Peptide-MHC class I stability is a stronger predictor of CTL immunogenicity than peptide affinity

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Introduction

- Peptide-MHC Class I interaction has primarily been investigated in terms of affinity.
- >100.000 peptide-MHC class I affinity binding data
- netMHCPan: Prediction of peptide binding to MHC Class I

  "NetMHCpan, a method for quantitative predictions of peptide binding to any HLA-A and -B locus protein of known sequence"

  -Nielsen, M. et al. PLoS One, 2007
- Only 10-20% turns out to be immunogenic…
- Something is missing.

Stability and immunogenicity

Has been shown to correlate for:

- MHC-I:

  "Immunogenicity of peptides bound to MHC class I molecules depends on the MHC-peptide complex stability"

  - *van der Burg, S. H. et al. J Immunol, 1996*

  "MHC class I/peptide stability: implications for immunodominance, in vitro proliferation, and diversity of responding CTL"

  - *Busch, D. H. et al. J Immunol, 1998*

- And MHC-II:

  "The kinetic stability of MHC class II:peptide complexes is a key parameter that dictates immunodominance"

  - *Lazarski, C. A. et al. Immunity 2005*

- But only investigated small scale

Measuring Peptide-MHC-I stability

- Requires either:
  - labeling peptides
  - cumbersome separation steps

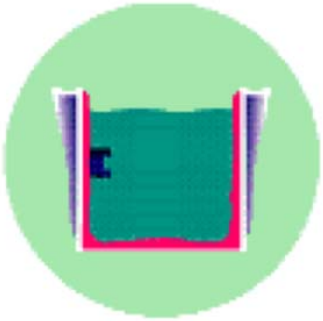
Observation made by KC Parker:

"The beta 2-microglobulin dissociation rate is an accurate measure of the stability of MHC class I heterotrimers and depends on which peptide is bound"

- Parker, K. C. et al. J Immunol 1992

- Radiolabeling of β_2m.
- Scintillation proximity assay

Scintillation proximity assay

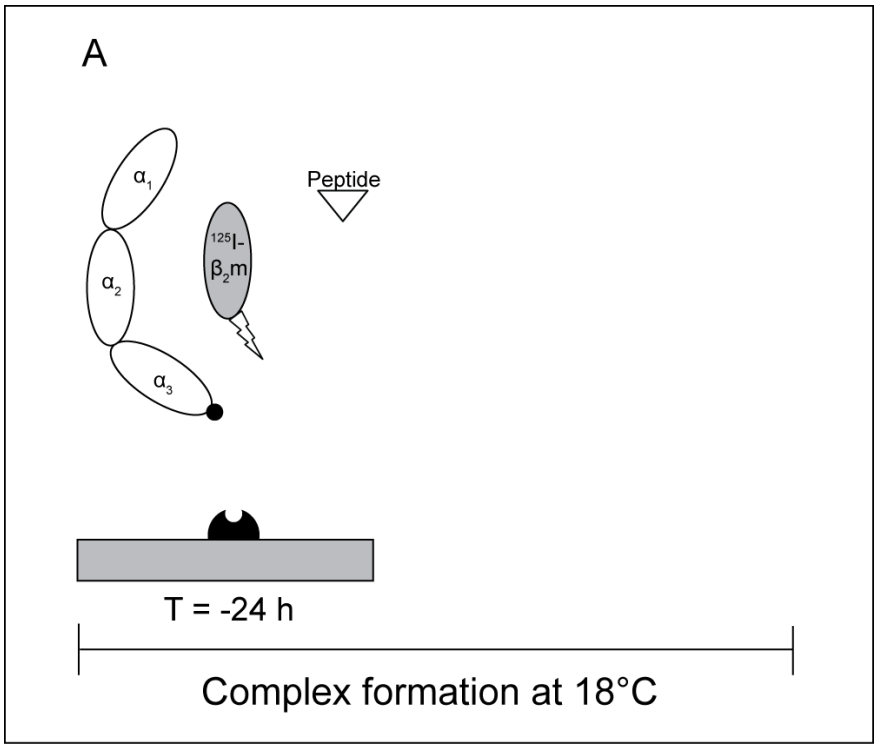


Step 1: Capture molecule (target) is coated onto the wells.

