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DRUG DISCOVERY

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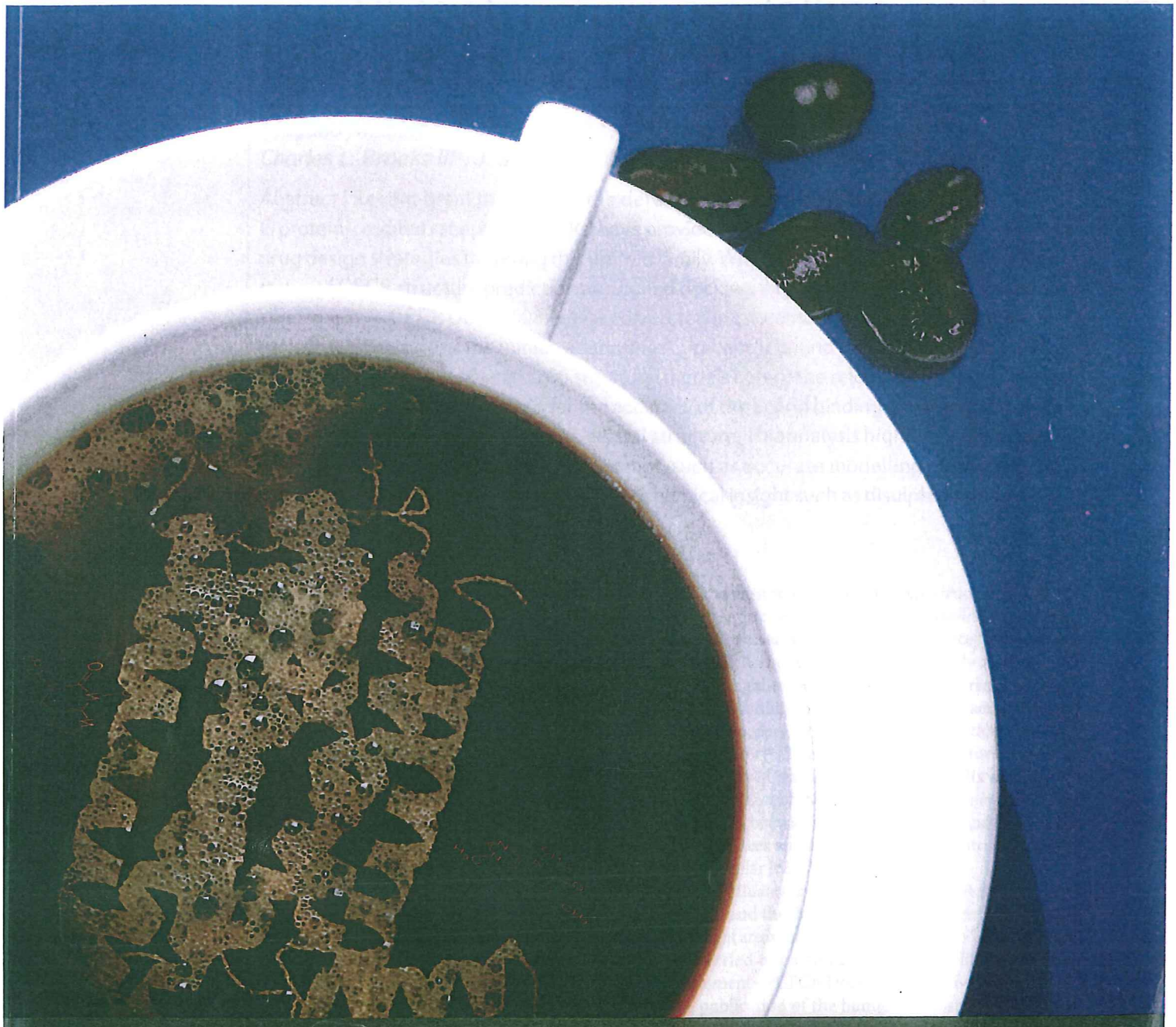

KINASES IN INFLAMMATION

Potential targets for a new generation of oral anti-inflammatory drugs

GPCR structure modelling and ligand docking

A community-wide assessment

Community-wide assessment of GPCR structure modelling and ligand docking: GPCR Dock 2008

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Abstract | Recent breakthroughs in the determination of the crystal structures of G protein-coupled receptors (GPCRs) have provided new opportunities for structure-based drug design strategies targeting this protein family. With the aim of evaluating the current status of GPCR structure prediction and ligand docking, a community-wide, blind prediction assessment — GPCR Dock 2008 — was conducted in coordination with the publication of the crystal structure of the human adenosine A_{2A} receptor bound to the ligand ZM241385. Twenty-nine groups submitted 206 structural models before the release of the experimental structure, which were evaluated for the accuracy of the ligand binding mode and the overall receptor model compared with the crystal structure. This analysis highlights important aspects for success and future development, such as accurate modelling of structurally divergent regions and use of additional biochemical insight such as disulphide bridges in the extracellular loops.

