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VALIDATION OF A RECOMBINANT ANTIBODIES REPERTOIRE TO SELECT INTRACELLULAR REAGENTS FOR FUNCTIONAL PROTEOMIC APPLICATIONS

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Antibodies can mediate phenotypic knock-out of physiological and pathological functions. One of the major drawbacks in the use of antibodies as vehicles for *in vivo* interference is represented by the difficulty to functionally express in the cell cytoplasm these proteins that are naturally secreted. In fact, in this reducing compartment disulphide bonds cannot be formed and folding enzymes are absent, often resulting in insoluble protein aggregates that are rapidly degraded. Since disulphide bonds appear to contribute significantly to antibody stability, folding of most antibodies under reducing conditions does not normally lead to functional proteins. This is a serious restraint for applications that require a specific interference to cytoplasm-resident proteins.

A repertoire of intrinsically stable antibodies has been constructed in our lab, using the scaffold of a scFv antibody (scFvF8) with remarkable molecular properties. This molecule, derived from a mouse monoclonal antibody, was functionally expressed in the cytoplasm of bacteria, yeasts and plant cells and showed a long *in vivo* half-life. The thermodynamic characterisation of scFvF8 also demonstrated a very high intrinsic stability of this molecule. The derived repertoire ('F8 library') was generated according to a modelling-assisted design, resulting in a collection of 5×10^7 independent clones. Recombinant antibodies with new specificities and biochemical characteristics similar to the cognate antibody (high stability and solubility in the cytoplasm) have been selected from the 'F8 library'.

The aim of the present work was to assess if all single chain antibodies isolated from this library could really perform as "intrabodies", being able to recognize the antigen when expressed in the cytoplasm. The data reported highlight the peculiarity of this molecular repertoire from which it is always possible to isolate effective intrabodies, to be used in most functional proteomics applications.