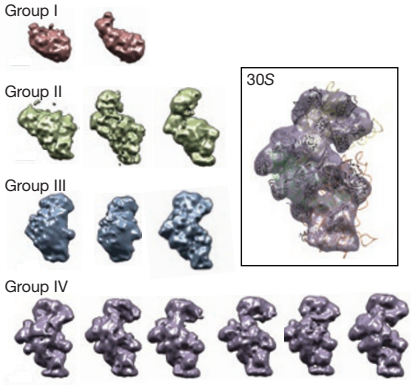
IMAGING

The birth of a ribosome

A team of researchers applied a 'discovery single-particle profiling' experimental strategy to visualize the assembly of the ribosome via time-resolved electron microscopy.

Being interested in understanding ribosome biogenesis, several years ago James Williamson of The Scripps Research Institute thought about using electron microscopy to study ribosome assembly, a poorly understood process. This was an extremely challenging experimental question in need of a good method. Meanwhile, Bridget Carragher and Clinton Potter, also at Scripps, who direct the National Resource for Automated Molecular Microscopy (NRAMM, funded by the National Center for Research Resources), were looking for interesting collaborative projects through which to showcase their automated electron microscopy imaging and data processing pipeline. "Finally, there was a convergence," says Williamson. "The planets aligned; they had the horsepower, and we had this wonderful problem, and we put it together."



Fourteen distinct ribosome assembly intermediate structures were classified by DSP into four groups. The earliest intermediates are the smallest (group I); the latest intermediates are the largest (group IV) and most resemble the intact 30*S* subunit (boxed). Reprinted with permission from the American Association for the Advancement of Science.

The success of the collaboration is reflected in the exciting new insights into 30*S* ribosome subunit assembly that the researchers recently obtained. Using time-resolved electron microscopy, they identified 14 distinct, partially assembled 30*S* subunit structures that could be assigned to one of four timepoint groups. They observed the populations of these intermediates changing over time, and surprisingly, they found that there was more than one way to build a ribosome. "Sometimes one set of proteins would bind first, and sometimes another set would bind first," explains Williamson.

In a typical single-particle electron microscopy experiment to determine the structure of a biological entity, samples are carefully purified to obtain a homogenous population of identical particles. But using this approach to try to tackle the mechanism of a complex, multistep assembly process such as ribosome biogenesis, purifying identical assembly intermediates and analyzing them individually by electron microscopy would have been so labor-intensive so as to be nearly impossible. The Scripps team thus took a different tactic, implementing a new experimental concept called 'discovery single-particle profiling' or

SYSTEMS BIOLOGY

A MODEL BRAIN

A predictive model of intercellular metabolic interactions in the human brain is reported.

A complex tissue such as the brain cannot be completely modeled without taking into account the fact that it is spatially organized into different regions and that it consists of multiple different cell types.

A few years ago, researchers from the laboratory of Bernhard Palsson, at the University of California, San Diego, reported a genome-scale reconstruction of the human metabolic network, and subsequent refinements of this model included tissue-specific information as well. But cell-specific information was still lacking. In a recent paper, Nathan Lewis, Palsson and colleagues have now taken this work to a still finer-grained level, modeling metabolic coupling between different cell types in the human brain. They examined metabolic coupling between astrocytes and three different types of neurons—glutamatergic, GABAergic and cholinergic neurons.

To do this, they used the existing human metabolic network (named Recon 1) as the context for cell-specific information curated from the literature. "Basically, we used proteomic and transcriptomic data to help us understand what metabolic pathways are active in the brain," says Lewis. "Then we parsed this out into individual cell types and coupled the cells with known transporters that go between the cell types." This needed both manual curation of cell type–specific information from the literature, as well as the use of databases like the Human Protein Reference Database, the Human Protein Atlas and the Human Proteome Organization (HUPO) brain proteome project. What resulted were mathematical models that describe metabolic interactions between the defined cell types and that can be used, in simulations, to make predictions about systems-level properties of the brain.

The fluxes in the models, the researchers report, were consistent with what is known from experimental measurements, giving confidence that they are reasonably accurate representations of reality. What is more, the models could predict the empirically known protection of GABAergic neurons from cell death in early stages of Alzheimer's disease, as compared to glutamatergic or cholinergic neurons. These differences could be described in terms of the response to changes in central metabolic enzymes in individuals with the disease.

As Palsson points out, genome-scale reconstructions are integrations of hard biological data and are thus particularly useful for generating mechanistic understanding. "Most analysis

DSP, which, according to graduate student and first author Anke Mulder, "demonstrates the power of high-throughput electron microscopy as a tool for discovery-based science."

The basic idea of DSP is that you let the data tell you what is there, Mulder explains. The team took more than a million snapshots of a mixture of growing 30*S* ribosome subunits as they self-assembled from the 20 individual purified protein components and the single 16*S* rRNA molecule. They merged all of the snapshots from different time-points into a single dataset and then applied classification algorithms to sort out distinct structure populations. They then mapped how the populations had changed over time, from the very beginning of the assembly process to completion, the fully assembled 30*S* subunit.

