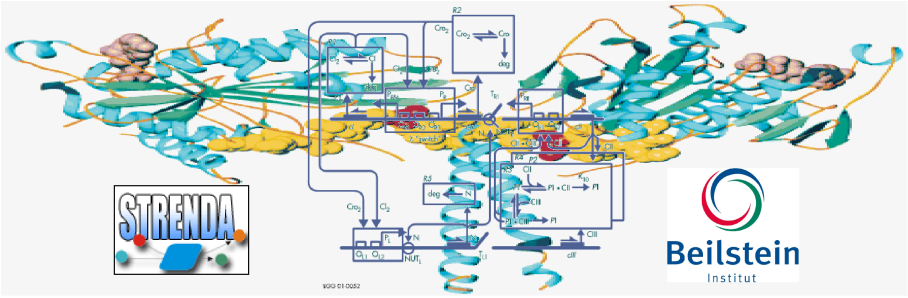

Minutes of the 3rd STRENDA Meeting

The 2nd Int'l Symposium on Experimental Standard Conditions of Enzyme Characterizations took place in Ruedesheim, Germany, 19th - 23rd March, 2006

These meeting minutes have been written as part of the STRENDA activities during the 2nd ESCEC symposium. The first part of the minutes below relates to a brief one hour meeting on the evening of Tuesday 21st when the STRENDA commission discussed the requirements of data repository in journals and databases.
The second part of these minutes lists suggestions - as requested by the organizers - from the speakers during their talks. These suggestions are intended to be considered by the STRENDA group when extending the checklists. CK gathered this input from his own minutes and from the provided presentations, checked it for its inclusion in the existing checklists and added some comments.
The third part of this documents contains the results of the „round table" discussion on Monday, 20th, to which all the participants of the ESCEC symposium were invited. The purpose of this meeting was to collect comments and suggestions on the STRENDA checklists from a broader community to strengthen the impact of these lists. Actually this part consists of the updated checklists. Fortunately, only slight changes were necessary. These changes are labeled.

Part One

Besides the creation of checklists to set recommendations on Good Publication Practice the definition of requirements for the deposition of enzyme data in journals and databases is another project of STRENDA. The idea is that authors submit their data in a machine-readable format to journals and/or database providers after their manuscripts have been accepted. A successful cooperation with the journals and database providers could result in a prospective design that enables them to capture enzyme data electronically.

Some conditions have to be fulfilled for a successful realization of this aim:

i. announcement of this project to the broader community (by e.g. advertising) and to ensure that this project is relevant for both the scientific community and those involved in data collection and dissemination;
ii. cooperation from journals and database providers must be sought;
iii. creation of a kind of a work flow from creation to submission of enzyme data which includes the implementation of appropriate technical structures at the journals and database sites;

With regards to the first point:

This appears to be unsolved and seems to be a typical chicken-and-egg problem because an announcement is useless since currently there is neither a mock-up is nor a detailed description of the process.
A suggestion to start a „road show“ to present the idea before sending out the letter to editors was not adopted.

Question: Who is willing to create this description? It was suggested that this could be done jointly with Dietmar (s. below)

With regards to the second point:

Communication with journal editors and publishers is required. The question remains which journals to contact in addition to those whose editorial boards are not covered by STRENDA members. The closing decision was to contact initially the most relevant journals (i.e. the most cited journals) for BRENDA (s. list below).
A letter to the editors will be drawn up briefly explaining the intentions of such data repository in a few words. This letter should also contain a first draft on the time table of the implementation/realization of this project. It was suggested to take Keith's draft letter from the April 2005 STRENDA meeting (see appendix) and extend it by including a little more information on BRENDA, and the NC-IUBMB approval of the STRENDA checklists as well.

Question: Who is willing to write this letter? Who will be contacted?

Tab. 1 List of journals of highest impact for BRENDA

Journals
J. Biol. Chem.
Biochim. Biophys. Acta
Biochemistry
Eur. J. Biochem.
Arch. Biochem. Biophys.
Biochem. J.
J. Bacteriol.
J. Biochem.
Phytochemistry
Plant Physiol.
Biochem. Biophys. Res. Commun.
FEBS Lett.

With regards to the second point:

Dietmar Schomburg is ready to prepare the BRENDA database system to receive enzyme data from authors. Prerequisite: updated STRENDA checklists (s. below).
The discussion if SABIO (Isabel Rojas-Mujica, EML Research gGmbH) could also be involved resulted in the finding that this database is a.o. less suited for this project since the long-term funding situation of SABIO is not yet been resolved. In contrast, there is a definite assurance of funding for BRENDA for at least next 5 years. However, the question remains if the scientific community would accept BRENDA as the exclusive data repository.

Question: Is there a preliminary schedule for the implementation/adaption in BRENDA?

