ARC Centre for Complex Systems, Australia

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Modelling the Import of Nuclear Proteins

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Introduction

- After being manufactured in the cytosol, many proteins undergo transport to organelles.
- The transport processes rely on signals within the protein.
- The signals are recognised by organelle specific mechanisms, which then effect transport.
- Ideally we would like to be able to predict the localisation of a protein from primary structure alone.

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Nuclear Import and the Regulatory Proteome

- Other organelles tend to have stable protein content
- The nucleus has a far more transitory protein complement
  - Due to the fact that localisation is one of the mechanisms of regulating interaction with the genome
- Hence accurate nuclear localisation prediction must form part of our understanding of the regulatory proteome.

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Nuclear Localisation

- Nuclear Localisation is unusual
  - Unlike some other localisation processes does not use a cleaved targeting peptide.
  - Large number of signals with no defined position.
  - Proteins are imported in their mature folded state.
- Transport requires a large channel through the nuclear membrane.
  - A macromolecule called the Nuclear Pore Complex

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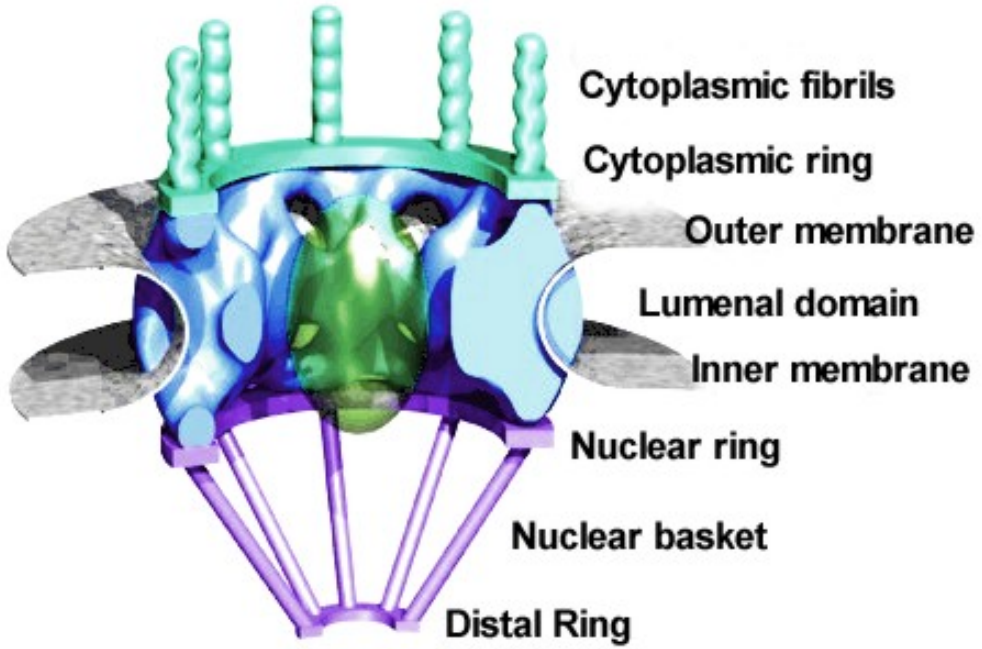
Nuclear Pore Complex (NPC)

- Spans the membrane between nucleus and cytoplasm.
- Passive diffusion of small molecules
- Large molecules require assistance.



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Getting Imported

- Mediated import through the NPC requires the assistance of proteins called Importins.
- These recognise the Nuclear Localisation Signal (NLS) on the ‘cargo’ molecule and bind to it.
- The importin-cargo complex binds to the cytoplasmic fibrils.
- Conformational change in the fibrils effects the transport of the importin-cargo complex.

Classical Nuclear Localisation Signals

- Usually short stretches of basic amino acids.
- SV40 large T-antigen like NLSs:
  - Pro-Lys-Lys-Lys-Arg-Lys-Val
- Xenopus nucleoplasmin like NLSs:
  - Two basic regions separated by a spacer.
- Mat α2 amino terminal signal:
  - Lys-Ile-Pro-Ile-Lys

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Ambiguities

- Matching to the ‘classical’ NLS is very flexible.
- Likely to occur reasonably frequently
- Hence not a reliable prediction method.
- In recent years numerous other highly specialised NLSs have been discovered.

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Non-Classical NLSs

- Christophe *et al* (2000) Give a listing of non-classical sequences known to be involved in nuclear localisation.
  - varying lengths up to 38 residues.
  - None of which are particularly high in basic residues.
  - One example they site is high in acidic residues.
- NLSdb – Nair, Carter & Rost (2003)
  - Contains 308 entries.
    - 114 Experimentally determined NLSs
    - 194 Discovered through *in-silico* mutagenesis

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Machine Learning Models

- Models that predict Nuclear Localisation rely on information other than the presence of these known NLSs.
- Some form of sequence summary
  - K-mer
    - Amino Acid composition
    - Di-peptide composition
- In recent years a range of sophisticated ensemble models have emerged that include the nucleus among their predicted localisations.

