



FAIRDOM: hands-on basic training



Using SEEK to manage your research assets

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Tasks



- **T1.** Creating and interlinking ISA elements
- **T2.** Downloading spreadsheet templates based data from SEEK, editing it and uploading to own ASSAY created in T1.
- **T3.** Creating SOP (as link to Nature protocols or local file to upload), link it to data file
- **T4.** Registering publications (with DOI or PubmedID), linking it to ISA, data file.
- *T5. Creating/using sample types and (bio)samples*
- *T6. Create model, upload image representing e.g. pathway your model describes*

Pre-task



- Go to the SEEK instance set for training
<https://sandbox4.fairdomhub.org/>
- Log in <https://sandbox4.fairdomhub.org/> (username/password : **guest11 – guest30**)
- Go to <https://sandbox4.fairdomhub.org/events/3>
- Find **Help** documents <http://docs.seek4science.org/help/>
- Find **Presentation** “HandsOn_SEEK_Instructions”
<https://sandbox4.fairdomhub.org/presentations/7>

Instructions



In the hands-on session you can either

- (i) use your own data, SOPs, to set up an ISA structure in SEEK
- (ii) use examples from FAIRDOMHub (e.g. templates with example data , SOPs, available to you

Please note that the assets you create in a training SEEK instance will be available for the duration of the workshop (+ one week) and deleted afterwards the workshop

....organised in an ISA (Investigation, Study, Assay/Analysis) format.



FAIRDOM Browse - Help - Search here... Search

Home / Investigations Index / Glucose metabolism in Plasmodium falciparum trophozoites

Glucose metabolism in Plasmodium falciparum trophozoites

The investigation entails the construction and validation of a detailed mathematical model for glycolysis of the malaria parasite Plasmodium falciparum in the blood stage trophozoite form.

ID:50

Projects: Whole body modelling of glucose metabolism in malaria patients

Selected item: Investigation: Glucose metabolism in Plasmodium falciparum trophozoites [Full graph](#)

Investigation

Investigation: Glucose metabolism in Plasmodium falciparum trophozoites

Study

- Study: Model construction
- Study: Model validation
- Study: Model analysis
- Publication: Construction and validation of a detailed kinetic model of glycolysis in Plasmod...

Related Items

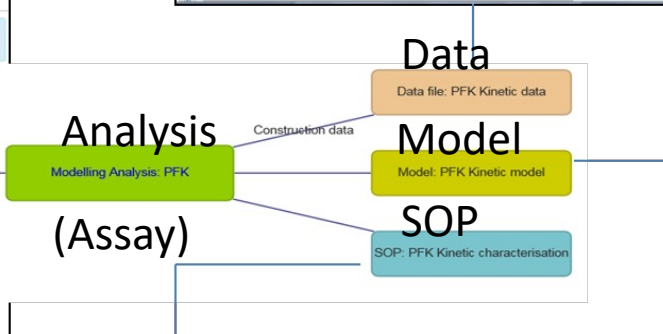
People (1) Projects (1) Studies (3) Assays (24) Data files (16) Models (19) SOPs (13) Publications (1)

David Van Niekerk

Projects: SysMO DB, Whole body modelling of glucose metabolism in malaria patients
Institutions: University of Stellenbosch

Disciplines: Miositer
Roles: Not specified
Expertise: Not specified
Tools: Not specified

	Values (examples)			
18	Assay Title	PFK Kinetic Data		
19	Uploader	Derick van Niekerk		
20	Uploader SEEK ID			
21	Project			
22	Assay			
23	Assay SEEK ID	0		
24	Assay Date	PFK		
25	Assay Type	Microplate Assay		
26	Technology Type	well-plate experiment		
27	Description	Kinetic characterisation of PFK, Enzym		
28	Experimentation	Creed Pradhan		
29	Date			
30	DOI			
31	Publication (optional)			
32	Experimental conditions			
33	Component (of concentration)	temperature	pH	buffer
34	Unit	°C		HEPES
35	Value (optional)	37	7.17	mM
36	End_value (optional)			30
37	Comments			
38	Culture growth	Batch		
39	Factors studied			
40	None	substration	concentration	concentration
41	Component (of concentration)	ADP	ATP	FBP
42	Unit	mM	mM	mM
43	Value (optional)	0	0	0
44	End_value (optional)	0.214713	0	0
45	Comments			
46	DOI (optional)			



PFK Kinetic model

Mathematica notebook for the parameterisation of the PFK rate equation based on SEEK ID 50

1 item (and an image) are associated with this Model:

- PFK-SEEK ID (Mathematica Notebook - 202 KB)

Organism: Not specified

Model type: Ordinary differential equations

Model format: Mathematica

Execution or visualisation environment: Not specified

Model image: [Click on the image to zoom](#)

$$v_{PFK} = \frac{V_{max} \cdot \frac{atp}{K_{ATP}} \cdot \frac{fbp}{K_{FBP}}}{(1 + \frac{atp}{K_{ATP}}) \cdot (1 + \frac{fbp}{K_{FBP}} + \frac{fbp}{K_{FBP}}) \cdot (1 + \frac{atp}{K_{ATP}} + \frac{atp}{K_{ADP}})}$$

Selected item: Model: PFK Kinetic model

PFK SOP

Specific activity of the glycolytic enzymes were measured in NADPH/NADP⁺ linked enzyme assays that were adapted from Teokin et al. [1] and measured at 340 nm in 96-well plates (Flat Bottom microplate, Greiner Bio-One, Kriemundsmir, Austria) on a spectrophotometer (Microplate microplate reader, Thermo Electron Corporation, Waltham, Massachusetts, USA). The same buffer, (20 mM HEPES, 20 mM MgCl₂, 10 mM KCl and 20 mM NaCl), was used for all assays, with a pH set to 7.17, matching the cytosolic pH of P. falciparum [2]. All of the linking enzymes were used at a non-limiting, final concentration of 5 U/ml. All reagents and enzymes were obtained from Sigma-Aldrich, St. Louis, Missouri, USA.

