Characterisation of the Nuclear Localisation Signals of Cytomegalovirus Major Immediate Early Proteins

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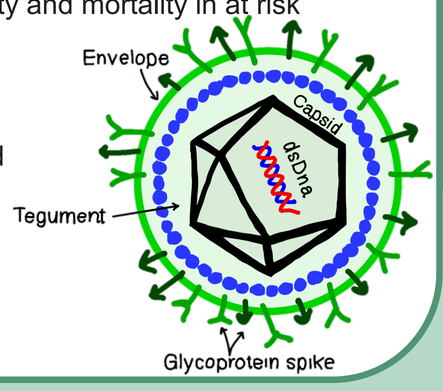
Background

What is HCMV?

- Human Cytomegalovirus (HCMV) is a ubiquitous pathogen belonging to the Herpesviridae, *Betaherpesviridae* subfamily
- Medically significant pathogen associated with a high disease burden and significant morbidity and mortality in at risk populations
- Limited treatment – antivirals associated with resistance and severe side effects
- No currently licensed vaccine



HCMV Immediate Early Proteins

- Events at the onset of replication are a novel therapeutic target
- Transcription of genome tightly regulated temporal cascade: Immediate Early (IE), Early, Late
- Initiation of lytic expression by immediate early proteins, IE1 and IE2
- Multifunctional nuclear phosphoproteins which act as promiscuous transactivators of viral and host genes

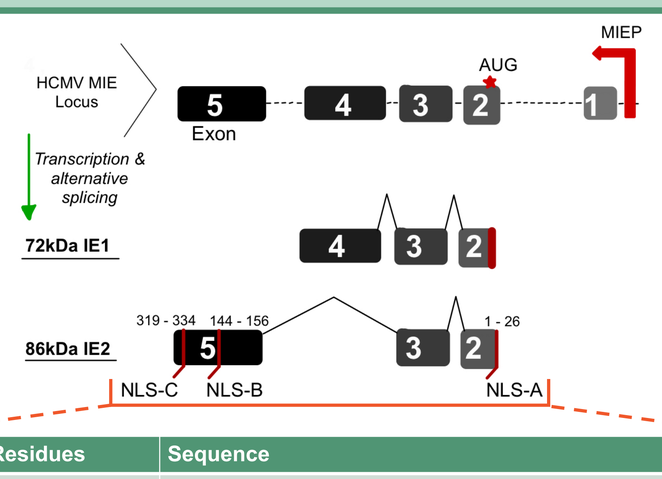
Nuclear Import of IE Proteins

- The nucleus is the site of HCMV replication and capsid assembly
- Entry of HCMV virion and IE1/2 proteins critical for successful infection
- IE proteins are known to affect functions within the nucleus and putative NLS have been identified
- Proteins larger than 40kDa are actively transported, usually via the classical nuclear transport pathway mediated by importins

Results

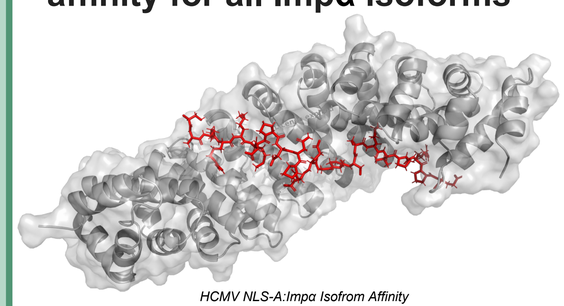
Three classical nuclear localisation signals were identified within immediate early protein 2 (IE2)

- Three classical nuclear localisation signals (cNLS) were identified using cNLS mapper
- Specific affinity of predicted NLS with importin alpha (Impα) isoforms was determined using fluorescence polarization assays
- All three NLS within IE1/2 bind all Impα isoforms tested, with highest affinity for Impα3
- The structure of these NLS were resolved in complex with Impα2
- HA-tagged full-length IE1 and IE2 localise to the nucleus. Mutation of key residues within NLS-A was sufficient to abolish nuclear localisation of IE1 but not IE2
- Additional IE2 specific NLS (NLS-B/C) must be sufficient to drive nuclear localisation of this essential viral protein



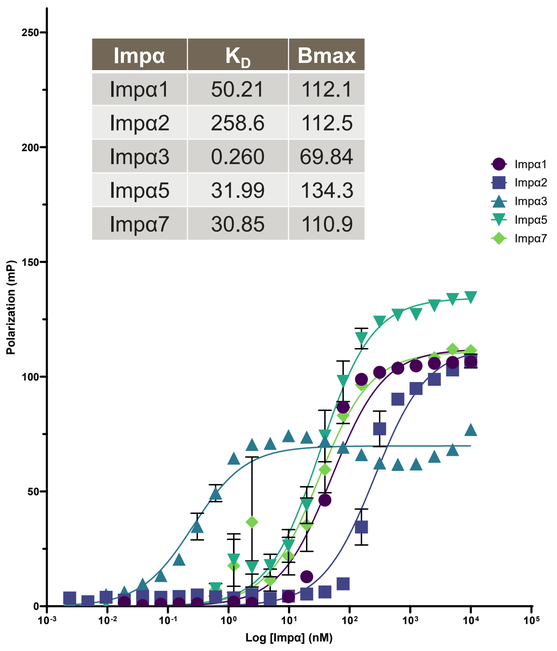
	Residues	Sequence	cNLS Type
NLS-A	1 - 26	MESSAKRKMDPDNPDEGPSSKVPRPE	Bipartite
NLS-B	144 -156	SPRKKPRKTTRPF	Monopartite
NLS-C	319 - 334	STGPRKKKSKRISELD	Monopartite

NLS-A functions as a bipartite NLS within IE1 and IE2 with affinity for all Impα isoforms