The robust data-processing pipeline already in place at NRAMM was crucial for implementing DSP. "One of the roles of automation is to free people up to do whatever experiment they need to do because they're not limited by the data collection," explains Carragher. This DSP approach will certainly be applicable for studying the assembly process of other complex cellular structures that can self-assemble *in vitro*. "At the beginning, taking on this project seemed like a totally daunting task," says Carragher. "Now, with all the infrastructure in place, there are all kinds of interesting biological experiments that become possible."

In terms of furthering our understanding of ribosome biogenesis, Williamson is interested in following up using other methods to determine whether the *in vitro* ribosome assembly mechanism deduced by using DSP is how it also works *in vivo*.

Allison Doerr

RESEARCH PAPERS

Mulder, A.M. *et al.* Visualizing ribosome biogenesis: parallel assembly pathways for the 30*S* subunit. *Science* **330**, 673–677 (2010).

[of systems-level data] is statistically driven, which means it gives you clusters and correlations and a lot of inference-type information," he says. But when you add 'omics' data to a genome-scale mathematical model, as the researchers did in this study, "you suddenly start to see the mechanisms at work, for example, that underlie the generation of an expression profile," he says. In their analysis of cell type–specific metabolic changes in Alzheimer's disease, for instance, Lewis, Palsson and colleagues used the model to generate precise, molecular and testable hypotheses about the underlying basis for the difference between the neuronal types. Thus these models provide a mechanistic 'context' for high-throughput data 'content'.

One of the reasons it has been possible to build predictive models for metabolic networks is that there is a great deal known about the function of the gene products involved (these number about 1,500 open reading frames in *Homo sapiens* and about 1,300 in *Escherichia coli*), but there are more and more such data becoming available for other biological processes, such as transcriptional regulation, as well. Coupled with a shift in emphasis from data generation to data integration and analysis, we may be heading for a time when models much more routinely lead to a mechanistic understanding of biological processes.

Natalie de Souza

RESEARCH PAPERS

Lewis, N.E. *et al.* Large-scale *in silico* modeling of metabolic interactions between cell types in the human brain. *Nat. Biotechnol.* **28**, 1279–1285 (2010).

NEWS IN BRIEF

IMAGING

Video-rate stimulated Raman scattering

Stimulated Raman scattering (SRS) microscopy is a label-free optical imaging technique based on the detection of signature molecular bond vibrations. Saar *et al.* now report technical developments that greatly increase the SRS imaging speed, allowing video-rate SRS microscopy. These advances facilitated SRS imaging of the skin and of small molecule drug penetration into the skin of living mice and humans, highlighting the potential utility of SRS for clinical imaging.

Saar, B.G. *et al. Science* **330**, 1368–1370 (2010).

GENOMICS

It takes 1000 Genomes

The three pilot-phase projects of the international 1000 Genomes Project—whole-genome, high-throughput sequencing of two family trios, one of African and another of European ancestry, whole-genome sequencing at low coverage of 179 individuals from four populations, and exon sequencing in genomes of 697 individuals from seven populations—are now complete. The goal of the entire project is to decipher how genetic variation affects phenotype; the aim of the pilot phase was to compare sequencing strategies and catalog over 95% of commonly found variants in any individual.

The 1000 Genomes Project Consortium. *Nature* **467**, 1061–1073 (2010).

SYSTEMS BIOLOGY

Monitoring metabolite-protein interactions

The metabolome has received far less attention than the genome, the transcriptome and the proteome. Li *et al.* now present a large-scale, *in vivo* analysis of metabolite-protein interactions in yeast, demonstrating that metabolites may have more important regulatory roles than currently appreciated. Using an affinity purification–mass spectrometry approach, they found that 16 of 21 enzymes in the ergosterol biosynthesis pathway specifically associated with metabolites, as did 21 of 103 protein kinases.

Li, X. *et al. Cell* **143**, 639–650 (2010).

MOLECULAR BIOLOGY

Robust minicircle DNA production

In contrast to standard plasmid vectors, minicircle DNA vectors (which lack the bacterial plasmid DNA backbone) allow more efficient and long-term transgene expression. Minicircle DNA vectors have not been widely used, however, because they are challenging to produce. Kay *et al.* now describe an approach to improve minicircle production to the level of plasmid DNA preparation, based on a modified *Escherichia coli* that stably expresses inducible minicircle assembly enzymes.

Kay, M.A. *et al. Nat. Biotechnol.* **28**, 1287–1289 (2010)

BIOPHYSICS

A database for dynamics

Proteins are flexible molecules in constant motion; this motion is crucial for function. Experimental detection of the conformations that a protein samples is very challenging, but molecular dynamics simulations can help fill in some of the gaps. Meyer *et al.* present their molecular dynamics extended library (MoDEL) database, which contains native-state atomistic trajectories of more than 1,700 soluble proteins. The database is available at http://mmb.pcb.ub.es/MoDEL/.

Meyer, T. *et al. Structure* **18**, 1399–1409 (2010).