For phosphofructokinase (PFK) activity, the phosphorylation of FBP (0 - 30 mM) by ATP (0 - 5 mM) as well as inhibition by ADP (0 - 5 mM) was linked to the oxidation of NADH (0.6 mM) via acetylP₄ ALD. PFK Product inhibition by F16BP (0 - 60 mM) was assayed by linking the production of ADP to the oxidation of NADH (0.6 mM) via LDH. PFK in the presence of FBP (2 mM). Since PFK exhibited substrate inhibition, the enzyme rates could not be normalised to maximal specific activity at saturating substrate concentrations. A control rate was determined at 1.25 mM ATP and 1 mM FBP.

[1] Teokin B, Passarge J, Rejzinger C, Eggshado E, van der Weijden C, et al. (2009) Can yeast glycolysis be understood in terms of an enzyme kinetics of the constituent enzymes? testing biochemistry. Eur J Biochem 281: 5313-5329.

[2] Wörnsch S, Sanchez C, Gekle M, Grosse-Wilde L, Wiesner J, et al. (1998) Differential stimulation of the Na⁺/H⁺ exchanger determines citosolic uptake in Plasmodium falciparum. J Cell Biol 140: 335-345.

Task 1. Creating new ISA








- Create new Investigation, associate it to your project (FAIRDOM training) , share it with own project
- On created **Investigation page** add a new **Study**, associate it to your project (FAIRDOM training) , share it with own project
- Scroll down to the I-S-A graph, navigate to the **Study** page
- Add new **experimental Assay** to the **Study** created
- Define assay type and appropriate technology type (choose from the drop-down lists)
- Assign your **Assay** to the existing **Study**
- Define the access rights for your **Assay** – e.g. sharing it with own project, or with single person
-


Access Permissions : Just Enough Sharing

Sharing ▾

Here you can specify who can **view** the summary of, **get** access to the content of, and **edit** the Data file.

	No Access	View	Download	Edit	Manage	
 Public	✗	○	○	▨	▨	
 Kinetics on the move - Workshop 2016	○	✓	✓	○	○	
 Martin Siemann-Herzberg	○	✓	✓	✓	○	✗
 SysMO-LAB @ University of Amsterdam	○	✓	○	○	○	✗
 EmPowerPutida	○	✓	✓	○	○	✗

 Share with a person

 Share with a project/institution

Task 1a. Creating new ISA



- Navigate back to the **Study** page (via menü or using I-S-A graph)
- Add new **modelling analysis Assay** to your **Study**
- Define **Model type** , **Model format**, and **Preferred execution or visualisation environment** (choose from the drop-down lists)
- Assign your **Assay** to the existing **Study**
- Define the access rights for your **Assay** – e.g. sharing it with own project, or with single person

Task 2. 1 Downloading and uploading data



- Go to https://fairdomhub.org/data_files/927/
- Visualise data content, look for spreadsheet structure and format
- Download this **data file** and open it
- Fill in some reasonable example data. This spreadsheet has been created using RightField, so you can select values from the drop-down lists in the spreadsheet
- Save the **data file** with a new name on your PC
- Upload (create new **data file**) **this file** to <https://sandbox4.fairdomhub.org/>
- Describe your data
- Link the data file to own experimental **assay** created in T1.

Task 2.2 Sharing data file



- Share **data file** with (i) project (ii) certain person (iii) public
- Define a temporary public link to your **data file** (expiring e.g. at end of June 2017)
- Logout and check whether you can access the **file** directly via **temporary URL**
- Subsequently make the **file** public
- Logout and check whether you can find and access the **data file** by browsing

Task 3. Creating SOP



- Go to example **SOP** <https://fairdomhub.org/sops/253> , view content
- Go to <http://www.nature.com/protocolexchange/protocols/> and choose any protocol (e.g. <http://www.nature.com/protocolexchange/protocols/4761>)
- Go to FAIRDOMHub , create new SOP as
 - a) external link to Nature protocols (URL)
 - b) uploading local file (downloaded from FAIRDOMHub or your own prepared SOP)
- Share it with own project, or with single person
- Link **SOP** to experimental **Assay** created in T1.
- Link **SOP** to the **data file** created in T2.

Task 4. Registering publications



- Go to Pubmed and choose any publication of your interest, copy PubMedID
(e.g. <https://doi.org/10.1371/journal.pcbi.1003186>)
- Go to FAIRDOMHub and register new **Publication** (using PubMedID or DOI)
 - select **Publication** from Create menu
 - choose PubMed ID or DOI – insert the corresponding ID, click “Fetch” button
 - confirm abstract and authors list, finish
- Link **publication** to **data file** created in T2.
- Link **publication** to **SOP** created in T3.