Skinner Lab Meeting

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Amyloid bioinformatics project

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Introduction

Questions:

How are amyloid diseases the same, and how are they different?

More specifically:

1) What molecular pathways are common to many or all amyloid diseases?

2) What molecular pathways are unique to certain amyloid diseases (such as prion disease or Alzheimer's disease?)

Rationale:

A bioinformatics approach allowing comparisons of gene expression, gene annotation, and protein-protein and protein-DNA interaction data may answer these questions and provide leads for biomarker discovery

Aims and Methods

1) Comparative microarray analysis:

Create a unified amyloid bioinformatics database using gene transcription data

Outcome: two target lists of genes of interest – by their expression across many amyloid diseases, or by expression unique to individual amyloid diseases

2) Ontology annotation and data integration:

Create a semi-automated text mining and gene annotation tool using known ontologies, as well as with associated gene-disease, protein-DNA and protein-protein interaction data

Outcome: function annotation and clustering of genes in the target lists above

3) Data visualization and network inference:

Create a data visualization and network inference tool, using as input the unified amyloid database and the ontology annotation tool described above

Outcome: concise data visualization and network inference to prioritize target lists of genes and gene products for further analysis as biomarkers

Next steps

Structure project as a Java plug-in for Cytoscape

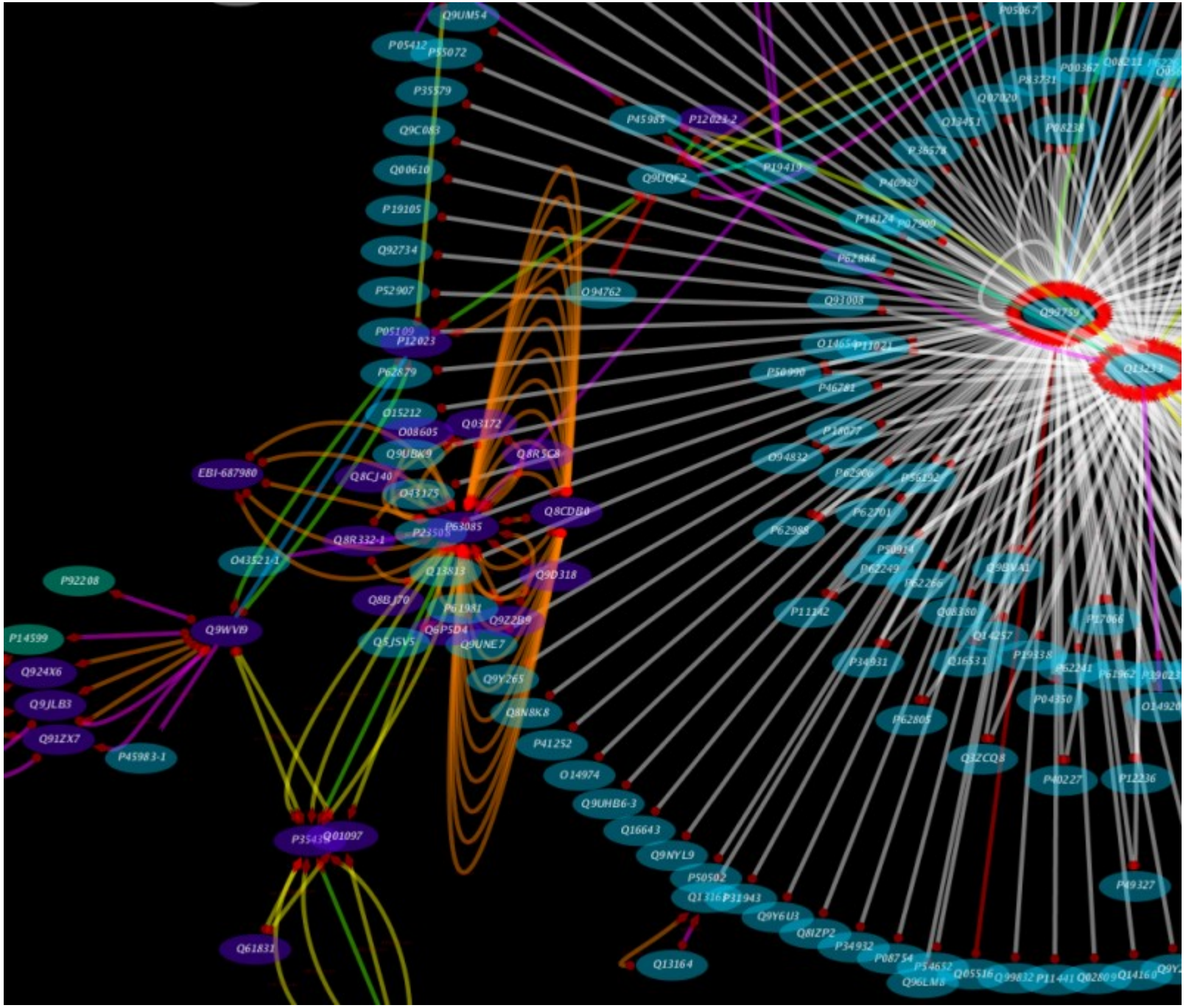
- Java and Cytoscape training

Identify and begin aggregating data

- GEO – Gene Expression Omnibus at NIH/NCBI
- Gene Ontologies (GO)
- Protein-protein/DNA interaction databases such as the Reactome Knowledgebase

Design database

Example of Cytoscape network visualization:



Amyloid diseases

- Amyloid diseases are characterized by extracellular deposition of insoluble fibrillar protein
  - all amyloid protein has a specific cross-beta core ultrastructure (reason for birefringence), with associated serum amyloid P (SAP) protein...
