to identify a spurious 'homology' between regions responsive to motion and body parts in monkeys and faces, prosody and social cognition in humans. This phenomenon provides a rival explanation for some of the putative homologies Mantini *et al.*[1] identify across anatomically divergent regions.

The susceptibility of the ISAC method to these pitfalls will no doubt be investigated in future studies, as will its ability to provide information on homology outside the visual system. Subsequent studies could replicate interspecies activity correlations across different types of stimulus sets, including those that monkeys and humans are more likely to perceive in similar ways. In addition, putative homologies could be corroborated by follow-up fMRI or electrophysiological studies attempting to find the critical features that drive regional activity across species.

Another exciting future direction is the hybridization of the ISAC method with established techniques for retinotopic and functional mapping so that candidate homologous regions may be jointly constrained by interspecies activity correlations and similarity in functional responses. Ultimately, this type of approach could be used to integrate other types of information as well, including patterns of gene expression and cytoarchitecture. This strategy may be supplemented by methodological developments. Multivariate methods[6] can be used to both bypass the need for a priori definition of 'seed' regions based on anatomy and simultaneously consider multiple sources of information. Pattern-based methods could be used to identify regions that code for similar types of information across species[4].

In sum, the ISAC approach will lead to new opportunities, new challenges and new conflicts to be resolved, as any new technique does. It comes at a crucial and exciting time in the neurosciences, as new techniques including fMRI can finally be used to directly map brain function in humans and other species, and this wealth of parallel information must be integrated to bring insights from animal models to bear on the human condition in increasingly precise ways.

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1. Mantini, D. *et al. Nat. Methods* **9**, 277–282 (2012).
2. Ongur, D. & Price, J.L. *Cereb. Cortex* **10**, 206–219 (2000).
3. Sereno, M.I. & Tootell, R.B. *Curr. Opin. Neurobiol.* **15**, 135–144 (2005).
4. Kriegeskorte, N. *et al. Neuron* **60**, 1126–1141 (2008).
5. Schneider, K.A. & Kastner, S. *J. Neurosci.* **29**, 1784–1795 (2009).
6. Krishnan, A., Williams, L.J., McIntosh, A.R. & Abdi, H. *Neuroimage* **56**, 455–475 (2011).

Modeling cellular signaling: taking space into the computation

Michael W Sneddon & Thierry Emonet

In living systems, chemical reactions and the geometry of cells feed back on each other. Methods for computational modeling are beginning to take this complexity into account.

We often describe cellular signaling networks in terms of circuits or wiring diagrams, but that view is incomplete. In a computer, computation takes place on a static network of electric wires. In biological systems, computation takes place in networks of biochemical reactions. A key difference is that biochemical reactions actively change the shape of cells and organelles over time, directly affecting the spatial position of molecules and the possible biochemical reactions that can occur. In other words, cells and tissues constantly remodel the structure of the computing networks—the 'wires'—by altering the spatial organization of the reactants. Hence, in biology the remodeling of the network is as important to the computation as the reactions taking place. A new computational method described in this issue of *Nature Methods*[1] now makes it easier for biologists to model such feedback between chemical reactions and geometry.

Taking into account this feedback in computational models requires addressing two key challenges. First, one must track the effects of chemical reactions on the mechanics and geometry of cells and tissues[2]. Second the network of possible biochemical reactions must be locally reconstructed as the cellular geometry changes, a task that is computationally expensive because of the combinatorial complexity of signaling networks[3].

Angermann *et al.*[1] describe a new computational approach that addresses these challenges by combining advanced spatial simulation techniques with a rule-based description of molecular interactions (**Fig. 1**). They implement this hybrid approach in the general-purpose software platform Simmune[4], which provides a flexible graphical interface for constructing and simulating such models. Together, the new methods and graphical modeling environment of Simmune should expand the scope of spatial models of cellular signaling and accelerate the construction of new models.

One of the standard methods for modeling the spatial localization of biochemical reactions is partial differential equations (PDEs). With this approach, three-dimensional (3D) spatial compartments, such as cells and organelles, are subdivided into small volumes, called voxels. PDEs are then used to compute how the average concentration of every molecular species in each voxel changes over time. As the shape of the compartment changes, voxels can be deformed, added or removed. Some simulation tools are starting to provide these spatial capabilities, such as CompuCell3D (ref. 5) and Virtual Cell[6]. A fundamental limitation of PDE models is that a separate equation and variable is required for every potential molecular species. Taking protein complexes and post-translational modifications into account typically creates an overwhelming number of possible molecular species. This difficulty has been termed the problem of combinatorial complexity[3].