Step 2: Radioligand and samples are added and incubated.

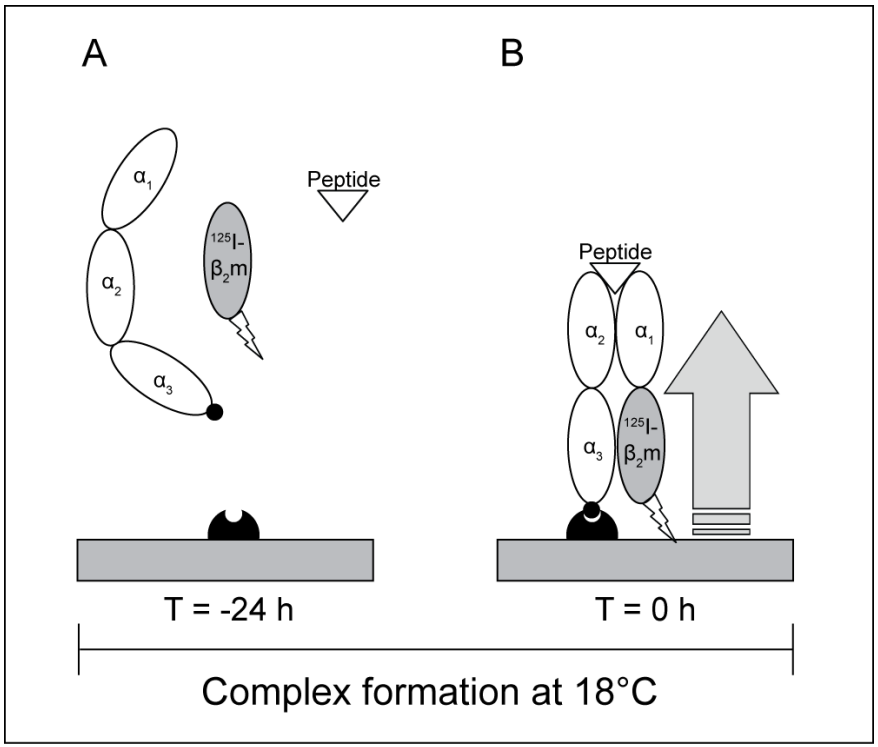
Step 3: Unbound radioligand is not detected. Radioligand bound to target is detected.

High-throughput dissociation assay (I)



