and cell fate (Benoit et al. 2008; Frith et al. 2014; Zhu et al. 2015), while other studies have even functionalised hydrogels with larger proteins such as growth factors (Fan et al. 2007; Mehta et al. 2010) and recombinant extracellular matrix (ECM) proteins (Connelly et al. 2011; Martino et al. 2011; Martino et al. 2013).

The specific cross-linking strategies employed for bio-functionalization depends both on the chemistry of the material and the biomolecule itself. These reactions require good specificity, efficiency and stability in order to link a sufficient number of molecules to the right location on the hydrogel polymer and for the linkage to remain intact under physiologic conditions. Common reactive groups on the polymer include hydroxyl (Trmcic-Cvitas et al. 2009; Petrie et al. 2006; Costa et al. 2014), carboxyl (Rowley et al. 1999), and acrylate groups (Shin et al. 2002; Mann et al. 2001; Lee et al. 2008), while peptides are often accessed through primary amines or thiols (DeForest et al. 2009; Mann et al. 2001; Rowley et al. 1999; Lutolf et al. 2003). The carboiimide EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) reacts with carboxyl groups under aqueous conditions, and when combined with amine-reactive sulfo-N-hydroxysuccinimide (sulfo-NHS) (Hermanson 2008), it can be used to couple peptides to carboxyl-containing hydrogels (e.g., sodium alginate (Rowley et al. 1999)). Similarly, the compounds 4-nitrophenyl chloroformate (NPC) and N,N-disuccinimidyl carbonate (DSC) react with hydroxyl groups and can be used to couple primary amines of peptides to polymers (Hermanson 2008). Another key bio-functionalization reaction is the Michael-type addition of thiol groups in cysteine-containing peptides to methyacrylate groups in PEG-based polymers (Heggli et al. 2003). Likewise, derivatization of hydrogel materials with maleimide groups supports highly efficient coupling with thiols (Phelps et al. 2012; Hermanson 2008). These reactions, although not an extensive list, are some of the most common strategies for bio-functionalization and highlight the diversity of chemistries available for tuning hydrogel bioactivity.

Efficiency and specificity are key considerations when selecting a functionalization strategy, and recent developments in 'click-chemistry' reactions have made significant advances in this area. 'Click chemistry' refers to a class of reactions characterised by high selectivity, minimal by-products, and high efficiency under mild reaction conditions (e.g., physiological temperature and pressure). They include the azide-alkyne cycloaddition (Rostovtsev et al. 2002), as well as the copper-free variation (Baskin et al. 2007; Agard et al. 2004), which uses strained alkynes to avoid the need for toxic copper catalysts. In addition, radical-mediated thiol-ene reactions between free thiol groups and unstatured carbon-carbon bonds are also considered click chemistry type reactions and have been to functionalise a range of different biomaterials (DeForest et al. 2009; Costa et al. 2014; Hensarling et al. 2009). Recent work using norbornene reactive groups to covalently bind thiols and tetrazines have also shown great promise for dual hydrogel cross-linking and functionalization (Gramlich et al. 2013). Thus, there is currently a wide range of strategies for functionalization of hydrogels with biomolecules, and this toolbox provides great flexibility for engineering bio-active hydrogels.

The ultimate goal for bio-functionalization is to specifically control cellular responses to the hydrogel. For example, the presentation of cell adhesive ligands, such as the RGD motif, is particularly important for cell viability within hydrogels