The new methods implemented in Simmune address the problem of combinatorial complexity by using an established rule-based approach[3,7,8] to define the biochemistry of the system. Rules describe only the minimal conditions required for a reaction to occur. Thus, a small set of interaction rules can succinctly represent, without approximation, a much larger reaction network composed of many

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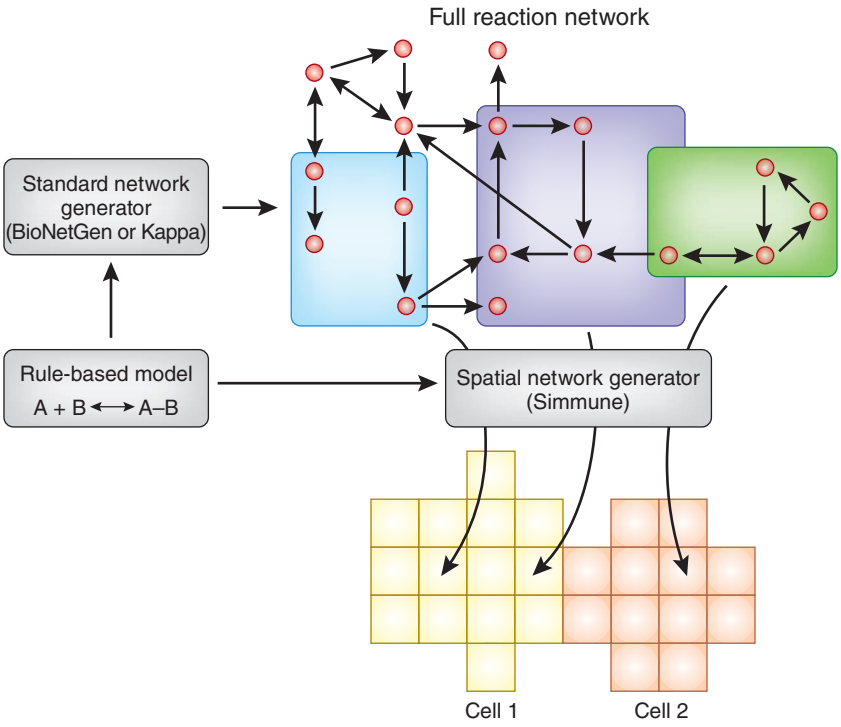

Figure 1 | Combination of rule-based modeling with dynamic cellular geometries. Existing network generators yield the full reaction network given a set of interaction rules, typically without spatial information. A spatial reaction network generator uses rules to map possible reactions to specific cellular locations. The reactions can then be simulated and voxels added or removed to model changes in cellular geometry.

distinct molecular species. Rules also facilitate incremental model building because molecular details can be incorporated as they become available from new experimental results, without major revisions of the model[9].

Software such as BioNetGen and Kappa can then convert a set of rules into the complete set of reactions[7,8]. However, generating the full reaction network for spatial simulations is not required because many reactions can only occur in certain cellular locations. The key advance of Simmune is a new reaction network generator that intelligently decides which voxels require which subset of reactions (**Fig. 1**). For instance, if a molecular interaction can never occur on the membrane surface, then the reaction is not added to the membrane voxels. This provides two important advantages: first, the burden of constructing a new spatial model is reduced because researchers only need to specify a relatively small set of rules. Second, simulations run more efficiently because molecular interactions that do not occur in a particular cellular location are never considered during the simulation.

Angermann *et al.*[1] demonstrate these advantages by constructing a model of the mitogen-activated protein kinase (MAPK) signaling pathway involved in the pheromone response of yeast. Like many eukaryotic signaling networks, the MAPK response is inherently a spatial process in which a localized signal generates an internal concentration gradient of activated kinase. Additionally, signaling molecules in the pathway can form large complexes and can be phosphorylated on multiple sites, requiring a model that accounts for more than 150 reactions in some voxels. The model of Angermann *et al.*[1] reproduced the complex spatial pattern of intracellular kinase activation observed in recent experiments.

