March 2024

# *Südhof Laboratory Manual*
## *Expectations, Rules & Regulations*

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## 1. General

In our lab, all members cooperate and collaborate. Projects are proprietary and don't overlap, but reagents, equipment, and ideas are not owned by anyone. Everyone has a separate project that is often related and adjacent to what other lab members do enabling collaborations without competition.

I ask everybody to keep a spirit of mutual help and cooperation, and I request that everybody contributes to the general progress and atmosphere of the lab. Moreover, our lab comprises a diverse group of people with a vast range of cultural, religious, and ethnic backgrounds. Everyone has to respect everyone else's cultures, beliefs, and boundaries – we will not tolerate inappropriate behaviors. Everyone has to follow the general rules for scientific practices and personal behaviors outlined here. Severe violations of lab standards can be a cause for termination.

Since we are a medium-sized, horizontally organized lab without much technical support, we need to distribute duties among lab members. Although individual lab duties are assigned to specific persons, everybody participates in keeping the lab running via a **collective responsibility**. Thus, everybody is expected to help everybody else as needed. Moreover, if anybody notices that something is broken, that there is misuse of equipment or a lack of cleanliness, or that an item needs to be ordered, he/she has the responsibility to deal with the problem immediately. I am particularly worried about people who ignore problems that do not directly impact their work – this is unacceptable. Please help to keep the whole lab running! I am forced to stop the project of anybody who does not do her/his share, no matter how important the project, because the functionality of the overall lab is more important than any individual project.

As you all know, I value data; I think they are what makes science fun. However, what for me personally is more important than data is **that everybody likes being in the lab**, that there are no non-scientific conflicts (as opposed to scientific differences in opinion that are often productive and enjoyable), and that people feel free to pursue their ideas. It makes doing science difficult if a lab member is inconsiderate, behaves egoistically, is messy, non-communicative, and/or does not use lab equipment correctly etc. I think that as a lab, we cannot accept people who are not willing to make a 100% effort for the overall lab and the people in it, no matter how good they are scientifically and how many data they produce.

Although this should not be necessary to mention explicitly, I expect everybody to behave courteously. Apart from the obvious absolute necessity of complying with the US rules against sexual harassment and other forms of inappropriate behavior (remember that not everything you think is funny is necessarily funny in the eyes of someone else), I require that people deal with each other respectfully and kindly, and that they assist and help each other. For me personally, it is more important that people in the lab like their work than that they are successfully producing data.

Another aspect of lab work that is crucial for the success of a scientist are work habits. I will not monitor when people come and leave, whether people work on weekends, or what vacations people take, although I appreciate it if everybody could be here by 10 am on weekdays and could tell me about prolonged absences. The amount of time spent in the lab is not the major determinant of success, it is how the time is spent that determines success! I urge you to be organized, not to have messy desks and lab benches, and to plan your work so that your projects move forward efficiently. **Your success depends on what you can actually do, i.e. what you discover and publish – quality not quantity counts, not where papers are published or how many papers but what is in the papers** (please also see section 8 on job applications below)! For me, your success in my lab provides many levels of satisfaction, including the pleasure of seeing you happy, the validation that I feel by having successful people come out of my lab, and finally the fact that we are both credited with a particular discovery, not only you or I. Both when you are in my lab and long after you leave, our careers are intertwined.

Finally, remember that a lab is also a social community. We have people from all over the world spanning many cultural and religious identities, and many of us are immigrants. Talk to each other in whatever language you feel comfortable without intruding on others. If you speak another language besides English, use it only when no one is there who doesn't understand that particular language. It is a question of being considerate – you shut people out if you speak a language they don't understand. Please use your judgement and be aware of the multicultural nature of our lab. It is this multiculturism that makes our shared experiences in science so rich and rewarding!

## 2. Science etiquette & publication policies

### 2.1 Key rules for experiments

- All experiments involving comparisons between samples have to be carried out blindly whenever humanly possible. This is not only required for behavioral or physiology experiments where it is crucial that the samples are blinded, but also for culture assays, ELISAs etc.
- All experiments must be performed at least three times independently (for example: immunoblot analyses of KO vs. wild-type mice require at least three littermate pairs, the same three pairs must be separately but identically analyzed). We distinguish between true replicates (independent experimental repetitions) and pseudo-replicates (multiple points in the same experiment)
- All experiments must be documented in digital lab notebooks with dates and descriptions and brief summaries of outcomes – even if an experiment failed (e.g., contamination etc)
- All raw data must be converted into electronic form and deposited in your section of Stanford box
- No selection of subsets of data for analysis is allowed; exclusion of datapoints is only acceptable using prestated criteria
- All reagents produced by the lab are COMMON; specifically: oligos, antibodies, clones, vectors, and mice, and must be registered in our central databases
- All renewable reagents (plasmids, mice) need to be archived, adequately documented, and distributed
- All raw data for a paper must be publicly deposited in the SDR (as of 2024)

### 2.2 Authorship policy

Authorship credit is based on three components: 1) substantial contributions to the conception and/or design of experiments, and/or to the acquisition, analysis, or interpretation of data; 2) drafting the article and/or revising it critically for intellectual content; and 3) final assessment and approval of the version to be published. Authors should meet conditions 1 and/or 2, and always 3 (see "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication by the International Committee of Medical Journal Editors" (http://www.icmje.org/ index.html)).

Each author should have participated sufficiently in the work to take public responsibility for its entire content. The PI ultimately decides the order of authors based on discussions with co-authors. The fundamental rule is that the person(s) who performed most of the work will be first author(s), and that the person(s) responsible for the conception/organization/submission of the paper and for organizing the work's funding will be corresponding author(s). Please note that this rule can be superseded/changed if a person leaves the lab before a study is completed and/or published. Especially if additional work, including revisions in response to reviewers' criticisms, is required to publish a paper, the authorship list may be led by who worked on the project last, and the person who did most of the work will not necessarily continue to be the first author.

For papers, all figures will be drawn by respective investigators in Adobe Illustrator. For all accepted manuscripts, raw data and reagents have to be deposited as described under 'Records' below.

There is no expectation that more than one author of a paper will independently numerically analyze all raw data because some analyses are so labor intensive that they require a full-time commitment and because some analyses require specialized expertise that is available to only a subset of authors. However, the corresponding authors of all papers are required to have seen the raw data and discussed them with the person generating the raw data.

