

antibody might be similar to the signal generated from a small amount of very high affinity antibody. Two color detection can allow one to simultaneously measure, for example, the amount of secreted antibody and the amount of peptide bound by that antibody. By normalizing the bound peptide signal against the amount of antibody in the droplets, it is possible to accurately rank the antibodies according to binding affinity in some cases.

[0082] The present invention provides, in another aspect, a variety of assays and other applications of manipulating droplets containing cells that can secrete various species, such as antibodies, for example, hybridoma cells or non-immortal antibody-producing cells. For instance, droplets may be identified, determined, sorted, split, coalesced with other droplets, reacted, assayed, or the like, and other species may be added to the droplets in some cases. In some cases, such techniques will involve signaling entities or the like, as previously described.

[0083] As an example, in one set of embodiments, relatively similar molecules may be differentiated using antibodies or other species. It should be understood that, although cells are described in the context of secreting antibodies, that is only by way of example, and in other embodiments, other cells able to secrete other species (e.g., insulin, neurotransmitters, proteins, hormones, etc.) may be used instead of antibodies and antibody-producing cells.

[0084] In one embodiment, an antibody (or other species) may preferentially bind to a first target relative to a second target, even if the targets are substantially similar. For instance, an antibody-producing cell may be co-encapsulated in a fluidic droplet with a first target and a second target, where the antibody-producing cell secretes antibodies having an affinity to the first target and/or the second target. The targets may each be any potentially suitable target for the antibody, for example, a cell, a protein, an enzyme, a virus, or the like. In some cases, a difference in affinity between the antibody and the first target, and the antibody and the second target, may be desirable, and a plurality of fluidic droplets, some of which may contain antibody-producing cells, may be screened to determine those antibody-producing cells having a preferential affinity to the first target relative to the second target.

[0085] In one set of embodiments, fluidic droplets that contain at least two different, yet related targets (e.g., steroids with different chemical structures, or phosphorylated versus non-phosphorylated proteins or peptides) may be determined using antibodies or other species. The droplets may contain a species (e.g., an antibody) which can potentially bind to one or more of the targets. A first species may be determined that has a high affinity for one target (e.g., a desired target) but not to a second target (e.g., a competitive binding site that has a similar structure but is inactive). A variety of species (e.g., antibodies) may be tested, e.g., by using a variety of distinguishable cells that secrete the species. For instance, a first droplet may contain a first antibody-producing cell that secretes a first antibody, while a second droplet may contain a second antibody-producing cell that secretes a second antibody distinguishable from the first antibody, e.g., by configuration, sequence, structure, etc. Because each of the species are isolated (e.g., contained in separate droplets), a selectively-binding first species (e.g., that preferentially binds to the first target relative to the second target) can be distinguished from a second species that binds to both targets substantially equally, which may be undesirable. Accord-

ingly, the relative specificity of the species may be determined in some embodiments of the invention.

[0086] In one embodiment, droplets containing a species such as an antibody (e.g., produced by an antibody-producing cell) are determined, where the antibody may bind a first target preferentially relative to a second target. For instance, a plurality of droplets may be provided, where at least some of the droplets contain a single B-cell that secretes an antibody (or other species). The secreted antibody may be labeled with a first signaling entity (e.g., a tagged secondary antibody). The droplets may also contain two, three, four, or more target antigens that have a different characteristic, but which may potentially bind to the antibody secreted by the cell. The target antigens may each be labeled with a second signaling entity. In some cases, each of the targets is tagged with a different signaling entity.

[0087] To determine whether an antibody in a droplet has a high specificity for a desired target, one can observe the co-localization of signals produced by the signaling entities in each of the droplets. For example, co-localization of the first signaling entity (associated with the secreted antibody) and a second signaling entity associated with a first, desired target indicates that the antibody in this droplet has a high affinity for the desired target. If there are no other co-localized signals in this droplet, this may indicate that the antibody has high selectivity. On the other hand, if the droplet additionally contains co-localization of the first signaling entity with a signaling entity associated with a second target, this may show that the antibody has high affinity but low selectivity. Highly selective species, and cells that secrete such species, can be identified in this manner and then further manipulated if desired. For example, the cells producing such species may be ruptured and the DNA extracted and manipulated to generate replicated antibodies having both high affinity and selectivity for a target, as described herein.

[0088] For screens involving cells that secrete antibodies, the cells isolated by this type of screen may produce antibodies that are better functionally-characterized (e.g., have more selective affinity) than, for example, the cells that are isolated after the first steps of a typical hybridoma screen. More complex assays, resulting in more complete antibody characterization, can also be performed. For example, the target protein may be embedded in a lipid bilayer or in a cell membrane and cells can be selected only if the secreted antibodies performed in this context.

[0089] In another example, fluidic droplets may contain both a full-length wild-type target protein (e.g., labeled with cy3 dye) and mutant version of the target protein (e.g., a mutant at a key residue in the antibody binding site and labeled with cy5). The screen can identify and select droplets containing cells that secrete an antibody that binds the wild-type protein without binding the mutant protein (in these droplets, the cy3 dye may be concentrated on the protein bead and the cy5 dye may remain diffuse).

[0090] In embodiments in which there are at least two different targets inside a fluidic droplet, the targets may be related or non-related. Related targets may include, for example, a first protein or nucleic acid having at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% homology to a second protein or nucleic acid. For instance, a method of the invention may involve providing a fluidic droplet containing two targets, e.g., a first protein and a second protein having at least about 70%, at least about 80%, at least about 90%, at