

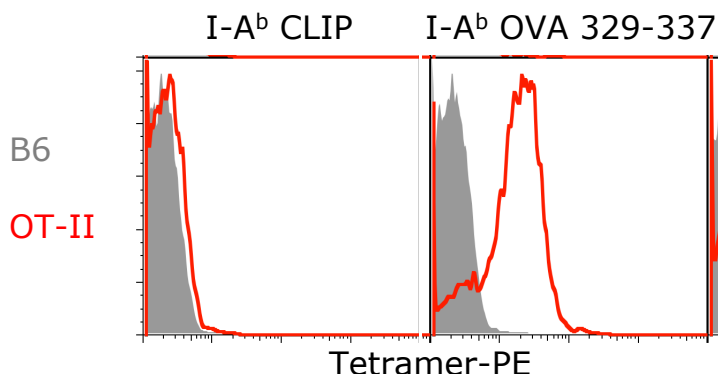
I-A^b OVA 323-339 tetramers, version 2.0

- Several groups have shown that the dominant OVA 323-339 epitope binds to I-A^b and I-A^d in multiple registers. This results in tetramers that are not multivalent, and therefore do not stain well.
- Luc Teyton's lab recently published new tetramer designs that fix this. OVA 323-339 is expressed as shorter peptides tethered to the I-A^b or I-A^d beta chain by a short, 7 amino acid linker.

Register#1 (324-332)	I	S	Q	A	V	H	A	A	H	A	E	I	N	E	A	G	R
Register#2 (329-337)	I	S	Q	A	V	H	A	A	H	A	E	I	N	E	A	G	R
Register#3 (326-334)	I	S	Q	A	V	H	A	A	H	A	E	I	N	E	A	G	R
Register#4 (327-335)	I	S	Q	A	V	H	A	A	H	A	E	I	N	E	A	G	R

Landais, et al. J. Immunol. 183:7949, 2009

- The Tetramer Facility has produced I-A^b tetramers with OVA 329-337 (register #2). This is the register that is recognized by the OT-II TCR (and DO11.10 in I-A^d). These tetramers stain OT-II T cells (below), and we expect them to stain cells from I-A^b mice immunized with OVA 323-339 or whole OVA protein but this has not yet been tested.
- Please optimize staining in your system according to our class II staining guidelines. In our hands, 37°C incubation was required.**
- It is important to note that immunization with OVA 323-339 or whole OVA protein will generate T cell responses against some of the other registers. This tetramer will most likely NOT stain these T cells. We are working on tetramers that will recognize these registers as well as I-A^d versions.
- Contact Rick Willis, Technical Director (richard.willis@emory.edu, 404-727-7215) with questions.



Spleen cells from B6 or OT-II mice were stained at 37°C with tetramers as shown. Histograms were gated on CD4⁺ Thy1⁺ events. **Staining was not observed at 4°C or room temperature.** Thanks to Pablo Romagnoli in the Altman lab for staining.