### 2.3 Record keeping (see also below in section 3)

All raw data have to be recorded electronically and stored both on your personal computers and 'in the cloud'. Once a paper is submitted, all raw data and analysis records have to be deposited in two places for security: the publicly accessible Stanford Data Repository (SDR) and our personal cloud storage on Stanford Box. In addition to that, I recommend that the first author(s) keep a copy of all raw data in case questions arise.

## 2.31 Experimental phase

Everybody has to maintain three types of records:

- An electronic daily lab notebook which contains for every day in the lab an entry of what experiments / procedures were done – even if only 'analyzed data on …[experiment, record locator]...'. The daily lab notebook entries should include considerations such as assessing needed sample sizes, references to protocols and electronic data, and a summary of original raw data.
- Electronic descriptions of all experimental protocols. Nothing should be scribbled down somewhere – everything needs to be documented electronically. This can be included in the daily lab notebook but each experimenter should have a standard reference for specific methods.
- Electronic records of all raw data and all analyses should be maintained and regularly transferred to the 'cloud'. Even raw data that are not electronic in nature (e.g., cell counts) should be converted to electronic form by documenting them in the daily notebook. It is important to have a good description of key reagents such as plasmids and use exact labeling systems. ALL raw data should be kept –complete blots for example– and everything needs to be dated and conditions need to be identified for each set of data.

## 2.32 When a paper is submitted for publication

- The first author(s) is/are responsible for assembling and depositing into our 'box' and into the SDR a folder containing:
    1. All raw data from all co-authors, organized into figures and panel numbers, with raw data shared between figures/panels clearly identified
    2. All experimental protocols describing the precise methods of different experiments
- The first author is responsible for depositing key plasmids + sequences with the plasmid collection/database, depositing the protocols in the protocol database, etc.

Data deposition is not required for the initial submission but is required upon submitting a revised paper.

## 2.33 Records when a person leaves the lab

Everybody leaving has to complete the 'off-boarding' check list that includes a description of the data deposition requirements. Basically, everyone leaving the lab needs to deposit in Stanford Box a complete set and accounting of all unpublished raw data in addition to obviously fulfilling the requirements for published data.

## 2.4 Pre-peer review and pre-print publications (BioRxiv)

Some funders encourage preprint publications on BioRxiv (e.g., HHMI). In general, this is an excellent approach to pre-publicize important findings. However, there are associated risks. Once a paper is deposited on BioRxiv, it cannot be withdrawn. It can be modified, but the original version remains accessible. Different from a paper submission that allows corrections if we as authors realize we made mistakes, mistakes in BioRxiv papers become part of the official record. As a result, we will only publish papers on BioRxiv after they have been thoroughly wetted, and not as trial balloons to test our colleagues' reactions. A second risk, that we may be scooped and alert others to our pending papers, is much less significant but worth considering in selected cases.

## 2.5 Co-authorships in collaborations

We never request or accept co-authorships for provision of published reagents, but as a courtesy accommodate similar requests by others on our papers. I greatly encourage collaborations that enhance interactions and provide access to new techniques and concepts but also would like to counsel caution in that all collaborations should be guided by the quality of all collaborators – we should only engage in collaborations that fit to the mission of the lab and involve labs of recognized quality. Whenever there is any doubt, we withdraw our contributions.

# 3. Data Management ('Digital')

## 3.1 Electronic Notebooks

EVERYONE in the lab has to keep an electronic notebook on Stanford Box. You can use Benchling and save pages onto the Stanford box once a week but it has to be on Stanford box and it needs to have daily entries. This is an essential tool for a contemporary lab – no more paper notebooks except if you want to ALSO keep a physical notebook in addition to the electronic notebook.

## 3.2 Data Storage on Stanford Box

Our files in Stanford Box are organized as follows:

-Specific files for each lab member that are accessible only to the lab member, me, and the lab administrator

-Shared files that are accessible for all lab members but are editable only by designated lab members. These shared files include our databases (see below) and other resources.

## 3.3 Raw Data Deposition for Publication in the SDR (Stanford Data Repository)

All raw data used in a paper need to be deposited in the SDR together with the final submission of the paper (website: sdr.stanford.edu; log in requires a SUNet ID and password). SDR contact:

Amy E. Hodge, PhD
*Science Data Librarian*
Stanford University Libraries
amyhodge@stanford.edu & sdr-support@jirasul.stanford.edu

Deposited data are publicly available at its own persistent URL, such as this one: https://purl.stanford.edu/hm844rq5533. In addition, for each set of deposited data we can obtain a DOI, which for the example cited above is https://doi.org/10.25740/hm844rq5533; the DOI redirects a user to the PURL page.

SDR has no requirements for data format. Any type of file can be deposited. There is a metadata form that must be filled out, but because SDR supports deposits from the full spectrum of academic disciplines, it is quite minimal. The required fields are:

- Title
- Contact email
- At least one author. ORCID can be used to automatically retrieve author names for entry.
- Abstract/description
- At least one keyword, which can be selected from the OCLC FAST vocabulary or free text
- Designation of who has permission to access and download the files: options are Stanford only or anyone in the world. For publications it is 'anyone in the world'.
- Selection of either the immediate release option or an embargo (depends on when your paper is published)
- Agreement to the Terms of Deposit by checking a box.

There are additional optional fields as well as the ability to assign a DOI and a license, etc. There is no curatorial review, so deposits are available at purl.stanford.edu pages online as soon as the content has made its way into the repository. For larger content this takes longer, naturally.

Three file upload options available via the web app. The third option is for content over 10 GB and uses an integration with Globus to get the files to the SDR. If your content is on Oak or Sherlock, there are existing endpoints for those services that make the Globus transfer even easier.

There is no fee for individual deposits of less than 1 TB. For individual deposits of 1TB or more, the typical charge is a one-time fee of $500/TB.

### 3.4 Bioinformatics

Data management includes data analyses, often subsumed under 'Bioinformatics'. This category comprises, among others, image analyses, RNAseq analyses, databank searches, and many other procedures. Bioinformatics is becoming increasingly important owing to the wide availability of excellent databases for transcriptomics, genomics, proteomics etc. The vast information that is out there can be harnessed to enormous benefit for virtually any project.

