Computational biology exploration of the enzymatic diversity of an uncharacterised prokaryotic protein family

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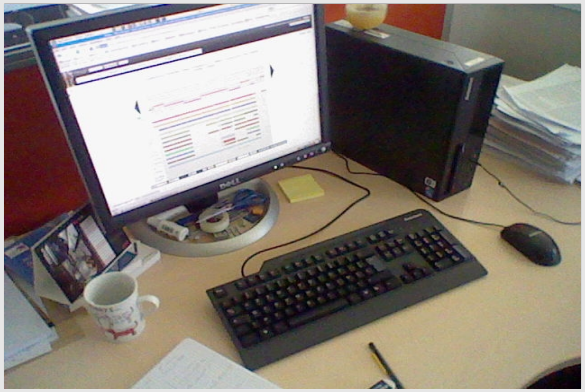
Discovery of a gene for an orphan activity

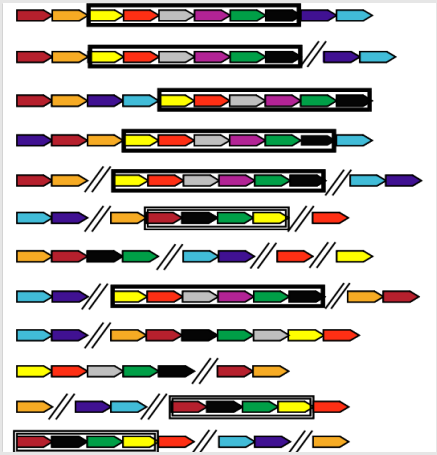
Approx. 30% of EC numbers defined by the IUBMB are still sequence-orphans.

The Genoscope has started a transversal initiative to identify genes for these activities.

1 pilot project



Bioinformatics analysis of an incomplete metabolic pathway led to ...



...the discovery of a conserved genomic context, hinting a possible activity for an uncharacterised protein family...

biochemical assays

What about the others? (approx. 660)

... which was then experimentally confirmed on **some** (12) family members.

Overview of the BKACE Pfam family

DATA :
Family of proteins defined by the presence of a conserved Pfam domain.
Illustrated : alignment of DOMAIN over 663 proteins

OBJECTIVE :
explore metabolic diversity of all proteins

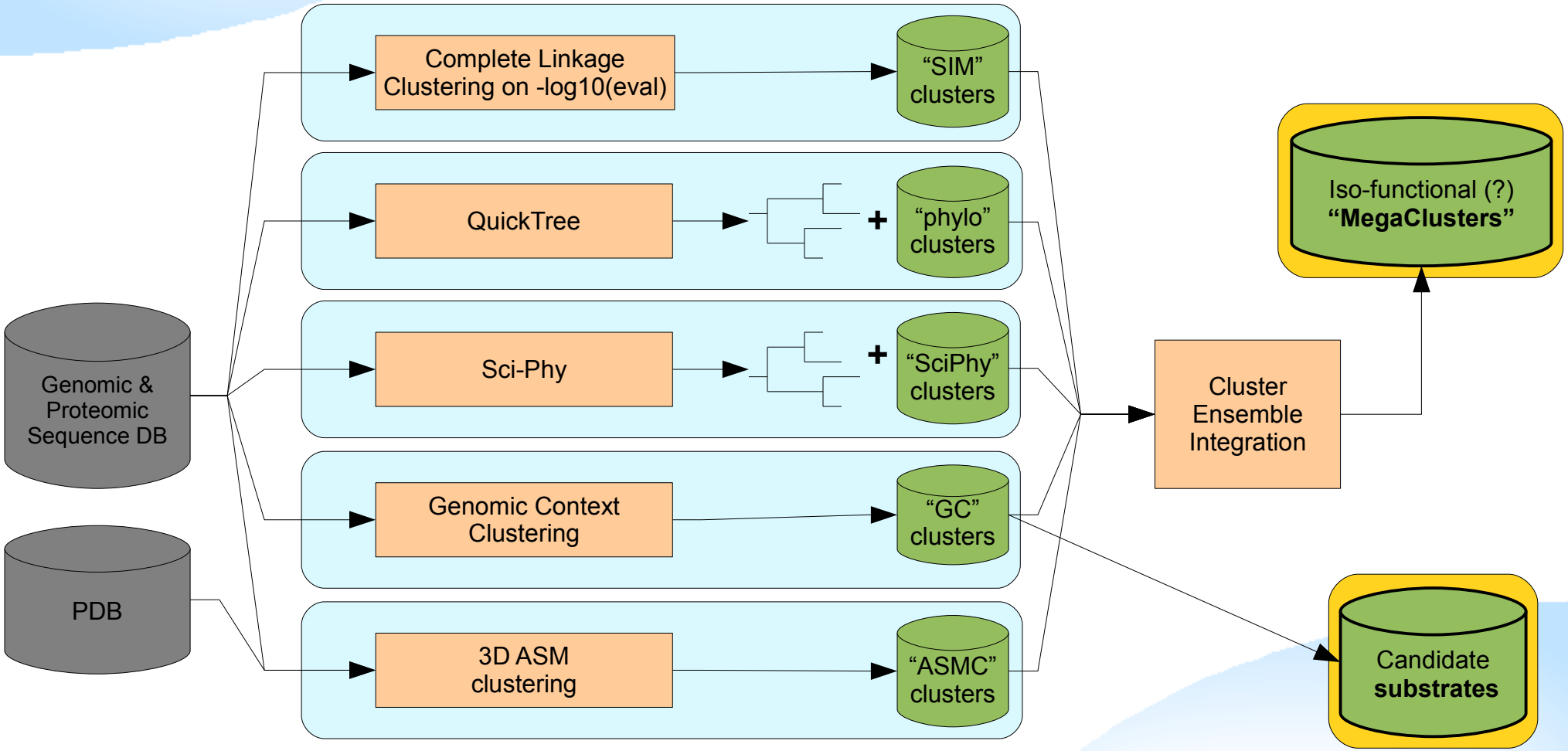
GENERAL STRATEGY :