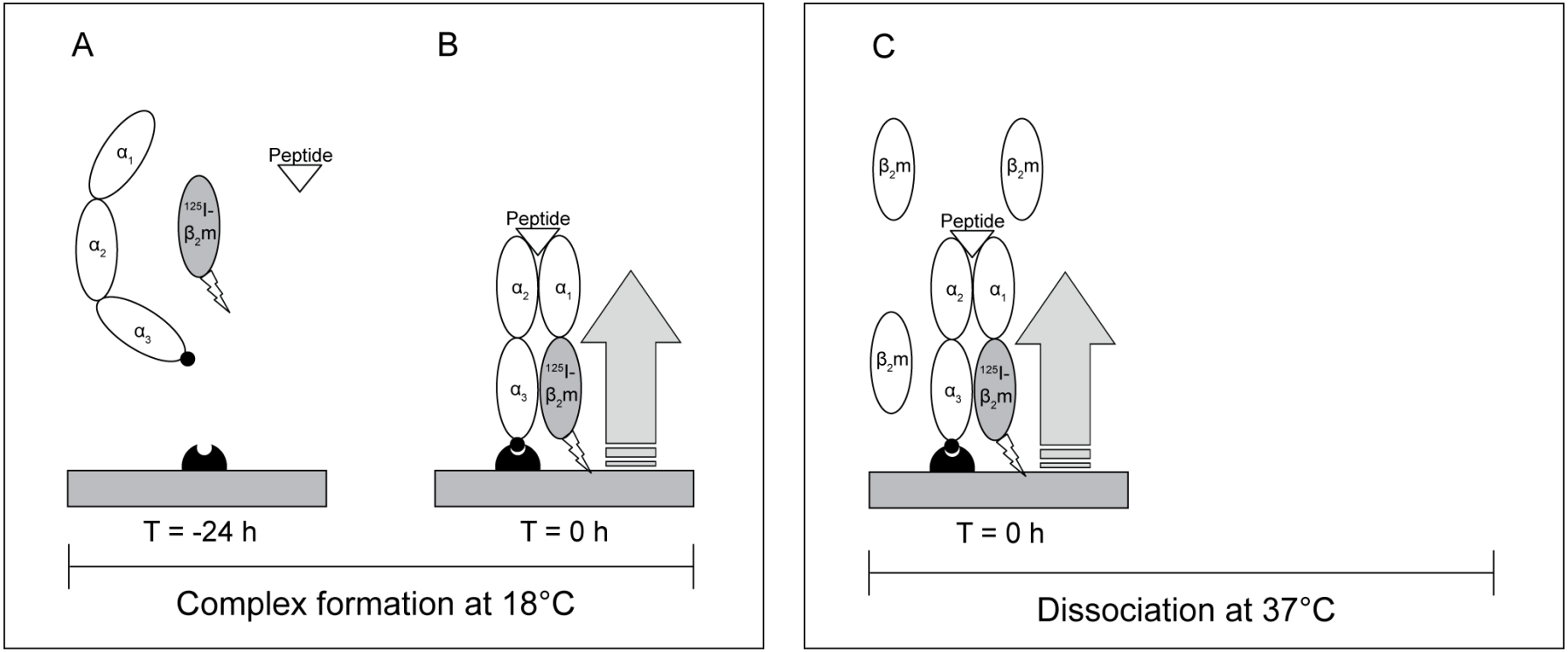
Harndahl, M, *et al., J. Immunol. Methods*, **2010**.

High-throughput dissociation assay (II)



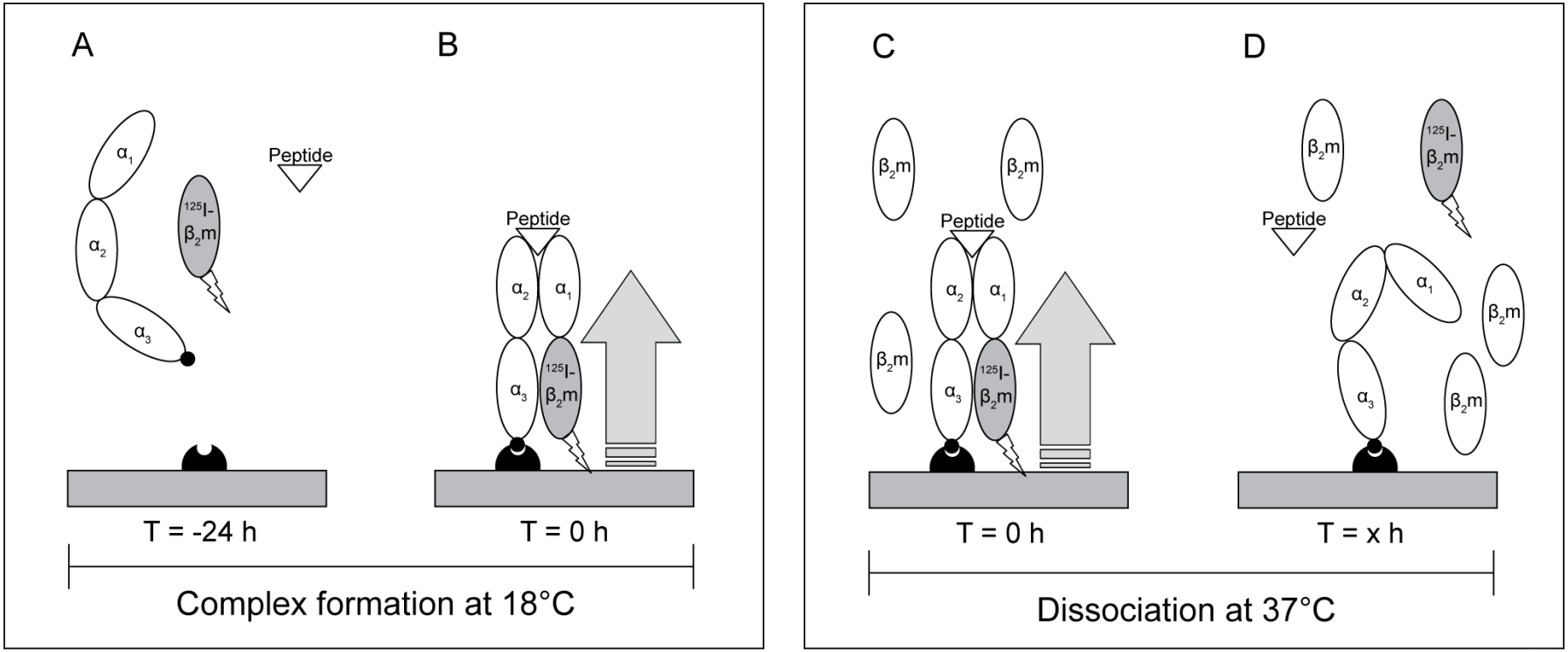
Harndahl, M, *et al., J. Immunol. Methods*, **2010**.