  - ...but the causes of amyloidosis are heterogeneous...
  - ...and about 35 different amyloid-forming proteins have been linked to human or animal diseases, either by their defective folding, or in their native state in high enough concentrations
- Can be inherited or acquired, localized or systemic, lethal or incidental
- Amyloid deposition may be reversible, by treating underlying cause
- In this patient, the amyloid was likely from monoclonal immunoglobulin light chains arising from her multiple myeloma (plasma cell tumors) – resulting in “AL amyloidosis”

Pepys MB. Phil Trans R Soc Lond B 2001;356:203-211.
Lachmann HJ & Hawkins PN. Chron Respir Dis 2006;3;203-214.
Rosai and Ackerman’s Surgical Pathology, 9th ed. Elsevier, 2004.

AL amyloidosis

- 60% of all systemic amyloidosis
- from monoclonal immunoglobulin light chains
- myeloma, and other lymphoproliferative disorders can cause it
  - Sjogren's syndrome, Castleman's disease, Waldenstrom's macroglobulinemia
  - about 10% of myeloma patients develop amyloidosis
- systemic AL: only about 10% 5-year survival, death usually by cardiac (50%), kidney or autonomic failure
- *dyspnea usually due to cardiac, not lung deposits*
- 30-90% of systemic AL have histologic pulmonary involvement shown at post mortem exam
  - may have restrictive pulmonary function pattern
    - rarely, reduced gas exchange

Cordier J-F. Sem Respir Crit Care Med 2005;26:502-515.
Lachmann HJ & Hawkins PN. Chron Respir Dis 2006;3;203-214.

AA amyloidosis

- ‘reactive systemic’ amyloidosis
  - as a result of chronic inflammatory states – MANY different causes of this
- via fragments of an acute phase reactant, serum amyloid A protein (SAA)
- common kidney involvement - nephrotic syndrome

Cordier J-F. Sem Respir Crit Care Med 2005;26:502-515.
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Table 2 Conditions associated with systemic AA (secondary) amyloidosis

Inflammatory arthritis
- Adult Still's disease
- Ankylosing spondilitis
- Juvenile idiopathic arthritis
- Psoriatic arthropathy
- Reiter's syndrome
- Rheumatoid arthritis
- Gout

Chronic infections
- Bronchiectasis
- Chronic cutaneous ulcers
- Chronic pyelonephritis
- Leprosy
- Osteomyelitis
- Q fever
- Subacute bacterial endocarditis
- Tuberculosis
- Whipples disease

