%	0.93	89.14

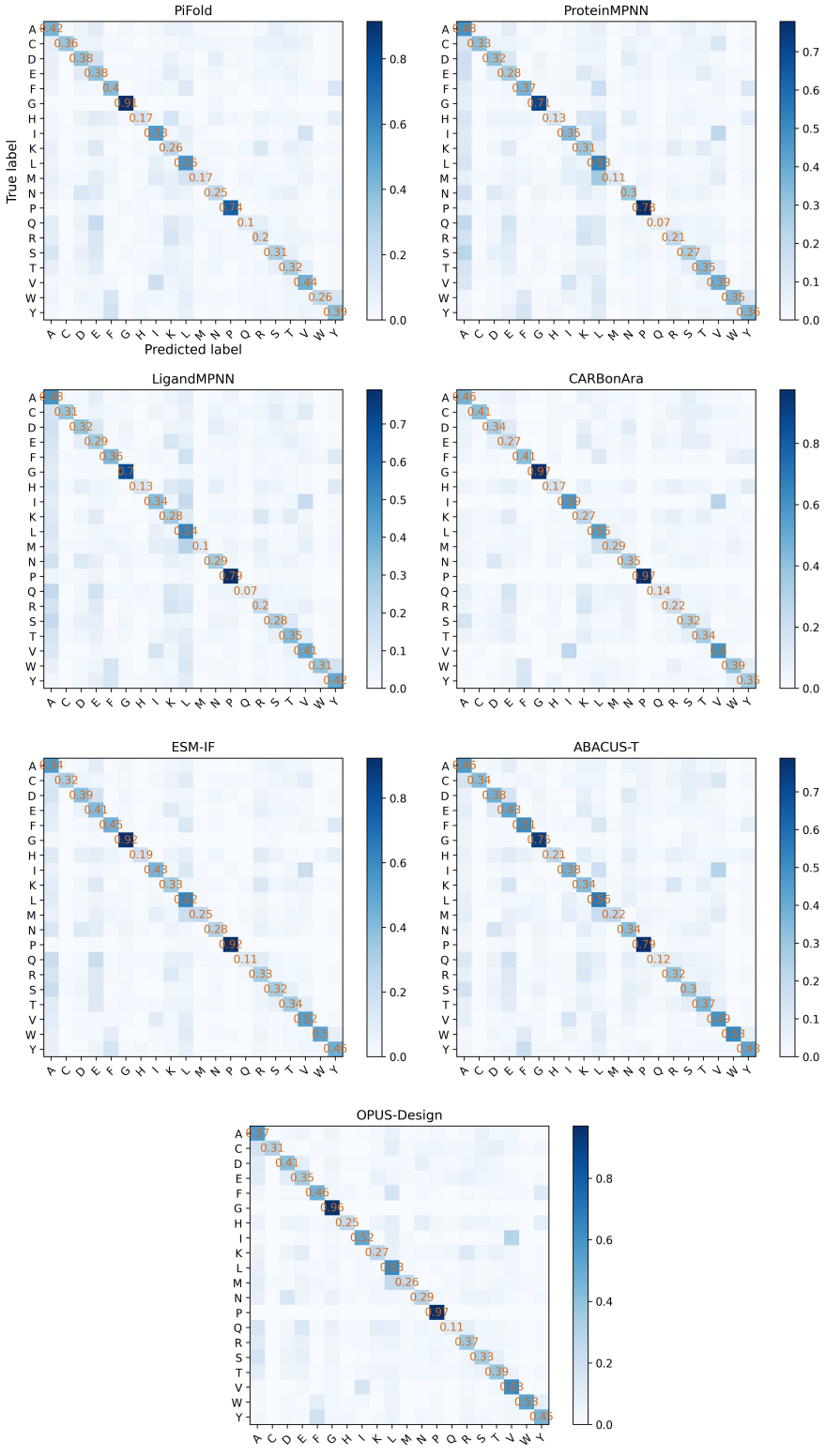

Figure A1 The confusion matrices for each method on the CASP15 dataset, evaluated across all residues.