High-throughput dissociation assay (III)



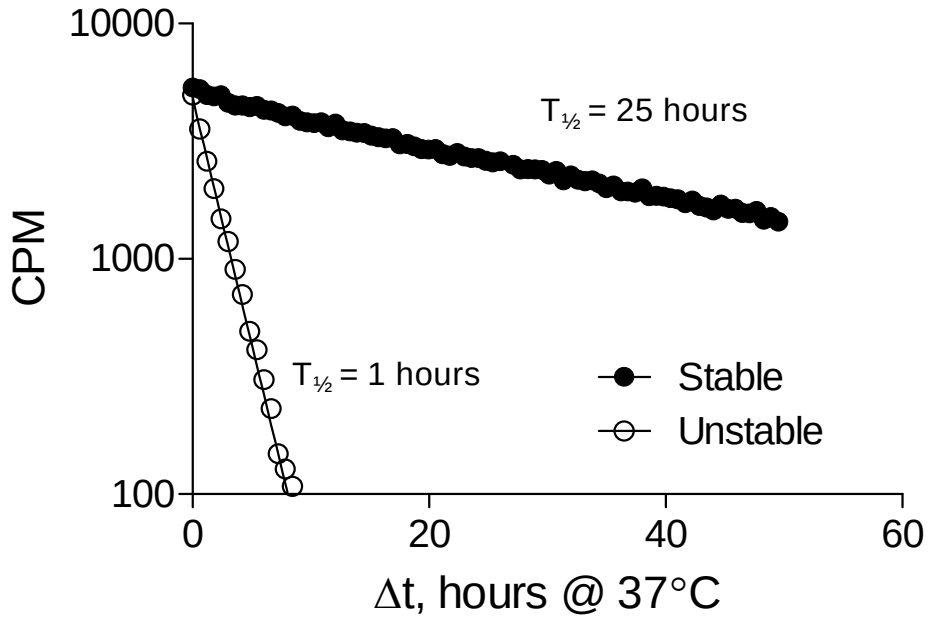
Harndahl, M, *et al., J. Immunol. Methods*, **2010**.