Immunodeficiency states
- Common variable immunodeficiency
- Cyclic neutropenia
- Hyperimmunoglobulin M syndrome
- Hypogammaglobulinaemia
- Sex linked agammaglobulinaemia
- HIV/AIDS

Other conditions predisposing to chronic infections
- Cystic fibrosis
- Epidermolysis bullosa
- Injected drug abuse
- Jejuno-ileal bypass
- Kartagener's syndrome
- Paraplegia
- Sickle cell anaemia

Hereditary periodic fevers
- Familial cold urticaria
- Familial Mediterranean fever
- Hyperimmunoglobulin D syndrome
- Muckle-Wells syndrome
- TNF receptor associated periodic syndrome

Inflammatory bowel disease
- Crohn's disease
- Ulcerative colitis

Neoplasia
- Adenocarcinoma of the lung, gut, urogenital tract
- Basal cell carcinoma
- Carcinoid tumour
- Castleman's disease
- Gastrointestinal stromal tumour
- Hairy cell leukaemia
- Hepatic adenoma
- Hodgkin's disease
- Mesothelioma
- Renal cell carcinoma
- Sarcoma

Systemic vasculitis
- Behcet's disease
- Giant cell arteritis
- Polyarteritis nodosa
- Polymyalgia rheumatica
- Systemic lupus erythematosis
- Takayasu's arteritis

Other
- Atrial myxoma
- Inflammatory abdominal aortic aneurism
- Retroperitoneal fibrosis
- SAPHO syndrome
- Sarcoidosis
- Sinus histiocytosis with massive lymphadenopathy

Lachmann HJ & Hawkins PN. Chron Respir Dis 2006;3;203-214.

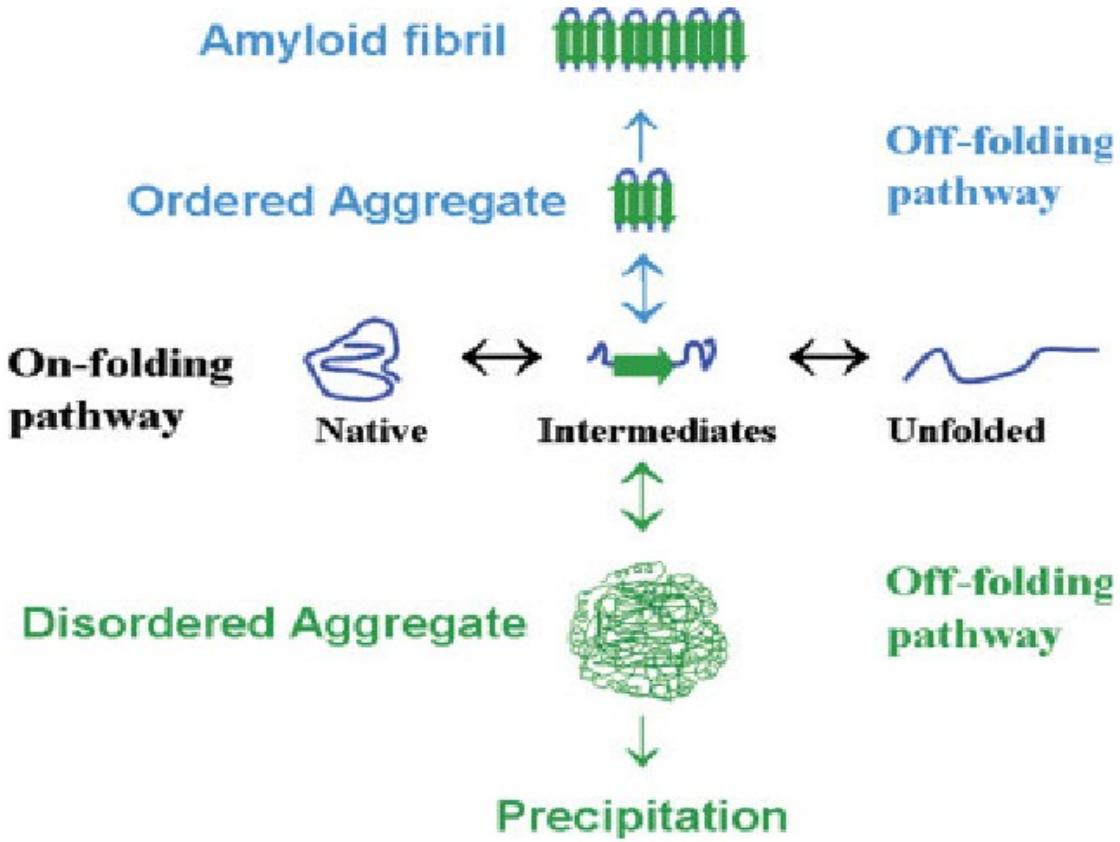
There are many amyloid diseases other than AL and AA:

Table 1
Some of the diseases associated with amyloid fibril formation and the main protein component of the aggregates formed

Disease	Main component of aggregates associated with disease
Alzheimer's disease	Aβ peptides, Tau
Frontal-temporal dementias	Tau
Parkinson's disease	α-Synuclein
Dementia with Lewy bodies	α-Synuclein
Transmissible spongiform encephalopathies (*e.g.* Creutzfeldt-Jakob disease and Mad Cow)	Prion
Huntington's disease	Huntingtin
Type II diabetes	Amylin
Senile systemic amyloidosis	Transthyretin
Familial amyloid polyneuropathy I	Transthyretin
Familial amyloid polyneuropathy III	Apolipoprotein AI
Haemodialysis-related amyloidosis	β_2-Microglobulin
Injection-localized amyloidosis	Insulin
Hereditary nonneuropathic systemic amyloidosis	Lysozyme
Spinocerebellar ataxias	Ataxins
Spinocerebellar ataxia 17	TATA-box binding protein
Primary systemic amyloidosis	Ig light chains
Secondary systemic amyloidosis	Serum amyloid A
Amyotrophic lateral sclerosis	Superoxide dismutase I
Medullary carcinoma of the thyroid	Calcitonin

Ecroyd H, Carver JA. Life 2008;60(12):769-774.

Amyloid formation



- A protein folds to its native state via partially folded intermediates
- Under conditions in which partially folded intermediates persist (e.g. during times of cellular stress or due to mutation), they can mutually associate via exposed hydrophobic regions normally buried in the core of the protein.
- When this occurs, intermediates aggregate via either a disordered or ordered mechanism, leading to the formation of amorphous (disordered) precipitates or ordered amyloid fibrils

