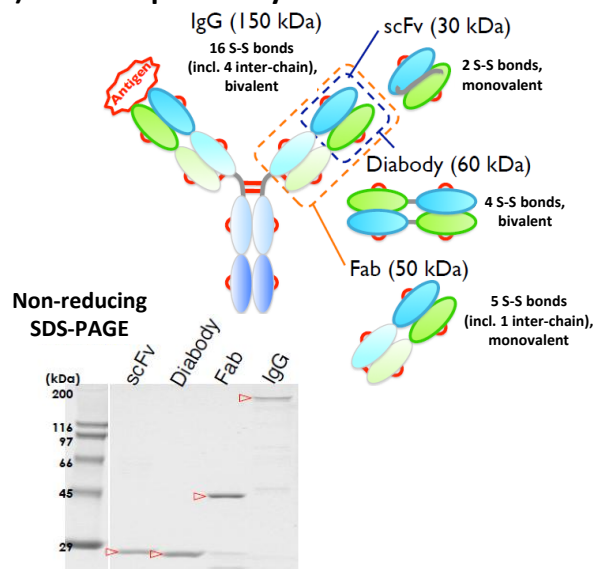


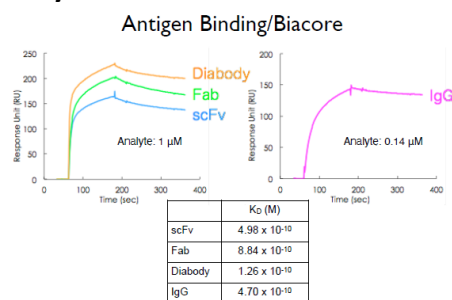
Application Note

Tocilizumab ScFv – NMR analysis

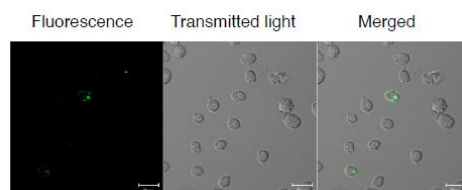
(a) Cell-free protein synthesis



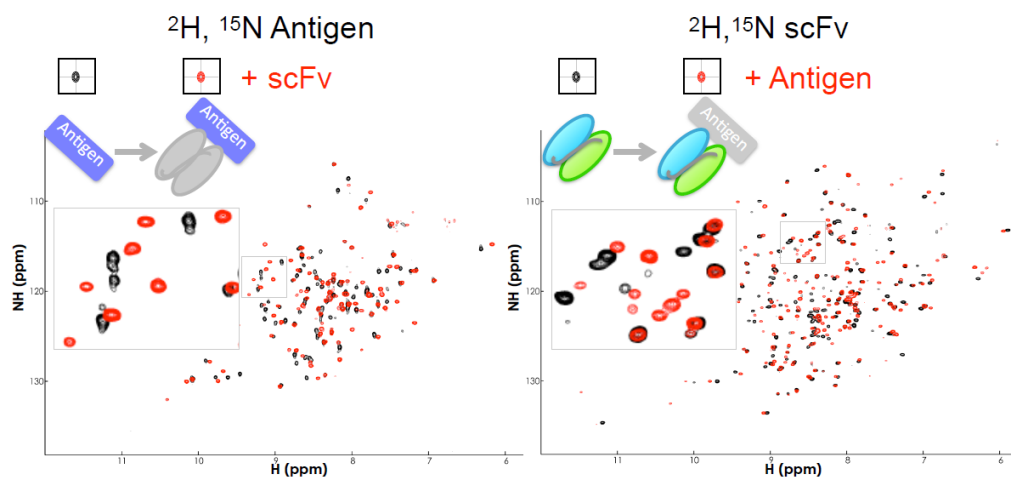
(b) SPR analysis



(c) Cells stained with fluorescently labeled scFv



(d) Antigen-scFv interaction analysis by NMR



Data provided by Dr. Kigawa (RIKEN); used with permission.



Summary

ScFv, diabody, Fab, and IgG formats of tocilizumab were produced by cell-free protein synthesis and evaluated. Antigen–scFv interactions were characterized by NMR.

- Cell-free protein synthesis was carried out under conditions optimized for disulfide bond formation containing 2% PEG, 0.4 mg/mL DsbC, 1 mM GSH and 4 mM GSSG. For Fab and IgG production, heavy and light chains were co-expressed. The synthesized proteins were purified and analyzed by non-reducing PAGE.
- Affinity for the antigen, IL-6R α domain 3(sIL-6R), was assessed by SPR using Biacore, revealing subnanomolar dissociation constants (K_D).
- Binding of the scFv to U266B1 cells (IL-6R positive) was evaluated using Alexa-488 labeled scFv. Cell staining images were acquired to visualize antigen-dependent binding.
- Antigen (sIL-6R)–scFv interaction was analyzed by NMR chemical shift perturbation experiments. The left panel shows the ^1H – ^{15}N TROSY-HSQC spectrum of ^2H , ^{15}N -labeled antigen alone (black) overlaid with that of the labeled antigen in the presence of unlabeled scFv (red). The right panel shows the ^1H – ^{15}N TROSY-HSQC spectrum of ^2H , ^{15}N -labeled scFv alone (black) overlaid with that of the labeled scFv in the presence of unlabeled antigen (red). Spectra were acquired with NS=16 at 700MHz and 298K using 0.1 mM ea. solutions of each protein.