RNAseq databases include (incomplete)

- Allen Brain Map single-cell RNA-Seq data – https://celltypes.brain-map.org/rnaseq/mouse_ctx-hpf_10x
- Dorsoventral gene expression patterns in hippocampal principal neurons – https://hipposeq.janelia.org/
- Gene expression and alternative splicing in inhibitory and excitatory populations from RiboTag translatomics datasets
  The Splice code database – https://scheiffele-splice.scicore.unibas.ch/
- Hippocampal subclass-specific gene expression – http://dropviz.org
- Single-nucleus RNA-seq (sNuc-Seq) database https://singlecell.broadinstitute.org/single_cell/study/SCP1/-single-nucleus-rna-seq-of-cell-diversity-in-the-adult-mouse-hippocampus-snuc-seq
- Alternative Splicing & Gene Expression Summaries of Public RNA-Seq Data – http://ascot.cs.jhu.edu/
- Allen Institute MERSCOPE (~4 million cells): https://knowledge.brain-map.org/data/LVDBJAW8BI5YSS1QUBG/explore?filterOptions%5Ba%5D=&layoutState=Single&visualizations=a~ZI3RR0FXL3HYXGVE2S5%3A2dBrainSectionRotated~METADATA%3AH5T3R1K3N0KO7SM9D9F%3Anull%3Anull%3Anull%3Afalse%3A0.5
- Allen Institute 10X Genomics, whole brain (~4 million cells): https://knowledge.brain-map.org/data/LVDBJAW8BI5YSS1QUBG/explore?filterOptions%5Ba%5D=&layoutState=Single&visualizations=a~AP8JNN5LYABGVMGKY1B%3AwholeBrainID~METADATA%3AH5T3R1K3N0KO7SM9D9F%3Anull%3Anull%3Anull%3Afalse%3A0.5

We have bioinformatics expertise in the lab and collaborate with other expert labs on bioinformatics. Everyone should feel free to seek out people with expertise for help, not just to analyze their own data, but also to determine expression patterns, regulation etc of genes they might work on, or to assess the limitations of a given database (e.g., GO analyses, literature analyses). Remember that although there are many reliable databases, there is also a lot of 'bullshit' (sorry) – for example, so-called protein-interaction databases are created based solely on limited approaches that create so many false-positives that they are completely unreliable.

### 3.5 Artificial intelligence (AI) – machine learning

Our goal is to introduce AI approaches to all analyses of larger datasets. For this to work, we need to use established algorithms (ideally open source software since commercial software is becoming increasingly expensive – rent-seeking is astounding here, as is evidenced by simple programs such as Adobe Illustrator that are astonishingly expensive and have amazing features we never use) or do our own coding (sometimes facilitated by ChatGPT). AI will be indispensable for image analysis in future, and likely other approaches as well.

Stanford has its own large language model AI only accessible for Stanford employees.

# 4. Rules: Personal safety

## 4.1 Personal protection – lab safety

Fundamental rules everybody is required to follow:

a. Do not wear clothes in the lab with the potential to expose skin to chemical or glass injuries (e.g., sandals, or short pants or skirts) - please wear lab-appropriate clothing (e.g., long pants and closed toed shoes)
b. Wash hands after completion of work
c. Wear a lab coat when doing lab work, in particular when using any chemical/biohazardous agents that may be harmful
d. Wear a face shield and/or safety glasses or goggles when pipetting liquids that could be hurtful to your eyes and face – for example, when pH-ing a solution with acid or base. The same applies to all procedures with potential for glass breaks or spills of boiling solutions, including water
e. Wear gloves and glasses whenever working with radioactivity, potentially infectious samples, or otherwise potentially harmful materials
f. Use the fume hoods for all organic solvents, concentrated acids or bases, toxic chemicals, or any material that may produce vapors
g. Perform all work with viruses with a lab coat, gloves and in a Biological safety cabinet or using face protection
h. Properly dispose of waste in appropriate waste containers, like biohazardous bins, sharp containers, chemical waste collection containers and so on
i. In case of any incident, like a chemical or biological exposure, contain spill and remove contaminated clothing if applicable, wash exposed area with water and soap for 15 minutes. Seek medical attention by calling 911 or the Stanford occupational health department and notify me and the lab administrator

The most important rule: use common sense! Whenever there is a possible danger, treat it like a real danger and protect yourself

## 4.2 Undergraduates in the lab

Students and postdocs are encouraged to mentor undergraduates but have to follow a number of basic rules. Working with an undergraduate in the lab requires a firm commitment on the part of both the lab's mentor and the undergraduate. The mentor commits to teach and advise the undergraduate student and to involve the students in scientific processes both at the conceptual and the technical level. A lab member should only take on an undergraduate if he/she/they have the time to actually mentor the undergraduate. The undergraduate student, in turn, commits to make a sincere effort in contributing to the mentor's lab project. An undergraduate should only join, again, if he/she/they have the time available in their schedule to spend in the lab. By mentoring undergraduates, we as a lab also commit to help the student in her/his/their career development, provide educational credits where needed, and write recommendation letters, but this obviously depends on the undergraduate student's contribution.

Key rules:

- Undergraduates are partners and volunteers, not employees. Undergraduates don't have to listen to orders but have to follow directions in scientific issues. If there is a disagreement between mentors and undergraduates, it should be discussed with me
- As a PI, I will always be available for undergraduates for discussions on lab or career issues, and will meet regularly with undergraduates for a common conversation
- Undergraduates have to follow the safety rules and other rules of the lab carefully with no exceptions
- Undergraduates should never be allowed to perform procedures in which they were not trained

- Undergraduates should not be left in the lab alone, for example in the night – there should always be somebody around when an undergraduate is in the lab

# 5. Rules: Institutional and governmental regulations

## 5.1 Required permits from mice to biosafety to chemicals

We are facing an increasing number of regulations, inspections, and trainings. Some of these regulations make eminent sense whereas for others one really has to dig deep to find a rationale. At present, we are required to have the following permits for the lab:

5.11 **Animal use & care permit (IACUC or APLAC at Stanford)**. The mice we use are our partners in discoveries benefitting human health. We have to take care of them humanely and with appropriate SOPs. To be allowed to do this, we maintain up-to-date approved animal protocols. Anybody found to be negligent with respect to mouse care and protocol rules will be banned from working with mice.