High-throughput dissociation assay (IV)

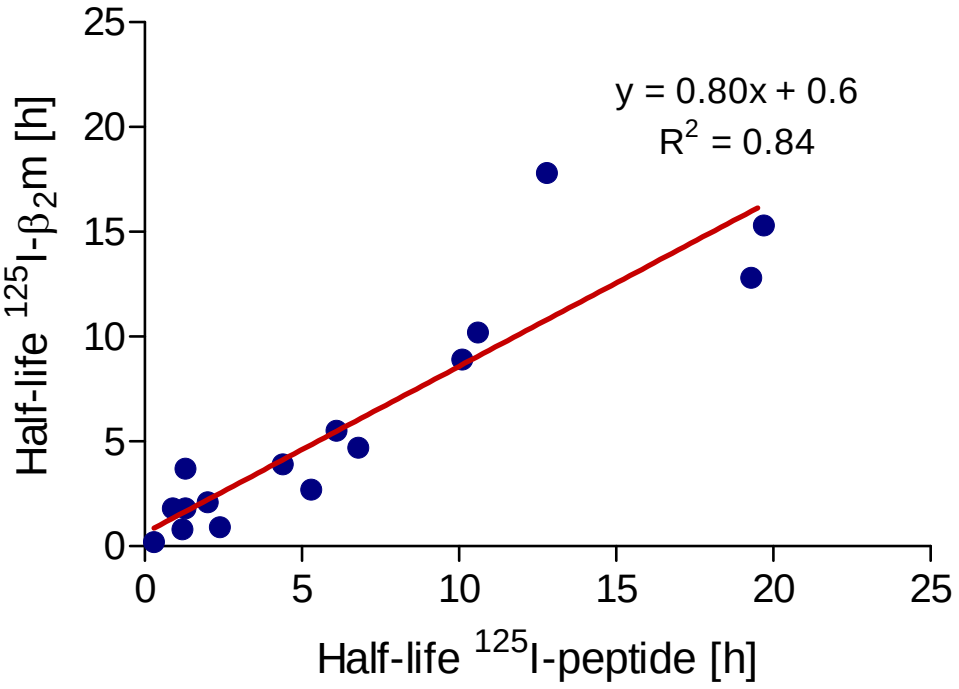


Harndahl, M, *et al., J. Immunol. Methods*, **2010**.