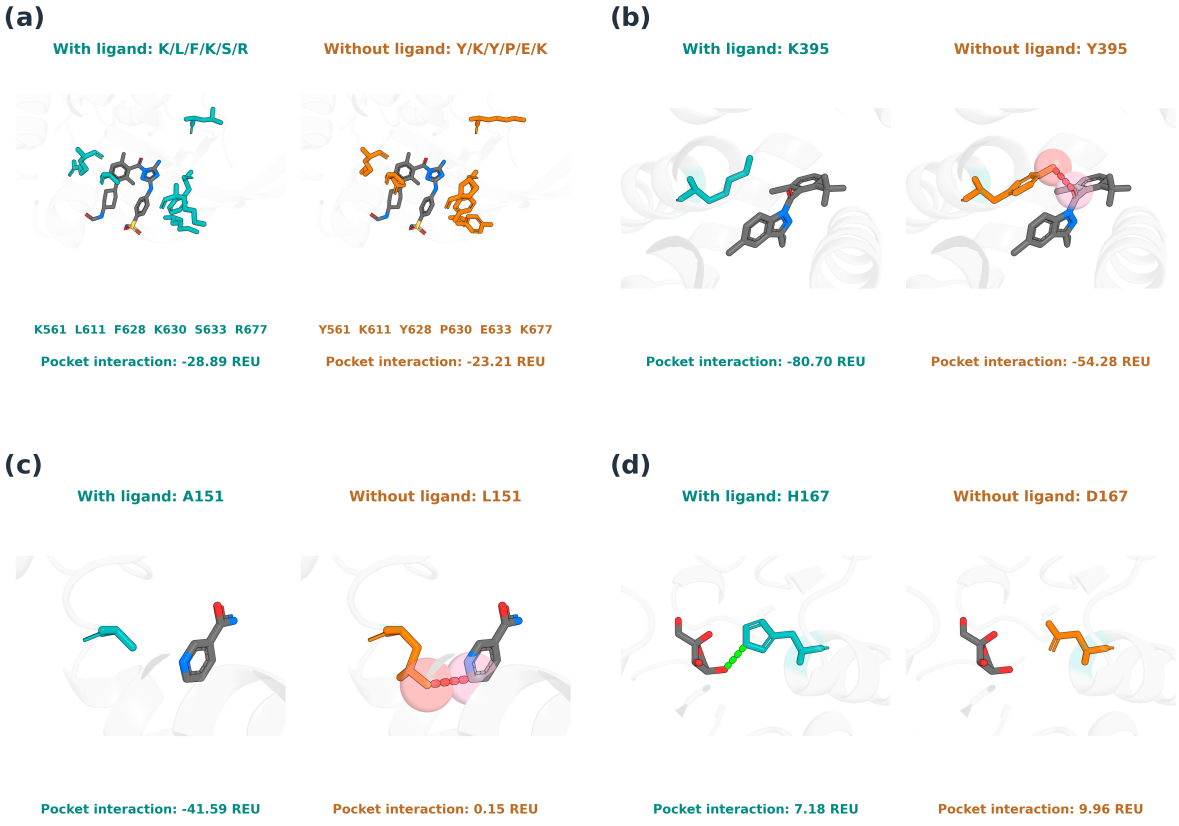

Figure A2 Ligand-dependent changes in four OPUS-Design pocket examples: (a) 9nd3, (b) 9gw3, (c) 9i3y, and (d) 9i1m. The ligand is shown in both views but is provided to OPUS-Design only in the "With ligand" condition. Panel (a) labels all six changed ligand-related residues, (b-d) highlight chain A residues 395, 151, and 167, respectively. Red marks indicate short modeled contacts in (b) and (c), and the green dashed line indicates a possible hydrogen bond in (d). "Pocket interaction" is the unnormalized Rosetta cross-energy (REU) between ligand-related residues. It is not the energy of a single contact or a measure of experimental binding affinity.