5.12 **Stem cell permit (SCRO)**. The rationale for such permits is no longer clear but they have to be maintained. Moreover, for NIH grants we need to list the ES (but not the iPS) cell lines we use.

5.13 **Chemical safety permit**. We are required by law to maintain an on-line inventory of chemicals (chemtracker), to label and date all solutions, and to store flammable liquids in fire-proof cabinets. Moreover, strict rules govern the disposal of chemically contaminated waste and liquids. Most obviously, never pour a chemical solution down the sink – all chemical waste has to be discared in labeled special containers and for pick-up. These rules make eminent sense and should be meticulously followed.

5.14 **Biosafety permit (APB)**. Biological agents at risk are classified into 4 biosafety levels according to their perceived risk. We only use BSL1 (regular molecular biology procedures; AAVs) and BSL2 (e.g., lentiviruses). Not all classifications are sensical but they are the law.

5.15 **Controlled substances**. The law lists 5 basic types of controlled substances (hallucinogens, anabolic steroids, narcotics, depressants, and stimulants) that are classified into schedules (schedule 1, drugs with a high abuse risk; schedule 2, drugs with a high abuse risk that also have safe accepted medical uses; schedule 3, drugs with a lesser abuse risk; schedule 4 & 5, drugs with little risk). All controlled substances in schedule 1-3 have to be individually approved, have to be stored in locked containers, and their use has to be documented. We use the following controlled substances as analgesics and anesthetics for animal experiments as described in animal protocols and occasionally for in vitro studies:

| Substance | Schedule | Stock unit info | Dosage for mice | Route |
|---|---|---|---|---|
| Ketamine HCl injection | III | 100 mg/ml, 10 ml/bottle | 80-100 mg/kg | Intraperitoneal (IP) |
| Buprenorphine SR | III | 0.5 mg/ml (for mouse), 5 ml/vial | 0.5-1 mg/kg | Subcutaneous (SQ) |
| Ethiqa XR | III | 1.3 mg/ml, 3 ml/vial | 3.25 mg/kg | Subcutaneous (SQ) |

*For standard use, the recommended Buprenorphine-SR dose is:* ***Buprenorphine-SR (sustained release)*** *0.3 - 1.0 (0.5 recommended) mg/kg SC* ***once, undiluted given 30 minutes pre-procedure.*** VSC *handout on buprenorphine SR: http://med.stanford.edu/content/dam/sm/vsc/documents/vet-services/BuprenorphineSR_Rats_and_Mice_201803.pdf*

*The same dose is used for neonatal and adult mice – see study on the efficacy and safety of Bup-SR in neonatal rats: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0246213*

*Buprenorphine-SR was recently renamed as buprenorphine-ER without a change in the formulation or release technology in order for Zoopharm to remain compliant with current regulatory guidance.*

*When using Buprenorphine-ER (or Buprenorphine-SR):*

- *The dosage recommendations and route of administration (subcutaneous) remain unchanged*
- *The same concentrations of Bup-ER are available (0.5 mg/mL and 1 mg/mL)*
- *Administer Buprenorphine-ER using a 22g needle and a Luer-lock 1 ml tuberculin syringe*
- *Expiration guidance remains unchanged*

*An alternative for Buprenorphine-SR is Ethiqa-XR (another extended-release buprenorphine). For mice the dose is 3.25 mg/kg for Ethiqa-XR: https://med.stanford.edu/content/dam/sm/vsc/documents/vet-services/Ethiqa-XR-Rats-and-Mice-2-.pdf*

Online forms for ordering and disposal requests of controlled substances, as well as for managing authorized researchers are here:

**CSP Form 1a – Purchase Request Application:**
https://stanforduniversity.qualtrics.com/jfe/form/SV_esyr1yZRgEj8bFX
**CSP Form 1b - Re-ordering Request:**
https://stanforduniversity.qualtrics.com/jfe/form/SV_6gtTeCfqCiONlHL
**CSP Form 1c - Addition or Deletion of Authorized Researchers:**
https://stanforduniversity.qualtrics.com/jfe/form/SV_0olLrEzixbmhZK5
**CSP Form 2 - Authorized Researcher Application:**
https://stanforduniversity.qualtrics.com/jfe/form/SV_5nE71n8ahd0dbTv
**CSP Form 3 - SU Controlled Substances Usage Log**
https://ehs.stanford.edu/wp-content/uploads/formCSP3.pdf?1688924519
**CSP Form 6 - Controlled Substances Disposal Request Form:**
https://stanforduniversity.qualtrics.com/jfe/form/SV_9ZwmxsqSKuRKrUV.edu.
**CSP Form 7 - SU Controlled Substance Periodic Inspection Checklist**
https://ehs.stanford.edu/wp-content/uploads/formCSP7.pdf?1688924585

After submission, forms 1a, 1b, and 1c will be sent directly to the PI for certification. After PI certification, the form is sent to EH&S for processing. Each submitter will receive an email detailing at which step of processing their form is along with a copy of their submission. Please keep a look-out for emails from the Stanford University Controlled Substances Program with "SECURE:" in the subject line. Forms 2 and 6 will be sent directly to EH&S for processing after submitting.

5.16 **Select agents**. The government defines as 'select agents' substances that could potentially be used by terrorists. Inexplicably, tetrodotoxin (TTX; naturally produced by pufferfish but nearly impossible to use as a poisonous agent) is included. We are allowed to store for electrophysiology maximally 500 mg tetrodotoxin in a lockbox in a locked refrigerator. Moreover, TTX needs to be included in the online ChemTracker inventory, users need to be specifically approved, and everyone needs to sign in/out in a specific log book for use of TTX and note how much TTX is used. Here is link on how to manage select agents:https://ehs.stanford.edu/topic/hazardous-materials/select-agent-toxins#:~:text=Select%20Agents%20and%20Toxins%20are,to%20animal%20or%20plant%20products.

5.17 **Laser safety permit**. Several instruments in our lab (microscopes, scanners, microtiter plate readers) contain integrated lasers whose safety needs to be regularly documented.

5.18 **Radioactivity permit**. We no longer use radioactivity in the lab.

