

# One gene $\neq$ one protein

- Post-translational modifications
  - Phosphorylation, methylation, sulfation, ubiquitination, glycosylation, acetylation, poly ADP ribosylation, phosphoadenylation, palmitoylation
- Alternative splicing
  - Different exons can have different functions, e.g. Bcl-X<sub>L</sub> and Bcl-X<sub>S</sub>
  - Introns used as exons (PSA and PSA-LM: only share promoter and signal sequence, PSA-LM cds is fourth intron of PSA, no homology at all)
- Alternative and intein processing
  - Dentin phosphoprotein and dentin sialoprotein, two proteins cleaved from a single precursor protein, dentin sialophosphoprotein
  - obestatin and ghrelin both derived from pro-ghrelin opposite effects on appetite
- Antisense translation
  - 3 proteins encoded within the antisense strand of a neurofibromin gene
- Transplicing
  - *Modifier of mdg4* in drosophila transcribes 4 exons from one strand, 2 exons from the antisense strand joined together via region of homology
- Overlapping genes
  - 44 of 464 genes in Guillarda are overlapping
- RNA editing
  - RNA editing of apoB introduces a stop codon, leading to two forms of the protein, one liver, the other intestine specific

# Why use in vitro selected binders? or 21st vs 20th century technology

- Sequence the gene
  - Forever available and archivable (sequence)
  - Web based distribution (sequence)
  - Downstream uses (next slide)
  - In vivo protein knockouts
- Can affinity mature to picomolar
- Truly monoclonal, make multimeric or polyclonal or oligoclonal as desired
- Avoid animal welfare issues (Europe)
- Provide additional properties
  - Enzyme activity, fluorescence
  - Modulate properties with binding
- Not limited to epitopes recognized by immune system
  - Generic post-translational modifications
  - Greater variety of binders
- Can target specific epitopes both positively and negatively
- Molecularly homogenous
- Eventually cheaper?
- Higher throughput
- Standard selections yield  $\geq 10$  binders per target

# Downstream use

- Intracellular expression vectors
  - Functional knock-outs
- Functional domains
  - E.g. GFP, alkaline phosphatase, dimerization, multimerization, coiled coils
  - Fc fusions - will access all presently available IgG based technology
- C terminal cysteines
  - Targeting, biosensors
- Tandem affinity purification vectors
  - Immunoprecipitation and MS
- Tethering / immobilization domains
  - Arrays