Part Two: Suggestions to STRENDA from 2nd ESCEC symposium

Suggestions	*Included in checklists*	*To be discussed / comment*
assay conditions in extremophiles (determination of optimal growth conditions)		experimental standards for model organisms as started with level 2 list. Are there any extremophile model organisms?
clear description of conditions used	√	
Information missing in publications about assay proceedings, experimental conditions or complete reactions (with all reactants)	√	
presentation of k_{cat} to describe how the amount of functional enzyme has been determined	√	see list level 1B
presentation of the unit for k_{cat} as $time^{-1}$ which should require that the amount of functional enzyme was determined through active site titration	√	?
names of proteins often are gene names		nomenclature problem
„chaos“ in enzyme nomenclature (EC number problem) --> time consuming when searching for information --> standardized enzyme nomenclature required		nomenclature problem
• - different nomenclature systems used in biochemistry; • - commonly used trivial names vs. recommended systematic names; • - functional classification of enzymes vs. physical enzyme proteins (e.g. only 1 EC-class for hundreds of protein kinases) •		nomenclature problem
purity control of enzyme fraction		see list level 1A

Suggestions	*Included in checklists*	*To be discussed / comment*
enzyme characterization including sequence coverage and post-translational modifications	√	see list level 1A
control of identity of proteolytic reaction products		good point: what about expanding list level 1A by „products determined“ under „Methodology“?
lack of reproducibility --> no common standards --> no enforcement in reporting molecular modelling results reporting „experimental“ conditions		general problem of reporting the experimental conditions
incomplete descriptions of models in literature		the same problem as with the reporting of experimental conditions. Suggestion: List level 3, required data for model descriptions
standards required which provide descriptions but not prescriptions		important issue to be considered when propagating the checklists
standards for normalizing methods		use of standard enzymes as often done in clinical enzymology? This is not an issue for STRENDA, isn't it?
availability of raw experimental data is prerequisite to model development and validation		added to list level 1B, issue for discussion on data repository discuss
reporting molecular modeling results		same as incomplete description of models in literatur --> list level 3?
organism (Mammals, Rat or even no organism)	√	
environmental data (missing pH, temperature, buffer composition, substrate concentration assayed)	√	

Suggestions	*Included in checklists*	*To be discussed / comment*
identification of compounds/reactions (Multiple names - one compound)		usual (bio)chemical problem --> suggestion: list level 1A, IUPAC names only for compounds?

Part Three:

STANDARD REQUIREMENTS FOR REPORTING ENZYME ACTIVITY DATA

http://www.strenda.org/

Version 1.1
Date: April 5th, 2006

The Beilstein Institute created a working group after the 1st ESCEC symposium in 2003, called STRENDA. The name represents "Standards for Reporting Enzymology Data".
Much reported enzyme data is of limited use to others attempting to apply those data, because the conditions under which they were obtained are insufficiently documented. The members of the STRENDA commission have recently described these difficulties more fully (*Trends Biochem. Sci.*, 2005: **30**:11-12; PMID: 15653320), and this Commission, in consultation with the wider scientific community, plans to address them, in the hope that future publications will more readily yield the sort of information that researchers hope to find. This list was compiled, as a service to the community, by the STRENDA Commission to define the minimum amount of information that should accompany any published enzyme activity data.

Level 1A: required data for e.g. materials&methods section

Identity of the enzymes

- Enzyme name, EC-number and organism *(or, if not available, sequence or reaction catalysed)*
- Isoenzyme

Conditions

- measured reaction
- defined assay temperature
- defined assay pH
- buffer & concentrations *(e.g., 100 mM Tris-HCl, 200 mM potassium phosphate)*
- metal salt(s) & concentrations *(e.g., 10 mM KCl, 1.0 mM $MgSO_4$)*

• other assay components & concentrations *(e.g., 1.0 mM EDTA, 1.0 mM dithiothreitol,* 0.1 % Triton X-100, coupled assay components)
• substrates & concentrations *(e.g., 100 mM glucose, 5 mM ATP)*
• enzyme/protein concentration *(e.g., nmol ml^{-1} or mg ml^{-1})*

Methodology
• assay method *(a literature reference may suffice for an established procedure)*
• type of assay *(e.g., continuous or discontinuous, direct or coupled)*
• reaction stopping procedure (*in the case of discontinuous assays)*
• direction of the assay *(e.g., NAD reduction, glucose phosphorylation)*
• reactant determined *(e.g., NADH formation, O_2 utilization)*
• reaction stoichiometry *(e.g., 2 mol substrate oxidized per mol O_2 consumed)*

Preparation
• description *(e.g., commercial source, procedure used or reference)*
• artificial modification *(e.g. Truncated, His-tagged, fusion protein, lacking native glycosylation)*
• purity *purity defined by which criteria?* *(e.g., apparently homogeneous, crude mitochondrial fraction)*

Additional information
• tissue/organelle
• localization
• post-translational modification

Nice to have
- total assay mixture ionic strength
- free concentrations of Mg^{2+} and Ca^{2+}

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Level 1B: Reporting enzyme data - *Preliminary draft*

Enzyme Activity
• proportionality between initial velocity and enzyme concentration
• initial rates of the reaction measured

Comments: these were originally in List A but appear to have got lost. They are far too important not to be stressed.