Using rules to automatically assign reactions to specific voxels has yet another key advantage: it allows voxels to be efficiently added during simulation (because a computationally costly step of creating new voxels is in deciding which reactions should be modeled within each new voxel). This feature allows the shape of a cell to change dynamically during simulation. To correctly model changes in cell morphology based on physical forces, Angermann *et al.*[1] use a 3D cellular Potts model[10], a type of model created by defining an energy cost for adding new connected voxels or removing existing voxels on the cell surface. If adding or removing a voxel is energetically favorable, then the action is carried out with some probability. The energy function can be defined both in terms of physical forces and the biochemical state of the system, which allows the signaling network to feed back on cellular morphology.

One important application of this method is the study of cellular adhesion, a process that remains challenging to simulate in detail because of the complexity of adhesion complexes and the dynamic shape of the cell. With Simmune, Angermann *et al.*[1] built a model of E-cadherin–mediated cell adhesion that demonstrated the importance of considering the dynamic feedback between the biochemistry and the shape of the cell. Only a dynamic cellular geometry could properly account for the distribution of E-cadherin complexes on the surface of the interacting cells.

The new features of Simmune are a welcome addition to the computational modeling toolbox, but there are still many unresolved challenges in building spatial models of cellular signaling. For instance, in many situations, such as when details of receptor aggregation are considered, particle-based approaches[11,12]—which track the individual 3D location of each molecule and simulate rules directly to avoid full network generation—are needed to properly account for system dynamics. Future research is necessary to determine how exactly to combine PDE-based methods with existing rule-based particle methods[7,9]. Additionally, as the interpretation of new experiments requires larger and more complicated computational models, simulation efficiency of spatial models becomes a key bottleneck. Much work is still required to devise efficient methods for running spatial simulations of cellular signaling on multicore processors and parallel supercomputers.

The methods implemented in Simmune signify a growing trend toward multiscale, hybrid approaches that combine the features of multiple modeling methods. We expect that this trend will greatly expand the scope and accessibility of biological models in the near future but will require continued development and support of advanced software applications such as Simmune.

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1. Angermann, B.R. *et al. Nat. Methods* **9**, 283–289 (2012).
2. Mogilner, A. & Odde, D. *Trends Cell Biol.* **21**, 692–700 (2011).
3. Hlavacek, W.S. *et al. Sci. STKE* **2006**, re6 (2006).
4. Meier-Schellersheim, M. *et al. PLOS Comput. Biol.* **2**, e82 (2006).

5. Izaguirre, J.A. *et al. Bioinformatics* **20**, 1129–1137 (2004).
6. Moraru, I.I. *et al. Systems Biol. IET* **2**, 352–362 (2008).
7. Danos, V., Feret, J., Fontana, W. & Krivine, J. *Lect. Notes Comput. Sci.* **4807**, 139–157 (2007).
8. Faeder, J.R., Blinov, M.L. & Hlavacek, W.S. *Methods Mol. Biol.* **500**, 113–167 (2009).
9. Sneddon, M.W., Faeder, J.R. & Emonet, T. *Nat. Methods* **8**, 177–183 (2011).
10. Glazier, J.A. & Graner, F. *Phys. Rev. E Stat. Phys. Plasmas Fluids Relat. Interdiscip. Topics* **47**, 2128–2154 (1993).
11. Andrews, S.S., Addy, N.J., Brent, R. & Arkin, A.P. *PLoS Comput. Biol.* **6**, e1000705 (2010).
12. Takahashi, K., Tănase-Nicola, S. & ten Wolde, P.R. *Proc. Natl. Acad. Sci. USA* **107**, 2473–2478 (2010).

Small is beautiful

Keith Moffat

Using two independent methods, researchers show that *in vivo*–grown crystals of soluble proteins and of membrane proteins grown in the lipidic sponge phase can be analyzed by serial femtosecond crystallography on an X-ray free electron laser.

X-ray scattering by electrons has been a brilliantly successful technique for three-dimensional structure determination. The atomic-scale imaging afforded by X-ray scattering provides a 'seeing is believing' route that powerfully addresses questions in disciplines ranging from physics and chemistry through materials science to geophysics and structural biology. Two approaches by Duszenko and colleagues[1] and Neutze and colleagues[2] make use of short X-ray pulses of exceptionally high intensity to advance the way crystals are prepared and processed for X-ray diffraction analysis, and thus to yield more structural information from much smaller crystals.