## 5.2 General lab practice regulations

5.21 **Waste disposal**

There are multiple waste categories: regular waste (landfill, compost, and recycling), liquid potentially hazardous waste (any organic fluid or potentially toxic chemical solutions), biological waste (any solid bacteria or cell culture or animal remnants or any material that may be contaminated with a potentially biohazardous agent, sharps (glass pipettes, syringe needles), and lab glass for recycling)

- Only well-defined non-toxic liquids can be poured down the drains, and only innocuous waste can be discarded in one of the three bins (landfill, compost, and recycling). Biological waste has to be separated and disposed separately with autoclaving. All other waste, liquid or solid, has to be separately discarded in well-labeled containers
- Glass Recycle Boxes should only contain lab glassware (no liquids, no beer bottles or other types of non-lab glassware).
- Glass pipettes have to be disposed of in a sharps container.

- Waste tags need to be 'completely' labeled
- All biohazardous liquid waste must be mixed with bleach to a final concentration of 10% for 30 minutes before disposal down the sink.

5.22 **Reagent storage**
- Flammables/chemicals need to be properly segregated in refrigerators and freezers
- All chemicals, buffers etc need to be properly labeled and dated and stored in a secondary container
- Flammables can only be stored in appropriate fire-safe cabinets
- Chemical bottles need full chemical names
- A chemical inventory needs to be maintained and continuously updated
- Self-evidently, no food, drink etc is allowed to be stored in lab refrigerators or cold rooms

5.23 '**Self inspections**' and **inventories**
- We have to do quarterly Lab self-inspections that have to be documented
- Chemical Inventories need to be continuously 'self-inspected', especially for gas cylinders and toxic chemicals.

## 5.3 Computers, software & IT security

We have more than 30 computers in the lab since everyone has a personal computer on their desks and various instruments (ephys setups, microscopes etc) also have dedicated computers. All of these computers are connected to the internet. Stanford continuously updates various pieces or software and operating systems. Sadly, often incompatibilities arise between updates and programs we run on our computers.

Multiple rules apply:
- All software needs to be legal – no pirated software is allowed
- Whenever possible, use open-source software and use software that is actually installed on the computer instead of software operating in some cloud. Software companies are extracting increasingly higher user fees (often extortionist in nature) with annual updates that are expensive but useless. Moreover, companies try to gain control of data in the cloud and render users dependent on them to extract even more money. Annual software expenses for the lab, ranging from simple office procedures (GraphPad, Adobe Illustrator etc) to sophisticated image analysis programs, are ballooning. We have to try everything to control both the costs and our autonomy in this area.
- All computers must conform to Stanford rules, even though these rules are often difficult to understand.
- Please recall that nothing connected to Stanford, even your private laptop, is private – in the US, Stanford has the right to monitor or survey or analyze anything that is on your computer. Only your private gmail is not accessible to Stanford.

The biggest challenge we face as a lab is to maintain excellence in the computational analysis of 'big data' (imaging, transcriptomics etc) in a manner that we don't completely depend on specialists.

# 6. Rules: Lab resources

Our databases are a non-public lab resource to be used by everyone in the lab.

## 6.1 Plasmid clones

a. Every lab member must keep a "plasmid log" and maintain a personal plasmid database that conforms to the overall lab plasmid database and can be merged with the lab database (see below). The plasmid log and database should contain lists of all clones generated, including their names, construction, sequences and utility.
b. Plasmids should not have colorful names but be labeled systematically. I suggest the following system: A person's initials followed by the last two digits of the year, a dash, and then a running number.
c. All major plasmids have to be restriction mapped and sequenced.
d. All major plasmids have to be deposited with a full description AND a full sequence in the lab's plasmid database and repository

Plasmid database/repository:

The lab's plasmid database and plasmid collection are administered as a lab duty. Old plasmids are stored as glycerol stocks but all new plasmids are stored spotted on filter paper in multiple aliquots and kept in sheet protectors. In addition, for all plasmids we maintain stocks of DNA in TE at 4 and at -20 degrees.

Every entry into the plasmid database has to contain the following information:

1. The full plasmid sequence
2. Vector identification, promoter identity, specific features (WRE sequence yes/no etc)
3. Reference to the paper if published
4. Utility remarks that explain the use of the plasmid and how well does it work

Please remember, reagents in the Südhof lab are **communal** properties; they do not belong to anybody. Only projects are proprietary, not reagents.

## 6.2 Antibodies

The lab's antibody databank is also administered as a lab duty. The database and storage includes commercial as well as homemade antibodies. The database should contain the following information:

1. precise antigen for an antibody (with sequences, species information etc)
2. usefulness/specificity/dilutions for immunoblotting, immunoprecipitation, and immunocytochemistry – best with original data
3. references to the original papers

Everybody in the lab has to inform the person whose lab duty is to maintain the antibody database about the properties of an antibody.

Antibodies are stored in the locked -80 C freezers. Aliquots for general use are kept alphabetically by antigen in the lab refrigerator/freezer. Only the responsible lab member can stock up the accessible aliquots when they run out. Nobody is allowed to keep their own antibodies separately. If an antibody is found not to work (and not only because the secondary antibody was at fault), this observation should be communicated and the vial should be removed. If necessary, the antibody entry in the database should be appropriately annotated.

## 6.3 Nanobodies

We are building a nanobody databank that includes information such as the sequences, affinities, and specificities of published nanobodies as well as the applicability.

## 6.4 Mouse database

General. All mice are described in the mouse database that includes a comprehensive description of the various mutations or transgenes, phenotype summaries, and genotyping protocols with oligo sequences. In addition, the database contains the complete sequences of the wild-type and mutant alleles and information about potential breeding problems. Note that mouse costs at Stanford are likely the highest in the world but the level of service is not the best compared to other institutions.

Individual responsibilities. Everybody has to keep a running log as an excel file for EVERY cage for which he/she/they is/are responsible, with a list of the DOB and genotype of EVERY mouse in that cage. This log is updated whenever a change occurs, at least weekly. The log is considered an experimental record (i.e., non-compliance basically means the same as faking data). Also, everybody is responsible for the mating/weaning/genotyping/sacrificing of their own mice. So, whenever a change occurs, everybody updates their log.

Help. Although our permanent staff provides limited assistance, most of the mouse work will have to be done by individual postdocs and students.

Wild-type mice. A wild-type CD1 mouse colony is maintained as a lab duty, and will be expanded or contracted according to needs. The lab duty includes the need to visit the colony daily and mark new births. Wild-type CD1 mice are primarily used for mixed neuron-glia cultures or glia only cultures from newborn pups, but are also required for some breeding and behavioral experiments. People who need mice at a particular age can reserve cages for their use on a first-come-first-serve basis. Each reserve card has to contain a date and name. If mice remain unclaimed after the date on the card is passed, the mice can be sacrificed or used by anybody.