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Independent Test Results

Model	Dataset	Accuracy	Sensitivity	Specificity	MCC
Nucleo V1.0	Dual	0.64	0.68	0.62	0.28
	Non-Dual	0.71	0.80	0.62	0.42
	All	0.70	0.76	0.62	0.38
LOCtree	Dual	0.64	0.55	0.68	0.22
	Non-Dual	0.66	0.64	0.68	0.32
	All	0.64	0.61	0.68	0.29
P2SL	Dual	0.62	0.35	0.74	0.10
	Non-Dual	0.62	0.49	0.74	0.24
	All	0.57	0.45	0.74	0.19
HSLPred	Dual	0.67	0.56	0.71	0.25
	Non-Dual	0.57	0.43	0.71	0.14
	All	0.57	0.46	0.71	0.18
predictNLS	Dual	0.71	0.21	0.93	0.20
	Non-Dual	0.62	0.30	0.93	0.29
	All	0.54	0.27	0.93	0.25
SubLoc	Dual	0.57	0.66	0.53	0.17
	Non-Dual	0.64	0.75	0.53	0.29
	All	0.64	0.72	0.53	0.25

Problems

- Incomplete domain knowledge
- Existing predictors often use training sets consisting of exclusively nuclear localised proteins.
  - However 20% of known nuclear proteins are dual localised.
- Some models rely on protein homology
  - Hence have questionable capacity to generalize to truly novel sequences

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An Ideal Model

- An ideal model for Nuclear prediction would be able to base its predictions on the correct identification of the NLSs
- To do this the model needs to be able to
  - Recognise the motifs in the sequence
  - Regardless of position
  - Recognise positional context
  - Allow for dependencies between regions

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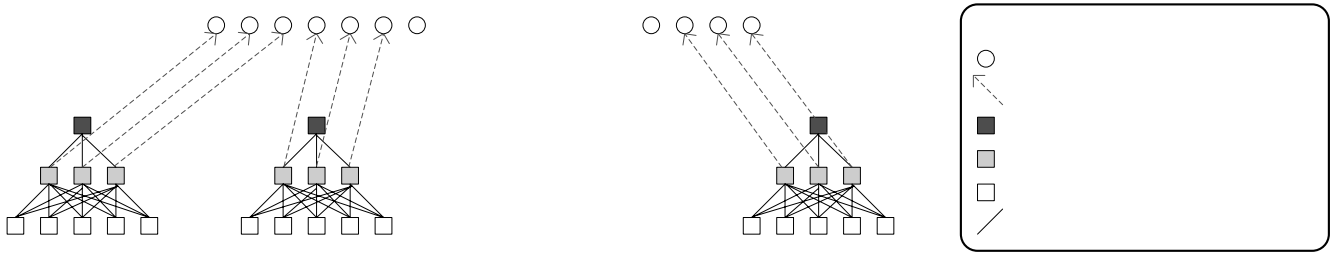
The PALFE Framework

- Proportionally Assigned Local Feature Extraction framework of neural networks
- Using an array of neural networks
- Each assigned a ‘local’ window of the sequence
  - Based on a proportion of the sequence length
- Each networks is trained to attempt the global task
  - Thus learning the ‘local’ features most pertinent to the ‘global’ task

The PALFE Framework cntd

- Each ‘local’ feature extraction network generates a feature vector in the hidden node activations of the network
- The feature vectors are concatenated to produce a global feature vector for the sequence



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Simulation Protocol

- Use feature vector to train SVM
- Five fold cross validation
- Compared against:
  - A set of standard sequence kernels
  - A normalised sequence fed into an SVM
  - A null model consisting of the concatenated input windows fed into an SVM

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Cross Validation Results

Model	Configuration	Accuracy	Sensitivity	Specificity	MCC
Mismatch	k=3,m=1	0.675	0.662	0.689	0.351
Substitution	k=3	0.686	0.671	0.703	0.374
Local Alignment		0.676	0.715	0.634	0.35
Spectrum	k=4	0.711	0.669	0.751	0.427
Spectrum	k=5	0.704	0.642	0.753	0.415
Composite Spectrum	k=1,4,5	0.749	0.759	0.76	0.497
Normalised	L=10	0.717	0.775	0.709	0.433
NULL-PALFE	10*31	0.658	0.72	0.657	0.313
PALFE FFNN	10*31	0.673	0.701	0.681	0.343
PALFE BiD-RNN	10*31	0.824	0.837	0.828	0.648

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PALFE Performance

- The BiD-RNN PALFE networks provide superior performance to all other models
- The FFNN PALFE networks perform only marginally better than the NULL model
  - And worse than the normalised sequence
- We have conducted several analyses of the structure of the BiD-RNN feature space
  - Suggests that they are better equipped to accommodate movement in the position of the motifs

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Independent Test Again

Model	Dataset	Accuracy	Sensitivity	Specificity	MCC
Nucleo V2.0	Dual	0.67	0.86	0.59	0.42
	Non-Dual	0.78	0.97	0.59	0.60
	All	0.79	0.93	0.59	**0.57**
Nucleo V1.0	Dual	0.64	0.68	0.62	0.28
	Non-Dual	0.71	0.80	0.62	0.42
	All	0.70	0.76	0.62	0.38
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Conclusion

- Our Final Model,
  - Accuracy of 0.79
  - MCC of 0.57
- According to our independent test it is the best current model of nuclear import prediction.
- We are now approaching the task of distinguishing between the dual and statically localised nuclear proteins.
- This will allow use to begin to focus in on predicting the regulatory proteome

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