

Human Antibody Initiative (HAI)

Proposal to HUPO council

Mathias Uhlen, April, 2005

Summary

The Human Antibody Initiative (HAI) aims to promote and facilitate the use of antibodies for proteomics research. The initiative consists of two separate activities; (1) the generation of a catalogue of validated antibodies from many different sources and (2) a protein atlas for the expression and localization of human proteins in normal and disease tissue. The two separate activities have as their primary deliverables to generate databases with free public accessibility. The Antibody Resource database is aimed to produce a comprehensive catalogue of validated antibodies towards human proteins. This initiative depends on input from a large number of academic groups and commercial companies. A pilot phase will be initiated Q3 2005. From Q1 2007 this initiative will start the “production phase” and the objective is to have quarterly releases with all data approved by curator. The Protein Atlas initiative is aimed to provide comprehensive and annotated database of high-resolution images showing tissue profiles in normal and cancer tissues. The first release of the database will be at the fourth annual HUPO conference (August 2005 in Munchen) with 300,000 images representing 600 human proteins. An updated release of the database will be done thereafter in conjunction with the annual HUPO conference with at least 500,000 additional images (1000 proteins) each time. Both databases will be open to the public without restriction (no passwords).

The HAI will be chaired by Mathias Uhlen until Q3 2007. An executive committee with representatives (co-chairs) from all HUPO regions (Europe, Asia/Pacific and North America) and the HUPO headquarter will act as an advisory board. An annual workshop will be arranged to discuss and approve guide-lines and objectives for the initiative. The chair, co-chairs and other members of the executive committee will be decided on a “rolling two-year” basis by the HUPO council in conjunction with the HUPO annual conference each year.

Vision

The vision is to enable, within 10 years, the generation of uniform antibody collections against every human protein (non-redundant set) and to use these reagents to functionally explore the corresponding proteins, protein variants (isoforms) and protein interactions.

The proteome is here defined as the “non-redundant” set of proteins, which is a representative protein from every gene locus. For the multitude of proteins with splice variants, isoforms or other protein variants, the preferred target for the antibody (if possible) is one of the common, non-variable regions of the protein. At present, the non-redundant proteome, as defined by Ensembl (www.ensembl.org), consists of approximately 22,000 proteins

Motivation for a HUPO-endorsed antibody initiative

- Affinity reagents are absolutely critical in proteomics, molecular and cell biology
- Researchers make antibodies against the same set of human gene products, resulting in huge duplication of effort.
- Standards are needed to provide a comprehensive proteome-wide resource
- Proteomic efforts require the provision of large sets of reagents such as antibodies to study a collection of proteins, rather than a few individual proteins.

Scientific objectives

The initiative consists of two separate activities but with considerable synergy and overlap. In both cases, the primary deliverables are databases with free public access aimed to promote the use of antibodies for proteomics research.

1. Antibody Resource Database

The objective is to create a HUPO-endorsed database with links to validated antibodies. Each antibody should be available to the public either through a commercial source or a public repository. The aim is to set-up a web-page with a list of all available antibodies with a description, including:

- Gene Id of target (Swissprot, Ensembl etc)
- Description of target used for the antibody generation
- Description of type of antibody (pAb, mAb or recAb)
- Results from quality assurance (at least one)
- Information about the availability of the antibody (web-link)

The antibodies should be quality assured by at least one of the following assays (exact rules will be defined by the antibody initiative workshops):

- Protein assay (ELISA, protein array etc)
- Western blot (with relevant human tissue extracts)
- Immunohistochemistry (preferably Tissue microarray)

- Immunocapture results (“pull-down” followed by MS-analysis)

A web-based submission scheme will be developed. The submissions will be approved by a curator and subsequently published on the public database. The intention is to subsequently incorporate experience from the public (users) into the database to indicate if antibodies work or not work for specific applications.

The long-term objective (10 year) is to enable at least two independent antibodies to all human proteins. In addition, the generation of a resource of isoform-specific antibodies (splice-forms etc) will be promoted.

2. Human Protein Atlas database

The objective is to create a HUPO-endorsed database with annotated images of the expression and localization of proteins in normal and cancer tissues. A set of standardized tissue micro arrays (TMAs) has been produced to allow for rapid screening of a multitude of different cells and tissues using immunohistochemistry. Tissue micro array blocks have been produced containing 48 different normal human tissues in triplicate and cancer tissue from 216 individually different tumors in duplicate. Digital images for annotation of expression profiles are generated using a semi-automated approach. 576 images and annotation data corresponding to a total of 30 Gbytes of data are collected for each antibody.