Mouse cage count. A mouse count is done at the end of every month.

Animal trax: All mouse orders, transfers and barcode sheet orders need to be filled out in the lab google ordering sheet and orders need to be send to the lab administrator for administrative approval. The Animal Trax system is recording mouse cages numbers and locations, every cage from day 1 to check-out day. Charges are based on days of service. The system is automatically updated every day and allows us to obtain daily or monthly reports for every person. Innumerable errors occur because the Stanford staff does not always accurately count cages.

## 6.5 Protocols

The protocol database is administered by the lab administrator in Stanford Box. The database serves three purposes:

a. to allow references to specific experimental procedures in our papers
b. to standardize lab procedures as much as possible. Thus, there need to be updates when we improve something, but it is of fundamental advantage when everybody does experiments in the same way.
c. to facilitate doing experiments for everybody – people don't have to shop around for information on how to do something, but they can just go to the protocol database first ….

To be useful, the database should just identify the person who provided the information, the date of updates etc, and hints about 'tricks'. You all benefit when you leave.

For all papers, protocols have to be submitted to the protocols database with a description of the figure numbers of the paper in which the protocol was used.

# 7. Data analyses, statistics and replication efforts

Our lab's basic rule is that all experiments have to be performed and analyzed 'blindly' (even exploratory experiments to assess effect sizes), i.e. with anonymized samples, animal subjects, or data, and that all experiments that are quantitatively evaluated have to be performed in at least 3 true biological replicates.

Here, we define as n's the true number of experiments (replications), and as pseudo-n's the number of replicates within an experiment (pseudo-replicates, for example ROI's in an imaging experiment). The need for true replicates as a rule is not yet considered necessary by journals – even high-ranked journals inexplicably still publish studies where the true number of replicates is n = 1 and where statistical significance is achieved by using the pseudo-replicates of a single experiment as the basis for the statistical 'n'. One complicating factor is that different types of experiments have distinct traditional standards to which we need to adhere to make experiments understandable. Here is a non-exhaustive list of our lab's current practice:

Culture experiments: Three independent cultures are essential. If both replicates and pseudo-replicates are used for statistical analyses (e.g., for imaging or ephys), data from each culture needs to contribute to a similar extent to the analysis.

Slice electrophysiology: Slices from at least three mice need to be analyzed. If a biological sex effect is observed, at least three mice of each gender need to be analyzed.

Experiments involving imaging of mouse brain sections: Same as for slice electrophysiology.

RNAseq experiments: RNAseq experiments should be performed three times (not in three replicates at once = these are pseudo-replicates!), even though this is not the standard of the field.

Mouse behavior experiments: A single cohort of mice that count as n's should be analyzed according to standard procedures. If a robust effect trend without statistical significance is observed, mice should not just be added to the first experiment but a new experiment with a larger n should be started. This approach minimizes the number of mice used without compromising experimental rigor. Moreover, we will always aim to independently validate key conclusions with a different experimental approach.

Biochemical experiments: Three true independent replicates are essential.

Protein structures: Traditionally they are performed once but should be validated independently.

## 7.1 Statistics

Sophisticated statistical analyses are increasingly required even though for clearcut results, these analyses can have an obfuscating effect.

In grant applications and papers, we are often asked to perform a power analysis. Power analyses enable a prediction of the required 'n' number for an experiment to be statistically definitive. Power analyses are based on an expected effect size and are useful when a reasonable guess of the effect size is possible, but they cannot be applied when a particular experiment is performed for the first time and effect sizes are unknown. In many experiments, the effect size turns out to be so large in the first set of experiments that the experiments simply need to be replicated multiple times for validation without a new set of experiments. Thus, to avoid p-hacking, any exploratory effect-searching experiments should be prespecified with the number of true replications (usually n = 3) and samples per experiment to prepare for the situation in which the effect size is sufficiently large for the exploratory n to test statistical significance.

Rule of thumb: understand what comparison you are performing, the nature of the data you have, and the assumptions/goals for different statistical models, to decide which statistical model is most appropriate for your comparison.

**Some basic principles:**

1. Understand the number of “independent variables” in the comparison. For example, comparing different genetic manipulations in one mouse line has only one independent variable, whereas comparing different genetic manipulations in 2 mouse lines has two independent variables.

2. Understand whether samples in different groups are “dependent”. For example, measuring the phenotype of one mouse before and after a treatment produced dependent groups (before/after).

3. Understand whether data follow a “normal distribution”. For example, RNAseq read count is well-known not following a normal distribution; thus, the statistical model must not assume a normal distribution.

**Some practical advice:**

1. If we are comparing the synapse density of two groups and if the data are normally distributed, an independent t-test is appropriate. If the groups are dependent (e.g., before and after a treatment for the same mouse), paired t-test is appropriate.

2. If we want to know if a genetic manipulation (WT, KO, KO+rescue1, KO+rescue2) has any significant effect on the synapse density in a cKO line (in other word, is there any difference of synapse density among the genetic manipulations in a cKO line?), you can generally assume the data are normally distributed and use one-way ANOVA.

3. If we want to know if a mouse line (BL6 vs cKO line) and genetic manipulation (dcre vs. cre) and the interaction of mouse line and genetic manipulation has any significant effect on the synapse density. Assume all data are normally distributed. Use two-way ANOVA.

4. If we want to obtain the p-value by comparing more than 2 groups: WT, KO, KO+rescue1, KO+rescue2 in a cKO line, and all data are normally distributed. Due to family-wise error introduced during multiple comparisons, the p-value needs to be corrected/adjusted.

(1) If the goal is to compare all 4 groups in pairwise manner (6 comparisons): Tukey’s test.

(2) If the goal is to compare all groups to WT only (WT vs KO, WT vs KO+rescue1, WT vs KO+rescue2): Dunnett’s test.

(3) If the goal is to compare selected groups: independent t-test followed by Holm-Sidak test.

**Because family-wise error increases with the number of comparisons, scenario (1) naturally results in higher p value than (2) or (3).

5. Compare if the expression level of a gene is significantly different in two groups in a RNAseq experiment. DEseq2, EdgeR or other non-parametric tests (e.g. Wilcoxon-Rank sum).