Whether a particular scattering experiment is in fact feasible depends on the properties of the X-ray source itself. Electrons do not scatter hard X-rays efficiently. Even the most brilliant of today's storage ring X-ray sources require quite large crystals (1–50 micrometers containing 10^{10}–10^{12} molecules) to scatter sufficient X-rays to generate high-quality diffraction patterns, from which structural information can be obtained. If a much more brilliant X-ray source was available, smaller samples containing fewer electrons could in principle be examined.

The revolutionary advance of using an X-ray free-electron laser (XFEL; **Fig. 1**) as an effective source of hard X-rays has recently occurred[3]. XFELs constitute disruptive technology: they are distinguished from storage ring sources by their ten-orders-of-magnitude greater brilliance, three-to-four-orders-of-magnitude shorter X-ray pulses, nearly monochromatic nature and coherence. In principle, enough X-rays can be scattered by the electrons in a single cell, a nanocrystal or even a single protein to permit determination of its structure. However, the greater brilliance also means that all samples are ultimately destroyed by the X-rays, which led to proposal of a 'diffract and destroy' approach exploiting the fact that diffraction precedes destruction[4].

The first hard XFEL in the world known as the Linac Coherent Light Source (LCLS) has recently begun operation at the SLAC National Accelerator Laboratory at Stanford University. In two papers in this issue[1,2], two groups present the results of preliminary experiments at the LCLS. Both groups introduced their crystals into a beam of hard X-ray pulses of femtosecond duration, obtained diffraction patterns and began to extract quantitative structural information. Their results thus validate the 'diffract and destroy' approach[4] and point the way to developing new classes of experiments that are impossible at storage ring sources.

Duszenko and colleagues noticed needle-shaped microstructures, which electron microscopy suggested were single protein crystals, in intact Sf9 insect cells[1]. Crystallization of proteins *in vitro* is an arduous process with uncertain outcome that normally requires molecular homogeneity. It was not clear whether these *in vivo* microstructures in the highly heterogeneous, crowded environment of a cell were well-ordered, single protein crystals and, if so, whether structural information could be obtained from them. Researchers had earlier reported isolated occurrences of *in vivo* crystallization of seed-storage proteins, insulin granules[5] and subunits of calcineurin[6] in the Sf9 heterologous protein expression system. Koopmann *et al.*[1] collected stable, intact crystals of a partially glycosylated cathepsin by lysis of the Sf9 cells and differential centrifugation. However, these did not yield single-crystal diffraction at a storage ring beamline, probably owing to their small size rather than lack of crystalline order.

In a step that makes conventional crystallographers shudder, they crushed these crystals to obtain nanocrystalline fragments, which they injected into the brilliant X-ray beam at the LCLS, using the ingenious liquid jet injector devised by DePonte, Spence and colleagues[7] that allows smooth injection of hundreds of thousands of nanocrystals or crystal fragments. Only a small fraction of droplets in this jet contained a crystal and, when hit by an X-ray pulse, generated a very limited diffraction pattern. However, from

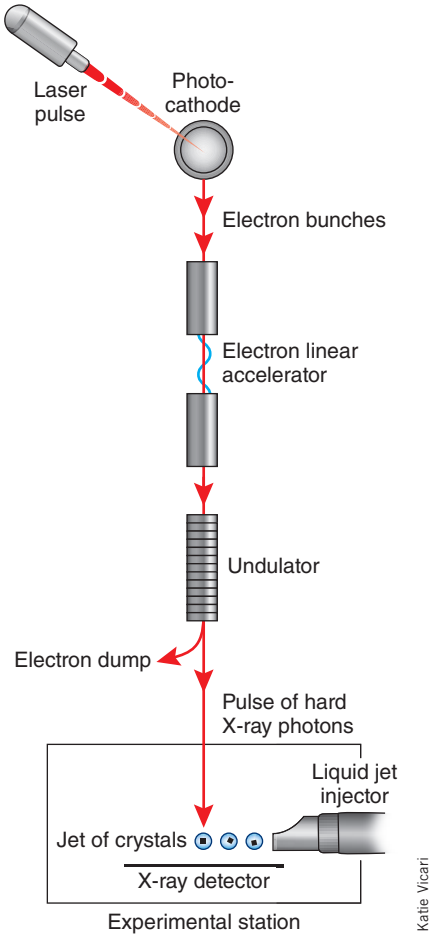

Figure 1 | Schematic of an XFEL–based beamline.

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