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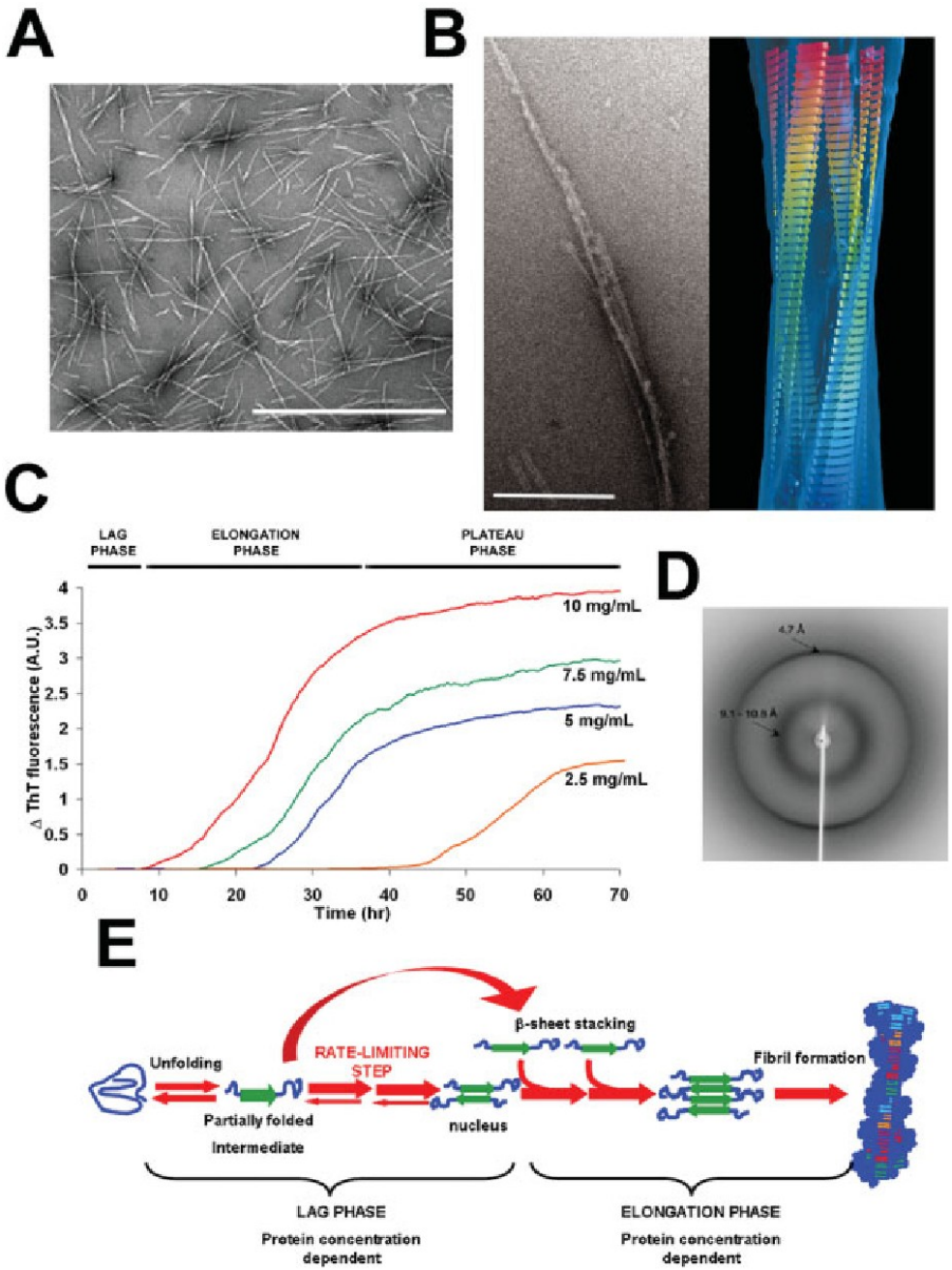

Figure 2. Monitoring the formation of amyloid fibrils and their generic core architecture.

(A) The typical structure of amyloid fibrils as viewed by transmission electron micrograph showing them as long, unbranched, rope-like fibers. Scale bar is 1 um.

(B) In the left panel, a magnified view of an a-synuclein fibril highlighting its internal protofilament substructure (scale bar is 200 nm). In the right panel, a schematic view of an amyloid fibril formed from insulin. This model shows the core structure of each filament, that is, the typical cross b-sheet array formed from sheets of b-strands lying perpendicular to the axis of the fibril and the aligning of these b-sheets into individual filaments.

(C) Monitoring amyloid fibril formation via the change in fluorescence of the amyloidogenic dye thioflavin T upon its binding to the fibril. The kinetics of fibril formation include a lag phase, elongation phase, and plateau phase. Typically, as the concentration of protein increases, the lag phase of the reaction decreases and the rate of fibril elongation increases.

(D) X-ray fiber diffraction of amyloid fibrils showing the diagnostic meridional and equatorial reflections which form the ‘‘cross b-sheet’’ pattern.

(E) The standard nucleation-dependent model of amyloid fibril formation. Fibril formation commences with the unfolding of a native protein, forming a pool of partially folded intermediates, a process that is reversible. The partially folded intermediates are able to associate with each other until they reach a critical size/mass at which a stable nucleus is formed. The formation of this nucleus from the partially folded intermediates is slow and rate-limiting in the overall process of fibril formation (lag phase). Fibril elongation then proceeds via the addition of intermediates to the growing nucleus. The mechanism also explains how seeding the reaction increases the reaction rate and decreases the lag phase because addition of preformed fibrils overcomes the time required to form nuclei.

Ecroyd H, Carver JA. Life 2008;60(12):769-774.