Useful links:
https://www.youtube.com/watch?v=u9h8qjxMV-Y
https://www.youtube.com/watch?v=5pPd2rLS1GU
https://www.youtube.com/watch?v=l4yVt_Dht4U

## 7.2 Replication strategy

There are two principal types of replication, the exact repetition of an experiment and the testing of the conclusion of an experiment by an independent approach. The former is often inconclusive because for many biological experiments it is virtually impossible to exactly repeat them. The latter, thus, is the way to go.

It is our lab’s policy to test all major conclusions by independent approaches. This policy is occasionally difficult to follow because journals are hesitant to consider papers that confirm or question a previous conclusion – in the first case, the editors call it ‘incremental’, in the second, they generally state that since the exact experiment wasn’t repeated, the differences between conclusions might be circumstantial.

These difficulties notwithstanding, our lab will try to follow this policy even if it requires sacrifices. In the long run, we need to build more than one of the walls of our scientific edifice, even if the second wall looks the same as the first.

# 8. Joining and leaving the Südhof lab

## 8.1 Joining our lab

### 8.11 **Recruiting undergraduates, students and postdocs**

Postdocs: We select applicants for interviews based on whether we have positions available and whether an applicant appears to fit the mission of the lab. Postdocs are then interviewed and the best suitable candidate is offered a position.

Students: At Stanford, we can accept only graduate students who are enrolled in a Stanford program. Since Stanford charges graduate student tuition that neither the students nor PIs can pay, Stanford graduate programs are very small because students need to be supported by training grants or philanthropy. We welcome any student for a rotation in our lab and after a rotation decide mutually with the student if he/she/they and the lab are a good fit.

Undergraduates. Working with undergraduates is a commitment for both the lab mentor and the undergraduate. Undergraduates are recruited by individual lab members with my agreement.

### 8.12 **On-boarding**

All incoming lab members need to undergo a formal 'on-boarding' process that includes extensive Stanford-mandated trainings as well as lab-specific instructions and that is coordinated by the lab manager and lab administrator.

## 8.2 Strategies for job applications

Our lab does not intend to train people for a specific career path such as academic research or teaching or a biotech position, but instead aims to help people develop the technical and theoretical expertise for different jobs, depending on a person's interests. Our goal in particular is to foster a person's ability for critical thinking and for creative explorations, abilities that are crucial for most types of careers.

### 8.21 **Academic job searches**

#### 8.211 ***When are you ready?***

Fundamentally, people have the greatest chance of success in applications for an academic position if they fulfill three criteria:

**a**. they have a body of publications – 1-3 high-profile journal papers or a string of specialty journal papers that form a story and together represent a significant advance in the field. Note that a Cell/Nature/Science paper is not a ticket to a job! Some of the best jobs in the country are filled with people who have no CNS paper. The overall scientific narrative is **more** important than the journal in which it is published.

**b**. they are trained for a time of 5-8 years in a first-rate lab with a great interdisciplinary environment that exposes them to a rich intellectual culture

**c**. they are scientifically mature, have an idea of what they want to work on, and have learned enough techniques and gained enough conceptual insights to lead a lab – typically qualities that are not apparent from a c.v. but are revealed in an interview

It is important to form a clear view of what type of academic job/institution you would like to target. The targeted jobs have to be both realistic and at places at which you would want to be. There is nothing wrong with applying to places that might be unlikely to hire you if you really would love to go there, but it is important not to take rejections personally. Conversely, it is pointless to apply to lots of places where you would never want to go – if you feel you need some 'secure' places, choose them carefully.

#### 8.212 ***The process***

Any job application has three components:

**a. the application itself** (c.v., 'research interests', diversity statement, teaching statement) – this is the most important component to get you an interview

**b. associated references/recommendations**

**c. the visit/interview** (first a zoom interview, then an in-person visit) – this is what decides whether you get an offer

Some more detailed remarks:

8.213 **The application** (c.v., 'research interests', diversity statement, teaching statement)

The c.v.: The first thing committees look at usually.

1. Don't list abstracts, hobbies, vacations, etc on your c.v. but do list extracurricular activities that are relevant (e.g., service in a university society, teaching at community colleges, etc)

2. Having a demonstrable ability to get a fellowship or a grant helps tremendously – a K99 award is not a prerequisite but having never gotten any grant award is a detriment

3. The number of publications is less important than their quality impact. Ideally your work should have a theme, a consistent subject – you need a story in which you can build in your independent career

Research plans: These are crucial. They need to be fundable, interesting, realistic, and neither pedestrian nor overambitious. The 'Research plans' need to intrigue people, make them want to have you as a colleague, but not appear contrived or dictated by fashion.

A key issue is the relation of your future plan to our lab: don't over- or underemphasize it. Our lab's general policy is that we encourage former lab members to continue their postdoctoral research projects independently (in discussions, but not necessarily in collaboration with us) or to branch out from these projects. We encourage continuation of postdoctoral projects because there is always more to do in our field than our lab can possibly pursue, but we equally support a former lab member's initiative to do something new. Thus: emphasize that the projects our lab pursues are so broad with so many ramifications and implications that taking an important piece with you is straightforward and that I am in full agreement with this. Confront the issue by stating that the project represents an extension of what you did in my lab but is not overlapping with what will be carried out here.

Finally, please make sure that your application is well written and does not raise concerns about your ability to express your thoughts. Most of us are immigrants to the US who are not native English speakers. I suspect that in future many applications will be written by ChatGPT, which is a useful tool but can lead to rather similar sounding 'cookie cutter' type of write-ups that should be avoided.

Diversity: The major change that has happened in the last decade is an increasing emphasis on diversity. In some universities the first round of screening for faculty job applicants involves a review of the diversity statement, which is thus of crucial importance.

The diversity statement will generally be scored based on three categories: knowledge of DEI (diversity, equity, and inclusion), experience, and diversity plans.