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Molecular modelling has an important role in rational drug design[1,2]. Reliable three-dimensional models can provide valuable insights into basic principles of molecular recognition and aid in structure-based approaches to lead discovery and optimization[3]. G protein-coupled receptors (GPCRs) are membrane proteins involved in signal transduction pathways and are important therapeutic targets for numerous diseases[4,5]. As such, significant structure prediction efforts using methods ranging from *de novo* to homology-based approaches have been applied to members of the GPCR family[6,7].

Until recently, most GPCR homology modelling efforts have been based on the templates of bovine rhodopsin and bacteriorhodopsin, with refinement of the models achieved through molecular dynamics simulations, ligand docking and incorporation of additional biochemical and biophysical data[8–12]. The refinement step is necessary in building accurate models, especially around the ligand-binding site, owing to the expected structural differences among members of the family. These differences result from the generally low sequence identity and the large diversity of ligands accommodated within the family[7,13–15], and from the various conformational states that are associated with different levels of ligand efficacy[16–18].

The most recently solved GPCR structure is the 2.6 Å crystal structure of the human adenosine A_{2A} receptor bound to an antagonist[19]. Adenosine receptors belong to the class A rhodopsin-like GPCR family and represent promising therapeutic targets in a wide range of conditions, including cerebral and cardiac ischaemic diseases, sleep disorders, immune and inflammatory disorders, and cancer[20]. The A_{2A} receptor structure shows an overall seven transmembrane (TM) helix architecture similar to that of the rhodopsin and adrenergic receptor structures, but with shifts in the positions and orientations of the helices and a markedly different structure of the extracellular loops[19].

To evaluate current progress in GPCR structure prediction and the docking of potential ligands, as well as highlight areas for future efforts in method development, we carried out a community-wide, blind prediction assessment — GPCR Dock 2008 — in coordination with the publication of the human adenosine A_{2A} receptor structure in October 2008 (REF. 19). GPCR Dock 2008 was organized in a similar manner to the previous CASP (Critical Assessment of methods of Protein Structure) and CAPRI (Critical Assessment of PRediction of Interactions) studies[21,22]. In this paper, we report the

Rhodopsin and bacteriorhodopsin
These two light-activated membrane proteins have a seven transmembrane alpha-helical bundle architecture that is similar to the general structure of the larger GPCR family.

outcome of the assessment together with our analysis of the current status of GPCR structure and ligand docking predictions.

GPCR Dock 2008

In August 2008, before the publication of the human adenosine A_{2A} receptor structure in October 2008 (REF. 19) and public release of the three-dimensional coordinates, participants were asked to predict and submit up to ten ranked models of the human A_{2A} receptor in complex with the ligand ZM241385, starting from the amino acid sequence of the receptor and a two-dimensional structure of the ligand (see BOX 1 for list of GPCR Dock 2008 participants). A total of 63 different groups initially registered, with 206 models submitted by 29 different groups in the final data set (see Supplementary information S1 (box) for details). Of the 206 submitted models, 37 were either missing the ligand or had incorrect bond connectivity for the ligand. We assessed the remaining 169 models for the prediction accuracy of the ligand binding mode, and all 206 models were assessed for the prediction accuracy of the receptor alone.