• units (given as ~~concentration~~ amount per time, e.g. (micromol product formed/min)/mg enzyme protein (sometimes referred to as enzyme unit or international unit), or katal: conversion factor 1 unit = 16.67 nkat)

Comments: The old enzyme unit was µmol/min/mg protein. It is satisfactory provided it is defined as such.

• specify whether relative to subunit or native enzyme (oligomeric form)

Note: the term turnover number should not be used unless this is specified.

Kinetic parameters (V_{max}, k_{cat}, K_m, k_{cat}/K_m, $S_{0.5}$ with ~~dimensions~~ units, Hill coefficient etc.)
• How parameter was obtained (e.g. non-linear curve fitting using least squares, non-parametric method such as direct linear plot, linear regression to transformed form of rate equation)
• explanation why the chosen model is considered to be the „right" model
• s/K_m range used (e.g. 0.1 to 10)

• number of repeats (also indication of problems of repeats)
• indication of accuracy
(e.g. standard error of the mean, standard deviation, confidence limits, quartiles)
• high-substrate inhibition with K_I value if observed

Inhibition
• time-dependence and reversibility
• reversibility
• for reversible inhibitors -
• type (e.g. competitive, uncompetitive, mixed)

Note: Consider the sensitivity to „mixed inhibition“ of some experts!

• K_I values (with dimensions) and how determined.
• for tight-binding inhibitors
• association/dissociation rates
• type and K_I values (with dimensions)
• for irreversible inhibitor -
• type (e.g., non-specific, mechanism-based, "suicide substrate", with appropriate parameters)

Note: IC_{50} may be used for both reversible or irreversible inhibition, but the meaning depends on the type of inhibition.

Comments: These are many alternative parameters here; too many to list in a brief document. The reference to a quite comprehensive source is recommended: Enzymes: Irreversible Inhibition. K.F. Tipton In: *Nature Encyclopedia of Life Sciences*. Nature Publishing Group, London. http://www.els.net/ [doi:10.1038/npg.els.0000601] (2001), but then I would think it is a splendid account.

Activation
Similar requirements to those for inhibition.

Nice to have
• *Kinetic mechanism (e.g. ordered bi-bi, Monod-Wyman-Changeux, reversible Hill)*

Comments: There is conflicting material here. It implies that reversibility only occurs with Hill, whereas it is important fo all mechanisms. Furthermore, Hill describes an equation that is known to be based on an invalid model, and not a mechanism such as those in the other examples. Finally, apart form hydrolases and some isomerases, most reactions involve more than one substrate and it should not be implied that reactions such as the Monod-Wyman-Changeux are necessarily single substrate mechanisms. Since this item is of a different level of complexity from the others, it is suggested to put it in, yet another, list of requirements for reporting mechanistic data.

• time-dependency of enzyme reactions
• example of at least one experiment together with raw data

Appendix:

Keith's suggestions for

STRENDA LETTER TO JOURNAL EDITORS

Dear....
The STRENDA Commission was formed, with the support of the Beilstein Institute, to address the problem of standards in the reporting of enzyme data. Further details of the goals and composition of the Commission can be found at www.strenda.org.

In the first instance we devoted our attention to the problem that much of the data reported in the literature is deficient in that they are insufficiently documented. This means that they can be of little value to those trying replicate and extend the work or to use them for systems biology applications. Therefore, we have compiled a list of the minimum amount of information that should accompany such data. This is attached as an annotated list and also as a draft check list might be of use to Editors and reviewers.

*[We have written separately to xx, yy, zz from your Editorial (Advisory) Board since we believe their expertise makes advice from the particularly valuable for this work, and would be grateful if you would bring it to the attention of any other members of your Board who have particular interest in this area.]

‡{Since we are aware of th critical importance of standards for the Clinical Biochemists (Chemical Pathologists, Medicinal Chemists,...) and we would particularly welcome your input.}

We would value your comments and suggestions on this, because when the contents are fully agreed, we hope you will consider making the provision of such data, either within the paper itself or for on-line deposition, a requirement for acceptance. It would be of invaluable service to those interested in all aspects of enzyme work if that were done.

Please let me know if you would like any further information at this stage.

Yours sincerely