- Knowledge: Avoid vague terms such as "diversity is important for STEM". Be specific about your understanding and experience of diversity that result from different identities (ethnic, socioeconomical, racial, gender, sexual orientation, disability and cultural). Show your awareness of demographic data related to diversity in higher education. List for each institution to which you apply how you would fit into THEIR specific diversity efforts. Understand the challenges faced by underrepresented individuals, and discuss diversity, equity and inclusion as core values of higher education. Note that although this is not universally accepted, at least some committees also consider a background of poverty as a diversity criterion.
- Experience: Be genuine, relate to your personal experience, both in terms of your own personal life as well as in terms of what you might have done to help alleviate inequities (for example, by mentoring an undergraduate from a diversity background, or teaching in a minority-dominant school). Describe multiple activities in depth, try to show your track record in engaging in these activities. You can go back to your undergraduate, graduate and postdoc years for these activities.
- Plans: Show clear and detailed ideas of how you would get involved in the existing DEI programs at the institution to which you are applying and/or outline plans for starting new programs. Indicate your intention of being a strong advocate for diversity, equity and inclusion. Again, such write-ups can appear to be fake, so try to be authentic and admit limitations in this area.

This URL has some examples of diversity statement that might inspire you:

https://careerservices.upenn.edu/application-materials-for-the-faculty-job-search/diversity-statements-for-faculty-job-applications/

I think most of us agree that, in order to enable people from every segment of our population to achieve their personal potential, it is essential to eliminate barriers that exist for many, such as people from a poor economic background or a racial and/or ethnic minority. Whether or not you agree with the current approaches to eliminate these barriers is not the issue, you have to show an understanding of the problem and you have to demonstrably and authentically work towards solving it.

Teaching: It helps if you are genuinely interested in teaching, have mentored an undergraduate or trained somebody in the lab, or have done some teaching. Remember that for most institutions, teaching is as important as research. It is a skill that is both sophisticated and difficult and should not be taken lightly.

In general, institutions want to hire well-rounded individuals, not specialists. Ideally an institution would like to recruit a future star, someone who will develop into a brilliant scientist and beloved teacher, but institutions are realistic about the chances of identifying such candidates and would rather have someone who will make scientific advances, teach well, and support the mission of a university than someone who is good at publishing papers but nothing else.

### 8.214 **References & recommendations**

It is not only important what is in letters of recommendation, but also who writes them and what is NOT in the letters. Nobody writes a truly negative letter – thus, what is left out is telling. People always write supportive letters because nobody wants to willfully hurt someone and because people are afraid of getting sued. As a result, letters that are generally supportive without being enthusiastic have an adverse impact on your application – so don't ask for letters from just anybody. Ideally, reference letters should be from your PhD mentor, your postdoc mentor(s), and one or two well-known scientists who know you and your work well. For this reason, outside collaborations can be extremely useful. Most applications require three letters; having more than five letters can be counterproductive.

### 8.215 **The zoom interview and in-person visit**

Once a committee has reviewed your application, it might invite you to a zoom interview and possibly to an in person visit. These interviews are key. Their success depends only on you and decides your chances. The minute you interview for a job, the impression you make is more important than your c.v., references, and grants/fellowships.

The zoom interview

Almost all places do these now. You have 15 minutes to impress a search committee, which is challenging! Usually you are asked to summarize your work in 5-10 minutes and then there will be a brief discussion. The research summary should focus on ONE major finding that showcases your abilities and conceptual thinking. Show slides, explain your work with data, not just summary slides. In the discussion, be prepared to answer questions such as what your first R01 grant would look like, what kind of teaching you are interested in, what faculty at the institution you would collaborate with, and how you would fit into their DEI programs.

The in-person visit

If the zoom interview is successful, you will be asked to visit in person, give a seminar, talk to faculty, and present a 'chalk talk' (without chalk)

1. The **seminar** = be lucid, be open, be understandable. It is about impressing and charming people, not about confronting them. The slides need to be clear and simple; always come back to the question you want to answer so that you don't lose people. Be prepared for disruptive questions, always answer patiently, never give the impression that a question is dumb, or that you want to avoid it. At the end, talk 5-10 minutes about your future plans.

2. The **'visit'** = talking to people, going to dinner … Always aim to convince the person you talk to that you are their best possible colleague! Prepare by looking up what people do before your visit and mention ways of how you could collaborate with them. In one-on-one discussions, most people will show you their data – be enthusiastic but discerning, don't just say 'this is great' but prepare your visit so you can make some interesting comments. Be authentic even if that exposes your limitations because everyone has limitations – there is nothing like a perfect candidate.

3. The **chalk talk** = this part of an interview gives an institution to evaluate your conceptual abilities and interaction qualities and enables it to assess whether you can teach and get grants. Often you will be asked to explain the specific aims of your first planned R01 grant but the idea is not just to assess grant plans. The committee wants to evaluate your familiarity with a subject matter, your ability to think on your feet (there will be lots of disruptive questions), your passion, and your creativity. Be realistic and have a vision without being overreaching! React to questions thoughtfully but quickly – don't ramble.

You HAVE to practice the seminar and the chalk talk – over and over. This is important. Our lab will always be happy to listen to and to critique your presentations.

### 8.22 **Industry job searches**

Industry jobs are different but no less demanding than academic jobs. They can be very rewarding when they lead to the development of an effective new therapy that will truly help patients and they are financially more lucrative than academic jobs, but industry jobs offer no freedom in terms of the research subject and types of experiments and no job security.

The first step towards industry jobs is to identify what kind of company you want to work for (big or small, biologics or small molecules, therapeutic areas etc) and what your long-term career plans are (bench scientist, manager, etc). You need to identify a 'head hunter' who can help you find the right job in addition to applying directly to advertisements. You need to draft a professional c.v. that is different from that in academia since companies do care about technical expertise, teamwork, and management experience.

Once you interview for a job, it is important to emphasize that your interest is NOT in freewheeling research but in developing applications, and that you love to be part of a team. You also have to convince the people who interview you that you would be a great colleague, that you know 'your stuff' (i.e., are technically well-versed), and that you have the right mix of ambition and drive on the one hand and desire to collaborate on the other hand.

### 8.23 **Alternative career paths (journalism, consulting, editors, administration)**

There are many, among which science administration and scientific publishing is likely the most common. These are truly great and rewarding options for those individuals whose career goal is to help others do great science. These are also demanding and challenging careers – we all know how difficult it is to be a good editor or science administrator since we have dealt with so many who are not! Please do consider these options as serious alternatives for a career in science that is truly essential for others to succeed.

## 8.3 Leaving our lab

We have a standardized off-boarding procedure to ensure a smooth departure. Our lab's administrator is in charge of off-boarding.