Box 1 | GPCR assessment participants

- Arthur Olson: Department of Molecular Biology, The Scripps Research Institute, USA
- Wiktor Jurkowski and Arne Elofsson: Center of Biomembrane Research, Department of Biochemistry & Biophysics, Stockholm University, Sweden
- Slawomir Filipek: Laboratory of Biomodelling, International Institute of Molecular and Cell Biology, Poland
- Irina Pogozheva and Andrei Lomize: Peptide Synthesis and Molecular Recognition Laboratory, University of Michigan, USA
- Bernard Maigret: Orpailleur team, LORIA, Nancy University, France
- Jeremy Horst, Brady Bernard, Shyamala Iyer and Ram Samudrala: Computational Biology Group, University of Washington, USA; Ambrish Roy and Yang Zhang: Department of Molecular Biosciences, Center for Bioinformatics, University of Kansas, USA
- Osman Ugur Sezerman: Biological Science and Bioengineering, Sabanci University, Turkey
- Gregory V. Nikiforovich: MolLife Design LLC, USA; Christina M. Taylor: Department of Biochemistry and Molecular Biophysics, Washington University, USA
- Stefano Costanzi: Laboratory of Biological Modeling, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, USA
- Y. Vorobjev, N. Bakulina, and V. Solovyev: Department of Computer Science, Royal Holloway, University of London and Softberry Inc., UK
- Kazuhiko Kanou, Daisuke Takaya, Genki Terashi, Mayuko Takeda-Shitaka and Hideaki Umeyama: School of Pharmacy, Kitasato University and RIKEN Systems and Structural Biology Centre, Japan
- William A. Goddard III, Youyong Li, Soo-Kyung Kim, Bartosz Trzaskowski, Ravinder Abrol and Adam Griffith: Materials and Process Simulation Center, California Institute of Technology, USA
- Vsevolod Katritch, Manuel Rueda and Ruben Abagyan: Molsoft LLC, USA
- Ian Davis, Patrick Barth and David Baker: Department of Biochemistry, University of Washington, USA
- Michael Feig: Department of Biochemistry and Molecular Biology, Michigan State University, USA
- Michal Brylinski, Hongyi Zhou, Seung Yup Lee and Jeffrey Skolnick: Center for the Study of Systems Biology, Georgia Institute of Technology, USA
- Liliana Ostopovici-Halip and Cristian Bologa: Division of Biocomputing, University of New Mexico, USA
- Polo Lam and Ruben Abagyan: Department of Molecular Biology, The Scripps Research Institute, USA
- Eric S. Dawson, Kristian Kaufmann, Nils Woetzel and Jens Meiler: Center for Structural Biology, Vanderbilt University, USA
- Feng Ding, Adrian Serohijos, Shuangye Yin and Nikolay V. Dokholyan: Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, USA
- David Rodriguez and Hugo Gutiérrez-de-Terán: Fundación Pública Galega de Medicina Xenómica, Complejo Hospitalario Universitario de Santiago de Compostela, Spain
- Henri Xhaard: Center for Drug Research, Faculty of Pharmacy, University of Helsinki, Finland

For full details, see Supplementary information S1 (box).

Assessment criteria. Assessment criteria are dependent on the purpose of the generated models. Given the value of the GPCR structural models in expanding our knowledge in basic molecular recognition and their potential use in the design and development of new small molecules, the quality of the models was primarily assessed by the accuracy of the ligand binding mode. Particular attention was given to the fact that the crystal structure is a static structure with positional errors, and the value of modelling is ultimately to guide drug discovery and provide biological insight. Our numerical measure of accuracy for the ligand binding mode was based on two metrics: ligand root mean square deviation (RMSD) and the number of correct receptor–ligand contacts. Neither metric alone was sufficient to capture the accuracy of prediction around the ligand binding site; hence, both were used and combined into a z-score to rank the models.

The ligand RMSD between the model and the crystal structure was calculated as the coordinate RMSD for the 25 non-hydrogen atoms of ZM241385 after superimposing the Cα atoms of the protein in the model and the crystal structure. In addition, the ligand RMSD is also calculated excluding the phenoxy group of ZM241385 that has high B-factor values. The number of correct contacts is counted as the number of correctly predicted native contacts observed between protein atoms and the ligand. A native contact is defined as any interatomic distance within 4 Å of the ligand in the crystal structure. There are 75 such receptor–ligand contacts, and an additional 15 contacts formed with water.

The models were ranked by assigning a combined mixed z-score to each model. The combined z-score was calculated as the average of z-scores for ligand RMSD and the number of correct contacts:

$$Z_{combined} = (-Z_{ligand\ RMSD} + Z_{Number\ of\ correct\ contacts})/2.$$

The z-scores for ligand RMSD and the number of correct contacts were computed by the following steps. First, a z-score was assigned to each model using the average and standard deviation (SD) values from all models. Second, the average and SD was re-computed excluding models with z-scores that were more than two SDs above (for ligand RMSD) or below (for the number of correct contacts) the average. Third, a z-score was reassigned to each model using the revised average and SD values obtained in step two. The best model — that is, the model with the highest combined z-score — from each group was analysed.