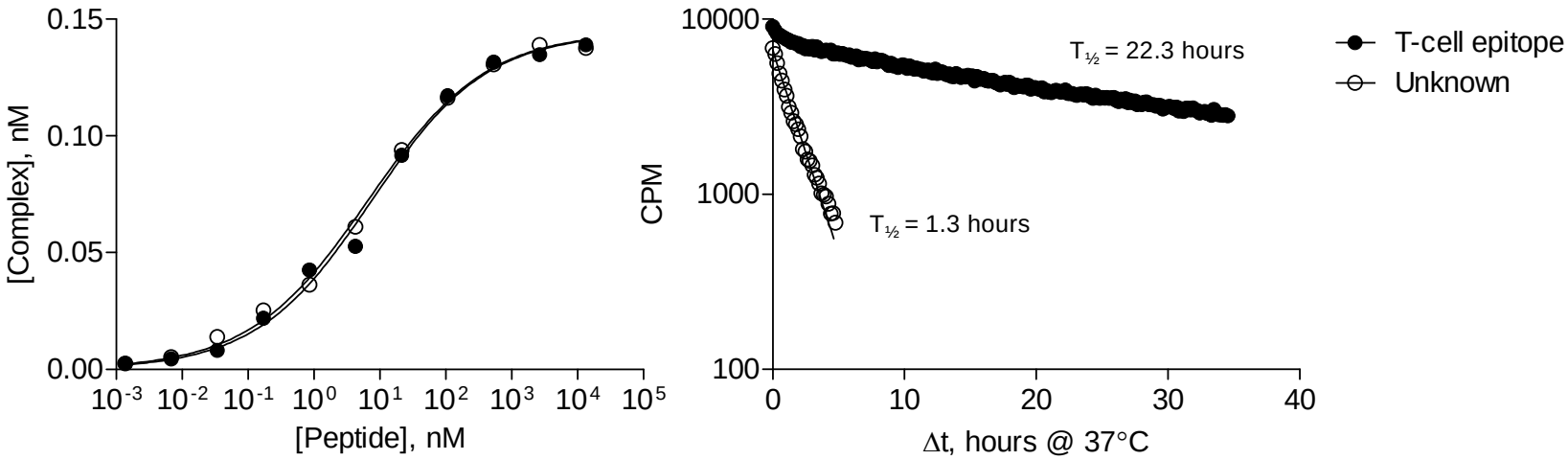
Dissociation curves



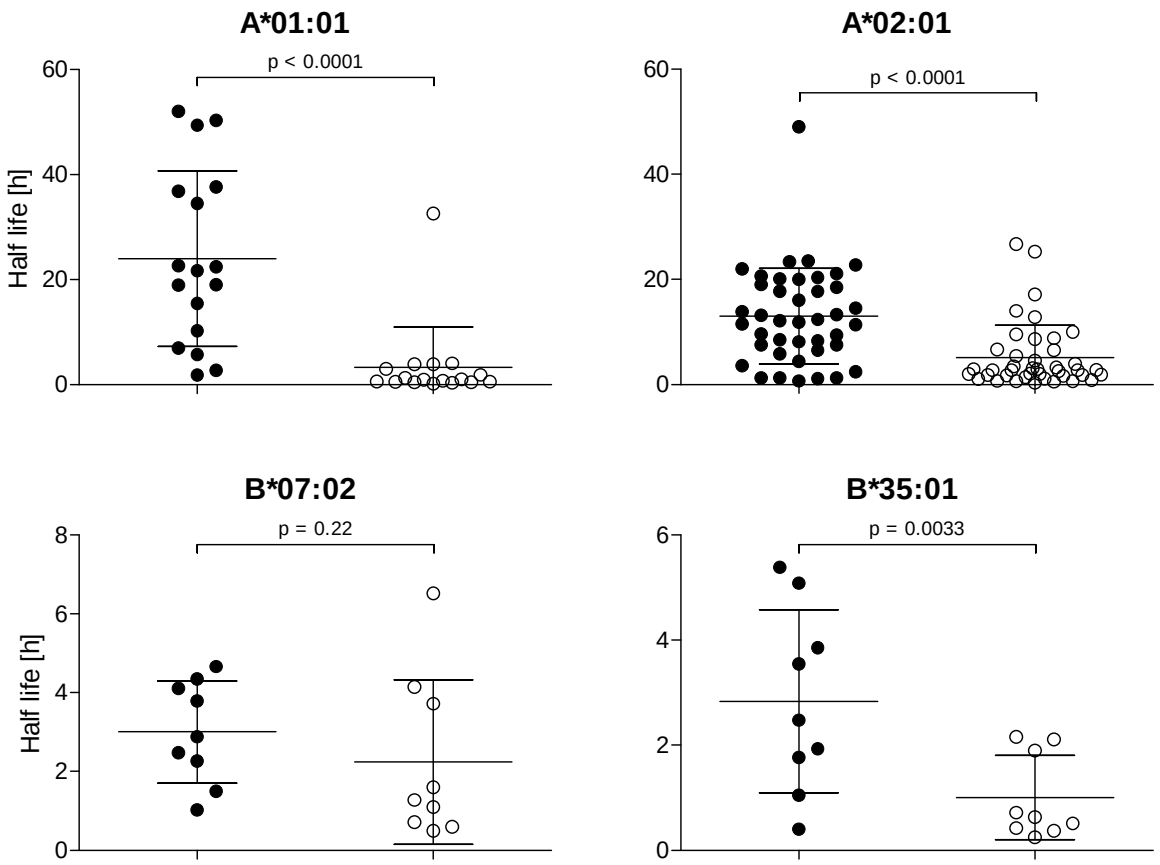
Correlation between labeled peptide and labeled β_2m



Same affinity. Different stability



Same affinity. Different stability



Affinity-matched pairs:

- ● Immunogenic
- ○ Non-immunogenic

Harndahl, M, *et al., European J. of Immunology. Paper accepted*

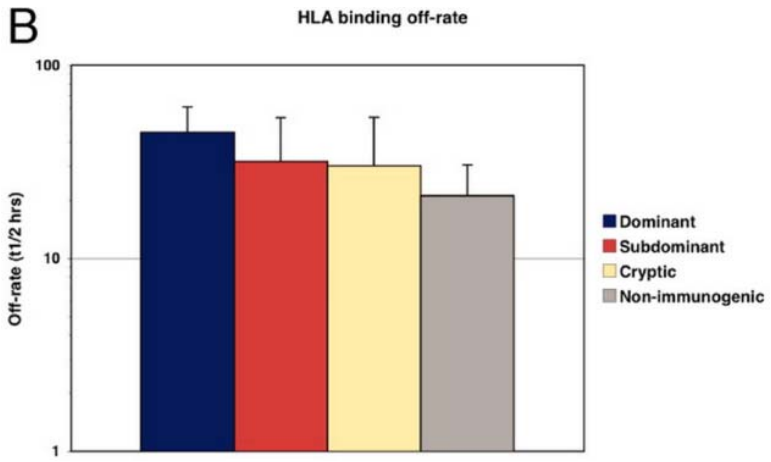
A Quantitative Analysis of the Variables Affecting the Repertoire of T Cell Specificities Recognized after Vaccinia Virus Infection[1]

Erika Assarsson,* John Sidney,* Carla Oseroff,* Valerie Pasquetto,* Huynh-Hoa Bui,* Nicole Frahm,† Christian Brander,† Bjoern Peters,* Howard Grey,* and Alessandro Sette[2]*

Many components contribute to immunodominance in the response to a complex virus, but their relative importance is unclear. This was addressed using vaccinia virus and HLA-A*0201 as the model system. A comprehensive analysis of 18 viral proteins recognized by $CD8^+$ T cell responses demonstrated that approximately one-fortieth of all possible 9- to 10-mer peptides were high-affinity HLA-A*0201 binders. Peptide immunization and T cell recognition data generated from 90 peptides indicated that about one-half of the binders were capable of eliciting T cell responses, and that one-seventh of immunogenic peptides are generated by natural processing. Based on these results, we estimate that vaccinia virus encodes ~150 dominant and subdominant epitopes restricted in by HLA-A*0201. However, of all these potential epitopes, only 15 are immunodominant and actually recognized in vivo during vaccinia virus infection of HLA-A*0201 transgenic mice. Neither peptide-binding affinity, nor complex stability, nor TCR avidity, nor amount of processed epitope appeared to strictly correlate with immunodominance status. Additional experiments suggested that vaccinia infection impairs the development of responses directed against subdominant epitopes. This suggested that additional factors, including immunoregulatory mechanisms, restrict the repertoire of T cell specificities after vaccinia infection by a factor of at least 10. ***The Journal of Immunology,*** **2007, 178: 7890–7901.**

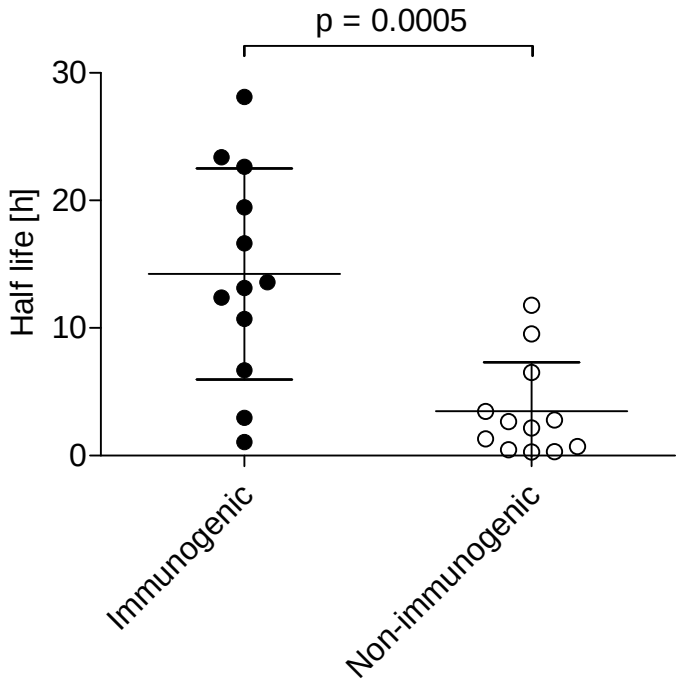
A Quantitative Analysis of the Variables Affecting the Repertoire of T Cell Specificities Recognized after Vaccinia Virus Infection[1]