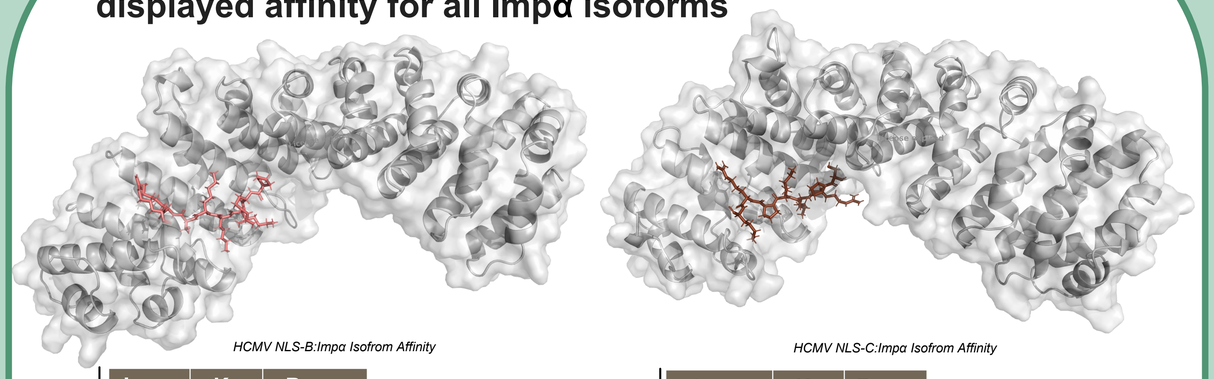


HCMV NLS-A:Impα Isofrom Affinity

Impα	K_D	Bmax
Impα1	50.21	112.1
Impα2	258.6	112.5
Impα3	0.260	69.84
Impα5	31.99	134.3
Impα7	30.85	110.9

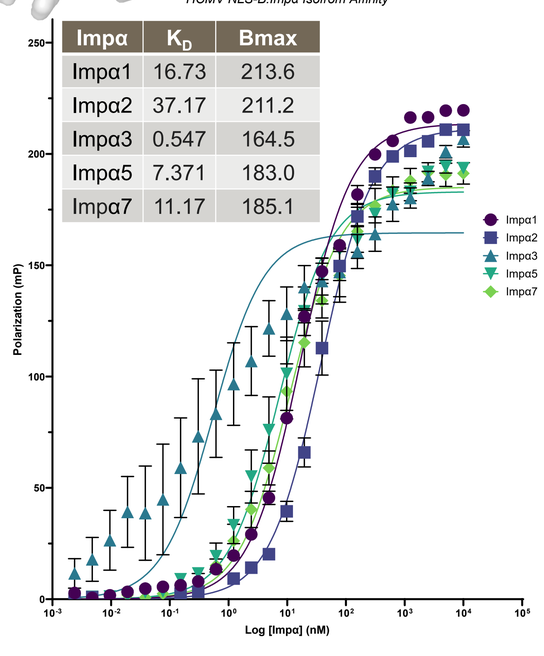


Two IE2 specific NLS were identified as monopartite and displayed affinity for all Impα isoforms



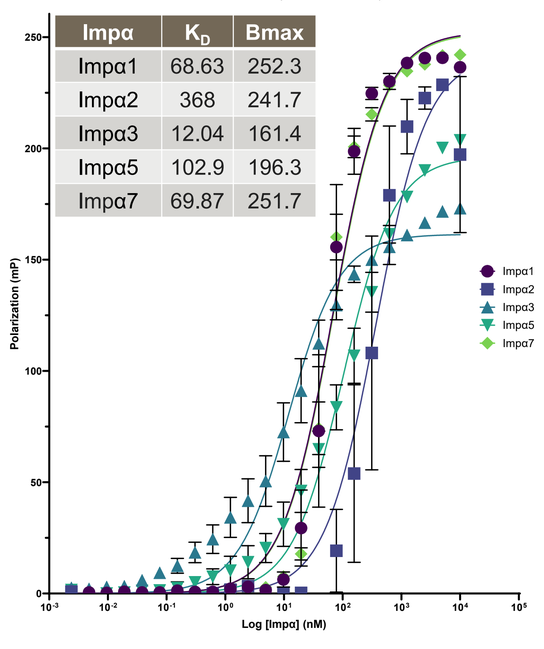
HCMV NLS-B:Impα Isofrom Affinity

Impα	K_D	Bmax
Impα1	16.73	213.6
Impα2	37.17	211.2
Impα3	0.547	164.5
Impα5	7.371	183.0
Impα7	11.17	185.1



HCMV NLS-C:Impα Isofrom Affinity

Impα	K_D	Bmax
Impα1	68.63	252.3
Impα2	368	241.7
Impα3	12.04	161.4
Impα5	102.9	196.3
Impα7	69.87	251.7



IE2 specific NLSs are sufficient to drive nuclear localisation in the absence of functional NLS-A

Mutations within NLS-A of full-length IE	
IE-ΔNLS	MESSA**AAA**MDPDNPDEGPSS**A**VP**A**P**A**

Conclusions

- Nuclear import of HCMV immediate early proteins IE1 and IE2 utilizes the classical Impα/β mediated import pathway. All three NLS predicted within these proteins showed highest affinity for Impα3, and overall strong affinity for all Impα (K_D <100nM).
- In the absence of the shared N-terminal NLS-A, IE1 is cytoplasmic while IE2 is still strongly nuclear localised. NLS-B and -C within IE2 appear functional and sufficient to mediate nuclear localisation of this essential viral protein.

The functional redundancy of NLS within IE2 highlights the critical importance of this protein and significance of this host:pathogen interaction for successful infection.

References