Table 1 | **Summary of results for the best models from the top ranking groups**

Group name	Rank (total number of models)	Ligand RMSD (Å)	Ligand RMSD without phenoxy group (Å)	Number of correct contacts	Binding site residues RMSD (Å)	Protein Cα RMSD (Å)	TM I–VII Cα RMSD (Å)	ECL2 Cα RMSD (Å)	Combined z-score (average ± SD)
Costanzi	2 (4)	2.8	2.7	34	3.4	3.0 (266)	2.5 (212)	3.8 (8)	3.02 (0.86 ± 1.48)
Katritch & Abagyan	1 (10)	6.2	4.0	40	3.5	4.0 (283)	2.7 (214)	8.9 (23)	2.76 (1.89 ± 1.13)
Lam &Abagyan	1 (3)	5.7	3.6	33	3.3	4.1 (283)	3.6 (214)	7.3 (23)	2.42 (0.88 ± 1.34)
Davis, Barth & Baker	4 (5)	5.8	5.4	18	4.0	3.5 (283)	2.1 (214)	8.4 (23)	1.46 (0.16 ± 0.86)
Maigret	8 (10)	2.6	2.1	5	7.3	5.1 (283)	4.1 (214)	9.1 (23)	1.23 (0.05 ± 0.57)
Jurkowski & Elofsson	2 (8)	5.3	5.2	10	3.9	6.2 (283)	2.9 (214)	12.7 (23)	1.04 (–0.02 ± 0.98)
Kanou	7 (10)	5.4	5.5	8	6.9	3.5 (279)	2.8 (214)	7.1 (23)	0.91 (0.66 ± 0.11)
Goddard	8 (10)	5.0	3.9	5	4.8	4.3 (284)	2.5 (214)	10.7 (23)	0.78 (0.16 ± 0.37)
Bologa	3 (10)	6.7	2.8	9	3.9	3.4 (278)	2.5 (213)	7.2 (19)	0.72 (–0.14 ± 0.39)
Olson	1 (9)	4.8	4.7	3	5.8	3.5 (284)	2.3 (214)	7.5 (23)	0.69 (–0.14 ± 0.58)

Participants were allowed to submit up to 10 models. Rank indicates the ranking that the participant assigned to their best model as determined in the GPCR Dock 2008 study with the total number of models submitted by that participant in parentheses. The root mean square deviation (RMSD) values were calculated for the heavy atoms of the ligand ZM241385 (all 25 atoms and partially without the phenoxy group), heavy atoms of the binding site residues (F168$^{5.29}$, E169$^{5.30}$, M177$^{5.38}$, W246$^{6.48}$, L249$^{6.51}$, H250$^{6.52}$, N253$^{6.55}$, H264$^{6.66}$, M270$^{7.35}$), Cα atoms of all residues, Cα atoms of residues in the transmembrane helices (TM) I to VII (helix I: 6–34; helix II: 40–67; helix III: 73–107; helix IV: 117–142; helix V: 173–205; helix VI: 222–258; helix VII: 266–291), and Cα atoms of resides in extracellular loop 2 (ECL2) (143–172 excluding 149–155 that are missing in the crystal structure). All RMSD values were obtained after the models were superimposed to the crystal structure, using the protein Cα atoms in PyMOL (version 1.0r2, www.pymol.org). The assignment of residues in the ligand-binding site and the secondary structure elements is from the Protein Data Bank header section (PDB ID: 3EML). The number of residues used in the RMSD calculation is in brackets. The combined z-score value for the best model, as well as the average and standard deviation (SD) values for all models submitted by each group, are shown.

Other sources of error include not modelling the water molecules that are either structurally important or directly involved in ligand binding interactions[3]. The ligand binding cavity in the A_{2A}–ZM241385 structure has four ordered water molecules[19], yet none of the submitted predictions included water molecules. We tried re-docking the ligand to the crystal structure using ICM[28] and found that a native-like binding pose (within 1 Å heavy atom RMSD for the bicyclic ring and the furanyl substituent of the ligand, and less than 3 Å overall ligand RMSD) can be recovered without any water molecules, which suggests that water may not be critical for accurately predicting the ligand interactions. However, modelling water molecules together with the ligand might contribute to a better prediction of the ligand binding pose or affinity. Additional re-docking studies with the docking protocols used by the participating methods would help assess the effect of the water molecules, and the accuracy of the docking methods separately from that of the receptor modelling methods.

Finally, it is interesting that the best model was from the S. Costanzi group, which has previously worked on adenosine receptor modelling and docking. Their domain knowledge on the adenosine receptor is likely to have been crucial for the evaluation and interpretation of the mutagenesis and ligand interaction data.

Conclusions

Accurate prediction of GPCR structure and ligand interactions remains a challenge, and the approach will improve with the recent availability of experimentally solved GPCRs. Assessment of these predictions highlights similar issues addressed by the CASP predictions for template-based modelling targets; that is, the difficulty in loop modelling, refinement and improvement over the best available template and model ranking. Accurate modelling of the structurally divergent regions (such as the extracellular loops that form defined architectures), and disulphide bond formation affecting helix residue registry and helical shifts in the TM region seem to be crucial for accurately predicting the key ligand interactions in GPCRs, and this area is perhaps the most in need of technological development. Progress in GPCR modelling and docking will require further improvements in the current prediction methods to enhance the best available templates and generate models that will be more useful for applications in structure-based drug design.