- Using radiolabeled peptide (4 immunogenic and 3 negative)
- Correlation between immunogenicity and stability, but not significant



A Quantitative Analysis of the Variables Affecting the Repertoire of T Cell Specificities Recognized after Vaccinia Virus Infection[1]

- Using radiolabeled β_2m more peptides can be analyzed
- Affinity matched pairs



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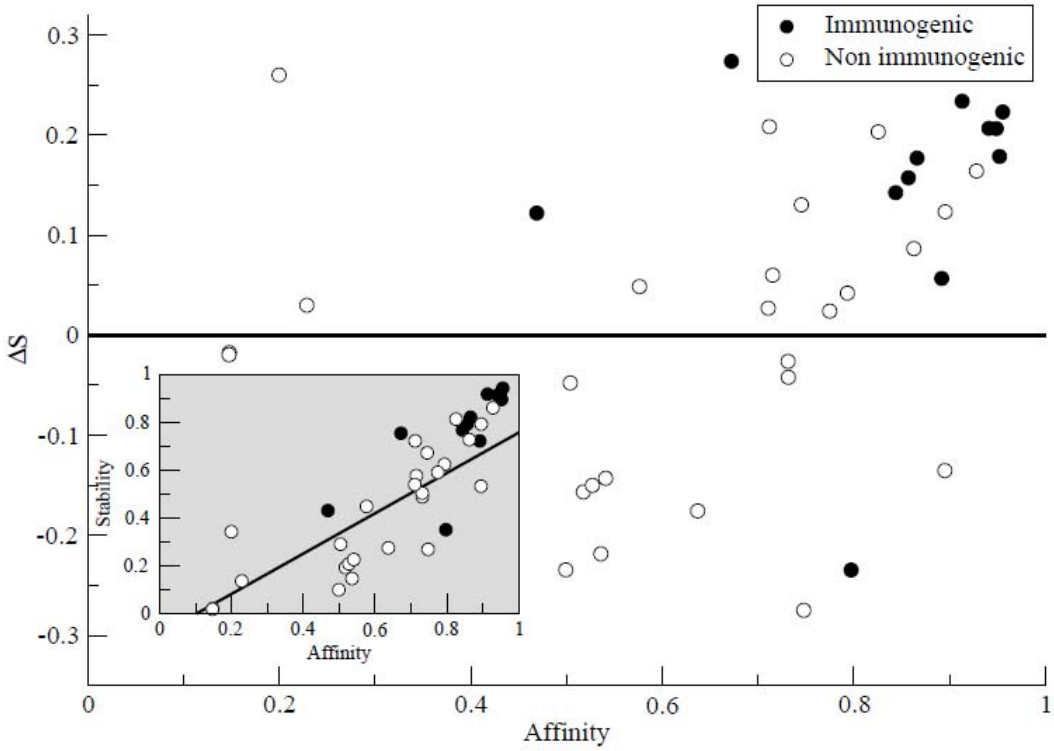
Predicting stability

Predictor based on 740 A*02:01 stability or affinity measurements



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Predicting stability

- Affinity-balanced analysis (e.g. neutralizing affinity and isolating the effect of stability) – highly significant correlation between stability and immunogenicity
- Stability-balanced analysis (e.g. neutralizing stability and isolating the effect of affinity) – no correlation between affinity and immunogenicity

Outlook

- Large scale stability data for more MHC-I alleles
- Incorporate pMHC-I stability into bioinformatic predictors
- MHC-II

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