The database will be based on open source architecture. All original TIFF images will be stored in duplicate. Public access to the data will be ensured through a web-server with high capacity and high-speed access. All images will be text annotated by manual and/or automated methods to allow simple searches and queries.

Collaborators will be invited to submit their own data to the database. The format set-up by the HAI executive committee and the quality assurance rules must be followed. Antibodies from both commercial and academic sources will be accepted. Data will be release independent of IPR status of the respective antibody. All clones used for recombinant expression of proteins must be sequence verified. All proteins used for generation of antibodies must be size determined by mass spectrometry. All antibodies used for tissue profiling must be validated by at least one of the assays defined in the Antibody Resource database guide-lines.

Time plan and deliverables (two-year plan)

- Proposal to HUPO executive committee for approval (April 2005)
- Launch of the Human Protein Atlas (August 2005)
- Workshop to set-up rules for Antibody Resource database (August 2005)
- Launch of the pilot version of the Antibody Resource database (Q1 2006)
- Second release of Protein Atlas (Q3 2006)
- Workshop to review both efforts (Q3 2006)
- Launch of Antibody Resource database with curator (Q1 2007)

- Third release of Protein Atlas (Q3 2007)
- Workshop to review both efforts (Q3 2007)

Data sharing plan

The first launch of the protein atlas is suggested to take place at the HUPO conference in Munchen, August 29, 2005. The objective is to release approximately 300,000 images representing at least 600 proteins at this time. A new annual release of the database will be done thereafter in conjunction with the annual HUPO conference with at least 500,000 additional images (1000 proteins) each time. The database will be open to the public without restriction (no passwords). Discussion has been initiated with the other public databases (such as EBI and Ensembl) to cross reference the Protein Atlas database.

The launch of the pilot version of the Antibody Resource database will be done in January 2006. The database will be updated on a quarterly basis. The pilot project will be evaluated at the fifth annual HUPO conference and the aim is to launch an “executive” version of the database in the beginning of 2007. The database needs from this launch onwards, one to two full-time curators and the location and funding of these persons need to be decided in conjunction with the fifth annual conference.

Leadership and management

The initiative will be chaired by Mathias Uhlen until Q3 2007. An executive committee with representatives (co-chairs) from all HUPO regions (Europe, Asia/Pacific and North America) and the HUPO headquarter will act as an advisory board. An annual workshop will be arranged to discuss and approve guide-lines and objectives for the initiative. The chair, co-chairs and other members of the executive committee will be decided on a “rolling two-year” basis by the HUPO council in conjunction with the annual HUPO conference each year.

The chair will manage the development of the two databases, organize workshops and be responsible for quarterly reports. Co-chairs will be appointed by the HUPO council after recommendations from the Antibody workshop in August 2005. The chair and the co-chairs together with the Director of the HUPO headquarter constitute the HAI executive committee, which meets at least once a year. The responsibility of the committee is:

- To define the rules for HUPO-endorsed antibody projects and how to coordinate these
- To define quality control specifications (clones, proteins and antibodies)
- To define public accessibility to information (databases) and resources (reagents)
- To organize a workshop at least annually (in conjunction with the HUPO conference)

Reporting

A short report on the progress of the initiative will be sent to the HUPO headquarter on a quarterly basis. A representative of the antibody initiative (preferably the chair person) will participate in each annual HUPO initiative meeting (spring time) as well as the annual HUPO meeting (fall).

Workshops

Each year in conjunction with the annual HUPO meeting, there will be a workshop organized by the chair of the initiative.

Interaction with existing initiatives

An important function of the Antibody Initiative is to promote the generation of antibodies to proteins of particular interest for the different HUPO initiatives. The generation of antibodies to proteins of interest for HUPO initiative will be of high priority.

Budget

For the first two years, the databases will be set-up and managed by the Albanova University Centre in Stockholm (www.hpr.se). Funding to manage the Protein Atlas database for the two-year period, starting September 2005, has been obtained from the non-profit organization Knut and Alice Wallenberg Foundation, Sweden. Funding to set-up the Antibody Resource database and to run a pilot project until end of 2006 has also been secured from the same source. Funding to manage and curate the database (1-2 FTEs) following the pilot phase must be resolved during 2006.