- using computational biology :
  - cluster proteins into **iso-functional** groups
  - use diverse functional homology clues
  - propose **candidate substrates** for each cluster

- biochemical assays :
  - select representatives for each cluster
  - test known & proposed activities

Pfam : http://pfam.sanger.ac.uk/
"The Pfam protein families database" R.D. Finn & al., Nuc. Acids Res. (2010) Database Issue 38:D211-222

Clustering strategies applied to the BKACE family



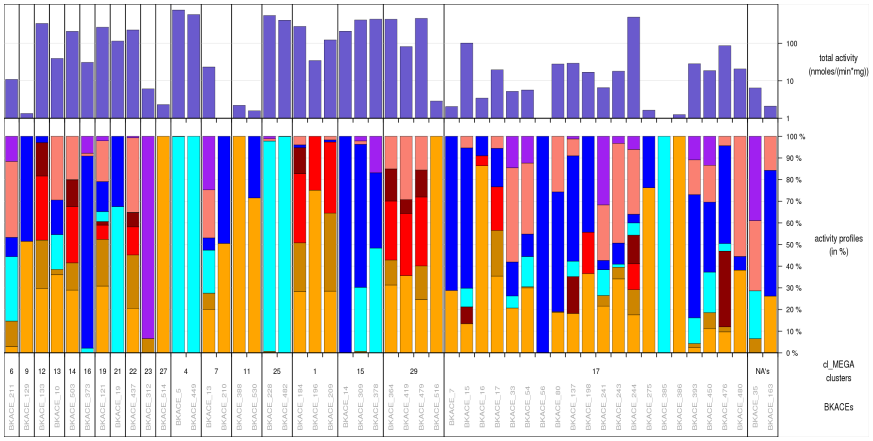
Clustering & Biochemical results

Proposed candidate substrates :

- 4 new candidates proposed using metabolic contexts
- 7 new candidates proposed using substrate similarity
  - ➔ 8 found as potential substrates so far

MegaClustering :

- 88 MegaClusters
- heterogeneous cluster sizes (3-130); no singletons
- 1 “dustbin” cluster



Biochemistry :

- 130 BKACE attempted
- 62 BKACE cloned and tested for proposed activities
- 52 positive hits
- too few cloning successes (high GC content) per MegaCluster
  - ➔ Impossible to validate MegaClustering strategy
  - ➔ New candidates currently being tested

Thankfully :

- ➔ coherence of various clusterings i.r.t. biochemistry
- ➔ explored a varied part of family's functional space

Perspectives

- Finish new clones & biochemical assays

- Assess coherence of different clusterings (GC, ASMC, MegaClustering)

- Assess usefulness of metabolic context pooling for substrate proposition

- Establish generalised strategy / protocol

- Try out on new families

Many thanks to ...

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Hungry for more details ?

Come discuss the strategy in front of our poster (#23)

Thank you for your attention !