time-trace determination (Fig. 1b), detection of bleaching, determination of correction factors, molecule filtering and grouping (Fig. 1c), dwell-time analysis and distribution analysis (Fig. 1d). The program is distributed in compiled and open-source versions at http://isms.au.dk.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper (doi:10.1038/nmeth.3435).

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InferenceMAP: mapping of single-molecule dynamics with Bayesian inference

To the Editor: Single-particle tracking (SPT) grants unprecedented insight into cellular function at the molecular scale1. Throughout the cell, the movement of single molecules is generally heterogeneous and complex. Hence, there is a strong need to understand the multiscale nature of single-molecule dynamics in biological systems. We have previously shown that with high-density SPT, spatial maps of the parameters that dictate molecule motion can be generated to intricately describe cellular environments2,3. To date, however, there exist no publicly available tools that reconcile trajectory data to generate such maps. We address this void with InferenceMAP, an interactive software package that uses a power-Bayesian method to spatially map the dynamics of individual biomolecules (Supplementary Software 1 and 2).

High-density SPT methods, such as sptPALM4 and uPAINT5, capture thousands of molecule trajectories in a few minutes of acquisition at high spatiotemporal resolution. As input, InferenceMAP accepts trajectories reconstituted using one of the many available particle-tracking algorithms6. These massive trajectory data are treated with a Bayesian-inference mapping algorithm that, notably, imposes no constraints on trajectory lengths2,3. Our algorithm is compatible with different models of single-molecule motion, including hopping diffusion (Fig. 1a), active processes, confinement, and interaction energy–driven systems (Fig. 1b). Model-specific physical processes are distinguished and mapped, revealing rich landscapes of molecule dynamics.

Generating dynamical maps from single-particle trajectories is critically dependent on the meshing utilized. As local diffusivities may vary by orders of magnitude over a few hundred nanometers, meshes should locally adapt to match the characteristic size of molecule displacements. To this end, InferenceMAP offers adaptive meshing techniques that users may tune to fit the spatial organization of their single-molecule trajectories (Fig. 1a,c). In each zone of a mesh, dynamic parameters are inferred to give rise to a parameter landscape (Supplementary Software 1 and 2 and Supplementary Note).

Calculations can be performed in an automated fashion irrespective of the biological system, or parameters may be carefully adjusted to conform to desired mapping resolution and optimization constraints. Additionally, prior knowledge of the biological system can be incorporated in calculations via user-defined prior probabilities (Supplementary Note). Furthermore, a randomized optimization algorithm is available for exceptionally large problems, permitting mapping of entire cells (as in Fig. 1b). Localized analysis in
subregions is easily performed through a custom-selection macro. Resulting inferred data are exportable in image and ASCII formats. InferenceMAP offers a host of features to address a major concern of the SPT community: it reveals the parameters that dictate the motion of molecules. Moreover, in the burgeoning age of ‘big data’ experimental biophysics, it enables the extraction of sensible results from otherwise dense and complicated observations using a robust Bayesian method. InferenceMAP is controlled with a user-friendly interface and is compatible with Mac OS X and Windows. The software is freely available for academic use (source code is available upon signing a material transfer agreement), and updated versions may be downloaded from http://umr168.curie.fr/en/research-groups/locco/software and http://www.pasteur.fr/en/research/genomes-genetics/units-groups/jean-baptiste-masson.

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Chemically defined, albumin-free human cardiomyocyte generation

To the Editor: Human pluripotent stem cell (hPSC)-derived cardiomyocytes are important tools for cardiovascular research and have substantial therapeutic potential. Several efficient differentiation strategies have been devised to generate cardiomyocytes from hPSCs. Recently, we2,3 and Burridge et al.4 published defined, growth factor–free protocols for differentiating hPSCs to cardiomyocytes. Our Gsk3 inhibitor and Wnt inhibitor (GiWi) method (Fig. 1a) applies two small molecules at precise developmental stages to sequentially promote mesoderm formation and cardiomyocyte specification2–5. Although the medium—RPMI with B27-ins (B27 without insulin)—used in the GiWi protocol lacks animal sera and growth factors, the inclusion of bovine serum albumin (BSA) increases the cost and adds xenogeneic components. Recently, Burridge et al.4 described modifications to the GiWi method, including replacing B27-ins with recombinant human albumin and L-ascorbic acid 2-phosphate. They reported that albumin and L-ascorbic acid 2-phosphate are necessary for cardiomyocyte differentiation with high yield and purity.

We also simplified the GiWi protocol and developed an albumin-free cardiomyocyte differentiation platform (Supplementary Methods). First, we compared B27-ins (Supplementary Table 1) with other published recipes for cardiomyocyte differentiation5–9 and identified five commonly shared differentiation media supplements (transferrin, sodium selenite, progesterone, putrescine and BSA). RPMI containing these five components (5F) supported hPSC differentiation to cardiomyocytes: more than 90% of the cells expressed cardiac troponin T (cTnT+) compared to the purity of cardiomyocytes generated in RPMI/B27-ins (Fig. 1b). Removal of transferrin (4F) also produced 90% cTnT+ cells.

However, removal of BSA from 4F medium resulted in virtually no cardiomyocytes. Treatment with 12 µM CHIR99021 (CH) caused proliferous cell death in the absence of BSA. However, 6 µM CH produced >90% cTnT+ cells in the absence of albumin (Fig. 1b and Supplementary Fig. 1a). In addition, 2.5 µM IWP2 was sufficient to induce >90% cTnT+ cells (Supplementary Fig. 1b), which is lower than the 5 µM IWP2 required in the presence of BSA. Thus, in contrast to the observation of Burridge et al., we found that albumin is not necessary for cardiomyocyte differentiation and that in fact its presence diminishes activity of small-molecule agonists and antagonists of Wnt signaling. Basal RPMI lacking supplements supported hPSC differentiation to cardiomyocytes when we used the GiWi method (Supplementary Fig. 1c). DMEM, DMEM/F12 and MEM also supported cardiomyocyte differentiation, but RPMI outperformed these media (Supplementary Fig. 1d).

6 µM CH in albumin-free RPMI induced robust brachyury expression in hPSCs (Fig. 1c and Supplementary Fig. 2). However, 1% BSA or human recombinant albumin (HRA) completely blocked brachyury expression at CH concentrations up to 6 µM, demonstrating Wnt activation induced by Gsk3-inhibitor treatment is more efficient in media lacking albumin. 30 µM CH induced brachyury expression in medium containing 1% HRA (Fig. 1c).

This albumin-free GiWi (named GiWi2) protocol produced 88–98% cTnT+ cells with yields of greater than 106 cardiomyocytes/cm2 in multiple human embryonic stem cell (hESC) and induced pluripotent stem cell (iPSC) lines (Supplementary Table 2 and Supplementary Fig. 3). The GiWi2 protocol was equally effective with cells maintained in E8 or mTeSR1 media (Supplementary Fig. 4). These cardiomyocytes exhibited spontaneous contraction for more than 8 months (Supplementary Video 1).

These chemically defined, albumin-free conditions supported cardiac induction from hPSCs, as determined by cTnT (Fig. 1d), cardiac troponin I (cTnI; Fig. 1e), sarcomeric myosin heavy chain, α-actinin and Nkx2.5 expression (Supplementary Fig. 5). α-actinin showed clear Z-line localization (Fig. 1e), and connexin-43 localized to cell-cell junctions (Fig. 1f). The earliest wave-like spontaneous contractions were observed on day 7, and robust beating was observed by day 10 (Supplementary Video 1).